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SELF-ASSEMBLED QUANTUM DOTS ON AU AND THE INTERFACE FLUORESCENCE

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In this paper, amino capped CdSe/ZnS quantum dots (QDs) were immobilized on the 11-mercaptoundecanoic acid (MUA) self-assembled Au surface (SAM/Au) by 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC). Atomic force microscopy (AFM), fluorescence imaging and electrochemistry were employed to characterize the surface. The results showed that CdSe/ZnS QDs were immobilized on the surface of SAM/Au successfully. Based on this method, the fluorescence of the QDs on the SAM/Au was monitored on-line.

Keywords: Self-assembled; quantum dots; interface fluorescence.

1. Introduction

Quantum dots (QDs) are stable and zero-dimension semiconductor nanoparticles composed of II-VI or III-V elements with diameters on the order of 2–10 nm. Due to their quantum limitation effect, generated by physical sizes that are smaller than the bore radius of exaction, they have special electronic and optical properties. QDs are being broadly used in many scientific fields, including homogenous and solid-state assays.[1,2]

In the field of biosensor fabrication involving QDs, researchers are interested in homogenous and solid-state assays of QDs. QDs have recently appeared as a donor in homogenous assays that involve fluorescence detection of QDs.[3] A vast literature reported the DNA detection by fluoroscopy that involves solid-state assays.[4] But, to the best of our knowledge, no paper about solid-state assay of QDs was found, because it is difficult to measure the fluorescence of QDs on solid-state substrate.

In this paper, a novel method about measuring solid-state assay of QDs on Au substrate has been proposed. Briefly, MUA was self-assembled on Au surface and conjugated with QDs catalyzed by EDC. The structure of the obtained interface was characterized by AFM, electrochemistry and fluorescent spectra. The results show that QDs have been successfully immobilized on SAM/Au film. Fluorescent spectra of QDs have been collected on solid substrate on-line. This study provides a novel method for immobilizing QDs on solid substrate and for on-line monitoring of the fluorescence of QDs.

2. Materials and Methods

2.1. *Reagents and chemicals*

$Cd(Ac)_2$, $Zn(Ac)_2$ and Selenium (Se) powder was purchased from Acros Organics. Mercaptamine hydrochloride (MEA), MUA, EDC, tri-*n*-octylphosphine oxide (TOPO), hexadecylamine (HDA), tri-*n*-octylphosphine (TOP) and hexamethyldisilathiane ($(TMS)_2S$) were obtained from Sigma-Aldrich. Mica sheets were obtained from Shenshi reagent corporation. The other reagents are of analytical grade. PBS is phosphate buffer solution (100 mmol L^{-1}; pH 7.0). All solutions were prepared with double distilled water.

2.2. *Apparatus*

Absorption spectra and fluorescent spectra were collected by UV-2550 UV-Vis Spectrum Spectrometer (Shimadzu Co., Japan) and LS-55 fluorometer (PerkinElmer Inc., USA) respectively at room temperature. Electrochemical experiments were carried out on electrochemistry CHI660 workstation (Chenhua Co., China). The contact angle was measured by Processor Tensiometer K-12 dynamic surface energy analyzer (Krüss Co., Germany).

2.3. *Preparation of QDs*

Oil-soluble CdSe/ZnS was prepared according to the literature.[5] Briefly, 0.125 g of $Cd(Ac)_2$ and 10 g of TOPO were loaded into a three-necked flask and heated to 260°C for 2 h under Ar flow. Then, 42.86 mg of Se powder was dissolved in 5 g TOP to get Se stock solution. After the complex solution in the three-necked flask was heated to 330°C and the temperature was stable for 30 min, Se stock solution was swiftly injected into the reaction flask for the growth of nanocrystal. After about 3 min, the heating device was removed and xylene was added to decrease the reaction temperature till it was lower than 140°C; CdSe solution was obtained after the device cooled down. CdSe was obtained by precipitation with methanol, and finally, was dissolved in chloroform.

For coating of ZnS, 5 g TOPO, 2.5 g HDA, 0.1 g $Zn(Ac)_2$ mixture was heavily stirred with prepared CdSe in the three-necked flask and heated up to 220°C for 2 h in an inert atmosphere glove box. The precursor S was prepared by dissolving 2 g trioctylphosphine oxide (TOPO) in 90 mL $(TMS)_2S$. Then S was injected into the three-necked flask gradually every 5 s and then stirred for 2 h at 90°C. Purified CdSe/ZnS QDs in chloroform were obtained by centrifugation and precipitation by methanol and redispersed in hexane, and then, the product was diluted by chloroform.

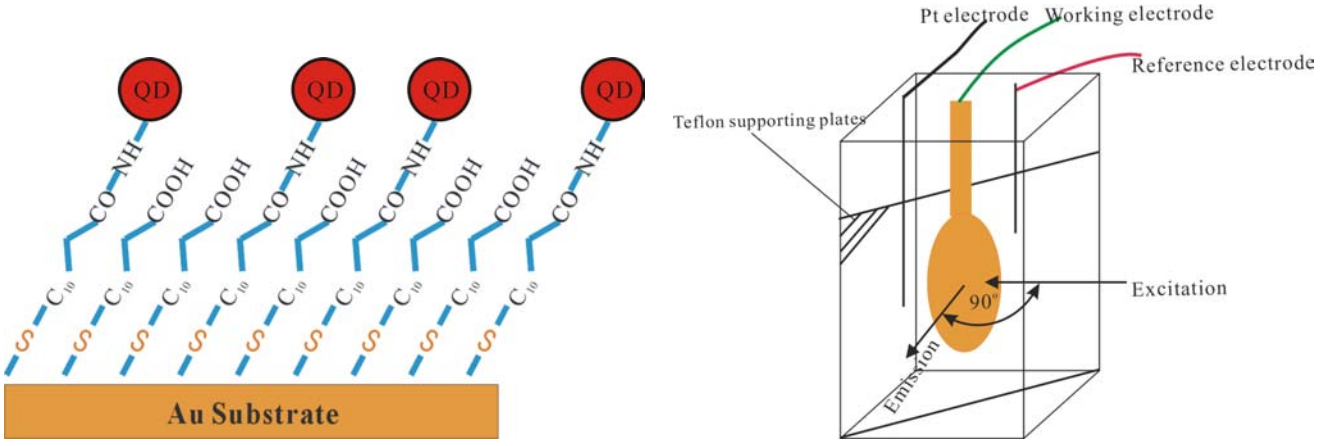
Water-soluble amino capped CdSe/ZnS QDs were prepared. After a mixture of 100 mg MEA and 500 μL 1.72×10^{-4} mol L^{-1} were vibrated for 12 h, amino capped CdSe/ZnS QDs were centrifuged by using water and acetone respectively for three times. And the emission wavelength of the QDs is 586 nm.

2.4. *Characterization of gold electrode*

Au was boiled in nitric acid for 5 min, and then carefully abraded with emery paper, polished on chamois leather containing 0.05 μm alumina slurry, and then washed ultrasonically in water, acetone, and then water, for 5 min respectively. The cleaned Au was preserved in 0.5 mol L^{-1} MUA solution with ethanol at 4°C refrigerator for 12 h to obtain SAM/Au. Then it was immersed in ethanol for 4 h to remove the surplus MUA and then put into amino capped CdSe/ZnS QDs activated by 10 μL of 10 mg mL^{-1} EDC for 12 h at room temperature to obtain QDs/SAM/Au, and the model was shown in Scheme 1(a). A conventional three-electrode cell with Au or SAM/Au or QDs/SAM/Au as a working electrode, Pt as a counter electrode and Ag/AgCl as a reference electrode was used for cycle voltammetry (CV).

The construction of the fluorescence-electrochemistry detection cell was shown in Scheme 1(b). A 1 cm × 1 cm × 3.6 cm quartz cell was shown; the excitation and emission refer to excitation light and emission light, respectively, which was detected by a LS-55 fluorometer. Au working electrode, Pt electrode, Ag/AgCl reference electrode and teflon supporting plates were shown in the quartz cell; the angle between the excitation and emission light is 90°.

The PicoScan system was used in AFM and the data were collected by commercial MAClever Type II Si detect pin (Molecular Imaging Inc., USA) in the tip mode, whereby the tip elastic force constant was about 0.34 N/m and the scan rate controlled at 1 lines/s. Au vapor was deposited on fresh mica sheet in vacuum by using IB-3 ion coater (Giko Engineering Co., Japan) as substrate for AFM characterization.



(a) (b)

Scheme 1. Illustration of (a) QDs/SAM/Au and (b) fluorescence-electrochemistry detection cell.

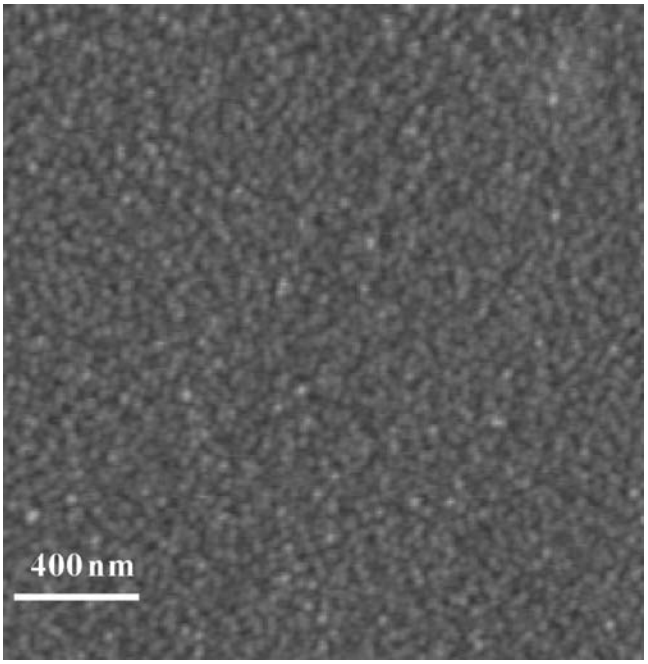
3. Results and Discussion

Self-assembly is a usual way for modification of heavy metal surface,[6] which can form a compact single molecular layer, and can bring needed active group[7] to connect with other molecules, like DNA[8] and protein.[9] The contact angles (CA) of Au were measured before and after modification. The result showed that CA of bare Au is 55° and SAM/Au is 51.5°, indicating that hydrophilicity of SAM/Au was better than bare Au because of MUA immobilization on Au with exposure of hydrophilic carboxyl groups at surface, changing the properties of the surface and intensifying affinity between Au and water.[10] The result indicated that MUA had already been immobilized on Au surface to form the MUA/Au.

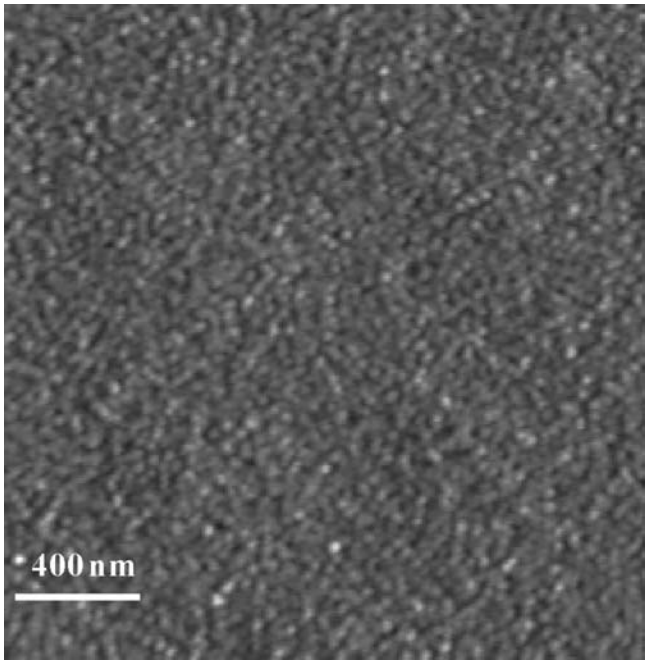
AFM was employed for characterization of bare Au, SAM/Au and QDs/SAM/Au. It was observed that the diameter of particles on SAM/Au surface was about 4.3±0.2 nm [Fig. 1(b)], which was almost the same as those on bare Au [Fig. 1(a)], indicating that the surface was flat due to our ion sputtering equipment. The carboxyl group at the end of SAM is easily combined with the amino group on the surface of QDs, so the surface of SAM provided a comfortable environment for immobilization of QDs. By observing the surface of QDs/SAM/Au, nanoparticles with diameter of 13.7 ± 0.5 nm were found [Fig. 1(c)], considering the broadening effect of tip, which agreed well with the size of QDs. And the experiment proved that QDs were successfully immobilized on the surface of SAM.

With the advantage of high sensitivity, the fluorescent spectrum was combined with electrochemistry for real-time data collection on the solid substrate. The fluorescence of QDs at SAM/Au was also detected by a self-constructed fluorescence-electrochemistry detection cell.[11] The result showed that there was no fluorescent signal on bare Au (Fig. 2, graph a) and SAM/Au (Fig. 2, graph b). However, there was a fluorescent peak at 586 nm on detection of QDs/SAM/Au (Fig. 2, graph c), which is the same as the excitation wavelength of the QDs (586 nm) (Fig. 2, insert). The result further proved that QDs were definitely conjugated on the surface of electrode.

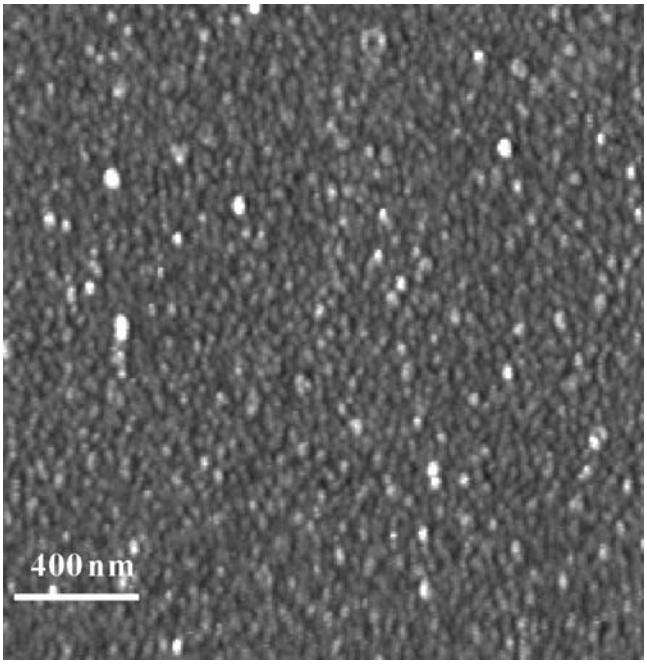
Further studies on self-assembled modification of Au and the process of QD immobilization were carried out by using $K_3Fe(CN)_6$ as a detection probe. The results showed that $K_3Fe(CN)_6$ had a pair of reversible redox peak (Fig. 3, graph a) with peak separation ΔE of 65 mV; however, no peak currents were present at SAM/Au (Fig. 3, graph b). There might be two reasons: one is a dense film of MUA forming on Au hindering the electron transfer, the other is the negatively charged carboxyl group at the end of SAM electrostatic repulsing the same charged $Fe(CN)_6^{3-}$, making it difficult for redox reaction to occur on the electrode. However, after QDs were conjugated on the surface of SAM/Au, a pair of irreversible redox peaks were observed (Fig. 3, graph c), but the peak currents (1.2 μA) were lower than that on bare Au (5.0 μA), and the peak separation ΔE, 523 mV, was apparently larger than that on bare Au. The probable reason is that part of carboxyl ended SAM/Au conjugated with amino capped QDs decreased the charge density of the surface and led to decreased



(a)



(b)



(c)

Fig. 1. AFM images of (a) Au, (b) SAM/Au, (c) QDs + SAM/Au. Scan range: $2\,\mu$m $\times$ $2\,\mu$m. Z is 15 nm.

impulsion from $Fe(CN)_6^{3-}$ and promoted the charge transfer on the electrode. Due to the presence of a large number of free carboxyl groups, it is difficult for $Fe(CN)_6^{3-}$ to be involved in redox reactions on electrode.

At last, with different potentials, changes of fluorescent intensity on QDs/SAM/Au were observed in real time as shown in Fig. 4. In the range of $0 \sim 1.0$ V [Fig. 4(a)] and $-1.0 \sim 0$ V [Fig. 4(b)], fluorescent intensities of electrode surface decreased gradually with increasing potentials. By changing the potentials in the range of $0 \sim 1.0$ V or $-1.0 \sim 0$ V, the fluorescent peak did not redshift or blueshift obviously. After changing the potential from 0 V to

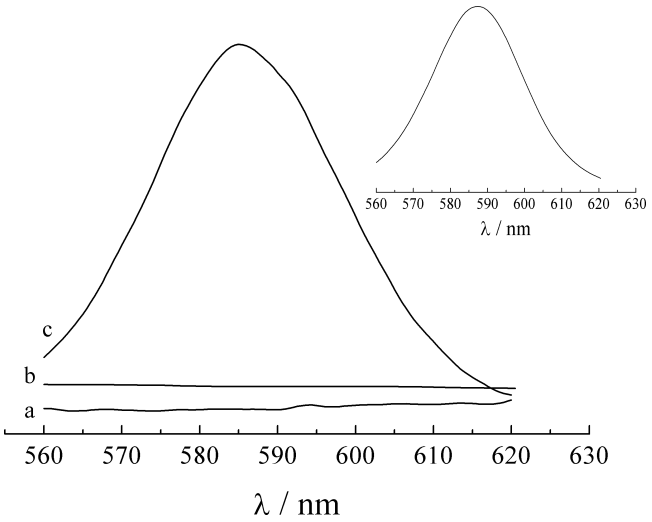


Fig. 2. Fluorescence of (a) Au, (b) SAM/Au, (c) QDs + EDC + SAM/Au. Excitation: 350 nm. Insert: emission light of amino modified water soluble CdSe/ZnS QDs.

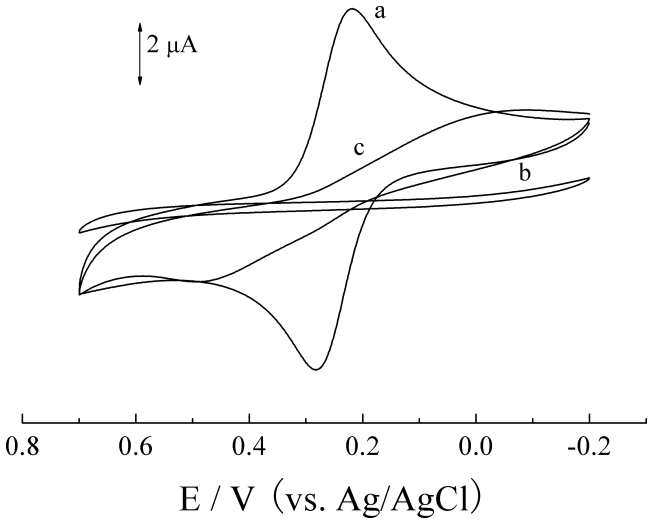


Fig. 3. CVs of bare Au (a), SAM/Au (b), SAM/QDs/Au (c) in 0.5 mmol L^{-1} $K_3Fe(CN)_6$ (1 mol L^{-1} KCl), scan rate 100 mV s^{-1}.

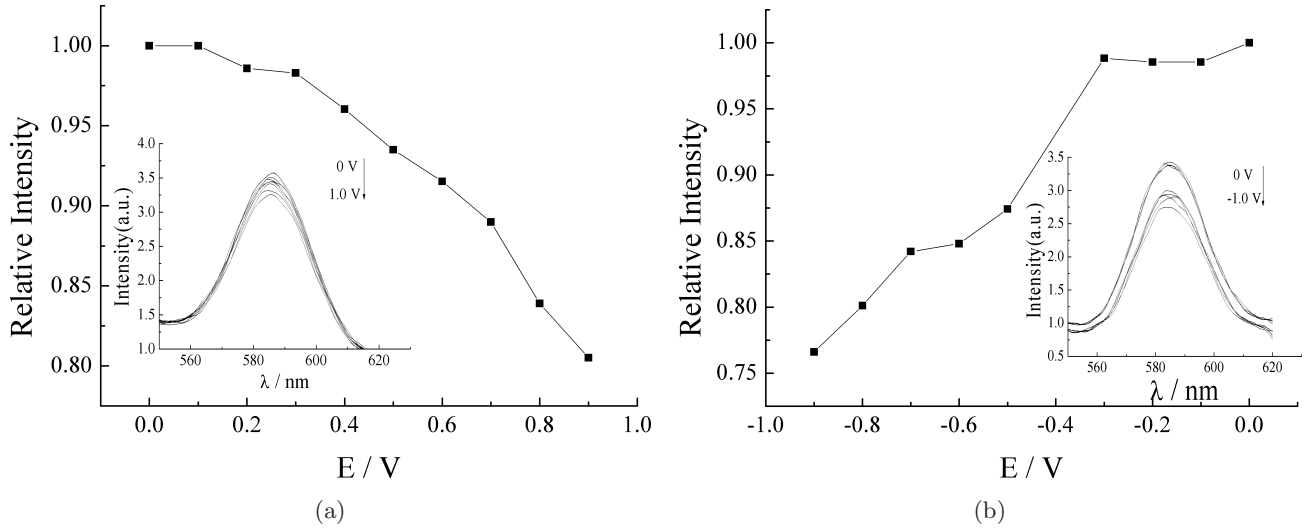


Fig. 4. Relationship between fluorescent intensity on QDs/SAM/Au and changing of potentials. (a) 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 V; (b) 0, −0.1, −0.2, −0.3, −0.4, −0.5, −0.6, −0.7, −0.8, −0.9 V. Insert: fluorescent spectra of QDs/SAM/Au.

1.0 V, or 0 V to −1.0 V, the potential was reversed, scanning from 1.0 V to 0 V, or −1.0 V to 0 V, respectively, but the fluorescent intensities could not be recovered. The probable reason was that the SAM film was destroyed gradually by increasing potentials on QD/SAM/Au, and then QDs on SAM were also gradually detached from the surface, resulting in the decease of fluorescent intensity.

4. Conclusion

QDs were directly immobilized on SAM/Au, the surface structure of which was characterized by AFM, fluorescent spectrum and electrochemistry, and the interface fluorescence of QDs was monitored on-line. The results indicated that QDs were successfully immobilized on Au and the fluorescent intensity of QDs/SAM/Au changed with different potentials charged on Au. This method achieved the immobilization and manipulation of QDs on Au and provided a new way for solid-state fluorescence detection in biosensor construction.

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