Supporting Information

A Redox Responsive, Fluorescent Supramolecular Metallo-hydrogel Consists of Nanofibers with Single-Molecule Width

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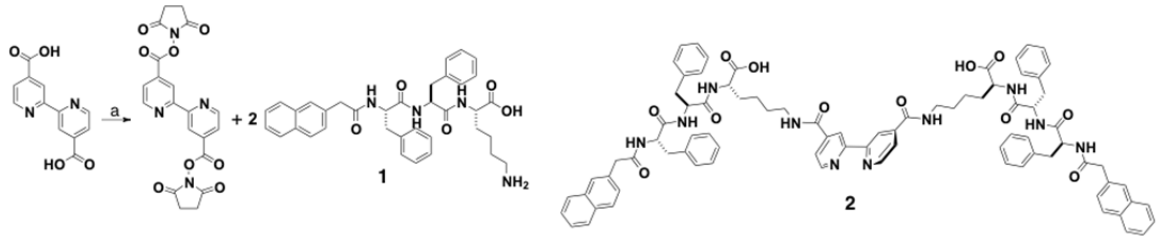
Contents

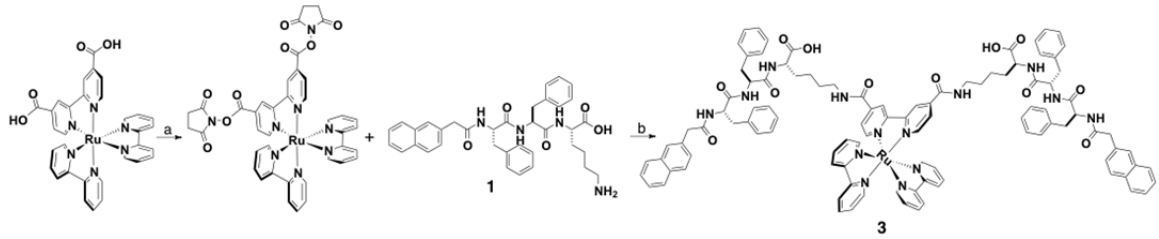
S1 Synthesis of ligand 2 and metallo-hydrogelator 3

Materials and methods

Anhydrous DMF, anhydrous Acetonitrile, 4,4'-dimethyl-2,2'-bipyridine (99.5%), *N*,*N*-diisopropylcarbodiimide (DIC) (99.0%), *N*,*N*-diisopropylethylamine (DIEA) and bis(2,2'-bipyridine)dichlororuthenium(II) hydrate were purchased from Sigma-Aldrich. Hydroxybenzotriazole (HOBt) was purchased from GL Biochem. All reagents were used without further purification. NMR spectra were recorded on a 400 MHz Varian NMR spectrometer.



Scheme S1. The synthetic procedures of ligand **2**.



Scheme S2. The synthetic procedures of metallo-hydrogelator **3**.

2: 4,4'-dicarboxyl-2,2'-bipyridine (0.5 mmol, 122 mg) was charged in a round bottom flask, and anhydrous DMF (5 mL) was added into the flask. DIC (1.0 mmol, 0.19 mL) and NHS (1.0 mmol, 115 mg) were added into the flask and the mixture was stirred at 40°C overnight until the mixture became a clear solution. Motif **1** (1.0 mmol, 608 mg) and DIEA (1.0 mmol, 0.17 mL) were dissolved in anhydrous DMF (10 mL), and the solution was added dropwise into the transparent solution in previous mentioned flask. After overnight reaction, DMF was removed from the reaction mixture and HPLC was applied to obtain ligand **2** as white powder in 56% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.97 (s, 2H), 8.82 (m, 2H), 8.42 (d, J = 7.2 Hz, 2H), 8.27 (d, J = 8.2 Hz, 2H), 8.18 (d, J = 7.2 Hz, 2H), 7.83 (d, J = 5.6 Hz, 4H), 7.75 (dd, J = 8.4, 15.2 Hz, 4H), 7.58 (s, 2H), 7.45 (m, 4H), 7.22 (m, 20H), 4.59 (m, 2H), 4.51 (m, 4H), 4.26 (m, 2H), 3.3 (m, 4H), 2.99 (m, 6H), 2.75 (m, 6H), 1.77-1.54 (m, 8H), 1.37 (m, 4H) ppm.

3: Ru(II)(bipy)$_2$(4,4'-dicarboxyl-2,2'-bipyridine)dicholoride (215 mg, 0.22mmol) was dissolved in anhydrous acetonitrile (10 mL). DIC (0.45 mmol, 0.07 mL), and NHS (0.47 mmol, 54.5 mg) were added into the solution. The mixture was stirred in an

ice bath for 48 h in the dark. The filtrate of the crude produce was added to anhydrous 2-propanol (75 mL) to precipitate at 0°C for 4 h. The orange-colored NHS ester was collected by filtration and washed with dry ether (25 mL) three times. The dry NHS ester was dissolved in anhydrous DMF (5 mL), and the solution was added dropwise to a stirred water solution of **1** (0.45 mmol, 274 mg) at pH = 8 and room temperature. After overnight reaction, NH_4PF_6 solution was added, and the precipitate was collected. The dry product **3** was obtained as orange powder in 44% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (m, 4H), 8.26 (d, *J* = 8.0 Hz, 2H), 7.94 (s, 6H), 7.85 (d, *J* = 8.4 Hz, 4H), 7.76 (dd, *J* = 7.2, 16.8 Hz, 6H), 7.59 (s, 4H), 7.49 (m, 6H), 7.29-7.15 (m, 24H), 4.58 (d, *J* = 7.2 Hz, 2H), 4.49 (d, *J* = 4.49, 2H), 4.21 (m, 2H), 3.54 (dd, *J* = 14.0, 42.0Hz, 4H), 3.07 (d, *J* = 7.2 Hz, 2H), 3.49 (d, *J* = 14.0 Hz, 2H), 2.88-2.68 (m, 8H), 1.77-1.57 (m, 8H), 1.38 (m, 4H) ppm.

S2 Characterization of the self-assembly properties of 1, 2 and 3

S2.1 Optical images and Transmission Electron Microscopy (TEM)

TEM micrographs were obtained with a Morgagni 268 transmission electron microscope by the negative-staining method with uranyl acetate solution (2%) as the staining agent.

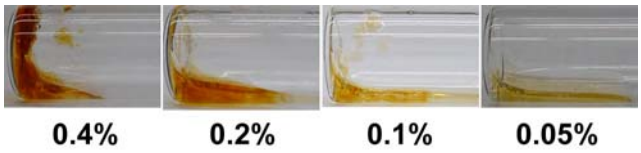


Figure S1. Optical images of metallo-hydrogelator **3** in water (pH = 1) with four different concentrations (w/v) under normal light.

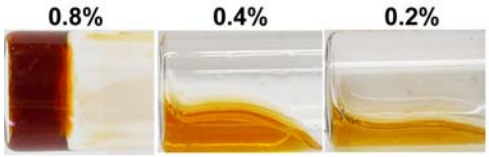


Figure S2. Optical images of metallo-hydrogelator **3** in water (pH = 7) with three different concentrations (w/v) under normal light.

At concentrations equal to or lower than 0.05%, no hydrogel was formed. At concentration of 0.05%, the solution became viscous. And small pieces of hydrogel appeared at concentration of 0.1%. At concentration of 0.4%, a stable red hydrogel formed. TEM images of **3** in water with various concentrations at pH = 1 show that nanofibrous structure formed since the concentration reaches 0.00625% (w/v). So, before the formation of hydrogels, the molecular nanofibers are able to form at much lower concentrations.

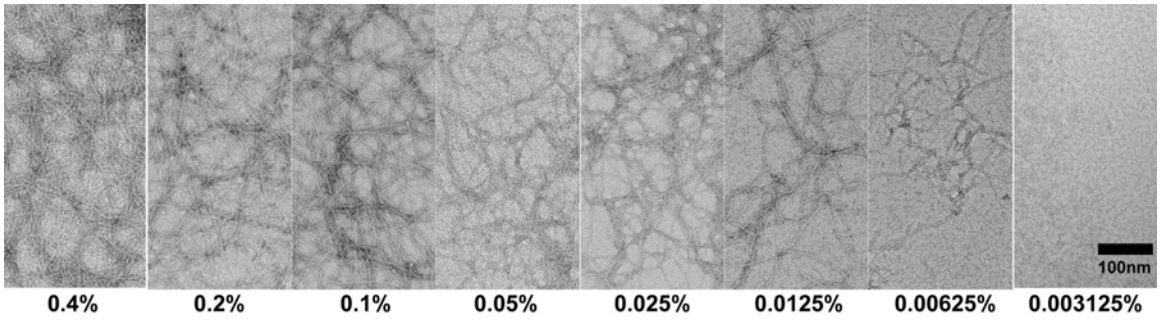


Figure S3. TEM images of metallo-hydrogelator **3** in water (pH = 1) at different concentrations (w/v). The TEM images are corresponding to the optical images in Figure S1.

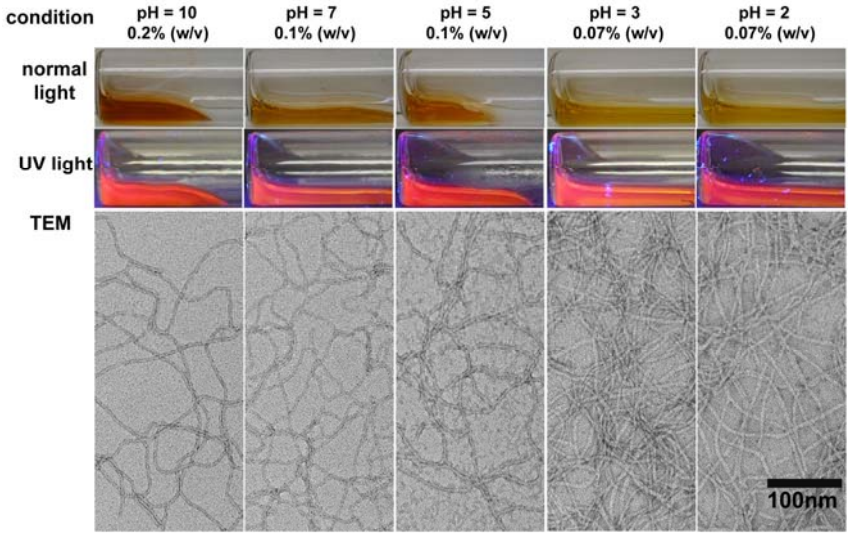


Figure S4. Optical images of metallo-hydrogelator **3** in water with different concentrations and different pH under normal light and UV (long wavelength) light and corresponded TEM images.

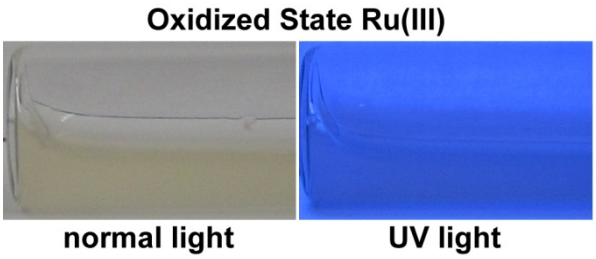


Figure S5. Optical images of **3** in water (0.8% w/v, pH = 1) at oxidized state (after oxidized by $Ce(SO_4)_2$) under normal light and UV light.

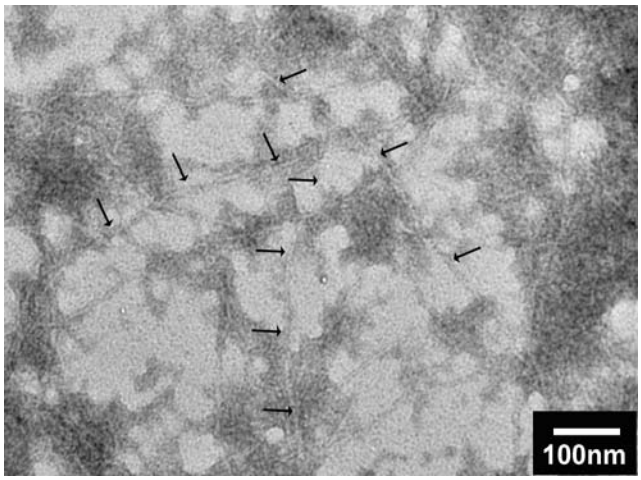


Figure S6. TEM image of metallo-hydrogelator **3** in cell culture media (pH = 7.4-7.6) at 200 μM that used for cell imaging test in Fig.4. The nanofibers formed by self-assembly of **3** tangled with proteins in the culture media. Black arrows were applied here for pointing out the location of some nanofibers.

S2.2 High Resolution Transmission Electron Microscopy (HRTEM)

HRTEM micrographs were obtained with TITAN 80-300 FEG Scanning Transmission Electron Microscope.

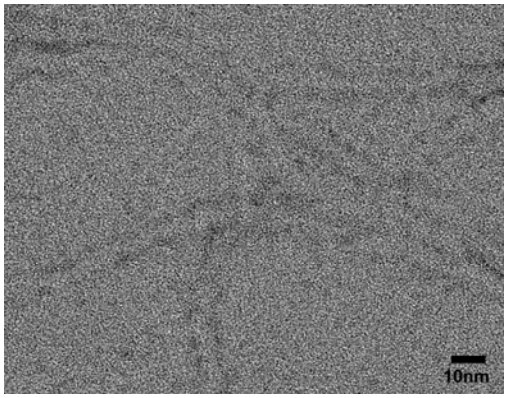


Figure S7. HRTEM image of nanofibers in hydrogel formed by motif **1** (0.8%, w/v, pH = 7).

S2.3 UV-vis Spectroscopy

Emission spectra were recorded on SHIMADZU RF-5301PC spectrofluorophotometer. UV-vis spectra were recorded on VARIAN 50 Bio UV-visible spectrophotometer.

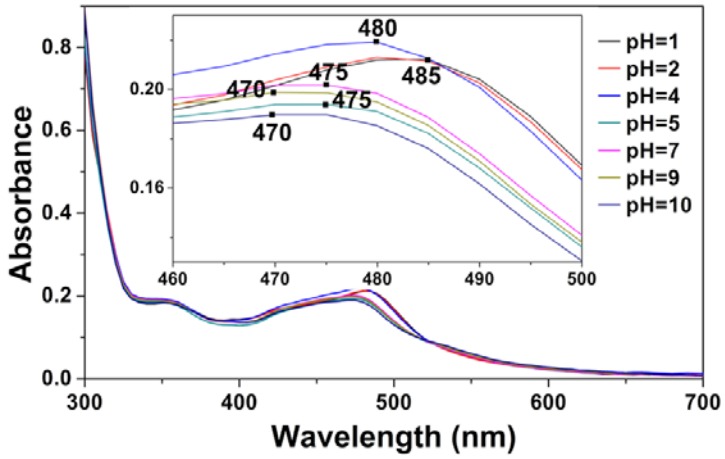


Figure S8. UV-vis spectra of **3** in water (0.025% w/v) at different pH.

S2.5 Rheology

Rheology experiments were performed in the ARES-G2 rheometer with cone plate geometry (25 mm diameter, the cone angle 0.099 rad). Metallo-hydrogel of **3** was prepared at pH = 7 with a concentration of 0.4% (w/v). Hydrogel-1 that motif **1** was applied as hydrogelator was prepared under the same condition and concentration.

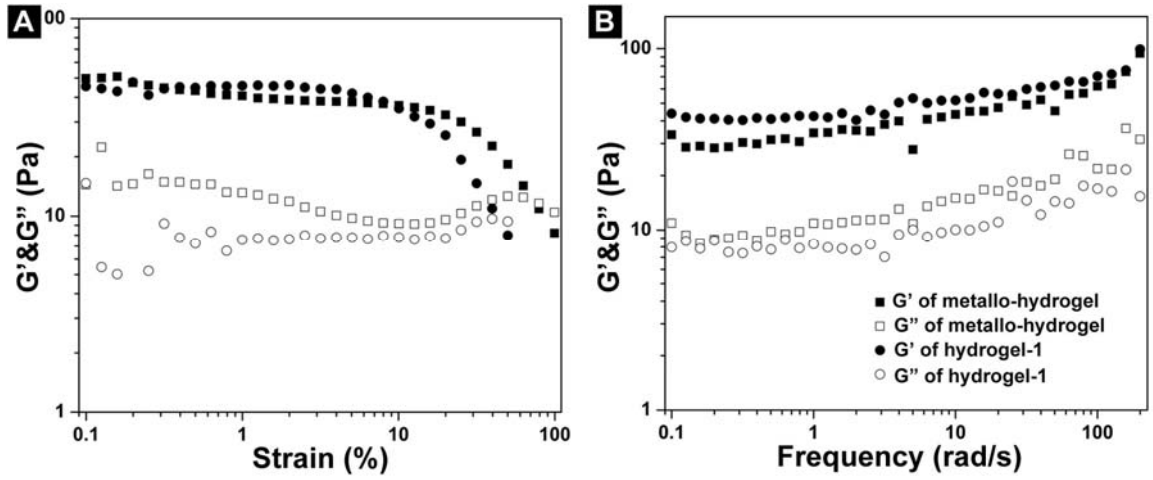


Figure S9. (A) Strain and (B) Frequency dependences of dynamic storage modulus (G') and loss modulus (G") of metallo-hydrogel (0.4% w/v, pH = 7) and hydrogel-1 (0.4% w/v, pH = 7).

S3 DNA binding test and cell viability test of **3**

The luminescence measurements were conducted by using a SHIMADZU RF-5301PC spectrofluorophotometer at 20 °C. Samples were excited at 470 nm, and the emission was monitored at 630 nm. In the experiments, small aliquots of concentrated solutions of the double strand DNA were added to soft hydrogels formed by **3** (0.4% w/v, pH = 7) in 2 mL volumes and mixed well without changing

the concentration of **3**. All soft gels were allowed to equilibrate thermally for ~15 min before measurements were made.

DNA sequence: 5'-ACT GGC TCA ATG TCG A-3'

3'-TGA CCG AGT TAC AGC T-5'

Cell viability assay (MTT): Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 50,000 cell/well. The cells were allowed to attach to the wells for 24 h at 37 ºC, 5% CO_2, then the culture medium was removed and 100 μL new culture medium containing **1** at a series of concentrations was placed into each well. After culturing at 37 ºC, 5% CO_2 for desired time, each well was added by 10 μL of 5 mg/mL MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and the plated cells were incubated at dark for 4 h. 100 μL 10% SDS with 0.01 M HCl was added to each well to stop the reduction reaction and to dissolve the purple. After incubation of the cells at 37 ºC for overnight, the viability is measured. Data represent the mean ± standard deviation of three independent experiments.

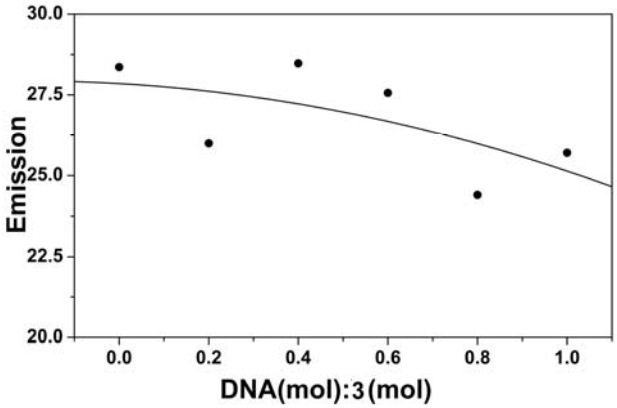


Figure S10. Emission spectrum of hydrogel formed by **3** (0.4%, w/v, pH = 7) mixed with double strand DNA at different molar ratio at 630 nm excited at 470 nm.

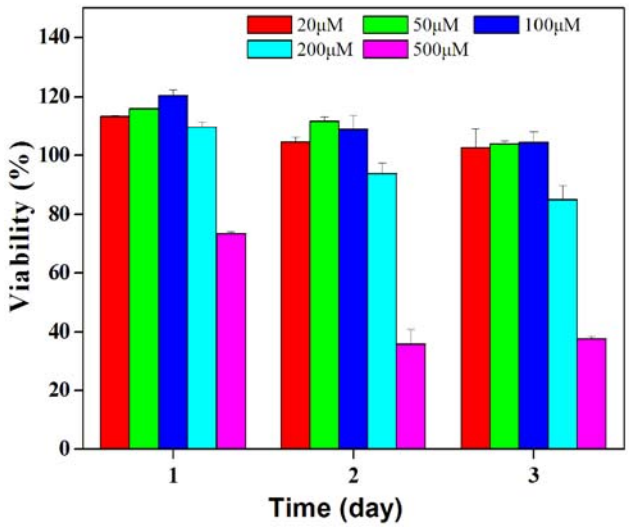


Figure S11. 72h cell viability test of metallo-hydrogelator **3**.

S4 CPK models and 3D-dimention information of **1** and **2**

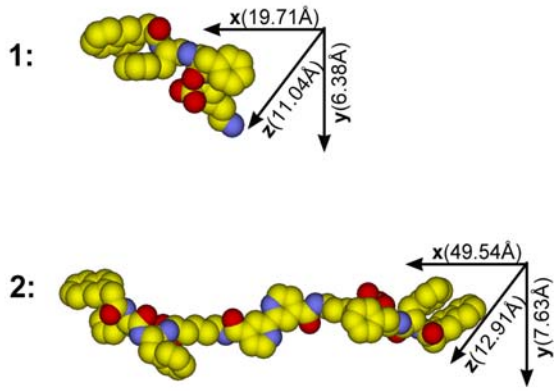


Figure S12. CPK models of motif **1** and ligand **2**.