Oriented assembly of Au nanorods using biorecognition system

Jia-Yaw Chang,[ab] Huimeng Wu,[a] Hui Chen,[a] Yong-Chien Ling[b] and Weihong Tan*[a]

Received (in Columbia, MO, USA) 11th September 2004, Accepted 26th October 2004
First published as an Advance Article on the web 5th January 2005
DOI: 10.1039/b414059a

The design and formation of a linear assembly of gold nanorods using a biomolecular recognition system are described. Anti-mouse IgG was immobilized on the {111} end faces of gold nanorods through a thioctic acid containing a terminal carboxyl group. The biofunctionalized nanorods can be assembled with the desired length using mouse IgG for biorecognition and binding. The gold nanorods can be assembled to extended nanorod chains, which can be as long as 3 μm. These assembled nanostructures may be used as the precursors for future nanodevices.

The assembly of nanomaterial across extended length scales is a key challenge to the integration of functional nanodevices and nanomaterials. The real value of nanotechnology is to develop advanced nanodevices of superior function and properties. While most of the ordered structures published recently are based on colloidal particles,[1–6] nanorods with anisotropic properties and longitudinal mode on optical excitations have also attracted great attention. On the other hand, extensive studies have been performed on the synthesis of nanorods;[7–10] comparatively, the oriented assembly of nanorods is rare.[11,12] Bioconjugation is a promising approach towards nanoassembly as biomolecules usually have high binding specificity. Biomolecules, such as oligonucleotides, antibody–antigen, avidin–biotin and aptamer–protein, have received much attention due to their specific recognition and selective binding on a molecular level. This receptor–ligand mechanism combined with the physical properties of nanomaterials makes biomolecule–nanomaterial conjugates unique. For example, it has been found that biomaterials with several receptor sites, such as the two Fab fragments of antibodies, and the four binding domains of streptavidin or concanavalin, can be an effective template for multi-directional growth of nanomaterials for biomedical usage.

In this work, we report a feasible approach for the oriented assembly of gold nanorods in solution by a biomolecular recognition system, where antigens specifically bind to antibodies. Fig. 1 illustrates the chemistry used to achieve immunoreagent directed assembly of gold nanorods. Briefly, thioctic acid (TA) molecules self-assembled onto gold nanorods. The presence of both a disulfide and a ring structure in TA leads to a rigid conformation, which makes TA molecules preferentially bind to the ends of gold nanorods. Self-assembly of TA molecules at the end of gold nanorods can facilitate the conjugation of nanorods with ligand molecules which specifically bind with receptors. Following the self-assembly step, the carboxyl groups of adsorbed TA molecules are chemically activated by treating with sulfo-*N*-hydroxysuccinimide (sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). EDC reacts with carboxylic acid and EDC-derived ester intermediate often undergoes hydrolysis rapidly; however, in the presence of excess sulfo-NHS, a water stable sulfosuccinimidyl intermediate is formed.[14,15] Subsequently, the succinimidyl intermediate undergoes nucleophilic substitution with the primary amine group on the surface of the anti-mouse IgG. The addition of mouse IgG which has two binding sites initiates the linking of two gold nanorods. Driven by the biomolecular recognition, gold nanorods can be assembled to nanorod chains, with different orientations. The control of the concentrations of IgG and the functionalized nanorods and the assembling procedures will determine the structure of the nanorod chains.

Gold nanorods were prepared using a seed-mediated growth method in the presence of a shape-directing surfactant, cetyltrimethylammonium bromide (CTAB), as previously described.[13] 100 μL of 1 μM thioctic acid in ethanol was added into 10 mL of gold nanorods which were capped with a CTAB bilayer and suspended in an aqueous solution at room temperature for 4 h. Following centrifugation at 5000 rpm for 10 min to remove excess CTAB and TA, gold nanorods were dispersed in 1 mL of 10 mM phosphate buffer containing 10^{-4} M EDC and 10^{-4} M sulfo-NHS for 30 min. Then 1 μL of 10 μM anti-mouse IgG and gold nanorods modified with bifunctional linkers were allowed to react for 30 min at room temperature. The mixture was centrifuged at 5000 rpm for 10 min again and the supernatant was removed. Gold nanorods derivatized with anti-mouse IgG were incubated with 1 μL of 2 μM mouse IgG for 3 h. A 5 μL sample of assembled gold nanorods was spotted on a carbon-coated copper grid and left to dry at room temperature and then examined on a Hitachi H-7000 transmission electron microscope (TEM) at an operating voltage of 75 kV.

Fig. 2a shows the UV-Vis spectrum of the gold nanorods in solution. The band at 520 nm is referred to as the transverse plasmon resonance, while another one centered at 720 nm is identified as the longitudinal plasmon absorption. It is indicated that the primitive gold nanorods (Fig. 2a, inset) have an aspect ratio of 3 ± 0.5. The dimension of gold nanorods was measured to be 20 nm in diameter and 60 nm in length. In the seed mediated growth method, CTAB can form a bilayer around gold nanorod rather than a micellar form and prefers to bind with the {100} longitudinal side surface of the gold nanorod rather than the {111} end surface.[16,17] The exposed {111} end surface of gold nanorods with high active sites therefore have preferential tendency to bind with the disulfide groups of TA. As indicated by Murphy's group,[11] the fewer CTAB surfactants at the ends of nanorods

*tan@chem.ufl.edu

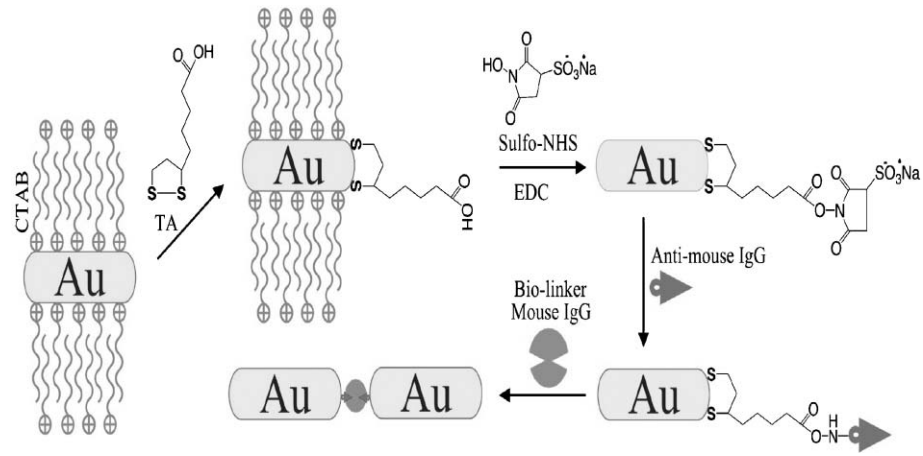

Fig. 1 Schematic illustration of antibody–antigen directed assembly of gold nanorods. Gold nanorods modified with anti-mouse IgG can be assembled in the presence of mouse IgG to different nanostructures with desired orientations by controlling the concentrations of the biomolecules.

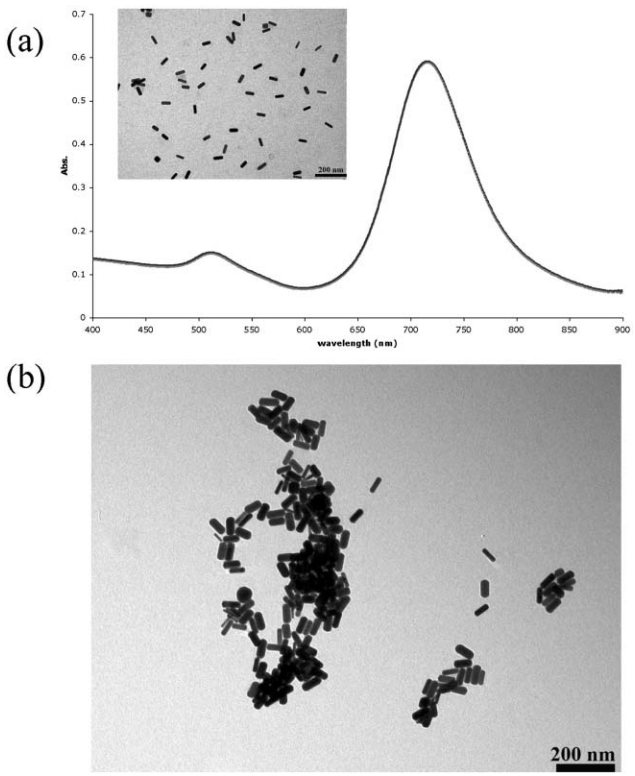

Fig. 2 (a) UV-vis spectrum of gold nanorods with an aspect ratio of 3 ± 0.5. The inset shows the TEM image of gold nanorods prepared by the seed mediated growth method. (b) TEM image of irregular aggregation of gold nanorods without CTAB on the longitude end surfaces of the nanorods.

makes it easier for thiol–gold bonding. The longitudinal side surfaces of gold nanorods, formerly capped with CTAB bilayers, facilitate the selective thiol–gold bonds at the end surfaces of the nanorods. In control experiments, nanorods with thiol groups nonspecifically absorbed at all surfaces of the gold nanorods (in the absence of CTAB on the longitudinal side surfaces of nanorods), led to irregular aggregation of nanorods as shown in the Fig. 2b.

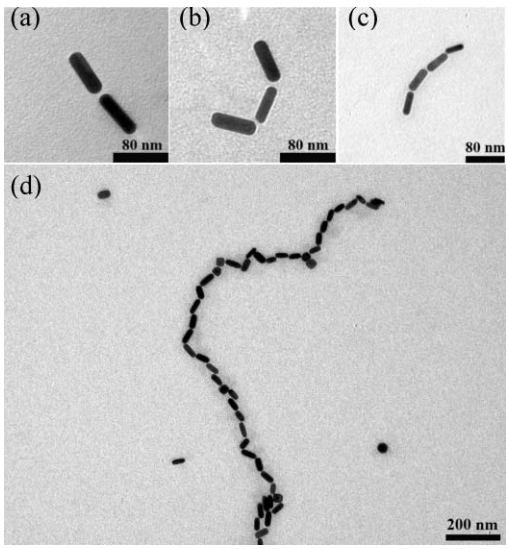

Fig. 3 TEM images of (a) di-, (b) tri-, and (c) tetra-gold nanorods assembly. The anti-mouse IgG provides an anchoring site at the end surface of nanorod and interacts with mouse IgG for assembly. (d) Gold nanorods were assembled into successive chains *via* stepwise increasing concentration of mouse IgG.

Fig. 3 shows TEM images of the assembly of gold nanorods due to the highly selective molecular recognition of immune receptor–ligand system. Fig. 3a shows the TEM image of the preliminary di-nanorod formation which attributes to specific interaction between anti-mouse IgG and mouse IgG, which can continue to form tri- and tetra-nanorod chain-like structures as shown in Fig. 3b and 3c. The distance between successive nanorods is 10–15 nm, due to the steric effect of immunoglobulin. In order to optimize the desirable assembly of gold nanorods, mouse IgG concentration was increased to raise the collision probability and therefore the biorecognition and binding. However, at a high concentration (10 μM) of mouse IgG, gold nanorods aggregated instead of regular organization. To avoid aggregation, the total 10 μL mouse IgG solution was added into the solution of gold nanorods evenly in five consecutive times with 3 h intervals

for incubation, then a desirable linear organization was assembled by individual gold nanorods as shown in Fig. 3d. It was assumed that abundant mouse IgG molecules were nonspecifically wrapped on the longitude surfaces of gold nanorods rather than specifically interacted with anti-mouse IgG molecules on end surfaces in the case of the one time addition of the total of 10 μL (10 μM) solution of mouse IgG. If the concentration of mouse IgG was gradually increased, anti-mouse IgG molecules immobilized at the end surfaces of nanorods were allowed to only bind with anti-mouse IgG, minimizing nonspecific binding with the longitude surfaces of nanorods to generate random aggregates.

The work presented herein illustrates a proof-of-concept new method for utilizing biomolecules and their capabilities of molecular recognition to guide the assembly of nanorod building blocks for bionanomaterials. The formation of a TA monolayer containing a terminal carboxyl group at the end surface of gold nanorod gives rise to conjugation with various immunoglobulins, or other biomolecules. The control of concentrations of the nanorods and the biomolecules has led to a controlled assembly of nanomaterials. Stepwise increasing concentration of anti-mouse IgG allows a long linear assembly of gold nanorods. These results provide a feasible foundation for the antibody–antigen system to be used directly for the assembly of nanostructures in a predictable fashion, which may be exploited in the application of DNA/protein assay and in the study of biomolecule based nanodevices.

The financial support by the NIH grants, NSF NIRT and Packard Foundation grant is acknowledged.

Jia-Yaw Chang,[ab] Huimeng Wu,[a] Hui Chen,[a] Yong-Chien Ling[b] and Weihong Tan*[a]

[a]Department of Chemistry and Shands Cancer Center, UF Genetics Institute, University of Florida, Gainesville, FL 32611-7200. E-mail: tan@chem.ufl.edu; Fax: 352 846 2410; Tel: 352 846 2410

[b]Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30043, R. O. C.

Notes and references

1 C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607.
2 C. M. Niemeyer, W. Burger and J. Peplies, *Angew. Chem., Int. Ed.*, 1998, **37**, 2265.
3 J. J. Storhoff and C. A. Mirkin, *Chem. Rev.*, 1999, **99**, 1849.
4 C. B. W. Garcia, Y. Zhang, S. Mahajan, F. DiSalvo and U. Wiesner, *J. Am. Chem. Soc.*, 2003, **125**, 13310.
5 A. D. Ormonde, E. C. M. Hicks, J. Castillo and R. P. Van Duyne, *Langmuir*, 2004, **20**, 6927.
6 H. Lee, A. M. Purdon, V. Chu and R. M. Westervelt, *Nano. Lett.*, 2004, **4**, 995.
7 C. J. Murphy and N. R. Jana, *Adv. Mater.*, 2002, **14**, 80.
8 B. Nikoobakht and M. A. EI-Sayed, *Chem. Mater.*, 2003, **15**, 1957.
9 T. K. Sau and C. J. Murphy, *J. Am. Chem. Soc.*, 2004, **126**, 8648.
10 K. K. Caswell, J. N. Wilson, U. H. F. Bunz and C. J. Murphy, *J. Am. Chem. Soc.*, 2003, **12**, 13914.
11 K. K. Caswell, J. N. Wilson, U. H. F. Bunz and C. J. Murphy, *J. Am. Chem. Soc.*, 2003, **125**, 13914.
12 Y. Chang and H. C. Zeng, *Cryst. Growth Des.*, 2004, **4**, 397.
13 N. R. Jana, L. Gearheart and C. J. Murphy, *J. Phys. Chem. B*, 2001, **105**, 4065.
14 Z. Grabarek and J. Gergely, *Anal. Biochem.*, 1990, **185**, 131.
15 J. V. Staros, R. W. Wright and D. M. Swingle, *Anal. Biochem.*, 1986, **156**, 220.
16 C. J. Johnson, E. Dujardin, S. A. Davis, C. J. Murphy and S. Mann, *J. Mater. Chem.*, 2002, **12**, 1765.
17 J. Gao, C. M. Bender and C. J. Murphy, *Langmuir*, 2003, **19**, 9065.

