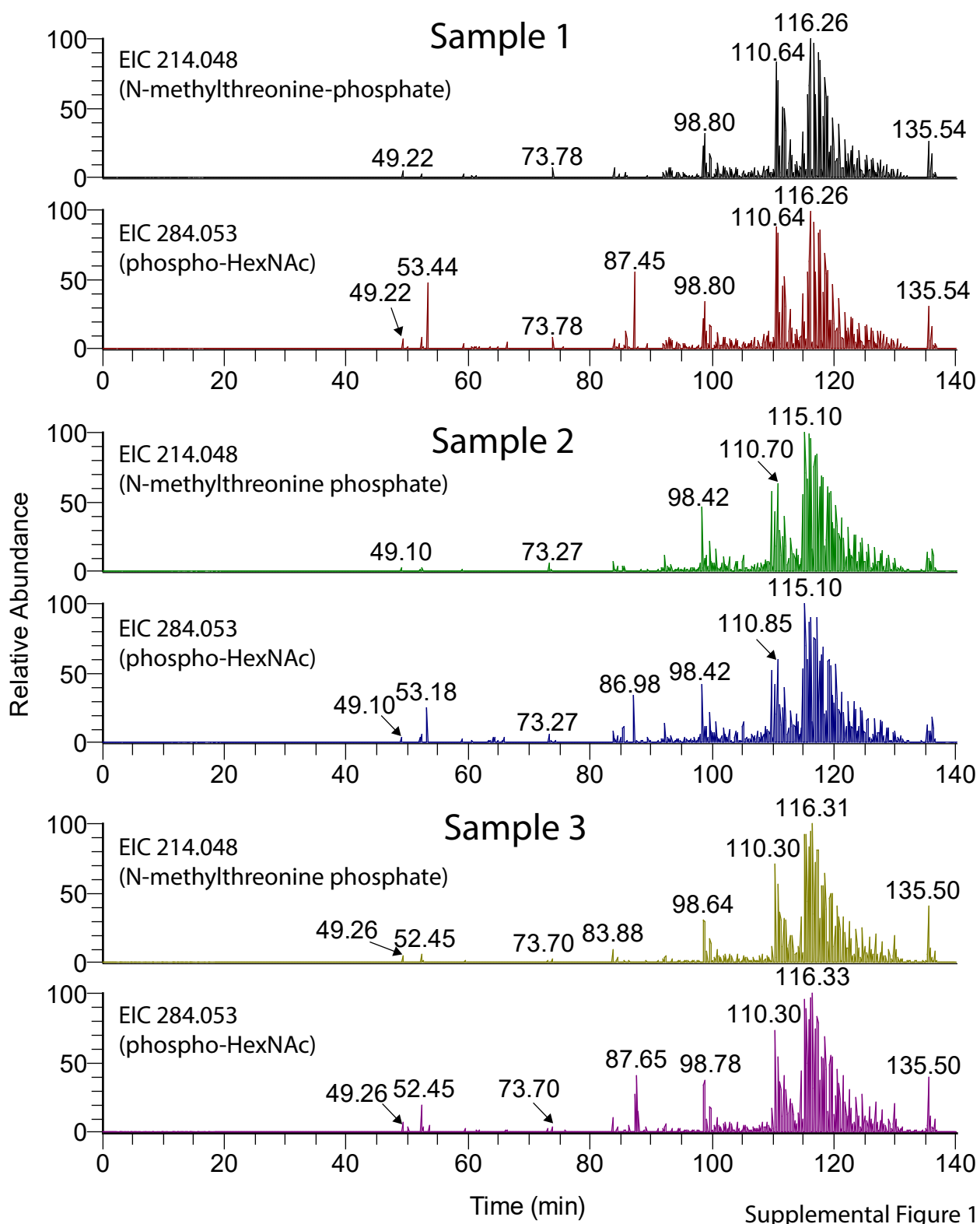


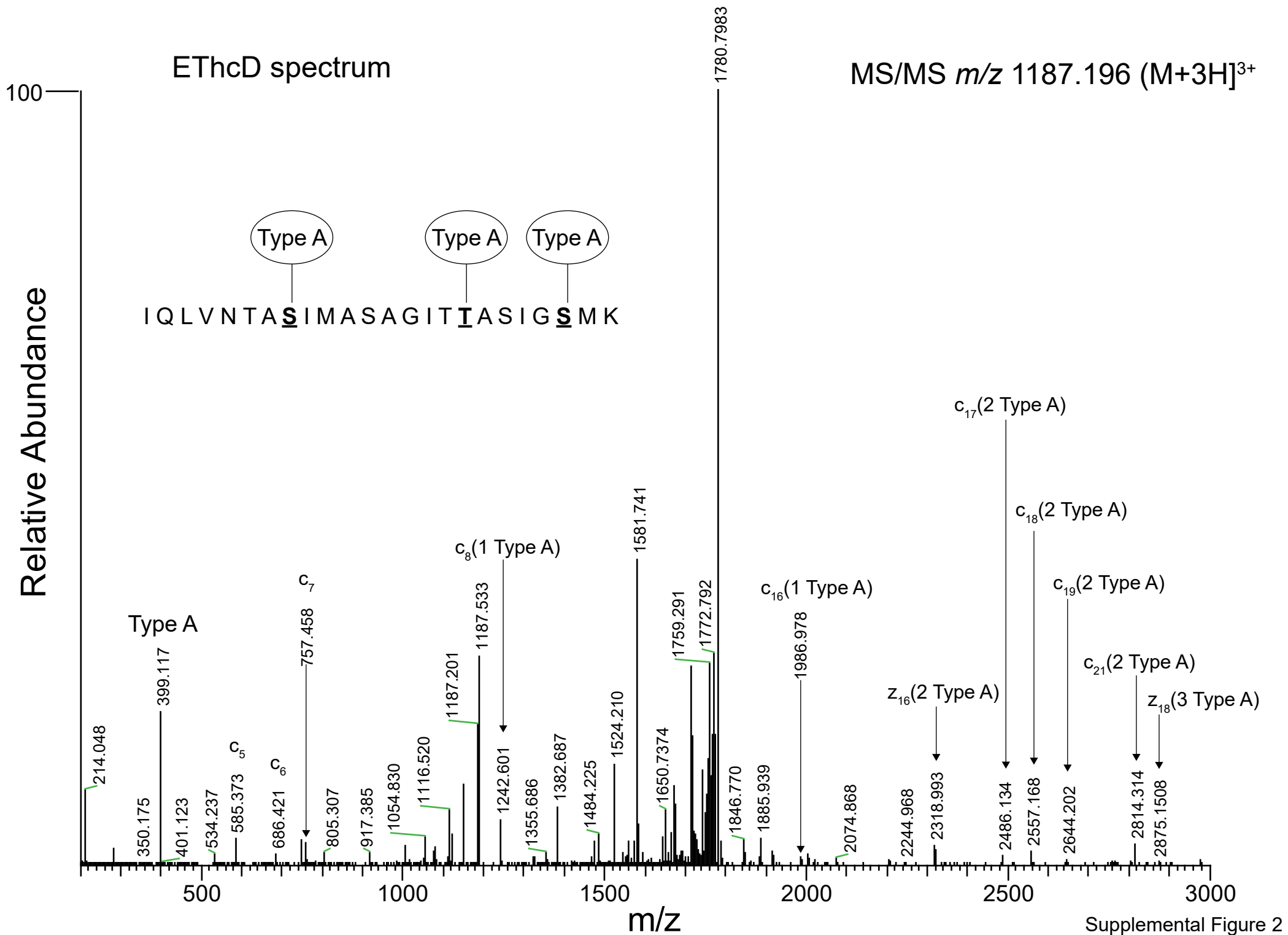
Supporting Information

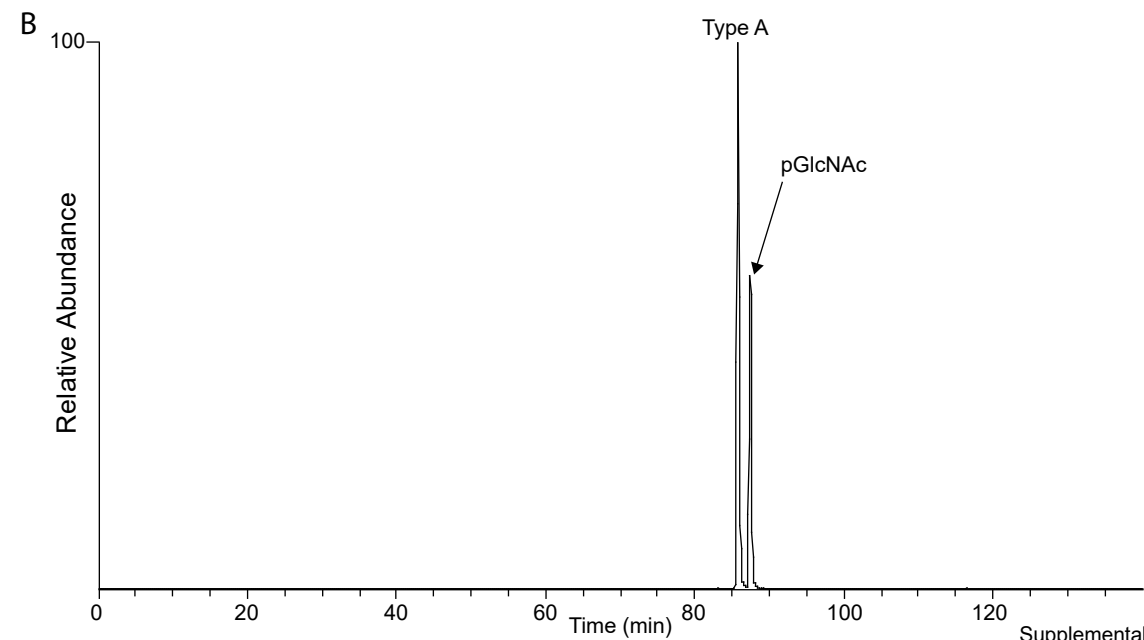
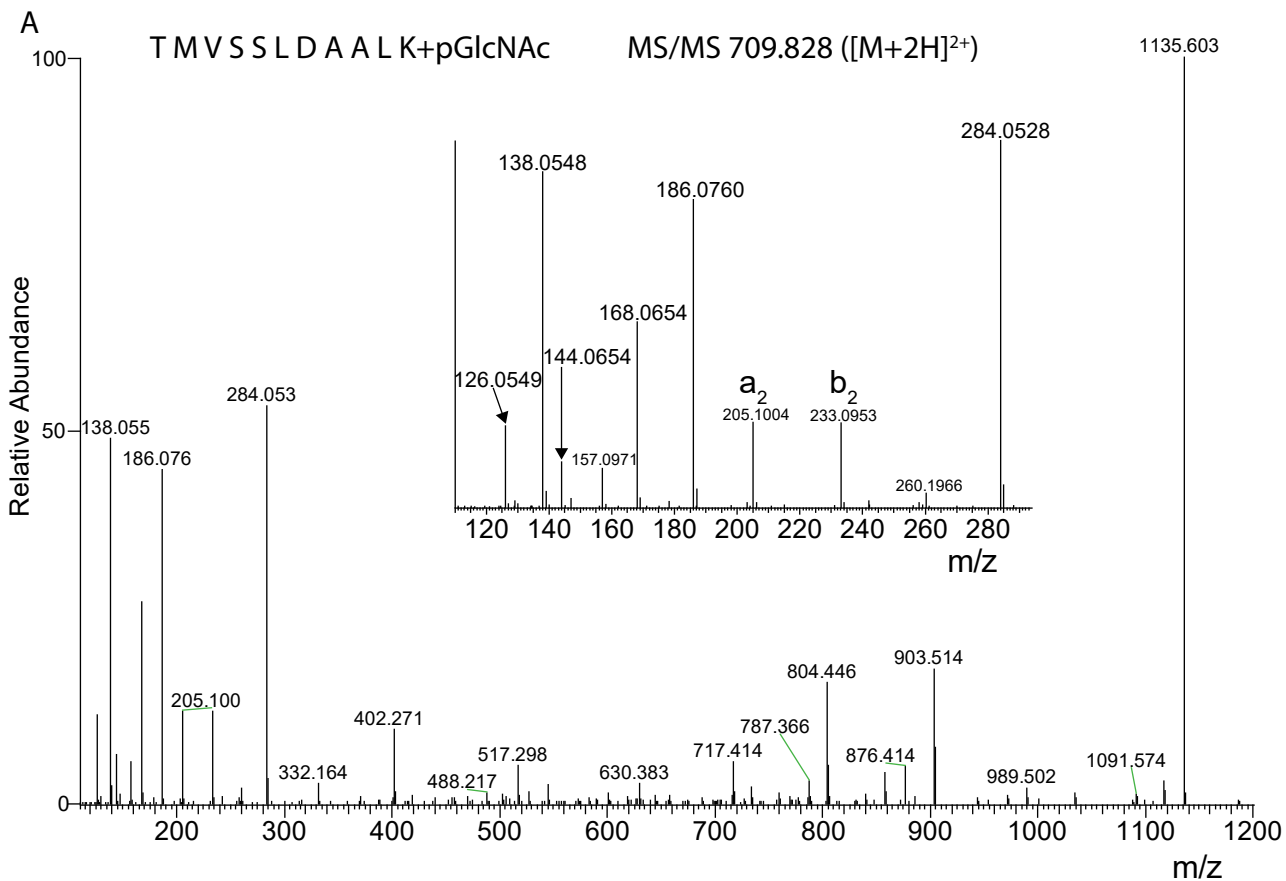
New insights into *C. difficile* strain 630 flagellin C modifications by
phosphoproteomics analysis

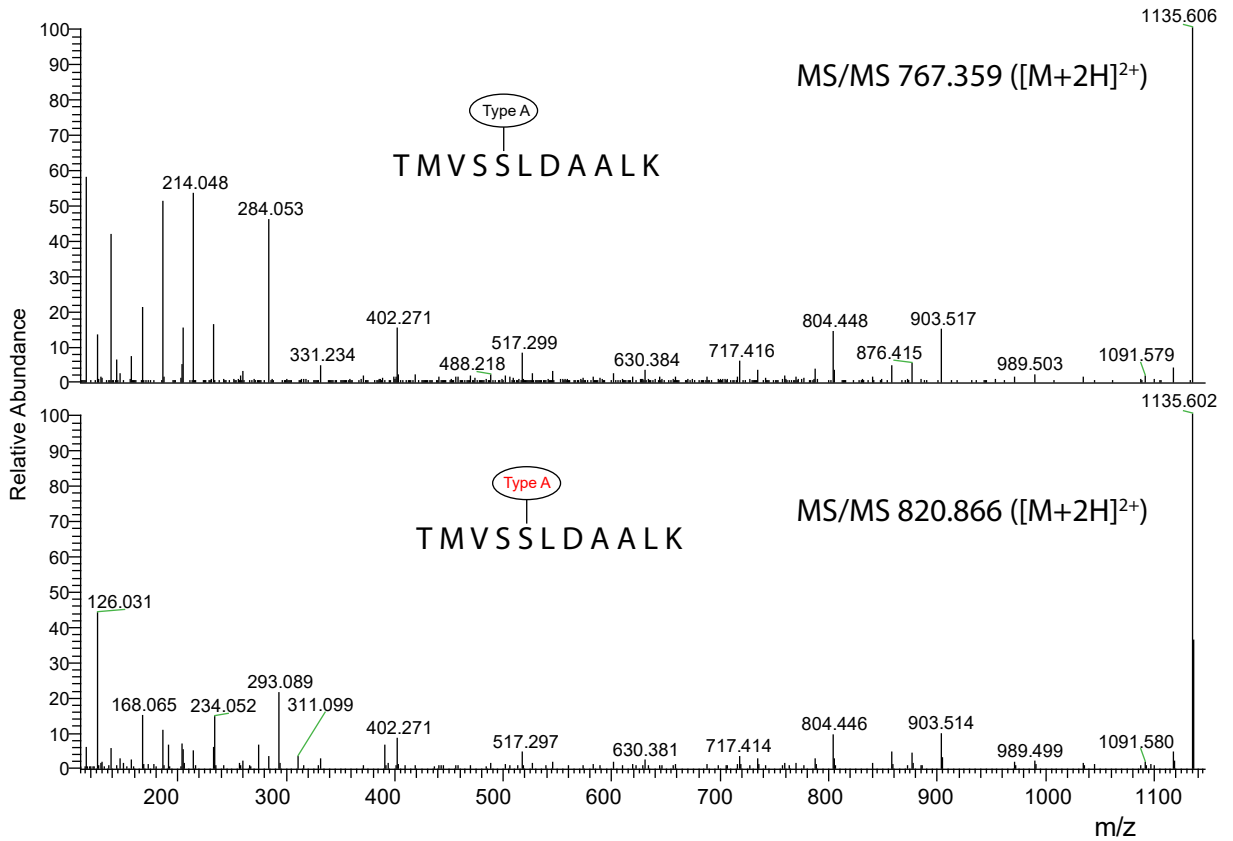
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Peter A. van Veelen[†]

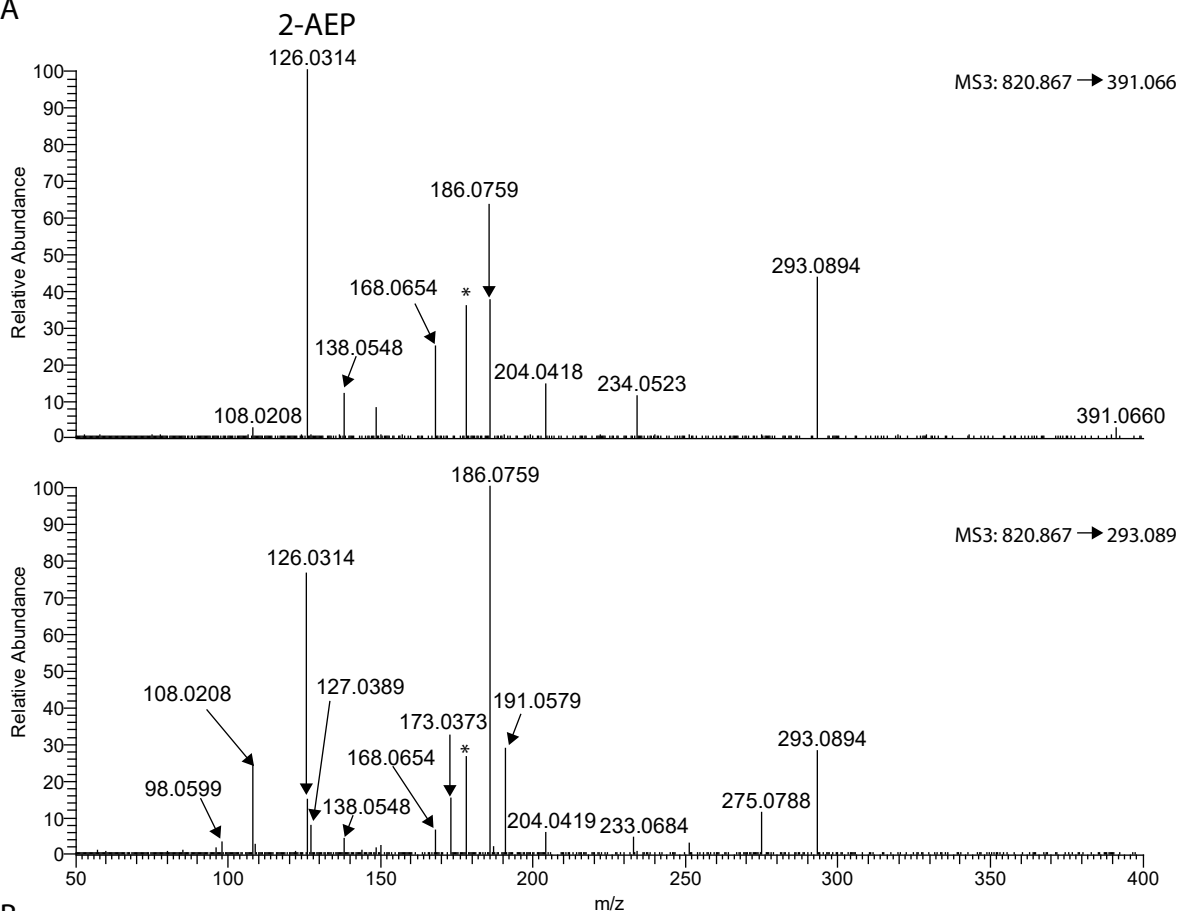








A



B

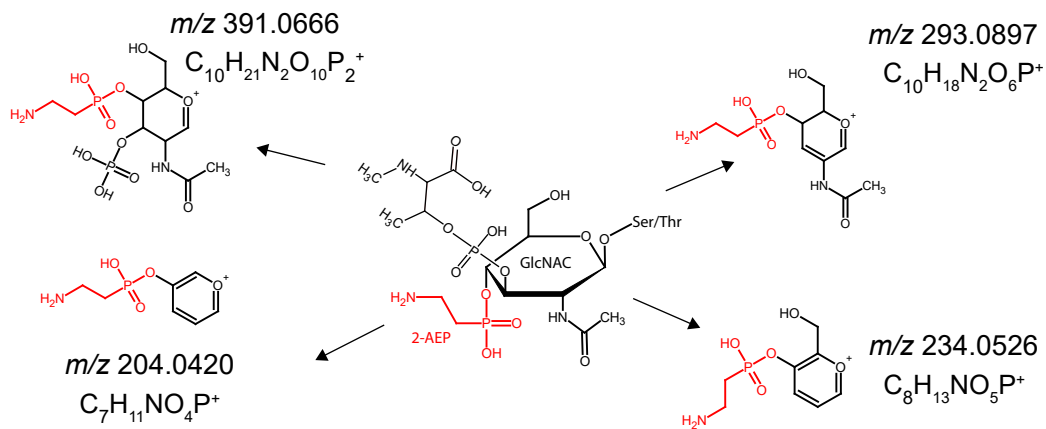


Figure legend Supplemental Figures

Supplemental Figure 1. Comparison of extracted ion chromatograms of Type A specific fragment ions in LC-MS/MS data from IMAC purified peptides from three independent *C. difficile* cultures.

Supplemental Figure 2. EThcD of the flagellin C tryptic peptide IQLVNTASIMASAGITTASIGSMK carrying three Type A structures (see Figure 2A).

Supplemental Figure 3. A. MS/MS spectrum of the flagellin C tryptic peptide TMVSSLDAALK carrying a phospho-GlcNAc (pGlcNAc). B. Chromatographic profile of the tryptic peptide TMVSSLDAALK modified with either a Type A structure or a pGlcNAc.

Supplemental Figure 4. Comparison of the MS/MS fragmentation spectrum of the flagellin C tryptic peptide TMVSSLDAALK carrying either a Type A structure (upper panel) or an alternative Type A structure (indicated in red) with an additional mass of 107.013 Da (lower panel, fully annotated in Figure 4). Subsequent analyses showed that this alternative Type A structure corresponds to 2-AEP-Type A (see Figure 4 for further details).

Supplemental Figure 5. A. MS3 fragmentation of the flagellin C tryptic peptide TMVSSLDAALK carrying a 2-AEP-Type A structure, where either the fragment ion at m/z 391.066 (upper panel) or 293.089 (lower panel) were selected for additional fragmentation. See Figure 4A for the MS2 fragmentation spectrum. B. Tentative assignment of 2-AEP containing fragments observed in the MS2 and MS3 spectrum of the flagellin C tryptic peptide TMVSSLDAALK carrying a 2-AEP-Type A structure. The signal indicated with an asterisk (*) at m/z 178.243 is a background signal of unknown origin which was observed in all fragmentation spectra.