# *Staphylococcus aureus* sortase A mediated incorporation of peptides: effect of peptide modification on incorporation


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## *Introduction*

Endogenous *Staphylococcus aureus* **Sortase A** covalently anchors bacterial **surface proteins** equipped with a specific recognition motif (LPXTG) into the **peptidoglycan** layer of the cell wall:

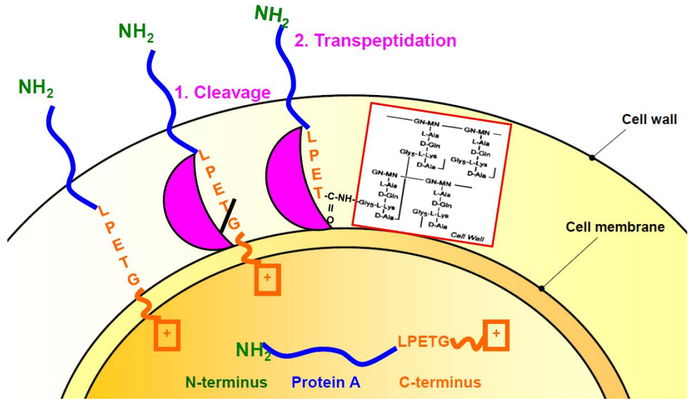


***Figure 1**: LPETG containing S. aureus **surface protein** incorporation into the staphylococcal **peptidoglycan**.*

**Previously, we showed:**

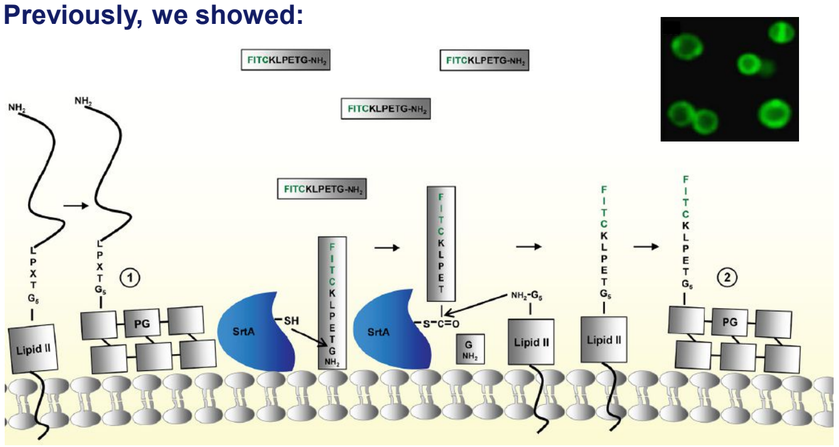


***Figure 2**: A proposed model of SrtA synthetic substrate incorporation We propose that the exogenously added SrtA synthetic substrates, such as a FITC-K-LPETG-amide are recognized and processed in the same manner as the native CWA proteins (as described in Fig. 1). CWA proteins are covalently incorporated into the bacterial cell-wall (**1**) in addition to native SrtA substrates (**2**).*

***Aim of this study:***
***Since high concentrations of the synthetic FITC-K-LPETG-amide substrate were required (1mM), we aimed in this study to improve the incorporation efficiency by modification of the substrate.***

## *Materials & Methods*

*S. aureus* strains (WT and *ΔSrtA*) were exposed to (fluorescently) labelled synthetic sortase substrates (Table 1) during growth in a Luria broth (LB) medium and the fluorescence was determined with Facs scan.

***Table 1**: Substrates used in this study:*

| Substrate number | N-term | Substrate sequence | C-term |
|---|---|---|---|
| 1 | K(FITC) | LPETG | amide |
| 2 | K(FITC) | EGTLP | amide |
| 3 | K(FITC) | AKKSELPETGGEESTNKGMLFGGLFSILGLALLRRNKKNHK | amide |
| 4 | K(FITC) | AKKSELPETGGEESTNKRRNKKNHKAGMLFGGLFSILGLALL | amide |
| 5 | K(FITC) | AKKSELPETGGEESTNKRRNKLKNHK | amide |
| 6 | K(FITC) | LPETGGEESTNKRKKW | amide |
| 7 | K(FITC) | LPMTG | amide |
| 8 | K(FITC) | K(Vancomycin)LPMTG | amide |
| 9 | K(FITC) | K(Vancomycin)MGTLP | amide |
| 10 | K(FITC) | LPMTGG-K(vancomycin) | amide |
| 11 | K(FITC) | Vancomycin-K-AKKSELPETGGEESTNKRRNKLKNHK | amide |
| 12 | | K(Vancomycin)LPMTG | amide |
| 13 | | K(Vancomycin)MGTLP | amide |

## *Results*

The elongation of the FITC-K-LPETG-amide substrate with the positively charged sequence derived from membrane spanning domain of physiological SrtA substrates resulted in a 20-fold higher incorporation.

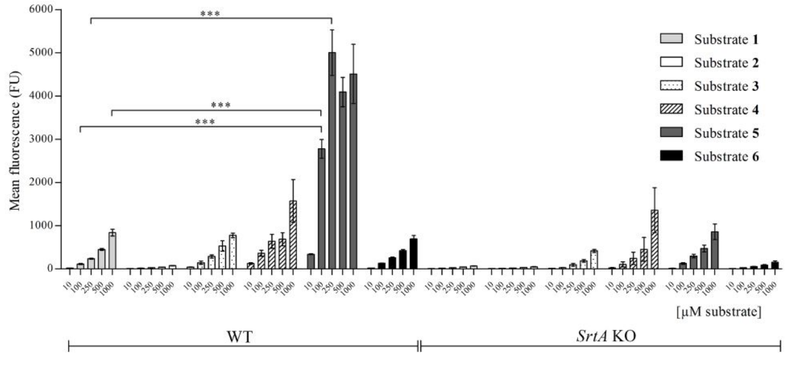


***Figure 3**: Titrations of the SrtA substrates.*

FITC-K-LP**M**TG-amide is incorporated three and half times more efficiently into the WT *S. aureus* bacteria than the FITC-K-LP**E**TG-amide.

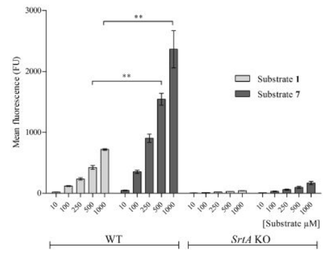


***Figure 4**: Titration of the FITC-K-LPMTG-amide substrate.*

Covalent coupling of vancomycin to FITC-K-LPMTG-amide resulted in the same incorporation at a 500-times lower substrate concentration. However, this incorporation did not result in an increased vancomycin efficiency.

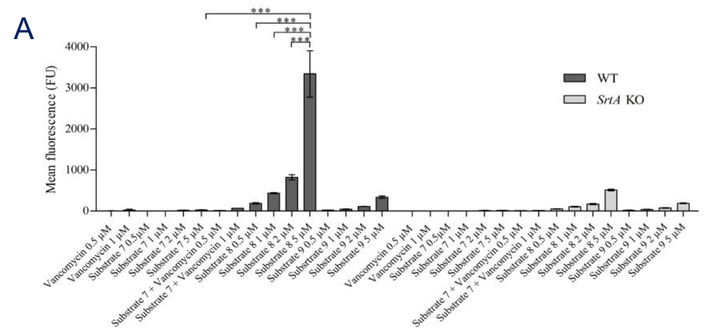


***Figure 5**: (A)Titration of the FITC-K-LPMTG-amide coupled to vancomycin and (B)MIC determination.*

## *Conclusions*

These newly developed synthetic substrates can potentially find broad applications in for example the *in situ* imaging of bacteria; the targeted delivery and covalent incorporation of antimicrobial compounds into the bacterial cell wall.