Supporting Information for

Branched Peptides for Enzymatic Supramolecular Hydrogelation and Rapid Delivery Targeting Mitochondria

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S1. Materials and instruments

Materials

All amino acid derivatives involved in the synthesis were purchased from GL Biochem (Shanghai) Ltd. N, N-diisopropylethylamine (DIPEA), O-benzotriazole-N,N,N',N'-tetramethyluronium-hexafluorophosphate (HBTU) were purchased from Fisher Scientific. The synthesis of all peptide fragments was based on solid-phase peptide synthesis (SPPS). The branched peptides were made via the combination of SPPS and liquid phase synthesis. All crude compounds were purified by HPLC with the yield of 70-80%. All reagents and solvents were used as received without further purification unless otherwise stated.

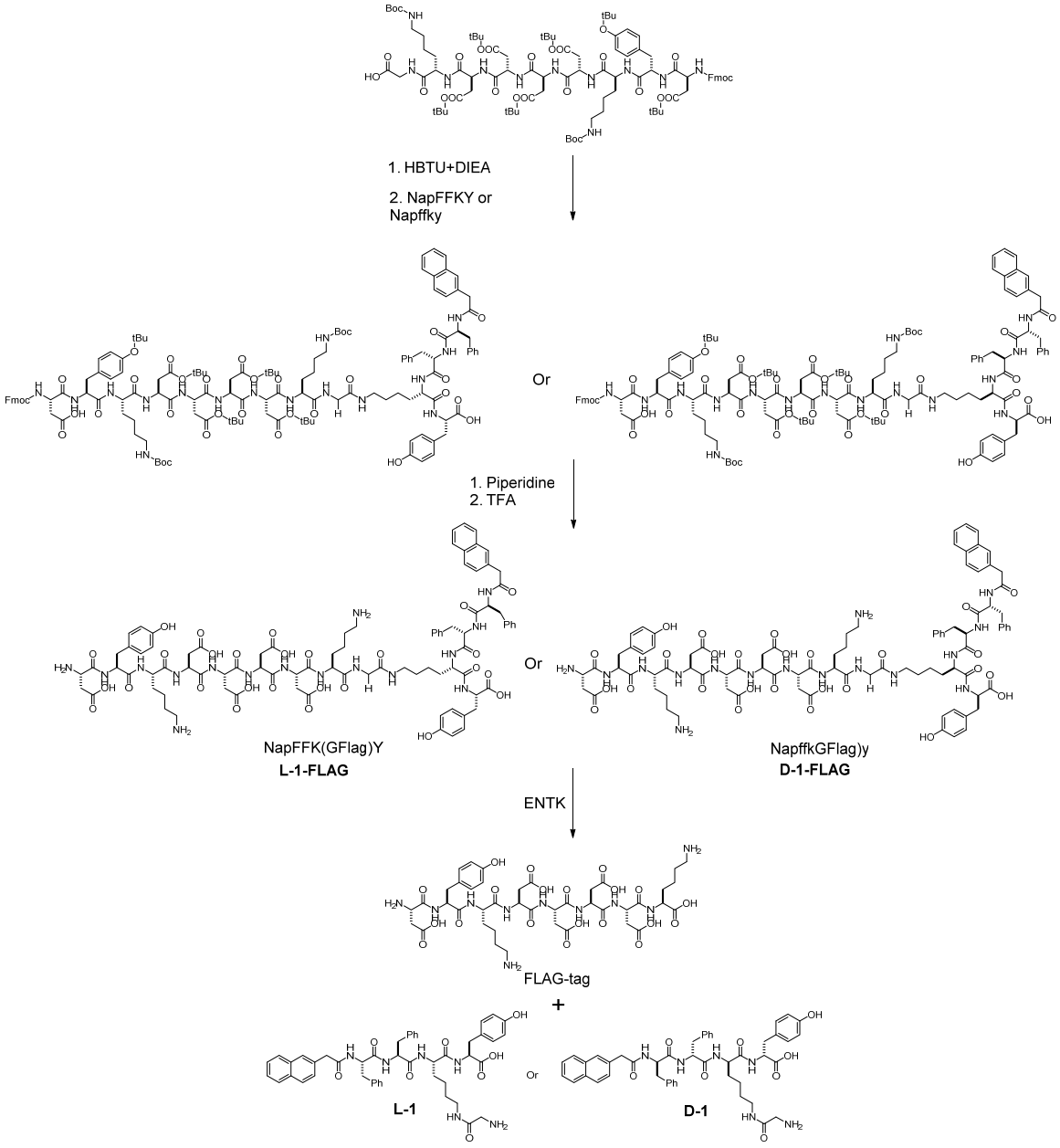
Instruments

All peptides were purified by Water Delta600 HPLC system, equipped with an XTerra C18 RP column. LC-MS was operated on a Waters Acquity Ultra Performance LC with Waters MICRO-MASS detector. ^{1}H-NMR spectra were gained on Varian Unity Inova 400 with Deuterated DMSO as solvent. Transmission electron microscope (TEM) images were taken on Morgagni 268 transmission electron microscope. Circular dichroism spectra were

obtained by Jasco J-810 spectropolarimeter. Fluorescent analysis was performed on Shimadzu RF-5301-PC fluorescence spectrophotometer. Rheological tests were conducted on TA ARES-G2 rheometer at 25□. Dynamic light scattering data was obtained using ALV/DLS/SLS-5000 System at ambient condition. The diameter distribution was determined from DLS data based on the spherical model discussed by Chu, Benjamin[1]:

$$r_h = \frac{kT}{6\pi\eta D} \tag{1}$$

Where k is Boltzmann's constant, T is absolute temperature, η is the solvent viscosity, and D is the diffusion constant for particles (determined from DLS experiments).



Scheme S1. Synthetic route of the branched peptides and ENTK hydrolysis products

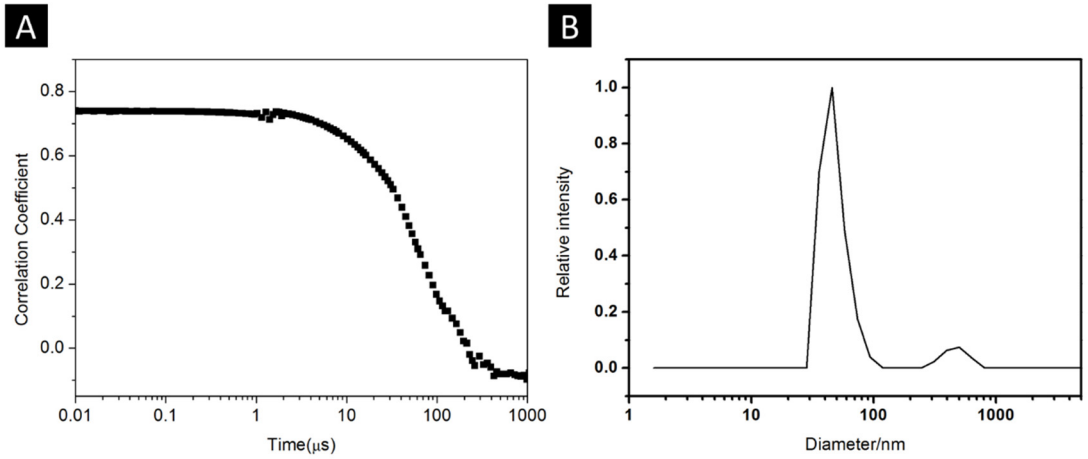


Fig. S1. (A) Correlogram and (B) hydrodynamic diameter distrubutions of L-**1₸FLAG** micelles (200 μM) in PBS buffer deduced from dynamic light scattering (DLS) measurement.

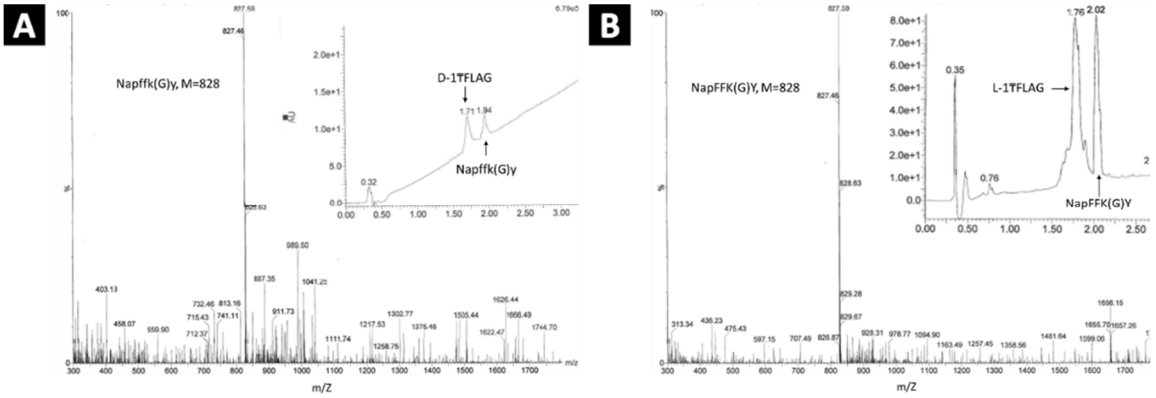


Fig. S2. LC-MS evidences of the hydrolysis product (NapFFK(ᵋG)Y and Napffk(ᵋG)y, M=828) of L-**1₸FLAG** and D-**1₸FLAG** (2.5 wt%) by enterokinase (10U/ml) after 48 hours at ambient condition.

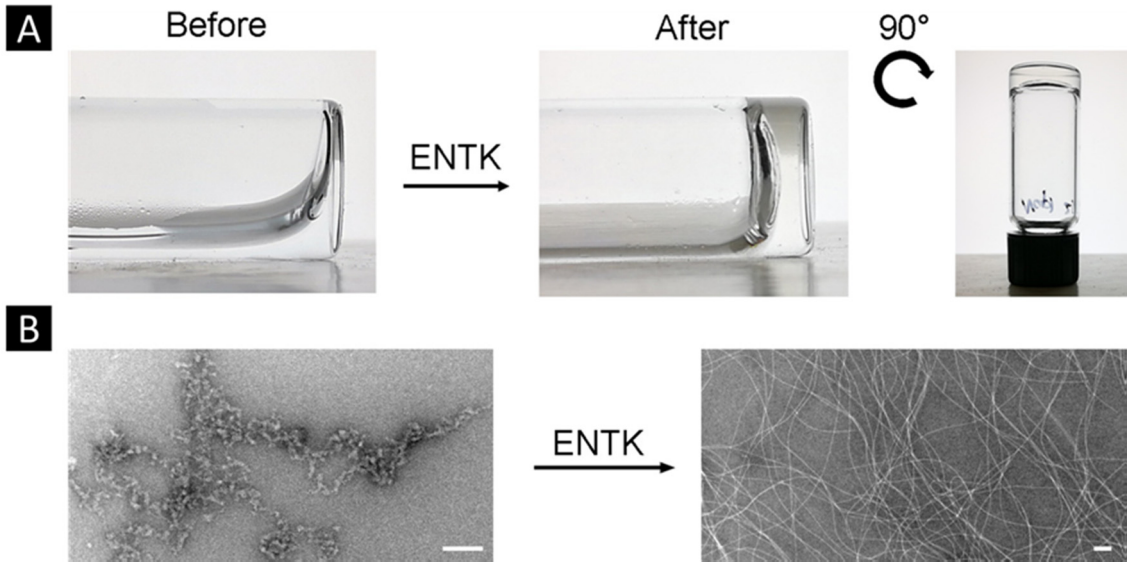


Fig. S3. (A) Optical (2.5 wt%) and (B) TEM (200 μM) image of the solution of D-**1₸FLAG** before (left) and after (right) adding ENTK (10U/ml, 24 h, ambient condition). scale bar = 100 nm.

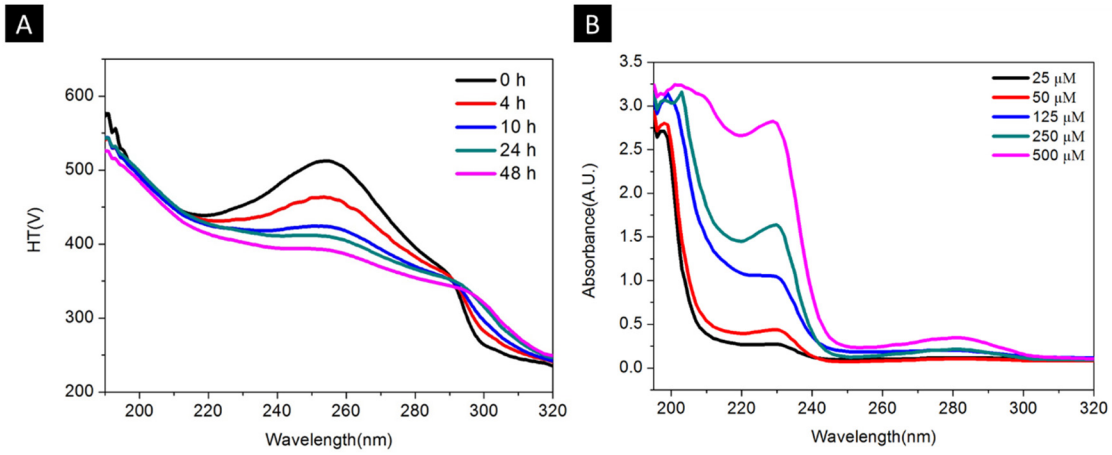


Fig. S4. (A) Time-dependent high tension (HT) data of L-**1₸FLAG** (500 μM) incubated with ENTK (10 U/mL), and (B) UV spectra of L-**1₸FLAG** in different concentrations. The maximum HT value and the agreement of HT curve and UV spectrum exclude the possibility of scattering artifacts.

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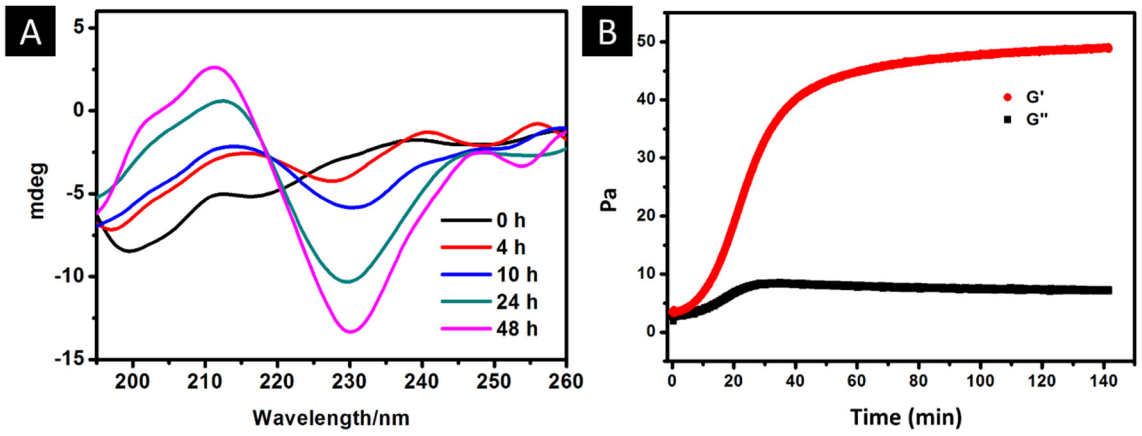


Fig. S5. CD spectra and time dependent dynamic storage moduli (G′) and loss moduli (G′′) of D-**1₸FLAG** (500 μM) after adding ENTK (10 U/mL) at 25 ºC.

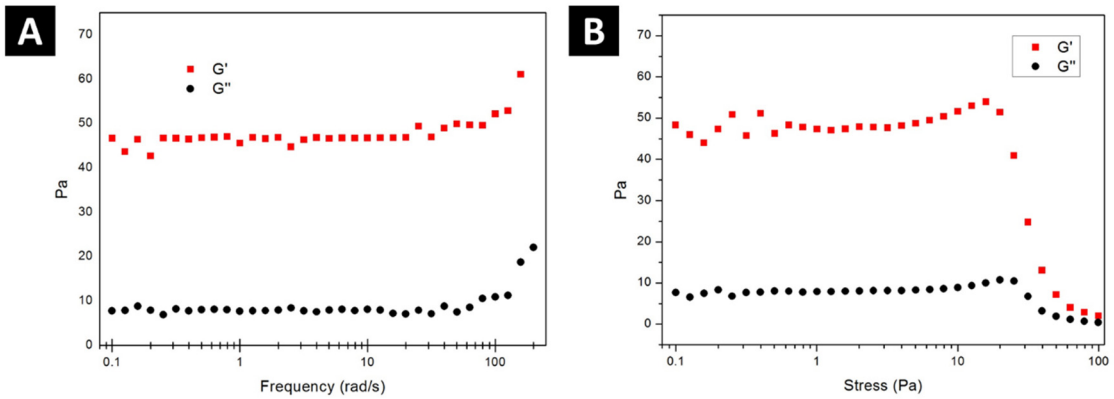


Fig. S6. Frequency sweeps and strain sweeps of D-**1₸FLAG** (500 μM) with ENTK (10U/ml) after time-dependent dynamic storage moduli and loss moduli measurement (2.5 h). These results confirm that the time sweeps were conducted under appropriate conditions.

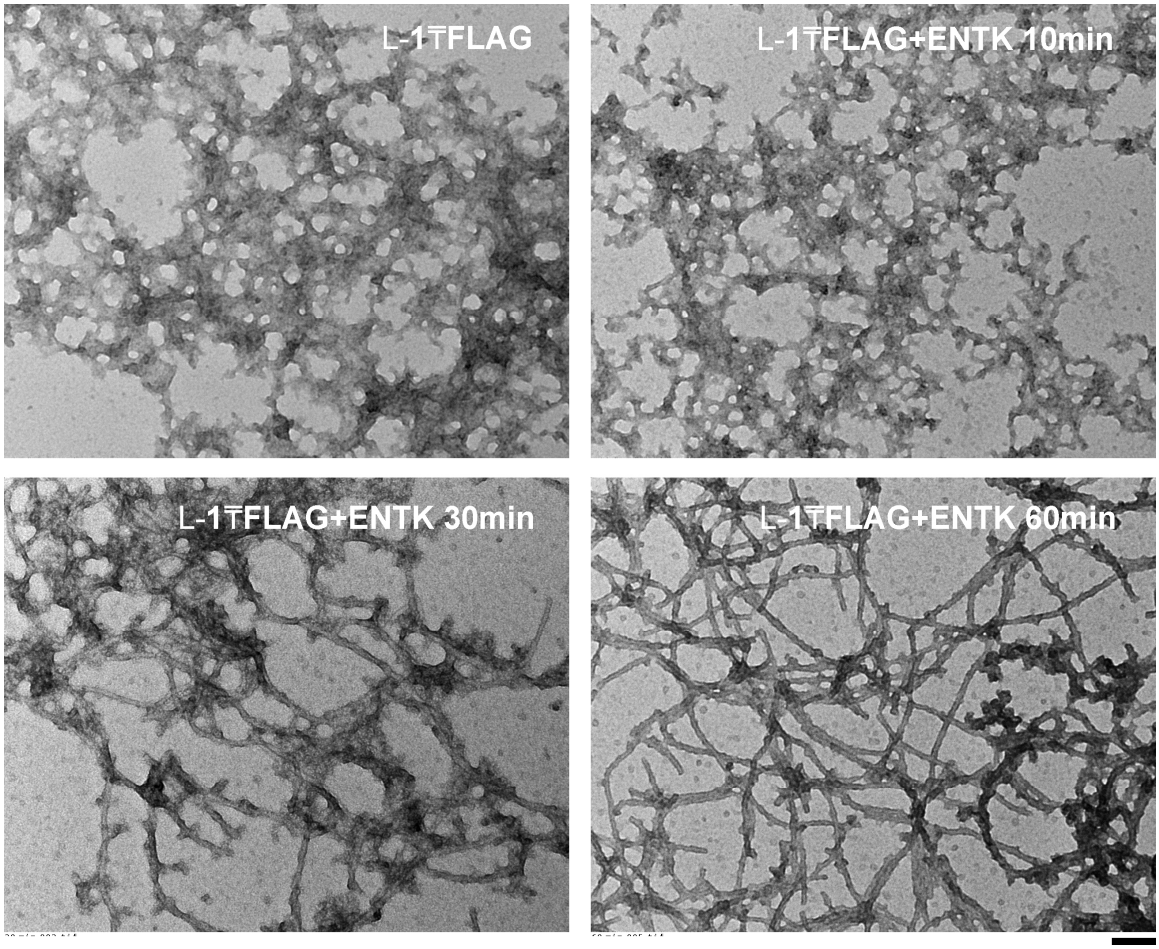


Fig. S7. Time-dependent TEM images of L-**1-FLAG** (500 μM) incubated with ENTK (10 U/ml). Scale bar=100 nm.

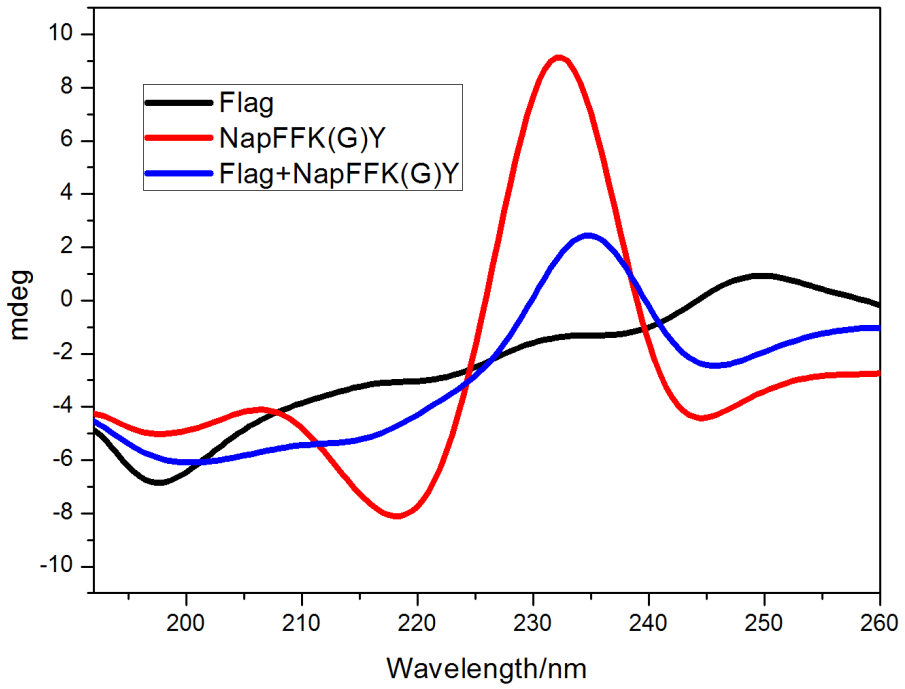


Fig. S8. CD spectra of L-**1** (250 μM), FLAG-tag (250 μM), and the mixture of L-**1** (250 μM) and FLAG-tag (250 μM). L-**1** has β-sheet like conformation while FLAG-tag has random coil.

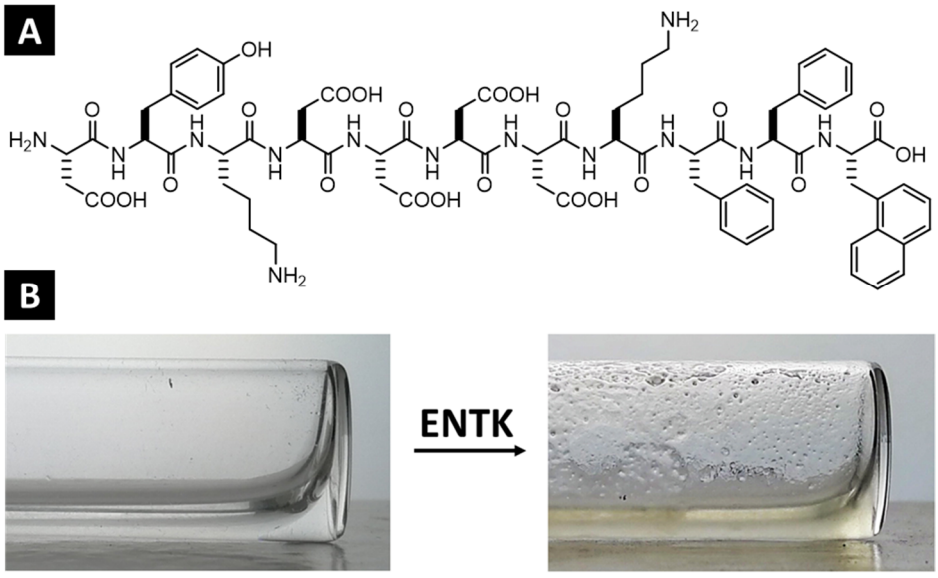
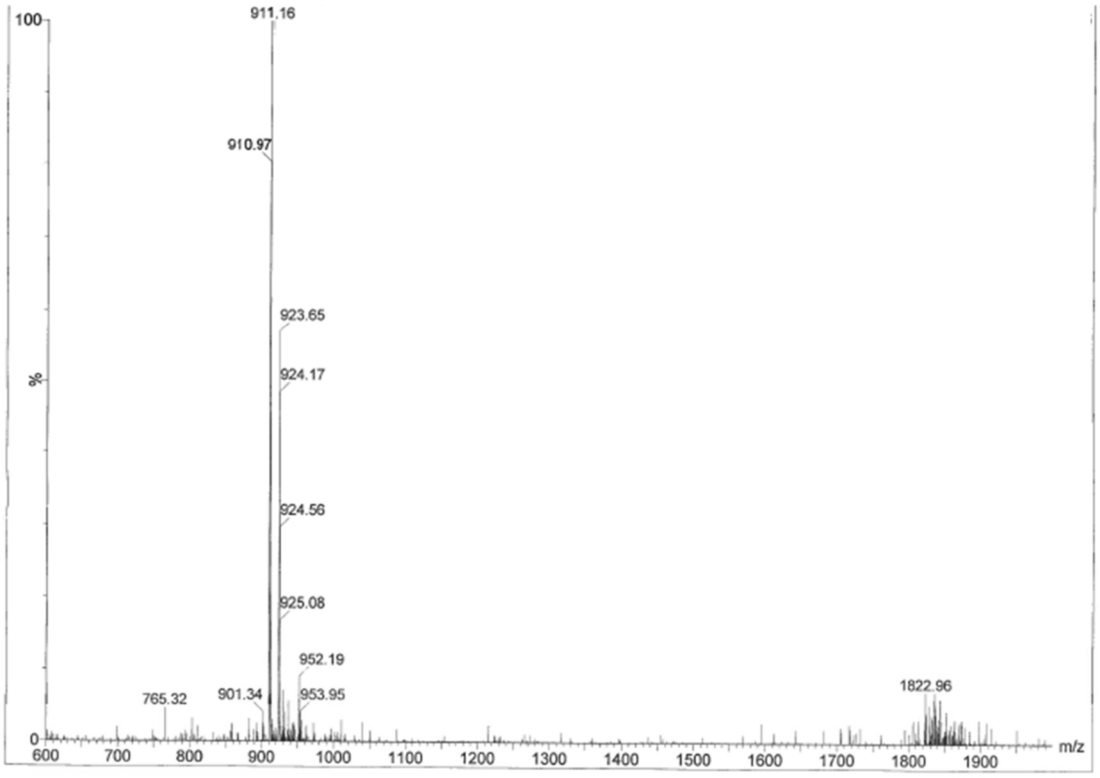


Fig. S9. (A) Molecular structure of linear control L**-1-FLAG**. (B) Optical images of the lineal control (2.5 wt%) before and after the addtion of ENTK (10U/ml, 24h, ambient condition).


100
%
0
911.16
910.97
923.65
924.17
924.56
925.08
952.19
953.95
901.34
765.32
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700
800
900
1000
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1200
1300
1400
1500
1600
1700
1800
1900
m/z

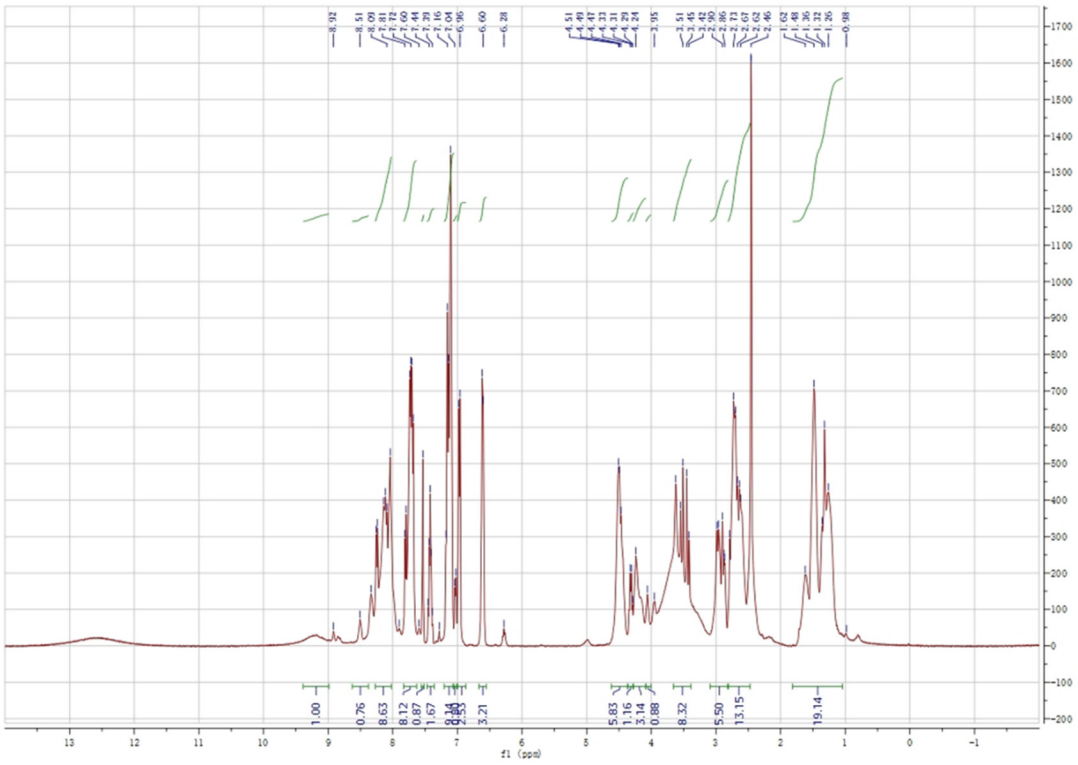
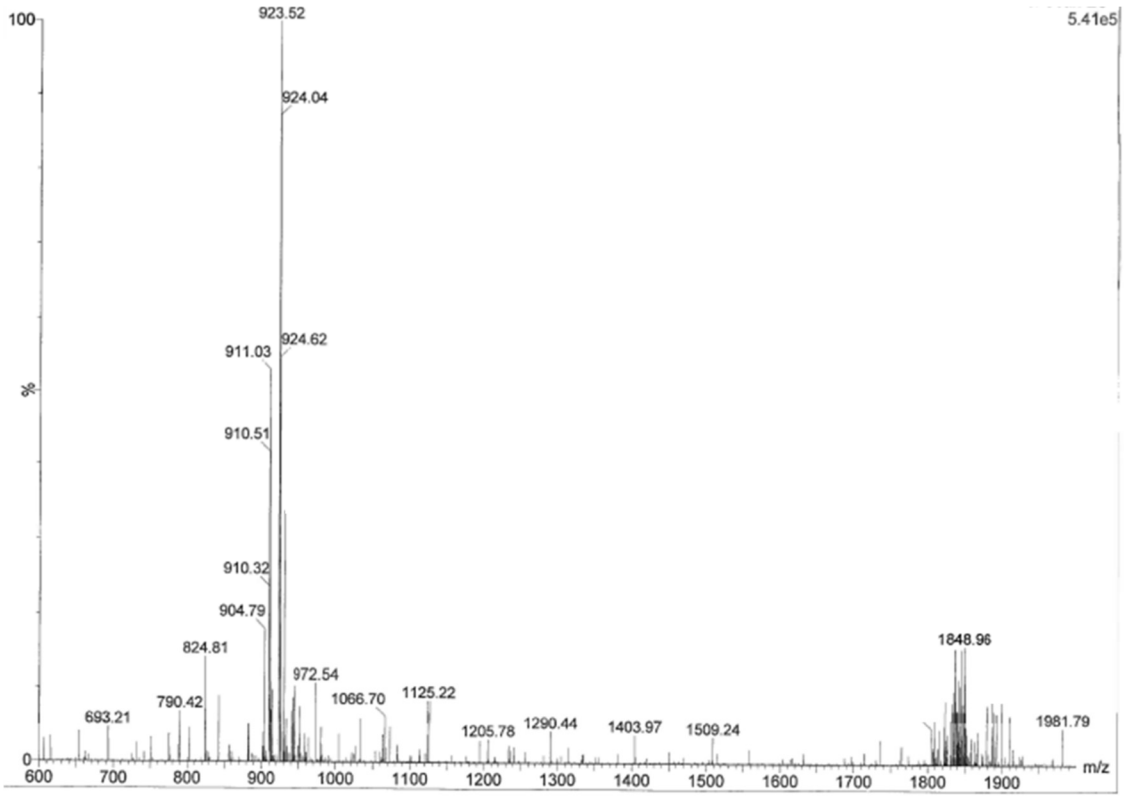

f1 (ppm)

Fig. S10. ^{1}H-NMR spectrum in DMSO-d_6 and LC-MS data of L-**1₸FLAG**. The calculated molecular weight (Mw) of L-**1₸FLAG** is 1822.77. The observed m/z (911.16 and 923.65) is the ½ Mw of L-**1₸FLAG** and its mono-sodium salt (Mw=1845.75). The observed m/z = ½ Mw is due to the ionization of two proton (z = -2).


100
%
0
923.52
924.04
924.62
911.03
910.51
910.32
904.79
824.81
972.54
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693.21
1066.70
1125.22
1205.78
1290.44
1403.97
1509.24
1848.96
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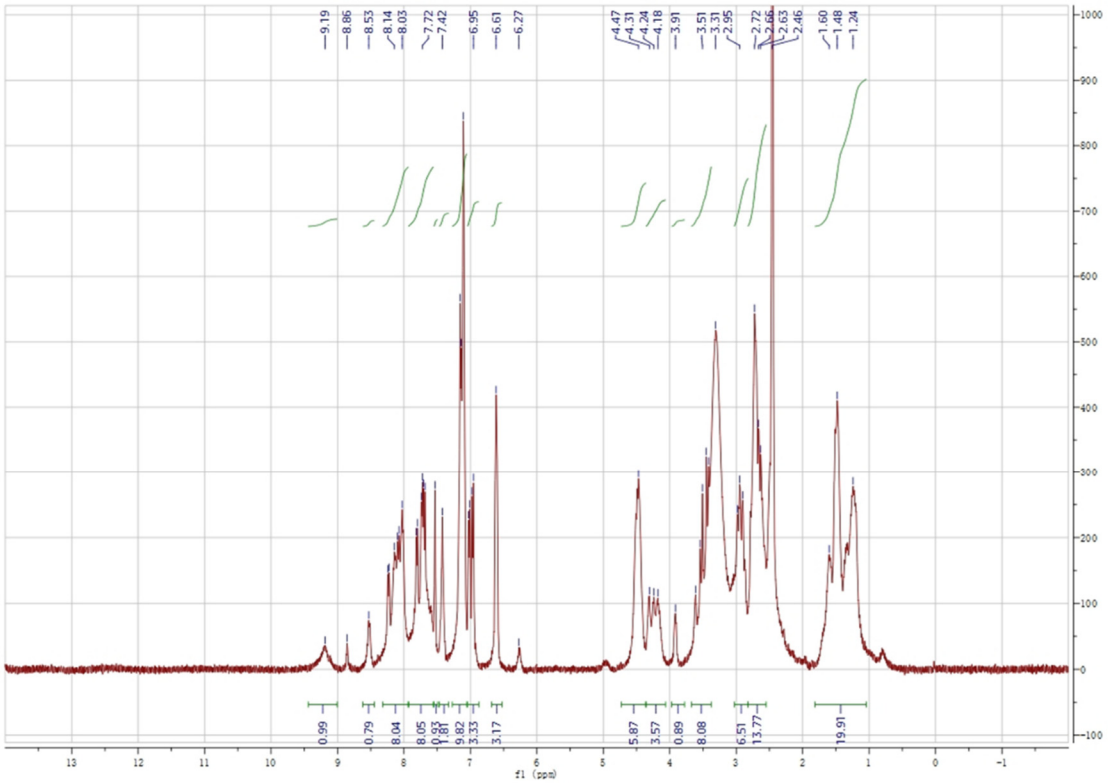
1300
1400
1500
1600
1700
1800
1900
m/z


9.19
8.86
8.53
8.14
8.03
7.72
7.42
6.95
6.61
6.27
4.47
4.31
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4.18
3.91
3.51
3.31
2.95
2.72
2.66
2.63
2.46
1.60
1.48
1.24
0.99
0.79
8.04
8.05
0.87
9.82
3.33
3.17
5.87
3.57
0.89
8.08
6.51
13.77
19.91
f1 (ppm)

Fig. S11. LC-MS data and ^{1}H-NMR spectrum in DMSO-d_6 of D-**1₸FLAG**. The calculated molecular weight (Mw) of D-**1₸FLAG** is 1822.77. The observed m/z (911.03 and 923.52) is the ½ Mw of D-**1₸FLAG** and its mono-sodium salt (Mw=1845.75). The observed m/z = ½ Mw is due to the ionization of two proton (z = -2).

Reference:

1. B. Chu, *Laser light scattering: basic principles and practice*, Courier Corporation, 2007.