Supplementary Online Material for

THE SORTASE A ENZYME THAT ATTACHES PROTEINS TO THE CELL WALL OF *BACILLUS ANTHRACIS* CONTAINS AN UNUSUAL ACTIVE SITE ARCHITECTURE

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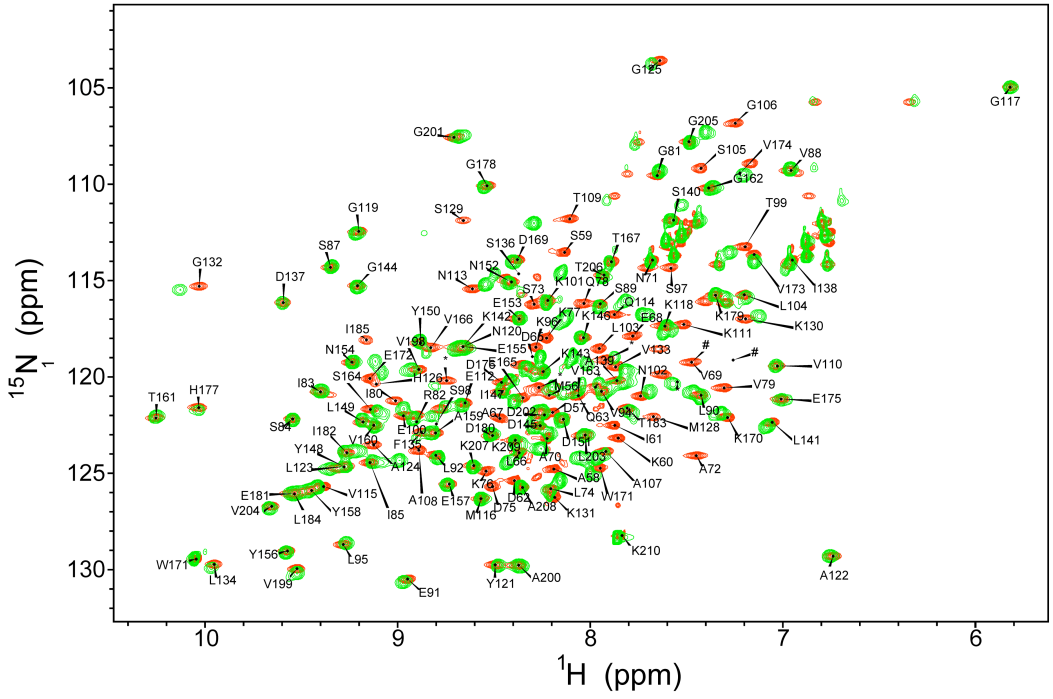

Fig. S1: ^{1}H-^{15}N HSQC of Ba-SrtA$_{\Delta 56}$ (red) and Ba-SrtA$_{\Delta 64}$ (green). Chemical shift assignments are taken directly from Ba-SrtA$_{\Delta 56}$. An '*' indicates cross peaks that could not be assigned in Ba-SrtA$_{\Delta 56}$. A '#' indicates cross peaks that are folded into the spectra and unlikely to be backbone amides. This data shows residues Asp57-Pro64 can be deleted without significant structural disruptions.

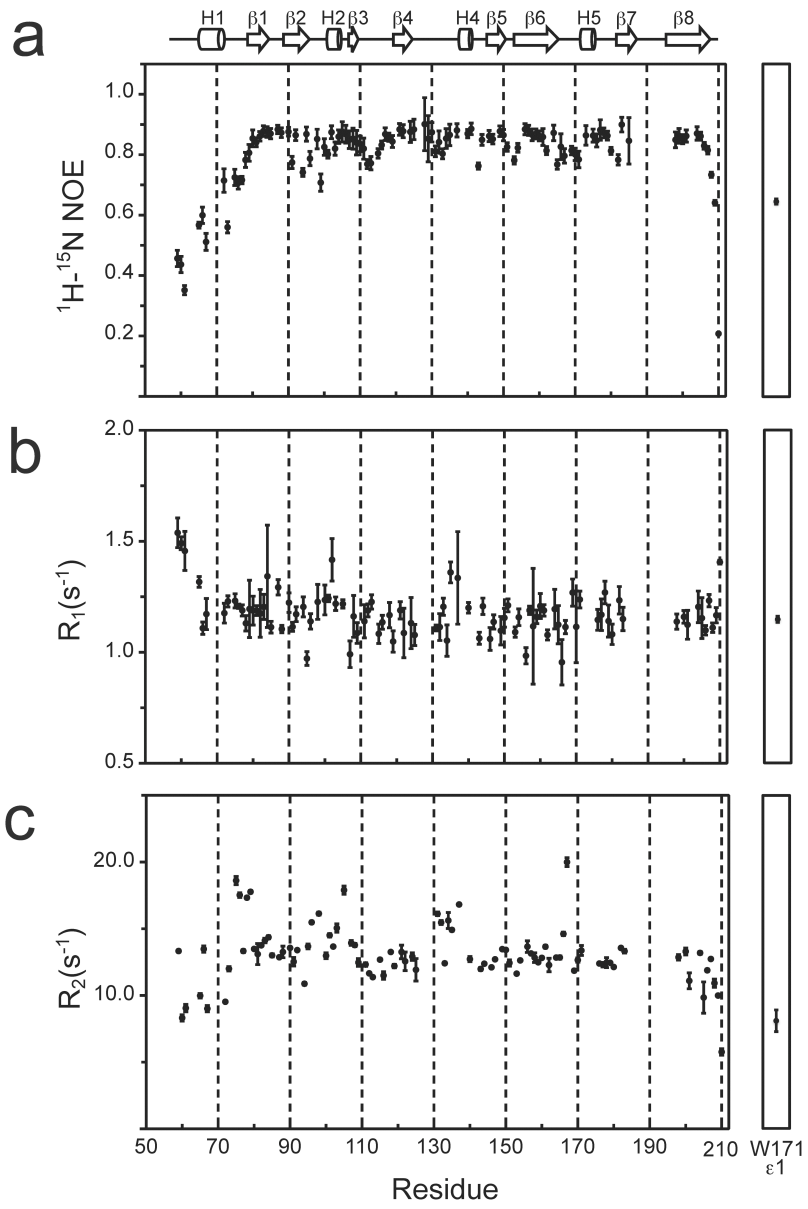

Fig. S2: Backbone and Trp171 Nε1 side-chain amide (A) ^{1}H-^{15}N NOE, (B) R_2 and (C) R_1 spin relaxation data for Ba-SrtA$_{\Delta 56}$. The right panels show data for the rp171 side chain Nε1 atom. Secondary structure is displayed at the top of panel A.

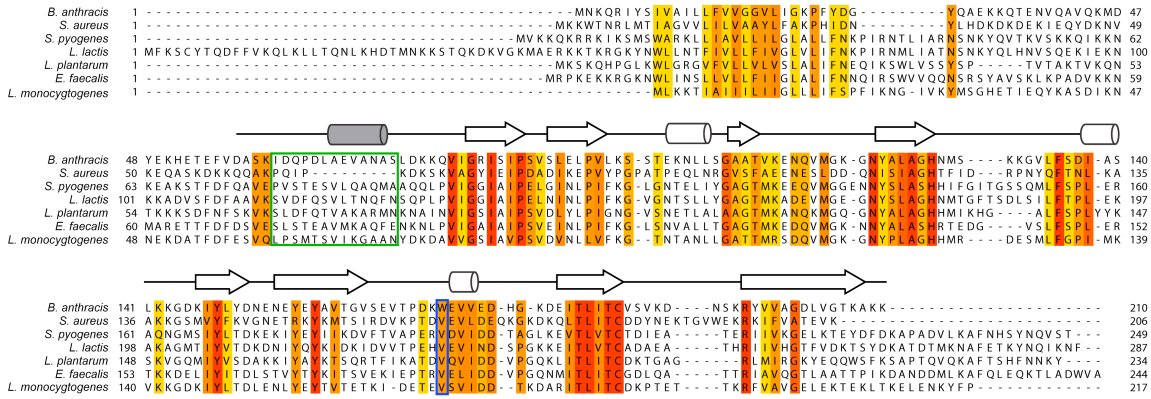

Fig. S3: Multiple sequence alignment of SrtA-family enzymes. Secondary structure of *B. anthracis* SrtA is shown on top of the alignment. The structurally analogous N-terminal helix of Ba-SrtA (shaded) is not conserved in Sa-SrtA or Sp-SrtA (green box), but may be present in other SrtA enzymes. Trp171 appears to be unique to *B. anthracis* SrtA, as other sortases contain a valine residue in this position (blue box). Alignment was made using the Structure Based Sequence Alignment Program (STRAP) (1) based on the Ba-SrtA, Sa-SrtA (2), and Sp-SrtA (3) structures. The figure was edited using Jalview (4).

Table S1: Model-free parameters calculated for Ba-SrtA$_{\Delta 56}$

Residue	Model	S^2	S^2_f	S^2_s	t_e	R_{ex}

60	5	0.495 ± 0.020	0.870 ± 0.016	0.569 ± 0.019	1299.4 ± 67.3	
61	5	0.558 ± 0.024	0.915 ± 0.032	0.610 ± 0.026	1044.7 ± 74.2	
65	5	0.640 ± 0.013	0.854 ± 0.012	0.750 ± 0.13	1265.5 ± 55.7	
66	4	0.795 ± 0.021			50.8 ± 8.6	1.750 ± 0.404
67	5	0.580 ± 0.021	0.778 ± 0.026	0.746 ± 0.036	1085.2 ± 142.7	
72	5	0.614 ± 0.014	0.753 ± 0.016	0.815 ± 0.025	1989.8 ± 1205.5	
73	5	0.805 ± 0.012	0.913 ± 0.012	0.882 ± 0.013	596.8 ± 117.1	
75	4	0.910 ± 0.024			57.4 ± 35.4	5.221 ± 0.488
76	4	0.888 ± 0.008			53.3 ± 9.2	4.446 ± 0.208
77	2	0.902 ± 0.007			55.5 ± 8.6	
78	3	0.855 ± 0.027				4.769 ± 0.412
79	3	0.904 ±0.085				4.494 ± 1.248
80	1	0.918 ± 0.003				
81	1	0.898 ± 0.015				
82	1	0.937 ± 0.005				
83	1	0.958 ± 0.011				
84	1	0.978 ± 0.008				
85	1	0.880 ± 0.007				
88	1	0.846 ± 0.012				
90	1	0.924 ± 0.004				
91	1	0.846 ± 0.013				
92	1	0.912 ± 0.002				
94	5	0.717 ± 0.010	0.817 ± 0.013	0.878 ± 0.021	1882.1 ± 330.5	
95	3	0.735 ± 0.024				2.884 ± 0.397
96	3	0.862 ± 0.027				2.827 ± 0.409
98	3	0.928 ± 0.057				2.510 ± 0.853
100	1	0.888 ± 0.014				
101	1	0.969 ± 0.007				
102	1	0.931 ± 0.005				
103	3	0.922 ± 0.022				1.516 ± 0.474
105	3	0.921 ± 0.014				4.366 ± 0.352
107	3	0.750 ± 0.046				2.929 ± 0.686
108	1	0.937 ± 0.008				
109	1	0.843 ± 0.018				
111	1	0.839 ± 0.009				
112	5	0.779 ± 0.006	0.842 ± 0.009	0.926 ± 0.013	1801.8 ± 600.0	
113	5	0.751 ± 0.014	0.837 ± 0.011	0.897 ± 0.020	2343.4 ± 1638.1	
115	1	0.862 ± 0.006				
116	1	0.813 ± 0.014				
118	1	0.903 ± 0.003				
119	1	0.829 ± 0.008				
121	1	0.901 ± 0.024				
122	1	0.847 ± 0.041				
124	1	0.878 ± 0.019				
125	1	0.814 ± 0.031				
131	3	0.839 ± 0.009				3.795 ± 0.193
132	3	0.842 ± 0.048				3.096 ± 0.716
133	1	0.848 ± 0.006				

134	3	0.796 ± 0.053				3.928 ± 0.974
135	1	1.000 ± 0.003				
137	1	1.000 ± 0.003				
140	1	0.881 ± 0.011				
143	2	0.812 ± 0.007			14.4 ± 2.5	
144	1	0.844 ± 0.003				
146	1	0.824 ± 0.003				
147	1	0.865 ± 0.005				
149	1	0.917 ± 0.002				
150	1	0.913 ± 0.004				
151	1	0.871 ± 0.013				
153	2	0.793 ± 0.004			9.3 ± 2.5	
154	1	0.860 ± 0.005				
157	1	0.896 ± 0.004				
158	1	0.874 ± 0.024				
159	1	0.850 ± 0.002				
160	1	0.873 ± 0.005				
161	1	0.926 ± 0.007				
162	1	0.820 ± 0.016				
164	1	0.874 ± 0.003				
165	2	0.873 ± 0.008			21.2 ± 5.9	
166	3	0.722 ± 0.075				4.007 ± 1.105
167	3	0.842 ± 0.024				7.624 ± 0.490
169	1	0.811 ± 0.006				
170	1	0.861 ± 0.011				
171	1	0.922 ± 0.019				
176	1	0.844 ± 0.005				
177	1	0.840 ± 0.011				
178	1	0.882 ± 0.021				
179	1	0.848 ± 0.004				
180	1	0.826 ± 0.005				
182	1	0.924 ± 0.005				
183	1	0.905 ± 0.009				
198	1	0.872 ± 0.013				
200	1	0.894 ± 0.013				
201	1	0.794 ± 0.032				
204	1	0.898 ± 0.007				
205	1	0.786 ± 0.052				
206	1	0.812 ± 0.006				
207	1	0.870 ± 0.004				
208	5	0.731 ± 0.025	0.796 ± 0.016	0.919 ± 0.017	1288.4 ± 252.4	
209	5	0.655 ± 0.005	0.791 ± 0.010	0.828 ± 0.015	1274.4 ± 93.1	
210	5	0.310 ± 0.019	0.790 ± 0.012	0.393 ± 0.019	1114.2 ± 19.9	

Table S2: Intramolecular NOEs used to calculate the Ba-SrtA-LPAT* model and corresponding intramolecular NOEs used to calculate the experimentally determined structure of Sa-SrtA-LPAT* preveriously reported.

LPAT*	Ba-SrtA	Sa-SrtA
Leu-Hα	V174-Hγ	L169-Hδ
Leu-Hα	I185-Hδ	I182-Hδ
Leu-Hβ	P168-Hα	P163-Hα
Leu-Hβ	W171-Hα	V166-Hα
Leu-Hβ	V173-Hγ	V168-Hγ
Leu-Hγ	P168- Hα	P163-Hα
Leu-Hγ	K118-HN	D165-HN
Leu-Hγ	W171- Hα	V166-Hα
Leu-Hγ	W171-HN	V166-HN
Leu-Hγ	V173-Hγ	V168-Hγ
Leu-Hγ	R196-Hγ	R197-Hγ
Leu-Hδ	P168- Hα	P163-Hα
Leu-Hδ	P168-Hβ	P163-Hβ
Leu-Hδ	K170-HN	D165-HN
Leu-Hδ	W171- Hα	V166-Hα
Leu-Hδ	W171-HN	V166-HN
Leu-Hδ	E172-HN	G167-HN
Leu-Hδ	V173-Hβ	V168-Hβ
Leu-Hδ	V173-Hδ	V168-Hδ
Leu-Hδ	R196-Hδ	R197-Hδ
Leu-Hδ	R196-Hε	R197-Hε
Pro-Hβ	V110- Hα	A104-Hα
Pro-Hβ	A124-Hβ	A118-Hβ
Pro-Hγ	V110- Hα	A104-Hα
Pro-Hγ	A124-Hβ	A118-Hβ
Pro-Hγ	V174-Hγ	L169-Hδ
Pro-Hγ	I185-Hγ2	I182-Hγ2
Pro-Hγ	I185-Hδ	I182-Hδ
Pro-Hδ	V173-Hγ	V168-Hγ
Pro-Hδ	V174-Hγ	L169-Hδ
Pro-Hδ	I185-Hγ2	I182-Hγ2
Pro-Hδ	I185-Hδ	I182-Hδ
Ala- Hα	L103-Hδ	L97-Hδ
Ala-Hβ	L103-Hδ	L97-Hδ
Ala-HN	L103-Hδ	L97-Hδ

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