Electronic Supplementary Information (ESI)

Diffusion of Small Molecules inside a Peptide Hydrogel

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General information:

Analytical grade reagents and solvents were purchased from Sigma Aldrich, Inc., Alfa Aesar, Inc., Amresco, Inc., and used without further purification. H-Phe(4-CF_3)-OH was purchased from Chem-impex, Inc.

Rink amide MBHA resin for SPPS was purchased from Chem-impex, Inc. Fmoc-protected amino acids were purchased from Aapptec, Inc. Peptides were synthesized on a CEM Liberty microwave peptide synthesizer.

Purifications of fluorinated amino acids and peptides were conducted on Agilent 1100 HPLC system with a VWD detector. Column: Agilent ZORBAX 300SB-C18 PrepHT (21.2 × 250 mm, 7 micron particle size). Flow rate: 5 mL/min. Eluents: A: H2O (0.1% HCl), B: MeOH (0.1% HCl), unless otherwise specified.

Analytical characterization:

Purity of all compounds was verified on Agilent 1100 HPLC with a DAD detector. Column: Agilent ZORBAX 300SB-C18 (4.6 × 250mm, 5 micron particle size). Flow rate: 1 mL/min. Eluents: A: H2O (0.1% TFA), B: MeOH (0.1% TFA), unless otherwise specified.

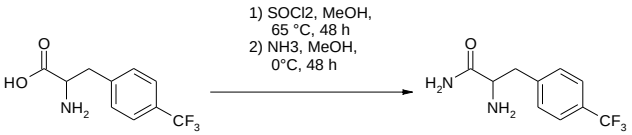
All Mass spectrometric analyses of the samples were carried on a Finnigan LCQ mass spectrometer.

All NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer, equipped with a $^{1}H/^{19}F\{^{13}C/^{15}N\}$ 5mm Triple resonance PFG probe.

The concentration of peptide solutions were calculated according to the UV absorption of Trp residues in peptide sequences ($\varepsilon_{280,Trp}$ = 5690 $M^{-1}\cdot cm^{-1}$, here the peptide have 2 Trp residues, $\varepsilon_{280,peptide}$ = 11380 $M^{-1}\cdot cm^{-1}$).[1] A Thermo Scientific Evolution 60S UV-Visible Spectrophotometer was used.

Dynamic rheological measurements were performed using a NOVA Rheometer (REOLOGICA Instruments, Inc., Sweden) featuring a null balance system which allows for nano-torque and nano-strain measurement control and analysis. The instrument is equipped with a sealed-cell geometry which prevents dehydration of the water-based samples during prolonged measurements. In order to exclude possible dehydration of the sample at 25 °C, a simple in-house designed system was used to humidify the incoming air used for the sealed-cell. The NOVA rheometer also features a temperature-control unit, and rheological characterizations of the gels were performed using a 25-mm diameter cone-and-plate steel geometry.

Synthesis and purification of H-Phe(4-CF_3)-NH_2 (Compound 2)



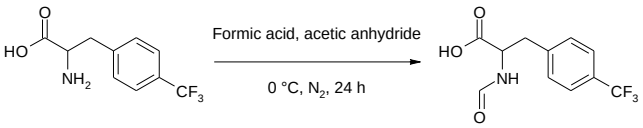
1 **2**

H-Phe(4-CF_3)-OH (**1**, 300 mg, 1.29 mmol) was dissolved in 250 mL of MeOH and stirred at r.t. for 10 min. Thionyl chloride (3.0 mL, 41.1 mmol) were added dropwise to the mixture. The solution was refluxed for 24 h, and then concentrated by rotary evaporation of the solvent under reduced pressure. The resulting white solid was further washed with anhydrous diethyl ether (25 mL). The crude solid was redissolved in MeOH (5 mL) and thionyl chloride (1.0 mL, 13.7 mmol) was added. The resulting solution was refluxed for another 24 h, after which it was concentrated by rotary evaporation under reduced pressure. The resulting solid was washed with anhydrous diethyl ether (3 × 50 mL), and air-dried for 5 min to give 302 mg of H-Phe(4-CF_3)-OMe in a total yield of 95%. Analytical HPLC analysis of H-Phe(4-CF_3)-OMe showed it to be 98% pure, as indicated by A_{254} detection with a retention time of 21.4 min. Low-resolution ES^+ MS analysis of the product in a solvent of MeOH gave the following m/z peak $[M+H]^+$ = 248.1 (calcd. $C_{11}H_{12}F_3NO_2$, M = 247.08).

H-Phe(4-CF_3)-OMe was used without further purification. H-Phe(4-CF_3)-OMe was then dissolved in MeOH at 0 °C. Ammonia gas was pumped into the reaction mixture throughout a period of 4 h. Ammonia gas was produced by gently heating calcium hydroxide (44.0 g, 593 mmol) and ammonium chloride (64.0 g, 1.20 mol) and dried by passing the gas through a column of potassium hydroxide (10 g). The solution was stirred at 0 °C for 24 h, after which the reaction was judged to be complete by HPLC analysis. The solution was concentrated by rotary evaporation under reduced pressure, and the resulting solid was washed with anhydrous diethyl ether (2 × 25 mL). The product was air-dried for 10 min to give 294 mg of compound **2** that corresponds to an overall 98.2% yield by weight. Analytical HPLC analysis of compound **2** showed it to be 96.2% pure with a retention time of 17.1 min. The product was further purified by preparatory HPLC. Removal of TFA salt from the prep-HPLC eluent was done by adding 5.0 mL of 0.1M HCl to the dried product, and the resulting solution was lyophilized to yield pure **2**. Low-resolution ES^+ MS analysis of the product in a solvent of MeOH gave the following m/z peak $[M+H]^+$ = 233.1 (calcd. $C_{10}H_{11}F_3N_2O$, M = 232.08). ^{1}H NMR (MeOH-d_4) δ = 3.17 (dd, 1H, *J* = 7.8, 7.1), 3.31 (m, 1H), 4.16 (t, 1H, *J* = 7.1), 7.51 (d, 2H, *J* = 7.8), 7.66 (d, 2H, *J* = 7.8); ^{13}C NMR (MeOH-d_4) δ = 38.4, 55.3, 127.0, 131.5, 140.5, 171.6 ppm; ^{19}F NMR (MeOH-d_4) δ = -63.7 ppm.

Compound **2** (30 mg, 0.13 mmol) was dissolved in a solution of 1:1 H_2O/MeOH (1.0 mL). Preparative chromatographic method: 0-60% B in 0-20 min; 60-100%B in 20-60 min with linear gradient. The fractions containing **2** were collected and combined. The solvent was removed by rotary evaporation under reduced pressure. The resulting solution was lyophilized to yield compound **2**.

Synthesis and purification of CHO-Phe(4-CF_3)-OH (Compound 3)

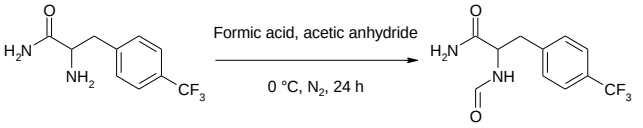


1 **3**

H-Phe(4-CF_3)-OH (**1**, 300 mg, 1.29 mmol) were dissolved in formic acid (3.60 mL, 95.4 mmol) at 0 °C. The mixture was stirred under nitrogen for 20 min. Acetic anhydride (1.20 mL, 12.6 mmol) was added dropwise over a period of 3 min. The reaction was stirred at 0 °C under nitrogen overnight. The compound was then dried by rotary evaporation under reduced pressure to yield a white solid compound. This procedure produced 294 mg of CHO-Phe(4-CF_3)-OH for an apparent yield of 87.6%, as indicated by the total weight of the material. Analytical HPLC analysis of **3** indicated the material was actually 96% pure with a retention time of 24.1 min. The compound was further purified by preparatory HPLC (see method below) to give 208 mg of **3** in an overall of 62.0% yield. Analysis by analytical HPLC indicated the compound was 100% pure. Low-resolution ES^- MS in a solvent of MeOH showed a charged m/z peak $[M-H]^-$ = 260.2 (calcd. $C_{11}H_{12}F_3N_2O_2$ = 261.1). ^{1}H NMR (MeOH-d_4) δ = 3.05 (dd, 1H, *J* = 8.8, 13.9), 3.27 (m, 1H), 4.78 (t, 1H, *J* = 6.6), 7.38 (d, 2H, *J* = 7.8), 7.54 (d, 2H, *J* = 7.8), 7.99 (s, 1H); ^{13}C NMR (MeOH-d_4) δ = 38.4, 53.4, 126.4, 127.0, 130.2, 130.5, 131.2, 131.5, 142.9, 163.6, 173.8 ppm; ^{19}F NMR (MeOH-d_4) δ = -63.6 ppm.

Compound **3** (10 mg, 0.038 mmol) was dissolved in a solution of 1:1 H_2O/MeOH (1.0 mL). Preparative chromatographic method: 0-60% B in 0-30 min; 60-100% B in 30-60 min with linear gradient. The fractions containing **3** were collected and combined. The solvent was removed by rotary evaporation under reduced pressure. The resulting solution was lyophilized to yield compound **3**.

Synthesis and purification of CHO-Phe(4-CF_3)-NH_2 (Compound 4)



2 **4**

H-Phe(4-CF_3)-NH_2 (**2**, 300 mg, 1.29 mmol) was dissolved in formic acid (3.60 mL, 95.4 mmol) at 0 °C. The mixture was stirred under nitrogen for 20 min. Acetic anhydride (1.20 mL, 12.6 mmol) was added dropwise over a period of 3 min. The reaction was stirred at 0 °C under nitrogen overnight. The compound was then dried by rotary evaporation under reduced pressure to yield a white solid compound. This procedure produced 309 mg of CHO-Phe(4-CF_3)-NH_2 for an apparent yield of 92.1%, as indicated by the total weight of the material. Analytical HPLC analysis of **4** indicated the material was actually 98% pure with a retention time of 21.8 min. Low-resolution ES^- MS in a solvent of MeOH showed a charged m/z peak $[M+Cl]^-$ = 295.0 (calcd. $C_{11}H_{11}F_3N_3O_2$ = 260.08). ^{1}H NMR (MeOH-d_4) δ = 3.00 (dd, 1H, *J* = 4.9, 8.8), 3.24 (dd, 1H, *J* = 5.8, 8.3), 4.77 (t, 1H, *J* = 7.3), 7.44 (d, 2H, *J* = 7.8), 7.58 (d, 2H, *J* = 8.3), 8.01 (s, 1H); ^{13}C NMR (MeOH-d_4) δ = 38.0, 54.0, 126.4, 131.2, 143.1, 163.6, 175.3 ppm;

^{19}F NMR (MeOH-d_4) δ = -63.6 ppm.

Compound **4** (30 mg, 0.115 mmol) was dissolved in a solution of 1:1 H_2O/MeOH (3.0 mL). Preparative chromatographic method: 0-60% B in 0-30 min; 60-100% B in 30-60 min with linear gradient. The fractions containing **4** were collected and combined. The solvent was removed by rotary evaporation under reduced pressure. The resulting solution was lyophilized to yield compound **4**.

1-octanol/water partition coefficients (P_{oct}) of compounds 1, 2, 3 and 4

1-octanol/water partition coefficients (P_{oct}) of compounds were measured by the UV absorption method. About 5 mg of each compound was weighted and dissolved in 1 ml PBS buffer (or D_2O), and the pH was adjusted to 7.4. The solutions were centrifuged for 2 minutes and the supernatant was taken as stock solutions. Then, 400 µl stock solution of each compound was mixed with 400 µl 1-octanol and vortexed for 3 minutes. The mixture stood overnight at room temperature to let the two phases separate and equilibrate.

The UV absorption of compounds in stock solution and in water phase was measured at 263 nm (for compounds **1**, **2** and **3**), 213 nm (compound **4**) and 280 nm (K11 and E11). P_{oct} was calculated as:

$$P_{oct} = C_{octanol}/C_{water} = (C_{stock} - C_{water})/C_{water} \quad (1)$$

where $C_{octanol}$, C_{water} and C_{stock} are the concentration of the compound in 1-octanol, aqueous phase and stock solution, respectively.

Synthesis and purification of peptide K11 and E11

K11 and E11 were synthesized on Rink-amide MBHA resin using a CEM microwave synthesizer with standard method. The *N*-terminal of both peptides was acetylated by acetic anhydride.

The crude peptide was cleaved by a TFA/TIS/H_2O (95/2.5/2.5) cocktail for 2 × 2 hours and the side chain protect group were remove at the same time. TFA was removed by rotary evaporation under reduced pressure, and then the crude peptides were precipitated and washed twice by cold ethyl ether.

The crude peptides were dissolved in water and lyophilized before purification. Preparative reverse-phase HPLC method was used to purify the crude peptides. In purification of K11, solvent A is 0.1% HCl in water and solvent B is 0.1% HCl in MeOH; in E11 purification, solvent A is 20 mM NH_4HCO_3 in water (pH=7.0), solvent B is 20 mM NH_4HCO_3 (pH 7.0) in MeOH/water (8:2). Chromatographic method of peptide purification: 0-40% B in 0-60 min, 40-100% B in 60-90 min with linear gradient for each segment. The purity of K11 and E11 showed in Figure S17 and S18 was verified by reverse-phase HPLC analysis. The solvents used are the same as the preparative HPLC run. 0-50% B in 0-20 min. with linear gradient (2.5% B/min) was used and followed with 50-100% B in 20-25 min. The MS (ESI) results were shown in Figure S19 (K11, calculated M.W. 1,413 Da) and Figure S20 (E11, calculated M.W.

1,419, Da, operate under negative mode).

Rheology experiment

Sample Preparation. 16 mM stock solutions of each peptide were prepared in PBS buffer at pH 7.4 or in H_2O. After stock solutions were equilibrated at room temperature, 200 μL of each peptide solution were centrifuged for 10 min at 8000 rpm and two peptides were mixed by simultaneous pippeting out two solutions through Y-shaped connector into the cell of cone-and-plate geometry of the rheometer.

Rheological Measurements. Time-sweep measurements were conducted at 0.2% strain amplitude and 1 rad/s angular frequency. The data points were taken once every 180 s after two integrations with 5 s delay time, between the start of application of the respective stress and the start of data acquisition for calculations. This delay is necessary for equilibration. Strain-sweep measurements were performed with single integration cycle at 1 rad/sec frequency, within the range of strain amplitudes from 0.1% to 100% in a log mode with 23 data point per decade. The strain sweep and yield sweep experiments were showed in Fig. S21 and S22.

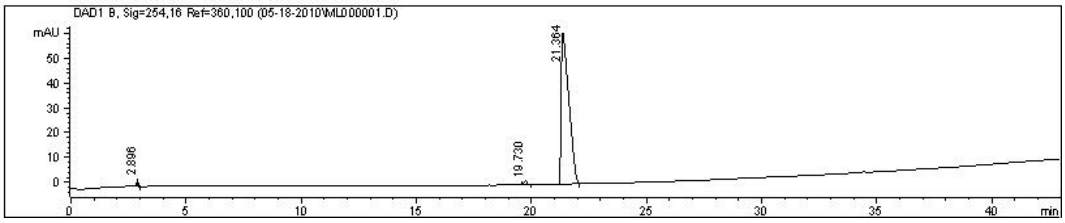


Figure S1 HPLC Chromatogram of H-Phe(4-CF_3)-OMe.

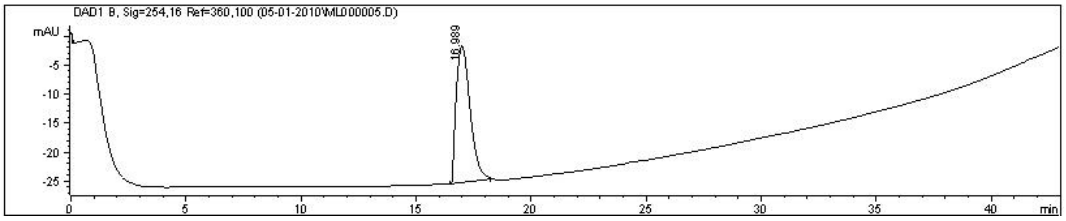


Figure S2 HPLC Chromatogram of H-Phe(4-CF_3)-NH_2, Compound **2**.

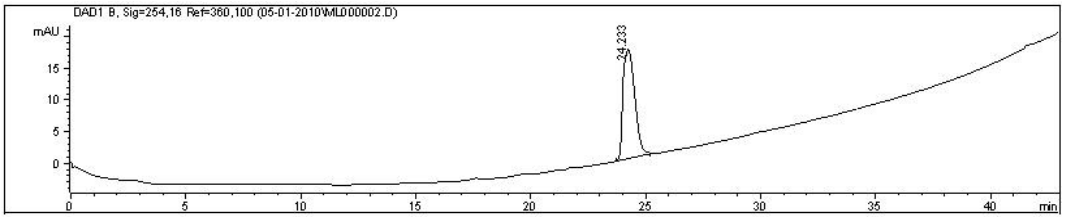


Figure S3 HPLC Chromatogram of CHO-Phe(4-CF_3)-OH, Compound **3**.

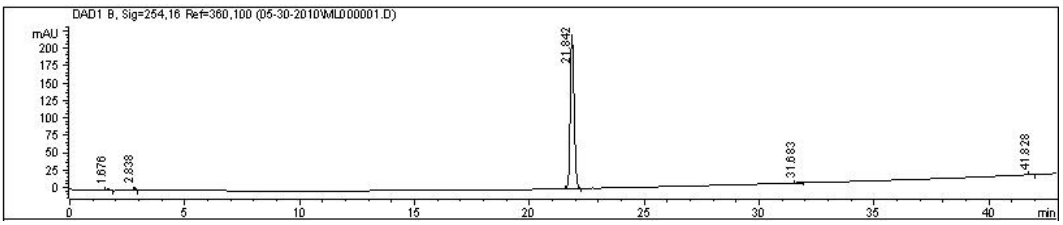


Figure S4 HPLC Chromatogram of CHO-Phe(4-CF_3)-NH_2, Compound **4**.

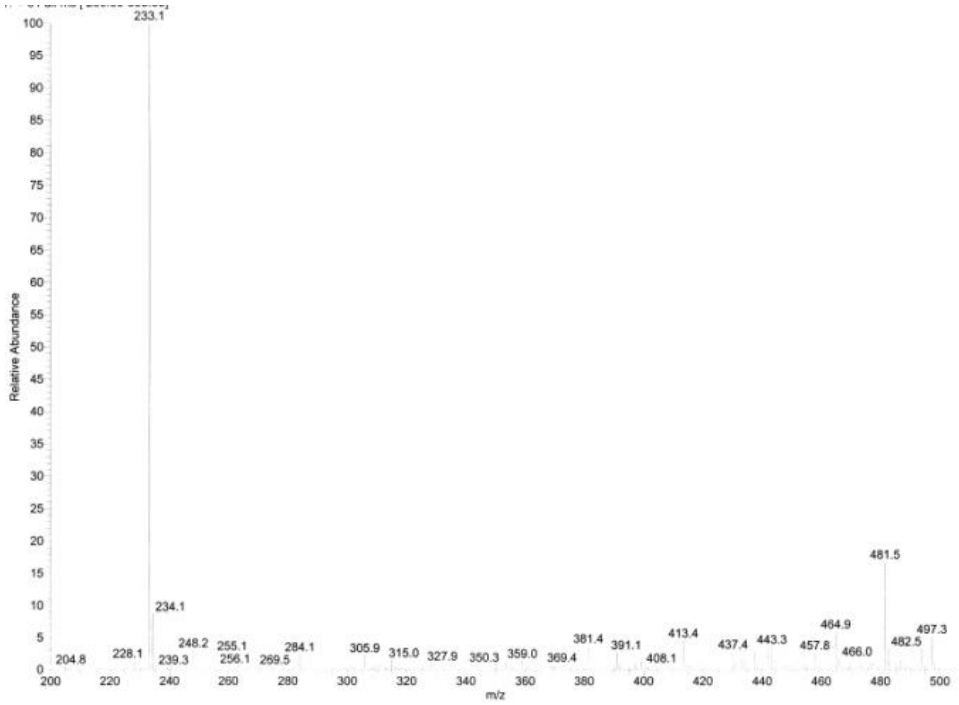


Figure S5 ESI-MS of Compound **2**.

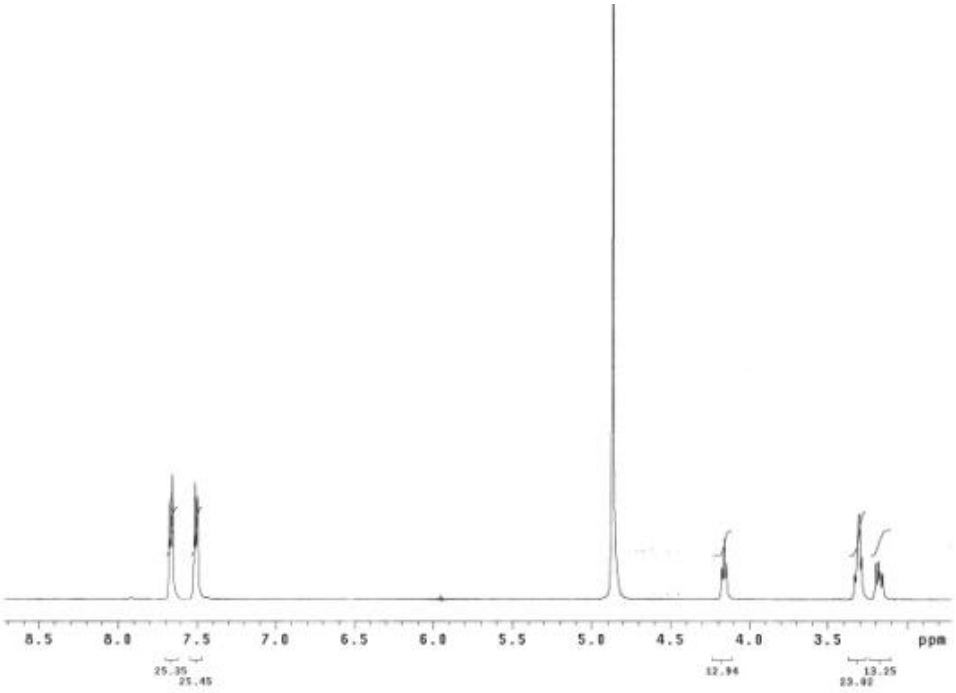


Figure S6 ^{1}H NMR Spectrum of Compound **2** in CD_3OD.

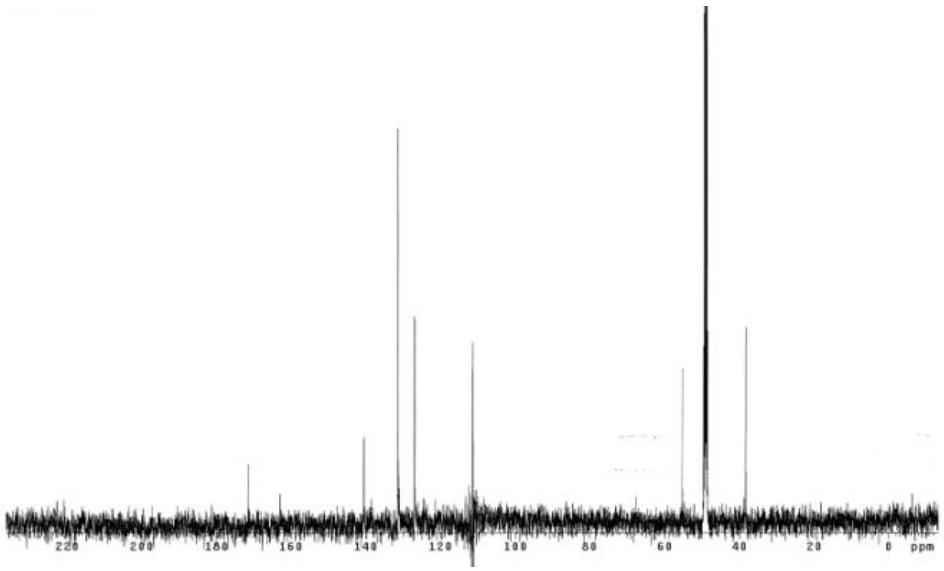


Figure S7 ^{13}C NMR Spectrum of Compound **2** in CD_3OD

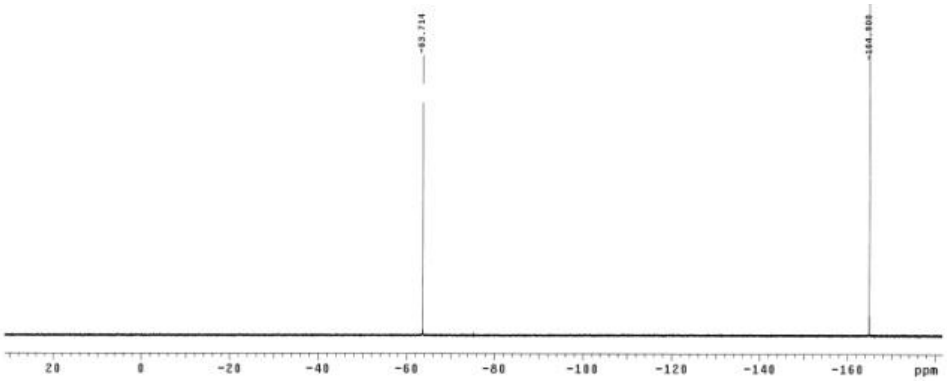


Figure S8 ^{19}F NMR Spectrum of Compound **2** in CD_3OD (the singlet peak at -164.5 is the chemical shift reference, C_6F_6).

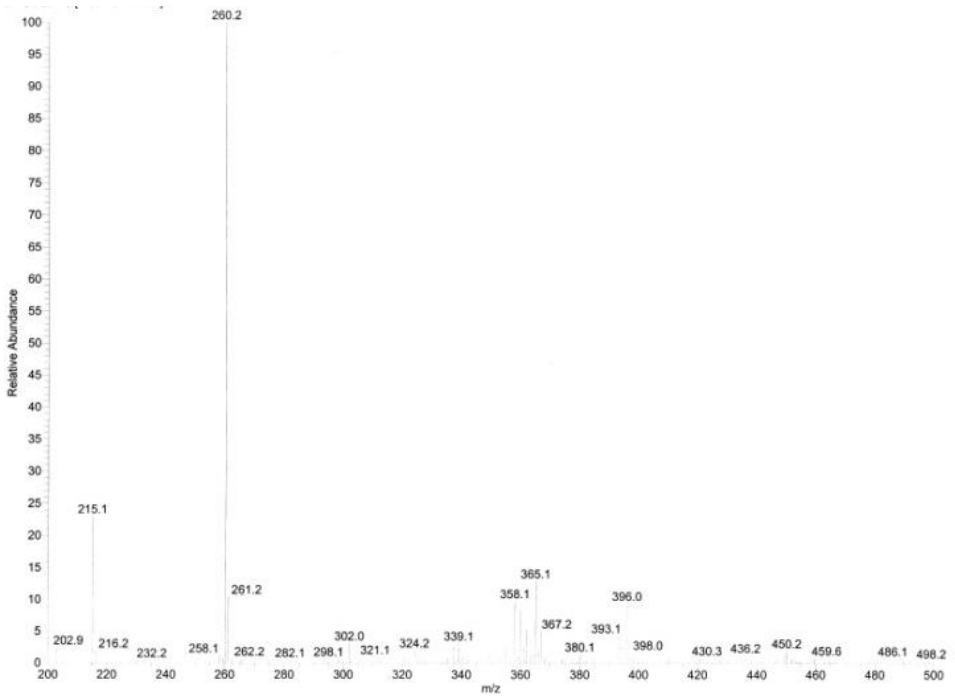


Figure S9 ESI-MS of Compound **3**.

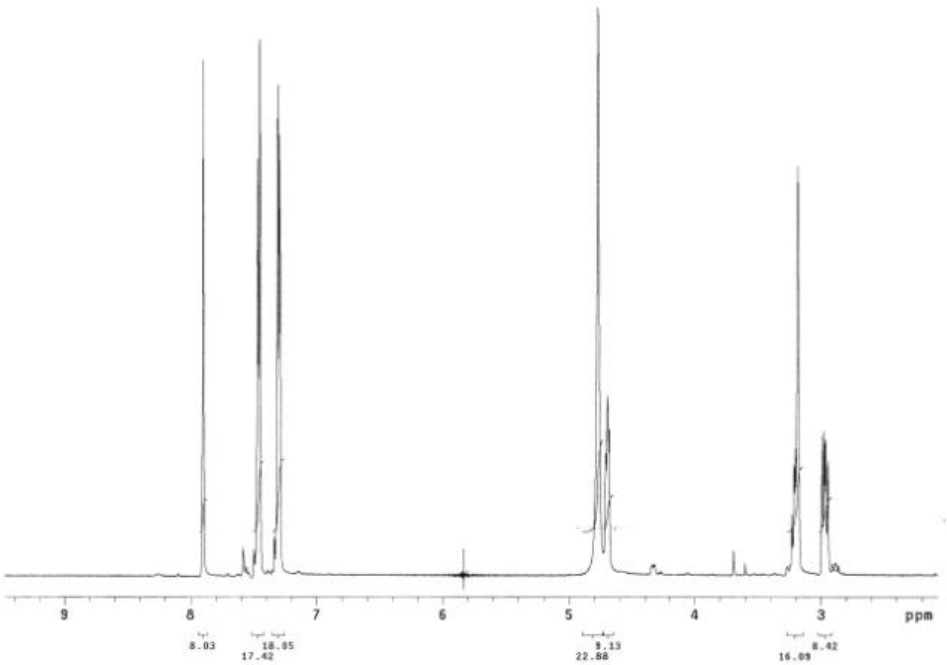


Figure S10 ^{1}H NMR Spectrum of Compound **3** in CD_3OD.

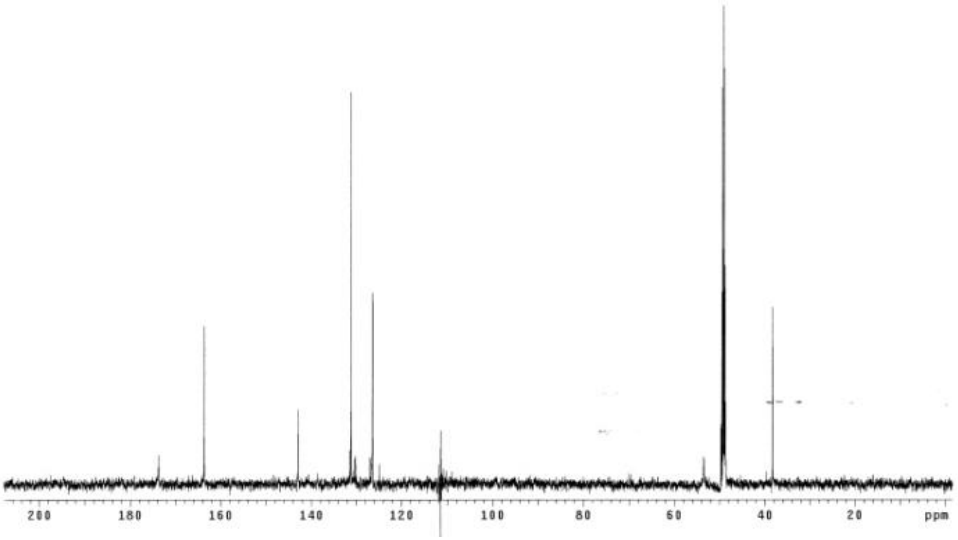


Figure S11 ^{13}C NMR Spectrum of Compound **3** in CD_3OD.

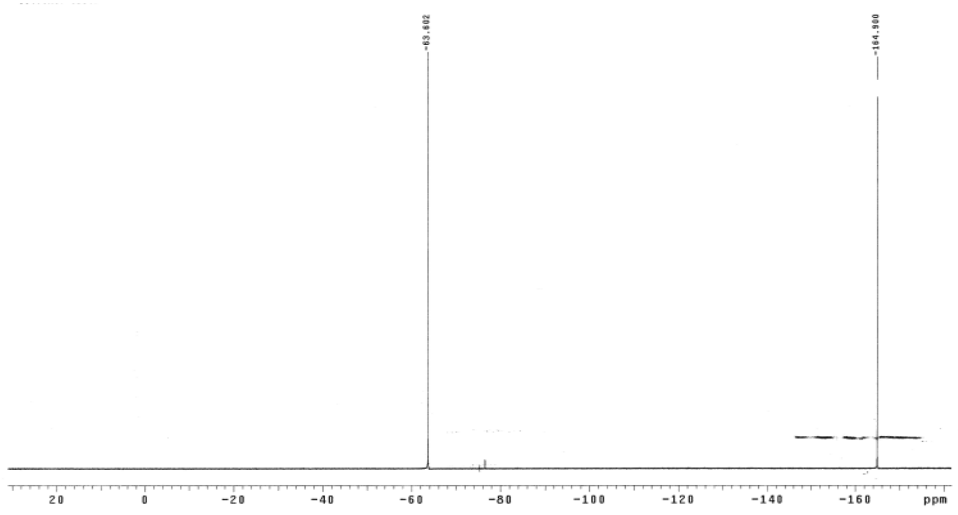


Figure S12 ^{19}F NMR Spectrum of Compound **3** in CD_3OD (the singlet peak at -164.5 is the chemical shift reference, C_6F_6).

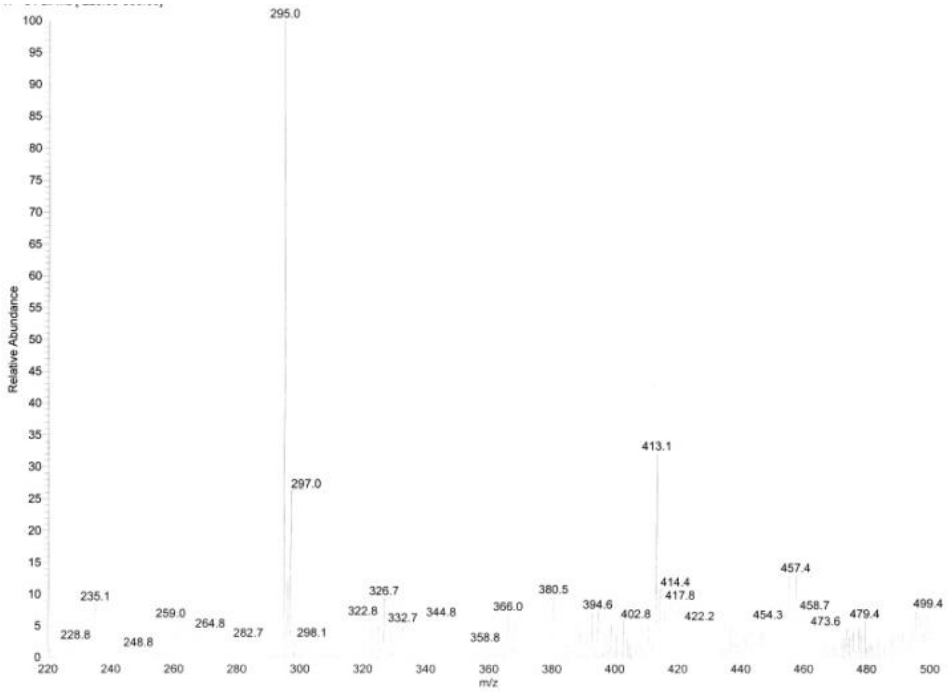


Figure S13 ESI-MS of Compound **4**.

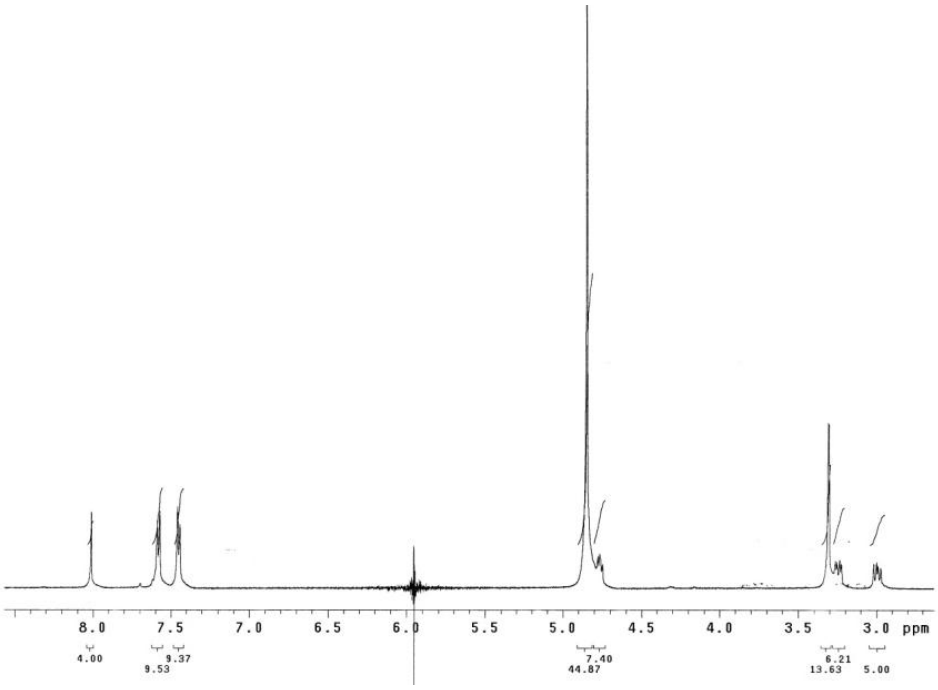


Figure S14 ^{1}H NMR Spectrum of Compound **4** in CD_3OD.

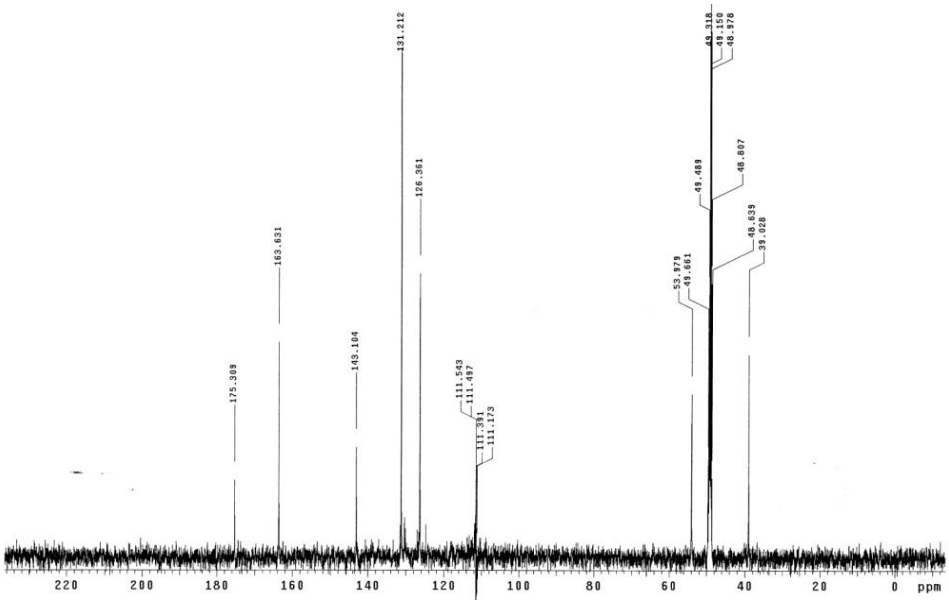


Figure S15 ^{13}C NMR Spectrum of Compound **4** in CD_3OD.

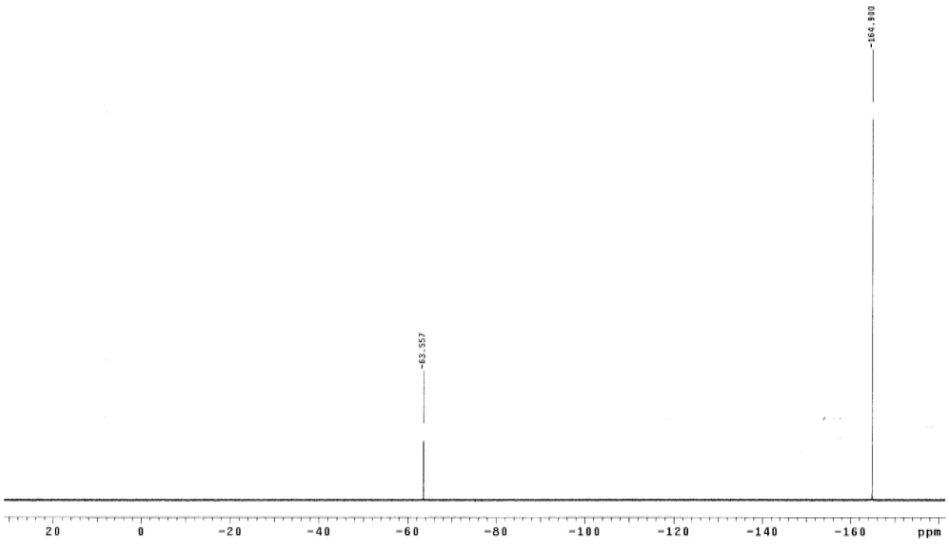


Figure S16 ^{19}F NMR Spectrum of Compound **4** in CD_3OD (the singlet peak at -164.5 is the chemical shift reference, C_6F_6).

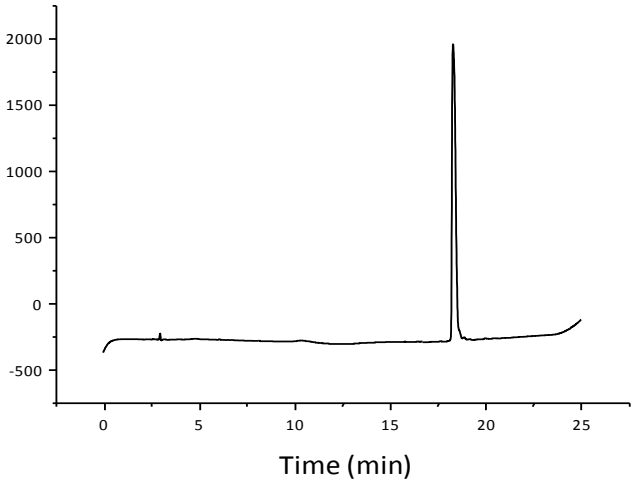


Figure S17 Analytical chromatogram of peptide K11. Solvent A: 0.1% HCl in water, solvent B: 0.1% HCl in MeOH.

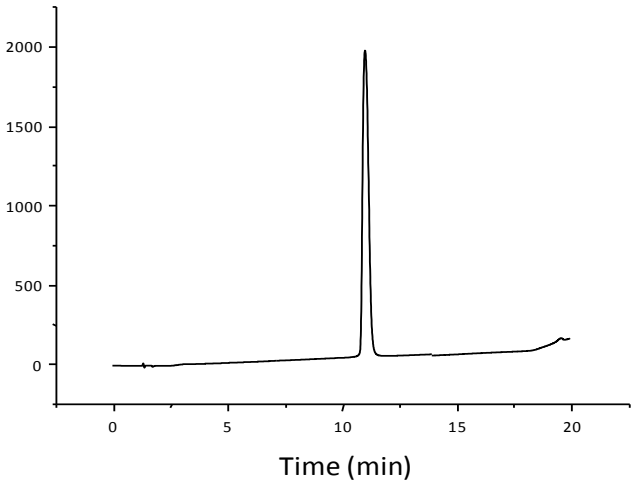


Figure S18 Analytical chromatogram of peptide E11. Solvents A: 20 mM NH_4HCO_3 in water (pH=7.0), solvent B is 20 mM NH_4HCO_3 (pH 7.0) in MeOH/water (8:2).

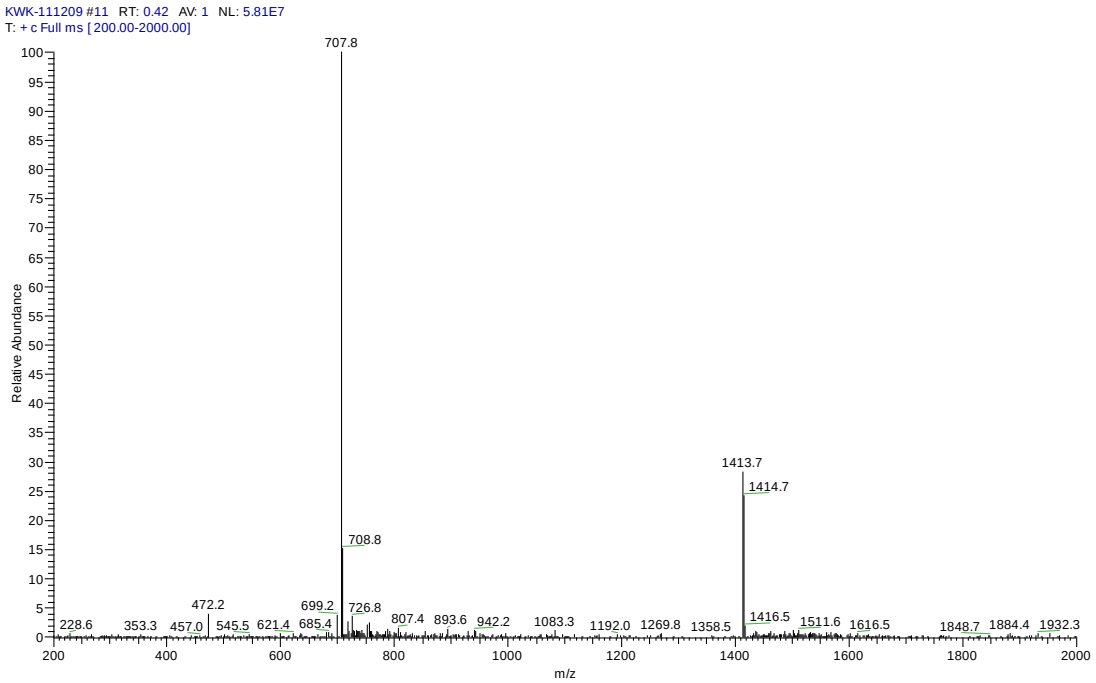


Figure S19 ESI-MS of peptide K11.

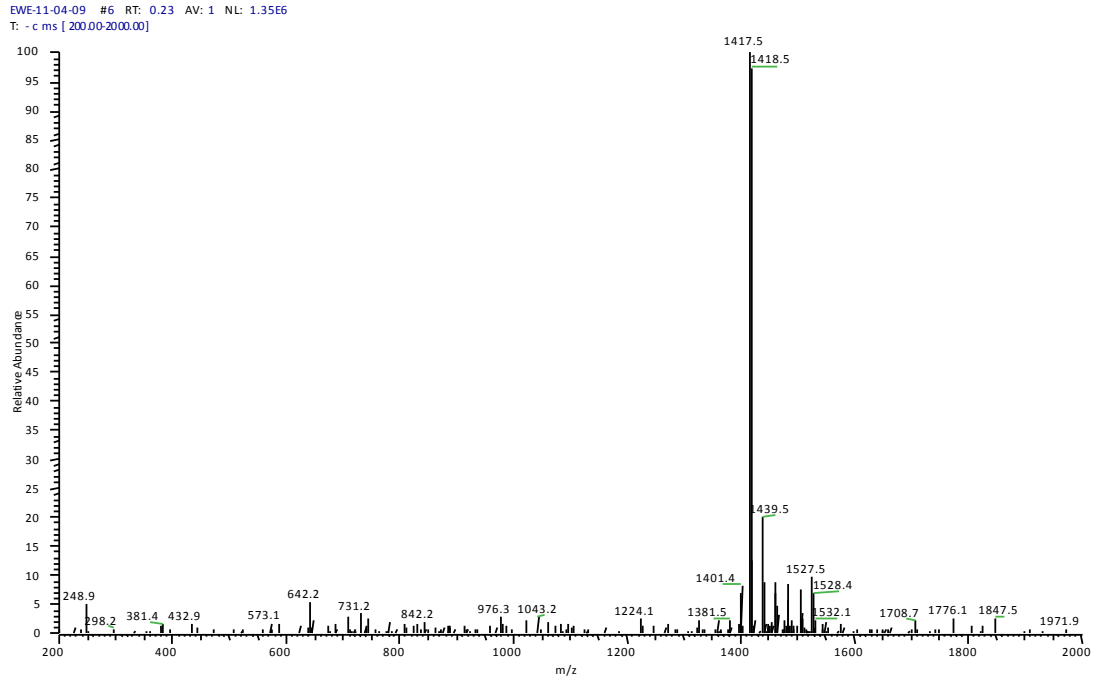


Figure S20 ESI-MS of peptide E11.

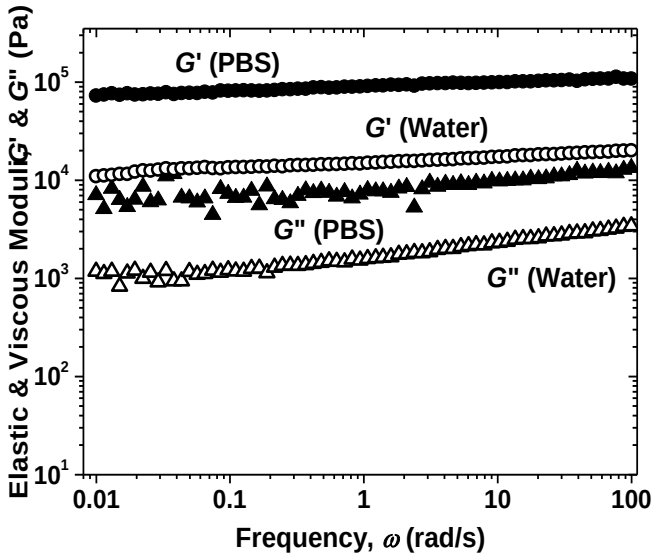


Figure S21 Frequency sweep of hydrogel in PBS and water system.

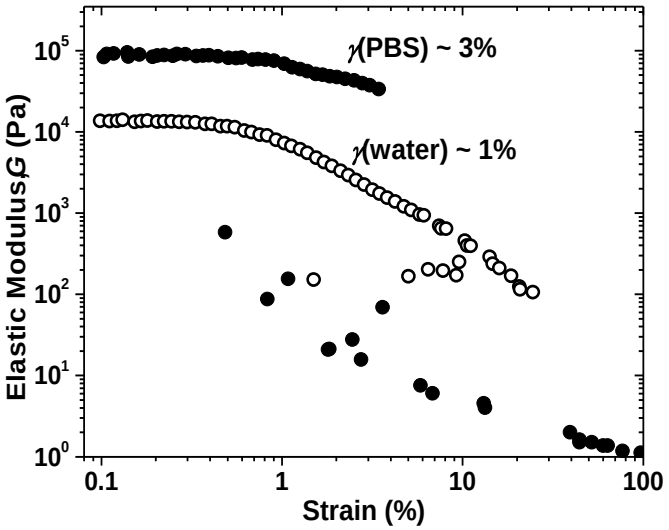


Figure S22 Strain sweep of hydrogel in PBS and water system.

Table S1 Diffusion coefficients (D, in 10^{-10} m^2s^{-1}) of **1**, **2**, **3** and **4** in different media.

meida \ diffusants		**1** (+, –)	**2** (+, 0)	**3** (0, –)	**4** (0, 0)
PBS series	PBS (0, 0)	4.71	5.01	4.50	4.98
	K11 sol (+, 0)	4.37	4.65	4.13	4.52
	E11 sol (0, –)	4.30	4.45	4.40	4.42
	Hydrogel (+, –)	4.09	4.30	3.98	4.25
D2O series	D_2O (0, 0)	5.09	5.18	4.89	5.15
	K11 sol (+, 0)	4.89	4.97	4.30	4.96
	E11 sol (0, –)	4.94	4.74	4.67	4.88
	Hydrogel (+, –)	4.71	4.77	4.54	4.72

Table S2 Diffusion coefficient (D, in10^{-10} m^2s^{-1}) of H_2O in different media.

	PBS	D_2O
in **1** + solvent	18.36	18.66
in **2** + solvent	18.40	18.56
in **3** + solvent	18.44	18.61
in **4** + solvent	18.37	18.53
in **1** + K11	17.70	18.23
in **2** + K11	17.74	18.20
in **3** + K11	17.91	18.31
in **4** + K11	17.79	18.33
in **1** + E11	17.52	18.31
in **2** + E11	17.77	18.17
in **3** + E11	17.94	18.42
in **4** + E11	17.60	18.30
in **1** + hydrogel	17.04	18.04
in **2** + hydrogel	16.97	17.97
in **3** + hydrogel	17.13	17.96
in **4** + hydrogel	16.92	17.83

Reference:

1. S. C. Gill and P. H. Von Hippel, *Anal. Biochem.*, 1989, **182**, 319