Supplementary Material (ESI) for Chemical Communications

Supporting information for:

Preparation of 1-D Nanoparticle Superstructures with Tailorable Thicknesses using Gold-Binding Peptide Conjugates: New Insights into Fabrication Process and Mechanism

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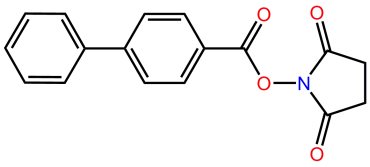
1. Materials and methods

All solvents and chemicals were obtained from commercial sources and used without further purification. 0.1M HEPES (4-(2-hydroxyethyl)-piperazineethanesulfonic acid) buffer was made by directly diluting 1.0M HEPES buffer (pH=7.3±0.1; Fisher Scientific) with water (NANOpure, Barnstead Diamond^{TM} System.; 18.2 MΩ). PEP_{Au} (AYSSGAPPMPPF) and AYSS-PEP_{Au} (AYSSAYSSGAPPMPPF) were synthesized and purified by New England Peptide (NEP). ^{1}H-NMR spectra were recorded on a Bruker DRX 300 spectrometer at room temperature. Chemical shifts were recorded in ppm (parts per million) based on residual solvent peaks as internal reference [$CDCl_3$ δ: 7.26 (^{1}H)]. Reverse-phase high pressure liquid chromatography (HPLC) was performed at ambient temperature with an Agilent 1200 liquid chromatographic system equipped with diode array and multiple wavelength detectors using a Grace Vydac protein C4 column (214TP1010, 1.0 cm × 25 cm). Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained on an Applied Biosystem Voyager System 6174 MALDI-TOF mass spectrometer using α-cyano-4-hydroxy cinnamic acid (CHCA) as the matrix. Samples for atomic force microscopy (AFM) were prepared on freshly peeled MICA substrates that were treated with a 1% solution of 3-aminopropyl trimethoxysilane. [1] Tapping-mode AFM was performed on a Veeco Dimension VSPM. Transmission electron microscopy (TEM) samples were prepared by pipetting one drop of solution onto a 3-mm-diameter copper grid with carbon film; 2% aqueous phosphotungstic acid was used for negative staining. TEM was conducted on a JEOL 200CX instrument operated at 200kV and images were collected using a Gatan CCD image system. Scanning transmission electron microscopy (STEM) was used in TEM mode. STEM was conducted on a JEOL 2000-FX instrument operated at 200 kV and images were collected using a Gatan CCD camera with digital micrograph software program.

2. Preparation of N-hydroxyl-succinimide esters and peptide conjugates

BP-PEP_{Au} and BP-AYSS-PEP_{Au} were synthesized and purified by established methods.[2]

Preparation of N-hydroxyl-succinimide esters:



Preparation of N-hydroxyl-succinimide esters. 4-phenylbenzoic acid (500.0 mg, 2.52 mmol) and N-hydroxysuccinimide (290.2 mg, 2.52 mmol) were dissolved in 15 ml anhydrous dimethylformamide (DMF) in an argon atmosphere. The solution was stirred for 5 min at room temperature and then cooled to 0 ^{o}C in an ice bath. After addition of dicyclohexyl carbodiimide (DCC) (562.1 mg, 2.72 mmol) at 0ºC, the solution was stirred overnight at room temperature. The reaction mixture was processed by removing the precipitate via filtration. The solvent was removed under reduced pressure and the crystalline residue recrystallized from isopropanol to yield the N-hydroxyl-succinimide ester (585.9 mg, 1.984 mmol, 79 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23-8.20 (dd, 2H, J = 5.1 Hz, J = 6.3 Hz), 7.75-7.72 (dd, 2H, J = 4.8 Hz, J = 6.6 Hz), 7.65-7.62 (m, 2H), 7.496-7.454 (m, 3H), 2.92 (s, 4H).

General preparation of biphenyl peptide conjugates: BP-PEP_{Au} and BP-AYSS-PEP_{Au}. Either PEP_{Au} (AYSSGAPPMPPF: 1.23 mg, 1.0×10^{-3} mmol) or AYSS-PEP_{Au} (AYSSAYSSGAPPMPPF (1.3 mg, 8.0×10^{-4} mmol) was dissolved in 0.1 ml anhydrous DMF. 0.1M N-hydroxyl-succinimide ester in DMF (60 µl, 1.2×10^{-3} mmol) and 1µl triethylamine were added to the peptide solution. 1day after stirring at room temperature, the reaction mixture was separated by reversed-phase HPLC eluting with a linear gradient of 0.05% formic acid in CH_3CN and 0.1% formic acid in nanopure water (5/95 to 95/5 over 32 min for BP-PEP_{Au} and

5/95 to 95/5 over 35 min for BP-AYSS-PEP_{Au}). The concentrations of the peptide conjugates were determined spectrophotometrically in water/acetonitrile (1:1) using a molar extinction coefficient of tyrosine (1280 $M^{-1}cm^{-1}$) at 280 nm.

3. Preparation of gold nanoparticle superstructures

Lyophilized BP-PEP_{Au} peptide conjugate (7.13×10^{-8} mol) or BP-AYSS-PEP_{Au} peptide conjugate (7.73×10^{-8} mol) was completely dissolved in 0.5 ml of 0.1M HEPES buffer in a plastic vial. This solution was allowed to incubate at room temperature for 30 min (note: for BP-AYSS-PEP_{Au}, a second solution was allowed to incubate for 6 d at room temperature). Thereafter, 2 μl of a freshly prepared solution of 0.1M chloroauric acid ($HAuCl_4$) in 1.0 M triethylammonium acetate (TEAA) buffer was added to the peptide conjugate solution. The mixture was vortexed for a few seconds as soon as the $HAuCl_4$ solution was added and then left undisturbed at room temperature.

4. Supplementary data

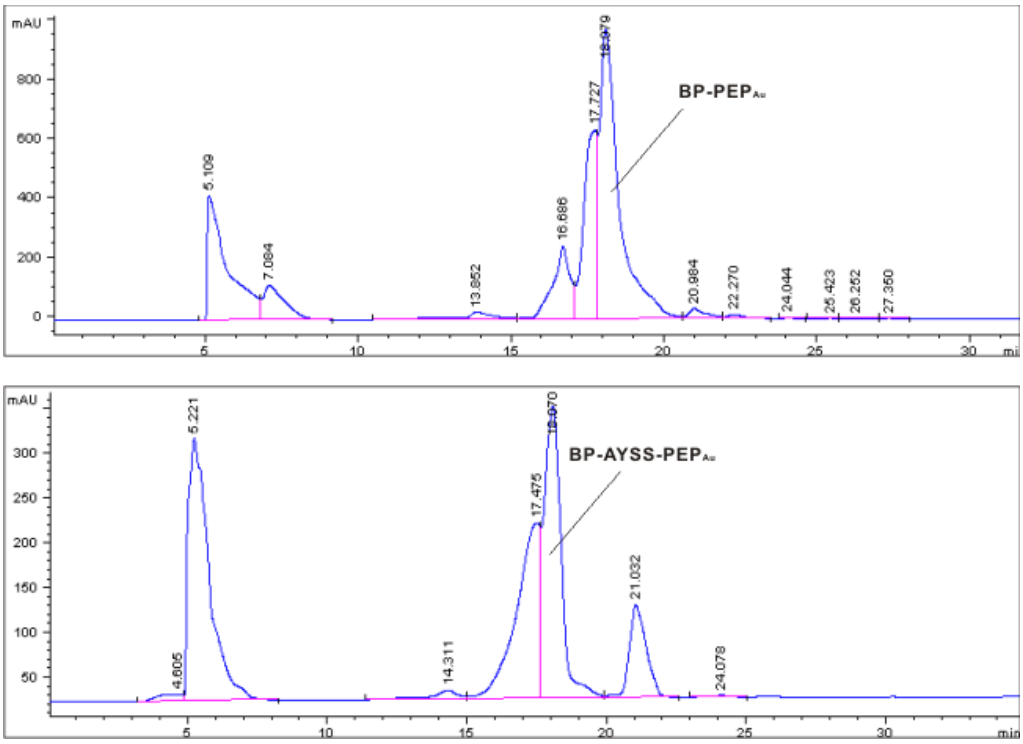


Figure S1. Reverse-phase HPLC chart for the coupling reaction between either BP-PEP$_{Au}$ or BP-AYSS-PEP$_{Au}$ with biphenyl N-hydroxyl-succinimide ester.

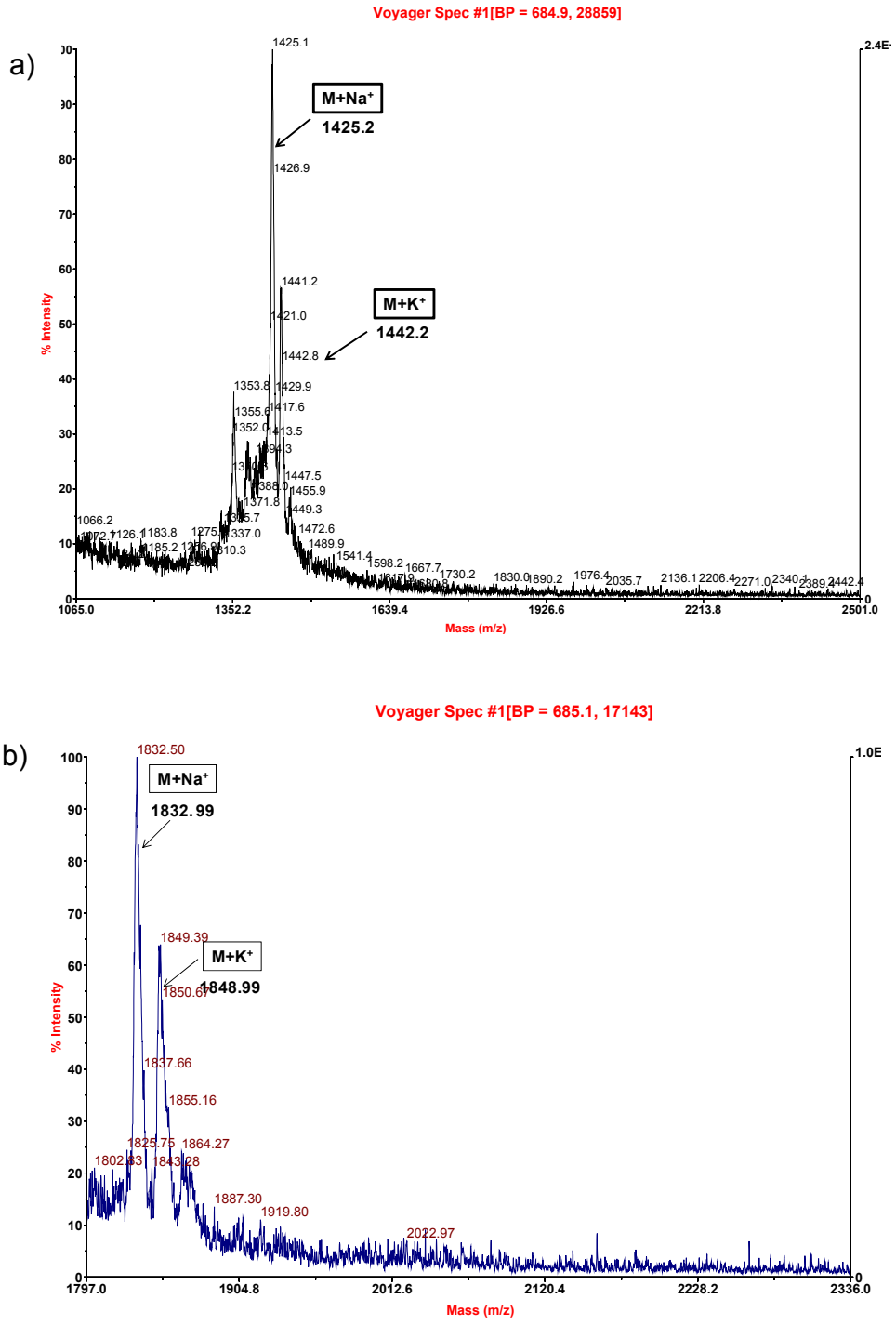


Figure S2. MALDI-TOF mass spectrum of purified BP-PEP_{Au} (Calcd. Mw. = 1402.2) (a) and BP-AYSS-PEP_{Au} (Calcd. Mw. = 1809.99) (b).

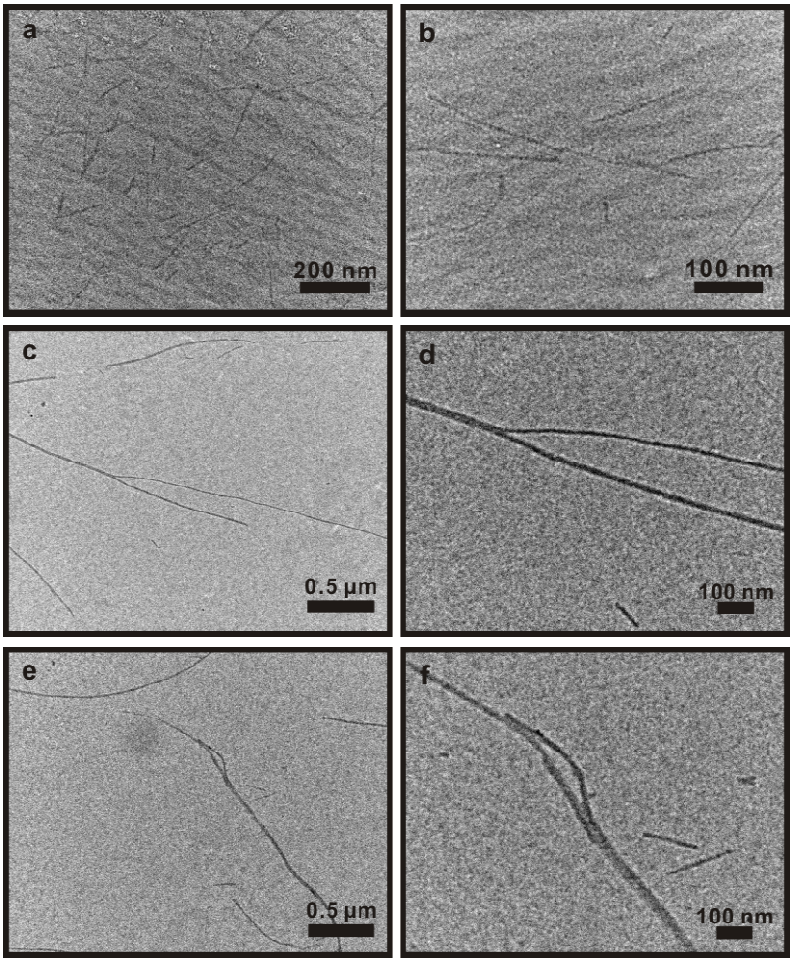


Figure S3. TEM images of BP-AYSS-PEP_{Au} fibers obtained after BP-AYSS-PEP_{Au} conjugates were incubated in 0.1M HEPES buffer (pH 7.3) for 30 min (a-b). STEM images of BP-AYSS-PEP_{Au} fibers obtained after BP-AYSS-PEP_{Au} conjugates were incubated in 0.1M HEPES buffer (pH 7.3) for 6 days (c-f). In all cases, the solution was stained with 2% phosphotungstic acid.

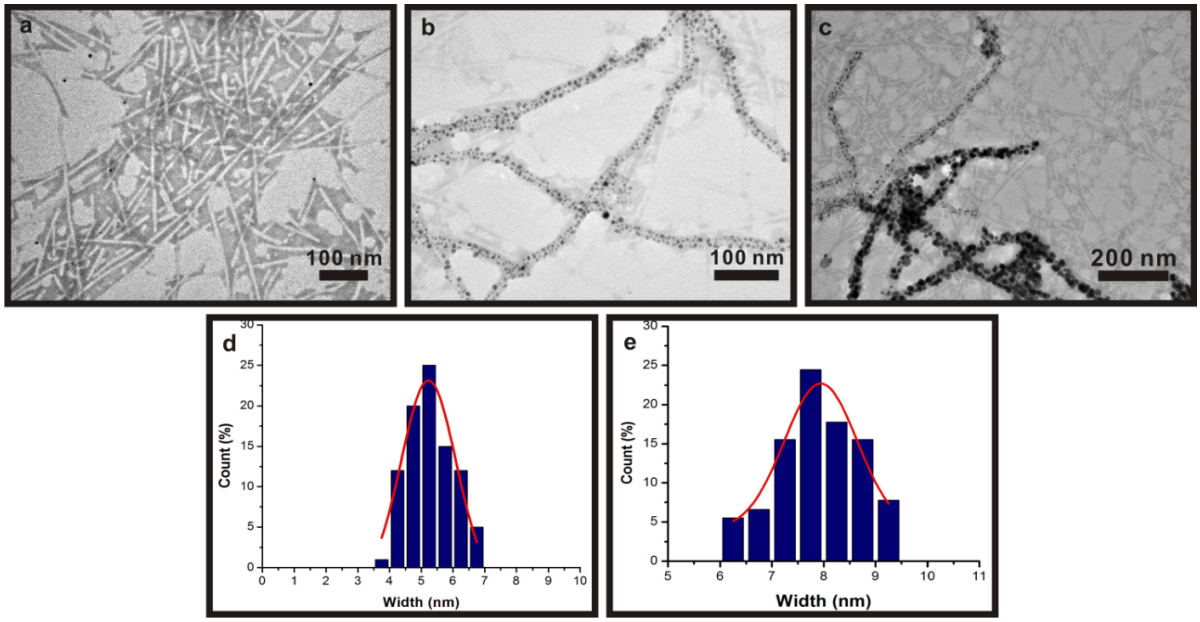


Figure S4. Negative stained TEM images of BP-AYSS-PEP_{Au} fibers and 1-D gold nanoparticle superstructures (a-c) and the width distribution of the BP-AYSS-PEP_{Au} fibers (d) and the gold nanoparticle superstructures (e). The samples used for these images were produced in the following way: 1) BP-AYSS-PEP_{Au} was incubated for 30 min in HEPES buffer and 2) the $HAuCl_4$ solution was added to the BP-AYSS-PEP_{Au} solution and the resulting mixture was allowed to incubate for 30 min. The images show that there is a mixture of fibers decorated with nanoparticles and fibers without any gold nanoparticles assembled onto them. It is clear from images b) and c) that nanoparticles of various sizes are produced. The width of the BP-AYSS-PEP_{Au} fibers is 5.2 ± 0.1 nm, based on 100 counts (d). The width of the gold nanoparticle superstructures is 7.9 ± 0.1 nm, based on 90 counts (e).

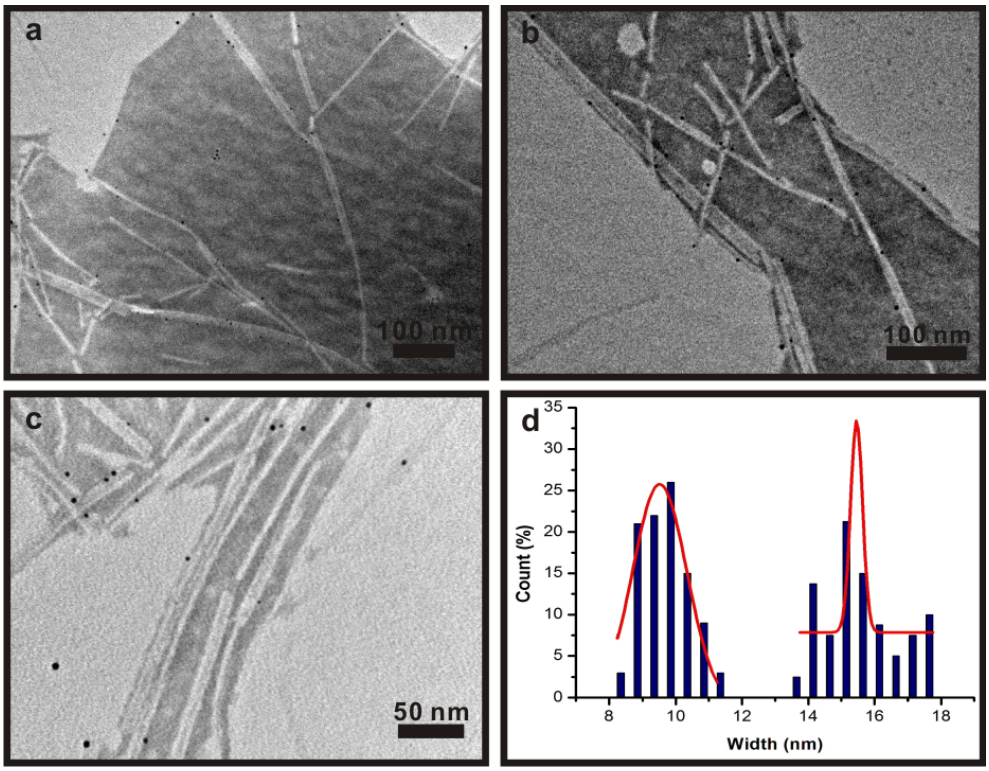


Figure S5. Negative stained TEM images (a-c) BP-AYSS-PEP$_{Au}$ fibers. The samples used for these images were produced in the following way: 1) BP-AYSS-PEP$_{Au}$ was incubated for 6 d in HEPES buffer and 2) the $HAuCl_4$ solution was added to the BP-AYSS-PEP$_{Au}$ solution and the resulting mixture was allowed to incubate for 15 min. The images (a-c) reveal that the fibers begin to stack and align along their longitudinal axes to form bundles of 2-3 fibers. The distribution of the widths of the 2-fiber bundles is 9.5 ± 0.1 nm, based on 100 counts, and the distribution of the widths of the 3-fiber bundles is 15.5 ± 1.7 nm, based on 80 counts (d).

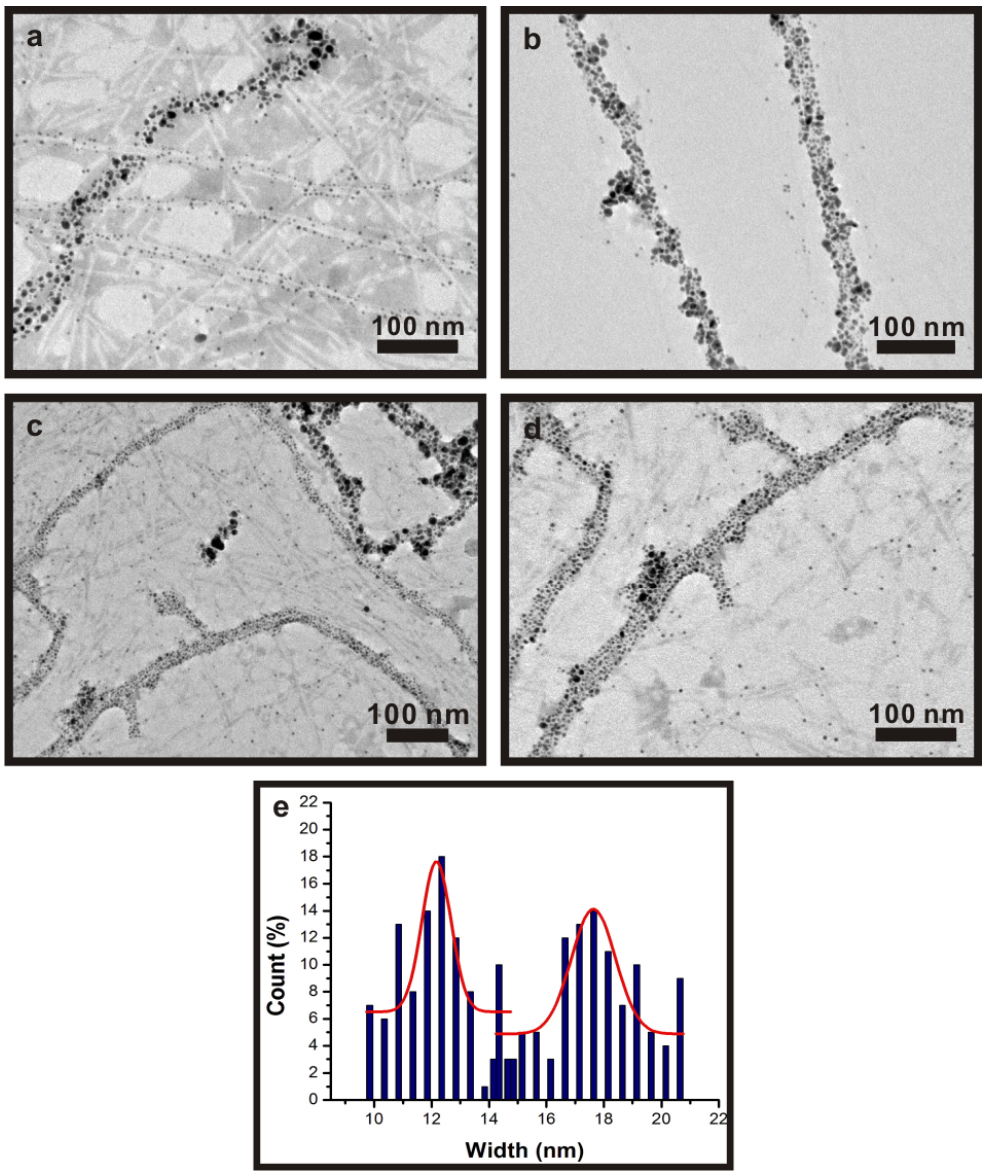


Figure S6. Negative stained TEM images of BP-AYSS-PEP$_{Au}$ fibers and 1-D gold nanoparticle superstructures (a-d) and their width distributions (e). The samples used for these images were produced in the following way: 1) BP-AYSS-PEP$_{Au}$ was incubated for 6 d in HEPES buffer and 2) the $HAuCl_4$ solution was added to the BP-AYSS-PEP$_{Au}$ solution and the resulting mixture was allowed to incubate for 15 min. The images show that there is a mixture of fibers decorated with nanoparticles and fibers without any gold nanoparticles assembled onto them. Because a mixture

of 2- and 3-fiber bundles form under this condition, gold nanoparticle superstructures of different widths are observed. Some structures had widths of 12.2 ± 0.2 nm, based on 100 counts,. Other structures had widths of 17.6 ± 0.2 nm, based on 100 counts, which is consistent with templation by the 3-fiber bundles.

5. References

1. J. Hu, M. Wang, H. U. G. Weier, P. Frantz, W. Kolbe, D. F. Ogletree and M. Salmeron, *Langmuir*, 1996, **12**, 1697-1700.
2. C.-L. Chen, P. Zhang and N. L. Rosi, *J. Am. Chem. Soc.*, 2008, **130**, 13555-13557.