Evaluating Nucleophile and Substrate Specificities of Sortase A Homologs for Orthogonal Reactivity

Nicholas Horvath  
*Western Washington University*

Jesse Prelesnik  
*Western Washington University*

Follow this and additional works at: [https://cedar.wwu.edu/scholwk](https://cedar.wwu.edu/scholwk)

Part of the [Chemistry Commons](https://cedar.wwu.edu/scholwk)

Horvath, Nicholas and Prelesnik, Jesse, "Evaluating Nucleophile and Substrate Specificities of Sortase A Homologs for Orthogonal Reactivity" (2016). Scholars Week. 3.  
[https://cedar.wwu.edu/scholwk/2016/Day_one/3](https://cedar.wwu.edu/scholwk/2016/Day_one/3)

This Event is brought to you for free and open access by the Conferences and Events at Western CEDAR. It has been accepted for inclusion in Scholars Week by an authorized administrator of Western CEDAR. For more information, please contact westerncedar@wwu.edu.
Enzymes have become an attractive option for protein modification chemistry due to the remarkable site-specificity they afford. Of particular interest is sortase A from Staphylococcus aureus (SrtA_{S.a.}), which has garnered attention for its ability to install a variety of non-natural modifications to a conserved oligopeptide substrate. In addition to SrtA_{S.a.}, it has become apparent that sortase A homologs exist in other bacterial strains, each of which is potentially a novel catalyst for protein engineering. Previous work has demonstrated that eight representative sortase A homologs exhibit unique specificities for synthetic peptide substrates, capable of identifying characteristic combinations of amino acids in the "sortase motif." Presented here is a nucleophile profile of the most promiscuous sortase A homology investigated, that from Streptococcus pneumoniae (SrtA_{S.p.}). Exhibiting unique specificities, this SrtA variant may enable unique protein modification chemistry.

**Abstract**

Sortase A is a ligase found in many gram-positive bacteria that has recently risen to prominence due to the specificity and efficiency with which it transfers nucleophilic amino to target peptides both in vitro and in vivo. The best studied example is that of Staphylococcus aureus, SrtA_{S.a.}, which selectively recognizes the oligopeptide 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 nucleophilic substrate, typically glycine, and the enzyme is released. Due to the simple catalytic mechanism by which this occurs, SrtA_{S.a.} ligation have found use in vitro for the appendage of a wide range of non-natural functional groups to polypeptides that contain the "sortase motif" LPXTG.

**Background**

Enzymes have become an attractive option for protein modification chemistry due to the remarkable site-specificity they afford. Of particular interest is sortase A from Staphylococcus aureus (SrtA_{S.a.}), which has garnered attention for its ability to install a variety of non-natural modifications to a conserved oligopeptide substrate. In addition to SrtA_{S.a.}, it has become apparent that sortase A homologs exist in other bacterial strains, each of which is potentially a novel catalyst for protein engineering. Previous work has demonstrated that eight representative sortase A homologs exhibit unique specificities for synthetic peptide substrates, capable of identifying characteristic combinations of amino acids in the "sortase motif." Presented here is a nucleophile profile of the most promiscuous sortase A homology investigated, that from Streptococcus pneumoniae (SrtA_{S.p.}). Exhibiting unique specificities, this SrtA variant may enable unique protein modification chemistry.

**Peptide Library Synthesis Using Isokinetic Coupling**

**Nucleophile Specificity of SrtA_{pneu}**

**Site-Specific Modification of CRF**

**Conclusion**

- Sortase A from S. pneumoniae is capable of recognizing a host of nucleophiles in addition to its substrate promiscuously previously demonstrated.
- Includes many amino acid nucleophiles not recognized by SrtA_{S.a.}
- Synthetic primary amine permits less engineering in applications.
- Corticotropin-releasing hormone (CRF) used as a nucleophile in Sortase A-mediated transpeptidation reactions has allowed for a broader nucleophile application regarding in-vivo conjugates.

**Acknowledgements**

The authors thank Erin Macri and Charles Wandler at WWU Scientific Technical Services for assistant with mass spectrometry. Funding was provided by WWU, WWU Research and Sponsored Programs, and Research Corporation for Scientific Advancement (CCSA).