Supporting Information

Stapling of Short Cell-Penetrating Peptides for Enhanced Tumor Cell-and-Tissue Dual-Penetration

Mengzhen Shi[a], Zherui Jiang[a], Yao Xiao[b], Rui Tang[a], Ling Zhang[b], Yue Song[a], Jianfeng Huang[a], Yuan Tian[a]*, Shaobing Zhou[b]*

a Sichuan Engineering Research Center for Biomimetic Synthesis of Natural Drugs, School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan 610031, P.R. China

b Key Laboratory of Advanced Technologies of Materials, Ministry of Education, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan 610031, P.R. China

---

Email address: ty@swjtu.edu.cn (Yuan Tian); shaobingzhou@swjtu.edu.cn (Shaobing Zhou)

Supplementary Figure Captions:

Table S1. Peptide sequence, chemical formula and mass spectrometry analysis.

Figure S1. Representative synthetic route for the construction of stapled cell-penetrating peptides.

Figure S2. Flow cytometry analysis of B16F10 cells treated with FITC-labelled peptides in the presence and absence of Trypan blue and Triton.

Figure S3. B16F10 Cell penetration of the peptides.

Figure S4. HeLa Cell penetration of the peptides.

Figure S5. 4T1 Cell penetration of the peptides.

Figure S6. A) CD spectra of the peptides in ddH2O and in 30 mM SDS solution. B) Serum stability of the peptides in the presence of 25% FBS.

Figure S7. Hemolytic activity of the stapled cell-penetrating peptides.

Figure S8. Mean fluorescence of B16F10 cells after incubation with 3 μM FITC-labelled stapled CPPs in the presence of different endocytosis inhibitors.

Figure S9. Schematic illustration for in vitro experimental models of transcellular penetration of B16F10 cells (A), HeLa cells (B) and 4T1 cells (C).

Figure S10. Exocytosis of the stapled cell-penetrating peptides.

Figure S11. Fluorescence microscopic images of 4T1 tumors explants after incubation with FITC-labelled peptides

Figure S12. Fluorescence microscopic images of 4T1 tumors sections after intratumorally injection with 40 μM FITC-labelled Tat and sTat-4 peptides for 4 hours before harvesting of the tumors.

Material and methods

All solvents and reagents were purchased from commercial suppliers, including GL Biochem (Shanghai) Ltd., Energy Chemical Co., or Chron Chemicals Co., and were used without further purification unless otherwise stated. All animal procedures were approved and carried out according to the Institutional Animal Care and Use Committee of Southwest Jiaotong University, China.

1. Peptide synthesis

The peptides were synthesized manually according to a standard solid phase peptide synthesis protocol. Briefly, the Rink Amide MBHA resin (loading amount: 0.572 mmol/g) was pre-swelled in DMF for 30 minutes. The Fmoc protecting group was removed by 60 % morpholine in DMF for 35 min (2 times). The resin was then thoroughly washed with DMF (3 times), DCM (3 times) and DMF (3 times). According to the initial loading of the resin, 5.0 equivalents of Fmoc-protected amino acids and 4.9 equivalents of HATU were dissolved in DMF, and then 10.0 equivalents of DIPEA was added to the mixture, followed by reacting with the resin for 2 hours. After that, the resin was sequentially washed with DMF (3 times), DCM (3 times), and DMF (3 times). Peptide stapling was performed by reacting the small molecular crosslinking reagents (α, α'-dibromo-m-xylene, 4, 4'-Bis(bromomethyl)biphenyl, perfluorobenzene, decafluorobiphenyl) with the cysteines in the peptides on resin. Briefly, the Trt protecting groups in cysteines were removed on resin by a solution of 3% TFA and 5% TIS in DCM until the disappearance of the yellow color. Then, 2.0 equivalents of crosslinking reagent and 4.0 equivalents of DIPEA were mixed in DMF, and the mixture was added to the resin for 4 hours at room temperature. For FITC labelling, a mixture of 5.0 equivalents of FITC and 10.0 equivalents of DIPEA were dissolved in DMF, followed by reacting with the resin in dark for 8 hours. The peptides were finally cleaved from the resin with a mixture of 95% TFA, 2.5% H2O, and 2.5% TIS for 3 hours. The crude peptides were then precipitated with Et_2O and dried collected by centrifugation. Then, the crude peptides were purified by reverse-phase HPLC and analyzed by LC-MS. The concentration of the purified peptides was quantified by absorbance of FITC at 494 nm with an extinction coefficient of 77,000 M^{-1} cm^{-1}.

2. HPLC and Mass spectrometry

The crude peptides were purified by Agilent 1260 using a C18 column (Agilent ZORBAX SB-Aq, 4.6 × 250 mm, 5 μm, flow rate 1.0 mL/min) with a linear gradient of acetonitrile and H_2O

(containing 0.1% TFA). LC-MS were carried out on Waters e2695-ACQUITY Qda.

3. Flow cytometry analysis

The cells (B16F10, HeLa, 4T1) were cultured in a 12-well plate at a density of 5×10^5 cells per well at 37 ℃ for 24 hours. The cells were then treated with 3 μM FITC-labelled peptides in serum-free medium for 1 hour. After removal of the peptides, the cells were then washed with PBS and digested with 0.25 % trypsin for 5 min to remove the peptides bound to the cell surface. The cells were then washed with PBS and treated with trypan blue for 3 minutes to quench extracellular fluorescence prior to flow cytometry analysis.

4. Live-cell fluorescence microscopy imaging

The cells (B16F10, HeLa, 4T1) were seeded on 12-well cover glass (Nest) and cultured overnight. The cells were then incubated with 3 μM FITC-labelled peptides in serum-free medium for 1 hour followed by thoroughly washing with PBS. Then, the cells were subsequently stained with Hochest 33258 for 15 minutes followed by imaging with a Carl Zeiss Apotome 2 fluorescence microscope.

5. Hemolytic activity

The fresh rabbit red blood cells (RBCs) were collected by centrifugation at 1000 rpm for 10 minutes. Then, the RBCs were thoroughly washed with 0.9 % NaCl. After that, 200 μL of RBCs suspension was mixed with the peptide at a final concentration of 5 μM, 10 μM, 20 μM, and 40 μM. 0.9 % NaCl and H_2O were employed as negative and positive controls, respectively. After incubation at 37 °C for 1 hour, all the samples were centrifuged at 12,000 rpm for 5 minutes, and then the absorbance at 541 nm of the supernatant was subsequently measured. The percent hemolysis was calculated by the following formula: Percent hemolysis = (OD sample – OD negative) / (OD positive – OD negative) × 100%.

6. Circular Dichroism spectroscopy (CD)

The peptide was dissolved in ddH_2O or 30 mM sodium dodecyl sulfate (SDS) at a concentration of 1×10^{-5} M. CD spectra were collected using a Jasco J-810 Circular Dichroism Spectrometer. The detection conditions are as follows: step resolution is 0.5 nm, speed is 20 nm/s, accumulated 10 times, response time is 1 s, bandwidth is 1 nm, the path length is 10 mm. All data are presented as mean residual elipticity [θ] in $deg\cdot cm^2\cdot dmol^{-1}$.

7. Serum stability

Serum stability was performed by incubation of the peptides with 25% FBS (v/v) at a peptide

concentration of 100 μM at 37 ˚C for indicated periods of time. Aliquots of the mixture (10 μL) were taken at 0 h, 1 h, 2 h, 4 h followed by adding 150 μl 12% trichloroacetic acid in H_2O/CH_3CN (1:3) to precipitate serum proteins. After that, the samples were centrifuged at 12000 rpm for 5 min. The supernatant was then analyzed by HPLC.

8. In Vitro Penetration of Tumor Spheroids.

To construct the 4T1 tumor spheroids, 1% agarose (w/v) solution was autoclaved and dispensed into a 12-well plate with 1.0 ml per well. The 12-well plate was then kept at room temperature until the agarose was solidified. 4T1 cells (1×10^5) were seeded on the gel and incubated for 5-7 days to form tumor spheroids. Then, the tumor spheroids were treated with 20 μM FITC-labelled peptides in serum-free medium at 37 °C for 12 hours. After removal of the peptides, the tumor spheroids were washed with PBS and fixed with 2.5% glutaraldehyde and imaged by a confocal laser scanning microscopy.

9. The cellular uptake mechanism study

B16F10 cells were seeded in a 12-well plate and grown for 24 hours. The medium was removed and washed 3 times with PBS. The cells were pretreated with the following inhibitors in serum-free medium for 1 hour: (1) chlorpromazine (10 μM); (2) amiloride (10 μM); (3) genistein (200 μM); (4) Methyl-β-cyclodextrin (5 mM); (5) Heparin sodium (0.5 mg/ml). Then, the cells were incubated with 3 μM R8, sR8-4, Tat and sTat-4 in serum-free 1640 medium for 1 hour at 37°C in the presence of inhibitors. The cells were then washed with PBS and digested with 0.25 % trypsin for 5 min to remove the peptides bound to the cell surface. The cells were then washed with PBS and treated with trypan blue for 3 minutes to quench extracellular fluorescence prior to flow cytometry analysis.

10. Ex vivo and in vivo penetration of tumor tissues

All animal procedures were approved and carried out according to the Institutional Animal Care and Use Committee of Southwest Jiaotong University, China. Fresh tumor tissues were excised from 4T1 tumor-bearing BALB/c mice and incubated with 40 μM FITC-labelled peptides for 4 hours at 37 °C. After removal of the peptides, the tumor explants were washed with PBS and fixed with 4% paraformaldehyde. The tumor tissues were sectioned and stained with DAPI followed by observation with a Carl Zeiss Apotome 2 fluorescence microscope. For the in vivo penetration of tumor tissues, the 4T1 tumor-bearing BALB/c mice were intratumorally injected with 40 μM FITC-labelled peptides. At 4 h post-injection, the 4T1 tumors were subsequently excised and fixed with

4% paraformaldehyde. Four sections (300 μm spacing between two adjacent slices) of the tumor tissue were obtained from the center to the edge of the tumor tissue and stained with DAPI. The tumor sections were then imaged with a Carl Zeiss Apotome 2 fluorescence microscope.

11. Transwell study

The cells were seeded in a 12-well Transwell insert (polyester membrane, 0.4 μm pore, Corning) at a density of 1×10^5. The cells in the Transwell insert were incubated with 10 μM FITC-labelled peptides in serum-free 1640 medium for 1 hour. After removal of the peptides, the cells in the Transwell insert were thoroughly washed with PBS. After that, the Transwell insert was co-incubated with the cells at the bottom of the cell plates for another 2 hours. Then, the cells at the bottom were fixed with 2.5 % glutaraldehyde and stained with DAPI followed by imaging with a Carl Zeiss Apotome 2 fluorescence microscope.

12. Exocytosis of the peptides

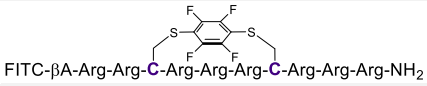
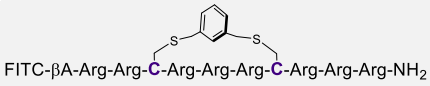
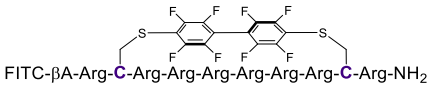
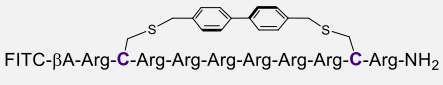
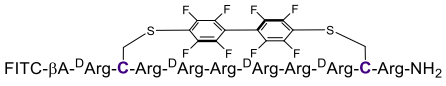
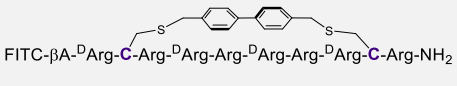
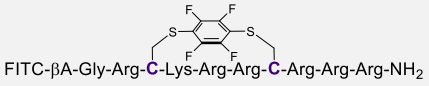
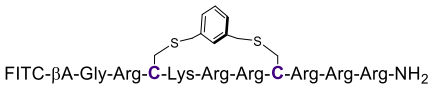
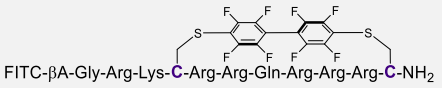
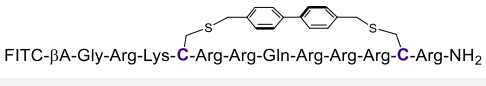
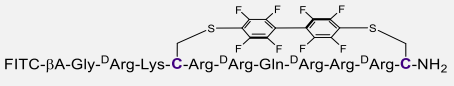
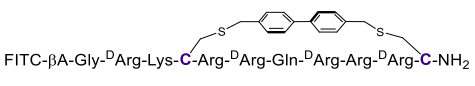
B16F10 cells were seeded in a 12-well plate at a density of 1×10^5 cells per well and cultured overnight. To confirm the exocytosis of the peptides, the cells were first incubated with FITC-labelled peptides in a serum-free medium for 1 hour followed by removing of the peptides and washing with PBS. Then, the cells were incubated in fresh medium for additional 2 hours followed by flow cytometry analysis. For the Exo1 or nocodazole group, the cells were first incubated with FITC-labelled peptides in a serum-free medium for 1 hour followed by removing of the peptides and washing with PBS. Then, the cells were incubated with Exo1 (50 μM) or nocodazole (10 μM) for additional 2 hours followed by flow cytometry analysis.

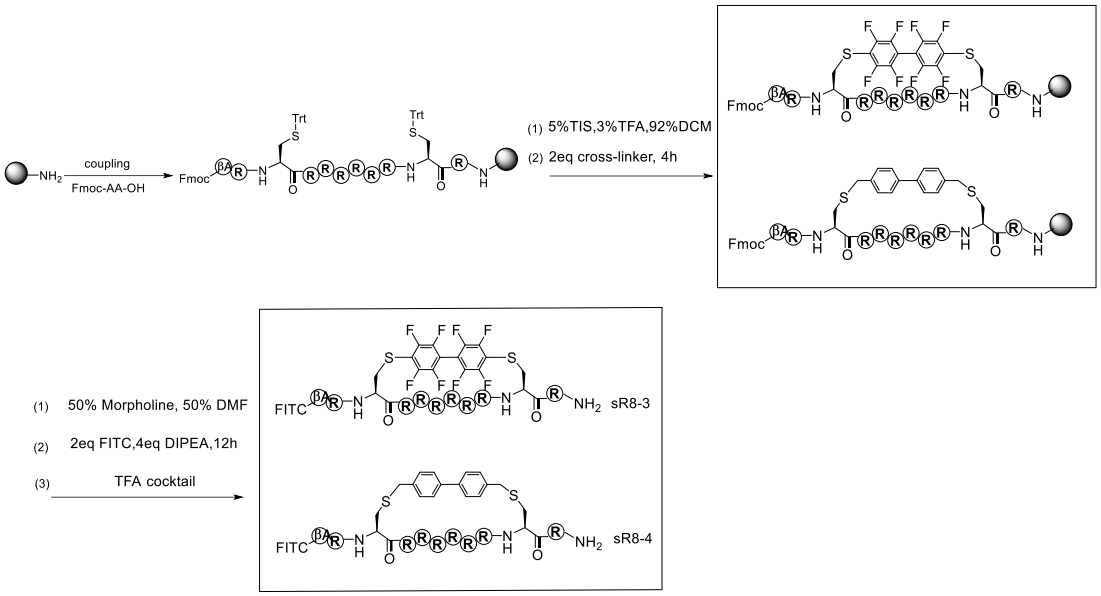
13. Statistical Analysis

SPPS was used to analyze the data, One-way analysis of variance (ANOVA) was performed to determine statistical significance of the data, the data were expressed as the mean ± SD. $P<0.05$ indicates a statistical difference, $P<0.01$ indicates a significant difference, and $P<0.001$ indicates a very significant difference.

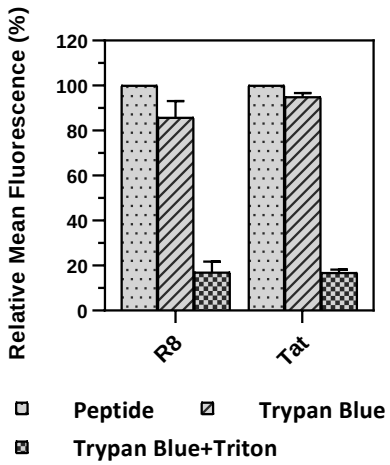
Tables and Figures

Supporting Table S1. The peptide sequence, chemical formula and mass spectrometry analysis.

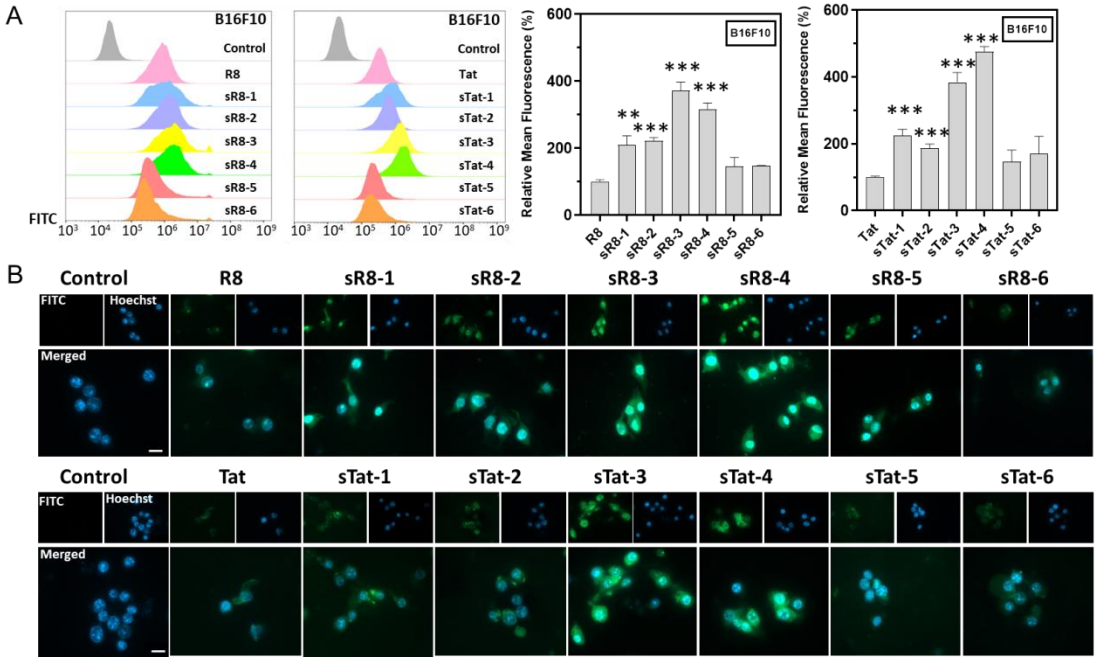
Peptide	Sequence	Chemical Formula	Calculated m/z	Found m/z
R8	FITC-βA-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-NH_2	$C_{72}H_{115}N_{35}O_{14}S$	576.30/432.48/ 346.18/288.65	576.63/432.72/ 346.39/288.85
sR8-1		$C_{84}H_{123}F_4N_{37}O_{16}S_3$	693.63/520.48/ 416.58/347.32	693.92/520.73/ 416.86/347.55
sR8-2		$C_{86}H_{131}N_{37}O_{16}S_3$	678.99/509.49/ 407.79/340.00	679.38/509.77/ 408.09/340.19
sR8-3		$C_{90}H_{123}F_8N_{37}O_{16}S_3$	742.97/557.48 446.18/371.98	743.37/557.73/ 446.44/372.19
sR8-4		$C_{92}H_{135}N_{37}O_{16}S_3$	704.33/528.50/ 423.00/352.67	704.74/528.77/ 423.34/352.91
sR8-5		$C_{90}H_{123}F_8N_{37}O_{16}S_3$	742.97/557.48 446.18/371.98	743.35/557.74/ 446.47/372.24
sR8-6		$C_{92}H_{135}N_{37}O_{16}S_3$	704.33/528.50/ 423.00/352.67	704.75/528.74/ 423.30/352.82
Tat	FITC-βA-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-NH_2	$C_{79}H_{126}N_{34}O_{17}S$	619.33/464.75/ 372.00/310.16	619.62/465.01/ 372.22/310.35
sTat-1		$C_{80}H_{114}F_4N_{32}O_{16}S_3$	651.27/488.71/ 391.16/326.14	651.64/488.97/ 391.44/326.31
sTat-2		$C_{82}H_{122}N_{32}O_{16}S_3$	636.63/477.72/ 382.38/318.82	637.03/478.02/ 382.67/319.01
sTat-3		$C_{91}H_{122}F_8N_{34}O_{18}S_3$	743.29/557.72/ 446.37/372.15	743.68/557.97/ 446.66/372.47
sTat-4		$C_{93}H_{134}N_{34}O_{18}S_3$	704.66/528.75/ 423.20/352.83	704.97/529.01/ 423.50/353.04
sTat-5		$C_{91}H_{122}F_8N_{34}O_{18}S_3$	743.29/557.72/ 446.37/372.15	743.69/557.96/ 446.65/372.38
sTat-6		$C_{93}H_{134}N_{34}O_{18}S3$	704.66/528.75/ 423.20/352.83	705.07/529.02/ 423.54/353.04



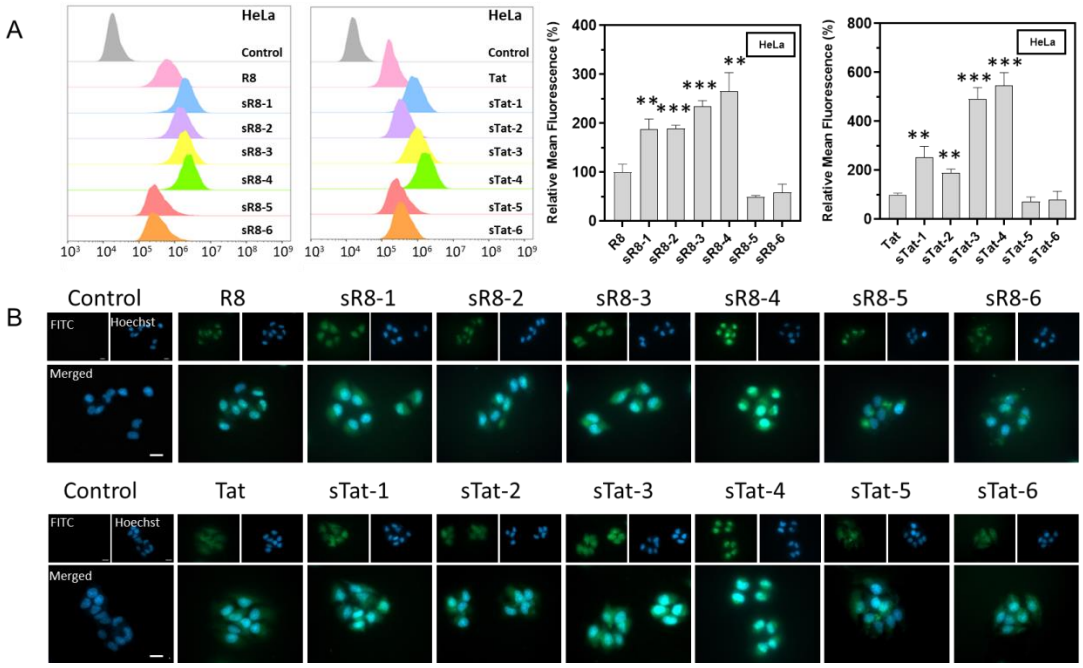
Supporting Figure S1. Representative synthetic route for the construction of stapled cell penetrating peptides.



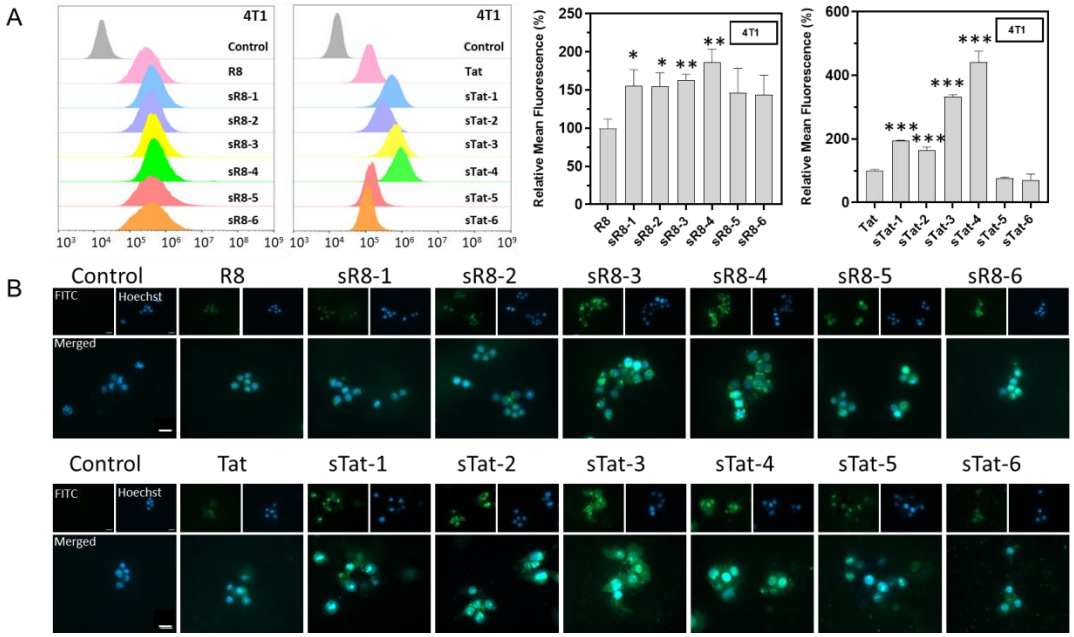
Supporting Figure S2. Flow cytometry analysis of B16F10 cells treated with FITC-labelled peptides in the presence and absence of Trypan blue and Triton. Mean fluorescence, mean ± s. d., and n=3.



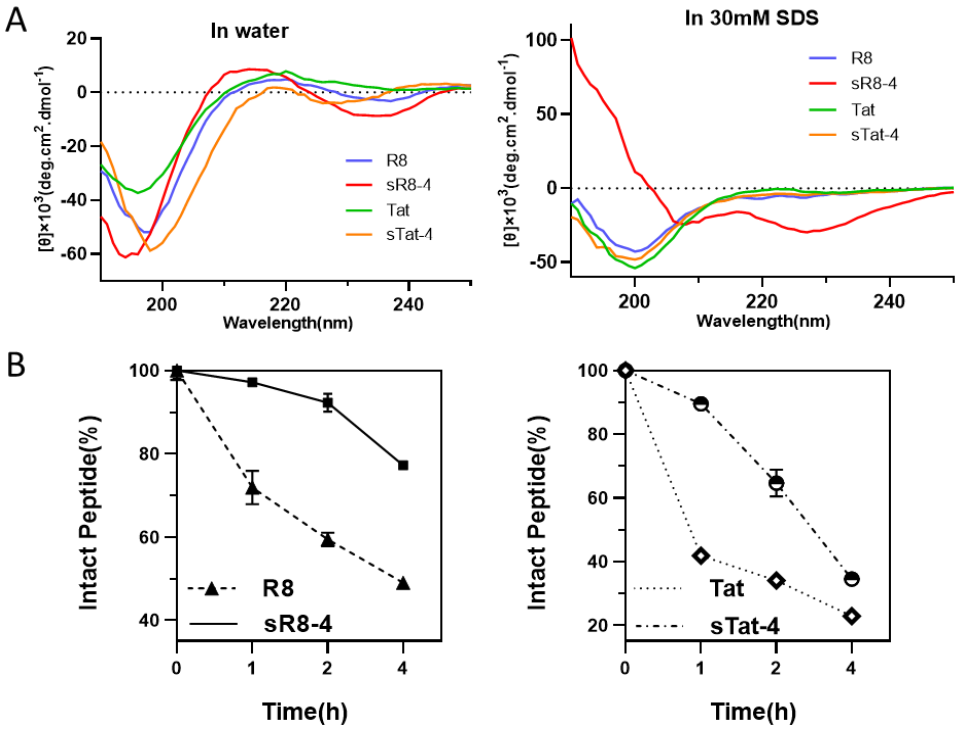
Supporting Figure S3. Cellular uptake of the peptides. A) Flow cytometry analysis of B16F10 cells after incubation with 3 μM FITC-labelled peptides for 1 hour. Relative mean fluorescence mean ± s. d., and n=3; B) Live-cell fluorescence microscopic images of B16F10 cells after incubation with 3 μM FITC-labelled peptides for 1 hour. FITC (green), Hoechst (blue). Scale bar, 20 μm. ** P<0.01, *** P<0.001.



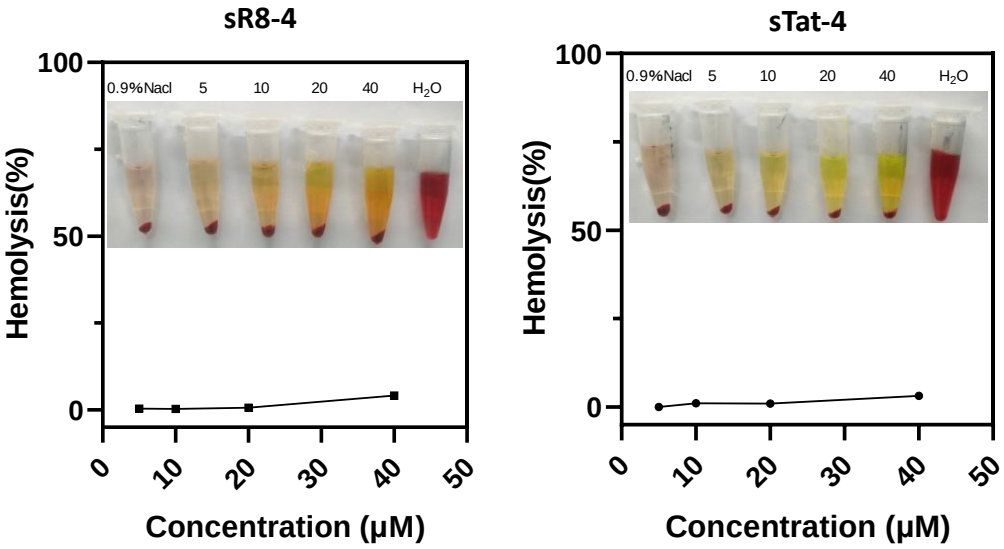
Supporting Figure S4. Cell penetration of the peptides. A) Flow cytometry analysis of HeLa cells after incubation with 3 μM FITC-labelled peptides for 1h. Relative mean fluorescence, mean ± s. d., and n=3; B) Live-cell fluorescence microscopic images of HeLa cells after incubation with 3 μM FITC-labelled peptides for 1h. FITC (green), Hoechst (blue). Scale bar, 20 μm. ** P<0.01, *** P<0.001.



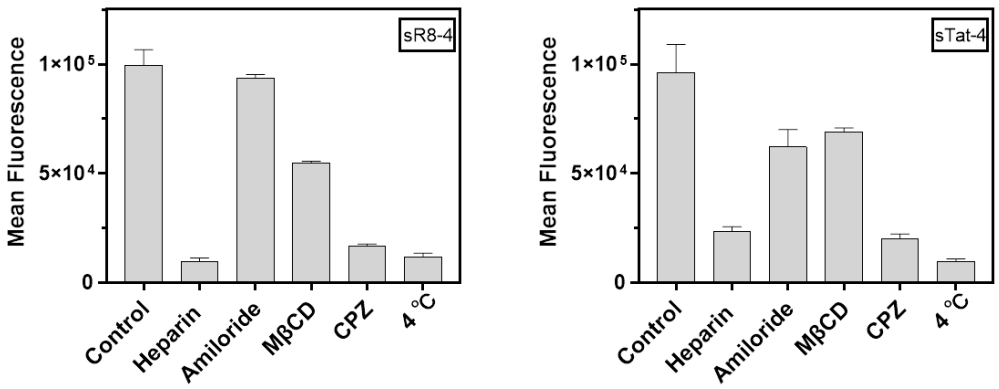
Supporting Figure S5. Cell penetration of the peptides. A) Flow cytometry analysis of 4T1 cells after incubation with 3 μM FITC-labelled peptides for 1h. Relative mean fluorescence, mean ± s. d., and n=3; B) Live-cell fluorescence microscopic images of 4T1 cells after incubation with 3 μM FITC-labelled peptides for 1h. FITC (green), Hoechst (blue). Scale bar, 20 μm. * P<0.05, ** P<0.01, *** P<0.001.



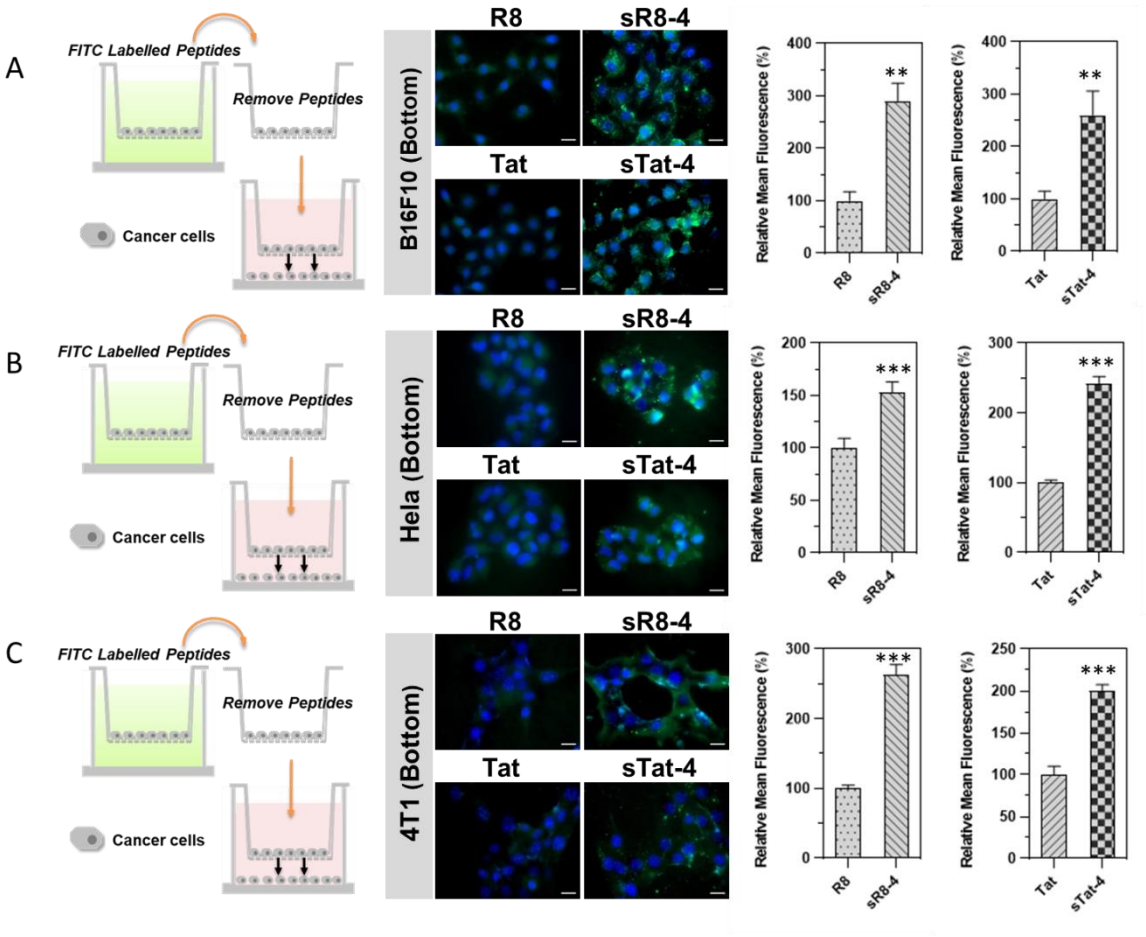
Supporting Figure S6. A) CD spectra of the peptides in ddH_2O and in 30 mM SDS solution. B) Serum stability of the peptides in the presence of 25% FBS. Percentage intact, mean ± s. d., and n=3.



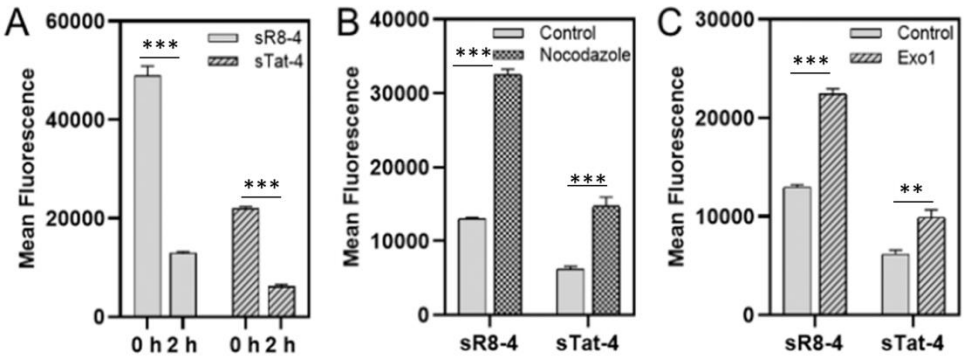
Supporting Figure S7. Hemolytic activity of the stapled cell penetrating peptides. mean ± s. d., and n=3.



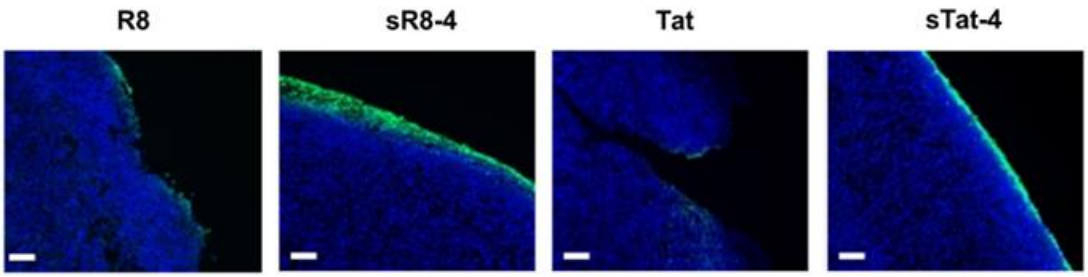
Supporting Figure S8. Mean fluorescence of B16F10 cells after incubation with 3 μM FITC-labelled stapled CPPs in the presence of different endocytosis inhibitors. Mean fluorescence, mean ± s. d., and n=3.



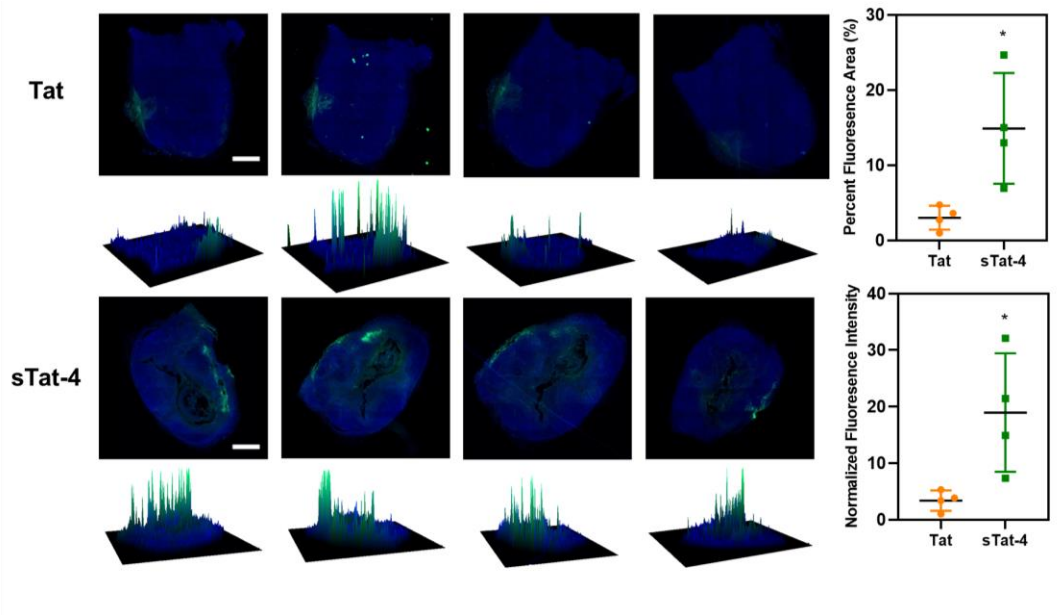
Supporting Figure S9. Schematic illustration for in vitro experimental models of transcellular penetration of B16F10 cells (A), HeLa cells (B) and 4T1 cells (C). Fluorescence microscopic images of tumor cells in the bottom chamber. Scale bar, 20 μm. ** P<0.01, *** P<0.001. Relative mean fluorescence, mean ± s. d., and n=3;



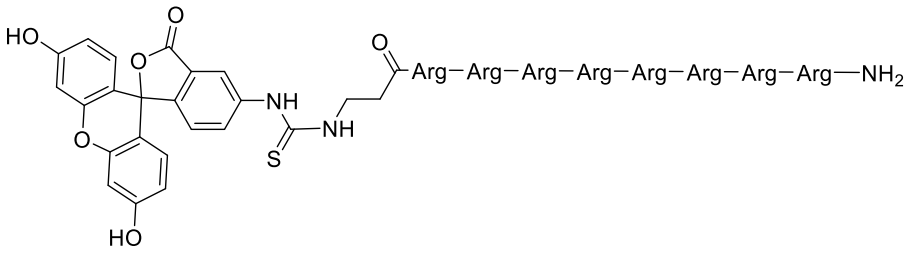
Supporting Figure S10. Exocytosis of the stapled cell-penetrating peptides. Flow cytometry analysis of B16F10 cells treated with FITC-labelled peptides for 1 hour, washed with PBS, and incubated in fresh medium for additional 2 hours (A). Mean fluorescence, mean ± s. d., and n=3; Flow cytometry analysis of B16F10 cells treated with FITC-labelled peptides for 1 hour, washed with PBS, and incubated in fresh medium for additional 2 hours in the presence and absence of inhibitor nocodazole (B) or Exo 1 (C). ** $P<0.01$, *** $P<0.001$. Mean fluorescence, mean ± s. d., and n=3.



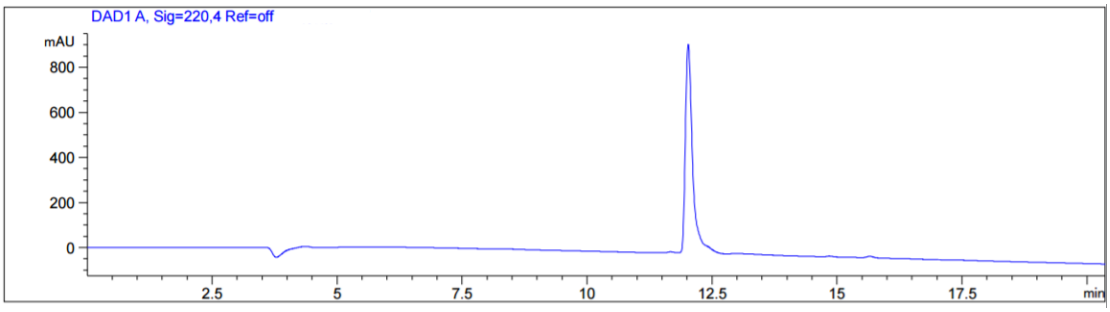
Supporting Figure S11. Fluorescence microscopic images of 4T1 tumors explants after incubation with FITC-labelled peptides. Nuclei were stained with DAPI (blue). Scale bar, 200 μm.

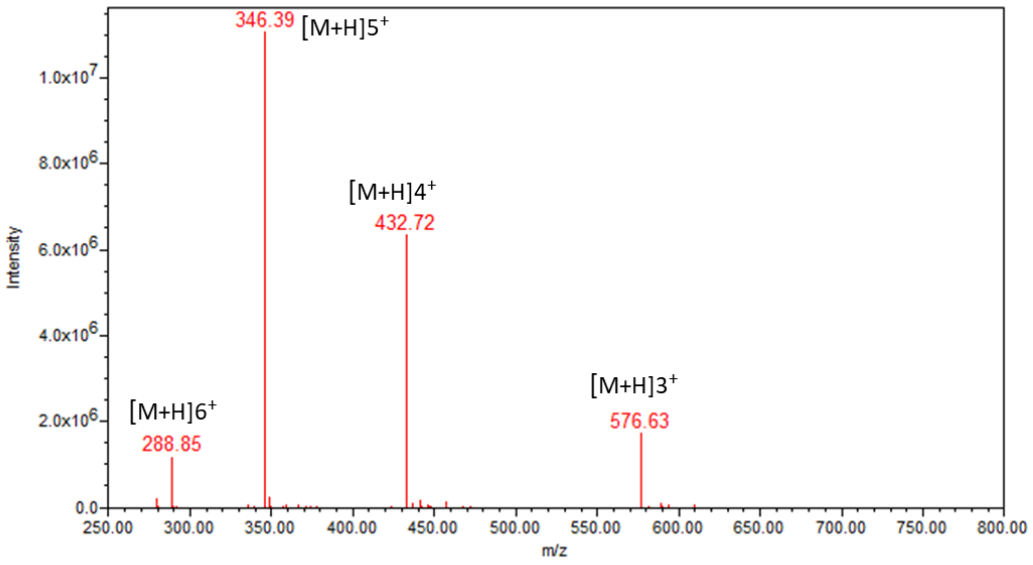


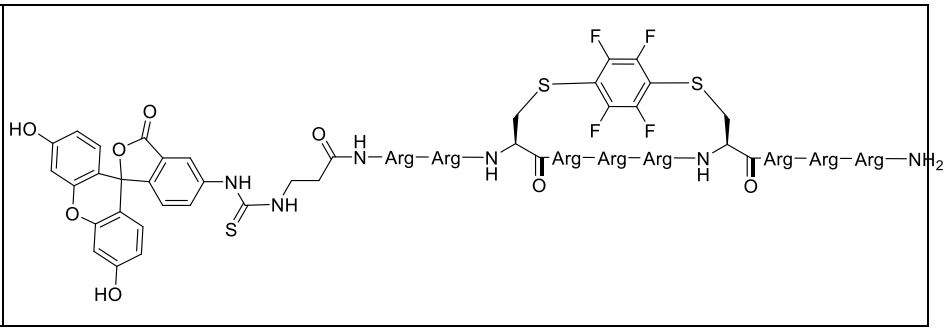
Supporting Figure S12. Fluorescent images of 4T1 tumors sections after intratumorally injection with 40 μM FITC-labelled Tat and sTat-4 peptides for 4 h before harvesting of the tumors. The space between adjacent sections is 300 μm. Scale bar, 1000 μm. * $P<0.05$. Fluorescence area and intensity, mean ± s. d., and n=4.

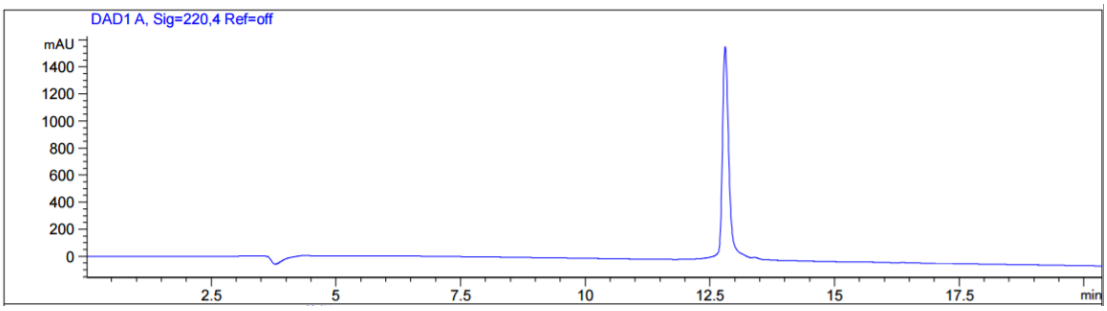
R8	

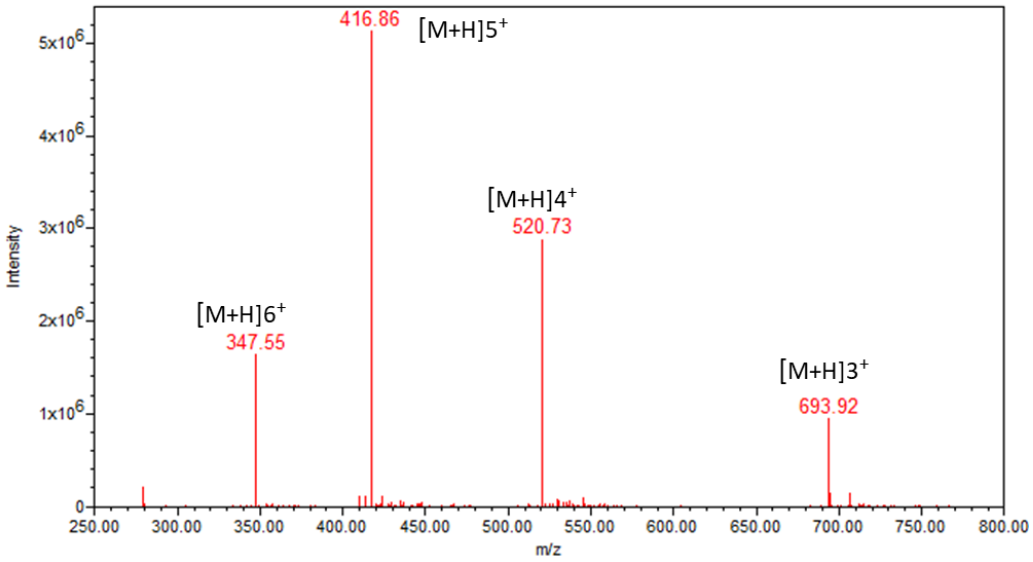
HO
O
O
O
NH
NH
S
O
Arg—Arg—Arg—Arg—Arg—Arg—Arg—Arg—NH2
HO

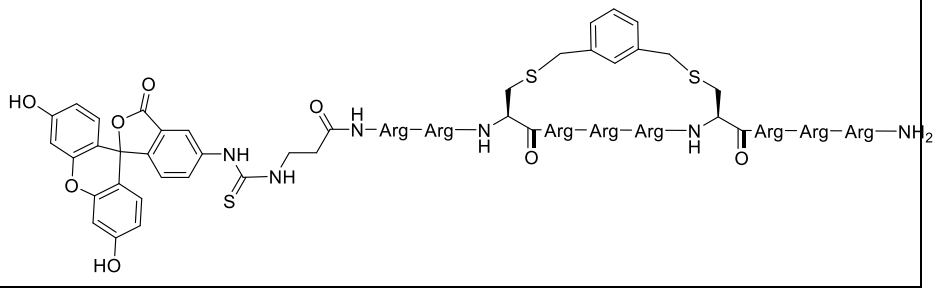

DAD1 A, Sig=220,4 Ref=off
mAU
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
min

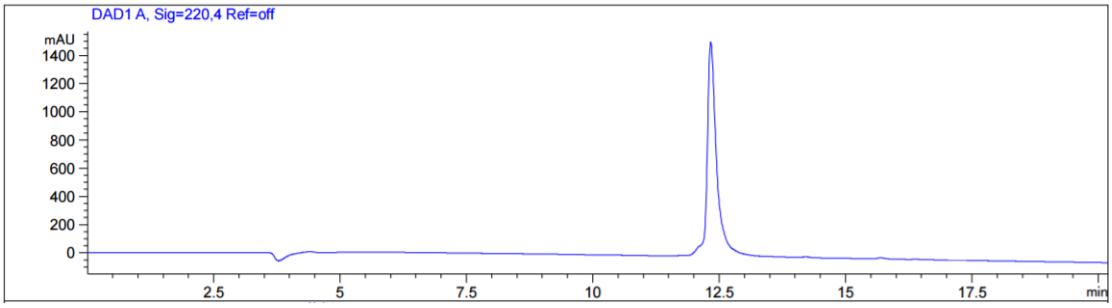

346.39
[M+H]5+
1.0x10^7
8.0x10^6
[M+H]4+
432.72
6.0x10^6
Intensity
4.0x10^6
[M+H]3+
576.63
2.0x10^6
[M+H]6+
288.85
0.0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

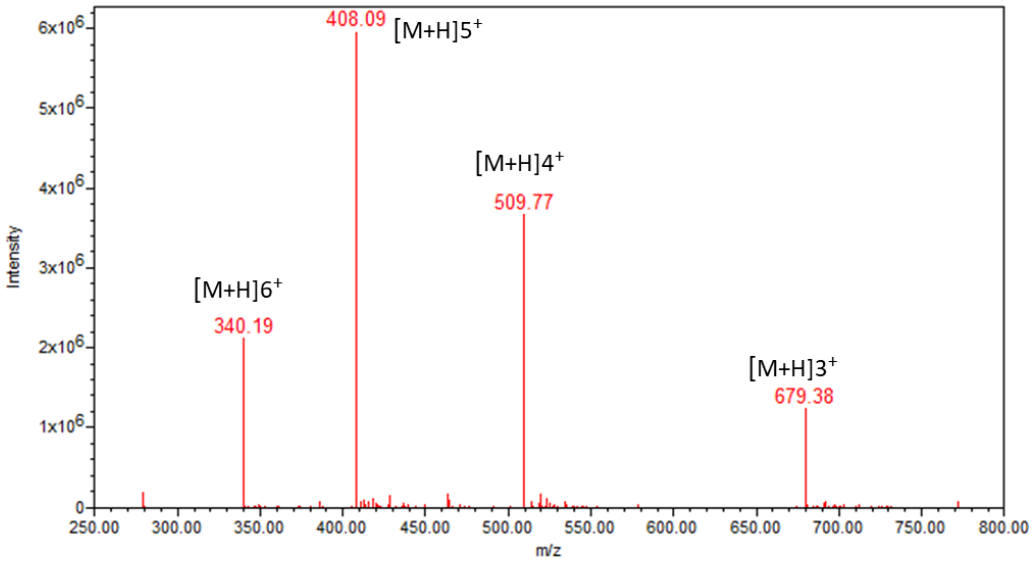
sR8-1	

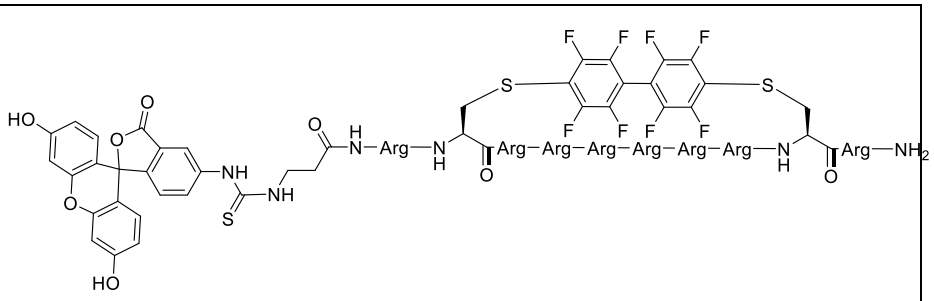

DAD1 A, Sig=220,4 Ref=off
mAU
1400
1200
1000
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
min

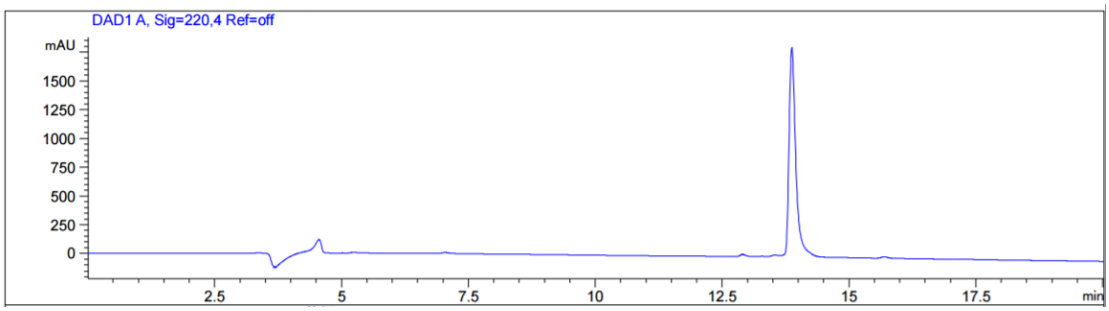

416.86
[M+H]5+
[M+H]4+
520.73
[M+H]6+
347.55
[M+H]3+
693.92
Intensity
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

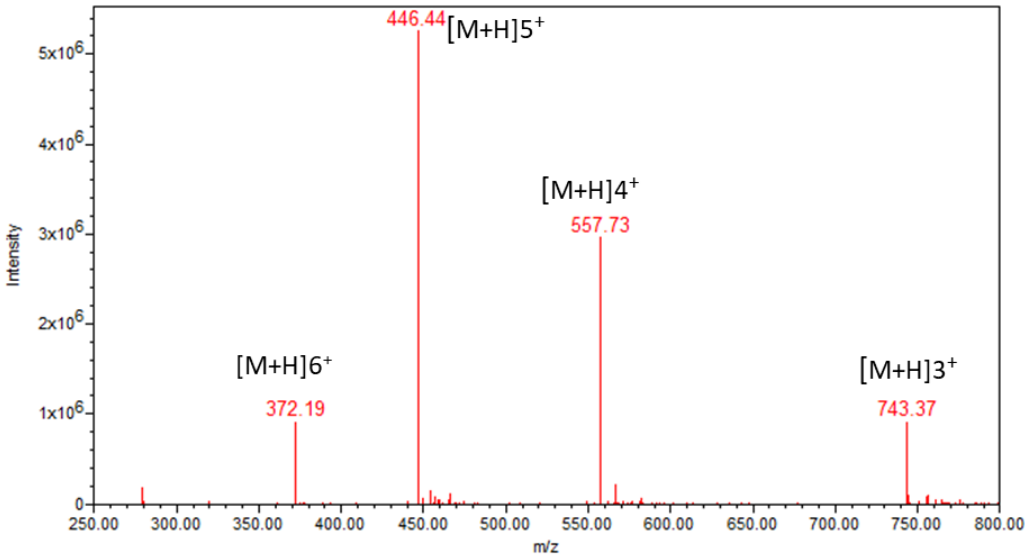
sR8-2	

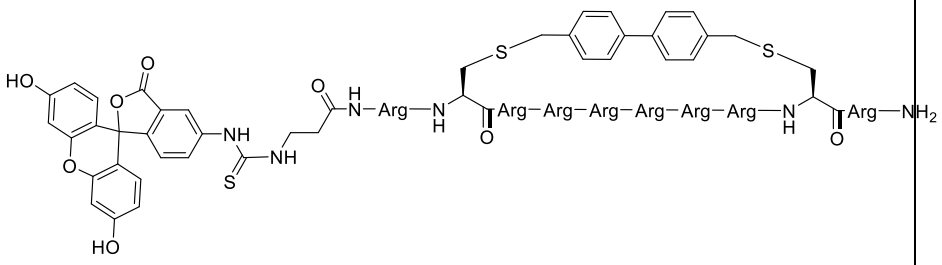

DAD1 A, Sig=220,4 Ref=off
mAU
1400
1200
1000
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
min

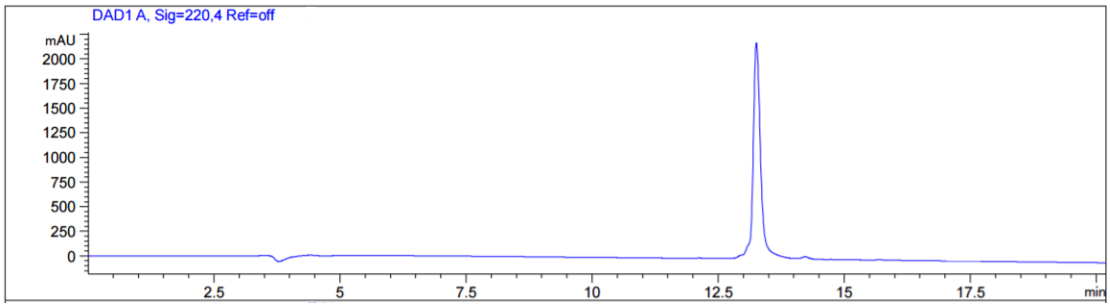

408.09
[M+H]5+
[M+H]4+
509.77
[M+H]6+
340.19
[M+H]3+
679.38
Intensity
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

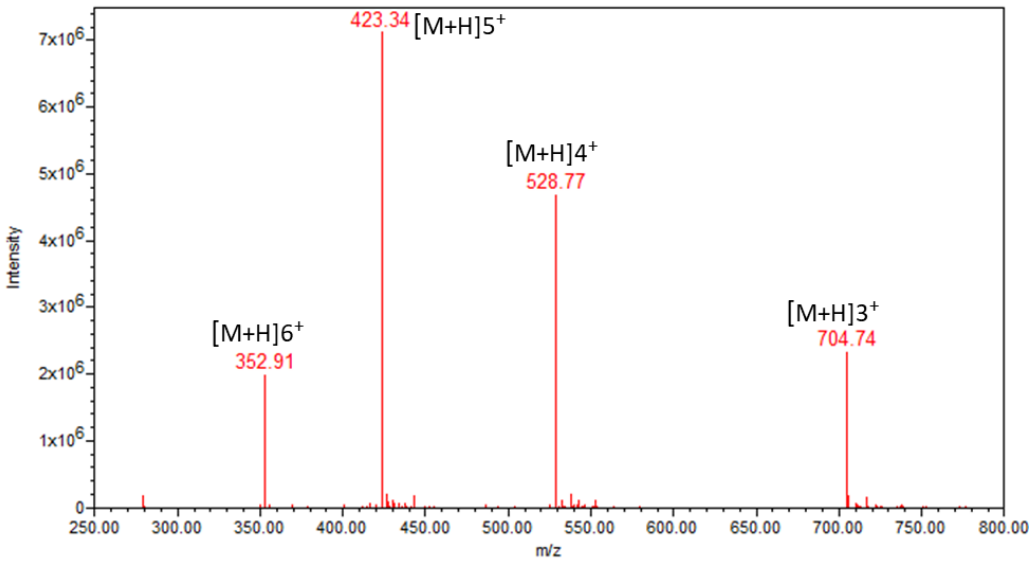
sR8-3	

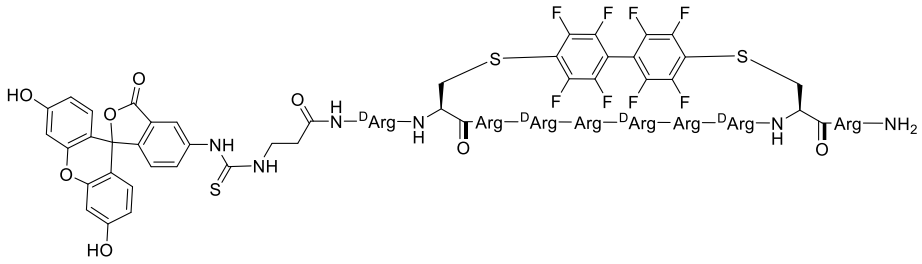

DAD1 A, Sig=220,4 Ref=off
mAU
1500
1250
1000
750
500
250
0
2.5
5
7.5
10
12.5
15
17.5
min

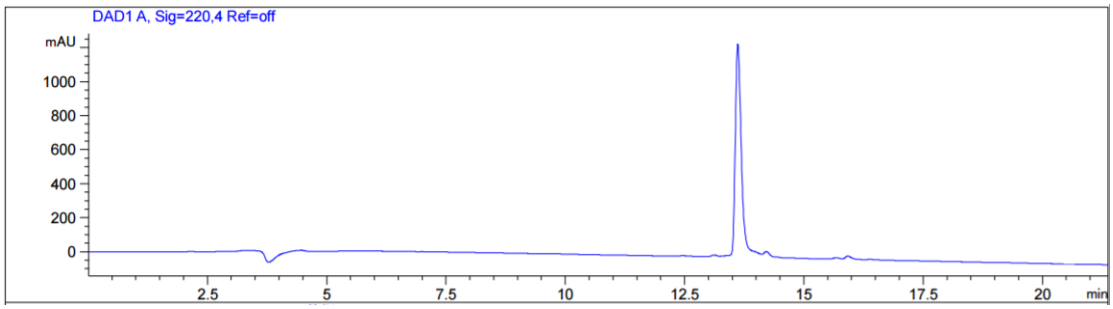

446.44
[M+H]5+
[M+H]4+
557.73
[M+H]6+
372.19
[M+H]3+
743.37
Intensity
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

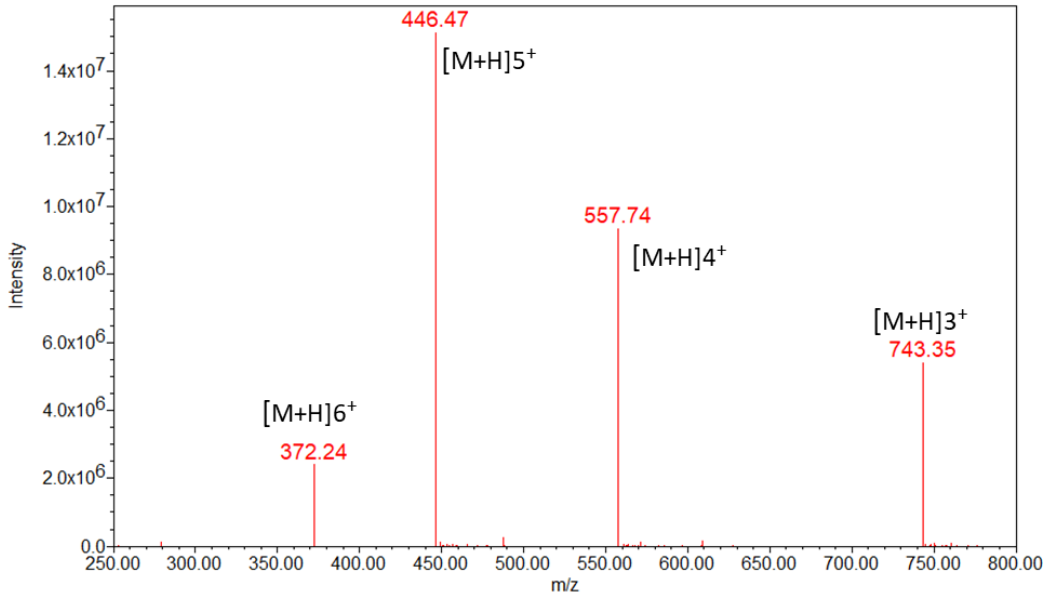
sR8-4	

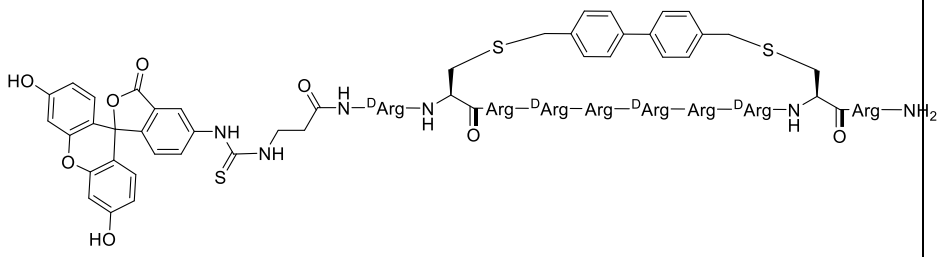

DAD1 A, Sig=220,4 Ref=off
mAU
2000
1750
1500
1250
1000
750
500
250
0
2.5
5
7.5
10
12.5
15
17.5
min

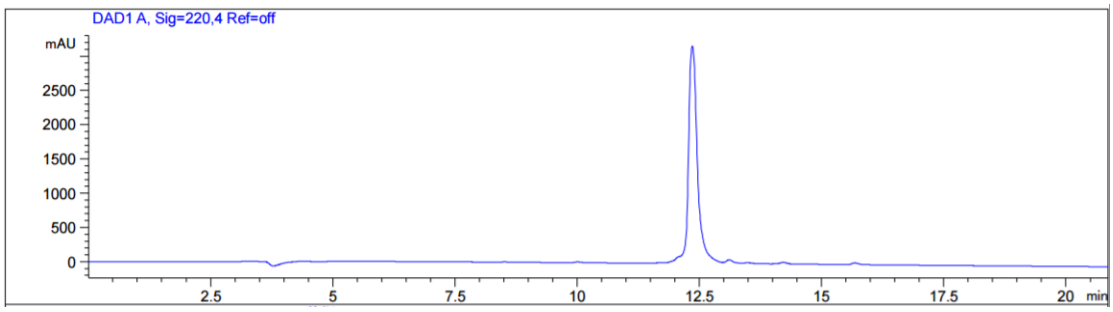

423.34 [M+H]5+
[M+H]4+
528.77
[M+H]3+
704.74
[M+H]6+
352.91
Intensity
7x10^6
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

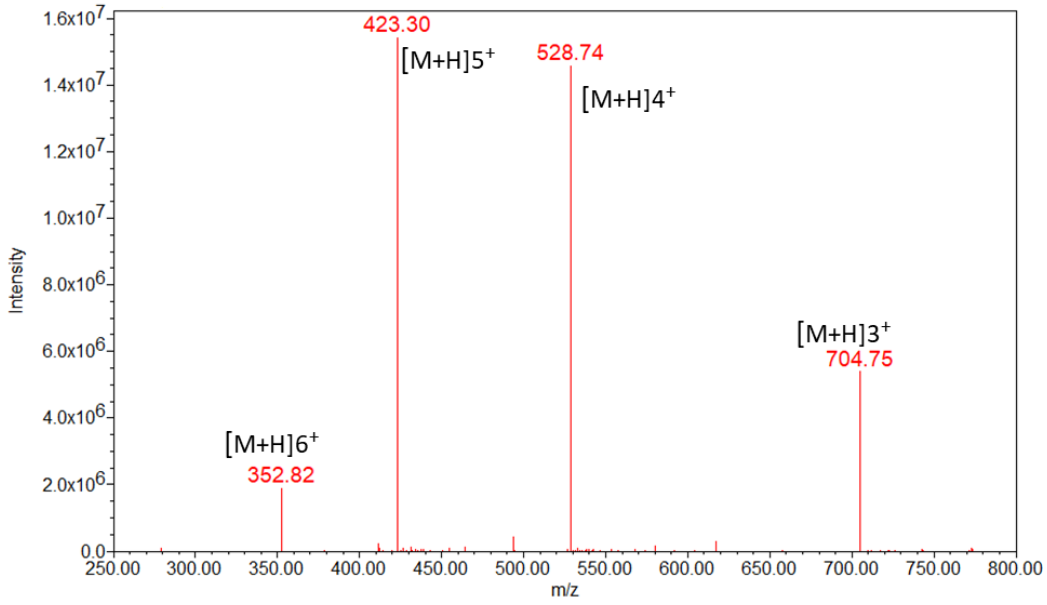
sR8-5	

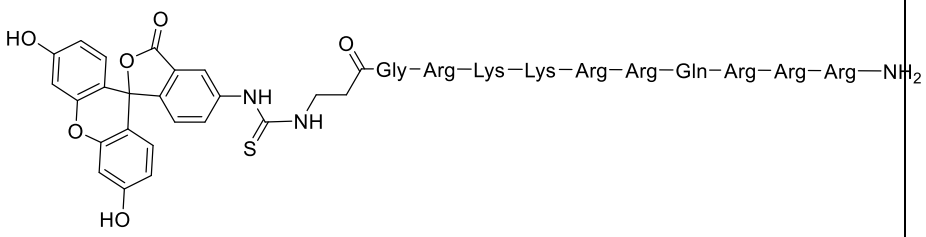

DAD1 A, Sig=220,4 Ref=off
mAU
1000
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
20
min


446.47
[M+H]5+
557.74
[M+H]4+
[M+H]3+
743.35
[M+H]6+
372.24
Intensity
1.4x10^7
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

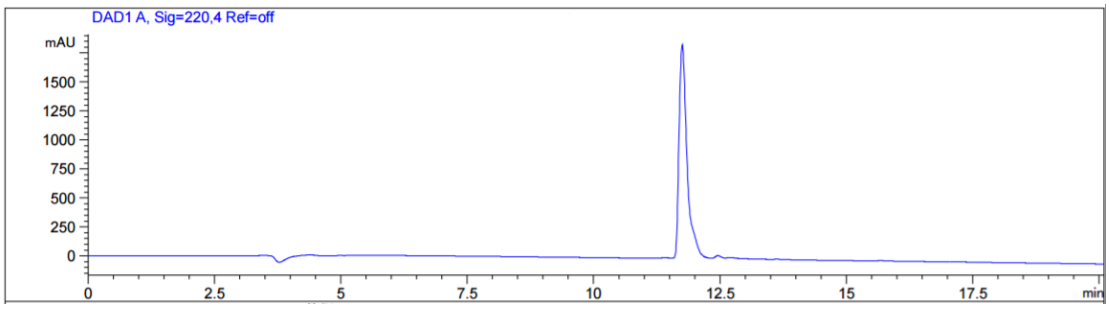
sR8-6	

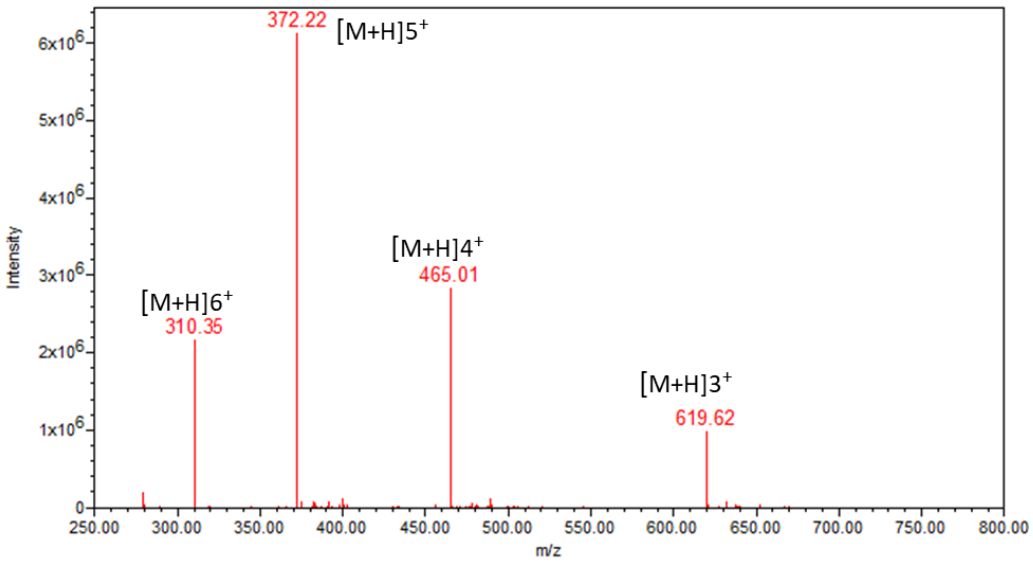

DAD1 A, Sig=220,4 Ref=off
mAU
2500
2000
1500
1000
500
0
2.5
5
7.5
10
12.5
15
17.5
20
min

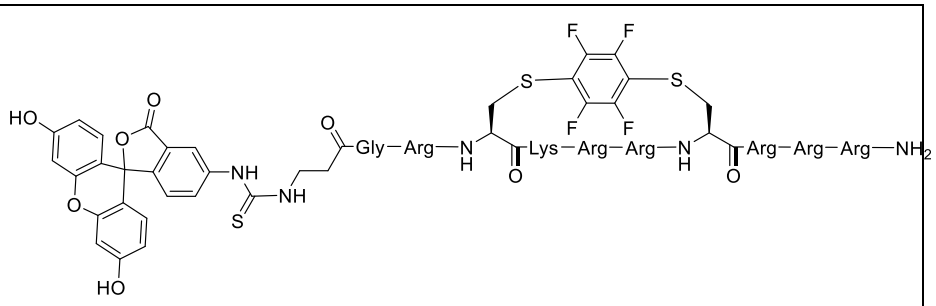

423.30
[M+H]5+
528.74
[M+H]4+
[M+H]3+
704.75
[M+H]6+
352.82
Intensity
1.6x10^7
1.4x10^7
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

Tat	

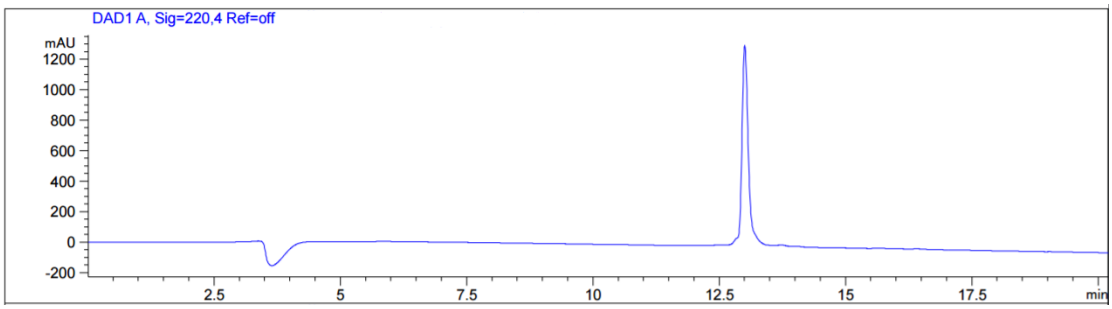
HO
O
O
NH
NH
S
O
Gly–Arg–Lys–Lys–Arg–Arg–Gln–Arg–Arg–Arg—NH2
O
HO

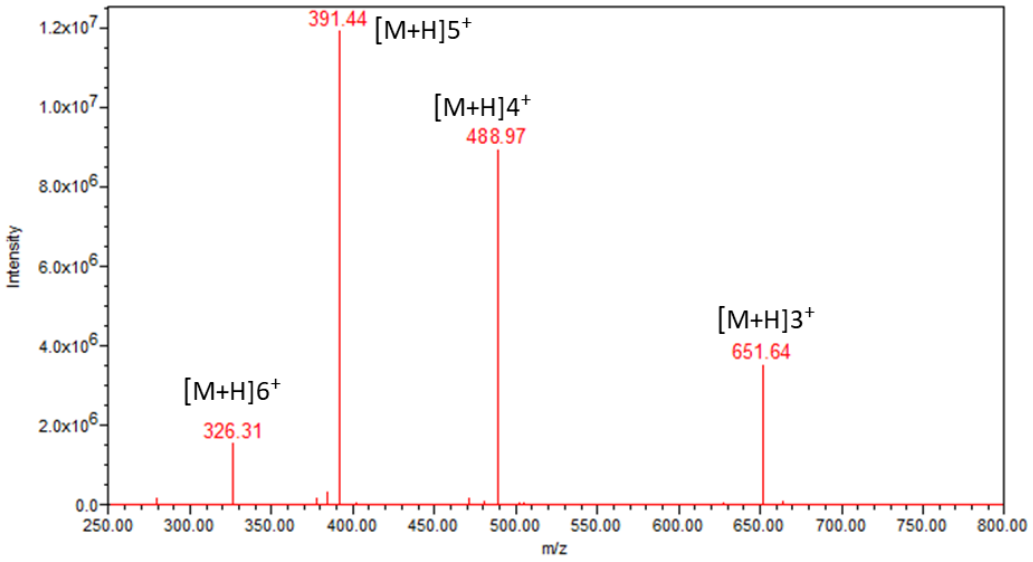

DAD1 A, Sig=220,4 Ref=off
mAU
1500
1250
1000
750
500
250
0
0
2.5
5
7.5
10
12.5
15
17.5
min

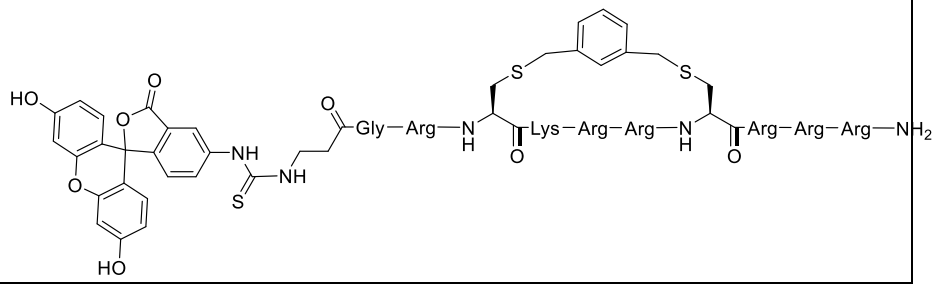

372.22
[M+H]5+
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
Intensity
[M+H]4+
465.01
[M+H]6+
310.35
[M+H]3+
619.62
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

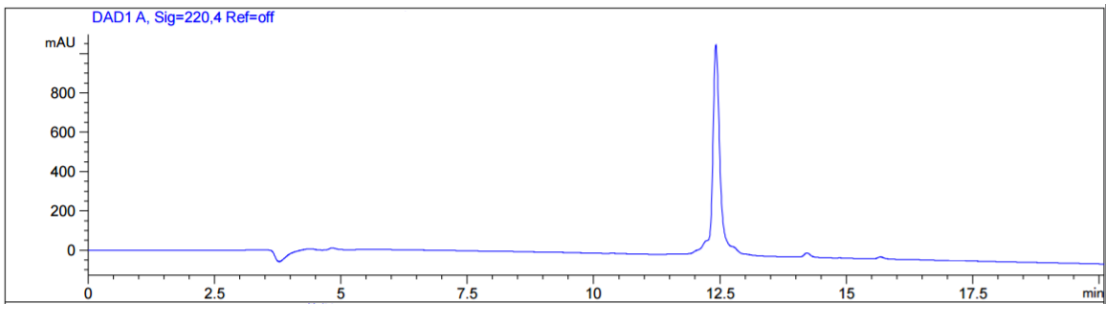
sTat-1	

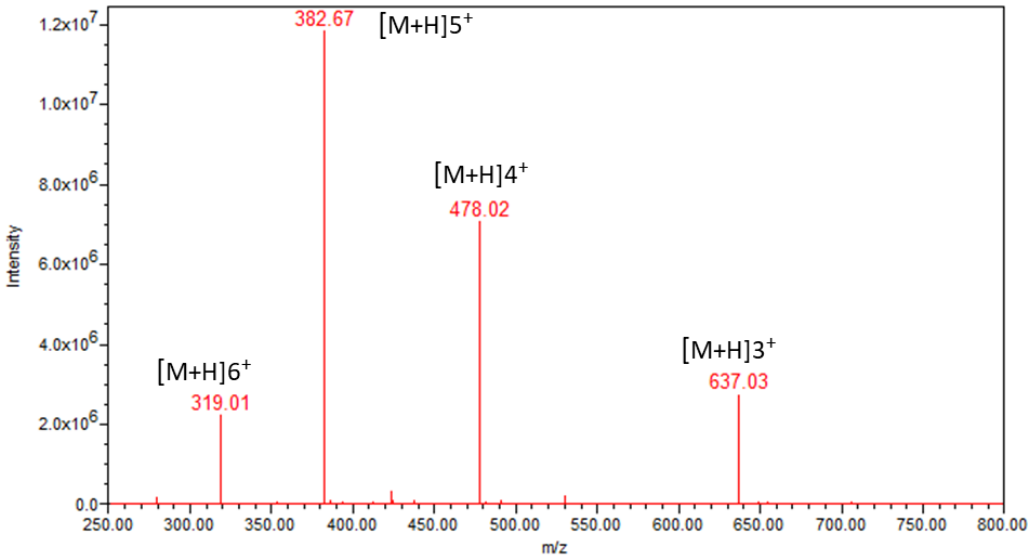
HO
O
O
O
HO
NH
S
NH
O
Gly—Arg—N
H
O
F
F
S
S
F
F
Lys—Arg—Arg—N
H
O
Arg—Arg—Arg—NH2

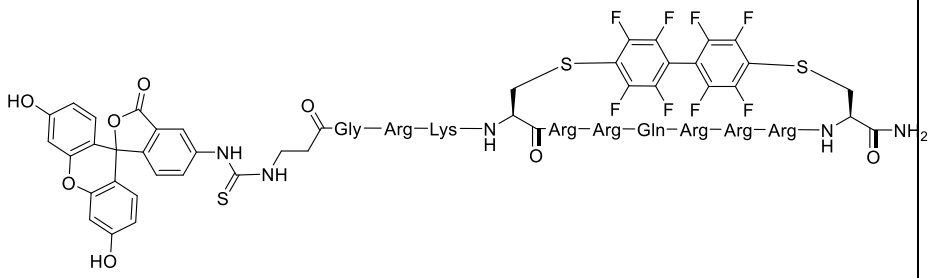

DAD1 A, Sig=220,4 Ref=off
mAU
1200
1000
800
600
400
200
0
-200
2.5
5
7.5
10
12.5
15
17.5
min

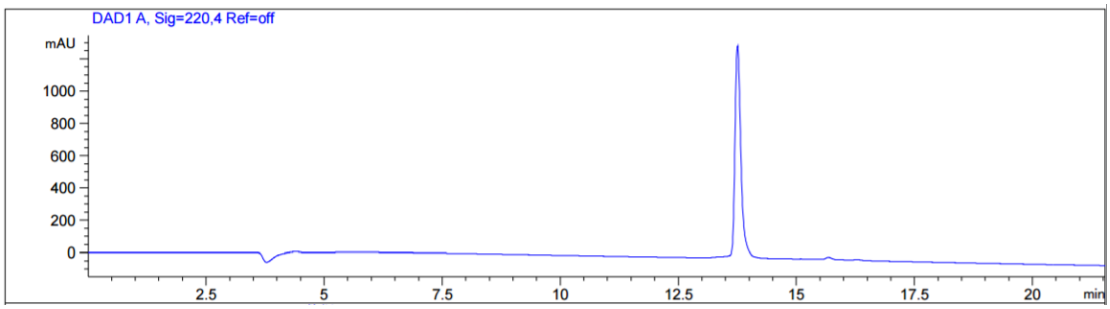

391.44
[M+H]5+
[M+H]4+
488.97
[M+H]3+
651.64
[M+H]6+
326.31
Intensity
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

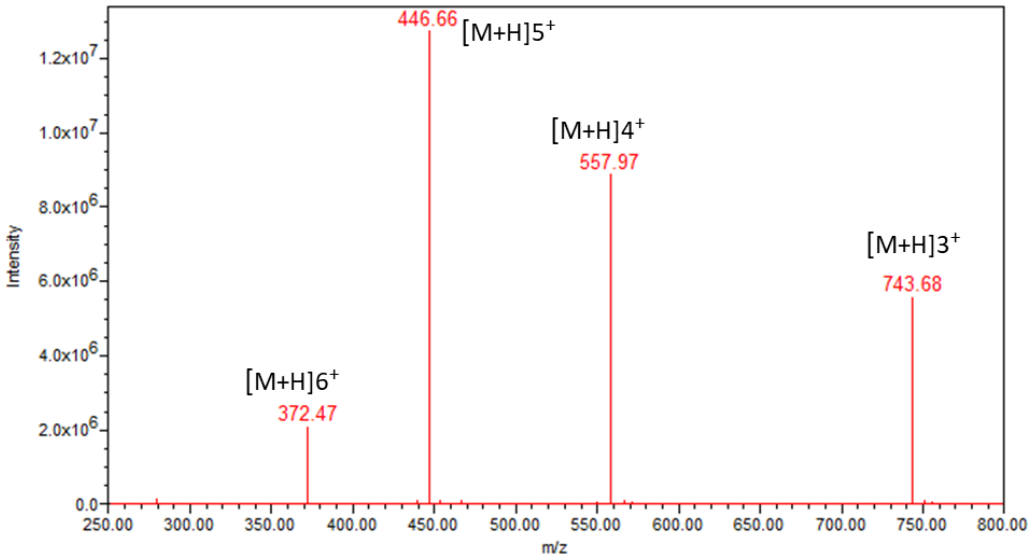
sTat-2	

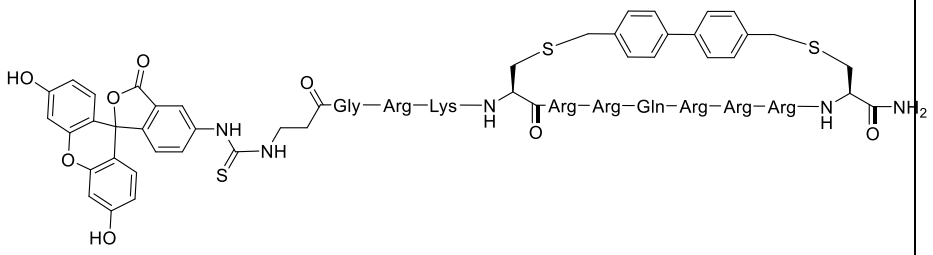

DAD1 A, Sig=220,4 Ref=off
mAU
800
600
400
200
0
0
2.5
5
7.5
10
12.5
15
17.5
min


382.67
[M+H]5+
[M+H]4+
478.02
[M+H]6+
319.01
[M+H]3+
637.03
Intensity
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

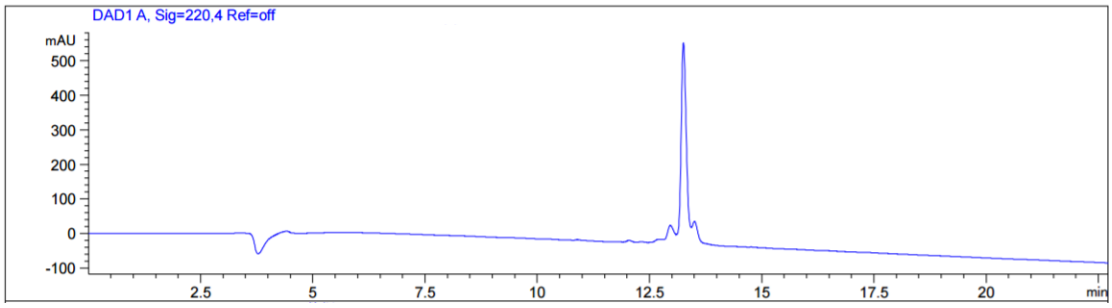
sTat-3	

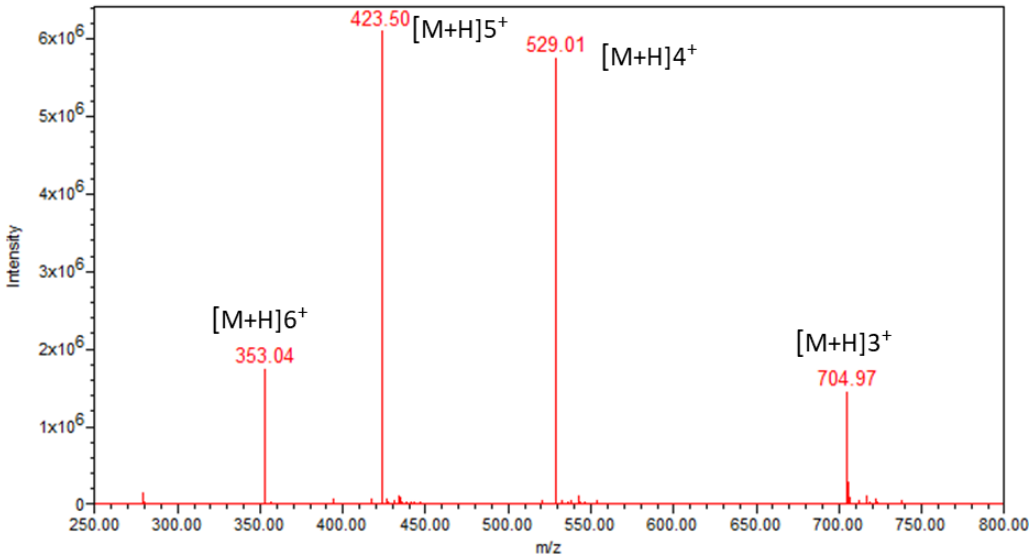

DAD1 A, Sig=220,4 Ref=off
mAU
1000
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
20
min

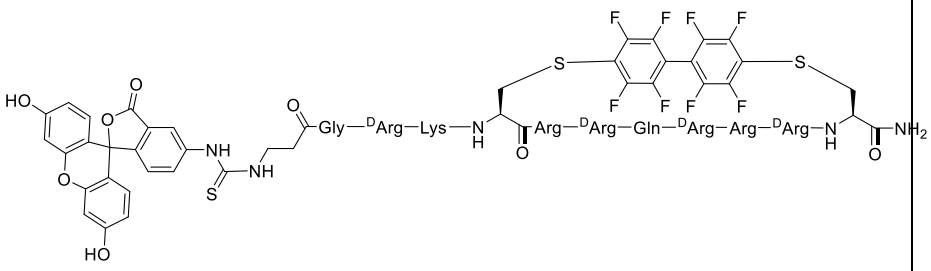

446.66
[M+H]5+
[M+H]4+
557.97
[M+H]3+
743.68
[M+H]6+
372.47
Intensity
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

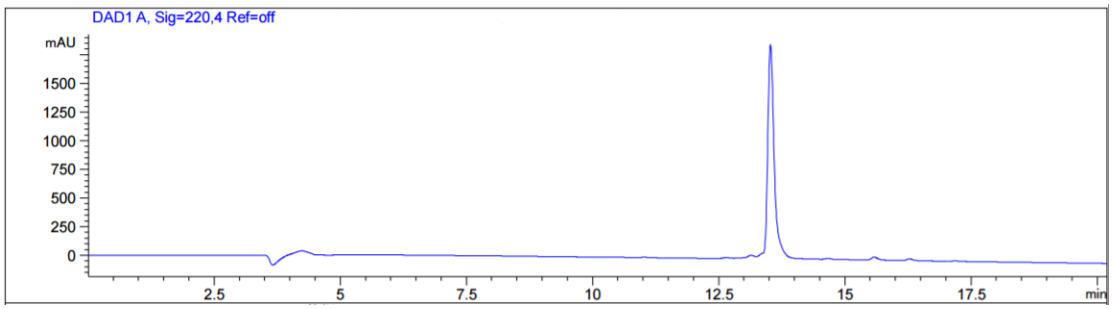
sTat-4	

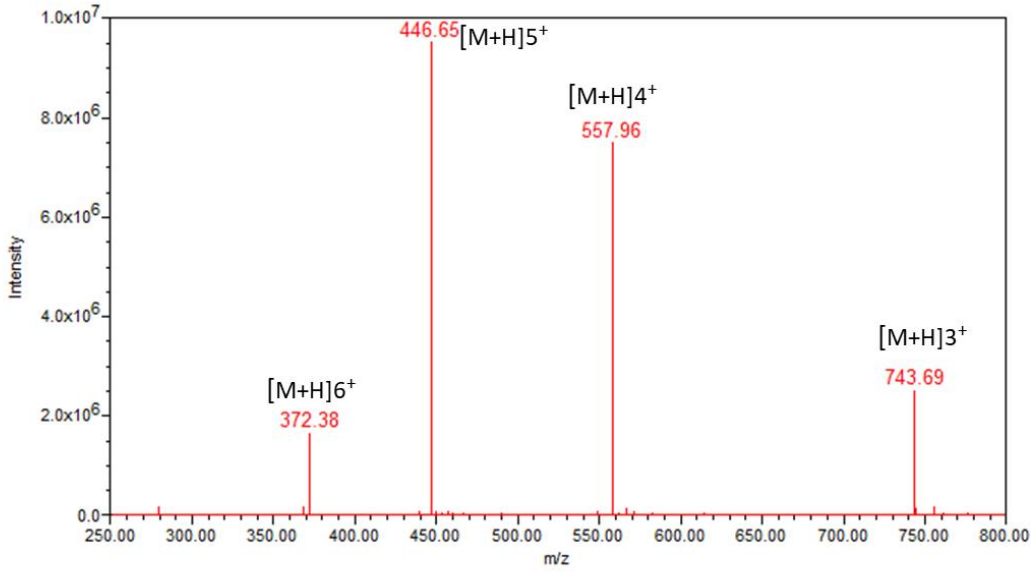
HO
O
O
O
HO
NH
NH
S
O
Gly—Arg—Lys—N
H
S
S
Arg—Arg—Gln—Arg—Arg—Arg—N
H
O
NH2

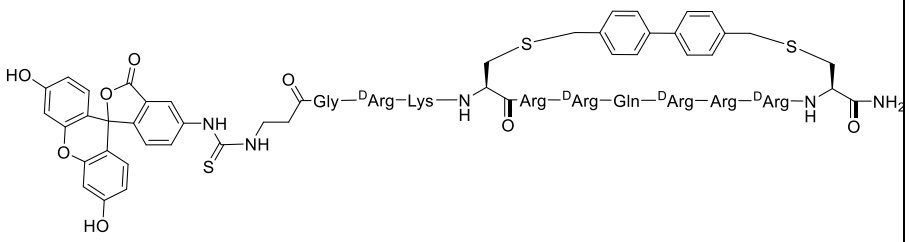

DAD1 A, Sig=220,4 Ref=off
mAU
500
400
300
200
100
0
-100
2.5
5
7.5
10
12.5
15
17.5
20
min

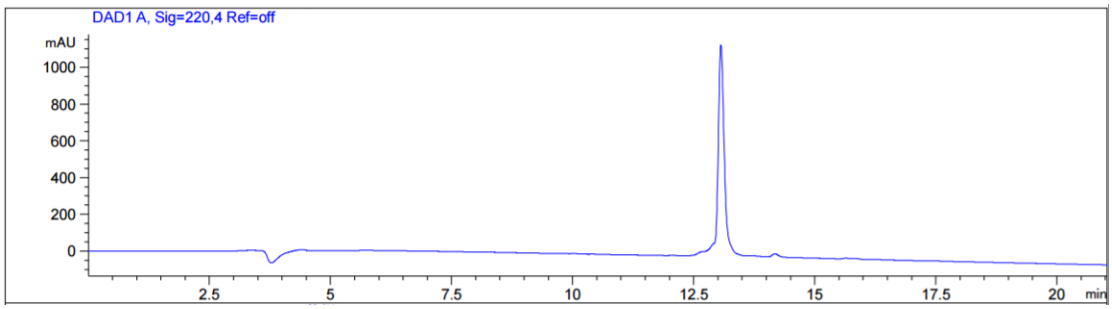

423.50
[M+H]5+
529.01
[M+H]4+
[M+H]6+
353.04
[M+H]3+
704.97
Intensity
6x10^6
5x10^6
4x10^6
3x10^6
2x10^6
1x10^6
0
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

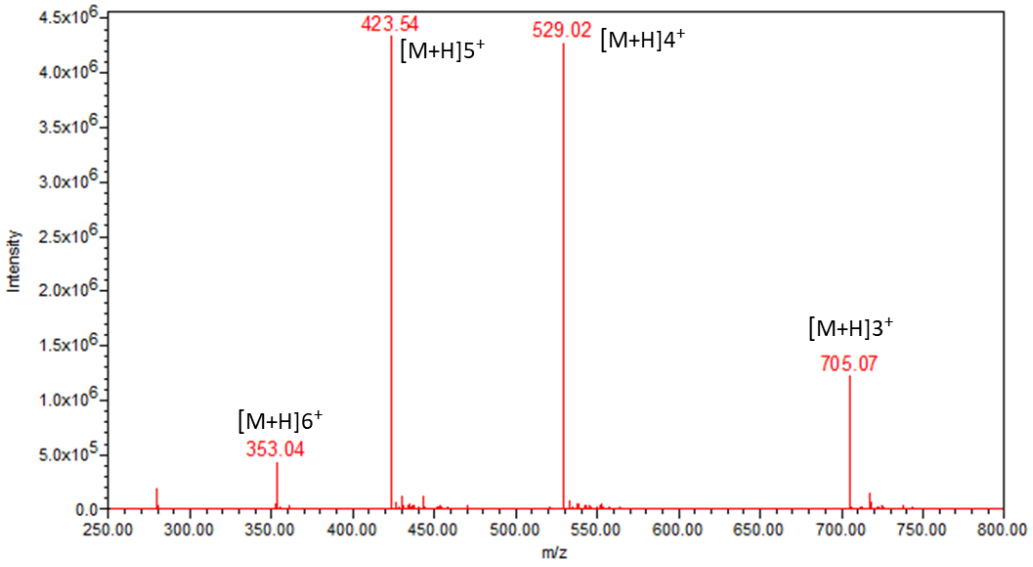
sTat-5	


DAD1 A, Sig=220,4 Ref=off
mAU
1500
1250
1000
750
500
250
0
2.5
5
7.5
10
12.5
15
17.5
min


1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
Intensity
446.65
[M+H]5+
[M+H]4+
557.96
[M+H]3+
743.69
[M+H]6+
372.38
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z

sTat-6	


DAD1 A, Sig=220,4 Ref=off
mAU
1000
800
600
400
200
0
2.5
5
7.5
10
12.5
15
17.5
20
min


Intensity
4.5x10^6
4.0x10^6
3.5x10^6
3.0x10^6
2.5x10^6
2.0x10^6
1.5x10^6
1.0x10^6
5.0x10^5
0.0
423.54
[M+H]5+
529.02
[M+H]4+
[M+H]3+
705.07
[M+H]6+
353.04
250.00
300.00
350.00
400.00
450.00
500.00
550.00
600.00
650.00
700.00
750.00
800.00
m/z