Supporting Information

Materials and general methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai, China). Rhodamine B was received from J&K. All the other Starting materials were obtained from *Alfa*. Chemical reagents and solvents were used as received from commercial sources.

General methods: ^{1}H NMR spectra were obtained on Bruker ARX 400; HR-MS were received from VG ZAB-HS system (England). HPLC was conducted at LUMTECH HPLC (Germany) system using a C_{18} RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents; SEM images were obtained at QUANTA 200 (America). LC-MS was conducted at the LCMS-20AD (Shimadzu) system, and rheology was performed on an AR 1500ex (TA instrument) system using a parallel plates (25 mm) at the gap of 350 μm.

***Protein expression and purification*:** Expression and purification of proteins using standard recombinant protein technology as previously reported.[20] Briefly, DNA fragments corresponding to protein sequences were cloned into an in-house modified version of the pET32a (Novagen) in which the S-tag and the thrombin recognition site were replaced with a sequence encoding a PreScission protease-cleavable segment (Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro). The resulting protein contained a Trx-His_6-tag in its N-terminus.

BL21(DE3) CodonPlus *Escherichia coli* cells harboring the expression plasmid were grown in LB medium at 37 °C until the OD_{600} reached 0.6 and then induced with 0.3 mM isopropyl-β-D-thiogalactoside at 16°C for about 16-18 h. Then collected E. coli cells via centrifuged and resuspended in $T_{50}N_{500}I_5$ buffer (50 mM Tris-HCl pH 7.9, 500 mM NaCl and 5 mM imidazole) supplemented with 1 mM phenylmethylsulfonyl fluoride, 1μg/mL leupeptin, and 1 μg/mL antipain. The cells were then lysed by

sonication. After the lysates had been centrifuged, the supernatant was loaded onto a Ni-NTA agarose column (Qiagen) and size-exclusion column (GE Healthcare). After digestion with PreScission Protease to cleave the N-terminal Trx-His_6-tag, the target protein was purified on a Hiprep Q FF 16/10 anion-exchange column. The final purification step was size-exclusion chromatography on a HiLoad 26/60 Superdex 200 column in 50 mM PBS pH 7.4, 100 mM NaCl, and 0.05 mM EDTA. Typical protein yields were 70–130 mg from one liter cell culture.

Analytical gel filtration: Size-exclusion chromatography was performed on an AKTA FPLC system using a Superose 12 10/300 column (GE Healthcare) for all the proteins. Protein samples were dissolved in buffer containing 50 mM PBS pH 7.4, 100 mM of NaCl, and 0.05 mM of EDTA.

Analytical ultracentrifugation: Sedimentation velocity (SV) was performed in a Beckman/Coulter XL-I analytical ultracentrifuge using double-sector. An additional protein purification step on a HiLoad 26/60 Superdex 200 size exclusion column in 50 mM PBS pH 7.4, 100 mM of NaCl, and 0.05 mM of EDTA was performed before the experiments. The experiments were conducted at 42,000 rpm (30,000 rpm for ULD-CaM) and 4℃ using interference detection and double-sector cells loaded with 1 mg/mL of the protein. The buffer composition (density and viscosity) and protein partial specific volume (V-bar) were obtained using the program SEDNTERP (http://www.rasmb.bbri.org/). The data were analyzed using the programs SEDFIT and SEDPHAT.

Preparation of hydrogels: 100 mg/mL of 4-armed PEG-Mal in PBS buffer (pH=7.4) and 80 mg/mL of protein ULD in PBS buffer were used as stock solutions (for proteins of ULD-RGD, ULD-Tip1 and ULD-CaM, the stock concentration were 75, 85 and 186 mg/mL, respectively). For the gel of protein ULD, 50 μL of stock solution of 4-armed PEG-Mal, 325 μL PBS, and 125μL stock solution of ULD protein were mixed together. Gels would form after being kept at room temperature (22-25 ^{0}C) within one minute (the final concentration of 4-armed PEG-Mal was 1.0 wt% and 2.0

wt% for ULD). The other protein hybrid hydrogels were also formed using the same method.

Cell proliferation and live-dead assay: CCK-8 assay was conducted to quantify cell proliferation inside the cell-gel constructs at different times. A 3D Culture standard was made by encapsulating cells (final concentration was 10^6 cells/mL) into hydrogels. Briefly, cells in culture medium without FBS and 4-armed-PEG-Mal stock solution in PBS were firstly mixed together. Protein in PBS was then added to the above solution. The final solution was transferred to 96 well plates (50 μL per plate) within one minute. To perform the CCK-8 assay, each cell-gel construct was washed with complete cell culture medium twice, and then incubated with 10 μL of CCK-8 stock solution in 100μL complete cell culture medium per plate for 4h in an incubator. The absorbance at 450 nm was determined using the microplate reader (MultiskaniMark, Bio-Rad, USA).

Cell viability and spreading were performed by a Live–Dead assay (Sigma-Aldrich). At designated times, the cell–gel constructs were washed three times with PBS buffer, and then 100 μL of the Live/Dead solution which contains 4 μM EthD-1 (ethidium homodimer-1) and 2 μM calcein AM was added on top of each sample for 30 min in an incubator at 37℃ with 5% CO_2. After removing the staining solution, Nikon Eclipse E600 fluorescence microscope was then used to view the sample with excitation filters of 450–490 nm (green, Calcein AM) and 510–560 nm (red, EthE-1).

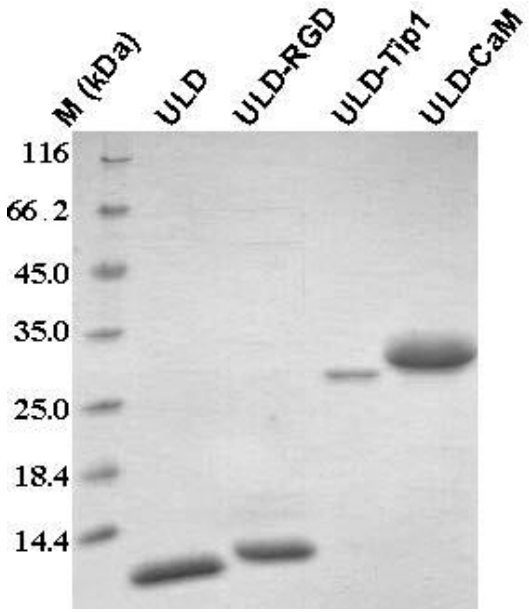


Fig. S-1. SDS-PAGE gel showing the final protein purities

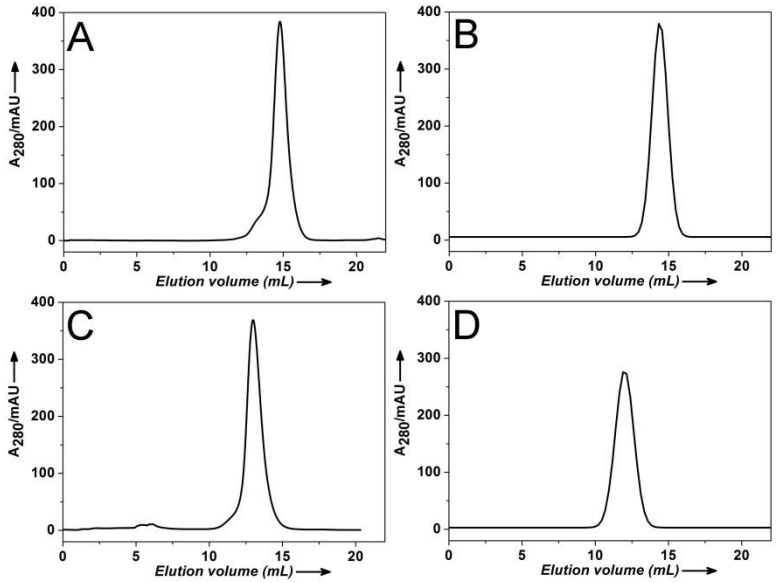


Fig. S-2. Analytical gel filtration profiles of different proteins, all concentration is 5 mg/mL: A) protein of ULD, B) protein of ULD-RGD, C) protein of ULD-Tip-1, and D) protein of ULD-CaM. These results indicating that these proteins exist in a unique form of oligomers

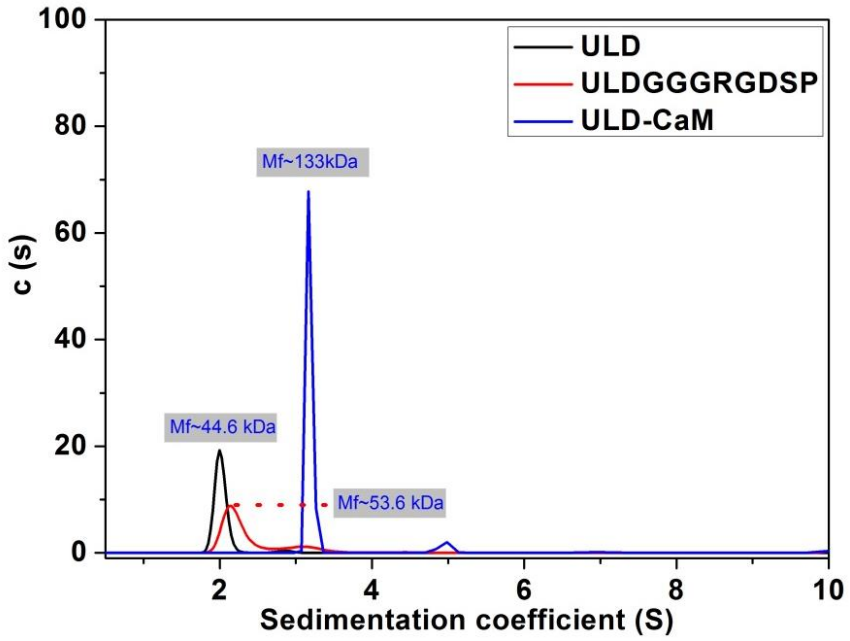


Fig. S-3. c(S) distributions from SV runs for different proteins: these results suggesting that all the proteins are mono-disperse, and it is estimated to be a stable tetramer in solution. The theoretical molecular weight (MW) of ULD, ULD-GRD, and ULD-CaM is 11.58 kDa, 12.26 kDa, 28.57 kDa, respectively.

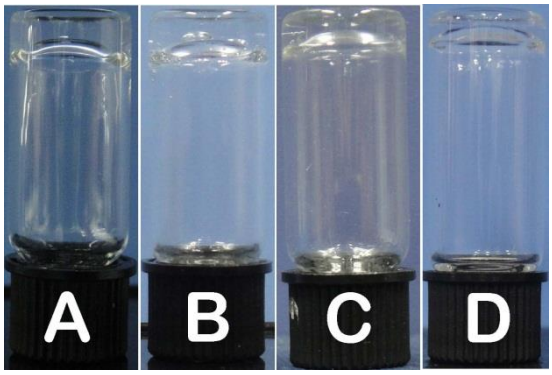


Fig. S-4. Optical images of the hydrogels formed by different proteins. Hydrogel formed by A) protein of ULD, B) protein of ULD-RGD, C) protein of ULD-Tip-1, and D) protein of ULD-CaM (the final concentration of 4-armed PEG-Mal was 1.0 wt% and 2.0 wt% for protein).

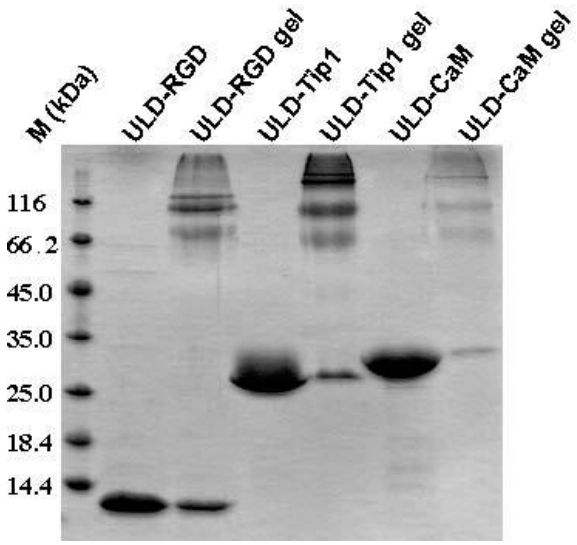


Fig. S-5. SDS-PAGE gel of proteins and the corresponding hydrogels at 12h time point

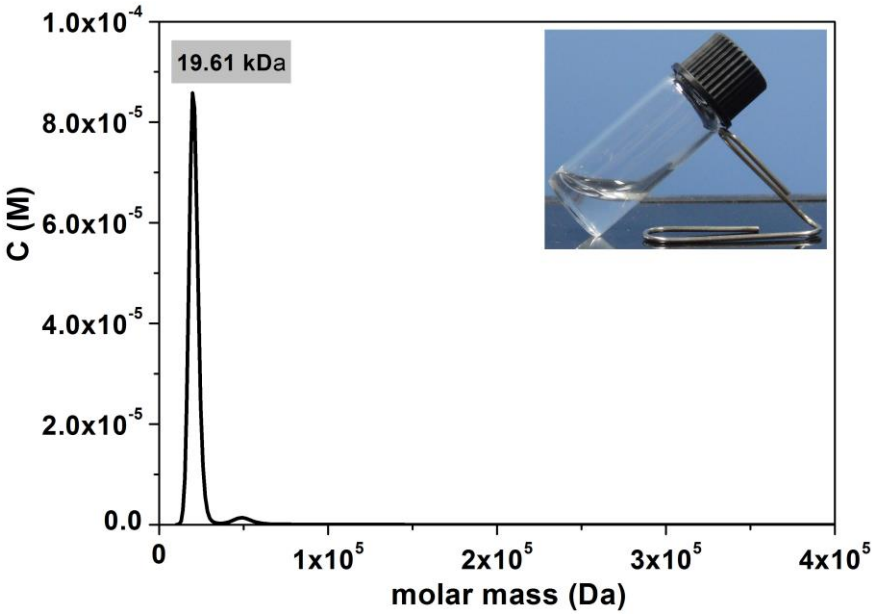


Fig. S-6. The molecular weights of the purified proteins were measured by analytical ultracentrifugation sedimentation velocity (SV), indicating that ULD mutant protein, in which all three residues (Lys137, Trp138, and Asn138) mutated to Ala), formed into a dimer. The theoretical molecular weight (MW) of ULD is 11.43 kDa.

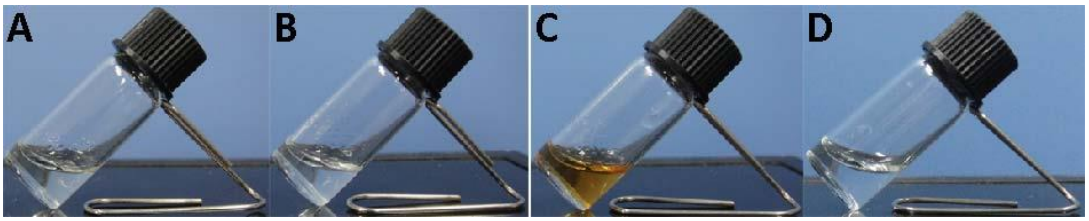


Fig. S-7. Optical images of samples in PBS buffer (pH = 7.4) of A) 5.0 wt% BSA with 2.0 % 4-armed-PEG-Mal, B) 5.0 wt% trypsin with 2.0 % 4-armed-PEG-Mal, C) 5.0 wt% phosphatase with 2.0 % 4-armed-PEG-Mal, and D) 5.0 wt % mULD with 2.0 % 4-armed-PEG-Mal.

Rheology: Rheology test was done on an AR 1500ex (TA instrument) system, 25 mm parallel plates was used during the experiment at the gap of 350 μm. The dynamic time sweep was conducted at the frequency of 1 rad/s and the strain of 1%. Dynamic strain sweep was performed and the strain values within the linear range were chosen for the following dynamic frequency sweep. The gels were also characterized by the mode of dynamic frequency sweep in the region of 0.1-100 rad/s at the strain of 1%.

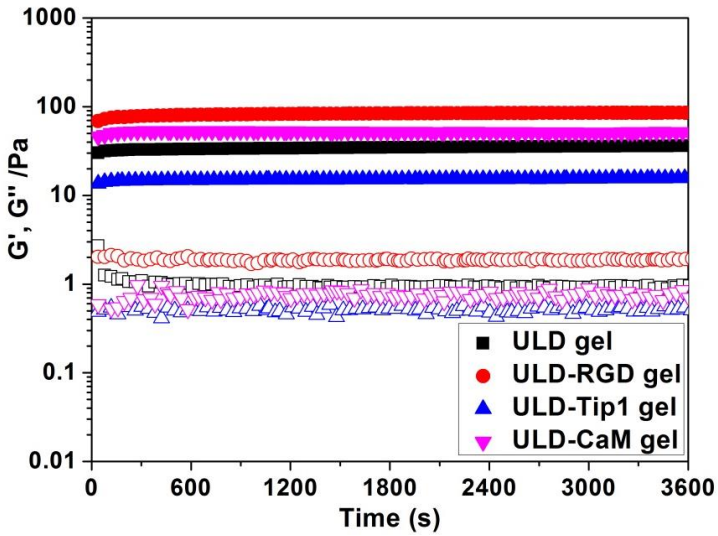


Fig. S-8. Rheological measurement in dynamic time sweep mode for different gels at the frequency of 1 rad/s and strain of 1%. Closed symbols: G' and open symbols: G")

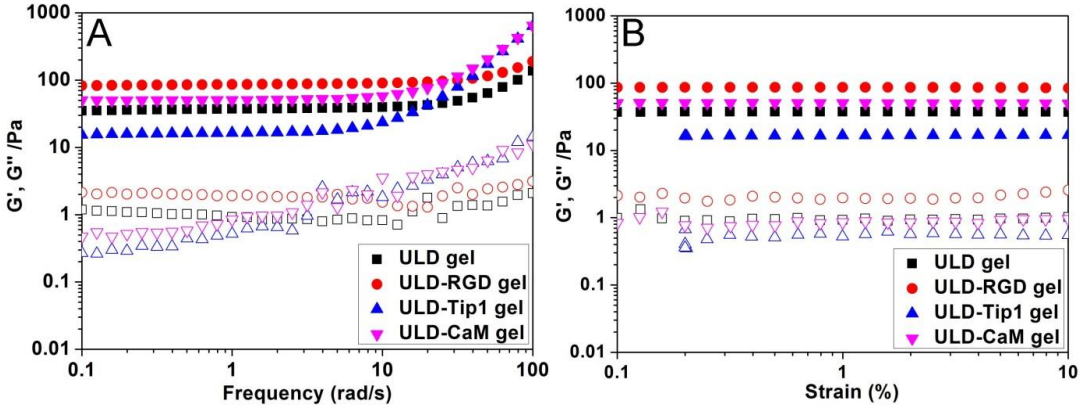


Fig. S-9. Rheological measurements with the mode of A) dynamic frequency sweep at the strain of 1% and B) dynamic strain sweep at the frequency of 1 rad/s. Closed symbols: G' and open symbols: G")

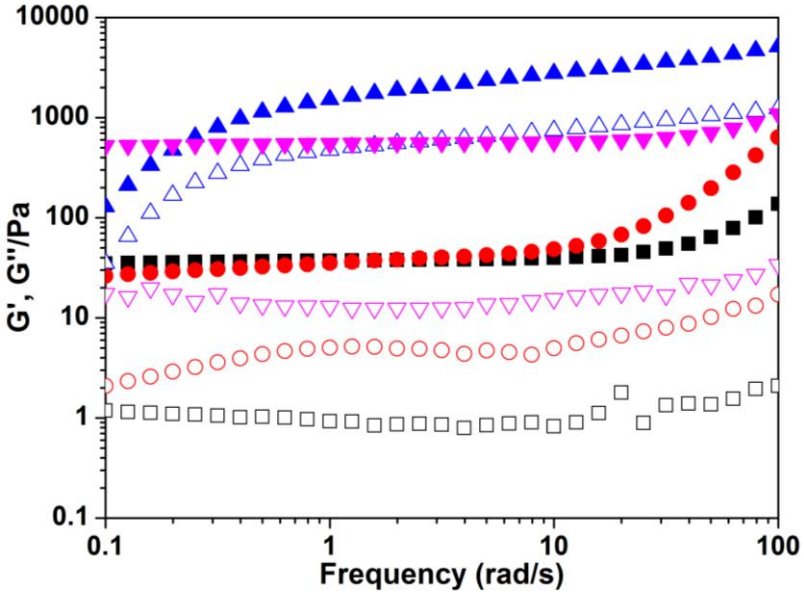


Fig. S-10. Rheological measurements with the mode of dynamic frequency sweep at the strain of 1.0% of ULD gel formed at different conditions (filled symbols: G' and open symbols: G"). (squares in black: 2.0 % of protein ULD and 2.0 % of 4-armed PEG, 25 ℃, 150 mM of NaCl; triangles in blue: 2.0 % of protein ULD and 2.0 % of 4-armed PEG, 25 ℃, 200 mM of NaCl; triangles in pink: 3.0 % of protein ULD and 3.0 % of 4-armed PEG, 25 ℃, 150 mM of NaCl; and circle in red: 2.0 % of protein ULD and 2.0 % of 4-armed PEG, 37 ℃, 150 mM of NaCl)

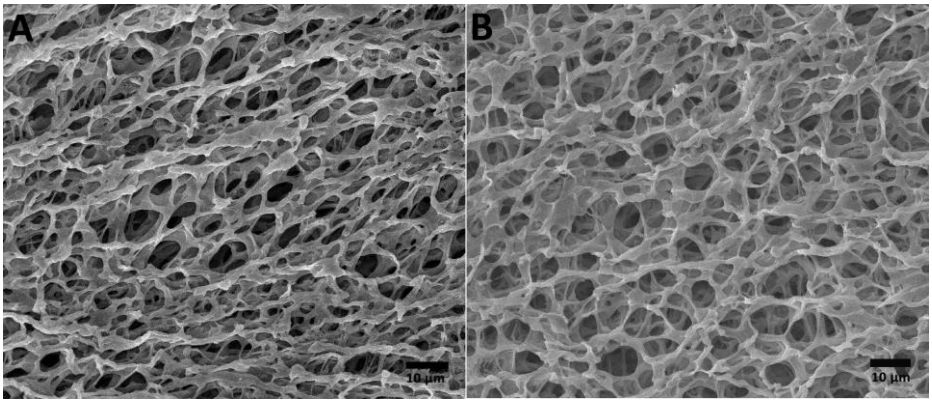


Fig. S-11. SEM images of A) hydrogel of 2.0 wt% ULD-GGGRGDSP with 1.0% 4-armed-PEG-Mal; B) hydrogel of 5.0 wt% ULD-CaM with 2.0% 4-armed-PEG-Mal.

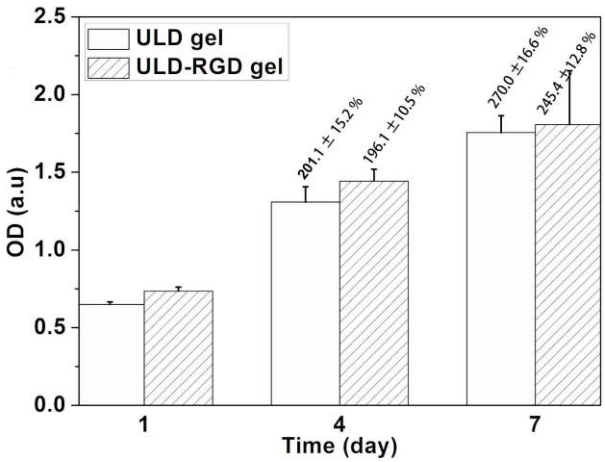


Fig. S-12. Cell proliferation rate of 3T3 cells cultured in hydrogels. ULD vs ULD-RGD gel, ***p<0.0001

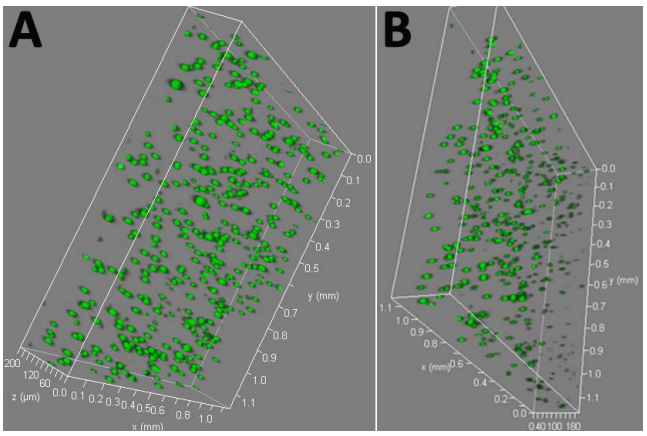


Fig. S-13. 3D cell culture in the gels. A) ULD gel; B) ULD-RGD gel. ((3D reconstruction of confocal images acquired through the 200 μm thickness of the gel)

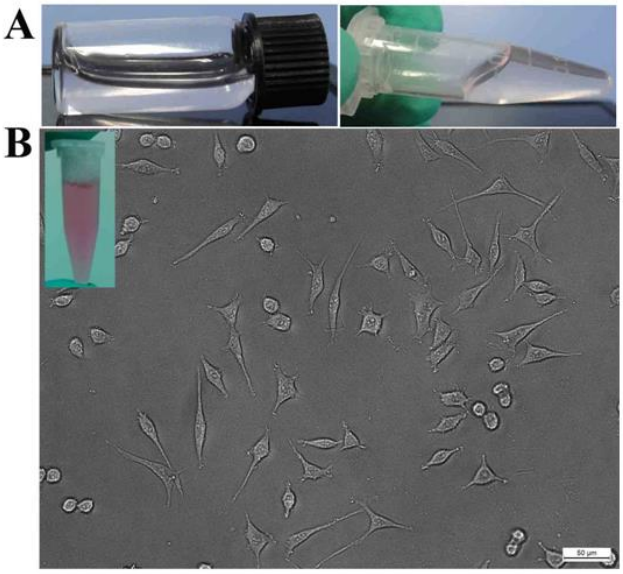


Fig. S-14. A) left: 200 uL of the hydrogel was added with 100 uL of Trypsin (2.5 ug/mL) for 3 minutes, and then added with 200 uL of PBS and right: the solution was then centrifuged at 1000 rpm for 5 minutes and no precipitate could be observed; B) cells separated from the cell-gel construct by adding Trypsin and then being centrifuged. They grew well on a conventional 96-well tissue culture plate (the scale bar represents 50 μm).

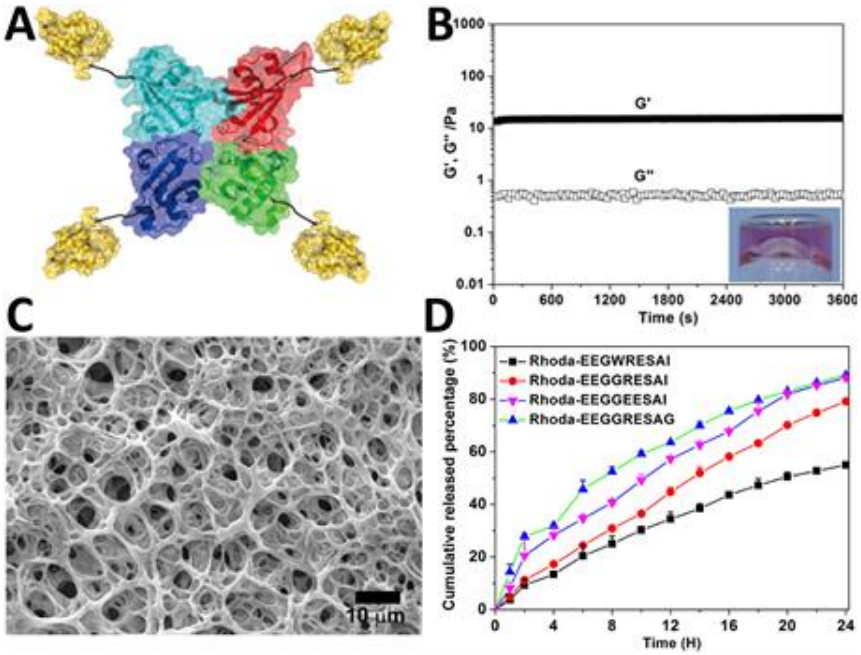


Fig. S-15. A) A schematic diagram of ULD-TIP-1 protein based on the crystal structure of ULD and TIP-1 proteins, B) rheological measurement with the mode of dynamic time sweep of the hydrogel (both PEG and ULD-TIP-1 concentrations are

2.0 wt%, insert: an optical image of the hydrogel formed in complete cell culture medium), C) SEM image of the hydrogel, and D) drug release profile of Rhoda-peptides from hydrogels (higher binding affinity of peptides to TIP-1 protein results in lower release speed, and vice versa)

Release profile: 0.4 mL of ULD-Tip1 hydrogel containing different Rhodamine-peptide conjugates were formed as described above (the final concentration of the protein is 2.0 wt% and 4-armed-PEG-Mal is 2.0 wt%). After 8hrs, each of the gels was treated with 0.4 mL of fresh PBS buffer solutions (pH = 7.4). 0.4 mL of the upper buffer solution was taken out and used to tested by UV-Vis Spectrophotometer at the wavelength of 554 nm at designated times. A fresh 0.4 mL of PBS was added back to the gel. Standard curve of all model drugs were drew before the test. The experiment was conducted in 3 parallel experiments.

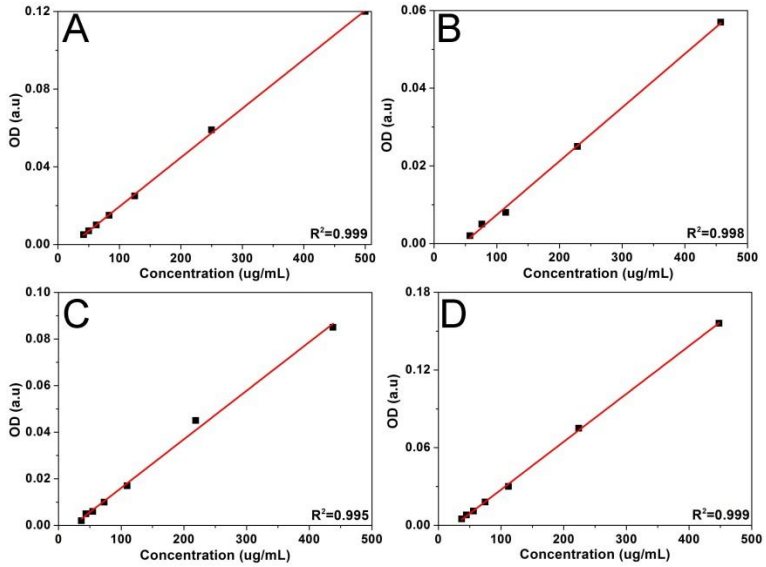
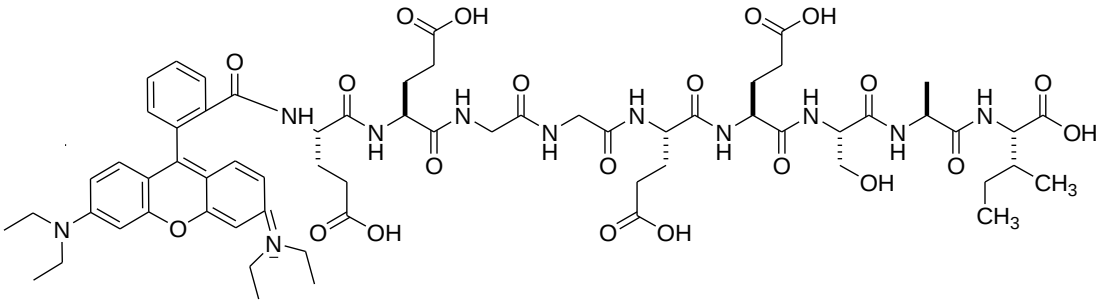


Fig. S-16. Standard curves of different model drugs: A) Rhoda-EEGWRESAI, B) Rhoda-EEGGRESAI, C) Rhoda-EEGGRESAG, D) Rhoda-EEGGEESAI

Synthesis and characterizations:

Peptide systhesis: The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.5 mmol/g.

20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, Rhodamine B was used to attach on the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 1 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TMS and 2.5% of H_2O for 1 hours. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was centrifuged for 10 min at 4 ^{0}C at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation.



Scheme S-1. Chemical structure of **Rhoda-EEGGEESAI**

Rhoda-EEGGEESAI: ^{1}H NMR (400MHz, DMSO-d_6) δ 7.51-7.55 (m, 2H), 7.26 (s, 1H), 7.14 (s, 1H), 7.01 (s, 1H), 6.19-6.42 (m, 3H), 4.12-4.38 (m, 8H), 3.92-3.95 (m, 2H), 3.51-3.82 (m, 5H), 3.16-3.41 (m, 4H), 2.21-2.33 (m, 3H), 1.97-2.16 (m, 2H), 1.80-1.83 (m, 2H), 1.50-1.79 (m, 3H), 1.30-1.49 (m, 1H), 1.21 (d, J=6.99Hz, 3H), 1.05-1.10 (m, 5H). MS: calc. M^+ = 1344.60, obsvd. HR-MS: $(M)^+$= 1344.5990.

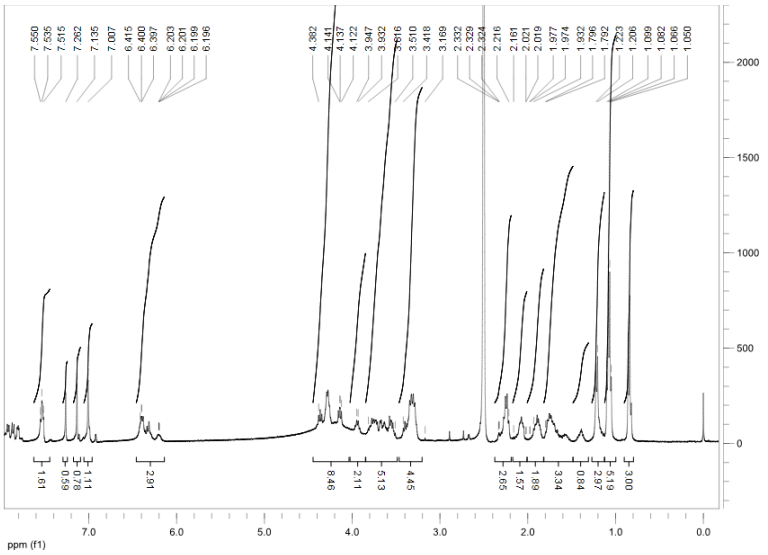


Fig. S-17. ^{1}H NMR of **Rhoda-EEGGEESAI**

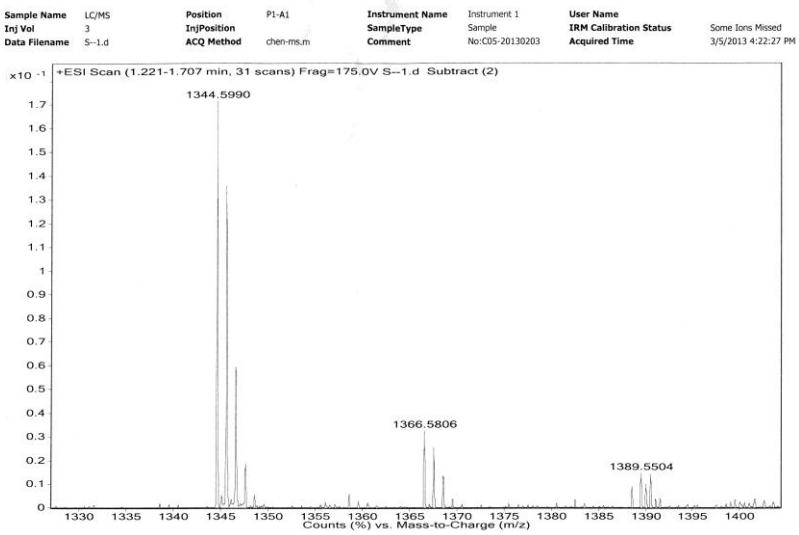
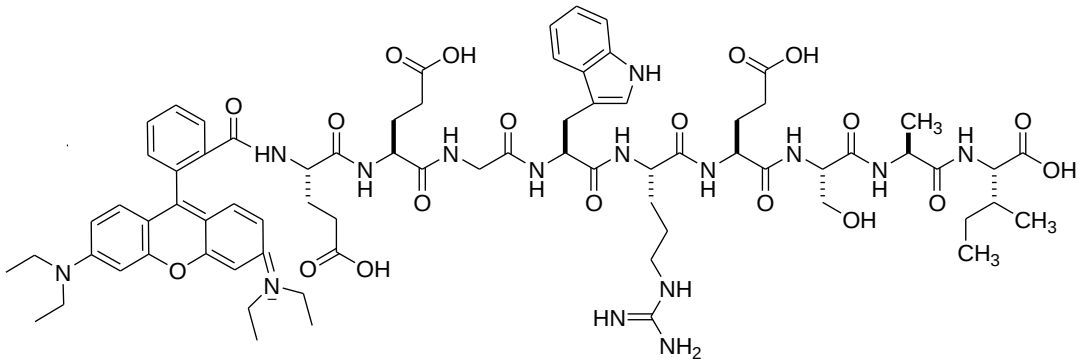


Fig. S-18. HR-MS of Compound **Rhoda-EEGGEESAI**



Scheme S-2. Chemical structure of **Rhoda-EEGWRESAI**

Rhoda-EEGWRESAI: ^{1}H NMR (400MHz, DMSO-d_6) δ 7.89-7.97 (m, 1H), 7.74-7.86 (m, 1H), 7.40-7.70 (m, 3H), 7.33 (d, J=7.98 Hz, 2H), 7.26 (s, 1H), 7.10-7.23 (m, 2H), 7.00-7.09 (m, 2H), 6.38-6.45 (m, 1H), 6.14-6.34 (m, 1H), 4.50-4.65 (m, 1H), 4.22-4.44 (m, 2H), 4.10-4.20 (m, 1H), 3.84-4.00 (m, 2H), 3.52-3.60 (m, 2H), 3.40-3.50 (m, 2H), 3.20-3.38 (m, 3H), 3.00-3.14 (m, 2H), 2.86-2.98 (m, 1H), 2.20-2.42 (m, 2H), 1.86-2.18 (m, 3H), 1.60-1.84 (m, 3H), 1.28-1.58 (m, 3H), 1.12-1.26 (m, 3H), 0.98-1.10 (m, 4H), 0.78-0.90 (m, 3H). MS: calc. M^{+} = 1500.72, obsvd. HR-MS: $(M)^{+}$= 1500.7163.

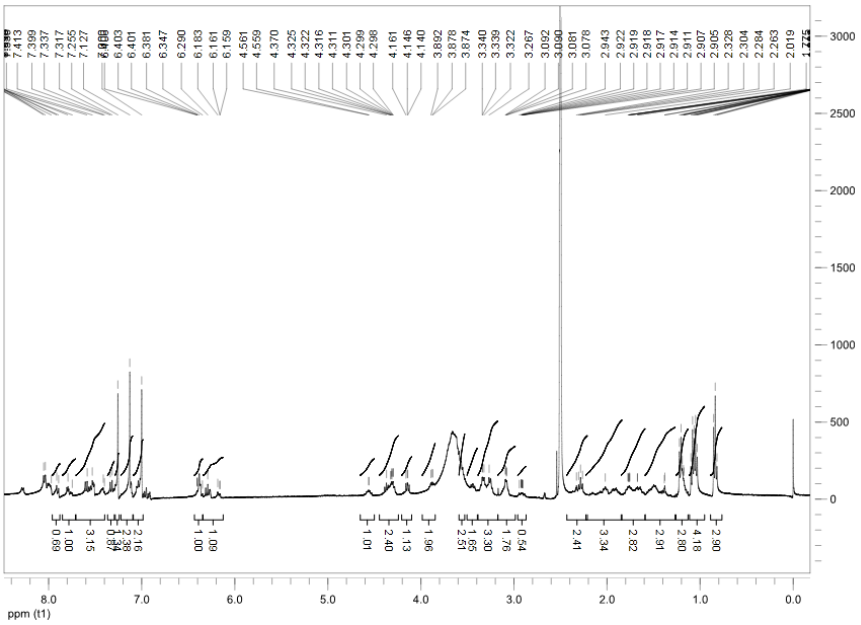


Fig. S-19. ^{1}H NMR of **Rhoda-EEGWRESAI**

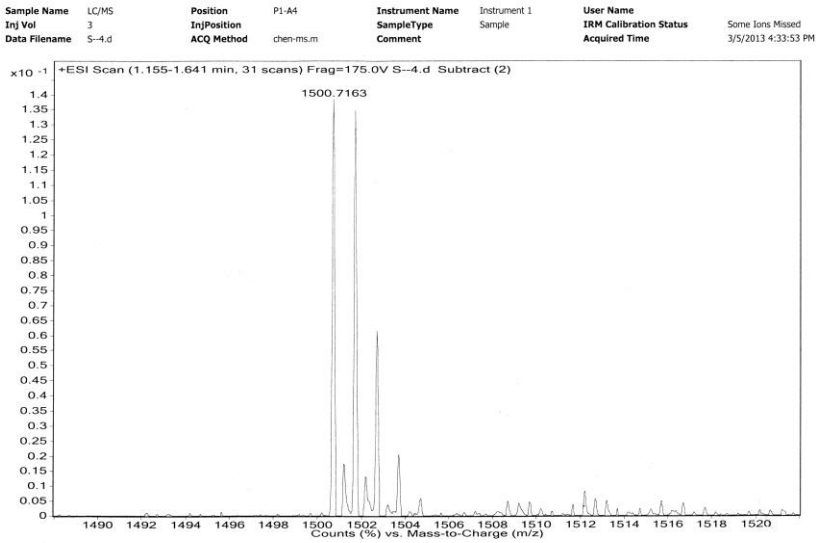
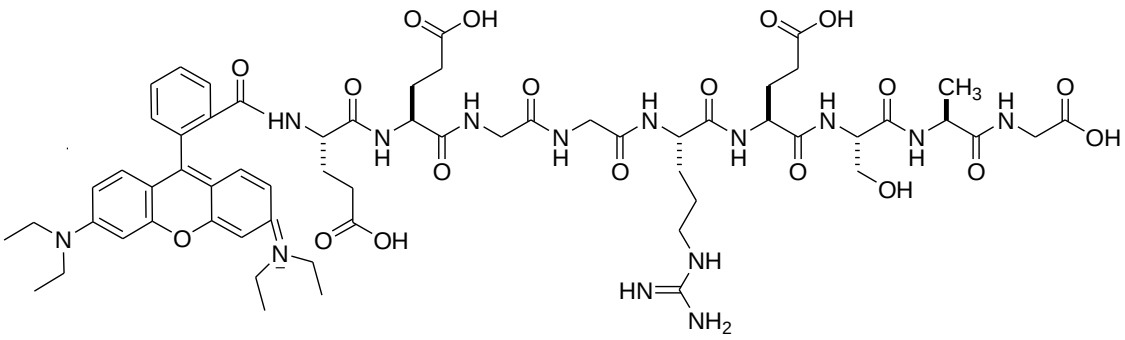


Fig. S-20. HR-MS of Compound **Rhoda-EEGWRESAI**



Scheme S-3. Chemical structure of **Rhoda-EEGGRESAG**

Rhoda-EEGGRESAG: ^{1}H NMR (400MHz, DMSO-d_6) δ 7.81-7.86 (m, 1H), 7.42-7.59 (m, 3H), 7.24 (s, 1H), 7.11 (s, 1H), 6.98-7.02 (m, 2H), 6.40-6.41 (m, 2H), 6.30-6.38 (m, 1H), 4.28-4.33 (m, 6H), 3.52-3.92 (m, 9H), 3.28-3.50 (m, 6H), 3.08 (d, J=5.41, 1H), 2.54 (s, 1H), 2.24-2.33 (m, 3H), 2.04-2.08 (m, 2H), 1.85-1.93 (m, 2H), 1.54-1.57 (m, 3H), 1.23 (d, J=7.11, 3H), 1.05-1.10 (m, 8H). MS: calc. M^+ = 1315.60, obsvd. HR-MS: $(M)^+$= 1315.5966.

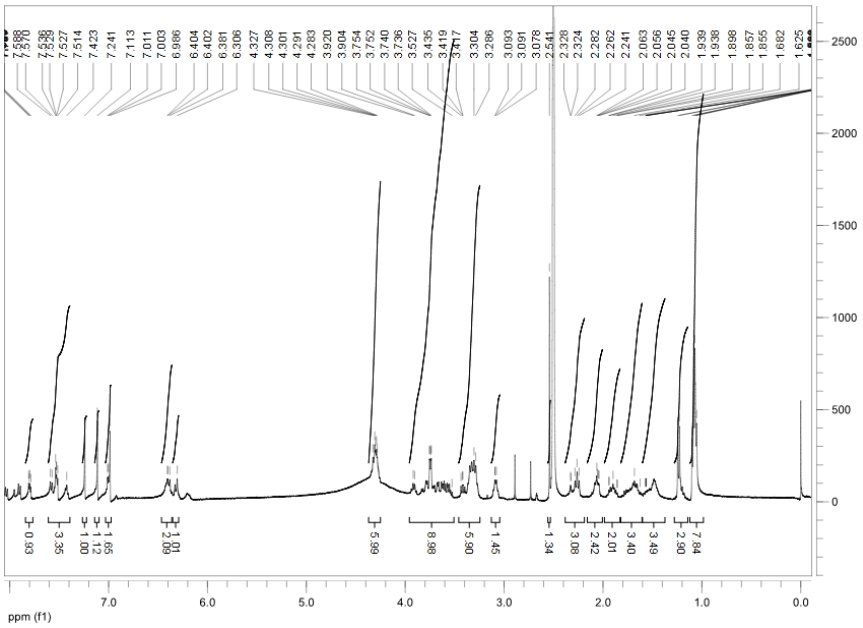


Fig. S-21. ^{1}H NMR of **Rhoda-EEGGRESAG**

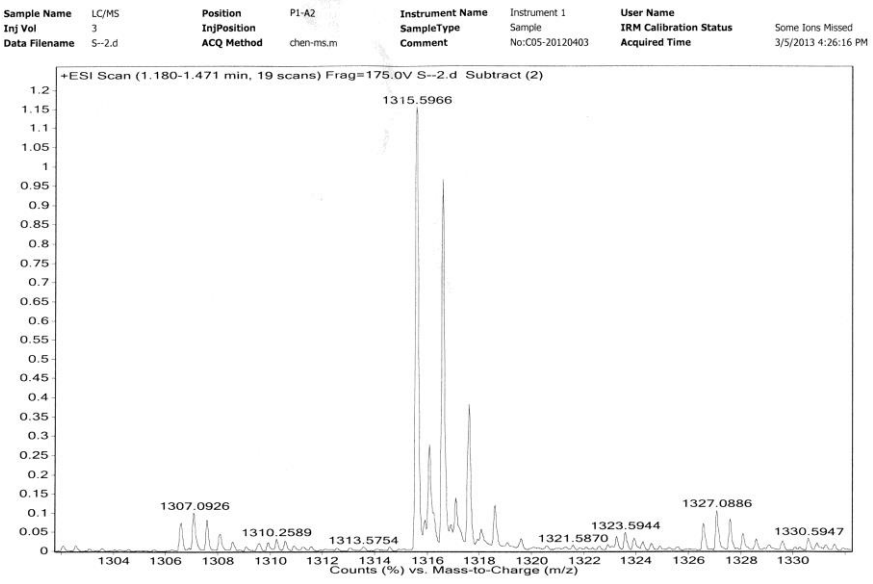
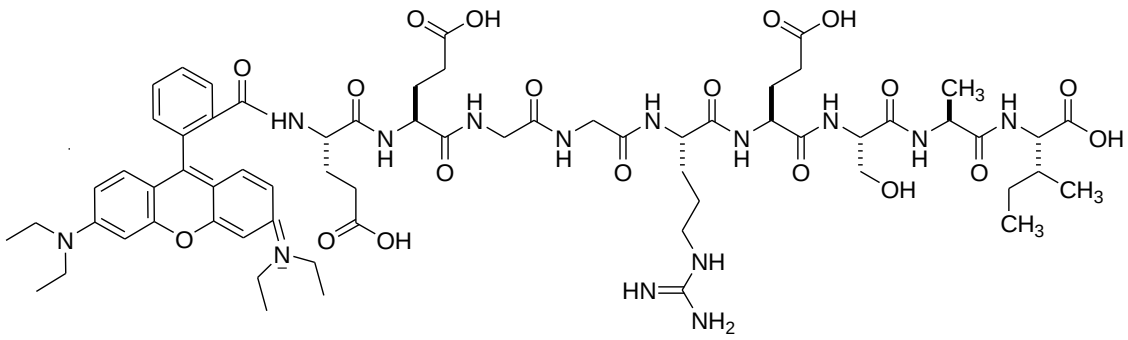


Fig. S-22. HR-MS of Compound **Rhoda-EEGGRESAG**



Scheme S-4. Chemical structure of **Rhoda-EEGGRESAI**

Rhoda-EEGGRESAI: ^{1}H NMR (400MHz, DMSO-d_6) δ 7.55-7.57 (m, 1H), 7.43 (s, 2H), 7.31 (s, 1H), 7.18 (s, 1H), 7.10-7.13 (m, 1H), 6.98-7.09 (m, 1H), 6.92 (m, 1H), 6.18-6.37 (m, 2H), 5.24 (s, 9H), 3.90-4.37 (m, 5H), 3.52-3.80 (m, 8H), 3.30-3.42 (m, 6H), 3.08 (d, 2H), 1.40-2.33 (m, 10H), 1.18-1.23 (t, 5H), 1.05-1.10 (q, 3H), 0.81-0.86 (m, 3H). MS: calc. M^+ = 1371.66, obsvd. HR-MS: $(M+H)^+$= 1371.6570.

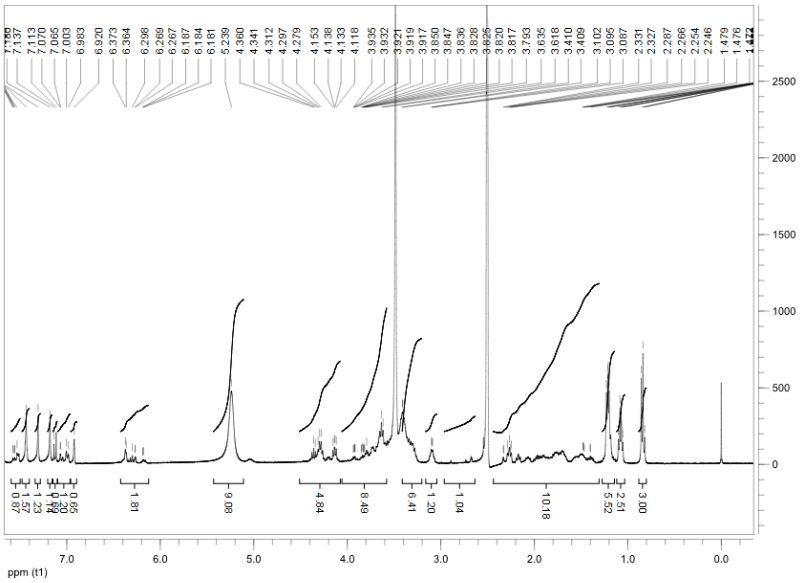


Fig. S-23. ^{1}H NMR of **Rhoda-EEGGRESAI**

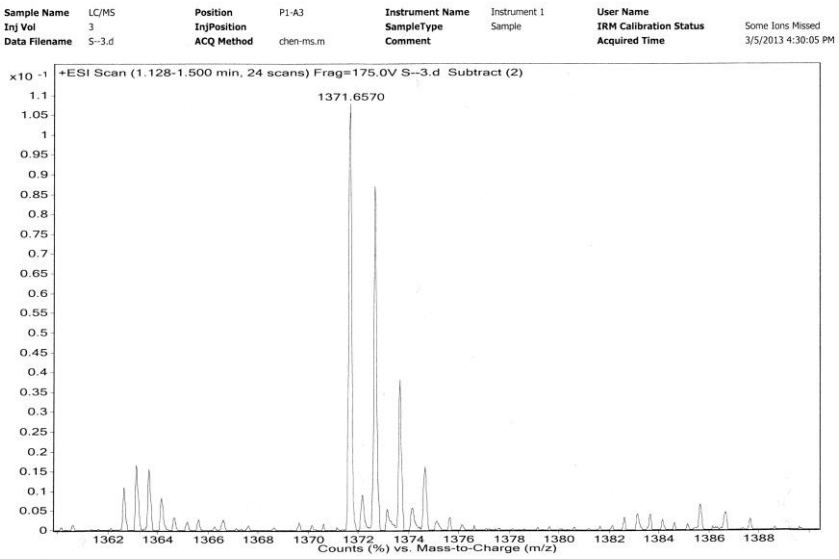


Fig. S-24. HR-MS of Compound **Rhoda-EEGGRESAI**