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4th Edition
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e-BOOK OF ABSTRACTS

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PLENARY LECTURES

Exploring the nano-bio interface of biomimetic materials

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Recent advances in the field of material science have shown that biomaterials and biomimetic approaches can be employed to improve the functional properties of medical technologies. By controlling the biochemistry of the nano-bio interface of synthetic materials, it is possible to create biomedical platforms able to better interact with the complex biology of the human body. Through the cross-talk with endothelial, immune and stem cells and the regulation of local and systemic inflammation, we demonstrated that is possible to drive the accumulation of therapeutic payloads at the target site and to increase the functional recovery of tissues after trauma or degeneration. In particular, the seminar will discuss: 1- the development of nanocarriers endowed with a biological identity to improve circulation time, targeting, and drug delivery, and 2- the synthesis of scaffolds that mimic the composition and structure of native tissues to boost regenerative medicine applications.

How PROTAC degraders work: Molecular recognition and design principles

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Our laboratory uses molecular information on protein-protein interactions and protein degradation to discover novel therapeutics. Degrader molecules, also known as PROTACs (PROteolysis-Targeting Chimeras) recruit proteins to E3 ligases for targeted protein degradation. Formation of a ternary complex between the PROTAC, the E3 and the target leads to the tagging of the target protein by ubiquitination, and subsequent proteasomal degradation. In cancer, one such drug target is the E3 ubiquitin ligase VHL, which can be hijacked by PROTACs. Our lab solved crystal structures of VHL bound to fragments of its natural substrate and analysed it to design and synthesize novel small molecule ligands of VHL. We tethered the VHL ligand to a small molecule inhibitor targeting BRD4, a protein frequently deregulated in leukemia. The resulting PROTAC MZ1 bridges BRD4 with VHL and removes BRD4 from leukemic cells. Solving the structure of the ternary bridging complex, we unravelled how the PROTAC MZ1 glue BRD4 to VHL, illuminating structural and biophysical insights into PROTAC molecular recognition and mechanism of action. This fundamental understanding has enabled us to develop further small molecules for hard to target proteins and shown how to improve PROTAC activity.

Porous materials for biomolecules delivery

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Delivering nucleic acids and biomolecules for therapeutic purposes remains a great challenge because of their low cell uptake, instability in blood, difficulties in crossing body barriers.[1]
COVID-19 has shown that RNA delivery can be efficiently achieved using liposomes and a vaccine based on nanoparticles has been formulated. But what other type of porous materials can be used for biomolecules delivery? The talk will drive the participants through a short trip touching porous silicon nanostructures and hybrid silica nanostructures highlighting the problems and advantages of each choice.
Porous silicon nanoparticles have been recently explored for such use and their *in vivo* applications have shown their possible role as carriers also in view of their biocompatibility. The high porosity of these structures allows large amount of RNA entrapment and the structures prevent the degradation of the biomolecule and allow the specific drug release in a targeted area [2].
In contrast with such high porous silicon materials silica nanoparticles incorporating synthetic nucleic acids and analogs as constitutive components of the organosilica structures are discussed. Interestingly different nanomaterials containing single-stranded nucleic acids that are covalently embedded in the silica network, that respond to various biological, physical, and chemical inputs through detectable physicochemical changes.[3] Also supramolecular organo-silica systems based on PNA- derivatives that can self-assemble through direct base paring or can be joined through a bridging functional nucleic acid, such as the ATP-binding aptamer are reported.

References

[1] Zehua Liu, Hélder A. Santos, et al. *Advanced Drug Delivery Reviews*, **2021**, *174*, 576-612,
[2] Byungji Kim, Ji-Ho Park, Michael J. Sailor Adv. Mater., **2019,** *49*, e1903637.
[3] Pierre Picchetti, Luisa De Cola et al. *J. Am. Chem. Soc*. in press.

INVITED LECTURES

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Beating organs-on-chip as innovative technological platforms for drug discovery: advanced in vitro models of human physiology and pathology

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Mimicking *in vitro* relevant mechanical cues still represents a breakthrough to improve the modelling of cardiac pathophysiological states, with the aim to elucidate pathological mechanism and increase efficiency of the drug developmental pipeline. Here we present uBeat® Platform[1], the first beating organ-on-chip providing a finely controlled mechanical stimulation to 3D microtissues. uBeat® was exploited to: *i)* generate a physiological beating cardiac model (uHeart) for screening drug cardiotoxicity and *ii)* a scar-like pathological model (uScar) for studying cardiac fibrosis.

A heartbeat-like mechanical stimulation (10-12% uniaxial strain, 1Hz) was exploited to mature physiological cardiac microtissues, generated from human induced pluripotent stem cell derived cardiomyocytes (75%) and human dermal fibroblasts (25%). Microtissues' functionality was evaluated by on-line measuring electrophysiological properties through an innovative integrated electrical system, µECG[2]. Cardiac microtissues spontaneously beat as a syncytium after 7 days (RR of 1.7±0.45s, field potential duration FPD of 0.6±0.2s). Drug screening studies were successfully conducted on 11 compounds to calibrate uHeart as predictor of functional cardiotoxicity[3]: as an example, aspirin did not change the FPD, while Sotalol (from 15µM) prolonged and Verapamil (from 50nM) shorten cardiac repolarization.

By applying the same mechanical stimulation to a 3D model encompassing only cardiac fibroblasts[4], key traits of scar formation were successfully recapitulated, without the need of supra-physiological doses of pro-fibrotic TGFβ1. Mechanical stimulation was sufficient to increase cell proliferation, promote α-SMA expression (90.2±3.7%) indicating fibroblasts-to-myofibroblasts transition and increase production of scar-like ECM rich in collagen type-I and fibronectin. Standard of care anti-fibrotic drugs (i.e. Pirfenidone and Tranilast) were confirmed to be efficient in preventing the onset of fibrotic traits in uScar. Conversely, the mechanical stimulation applied to the microtissues limited the ability of a miRNA therapy[5] to directly reprogram fibroblasts into cardiomyocytes, despite its proved efficacy in 2D models. Such results demonstrate the importance of incorporating in vivo-like stimulations to generate more representative 3D in vitro models able to predict therapies' efficacy in patients.

Integration of 3D mechanical microenvironment resulted in cardiac miniaturized models with enhanced functionality and resemblance to pathological states. uBeat® is highly versatile and applicable to any organ in which mechanical stimulation exerts a pathophysiological state[6], thus representing a new powerful tool for in vitro drug screening and disease modelling.

References

[1] Marsano et al., *LabChip*, 2016
[2] Visone et al., *Biofabrication*, 2021
[3] Visone et al., *Toxicological sciences*, 2023
[4] Occhetta et al., *Integrative Biology*, 2018.
[5] Paoletti et al., *Cells*, 2022
[6] Occhetta et al., *Nature Biomed Eng*, 201

Non-conventional targeting of non-conventional targets

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G-quadruplex (G4) are non-canonical DNA or RNA secondary structures, which are emerging as prosing targets in the development of innovative anticancer agents. In the search of selective G4-targeting chemotypes, natural compounds have been thus far poorly explored, though representing appealing candidates due to the high structural diversity of their scaffolds. A high diversity in house library composed of ca. one thousand individual natural products was investigated through a combination of molecular modelling and experimental assays. Five hit binders of telomeric and oncogenic G4s, i.e., Bulbocapnine, Chelidonine, Ibogaine, Rotenone and Vomicine were identified. Biophysical studies unambiguously demonstrated the selective interaction of these compounds with G4s compared to duplex DNA. The rationale behind the G4 selective recognition was suggested by molecular dynamics simulations. From biological assays, Chelidonine and Rotenone emerged as the most active compounds of the series against cancer cells, also showing good selectivity over normal cells. In a follow-up optimization study, some analogues of bioactive G4 binders were tested. Among them, Dicentrine was found to thermally stabilize telomeric and oncogenic G-quadruplexes without affecting the control duplex. Molecular dynamics simulations indicated that Dicentrine preferentially binds the G-quadruplex groove or the outer G-tetrad for the telomeric and oncogenic G4s, respectively. Finally, biological assays proved that Dicentrine is highly effective in promoting potent and selective anticancer activity by inducing cell cycle arrest through apoptosis, preferentially targeting G-quadruplex structures localized at telomeres. Taken together, these data validate a few natural products as putative anticancer candidates that target selectively cancer-related G4 structures.

References

[1] Int J Mol Sci. 2023 Feb 17;24(4):4070
[2] Pharmaceutics. 2021 Oct 3;13(10):1611

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Selective Targeting of the PI3K-mTOR Pathway for Cancer Treatment

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Keywords: kinase inhibitors, selectivity, stability, mTOR inhibitors, covalent molecules

Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/Akt) - mechanistic target of rapamycin (mTOR) axis are considered valuable assets in cancer therapy. Despite many pan-PI3K inhibitors have been investigated as anticancer agents, most of them failed in clinical trials due to on-target metabolic side effects such as hyperglycemia and hyperinsulinemia. To overcome the limitations of pan-PI3K inhibitors we developed (i) highly selective mTOR inhibitors, and (ii) selective PI3Kα covalent molecules.

In 2018, we discovered PQR620, the first-in-class brain-penetrant ATP-competitive mTOR inhibitor (TORKi) able to attenuate epileptic seizures in a mouse model of Tuberous Sclerosis Complex (TSC).[1] Despite promising results in rodent disease models, the limited stability of PQR620 in human hepatocyte assays and short half-live in pharmacokinetic studies in Cynomolgus monkeys, prevented its entry into clinical development. Aiming to develop follow up compounds for PQR620, we have disclosed a conformational restriction strategy and discovered the first pyrimido-pyrrolo-oxazine highly selective TORKi (PQR617).[2] While the first-generation tricyclic compounds displayed a limited brain penetration, investigation on the heteroaromatic ring led to second generation pyrimido-pyrrolo-oxazines with predicted BBB permeability.[3] In addition, we combined pharmacophore features of PQR620 and PQR617, and identified PQR626. PQR626 displayed an excellent brain penetration, very good tolerability in mice and was able to significantly prevent mortality in *Tsc1*^{GFAP}CKO mice.[4] Recently, we explored 3,6-dihydro-2H-pyran and tetrahydro-2H-pyran as isosteres of the morpholine moiety to unlock a novel chemical space for TORKi generation.[5] Overall, we exploited a variety of chemical strategies to identify metabolically stable mTOR inhibitors for the treatment of cancers and neurological disorders.

In parallel, we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309, bimiralisib), into highly selective PI3Kα-covalent inhibitors aiming to minimize the on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase target selectivity by covalently targeting a PI3Kα non-conserved nucleophilic amino acid side chain, namely Cys862. A reactive moiety, so called warhead, has been introduced into a chemically modified bimiralisib. The development of isoform-selective covalent compounds represents a major step towards an increased local and temporal control of PI3K in precise and innovative cancer therapy.[6]

References

[1] Rageot, D. et al. Discovery and Preclinical Characterization of 5-[4,6-Bis({3-oxa-8-azabicyclo[3.2.1]octan-8-yl})-1,3,5-triazin-2-yl]-4-(difluoromethyl)pyridin-2-amine (PQR620), a Highly Potent and Selective mTORC1/2 Inhibitor for Cancer and Neurological Disorders. J Med Chem. 2018, 61 (22), 10084-10105.
[2] Borsari, C. et al. A Conformational Restriction Strategy for the Identification of a Highly Selective Pyrimido-pyrrolo-oxazine mTOR Inhibitor. J Med Chem. 2019, 62 (18), 8609-8630.
[3] Borsari, C. et al. Second-generation tricyclic pyrimido-pyrrolo-oxazine mTOR inhibitor with predicted blood-brain barrier permeability. RSC Med Chem. 2021, 12 (4), 579-583.
[4] Borsari, C. et al. 4-(Difluoromethyl)-5-(4-((3*R*,5*S*)-3,5-dimethylmorpholino)-6-((*R*)-3-methylmorpholino)-1,3,5-triazin-2-yl)pyridin-2-amine (PQR626), a Potent, Orally Available, and Brain-Penetrant mTOR Inhibitor for the Treatment of Neurological Disorders. J Med Chem. 2020, 63 (22), 13595-13617.
[5] De Pascale, M. et al. Investigation of morpholine isosters for the development of a potent, selective and metabolically stable mTOR kinase inhibitor. Eur J Med Chem. 2023, 248, 115038.
[6] Borsari, C. et al. Covalent Proximity Scanning of a Distal Cysteine to Target PI3Kα. J Am Chem Soc. 2022, 144 (14), 6326-6342.

ORAL COMMUNICATIONS

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Peptides as a targeting strategy to eradicate Pancreatic Ductal Adenocarcinoma (PDAC)

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Keywords: Pancreatic Ductal Adenocarcinoma, Peptides, Mitochondria, Ion channels

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a devastating neoplasm whose incidence is continuously rising. Available therapeutic options obtain only a disappointing increase in overall survival and chemo-resistance is frequently developed. One of the main causes of therapeutic failure is the presence of a dense stroma, which prevents the drug from reaching the optimal concentration. A promising strategy to increase the levels of the drug in the tumor site and to specifically target cancer relies on the use of peptides recognizing proteins over-expressed by cancer cells. We previously exploited this technique for the brain delivery of PAPTP, a novel anti-cancer drug acting as an inhibitor of the voltage-gated potassium channel 1.3 (Kv1.3) [1]. Results obtained in an orthotopic PDAC model showed that PAPTP, is able to reduce tumor volume and mass by 70% and 64%, respectively [2].

AIM OF THE PROJECT

To synthesize different PAPTP conjugates with PDAC-targeting peptides and to test *in vitro* and *in vivo* their targeting potential (i.e., increased concentration/selective accumulation in the tumor).

METHODS AND RESULTS

The workflow of the project is based on the continuous synthesis and screening of the conjugates, in order to identify the most efficient ones for the delivery to cancer cells. The screening performed so far involved the targeting of the following proteins: Neuropilin-1 (NRP-1) and its co-receptors VEGFR-2 or Integrin-β5, Low-Density Lipoprotein Receptor (LDLR), Gastrin Receptor (GR) and Gastrin Releasing Peptide-1 Receptor (GRP-1R). For each protein we tested its expression in different PDAC cell lines and in tumor tissue from Pan02 orthotopic mouse tumor; peptides were conjugated to PAPTP through a bio-reversible carbamate bond. The conjugates were screened for their blood stability and for their possible hemolytic effect. Fluorescent-tagged conjugates were used to study cellular uptake [3]. At the end of this screening, *in vivo* tissue distribution of the most suitable candidates was assessed.

Among the several conjugates tested, the one bearing a GR-targeted peptide is giving the best results and is under investigation in a mouse model of orthotopic PDAC. The pharmacokinetic analysis conducted so far show that this conjugate is well accumulated in the tumor tissue, sparing the other organs such as heart and liver. Moreover, kinetics of distribution and elimination of the conjugate were interestingly slower compared to PAPTP. These results set the bases for efficacy studies, which are currently ongoing.

CONCLUSION

The conjugation of PAPTP with peptides targeting proteins selectively expressed by PDAC seems to be a good strategy to improve the pharmacokinetic profile of this drug, which has already shown promising results against this cancer.

References

[1] Parrasia, S., Rossa, A., Varanita, T., Checchetto, V., De Lorenzi, R., Zoratti, M., ... & Biasutto, L. (2021). An Angiopep2-PAPTP Construct Overcomes the Blood-Brain Barrier. New Perspectives against Brain Tumors. Pharmaceuticals, 14(2), 129.
[2] Li, W., Wilson, G. C., Bachmann, M., Wang, J., Mattarei, A., Paradisi, C., ... & Patel, S. H. (2022). Inhibition of a mitochondrial potassium channel in combination with gemcitabine and abraxane drastically reduces pancreatic ductal adenocarcinoma in an immunocompetent orthotopic murine model. Cancers, 14(11), 2618.

[3] Parrasia, S., Rossa, A., Roncaglia, N., Mattarei, A., Honisch, C., Szabò, I., ... & Biasutto, L. (2023). DA7R: A 7-Letter Zip Code to Target PDAC. Pharmaceutics, 15(5), 1508.

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Development of PET imaging tracers for c-Met aberrant tumors using ^{68}Ga chelators

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Keywords: c-Met; Gallium-68 tracers; HGF; peptides, PET imaging

c-Met is a receptor tyrosine kinase which, after activation by its ligand, the hepatocyte growth factor (HGF), mediates a range of intracellular signalling pathways including some related to proliferation, motility, migration and invasion of cancer cells. Aberrant HGF/c-Met signalling is involved in the development and metastatic progression of several tumor types [1]. Thus, this protein receptor is a key player in cancer initiation and progression. For this reason, monitoring of c-Met expression in real time is likely to assist in the diagnosis and the monitoring of response to therapy [2]. Positron emission tomography (PET) imaging represents one of the most promising clinical tools for the in vivo real-time monitoring of abnormal alterations of c-Met and for the diagnosis of c-Met related cancers. Here we present the rational design and synthesis of a library of novel peptide-chelator bioconjugates potentially able to effectively target c-Met and to efficiently bind gallium-68, and therefore to make the cells visible in the positron emission tomography. In fact, the main feature of ^{68}Ga is that it undergoes a spontaneous radioactive decay and releasing a positron, which is then measured by common hospital PET scanner. Moreover, the use of ^{68}Ga as radioactive species would abolish the costly infrastructures related with ^{18}F and ^{11}C production and the more complex synthetic chemistry procedures involved in radiolabeling [3]. Non-invasive PET imaging with the developed tracers will support powerful tools for the detection and monitoring of the most common and lethal cancers among Europe and worldwide.

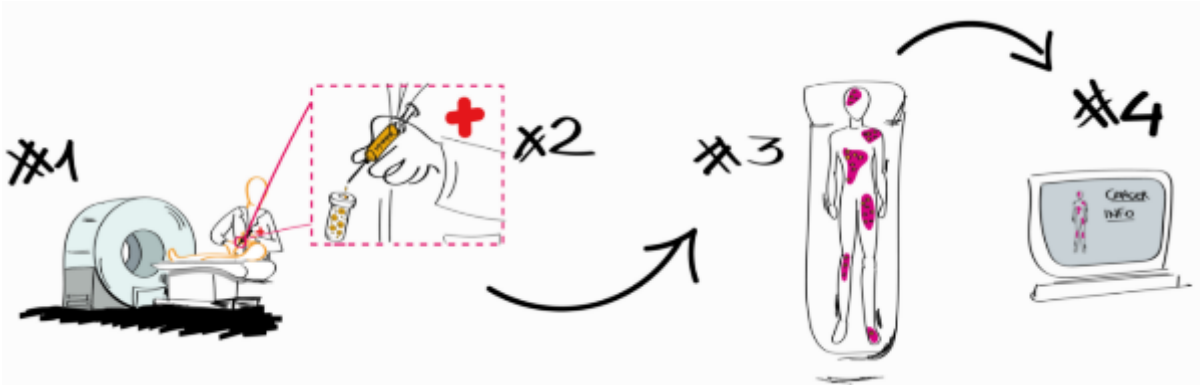

Fig. 1. Graphical representation of how our radiotracers should work.

References

[1] Organ S. L., Tsao M. S. An overview of the c-MET signaling pathway. Ther. Adv. Med. Oncol. 2011, 3, S7-S19.
[2] Yang J.J. et al. Soluble c-Met protein as a susceptible biomarker for gastric cancer risk. Int. J. Cancer. 2013, 132(9), 2148-2156.
[3] Klein P.J. et al. Synthesis, radiolabeling, and evaluation of novel amine guanidine derivatives as potential PET tracers for the ion channel of the N-methyl-d-aspartate receptor. Eur. J. Med. Chem. 2016, 18, 143-160.

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Self-assembling nanoparticles for miRNA delivery towards precision medicine against metastatic melanoma

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Keywords: lipid nanoparticles, RNA delivery, melanoma, precision medicine

Metastatic melanoma is a highly aggressive tumor that can be fatal within 18 months of diagnosis [1]. The identification of a subset of patients whose mitogen-activated protein kinase (MAPK) pathway is overactivated has led to development of targeted therapy, which is able to inhibit this pathway. However, the long-term efficacy of targeted therapy is compromised by the onset of drug resistance. We have recently identified a panel of oncosuppressor microRNAs (miRNAs) able to prevent the development of drug resistance to targeted therapy [2,3]. Systemic miRNA delivery requires the use of nanocarriers to prevent degradation by endogenous nucleases and to facilitate intracellular delivery [4]. Furthermore, due to the rapid development of therapies based on precision medicine, there is an urgent need to develop a versatile nanoparticle platform enabling miRNA loading and delivery at the point of care. In order to address these challenges, we have developed self-assembling lipid nanoparticles (SANP) with a calcium phosphate core enclosed by a lipid shell [5] as a novel miRNA delivery platform against drug-resistant melanoma. SANP are prepared before use and offer the possibility to encapsulate the miRNAs required for the specific patient, paving the way to personalized RNA-based therapies. We optimized the lipid shell composition and mixing ratios to achieve miRNA-loaded SANP, which showed hydrodynamic diameters below 200 nm, high miRNA encapsulation efficiencies, good colloidal stability in serum, and low hemolytic activity. *In vitro*, selected miRNA-loaded SANP formulations were able to effectively deliver miRNA, to inhibit the release of soluble tumor-promoting factors (*i.e.*, transforming growth factor-β1 and vascular endothelial growth factor a), and to prevent the proliferation of two different cell lines of metastatic melanoma. When used in combination with targeted therapy, miRNA-loaded SANP formulations were able to inhibit cancer cell proliferation in a dose-response manner. These results demonstrate the potential of the SANP technology as a platform for miRNA delivery against metastatic melanoma with unprecedented design flexibility and great potential for rapid clinical translation which can be used in combination with targeted therapy to prevent the development of drug resistance.

References

[1] F. Valenti, *et al.* Precision Medicine and Melanoma: Multi-Omics Approaches to Monitoring the Immunotherapy Response. Int. J. Mol. Sci. 2021, 22.
[2] L. Fattore, *et al.* Reprogramming miRNAs global expression orchestrates development of drug resistance in BRAF mutated melanoma. Cell Death Differ. 2019, 26, 1267.
[3] L. Fattore, *et al.* Oncosuppressive miRNAs loaded in lipid nanoparticles potentiate targeted therapies in BRAF-mutant melanoma by inhibiting core escape pathways of resistance. Oncogene 42, 293–307 (2023).
[4] S. W. L. Lee, *et al.* MicroRNA delivery through nanoparticles. J. Control. Release 2019, 313, 80.
[5] V. Campani, *et al.* Hybrid lipid self-assembling nanoparticles for brain delivery of microRNA. Int. J. Pharm. 2020, 588, 119693.

Paper-Based Electrochemical Platform to Detect miRNA-652 associated to Triple-Negative Breast Cancer

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Keywords: Triple-negative breast cancer; Paper-based; MicroRNAs; Screen-printed electrodes

Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer that is often characterized by early metastasis and poor patient prognoses [1]. TNBC is a clinical diagnosis characterized by the absence of progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2). This breast cancers represent approximately 10-20% of all cases. In comparison to other types, TNBC poses a greater risk of recurrence, metastasis, and mortality [2]. The necessity for detecting early-stage TNBC (ES-TNBC) has become crucial due to its aggressive nature and low probability of survival: the early diagnosis increases the chances of patients survival up to 30% [3]. For this reason, the main challenge in the diagnosis of TNBCs is the development of non-invasive platforms for the early diagnosis of cancer. To this regard, circulating nucleic acids represent promising biomarkers, even of their quantitation is usually carried out through molecular biology techniques, i.e. enzyme ligation assays, Northern blot, microarray, quantitative real-time polymerase chain reaction, etc., which are expensive and time-consuming approaches, also requiring skilled personnel [4]. In this context, portable (bio)sensors, with a major focus on electrochemical ones have recently received considerable interest due to their multiple advantages, such as excellent selectivity, cost-effectiveness, non-invasive process and easy interpretation of results [5] in particular the use of microRNAs (miRNAs) as promising biomarkers is becoming more frequent. The potential of utilizing miRNAs as effective cancer biomarkers is significantly hampered due to the unavailability of reliable, rapid and cost-effective diagnostic tests for their identification in biofluids. The present work has been focused on the development of a paper-based electrochemical device which is capable of detecting miRNA-652, which is involved in the pathogenesis of TNBCs. This innovative detection mechanism was developed using gold nanoparticles (AuNPs) and an anti-miRNA-652 probe, labeled with a redox mediator, namely Methylene Blue (MB). The adoption of Whatman No.1 chromatography paper resulted in an enhanced ability to detect miRNA-652 with greater sensitivity, as the material's pore structure facilitated pre-concentration of the species. Therefore, this marks a significant progression towards more accurate quantification of miRNAs. The detection system is known as "signal off". Without the target, MB can quickly interchange electrons with the electrode's surface. However, when forming a duplex between probe and target, it restricts system flexibility and diminishes signal quality by reducing MB electron exchange. This platform has been validated with controlled optimisation experiments and is highly reliable, sensitive, and specific. This platform successfully detected miRNA-652 in both standard and serum solutions with low nanomolar limits of detection. This device has proven to be extremely advantageous for assessing miRNA-652 levels as a TNBC biomarker due to its high sensitivity and selectivity, cost-effectiveness, short procedure time, simplicity, ability to be miniaturized and high potential for applicability in complex biological matrices.

References

[1] E.M. Grasset, M. Dunworth, G. Sharma, M. Loth, J. Tandurella, A. Cimino-Mathews, M. Gentz, S. Bracht, M. Haynes, E.J. Fertig, A.J. Ewald, Triple-negative breast cancer metastasis involves complex epithelial-mesenchymal transition dynamics and requires vimentin, Science Translational Medicine. 14 (2022) eabn7571. https://doi.org/10.1126/scitranslmed.abn7571.
[2] R. Kedmi, D. Peer, RNAi nanoparticles in the service of personalized medicine, Nanomedicine. 4 (2009) 853–855. https://doi.org/10.2217/nnm.09.74.

[3] M. Sharifi, A. Hasan, F. Attar, A. Taghizadeh, M. Falahati, Development of point-of-care nanobiosensors for breast cancers diagnosis, Talanta. 217 (2020) 121091. https://doi.org/10.1016/j.talanta.2020.121091.
[4] A.-M. Chiorcea-Paquim, Advances in Electrochemical Biosensor Technologies for the Detection of Nucleic Acid Breast Cancer Biomarkers, Sensors. 23 (2023) 4128. https://doi.org/10.3390/s23084128.
[5] S. Singh, A. Miglione, A. Raucci, A. Numan, S. Cinti, Towards sense and sensitivity-based electrochemical biosensors for liquid biopsy-based breast cancer detection, TrAC Trends in Analytical Chemistry. 163 (2023) 117050. https://doi.org/10.1016/j.trac.2023.117050.

Metabolic changes induced by Aurothiomalate in A2780 ovarian cancer cells

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Keywords: NMR metabolomics, Aurothiomalate

In recent years, numerous studies have shown that gold compounds are potential candidates as new anticancer drugs [1]. Auranofin(AF), an FDA-approved antiarthritic gold drug with remarkable antiproliferative properties, has become the lead compound for this class of anticancer agents [2]. Many investigations have been carried out so far to disclose the actual modes of action of anticancer gold(I) compounds. A widely accepted mechanistic hypothesis for AF postulates that the large increase in intracellular oxidative stress observed upon treatment is the consequence of strong inhibition of the selenoenzyme thioredoxin reductase (TrxR); in turn, such severe oxidative stress causes profound mitochondrial dysfunction ultimately leading to apoptosis [3-5]. Although the molecular mechanisms underlying the action of these compounds remain largely unknown, the modes of action and targets appear to be multiple and especially different from those of platinum compounds (e.g., cisplatin) [6]. NMR metabolomics is a powerful tool to characterize the changes in cancer cell metabolism elicited by anticancer drugs. The use of a metabolomic approach may be effective in assessing tumor response to drugs and provide more information about pharmacological mechanisms of action. Indeed, changes in intracellular and extracellular metabolites induced by drug treatment may be directly related to dysregulation of specific biochemical pathways [7,8]. Recently, this method of investigation has been applied to characterize the metabolic perturbations induced by AF in a reference tumor cell line [6,9]. The analysis of cellular alterations caused by AF in A2780 ovarian cancer cells revealed a large increase of glutathione intracellular concentration as the main effect of the treatment [5]. These results testify to the onset of a metabolic shift and redox imbalance induced by AF in the cell model used. NMR metabolomics was also used to analyze the metabolic changes brought about by the treatment of A2780 cancer cells with Aurothiomalate, another gold-based compound used to treat rheumatoid arthritis but recently discovered as a potential anticancer drug for ovarian cancer. Results were compared with those previously obtained for AF treatment [6]. Interestingly, results highlight that aurothiomalate induces metabolic perturbations in A2780 cells that are distinctly different from those caused by AF, reflecting large differences in the mechanism of action of the two gold-based drugs. Specifically, the metabolomic study showed significant intracellular accumulation of succinate and decreased fumarate in aurothiomalate-treated A2780, identifying the mitochondrion as a primary target and suggesting a severe alteration at the Krebs cycle level. Besides, it was demonstrated that aurothiomalate causes in A2780 tumor cells a reduction in the levels of the A subunit of the mitochondrial enzyme succinate dehydrogenase, the enzymatic subunit responsible for the conversion of succinate to fumarate. In addition, aurothiomalate promotes drastic alterations in the expression level of mitochondrial oxidative phosphorylation OXPHOS complexes, however, not impacting the respiratory capacity of tumour cells. These results are in agreement with metabolomic findings and testifies how aurothiomalate can probably exert its action by primarily targeting the mitochondrial functional status of ovarian cancer cells A2780.

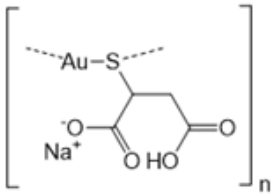

Figure 1. The chemical structure of Aurothiomalate.

References

[1] Nobili S, Mini E, Landini I, Gabbiani C, Casini A, Messori L. Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. Med Res Rev. 2010;30(3):550-580.
[2] Gamberi T, Chiappetta G, Fiaschi T, Modesti A, Sorbi F, Magherini F. Upgrade of an old drug: Auranofin in innovative cancer therapies to overcome drug resistance and to increase drug effectiveness. Med Res Rev. 2022;42(3):1111-1146.
[3] Abdalbari FH, Telleria CM. The gold complex auranofin: new perspectives for cancer therapy. Discov Oncol. 2021;12(1):42. Published 2021 Oct 20.
[4] Roder C, Thomson MJ. Auranofin: repurposing an old drug for a golden new age. Drugs R D. 2015;15(1):13-20.
[5] Chiappetta G, Gamberi T, Faienza F, et al. Redox proteome analysis of auranofin exposed ovarian cancer cells (A2780). Redox Biol. 2022; 52:102294.
[6] Ghini V, Senzacqua T, Massai L, Gamberi T, Messori L, Turano P. NMR reveals the metabolic changes induced by auranofin in A2780 cancer cells: evidence for glutathione dysregulation. *Dalton Trans*. 2021;50(18):6349-6355.
[7] Eraslan Z, Cascante M, Günther UL. Metabolomics in Cell Biology. Handb Exp Pharmacol. 2023; 277:181-207.
[8] Cuperlović-Culf M, Barnett DA, Culf AS, Chute I. Cell culture metabolomics: applications and future directions. Drug Discov Today. 2010;15(15-16):610-621.
[9] Ghini V, Magherini F, Massai L, Messori L, Turano P. Comparative NMR metabolomics of the responses of A2780 human ovarian cancer cells to clinically established Pt-based drugs. Dalton Trans. 2022;51(33):12512-12523. Published 2022 Aug 23.

Investigation of the mechanism of action of CFTR corrector ARN23765 via Photo-Affinity Labeling (PAL) approach

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Keywords: cystic fibrosis, photo-affinity labeling, chemical probe, target ID

Cystic Fibrosis (CF) is a rare genetic disease characterized by deficiencies in the synthesis or function of the CF transmembrane conductance regulator (CFTR) anion channel, caused by mutations in the CFTR gene. Small-molecule compounds addressing the basic defect of the disease have been described, and are referred to as CFTR modulators.[1] Among these, ***ARN23765***, a F508del-CFTR corrector discovered by our group, showed high potency in rescuing the function of mutant CFTR in primary human bronchial epithelial cells derived from CF patients homozygous for the F508del mutation.[2]
Despite the validated pharmacological effects, **ARN23765** mechanism of action has not yet been conclusively defined.[3] CFTR correctors could act either directly by binding to CFTR or by interacting with the machinery responsible for protein synthesis, trafficking and maturation. However, no data are so far available disclosing the interaction of modulators with CFTR (either wt- or F508del mutant) in a native cellular environment.
Our project aims at investigating the biological target(s) and mechanism of action of ***ARN23765*** in living cells expressing CFTR.
To this purpose, a Photo-Affinity Labeling (PAL)[4,5] approach has been pursued as a convenient strategy to enable the cross-linking of the small molecule to target proteins, allowing their subsequent identification. A set of photo-affinity probes (PAPs) was designed and synthesized by introducing a small photo-reactive moiety and a reporter/purification tag (or a chemical handle suitable for conjugation to such a tag) on the scaffold of ***ARN23765***. The diazirine was selected as convenient photo-reactive moiety, since its activation by UV light produces highly reactive transient chemical species that crosslink in a covalent manner to bio-molecules in close proximity.[6] On the other hand, suited tags or chemical handles were employed to allow probe-target adducts identification in biochemical studies.
After a preliminary evaluation of their activity in rescuing F508del-CFTR function, novel PAPs were employed in PAL studies, demonstrating their in situ binding to wild type and mutant F508del-CFTR in live cells.
Next, biochemical studies on CFTR domains were performed to detail the mechanism of action of ***ARN23765***. Furthermore, docking analyses highlighted the relevant residues needed for a proper interaction of **ARN23765** to CFTR, which will contribute to outline the binding site of our corrector through tailored mutagenesis experiments.
To the best of our knowledge, our study is the first to disclose the interaction of a corrector probe to wild type and mutant F508del-CFTR in an intact cellular setting.

References

[1] M. Lopes-Pacheco, N. Pedemonte, G. Veit. Discovery of CFTR modulators for the treatment of cystic fibrosis. Expert Opin. Drug Discov. 2021, 21, 897-913.
[2] N. Pedemonte, F. Bertozzi, E. Caci, F. Sorana, P. Di Fruscia, V. Tomati, L. Ferrera, A. Rodrìguez-Gimeno, F. Berti, E. Pesce, E. Sondo, A. Gianotti, P. Scudieri, T. Bandiera, L.J.V. Galietta. Discovery of a picomolar potency pharmacological corrector of the mutant CFTR chloride channel. Sci. Adv. 2020, 6, eaay9669.
[3] S. Pankow, C. Bamberger, D. Calzolari, S. Martínez-Bartolomé, M. Lavallée-Adam, W.E. Balch, J.R. Yates. ΔF508 CFTR interactome remodelling promotes rescue of cystic fibrosis. Nature 2015, 528, 510-516.
[4] L. Dubinsky, B.P. Krom, M.M. Meijler. Diazirine based photoaffinity labeling. Bioorg. Med. Chem. 2012, 20, 554-570.
[5] E. Smith, I. Collins. Photoaffinity labeling in target- and binding-site identification. Future Med. Chem. 2015, 7, 159-183.
[6] J.R. Hill, A.A.B. Robertson. Fishing for drug targets: a focus on diazirine photoaffinity probe synthesis. J. Med. Chem. 2018, 61, 6945-6963.

Exploring ROS formation from ZnO/cellulose acetate composites films in Aqueous Solution: Light vs. Darkness

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Keywords: flexible devices; ZnO/cellulose composites; reactive oxygen species; impedance; photocatalysis.

The spectacular evolution of wearable/implantable medical devices in the last two decades has resulted in efficient medical treatments, higher life quality and expectancy. A threat to this bright future is the misuse/overuse of antibiotics resulting in antimicrobial resistance to pathogens [1]. A solution to this issue is the design of systems able to respond to microbe pathogens, by formulating materials composites coupling biocompatibility with switchable antibacterial properties. Accordingly, this work shows ZnO nanostructures (n-ZnO)/cellulose acetate composites that can produce bactericidal Reactive Oxygen Species (ROS) under simulated solar light. Both ZnO and cellulose acetate are bactericidal and biocompatible, having been found numerous applications in biomedical sciences [2]. The n-ZnO are synthesized by a rational approach (85°C, 60 hours) leading to n-ZnO assembled in micrometric flower-shaped grains. Different instrumental techniques (SEM, XPS, XRD, UV-Vis, thermogravimetry, cyclic-voltammetry and ζ-potential) permit to analyze their morphology and physico-chemical properties. n-ZnO/cellulose acetate composites films are prepared from n-ZnO dispersed at different weight percentages (1-15 w/w %) in cellulose acetate dissolved at 2 mg/mL in ethyl acetate. The dispersion is drop casted in a 4 cm diameter beaker and placed at 80°C for three hours to evaporate ethyl acetate, resulting in self-standing micrometer thick flexible films. Accordingly, n-ZnO fillers tune the water contact angle and the dielectric properties of the composites. The formation of ROS under simulated solar light is demonstrated by the photocatalytic degradation of the dye methylene blue (MB), showing an apparent degradation kinetics of 0.029 ± 0.002 min^{-1} (at 10 % W/W n-ZnO), along with an excellent reusability at the second and third cycles (0.041 ± 0.002 and 0.039 ± 0.002 min^{-1}, respectively), whereas control experiments in the absence of light show a negligible MB photodegradation. Electrochemical Impedance spectroscopy (50 kHz - 1 Khz) is carried out on the aqueous solutions where the composites are soaked to monitor the release of ionic species in dark. The higher the n-ZnO filler concentration, the lower is the solution bulk impedance, as a result of zinc ionic species leaching in solution.

References

[1] E. Seebacha and K. Kubatzky, Chronic Implant-Related Bone Infections-Can Immune Modulation be a Therapeutic Strategy? Frontiers in Immunology, 2019, 10, 1724.
[2] G. Arrabito, A. Delisi A., G. Giuliano, G. Prestopino, P.G. Medaglia, V. Ferrara, F. Arcidiacono, M. Scopelliti, D.F. Chillura Martino, B. Pignataro, Self-Cleaning Bending Sensors Based on Semitransparent ZnO Nanostructured Films, ACS Applied Engineering Materials, 2023, 1, 1384.

Acknowledgements

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AMYC BIOMED 2023

Enhancing Analytical Sensing with DNA-Based Nanoswitches for Dual-Signal Electrochemiluminescence at the Nanoscale

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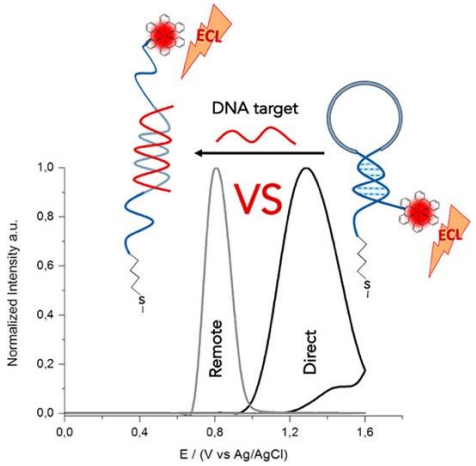
Keywords: Electrochemiluminescence, Nanotechnology

ECL is luminescence generated by electrochemical reactions, and for this reason it possesses better spatio-temporal control and low background in comparison with photoluminescence or other optical methods that rely on external light illumination. In the last 20 years ECL has proved to be a versatile and powerful analytical technique, widely used in different fields, ranging from fundamental research to commercial clinical and biological applications [1]. ECL is indeed a surface-confined process and a comprehensive control of spatial distribution of the ECL signal at nanometric distances is critically important in view of its applications in sensing devices [2,3]. Herein I will present a recent strategy in which nanomaterials are used as tool for exploring the mechanism underlying ECL signal generation at nanoscale using luminophore-reporter-modified DNA-based nanoswitches (i.e., molecular beacon) with different stem stabilities [4]. ECL is generated according to the "oxidative-reduction" strategy using tri-n-propylamine (TPrA) as a coreactant and $Ru(bpy)^{3}{}_{2+}$ as a luminophore. Our findings suggest that by tuning the stem stability of DNA nanoswitches we can trigger different ECL mechanisms (direct and remote). As a result, we successfully achieved a significantly increased signal gain (up to 10 times) compared to the conventional "signal-off" electrochemical readout method. Our study showcased the coexistence of two distinct ECL generation mechanisms at the nanoscale, presenting new possibilities for developing tailored DNA devices capable of generating highly efficient dual-signal-output ratiometric-like ECL systems.



Normalized ECL signals showing the two different mechanisms for the ECL generation obtained through DNA-based Nanoswitches.

References

[1] Sojic, N., Analytical Electrogenerated Chemiluminescence, (2019) Ed.; Detection Science; Royal Society of Chemistry.
[2] Zanut, A.; Fiorani, A.; Canola, S.; Saito, T.; Ziebart, N.; Rapino,S.; Rebeccani, S.; Barbon, A.; Irie, T.; Josel, H.-P.; Negri, F.;Marcaccio, M.; Windfuhr, M.; Imai, K.; Valenti, G.; Paolucci, F. Insights into the mechanism of coreactant electrochemiluminescence facilitating enhanced bioanalytical performance, Nat. Commun. 2020, 1–9.
[3] Zanut A., Rossetti M., Marcaccio M., Ricci F., Paolucci F., Porchetta A., Valenti G., DNA-Based Nanoswitches: Insights into Electrochemiluminescence Signal Enhancement, Anal. Chem. 2021, 93, 30, 10397–10402

[4] Ranallo S., Porchetta A., Ricci F., DNA-Based Scaffolds for Sensing Applications, Anal. Chem. 2019, 91, 1, 44–59

A General Organophotoredox Strategy to Difluoroalkyl Bicycloalkane Hybrid Bioisosteres

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Keywords: bioisosteres, organic synthesis, photocatalysis, photochemistry, radicals

The bioisosteric replacement is a fundamental strategy in modern medicinal chemistry.[1] This concept is based on the replacement of a chemical motif within a molecule with a different functionality to improve its physicochemical properties and increase its biological activity.
Here, we report our efforts towards to the preparation of difluoroalkyl bicycloalkane (CF2-BCA) hybrid isosteres **1**, as structural surrogates of aryl ketones and aryl ethers **2**. This new class of molecules combines a difluoromethylene group (CF2), an isostere of a carbonyl group (C=O) or an oxygen atom able to impart increased lipophilicity and metabolic stability,[2] with a bicycloalkane (BCA) moiety, an aryl ring bioisostere with increased drug-like properties (Figure 1a).[3] Our synthetic approach is based on a light-driven protocol that allows the difluoroalkylation of propellanes **4** using bromodifluoro derivatives **3** to give CF2-BCAs **5** under mild conditions (Figure 1b). Capitalizing on an acridine photocatalyst PC recently developed within our group,[4] this process gives access to a variety of biorelevant hybrid isosteres, whose structural features were investigated by single crystal X-ray analysis and docking studies.

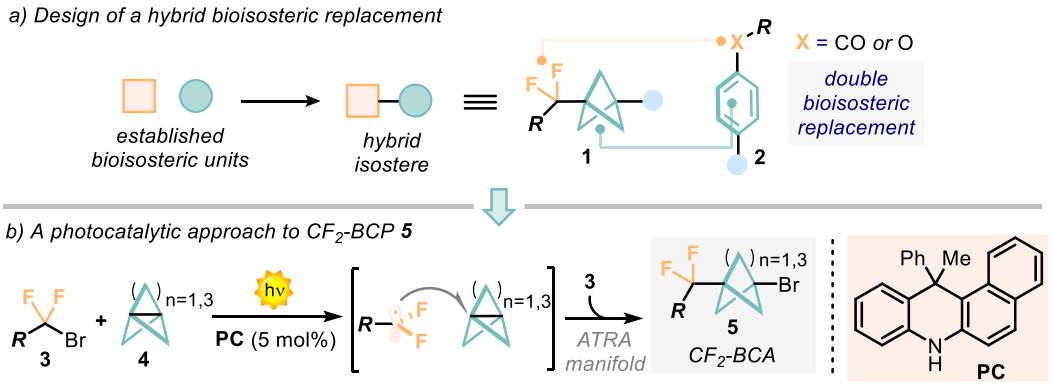

Figure 1: a) design and b) synthetic plan for the preparation of CF2-BCA **5** as a new class of hybrid isosteres.

References

[1] N. A. Meanwell. Synopsis of some recent tactical application of bioisosteres in drug design. *J. Med. Chem.* **2011**, 54, 2529 – 2591.
[2] N. A. Meanwell. Fluorine and fluorinated motifs in the design and application of bioisosteres for drug design. *J. Med. Chem.* **2018**, 61, 5822 – 5880.
[3] P. K. Mykhailiuk. Saturated bioisosteres of benzene: where to go next? *Org. Biomol. Chem.* **2019**, 17, 2839 – 2849.
[4] T. Bortolato, G. Simionato, M. Vayer, C. Rosso, L. Paoloni, E. M. Benetti, A. Sartorel, D. Leboeuf, L. Dell'Amico. The rational design of reducing organophotoredox catalysts unlocks proton-coupled electron-transfer and atom transfer radical polymerization mechanisms. *J. Am. Chem. Soc.* **2023**, 145, 1835 – 1846.

Fipronil Detection with a Smartphone-based Aptasensor: Addressing the Small Molecule Challenge

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Keywords: Aptasensor, Smartphone-based sensor, pesticide, aptamer, Fipronil

The detection of small molecules with aptasensors can be challenging due to several factors. In fact, small molecules typically have low molecular weights and simpler structures compared to larger biomolecules such as proteins or nucleic acids. Their small size makes it difficult to design aptamers that can bind to them with high specificity and affinity [1]. Aptamers are short, single-stranded nucleic acid sequences that can fold into three-dimensional structures to bind target molecules. Designing aptamers that can selectively bind to small molecules and discriminate them from structurally similar compounds is a complex task. Aptasensors typically rely on various detection methods to signal the binding event between the aptamer and the target molecule. These methods can include fluorescence, electrochemical, or mass-based detection techniques. However, many of these detection methods require modifications or labeling of the small molecule targets to generate a measurable signal, which can be technically demanding and affect the target molecule's behavior and binding properties. For this reason, the development of new simple approaches is of high interest.
Pesticides are among the analytes that attract the greatest attention in the field of food control, and among them Fipronil, which is a toxic small molecule that poses a significant risk both to humans and animals [2]. Hence, detecting Fipronil in the environment is important to ensure its safe use and prevent harm to people and wildlife. Fipronil analysis is typically performed using advanced analytical techniques such as gas chromatography and mass spectrometry. In the European Union, the maximum residue limit (MRL) for fipronil in food products is 0.005 milligrams per kilogram (mg/kg). This limit applies to a wide range of food products, including eggs, meat, dairy, and certain fruits and vegetables.
Portable sensors could be useful tools for detecting Fipronil in the field, as they could provide rapid, on-site results without the need for laboratory analysis.
Here, we present a smartphone-based aptasensor that exploits a competitive replacement assay approach. Standard solutions of Fipronil and real food samples were analyzed, with the aim of providing a tool capable of quickly, easily, and accurately monitoring the presence of traces of this pesticide. In Figure 1, it is shown the modification of the screen-printed electrode and the calibration with standard solution in the range of 2-15 ppb.

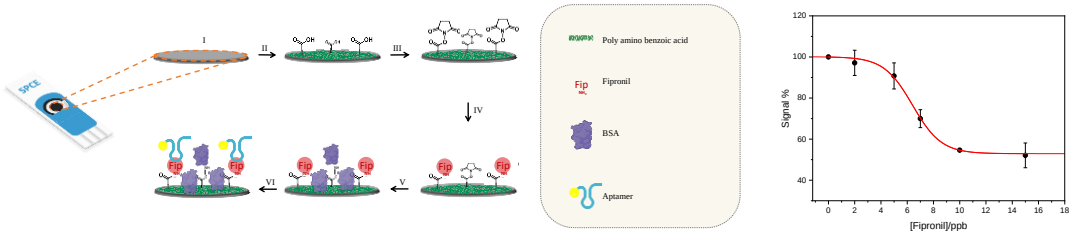

Figure 1. Aptasensor assembly and calibration curve.

This smartphone-based sensor has the potential to improve the way food is analyzed and provide consumers with real-time information about the safety of the food they eat.

References

[1] Sanford, A. A., Manuel, B. A., Romero-Reyes, M. A., & Heemstra, J. M.. Combating small molecule environmental contaminants: detection and sequestration using functional nucleic acids. Chemical Science, (2022), 13(26), 7670-7684.

[2] Li, X., Ma, W., Li, H., Zhang, Q., & Ma, Z.. Determination of residual fipronil and its metabolites in food samples: A review. Trends in Food Science & Technology, (2020), 97, 185-195.

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Evidence of amino-thiadiazoles as innovative inhibitors of human glutaminyl cyclase, validated target for neurodegenerative disorders

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Keywords: human glutaminyl cyclase, neurodegenerative disorders, amino-thiadiazole inhibitors, X-ray crystallography

Human glutaminyl cyclase (hQC) is a zinc-dependent enzyme belonging to the class of acyltransferases that catalyzes the intramolecular cyclization of the N-terminal glutaminyl or glutamyl of peptides and proteins into pyroglutamic acid (pGlu). This post-translational modification stabilizes and protects macromolecules from proteolytic degradation and assists them to develop the proper conformation (1). However, since their tendency to rapidly aggregate, pGlu-modified peptides are highly neurotoxic, promoting the insurgence and progression of various neurodegenerative pathologies, such as Alzheimer (AD) and Huntington diseases (HD) (2,3). Former studies have reported the upregulation of hQC in these neurodegenerative disorders, thus this enzyme represents an attractive target to develop novel drugs for these pathologies, still lacking effective treatments. In this study, we have investigated a series of molecules relying on the amino-thiadiazole core as new Zn(II)-binding moiety to probe their inhibition effects on hQC. This library of amino-thiadiazole derivatives was formerly developed by us to target the parasite enzyme, Trypanosoma brucei pteridine reductase 1 (TbPTR1) (4). Notably, these compounds showed a safe profile in a panel of early toxicity assay comprising cytotoxicity, mitochondrial toxicity, hERG toxicity, CYP isoforms, and Aurora B kinase. Thus, the amino-thiadiazole scaffold has a well-tolerated liability profile and it can be further explored for inhibitor development also towards other targets, such as hQC. For the present investigation, a set of twenty-four compounds have been selected from this library and tested towards hQC, leading to the identification of three inhibitors having Ki values in the high nM range. To unveil the binding mode of the most active compounds of this series, X-ray crystallography experiments have been performed using the hQC double mutant Y115E-Y117E (hQC-2X), formerly validated by us as soluble protein variant exploitable for drug discovery purposes (5). The structural information achieved on the complexes of hQC-2X with fourteen amino-thiadiazole derivatives has allowed us to evaluate the structure-activity relationship of these inhibitors, obtaining key insights to evolve new hQC inhibitors based on this innovative Zn(II)-binding motif.

References

[1] W. H. Fischer, J. Spiess, Proc Natl Acad Sci U S A. 1987, 84, 3628.
[2] O. Wirths, A. Hillmann, L. Pradier, W. Härtig, T. A. Bayer, J Alzheimers Dis 2013, 35, 741.
[3] M. Jimenez-Sanchez, W. Lam, M. Hannus, B. Sönnichsen, S. Imarisio, A. Fleming, A. Tarditi, F. Menzies, T. E. Dami, C. Xu, E. Gonzalez-Couto, G. Lazzeroni, F. Heitz, D. Diamanti, L. Massai, V. P. Satagopam, G. Marconi, C. Caramelli, A. Nencini, M. Andreini, G. L. Sardone, N. P. Caradonna, V. Porcari, C. Scali, R. Schneider, G. Pollio, C. J. O'Kane, A. Caricasole, D. C. Rubinsztein, Nat Chem Biol. 2015, 11, 347.
[4] P. Linciano, A. Dawson, I. Pöhner, D. M. Costa, M. S. Sá, A. Cordeiro-da-Silva, R. Luciani, S. Gul, G. Witt, B. Ellinger, M. Kuzikov, P. Gribbon, J. Reinshagen, M. Wolf, B. Behrens, V. Hannaert, P.A. M. Michels, E. Nerini, C. Pozzi, F. Di Pisa, G. Landi, N. Santarem, S. Ferrari, P. Saxena, S. Lazzari, G. Cannazza, L.H. Freitas-Junior, C. B. Moraes, B. S. Pascoalino, L. M. Alcântara, C. P. Bertolacini, V. Fontana, U. Wittig, W. Müller, R. C. Wade, W. N. Hunter, S. Mangani, L. Costantino, M. P. Costi, ACS Omega. 2017, 2, 5666.
[5] C. Castaldo, S. Ciambellotti, R. de Pablo-Latorre, D. Lalli, V. Porcari, P. Turano, PLoS ONE 2013, 8, e71657.

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Ebselen Analogues as Human Neuthophil Elastase (HNE) Inhibitors and Antioxidant Agents

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Keywords: human neutrophil elastase, HNE inhibitors, selenorganic compounds, respiratory diseases

Human neutrophil elastase (HNE) is a serine protease stored in neutrophils and is involved in several respiratory diseases, such as chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS), acute lung injury (ALI) and cystic fibrosis, but also in cancer, rheumatoid arthritis and psoriasis [1,2]. Moreover, the involvement of the enzyme in Covid-19 has been recently highlighted, since the excessive HNE proteolytic activity in the lungs is responsible for the exacerbation of the severe respiratory complications related to the disease [3,4]. Our research group has been working in the field of HNE inhibitors for many years, obtaining potent compounds with nanomolar activity. These compounds are characterized by the presence of an N-CO function which attacks the OH group of Ser195 in the catalytic triad. In further project development, a series of benzo[*d*][1,2]selenazol-3(2H)-ones as Ebselen analogues have been synthesized. The aim was to obtain compounds with anti-HNE and antioxidant effects, the latter due to selenium, leading to compounds with dual action, which are particularly attractive for the treatment of respiratory diseases (Figure 1).

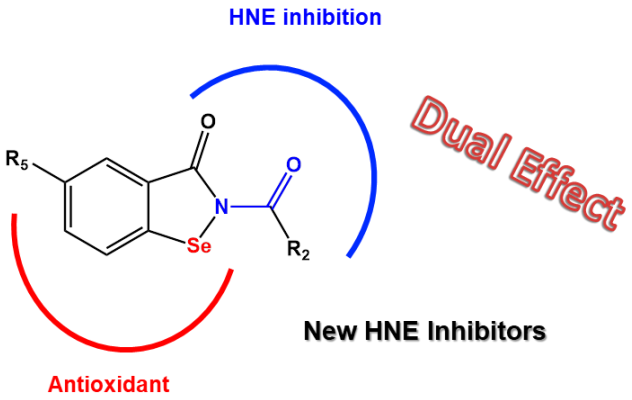

Figure 1. Aim of the work

References

[1] B. Korkmaz, S.M. Horwitz, D.E. Jenne, F. Gauthier. *Pharmacol. Rev.* **2010**, *62*, 726-759
[2] L. Crocetti, M.T. Quinn, A.I. Schepetkin, M.P. Giovannoni. *Expert Opin. Ther. Pat.* **2019**, *29*, 555-578
[3] H.M. Al-Kuraishy, A.I. Al-Gareeb, H.A. Al-Hussaniy, N.A.H. Al-Harcan, A. Alexiou, G.E. Batiha. *Int. Imm.* **2022**, *104*, 108516
[4] M. Laforge, C. Elbim, C. Frère, M- Hémadi, C. Massaad, P. Nuss, J.J. Benoliel, C. Becker. *Nat. Rev.* **2020**, *20*, 515-516

AMYC BIOMED 2023

Targeting 3-chymotrypsin-like protease of SARS-CoV-2 by protein degradation: the first stirrings of PROTAC

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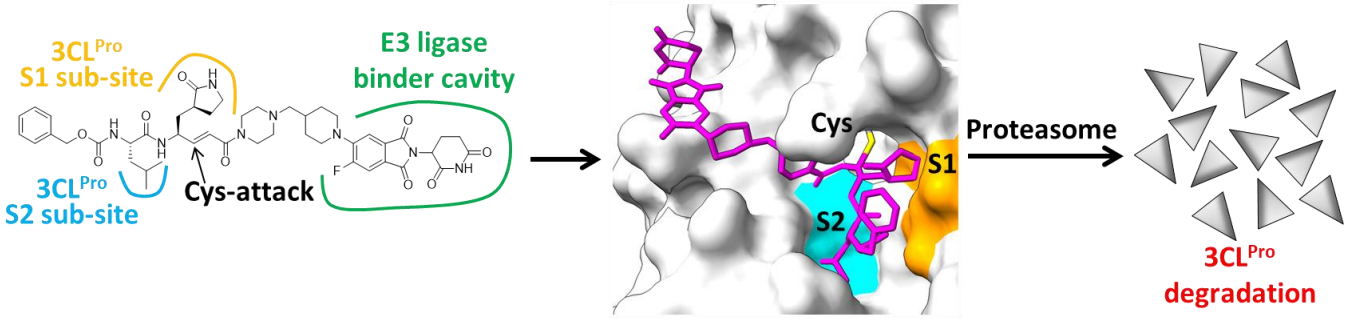
Keywords: 3-Chymotrypsin-like protease; Medicinal chemistry;Peptidomimetics; PROTAC; SARS-CoV-2

Coronaviruses have been recognized for decades as human pathogens and since 2003 an escalation of their virulence has occurred. This compels worldwide scientific community to search for solutions against future outbreaks. In this regard, we have here applied a PROteolysis TArgeting Chimeras (PROTAC)-based strategy to obtain a peptidomimetic molecule able to trigger the degradation of the main protease of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 3CL ^{Pro}), one of the most viable targets for COVID-19 treatment. The recently discovered PROTAC technology provides an attractive approach that exploits the cell's protein degradation machinery as a vehicle to reduce the cellular level of a pathological target. [1-3]
The PROTAC molecule was obtained conjugating, through a piperazine-piperidine linker, a SARS-CoV-2 3CL ^{Pro} dipeptidyl ligand to a pomalidomide moiety, which is well suited to bind to the E3 ligase substrate receptor Cereblon (CRBN). The latter is one of the substrate receptors of the CRL4 E3 ubiquitin ligase complex responsible for the poly-ubiquitination of substrates that can then be degraded by the proteasome. [4,5]
NMR and crystallographic data complemented with an enzymatic activity assay showed that the dipeptidyl moiety of the synthetized PROTAC molecule specifically and tightly binds to the active site of the dimeric SARS-CoV-2 3CL ^{Pro} enzyme forming a reversible covalent bond with the sulfur atom of the catalytic Cys145. On the contrary, the linker and the pomalidomide moiety protrude from the protein and are totally solvent exposed, displaying a very high degree of mobility as well as the absence of interactions with other regions of the protein. These structural features successfully allow the synthetized PROTAC molecule to activate at the cellular level SARS-CoV-2 3CL ^{Pro} protein degradation, as experimentally validated by immunoblotting analysis. This study paves the way for the future applicability of peptidomimetic PROTACs to attack 3CL ^{Pro} -dependent viral infections.



Schematic view of the PROTAC strategy. The molecule binds the catalytic pocket of SARS-CoV-2 $3C^{pro}$ at two different subsites S1 in yellow and S2 in blue, whereas in green is highlighted the moiety that can be employed to drive the protein towards the proteasomal degradation path way.

References

[1] Li, K.; Crews, C. M., PROTACs: past, present and future. Chem Soc Rev 2022, 51 (12), 5214-5236.
[2] Cowan, A. D.; Ciulli, A., Driving E3 Ligase Substrate Specificity for Targeted Protein Degradation: Lessons from Nature and the Laboratory. Annu Rev Biochem 2022, 91, 295-319.
[3] Toure, M.; Crews, C. M., Small-Molecule PROTACS: New Approaches to Protein Degradation. Angew Chem Int Ed Engl 2016, 55 (6), 1966-73
[4] Yang, Q.; Zhao, J.; Chen, D.; Wang, Y., E3 ubiquitin ligases: styles, structures and functions. Molecular biomedicine 2021, 2 (1), 23.
[5] Pickart, C. M., Mechanisms underlying ubiquitination. Annu Rev Biochem 2001, 70, 503-33.

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Quinazolin-4(3*H*)-one-based modulators of *Pseudomonas aeruginosa* virulence factors

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Keywords: *Pseudomonas aeruginosa*, virulence factors, quorum sensing, nitrogen-based compounds

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen, responsible for both acute and chronic infections. In particular, it is associated with high morbidity and mortality in many groups, including individuals with healthcare-associated pneumonia or cystic fibrosis (CF), thus outlining the urgent need for the development of new antibiotics.[1]
P. aeruginosa resistance to a plethora of antibiotics makes the treatment of infections particularly challenging and can be be classified into intrinsic, acquired and adaptive. Low outer membrane permeability accounts for the intrinsic resistance, while acquired resistance involves mutation or horizontal transfer of resistance genes. Notable example of this latter mechanism is represented by beta-lactamase enzymes. Finally, adaptive resistance involves the formation of biofilm in the lungs such as in CF patients.[2]
Bacterial quorum sensing (QS) is a cell-to-cell communication system that guides the expression of a wide range of genes involved in bacterial–host interactions, including virulence factors production, antibiotic resistance and biofilm formation.[3]
To facilitate the establishment of infection, *P. aeruginosa* produces a remarkable assortment of both cell-associated and extracellular virulence factors (*i.e.* extracellular proteases, iron chelators, efflux pumps expression and biofilm development), whose expression is regulated by two acyl-homoserine lactone (AHL) QS systems (LasI-LasR and RhlI-RhlR) and by *Pseudomonas* quinolone system (*pqs*). This latter utilizes 2-alkyl-4-quinolone-based autoinducers to bind the transcriptional regulator protein receptor PqsR, which triggers the synthesis and release of a variety of virulence factors including pyocyanin, rhamno-lipids, pyoverdine, elastase and hydrogen cyanide.[4]
In this context, the development of compounds able to impair the production of QS-regulated virulence factors represents a promising strategy to overcome *P. aeruginosa* pathogenicity and resistance.
Nitrogen heterocycles constitute a large group of compounds with a vast array of pharmacological properties, including anti-bacterial activity. Notably, quinazolinone derivatives have been extensively studied as PqsR antagonists due to their structural similarity with natural agonists.[5] We have designed and synthesized a novel series of quinozalin-4(3*H*)-one compounds characterized by the presence of various substituted aromatic residues at N3. These compounds have demonstrated intriguing phenotypic activity as QS modulators. Furthermore, in recent years, the development of drug candidates with multitargeting properties has been recognized as a promising strategy in combating antimicrobial resistance. Building upon this strategy, our objective was to interfere with metalloenzymes and metal-mediated metabolic pathways. To achieve this, we have also synthesized a library of quinazolinones linked to molecular scaffolds specifically designed to interact with different metal ions.

References

[1] I. Jurado-Martin, M. Sainz-Mejías, S. McClean. Pseudomonas aeruginosa: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors. Int J Mol Sci. 2021, 27, 2439.
[2] R. Frèdi Langendonk, D.R. Neill, J.L. Fothergill. The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol. 2021, 11, 6657759.
[3] Y. Xie, J. Chen, B. Wang, AY. Peng, ZW. Mao, W. Xia. Inhibition of Quorum-Sensing Regulator from Pseudomonas aeruginosa Using a Flavone Derivative.Molecules. 2022, 27, 2439.
[4] M. Kostylev, D.Y. Kim, N.E. Smalley, A.A. Dandekar. PNAS. 2019, 16, 7027.
[5] S. Sabir, T. Das, R. Kuppusamy, T. Tin Yu, M. Dp Willcox, D. StC Black, N. Kumr. Novel quinazolinone disulfide analogues as pqs quorum sensing inhibitors against Pseudomonas aeruginosa. Bioorg Chem. 2023, 130, 106226.

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Monitoring strategies of the immune response to SARS-CoV-2 after vaccination/recovery by lateral flow immunoassay

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Monitoring the efficacy of vaccination against COVID-19 and follow the immune response of vaccinated and/or recovered people is crucial for the return to normalcy [1]. High performance laboratory techniques cannot manage such large number of involved samples. Therefore, antibody detecting point-of-care (POC) testing, by means of the lateral flow immunoassay (LFIA) technique can fulfil this need. Well-established as a widely employed POC test for COVID19 antigen and serological diagnosis for antibody detection [2]. A typical serological LFIA includes a strip where capture immunoreagents are anchored in delimited reactive lines (test and control lines) and a glass fiber reservoir where the labelled detection reagent is dry stored. The serum is collected by a cellulose fiber pad, resuspend the labelled reagents and flows by capillarity through the nitrocellulose membrane encountering the reactive lines. Generally diagnostic tests are developed to increase indiscriminately the sensitivity, while to follow up the immune response progression in time, more than a sensitive qualitative test, a quantitative correlation with the reference serological laboratory method (ELISA) should be preferred. In this work we developed and tried several mono- and multi-target serological LFIA devices to detect antibody against the Spike protein and the Nucleocapsid protein from SARS-CoV2 to follow up the immune response given by recovery from infection or promoted by the vaccination. The devices were tested with more than 50 human sera, and the color intensity of the test lines was correlated with ELISA antibody response. The LFIAs for SARS-CoV2 antibody detection include capture antigens, recombinant Spike (S) and Nucleocapsid (N) proteins, and detection reagents, labelled with ruby red gold nanoparticles, different in the three main formats and several variants (LFIA-1, LFIA-2, LFIA-3). In the LFIA-1 the recombinant antigens were labelled with AuNPs (Ag*) to promote a double antigen sandwich Ag*-Ab-Ag, to avoid random antibody saturation with double specificity. In the LFIA-2 staphylococcal protein A (SpA) is the detection reagent, to increase the sensitivity due to the 5 binding domains for the antibodies. The LFIA-3 employs secondary anti-human G immunoglobulins antibodies (anti-hIg). The LFIA-2 format resulted as the most sensitive (Se=85.2%) but showed low correlation (r_s=0.47). Three variants, based on the multi line strategy, were adopted to discriminate "low" and "high" positives with a "traffic light" interpretation. No discrimination was possible, due to the interpatient variability and uncontrolled lattice of labelled bioreagents with random immunoglobulins. Otherwise, the LFIA-1 evidenced the highest correlation (r_s=0.84), and the use of a multi-line strategy allowed for a better discrimination of the immune response of the individuals with appearance of three, two, or just one of the test lines.

References

[1] J. Li, P.Giabbanelli, JMIR Med Inform, 2021, 9, 4, 2291-9694, 10.2196/27419
[2] https://www.finddx.org/tools-and-resources/dxconnect/test-directories/covid-19-test-directory/

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Promising green solvents as alternatives to acetonitrile in RP-LC for biopharmaceuticals applications

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Keywords: Green solvents, reversed-phase liquid chromatography, biopharmaceuticals analysis, preparative chromatography

Biopharmaceuticals are currently establishing as a promising class of drugs, thanks to their unique medical properties. Regardless of the synthetic technique chosen for their production, nevertheless, very complex mixtures are obtained, where minor species having structures similar to the active pharmaceutical ingredient coexist with the main product. These unwanted species are called "impurities" and must be removed for the product to be approved by regulatory agencies, which request a deep characterization of the risks associated with the residual impurities. Therefore, it is clear that the downstream processing development (namely the purification procedures) represents a key point to boost the product quality and purity [1].
Purification of biopharmaceuticals at laboratory and industrial scale is usually conducted through preparative liquid chromatography, which requires considerable amounts of solvents. Since often these compounds are hydrophobic, the chromatographic mode most often employed is Reversed-Phase LC, where an organic solvent (generally acetonitrile, ACN) is used together with an aqueous buffer. ACN shows excellent chemical-physical properties suitable for RP-LC applications, such as elution strength, viscosity, miscibility with water, and UV transparency, but it can also result to be potentially toxic if adsorbed by the human body since it is converted into cyanide, which is the source of said toxicity.
Given these premises, ACN is now classified as a solvent which is recommended to be substituted. Good candidates for its replacement could be traditional alcohols, especially ethanol and isopropanol, which have already been deeply studied for RP-LC applications, but rarely used for preparative biopharmaceuticals purification. On the other side, unconventional and innovative solvents are being tested as green alternatives for chromatographic application. One example is dimethyl carbonate (DMC), which up to now has mainly been employed for batteries, except for one application in HPLC-ICP-MS related to small molecules [2]. In this work, promising chromatographic performance of DMC will be shown, not only regarding small molecules but also for some peptides, both on analytical and on preparative scale, to corroborate its applicability for biopharmaceuticals analysis and purification. Its elution properties will also be compared to other potential alternative solvent such as some alcohols.

References

[1] L. Ferrazzano, M. Catani, A. Cavazzini et al. Sustainability in peptide chemistry: current synthesis and purification technologies and future challenges. Green Chem. 2022, 24, 975-1020.
[2] B. Lajin, W. Goessler. Introducing dimethyl carbonate as a new eluent in HPLC-ICPMS: stronger elution with less carbon. J Anal At Spectrom. 2021, 36, 1272-1279.

AMYC BIOMED 2023

Redesigning and improving the synthesis of coumarin-based TADF dyes for bioimaging applications

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Keywords: Coumarin, Fluorescence, theranostics

The development of new fluorophores for in vivo and in vitro bioimaging applications is a very lively research field which consists in the study of high efficiency and selectivity fluorescent molecular probes[1]. Studies in this field are limited by the low yields of the syntheses that allow access to these compounds[2]. Recently our research group has undertaken a series of studies aimed at the identification of new molecular scaffolds with TADF properties to be employed in technological and diagnostic applications[3,4]. Herein we report a new strategy for the synthesis of TADF coumarins and the realization of a library of derivatives which were photophysically characterized and evaluated as fluorescent probes *in vitro* tumour cells screening.
By means of a computer-aided approach we have been able to identify a series of coumarins with delayed fluorescence (Cmr-Cz), associated with low levels of cytotoxicity and excellent cell permeability. Furthermore, selected coumarins endowed with high quantum efficiency value were selected for the study of the optical sensitivity to oxygen, showing low oxygen sensitivity. Overall, these compounds showed potential applications as oxygen sensors as well as for diagnostic and therapeutic purposes.

Figure 1: Representative fluorescence and in vitro assay of TADF coumarin Cmr-Cz derivatives
References

References

[1] F. Fang, L. Zhu, M. Li, Y. Song, M. Sun, D. Zhao, J. Zhang, Adv. Mat., 2021, 8, 2102970-2102989.
[2] J.-X. Chen, W. Liu, C.-J. Zheng, K. Wang, K. Liang, Y.-Z. Shi, X.-M. Ou, X.-H. Zhang, ACS Appl. Mater. Interfaces 2017, 9, 10, 8848–8854
[3] A. Cocco, P. Caria, G. Sanna, L. Stagi, E. Cadoni, R. Corpino, P.C. Ricci, C.M. Carbonaro, F. Secci, ACS Omega 2021, 6, 33708-33716.

[4] T. Dettori, G. Sanna, A. Cocco, G. Serreli, M. Deiana, V. Palmas, V. Onnis, L. Pilia, N. Melis, D. Moi, P. Caria, F. Secci. Molecules, 2022, 27, 8897.

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Hybrid compounds natural products based as multifunctional agents against Alzheimer's disease

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Keywords: neuroprotection; amyloid β (Aβ); chelating agents; antioxidants

Alzheimer's disease (AD) is a neurodegenerative disorder that leads to progressive loss of memory, decline in language skills and other cognitive functions inducing a downfall in patient's ability to live in society [1]. Much experimental evidence has suggested that AD is a multifactorial illness characterized by a detrimental reduction of acetylcholine levels combined with high oxidative stress (OS) and dyshomeostasis of biometals [2]. One of the main hallmark of AD is the extracellular senile plaques formation, characterized by β-amyloid (Aβ). Natural bioactive compounds extracted from plants and organisms, commonly named nutraceuticals, have been used in traditional medicine, handing over their use from one generation to another. The multifunctional character of natural products has drawn the attention of the researchers because these molecules, and their derivatives, are able to simultaneously act on several targets of AD. Recently, many natural products are placed under investigation in pre-clinical and clinical trials in the treatment of AD [3].
In this contest, a series of new derivatives **1a-c**, **2a-c**, **3a-c**, **4a-c**, **5a-c**, inspired by nature, were synthesized and studied as multifunctional agents for the treatment of Alzheimer's disease (AD). These compounds were designed merging together the previously identified trifluoromethyl benzyloxyamidic scaffold, with different moieties present in natural compounds. The ability of the synthesized compounds to chelate biometals, such as Cu^{2+}, Zn^{2+} and Fe^{2+}, was studied by UV-vis spectrometer and, a preliminary screening to evaluate their antioxidant activity was done by DPPH and ABTS free radical methods. Then, few selected compounds were tested on post mortem rat brain using antioxidant TBARS assay. Combining the results, compounds **2a-c**, the strongest antioxidant and biometal chelators, were studied for their ability to contrast Aβ aggregation.

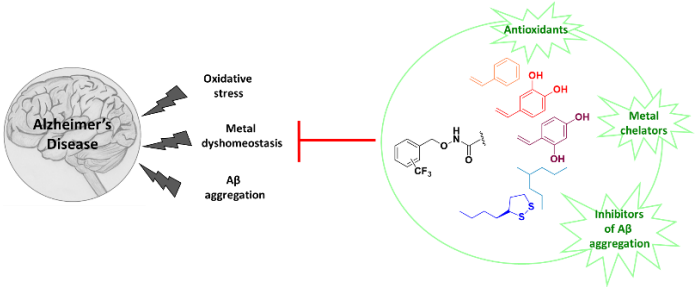

Figure 1. Overview of hybrid compounds natural products based as potential agents against AD.

References

[1] Knopman, D.S.; Amieva, H.; Petersen, R.C.; Chételat, G.; Holtzman, D.M.; Hyman, B.T.; Nixon, R.A.; Jones, D.T. Alzheimer Disease. Nat Rev Dis Primers **2021**, 7, 1-21
[2] Aaseth, J.; Skalny, A.V.; Roos, P.M.; Alexander, J.; Aschner, M.; Tinkov, A.A. Copper, Iron, Selenium and Lipo-Glycemic Dysmetabolism in Alzheimer's Disease. Int. J. of Mol. Sci. **2021**, 22, 9461

[3] Andrade, S.; Ramalho, M.J.; Loureiro, J.A. Natural Compounds for Alzheimer's Disease Therapy: A Systematic Review of Preclinical and Clinical Studies. Int. J. Mol. Sci. **2019**, 41.

AMYC BIOMED 2023

A double steroid chemiluminescence immunoassay employing reusable microchannel and functionalized superparamagnetic microbeads

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Keywords: Chemiluminescence; Cortisol; DHEA; Immunoassay; Magnetic beads; Microfluidics

Portable and simple analytical devices to be used for routinary self-diagnostic applications are particularly attractive for avoiding high-cost and time-consuming analysis. Lab-on-chip (LOC) approach combining a microfluidic platform with ultrasensitive miniaturized detector, perfectly fit this purpose and the possibility to make this kind of tool reusable and suitable for the analysis of different target analytes can be a great step forward for Point-of-Care applications.
The proposed work in the frame of the project APHRODITE (Autonomous PHotosensing Reusable Onboard Device for Immunological Tests Execution) selected and financed by Italian Space Agency (ASI) focused on the optimization of a dual-chemiluminescent immunoassay implemented onto a portable and easy-to-use platform for the quantification of two salivary hormones, cortisol and dehydroepiandrosterone (DHEA). Cortisol and DHEA are two most abundant steroid hormones produced by the zona fasciculata and zona reticularis of the adrenal cortex in the adrenal gland, respectively. They play a role in the stress response and they are related to immune system dysfunctions [1]. Several assay techniques have been used to measure salivary cortisol, including radioimmunoassay and more recently liquid chromatography–tandem mass spectrometry [2].
The developed method is based on the use of immunological technique combined with chemiluminescent (CL) detection exploiting a microfluidic channel integrated with a dedicated detector. In this approach, two aliquots of magnetic beads (MBs) functionalized respectively with anti-cortisol and anti-DHEA antibodies, were entrapped by magnets in two different areas along the microchannel. Since the competitive immunoassays format was employed, a solution containing the sample and the peroxidase-conjugates of both DHEA and cortisol was injected to enable the competition for binding the antibodies immobilized on the MBs surfaces. After washing step, by adding the proper CL substrate it was possible to monitor the CL signal in real time employing an array of hydrogenated amorphous silicon (a-Si:H) photosensors (Figure 1). Once the assay was completed, by removing the magnets and performing washing step, the microchannel was clean and ready to run a new assay. With this system, taking advantage of the possibility of confine magnetically the immunoprobes, chemiluminescence detectability, and photosensor sensitivity, accurate quantification of target analytes down to 0.1 ng mL^{-1} for cortisol and 0.05 ng mL^{-1} for DHEA were obtained with high specificity and multiplexing ability.
Results confirmed the good detection capabilities and assay applicability of the proposed system prompting the development of innovative universal tool for multiplex assays that allow to monitor simultaneously health-related panel of biomarkers through a single analysis.

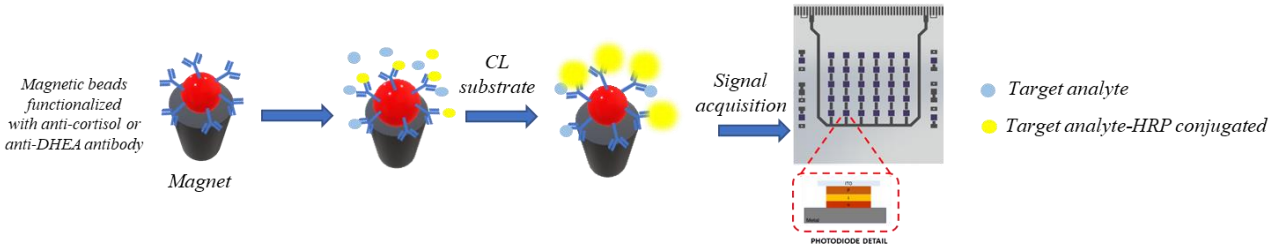

Figure 1: Scheme of the competitive CL immunoassay on magnetic beads

References

[1] Rooney BV et al., Herpes Virus Reactivation in Astronauts During Spaceflight and Its Application on Earth. Front. Microbiol.2019

[2] Warrick J. Inder, Goce Dimeski, and Anthony Russell, Measurement of salivary cortisol in 2012 – laboratory techniques and clinical indications

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Combining EPR and NMR spectroscopies to investigate the function and dynamics of XRCC4 protein involved in the non-homologous DNA end joining pathway

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Keywords: DNA-repairing process, NMR spectroscopy, EPR spectroscopy, protein-protein interactions, protein-DNA-interactions

DNA double strand breaks (DSBs) are one of the most dangerous types of DNA damages because they can result in the loss of large chromosomal regions. In mammals, DSBs are predominantly repaired by the *non-homologous* DNA *end joining* (NHEJ) pathway where different proteins, with specific functions, participate forming a large complex [1]. In this process, the DSBs are initially detected by the three proteins (Ku70/Ku80 and DNA-PKcs), that help the recruitment of the other components. After processing of the DNA ends, the final joining step is catalyzed by DNA Ligase IV (LIG4) in association with the *X-ray repair cross complementing 4* protein (XRCC4). XRCC4 is a homodimeric protein (2x38 kDa) whose monomer is formed by a folded head domain (*a.a.1-117*) that interacts with XLF, a stalk helical domain (*a.a.116-203*) which interacts with LIG4, and a C-terminal intrinsically disordered region (IDR) (*a.a. 204-336*). Moreover, the activity of LIG4/XRCC4 is further stimulated by XRCC4-like factor (XLF) which interacts directly with both XRCC4 and Ku proteins [2].
Despite several investigations of the folded domain of XRCC4, little is known about the function of its IDR [3]. In this study, we combined EPR and NMR spectroscopy to provide new structural insights about XRCC4. After site directed labelling reactions we performed cw-EPR, 4p-DEER and PRE-NMR experiments in order to characterize the XRCC4 IDR dynamics and also its interactions with LIG4, XLF and DNA. Using the same combinatorial experimental approach, we extended our analysis to the folded head domain to provide a finer understanding of its structural dynamics under its intra- and inter-molecular interactions. Altogether our results demonstrate a high degree of structural flexibility of the XRCC4 IDR and suggest how it could interact with its folded domains to modulate recruitment of multiple partners including DNA during DSB repair by NHEJ.

References

[1] Chang, H. H. Y. *et al.*, *Nat. Rev.* 18, 495–506 (2017).
[2] Chen, S. *et al.*, *Nature* 593, (2021).
[3] Normanno *et al.*, *Elife* 4, 1–28 (2017).

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TEMPO-labeled polyethyleneimines for gene-delivery with antioxidant perspectives

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Keywords: TEMPO, polyethylenimine, antioxidant activity, gene-delivery

The 2,2,6,6-tetramethylpiperidin-1-oxyl molecule, commonly known as TEMPO, is one of the most popular stable nitroxyl radicals. Among its various applications, it has already proved to be useful as spin probe and magnetic resonance imaging (MRI) contrast agent, as well as antioxidant in biological systems. [1] The aim of this work was to exploit this last function, the antioxidant ability, to obtain an anti-inflammatory and cytoprotective effect during the use of polyethyleneimines (PEIs) as non-viral carriers in the field of gene delivery. In fact, PEI has been detected as the golden standard for this application; [2] as a cationic polymer, it can indeed spontaneously assemble with anionic exogenous nucleic acids (NAs) to form polyplexes in high yields, able to deliver their payload intracellularly. [3] Nonetheless, it is of utmost urgency to design more effective transfection reagents: polyplexes might suffer from aggregation upon systemic administration, and unfortunately, their loading efficacy (N/P value) and cytotoxicity are directly proportional. [4]
To assess if the introduction of the antioxidant TEMPO moiety could reduce the cytotoxic effect intrinsic in the cationic polymers used as a vector, TEMPO-PEIs with different grafting degrees were designed and tested, looking for a dose-response correlation. To accomplish that, few linkers were selected and reacted with TEMPO, then the synthesized TEMPO-derivatives were grafted with different PEIs with the aim of obtaining a biocompatible material to be tested for its intrinsic antioxidant properties. Linear and branched PEIs were therefore reacted with the TEMPO-derivatives to get polymers with different grafting degree. The different compounds obtained were characterized via NMR and elemental analyses, whereas EPR analyses were performed to determine the effective grafting degree of TEMPO on each polymer. Titration of the free amino groups were used for the N/P ratio calculation before the NAs pairing for the transfection assays. Cytotoxicity tests, on two different cell lines (HeLa cells and L929 cells) were carried out on the different PEIs functionalized with TEMPO. Given the good performances registered in preliminary assays, the synthesized materials are being studied as possible non-viral vectors in gene-delivery.

References

[1] E. Mauri, E. Micotti, A. Rossetti et al. Microwave-assisted synthesis of TEMPO-labeled hydrogels traceable with MRI. *Soft Matter*, **2018**, *14*, 558-565; B. P. Soule, F. Hyodo et al. The chemistry and biology of nitroxide compounds. *Free Radical Biology and Medicine*, **2007**, *42*, 11, 1632–1650; C. Prescott, S. E. Bottle. Biological relevance of free radicals and nitroxides. *Cell Biochemistry and Biophysics*, **2017**, *75*, 2, 227–240.
[2] D. Pezzoli, E. K. Tsekoura, K.C. R. Bahadur, G. Candiani et al. Hydrophobe-substituted b-pei derivatives: boosting transfection on primary vascular cells. *Science China Materials*, **2017**, *60*, 6, 529–542.
[3] G. Protopapa, N. Bono, R. Visone et al. A new microfluidic platform for the highly reproducible preparation of non-viral gene delivery complexes. *Lab Chip*, **2023**, *23*, 136-145.
[4] N. Bono, B. Coloma Smith, F. Moreschi et al. *In silico* prediction of the *in vitro* behavior of polymeric gene delivery vectors. *Nanoscale*, **2021**, *13*, 17, 8333–8342.

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Quality by Design tools in early-stage nanopharmaceuticals development

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Keywords: Optimization, nanomedicine, reproducibility, design, quality

According to Quality by Design (QbD) approach, quality should be built into product during pharmaceutical development. The guidelines for current good manufacturing practice (cGMP) edited by Food and Drug Administration (FDA) and European Medicinal Agency (EMA) introduced QbD during the pharmaceutical development at industrial level. Nowadays, academic researchers whose ambitions lie in translational science are focused on applying QbD to nanomedicine research. The application of QbD tools guarantees final product with high-quality target profile, emphasizing product and process understanding to speed up the research timeline and reduce the gap from lab bench into applicable therapeutics [1]. The rational design of nanomedicine is a laborious and time-consuming process since several input factors may affect the product outcome. In addition to the selection of the raw materials (i.e. type, concentration, ratio), other factors such as the process parameters (i.e. time, temperature, pH) can influence the quality attributes and the physico-chemical properties of the final product (i.e. size, shape, surface properties, stability) and need to be tuned. Therefore, identifying the critical material attributes (CMAs) and the critical process parameters (CPPs) in terms of their impact on product performance, safety and quality are of utmost importance. Within this context, computer-based models offer many benefits, specially at early-stage pharmaceutical product development.
The Design of Experiment (DoE), for example, is a methodology that allows the experimenter to systematically and simultaneously vary multiple input variables (X) to investigate its effects on a measured response (Y) and find the optimum of a process/product through mathematical models and statistical parameters [1]. This multivariate approach allows to evaluate the interactions among all variables in a reduced number of experiments obtaining the best information with minimum effort in terms of time and cost. Additional consideration includes the evaluation of batch-to-batch consistency to ensure a successful reproducible manufacturing process. A detailed overview of different nanoplatforms (nanocrystals; polymeric micro- and nanoparticles; nanogel) designed and optimized following the QbD criteria will be presented. Each nanoformulation has been designed using different type of DoE (Box Benken-; D-Optimal-; I-Optimal Design) on the basis of specific active payload such as: biomolecules (Lacticaseibacillus rhamnosus GG; ovalbumin), natural compound (berberine; curcumin) or synthetic drug (carbamazepine) for various applications (functional food; vaccine delivery; brain delivery) [2-6]. Overall, our results, showed the applicability and reliability (error % <10) of DoE technology to implement nanopharmaceuticals development to obtain a product with fulfills attributes and quality profile, achieving a robust manufacturing process.

References

[1] A. Bonaccorso, G. Russo, F. Pappalardo, C. Carbone, G. Puglisi, R. Pignatello, T. Musumeci. Quality by design tools reducing the gap from bench to bedside for nanomedicine. Eur J Pharm Biopharm. 2021, 169:144-155.
[2] A. Bonaccorso, N. Russo, A. Romeo, C. Carbone, M.A. Grimaudo, C. Alvarez-Lorenzo, C. Randazzo, T. Musumeci, C. Caggia. Coating Lacticaseibacillus rhamnosus GG in Alginate Systems: an Emerging Strategy Towards Improved Viability in Orange Juice. AAPS PharmSciTech. 2021, 22(3):123.
[3] A. Bonaccorso, C. Carbone, B. Tomasello, P. Italiani, T. Musumeci, G. Puglisi, R. Pignatello. Optimization of dextran sulfate/poly-l-lysine based nanogels polyelectrolyte complex for intranasal ovalbumin delivery. J Drug Deliv Sci Technol. 2021, 65, 102678
[4] T. Musumeci, A. Bonaccorso, C. Carbone, G. Russo, F. Pappalardo, G. Puglisi. Design and optimization of PEGylated nanoparticles intended for Berberine Chloride delivery. J Drug Deliv Sci Technol. 2019, 52: 521-530.

[5] A. Bonaccorso, M.R. Gigliobianco, R. Pellitteri, D. Santonocito, C. Carbone, P. Di Martino, G. Puglisi, T. Musumeci. Optimization of curcumin nanocrystals as promising strategy for nose-to-brain delivery application. Pharmaceutics. 2020, 12, 476.
[6] A. Bonaccorso, M.R. Gigliobianco, R. Lombardo, R. Pellitteri, P. Di Martino, A. Mancuso, T. Musumeci. Nanonized carbamazepine for nose-to-brain delivery: pharmaceutical formulation development. Pharm Dev Technol. 2023, 28(2):248-263.

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Molecular modelling for (bio)technologies

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Keywords: Computational chemistry, molecular dynamics, density functional theory, metal-dependent systems

Molecular modelling is a research strategy based on the use of modern tools, algorithms and workflows provided by the theoretical and computational chemistry. It is adopted for the investigation of different chemical properties, such as reactivity, chemical-physics parameters and structural characterization, of molecules and complex chemical systems. Molecular modelling, in addition, gives the opportunity to look at the molecules at atomistic level, providing information and an unrivalled point of view of the investigated system. With such knowledge level, coupled to that arising from the experimental observations, it is possible to design rationally, and customize, new molecular properties of targeted systems. At this purpose, the presentation aims to show the efforts carried out by our group in contributing/extending the use of molecular modelling tools to systems with (bio)technological relevance. A brief introduction of the schemes and workflows provided by state-of-art computational chemistry approaches routinely used in our lab will be initially given, highlighting, during the course of the presentation, the results obtained for some representative investigated systems. In particular, will be provided results on the application of molecular modelling approach to the in-depth understanding of the activity of three systems: the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), the plastic degrading fastPETase and 4f/5f-dependent proteins.

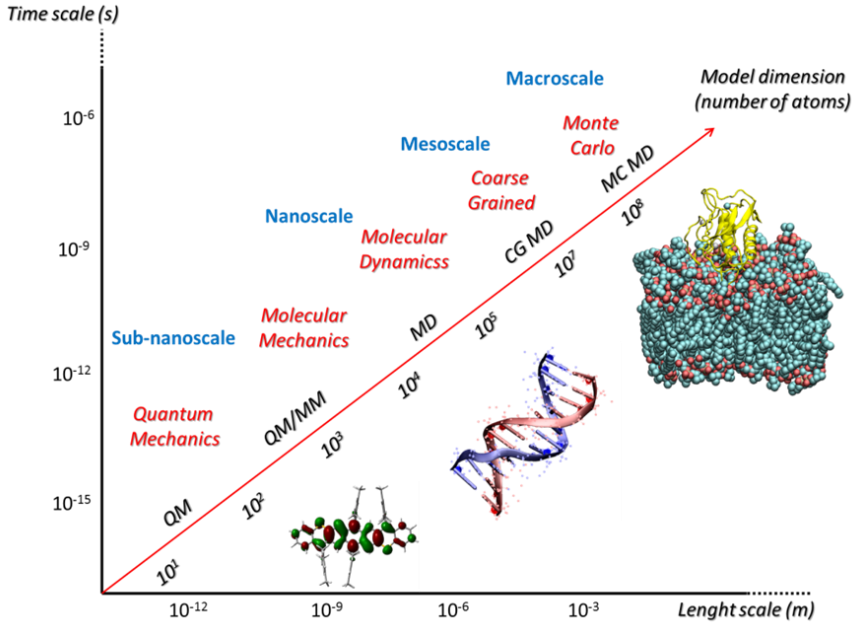

Figure 1. Molecular modelling strateg.

Paper substrates integrating laser-induced platinum nanostructures with nanozyme activity for smartphone-based ascorbic acid determination

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Keywords: CO_2-laser, one-shot nanodecoration, colorimetric assay, paper-based platform, oxidase-mimicking activity

Pt-based nanomaterials (nPt) are widely employed as heterogeneous catalysts in energy, medicine, chemical, environmental and (bio)sensors. The latter mainly exploits colloidal nPt due to the oxidase-mimicking activity, which allows catalyzing oxidation-reaction in the presence of O_2 [1]. In this framework, the nPt-nanozyme performance is strictly related to the nPt chemistry, size, morphology, and surface; the presence of surfactants and/or stabilizing agents often reduces/masks this activity. In this framework, several analytical efforts are devoted to avoiding the use of external stabilizers to synthesize nPt and integrate catalytic nPt onto solid substrates. In particular, in the (bio)sensors field, the search for alternative strategies to customize the Pt nanodecoration of flexible supports (i.e., cellulosic, flexible plastics, textiles, etc.) and to assemble the same in all-in-one devices is still an open challenge [2].
Herein, a new CO_2 laser-based strategy to in-situ synthesize nacked Pt nanostructures (PtNtr) with micrometric-resolution patterns, directly onto cellulosic substrates, will be presented. An office-grade CO_2 laser plotter was used to achieve the direct paper nano-structuration by triggering the metal reduction and nucleation, without the need for external reducing/stabilizing agents. Noteworthy, the PtNtr-formation occurs according to tailored designs means the use of a design-software, making this strategy able to give rise to customizable patterns. The as-obtained PtNtr-paper substrates were carefully studied and morpho-chemically characterized; eventually, the paper nanodecored substrates were combined with office instruments to realize a flip-flop paper device (FF-PD).
The FF-PD, composed by PtNtr-paper interfaced to a colorimetric layer made of fiberglass containing 3,3',5,5'-tetramethylbenzidine (TMB) (Fig. A), was employed for the fast determination of ascorbic acid (AA). The sensing principle relies on the PtNtr oxidase-like activity that allows the TMB color to switch from colorless to blue; the analytical strategy relies on the AA's ability to inhibit TMB oxidation and relative color formation (Fig. B). The FF-PD, conceived to favor the sample fluidic and maximize the PtNtr-paper catalytic activity, allow obtaining reliable colorimetric readout just using a simple smartphone. The herein-presented FF-PD allows the multiple, selective, and immediate determination of AA in various food and biological samples. Herein for the first time, the in-situ laser-writing of PtNtr onto cellulosic substrates is proposed; this strategy is a captivating tool for the manufacturing of within everyone's reach (bio)sensing paper-based colorimetric devices.

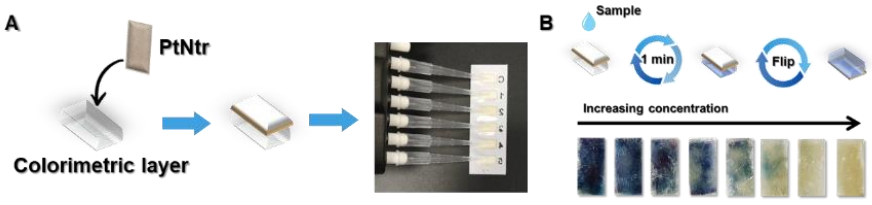

Figure. (A) Flip-flop paper-device assembling; (B) assay format and colorimetric response obtained with increasing AA concentration.

References

[1] Q. Cheng, Y. Yang, Y. Peng, M. Liu. Pt nanoparticles with high oxidase-like activity and reusability for detection of ascorbic acid. Nanomaterials. 2020. 10, 6, 1015.
[2] A. Scroccarello, F. Della Pelle, D. Compagnone. Optical plasmonic sensing based on nanomaterials integrated in solid supports. A critical review. ACA. 2023, 1237, 340954.

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From MG624 to selective antagonists of α9α10 nicotinic acetylcholine receptor

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Keywords: nicotinic acetylcholine receptor (nAChR); α9α10-nAChR; α7-nAChR; Structure-activity relationships (SAR)

The 2-triethylammonium ethyl ether of 4-stilbenol (**MG624**) is an unselective antagonist of the α7-nAChR (1.99 μM IC_{50}) and α9α10-nAChR (6.68 μM IC_{50}), with moderate and high selectivity over the β4 and β2 containing nAChRs respectively [1,2,3]. This compound was extensively studied in the recent past because dual inhibitors of α7- and α9α10-nAChRs could be used as potential anticancer drug in adenocarcinoma models [3]. On the other hand, selective inhibition of α9α10-nAChR could be desirable to target neuropathic pain, as demonstrated by *in vivo* animal studies of several selective conotoxin [4]. Moreover, selective activation of the metabotropic function of α9α10-nAchR in the immune cell system could result in new anti-inflammatory agents [4].
In this scenario, aiming to increase the functional selectivity toward α9α10- over the α7-nAChR subtype, the synthesis of more than sixty MG624 analogues was performed. All the MG624 analogues were characterized for binding affinity at the α7-nAChRs, and a selection of these compounds was assayed in electrophysiological experiments using *Xenopus laevis* oocytes expressing human α7- or α9α10-nAChRs [2,5]. The SAR study allowed to define that selective inhibition of ACh-evoked currents at α9α10-nAChR could be achieved by modifying different portion of the lead compound MG624 (*i.e.*, the ammonium head, the linker, and the stilbene moiety, Figure 1). Moreover, compound **1** was identified as one of the most interesting compounds of the series [2], being a potent and selective antagonist of α9α10-nAChR (5.74 μM IC_{50}), with very low affinity at α7-nAChR, and completely devoid of activity at the α7-nAChR subtype at the maximum tested concentration (100 μM). For these features, compound **1** and analogues will be investigated as potential anti-inflammatory agents *in vitro*.

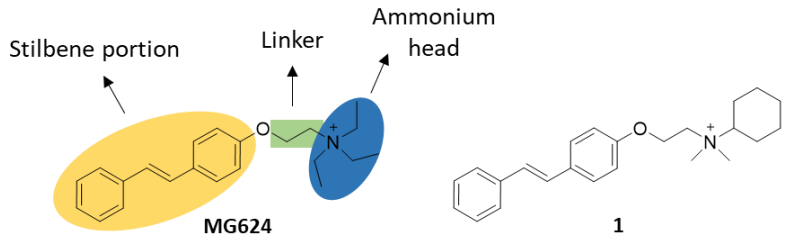

Figure 1. *Representation of SAR study on* ***MG624*** *and structure of compound* ***1****.*

References

[1] C. Gotti, B. Balestra, M. Moretti, G.E. Rovati, L. Maggi, G. Rossoni, F. Berti, L. Villa, M. Pallavicini, F. Clementi, 4-Oxystilbene compounds are selective ligands for neuronal nicotinic αBungarotoxin receptors, Br J Pharmacol. 124 (1998) 1197–1206.
[2] F. Bavo, M. Pallavicini, S. Pucci, R. Appiani, A. Giraudo, B. Eaton, L. Lucero, C. Gotti, M. Moretti, P. Whiteaker, C. Bolchi, From 2-Triethylammonium Ethyl Ether of 4-Stilbenol (MG624) to Selective Small-Molecule Antagonists of Human α9α10 Nicotinic Receptor by Modifications at the Ammonium Ethyl Residue, J Med Chem. 65 (2022) 10079–10097.

[3] V. Mucchietto, F. Fasoli, S. Pucci, M. Moretti, R. Benfante, A. Maroli, S. Di Lascio, C. Bolchi, M. Pallavicini, C. Dowell, M. McIntosh, F. Clementi, C. Gotti, α9- and α7-containing receptors mediate the pro-proliferative effects of nicotine in the A549 adenocarcinoma cell line, Br J Pharmacol. 175 (2018) 1957–1972.
[4] A. Giraudo, M. Pallavicini, C. Bolchi, Small molecule ligands for α9 * and α7 nicotinic receptors: A survey and an update, respectively, Pharmacol Res. 193 (2023) 106801.
[5] F. Bavo, M. Pallavicini, S. Pucci, R. Appiani, A. Giraudo, H. Oh, D.L. Kneisley, B. Eaton, L. Lucero, C. Gotti, F. Clementi, P. Whiteaker, C. Bolchi, Subnanomolar Affinity and Selective Antagonism at α7 Nicotinic Receptor by Combined Modifications of 2-Triethylammonium Ethyl Ether of 4-Stilbenol (MG624), J Med Chem. 66 (2023) 306–332.

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Beyond Fluorescent Signal Readouts: New Design Principles for Cucurbit[*n*]uril-Based Chemosensors

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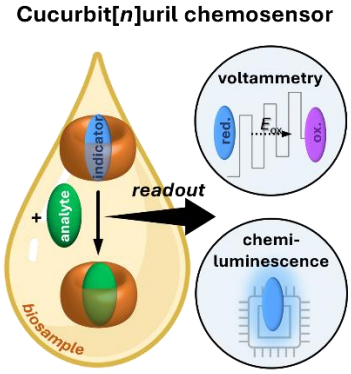
Keywords: Chemosensor, chemiluminescence, electrochemistry, biofluids, drugs

Supramolecular chemistry has enabled the design and preparation of artificial receptors that have potential applications in various fields such as environmental and medical diagnostics.[1] Chemosensors are functional receptor-like molecules that bind to analytes through noncovalent interactions.[2]
Among synthetic macrocyclic receptors known to bind small organic molecules, cucurbit[*n*]uril (CB*n*)[3] receptors exhibit exceptional binding affinity for many biomolecules and drugs.[4] For this reason, CB*n* have attracted much interest in the development of fluorescence-based chemosensor assays. Although conceptually simple, fluorescence-based signal readout has several drawbacks, such as suboptimal signal-to-noise ratio, or poses challenges for the development of miniaturized and portable sensors.[5]
In this work, new design principles are presented for implementing chemiluminescent[6] or electrochemical[7] readouts using CB*n*-based chemosensors for the detection of drugs in biofluids. Both cucurbit[8]uril (CB8) and cucurbit[7]uril (CB7) serve as macrocyclic receptors, while adamantyl-containing phenoxy-1,2-dioxetanes and a platinum-triazolylpyridine complex are used as chemiluminescent and electrochemically active indicators, respectively. The operating principle of these new chemosensors is based on the modulated chemiluminescent or electrochemical properties of the indicators once they form a host-guest inclusion complex with CB*n*. By setting up an indicator displacement assay, drugs can be detected at low micromolar concentrations in water and biofluids, such as urine or serum. The presented CB*n*-based chemosensors are a useful alternative to existing fluorescence-based sensors as they enable new types of signal readout relevant to point-of-care applications and the design of wearable sensors.



Cucurbit[*n*]uril-based chemosensors with electrochemical or chemiluminescent readout for the detection of drugs in biofluids

References

[1] [a] J.-M. Lehn. Supramolecular Chemistry – Scope and Perspectives Molecules, Supermolecules, and Molecular Device. *Angew. Chem. Int. Ed.* **1988**, 27, 89-112; [b] L. You, D. Zha, E. V. Anslyn. Recent Advances in Supramolecular Analytical

Chemistry Using Optical Sensing. *Chem. Rev.* **2015**, *115*, 7840-7892; [c] J. Krämer, R. Kang, L. M. Grimm, P. Picchetti, F. Biedermann. Molecular Probes, Chemosensors, and Nanosensors for Optical Detection of Biorelevant Molecules and Ions in Aqueous Media and Biofluids. *Chem. Rev.* **2022**, *122*, 3459-3636.
[2] T. W. Bell, N. M. Hext. Supramolecular Optical Chemosensors for Organic Analytes. *Chem. Soc. Rev.* **2004**, *33*, 589-598.
[3] [a] R. Behrend, E. Meyer, F. Rusche. Ueber Condensationsproducte aus Glycoluril und Formaldehyd. *Liebigs Ann. Chem.* **1905**, *339*, 1; [b] W. A. Freeman, W. L. Mock, N. Y. Shih. Cucurbituril. *J. Am. Chem. Soc.* **1981**, *103*, 7367-7368; [c] J. Kim, I.-S. Jung, S.-Y. Kim, E. Lee, J.-K. Kang, S. Sakamoto, K. Yamaguchi, K. Kimoon. New Cucurbituril Homologues: Syntheses, Isolation, Characterization, and X-ray Crystal Structures of Cucurbit[*n*]uril (*n* = 5, 7, and 8). *J. Am. Chem. Soc.* **2000**, *122*, 540-541.
[4] F. Biedermann, V. D. Uzunova, O. A. Scherman, W. M. Nau, A. De Simone. Release of High-Energy Water as an Essential Driving Force for the High-Affinity Binding of Cucurbit[*n*]urils. *J. Am. Chem. Soc.* **2012**, *134*, 15318-15323.
[5] S. Sinn, F. Biedermann. Chemical Sensors Based on Cucurbit[*n*]uril Macrocycles. *Isr. J. Chem.* **2018**, *58*, 357-412.
[6] [a] A. Simeonov, Davis, M. I. Interference with Fluorescence and Absorbance; Eli Lilly & Company and the National Center for Advancing Translational Sciences. 2015 URL: https://www.ncbi.nlm.nih.gov/books/NBK343429/. [b] H. C. Ates, P. Q. Nguyen, L. Gonzalez-Macia, E. Morales-Narváez, F. Güder, J. J. Collins, C. Dincer. End-to-End Design of Wearable Sensors. *Nat. Rev. Mater.* **2022**, *7*, 887-907.

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A drug repurposing strategy to treat an ultra-rare IAHSP form

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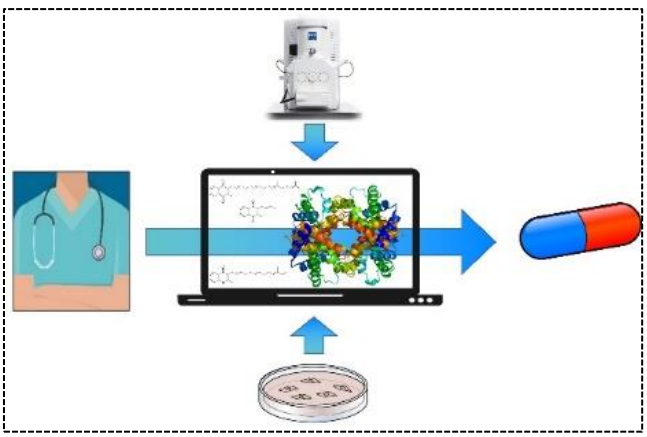
Keywords: Drug Repurposing, Cryo-EM, Virtual Screening, Hereditary Spastic Paraplegia, Rare Disease

Infantile Ascending Hereditary Spastic Paralysis (IAHSP) is an infantile ultra-rare disease with autosomal recessive transmission pattern originating from mutations to the gene ALS2, coding for the protein Alsin known for its implications in the pathogenesis of other hereditary spastic paraplegia forms (HSPs, e.g., Amyotrophic lateral Sclerosis). Alsin is essential for the maintenance of motor neurons, and it has been implicated in cell mechanisms such the vesicular transport, as well as in mitochondrial fusion/fission dynamics.[1]
Pathogenic mutations encompass early truncations and missense, leading to amino acids substitutions. Some of those mutants have been investigated by means of molecular biology and biophysics techniques, understanding that the active, cytosolic form of Alsin is a tetramer.[2] However, the 3D molecular structure of Alsin and its molecular aspects are unknown, impeding medicinal chemistry strategies to develop specific treatments.
In this work, we investigated the aggregation status of Alsin with the application of computational tools. We started by analyzing the AlphaFold model of human Alsin and applied protein docking techniques to obtain dimer and tetramer models compatible with the biochemical literature data.[3] Then, in an extensive collaborative effort, we expressed and purified WT Alsin. This served as basis launch to resolve Alsin's structure with high resolution. This is the first time that the structure of Alsin is experimentally resolved, and this result allowed us to benchmark our computational predictions, as well as to better understand the exact role of selected amino acids. Furthermore, we could generate missense mutant models, make founded hypotheses about their pathogenic mechanisms, and predict their druggability.
To demonstrate the usefulness of this molecular knowledge, we synthetically report a patient case harboring a R1611W missense mutation. Computational predictions indicate that the mutant protein forms abnormal oligomers, impeding further protein functions. A virtual screening aimed at repurposing molecules binding the mutant residue identified MK4, a safe, therapy approved compound.[4] We generated a skin fibroblast cell line from the patient and performed proof-of-concept experiments confirming its safety and beneficial effect. These results were enough to obtain a compassionate treatment approval in Italy, and the patient is currently under treatment.
Our results show that the application of computational techniques can drive personalized drug discovery programs in a time and cost-efficient manner. This is supported by the structural experimental data and the in vitro tests. Overall, we report that a balanced approach between experiments and predictions can have a concrete impact on the life of patients suffering from rare diseases.



References

[1] Lesca, G. et al. Infantile ascending hereditary spastic paralysis (IAHSP): Clinical features in 11 families. Neurology 60, 674–682 (2003).
[2] Sato, K. et al. Altered oligomeric states in pathogenic ALS2 variants associated with juvenile motor neuron diseases cause loss of ALS2-mediated endosomal function. Journal of Biological Chemistry 293, 17135–17153 (2018).
[3] Rossi Sebastiano, M., Ermondi, G., Hadano, S. & Caron, G. AI-based protein structure databases have the potential to accelerate rare diseases research: AlphaFoldDB and the case of IAHSP/Alsin. Drug Discov Today xxx, (2021).
[4] Rossi Sebastiano, M. et al. Personalized Treatment for Infantile Ascending Hereditary Spastic Paralysis Based on In Silico Strategies. Molecules 27, 7063 (2022).

AMYC BIOMED 2023

Structure-Activity Relationship Investigation into the Antitubercular Potential of *N*-aryl-2,5-dimethylpyrroles

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Keywords: Tuberculosis, MDR-TB, Antimycobacterial, Pyrroles, SAR

Tuberculosis (TB) is becoming a major challenge for the World Healthcare Systems and it is now the second preeminent cause of mortality from a single infectious agent, after COVID-19. Due to the continuous emergence of multidrug-resistant (MDR) tuberculosis strains, there is an urgent need to develop novel anti-tubercular agents to stem TB and potentiate the drug discovery pipeline.
We recently adopted a molecular hybridization strategy of the antitubercular drugs BM212 and SQ109 which led to the discovery of *N*-aryl-2,5-dimethylpyrrole **1** (Fig. 1) that showed a high potency against *M. tuberculosis* and intracellular mycobacteria.[1-3] Herein, in efforts to further identify novel antitubercular compounds eligible for hit-to-lead development, we designed, synthesised and biological evaluated a series of 2,5-dimethylpyrroles derivatives.[4]
Structure-Activity Relationship (SAR) considerations, deduced from our previous work, only underscored the criticality of the 2,5-dimethylpyrrole scaffold of compound **1** for the antimycobacterial activity; next we sought to expand the scope of the investigation focusing on the *N*1 and C3 positions of the pyrrole nucleus as key sites for diversification. Analogues incorporating a cyclohexanemethyl group on the methylenamine side chain at C3 of the pyrrole core exhibited potent inhibitory effects against the *M. tuberculosis* strains, substantiating the essentiality of the moiety to their antimycobacterial activity. Among the tested compounds, selected derivatives showed promising cytotoxicity profiles against human pulmonary fibroblasts and/or murine macrophages. Furthermore, the most active derivatives demonstrated promising activity against a panel of MDR-TB clinical isolates, notable growth restriction of intracellular mycobacteria, and presented either bactericidal or bacteriostatic effects comparable to **1**. Finally, molecular docking studies have been carried out on the mycolic acid transporter MmpL3 as the putative target of these compounds, suggesting a binding mode similar to SQ109. Three novel compounds, together with the hit pyrrole **1**, will be used in follow-on pre-clinical studies.

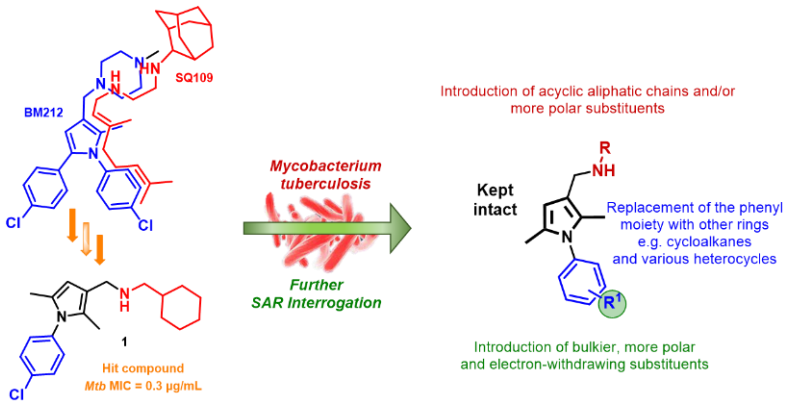

Fig.1 Rationale behind this work and the strategy adopted in designing analogues of the hit compound **1**

References

[1] M. Touitou, F. Manetti, C.M. Ribeiro, F.R. Pavan, N. Scalacci, K. Zrebna, N. Begum, D. Semenya, A. Gupta, S. Bhakta, T.D. McHugh, H. Senderowitz, M. Kyriazi, D. Castagnolo. Improving the Potency of *N*-Aryl-2,5-dimethylpyrroles against Multidrug-Resistant and Intracellular Mycobacteria. ACS Med. Chem. Lett., 2020, 11, 638-644.
[2] D. Masci, C. Hind, M.K. Islam, A. Toscani, M. Clifford, A. Coluccia, I. Conforti, M. Touitou, S. Memdouh, X. Wei, G. La Regina, R. Silvestri, J.M. Sutton, D. Castagnolo. Switching on the activity of 1,5-diaryl-pyrrole derivatives against drug-resistant ESKAPE bacteria: Structure-activity relationships and mode of action studies. Eur. J. Med. Chem., 2019, 178, 500-514.
[3] S. Bhakta, N. Scalacci, A. Maitra, A.K. Brown, S. Dasugari, D. Evangelopoulos, T.D. McHugh, P.N. Mortazavi, A. Twist, E. Petricci, F. Manetti, D. Castagnolo. Improving the Potency of *N*-Aryl-2,5-dimethylpyrroles against Multidrug-Resistant and Intracellular Mycobacteria. J. Med. Chem., 2016, 59, 2780-2793.
[4] D. Semenya, M. Touitou, D. Masci, C.M. Ribeiro, F.R. Pavan, G.F. Dos Santos Fernandes, B. Gianibbi, F. Manetti, D. Castagnolo. Tapping into the antitubercular potential of 2,5-dimethylpyrroles: A structure-activity relationship interrogation. Eur. J. Med. Chem., 2022, 237, 114404.

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High-selective Molecular Imprinted Polymers for low-trace food contaminants detection

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Keywords: Molecular Imprinted Polymers, low-trace detection, Organic Electrochemical Transistors

Molecular imprinted polymers (MIPs) played an important role in the development of reliable and stable sensors, considering the biomimicking nature of this platform with respect to several biorecognition elements like antibodies, DNA or enzymes, for instance enclosing nanomaterials as nanozymes.[1,2] MIP synthesis is typically carried out through a polymerization method to create semi-specific positions for targets in the molecular dimensions by using a monomer (e.g., o-phenylenediamine, pyrrole etc.) and a target molecule, which acts as template.[3]

In this work, we developed a MIP based electrode to sense 2,4-dichlorophenossiacetic acid (2,4-D) at ultralow/traces concentrations combining electrochemical techniques and electronic measurements, carried out using an Organic Electrochemical Transistor (OECT) biosensor. At first, we characterised the modified electrode by using several electrochemical techniques (Cyclic Voltammetry, Square Wave Voltammetry, Electrochemical Impedence Spectroscopy) to demonstrate the effective removal of the templating molecule and the possibility to perform the rebinding/washing step multiple times, hence proving the stability of the prepared electrode surface. Furthermore, we performed a spectroscopic characterisation of the modified surface by means of X-Ray Photoelectron Spectroscopy (XPS) to prove the efficient template removal. Finally, the platform was also used to perform low-trace sensing measurements using OECT biosensors.

References

[1] C. Malitesta, E. Mazzotta, R.A. Picca, A. Poma, I. Chianella, S.A. Piletsky, Anal. Bioanal. Chem., 402 (2012), 1827-1846.
[2] Z. Zhang, Y. Li, X. Zhang, J. Liu, Nanoscale, 11(2019), 4854-4863.
[3] F. Perniachi, S. Kashanian, M. Nazari, C. Santoro, P.Bollella, K. Varmira, Microchem. J., 168 (2021), art. No. 106367.

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Chemical modification of clay minerals for application in life science

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Keywords: clay minerals, halloysite, organic molecules, chemical modification

The use of clay minerals in biomedical applications is known from ancient times and they are regaining attention in recent years. Due to their naturally availability, low-cost, biocompatibility and physico-chemical features, clay minerals have been found applications in health science ranging from their use as active principles or excipients in pharmaceutical preparations, drug carrier and delivery systems to tissue engineering.
In addition, the design and development of innovative nanomaterials based on clay minerals provide new frontiers for more and more smart systems for application in health science.
Among the different clay minerals that can used for these purposes, halloysite with a typical hollow tubular structure, is an emerging nanomaterial which, because of its interesting properties has been recently modified for the synthesis of smart systems for the treatment of several diseases [1].
In this communication an overview of our recent developments on the modification, covalent and supramolecular, of clay minerals surface for health application is reported.
The obtained nanomaterials found application in cancer treatment, genetic material delivery and so on [2,3].

References

[1] M. Massaro, R. Noto, S. Riela. Past, present and future perspectives on halloysite clay minerals. Molecules 2020, 25, 4863.
[2] M. Massaro, P. Poma, G. Cavallaro, F. García-Villén, G. Lazzara, M. Notarbartolo, N. Muratore, R. Sánchez-Espejo, C. Viseras Iborra, S. Riela. Prodrug based on halloysite delivery systems to improve the antitumor ability of methotrexate in leukemia cell lines. Coll. Surf. B. 2022, 213, 112385.
[3] M. Massaro, E. Licandro, S. Cauteruccio, G. Lazzara, L. F. Liotta, M. Notarbartolo, F. M. Raymo, R. Sánchez-Espejo, C. Viseras-Iborra, S. Riela. Nanocarrier based on halloysite and fluorescent probe for intracellular delivery of peptide nucleic acids. J. Coll. Interf. Sci.. 2022, 620, 221-233.

Development and application of a colorimetric device for urinary zinc detection to improve prostate cancer diagnosis

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Keywords: colorimetric device, zinc ions, urine, prostate cancer, POCT

Prostate cancer (PCa) is the most common cancer diagnosed in men and represents the second cause of cancer-related death for men worldwide [1].
The process of diagnosing prostate cancer (PCa) mainly involves the measurement of prostate-specific antigen (PSA) levels in the blood and a digital rectal examination (DRE). Detecting PCa at an early stage is crucial for effective treatment with minimal complications [2], emphasizing the importance of urological consultations. Currently, there is a vigorous debate surrounding the use of the serum PSA test as a screening tool for PCa due to its lack of specificity and the high incidence of over-diagnosis and over-treatment [3]. Consequently, it is imperative to shift our attention towards alternative molecular markers that can assist in the diagnosis and management of the disease [4].
Recently, Maddalone et al. observed that, that the average amount of zinc detectable in urine after a prostatic massage is lower in patients with prostate cancer than in healthy subjects. [5].
Atomic Absorption is the most common analytical methodology to detect metal ions in clinical laboratory. However, the procedure is typically time consuming and expensive. Therefore, a decentralized rapid diagnostic test for urinary zinc ions detection is needed and can be an easy and affordable way to screen the whole male population at risk.
In this communication, the development of an easy-to-use colorimetric device to detect urinary zinc ions will be presented. The device has been successfully applied to analyze 238 patient samples showing better diagnostic performances in comparison to the standard of care.

References

[1] R. L. Siegel, K. D. Miller, H. E. Fuchs, A. Jemal. Cancer statistics, 2022. CA Cancer J Clin. 2022, 72, 7-33.
[2] C.A. Salinas, A. Tsodikov, M. Ishak-Howard, K.A. Cooney. Prostate cancer in young men: An important clinical entity. Nat. Rev. Urol. 2014, 11, 317–323.
[3] S. Loeb, M.A. Bjurlin, J. Nicholson, T.L. Tammela, D.F. Penson, H.B. Carter, P. Carroll, R. Etzioni. Overdiagnosis and Overtreatment of Prostate Cancer. Eur. Urol. 2014, 65, 1046–1055.
[4] S. Saini. PSA and beyond: Alternative prostate cancer biomarkers. Cell. Oncol. 2016, 39, 97–106.
[5] M.G. Maddalone, M. Oderda, G. Mengozzi, I. Gesmundo, F. Novelli, M. Giovarelli, P. Gontero, S. Occhipinti. Urinary Zinc Loss Identifies Prostate Cancer Patients. Cancers. 2022, 14, 5316.

On-site electrochemical detection of Zinc (II) in biological samples by Bismuth-MXene nanocomposites

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Keywords: Heavy metal ions; electrochemical detection; Zinc (II); Bismuth-MXene nanocomposites

The existence of heavy metal ions in water causes harmful pollution that persists over time, raising significant worries among both academic communities and the public at large. Heavy metals are dangerous because they're highly toxic, don't degrade easily, and threaten the health of humans and animals alike. Zinc [Zn(II)] is extensively used in various industrial fields such as galvanization, alloy and battery production, petroleum refining, lubrication and pigment manufacturing. While its utility across diverse sectors cannot be overstated, there persists an alarming issue of groundwater contamination that looms large over its extensive usage. The untreated wastewater generated by these industries can potentially discharge harmful zinc ions into waterways or permeate soil layers thereby putting our invaluable groundwater and sea water at risk. The persistence and bioaccumulative properties of Zn(II) amplify the potential risks they pose to both ecosystems and human health. Consequently, it becomes imperative to address this critical concern with utmost urgency and diligence. In this study, bismuth and MXene (Bis-MXene) nanocomposites materials is used as an point-of-care testing (POCT) to detect Zn(II) in different water sample is presented. Under optimized conditions, this approach achieves a dynamic linear range of 3 µg/mL with a LOD of 5 ng/mL. To sum up, the analysis of Zn(II) in biological samples like saliva and serum was effectively carried out using Bis-MXene nanocomposites. The developed sensor exhibited satisfactory characteristics such as reliability, repeatability, applicability, reproducibility as well as remarkable anti-interference properties for detecting zinc ions. The demonstration of this principle marks a significant milestone in advancing the development of an assay that is incredibly sensitive and accurate to detect zinc.

In vitro investigation of the impact of protein corona on the biological effects of gold nanoparticles against human breast cancer cells

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Keywords: protein corona, gold nanoparticles, breast cancer cells, proteomics

Gold nanoparticles (AuNPs) are emerging as elective candidates for selective breast cancer damage [1]. However, their use in clinical practice remains limited due to an incomplete understanding of the factors at the bio-nano interface both *in vitro* and *in vivo*. Upon exposure to biological fluids, proteins rapidly adsorb to AuNPs and form protein corona (PC), which can modify the biological identity of NPs [2]. To this end, we hypothesized that the PC governs AuNPs-breast cancer interactions. To test this hypothesis, PC was formed by incubating AuNPs (sphere- or star-shaped) in the cell culture medium (supplemented with 10% fetal bovine serum) of SK-BR-3 and MCF7 breast cancer cell lines at 37°C and at different incubation times. PC formation was assessed by dynamic light scattering (DLS), zeta potential measurements, UV-Vis spectrophotometry, SDS-PAGE electrophoresis, Cryo-EM, and bicinchoninic acid (BCA) assay. Mass spectrometry (MS)-based proteomic analysis was used to assess PC composition. Both differently shaped gold nanoparticles without PC significantly reduced the viability of cancer cells by altering the expression of apoptotic proteins. Interestingly, PC reversed these effects. Similarly, the presence of PC affected the uptake of AuNPs by decreasing the level of internalization in breast cancer cells. Preliminary proteomic data revealed unique protein patterns based on the shape of AuNPs. Bioinformatic analyses are underway to identify proteins related to the uptake and biological effects of AuNPs. These findings are expected to have implications for the future development of AuNPs-based anticancer therapies.

References

[1] Bloise N, Strada S, Dacarro G, Visai L. Gold Nanoparticles Contact with Cancer Cell: A Brief Update. Int J Mol Sci. 2022 Jul 12;23(14):7683.
[2] Ahsan SM, Rao CM, Ahmad MF. Nanoparticle-Protein Interaction: The Significance and Role of Protein Corona. Adv Exp Med Biol. 2018;1048:175-198.

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Decorated nanogels as promising tools for selective drug delivery in spinal cord injury

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Keywords: central nervous system, colloids, drug delivery, nanoparticles, polymer

INTRODUCTION

Spinal cord injury (SCI) is an invalidating disease that involves the damage of the spinal cord or the nerves connecting the spine to the central and peripheral nervous system [1]. This pathology is characterized by the primary SCI, that is the consequence of the traumatic event, and by the subsequent inflammatory response, characterized by the activation of microglia/macrophages/astrocytes, that leads to an aggravation of the pathology and to neurodegeneration [2]. A possible therapeutic approach is represented by the possibility to modulate the inflammatory response through the release of drugs in the damaged zone selectively within different cell lines. Recent advances in polymer science and nanotechnologies showed an increased interest for the nanogels (NGs), a new class of colloidal systems that, if properly functionalized, can be used as carriers of drugs to treat SCI.

MATERIAL AND METHODS

Nanogels were synthesized using polyethylene glycol (PEG) and polyethylenimine linear (PEI), after having functionalized PEI with a chromophore using a "click" reaction [3, 4]. This PEI functionalization is essential for being able to constantly trace the nanogels during the biological assays. Many different coating strategies of the nanogels were analyzed: in fact, the surface functionalization is essential to tune the characteristics, and the biological behavior, of the final system.

RESULTS

The NGs underwent characterization through dynamic light scattering analyses and drug release tests together with *in vitro* and *in vivo* biological assays.

DISCUSSION

Biological tests proved that functionalized nanogels were able to be selectively internalized in mouse microglia or astrocytes depending on their surface decoration, that their degradation promoted drug release and the use of anti-inflammatory molecules as delivered drug were able to mitigate the pain state [5, 6]. *In vivo* subsequent assays on diseased mouse confirmed the result obtained *in vitro* and the potentiality of this kind of surface functionalization (Figure 1). Nanogels are for sure effective devices in controlled drug delivery and here we showed their potentialities as targeted drug delivery systems in SCI inflammatory state treatment.

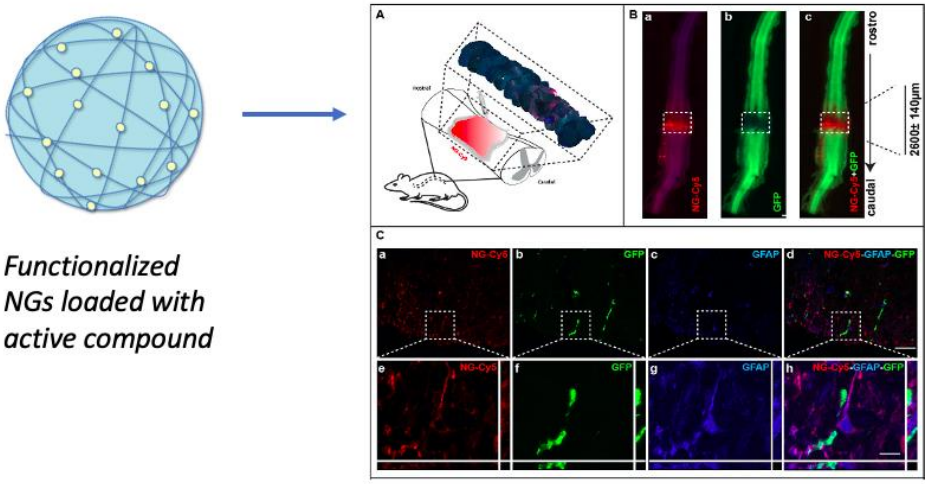

Figure 1. Schematization of the functionalized NGs loaded with active compound for the spinal cord injury treatment. *In vivo* biological assays (Fig.A) are reported: it is possible to see the co-localization of NGs and astrocytes markers (Fig. B and C) confirming their internalization.

References

[1] S. Thuret, L. D. F. Moon, F. H. Gage. Therapeutic interventions after spinal cord injury. 2006, 7, 628-643.
[2] S. Papa, V. Veneruso, E. Mauri, G. Cremonesi, X. Mingaj, A: Mariani, M. De Paola, A. Rossetti, A. Sacchetti, F. Rossi, G. Forloni, P. Veglianese. Functionalized nanogel for treating activated astrocytes in spinal cord injury. J. Control. Release. 2021, 330, 218-228.
[3] F. Pinelli, F. Pizzetti, A. Rossetti, Z. Posel, M. Masi, A. Sacchetti, P. Posocco, F. Rossi. Effect of surface decoration on properties and drug release ability of nanogels. Coll. Surf. A. 2021, 614, 126164.
[4] F. Pinelli, M. Saadati, A. Rossetti, F. Rossi, A. Sacchetti. On the influence of polyethyleneimine modification in nanogel-driven drug delivery. Coll. Surf. A. 2023, 658, 130623.
[5] I. Vismara, S. Papa, Valeria Veneruso, E. Mauri, A. Mariani, M. De Paola, R. Affatato, A. Rossetti, M. Sponchioni,D. Moscatelli, A. Sacchetti, F. Rossi, G. Forloni, P. Veglianese. Selective modulation of A1 astrocytes by drug-loaded nano-structured gel in spinal cord injury. ACS Nano. 2020, 14, 360-371.
[6] S. Papa, F. Rossi, R. Ferrari, A. Mariani, M. De Paola, I. Caron, F. Fiordaliso, C. Bisighini, E. Sammali, C. Colombo, M. Gobbi, M. Canovi, J. Lucchetti, M. Peviani, M. Morbidelli, G. Forloni, G. Perale, D. Moscatelli, P. Veglianese. Selective nanovector mediated treatment of activated proinflammatory microglia/macrophages in spinal cord injury. ACS Nano. 2013, 7, 9881-9895.

Design, synthesis and assessment of a new series of ppGpp synthetase inhibitors against bacterial persistence

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Keywords: bacterial persistence, phenotypic resistance, (p)ppGpp, AMR

Bacteria are continuously evolving to survive against antibiotic treatment by utilizing genetic and phenotypic resistance.[1,2] Although researchers have explored genetic resistance, they are just starting to delve into phenotypic resistance. Bacterial persisters are an antibiotic tolerant, reversible, sub-population phenotype. The accumulation of (p)ppGpp (guanosine tetra- or penta-phosphate) seems to play a pivotal role in persisters formation.[3] Intracellular levels of (p)ppGpp are regulated by superclass of enzymes widespread in the bacterial kingdom: the RelA/SpoT homologue (RSH) proteins. These enzymes catalyse the synthesis and/or the hydrolysis of (p)ppGpp. The discovery of new molecules targeting (p)ppGpp synthesis inhibition might, therefore, hamper persisters formation.
After having identified three chemotypes for Rel_{Seq} (*Seq*, *Streptococcus dysgalactiae* subsp. *Equisimilis*) synthetase catalytic site,[4,5] we now show growth of the first fragment chemotype into a small family of ppGpp synthetase ligands/inhibitors. In particular, we report their synthesis, their affinity measurement for the target protein (TSA), and the quantification of their effect on the enzyme catalytic activity.

References

[1] D. Wilmaerts, E. M. Windels, N. Verstraeten, J. Michiels. General Mechanisms Leading to Persister Formation and Awakening. *Trends Genet.* 2019, 35, 401–411.
[2] S. M. Schrader, J. Vaubourgeix, C. Nathan. Biology of antimicrobial resistance and approaches to combat it. *Sci. Transl. Med.* 2020, 12, eaaz6992.
[3] S. H. Jung, C. M. Ryu, J. S. Kim. Bacterial persistence: Fundamentals and clinical importance. *J. Microbiol. 2019*, 57, 829–835.
[4] M. Civera, S. Sattin. Homology Model of a Catalytically Competent Bifunctional Rel Protein. *Front. Mol. Biosci.* 2021, 8, 628596.
[5] C. Coppa, L. Sorrentino, M. Civera, M. Minneci, F. Vasile, S. Sattin. New Chemotypes for the Inhibition of (p)ppGpp Synthesis in the Quest for New Antimicrobial Compounds. *Molecules* 2022, 27, 3097.

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Metabolomics in Human Ageing: studying Alzheimer's disease evolution via a personalized serum NMR-based approach

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Keywords: Alzheimer's Disease, Metabolomics, NMR, Precision Medicine, Prognosis

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder that slowly destroys memory, thinking skills, and ultimately the ability to accomplish even the simplest daily tasks. It is the most common neurodegenerative disorder in the elderly population, affecting about 5% to 7% of the population over 60 years of age[1]. In clinical practice, brain MRI coupled with abnormal levels of the cerebrospinal fluid (CSF) core AD biomarkers, total tau (t-tau), phosphorylated tau (p-tau) proteins and amyloid beta 1-42 peptide(αβ42), enable the identification of patients affected by AD. Nevertheless, the molecular pathways involved in AD onset and progression are not fully understood.
We have hypothesized that the metabolic alterations present at the level of the central nervous system could be reflected at a systemic level not only in the CSF[2] of patients but also in their blood serum. This study proposes a serum metabolomic investigation via nuclear magnetic resonance (NMR) spectroscopy in a population that covers the entire spectrum of cognitive impairment, from mild cognitive impairment (MCI), to MCI due to AD (MCI-AD), and to AD dementia (ADdem). The ^{1}H NMR spectra of serum samples were acquired at 310 K using a 600 MHz spectrometer (Bruker BioSpin). A standard NOESY 1Dpresat pulse sequence was applied to detect signals of both low- and high-molecular weight molecules[3]. A panel of 26 metabolites and of 112 lipoprotein-related parameters was quantified using the Bruker IVDr Quantification platform in Plasma/Serum (B.I.Quant-PS 2.0.0, B.I. Lisa 1.0.0).
Using a LASSO regression approach, we found the best combination of metabolites and lipoproteins to discriminate AD and MCI patients. The covariates were selected maximizing the area under the receiver operating characteristic curve (AUROC) and minimizing the number of variables included in the model. The calculated logistic model (which is corrected for age and sex) classifies AD and MCI patients with an AUROC of 0.89 and 83% accuracy. This model was then used to predict the risk of AD evolution of MCI-AD patients: patients classified as AD were deemed as high risk of AD progression, whereas those predicted as MCI were considered low risk. Following this approach, 6 MCI-AD patients were identified as low risk and 17 as high risk. Interestingly, this metabolomic stratification seems to reflect the levels of CSF t-tau proteins and the trend of cognitive decline.
These results based on the circulating metabolome provide crucial information on the underlying metabolic causes of Alzheimer's disease at a molecular level. The identification of potential novel peripheral biomarkers of Alzheimer's disease, as proposed in this study, paves the way for an innovative and minimally invasive method to diagnose AD in its very early stages. Moreover, the proposed metabolomic model appears to be able to sub-stratify MCI-AD patients identifying those associated with a worse and faster rate of clinical progression.

References

[1] Foley, S. F.; Tansey, K. E.; Caseras, X.; Lancaster, T.; Bracht, T.; Parker, G.; Hall, J.; Williams, J.; Linden, D. E. J. Multimodal Brain Imaging Reveals Structural Differences in Alzheimer's Disease Polygenic Risk Carriers: A Study in Healthy Young Adults. Biological Psychiatry 2017, 81 (2), 154–161.
[2] Vignoli, A.; Paciotti, S.; Tenori, L.; Eusebi, P.; Biscetti, L.; Chiasserini, D.; Scheltens, P.; Turano, P.; Teunissen, C.; Luchinat, C.; Parnetti, L. Fingerprinting Alzheimer's Disease by 1H Nuclear Magnetic Resonance Spectroscopy of Cerebrospinal Fluid. J. Proteome Res. 2020, 19 (4), 1696–1705.
[3] Vignoli, A.; Ghini, V.; Meoni, G.; Licari, C.; Takis, P. G.; Tenori, L.; Turano, P.; Luchinat, C. High-Throughput Metabolomics by 1D NMR. Angew. Chem. Int. Ed. Engl. 2019, 58 (4), 968–994.

AMYC BIOMED 2023

Atomic Force Microscopy applied to the study of the CSF of Alzheimer's disease patients

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Keywords: Alzheimer's disease; Atomic Force Microscopy; cerebrospinal fluid; protein aggregation; biomarkers of neurodegeneration

Alzheimer's disease (AD) is the most common neurodegenerative condition that affects tens of millions of people worldwide, although its pathogenic mechanism is still poorly understood [1]. The aggregation of the amyloid-β peptide, which forms amyloid plaques, and of the tubulin-associated unit (tau), which forms neurofibrillary tangles, in the parenchymal and cortical brain constitute the main hallmarks of the disease [2,3]. AD is typically identified when irreparable brain damage has occurred and the definite diagnosis of AD can only be made post-mortem, upon the detection of the aforementioned aggregates [3]. Therefore, one of the main challenges in the research in this field is to develop a sensitive and non-invasive approach for AD diagnosis that would enable an early therapeutic intervention [4]. In order to understand the biological complexity of the disease, it becomes increasingly clear that it is fundamental to study the extracellular environment of neurons and glial cells. We must comprehend how alterations in the brain's micro-environment lead to neuronal dysfunction and inflammatory activity, in order to identify the molecular determinants of the neurodegenerative process and to identify the biomarkers of the disease. The cerebrospinal fluid (CSF), is continuously produced by ultrafiltration of plasma and it is contained in the ventricles of the brain and the subarachnoid spaces [5]. Since the CSF interacts with extracellular brain regions, it can reflect neuropathological alterations and thus provides a valuable source for identifying AD biomarkers. Recently, the presence of amyloid-β aggregates has been detected in the CSF through atomic force microscopy (AFM) [6,7]. AFM, due to its capability for the structural analyses of individual macromolecules, has extensively demonstrated to be a powerful technique for the study of the fibrillogenesis of amyloid-β *in vitro* [8,9], as well as for the morphological characterization of the amyloidogenic aggregates, such as oligomers and fibrils [10,11]. Consequently, we decided to employ AFM to examine directly the CSF collected from well-characterized AD patients, identifying the presence of peculiar fibrillar structures (**Figure 1**). We analyzed the morphology of such fibrils at nanometric resolution, tracking their development and comparing their structure with that of amyloid fibrils produced *in vitro*. Our findings emphasize the importance of further investigations into the macromolecules found in CSF of AD patients, which can help to gather essential information on the molecular pathophysiology of the disease and to identify the biomarkers that may result in the development of an early diagnostic method.

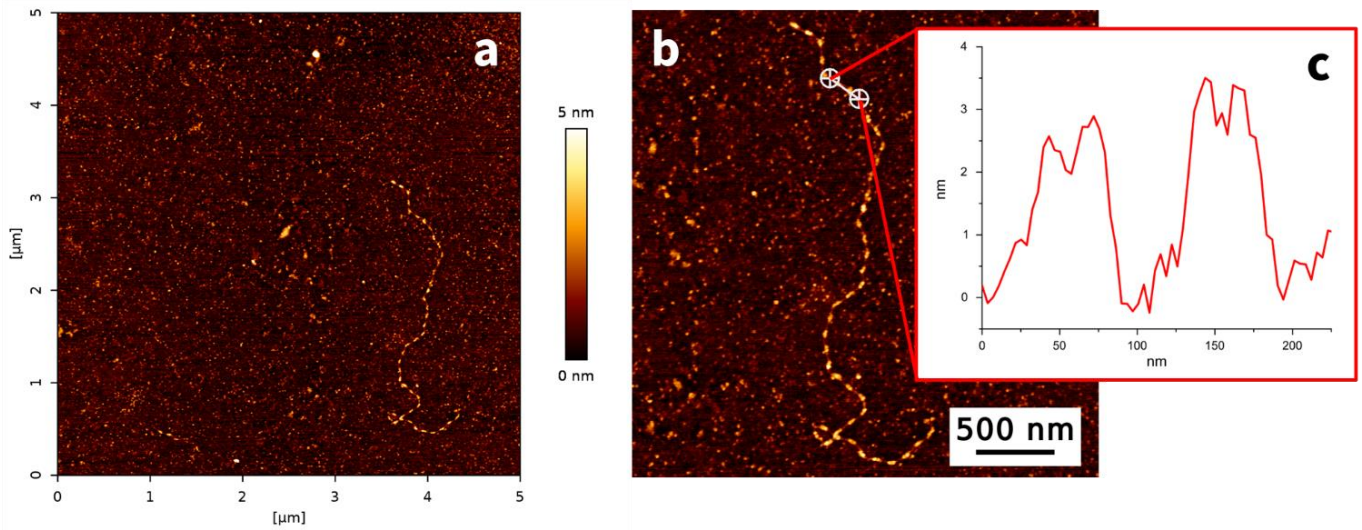

Figure 1: (**a**) Topographic image acquired in tapping mode on the CSF sample of patient AD#1056 deposited on mica (colorimetric height bar shown on the right). (**b**) High resolution detail of "a". (**c**) Height measured along the segment shown in "b".

References

[1] Patterson, C. (2018) *World Alzheimer report 2018*, Alzheimer's Disease International.
[2] Chiti, F. and Dobson, C.M. (2017) Protein Misfolding, Amyloid Formation, and Human Disease: A Summary of Progress Over the Last Decade. *Annual Review of Biochemistry* 86, 27–68.
[3] Bistaffa, E. *et al.* (2020) Contributions of Molecular and Optical Techniques to the Clinical Diagnosis of Alzheimer's Disease. *Brain Sci* 10, E815.
[4] P. Polykretis, M. Banchelli, C. D'Andrea, M. de Angelis, P. Matteini, Raman Spectroscopy Techniques for the Investigation and Diagnosis of Alzheimer's Disease. Frontiers in Bioscience-Scholar. 14, 22 (2022).
[5] Telano, L.N. and Baker, S. (2022) Physiology, Cerebral Spinal Fluid. In *StatPearls*, StatPearls Publishing.
[6] De, S. *et al.* (2019) Soluble aggregates present in cerebrospinal fluid change in size and mechanism of toxicity during Alzheimer's disease progression. *Acta Neuropathologica Communications* 7, 120.
[7] Nirmalraj, P.N. *et al.* (2023) Protein fibril length in cerebrospinal fluid is increased in Alzheimer's disease. *Commun Biol* 6, 1–9.
[8] Arimon, M. *et al.* (2005) Fine structure study of Aβ1–42 fibrillogenesis with atomic force microscopy. *The FASEB Journal* 19, 1344–1346.
[9] Drolle, E. *et al.* (2014) Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer's disease. *Drug Metabolism Reviews* 46, 207–223.
[10] Breydo, L. *et al.* (2016) Structural differences between amyloid beta oligomers. *Biochemical and Biophysical Research Communications* 477, 700–705.
[11] Stylianou, A. *et al.* (2019) Atomic Force Microscopy on Biological Materials Related to Pathological Conditions. *Scanning* 2019, 8452851.

AMYC BIOMED 2023

Plasma protein profiling reveals novel specific biomarkers reflecting the multifactorial nature of Alzheimer´s disease continuum

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Keywords: biomarkers, plasma, neurodegenerative diseases, proximity extension assays, Alzheimer's disease

BACKGROUND
Plasma-based biomarkers able to depict the molecular diversity specifically associated to Alzheimer´s disease (AD) are needed to better track disease progression and for the development of biological therapies targeting different pathomechanisms. Here we aimed to identify plasma protein changes covering different biological pathways along the full AD continuum and specific for this dementia type.
METHOD
Proximity extension-based multiplex immunoassays (Olink® Explore panels)[1,2] were used to analyse 1572 proteins in 1003 plasma samples collected from 6 international centres (JPND bPRIDE consortium), including 330 cognitively unimpaired controls (CTRL) Aβ-, 164 CU Aβ+ (preAD), 129 MCI due to AD (MCI-AD), 137 AD at dementia stage (AD-dem), 171 Dementia with Lewy Bodies (DLB) and 170 Frontotemporal dementia patients. Data was analysed using nested linear models adjusted for multiple testing and penalized regressions.
RESULTS
We detected >200 proteins differentially regulated along the AD continuum compared to controls ($q<0.05$). More than 300 of the proteins differentially regulated at AD-dem were also changed between AD-dem and FTD or DLB (e.g., CALCOCO1, MAP3K5, BIN2, GFAP). Enrichment in pathways related to autophagy, ubiquitination, mitotic cell cycle and protein kinase activity was detected along the full AD continuum. GFAP showed the strongest performance to discriminate any AD stage from controls (AUCs 0.68-0.82) as well as AD from FTD (0.75 AUC). SUSD1 and ITGAV showed the strongest performances to discriminate AD from DLB (AUC = 0.77 and 0.81, respectively). Multivariate analysis identified panels of plasma proteins (17-21 proteins) able to discriminate MCI-AD or AD from controls and AD from FTD or DLB with AUCs>0.80. All the proteins

well discriminating both MCI-AD and AD-dem from CTRL (Figure 1, e.g., GFAP, MAP3K5, CALCOCO1, FLT3LG) showed an incremental increase/decrease from preclinical to dementia AD stages. The proteins within the panels are associated to different biological functions (e.g., phagocytosis, myelination, endolysosomal system, Calcium signalling, energy metabolism).

CONCLUSION

This unprecedent large proteome study unveils novel plasma protein changes along the full AD continuum associated to processes involved in AD pathophysiology. We identified plasma protein panels depicting the biological diversity of this dementia type, which can now be translated into customized assays for widespread validation in clinical settings and trials.

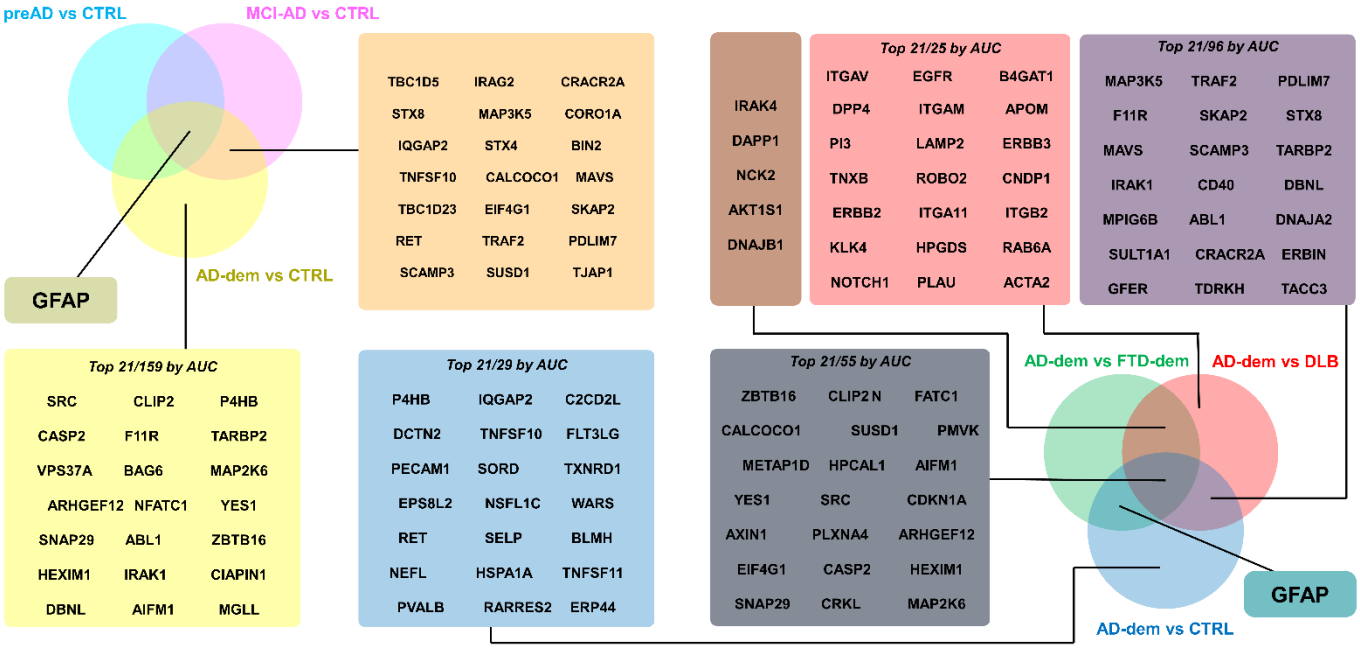

Figure 1. *Venn's diagrams summarizing the most promising biomarkers selected from the univariate analysis of AD-continuum vs CTRL and from AD-dem vs FTD and DLB. Promising biomarkers were chosen by selecting those providing a p-value adjusted for age, gender and FDR < 0.05, an AUC > 0.68 and an effect size (Cohen's D) > 0.6 for each comparison. For viewing purposes, when more than 21 biomarkers were selected in this way, only the top-21 biomarkers with the highest averaged AUCs are shown.*

References

[1] Gaetani, L., Bellomo, G., Parnetti, L., Blennow, K., Zetterberg, H. & Di Filippo, M. Neuroinflammation and Alzheimer's Disease: A Machine Learning Approach to CSF Proteomics. *Cells* **10,** 1930 (2021)

[2] del Campo, M., Peeters, C. F. W., Johnson, E. C. B., Vermunt, L., Hok-A-Hin, Y. S., van Nee, M., Chen-Plotkin, A., Irwin, D. J., Hu, W. T., Lah, J. J., Seyfried, N. T., Dammer, E. B., Herradon, G., Meeter, L. H., van Swieten, J., Alcolea, D., Lleó, A., Levey, A. I., Lemstra, A. W., Pijnenburg, Y. A. L., Visser, P. J., Tijms, B. M., van der Flier, W. M. & Teunissen, C. E. CSF proteome profiling across the Alzheimer's disease spectrum reflects the multifactorial nature of the disease and identifies specific biomarker panels. *Nat Aging* **2,** 1040–1053 (2022)

AMYC BIOMED 2023

Harmaline-based scaffold as Human Caseinolytic Protease P (hClpP) inducers for Diffuse Intrinsic Pontine Glioma (DIPG) treatment

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Keywords: DIPG; hClpP; ONC201; natural products; drug screening

Diffuse intrinsic pontine glioma (DIPG) is a highly aggressive pediatric brainstem tumor which accounts for about 10% to 20% of all childhood brain tumors[1], with a peak of incidence between 6 and 8 years. Prognosis is extremely poor, due to localization and inoperability. Indeed, surgery cannot be performed, and focal radiotherapy remains the standard of care currently that has demonstrated clinical efficacy. Over the last decade, preclinical studies identified ONC201, an experimental anticancer drug from the imipridone class to be endowed with cytotoxic activity against multiple human cancer cell lines, including DIPG. Only recently, the X-ray analysis of the complex of the human mitochondrial caseinolytic serine protease type C (hClpP) and ONC201 (PDB ID: 6DL7, Figure 1)[2], has allowed to identify hClpP as its main direct target. This provided the rationale to evaluate the hClpP activity in patients recruited in ongoing clinical trials using ONC201 as a drug. Downstream of target engagement, hClpP plays a pivotal role in the quality control of mitochondrial proteins involved in important cellular pathways.
The hyperactivation of hClpP, due to the interaction with ONC201, alters the structure and mitochondrial function, causes the death of cancer cells, without affecting healthy cells. To date, a computational pipeline was applied to perform a FLAP virtual screening of 1500 commercial natural products (CNPs) followed by Volsurf analysis to identify a novel original scaffold as hClpP inducers able to cross blood-barrier brain. *In silico* investigation identifies Harmaline, a fluorescent indole alkaloid, with anti-inflammatory and anti-cancer properties. Harmaline chemical structure has been opportunely modified to find out structural determinants for the hClpP induction. The Structure Activity Relationship Study results will be presented and discussed.

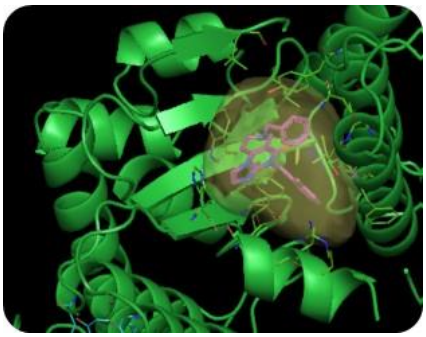

Figure 1. Crystallographic structure of hClpP: ONC201 (PDB ID: 6DL7).

References

[1]. MG. Perrone, A. Ruggiero, A. Centonze, A. Carrieri, S. Ferorelli, A. Scilimati. Diffuse Intrinsic Pontine Glioma (DIPG): Breakthrough and Clinical Perspective. Curr Med Chem. 2021, 28, 3287-3317.

[2]. J. Ishizawa, SF. Zarabi, RE. Davis, et al. Mitochondrial ClpP-Mediated Proteolysis Induces Selective Cancer Cell Lethality. Cancer Cell. 2019, 35, 721-737.e9.

AMYC BIOMED 2023

Rational design and synthesis of a water-soluble resorcarene-based linker for immunosensors development

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Keywords: Resorcarenes, Macrocycles, Immunosensors, Site-directed immobilization, Screen-printed electrodes

In recent years, several efforts have been made to develop selective, sensitive, fast response, and miniaturized immunosensors with improved performance for the monitoring and screening of analytes in several matrices, significantly expanding the use of this technology in a broad range of applications.[1] However, one of the main technical challenges in developing immunosensors is overcoming the complexity of binding antibodies (Abs) to the sensor surface. Most immobilizing approaches lead to a random orientation of Abs, resulting in lower binding site density and immunoaffinity. In this context, supramolecular chemistry has recently emerged as a suitable surface modification strategy for obtaining pre-organized and ordered surfaces, in which the bioreceptors are guided to assume an oriented arrangement, allowing a clear improvement of the functional properties of the system and of the self-assembled monolayers (SAMs). Among the large pool of macrocycles available, resorc[4]arenes are characterized by a unique three-dimensional surface that can be functionalized at both the upper and lower rims with several functional groups to tailor their recognition properties towards a specific class of analytes.[2] In previous work, we demonstrated that a gold SPR sensor chip surface modification by suitably functionalized resorc[4]arenes represents a potentially powerful system to improve sensitivity, providing new insight into sensor development.[3] Herein, a supramolecular chemistry/nanotechnology-based platform was conceived to develop sensitive label-free electrochemical immunosensors, by using a resorcarene macrocycle as an artificial linker for the oriented Abs immobilization. To this aim, a bifunctional resorc[4]arene derivative RW, decorated at the upper rim with eight hydrophilic carboxylate groups and featuring long thioether alkyl chains at the lower rim, was designed and synthesized to anchor the gold-coated magnetic nanoparticles (Au@MNPs) and to maximize the amount of the active immobilized Ab in the proper "end-on" orientation. The resulting supramolecular chemistry-modified nanoparticles, RW@Au@MNPs, were deposited onto graphite screen printed electrodes (SPEs) which then were employed to immobilize three different Abs. Furthermore, to confirm the technological platform's validity, an immunosensor for atrazine (ATZ) analysis was developed and characterized by differential pulse voltammetry technique (DPV) in ATZ standard solutions and water samples fortified with ATZ. The RW based-immunosensor improved AbATZ loading on Au@MNPs and sensitivity towards ATZ by almost 1.5 times compared to the random platform. This study outlines the successful application of the resorc[4]arene-based sensor design strategy to develop label-free and miniaturized electrochemical immunosensors. Indeed, the resorc[4]arene-based immunosensor resulted in a versatile platform that can be adequately modified, extending its application to different bio-traducers.

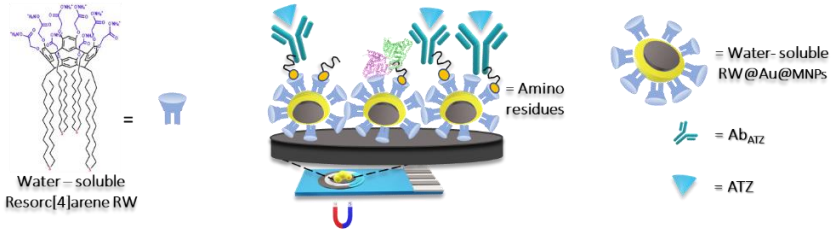

Figure: Schematic representation of the resorc[4]arene-based ATZ immunosensor.

References

[1] P. Bollella, E. Katz. Biosensors—Recent Advances and Future Challenges. Sensors. 2020, 20 (22), 6645.
[2] D. Quaglio, F. Polli, C. Del Plato, G. Cianfoni, C. Tortora, F. Mazzei, B. Botta, A. Calcaterra, F. Ghirga. Calixarene: a versatile scaffold for the development of highly sensitive biosensors. Supramolecular Chemistry. 2021, 33 (7), 345-369.
[3] D. Quaglio, L. Mangiardi, G. Venditti, C. Del Plato, F. Polli, F. Ghirga, G. Favero, M Pierini, B. Botta, F. Mazzei. Site-Directed Antibody Immobilization by Resorc[4]arene-Based Immunosensors. Chemistry - A European Journal. 2020, 26 (38), 8400-8406.

SLIDE & TALKS

AMYC BIOMED 2023

Rational design and synthesis of anthraquinone-based SMO inhibitors for the treatment of hedgehog-dependent tumors

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Keywords: Hedgehog, SMO, Anthraquinones, CSC

Aberrant hyperactivation of HH signaling is responsible for the tumorigenesis of several disparate human cancers, including medulloblastoma (MB), basal cell carcinoma (BCC) and acute myeloid leukemia (AML), thus emerging as an attractive target for anticancer therapy. Extensive efforts were mostly focused on the development of HH modulators acting as antagonists of the upstream receptor SMO. [1] Although some of them have moved into clinical trials for the treatment of HH-dependent tumors and vismodegib, sonidegib, and glasdegib have been already approved by the Food and Drug Administration (FDA) for the treatment of BCC and AML, respectively, several side effects and pitfalls, including the onset of SMO drug-resistant mutations, limit their use. Currently, one of the most promising strategies to overcome the major limitations of the small molecules able to block the HH pathway at the upstream receptor SMO is the design of compounds able to antagonize the D473H drug-resistant form of SMO. [2]
Notably, natural products (NPs) have played a crucial role in understanding HH signaling and its pharmacological modulation. [3] Plants provide a large number of secondary metabolites that are characterized by selective biological activities, enormous chemical diversity and complexity raised during biosynthesis, in addition to more advantageous ADME/T properties (Adsorption, Distribution, Metabolism, Excretion/Toxicity). In this context, an alternative approach to discover new biologically relevant compounds starts from natural chemotypes and employs the combination of the basic skeleton of a NP with a molecularly targeted drug bullet. [4] Among the different classes of natural products, anthraquinone emerged as a potentially privileged scaffold for the treatment of various types of tumors. [5] In this regard, this project aims at providing innovative exploitation of the chemical versatility as well as the affinity for the HH pathway of the anthraquinone scaffold by combining it with the active portion of such well-established and known SMO inhibitors capable of stably binding this receptor even in the presence of mutations that induce drug resistance (Taladegib and Anta XV). Our strategy implied the design, supported by computer-aided methods, and synthesis of a library of anthraquinone derivatives, featuring variously substituted piperazine linkers at C3 position, able to interact *in silico* preferentially with the SMO receptor, and the investigation of the HH inhibitory properties of the synthesized compounds, to validate the computational modeling predictions. This work provides an alternative approach to turn natural products from nontargeted to targeted anticancer therapies, by exploiting natural sources and combining them with modern technologies for guided lead identification and synthesis development.

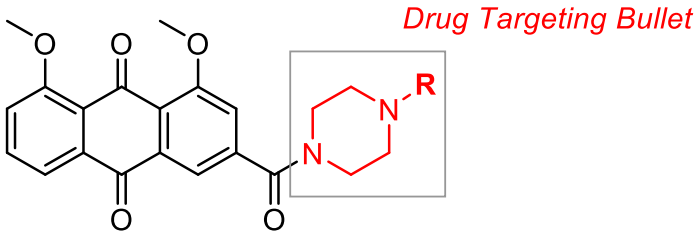

Figure: Rational design of anthraquinone derivatives as potential SMO inhibitors.

References

[1] D. Quaglio, P. Infante, L. Di Marcotullio, B. Botta, M. Mori. Hedgehog signaling pathway inhibitors: an updated patent review (2015–present). *Expert Opin Ther Pat.* 2020, 30(4), 235–250.
[2] J. George, Y. Chen, N. Abdelfattah, K. Yamamoto, T.D. Gallup, S.I. Adamson, K. Yun. Cancer stem cells, not bulk tumor cells, determine mechanisms of resistance to SMO inhibitors. *Cancer Treat Res Commun*. 2022, 2(6), 402-416.
[3] R. Palermo, F. Ghirga, M.G. Piccioni, F. Bernardi, N. Zhdanovskaya, P. Infante, M. Mori. Natural products inspired modulators of cancer stem cells-specific signaling pathways notch and Hedgehog. *Curr Pharm Design*. 2018; 24(36): 4251–4269.
[4] M. Grigalunas, S. Patil, A. Krzyzanowski, A. Pahl, J. Flegel, B. Schölermann, H. Waldmann. Unprecedented Combination of Polyketide Natural Product Fragments Identifies the New Hedgehog Signaling Pathway Inhibitor Grismonone. *Chem. Eur. J*. 2022, 28(67), e202202164.
[5] S. Henamayee, K. Banik, B.L. Sailo, B. Shabnam, C. Harsha, S. Srilakshmi, N. Vgm, S.H. Baek, K.S. Ahn, A.B. Kunnumakkara. Therapeutic emergence of rhein as a potential anticancer drug: A review of its molecular targets and anticancer properties. *Molecules* 2020**,** *25* (10), 2278.

AMYC BIOMED 2023

Combining printed electrodes with lateral flow test strip for enhanced applications in cancer detection

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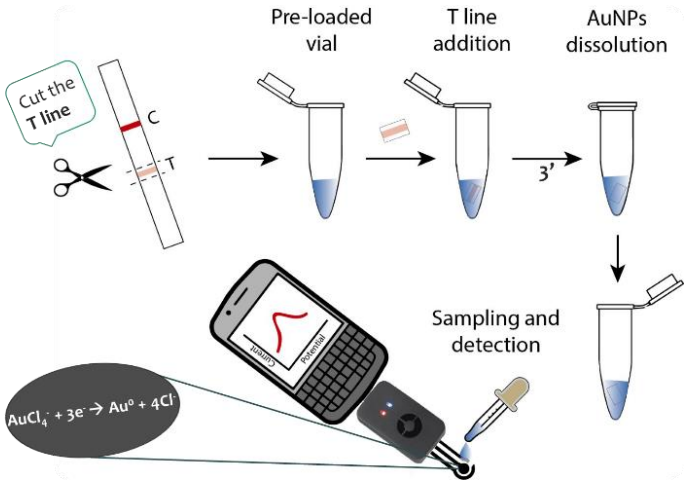
Keywords: Lateral flow assay, electroanalysis, screen printed electrodes, diagnostic tools, biomarkers

Lateral flow assays (LFAs) are the most well-known point-of-care (POC) devices that enable rapid detection of relevant bio- or chemical markers in a simple and low-cost way by non-specialized users. However, these test strips rarely provide the quantitative detection of biomarkers in clinical samples. In contrast, electrochemical detection enables quantitative detection in immunoassays. Electrochemical transduction is attractive to be integrated into LFAs due to its simplicity, high sensitivity, fast signal generation, and cost-effectiveness. In particular, the advantages associated with use of screen-printed electrodes (SPEs) consist in very small sample volumes used, improved performance, unlike optically based detection where miniaturized system can lead to poorer sensitivity. Moreover, the electrochemical performance can be further improved through developing signal tracer in similar manners achievable for naked-eye detection, such as utilizing gold nanoparticles (AuNPs) that remains a gold standard in LFA that allows detection of the color change by naked eyes [1].
In this work, an integrated lateral flow test strip with a screen-printed electrochemical sensor was developed for the rapid and sensitive detection of prostate-specific antigen (PSA), testing different type of AuNPs as signal tracers in both colorimetric and electrochemical techniques. After setting up the lateral flow test for PSA and running the test, the area close to the test line was cut and immersed in a plastic tube containing concentrated HCl. After three minutes, the test line disappeared and the solution, containing tetrachloroaurate ions, was diluted to allow electrochemical analysis by differential pulse voltammetry (DPV), which indirectly gave the measurement of bound PSA on the test line. Preliminary results have shown an improved sensitivity up to 0.01 ng/mL and a quite reproducibility. This method is rapid, clinically practical, and less expensive than other diagnostic tools for PSA, making the basis for simple, sensitive, quantitative point-of-care testing of disease-related protein biomarkers [2].



Schematic representation of the analytical detection method of the LFIA-SPE integrated system

References

[1] Perju A., Wongkaew N. Integrating high-performing electrochemical transducers in lateral flow assay. Anal Bioanal Chem 413, 5535–5549, 2021.
[2] Lin Y-Y, Wang J, Liu G, Wu H, Wai C.M, Lin Y, A nanoparticle label/immunochromatographic electrochemical biosensor for rapid and sensitive detection of prostate-specific antigen. Biosensors and Bioelectronics, 23, 11, 2008.

AMYC BIOMED 2023

Lipid nanoparticles encapsulating miR182-3p for breast cancer Treatment

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Keywords: miR182-3p, lipid nanoparticles, target therapy, breast cancer, triple-negative breast cancer

Triple-negative breast cancer (TNBC)represents about 15-20% of the total breast cancer and remains responding exclusively to the chemotherapy while more advanced therapeutic approaches are inefficient. In the research of novel therapeutic approaches for TNBC, abnormal levels of telomeric repeat-binding factor 2 (TRF2), a protein with a key role in the maintenance of telomere structure and function, have been correlated to the tumor development and progression. Interestingly, TRF2 levels can be modulated by a miRNA, named miR-182-3p, suggesting novel RNA-based anti-cancer therapies against TNBC [1]. In this perspective, lipid nanoparticles (LNPs) can be used for overcoming the poor biopharmaceutical profile of miRNA.
In this study we developed lipid nanoparticles (LNPs) encapsulating miR-182-3p to ensure its efficient delivery in vivo. LNP- miR-182-3p were prepared and fully characterized in terms of size polydispersity index (PI), superficial charge (ZP) and miRNA encapsulation efficiency. MiR-182-3p and LNP- miR-182-3p were tested in vitro in human cancer cells and finally delivered in vivo in TNBC mouse models.
Results showed that LNPs- miR-182-3p had a hydrodynamic diameters < 200 nm and high miRNA encapsulation. Moreover, miR-182-3p was able to abrogate TRF2 expression, thus activating DNA damage and inducing apoptosis in vitro. Finally, the intravenous injection of LNPs- miR-182-3p impairs tumor growth in TNBC without important adverse effects being also able to cross the blood-brain barrier and reduce intracranial tumors, representing a powerful tool to treat metastatic brain lesions [1].

References

[1] Dinami R., et al. MiR-182-3p targets TRF2 and impairs tumor growth of triple-negative breast cancer. EMBO Mol Med. 2023 Jan 11;15(1)

AMYC BIOMED 2023

Circularly Polarized activity from two photon excitable Europium and Samarium chiral bioprobes

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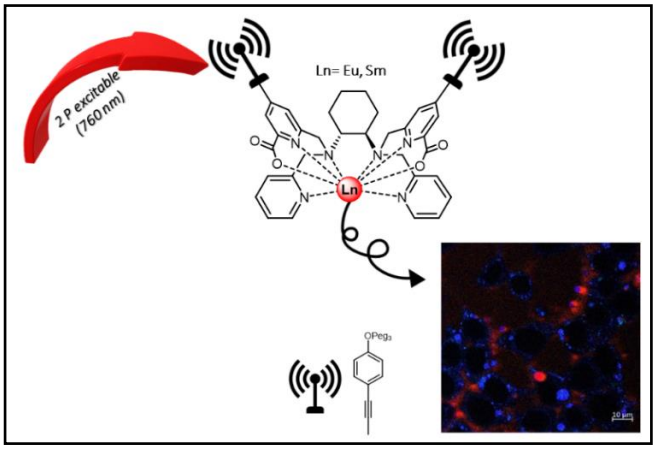
Keywords: Lanthanides, bioimaging, luminescence, circularly polarized luminescence, two photon absorption

In this contribution1, we synthesized and spectroscopically characterized in polar protic solvents (water, methanol) two couples of cationic enantiomeric complexes [(R,R)-[LnL]Cl and (S,S)-[LnL]Cl, with Ln = Sm and Eu and L = N, N'-bis(2-pyridylmethyl)- 1,2-(R,R or S,S)- cyclohexanediamine functionalized at sp3 N with the picolinate antennae]2. Both complexes are highly stable in aqueous solution (logK = 20.13 for the EuL species chosen as representative) and only one main species is present at physiological pH (7.4). The complexes exhibit a good CPL activity, in particular for the magnetic dipole (MD) allowed transitions, [4G5/2→6H5/2 (564 nm) of Sm(III) and 5D0→7F1 (593 nm) of Eu(III)]. Since the luminescence of both Sm(III) and Eu(III) complexes can be sensitized upon 1P and 2P excitation of the chromophoric antenna, they can be considered promising candidates as NIR-to-RED in cellulo chiroptical bioprobes. In fact, preliminary biphotonic imaging experiments on (S,S)-[EuL]Cl complex reveal that it can be easily internalized in two different cell lines (293T cancer cells and THP-1 macrophages).



Chemical structure of Sm and Eu complexes that we described in this contribution and the 2P-microscopy imaging of living cells stained with (S,S)-[EuL]Cl

References

[1] S. Mizzoni; et al. Circularly polarized activity from two photon excitable europium and samarium chiral bioprobes. J. Mater. Chem. C. 2023,11,4188-4202.

[2] J.-C. G. Bunzli, C. Piguet. Taking advantage of luminescent lanthanide ions. Chem. Soc. Rev. 2005, 34,1048-1077.

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Targeted gold nanoparticles with heating efficiency for Endometriosis Photothermal Therapy

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Keywords: gold nanoparticles, endometriosis, photothermal therapy

INTRODUCTION

Endometriosis is an estrogen-dependent inflammatory condition that affects women in their reproductive period. Since endometriosis and cancer share many pathophysiological features, some fundamental principles of cancer nanomedicine can be adapted to develop novel nanoparticle-based strategies for the treatment and imaging of endometriosis.[1] The study aims to evaluate an active targeting of CD44 (transmembrane glycoproteins) overexpressing endometriosis cells by using gold nanoparticles conjugated with anti-CD44 antibody with and without photothermal therapy (PPT).

EXPERIMENTAL METHODS

Gold nano-stars (GNS) and gold nanospheres (GNP) were synthesized, pegylated (PEG), and conjugated to anti-CD44 antibody (GNS@antiCD44 and GNP@antiCD44) using the Maleimide chemistry.[2] They were characterized using UV-visible spectroscopy (UV-Vis), DLS, Bicinchoninic Acid (BCA), and dot blot analyses.

The *in vitro* tests were performed to verify the biocompatibility (MTT assay), the receptor recognition and the internalization (confocal microscopy (CLSM) and inductively coupled plasma-mass spectrometry (ICP)) of the nanoconjugate against three different cell lines: CD44 overexpressing cells (Z12) and compared to CD44 low expressing cells (Thesc) and normal fibroblast cell line (NIH-3T3). Viability studies with/without laser treatment were performed to verify the efficiency of nanosystems.

RESULTS AND DISCUSSION

DLS and Zeta potential showed that the conjugation increased particle size reduced negative zeta potential. The UV-Vis analysis evidenced a spectrum shifted with respect to PEG-GNSs and PEG-GNPs. The antibody's presence on the surface of the conjugated nanoparticles was also assessed by dot blot and BCA studies. The viability assays showed the biocompatibility of the obtained conjugated to all the three cell lines. CLSM and ICP data revealed that nanoconjugate better recognized the receptor on Z12 cells (the one overexpressed CD44), in which the internalization was higher compared to the other cell lines. The photothermal activity of the nanosystem was observed by *in vitro* studies only on Z12 cells.

CONCLUSION

Further investigations on 2D and 3D *in vitro* models and *in vivo* studies are required to assess the toxicity and efficacy of this PTT nanoplatform against endometriotic cells overexpressing CD44

References

[1] Moses, A.S., et al., nanomedicines for endometriosis: lessons learned from cancer research small, 17(7): p. e2004975, 2021.
[2] Lahnsteiner, m., et al., improving the stability of maleimide–thiol conjugation for drug targeting. chem. eur. j. 2020, 26, 15867 – 15870

Acknowledgments

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AMYC BIOMED 2023

HDAC6 inhibitors as therapeutic option in cystic fibrosis: the first proof of concept

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Keywords: cystic fibrosis, inflammation, proof of concept, murine model, HDAC6 inhibitors

Dysregulation in epigenetic mechanisms can significantly contribute to the origin and progression of several human cancers and non oncological diseases, like Cystic Fibrosis (CF), whose epigenetic bases have been unveiled in the onset of mutations related to CFTR gene. Dysregulation of inflammatory mediators in the airway underlying CF physio-pathology also has epigenetic basis, with particular reference to the role of histone deacetylase 6 (HDAC6) in inflammatory and fibrotic phenotypes[1]. Herein we provide the first *in vivo* proof-of-concept for the efficacy of a selective HDAC6 inhibitor in contrasting inflammatory processes in a CF-related murine model of acute and chronic *P. Aeruginosa* (PA) infection. Three tool compounds were selected for their pharmacokinetic, selectivity and potency characteristics, and for their synthetic feasibility. Mainly, solubility was a key selection parameter in order to allow intratracheal administration using water as the vehicle (Penn Century® aerosolizer). After their re-synthesis, compounds' efficacy (in enzymatic and cell-based assays) and aqueous solubility were evaluated in house in order to confirm literature data, identifying compound **1**[2] (Figure 1) as the best inhibitor to be tested for its efficacy in the in vivo model[3]. A first assay was performed in a murine model of acute PA infection, confirming the lack of toxicity of compound **1**. A second experiment was performed on a chronic model of airway infection, obtained treating mice with PA strains embedded in agar beads and 7 days of aerosol treatment by Penn Century of compound **1** at different concentration or vehicle. After this time all mice were scarified and total inflammatory cells count was evaluated in bronchoalveolar lavage fluid (BALF), showing a statistically significant dose-dependent reduction in inflammatory markers. Furthermore, Bioplex Multiplex confirmed a robust reduction in the levels of interleukins and other inflammatory markers. These results confirm the efficacy of selective HDAC6 in reducing CF-related inflammatory process, laying the basis for innovative therapeutic approaches in the treatment of the complex clinical picture associated to the pathology and encouraging the development of more potent and selective HDAC6 inhibitors.

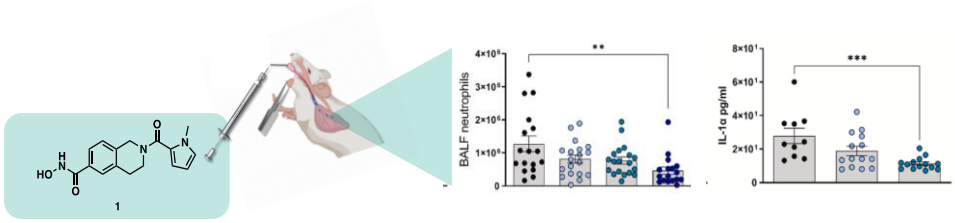

Figure 1: Evaluation of inflammatory cells and markers after seven days of treatment with compound **1** in a chronic PA infection mouse model.

References

[1] S. Barone, E. Cassese, A.I. Alfano, M. Brindisi, V. Summa, J Med Chem 2022, 65, 3080–3097.
[2] C. Blackburn, C. Barrett, J. Chin, K. Garcia, K. Gigstad, A. Gould, J. Gutierrez, S. Harrison, K. Hoar, C. Lynch, R.S. Rowland, C. Tsu, J. Ringeling, H. Xu, J Med Chem 2013, 56, 18, 7201–7211.
[3] M. Brindisi, S. Barone, A. Rossi, E. Cassese, N. Del Gaudio, A.J. Feliz Morel, G. Filocamo, A. Alberico, I. De Fino, D. Gugliandolo, M. Babaei, G. Bove, M. Croce, C. Montesano, L. Altucci, A. Bragonzi, V. Summa, Eur J Pharmacol 2022, 936, 175349.

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Discovery of NDM-1 non-conventional degraders with a novel mechanism of action to fight antibiotic resistance

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Keywords: NDM-1, antibiotic resistance, Pharmacological Protein Inactivation by Folding Intermediate Targeting, non-conventional degraders

One of the most common mechanisms of bacteria resistance to antibiotics is the hydrolysis of β-lactam antibiotics, which is catalyzed by β-lactamases [1]. In particular, the rapid worldwide spread of New Delhi Metallo-β-lactamase-1 (NDM-1) is causing an increased alarm within the scientific community due to its broad-spectrum of action, selection of variants, and transferability, representing the highest risk of a new bacterial pandemic [2]. Although various Serine-β-lactamases (SBLs) mechanism-based inhibitors are currently available in therapy (Clavulanic Acid, Sulbactam, Tazobactam, and Avibactam), there are no specific and effective inhibitors against NDM-1 on the market or in clinical practice [3]. Therefore, there is an urgent need to identify clinically useful inhibitors of Metallo-β- lactamases (MBLs) to be co-administered with existing β-lactams to restore their antibacterial activity. In order to tackle this rising issue, we used the Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT) technology [4] to identify NDM-1 non-conventional degraders. PPI-FIT is a novel and advanced drug discovery approach based on the rationale of negatively regulating protein levels by targeting folding intermediates. We applied PPI-FIT to NDM-1 and identified putative druggable pockets in a relevant folding intermediate, not present in the native conformation and different from the catalytic site. A structure-based virtual screening was performed, leading to the identification of virtual hits. Here, we report our rationale based on a combined program of medicinal chemistry, structural biology, biochemical testing, and microbiological profiling to identify novel NDM-1 non-conventional degraders.

References

[1] Santajit, S. & Indrawattana, N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. 2016, (2016).
[2] Li, X., Zhao, D., Li, W., Sun, J. & Zhang, X. Enzyme inhibitors: The best strategy to tackle superbug ndm-1 and its variants. Int. J. Mol. Sci. 23, (2022).
[3] Li, R., Chen, X., Zhou, C., Dai, Q. & Yang, L. Recent advances in β -lactamase inhibitor chemotypes and inhibition modes. Eur. J. Med. Chem. 242, 114677 (2022)
[4] Spagnolli, G. et al. Pharmacological inactivation of the prion protein by targeting a folding intermediate. Commun. Biol. 4, 1–16 (2021)

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Design, synthesis and selection of INF200, a new non-sulfonylurea based NLRP3 inhibitor

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Keywords: NLRP3 inhibitors, NLRP3 inflammasome, chronic inflammation, molecular simulation, metaflammation

The nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain containing protein 3 (NLRP3) inflammasome is a cytosolic multiprotein complex involved in the intracellular activation of the innate immune system after exposure to endogenous or exogenous noxious stimuli. Once activated and assembled, the NLRP3 inflammasome triggers the auto-proteolytic cleavage of pro-caspase-1 into the active caspase-1, converting the pro-inflammatory cytokines pro-interleukin (IL)-1β and pro-IL-18 into their active forms and causing pyroptotic cell death. Aberrant activation of NLRP3 is implicated in a variety of diseases, such as auto- and chronic inflammatory, neurodegenerative, metabolic, or infectious diseases, as well as in certain forms of cancer. Different types of NLRP3 inhibitors have been synthesized; among them, the sulfonylurea-based compounds are the largest and most studied class, with the potent NLRP3 inhibitor, MCC950, being the best pharmacologically characterized compound. However, its in vivo use in humans has been limited due to hepatic toxicity.[1,2] Inspired by the recent discovery of the binding mode of sulfonylurea inhibitors to the NLRP3 sensor protein,[3] we developed a new series of non-sulfonylurea-based compounds by replacing this moiety with different heterocycles, focusing on the 1,2,4-oxadiazoles, the 1,3,4-oxadiazol-2-ones, and the 1,3,4-thiadiazoles. Computational studies evidenced that some of the designed compounds were able to maintain important interactions within the NACHT domain of the target protein similarly to the most active sulfonylurea-based NLRP3 inhibitors. In vitro biological evaluations allowed to select the 1,3,4-oxadiazol-2-one derivative **INF200** that showed the most promising results being able to prevent NLRP3-dependent pyroptosis triggered by LPS/ATP and LPS/MSU by 66.3 ± 6.6% and 61.6 ± 11.5% (at 10 μM) and to reduce IL-1β release with an IC_{50} of 16.6 ± 2.6 μM in human macrophages. Supported by these results, **INF200** was tested in an in vivo model of high-fat-diet (HFD)-induced metaflammation in rats to evaluate its cardiometabolic effects. **INF200** proved able to reverse the unfavorable cardiometabolic dysfunction associated with obesity and to reduce systemic inflammation and anthropometric changes in HFD rats.[4]

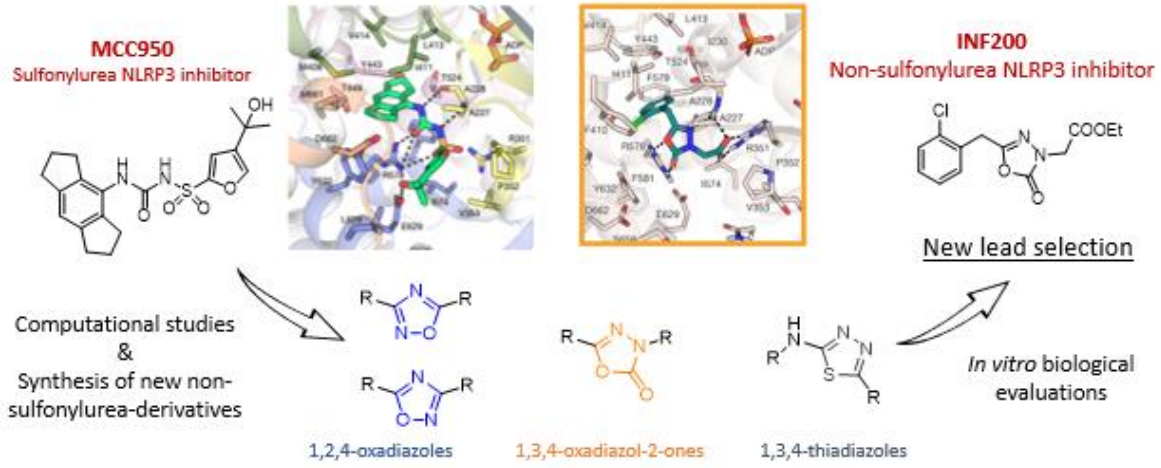

Figure 1. Rationale of the work, structures of the investigated scaffolds and of the selected new NLRP3 inhibitor, **INF200**.

References

[1] Swanson, K.V., Deng, M. & Ting, J.PY. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nat Rev Immunol.* 2019, **19**, 477–489.
[2] Blevins, H.M., Xu, Y., Biby, S. & Zhang, S. The NLRP3 Inflammasome Pathway: A Review of Mechanisms and Inhibitors for the Treatment of Inflammatory Diseases. *Front Aging Neurosci.* 2022, 14, 879021.
[3] Dekker, C., Mattes, H., Wright, M., Boettcher, A., Hinniger, A., Hughes, N., Kapps-Fouthier, S., Eder, J., Erbel, P., Stiefl, N., Mackay, A., Farady, C.J. Crystal structure of NLRP3 NACHT domain with an inhibitor defines mechanism of inflammasome inhibition. *J. Mol. Biol*. 2021, 433, 167309.
[4] Gastaldi, S., Rocca, C., Gianquinto, E., Granieri, M.C., Boscaro, V., Blua, F., Rolando, B., Marini, E., Gallicchio, M., De Bartolo, A., Romeo, N., Mazza, R., Fedele, F., Pagliaro, P., Penna, C., Spyrakis, F., Bertinaria, M. & Angelone, T. Discovery of a novel 1,3,4-oxadiazol-2-one-based NLRP3 inhibitor as a pharmacological agent to mitigate cardiac and metabolic complications in an experimental model of diet-induced metaflammation. *Eur. J. Med. Chem*. 2023, 257, 115542.

New piperazine derivatives as dual P-gp and hCA XII inhibitors to overcome multidrug resistance

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Keywords: MDR reversers, multitarget ligands, P-gp modulators, hCA XII inhibitors, dual P-gp/hCA XII inhibitory activity

Antineoplastic treatments are often compromised by multidrug resistance (MDR). P-glycoprotein (P-gp) is overexpressed in many resistant tumors, and it is the first efflux pump, belonging to the ABC transmembrane protein family, to be discovered playing a role in MDR[1]: P-gp actively transports the anticancer drugs outward, reducing their intracellular concentration below their active dose[1]. Interestingly, on the membrane of several resistant cancer cells P-gp is co-localized and physically associated to the isoform XII of human carbonic anhydrase (hCA XII)[2]. Carbonic anhydrases are metalloenzymes that catalyze the conversion of carbon dioxide to bicarbonate and a proton. In particular, the pharmacological inhibition of hCA XII alters the optimal pH for the ATPase activity of P-gp, reducing its efflux effect[2]. As a continuation of our research on dual P-gp/hCA XII inhibitors with a synergistic mechanism to overcome P-gp-mediated MDR in resistant cancer cells overexpressing both proteins[3,4], in the present study we designed and synthesized new piperazine derivatives. Especially, these new compounds carry the *N*-(methoxysubstituted)aryl piperazine scaffold, found in potent P-gp modulators[5], and four different coumarin residues (**I**-**IV**) to selectively target hCA XII, linked to the piperazine ring by a 2, 3 or 4 methylene chain (Figure 1).

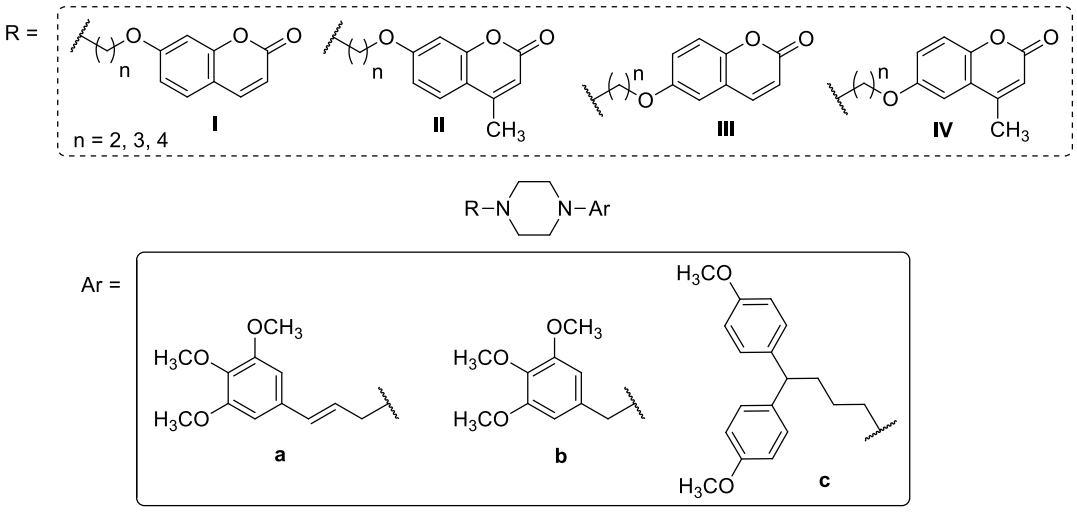

Figure 1. Structure of new piperazine derivatives as dual P-gp/hCA XII inhibitors.

First, the synthesized compounds were tested for their inhibitory effect on the two targets, P-gp and hCA XII, taken individually. In general, derivatives characterized by the **a** and **c** aryl residues are good P-gp modulators, since they enhanced the cytotoxicity of doxorubicin, that is a P-gp substrate, on K562/DOX cells overexpressing only P-gp. Moreover, the hCA inhibition activity was evaluated on four different hCA isoforms: the cytosolic hCA I and hCA II, and the tumor-associated transmembrane hCA IX and hCA XII isoforms. Results showed that the interaction with hCA XII seems to be mainly influenced by the coumarin residue, indeed the **IV** group reduced the activity on hCA XII. However, the other coumarin derivatives inhibited the hCA XII isoform, showing in some cases an outstanding potency, with K_i values in the low nanomolar range.

Based on these results, selected compounds have been studied to evaluate their synergistic activity on the MDR reversal effect due to their dual P-gp/hCA XII inhibition in doxorubicin-resistant human adenocarcinoma colon cells (HT29/DOX) and in doxorubicin-resistant non-small cell lung cancer cells (A549/DOX), that overexpress both P-gp and hCA XII[2].
The synthesis and the promising preliminary results of the new molecules will be reported and discussed.

References

[1] M.M. Gottesman, T. Fojo, S.E. Bates. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev. Cancer. 2002, 2, 48-58.
[2] J. Kopecka, I. Campia, A. Jacobs, et al. Carbonic anhydrase XII is a new therapeutic target to overcome chemoresistance in cancer cells. Oncotarget. 2015, 6, 6776-6793.
[3] E. Teodori, L. Braconi, S. Bua, et al. Dual P-Glycoprotein and CA XII Inhibitors: A New Strategy to Reverse the P-gp Mediated Multidrug Resistance (MDR) in Cancer Cells. Molecules. 2020, 25, 1748.
[4] L. Braconi, E. Teodori, C. Riganti, et al. New Dual P-Glycoprotein (P-gp) and Human Carbonic Anhydrase XII (hCA XII) Inhibitors as Multidrug Resistance (MDR) Reversers in Cancer Cells. J. Med. Chem. 2022, 65, 14655-14672.
[5] S. Dei, M. Coronnello, G. Bartolucci, et al. Design and synthesis of new potent *N*,*N*-bis(arylalkyl)piperazine derivatives as multidrug resistance (MDR) reversing agents. Eur. J. Med. Chem. 2018, 147, 7-20.

AMYC BIOMED 2023

Expression of Nucleocapsid protein (N) from SARS-CoV-2 and its characterization through high-field NMR spectroscopy

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Keywords: Nucleoprotein; SARS-CoV 2; Covid-19; IDP; NMR

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) is responsible for one of the most significant global public health crises of our century. One of the structural proteins of coronavirus 2, the N protein, appears to be genetically stable, making it an excellent candidate for the development of antiviral drugs. N is the most highly expressed of the four structural proteins of the virus and its main role is to organize the ribonucleo-protein (RNP) complex formed by the interaction of N with the genomic RNA. N is a multi-domain protein composed by 419 amino acids. It is organized into an N-terminal RNA-binding domain (NTD), a C-terminal dimerization domain (CTD) and three intrinsically disordered regions (IDR1, IDR2 and IDR3) that comprise almost 40 per cent of the protein's primary sequence (1). Our central goal is to characterize the Full Length (FL) N protein and to study its interaction with RNA in its entirety. We want to elucidate how the interaction with RNA changes as the complexity of the system increases, moving from single RNA binding domain (44-180) to the FL (1-419) also considering a construct that comprises the NTD and the flanking IDRs (NTR 1-248). Indeed, studies conducted on NTR revealed that intrinsically disordered regions play an important role in the interaction of N with RNA (2). Nuclear magnetic resonance spectroscopy (NMR), with the support of other biophysical techniques, can provide the information needed to study the disordered components of the protein.
In particular, to study modular proteins with intrinsically disordered regions, ^{13}C detection is a key technique. ^{13}C-NMR provides a wide chemical shift dispersion, which is crucial for obtaining highly resolved spectra (3). Furthermore, this technique overcomes the solvent exchange problem for amide proton signals when approaching physiological conditions. To study systems with very different structural and dynamic properties, composed of globular domains and disordered regions, it is necessary to filter out the resonance of the globular domains. Indeed, the flexibility of IDRs allows us to visualize them by NMR even in the context of a large protein complex. Thanks to these experiments, it was possible to analyze the differences in the IDRs between the entire N protein and the previously studied constructs (NTR, NTD).

References

[1] L. Pontoriero, M. Schiavina, S. M. Korn, A. Schlundt, R. Pierattelli, I. C. Felli, *Biomolecules* **12**, 929 (2022)
[2] M. Schiavina, L. Pontoriero, G. Tagliaferro, R. Pierattelli, I. C. Felli, *Biomolecules* **12**, 1302 (2022)
[3] M. Schiavina, M. G. Murrali, L. Pontoriero, V. Sainati, R. Kümmerle, W. Bermel, R. Pierattelli, I. C. Felli, *Biophys J* **117**, 46–55 (2019)

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AMYC BIOMED 2023

An antiviral agent triggering the proteolysis of the main protease from *Coxsackievirus B3*

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Keywords: Coxsackievirus B3, 3C protease, proteolysis, crystal structure, NMR

Some positive-sense RNA viruses can be classified into the picornavirus-like supercluster, which includes viruses belonging to the Picornaviridae, Caliciviridae, and Coronaviridae families [1]. These viruses possess a viral 3C or 3C-like protease ($3C^{Pro}$ or $3CL^{Pro}$, respectively), which share several structural common characteristics at the active site and are responsible for cleavages of the viral polyproteins [2]. Coxsackievirus B3 (CVB3) 3C protease plays an essential role in the viral replication of CVB3, which is a cardiotropic virus belonging to Enterovirus genus within Picornaviridae family [3]. CVB3 $3C^{Pro}$ has been retained as an attractive target for the development of antiviral agents, as there is no effective therapeutic strategy for the prevention and treatment of diseases caused by CVB3 infection. In this regard, here we consider a peptidomimetic based strategy to obtain a molecule able to trigger the degradation of the CVB3 main protease. The peptidomimetic molecule (FT235) consists of a dipeptidyl ligand (FT234) conjugated to a pomalidomide moiety able to bind the E3 ligase substrate [4]. By solution NMR spectroscopy and X-ray diffraction, we characterize the interaction of CVB3 $3C^{Pro}$ with FT235 and with its precursor. The data showed that $3C^{Pro}$ from CVB3 forms a complex with FT235 where the α, β unsaturated moiety of the peptidomimetic molecule is covalently bound to the catalytic Cys147.

References

[1] Kim, Y.; Lovell, S.; Tiew, K.-C.; Mandadapu, S.R.; Alliston, K.R.; Battaile, K.P.; Groutas, W.C.; Chang, K.-O. Broad-Spectrum Antivirals against 3C or 3C-Like Proteases of Picornaviruses, Noroviruses, and Coronaviruses. *J Virol* **2012**, *86*, 11754–11762
[2] Zhang, L.; Lin, D.; Kusov, Y.; Nian, Y.; Ma, Q.; Wang, J.; von Brunn, A.; Leyssen, P.; Lanko, K.; Neyts, J.; et al. α-Ketoamides as Broad-Spectrum Inhibitors of Coronavirus and Enterovirus Replication: Structure-Based Design, Synthesis, and Activity Assessment. *J Med Chem* **2020**, *63*, 4562–4578
[3] Massilamany, C.; Gangaplara, A.; Reddy, J. Intricacies of Cardiac Damage in Coxsackievirus B3 Infection: Implications for Therapy. *Int J Cardiol* **2014**, *177*, 330–339
[4] Bricelj, A.; Steinebach, C.; Kuchta, R.; Gütschow, M.; Sosič, I. E3 Ligase Ligands in Successful PROTACs: An Overview of Syntheses and Linker Attachment Points. *Front Chem* **2021**, *9*

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Role of novel synthetized caffeic acid phenethyl ester derivatives as HO-1 inducers and ferroptosis effectors for anticancer therapy

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Keywords: Ferroptosis, cancer, heme-oxygenase, antioxidants

Heme oxygenase (HO) is an intracellular enzymatic system responsible for heme degradation in stoichiometric amounts of ferrous iron, carbon monoxide and biliverdin. Among the two main isoforms identified so far, HO-1 is strongly induced under cellular stressful conditions and its expression is transcriptionally regulated by the Nrf2-Keap1 axis. HO-1 has been demonstrated to be a useful target for the treatment of pathologies related to an unbalanced oxidative stress status. Moreover, HO-1 can exert either a cytoprotective or a detrimental action in cancer [1,2], depending on the specific cellular conditions. Recent findings [3] suggest a prominent role of HO-1 induction in ferroptosis, a newly discovered form of cellular death triggered by iron accumulation and lipid peroxidation. Herein we report biological evaluation on breast cancer cells of novel HO-1 inducers whose chemical structure derives from the natural compound caffeic acid phenethyl ester (CAPE), including analysis of HO expression and enzymatic activity, and assessment of main ferroptotic features. Preliminary data showed CAPE derivatives ability to induce cancer cells death in a ferroptotic related manner, highlighting their potential for further cancer research applications.

References

[1] V. Sorrenti, A.G. D'amico, I. Barbagallo, V. Consoli, S. Grosso, L. Vanella. Tin Mesoporphyrin Selectively Reduces Non-Small-Cell Lung Cancer Cell Line A549 Proliferation by Interfering with Heme Oxygenase and Glutathione Systems. Biomolecules. 2021, 11 (6), 917.
[2] A.N. Fallica, V. Sorrenti, A.G. D'amico, L. Salerno, G. Romeo, S. Intagliata, V. Consoli, G. Floresta, A. Rescifina, V. D'agata, L. Vanella, V. Pittalà. Discovery of Novel Acetamide-Based Heme Oxygenase-1 Inhibitors with Potent In Vitro Antiproliferative Activity. J. Med.Chem. 2021, 64 (18), 13373-13393.
[3] V. Consoli, V. Sorrenti, V. Pittalà , K. Greish, A.G. D'amico, G. Romeo, S. Intagliata, L. Salerno, L. Vanella. Heme Oxygenase Modulation Drives Ferroptosis in TNBC Cells. Int. J. Mol. Sci. 2022, 23 (10), 5709.

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Exploring unripe Datterini tomatoes as a promising nutraceutical for sarcopenia prevention

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Keywords: aging, sarcopenia, unripe tomatoes, Datterini tomatoes, nutraceutical

Sarcopenia is the most common musculoskeletal aging-related disease, characterized by the loss up to 50% of muscle mass. The consequences of sarcopenia include several adverse health outcomes, as loss of function, disability, and frailty. Recent research has focused on identifying molecular pathways involved in the aging process and sarcopenia pathogenesis. Among these pathways, mammalian target of rapamycin (mTOR) and activating transcription factor 4 (ATF_4) had been identified as critical targets in muscle atrophy processes.[1] Simultaneous activation of mTOR and inhibition of ATF_4 stimulate protein synthesis and promote muscle growth. Therefore, out attention was focused on identifying natural compounds for the development of nutraceuticals for sarcopenia prevention. Based on literature data, we have selected tomatidine as a molecule able to act on both targets. Tomatidine is the aglyconic component of α-tomatine, a glycoalkaloid isolated from unripe tomatoes (*Solanum Lycopersicum* L.). The *in vivo* metabolic mechanism responsible for the conversion of α-tomatine to tomatidine has not yet been elucidated.[2] The nutraceutical development requires several steps, including food matrix selection, qualitative and quantitative profile analysis, pharmacological studies to evaluate the biological activity, and finally formulation development (Fig. 1). A screening of seven unripe tomatoes cultivars was performed to investigate their phytochemical profile. The characterization results led to the selection of Datterini tomatoes (DT) as the cultivar with the most promising glycoalkaloid and polyphenol content (α-tomatine: 33.7 ± 1.4 mg/g DW; chlorogenic acid: 1.41 ± 0.01 mg/g DW; rutin: 996 ± 4 µg/g DW). Subsequently, DT cultivar was evaluated through *in vitro* and *in vivo* assays to confirm the potential of unripe tomatoes in sarcopenia prevention. *In vitro* assays were performed using tomatidine on C2C12 muscle cell cultures to test its ability to modify protein synthesis and mitochondrial metabolism. These results confirmed that tomatidine increases protein synthesis, mitochondrial activity and mitochondrial biogenesis by activating mTORC1. To assess the DT potential in sarcopenia prevention, an *in vivo* proof of concept to increase fatigue resistant in elderly mouse was carried out. The method for the incorporation of the matrix in the feed was validated. After 50 weeks of administration, a treadmill test on 12 mice was carried out, indicating that the mice feed with DT supplemented chow show a better muscle performance. They performed the test by spending more time on the treadmill, with a reduction of the number of light beams, and moving a greater distance, demonstrating a greater muscle endurance than the control group. These results indicated DT as a possible food matrix for the development of new nutraceuticals for the prevention of muscle mass loss. Future goal will include a clinical trial in elderly patients to verify DT antisarcopenic properties.

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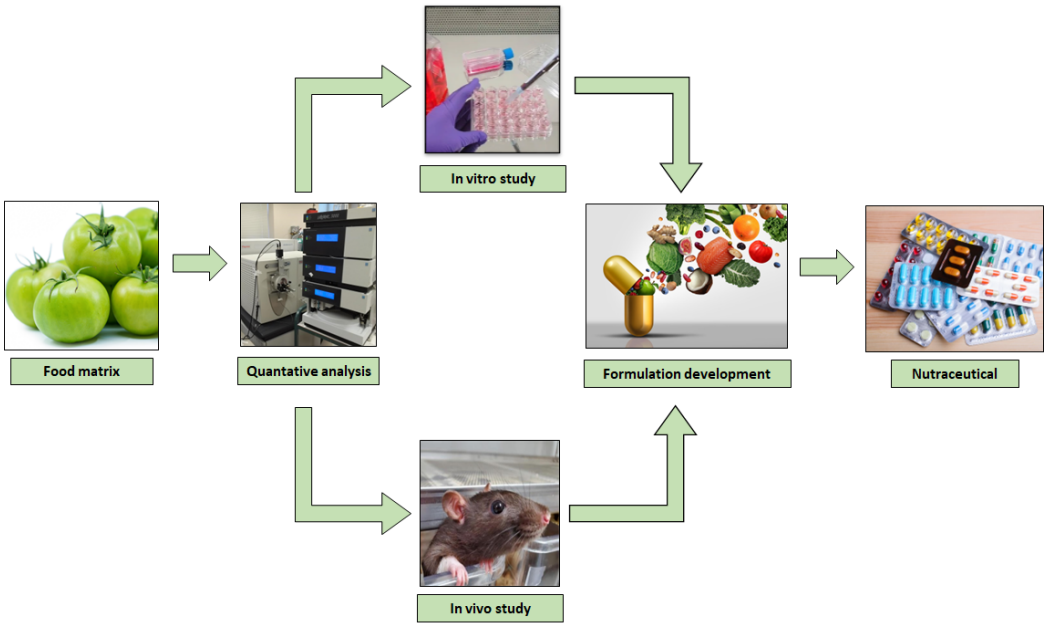

Figure 1: Workflow of DT nutraceutical development.

References

[1] Ebert, S.M. et al. Activating transcription factor 4 (ATF4) promotes skeletal muscle atrophy by forming a heterodimer with the transcriptional regulator C/EBPβ. Molecular bases of disease, 2020, 295(9) 2787-2803.
[2] Cichon, M.J. et al. Plasma metabolomics reveals steroidal alkaloids as novel biomarkers of tomato intake in mice. Mol Nutr Food Res, 2017, 61(12).

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Extraction of α-tomatine from food waste of tomato processing

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Keywords: tomato, extraction, tomatine, food waste

Nowadays, there has been considerable attention towards the recovery of food waste, in particular the plant matrices, as possible sources of functional compounds with health properties [1]. It has been shown that the main by-products of tomato processing contain different bioactive molecules and, for this reason, they can be recovered and re-used in various ways. Tomato is a plant belonging to the *Solanaceae* family and it is known for the many beneficial properties (antioxidant, anti-inflammatory, anti-proliferative, anticancer) due to its bioactive constituents. In the agri-food sector, tomato is widely processed to obtain juices and purees, thus generating a huge amount of food waste. It has been shown that these tomato by-products, such as the leaves, represent a good source of phytochemical compounds with health properties, such as α-tomatine. Tomatine (α-TM) is a steroidal glycoalkaloid that possesses numerous beneficial properties such as antioxidant, antiviral, anti-inflammatory, and anticancer [2]. In particular, the latter has been demonstrated in many cancers, like colon, breast, liver, lung, and prostate cancer.
Therefore, the purpose of this study was to evaluate the health properties of leaves extracts from two tomato Sicilian cultivars, the *Datterino* and *Piccadilly*, related to the tomatine content. Leaves fractions were subjected to drying in a vacuum oven for 24 h at 60°C and subsequently pulverized. The powders obtained were extracted using two different experimental procedures: one was carried out with a mixture of ethanol: acetic acid (95:5, v/v) [3] and the other with water; both were stirred for 72 and 48 hours, respectively. After freeze-drying, the extracts were analyzed by UHPLC-MS/MS. The obtained results showed that ethanolic extraction proved to be more suitable for α-TM extraction. In particular, the *Piccadilly* cultivar showed a high α-TM content. Based on the obtained results, *Piccadilly* leaves extract could be regarded as source of α-TM, an interesting molecule for a nutraceutical and pharmaceutical fields.

References

[1] D. Santonocito, et al. Carob Seeds: Food Waste or Source of Bioactive Compounds? Pharmaceutics. 2020 Nov 13;12(11):1090.
[2] C. Bailly. The steroidal alkaloids α-tomatine and tomatidine: Panorama of their mode of action and pharmacological properties. Steroids. 2021, 176:108933.
[3] N.P. Silva-Beltrán, et al. Total Phenolic, Flavonoid, Tomatine, and Tomatidine Contents and Antioxidant and Antimicrobial Activities of Extracts of Tomato Plant. Int J Anal Chem. 2015; 2015:284071.

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Purification of Oligonucleotides

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Keywords: Oligonucleotides, purification

Therapeutic oligonucleotides represent a recent breakthrough in the pharmaceutical industry, they are short nucleic acid chains, and they are emerging as a major category of therapeutic drugs, due to their ability to regulate gene expression with great specificity [1].
Nowadays the most established technology for the production of oligonucleotides is the solid phase synthesis, a cyclic approach where the progressive addition of nucleotide monomers results in a polymeric chain. However, this technique leads not only to the main product but also results in many structurally similar impurities such as deletion or addition of one nucleotide resulting in the so called shortmers (n-1) and longmers (n+1) impurities [1,2,3].
Therapeutic oligonucleotides require extensive chromatographic purification to ensure that a high-quality API (Active Pharmaceutical Ingredient) is manufactured, and to fulfill the strict purity requirements imposed by the regulatory agencies. In the biopharma industries the purification of oligonucleotides is usually carry out through single column preparative liquid chromatography, in particular the main technique used for this purpose are Anion Exchange Chromatography (AEX) and Ion Pair liquid chromatography (IP-RP) [1,2,3].
Anion Exchange chromatography takes advantage of the charge on the phosphate linkage on the oligonucleotide and ensures a fine separation between the oligos and shortmers and longmers. The elution is generally performed with sodium salt, in this way the purified product is already in its desired sodium form. On the other hand, Ion Pair Reverse Phase chromatography ensures not only charged based residues but also charge neutral lipophilic impurities are discriminated.
However, because of the use of a counterion, an additional salt-exchange step is required to yield the oligonucleotide in its sodium form [4].
Nevertheless, single column purifications techniques often suffer from a yield-purity trade-off. To improve the purity of the target a Multicolumn Countercurrent continuous chromatography approach can be used. This approach is based on a twin-column system, working either interconnected or in parallel, in this way internal recycling takes place. Furthermore, the whole process is fully automated [1,2].
This work will describe the different approaches used for the purification of oligonucleotides.

References

[1] R. Weldon et al., Purification of a GalNAc-cluster-conjugated oligonucleotide by reversed-phase twin-column continuous chromatography, Journal of Chromatography A 1663, 2022, 462734.
[2] M. Catani et al., Oligonucleotides: Current Trends and Innovative Applications in the Synthesis, Characterization, and Purification, Biotechnology Journal, 2020, 15, 1900226.
[3] G. Lievore et al., Enrichment and recovery of oligonucleotide impurities by N-Rich twin-column continuous chromatography, Journal of Chromatography B, 2022, 1209, 123439.
[4] E. Paredes et al., Manufacturing of Oligonucleotides, 2017, Elsevier.

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Production of recombinant KHSRP and NMR study of its interaction with *c-myc* oncogene promoter G-quadruplex

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Keywords: KHSRP, *c-myc* oncogene, G-quadruplexes, 1D ^{1}H and 2D ^{1}H-^{15}N HSQC NMR, Protein-DNA interactions

Proteins that bind nucleic acids regulate many cellular processes, including transcription, translation, gene silencing, microRNA biogenesis and telomere maintenance. Some of these, known as DNA- and RNA-binding proteins (DRBPs), have domains able to bind both DNA and RNA with different specificity and manner. One of the known bivalent domains present in DRBPs is the K homology (KH) domain [1-2]. KHSRP (also known as KSRP or FBP2) is a multifunctional nucleic acid binding protein, present in the nucleus and cytoplasm [3]. It regulates transcription, mRNA translation, miRNA biogenesis, and modulates diverse cellular functions, including cell differentiation/proliferation and innate immunity, playing a key role in immune cell function and tumour progression [3]. Particularly, the over-expression of KHSRP has been shown to promote *c-myc* transcription, a well-known proto-oncogene involved in a broad spectrum of human cancers [4-6]. KHSRP (1-711) is composed of a structured central nucleic acid binding region that includes four KH domains (130-503) and two N- (1-129) and C-terminal (504-711) unfolded regions [3-8]. This particular structure and its ability to bind to nucleic acids have made its biochemical, biophysical, and structural studies very difficult. To date, KHSRP expression and purification have shown several limitations. Here, we describe, for the first time, a new approach to express and purify the human KHSRP using the *Escherichia Coli* system and a different purification method without precipitation and refolding, to obtain the protein in its native folding. In addition, 1D ^{1}H and 2D ^{1}H-^{15}N HSQC NMR experiments have been performed to confirm the correct folding of KHSRP and validate our protein production method, as well as to detect protein interaction with a G-quadruplex (G4)-forming DNA sequence from the promoter region of *c-myc* oncogene. Our results offer an exciting outlook for the future of KHSRP/G4 interactions studies in cancer, which have so far been poorly analysed. This work wants to provide useful and updated methods to bring new opportunities to this rapidly expanding field of medicinal chemistry.

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References

[1] Hudson WH, Ortlund EA. Nat Rev Mol Cell Biol. 2014, 15, 749-60.
[2] García-Mauriño SM, Rivero-Rodríguez F, Velázquez-Cruz A, Hernández-Vellisca M, Díaz-Quintana A, De la Rosa MA, Díaz-Moreno I. Front Mol Biosci. 2017, 4, 71.
[3] Duncan R, Bazar L, Michelotti G, Tomonaga T, Krutzsch H, Avigan M, Levens D. Genes Dev. 1994, 8, 465-80.
[4] Briata P, Chen CY, Ramos A, Gherzi R. Biochim Biophys Acta. 2013, 1829, 689-94.
[5] Briata P, Bordo D, Puppo M, Gorlero F, Rossi M, Perrone-Bizzozero N, Gherzi R. Wiley Interdiscip Rev RNA. 2016, 7, 227-40.
[6] Palzer KA, Bolduan V, Käfer R, Kleinert H, Bros M, Pautz A. Cells. 2022, 11, 1482.
[7] Ramdzan M. Zubaidah, Gek San Tan, Sandra B. E. Tan, Seng Gee Lim, Qingsong Lin and Maxey C. M. Chung. Proteomics 2008, 8, 5086–5096.
[8] Gherzi R, Chen CY, Ramos A, Briata P. Semin Cell Dev Biol. 2014, 34, 2-8.

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DNA Electrochemical Biosensing using a Versatile and Automatable Microfluidic Platform

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Keywords: electrochemical biosensors, microfluidic platform, magnetic beads, DNA detection, lab-on-a-chip

Conventional bioanalytical approaches for detecting target environmental and biological DNA markers often require substantial effort, time, and training to ensure accurate and reliable results. Thus, the need for simple, portable, and easily-operable procedures able to streamline detection protocols continuously increases.
To address these requirements, this work aimed to adapt and integrate all steps of a DNA detection scheme to work in a continuously-controlled microfluidic flow inside an easily-produced electrochemical platform, thus minimising manual intervention and reducing sample volumes.
DNA-functionalised superparamagnetic microbeads are employed to facilitate the capture and isolation of target DNA fragments. These particles are introduced into the system's microchannel and positioned using a magnet. Through the microfluidic flow, controlled by a peristaltic pump, the beads are exposed to the various reagents, including the analyte of interest and a signalling DNA probe carrying an enzyme. Following an experimentally-optimised protocol, post-hybridization the beads are dislodged from their position and directed inside an electrochemical cell, where the target oligonucleotide is electrochemically detected at low concentrations exploiting an enzymatic signal amplification reaction. Efficiency and sensitivity improvements were achieved by optimising the workflow, focusing on reducing reagent volumes and total experimental runtime. The platform was tested in the proof-of-concept analysis of short DNA fragments, completing the assay in less than one hour.
Notably, this system has the potential to be seamlessly applied in the detection of DNA with applications ranging from environmental to food and clinical specimens, with the prospect of incorporating software-controlled mechanisms to easily automate all steps. Moreover, the integration of DNA amplification techniques within the microfluidic chip to further enhance the sensitivity is also expected.

References

[1] C. Patiti, P.S. Sfragano, S. Laschi, S. Pillozzi, A. Boddi, O. Crociani, A. Bernini, I. Palchetti. Chip-Based and Wearable Tools for Isothermal Amplification and Electrochemical Analysis of Nucleic Acids. Chemosensors. 2022, 10(7), 278.
[2] P. Khashayar, S. Al-Madhagi, M. Azimzadeh, V. Scognamiglio, F. Arduini. New frontiers in microfluidics devices for miRNA analysis. TrAC Trends in Analytical Chemistry. 2022, 156, 116706.

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Protease detection using activity-based CRISPR-Cas amplification

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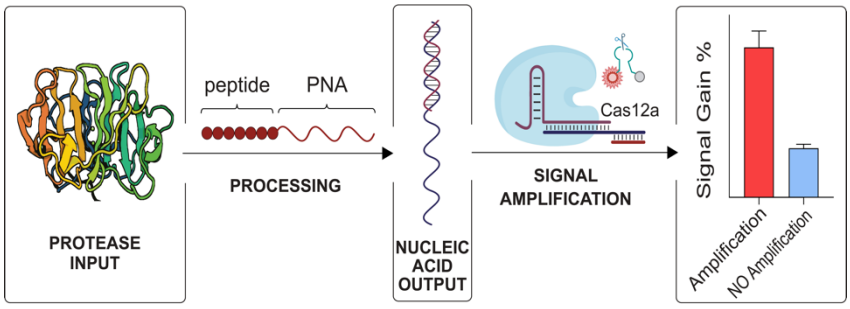
DNA nanotechnology provides unparalleled opportunities for creating artificial communication pathways among biomolecules through programmable interactions involving nucleic acids. This can be harnessed to engineer sensing platforms for specific proteins. Leveraging this potential, our study introduces a novel activity-based detection strategy for MMP2, an important protease biomarker for various types of cancer [1]. This strategy involves the design of a DNA-based mechanism responsive to the typical proteolytic activity of MMP2 and its integration with a CRISPR-Cas12a-assisted amplification process, thus realizing artificial communication between peptide- and DNA-based processes. We have devised a chemical translator composed of two distinct functional units—a peptide and a peptide nucleic acid (PNA)—integrated together. The peptide unit presents the substrate sequence of the target protease, while the PNA unit enables the conversion of protein-based input, namely peptide cleavage, into a nucleic acid output that can be subsequently processed and amplified. We have evaluated the efficiency of the proteolytic cleavage executed by MMP2 on this artificial substrate by mass spectrometry, and then immobilized this latter on micrometric magnetic beads as supportive materials, investigating the cleavage kinetics and platform specificity for the chosen protease. Subsequently, we have incorporated into our design a single-stranded DNA partially hybridized with the PNA sequence of the translator. The free region of this DNA strand is complementary to the RNA sequence of a CRISPR Cas12a/crRNA complex. Upon hybridization, the nuclease trans-cleavage activity of Cas12a is activated, resulting in the degradation of FRET-labeled DNA reporters [2]. An amplified fluorescence signal is therefore generated that is proportional to the proteolytic activity of MMP2. Through this approach, we can successfully detect MMP2 in the low picomolar range, relying on its enzymatic activity and achieving a limit of detection several orders of magnitude lower than that of commercially available peptide-based kits. Beyond the immediate implications, our study provides essential principles for molecular design that can broaden the applicability of this approach to various target proteases and to other DNA nanotechnology-enabled amplification systems. This platform exhibits promising potential for detecting biomarker proteases in cell cultures and tissue samples, thereby offering significant prospects for biomedical research and diagnostics.



References

[1] Kwon, E. J., Lo, J. H., & Bhatia, S. N. Smart nanosystems: Bio-inspired technologies that interact with the host environment. 2015, Proc..Natl. Acad. Sci, 14460-14466
[2] Rossetti M., Merlo R., Bagheri N., Moscone D., Valenti A., Saha A., Arantes P.,Ippodrino R., Ricci F., Treglia R., Delibato E., van der Oost J., Palermo G., Perugino G., Porchetta, A. Enhancement of CRISPR/Cas12a *trans*-cleavage activity using hairpin DNA reporters. 2022, Nucleic acids res, 8377-8391.

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Ad hoc tools to monitor bRo5 chameleonicity

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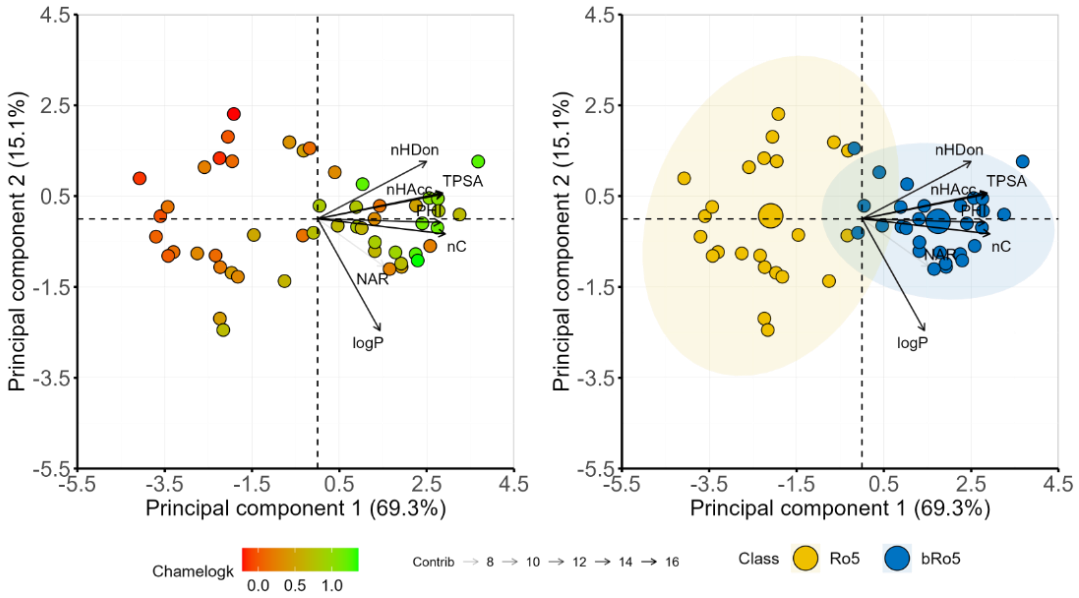
Keywords: PROTAC, chameleonicity, ADME

Since the turn of the century, the chemical region beyond Lipinski's Rule of Five (bRo5) has become very popular. In fact, molecules governing this space are gaining a huge interest in drug discovery since they can act over difficult-to-drug targets involved in cancer and other untreated diseases. Nevertheless, their complex structure is often related to pharmacokinetic limitations, mainly related to solubility and permeability issues. Thus, despite their therapeutic potential, their future as oral therapies is compromised. In addition, property-based strategies to overcome these drawbacks are not yet available and structural improvements are still under study. To date, cyclosporine is the best well-known outlier currently known to be orally accessible. It is able to undergo structural and physicochemical changes in an environment-dependent manner to address solubility and cell permeability issues. This behavior is often referred to as "molecular chameleonicity," and it represents a cutting-edge method for creating new oral bRo5 drugs.

However, the main challenge is still the experimental assessment of chameleonicity and subsequently the *in silico* prediction. Thus, we created a high-throughput (HT) method based on a chromatographic descriptor (HPLC) that allows us to rank and measure the chameleonic potential of bRo5 molecules, primarily PROTACs and macrocycles.[1] The "Chamelogk" tool offers a number of benefits over other well-known chameleonicity assessment techniques like NMR, X-Ray, and ChamelogD. We confirmed that bRo5 compounds are more chameleonic compounds than regular Ro5 ones and observed several patterns and differences among their subclasses (PROTACs, macrocycles, etc.).

Finally, we attempted to rationalize Chamelogk values with computational tools such as machine learning, conformational sampling (CS) and steered molecular dynamics (SMD) . Then, a thorough conformer analysis was performed to understand the molecular mechanisms underlying the experimental evidence.[2]



Graphical abstract: driving factors of bRo5 chameleonicity

References

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[1] Ermondi, G.; Vallaro, M.; Caron, G. Degraders Early Developability Assessment: Face-to-Face with Molecular Properties. *Drug Discov. Today* **2020**, 25 (9), 1585–1591.
[2] Rossi Sebastiano, M.; Garcia Jimenez, D.; Vallaro, M.; Caron, G.; Ermondi, G. Refinement of Computational Access to Molecular Physicochemical Properties: From Ro5 to BRo5. *J. Med. Chem.* **2022**, 65 (18), 12068– 12083.

AMYC BIOMED 2023

A new microfluidic cartridge to prepare non-viral gene delivery complexes

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Keywords: non-viral gene delivery, microfluidic cartridge, polyplexes, transfection, reproducibility

Non-viral gene delivery (also named transfection) describes the process of introducing exogenous nucleic acids (NAs) into target cells utilizing non-viral means [1]. In the last couples of decades, scientists have been doing their best to design and develop ever more effective transfection tools and reagents [2, 3]. Cationic lipids and polymers, collectively called transfection reagents, are very well liked because they are reasonably functional in transfecting cells. These are mixed with NAs in a vial to give rise to gene delivery complexes, i.e., lipoplexes and polyplexes, respectively, which are next dripped onto cell cultures to deliver their payload intracellularly [4]. Although the preparation of non-viral gene delivery complexes is basically an easy task, slight yet often unintended changes in its execution may result in disparate transfection behavior and, thus, considerable heterogeneity of results across studies [5]. Moreover, because of the clinical quest for an ever-larger quantity of nanocomplexes, the development of tools for scaling up and standardizing the production of gene delivery particles cannot be delayed further [2, 3, 6]. As microfluidics allows the most accurate manipulation of small amounts of fluids by simply tuning the experimental parameters such as species concentrations and flow rates, this technology has thus been extensively used to produce optimally tailored gene delivery nanoassemblies [7, 8]. Unfortunately, although microfluidics offers distinct advantages over the canonical approaches to preparing nanoparticles, to the best of our knowledge, the systems available do not address the most frequent and practical quest for the simultaneous generation of multiple polymer-to-NA ratios (N/Ps), i.e., the mole ratio of the amino groups (N) borne by the cationic polymer used to complex the phosphate groups (P) of a given amount of NA [5]. Driven by the need to fill this gap and motivated to address the lack of standardization in test procedures underpinning the low reproducibility of results [9], we developed and validated a user-friendly polydimethylsiloxane (PDMS)-made microfluidic cartridge to repeatably prepare non-viral gene delivery nanoparticles and screen across a range of seven N/Ps at once or significant volumes of polyplexes at a given N/P [10]. The microchip (Figure 1) is equipped with (i) a chaotic serial dilution generator for the automatic linear dilution of the polymer to the downstream area, which encompasses (ii) the NA divider to dispense equal amounts of DNA to (iii) the mixing area, enabling the formation of particles at seven N/Ps eventually collected in (iv) individual built-in tanks. This is the first example of a stand-alone microfluidic cartridge for the fast and repeatable preparation of non-viral gene delivery complexes at different N/Ps and their recovery and storage until use.

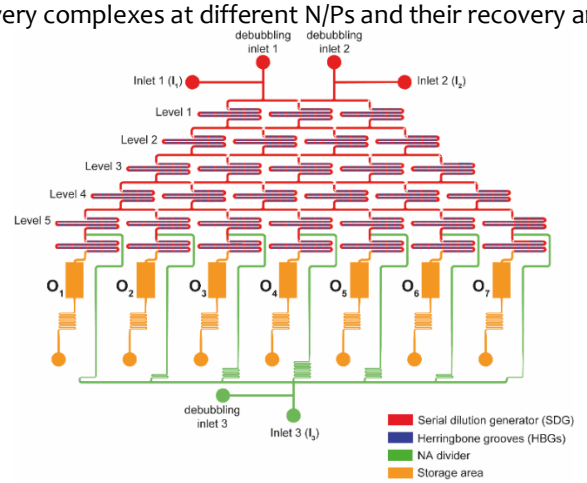

Figure 1. The microfluidic cartridge consists of *i)* an upstream 5-level serial dilution generator (SDG) (red) integrated with herringbone grooves (HBGs) (blue), *ii)* a NA divider (green), *iii)* a mixing area, and *iv)* a storage area. The polymer solution and the water-based medium are injected into the two main

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inlets (inlets 1 and 2, I_1 and I_2) through the SDG and moved to the downstream area. An additional inlet (I_3) allows for the addition and distribution of the DNA solution to the seven mixing units, where the polymer and DNA are mixed to give rise to polyplexes. These are collected into the storage tanks

References

[1] H. Zu, D. Gao. Non-viral Vectors in Gene Therapy: Recent Development, Challenges, and Prospects. AAPS J. 2021, 23, 78.
[2] R. Yang, Y. Deng, B. Huang, L. Huang, A. Lin, Y. Li, W. Wang, J. Liu, S. Lu, Z. Zhan, Y. Wang, W. Wang, P. Niu, L. Zhao, S. Li, X. Ma, L. Zhang, Y. Zhang, W. Yao, X. Liang, J. Zhao, Z. Liu, X. Peng, H. Li, W. Tan. A core-shell structured COVID-19 mRNA vaccine with favourable biodistribution pattern and promising immunity. Signal Transduct. Target. Ther. 2021, 6, 213.
[3] A. Tejeda-Mansir, A. García-Rendón, P. Guerrero-Germán. Plasmid-DNA lipid and polymeric nanovaccines: a new strategic in vaccines development. Biotechnol. Genet. Eng. Rev. 2019, 35, 46–68.
[4] S. Mirón-Barroso, E. B. Domènech, S. Trigueros. Nanotechnology-based strategies to overcome current barriers in gene delivery. Int. J. Mol. Sci. 2021, 22, 8537.
[5] N. Bono, F. Ponti, D. Mantovani, G. Candiani. Non-viral in vitro gene delivery: It is now time to set the bar!. Pharmaceutics. 2020, 12, 1–23.
[6] M. Lim, A. Z. M. Badruddoza, J. Firdous, M. Azad, A. Mannan, T. A. Al-Hilal, C. S. Cho, M. A. Islam. Engineered nanodelivery systems to improve dna vaccine technologies. Pharmaceutics. 2020, 12, 1–30.
[7] F. Tian, L. Cai, C. Liu, J. Sun. Microfluidic technologies for nanoparticle formation. Lab Chip. 2022, 22, 512–529.
[8] M. A. Tomeh and X. Zhao. Recent Advances in Microfluidics for the Preparation of Drug and Gene Delivery Systems. Mol. Pharm. 2020, 17, 4421–4434.
[9] A.L. Plant, R.J. Hanisch. Reproducibility and Replicability in Science, A Metrology Perspective. Harvard Data Sci. Rev. 2018, 1–21.
[10] G. Protopapa, N. Bono, R. Visone, F. D'Alessandro, M. Rasponi, G. Candiani. A new microfluidic platform for the highly reproducible preparation of non-viral gene delivery complexes. Lab Chip. 2023, 23, 136–145.

Lab-made nanostructured third-generation biosensors for fructose determination in food and biological samples

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Keywords: Fructose; urine; diabetes; biosensor; stencil-printing

The consumption of food and beverages with high sugar content significantly increases the risk of developing metabolic syndromes, obesity, and cardiovascular diseases; indeed, high levels of free fructose intake can promote insulin resistance in the liver, leading to diabetes conditions [1]. Therefore, the development of rapid and in situ strategies for fructose determination have critical importance, both in food products and biological fluids, for the implication related to the diet and to monitoring health conditions, respectively [2].
In this work Fructose dehydrogenase (FDH) based electrochemical biosensors have been developed and applied for the fructose determination in food and biological samples; three different beverages types and honey varieties were considered, whereas urines were collected from healthy volunteers and then spiked at pathological and sub-pathological levels of fructose, to simulate different diabetes severities [3]. To this aim, the electrochemical platform was built-up via stencil-printing (StPE), employing office-grade technologies and substrates. The electrodes were then modified with 0D carbonaceous nanomaterials, namely carbon black (CB) and mesoporous carbon (MS), previously prepared in water-phase dispersions through a sonochemical approach; both materials exhibited enhanced electro-catalytic features, promoting the direct electron transfer (DET) reaction between the FDH and the sensing surface, thus the nano-StPEs were exploited for fructose electro-sensing via chronoamperometry. CB-StPE and MS-StPE biosensors displayed appreciable sensitivity (~150 μA cm^{-2} mM^{-1}), along with sub-μmolar LODs (0.35 and 0.16 μM, respectively) and extended linear ranges (2 – 500 and 1 – 250 μM, respectively). Moreover, the biosensors demonstrated reliable analytical efficacy for real sample analysis, showing remarkable selectivity in the presence of potentially interfering species thanks to the low working overpotential (0.15 V), along with satisfactory accuracy (recoveries between 95–116%) and reproducibility (RSD ≤ 8.6%).
Herein, the marriage between the versatility of the manufacturing strategy with the electro-catalytic properties of the water-based carbon nanomaterials provides new paths for the fabrication of smart and customizable FDH-based electronics, suitable even for the on-site fructose monitoring in food and clinical scenarios.

References

[1] Bray, G.A., Popkin, B.M. Calorie-sweetened beverages and fructose: what have we learned 10 years later Pediatr. Obes. 2013. 242–248.
[2] Sloboda, D.M., Li, M., Patel, R., Clayton, Z.E., Yap, C., Vickers, M.H. Early Life Exposure to Fructose and Offspring Phenotype: Implications for Long Term Metabolic Homeostasis. J. Obes. 2014. 203474.
[3] Kawasaki, T., Igarashi, K., Ogata, N., Oka, Y., Ichiyanagi, K., Yamanouchi, T. Markedly increased serum and urinary fructose concentrations in diabetic patients with ketoacidosis or ketosis. Acta Diabetol. 2012. 49, 119–123.

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Click Chemistry for advancing the development of dual RGD integrin/MMP ligands

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Keywords: Tumor angiogenesis, Dual inhibition, Integrins, Matrix Metallo-Proteases (MMPs), Click Chemistry

Through the process of angiogenesis, tumor cells generate new blood vessels to ensure supply and a route of elimination of waste metabolites. So, tumor cells can grow and spread out to the body generating metastasis. Integrin receptors and Matrix Metallo-Proteases (MMPs) are proteins deeply involved in tumor cell invasion and metastatic diffusion, interacting each other to facilitate these processes [1]. Indeed, Integrins are responsible of cellular adhesion with the extracellular matrix (ECM), while MMPs have a role in tissue remodeling and in the degradation of several structural proteins in the ECM.

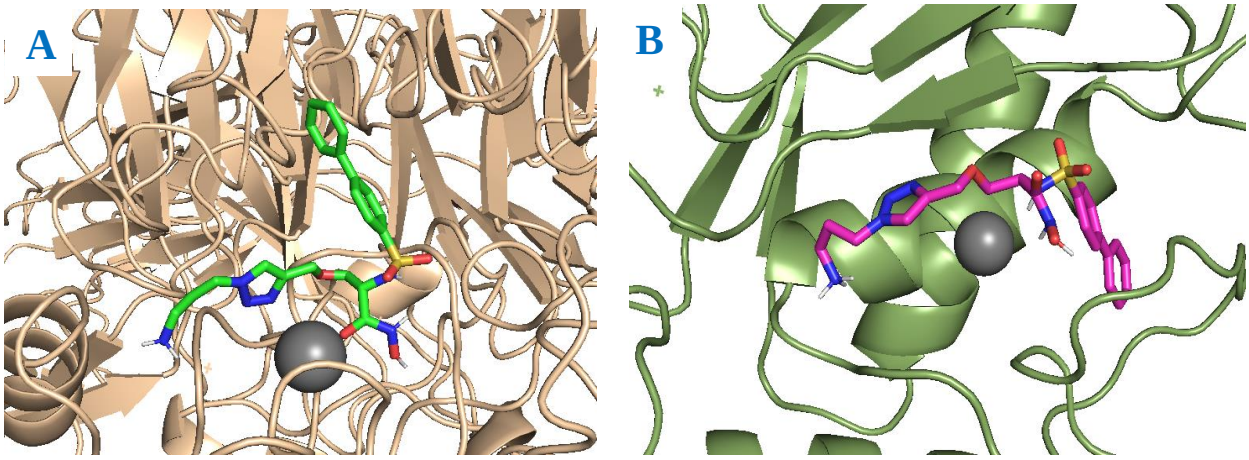

Figure 1 – Molecular Docking studies of a selected ligand toward $\alpha_v\beta_3$ Integrin receptor (Figure A) and MMP-2 (Figure B)

Recently, we developed *L*-tyrosine-derived dual RGD integrin/MMP ligands [2], that demonstrated the similarity of the active sites of these two proteins and the possibility of targeting both with a ligand possessing three main Functional Groups (FGs): a chelating FG for interacting with the metal ion, a basic FG for establishing a salt bridge with acidic amino acid residues, and a hydrophobic moiety for penetrating a lipophilic pocket. In this view, we reasoned exploiting the click chemistry for synthesizing novel compounds as dual RGD integrin/MMP ligands possessing the aforementioned interacting FGs and the triazole ring as central scaffold, resulting in the identification of a serine-derived compound with significant bioactivity.

References

[1] S. Quintero-Fabián, R. Arreola, E. Becerril-Villanueva, J.C. Torres-Romero, V. Arana-Argáez, J. Lara-Riegos, M.A. Ramírez-Camacho, M.E. Alvarez-Sánchez. Role of Matrix Metalloproteinases in Angiogenesis and Cancer. *Front. Oncol.* **2019**, 9, 1370.
[2] L. Baldini, E. Lenci, F. Bianchini, A. Trabocchi. Identification of a Common Pharmacophore for Binding to MMP2 and RGD Integrin: Towards a Multitarget Approach to Inhibit Cancer Angiogenesis and Metastasis. *Molecules*. **2022**, 27, 1249.

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Surface Plasmon Resonance biosensor to detect and quantify drug and anti-drug antibodies in patients treated with the therapeutic monoclonal antibody Adalimumab

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Keywords: biotherapeutic, Adalimumab, anti-drug antibodies, surface plasmon resonance, biosensor

Adalimumab (ADL) is a Tumor Necrosis Factor α (TNF-α)-blocking monoclonal antibody approved for the treatment of autoimmune diseases, *e.g.*, Non-infectious Uveitis, Juvenil Idiopathic Arthritis, and Rheumatoid Arthritis. The occurrence of anti-Adalimumab antibodies (ADAbs) in treated patients is reported at a rate up of 8% after 8 weeks and 24% after 60 weeks, despite ADL is a fully human antibody. The presence of such type of ADAbs is supposed to be correlated with treatment failure and adverse effects.[1,2] Although different studies demonstrated a correlation between ADAbs presence and higher disease activity, the comparison among these studies is challenging because of the different ADAbs detection assays used.[3,4] The methodologies employed may break down at the lower end of the detectable range and, consequently, the development of more sensitive drug-tolerant assay is fundamental to evaluate the correlations between ADAbs and disease outcome, allowing the personalization of the therapy depending on each patient response to the treatment, leading to benefits both for patient's health and at the financial level.[5]
With this study we propose a method to measure and quantify independently ADL and ADAbs using a Surface Plasmon Resonance (SPR)-based optical biosensor, which allows to measure in real-time mass variations following the binding of the analyte flowing onto a biochip surface bearing an immobilized ligand. Particularly, the immobilization of Adalimumab on the chip surface allowed the direct quantification of ADAbs in patients' sera, without any particular sample preparation procedure. Moreover, the immobilization of an anti-ADL monoclonal antibody made it possible to directly detect and quantify the free ADL in sera samples, another key factor in the evaluation of the therapeutic treatment efficacy. Both these methods were optimized to overcome relevant problem caused by the presence of non-specific binding of the sera samples, which was addressed using an irrelevant antibody as blank, thus completely removing the non-specific signal without affecting the specific response. Another main issue to be solved was the correct chip regeneration after each analysis, in order to reuse the same chip for a large number of samples. This crucial step was accomplished through a first injection at low pH, followed by two injections at high pH.
The optimized methods for ADL and ADAbs were successfully validated, testing positive and negative control sera previously characterized using a commercial ELISA kit. The data obtained show a good agreement between ELISA and SPR in both Adalimumab and anti-Adalimumab antibodies detection and quantification, opening the door to a possible routine use of SPR-based biosensor for disease monitoring in clinical practise.

References

[1] van Schouwenburg, P. A.; van de Stadt, L. A.; de Jong, R. N.; van Buren, E. E. L.; Kruithof, S.; de Groot, E.; Hart, M.; van Ham, S. M.; Rispens, T.; Aarden, L.; Wolbink, G. J.; Wouters, D. *Ann Rheum Dis* **2013**, 72 (1)

[2] Imagawa, T.; Takei, S.; Umebayashi, H.; Yamaguchi, K.; Itoh, Y.; Kawai, T.; Iwata, N.; Murata, T.; Okafuji, I.; Miyoshi, M.; Onoe, Y.; Kawano, Y.; Kinjo, N.; Mori, M.; Mozaffarian, N.; Kupper, H.; Santra, S.; Patel, G.; Kawai, S.; Yokota, S. *Clin Rheumatol* **2012**, 31 (12), 1713–1721
[3] Bloem, K.; van Leeuwen, A.; Verbeek, G.; Nurmohamed, M.; Wolbink, G.; van der Kleij, D.; Rispens, T, *J Immunol Methods* **2015**, 418, 29-38
[4] Rusche, H.; Marrani, E.; Real-Fernandez, F.; Ponti, R.; Terzani, F.; Maccora, I.; Monasson, O.; Mastrolia, M.V.; Peroni, I.; Pagnini, I.; Cimaz, R.; Papini, A.M.; Simonini, G.; Rovero, P. *Sci Rep* **2021**, 11, 16393.
[5] Garcês, S.; Demengeot, J.; Benito-Garcia, E. *Ann Rheum Dis* **2013**, 72 (12), 1947–1955.

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Lipid mesophases-based beads as new platform for sustained release of TOFA

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Keywords: TOFA, sustained release, lipid mesophases, beads

Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affects joints, causing inflammation, pain, fatigue and - over time - joint damage. There is no cure for this disease, but early treatment with certain drugs can improve the long-term outcome. Tofacitinib citrate (TOFA, Figure 1A), taken as tablets, is a Janus kinase inhibitor that works on the immune system, and it's commonly prescribed to treat RA, although severe adverse effects such as an increased risk of viral infection, deep vein thrombosis, pulmonary embolism, and anemia have been reported[1].
To reduce the side reactions caused by systemic administration of TOFA, drug delivery systems may be used: they can extend the bioavailability and the duration of action of a drug. Among all the different classes of drug carriers, lipid mesophases show unique properties. They form when lipid molecules are mixed with aqueous solvents. Different temperature and water compositions lead to the formation of different geometries[2]: lamellar phase (consisting of flat lipid bilayers), bicontinuous cubic phases (consisting of a continuous lipid bilayer, which subdivides space into two interpenetrating, but not connected, water networks), inverted hexagonal phase (consisting of hexagonally packed water channels surrounded by lipid) and inverse micellar phase (consisting of 3D packings of discrete inverse micellar aggregates).
In order to provide a drug delivery system that could be locally administered, we prepared lipid mesophases-based beads loaded with different percentages of TOFA. We characterized them with small-angle X-ray scattering (SAXS) kinetics (Figure 1B), wide-angle X-ray scattering (WAXS), rheology, differential scanning calorimetry (DSC) and microscopy and we evaluated the release profile of the drug. We also added Vitamin E to the formulation, forming different structures with lower diffusion coefficient and more sustained release profile. At the end, we proved that the beads could be injected using an 18G needle.

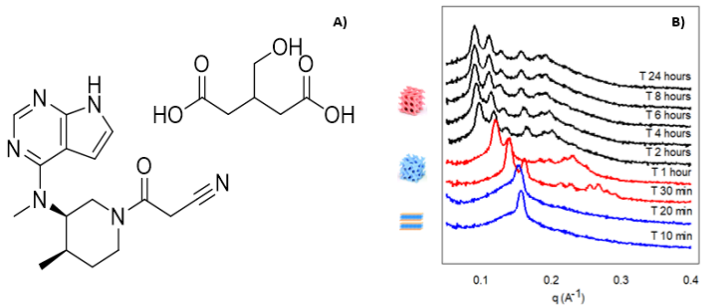

Figure 1: A) TOFA structure and B) kinetic profile of the beads

References

[1] Molander, V.; Bower, H.; Frisell, T.; Delcoigne, B.; Di Giuseppe, D.; Askling, J. Venous Thromboembolism with JAK Inhibitors and Other Immune-Modulatory Drugs: A Swedish Comparative Safety Study among Patients with Rheumatoid Arthritis. *Ann. Rheum. Dis.* **2022**, *82* (2), 189–197. https://doi.org/10.1136/ard-2022-223050.
[2] Aleandri, S.; Mezzenga, R. The Physics of Lipidic Mesophase Delivery Systems. *Phys. Today* **2020**, *73* (7), 38–44. https://doi.org/10.1063/PT.3.4522.

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Ruthenium (II) metal complexes: synthesis and binding with mitochondrial G-quadruplex DNA

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Keywords: ruthenium complexes, G-quadruplexes, mitochondrial targeting, anti-cancer agents

Recently, ruthenium-based anticancer drugs have been making their way into clinical trials, unveiling exceptionally promising outcomes. Ruthenium complexes offer diverse possibilities in their mode of action and targets. Some ruthenium compounds, including the ones bearing polypyridyl ligands, localize to mitochondria and trigger apoptosis or necrosis in cells.[1] Indeed, mitochondria play a critical role in cellular metabolism, and the dysfunctions of this organelle have been closely associated with several hallmarks of cancer. In this context, G-quadruplexes (G4) are secondary DNA structures involved in different biological processes, such as transcription, translation, genome instability and cancer. G4s are overrepresented in significant portions of human genome, such as oncogene promotors and telomeres.[2] Lately, however, it has been reported that G4 formation is possible also in mitochondrial DNA (mtDNA), suggesting a possible regulation role.[3] Metal complexes containing dipyrido[3,2-a:2',3'-c]phenazine (dppz) ligand can interact with nuclear G4s in vitro.[4] Additionally, the incorporation of the triphenyl phosphonium moiety to metal complexes has been associated to specific targeting of the mitochondria. In order to assess the potential impact of G4 interacting compounds with mtDNA G4s, we synthesized Ru(II) complexes of the type [Ru(bipy)2(L)] and [Ru(phen)2(L)], where L stands for a dppz moiety functionalized with a triphenyl phosphonium salt (Fig.1). Furthermore, considering the promising anticancer properties of metal complexes of oxadiazole ligands,[5] we decided to investigate also the G4 interaction of new complexes of 1,2,4-oxadiazoles ligands with Ru(II), still unexplored in the literature. We tested the interaction of the novel synthesized metal complexes with mitochondrial G4s sequences using spectroscopical techniques such as UV-Vis, fluorescence, circular dichroism and FRET.

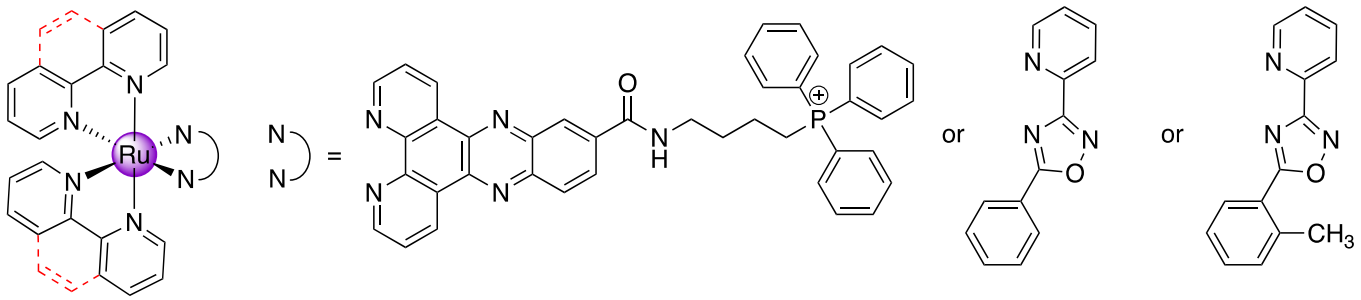

Figure 1: Structure of the newly synthesized ruthenium compounds with the general formula [Ru(bipy)2(L)] and [Ru(phen)2(L)].

References

[1] L. Zeng et al. The development of anticancer ruthenium(II) complexes: from single molecule compounds to nanomaterials. Chem. Soc. Rev., 2017, 46, 5771.
[2] D.Varshney et al. The regulation and functions of DNA and RNA G-quadruplexes. Nat Rev Mol Cell Biol. 2020, 21, 459–474.
[3] V.J. Sahayasheela et al. Mitochondria and G-quadruplex evolution: an intertwined relationship. Trends in Gen. 2023, 39, 15-30.
[4] K. McQuaid et al. Three thymine/adenine binding modes of the ruthenium complex Λ-[Ru(TAP)$_2$(dppz)]$^{2+}$ to the G-quadruplex forming sequence d(TAGGGTT) shown by X-ray crystallography. Chem. Commun., 2019,55, 9116-9119.
[5] G. Salassa et al. Metal Complexes of Oxadiazole Ligands: An Overview. Int. J. Mol. Sci., 2019, 20(14), 3483

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Novel dimolybdenum paddlewheel complexes bearing NSAID ligands. Synthesis, characterization and possible biological applications

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Keywords: Bioinorganic Chemistry; Dimolybdenum; Anti-Inflammatory; Anticancer

Multinuclear complexes are a class of metal compounds endowed with interesting potential for biological applications. The additive effect of the individual adjacent metal centres can lead to reactivity patterns not commonly observed with mononuclear species. In particular, the M_2L_4-type paddlewheel dinuclear complexes having monoanionic bridging ligands, are featured by the simplest metal cluster motif. In the last decades molybdenum has been extensively studied regarding the formation of multiple-bonded M_2^{n+} compounds, due to the complexes' unique scaffold, redox behavior, spectroscopic properties as well as catalytic applications. Its biological properties, however, are much less explored and only sporadic studies have been carried out.[1] In general, there are only a few examples of dimetallic paddlewheel complexes with biological active molecules as ligands. Among the most interesting there are certainly the diruthenium(II,III) paddlewheel complexes bearing non-steroidal anti-inflammatory drugs (NSAID), that together with the complex $[Ru_2(EB776)_4Cl]$ (recently synthesized and studied by us), represent a series of dimetallic metallodrugs with a promising anticancer activity.[2] Coordinating active molecules to a metal scaffold is a widely used strategy to create effective dual drugs.[3] Hence, in this work, we synthesized a series of novel dimolybdenum paddlewheel complexes with formula $Mo_2(\mu\text{-}O_2CCR)_4$ bearing different NSAID ligands (Figure 1). The products were extensively characterized using NMR, IR and Raman spectroscopy, and their stability was studied using both an experimental and computational approach. Finally, their anticancer activity, as well as their anti-inflammatory properties, have been preliminary assessed, together with the interaction with proteins. Overall, interesting results emerged for this family of unconventional compounds, both from a biological and mechanistic point of view.

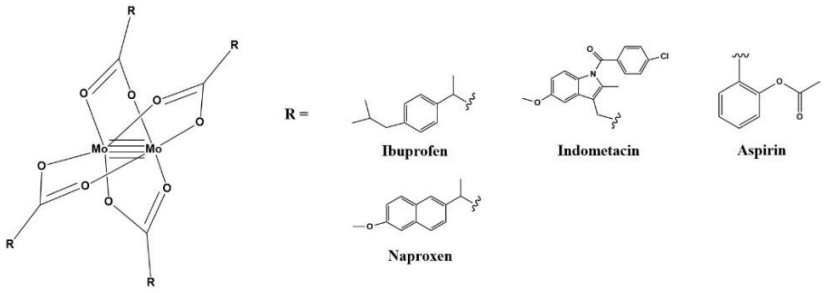

Figure 2 *Structures of the Mo2(μ-O2CCR)4 complexes synthesized in this work.*

References

[1] A.T. Odularu, P.A. Ajibade, J.Z. Mbese. *Bioinorganic Chemistry and Applications*, 2019, vol. 2019, 1-9.
[2] E. Barresi, I. Tolbatov, A. Pratesi, V. Notarstefano, E. Baglini, S. Daniele, S. Taliani, N. Re, E. Giorgini, C. Martini, F. Da Settimo, T. Marzo, D. La Mendola, *Dalton Trans.,* 2020, 49, 14520-14527.
[3] A. K. Renfrew, *Metallomics*, 2014 vol.6,8, 1324–1335.

AMYC BIOMED 2023

Exploring the impact on metabolism of ovarian cancer treatment with gold-based drugs using a context-specific genome-scale metabolic model

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Keywords: Genome scale metabolic model (GEM), epithelial ovarian cancer, gold-based drugs

A genome scale metabolic model (GEM) is a mathematical formalization of metabolism. There are several applications such as analyzing the organism's metabolism under different conditions by simulating the metabolic fluxes and the growth rate. In this work, we used such approach to study the response of ovarian cancer metabolism to gold-based drugs treatments. First, we created a A2780-cell line specific GEM by pruning the reactions present in the reference human metabolic reconstruction (Recon3D) according to transcriptomic data retrieved from the cancer cell line encyclopedia (CCLE). Afterwards, we integrated such reconstruction with metabolomic data (from nuclear magnetic resonance spectroscopy) to create condition-specific models and identified possible gold-based drugs targets. Together with the classical flux balance analysis (FBA) approach, we used random flux sampling analysis that ensure to possibility not to assume a specific objective function (i.e. the most likely cellular target in a given condition) during simulations. This approach led us to explore the feasible flux solutions in the metabolic network by generating the probability distributions of steady-state reaction fluxes, resulting in a wide landscape of the metabolic network before and after each treatment. As a results, we depicted the most likely metabolic rearrangements of ovarian cancer cells following the exposure to gold-based drugs. Such predictions will be used to elucidate the mechanistic details of the action of these drugs on cancer cells and, consequently, find strategies to improve their actions *in vivo.*

References

[1] Nobili S, Mini E, Landini I, Gabbiani C, Casini A, Messori L. Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. *Med Res Rev*. 2010;30(3):550-580. doi:10.1002/med.20168

AMYC BIOMED 2023

Reactions of Auranofin and Medicinal Gold(I) Compounds with Human Thioredoxin Reductase through ESI-Q-TOF MS

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Keywords: Auranofin**,** Anticancer Compounds, Thioredoxin Reductase, Mass Spectrometry

Owing to the great success of Pt-based drugs in anticancer chemotherapy, the study of metal complexes has gained a lot of interest in view of the discovery of innovative antitumor agents. Gold complexes form an interesting class of medicinal compounds of potential application for cancer treatment. The interactions of gold compounds with nucleic acids are relatively weak; currently, it is known that the main targets of anticancer Au(I) complexes are proteins. Owing to the high affinity of gold toward sulfur and selenium, gold complexes can strongly react with the activated cysteine or selenocysteine residues of enzymes by Au-S/Se bond formation [1]. For instance, it was demonstrated that thioredoxin reductase is an effective target for gold(I) compounds upon gold coordination to active site selenocysteine [2]; numerous interactions between cysteine residues and gold complexes via Au-S bond were documented by ESI-MS [3]. However, the precise modes of action of gold compounds remain largely unexplored.
One of the most studied gold complexes is Auranofin; it consists of a gold(I) center linearly coordinated to a triethylphosphine and a tetracetylthioglucose ligand. AF behaves like a typical prodrug that requires chemical activation, i.e. the release of the thiosugar moiety, to perform its biological actions. Accordingly, the ESI-MS approach has been applied to a small panel of experimental and clinically established anticancer gold(I) compounds i.e. Auranofin, its analogues, Aurothiomalate, Au(NHC)Cl and the corresponding bis-carbene complex $[Au(NHC)_2]PF_6$ (where NHC is a N-heterocyclic carbene ligand). Several different experimental conditions were tested to obtain detailed information on the binding mode between the selected Au compounds and h-TrxR.
Generally, the analysis of the obtained mass spectra revealed the formation of stable adducts between gold compounds and h-TrxR and allowed us to characterize the nature of the different metal fragments that bind the protein. Overall, the present results set the stage to better understand the mechanism of action of these gold compounds and elucidate at the atomic level their mechanism of interaction with human thioredoxin reductase.

References

[1] D. Krishnamurthy, MR. Karver, E. Fiorillo, V. Orrú, SM. Stanford, N. Bottini, M. Barrios. Gold(I)-mediated inhibition of protein tyrosine phosphatases: a detailed in vitro and cellular study. J. Med. Chem, 2008, 51, 4790–4795.
[2] A. Bindoli, MP. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori. Thioredoxin reductase: A target for gold compounds acting as potential anticancer drugs. Coor. Chem. Rev., 2009, 253(11-12), 1692-1707.
[3] C. Zoppi, L. Messori, A. Pratesi. ESI MS studies highlight the selective interaction of Auranofin with protein free thiols. Dalton Trans, 2020, 49(18), 5906-5913.

AMYC BIOMED 2023

NMR of Paramagnetic Proteins: New Routes for ^{13}C Direct Detection in Challenging Iron-Sulphur Proteins

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Keywords: solution NMR, biomolecular NMR, theory and methods, biomolecules, iron-suphur proteins

For Iron-Sulfur Proteins, the robustness of NMR coherence transfer in proximity of the cluster depends on the relaxation properties of the nuclei involved, therefore recording the same experiment with different pulse schemes or different parameter sets provides often complementary results. The integrative effect of ^{1}H start and ^{13}C start CACO experiments will be discussed in practice attending a non-canonical sequence-specific assignment of revived Cisd3[1] C^{α}/C' correlations in the spectra. Additionally, in paramagnetic metalloproteins, longitudinal relaxation rates of $^{13}C'$ and $^{13}C^{\alpha}$ nuclei can be measured using ^{13}C detected experiments and converted into electron spin-nuclear spin distance restraints (Figure 1), also known as Paramagnetic Relaxation Enhancements Restraints (PRE). We will discuss the complementarity of ^{13}C PRE restraints with ^{1}H PRE restraints in the case of the High Potential Iron Sulfur Protein (HiPIP) PioC[2] and how ^{13}C R_1 values can be measured also at very short distances from the paramagnetic center. The obtained set of ^{13}C based restraints can be added to ^{1}H PREs and to other classical and paramagnetism based NMR restraints.

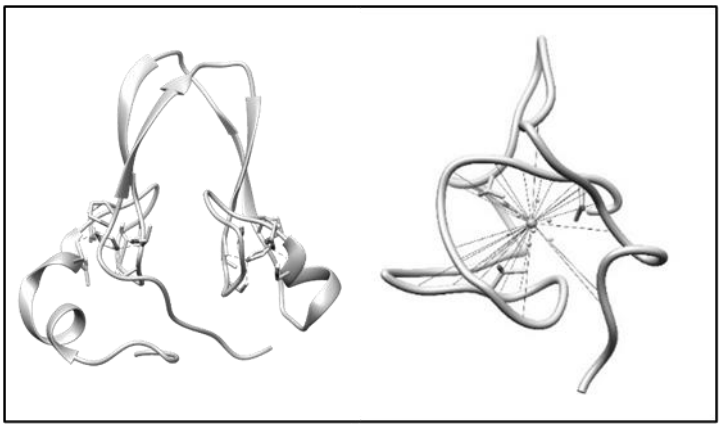

Figure 1: 3D structure of Cisd3 (right) and PioC (left). Lines in PioC structure represent C'/C^{α}-to-metal distances calculated from longitudinal relaxation rates, R_1

References

[1] Querci, L.; Grifagni, D.; Trindade, IB.; Silva, JM.; Louro, RO.; Cantini, F.; Piccioli M. Paramagnetic NMR to study Iron-Sulphur Proteins: the revival of signals in ^{13}C detected experiments. (Submitted)
[2] Querci, L.; Trindade, I. B.; Invernici, M.; Silva, J. M.; Cantini, F.; Louro, R. O.; Piccioli, M. NMR of Paramagnetic Proteins: 13C Derived Paramagnetic Relaxation Enhancements Are an Additional Source of Structural Information in Solution. *Magnetochemistry* **2023**, *9* (3), 66.

AMYC BIOMED 2023

Bioorganometallic platinum(II)-peptide nucleic acid conjugates

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Keywords: peptide nucleic acids, platinum complex, bioorganometallic compounds, photodynamic therapy

Peptide nucleic acids (PNAs) are mimics of natural nucleic acids able to target complementary DNA or RNA strands with high sequence specificity and affinity, and are therefore potential excellent candidates in diagnostics, antisense and antigene therapy.[1] In place of the ribose phosphodiester backbone of DNA and RNA, PNAs contain a pseudopeptide backbone, composed of *N*-(2-aminoethyl)glycine units, on which the four nucleobases are inserted. PNAs display high chemical and enzymatic stability towards nucleases, but unmodified PNAs often exhibit low cellular uptake and this feature constitutes a drawback towards its effective use in therapy. One of the strategies to overcome this problem is the conjugation of PNA to metal complexes that can modify their intrinsic physico-chemical and spectroscopic properties.[2] In our ongoing studies on PNAs, we have focused our attention on the development of novel bioorganometallic metal-PNA conjugates that can be employed as dual activity agents for the synergic treatment of cancer or bacterial infections, combining antisense therapy based on PNAs and photodynamic therapy (PDT) related to the use of the metal complex as the photosensitizer able to generate cytotoxic singlet oxygen ($^1O_2$) under appropriate excitation light.[3] Among others, Pt(II)complexes with a cyclometallated terdentate ligand of the NCN type which affords the platinum center a rigid coordination environment represent promising photosensitizers, being the most luminescent Pt emitters in solution at room temperature. In this communication we will report our first studies on the innovative bioorganometallic metal-PNA conjugates formed by model PNA sequences covalently linked to Pt(II)- chloride complexes with a 1,3-di(2-pyridyl)benzene ligand, (**Figure 1**). The synthetic strategies to prepare these innovative PNA-conjugates will be described along with their photophysical characterization including absorption and emission studies.

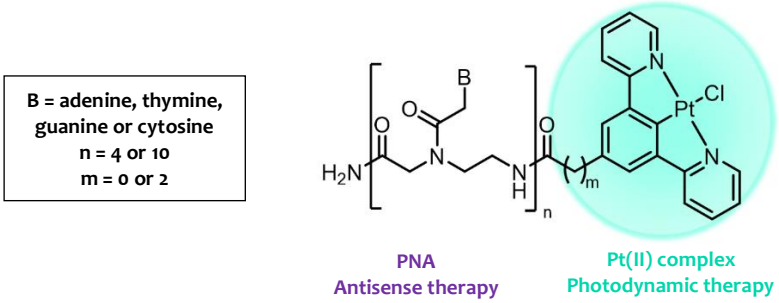

Figure 1: *PNA conjugated to luminescent cyclometalated Pt(II) complex*

References

[1] P. E. Nielsen, M. Egholm, R. H. Berg, O. Buchardt. Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science*, **1991**, *254*, 1497–1500.
[2] S. Cauteruccio, M. Panigati, L. Veronese, N. Zaffaroni, M. Folini, E. Licandro. Luminescent dinuclear rhenium(I)–PNA conjugates for microRNA-21 targeting: Synthesis, chemico-physical and biological characterization. *J. Organomet. Chem.* **2019**, *887*, 32-39.

[3] Y. Wu, S. Li, Y. Chen, W. He, Z. Guo. Recent advances in noble metal complex based photodynamic therapy, *Chem. Sci.* **2022**, 13, 5085-5106.

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Poly(diglycerol adipate) Variants as Enhanced Nanocarrier Replacements in Drug Delivery Systems

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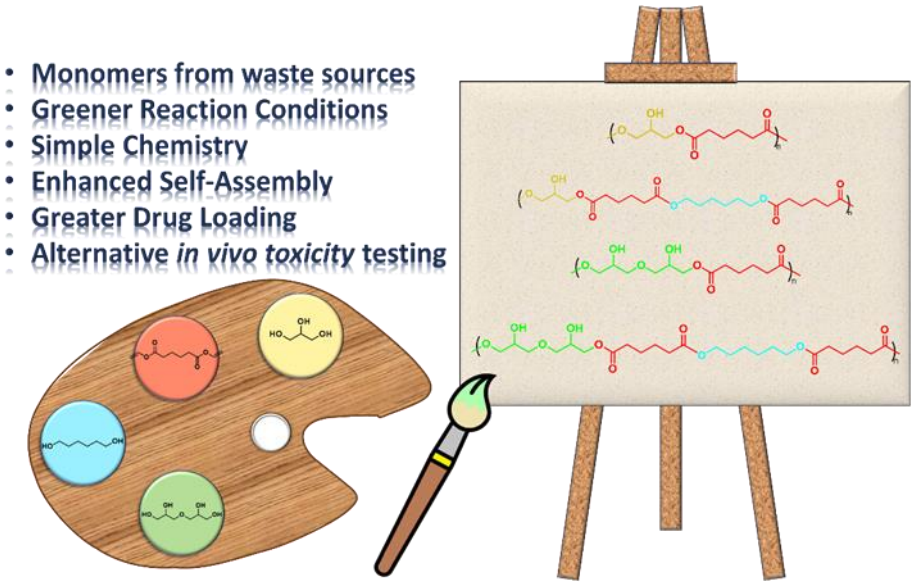
Keywords: Drug-delivery, biomaterials, polyesters, nanoparticles, *in vivo*



Sustainably derived poly(glycerol adipate) (PGA) exhibits the key features expected in a polymeric scaffold for drug-delivery. It is biodegradable, biocompatible, capable of self-assembly into nanoparticles (NPs) and has a functionalisable pendant group. Despite exhibiting these advantages over commercial alkyl polyesters, PGA suffers from drawbacks caused by poor amphiphilic balance leading to weak drug-polymer interactions, low drug-loading in NPs, and low NPs stability. In this work, we demonstrate the modification of PGA in a one-pot reaction, introducing new functionalities and stabilising groups into the polymer backbone, maintaining mild reaction conditions.[1]

The 1,6-*n*-hexanediol (Hex) modified polymer demonstrated excellent self-assembling ability into NPs, enhanced drug encapsulation and improved stability in a range of media. Glycerol was replaced with the more hydrophilic diglycerol to investigate the effect of the modified amphiphilicity on the self-assembling ability of the polymer. The synergistic effect of combining both Hex and diglycerol has been investigated and compared with unmodified PGA based polyesters. Poly(diglycerol adipate) (PDGA) showed improved water solubility and diminished self-assembling ability, while the Hex variation (PDGAHex) demonstrated enhanced features as a nanocarrier. PDGAHex NPs were tested for their stability in different environments and for their ability to enhance drug loading. Moreover, these materials have shown good

biocompatibility in both *in vitro* and *in vivo* experiments. Finally, Coumarin-6 encapsulated PDGAHex NPs were used to track uptake in vivo, in nematodes, showing successful coloured-NPs delivering across *C. elegans* intestinal membrane.[2]

References

[1] P. L. Jacob, L. A. Ruiz Cantu, A. K. Pearce, Y. He, J. C. Lentz, J. C. Moore, F. Machado, G. Rivers, E. Apebende, M. R. Fernandez, I. Francolini, R. Wildman, S. M. Howdle and V. Taresco, Polymer, 2021, **228**, 123912.
[2] P. L. Jacob, B. Brugnoli, A. Del Giudice, H. Phan, V. M. Chauhan, L. Beckett, R. B. Gillis, C. Moloney, R. J. Cavanagh, E. Krumins, M. Reynolds-Green, J. C. Lentz, C. Conte, V. Cuzzucoli Crucitti, B. Couturaud, L. Galantini, I. Francolini, S. M. Howdle and V. Taresco, J. Colloid Interace Sci., 2023, **641**, 1043–1057.

AMYC BIOMED 2023

Engineered poly(lactide-co-glycolide) nanoparticles for co-delivery of antigens and immune adjuvants to the respiratory mucosa

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Keywords: Mucosal vaccination, Immunization, Nanoparticles, Lung

Mucosal vaccination *via* the respiratory tract could be an interesting strategy for both preventive and therapeutic immunization [1]. In fact, it allows to induce high immune response locally and systemically as well as a good activation of B- and T-cells [1]. To successfully exploit these advantages, an effective design of vaccine formulations is needed since the immunization of respiratory mucosa is still hampered by a number of limitations imposed by the administration route [2]. Our research group developed inhalable nanoparticles (NPs) composed of poly(lactic-co-glycolic) acid (PLGA) as a platform for the pulmonary delivery of a model antigen, namely ovalbumin (OVA). A single-stranded microbial DNA fragment with unmethylated deoxycytidylyldeoxyguanosine dinucleotide (CpG) motifs was co-incapsulated as vaccine adjuvant [3]. An in-depth formulation study was carried out engineering the NPs surface with different moieties: 1,2 distearoil-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG), poly(ethylene glycol) (PEG) and monophosphoryl lipid A (MPLA). The last is a well-known TLR4 agonist, used as vaccine adjuvant to stimulate a strong Th1 response and to enhance an antigen-presenting cell maturation [4]. When needed, polyethylenimine (PEI), a cationic polymer with proved immune-activating activity, was added to impart a positive charge to the NPs. In so doing, a panel of OVA/CpG-loaded NPs was produced and fully characterized in terms of size (D_H), PDI and ζ-potential. All the NPs were homogeneously dispersed with a size lower than 250 nm and displayed negative/positive surface charge depending on composition. To gain more information related to the architecture of the NPs, specific analytical assays were carried out, including the quantitation of PEG by enzymatic immunosorbent assay, MPLA by a Chromogenic Endotoxin Quant-IT kit and PEI by complexation with copper (II). The entrapment efficiency of OVA and CpG was evaluated and resulted respectively around 70% and 95% of the amounts initially added to the formulation. The ability of the NPs to slowly release the entrapped payloads was verified, as well. Optimized formulation showing the most promising technological features underwent *in vitro* uptake studies and antigen presentation tests on murine dendritic cells. Meanwhile, their pro-inflammatory potential was evaluated on human monocytes isolated from the peripheral blood of healthy donors. Preliminary results highlight the potential of the developed approach to successfully deliver antigens to the respiratory mucosa.

References

[1] Hellfritzsch M, Scherließ R. Mucosal Vaccination via the Respiratory Tract. *Pharmaceutics*. 2019, 11(8):375.
[2] Liang W, Pan HW, Vllasaliu D, Lam JKW. Pulmonary Delivery of Biological Drugs. *Pharmaceutics*. 2020, 12(11):1025.
[3] Fransen, F., C. J. Boog, J. P. van Putten, and P. van der Ley. 'Agonists of Toll-like receptors 3, 4, 7, and 9 are candidates for use as adjuvants in an outer membrane vaccine against Neisseria meningitidis serogroup B', *Infect Immun*, 2007, 75: 5939-46.
[4] Rhee, E. G., R. P. Kelley, I. Agarwal, D. M. Lynch, A. La Porte, N. L. Simmons, S. L. Clark, and D. H. Barouch. 'TLR4 ligands augment antigen specific CD8+ T lymphocyte responses elicited by a viral vaccine vector', *J Virol*, 2010, 84: 10413-9.

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Vesicular nanosystems for the transdermal delivery of mangiferin to counteract the O_3-induced cutaneous damage

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Keywords: human skin, vesicular nanosystems, air pollutants, Ozone, OxInflammation

Human skin is everyday exposed to a variety of environmental insults as air pollutants. Exposure to these toxic agents can promote an alteration in skin homeostasis in term of oxidative stress and inflammatory reactions (OxInflammation), that can lead to the onset of a variety of skin conditions. Thus, in the past years, the use of natural antioxidants and anti-inflammatory compounds has been employed to counteract the cutaneous damage induced by environmental pollutants. However, the use of these compounds to prevent skin disorders is limited by their low solubility and stability as well as by the particular structure of the skin. Thus, the present work aimed to develop new formulative strategies to better vehicle natural antioxidant compounds as mangiferin, within the skin tissue. Here we describe a formulative study for the development of vesicular systems for mangiferin delivery, based on phosphatidylcholine and the block copolymer pluronic. Plurethosomes were designed for mangiferin transdermal administration and compared to ethosome and transethosome. Particularly, the effect of vesicle composition was investigated on size distribution, inner and outer morphology by photon correlation spectroscopy, and transmission electron microscopy. The potential of selected formulations as vehicles for mangiferin was studied, evaluating encapsulation efficiency and in vitro diffusion parameters by Franz cells. Moreover, the rheological performance of plurethosomes was investigated. Moreover, the antioxidant and anti-inflammatory potential effects of mangiferin against pollutants was evaluated on 3D reconstructed human epidermis models (RHE) exposed to ozone (O3), one of the most toxic pollutants agents. We found that plurethosomes and transethosomes loaded with mangiferin could protect the cutaneous oxidative (4-hydroxynonenal) and Inflammatory (IL-1beta cytokine release) damage induced by O3 exposure in RHE , suggesting that they represent good transdermal delivery system to enhance the delivery of natural compounds within the cutaneous tissue and protect from the pollutants induced skin damage.

AMYC BIOMED 2023

Preliminary approaches to detect targets enclosed by the lipid bilayer via electrochemical reading

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Keywords: bilayer lysis, liposomes, electrochemical detection, liquid biopsy, biosensor

Extracellular vesicles are a population of nanosized vesicles enclosed by a lipid bilayer secreted into the extracellular matrix by all living cells. Exosomes (Exos) represent the smallest group of this vesicular population (30-150 nm) and originate in the endosomal pathway forming late endosomes or multivesicular bodies. Exos have an emerging role in medicine as they have proven to be excellent biomarkers reflecting the state of the cell of origin, containing a wide range of bioactive molecules such as nucleic acids, proteins, and lipids[1], [2]. Tumor-derived Exos enrich all human biofluids in large quantities, therefore their identification and determination are very promising for the development of liquid biopsy techniques for cancer diagnosis and prognosis. Effective and rapid techniques to identify biomolecular contents within Exos are still needed to translate detection into clinical practice. Some issues hinder the application of Exos in clinical medicine, such as the need for standardized protocols to lyse exosomes and portable platforms to reveal their contents quickly and easily. Effective lysis protocols are needed to distinguish between markers present inside and outside the membrane by breaking down the lipid bilayer membrane allowing for more precise and accurate characterization of Exos[3].
Although numerous efforts have been made to build a device capable of detecting Exos, creating a platform capable of lysing them and determining their contents still remains a challenge.
Here we discuss a proof of concept towards the establishment of an integrated electrochemical platform that would be able to break exosomes and to analyze specific markers that are released. Lysis of Exos is a crucial step that could compromise the detection of sought biomarkers or create false positives. To overcome this criticality, we first examined the possibility of membrane disruption using various liposomal nanoparticles that mimicked the structure of the Exos, using electrochemical mediators as the target analyte to be detected via electrochemical reading. Various approaches have been evaluated, both investigating liposomes' composition, lysis protocol, electrochemical technique and electrochemical mediators used as the model target to be recognized. The preliminary results highlight the complexity of the entire system, but they represent the starting point towards the development of integrated and portable devices to be applied towards exosomal characterization.

References

[1] S. Romanò *et al.*, 'Label-free spectroscopic characterization of exosomes reveals cancer cell differentiation', *Anal Chim Acta*, vol. 1192, p. 339359, 2022.
[2] S. Romanò *et al.*, 'Fourier Transform Infrared Spectroscopy as a useful tool for the automated classification of cancer cell-derived exosomes obtained under different culture conditions', *Anal Chim Acta*, vol. 1140, 2020.
[3] S. Romanò and S. Cinti, 'Extracellular Vesicles lysis: a guide for the release of biomarkers to be used in cancer

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NMR-based investigation of intrinsically disordered regions of modular proteins for tailored design of interacting peptides

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Keywords: Modular proteins, peptides, solution NMR titrations

Several RNA-binding proteins (RBPs) are characterized by modular structures encompassing folded domains and intrinsically disordered regions (IDRs). Investigating the role of these domains and their possible crosstalk is crucial for understanding protein function and possible strategies to interfere with it.
The Nucleocapsid protein (N) of SARS-CoV-2 is a pivotal example of RBP. It is one of the most important proteins encoded by the virus involved in viral genome packaging and facilitating its replication inside the host cell. Interestingly, the N protein has recently been proposed as an alternative drug target because it is characterized by a low rate of mutations. Its complex structure encompasses two folded domains and three IDRs. In particular, the globular N-terminal domain (NTD) is responsible for the viral RNA interaction and the two flanking IDRs play an important synergic role [1].
The aim is the design and synthesis of peptides able to interfere with its function, monitoring the interaction through solution NMR titrations. Five rationally designed peptide sequences have been synthesized to understand the residues and/or conformational motifs essential for the interaction with the protein target. This collection of peptides has been tested by NMR titrations (^{1}H-^{15}N HSQC experiments) to identify the sequence displaying the highest affinity with NTD. The most promising one has been finally used to monitor the interaction with the NTR protein construct, which includes two IDRs flanking the globular NTD, and to evaluate a possible enhanced interaction.
The final aim is to develop de novo designed antiviral peptides able to displace the nucleic acid from the protein, to be further stabilized *in vivo* by innovative stapling synthetic strategies [2].

References

[1] Pontoriero, L.; Schiavina, M.; Korn, S.M.; Schlundt, A.; Pierattelli, R.; Felli, I.C. Biomolecules **12**, 1 (2022).
[2] Quagliata, M.; Stincarelli, M.A.; Papini, A.M.; Giannecchini, S.; Rovero, P.. ACS Omega (2023), DOI: 10.1021/acsomega.3c01436.

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Towards the total synthesis of pentacaronic acid

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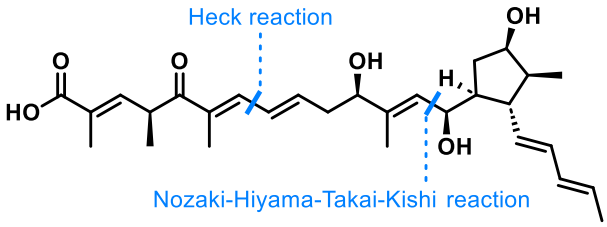
Keywords: pentacaronic acid, polyketide, total synthesis

Polyketides are a class of acetate derived natural compounds exhibiting a wide range of functional and structural diversities. These natural products can be isolated from diverse microorganisms and they show antibiotic, anticancer, antifungal, antiparasitic and immunosuppressive properties. [1] In the past few years, an attempt of isolation of natural compounds from myxobacteria allowed to isolate pentacaronic acid (Figure 1) from *Sorangium cellulosum*. This polyketide has a 24 carbon atoms skeleton featuring 6 *E*-configured double bonds and 7 stereocenters, 5 of which are contiguous, delimiting a 5 member ring. The absolute configurations of each stereocenter have been defined except for the one in C4, which is assumed to be S-configured, due to the structural similarity between pentacaronic acid and maltepolide E. [2] The synthesis of this compound reckon on the coupling of 3 major chiral fragments: the eastern fragment can be connected by a Nozaki-Hiyama-Takai-Kishi reaction whereas the eastern fragment can be attached by exploiting a Heck coupling. The eastern fragment, containing the 5 member ring can be prepared by a SOMO-activated radical cyclization reaction catalyzed by an Ir (III)-photocatalyst, [3] whereas the rest of the stereochemistry of the fragment is built up by an Oppolzer aldol reaction.



pentacaronic acid

Figure 1. Structure of pentacaronic acid.

References

[1] J. Staunton, K. J. Weissman. Polyketide biosynthesis: a millennium review. Nat Prod Rep. 2001, 18, 380-416.
[2] H. Irschik, P. Washausen, F. Sasse, J. Fohrer, V. Huch, R. Müller, E. V. Prusov. Isolation, Structure Elucidation, and Biological Activity of Maltepolides: Remarkable Macrolides from Myxobacteria. Angew. Chem. Int. Ed. 2013, 52, 5402 –5405.
[3] A. G. Capacci, J. T. Malinowski, N. J. McAlpine, J. Kuhne, D. W. C. MacMillan. *Nat. Chem.* **2017**, 9, 1073–1077.

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Molecular basis of the Multiple Mitochondrial Dysfunctions Syndrome 2: the pathogenic His96Arg BOLA3 mutation

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Keywords: BOLA3; GLRX5; multiple mitochondrial dysfunctions syndrome; iron-sulfur cluster biogenesis; ISC machinery

Iron-sulfur (Fe/S) clusters are simple inorganic protein cofactors composed by ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron and sulfide (S^{2-}) ions. They are ancient, ubiquitous prosthetic groups, involved in several cellular processes. The clusters biosynthesis and incorporation into scaffold proteins are guaranteed by different biogenesis machineries, specific for each cellular compartment [1-2]. The Fe/S clusters biosynthesis starts in the mitochondrion, where Fe/S proteins take part in important cellular pathways such as oxidative phosphorylation, lipoic acid synthesis and iron metabolism, contributing to the main function of this organelle, which is the production of cellular energy.
Mutations in genes encoding for some components of the mitochondrial ISC machinery, namely NFU1, BOLA3, IBA57, ISCA2 and ISCA1 proteins, lead to a group of rare syndromes which cause autosomal recessive diseases, and which have been identified, since 2011, as Multiple Mitochondrial Dysfunctions Syndromes (MMDS) types 1 to 5. These rare disorders appear early in life with severe weakness, respiratory failure, lactic acidosis, lower energy metabolism and consequent impairment in neurologic development.
Bi-allelic variants in the gene encoding for BOLA3 cause MMDS type 2, which typically appears in early childhood with encephalopathy, leukodystrophy, nonketotic hyperglycinemia, cardiomyopathy, and death [3-4]. A homozygous His96Arg (c. 287 A > G) mutation in the BOLA3 gene involves a fully conserved residue of the bola-like protein family, previously identified as a [2Fe-2S] cluster ligand in the BOLA3-[2Fe-2S]-GLRX5 heterocomplex. The mutation is responsible for severe lactic acidosis and combined respiratory chain complex deficiencies, with the implication of a wide range of organs. The molecular basis of the pathogenicity of the His96Arg mutation have not been investigated yet [5-6].
Here we present the results of the spectroscopic and biochemical characterization of the His96Arg BOLA3 mutant and of its interaction with the partner monothiol glutaredoxin GLRX5, in the formation of BOLA3-[2Fe-2S]-GLRX5 complex. Moreover, we investigated the effect of the mutation on the heterocomplex ability in assembling a [4Fe-4S] cluster on NFU1.

References

[1] Ciofi-Baffoni S, Nasta V, Banci L. Protein networks in the maturation of human iron-sulfur proteins. *Metallomics*. 2018, 10(1):49-72.
[2] Braymer JJ, Freibert SA, Rakwalska-Bange M, Lill R. Mechanistic concepts of iron-sulfur protein biogenesis in Biology. *Biochim Biophys Acta Mol Cell Res*. 2021, 1868(1):118863.
[3] Saudino G, Suraci D, Nasta V, Ciofi-Baffoni S, Banci L. Molecular Basis of Multiple Mitochondrial Dysfunctions Syndrome 2 Caused by CYS59TYR BOLA3 Mutation. *Int J Mol Sci*. 2021, 22(9):4848.
[4] Lebigot E, Schiff M, Golinelli-Cohen MP. A Review of Multiple Mitochondrial Dysfunction Syndromes, Syndromes Associated with Defective Fe-S Protein Maturation. *Biomedicines*. 2021, 9(8):989.
[5] Camponeschi F, Ciofi-Baffoni S, Calderone V, Banci L. Molecular Basis of Rare Diseases Associated to the Maturation of Mitochondrial [4Fe-4S]-Containing Proteins. *Biomolecules*. 2022, 12(7):1009.
[6] Nishioka M, Inaba Y, Motobayashi M, et al. An infant case of diffuse cerebrospinal lesions and cardiomyopathy caused by a BOLA3 mutation. *Brain Dev*. 2018, 40(6):484-488.

Tip-tip filtration as a non-conventional tool for the improvement of single-phase lipids extraction

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Keywords: Lipid extraction, mass spectrometry, sample pre-treatment, untargeted lipidomics

The human lipidome is a large and complex system of molecules, that vary in both chemical structure and concentration in biological fluids. Its analysis now mainly relies on LC-HR-MS/MS, but its heterogeneity represents a crucial challenge for an accurate separation in liquid chromatography and a proper handling of the sample, prior to analysis [1,2]. For this reason, we compared three different commonly performed methods for the extraction of lipids from human plasma using different percentages of methanol and chloroform, that still represent the gold standard solvents for the extraction of non-polar compounds: (A) methanol/chloroform (2:1, v/v); (B) methanol/chloroform (1:1, v/v); and (C) methanol/chloroform/tert-butyl methyl ether (1.5/1/1, v/v) [3–5]. We also evaluated whether a filtration of the sample could be beneficial to lipidomics studies (D).

Lipids were single-phase extracted from a diluted pool of healthy human donors' plasma following the aforementioned protocols (A-C); before the extraction, samples were added with the internal standard (IS, phosphatidylcholine (15:0-18:1) d7 25 µg/mL, 6 µL). Protocol (D) added a *tip-tip* filtration step of the redissolved sample before analysis to protocol (A): the extract was aspirated through a 20 µL tip polyethylene filter and the clarified solution from the part overlying the filter was withdrawn with another tip directly into the vial (**Figure 1A**). The usage of syringes was avoided as its application is quite expensive, time-consuming, and needs high volume samples.

According to Deming Linear Regression test [6], methods (A) and (B) are comparable (R^2 = 0.98), while method (C) is different from the others (R^2 = 0.81 A vs C, 0.80 B vs C). The total lipids extraction from these three methods can be appreciated in **Figure 1B**: even though there is no statistically significant difference among them all, a slightly higher yield can be attained using protocol A. The recovery of the IS mirrors the behaviour of all lipid species. Reproducibility was evaluated comparing the percent CV% of the three methods. Overall, protocol (A) seemed the most promising, as it lent the minor CVs% in both positive (A vs B and C: 21% vs 23% and 26%) and negative (A vs B and C: 5% vs 15% and 17%) ionizations. Using protocol (D), the extraction yield calculated on total lipids slightly improved in ESI+, while it remained the same in ESI-, and the recovery of the internal standard was remarkably improved (**Figure 1C**). An improvement in the variability could also be appreciated (21% vs 13%).

Our results demonstrate a noticeable superiority of method (A), in terms of both better reproducibility and rate of extraction. Furthermore, *tip-tip* filtration led to improved results compared to the aforementioned method; along with a minimal loss of lipids, it allows a higher purification of the samples, an increased column lifetime, and a sharp cut in costs.

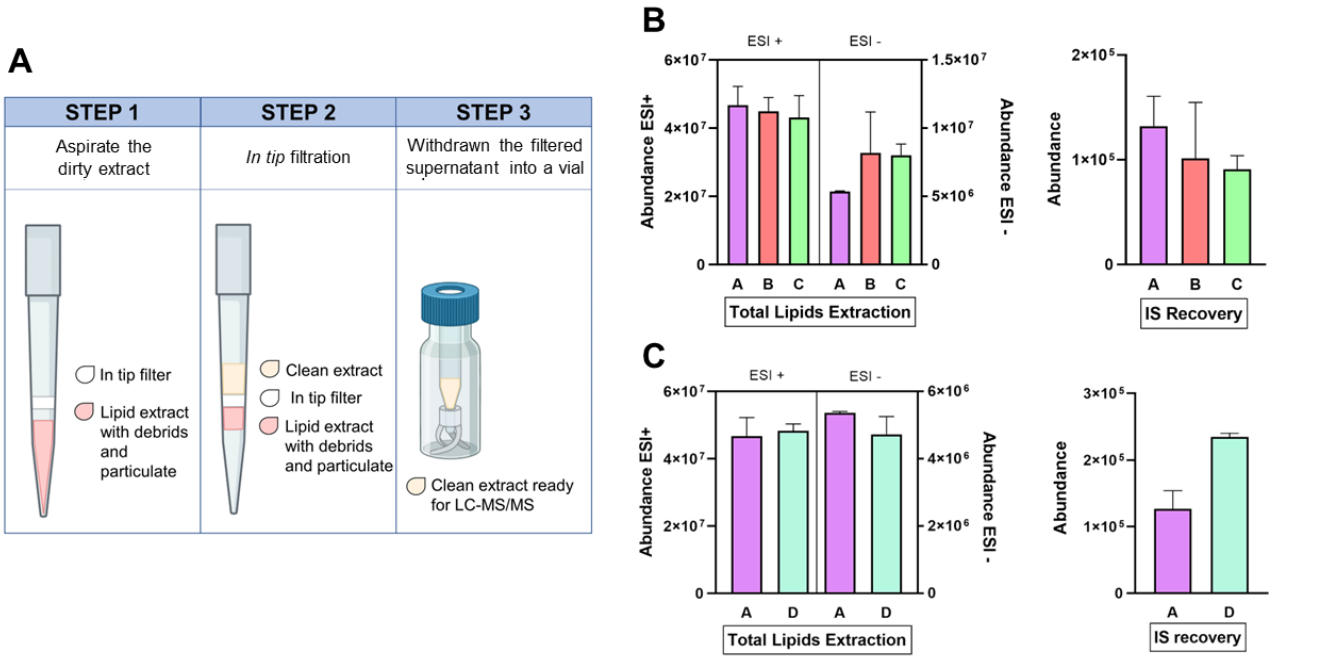


Figure 1 (Panel A) Scheme for tip-tip filtration protocol of lipid extracts. (Panel B) Sum of total lipids abundance in both the polarities and recovery of internal standard (phosphatidylcholine (15:0-18:1) d7, 1.5 µg/mL) by using (A) methanol/chloroform (2:1, v/v); (B) methanol/chloroform (1:1, v/v); (C) methanol/chloroform/tert-butyl methyl ether (1.5:1:1, v/v/v) as extraction solvents. (Panel C) Sum of total lipids abundance in both the polarities and recovery of the recovery of internal standard (phosphatidylcholine (15:0-18:1) d7, 1.5 µg/mL) without (A) and with (D) the tip-tip step.

References

[1] O. Quehenberger, A.M. Armando, A.H. Brown, S.B. Milne, D.S. Myers, A.H. Merrill, S. Bandyopadhyay, K.N. Jones, S. Kelly, R.L. Shaner, C.M. Sullards, E. Wang, R.C. Murphy, R.M. Barkley, T.J. Leiker, C.R.H. Raetz, Z. Guan, G.M. Laird, D.A. Six, D.W. Russell, J.G. McDonald, S. Subramaniam, E. Fahy, E.A. Dennis Lipidomics reveals a remarkable diversity of lipids in human plasma1, *J. Lipid Res.* 2010, 51, 3299–3305

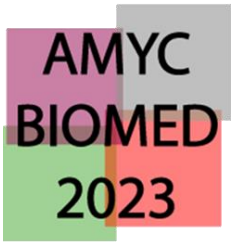
[2] M. Holčapek, G. Liebisch, K. Ekroos Lipidomic Analysis, *Anal. Chem.* 2018, 90, 4249–4257

[3] M.L.G.S. J Folch A simple method for the isolation and purificatioin of total lipids from animal tissues, *J Biol Chem*. 1957, 1, 497–509

[4] E.G. BLIGH, W.J. DYER A rapid method of total lipid extraction and purification., *Can. J. Biochem. Physiol.* 1959, 37, 911–917

[5] R.M. Pellegrino, A. Di Veroli, A. Valeri, L. Goracci, G. Cruciani LC/MS lipid profiling from human serum: A new method for global lipid extraction, *Anal. Bioanal. Chem.* 2014, 406, 7937–7948

[6] M.W.K. Wong, N. Braidy, R. Pickford, P.S. Sachdev, A. Poljak Comparison of single phase and biphasic extraction protocols for lipidomic studies using human plasma, *Front. Neurol.* 2019, 10, 1–11



POSTERS

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Exploiting induced Circular Dichroism to probe biomolecule's conformations

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Keywords: Porphyrinoids, chirality, DNA, micro-RNA, Circular Dichroism

Biomacromolecules such as polynucleotides and peptides always are arranged in several conformations. Most of the secondary structures adopted by biomacromolecules depend on the functionality of the target, indicating relationship between structure and activity. Therefore the possibility to identify the conformations allows interfering with biological processes, paving the way to wide biomedical applications. However up today, the biological role of most of the conformations studied is yet unknown. Circular dichroism (CD) is a powerful technique to obtain information on electronic transitions and has been used extensively for studies on biomolecule's conformations exhibiting supramolecular chirality [1]. However even if most of the secondary structures present different and specific CD spectra, it is not possible recognize between two or more conformation by CD spectra due to spectroscopic interferences by others biomolecules absorbing in the same spectroscopic region. Therefore to detect a particular conformation it is necessary to find a molecule that interacts specifically with this structure and shows a spectroscopic signal in another region far from biomolecule absorption region. In this contest, porphyrins are ideal compounds to work as conformational probe for several particular spectroscopic characteristics: i) huge extinction coefficients, ii) synthetic versatility, iii) absorption region in the visible range [2]. In addition to these interesting features, the porphyrins used in our works are not chiral, allowing to exploit the induced chirality arose in the porphyrin absorption region after the interaction with biomolecules. Indeed not chiral porphyrins are silent to CD spectroscopy, however interacting with chiral matrix as DNA, an induced CD signal is observed in porphyrins absorption region, which is far from the absorbance of other biomolecules (that means spectroscopic signals free from interference). The detected induced CD signals are specific for each type of interaction.
Here we show some examples of designing of chiroptical porphyrin-based probe for biomolecule's conformation. In details we have proposed some metalloporphyrins as chiroptical probe for unusual DNA conformation (Z-DNA), demonstrating the ability of porphyrins to sense the DNA helix handedness, even in competitive environment [3]; we investigated the potency of porphyrins as inhibitor and modulator of proteasome, which is the protein involved in many biological processes [4]. In addition we have exploited the stabilizing ability of spermine porphyrin derivatives, towards G-quadruplex structures, obtaining some interesting results [5]. Finally we characterized the conformation and structures adopted by several microRNA in physiological conditions highlighting the important role played by the secondary structures to fulfill the biological function. [6]

References

[1] J. Kypr et al Nucleic Acids Research, **2009**, 37/6 1713;
[2] A. D'Urso, M.E. Fragalà, Roberto Purrello. Non-Covalent Interactions of Porphyrinoids with Duplex DNA in Top Heterocycl Chem DOI: 10.1007/7081_2013_113 Ed. Springer-Verlag Berlin Heidelberg **2013**.
[3] a) M. Balaz, et al. *Angew. Chem. Int. Ed.* **2005**, 44, 4006; b) A. D'Urso, et al. *J. Am. Chem. Soc.* **2009**, 131, 2046; c) A. D'Urso, et al. *J. Am. Chem. Soc.* **2013**, 135, 8632;
[4] a) A. M. Santoro, et al. *J. Am. Chem. Soc.,* **2012**, 134, 10451; b) A. M. Santoro, et al. *Chem Sci* **2016,** 7, 1286–1297; c) A. Di Dato, et al. *Scientific REPORTS* **2017**, 7: 17098; d) .
[5] a) N.C. Sabharwal, et al. *Int. J. Mol. Sci.,* **2018**, 19, 3686; b) A. D'Urso et al. *Phys. Chem. Chem. Phys.,* **2017**, 19, 17404.
[6] C.M.A. Gangemi, et al. *Scientific Reports* 2020, 10, 1.

Molecular dynamics simulations of the interaction between antibacterial small molecules and the Staphylococcus Aureus lipid bilayer

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Keywords: Molecular Dynamics, Antibacterial, *S. aureus*, Lipid Bilayer

BACKGROUND/AIM
Bacterial resistance to antimicrobial drugs is one of the biggest threats to human health. Several clinically relevant organisms are rapidly evolving towards multidrug and even pandrug-resistant phenotypes, whose global spread represents a significant Public Health issue.(1) The increasing occurrence of infections is associated with high mortality and morbidity. As conventional antibiotics are not effective in the treatment of infections caused by such bacteria, novel antibacterial therapeutics are urgently required. Herein, we used molecular dynamics (MD) simulations to investigate the mechanism of action of antibacterial small molecules at the level of *S. aureus* cytoplasmic membrane.
METHOD
The symmetric lipid bilayer mimicking *S. aureus* membrane was built using the CHARMM-GUI(2,3) Membrane Builder tool. All-atom MD simulations were performed with AMBER18 using the CHARMM36m38 force field.(4) Two independent MD replicas of 500 ns each were run for each membrane/small molecule complex. The interaction of *S. aureus* membrane with two molecules endowed with opposite bioactivity profile but high chemical similarity was simulated.
RESULTS
MD results of the bioactive hit 1 show a marked propensity of the molecule to bind the *S. aureus* membrane model. The distribution of the ligand mass density along MD simulations demonstrates that 1 is fully embedded in the membrane from 70 ns to the end of the MD simulation. On the contrary, the inactive compound 2 shows less stable interactions with the phospholipid bilayer. Mass density analysis highlights a ligand peak at around 30 Å from the bilayer center, which suggests that the compound is predominantly outside the membrane. Based on these results, we assume that the membrane of *S. aureus* might be the site of action of 1, in agreement with experimental data, and that slight changes in chemical structure might have a deep impact on the interaction with the membrane.
CONCLUSION
MD simulations are profitable tools in the study of small molecule interaction with the *S. aureus* membrane, which can be further used in lead optimization of antibacterial compounds.

References

[1] Mancuso, G., Midiri, A., Gerace, E., & Biondo, C. Bacterial Antibiotic Resistance: The Most Critical Pathogens. Pathogens (Basel, Switzerland). 2021, 10(10), 1310.
[2] Jo, S., Kim, T., Iyer, V. G., & Im, W. CHARMM-GUI: a web-based graphical user interface for CHARMM. Journal of computational chemistry. 2008, 29(11), 1859–1865.
[3] Li, Y., Liu, J., & Gumbart, J. C. Preparing Membrane Proteins for Simulation Using CHARMM-GUI. Methods in molecular biology (Clifton, N.J.). 2021, 2302, 237–251.

[4] Lee, J., Hitzenberger, M., Rieger, M., Kern, N. R., Zacharias, M., & Im, W. CHARMM-GUI supports the Amber force fields. The Journal of chemical physics. 2020, 153(3), 035103.

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Plasmon-assisted organic electrochemistry – towards selective hydrogenation

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Keywords: Electroorganic synthesis, Photoelectrochemistry, Plasmon-assisted chemistry

Photocatalysis and electrochemistry are two useful strategies of organic synthesis, each favored by their advantages, which can be combined in a photoelectrochemical approach [1]. Utilization of photoelectrochemistry in organic synthesis allows the elimination of the use of hazardous and toxic substances and can significantly increase the reaction rate and selectivity [2]. In addition, photoelectrochemical synthesis can be performed with the involvement of a green and renewable energy source – sunlight [3]. Photoelectrochemical approach can be used in the synthesis of complex organic molecules, including bioactive pharmaceutical compounds, with improved efficiency and selectivity. Our work is focused on the photoelectrochemical reduction of organic compounds, performed with the utilization of plasmon-assisted chemistry. Unlike previously reported approaches, we used the bimetallic electrode, which combines the plasmon and redox activities. The plasmonic properties of electrodes allow us to efficiently absorb the incident light and convert it into surface plasmon, with the corresponding subdiffraction focusing of photon(s) energy. In turn, the presence of catalytically active metal ensures efficient surface sorption of reagent molecules and their reduction. Finally, the charge transfer (and related reduction processes) were significantly enhanced by surface plasmon excitation. We demonstrated the advantages of the proposed approach using the electrochemical conversion of acetophenone to 1-phenylethanol as a model reaction. The efficient reaction proceeding with high rate, conversion, and yield was demonstrated. The apparent contribution of the surface plasmon to the reaction kinetics was also observed.

References

[1] J. Liu, L. Lu, D. Wood, S. Lin. New Redox Strategies in Organic Synthesis by Means of Electrochemistry and Photochemistry. ACS Cent. Sci. 2020, 6 (8), 1317-1340.
[2] T. Ali, H. Wang, W. Iqbal, T. Bashir, R. Shah, Y. Hu. Electro-Synthesis of Organic Compounds with Heterogeneous Catalysis. Adv. Sci. 2023, 10 (1), 2205077.
[3] L. Qian, M. Shi. Contemporary photoelectrochemical strategies and reactions in organic synthesis. ChemComm. 2023, 59 (24), 3487-3506.

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Towards improved anti-HMGB1 G-quadruplex-forming aptamers: studies on dimerization

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Keywords: G-quadruplex, Aptamers, Cancer, Theranostics

High-Mobility Group Box 1 (HMGB1) is an abundant, highly conserved, non-histonic nuclear protein present in almost all eukaryotic cells[1,2]. It is also a DNA binding protein, involved in critical biological processes, such as DNA transcription, replication, repair, and recombination[3]. In an inflammatory state, HMGB1 is actively secreted from immune cells in the extracellular matrix, where it behaves as a proinflammatory cytokine[4], contributing to the pathogenesis of various chronic inflammatory and autoimmune diseases as well as cancer[5]. Given the multiple roles of the protein in these pathologies, identification of HMGB1-inhibitors is of considerable interest[6,7].
Considering the ability of this protein to induce bending in double-stranded DNA[8,9], as well as the identification of HMGB1 as a telomeric and non-telomeric G-quadruplex (G4)-interacting protein[10,11], in a recent work we identified a set of G4-forming aptamers from a focused library of G-rich oligonucleotides able to interact with high affinity with the protein and also inhibit the HMGB1-induced cell migration[12]. A more in-depth biophysical and biological characterization of one of the best anti-HMGB1 aptamers revealed that its efficacy was mostly due to its ability to spontaneously form dimeric species. Thus, the aim of this work is the design and the evaluation of the biophysical properties of covalent dimers of the best G4-forming anti-HMGB1 aptamer, obtained using linkers with different features, to develop optimized constructs that can better interact with HMGB1 and also better inhibit the protein pathological activities.

References

[1] G. H. Goodwin, C. Sanders, E. W. Johns, P. Electrophoresis, Eur. J. Biochem. 1973, 38, 14–19.
[2] G. H. Goodwin, E. W. Johns, Method Cell Biol. 1977, 16, 257–267.
[3] T. Ueda, M. Yoshida, BBA-Gene Regul Mech. 2010, 1799, 114–118.
[4] U. G. Andersson, K. J. Tracey, J. Intern. Med. 2004, 255, 318–319.
[5] R. Kokkola, Å. Andersson, G. Mullins, T. Östberg, C. J. Treutiger, B. Arnold, P. Nawroth, U. Andersson, R. A. Harris, H. E. Harris, Scand. J. Immunol. 2005, 61, 1–9.
[6] D. Musumeci, G. N. Roviello, D. Montesarchio, Pharmacol Ther. 2014, 141, 347–357.
[7] E. Venereau, F. De Leo, R. Mezzapelle, G. Careccia, G. Musco, M. E. Bianchi, Pharmacol. Res. 2016, 111, 534–544.
[8] C. M. Read, P. D. Cary, C. Crane-robinson, P. C. Driscoll, D. G. Norman, Nucleic Acids Res. 1993, 21, 3427–3436.
[9] C. H. Hardman, R. William Broadhurst, A. R. C. Raine, K. D. Grasser, J. O. Thomas, E. D. Laue, Biochemistry-US. 1995, 34, 16596–16607.
[10] J. Amato, T. W. Madanayake, N. Iaccarino, E. Novellino, A. Randazzo, L. H. Hurley, B. Pagano, Chem. Commun. 2018, 54, 9442–9445.
[11] J. Amato, L. Cerofolini, D. Brancaccio, S. Giuntini, N. Iaccarino, P. Zizza, S. Iachettini, A. Biroccio, E. Novellino, A. Rosato, M. Fragai, C. Luchinat, A. Randazzo, B. Pagano, Nucleic Acids Res. 2019, 47, 9950–9966.
[12] E. Napolitano, A. Criscuolo, C. Riccardi, C. L. Esposito, S. Catuogno, G. N. Roviello, D. Montesarchio, D. Musumeci, *submitted*.

Skin anti-aging carrageenan gel containing SNEDDS loaded with Sichuan pepper extract

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Keywords: gel, Sichuan pepper, carrageenan, SEDDS, anti-aging

Numerous studies have been published on drug delivery systems containing bioactive ingredients. These systems should prevent the activity of the drug, but also increase its bioavailability. Carrageenan is a versatile polymer because is more stable than other encapsulation material such as pectin and has a great electronegativity responsible for the protection of bioactive compounds. Most of the works based on carrageenan nutraceutical drug delivery systems focuses on the use of just one vehicle, however it should be convenient to develop nutraceutical formulations containing two or more vehicles with synergic effect [1]. For example, hydrogels of carrageenan should be combined with nano-vesicular systems to protect the bioactive from degradation and simultaneously increase its biopharmaceutic characteristic. Self-nano emulsifying drug delivery systems (SNEDDS) are mixtures of drugs, lipids/oils, cosolvents and surfactants that form nanoemulsions following dilution in an aqueous medium *in vivo.* SNEDDS, if topically applied, are diluted from the aqueous phase of the skin that comes from secretion, sweat or trans epidermal water loss and consequently they form an occlusive topic system with high driving thermodynamic force for cutaneous delivery [2]. The presence of an oil phase enhances the solubility of lipophilic compounds such as α-hydroxysanshool (HAS). HAS is the active alkylamide present in the extract of *Zanthoxylum piperitum* (also called Sichuan pepper), but its use is quite limited for its low water solubility and stability. Therefore, the aim of this work was to prepare and characterize a carrageenan-based hydrogel containing SNEDDS loaded with Sichuan pepper extract (SPE) for cutaneous application as anti-aging. SNEDDS were prepared solubilizing separately, SPE in a solution of water and glycerin (2:3 ratio) and mixing Tween 80 and Capmul in ratio 5:2. The aqueous phase was then added to the oil phase and the resulting SNEDDS were characterized for dimension, ζ-potential values and thermodynamic stability. For the preparation of the gel, κ-carrageenan was solubilized in water (1% w/v) for 2 h at 70 per and after the temperature was reduced to 40°C, SNEDDS were added under continuous stirring. Interaction studies performed on carrageenan-based hydrogel containing SNEDDS loaded with SPE demonstrated the ability of the polymer to bind the extract. Subsequently, to evaluate the stability and absorptive properties of the hydrogel, swelling studies in phosphate buffer saline (PBS) were carried out. The swelling degree of the gel was 200 % circa after 90 minutes of incubation in PBS and this degree decreased after 120 minutes and lasted constant up to 240 minutes. The water sorption ability of carrageenan is probably due to the presence of highly polar sulfate groups in the structure [3] that make carrageenan promising in a wide range of applications. Evaluation of lifting properties of the formulation *in vitro* are under investigation.

References

[1] Dong, Y., Wei, Z., & Xue, C. (2021). Recent advances in carrageenan-based delivery systems for bioactive ingredients: A review. *Trends in Food Science & Technology*, *112*, 348-361.
[2] Ponto T., Latter, G., Luna, G., Leite-Silva, V. R., Wright, A., & Benson, H. A. (2021). Novel self-nano-emulsifying drug delivery systems containing astaxanthin for topical skin delivery. *Pharmaceutics*, *13*(5), 649.
[3] Zia, K. M., Tabasum, S., Nasif, M., Sultan, N., Aslam, N., Noreen, A., & Zuber, M. (2017). A review on synthesis, properties and applications of natural polymer based carrageenan blends and composites. *International journal of biological macromolecules*, *96*, 282-301.

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AMYC BIOMED 2023

Chitosan-sodium usnate nano-assemblies activity against osteosarcoma 143B cells

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Keywords: chitosan, sodium usnate, self-assembly, osteosarcoma

Osteosarcoma (OS) is the most common primary malignant bone cancer disease in pediatric and adolescent patients. Chemotherapy remains the main therapeutic strategy against OS but, unfortunately, causes alterations in cellular growth not only in cancerous cells but also in healthy ones [1]. Drug encapsulation in polymer nanoparticles is a strategy commonly employed to mitigate inconvenience associated with toxic drugs, including poor solubility and systemic toxicity [2].
In this study, chitosan (CS) nanoparticles (NPs) were investigated as carriers for the anticancer drug sodium usnate (NaU). Self-assembled CS NPs were obtained by ionotropic gelation and loaded with NaU, without the use of organic solvents or surfactants. A high drug encapsulation efficiency was obtained thanks to the establishment of drug/polymer ionic interactions. Hepatotoxicity of free and encapsulated drug was evaluated against HepG2 cells. The efficacy of the formulations against osteosarcoma 143B cells was investigated by both cell viability assay and evaluation of the Maspin protein production, a tumor suppressor agent. A significant reduction in NaU hepatotoxicity when encapsulated in CS NPs compared to free NaU was evidenced and, for the first time, a relevant synergistic activity of CS and NaU in promoting Maspin stimulation in 143B cells was found. The safety of NaU-loaded CS NPs observed in in vivo biocompatibility tests on nematodes further validates these systems as suitable nano-formulations to deliver toxic anticancer agents.

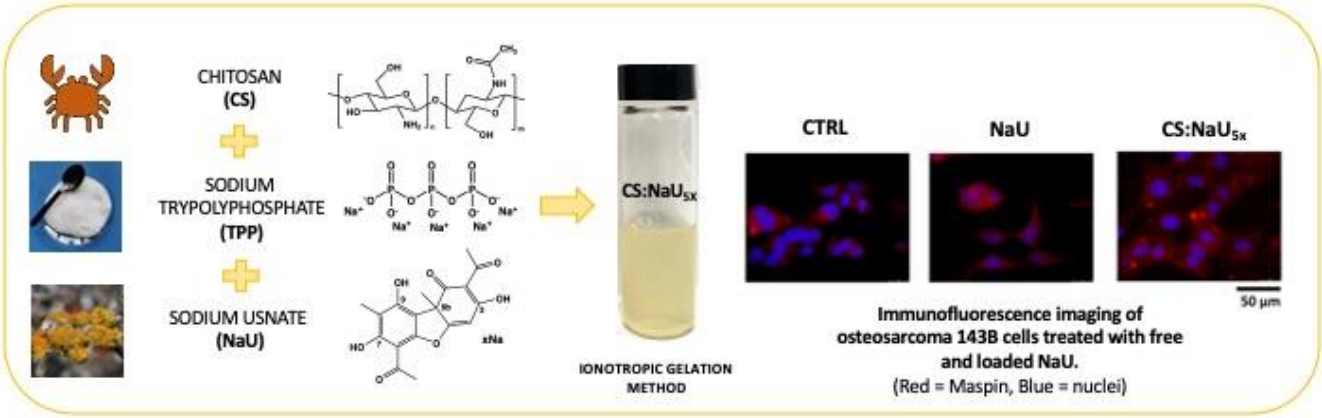

Figure 1. Preparation and immunofluorescence imaging of CSNaU5x against osteosarcoma 143B cells

References

[1] Li, S., Zhang, H., Liu, J., & Shang, G. *Journal of cancer research and clinical oncology*, 2023
[2] Francolini, I., Taresco, V., Crisante, F., Martinelli, A., D'Ilario, L., & Piozzi, A. *International journal of molecular sciences*, 14(4), 7356–7369, 2013

AMYC BIOMED 2023

Effect of Gd-based contrast agents on endogenous Chemical Exchange Saturation Transfer (CEST) signals: a new tool for investigating water dynamics across cell membrane in tumor by MRI

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Keywords CEST- MRI; Gadolinium-based Contrast Agents; Water permeability

In this work an innovative MRI tool for imaging tumor phenotype and metabolism is presented. This method exploits Chemical Exchange Saturation Transfer-Magnetic Resonance Imaging (CEST-MRI) of endogenous intracellular small molecules to investigate water dynamics in cancers. The reported technique investigates the effect of clinically approved Gd-based contrast agents on intracellular endogenous 1H-CEST signals of small metabolites, whose resonance frequency is at 2ppm with respect to bulk water signal (e.g. amine groups of glutamine and/or creatine [1]), named CEST@2ppm effect. When their resonance is saturated by a proper rf pulse, since they are in exchange with bulk water proton pool, it is possible to observe a reduction in MRI water signal intensity, due to a saturation transfer (ST) mechanism, that can be quantified through the ST% parameter. The ST% reduction is dependent on two key factors: the concentration of contrast agent in the extravascular/extracellular compartment and on the exchange rate of water molecule across cell membrane able to transfer the relaxation from the extra- to the intra-cellular compartment. In fact, in vivo, GBCAs remain confined in the extravascular/extracellular tumor compartment and their effect on intracellular signals is strictly dependent on the tumor perfusion and tumor cells' water permeability (Fig.1). We tested in vitro this hypothesis with a set of different experiments. By placing GBCAs directly in contact with CEST agent (e.g. creatine) or separated by different kinds of membranes (LipoCEST, RBC, tumoral cells), we observed differences in exponential decay constant of ST%. In vivo we tested for the effect in murine breast cancer models with increasing degrees of malignancy (namely 4T1, TS/A and 168FARN), and performed MRI acquisition pre and post administration of a GBCA. Results confirmed that this method can report about differences in tumor perfusion and water dynamics. Highly aggressive tumors show a higher water permeability respect to less aggressive ones. Water permeability MRI maps were obtained, considering the wash-in/washout kinetics of GBCAs in the tumor. In conclusion, we developed a new method to obtain quantitative maps of water permeability across cancer cell membrane using CEST-MRI in presence of exogenous GBCA. This technique allows improving the capability of MRI to phenotyping tumor using water permeability as new biomarker obtaining functional information about tumor aggressiveness and also reducing 6-fold the administered dose of GBCA to the patient, because of the higher sensitivity in comparison to clinically used T1- weighted MRI sequences. Fig.1 Rationale of the method: effect of GBCAs on intracellular CEST signals.

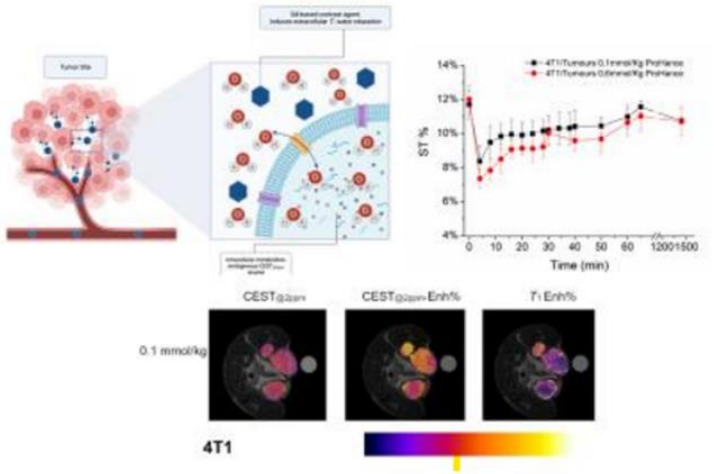

Figure.1 Rationale of the method: effect of GBCAs on intracellular CEST signals

References

[1] Cai K. et al., CEST signal at 2ppm (CEST@2ppm) from Z-spectral fitting correlates with creatine distribution in brain tumor. NMR Biomed. 2015 Jan;28(1):1-8. doi: 10.1002/nbm.3216

AMYC BIOMED 2023

Development of magnetic molecular imprinted polymer systems for biomedical applications

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Keywords: Molecularly imprinted polymers, magnetic nanoparticles, drug delivery

In recent years, molecularly imprinted polymers (MIPs) have received increasing attention due to their unique characteristics such as high stability, simple preparation, robustness, specificity in molecule capture, and low cost of production. The molecular imprinting approach has allowed the development of promising systems to be used both in environmental applications for capture of pollutants [1], and in biomedical field for drug administration, to remove undesirable substances from the body or as diagnostic sensors [2]. MIPs can also be combined with different nanomaterials such as silica (SiO_2), iron oxide (Fe_3O_4), gold (Au) and silver (Ag) to produce multifunctional composite systems with improved properties [3]. Moreover, the functionalization with small molecules may make MIPs a powerful tool for drug delivery. In this work, innovative magnetic MIPs for ciprofloxacin (CPR) delivery were developed. CPR, a fluoroquinolone antibiotic of second-generation having activity against Gram-positive and Gram-negative microorganisms, is frequently used for treating bacterial infections. We developed a nanostructured magnetic composite system consisting of an inorganic core (Fe_3O_4 nanoparticles) and 2 polymeric shells, the first composed of chitosan (CS) to avoid nanoparticle aggregation phenomena, and the second of a molecular imprinted acrylic polymer. While the magnetic nanoparticles were chosen for their easy recovery through the application of a magnetic field, and for relatively low manufacturing costs relatively low, CS was preferred for its biocompatibility, good antimicrobial activity, and good absorbent capacity [4].
To obtain the MIP system an in situ polymerization of methacrylic acid (MAA) with ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent and CPR as template were used. Particularly, different concentrations of MAA, EDGMA and CPR were investigated. Finally, to evaluate the effectiveness of nanostructured magnetic MIPs, non-imprinted systems (NIPs) were also produced. UV-vis spectroscopic analysis highlighted the greater ability of imprinted systems compared to NIPs to bind CPR. Biological tests are underway to verify the possible application of these systems in drug release.

References

[1] Silvestro, I.; Fernández-García, M.; Ciarlantini, C.; Francolini, I.; Girelli, A.; Piozzi, A. Molecularly Imprinted Polymers Based on Chitosan for 2,4-Dichlorophenoxyacetic Acid Removal. *Int. J. Mol. Sci.* **2022**, *23*, 1–18, doi:10.3390/ijms232113192.
[2] Ayankojo, A.G.; Borozniak, R.; Reut, J.; Öpik, A.; Syritski, V. Molecularly Imprinted Polymer Based Electrochemical Sensor for Quantitative Detection of SARS-CoV-2 Spike Protein. *Sensors Actuators B Chem.* **2022**, *353*, doi:10.1016/j.snb.2021.131160.
[3] Zaidi, S.A. Molecular Imprinting Polymers and Their Composites: A Promising Material for Diverse Applications. *Biomater. Sci.* **2017**, *5*, 388–402, doi:10.1039/c6bm00765a.
[4] Aranaz, I.; Alcántara, A.R.; Civera, M.C.; Arias, C.; Elorza, B.; Caballero, A.H.; Acosta, N. Chitosan: An Overview of Its Properties and Applications. *Polymers (Basel).* **2021**, *13*, 1–27, doi:10.3390/polym13193256.

AMYC BIOMED 2023

Determination of Fipronil in Honey Samples by an electrochemical Competitive replacement Assay

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Keywords: Aptasensor, Competitive replacement Assay, pesticide, Fipronil, honey

Fipronil, an highly effective insecticide used worldwide for the control of a wide range of insects in crops, public hygiene but also domestic and veterinary services, is an ubiquitous environmental pollutant with serious effects on human health [1].Currently, the main methods employed to determine most of the pesticides are HPLS-MS, GC-MS and ELISA. HPLS-MS and GC-MS are expensive equipment and purification of samples is required prior to assay. On the other hand, immunoassays are highly sensitive, but they involve the use of antibodies which are both difficult to obtain and expensive. For these reasons, considering the small molecular size of Fipronil we have developed on screen printed electrodes a biosensor based on the displacement of surface bound aptamers by the analyte (Fipronil). A scheme of the biosensor assembly is shown in Figure 1.

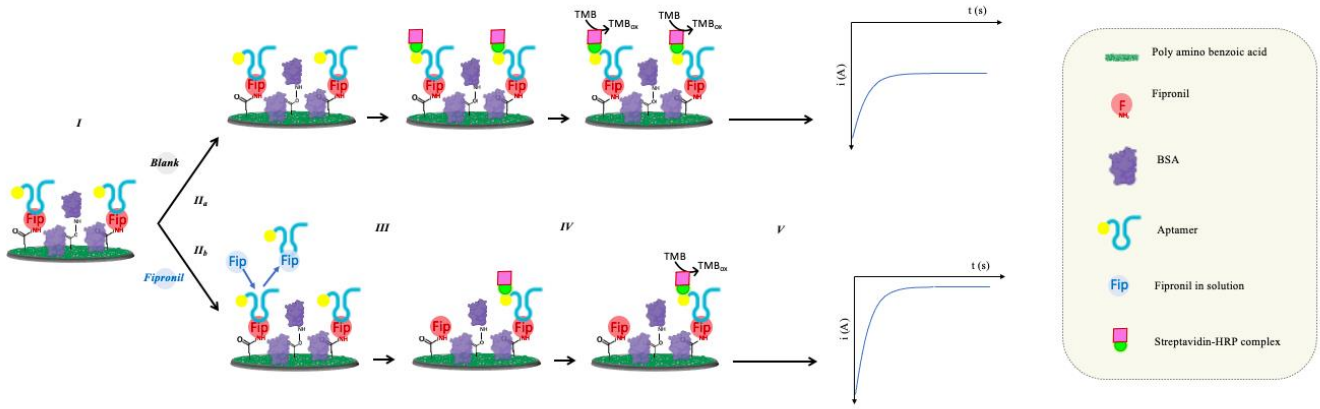

Figure 1. Schematic representation of the biosensor assembly for fipronil detection. I: modified SPCE, II_a: blank sample incubation, II_b: fipronil spiked sample incubation, III: incubation with streptavidin-HRP, IV: TMB deposition, V: chronoamperometric measurement and response to the blank sample (above) and to the spiked sample (below).

Here, after acquisition of the most relevant analytical parameters: calibration curve in a range of 2-15 ppb with a 0.999 of correlation coefficient, detection limit of 1 ppb and a good selectivity tested towards atrazine, we present the results obtained in the quantification of fipronil in honey samples. The legislation governing the Maximum Residual Limits applies, a provisional fipronil MRL in honey of 5 ppb as the sum of fipronil and sulfone metabolite [2]. An analytical comparison with the most commonly HPLS-MS techniques has been performed.

References

[1] X. Li, W. Ma, H. Li, Q. Zhang, Z. Ma. Determination of residual fipronil and its metabolites in food samples: A review. Trends in Food Science & Technology. (2020), 97, 185-195.
[2] Regulation (EC) n. 396/2005.

AMYC BIOMED 2023

Effect of Hydrophobicity Alterations and Purification Nature on Poly(glycerol adipate) Self-Assembling Behaviors

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Keywords: Biodegradable, Poly (glycerol adipate), self-assembling, purification, hydrophobicity

Nowadays, biodegradable polymers are emerging as appealing polymeric systems for biomedical applications. In this regard, polyesters appear as one of the most promising platforms for these applications owing to their tendency to be hydrolyzed/degraded under physiological conditions. Although they have been used for many years for biomedical and pharmaceutical applications, improvements are still needed due to their lack of biological clues and chemical handles. To justify, biodegradable biopolymers, such as Poly(lactic-co-glycolic) Acid (PLGA), Poly(lactic acid) (PLLA), and Poly(ε-caprolactone) (PCL), are either completely deficient in hydrophilic functional groups or comprise of only one, mainly the terminal hydroxyl group (-OH), and therefore require conjugation with hydrophilic systems like PEG. For this reason, Poly(glycerol adipate) (PGA), as seen in *Figure 1*, has been investigated as a bio-derived, biodegradable alternative and thanks to its intrinsic amphiphilicity and it can self-assemble into nanoparticles (NPs) without the addition of any stabilizers. It has also been shown to be able to encapsulate small- and macro-active ingredients. PGA's chemo- and regioselective enzymatic synthesis leaves the secondary hydroxyl group as an available pendant, useful for further functionalization and modifications. To illustrate, as a result of PGA's ability to be functionalized, a range of PGA variants have been introduced and studied. To start with, by including the hydrophobic 1,6-Hexanediol in the synthesis mixture, a Hex-functionalized variation can be produced; Poly(glycerol adipate)-hexanediol (PGA-Hex). Moving further, by replacing glycerol with diglycerol, Poly(diglycerol adipate)(PDGA) can be synthesized, and accordingly, when combining these two modifications Poly(diglycerol adipate)-hexanediol (PDGA-Hex) is generated. Although these modifications have been extensively investigated, variation in hydrophobicity and its impact on this polymeric system is relatively underexplored. For this reason, in this project, we focused on the effect of the incorporation of a series of hydrophobic segments (1,6-Hexanediol) in different ratios with respect to glycerol. We unveiled the effect of hydrophobicity variation upon the self-assembling properties of PGA into NPs, including size, shape, surface nature, and stability. In addition, we also studied the effect of polymer purification on self-assembly. In particular, the purification step provided insight into shorter chain entities upon self-assembling. Currently, in the literature, only a few studies investigate the effect of polymer purification on polymer self-assembling and NPs properties. For this reason, this project aims to examine the changes in NPs' behavior after the polymer purification process. All polymers were analyzed by ^{1}H-NMR and Gel Permeation Chromatography (GPC) while the produced NPs, stability, and surface charges were examined by DLS and zeta potential analysis. Purification of the polymers was performed by the precipitation step.

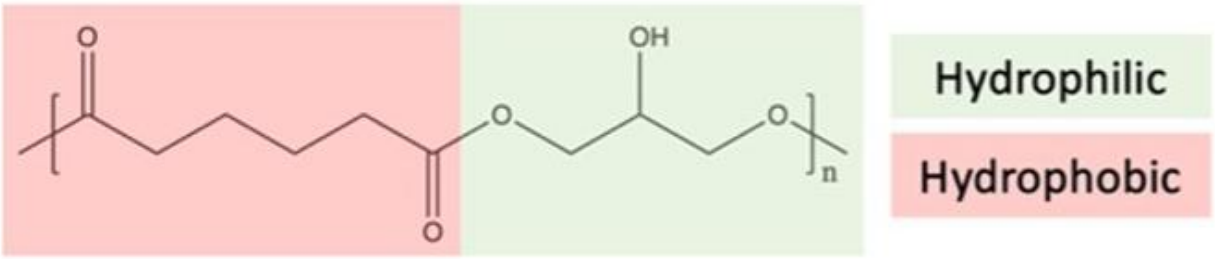

Figure 2. *Structure of poly(glycerol adipate) (PGA). PGA acquires both hydrophilic and hydrophobic parts, and for that reason, it can be described as an amphiphilic polymer.*

Figure 3. *Illustration of purification steps. Purification occurs via precipitation. The polymer solution is dropwise pipetted into the solvent, which is already placed in an ice bath. After centrifugation and removal of the supernatant, the polymer is now purified.*

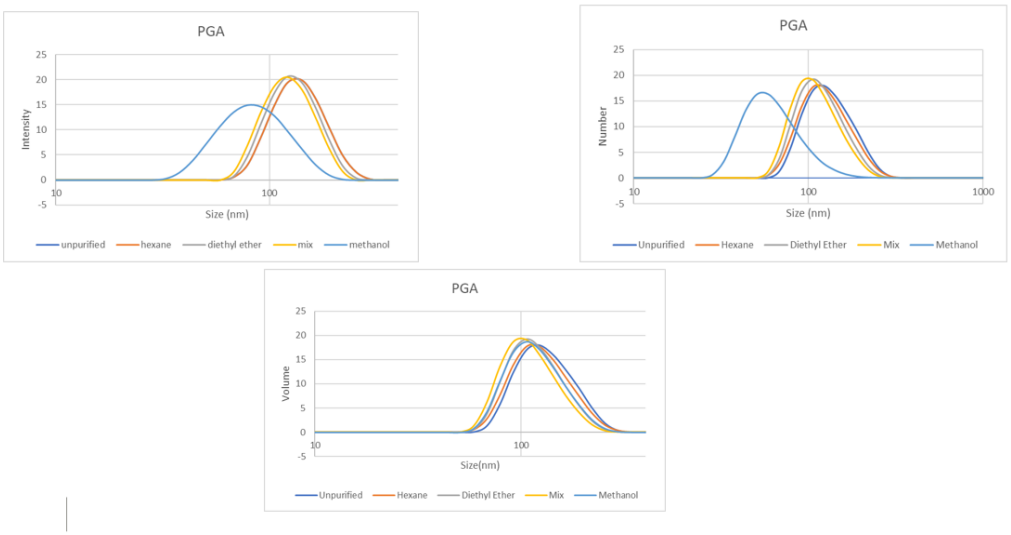

Figure 4. *Example of presenting DLS results for the PGA purification. There are three ways of presenting the DLS data, plotting the graphs by intensity, number, or volume. Purification with methanol is affecting the NP's sizes, while the use of other solvents is creating NPs with the same sizes as the unpurified PGA.*

References

[1] Jacob, P. L. et al., Polymer (Guilford). 2021, 228, 123912.
[2] Gordhan et. al., Journal of pharmaceutical sciences, 2020, 109 (3), 1347-1355.
[3] Dirauf M. et. al., Progress in Polymer Science, 2022, 129, 101547.

AMYC BIOMED 2023

Toolbox to hijack the E3 ligase in viral infections

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Keywords: PROTAC, anti-microbial resistance problem, virus, flavivirus, E3 ligase

Antimicrobial resistance (AMR) is a serious global problem that poses a major threat to human health. Despite that, there is an innovation stuck in the pipeline of novel drugs in this field. To tackle this problem, we aim to exploit a novel emerging concept in drug discovery: the proteolysis targeting chimera (PROTAC) approach. [1] A PROTAC is a heterobifunctional molecule constituted by a ligand able to engage the protein of interest (POI) bridged to a binder for the E3 ligase by a suitable linker. This tri-component molecule induces degradation of the POI *via* the formation of a ternary complex able to activate the ubiquitin system. The choice of the right length, geometry and polarity of the linker play an essential role not only in the formation of the ternary complex but will also drastically influence the drug-like properties of the final PROTAC. [2] Therefore, subset of different matched pairs linker-E3ligase binders have been designed and synthesized and will be ultimately conjugated to the POI ligand. We expect that tuning the physicochemical properties of our PROTAC since the beginning with a careful and rational design of the linker, will positively affect aqueous solubility and cell permeability.
Here, in the case of viral infections, we take advantage of the eukaryotic ubiquitin system and use the well-studied E3 ligase binders (e.g. thalidomide, von Hippel–Lindau). Specifically, our POI ligand is intended to bind to the NS2B-NS3 protease present in the *Flaviviruses* family of RNA viruses. [3] Homology analysis showed that this protease is quite well conserved among the Dengue, Zika and West Nile viruses, broadening the spectrum of action of our final molecules.

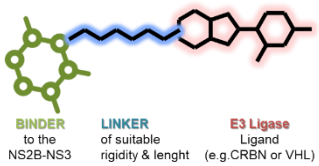

Figure 1. Schematic representation of a PROTAC

References

[1] a) Salerno, A.; Seghetti, F.; Caciolla, J.; Uliassi, E.; Testi, E.; Guardigni, M.; Roberti, M.; Milelli, A.; Bolognesi, M. L. Enriching proteolysis targeting chimeras with a second modality: when two are better than one J. Med. Chem. 2022, 65, 9507–9530. b) Espinoza-Chávez, R. M.; Salerno, A., Liuzzi, A.; Ilari, A.; Milelli, A.; Uliassi E.; Bolognesi, M. L. Targeted protein degradation for infectious diseases: from basic biology to drug discovery. ACS Bio Med Chem Au 2023, 3, 1, 32–45.
[2] T. Ishida, A. Ciulli. E3 Ligase ligands for PROTACs: how they were found and how to discover new ones. SLAS Discovery. 2021, 26(4) 484–502
[3] Yao, Y.; Huo, T.; Lin, Y.-L.; Nie, S.; Wu, F.; Hua, Y.; Wu, J.; Kneubehl, A. R.; Vogt, M.B.; Rico-Hesse, R.; Song, Y. Discovery, X-ray Crystallography and Antiviral Activity of Allosteric. Inhibitors of Flavivirus NS2B-NS3 Protease J. Am. Chem. Soc. 2019, 141, 6832–6836.

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Development of PROTACs for Cancer Treatment

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Keywords: PROTAC, organic synthesis, structural characterization, bromodomains

Bromodomain Adjacent To Zinc Finger Domain 2A (BAZ2A), also known as TIP5, is a large multi-domain protein involved in the silencing of a fraction of mammalian rRNAs. Such protein is the largest subunit of the nucleolar remodeling complex containing SNF2H (NoRC), and it is directly involved in the interaction with rDNA. NoRC functioning relies on the recognition of acetylated lysine 16 in histone 4 (acH4K16) by BAZ2A bromodomain, which cooperates with the adjacent PHD finger to recruit HDAC1, DNMT1, DNMT3, and SNF2H on rDNA and to establish repressed chromatin features[1][2]. Recently, TIP5 was found to be upregulated in late-stage prostate cancer. The pathological role of this protein can be ascribed to epigenetic alterations favoring aggressive phenotype and promoting stem cell-like features[3][4]. Thus, blocking the recognition of Kac exerted by the BAZ2A bromodomain can represent a valid strategy for treating late-stage prostate cancer (PCa). In this context, we developed two classes of BAZ2A bromodomain inhibitors through a structure-based drug design approach[5][6]. Although compounds presented a good target affinity, they did not show any relevant antiproliferative effect on the PCa cell population.Further experiments revealed that simple TIP5 bromodomain inhibition did not affect cellular growth, while complete ablation of such protein by siRNA slowed down cancer cell proliferation and impaired migration and invasion[3]. As a consequence, BAZ2A depletion results in a novel therapeutic approach for late-stage PCa, and it could be easily obtained by exploiting Proteolysis Targeting Chimeras (PROTACs). PROTAC technology takes advantage of a heterobifunctional molecule binding to a protein of interest (POI) and E3 ubiquitin ligase. By forcing the contact between the POI and an E3 ligase, degraders mediate target ubiquitination and consequent degradation through the 26S proteasome[7][8]. In recent years, a huge number of degraders were developed for bromodomains, suggesting a high adaptability of this class of proteins for PROTAC technology[9][10][11]. Thus, we employed the previously discovered hit compound[6] to develop the first BAZ2A PROTAC degrader, recruiting CRBN ubiquitin ligase. Currently, the organic synthesis of other degraders bearing different combinations of linkers and recruiters is ongoing. In the future, developed PROTACs will be structurally characterized in complex with BAZ2A bromodomain and E3 ubiquitin ligase. At the moment, CRBN and DDB1 are expressed in H5 insect cells; such proteins will be exploited in the X-ray crystallographic analysis. Molecules will be then tested for their therapeutic activity in prostate cancer cell lines, and degradation will be verified by western blotting. In conclusion, we developed the first BAZ2A PROTAC degrader that represents a valid alternative to reduce metastatic potential and avoid disease reoccurrence in late-stage prostate cancer.

References

[1] Y. Zhou, I. Grummt. The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing. *Curr Bio*. 2005, 15(15), 1434-1438.
[2] C. Tallant, E. Valentini, O. Fedorov, L. Overvoorde, F.M. Ferguson, P. Filippakopoulos, D.I. Svergun, S. Knapp, A. Ciulli. Molecular basis of histone tail recognition by human TIP5 PHD finger and bromodomain of the chromatin remodeling complex NoRC, *Structure*. 2015, 23,1, 80-93.[3] L. Gu, S.C. Frommel, C.C. Oakes, R. Simon, K. Grupp, C.Y. Gerig, D. Bär, M.D. Robinson, C. Baer, M. Weiss, Z. Gu, M. Schapira, R. Kuner, H. Sültmann, M. Provenzano, M.L. Yaspo, B. Brors, J. Korbel, T. Schlomm, R. Santoro. BAZ2A(TIP5) is involved in epigenetic alterations in prostate cancer and its overexpression predict disease reoccurrence. *Nat Gen*. 2015, 47(1), 22-30.

[4] R. Pena-Hernández, R. Aprigliano, S. Carina Frommel, K. Pietrzak, S. Steiger, M. Roganowicz, L. Lerra, J. Bizzarro, R. Santoro. BAZ2A-mediated repression via H3K14ac-marked enhancers promotes prostate cancer stem cells. *EMBO Rep*. 2021, 22(11).
[5] A. Dalle Vedove, G. Cazzanelli, J. Corsi, M. Sedykh, V.G. D'Agostino, A. Caflisch, G. Lolli. Identification BAZ2A Bromodomain Hit Compound by Fragment Joining. *ACS bio & med chem Au*. 2021, 1(1), 5-10.
[6] A. Dalle Vedove, G. Cazzanelli, L. Batiste, J.R. Marchand, D. Spiliotopoulos, J. Corsi, V.G. D'Agostino, A. Caflisch, G. Lolli. Identification of BAZ2A-Bromodomain Hit Compound by Fragment Growing. *ACS Med Chem Lett*. 2022, 13(9), 1434-1443.
[7] S. Zeng, W. Huang, X. Zheng, C. Liyan, Z. Zhang, J. Wang, J. Shen. Proteolysis targeting chimera (PROTAC) in drug discovery paradigm: Recent progress and future challenges. Eur J Med Chem. 2021, 210, 112981.
[8] K. Li, C.M. Crews. PROTACs: past, present and future. *Chem Soc Rev*. 2022, 51(12), 5214-5236.
[9] V. Zoppi, S.J. Hughes, C. Maniaci, A. Testa, T. Gmaschitz, C. Wieshofer, M. Koegl, K.M. Riching, D.L. Daniles, A. Spallarossa, A. Ciulli. Iterative Design and Optimization of Initially Inactive Proteolysis Targeting Chimeras (PROTACs) Identify VZ185 as Potent, Fast, and Selective von Hippel-Lindau (VHL) Baed Dual Degrader Prove of BRD9 and BRD7. *J Med Chem*. 2019, 69(2), 699-726.
[10] C. Wang, Y. Zhang, S. Yang, W. Chen, D. Xing. PROTACs for BRDs proteins in cancer therapy: a review. J Enzyme Inhib Med Chem. 2022, 37(1), 1694-1703.
[11] Y. Shi, Y. Liao, Q.Liu, Z. Ni, Z. Zhang, M. Shi, P. Li, H. Li, Y. Rao. BRD4-targeting PROTAC as a unique tool to study biomolecular condesates. *Cell Discov*. 2023, 9(1), 47.

AMYC BIOMED 2023

Development of polysaccharide-based nano-systems containing Lactoferrin for treatment of respiratory infections

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Keywords: Alginate, Hyaluronic acid, Lactoferrin, Nanocomplexes, Respiratory infections

Viral respiratory infections affect people of all ages, the emergence of new viruses and the selection of drug-resistant viral strains have prompted the development of new therapeutic strategies. The advent of nanotechnology in biomedicine has opened the way to new therapies for the delivery of drugs, proteins and nucleic acids. The small size provides these systems with excellent chemical-physical and pharmacokinetic properties, making it possible for them to transit inside small capillaries and cross biological barriers [1]. Among nano-carriers, the most promising in the biomedical field are those based on polysaccharides thanks to their biodegradability, bioactivity and biocompatibility as well as the presence of many functional groups that can be used to improve drug loading. This work was aimed at developing innovative therapies for the treatment of infection by respiratory viruses, such as influenza virus and adenovirus, and of the inflammatory processes caused by them. Lactoferrin (Lf) possesses many biological functions including antimicrobial, antitumor, anti-inflammatory activities [2]. Many studies have shown protective effects of lactoferrin against common viral infections [3]. Polysaccharide-based nanocarriers were developed in order to improve the lactoferrin therapeutic index. Alginate and hyaluronic acid were used to prepare two different nano-complexes for protein delivery using the complex coacervation method. The obtained systems were characterized by DLS technique to investigate their dimensions, polydispersity and stability. Morphological analysis, performed by scanning electron microscopy (SEM), confirmed the presence of nanostructures while thermal analysis (TGA) evidenced the formation of polysaccharide-protein interactions with an improvement in thermal stability (increase in T_D) of the nanocomplexes as protein concentration increased. Circular dichroism measurements displayed no conformational change of lactoferrin after sonication treatment or interaction with alginate and hyaluronic acid. By physicochemical characterization were identified the most promising complexes then subjected to cytotoxicity analysis by MTT test on two different cell lines (A549 and MDCK). Preliminary results were positive. Further tests are ongoing to evaluate the anti-inflammatory and antiviral activity of the nanocomplexes.

References

[1] J Solaro R., Chiellini F., Battisti A., 2010, Materials, **3**, 1928-1980.
[2] F. Superti, *Nutrients*, 2020,**12**, 2562. doi: 10.3390/nu12092562.
[3] Superti F., Ammendolia M. G., Valenti P., Seganti L., 1997, Med. Microbiol. Immunol., **186**, 83-91.

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Targeting the adenosinergic axis in cancer immunotherapy: design and synthesis of small molecules as CD73 inhibitors

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Keywords: ecto-5'-nucleotidase, CD73, small molecule inhibitors, cancer immunotherapy

In recent years, the blockade of the adenosinergic pathway has attracted a great deal of attention as an innovative strategy in the fight against cancer. It is well known that hypoxia and extracellular stress induce a series of metabolic and immunological modifications within the tumor microenvironment that contribute to tumor growth and development. Oxygen deficiency, for example, leads to the extracellular accumulation of ATP and, consequently, of adenosine. The conversion of ATP to adenosine is catalyzed by the ectonucleotidases CD39 (ectonucleoside triphosphate diphosphohydrolase 1), and CD73 (ecto-5'-nucleotidase). In particular, CD73 catalyzes the dephosphorylation of AMP to adenosine, which binds to its A_{2A} and A_{2B} receptors, stimulating profound immunosuppressive and anti-inflammatory responses. Thus, tumor cells exploited these immunosuppressive effects of adenosine to escape the antitumor immune responses. Furthermore, adenosine receptors are expressed on tumor cells, where their stimulation induces and increases cell proliferation and migration. Indeed, among various strategies, drugs targeting adenosine receptors involved in tumor development and/or adenosine-generating enzymes could represent a therapeutic breakthrough as they can interfere with negative feedback mechanisms activated by the tumor on immune cells. Preclinical studies on monoclonal antibodies (MAbs) or small molecules demonstrated that inhibition of CD73 reduces tumor growth and migration [1]. Considerable advances have been made in the design of nucleotide-based CD73 inhibitors, but only a few classes of less-acidic, non-nucleotide small molecules targeting this enzyme are available to date. Although X-ray crystal structures of CD73 in complexes with various inhibitors were reported, the large structural variability of these compounds and the paucity of in-depth structure-activity relationships (SAR) studies make it difficult to identify recurring structural features important for highly potent inhibitors [2]. Thus, on the basis of the data reported in the literature, novel heterocyclic compounds belonging to different chemical classes were designed and synthesized. These compounds are endowed with a heterocyclic core that mimics the adenine portion of AMP for engaging π-π interactions with Phe500 and Phe417 at the CD73 binding site. Moreover, the aromatic core was decorated with suitable substituents to expand the interaction pattern with key amino acids. The Malachite Green Phosphate assay was used to evaluate the CD73 inhibitory activity of the newly synthesized compounds. Some of the compounds tested showed detectable inhibitory activity towards CD73, thus this preliminary study can be considered a good starting point for the development of more active compounds.

References

[1] D. Vijayan, A. Young, M.W.L. Teng, M. J. Smyth. Targeting immunosuppressive adenosine in cancer. Nat. Rev. Cancer. 2017, 17, 709-724.
[2] A. Nocentini, C. Capasso, C. T. Supuran. Small-molecule CD73 inhibitors for the immunotherapy of cancer: a patent and literature review (2017-present). Expert. Opin. Ther. Pat. 2021, 31, 867-876.

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Exploiting the potential of hybrid nanoparticles as nucleic acid carrier in pulmonary diseases

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Keywords: hybrid nanoparticles, nucleic acid, polycations, nano-embedded microparticles

In recent years the absence of effective therapeutic treatments in the case of chronic respiratory diseases has pushed the research interest toward the development of innovative therapeutic approaches based on the administration of nucleic acids *via* inhalation (1). In fact, this administration route offers many advantages that can help to overcome the limitation imposed by the physiochemical nature of nucleic acids (NAs). For instance, a specific delivery to the pulmonary tissue where the pharmacological target is located, with high availability of the drug at the target site and limited systemic toxicity. With this idea in mind, our group has successfully developed hybrid nanoparticles constituted with a poly (lactic-co-glycolic acid) (PLGA) core and a shell of dipalmitoyl phosphatidyl choline (DPPC) one of the major components of the pulmonary surfactant to increase the biocompatibility of the system for the delivery a siRNA against the NFkB gene (2,3)
To exploit the full potential of hNPs, the aim of this work is the validation of the developed nanoplatforms for delivery of high molecular weight RNAs to the lungs. To this purpose, a formulation study was carried out to adapt the production method to the encapsulation of a model mRNA and circular RNA, adding different polycations to the system as encapsulation enhancers. The nanoparticles were produced through an emulsion-solvent diffusion technique and were fully characterized in terms of size, PDI and ζ potential as well as for the encapsulation efficiency. All the formulations showed a size around 140 nm with a very low PDI value and a negative charge. The addition of polycation in the hNPs resulted in a significant improvement of both the encapsulation efficiency and the release kinetics of the entrapped NAs. From a translational perspective, a comparative study between freeze-drying and spray-drying techniques was carried out to find the most suitable conditions to obtain a stable dry product to be reconstituted in media for pulmonary administration through nebulizers. Nano-embedded microparticles (NEM) were achieved in the presence of different inert carriers, that is mannitol or trehalose. Their potential for pulmonary delivery was fully demonstrated upon in vitro aerosolization test strongly supporting their advancement towards evaluation *in vitro*.

References

[1] Chow MYT, Qiu Y, Lam JKW. Inhaled RNA Therapy: From Promise to Reality. Trends Pharmacol Sci. 2020; 41(10):715-729.
[2] d'Angelo I, Costabile G, Durantie E, Brocca P, Rondelli V, Russo A, Russo G, Miro A, Quaglia F, Petri-Fink A, Rothen-Rutishauser B, Ungaro F. Hybrid Lipid/Polymer Nanoparticles for Pulmonary Delivery of siRNA: Development and Fate Upon In Vitro Deposition on the Human Epithelial Airway Barrier. J Aerosol Med Pulm Drug Deliv. 2018 Jun;31(3):170-181.
[3] Conte G, Costabile G, Baldassi D, Rondelli V, Bassi R, Colombo D, Linardos G, Fiscarelli EV, Sorrentino R, Miro A, Quaglia F, Brocca P, d'Angelo I, Merkel OM, Ungaro F. Hybrid Lipid/Polymer Nanoparticles to Tackle the Cystic Fibrosis Mucus Barrier in siRNA Delivery to the Lungs: Does PEGylation Make the Difference? ACS Appl Mater Interfaces. 2022 Feb 16;14(6):7565-7578.

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Delivery of AuNCs into extracellular vesicles using fusogenic liposomes cargos

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Keywords: extracellular vesicles; fluorescent nanoclusters; drug delivery system; imaging; membrane modification

Extracellular vesicles (EVs) are well-known membrane-limited particles secreted by healthy and cancerous cells. EVs have heterogeneous size and three subtypes are described depending on the location of secretion: microvesicles, myelinosomes and exosomes. EVs are identified in human follicular fluid as a mode of communication in the ovarian follicle.[1] In addition EVs involved in cell-cell communication are considered as biomarkers for early cancer diagnosis. The analysis of their content and their labeling with easily detectable nanoparticles could enable the development of a powerful tool for the early diagnosis of specific diseases

In this view, gold nanoclusters (AuNCs) appear as a recent class of non-toxic fluorophores. Their brightness, their ultrasmall size (< 2 nm) and large window of fluorescence lifetime (1ns - 1µs) and their good biocompatibility make them an attractive alternative as fluorescent probes for biological labeling and bioimaging.[2-5] In addition, their ultra-small size facilitates their clearance when they are injected into the body. Their small size makes them attractive to encapsulate them into liposomes without damaging the compartment integrity and then to be delivered into the extracellular vesicles. We previously demonstrate that their direct attractive electrostatic interaction with extracellular vesicles result into the labelling of their membrane or disruption of the EVs into multilamellar structures.[6]

We investigated encapsulation of ultra-small-sized red and blue emitting Au NCs into liposomes of various sizes and chemical compositions. The efficiency of the process was correlated to the structural and morphological aspect of the AuNCs encapsulating vesicles via complementary analyses by SAXS, cryo-TEM, and confocal microscopy techniques. We confirmed the possible encapsulation of ultra-small-sized red and blue emitting Au NCs into liposomes of various sizes and chemical compositions. Cell-like-sized vesicles (GUVs) encapsulating red or blue Au NCs were successfully obtained by an innovative method using emulsion phase transfer. Finally, exosome-like-sized vesicles (LUVs) containing Au NCs were obtained with an encapsulation yield of 40%, as estimated from ICP-MS.[7]

Herein, we investigate the interaction of fusogenic liposomes encapsulating the AuNCs with EVs extracted from human seminal fluids. The composition of the membrane was optimized to improve the fusion properties of the liposomes. The mixture of the liposomes and EVs results in lipid exchange as demonstrated by FRET experiments and in increased size revealed by flux cytometry. The resulting fused hybridosomes were successfully isolated by size exclusion chromatography. They exhibit typical fluorescence of the AuNCs together with an increased size in agreement with the expected size of the fused exosomes. Besides their interest to analyze the EVs content, such hybridosomes could serve as drug or macromolecule carriers or nanosensors in living organisms.

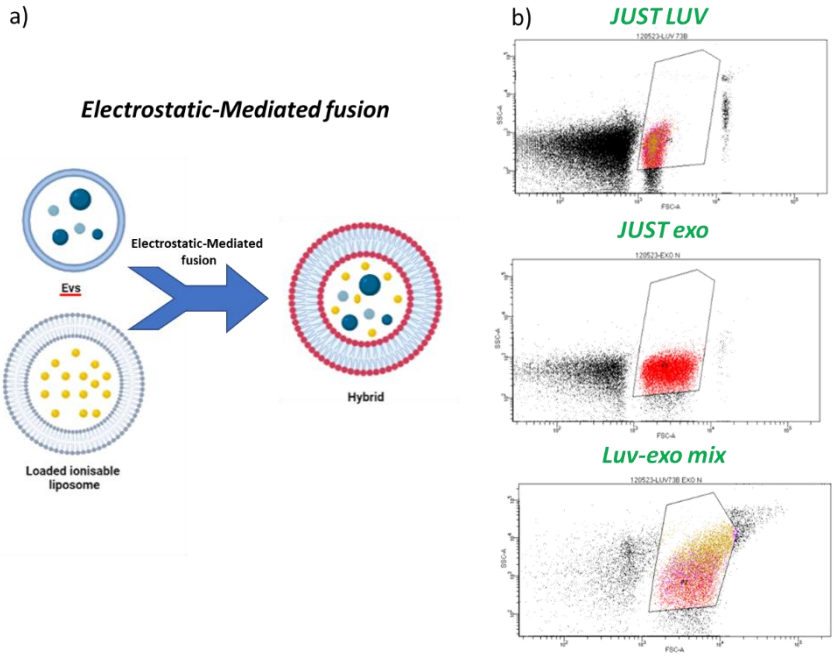

Figure 1. *a) Schematic approach of the fusion approach b) Preliminary results observed by Flux-Cytometry*

References

[1] A.S. Neyroud, R. M. Chiechio, M. Yefimova, J.M. Lo Faro, N. Dejucq-Rainsford, S. Jaillard, P. Even-Hernandez, V. Marchi, C. Ravel. Extra-cellular vesicles of the male genital tract: new actors in male fertility. Basic and Clinical Andrology. 2021, 31, 25.
[2] R. M. Chiechio, R. Le Guevél, S. Ducarre, H. Solhi, S. Dutertre, X. Pinson, J.P. Bazureau, O. Mignen, P. Even-Hernandez, P. Musumeci, C. Ravel, M. J. Lo Faro, V. Marchi. Active U11 Peptide Luminescent Gold Nanoclusters for Pancreatic Tumor Cell Targeting. ACS Appl. Nano Mater. 2023, 6, 10, 8971–8980.
[3] J. Wang, J. Ye, H. Jiang, S. Gao, W. Ge, Y. Chen, C. Liu, C. Amatore, X. Wang. Simultaneous and Multisite Tumor Rapid-Target Bioimaging through in Vivo Biosynthesis of Fluorescent Gold Nanoclusters. RSC Adv. 2014, 4, 71, 37790–37795.
[4] Nonappa. Luminescent gold nanoclusters for bioimaging applications. Beilstein J. Nanotechnol. 2020, 11, 533–54.
[5] H. Cui, Z.S. Shao, Z. Song, Y.B. Wang, H.S. Wang. Development of Gold Nanoclusters: From Preparation to Applications in the Field of Biomedicine. J. Mater. Chem. C, 2020, 8, 14312—14333.
[6] R. M. Chiechio, S. Ducarre, G. Moulin, A. Dupont, C. Marets, P. Even-Hernandez, F. Artzner, P. Musumeci, G. Franzò, C. Ravel, M. J. Lo Faro, V. Marchi. Luminescent Gold Nanoclusters Interacting with Synthetic and Biological Vesicles. J. Phys. Chem. Lett. 2022, 13, 30, 6935–6943
[7] R. M. Chiechio, S. Ducarre, C. Marets, A. Dupont, P. Even-Hernandez, X. Pinson, S. Dutertre, F. Artzner, P. Musumeci, G. Franzò, C. Ravel, M. J. Lo Faro, V. Marchi. Encapsulation of Luminescent Gold Nanoclusters into Synthetic Vesicles. Nanomaterials. 2022,12, 21, 3875.

AMYC BIOMED 2023

Low-cost benchtop approaches for the manufacturing of nanostructured platforms for bioanalytical applications

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Keywords: Lab-made devices; nanomaterials; electrochemical sensors, biosensors

In the electro(bio)analytical scenario the use of commercial screen-printed electrodes is highly widespread, nevertheless, they present limitations regarding their default design, rigid substrate, and analytical performance. Recently, different low-cost strategies to fabricate (bio)devices have emerged as smart alternatives to the more expensive and cumbersome clean-room and lithography-based methods. In particular, the manufacturing of tailored analytical devices integrating nanomaterials (NMs) based conductive films is still a hot topic; to overcome tedious, expensive, and not sustainable conventional fabrication techniques, several efforts are devoted to implementing effective and affordable technologies to produce nanostructured analytical devices. In this framework, low-cost substrates/materials and emerging manufacturing technologies, represent a captivating opportunity, and although much progress has been made, there are still unexplored rooms.
This presentation will be focused on using functional NMs, mainly produced by avoiding the use of solvents and pollutant chemicals, as building blocks for the implementation of completely lab-made analytical devices. An overview of nanomaterials' sustainable production, nano architectures assembling, and their integration into freestanding conductive films, flexible sensors and biosensors, and all-in-one devices will be given. Attention will be paid to the fabrication of devices integrating 0D, 1D, and 2D NMs using low-cost substrates as flexible polymers, thermoplastic sheets, cellulosic components, and benchtop microfabrication technologies as xurography, thermal lamination, and CO_2 laser scribing and cutting. The development of completely lab-made second- [1] and third generation [2] flexible enzymatic biosensors, and laser-nanostructured paper-based devices hosting live cells will be presented, together with their application in agri-food and biological field. Will be shown the application of the proposed devices for the determination of (i) pesticides, (ii) fructose in foods and urines, and the (iii) enzyme-free real-time detection of hydrogen peroxide released by chemically stimulated cancer cells.
The main goal of this presentation is to prove how NMs produced using emerging sustainable strategies can be easily integrated into tailorable cutting-edge bioanalytical devices manufactured via within everyone's reach technologies.

References

[1] Silveri, F., Della Pelle, F., Scroccarello, A., Bukhari, Q. U. A., Del Carlo, M., & Compagnone, D. (2022). Modular graphene mediator film-based electrochemical pocket device for chlorpyrifos determination. Talanta, 240, 123212.
[2] Silveri F., Paolini D., Della Pelle F., Bollella P., Scroccarello A., Suzuki Y., Fukawa E., Sowa K., Di Franco C., Torsi L., Compagnone D. (2023). Lab-made flexible third-generation fructose biosensors based on 0D-nanostructured transducers. Biosensors and Bioelectronics, 115450, DOI: 10.1016/j.bios.2023.115450

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Design of plasmonic nanoparticles as functional fillers for 3D printable biocompatible photopolymers

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Keywords: gold nanoparticles; photo-curable polymers; stereolithography; plasmonic nanocomposites; micro-structurable plasmonic hydrogel

To date, noble-metal nanoparticles (NPs) have gained significant attention in the biomedical sector due to their unique combination of chemical and physical properties, that can accomplish both diagnostic (exploiting localized surface plasmon resonance) and therapeutic (exploiting plasmonic hyperthermic effect) functions for a wide range of diseases. Among all, gold NPs (AuNPs) are one of the most widely studied nanosystems thanks to their biocompatibility chemical stability and easy synthesis at low cost with different size and shape, thus enabling to tune their plasmonic properties. In addition, standard functionalization protocols can be implemented to engineer gold NPs outer surfaces, in order to guarantee high affinity towards specific biomolecules as well as high colloidal stability, even in highly complex matrices such as biological fluids. [1]

More recently, the research on gold NPs applied to the biomedical field has been focusing on structural combination of polymer hydrogels network with NPs, with the promise of provide biocompatible composite materials, showing enhanced cell adhesion and differentiation, [2] photo-switchable conductivity and swelling properties. [3] As a step forward, NPs can be loaded in photo-curable polymer matrix, toobtainphoto-curable nanocomposites, that can be nano/micro-structured through 3D printing approaches, thus enabling high design versatility of functional microstructures. [4] For this purpose, efforts are still needed to engineer NPs surface, in order to guarantee plasmonic properties retention upon interaction with polymer matrices, as well as to finely control NPs trapping on the matrix.

In this scenario, the present work is focused on the development and optimization of AuNPs-loaded photopolymers, aimed at obtaining 3D printable hybrid materials with plasmonic properties, and switchable features, for the fabrication of biocompatible structures for cell culture supports and sensing. To this end, AuNPs of different morphologies, ranging from isotropic nanospheres to hyperbranched nanostars, were synthesized and further engineered to have photo-curable pendant moieties, guaranteeing at once colloidal stability and NPs chemical trapping via co-polymerization with the hydrogel matrix. NPs functionalization was achieved by implementing one-step and two-steps strategies, involving ligand-exchange properties based on thiol-gold high chemical affinity. Morphological and chemical characterization attested that NPs engineering was successfully achieved avoiding morphological changes, a critical point especially for multi-branched stars. Finally, to demonstrate their feasibility as filler for 3D structurable materials, AuNPs with photocurable pendant have been loaded at different concentration with a biocompatible synthetic hydrogel (polyethylene glycol diacrylate, PEGDA) processed by laser-based 3D stereolithography to obtain proof-of-concept NPs-loaded structures.

References

[1] B. Sharma et al. Materialstoday, 2012, 15 (1-2), 16-25
[2] G.Kaur et al. RSC Adv., 2015, 5, 37553-37567
[3] A. G. Skirtach et al. Nano Lett. 2005, 5, 1371
[4] C. García-Astrain et al. Adv. Funct. Mater. 2020, 30, 2005407

If you get the switch you get the control: OSCP subunit as an emerging therapeutic target for the PTP modulation

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Keywords: Protein structure, NMR, ATP synthase

The Permeability Transition Pore (PTP) is a Ca^{2+}-triggered channel, detected in the inner mitochondrial membrane, whose opening leads to cellular death through apoptosis [1]. PTP dysregulated activity is directly related to aging and several human diseases as Alzheimer's and cancer [2]. Even though the molecular structure of PTP has not been fully elucidated, experimental evidences indicate that ATP synthase is most likely the PTP main component [3].
The ATP synthase subunit OSCP (Oligomycin Sensitivity Conferral Protein) is an emerging therapeutic target for its putative role as the molecular switch for the regulation of PTP [3]. In fact, OSCP is the binding site for Cyclophilin D (CypD), the immunomodulatory benzodiazepine Bz423 and the mitochondrial inhibitor IF1 [4] which are three PTP modulators. Targeting the OSCP to achieve a drug-based control of the PTP opening represents an interesting therapeutic perspective. A huge limit in this sense is made by the possibility to manage the isolated OSCP in solution. Unfortunately, OSCP is described to be very prone to non-specific aggregation when it is disconnected from the rest of the ATP synthase, as well as its C-terminal domain OSCP-CT [5]. Only the N-terminal domain of the protein was obtained in solution and then characterized by NMR spectroscopy [5].
Here we report the first biophysical characterization of both OSCP-CT and OSCP subunit in solution, due to the synergic use of NMR spectroscopy, Bio-SAXS and native-mass spectrometry. Unlike that reported in literature, we obtained the recombinant C-terminal domain and the full protein through pET SUMO expression strategy. In this way, we found that both protein constructs do not aggregate in solution but they are involved in an unexpected oligomerization equilibrium in which the monomeric state seems to be not highly populated. We therefore designed a peptide for stabilizing the monomeric OSCP-CT as well as the full protein. Overall, our results make the OSCP suitable for further interactions studies in solution, with both novel and old interactors. Our work will help to strengthen the role of OSCP as the first protein target for the regulation of PTP activity.

References

[1] P.Bernardi et al. From ATP to PTP and back. A dual function for the mitochondrial ATP synthase. Circ. Res. 2015, **116**, 1850-1862.
[2] C.Galber et al. The ATP Synthase Deficiency in Human Diseases. Life. 2021, **11**, 1-19.
[3] V.Giorgio et al. Cyclophilin D Modulates Mitochondrial F0F1-ATP Synthase by Interacting with the Lateral Stalk of the Complex. The J. of Bio. Chem. 2019, **284**, 33982-33988.
[4] S.Fabbian et al. The mitochondrial inhibitor IF1 binds to the ATP synthase OSCP subunit and protects cancer cells from apoptosis. Cell Death & Dis. 2023, **14**.
[5] R.J.Carbajo et al. Structure of the F1-binding Domain of the Stator of Bovine F1Fo-ATPase and How it Binds an α-Subunit. JMB. 2005, **351**, 824-838.

AMYC BIOMED 2023

Physicochemical and technological studies of nanoparticles loaded with rutin/2-hydroxypropyl-β-cyclodextrin inclusion complex

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Keywords: Natural products, cyclodextrin, thermodynamics, nanoparticles, drug delivery

Rutin (RUT), a glycoside of the flavonoid quercetin, is found in many plants and fruits, especially buckwheat, apricots, cherries, grapes, grapefruit, plums, and oranges. Despite being endowed with the ability to elicit a strong inhibitory effect on the growth of several tumour cell lines, RUT application as an active molecule per se is severely restricted by its inherent hydrophobicity and consequent low *in vivo* bioavailability. A possible way to unravel the therapeutic potential of RUT is to enhance its solubility by forming a host–guest complex with a suitable cyclodextrin. Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic cavity [1,2]. In this context, the aim of this work was to create a between RUT/semisynthetic 2-hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complex. A thorough physicochemical study of RUT-HPβCD complex formation has been performed by means of phase solubility, differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC) [3]. Thereafter, the complex was encapsulated within amphiphilic polymeric nanoparticles (NPs) based on poly(lactic-coglycolic acid) (PLGA) using nanoprecipitation technique. NPs were endowed with active targeting ability by coating the surface with hyaluronic acid (HA), which was chosen based on its tropism toward CD-44 receptor that is overexpressed in a wide array of solid tumours [4,5].
Outcomes of the physicochemical investigations suggest the formation of host-guest inclusion complex with a 1:1 stoichiometry. The mean size of both control and RUT-loaded NPs formulated was < 220 nm with a narrow size distribution (IP < 0.2) and a negative zeta potential (-20 ÷ -40 mV). The produced NPs will be tested for their release kinetics and *in vitro* biological activity toward selected tumour cells, using healthy cells as a control.

References

[1] Naeem A, Yu C, Zang Z, Zhu W, Deng X, Guan Y. Synthesis and Evaluation of Rutin-Hydroxypropyl β-Cyclodextrin Inclusion Complexes Embedded in Xanthan Gum-Based (HPMC-g-AMPS) Hydrogels for Oral Controlled Drug Delivery. Antioxidants. 2023,12(3):552
[2] Satari A, Ghasemi S, Habtemariam S, Asgharian S, Lorigooini Z. Rutin: A Flavonoid as an Effective Sensitizer for Anticancer Therapy; Insights into Multifaceted Mechanisms and Applicability for Combination Therapy. Evid Based Complement Alternat Med.2021,2021:9913179.
[3] Usacheva, T., Kabirov, D., Beregova, D. et al. Thermodynamics of complex formation between hydroxypropyl-β-cyclodextrin and quercetin in water–ethanol solvents at T = 298.15 K. J Therm Anal Calorim.2019; 417–424 (2019).
[4] Serri C, Quagliariello V, Iaffaioli RV, Fusco S, Botti G, Mayol L, Biondi M. Combination therapy for the treatment of pancreatic cancer through hyaluronic acid-decorated nanoparticles loaded with quercetin and gemcitabine: A preliminary in vitro study. J Cell Physiol. 2019,234(4):4959-4969
[5] Yunqi Zhao, Ti Zhang, Shaofeng Duan, Neal M. Davies, M. Laird Forrest. CD44-tropic polymeric nanocarrier for breast cancer targeted rapamycin chemotherapy. Nanomedicine: Nanotechnology, Biology and Medicine.2014, Volume 10, Issue 6,Pages 1221-1230

AMYC BIOMED 2023

In silico analysis of Fibroblast Activation Protein ligand selectivity

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Keywords: FAP, Molecular Modeling, SAR, and ligand selectivity

Fibroblast activation protein alpha (FAP) is a serine protease belonging to the dipeptidyl peptidase (DPP) protein family. FAP has no crucial roles in physiological processes and is over-expressed in tumors, where it is involved in angiogenesis and remodeling of the structure and composition of extracellular matrix. It is therefore a promising target for developing selective covalent inhibitors with antitumoral activity, among which radiopharmaceuticals with theragnostic activity useful for tumor imaging [1]. Although FAP presents a high sequence homology (>70% in the active site) with DPP-IV, a common target for anti-diabetic drugs, it is also characterized by an endopeptidase activity that can be exploited for ligand selectivity [2]. Through an *In-Silico* analysis of more than 90 experimental 3D structures in complex with both small molecules and peptide-mimetic covalent and non-covalent inhibitors, we rationalize the available ligand structure-activity relationships and the observed FAP inhibitor selectivity.

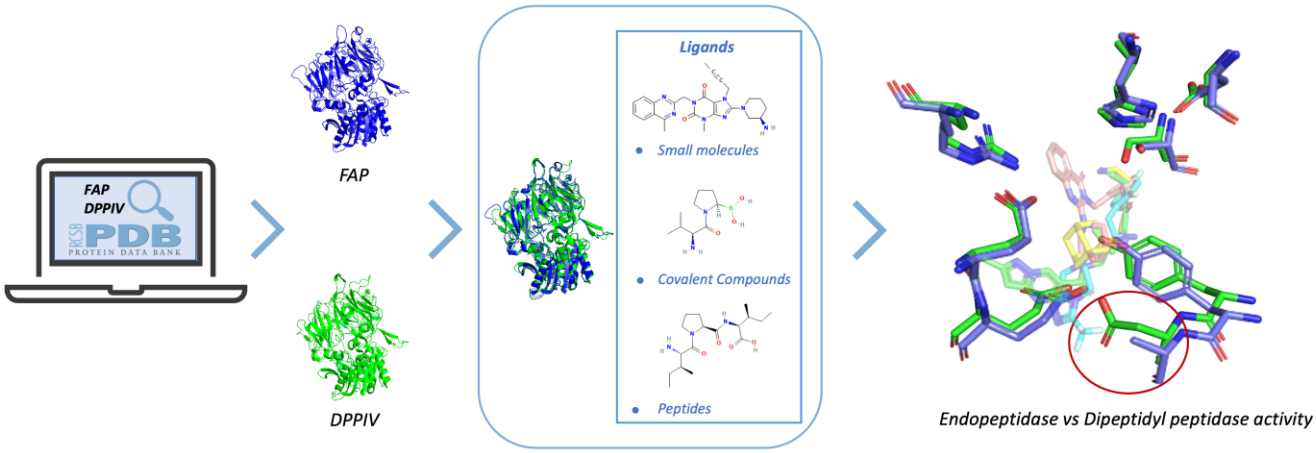

Figure 1 Graphical Abstract: FAP and DPPIV X-Ray structures were retrieved from the Protein Data Bank. A In-Silico study of interactions with different ligands (small molecules, covalent compounds, and peptides) revealed new structural insights into ligand recognition and selectivity.

References

[1] A. Šimková, P. Bušek, A. Šedo, and J. Konvalinka, "Molecular recognition of fibroblast activation protein for diagnostic and therapeutic applications," *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, vol. 1868, no. 7, p. 140409, Jul. 2020, doi: 10.1016/j.bbapap.2020.140409.

[2] E. J. Hamson, F. M. Keane, S. Tholen, O. Schilling, and M. D. Gorrell, “Understanding fibroblast activation protein (FAP): Substrates, activities, expression and targeting for cancer therapy,” *Proteomics Clin Appl*, vol. 8, no. 5–6, pp. 454–463, Jun. 2014, doi: 10.1002/prca.201300095.

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Solid Lipid Nanoparticles Hydroquinone-Based for the Treatment of Melanoma: Efficacy and Safety Studies

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Keywords: melanoma; solid lipid nanoparticles; hydroquinone; stearic acid

Classical melanoma therapy has several side effects that are responsible for a decrease in the final therapeutic efficacy. It is possible that the drug is degraded before reaching the target site and is metabolized by the body itself, resulting in repeated doses being administered throughout the day and a decrease in patient compliance [1,2]. Drug delivery systems avoid degradation of the active ingredient, improve release kinetics, prevent the drug from being metabolized before reaching the site of action, and improve the safety and efficacy profiles of adjuvant cancer therapy [3,4,5]. The solid lipid nanoparticles (SLNs) based on hydroquinone esterified with stearic acid realized in this work represent a chemotherapeutic drug delivery system that is useful in the treatment of melanoma. The starting materials were characterized by FT-IR and ^{1}H-NMR, while the SLNs were characterized by dynamic light scattering. In efficacy studies, their ability to influence anchorage-dependent cell proliferation was tested on COLO-38 human melanoma cells. Furthermore, the expression levels of proteins belonging to apoptotic mechanisms were determined by analyzing the role of SLNs in modulating the expression of p53 and p21WAF1/Cip1. Safety tests were conducted to determine not only the pro-sensitizing potential but also the cytotoxicity of SLNs, and studies were conducted to assess the antioxidant and anti-inflammatory activity of these drug delivery.

References

[1] Saginala, K.; Barsouk, A.; Aluru, J.S.; Rawla, P.; Barsouk, A. Epidemiology of Melanoma. Med. Sci. 2021, 9, 63.
[2] Zeng, H.; Li, J.; Hou, K.; Wu, Y.; Chen, H.; Ning, Z. Melanoma and Nanotechnology-Based Treatment. Front. Oncol. 2022, 12, 858185.
[3] Attia, M.F.; Anton, N.; Wallyn, J.; Omran, Z.; Vandamme, T.F. An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J. Pharm. Pharmacol. 2019, 71, 1185–1198
[4] Cassano, R.; Cuconato, M.; Calviello, G.; Serini, S.; Trombino, S. Recent Advances in Nanotechnology for the Treatment of Melanoma. Molecules 2021, 26, 785.
[5] Cassano, R.; Serini, S.; Curcio, F.; Trombino, S.; Calviello, G. Preparation and Study of Solid Lipid Nanoparticles Based on Curcumin, Resveratrol and Capsaicin Containing Linolenic Acid. Pharmaceutics 2022, 14, 1593

Synthesis of fluorescent nanomaterials based on halloysite for potential fluorescence imaging

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Keywords: halochromic switch, halloysite nanotubes, covalent modification, bottom-up synthesis, top-down approach

In the last years, nanomaterials have attracted increasing interest for biomedical applications. Nanomaterials are particularly attractive for being tunable systems, with the possibility to add novel functionalities that open up their use both in drug delivery and therapy for diagnostic.
In particular the latter is an emerging technique to fight cancer. Commonly for this purpose several organic fluorophores have been used. However, most of the commonly used chromophores are hydrophobic and therefore their use in the biological field is limited. To overcome this problem, several carrier systems were often employed. Recently, natural inorganic carriers such as halloysite nanotubes (HNTs) are attracted considerable attentions for applications in bioimaging since this clay increases the chromophore solubility in physiological conditions and boosts the fluorescent properties.[1] In addition, compared to other nanoparticles, halloysite is naturally occurring, eco and bio-compatible. In the last years, the supramolecular loading of fluorescent molecules into HNTs lumen or onto the external surface was reported.[2]
Herein we report two different approaches for the covalent modification of HNTs external surface with an halochromic switch to develop biological probes. The first approach is a top-down one in which the probe was first synthesized and, then linked to previously modified HNTs. Conversely, the second approach foresees the synthesis of the halochromic switch directly onto halloysite external surface.
The obtained nanomaterials were characterized by different physical-chemical techniques and their morphology was investigated by transmission electron microscopy (TEM) as well. Furthermore, the photoluminescent properties of the nanomaterials were investigated both in solution and in solid state.

References

[1] M. Massaro, M. Notarbartolo, F. M. Raymo, G. Cavallaro, G. Lazzara, M. A. Mazza, C. Viseras-Iborra, S. Riela. Supramolecular Association of Halochromic Switches and Halloysite Nanotubes in Fluorescent Nanoprobes for Tumor Detection. ACS Appl. Nano Mater. 2022, 5, 10, 13729–13736.
[2] S. Riela, A. Barattucci, D. Barreca, S. Campagna, G. Cavallaro, G. Lazzara, M. Massaro, G. Pizzolanti, T. M. G. Salerno, P. Bonaccorsi, F. Puntoriero. Boosting the properties of a fluorescent dye by encapsulation into halloysite nanotubes. Dyes Pigm. 2021, 187, 109094.

Comparison of the biological effects of Gadodiamide (Omniscan) and Gadoteridol (ProHance) by means of multi-organ and plasma metabolomics

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Keywords: Gadolinium-based contrast agents (GBCA), multi-organ metabolomics, plasma metabolomics, multivariate data analysis

Among the metal ion-based contrast agents, Gadolinium ones are the most massively employed in radiology to increase the sensibility of magnetic resonance imaging, giving the strong paramagnetic properties of gadolinium ions.[1] During the past decades, increasing warnings about the release of free gadolinium cations from linear Gadolinium-based contrast agents (GBCAs) have been raised, questioning the use of these compounds without any potential harmful effects.[2] However, investigations aiming at detecting possible metabolic perturbations or potential adverse health effects due to gadolinium deposition are still lacking. Thus, the aim of the present work was to exploit a multi-organ (liver, kidney, spleen, cerebrum and cerebellum) and plasma metabolomics approach to investigate the effects of multiple administrations of one linear and less stable, Omniscan (gadiodiamide), and one macrocyclic and more stable GBCA, ProHance (gadoteridol), on the main metabolic pathways in healthy mice processes in the context of tissues Gadolinium retention. Aqueous organs extracts were analyzed by GC-MS while plasma samples analysis was carried out by ^{1}H-NMR. Multivariate analysis on the collected data revealed that plasma metabolome was not differently perturbed by the two GBCAs. For this reason, plasma analysis alone cannot be considered exhaustive enough to understand the alteration of the physiological state of the mouse model. On the contrary, the multiorgan metabolomics analysis displayed a clear separation of the Omniscan-treated group from the control and the ProHance-treated ones, suggesting that treatment with Omniscan actually perturbs the physiological state of the mouse model. Interestingly, the observed biochemical alterations seemed to be organ-related since some organs where most affected than others by the Omniscan treatment. Indeed, in brain, cerebellum and liver an up-regulation of the energetic pathways, as well as a dysregulation of the amino acids and nucleotide metabolism was observed. The results of this study clarify, for the first time, the toxic effects related to the use of a linear GBCA vs macrocyclic one, by identifying the organs most affected by the administration of the investigated chelates. Thus, this work paves the way both to the safest use of the commercially available GBCAs and to the development of new GBCAs characterized by lower general toxicity. Moreover, it suggests that the metabolomics approach can be considered as a valid additional tool for the evaluation of the toxicity of the investigated GBCAs.

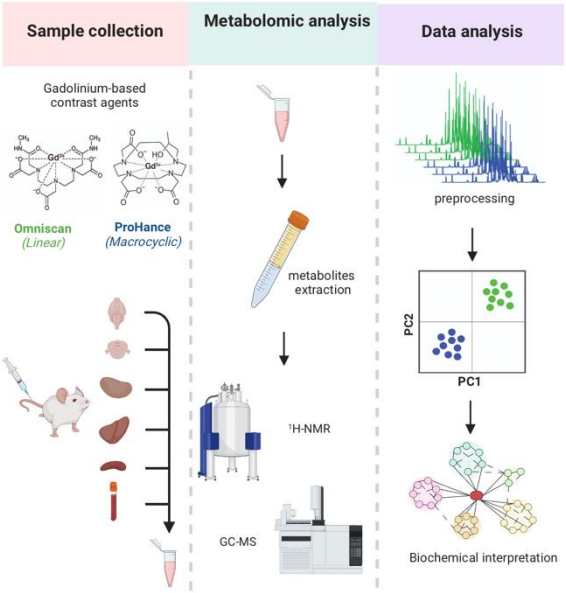

Figure. Workflow employed for the metabolomics analyses.

References

[1] C. Do, J. DeAguero, A. Brearley, X. Trejo, T. Howard, G. P. Escobar, B. Wagne. Gadolinium-Based Contrast Agent Use, Their Safety, and Practice Evolution. Kidney 360. 2020, 1, 561-568

[2] M. A. Sieber, H. Pietsch, J. Walter, W. Haider, T. Frenzel, H. Weinmann. A Preclinical Study to Investigate the Development of Nephrogenic Systemic Fibrosis: A Possible Role for Gadolinium-Based Contrast Media. Invest. Radiol. 2008, 43, 65-75

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Structural studies of two anticancer metallodrugs with B-DNA double helical dodecamer

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Keywords: antitumor drug, platinum, DNA interaction, rhodium complex

Cisplatin and its analogues carboplatin and oxaliplatin continue to be extensively used, as single agents or in combination with other drugs, for the treatment of different types of cancer. [1] The cytotoxic activity of these Pt-based drugs is due to their interaction with DNA. Indeed, these molecules covalently bind two adjacent guanines in the major groove, kink the DNA duplex, interfering with DNA replication and transcription processes. [2] Unfortunately, the use of Pt-based anticancer agents is associated with undesirable side effects. [3] As a result, new Pt-based drugs or agents based on different metals have been studied. In this frame, arsenoplatin, AP-1 (with a Pt-As bond), [4] and dirhodium tetraacetate, $[Rh_2(\mu\text{-}O_2CCH_3)_4]$ (with a Rh-Rh bond), [5] are of particular interest. Although there are studies in the literature on the interaction between these metallodrugs and model proteins, [4,6] information on their interaction with DNA is very poor. To obtain insights into the mechanism of action and the mode of binding of these two compounds with DNA, the X-ray structures of the adducts formed upon reaction of AP-1 and of $[Rh_2(\mu\text{-}O_2CCH_3)_4]$ with a B-DNA double helical dodecamer were solved and refined. Two structures of the AP-1/DNA adduct at different soakings times were obtained. In the adduct formed after a soaking of 4h, two binding sites were identified: close to Gua14, the Pt-As bond is preserved, while close to Gua10, the Pt-As bond is broken. In contrast, in the adduct formed after 48h soaking, close to Gua2 the Pt-As bond is intact, while close to Gua10 and Gua14 only Pt atoms were found. These results indicate that Pt-As bond can break upon the interaction with the double helix. The X-ray structure of dirhodium/DNA adduct reveals a bimetallic centre bound to an adenine via axial coordination. [7] A search in the Protein Data Bank reveals that this structure represents the first structural example of a Rh/DNA adduct and a rare example of a Rh compound/DNA complex. Overall, these findings highlight how two anticancer metallodrugs can differently react with the DNA double helix.

References

[1] S. Dasari, and P. B. Tchounwou. Cisplatin in cancer therapy: Molecular mechanisms of action. European Journal of Pharmacology. 2014, 740, 364-378.
[2] E. R. Jamieson and S. J. Lippard. Structure, Recognition, and Processing of Cisplatin–DNA Adducts. Chem. Rev. 1999, 99, 2467–2498.
[3] F. Muggia. Platinum compounds 30 years after the introduction of cisplatin: Implications for the treatment of ovarian cancer. Gynecologic Oncology. 2009, 112, 275-281.
[4] Đ. Miodragović, A. Merlino, E. P. Swindell, A. Bogachkov, R. W. Ahn, S. Abuhadba, G. Ferraro, T. Marzo, A. P. Mazar, L. Messori, and T. V. O'Halloran. Arsenoplatin-1 Is a Dual Pharmacophore Anticancer Agent. J. Am. Chem. Soc. 2019, 141, 16, 6453–6457.
[5] R. Hrdina. Dirhodium (II, II) Paddlewheel Complexes. Eur. J. In. Chem. 2021, 6, 501.
[6] G. Ferraro, A. Pratesi, L. Messori, and A. Merlino. Protein interactions of dirhodium tetraacetate: a structural study. Dalton Trans. 2020, 49, 2412-2416.
[7] G. Tito, R. Troisi, G. Ferraro, A. Geri, L. Massai, L. Messori, F. Sica, and A. Merlino. Dirhodium tetraacetate binding to a B-DNA double helical dodecamer probed by X-ray crystallography and mass spectrometry. Dalton Trans. 2023, 52, 6992-6996.

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Macromolecular crowding effects on enzymatic kinetics and diffusion in the extracellular matrix

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Keywords: Extracellular matrix, enzymatic kinetics, macromolecular crowding, Human neutrophil elastase

The extracellular matrix (ECM) is the non-cellular component that builds up all tissues and organs, composed of water and macromolecules, such as elastin [1]. Fundamental to an organism's health, ECM must be continuously remodelled, mainly through the action of enzymes. So densely populated by macromolecules, ECM is an example of crowded environment, a complex system whose properties are hardly reproducible in vitro. Shedding light on how crowding affects enzymatic activity in the ECM is the objective of X-Crowd, a project the authors of this work are part of.
It's an international project funded by the French institution Agencie Nationale de la Recherche (ANR), which involves physicists, biologists and chemists working side by side. Some of its members had previously developed the first comprehensive kinetic theory of the late stage of hyaluronic acid degradation [2].
X-Crowd focuses on investigating the kinetics of key ECM enzymes under controlled substrate concentrations and crowding conditions. By performing enzyme kinetics and diffusion experiments, the goal is to understand how crowding affects the dynamics and explore potential explanations, such as excluded-volume effects and substrate-environment interactions.

This work is focused on the kinetic behaviour of human neutrophil elastase (HNE), a serine protease present in the ECM, responsible for the degradation of elastin. Experimental tests were conducted using spectrophotometry to monitor the degradation by HNE of a synthetic small substrate (MeoSuc-Ala-Ala-Pro-Val-AMC), whose diffusion properties were examined through NMR spectroscopy. Both experiments were carried out with and without crowders, such as dextrans.
To assess the impact of crowding, a model capable of predicting long-term elastase kinetics for different substrate concentrations was developed. While the serine protease enzymatic reaction is generally understood as a three-step mechanism [3], unexpected dynamic behavior was observed even in the absence of crowders when fitting long time data series at different substrate concentrations simultaneously. Not questioning the well understood three-step mechanism, this led to the introduction of kinetic models relying on multiple enzymatic conformations, an idea supported by existing evidence in the literature [4], or further substrate-enzyme interactions.
These models help explain the observations and investigate the impact of crowding on diffusion and kinetics experiments. An attempt is made to establish a comprehensive understanding of how crowding influences these experimental outcomes.

References

[1] C. Frantz, K. M. Stewart, V. M. Weaver . The extracellular matrix at a glance. J. Cell. Sci. 2010, 123, 4195-4200.
[2] R. Nehmé, R. Nasreddine, L. Orlic, C. Lopin-Bon, J. Hamacek, F. Piazza. Kinetic theory of hyaluronan cleavage by bovine testicular hyaluronidase in standard and crowded environments. Biochim Biophys Acta. 2021, 1865, 3.
[3] L.Hedstrom. Serine Protease Mechanism and Specificity. Chem. Rev. 2002, 102, 4501–4523.
[4] R. L. Stein. Catalysis by Human Leukocyte Elastase. 4.1 Role of Secondary-Subsite Interactions. J. Am. Chem. Soc. 1985, 107, 5767-5775.

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Synthesis and pharmacological evaluation of hybrid molecules containing fibrates and the semi-synthetic bile acid derivative BAR502

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Keywords: BAR 502, GPBAR1, FXR, PPAR-α, NASH

The development of hybrid compounds represents an interesting approach for the discovery of new therapeutic strategies, allowing to obtain a single synergistic "*super molecule*", that retains the pharmacological actions of each counterpart or is endowed with higher effect than the sum of each individual parts.[1] In this poster, we will report the synthesis and the biological evaluation of a library of hybrid compounds prepared by combining, through a condensation reaction, some fibrates, famous/known PPAR-α ligands, with **BAR502**, a semisynthetic bile acid analogue; active as dual FXR/GPBAR1 agonist and represents a promising lead for the treatment of cholestasis and NASH.[2,3] The aim of this project is to combine the effects of these two pharmacologically active entities/components for the treatment of complex pathology for example NASH or NAFLD, through the modulation of multiple receptors involved in these diseases. The library of BAR502-fibrate conjugates was prepared exploiting the hydroxyl groups on **BAR502** and the carboxylic moiety of fibrates as conjugation points through a condensation reaction, obtaining dimers or monomers (Figure 1). Compound **1**, obtained by conjugation of clofibric acid at C-23 of BAR502, resulted the most promising molecule of the series, and was subjected to further pharmacological investigation, together with stability evaluation and cell permeation assessment. We have proved by LC-MS analysis that compound 1 is hydrolyzed in mice releasing clofibric acid and BAR505, the oxidized metabolite of BAR502, endowed with retained dual FXR/GPBAR1 activity.

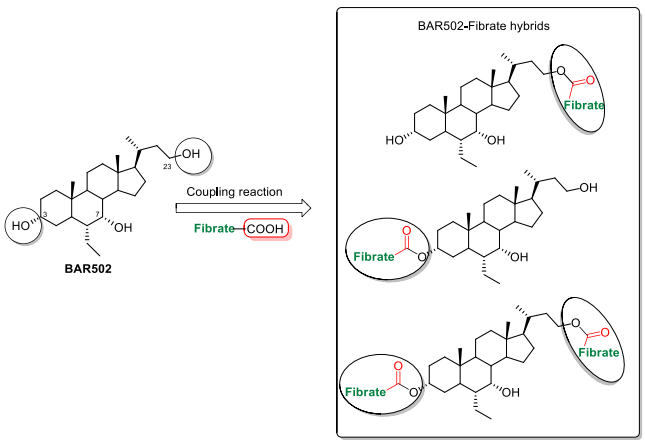

Figure 1. BA-fibrate hybrids sketch

References

[1] Navacchia, M. L.; Marchesi, E.; Perrone, D. Bile acid conjugates with anticancer activity: most recent research. *Molecules* **2021**, 26, 25.
[2] Carino, A. et al. BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. *Sci. Rep.* 7, 42801; **2017.**
[3] Festa, C. et al. Exploitation of cholane scaffold for the discovery of potent and selective farnesoid X receptor (FXR) and G-protein coupled bile acid receptor 1 (GP-BAR1) ligands. *J* Med Chem 57, 8477−95; **2014**.

AMYC BIOMED 2023

Highly efficient electrochemiluminescence from imidazole based thermally activated delayed fluorescence emitters

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Keywords: Electrochemiluminescence, Thermally Activated Delayed Fluorescence

Electrochemiluminescence (ECL) is a process that uses an applied electrical bias to generate reactive species at an electrode surface that undergo subsequent electron-transfer reactions to generate luminescent species [1]. This process could be used to amplify signals of analytes, making this technique the most powerful tool for biomedical applications.
However, due to spin statistics only phosphorescent emitters such as Ru(II) [2] complexes could be used efficiently to harvest triplet states thanks to the strong spin-orbit coupling which favors intersystem crossing. Recently a new "metal-free" strategy was proposed to harvest triplet excitons to enhance Internal Quantum Efficiency (IQE): the Thermally Activated Delayed Fluorescence (TADF) [3], which is a phenomenon shown by molecules that possess a tilted donor-acceptor structure where HOMO and LUMO are spatially separated. From this spatial separation follows a small energy gap between S_1 and T_1 states that makes possible reverse intersystem crossing and enhanced IQE, making these molecules intriguing not only for OLED applications [3], but also for solution-state ECL [4,5].
However, almost all examples about ECL of TADF molecules are performed in the so-called "annihilation way", where compounds are subsequently oxidized and reduced, while there are only few examples of ECL of TADF molecules in presence of a coreactant [4,5].
Herein we present a family of novel TADF emitters bearing a fixed acceptor moiety, 4-(1H-imidazol-1-yl)benzonitrile, where donors were systematically varied in order to correlate photophysical and electrochemical properties with their performance in electrochemiluminescence in presence of benzoyl peroxide as coreactant.
Moreover, the imidazole moiety can be efficiently alkylated with chains bearing functional group, such as tert-butyl esters, enabling practical applications.

References

[1] M. M. Richter. Electrochemiluminescence. Chem. Rev. 2004, 104, 3003-3006.
[2] N. E. Tokel, A. J. Bard. Electrogenerated chemiluminescence. IX. Electrochemistry and emission from systems containing tris(2,2'-bipyridine)ruthenium(II) dichloride. J. Am. Chem. Soc. 1972, 94, 2862-2863.
[3] H. Uoyama, K. Goushi, K. Shizu, H. Nomura, C. Adachi. Highly efficient organic light-emitting diodes from delayed fluorescence. Nature, 2012, 492, 234-238.
[4] P. Huang, X. Zou, Z. Xu, Y. Lan, L. Chen, B. Zhang, L. Niu. Studies on Annihilation and Coreactant Electrochemiluminescence of Thermally Activated Delayed Fluorescent Molecules in Organic Medium. Molecules, 2022, 27, 7457.
[5] S. Kumar, P. Tourneur, J. R. Adsetts, M. Y. Wong, P. Rajamalli, D. Chen, R . Lazzaroni, P. Viville, D. B. Cordes, A. M. Z. Slawin, Y. Olivier, J. Cornil, Z. Ding, E. Zysman-Colman. Photoluminescence and electrochemiluminescence of thermally activated delayed fluorescence (TADF) emitters containing diphenylphosphinechalcogenide-substituted carbazole donors. J. Mater. Chem. C, 2022, 10, 4646-4667.

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Functional biofilms based on alginate polymer filled with modified halloysite for active food packaging

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Keywords: Active Food Packaging, Halloysite Nanotubes, Antimicrobial, Antioxidant, Alginate

Nowadays, food spoilage due to microbial contamination represents a significant problem, which every year causes enormous economic loss. To prevent bacteria, biofilms based natural polymers with antimicrobial properties have been prepared for active food packaging applications.[1]
Lately, the possibility to increase the physico-chemical properties of the biofilm was investigated by adding fillers to the polymeric matrix. Among the different fillers which can be used, inorganic nanoparticles have attracted considerable attention.[2]
Halloysite nanotubes (HNTs) are natural clay minerals with a hollow tubular structure, consisting of a negatively charged outer surface composed of siloxane groups (Si-O-Si), and a positively charged lumen composed of aluminol groups (Al-OH). Due to the different chemical composition and presence of an empty lumen, HNTs can be selectively modified onto the surfaces obtaining nanocarriers and nanofillers with improved interfacial properties with different biopolymers.[3,4]
Herein we report the synthesis and characterization of alginate based bionanocomposites with antioxidant and antimicrobial properties for potential application in the active food packaging field. Firstly, HNTs, chosen as fillers, were modified *ad hoc* with suitable organic molecules both to increase the mechanical properties of the biopolymer and to confer antioxidant and antimicrobial properties to the final bionanocomposite. Furthermore, the possibility to load biologically active molecules into HNTs was also exploited.

References

[1] F. Versino, F. Ortega, Y. Monroy, S. Rivero, O.V. López, M.A. García. Sustainable and Bio-Based Food Packaging: A Review on Past and Current Design Innovations. *Foods.* 2023, *12*, 1057.
[2] O.M. Atta, S. Manan, A. Shahzad, M. Ul-Islam, M.Y. Ullah, G. Yang. Food Packaging Materials with Special Reference to Biopolymers-Properties and Applications. Food Hydrocoll. 2022, *125*, 107419.
[3] M. Massaro, R. Ciani, G. Cinà, C.G. Colletti, F. Leone, S. Riela. Antimicrobial Nanomaterials Based on Halloysite Clay Mineral: Research Advances and Outlook. *Antibiotics.* 2022, *11*, 1761.
[4] L. Qingqing, R. Tian, P. Perkins, H. Xiaofeng, W. Xiaolong. Applications of halloysite nanotubes in food packaging for improving film performance and food preservation. Food Control. 2021, *124*, 107876.

Development of potential HDAC6-targeting PROTACs for the treatment of idiopathic pulmonary fibrosis

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Keywords: PROTACs, HDAC6, epigenetics, IPF, medicinal chemistry

Proteolysis-targeting chimeras (PROTACs) are emerging as a promising class of "beyond rule of five" degraders, capable of triggering targeted protein degradation (TPD) by hijacking the ubiquitin-proteasome system. This strategy overcomes the traditional target inhibition-associated limitations, granting a new therapeutic approach to tackle complex diseases. Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease, characterized by a poor prognosis. The underlying fibrogenic process leads to irreversible tissue degeneration and stiffening, resulting in chronic hypoxia. Histone deacetylase 6 (HDAC6) belongs to the HDAC superfamily of epigenetic erasers and its overexpression has been highlighted in the IPF phenotype [1,2]. Indeed, this peculiar cytosolic isoform plays a pivotal role in the onset and progression of the disease. Evidence showed that **NF2376** - an indoline-based selective HDAC6 inhibitor (HDAC6i) - had beneficial effects in reverting fibrogenesis [2]. Given that PROTAC-induced TPD has proved to be valuable for its antiproliferative effect on several cancer cell lines, we decided to explore its capability in an IPF fibrotic phenotype, which shares several key features with tumor cells. An explorative library of potential HDAC6-targeting PROTACs was designed exploiting **NF2376**, a selective in-house HDAC6i as the protein of interest (POI) ligand, and pomalidomide as the cereblon (CRBN)-recruiting E3 ligase ligand. Different linkers were selected to screen the optimal length and composition needed to achieve significant HDAC6 degradation. Herein, we report the design and synthesis of **NF2376-based HDAC6-targeting PROTACs**, and the preliminary results of their ongoing biological evaluation. Enzymatic inhibition and immunoblotting assays were carried out to assess their ability to inhibit and/or degrade the POI. Most PROTACs were able to induce HDAC6 degradation in the A549 cell line and the most interesting ones completely abolished HDAC6 expression after 30 h incubation. Further investigations regarding the compounds' mode of action and selectivity profile are currently underway and will lead to the identification of the most promising derivatives.

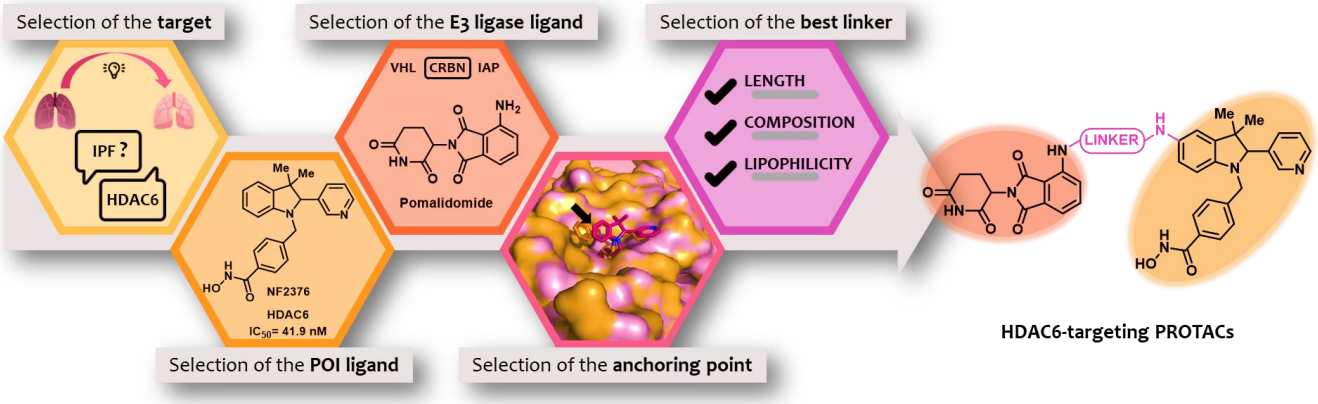

Figure 1. Design of **NF2376-based HDAC6-targeting PROTACs**.

References

[1] M. Korfei, *et al*. Targeting Histone Deacetylases in Idiopathic Pulmonary Fibrosis: A Future Therapeutic Option. Cells. 2022, 11(10), 1626.

[2] G. Campiani, *et al*. Harnessing the Role of HDAC6 in Idiopathic Pulmonary Fibrosis: Design, Synthesis, Structural Analysis, and Biological Evaluation of Potent Inhibitors. J Med Chem. 2021, 64(14), 9960–9988.

AMYC BIOMED 2023

Synthesis of covalent inhibitors targeting m6a reader proteins

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Keywords: Covalent drug, ebselen, warhead, reader proteins, m6A

N6-Methyladenosine (m6A) is a highly prevalent and conserved mRNA modification controlled by a wide number of methyltransferases (writers) and demethylases (erasers). Additionally, a group of proteins known as readers can recognize and selectively bind the modified m6A RNAs regulating their biological functions. Among these, YTHDF proteins share a highly conserved and hydrophobic pocket, known as the YTH domain and responsible of the recognition of the m6A modification.
Despite being generally considered undruggable, in the last year the very first inhibitors were published among which we reported the first covalent inhibitor for DF1 and DF2 proteins. [1]
My Ph.D. project aims to design and synthesize a library of selective and potent compounds that can disrupt the m6A recognition process in YTHDF protein. Derived from an HTS screening, we identified Ebselen, an organoselenium compound, as a promising lead compound.
Ebselen (figure 1a) is an organoselenium compound that contains an electrophilic selenium atom able to form a Se-S bond with free thiol of cysteine residues. X-ray analysis on the YTHDF1-Ebselen complex shows that the formation of the covalent bond (figure 1c) causes a conformational rearrangement of part of the protein disrupting the YTH-m6A recognition pocket (figure 1b).

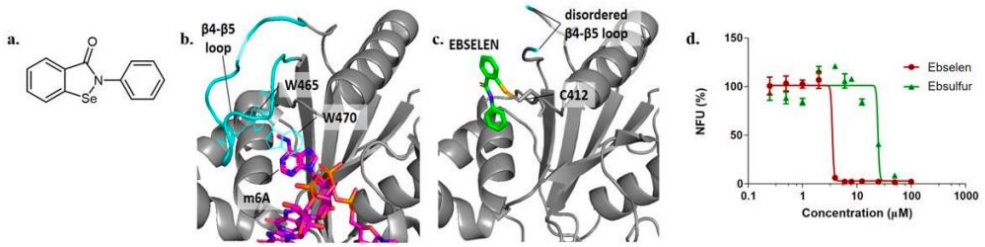

Figure 1. a) Ebselen b) Methylated RNA (purple) bound by YTHDF1. The recognition pocket is a hydrophobic region mainly composed of tryptophan residues (blue residues) – PDB 4RCJ; c) Ebselen bound to the cysteine near m6A recognition binding pocket – PDB 7PCU; d) Ebselen and its sulfur analog Ebsulfur vs YTHDF1 in Tryptophan Fluorescence Quenching assay. [1]

Due to its covalent mechanism of action, the optimization of Ebselen must consider both the strength of its electrophilic moiety and the specific interactions it can establish with the target protein. To decrease warhead reactivity, I initially replaced the selenium warhead with a sulfur atom which forms with Cys412 an S-S covalent bond and, even in this case, this disrupts the capacity of YTHDFs to recognize and bind m6A-modified RNAs (figure 1d.). Thus, guided by docking calculation, I have synthesized a small library of selenium and sulfur analogs which have been evaluated for their affinity to YTHDF1 protein by X-ray and orthogonal biochemical assays. Crystallographic data has revealed the influence of various substituents on the relative position of the analogs within the YTH domain, providing valuable insights for the ongoing development of novel analogs. Besides, biochemical assays have further confirmed the ability of these analogs to interfere with the m6A-YTHDF recognition process.

References

[1] M. Micaelli, et al. Small-Molecule Ebselen Binds to YTHDF Proteins Interfering with the Recognition of N6-Methyladenosine-Modified RNAs; ACS Pharmacol. Transl. Sci., 5, 2022, p. 872-891.

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Drug-repurposing campaign targeting the fatty acids pocket of SARS-CoV-2 Spike protein

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Keywords: SARS-CoV-2; RBD; Fatty Acids pocket; Docking; Molecular Dynamics

The Severe Acute Respiratory Syndrome-CoronaVirus-2 (SARS-CoV-2) infection has forced the scientific community to unprecedented efforts to explore all possible approaches against COVID-19 and the virus's evolving variants [1]. One of the main antiviral strategies was pursued to inhibit the viral entry, which is mediated by the binding of trimeric Spike proteins' Receptor Binding Domain (RBD) to the Angiotensin-Converting Enzyme 2 (ACE2) receptor PD domain [2]. Our research group has previously identified a series of natural and semisynthetic steroids targeting predicted RBD drug binding sites that are less likely to mutate [3]. Subsequently, Toelzer et al. have uncovered a hydrophobic, highly-conserved binding site named the Fatty Acids (FA) pocket able to allocate free fatty acid molecules, as Linoleic Acid [4]. Such FA-pocket is crucial to maintain the RBD's domain "closed state", thus indirectly hampering the RBD/ACE2 interaction. Given such findings, we have conducted a second Drug-Repurposing campaign of an in-house library followed by long Molecular Dynamics simulations (MDs) to understand if the FA pocket could be a putative binding site also for natural and semisynthetic steroids and to assess the stability of their binding mode. Based on the MDs analyses, it appears that the two most stable families of natural steroids are UDCA and UDCOH derivatives, as observed during 1μs simulations. Through in vitro experiments, we have confirmed our initial findings and used them to design and synthesize potentially more powerful steroidal derivatives.

References

[1] R. Cannalire, I. Stefanelli, C. Cerchia *et al.* SARS-CoV-2 Entry Inhibitors: Small Molecules and Peptides Targeting Virus or Host Cells. Int. J. Mol. Sci. 2020, 21, 5707.
[2] N.M. Beeraka, S.P. Sadhu, S.V. Madhunapantula *et al.* Strategies for Targeting SARS CoV-2: Small Molecule Inhibitors-The Current Status. Front. Immunol. 2020, 11, 552925.
[3] A. Carino, F. Moraca, B. Fiorillo *et al.* Hijacking SARS-CoV-2/ACE2 Receptor Interaction by Natural and Semi-synthetic Steroidal Agents Acting on Functional Pockets on the Receptor Binding Domain. Front. Chem. 2020, 8, 572885.
[4] Toelzer, C., Gupta, K., N. Yadav, Free fatty acid binding pocket in the locked structure of SARS-CoV-2 spike protein. Science. 2020, 370(6517), 725–730.

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Enzyme-Based Catalytic Methods for Real-Time Monitoring of Urea

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Keywords: Urea, Biosensors, FIA, Potentiometry, Bioreactor

Enzymatic catalytic processes have the potential to reduce pollutants in wastewater treatment facilities and promote sustainable bioremediation strategies. Monitoring urea levels in wastewater is crucial due to its widespread use as a fertilizer and cattle feed supplement[1], which contributes to environmental contamination through soil runoff, leading to algal blooms and eutrophication[2]. In this study, we propose a real-time urea measurement method that combines enzyme reactors with flow injection analysis (FIA) potentiometric analysis. FIA-based bioreactors are ideal for continuous monitoring and automated sample processing. Various bioreactors were constructed by immobilizing enzymes onto solid-phase materials (e.g., glass beads, plastic tube inner walls)[3] and connected to solid-state ammonium sensors for electrochemical readings. Analytical performance was assessed based on factors such as cross-linker concentration, immobilized enzyme quantity, and flow rate. The FIA system was optimized to achieve maximum signal in minimal time, and key analytical parameters were evaluated. Ultimately, the FIA system should be fully automated, enabling its installation at strategic locations within wastewater treatment plants for real-time monitoring.

References

[1] S.N. Botewad, D.K. Gaikwad, N.B. Girhe, H.N. Thorat and P.P. Pawar. Urea biosensors: A comprehensive review. Biotechnol. Appl. Biochem. 2021, 70(2), 485-501.
[2] P.M. Glibert, J. Harrison, C. Heil, and S. Seitzinger. Escalating worldwide use of urea–a global change contributing to coastal eutrophication. Biogeochemistry. 2006, 77, 441-463.
[3] K. S. Mangaldas, Y. S. Rajput, and R. Sharma. Urease immobilization on arylamine glass beads and its characterization. J. Plant Biochem. 2010, 19, 73-77.

AMYC BIOMED 2023

Red emitting thermally activated delayed fluorescence emitters for electrochemiluminescence applications.

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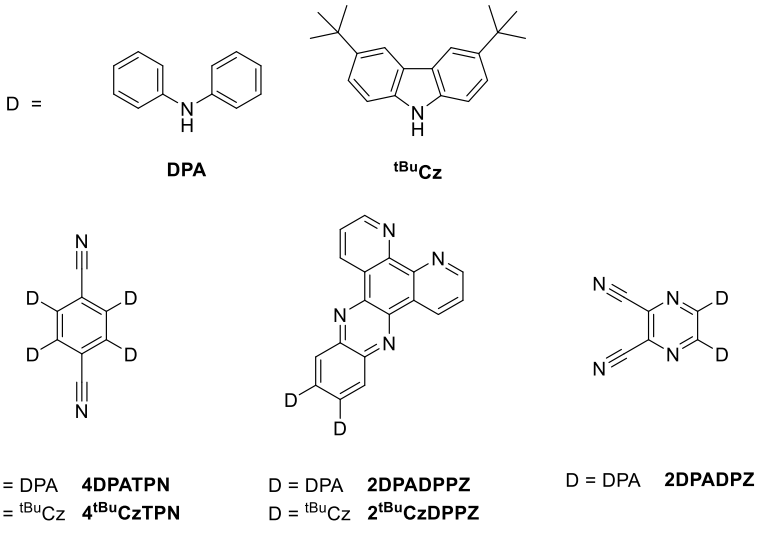
Keywords: Electrochemiluminescence, TADF, red emitters, bioimaging

Electrochemiluminescence (ECL) can be considered one of the most powerful analytical techniques going from environmental analysis[1] to (bio)markers[2], to single molecule imaging[3]. Nevertheless, in a ECL system, the excitons are produced through a non-radiative, electrochemical process, resulting in a statistical population of singlet and triplet states in a 1:3 ratio, limiting the efficiency of purely fluorescent compounds. Phosphorescent transition metal complexes represent efficient emitters thanks to the strong spin orbit coupling that favors intersystem crossing allowing both singlet and triplet excited states to contribute to the emission[4]. Recently, a new strategy to harvest triplet excitons was reported, namely Thermally Activated Delayed Fluorescence (TADF). Typically, organic TADF emitters consist in a twisted donor-acceptor (D-A) structure, that gives rise to a small singlet-triplet energy gap (ΔE_{ST}) allowing theoretically 100% internal quantum efficiency thanks to thermally activated reverse intersystem crossing (RISC). Since the seminal work of Adachi and colleagues[5], this new class of emitters have gained increasing attention as an alternative to heavy metal phosphorescent complexes for several applications, such as OLEDs[6] and photocatalysis[7], and only recently for ECL applications[8,9,10]. Starting from a previous work[11], diphenylamine (DPA) and terbutyl carbazole (^{tBu}Cz) have been chosen as donor moieties, since they displayed electrochemical reversibility towards oxidation. As acceptor moieties, terephthalonitrile (TPN), dipyridilphenazine (DPPZ) and dicyanopyrazine (DPZ) have been tested to obtain reduction potentials suitable for the use of tripropylamine (TPrA) as co-reactant ($E°_{TPrA*}$ = -1.7 V vs Ag/AgCl). The emitters have been synthetized towards a unified metal-free procedure (Scheme 1).



Scheme 1 Donor precursors and synthetized compounds.

Cyclic voltammetries, photoluminescence quantum yields and excited state lifetimes of the compounds in solution were recorded. Finally, the compounds were tested as dyes in ECL using TPrA as co-reactant and their efficiencies have been determined in referment to the gold standard $Ru(bpy)_3(PF_6)_2$. The ECL performances of the compounds have been correlated with their optical and electrochemical properties. Interestingly, 4DPATPN, was found to be the best performing luminophore, with ECL efficiency double the one of the standard.

References

[1] Z. Wang, J. Pan, Q. Li, Y. Zhou, S. Yang, J. J. Xu, D. Hua. Improved AIE-Active Probe with High Sensitivity for Accurate Uranyl Ion Monitoring in the Wild Using Portable Electrochemiluminescence System for Environmental Applications. Adv. Funct. Mater., 2020, 30, 2000220.
[2] P. Nikolaou, E. L. Sciuto, A. Zanut, S. Petrarca, G. Valenti, F. Paolucci, L. Prodi, S. Conoci. Ultrasensitive PCR-Free Detenction of Whole Virus Genome by Electrochemiluminescence. Biosens Bioelectron., 2022, 209, 114165
[3] Y. Liu, H. Zhang, B. Li, J. Liu, D. Jiang, B. Liu, N. Sojic. Single Molecule Imaging by Electrochemiluminescence. J. Am. Chem. Soc., 2021, 143 (43), 17910-17914.
[4] C. Adachi, M. A. Baldo, M. E. Thompson, S. R. Forrest. Nearly 100% internal phosphorescence efficiency in organic light-emitting device. J. Appl. Phys., 2001, 90 (10), 5048-5051.
[5] H. Uoyama, K. Goushi, K. Shizu, H. Nomura, C. Adachi. Highly Efficient Organic Lught-Emitting Diodes from Delayed Fluorescence. Nature, 2012, 492 (7428), 234-238.
[6] M. Y. Wong, E. Zysman-Colman. Purely Organic Thermally Activated Delayed Fluorescence Materials for Organic Light-Emitting Diodes. Adv. Mater., 2017, 29 (22), 1605444.
[7] M. A. Bryden, E. Zysman-Colman. Organic Thermally Activated Delayed Fluorescence (TADF) Compounds Used in Photocatalysis. Chem. Soc. Rev., 2021, 50, 7587-7680.
[8] R. Ishimatsu, S. Matsunami, T. Kasahara, J. Mizuno, T. Edura, C. Adachi, K. Nakano, T. Imato. Electrogenerated CHemiluminescence of Donor-Acceptor Molecules with Thermally Activated Delayed Fluorescence. Angew. Chem. Int. Ed., 2014, 53 (27), 6993-6996.
[9] P. Huang, X. Zou, Z. Xu, Y. Lan, L Chen, B. Zhang, L. Niu. Studies on Annihilation and Coreactant Electrochemiluminescence of Thermally Activated Delayed Fluorescent Molecules in Organic Medium. Molecules, 2022, 27 (21), 7457.
[10] Z. Chu, J. R. Adsetts, Z. Whitworth, S. Kunar, E. Zysman-Colman. Elucidation of an Aggregate State in th Electrochemiluminescence and Chemiluminescence of a Thermally Activated Delayed Fluorescence (TADF) Emitter. Langmuir, 2023, 39, 2829-2837.
[11] Submitted article.

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Towards cost-effective side-chain isotope labelling of proteins expressed in human cells

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Keywords: Isotope labelling, Side-chain labelling, In-cell NMR, Mammalian protein expression

Side chain isotope labelling is a powerful tool to study protein structure and interactions [1]. $^{1}H,^{13}C$ labelling of side-chain methyl groups in a deuterated background allows for extremely sensitive NMR experiments, useful to study large molecules that would be otherwise undetectable. On the other hand, side-chain aromatic groups are highly sensitive to the interactions with ligands, drugs, and other proteins. Moreover, such non-uniform labelling schemes greatly simplify the spectra of large proteins [2].
In *E. coli*, side chain labelling is typically performed by substituting amino acids with the cognate labeled precursors to the growth medium, as labeled precursors are more cost effective than labeled amino acids [2] [3] [4] [5]. Precursors (α-ketoacids) are carboxylic acids with a ketone group in the α position. The precursors are converted into the respective amino acids by bacterial transaminases, which substitute of the ketone group with an amino group.
Although this method is extremely effective, some proteins can only be produced in mammalian cells, which need to be supplemented with expensive isotope-labelled amino acids. Some transaminases are known to be expressed in mammalian cells [6] [7]. Therefore, we sought to establish a simple and cost-effective method to label side chains in mammalian cells which exploits the reversible reaction catalysed by the transaminases. We accomplished this by providing human HEK293T cells with culture medium enriched with a labelled α-ketoacid precursor and lacking the corresponding amino acid. We then tested CAII heterologous expression in the above custom-made medium and compared it with that obtained with regular culture medium, to assess the quantity of precursor needed to obtain the same expression levels. The amino acid incorporation was then confirmed via NMR spectroscopy both in intact cells and in cell lysates. Three proteins were investigated: two globular proteins, carbonic anhydrase II (CAII) and the deglycase DJ-1, and an intrinsically disordered protein, α-synuclein.
The resulting in-cell NMR spectra showed well-resolved peaks corresponding to the side chain-labelled amino acids incorporated in the protein, which were better resolved in the lysate NMR spectra. Conversely, the signals from α-synuclein were highly overlapped with the cellular background signal, due to the poor chemical shift dispersion typical for intrinsically disordered proteins. We furthermore validated the efficacy of the method for the combination of multiple precursors in the same culture medium, showing that the expression and incorporation levels remained unchanged.

References

[1] R. A. Rodriguez-Mias and M. Pellecchia, 'Use of Selective Trp Side Chain Labelling To Characterize Protein–Protein and Protein–Ligand Interactions by NMR Spectroscopy', *J. Am. Chem. Soc.*, 2003, vol. 125, no. 10, pp. 2892–2893.
[2] R. Lichtenecker, M. L. Ludwiczek, W. Schmid, and R. Konrat, 'Simplification of Protein NOESY Spectra Using Bioorganic Precursor Synthesis and NMR Spectral Editing', *J. Am. Chem. Soc.*, 2004 vol. 126, no. 17, pp. 5348–534.
[3] N. K. Goto, K. H. Gardner, G. A. Mueller, R. C. Willis, and L. E. Kay, 'A robust and cost-effective method for the production of Val, Leu, Ile (δ1) methyl-protonated 15N-, 13C-, 2H-labeled proteins', *J. Biomol. NMR* , 1999, vol 13, pp. 369-374.

[4] R. J. Lichtenecker, N. Coudevylle, R. Konrat, and W. Schmid, ‘Selective Isotope Labelling of Leucine Residues by Using α-Ketoacid Precursor Compounds’, *ChemBioChem*, 2013, vol. 14, no. 7, pp. 818–821.
[5] R. J. Lichtenecker, K. Weinhäupl, W. Schmid, and R. Konrat, ‘α-Ketoacids as precursors for phenylalanine and tyrosine labelling in cell-based protein overexpression’, *J. Biomol. NMR*, 2013, vol. 57, no. 4, pp. 327–331.
[6] F. Caligiore *et al.*, ‘Human cytosolic transaminases: side activities and patterns of discrimination towards physiologically available alternative substrates’, *Cell. Mol. Life Sci.*, 2022, vol. 79, no. 8, p. 421.
[7] A. Peracchi and E. Polverini, ‘Using Steady-State Kinetics to Quantitate Substrate Selectivity and Specificity: A Case Study with Two Human Transaminases’, *Molecules*, 2022, vol. 27, no. 4, p. 1398.

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Binding to lysozyme of $V^{IV}O$–pyranonato and $V^{IV}O$–pyridinone

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Keywords: metallodrugs, protein metalation, biologically active vanadium compounds, metal-protein interactions

Vanadium complexes (VCs) with organic derivatives have been suggested for the treatment of different types of diseases, such as diabetes and cancer. However, none of those compounds is currently used in clinics. [1] The use of VCs as drugs is primarily hampered by the limited knowledge of their transformation in the organism, which is related to the interaction with bioligands. In fact, the interaction of the active species of VCs with biological macromolecules, like proteins, is of great importance for their pharmacological activity. [2,3] Among the most promising VCs, bis(maltolato)oxidovanadium(IV) ($[V^{IV}O(malt)_2]$, where malt is 3-hydroxy-2-methyl-4H-pyran-4-onato), and $[V^{IV}O(empp)_2]$ (where Hempp is 1-methyl-2-ethyl-3-hydroxy-4(1H)-pyridinone), show important biological activity. The binding of $[V^{IV}O(malt)_2]$ and $[V^{IV}O(empp)_2]$ with the model protein hen egg white lysozyme (HEWL) was studied by X-ray crystallography.
The $[V^{IV}O(malt)_2]$/HEWL system shows non-covalent binding of *cis*-$[VO(malt)_2(H_2O)]$ and $[VO(malt)(H_2O)_3]^+$, and covalent binding of $[VO(H_2O)_{3\text{-}4}]^{2+}$, *cis*-$[VO(malt)_2]$ and other V-containing fragments to the side chains of Glu35, Asp48, Asn65, Asp87, and Asp119 and to the C-terminal carboxylate. [4]
For the $[V^{IV}O(empp)_2]$/HEWL system, crystallographic data show covalent binding of $[V^{IV}O(empp)(H_2O)]^+$ to the side chain of Asp48, and non-covalent binding of *cis*-$[V^{IV}O(empp)_2(H_2O)]$, $[V^{IV}O(empp)(H_2O)]^+$, $[V^{IV}O(empp)(H_2O)_2]^+$, and of an unusual trinuclear oxidovanadium(V) complex, $[V^{V}_3O_6(empp)_3(H_2O)]$, with accessible sites on the protein surface. [5]
The comparison between the binding modes of $[V^{IV}O(malt)_2]$ and $[V^{IV}O(empp)_2]$ to HEWL reveals similarities and differences that can be related to the ligand structure, the stability of the $V^{IV}O^{2+}$ species, the possibility of formation of covalent and/or non-covalent bonds, and the stabilization of the adducts through secondary interactions. The oxidation of V^{IV} to V^{V} with formation of the trinuclear species $[V^{V}_3O_6(empp)_3(H_2O)]$ is observed in the $[V^{IV}O(empp)_2]$/HEWL system, while in the $[V^{IV}O(malt)_2]$/HEWL system, no similar adducts were found. [5]
The reactivity of $[V^{IV}O(malt)_2]$ and $[V^{IV}O(empp)_2]$ with HEWL could help in understanding of transport and mechanisms of action of vanadium and of other metal-based drugs, promoting the development of new compounds as potential therapeutic agents.

References

[1] J. C. Pessoa, S. Etcheverry, D. Gambino. Vanadium compounds in medicine. Coord. Chem. Rev. 2015, 301-302, 24-48
[2] K. D Mjos, C. Orvig. Metallodrugs in medicinal inorganic chemistry. Chem. Rev. 2014, 114, 4540-4563
[3] V.M. Miranda. Medicinal inorganic chemistry: an updated review on the status of metallodrugs and prominent metallodrug candidates. Rev. Inorg. Chem. 2022, 42, 29-52
[4] G. Ferraro, M. Paolillo, G. Sciortino, E. Garribba, A. Merlino. Multiple and Variable Binding of Pharmacologically Active Bis(maltolato)oxidovanadium(IV) to Lysozyme. Inorg. Chem. 2022, 61, 16458-16467
[5] G. Ferraro, M. Paolillo, G. Sciortino, F. Pisanu, E. Garribba, A. Merlino. Implications of Protein Interaction in the Speciation of Potential $V^{IV}O$–Pyridinone Drugs. Inorg. Chem. 2023, 62, 8407−8417

AMYC BIOMED 2023

Exploring green alternatives to acetonitrile in reversed-phase liquid chromatography: from small molecules to biomolecules

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Keywords: liquid chromatography, dimethyl-carbonate, green chemistry

Reversed phase liquid chromatography (RPLC) is the more common separation technique used in the biopharmaceutical industry to separate, purify and analyze biomolecules of interest such as proteins, peptides and nucleic acids. Its popularity is mainly due to the high resolution between peaks and the large loading capacity that allows to handle high sample throughput. Acetonitrile (ACN) is the most commonly used organic modifier in RPLC and its wide employment is due to its excellent miscibility with water and/or other organic solvents, low viscosity which results in low backpressure and high elution strength. In addition, it allows the elution of a wide range of compounds and it has good chemical stability and low reactivity which makes it extremely compatible with sensitive compounds. On the other hand, however, it has some disadvantages including a higher cost than other organic solvents such as methanol or ethanol and it is a toxic, hazardous solvent as it can be converted to cyanide by the body [1].

Therefore, the decision to adopt greener solutions than acetonitrile in RPLC was mainly motivated by the need to reduce the environmental impact of biopharmaceuticals production as well as to promote sustainable industrial activity and ensure the safety of operators. Therefore, efforts are being made to develop new "green" organic solvents that are equally effective and safe, but with a reduced environmental footprint [2].

Recent attention has been given to solvents beyond the well-known physico-chemical properties of alcohols. Among these solvents is dimethyl carbonate (DMC), which has been included in the list of "green chemicals" established by the U.S. Environmental Protection Agency (EPA) thanks to its low toxicity and high biodegradability [1]. Currently, dimethyl carbonate (DMC) finds employment as a solvent in the field of energy storage (such as in lithium batteries production), but also for the synthesis of polymers, paints, and coatings. it is also used both in the biological field as an excellent extraction agent in the separation of organic compounds from complex matrices and for environmental applications as a solvent for analysis of contaminants. DMC has also been recently introduced as organic modifier for chromatographic separations, with a pioneer applicative work showing its use for separations in high performance liquid chromatography hyphenated to inductively coupled plasma mass spectrometry [3]. The purpose of this work is to investigate the use of DMC as a possible new organic modifier in RPLC from a fundamental perspective through a series of case studies, carried out first on some small molecules and then on peptides, by using a liquid chromatography instrument with UV-Vis detection. the study included fundamental studies on retention and efficiency under linear conditions and then the application of DMC on preparative-scale applications with the goal of purifying pharmaceutically relevant peptides.

References

[1] www.epa.gov
[2] M. Yabré et al., Greening Reversed-Phase Liquid Chromatography Methods Using Alternative Solvents for Pharmaceutical Analysis. Molecules. 2018, 23, 1065.
[3] B. Lajin et al., Introducing dimethyl carbonate as a new eluent in HPLC-ICPMS: stronger elution with less carbon. J. Anal. At. Spectrom. 2021,36, 1272

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Synthesis of polymers with core-shell morphology in supercritical carbon dioxide.

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Keywords: Core-shell, Polymer, Supercritical CO_2, Additive

Polymeric core-shell particles have a variety of uses ranging from catalysis, pollution control, and performance enhancing polymer additives to drug delivery.[1] The conventional synthesis of core-shell particles however generates large quantities of contaminated water and necessitates the use of extensive energy consuming drying processes.

Supercritical carbon dioxide ($scCO_2$) is a highly versatile solvent that is underutilized in the field of polymer synthesis and can overcome many of these drawbacks. The critical point of CO_2 of 73.8 bar and 31.1°C is easily attainable,[2] and $scCO_2$ possesses the additional beneficial property of being a good solvent for most monomers, whilst being a poor solvent for most polymers. This makes $scCO_2$ ideal for dispersion polymerization.[3] Following polymerization using $scCO_2$ as a solvent, the product is often obtained as a dry powder, with the CO_2 reverting to a gas as the pressure and temperature are returned to ambient.

In this work we report the first synthesis of core-shell particles in $scCO_2$, a system of a polystyrene (PS) core and a poly(methyl methacrylate) (PMMA) shell. We further demonstrate that it is possible to adjust the ratio of PS to PMMA from 1:1 to a maximum of 1:8. Figure 1 shows the procedure utilized for the synthesis.

The structures were confirmed to be as predicted using scanning electron microscopy and transmission electron microscopy (SEM and TEM), and it was verified that the sizes of the particles matched with our predictions using SEM and dynamic light scattering (DLS). Dynamic mechanical analysis (DMA) was used to confirm that the PS and PMMA were present in distinct phases, and not as a blend.

By adjusting the synthesis of the PS core, we were able to generate larger PS particles, with the addition of the PMMA shell proceeding as previously. Further adjustment of the PS core synthesis added a crosslinker (divinyl benzene, DVB), which generated crosslinked PS particles. Again the PMMA shell was able to be added to these particles, generating core-shell particles with an insoluble core. The addition of allyl methacrylate (AMA) was used to crosslink the PMMA shell in a similar reaction.

This work has reported the first synthesis of core-shell particles in $scCO_2$, and demonstrated facile tailorability of size of both core and shell. The capacity to add crosslinking, affecting the phase stability and solubility, was also shown in both core and shell, increasing the potential applications for these materials.

These hybrid core-shell materials show promising properties with potential applications including as biomaterials.[4] Specifically, there is potential for the PS-PMMA core-shell particles to be used as fillers for orthopedic prostheses, with hydrophobic drugs embedded in the core.

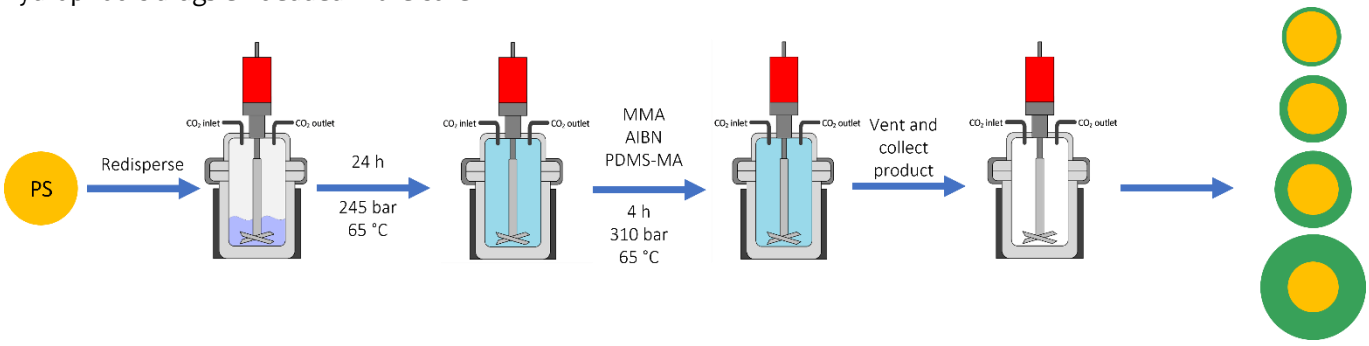

Figure 1: *Synthesis of core-shell particles, staring from pre-formed PS core*

References

[1] Ramli, R. A.; Laftah, W. A.; Hashim, S., Core–shell polymers: a review. RSC Adv. 2013, 3 (36), 15543.
[2] Cooper, A. I., Polymer synthesis and processing using supercritical carbon dioxide. J. Mater. Chem. 2000, 10 (2), 207-234
[3] McAllister, T. D.; Farrand, L. D.; Howdle, S. M., Improved Particle Size Control for the Dispersion Polymerization of Methyl methacrylate in Supercritical Carbon Dioxide. Macromol. Chem. Phys. 2016, 217 (20), 2294-2301
[4] Saunders, B. R.; Laajam, N.; Daly, E.; Teow, S.; Hu, X.; Stepto, R., Microgels: From responsive polymer colloids to biomaterials. Adv. Colloid Interface Sci. 2009, 147-148, 251-62.

Neolectins, towards new tools for selective sugar targeting

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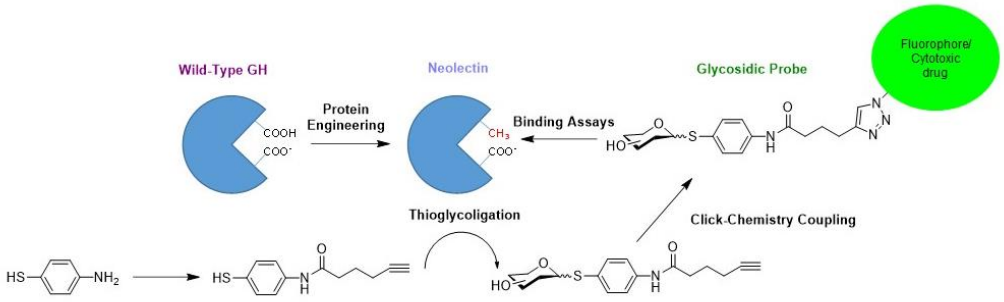
Keywords: neolectin, glycoside hydrolase, thioglycoligase

Lectins are carbohydrate-binding proteins specific for sugar molecules incorporated in glycosides or glycans at cell surface, playing a critical role in recognition processes at cellular and molecular levels. Engineering of lectins has been a challenge for many years. In our laboratory, a synthetic lectin has recently been developed by engineering a galactofuranosidase, able to recognize and hydrolyse the galactofuranose entity, by removing its catalytic activity and retaining its sugar binding ability, resulting in a "neolectin".[1] Based on this result, we aim at developing a library of neolectins, by selecting glycoside hydrolase selective for targeted carbohydrate structures and turning them into a "neolectin" by site-directed mutagenesis. However, to determine their binding affinity and sugar selectivity, a corresponding library of glycosides has to be synthesized to serve as chemical probes for *in-vitro* assays. In this context, an original biocatalytic approach has been chosen as the engineered neolectins are also thioglycoligase able to catalyze the formation of the corresponding *S*-glycosides.[2,3] Thus, the neolectins to be assayed as sugars receptors will also serve as biocatalysts to generate their own dedicated chemical probes. Several examples of chemoenzymatic synthesis of such *S*-glycosides bearing either fluororescent moiety or biorthogonal functions for subsequent coupling will be presented.



References

[1] R. Daniellou, P. Lafite, M. Seničar, B. Roubinet, L. Landemarre. Patent WO2022096829, 2022.
[2] C. Peyrot, B. Didak, L. Guillotin, L. Landemarre, P. Lafite, L. Lemiègre, R. Daniellou. Enzymatic synthesis of a series of thioglycosides: analogs of arbutin with efficient antipigmentation properties. *Eur. J. Org. Chem.* 2021, 3812-3818.
[3] M. Kurdziel, M. Kopeć, A. Pâris, K. Lewinski, P. Lafite, R. Daniellou. Thioglycoligation of aromatic thiols using a natural glucuronide donor. *Org. Biomol. Chem.* 2020, *18*, 5582-5585.

Targeting the undruggable: Developing pharmacological degraders for the Cellular Prion Protein

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Keywords: Prion disease, protein folding, medicinal chemistry, HTS

Prion diseases are rare and fatal neurodegenerative diseases which find their origin in protein misfolding. The interaction between the normal protein isoform (PrP^{C}) and the pathogenic one (PrP^{Sc}) causes PrP^{c} to be converted into PrP^{Sc}, leading to the accumulation of the abnormal protein and ultimately to the disease [1]. Although PrP is considered an undruggable protein by classical pharmacology, our previous work shows how it could be possible, by stabilizing a PrP folding intermediate, to send it to degradation and halt the cascade which leads to the fatal aggregation.
This new approach for selectively reducing the level of target proteins by impairing their folding process rather than targeting their native conformations, called Pharmacological Protein Inactivation by Folding Intermediate Targeting (PPI-FIT), is made possible by computational algorithms allowing the full atomistic reconstruction of folding and misfolding processes of polypeptides. The rationale underlying PPI-FIT is that targeting a folding intermediate with small ligands could promote its degradation by the cellular quality control machinery, which recognizes such artificially stabilized intermediates as improperly folded species. We have applied PPI-FIT to target the cellular prion protein (PrP), and identified a pharmacological degrader (named SM875) capable of dose-dependently suppressing the expression of the protein [2].
A general synthetic plan for SM875 was designed, more than 40 analogs were synthesized, purified and characterized in order to test them for biological activity. To improve the testing efficiency, a new image-based screening method was developed and validated on Western Blot results of the *in-house* library. This innovative screening method is fundamental during the *in-vitro* studies, as it accelerates the process of compound optimization for drug development. Dose-dependent analysis of each molecule allowed us to draw a first structure-activity relationship for SM875, which was used to refine the docking model of the compound-pocket interaction. To have an image of the folding intermediate pocket, different co-crystallization experiments between PrP and SM875 were tried on Earth. Unfortunately, they all failed leading us to expand our limits and setting up an experiment on the International Space Station (ISS). These results represent fundamental steps along the SM875 optimization pipeline and encourage searching for additional analogs and completely new structures with improved pharmacological and pharmacokinetic properties.

References

[1] J. C. Watts, *et al.*, The function of the cellular prion protein in health and disease, Acta Neuropathol., 2018, 135, 159–178, doi: 10.1007/s00401-017-1790-y.
[2] G. Spagnolli *et al.*, Pharmacological inactivation of the prion protein by targeting a folding intermediate, Commun. Biol., 2021, 4, 1–16, doi: 10.1038/s42003-020-01585-x.

AMYC BIOMED 2023

Linear polymers of Hyaluronan-Cyclodextrin as carriers for Sorafenib

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Keywords: Cyclodextrins, Hyaluronic acid, drug delivery

Cyclodextrins are oligosaccharides widely used to increase drug delivery overcoming the drug side effects and improving its solubility and bioavailability [1]. In order to increase the drug internalization in a specific tissue, nanoparticles based on cyclodextrins have been synthesized by grafting them to a polymeric backbone [2]. In some cases is possible to use the backbone for passive but also active targeting [3].
The active targeting may be exploited by selecting molecules that can bind over-expressed receptors in cancer cells, for example hyaluronic acid (HA), a linear glycosaminoglycan. HA is a component in the extracellular matrix. It may be recognized by specific receptors including CD44 and RHAMM overexpressed in cancer cells [4].
Recently we have synthesized polymers based on hyaluronic acid with β-cyclodextrin. We found that the polymers were able to increase the solubility of doxorubicin in water and improved the antiproliferative activity compared to the drug alone [5].
Taking into consideration the results obtained, here we report the design of two new polymers based on HA at different molecular weights (11 kDa and 45 kDa) conjugated with γ-cyclodextrins. The different dimensions of the γ-cyclodextrin cavity can increase the interaction with the guest and also their bioavailability and solubility. We have tested these polymers as a drug delivery system for Sorafenib, an anticancer drug which is an inhibitor of kinase proteins with very low water solubility [6]. The antiproliferative activity of the inclusion complexes was carried out in three different cancer cell lines: A2780 (ovarian cancer), SK-HeP-1 (adenocarcinoma) and MDA-MB-453 (breast cancer). Data showed a significatively improvement (up to 70%) in antiproliferative activity of the inclusion complexes of Sorafenib compared to the free drug, especially in the case of the polymer at lower molecular weight.

References

[1] P. Jansook, N. Ogawa, & T. Loftsson. Cyclodextrins: structure, physicochemical properties and pharmaceutical applications. Int. J. Pharm., (2018), 535(1-2), 272-284.
[2] I. Gritli ; E. Garmey; S. Eliasof; A. Tellez; M.E Davis; & Y. Yun. Polymeric nanoparticles and cancer: Lessons learnt from CRLX101. RSC-BMCS, 2016; pp. 199–232
[3] D. Vyas, M. Patel, & S. Wairkar. Strategies for active tumor targeting-an update. Eur. J. Pharmacol., (2022). 915, 174512.
[4] C. Buckley, E. J. Murphy, T. R. Montgomery, & I. Major. Hyaluronic acid: A review of the drug delivery capabilities of this naturally occurring polysaccharide. Polym. J. (2022), 14(17), 3442.
[5] N. Bognanni, M. Viale,L. La Piana, S. Strano, R. Gangemi, C. Lombardo, ... & G. Vecchio. Hyaluronan-Cyclodextrin Conjugates as Doxorubicin Delivery Systems. Mol. Pharm. (2023), 15(2), 374.
[6] Y. Pang, A. Eresen, Z. Zhang, Q. Hou, Y. Wang, V. Yaghmai, & Z. Zhang. Adverse events of sorafenib in hepatocellular carcinoma treatment. Am. J. Cancer Res., (2022). 12(6), 2770.

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Design of novel antiproliferative nanosystems based on hyaluronan-terpyridine conjugates

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Keywords: terpyridine, metal complexes, antiproliferative activity, polymer

Hyaluronic acid (HA) is a versatile building block to fabricate therapeutic platforms, owing to biocompatibility, biodegradability and CD44-targetability.[1]
In the current study, HA has been chemically modified introducing terpyridine (tpy) units. It is a tridentate pincer exhibiting cytotoxic properties and allowing the decoration of HA-based NPs via coordination chemistry, as described for similar systems.[2]
Here, we reported the synthesis and antiproliferative activities of a novel nano-based hyaluronic acid-terpyridine (HAtpy) conjugate and its metal complexes. The work aimed to evaluate their potentiality as tumor-targeting nanoparticles.
The presence of HA confers water solubility to the nanosystems and thus improves tpy biological applications. HAtpy form NPs in water in phosphate buffer at pH 7.4. The DLS spectra showed a main population with a hydrodynamic diameter of about 8 nm.
Cu (II) and Fe (II) ternary complexes of Hatpy with TpyCl were synthesised and investigated on four cancer cell lines by MTT assay. Significant outcomes in *in vitro* studies have been observed for Cu (II) ternary complexes, which showed cytotoxicity in the nM range. Additionally, they exhibited higher antiproliferative activity than Doxorubicin in the metastatic breast cancer cell line (MDA-MB-453).

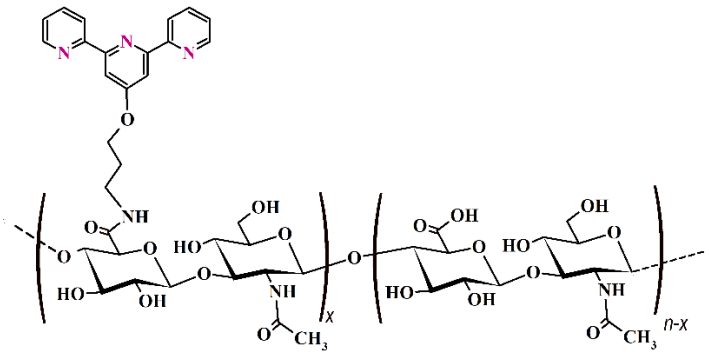

Figure 1: *hyaluronan-terpyridine conjugate (n=30, x=7)*

References

[1] N. Sahiner, R.S. Ayyala, S.S. Suner. Nontoxic Natural Polymeric Particle Vehicles Derived from Hyaluronic Acid and Mannitol as Mitomycin C Carriers for Bladder Cancer Treatment. ACS Applied Bio Materials. 2022, 5(12), 5554-5566.
[2] R. Panebianco, M. Viale, N. Bertola, F. Bellia, G. Vecchio. Terpyridine functionalized cyclodextrin nanoparticles: metal coordination for tuning anticancer activity. Dalton Trans., 2022, 51(13), 5000-5003

AMYC BIOMED 2023

Transdermal Delivery of Phloretin by Gallic Acid Microparticles

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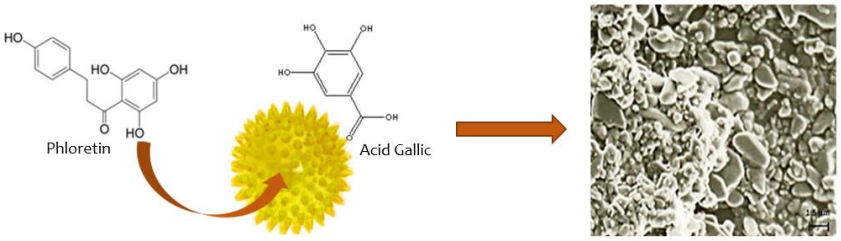
Keywords: gallic acid; phloretin; microspheres; antioxidant; transdermal delivery



Representation of phloretin-loaded in gallic acid microspheres

Exposure to ultraviolet (UV) radiation causes harmful effects on the skin, such as inflammatory states and photoaging, which depend strictly on the form, amount, and intensity of UV radiation and the type of individual exposed [1]. Fortunately, the skin is endowed with a number of endogenous antioxidants and enzymes crucial in its response to UV radiation damage. However, the aging process and environmental stress can deprive the epidermis of its endogenous antioxidants. Therefore, natural exogenous antioxidants may be able to reduce the severity of UV-induced skin damage and aging [2]. Several plant foods constitute a natural source of various antioxidants. These include gallic acid [3]and phloretin [4], used in this work. Specifically, polymeric microspheres, useful for the delivery of phloretin, were made from gallic acid, a molecule that has a singular chemical structure with two different functional groups, carboxylic and hydroxyl, capable of providing polymerizable derivatives after esterification. Phloretin is a dihydrochalcone that possesses many biological and pharmacological properties, such as potent antioxidant activity in free radical removal, inhibition of lipid peroxidation, and antiproliferative effects [5]. The obtained particles were characterized by Fourier transform infrared spectroscopy. Antioxidant activity, swelling behavior, phloretin loading efficiency, and transdermal release were also evaluated. The results obtained indicate that the micrometer-sized particles effectively swell, and release the phloretin encapsulated in them within 24 h, and possess antioxidant efficacy comparable to that of free phloretin solution. Therefore, such microspheres could be a viable strategy for the transdermal release of phloretin and subsequent protection from UV-induced skin damage.

References

[1] P.H. Hart, M. Norval, S.N. Byrne, L.E. Rhodes. Exposure to Ultraviolet Radiation in the Modulation of Human Diseases. Annu.Rev. Pathol. Mech. Dis. 2019, 14, 55–81.
[2] S. SYin, Y. Wang, N. Liu, M. Yang, Y. Hu, X. Li, Y. Fu, M. Luo, J. Sun, X. Yang. Potential skin protective effects after UVB irradiation afforded by an antioxidant peptide from Odorrana andersonii. Biomed. Pharmacother. 2019, 20, 109535–109547.
[3] J. Bai, Y. Zhang, C. Tang, Y. Hou, X. Ai, X. Chen, Y. Zhang, X. Wang, X. Meng. Gallic acid: Pharmacological activities and molecular mechanisms involved in inflammation-related diseases. Biomed. Pharmacother. 2021, 133, 110985–110998.
[4] A.V.A. Mariadoss, R. Vinyagam, V. Rajamanickam, V. Sankaran, S. Venkatesan, E. David. Pharmacological Aspects and Potential Use of Phloretin: A Systemic Review. Mini Rev. Med. Chem. 2019, 19, 1060–1067.

[5] M. Hytti, J. Ruuth, I. Kanerva, N. Bhattarai, M.L. Pedersen, C.U. Nielsen, A. Kauppinen. Phloretin inhibits glucose transport and reduces inflammation in human retinal pigment epithelial cells. Mol. Cell. Biochem. 2022, 478, 215–227.

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Monitoring stress conditions with optical sensor array

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Keywords: Sensor array, stress biomarkers, medical diagnosis

The development of sensors that can quantitatively measure stress biomarkers in our fluids, such as blood, urine, saliva, and sweat, have a significant impact on the field of medical diagnosis.[1] The release of neurotransmitters and hormone by the nervous and endocrine systems, respectively, is one of the essential reactions of our body to stress exposure. Therefore, primary stress markers include dopamine (DA) and cortisol. [2] Our target is to develop sensoristic devices to detect stress biomarkers in harsh and adverse environments, such as **space missions**.[3] We have chosen to exploit the Array technology for the realization of the sensor device, due to its peculiar characteristics: i) easy to use, ii) extremely selective, iii) sensitive; iv) easily implementable.[4] Array was designed considering an optical output. In particular, we selected and synthetized some organic fluorophores (probes) considering two important features: 1) spectral properties of the transductor, in particular due to the absorption/emission range of the probe; 2) interactions with the analyte by non-covalent interactions. Taking into account these considerations, the selected and synthetized fluorescent probes are BODIPY's, Naphtalimides, Rodhamines, macrocyclic receptors (Figure 1). The interaction between each probe and the stress marker has been tested previously in solution, then the Array response has been analyzed by using a commercial smartphone or optical fibre as detector. [5] In addition, the possibility to perform sensing in real field by using a simple methodology to acquire and elaborate the data of the array should be extremely useful for practical applications. The use of a smartphone or optical fibre for this purpose opens the possibility of performing rapid analysis in the field, avoiding complicated sampling and the need for highly specialized personnel.

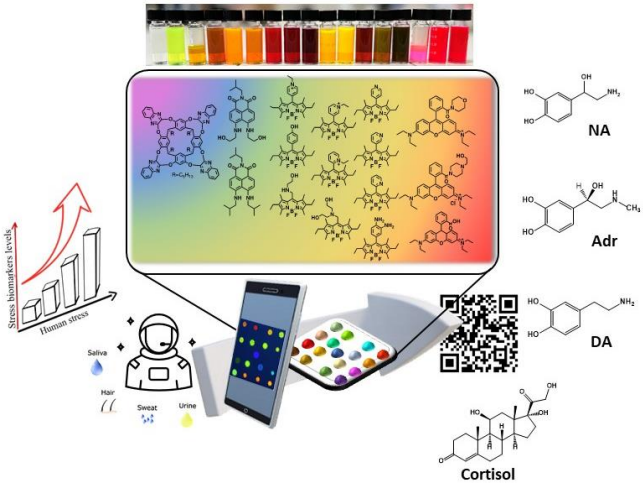

Figure 2. Real sample of the sensoristic device

References

[1] J. P. Ulrich-Lai Y.M.,Herman; Nat. Rev. Neurosci. 2009, 10, 397– 409.
[2] S. Nishitani; T. Sakata; ACS Appl. Mater. Interfaces 2020, 12, 14761–14769.
[3] V. Sharma; T. K. Sharm; I. Kaur, Applied Nanoscience, 2021, 11, 2577–2588.
[4] L. Zheng; K. S. Suslick; Acc. Chem. Res. 2021, 54, 950–960.

[5] R. Santonocito; N. Tuccitto; A. Pappalardo; G. Trusso Sfrazzetto; Molecules 2022, 27, 21, 7503.

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SUSTAINABLE POLYHYDROXYALKANOATES AMINOLYSIS BY USING CHOLINE-TAURINATE IONIC LIQUID FOR NANOPARTICLES PREPARATION

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Keywords: Poly-hydroxyalkanoates, nanoparticles, drug delivery, ionic liquids

Polymer nanoparticles (NPs) have gained interest in various fields for environmental, nano-sensing, and biomedical applications. As far as the latter, nanostructured systems can encapsulate and vehiculate drugs or bio-active molecules that are toxic in systemic administration or not soluble in physiological fluids, such as hydrophobic ones (i.e., corticosteroids, steroids, and cortisone). In the design of a drug delivery system, biocompatibility is a key factor that drives the choice of materials, chemicals, and processing to avoid or reduce possible harm to the body. Bio-polyesters, like polyhydroxyalkanoates (PHAs), are a class of polymers that are very appealing in this field due to their biodegradability in nontoxic products. However, there are many drawbacks related to their high hydrophobicity which favours nano-particles aggregation and can drastically reduce the compatibility towards living systems. Chemical modifications of these polyesters by acid or basic hydrolysis, or amynolysis with di- or multifunctional amines lead to the introduction of functional groups able to increase hydrophilicity and improve nanostructures stability in physiological environment. Unfortunately, the use of toxic solvents (chlorinated solvents) or reactants (di- or multifunctional amines, such as ethylene diamine or hexamethylene diamine) as well as surfactants often used for polymer functionalization and nano-particles preparation, implies careful purification steps in the production process. In this context, two PHAs, poly-3hydroxybutirate-co-3hydroxyhexanoate and poly-3hydroxynonanoate-co-3hydroxyheptanoate-co-3hydroxyvalerate were partially aminolyzed by choline taurinate (Ch-Tau), an ionic liquid soluble in water and ethanol, which shows no cytotoxicity. Aminolysis reaction was carried out in non-toxic solvents by adding Ch-Tau ethanol solution to ethyl acetate PHAs solution. In this way the reaction products consisted of hydrophobic oligomers with an SO^{3-} polar head which gave them a surfactant behavior, making possible the formation of stable nanoparticles by a simple and rapid process without the use of external surfactants which otherwise should be removed. Then, NPs were obtained by adding the aminolyzed PHAs solution to water under vigorous stirring and solvent evaporation. The safety of the chemicals and solvents used has made it possible to reduce or eliminate the time-consuming and complex purification process. The nanoparticles were characterized by dynamic Light Scattering (DLS) experiments, infrared spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Preliminary results demonstrate the effectiveness of one-pot aminolysis and NPs production. Additional studies are now in progress to evaluate water-insoluble drug loading and release.

AMYC BIOMED 2023

Design and synthesis of edaravone-based thiazolo[5,4-d]pyrimidine derivatives. New antioxidant A_{2A} and A_{2B} adenosine receptor antagonists as multitarget agents against demyelinating diseases

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Keywords: demyelinating diseases; multitarget strategy; antioxidants; adenosine antagonists

Demyelinating diseases, such as multiple sclerosis (MS) and cerebral ischemia, are characterized by neurodegeneration, neurological symptoms, and cognitive impairment. These diseases are complex pathologies due to the alteration of different processes, some of which are modulated by adenosine and its receptors (R) (named A_1, A_{2A}, A_{2B}, A_3). In particular, the blockade of $A_{2A}R$ and $A_{2B}R$ seems to favor the remyelination process [1]. Furthermore, since oxidative stress is involved in inflammatory demyelinating progression, treatment with antioxidants is being studied as well [2].
This project aims to find novel compounds capable of blocking $A_{2A}R$ and/or $A_{2B}R$ and endowed with antioxidant properties because they might possess potentiated remyelinating and neuroprotective effects, compared to the single targeted agents. To obtain the desired compounds, previously reported $A_{2A}R/A_{2B}R$ antagonists, or their new analogs, were hybridized with the antioxidant Edaravone (EDA), a drug in the market for the treatment of cerebral ischemia which proved to be effective as a neuroprotective agent in animal models of MS [5].
Potent $A_{2A}R/A_{2B}R$ antagonists, belonging to our thiazolo[5,4-d]pyrimidine series [3,4], were chosen as lead compounds and were properly functionalized and merged with the EDA counterpart to get the desired hybrid derivatives (*Figure 1*).
The new compounds were tested to evaluate their affinity at human (h) A_1, hA_{2A}, hA_3 adenosine receptors and potency at the $hA_{2B}R$ subtype. The available biological data at ARs showed that we obtained an EDA-based thiazolopyrimidine (compound **1**, linker= $CONH(CH_2)_2O$ at position 3, *Figure 1*) endowed with high $A_{2A}R$ affinity (Ki= 36.3 nM) and selectivity *vs* the other ARs. Antioxidant studies (MTT assay) are in progress on compound **1** to assess its radical scavenging activity. Interestingly, some intermediates of the synthesis showed nanomolar $A_{2A}R$ and $A_{2B}R$ affinity and different degrees of selectivity, confirming that the thiazolo[5,4-d]pyrimidine nucleus is a robust scaffold to obtain $A_{2A}R/A_{2B}R$ antagonists.

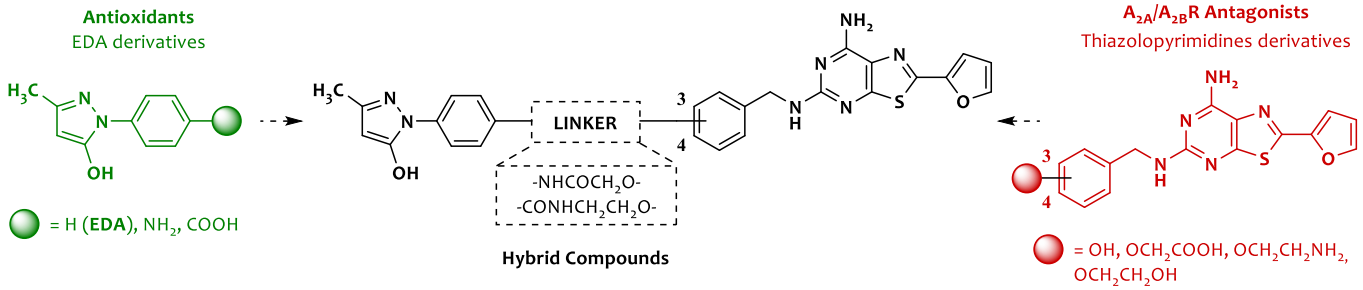

Figure 3 Hybridization approach.

References

[1] A. Melani, A.M. Pugliese, F. Pedata. Adenosine receptors in cerebral ischemia. Int. Rev. Neurobiol. 2014, 119, 309-348.
[2] V. Pegoretti, K.A. Swanson, J. R. Bethea, L. Probert, U. L. Eisel, R. Fischer. Inflammation and oxidative stress in multiple sclerosis: consequences for therapy development. Oxid. Med. Cell. Longev. 2020, 2020.
[3] F. Varano, V. Colotta, D. Catarzi, K. Varani, P.A. Borea, F. Vincenzi. U.S. Patent Application No. 16/310,844. 2020.
[4] F. Varano, D. Catarzi, F. Vincenzi, M. Betti, M. Falsini, A. Ravani, P. A. Borea, V. Colotta, K. Varani. Design, synthesis, and pharmacological characterization of 2-(2-furanyl) thiazolo [5, 4-d] pyrimidine-5, 7-diamine derivatives: new highly potent A2A adenosine receptor inverse agonists with antinociceptive activity. J. Med. Chem. 2016, 59(23), 10564-10576.
[5] D. Michaličková, H. Kubra Ozturk, J. Hroudova, M. L'uptak, T. Kucera, T. Hrncir, N. Kutinova Canova, M. Sima, O. Slanar. Edaravone attenuates disease severity of experimental auto-immune encephalomyelitis and increases gene expression of Nrf2 and HO-1. Physiol. Res. 2022, 71.1, 147.

Development of a Chemiluminescent Method for the Analysis of Antioxidant Activity in Cosmetic Products

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Keywords: Chemiluminescence, Cosmetic Products, Total Antioxidant Activity, Point-of-Care

New cosmetic formulations are continuously requested by the market and the ingredients used in cosmetic products are constantly evolving. The application of antioxidants in cosmetics has been increasing as active ingredients as well as stabilizers [1]. In this context, analytical methods able to quickly and easily assess the antioxidant activity of cosmetics would allow to carry out analyses on new formulations even within the manufacturing process without the need for specialized laboratories and personnel thus evaluating directly on-site the effectiveness and the shelf-life of the products [2,3].

In this work, a chemiluminescent inhibition assay was developed and optimized to determine the total antioxidant capacity in cosmetic products. The method was based on the luminol/enhancers/hydrogen peroxide/horseradish peroxidase chemiluminescent (CL) system, which generates light signals measurable through simple and compact instrumentation. The formation of the chemiluminescent signal is inhibited by the presence of antioxidant substances while it is restored once all the antioxidant molecules have been oxidized. The total antioxidant capacity (TAC) of the sample is estimated by measuring the time of appearance of the light signal.

The method developed involved the use of an integrated Lab-On-Chip platform in which the assay was carried out exploiting a device composed by a microwell-plate coupled to a matrix of a-Si:H photosensors, which convert the incident light signal into electric current signals. This system was used to measure the light emission resulting from CL reactions and follow their kinetics in real time.

The method was optimized in terms of concentration and volumes of the required reagents. A calibration curve was then generated using ascorbic acid as the reference antioxidant substance. Procedures for the pretreatment of the sample were also evaluated taking into consideration the great variability of the cosmetic matrices and the fact that these procedures must be performed by unqualified personnel and outside specialized laboratories. To this aim, several formulations were prepared and tested exploiting different pre-treatment protocols in order to find the most rapid and easy-to-use method for the TAC measurements. The method developed was finally applied for determining the TAC of cosmetic products currently on the market claimed for their antioxidant properties expressed in terms of ascorbic acid equivalents.

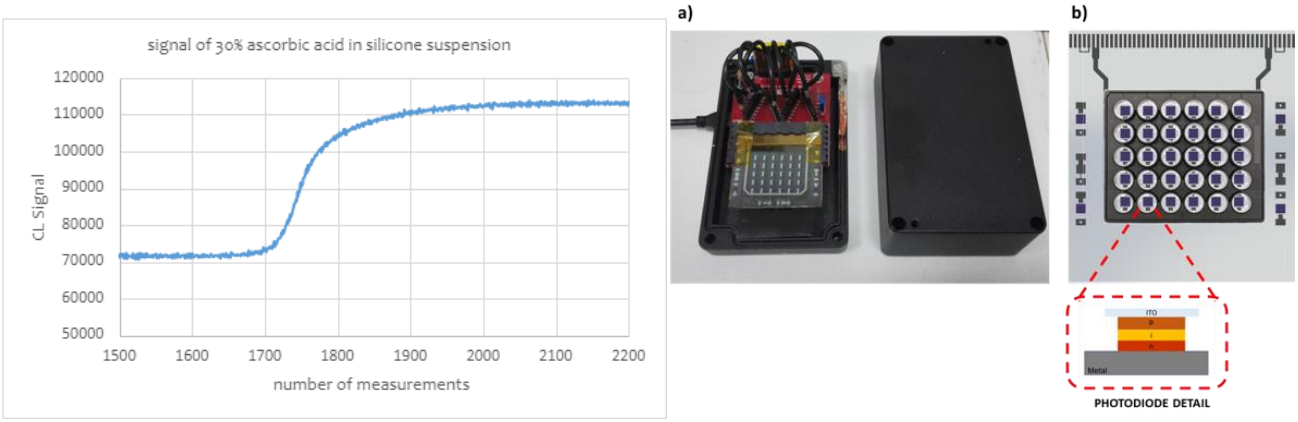

Figure 1. - left: Chemiluminescent signal of a 30% vitamin C silicone suspension; - right: Amorphous silicon hydrogenated photosensors integrated with microwell plate enclosed in a mini-dark box

References

[1] Hoang, H. T., Moon, J. Y., & Lee, Y. C. Natural Antioxidants from Plant Extracts in Skincare Cosmetics: Recent Applications, Challenges and Perspectives. Cosmetics, 2021, 8(4), 106.
[2] Munteanu, I.G.; Apetrei, C. Analytical methods used in determining antioxidant activity: A review. Int. J. Mol. Sci. 2021, 22, 3380.
[3] Calabria, D., Guardigli, M., Severi, P., Trozzi, I., Pace, A., Cinti, S., ... & Mirasoli, M., A smartphone-based chemosensor to evaluate antioxidants in agri-food matrices by in situ AuNP formation. Sensors, 2021,21(16), 5432.

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Nitrative stress in synthetic models of neuromelanins

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Keywords: Neuromelanins, nitrative stress, Parkinson's disease, NMR spectroscopy, mass spectrometry

Parkinson's disease (PD) is a neurodegenerative pathology related with mitochondrial dysfunction and oxidative and nitrative stress and characterized by progressive loss of dopaminergic neurons in *substantia nigra* (SN) [1]. Despite the PD arising mechanism has not been fully clarified yet, it has been established that PD is associated to high levels of neuromelanins (NMs) [2]. NMs are dark pigments with melanic, lipidic and peptide components linked together by covalent bonds, which accumulate during aging and exhibit both neuroprotective, since their synthesis prevent dopamine (DA) accumulation, and neurodegenerative effects [2,3]. As regards the NMs found in the SN, the melanic part is constituted by DA, which can be oxidized to quinone and then polymerize (Figure 1) [3,4].

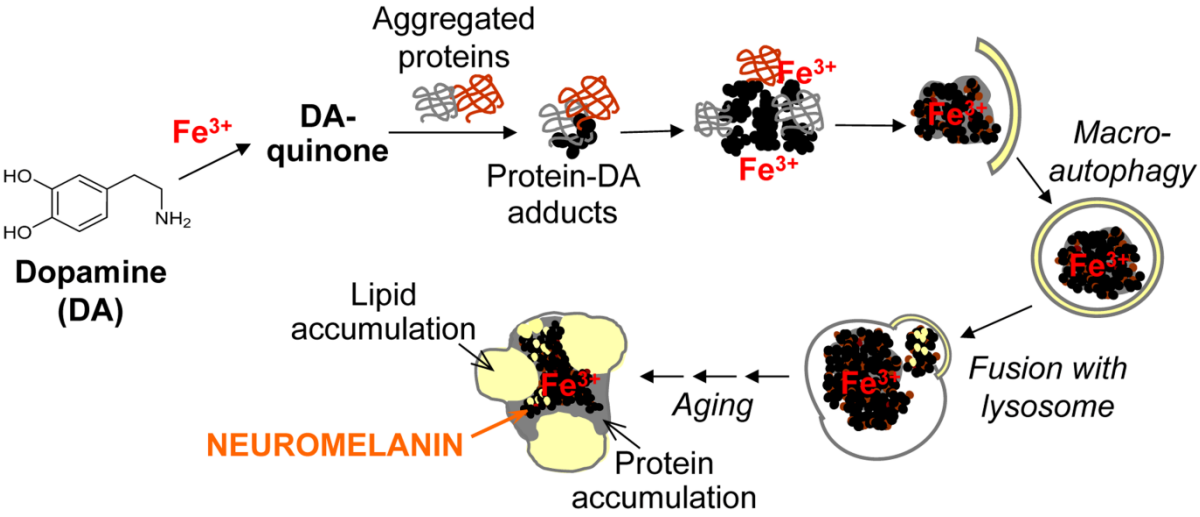

Figure 1. proposed mechanism for NM formation [4]

Only a small amount of NM can be isolated from the human brain [3], so it is very important to develop synthetic models of NM for research purposes. In this study, we prepare synthetic NMs from DA and β-lactoglobulin (BLG), a whey and milk protein of ruminants, belonging to the lipocalins family, which is constituted by 162 amino acids and 8 antiparallel β-barrels with 3 α-helices on its surface [5]. Moreover, in this study we take into consideration the nitrative stress, which is related to PD, as mentioned above. In fact, under pathological conditions, nitric oxide (NO), a signaling molecule generated by nitric oxide synthase, can interact with reactive oxygen species (ROS) to generate reactive nitrogen species (RNS) [1].
The aim of this work is to investigate the effect of the nitration reaction in the presence of H_2O_2 and NO_2^-, either on the protein or the melanic portion of the synthetic NMs. First, our mass spectrometry data show that the nitration sites on BLG are tyrosines Tyr_{42} and Tyr_{99}, and that the nitration yield increases with the fibrillated protein, fBLG, since in its structure the tyrosines which undergo nitration are more solvent-exposed. Moreover, tyrosine nitration has been confirmed also in the protein treated with the H_2O_2/NO_2^- system after the melanization reaction, but it is to confirm whether the nitration occurs on the melanized protein or on the non-reacted portion. Then, since it was previously assessed that melanization reaction can't occur in presence of the only nitrated DA (6-nitrodopamine, 6-NDA), synthetic NM samples have been prepared with a DA/6-NDA mixture and analyzed by NMR spectroscopy. The protein peaks broadening in NMR spectra confirms the melanin formation; moreover, since these spectral modifications are more evident in the sample with BLG with respect to fBLG, we may hypothesize that either fBLG possesses a less tendency to melanize, or the melanization process with the fibrillated protein is faster but insoluble products, which cannot be seen in the NMR spectra, form.

As future perspective, this study could be completed by testing synthetic NM samples pre-nitrated on both the protein and the melanic portions.

References

[1] A.H.K. Tsang, K.K.K. Chung. Oxidative and nitrosative stress in Parkinson's disease. Biochim. Biophys. Acta. 2009, 1792, 643-650.
[2] F.A. Zucca, J. Segura-Aguilar, E. Ferrari, P. Muñoz, I. Paris, D. Sulzer, T. Sarna, L. Casella, L. Zecca. Interactions of iron, dopamine and neuromelanin pathways in brain aging and Parkinson's disease. Prog. Nuerobiol. 2017, 155, 96-119.
[3] A. Capucciati, E. Monzani, M. Sturini, S. Nicolis, F.A. Zucca, L. Bubacco, M. Bortolus, L. Zecca, L. Casella. Water-solubke melanin-protein-Fe/Cu conjugates derived from norepinephrine as reliable models for neuromelanin of human brain Locus Coeruleus. Angew. Chem. 2022, e202204787.
[3] F.A. Zucca, E. Basso, F.A. Cupaioli, E. Ferrari, D. Sulzer, L. Casella, L. Zecca. Neuromelanin of the human substantia nigra: an update. Neurotox. Res. 2014, 25, 13-23.
[4] G. Kontopidis, C. Holt, L. Sawyer. β-lactoglobulin: binding properties, structure and function. J. Dairy Sci. 2004, 87, 785-796.

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Computational and spectroscopic study of phosphorene interactions with protein models to foster biomedical applications

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Keywords: 2d Nanomaterials, Phosphorene, Proteins, Molecular recognition, Molecular Dynamics

As a novel member of the two-dimensional nanomaterial family, phosphorene (PP) has emerged as one of the most significant materials in the post-graphene era. PP is composed of puckered honeycomb layers of phosphorus atoms, which are connected via strong intra-layer P–P bonding and weak inter-layer van der Waals forces. This distinct structural feature leads to unique physical and mechanical properties.[1] Further, non-toxic degradation products make phosphorene an attractive candidate for biomedical applications, such as photothermal agent in cancer treatment, bio-sensing transistor or a drug delivery system.[2] Yet, the development of novel biomedical applications is currently hindered by the need to elucidate the mechanism of interaction of PP with biomolecules. Proteins, in particular, can bind to a nanomaterial forming a tightly bound, but dynamic surface coating (protein corona) which can dramatically alter the properties of nanomaterials. On the other hand, the conformation of biomolecules can be significantly perturbed, leading to unwanted and off-target toxicity.[3] In this scenario, we used an integrated computational-experimental approach to develop an interaction model able to rationalize the effect of PP on a protein system. As so, a variety of spectroscopic techniques were used to investigate the interaction of PP with lysozyme proteins (Lys). The experimental observable was then integrated with the atomistic description of the interaction by means of molecular dynamics (MD). We performed extensive MD simulations on both the real system (Lys) and two theoretical models with a unique secondary structure (alpha-helix or beta-strands) in order to disentangle the complexity and variability of the experimental observables into a few primary drivers of the proteins-PP interaction. We demonstrated that secondary structure of the protein does not significantly influence the adsorption mode on the phosphorene surface, but does influence the resulting conformation changes. Indeed, while the beta structure interacts mainly through unfolded regions, the alpha fold favours PP binding through structured clusters of residues, leading to more significant structural and dynamic perturbations. This model provided valuable insights into the biocompatibility of phosphorene and its molecular recognition mechanism with protein systems, which will assist in the design of improved PP-based devices.

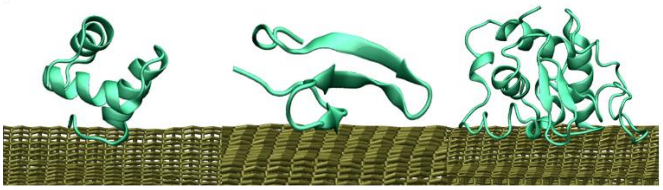

Figure 1. Representative binding poses from MD simulations on protein models

References

[1] Kou Liangzhi at al. Phosphorene: fabrication, properties, and applications. J. Phys. Chem, 2015, 6,.14.
[2] Tatullo Marco et al. Phosphorene is the new graphene in biomedical applications. Materials. 2019, 12,14.
[3] Ou L et al. Toxicity of graphene-family nanoparticles: a general review of the origins and mechanisms. Particle and fibre toxicology. 2016,13,1.

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Functionalization of electrospun poly(L-lactic acid) scaffold with a selective β-lactam integrin agonist for tissue regeneration

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Keywords: electrospinning, integrin agonists, tissue regeneration, diabetic mice, PLLA

Regulating cell adhesion and growth onto functionalized biomaterial scaffolds is an important issue in the field of tissue engineering and regenerative medicine. New bio-active three-dimensional nanofibrous scaffolds based on poly (L-lactic acid) (PLLA), as bioresorbable polymer, incorporating a β-lactam compound as a selective integrin ligand (Figure 1) were realized by electrospinning technology and fully characterized. These new functional biomaterials were proven to give a sustained release of the bioactive β-lactam compound and showed enhanced adhesion properties on h-BMMSC cells due to the action of the integrin ligand,[1] suggesting a possible application in tissue repair and regeneration. In fact, integrins regulate crucial aspects of cellular functions, including adhesion, migration, differentiation, growth, and survival, by communicating bidirectional signals between the extracellular and intracellular environment.[2]
In this work, PLLA scaffolds functionalized with GM18, a potent selective agonist toward integrin $\alpha_4\beta_1$, were further investigated. A pilot study focusing on the adhesion ability of equine adult (adipose tissue) and fetal adnexa (Wharton's jelly) derived MSCs to GM18 and PLLA-GM18 was conducted.[3] Finally, an *in vivo* experiment on a murine model of diabetic foot ulcer, showed the effectiveness of PLLA-GM18 in reducing wound healing timing.[4]

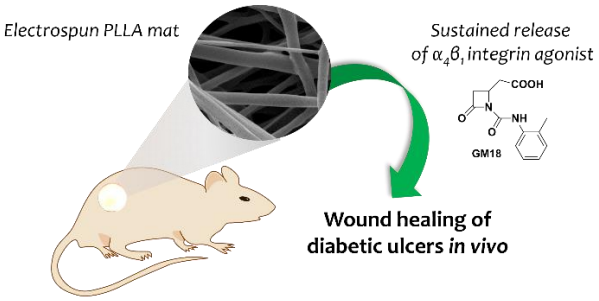

Figure 1: PLLA nanofibrous scaffold releasing GM18 promotes wound healing in a pressure ulcer model of diabetic mice *in vivo*.

References

[1] G. Martelli, N. Bloise, A. Merlettini, G. Bruni, L. Visai, M.L. Focarete, D. Giacomini. Combining Biologically Active β-Lactams Integrin Agonists with Poly(L-lactic acid) Nanofibers: Enhancement of Human Mesenchymal Stem Cell Adhesion. Biomacromolecules. 2020, 21, 1157-1170.
[2] M. Baiula, P. Galletti, G. Martelli, R. Soldati, L. Belvisi, M. Civera, S.D. Dattoli, S.M. Spampinato, D. Giacomini. New β-Lactam Derivatives Modulate Cell Adhesion and Signaling Mediated by RGD-Binding and Leukocyte Integrins. J. Med. Chem. 2016, 59, 9721-9742.
[3] B. Merlo, V.A. Baldassarro, A. Flagelli, R. Marcoccia, V. Giraldi, M.L. Focarete, D. Giacomini, E. Iacono. Peptide Mediated Adhesion to Beta-Lactam Ring of Equine Mesenchymal Stem Cells: A Pilot Study. Animals. 2022, 12, 734.

[4] V.A. Baldassarro, V. Giraldi, A. Giuliani, M. Moretti, G. Pagnotta, A. Flagelli, P. Clavenzani, L. Lorenzini, L. Giardino, M. L. Focarete, D. Giacomini, L. Calzà. Poly (L-lactic acid) Scaffold Releasing an $\alpha_4\beta_1$ Integrin Agonist Promotes Non-Fibrotic Skin Wound Healing in Diabetic Mice. ACS Appl. Bio Mater. 2023, 6, 296–308.

AMYC BIOMED 2023

Optimizing vaccine design for prevention of neonatal sepsi

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Keywords: Vaccine design; MrkA protein antigen; *Klebsiella pneumoniae*; self-assembling protein nanoparticles; structural characterization

Neonatal sepsis is a major cause of death across low- and middle-income countries (LMICs). These infections are acquired both in communities and in health-care facilities. Coupled with the high rate of multi-drug resistant strains causing such diseases, such as Klebsiella pneumoniae (Kp), there is a strong argument for a maternal vaccination approach [1]. With this aim, innovative vaccine designs are needed to allow a strong response after only one immunization and to enable optimal combinations of protein and polysaccharide antigens. Over the last years, protein nanoparticles (NP) have raised increasing interest as powerful vaccine platform and have been demonstrated to be highly effective also against SARS-CoV-2. Protein NP are naturally occurring molecules that have intrinsic ability to self-assemble into highly stable and symmetric structures and they have been largely investigated as a platform to display vaccine antigens in multicopy ordered patterns with the overall aim to enhance their immunogenicity [2].
In this work type 3 fimbriae major subunit MrkA belonging to Kp was selected as model antigen. It is a well conserved protein expressed in approximately 60% of Kp strains, including hypervirulent ones [3]. MrkA monomer has been proposed as vaccine antigen and demonstrated able to stimulate a protective immune response in vivo. However, its 3D-molecular structure is not yet known. We are performing an in-depth structural investigation of MrkA by X-ray crystallography and Nuclear Magnetic Resonance (NMR) to inform about the optimal antigen design and to solve its structure. Indeed, MrkA subunits naturally assemble into long polymers and limited information is available about the antigen conformation and antigenic epitopes recognized by functional antibodies. Also, NP was used successfully as innovative technology to improve MrkA monomer immunogenicity, as shown by immuno results in mice.
In conclusion, this work demonstrates and supports the capacity of self-assembling NP to increase the immune response of a bacterial protein antigen like MrkA and will contribute to better characterize it from a structural point of view.

References

[1] Assoni L. et al; Infectious diseases and therapy, 2021; vol. 10,4: 2157-2175.
[2] Nguyen B, Tolia NH.; NPJ Vaccines, 2021; 6(1): p. 70.
[3] María D. Alcántar-Curiel et al.; Virulence, 2013; 4:2, 129–138.

Analytical Characterization of (h-PG) D-(-)-Fructose Amperometric Biosensor based on Self-Templated Highly Porous Gold

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Keywords: enzyme amperometric biosensors; highly porous gold; electrodeposition; fructose

In the last decades, nanomaterials have shown great advantages in terms of functional properties for a wide range of technological applications [1]. Metal nanoparticles provide a lot of advantages compared to macroelectrodes, such as enhancement of mass transport, catalysis, high effective surface area and control on the electrode conductive microenvironment. Highly porous gold (h-PG) can be synthesized by using a chemical approach and electrodeposition. The main advantages of the electrodeposition method are the thickness, roughness and size control of the h-PG layer [2]. In this work h-PG was directly electrodeposited onto a gold electrode (AuE) by using a two-steps method: 1) sweeping the potential in a 10 mM $HAuCl_4$ solution (supporting electrolyte 2.5 M NH_4Cl); 2)applying a potential -3 V vs. $Ag|AgCl_{sat}$ [3,4]. Initially we optimized the different parameters that can affect the electrodeposition process, such as scan numbers, concentration of precursor solution, etc. Afterwards the nanostructured electrodes were characterized by scanning electron microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS). The so modified electrodes were further characterized with $Fe(CN)_6^{3-/4-}$ by cyclic voltammetry and electrochemical impedance spectroscopy, and compared to the naked gold electrode (AuE) in order to determine the electroactive area (AEA), electron transfer rate constant (k0), real surface area (Areal) and the roughness factor (ρ) [5]. Finally, the electrodes were modified with FDH and poly(vinyl alcohol), N-methyl-4(4'-formylstyryl)pyridinium methosulfate acetal (PVA-SbQ) to detect D-(-)-fructose in fruit juice samples as potential on-line biosensor for food industrial production processes [6].

References

[1] S.D. Minteer, Top. Catal. 55 (2012) 1157-1161.
[2] J. Zhang, M. Oyama, Anal. Chim. Acta 540 (2005) 299-306.
[3] X. Dai, R.G. Compton, Anal. Sciences 22 (2006) 567-570
[4] G. Sanzó, I. Taurino, R. Antiochia, L. Gorton, G. Favero, F. Mazzei, G. De Micheli, S. Carrara, Bioelectrochemistry 112 (2016) 125-131.
[5] I. Lavagnini, R. Antiochia and F. Magno, Electroanalysis 16 (2004) 505-506.
[6] R. Antiochia, I. Lavagnini, F. Magno, Anal. Letters 37 (2004) 1657-1669.

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Alginate hydrogels to deliver plasma generated RONS for biomedical purposes

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Plasma can be defined as a totally or partially ionized gas that contains excited atoms and molecules, free electrons, radicals, ions and UV-Vis radiation. The accomplishment of plasma sources able to operate at atmospheric pressure and at temperatures close to room temperature fostered the development of a research field named Plasma medicine [1]. It is based on the use of direct or indirect cold atmospheric plasma (CAP) treatments of cells, tissues, or organs. CAPs have been indeed evaluated as an effective tool for different biomedical uses such as sterilization, wound healing, blood coagulation and cancer treatment [2,3]. The efficacy of CAP in these fields primarily derives from their ability to deliver gaseous blends of reactive oxygen and nitrogen species (RONS) acting as redox modulators and oxidative stress inducers in redox biology [1-3].
Particularly, CAPs are emerging as a technology to enrich liquids with stable RONS (e.g., H_2O_2, NO_2^-, NO_3^-), to be incubated with cells in vitro or injected into tissues or organs in vivo [3,4]. In fact, the obtained liquids revealed often as efficacious as the direct CAP applications for biomedical purposes opening the door for minimally invasive therapies [5,6].
However, the direct contact of plasma treated liquids with the biological sites can result in a fast diffusion, dilution and washing away due to presence of other fluids. Therefore, the design of biomaterials acting as vehicle for the local confinement and delivery of RONS represents a very innovative frontier in the scientific research. Hydrogels can be an asset for this aim, due to their high liquid content and swollen three-dimensional network that are key features in the design of advanced biomaterials [7-9]. Specifically, alginate hydrogels are evaluable for biomedical applications because they are based on a natural polymer, biocompatible and biodegradable [9].
This contribution focuses on the use of CAPs to realize alginate hydrogels containing RONS. An atmospheric pressure plasma jet with a coaxial cylindrical dielectric barrier discharge configuration has been utilized. Helium has been used to feed the discharges and different experimental approaches have been investigated to produce the RONS-containing hydrogels. The plasma-generation and release of RONS from the samples has been evaluated by means of release tests performed in the phosphate buffered solution (PBS). The H_2O_2 and NO_2^- concentrations have been quantified with colorimetric assays. Results show that the application of CAPs in different experimental strategies is an effective method to realize hydrogels able to modulate the release of the reactive species. In particular, the RONS release varies in the time and CAP treatment of the alginate solution allow to generate a higher concentration of H_2O_2, while the CAP treatment of the hydrogel favours the production of NO_2^-.

Figure 1. *Schematic representation of the experimental work*

References

[1] G. Fridman, A. Gutsol, A. B. Shekhter, V. N. Vasilets, A. Fridman. Applied Plasma Medicine. Plasma Process. Polym. 2008, 5, 503-533.

[2] P. Favia, E. Sardella, H. Tanaka. Special issue: Advanced applications of plasmas in LifeSciences 2020. Plasma Process Polym. 2020, 17, 1-4.
[3] V. Armenise, V. Veronico, S. Cosmai, D. Benedetti, R. Gristina, P. Favia, F. Fracassi, E. Sardella. Plasma Process Polym. 2023, e2200182.
[4] P. J. Bruggeman, M. J. Kushner, B. R. Locke, J. G. E. Gardeniers, W. G. Graham, D. B. Graves, R. C. H. M. Hofman-Caris, D. Maric, J. P. Reid, E. Ceriani, D. Fernandez Rivas, J. E. Foster, S. C. Garrick, Y. Gorbanev, S. Hamaguchi, F. Iza, H. Jablonowski, E. Klimova, J. Kolb, F. Krcma, P. Lukes, Z. Machala, I. Marinov, D. Mariotti, S. Mededovic Thagard, D. Minakata, E. C. Neyts, J. Pawlat, Z. L. Petrovic, R. Pflieger, S. Reuter, D. C. Schram, S. Schröter, M. Shiraiwa, B. Tarabová, P. A. Tsai, J. R. R. Verlet, T. von Woedtke, K. R. Wilson, K. Yasui, G. Plasma–liquid interactions: a review and roadmap. Plasma Sources Sci. Tec. 2016,25, 053002-053061.
[5] E. Sardella, V. Veronico, R. Gristina, L. Grossi, S. Cosmai, M. Striccoli, M. Buttiglione, F. Fracassi, P. Favia. Plasma Treated Water Solutions in Cancer Treatments: The Contrasting Role of RNS. Antioxidants.2021, 10, 605-625.
[6] E. Sardella, M. G. Mola, R. Gristina, M. Piccione, V. Veronico, M. de Bellis, A. Cibelli, M. Buttiglione, V. Armenise, P. Favia, G. P. Nicchia. A Synergistic Effect of Reactive Oxygen and Reactive Nitrogen Species in Plasma Activated Liquid Media Triggers Astrocyte Wound Healing. Int. J. Mol. Sci. 2020, 21,3343-3356.
[7] C. Labay, M. Roldan, F. Tampieri, A. Stancampiano, P. E. Bocanegra, M. P. Ginebra, C. Canal. Enhanced Generation of Reactive Species by Cold Plasma in Gelatin Solutions for Selective Cancer Cell Death. ACS Appl. Mater. Interfaces. 2020, 12, 47256-47269.
[8] K. L. M. Taaca, E. I. Prieto, M. R. Vasquez. Current Trends in Biomedical Hydrogels: from traditional crosslinking to plasma-assisted synthesis. Polymers, 2022, 14, 2560-2572.
[9] B. Reig-Vano, B. Tylkowski, X. Montané, M. Giamberini. Alginate-based hydrogels for cancer therapy and research. Int. J. Biol. Macromol.,2021, 170,424–436.
[10] V. Veronico, P. Favia, F. Fracassi, R. Gristina, E. Sardella. Validation of colorimetric assays for hydrogen peroxide, nitrate and nitrite ions in complex plasma-treated water solutions. Plasma Processes Polym., 2021, e2100062.

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Nucleic acid-based portable device for liquid biopsy application

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In the field of clinical diagnostics, microRNAs (miRNAs) have emerged as indispensable biomarkers for detecting and tracking a spectrum of human ailments. Their significance is especially pronounced in the case of cancer detection [1]. Liquid biopsy samples, comprising different bodily fluids, have proven effective in detecting them and their occurrence can substantially aid in the timely diagnosis and prediction of cancer [2]. The medical community urgently requires simple and effective analytical methods to detect miRNAs, which can offer significant advantages for patients and healthcare providers [3]. It is important to consider the distinctive physicochemical traits of the specimen under analysis as it can affect how miRNA targets are determined. Within this endeavour, a new technique for detecting miRNA using a gold electrode screen printing platform has been developed. miRNA-21 was chosen as a biomarker for detection due to its significant involvement in various cancers, such as prostate, breast, colon, pancreatic and liver. To facilitate the detection process, a customized DNA sequence modified with methylene blue (MB) was attached to the electrochemical strip [4], enabling precise identification of miRNA-21. Following the optimization of various parameters in standard solutions, including pH, presence of interferent species, and NaCl salt concentration, the square-wave voltammetry (SWV) technique was successfully employed for detecting miRNA-21 at remarkably low concentrations, reaching a detection limit of around 2 nM. Subsequently, the developed device was utilized for analyzing multiple urine samples. Remarkably, the device demonstrated exceptional selectivity in the presence of complex matrices, exhibited satisfactory repeatability, and achieved a limit of detection in the nanomolar range, similar to the results obtained with standard solutions.

References

[1] Y. Huang, XJ. Shen, Q. Zou, SP. Wang, SM. Tang, GZ. Zang. Biological functions of microRNAs; A review. J Physiol Biochem. 2011, 67, 129-139.
[2] TA. Farazi, JI. Spitzer, P. Morozov, T. Tuscl. MiRNAs in human cancer. J Pathol. 2010, 223, 102-115
[3] V. Shah, J. Shah. Recent trends in targeting miRNAs for cancer therapy. J Pharm Pharmacol. 2020, 72, 1732-1749
[4] S. Cinti, E. Proietti, F. Casotto, D. Moscone, F. Arduini. Paper-Based strips for the Electrochemical dection of single and double stranded DNA. Anal Chem. 2018, 90, 13680-13686.