### 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.

#### 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 (>10^3) 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.

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**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 a mechanism of action can be challenging.

**Bicycles**

- Target-based discovery.
- Huge diversity (>10^3).
- Chemically synthesised.
- Established pathway to optimise for affinity and pharmacokinetics.
- High (~80%) hit rate across diverse target classes.
- Can be used to find binders to low "ligandability" targets.

**Polymyxin**

- Renal elimination.
- Potentially minimising toxicological burden on liver and gut.
- Moderate molecular weight (1.5-2.5kDa), delivering rapid tissue penetration and tunable "PK.

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**Discovery workflow**

**Phage Screening**

- Target
- Crystallisation
- Incorporate non-natural amino acids

**Affinity Optimisation**

- "Naked" Bicycles cannot enter cells
- Porinated cells are used to demonstrate bacterial killing
- Bicycles "conjugated" to vector molecule to enter and kill bacteria

**Demonstrate antimicrobial activity**

- Bicycle conjugates given matrix stability by unnatural amino acids and modified vectors
- Bicycle conjugates show efficacy in standard mouse infection models

**In vivo Efficacy**

- In vivo proof of concept molecules

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**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 spectra 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 antibiotics, BarmA and proteins of the LPS transport system.

**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 vivo efficacy models and appears promising.