Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information for

Multivalent Peptides Displayed on OEGMA-based Copolymers for the Modulation of Protein-Protein Interactions

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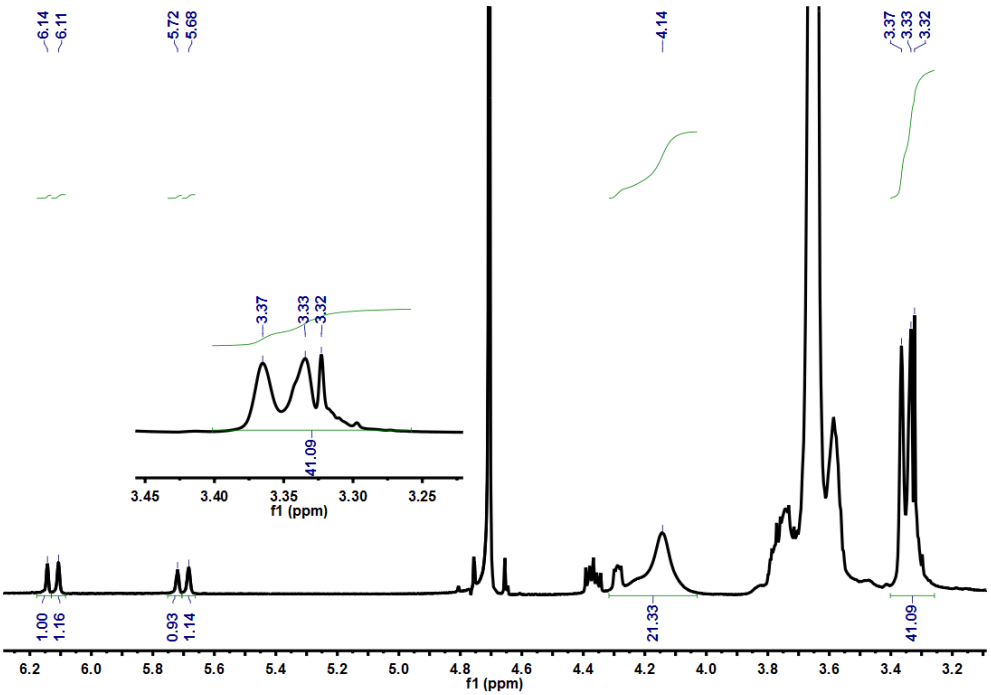
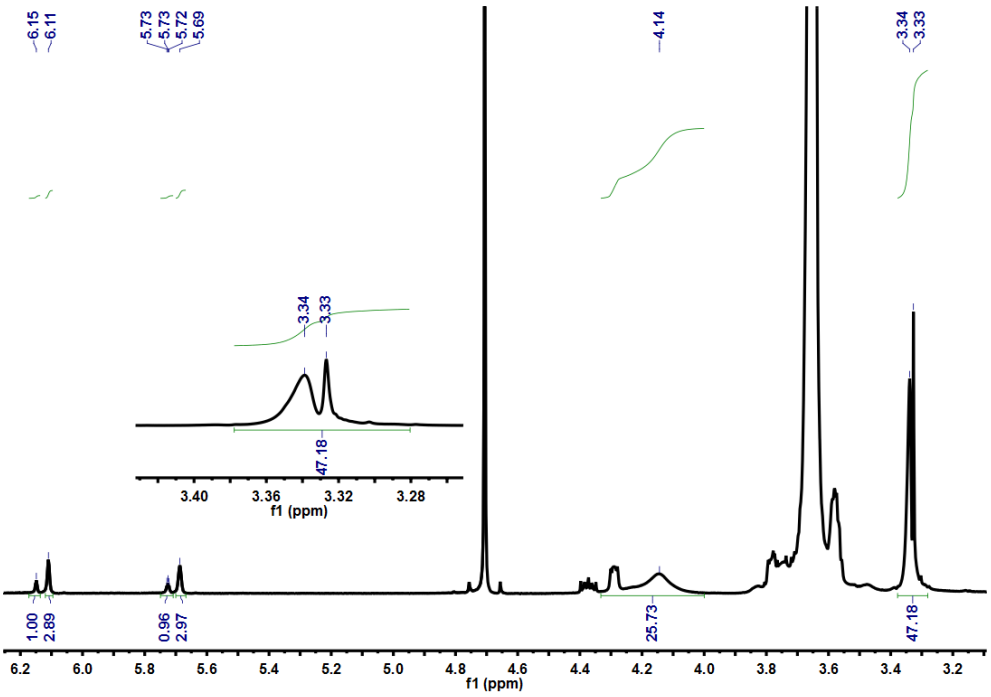
1. ^{1}H NMR of P1 (a), P2 (b), P1-SPDP-(20) (c), P1-SPDP-(17) (d) and P1-SPDP-(46) (e)

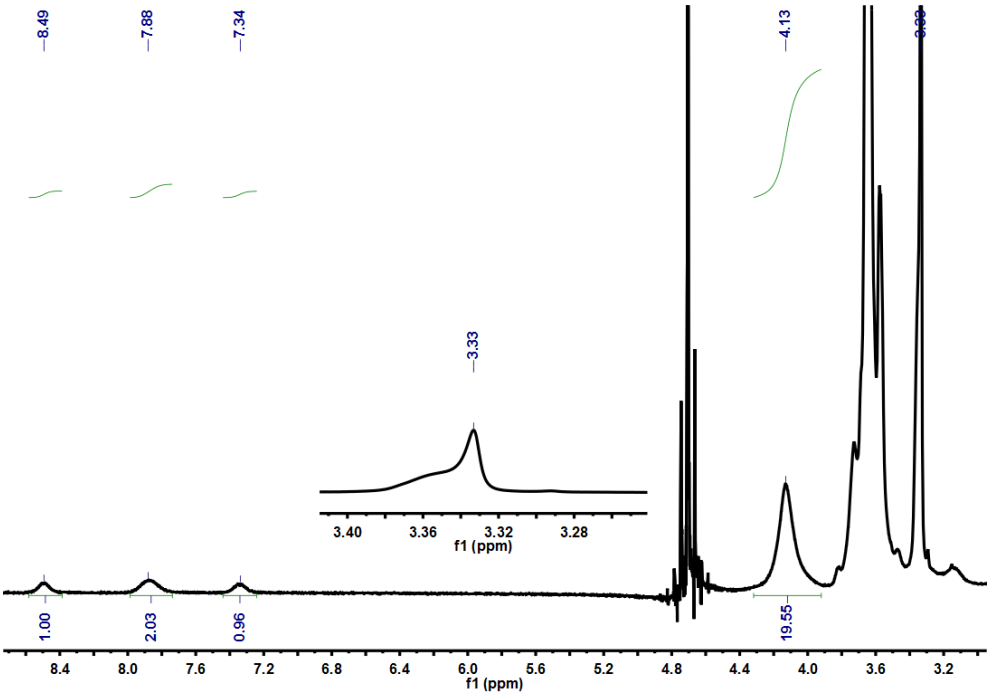
a) **P1** (before purification by dialysis)



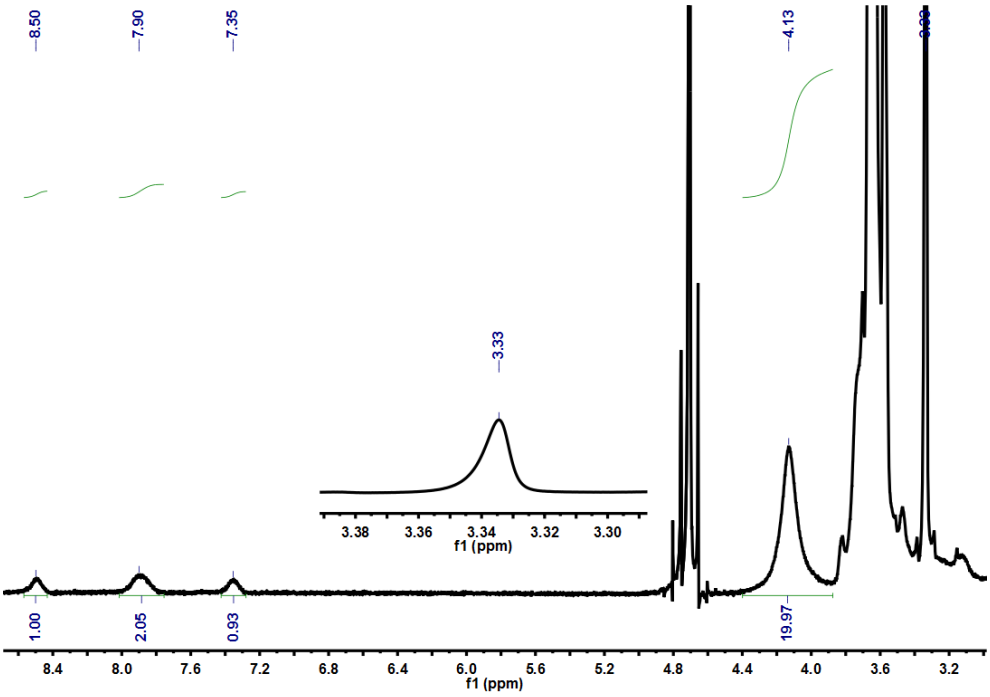
b) **P2** (before purification by dialysis)



c) **P1-SPDP-(20)** (after purification by dialysis, ~20 SPSP per polymer)



d) **P2-SPDP-(17)** (after purification by dialysis, ~17 SPSP per polymer)



e) **P1-SPDP-(48)** (after purification by dialysis, ~48 SPSP per polymer)

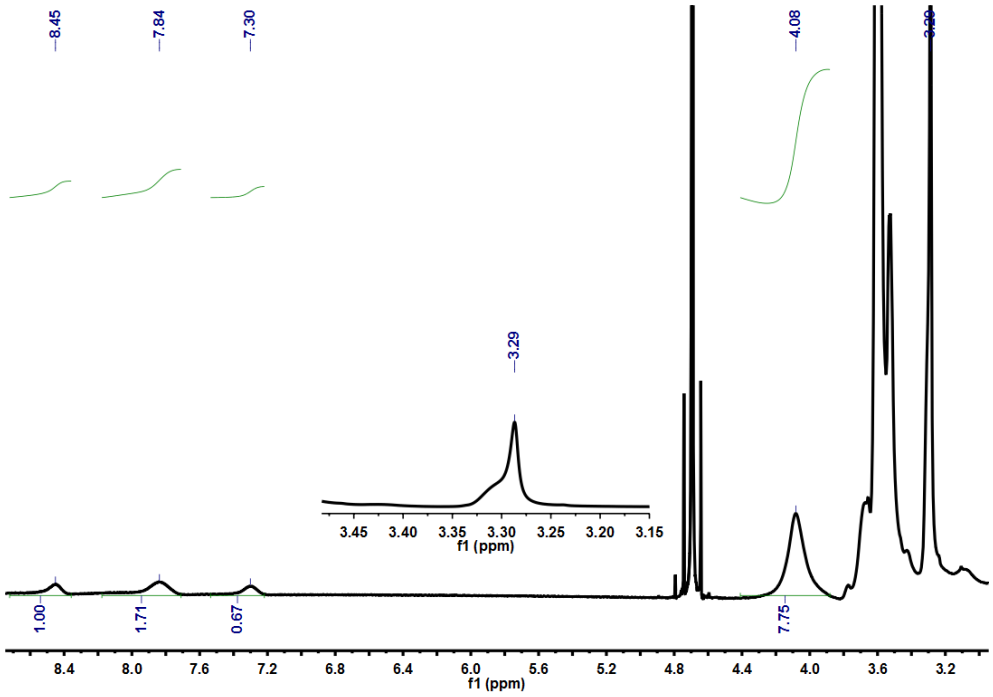
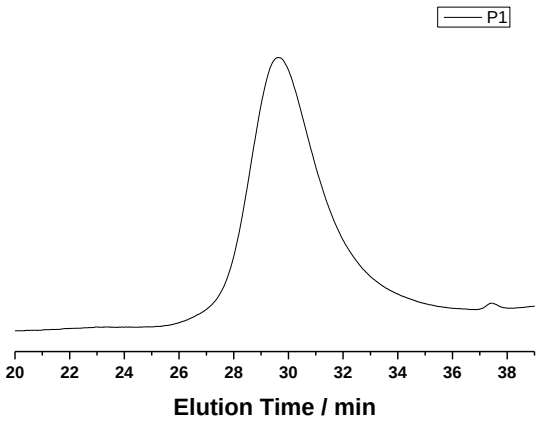


Figure S1. ^{1}H NMR of **P1** (a), **P2** (b), **P1-SPDP-(20)** (c), **P2-SPDP-(17)** (d), and **P1-SPDP-(48)** (e) dissolved in D_2O. The conjugation of SPDP was confirmed by the appearance of three peaks at 7.30 (1H, aromatic proton, ortho-disulfide linkage), 7.84 (2H, aromatic proton meta-N and para-N), and 8.45 (1H, aromatic proton ortho-N) ppm (c, d and e). The SPDP content can be determined by integrating the CH2OC(O) protons at 4.08 ppm in the oligo (ethylene glycol) unit and the aromatic proton (ortho-N) at 8.45 ppm in the pyridine unit.

2. GPC traces of the purified P1 and P2

The polydispersity of **P1** and **P2** were determined by aqueous GPC at 30 ℃ using a Shodex OHpak SB-804 HQ and an OHpak SB-803 HQ column connected in series to SHIMADZU RID-10A refractive index detector. The eluent was 0.2 M HAc-NaAc buffer (pH 7.4) at a flow rate of 0.5 mL/min. The polydispersity was determined with a calibration curve based on eight pullulan standards.

a)



b)

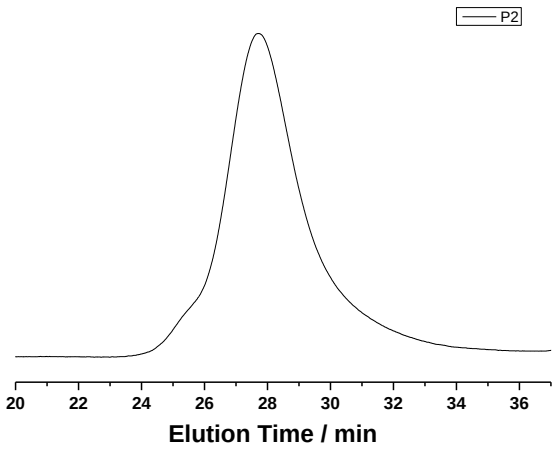


Figure S2. GPC traces of **P1** (a) and **P2** (b).

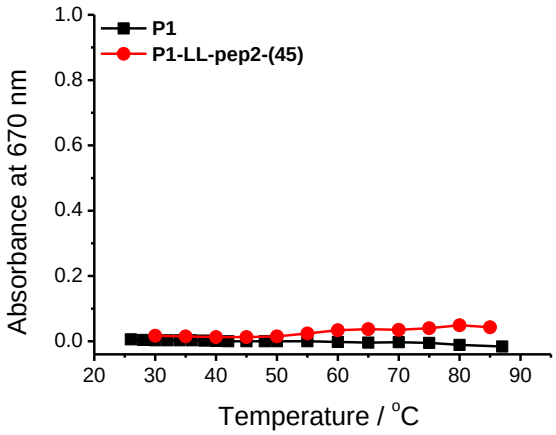


Figure S3. Absorbance (at 670 nm) as a function of temperature for **P1** (~1 wt%) and **P1-LL-pep2-(45)** (50 μM; peptide basis) dissolved in aqueous solution.

3. Table S1. Composition, molecular weight and PDI of copolymers **P1** and **P2**

Polymer	Monomer ratio[a]	Monomer/Initiator[b]	Conv. / % (^{1}H NMR)	M_n (^{1}H NMR)	M_n (GPC)[c]	M_w/M_n (GPC)[c]
P1	4/5/5	280/1	92%	72503	18401	1.45
P2	4/0/10	280/1	82 %	90890	42616	1.30

[a] ratio of monomer AEMA, OEG^{2}MA and OEG^{9}MA in mol/mol; [b] ratio of monomer to ATRP initiator for the synthesis of copolymers; [c] polydispersity index (PDI) determined by aqueous GPC using pullulan standards.

4. Quantification of the peptide incorporated into the copolymer-peptide conjugates

The number of peptide conjugated to one polymeric chain was determined using the following formula:

$$N_{\mathrm{pep/chain}} = \frac{n_{\mathrm{pep}}}{n_{\mathrm{polymer}}} \div 98\% = \frac{n_{\mathrm{pep}}}{n_{\mathrm{thiol}}/N_{\mathrm{thiol/chain}}} \div 98\% = \frac{C_{\mathrm{pep}} \cdot V_{\mathrm{pep}}}{n_{\mathrm{thiol}}/N_{\mathrm{thiol/chain}}} \div 98\% \quad (2)$$

$N_{\mathrm{pep/chain}}$ is the number of peptide in copolymer-peptide conjugates;

n_{pep} is moles of peptide in copolymer-peptide conjugates after purification by ultrafiltration;

n_{polymer} is moles of copolymer used for the conjugation reaction, which is equal to $n_{\mathrm{thiol}}/N_{\mathrm{thiol/chain}}$;

98% is the percent recovery of ultrafiltration for the purification of copolymer-peptide conjugates;

n_{thiol} is moles of thiol group (or SPDP in polymer) in the conjugation reaction;

$N_{\mathrm{thiol/chain}}$ is the number of the SPDP incorporated into the copolymer determined by ^{1}H NMR;

C_{pep} is the molar concentration of peptide in the solution measured by absorbance at 280 nm;

V_{pep} is the volume of the copolymer-peptide conjugate solution.

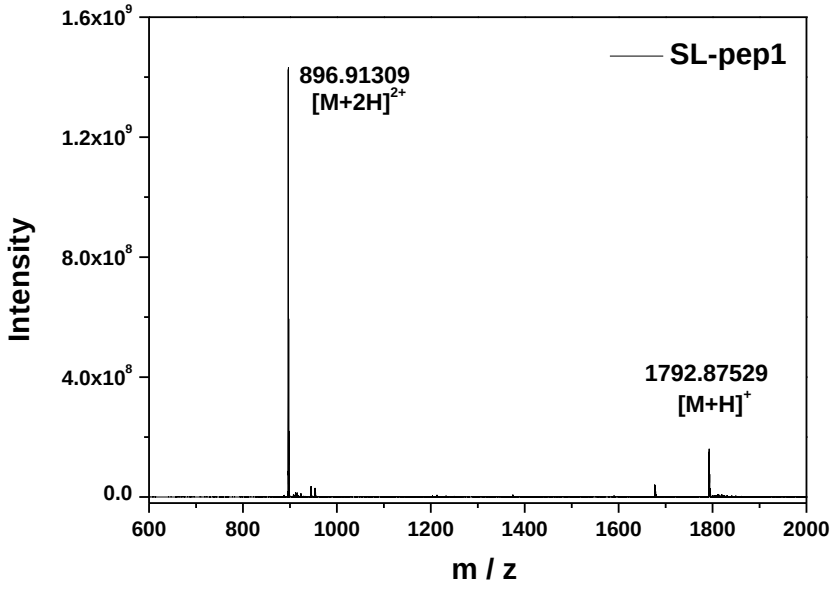
5. **Table S2.** Composition of the obtained copolymer-peptide conjugates, including the number of amine groups before and after the conjugation of SPDP, the number of free thiol groups after the conjugation of peptides, and the number of the conjugated peptides per polymeric chain.

Copolymer-Peptide Conjugate	Number of $-NH_2$ Per Chain[a]	Number of Free $-NH_2$ Per Chain[b]	Number of Free $-SH$ Per Chain[c]	Number of Peptide Per Chain[d]
P1-SL-pep1-(1)	73	53	19	1
P1-ML-pep1-(3)	73	53	17	3
P1-LL-pep1-(2)	73	53	18	2
P1-LL-pep1-(14)	73	53	6	14
P1-LL-pep1-(35)	73	25	13	35
P2-LL-pep1-(3)	66	49	14	3
P2-LL-pep1-(15)	66	49	2	15
P1-LL-pep2-(1)	73	53	19	1
P1-LL-pep2-(5)	73	53	15	5
P1-LL-pep2-(9)	73	53	11	9
P1-LL-pep2-(12)	73	53	8	12
P1-LL-pep2-(18)	73	53	2	18
P1-LL-pep2-(33)	73	25	15	33
P1-LL-pep2-(45)	73	25	3	45

[a] the number of amine groups on each copolymer chain before SPDS-conjugation quantified by ^{1}H NMR; [b] the number of amine groups remaining on each copolymer chain after SPDS-conjugation (the number of side-chain SPDP on each copolymer chain was determined by ^{1}H NMR); [c] the number of free thiols remaining on each copolymer-peptide conjugates after peptide-conjugation. Of note, the residual thiols in copolymers can be partially oxidized to disulfides after the purification. However, we found that the presence of dithiothreitol in buffers, which was used to reduce the possibly formed disulfide bonds, does not obviously affect the dissociation constants. [d] the number of peptide conjugated to each copolymer chain calculated using eq. 2.

6. MS characterization of maleimide-bearing pep1 and pep2

a)



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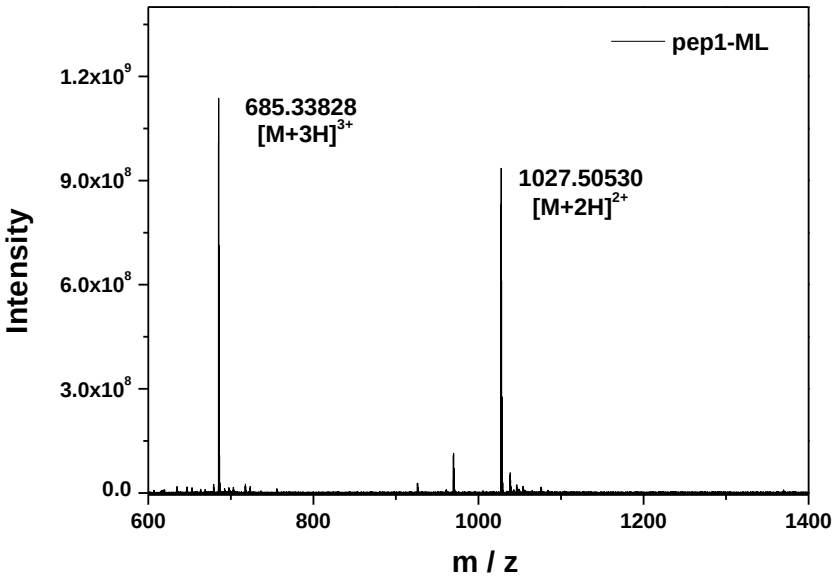
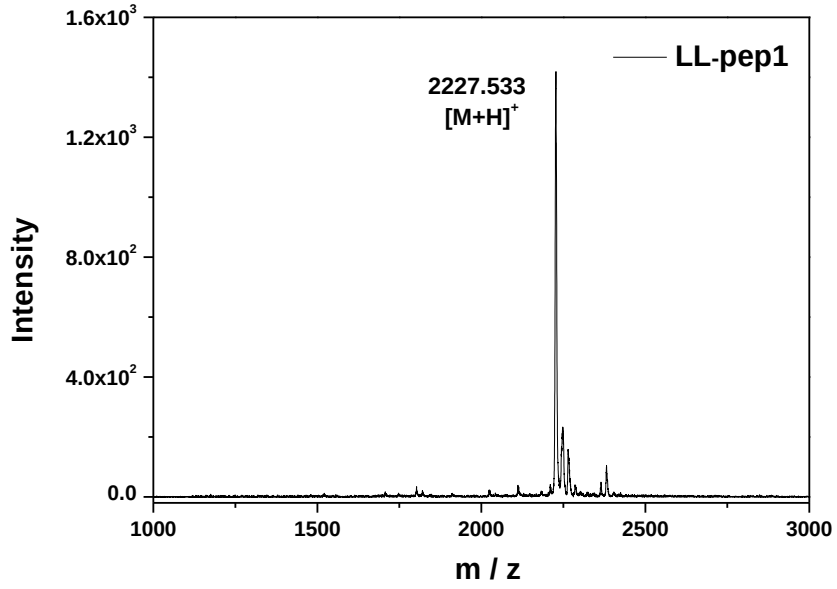


c)



d)

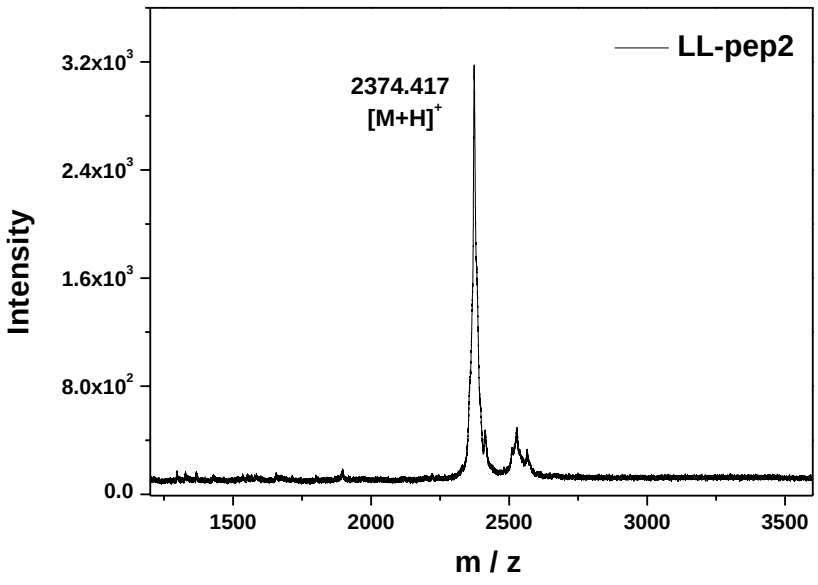


Figure S4. MS spectra of the peptide-crosslinker conjugates; a): **SL-pep1**, b): **ML-pep1**, c): **LL-pep1**, and d): **LL-pep2**.

7. Fluorescence polarization competition assay

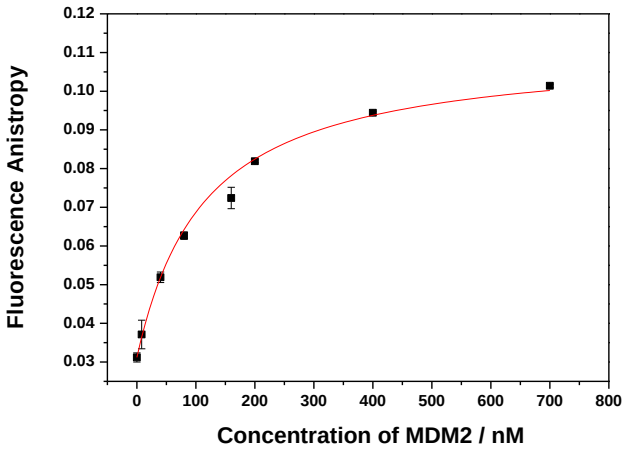


Figure S5. A representative titration curve for the binding of FITC-**pep1** to Mdm2; the concentration of FITC-**pep1** is 8 nm; the experiment was performed at room temperature in 1×PBS (10 mM, pH 7.4).

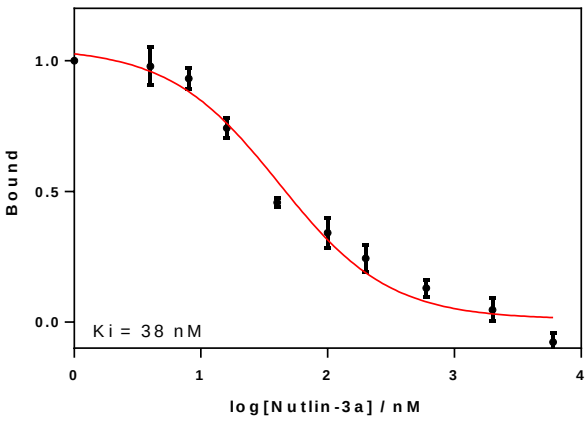


Figure S6. Binding affinity of Nutlin 3a to Mdm2 (K_i = 38 nM) measured by fluorescence polarization competition assay at room temperature in 1×PBS (10 mM, pH 7.4). The solid line is a curve obtained from nonlinear regression analysis using GraphPad Prism.

8. Isothermal Titration Calorimetry (ITC)

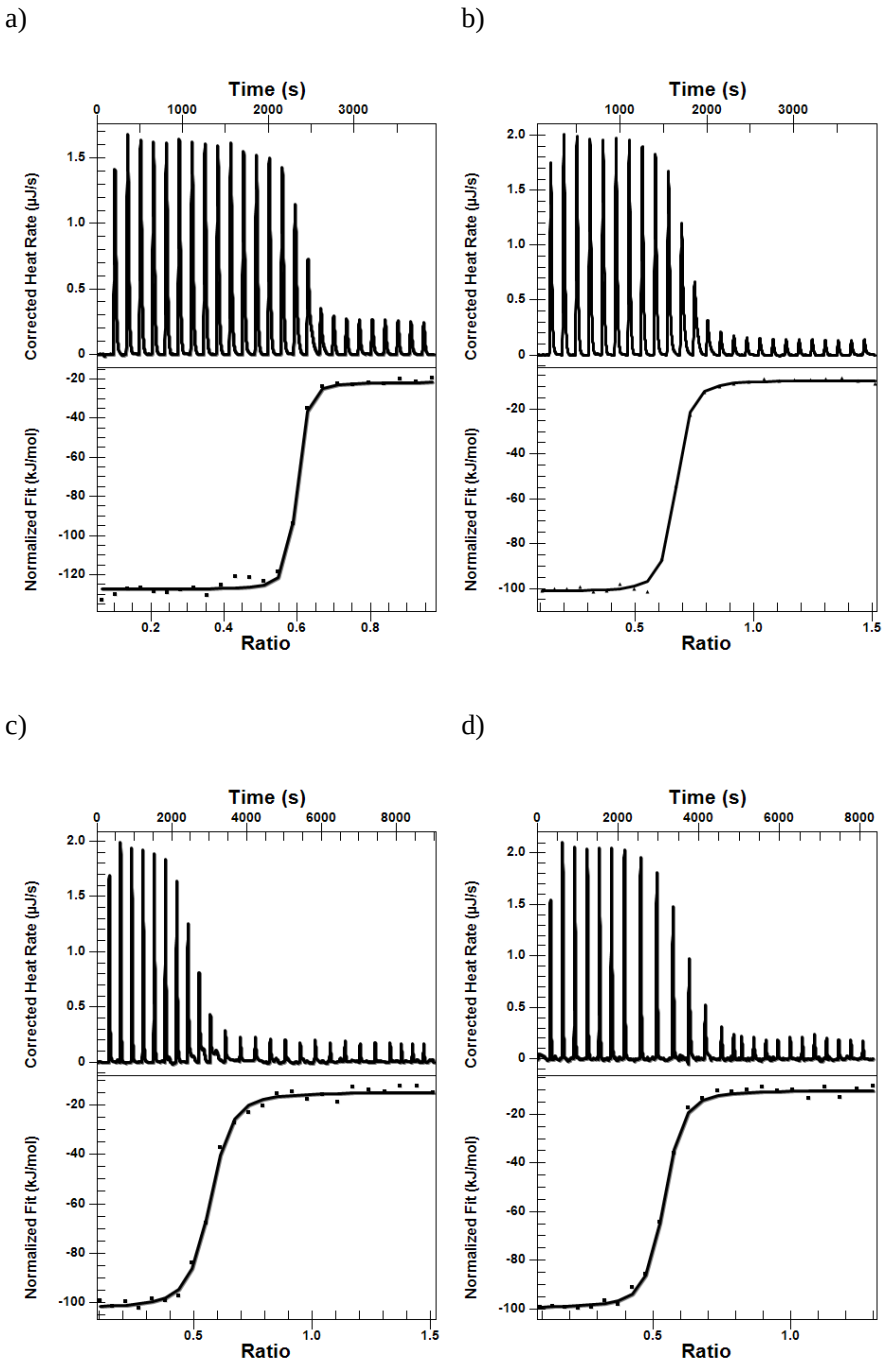


Figure S7. Isothermal titration calorimetry profiles. a) Titration of 0.15 mM **pep1** into 0.052 mM Mdm2; b) titration of 0.117 mM **P1-LL-pep1-(2)** into 0.052 mM Mdm2; c) titration of 16.7 µM **P1-LL-pep1-(14)** into 0.052 mM Mdm2; d) titration of 6.69 µM **P1-LL-pep1-(35)** into 0.052 mM Mdm2.

9. CD spectral characterization of pep1 and copolymer-pep1 conjugates

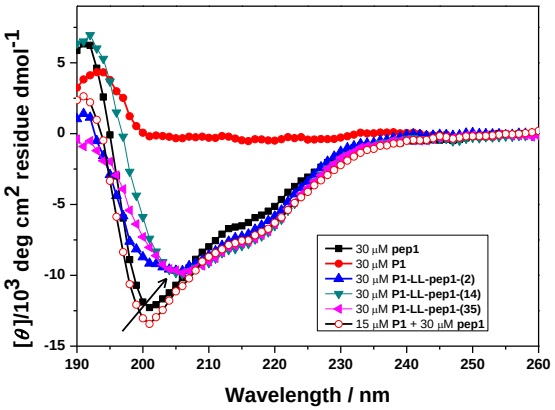


Figure S8. CD spectra of **pep1** (black), **P1-LL-pep1-(2)** (blue), **P1-LL-pep1-(14)** (dark cyan), **P1-LL-pep1-(35)** (magenta), mixture of **pep1** and **P1** (black line with red circle), and **P1** (red) recorded in 1×PBS (10 mM, pH 7.4) at room temperature. The final concentration of **pep1** in each solution is 30 μM.

10. Gel filtration chromatograms of P1-LL-pep2-(n) (n=1, 5, 9, 12, 18, 45) and their complexes with Mdm2

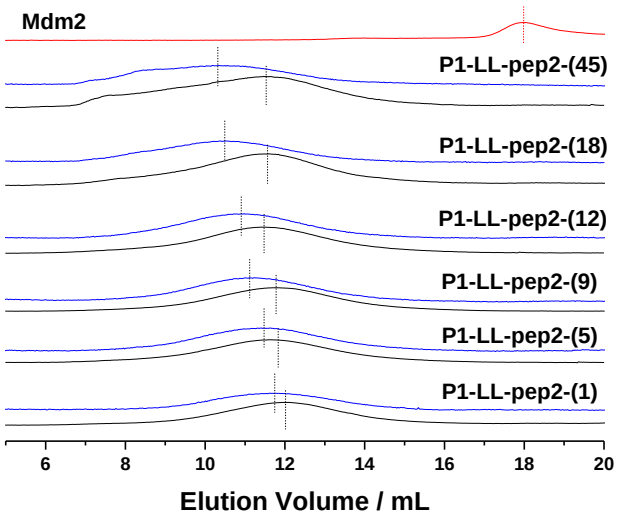
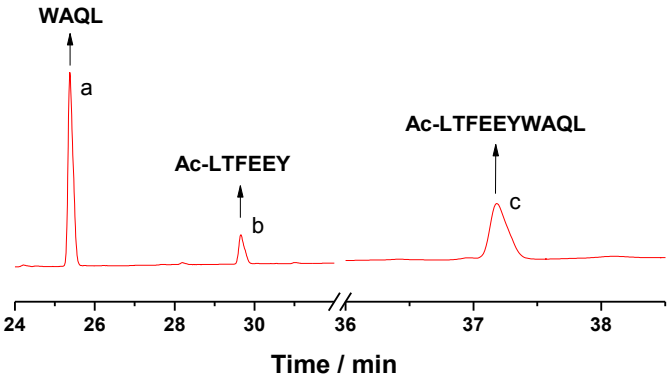
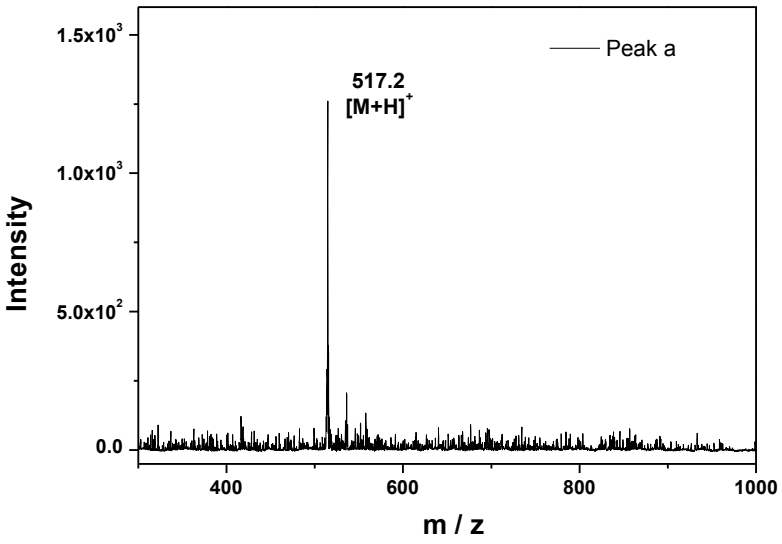
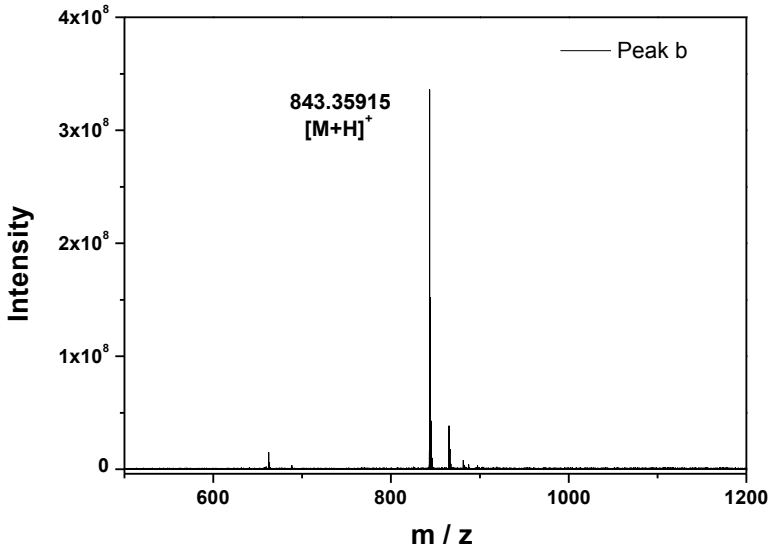


Figure S9. Gel filtration chromatograms show the elution of **P1-LL-pep2-(n)** (n = 1, 5, 9, 12, 18 and 45) before (black line) and after (blue line) incubation with Mdm2 (molar feed ratio of **pep1**/Mdm2 = 1:1) on Superdex-200 column. Samples were eluted by 1×PBS, pH 7.4, at a rate of 0.45 mL/min at 4 ℃.

11. HPLC and MS analysis of the digestion of pep2 by chymotrypsin







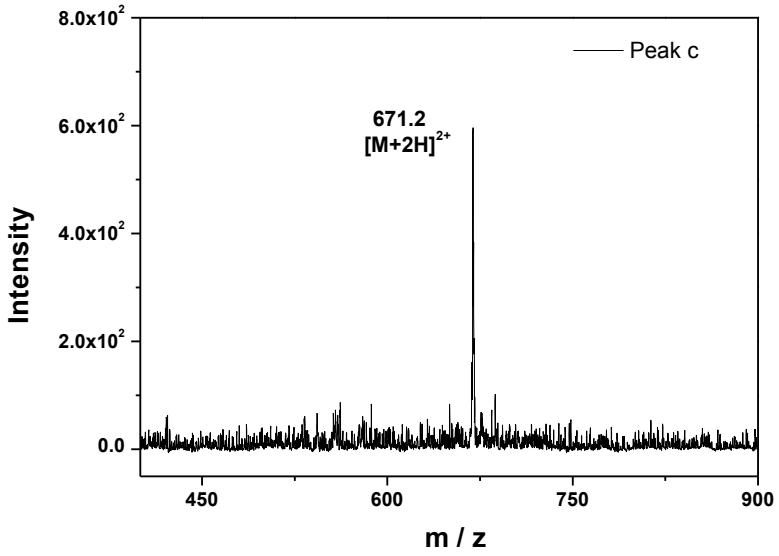


Figure S10. HPLC and mass spectrometry analysis of **pep2** fragments formed in chymotrypsin digestion solution. **Pep2** was digested with chymotrypsin and purified by HPLC. Then, the isolated peptide fragments were analyzed by mass spectrometry. From top to bottom: chromatogram of digested sample in UV channel (280 nm), mass spectra of peaks a, b and c labeled in the chromatogram.

Assignment of the isolated peptide fragments:

Peak No.	**Sequence**	**m/z $(M+H)^+$ expected**	**m/z $(M+H)^+$ found**
a	WAQL	516.61	517.2
b	Ac-LTFEEY	842.8	843.4
c	Ac-LTFEEYWAQL	1341.41	671.3 $(M+2H)^{2+}$

Peptide-fragment analysis by MS identifying the sites of chymotrypsin proteolysis:

Site-2 Site-1

Ac-LTFEEY ↓ WAQL ↓ TSAAK-NH_2

12. Fluorescence imaging of cells incubated with fluorescein-labeled P1-LL-pep2-(45)

a) b) c)

Figure S11. Fluorescence imaging of MCF7 cells incubated with fluorescein-labeled **P1-LL-pep2-(45)**; a) bright field, b) fluorescein channel, c) LysoTracker red DND-99 channel for lysosome tracking.

Protocol: 1) fluorescein-labeled **P1-LL-pep2-(45)** was prepared through the conjugation of the free amino groups of **P1-LL-pep2-(45)** with FITC in DMSO/200 mM phosphate buffer (*v*/*v* = 10/1), and was purified by repeated centrifugal ultrafiltrations. It was estimated that ~9 fluorescein molecules can be successfully conjugated onto each copolymer chain (determined by UV-Vis absorption). 2) fluorescein-labeled **P1-LL-pep2-(45)** at an overall peptide concentration of 1.0 μM was incubated with MCF7 cells for 20 h.