

Using Congo red to report intracellular hydrogelation resulted from self-assembly of small molecules

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Supporting Information

General.

Chemical reagents and solvents were used as received from commercial sources. Alkali phosphatase: 1 U = cleaving 1 μmol of phosphate group from 4-nitrophenyl phosphate/minute. ^1H NMR and ^{31}P NMR spectra were obtained on a 300 MHz Varian XL-300 using $\text{DMSO-}d_6$ as the solvent, mass-spectra on Finnigan TSQ7000 System, emission spectra on a Perkin-Elmer LS-55 luminance spectrometer, and cell images on a Nikon Edipse TE2000-U microscope with epifluorescence and a phase contrast system. MTT results were recorded on an IEMS Analyzer (Lab-system, Type 1401).

Syntheses and characterizations.

Nap-D-Phe-D-Phe-tyrosine phosphate (**2**): 203.5 mg (0.424 mmol) of Nap-D-Phe-D-Phe and 49.1 mg (0.427 mmol) of NHS were dissolved in 12 ml of chloroform. 128.6 mg (0.623 mmol) of DCC was added to the above clear solution. The total mixture was stirred at room temperature for 3 hour, the resulting precipitate was separated by filtration, and then the filtrate was dried by a rotary evaporator. The resulting solid was used for next reaction without further purification.

111.1 mg (0.426 mmol) of tyrosine phosphate was dissolved in 15 ml of water containing 164.0 mg (1.95 mmol) of NaHCO_3 . The crude compound obtained from previous step was dissolved in 25 ml of acetone, added to the above aqueous solution. The resulting solution was stirred at room temperature overnight. The solvent was removed by compressed nitrogen and the resulting solid was re-dissolved in 30 ml of water, the precipitate, which could not dissolve in water, was separated by filtration, and then the aqueous solution was acidified to pH~1 by conc. HCl, the resulting solid was got by filtration. The crude product was purified by reversed-phase HPLC (RP-HPLC) to give 164.9 mg (0.228 mmol) of pure product (yield: 53.8%). $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$): 8.45 (d, 1H), 8.40 (d, 1H), 8.20 (d, 1H), 7.82 (d, 1H), 7.74-7.69 (m, 2H), 7.57 (s, 1H), 7.46-7.43 (m, 2H), 7.28-7.05 (m, 13H), 6.93 (d, 2H), 4.60 (t, 1H), 4.51 (t, 1H), 4.42 (t, 1H), 3.50 (dd, 2H), 3.06 (dd, 1H), 2.98 (dd, 1H), 2.85 (dd, 1H), 2.69 (dd, 1H), 2.57 (dd, 1H), 2.40 (dd, 1H); $^{31}\text{P-NMR}$ (300 MHz, $\text{DMSO-}d_6$): -6.24; MS: calc. $M^+ = 723.2$, obsvd. $(M+1)^+ = 724.2$.

The preparation of Nap-Phe-Phe-NH(CH₂)₂OCO(CH₂)₂COOH (**4**) was followed the literature methods [1].

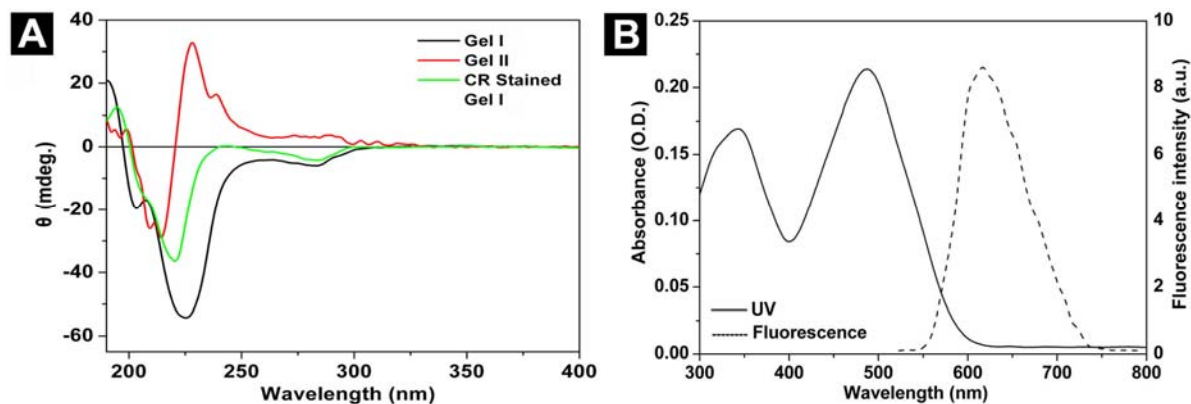


Fig. S1. (A) CD spectra of Gel I of **3**, Gel II of **5** and Congo red stained Gel I; (B) UV and Fluorescence spectra of Congo red.

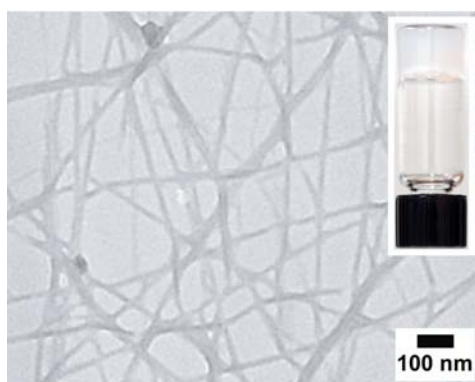


Fig. S2. TEM images of Gel II (inset: optical image) formed by **5** via enzymatic gelation in water (conc. = 0.5 wt%, pH = 8.0).

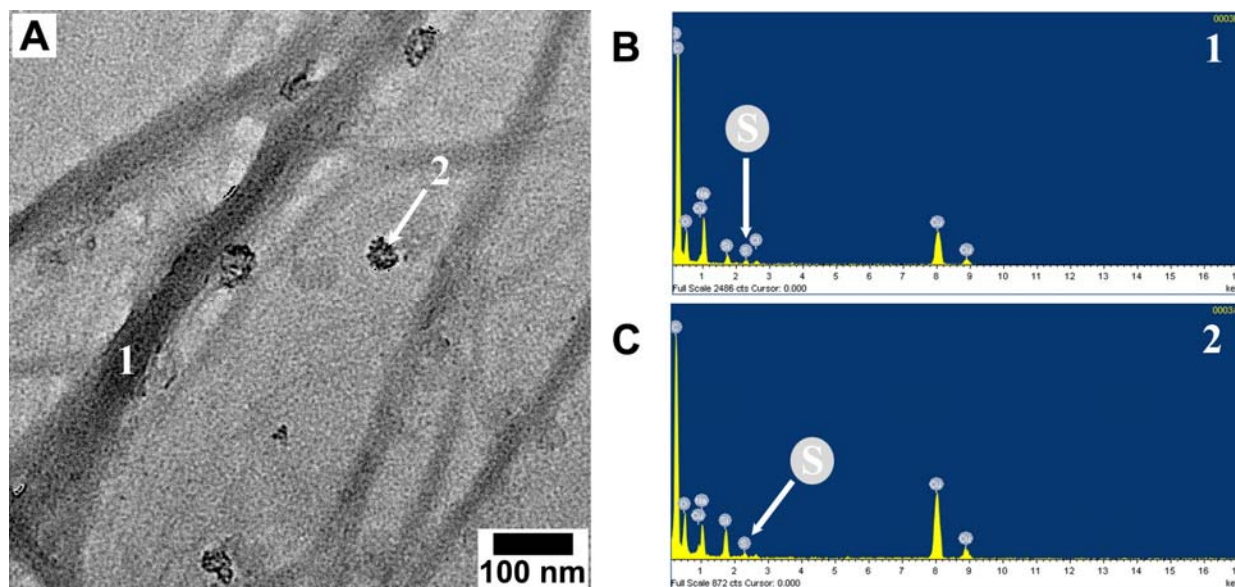


Fig. S3. (A) TEM image of Congo red stained Gel I of **3**; (B) EDX analysis of the nanofiber part; and (C) EDX analysis of black particles.

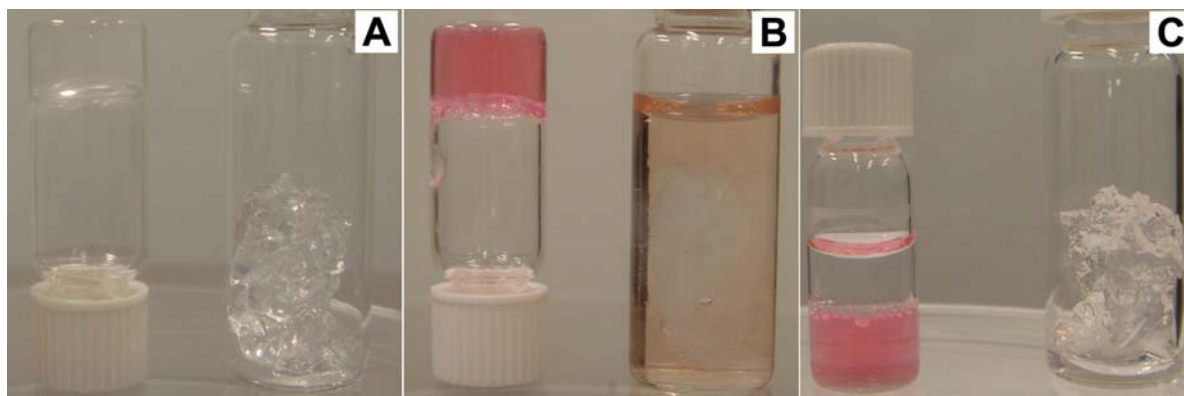


Fig. S4. (A) Supramolecular hydrogel of Gel I (left) and polymer hydrogel polyacrylamide (right) for Congo red staining; (B) Gel I and polyacrylamide stained by Congo red; (C) Congo red stained Gel I and polyacrylamide after washing by PBS buffer.

Cytotoxicity test.

HeLa (HepG-2) cells were seeded into a 96-well plate at a concentration of 3×10^3 cells/well in 100 μ L MEM medium with 10% FBS. Compounds **2**, and **4** at different concentrations were added when cells were plated. Then, the cell cultures were incubated for 24 hours at 37 °C and 5% CO₂. For cell survival assay, MTT assays were carried out to measure the proliferation of HeLa cells. The optical density (OD) of the dissolved formazan crystals was measured at wavelengths of 595 nm. The percentage of cell viability was calculated using the following equation: $\text{viability (\%)} = \text{OD}_{\text{treatment group}} \times 100 / \text{OD}_{\text{control group}}$, where $\text{OD} = \text{OD}_{595 \text{ nm}}$.

Cytotoxicity of **2** and **4**.

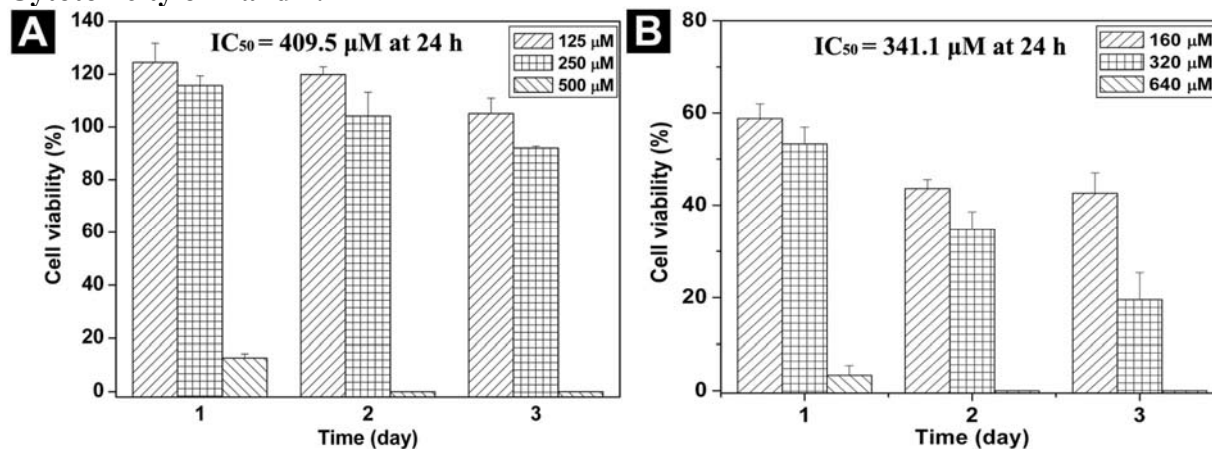


Fig. S5. (A) MTT assay of **2** on HeLa cell; (B) MTT assay of **4** on HepG2 cell.

Extracellular Congo red staining.

Before Congo red staining, **2** were dissolved in PBS buffer to final concentrations of 8.3 mM (pH 7.4). After adding of alkaline phosphatase (500 u/mL), transparent hydrogel was obtained (Fig. 2A) in a few minutes. After the Congo red stock (100 \times) was added to the hydrogel to a calculated concentration of 10 μ M, the hydrogel was slowly stained by Congo red during the permeation of Congo red solution (Fig. 2B)

Intracellular Congo red staining.

Particularly, after incubation with cells for 24h, the culture medium with the precursors were removed, and the cells were washed with PBS for three times. Then the cells were incubated with 10 μM of **1** in culture medium at 37 $^{\circ}\text{C}$, 5% CO_2 and a humid environment for 30 min. After staining, the PBS buffer containing **1** was replaced with fresh PBS buffer and cell images were taken on a reverse fluorescence microscope. For the cells as positive control, they were incubated with 1% Triton X-100 at 37 $^{\circ}\text{C}$, 5% CO_2 and a humid environment for 1 min before Congo red staining.

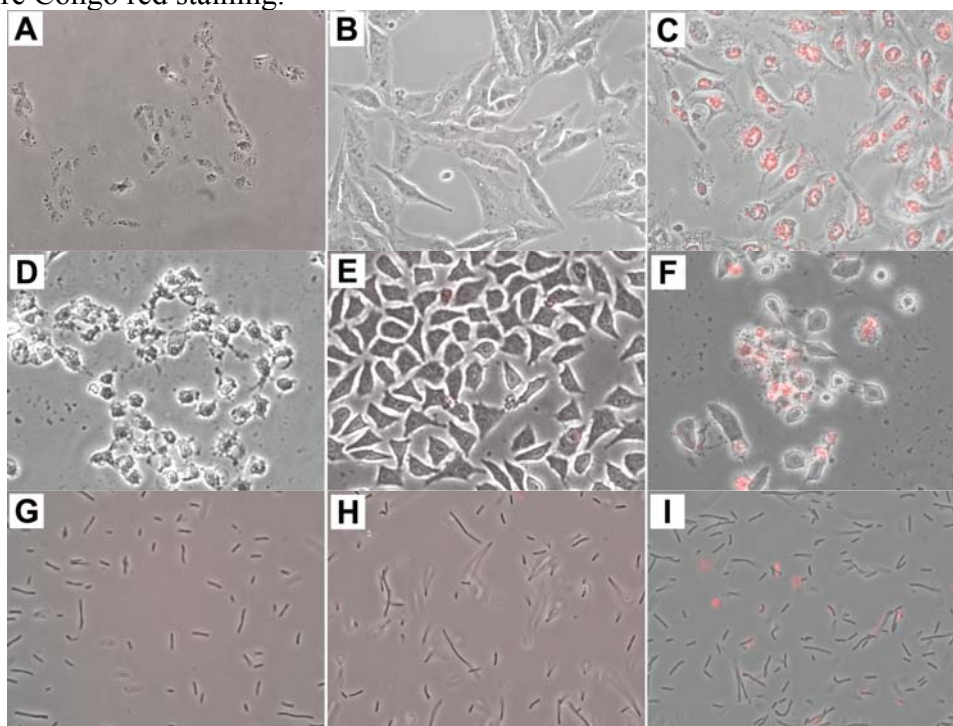


Fig. S6. (A, B, C) Merged microscope images (optical and fluorescence) of HeLa cells stained by Congo red: (A) Triton X-100 treated for 1 min before staining; (B) 250 μM and (C) 1 mM of **2** treated for 24 h; (D, E, F) Merged microscope images (optical and fluorescence) of HepG2 cells stained by Congo red: (D) Triton X-100 treated for 1 min before staining; (E) 160 μM and (F) 640 μM of **4** treated for 24 h; (G, H, I) Merged microscope images (optical and fluorescence) of phosphatase overexpressed *E. coli* stained by Congo red: (G) Control; (H) 1.73 μM and (I) 3.46 μM of **2** treated for 24 h at 18 $^{\circ}\text{C}$.

[1] Z. M. Yang, K. M. Xu, Z. F. Guo, Z. H. Guo, and B. Xu, *Adv. Mater.*, 2007, In press.