Towards the Discovery of New Antimicrobials: the Bifunctional Penicillin Binding Proteins

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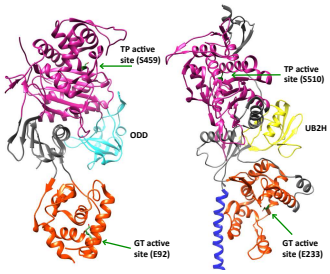
WARWICK

INTEGRATE
ANTIMICROBIAL RESISTANCE

Structure and function of PBPs

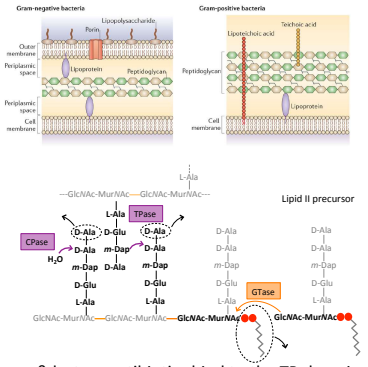
The major component of the bacterial cell wall is a cross-linked glycopeptide polymer called peptidoglycan (PG). The PG layer is an important antimicrobial target, due to its essentiality and uniqueness to bacteria [1].



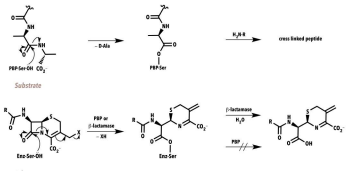
PBP1a
Acinetobacter baumannii
(PDB id: 3UDX)

PBP1b
Escherichia coli
(PDB id: 3VMA)

The bifunctional penicillin-binding proteins (PBPs) function in the last stage of the PG biosynthetic pathway. Here, the precursor Lipid II (undecaprenyl-pyrophosphoryl-MurNAc(pentapeptide)-GlcNAc) is polymerized and cross-linked into PG by the glycosyltransferase (GT) and transpeptidase (TP) domain, respectively. The TP domain can also catalyze DD-carboxypeptidase reactions, resulting in release of the terminal D-Ala [2].



β-lactam antibiotics bind to the TP domain, causing weakening of the cell wall and eventually bacteria lysis. To escape the action of β-lactams bacteria have evolved resistance mechanisms, for instance the the production of β-lactamases in Gram-negative bacteria [3].



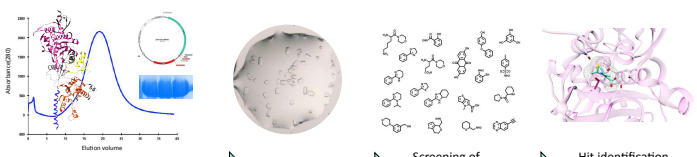
Aim of the project

Structural and kinetic characterization of bifunctional PBPs in Gram-negative bacteria, with a primary focus on *Pseudomonas aeruginosa (Pa)* and *Acinetobacter baumannii (Ab),* two pathogens responsible for the majority of nosocomial infections and with reported increasing multi-drug resistance [4].

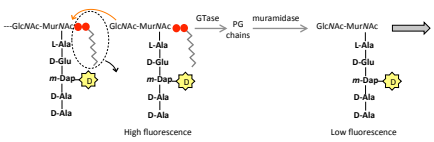
This research will provide *in vitro* data that will help better elucidate the mechanistic basis of *in vivo* catalyzed reactions and regulation of PBPs and, ultimately, guide the optimization of validated screening assays aimed at discovering new PBP inhibitors.

Methods

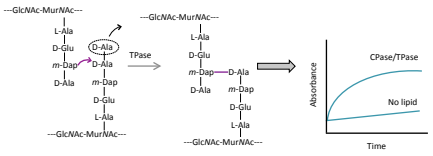
Fragment-based screening by protein crystallography



In vitro PG synthesis assays

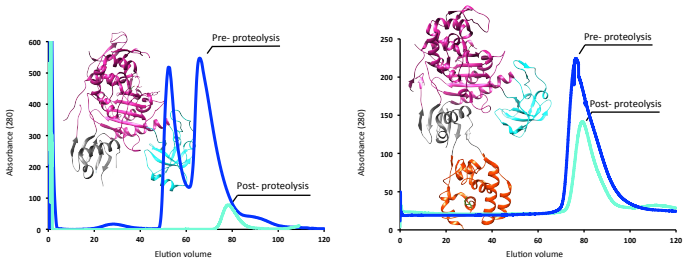


Continuous GTase assay with dansylated Lipid II substrate [5].

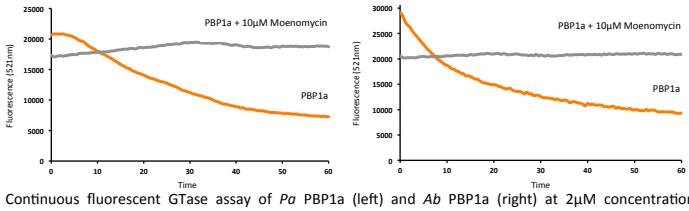


Continuous CPase/TPase assay with Lipid II *m*-DAP substrate developed by Dr. Adrian J. Lloyd.

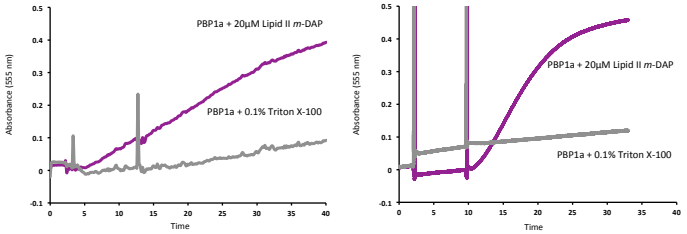
Results



Purification of *Pa* PBP1a (left, PDB id: 4OON) and *Ab* PBP1a (right, PDB id: 3UDX). Crystallization of these proteins requires a poorly controlled proteolysis step [6-7].



Continuous fluorescent GTase assay of *Pa* PBP1a (left) and *Ab* PBP1a (right) at 2μM concentration. Moenomycin is a nM affinity inhibitor of the GT domain, here used as a control.



Continuous spectrophotometric TPase assay of *Pa* PBP1a (left) and *Ab* PBP1a (right) at 3μM concentration. 0.1% Triton X-100 is the detergent used to solubilize Lipid II *m*-DAP, here used as a control.

Conclusions and future work

The published crystallization conditions for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* PBP1a have been tried, but no crystals have grown so far. Next, crystallization attempts will be focused on the GT domain of these proteins.

The soluble constructs of PBP1a from *Pseudomonas aeruginosa* and *Acinetobacter baumannii* have shown to be active in both the GTase and TPase assays.
Next, the full-length versions of PBP1a will be investigated, and whether or not the presence of the transmembrane helix affects the activity of PBP1a *in vitro* will be evaluated.

References

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Acknowledgements

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MIBTP Midlands Integrative Biosciences Training Partnership