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SQUARAINE DYES

FLUORESCENCE "TURN-ON" FOR PROTEIN DETECTION AND QUANTIFICATION

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INTRODUCTION

In the last decades great attention was dedicated to the development of new dyes as markers in biological applications. **Polymethine dyes** (i.e. squaraines and cyanines) deserve to be counted among innovative potential photosensitizers (PSs) because they offer numerous advantages such as their easiness in designing NIR molecules simply by elongating the central bridge and/or tuning the lateral functional groups [1], providing NIR compounds with absorption that perfectly match the phototherapeutic window (650-850 nm). However, in physiological conditions, their chemical instability and **self-aggregation** properties limit their widely applications [2]. Several squaraines exhibit a fluorescence "turn-on" when bound to proteins which translate into an increase in fluorescence quantum yield and lifetimes due to the changes in the environment (Figure 1) [3].

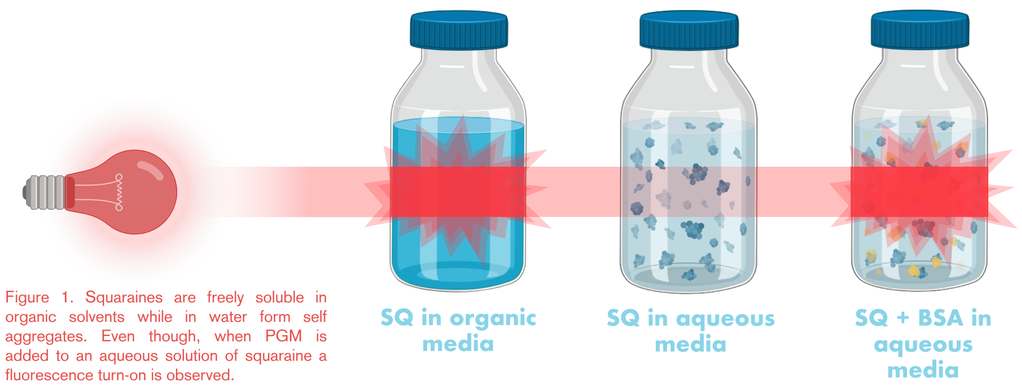
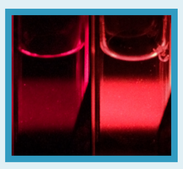


Figure 1. Squaraines are freely soluble in organic solvents while in water form self aggregates. Even though, when PGM is added to an aqueous solution of squaraine a fluorescence turn-on is observed.

AIMS

There is an urgent need and challenge for the development of novel **fluorescent probes** to serve as a platform for a variety of biological applications.



Squaraine dyes that exhibit a **fluorescence turn-on** when bound to proteins could be potentially employed as fluorescent markers for biological applications [3].

Herein we investigate from a spectroscopic point of view the interaction between several proteins and a series of squaraine dyes with different substitutions (Figure 2).

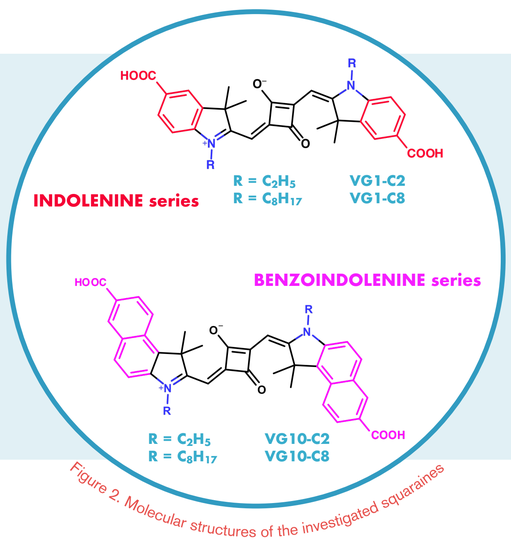


Figure 2. Molecular structures of the investigated squaraines

EXPERIMENTAL PART

- **Spectroscopic characterization**: UV/Vis absorption and steady-state fluorescence spectra of a constant concentration of the squaraine dye were recorded upon increasing the concentration of the protein.
- **Time-domain lifetime** measurements of the Protein-Squaraine adducts.
- **Kinetic measurements** of the formation of Protein-Squaraine complexes.
- **Quantum yield** measurements.
- **Transmission electron microscopy** (TEM) characterization of the adducts.

RESULTS AND DISCUSSION

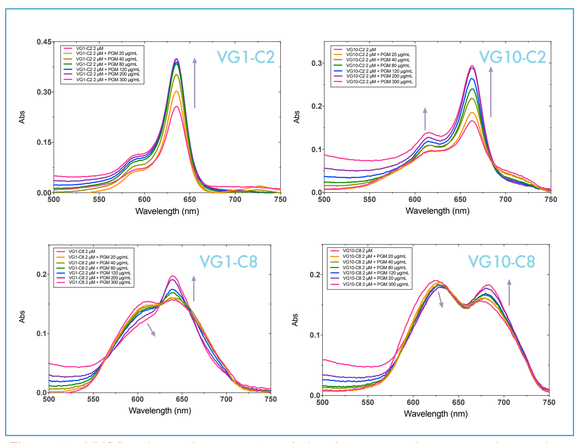


Figure 3. UV/Vis absorption spectra of the four squaraines upon increasing concentrations of PGM.

UV/Vis absorption spectroscopy

Upon addition of protein the UV/Vis absorption spectra of squaraine changes due to the interactions established between the two species.

Addition of increasing concentrations of PGM to a constant concentration of squaraine results in a **disaggregation effect** with a greater amount of solubilized squaraine (Figure 3).

Steady-state fluorescence spectroscopy

Squaraine molecules are almost non non emissive when they are suspended in water, however a gradual addition of protein (such as BSA or PGM) gave an **enhancement** in fluorescence intensity (turn-on), sometimes with a bathochromic shift of about 11-12 nm for all the studied squaraines (Figure 4).

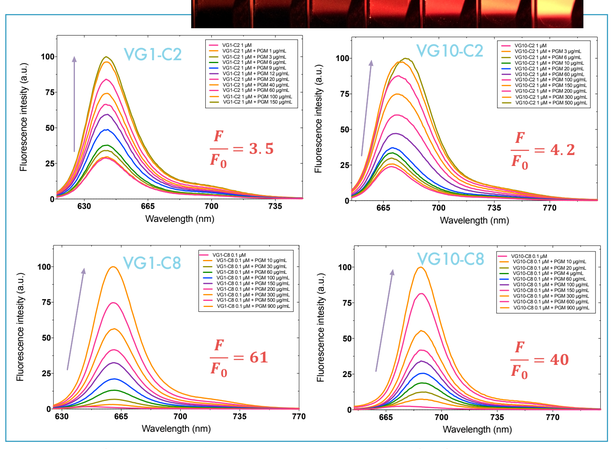


Figure 4. Steady-state fluorescence spectra of VG1-C8 upon increasing concentrations of PGM.

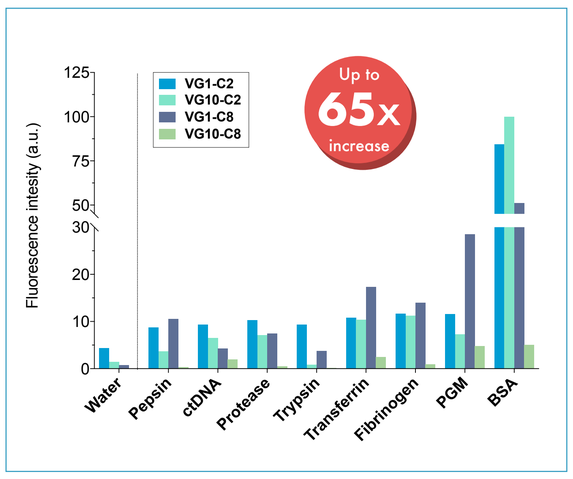


Figure 5. Maximum of the fluorescence intensity of the four squaraines alone and in presence of different proteins.

Fluorescence "turn-on"

The addition of the proteins to a water solution of squaraine generally yielded a significant increase of the fluorescence intensity (Figure 5).

Particularly, the greater enhancement of fluorescence intensity was observed especially in presence of bovine serum albumin (BSA) and porcine gastric mucin (PGM).

Kinetics of interaction

The dye backbone structure as well as the length of the alky chain play a crucial role in the kinetics of the interaction with PGM. The bulkier the dye's molecular structure the slower the interaction (Figure 6).
VG1-C2 formed an immediately stable complex, while VG10-C2 and VG1-C8 showed similar behaviour with a reaction time within 20 minutes. VG10-C8 requested the longer time to form a steady complex.

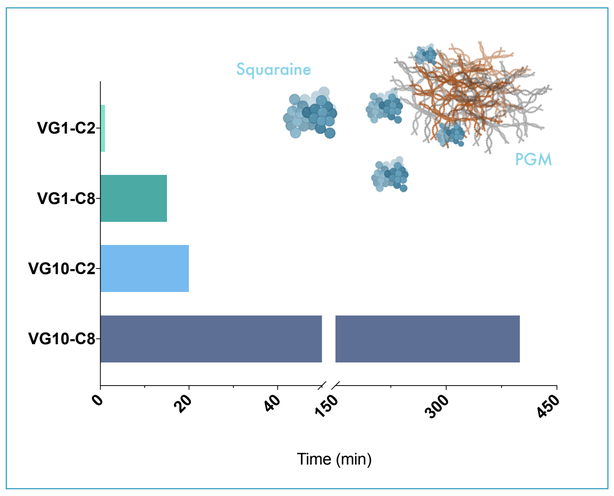


Figure 6. Kinetic behavior of the squaraine-PGM complexes formation.

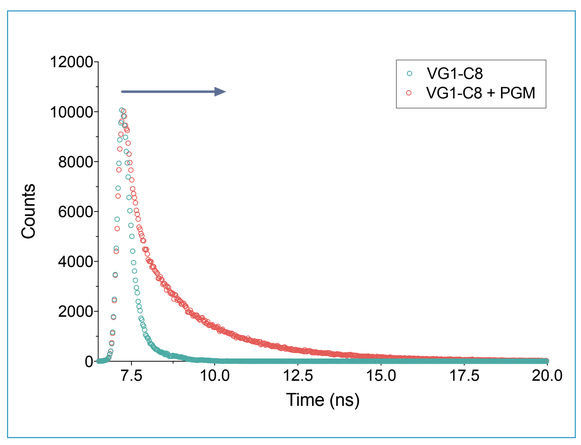


Figure 7. Time-domain lifetime of VG1-C8 alone and in presence of increasing concentrations of PGM.

Lifetime measurements

The complex formation between VG1-C2 and VG1-C8 with PGM was further confirmed by time-correlated single photon counting (TCSPC).
For instance, time-resolved fluorescence analysis indicated that VG1-C8 alone exhibits a mono-exponential decay, whereas bi-exponential decay with significantly increased lifetimes were observed in the presence of PGM (Figure 7).

TAKE HOME MESSAGE

Squaraine dyes have a **structure-relationship influence** on the kinetic interaction with PGM (and BSA [3]).
Squaraine showed a significant **increase of fluorescence intensity** when proteins (especially BSA and PGM) were added probably due to the interactions established with the hydrophobic domains of the protein.
Protein-dyes adducts could be employed as potential probes or photosensitizers for different applications (bioimaging, photodynamic therapy, etc).

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24-26 June 2019, Lipari

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