Improved Sortase A-Catalyzed Transpeptidation by Selective Electrostatic-Assisted Aminolysis Trapping

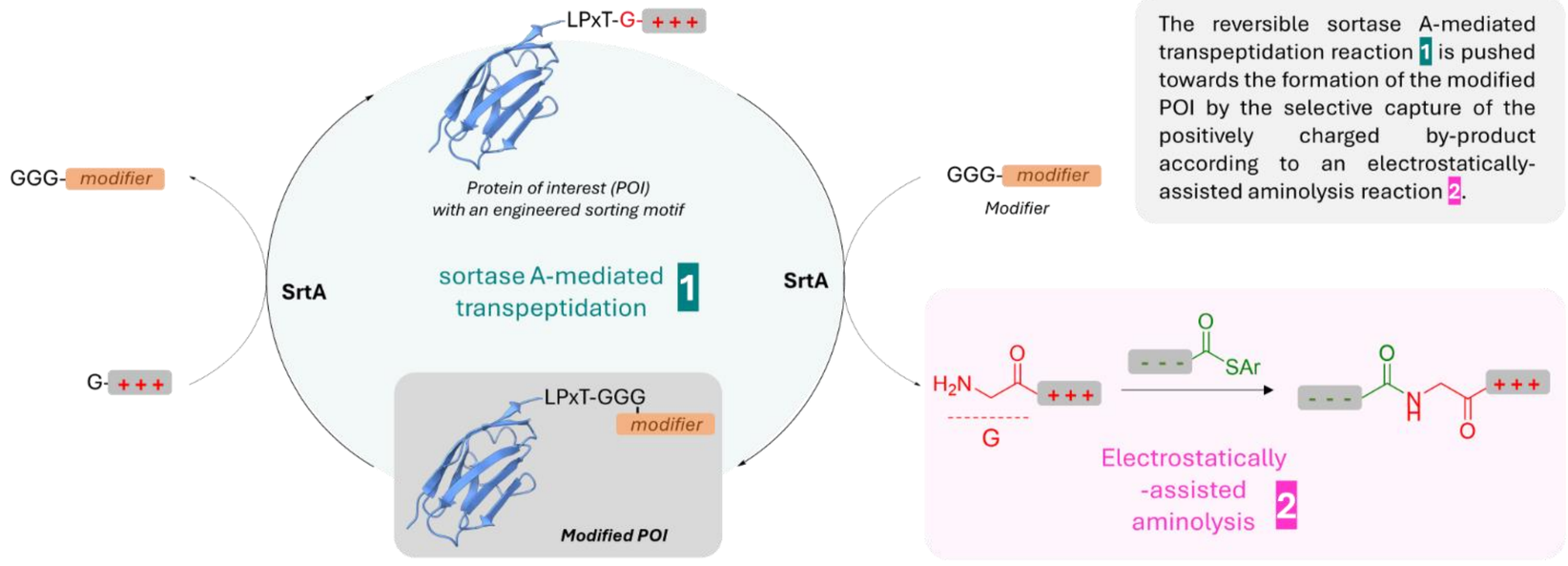
Chen Wang,[a, b] Benoît Snella,[a] Rémi Desmet,[a] Oleg Melnyk,[a] Vangelis Agouridas[a,b]

[a] Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 9017 - CIIL
Center for Infection and Immunity of Lille, F-59000, Lille, France
[b] Centrale Lille, F-59000, Lille, France

Introduction

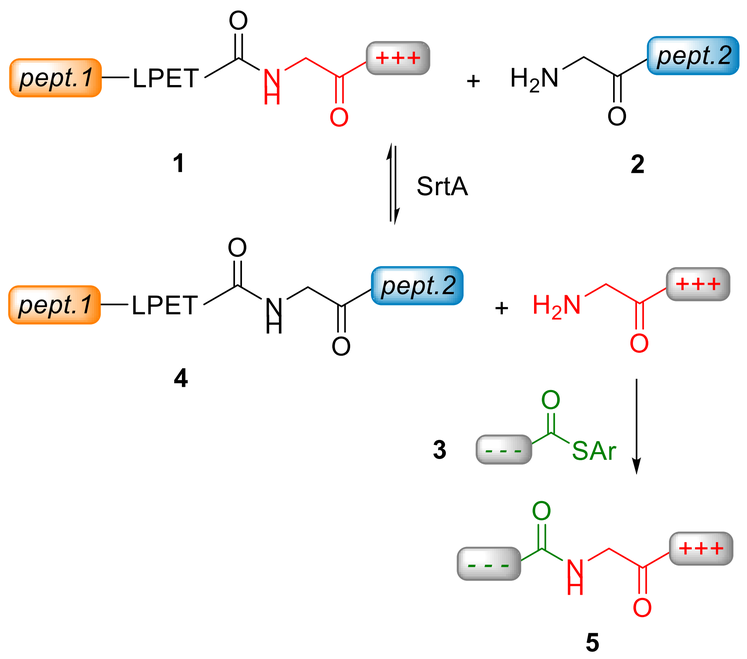
Sortase A (SrtA) and some variants thereof have been used in a wide range of applications including fluorescent labeling, protein cyclization and immobilization due to their mild reaction conditions and high specificity.[1] However, SrtA-catalyzed transpeptidation suffers the inherent limitation of being a reversible process which therefore requires an excessive amount of substrate to drive the reaction towards completion. Such an issue can prove prohibitive, especially in the case of high value-added substrate molecules.[2, 3]

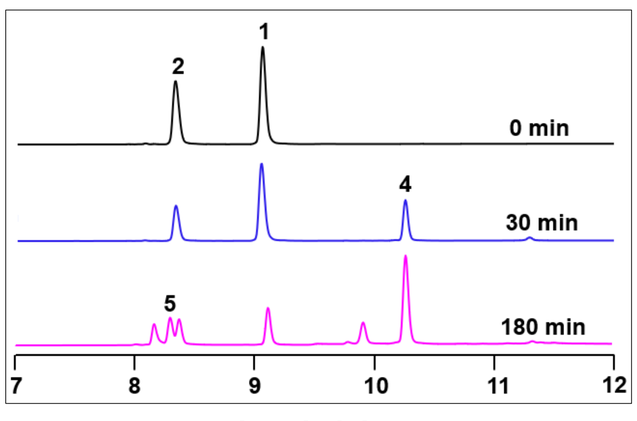
In this context, we disclose a novel substrate engineering strategy that enables to achieve high levels of SrtA-mediated protein modification with nearly stoichiometric amounts of substrate. **Extension of the consensus sorting motif LPXTG with a positively charged peptidic module** allows to achieve sequence-specific removal of by-products by applying the concept of electrostatic-assisted aminolysis reaction recently described by our group.[4] The reaction equilibrium is driven to favor product formation, thereby greatly improving reaction yield.

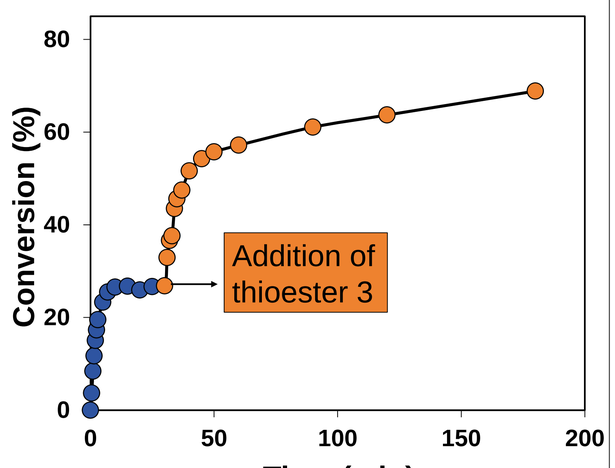


Proof-of concept

The model peptide **1** containing an Arg-rich positively charged module is incubated with the nucleophilic peptide **2** (1.2 equiv.) for 30 minutes. Thioester **3** equipped with a negatively charged module is added after 30 min (1.5 equiv.).







- ✓ In the first 30 minutes, the reaction plateaus at 26% conversion showing poor conversion to the transpeptidated product (yield calculation from Trp UV absorption at 280 nm).
- ✓ Addition of thioester **3** at 30 min immediately promotes peptide balance toward the product forming direction with a fast and instant conversion increase. The formation of the aminolysis product **5** can be observed by HPLC at 215 nm.
- ✓ After 3 additional hours, the conversion has almost tripled.

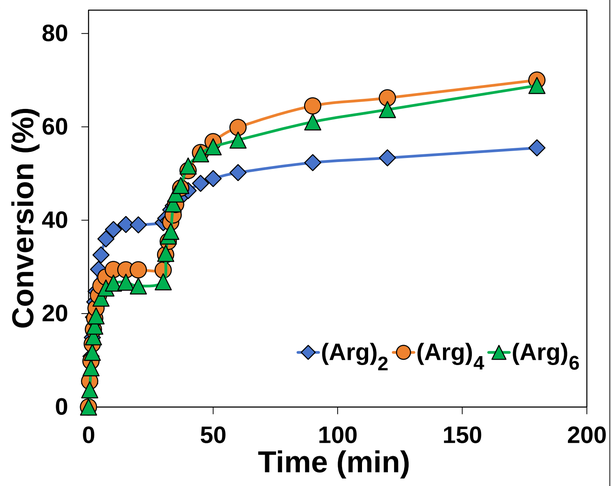
This experiment shows that the ligation byproducts are obviously removed and the yield is significantly increasing by the electrostatically assisted aminolysis reaction.

Optimization

1) Influence of the number of positive charges in the Arg-rich module

The number n of Arg residues was varied in the positively charged module with n = 2, 4, 6.

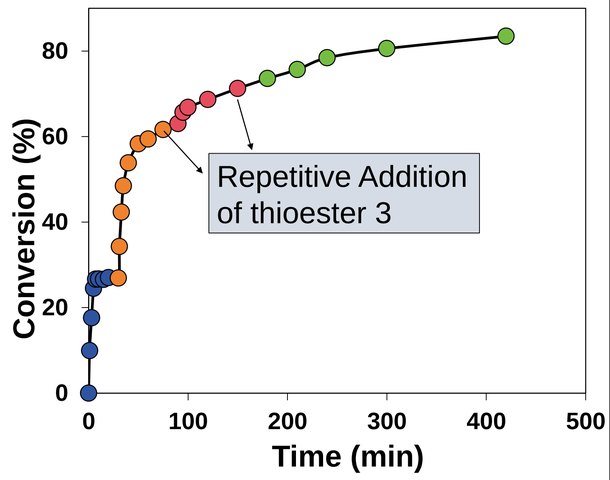
Result: $(Arg)_4$ and $(Arg)_6$ containing modules show similar results in the SrtA and electrostatically assisted reactions, both reaching 70% conversion after 180 minutes of reaction.
$(Arg)_2$ is less efficient in driving the SrtA transpeptidation equilibrium to high conversions despite reaching a higher plateau after 30 min. In this case, the electrostatically-assisted aminolysis is much less efficient.



2) Repetitive additions of thioester 3.

The negatively charged thioester **3** is completely consumed one hour after the addition and cannot further promote the progress of the SrtA-catalyzed trans peptidation reaction. Therefore, repetitive additions of thioester **3** at 90 min and 180 min were tested.

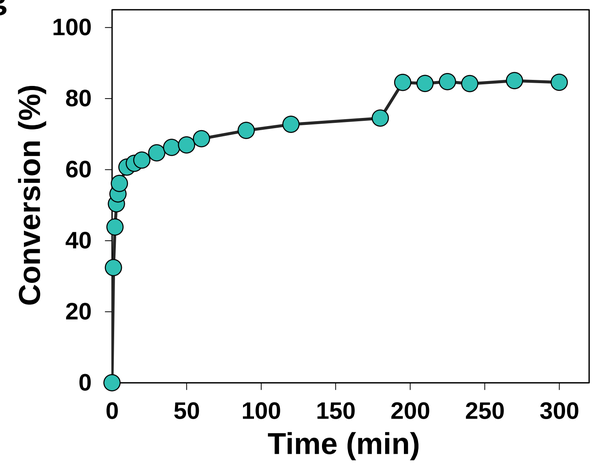
Result: SrtA-mediated ligation is further displaced toward product formation after each thioester addition, resulting in 83% conversion after three additions.



3) Addition of the thioester the beginning of the reaction

Peptide **1** (500 µM), peptide **2** (1 mM) and thioester **3** (750 µM) are added all together at the beginning of the reaction.

Result: the conversion reaches 74% after 3 hours. A second addition allows to increase the yield up to 85% after 2 additional hours of reaction.



Conclusion

We have developed a straightforward method to significantly improve the efficiency of SrtA-mediated ligation by incorporating an electrostatically-assisted aminolysis to inactivate the reaction byproduct. Since positively charged sequences can be easily programmed into recombinant protein, this strategy enables efficient preparation of proteins with various modifications. The approach is currently being further developed and applied on protein models.

References

[1] Mao, H. et al. J. Am. Chem. Soc., **2004**, 126, 2670-2671.
[2] Morgan, H. E. et al. Chem. Soc. Rev., **2022**, 51, 4121-4145.
[3] Zou, Z. et al. Angew. Chem. Int. Ed. **2024**, 63, e202310910.
[4] Ollivier, N. et al. Nat. Commun. **2022**, 13, 6667.