Supporting Information

Cell-Permeable Cyclic Peptidyl Inhibitor against the Keap1-Nrf2 Interaction

*Heba Salim,[†] Jian Song,[†,‡] Ashweta Sahni,[†] and Dehua Pei [†,]**

[†]Department of Chemistry and Biochemistry, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210, USA.

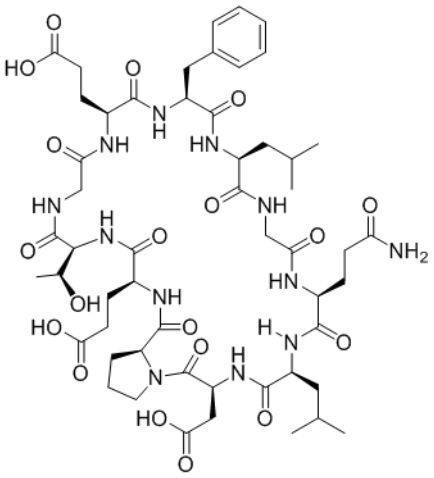
[‡]School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, Guangdong Province 510006, P. R. China

*Corresponding author. Phone: 614-688-4068; E-mail: pei.3@osu.edu

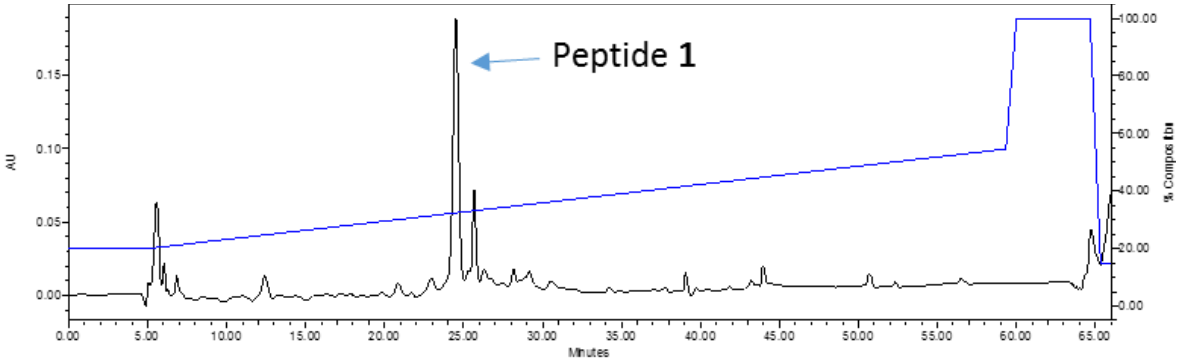
Table of Contents

Figure S1. Structures, purity (as assessed by reversed-phase analytical HPLC), and HR-MS (MALDI FT-ICR) of peptides used in this work. Note: Some of the NF-labeled peptides eluted as two separate peaks, because the commercially available NF dye is a mixture of 5- and 6-carboxy isomers. The mixtures of two isomers were used in all experiments.

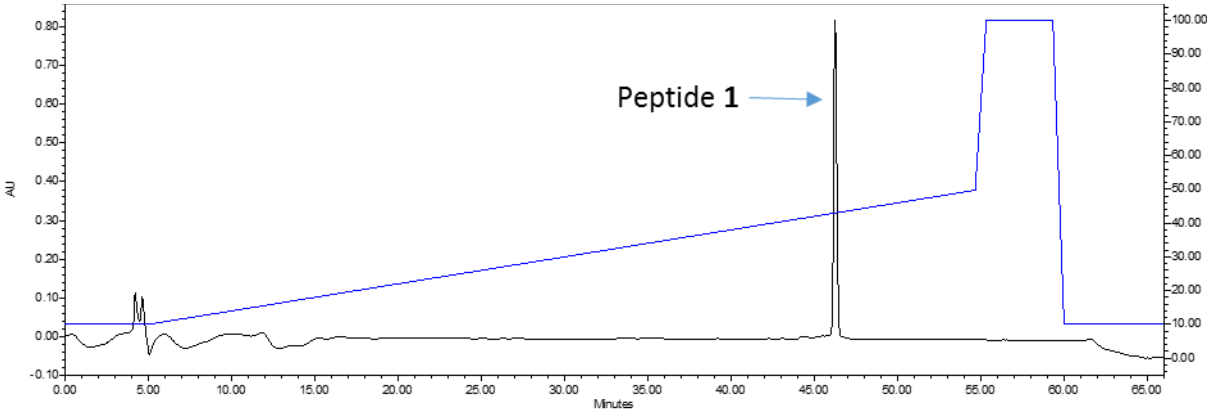
Peptide **1**



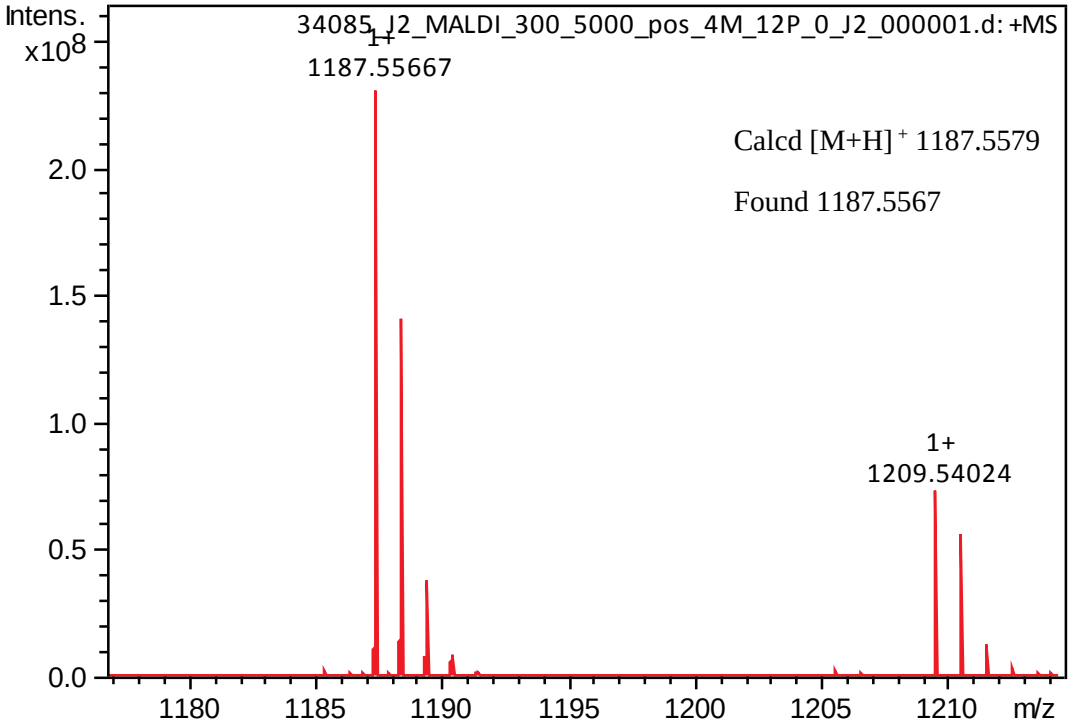
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



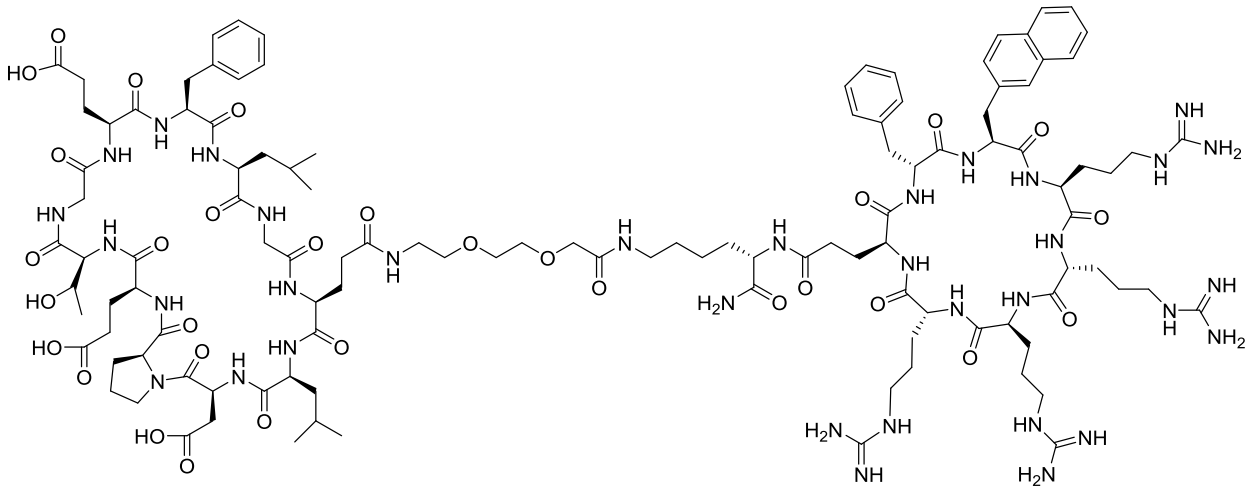
Purity check on analytical reversed-phase HPLC (214 nm):



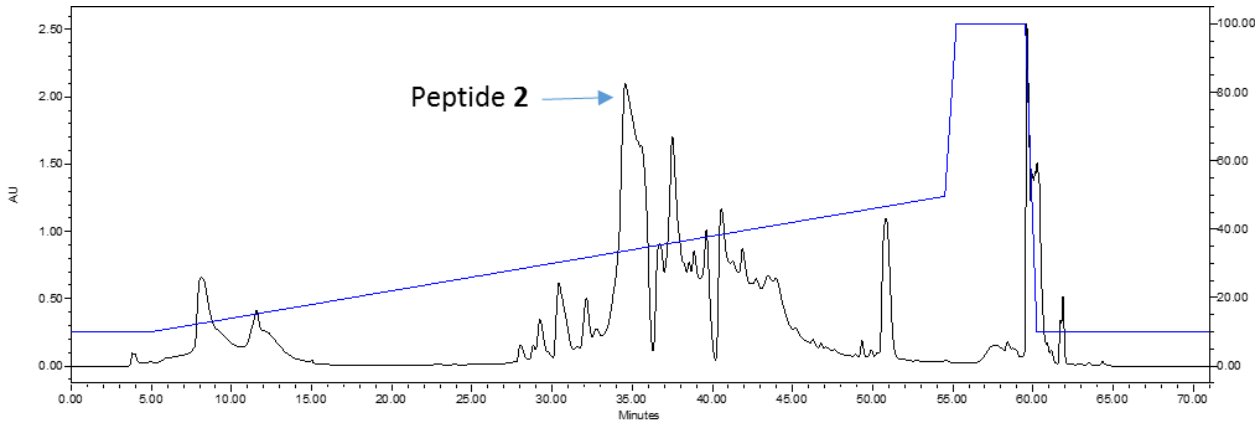
HRMS:



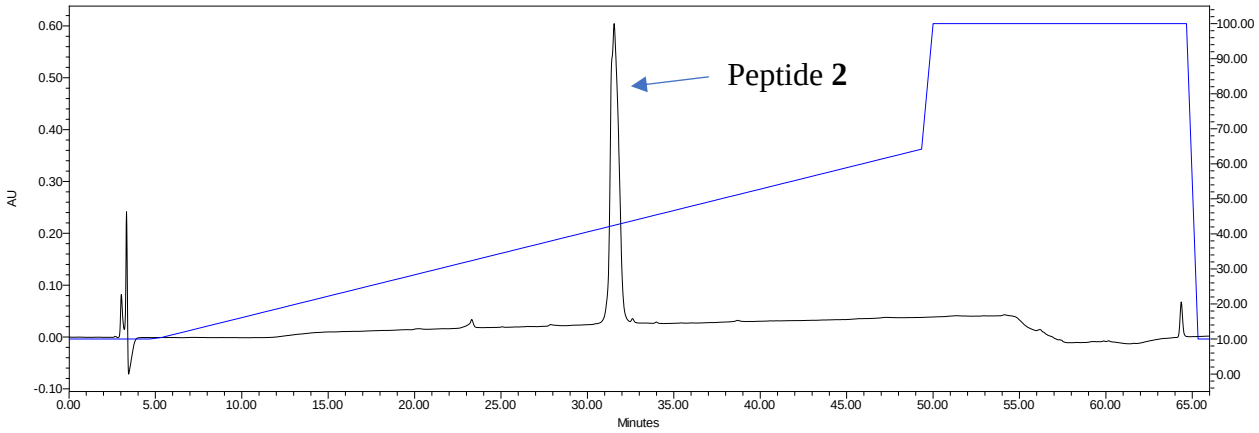
Peptide **2**



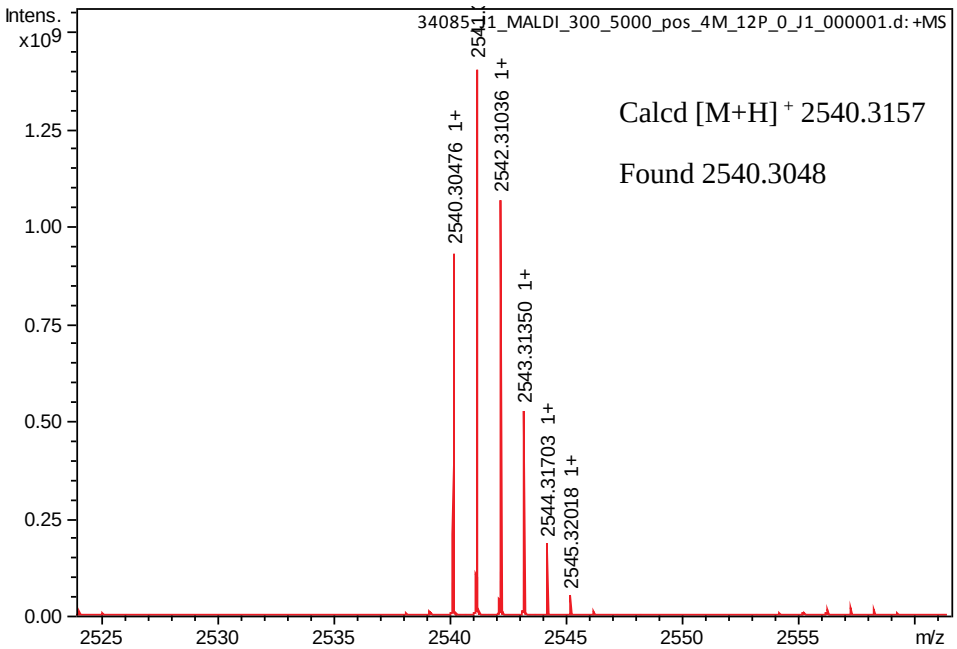
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



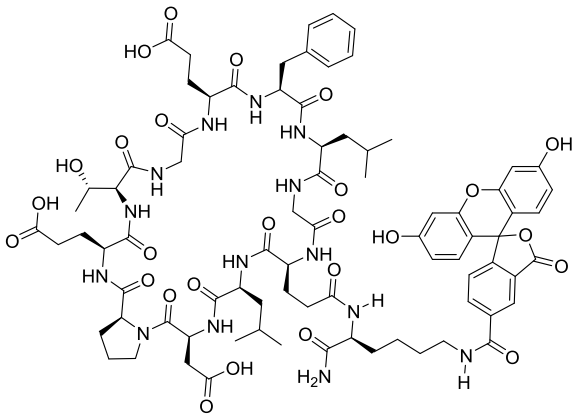
Purity check (analytical reversed-phase HPLC at 214 nm):



HRMS:

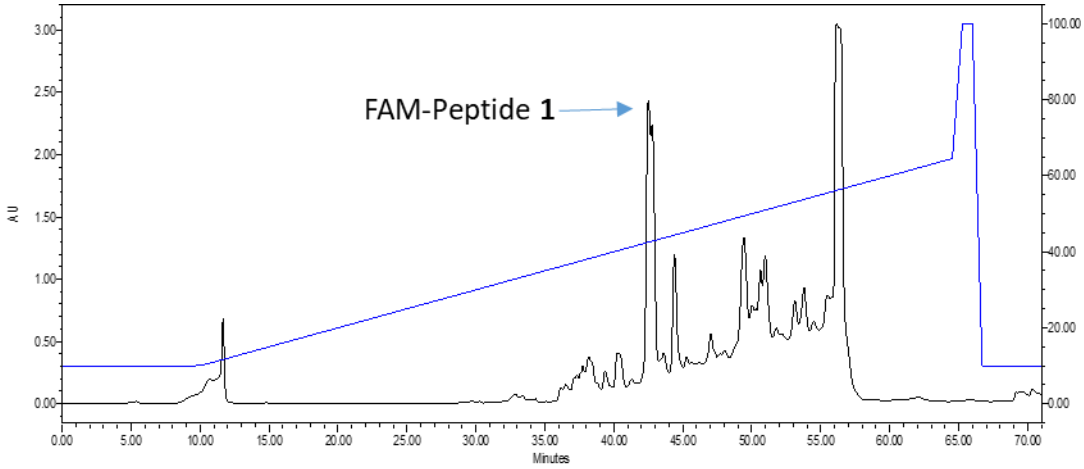


FAM-Peptide **1**

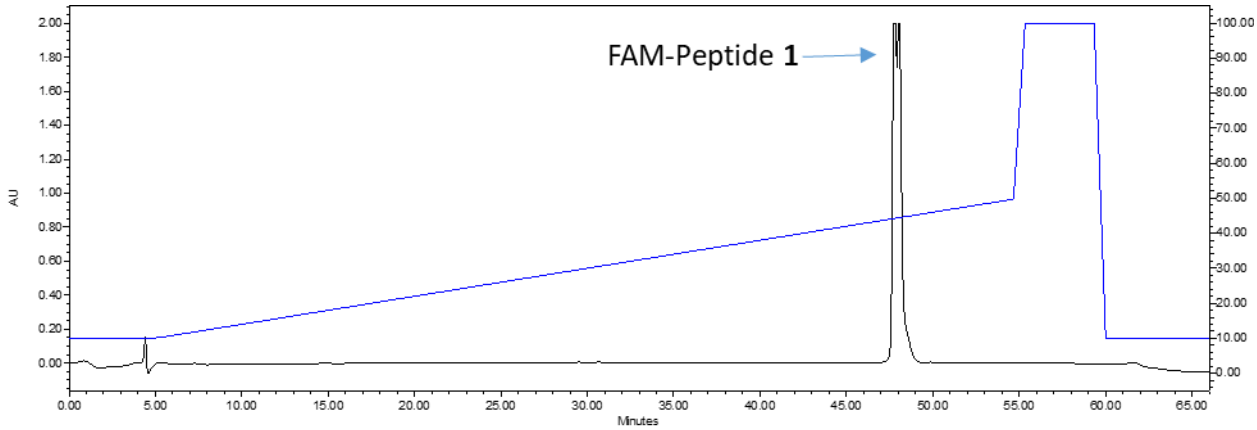


Chemical Formula: $C_{80}H_{100}N_{14}O_{26}$
Exact Mass: 1672.69332

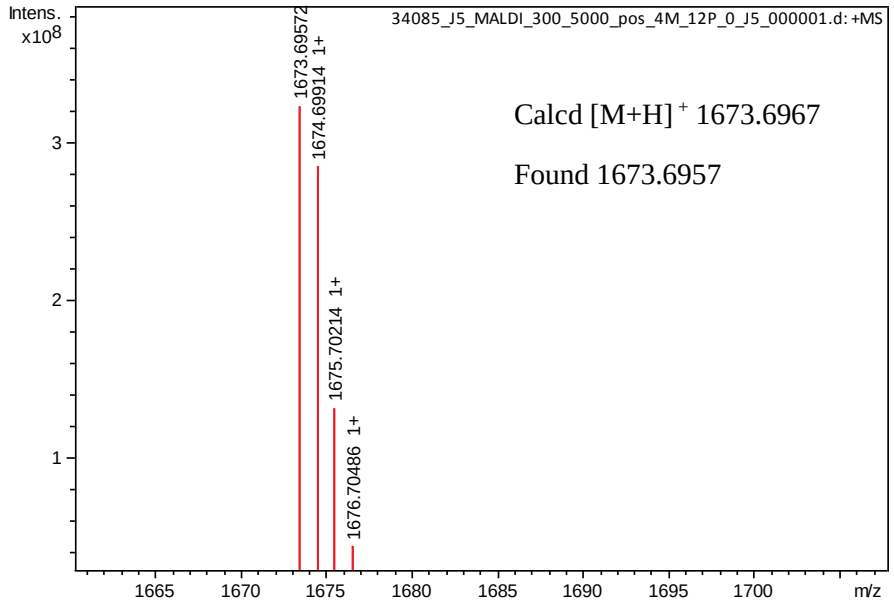
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



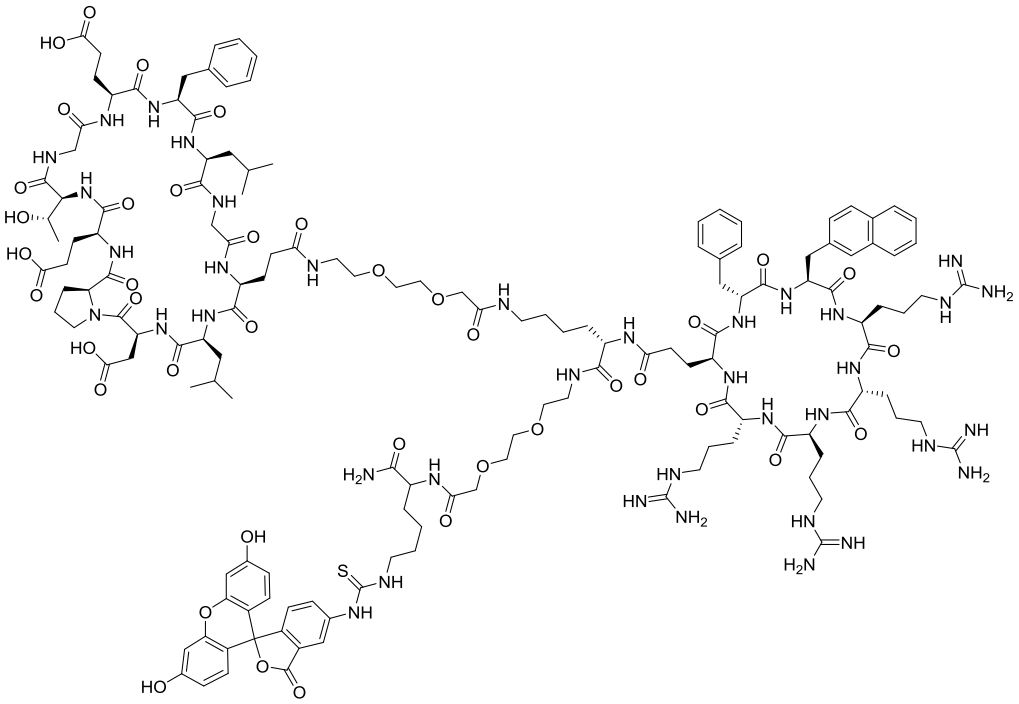
Purity check on analytical reversed-phase HPLC (214 nm):



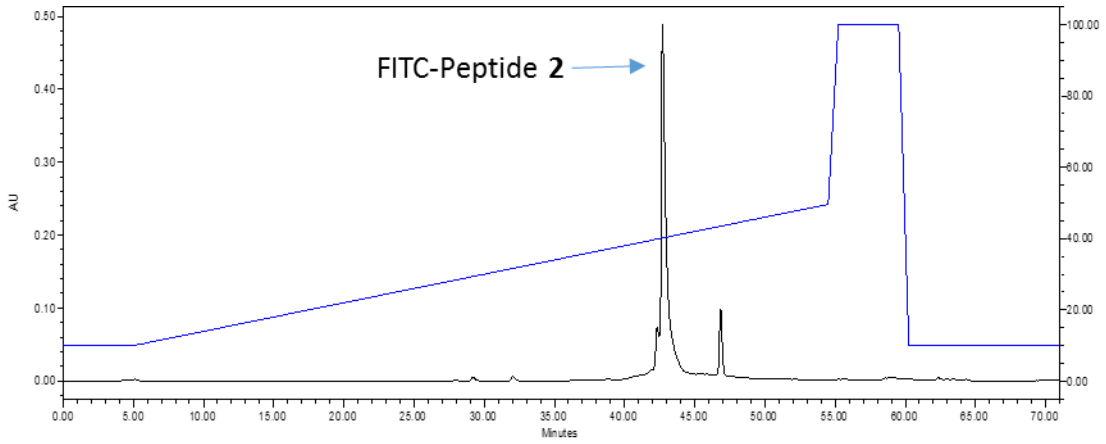
HRMS:



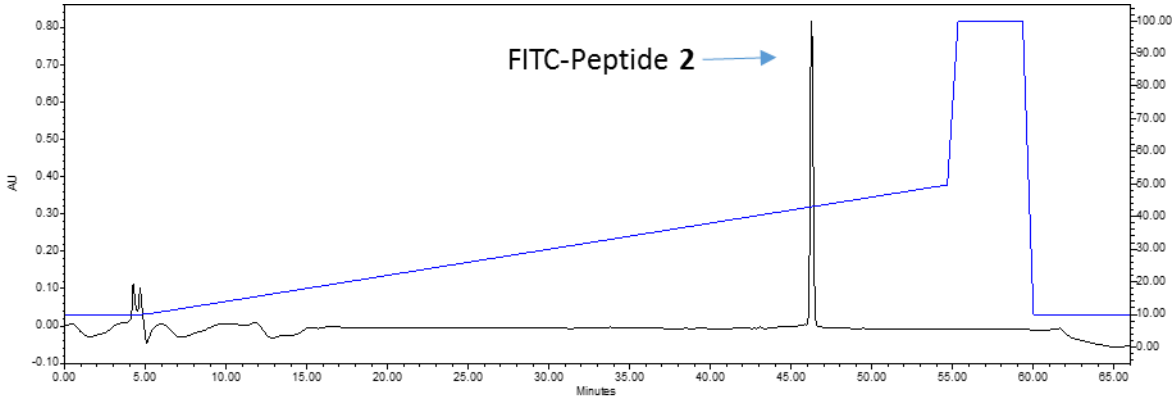
FITC-Peptide **2**



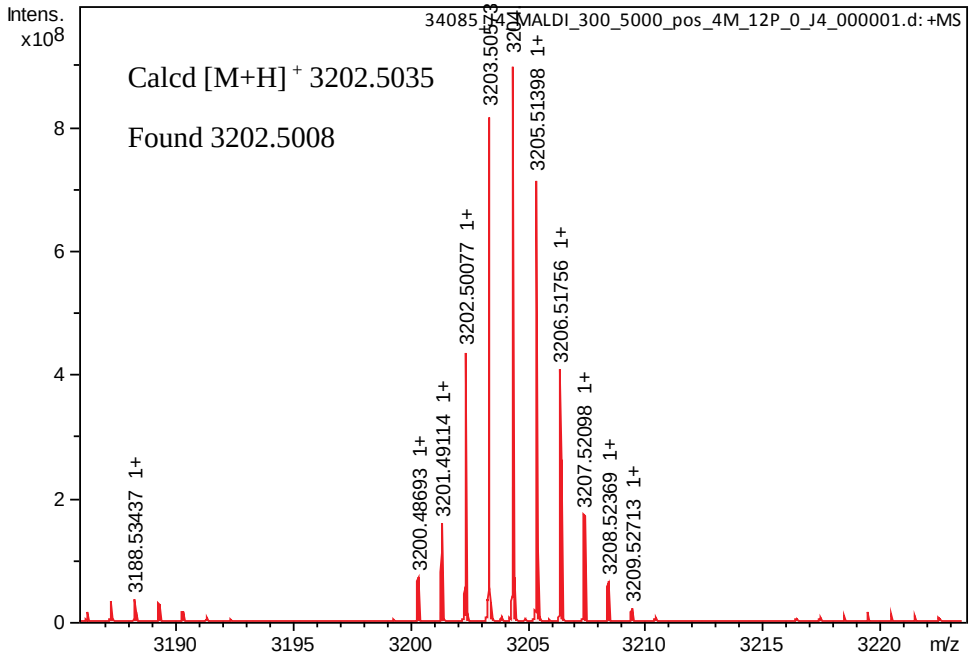
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



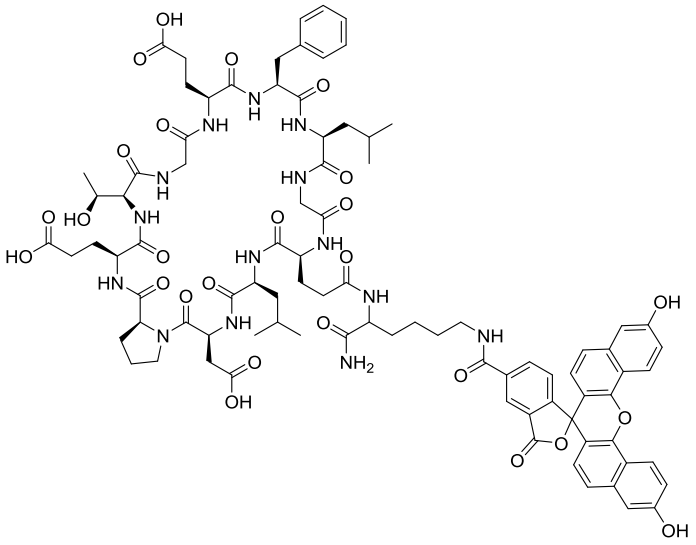
Purity check on analytical reversed-phase HPLC (214 nm):



HRMS:

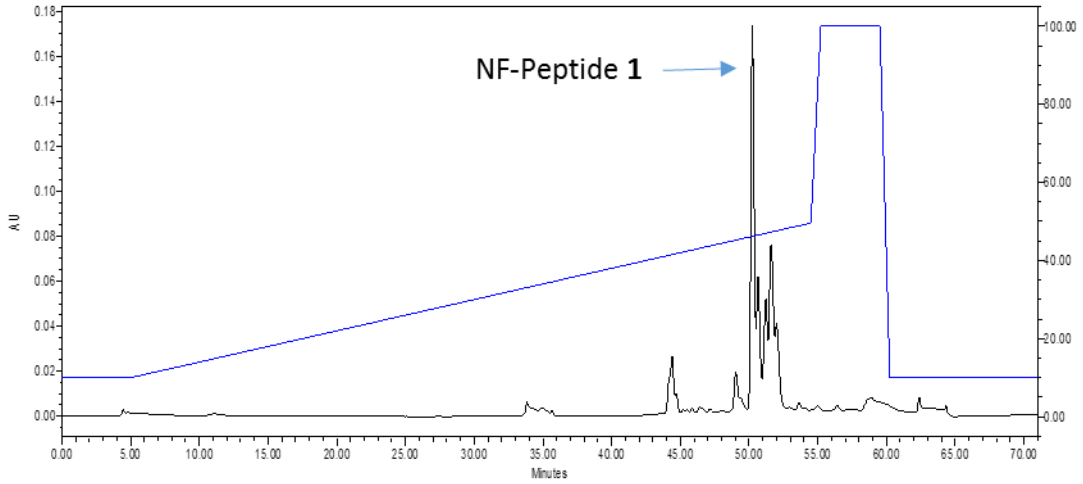


NF-Peptide **1**

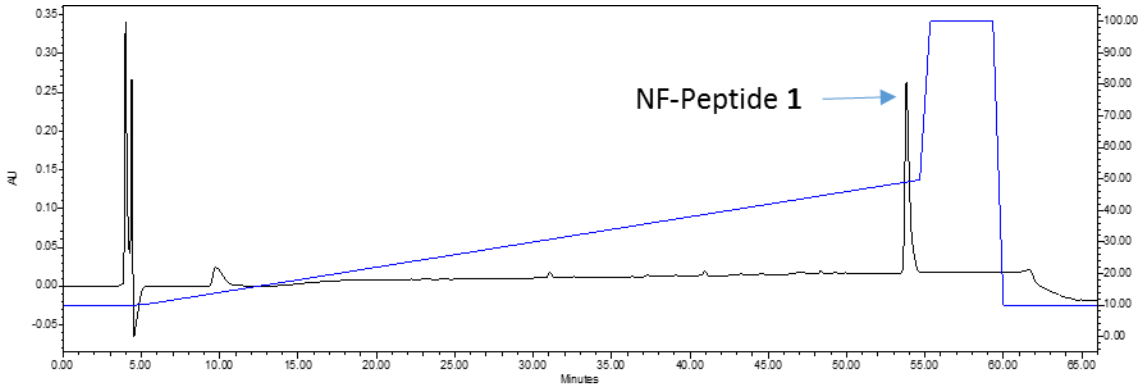


Chemical Formula: $C_{88}H_{104}N_{14}O_{26}$
Exact Mass: 1772.72462

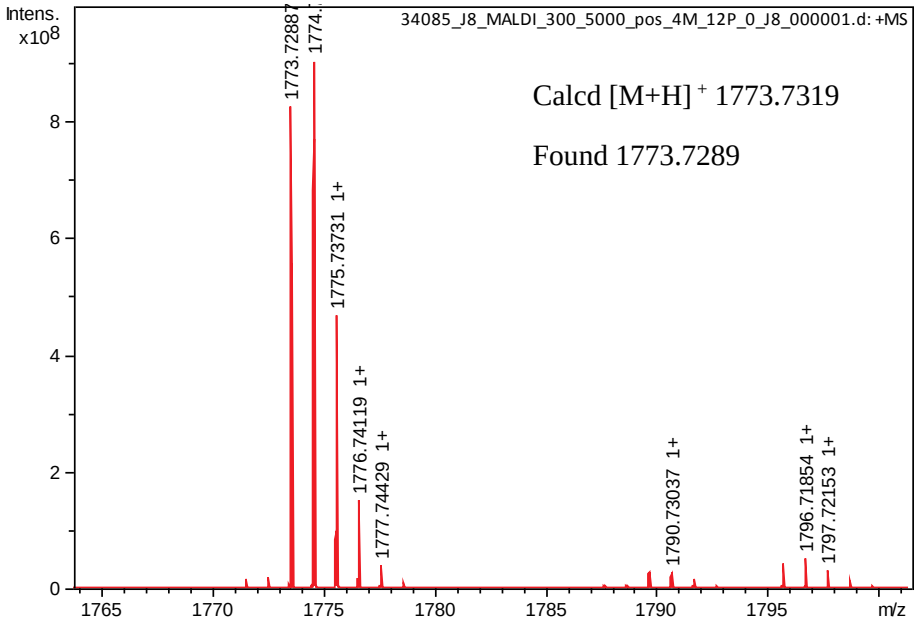
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



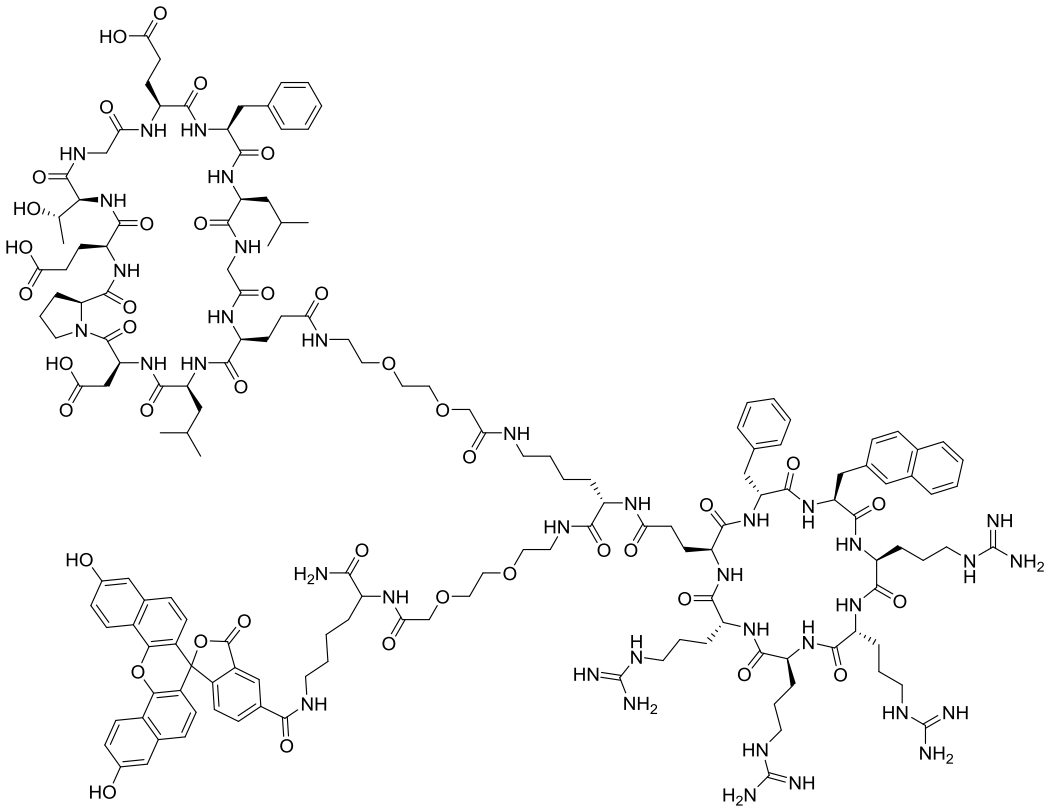
Purity check on analytical reversed-phase HPLC (214 nm):



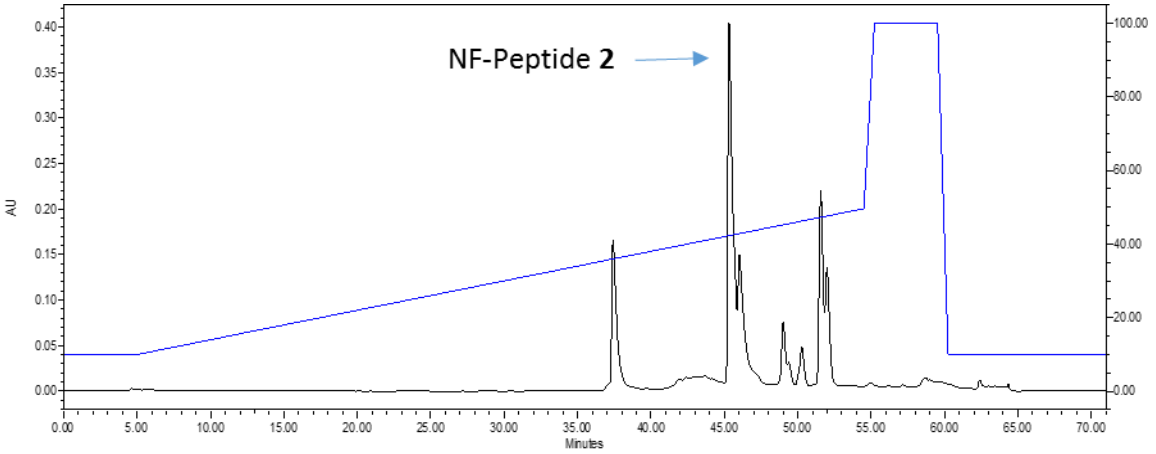
HRMS:



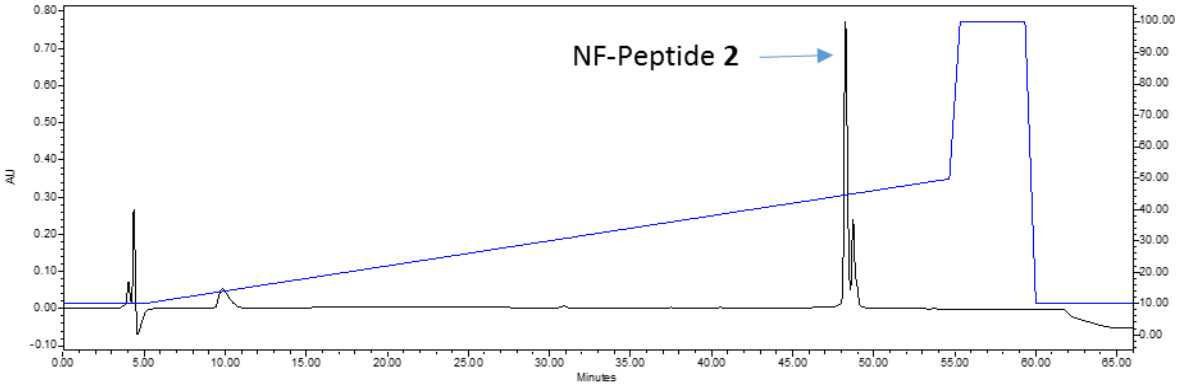
NF-labeled Peptide **2**



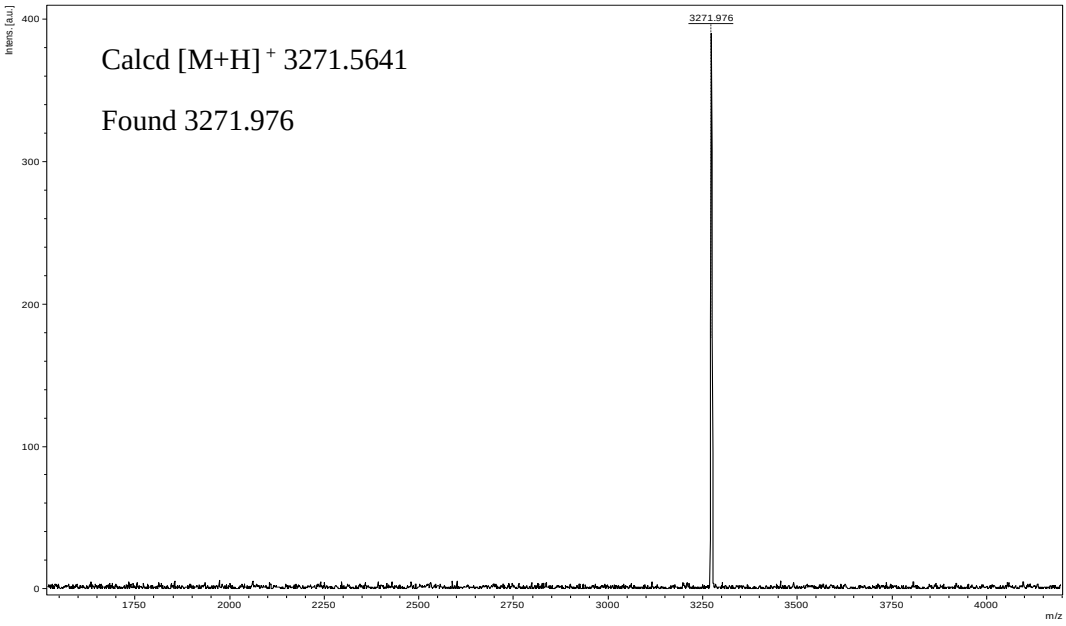
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



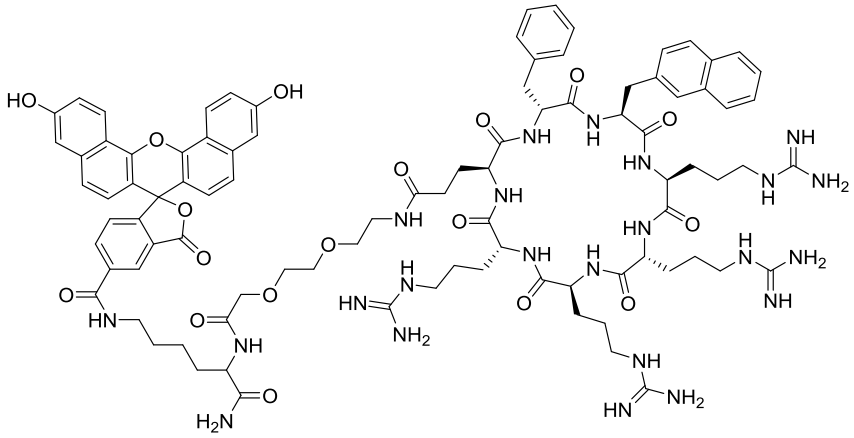
Purity check on analytical reversed-phase HPLC (214 nm):



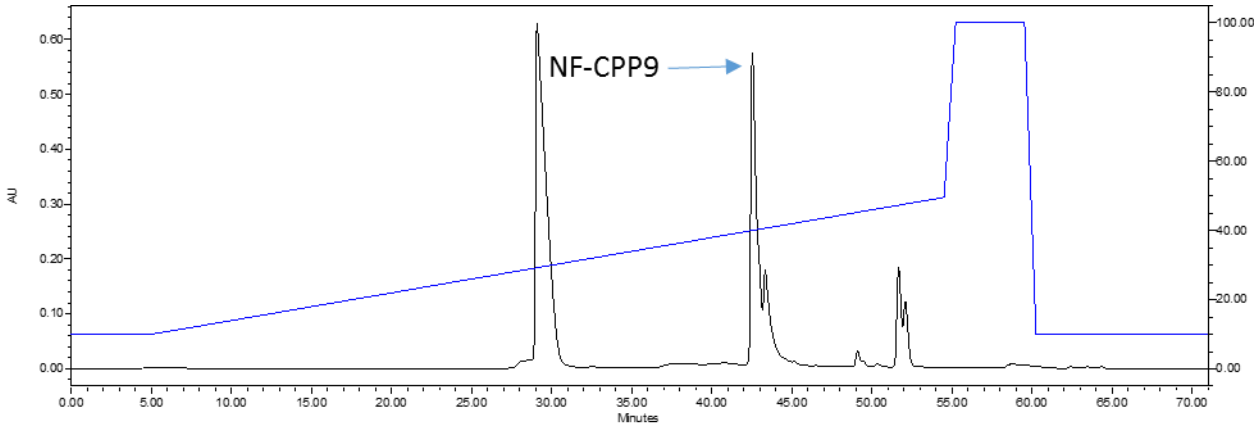
MS (low-resolution):



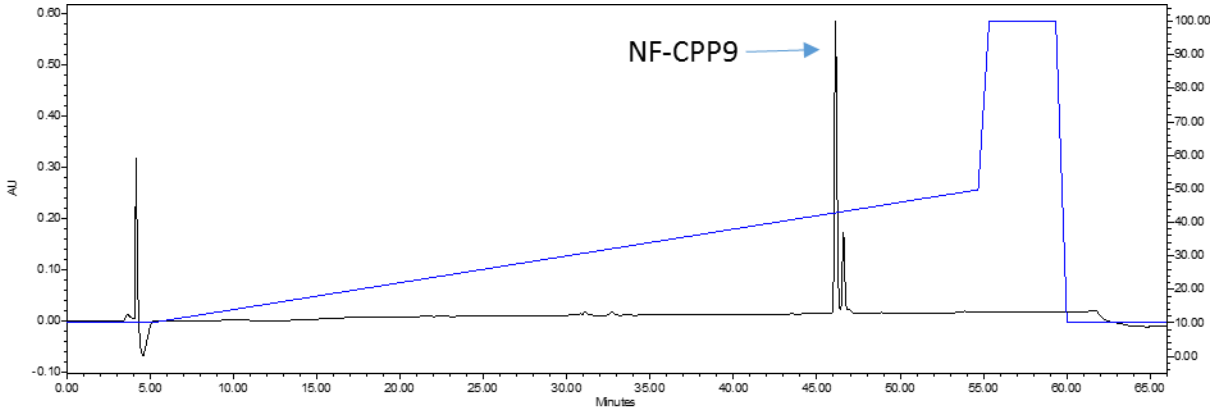
NF-CPP9



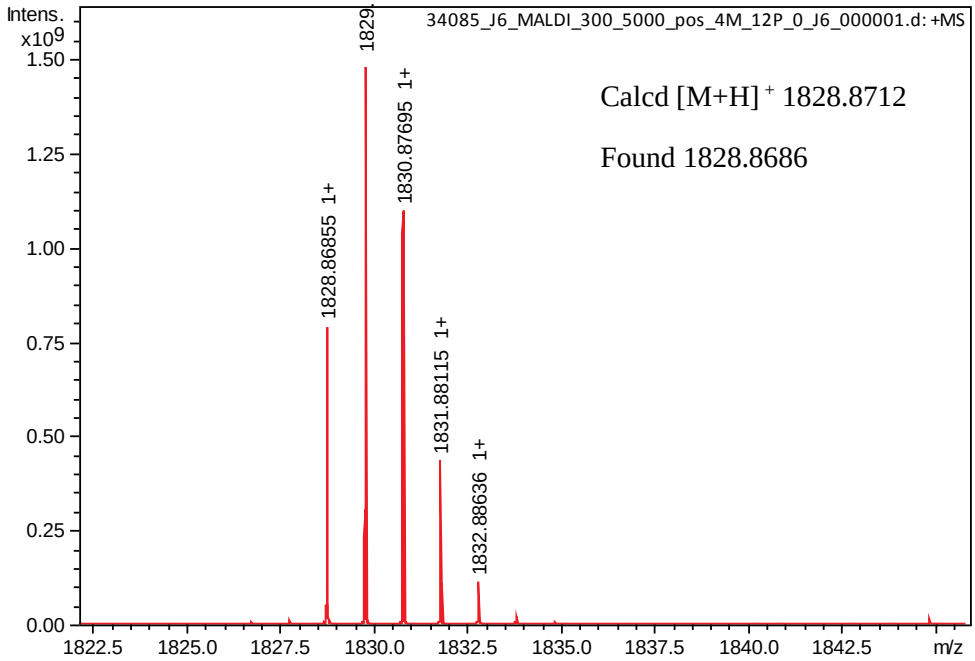
Crude peptide on semi-preparative reversed-phase HPLC (monitored at 214 nm):



Purity check on analytical reversed-phase HPLC (214 nm):



HRMS:


34085_J6_MALDI_300_5000_pos_4M_12P_0_J6_000001.d: +MS
Intens.
x10^9
1.50
1.25
1.00
0.75
0.50
0.25
0.00
1828.86855 1+
1829.
1830.87695 1+
1831.88115 1+
1832.88636 1+
Calcd [M+H]+ 1828.8712
Found 1828.8686
1822.5
1825.0
1827.5
1830.0
1832.5
1835.0
1837.5
1840.0
1842.5
m/z

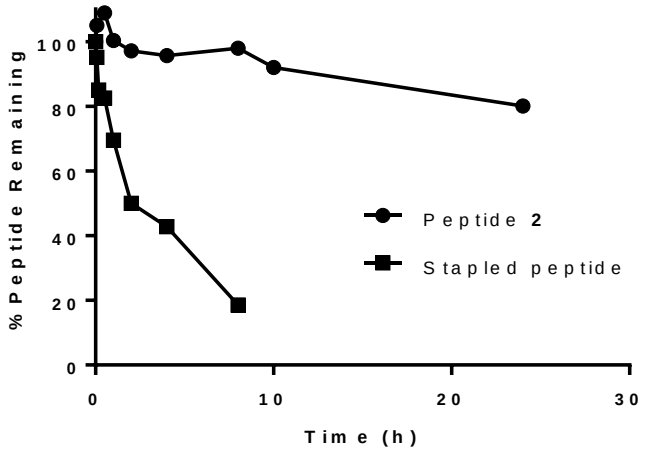


Figure S2. Serum stability of peptide **2** and a stapled peptide, Ac-GGYPED*ILDK*HLQRVIL-$(miniPEG)_2$-Dap-CPP9, in which an amide bond is formed between the side chains of D* and K*.

Procedure: Human serum was diluted to 25% in sterile DPBS and incubated at 37 °C for 15 min. Peptide was added to the diluted serum to a final concentration of 100 µM and incubated at 37 °C with gentle mixing. Aliquots of 100 µL were withdrawn at various time points and mixed with 100 µL of 15% trichloroacetic acid in MeOH (w/v) and 100 µL of acetonitrile to quench the reaction. After overnight storage at 4 °C, aliquots were centrifuged at 15000 g for 5 min at 4°C and analyzed by RP-HPLC on a C18 column. The amount of remaining peptide was assessed by integration of the area under the peak in the HPLC chromatogram and comparing it with that at zero-time point.

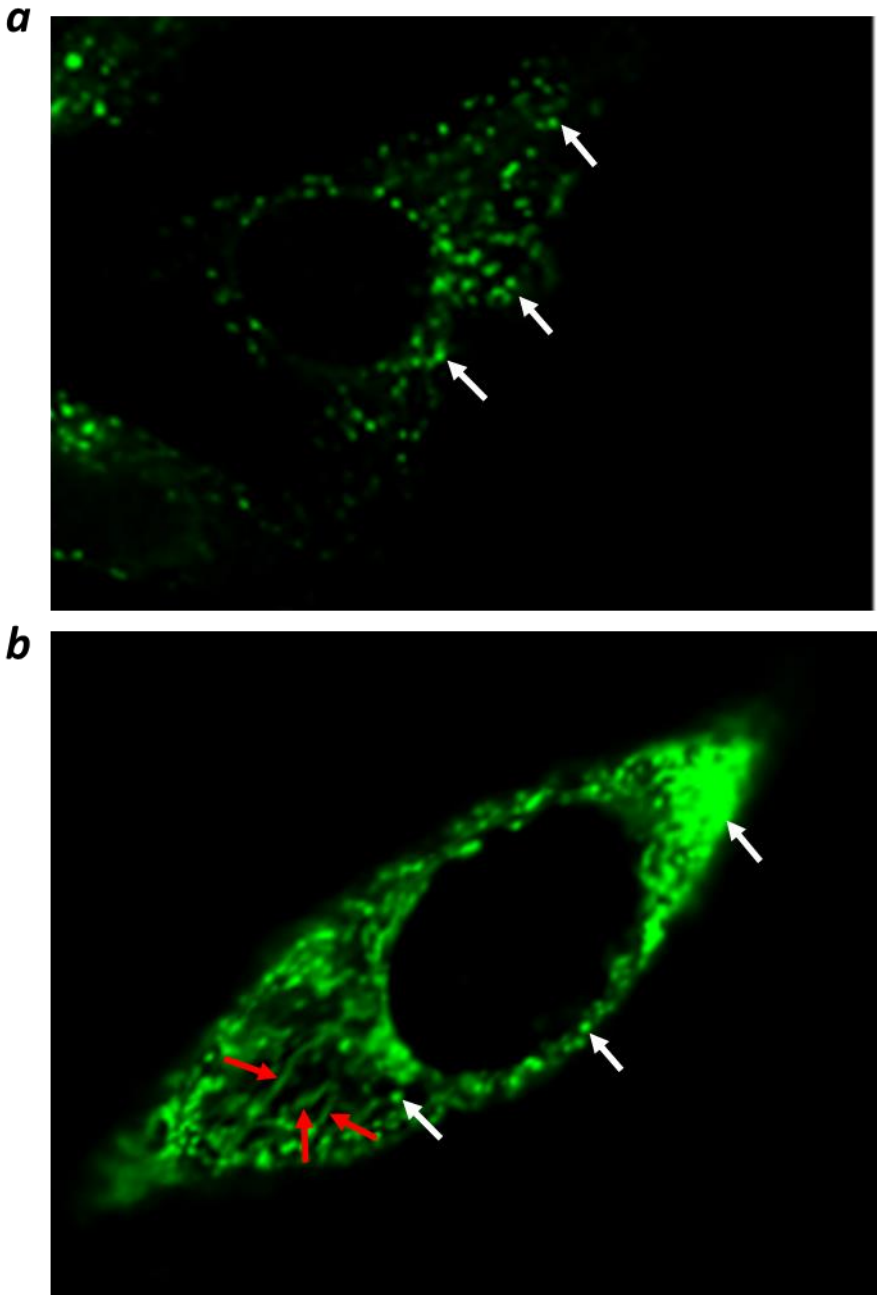


Figure S3. Enlarged confocal microscopy images from Figure 2 (main text) to show the differential intracellular localization of peptides **1** (*a*) and **2** (*b*). Representative round, intensely fluorescent structures (presumably endosomes and lysosomes) are indicated by white arrows, whereas the fibrous structures in (*b*) are marked with red arrows.

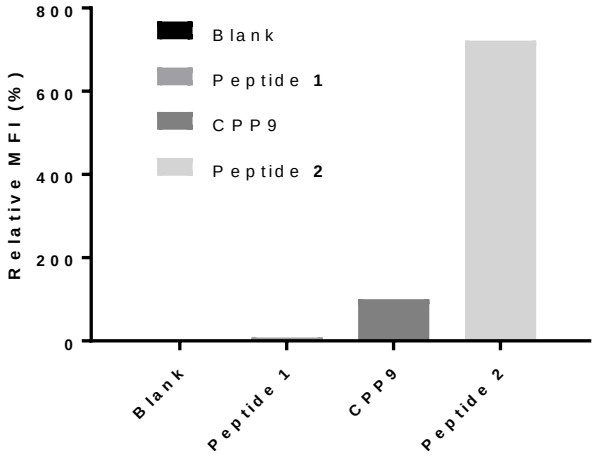


Figure S4. Cytosolic entry efficiencies of peptides **1** and **2**. HeLa cells were treated with 5 μM NF-labeled peptide for 2 h in the presence of 10% FBS, washed, and suspended in a pH 6.5 buffer immediately before flow cytometry analysis. All MFI values are relative to that of CPP9-NF (100%).

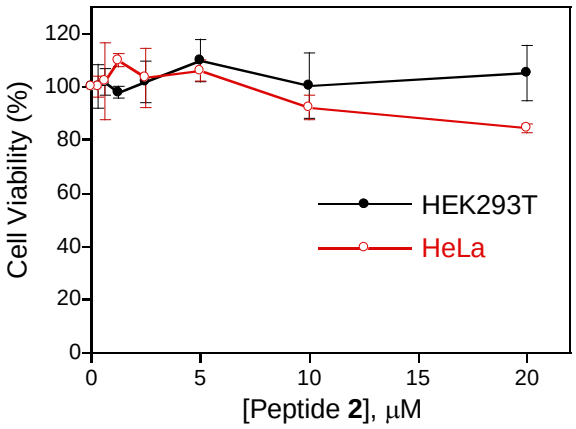


Figure S5. Effect of peptide **2** on the viability of HeLa and HEK293T cells as monitored by the MTT assay.