UBC Faculty of Medicine, Department of Biochemistry & Molecular Biology

Structural and Thermodynamic Characterization of a Toxin-Antitoxin System

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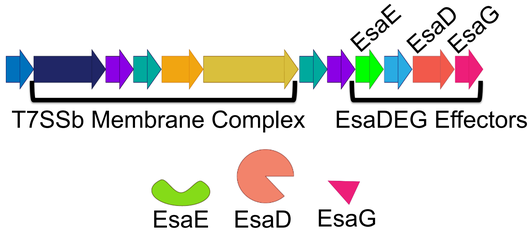
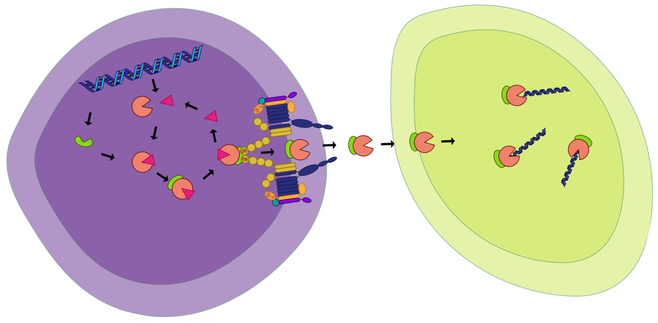
S. aureus Resistance & Virulence

Staphylococci are one of the most prevalent nosocomial and increasingly community-based infections. *S. aureus* has acquired resistance to a broad spectrum of nearly all classic antibiotics[1]. A virulence factor, the *S. aureus* Type VII Secretion System (T7SSb), is implicated in having key roles in infections and interbacterial competition[2]. Specifically, the T7SSb secreted effectors EsaD, an endonuclease, EsaE a chaperone required for secretion, and EsaG, EsaD's immunity protein or "anti-toxin" are of particular interest. EsaD's nuclease activity has proved to be involved in interbacterial competition allowing for acquisition of required nutrients for persistent infections[3]. Inhibition of this nuclease toxin system could be a novel antivirulent target for therapeutics in the constant battle against *S. aureus* resistance.

EsaD Secretion Mechanism



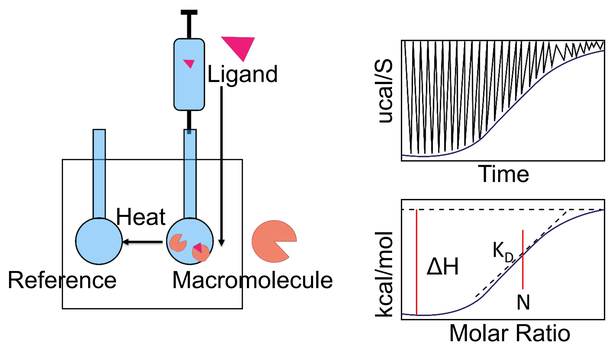


S. aureus cell

Target/Competitor cell

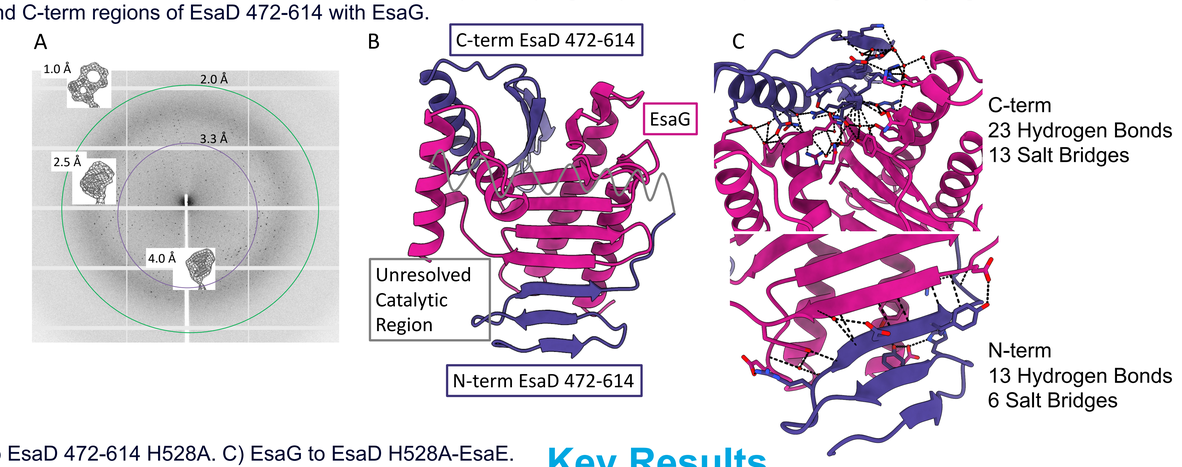
Iso Thermal Calorimetry (ITC)

Overview of ITC, ligands are titrated from a syringe to the sample cell containing the binding partner. Heat release or absorption is measured in comparison to a reference cell.



X-ray Crystallography

A) Representative diffraction pattern from EsaD 472-614 X-ray data collection with examples of data quality at different resolutions. B) overall model of EsaD 472-614 (blue) EsaG (magenta) at 2.15 Å. C) Salt bridges and hydrogen bonds in N and C-term regions of EsaD 472-614 with EsaG.



Example ITC titration thermograms. A) EsaG to EsaD 472-614. B) EsaG to EsaD 472-614 H528A. C) EsaG to EsaD H528A-EsaE. D) DNA to EsaD 472-614.

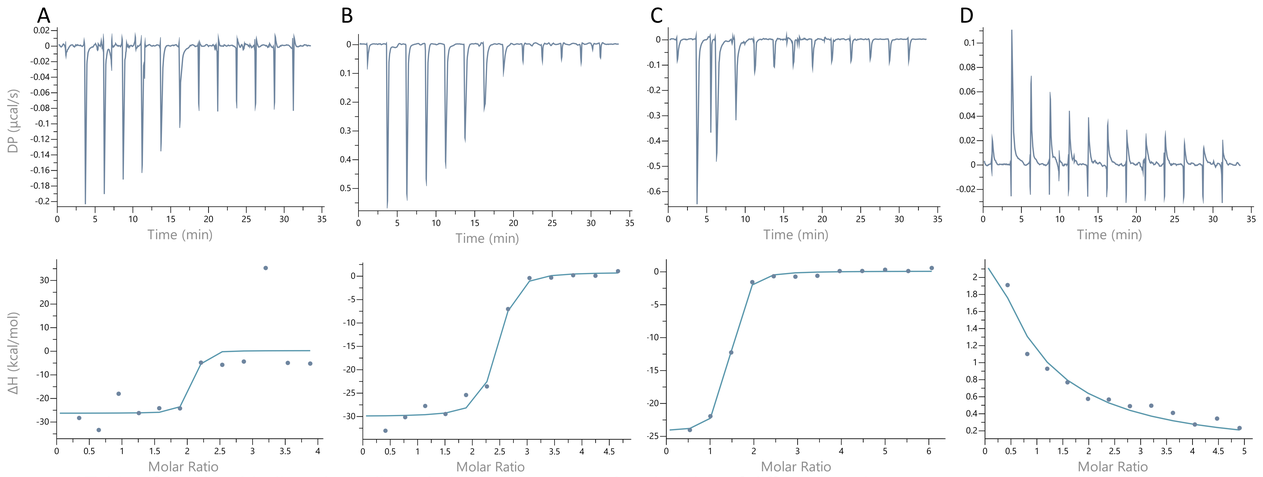


Table of number binding sites, dissociation constants, and enthalpies of different nuclease constructs when titrated with either its antitoxin EsaG or different double stranded DNA sequences.

Sample (Cell)	Ligand (Syringe)	N (sites)	KD	ΔH (kcal/mol)
EsaD 472-614	EsaG	1.87 ± 0.27	1.4 nM ± 1.0 nM	-24.3 ± 6.09
EsaD472-614 H528A	EsaG	2.23 ± 0.03	16.8 nM ± 8.6 nM	-29.6 ± 1.01
EsaD H528A EsaE	EsaG	1.00 ± 0.03	18.8 ±15.3 nM	-21.8 ± 0.88
EsaD 472-614	8 bp Palindrome DNA*	**	uM	Endothermic
EsaD 472-614	Recessed End DNA*	**	uM	Endothermic
EsaD 472-614	18 bp Palindrome*	**	uM	Endothermic

*Not run in triplicate

** Data obtained is inadequate for accurate determination of apparent N sites, KD, and ΔH

Key Results

- ITC binding analysis of EsaD-EsaG displays exothermic, high nM affinity binding.
- EsaD-DNA binding assessment revealed a lower endothermic, uM affinity.
- X-ray crystallographic structural characterization of EsaD 472-614 EsaG complex shows a unique binding mode with disordering of the catalytic region

Reference / Bibliography

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2. Ohr, R. J.; Anderson, M.; Shi, M.; Schneewind, O.; Missiakas, D. EssD, a Nuclease Effector of the *Staphylococcus aureus* ESS Pathway. *J. Bacteriol.* **2017**, *199* (1). https://doi.org/10.1128/JB.00528-16.
3. Burts, M. L.; DeDent, A. C.; Missiakas, D. M. EsaC Substrate for the ESAT-6 Secretion Pathway and Its Role in Persistent Infections of *Staphylococcus aureus*. *Mol. Microbiol.* **2008**, *69* (3), 736–746. https://doi.org/10.1111/j.1365-2958.2008.06324.x.

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