Supporting Information

Metal ions modulating self-assembly of short peptide conjugating nonsteroidal anti-inflammatory drugs (NSAIDs)

Wenjiao Fu, [a] Zeinab Farhadi Sabet, [b,c] Jiaming Liu, [b] Min You, [b] Huige Zhou, [b] Yaling Wang, [b] Yuan Gao, [b] Jiayang Li, [*b] Xiaomei Ma [*a] and Chunying Chen [*b,c]

[a] College of Chemistry and Chemical Engineering, State Key Laboratory of Bio-Fibers and Eco-Textiles, Qingdao University, Qingdao 266071, China. E-mail: mxm@qdu.edu.cn
[b] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety and CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Beijing 100190, People's Republic of China. E-mail: lijiayang@nanoctr.cn; chenchy@nanoctr.cn
[c] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

Peptide derivatives synthesis

Naproxen-FF (**P1**) and nap-FF (**P2**) were prepared by the standard solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (0.8-1.3 mmol/g) and the corresponding N-fmoc protected amino acids. The resin (1 g) was first swelled in anhydrous dichloromethane (DCM) by bubbling it with nitrogen gas (N_2) for 30 min, and was washed with 5 ml anhydrous N, N-dimethylformamide (DMF) for three times. Then the first amino acid fmoc-phe-OH was loaded onto the C-terminal of resin by bubbling the resin in a DMF solution of fmoc-protected amino acid (2 equiv.) and 1 ml of N, N-diisopropylethylamine (DIPEA) for 1 hours. After washed with 5 ml of DMF for three times, the unreacted sites in resin were quenched with blocking solution (16:3:1 of DCM/MeOH/DIPEA) for 15 min for two times. Then the resin was treated with 20% piperidine (in DMF) for 30 min to remove the protecting group, followed by washing the resin for five times. Then we conjugated the sequent fmoc-phe-OH (2 equiv.) to the free amino group on the resin using DIPEA/*O*-benzotriazole-N, N, N', N'-tetramethyl-uroniumhexafluoro-phosphate (HBTU) (2 equiv.) as the coupling reagent. Npx can be used as amino acid to elongate the peptide chain carried out by the standard fmoc SPPS protocol. At the final step, **P1** and **P2** were cleaved with TFA (10 mL) for 2 hours and the resulted crude products were purified by reverse phase HPLC.

Characterization of the peptide derivatives

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.95 (d, *J* = 8.0 Hz, 0H), 7.83 (d, *J* = 8.4 Hz, 0H), 7.71 – 7.60 (m, 3H), 7.31 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.22 – 7.07 (m, 8H), 7.07 – 6.97 (m, 5H), 4.71 – 4.60 (m, 1H), 4.60 – 4.50 (m, 1H), 3.88 (s, 3H), 3.70 (q, *J* = 7.1 Hz, 1H), 3.09 (dd, *J* = 13.9, 5.2 Hz, 1H), 3.01 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.81 (dt, *J* = 13.9, 9.3 Hz, 2H), 1.36 (d, *J* = 7.1 Hz, 3H). MS (ESI) (m/z): C32H32N2O5 calc. 524.22709; found 523.22358 [M-H]$^{-}$.

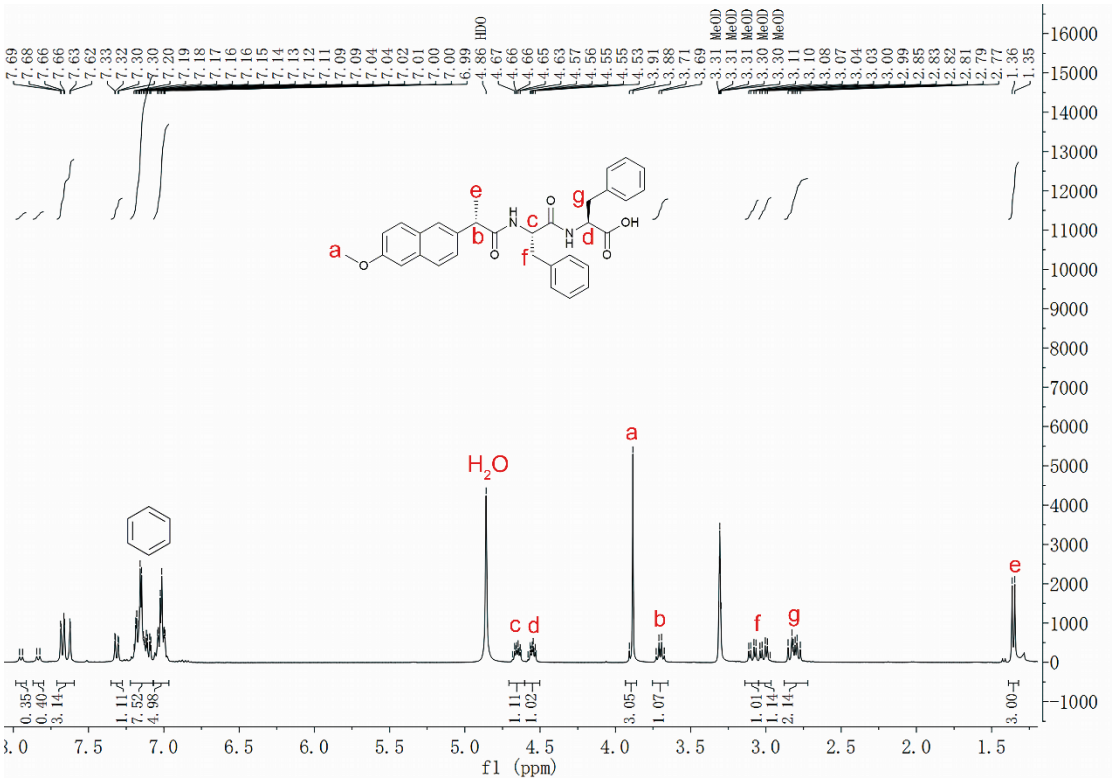


Fig. S1 ^{1}H NMR spectrum of naproxen-FF (**P1**).

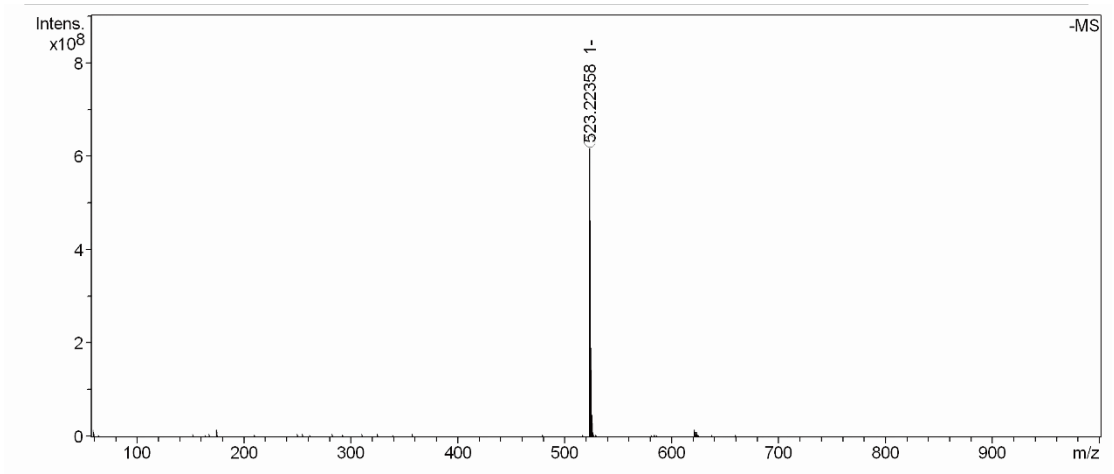


Fig. S2 ESI-MS spectrum of **P1**.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.84 – 7.77 (m, 1H), 7.77 – 7.68 (m, 2H), 7.58 (d, *J* = 1.7 Hz, 1H), 7.50 – 7.39 (m, 2H), 7.24 – 7.05 (m, 11H), 4.66 (td, *J* = 8.7, 8.0, 5.1 Hz, 2H), 3.65 – 3.52 (m, 2H), 3.13 (ddd, *J* = 30.0, 14.0, 5.1 Hz, 2H), 2.96 (dd, *J* = 13.9, 8.2 Hz, 1H), 2.82 (dd, *J* = 14.0, 9.6 Hz, 1H). MS (ESI) (m/z): C30H28N2O4 calc. 480.20279; found 479.19722 [M-H]$^-$.

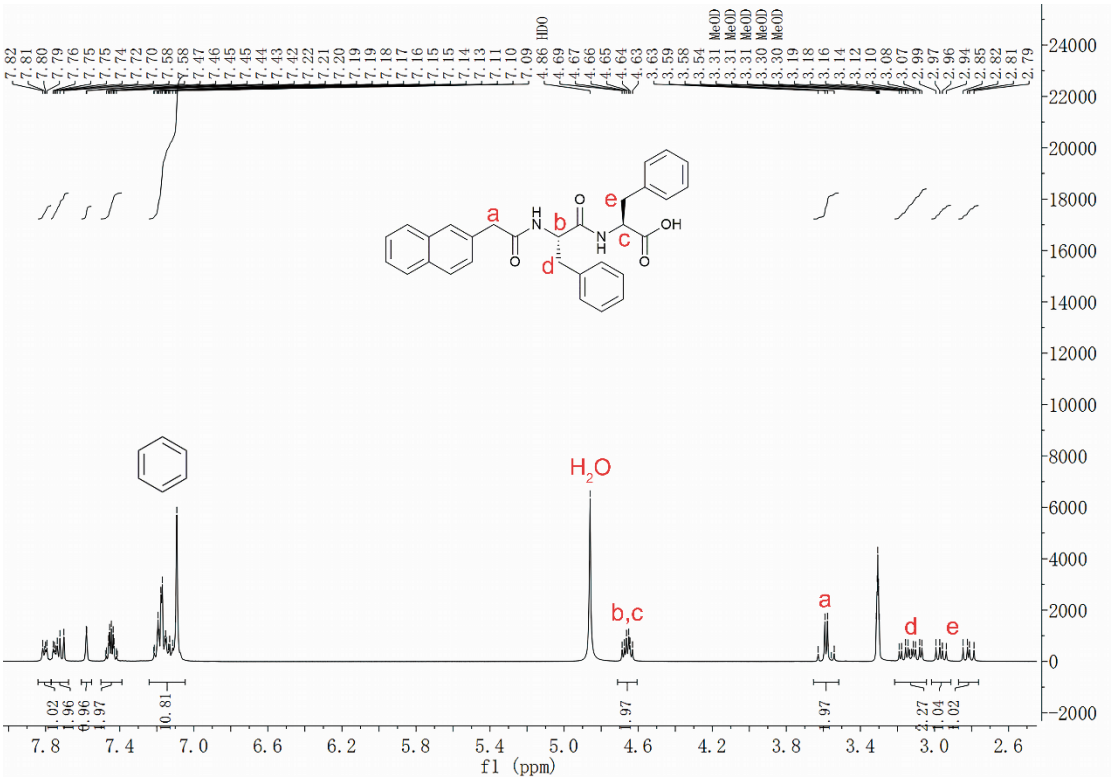


Fig. S3 ^{1}H NMR spectrum of nap-FF (**P2**).

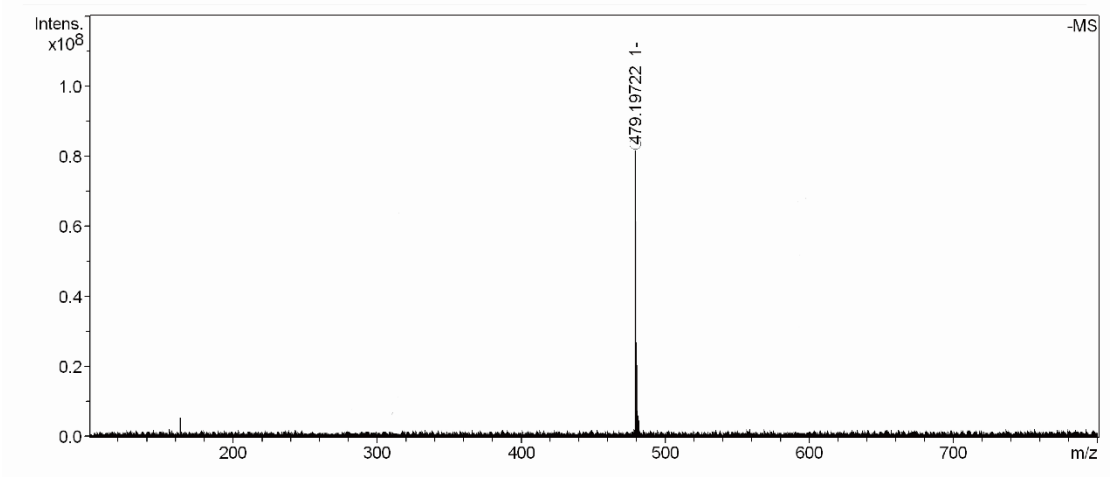


Fig. S4 ESI-MS spectrum of **P2**.

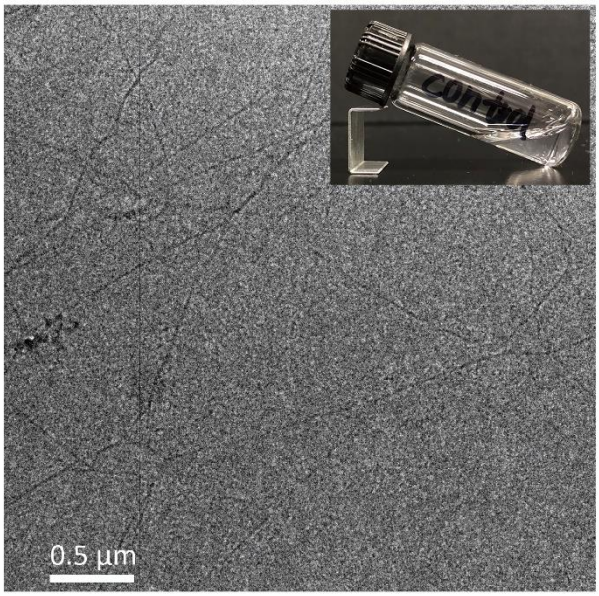


Fig. S5 The optical and TEM image of **P1** solution in the absence of metal ions. Scale bar: 0.5 μm.

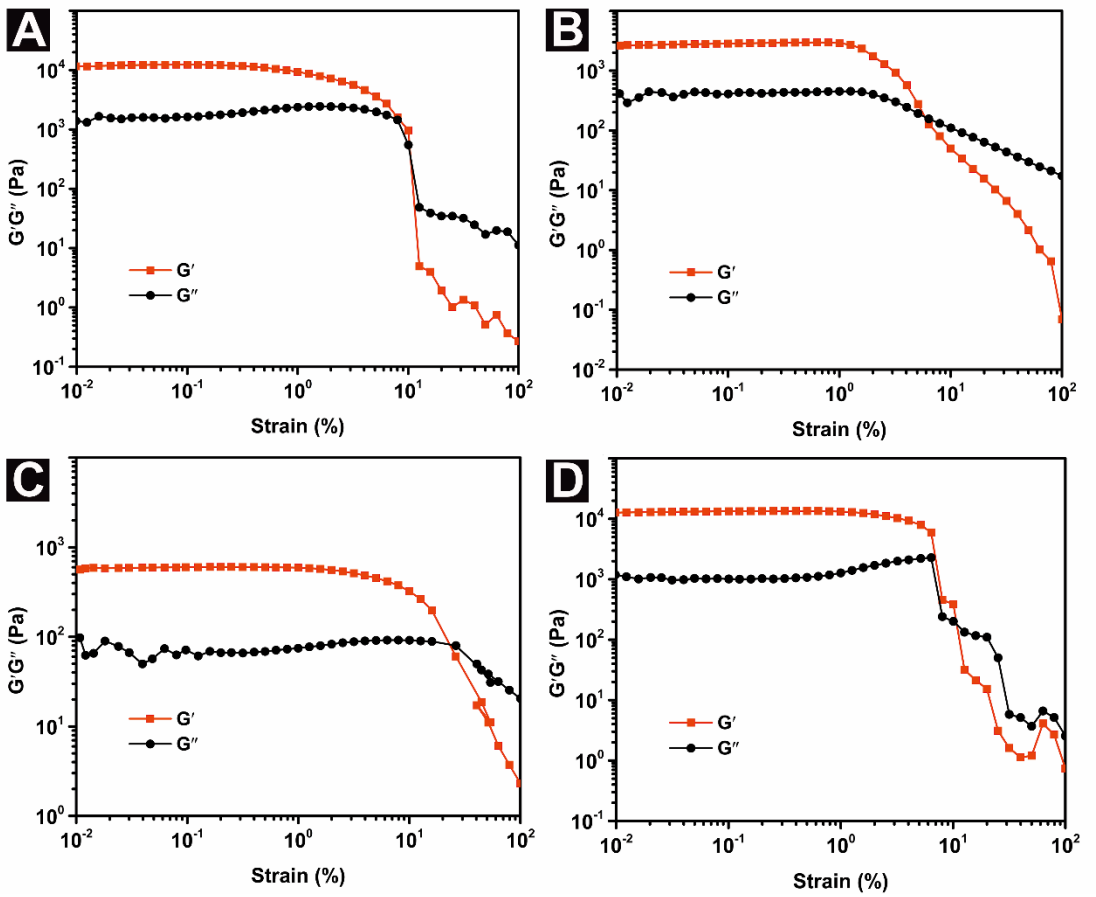


Fig. S6 Strain-dependent oscillatory shear rheology of **P1** hydrogels at a frequency of 6.283 rad/s. (A) $MgCl_2$. (B) KCl_2. (C) $CaCl_2$. (D) $NiCl_2$.

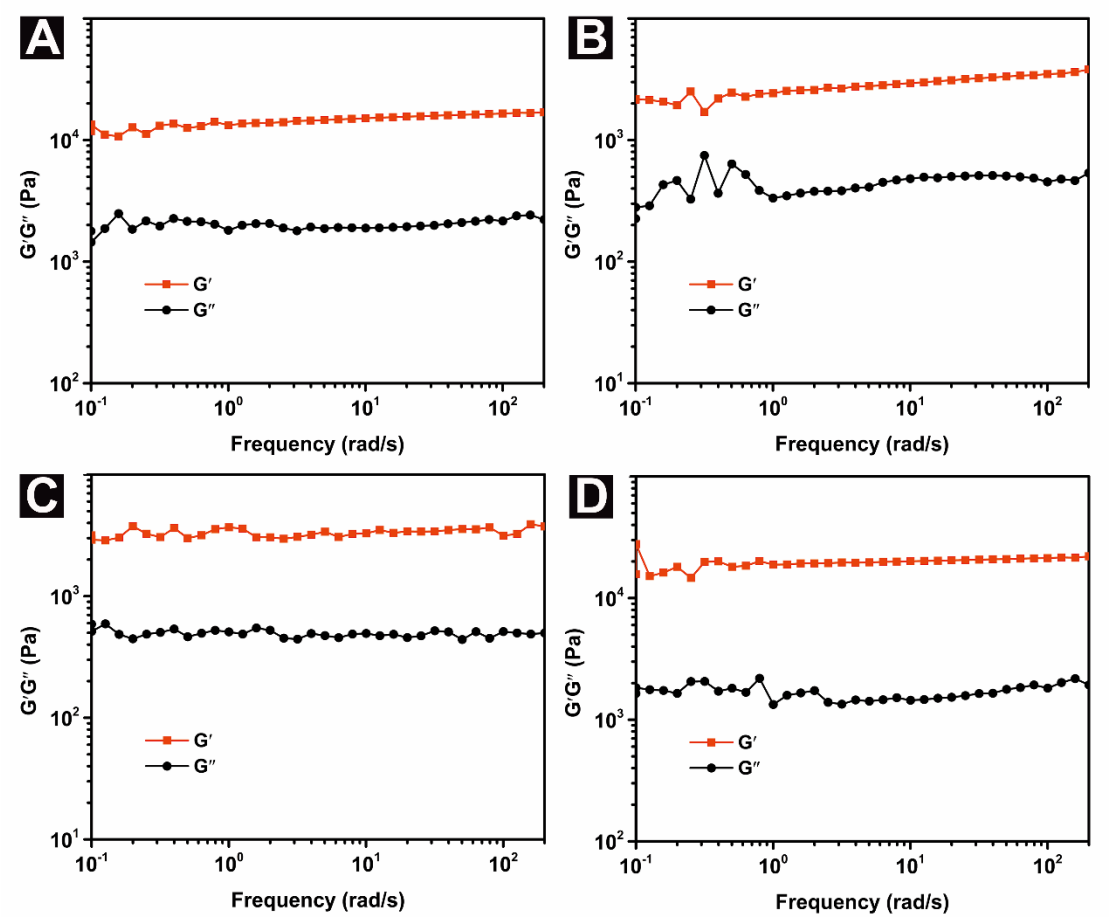


Fig. S7 Dynamic frequency sweep of **P1** hydrogels at a strain of 0.5%. (A) $MgCl_2$. (B) KCl_2. (C) $CaCl_2$. (D) $NiCl_2$.

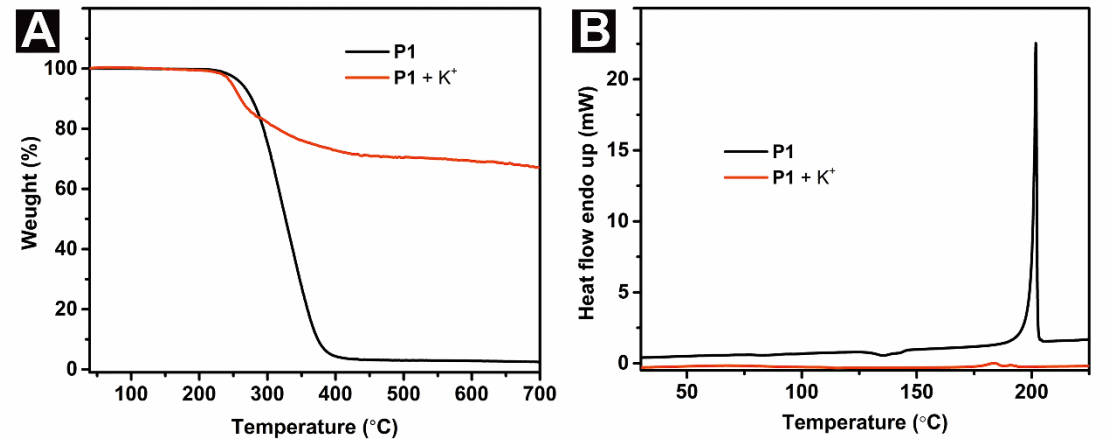


Fig. S8 Thermal stability of **P1** and the K-triggered hydrogel in nitrogen atmosphere. (A) Thermogravimetric analysis (TGA). (B) Differential scanning calorimetry (DSC) heating curves.

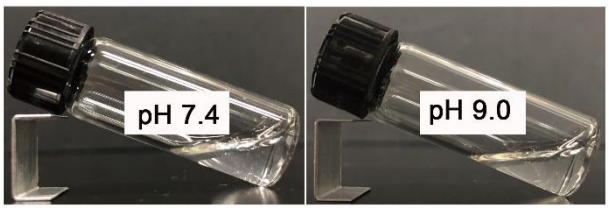


Fig. S9 The optical images of **P2** samples in 2% DMSO in H2O (v/ v) at pH 7.4 / pH 9.0 with the concentration of 5 mg/mL.

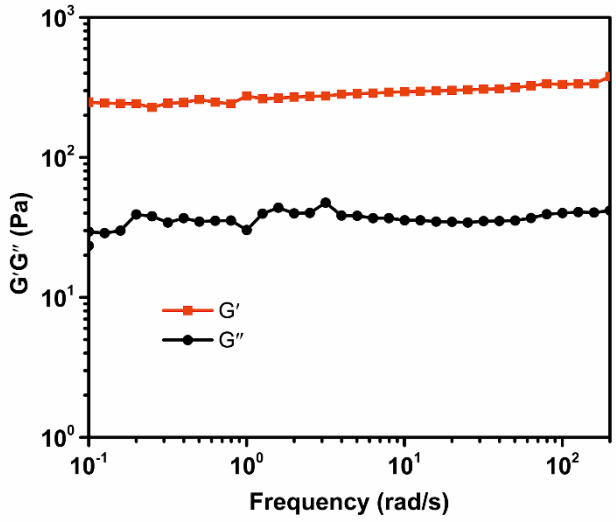


Fig. S10 Oscillatory rheology dynamic frequency sweeps of the K-triggered hydrogel at the pH value of 9.0 with the strain 0.5%.

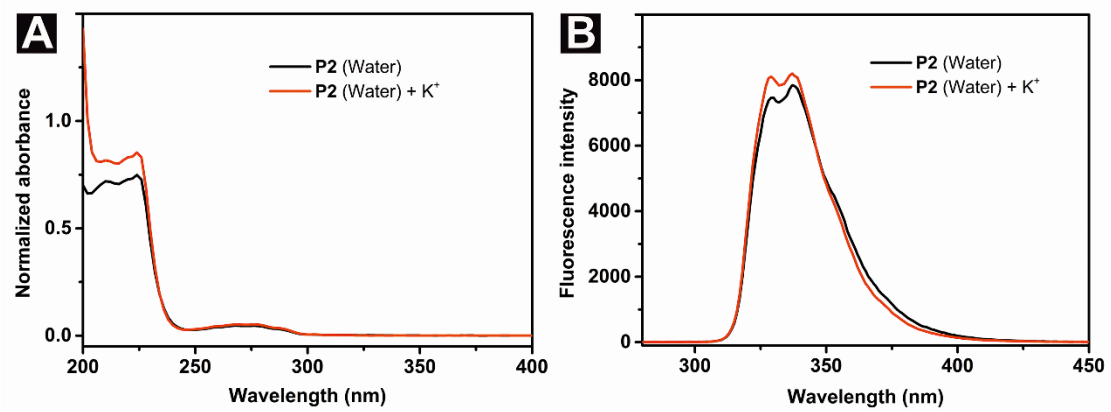
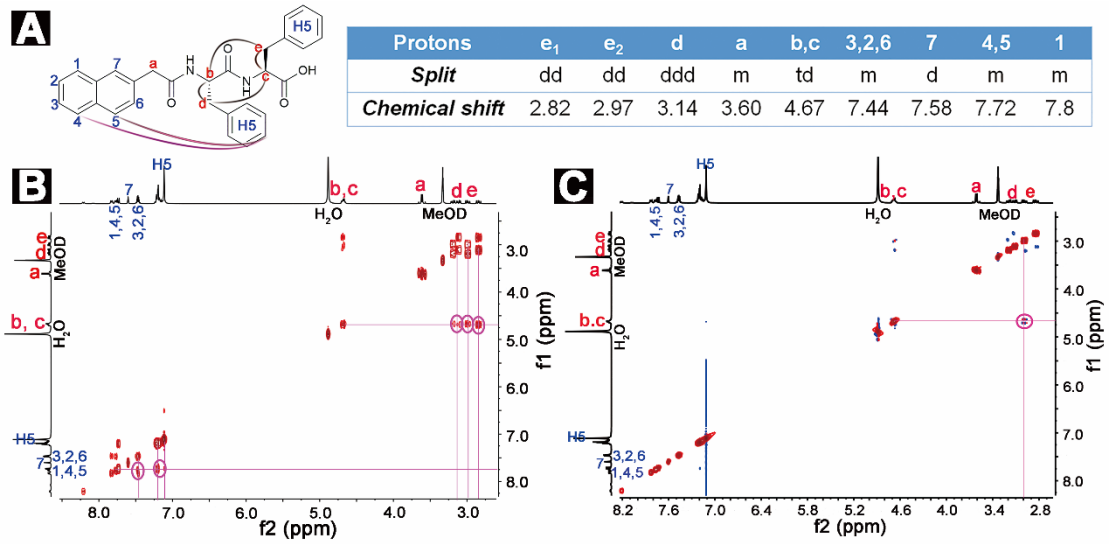


Fig. S11 The spectra analysis of the **P2** solution in the absence/presence of K^+. (A) UV/Vis spectrum. (B) FL spectrum.



Protons	e_1	e_2	d	a	b,c	3,2,6	7	4,5	1
Split	dd	dd	ddd	m	td	m	d	m	m
Chemical shift	2.82	2.97	3.14	3.60	4.67	7.44	7.58	7.72	7.8

Fig. S12 The assignment of protons. (A) The interactions diagram of proton in **P2** and its chemical shifts. Black colour: the interactions of groups protons, pink colour: the interactions of π-π staking. (B) 2D COSY spectra of **P2**. (C) 2D ROESY spectra of **P2**.

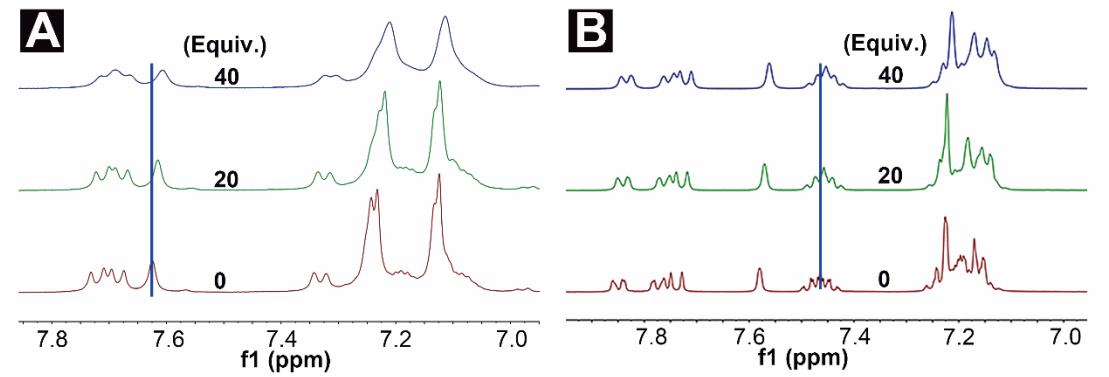


Fig. S13 Partial stacked 1H NMR spectra during titration by adding the different molar ratio of K^+ in d_6-DMSO. (A) **P1**. (B) **P2**.