Experimental Method

Materials

4-Methylbenzhydrylamine (MBHA) rink amide resin, Fmoc-protected amino acids, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 5(6)-carboxylfluoresceinpiperidine, diisopropylethylamine (DIPEA), 6-(7-nitrobenzofurazan-4-ylamino) hexanoic acid (NBD-hexanoic acid), Active Human MMP-2 enzyme and Congo red were purchased from Sigma-Aldrich. Trifluoroacetic acid (TFA), triisopropylsilane (TIS), acetonitrile (CH_3CN), dimethylformamide (DMF), dichloromethane (DCM), methanol, hexane, ethyl acetate, acetic anhydride, 3-mercaptopropionic acid (MPA), 2,2-dithiodipyridine (Py-SS-Py), Dulbecco's modified Eagle medium (DMEM), Gibco Roswell Park Memorial Institute (RPMI) 1640 Medium, Ham's F-12 Nutrient Mixture and glutathione (reduced) were purchased from Fisher Scientific and used as received. Doxorubicin hydrochloride was purchased from Avachem Scientific. Dimethyl sulfoxide-d_6 was purchased from Millipore Sigma. Hoechst 33342 was purchased from Life Technologies. Fetal Bovine Serum (FBS) was purchased from VWR. SensoLyte ® 520 MMP - 2 Assay Kit *Fluorimetric* was purchased from AnaSpec, Inc. CCK8 assay kit was obtained from Dojindo Molecular Technologies (Rockville, MD). Transmission Electron Microscopy (TEM) grids and uranium acetate dihydrate were purchased from Ted Pella, Inc.

Peptides synthesis and purification. The synthesis was performed on on a *Prelude*® peptide synthesizer at a 30 μmol scale using MBHA rink amide resin as the solid support. The synthetic procedure followed the standard Fmocs solid-phase peptide synthesis method. The Fmoc group

was deprotected in the presence of 2 mL of 20% (V/V) piperidine/DMF for 5 minutes and repeated once. 1 mL of 120 mM Fmoc-protected amino acids in DMF were mixed with 0.3 mL of 400 mM HBTU and 0.3 mL of 800 mM of DIPEA in DMF. The mixture was added to the reaction vessel and the coupling reaction was carried out for 30 mins. After the completion of the synthesis, the N-terminus of the MDPs was acetylated using 50 μL of DIPEA and 330 μL of acetic anhydride in DMF (2 mL) for 1 hr. A Kaiser test was performed to confirm the completion of the acetylation reaction. The acetylated peptide was cleaved in a mixture of 2 mL of TFA/TIS/ H_2O (95/2.5/2.5 by volume) for 3 hrs. The cleavage solution was filtered, and the filtrates were collected. The resin was washed three times with neat TFA, and all filtrate solutions were combined and evaporated under airflow. The residual peptide solution was precipitated in cold diethyl ether, followed by centrifugation and washing with cold diethyl ether three times. The crude peptide was dried under vacuum overnight for HPLC purification. Peptides were purified using a preparative reverse phase C4 column with a linear gradient of H_2O/CH_3CN (5% to 95% of CH_3CN in 30 mins) containing 0.05% TFA and the elution was monitored at both 230 nm and 280 nm. The HPLC fractions were collected, combined and lyophilized for 2 days yielding while powders with an overall yield at ~ 40%. NBD labeled peptides were synthesized as follows. After the final deprotection of the Fmoc group, peptide resin (typically 15 μmol based on the loading number of the original amino groups on the MBHA resin) was treated with 4 equivalents (i.e. 60 μmol) of 6-(7-nitrobenzofurazan-4-ylamino) hexanoic acid (NBD-hexanoic acid), 4 equivalents of HBTU and 8 equivalents of DIPEA in 2 mL of DMF. The reaction mixture was stirred overnight. The completion of the coupling reaction was confirmed by the Kaiser test. The cleavage and purification steps

followed the same procedure as described above yielding yellow powders with an overall yield of ~ 20%. The molecular weight of each peptide was characterized and confirmed by ESI mass spectrometry.

Table S1. List of peptides used in the study

Name	Peptide Sequence	Mass calculated $[M+3H]^+$	Mass found $[M+3H]^+$
CS-MDP	Acetyl-$K_{10}(QW)_6E_3G_3$PLGLAGK_5	1645.3	1645.8
NS-MDP	Acetyl-$K_{10}(QW)_6E_3G_3$LALGPGK_5	1645.3	1645.8
NBD-CS-MDP	NBD-$K_{10}(QW)_6E_3G_3$PLGLAGK_5	1723.3	1723.9
NBD-NS-MDP	NBD-$K_{10}(QW)_6E_3G_3$LALGPGK_5	1723.3	1723.8
DOX-CS-MDP	DOX-SS-C$K_{10}(QW)_6E_3G_3$PLGLAGK_5	1890.0	1890.1
DOX-NS-MDP	DOX-SS-C$K_{10}(QW)_6E_3G_3$LALGPGK_5	1890.0	1890.2
DOX-(NBD)CS-MDP	DOX-SS-C(NBD)$K_{10}(QW)_6E_3G_3$PLGLAGK_5	1967.7	1968.3
DOX-(NBD)NS-MDP	DOX-SS-C(NBD)$K_{10}(QW)_6E_3G_3$LALGPGK_5	1967.7	1968.2

Synthesis of 2-pyridyl-2-carboxyethyl disulfide (Py-SS-MPA). Py-SS-MPA was synthesized and purified according to a literature method.[1] 0.2 mL of acetic acid was added to a 10 mL of ethanol solution of Py-SS-Py (1 mmol, 220 mg). 42 μL (0.5 mmol) of MPA was dissolved in 10 mL of ethanol and added dropwise through an addition funnel. The reaction mixture was stirred at room temperature for 2 hrs. The solvent was evaporated with a rotary evaporator. The crude product was purified on a silica gel column using hexane/ethyl acetate (v/v = 3/2) as the eluent to afford the product. After removing the solvent with a rotary evaporator, a colorless viscous oil was produced with a yield of 45%. ^{1}H-NMR spectra were recorded on a JEOL ECA 500 MHz spectrometer and referenced to the internal solvent (2.5 ppm in DMSO-d_6). NMR data are reported as: chemical shift (in ppm, δ), integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (in Hz, *J*). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.56 – 8.30 (m, 1H), 7.89 – 7.63 (m, 2H),

7.34 – 7.06 (m, 1H), 2.97 (t, J = 6.9 Hz), 2.61 (t, J = 6.8 Hz). As additional notes, the product contains trace amount (less than 5%) of 2,2'-dithiol dipyridine (2,2'-DTDP) as shown at 7.64 ppm. This observation was reported in another work where trace amounts of 2,2'-DTDP were found in Py-SS-MPA using silica flash chromatography.[2] While its complete removal is possible using multi-step acid-base extractions and anion exchange chromatography, the product was used without further purification because 2,2'-DTDP is unlikely to cause side reactions during the synthesis of Py-SS-DOX and any side product can be removed by HPLC after the synthesis of DOX-peptide conjugate.

Synthesis of Py-SS-DOX. Py-SS-DOX was synthesized according to a literature method.[1] Py-SS-MPA (21.5 mg, 0.1 mmol) and HBTU (37.9 mg, 0.1 mmol) were dissolved in 5 mL of DMF and stirred for 30 mins to activate the carboxyl group on the Py-SS-MPA. DOX•HCl (87 mg, 0.15 mmol) was dissolved in 5 mL of DMF and was added dropwise to the reaction mixture. 0.2 mL of DIPEA was added to the reaction mixture. The reaction mixture was stirred at room temperature for 3 hrs and was precipitated in ~ 300 mL of cold anhydrous diethyl ether. The crude product was purified on a silica gel column using DCM/methanol (30/1) as the eluents with an overall yield at ~ 20%. Residual DIPEA was found in the product after silica column purification and further removed by a reverse phase C4 column using H_2O/CH_3CN containing 0.05% TFA (with a linear gradient of 5% to 95% of CH_3CN in 30 mins). The final product was lyophilized and appeared as red powders. The structure of the product was characterized and confirmed by 1D ^{1}H NMR (referenced to the internal trimethyl silane at 0 ppm), 2D COSY ^{1}H-^{1}H NMR and ESI/MS (**Fig. S11**). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (d, J = 4.8 Hz, 1H), 7.81 – 7.75 (m, 2H), 7.71 – 7.58 (m, 3H), 7.52 (d, J = 7.3 Hz, 1H), 7.15 (s, 1H), 7.12 – 7.08

(m, 1H), 7.04 (s, 1H), 6.94 (s, 1H), 5.12 (m, 1H), 4.82 (m, 1H), 4.48 (s, 2H), 4.07 (m, 1H), 3.87 (m, 4H), 2.92 – 2.76 (m, 4H), 2.15 – 1.96 (m, 2H), 1.74 (dt, *J* = 13.0, 4.1 Hz, 1H), 1.33 (m, 1H), 1.03 (d, J = 6.4 Hz, 3H). ESI/MS: expected 740.8, observed 741.3.

Synthesis of DOX-peptide conjugates. DOX-CS-MDP and DOX-NS-MDP were prepared through a thiol-disulfide reaction between Py-SS-DOX and a cysteine containing MDP, denoted as Cys-CS-MDP or Cys-NS-MDP. Experimentally, 7.0 mg (1.6 μmol) of Py-SS-DOX and ~ 1.0 mg (1.2 μmol) of Cys-CS-MDP or Cys-NS-MDP were dissolved in 1 mL of DMF. The reaction was stirred at room temperature for 24 hrs. The reaction mixture was directly injected on a semi-preparative reverse phase HPLC using a C4 column and a linear gradient of H_2O/CH_3CN (5% to 95% of CH_3CN in 30 mins) containing 0.05% TFA. The HPLC elutions as monitored by UV at 230 nm and 280 nm were collected for lyophilization, which yielded red powder products with an overall yield at ~ 33%. The product was characterized and confirmed by ESI/MS. For the synthesis of DOX-(NBD)CS-MDP and DOX-(NBD)NS-MDP, NBD was first conjugated on the N-terminus of Cys-CS-MDP following the procedure described in the section of "Peptide Synthesis and Purification". DOX was attached using the same procedure through thiol-disulfide reaction to generate DOX-(NBD)CS-MDP and DOX-(NBD)NS-MDP. The reaction mixture was directly injected on a semi-preparative reverse phase HPLC using a C4 column and a linear gradient of H_2O/CH_3CN (5% to 95% of CH_3CN in 30 mins) containing 0.05% TFA. The HPLC elutions as monitored by UV at 230 nm and 280 nm were collected for lyophilization, which yielded red powder products with an overall yield at ~ 15%.

Determination of the MMP-2 cleavage efficiency. Solutions of CS-MDP or NS-MDP were freshly prepared at a concentration of 100 μM in 1 mL of buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4). 6.5 μL of 0.1 μg/ μL active MMP-2 enzyme stock solution was added to the peptide solution to reach a final concentration of 10 nM. The reaction mixture was incubated at 37 °C for 4 hrs and 24 hrs and then injected onto the HPLC. The area of the elution peaks corresponding to the intact peptide and the fragment were integrated and used to calculate the cleavage efficiency.

Circular Dichroism (CD) Spectroscopy. The CD spectra were collected on a Jasco-J710 spectrometer. To prepare samples for CD measurements, stock solutions of CS-MDP or NS-MDP (~ 1 mM) were dilute to 100 μM in the following buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4). Active MMP-2 enzyme stock solution in the same buffer (0.1 μg/ μL) was added to the peptide solution to reach a final concentration at 10 nM. After incubation for 4 hrs and 24 hrs, peptides were further diluted to 20 μM in the same buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4) for CD spectra acquisition. CD spectra were collected from 250 nm to 190 nm at room temperature using a 2 mm cuvette, a bandwidth at 0.1 nm, scan rate at 100 nm/min and a response time of 2 sec. The final CD spectrum is the average of five consecutive scans. The mDeg of rotation was converted to the mean residual ellipticity using the following formula $\theta = \frac{1000 \times mDeg}{c * n * l}$, where c is the concentration of the peptide solution in mM, n is the number of amino acids in the peptide sequence and l is the path length of the cell used in mm.

Transmission Electron Microscopy (TEM). TEM was performed on a Hitachi H-9500 High-resolution TEM instrument. For TEM sample preparation, stock solutions of CS-MDP or NS-

MDP (~ 1 mM) were dilute to 100 μM in the following buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4). Active MMP-2 enzyme stock solution in the same buffer (0.1 μg/ μL) was added to the peptide solution to reach a final concentration at 10 nM. After incubation for 4 hrs and 24 hrs, 10 μL of the respective peptide solution was pipetted onto a holey carbon grid (TED PELLA 01824). After 2 mins, the excess solution was carefully removed with filter paper. 10 μL of 2 wt% uranyl acetate solution was dropped onto the grid for negative staining. The excess staining solution was removed with filter paper after 2 mins. The TEM sample was air-dried overnight before imaging.

Analytical Ultracentrifugation (AUC). To prepare samples for AUC measurements, stock solutions of CS-MDP or NS-MDP (~ 1 mM) were dilute to 100 μM in the following buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4). Active MMP-2 enzyme stock solution in the same buffer (0.1 μg/ μL) was added to the peptide solution to reach a final concentration at 10 nM. After incubation for 4 hrs and 24 hrs, peptides were further diluted to 20 μM in the same buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4) prior to measurements. Sedimentation velocity experiments were performed on a Beckman-Coulter Optima XL-I analytical ultracentrifuge equipped with an An-50 Ti 8-hole rotor. Double-sector centerpieces sandwiched between sapphire windows in a standard cell housing were loaded with 400 μL of sample (in the sample sector) and an equal volume of reference buffer , i. e. Tris buffer (20 mM, pH = 7.4) containing 5 mM of $CaCl_2$ and 20 mM of $ZnCl_2$ (in the reference sector). After 2 hrs of equilibration under vacuum at the experimental temperature (20 °C), samples were centrifuged at 50,000 rpm or 15,000 rpm. Data were acquired using UV absorbance optics tuned to 280 nm for each sample. Sedimentation velocity data was fitted to a continuous *c(s)*

distribution model using SEDFIT. The buffer density and viscosity at room temperature were determined to be 0.99939 g/mL and 0.01009 cP, respectively using SEDNTERP. The partial-specific volume was estimated at 0.75613 mL/g. A resolution of 100 was utilized with a regularization level of 0.68. Time-invariant noise elements were removed from the data. All figures featuring *c(s)* distributions were generated in GUSSI.[3]

Cell culture. HeLa cells were maintained in DMEM containing 10% FBS. A549 cells were maintained in RPMI1640 containing 10% FBS. KYSE-30 cells were maintained in a medium mixture of 45% RPMI1640, 45% Ham's F-12 and 10% FBS.

MMP-2 activity assay. SensoLyte ® 520 MMP - 2 Assay Kit *Fluorimetric* was standardized with an active MMP-2 enzyme. The active MMP-2 enzyme was prepared in 25 μL of analysis buffer with the following working concentration (0.2 nM, 0.4 nM, 0.8 nM, 1.6 nM and 3.0 nM) (three replicates for each concentration) in a 96 well-plate. 25 μL of MMP-2 substate was added to each well. The reaction mixture was incubated at 37 °C for 1 hr followed by fluorescence measurement at ex/em = 490 nm / 520 nm. A standard curve for MMP-2 quantification was generated by plotting the fluorescence intensity against the MMP-2 working concentrations (Fig. S7a). To quantify the endogenous MMP-2 concentrations in different cell cultures, HeLa cell, A549 cell and KYSE-30 cell were seeded onto a 96-well plate at a density of 10^4 cells/well and incubated at 37 °C in an incubator with 5% of CO_2. After 24 hrs, HeLa cells were refreshed with DMEM medium, A549 and KYSE-30 cells were refreshed with RPMI1640 medium. After 24 hrs of incubation at 37 °C in an incubator with 5% of CO_2, the culture medium was removed for MMP-2 activity assay following the same procedure detailed above. Three replicates were performed for each cell line.

Cell uptake. For the cell uptake study, cells were seeded onto a petri dish with a cover glass on the bottom at a density of 10^5 cells/well and incubated overnight at 37 °C in an incubator with 5% of CO_2. After 24 hrs, HeLa cells were refreshed with 180 μL of DMEM medium, and A549 and KYSE-30 cells were refreshed with 180 μL of RPMI1640 medium. 20 μL of 200 μM NBD-CS-MDP or NBD-NS-MDP (in Tris buffer (20 mM, pH 7.4)) were added to the culture medium to reach a final concentration at 20 μM. For samples with the addition of exogenous MMP-2, 0.4 μL of 0.1 μg /μL active MMP-2 enzyme was added to the culture medium to reach a final concentration of 3 nM before adding the peptide. After 24 hrs of incubation, the culture medium was removed and washed three times with PBS buffer. Cells were stained with nucleus staining dye, Hoechst 33342, at 37 °C for 15 min and washed with PBS buffer three times. Cells were imaged by an Ti2 inverted A1R HD25 confocal laser scanning microscope (Nikon, Japan) with a 40X objective (NA 1.3) and fluorescence images were processed using ImageJ.

Congo red (CR) staining study. A549 cells were seeded in a petri dish with a cover glass bottom at a density of 10^5 cells/well and incubated at 37 °C for 24 hrs. 20 μL of 200 μM NBD-CS-MDP or NBD-NS-MDP (in Tris buffer (20 mM, pH 7.4)) was mixed with 180 μL of fresh cell culture RPMI1640 medium. After incubation at 37 °C for 24 hrs, the culture medium was removed and washed three times with PBS buffer. Cells were stained Hoechst 33342 at room temperature for 15 min and followed by washing three times with PBS buffer. 200 μL of 5 μM CR solution was added to cell culture for staining at 37 °C for 1 hr. After removing CR solution and washing three times with PBS buffer, cells were imaged by an Ti2 inverted A1R HD25

confocal laser scanning microscope (Nikon, Japan) with a 40X objective (NA 1.3) and fluorescence images were processed using ImageJ.

Flow cytometry. Cells were seeded onto a 24-well plate at a density of 10^5 cells/well and incubated for 24 hrs at 37 °C with 5% of CO_2. HeLa cells were refreshed with 450 μL of DMEM medium while A549 and KYSE-30 were refreshed with 450 μL of RPMI1640 medium. 50 μL of NBD-CS-MDP or NBD-NS-MDP solutions (200 μM in Tris buffer (20 mM, pH 7.4)) were added. Three replicates was performed for each sample. After 24 hrs, the culture medium was removed and cells were washed with PBS buffer for three times. Cells were digested with trypsin and washed twice with PBS buffer. 2% paraformaldehyde was used for cell fixation. The mean fluorescence intensity of cells upon different peptide treatments was quantified using the BD LSR II flow cytometer. A minimum of 10,000 events per sample was analyzed and data were processed using FlowJo software.

Cytotoxicity measurement. Cells were seeded onto a 96-well plate at a density of 10^4 cells/well and incubated at 37 °C in an incubator with 5% of CO_2. After 24 hrs, HeLa cells were refreshed with 90 μL of DMEM medium and A549 and KYSE-30 were refreshed with 90 μL of RPMI1640 medium. 10 μL of freshly prepared DOX, DOX-CS-MDP, DOX-NS-MDP (200 μM), CS-MDP or NS-MDP were added to each reach a final concentration at 20 μM. Cells incubated with 10 μL of Tris buffer (20 mM, pH 7.4) were used as a control group. The culture medium was refreshed after 24 hrs. After 48 hrs of incubation, the culture medium was removed and the mixture of 90 μL fresh medium and 10 μL of CCK8 assay solution was added to each well.[4] After 1 hr of incubation at 37 °C, UV absorbance was measured at 450

nm and the cell viability was calculated using the following equation. All experiments were performed in four replicates.

$$\% \text{ cell viability} = (A_{peptide} - A_{Tris\ control}) / A_{Tris\ control} \times 100$$

Statistical analysis. All data were expressed as means ± standard deviation (SD). The statistical analysis was performed using Student's T-test and one-way analysis of variance (ANOVA) at confidence levels of 95%.

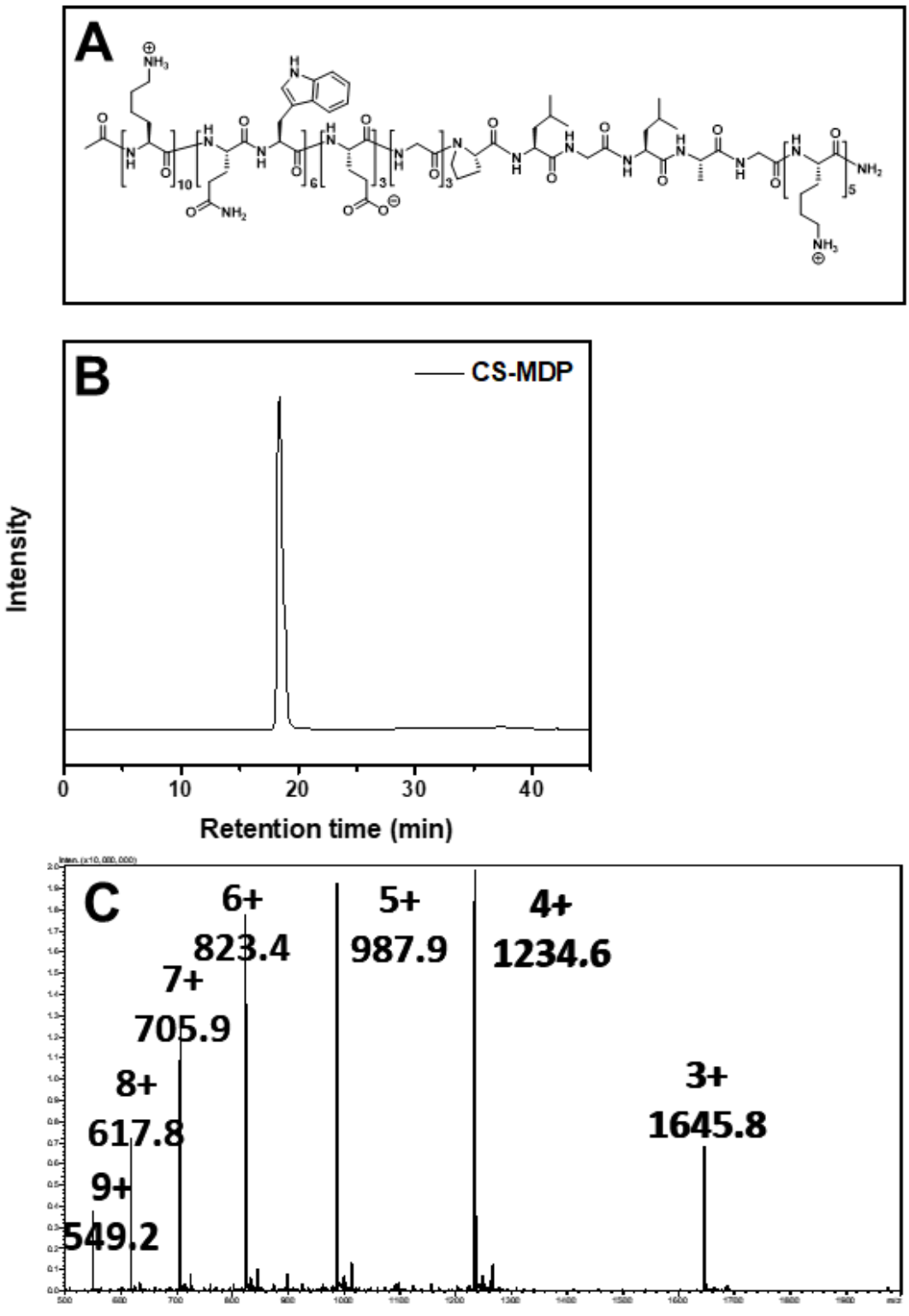

Figure S1(1). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of CS-MDP.

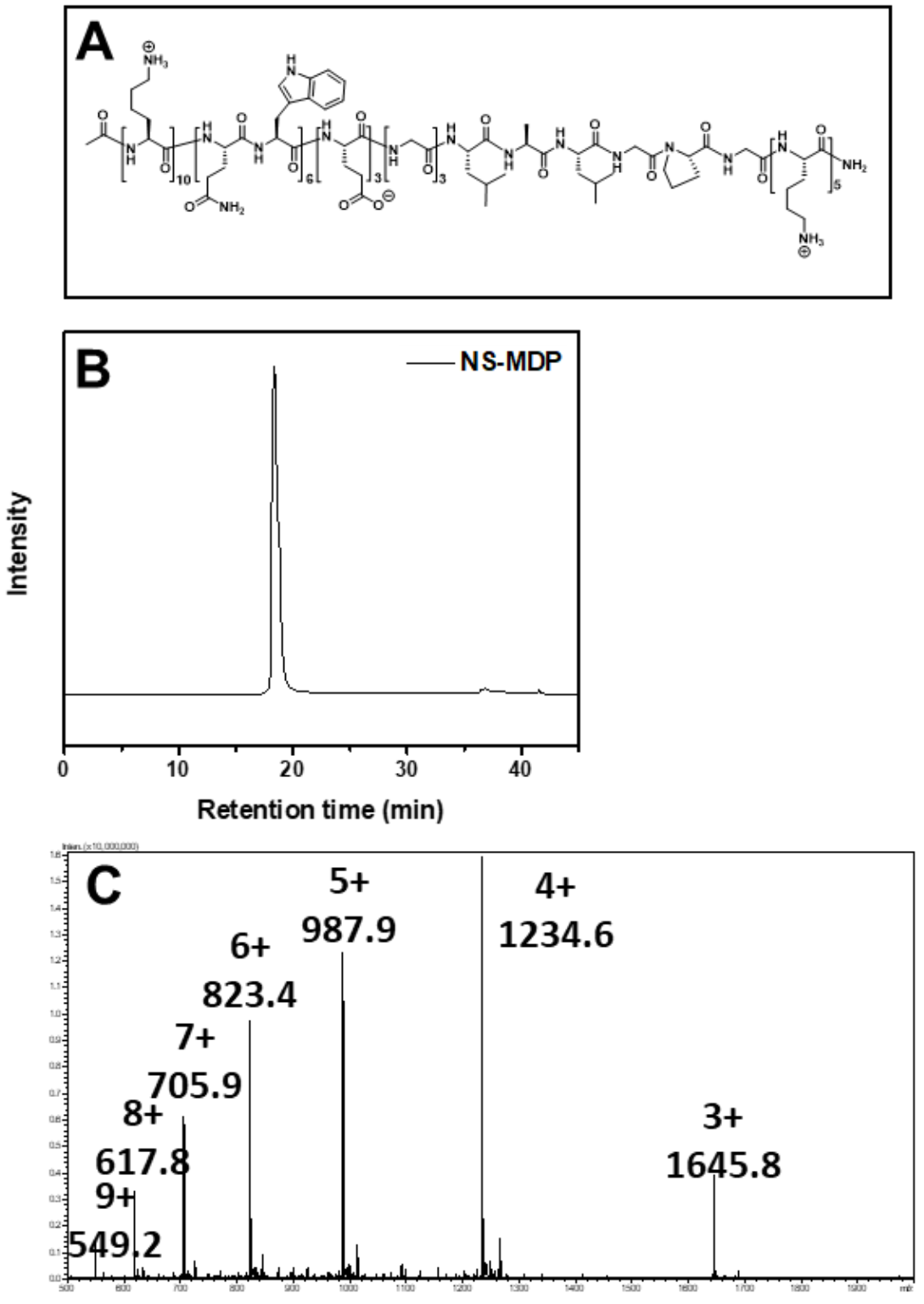

Figure S1(2). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of NS-MDP.

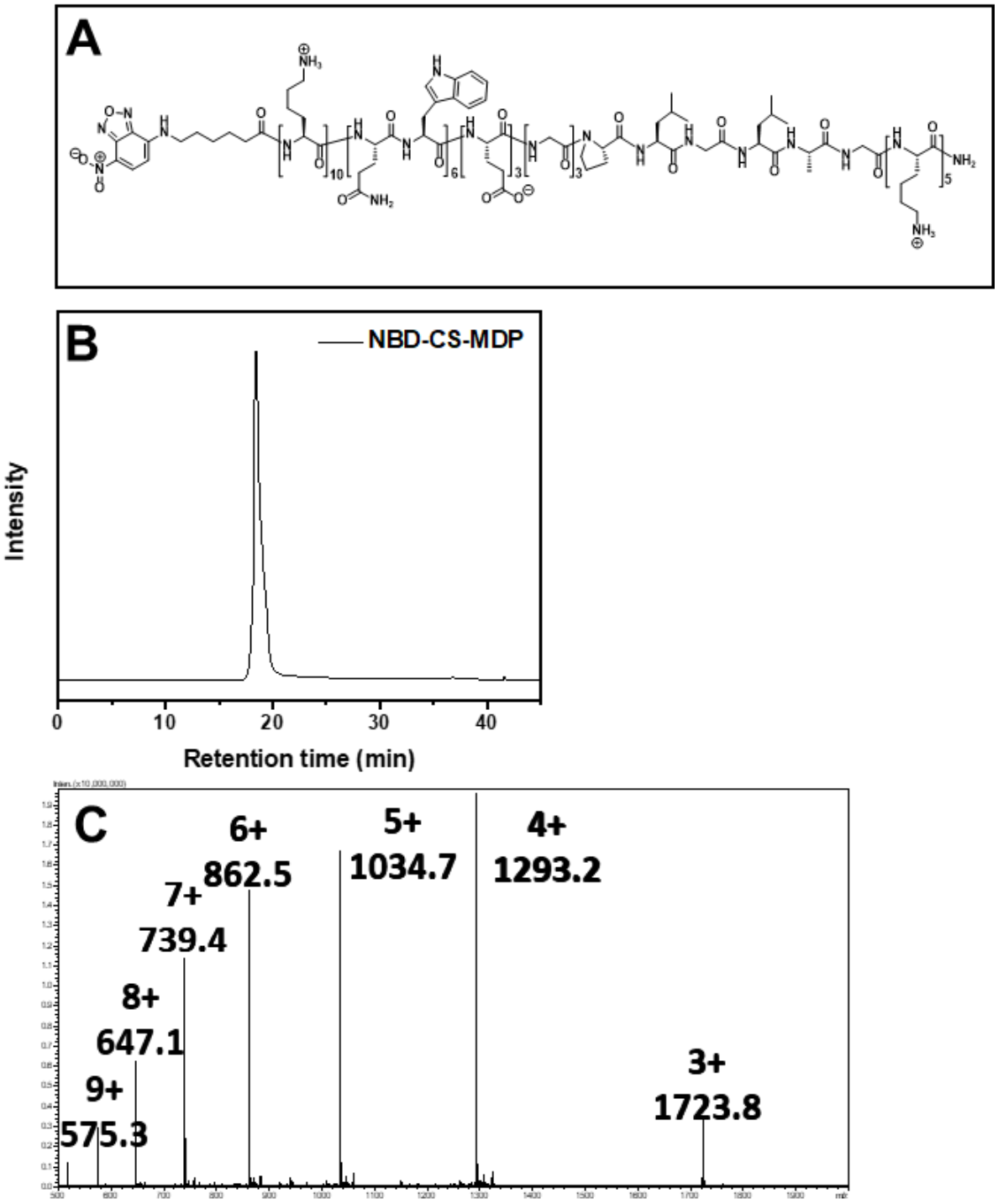

Figure S1(3). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of NBD-CS-MDP.

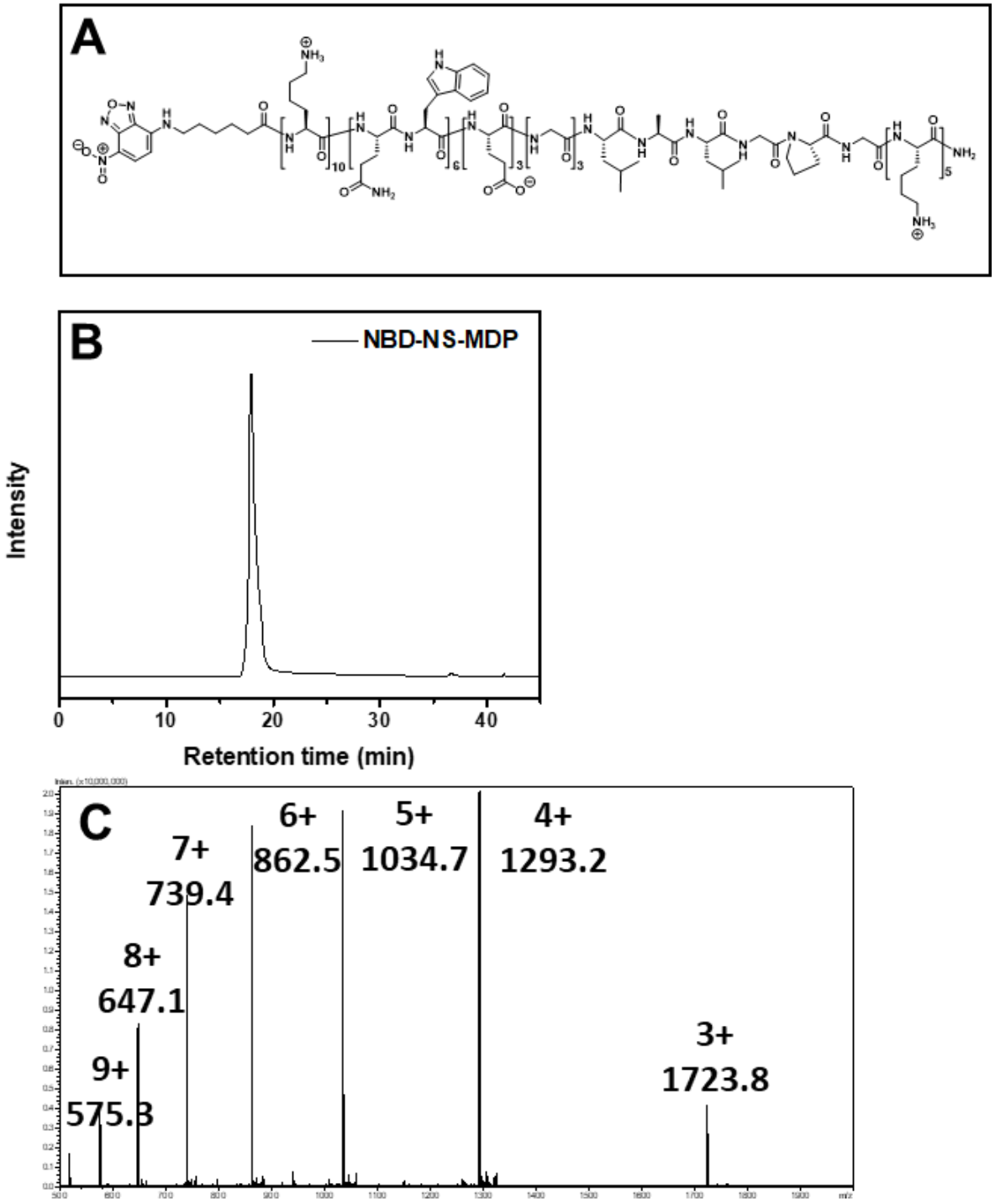

Figure S1(4). (**A**) Chemical structure , (**B**) HPLC and (**C**) ESI/MS of NBD-NS-MDP.

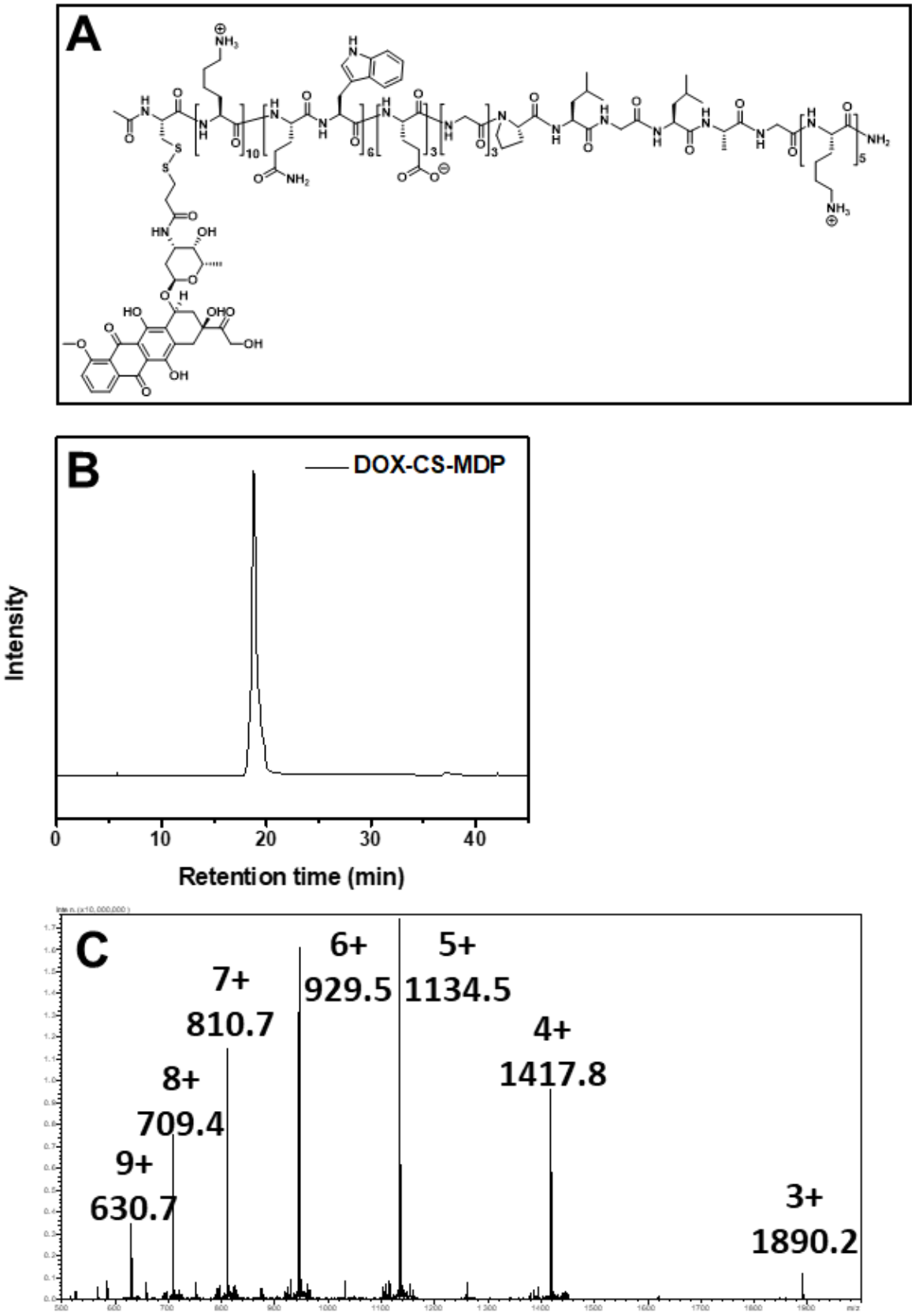

Figure S1(5). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of DOX-CS-MDP.

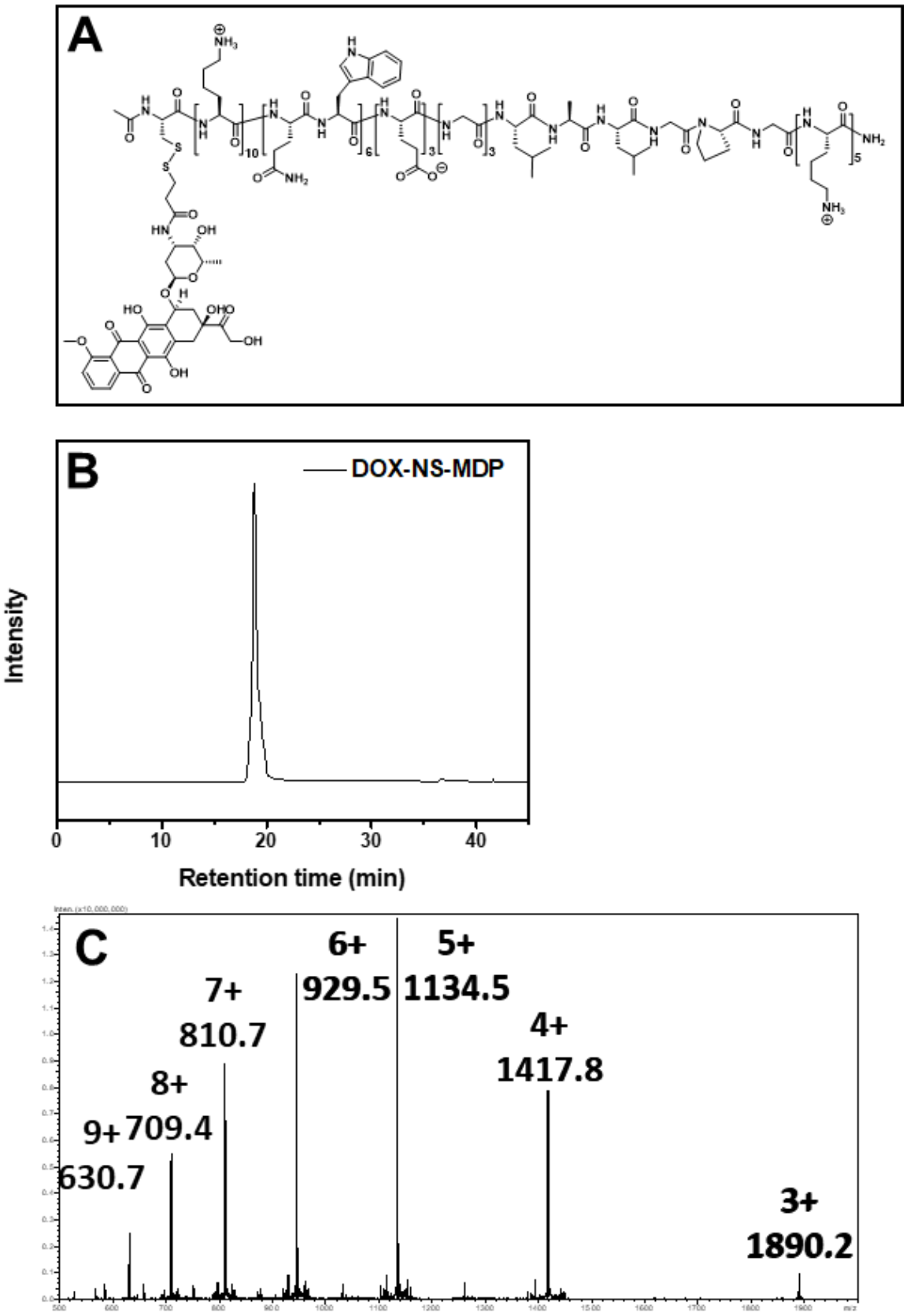

Figure S1(6). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of DOX-NS-MDP.

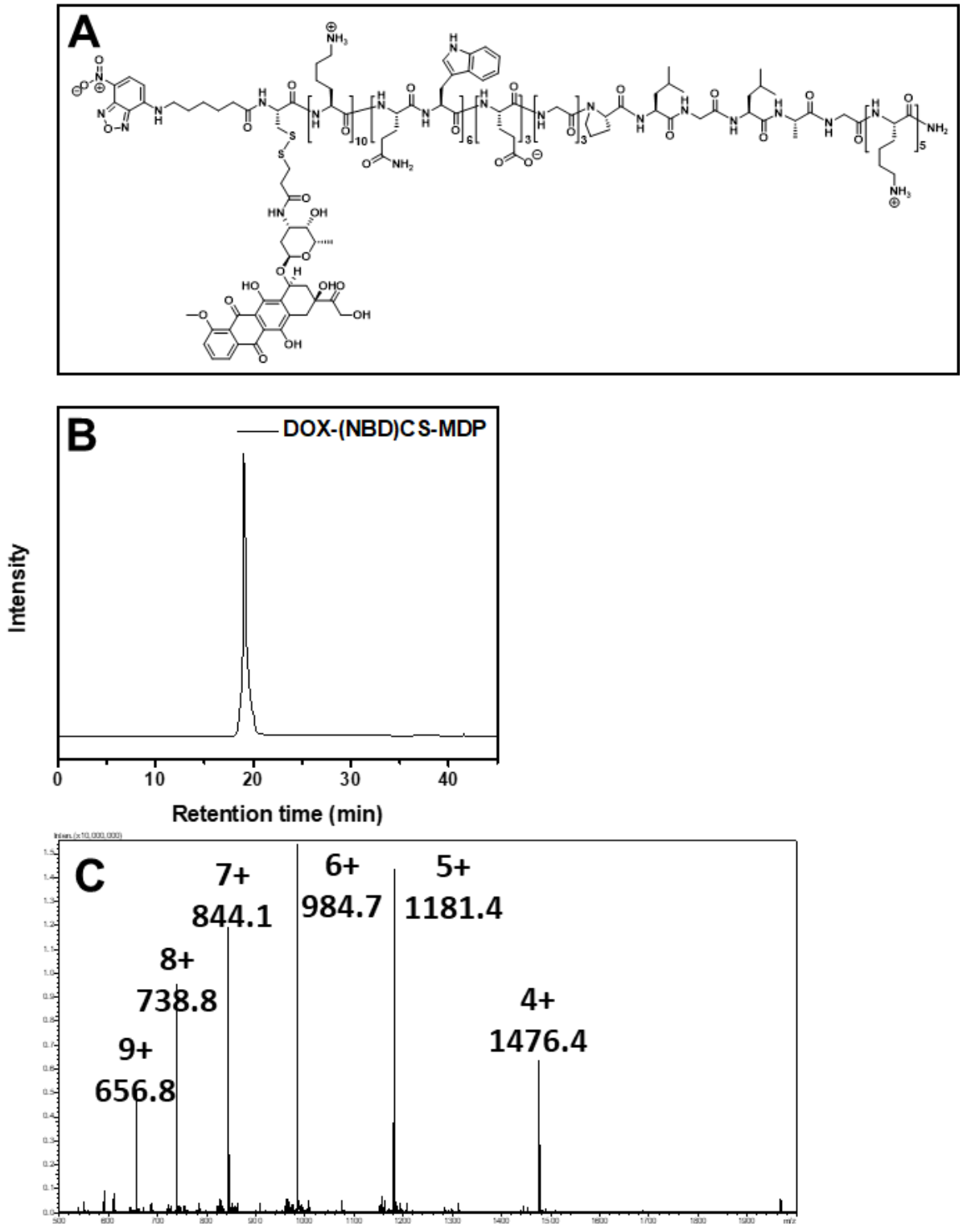

Figure S1(7). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of DOX-(NBD)CS-MDP

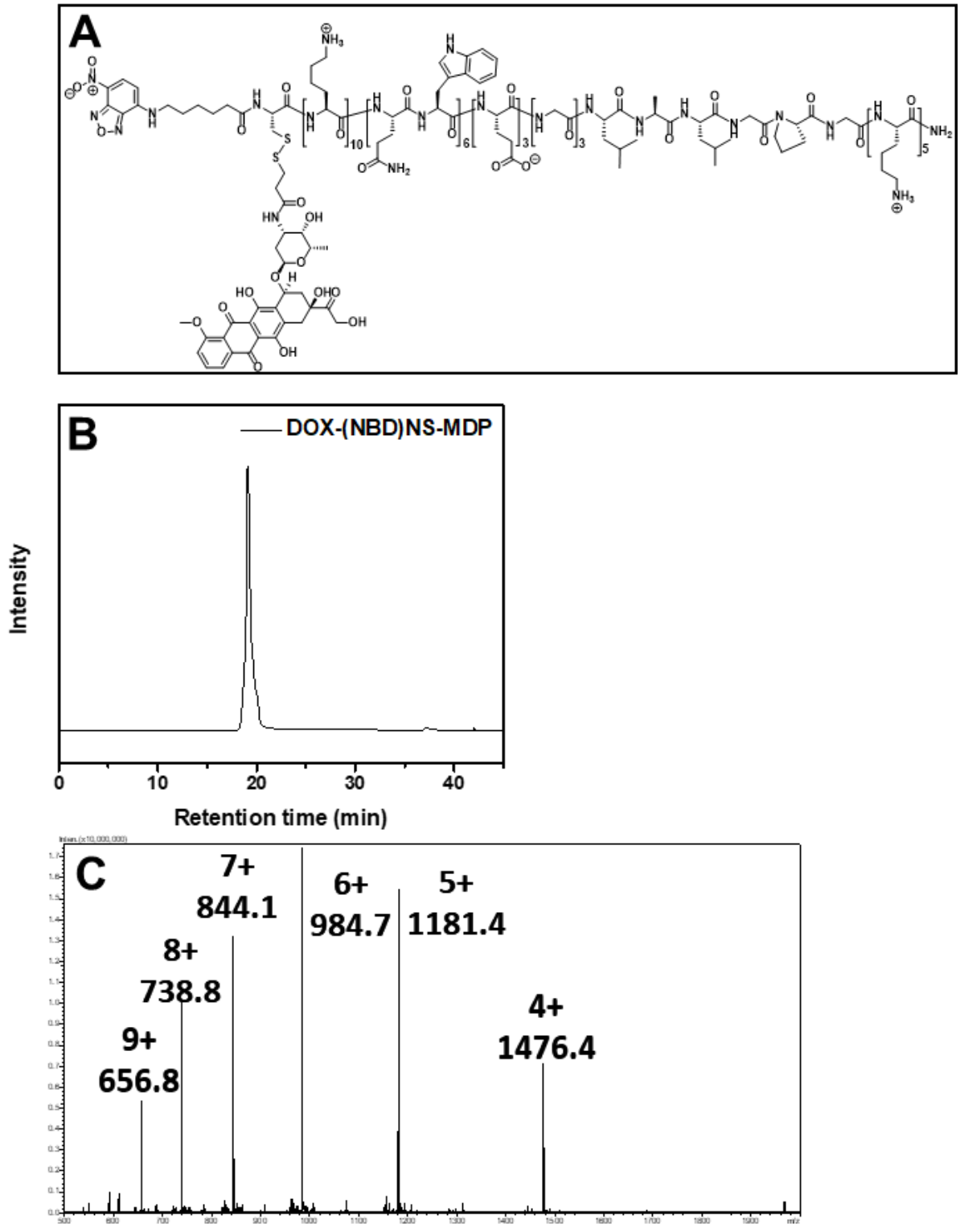

Figure S1(8). (**A**) Chemical structure, (**B**) HPLC and (**C**) ESI/MS of DOX-(NBD)NS-MDP

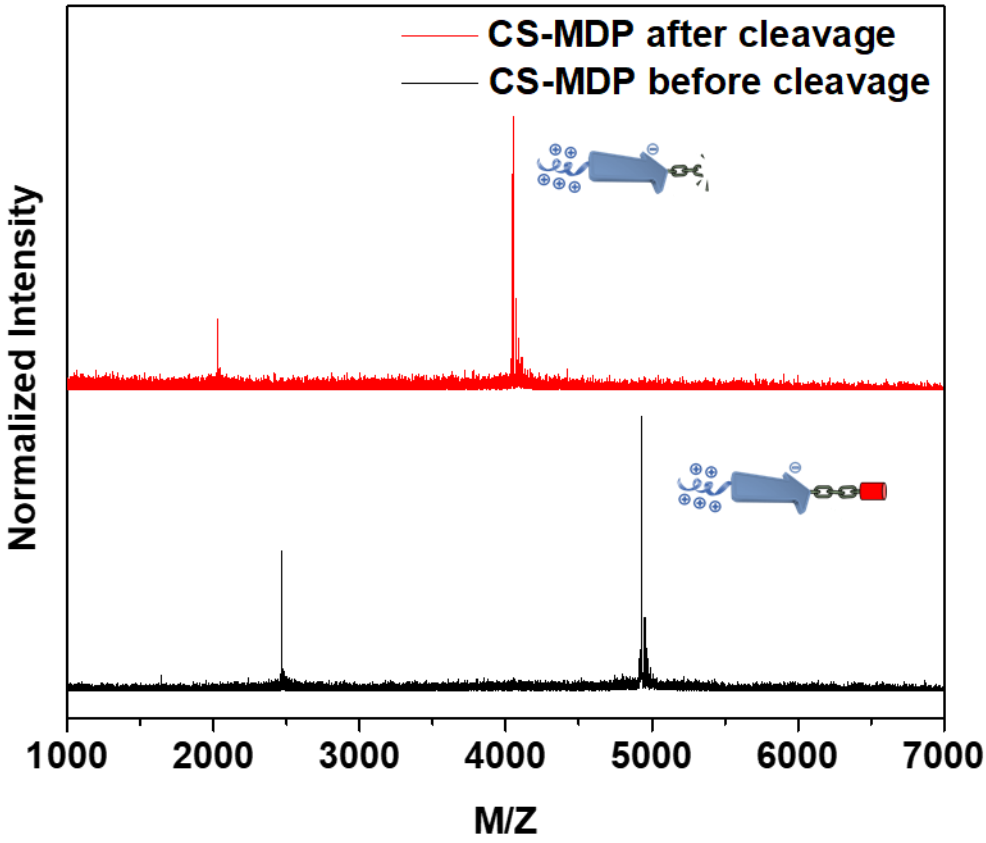

Figure S2. MALDI mass spectrometry characterization of CS-MDP before and after MMP-2 cleavage in buffer (Tris 20 mM, $CaCl_2$ 5 mM, $ZnCl_2$ 20 mM, pH = 7.4).

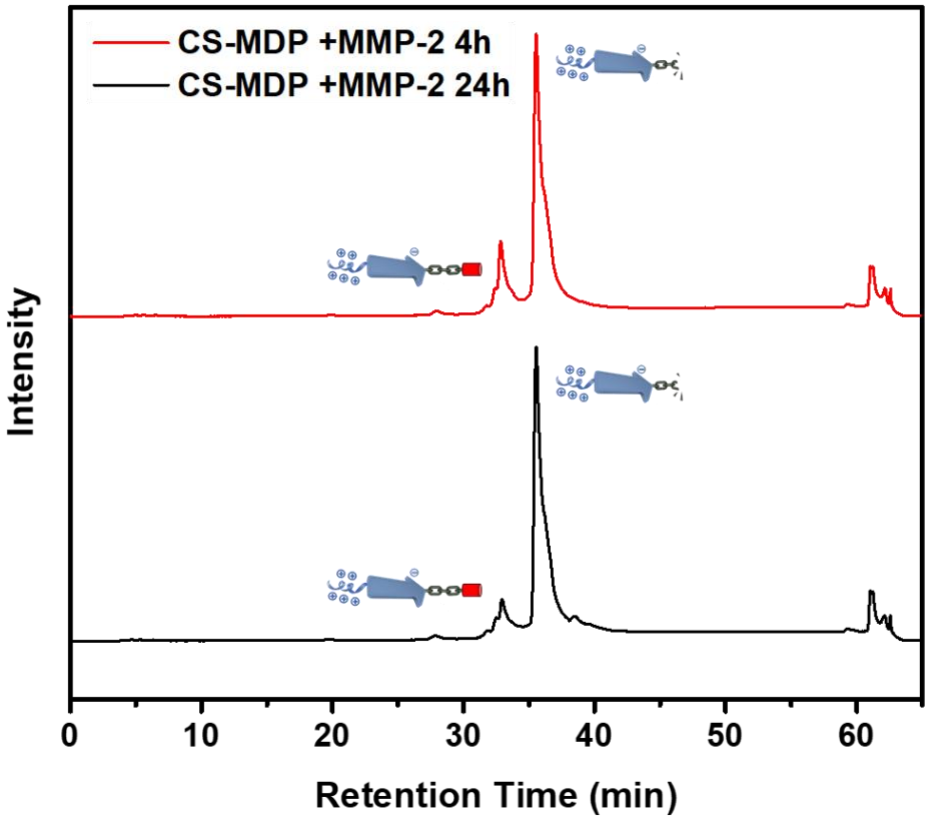

Figure S3. HPLC elution profiles of CS-MDP after MMP-2 treatment for 4 hrs and 24 hrs in Tris buffer (20 mM, pH = 7.4) containing 5 mM $CaCl_2$ 5 mM and 20 mM $ZnCl_2$.

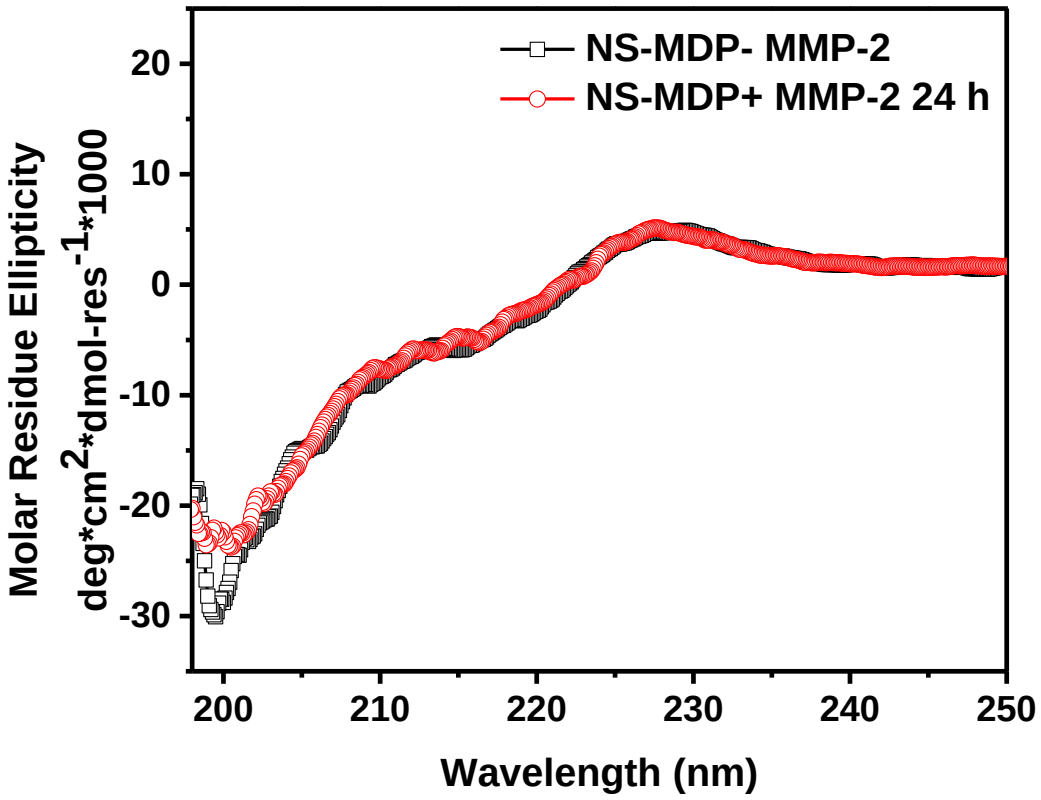

Figure S4. Circular dichroism spectroscopy of NS-MDPs with and without MMP-2 treatment. Peptide concentration: 20 μM.

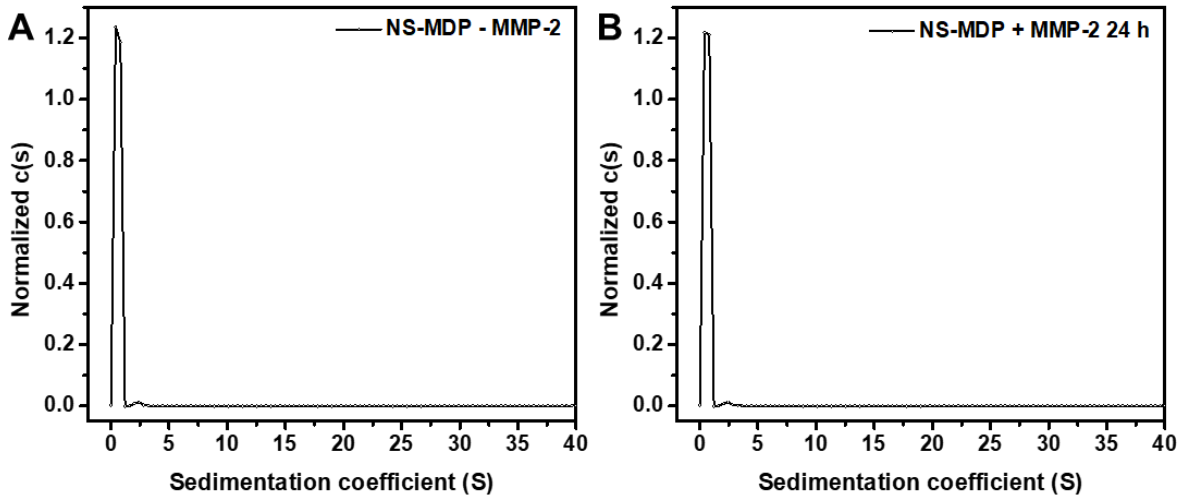

Figure S5. Continuous sedimentation coefficient distribution, c(s) curve of NS-MDP **A)** in the absence of MMP-2. **B)** upon MMP-2 (10 nM) treatment at 37 ℃ for 24 hrs. Peptides were prepared in Tris buffer (20 mM, pH=7.4) containing 5 mM $CaCl_2$ and 20 mM $ZnCl_2$ with a final peptide concentration at 20 μM.

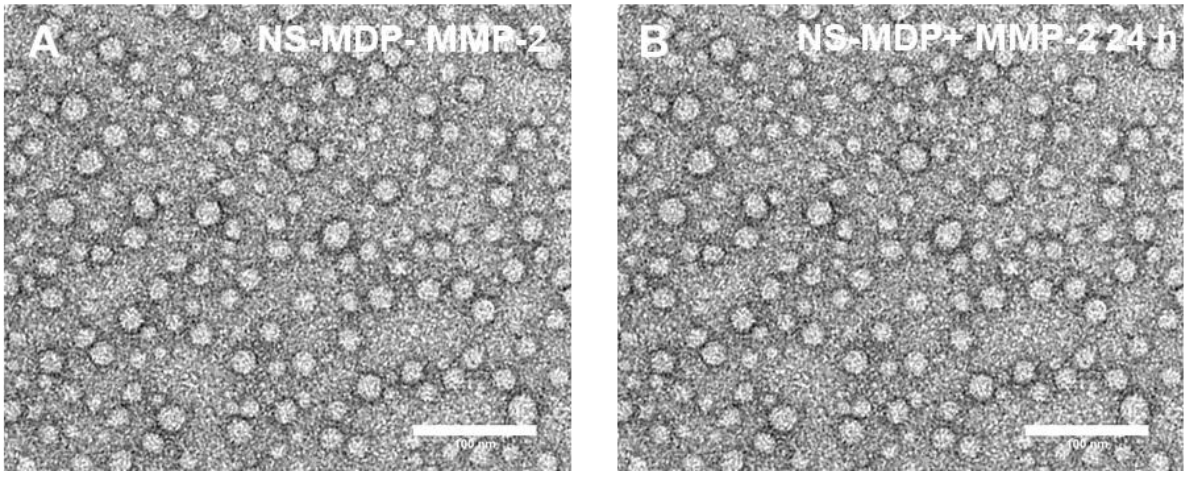

Figure S6. TEM images of NS-MDP **A**) in the absence of MMP-2. **B**) upon MMP-2 (10 nM) treatment at 37 ℃ for 24 hrs. Peptide concentration: 100 μm. Scale bar: 100 nm.

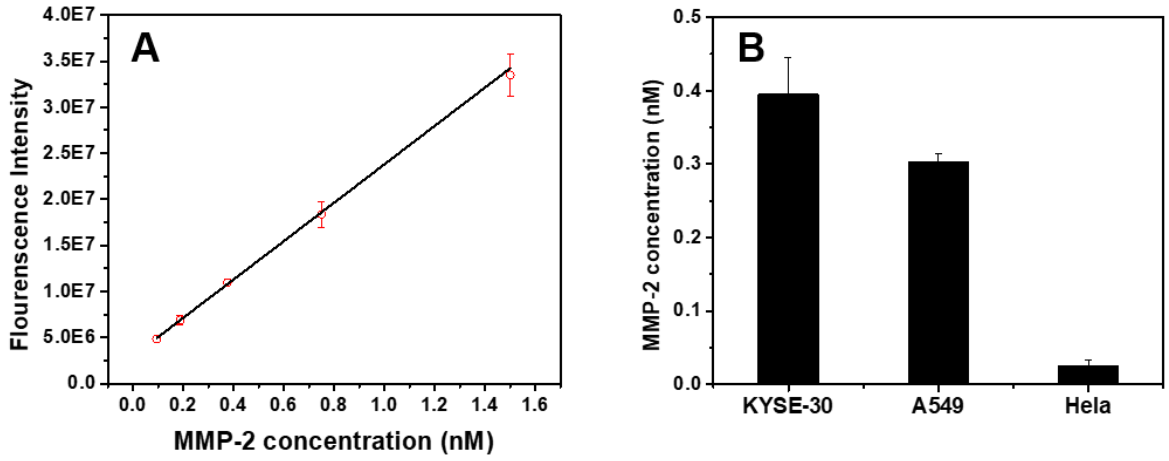

Figure S7. A) standard curve of MMP-2 concentration determined by SensoLyte MMP-2 activity assay. **B**) MMP-2 expression level in culture medium of KYSE-30, A549 and HeLa cells upon 24 hrs incubation.

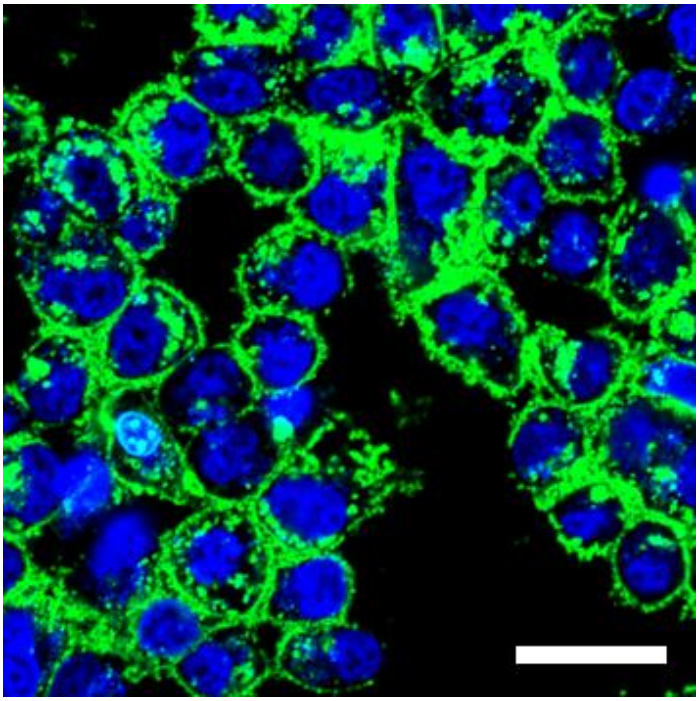

Figure S8. CLSM images of HeLa with 3 nM exogenous MMP-2 upon incubation with NBD labeled CS-MDP. Incubation time: 24 hrs. Final peptide concentration in the culture medium: 20 μM. Scale bar: 50 μm.

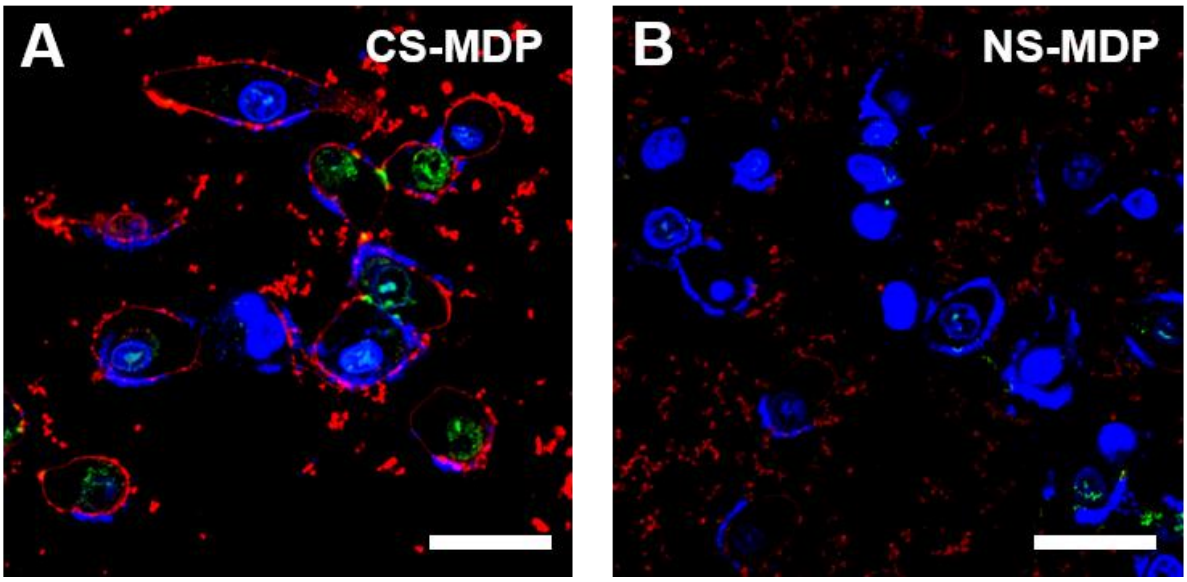

Figure S9. CLSM images of A549 cells after incubation with **A**) NBD-CS-MDP and **B**) NBD-NS-MDP followed by Congo red staining. Peptide concentration: 20 μm. Congo red concentration: 5 μm. Scale bar: 50 μm.

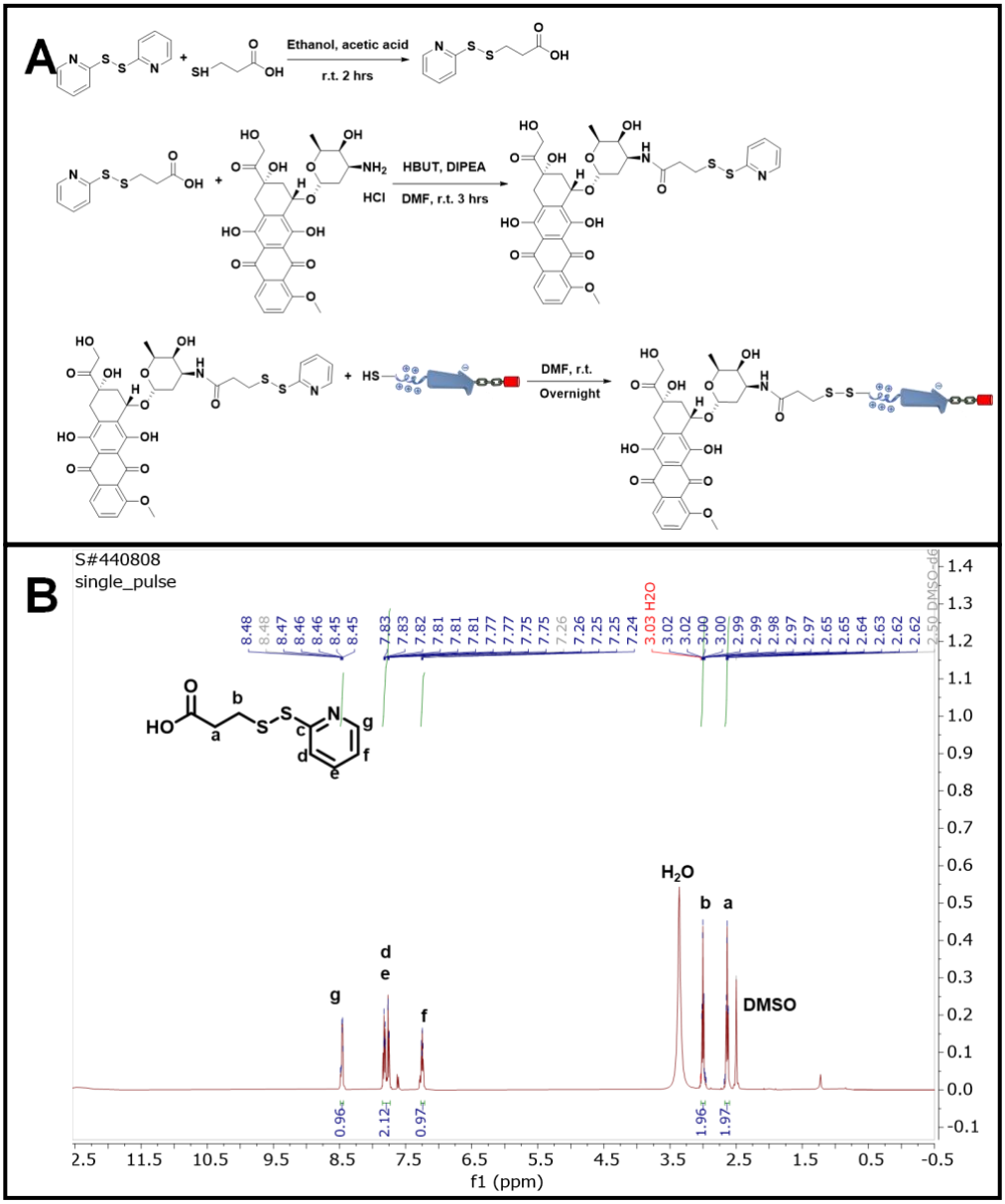

Figure S10. (**A**) Synthetic routes for the synthesis of DOX-CS-MDP through thiol-disulfide exchange reaction. (**B**) ^{1}H-NMR spectrum of Py-SS-MPA in DMSO-d_6.

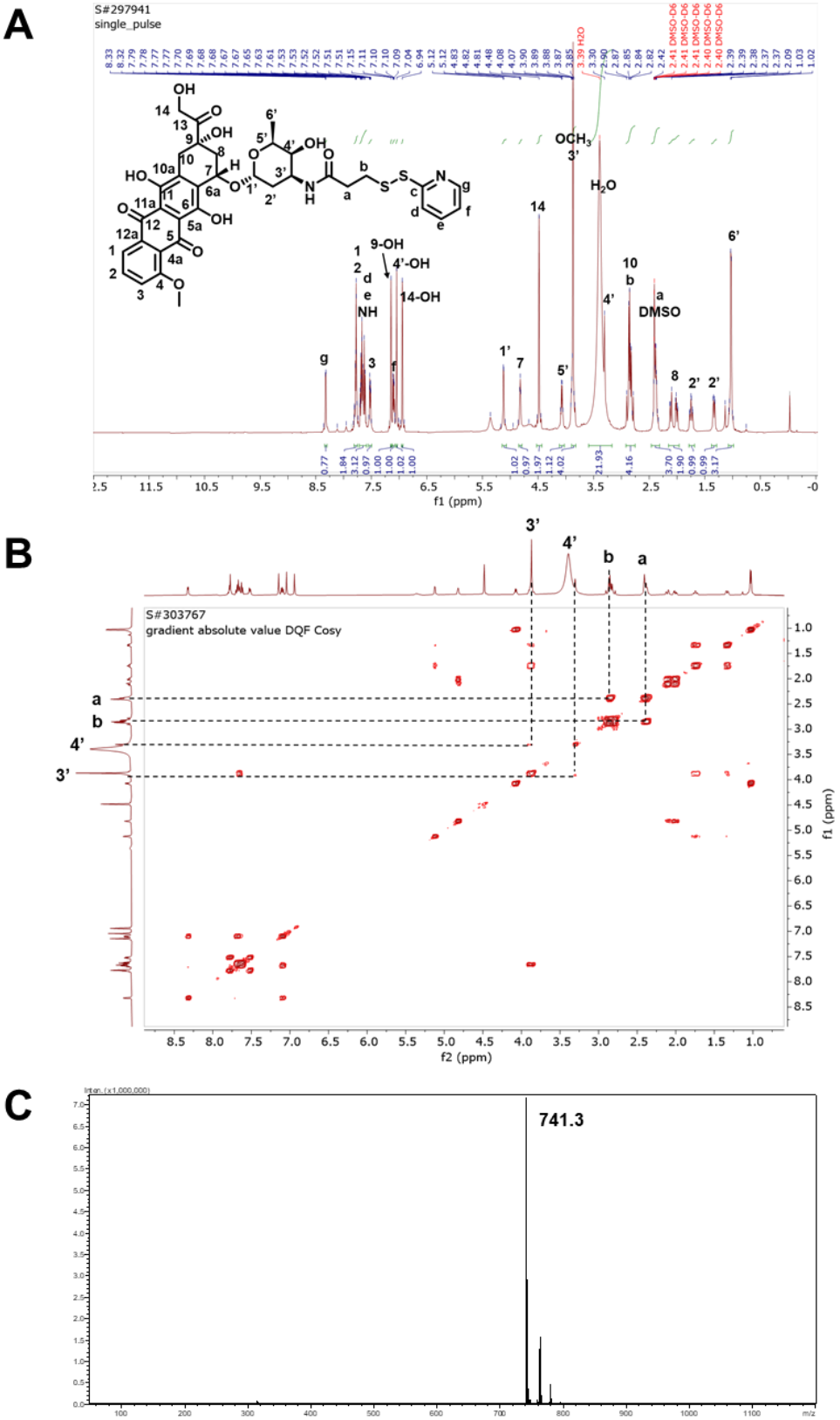

Figure S11. (**A**) The 1D ^{1}H-NMR of Py-SS-DOX in DMSO-d_6. (**B**) The 2D ^{1}H-^{1}H COSY NMR spectrum of Py-SS-DOX in DMSO-d_6 to further confirm the structure. Notably, H(4') and H(a) that were overlapped with solvents are identified through the correlation with vicinal protons. (**C**) ESI mass spectrum of Py-SS-DOX.

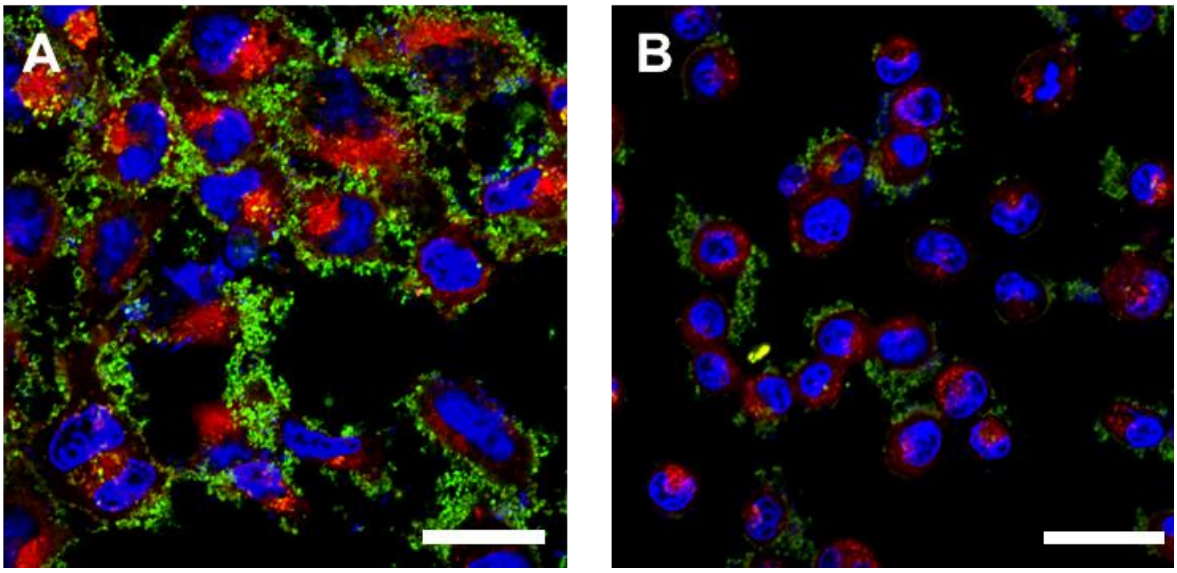

Figure S12. CLSM images of (**A**) KYSE-30 and (**B**) A549 with 3 nM exogenous MMP-2 upon incubation with NBD labeled DOX conjugated CS-MDP Incubation time: 24 hrs. The final peptide conc in the culture medium: 10 μM. Scale bar: 50 μm.

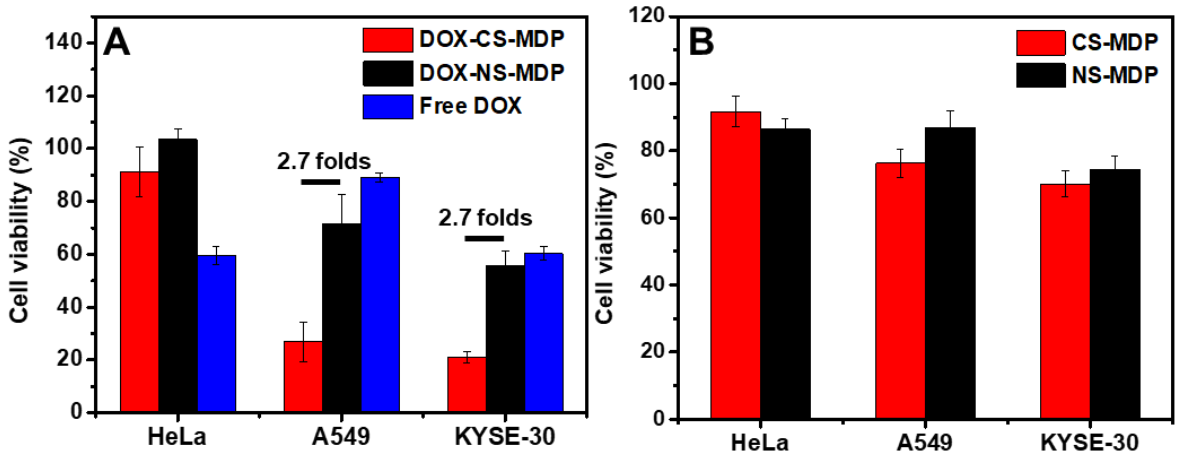

Figure S13. **A**) Cell viability assay of HeLa cell, A549 cell and KYSE-30 cell upon incubation with free DOX, DOX conjugated CS-MDPs and NS-MDPs. B) Cell viability assay of HeLa cell, A549 cell and KYSE-30 cell upon incubation with CS-MDPs and NS-MDPs. The assay was performed after 48 hrs of incubation of cells with peptides with a total peptide concentration of 20 μM.

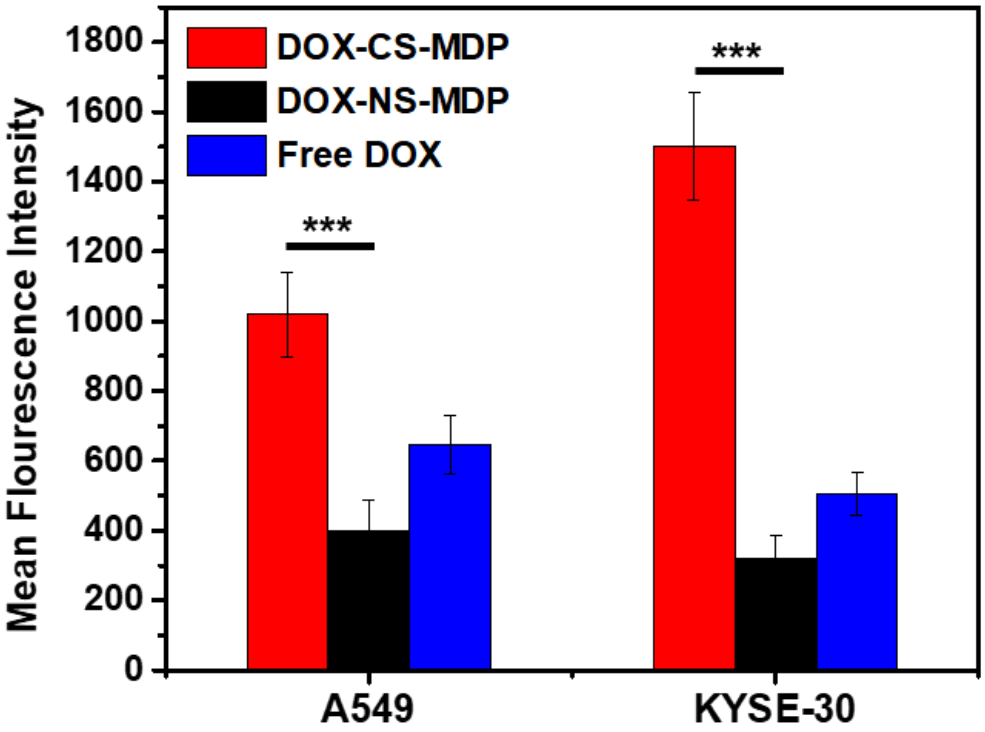

Figure S14. Flow cytometry of of HeLa cell, A549 cell and KYSE-30 cell treated with DOX conjugated CS-MDP and NS-MDP. Incubation time: 24 hrs. Final peptide conc in the culture medium: 20 μM. Statistic significant difference is indicated by ***$p < 0.001$.

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