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A lateral flow immunoassay for straightforward determination of fumonisin mycotoxins based on the quenching of the fluorescence of CdSe/ZnS quantum dots by gold and silver nanoparticles

Laura Anfossi[1]*, Fabio Di Nardo[1], Simone Cavalera[1], Cristina Giovannoli[1], Giulia Spano[1], Elena S. Speranskaya[2], Irina Y. Goryacheva[2], Claudio Baggiani[1]

[1] Department of Chemistry, University of Turin. Via Giuria, 5, I-10125 Turin, Italy

[2] Department of General and Inorganic Chemistry, Chemistry Institute, Saratov State University, Astrakhanskaya 83, 410012 Saratov, Russia

EXPERIMENTALS

Chemicals and materials

Gold (III) chloride trihydrate, silver nitrate, Fumonisin B1 (FMB1) Oekanal standard solution, bovine serum albumin (BSA), poly(maleic anhydride-alt-1-octadecene) (PMAO), Jeffamine 100, and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydro-chloride (EDC) were obtained from Sigma–Aldrich (St. Louis, MO, USA https://www.sigmaaldrich.com). FMB1 powder was purchased from Fermentek (Jerusalem, Israel). Tween 20, sodium borohydride, and other chemicals were purchased from VWR International (Milan, Italy, https://it.vwr.com/).

The anti-FMB1 antibody (rabbit polyclonal antiserum raised against FMB1, relative cross-reactivity towards fumonisin B2, FMB2, 97%) was kindly supplied by EuroClone Spa (Milano, Italy, www.euroclonegroup.it/). The γ-globulin fraction was isolated by ammonium sulphate precipitation and used without any additional treatments. The goat anti-rabbit immunoglobulin antibody was purchased from AbCam (Cambridge, UK, www.abcam.com/).

Millipore High-Flow 180, absorbent cellulose pad and glass fibre conjugate pad were obtained from Merck Millipore (Billerica, MA, USA, https://www.merckmillipore.com/IT/it/products/ivd-oem-materials-reagents/)

QD synthesis

QDs were synthesized via a hot injection method according to Beloglazova et al. [36] . Briefly, using the Se precursor solution (0.1 M), in ODE and the Cd precursor solution (0.1 M), prepared by combining CdO and oleic acid in a 1:8 molar ratio with ODE. A mixture of Cd precursor solution (5 mL) and octadecene (13 mL) in a three-neck flask was heated to 260 °C under nitrogen. At this temperature, 5 mL of the Se precursor solution was quickly injected into the reaction mixture. The temperature was lowered to 235 °C and retained at this value throughout the course of CdSe quantum dot growth. To isolate CdSe quantum dots, an equal volume of ethanol/butanol mixture (1/1, v/v) was added to the QD solution. Then, ethanol was added until an opalescent solution was formed. This turbid solution was centrifuged for 7 min at 2,700×g. Afterwards, the precipitate was dissolved in toluene and precipitated again with ethanol following with dispersion in toluene. Obtained CdSe cores had diameter of ~2.5 nm. For CdSe shelling, zinc sulphide (ZnS) shell growth and precursor preparation were carried out under nitrogen atmosphere. The Zn precursor solution (0.1 M) was

prepared by dissolving zinc acetate in a mixture of oleylamine (OLA/Zn 8:1 molar ratio) and octadecene. 2.98 mL of both Zn and S precursors was mixed with 4 mL of ODE. The toluene solution of CdSe QDs (0.3 mL, 1×10^{-4} mmol was added to the precursor mixture and the temperature was increased up to 140 °C and kept constant for 2.5 h. For purification acetone was added to the CdSe/ ZnS solution dropwise until a turbid solution was obtained. The samples were centrifuged at 2,700 × g for 4 min. The QDs were redissolved in toluene.

For hydrophilization amphiphilic polymer poly(maleicanhydride-alt-1-octadecene) (PMAO) and olyoxyethylene/ polyoxypropylene block-copolymer, contained one primary amine group (Jeffamine M1000), was synthesized [30]. For encapsulation, QDs and the amphiphilic polymer were mixed in chloroform and stirred overnight at room temperature (molar ratio QD:polymer was 1:40). Chloroform was evaporated; residue was dissolved in KOH water solution. To completely remove any polymer residues, ultracentrifugation at 200,000 g in sugar gradient was used.

Metal nanoparticle synthesis

Gold nanoparticles with SPR band at 525 nm were prepared by the citrate reduction method, as previously reported [33]. A typical protocol consists in adding 1 mL of 1% sodium citrate to 100 mL of boiling 1% tetrachloauric acid under vigorous stirring. In few minutes, the colour of the solution turns from blue-greyish to ruby red indicating successful formation of GNPs. After cooling down, the pH of the colloid is adjusted to 8 by means of sodium carbonate-sodium bicarbonate buffer (50 mM pH 9.6) and stored at 4 °C for subsequent conjugation to biomolecules.

Silver nanoparticles with SPR band at 372 nm were obtained by reducing silver nitrate with $NaBH_4$ using citrate as a capping agent, according to Bao et al. [37]. In details, 10 mL of silver nitrate (2×10^{-3} M) were mixed with 10 mL of sodium citrate (6×10^{-3} M) and vigorously stirred. 0.5 ml of $NaBH_4$ (25 mM) were slowly added to the mixture that turned initially grey-black and then brilliant yellow, which confirmed the successful obtainment of SNPs. The colloidal solution was kept for further 10 minutes under stirring, followed by pH adjusting to 8 as described for GNPs.

Antigen preparation

The BSA-FMB1 conjugate was synthesized as described in [32]. Briefly, 6.5 mg of FMB1 and 3 mg of BSA were dissolved in MES buffer (0.1M, 0.9M NaCl, pH = 4.7). 0.7 mL of a freshly prepared EDC aqueous solution (10 mg mL^{-1}) was added dropwise and reacted for 2 hours at room temperature under stirring. The BSA-FMB1 product was recovered by size-exclusion chromatography on Sephadex G-25 cartridges (GE Healthcare Life Sciences, UK, www.gelifesciences.com/), following manufacturer's instructions.

QD functionalization with antigen

The BSA-FMB1/QD conjugates (QD-Ag) were prepared using the activated ester approach, according to the protocol used in Di Nardo et al. [12], with the following minor modifications: 0.1 mL of QDs diluted in 1 mL of phosphate buffer, was pre-activated for 20 min at room temperature by 0.05 mL of EDC as described for antigen preparation. Then, 0.66 mg of the antigen was added to the pre-activated QDs and reacted for 30 min at room temperature. A second aliquot of EDC was added and the tube was transferred to 4 °C for a further overnight incubation. QDs-Ag were separated from unreacted antigen and other by-products by repeated centrifugations (14000 rpm, 30 min).

Spectroscopic characterization of nanoparticles

Absorption spectra of NPs were recorded on a Varian Cary 1E spectrophotometer (Agilent Technologies, USA, http://www.agilent.com/) and characteristic SPR bands of the NPs were confirmed.

Photoluminescence of QDs and QDs - metal NPs mixed solutions was measured by registering emission spectra of QDs (400-600 nm) on a Varian Cary Eclipse Fluorescence spectrophotometer (Agilent Technologies, USA, http://www.agilent.com/). Excitation was set at 365 nm (10 nm bandwidth).

Electron micrographs were obtained using a Jeol 3010-UHR (Jeol Ltd, Japan, https://www.jeol.co.jp/en/) high resolution transmission electron microscope (HRTEM) equipped with a LaB6 filament operating at 300 kV and with an Oxford Inca Energy TEM 300 X-ray EDS analyser. A drop of the metal NPs aqueous suspension was put on a copper grid covered with a lacey carbon film for the analysis.

Preparation of strips for the FQICST

Strips comprised (from bottom to up): a glass fibre sample pad, the nitrocellulose membrane (Hi-flow plus 180), and a cellulose adsorbent pad. Immunoreagents were applied onto the membrane by means of the XYZ3050 dispenser (Biodot, Irvine, CA). The Test line was composed of QDs (3 µM) mixed withBSA-FMB1 (0.1 mg/ml) and the Control line comprised a goat anti-rabbit antibody (1 mg/ml) using phosphate buffer as the diluent. Reagents were applied at a flow rate of 1 µL cm^{-1}, keeping a distance of 4 mm between the lines. Membranes were dried at 37 °C for 60 minutes under vacuum, cut into strips (4 mm width) by means of a CM4000 guillotine (Biodot, Irvine, CA, USA, https://www.biodot.com/) and inserted into plastic cassettes (Kinbio, China, www.kinbio.com/).

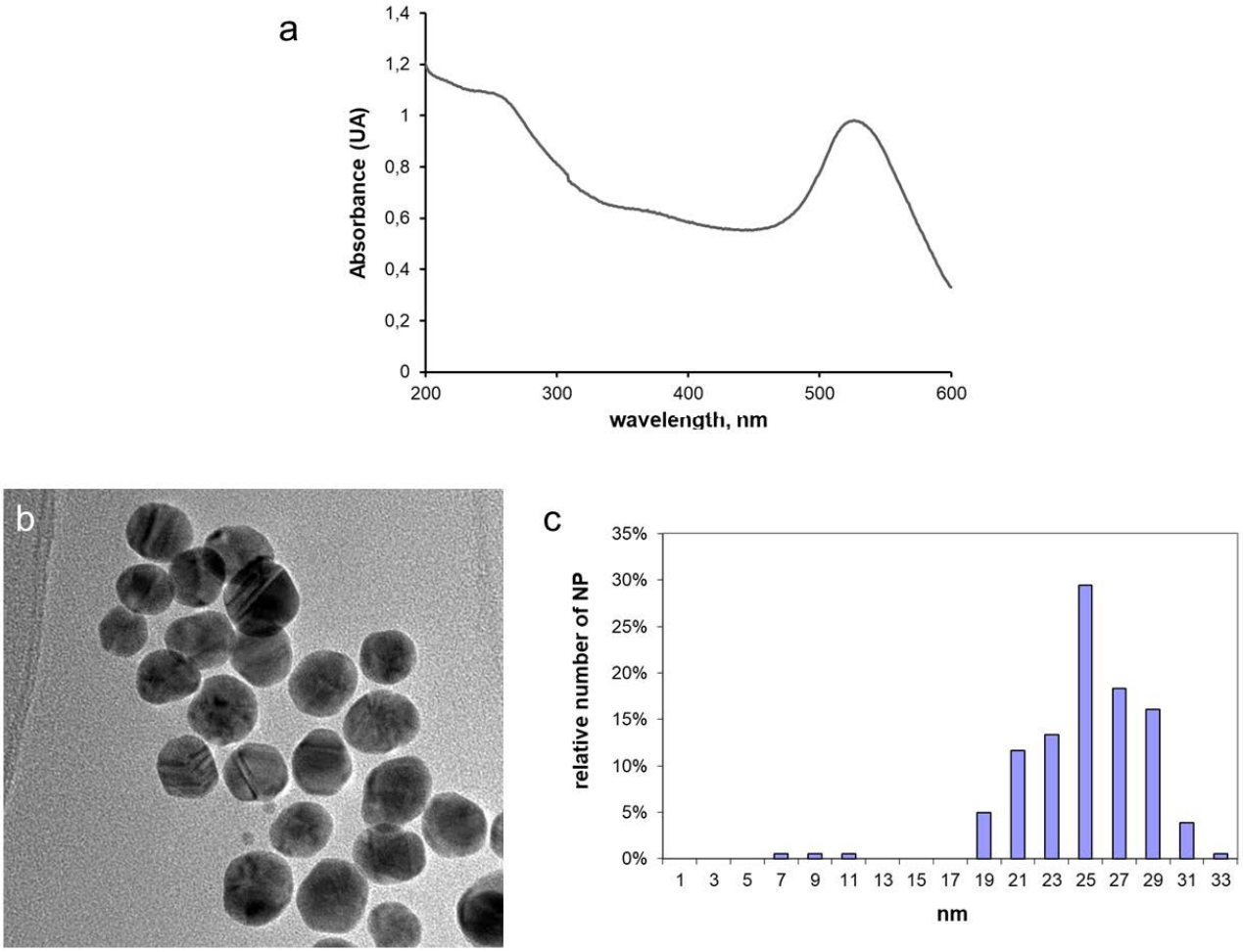
Processing of strip images for quantifying photoluminescence

Pictures of the fluorescence ICST were obtained through a super charge-coupled device camera (Fujifilm, Tokyo, Japan). Digital processing of Tagged Image File Format comprised the following steps: (1) selecting the green component from RGB image, (2) reversing black/white, (3) quantifying black pixels with QuantiScan 3.0 software (Biosoft, Cambridge, UK, www.biosoft.com/).

Spectroscopic characterization of metal nanoparticles.

The SPR band of GNPs was confirmed at 525 nm (Fig S1a). TEM images revealed that GNP were almost spherical in shape (Fig S1b) and had mean diameters of 25 ± 4 nm (Fig S1c).

Figure S1. UV-vis absorption spectrum (a) and TEM image (b) of GNPs with SPR band at 525 nm. Mean diameter of GNPs was calculated as 25 ± 4 nm, based on averaging dimension of 180 NPs (c).



Silver nanoparticles showed a strong SPR band at 372 nm (Fig S2a). The morphology investigated by TEM imaging revealed a less homogenous preparation compared to GNP. However SNP were roughly spherical in shape (Fig S2b), with lower dimension respect to GNPs (diameter < 20 nm)

Figure S2. UV-vis absorption spectrum (a) and TEM image (b) of SNPs with SPR band at 372 nm.