Supporting Information for:

# Vinyl Sulfones: Inhibitors of SrtA, a Transpeptidase Required for Cell Wall Protein Anchoring and Virulence in *Staphylococcus aureus*

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**General.** The sortase substrate Abz-LPETG-Dap(Dnp)-$NH_2$ was synthesized by the Fmoc/piperidine strategy on PAL resin using an Applied Biosystems 433A synthesizer, purified by HPLC to $\geq$ 98% purity and analyzed by MALDI-TOF MS. Vinyl sulfone inhibitors were obtained from Aldrich and used without further purification. Stock solutions were prepared fresh before use in 100% DMSO. Buffer salts were from Sigma.

**Expression and purification of SrtA.** Recombinant SrtA lacking the 24 amino acid membrane anchor was expressed and purified to homogeneity from *E. coli* BL21(DE3) harboring the plasmid pET15bSrtA$_{\Delta 24}$ [1]. Briefly, cells were grown in Luria Broth at 37 $^{o}$C with 100 μg/mL ampicillin to an $OD_{580}$ of 0.8. IPTG (1 mM) was added to induce expression of SrtA and cells were harvested after 3 h. Cells were resuspended in 150 mM NaCl, 50 mM Tris, 5 mM imidazole, 10% glycerol, pH 7.5 and lysed using an EmulsiFlex®-C5 high-pressure homogenizer (Avestin, Inc.). The resultant lysate was clarified by centrifugation and applied to a chelating sepharose fast flow column. SrtA was eluted with a linear gradient from 5 to 500 mM imidazole over 1 h. Fractions containing SrtA were pooled, concentrated, and loaded onto a HiPrep 26/60 Sephacryl S-200 gel filtration column previously equilibrated with 150 mM NaCl, 50 mM Tris, 5 mM $CaCl_2$, 0.1% β-mercaptoethanol, 10% glycerol, pH 7.5. Pure SrtA fractions were concentrated to 100 μM using an Amicon stirred cell concentrator. The concentration was determined using the calculated extinction coefficient ($\varepsilon_{280}$ = 17420 $M^{-1}cm^{-1}$).

**Standard HPLC assay for sortase activity.** In each standard assay recombinant SrtA (1 μM) was incubated with Abz-LPETG-Dap(Dnp)-$NH_2$ (0.23 mM), $Gly_5$ (2 mM) and the indicated amount of inhibitor at 37 $^oC$ in 2% DMSO, 150 mM NaCl, 5 mM $CaCl_2$ and 300 mM Tris-Cl, pH 7.5. Reactions were either initiated by the addition of SrtA or by the addition of a mixture of Abz-LPETG-Dap(Dnp)-$NH_2$ and $Gly_5$. At appropriate times, aliquots were removed from the reaction tube and quenched by mixing with ½ volume of 1 N HCl. Next, 40 μL of the quenched reaction mixture was injected on a Vydac reversed-phase C-18 fast analytical HPLC column (4 mL/min) and separated using a linear gradient of 0 to 45% $CH_3CN$/0.1% TFA over 5 min. Elution of Dnp-containing substrate (Abz-LPETG-Dap(Dnp)-$NH_2$) and product ($NH_2$-G-Dap(Dnp)-$NH_2$) was monitored at 355 nm. Integrated areas of peaks corresponding to substrate and product were used to calculate the percent of substrate converted to product.

**$IC_{50}$ determination.** The indicated concentrations of vinyl sulfones were preincubated with SrtA (1 μM) in assay buffer for 30 min at 37 $^oC$. Reactions were initiated with the addition of a mixture of Abz-LPETG-Dap(Dnp)-$NH_2$ and $Gly_5$ for a total volume of 100 μL. After 30 min, 90 μL of the reaction mixture was removed from the reaction tube, quenched by mixing with 1 N HCl (45 μL) and analyzed by HPLC as described above. Measurements were performed in triplicate. Plots of fractional activity remaining versus inhibitor concentration were fit using GraFit (Erithacus Software) to a variation of Equation S1, where $v_i$ is the initial velocity in the presence of inhibitor at concentration [I] and $v_o$ is the initial velocity in the absence of inhibitor.

$$\frac{v_i}{v_o} = \frac{1}{1+\left(\frac{[\mathrm{I}]}{\mathrm{IC}_{50}}\right)} \qquad \text{(Equation S1)}$$

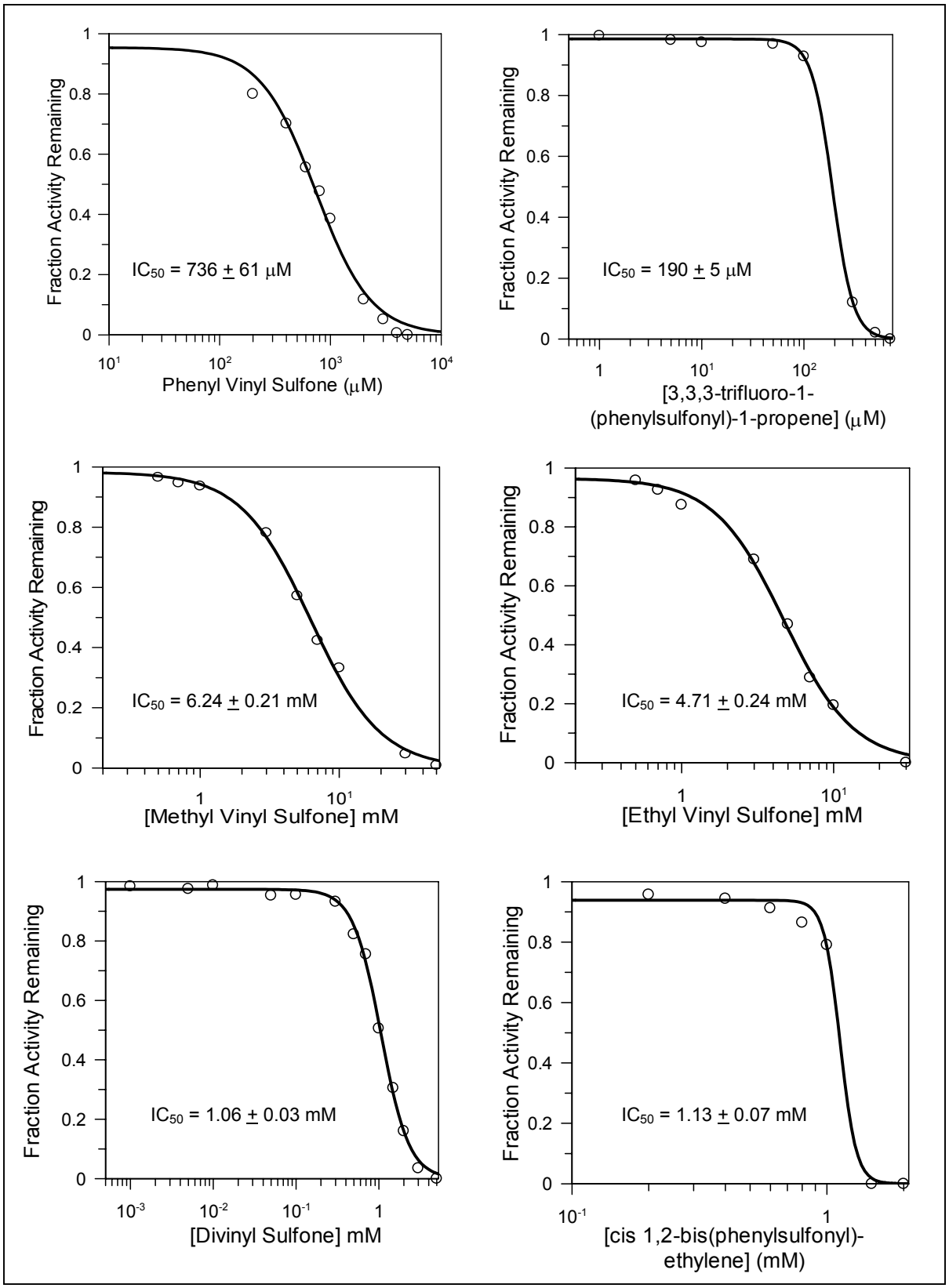



**Figure S1. $IC_{50}$ curves for SrtA treated with vinyl sulfone.**

**Progress curve analysis.** Reaction volumes of 400 μL were assembled as described above and initiated with the addition of SrtA to a final concentration of 1 μM. After 10, 20, 30, 40, 50 and 60 min reaction times, aliquots of 60 μL were removed from the reaction tubes and quenched by mixing with 1 N HCl (30 μL). Samples were analyzed by HPLC as described above. The resulting progress curves (product vs. time) were fit, using GraFit, to Equation S2 where P is product concentration, $A$ is amplitude, $k_{obs}$ is the observed rate constant for inactivation, and $t$ is time.

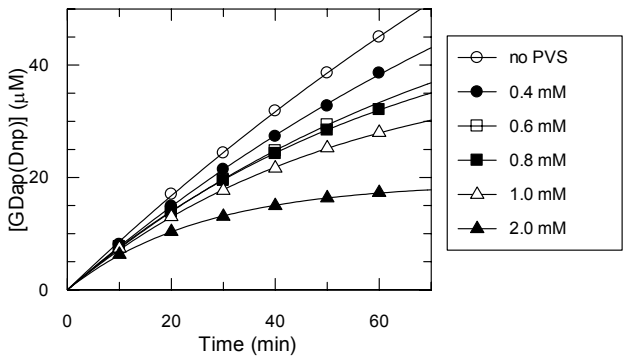
$$P = A\left(1\text{-}e^{-k_{obs}t}\right) \qquad \text{(Equation S2)}$$

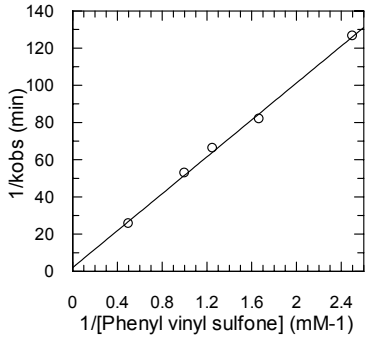
In several cases, due to either a lack of well-defined curvature in the observed progress lines, or to sensitivity limitations of the sortase assay, outlying $k_{obs}$ values were obtained. In order to improve the accuracy of further analysis, these outliers, mostly observed at low inhibitor concentrations, were discarded. The $k_{obs}$ values were then used to construct double reciprocal plots of $1/k_{obs}$ vs. 1/[I]. The reported $k_{inact}/K_i$ values were calculated according to the relationship described by Equation S3 for an irreversible inhibitor [2].

$$\frac{1}{k_{obs}} = \frac{K_i}{k_{inact}}\left(\frac{1}{[\mathrm{I}]}\right) + \frac{1}{k_{inact}} \qquad \text{(Equation S3)}$$

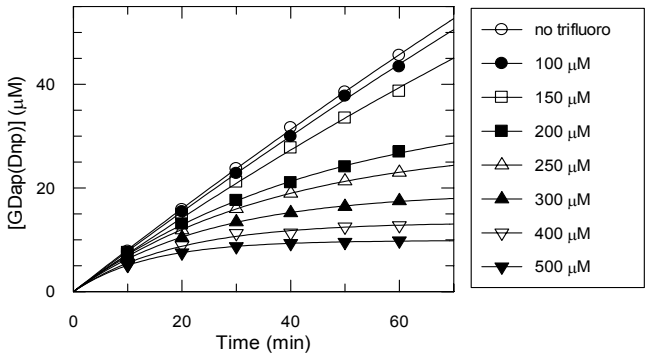
The progress curves and double reciprocal plots are shown in Figure S2. This analysis could not be performed for cis-1,2 bis(phenylsulfonyl)-ethylene due to solubility limitations in the sortase assay buffer.

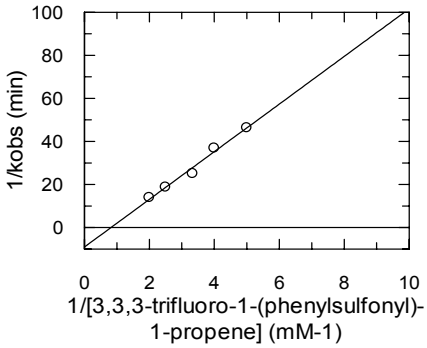
**A. Phenyl vinyl sulfone**



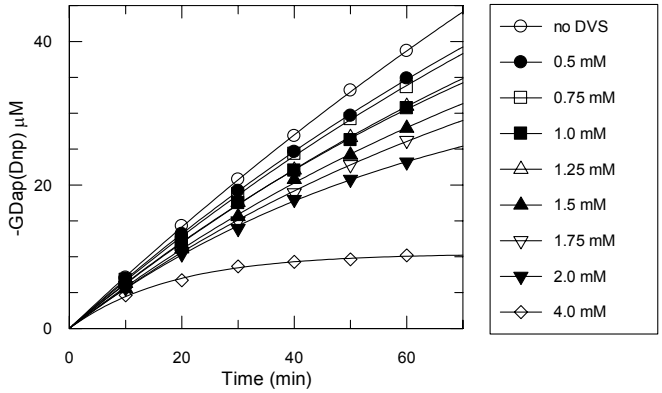




**B. 3,3,3-trifluoro-1-(phenylsulfonyl)-1-propene**




**C. Divinyl sulfone**



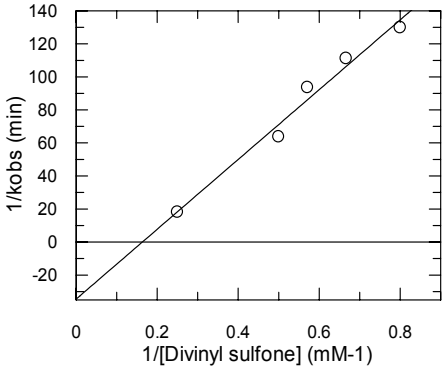




**Figure S2. Progress curve analysis of vinyl sulfone inhibitors.**

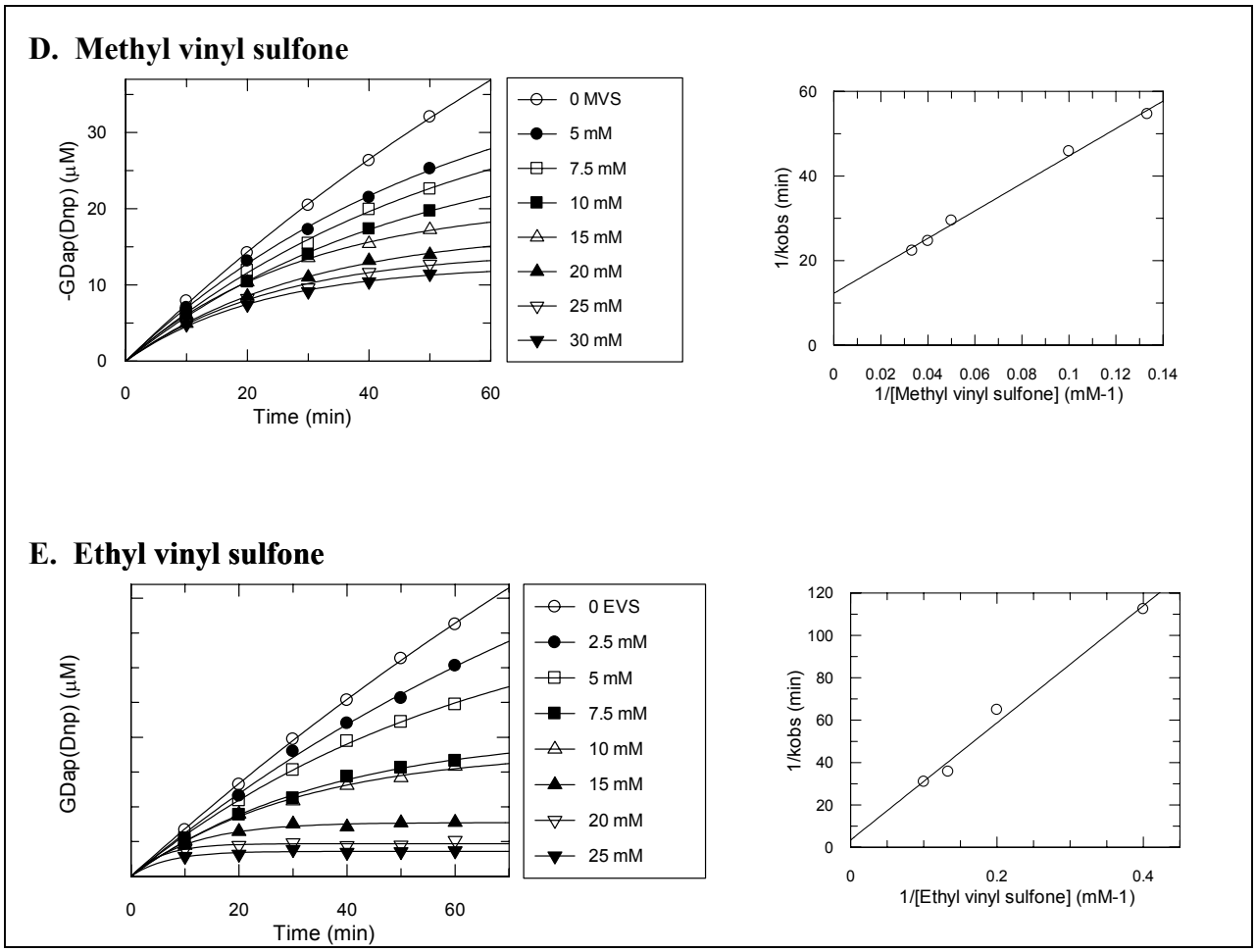



**Figure S2 cont'd. Progress curve analysis of vinyl sulfone inhibitors**

**Measurement of Minimal Inhibitory Concentration (MIC).** A culture of *S. aureus* Newman strain (5 mL) in Tryptic Soy Broth (TSB) was grown to saturation at 37 $^{o}$C and diluted to an $OD_{600}$ of 0.01. The culture was incubated for an additional 2 h and diluted to an $OD_{600}$ of 0.005. In each well of a 96 well plate, 180 μL of cells were mixed with 20 μL of a 10X concentrated vinyl sulfone solution in 10% DMSO for a final DMSO concentration of 1%. Culture plates were incubated overnight at 37 $^{o}$C and $OD_{600}$ was measured using a Multiskan Spectrum spectrophotometer (Thermo Labsystems). MIC values were determined as the lowest

concentration of vinyl sulfone that inhibited cell growth. MIC measurements were performed in triplicate.

**Mass spectrometry.** SrtA (9.6 μM) was incubated with phenyl vinyl sulfone (0, 1.25, 2.5 and 5.0 mM) in 2% DMSO, 150 mM NaCl, 5 mM $CaCl_2$ and 300 mM Tris-Cl, pH 7.5 for 1 hour at 37 $^{o}$C. A portion of the incubation mixture was loaded on an SDS-PAGE gel and the band corresponding to SrtA was excised and submitted to the University of Pennsylvania Proteomics Facility. Tryptic digests, MALDI-TOF MS/MS and ESI MS/MS sequencing were performed using standard procedures. MALDI-TOF MS/MS of tryptic fragments revealed the presence of a single peptide whose mass increased by 168.05 Da in samples treated with phenyl vinyl sulfone, consistent with the formation of a covalent adduct. The observed mass of 1555.73 Da (1723.78 Da after modification) corresponds to the peptide fragment QLTLITCDDYNEK, which contains the C184 nucleophile. No other mass shifts were observed regardless of phenyl vinyl sulfone concentration. Subsequent ESI MS/MS sequencing of this peptide (Figure S3) unequivocally localized the modification to C184.

**Fibronectin binding assay.** The ability of *S. aureus* Newman strain treated with phenyl vinyl sulfone to bind fibronectin was determined using a microtitre plate assay [3]. Overnight cultures of *S. aureus* were used to inoculate fresh TSB, and were grown at 37 $^{o}$C to mid log phase ($OD_{600}$ = 0.5). The culture was split into 5 mL aliquots, and phenyl vinyl sulfone, or control treatment, was added as indicated. Every 30 minutes for 2.5 h following the addition of phenyl vinyl sulfone, 0.65 mL cell suspension was removed and pelleted by centrifugation (10,000 rcf for 10 min). After storing overnight at –20 °C, pellets were resuspended in 0.65 mL TSB and distributed in 100 μL aliquots to individual wells of fibronectin coated flat-bottomed 96 well microtitre plates (Biocoat Cell Environments; Becton Dikinson Labware). Following a 2-

hour incubation at 37 °C, the cell suspension was removed and wells were washed with 0.15 mL Phosphate Buffered Saline (PBS). Bound cells were then fixed by incubation for 30 min with 2%(v/v) glutaraldehyde. Following a second wash with PBS, cells were stained for 15 min with 0.1 mL crystal violet dye (12.5 g/L). Plates were washed again with PBS, covered with aluminum foil, and allowed to dry overnight (12-16 h). The absorbance at 570 nm was subsequently measured using a microtitre plate reader (Thermo Labsystems Multiskan Spectrum).

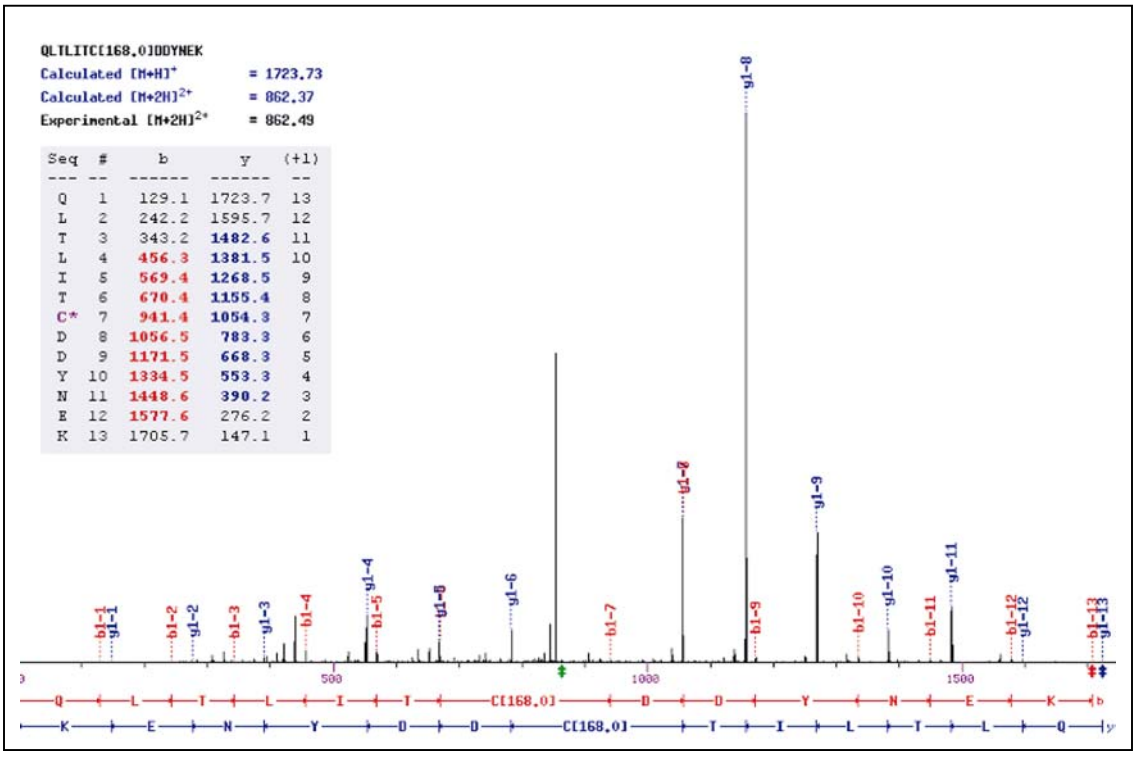



**Figure S3. ESI MS/MS Sequencing of SrtA peptide QLTLITCDDYNEK.**


(1) Ton-That, H.; Liu, G.; Mazmanian, S. K.; Faull, K. F.; Schneewind, O., *Proc Natl Acad Sci U S A* 1999, 96, (22), 12424-9.
(2) Kitz, R.; Wilson, I. B., *J Biol Chem* 1962, 237, 3245-9.
(3) Elgalai, I.; Foster, H. A., *J Appl Microbiol* 2003, 94, (3), 413-20.