Fabrication of Coaxial Metal Nanocables Using a Self-Assembled Peptide Nanotube Scaffold

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Supporting Online Material

This supplement contains:

Materials and Methods

Materials and methods

Preparation of peptides. The Cys-Gly-Ser-Gly-Phe-Phe (CGSGFF) and Phe-Phe-Cys (FFC) Linker peptides were purchased from Bachem (Bubendorf, Switzerland). The Cys-Phe-Phe (CFF) peptide was purchased from SynPep (Dublin, CA). Fresh stock solutions of diphenylalanine (FF) were prepared by dissolving the peptides in lyophilized form in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) at a concentration of 100 mg/ml. Stock solutions of CFF, CGSGFF, and FFC Linker peptides were prepared by dissolving the lyophilized form of the peptide in 0.25 M dithiothreitol to a final concentration of 25 mg/ml. To avoid any pre-aggregation, fresh stock solutions were prepared for each experiment. The diphenylalanine peptide stock solution was diluted to a final concentration of 2 mg/ml in ddH₂O. The peptide linker / FF peptide solution had a 1:10 concentration.
\textit{\textsuperscript{1}H NMR spectroscopy}. Diphenylalanine (2 mg/ml) in lyophilized form was dissolved in 500 μL deuterium oxide (Sigma Chemicals Co., USA) at 60°C to give a 6.8 mM solution. The sample was put into the spectrometer, which was maintained at the same temperature. The temperature in the spectrometer's probe was reduced to 32°C, stabilized, and homogeneity was achieved after 10 minutes. Subsequently, one-dimensional spectra were acquired at 1-min intervals for 220 minutes. The NMR experiments were performed on a Bruker Avance 600-MHz DMX spectrometer operating at a proton frequency of 600.13 MHz, using a 5-mm broadband probe. The transmitter frequency was set on the HDO signal. The spectral width was 13 ppm and the relaxation delay was set to 2 seconds.

Spectra were processed with XWINNMR (Bruker Analytische Messtechnik GmbH). Zero filling was applied prior to Fourier transformation, without a window function. The baseline was further corrected using the automatic phase correction protocol in the program, which also gave the absolute integral of the peak regions (the aromatic region, each of the two Hα peaks, and the Hβ region of the diphenylalanine, as well as the water peak – only the latter two are shown, but the kinetics was the same in all regions). The kinetics curves were fit using EXCEL.

\textit{Quantification of cysteine residues using ellman's reagent}. Quantification of bounded linker peptides was performed with ellman's reagent (Sigma Chemicals Co., USA). Peptide nanotubes solution was prepared and incubated with linker peptides as described above. The solution was then centrifuged for 30 min at 18,000g and washed in order to remove any unbound linker peptides. The samples were then tested by ellman's reagent according to standard protocol.\textsuperscript{29}
**Transmission Electron microscopy.** In the negative staining experiments, the grid was stained with 2% uranyl acetate in water, and after two minutes, excess fluid was removed from the grid. Images of silver-filled nanotubes were achieved without staining. Samples were viewed using a JEOL 1200EX electron microscope operating at 80 kV. High-resolution samples were viewed and analyzed with a Philips Tecnai F20 Field Emission Gun electron microscope operating at 200 kV. Energy dispersive X-ray analysis analysis was performed with an EDAX detector. A 10-µL aliquot of a peptide solution aged for one day was placed on a 400-mesh copper grid. After 1 minute, excess fluid was removed. In the linker binding-experiments, samples were examined by TEM only after they had been washed with ddH₂O, in order to remove unbound gold particles from the grid.

**Examination of the linker peptides.** Each of the three linker-peptides was examined separately by TEM with a negative stain. The peptides were prepared at different concentrations (0.2 mg/ml, 2 mg/ml, and 20 mg/ml) and at different time intervals (5 min, 1 hr, 24 hr, and 1 week).

**Binding of gold nanoparticles.** Gold colloid with a particle diameter of 20 nm was purchased from Sigma. The covalent binding of the gold nanoparticles was done using a 10% gold colloid solution, incubating for 30 min.

**Electroless deposition.** Monomaleimido nanogold with a particle diameter of 1.4 nm, which was purchased from Nanoprobes (Yaphank, NY), was covalently cross-linked to linker peptides for over 18 hrs at 4°C, according to the manufacturer's protocol. The Nanogold particles act as promoters for reducing the silver ions in the solution.
The Nanogold-labeled peptide nanotubes were subjected to gold enhancement with GoldEnhance LM (Nanoprobes). Enhancement was performed according to the manufacturer's protocol; these experiments were done only with the FFC and the CGSGFF linkers, which are more soluble than the CFF linker.

*Casting silver nanowires in the peptide nanotubes inner-space.* A 90-µL aliquot of nanotube solution (aged for one day) at a concentration of 2 mg/ml was added to 10 µL of a boiling solution of 10 mM AgNO₃. Citric acid was then added to a final concentration of 0.038% to serve as a reducing agent.