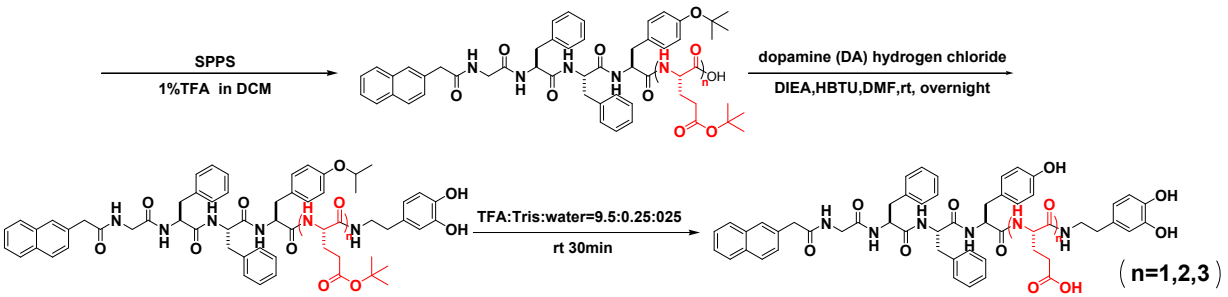
Supporting Information

Materials and methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai). HBTU and tris(2-carboxyethyl) phosphine Hydrochloride (TCEP) were purchased from Aladdin Chemistry CO. Ltd. Bortezomib (BTZ) were obtained from School of pharmaceutical and life sciences of Changzhou University. 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Invitrogen (Grand Island, NY). Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 400). ESI-MS and MALDI-TOF-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System and AutoflexIII LRF200-CID System, respectively. 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. TEM images were done on a Tecnai G2 F20 system, operating at 200 kV. Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500μm. Bio-RAD iMarkTM Microplate Reader (Bio-Rad, America) was used in the MTT assay.

Syntheses and characterization



Scheme S-1. Synthesis of compounds ***NapGFFYE$_n$-Cat (n=1, 2, 3)***

Peptide systhesis: The peptide derivative was synthesized by solid phase peptide synthesis (SPPS) method using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by different group. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 1.2 mmol/g. 20% piperidine in anhydrous N, N'-dimethylformamide (DMF) was used during the 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, 2-naphthylacetic acid 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 dichloromethane (DCM) for 2 min (5 mL per gram of resin). The peptide derivatives were cleaved using 1%TFA in DCM for 10 times (1 min/time, 5 mL per gram of resin). The solution was stirred at room temperature for 30min and the solvent was evaporated under vacuum. 20mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was filtrated and washed by ice-cold diethylether. Then the supernatant was decanted and the resulting solid was directly used in the next synthesis steps without further purification.

Synthesis of peptide-Cat: The mixture of peptide (1 mmol), HBTU (1mmol), DIEA (2 mmol) and dopamine (Cat) hydrogen chloride (1.2 mmol) in 1ml DMF was stirred at room temperature overnight. Then 95% of trifluoroacetic acid with 2.5% of TMS and 2.5% of H_2O was added to the mixture and stirred at room temperature for 30 minutes. The solution evaporated under vacuum about 2ml and 20ml ether was added. The filtrate was added to ice water and the precipitate was obtained by a further filter. The crude product was purified by HPLC. After lyophilization of purfied Nap-GFFY(E)n-Cat, residual trifluoroacetic acid counterion were exchanged by sublimation from HCl (10mM) in order to improve biocompatibility. Nap-GFFY(E)n-Cat were then resolubilized in deionized water, lyophilized, and stored at −20°C until used.

Formation of nanospheres and hydrogels:

Hydrogel of Nap-GFFY(E)$_n$-Cat: 1.25mg of Nap-GFFY(E)$_n$-Cat and n equiv. of Na_2CO_3 were firstly suspended in 0.25 mL phosphate buffer saline (PBS, pH = 7.4, n=1,2,3), and then the suspensions were heated to form clear solutions. The resulting clear solution was cooled down to room temperature to form the hydrogel or not.

Hydrogel of Nap-GFFY(E)$_n$-Cat-BTZ: 1.25 mg of Nap-GFFY(E)$_n$-Cat and n equiv. of Na_2CO_3 were firstly suspended in 0.25ml phosphate buffer saline (PBS, pH = 7.4 ,n=2,3), and then the suspensions were heated to form clear solutions. After this step, 1 equiv. of bortezomib was added to the solution and ultrasonic to promote the conjunction of peptide and bortezomib.

However, when n=1 and 3, there were no hydrogels formed in PBS buffer solution as shown in Fig.S-1.

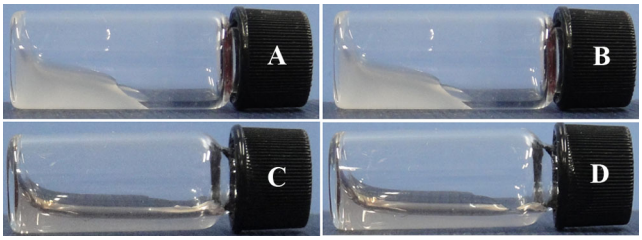


Fig.S-1. Optical images of (A) the suspension formed from 0.5% ***Nap-GFFYE-Cat*** in PBS solution (B) the suspension formed from 0.5% ***Nap-GFFYE-Cat*** with 1 equiv. of ***BTZ*** in PBS solution (C) the clear solution formed from 0.5% ***Nap-GFFYEEE-Cat*** in PBS solution (D) the clear solution formed from 0.5% ***Nap-GFFYEEE-Cat*** with 1 equiv. of ***BTZ*** in PBS solution.

Compound Nap-GFFYE-Cat: ^{1}H NMR (400 MHz, d^{6}-DMSO) δ 9.16 (s, 1H), 8.70 (s, 1H), 8.62 (s, 1H), 8.22 (s, 1H), 8.12 (dd, J = 16.5, 5.3 Hz, 3H), 8.00 (s, 2H), 7.88 – 7.79 (m, 3H), 7.74 (s, 1H), 7.67 (s, 1H), 7.52 – 7.36 (m, 4H), 7.17 (d, J = 17.4 Hz, 9H), 7.04 (d, J = 8.4 Hz, 2H), 6.62 (dd, J = 22.1, 9.5 Hz, 4H), 6.42 (d, J = 10.7 Hz, 2H), 4.49 (t, J = 12.7 Hz, 4H), 4.25 – 4.16 (m, 2H), 3.72 – 3.55 (m, 6H), 3.21 – 3.10 (m, 3H), 2.94 (dd, J = 30.6, 12.1 Hz, 4H), 2.76 (dd, J = 17.9, 4.2 Hz, 3H), 2.23 – 2.16 (m, 2H). HR-MS: calc. M = 964.40, obsvd. $(M+H)^{-}$ =965.4096.

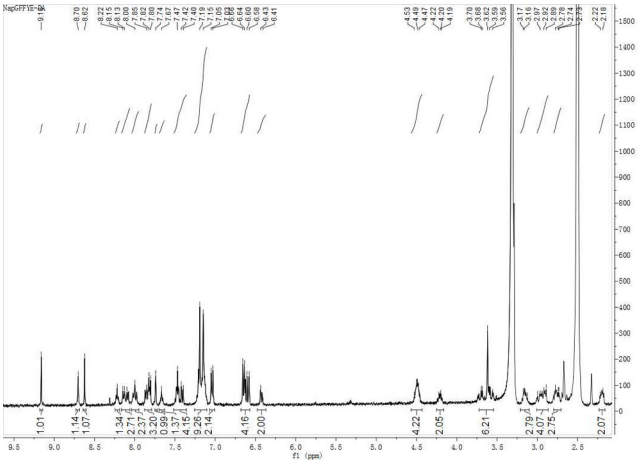


Fig. S-2. ^{1}H NMR of Compound ***NapGFFYE-Cat***

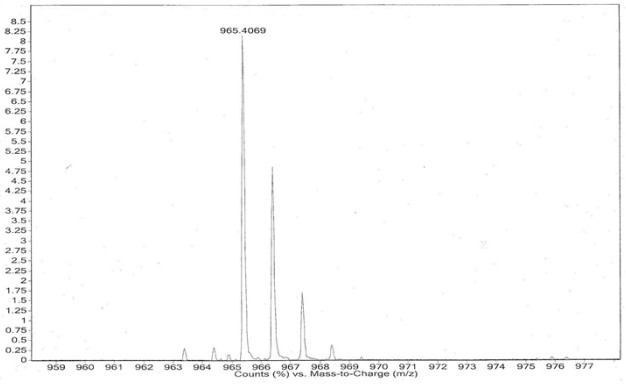


Fig. S-3. HR-MS of Compound ***NapGFFYE-Cat***

Compound Nap-GFFYEE-Cat: ^{1}H NMR (400 MHz, D_2O): δ 7.87 – 7.72 (m, 5H), 7.45 (d, J = 13.1 Hz, 3H), 7.36 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 12.1 Hz, 8H), 6.99 (s, 5H), 6.91 (s, 3H), 6.79 – 6.63 (m, 5H), 6.58 (d, J = 8.1 Hz, 1H), 4.39 (s, 4H), 4.21 – 3.98 (m, 5H), 3.76 (d, J = 16.8 Hz, 6H), 3.57 (s, 1H), 3.37 – 3.28 (m, 2H), 2.93 – 2.82 (m, 3H), 2.74 (d, J = 9.5 Hz, 4H), 2.61 (dd, J = 13.1, 7.0 Hz, 4H). HR-MS: calc. M = 1094.4503, obsvd. $(M+H)^-$ = 1095.4530.

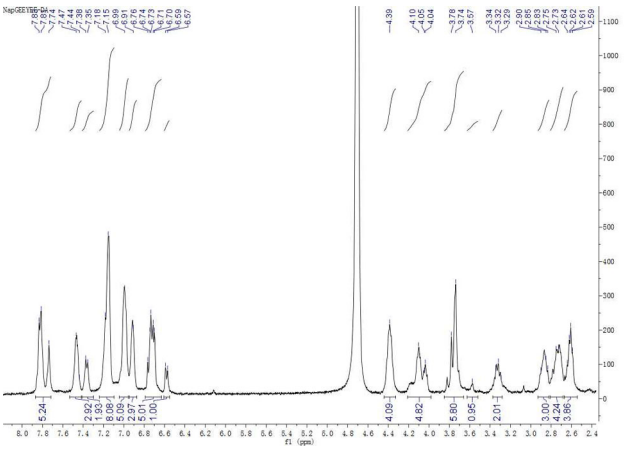


Fig. S-4. ^{1}H NMR of Compound ***NapGFFYEE-Cat***

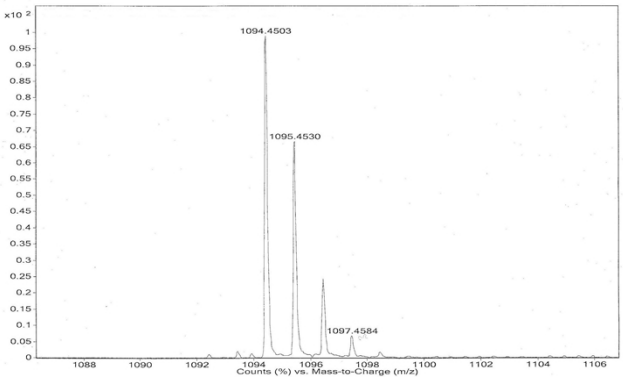


Fig. S-5. HR-MS of Compound ***NapGFFYEE-Cat***

Compound Nap-GFFYEEE-Cat: ^{1}H NMR (400 MHz, d^6-DMSO): δ 9.16 (s, 1H), 8.75 (s, 1H), 8.66 (s, 1H), 8.27 – 8.17 (m, 2H), 8.13 (d, J = 7.4 Hz, 2H), 8.08 – 7.98 (m, 3H), 7.95 (d, J = 4.5 Hz, 1H), 7.89 – 7.84 (m, 1H), 7.84 – 7.79 (m, 2H), 7.74 (s, 1H), 7.51 – 7.45 (m, 2H), 7.41 (dd, J = 8.4, 1.5 Hz, 1H), 7.23 – 7.17 (m, 4H), 7.15 – 7.08 (m, 6H), 7.05 (d, J = 8.4 Hz, 2H), 6.63 (dd, J = 11.6, 8.2 Hz, 3H), 6.57 (d, J = 1.9 Hz, 1H), 6.42 (dd, J = 8.0, 1.8 Hz, 1H), 4.53 – 4.45 (m, 3H), 4.33 – 4.23 (m, 2H), 4.17 (dd, J = 13.6, 7.9 Hz, 1H), 3.70 (dd, J = 16.8, 5.6 Hz, 1H), 3.64 – 3.52 (m, 4H), 3.25 – 3.07 (m, 3H), 3.02 – 2.86 (m, 4H), 2.79 – 2.61 (m, 4H), 2.34 – 2.16 (m, 7H), 1.95 – 1.84 (m, 3H), 1.81 – 1.70 (m, 3H). HR-MS: calc. M = 1223.4921, obsvd. $(M+H)^-$ = 1224.4995.

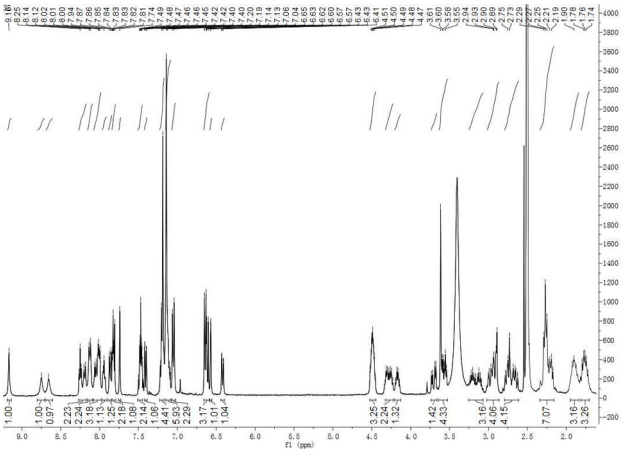


Fig. S-6. ^{1}H NMR of Compound ***Nap-GFFYEEE-Cat***

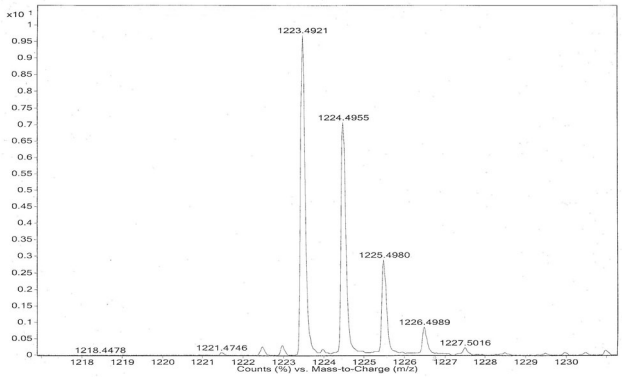


Fig. S-7. HR-MS of Compound ***Nap-GFFYEEE-Cat***

^{1}H NMR analysis of interaction of bortezomib and Nap-GFFYEE-Cat conjugates: Bortezomib (BTZ) was dissolved in d^{6}-DMSO at 2M concentration. Nap-GFFYEE-Cat was dissolved in D_2O at 0.2M， and pH of such solution of was adjusted with 0.1M Na_2CO_3 in D_2O to 7.4. Then BTZ and Nap-GFFYEE-Cat were mixed to give a stock solution of Nap-GFFYEE-Cat-BTZ conjugate at 0.1 M in D_2O. D_2O was used to dilute the Nap-GFFYEE-Cat-BTZ stock to 1 mM, and 0.2M of Nap-GFFYEE-Cat was diluted to 1mM by D_2O. These solutions were analyzed an hour after preparation on ^{1}H NMR (Bruker ARX 400) and peak integrals in the range of 7.2 to 5.5 ppm and range from 8.3 to 9.0 ppm were used for quantifying the degree of dissociation of Cat-BTZ presented in Fig.S-8.

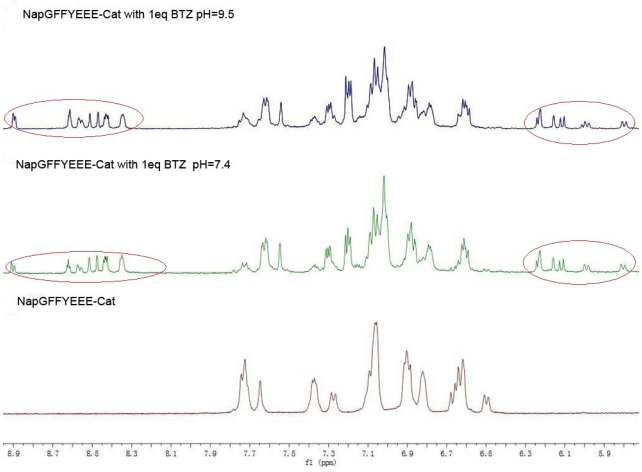


Fig. S-8. ^{1}H NMR spectrum of ***Nap-GFFYEE-Cat*** and ***Nap-GFFYEE-Cat*** with ***BTZ*** mixture.

Rheology: Rheology test was done on an AR 1500ex (TA instrument) system, 25 mm parallel plates was used during the experiments at the gap of 400μm. 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 0.1-100rad/s at the strain of 1%.

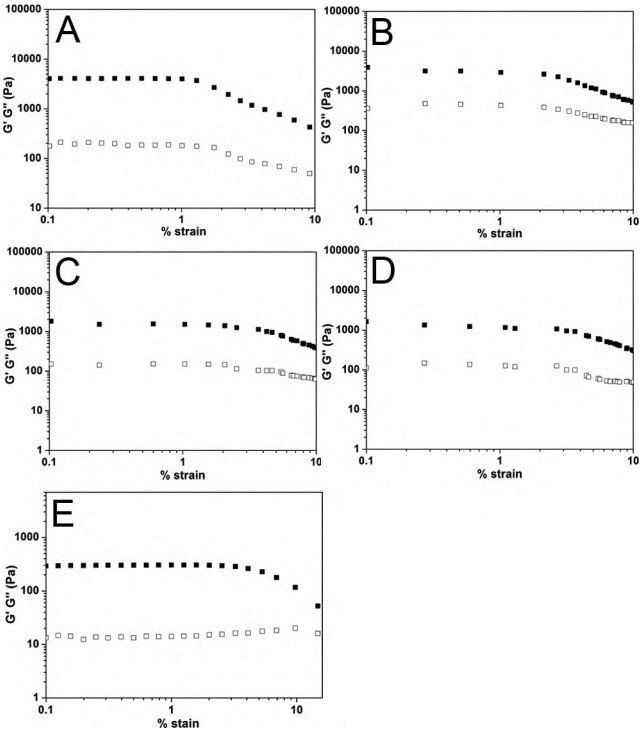


Fig.S-9. Dynamic strain sweep of the gels formed by PBS solution of Nap-GFFYEE-Cat (0.5 wt%) A) without BTZ, B) with 0.125 equiv. of BTZ, C) with 0.25 equiv. of BTZ, D) with 0.5 equiv. of BTZ, and E) with 1 equiv. of BTZ at the frequency of 1rad/s (the solid symbols represent elasticity (G') and the hollow ones represent viscosity (G'')).

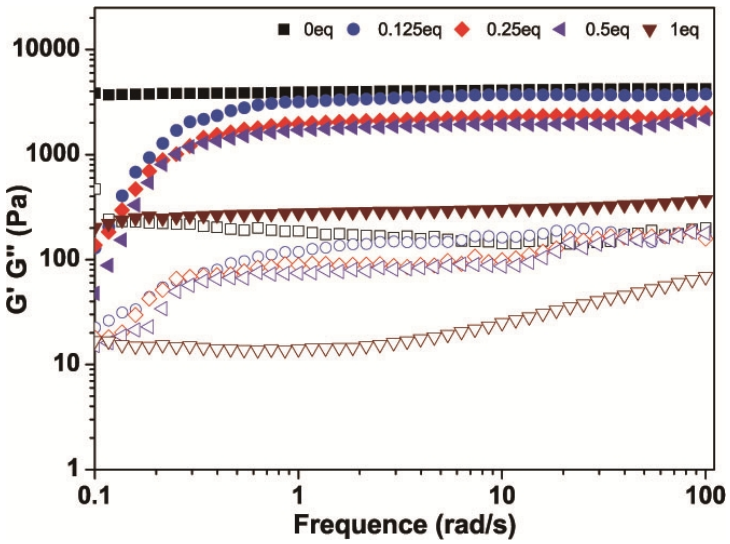


Fig. S-10. Dynamic frequency sweep of the gel formed by PBS solution of Nap-GFFYEE-Cat (0.5 wt%) without BTZ (■), with 0.125 equiv. of BTZ (●), 0.25 equiv. of BTZ(♦), 0.5 equiv. of BTZ (◀), and 1 equiv. of BTZ (▼).

In vitro release of BTZ from peptide derivatives: A hydrogel in PBS (pH =7.4) solution containing 0.5wt% of Nap-GFFYEE-Cat and contain 1, 0.5, 0.25 and 0.125 equiv. of bortezomib, then was formed in an eppendorf tube at 25°C. After 24 h, we added 0.25 mL of PBS on the surface of the hydrogels, 0.2 mL solution was taken out at the desired time point and 0.2mL PBS was added back. For the following time points, 0.2 mL of PBS was taken out and 0.2 mL of PBS was added back at each point. We then monitored and calculated the release profile from the gel formed by a LCMS-2020 (Shimadzu) system. The experiment was performed at 37 °C.

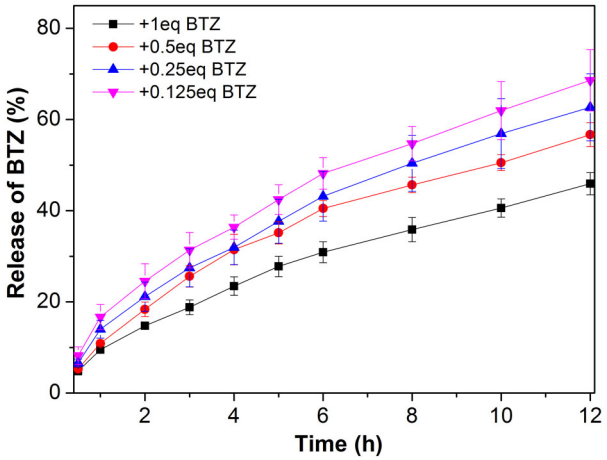


Fig. S-11. The release of BTZ from hydrogels to PBS buffer solution at 37℃.

Preparation of TEM samples of compounds:

Solutions of Nap-GFFYEE-Cat and Nap-GFFYEE-Cat with 1equiv. of bortezomib were prepared as described above. Next, 10 μL samples of each were placed on a carbon-coated copper grid and incubated

for 30 seconds to allow the peptide nanostructures to adhere to the substrate, then rinsed twice with ultrapure water. The samples were then stained with a saturated uranyl acetate solution and placed in a desiccator overnight prior to analysis.

Preparation of SEM samples of compounds:

First, the hydrogels of Nap-GFFYEE-Cat and Nap-GFFYEE-Cat with 1 equiv. of bortezomib were prepared as described above. Second, they were freeze-dried by a lyophilizer. Third, the freeze-dried powders were adhered to a copper plate. Then the copper plate was sprayed by metal gold prior to analysis.

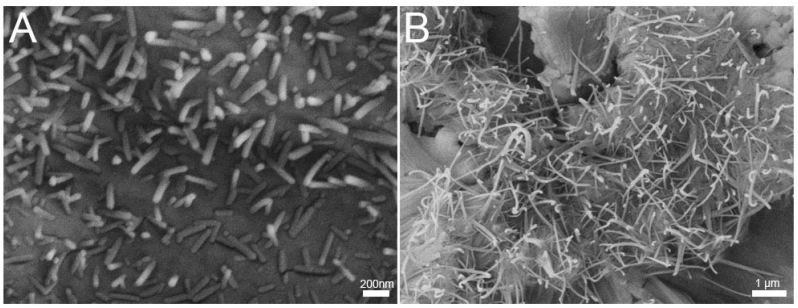


Fig. S-12. The SEM images of hyrogels formed by A) Nap-GFFYEE-Cat (0.5 wt%) with 1 equiv. of BTZ, B) Nap-GFFYEE-Cat (0.5 wt%).

Preparation of peptide-bortezomib conjugates for cytotoxicity assays:

For all the experiments where peptide-bortezomib conjugates were tested, the following sample preparation method was used. Peptide-bortezomib and pure BTZ solutions (100 mL) in DMEM at a final equivalent BTZ concentration of 1mg/L were prepared, and then they were diluted to use for the cell culture (the coefficient of dilution =0.5).

Determination of IC_{50} values on different cells: Three cells of 3T3, HepG2, and HeLa were seeded in a 96-well plate with the density of 10,000 cells per-well (total medium volume of 100 µL). 24 hours post seeding, the solutions with a serial of concentrations (8 concentrations) of the compound in 100µL of medium were added to each well (five wells for each concentration). Cells without the treatment of the compound were used as the control. The MTT assays were performed after an extra culture time of 48 hours. All compounds were removed and 90 µL fresh medium was added for each well, 10 µL of MTT solution (5 mg/mL) was added and incubated for 4 hours in 37^{o}C. Pipette out the spent media, formazon crystals at the bottom of each well were dissolved in 100 µL of DMSO. After 15 minutes at room temperature, absorbance at wavelength of 490 nm was tested using a microplate reader (BIO-RAD, iMark TM). IC_{50} values for the inhibition of cell viability were calculated from pharmacological inhibitory

response curves using software Prism 5.0. The cell viability percent was calculated by the following formula:

$$\text{The cell viability percent (\%)} = OD_{sample}/OD_{control} * 100\%$$

The concentrations of the compounds when 50% of cell viability was recorded represented the IC_{50} values of the compounds. All experiments were conducted in triplicate.

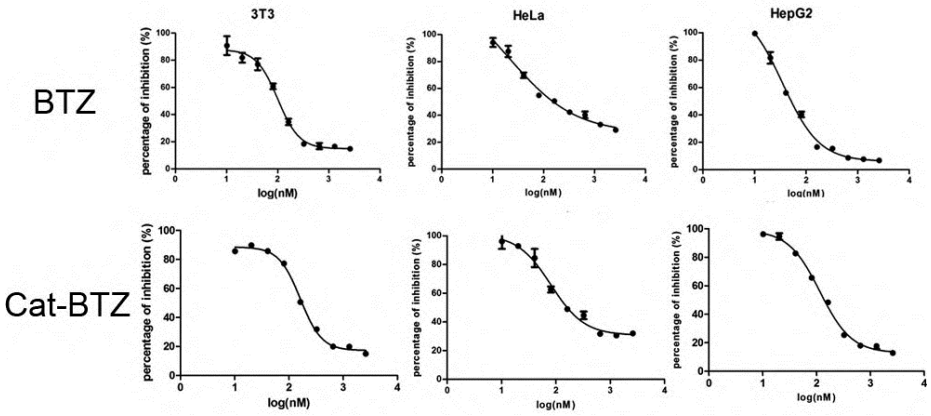


Fig.S-13. Congress curve of cell inhibition assay of different cells treated with different concentrations of BTZ and Nap-GFFYEE-Cat with 1equiv. of BTZ (n=3).