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Self-Assembly of Small Gold Colloids with Functionalized Gold Nanorods

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ABSTRACT

We present a general strategy to stabilize gold nanorod suspensions with mono- and bifunctional polyethylene glycol (PEG) and to attach a controlled number of nanoparticles or biomolecules. Characterization by gel electrophoresis, transmission electron microscopy (TEM), and optical dark-field microscopy show the specific binding of functionalized nanorods to their target while avoiding nonspecific binding to substrates, matrices, and other particles. Such nanorods are well suited for self-assembly of nanostructures and single-molecule labeling.

The wet chemical synthesis of inorganic nanoparticles of various shapes made from metals, semiconductors, magnetic materials, or insulators has made significant progress in recent years.[1,2] Besides fluorescence quantum dots, one of the most interesting nanoparticles for optical applications are gold nanorods due to the unusually strong light interaction at the plasmon frequency, leading to strong light absorption, scattering, and an enhanced localized electromagnetic field at the nanoparticles surface.[3] The use of gold nanorods in optical applications often benefits from a much weaker plasmon damping than that in spherical particles of the same size.[4] An increasing number of applications has emerged in the past years, including the use of gold nanoparticles as optical markers in single-molecule experiments,[5,6] as molecular rulers, and as local orientation sensors.[7] Metal nanoparticles also constitute sensitive biochemical nanosensors[8−10] and enhance Raman scattering of surface-bound molecules (SERS).[11] Recently, assemblies of many particles have been used to create new devices,[12] including the arrangement of metal and semiconducting nanoparticles to engineer a tactile sensor[13] or to design electronic flexible devices.[14]

The use of nanoparticles in most such applications relies on chemical modifications of the nanoparticle surface in order to link the nanoparticle to other particles, biological molecules, or substrates. Because biological ligand−receptor recognition is used in nature to assemble highly complex nanostructures, stable and reliable biofunctionalization of inorganic nanoparticles is a desirable goal both for biological labeling applications and as a powerful tool for nonbiological nanoparticle assemblies. Even though the biochemical functionalization of gold spheres has been extensively investigated,[15] functionalization of gold nanorods prepared in highly concentrated surfactant solutions remains a challenge. The goal is to attach a controlled number of target molecules, retaining their biological function while avoiding nonspecific interaction with surfaces and other particles in solution (aggregation). Usually, first, a stabilizing and protecting layer is attached to the nanoparticles, containing some chemical anchor points for further modification. Covalent chemical attachment offers high stability in different solvents and ionic environments. The reports on functionalized gold nanorods published to date rely either on noncovalent physisorption of the biomolecules on the surface of the rods,[16] the electrostatic anchoring of an additional polymeric layer,[17] or the use of short bifunctional cross-linkers attached to the gold surface, leading to low yield[18] or surface coverage.[19−21] Generally, these methods suffer from either difficult reproducibility, lack of anchor points for further modification, or a lack of characterization of the specificity of the functionalization toward binding of target molecules and toward avoiding nonspecific binding to substrates or nontarget molecules. The control of the surface density of anchor points is desirable in order to use the particles in applications requiring high coverage (for instance, in nanosensors) and low coverage (for instance in single-molecule biolabeling).

Here, we report on a general functionalization strategy involving a mixture of long-chain mono- and bifunctional polyethylene glycol (PEG), where the monofunctional PEG stabilizes the gold nanorods and the bifunctional PEG provides anchor points for further modification. The anchor points are used to attach either small gold nanoparticles or the biomolecule biotin, which is recognized specifically by small gold particles functionalized with the biotin-binding protein streptavidin (see Figure 1). The resulting self-assembled nanostructures are characterized using gel electrophoresis and transmission electron microscopy (TEM). The particles specifically bind to substrates functionalized

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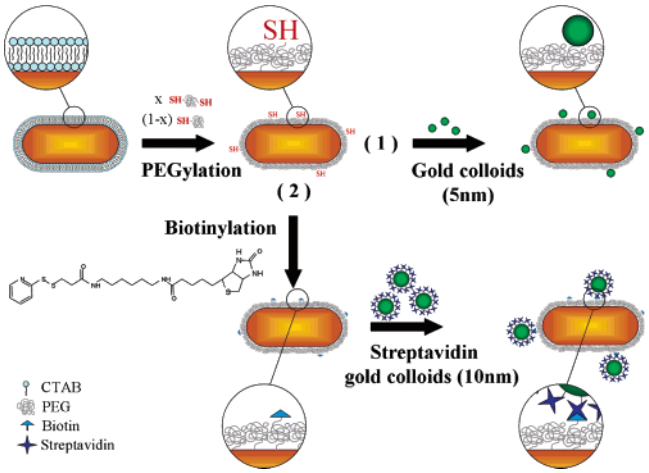

Figure 1. Schematic representation of the gold nanorod functionalization strategy: Mixed monofunctional and bifunctional PEG chains are first grafted on the surface of the rods (PEGylation). The free thiol groups are then reacted with either (1) small gold colloids (5 nm), leading to rods decorated with small colloids, or (2) a biotin derivative. Gold colloids (10 nm) coated with streptavidin can be then immobilized on the surface of the rods.

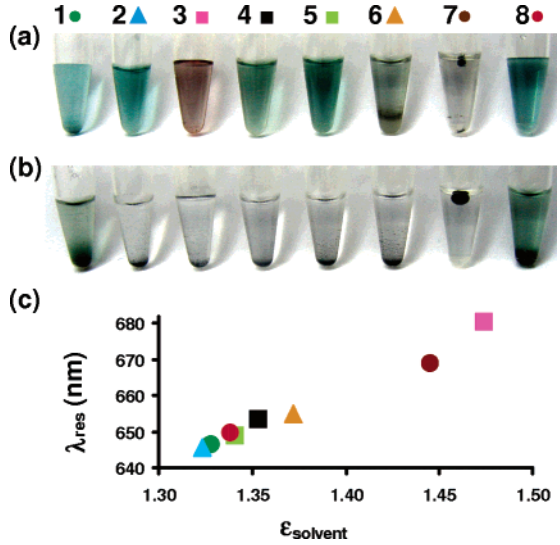

Figure 2. Images of gold nanorods in different solvents (1 water, 2 methanol, 3 DMSO, 4 acetone, 5 ethanol, 6 isopropanol, 7 chloroform, 8 NaCl (1 M)). PEGylated rods remain stable in suspension (a), whereas non-PEGylated rods precipitate (b). In the case of chloroform (7), the non-PEGylated rods are confined to the water phase. The PEGylated rods transfer partially to the organic phase. The linear relationship between the plasmon resonant wavelength λ_{res} of the PEGylated rods, and the dielectric permittivity of the solvent shown in (c) indicates an absence of aggregation.

with target molecules and show a low affinity to stick to blocked areas.

The gold nanorods are prepared according the seed growth method, as described by Nikoobakht et al.[22] and Jana et al.[23] Briefly, seeds are prepared by reducing 10 mL of an aqueous solution containing 0.25 mM gold tetrachloride ($HAuCl_4$) in 0.1 M hexadecyl-trimethyl-ammonium-bromide (CTAB, Sigma), adding 0.6 mL of 0.01 M sodium borohydride ($NaBH_4$). This seed solution (15 μL) is added to 10 mL of a growth solution consisting of 0.5 mM $HAuCl_4$ and 0.08 mM silver nitrate in 0.1 M CTAB mixed with 70 μL of 0.0788 M ascorbic acid. A strong color change indicates the formation of the gold nanorods after about 20 min. The rods are stored in the reaction mixture at room temperature for up to six months. Before further treatment, excess of surfactant and unreacted products from the growth solution are removed by two centrifugation steps.[24]

To stabilize and functionalize the gold nanorods, we first coat them with a mixture of monofunctional and bifunctional PEG (methoxy-PEG-thiol, mPEG-SH, MW 5682, and PEG-*bis*-thiol, SH-PEG-SH, MW 3878, respectively). Fresh mPEG-SH and SH-PEG-SH stock solutions (2 mM) are prepared just before use from freshly degassed water made by sonicating deionized water for 1 h. Both solutions are mixed in appropriate amounts to prepare solutions of the desired ratio. The fraction of bifunctional PEG x can be varied from 0 to 0.2. Higher fractions result in aggregation and precipitation of the gold nanorods. The mixed solution (100 μL) [$(1 - x)$ mPEG-SH/(x) SH-PEG-SH] is added to 900 μL of cleaned gold nanorod solution. After vortexing, the reaction mix is incubated and shaken at room temperature for 1−2 h. Excess of unreacted products is removed by one or two centrifugation steps.

The reaction of the gold nanorods with mono- and bifunctional PEG (PEGylation) results in a significant stabilization against aggregation in different solvents and in high-ionic-strength environments (Figure 2a). In contrast, non-PEGylated gold nanorods precipitate and the suspensions become colorless (Figure 2b). In addition, the resonance wavelength of the PEGylated gold nanorods shifts linearly with the dielectric permittivity of the solvent, as expected for nonaggregating nanorods (Figure 2c). Interestingly, the PEGylated nanorods also transfer partially into the organic phase in a chloroform−water system.

The stability in buffers allows using agarose gel electrophoresis for characterization and separation. Electrophoresis has been used successfully to separate small spherical gold nanocrystals conjugated with polymers according to the number of attached molecules.[25,26] Here, we observe that the mobility of PEGylated gold nanorods depends on the fraction of bifunctional PEG (Figure 3a), indicating successful attachment of both PEG species. The second functional group of the bifunctional PEG leads to a slower migration, likely due to a weak attractive interaction with the gel matrix. This hints at the availability of this group as an anchor point for further modification and the absence of "binding-loops", i.e., attachment of both SH groups of SH-PEG-SH to the same gold nanorod. To further test the availability of anchor points, we incubate the PEGylated gold nanorods with 5 nm gold spheres overnight to attach them to the anchor points (route 1 in the scheme in Figure 1) and subsequently separate nanorods and unreacted spheres by a gentle centrifugation step. The gel mobility of these gold nanorods decorated with gold colloids is shown in Figure 3b and strongly depends on the fraction of bifunctional PEG. The mobility of nanorods decorated with small nanospheres is slightly retarded compared with the PEGylated rods with the same fraction of bifunctional groups (Figure 3c), especially for higher fractions. This result suggests that indeed different numbers of

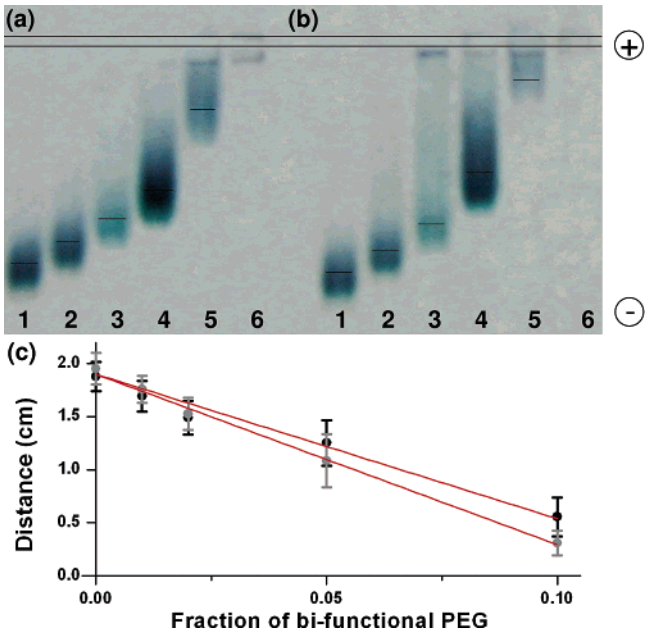

Figure 3. Electrophoretic mobility of functionalized gold nanorods in a 0.4% agarose gel. The lanes 1−6 correspond to different fractions of bifunctional PEG (0, 0.01, 0.02, 0.05, 0.1, and 0.2, respectively). (a) PEGylated gold nanorods. (b) Gold nanorods decorated with gold colloids. (c) Gel migration distance after 2 h 30 min at 130 V/15 cm as a function of the fraction of bifunctional PEG in the case of PEGylated rods (solid black circles) and of rods decorated with gold colloids (solid gray circles).

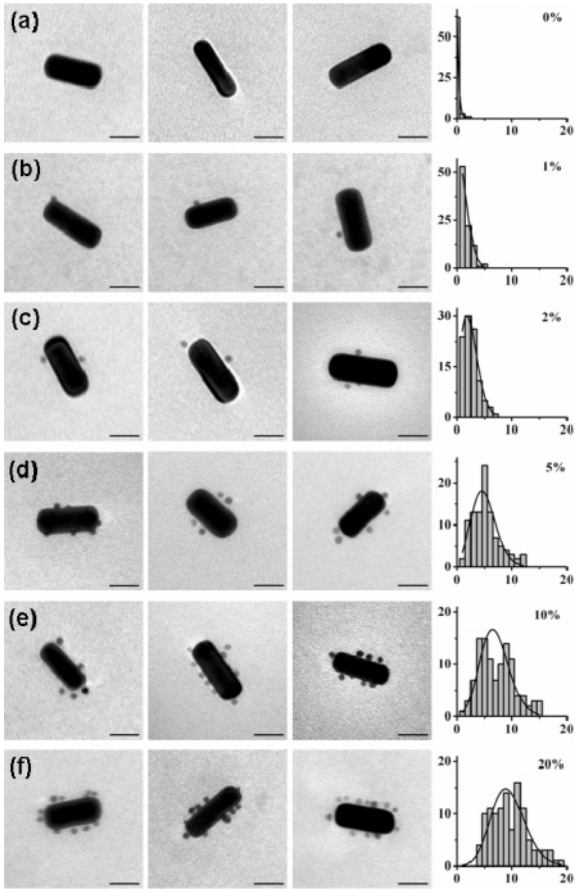

Figure 4. Self-assembly of PEGylated gold nanorods and gold colloids (5 nm) visualized by transmission electron microscopy. The lanes (a−f) present different fractions of bifunctional PEG corresponding to 0, 0.01, 0,02, 0.05, 0.1, and 0.2, respectively. The first three images from the left show individual rods (scale bar 20 nm). The fourth image presents an ensemble of rods (scale bar 60 nm). The histograms on the right show the number of gold colloids attached per nanorod as counted on approximately 100 images.

particles are attached to the gold nanorods depending on the number of anchor points present on the surface of the rods. A higher fraction of bifunctional PEG, resulting in a higher surface density of reactive groups or anchor points, leads to a greater number of small colloids attached to the rods. The attachment of small gold colloids to the surface of the gold nanorods results in a change in size of the combined cluster and reduces its mobility.

The retardation effect in gels is, however, too small to be considered a definite proof of the aggregate structure. To investigate more quantitatively the relationship between the fraction of bifunctional PEG and the number of particles attached to one single rod, we characterized our samples by transmission electron microscopy (TEM). After incubation of the gold colloids and the PEGylated gold nanorods, excess of small spherical particles is removed by adding 200 μL of water and centrifuging the solution once (500 g, 8 min). The pellet is resuspended in 50 μL of water and 5 μL of the sample is deposed on the TEM grid (Formvar/Carbon, 200 mesh) previously cleaned and charged by oxygen plasma etching (20 W, 30 s) and investigated in a Phillips CM12 microscope at 120 kV.

Representative images are shown in Figure 4 for different fractions of bifunctional PEG (a−f). About 100 individual, well-separated gold nanorods are investigated at 125k magnification for each fraction (shown in Supporting Information, Figures S1−S6) and the number of particles attached counted (Figure 4, rightmost column). The drying process during TEM sample preparation moves almost all small colloids to the sides of the gold rods (shown by a tilting series, Supporting Information, Figure S7) allowing a straightforward interpretation of these numbers as the true number of attached colloids. No gold colloids are found on the surface of the gold nanorods coated with monofunctional PEG (Figure 4a), proving the absence of unspecific attachment between these nanoparticles. As expected from gel electrophoresis, the mean number of gold colloids attached per gold nanorod increases with the fraction of bifunctional PEG x. As x is changed from 0.01 to 0.2, the average number of small colloids attached varies from 1 to 10 (Figure 3b−f). The number of colloids attached per nanorod N_q as a function of the fraction of bifunctional PEG follows nicely the Langmuir absorption model:

$$N_q = N_{\text{sat}} \frac{x \cdot K \cdot [q]}{1 + x \cdot K \cdot [q]} \qquad (1)$$

N_{sat} is the saturation number of attached colloids, K the equilibrium constant of the binding/unbinding reaction of gold colloids with the free thiol groups on the surface of the rods, and $[q]$ is the gold colloid concentration. A fit of eq 1

with the experimental data gives $N_{sat} = 14 \pm 0.5$, and $K = (2.5 \pm 0.2)\ 10^8\ mol^{-1}$ (see Supporting Information, Figure S8). The saturation number may seem surprisingly low and is probably an artifact due to steric hindrance of the 5 nm gold colloids or multiple attachments of one colloid to several anchor groups. The equilibrium constant corresponds to a free energy change of −47.8 kJ/mol, which compares well to the free energy change associated with the adsorption of thiolated DNA on the gold surface determined as −35.1 kJ/mol.[27] The total number of attached PEG molecules per nanorod can be deduced from the initial slope and gives a value of approximately 100, assuming the binding rate of bifunctional and monofunctional PEG to the rods is the same and all bifunctional PEGs are labeled by a small colloid. A rough estimation using the radius of gyration for PEG (~4 nm) gives comparable values. Another interesting observation is a small spectral shift of the plasmon resonance wavelength λ_{res} of the gold nanorods after attachment of small colloids. λ_{res} increases from 642 to 655 nm as the fraction of bifunctional PEG is increased from 0 to 20% (Supporting Information, Figure S9).

The experimental facts shown in Figures 3 and 4 (and the Supporting Information) conclusively demonstrate the ability to stabilize gold nanorods and to provide a controlled number of reactive groups (anchor points) on the surface available for further modification. The application of gold nanorods as markers in biological systems or for the biochemically assisted self-assembly of nanostructures requires the ability to attach biomolecules to those anchor points. To demonstrate this possibility, we chose the receptor−ligand system biotin−streptavidin because it is well characterized and frequently used as template. The gold nanorods are biotinylated using a thiol-reactive biotin derivative (EZ-Link biotin−HPDP, Pierce). Biotin−HPDP stock solutions (4 mM) are prepared in DMF, aliquoted, and stored frozen. Stock solution (10 μL) is added to 100 μL of PEGylated gold nanorods with 5% of bifunctional PEG. After vortexing, the reaction mix is incubated and shaken overnight, the unreacted biotin removed by two centrifugation steps, and finally resuspended in 100 μL of phosphate-buffered saline (PBS). These biotinylated gold nanorods in PBS are incubated with 100 μL of streptavidin-coated gold colloids (10 nm, 30 nM) for 3 h at room temperature and unbound particles removed by a gentle centrifugation step (500 g, 2 min). The pellet is resuspended in 50 μL of PBS and a drop of 5 μL is deposed on TEM grids as described previously. The resulting TEM images (Figure 5a and b) show the successful binding of streptavidin-coated gold colloids to the biotinylated nanorods, demonstrating the preservation of the high affinity between streptavidin and biotin and the ability to use biotinylated gold nanorods as versatile building blocks for designing nanostructures.

To use these biotinylated gold nanorods as markers in single-molecule experiments, the absence of nonspecific interactions with surfaces is of equal importance to the successful functionalization shown above. We checked this specific reactivity by immobilization of the biotinylated gold nanorods on a micropatterned glass surface prepared as described by Cuvelier et al.[28] The surface is partially streptavidin-coated, and the remaining parts are blocked with PEG. The substrates are incubated with the biotinylated gold nanorods for 45 min and rinsed with PBS (Figure 5c, inset). Optical characterization of the samples is carried out in a dark-field microscope[4] (Figure 5c, lower part). Biotinylated gold nanorods preferentially bind on the part of the substrate coated with streptavidin and avoid contact with the passivated part. The specificity, as evaluated by counting the number of rods found on both regions of the substrate, is about 5:1, which is a fairly high number compared to similar systems.[7]

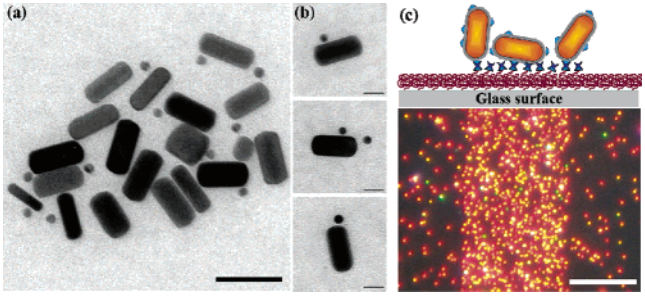

Figure 5. Functionalization of PEGylated gold nanorods with a biotin derivative. (a) Self-assembly of biotinylated gold nanorods and streptavidin colloids with 10 nm diameter characterized by TEM (scale bar is 60 nm). (b) TEM pictures of individual rods at higher magnification (scale bar is 20 nm). (c) Immobilization of biotinylated gold nanorods on a patterned streptavidin substrate (schematically represented on the upper figure) and visualized by dark-field microscopy (lower figure: scale bar is 10 μm). Approximately 150 rods per 100 μm^2 are found on the streptavidin part compared to approximately 30 rods per 100 μm^2 on the passivated part of the substrate.

Taken together, these results conclusively show that we are able to replace the CTAB surfactant layer of freshly prepared gold nanorods by coating the surface with thiolated PEG while avoiding aggregation of the rods. These PEGylated gold nanorods show a surprising high stability against aggregation under high ionic strength environments, a large pH range, and in different solvents. The use of bifunctional PEG allows us to provide anchor points for further modifications, demonstrated by direct immobilization of gold colloids and the reaction with biomolecules. The surface density of anchor points can be controlled by the fraction of bifunctional PEG in the reaction mix. The resulting versatile nanorods can be used to design self-assembled nanostructures to carry out single-molecule experiments.

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Supporting Information Available: Detailed protocols of the experimental procedures. Figures showing the number of small colloids adsorbed onto gold nanorods as TEM images of individual particles (Figures S1−S6), a tilting series of one such assembly (Figure S8), the average number

of attached colloids as a function of the ratio of bi- to monofunctional PEG (Figure S8), and the plasmon shift of the gold nanorods upon attachment of small colloids (Figure S9). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Perez-Juste, J.; Pastoriza-Santos, I.; Liz-Marzan, L. M.; Mulvaney, P. *Coord. Chem. Rev.* **2005**, *249*, 1870.
(2) Yin, Y.; Alivisatos, A. P. *Nature* **2005**, *437*, 664.
(3) Eustis, S.; El-Sayed, M. A. *Chem. Soc. Rev.* **2006**, *35*, 209.
(4) Sönnichsen, C.; Franzl, T.; Wilk, T.; von Plessen, G.; Feldmann, J.; Wilson, O.; Mulvaney, P. *Phys. Rev. Lett.* **2002**, *88*, 077402.
(5) Schultz, S.; Smith, D. R.; Mock, J. J.; Schultz, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 996.
(6) Nie, S.; Emory, S. R. *Science* **1997**, *275*, 1102.
(7) Sönnichsen, C.; Alivisatos, A. P. *Nano Lett.* **2005**, *5*, 301.
(8) Raschke, G.; Kowarik, S.; Franzl, T.; Sönnichsen, C.; Klar, T. A.; Feldmann, J.; Nichtl, A.; Kurzinger, K. *Nano Lett.* **2003**, *3*, 935.
(9) McFarland, A. D.; Van Duyne, R. P. *Nano Lett.* **2003**, *3*, 1057.
(10) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
(11) Campion, A.; Kambhampati, P. *Chem. Soc. Rev.* **1998**, *27*, 241.
(12) Shenhar, R.; Rotello, V. M. *Acc. Chem. Res.* **2003**, *36*, 549.
(13) Maheshwari, V.; Saraf, R. F. *Science* **2006**, *312*, 1501.
(14) Service, R. F. *Science* **2006**, *312*, 1593.
(15) Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: New York, 1996.
(16) Spetzler, D.; York, J.; Daniel, D.; Fromme, R.; Lowry, D.; Frasch, W. *Biochemistry* **2006**, *45*, 3117.
(17) Gole, A.; Murphy, C. J. *Langmuir* **2005**, *21*, 10756.
(18) Pan, B. F.; Ao, L. M.; Gao, F.; Tian, H. Y.; He, R.; Cui, D. X. *Nanotechnology* **2005**, *16*, 1776.
(19) Chang, J.-Y.; Wu, H.; Chen, H.; Ling, Y.-C.; Tan, W. *Chem. Commun.* **2005**, *8*, 1092.
(20) Caswell, K. K.; Wilson, J. N.; Bunz, U. H. F.; Murphy, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 13914.
(21) Salem, A. K.; Chen, M.; Hayden, J.; Leong, K. W. Searson, P. C. *Nano Lett.* **2004**, *4*, 1163.
(22) Nikoobakht, B.; El-Sayed, M. A. *Chem. Mater.* **2003**, *15*, 1957.
(23) Jana, N. R.; Gearheart, L.; Murphy, C. J. *Adv. Mater.* **2001**, *13*, 1389.
(24) A centrifugation step consists of spinning 1 mL of the suspension at 6000 g for 20 min to spin down the gold rods, removing the supernatant, and resuspending the pellet in clean water, buffer, or reactant mixture.
(25) Sperling, R. A.; Pellegrino, T.; Li, J. K.; Chang, W. H.; Parak, W. J. *Adv. Funct. Mater.* **2006**, *16*, 943.
(26) Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Alivisatos, A. P. *Nano Lett.* **2001**, *1*, 32.
(27) Yang, M.; Yau, H. C. M.; Chan, H. L. *Langmuir* **1998**, *14*, 6121.
(28) Cuvelier, D.; Rossier, O.; Bassereau, P.; Nassoy, P. *Eur. Biophys. J.* **2003**, *32*, 342.

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