

# **Supramolecular polymer/peptide hybrid hydrogels with tunable stiffness mediated by interchain acid-amide hydrogen bonds**

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## **Supporting Information**

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## 1. Experimental Methods

**General Methods:** Pentafluorophenyl acetic acid was purchased from Apollo Scientific and anhydrous grade solvents were purchased from Sigma-Aldrich and Acros Organics. *N*-isopropylacrylamide (NIPAM) (Sigma-Aldrich) and poly(ethylene glycol) diacrylate (PEG-DA) (Sigma-Aldrich) were purchased from a commercial source and used without further purification. The synthesis of tripeptides was performed by solid phase peptide synthesis (SPPS) using classical Fmoc protocol. The purity of the final compounds was confirmed using  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, and MS techniques. NMR spectra of the compounds were recorded at VARIAN AS500 MHz NMR instrument in deuterated solvents. High-resolution mass spectra were recorded on Micromass Q-TOF MS spectrometer. TEM images were obtained with a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV. Samples were visualized with a JEOL JSM-6700F scanning electron microscope at an accelerating voltage of 5 kV and a working distance of 6.3 mm.

**Preparation of polyacrylamide gel:** A solution of *N*-Isopropylacrylamide (NIPAM) and poly(ethylene glycol) diacrylate (PEGDA) (wt% 10:1) were dissolved in DMSO with the total concentration 150 mg/mL. Ammonium persulfate (3wt%) as the photoinitiator was added to the solution followed by exposure to UV light for 20 h at room temperature to form a pre-gel. Finally, the gel was dialyzed against distilled water for 3 days ( $M_w$  cut-off 3500) to remove the unpolymerized monomers and DMSO.

**Preparation of polymer-supramolecular double network gels:** PFB-FFD (2.4 wt%) or PFB-FFK (2.4 wt%) and acrylamide NIPAM with crosslinker PEGDA (wt% 10:1) were dissolved in a DMSO solution (0.5 mL) at a concentration of 24 mg mL<sup>-1</sup> and 150 mg mL<sup>-1</sup> respectively. Then ammonium persulfate was added to the mixture as a photoinitiator for polymerization. The solution

was irradiated with UV light for 20 h at room temperature and the pre-gel was obtained. The pre-gel was then treated with water (1 mL) to initiate self-assembly of the peptide molecules for 3 h. The peptide fibers formed upon addition of water to the DMSO sample. Finally, the pre-gel was dialyzed in DI water for 24 hours to remove the unpolymerized monomers and DMSO. (NIPAM: 81.8 mg, PEGDA: 8.2 mg, FFD: 14.4 mg, FFK: 14.4 mg, Photoinitiator: 18 mg, total volume: 0.6 mL). For the **PNIPAM-PEGDA:PFB-FFD:Ca<sup>2+</sup>** hydrogel, 10mM CaCl<sub>2</sub> solution was added to the monomer solution.

The aspartic acid group of PFB-FFD and the amide of NIPAM would induce the a strong hydrogen bond between acid and amide through molecular recognition process. Since PFB-FFK forms only weak hydrogen bonds, no double network structure is noticed.

**Transmission Electron Microscopy (TEM):** TEM 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.

**Scanning electron microscopy (SEM):** Hydrogels were applied directly onto silicon wafers and the samples were allowed to air dry. Samples were visualized with a JEOL JSM-6700F scanning electron microscope at an accelerating voltage of 5 kV and a working distance of 6.3 mm.

**Rheological tests:** Rheological tests were conducted on TA discovery. 20 mm cone plate was used during the experiment. During measurement, 500 mg of gel was taken. The geometry gap was set at 100  $\mu$ m. The rheology experiments were carried out using a frequency-sweep mode with

frequency of 1-100 rad s<sup>-1</sup> at a constant strain about 0.8% and a strain-sweep mode with strain range of 0.1-1000% at constant frequency of 1 rad s<sup>-1</sup>. Temperature-dependent rheological measurement was carried out with a frequency of 1 rad s<sup>-1</sup> at a constant strain about 0.8%. The critical strain ( $\gamma_c$ ) for every hydrogel is the strain where the G' values deviate by more than 5% from the linear region. The hydrogels showed a crossover transition between G' and G'' at high strain values, indicating a phase change from primarily elastic to primarily viscous. In many cases, the values were obtained directly from experimental data; however, in few cases, the values were derived by extrapolating the experimental results.

**Circular dichroism (CD) spectra:** 30mg of double network hydrogels were dissolved in 1.5 mL DI water and 250  $\mu$ M pure peptides using DI water as solvent were prepared for CD measurement. Then the CD spectra of all these samples were recorded using JASCO Corp., J-810 spectrophotometer. CD spectra were recorded in the UV-Vis region (200-350 nm) using a 0.1 mm quartz cuvette for all the hydrogelators.

**NMR spectra:** NMR spectra were recorded on VARIAN AS500 MHz NMR instrument at 298 K using partially deuterated solvents as internal standards for <sup>1</sup>H-NMR. Coupling constants (J) are denoted in Hz and chemical shifts ( $\delta$ ) in ppm. Multiplicities are denoted as follows: s = singlet, d = doublet, t = triplet, m = multiplet.

**Fourier Transfer Infrared Spectroscopy (FTIR):** The hydrogels were put into the freeze drying machine then freeze dried for 24 hours. Then, milling these solids into powder and mixed with potassium bromide (KBr) using bead machine to make them become a tablet. For pure peptide samples, we prepared 2wt% for each using DI water as solvent. The tablets and peptide solution were deposited on CaF<sub>2</sub> windows (thickness, 1.5 mm), noticed that peptide solution should be dried into film before putting the windows in to the instrument. The FTIR spectra of all these

samples were recorded using Perkin-Elmer spectrum 100 FT-IR spectrometer and were collected at a resolution of 4 cm<sup>-1</sup> using a detector by averaging scans (60 scans).

**Photoluminescence spectra (PL):** 30 mg of double network hydrogels were dissolved in 1.5 mL DI water and 250 μM pure peptides using DI water as solvent were prepared for PL measurement. The PL spectra of all these samples were measured by F-7000 FL Spectrophotometer. The experiments were carried out with emission mode. (Starting wavelength: 280 nm; Ending wavelength: 470 nm; Excitation slit: 10mm; Emission slit: 10mm; Photomultiplier voltage: 700V)

**UV-vis spectra:** 30 mg of double network hydrogels were dissolved in 1.5 mL DI water and 250 μM pure peptides using DI water as solvent were prepared for UV-vis measurement. The UV-vis spectra of all these samples were recorded by Jasco V-670 UV-visible spectrophotometer and scanned from 400 nm to 200 nm.

### **Thermo-gravimetric analysis**

The decomposition profile of the peptide-polymer hybrid hydrogels was characterized by thermogravimetric analysis (Mettler-Toledo, 2- HT). Samples were analyzed in a nitrogen atmosphere with a heating rate of 10 °C/min.

### **Cell viability assay<sup>1</sup>**

**Gel extraction medium:** The hydrogel pieces cut into 1cm\*1cm were immersed in 10 ml buffer solution (pH 7.4) and incubated at 37 °C for 24 h.

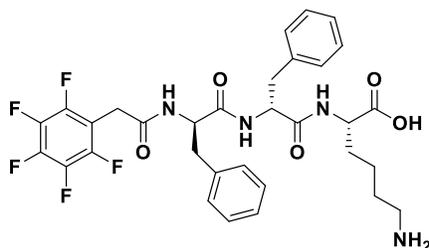
The cell viability was analyzed by MTT assay, 3A6 cells were seeded at a density of 4 X 10<sup>4</sup> cells in a 24-well tissue culture plate with growth medium and incubated at 37 °C under a 5% CO<sub>2</sub> atmosphere for 24 h. The medium was then removed, and the cells were incubated with the

hydrogel leachable (gel extraction medium). The medium was replaced at each time point with fresh medium containing the MTT reagent to a  $0.5 \text{ mg mL}^{-1}$  final concentration and incubated for another 4 h, then the MTT reagent was removed and DMSO was added to dissolve the formazan crystals, the 100 mL solution from each 24-well plate were transferred to 96-well plate and the optical density of the resulting solution was measured at 595 nm using a TECAN Infinite F50 microplate reader. Cells without the gel extract were used as the control.

### **General synthetic procedure for PFB-FFK and PFB-FFD tripeptides**

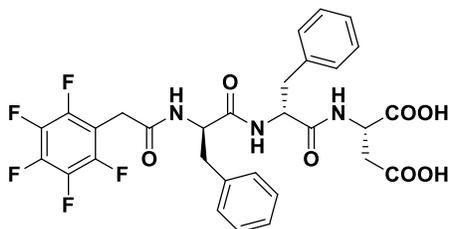
FFK and FFD tripeptides were prepared through the solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin. The resin (1.2 g) was pre-swelling with anhydrous THF under nitrogen atmosphere. After stirring at room temperature for 1 h, the solvent was removed and then Fmoc-Lys(Boc)-OH or Fmoc-Asp(OtBu)-OH (2 mmol) in anhydrous *N,N*-Dimethylformamide (DMF) was added to the resin along with *N,N*-Diisopropylethylamine (DIEA) (0.85 mL, 5 mmol). The mixture was allowed to react for 1 h. Then Fmoc deprotection was carried out with 20 % piperidine in DMF (30 min). The coupling of Fmoc-Phe-OH (0.75 g, 2 mmol) amino acid was then performed using 3-[Bis(dimethylamino)methyl]imidazolium hexafluoro phosphate (HBTU) (0.75 g, 2 mmol) and DIEA (0.85 mL, 5 mmol) in DMF for 1 h. The deprotection and coupling processes were repeated to build the tripeptides. Finally, phenylacetic acid (6 mmol) was coupled with the free amino group of resin bounded peptide using HBTU (2.28 g, 6 mmol) and DIEA (2.5 mL, 15 mmol) as coupling agents. A solution of 90% trifluoroacetic acid (TFA) in DI water was used for the peptide cleavage and global deprotection of acid-labile protecting groups. The TFA-peptide solution was concentrated under vacuum to get a viscous material, which was further treated with cold diethyl ether to precipitate the desired compound. The solid obtained was dried under vacuum to remove any residual solvents.

### 2,3,4,5,6-Pentafluorobenzyl-diphenylalanyllsine (PFB-FFK).



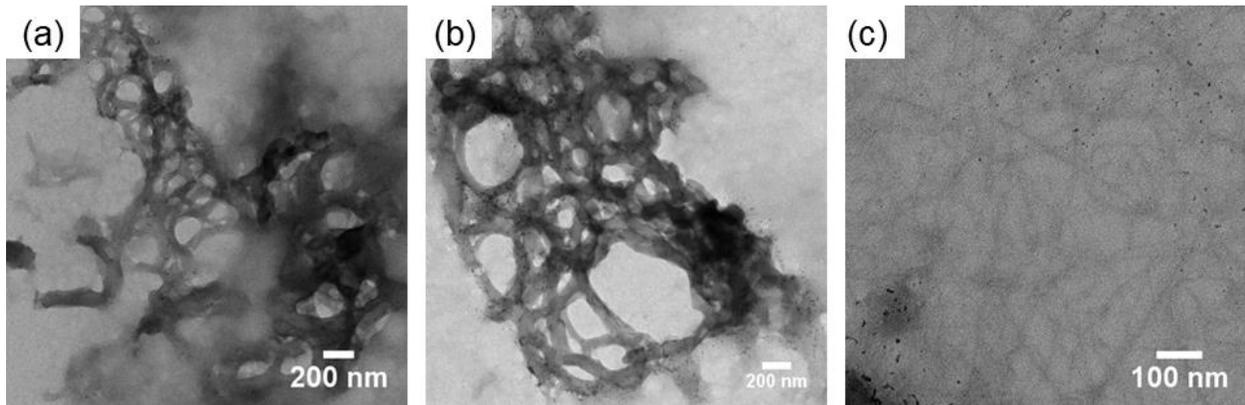
Yield (0.60 g).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ ,  $25^\circ\text{C}$ ):  $\delta$ =1.33-1.40 (m, 2H;  $\text{CH}_2$ ), 1.45-1.65 (m, 3H;  $\text{CH}_2$ ), 1.70-1.80 (m, 1H;  $\text{CH}_2$ ), 2.60-2.85 (m, 4H;  $2\text{CH}_2$ ), 2.97 (dd,  $J = 13.8, 3.6$  Hz, 1H), 3.06 (dd,  $J = 13.5, 3.6$  Hz, 1H), 3.53 (s, 2H;  $\text{CH}_2$ ), 4.20 (dd,  $J = 13.3, 8.4$  Hz, 1H), 4.45-4.60 (m, 2H; 2CH), 7.15-7.25 (m, 10H; 10CH);  $^{13}\text{C}$  NMR (125 MHz,  $\text{DMSO-}d_6$ ,  $25^\circ\text{C}$ ):  $\delta$ = 22.8, 27.0, 29.0, 30.9, 37.9, 52.1, 54.24, 54.45, 126.62, 126.71, 128.33, 128.44, 129.57, 129.71, 138.1, 166.8, 171.28, 171.46, 173.8; HRMS [ESI $^+$ ]:  $m/z$ (%): calcd. 649.2444; found 649.2452 [M+H] $^+$ .

### 2,3,4,5,6-Pentafluorobenzyl-diphenylalanylaspartic (PFB-FFD)

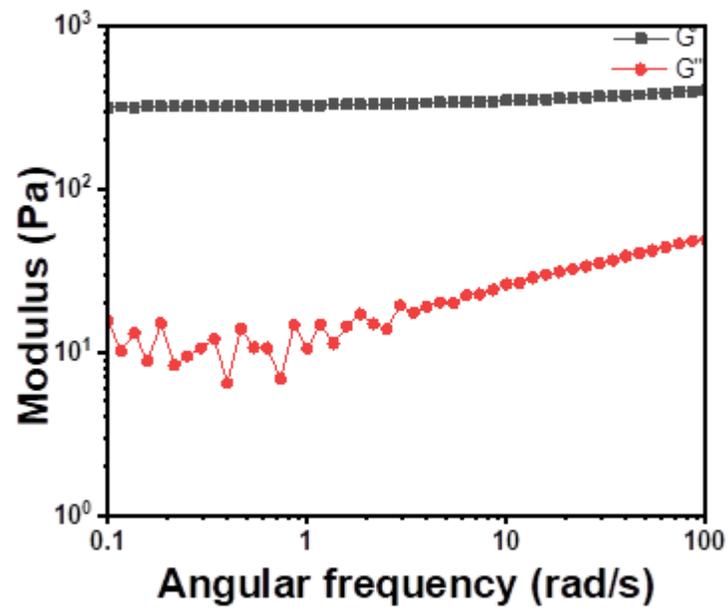


Yield (0.50 g).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-}d_6$ ,  $25^\circ\text{C}$ ):  $\delta$  = 2.60 (dd,  $J = 16.7, 6.5$  Hz, 1H), 2.65–2.74 (m, 2H), 2.80 (dd,  $J = 13.9, 9.4$  Hz, 1H), 2.99 (dd,  $J = 13.8, 3.9$  Hz, 1H), 3.06 (dd,  $J = 13.9, 4.3$  Hz, 1H), 3.38 (s, 2H;  $\text{CH}_2$ ), 4.50-4.60 (m, 3H; 3CH), 7.10-7.30 (m, 10H; 10CH);  $^{13}\text{C}$  NMR (125 MHz,  $\text{DMSO-}d_6$ ,  $25^\circ\text{C}$ ):  $\delta$  = 28.6, 36.1, 37.53, 37.64, 40.0, 48.7, 53.74, 53.90, 126.18, 126.27, 127.9, 128.0, 129.18, 129.27, 137.60, 137.65, 166.3, 170.77, 170.79, 171.7, 172.2; HRMS [ESI $^+$ ]:  $m/z$ (%): calcd. 636.1764; found 636.1771 [M+H] $^+$ .

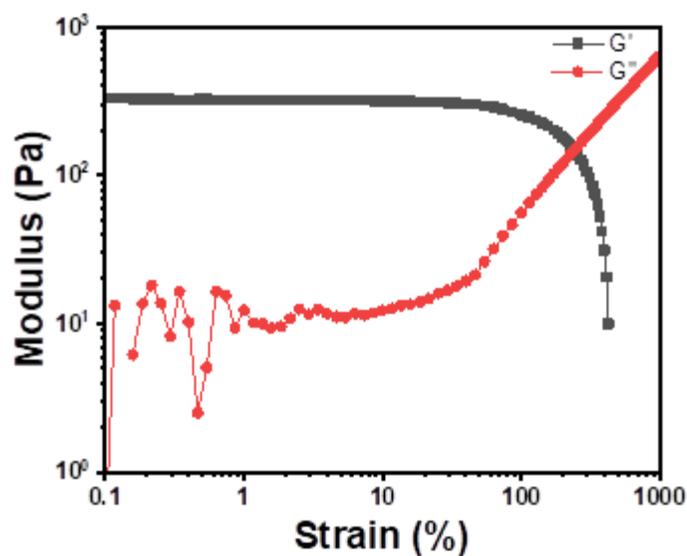
## 1. Supplementary Figures.



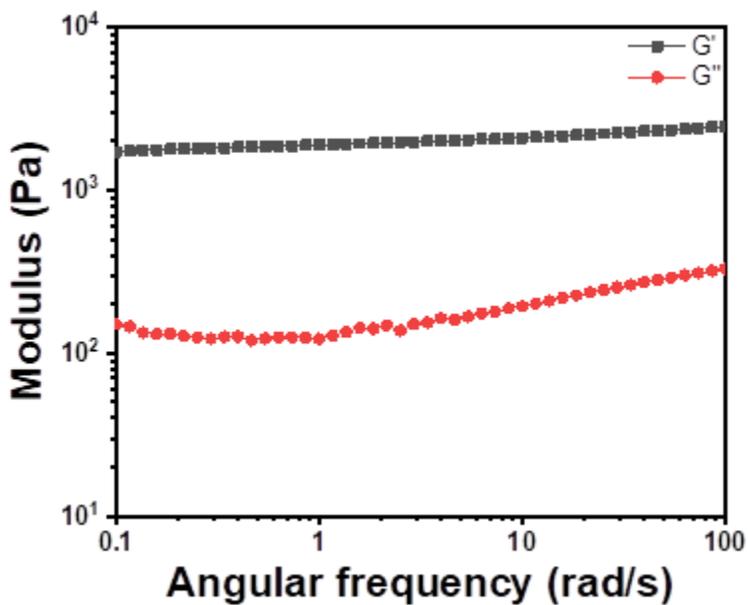
**Figure S1** TEM images of PNIPAM-PEGDA:PFB-FFD hydrogel.



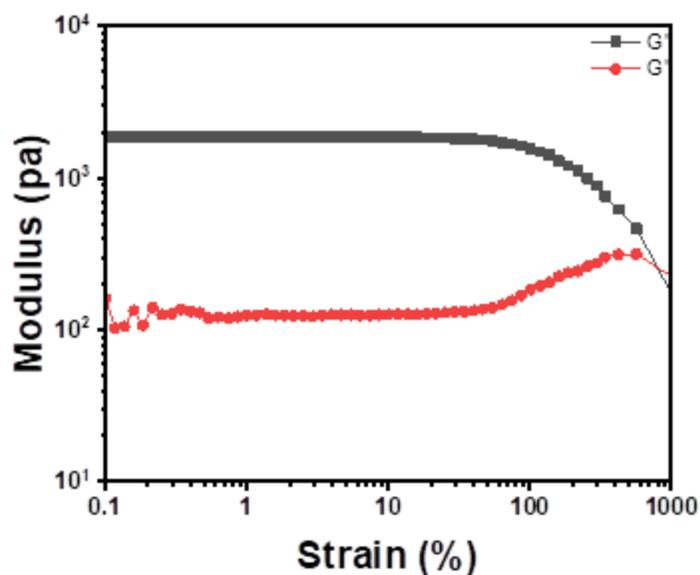
**Figure S2** Dynamic frequency sweep study of PNIPAM-PEGDA hydrogel measured at the strain of 0.8 %. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



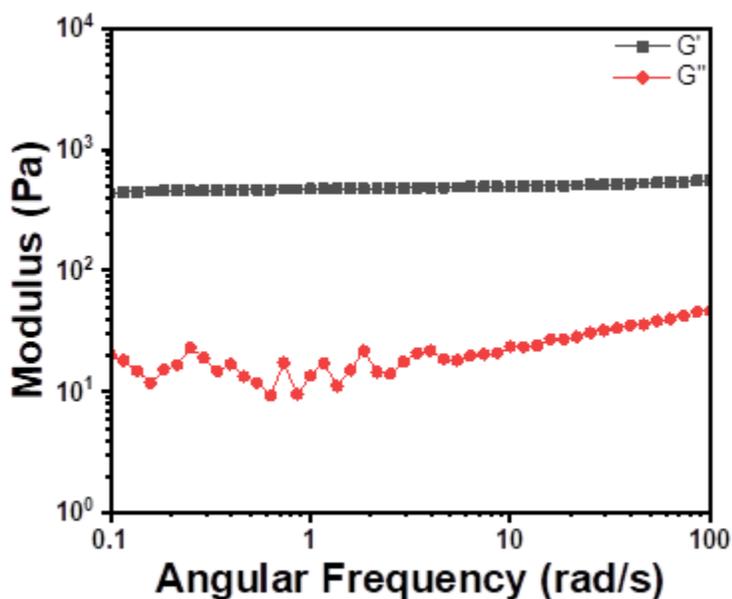
**Figure S3** Dynamic strain sweep study of **PNIPAM-PEGDA** hydrogel at the frequency of 1 Hz. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



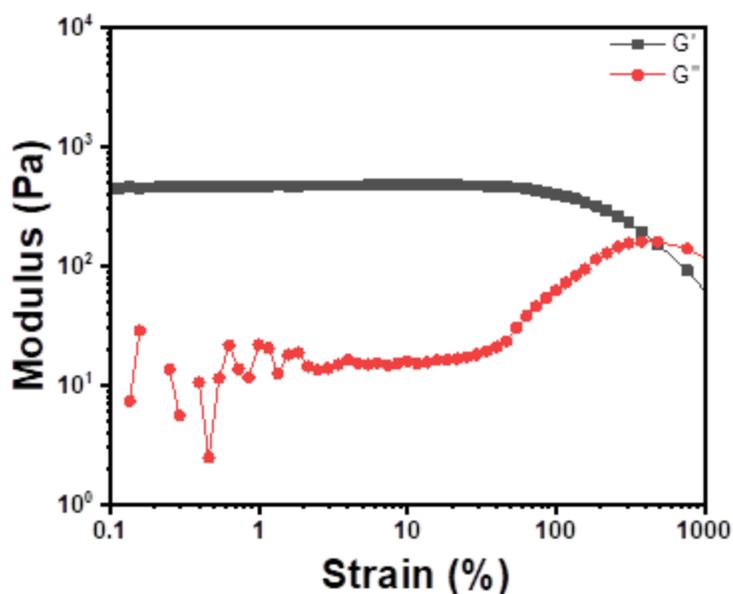
**Figure S4** Dynamic frequency sweep study of **PNIPAM-PEGDA:PFB-FFD** (peptide:NIPAM = 1:5.68) hydrogel measured at the strain of 0.8 %. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



**Figure S5** Dynamic strain sweep study of **PNIPAM-PEGDA:PFBD** (peptide:NIPAM = 1:5.68) hydrogel at the frequency of 1 Hz. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



**Figure S6** Dynamic frequency sweep study of **PNIPAM-PEGDA:PFBD** (peptide:NIPAM = 1:5.68) hydrogel measured at the strain of 0.8%. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).

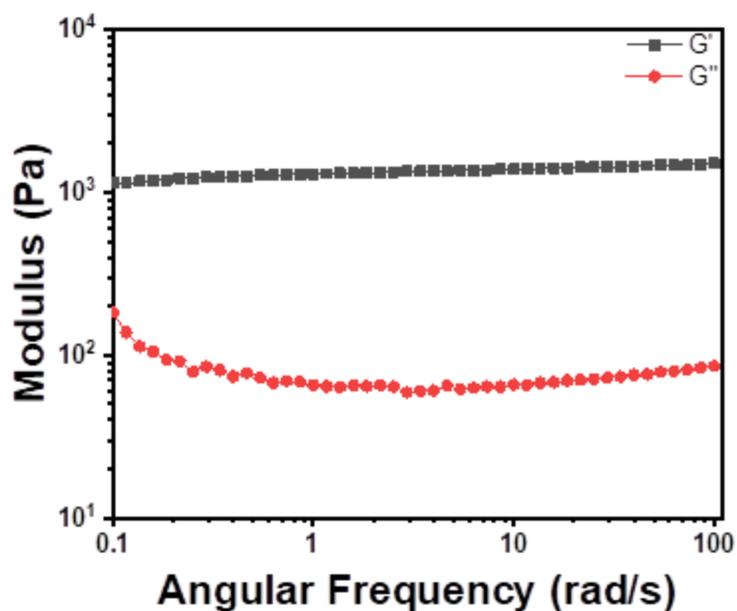


**Figure S7** Dynamic strain sweep study of **PNIPAM-PEGDA:PFB-FFK** (peptide:NIPAM = 1:5.68) hydrogel at the frequency of 1 Hz. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).

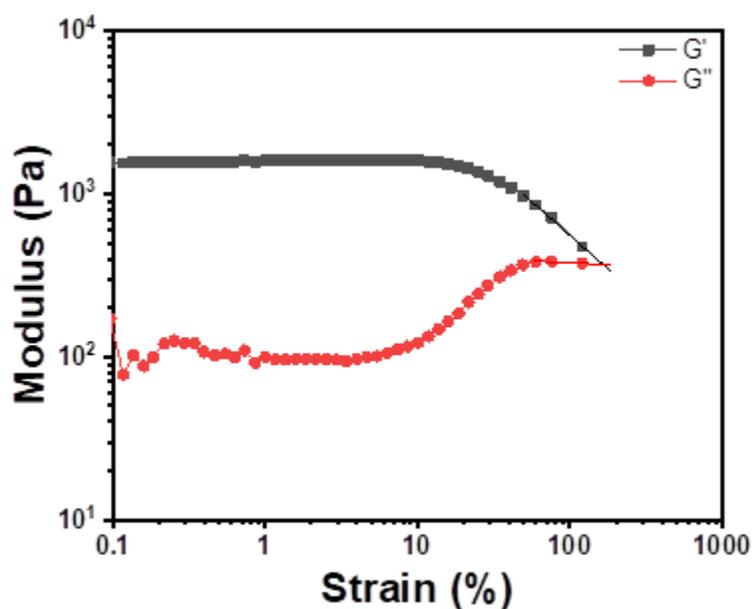
**Table S1** Rheological characteristics of PNIPAM:PEGDA hydrogels at various conditions.

Entry	Ratio (Peptide:NIPAM)	Addition of metal ion	Moduli <sup>a</sup> $G'$ , $G''$ (Pa)	Critical Strain <sup>b</sup> $\gamma_c$ (%)
PNIPAM-PEGDA:PFB-FFD	1:5.68	-	$2.1 \times 10^3$ , $1.9 \times 10^2$	46.6 (791)
PNIPAM-PEGDA:PFB-FFD	1:11.36	-	$1.4 \times 10^3$ , $0.65 \times 10^2$	15.9 (164)
PNIPAM-PEGDA: PFB-FFD	1:22.72	-	$0.89 \times 10^3$ , $0.37 \times 10^2$	13.6 (219)
PNIPAM-PEGDA: PFB-FFD: $\text{Ca}^{2+}$	1:5.68	$\text{Ca}^{2+}$ (10 mM)	$2.9 \times 10^3$ , $3.7 \times 10^2$	21.7 (163)

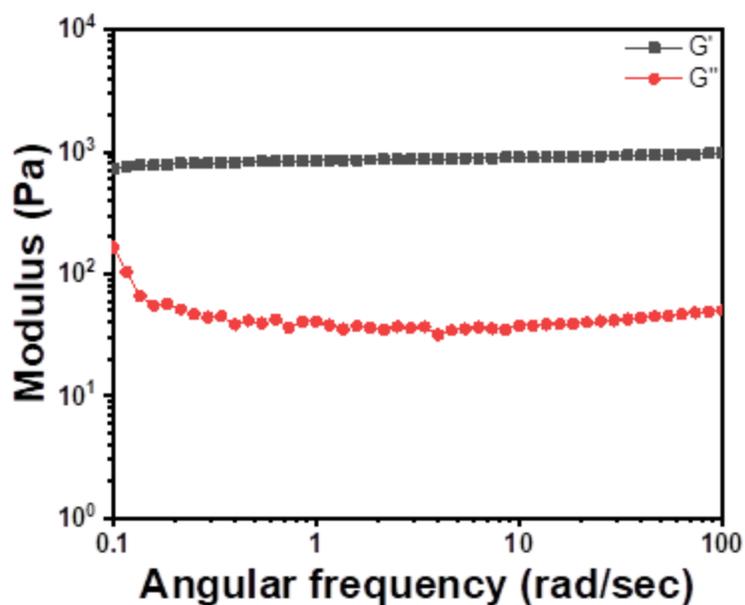
<sup>a</sup> The values at  $10 \text{ rad s}^{-1}$ . <sup>b</sup> The crossover transition point from primary elastic to primary viscous is shown in parenthesis



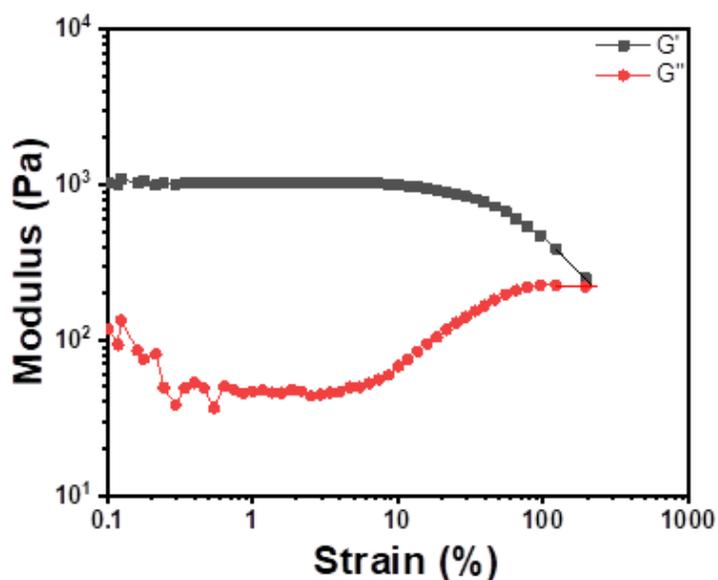
**Figure S8** Dynamic frequency sweep study of **PNIPAM-PEGDA:PFB-FFD** (peptide:NIPAM = 1:11.36) hydrogel measured at the strain of 0.8%. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



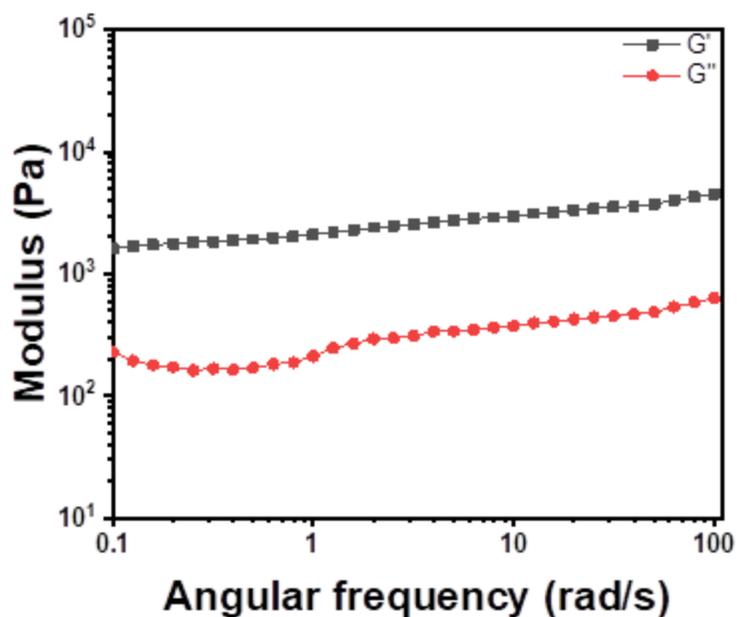
**Figure S9** Dynamic strain sweep study of **PNIPAM-PEGDA:PFB-FFD** (peptide:NIPAM = 1:11.36) hydrogel at the frequency of 1 Hz. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



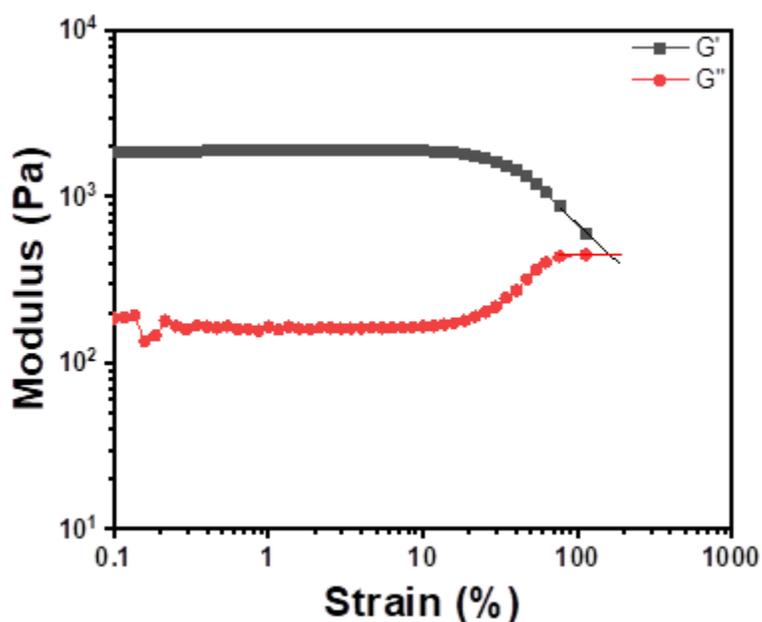
**Figure S10** Dynamic frequency sweep study of **PNIPAM-PEGDA:PFB-FFD** (peptide:NIPAM = 1:22.72) hydrogel measured at the strain of 0.8%. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



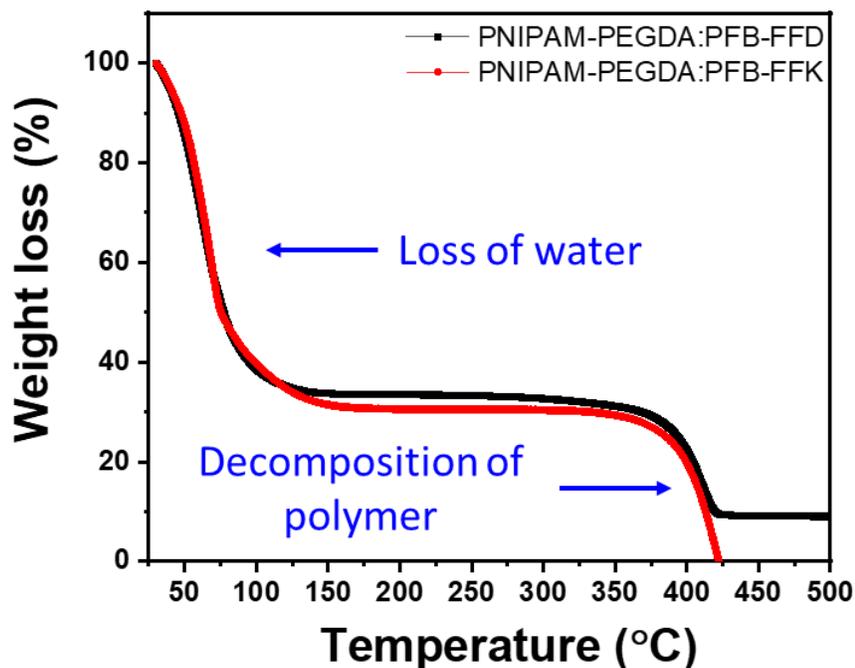
**Figure S11** Dynamic strain sweep study of **PNIPAM-PEGDA:PFB-FFD** (peptide:NIPAM = 1:22.72) hydrogel at the frequency of 1 Hz. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



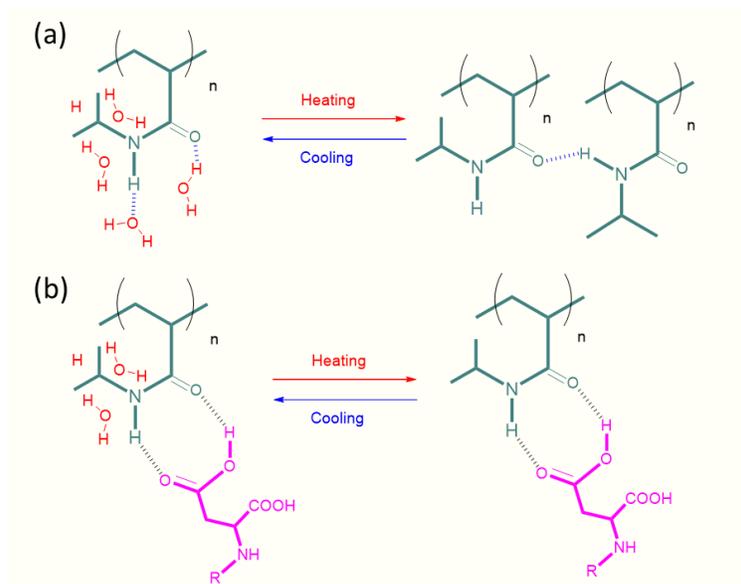
**Figure S12** Dynamic frequency sweep study of **PNIPAM-PEGDA:PFB-FFD:Ca<sup>2+</sup>** (peptide:NIPAM = 1:5.68) hydrogel measured at the strain of 0.8%. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



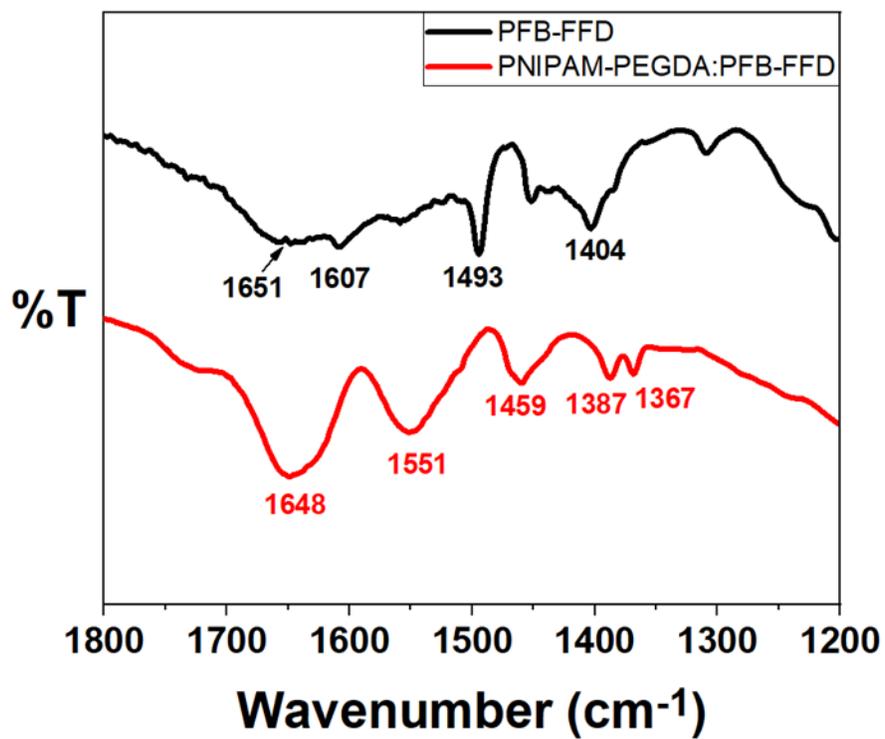
**Figure S13** Dynamic strain sweep study of **PNIPAM-PEGDA:PFB-FFD:Ca<sup>2+</sup>** (peptide:NIPAM = 1:5.68) hydrogel at the frequency of 1 Hz. Storage modulus ( $G'$ , black square) and loss modulus ( $G''$ , red circle).



**Figure S14** Thermal gravimetric analysis (TGA) scans of PNIPAM-PEGDA:PFB-FFD and PNIPAM-PEGDA:PFB-FFK hydrogels.

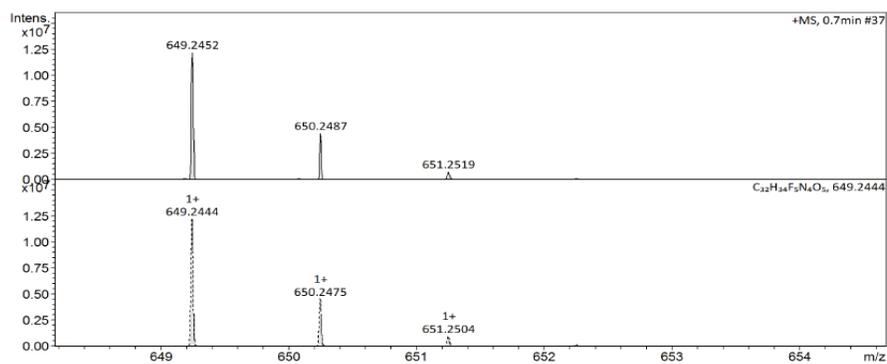


**Figure S15** (a) Schematic representation of the molecular interactions between PNIPAM and hydration water under aqueous condition. Hydrophobic hydration of the isopropyl group is associated with surface tension. The transition to PNIPAM occurs when the material is heated beyond the lower critical solution temperature (LCST).<sup>2,3</sup> (b) Schematic shows possible molecular interactions of PNIPAM-PEGDA:PFB-FFD hydrogels and their transition to higher temperatures.

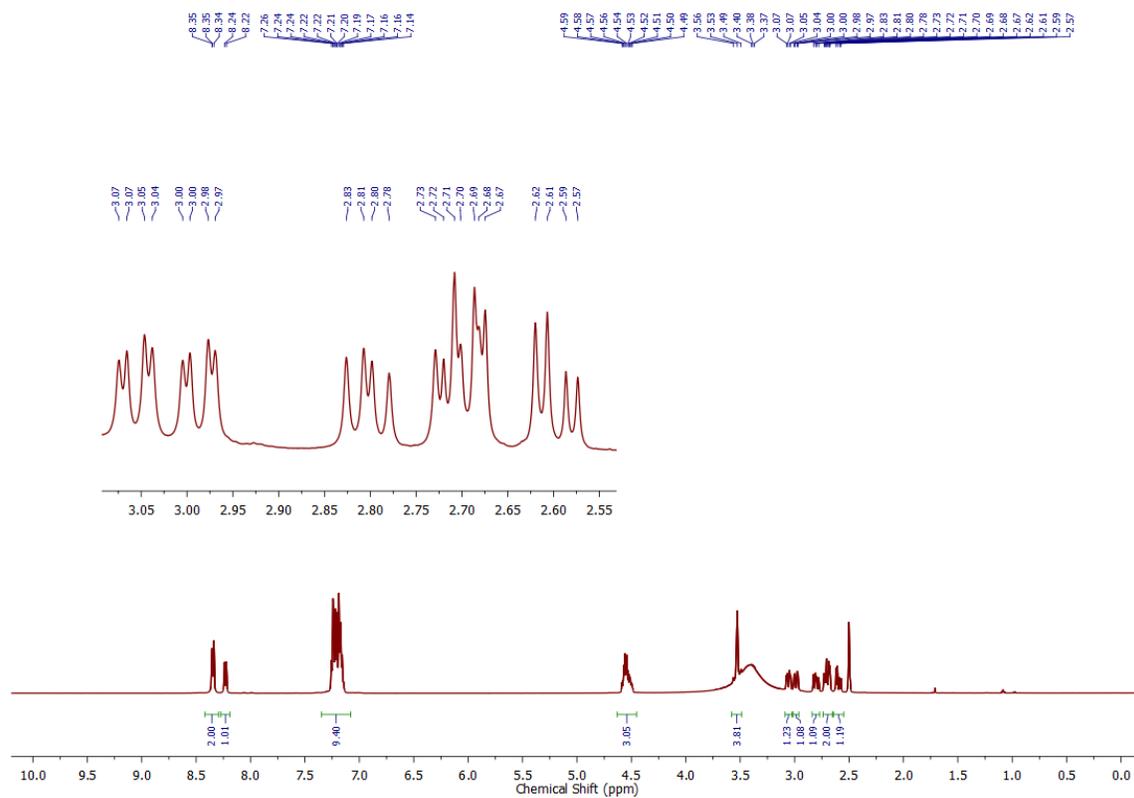


**Figure S16** FT-IR spectra of **PFB-FFD** and **PNIPAM-PEGDA:PFB-FFD** hydrogels.

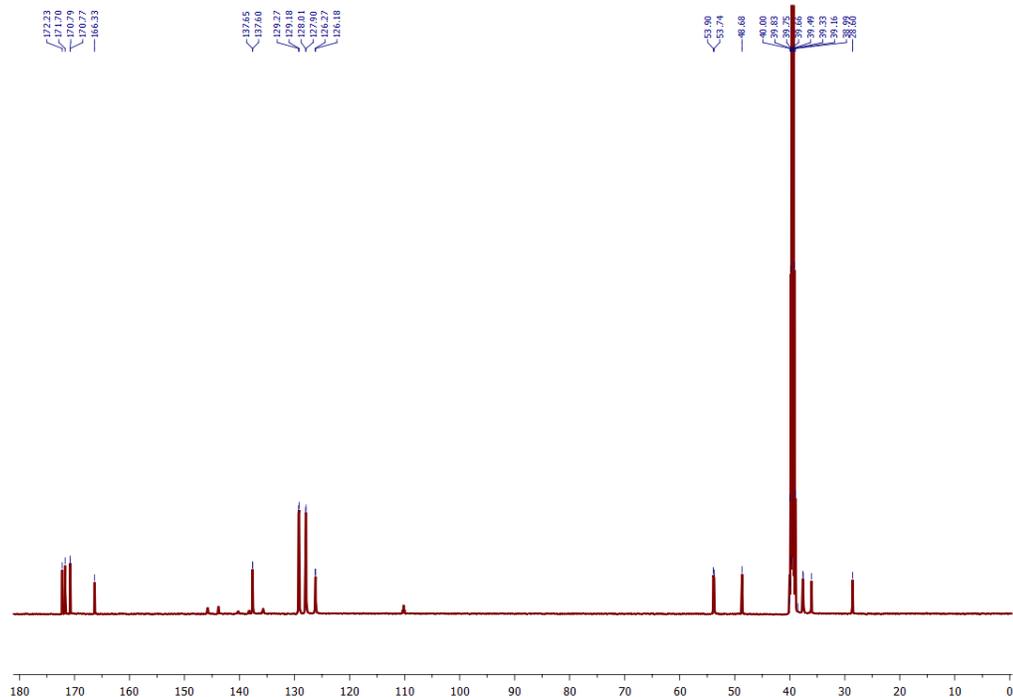




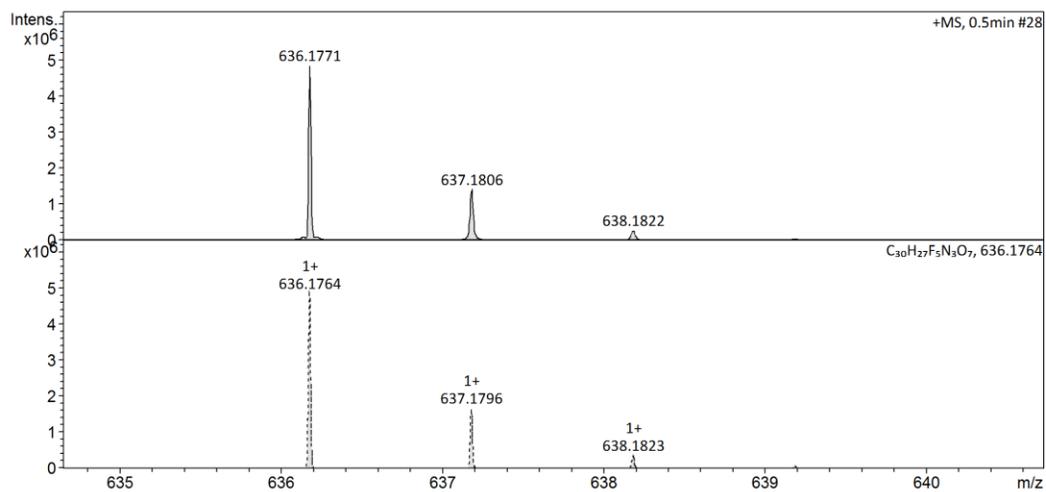
**Figure S19** The HRMS spectrum of **PFB-FFK**.



**Figure S20** The  $^1H$  NMR spectrum of **PFB-FFD** in  $DMSO-d_6$ . Multiplicity patterns are illustrated in the insert.



**Figure S21** The  $^{13}\text{C}$  NMR spectrum of **PFB-FFD** in  $\text{DMSO-}d_6$ .



**Figure S22** The HRMS spectrum of **PFB-FFD**.

#### Reference

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2. Y. Zhang, S. Furyk, L. B. Sagle, Y. Cho, D. E. Bergbreiter and P. S. Cremer, *J. Phys. Chem. C*, 2007, **111**, 8916-8924.
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