Au/SiO₂ core/shell nanoparticles enhancing fluorescence resonance energy transfer efficiency in solution†

Qinghui Zeng,[ab] Youlin Zhang,[ab] Xiaomin Liu,[ab] Langping Tu,[a] Yu Wang,[ab] Xianggui Kong*[a] and Hong Zhang*[ab]

Tailor-designed Au/SiO₂ core/shell nanoparticles are employed to enhance the efficiency of fluorescence resonance energy transfer based on quantum dots and R-phycoerythrin in solution.

Fluorescence resonance energy transfer (FRET) has been widely applied in the biological field. It can provide information on the spatial relationship between two fluorophore-labeled sites in biological structures and macromolecules.[1] In bioanalytical and biosensing applications, detection based on FRET is becoming more and more popular. An example is homogeneous immunoassays (HIA) by constructing a donor–acceptor (DA) system in aqueous solution.[2,3] HIA are important in modern medical diagnosis, owing to their critical advantage over heterogeneous immunoassays: there is no need for separation and purification of the labeled biological molecules, which often leads to a drop of the biological specificity of the labeled molecules and decreased sensitivity of the immune detection.[4] Obviously, the key to increase the sensitivity of FRET-based HIA is to enhance the FRET efficiency. Until now, most of these efforts are directed to the optimization of donor and acceptor properties, including increasing the fluorescent lifetime of the donor or the spectral overlap between the donor's emission and the acceptor's absorption, or decreasing the distance between the DA pair.[5,6] Recently, quantum dots (QDs) are often employed as FRET donors due to their superior optical properties, such as a wide absorption band, long luminescence lifetime, resistance to chemical degradation, excellent photostability, optical tunability with spatial size and high quantum yield (QY).[5,7,8]

Generally, the local electric field enhancement resulting from the surface plasmon resonance (SPR) near metal nanoparticles (NPs) is widely investigated and broadly applied on surface-enhanced spectroscopy,[9–11] super-resolution optical imaging,[12] and biosensing.[9] Since the last decade, with the robust development of nanoscience and nanotechnology, the role of SPR in enhancing the luminescence of QDs has been explored. Recently, the applications of SPR have been further extended, among which metal plasmon field enhanced (PFE) FRET is a focus of the attention. Most of these investigations intend to enhance the FRET efficiency by the construction of metal films to explore the mechanism.[13,14] So far, no report has appeared on the metal enhanced FRET in *colloidal solution*, although colloidal solution is directly related to the HIA application of QDs.

The interaction mechanism between metal NPs and nearby chromophores has been unravelled, including (i) photoluminescence (PL) quenching due to energy transfer (ET) or charge transfer (CT) processes,[15–18] and (ii) the PFE effect on the PL. Here, the distance is the key factor. When the distance exceeds the required value for the ET/CT, the quenching effect will be minimized and enhancement of the PL intensity of the nearby chromophores could then be significant. SiO₂ is known to be a good shell material based on several merits: firstly, SiO₂ is a chemically inert material and could help prevent aggregation. Secondly, the SiO₂ shell coating is often uniform and conformal. Thirdly, SiO₂ is a kind of optically transparent and dielectric material, which is not expected to influence the optical properties of the coated core or the adjacent fluorescent molecules. Klimov *et al.* have utilized SiO₂ to coat gold NPs and investigated the gold-based plasmon field enhanced PL of organic phase QDs.[19] Based on this result, we believe that the encapsulation of a SiO₂ layer on metal NPs' surface could facilitate FRET in solution effectively.

In this work, we turn to an approach, different from the existing ones, to enhance the FRET efficiency with the aid of the plasmonic field of the connecting tailor-designed Au particles. We utilized aqueous CdTe/CdS QDs as donors and R-phycoerythrin (RPE) as an acceptor to investigate whether the FRET efficiency between QDs and RPE could be increased when Au/SiO₂ core/shell NPs are in close proximity to the complex of CdTe/CdS QDs and RPE. Based on the result of ref. 19, the distance between Au and QDs, which was equal to the SiO₂ shell thickness, was chosen to be about 80 nm to eliminate the possible PL quenching of the QDs by Au NPs (Scheme S1, ESI†). It was expected that the localized surface plasmon oscillations of such constructed Au/SiO₂ core/shell NPs could enhance the efficiency of FRET from QDs to RPE. The principle of the design is depicted in Scheme 1.

Choosing RPE as the energy acceptor was primarily based on its broad applications and suitable optical properties. Besides, RPE could easily combine with NPs through non-specific adsorption. As shown in Fig. 1a, the absorption spectrum of the RPE acceptor overlaps well with the emission spectrum of the QD donor, enabling an efficient FRET process from the QDs to the RPE. So-designed Au/SiO₂ core/shell NPs have indeed enhanced the FRET efficiency (Fig. 1b). It is apparent

[a] Key Laboratory of Excited State Processes, Changchun Institute of Optics, Fine Mechanics and Physics, Chinese Academy of Sciences, 3888 Eastern South Lake Road, Changchun 130033, China. E-mail: xgkong14@ciomp.ac.cn; Tel: +86-431-86176313
[b] Van't Hoff Institute for Molecular Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. E-mail: h.zhang@uva.nl; Tel: +31-20-5256976
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=RPE ●=QDs ·=Au/SiO₂ NPs
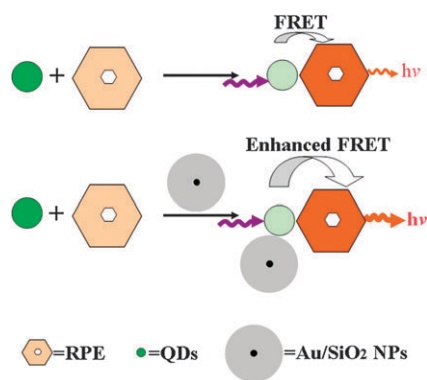
Scheme 1 Schematic depiction of the FRET process from QDs to RPE and the plasmonic field enhanced FRET. The sizes of the materials are not to scale to show the expression clearly.

that adding the RPE to QDs aqueous solution significantly enriches the emission of the RPE, at the expense of the QD emission energy—a typical FRET case. Strikingly, further adding the Au/SiO₂ core/shell NPs into the solution improves the PL intensity of both QDs and RPE. These two improvements do not follow the same mechanism. The QD emission enhancement benefiting from the Au/SiO₂ core/shell NPs is based on the PFE principle (*vide infra*), whereas RPE emission improvement comes from the more efficient FRET process from the QDs in the presence of Au/SiO₂ NPs or based on the PFE principle. To further prove the above results, the effect of acceptor concentration on the emission spectral variation of the contacted composites was studied, as shown in Fig. S1.† The QD–RPE composites demonstrated similar dependence on the RPE concentration of the emission spectral shape with and without the presence of Au/SiO₂ NPs. This verified also that in both cases the emission energy transfer dynamics of the composites were similar—*via* FRET. To confirm that enhancement of the fluorescence of RPE was *via* FRET, not *via* a direct PFE process, the fluorescence spectrum of RPE was also measured when the Au/SiO₂ NPs were mixed with the RPE in aqueous solution, where no fluorescence enhancement was observed (ESI,† Fig. S2). Therefore, the fact that the spectral intensity was significantly higher in the presence of Au/SiO₂ NPs (ESI,† Fig. S1d) implied that the efficiency of FRET is obviously increased by adding Au/SiO₂ core/shell NPs to the DA (QDs–RPE) solution. The details will be given later in the text.
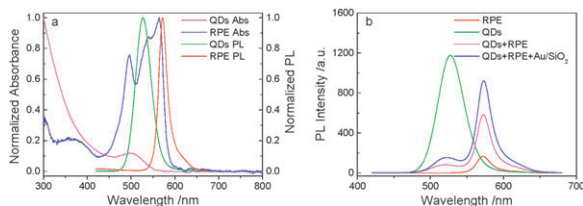

Fig. 1 Normalized absorption and PL spectrum of RPE and CdTe/CdS QDs, respectively (a). PL spectra of RPE, QDs, QDs–RPE and Au/SiO₂/QDs–RPE solution (b). The concentrations of QDs and RPE were kept constant as 100 and 80 nM respectively, and the excitation wavelength was 350 nm in all PL measurements. Because of a little absorption at 350 nm, the PL spectra of RPE can be observed with very low PL intensity.

To quantitatively assess the FRET efficiency improvement, we turned to the variation of acceptor fluorescence instead of donor emission. The FRET efficiency (Φ_{FRET}) can be described as:[6]

$$\Phi_{FRET} = A_A(\lambda_D)[F_{AD}(\lambda_A)/F_A(\lambda_A) - 1]/A_D(\lambda_D). \qquad (1)$$

Based on this relationship and the spectra in Fig. 1, the optimal FRET efficiency was determined to increase 1.9-fold from 37% without Au/SiO₂ NPs to 69% with Au/SiO₂ NPs.

The enhanced FRET efficiency depends on the formation of Au/SiO₂/QDs nanocomposites, which need to be constructed and proved evidently with the following experiments. The standard tetraethyl orthosilicate (TEOS) molecules could be easily connected with the gold NPs due to electrostatic interaction or van der Waals force.[20] The Au cores, without any surface activation, were coated with SiO₂ shells through the Stober method using TEOS as a silica source in ethanol.[21] The silica shell was then modified with 3-aminopropyltriethoxysilane (APTES) to functionalize the silica surface with positively charged amino groups. In this work, the thiol–carboxylic acid-capped CdTe/CdS core/shell QDs were prepared as described previously, which imparted the QDs with negative surface charge.[22] Hereby, formation of Au/SiO₂/QDs nanocomposites was easily realized *via* electrostatic adsorption without any substitution of surface ligands of the QDs.

The characterization of the tailor-designed Au/SiO₂ core/ shell NPs and self-assembled Au/SiO₂/QDs nanocomposites is verified by the field emission scanning electron microscopy (FE-SEM) images shown in Fig. 2, where the diameter of the gold core was determined to be $D = 20 \pm 5$ nm, which is in agreement with the FE-SEM result shown in Fig. S3a.† The silica shell thickness was determined to be 83 ± 8 nm. Because of the preferable manipulation of the Stober method, the Au/SiO₂ core/shell NPs were nearly spherical and monodisperse, and the encapsulation was mostly uniform. As can be seen in Fig. 2b, the surface of the Au/SiO₂ NPs was changed from smooth to rough due to adsorption of the QDs. Therefore, the Au/SiO₂/QDs nanocomposites are formed in this approach.

As mentioned above in this paper, the emission of the CdTe/CdS QDs ($\lambda_{em} = 528$ nm) could be quenched by the gold NPs (Fig. S3, ESI†).[15–18] The more Au NPs dropped into the CdTe/CdS QD solution, the less the PL intensity of the QDs. However, when the positive Au/SiO₂ core/shell NPs were dropped into the QDs solution, the absorption intensity
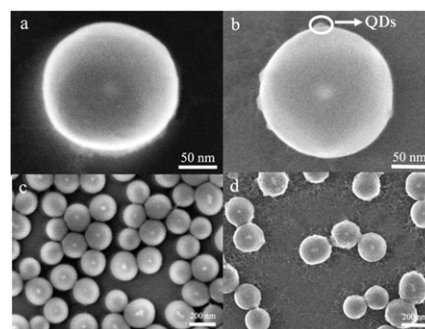

Fig. 2 FE-SEM images of Au/SiO₂ NPs (a, c) and Au/SiO₂/QDs nanocomposites (b, d).
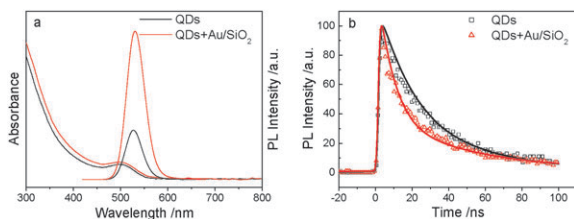
Fig. 3 Absorption, PL spectra (a) and fluorescent lifetime (b) of CdTe/CdS QDs before and after adding Au/SiO$_2$ NPs. The excitation wavelength was 350 nm.

had a slight increase (Fig. 3a), which is ascribed to the additional absorption of the Au/SiO$_2$ core/shell NPs. At the same time, the PL of the QDs was greatly increased. In this complex structure, two interaction mechanisms might exist between QDs and metal NPs: (1) the PFE effect applied to the QDs, which will ultimately lead to the enhancement of the PL efficiency of the QDs;[19,23,24] (2) PL quenching of QDs induced by ET/CT from the QDs to the metal NPs.[15–18] Since the critical distance for the ET/CT is in the range of 1–30 nm,[15,18,19,25] and in this case the distance between Au core and QDs was over 80 nm, the PFE effect should be dominant. The dependence of the QD PL on Au/SiO$_2$ NPs is given in Fig. S4.† Because the number of CdTe/CdS QDs adsorbed onto the Au/SiO$_2$ NPs is increased accompanied with the increase of the concentration of the Au/SiO$_2$ NPs, the gradual enhancement of PL was presented until saturation was reached. In this way, the QY can be lifted from 30% to 79% (2.6-fold). This phenomenon also explains the reason for the common PL enhancement of QD donor and RPE acceptor indicated in Fig. 1b. To confirm that the PL enhancement was uniquely originated from the SPR of the gold cores, a control experiment was performed where samples (SiO$_2$ NPs) were prepared and interacted with the aqueous QDs. No PL enhancement was observed when the SiO$_2$ NPs ($D = 180$ nm) were mixed with the CdTe/CdS QDs, as is shown in Fig. S5.†

In order to further manifest the PFE effect, time-resolved PL spectroscopy was carried out. As is shown in Fig. 3b, the PL of QDs decays non-exponentially for both CdTe/CdS QDs and Au/SiO$_2$/QDs nanocomposite solution. The decay traces can be well fitted with a bi-exponential model. The fast lifetime (τ_1), slow lifetime (τ_2), radiative rate (k_r), and QY are summarized in Table S1 (see details in ESI†). The origin of the fast and slow component in the CdTe/CdS QDs has been studied extensively.[22,26] As is shown in Fig. S6 and Table S1,† with the strengthening of the PFE effect by adding more Au/SiO$_2$ NPs in the QDs solution, the radiative rate is increased from 0.84×10^7 to 2.66×10^7 s^{-1}. Theoretically, the PL QY of QDs is related to the efficiency of FRET (see details in ESI†). During this calculation, the Φ_{FRET} can be deduced as 61% in the presence of Au/SiO$_2$ NPs, which is very close to 69% obtained as the Φ_{FRET} value calculated by eqn (1). This experiment provided additional evidence that FRET was indeed enhanced in the so-designed composite by the Au/SiO$_2$ NPs.

In summary, a new approach of using Au/SiO$_2$ core/shell nanoparticles to enhance FRET efficiency in colloidal solution was proven to be feasible. The FRET efficiency from aqueous QDs to RPE was found to increase until 1.9-fold when the QDs/RPE FRET system was connected with the Au/SiO$_2$ core/shell NPs. This result offers a new method to improve the FRET homogeneous immunoarrays based on QDs.

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