Immunoassay quantification using surface-enhanced fluorescence (SEF) tags†

S. A. Camacho,a,b R. G. Sobral-Filho,b P. H. B. Aoki,b,c C. J. L. Constantinoa and A. G. Brolo†a,b

Fluorescence-based immunoassays are widely used in several areas, ranging from basic biomedical research to disease diagnostics. A variety of new probes have been developed recently to address some limitations in typical assays performed with organic dyes. Ideally, new fluorescence tags that allow quantification with a low limit of detection are highly desired. In this work, the surface-enhanced fluorescence (SEF) phenomenon was explored in the development of tags for Immunoglobulin-M (IgM) detection. Shell-isolated gold nanoparticles (Au-SHINs) with 100 nm core size and a 10 nm silica shell were synthesized. These particles contain an outermost thin fluorescent layer of Nile blue (NB) that was further coated by another 5 nm of silica (SEF tags). The outer silica shell was then functionalized with antibodies to allow the detection of IgM as in typical immunological sandwich assays. IgM concentrations down to the 10 ng mL⁻¹ mark were successfully detected. A linear dependence between the average fluorescence intensity and the IgM concentration was found.

Introduction

Fluorescence-based methods are widely used in biomedical analysis, particularly in immunoassays.1–3 However, despite their large adoption and popularity, some common fluorescent labels have disadvantages in terms of emission efficiency, particularly for determinations at low concentrations. Therefore, there is a continuous quest for more sensitivity and stable probes4 to improve the performance of fluorescence-based immunoassays and imaging.

The advent of different classes of nanostructured materials has been providing interesting alternatives to organic dyes for optical analytical methods.5,6 For instance, quantum dots have been largely applied as fluorescence probes for biological analysis.7 Shell-isolated nanoparticles (SHINs) represent a new generation of nanomaterials used for enhanced spectroscopy. They were initially introduced as a new approach for surface-enhanced Raman scattering (SERS),8 in a technique named shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS). In SHINERS, the Raman signal from a sample of interest is amplified by metallic nanoparticles (Au, Ag and Cu) coated with an ultrathin silica shell (2–4 nm of thickness).8,9 The silica layer eliminates issues related to metal–molecule interactions, particularly related to chemical adsorption and formation of metal complexes. Furthermore, SHINs may be used over different types of surfaces (single crystal, flat materials, and others).8,9

The SHINs concept can also be applied to the engineering of high efficient labels for fluorescence spectroscopy. However, metallic surfaces are known to quench the emission of directly adsorbed dyes, which, in principle, could limit the applicability of plasmonics for enhanced fluorescence. On the other hand, it has been demonstrated that when the distance between the molecule and the nanostructure increases (controlled by an inert spacer, for instance) a continuous transition from fluorescence quenching to fluorescence enhancement is observed.10 A maximum enhanced observed at about 10 nm from the metallic surface.11 At a distance (emitter – metal) superior to 35 nm, the enhanced efficiency drops back to the levels observed for an unperturbed molecule.11 SHINs then appear as ideal platforms to promote surface-enhanced fluorescence (SEF).12–15 since the distance between a molecular emitter and the nanostructure can be easily controlled by tuning the silica shell thickness.12 Highly efficient fluorescence labels can be designed by tuning parameters, such as size, geometry, and composition of SHINs.

Herein we report on a new design of SHINs tags for SEF-based immunological assays. The goal is to take advantage of the enhanced fluorescence provided by SHINs, which has
shown to be orders of magnitude higher than the fluorescence signal collected from regular dyes, to improve the limit of detection for immunoassays. The Au-SHINs were synthesized with a 100 nm core size and a 10 nm silica shell, which was coated with a fluorescent layer of Nile blue (NB). The nanostructure (Au core–SiO₂ shell–NB dye layer) was further wrapped in a final silica shell (5 nm-thick) leading to a “SEF tag”. This outer silica shell is applied in order to: (i) protect the SEF tag from uncontrolled aggregation that could result in the lack of reproducibility and (ii) provide a handle for chemical functionalization, imparting the required specificity to the SEF tag for affinity tests. In our case, the SEF tag was functionalized with polyclonal IgM antibodies (anti-IgM) (“SEF tag – pAb”) and applied, as a proof of concept, for the detection of IgM. IgM is the first antibody recruited by the body to deal with new infections. IgM-based assays can then be extremely useful to diagnosis initial phases of infectious diseases, such as dengue and Zika. The SEF tags developed here allowed probing from highly diluted solutions of IgM biomarker (down to 10 ng mL⁻¹) with a limit of detection (LOD) of ~5.0 ng mL⁻¹ in a typical sandwich assay. Moreover, a linear dependence was found between the fluorescence signal intensities and the IgM concentrations. These results demonstrated a new and improved immunological assay based on the use of plasmonic nanotags.

**Experimental**

**Materials**

All glassware was cleaned with piranha solution (1:1 ratio of H₂SO₄/H₂O₂) and rinsed thoroughly with ultrapure water (resitivity = 18.2 Ω cm). Tetrachloroauric acid (HAuCl₄·3H₂O, MW = 393.83 g mol⁻¹), sodium citrate (C₆H₅Na₃O₇·2H₂O, MW = 294.10 g mol⁻¹), hydroxylamine hydrochloride (NH₂OH·HCl, MW = 69.49 g mol⁻¹), (3-aminopropyl)trimethoxysilane (APTMS, C₆H₁₇NO₃Si, MW = 179.29 g mol⁻¹, d = 1.027 g mL⁻¹), sodium silicate solution (10.6% Na₂O and 26.5% SiO₂, d = 1.39 g mL⁻¹), 3-(triethoxysilyl)propyl isocyanate ((C₂H₅O)₃Si (CH₃)₂NCO, MW = 247.36 g mol⁻¹, 95%), tetraethyl orthosilicate (TEOS, Si(OCH₃)₄, MW = 208.33 g mol⁻¹) and phosphate buffered saline (10× concentrate, suitable for cell culture) were purchased from Sigma-Aldrich and used without further purification. Nile blue A perchlorate (NB, C₁₃H₂₄O₆Si MW = 304.41 g mol⁻¹, d = 1.070 g mL⁻¹ 95%) was acquired from Gelest.

**Synthesis of gold shell-isolated nanoparticles (Au-SHINs)**

AuNPs-40 (gold nanoparticles with ~40 nm diameter) were prepared by sodium citrate reduction as described by Grabar et al.¹⁷ and Aroca et al.¹³ Briefly, in a round-bottom flask attached to a condenser, 100 mL of 0.01% HAuCl₄ were brought to boil with vigorous magnetic stirring. Once under reflux, 1.3 mL of 1% sodium citrate were added quickly and the boiling and stirring were kept for 10 min after the resulting solution became reddish-purple. The colloidal suspension was removed from the heat, stirred for another 15 min and cooled to room temperature. The AuNPs-100 (gold cores with ~100 nm diameter) were prepared according to Li et al.⁹ 4 mL of AuNPs-40 were placed into a round-bottom flask containing 53 mL of ultrapure water. Then, 0.9 mL of 1% sodium citrate was added and the resulting mixture was stirred for 3 min. 0.9 mL of 1% HAuCl₄ was then added and kept under stirring for another 8 min. The growth of the Au cores was initiated by adding 1.4 mL (1 × 10⁻¹⁰ mol L⁻¹) of hydroxylamine solution to the round-bottom flask under continuous stirring. The reaction was completed after 5 min, but the stirring continued for another hour at room temperature. In order to coat the 100 nm Au cores with silica, 15 mL of AuNPs-100 colloidal were diluted in 30 mL of ultrapure water and placed in a round-bottom flask. Then, 0.5 mL of APTMS at 5 × 10⁻⁴ mol L⁻¹ was added to the flask and the mixture was stirred for 20 min. Subsequently, 2.8 mL of 0.54% aqueous sodium silicate solution (pH = 10) were added and this mixture was kept under stirring for 3 min. The mixture was rapidly placed in a water bath with a controlled temperature between 90 and 95 °C, from which the Au-SHINs samples were collected after 6 h. The Au-SHINs were centrifuged at 6000g for 10 min, the supernatant was decanted and the Au-SHINs were subsequently redispersed in ultrapure water. This procedure was repeated at least five times to obtain clean and concentrated Au-SHINs, with a final concentration of approximately 1.1 × 10¹² nanoparticles per mL. The details on the determination of Au-SHINs concentration are provided in ESI (Fig. S1†).

The silica coating efficiency for fluorescence enhancement was confirmed by mixing NB solutions (10⁻⁵ mol L⁻¹) with uncoated AuNPs-100 achieving a final NB concentration of 10⁻¹⁰ mol L⁻¹. In this case, the fluorescence was quenched (633 nm excitation), but SERS features of NB were identified. A similar experiment using Au-SHINs yielded a strong fluorescence emission. The validation of silica coating efficiency for fluorescence enhancement is presented in Fig. S12 (ESI†).

**Fabrication of surface-enhanced fluorescence tags (SEF tags)**

A Silica-NB precursor was first prepared following the methodology proposed by Grandi et al.¹⁸ Briefly, 4 × 10⁻⁵ mol of NB (8.0 mL of 5 × 10⁻³ mol L⁻¹) was mixed with 4 × 10⁻³ mol of 3-(trimethoxysilyl)propyl isocyanate (10.45 µL of 2.75 mol L⁻¹), under vigorous stirring for 24 h at room temperature. The iso-thiocyanate group of 3-(trimethoxysilyl)propyl isocyanate covalently binds to the amino terminal of NB, forming the Silica-NB precursor.

SEF tags were fabricated as proposed by Lee et al.⁴ with minor modifications. In a first step, 250 µL of Au-SHINs (1.1 × 10¹² nanoparticles per mL) were diluted in 2.75 mL of ultrapure water and 20 mL of isopropanol. Then, the solution
was stirred with 9.2 µL of Silica-NB precursor (5 × 10⁻³ mol L⁻¹) for 30 min, followed by addition of 9.2 µL of TEOS (1 × 10⁻² mol L⁻¹) and 20 µL of NH₄OH (28–30%). The mixture remained under stirred for 4 days before centrifugation at 10 000g for 10 min. The supernatant was decanted and the SEF tags were subsequently redispersed in ultrapure water. This rinsing procedure was repeated at least five times in order to obtain clean and concentrated SEF tags. Fig. 1a shows a schematic illustration of each step for preparing the SEF tags. In addition, the variation of the fluorescence intensity of the SEF tags was followed at each rinsing step in order to guarantee that the excess of NB molecules was removed. The variation of SEF tags fluorescence intensity along the rinsing process is displayed in Fig. SI3 (ESI†).

**Antibody conjugation**

**SEF tags.** SEF tags conjugated with polyclonal anti-IgM antibodies (pAb) were prepared as described by Li *et al.*,19 with slight modifications. Fig. 1a exhibits an illustration of the SEF tags conjugation with pAb (anti-IgM). First, 200 µL of SEF tags (1.1 × 10¹² SEF tags per mL) were centrifuged at 10 000g for 10 min and redispersed into 200 µL of ethanol. The SEF tags were then incubated overnight (20 h) in 1.0 mL of TEPSA ethanol solution (0.12 mol L⁻¹) at room temperature, under stirring, in order to obtain a carboxyl group modified surface. After this step, the SEF tags were washed three times with PBS buffer and redispersed into 1.0 mL of PBS buffer containing NHS (5 × 10⁻² mol L⁻¹) and EDC (0.2 mol L⁻¹) in order to activate the carboxyl terminal groups. The SEF tags were incubated in the NHS/EDC PBS buffer for 2 h at room temperature, under stirring, followed by the rinsing procedure (3×) with PBS buffer. Then, 1.0 mL of a PBS buffer solution of polyclonal anti-IgM antibody (2 × 10⁻⁴ g mL⁻¹) was added onto the activated SEF tags and incubated overnight (20 h) at 10 °C. Unbound polyclonal anti-IgM antibodies were removed by centrifugation at 1500g for 10 min followed by 3× rinsing with PBS buffer. The resultant SEF tags were redispersed into 200 µL of PBS buffer for further use. Protein-coated tags followed the same storage guidelines as the antibodies.

![Schematic illustration of (a) SEF tag – pAb preparation: Au-SHINs (~100 nm core size + 10 nm silica shell thickness); Au-SHINs + a fluorescent layer of NB; Au-SHINs + a fluorescent layer of NB + a ~5 nm final silica shell (SEF tag); and conjugation onto polyclonal anti-IgM (SEF tag – pAb). (b) Cover slide conjugation to monoclonal anti-IgM antibodies: incubation in TEPSA to obtain COOH groups modified surface; immersion in NHS/EDC to activate the COOH groups to bind the amino groups of proteins; incubation with monoclonal anti-IgM antibodies (mAb); and incubation with skim milk to minimize the non-specific adsorption. (c) SEF immunoassay: IgM biomarkers of different concentrations were dropped onto the corresponding punched-well, incubated for 1 h, and rinsed with PBS buffer. (d) SEF tag – pAb was dropped onto the corresponding punched-well, incubated for 1 h, rinsed with PBS buffer, and measured with 633 nm laser line excitation. Rinsing with PBS buffer was performed at least 3× in each step of the procedure, as described in the Experimental section.](https://doi.org/10.1039/C7AN00905D)
Cover slides. Monoclonal anti-IgM antibodies (mAb) were immobilized onto cover slides following the procedure described by Li et al. A schematic illustration of the cover slide modification with functional monoclonal anti-IgM antibodies (mAb) is shown in Fig. 1b. Cover slides (25 mm length, 18 mm width and 1 mm thick) were cleaned by sonication 3× in ultrapure water and once in ethanol for 10 min each. After that, the slides were incubated overnight (18 h) at room temperature in TEPSA (0.1 mol L\(^{-1}\)) ethanol solution and then rinsed 3× with ethanol and 3× with PBS buffer in order to obtain a carboxyl group modified surface. The carboxyl groups were activated by immersing the slides in PBS buffer containing NHS (5 × 10\(^{-2}\) mol L\(^{-1}\)) and EDC (0.2 mol L\(^{-1}\)) for 2 h at room temperature. After rinsing 3× with PBS buffer, punched-wells PDMS masks of 5 mm in diameter were sealed onto the cover slides in order to pre-define the immunoassay regions. The immunoassay regions were activated by immersing the slides in PBS buffer containing NHS (5 × 10\(^{-2}\) mol L\(^{-1}\)) and EDC (0.2 mol L\(^{-1}\)) for 2 h at room temperature. After rinsing 3× with PBS buffer, punched-wells PDMS masks of 5 mm in diameter were sealed onto the cover slides in order to pre-define the immunoassay regions.

The immunoassay regions were incubated overnight (20 h) at 10 °C in a PBS buffer solution of the monoclonal anti-IgM antibody (2 × 10\(^{-4}\) g mL\(^{-1}\)). Finally, the non-specific adsorption was minimized by incubating the punched-wells in a PBS buffer solution of skim milk 1% for 2 h at 10 °C, followed by rinse steps with PBS buffer (3×). Experiments using bovine serum albumin (BSA) as blocking agent were also carried out, but skim milk provided a better performance for this specific assay.

**SEF immunoassay of IgM biomarker**

A schematic representation of the SEF immunoassay is depicted in Fig. 1d. Different concentrations of IgM (biomarker) were prepared in PBS buffer. The selected concentrations were: 10 ng mL\(^{-1}\), 100 ng mL\(^{-1}\), 250 ng mL\(^{-1}\), 500 ng mL\(^{-1}\), 5 µg mL\(^{-1}\), 15 µg mL\(^{-1}\), 50 µg mL\(^{-1}\) and 100 µg mL\(^{-1}\). These concentration values are within the range of clinically relevant concentrations of IgM detected in regular diagnostic kits for infectious diseases. 10 µL of each IgM biomarker concentration were dropped onto the corresponding punched-well of the SEF immunoassay region, and incubated for 1 h at 10 °C. After this period, the immunoassay regions were carefully rinsed 3× with PBS buffer in order to remove any unbound IgM biomarker. Then, 10 µL of SEF tags – pAb were dropped onto the corresponding wells and incubated for one more hour at 10 °C, followed by the rinsing steps with PBS buffer (3×).

**Characterization**

The UV-Vis extinction spectra of AuNPs-40, AuNPs-100, Au-SHINs and SEF tags were measured in a Varian spectrophotometer, model Cary 50, from 190 to 1100 nm. Transmission electron microscopy (TEM) images were carried out with a JEOL JEM-1400 transmission electron microscope equipped with a Gatan Orius SC1000 camera. The instrument has a 0.2 nm lattice resolution and magnification range from 200× to 1 200 000×. Normal Raman, SERS and SEF spectral acquisition was performed with an in-Via Raman microscope (Renishaw Inc., Hoffman Estates, IL, USA) with laser line at 633 nm, a 50× (NA = 0.75) dry objective (Leica Microsystems, Wetzlar, Germany). Spectral acquisition was carried out using 600 lines per mm diffraction grating, 1 s acquisition time, laser power at 17 mW attenuated to 10%. A 638.6–781.3 nm spectral window was recorded in the emission. Spectra were recorded with a thermoelectrically cooled iDus CCD detector (Andor Technology, Belfast, UK). All the spectral manipulation was performed with the software GRAMS AI®. The TEM images were processing using ImageJ®. The map of the intensity distribution of the fluorescence in the probed area was evaluated using the Wire 3.4® software (Renishaw).

**Results and discussion**

**Morphological and spectroscopic characterization of Au-SHINs and SEF tags**

As detailed in the Experimental section, SEF tags were fabricated from Au-SHINs. Fig. 2a shows a TEM image of the AuNPs-40 (40 ± 3.4 nm). The AuNPs-40 were then applied as seeds for the growth of AuNPs-100 (102 ± 3.8 nm) depicted in Fig. 2b. Fig. 2c shows the AuNPs-100 coated with the first silica layer. The silica shell thickness was tuned to 10 nm to provide

---

*Fig. 2* TEM images for (a) AuNPs-40 (40 ± 3.4 nm), (b) AuNPs-100 (102 ± 3.8 nm), and (c) Au-SHINs (102 ± 3.8 nm of core size and 9.0 ± 1.0 of silica shell thickness). (d) Extinction spectra for AuNPs-40, AuNPs-100, and Au-SHINs with maximum LSPR at 530, 570, and 577 nm, respectively.
an optimal distance between the metallic nanoparticle and the fluorescent layer. As pointed out in the literature, an increase in the thickness of silica shell tunes the metal-dye distance, allowing a continuous transition from fluorescence quenching to fluorescence enhancement. Additionally, we have recently demonstrated that Au-SHINs with larger cores (ca. 100 nm) produce higher fluorescence enhancements. The characteristic localized surface plasmon resonance (LSPR) of the AuNPs was dependent on both the core size and the silica coating thickness. Fig. 2d shows a significant LSPR shift from 530 nm to 570 nm as the AuNPs core size increased. The silica coating causes a further red-shift from 570 nm to 577 nm, as shown in Fig. 2d.

Fig. 3a and b illustrate the increase on the silica shell thickness from 9.0 ± 1.0 nm to 13.4 ± 1.4 nm as the final layer of silica coated the chemically bound NB. A slight red-shift on the LSPR extinction maximum, from 577 nm to 582 nm, is seen in Fig. 3c as a result of this final silica coating. Fluorescence emission spectra from 10⁻⁶ mol L⁻¹ NB aqueous solution and from SEF tags colloidal suspension were also obtained, and they are presented in Fig. 3d. The maximum in the NB solution fluorescence emission was observed at 667 nm, while the SEF tags emission showed a maximum at 670 nm (Fig. 3d). The spectral features in Fig. 3d are in agreement with the reported emission of NB in aqueous solutions.

The applicability of the SEF immunoassay for biomarker detection was verified by performing a proof-of-concept experiment determination of IgM biomarker. Fig. 4 shows the fluorescence signal from the SEF tags in the presence of different concentrations of IgM, using the sandwich configuration described in Fig. 1. Two types of control experiments were performed (results shown in Fig. 4a). The controls were: (i) A “blank” measurement, where the emission from monoclonal anti-IgM antibodies bounded onto the wells was recorded; and (ii) A “no biomarker” control, where the assay was performed as in Fig. 1d, but without IgM biomarkers to enable the sandwich configuration. The spectral profile of both controls is compared in Fig. 4a with the SEF signal from the sandwich immunoassay in presence of 100 µg mL⁻¹ of IgM biomarker. The results from Fig. 4a show that (1) residual emission from other species in the assay is negligible compared to the SEF emission; (2) the presence of IgM is required for a readable optical output in our experimental conditions.

Fig. 3  TEM images for (a) Au-SHINs and (b) SEF tags showing the increase on the silica shell thickness from 9.0 ± 1.0 nm to 13.4 ± 1.4 nm, respectively. (c) Extinction spectra of Au-SHINs and SEF tags. A red-shift on the LSPR extinction maximum from SEF tags (583 nm), compared to the Au-SHINs (577 nm), was observed. (d) Fluorescence spectrum from 10⁻⁶ mol L⁻¹ NB solution and SEF spectrum from colloidal suspension, respectively. The inset in (d) shows the NB molecular structure.
IgM biomarker (from 100 µg mL\(^{-1}\) to 10 ng mL\(^{-1}\)) is related to a proper sampling of the surface. In standard fluorescence assays using commercial microarray plate readers, a certain number of measurement points can be defined for each particular well, to both improve statistics and take into consideration the spatial heterogeneity of the fluorescence signal. Similarly, we have decided to perform spatial mappings of the detection wells by collecting point-by-point SEF spectra along an area of 40 µm × 40 µm with 3 µm step. A total of 784 spectra were collected for each concentration. The value of the area under the fluorescence spectrum was then plotted for each position, generating the mapped pictures shown in Fig. 4b–i. The SEF maps in Fig. 4 are color-coded, where brighter spots refer to higher emission intensities (areas). More uniform SEF images were observed for higher concentrations of IgM biomarker (Fig. 4b). The number of spots with strong SEF signal decreased as the concentration of IgM biomarker was reduced, indicating that a small amount of SEF tags was captured per well, as expected. It is worth mentioning the clinical relevance of the concentration range of IgM biomarker analyzed here.\(^{22,26}\) For instance, enzyme-linked immunoassays (ELISA) tests for dengue- and Zika-specific IgM have limits of detection down to 100 µg mL\(^{-1}\) for patients with primary and secondary Dengue/Zika infections.\(^{2,27,28}\) Although the immunoassay system designed here is based on PBS solutions of IgM, the low levels of biomarker (10 ng mL\(^{-1}\)) quantified highlighted the usefulness of the SEF phenomenon to enhance the detection limits in biosensing applications.

The average SEF responses were calculated from the maps in Fig. 4 and plotted against the concentrations of IgM biomarker in Fig. 5. A linear dependence between the average SEF intensity and IgM concentration was demonstrated for two concentration ranges (µg mL\(^{-1}\) and ng mL\(^{-1}\)), shown in Fig. 5a and b, respectively. Notice that although the calibration plots

---

**Fig. 4** (a) SEF spectral response for the “blank”, for the “no IgM biomarker” control, and for the sandwich immunoassay in presence of 100 µg mL\(^{-1}\) of IgM biomarker. (b)–(i) Area mapping (40 µm × 40 µm and step of 3 µm) showing the intensity distribution of the integrated fluorescence for different concentrations of IgM biomarker. Laser line excitation at 633 nm, laser power = 17 mW attenuated to 10%, a 50× (NA = 0.75) dry objective and acquisition time = 1 s per pixel.
are linear for each concentration range in Fig. 5, the linearity is lost when all data is plotted in the same graph. In another words, the dynamic range of the method is about 1 order of magnitude within each concentration range.

The error bars in Fig. 5 represent the calculated standard deviation (SD) for the spatial variation of the SEF signal for each IgM concentration. The variation of the error bars is randomly distributed for different IgM concentrations. Similar behavior was observed by Li et al.\textsuperscript{20} in their SERS response for cancer biomarkers in PBS buffer.

The LOD of the SEF immunoassay system was determined considering the average noise level (∼500 counts) and its standard deviation (\(SDBlank = \pm 50\) counts) obtained from a “blank” experiment. The expected minimum detectable SEF intensity would be ∼650 counts within a 95% confidence level (average noise + 3 × \(SDBlank\)).\textsuperscript{20} Since 10 ng mL\(^{-1}\) of IgM was recorded at ∼1500 counts (Fig. 5b), the LOD can be estimated at ∼5.0 ng mL\(^{-1}\). The low levels of IgM biomarker detected here are compatible with those reported in the literature. In a regular scenario, ELISA samples are diluted with buffers to concentrations in the micro- to nanogram range. In order to put the results from Fig. 5 further into perspective, the LOD found for dengue virus (DENV NS1) biomarker detection, for instance, is ca. 75 ng mL\(^{-1}\) in human serum.\textsuperscript{22} The SEF immunoassay designed here is comparable to ELISA in terms of cost and complexity, but presents a better performance in terms of limit of detection. Other diagnostic methods, such as reverse-transcription polymerase chain reaction (RT-PCR),\textsuperscript{30} are more expensive, time consuming and complex.\textsuperscript{28}

Conclusions

Surface-enhanced fluorescent (SEF) tags were successfully fabricated using the concept of gold shell-isolated nanoparticles (Au-SHINs). By tuning the core size (100 nm) and the silica shell thickness (10 nm), the fluorescence enhancement of the nile blue (NB) chemically-bonded layer was optimized. The nanostructures were subsequently wrapped in a silica shell (5 nm-thick), functionalized with anti-IgM antibodies, and applied as proof-of-concept in immunological assays for detecting IgM biomarkers. IgM solutions down to 10 ng mL\(^{-1}\) were successfully detected within a linear dependence between the average fluorescence intensity and IgM concentrations. The limit of detection (LOD ∼5.0 ng mL\(^{-1}\)) reached here is at least 1 order of magnitude lower than similar assays already available for infectious disease diagnosis, highlighting the potential usefulness of surface-enhanced phenomena for immunological applications.

Acknowledgements

We acknowledge funding provided by FAPESP and NSERC. We thank Dr Patrick Nahirney and Brent Gowen for their assistance and access on the TEM.

References