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Broadening the Scope of Sortase-Mediated Ligations using Natural Sortase Homologs

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Broadening the Scope of Sortase-Mediated Ligations using Natural Sortase Homologs

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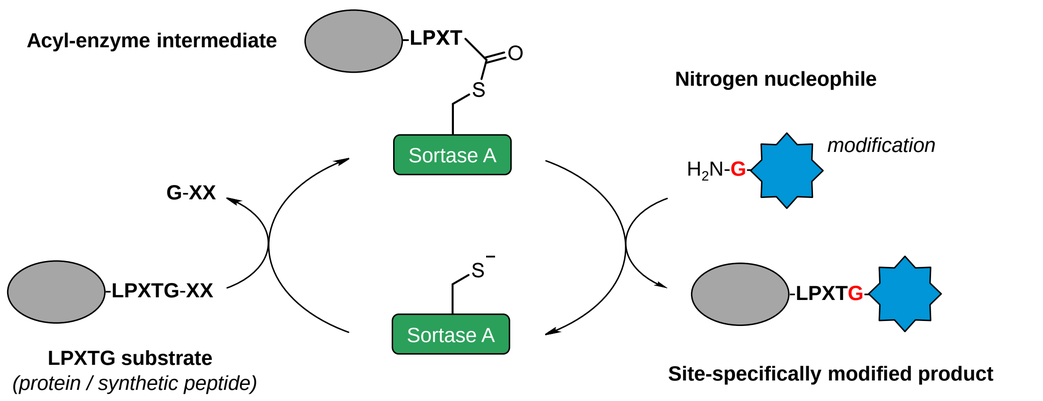
Abstract

Sortase-mediated ligations have become an attractive option for protein modification chemistry, enabling the synthesis of a wide range of non-natural polypeptide derivatives. In an effort to expand the scope of this methodology, we have been characterizing the *in vitro* reactivity of a panel of natural sortase homologs. Here we present our studies on the substrate and nucleophile tolerance of sortases from a range of bacterial species. Notable findings include that sortase A from *Streptococcus pneumoniae* ($SrtA_{pneu}$) shows a high degree of substrate promiscuity, allowing this enzyme to process a range of substrate variations that deviate from the LPXTG substrate motif typically associated with sortase-mediated methods. In addition, this enzyme has the ability to accept an expanded range of primary amine nucleophiles. To demonstrate the utility of this expanded substrate scope, we have also succeeded in using $SrtA_{pneu}$ to site-specifically modify the N-terminal serine residue of Dermcidin (DCD-1L). Overall, these results demonstrate that naturally occurring sortases represent a viable approach for the continued development of sortase-mediated protein modification.

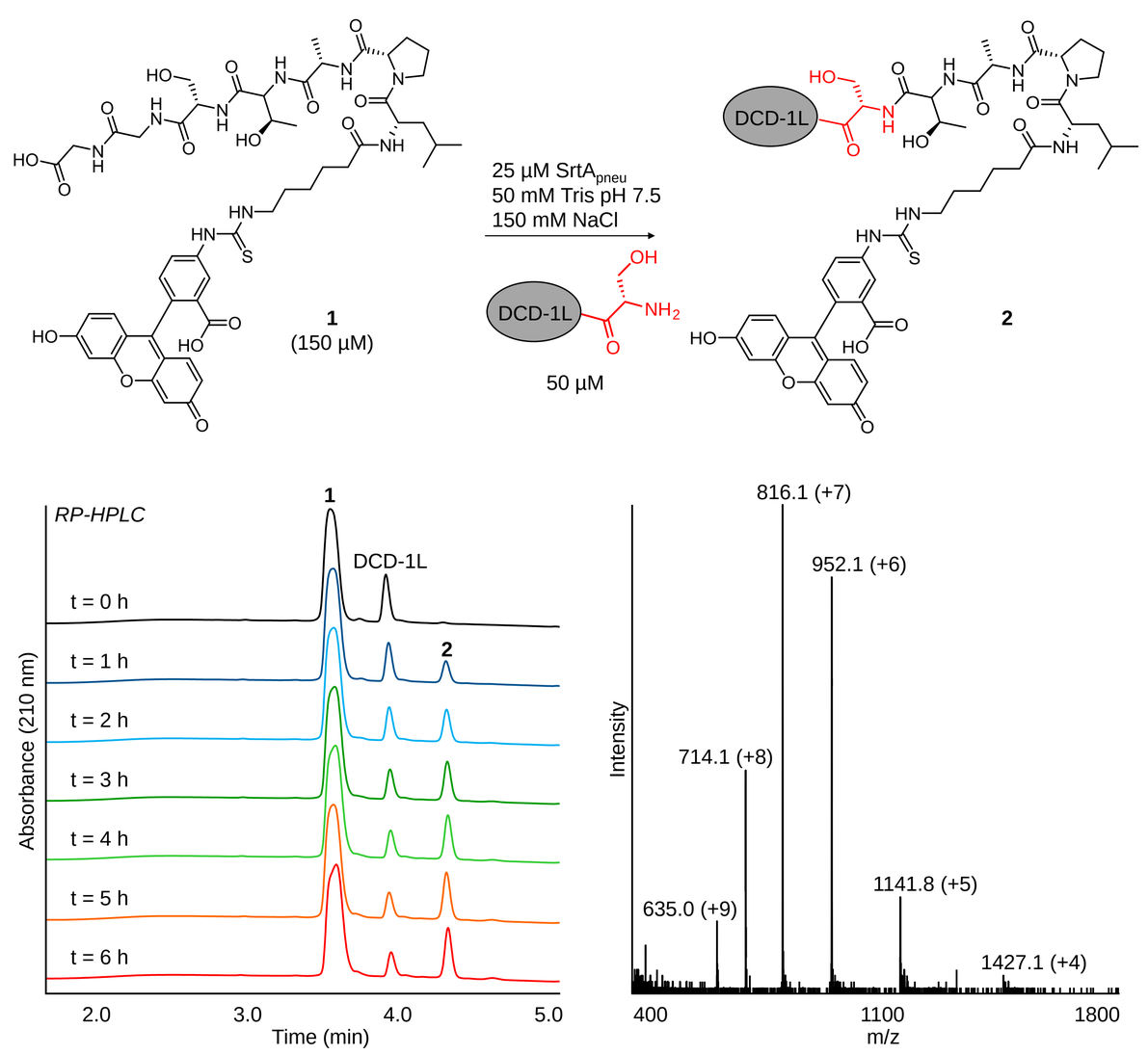
Background

Sortases are ligases found in many gram-positive bacteria that have recently risen to prominence due to their ability to selectively ligate nucleophilic amines to target peptides and proteins both *in vitro* and *in vivo*. The most thoroughly characterized example is sortase A from *Staphylococcus aureus* ($SrtA_{staph}$) which selectively recognizes the sequence LPXTG, where X denotes any amino acid. A nucleophilic cysteine in the enzyme active site attacks the carbonyl carbon of the threonine residue, ejecting the C-terminal fragment. This transient acyl-enzyme intermediate is then intercepted by an incoming nitrogen nucleophile, typically glycine, and the enzyme is released. Due to the simple catalytic mechanism by which this occurs, $SrtA_{staph}$ ligations have found use *in vitro* for the attaching a wide range of non-natural functional groups to polypeptides that contain the LPXTG "sorting motif".



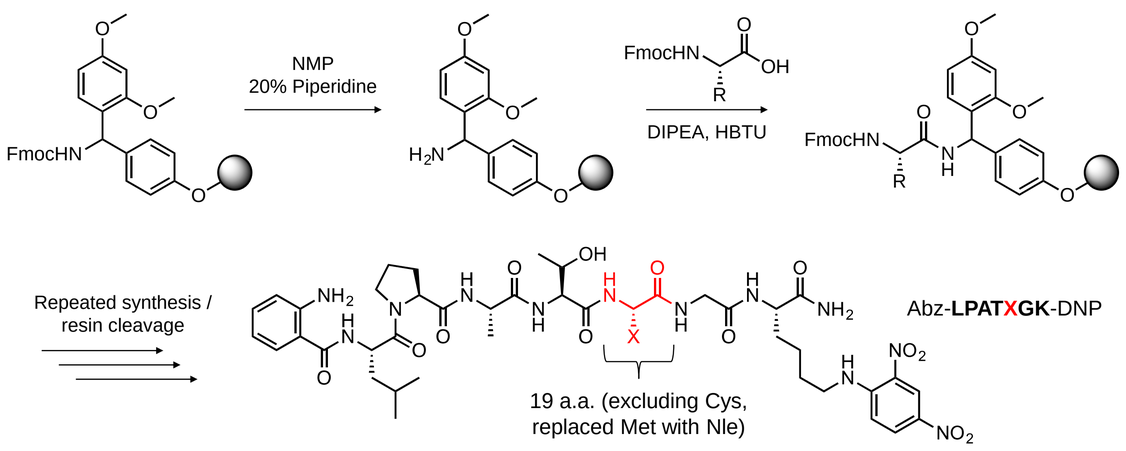
While useful, the LPXTG specificity of $SrtA_{staph}$ typically restricts the technique to substrates that inherently possess the LPXTG motif or those that have been engineered to display this sequence. To address this limitation in substrate scope, we are exploring the reactivity of sortase homologs from alternate bacterial species.

Site-Specific Modification of DCD-1L

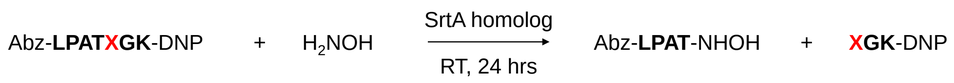


Peptide Substrates for Probing Substrate Tolerance

Substrate Synthesis



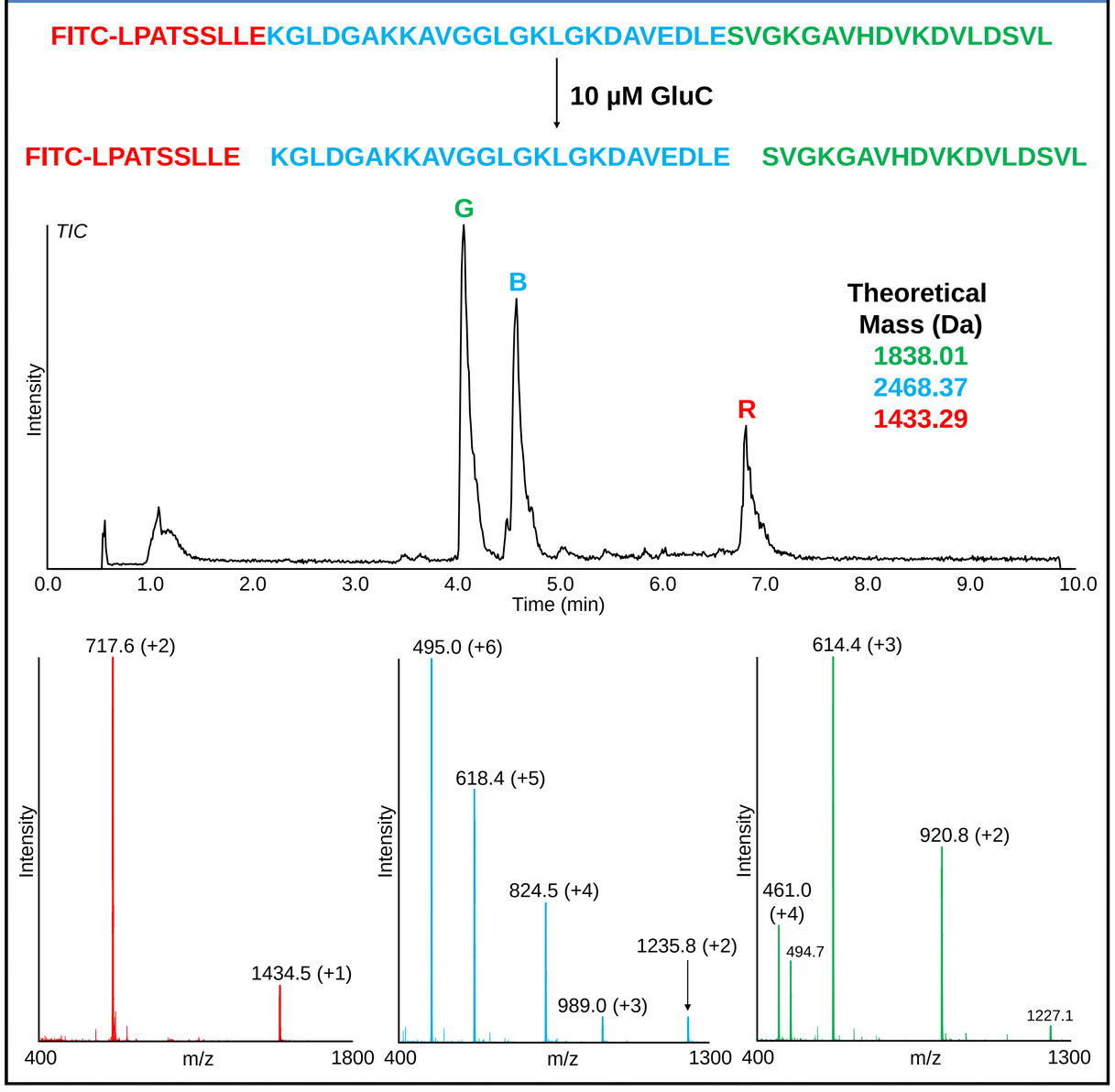
Sortase Reactivity



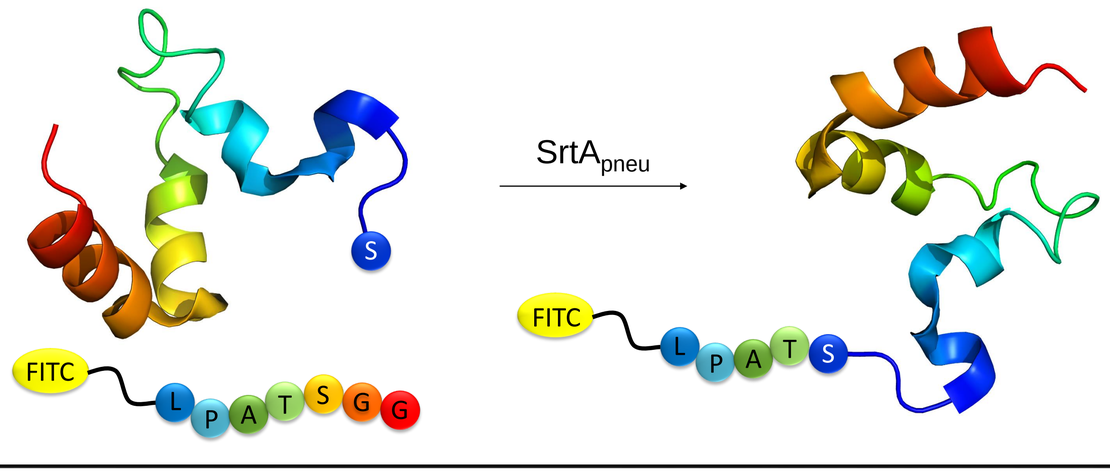
X =	G	S	A	C	N	Y	L	V	F	Nle	W	Q	I	D
staph	86	1	2	5	0	1	0	3	1	0	0	2	0	0
suis	71	67	74	73	58	2	0	2	3	2	0	3	0	0
oralis	74	54	46	48	3	1	0	2	2	1	0	6	0	0
pneu	81	84	92	76	48	14	41	39	29	14	11	11	3	0
mono	86	41	15	42	22	43	1	4	30	2	6	1	1	0
faec	7	7	5	10	6	0	0	1	3	1	0	1	0	0
lac	83	44	66	32	28	1	1	5	2	1	0	1	0	1
anth	42	8	22	17	17	2	0	1	1	1	0	0	0	2
plant	18	2	3	8	2	1	3	1	1	0	0	1	0	0

Reactivity of sortase A homologs with discretely synthesized peptides as evaluated by RP-HPLC. All reactions incubated for 24-26 hours at RT and included 25 μM sortase homolog, 200 μM peptide substrate, and 10 mM hydroxylamine in Tris buffer pH 7.5 with 10 mM Ca^{2+}. Reactions involving cysteine were supplemented with 100 mM DTT to retain a reductive environment. Conversion percentages were the average of three trials, each of which with a standard deviation < 10%.

Confirmation of Site-Specific Modification of DCD-1L



Application of $SrtA_{pneu}$ Substrate Tolerance



Conclusion

- Sortase A from *S. pneumoniae* is capable of recognizing multiple substrate variants other than LPXTG.
- *S. pneumoniae* sortase A has the potential to allow site-specific modification at a range of N-terminal residues, for example serine (DCD-1L).
- Naturally occurring sortase homologs provide a useful resource for expanding the scope of sortase-mediated protein engineering.

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