Self-Assembled Nanoparticles

One-Dimensional Assembly of Lipophilic Inorganic Nanoparticles Templated by Peptide-Based Nanofibers with Binding Functionalities**

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Spatially controlled assemblies of inorganic nanoparticles are of fundamental interest because of their unique electronic and optical properties.[1–3] To prepare one-dimensional systems, biological substrates[4] such as peptides,[5] viruses,[6]

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fungi,[7] and DNA[8] have been used recently as scaffolds for the assembly or growth of nanoparticles. Previous work has been limited to water-soluble nanoparticles because the templates used require water as a solvent. Thus, a large variety of well-characterized lipophilic nanocrystals that have been synthesized with excellent size and shape control have been excluded from these strategies. These include nanocrystals of II–VI[9] or III–V semiconductors,[10] metals,[11] and oxides.[12] We report herein a strategy for the creation of one-dimensional assemblies of lipophilic inorganic nanoparticles in apolar solvents by using peptide-based nanofibers with surface-binding motifs.

Nanofiber structures based on peptide β-sheet motifs have been widely studied[4,13,14] in analogy to physiological amyloid fibrils, the insoluble protein deposits related to Alzheimer's disease and other illnesses.[15] In our laboratory, we have been working on peptide amphiphiles that self-assemble in water to form supramolecular nanofibers containing β sheets for biomedical applications.[5a,16] To assemble nanofibers in apolar organic solvents capable of binding lipophilic nanoparticles, we started the study described herein with a tripeptide amphiphilic molecule of the type developed by Yamada and co-workers,[13] such as molecule **1**. It was previously demonstrated[13] that these molecules self-assemble into nanofibers in both water and apolar solvents such as benzene and carbon tetrachloride (CCl_4). For nanocrystals to bind specifically to the surfaces of the nanofibers, we designed molecule **2**, which contains a thymine moiety, and used it along with **1** in our previously described strategy of mixing two molecules to co-assemble nanofibers bearing two biological signals.[17] These surface-modified nanofibers of **1** and **2** formed in CCl_4 and were then mixed with lipophilic gold nanoparticles decorated with diaminopyridine (DAP) (Figure 1). The thymine–DAP base pairing was chosen in this case because of its chemical simplicity and high binding efficiency in aprotic environments.[18]

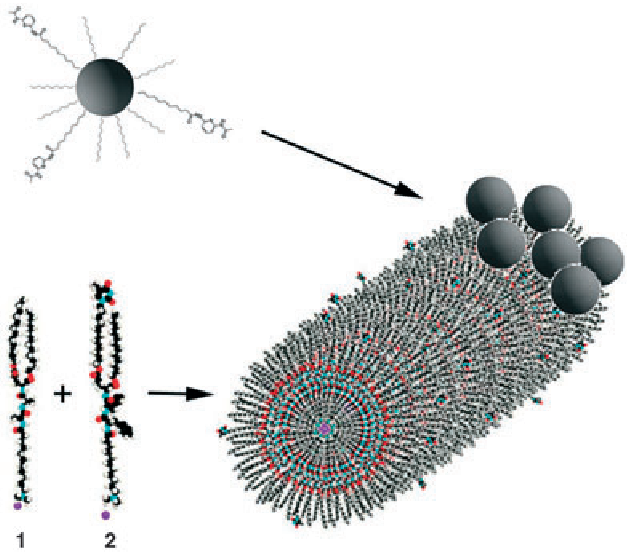
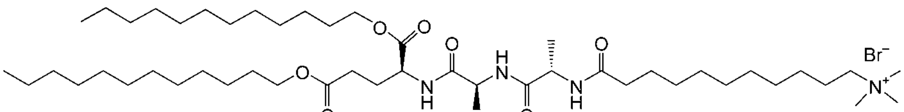


Figure 1. Schematic representation of the co-assembled nanofibers and binding of surface-modified nanoparticles. Molecules of **2** with a thymine moiety co-assemble into nanofiber matrices with a large excess of molecule **1**, providing binding sites on the surfaces of the fibers for its complementary base-pairing molecules, such as diaminopyridine functionalized molecules (e.g. DAP-SH **3**), which are bound to the nanoparticle surfaces.

Molecules **2** and **1** (1:10 molar ratio) were dissolved in a mixture of methanol and chloroform to ensure mixing, and the solution was subsequently dried. Excess of CCl_4 was then added, and the mixture was heated. A semitransparent gel formed when the mixture was cooled, and transmission electron microscopy (TEM) confirmed the formation of bundled nanofibers with diameters of ≈8 nm (see Supporting Information). Molecule **2** does not dissolve or cause gelation in CCl_4, but for entropy reasons, its mixing with a much larger amount of **1** should result in nanofibers that trap molecules of **2** and thus provide binding sites on their surfaces.

Lipophilic gold nanoparticles (≈3 nm diameter) were synthesized through the two-phase method developed by Brust et al.[11a] with octanethiol as the surface-stabilizing ligands. We then modified the gold nanoparticle surfaces with DAP-functionalized alkyl thiol **3**[18b] (DAP-SH) by stirring the nanoparticles in a DAP-SH/octanethiol solution (1:10 molar ratio) in dichloromethane at room temperature for 48 h[19] (NMR spectroscopy confirmed the attachment of the DAP-SH molecules on gold particles). When a solution of DAP-SH-coated gold particles (≈0.1 mg mL^{-1}) in CCl_4 (1.0 mL) was added to the thymine-modified nanofiber suspension (1.0 mL, ≈0.5 mg mL^{-1}), the aggregation of gold nanoparticles could be seen with the naked eye within 30 minutes at room temperature.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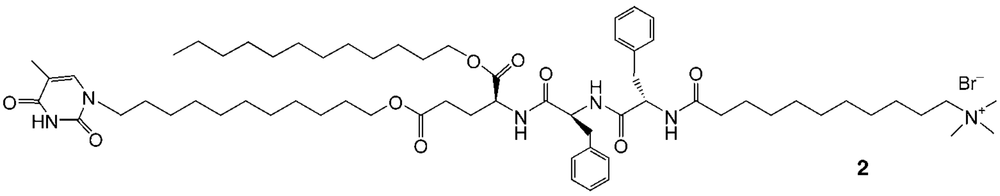
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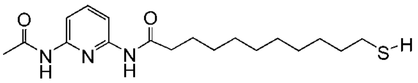
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The observed aggregates were diluted and collected with carbon-coated TEM grids. Under a transmission electron microscope, large numbers of linear arrays of gold nanoparticles were observed along with isolated particles. Figure 2 A shows a representative micrograph. The arrays of gold nanoparticles may extend for lengths of up to a few microns (Figure 2 C) and are made up of densely packed gold nanoparticles with an interparticle distance comparable to twice the length of the surface ligands (≈ 1 nm).

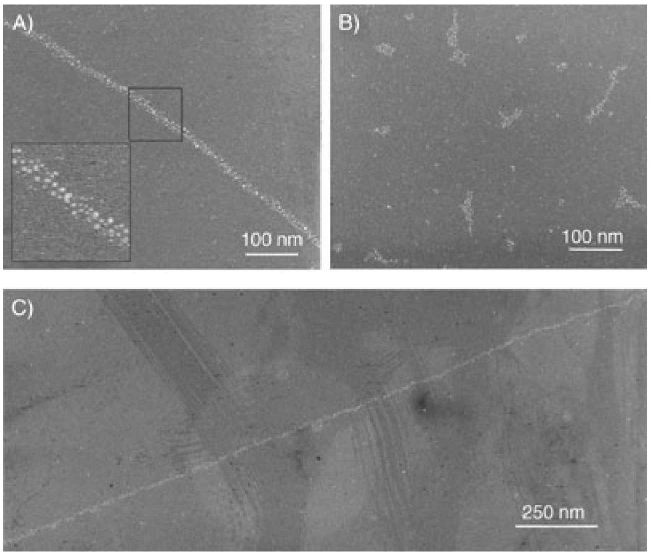

Figure 2. A) and C) Linear arrays of surface-modified gold nanoparticles in the presence of surface-modified peptide amphiphile nanofibers observed under TEM. B) For comparison, a typical image of gold nanoparticles when the surfaces of either the nanofibers or the nanoparticles are not modified. The inset (in A) is a zoom-in of the 100×100 nm^2 area enclosed in by the square.

To establish the relationship between the formation of the linear arrays of gold nanoparticles and chemical modifications on the surfaces of the nanoparticles and nanofibers, a series of control experiments were performed. Mixtures of gold nanocrystals and nanofibers without modification on either reagent did not show any precipitation in solution, nor were linear arrays of gold nanoparticles with a long persistence length observed by TEM (see Figure 2 B for a representative micrograph). In addition, a decrease in the molar ratio of thymine-functionalized amphiphile **2** in the fiber suspension or a decrease in the amount of DAH-SH on the gold particles results in a lower yield of the linear arrays and a longer incubation time required to observe precipitation from solution. These results indicate that formation of the linear arrays of gold nanoparticles is the result of specific binding between the nanoparticles and nanofibers.[20] The observations also prove indirectly the expected presence of trapped molecules of **2** in the nanofibers.

Whereas the long persistence length of the gold nanoparticle arrays is consistent with that of the nanofibers,[13a] their widths vary from a few (Figure 3 A) to tens of nanometers (e.g. Figure 2 A). In some cases the width is not uniform, even within the same array (Figure 3 B). This can be attributed to the strong tendency of nanofibers to bundle, which has been demonstrated in TEM and AFM studies.[13]

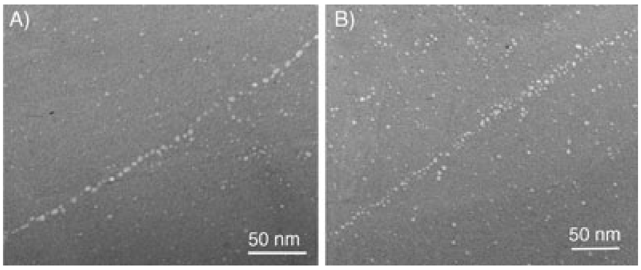

Figure 3. Variation in the width of nanoparticle arrays. A) An array made of a single file of gold nanoparticles. B) An array with variable width, presumably as a result of either bundling of the nanofibers or that of linear arrays.

Furthermore, the modified gold nanoparticles can link nanofibers through their binding to thymine sites, and therefore may also result in width variation of the arrays.

We have shown that peptide-based nanofibers can serve as scaffolds for the 1D assembly of lipophilic inorganic nanoparticles in apolar solvents. The method is simple and very general, and therefore could be extended to other surface-modified inorganic nanoparticles as well. In addition, because the scaffold molecules can be further modified with various organic functional groups such as chromophores and conducting oligomers, these nanofibers may prove to be a versatile scaffold for constructing various functional one-dimensional hybrid nanostructures.

Experimental Section

Molecule **1** and **2** were synthesized through the procedure described in reference [13a], except that 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole hydrate (HOBT) were used as coupling reagents in all the ester and amide formation, and that all the intermediates were purified by silica gel chromatography with dichloromethane/methanol (95:5 v/v) as eluent. To synthesize heterodiesters of glutamic acid, Boc-Glu(OBzl)-OH was used as one of the starting materials.

The intermediate 1-(ω-hydroxy-undecyl) thymine for molecule **2** was synthesized through a published procedure[18c] to make dodecyl uracil except that thymine and 11-bromo-1-undecanol were used instead. Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.24$ (s, 1H), 6.97 (s, 1H), 3.65 (m, 4H), 1.92 (s, 3H), 1.70–1.45 (m, 4H), 1.40–1.20 ppm (m, 14H); ESI MS (m/z): 297.5 $[M+1]$.

Molecule **2** was recrystallized from ethyl acetate as a white powder. ^{1}H NMR(500 MHz, $CDCl_3$): $\delta = 7.58$ (b, 1H), 7.25 (m, 10H), 6.97 (s, 1), 4.60 (m, 2H), 4.46 (m, 1H), 4.06 (m, 4H), 3.67 (m, 2H), 3.46 (m, 2H), 3.33 (s, 9H), 3.05 (m, 4H), 2.40–2.20 (m, 2H), 2.20–2.00 (m, 4H), 1.91 (s, 3H), 1.72 (m, 2H), 1.60 (m, 6H), 1.45 (m, 2H), 1.40–1.10 (b, 44H), 0.87 ppm (t, $J = 6.8$ Hz, 3H); ESI MS (m/z): 1113.8.

TEM was performed on a Hitachi H-8100 microscope operating at 200 kV.

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[20] As suggested by one of the reviewers of this manuscript, we treated DAP-SH modified gold nanoparticles with excess 1-[ω-(*N*-Boc-phenylalanyl)-undecyloxy]thymine in CCl_4, and then mixed them with a thymine-modified nanofiber suspension. We found that the binding affinity between the nanoparticles and the nanofibers decreased significantly.