Tackling antibiotic resistance by blocking signalling pathways

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1. Context

- BceRS is a two-component system (TCS) in *Bacillus subtilis*.
- BceRS enables *B. subtilis* to respond in the presence of bacitracin, a cell wall acting antibiotic.
- BceS is a histidine kinase and forms a key part of this signalling pathway.

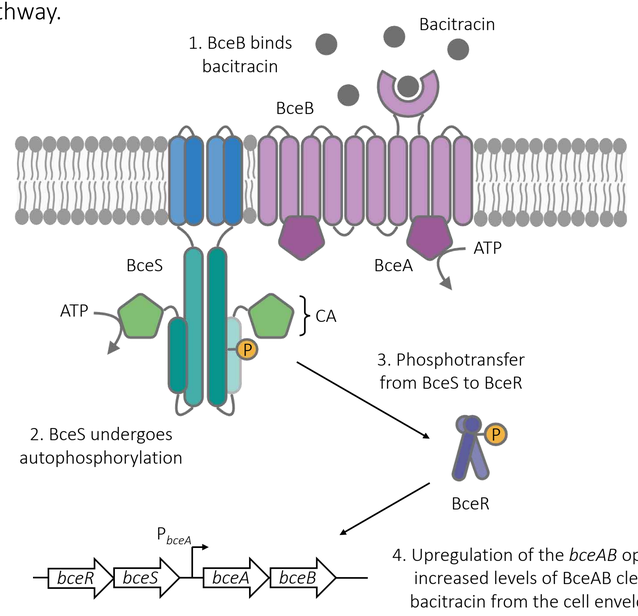


Figure 1: Schematic of the typical signalling pathway of the BceRS two-component system and its cognate transporter BceAB.[1]

2. Project Approach

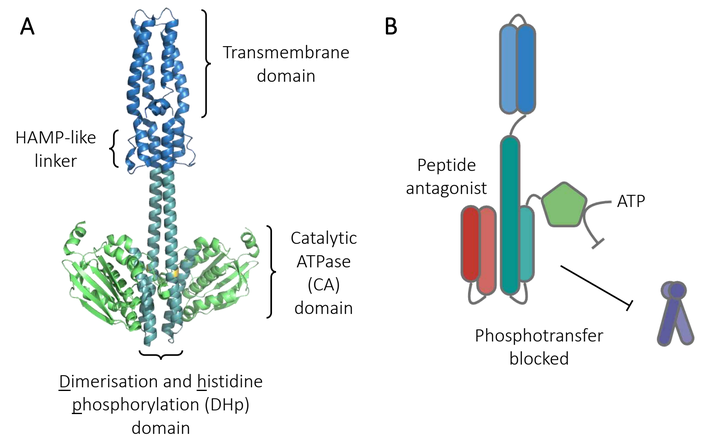


*Figure 2: **A)** Cryo-EM structure of BceS showing the domain architecture (PDB: 8G3A).[2] **B)** Schematic showing the project approach: the use of a peptide antagonist to block signalling through the BceRS pathway by disrupting BceS dimerisation.*

AIM: To disrupt BceS dimerisation and block BceRS signalling using a peptide antagonist, rendering *B. subtilis* unable to respond to bacitracin.

WHY: This constitutes a novel means of tackling AMR with reduced evolutionary pressure to develop resistance and leverages the sequence specificity of histidine kinase dimerisation domains.

IMPACT: There is the potential to translate this approach to other two-component systems in clinically relevant pathogens.

3. Screening Methodology

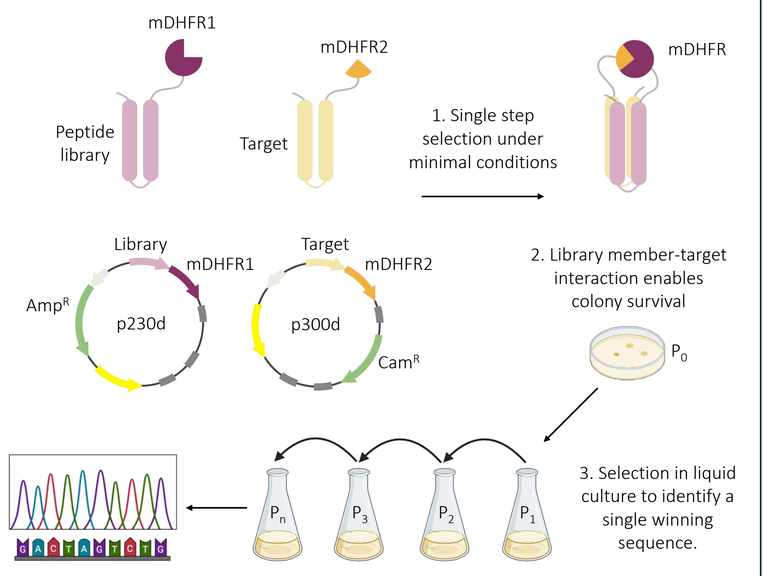
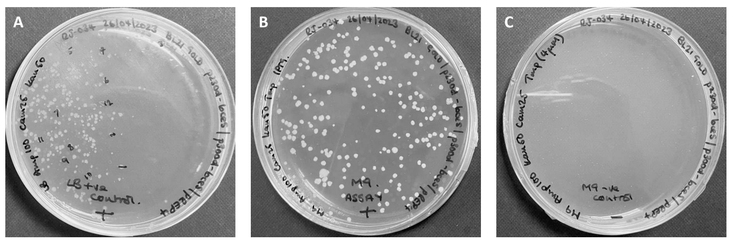


Figure 3: Schematic showing the protein-fragment complementation assay used to screen peptide libraries.

- Peptide libraries were screened using an **intracellular protein-fragment complementation assay** (PCA) based on murine dihydrofolate reductase (mDHFR).
- This methodology selects the strongest binder whilst also **profiling for biostability, solubility and selectivity**.[3,4]

4. Library Generation & Screening

- A DHp homodimerisation experiment showed colony survival under minimal conditions only in the presence of IPTG.
- This suggests the **BceS DHp** domain can **homodimerise** *in vivo* and is **amenable to PCA**.



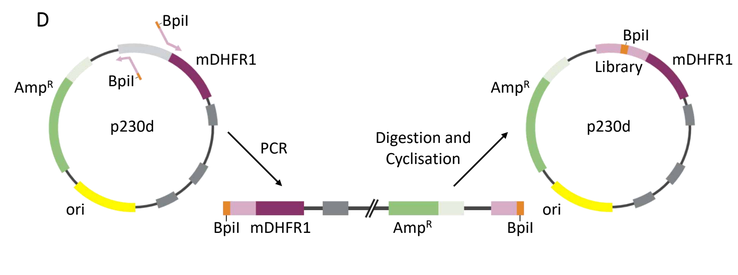


*Figure 4: Plate image from the PCA BceS DHp homodimerisation experiment. **A)** Positive control lacking trimethoprim. **B)** Assay conditions containing trimethoprim and IPTG. **C)** Negative control showing no growth in the absence of IPTG. **D)** Schematic describing library generation using whole plasmid PCR followed by restriction enzyme digest and intramolecular circularisation*

Initial *adg* Library

	1	2	3	4		5	6	7	8
Heptad:	*bcdef*	*gabcdef*	*gabcdef*	*gabc*	*ef*	*gabcdef*	*gabcdef*	*gabcdef*	*gab*
adg Lib.:	DELMA	WIHEVKT	PLTAMHL	IIDRMEDKA		LKSQLSY	EWLRIHL	LLDQQLH	QKR
	I	LN I	LI L	LN		II I	VL L	IQ L	L
		S L	Q	K			Q	Q	
				Q			L	K	
adg Hit:	DELMA	WIHEVKT	PLTALHL	IIDRMEDKA		IKSQLSY	LWLRIHL	LLDQLLH	LKR

- An initial **14.1-million-member library** containing randomisation at the ***a***, ***d*** and ***g*** positions was generated using whole plasmid PCR.[5]
- A single winning sequence was identified but exhibited **poor solubility**.

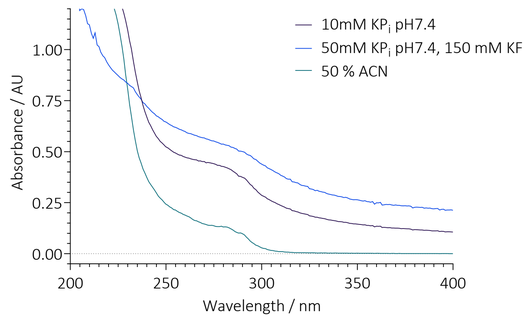


*Figure 5: Permutations included in the initial **adg** peptide library and the selected hit. This hit was synthesised with an additional C-terminal TAT sequence for cell permeability, but poor solubility was observed, as shown by the elevated baseline by UV-vis at 10 µM.*

Solubility Library

	1	2	3	4		5	6	7	8
Heptad:	*bcdef*	*gabcdef*	*gabcdef*	*gabc*	*ef*	*gabcdef*	*gabcdef*	*gabcdef*	*gab*
Sol Lib.:	DELMA	WIHEVKT	PLTALHL	IIDRMEDKA		IKSQLSY	LWLRIHL	LLDQLLH	LKR
	E	E R	K K	R E		R	H R	R E	R
	N	K Q	N R	N			N	N Q	
	K	T		K				K	
Sol2:	KELMA	WIQEVKK	PLKALHL	IIDRMEDKA		IKSQLSY	LWRRIHR	LLDQLLR	LKR
Sol3:	DELME	WIREVKT	PLTALHR	IINRMEDKA		IKRQLSY	LWLRIHR	LLEQLLR	LKR

- Next, an additional ~1-million-member **solubility library** was incorporated into the initial hit sequence.
- Two sequences showed an **increase** in the **number of hydrophilic residues** and gave a **strong helical response** when analysed by CD.

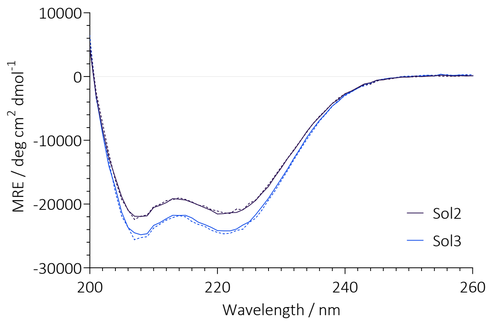


*Figure 6: Permutations included in the solubility library and the selected hits. CD analysis of the two solubility library peptides at 50 µM in 10 mM HEPES pH 7.4, 50 mM NaCl prior to (solid lines), and following (dashed lines), a 1-95 **°C melt**.*

5. Target Expression

A truncated **BceS construct** (Q97-V334) containing the **DHp and CA domains** was recombinantly expressed and purified for use in biophysical characterisation. It was shown to **exist as a homodimer**.

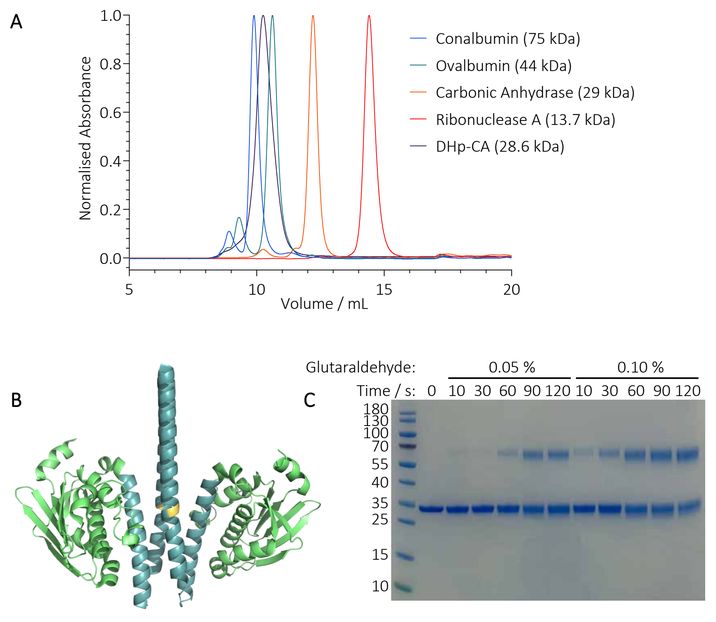
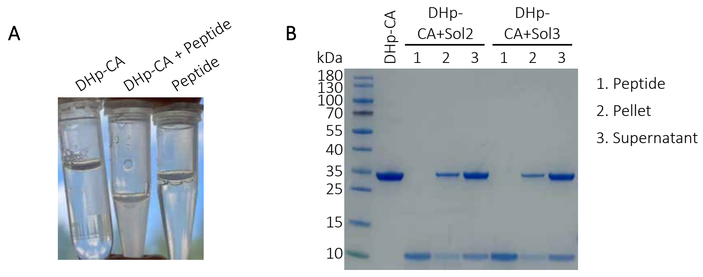


*Figure 7: **A)** Size exclusion chromatogram showing the DHp-CA target construct exists as a homodimer in solution. **B)** Predicted structure of the DHp-CA construct.[2] **C)** SDS-PAGE gel shows time and concentration dependent crosslinking of BceS Q97-V334 with glutaraldehyde, consistent with dimer formation.*

6. Binding Characterisation

- An equimolar combination of the DHp-CA target with a solubility library hit resulted in precipitation.



- Analysis of the supernatant showed the presence of a shoulder, potentially indicating disruption of homodimerisation.

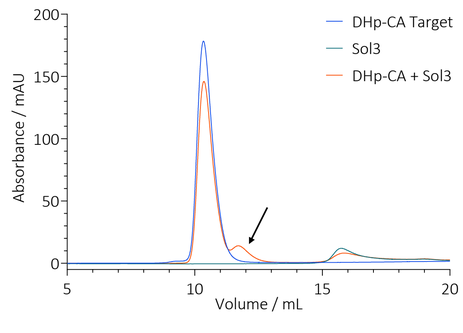


*Figure 8: **A)** Image showing the presence of precipitation having combined a PCA hit with the target. **B)** SDS-PAGE showing the composition of the pellet and supernatant following precipitation. **C)** Size exclusion chromatogram showing the presence of a shoulder, suggesting the formation of a complex of lower MW.*

7. Conclusions & Next Steps

- Two peptide libraries have been screened and hits identified.
- When combined with the target, precipitation was observed, but SEC analysis of the supernatant showed the presence of a shoulder.
- The composition of this shoulder should be confirmed.
- Further modification of the peptides, such as downsizing may facilitate easier handling.
- Investigate alternative buffer conditions to stabilise a potential antagonist-target complex.
- Expression of the DHp-CA BceS target construct is soluble, therefore the PCA hits could also be fused to the CA domain to assess binding, should peptide solubility continue to be an issue.

8. Selected References

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