

Supplementary Information

“Light-up” 1D supramolecular nanoprobe for silver ion based on an assembly of pyrene-labeled peptide amphiphiles: Cell imaging and antimicrobial activity

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Materials and Instruments. Rink Amide MBHA (100-200 mesh) and 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetra-methyluronium hexafluoro phosphate (HBTU) from Merck were used as received. Fmoc-His(Trt)-OH, Fmoc-Gly-OH, Fmoc-Lys(Fmoc)-OH, 1-pyrenebutyric acid (97%) were purchased from Beadtech, Merck, Sigma-Aldrich, and Alfa Aesar, respectively. N,N-dimethylformamide (DMF, 99.5%) from Fisher and dichloromethane (DCM, 99.5%), N-methylpyrrolidone (NMP, 99.7%), piperidine (99%) from Daejung Chemicals and N-Ethyldiisopropylamine (DIPEA, 99 %) from Merck were used as received. Trifluoroacetic acid (TFA, 99%) from Sigma-Aldrich and Methyl phenyl sulfide (99%) from Merck and 1,2-ethanedithiol (98%) from Merck were used as received. 12-aminododecanoic acid (12-ADDA) was prepared according to the procedures described previously.^{S1} NiSO₄·6H₂O, MgSO₄, MnSO₄·H₂O, 0.1M ZnSO₄, AgNO₃, CoCl₂·6H₂O, FeCl₂, Pb(NO₃)₂, CuSO₄, CaCl₂, NaBH₄ were purchased from Daejung Chemicals, Sigma-Aldrich, KANTO and Junsei.

Techniques. Peptide amphiphiles (PAs) were sequenced using CEM Focused MicrowaveTM Synthesis System, Discover and purified by using YL9100 HPLC equipped with a C-18 reverse phase chromatographic column. Mass spectrometry was performed on a Bruker Ultraflexextreme MALDI-TOF/TOF mass spectrometer using a matrix, α -cyano-4-hydroxycinnamic acid dissolved in acetonitrile : water = 1 : 1 mixed solution (0.1% TFA). UV/Vis absorption spectra were obtained from a UV-1800 Shimadzu UV spectrometer. The fluorescence spectra were obtained from a Perkin Elmer LS-55 fluorescence spectrometer. Dynamic light scattering (DLS) experiment was performed using ELS-Z (Otsuka Electronics, Japan). Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were recorded on ALPHA-P (Bruker Optic GmbH) using ZnSe pellet. Fluorescence microscopy was performed using a Nikon Eclipse Ti-E microscope. Transmission electron microscopy (TEM) images were

taken from 300 kV in-situ and 120 kV TEM (JEM-3011 HR and JEM-1400, respectively). Geometrical structure optimization of HG12 was carried out with Forcite, VAMP in Material Studio 6.0. The secondary structure was determined by a Jasco J-815 circular dichroism (CD) spectrometer (Jasco Inc., Japan) using a quartz cuvette (path length of ~1.0 cm) at 20 °C.

1. Synthesis of peptide amphiphiles (PAs) 1 and 2

Synthesis of 1. The pyrene-labeled **1** was synthesized on Rink Amide MBHA resin by conventional Solid Phase Peptide Synthesis procedure using CEM Focused MicrowaveTM Synthesis System, Discover. After the resin was washed with DCM, it was swollen in 1:1 mixed solution of DMF and DCM for over 30 min in shaking incubator. Then, the Fmoc-His(Trt)-OH (5 equiv.), Fmoc-Gly-OH (5 equiv.) were coupled to the N-terminus of the peptide on resin in a sequence of GHGGGHGHGGGH-Resin. **1** was synthesized by directly attaching of Fmoc-12-aminododecanoic acid to HG 12 peptide, followed by 1-pyrenebutyric acid. And then the resin was treated with a cleavage solution (TFA : 1,2-ethanedithiol : thioanisole = 95 : 2.5 : 2.5) for over 2 h and the mixture solution was triturated with *tert*-butyl methyl ether. **1** was purified by reverse phase HPLC on C18 column using linear gradient of water (0.1% TFA) and acetonitrile (0.1% TFA). The molecular weight of **1** was confirmed by MALDI-TOF/TOF mass spectrometry.

Synthesis of 2. The pyrene-labeled **2** was synthesized on Rink Amide MBHA resin by conventional Solid Phase Peptide Synthesis procedure using CEM Focused MicrowaveTM Synthesis System, Discover. After the resin was washed with DCM, it was swollen in 1:1 mixed solution of DMF and DCM for over 30 min in shaking incubator. Then, the Fmoc-His(Trt)-OH (5 equiv.), Fmoc-Gly-OH (5 equiv.) were coupled to the N-terminus of the peptide on resin in a sequence of GHGGGHGHGGGH-Resin. To synthesize **2** with branched alkyl chain, Fmoc-Lys(Fmoc)-OH was coupled to GHGGGHGHGGGH-Resin, followed by Fmoc-12-aminododecanoic acid and 1-pyrenebutyric acid. And then the resin was treated with a cleavage solution (TFA : 1,2-ethanedithiol : thioanisole = 95 : 2.5 : 2.5) for over 2 h and the mixture solution was triturated with *tert*-butyl methyl ether. **2** was purified by reverse phase HPLC on C18 column using linear gradient of water (0.1% TFA) and acetonitrile (0.1% TFA). The molecular weight of **2** was confirmed by MALDI-TOF/TOF mass spectrometry.

2. Fabrication of hybrid metal nanoparticles (NPs)-nanofibrils (NFs)

Fabrication of NFs and metal NPs-incorporated NFs. NFs were prepared by dissolving the PAs in distilled water. For the measurement of binding affinity of NFs toward various metal ions, the solution of metal ion was dropwise added to the PA solution and vigorously stirred for 5 min. After addition of 10 equiv. of reducing agent sodium borohydride (NaBH₄), the reaction mixtures were left vigorous stirring overnight under nitrogen to complete reduction of metal ions on the NFs, resulting in the formation of NPs-NFs.

Preparation of NFs, AgNPs and AgNPs-NFs. PA **2** was dissolved in distilled water to form NFs. The solution of silver nitrate (5 mM) was dropwise added to the NFs of **2** under vigorous stirring. After addition of 10 equiv. of reducing agent NaBH₄ (10 mM), the reaction mixtures were left vigorous stirring overnight under nitrogen to complete reduction of metal ions on the NFs, resulting in the formation of AgNPs-NFs.

Pure AgNPs were prepared in aqueous solution by the reduction of silver nitrate with NaBH_4 .^{S2} The AgNPs-NFs maintain stability for up to 3 months of storage at room temperature in air.

3. Characterization of NFs

Transmission electron microscopy (TEM). A drop of each sample in aqueous solution was placed on a formvar/carbon-coated copper grid and allowed to evaporate under ambient conditions. When sample was stained, a drop of uranyl acetate solution (2 wt%) placed onto the surface of the sample-loaded grid. The sample deposited about 1 min at least, and excess solution was wicked off by filter paper. The specimen was observed with a JEOL-JEM-3011 HR operating at 300 kV and JEM-1400 operating at 120 kV. The data were analyzed with Gatan Digital Micrograph program.

Cryogenic TEM (cryo-TEM). Cryo-TEM experiments were performed with a thin film of aqueous solution of sample (4 μL) transferred to a lacey supported grid by plunge-dipping method. The thin aqueous films were prepared at ambient temperature and with humidity of 97-99% within a custom-built environmental chamber in order to prevent evaporation of water from sample solution. The excess liquid was blotted with filter paper for 2-3 sec, and the thin aqueous films were rapidly vitrified by plunging them into liquid ethane (cooled by liquid nitrogen) at its freezing point. The specimen was observed with a JEOL-JEM-3011 HR operating at 300 kV. The data were analyzed with Gatan Digital Micrograph program.

Measurement for critical micellar concentration (CMC) of PAs.^{S3} Fluorescence measurements were carried out on a Perkin Elmer LS-55 Fluorescence Spectrometer with excitation wavelength of 340 nm and the emission spectrum was recorded from 370 nm to 600 nm. The fluorescence intensities of the peaks at ~ 396 nm (I_{III}) and ~ 378 nm (I_{I}) were extracted from the spectra and the $I_{\text{III}}/I_{\text{I}}$ value vs. concentration of the solution was used for CMC determination.

Circular dichroism (CD) measurement. CD spectra were measured using a JASCO J-815 spectropolarimeter. Spectra were monitored from 260 nm to 190 nm using a 1.0 cm light path-length cuvette, and scans were repeated three times and averaged. Molar ellipticity was calculated per amino acid residue.

Metal ion binding study by UV-Vis and fluorescence spectrometer. To examine optical properties upon the complexation of metal ions with **1** and **2**, a variety of metal ions, including Cu^{2+} , Fe^{2+} , Ni^{2+} , Co^{2+} , Mg^{2+} , Zn^{2+} , Mn^{2+} , Ag^+ , Ca^{2+} , and Pb^{2+} were added to PA solutions, respectively. All spectra were recorded from 200 nm to 900 nm with a 10 mm light path length of cuvette.

Determination of binding stoichiometry (Job's Plot). The binding stoichiometry of NFs-metal ion complexes was determined by Job's plot analysis. The fluorescence intensity at 396 nm was plotted against molar fraction of **1** and **2** under a constant total concentration. When the emission intensity reaches a maximum point, the molar fraction represents the binding stoichiometry of NFs-metal ion complexes.

4. Cell imaging and antimicrobial activity measurements

Cellular fluorescence imaging. HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). All cells were supplemented with an antibiotic–antimycotic solution (100 units/mL penicillin, 0.1 mg/mL streptomycin, and 0.25 mg/mL amphotericin B) and grown at 37 °C in standard cell culture conditions (5% CO₂, 95% humidity) for 12 h. Cl[−]-free buffer of 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, pH 6.8) containing 1.0 mM NaNO₃ was chosen to avoid the formation of insoluble AgCl precipitates.^{S4} Cells were first loaded with 10 μM of **2** in HEPES at 37 °C for 1 h and were washed three times with 20 mM HEPES buffer solution (1.0 mM NaNO₃, pH 6.8) to remove the free **2**. The weak fluorescent intensity of the cells was confirmed and then the cells were further incubated with 150 μM AgNO₃ for 30 min in 20 mM HEPES (pH 6.8) containing 1.0 mM NaNO₃.

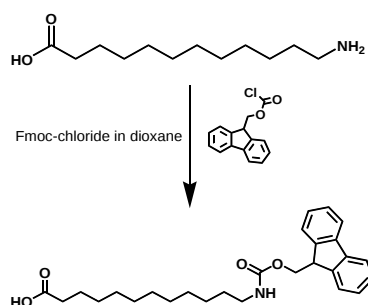
Cell viability test. For live/dead assay, HeLa cells (epithelial cervical cancer cell line) were incubated in cell culture plate with DMEM containing 10% FBS and 1% antibiotics at 37 °C and 5% CO₂ to allow cell attachment and spreading to occur. After incubation for 12 h, the solution of **2** and AgNPs-NFs were added to each culture plate and incubated for 1 h at 37 °C and 5% CO₂, respectively. Then, the assay solution containing 4 μM Calcein AM and 2 μM ethidium homodimer-1 (EthD-1) in PBS buffer which was prepared according to the live-dead assay (Molecular Probes #L3224) package instructions was added to each culture plate. After incubation for another 20 min, cellular viability was visualized using a fluorescence microscope (Nikon, eclipse TE-2000, Japan) equipped with a high-resolution CCD camera (Coolsnap, Roper Science, USA) with excitation filters of 494 nm (green, Calcein) and 528 nm (red, EthD-1).

Bacteria strain and cultivation.^{S5} Two types of bacteria were employed: *Bacillus subtilis* (*B. subtilis*) as the Gram-positive model, and *Escherichia coli* O157:H7 (*E. coli* O157:H7) as the Gram-negative model. The *E. coli* O157:H7 were transformed by electroporation with plasmid pMRP9-1, which constitutively expressed green fluorescent protein (GFP). The *E. coli* O157:H7 were grown in Luria Burtani (LB) medium supplemented with erythromycin (300 μg/ml) at 37 °C and 200 rpm, and the *B. subtilis* were grown in pure LB medium. After overnight incubation, bacteria were harvested by centrifugation at 5000 rpm, and bacterial pellet was resuspended with fresh LB media to conduct batch and microfluidic experiment.

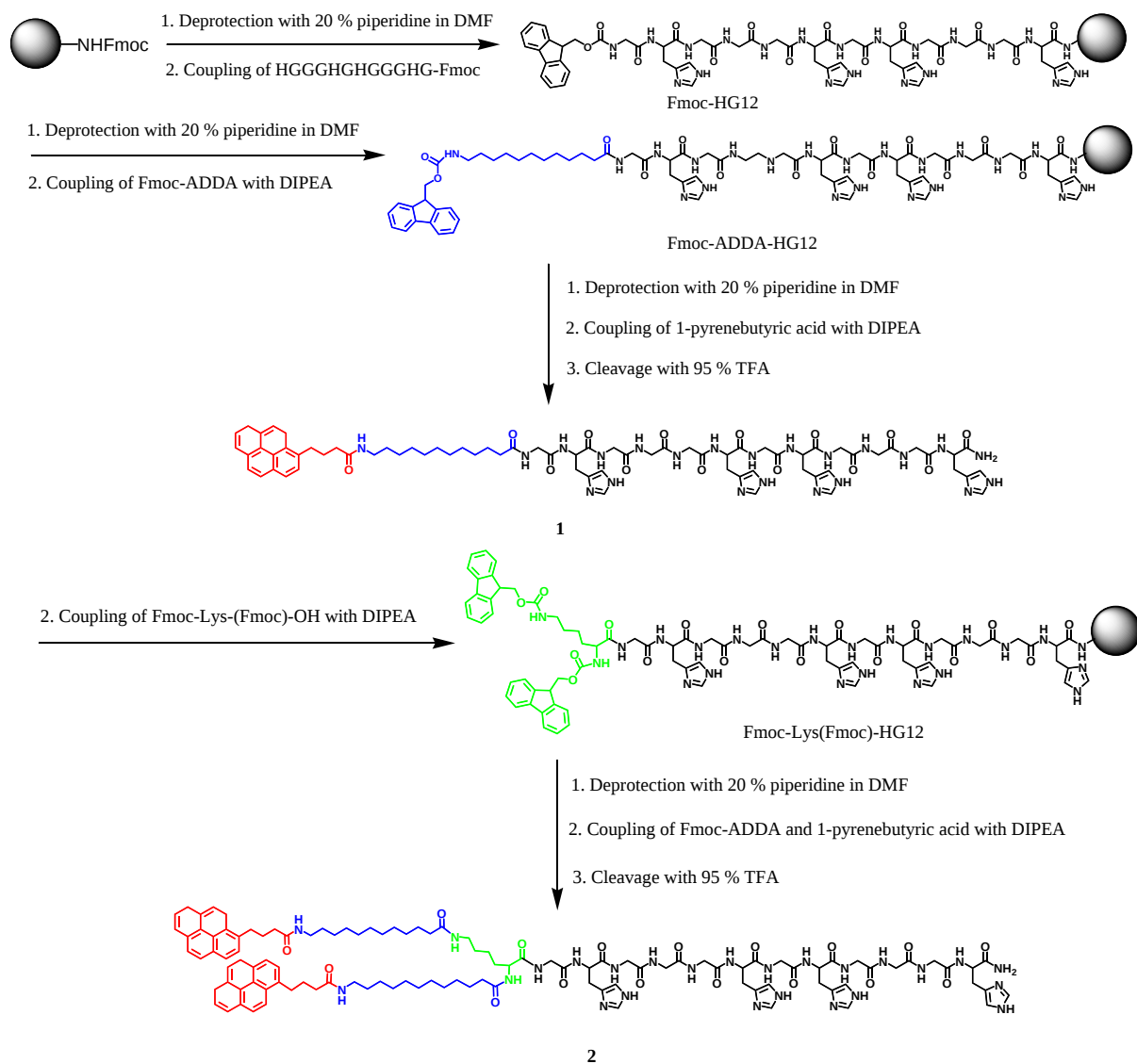
Bacteria kinetic test. For the growth-curve experiments, a starter culture of each strain was inoculated with fresh colonies and incubated for 14 h overnight in LB media. Fresh media were inoculated with the starter culture and grown to an OD₆₀₀ of 0.1 at 37 °C with continuous agitation at 200 rpm. LB broth containing bacteria was used as a positive control. Bacterial growth behaviors were determined by measuring the optical density at 600 nm using a UV spectrophotometer (UV-Vis 300, Thermo Scientific, USA).

Fabrication of microfluidic devices. Silicon master was fabricated with a SU-8 photoresist using photolithography. The microfluidic device was fabricated using a conventional soft lithography technique. Poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow-Corning, USA) prepolymer and its curing agent (10:1 ratio) were mixed and then degassed in a vacuum oven. The mixture was poured over the master mold and cured at 65 °C for 4 h. Finally, the multilayer of PDMS mold and a glass slide were rinsed sequentially with ethanol and distilled water, dried with nitrogen gas, exposed to oxygen plasma (PDC-002, Harrick, USA) for 30 sec, and bounded to each other.

Microfluidic experiment. To monitor antimicrobial effect in microchannel, the bacterial solutions ($OD_{600} = 0.1$) suspended in LB media are introduced into each inlet port in microchannel by syringe pump. Four types of microchannels were adopted for loading the bacterial solution, NFs, AgNPs and AgNPs-NFs solutions, respectively. All fluorescence images were acquired with a fluorescence microscope (NIKON, TE-2000, Japan) equipped with a high-resolution CCD camera (Coolsnap, Roper Science, USA).



Scheme S1 Synthesis of 12-aminododecanoic acid (12-ADDA).^{S1}



Scheme S2 Synthesis of PAs **1** and **2**.

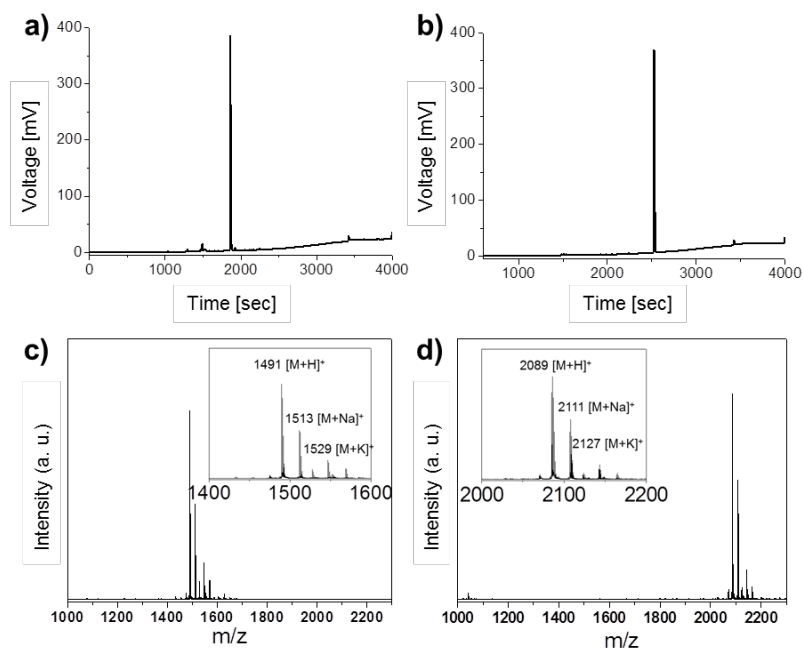


Fig. S1 (a,b) HPLC and (c,d) MALDI-TOF/TOF MS spectra of **1** and **2**, respectively.

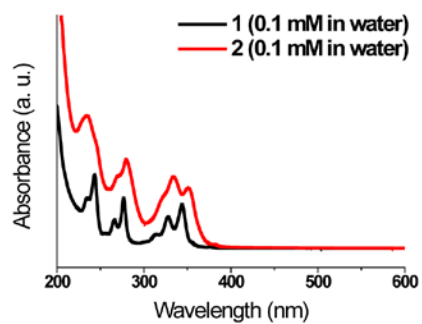


Fig. S2 Absorption spectra of **1** and **2**.

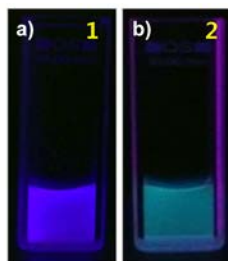


Fig. S3 Optical images of aqueous solution of (a) **1** and (b) **2** under a transilluminator when irradiated at 365 nm, respectively.

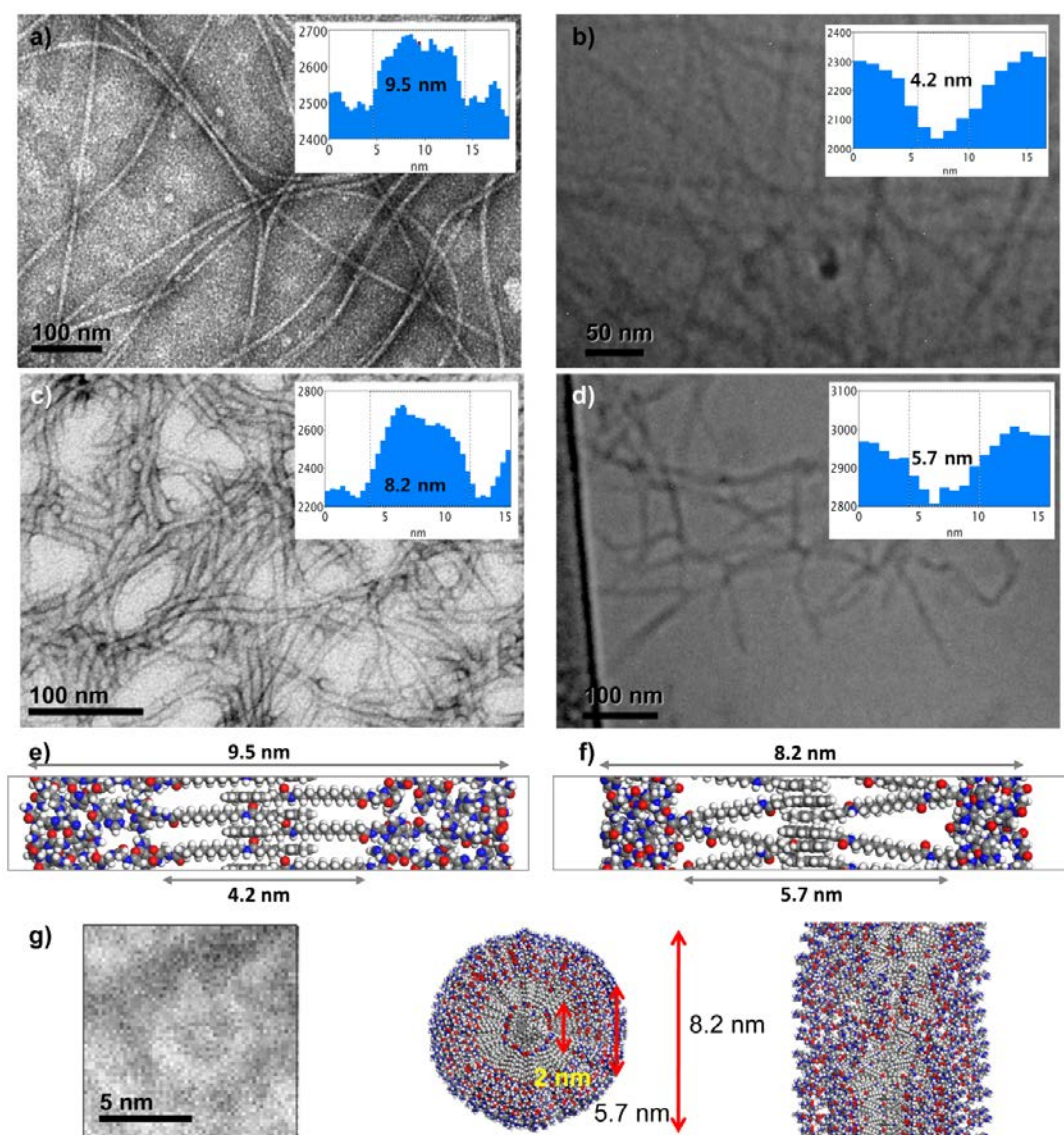


Fig. S4 (a,c) TEM and (b,d) cryo-TEM images of aqueous solution of **1** and **2**, respectively (inset, density profile indicating the width of NF). Proposed molecular packing within the NFs of (e) **1** and (f) **2** based on TEM and fluorescence spectroscopy. (g) Top-view TEM image of NF formed from **2** and the related modeling structure.

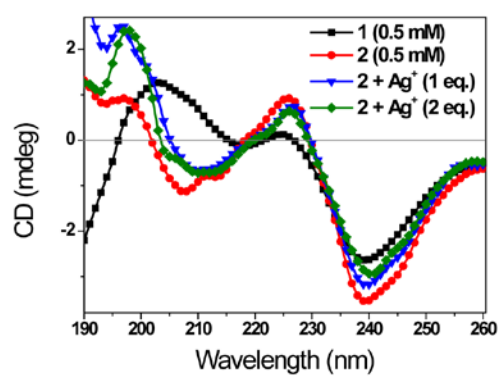


Fig. S5 CD spectra of **1**, **2** and **2** upon addition of Ag⁺.

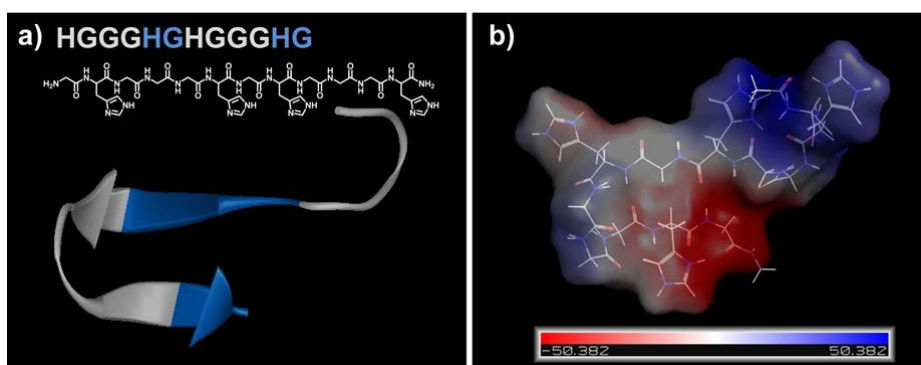


Fig. S6 β -turn structure of HG12 was drawn by using (a) DNA star and (b) PyMol modeling programs.

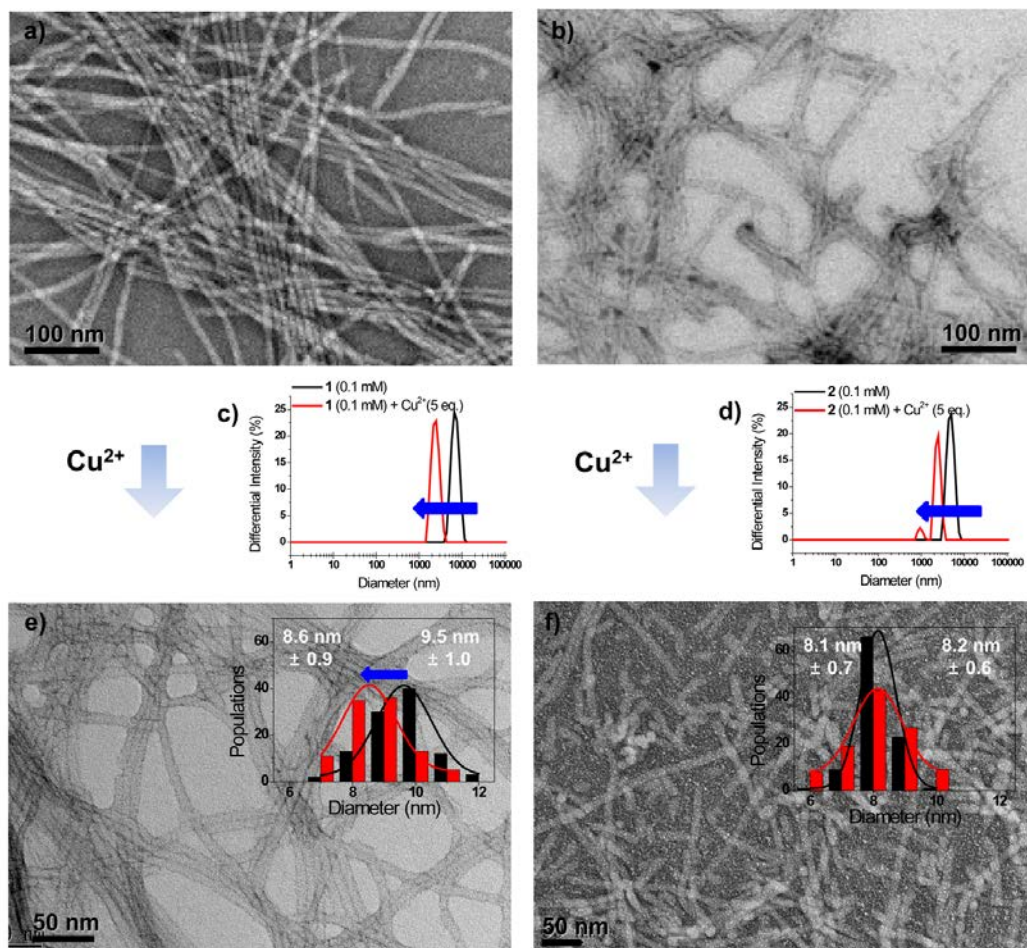


Fig. S7 Supramolecular complexes of NF-Cu²⁺ (0.1 mM). Negatively stained TEM images of bare NFs of (a) **1** and (b) **2**. The change in the average hydrodynamic diameters of (c) **1** and (d) **2** upon addition of 5 equiv. of Cu²⁺. TEM images of NFs of (e) **1** and (f) **2** after addition of Cu²⁺ (inset: change in the width averaged over 100 NFs).

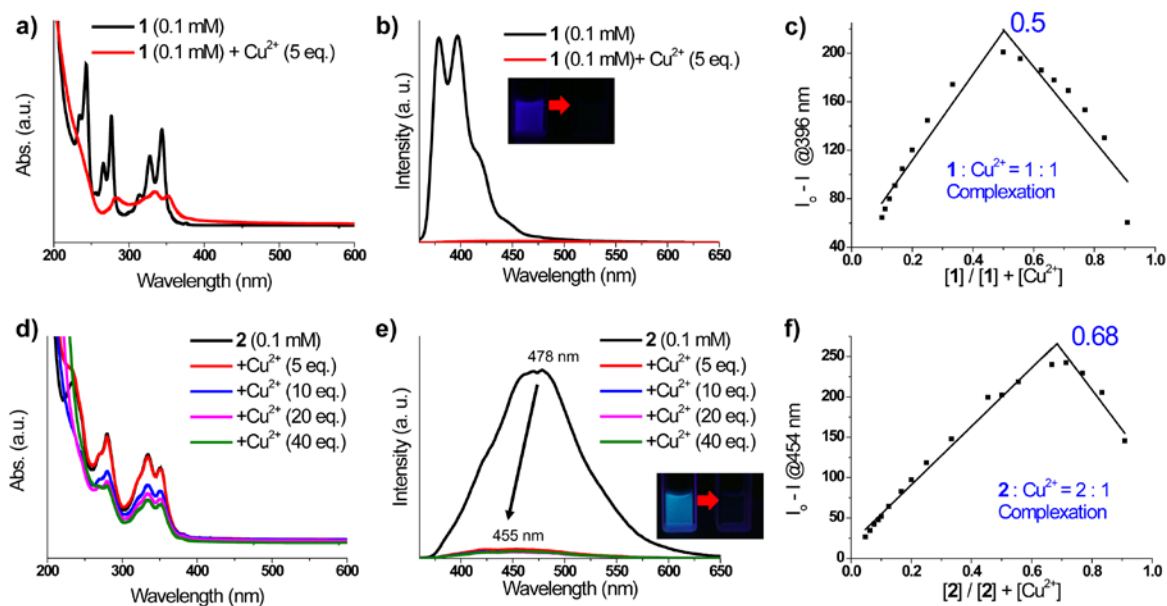


Fig. S8 (a,d) Absorption and (b,e) emission spectra of **1** and **2** upon addition of Cu^{2+} , respectively. (c,f) Job's plot analysis of PA- Cu^{2+} complex (in an aqueous solution of 0.1 mM).

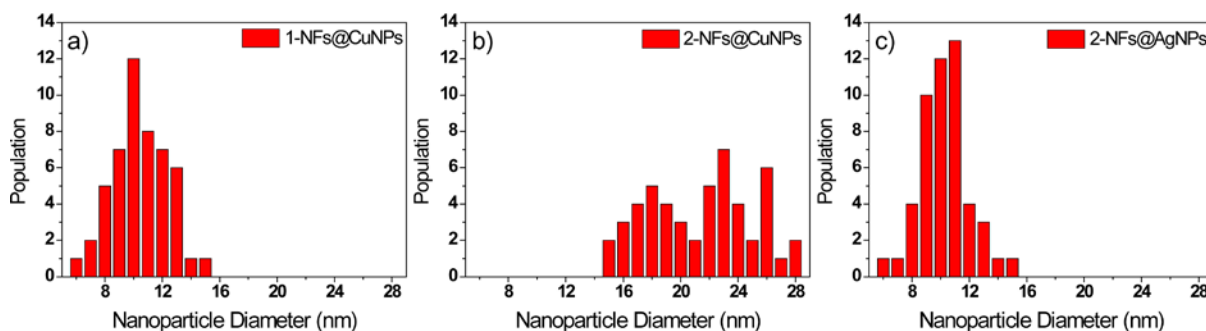


Fig. S9 The diameters of NPs on NFs observed in TEM image. Averaged over 50 NPs, the diameters of (a) CuNPs on 1-NFs, (b) CuNPs on 2-NFs, and (c) AgNPs on 2-NFs were determined to 11.9 ± 2.6 nm, 21.6 ± 3.7 nm, and 10.3 ± 1.7 nm, respectively.

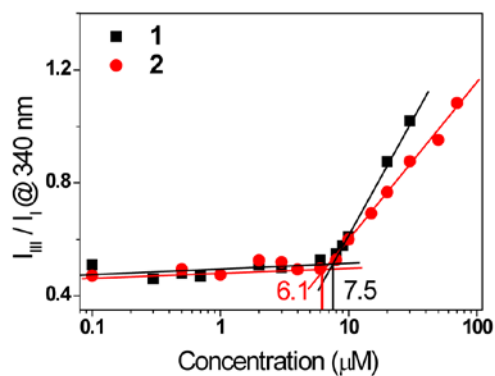


Fig. S10 Plots of pyrene I_{III}/I_I ratio versus concentration for **1** and **2**. The CMC was estimated by intersection of the two linear regression lines.

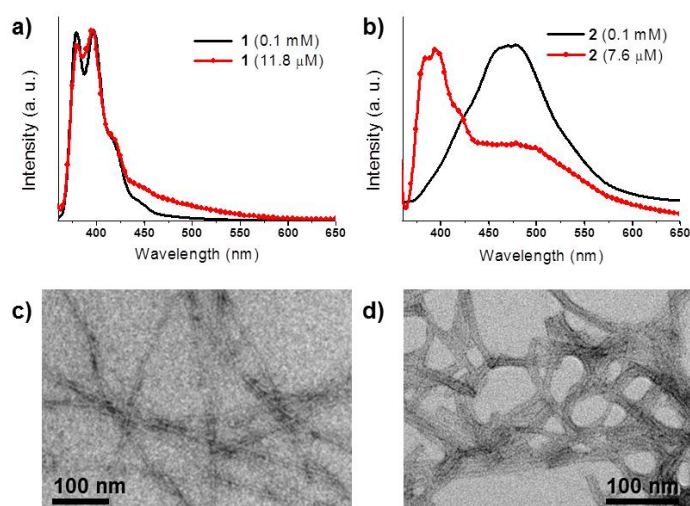


Fig. S11 Fluorescence emission spectra of (a) **1** and (b) **2** as varying the concentration. Negatively stained TEM images of aqueous solution of (c) **1** (11.8 μM) and (d) **2** (7.6 μM).

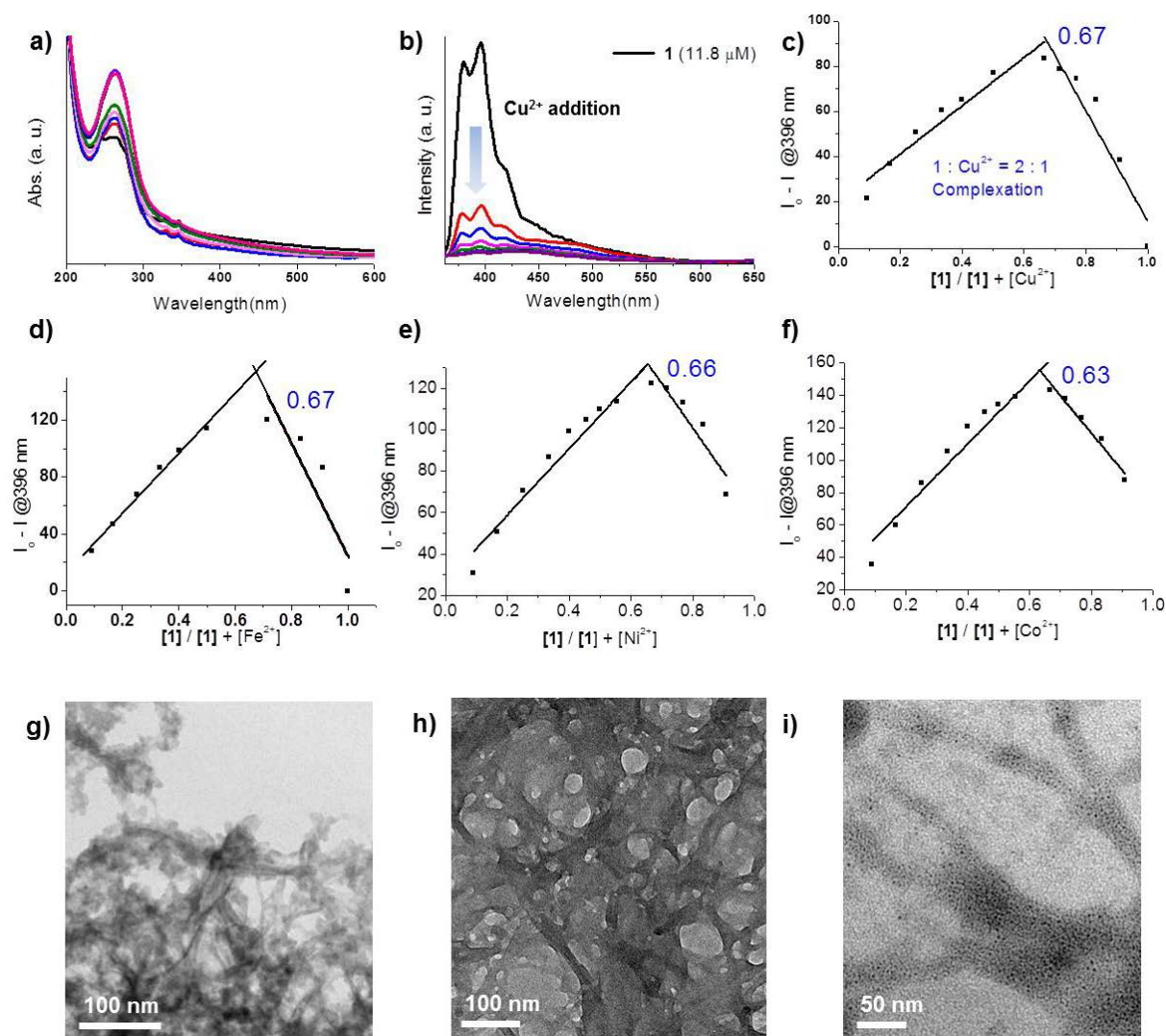


Fig. S12 (a) Absorption and (b) emission spectra of **1** upon addition of Cu^{2+} . Job's plots of **1** with (c) Cu^{2+} , (d) Fe^{2+} , (e) Ni^{2+} and (f) Co^{2+} complexes (in an aqueous solution of 11.8 μM). (g-i) TEM images of NFs of **1** after incubation with iron, nickel, and cobalt solutions and subsequent reduction with NaBH_4 .

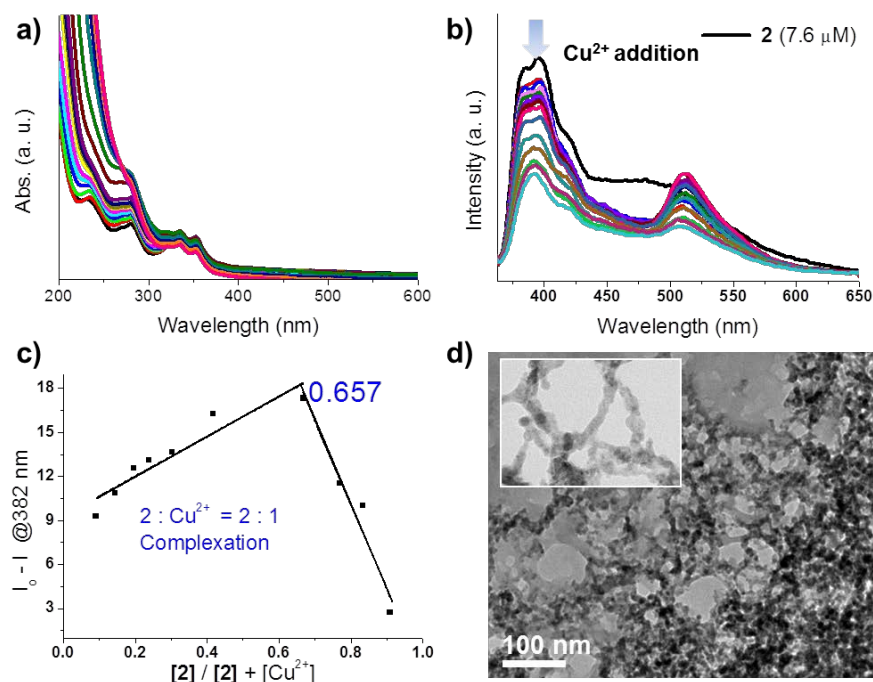


Fig. S13 (a) Absorption and (b) emission spectra of **2** upon addition of Cu^{2+} . Job's plot analysis of (c) PA- Cu^{2+} complex (in an aqueous solution of $7.6 \mu\text{M}$). (d) TEM images of NFs of **2** after incubation with a copper solution and subsequent reduction with NaBH_4 (Inset, enlarged image of hybrid NFs).

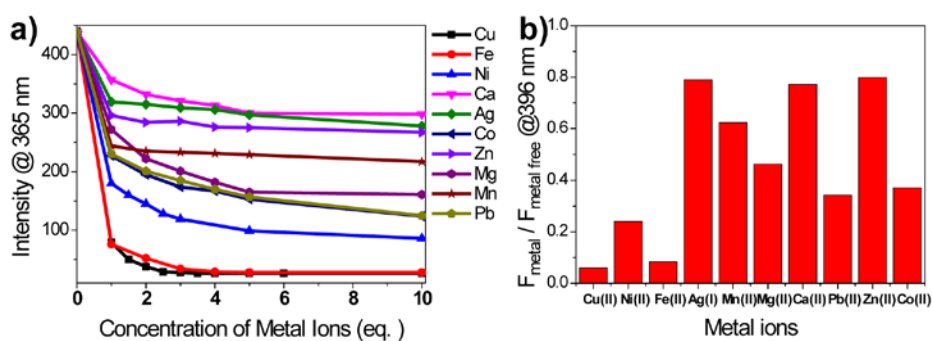


Fig. S14 (a) Change in the fluorescence intensity of **1** ($11.8 \mu\text{M}$) upon addition of various metal ions in water. (b) Fluorescence intensity ratios ($F_{\text{metal}}/F_{\text{metal free}}$) for **1** at 396 nm in the presence of 10 equiv. of various metal ions. The titration almost reached saturation with the addition of 10 equiv. of metal ion.

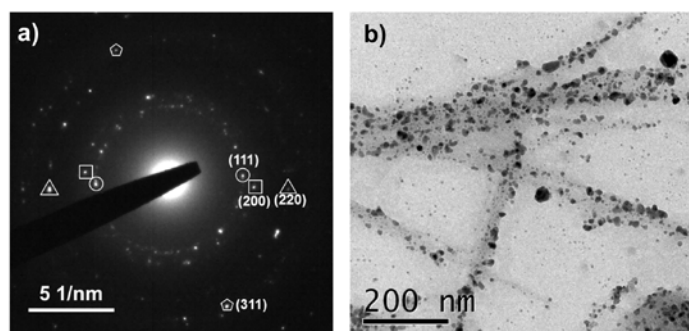


Fig. S15 AgNPs generation of NF of **2** at a concentration of 0.1 mM. (a) SAED confirms the silver face-centered cubic crystal lattice (d -spacing_{Ag} = 2.36 Å (111), 2.04 Å (200), 1.45 Å (220), 1.23 Å (311)).^{S6} (b) TEM image of AgNPs-decorated NFs (No staining was used).

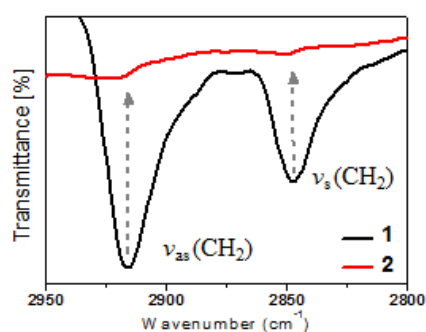


Fig. S16 FTIR spectra (2800-2950 cm⁻¹) of an aqueous solution of **1** and **2** (11.8 μM and 7.6 μM, respectively).

Ionic radius (Å)							
Ag ⁺	Pb ²⁺	Ca ²⁺	Fe ²⁺	Co ²⁺ Zn ²⁺	Cu ²⁺ Ni ²⁺	Mg ²⁺	Mn ²⁺
1.26	1.21	0.99	0.76	0.74	0.72	0.65	0.46

Fig. S17 Ionic radius of metal ions used in this study.

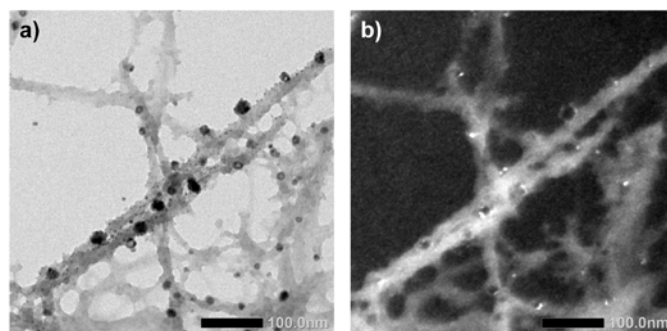


Fig. S18 (a) Bright and (b) dark field images of AgNPs-NFs.

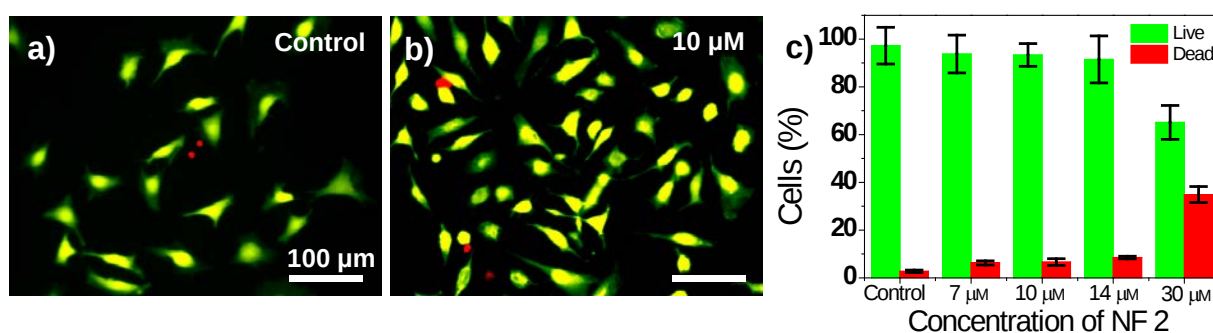


Fig. S19 Fluorescence live-dead assay on the HeLa cells incubated with different concentrations of NFs 2: (a,b) Scale bar represents 100 μm. Live cells were stained with Calcein AM (green fluorescence) while dead cells were stained with EthD-1 (red fluorescence). (c) Quantitative representation of cellular viability.

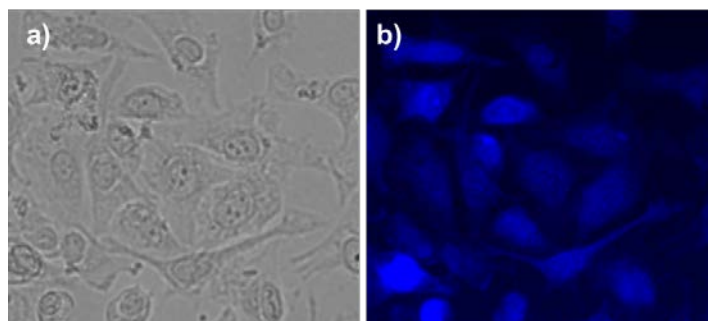


Fig. S20 (a) Bright field microscopy and (b) confocal laser scanning microscopy images of HeLa cells with 2 (10 μM) in the presence of AgNO₃ (150 μM), indicating that NFs 2 can penetrate HeLa cells.

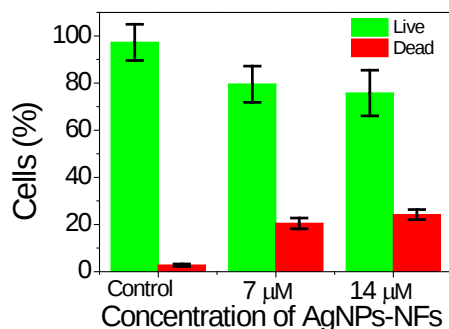


Fig. S21 Cell viability studies. Quantitative representation of cellular viability by a live-dead assay of the HeLa cells incubated with different concentrations (7 μ M and 14 μ M, respectively) of AgNPs-NFs.

5. Abbreviations

PA-peptide amphiphile

NPs-nanoparticles

NFs-nanofibrils

6. References for SI

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