Electronic Supplementary Material (ESI) for Molecular Systems Design & Engineering.

Supporting Information

Influence of the metal ion crosslinking on nanostructures, stiffness, and biofunctions of bioactive peptide hydrogels

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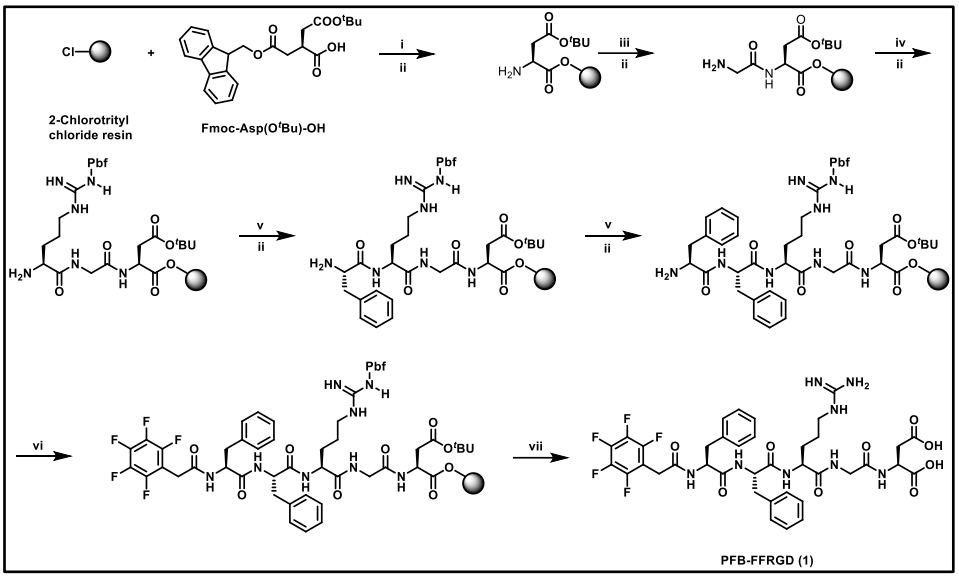
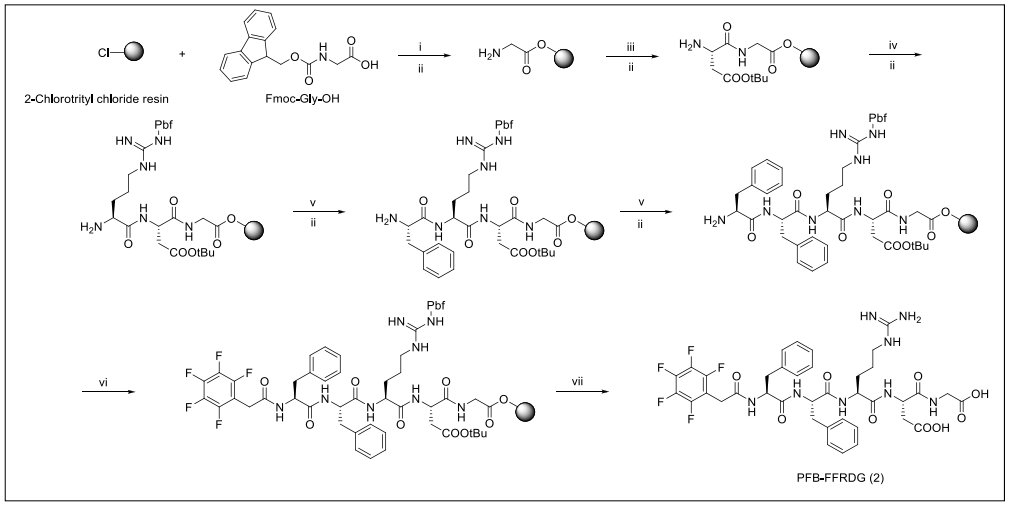
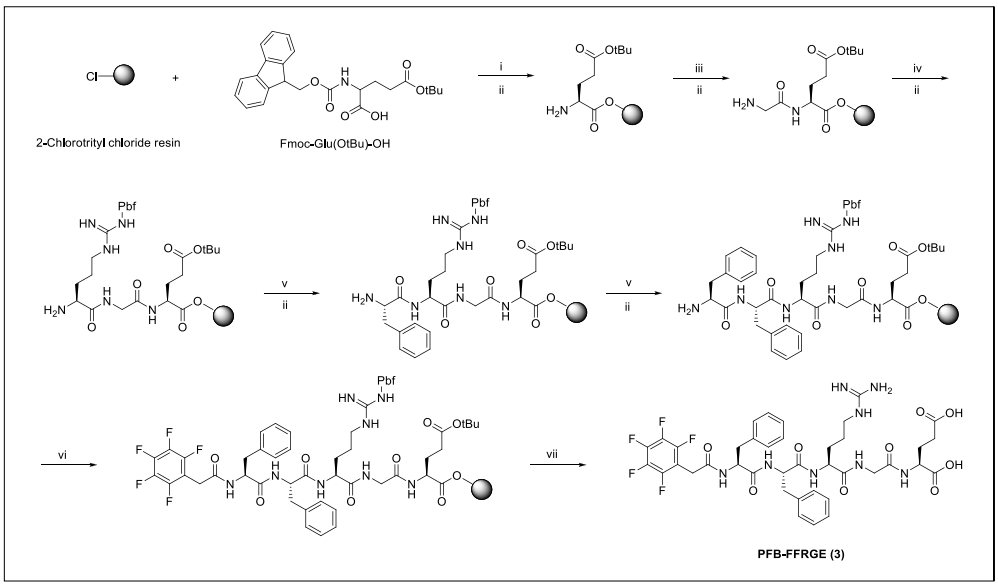
1. Experimental Section:



Scheme S1. Synthetic scheme for PFB-FFRGD: (i) DIEA; (ii) 20% Piperidine in DMF; (iii) Fmoc-Gly-OH, HBTU, DIEA; (iv) Fmoc-Arg(Pbf)-OH, HBTU, DIEA; (v) Fmoc-Phe-OH, HBTU, DIEA; (vi) PFB (Pentafluorophenylacetic acid), HBTU, DIEA; (vii) TFA: TIPS: water = 95:2.5:2.5 (v/v %).

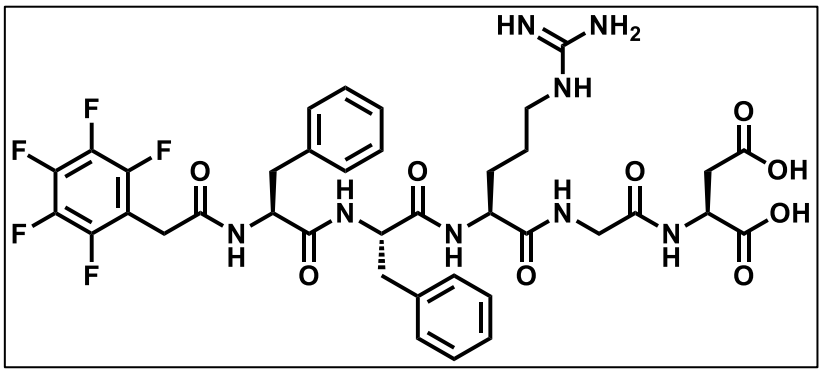


Scheme S2. Synthetic scheme for PFB-FFRDG: (i) DIEA; (ii) 20% Piperidine in DMF; (iii) Fmoc-Asp(O^{t}Bu)-OH, (iv) Fmoc-Arg(Pbf)-OH, HBTU, DIEA; (v) Fmoc-Phe-OH, HBTU, DIEA; (vi) PFB (Pentafluorophenylacetic acid), HBTU, DIEA; (vii) TFA: TIPS: water = 95:2.5:2.5 (v/v %).



Scheme S3. Synthetic scheme for PFB-FFRGE: (i) DIEA; (ii) 20% Piperidine in DMF; (iii) Fmoc-Gly-OH, HBTU, DIEA; (iv) Fmoc-Arg(Pbf)-OH, HBTU, DIEA; (v) Fmoc-Phe-OH, HBTU, DIEA; (vi) PFB (Pentafluorophenylacetic acid), HBTU, DIEA; (vii) TFA: TIPS: water = 95:2.5:2.5 (v/v %).

Synthesis of PFB-FFRGD (1)

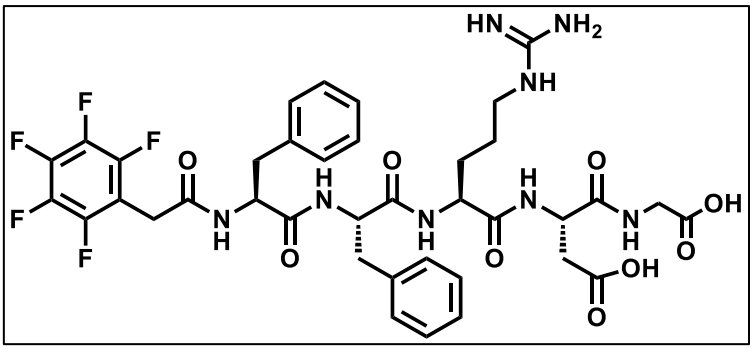


The peptide derivatives were synthesized by a solid phase peptide synthesis using 2-chlorotrityl chloride resin as the solid phase substrate. Briefly, the resin (1.2 g, 2.0 mmol) was suspended in anhydrous dichloromethane and allowed to swell for 30 minutes with continuous stirring. The first amino acid Fmoc-Asp(O^{t}Bu)-OH (0.823 g, 2.0 mmol) and *N,N*-diisopropylethylamine (DIEA) 5.0 mmol were dissolved in an appropriate amount of anhydrous dimethylformamide (DMF) and then added to the

resin. The reaction was carried out for 1 h to allow the amino acid to be attached to the resin. The Fmoc protecting group was then removed by reacting with 20% piperidine in DMF for 30 minutes, and the washing was repeated twice. Then, the second amino acid Fmoc-Gly-OH (0.743 g, 2.5 mmol) was coupled to amine using coupling agent *O*-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate, HBTU (0.948 g, 2.5 mmol) and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting 20% piperidine in DMF for an additional 30 minutes, and washing was repeated twice. The third amino acid Fmoc-Arg(Pbf)-OH (1.622 g, 2.5 mmol) was coupled to amine using coupling agent HBTU (0.948 g, 2.5 mmol) and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting with 20% piperidine in DMF for 30 minutes, and washing was repeated twice. The fourth amino acid Fmoc-Phe-OH (0.968 g, 2.5 mmol) was coupled to amine using coupling agent, HBTU (0.948 g, 2.5 mmol), and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the resulting solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting with 20% piperidine in DMF for 30 minutes, and washing was repeated twice. The fifth amino acid Fmoc-Phe-OH (0.968 g, 2.5 mmol) was coupled to amine using coupling agent, HBTU (0.948 g, 2.5 mmol), and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the resulting solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting 20% piperidine in DMF for 30 minutes, and washing was repeated twice. Finally, the capping agent, pentafluorophenyl acetic acid (1.356 g, 6.0 mmol), HBTU (2.276 g, 6.0 mmol), and DIEA (7.5 mmol) was dissolved in an appropriate amount of anhydrous DMF, and this solution was added to resin-bound amine and allowed to react for overnight. Finally, the solvent was removed and the

resin was cleaved by adding trifluoroacetic acid (TFA) 95% with deionized water 2.5% and TIPS 2.5% for 4 h. The resulting solution was air-dried, and then diethyl ether was added to precipitate the target product. The solid was dried under a vacuum to remove residual solvents. ^{1}H NMR (300 MHz, [d_6] DMSO): δ= 7.27-7.15 (m, 10H, Ar-H), 4.61-4.45 (m, 3H), 4.36-4.24 (m, 1H), 3.81-3.68 (m, 2H), 3.53 (s, 2H), 3.12-2.93 (m, 4H), 2.87-2.76 (m, 1H), 2.74-2.59 (m, 3H), 1.80-1.65 (m, 1H), 1.63-1.38 (m, 3H); ^{13}C NMR (75 MHz, [d_6] DMSO): δ= 173.3, 172.8, 172.3, 171.9, 171.8, 169.4, 167.3, 157.6, 147.4, 144.1, 138.7, 138.6, 136.0, 130.2, 130.1, 128.9, 128.8, 127.2, 127.1, 54.9, 53.1, 49.6, 42.5, 42.2, 38.5, 38.3, 37.3, 37.1, 30.2, 29.5, 28.8. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{38}H_{41}F_5N_8O_9$: 849.2989, obsvd: 849.3005.

Synthesis of PFB-FFRDG (2)

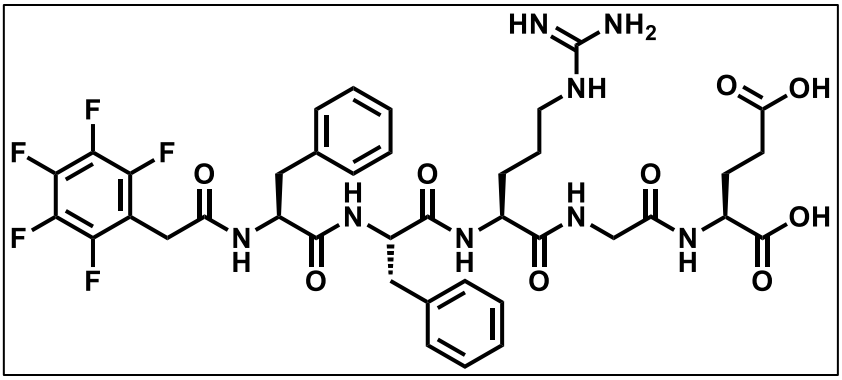


The peptide derivatives were synthesized by a solid phase peptide synthesis using 2-chlorotrityl chloride resin as the solid phase substrate. Briefly, the resin (1.2 g, 2.0 mmol) was suspended in anhydrous dichloromethane and allowed to swell for 30 minutes with continuous stirring. The first amino acid Fmoc-Gly-OH (0.594 g, 2.0 mmol) and *N,N*-diisopropylethylamine (DIEA) 5.0 mmol were dissolved in an appropriate amount of anhydrous dimethylformamide (DMF) and then added to the resin. The reaction was carried out for 1 h to allow the amino acid to be attached to the resin. The Fmoc protecting group was then removed by reacting with 20% piperidine in DMF

for 30 minutes, and the washing was repeated twice. Then, the second amino acid Fmoc-Asp(O^tBu)-OH (1.028 g, 2.5 mmol) was coupled to amine using coupling agent *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HBTU (0.948 g, 2.5 mmol) and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting 20% piperidine in DMF for an additional 30 minutes, and washing was repeated twice. The third amino acid Fmoc-Arg(Pbf)-OH (1.622 g, 2.5 mmol) was coupled to amine using coupling agent HBTU (0.948 g, 2.5 mmol) and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting with 20% piperidine in DMF for 30 minutes, and washing was repeated twice. The fourth amino acid Fmoc-Phe-OH (0.968 g, 2.5 mmol) was coupled to amine using coupling agent, HBTU (0.948 g, 2.5 mmol), and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the resulting solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting with 20% piperidine in DMF for 30 minutes, and washing was repeated twice. The fifth amino acid Fmoc-Phe-OH (0.968 g, 2.5 mmol) was coupled to amine using coupling agent, HBTU (0.948 g, 2.5 mmol), and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the resulting solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting 20% piperidine in DMF for 30 minutes, and washing was repeated twice. Finally, the capping agent pentafluorophenyl acetic acid (1.356 g, 6.0 mmol), HBTU (2.276 g, 6.0 mmol), and DIEA (7.5 mmol) was dissolved in an appropriate amount of anhydrous DMF, and this solution was added to resin-bound amine and allowed to react for overnight. Finally, the solvent was removed and the resin was cleaved by adding trifluoroacetic acid (TFA) 95% with deionized water 2.5% and TIPS 2.5% for 4 h. The resulting solution was air-dried, and then diethyl ether was added to

precipitate the target product. The solid was dried under a vacuum to remove residual solvents. ^{1}H NMR (300 MHz, [d_6] DMSO): δ= 7.30-7.08 (m, 10H, Ar-H), 4.64-4.46 (m, 3H), 4.35-4.28 (m, 1H), 3.70-3.59 (m, 2H), 3.52 (s, 2H), 3.14-3.00 (m, 4H), 2.99-2.95 (m, 1H), 2.86-2.80 (m, 1H), 2.73-2.65 (m, 2H), 1.83-1.75 (m, 1H), 1.61-1.47.(m, 3H); ^{13}C NMR (75 MHz, [d_6] DMSO): δ= 171.9, 171.4, 171.4, 171.3, 171.2, 170.9, 166.7, 156.9, 145.9, 143.9, 137.8, 137.8, 135.9, 129.4, 129.3, 128.2, 128.1, 126.5, 126.4, 54.3, 52.5, 49.6, 41.4, 40.7, 37.6, 37.3, 36.8, 36.3, 29.2, 28.7, 24.8. HRMS (ESI) *m/z*: $[M+H]^-$ calcd for $C_{38}H_{41}F_5N_8O_9$: 847.2844, obsvd: 847.2898.

Synthesis of PFB-FFRGE (3)

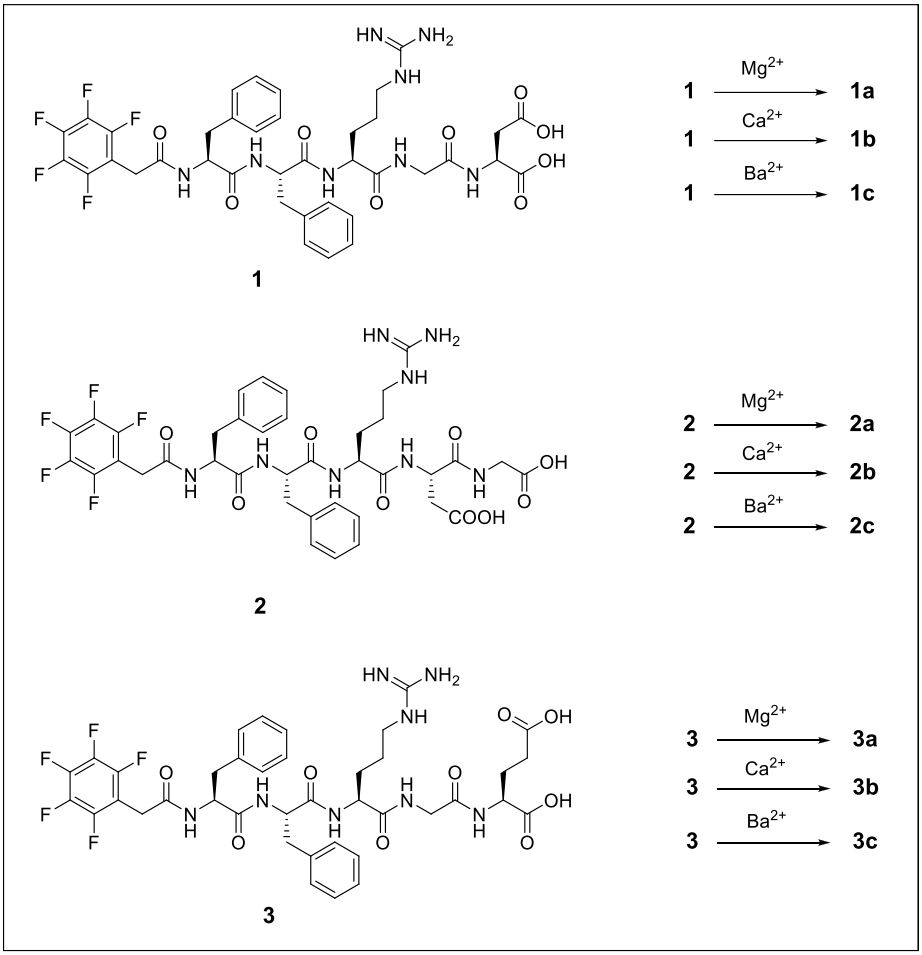


The peptide derivatives were synthesized by a solid phase peptide synthesis using 2-chlorotrityl chloride resin as the solid phase substrate. Briefly, the resin (1.2 g, 2.0 mmol) was suspended in anhydrous dichloromethane and allowed to swell for 30 minutes with continuous stirring. The first amino acid Fmoc-Glu(O^{t}Bu)-OH (0.851 g, 2.0 mmol) and *N,N*-diisopropylethylamine (DIEA) 5.0 mmol were dissolved in an appropriate amount of anhydrous dimethylformamide (DMF) and then added to the resin. The reaction was carried out for 1 h to allow the amino acid to be attached to the resin. The Fmoc protecting group was then removed by reacting with 20% piperidine in DMF for 30 minutes, and the washing was repeated twice. Then, the second amino acid Fmoc-Gly-OH (0.743 g, 2.5 mmol) was coupled to amine using coupling agent *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium

hexafluorophosphate, HBTU (0.948 g, 2.5 mmol) and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting 20% piperidine in DMF for an additional 30 minutes, and washing was repeated twice. The third amino acid Fmoc-Arg(Pbf)-OH (1.622 g, 2.5 mmol) was coupled to amine using coupling agent HBTU (0.948 g, 2.5 mmol) and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting with 20% piperidine in DMF for 30 minutes, and washing was repeated twice. The fourth amino acid Fmoc-Phe-OH (0.968 g, 2.5 mmol) was coupled to amine using coupling agent, HBTU (2.5 mmol, 0.948 g), and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the resulting solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting with 20% piperidine in DMF for 30 minutes, and washing was repeated twice. The fifth amino acid Fmoc-Phe-OH (0.968 g, 2.5 mmol) was coupled to amine using coupling agent, HBTU (0.948 g, 2.5 mmol), and DIEA (5.0 mmol) was dissolved in anhydrous DMF, and the resulting solution was added to the resin to react for 1 h. The Fmoc group was deprotected by reacting 20% piperidine in DMF for 30 minutes, and washing was repeated twice. Finally, the capping agent, pentafluorophenyl acetic acid (1.356 g, 6.0 mmol), HBTU (2.276 g, 3.0 mmol), and DIEA (7.5 mmol) was dissolved in an appropriate amount of anhydrous DMF, and this solution was added to resin-bound amine and allowed to react for overnight. Finally, the solvent was removed and the resin was cleaved by adding trifluoroacetic acid (TFA) 95% with deionized water 2.5% and TIPS 2.5% for 4 h. The resulting solution was air-dried, and then diethyl ether was added to precipitate the target product. The solid was dried under a vacuum to remove residual solvents. ^{1}H NMR (300 MHz, [d_6] DMSO): δ= 7.31-7.10 (m, 10H, Ar-H), 4.58-4.46 (m, 2H), 4.31-4.24 (m , 1H), 4.19-4.85 (m, 1H),

3.83-3.75 (m, 1H), 3.72-3.64 (m, 1H), 3.54 (s, 2H), 3.12-2.94 (m, 4H), 2.86-2.79 (m, 1H), 2.71-2.65 (m, 1H), 2.31-2.21(m, 2H), 2.00-1.92(m, 1H), 1.84-1.71(m, 2H), 1.62-1.45 (m, 3H); ^{13}C NMR (75 MHz, [d_6] DMSO): δ= 174.8, 174.1, 172.4, 172.0, 171.9, 169.6, 167.3, 157.7, 147.3, 144.1, 139.0, 138.6, 136.0, 130.2, 130.1, 129.0, 128.8, 127.2, 127.2, 54.9, 53.3, 53.2, 52.3, 42.6, 38.5, 38.2, 31.0, 30.8, 30.0, 29.5, 27.5, 25.7. HRMS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{43}F_5N_8O_9$: 863.3145, obsvd: 863.3146.

A schematic of the preparation of hydrogels with or without metal ions is given below.



Scheme S4. Chemical structures and schematic of the preparation of hydrogels with or without metal ions (Mg^{2+}, Ca^{2+}, Ba^{2+}).

2. Experimental Details:

Peptide synthesis

The desired peptides were synthesized by solid-phase peptide synthesis (SPPS) using classical Fmoc protocols. The compounds were purified by a reversed-phase HPLC. We measured the ^{1}H-NMR and ^{13}C-NMR spectra of the peptides using a VARIAN VNMRS-600 NMR spectrometer at 600 MHz and DMSO-d6 as solvent at room temperature. Mass data were acquired using a Micro Q-TOF MS spectrometer.

Hydrogel preparation

Hydrogels 2 wt% were prepared by weighing 8.0 mg of each compound into a screw-capped glass vial (2 mL). DI water (0.2 mL) was added to each vial, and then 1 M NaOH solution was added and sonicated to dissolve the peptides. A salt solution with a final concentration of 10 mM was added for the salt-induced hydrogels. The pH of the hydrogels was adjusted to neutral by adding 0.5 M HCl solution. DI water was added to increase the final volume to 0.4 mL. The hydrogels were allowed to self-assemble at room temperature for a few minutes, and their gelation was confirmed by the vial-inversion method.

Transmission electron microscopy (TEM)

The TEM images of hydrogels were obtained using a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV. Hydrogel samples were prepared by applying 20 µL of hydrogel directly onto 200 mesh carbon-coated copper grids. An excess amount of the hydrogel was carefully removed by capillary action using filter paper. The grid was then washed 3-4 times with DI water by touching it with the surface of the drops of DI water and the excess water was carefully wiped away with filter paper. The grids were then immediately stained with 2% uranyl acetate

for at least 30 seconds and excess amount of uranyl acetate stain was removed by capillary action and then copper grids were air dried for 48 h before imaging.

Rheology

The rheological properties were measured by Anton Paar rheometry. A 20 mm parallel plate was used to perform the experiment. Between the parallel plate geometry and a stationary bottom plate, 100 µL of hydrogel was placed and the storage modulus (G') and loss modulus (G'') were measured with angular frequency sweep test. Test range (0.1 to 100 rads-1 frequency, strain = 0.8 %), at temperature of 25°C.

Thioflavin T test

The peptide samples at different concentrations (500-3000 µM) were prepared in water. Then 20 µM ThT solution was added to the samples and incubated for 24 h. Then emission spectra of samples were measured using Hitachi F-4500 fluorescence spectrometer at an excitation wavelength of λ= 440 nm, with slit of 5 nm and PMT voltage of 600 V. The emission intensities detected at 484 nm respectively.

Congo red staining

The Congo red dye was added to freshly prepare saturated sodium chloride ethanol solution (80%) and vortexed vigorously before being filtered through a 0.45 micron filter. The Congo red dye solution 50 µL was placed onto the glass slide, and then 25 µL of hydrogel was placed in between. The hydrogel was stained for about a minute or two using Congo red dye. The stained hydrogel was covered with a coverslip and excess dye was removed by blotting. The hydrogel-containing slide was then placed between crossed polarizers, and images were obtained by Zeiss (Axio Vert.A1) microscope.

Circular dichroism (CD) spectroscopy

The CD spectra of the hydrogelators were obtained by using Jasco J-815 CD spectrometer in the UV-visible region (190-600 nm) using a quartz cuvette with a 0.1

mm diameter, and the total gelator concentration was 500 μM in each sample.

Spectroscopic studies

A JASCO- V670 spectrometer was used to measure absorption spectra, and a HITACHI F7000 was used to measure fluorescence spectra. A Bruker Vertex80v spectrometer was employed to measure FT-IR spectra with a spectral resolution of 2 cm-1 over an average of 200 scans.

Powder XRD analysis

A Powder XRD study was conducted to understand the periodic arrangement of molecules while they were self-assembling. Following preparation of the hydrogels, the samples were frozen and dried in a lyophilizer (FDM-2, UNISS) to obtain the xerogels. PXRD was used to analyse xerogel samples, the measurements were carried out on a Bragg–Brentano-type powder diffractometer (Bruker D8 Advance diffractometer, operated at 40 kV and 40 mA, with Cu Kα radiation, λ = 1.5418 Å). The data were collected in a 2θ range of 5–30° using a step size of 0.016.

Hydrogel degradation study

The hydrogel degradation studies were conducted on compound **1, 2**, and **3** with or without metal ions in phosphate buffered saline (PBS) under 37 °C. In brief, 0.4 mL of each hydrogel was prepared in a screw-capped 5 mL glass vial and PBS 4 mL (10 folds) was added on top of each hydrogel and incubated at 37 °C. At each designated time points 1 mL of PBS was removed from gels and replenished with fresh PBS 1 mL and incubated at 37 °C until seven days. After seven days of incubation, PBS was removed and samples were lyophilized. Dry weight (Wd) of each hydrogel was measured and the degradation percentage was calculated using the formula $(W_d - W_i)/W_i \times 100$ %, Wi is the initial weight of hydrogel on day 0.

Cell line and culture

Dr. Shih-Chieh Hung (China Medical University, Taiwan) provided us with human mesenchymal stem cells containing red fluorescence protein (3A6-RFP) which were cultured in Dulbecco's modified Eagle's medium (DMEM) low glucose (HyClone, Cat # SH30021.02) supplemented with 10% fetal bovine serum (HyClone, Cat #SH30071) and 100 IU/mL of penicillin and streptomycin. (HyClone Cat #SV30010).

Cell viability assay

The viability of the cells was assessed with a colorimetric assay (MTT). In a 24-well tissue culture plate, 3A6-RFP (hMSCs) and L929 (mouse fibroblasts) cells were seeded at density $5x10^4$ cells per well and incubated for 24 h at 37°C under 5% CO_2. Following 24 h. incubation, the medium was replaced with a freshly prepared medium containing samples at concentrations of 10 to 500 µM. Treated cells were incubated for 24, to 72 h. At the end of each time point, MTT reagent 0.5 mg/mL was added to each well and incubated for an additional 4 h and then formazan crystals were dissolved in DMSO. The 100 µL of each 24-well plates were transferred to a 96-well plate and the optical density of the resulting solution was measured at 595 nm using the TECAN Infinite F50 microplate reader. Cells without the treatment were used as the control.

Cell viability assay (hydrogel leachable)

The hydrogels 2 wt % were prepared under sterile conditions and then pH was adjusted neutral (≈ 7.4). The resulting hydrogels were then leached by complete medium (DMEM low glucose containing 10% fetal bovine serum supplemented with Penicillin/Streptomycin 100 IU/mL) under a humidified atmosphere of 5% CO_2 at 37°C. The 3A6-RFP cells were seeded onto a 24-well tissue culture plate at a density of $5×10^4$ cells in each well and incubated for 24 h. Following 24 h of incubation, the medium was removed and cells were then incubated with hydrogel extraction for 24 to 72 h. At

the end of each time point fresh medium containing MTT reagent 0.5 mg/mL was added and incubated for an additional 4 h. MTT reagent was removed after 4 h of incubation and DMSO was added to dissolve formazan crystals. The 100 µL of each 24-well plate was transferred to the 96-well plate and the optical density of the resulting solution was measured at 595 nm using the TECAN Infinite F50 microplate reader. Cells without the treatment of hydrogel extraction were used as the control.

3D cell culture

The human mesenchymal stem cells with a red fluorescent protein (3A6-RFP) $2x10^5$ cells were encapsulated in hydrogels 200 µL each were plated in 8-well chamber slides. The growth medium 200 µL was added on top of hydrogel encapsulated cells and cultured at 37 °C and 5% CO_2 atmosphere for 24 h. The cultured cells were then imaged using a Leica confocal microscopy.

3. Supplementary Figures:

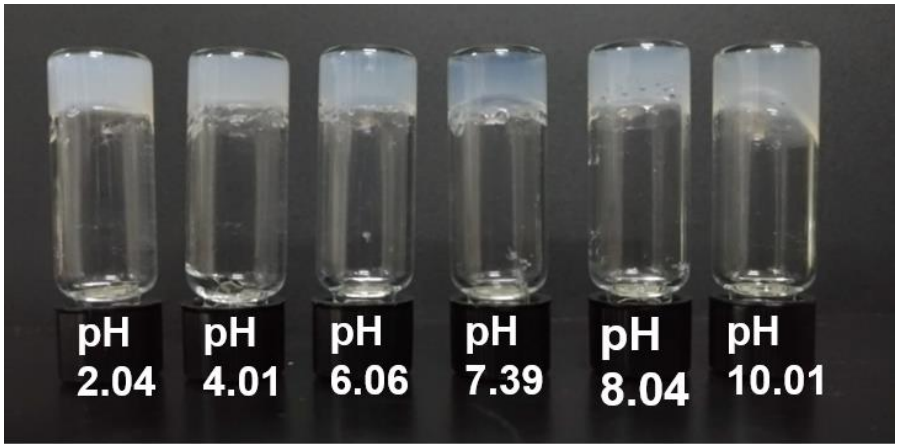


Fig. S1 Optical images of compound **1 (**PFB-FFRGD) hydrogels at various pH conditions (pH 2-10).

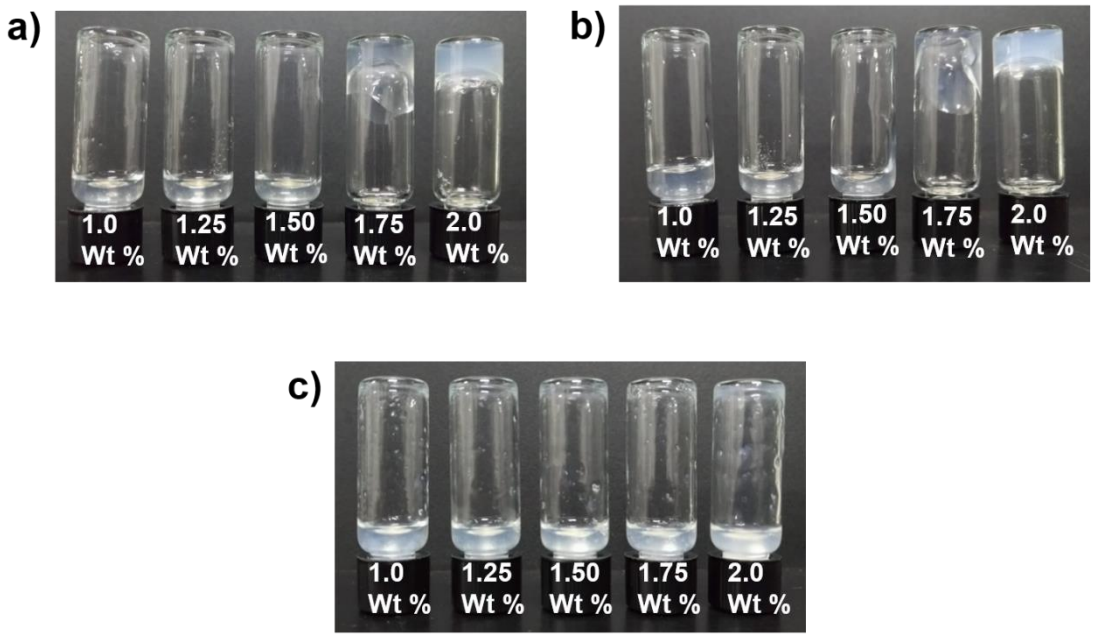


Fig. S2 Optical images of hydrogels at various concentrations (1 to 2 wt %) at neutral pH. a) PFB-FFRGD; b) PFB-FFRDG; c) PFB-FFRGE.

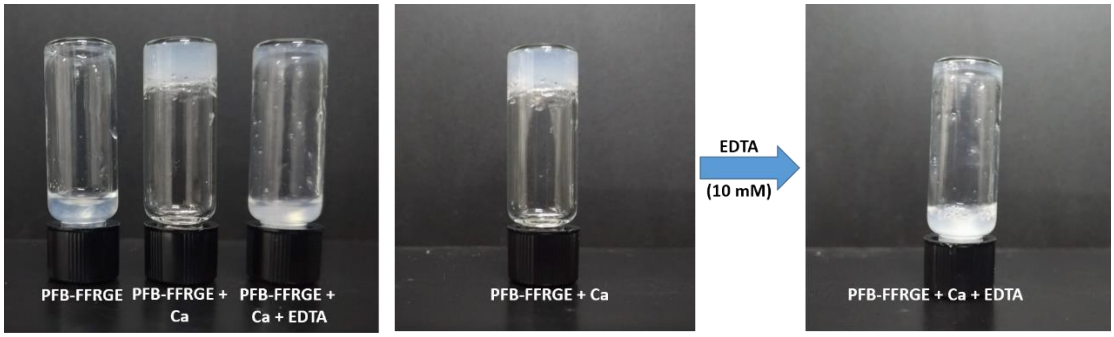


Fig. S3 Responsive behavior of hydrogelator **3** (PFB-FFRGE) under various conditions.

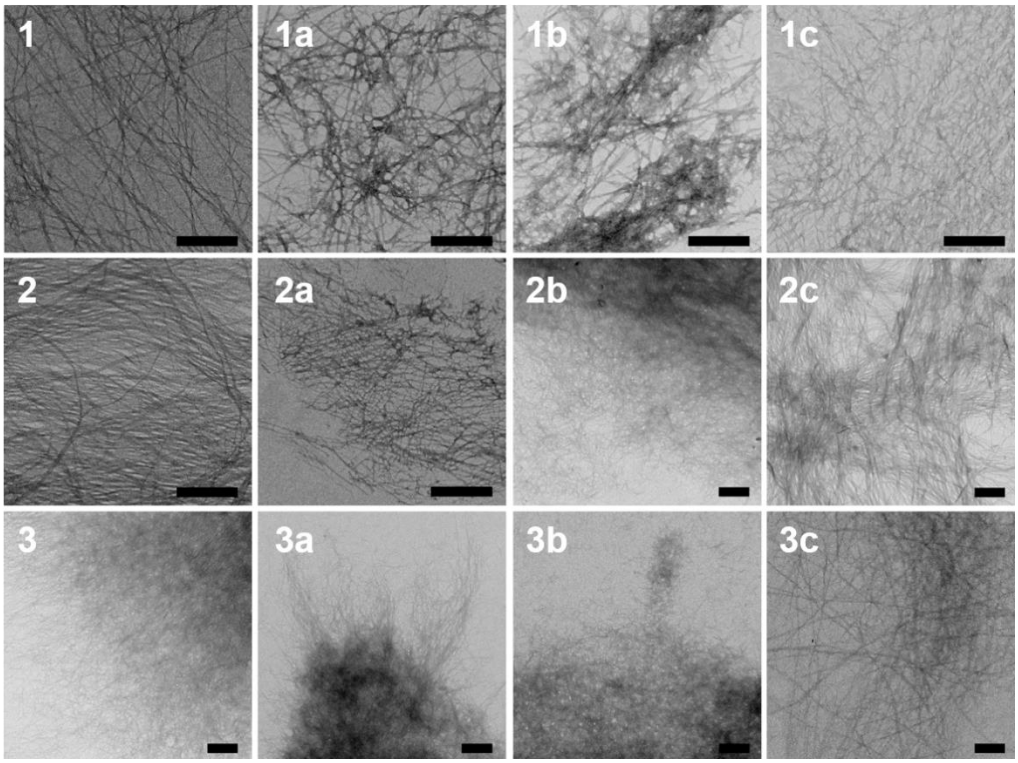


Fig. S4 Low magnification TEM images of 2 wt% hydrogels PFB-FFRGD (1); PFB-FFRDG (2); and PFB-FFRGE (3) with various metal ions (10 mM) [(a) Mg^{2+}, (b) Ca^{2+}, and (c) Ba^{2+}, (Scale bar : 500 nm)].

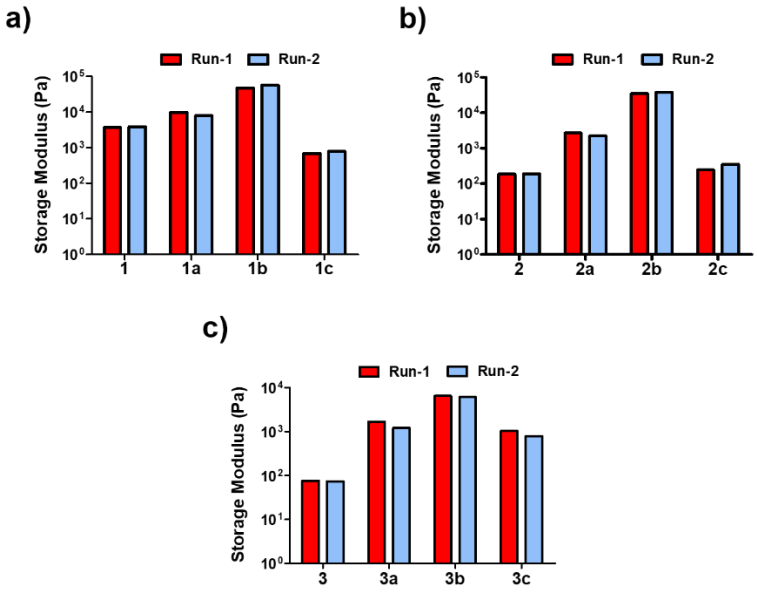


Fig. S5 Rheological measurements: average storage modulus (G') of 2 wt% hydrogels of compound **1**, **2**, and **3** with and without metal ions (**a-c**) at 25 ˚C and neutral pH. (Test range of 0.1–100 rad s^{-1} at a critical strain of 0.8%).

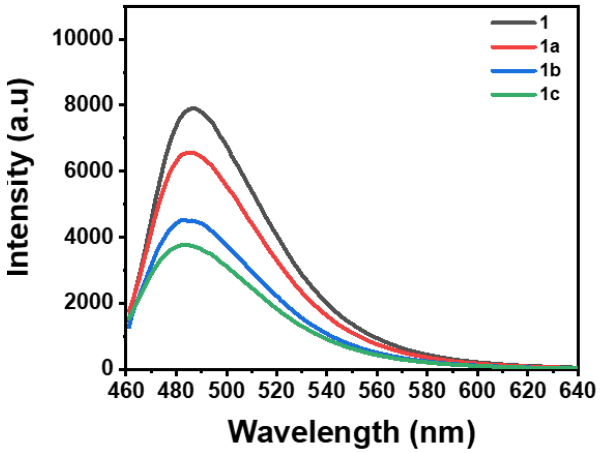


Fig. S6 Emission intensities of ThT (20 µM, λex = 440 nm) at 3000 µM concentration of compound **1** with or without metal ions: PFB-FFRGD (**1**), PFB-FFRGD/ Mg^{2+} (**1a),** PFB-FFRGD/ Ca^{2+} (**1b**), and PFB-FFRGD/ Ba^{2+} (**1c**)**.**

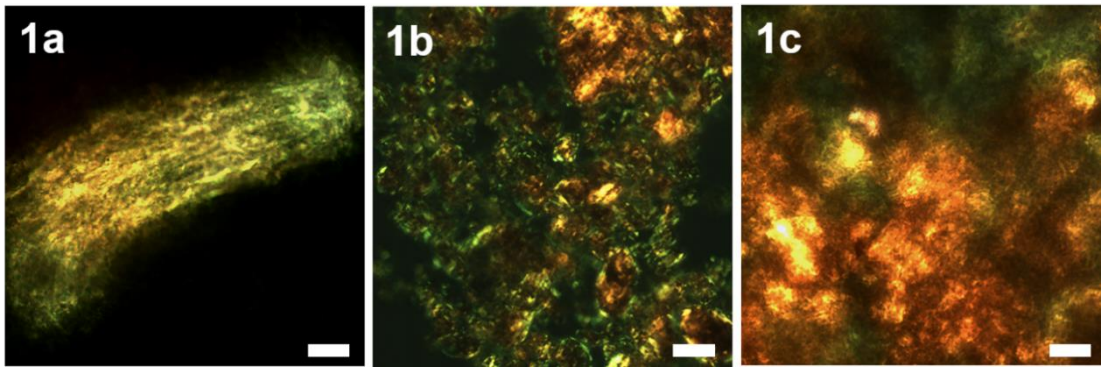


Fig. S7 Congo red staining: polarized optical microscopic image of compounds PFB-FFRGD/ Mg^{2+} (**1a),** PFB-FFRGD/ Ca^{2+} (**1b**), and PFB-FFRGD/ Ba^{2+} (**1c**)**.** (Scale bar: 100 µm).

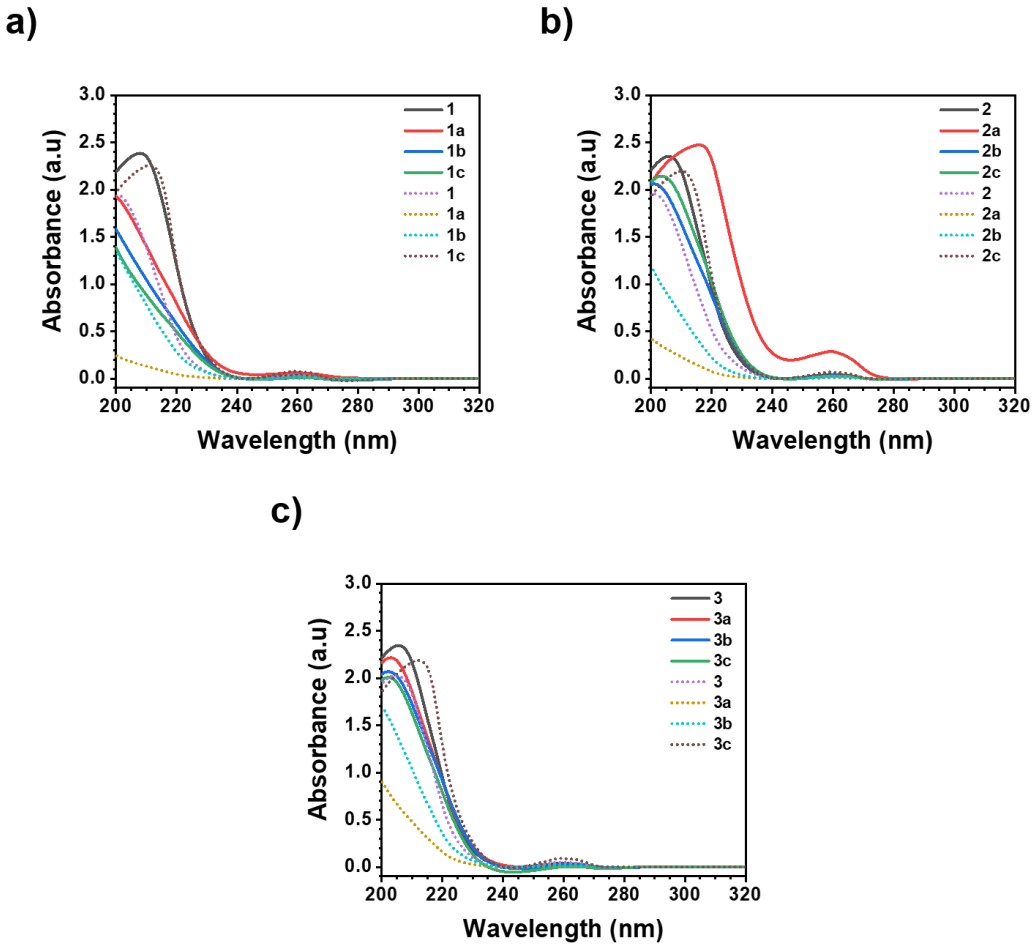


Fig. S8 UV-vis absorption spectra of gelators with or without metal ions at 500 µM concentration. (a) PFB-FFRGD (**1**); PFB-FFRGD/ Mg^{2+} (**1a**); PFB-FFRGD/ Ca^{2+} (**1b**); and PFB-FFRGD/ Ba^{2+} (**1c**), (b) PFB-FFRDG (**2**); PFB-FFRDG/Mg^{2+} (**2a**); PFB-FFRDG/Ca^{2+} (**2b**); and PFB-FFRDG/Ba^{2+} (**2c**), and (c) PFB-FFRGE (**3**), PFB-FFRGE/Mg^{2+} (**3a**); PFB-FFRGE/Ca^{2+} (**3b**); and PFB-FFRGE/Ba^{2+} (**3c**). Absorption spectra in water (solid lines) trifluoroethanol (dotted lines).

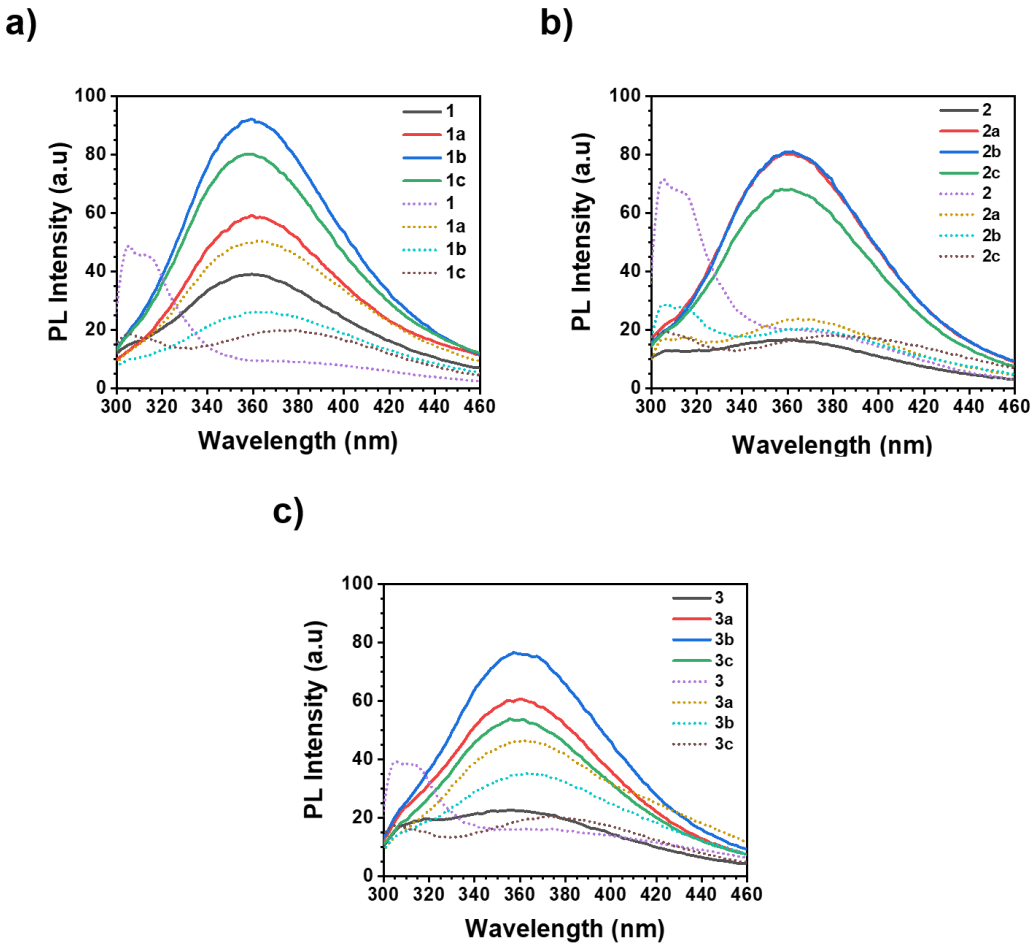


Fig. S9 Emission spectra of gelators with or without metal ions at 500 µM concentration. (a) PFB-FFRGD (**1**); PFB-FFRGD/ Mg^{2+} (**1a**); PFB-FFRGD/ Ca^{2+} (**1b**); and PFB-FFRGD/ Ba^{2+} (**1c**), (b) PFB-FFRDG (**2**); PFB-FFRDG/Mg^{2+} (**2a**); PFB-FFRDG/Ca^{2+} (**2b**); and PFB-FFRDG/Ba^{2+} (**2c**), and (c) PFB-FFRGE (**3**), PFB-FFRGE/Mg^{2+} (**3a**); PFB-FFRGE/Ca^{2+} (**3b**); and PFB-FFRGE/Ba^{2+} (**3c**). Emission spectra in water (solid lines) trifluoroethanol (dotted lines).

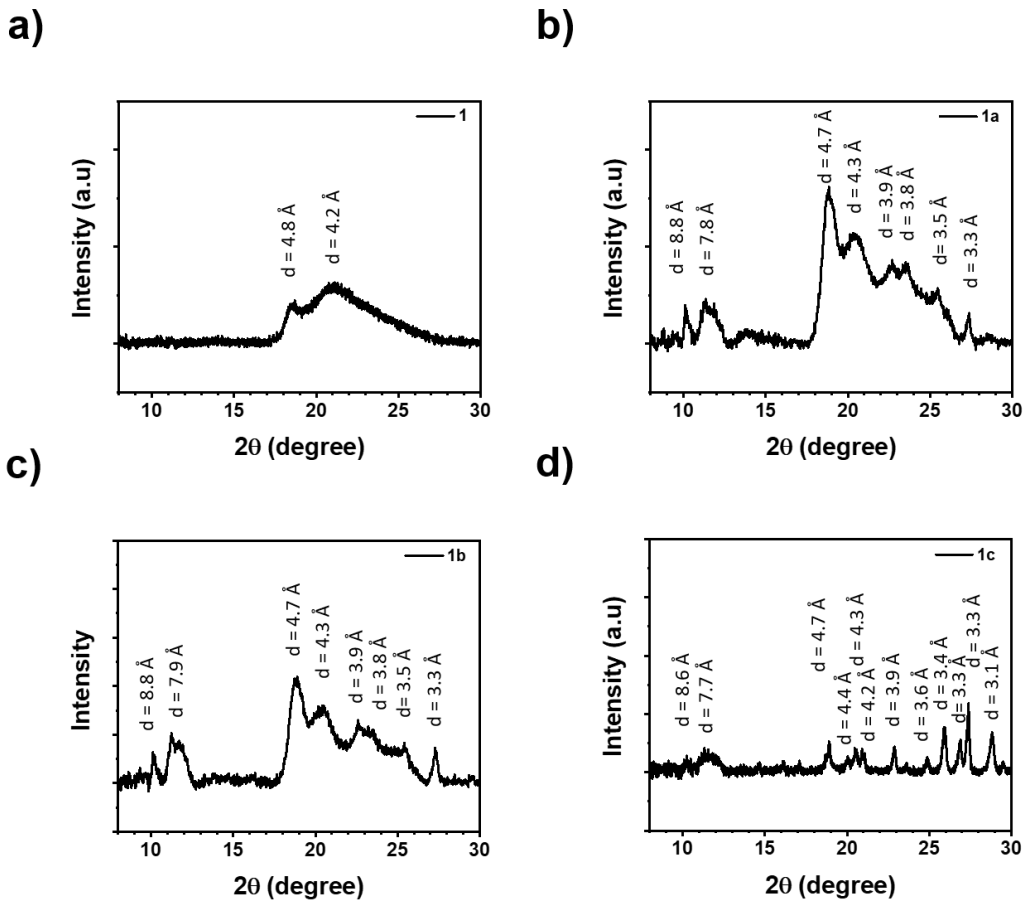


Fig. S10 Powder XRD of freeze-dried hydrogels (2wt %); (a) PFB-FFRGD (**1**), (b) PFB-FFRGD/Mg^{2+} (**1a**); (c) PFB-FFRGD/Ca^{2+} (**1b**); and (d) PFB-FFRGD/Ba^{2+}(**1c**).

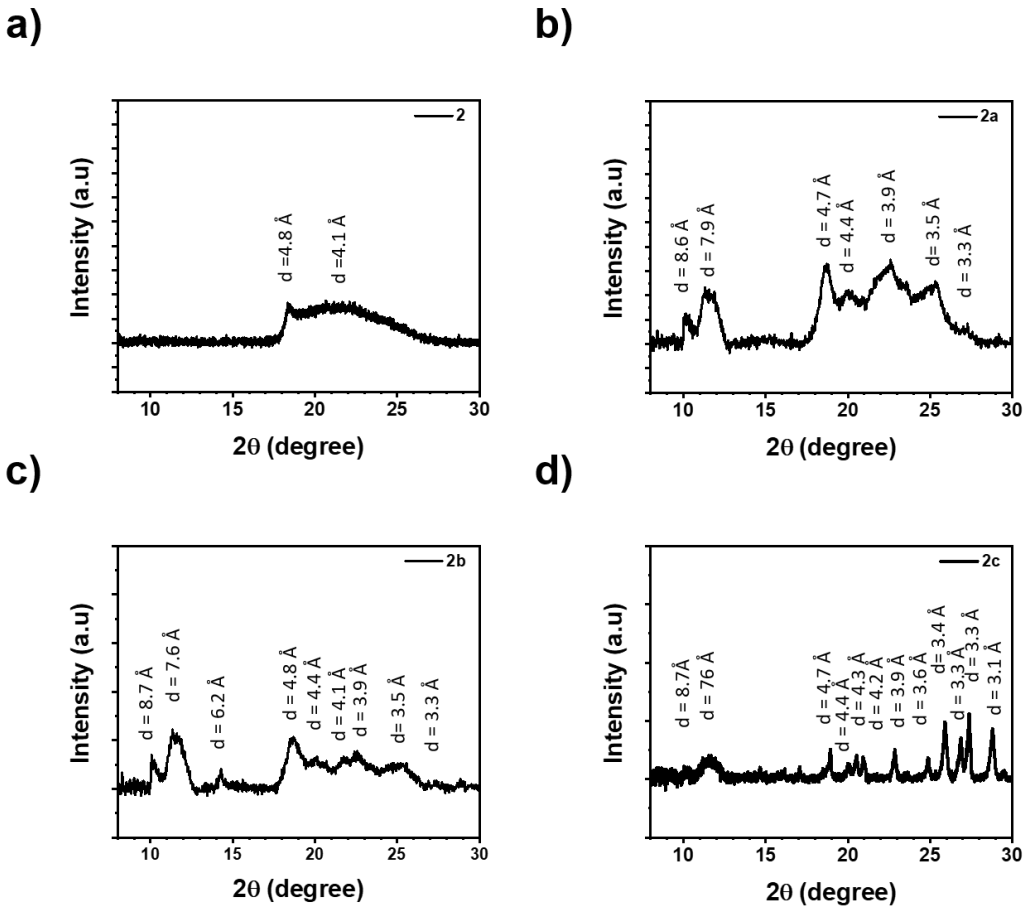


Fig. S11 Powder XRD of freeze-dried hydrogels (2wt %) (a) PFB-FFRDG (**2**), (b) PFB-FFRDG/Mg^{2+} (**2a**); (c) PFB-FFRDG/Ca^{2+} (**2b**); and (d) PFB-FFRDG/Ba^{2+} (**2c**).

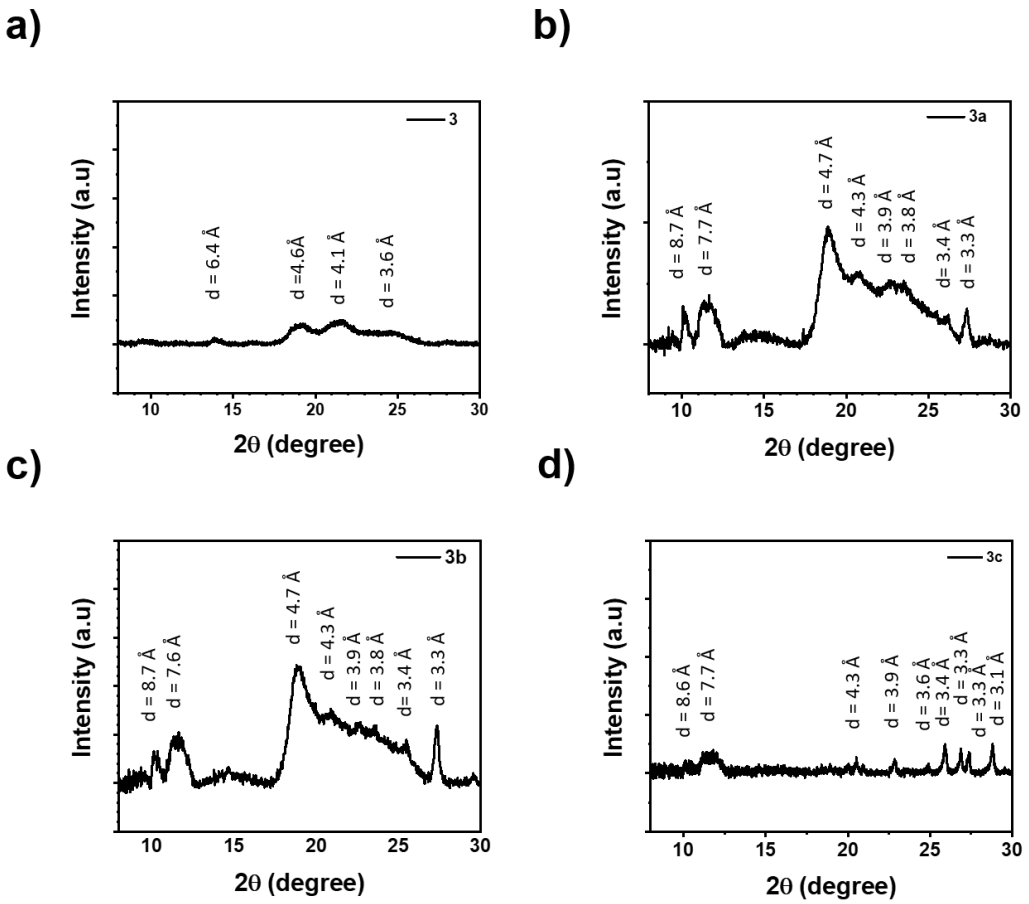


Fig. S12 Powder XRD of freeze-dried hydrogels (2wt %); a) PFB-FFRGE (**3**), (b) PFB-FFRGE/Mg^{2+} (**3a**); (c) PFB-FFRGE/Ca^{2+} (**3b**); and (d) PFB-FFRGE/Ba^{2+} (**3c**).

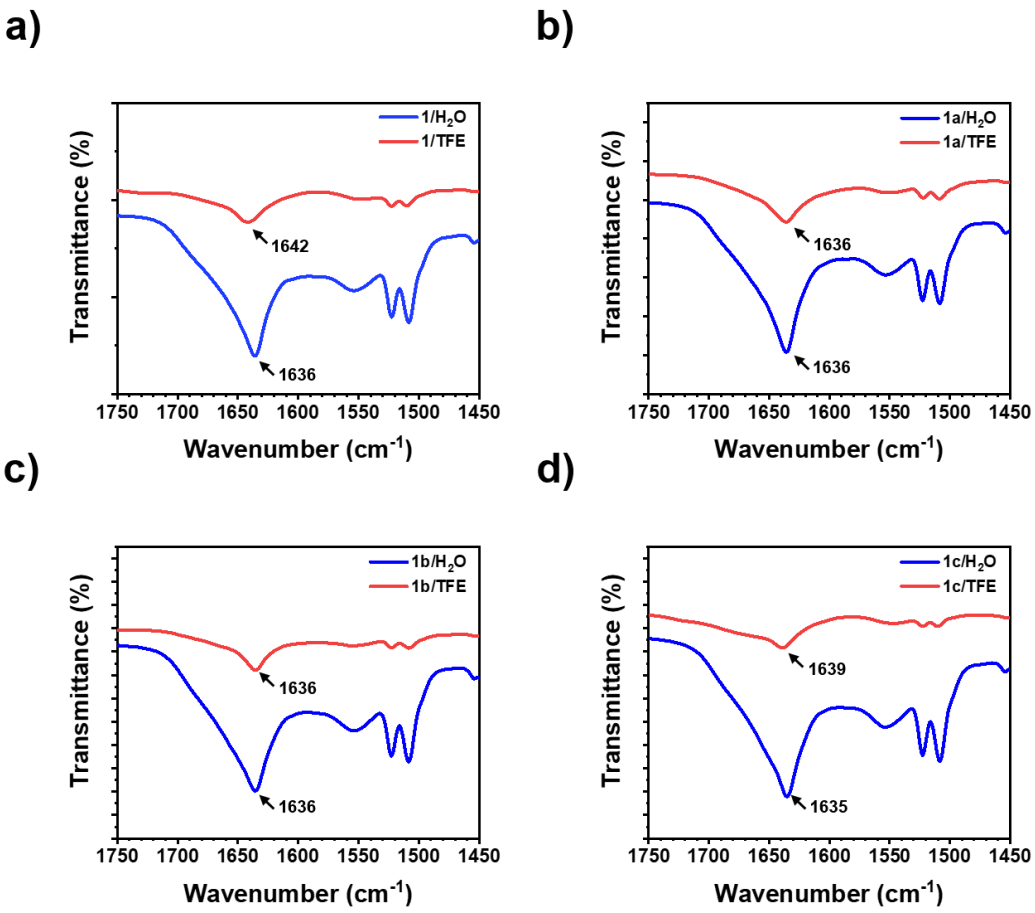


Fig. S13 FTIR spectrum of (a) PFB-FFRGD (**1**); (b) PFB-FFRGD/Mg^{2+} (**1a**); (c) PFB-FFRGD/Ca^{2+} (**1b**); and (d) PFB-FFRGD/Ba^{2+} (**1c**). FTIR spectra were collected in trifluoroethanol (red) and water (blue).

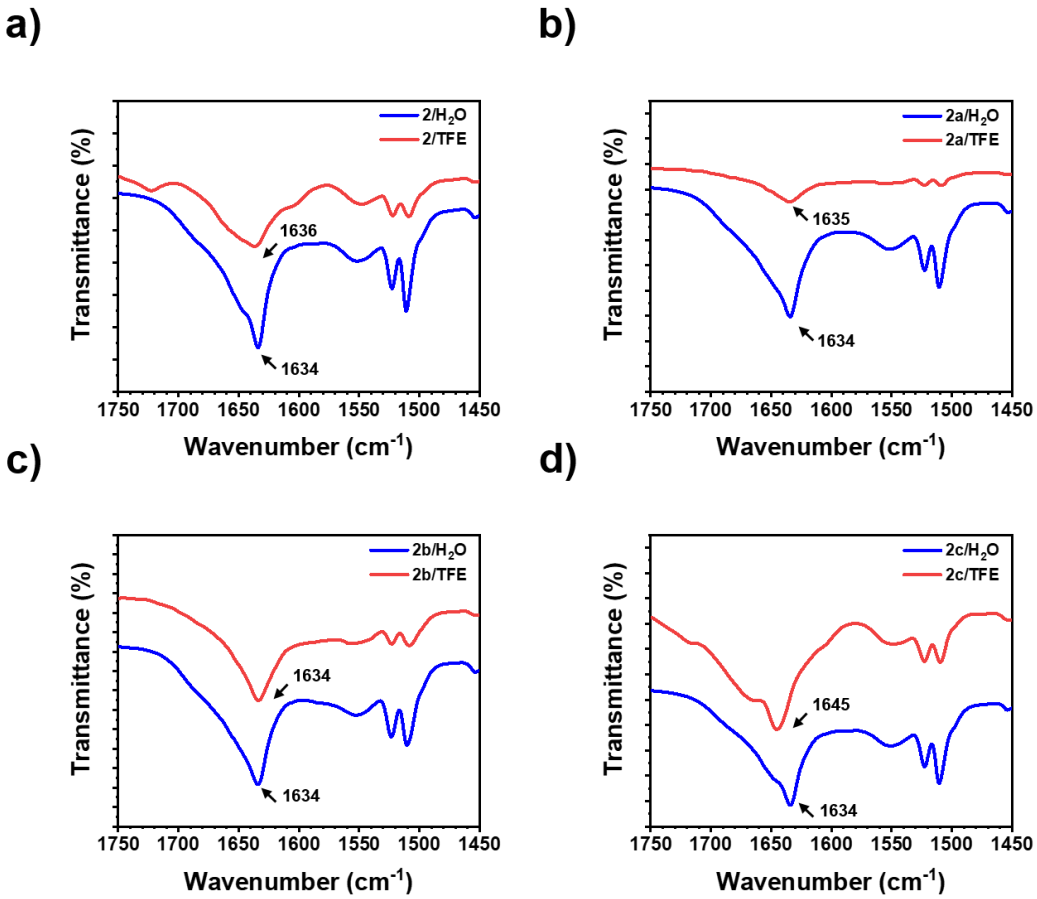


Fig. S14 FTIR spectrum of (a) PFB-FFRDG (**2**), (b) PFB-FFRDG/Mg^{2+} (**2a**); (c) PFB-FFRDG/Ca^{2+} (**2b**); and (d) PFB-FFRDG/Ba^{2+} (**2c**). FTIR spectra were collected in trifluoroethanol (red) and water (blue).

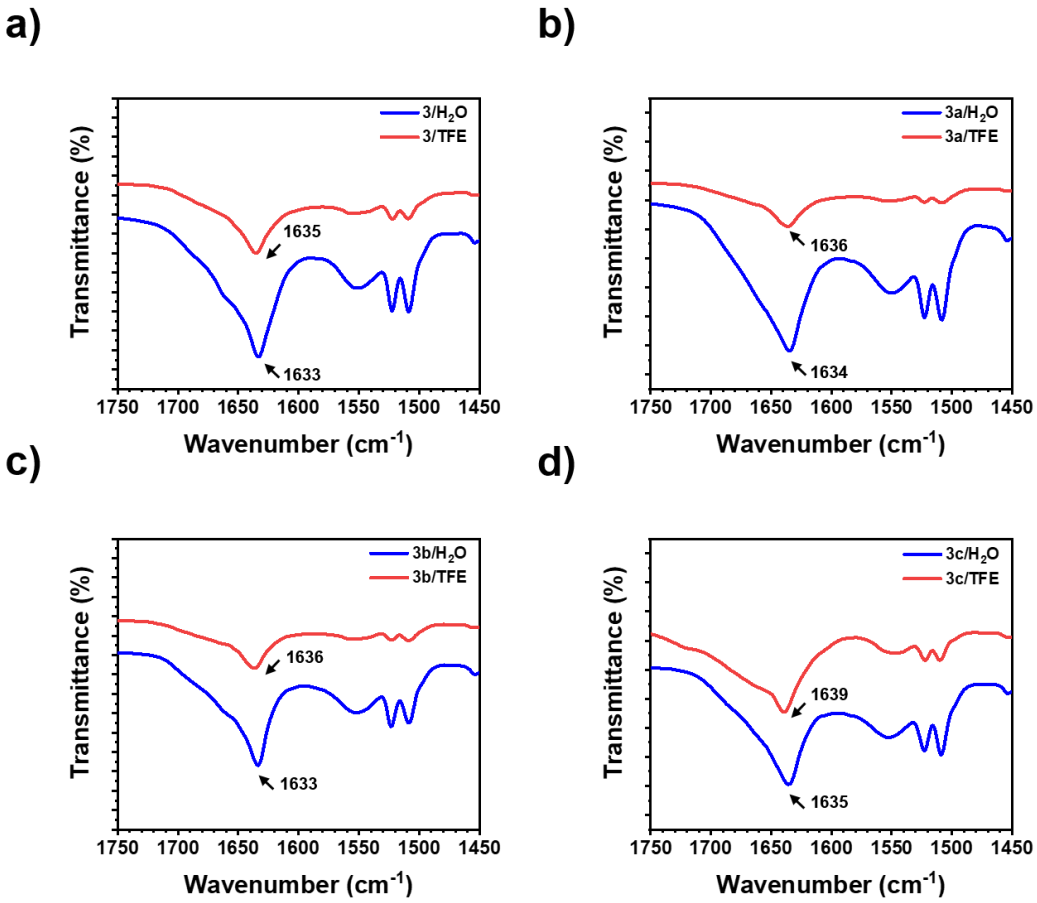


Fig. S15 FTIR spectrum of (a) PFB-FFRGE (**3**), (b) PFB-FFRGE/Mg^{2+} (**3a**); (c) PFB-FFRGE/Ca^{2+} (**3b**); and (d) PFB-FFRGE/Ba^{2+} (**3c**). FTIR spectra were collected in trifluoroethanol (red) and water (blue).

Fig.S16 Gel stability: Optical images of hydrogels before and after incubation with phosphate buffered saline (PBS) 7 days at 37 ºC and their corresponding xerogels. Compounds: PFB-FFRGD (**1)**; PFB-FFRGD/ Mg^{2+} (**1a)**; PFB-FFRGD/ Ca^{2+} (**1b**); PFB-FFRGD/ Ba^{2+} (**1c**)**,** PFB-FFRDG (**2**); PFB-FFRDG/Mg^{2+} (**2a**); PFB-FFRDG/Ca^{2+} (**2b**); PFB-FFRDG/Ba^{2+} (**2c**), PFB-FFRGE (**3**), PFB-FFRGE/Mg^{2+} (**3a**); PFB-FFRGE/Ca^{2+} (**3b**); and PFB-FFRGE/Ba^{2+} (**3c**).

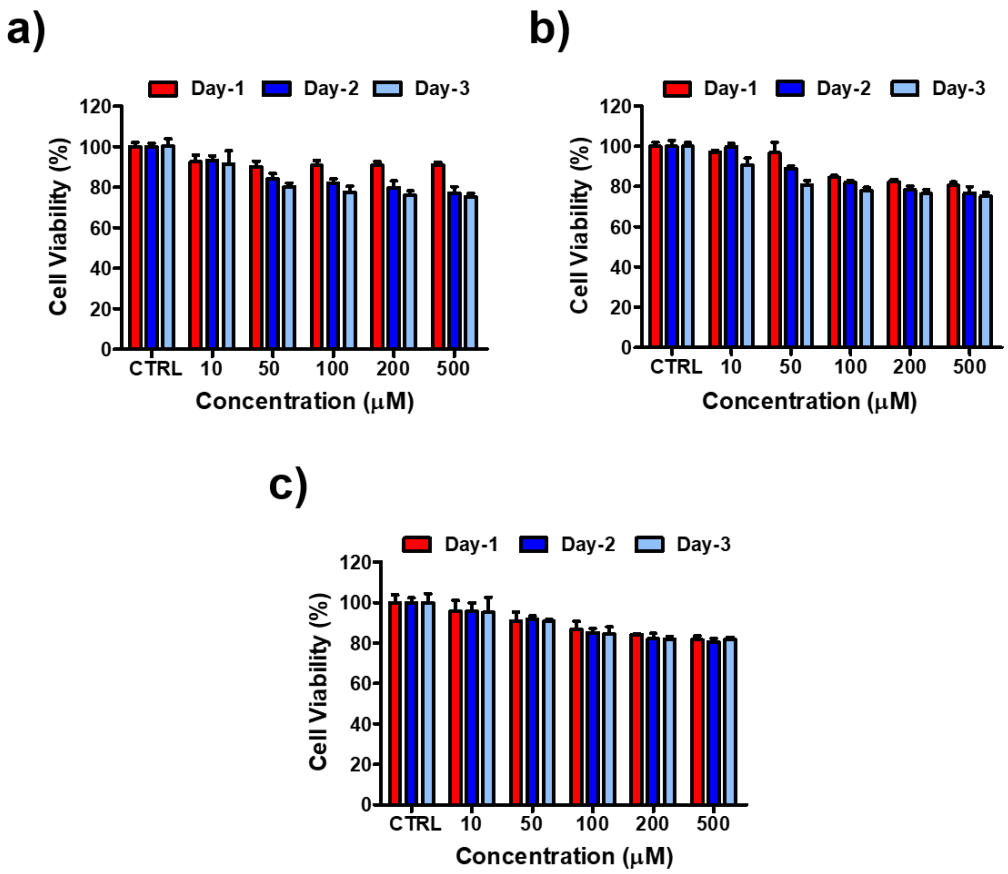


Fig. S17 Cell viability of 3A6-RFP cells after exposed at 10 to 500 μM concentrations of hydrogelators using MTT assay for 3 days. a) PFB-FFRGD (**1**); b) PFB-FFRDG (**2**); and c) PFB-FFRGE (**3**)**.**

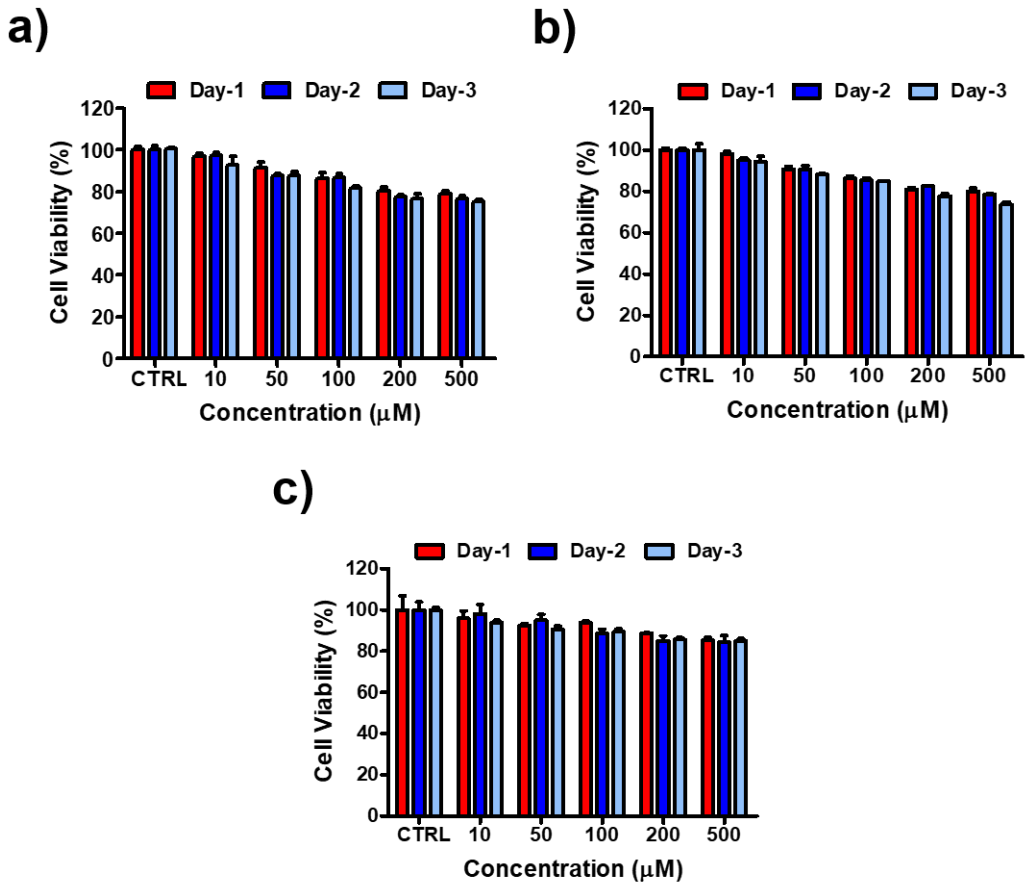


Fig. S18 Cell viability of L929 cells after exposed at 10 to 500 μM concentrations of hydrogelators using MTT assay for 3 days. a) PFB-FFRGD (**1**); b) PFB-FFRDG (**2**); and c) PFB-FFRGE (**3**).

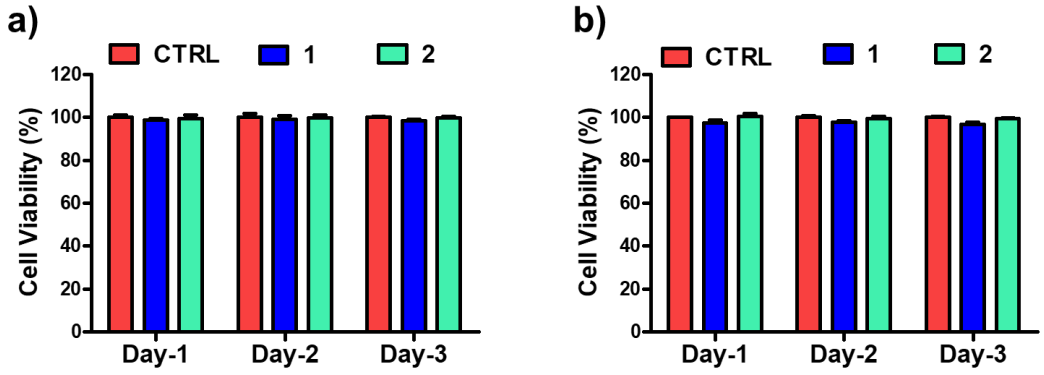


Fig. S19 Viability assay by trypan blue dye exclusion method. Cell viability of (a) 3A6-RFP cells and (b) L929 cells after exposed to hydrogels leachable PFB-FFRGD (**1**); and PFB-FFRDG (**2**) over 3 days.

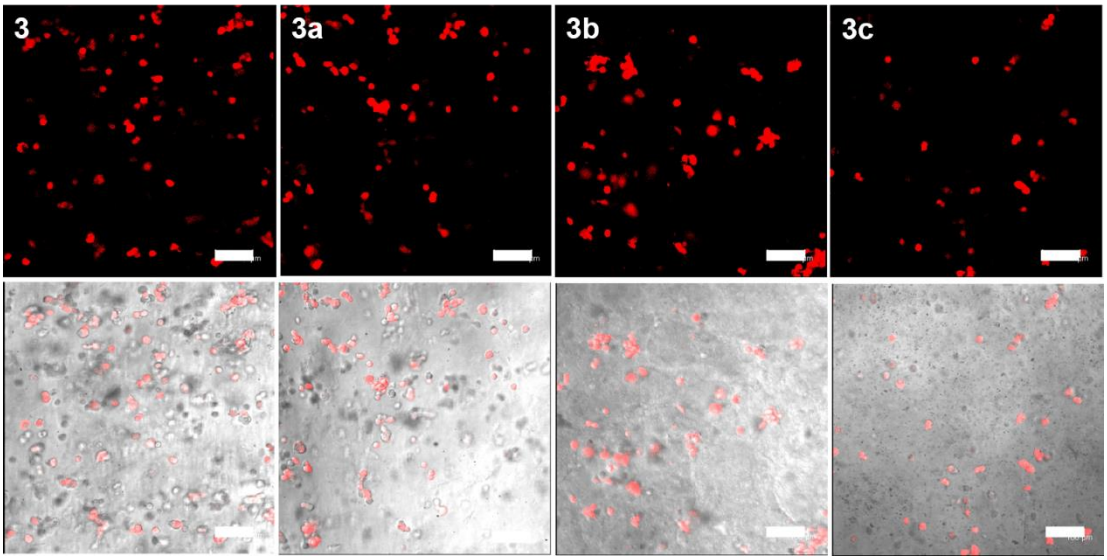


Fig. S20 Cell morphology of 3A6-RFP cells encapsulated in hydrogels **3** with or without metal ions for 24 h. PFB-FFRGE (**3**); PFB-FFRGE/Mg^{2+} (**3a**); PFB-FFRGE/Ca^{2+} (**3b**); and PFB-FFRGE/Ba^{2+} (**3c**). (Scale bar: 100 µm).

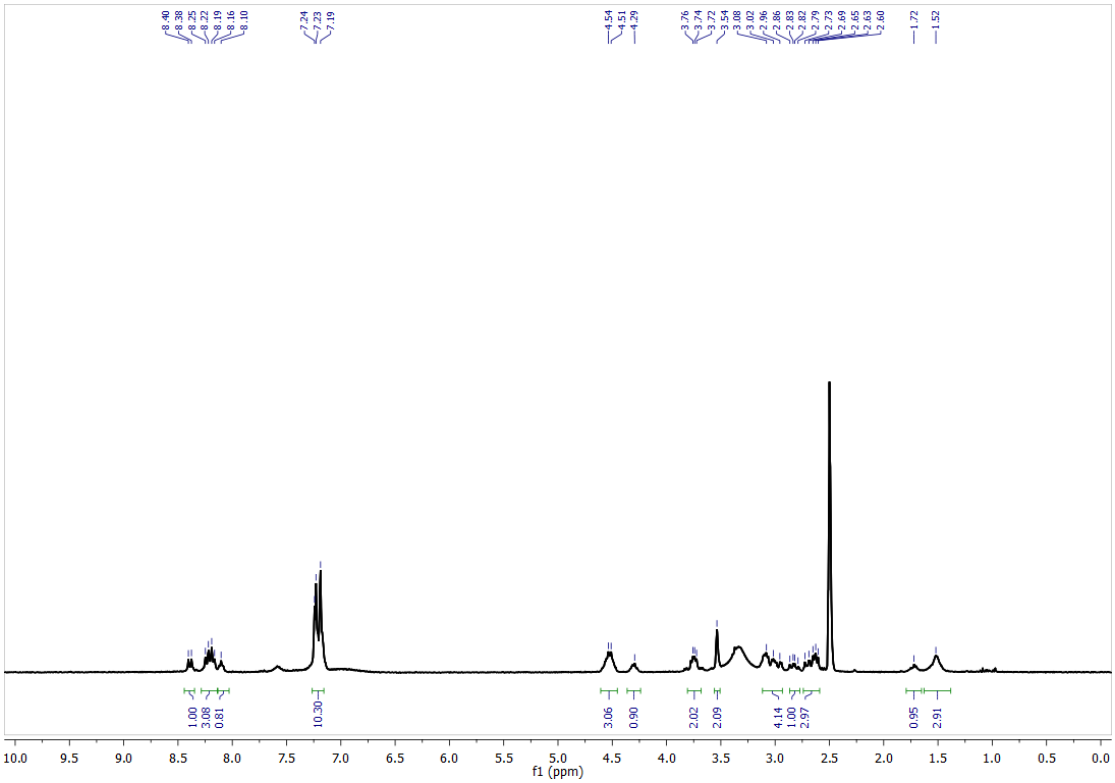


Fig. S21 ^{1}H NMR of PFB-FFRGD taken in DMSO-d_6.

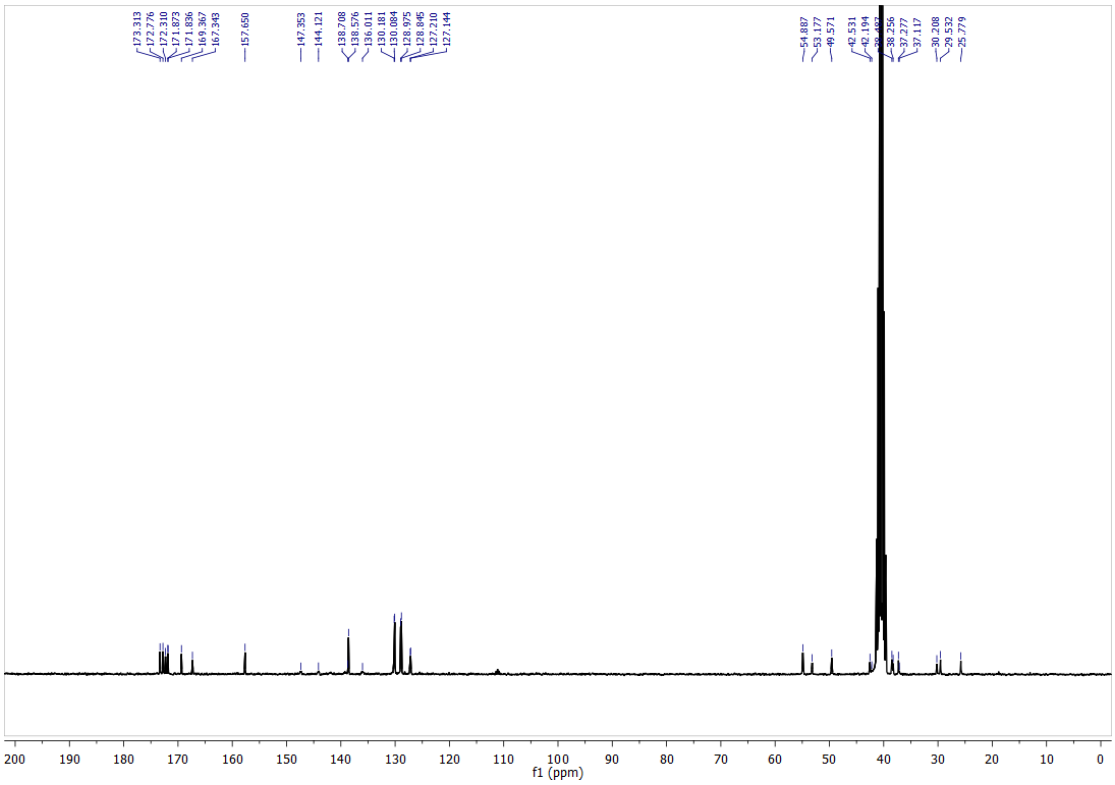


Fig. S22 ^{13}C NMR of PFB-FFRGD taken in DMSO-d_6.

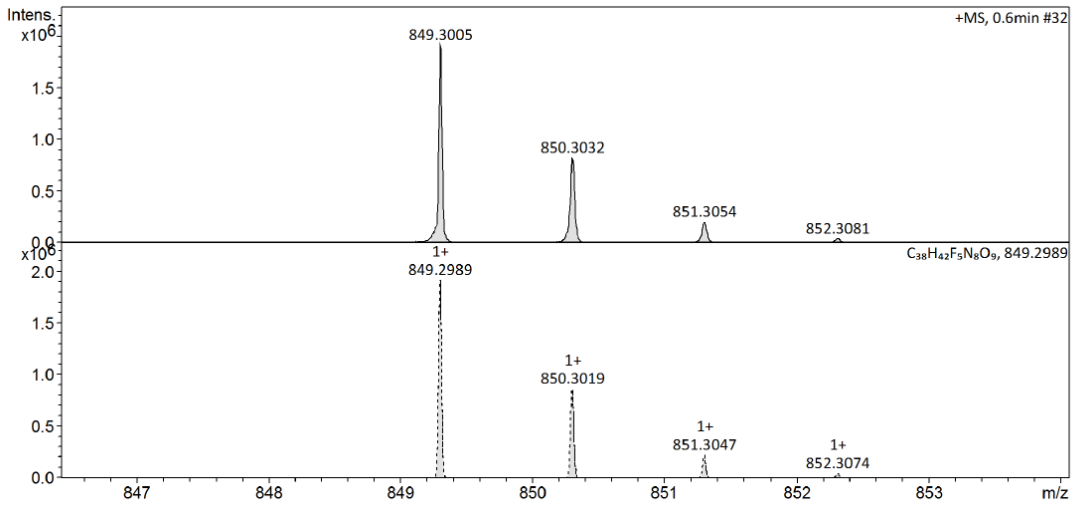


Fig. S23 High resolution mass spectrum of PFB-FFRGD.

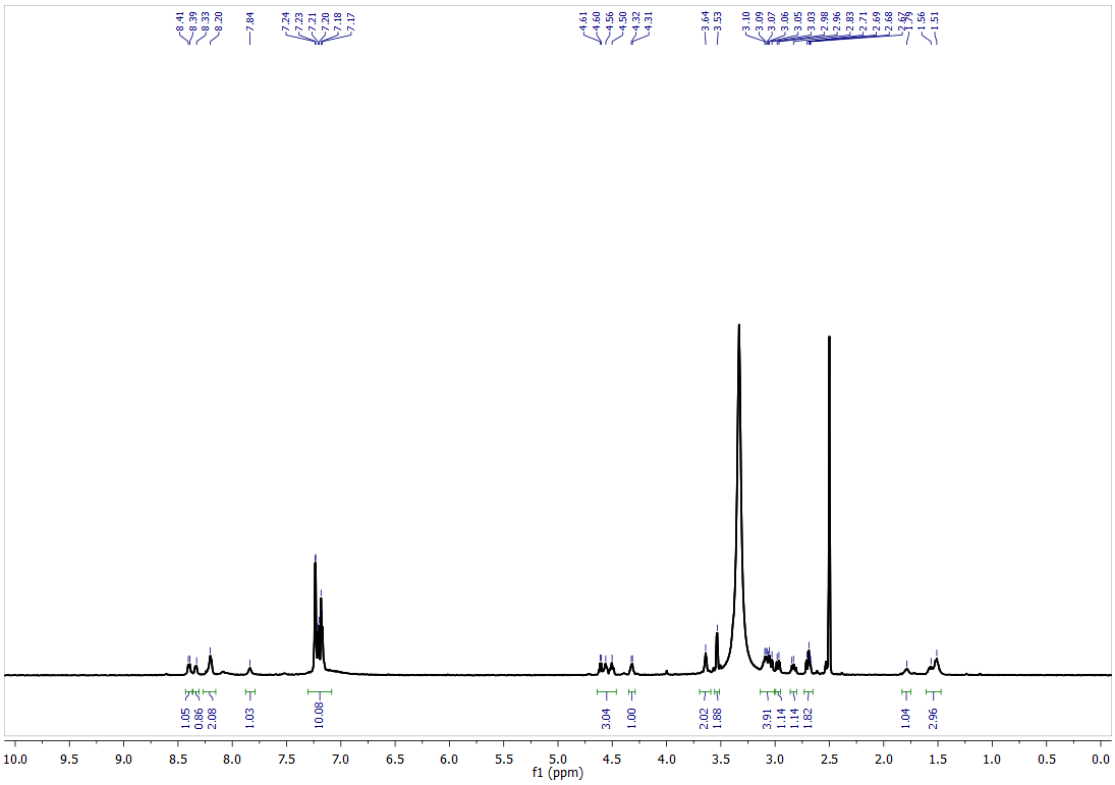


Fig. S24 ^{1}H NMR of PFB-FFRDG taken in DMSO-d_6.

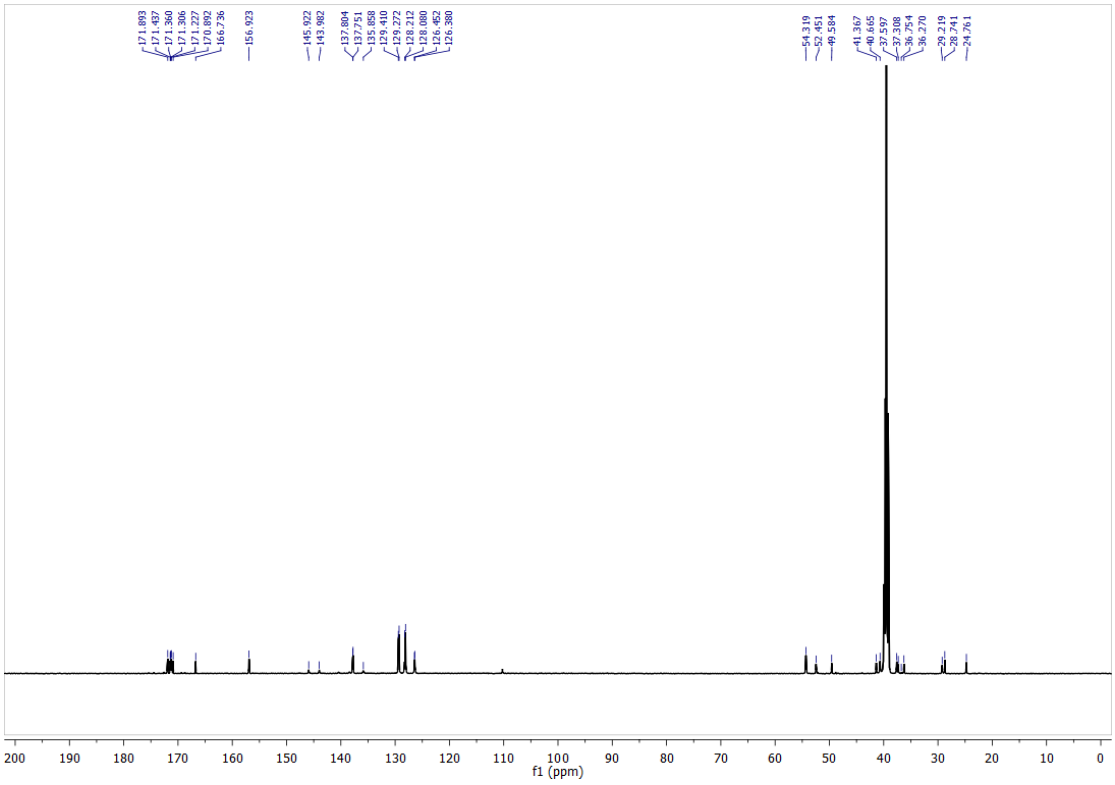


Fig. S25 ^{13}C NMR of PFB-FFRDG taken in DMSO-d_6.

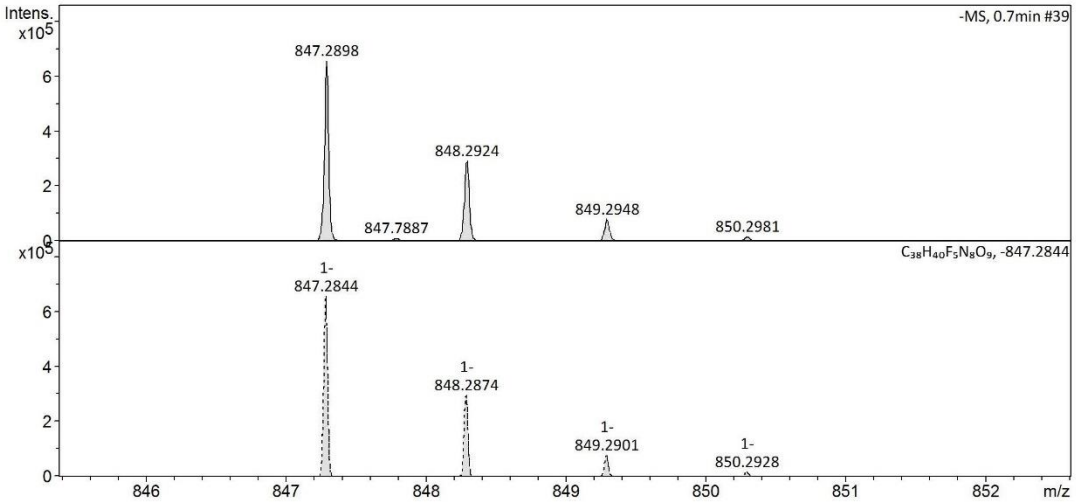


Fig. S26 High resolution mass spectrum of PFB-FFRDG

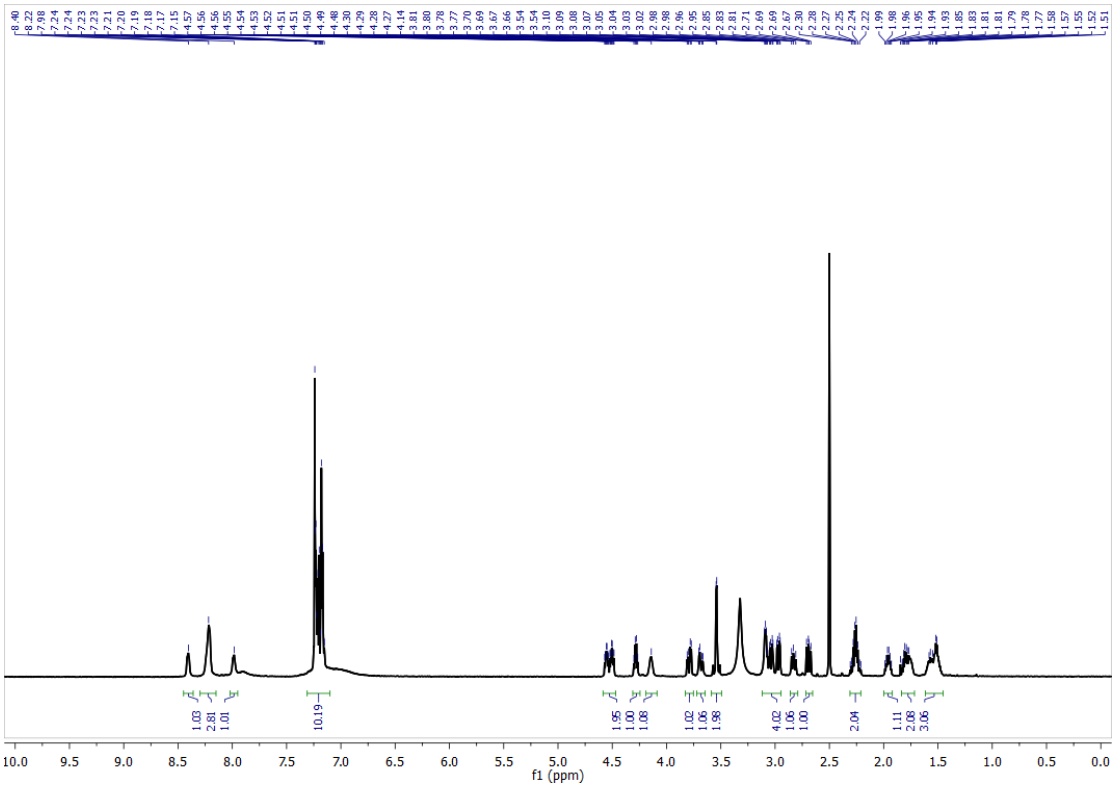


Fig. S27 ^{1}H NMR of PFB-FFRGE taken in DMSO-d_6.

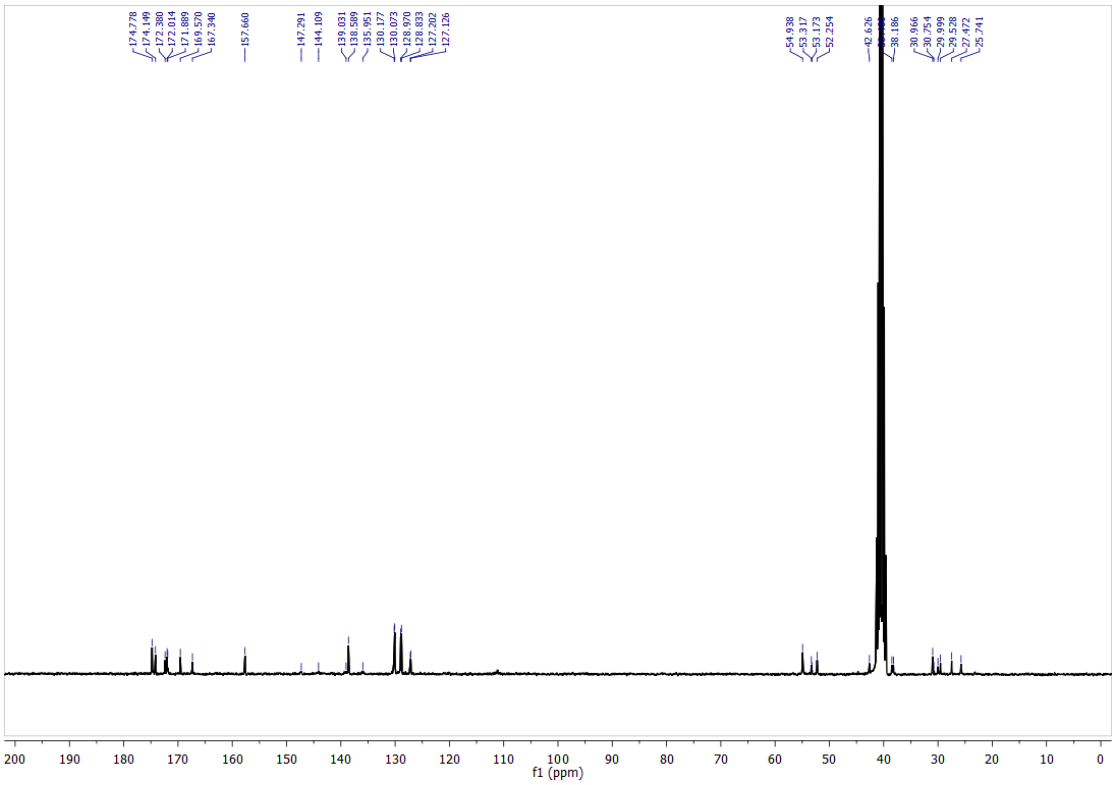


Fig. S28 ^{13}C NMR of PFB-FFRGE taken in DMSO-d_6.

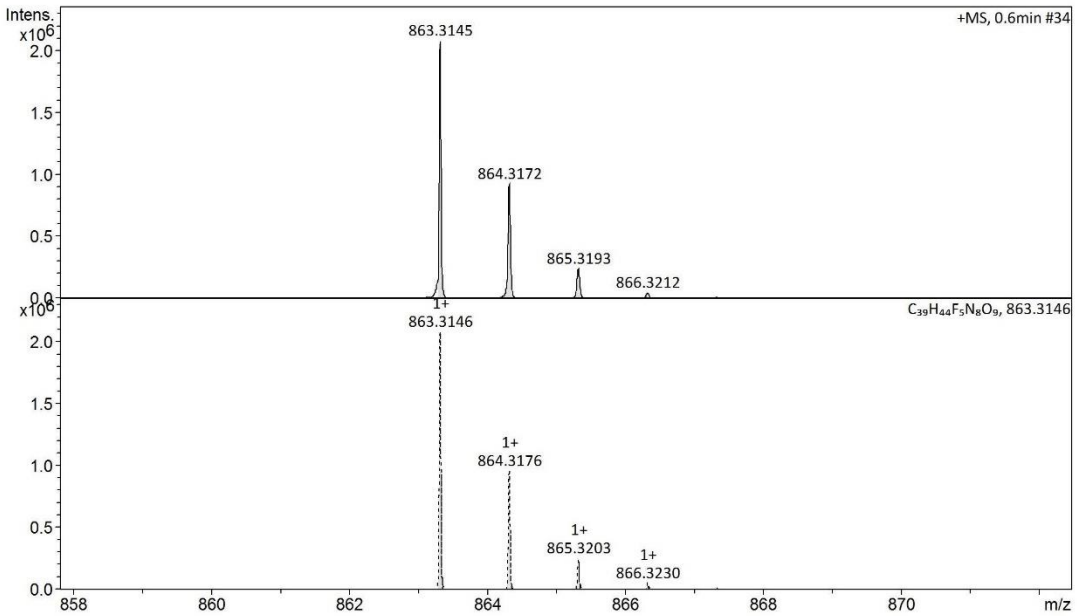


Fig. S29 High resolution mass spectrum of PFB-FFRGE.