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

Nicholas Horvath
Western Washington University

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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-native 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<sub>pneu</sub>) 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<sub>pneu</sub> to site-specifically modify the N-terminal serine residue of Dermaclad (DCD-1L). Overall, these results demonstrate that naturally occurring sortases represent a viable approach for the continued development of sortase-mediated protein modification.

**Peptide Substrates for Probing Substrate Tolerance**

**Substrate Synthesis**

- FITC-LPATSSSLLEKGLDGAKKAVGGLGKLDVEDELGSVKGAVHDVKDVLDSVL
- FITC-LPATSSSLLEKGLDGAKKAVGGLGKLDVEDELGSVKGAVHDVKDVLDSVL

**Site-Specific Modification of DCD-1L**

**Confirmation of Site-Specific Modification of DCD-1L**

**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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