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Mesoporous Silica Templated Biolabels with Releasable Fluorophores for Immunoassays

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A novel class of fluorescent immuno-biolabeling systems with extremely high F/P ratio (~1000−6000) were prepared by combining the template method and layer-by-layer (LbL) technique. Labels were constructed by loading organic dye fluorescein diacetate (FDA) molecules onto hollow periodic mesoporous organosilica (H-PMO) particles followed by ployelectrolyte encapsulation and antibody attachment. The labeling systems were stimuli-responsive to the addition of concentrated NaOH with the loaded dye molecules being released and detected in a well-controlled manner. When applied in sandwich immunoassays, results indicated that the biolabels were immuno-active and generated an optimal signal that was ~50 times higher than the conventional dye labelled antibody system.

Achieving ultrahigh sensitivity is a major goal in biological assays. Highly sensitive methods are urgently needed for disease diagnosis to detect markers present at ultralow levels during early stages of disease. Among the currently available analytical techniques, immunoassay is one of the most promising strategies for achieving high sensitivity and high specificity. In ultratrace fluorescent immunoassays (FIA), signal amplification is typically achieved by coupling fluorophores, such as organic dyes, to the antibody probes.[1] As the sensitivity of FIA is mainly determined by the number of light quanta emitted per analyte molecule, increasing the fluorescent dye to biomolecule ratio (i.e., the F/P ratio) is crucial for achieving signal amplification (and hence sensitivity). However, labeling antibodies with large numbers of fluorophores usually leads to reduced specificity and binding affinity as well as a reduced quantum yield due to dye self-quenching effects.[2] Thus the optimal dye/protein ratio is normally kept below ~4,[3] which greatly limits the sensitivity of FIA.

Several ways to increase the F/P ratio for immunoassays have been investigated. An effective route involves the encapsulation of fluorophores within nanoscale carriers like polystyrene latex beads,[4] silica nanoparticles,[5–7] and liposomes,[8] followed by functionalization with biomolecules. Alternatively, a higher F/P ratio (~1400) can be obtained by precipitating fluorescent perylene microparticles in the presence of the antibodies.[9,10] These particles contain a large number of fluorescent molecules and can be dissolved in a suitable solvent for detection after the immunoreaction. An analogous route for achieving highly sensitive immunoassays of protein, based on polyelectrolyte-encapsulated microcrystalline fluorescent material conjugated with antibodies, was reported by Trau and co-workers.[11] Because of the exceptional high F/P ratio of the biolabels (~51 000−190 000), a dramatically (70−2000-fold) amplified immunoassay was achieved. These two methods showed the advantages of high F/P ratio, high sensitivity, and controllability. However, a key limitation lies in the restricted number of materials that can be precipitated or crystallized for encapsulation. Given the limitations of existing fluorescence-based biochemical assays, the development of new strategies and biolabel systems is highly desirable.

The emergence of nanotechnology is opening new horizons for highly sensitive bioaffinity and biocatalytic assays.[12–14] Periodic mesoporous organosilicas (PMOs), with organic functional groups homogeneously distributed throughout the mesoporous silica walls, have attracted much attention recently.[15,16] Compared with other mesoporous materials, these kinds of material have highly ordered mesoporous structures, as well as better hydrothermal and mechanical stability. More interestingly, their surface composition and properties (e.g., charge, polarity, and hydrophobicity) can be tuned by changing the organic component, which makes them very attractive in many applications such as catalysis, sensors, drug delivery, and separation.

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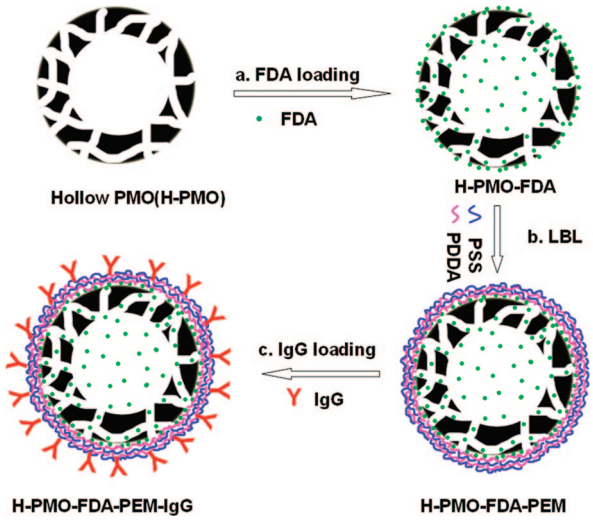

Figure 1. Schematic illustration of the preparation of the antibody coated H-PMO-FDA-PEM biolabels: (a) FDA loading into H-PMO particles; (b) LbL assembly of oppositely charged polyelectrolytes onto H-PMO-FDA particles; (c) further coating of IgG resulting in the formation of H-PMO biolabels.

Recently, our group has successfully synthesized hollow PMO (H-PMO) spheres with tunable wall thickness by a "dual templating" approach.[17] We also reported a new strategy for encapsulation of biomolecules by using mesoporous silica spheres as sacrificial templates.[18,19] Herein, we demonstrate a novel approach for the preparation of a novel class of fluorescent biolabels with high F/P ratio based on dye-loaded H-PMO particles. As illustrated in Figure 1, the biolabels were prepared by a stepwise process beginning with the loading of organic dye fluorescein diacetate (FDA) molecules onto H-PMO spheres. Ultrathin polyelectrolyte multilayers (PEM) were then deposited on the surface of the particles using the layer-by-layer (LbL) technique[20,21] to provide a suitable "interface" for further attachment of antibodies. The loaded FDA can be stimuli released and quantitatively determined by its fluorescence intensity. The ability of these novel antibody functionalized biolabels for immunoassay was further examined by performing a sandwich bioaffinity immunoassay. Compared with previous strategies, this approach has the following advantages: (1) Because of the capacity of mesoporous silica for immobilizing various materials, a large variety of dye molecules could be loaded into the particles, which vastly expands the selection of fluorescent dyes. (2) The high surface area of mesoporous material enables a high loading of dye molecules and therefore enables a high F/P ratio of the biolabel, which provides a promising way to increase the sensitivity of the bioassay. (3) The loaded dye molecules can be released from the biolabel in a controlled manner. (4) The released dye is detected in a large volume of the release reagent which can reduce the dye quenching problem normally arising from the high F/P ratio. In addition, this method also allows controlling the size and shape of the label systems by choosing suitable templates, and provides a promising way to prepare multidye label systems for particular applications.

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EXPERIMENTAL SECTION

Materials. Fluorescein diacetate (FDA), dimethyl sulfoxide (DMSO), cetyltrimethylammonium bromide (CTAB), 1,2-bis(trimethoxysilyl) ethane (BTME), poly(diallyldimethylammonium chloride) (PDDA), poly(sodium 4-styrenesulfonate) (PSS), β-morpholinoethansulfonic acid (MES) were purchased from Sigma-Aldrich and used as received. Affinity-purified polyclonal goat anti-mouse IgG (Gt α-M IgG (Fc specific)), affinity-purified polyclonal mouse IgG (M IgG), goat IgG (Gt IgG), Gt α-M IgG (whole molecule), Gt α-M IgG-FITC conjugate (catalog no. F0257, protein concentration 1.1 mg/mL, F/P ratio 4.2) and bovine serum albumin (BSA) were supplied by Sigma. $[C_3F_7O(CFCF_3CF_2O)_2$-$CFCF_3CONH(CH_2)_3N^+(C_2H_5)_2CH_3I^-]$ (FC_4) was purchased from Yick Vic Chemical Corp. (Hong Kong). The water used in all experiments was prepared in an Elga Water Unit with a resistivity higher than 18.2 MΩ cm.

Preparation of Hollow Periodic Mesoporous Organosilica (H-PMO) Spheres. H-PMO spheres were synthesized according to our previous reported method.[17] Briefly 0.5 g of FC_4 was dissolved in 96 mL of H_2O at room temperature and stirred for 1 h before addition of 0.2 g of CTAB and 0.7 mL of 2 M NaOH. An amount of 0.755 mL of BTME was introduced, and the whole system kept at 50 °C under vigorous stirring for 2 h. After washing with water and ethanol, the precipitates were washed in a mixture of ethanol and HCl at 50 °C for 24 h to remove the surfactant.

Loading of FDA onto H-PMO. Approximately 10 mg of H-PMO were incubated in 1.5 mL of 60 mg mL^{-1} FDA/DMSO solution for 30 min under sonication. The FDA loaded H-PMO (H-PMO-FDA) particles were then separated and washed three times with pH 3.5 water via centrifugation. An acidic wash was used to minimize desorption of FDA from the PMO particles.

Coating of Polyelectrolyte Multilayers. Polyelectrolyte multilayers were deposited on the H-PMO-FDA particles via adsorption from 5 mg mL^{-1} PDDA or PSS solutions (prepared in 0.5 M NaCl) for 10 min, with occasional shaking. Excess polyelectrolyte was removed by three centrifugation/redispersion cycles. This process was repeated until two (PDDA/PSS) bilayers were deposited.

Bioconjugation with Antibodies. An amount of 1 mg of H-PMO particles, after loading with FDA and PEM coating, were incubated in 1 mL of Gt α-M IgG (whole molecule) MES buffer solution (0.05 M pH 6.0) at 20 °C for 2 h. Three different antibody concentrations (50, 100, 200 μg mL^{-1}) were used. Following antibody immobilization, the particles were separated and washed with MES buffer twice via centrifugation (9000*g* for 5 min). The antibody surface coverage on the particles was determined by the difference of the UV absorption at 280 nm before and after adsorption. The IgG coated particles were then redispersed in MES buffer and stored at 4 °C for subsequent immunoassays.

Solid-Phase Sandwich Fluorescence Immunoassays. The immunoassay was carried out by the method described in the literature.[11] Briefly a Nunc Maxisorp 96-well microplate was coated with Gt α-M IgG (Fc Specific) by adding 100 μL/well of Gt α-M IgG solution (10 μg mL^{-1}, prepared in pH 9.6 carbonate/biocarbonate buffer). The plate was incubated at 20 °C for 2 h on a plate shaker and then at 4 °C overnight. After rinsing three times with washing buffer PBSTA (0.1 M PBS, 0.1% (w/v) BSA, 0.5% (w/v) Tween-20), the wells were blocked with 200 μL/well of 1.0%

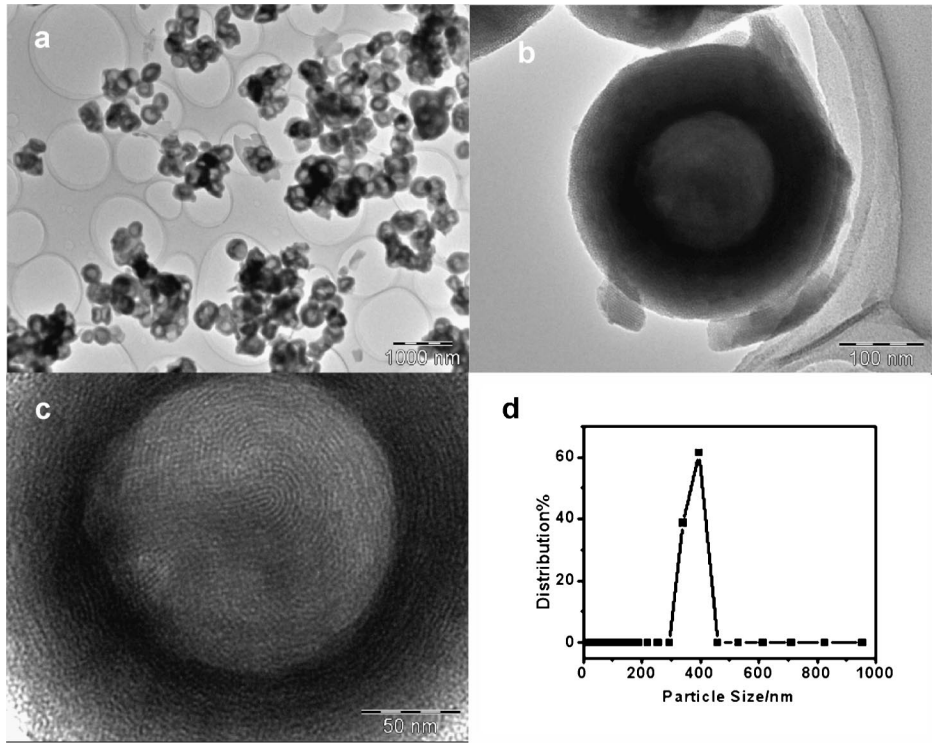

Figure 2. (a) Low and (b, c) high magnification TEM images of H-PMO particles. (d) Size distribution of as-synthesized H-PMO particles.

(w/v) BSA solution for 2 h at 20 °C and then washed three times with PBSTA. A stock M IgG solution (1 μg mL^{-1} in PBSTA) was serially diluted with PBSTA to give different concentrations of M IgG. In replicate ($n = 6$), 100 μL of each dilution of M IgG was added as analyte into each well. PBSTA alone was used as the blank control and Gt IgG of 500 ng mL^{-1} was added as a negative control. The plate was incubated with the samples at 20 °C for 2 h and subsequently washed three times. 0.1 mL Gt α-M IgG-coated H-PMO-FDA-PEM biolabels (with H-PMO concentration of 0.1 mg/mL) with different antibody surface coverage were added to the well, and the microplate was incubated again at 20 °C for 2 h. After incubation, excess biolabel conjugates were washed off by three washing cycles with buffer. FDA release was achieved by adding 100 μL of 40% NaOH per well and sonicated for 1 h. FITC-labeled Gt-α-M IgG dilutions (100 μL/well) of 1:64 were used for comparison. The intensity of fluorescence was measured at a Cary Eclipse microplate reader with an excitation/emission wavelength of 485/520 nm.

Characterization Methods. TEM analysis was performed using a FEI Tecnai 12 transmission electron microscope operated at 100 kV. The UV−vis absorbances were measured with a JASW V-550 spectrophotometer. Thermogravimetry analysis was recorded on a Shimadzu DTG-60 simultaneous DTA-TG apparatus at 5 °C min^{-1} in air. The ξ-potential was measured by a ZEN 3600 Nano-ZS Zetasizer nanoparticle analyzer (Malvern Instruments). The surface area and pore size distribution were measured on a Quadrasorb SI automatic surface area and pore size analyzer (Quantachrome Instruments).

RESULTS AND DISCUSSION

Loading of FDA onto H-PMO. The detailed synthesis, characterization, and formation mechanism of H-PMO spheres with a 3D pore network can be found in our recent report.[17] As shown in Figure 2, the as-prepared H-PMO particles have unique structure and surface features. The noticeable contrast between the core and the shell of the sphere indicates the hollow structure of PMO particles (Figure 2b). At higher magnification (Figure 2c), uniform well-defined mesostructures with pore size from 3.6 to 3.8 nm are clearly seen. The diameters of the particles are in the range from 300 to 450 nm with most particles having a diameter of 375 nm (Figure 2d) and a shell thickness of ∼50 nm. This unique structure provides the H-PMO particles with a high surface area of 844 m^2 g^{-1} and pore volume of 0.587 cm^3 g^{-1}, which makes them very attractive for material loading purposes.

Fluorescein diacetate (FDA, 15.37 Å in length, 7.80 Å in height, and 6.08 Å at the widest point[22]), the diacetate form of fluorescein, is chosen as a model organic dye for loading into the H-PMO. It is insoluble in water but can be easily turned into fluorescein by hydrolysis. While the insolubility of FDA will reduce possible leakage after loading, its hydrolysis feature enables potential controlled release. FDA loading was carried out by dispersing the dry H-PMO particles into the DMSO solution of FDA under sonication. Because of their small size, FDA molecules can be infiltrated into the mesopores of the H-PMO particles and it is also likely that some FDA molecules enter into the hollow part of the particle via the connected pores within the shell. The driving force for adsorbing FDA onto the particle surface are mainly physical interactions, for example, the hydrophobic interaction between the aromatic rings of FDA and the organic part of the H-PMO skeleton. Once the host−guest interactions are fulfilled, the additional FDA molecules occupy the leaving space of the cavity, with just FDA−FDA interactions.

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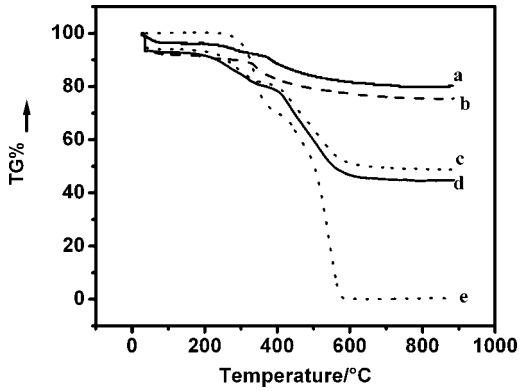

Figure 3. TGA curves of the (a) H-PMO, (b) H-PMO-PEM, (c) H-PMO-FDA, and (d) H-PMO-FDA-PEM particles and (e) pure FDA.

Coating of Polyelectrolyte Multilayers. After washing off the loosely adsorbed FDA, the FDA loaded H-PMO particles (H-PMO-FDA) were coated with two bilayers of poly(diallyldimethylammonium chloride) (PDDA)/poly(sodium 4-styrenesulfonate) (PSS) by the layer-by-layer self-assembly technique. The successful coating was monitored by measuring the ζ-potential of the coated particles.[11] The pure H-PMO particles exhibit an initial ζ-potential of -26.3 mV. After loading with neutral FDA molecules, the ζ-potential of the particles shifts positively to -20.5 mV. Alternating ζ-potentials were then observed for the subsequent deposition of positively charged PDDA (approximately 31 mV) and negatively charged PSS (approximately -26 mV), indicating the successful deposition of PDDA/PSS multilayer films on the H-PMO-FDA spheres. The polyelectrolyte multilayer coated particles are denoted as H-PMO-FDA-PEM. It was observed that the thickness of the four layer polyelectrolyte coating is only about 3−4 nm,[23] which did not cause any noticeable change in the morphology and size distribution of the PMO particles as observed by TEM. However, the polyelectrolyte network can effectively prevent desorption of FDA from the particle and more importantly, the outmost PSS layer provides the FDA-loaded particle a suitable surface for further antibody attachment (see below).

The FDA loading amount can be estimated by thermogravimetric analysis (TGA). Figure 3 shows the mass loss of (a) H-PMO, (b) H-PMO-PEM, (c) H-PMO-FDA, and (d) H-PMO-FDA-PEM particles and (e) pure FDA during heating from 20 to 900 °C. The different systems show different weight loss rates within the temperature range. The weight lost below 180 °C is attributed to the physical loss of water. Organosilica began to lose weight at around 300 °C while the weight loss of FDA and polyelectrolytes occurred after that. From the difference in weight loss between the H-PMO (curve a) and the H-PMO-FDA (curve c) system (~33.5%), the FDA loading amount on the H-PMO particles was calculated to be ~504 mg of FDA/g of H-PMO. This value is much higher than the loading amount of FDA on conventional mesoporous silica material MCM-41 (~140 mg of FDA/g of MCM-41).[22] The higher loading ability of H-PMO is probably due to its hollow structure and the hydrophobic feature of the organosilica that is favorable for the loading of organic dye molecules. After coating with polyelectrolyte film, the final FDA loading amount was calculated to be ~443 mg of FDA/g of H-PMO by comparing the weight loss difference between H-PMO-PEM (curve b) and the H-PMO-FDA-PEM (curve d) system (i.e., ~30.7%). The lower value compared to that of FDA adsorbed in the H-PMO system is probably due to the loss of some FDA molecules during the coating process and the introduction of polyelectrolyte coating to the system.

FDA Releasing. In order to be useful as a fluorescence biolabel, it is important that the loaded dye molecules can be released from the particles for detection. In the case of FDA, it is also necessary to convert the insoluble, nonfluorescent form of FDA into a soluble and fluorescent form. These two purposes can be achieved by adding 40% NaOH solution as a release reagent. The release reagent plays a double role here: First, it acts as a core-dissolving reagent to dissolve the silica particles, and free the loaded FDA molecules. Second, it acts as a hydrolysis catalyst and pH adjustor to turn the diacetate form of fluorescein into its dianionic form, which is fluorescent and soluble in water.[2] The structures of FDA, fluorescein and its dianionic form are shown in Figure 4a. The successful transfer can also be observed by the color change after the addition of NaOH. As shown in Figure 4b−d, as soon as 40% NaOH solution was added into the dispersion of H-PMO-FDA-PEM particles, the white solution became a red color (Figure 4b). The color became steadily darker and finally became black after 1 h of sonication due to the high concentration of dye molecules in the system (Figure 4c). Subsequent dilution resulted in a green color, which comes from the dianionic form of fluorescein (Figure 4d). The amount of FDA in the system can be quantitatively measured by fluorescence spectroscopy or UV−vis absorption. Our results indicate that around 90% of the loaded FDA can be released by this process. The above results are very interesting as they show that these types of fluorescence labels have a stimuli-release capability, i.e., the loaded FDA molecules are very stable in the particle but can be released and detected when needed.

Bioconjugation with Antibodies. Goat antimouse IgG molecules were then immobilized onto $(PDDA/PSS)_2$ multilayer coated H-PMO-FDA particles by physical adsorption. Adsorptive immobilization of antibody molecules onto polyelectrolyte-coated colloidal particles has been shown to be a simple and effective means for stably attaching biomolecules and retaining their specific immunorecognition ability.[11,24] The best pH for antibody adsorption is at pH 6.0 where the IgG molecule is at its isoelectric point and attaches to the outmost layer of PSS through mostly hydrophobic interactions. The adsorbed amount of antibody on the particles can be determined by monitoring the UV absorption difference at 280 nm of the antibody solution before and after adsorption. The results indicate that the adsorbed IgG amount is strongly dependent on the IgG concentration used for incubation. As presented in Table 1, the adsorbed IgG amount increases with increasing IgG concentration in the range 50−200 μg mL^{-1}. The approximate protein surface coverage of these three biolabels was calculated by assuming the polyelectrolyte coated particles are nonporous and by using H-PMO particle size of 375 nm (see above Figure 2) and H-PMO density of 0.51 g mL^{-1} (see Supporting Information for detailed calculation). The IgG surface coverage

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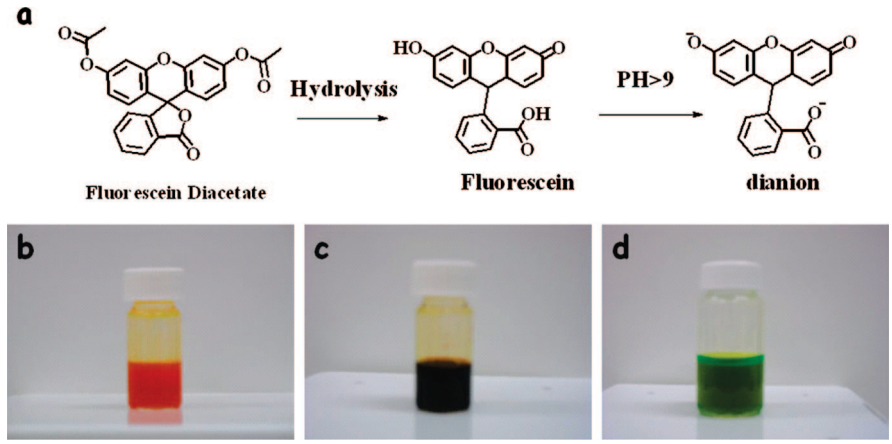

Figure 4. (a) The transfer of fluorescein diacetate to the dianionic form of fluorescein. Color changes after adding 40% NaOH into the H-PMO-FDA-PEM dispersion: (b) upon addition of NaOH; (c) after 1 h; and (d) after 10-fold dilution.

Table 1. IgG Surface Coverage on H-PMO-FDA-PEM Particles and the Corresponding F/P Ratios[a]

biolabels	a	b	c
incubation concentration of IgG (μg mL^{-1})	50	100	200
protein absorbed (μg mL^{-1})	28	73	161
protein surface coverage Γ (mg m^{-2})[b]	0.89	2.3	5.1
F/P ratio[c]	6046	2340	1055

[a] 1 mg of H-PMO particles, after FDA loading and PEM coating, incubated in 1 mL of IgG solution in pH 6.0 MES buffer. [b] Assuming H-PMO particles are round and nonporous. An average particle size of 375 nm and a density of 0.51 g mL^{-1} was used for calculation. [c] A loading amount of 443 mg of FDA/g of H-PMO was used for calculation.

of 0.89, 2.3, and 5.1 mg m^{-2} were obtained when the same mass of particles were incubated in 50, 100, and 200 μg mL^{-1} IgG solutions (denoted as biolabel a, b and c, respectively). Compared with the theoretically calculated surface coverage values for a closely packed Gt α-M IgG monolayer, which are in the range of 2.0−5.5 mg m^{-2}, depending on the different orientation of the IgG molecules,[11] the results suggest that it is likely that the IgG surface coverage of biolabel a may not reach monolayer coverage while a monolayer coverage may be formed for biolabel b and c. It is also likely that protein aggregates may occur on biolabel c.

By using the FDA loading amount of 443 mg of FDA/g of H-PMO (see above TGA results) and H-PMO particle size of 375 nm, the number of FDA molecules per particle is calculated to be 9.1×10^6. From this value and the protein surface coverage amounts, the F/P ratios can be calculated to be within a range of ∼1000−6000 (Table 1), much higher than the conventional covalently coupled fluorescent immunolabel, e.g., an IgG−FITC conjugate (F/P ratio ∼4−8). As F/P ratio is the key parameter for improving the sensitivity of fluorescent immunoassays, these novel biolabels have great potential to create highly amplified bioassays.

Solid-Phase Sandwich Fluorescence Immunoassays. The utility of these biolabel systems for immunoassays was examined by using a model sandwich immunoassay for mouse IgG (M IgG) detection. Figure 5 illustrates the scheme for performing the immunoassays. The capture antibody (Gt α-M IgG, Fc Specific) was preadsorbed on the microtiter plate. The analyte M IgG is added and immobilized by the capture antibody. After exposing to the biolabels and carefully washing off the unadsorbed biolabels, FDA in captured biolabels was released and converted into fluorescein molecules by adding 40% NaOH and sonicating for 1 h. The fluorescence intensity of the system was then measured by a fluorescence reader. The performance of three kinds of biolabels with different Gt α-M IgG surface coverage described in Table 1 was tested, and the fluorescence intensities in response to different analyte concentrations are shown in Figure 6. It was observed that for all three biolabels, the fluorescence intensity increases with the analyte concentration and finally reaches a plateau. No increase of background signal was observed when using 500 ng mL^{-1} goat IgG as a negative control. The results indicate that this method is immunoactive and has good selectivity. It is also noticed that among the three biolabels, biolabel system b, that possesses the intermediate IgG coverage, has the best performance by showing the highest fluorescence intensity and the widest linear detection range (∼20−100 ng mL^{-1}). These results indicate that a suitable antibody density on a biolabel is vital for achieving optimum immunoassay results. Increasing antibody density on the particles can increase the number of antibody/antigen interactions per particle thus increasing the assay avidity (as seen by the fact that the fluorescence response achieved by biolabel b is higher than that of biolabel a). However, an overcrowded antibody covered surface (e.g., biolabel c) decreases the F/P ratio and may also cause the steric constraint to prevent the interaction with analytes and thus is not favorable for performing immunoassay.[2]

We also compared the assay sensitivity in relative fluorescence intensity units (FIU) per analyte concentration change (in ng/mL; for measuring 40, 60, 80, and 100 ng/mL M IgG) between our particle biolabel system b and FITC labeled commercial antibody system (F/P ratio 4.2). Comparison resulted in a ∼10−52-fold higher sensitivity of the assays by using the particle biolabels, depending on the analyte concentration. Considering the much higher F/P ratio of biolabel b, from a theoretical viewpoint, the presented approach still has the potential to reach a higher amplification. The reasons might result from the steric constraint that prevents the particulate biolabels from freely interacting with analytes, the self-quenching problem of the dye, and the possibility that the current experimental conditions may not be fully optimized. With further development of this approach, considerable improvement in sensitivity can be expected.

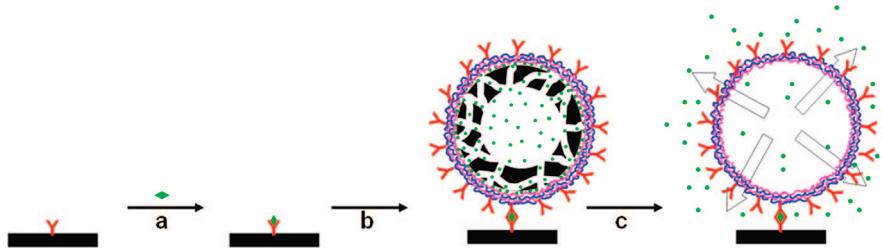

Figure 5. Scheme of a solid sandwich immunoassay using H-PMO biolabels. The analyte antigen is first immobilized by the capture antibody that is preadsorbed on the microtiter plate (a) and then exposed to FDA-loaded H-PMO biolabels (b). After washing off the unadsorbed biolabels, FDA in captured biolabels are released and converted into fluorescein molecules by adding NaOH (c). The fluorescence intensity of the system is proportional to the concentration of the analyte.

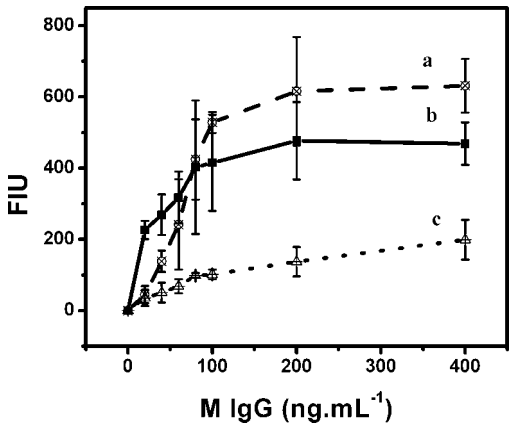

Figure 6. Sandwich fluorescent immunoassay of M IgG using three biolabels with different antibody surface coverage that are shown in Table 1: (a) biolabel b, (b) biolabel c, and (c) biolabel a. Error bars correspond to standard deviations of $n = 6$.

CONCLUSIONS

In summary, we have demonstrated a general and effective method based on utilization of hollow periodic mesoporous organosilica particles as templates for the preparation of a novel class of biochemical assay labeling systems. Because of the high surface area, hollow structure, and the hydrophobic surface nature of H-PMO, a high FDA loading amount (~443 mg of FDA/g of H-PMO) was achieved which resulted in the high F/P ratio (from ~1000 to 6000) of the resulting biolabels. The labeling systems are stimuli-responsive, i.e., the loaded FDA can be released and detected in a controlled manner (by adding 40% NaOH). The immunoassay experiments indicated that the biolabels were immuno-active and generated an optimal signal that was ~50 times higher than the conventional label. A further increment in sensitivity could be realized by increasing the dye loading amount and therefore increasing the F/P ratio, which can be achieved by utilizing H-PMO particles with larger mesopore size and vertical pore direction to facilitate the infiltration of dye molecules into the hollow part. Assay performance could also be improved by utilizing smaller size of mesoporous silica particles to reduce the steric effect and using quantum dots instead of fluorescent dyes to increase the fluorescence intensity and reduce self-quenching effect. These experiments are currently being undertaken in our laboratory. In addition, because of the immobilization capacity of mesoporous silica for various materials, this technique also has the potential to be extended to the loading of other interesting materials for preparing various kinds of label systems to meet the differing requirements in biochemical assays.

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SUPPORTING INFORMATION AVAILABLE

Calculation of the density of the H-PMO particles. This material is available free of charge via the Internet at http://pubs.acs.org.

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