PBP inhibitors discovered using a modified phage display platform (*Bicycles®*)

ABSTRACT#

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ABSTRACT

Cyclic peptides offer significant advantages over linear peptides as ligands for drug targets in terms of both affinity and pharmacokinetic properties. Such moieties are well-represented amongst successful antibiotics but have mostly been discovered from natural sources by laborious screening processes.

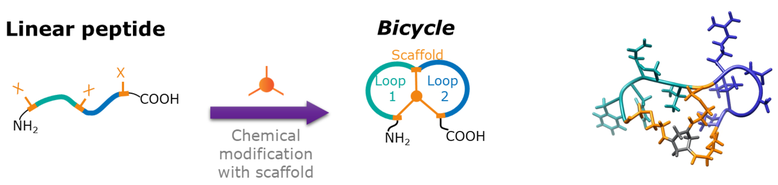
Bicycle Therapeutics' platform for discovery and optimization of bicyclic peptide drug leads has an over 80% success rate of delivering high affinity ligands, from screening over 90 drug targets. Two programmes have progressed to clinical studies.

We present here early studies in application of the platform to antibacterial targets, specifically the discovery of inhibitors of penicillin binding proteins.

BICYCLE PLATFORM

The Bicycle technology was originally developed in the laboratory of Sir Greg Winter, a pioneer of monoclonal antibodies, at the MRC Laboratory of Molecular Biology, Cambridge, UK and has been further developed and commercialized by Bicycle Therapeutics[1].

Bicycles are peptides of typically 8 – 20 amino acids with three strategically-placed cysteine moieties. These residues are reacted with a variety of trivalent scaffolds to form bicyclic structures.



Diversity in the Bicycle libraries comes from:

- The peptide sequence and length
- Positioning of the cysteines to yield symmetrical or asymmetrical Bicycles
- The nature of the scaffolding reagents

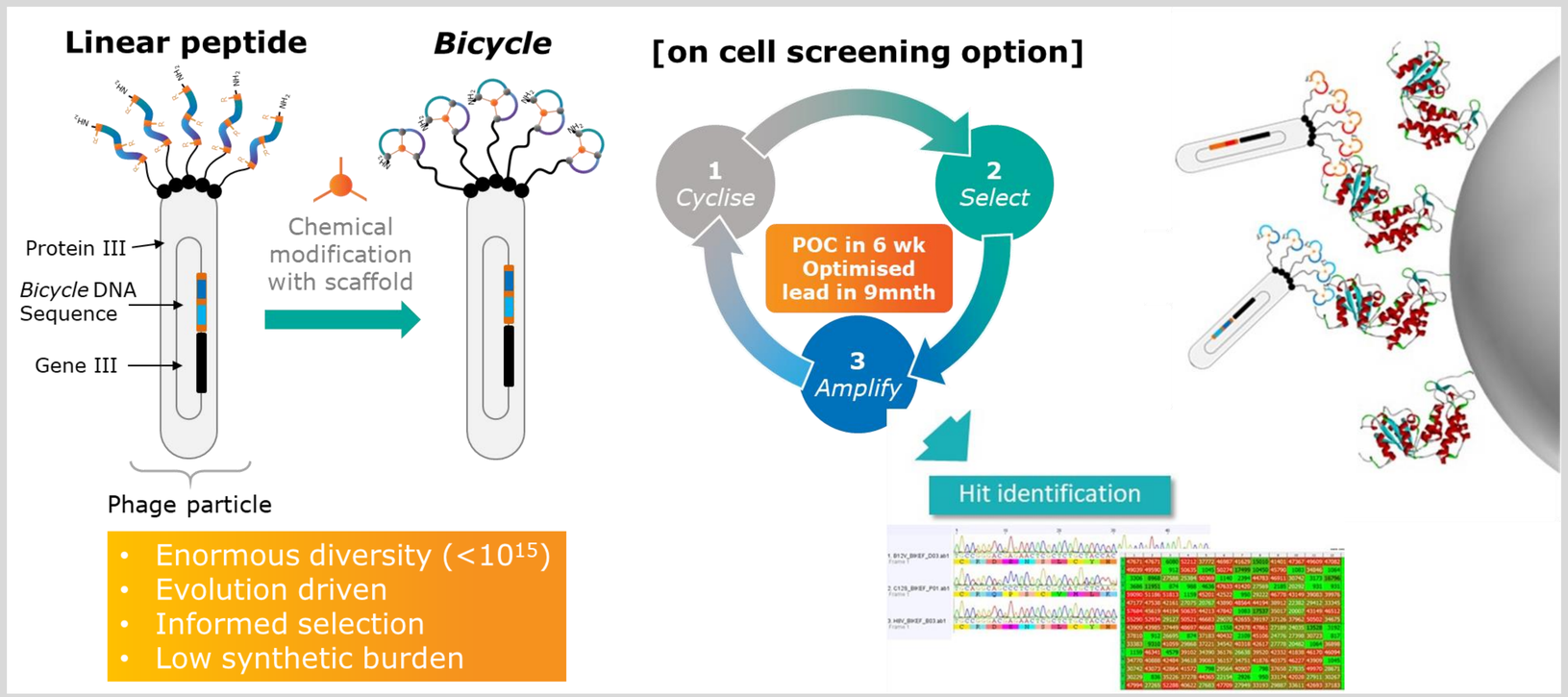
MATERIALS AND METHODS

- Penicillin binding proteins PBP1A from *Streptococcus pneumoniae* and PBP1B from *Escherichia coli* were cloned and expressed by Charles River Laboratories
- The purified proteins were biotinylated on surface lysine residues
- Target protein and phage (10^{12} cfu/ml) were mixed and incubated for 1hr before elution and propagation of binding phage in *E. coli*
- Phage output were sequenced prior to being ranked by AlphaScreen
- Binders were produced by automated solid-phase peptide synthesis
- Binding was quantified by SPR and/or fluorescence polarization
- Transpeptidase binding experiments were carried out by FP competition in the presence of ampicillin
- Transpeptidase inhibition was determined via a coupled enzyme assay, and carried out by Dr Adrian Lloyd

CONCLUSION/SUMMARY

- Bicycles offer a promising new therapeutic modality for antibacterial agents with a highly effective screening and optimisation process
- Promise has been demonstrated by rapid discovery of inhibitors of penicillin binding proteins
- Further maturation of affinity and optimisation of activity is in progress as well as investigation of other targets

Overall schematic of Bicycle platform



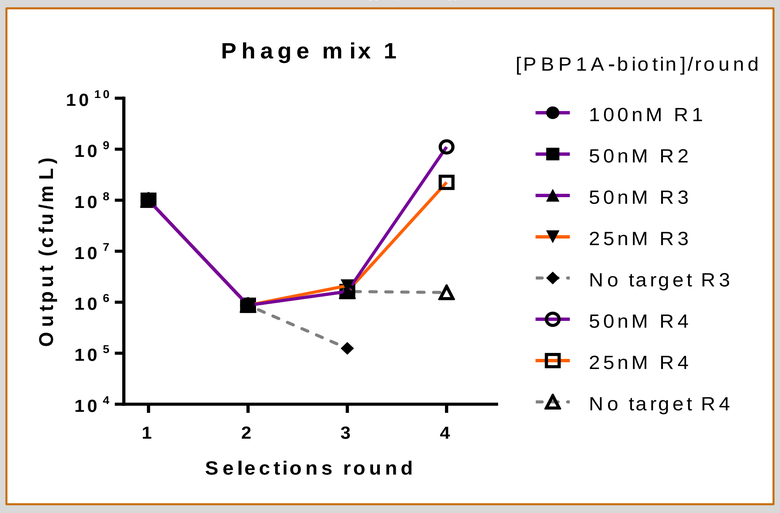
- Enormous diversity (<10^{15})
- Evolution driven
- Informed selection
- Low synthetic burden

Stages in the screening process:

1. Panning of Bicycle libraries against target proteins (or other cell structures)
2. Capture of target with bound phage e.g. using streptavidin beads with biotinylated target
3. Elution and amplification of phage
4. Repeat cycles with increasing stringency to select for tight binders
5. Sequencing of Bicycle 'hits' and ranking of affinity of phage using alpha screen
6. Synthesis of select binders by solid phase peptide synthesis for biochemical studies
7. Affinity maturation: further screening of custom libraries based on initial promising 'hits'
8. Structural studies of Bicycle-target interaction and further optimisation by conventional medicinal chemistry including incorporation of non-proteinogenic amino acids and/or non-peptidic fragments

PBP1A – *Streptococcus pneumoniae*

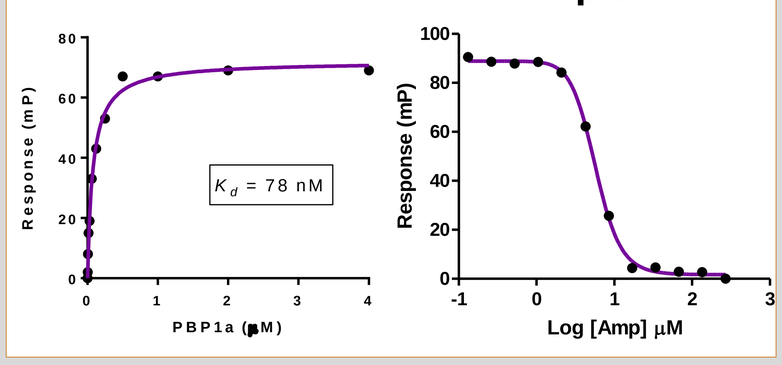
- Phage titre increases after four cycles of panning due to enrichment for binding phage



- Bicycles sequenced and affinity ranked by AlphaScreen
- Strong hits identified from 9 out of 26 libraries screened
- Selected Bicycles synthesised for further testing

Fluorescence polarisation assay:

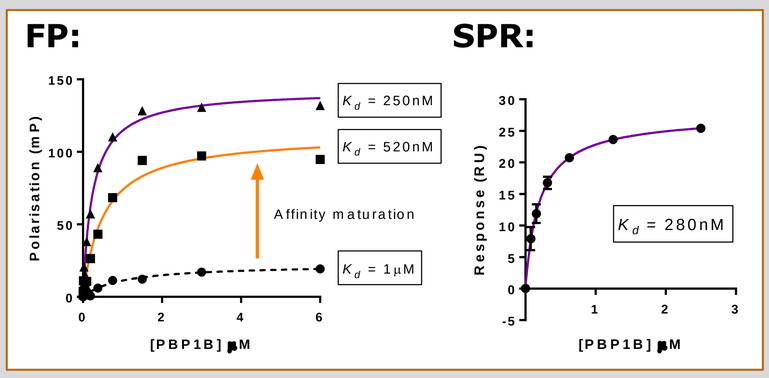
a) Direct binding **b) Competition with ampicillin**



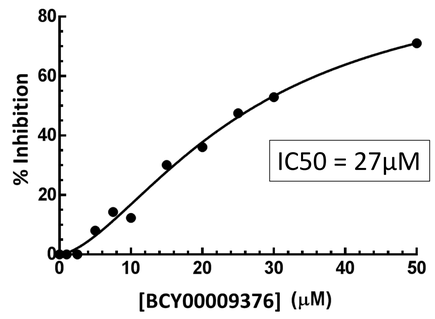
- Inhibition of peptide binding to PBP1a in the presence of ampicillin suggests binding to transpeptidase domain

PBP1B – *Escherichia coli*

- Bicycles identified with sub-μM binding affinities by fluorescence polarisation and surface plasmon resonance
- Affinity enhanced by affinity maturation on phage



Determination of an IC50 using a transpeptidase inhibition assay



- Bicycle shown to inhibit transpeptidase activity using lipid II substrate in coupled enzyme assay[2]

ACKNOWLEDGEMENTS

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REFERENCES

1. Heinis C, Rutherford T, Freund S & Winter G (2009) Nature Chemical Biology **5**, 502 – 507.
2. Transpeptidase assay

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