# Anti-Infectives Drug Discovery at Bicycle Therapeutics

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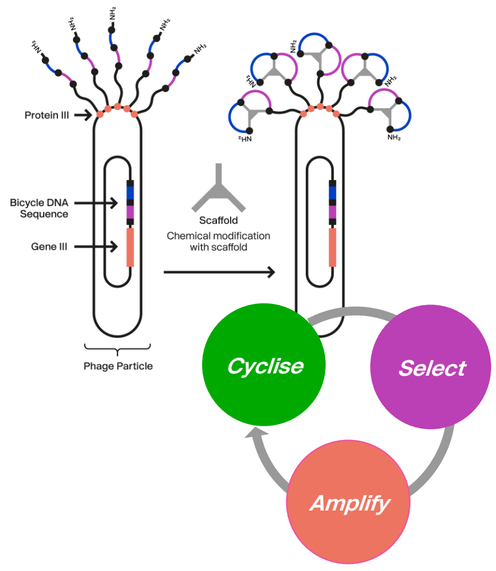
## Bicycle's Discovery Platform

*Bicycles* are formed by constraining short linear peptides into a stabilised bi-cyclic structure using a central chemical scaffold. They are discovered using the company's phage display platform which efficiently produces vast, diverse libraries which have identified hits for numerous target proteins, some of which have proved intractable to other modalities. Key features of the platform are described below

**Phage Display ▶**
Short (<20 aa) peptides are presented on the PIII protein of M13 phage:

1. The 3 cysteines of the peptide are cyclised by formation of 3 thioether bonds to a small molecule "scaffold"
2. Purified target protein is used to pull down and select bi-cyclic peptides with target affinity.
3. Selected phage are used to re-infect and amplify binding peptides
4. High affinity phage are sequenced and the corresponding *Bicycle* is chemically synthesised and tested

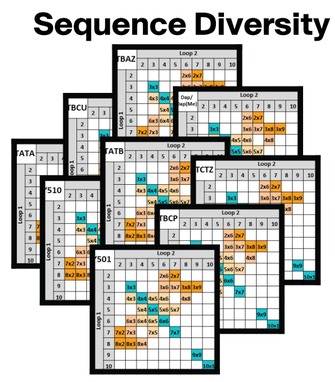
This process takes just 6 weeks, rapidly generating proof of concept molecules

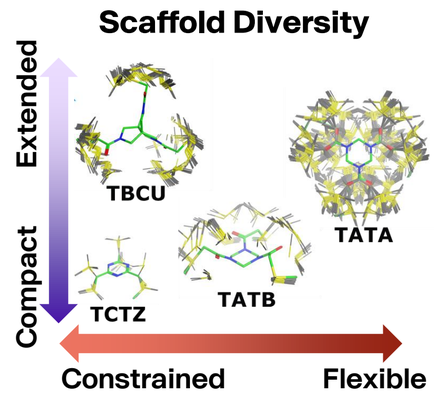



**Diversity ▶**
Diversity is derived from:

- Varying the sequence
- Varying the loop sizes
- Varying the loop symmetry
- Varying the chemical scaffold

The resultant diversity is a library of >$10^{20}$ unique *Bicycles*



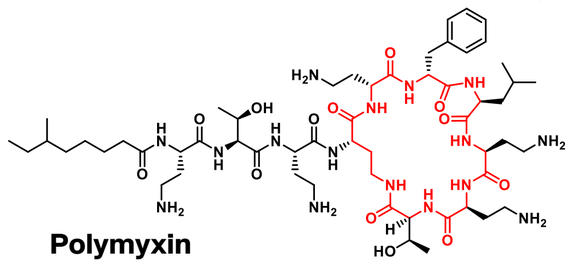




## Natural product-like cyclic peptide discovery

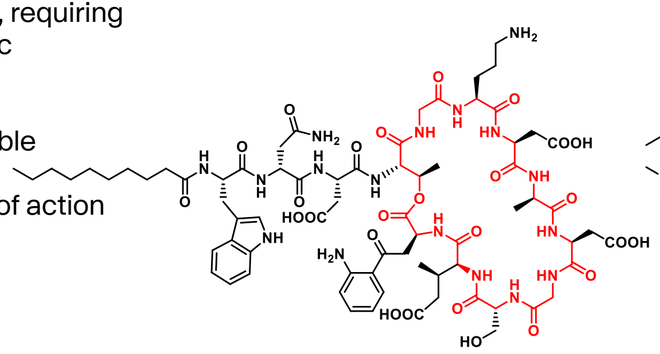
The failure of high-throughput screening of small molecule libraries to deliver new chemical matter in the antibiotic space is well documented. The majority of known antibiotics do not fit the traditional 'Lipinski chemical space' occupied by most small molecule drugs. The huge diversity (>$10^{20}$) offered by the Bicycle phage platform can generate molecules within the correct chemical space, which we believe have the potential to address the crisis in AMR

**Cyclic Peptide Natural Products ▶**
Bacteria produce various cyclic peptides as antibiotics, some of which are used as drugs.
However natural product cyclic peptides suffer from **several challenges**:

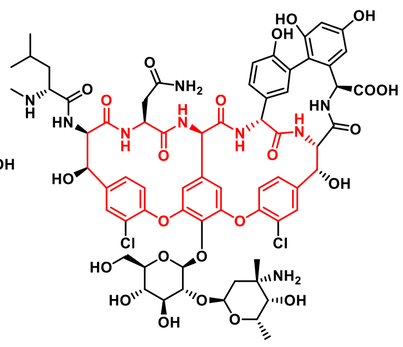
- Their synthesis is difficult, requiring fermentation or enzymatic modification
- This increases cost
- And often limits the possible derivations
- Establishing mechanism of action can be challenging



**Polymyxin**



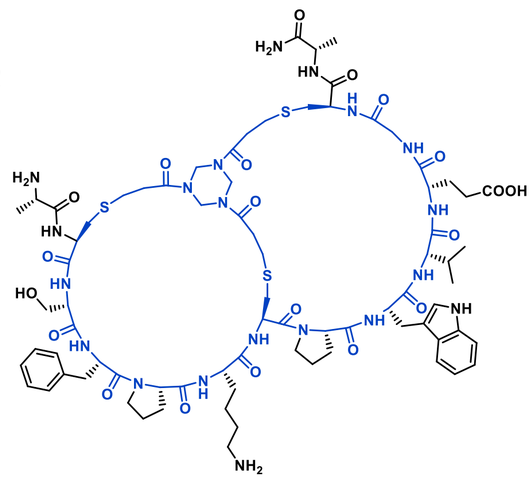
**Daptomycin**

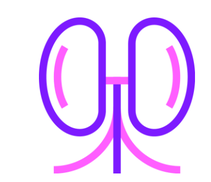


**Vancomycin**

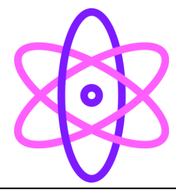
***Bicycles* ▶**

- Target-based discovery
- Huge diversity (>$10^{20}$)
- Chemically synthesised
- Established pathway to optimise for affinity and pharmacokinetics properties
- High (>80%) hit rate across diverse target classes:
- Can be used to find binders to low "ligandability" targets

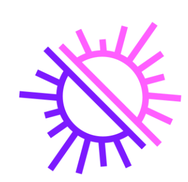




Renal elimination, potentially minimizing toxicological burden on liver and gut



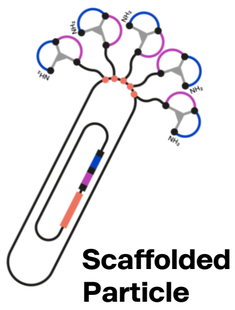
Moderate molecular weight (1.5-2.5kDa), delivering rapid tissue penetration and tuneable PK



Ability to multimerize together or conjugate to a range of therapeutic payloads

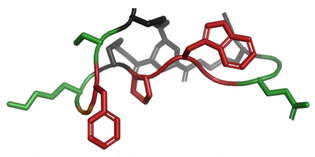
## Discovery workflow

### Phage Screening

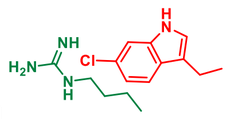
**Scaffolded Phage Particle**

### Affinity Optimisation

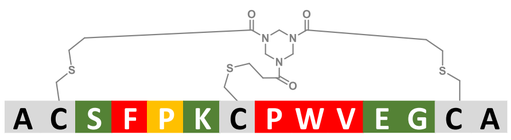


**Crystallography**

**Incorporate non-natural amino acids**



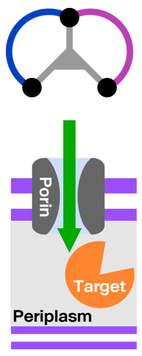
**Ala-scan**




▲ Easy synthesis enables Medicinal Chemistry cycles to optimise binding affinity

### Demonstrate antimicrobial activity

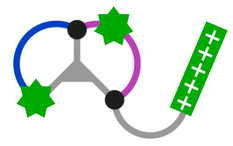

▲ "Naked" *Bicycles* cannot enter cells




▲ Porinated cells are used to demonstrate bacterial killing

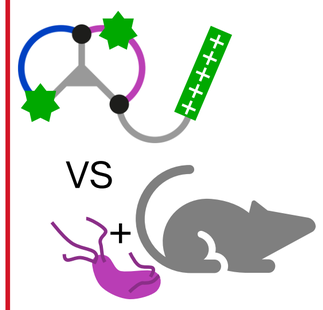

▲ *Bicycles* "conjugated" to vector molecule to enter and kill bacteria

### Stabilisation + optimisation of PK



▲ *Bicycle* conjugates given matrix stability by unnatural amino acids and modified vectors

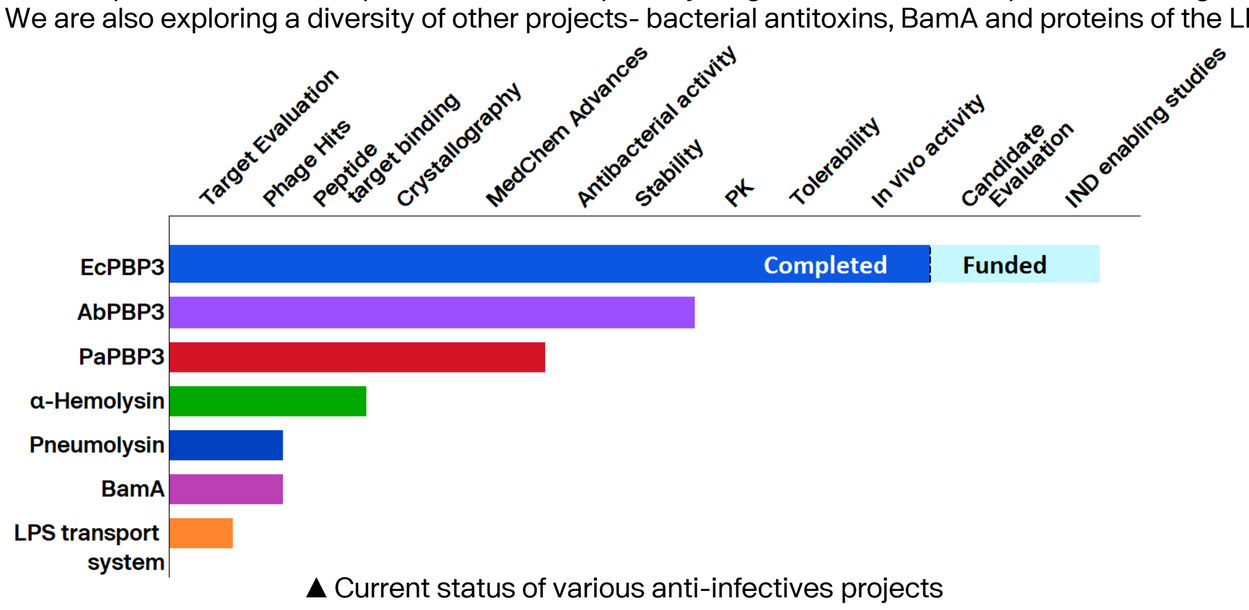
### *In vivo* Efficacy




▲ *Bicycle* conjugates show efficacy in standard mouse infection models

## Current Anti-infectives work at Bicycle

Currently our lead project is the *E. coli* PBP3 program. This is funded by an Innovate UK biomedical catalyst grant, with the goal of identifying an advanced molecule with a profile suitable for-IND filing. The lead compound has been shown to be efficacious in early *in vivo* mouse model studies (see Nik Bournakas' poster). This project has shown the initial potential for *Bicycles to be used* as antimicrobials.
Building on the success of this project, we have brought forward compounds targeting PBP3s from *A. baumannii* and *P. aeruginosa.* The high specificity of the *Bicycle*: protein interaction provides narrow spectrum to the compounds, with a unique *Bicycle* generated for each species. Work *against A. baumannii* is progressing well, with *in vitro* demonstration of antibacterial activity.
We are also exploring a diversity of other projects- bacterial antitoxins, BamA and proteins of the LPS transport system.




▲ Current status of various anti-infectives projects

## Summary

Bicycle Therapeutics is committed to drug discovery in the anti-infectives space. Using grant funding, academic collaboration and a dedicated team in-house, we have validated the phage-display platform as a tool for discovering antibiotic-like molecules. Our *E. coli* PBP3 program is advancing in to *in vivo* efficacy models and appears promising.

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