Content **Page No.**

Experimental Methods

Materials and General Methods

Fmoc-protected amino acids were purchased from Novabiochem. PL-Rink resin was purchased from Polymer Laboratories. 1H-Benzotriazolium 1-[bis(dimethylamino) methylene]-5chloro-hexafluorophosphate (1-),3-oxide (HCTU) was obtained from Peptides International, and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) was purchased from Chem Impex International. Trifluoroacetic acid was obtained from Acros organics, and 1,2-ethanedithiol was purchased from Fluka. Fluorescein-NHS was obtained from Pierce. Lab-Tek™ 4-well chambered #1 borosilicate glass slides, 24 well cell culture plates, Diethyl ether, N-Methylpyrrolidone (NMP) and sodium fluoride (NaF) were purchased from Fisher Scientific. Thioanisole, anisole, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), low-melting temperature agarose and 200 mM glutamine solution were obtained from Sigma-Aldrich. RPMI-1640 media and Hoechst 33342 trihydrochloride dye was purchased from Invitrogen. Heat inactivated fetal bovine serum (FBS) and trypsin EDTA were obtained from Hyclone Laboratory Inc. 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (PE), and rhodamine-labeled PE were purchased from Avanti Polar Lipids. HPLC solvents consisted of solvent A (0.1% TFA in water) and solvent B (0.1% TFA in 9:1 acetonitrile/water). All cancer cell lines were obtained from the NCI-60 repository. Athymic nu/nu mice were obtained from the NCI Animal Production Facility, and utilized for in vivo studies following all animal welfare regulations as detailed by the Animal Care and Use Committee (ACUC; animal safety protocol #13-313). All peptides utilized for experiments were prepared with an amidated C-terminus.

Peptide Synthesis and Fluorescent Labeling

Peptide synthesis was carried out via Fmoc-based solid-phase peptide chemistry with HCTU activation on PL-Rink resin using an automated ABI 433A peptide synthesizer. Synthesis of the R8 peptide required use of HATU during synthesis. Peptides were cleaved from the resin and simultaneously side-chain deprotected using a trifluoroacetic acid/thioanisole/1,2-ethanedithiol/anisole (90:5:3:2) cocktail for 2 hours under argon atmosphere. The crude product was precipitated with cold diethyl ether and then lyophilized, followed by reverse-phase HPLC equipped with a semi-preparative Vydac C18 column to purify the conjugates. Linear gradients of 0−100% solvent B over 100 min. was utilized to purify the CLIP2 – CLIP6 peptides. A gradient of 0-18% solvent B over 18 min., followed by 18-40% solvent B over an additional 44 min. was utilized to purify CLIP0. For purification of the TAT peptide, a linear gradient of 0−100% solvent B over 200 min. was employed. R8 was purified using a gradient of 0-4% solvent B over 16 min., followed by 4-30% solvent B over an additional 52 min. Transportan was purified using a gradient of 0-40% solvent B over 20 min., followed by 40-80% solvent B over an additional 40 min. The MAP peptide was purified using a gradient of 0-20% solvent B over 10 min., followed by 20-60% solvent B over an additional 40 min. Penetratin was purified using a gradient of 0-24% solvent B over 12 min., followed by 24-35% solvent B over an additional 44 min. All peptides were lyophilized to collect the pure product, and the purity verified by analytical HPLC-MS.

Fluorescently-labeled CPPs were prepared by reacting the N-terminal amine of the peptide on resin with Fluorescein(FI)-OSu (2 eq.) and DIEA (4 eq.) in NMP for 2 h while shaking at room temperature. All peptides were cleaved and purified following the conditions listed above for the unlabeled peptides. Analytical HPLC chromatograms and ESI (+) mass spectra for all of the pure peptides are shown in the Supporting Information, Figures S8 – S19.

Circular Dichrosim Spectroscopy

Circular Dichroism (CD) experiments were performed to determine the secondary structure of the peptides in aqueous buffer and in the presence of large unilamellar vesicles (LUV) as model lipid membranes. LUV of POPC and POPC/POPS (1:1) were prepared by extrusion techniques as previously described.[1] CD spectra of the peptides were obtained in aqueous solution (50 mM BTP, 100 mM KF, pH 7.4) and in the presence of LUV (final lipid concentration 2.5 mM). Peptide solutions (50 μM) were used to provide a peptide/lipid ratio of 1:50. Wavelength spectra were measured from 200 to 260 nm at 37 °C

using a 1 mm path length quartz cell. The mean residue ellipticity, [θ], was calculated from the equation $[\theta] = (\theta_{obs}/10lc)/r$, where θ_{obs} is the measured ellipticity (mdeg), *l* is the length of the cell (cm), *c* is the molar concentration, and *r* is the number of residues. CD spectra were collected on an AVIV model 420 circular dichroism spectrometer (AVIV Biomedical, Lakewood, NJ).

In Vitro Cytotoxicity

A549 and HeLa cells were plated at 5 x 10^3 cells/well, while MCF-7 cells were plated at 10 x 10^3 cells/well, in a 96 well plate and allowed to adhere overnight. The culture media was then removed and cells treated with 0.01 – 1000 µM concentrations of CPPs in serum-free media for 24 hours. Blank media or 20% DMSO containing media was used as a positive and negative control, respectively. After this incubation period cells were washed and 100 µL of fresh serum-containing media was added to each well. 10 µL of MTT solution (5 mg/mL in PBS) was added to each well and samples incubated for 2 hours for A549 and HeLa cell, or 4 hours for MCF-7 cells. The supernatant was then removed from each well and replaced with 150 µL of DMSO to dissolve the formazan product. Absorbance was then read at 540 nm using a UV plate reader (Biotek, Winooski, VT). The absorbance of the negative controls was subtracted from each sample as a blank, and percent viability calculated using the equation: $(Absorbance_{peptide\text{-}treated\ cells}/Absorbance_{untreated\ cells}) \times 100$. GraphPad Prism 5 software was used to fit cytotoxicity curves and calculate IC_{50} values employing a log(inhibitor) vs. normalized response non-linear regression model. Results shown represent the average of three independent experiments ± standard deviation.

Hemolysis and Serum Stability Assay

Hemolysis studies were performed by collecting fresh blood from healthy human volunteers in heparinized tubes, followed by immediate centrifugation at 3,460 rpm for 10 min. at 4°C. RBCs were washed three times with hemolysis buffer (10mM Tris, 150 mM NaCl, pH 7.4), and a 0.25% v/v solution of RBCs in hemolysis buffer was prepared. In a 96 well plate, 75 µL of the RBC solution was mixed with an equal volume of 2x peptide dissolved in hemolysis buffer to initiate the assay. Blank or 1% Triton-X100 containing buffer were used as negative and positive controls, respectively. Samples were incubated for 24 hours with gentle shaking, followed by centrifugation of the plates at 4,000 rpm for 10 min. at 4°C to pellet intact RBCs. 100 µL of the supernatant from each well was then removed and added to an empty 96 well plate. Absorbance was measured at 415 nm using a microplate reader (Biotek, Winooski, VT), and percent hemolysis was calculated using the following equation: $((Absorbance_{treated\ hRBC} - Absorbance_{untreated\ hRBC})/(Absorbance_{triton\text{-}X100\ treated\ hRBC} - Absorbance_{untreated\ hRBC})) \times 100$. Each treatment condition was performed in triplicate using RBCs from two independent blood donors. EC_{50} values were computed using the Graphpad 5.0 software package and represented as the average percent hemolysis ± standard deviation.

For serum stability studies, human blood was collected from healthy volunteers in non-heparinized tubes and kept at room temperature for 30 min. to clot. Blood was then centrifuged at 2,000 x g for 10 min. at 4°C, and serum either used immediately or stored as aliquots at -80°C before use. Peptides were weighed out as a dry lyophilized powder, and dissolved in 1:3 serum:buffer (20mM Tris-HCl, 100mM NaCl, pH 7.4) to a final concentration of 300 µM. Samples were then incubated at 37°C in a heated shaker. At 0, 0.5, 1, 2, 4 or 8 hours of incubation a 100 µL aliquot was removed and diluted with an equal volume of 15 wt% TCA in water, and the sample was kept on ice for 15 min. Samples were then centrifuged at 13,000 rpm for 10 min. and supernatant collected and subjected to LC/MS. Area under the curve for two characteristic ions of the parent CLIP6 (744.5 Da $[M + 3H]^{3+}$ & 558.6 Da $[M + 4H]^{4+}$) or TAT (574.0 Da $[M + 3H]^{3+}$ & 430.8 Da $[M + 4H]^{4+}$) peptides at each time point was measured from the mass TIC. Results were normalized to ion values at t = 0 h, and then averaged for three replicates, to determine the percent of intact peptide as a function of time.

Cellular Uptake of CPPs

Flow cytometry studies to determine the mechanism of CLIP6 uptake were performed by preconditioning cells before peptide addition. Briefly, 2.5 x 10^5 A549, HeLa or OVCAR-3 cells/well were prepared in a 24-well plate and allowed to adhere overnight under normal culture conditions. Cells were depleted of intracellular ATP by preincubation in glucose and serum free media containing 10mM sodium azide and 50mM 2-deoxy-D-glucose for 30 min.[2] To selectively prevent clathrin-mediated endocytosis cells were

alternatively pre-incubated in serum free media containing 0.45M sucrose for 1 h.[3] Samples were washed, and incubated with 10 µM of fluorescently-labeled peptide in serum-free media for 1 h. Uptake studies evaluating the effect of concentration and arginine-content on internalization of the CLIP peptides were performed in a similar manner, without the preconditioning steps. After treatment, cells were washed with cold PBS and incubated with 150µL of 0.25% trypsin-EDTA solution for 15 min. to both collect cells for analysis and digest any non-internalized peptide adsorbed to the surface of the cell based on a previously published procedure.[4] Cells were then pelleted by centrifugation at 2,000 rpm for 5 min. before suspending them in 1 mL of fresh PBS. Samples were analyzed by a Beckman Coulter FACsCalibur flow cytometer (488 nm excitation laser) with gating based on normalized fluorescence of untreated cells to evaluate the percentage of cells which internalized the fluorescently-labeled peptides. Uptake studies were performed in three independent experiments using three replicates for each experimental condition.

For live-cell imaging studies 2 x 10^4 HeLa cells/well were seeded in 4-well chambered glass slides and allowed to adhere overnight under normal culture conditions. Cells were washed with serum free RMPI-1640 media and incubated with 2 µg/mL Hoechst 33342 dye for 20 min. Glass slides were washed with serum free media and mounted onto a Zeiss LSM NLO510 Confocal Microscope with an environmental chamber to maintain 37°C and 5% CO_2 during live cell imaging experiments. Media was then removed from a selected well and replaced with 1mL of either serum-free or 10% serum media containing 10 µM FI-CLIP6. Samples were incubated for 5-60 min., followed by a final wash and addition of 1mL blank media before imaging. Cells were visualized using a Neofluar 40x oil objective, with planar and z-stack images collected on a selected cell utilizing a two-photon 800nm laser for the Hoechst signal (band pass emission filter 390nm – 465nm) and a single photon 488nm laser for the fluorescein and phase contrast signals (band pass emission filter 500nm – 550nm). Microscopy images were processed using LSM 5 software and shown as the overlaid fluorescein and Hoechst signals, with the borders of the cell outlined in white from the phase contrast signal for clarity. Percentage of total fluorescence at the membrane, within the cytoplasm or localized to the nucleus for each image was determined using ImageJ software and values normalized to background fluorescence.

CLIP6 Translocation into GUVs

Giant unilamellar vesicles (GUVs) were prepared using the agarose method, as detailed by Horger et al.,[5] with slight modification. Briefly, a solution of 1 wt% low-melting agarose was added to a 4-well chambered slide until it just covered the glass surface. The excess solution was drained and the slide allowed dried on a 45°C hotplate for 1 h. During drying additional agarose was added as needed to establish a uniform coating across the glass surface. Once the agarose film was established, 4 µL of a 3.75 mg/mL lipid solution in chloroform was deposited evenly across one side of the well. The lipid solution was then distributed across the agarose film by dragging a metal spatula carefully over the surface. This procedure was repeated once more in the reverse direction to obtain an even coating of the lipid solution, followed by drying under vacuum overnight. GUVs were prepared by swelling the dried lipid film in a 500 µL volume of pre-warmed (37°C) 150 mM KCl, followed by incubation for 15 minutes to allow for complete vesicle fusion. An aliquot of fluorescein-labeled CLIP6 in water was then added to the swelled GUV solution to achieve a final peptide concentration of 5 µM. Slides were mounted onto a Zeiss LSM NLO510 Confocal Microscope, equipped with environmental chamber, and imaged using a Neofluar 63x oil objective with a single photon 488nm (band pass emission filter 500nm – 550nm) and a 561nm (band pass emission filter 575nm – 615nm) laser for the fluorescein and rhodamine signals, respectively. Translocation ratio was calculated using ImageJ software, by dividing the extra-vesicular and intra-vesicular mean fluorescence intensities for three individual GUVs in the view field. Experiment was performed in duplicate

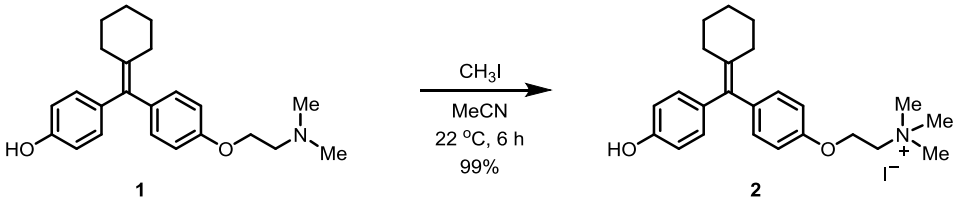
In Vivo MTD Studies

Before initiating animal studies, peptides were first purified of any contaminating endotoxin by loading 10 mL of a 10 mg/mL peptide solution in sterile water to a high capacity endotoxin removal spin column containing 1 mL of resin (Pierce, Rockford, IL), and shaken for 4 h. After centrifugation following the manufacturer's instructions, the retrieved peptide was lyophilized and tested for endotoxin levels using a LAL chromogenic endotoxin quantification kit (Pierce, Rockford, IL). Endotoxin levels were routinely found to be <2 EU/mL for the final pure peptides. MTD was determined by injecting the peptide prepared in

sterile HBSS to the tail vein of mice at 10 mL/kg volumes. The peptide solution was administered every other day for 7 days, totaling 3 injections. During this period, and for an additional week after treatment, animal body weight measurements and clinical observations of toxicity were performed daily. Dosing of the peptide was increased until any single animal showed weight loss greater than or equal to 20%, if they were unable to obtain food or water, had difficulty breathing, or appeared moribund; at which point the dosing level was considered to have exceeded the MTD.

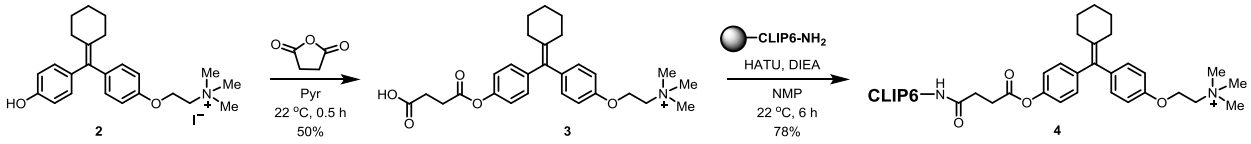
General Procedure for the Synthesis of 4-OHC$_Q$ and Its Coupling to the CLIP6 Peptide

Unless stated otherwise, reactions were conducted in oven-dried glassware under an atmosphere of argon using anhydrous solvents (passed through activated alumina columns). All commercial reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Analytical LC/MS was performed using a Shimadzu LCMS-2020 Single Quadrupole utilizing a Kinetex 2.6 µm C18 100 Å (2.1 x 50 mm) column obtained from Phenomenex, Inc. Runs employed a gradient of 0-90% MeOH/0.1% aqueous formic acid over 4.5 min at a flow rate of 0.2 mL/min. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometers (at 400 MHz and 100 MHz, respectively) and are reported relative to deuterated solvent signals. Data for ^{1}H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz), and integration. Data for ^{13}C NMR spectra are reported in terms of chemical shift.



1 2

(**2**): 4-hydroxycyclofen (4-OHC, **1**) was synthesized as previously reported.[6] To a solution of 4-hydroxycyclofen (100 mg, 0.28 mmol) in anhydrous acetonitrile was added methyl iodide (90 µL, 1.42 mmol). The resulting mixture was stirred at room temperature for 6 h. The solvent was evaporated to afford 4-OHC$_Q$ (**2,** 138 mg, 99% yield) as an off-white solid. ^{1}H NMR (CD$_3$CN, 400 MHz) δ 7.03 (dd, *J* = 7.8, 5.7 Hz, 3H), 6.94-6.85 (m, 4H), 6.80-6.72 (m, 2H), 4.38 (dq, *J* = 7.3, 2.6 Hz, 2H), 3.79-3.70 (m, 2H), 3.19 (s, 9H), 2.17 (q, *J* = 5.9 Hz, 4H), 1.63-1.49 (m, 6H); ^{13}C NMR (CD$_3$CN, 100 MHz) δ 156.30, 139.26, 138.05, 131.67, 131.52, 118.27, 115.71, 115.02, 62.79, 55.09, 55.05, 55.01, 33.01, 29.31, 27.46; ESI-MS [M+H]$^{1+}$: calcd. 366.2, obsvd. 366.3.



(**4**): 4-OHC$_Q$ (**2**, 52 mg, 0.11 mmol) was azeotroped twice with toluene to remove residual water, and dissolved in 1.05 mL of neat pyridine containing succinic anhydride (105 mg, 1.10 mmol). The reaction mixture stirred for 30 min. at room temperature, concentrated *in vacuo* and purified by reverse-phase HPLC using a gradient of 0-45% solvent B over 17 min., followed by 45-100% solvent B over an additional 55 min. to afford **3** (25.7 mg, 50% yield) as an off-white solid. ESI-MS [M+H]$^{1+}$: calcd 466.6; obsvd 278.1. Compound **3** (10.4 mg, 0.022 mmol) was then dissolved in 0.5 mL anhydrous NMP containing HATU (8.5mg, 0.022 mmol) and DIEA (2.71 µL, 0.051 mmol), and stirred for 5 min. at room temperature to activate. The mixture was then coupled to the N-terminal amine of CLIP6 by adding the activated mixture to the peptide on resin (74 mg, 0.015 mmol), and shaking at room temperature for 6 h. The conjugate was cleaved from the resin and simultaneously side-chain deprotected using a trifluoroacetic acid/triisoproylsilane/water (95:2.5:5.5) cocktail for 2 hours under argon atmosphere. The crude product was precipitated with cold diethyl ether and then lyophilized, followed by reverse-phase HPLC using a linear gradient of 0−100% solvent B over 100 min. to afford the pure CLIP6-4-OHC$_Q$

conjugate (**4**, 26.1 mg, 78% yield) as a fluffy white solid. The analytical HPLC chromatogram and ESI (+) mass spectra for the pure conjugate is shown in Figure S20.

MEF Activation

Primary mouse embryonic fibroblasts (MEFs) were isolated from E13.5 $Rosa^{CreER/mTmG}$ embryos as previously described,[7] and cultured in DMEM media containing 10% FBS, L-glutamine and gentamicin. For imaging studies cells were seeded at 15 x 10^3 cells/well in a 6 well plate and allowed to adhere overnight. The culture media was then removed and cells treated with serum-free media containing 10 μM of free 4-OHC_Q, the CLIP6 peptide alone, or the CLIP6-4-OHC_Q conjugate. Blank media, or a 1 μM solution of the cell-permeable 4-OHC compound, were included as negative and positive controls, respectively. It should be noted that a 10 μM concentration of 4-OHC was toxic towards MEFs, and therefore required us to utilize the 1 μM concentration of the positive control. Cells were incubated for 2 h, washed with serum-free media, and cells incubated for an additional 48 h in serum-containing media. Cells were then washed and incubated with 2 μg/mL Hoechst 33342 dye for 20 min., before being mounted onto an EVOS FL Auto fluorescent microscope (Life Technologies, Grand Island, NY) equipped with an environmental chamber to maintain 37°C and 5% CO_2 during experiments. Cells were imaged at 20x magnification using the manufacturer LED light cubes for DAPI (357/44 nm excitation, 447/60 nm emission), RFP (531/40 nm excitation, 593/40 nm emission) and GFP (470/22 nm excitation, 510/42 nm emission).

For analysis by flow cytometry, MEFs were treated using the same procedure as described above. Cells were then washed with cold PBS and incubated with 500μL of 0.25% trypsin-EDTA solution to remove them from the surface of the plates. After typsinization, cells were suspended in cold PBS and pelleted by centrifugation at 2,000 rpm for 5 min. Samples were analyzed by a Beckman Coulter FACsCalibur flow cytometer using an 488 nm excitation laser, and a 530/30 or 575/25 emission filter for EGFP and tdTomato, respectively. Signal compensation and gating was established based on the normalized fluorescence of untreated cells.

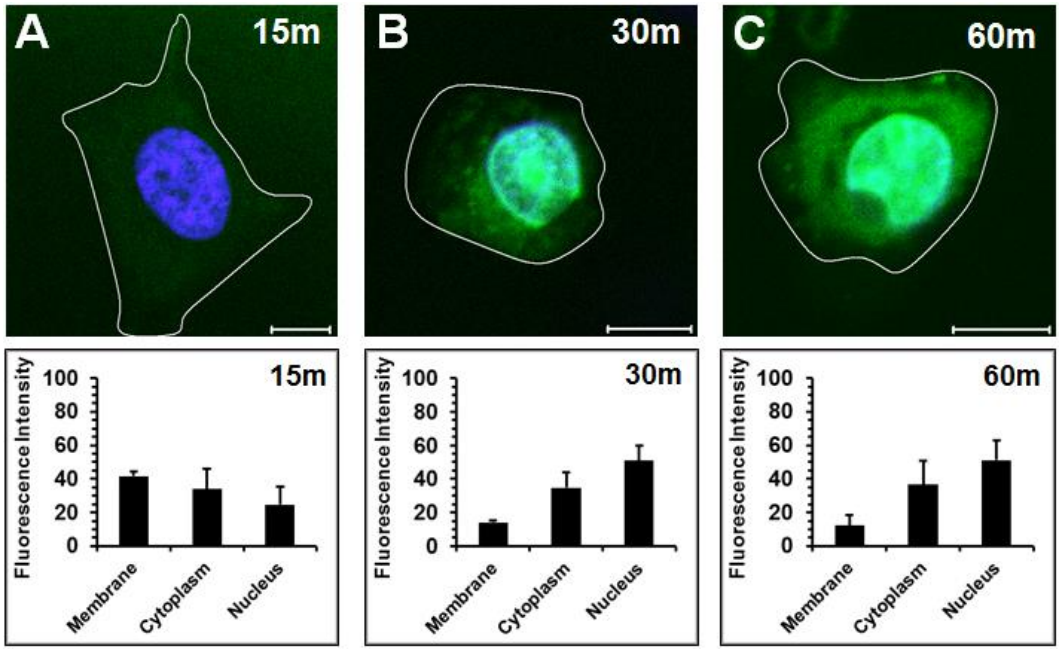


Figure S1: (**A-C**, *Top*) Live-cell imaging of HeLa cells as a function of time after addition of 10 μM fluorescein-labeled CLIP6 peptide in 10% serum-containing media (scale bar = 10 μm). (**A-C**, *Bottom*) Percentage of total cellular fluorescence measured at the membrane, within the cytoplasm or localized at the nucleus.

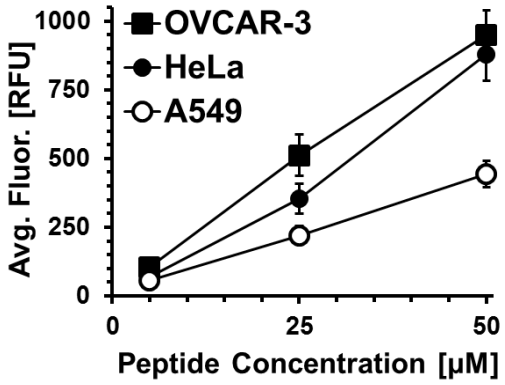


Figure S2: Average intracellular fluorescence of OVCAR-3, HeLa, or A549 cells (in relative fluorescence units) treated with increasing concentrations of the CLIP6 peptide.

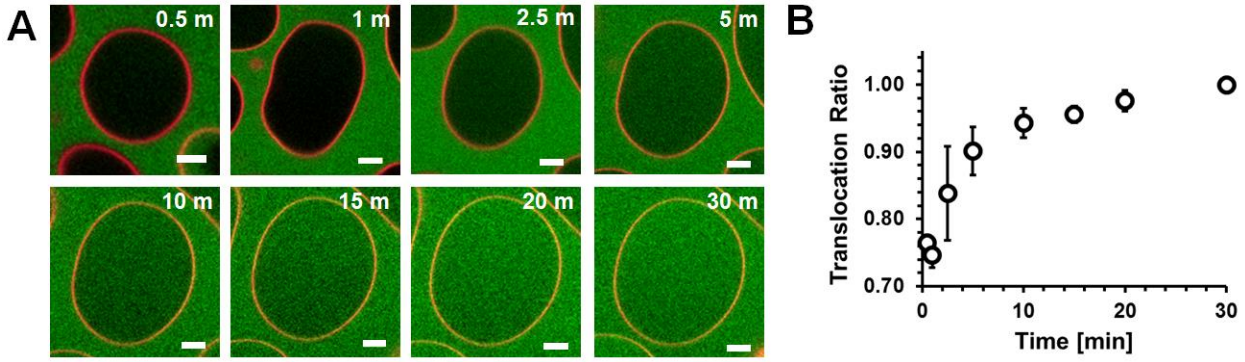


Figure S3: (**A**) Fluorescent confocal microscopy images showing the translocation of the fluorescein-labeled CLIP6 (green) peptide into giant unilamellar vesicles (GUVs) as a function of time (scale bar = 5 µm). GUVs were prepared at a 30:40:30 ratio of PS:PC:PE lipids, with 0.5 mol% of rhodamine-labeled PE included to aid in visualization (red). (**B**) Translocation ratio of CLIP6 as a function of time, calculated as the equilibration of the average fluorescence intensity from the extra-vesicular space to the interior of GUVs.

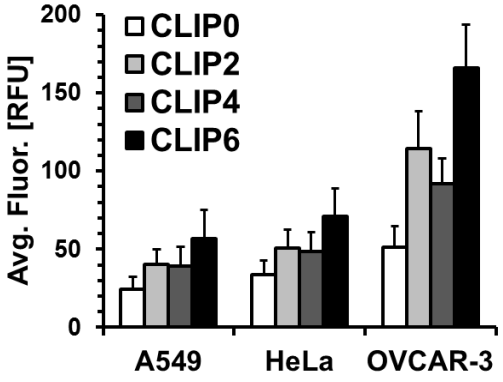


Figure S4: Influence of arginine-content on peptide cellular internalization. CLIP0 – CLIP6 peptides contain zero to six arginines in their sequence, respectively, with a corresponding substitution of these residues by lysine. See table S1 for exact sequence information.

Name	Sequence	No. of Residues	Charge
CLIP0	KVKVKVKV^{D}PPTKVKEKVK-NH_2	18	+8
CLIP2	KVKVRVKV^{D}PPTKVREKVK-NH_2	18	+8
CLIP4	KVRVRVKV^{D}PPTKVRERVK-NH_2	18	+8
CLIPΔ	KVRVRVRV^{D}PPTRVRVRVK-NH_2	18	+9

Table S1: Sequence of CLIP6 analogues and their charge under physiologic conditions.

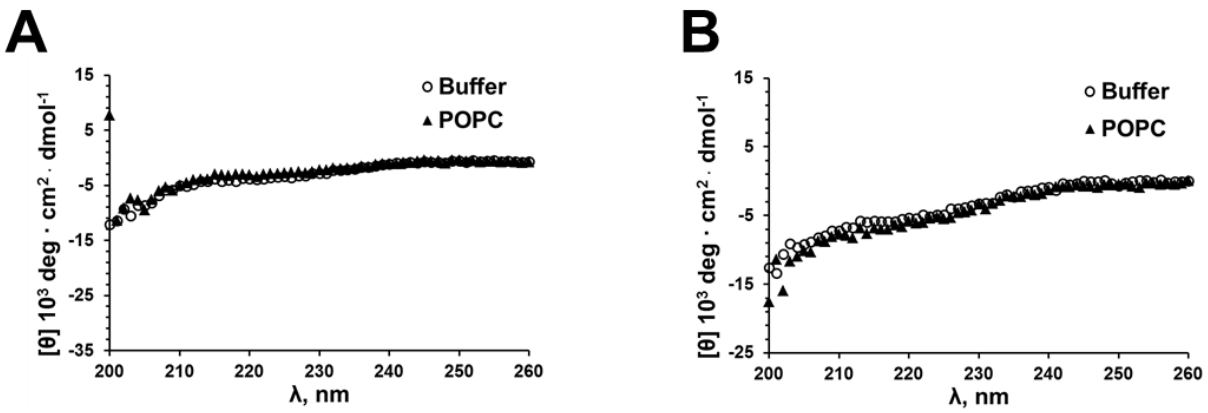


Figure S5: CD spectra of 50 µM (**A**) CLIP6 or (**B**) CLIPΔ peptides in the presence of (○) Buffer (50 mM BTP, 100 mM KF, pH 7.4) or (▲) Neutral POPC LUVs.

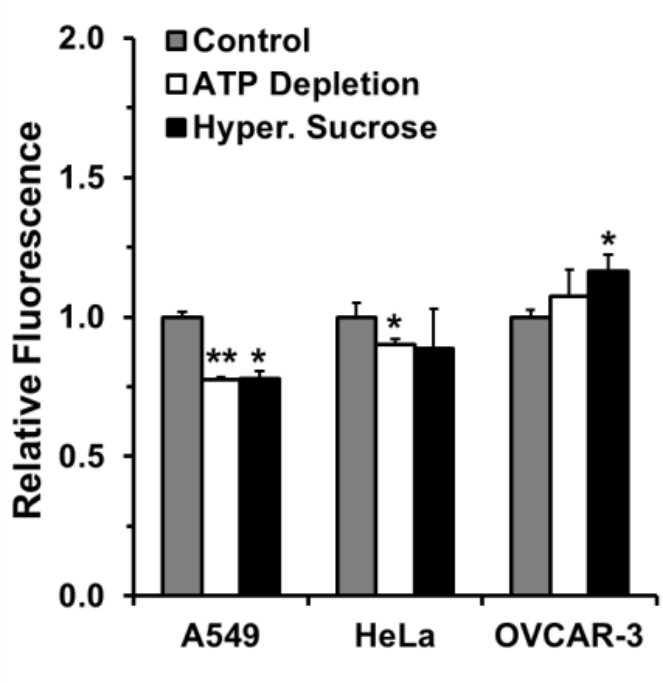


Figure S6: Mechanism of CLIPΔ uptake into cells after a 1 hour incubation with 10 μM of peptide alone (control, gray bars), or with ATP depletion (open bars) and hyperosmolar sucrose (black bars) preconditioning. Uptake results under ATP depletion and hyperosmolar sucrose conditions were compared to direct peptide incubation (gray bars) to determine statistical significance, which is denoted by * indicating $p \leq 0.05$, or ** indicating $p \leq 0.01$.

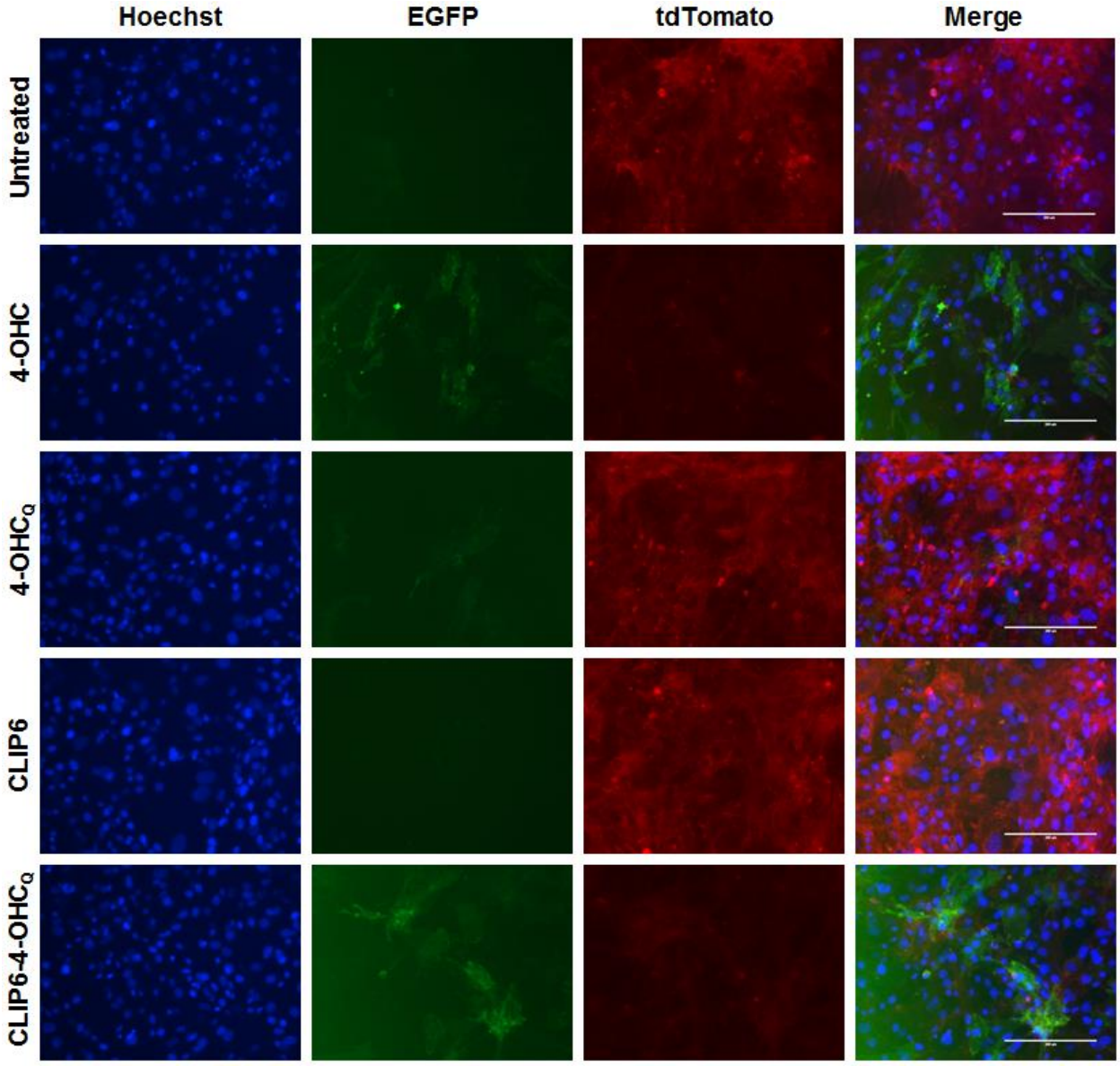
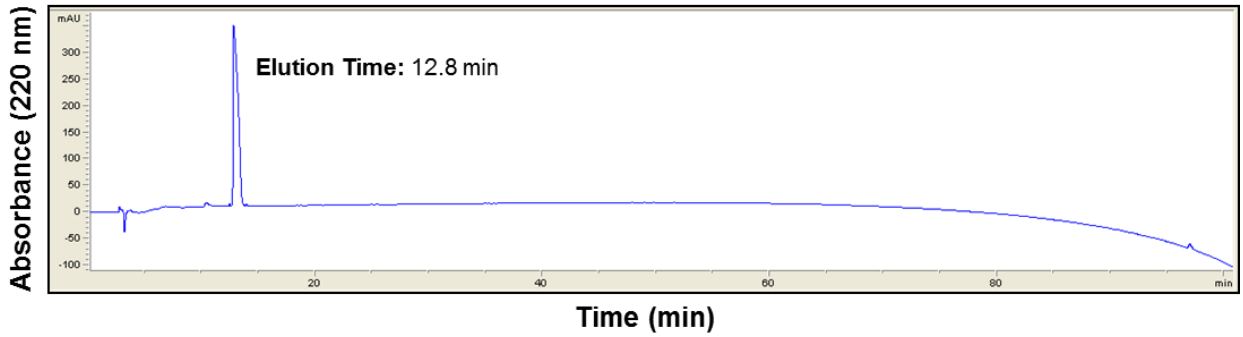


Figure S7: Individual fluorescence channels from live-cell microscopy performed on MEF cells incubated with the indicated compounds (scale bar = 200 µm).

A



B

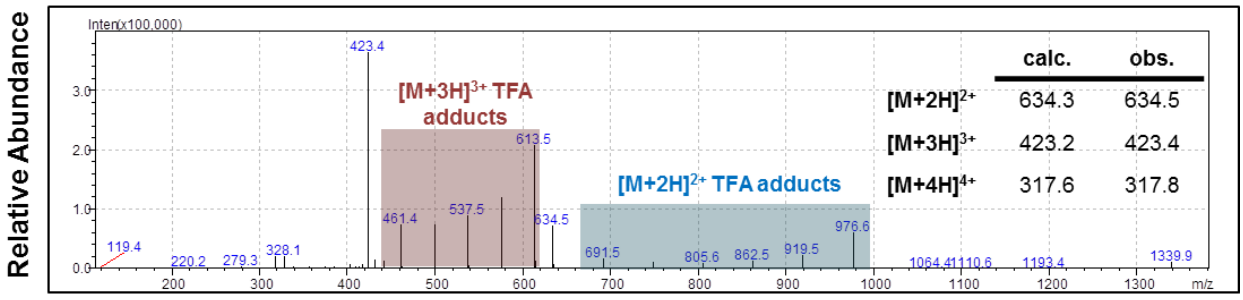


Figure S8: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified R8.

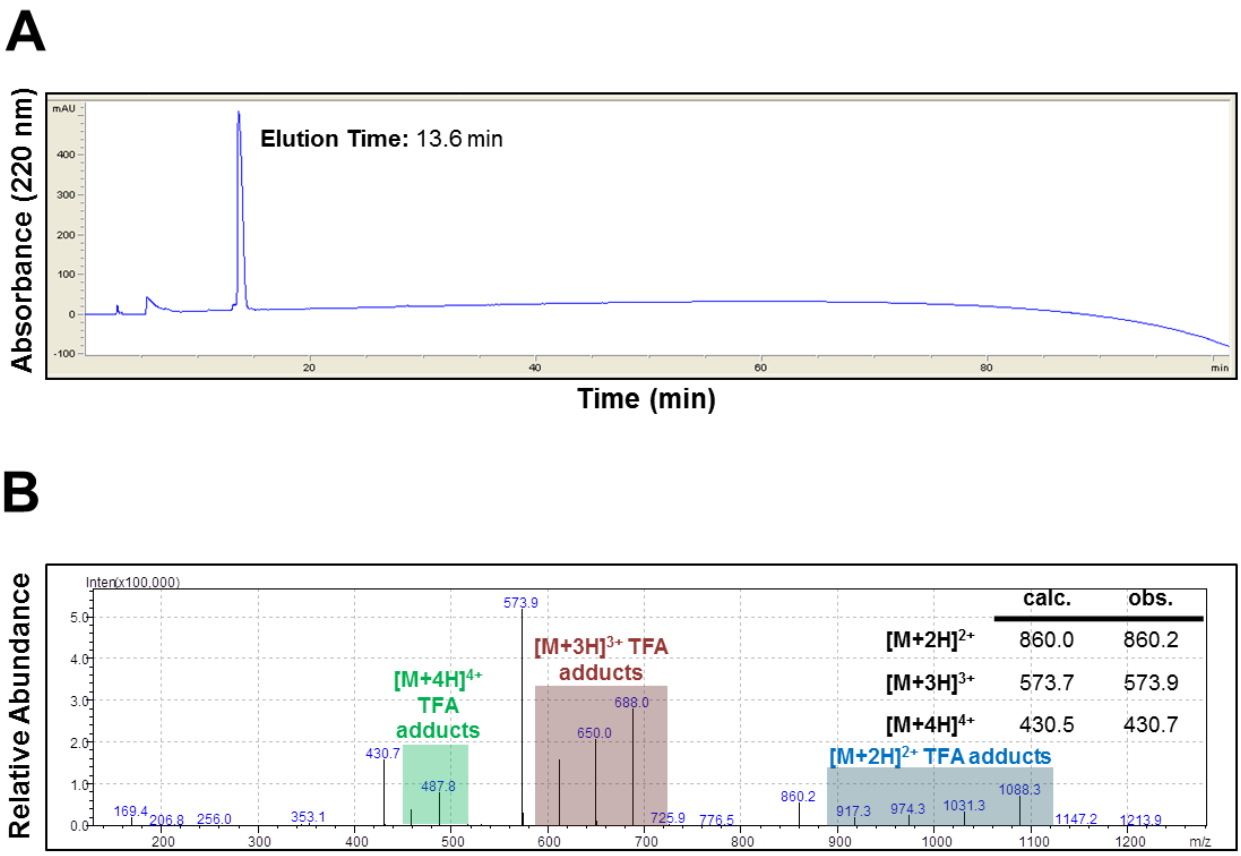
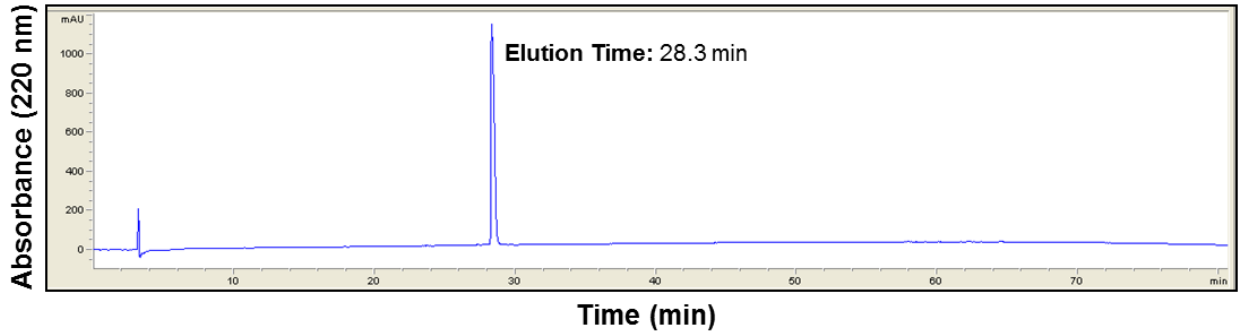


Figure S9: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified TAT.

A



B

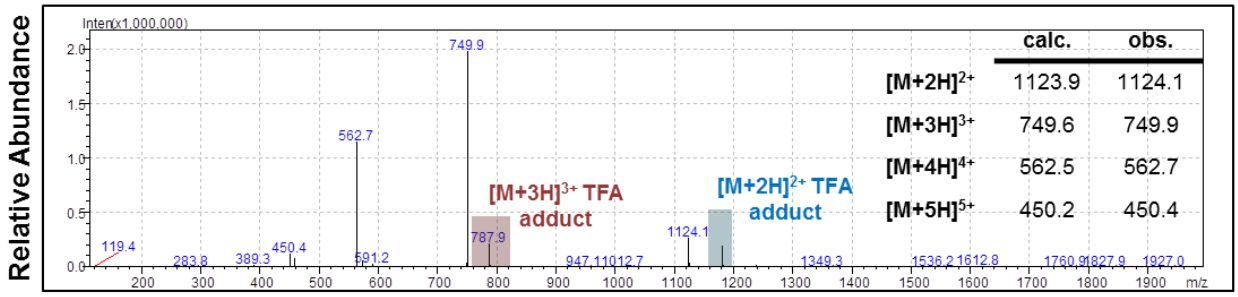
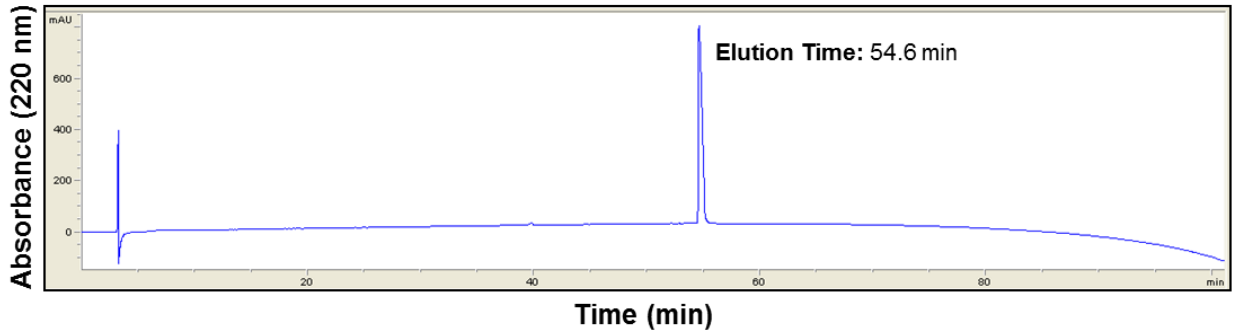


Figure S10: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified Penetratin.

A



B

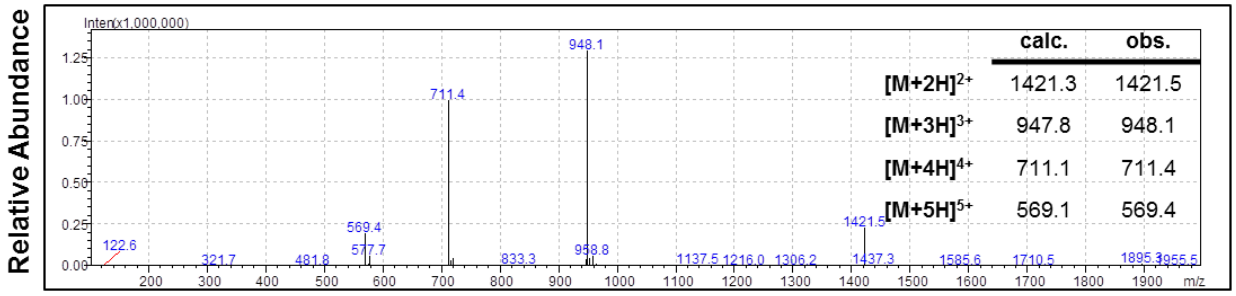
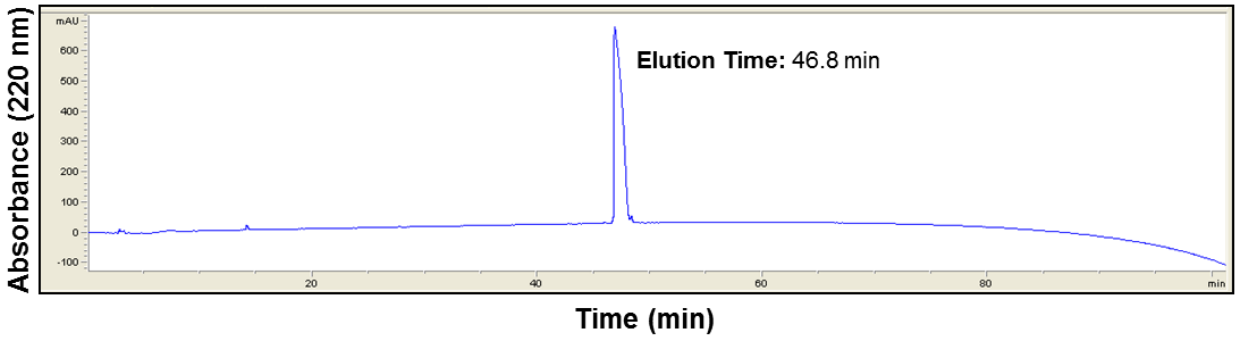


Figure S11: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified Transportan.

A



B

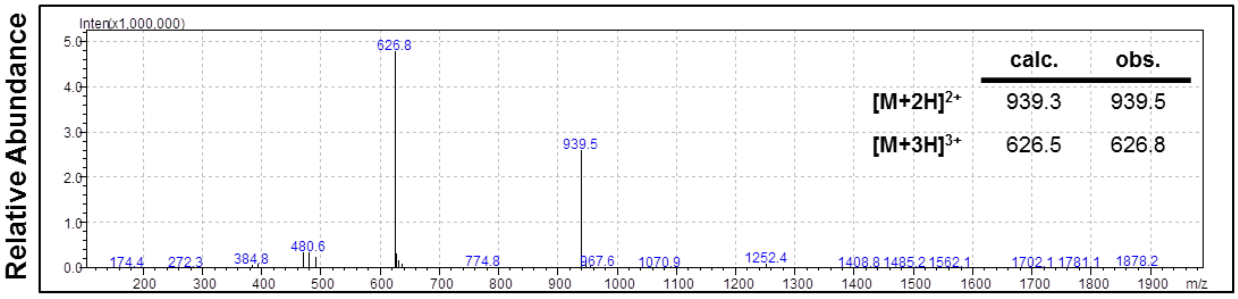


Figure S12: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified MAP.

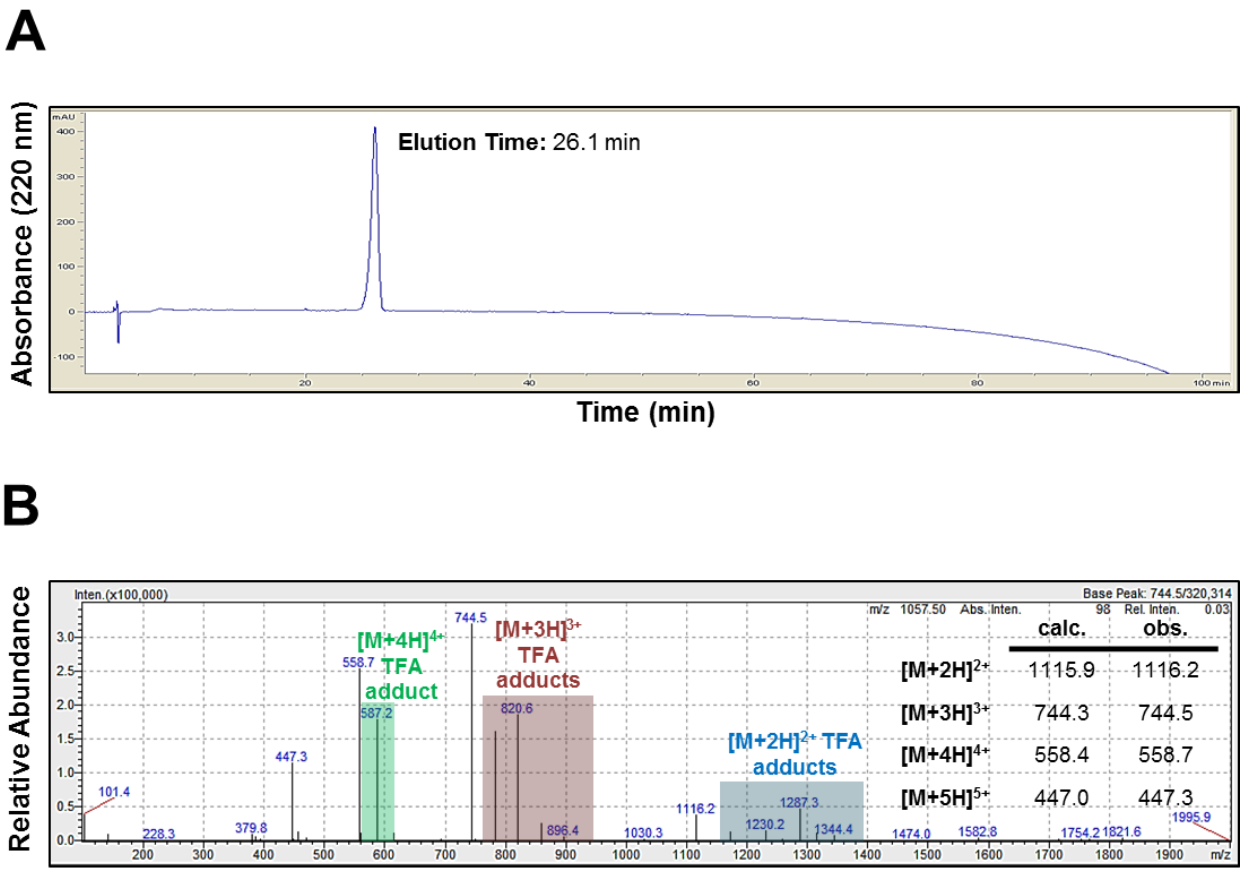
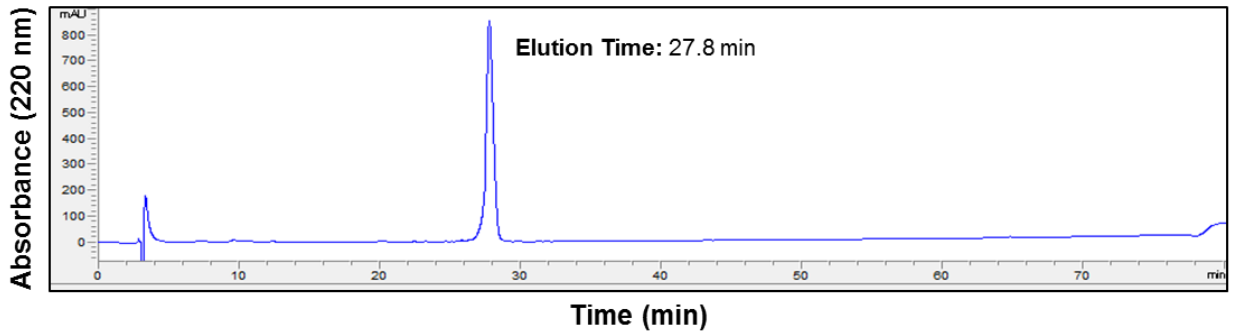


Figure S13: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified CLIP6.

A



B

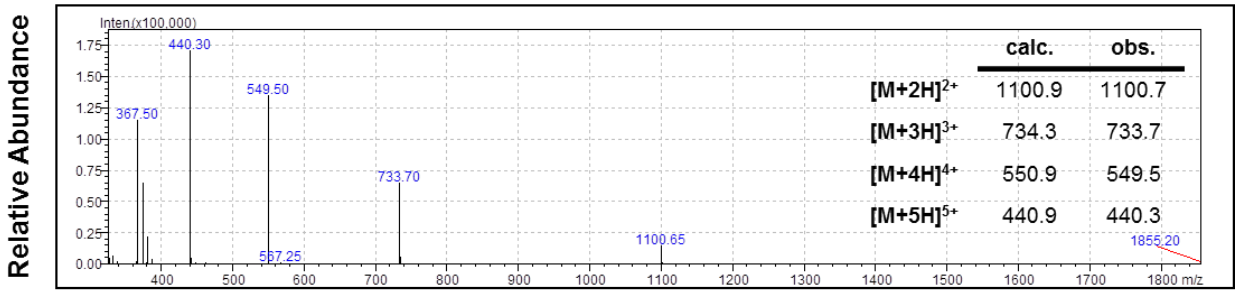
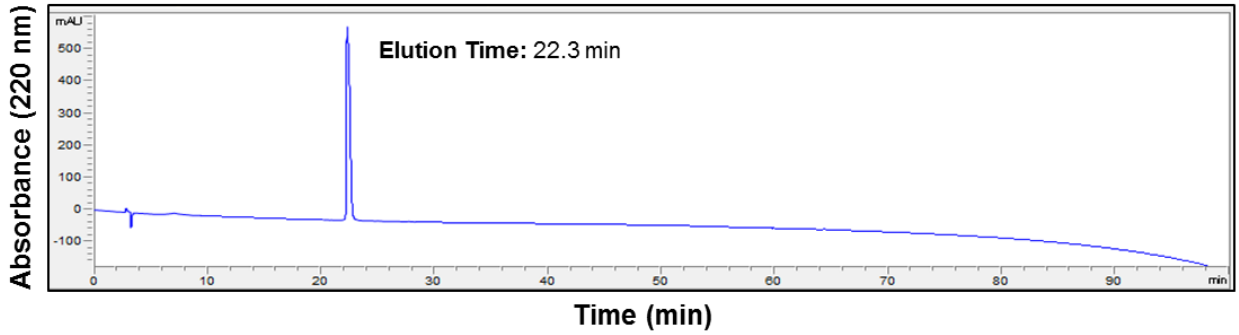


Figure S14: (**A**) Analytical HPLC (Vydac C18; 0% - 80% B over 80 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified CLIPΔ.

A



B

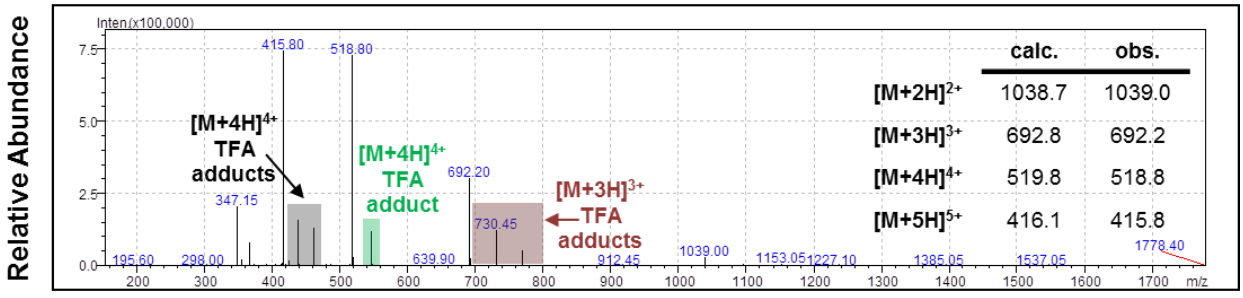
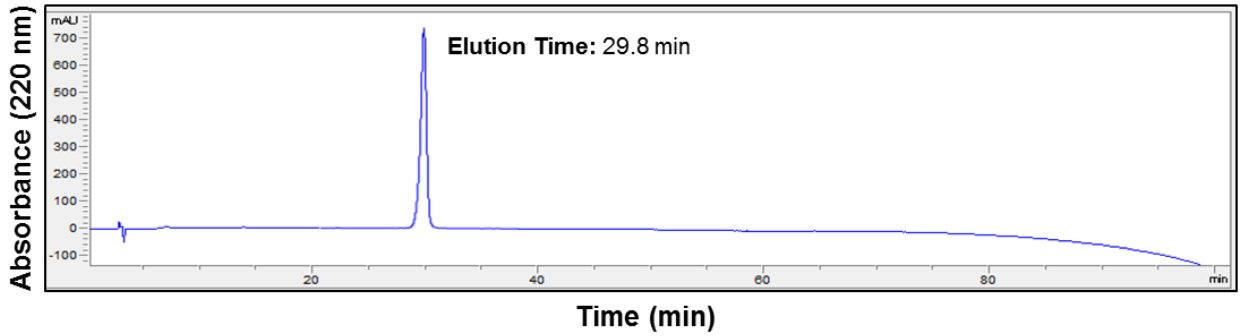


Figure S15: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified FI-TAT.

A



B

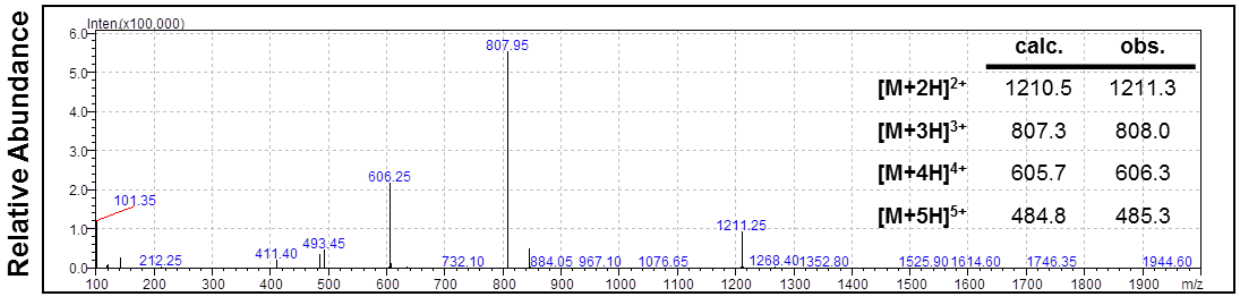


Figure S16: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified FI-CLIP0.

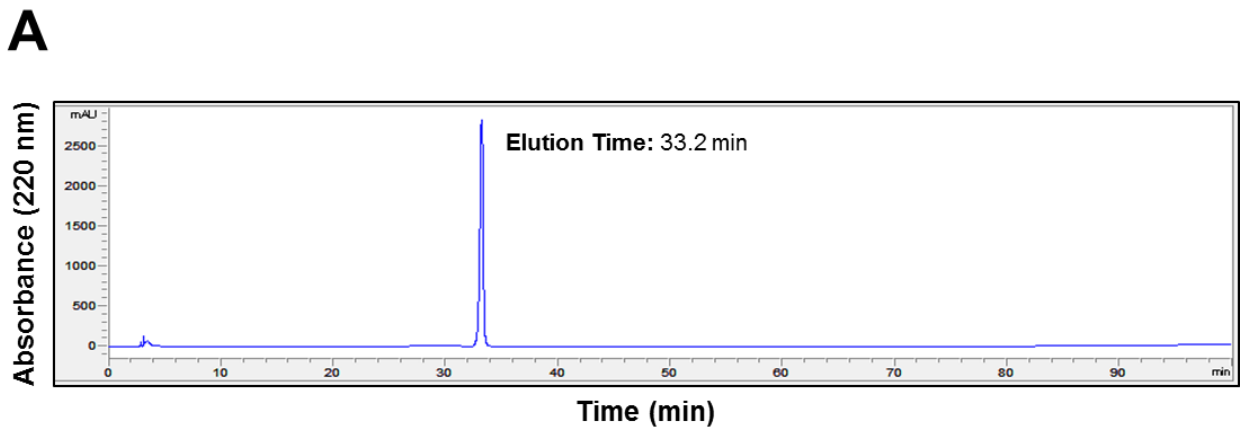


Figure S17: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified FI-CLIP2.

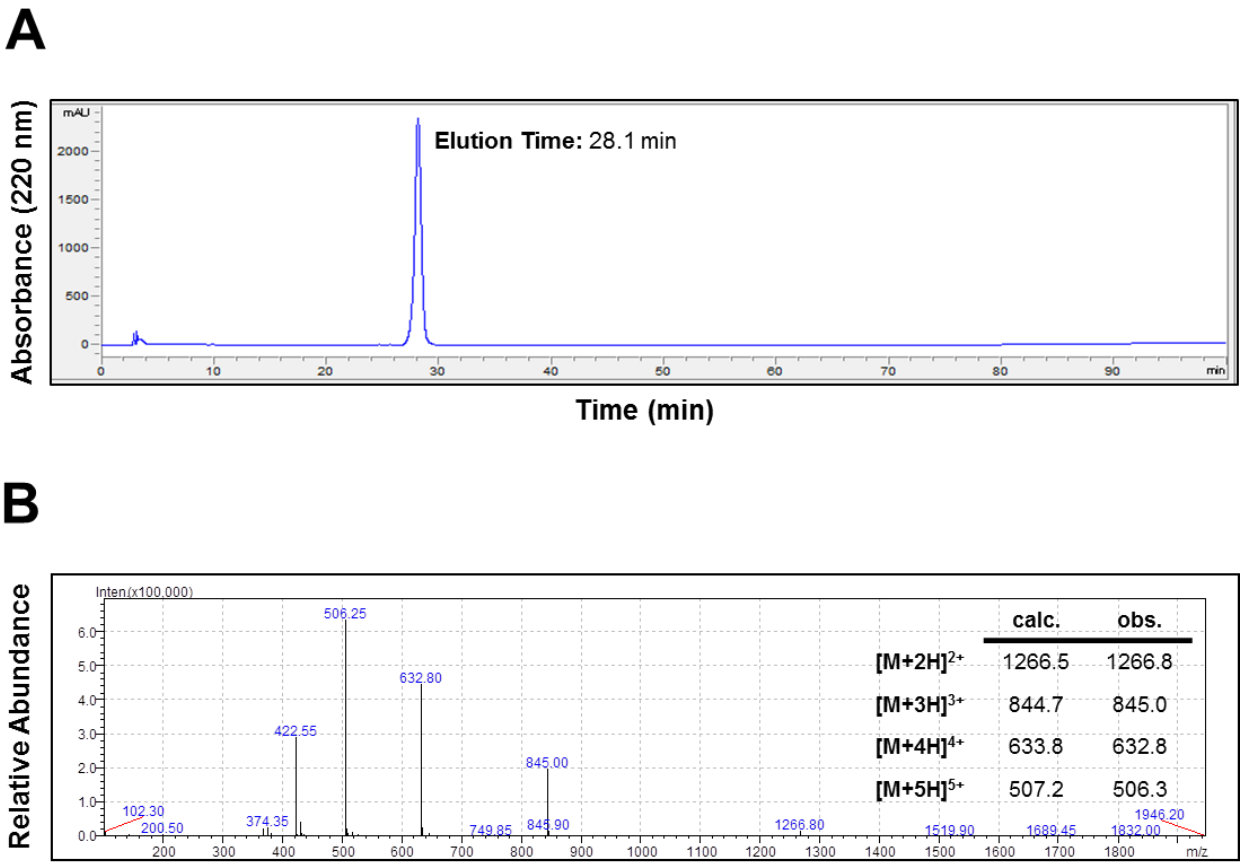
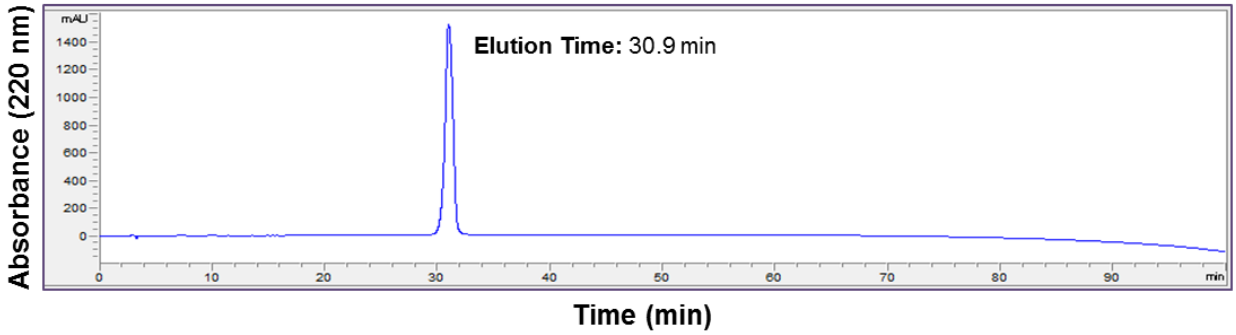


Figure S18: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified FI-CLIP4.

A



B

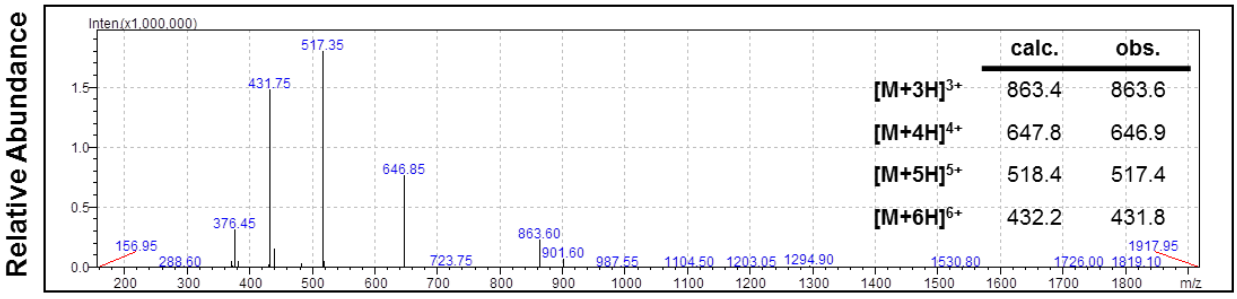


Figure S19: (**A**) Analytical HPLC (Vydac C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified FI-CLIP6.

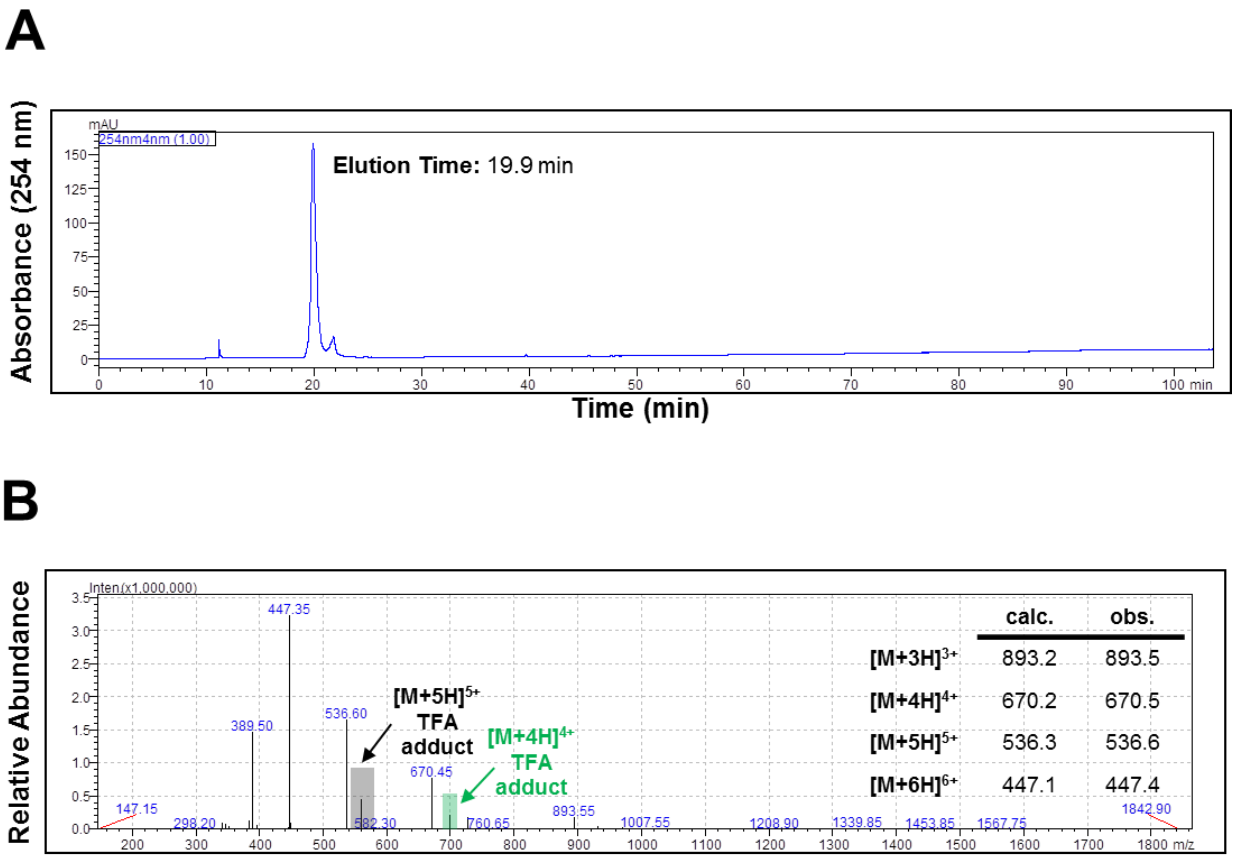


Figure S20: (**A**) Analytical HPLC (Phenomenex C18; 0% - 100% B over 100 min. at 40°C) and (**B**) ESI (+) mass spectrum of purified CLIP6-4-OHC$_Q$.

Supplemental References

[1] C. Sinthuvanich, A. S. Veiga, K. Gupta, D. Gaspar, R. Blumenthal, J. P. Schneider, *J. Am. Chem. Soc.* **2012**, *134*, 6210-6217.
[2] G. Drin, S. Cottin, E. Blanc, A. R. Rees, J. Temsamani, *J. Biol. Chem.* **2003**, *278*, 31192-31201.
[3] I. Massodi, G. L. Bidwell Iii, D. Raucher, *J. Controlled Release* **2005**, *108*, 396-408.
[4] J. P. Richard, K. Melikov, E. Vives, C. Ramos, B. Verbeure, M. J. Gait, L. V. Chernomordik, B. Lebleu, *J. Biol. Chem.* **2003**, *278*, 585-590.
[5] K. S. Horger, D. J. Estes, R. Capone, M. Mayer, *J. Am. Chem. Soc.* **2009**, *131*, 1810-1819.
[6] D. K. Sinha, P. Neveu, N. Gagey, I. Aujard, C. Benbrahim-Bouzidi, T. Le Saux, C. Rampon, C. Gauron, B. Goetz, S. Dubruille, M. Baaden, M. Volovitch, D. Bensimon, S. Vriz, L. Jullien, *Chem. Bio. Chem.* **2010**, *11*, 653-663.
[7] A. P. Gorka, R. R. Nani, J. Zhu, S. Mackem, M. J. Schnermann, *J. Am. Chem. Soc.* **2014**, *136*, 14153-14159.