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COMMUNICATION

Significant Metal Enhanced Fluorescence of Ag₂S Quantum Dots in the Second Near-Infrared Window

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The amplification of light in NIR-II from Ag₂S QDs via metal enhanced fluorescence (MEF) is reported for the first time. Significant fluorescence enhancement of over 100 times for Ag₂S QDs deposited on Au-nanostructured arrays, paves the way for novel sensing and imaging applications based on Ag₂S QDs, with improved detection sensitivity and contrast enhancement.

Biosensing and bioimaging in the second near-infrared window (NIR-II, 1.0–1.7 μm) are receiving increasing attention,^{1, 2} with significant advantages compared to the visible (400–750 nm) and conventional near-infrared (NIR, 750–900 nm) regions. In the NIR-II region, minimal light absorption from water (absorbing for wavelengths above 900 nm) and, at the same time, from hemoglobin (absorbing for wavelengths below 650 nm), offers high transparency when imaging soft tissues and blood. Therefore, detection in NIR-II allows for deeper tissue penetration, as well as a higher signal-to-noise ratio, as a result of reduced photon scattering and negligible autofluorescence from biomolecules.^{3, 4} However, only a limited number of NIR-II fluorescent probes have been reported to date, including several types of quantum dots (QDs; *e.g.* PbSe, PbS, CdHgTe), but their high toxicity raises concerns for *in vivo* applications.⁵ In contrast, the recently developed Ag₂S QDs^{5, 6} have emerged as attractive NIR-II fluorophores, owing to their high biocompatibility and tunability of emission to discrete wavelengths.^{6, 7} On the other hand, achieving bright emission with NIR-II fluorophores is generally much more difficult than with UV or visible dyes (Ag₂S QDs have an estimated quantum yield of <10%).⁴ Hence, amplification of light from NIR-II fluorophores by coupling to metal nanostructures is a highly desirable strategy for dramatically improving detection sensitivity, and can open up new routes for high-performance

diagnostic devices and photonics.^{8, 9}

Figure 1. Schematic diagram of fluorescence enhancement on Au nanostructured arrays. BSA: bovine serum albumin; B: Biotin; S: Streptavidin; QD: Ag₂S quantum dots.

Metal-enhanced fluorescence (MEF) has attracted interest over the past decade, for its applications in several areas of optics, medical diagnostics, and biological research.^{8, 10, 11} Most previous MEF studies, however, focused on fluorophores emitting in the visible region. In one of the few attempts investigating the NIR-II region, Hong *et al.* demonstrated MEF from single walled carbon nanotubes,¹² by only a 10-fold. In another work, Au nanoparticles enhanced the NIR-II fluorescence of PbS QDs by a factor of ~2.¹³ In the present work, significant fluorescence enhancement in the NIR-II of up to two orders of magnitude is demonstrated for the first time. The MEF of water-soluble Ag₂S QDs with tunable emission peaks was achieved by incorporating them on well-defined Au nanostructured arrays. Tunability of the arrays' plasmonic response in the NIR-II region was achieved simply through nanoscale control of the Au array structural characteristics. Immobilization of Ag₂S QD monolayers onto the arrays, and control of their separation distance, was attained *via* self-assembly of biotinylated bovine serum albumin (bBSA), followed by biotin-avidin interaction with streptavidin-functionalized Ag₂S QDs, as shown schematically in Figure 1. Ag₂S QDs of two different sizes (QD1 and QD2) were synthesized as reported by Zhang *et al.* (Supporting Information).⁶ Their size was controlled by varying the reaction temperature and growth time. Ligand exchange with dihydrolipoic acid rendered the Ag₂S QDs water soluble with a carboxylic acid group capping, also allowing ease of

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functionalization with biomolecules. Their morphology was examined by bright field transmission electron microscopy (BFTEM), which revealed spherical monodispersed nanoparticles (Figure 2a, d). The Ag₂S QD size distributions (Figure 2c, f) showed that the QDs had average sizes of 2.7 ± 1.1 nm (QD1) and 4.1 ± 1.3 nm (QD2). High resolution TEM (HRTEM) imaging (Figure 2a, d, insets) revealed the crystalline structure of the QDs, with lattice fringe spacings of 0.236 nm for QD1 and 0.223 nm for QD2, which correspond to the (-103) and (031) atomic planes of monoclinic Ag₂S (ref. #00-014-0072), respectively. TEM energy dispersive X-Ray (EDX) spectra collected from areas containing several QDs revealed the presence of Ag and S, confirming that the as-synthesized products were composed of Ag₂S.

Figure 2. Bright field transmission electron microscopy (BFTEM) images of 2.7 nm (QD1) and 4.1 nm (QD2) Ag₂S quantum dots (QDs; a, d; insets are high resolution TEM images), TEM energy dispersive X-Ray (EDX) spectra (b, e) and their size distributions (c, f). The curves in (c, f) represent the Gaussian fit to the data.

The optical properties of the Ag₂S QDs are shown in Figure 3. The absorption spectra for both QD1 and QD2 (Figure 3a), were featureless, with no first discrete absorption features.⁶ This is in contrast to II–VI QDs, which usually exhibit an evident discrete absorption feature, and has been attributed to the special electronic properties of Ag₂S QDs.⁶ The photoluminescence (PL) emission spectra (Figure 3b) of the QDs were measured using a 782 nm excitation laser and an InGaAs array near-IR detector. The 2.7 nm Ag₂S QDs had a fluorescence emission peak at 1100 nm, while the 4.1 nm Ag₂S QDs had an emission peak at 1200 nm, showing a red-shift in fluorescence with increasing QD size, in accordance to previous findings.^{6, 14} Zhang *et al.* experimentally determined the exciton Bohr radius of Ag₂S QDs as 2.2 nm.⁶ In their work, a continuous red-shift of the Ag₂S QD PL emission as their size increased from 2.4 to 4.4 nm was attributed to the quantum confinement effect, which narrows down the band gap energy as size increases.⁶ In contrast, the PL emission peak remained constant at ~1200 nm with increase of the Ag₂S size above 4.4 to 7 nm, which could be due to being out of the strong but in the weak quantum confinement regime.⁶ These optical properties of Ag₂S QDs are highly sought-after for applications in cellular labelling, deep-tissue imaging or assay labelling, and could help overcome many of the drawbacks of conventional organic fluorophores.¹⁵ Their broad absorption spectra allow for excitation at multiple wavelengths, while their narrow emission bands could be used for multicolour imaging. The tunability of their emission spectra without a large increase in size (<10 nm) is desirable for *in vivo* anatomical imaging with

fast clearance. Furthermore, Ag₂S QDs are extremely photostable, as previous studies have shown that their PL intensity remained constant over 30 days of storage at 4 °C.^{7, 14} Similar to our results, recent studies have adopted several approaches to obtain Ag₂S of well-controlled sizes, with distinct emissions in the NIR-II region.⁶ In order to overcome the drawback of weak fluorescence signals in the NIR-II and improve the applicability of Ag₂S QDs in biological applications, we proceeded to investigate the amplification of light from Ag₂S QDs through MEF.

Figure 3. UV-Vis-NIR absorption spectra (a) and photoluminescence (PL) emission spectra, at an excitation of 782 nm, of as synthesized Ag₂S QDs.

MEF is a well-recognized technology whereby the near-field interaction of fluorophores with metallic nanostructures will, under optimized conditions, lead to a substantial fluorescence enhancement.^{8, 11} Previous work in our group explored MEF using Ag nanoparticle patterned surfaces manufactured from colloidal lithography.⁹ The colloidal lithography technique allowed the fabrication of large area arrays of Ag triangular nanoparticles with controlled size, shape, and interparticle separation, and more importantly, with tunable optical features.⁹ In the present work, nanostructured arrays of Au rather than Ag were fabricated, in an attempt to tune their plasmonic response to the NIR-II region. Monolayers of polystyrene spheres (PS) with a diameter of 620 nm were directly deposited on to glass surfaces, using a self-assembly technique on a water surface (Supporting Information), a method which results in large areas of highly ordered hexagonal close-packed array (hcp) monolayers. These well-organized monolayers of PS enabled the fabrication of homogeneous Au nanotriangular-like arrays, following Au evaporation and template removal. Scanning electron microscopy (SEM) imaging of the Au nanostructured arrays showed nanotriangular-like arrays with the original hcp arrangement of the PS sphere template (Figure 4a-c). Using O₂ plasma to etch the PS templates for 5 s (sample PS620-Ox5; Figure 4b) or 15 s (sample PS620-Ox15; Figure 4c) before Au evaporation, led to progressively enlarged but shape controlled Au nanoparticles, with decreased tip-to-tip distances compared to the absence of O₂ plasma etching (sample PS620-Ox0; Figure 4a).

The in-plane width *a* (tip to tip dimensions), and interparticle distance *s* of the Au triangular-like nanoparticles produced using the PS sphere templates are shown in Table S1 (Supporting Information). The height of the Au nanoparticles was 50 nm, as measured by a quartz crystal monitor (QCM) in the Mantis evaporation system. The normalized extinction spectra of the nanostructured arrays (Figure 4d-f) presented several distinct resonance peaks which extended to the NIR-II

region, and whose wavelengths are summarized in Table S1. Multipole localized surface plasmon resonance (LSPR) modes (such as dipole, quadrupole, and octupole modes) on gold or silver nanostructures have been observed in previous studies, and have gained attention due to their potential applications in bio-sensing and fluorescence.¹⁶ The primary effects of LSPR excitation include selective photon absorption and scattering, and local electromagnetic field enhancement. The possibility of enhancing fluorescence in the NIR-II region, by exploiting the LSPR properties of the Au nanostructured arrays, was tested by immobilizing Ag₂S QD monolayers on the surface of these arrays.

Figure 4. Scanning electron microscopy (SEM) images (a-c) and normalized UV-Vis-NIR extinction spectra (d-f) of Au nanostructured arrays fabricated using 620 nm polystyrene (PS) sphere templates, without oxygen plasma etching (PS620-Ox0; a, d) or with 5 s (PS620-Ox5; b, e) or 15 s (PS620-Ox15; c, f) of oxygen plasma etching. (g) Normalized PL emission spectra of Ag₂S QDs (dashed curves) plotted against the plasmonic response (solid curves) of the Au nanostructured arrays. The dashed black line indicates the excitation wavelength. Please note various degrees of spectral overlap between plasmonic response and PL emission spectra of QD1 and QD2.

Ag₂S QD monolayers on Au nanostructured arrays and glass, as a control, were formed through biotin-avidin interaction, by first functionalizing the Ag₂S QDs with streptavidin using carbodiimide crosslinker chemistry (Supporting Information). Then, bBSA self-assembled monolayers were formed on glass and Au-nanostructured substrates. As has previously been reported, bBSA binds to both glass and Au surfaces, forming complete monolayers.¹⁷ The bBSA monolayer on glass surfaces is formed through non-covalent physisorption, while on metallic surfaces the bonding is thought to involve electrostatic affinity as well as covalent binding. Following pre-coating of the substrates with bBSA, the substrates were incubated with streptavidin-conjugated Ag₂S QDs (Supporting Information). The binding interaction between streptavidin and biotin is very strong ($K_a \approx 10^{15} \text{ M}^{-1}$) and results in a stable monolayer of streptavidin-QDs over the bBSA monolayer.⁹ bBSA, which has an ellipsoid shape with axes of 4 and 8 nm, also serves as a separation layer between the QDs and the Au surface with a thickness of 4-8 nm. Additionally, streptavidin, which is attached to the QDs, acts as an additional separation layer of about 4 nm in thickness, leading to a combined spacing of ~ 12 nm. Numerous studies have illustrated that MEF strongly depends on the distance between fluorophores and metal surfaces.^{13, 18} For instance, maximum fluorescence emission from PbS QDs coupled to Au NPs was obtained when the NPs were coated with a SiO₂ spacer of 10 nm.¹³ Since the electromagnetic field intensity decreases with increasing distance from the metal surface, fluorescence enhancement also decreases. On the other hand, non-radiative processes,

which are inversely proportional to the third power of the metal-fluorophore distance, dominate at very small distances (< 4 nm) and lead to fluorescence quenching.^{19, 20} However, in our experiments, the streptavidin-biotin conjugate provides a significantly larger spacing of ~ 12 nm, for which a significant increase in non-radiative decay rate is not anticipated.⁹

Figure 5. PL emission spectra of Ag₂S QD monolayers (QD1: a and QD2: b) on biotinylated bovine serum albumin (bBSA)-coated glass surfaces (grey) and PS620-Ox0 (red), PS620-Ox5 (green) or PS620-Ox15 (yellow) nanostructured arrays. bBSA-coated glass surfaces (black) were used as control samples.

Previous findings have shown that MEF largely depends on the spectral overlap between the LSPR in metal nanostructures with the spectral properties of the fluorophore.²¹ Consequently, maximum overlap of the QD emission spectra with the LSPR, may lead to higher fluorescence enhancement. Figure 4d shows the emission spectra of the Ag₂S QDs plotted against the plasmonic response of the Au nanostructured arrays. The wavelength (782 nm) used for the excitation of the samples for fluorescence measurements is shown by the dashed straight line. The fluorescence measurements of the two different sizes of QDs bound to glass substrates or Au nanostructures are shown in Figure 5. Glass substrates coated with bBSA were used as background controls. Similar to the PL emission of bare QDs measured in solution, the QD monolayers on substrates exhibit emission peaks at 1100 nm for QD1 and 1200 nm for QD2, respectively. The averaged fluorescence enhancement factors (E_f) were calculated as previously described,⁹ using:

$$E_f = [(E_{\text{Au/QD}} - E_{\text{Au/bBSA}}) / (E_{\text{glass/QD}} - E_{\text{glass/bBSA}})] \cdot (S_{\text{uncovered}} / S_{\text{total}})$$

and are summarized in Table 1, after normalising for surface area differences.

Sample	E_f – QD1	E_f – QD2
PS620-Ox0	11.9	103.2
PS620-Ox5	13.1	98.3
PS620-Ox15	7.6	20.4

Table 1. Average experimentally measured fluorescence enhancement factors (E_f) of Ag₂S QDs on Au nanostructured arrays.

As demonstrated in Table 1, all the samples exhibited enhanced fluorescence on Au nanotriangular arrays. Surprisingly, significant fluorescence enhancement up to 103 times was achieved. Previous findings have illustrated that MEF is a complex coupling process between fluorophore molecules and metal nanostructures.^{9, 18} On the one hand, MEF arises due to local enhancements of the electric field, induced by the LSPR of metal nanostructures. On the other hand, metal nanostructures are able to modify the radiative and non-radiative decay rates of nearby fluorophores,

changing both the fluorescence lifetime and quantum yield. Furthermore, these nanostructures may affect photostability, and increase energy transfer.²² Experimental results, also supported by theoretical calculations, have shown that fluorescence enhancement factors of metal nanostructures depend on particle size, shape, interparticle separation, the surrounding dielectric medium, as well as the particle arrangement geometry and distance between the metal and fluorophore.^{18, 19} Moreover, MEF critically depends on the spectral overlap between the LSPR in metal nanostructures with the spectral properties of the fluorophore.

In our results, the highest fluorescence enhancement was observed from QD2 on sample PS620-Ox0, giving up to two orders of magnitude higher fluorescence than that of QD2 monolayers deposited on glass. For both QD1 and QD2, higher enhancement factors were measured with samples PS620-Ox0 and PS620-Ox5, compared to sample PS620-Ox15. This is probably due to the higher degree of spectral overlap between the LSPR of these samples with the PL emission spectra of the QDs (Figure 4g), which is key to obtaining the maximum fluorescence enhancement. Despite the lower interparticle distance (*s*) between the nanotriangular-like particles in sample PS620-Ox5 compared to sample PS620-Ox0 (Table S1), both samples led to similar enhancement factors, for both QD sizes. This is in contrast to previous findings, which have demonstrated that the local electromagnetic field enhancement is highly dependent on the gap size between nanoparticles, with a smaller gap size causing plasmon coupling and dramatically enhancing the field close to the metal particle.²⁰ Moreover, much higher enhancement factors were obtained for QD2 than for QD1. This observation may be due to the relative position of the QD emission peaks compared to the LSPR of the Au nanostructured arrays. The plasmonic enhancement of single molecule fluorescence has been shown to be strongly frequency dependent: for molecules emitting to the blue of the LSPR, the fluorescence can be quenched, whereas for molecules emitting to the red of the LSPR, the fluorescence can be strongly enhanced.¹⁹ The finite-difference time-domain (FDTD) technique was previously used to calculate the absorption, scattering and extinction efficiencies for 80 nm Au and Ag nanospheres.¹⁸ Compared to Ag for which the scattering efficiency was dominant, Au was absorption-dominant.¹⁸ Since the scattering efficiency has been correlated to fluorescent enhancement, whereas absorption efficiency is linked to quenching,²² Au is expected to show some quenching below its LSPR peak.

Conclusions

In summary, we have demonstrated significant fluorescence intensity enhancement in the NIR-II region for Ag₂S QDs, using Au nanostructures produced by colloidal lithography. Ag₂S QDs are attractive NIR-II fluorophores, with high biocompatibility and ease of biomolecule functionalization, while their emission tunability without a large increase in size (<10 nm) allows for multiplexed analysis and fast *in vivo* clearance. Still, their application is limited by their relatively low fluorescence

quantum yield (<10%). Here, the potential to amplify fluorescence from Ag₂S QDs by 2 orders of magnitude through MEF is demonstrated. This work illustrates the possibility of fabricating effective MEF-platforms in the NIR-II region, paving the way for a wider application of Ag₂S QDs in sensing and imaging, with a dramatic improvement in their sensitivity.

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