

Au/Silica/Fe₂O₃ Nanohybrid Preparation and Biosensor Application

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Silica- and organic polymer insulated superparamagnetic nanoparticles have been studied and applied within a range of biomedical applications.^{1,2} Surface insulating layers can decrease the cytotoxicity of the internal magnetic particle core during *in vivo*- and *in vitro* applications. Besides the quenching effect on toxicity, surface coating layers can work as platforms to impart additional functionality. Especially, silica surface chemistry serves a good hydrophilic function and augments easy modification for tagging biomedical-functioning moieties.³⁻⁶

Reliable and rapid monitoring of bio-events and environments can provide early information of surroundings. Real-time sensing systems normally monitor changes in optical or electrical signals, allowing us to quickly recognize variations in our environment.^{7,8} Surface plasmonic resonance (SPR) is a colligative value of oscillation changes at a metal surface, and its oscillation frequency is much dependent on the refractive index of materials adsorbed onto the metal surface. For example, when biomolecules are adsorbed onto the metal surface, it would induce a local refractive index change, and thereby change the SPR extinction and frequency.⁹ Complementary base conjugated Au nanoparticle assembly has been applied to detect analyte by visual effects. However, there are scanty studies on recyclable Au nanohybrid sensors.¹⁰

In this study, we developed a real-time and recyclable optical biosensor using nanohybrids of Au and superparamagnetic nanoparticles. For utilizing SPR effects, Au nanoparticle was conjugated onto silica coated magnetic nanoparticles (see Figure 1). Controlled silica shell thickness caused tunable SPR effects.

First, monodisperse silica-coated Fe₂O₃ nanoparticles, hereafter noted as SiO₂/Fe₂O₃, were prepared. γ -Fe₂O₃ nanoparticles were coated with thin (~2 nm) or thick (~17 nm) silica shell employing water-in-cyclohexane reverse microemulsion technique. For a thin (2 nm-thick) silica shell, polyoxyethylene(5)nonylphenyl ether (0.56 mmol) was dispersed in cyclohexane (4.2 mL) by sonication. Then 400 μ L of Fe₂O₃ solution (0.8 mg/mL of cyclohexane) was added. The resulting mixture was vortexed, and ammonium

hydroxide (29.4%, 35 μ L) was added to form a transparent reverse microemulsion. Next, tetraethylorthosilicate (2 μ L, TEOS) was added, and the reaction was continued for 12 h at room temperature. In the case of thick silica shell (17 nm-thick) preparation, TEOS content and sol-gel aging time were increased to 20 μ L and 48 h, respectively. By adding methanol into the reaction solution, SiO₂/Fe₂O₃ nanoparticles were precipitated and collected by a magnet. They were functionalized with amine functionalities, which have been known to have strong affinity with transition metal nanoclusters, such as Fe₃O₄, and Au.^{11,12} In this study, 340 mg of SiO₂/Fe₂O₃ were functionalized with 0.075 mmol of *n*-(2-amino ethyl)-3-aminopropyltrimethoxysilane (AAPS, Gelest) by refluxing in toluene for 30 h. Next, Au was introduced as HAuCl₄ to the surface of SiO₂/Fe₂O₃ in DI water under microwave (250 W, 5 min). Figure 1 shows the overall schematic view of the preparation. The resulting primary amine-functionalized Au/SiO₂/Fe₂O₃ hybrids were further used for biotin conjugation experiments. *N*-hydroxysuccinimide ester (NHS) reacts with primary amines of Au/SiO₂/Fe₂O₃ hybrids to form stable amide bonds. The brief procedure is as follows: 5 mg of the Au/SiO₂/Fe₂O₃ hybrid powder was dispersed with 1.5 mL of borate buffer (pH 7.5) and was mixed with a NHS-PEG-biotin (Pierce, NHS-PEG12-Biotin) solution (1 mg/mL dimethylformamide). The resulting biotinylated Au/SiO₂/Fe₂O₃ hybrids were dialyzed after 2 h of



Figure 1. Schematic view of preparation of the biotinylated Au/SiO₂/Fe₂O₃ hybrid.

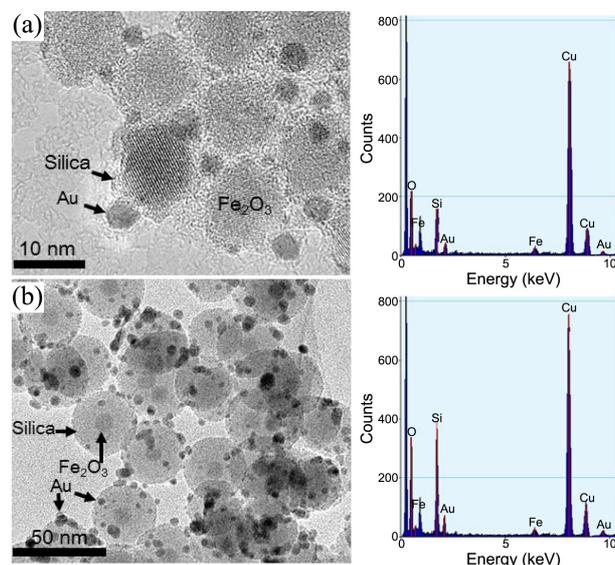


Figure 2. TEM and EDX data for (a) thin and (b) thick silica coated Au/SiO₂/Fe₂O₃ hybrids.

incubation and were preserved at 4 °C.

The Au/SiO₂/Fe₂O₃ hybrids were analyzed via transmission electron microscopy (TEM) and energy dispersive X-ray (EDX) spectra. The Au nanoparticle size was about 7 ± 4.2 nm (see Figure 2). TEM interfaced EDX analysis showed the existence of Au, Fe, O, and Si atoms (see Figure 2). From these data, it is clear that Au nanoparticles were well conjugated around the SiO₂/Fe₂O₃ particles. However the conjugated Au content showed difference for both thin and thick silica coated SiO₂/Fe₂O₃. Inductively coupled plasma-mass spectrometry (ICP-MS) elemental analyses showed 27.2 wt % Au for the thin SiO₂/Fe₂O₃ particles and 12.5 wt % Au for the thick SiO₂/Fe₂O₃. This can be caused by the difference of relative volume of SiO₂ shell which works as a substrate for holding Au particles. As noted early, different surroundings around metal induces variations of plasmon absorbance, and extinction. Figure 3(a) shows the corresponding UV-Vis absorbance spectra of the thin, and thick silica coated Au/SiO₂/Fe₂O₃, and bare Fe₂O₃ nanoparticles (~10 nm diameter). Thin silica coated Au/SiO₂/Fe₂O₃ hybrids were characterized by the absorbance peak at 531 nm, the Au nanoparticle plasmon band. However, as the thickness of the silica shell increases, the λ_{max} value was red-shifted. As explained in the previous literature, surface plasmon resonance is an electron oscillation phenomenon and induces an absorption band with the incident electromagnetic energy.¹³ Therefore, the absorption band properties are determined by the dielectric properties of the Au particles, geometries of Au/SiO₂/Fe₂O₃, and surrounding coating medium, here silica. In this work, the λ_{max} shift, by 31 nm, of the plasmon band was primarily induced by the thickness variation of the silica-coating medium and the consequent changes in dielectric properties. In the case of bare Fe₂O₃ nanoparticles, a prominent absorbance was not observed.

Further studies on the biosensor applications using thin silica coated Au/SiO₂/Fe₂O₃ hybrids were carried out. AAPS conjugated silica worked as an anchoring site for Au nanoparticle adsorption, and free primary amines, involved for capturing Au nanoparticles, formed amide bonding with NHS-biotin affording biotin exposed Au/SiO₂/Fe₂O₃ hybrids. It was well known that four biotins can combine with one avidin. Since the resulting Au/SiO₂/Fe₂O₃ hybrid has biotins on the surface, avidin can make specific bindings with Au/SiO₂/Fe₂O₃. An aqueous solution of avidin (67 kDa) (0.1 μmol/10 mL) was prepared, the Au/SiO₂/Fe₂O₃ hybrids (5 mg) were mixed with the avidin solution and stirred for 20 mins. The solution, analyzed by UV-Vis spectrometry, shows a reduced absorbance (Figure 3(b)) compared to the control experiment without avidin. This may be due to the bridging effects of avidin with biotins, which resulted in the aggregation and precipitation of Au/SiO₂/Fe₂O₃ hybrids (corresponding TEM image in SI). Au/SiO₂/Fe₂O₃ hybrids were collected with a magnet, and were re-dispersed in new batch of avidin aqueous solution with the same concentration, and stirred for 20 mins. Interestingly, the absorbance kept on decreasing (graph 3 and 4 in Figure 3(b)). If biotin conjugation on the hybrid were 100% then 1.06 μmol of biotin

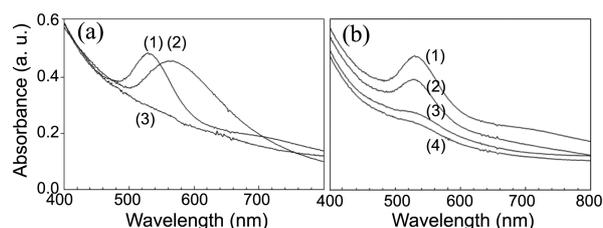


Figure 3. UV-Vis spectroscopy data for (a) thin (1) and thick (2) silica coated Au/SiO₂/Fe₂O₃ hybrids, and bare Fe₂O₃ particles (3), (b) recyclable biotin/Au/SiO₂/Fe₂O₃-avidin reaction, before reaction (1), after 1st (2), 2nd (3), and 3rd reaction (4).

will be available on the surface. Further, if most of avidin in the solution was involved in the binding reaction, biotin content on the hybrid can be quantified as about 1 mmol. The quantified content is comparable with the treated biotin concentration (1.06 μmol). Since biotin was conjugated on the bulky silica hybrid surface, it is quite likely that there will be non-bound free biotins due to hybrid particle-particle steric hindrance. Although precise quantification was not carried out, it was clear that the resulting Au/SiO₂/Fe₂O₃ hybrids can be applied to detect biomolecules in a real-time and recyclable manner at sub micromolar level.

In conclusion, a superparamagnetic core and controlled silica shell structure was prepared by a reverse-microemulsion method, and Au/SiO₂/Fe₂O₃ hybrids were produced using amine anchoring chemistry. The corresponding surface plasmonic properties were studied, and the controlled silica shell caused tunable SPR effects. For biosensor applications, biotin conjugation on the Au/SiO₂/Fe₂O₃ hybrids was performed. The resulting biotinylated Au/SiO₂/Fe₂O₃ hybrid is suitable for further application as a recyclable and real-time biosensor.

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