Multi-targeting of tRNA synthetases: A paradigm shift in combating antimicrobial resistance

Ricky Cain[1], Ramya Salimraj[1], Dom Bellini[1], Colin Fishwick[2], Lloyd Czaplewski[3], Christopher Dowson[1], Adrian Lloyd[1], David Roper[1]

[1] School of Life Sciences, University of Warwick, Coventry, CV4 7AL. [2] School of Chemistry, University of Leeds, Leeds, LS2 9JT. [3] Chemical Biology Ventures Limited, Abingdon, OX14 1XD.

WARWICK

INTEGRATE ANTIMICROBIAL RESISTANCE

Background and Aims

- Aminoacyl tRNA synthetases (aaRSs) catalyse the attachment of amino acids to their cognate tRNAs (Fig. 1).
- This process is essential for protein synthesis and hence cell survival making aaRSs good antibacterial targets.
- Mupirocin is the only aaRS inhibitor in clinical use to date.
- Aminoacyl sulfamoyl adenosines (aaSAs) are known potent inhibitors of aaRSs which mimic the adenylate intermediate.
- However these compounds are not viable drugs due to their lack of selectivity for bacterial enzymes over the human enzyme.

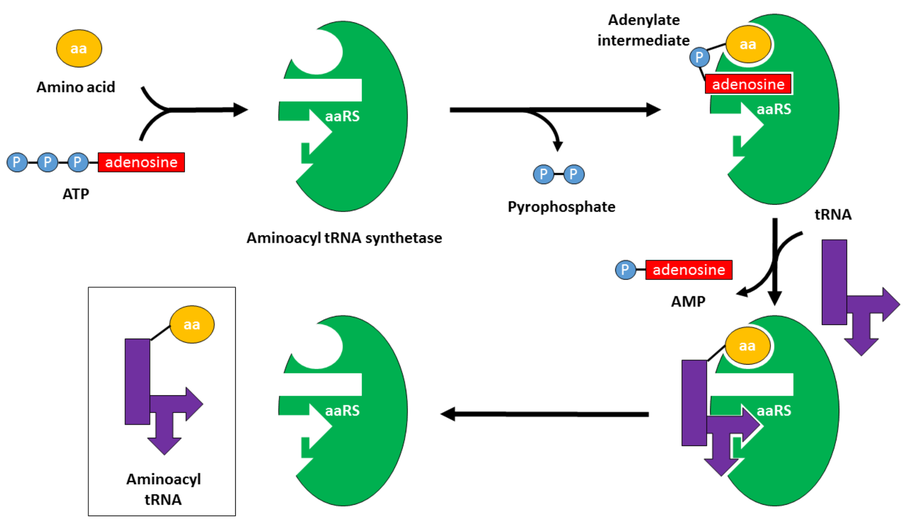

Figure 1: Catalytic mechanism of aminoacyl tRNA synthetases. An amino acid and ATP molecule bind the enzyme forming an adenylate intermediate with the release of pyrophosphate. Subsequently tRNA binds and covalent attachment of the amino acid occurs with release of AMP. The charged tRNA is then released by the enzyme.

This project aims to use structure-based drug design for the chemical modification of aminoacyl sulfamoyl adenosines to achieve selectivity for bacterial tRNA-synthetases over the human enzymes.

Crystallisation of aaRSs

- A limited number of structures exist for aaRSs from clinically relevant bacteria.
- Several aaRSs from such sources were cloned and overexpressed (Fig. 2A).
- A selection of these were purified and crystallised in the presence of aaSA (Fig. 2B).
- Affinity conferring interactions and space for the optimisation of inhibitors were identified (Fig 2C).
- Such structures also gave insight into how aaRSs can bind non-cognate amino acids (Fig. 3).

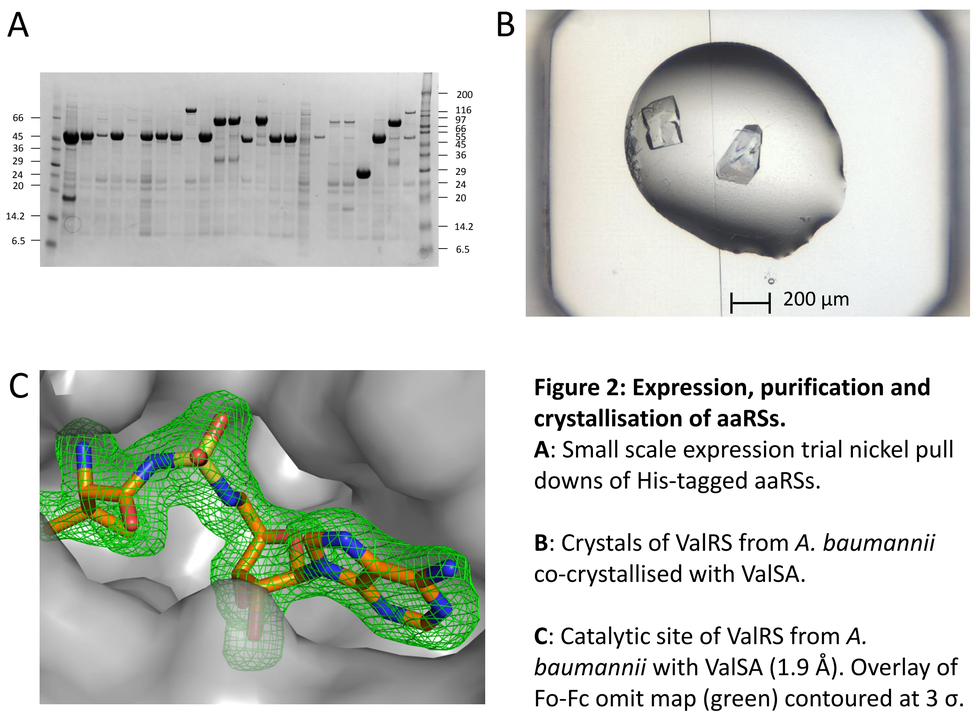

Figure 2: Expression, purification and crystallisation of aaRSs.
A: Small scale expression trial nickel pull downs of His-tagged aaRSs.

B: Crystals of ValRS from *A. baumannii* co-crystallised with ValSA.

C: Catalytic site of ValRS from *A. baumannii* with ValSA (1.9 Å). Overlay of Fo-Fc omit map (green) contoured at 3 σ.

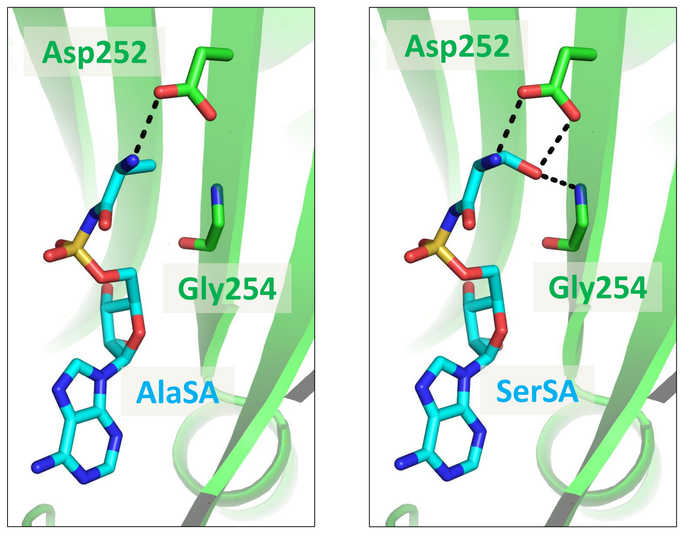

Figure 3: Differences in the interaction of AlaSA and SerSA with AlaRS. Catalytic domain of alanyl tRNA synthetase from *N. meningitidis* (green).
Left: AlaSA (cyan) bound structure (1.2 Å) with alanyl moiety interacting with an aspartic acid.
Right: SerSA (cyan) bound structure (1.1 Å) presents the seryl moiety interacting with an additional glycine residue.

Structure-Based Drug Design

- A computational structural overlay study of bacterial and human SerRS was conducted.
- A region of the structure was identified where selectivity could potentially be achieved.
- A small series of selectivity probes were designed based on the seryl sulfamoyl adenosine (SerSA) structure (Fig. 4).

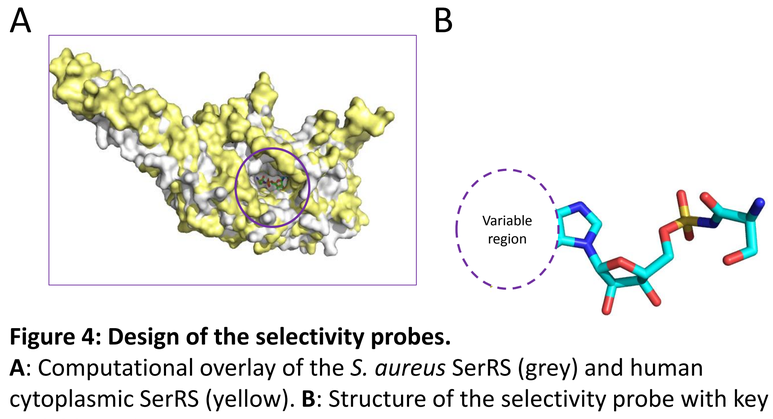

Figure 4: Design of the selectivity probes.
A: Computational overlay of the *S. aureus* SerRS (grey) and human cytoplasmic SerRS (yellow). **B**: Structure of the selectivity probe with key variable region.

- The designed compounds were synthesised.
- Each compound was tested against the *E. coli, S. aureus* and human cytoplasmic SerRS using a phosphate exchange assay (Fig. 5).

Figure 5: Chemical reactions of the phosphate exchange assay.

- Cleavage of Ap4A by an amino acid substrate and pyrophosphate yields two molecules of ATP.
- Production of ATP is continuously monitored by coupling to $NADP^+$ reduction at 340 nm.

Table 1: IC_{50} determination of SerSA and derivatives against SerRS enzymes.

	IC_{50} (µM)		
	E. coli SerRS	**S. aureus SerRS**	**Human Cytoplasmic SerRS**
SerSA	0.214 ± 0.0298	0.225 ± 0.490	2.17 ± 0.210
1	6.65 ± 0.643	6.34 ± 0.710	>1000 ± >100
2	17.7 ± 1.42	52.7 ± 4.81	>1000 ± >100

- Biological evaluation of the compounds reveals >100 fold selectivity for the bacterial enzymes over the human enzyme (Table 1).
- Crystallography is underway to determine the binding modes of the inhibitors.

Conclusions

- Crystal structures revealed differences in the catalytic binding pocket of SerRS from bacterial and human sources.
- Derivatives of the seryl sulfamoyl adenosine inhibitor were designed to target only the bacterial enzymes.
- Kinetic studies revealed >100-fold selectivity was achieved for the bacterial proteins over the human.

Acknowledgements

This work was funded by the Medical Research Council under Tackling AMR Theme 1: Understanding resistant bacteria in context of the host via an Innovation Grant and EPSRC Bridging the Gaps.

WARWICK THE UNIVERSITY OF WARWICK

EPSRC Engineering and Physical Sciences Research Council

MRC Medical Research Council

AMR Tackling Antimicrobial Resistance

CPPF-UK