Nanofibrous Hydrogels Self-Assembled from Naphthalene

Diimide (NDI)/Amino Acid Conjugates

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i) Fmoc-L-AA-R1, DIEA; ii) 20 % piperidine; iii) NDIA, HBTU, DIEA; iv) TFA:water=9:1 vol%

Scheme S1. The synthetic routes for NDI-Ser (1) and NDI-Lys (2).

Synthesis of NDI-Ser (1): The amino acid/dye conjugate derivative of 1 was prepared through SPPS using 2-chlorotrityl chloride resin, Fmoc-O-tert-Butyl-L-serine, and NDIA (Scheme S1). The resin (1.2 g) was swelled in anhydrous CH₂Cl₂ for 30 min and then Fmoc-O-tert-Butyl-L-serine (0.767 g, 2.000 mmol) was loaded onto the resin in anhydrous N,N-dimethylformamide and N,N-diisopropylethylamine (DIEA; 0.830 mL, 5.000 mmol) for 1 h. For deprotection of the Fmoc group, piperidine (20% in DMF) was added and the sample left for 20 min; this procedure was repeated twice (each time for 2 min). Finally, NDIA (0.873 g, 2.000 mmol) was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU; 0.759 g, 2.000 mmol) and DIEA (0.830 mL, 5.000 mmol) as coupling agents. After the reaction mixture had been stirred overnight, the peptide derivative was cleaved through treatment with CF₃CO₂H (90% in DI water) for 3 h. The resulting solution was dried by air and then Et₂O was added to precipitate the target product. The solid was dried under vacuum to remove residual solvent (dark red solid: 0.281 g). ¹H NMR (300 MHz, [D₆]DMSO, 25°C): δ =0.85-0.95 (m, 3H; CH₃), 1.20-1.45 (m, 10H; CH₂), 1.60-1.80 (m, 2H; CH₂), 3.60-3.70 (m, 1H; CH₂), 3.70-3.80 (m, 1H; CH₂), 4.07 (t, *J*=7.2 Hz, 2H; CH₂), 4.30-4.40 (m, 1H; CH), 4.83 (s, 2H; CH₂), 8.61 (d, *J*=7.8 Hz, 1H; NH), 8.65-8.75 (m, 3H; CH), 8.75-8.80 (m, 1H; CH) ; ¹³C NMR (75 MHz, [D₆]DMSO): δ =14.0, 22.1, 26.5, 27.3, 28.6, 28.7, 31.3, 42.4, 54.8, 61.5, 125.7, 126.0, 126.1, 126.2, 126.5, 130.4, 130.7, 130.8, 162.3, 162.4, 166.3, 171.8; MS [ESI⁻]: calcd. *m/z* 523.20, obsvd. 522.4 [*M*-H]⁻.

Synthesis of NDI-Lys (2): In a manner similar to that described above, the amino acid derivative of **2** was prepared by a mixture of N_{α} -Fmoc- N_{e} -Boc-L-lysine (0.937 g, 2.000 mmol), DIEA (0.830 mL, 5.000 mmol), 20 % piperidine in DMF, NDIA (0.873 g, 2.000 mmol), HBTU (0.759 g, 2.000 mmol) and DIEA (0.830 mL, 5.000 mmol) to obtain dark brown solid (0.422 g). ¹H NMR (300 MHz, [D₆]DMSO, 25°C): δ =0.85-0.95 (m, 3H; CH₃), 1.25-1.45 (m, 12H; CH₂), 1.55-1.90 (m, 6H; CH₂), 2.83 (t, *J*=7.5 Hz, 2H; CH₂), 4.10 (t, *J*=7.2 Hz, 1H; CH₂), 4.25-4.35 (m, 1H; CH) 4.79 (s, 2H; CH₂), 7.74 (br, 3H; NH), 8.63 (d, *J*=8.1 Hz, 1H; CH), 8.70-8.75 (m, 3H; CH), 8.75-8.80 (m, 1H; CH) ; ¹³C NMR (75 MHz, [D₆]DMSO): δ =14.0, 22.1, 22.4, 26.5,

26.6, 27.4, 28.6, 28.7, 30.6, 31.3, 35.9, 42.5, 51.9, 125.6, 125.87, 125.90, 126.1, 126.3, 130.4, 130.7, 162.2, 162.3, 166.4, 173.3; MS [ESI⁺]: calcd. *m/z* 564.26, obsvd. 565.2 [*M*+H]⁺.

Inverted tube method: Gelation tests were performed by weighing a compound (2.0 mg) in a screw-capped 2-mL vial (diameter: 10 mm), adding a solvent (0.20 mL), sealing the vial tightly, sonicating it until the compound had dissolved, and then cooling the vial to room temperature. Gelation was considered to have occurred when a solid-like material was obtained that did not exhibit gravitational flow (inverted test tube method) during a period of 5 min.

Rheological tests: Rheological tests were conducted using an Anton Paar rheometer and a 25-mm parallel plate. The hydrogel sample (200μ L, 1 wt %) was placed on the parallel plate for the angular frequency sweep test (test range: 0.1 to 100 rads⁻¹; strain, 0.8%; 13 points per decade; sweep mode, "log"; temperature,25 °C).

Transmission Electron Microscopy: Images were obtained with a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV. Hydrogels were applied directly onto 200 mesh carbon-coated copper grids. Excess amount of the hydrogel was carefully removed by capillary action (filter paper), and the grids were then immediately stained with uranyl acetate for 30 s. Excess stain was removed by capillary action, and the grids were allowed to air dry.

Cell viability tests: The biocompatibilities of different peptides were measured by the MTT cell viability test. The MCF-7 cells were seeded in 24-well plates at a density of 50000 cells per well with 0.5 mL medium (DMEM) contained 10 % FBS and 1 % Penicillin-streptomycin solution and incubated for 24 h. Compounds at different concentrations (10, 50, 100, 200, 500 µM) were added when cells were plated. 24 and 48 h later, replaced the medium with fresh medium supplemented with 0.5 mL of MTT reagent (4 mg mL⁻¹) per well. After another 4 h, the medium containing MTT was removed and DMSO (0.5 mL per well) was added to dissolve the formazan crystals. Each 24-well was transferred to 96 well plate. The optical density of the result solution was measured at 595 nm, using an absorbance microplate reader (Infinite F50, TECAN). Cells without the treatment of the compounds were used as the control. The cell viability percentage was calculated by the following formula: The cell viability percentage (%) = $OD_{sample} / OD_{control}$.

Cell Imaging: MCF-7 cells were seeded in a 35 mm Petri dish equipped with a glass cover slide. After 12 h, the cells were stained with the peptides (50 μ M) for 1.5 h. Prior to collecting imaging data, the cells were washed three times with phosphate-buffered saline (PBS, pH 7.4). Images were acquired using inverted fluorescence microscopy (Zeiss laser scanning microscope; DAPI filter; excitation: 350 nm; emission collected: 410–510 nm).



Fig. S1. ¹H NMR spectrum of **1** in D₆-DMSO.



Fig. S2. ¹H NMR spectrum of **2** in D₆-DMSO.







Fig S3. UV-vis spectra of (a) 1, (c) 2 and (e) a 1 : 1 blend of 1 and 2 at 0.05 wt% in water (black) and TFE (red), and (b), (d) and (f) are their normalized spectra, respectively.



Fig. S4. FTIR spectra of 1 (left) and 2 (right) at 2 wt% in water and their secondary derivative FTIR spectra (red).



Fig. S5. FTIR spectrum of a 1 : 1 blend of **1** and **2** at 2 wt% in water and its secondary derivative FTIR spectrum (red).



Fig. S6. Schematic representation of the self-assembled model of 1.