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

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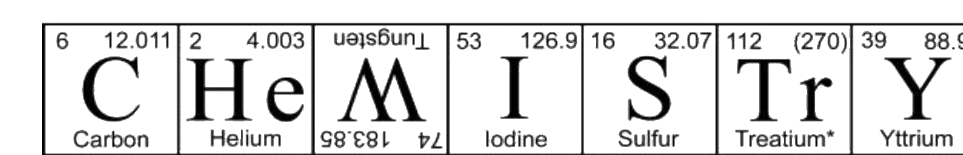
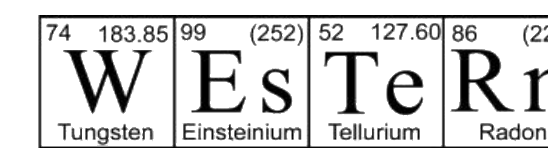
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# Evaluating Nucleophile and Substrate Specificities of Sortase A Homologs for Orthogonal Reactivity

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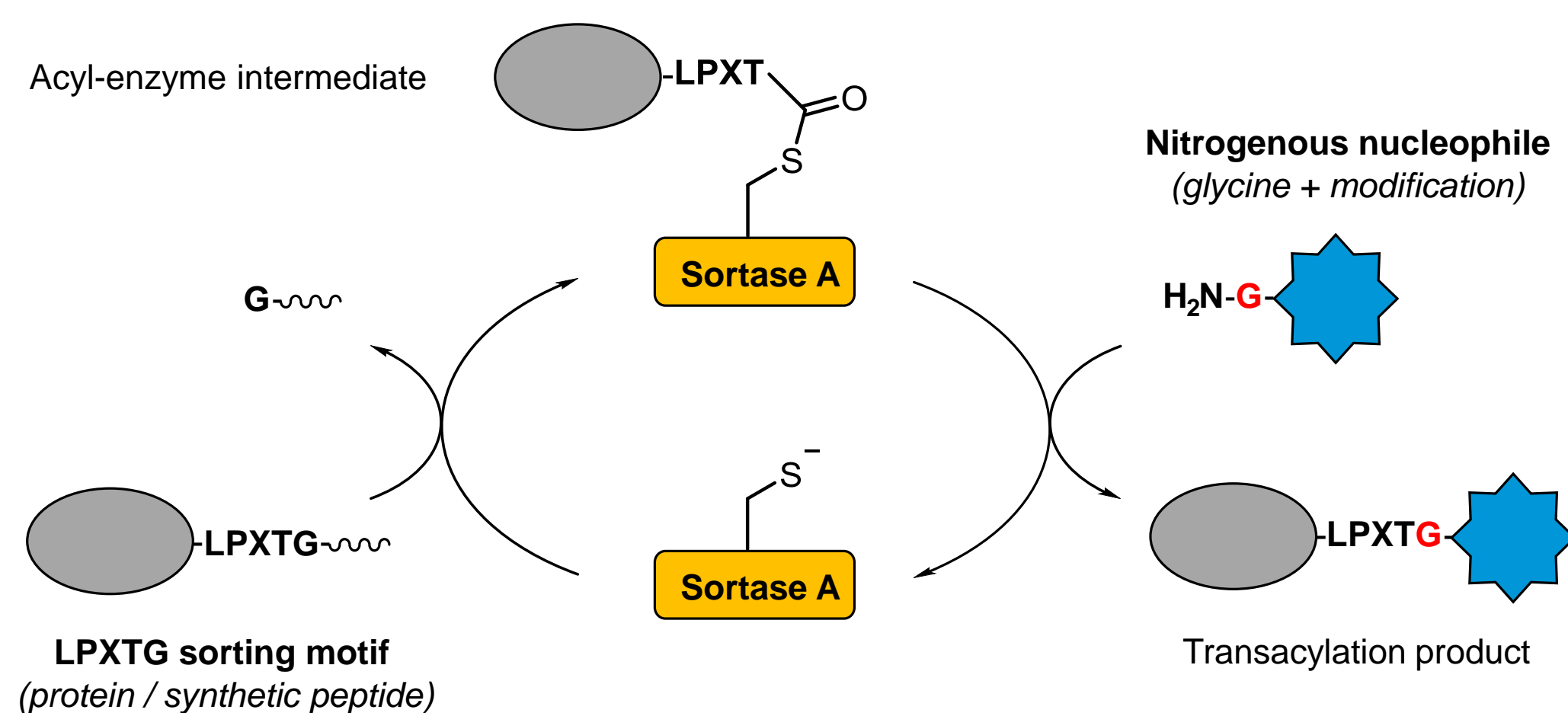
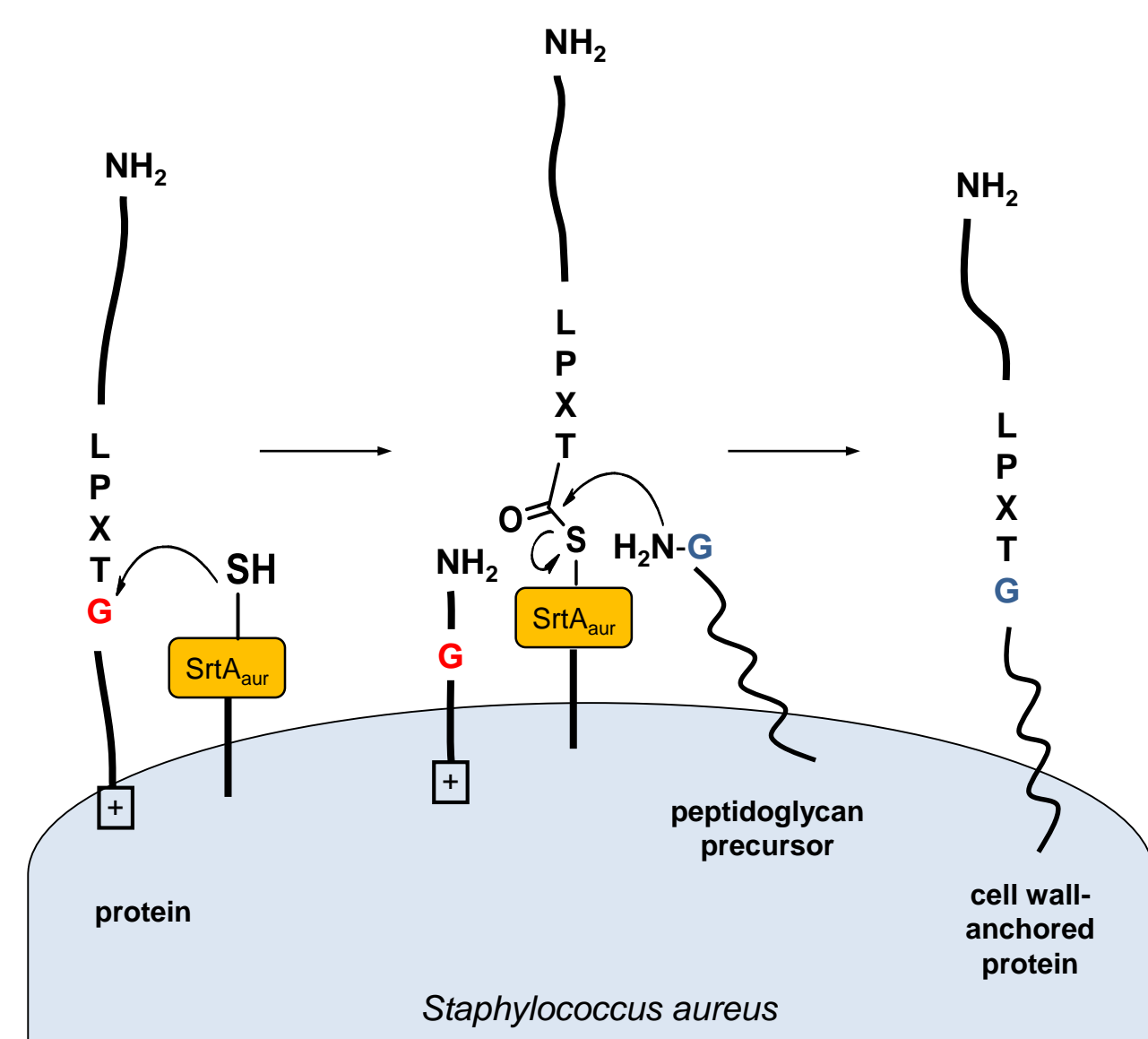


## Abstract

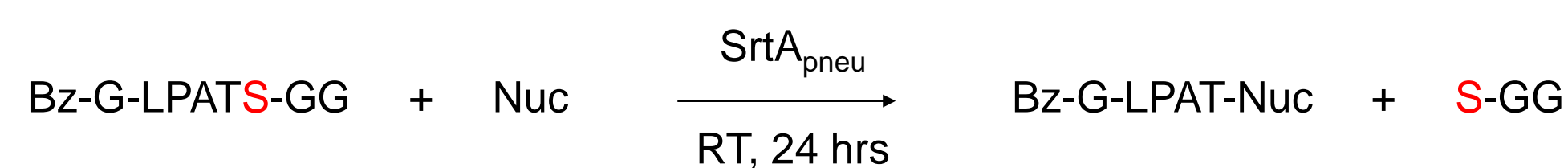
Enzymes have become an attractive option for protein modification chemistry due to the remarkable site-specificity they afford. Of particular interest is sortase A from *Staphylococcus aureus* (SrtA<sub>aur</sub>), which has garnered attention for its ability to install a variety of non-natural modifications to a conserved oligopeptide substrate. In addition to SrtA<sub>aur</sub> 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 "sorting motif." Presented here is a nucleophile profile of the most promiscuous sortase A homolog investigated, that from *Streptococcus pneumoniae* (SrtA<sub>pneu</sub>). Exhibiting unique specificities, this SrtA variant may enable unique protein modification chemistry.

## Background

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 amines to target peptides both *in vitro* and *in vivo*. The best studied example is that of *Staphylococcus aureus*, SrtA<sub>aur</sub>, 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 nitrogenous nucleophile, typically glycine, and the enzyme is released. Due to the simple catalytic mechanism by which this occurs, SrtA<sub>aur</sub> ligations have found use *in vitro* for the appendage of a wide range of non-natural functional groups to polypeptides that contain the "sorting motif" LPXTG.

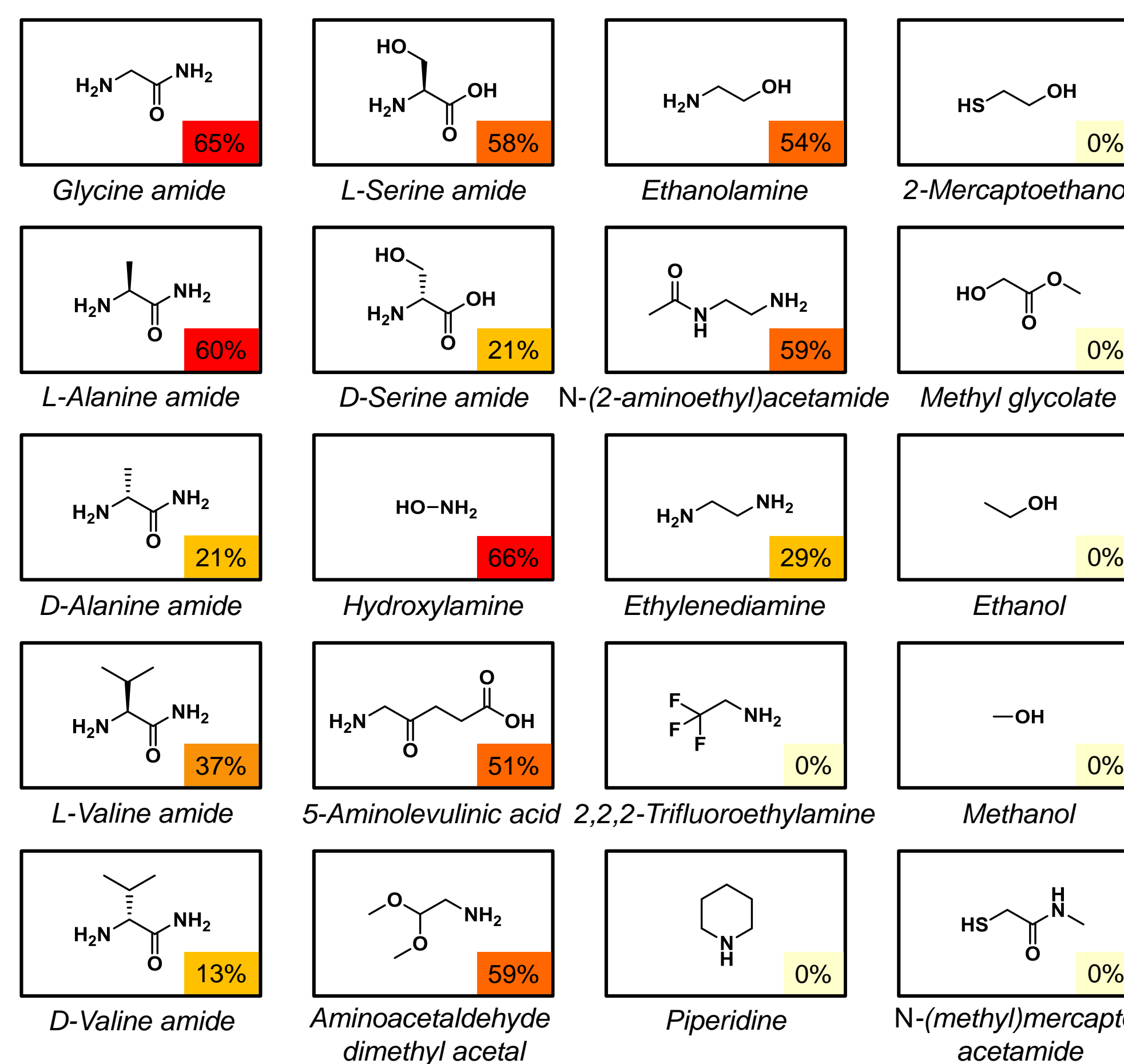
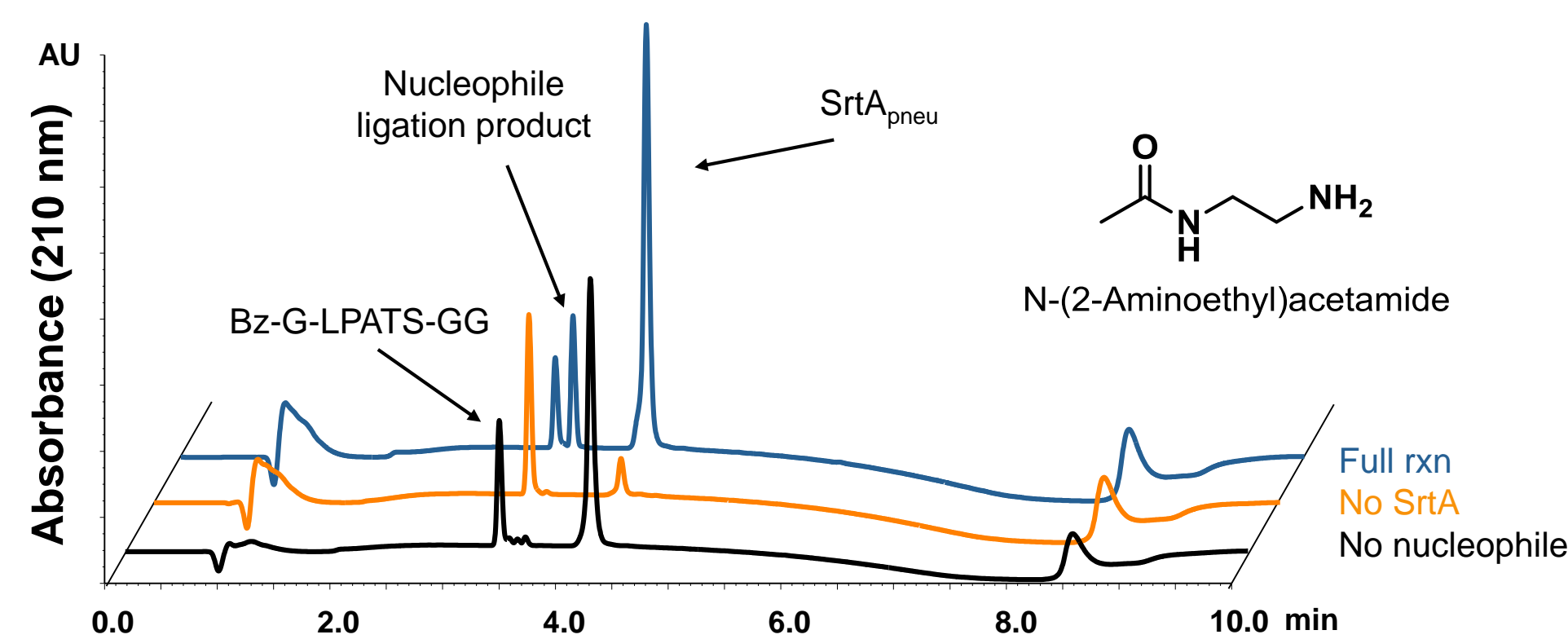


## Nucleophile Specificity of SrtA<sub>pneu</sub>

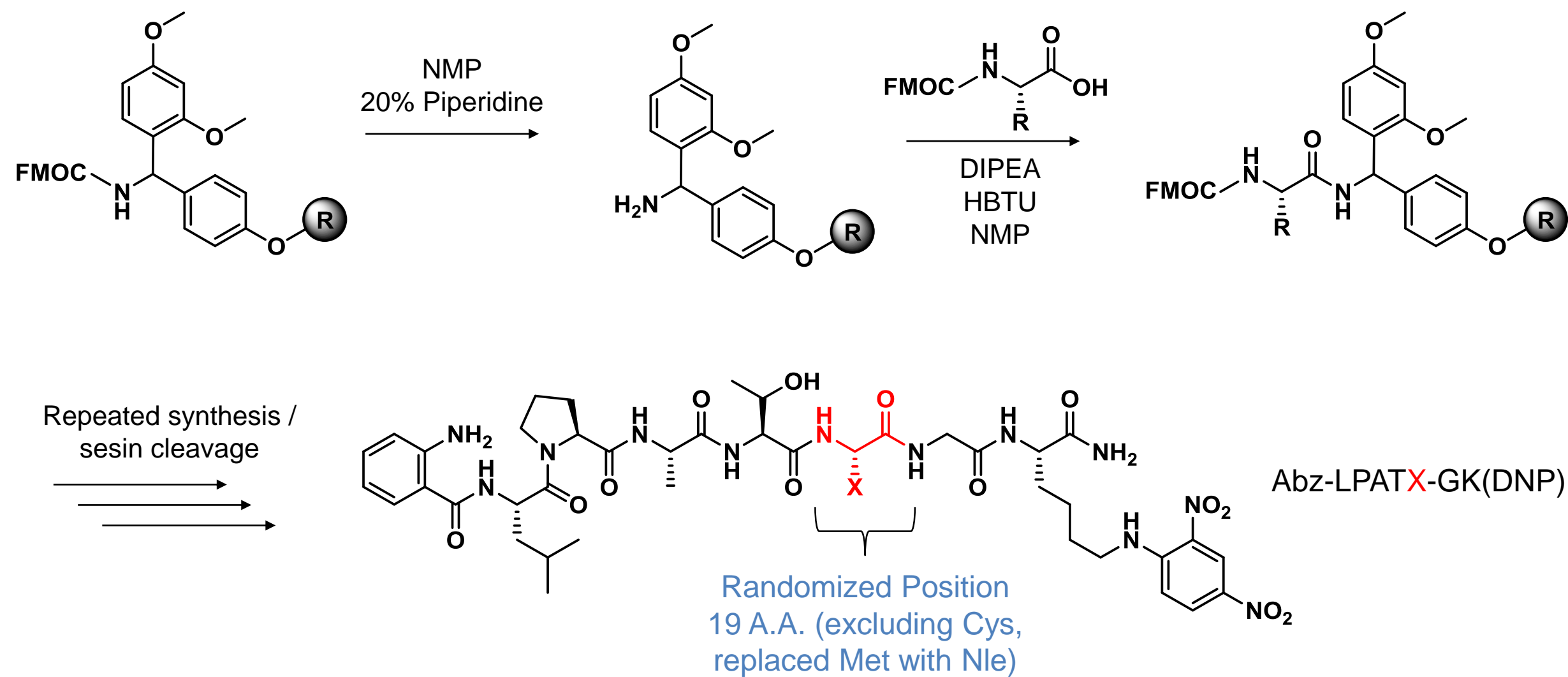
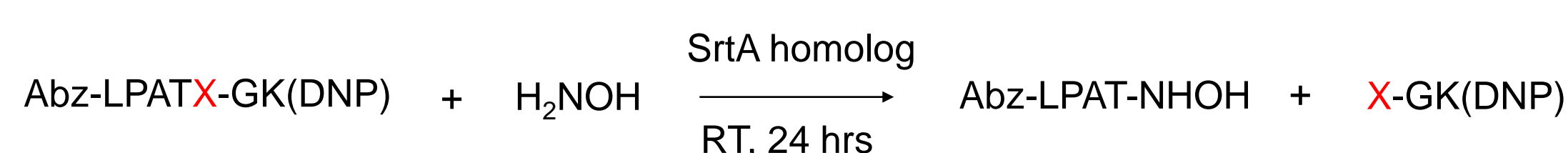


### Reaction Conditions

25  $\mu\text{M}$  SrtA<sub>pneu</sub>  
10 mM nucleophile  
200  $\mu\text{M}$  Bz-G-LPATS-GG  
Buffer:  
50 mM Tris pH 7.5  
150 mM NaCl  
10 mM CaCl<sub>2</sub>



## Peptide Library Synthesis Using Isokinetic Coupling

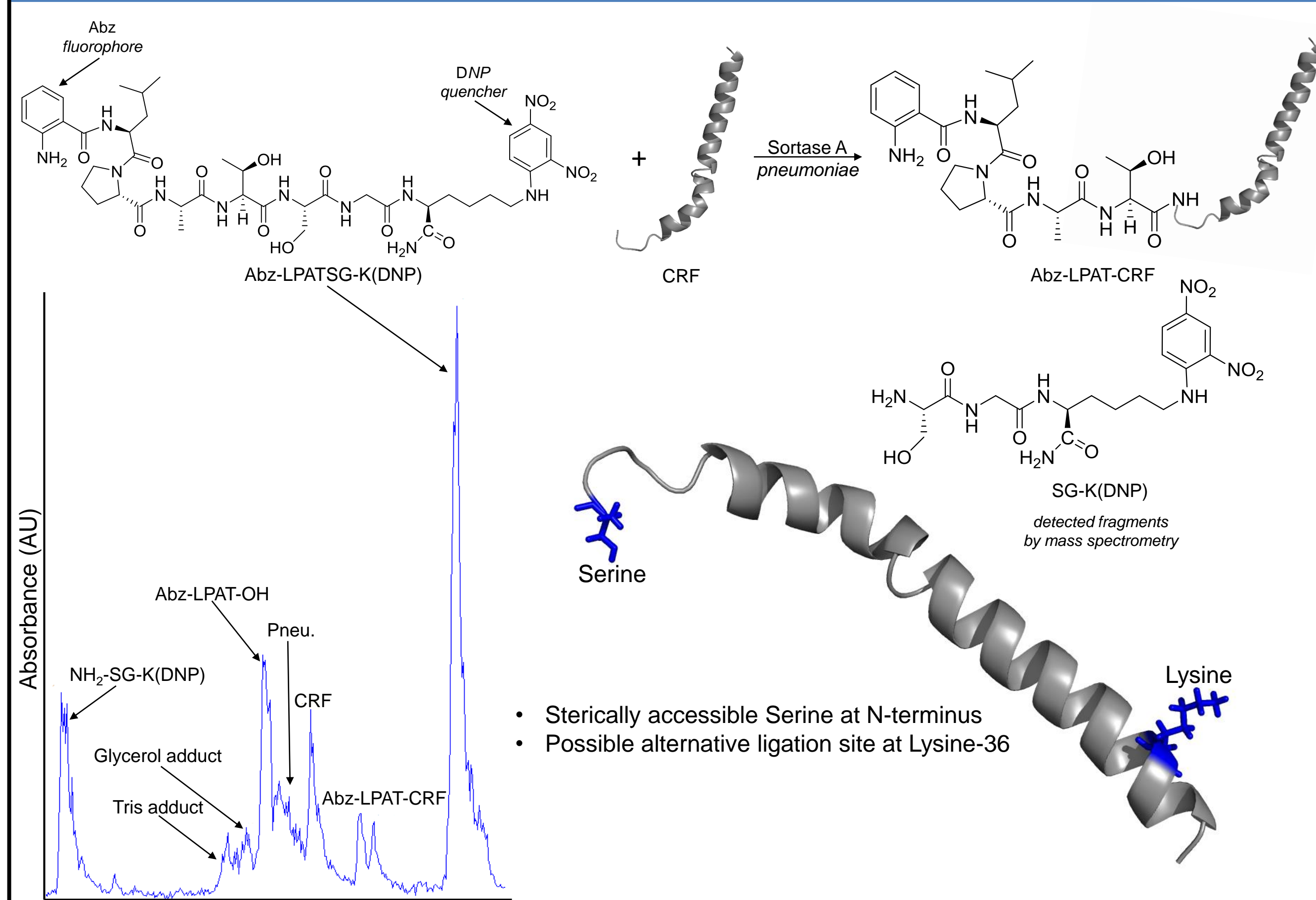


### Abz-LPATX-GK(DNP)

	V	Y	S	W	I	L	Nle	A	N	F	Q	G	C	D
SrtA <sub>aur</sub>	0	0	1	0	0	0	0	3	0	0	0	83	5	0
SrtA <sub>suis</sub>	2	1	63	0	0	0	0	80	17	0	2	67	73	0
SrtA <sub>oralis</sub>	3	0	8	0	0	0	0	12	2	0	0	11	48	0
SrtA <sub>pneu</sub>	36	11	84	13	3	7	4	91	40	10	15	72	76	0
SrtA <sub>mono</sub>	1	14	12	17	0	0	0	11	21	9	0	78	42	0
SrtA <sub>rae</sub>	1	0	1	0	0	0	0	5	5	0	0	6	10	0
SrtA <sub>fac</sub>	1	0	4	0	0	0	0	8	3	0	0	20	32	1
SrtA <sub>anth</sub>	0	3	28	0	0	0	0	23	11	0	0	82	17	2
SrtA <sub>plant</sub>	0	0	1	0	0	0	0	1	2	0	0	14	8	0

Activity of sortase A homologs with discretely synthesized peptides as evaluated by RP-HPLC. All reactions were incubated for 24 hours at RT and included 25  $\mu\text{M}$  sortase homolog, 200  $\mu\text{M}$  substrate, and 10 mM hydroxylamine in Tris buffer pH 7.5 with 10 mM Ca<sup>2+</sup>. 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%.

## Site-Specific Modification of CRF



- Sterically accessible Serine at N-terminus
- Possible alternative ligation site at Lysine-36

## Conclusion

- Sortase A from *S. pneumoniae* is capable of recognizing a host of nucleophiles in addition to its substrate promiscuity previously demonstrated
  - Includes many amino acid nucleophiles not recognized by SrtA<sub>aur</sub>
  - Synthetic primary amines permit 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).