

Three-Dimensional Directed Self-Assembly of Peptide Nanowires into Micrometer-Sized Crystalline Cubes with Nanoparticle Joints**

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In the last decade, nanoscale materials have been created with superior physical properties. The next challenge is to assemble nanoscale building blocks into complex and larger-scale superstructures so that these nanomaterials with unique properties can be integrated as macroscale components in solar cells, microelectronics, metamaterials, catalysis, and sensors.^[1] Recent trends in the complexity of device design demand the fabrication of three-dimensional (3D) superstructures from multi-nanomaterial components in precise configurations.^[2] Biomimetic assembly is an emerging technique in such pursuits, because living organisms are efficient, inexpensive, and environmentally benign material generators, allowing low temperature fabrication.^[3–7] Although DNA bionanotechnology has recently been used to precisely assemble 3D shapes,^[8–16] the methods to develop highly ordered macroscopic materials from these nanostructures remains limited,^[17] and for practical applications the production scale, size, and the yield of the assembled materials need to be amplified.^[18,19] Peptides are another of nature's building blocks with high specificity, versatility, and robustness for directed assembly that can be exploited to design novel 3D superstructures.^[20] Herein we applied the directed self-assembly of peptides and ligand-functionalized gold nanoparticle (AuNP) joints for the development of the micrometer-scale 3D cube-shaped hybrid superlattices, creating a physical framework for the proposed biomimetic assembly strategy. In this approach, we took advantage of the naturally robust assembling nature of collagen triple-helix peptides and used them as nanowire building blocks for 3D peptide–NP

superlattice generation. Assembling streptavidin-functionalized AuNPs and recombinant peptides that model a segment of the $\alpha 1$ chain of type I collagen specifically modified to be biotinylated at the N terminus in vivo, we created micrometer-sized cubes with peptide nanowires as frames and AuNPs as joints. These collagen-mimicking peptides can self-associate laterally,^[21,22] and this intrinsic peptide–peptide interaction allows us to create unit cells of various scales and also predicts the ability to create an extensive latticework of cubic microcrystals. The NPs can join the peptide nanowires through the streptavidin–biotin interaction to create cubic unit cells with extremely high yield and the resulting peptide–NP superlattices are ordered in the long range with the isotropic crystalline orientation. This simple and rapid fabrication method produces high yields of 3D materials in tailored shapes that are dependent on the geometry of the peptide–NP unit cells, thus promising ease and flexibility in manufacturing functional devices. To our knowledge, this is the first example of 3D NP superlattice assembly on a micrometer scale from peptide nanowires.

The collagen peptide is derived from the F877 peptide that was previously used as a nanowire template.^[23,24] This new BAP877 peptide is modified by the insertion of a 15 amino acid residue of biotin acceptor peptide (BAP) at the N terminus (Figure 1 a). Other design features of this collagen

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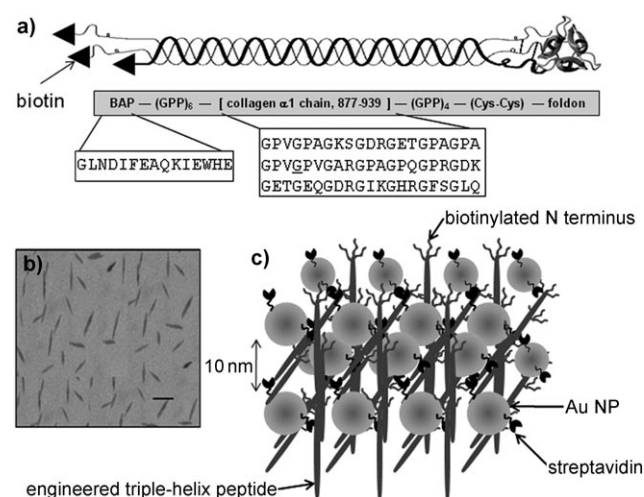


Figure 1. a) Structure of the engineered triple-helix peptide with biotin at the N terminus. b) TEM image of biotinylated triple-helix peptides. Scale bar: 50 nm. c) Proposed 3D superlattice structure of the biotinylated triple-helix peptides and streptavidin-functionalized AuNPs assembled by the streptavidin–biotin interaction and lateral peptide–peptide association through the collagen motif.

peptide include a triple-helix domain consisting of 63 residues from the $\alpha 1$ chain of type I collagen with additional repeating Gly-Pro-Pro sequences for increased stability, a bacteriophage T4 fibrin foldon domain at the C terminus serving as a nucleation site to facilitate the correct folding, and a Cys-knot sequence (Gly-Pro-Cys-Cys) to cross-link three polypeptide chains of the triple helix through a set of disulfide bonds (Figure 1a).^[23,24] This BAP site is designed to be biotinylated in vivo to generate the complementary binding motif for streptavidin-coated AuNPs. To accomplish this modification, BAP877 peptide was expressed as a fusion protein in *E. coli*, where endogenous bacterial biotin ligase BirA biotinylates the specific lysine residue within the BAP sequence.^[25,26] The purified biotinylated triple-helix peptide (Figure 1b) was monodisperse in size (4×40 nm²; for a size distribution, see the Supporting Information) and adapted to rigid conformation with no sign of bending.

In the proposed 3D peptide-NP superlattice assembly (Figure 1c), the biotin-streptavidin interaction joins the ends of triple-helix peptides to the AuNPs to form a unit cell for the cube microcrystal. The design of this triple-helix peptide mimics the staggered lateral association of natural collagen to scale these unit cells for the assembly of larger cubic crystals. As AuNPs with streptavidin on the surface bind the biotinylated triple-helix peptides, we anticipate the size ratio between the triple-helix peptide and the AuNP is important to define the shape of the unit cell of the peptide nanowires (Figure 1c). When 10 nm diameter AuNPs decorated with six streptavidin molecules were incubated with the biotinylated triple-helix peptide in solution for one hour, the peptides were assembled into a cubic structure (Figure 2a).

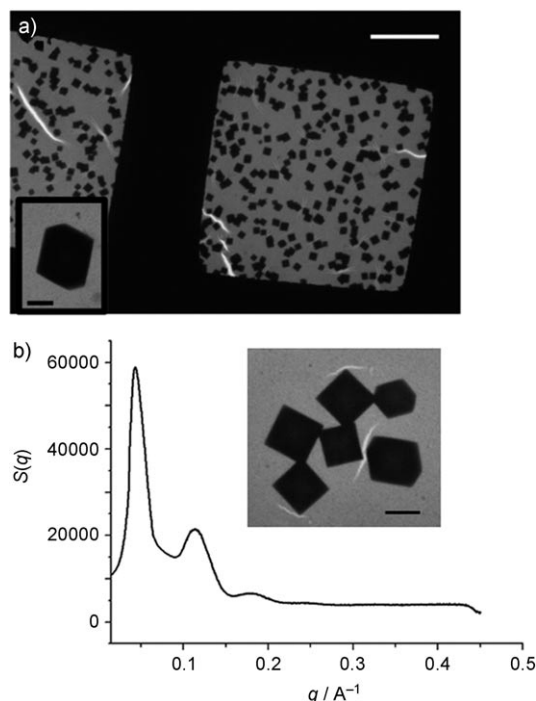


Figure 2. a) TEM image of the cubes assembled from the biotinylated triple-helix peptides and streptavidin-functionalized AuNPs. Scale bar: 20 μm. Inset: A tilted TEM image of the cubes in (a). Scale bar: 1 μm. b) SAXS pattern of the cubes. Scale bar (inset): 2 μm.

The yield of 1–2 μm cubes was extremely high with respect to uniformity in shape and dimension of the crystals, as seen in this TEM image. Repeating this assembly with a concomitant tenfold reduction of both the peptide and AuNP concentrations reduced the size of the cubes on the order of 100–200 nm (see the Supporting Information). When the TEM grid of the sample was tilted, the edges of the cubes become visible, indicating that these cubic assemblies are three-dimensional (Figure 2a, inset). A circular dichroism (CD) spectrum of these cubic superlattices indicates that the genetically engineered peptides maintain the triple-helical conformation with a small positive peak at about 225 nm and a deep negative peak at about 197 nm (see the Supporting Information).^[24] Figure 2b shows the small-angle X-ray scattering (SAXS) pattern of the cubes measured at 30 °C, with diffraction peaks located at $q = 0.06, 0.11$, and $0.18 \text{ \AA}^{-1}$, respectively. The q_x/q_1 ratios of these peaks are $1:2^{1/2}:5^{1/2}$, thus matching the characteristic diffraction pattern of a body-centered cubic structure (see the Supporting Information). On the basis of this SAXS spectrum, the interparticle distance of AuNPs in the diagonal direction of the body-centered cubic unit cell is 7 nm. A characteristic sharp increase in the scattering at small value of q in the SAXS profile indicates the long-range order of AuNPs in the cubes.^[27] To further confirm the arrangement of AuNPs in the cubic peptide-NP superlattices, we also imaged these cubes by polarized-light microscopy. The contrast for the cubic crystals was diminished with cross-polarized light (see the Supporting Information), supporting that the arrangement of AuNPs in the cube is isometric in the long range. In Figure 3a, high-resolution TEM (HRTEM) of the peptide cube reveals that the array of AuNPs with lattice fringes and crystalline faces of AuNPs are

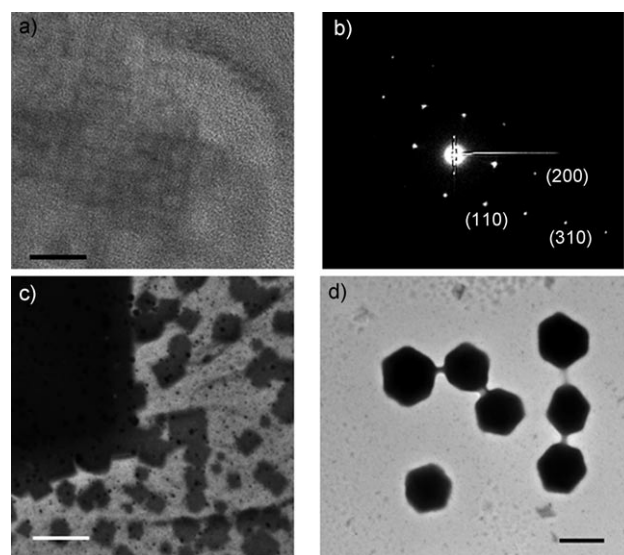


Figure 3. a) High-resolution TEM image of the cube assembled from biotinylated triple-helix peptides and AuNPs. Scale bar: 30 nm. b) An electron-diffraction pattern of the cube. c) TEM image of the cubes after the peptide frames were disassembled by lowering the pH. Scale bar: 100 nm. d) TEM image of the micrometer-sized hexagons assembled from larger AuNPs (diameter 30 nm) and the same triple-helix peptide nanowires (length 40 nm). Scale bar: 1 μm.

oriented in the isotropic direction. In this image, the periodic alignment of AuNPs and the peptide frame are visible when the peptides were stained by ammonium molybdate to increase the contrast of the peptide lattice in the cube. Electron diffraction of this cube (Figure 3b) matches the diffraction pattern of the single-crystalline gold, indicating that the crystalline orientation of AuNPs in the peptide frame is aligned in an isotropic direction (HRTEM image in Figure 3a). It should be noted that the same streptavidin-functionalized AuNPs mixed with non-biotinylated triple-helix peptides did not produce any peptide–NP aggregations (data not shown), demonstrating the crucial role of the biotin–streptavidin interaction for the assembly of the cube-shaped microcrystals. Previously, mesocrystals of calcium carbonate were observed to undergo the oriented assembly to form single crystals by dipolar interactions with polymer adsorption,^[5] and the NP alignment in an isotropic crystallographic direction in this peptide–AuNP cube could also be originated from the dipole interaction of peptides and NPs.^[27]

As collagen fibrils are known to be disassembled into triple-helix peptides in an acidic environment,^[21] it follows that cubic peptide–AuNP superlattice crystals should also be dismantled at lower pH. When the solution containing peptide–NP cubes was incubated overnight at a pH slightly lower than neutral, these peptide frames were slowly disassembled into smaller domains of the microcube, the sizes of which are in the range of 20 nm–80 nm (Figure 3c). When the pH of the solution with the cubes was decreased to 5.5, the micrometer-scale cubes were further disassembled into individual peptides and then these peptides followed their inherent reassembling process. After reassembly into the wire structures, these peptide wires were aligned side-by-side to form peptide sheets, thus resembling the natural collagen assembly (see the Supporting Information).

To further test our hypothesis that the shape of the peptide–AuNP assembly can be changed by altering the geometry of unit cell, larger 30 nm diameter AuNPs were assembled with the same triple-helix peptides, assuming that the size ratio between the diameter of AuNPs and the length of peptide nanowires can change the shape of unit cell. When the size of AuNPs was comparable to the length of the peptide nanowires, these AuNPs and peptides were assembled into micrometer-sized hexagons (Figure 3d). This result indicates that the size ratio indeed plays an important role in the assembly of the structure. It is plausible that the larger size of AuNPs changes the interaction and the structure of peptide–NP unit cells and the difference in the peptide–NP angle is responsible for the resulting shape change as these unit cells are assembled in a microscale.

In summary, a novel peptide-directed nanomaterial assembly technology is presented for the construction of macroscale multicomponent materials that still retain superior nanoscale domains and properties (that is, technology to bridge between nano- and macroassemblies). By applying this technique, nanomaterials can be assembled with peptides in the ordered dipole orientation and long periodicity, resulting in 3D peptide–inorganic superlattices with defined 3D shape. The unique features of molecular recognition by peptides and large-scale 3D self-assembly enable such multicomponent 3D

materials to be created in precise designs and high yields that are difficult to obtain by other templates, such as DNAs and polymers. The type of 3D peptide superlattice assembly with hybrid NP building blocks described herein shows potential to impact the future fabrication of complex 3D functional device building blocks, which demands precise long-range arrangement and periodicity of NPs. This programmable recognition-based assembly provides the flexibility to modify the NP arrangement and the final crystalline structure by altering the size ratio between triple-helix peptides and NPs and/or the number of ligands on NPs, as observed in the DNA–AuNP assembly.^[10] It is also expected that more systematic investigation of the robust large-scale assembly nature of peptides on 3D microcrystal structures will provide further insight into the design and fabrication of novel 3D crystals in practical sizes.

Experimental Section

The recombinant collagen-like fragment BAP877 peptide was generated using the original F877 bacteria expression construct^[24] with the insertion of a 15 amino acid sequence of biotin acceptor peptide (BAP)^[25,26,28] at the N terminus (Supporting Information, Figure S1). The insertion of BAP is achieved by PCR using the PrimeSTAR HS DNA polymerase (Takara Bio Inc) with the pET32a-877 (foldon) plasmid as the template and the forward and reverse primers described in the Supporting Information. The fusion protein was subcloned into a pCR4 blunt TOPO (Invitrogen, Carlsbad, CA) plasmid and the sequence was verified by DNA sequencing (ABI PRISM 3100-Avant Genetic Analyzer, Applied and Biosystems, Carlsbad, CA). The constructed plasmid was transformed into chemically competent *E. coli* BL21 (DE3). The protein in the supernatant was purified using nickel affinity chromatography, and the final protein was purified by RP-HPLC (Beckman Coulter) with C18 column (Vydac) with a 20–70% acetonitrile/water with 0.1% trifluoroacetic acid (TFA). To confirm in vivo biotinylation of fusion proteins, western blots were performed.

Gold nanoparticles (10 nm diameter) with bound streptavidin were purchased from Nanocs (New York, NY). The density of streptavidin is 4 to 6 per AuNP. The conjugated nanoparticle solution was diluted with 0.1 mM HEPES buffer (pH 7) in a 1:10 ratio. After the diluted streptavidin–gold conjugate was allowed to equilibrate for 30 min at room temperature, biotin-conjugated BAP877 peptide (10 mg mL^{-1}) was incubated in a molar ratio of 1:2 for 1 hour.

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