Building of multifluorescent mesoporous silica nanoparticles†

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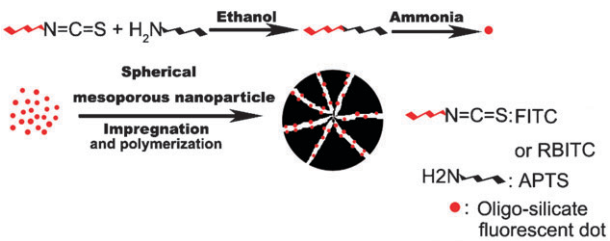
Bright and colourful nanocomposites are easily fabricated by doping and fixing one or two kinds of oligosilicate fluorescent dots encapsulated with dye molecules into the pore channels of mesoporous silica nanoparticles, which can engender abundant fluorescent signals with a single excitation wavelength.

Generally, the fluorescent markers used in aqueous biological systems should satisfy three conditions: strong and stable fluorescence intensity, biocompatibility and low non-specific adsorption.[1] However, most of the traditional dyes have shortcomings such as low fluorescence and easy photobleaching, which limit its effective application for bioassay. Recently, quantum dots have attracted wide attentions as biolabels due to their wide excitation spectra and tunable emission wavelengths.[2,3] However, the cytotoxicity of quantum dots and difficulties in obtaining biocompatible nanocrystals limit its applications.[4] Wiesner[5] and Tan[6,7] reported that the encapsulation of dye molecules into silica nanoparticles can form fluorescence particles much brighter and more stable than their constituent fluorophore. However, the adsorption and anchoring of dye molecules to silica particles is difficult and the effective encapsulation of dye molecules into the different layer of silica particles needs complicated procedures, which easily lead to the low fluorescent intensity of the composite.[6] Nevertheless, the silica nanoparticles still seem attractive as the host material of fluorescent nanocomposites mainly due to its excellent biocompatibility and colloidal stability. Silica-type materials with long-range ordered mesoporous channels between 2–10 nm may be ideal candidates for dye encapsulation,[8,9] which allows the incorporation of dye molecules into the pore channels by a simple impregnation method. However, the particles of mesoporous silica molecular sieves are generally irregular and agglomerate due to the using of long-chain surfactants, which can not be directly used as fluorescent labels since the applications for bioassay requires the single-particle monitoring or encoding of the targets such as protein and gene. Recently, some researchers have used modified Stöber method[10,11] or adopted novel surfactants[12–14] to prepare monodisperse silica particles or ribbons. Moreover, with the increasing biological data, the multiplexed bioassay becomes more and more important, which requires the building of a barcode library. The incorporation of dual-dye fluorescent resonance energy transfer pairs can engender abundant fluorescent signal by a single excitation wavelength.[15] In this communication, we report a novel multifluorescent nanocomposite using monodisperse mesoporous silica nanoparticles (named as MSPs) as the host, single or dual-dye pair as the guests.‡ This system achieves the purpose of obtaining multifluorescent silica nanoparticles excited with a single wavelength and has promising potential as the biolabel for cell and protein.

The preparation of fluorescent nanoparticles takes three steps: (1) the synthesis of MSPs; (2) the pre-modification of the dye molecules FITC (fluorescein isothiocyanate) or RBITC (rhodamine B isothiocyanate) with APTS ((3-aminopropyl)triethoxysilane) and the subsequent hydrolysis and polymerization of APTS to form an oligosilicate fluorescent dot (OSFD); (3) introducing OSFD into the MSPs pore channels by an impregnation method, then anchoring the OSFD onto the pore wall by silicohydroxyl polymerization (Scheme 1). To obtain multiple fluorescent signals with different colours and intensities, the above two different fluorescent dots were simultaneously introduced into the pore channels of MSPs with various ratios, which can form effective fluorescence energy transfer with a single excitation wavelength since the emission wavelength of FITC and the absorption wavelength of RBITC have great overlaps.

The TEM images of MSPs (Fig. 1) show that the particles are monodisperse and very uniform with average particle diameters of around 125 nm. This size is suitable for the application *in vivo* since larger particles will be captured by animal organs when applied as biolabels. The pore channels of the particles are aligned towards the center of the sphere and perpendicular to the surface with average pore size of 2.5 nm (see ESI).† This kind particle has several advantages used as the host material: the pore channels with the average diameter of 2.5 nm offer an adequate space for the entering of fluorescent dots; the unique diverging pore channel arrangement can provide unimpeded access for the fluorescent dots, leading to the high dispersion of dye guest; the abundant



Scheme 1 A novel doping procedure for dyes into monodisperse mesoporous silica particles.

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† Electronic supplementary information (ESI) available: The XRD and N_2 adsorption isotherms of MSPs and the photo of dye-doped MSPs samples under 365 nm UV illumination are given. See DOI: 10.1039/b900200f

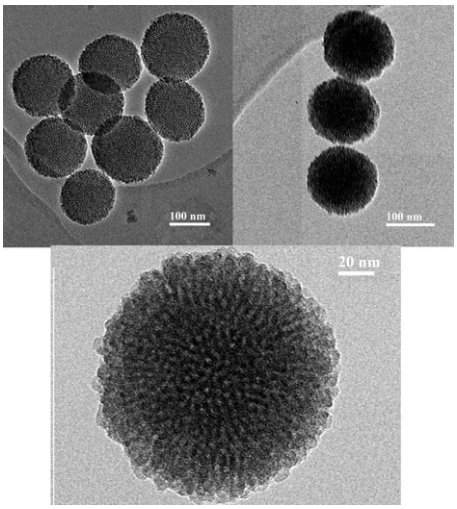

Fig. 1 TEM images of spherical mesoporous silica nanoparticles.

hydroxyl groups on the pore wall can effectively fix fluorescent dots by hydroxyl polymerization, thus reducing the energy loss of dye molecules caused by free movement. Basing on above advantages, the high loading amount of the dye guest and the strong fluorescent signal of the nanocomposite are expected by adopting the above preparation method. In fact, large amount of dye molecules (about 68.4% of total addition) were housed inside the MSPs calculated from the UV-vis absorption spectra of the filtration eluent. To compare the fluorescence intensity of a single MSP with a single fluorophore, the MSPs doped with FITC was investigated. First, the titration curve of the fluorescence intensity as a function of the FITC dye concentration was plotted, then the nanoparticle ($V_{\mathrm{sphere}} \approx 10^6$ nm^3 when the particle diameter is 125 nm) concentration was determined by drying and weighing a certain volume of nanoparticle solution. The fluorescence intensity of 10^{-15} M FITC-doped MSPs was measured, and the effective fluorescence intensity of a single particle is 50× brighter than a single dye molecule. This high signal amplification is essential to address the growing need for highly sensitive bioassays.

To form more colourfully fluorescent nanoparticles, we selected FITC and RBITC as the donor and acceptor molecules of FRET pairs, respectively, since the fluorescence wavelength of FITC and the absorption wavelength of RBITC have great overlaps (see Fig. 2(a)), which permits the energy transfer from the donor FITC to the acceptor RBITC and may

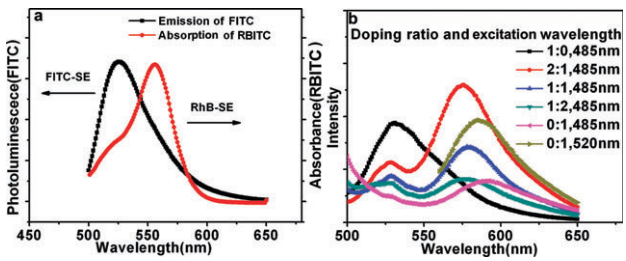

Fig. 2 (a) Fluorescence spectrum of FITC and absorption spectrum of RBITC; (b) fluorescence spectra of MSPs co-doped with FITC and RBITC with different doping ratios excited at 480 nm.

form various fluorescent signals by using a single excitation wavelength. The fluorescence spectra of the MSPs doped with both FITC and RBITC were shown in Fig. 2(b). Simultaneous appearance of the emissions of two dyes excited at 480 nm was obviously observed, which demonstrates the efficient FRET between the above two dye guests. The fluorescence emission shape varies with the doping ratio between the two dyes, indicating the finely controllable fluorescent signal is easily achieved. Moreover, the fluorescent intensity can also be adjusted by changing the particle size of MSPs and its pore diameter, and more specific data will be presented lately.

To elucidate the effective incorporation of dye molecules into the pore channels instead of onto the surface of the above nanoparticles, the fluorescent intensity of FITC and the FRET efficiency between FITC and RBITC dispersed on the surface of nonporous silica nanoparticle were investigated. The results show that the composites based on the nonporous nanoparticle has very weak fluorescence signal as well as low FRET efficiency, which implies heavily fluorescent quench of dye-densed oligosilicates dot and large physical separation distance between the FRET pairs anchored on the surface of nonporous nanoparticle. Moreover, we also found that the fluorescent signal of the dye-dense oligosilicates dot obtained from the first step for the preparation of MSPs also shows extremely low intensity. However, the encapsulation of these dots in the pore channels of MSPs can magnificently improve the fluorescent intensity as seen from Fig. 2(b). The great improvement of the fluorescence signal may be attributed to the high dispersion and fixing of the dye molecules in the pore channels, thus leading less contact with water molecules and energy loss caused by dye molecules movement and collision. Moreover, it is known that FRET efficiency is inversely proportional to the sixth power of the distance between the donor and acceptor.[16] When the donor and the acceptor is in close proximity (20–80 Å), the excited-state energy from the donor can be transferred to the acceptor *via* dipole–dipole coupling between the chromophores. Therefore, the 2.5 nm pore channels of MSPs offers the optimal space for the FRET pairs, which can effectively shorten the distance between donor FITC and acceptor RBITC, thus great improving the energy transfer between them.

The confocal fluorescence images of dye-doped MSPs were also shown. These particles exhibit different colours with 488 nm argon ion excitation on a confocal laser scanning microscope (Fig. 3). Single-dye-doped FITC MSPs (Fig. 3(a)) and single-dye-doped RBITC MSPs (Fig. 3(b)) show green and red light, respectively, while dual dye-doped particles with FITC/RBITC = 1 exhibit yellow light because of FRET. This model demonstrates the potential of multicolor MSPs used as tags for multiplexed bioassays. The numbers of the fluorescent signals with different emission wavelengths and intensities can be further enriched by varying the doping amount of the dye and the ratio between FRET pairs, or doping more complicated FRET pairs (such as triple or even more dyes) into the pore channel of MSPs.

In conclusion, we have successfully prepared multi-fluorescent mesoporous silica nanoparticles *via* a novel doping method. These multicolour silica nanoparticles provide a robust system for multiplexed bioassay. By using this

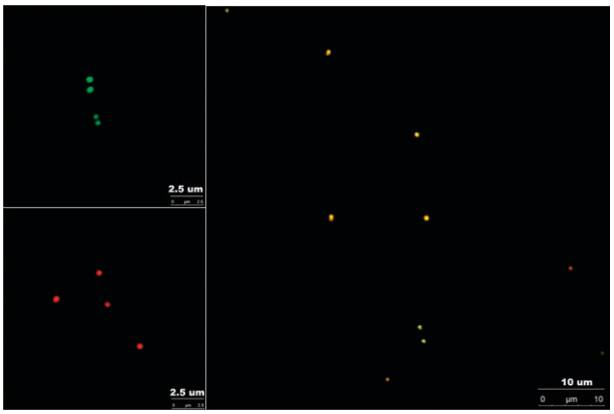

Fig. 3 Confocal fluorescence images of MSPs with different doping ratios between FITC and RBITC under 488 nm argon ion laser excitation: (a) 1 : 0; (b) 0 : 1; (c) 1 : 1.

nanocomposite, one can envision a dynamic, multicolour, colocalization methodology to follow proteins, nucleic acids, molecular machines, and assemblies within living systems.

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Notes and references

‡ Experimental procedure: monodisperse mesoporous silica nanoparticles were synthesized as described by Chen *et al.*[12] The Preparation of dye@APTS composites and their impregnation into pore channels was as follows: FITC and RBITC were individually dissolved in 125 mL of anhydrous ethanol, combined with an excess of APTS at a molar ratio of dye to APTS of 1 : 50, and stirred under a dry nitrogen atmosphere for 24 h avoiding light. Typically, 1 mL of ethanol was used per 0.1 g APTS. Then 9.55 mL ammonium hydroxide (28%) was added and the mixture was stirred for 24 h. 0.2 g MSPs samples were added afterward and stirred for 12 h. The dye doped nanocomposites were obtained after removing the residue dye by multiple centrifugation and wash treatment with water and ethanol. Taking FITC as an example ($n_{FITC} = 10^{-4}$ mol) and determined from the absorption spectroanalysis of the washing solution, about 68% dye molecules was introduced into the silica particle, leading to about 10 000 dye molecules per particle on average. As for the preparation of dual dye composites, it has identical ratio of reactants amount and the same procedure except that two kinds of oligosilicate fluorescent dots were mixed at different ratio before the addition of MSPs.

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