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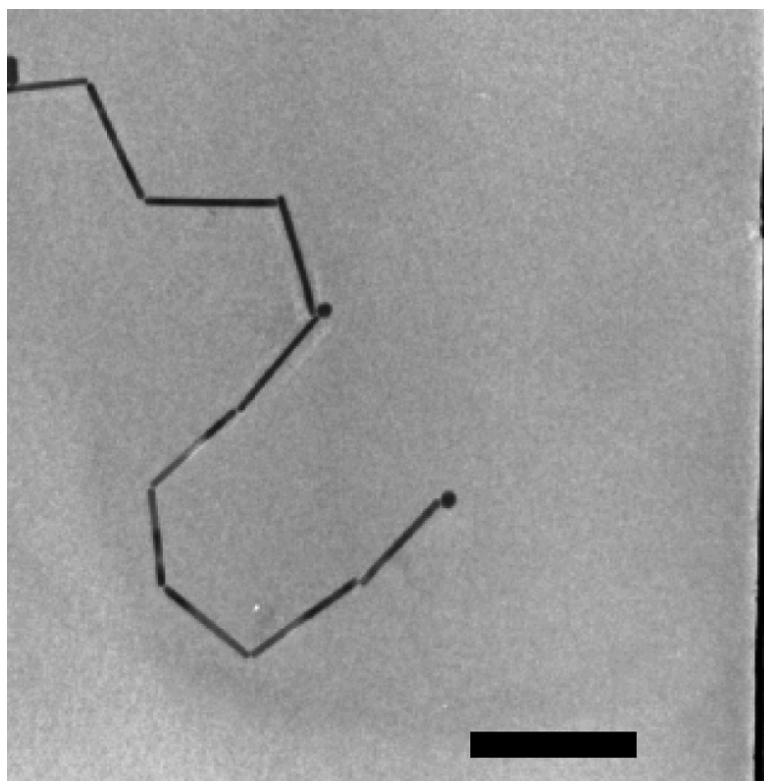
Preferential End-to-End Assembly of Gold Nanorods by Biotin–Streptavidin Connectors

K. K. Caswell, James N. Wilson, Uwe H. F. Bunz, and Catherine J. Murphy

500 nm

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Preferential End-to-End Assembly of Gold Nanorods by Biotin−Streptavidin Connectors

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The synthesis of inorganic materials with dimensions on the nanometer scale has greatly improved in the last 10 years.[1−7] Future applications in nanoscale electronic devices,[2] magnetic data storage systems,[3] optical devices,[8] and biological sensing[9−11] require monodisperse semiconductor or metallic materials of controlled size and shape. The next major challenge in the field is to assemble these nanoscale objects into functional devices. Self-assembly procedures governed by surface chemistry are already proving to be useful in this respect.[3] Here we report that gold nanorods, surface-functionalized with biotin, can be assembled preferentially in an end-to-end fashion upon the addition of a streptavidin linker (Figure 1). These results provide evidence that the chemical reactivity of nanomaterials is shape-dependent and may be useful in construction of future nanoscale assemblies.

Biotin, a small molecule, is well-known to bind tightly to the protein streptavidin, with four biotins binding per protein.[12] The synthesis of the biotin disulfide (Figure 1) was analogous to that reported by Connelly et al.[13] (Supporting Information). We also employed a commercially available biotin disulfide, EZ-Link Biotin HPDP (Pierce; Figure 1). Disulfides are known to bind gold surfaces to produce self-assembled monolayers very similar to those obtained with thiols, with some improvement in compositional control for mixed thiol species.[14−16] Other groups[13,17,18] have used biotin−streptavidin (or the related protein avidin) linkers to assemble inorganic nanospheres into three-dimensional (3D) structures, and Mann's group has shown that gold nanorods coated with DNA can be similarly assembled into 3D aggregates by the addition of a complementary linker DNA.[19] Thus, the spacing of the nanoparticles is governed by the size of the linker molecule.

We synthesized gold nanorods of aspect ratio 18 according to our seed-mediated growth approach in water in the presence of a shape-directing surfactant, cetyltrimethylammonium bromide (CTAB).[7,20,21] The nanorods thus prepared are covered with at least a bilayer of CTAB[22] and are stable and soluble in water. High-resolution crystallography on individual nanorods has shown that our nanorods are pentatetrahedral twins, with the {111} faces of gold at the ends, and {100} faces along the length of the rods.[23] We currently postulate that the CTAB preferentially binds to the {100} faces, along the length of the rods, compared to the end {111} Au faces, due to the size of the CTAB headgroup,[22,23] although the hydrocarbon tail of CTAB also plays a role in the growth of gold nanorods via a postulated "zipping" mechanism.[24]

Binding of the homemade biotin disulfide, in ethanol, to the gold nanorods was accomplished by adding 2.5 mL of 3.1×10^{-6} M biotin disulfide to 50 mL of the mixed solution of rods, stirring, sonicating, and allowing to set overnight. For the EZ-Link biotin HPDP, a mixed solution of rods (rehydrated to 14 mL), which had been centrifuged once to remove the majority of spheres, was
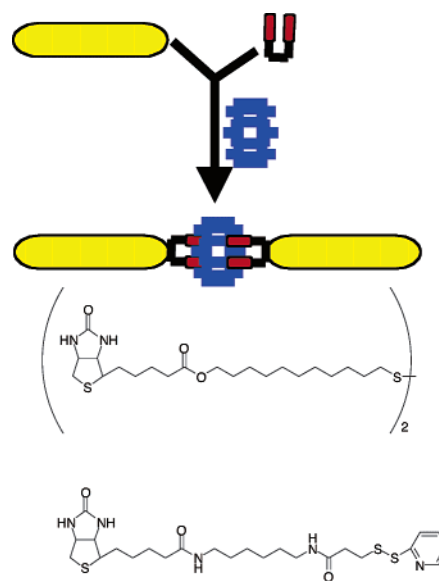

Figure 1. Scheme showing the assembly of gold nanorods (golden ovals) by surface functionalization with the biotin disulfide (red), and subsequent addition of streptavidin (blue), to produce aggregates of nanorods. The chemical structures of the two biotin disulfides are also shown, homemade (top) and commercial EZ-Link Biotin-HPDP (bottom).

functionalized by adding 1 mL of a 1×10^{-4} M stock solution of EZ-Link Biotin-HPDP. This mixture was stirred, shaken, sonicated, and allowed to set overnight. Nanoparticles with shapes other than rods and excess unbound biotin disulfide were purified away by a second round of centrifugation. This batch was split, and one-half was coated with a saturating amount of streptavidin. Excess, unbound streptavidin was centrifuged away. Aliquots of 2 μL, at a time, of the streptavidin-coated rods were titrated into the biotin-coated rods. This was mixed and repeated several times. A final centrifugation was then employed to separate the linked rods from unlinked.

The addition of streptavidin to the biotin-coated gold nanorods produced an unexpectedly high proportion of end-to-end-linked gold nanorods (Figure 2), which were separated by 4−5 nm spacings—the size of a streptavidin molecule ($\sim$4.5 × 4.5 × 5.8 nm³)—and at angles approximately what one would expect for the four biotin binding sites on the protein projected into two dimensions.[25] Control experiments in which no streptavidin, or no biotin, were used gave statistically distinct results (Figure 3). Both biotin derivatives worked, but the commercial linker was more water-soluble and easier to work with.

An average of multiple batches revealed that end-to-end linkages could be realized $\sim$30% of the time while other binding events comprised $\sim$34%. We defined binding as any rod within 5 nm of another; anything but clear end-to-end binding was called

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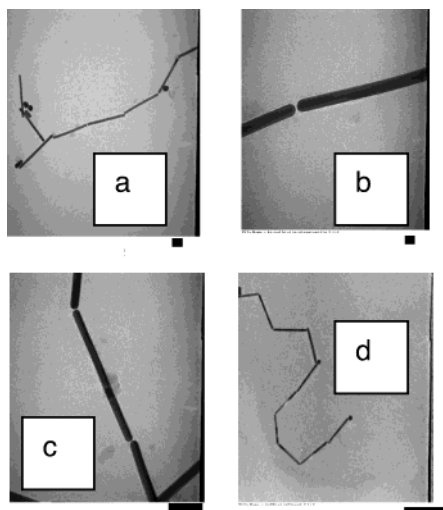
Figure 2. Transmission electron micrographs of gold nanorods, surface-derivatized with either biotin disulfide, after addition of streptavidin. Scale bars = 100 nm (a), 20 nm (b), 100 nm (c), and 500 nm (d).

Binding Events
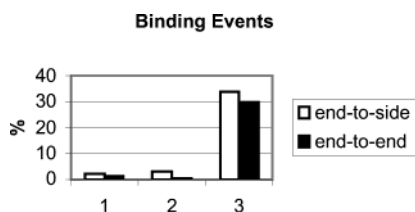
Figure 3. Summary of numbers of end-to-end and end-to-side linkages observed in transmission electron micrographs for experiments with (1) rods only, (2) rods, and streptavidin only, (3) biotinylated rods (commercial linker) and streptavidin. In each case 500 rods were counted and the interparticle distances measured.

"end-to-side." Crudely, one would expect on the basis of relative surface areas that side-by-side binding would predominate over end-to-end binding by 20-fold, should biotinylation and subsequent streptavidin linking be random. There were rather large "islands" on the TEM grids in control experiments (**1** and **2** from Figure 3) where distinctly liquid crystalline side-by-side behavior was observed, as documented previously, but not in **3**.[26] Control experiments with bovine serum albumin, which does not bind to biotin, produced results strikingly similar to **2** of Figure 3.

The mechanism behind the higher-than-expected proportion of end-to-end linkages of gold nanorods is still under investigation. There are two current hypotheses we are exploring: (i) the biotin disulfide was unsuccessful at displacing the CTAB bound to the length of the gold nanorods, and thus the biotin preferentially bound to the {111} ends of the rods, resulting in preferential end-to-end linkages upon the addition of streptavidin; the bilayer structure of CTAB on the nanorod surface may make the surfactant less easy to displace than one might expect;[22,24] (ii) the biotin did indeed bind all over the rods, but streptavidin is sterically constrained to bind to the ends of the rods. This second hypothesis is somewhat

contradictory to the observation of the closest-packed face of gold being at the ends of the nanorods[23] but is consistent with Fitzmaurice's notion of full biotin coverage blocking streptavidin sites.[13] Other workers have shown that the disordered sites at metallic interfaces yield more disordered molecular monolayers at those sites;[27] thus, it is possible that the biotin disulfide localizes preferentially to such sites at the pentahedral twin boundaries on the ends of the rods. Elemental analyses of biotinylated gold nanorods, of which unfortunately only small quantities are available, are consistent with a monolayer or less of the biotin disulfide on the surface, based on %Au and %S in the samples, and thus cannot distinguish between the hypotheses at present. Nonetheless, our current results provide evidence that chemical linkages can organize nanorods in a nonrandom fashion, which may be exploited for the assembly of higher-order arrays of nanomaterials.

Supporting Information Available: Details of the synthesis and characterization of the homemade biotin disulfide (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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