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BIOFUNCTIONALIZATION OF GOLD NANOSHELLS MONITORED BY SURPHASE PLASMON RESONANCE

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Abstract. Gold nanoshells (GNSs) were grown on monodispersed and aminopropyltriethoxysilane (APTES) functionalized silica nanoparticles (NPs) cores with varying sizes ranging from 60-180 nm, which were prepared by Stober route. Gold shells were deposited onto the surface of silica NPs by using tetrakis(hydroxymethyl) phosphonium chloride (THPC) and an electroless gold plating method. The coverage of the gold nanoshells on the surfaces of the silica NPs was evaluated using surface plasmon resonance (SPR) spectra and scanning electron microscope (SEM). The GNSs were also attached with biomolecules. The plasmon resonance wavelengths of this GNSs were tunable from visible to near infrared. The attachment process of biomolecules onto the GNSs surfaces was controlled by measuring both their SPR spectra and their zeta potentials.

Keywords: gold nanoshells, surface plasmon resonance, zeta potential.

Classification numbers: 2.1.1; 2.4.4; 2.5.1

1. INTRODUCTION

Gold nanoshells (GNSs) nanoparticles (NPs) consist of a spherical dielectric core coated with a concentric layer of gold [1]. Due to large extinction cross sections of GNSs between 700-1200 nm, it is possible to use them in biological applications such as hyperthermia agents, contrast agents biological imaging, controlled drug release, bioprobes in cell and tissue analysis, and for studying biological processes at the nanoscale [2, 3, 4, 5]. A critical factor limiting the applications of GNSs for therapeutic and diagnostic purposes is their short circulation time *in vivo* due to clearance by the body's reticuloendothelial system [6]. For the biomedical application, particularly for *in vivo* imaging it requires stable GNSs in biological media. Therefore, the surface biofunctionalization of the GNSs is necessary for their applications. General strategies are provided for the covalent and noncovalent mode of binding based on a combination of electrostatic and hydrophobic interactions of the biomolecules or biocompatible molecules onto the gold surface. These GNSs were attached with some kinds of alive biomolecules such as glutathione (GSH), protein bovine serum albumin (BSA), and anti-HER2

IgG monoclonal antibody [7]. Glutathione is an important tripeptide in all living organisms. It is one of important biological antioxidants, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. In a previous work, we reported about the binding of GSH onto the GNSs surfaces [8]. Serum albumin is a major protein component of blood plasma but is distributed to the interstitial fluid of the body tissues. Serum albumin is capable of binding to a wide variety of drugs, and there is strong interest in this abundant protein because of its effects on drug delivery. Therefore, immobilization and immunoreaction of a fundamental protein, such as serum albumin on gold NPs, is very important for bioapplications [7]. These GNSs were also conjugated with a biocompatible molecules, HS-PEG-COOH due to its stealth character with immune system [9]. Moreover, modified thiol–PEG (SH-PEG) is an excellent candidate for stabilizing gold NPs in physiological condition, and enabling long lasting circulation in the blood [10].

In this paper we focus on the preparation and the biofunctionalization of GNSs. The coverage of the GNSs on the surfaces of the silica NPs was evaluated using UV-VIS/NIR spectroscopy. The surphase plasmon resonance (SPR) wavelengths of these GNSs were tunable from visible to near infrared. Simultaneously, this work is to use the SPR spectra to monitor the biomolecules coating process onto GNNs NPs, on which we can determinate the suitable biomolecule amounts to obtain stability GNSs. The biofunctionalization of GNS NPs was estimated by dynamic light scattering (DLS) measurement. The results demonstrate the effects of the biomolecules layer on zeta potential values. This work also gives us how to determine the threshold of biomolecules binding onto the GNSs surfaces.

2. MATERIALS AND METHODS

2.1. Reagents

Tetraethylorthosilicate (TEOS, 99 %), ammonium hydroxide (NH_4OH, 29 % in water), and glutathione were purchased from Sigma–Aldrich. APTES (99 %); ethanol, tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9 %), Tetrakis(hydroxymethyl)phosphonium chloride (THPC, 80 % solution in water), sodium hydroxide (NaOH, 99 %), potassium carbonate (99 %), and formaldehyde (37 % solution in water) were purchased from Merck. Bovine serum albumin was obtained from Biochem. The heterobifunctional thiol polyethylene glycol acid with a molar weight of 1000 Dalton (HS-PEG-COOH) was purchased from Creative PEG works. The mouse anti-HER2 IgG monoclonal antibody (anti-HER2 IgG) (28-0003z) 1 mg/mL in PBS solution with pH 7.4 with 1 % BSA was purchased from Invitrogen. Deionized (DI) water was used in all experiments.

2.2. Preparation and biofunctionalization of gold nanoshells

GNSs were prepared by the seed-and-grow method which details were described in our previous work [11], using silica core with a diameter of 60-180 nm synthesized by Stober route. The particle surface was then terminated with amine groups by reaction with APTES. THPC gold NPs (1-2 nm) were prepared by the method of Duff et al. [12].

The size and stability of THPC gold were obviously improved by using citrate buffer of 4,6 mM. 1 ml of APTES functioned of silica NPs was then added to 3 ml of THPC gold NPs solution. THPC gold NPs were absorbed into the amine groups on the silica NPs surface resulting in an APTES functioned silica NP decorated with discrete of THPC gold NPs, called

the ‘seeds' NPs. The 10-20 nm gold shell layers were then grown on these ‘seeds' through the electroless gold plating process by using gold plating solution in the presence of formaldehyde. After the preparation of GNSs NP solution, in order to avoid the GNS NPs’s aggregation or to make the GNSs solutions more stable and to biofunctionalize these GNSs for the future biomedical applications, the GNS NPs were bioconjugated with GSH, BSA, HS-PEG-COOH and anti-HER2 IgG molecules.

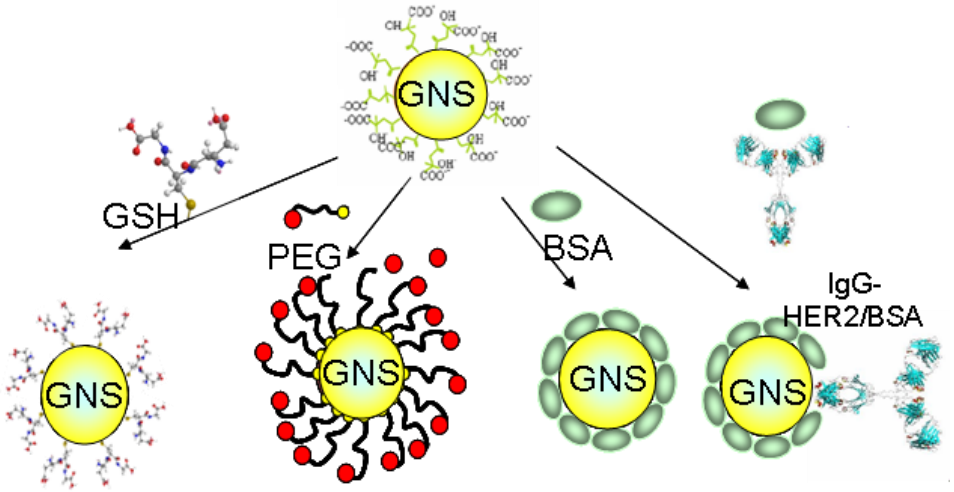

Figure 1. Schematic of GNS NPs was conjugated with biomolecules: GSH, HS-PEG-COOH, BSA and anti-HER2 antibody IgG.

Figure 1 presents the schematic of GNS NPs, which were conjugated with different biomolecules. The biofunctionalization process of each biomolecule was performed according to our previous works: with HS-PEG-COOH [4], with GSH [11]; with BSA [13,14], and with anti-HER2 IgG [14]. In brief, a number of above biomolecules was added into the GNS NPs solution to generate biomolecules-coated GNS NPs. The process was done by stirring the solution for 1 hour at room temperature. The suitable amounts of these biomolecules to coat GNSs NPs were estimated from a collection of their UV-VIS/NEAR spectra during their storage time in dark and at 4 ^{o}C.

2.3. Characterizations of gold nanoshells

The absorption spectra of GNS NPs and their biofunctionalized GNS NPs solutions were collected on a Shimadzu UV2600 spectrophotometer. Scanning electron microscopy (SEM) images of the GNPs NPs were obtained from HITACHI SEM S4800 microscope. The polydispersity index (PDI) values and zeta potentials of the bare and biofunctionalized GNS NPs were measured by dynamic light scattering (DLS) technique with an angle scattering of 173^{0} (Nano ZS, Malvern).

3. RESULTS AND DISCUSSION

3.1. The formation of gold nanoshells

The formation and characterization of GNSs with different sizes of a silica core coated with various coverage of gold shell were detailed in our previous work [11]. The SEM images in Figure 2 show the morphologic properties of GNS NPs obtained from silica core NPs with the corresponding sizes of 180 ± 5 nm and 70 ± 3 nm coated with gold shells with the thickness of about of 15 nm. The coverage was uniform regardless of silica NPs core sizes.

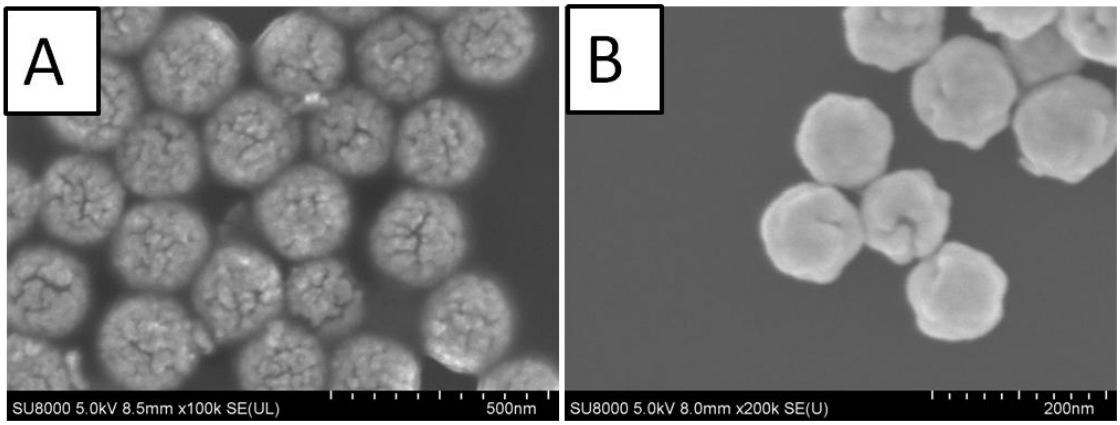

Figure 2. SEM images of silica NPs core with sizes of 180 ± 5 nm (A) and of 70± 3 nm (B) coated with gold shells.

3.2. Optical characterizations and biofunctionalization of gold nanoshells

Important information pertaining to the aggregation state and the stability of conjugated GNS NPs can be obtained from SPR spectra before and after the biofunctionalization process. Figure 3 shows the SPR absorption spectra of GNS NPs with sizes of 100 nm before (Fig. 3a) and after being coated with BSA molecules observed during two weeks of storage (Fig. 3b).

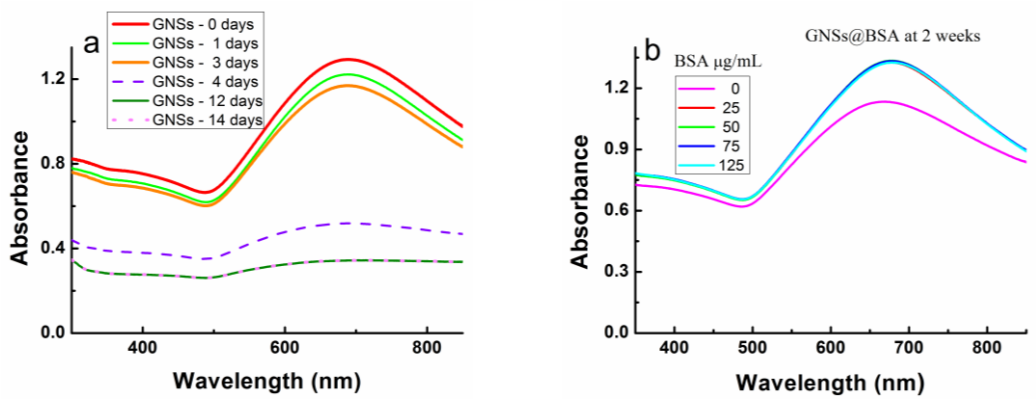

Figure 3. SPR spectra of 100 nm-size (a) bare GNSs NPs during 2 weeks storage; and (b) GNSs NPs coated with a different amount of BSA protein after two weeks storage.

As shown in Figure 3a, the absorbance of bare GNS NPs was strongly decreased after 3 days of storage. The previous work of Neoh and Kang reported that the absorbed BSA proteins on the gold NPs surface is $2.5 10^{16}$ BSA/m^2 [15]. Due to the surface of GNSs NPs can not be exactly determined, our GNS NPs were coated with a different amount of protein BSA, i.e. from

25 µg/mL-125 µg/mL. Their absorbances have insignificantly changed during two weeks of storage (Fig. 3b).

This result indicates that without BSA coating, the bare GNS NPs solution has a tendency of aggregation, as resulted from their instability, so the GNSs coated with a layer of BSA protein are expected to be prevented from the aggregation of GNSs NPs. In fact, we observed the GNS NPs@BSA proteins are more stable. According to this result, we can also determine the threshold or suitable amount of BSA to stabilize the GNS NPs solution.

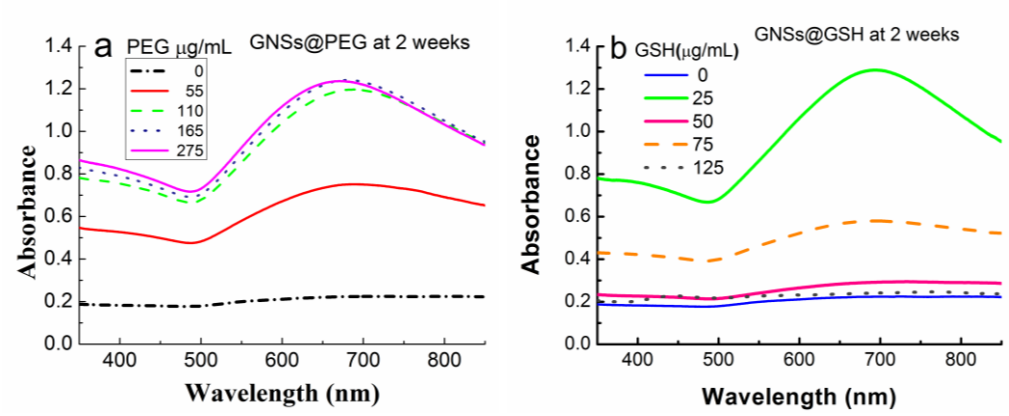

Figure 4. SPR spectra of GNSs NPs observed after two weeks storage of 100 nm in-size GNS NPs: (a) coated with different amount of PEG; (b) coated with different amount of GSH.

Owing to the strong binding between thiols to noble metals, the HS-PEG-COOH and GSH, thiol-containing molecules were frequently used to stabilize the gold NPs [15]. Similarly to our approach, different amounts of HS-PEG-COOH, GSH were used to coat the GNSs, and their SPR spectra were also measured during the storage time. The results showed that the suitable amounts to stabilize GNSs are about 25 µg/mL for GSH, and 110 µg/mL for HS-PEG-COOH, respectively, as indicated in Figures 4a and 4b. Observing the SPR spectra of the GNSs coated with biomolecules during storage time, we could determine their suitable amounts to stabilize the GNSs. These results fit with the previous report of Widrid *et al.* that the required n-alkanethiols binding onto the gold surface is about 5-6 molecules/nm^2 [16].

Figure 5 presents the SPR spectra of GNSs coated with the suitable amounts of different biomolecules as detailed in Table 1, corresponding to two different sizes shown in Figure 2. The results show that for the samples without coating the SPR intensity of GNSs decreases over storage time. In contrast, when GNSs were coated with these biomolecules, their SPR spectra shapes exhibited almost no change, while the SPR intensity increased by 5 to 10 percent (Figure 5a). In addition, their SPR spectra are slightly red-shift, i.e. of about 4 - 5 nm as shown in Figure 5b. These results correspond with the modeling of B. Dan and co-worker [17] on the SRP of a single protein conjugated GNS and with our previous studies [7] and, indicated that these biomolecules are successfully conjugated with GNS NPs. According to zeta potential measurement, the obtained data of GNSs coated with different above bimolecules in ultra pure water correspond with the above suitable amounts. Figure 6 presents the obtained zeta potential diagrams of bare GNS NPs and after being conjugated with BSA, GSH, PEG and IgG/BSA. The zeta potential of the GNS NPs increased from -31 mV (before binding) to -18 mV (after conjugation with biocompatible molecule HS-PEG-COOH).

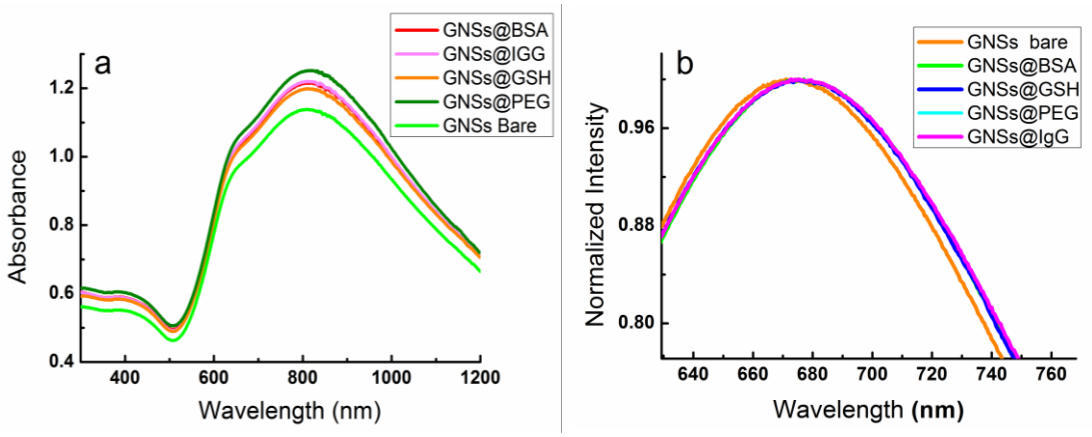

Figure 5. The SPR spectra of GNSs NPs of 200 mm size (a), and normalized SPR spectra of GNSs NPs of 100 nm size (b), coated by different biomolecules.

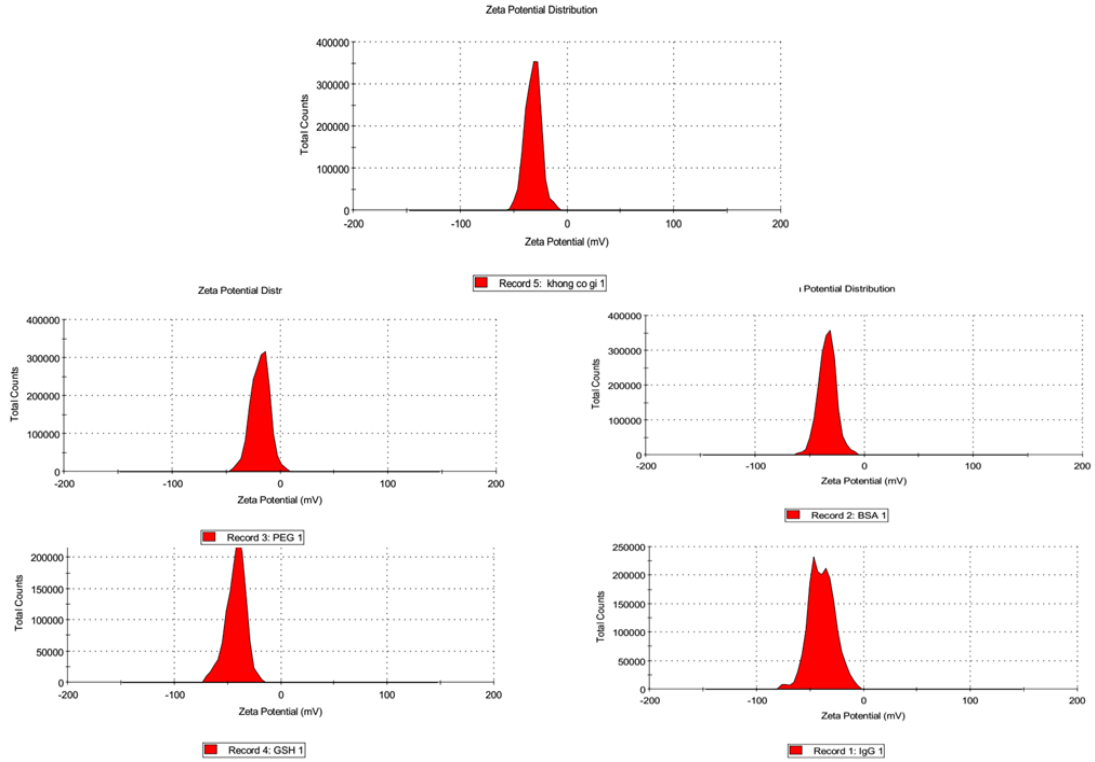

Figure 6. Zeta potential distribution of bare GNS NPs and after being conjugated with BSA, GSH, PEG and IgG/BSA.

Meanwhile, the binding of BSA, GSH or IgG/BSA onto the surface of GNSs NPs made their zeta potentials slightly decreased as shown in Table 1. Particularly, with the antibody IgG molecule stabilizer in BSA solution, the obtained zeta potential has two peaks at - 47 mV and - 30 mV. The zeta potential value of GNSs NPs solution depends on the nature of molecules bounded on the GNSs NPs surface. These results demonstrated that various functional groups on

the nanoparticles surface exhibited different surface zeta potential values. This result again demonstrated that these biomolecules are successfully conjugated with GNS NPs.

Table 1. Zeta potentials (ξ) of the GNSs conjugated with biomolecules and biocompatible molecules.

SamplesGNS NPs	Biomolecules and amount (µg)	ξ position (mV)
SiO2@Au	-	-31.9
SiO2@Au@BSA	50 µg BSA	-33.9
SiO2@Au@PEG-COOH	165 µg PEG	-18.6
SiO2@Au@GSH	25 µg GSH	- 42.3
SiO2@Au@IgG/BSA	25 µg BSA+2.5 µg IgG	-47 and -30

4. CONCLUSIONS

In conclusion, we report a simple way to form the GNSs from the silica NPs core of various sizes ranging from 70 to 180 nm. The SPR of the GNSs could be adjusted to any wavelength from the visible to the NIR region of the electromagnetic spectrum. The biofunctionalization process of bio-molecules onto the GNSs surfaces was controlled by observing their SPR spectra during two weeks storage. Simultaneously, the biofunctionalization process could help us to estimate the agent amount suitable to cover the GNSs NPs or particle density. The GNSs were successfully attached by various biomolecules or biocompatible molecules, making them ready for biomedical applications.

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