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Biomolecular Nanowires

Fabrication of Au Nanowires of Uniform Length and Diameter Using a Monodisperse and Rigid Biomolecular Template: Collagen-like Triple Helix**

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Recently, various metal and semiconductor nanowires have been developed as building blocks for electronics, optics, and sensors. Among these newly developed nanowires, nanowires grown on biomolecular templates such as DNA and peptide assemblies are advantageous since the molecular recognition functions of these biomolecules with specific ligands can be employed to immobilize nanowires onto specific locations to establish desired device geometries.[1–3] However, most of the biomolecular-nanowire templates made from DNAs or peptides do not possess suitable electric properties for those devices, and therefore there is an extensive effort in the field of bionanotechnology to coat these addressable biomolecular nanowires with metals and semiconductors.[4–16] Recently, the morphology of coating on these peptide–nanotube templates was shown to be controlled by means of changing the peptide sequences and conformations, thus fine-tuning the electronic structures of resulting nanowires for their device applications.[17–19]

While these biomolecular-nanowire templates appear to be promising building blocks for nanodevices, it is essential to have size monodispersity, strength, and mass producibility to impact real-world applications. For example, biomolecular templates self-assembled from peptidic monomers tend to yield polydisperse materials with heterogeneous diameters and uncontrolled length through the self-assembly process. The tobacco mosaic virus (TMV), a rod-shaped biomolecular template, has been applied for various metal coatings, however accurate control of the length with low dispersity is not an easy task.[20,21] The other type—DNA biomolecular templates—have defined lengths determined by the number of nucleic acids, however they lack conformational rigidity. The tendency of supertwisting of the double-helix DNA structure makes it difficult to obtain rigid and straight nanowires. Their production cost and time may also not be suitable for large-scale syntheses.

Herein we report a new application using a collagen-like triple helix as a template nanowire which appears to overcome some of the shortcomings of other biomolecular templates. The collagen-like triple helix is the genetically engineered polypeptide assembly that contains a fragment from the natural collagen sequence. Our study demonstrates that by using the recombinant technology, we can design and amplify a collagen-like triple helix that is monodisperse, easily mineralized with metal ions, and can, thus, be applied as rigid biomolecular templates for metal–nanowire fabrications. Collagens are the major components of extracellular matrices for bones, cartilages, skins, blood vessels, and corneas, and they are the most abundant proteins in higher organisms with superior mechanical properties.[22–24] The collagen-like triple helix is made of three polypeptide chains tightly twisted and bundled together to form a rigid, rod-shaped molecule that is suitable for applications in building blocks of nanodevices.

To explore the application of a collagen-like triple helix as a nanowire template, we studied the properties of two recombinant triple-helix molecules obtained from an *E. coli* expression system (Figure 1). The two recombinant triple helices, F877 and G901S, contain a foldon domain taken from bacteriophage T4 fibritin, which serves as the nucleation site for the formation of the triple helix.[25] The triple-helix domain of F877 consists of 63 amino acid residues modeling the region starting at position 877 (from the N terminus) of the α1 chain of type I collagen (*Homo sapien*). To increase the thermal stability of the triple helix, repeating $(Gly–Pro–Pro)_n$ sequences were added at the ends of the 63 residues. A pair of Cys residues were inserted at the interface of the foldon and the triple-helix domain to covalently link the three chains of the triple helix through a set of interchain disulfide bonds.[24] Triple helix G901S contains the same sequence as F877 but with the Gly→Ser substitution at position G901S (G in Figure 1). Replacing the obligatory Gly residue at every third position by any other amino acid residue with bulkier side chains, is known to affect the triple-helix conformation, and such mutations have been implicated in connective tissue diseases.[26] Both recombinant molecules form triple-helix conformations in solution (Figure 1a) with denaturation temperatures (T_m) of 42 and 30 °C, for F877 and G901S, respectively (inset of Figure 1a). The triple helix behaves as a trimer with no signs of further association based on the study

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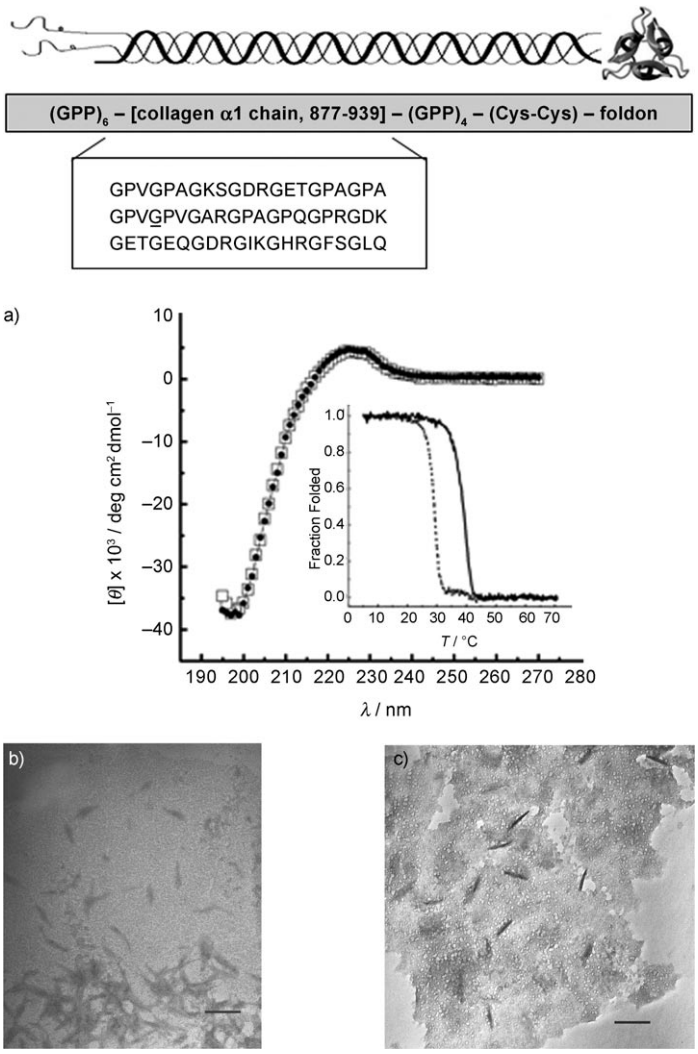

Figure 1. Illustrated structure of the collagen-like triple helices (top). An underlined G residue in the wild-type F877 helix was mutated to Ser in G901S. a) Circular dichroism (CD) spectra of F877 (□) and G901S (●) at 4 °C. Inset shows denaturation temperatures of F877 (——) and G901S (•••••). b) TEM image of the F877 triple-helix. c) TEM image of the G901S. Scale bars: 40 nm.

using analytical ultracentrifugation and gel filtration (see the Supporting Information).

The structure of the F877 triple helix was imaged by TEM as shown in Figure 1b. Triple-helix F877 formed monodisperse, straight nanowires with an average length of 40 nm and no bending, indicating a rather rigid conformation. The length of the triple helix observed under TEM agrees well with the value of the approximately 35 nm end-to-end distance of a single triple helix consisting of 93 amino acids and a foldon domain estimated from the triple-helix crystal structure.[27] The observed diameter of 4 nm appears to be larger than the 1–2 nm predicted from the crystal structure. This slightly larger diameter in the TEM image could be caused by swelling through hydration.[28] Triple-helix G901S has a similar dimension as the F877 but appears to disperse slightly more as shown in Figure 1c.

To examine feasibility in their application as building blocks for electronics, these triple helices were coated by Au. When the F877 triple helix (Figure 2a) is incubated with trimethylphosphinegold chloride ([$AuPMe_3Cl$]) for four days and then reduced by hydrazine hydrate for one day at 4 °C, Au crystals grow on the helix as shown in Figure 2b. This TEM image shows that the Au nanocrystal growth was localized on the helix surface. To grow Au on the triple helix more uniformly, we precoated the triple helix with a Au-mineralizing peptide, Ala-His-His-Ala-His-His-Ala-Ala-Asp (HRE), which has a high affinity for organic Au salts.[14,29] Our previous study demonstrated that the HRE binds to glycine–bolaamphiphile nanotubes through hydrogen bonding at the amide groups of the nanotube after a simple incubation process.[1] The subsequent Au electroless process on these HRE-bound nanotubes yields a uniform Au nanocrystal coating.[14] A similar enhanced, more uniformed mineralization is found for the triple helices, F877 and G901S, after incubation with HRE. When the F877 triple helix (Figure 2a) is incubated in the HRE peptide solution for 24 h, the triple helix is coated by the HRE peptide, as confirmed by FTIR spectrometry (see the Supporting Information). No significant changes in length, diameter, and shape are detected after the triple helix is coated by HRE, as shown in Figure 2c. The HRE peptide coating increases dispersion of the triple-helix nanowires, presumably because the coating of the HRE peptide contains clusters of positive charges, which reduces the potential attractive interaction between the triple helices. The reduction of Au on the HRE-coated triple helix produces uniform and highly crystalline Au coating on the surface, as shown in Figure 2d. The Au-coated triple helix in the inset of Figure 2d appears to be a ricelike shape, which could be due to the inhomogeneous coating of the HRE peptide at the ends. As shown in Figure 1, the foldon at the C-terminal end forms a three-stranded β-hairpin propeller, a conformation very different from that of the triple helix, while the helix fray at the N-terminal end of the triple-helix domain has been well-documented by structural studies of crystallography and NMR.[27] These conformation differences result in different binding of the HRE peptides and lead to less Au growth on those areas, compared to the middle part of triple helix, consistent with the rice-shape formation. Similarly, Au nanowires with identical features are obtained when the G901S triple-helix peptides are precoated by HRE.

While the Au growth is observed with both the HRE precoated and the neat (i.e. no HRE pretreatment) F877 triple helices, the reduction of Au with the neat G901S helix yields no Au nanowires. The Gly→Ser mutation included in the G901S is known to cause brittle bone disease as a result of the disruption of the triple-helix conformation.[26] While the G901S was shown to adapt to the triple-helix conformation at low temperature, the denaturation temperature (T_m) of this triple helix decreases by more than 10 °C (inset in Figure 1a) compared to F877, indicating the reduced stability of G901S with an altered conformation. It is unclear at this point whether the lack of Au deposit on the G901S is due to the

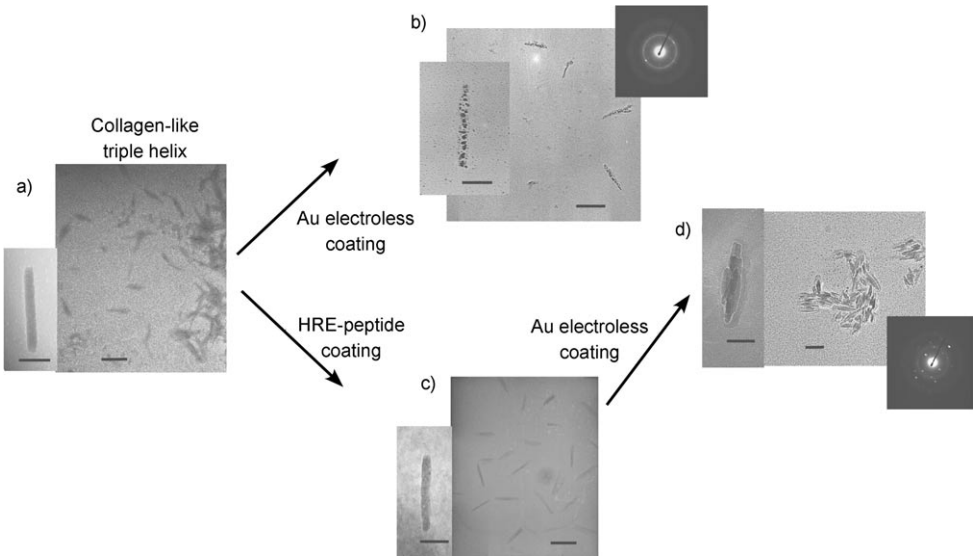

Figure 2. TEM images of the F877 triple-helix peptides: a) neat, b) coated by Au, c) coated by HRE-mineralizing peptides. d) TEM image of the HRE-coated triple-helix peptides in (c) coated by Au. Electron diffraction patterns of (b) and (d) are shown next to their TEM images. Scale bars: 40 nm. Insets are their HRTEM images; scale bars: 15 nm.

disruption of the charge distribution on the surface of the F877 triple helix or the altering of other structural features of the F877 triple helix caused by the Gly→Ser mutation. Nevertheless, the mutation-caused variations of the Au coating morphologies on the triple helix highlights the dependence of the properties of the nanowires on the molecular conformations of the helix template. Such sequence-dependent behavior also offers a practical means to produce nanowires with desired properties by modifying the sequence of the recombinant triple-helix molecules.

In summary, monodisperse Au nanowires with defined dimension of 4 nm × 40 nm were obtained by templating recombinant collagen-like triple helices from an *E. coli* expression system. The length of the nanowires can be easily controlled by the number of amino acid residues, and the production of triple helix can be scaled up by means of the cell multiplication. Thus, the unique molecular properties of collagen-like triple helix combined with the versatility of the recombinant technology offer a promising system to create biomolecular nanowires by design.

Experimental Section

The collagen fragments were cloned into a GPP-foldon vector built on the pET35a plasmid of Novagen (the original GPP-foldon vector was kindly provided by Dr. Jurgen Engel from the University of Basel, Switzerland). The product of this plasmid is a fusion protein with a 6 × His tag and thioredoxin as the carrier protein which can be removed by thrombin cleavage to produce the chimaeric protein containing the triple-helix domain and the foldon domain with the Cys knot inserted at the interface of the two domains. The protein was expressed in bacteria JM109 from Promega and purified by His-tag affinity column. After the His-tagged thioredoxin was removed by thrombin digestion and the second round of His-affinity column, the fragments were further purified by gel filtration to isolate the triple helix. The final samples are more than 97% pure as estimated by sodium dodecyl sulfate (SDS) electrophoresis and gel-filtration experiments.

To grow Au nanocrystals on F877 and G901S triple helices, first we mixed the triple-helix solutions (200 μL, 0.2 mg mL^{-1}) with Tris buffer solutions (535 μL, pH 8.6, 0.01 mol L^{-1}). The resulting mixture was then vortexed for 10 s and left 1 day at 4 °C. [AuPMe$_3$Cl] (1.8 mg, Sigma) was incubated for 4 days at 4 °C and then the supernatant of the solution containing unattached Au salts was removed by a pipette. Hydrazine hydrate (5 μl, Sigma) was then added to reduce Au salts on the triple helices. To coat the HRE peptides (GenScript Corp., NJ) onto the triple helices, the triple-helix solutions (200 μL) were mixed with the HRE solutions (535 μL, 3.9×10^{-4} mg mL^{-1}) in the pH 8.6 Tris buffer solutions for 1 day at 4 °C. After immobilization of HRE was confirmed by FTIR, we applied the same Au-growth procedure described above to coat Au on the HRE-immobilized triple helices. After one day of reduction at 4 °C, the triple-helix solutions (3 μL) were dried on carbon-coated copper TEM grids as excess solutions were removed by filter papers. These dried samples were then studied by TEM and electron diffraction (JOEL 1200 EX) at an acceleration voltage of 100 kV.

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