Supporting information for

anaerobic Sulfatase-Maturating Enzymes (anSME), radical SAM enzymes able to catalyze *in vitro* sulfatase post-translational modification

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General procedures

- **Bacterial strains, plasmids and DNA manipulations:** *C. perfringens* strain used in this study was ATCC 13124 strain. *E. coli* DH5α was used for routine DNA manipulations and *E. coli* BL21 (DE3) (Stratagene) was used for overexpression of the CPE0635 gene encoding the maturation enzyme. T4 DNA ligase was from Promega, Inc. Plasmid DNA purification kit (QIAprep spin) were from Qiagen, Inc. DNA fragments were extracted from agarose gel and purified with Wizard SV Gel and PCR clean up system Kit (Promega, Inc.). DNA sequencing was performed by VWR.

- **Cloning and construction of the pET-6His-CPE0635 overexpressing plasmid**: *C. perfringens* was grown anaerobically in BHI medium, pH 7.0, and cells harvested to extract their genomic DNA using the Wizard Genomic Kit from Promega. The CPE0635 gene encoding the maturation enzyme was amplified by a Polymerase Chain Reaction-based method (PCR) using genomic DNA as a template and the following primers: 5'cat atg cca cca tta agt ttg ctt att aag cca3' and 5'ctc gag tta tta ttt aat att gtt ggc aac att att tat3'. PCR was run on a Perkin Elmer Gene Amp PCR System 2400 as follows: genomic DNA (1 µg) in the presence of primers (0.5 µM each) and the Hot Start Kit (Promega) solutions were mixed according to manufacturer's instructions. 30 cycles of PCR were performed (1 min at 95°C, 1 min at 50°C, 1.5 min at 72°C), followed by a final 10 min elongation step at 72°C. The PCR product was digested with NdeI and XhoI and then ligated with T4 DNA ligase into pET-28(a) plasmid previously digested with the same restriction enzymes. The entire cloned gene was sequenced to ensure that no error was introduced during the PCR reaction.

- **Protein expression and purification**: *E. coli* BL21 (DE3) was transformed with pET-6His-CPE0635, then grown overnight at 37°C in LB medium (100 mL) supplemented with kanamycin (50 $\mu g.mL^{-1}$). The overnight culture was then used to inoculate fresh LB medium (1 L) supplemented with the same antibiotic and bacterial growth proceeded at 37°C until the OD_{600} reached 0.6. Protein expression was induced by adding 500 µM of IPTG. After 16 hours of culture at 25°C, the cells were collected by centrifugation at 4,000 x g for 30 min. After resuspension in 0.1 M Tris-HCl buffer, pH 7, containing 150 mM KCl, the cells were disrupted by sonication and centrifuged at 220,000 x g at 4°C for 1 hour. The solution obtained was then loaded onto a Ni-NTA Sepharose column previously equilibrated with 0.1 M Tris-HCl buffer, pH 7, 150 mM KCl. The column was washed extensively with the same buffer. Some of the adsorbed proteins were eluted by washing steps with 25 and 100 mM imidazole, and the over-expressed protein was eluted by applying 500 mM imidazole. DTT (5 mM) was immediately added to fractions containing the protein, further concentrated in Ultrafree cells (Millipore) with a molecular cut-off of 10 kDa. The *C. perfringens* anSME was further purified using a size exclusion chromatography step. A Superdex 200 HR (Pharmacia) column was equilibrated with 0.1 M Tris-HCl buffer, pH 7, 150 mM KCl and 5 mM DTT. The fractions containing anSME were then concentrated and stored at -80°C.

- **Spectrophotometric measurement:** UV-visible absorption spectra were recorded on a Beckman DU 640 spectrophotometer.

- **Protein analysis:** Protein concentration (by monomer) was determined by the method of Bradford (Bradford, M. M. (1976) *Anal Biochem* 72, 248-54). Protein-bound iron was determined according to standard procedures (Fish, W. W. (1988) *Methods Enzymol* 158, 357-64). Briefly, after acidic denaturation of the protein with perchloric acid (1M), the amount of released iron was spectrophotometrically assayed at 535 nm (corrected of the absorption at 680 nm) with bathophenantroline disulfate under reducing conditions. The measured OD was plotted on a standard calibration curve ranging from 0 to 150 µM Fe(II). A minimum of three determinations were made for each assay.

- **Iron binding to anSME**: The following procedure was used inside an anaerobic glove box. anSME CPE0635 (65 µM monomer) was treated with 5 mM of DTT and incubated overnight with a 10-fold molar excess of both Na_2S (Fluka) and $(NH_4)_2Fe(SO_4)_2$ (Aldrich). The protein was desalted on Sephadex G-25 (80 mL, elution with 0.1 M Tris-HCl buffer, pH 7, containing 150 mM KCl and 5 mM DTT) and the colored fractions concentrated on Microcon YM10 (Millipore).

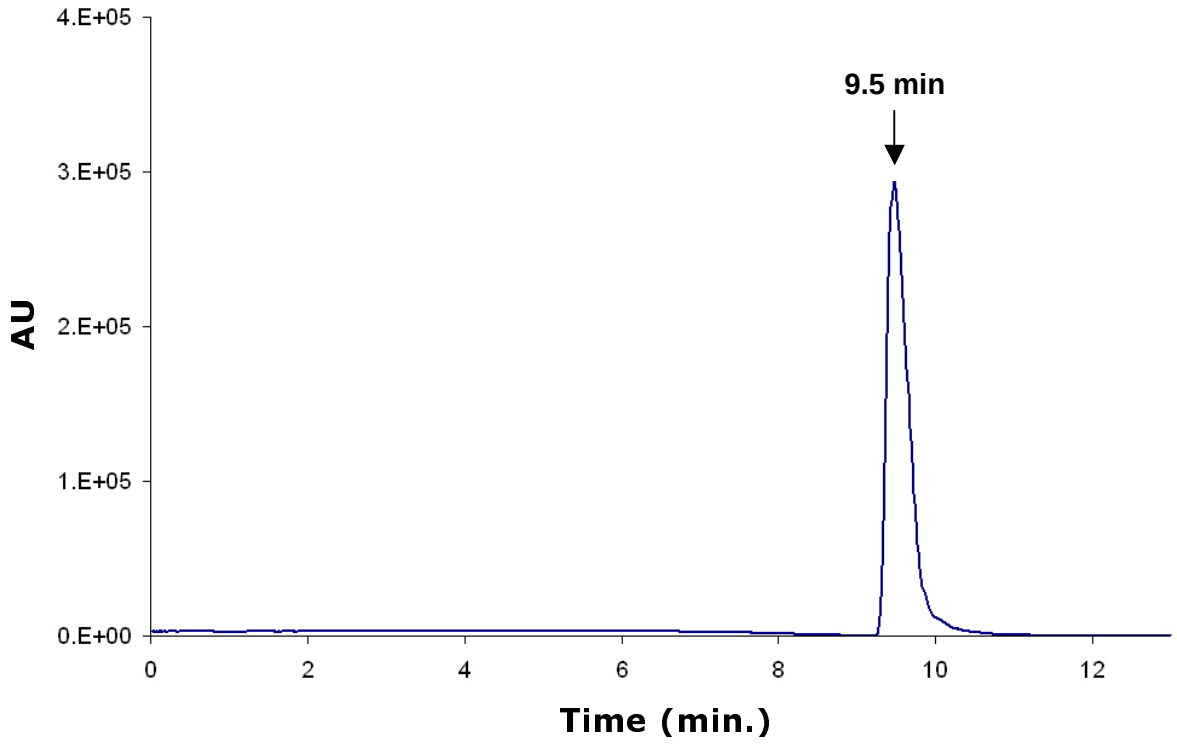
- ***S*-adenosyl-L-methionine reductase activity:** The cleavage of *S*-adenosyl-L-methionine (SAM) was monitored by HPLC. The reaction mixture (200 µL), in an anaerobic glove box, contained 40 µM of *C. perfringens* anSME in 0.1 M Tris-HCl buffer, pH 7, containing 150 mM KCl, 5 mM DTT, 3 mM dithionite, and 1 mM SAM. The reaction was initiated by addition of the enzyme. At given time intervals, a 20 µL aliquot was removed and treated with 2 µL of TCA 10%; the solution was then centrifuged 10 min at 10,000 g. The supernatant was completed to 180 µL with water and trifluoroacetic acid (TFA) 0.1% and analyzed by HPLC. 50 µL was injected onto an HPLC Zorbax SB-C18 column equilibrated with 0.1% TFA. A linear gradient from 0 to 30% acetonitrile in 0.1% TFA was performed at 1 mL/min over 15 min. AdoH, detected from its absorption at 260 nm, eluted after 9.5 min and SAM at 4.5 min.

- **Peptide synthesis:** The following 23-mer peptides (with the critical cysteine in bold): Ac-FENAYTAVPS**C**IASRASILTMSQ-NH_2 and Ac-FENAYTAVPS**A**IASRASILTMSQ-NH_2 were synthesized (0.1-mmol scale) by the solid phase methodology on a Rink amide 4-methylbenzhydrylamine resin (Biochem, Meudon, France) by using a 433A Applied Biosystems peptide synthesizer (Applera-France, Courtaboeuf, France) and the standard Fmoc manufacturer's procedure. The synthetic peptides were purified by reversed-phase HPLC on a 2.2 × 25-cm Vydac 218TP1022 C18 column (Alltech, Templemars, France) by using a linear gradient (10-50% over 45 min) of acetonitrile/trifluoroacetic acid (99.9 : 0.1 ; v/v) at a flow rate of 10 ml/min. Analytical HPLC, performed on a 0.46 × 25-cm Vydac 218TP54 C18 column (Alltech), showed that the purity of the peptides was >99.1%. The purified peptides were characterized by MALDI-TOF mass spectrometry on a Voyager DE PRO (Applera, France) in the reflector mode with α-cyano-4-hydroxycinnamic acid as a matrix.

- **anSME activity with peptides**: The N-acetylated and C-amidated 23-mer peptides were diluted to 7 mM in 50% acetonitrile and 0,1%TFA. 10 µL of this stock solution was added to a 200 µL reaction mixture containing anSME, SAM, DTT and dithionite as described above. The reaction was initiated by addition of the enzyme. At given time intervals, a 20 µL aliquot was removed and analyzed with MALDI-TOF MS. Equal volumes (1 µL) of matrix and sample were spotted onto the MALDI-TOF target plate. The α-cyano-4-hydroxycinnamic acid matrix (CHCA) was prepared at 4 mg.mL-1 in 0.15% TFA, 50% acetonitrile. The 2,4-dinitrophenylhydrazone acid matrix (DNPH) was prepared at 1.3 mg.mL-1 in 0.5% TFA, 50% acetonitrile. MALDI-TOF analysis was then performed on a Voyager DE STR Instrument (Applied Biosystems, Framingham, CA, USA). Spectra were acquired in the reflector mode with: 20 kV accelerating voltage, 62% grid voltage and a 120 ns delay.

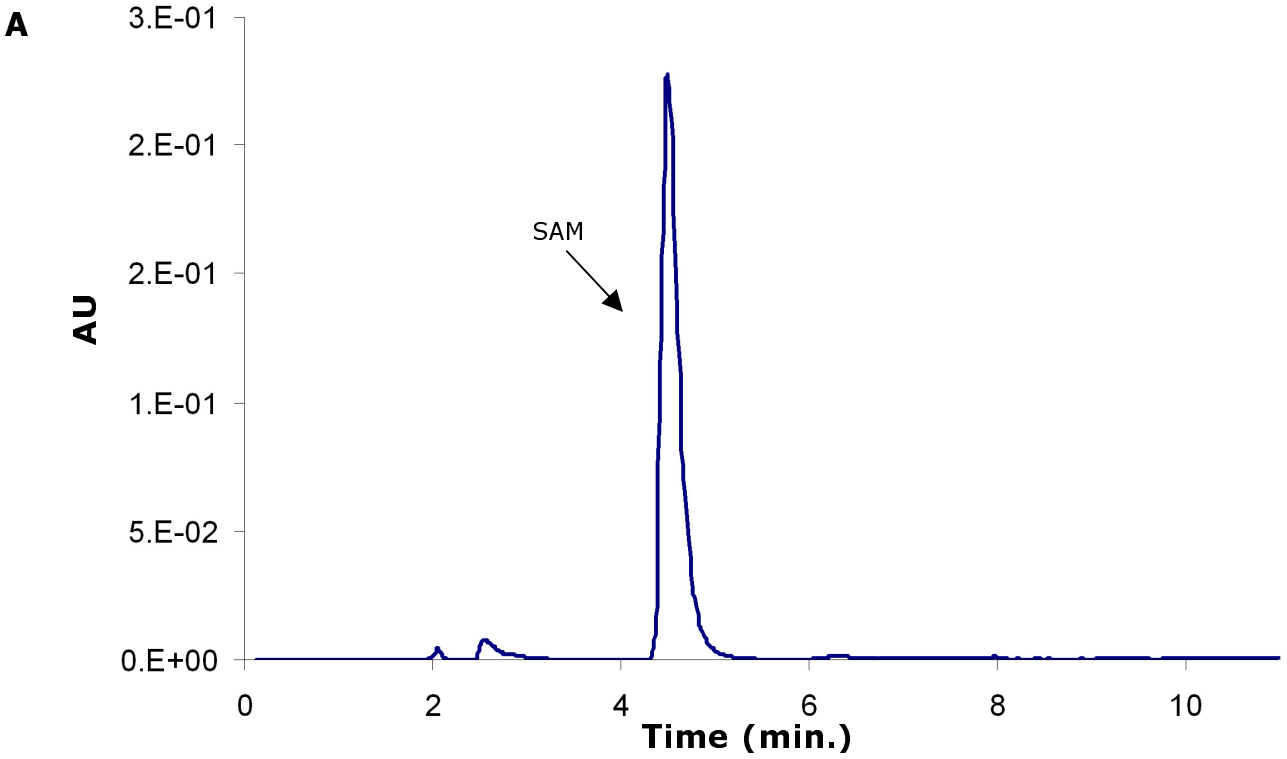
Reversed phase HPLC (C18) analysis of standard 5'-deoxyadenosine (AdoH)

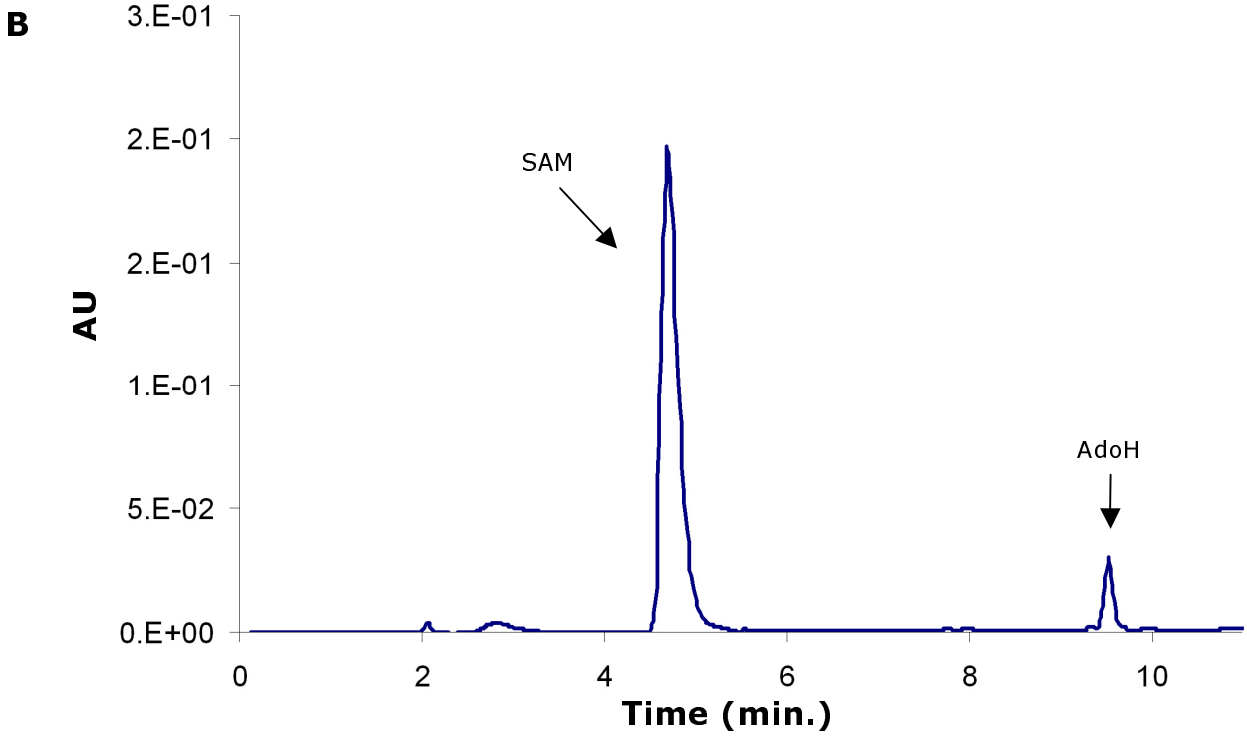
20 µL of a 5'-deoxyadenosine (Sigma) 10 µM solution was load on a reversed phase HPLC. Equilibration was made with H2O containing 0.1% TFA followed by a 15 min linear gradient from 0 to 30% acetonitrile in 0.1% TFA. Detection was performed at 260 nm.



HPLC analysis of the anSME CPE0635 reaction with S-adenosyl-L-methionine after 6 hours of incubation at 25°C in anaerobic conditions

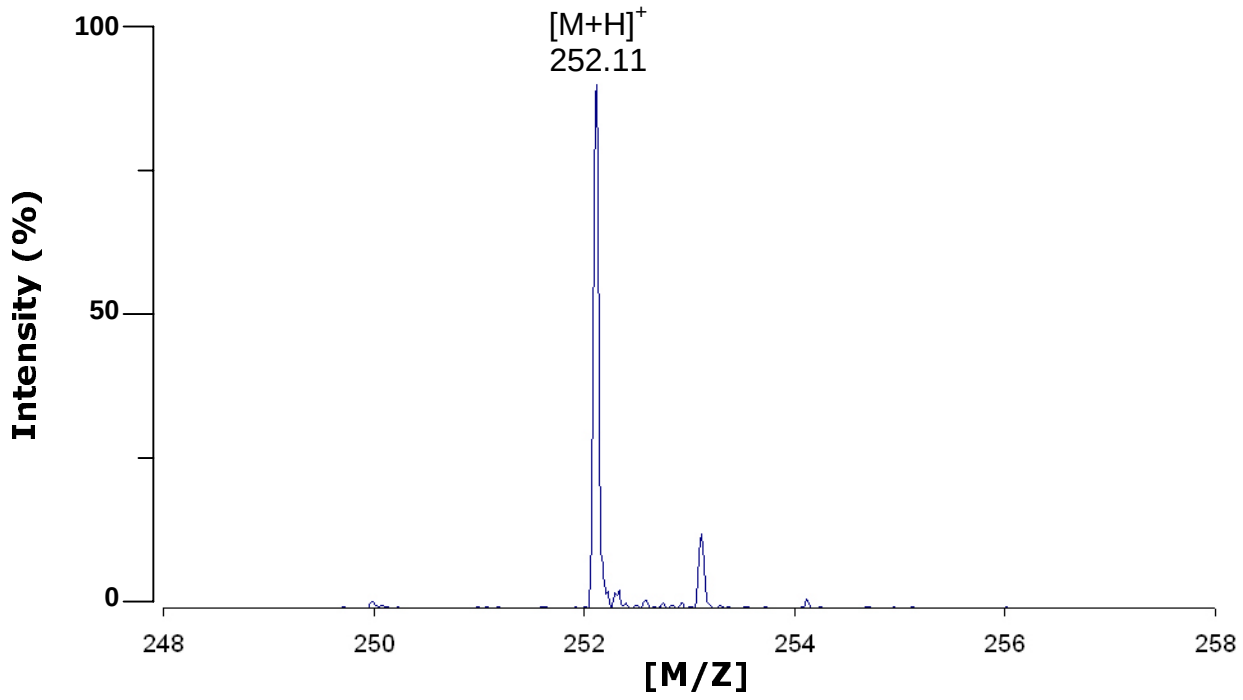
Incubation of 1 mM SAM in 0.1 M Tris-HCl buffer, pH 7, containing 150 mM KCl, 5 mM DTT, 3 mM dithionite in the absence (A) or presence of the anSME (B). Detection was performed at 260 nm.





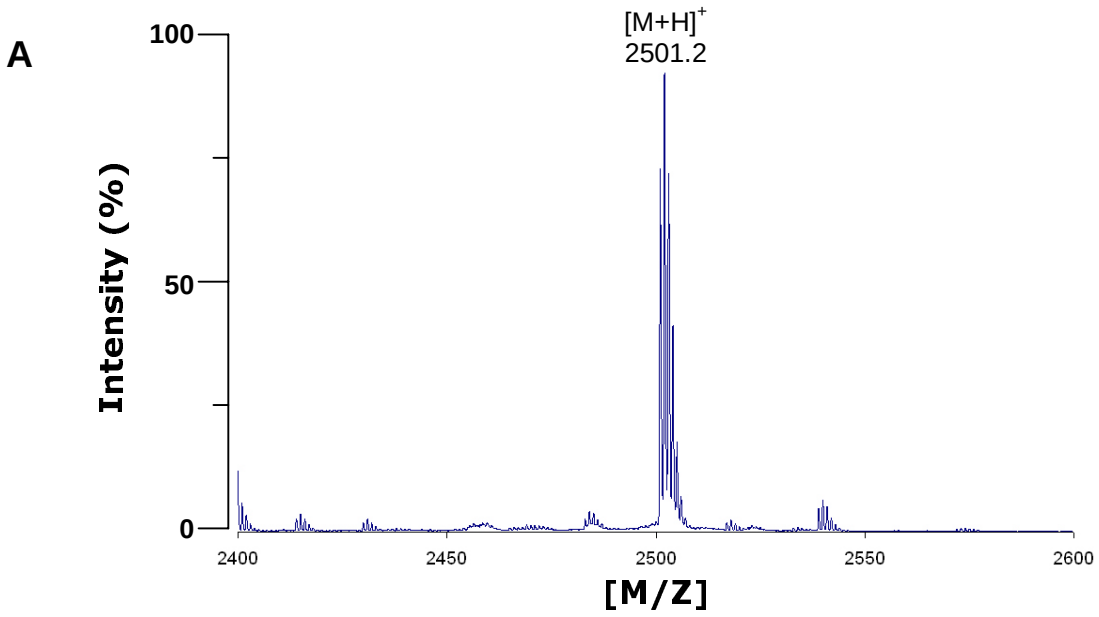
MALDI-TOF analysis of the 9.5 min eluted compound (AdoH)

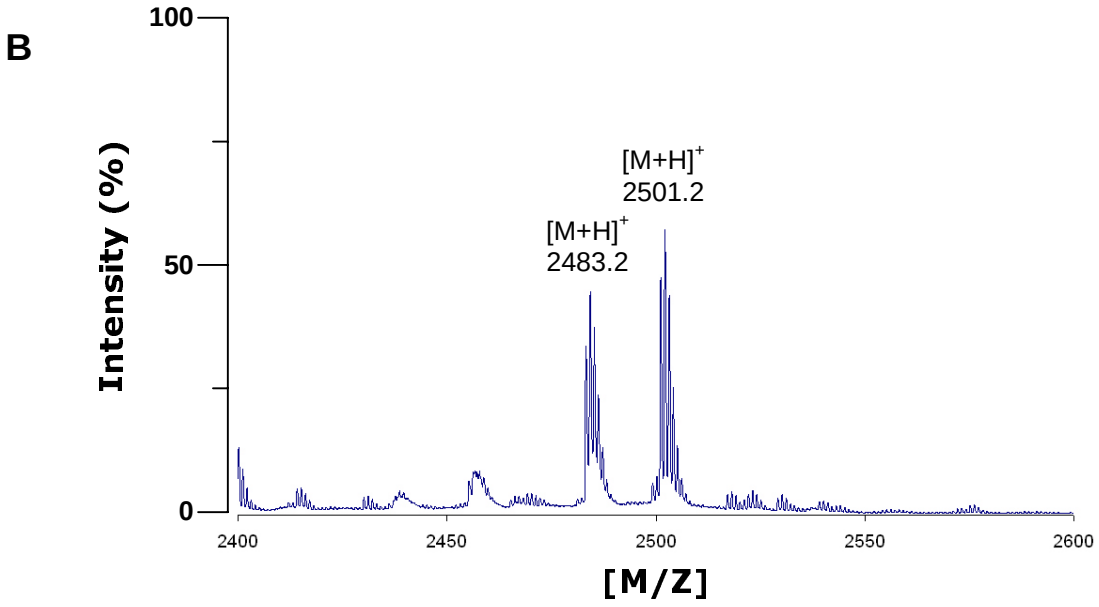
The compound eluted from HPLC with a retention time of 9.5 min was collected and further concentrated then analyzed with a MALDI-TOF spectrometer (Voyager DE STR Instrument- Applied Biosystems, Framingham, CA, USA) using α-cyano-4-hydroxycinnamic acid matrix (CHCA). Equal volumes (1 µL) of matrix and sample were spotted onto the MALDI-TOF target plate. MALDI-TOF analysis was then performed. Spectra were acquired in the reflector mode with the following parameters: 20 kV accelerating voltage, 62% grid voltage, 120 ns delay.



MALDI-TOF analysis of anSME reaction with the 23-mer peptide: Ac-FENAYTAVPS**C**IASRASILTMSQ-NH_2

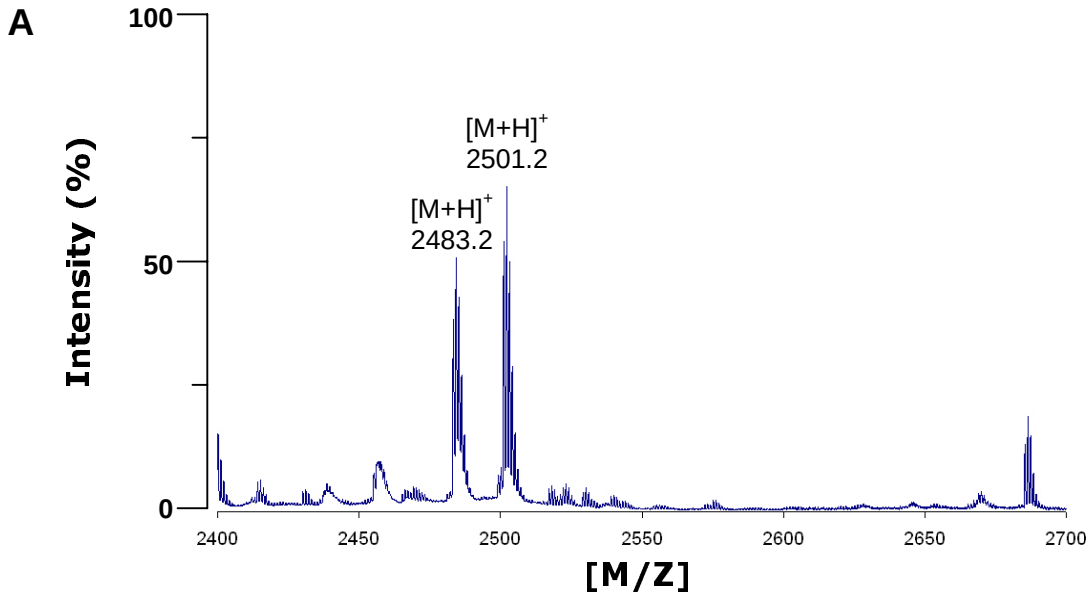
Incubations with the 23-mer peptide and *C. perfringens* anSME were analyzed with a MALDI-TOF spectrometer (Voyager DE STR Instrument-Applied Biosystems, Framingham, CA, USA) using α-cyano-4-hydroxycinnamic acid matrix (CHCA) at T=0 (**A**) and T=6 h (**B**). Equal volumes (1 µL) of matrix and sample were spotted onto the MALDI-TOF target plate. MALDI-TOF analysis was then performed. Spectra were acquired in the reflector mode with the following parameters: 20 kV accelerating voltage, 62% grid voltage, 120 ns delay.

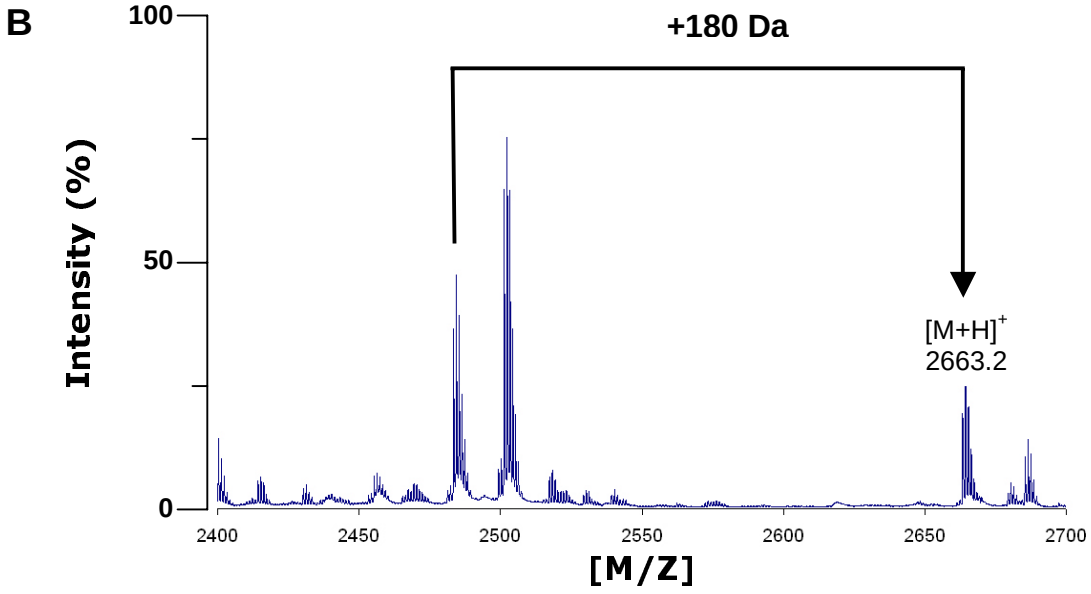




MALDI-TOF analysis of anSME reaction with the 23-mer peptide (Ac-FENAYTAVPS**C**IASRASILTMSQ-NH_2**) at T= 6 h with CHCA or DNPH matrix**

Incubations with the 23-mer peptide and *C. perfringens* anSME were analyzed with a MALDI-TOF spectrometer (Voyager DE STR Instrument-Applied Biosystems, Framingham, CA, USA) using α-cyano-4-hydroxycinnamic acid (**A**) or dinitrophenyl hydrazone matrix (**B**). Equal volumes (1 µL) of matrix and sample were spotted onto the MALDI-TOF target plate. MALDI-TOF analysis was then performed. Spectra were acquired in the reflector mode with the following parameters: 20 kV accelerating voltage, 62% grid voltage, 120 ns delay.





MALDI-TOF analysis of anSME reaction with the 23-mer peptide: Ac-FENAYTAVPS**A**IASRASILTMSQ-NH_2

Incubations with the 23-mer peptide and *C. perfringens* anSME were analyzed with a MALDI-TOF spectrometer (Voyager DE STR Instrument-Applied Biosystems, Framingham, CA, USA) using α-cyano-4-hydroxycinnamic acid matrix (CHCA) at T=0 (**A**) and T=6 h (**B**). Equal volumes (1 µL) of matrix and sample were spotted onto the MALDI-TOF target plate. MALDI-TOF analysis was then performed. Spectra were acquired in the reflector mode with the following parameters: 20 kV accelerating voltage, 62% grid voltage, 120 ns delay