Advanced Materials Research Vols 194-196 (2011) pp 416-420 *Online: 2011-02-21*
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doi:10.4028/www.scientific.net/AMR.194-196.416

Controllable Assembly of Au Nanorods through Recognition of H-IgG and Anti-h-LgG Fab

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Keywords: Au Nanorod, Assemble

Abstract. We present a strategy to fabricate discrete Au nanorods (AuNRs) into controllable side-by-side (SS) and end-to-end (EE) assemblies through bio-recognition of h-IgG and anti-hIgG Fab. Due to anisotropic properties of AuNRs, bifunctional PEG (SH-PEG-COOH) with low concentration preferentially bound to the ends of AuNRs while high concentration and large amount resulted in binding on the side surfaces and then proteins were covalently conjugated to either surface through EDC/NHS. Thus, controllable SS and EE assembly of AuNRs were obtained through antibody-antigen recognition.

Introduction

Over the years, great achievements have been made in study of metal nanocrystals due to their interesting optical, electronic and other properties. Further applications of nanomaterials require integration of nanocrystals into ordered assemblies which exhibit collective properties through coupling among neighboring nanocrystals, and can be used to construct future nanodevices [1-2].

Among metal nanocrystals, the anisotropic Au nanorods (AuNRs) with unique properties and various applications have attracted substantial attention. Ways have been developed to assemble AuNRs, such as interactions with polymers, antibody–antigen reaction, hydrogen bonding and DNA [3-6]. However, few reports construct the controllable end to end (EE) and side by side (SS) assembly of AuNRs in one reaction system at the same time. To achieve this goal, we took advantage of anisotropic properties of AuNRs to selectively attach low and high concentration of SH-PEG-COOH respectively on the end or side surfaces of AuNRs following conjugation of h-IgG and anti-IgG Fab onto either surfaces. Finally, through the bio-recognition of h-IgG and anti-hIgG Fab, controllable EE and SS AuNRs assembly motifs were obtained which show great promise in potential applications in optoelectronics.

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Experimental

Chemicals: Chloroauric acid ($HAuCl_4 \cdot 4H_2O$), sodium borohydride ($NaBH_4$), cetyltrimethylammounium bromide (CTAB), L-ascorbic acid (AA) and silver nitrate ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Co. Ltd, while other chemicals were obtained from Aldrich. All aqueous solution were prepared using Ultrapure water (18 $M\Omega \cdot cm^{-1}$).

Synthesis of AuNRs: Au nanorods (AuNRs) were synthesized via a seed-mediated protocol that had been reported by Murphy [7]. First, Au seeds were prepared by adding $NaBH_4$ (0.6 mL, 0.01 M) to a mixture of $HAuCl_4.4H_2O$ and CTAB (7.5 mL, 0.1 M). Then, 0.2 mL of seed solution was added to the growth solution containing CTAB (45 mL, 0.1 M), $HAuCl_4.4H_2O$ (2 mL, 0.01 M), $AgNO_3$ (0.35 mL, 0.01 M) and ascorbic acid (0.32 mL, 0.1 M). Finally, AuNRs were left undisturbed for at least 3 h.

Side by side assembly of AuNRs: A mixture of bifunctional PEG (SH-PEG-COOH, 10 μM) and SH-PEG (10 μM) were added to AuNRs solution for several hours. AuNRs after one centrifugation were redispersed in 0.01 M PBS buffer solution (PH=7.2) containing 0.4 M EDC and 0.1 M sulfo-NHS After reacting for 30 min, the purified solution was split into two batches. One-half was coated with certain amount of h-IgG, the other one was modified with anti-h-IgG Fab. Finally, the two batches were mixed together and incubated at 37 ^oC for 3 h.

End to end assembly of AuNRs: SH-PEG-COOH (200 μL, 1 μM) was added to 2 mL nanorods solution for 40 min. After centrifugation, AuNRs solution was resuspended in 2 mL PBS buffer. h-IgG and anti-h-IgG Fab were respectively immobilized onto the end faces of two batches of AuNRs by EDC/NHS coupling step the same as that practiced in SS assembly. Finally both proteins modified AuNRs were mixed together.

Measurements and Instrumentation

The zeta potential measurement was performed on a Malvern Zetasizer ZEN3600 instrument. Transmission electron microscopy (TEM) images were obtained using Tecnai F30 operating at 300 kV. The UV-Vis absorption data were collected on a DU 800 UV-Vis spectrophotometer.

Results and Discussion

Characterization of CTAB-AuNRs:

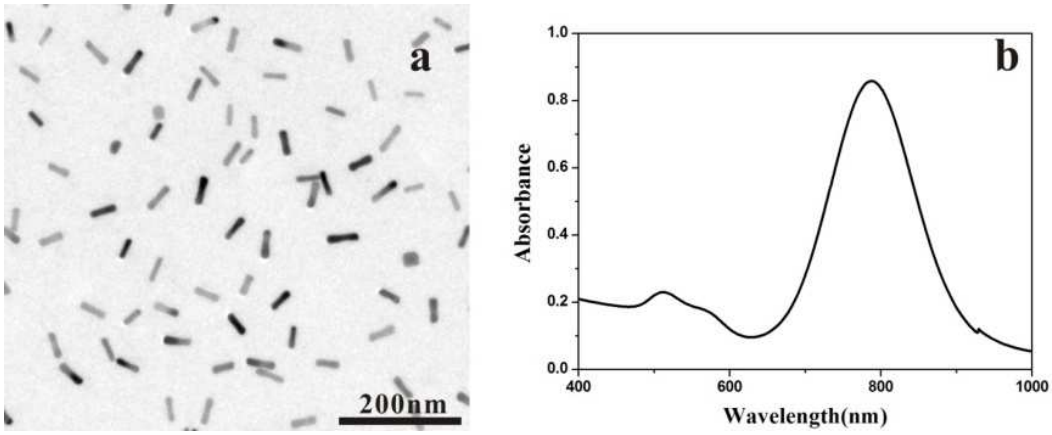

Fig. 1 TEM image (a) and UV-Vis spectra (b) of as-prepared AuNRs

The dimension of CTAB stabilized AuNRs prepared in this study was measured to be about 10 nm in diameter and about 36 nm in length with an aspect ratio of about 3.6 as shown in TEM

imaging (Fig.1a). UV-Vis spectroscopy (Fig. 1b) shows two absorption bands of AuNRs which contains transverse (512 nm) and longitudinal plasmon absorption (785 nm). Capping surfactant CTAB forms a bilayer on the surface of AuNRs which confers stability and good dispersion of AuNRs.

Side-by-Side Assembly of AuNRs. Bifunctional PEG (SH-PEG-COOH) was bound to AuNRs by Au-S bonding and provided active group (COOH) to tether antibodies through EDC/NHS chemistry. COOH-PEG-HS with higher concentration (10 μM) and large amount predominately attached onto side faces of AuNRs which have very large surface areas and eventually led to proteins conjugation mainly on these surfaces. Consequently, side-by-side assembly of AuNRs was achieved. As illustrated in zeta potential value of AuNRs in Table 1.

Table 1. Zeta potential value of AuNRs before and after coated with SH-PEG-COOH and SH-PEG on the side faces of AuNRs

AuNRs	Zeta potential value (mV)
AuNRs stabilized with CTAB	+49.5±3.2
PEG modified AuNRs	-13.7±2.8

AuNRs capped with a cationic bilayer of CTAB are positively charged with zeta potential of about +49.5±3.2 mV. However, after coated with both SH-PEG-COOH and SH-PEG, it decreased to -13.7±2.8 mV which suggested their successful attachment on the side faces of AuNRs.

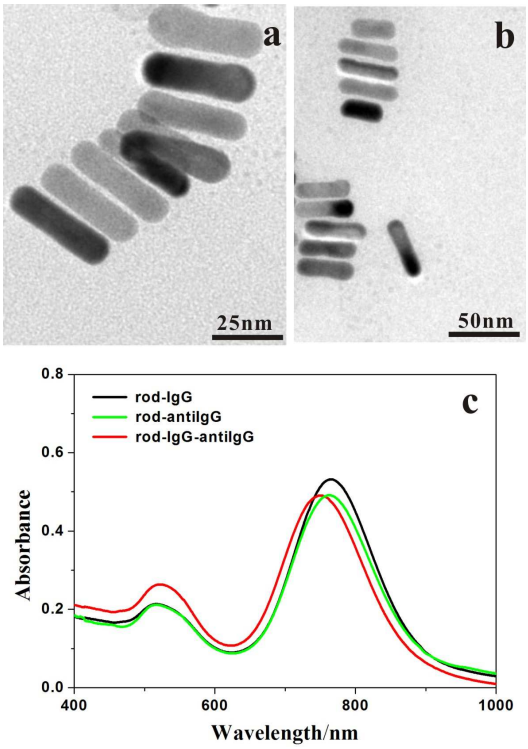

Fig. 2 TEM images of finally assembly motif (a, b) and UV-Vis spectra (c) of h-IgG-AuNRs and anti-h-IgG Fab-AuNRs before (black and green line) and after (red line) they were mixed together in 3 h

TEM images of the assembly of gold nanorods were shown in Fig.2 (a, b) indicating that h-IgG-AuNRs and anti-h-IgG Fab-AuNRs assembled in side-by-side fashion within 3 h by specific antibody–antigen binding process. Further evidence of the assembly is obtained by UV-Vis

spectroscopy (Fig.2c). The longitudinal surface plansmon resonance (SPR) peak of h-IgG and mouse anti-h-IgG Fab modified AuNRs occurred at 765 nm and 764 nm respectively, a blue-shift to 751 nm was observed after they mixed in 3 h. This obvious blue shift can be attributed to formation of side-by-side assembly of AuNRs, which agrees well with research focused on SS assembly of AuNRs by means of theoretical calculation [8].

End to End assembly of AuNRs. We utilize the similar antibody–antigen model to achieve controllable end-to-end assembly of AuNRs, except that SH-PEG-COOH located on the ends of AuNRs which led to the following conjugation of proteins on end faces. Compared with SS assembly, a relatively low concentration of SH-PEG-COOH (1 μM) was utilized to preferentially bind to the end faces of anisotropic AuNRs. Zeta potential measurement in Table 2 confirmed successful attachment of SH-PEG-COOH to the ends as the zeta potential decreased from +49.5±3.2 mV to +19.8±2.2 mV.

Table 2. Zeta potential value of AuNRs before and after coated with SH-PEG on the end faces of AuNRs

AuNRs	Zeta potential value (mV)
AuNRs stabilized with CTAB	+49.5±3.2
HS-PEG-COOH modified AuNRs on ends	+19.8±2.2

TEM images of the assembly of AuNRs were shown in Fig.3 (a, b), from which chain-like assembly fashion was observed within 3 h, demonstrating end-to-end assemble of AuNRs have been successfully achieved by specific h-IgG and anti-h-IgG Fab binding process.

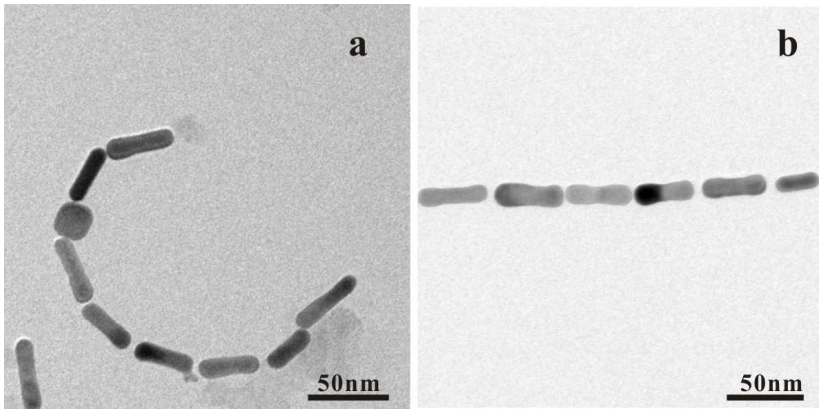

Fig. 3 TEM images (a, b) of finally assembly motif of end modified h-IgG-AuNRs and anti-h-IgG Fab-AuNRs after they were mixed together in 3 h

Conclusion

In summary, we have successfully achieved controllable end-to-end and side-by-side assembly of AuNRs by means of specific recognition of h-IgG and anti-h-IgG Fab. Both assembly motifs are demonstrated via zeta potential value, TEM imaging and UV-Vis spectroscopy which sensitively reflected the assembly process in their SPR bands. The scheme provides a feasible approach for fabricating assembly of nanostructures in a predictable fashion.

Acknowledgments

This project was supported by the Natural Science Foundation of China (No. 90923042) and the Natural Science Foundation of Fujian Province of China (No. 2010J01046), Research Fund for the Doctoral Program of Higher Education of China (No. 20100121120038), the Fundamental Research Funds for the Central Universities (No. 2010121023), and Open fund of the key laboratory of Biomedical Material of Tianjin.

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Advanced Engineering Materials
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