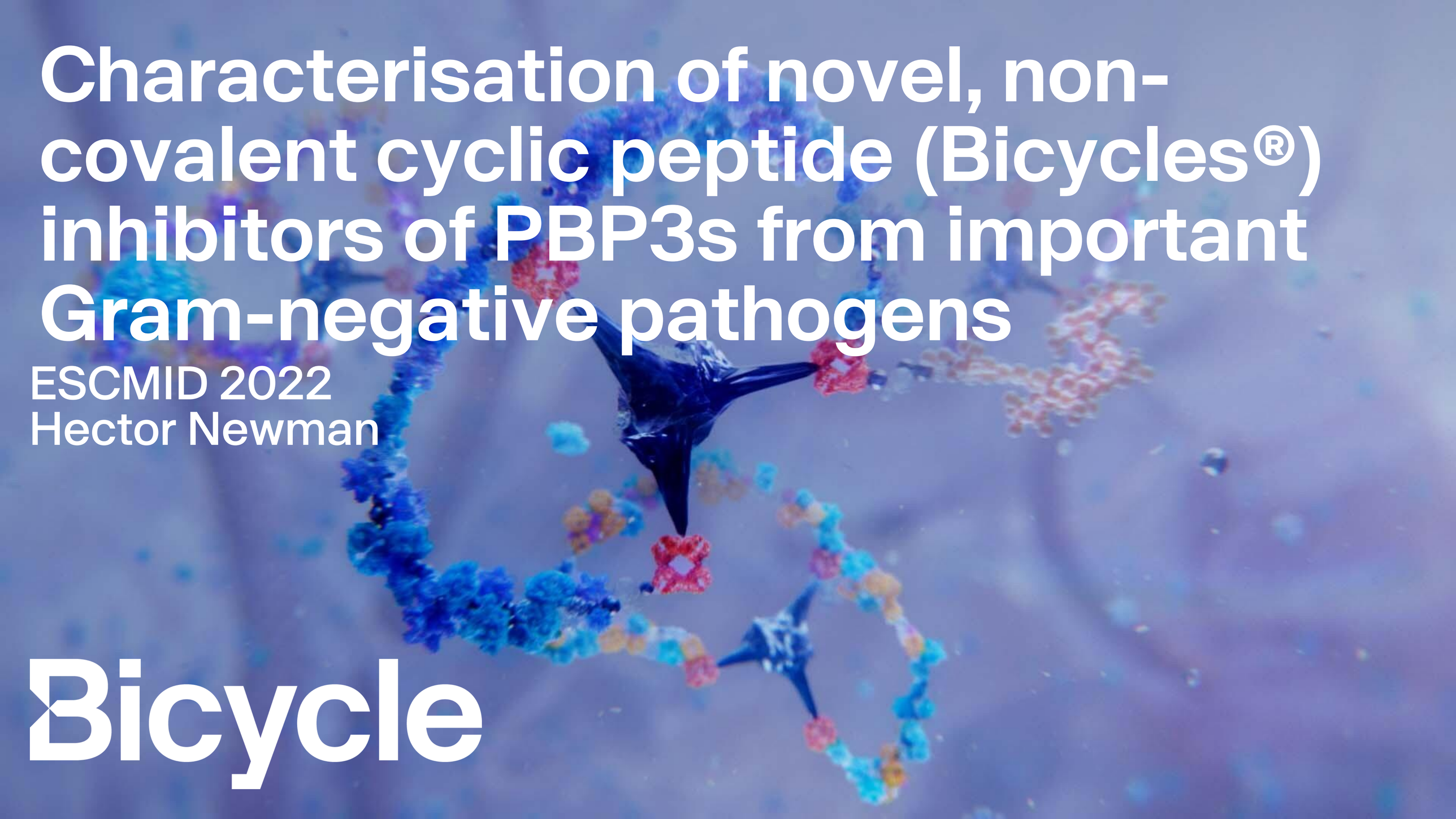
Characterisation of novel, non-covalent cyclic peptide (Bicycles®) inhibitors of PBP3s from important Gram-negative pathogens



ESCMID 2022

Hector Newman

Bicycle

Forward-looking statements

This presentation may contain forward-looking statements made pursuant to the safe harbor provisions of the Private Securities Litigation Reform Act of 1995. These statements may be identified by words such as “aims,” “anticipates,” “believes,” “could,” “estimates,” “expects,” “forecasts”, “goal,” “intends,” “may” “plans,” “possible,” “potential,” “seeks,” “will,” and variations of these words or similar expressions that are intended to identify forward-looking statements. All statements other than statements of historical facts contained in this presentation are forward-looking statements, including statements regarding the breadth of potential therapeutic applications of our platform technology, the potential applicability of Bicycles to targets other than PBP3, our current and prospective product candidates, and the timing and success of our development of our anticipated product candidates.

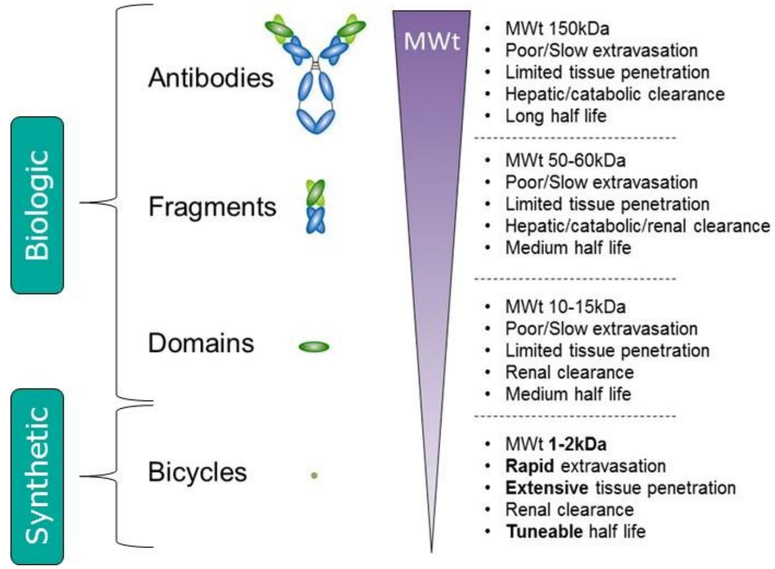
Forward-looking statements are neither historical facts nor assurances of future performance. Instead, they are based on our current beliefs, expectations and assumptions regarding the future of our business, future plans and strategies, our development plans, our preclinical results, our plans to initiate clinical trials, and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in or implied by such forward-looking statements. These risks and uncertainties include, but are not limited to, risks related to the ongoing COVID-19 pandemic, the risk that any one or more of our product candidates will not be successfully developed or commercialized, and the risk that we may not realize the intended benefits of our technology. For a discussion of these and other risks and uncertainties, and other important factors, any of which could cause our actual results to differ from those contained in the forward-looking statements, see the section entitled “Risk Factors” in our Quarterly Report on Form 10-Q, filed with the Securities and Exchange Commission on August 4, 2022, as well as in other filings we may make with the SEC in the future, as well as discussions of potential risks, uncertainties and other important factors in our subsequent filings with the Securities and Exchange Commission. New risks and uncertainties may emerge from time to time, and it is not possible to predict all risks and uncertainties. Except as required by applicable law, we do not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

Bicycle Therapeutics

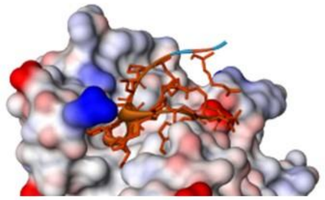
- Drug discovery and development Biopharma

- Unique potential therapeutic modality
  - platform with potential applications across all therapeutic areas

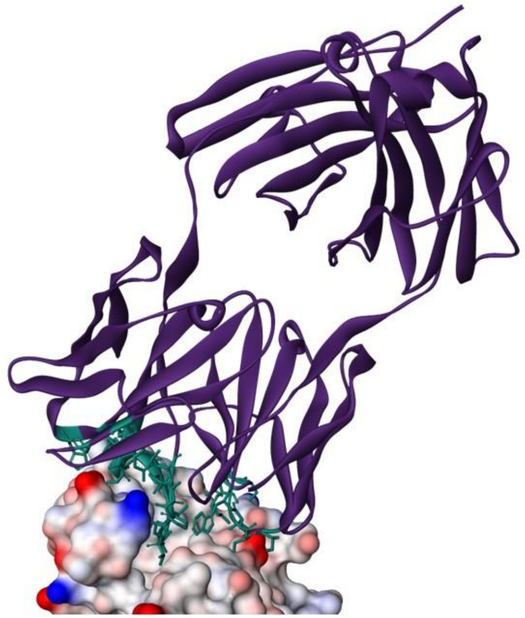
- Five molecules in clinic



	Bicycles	Fab
Weight	2.3kDa	48kDa
Size	19aa	445aa
Binding residues	16aa (85%)	24aa (5%)

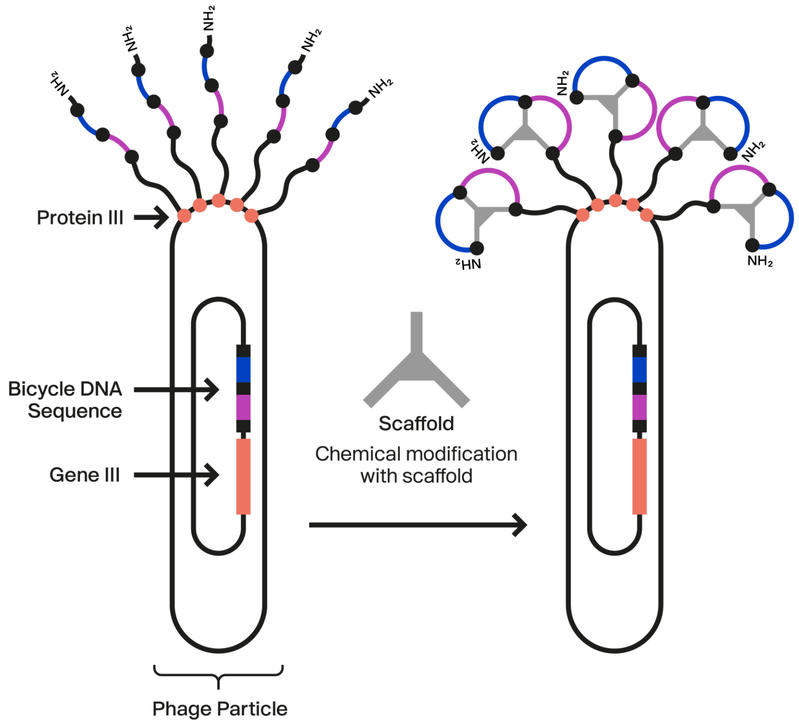


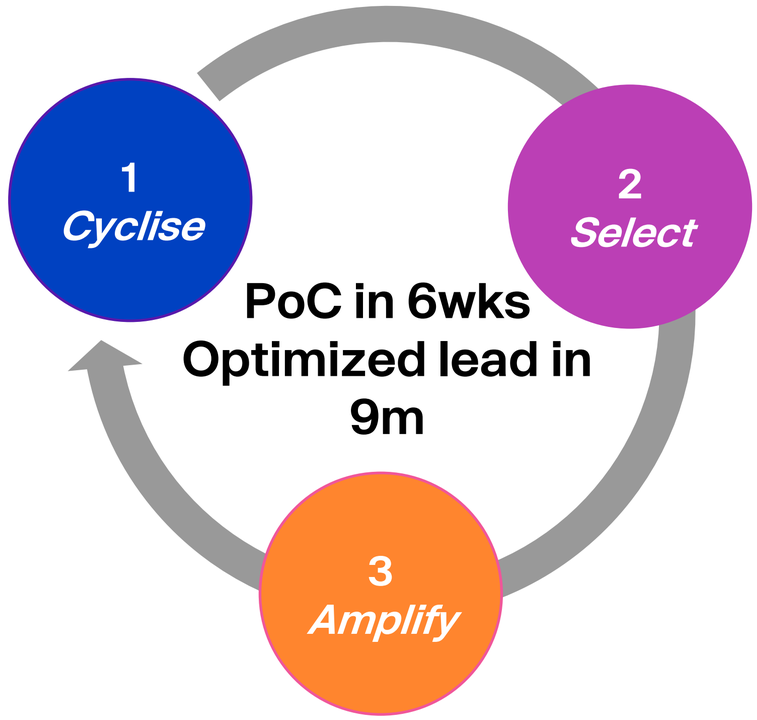
EphA2-binding
Bicycle



EphA2-binding
Fab

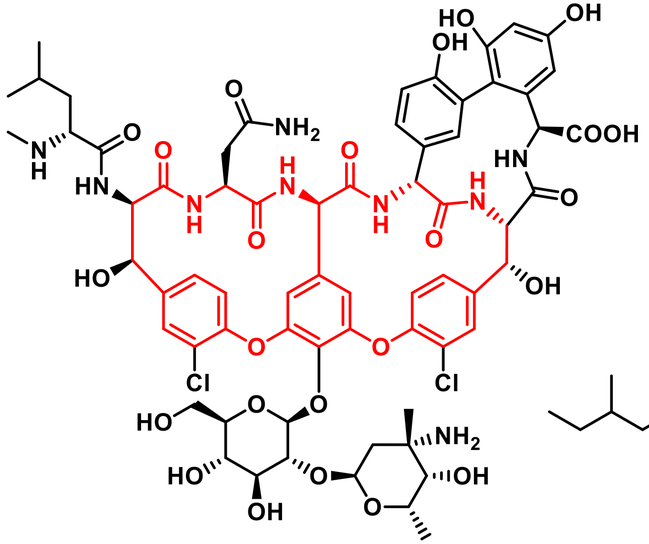
Bicycles: a potential new therapeutic modality



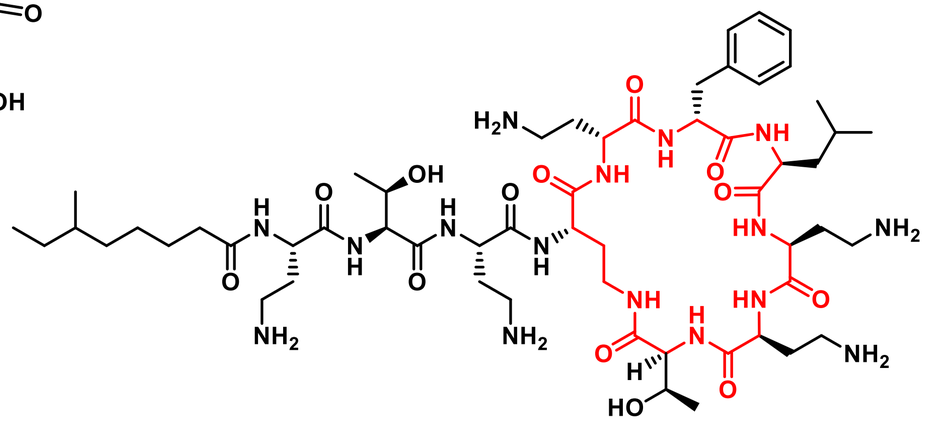


- **Highly constrained:** high affinity, exquisite selectivity, excellent stability
- **Large binding footprint:** disrupt protein-protein interactions
- **Fully synthetic:** NCE classification and synthetic control
- **Highly flexible modality:** modular building blocks retain pharmacology
- **Adjustable PK:** tissue penetration, renal elimination, tuneable $T_{1/2}$

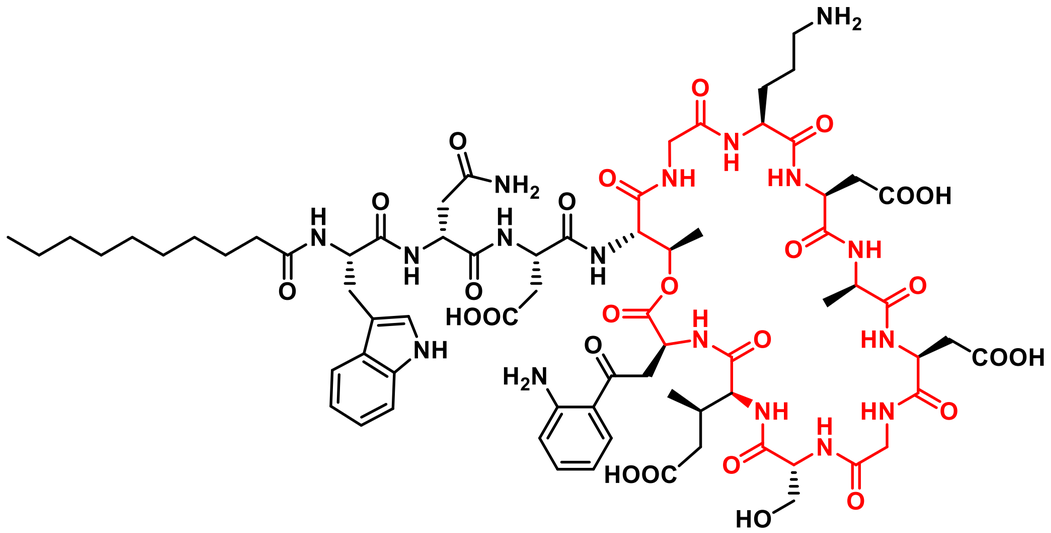
Natural Product Cyclic Peptides



Vancomycin



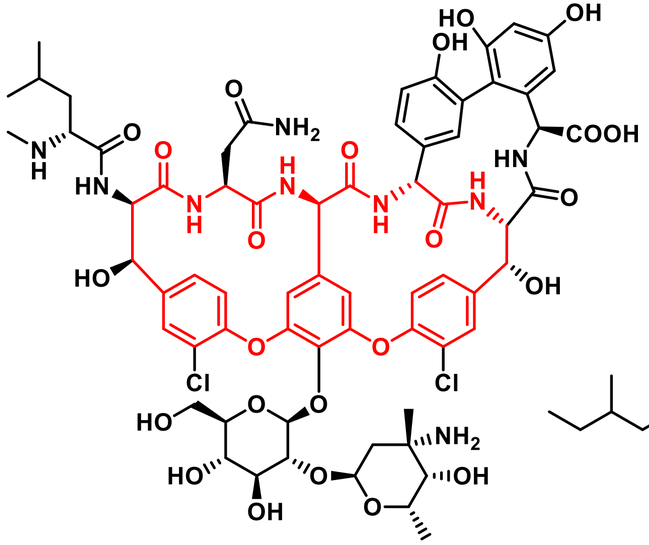
Polymyxin



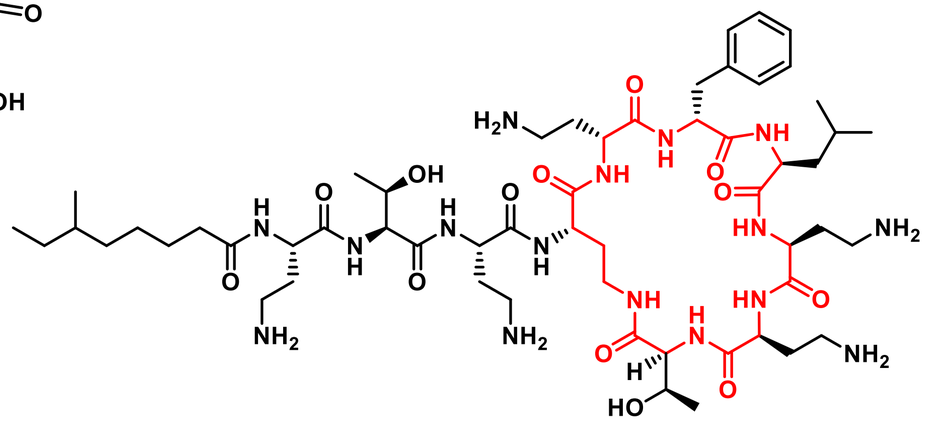
Daptomycin

◀ **Cyclic Peptide Natural Products**
Challenging synthesis and optimisation

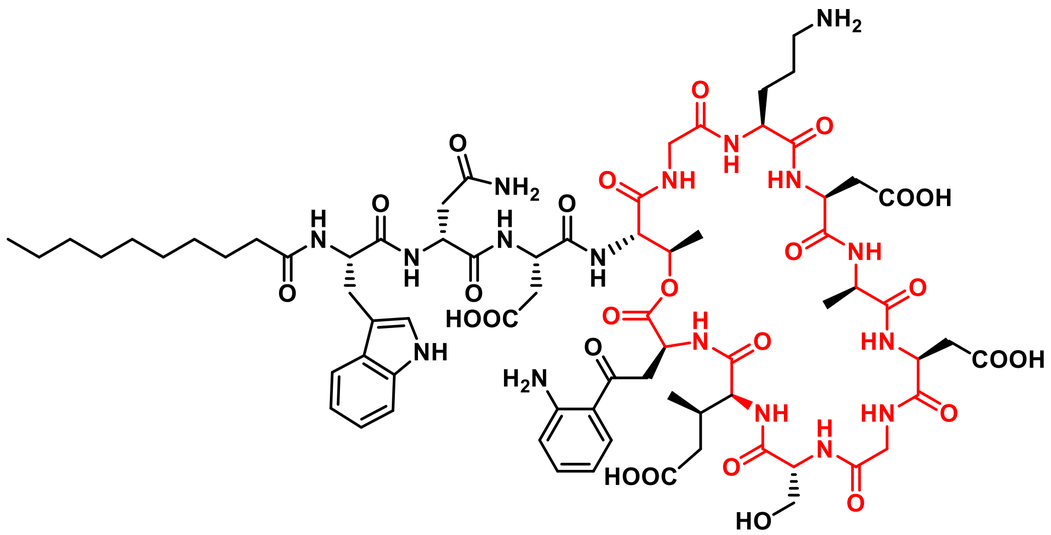
A platform for bi-cyclic (Bicycle®) peptide discovery



Vancomycin



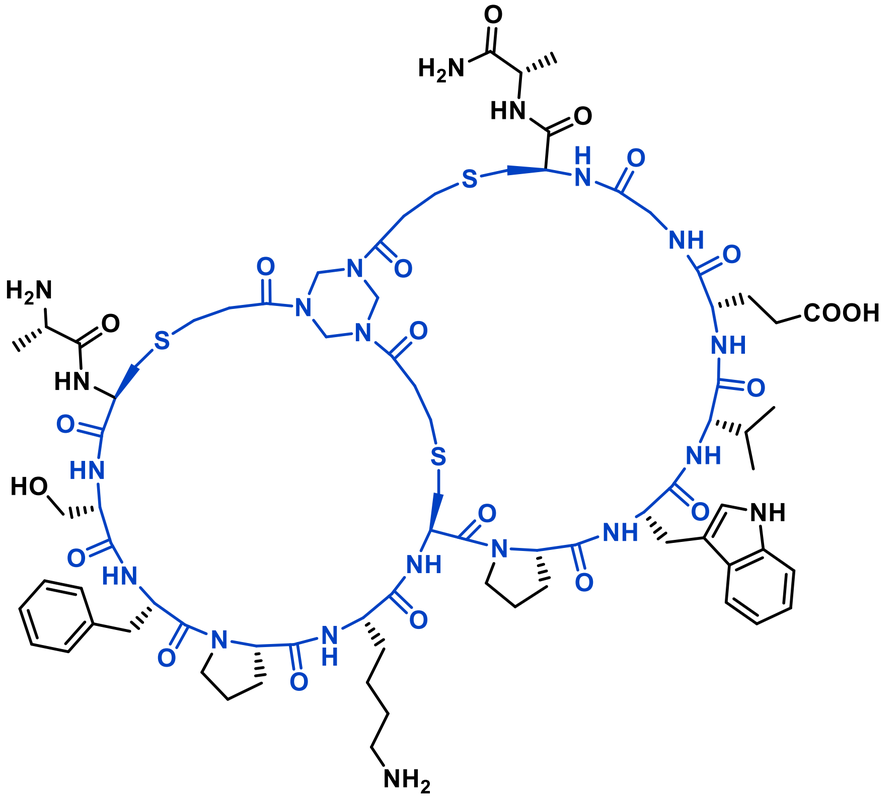
Polymyxin



Daptomycin

◂ **Cyclic Peptide Natural Products**
Challenging synthesis and optimisation

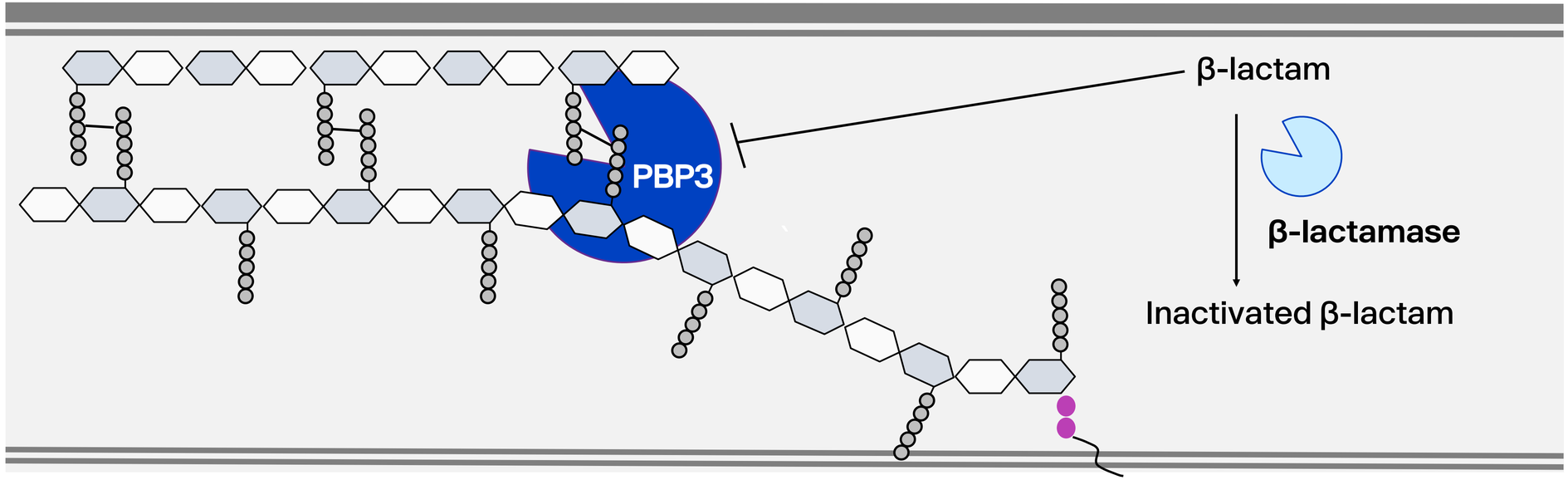
Bicycles ▸
- Target based discovery
- Huge diversity (>10^{20})
- Chemically synthesised
- Established pathway to optimise affinity and pharmacokinetics



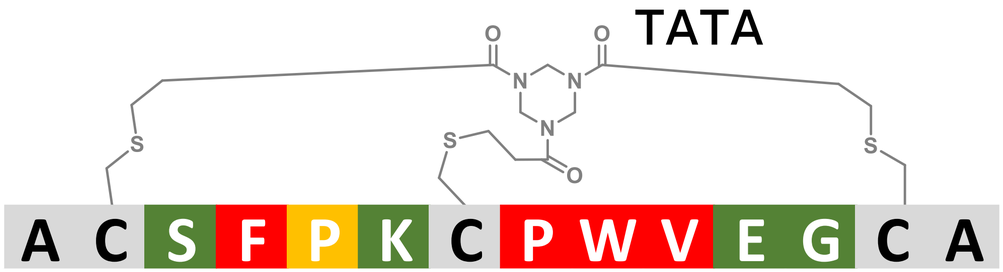
Bicycle

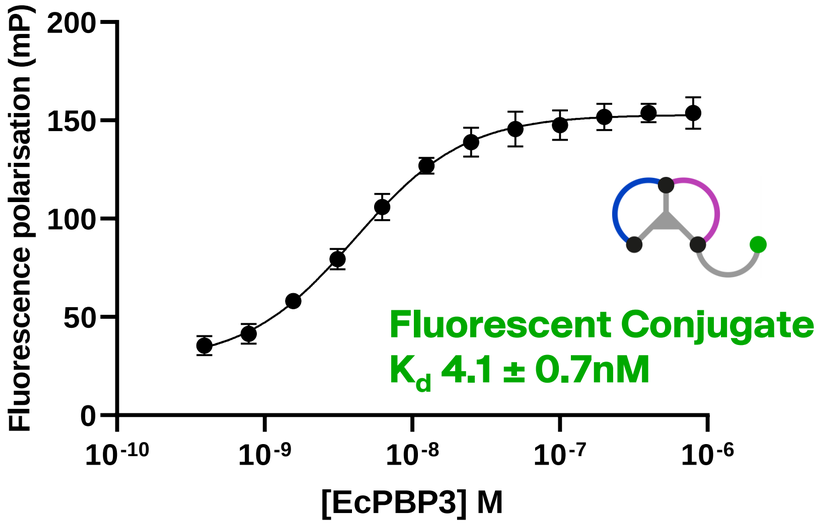
Penicillin binding proteins (PBPs) in bacterial cell wall synthesis

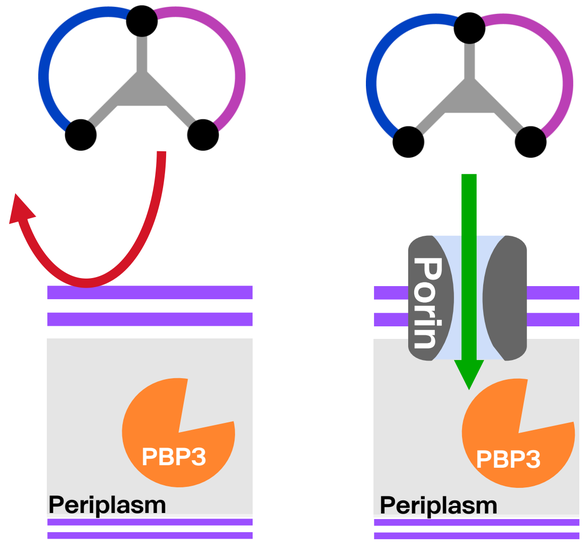
- Assemble peptidoglycan in cell walls – multiple classes with varying function
- *E. coli* PBP3 is an essential transpeptidase specific for cell division
- Non-β-lactam PBP inhibitors would be highly desirable



A promising antimicrobial *Bicycle*® against EcPBP3







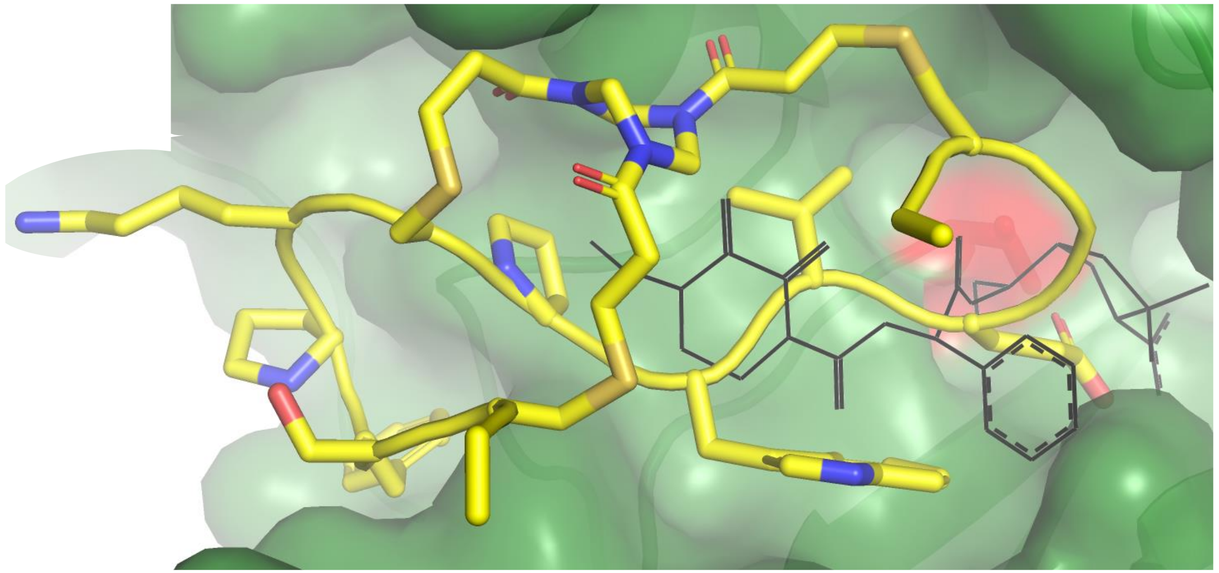
GKCW101 *GKCW102*

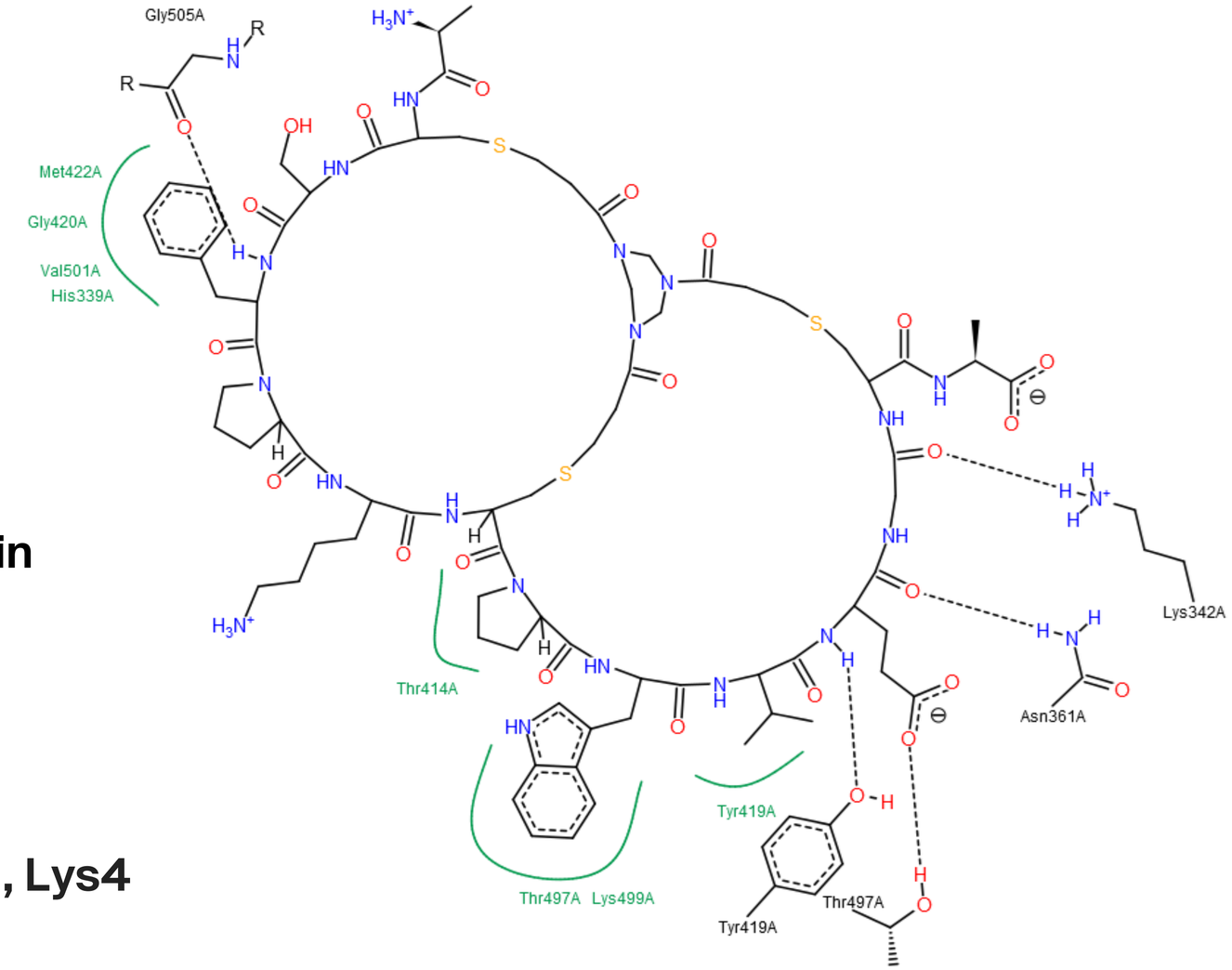
Compound	MIC (µg/ml)	
	E. coli GKCW101 (WT)*	E. coli GKCW102 (Ec-Pore)*
Bicycle	> 128	0.5 - 2
Carbenicillin	16	1

*Hyperporinated strains: Krishnamoorthy et al. (2016) AAC

Potent binder identified – conjugation strategy needed to achieve outer membrane uptake

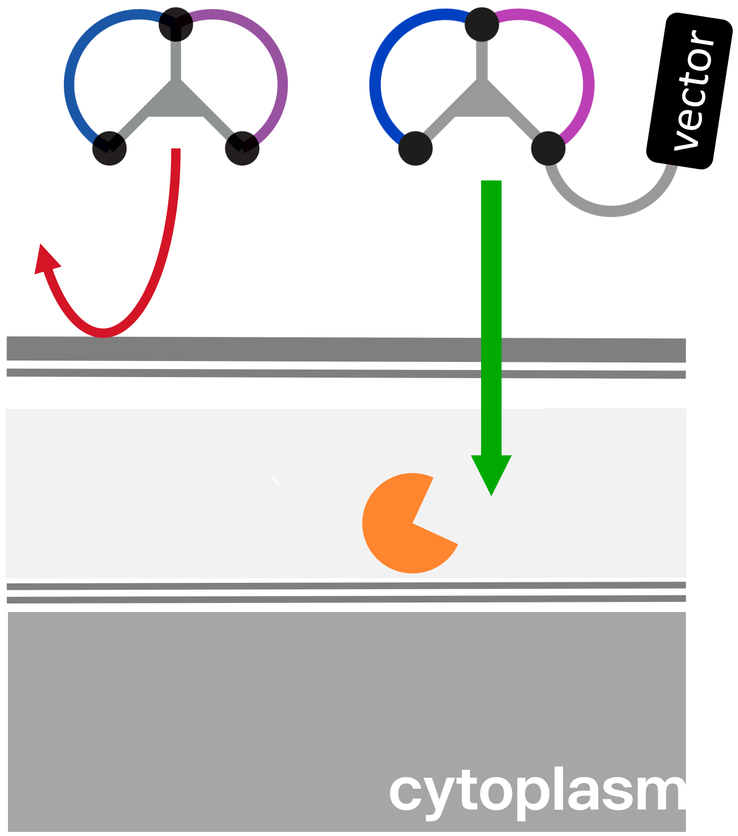
Insights from crystallography of EcPBP3 in complex with *Bicycle*



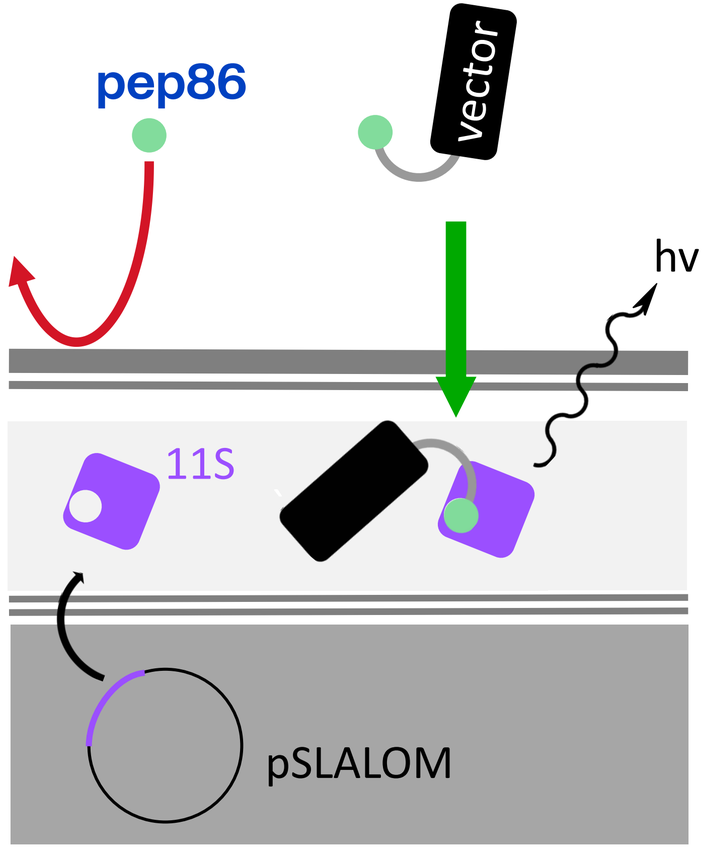


- **Co-crystal structure with transpeptidase domain solved at 1.5Å**
- ***Bicycle®* binds in the active site cleft**
- **Key interactions via Phe2 and Trp6, whilst substitutions for stability were tolerated at Ser1, Lys4**

Bicycle® -vector tandems can cross the outer membrane barrier



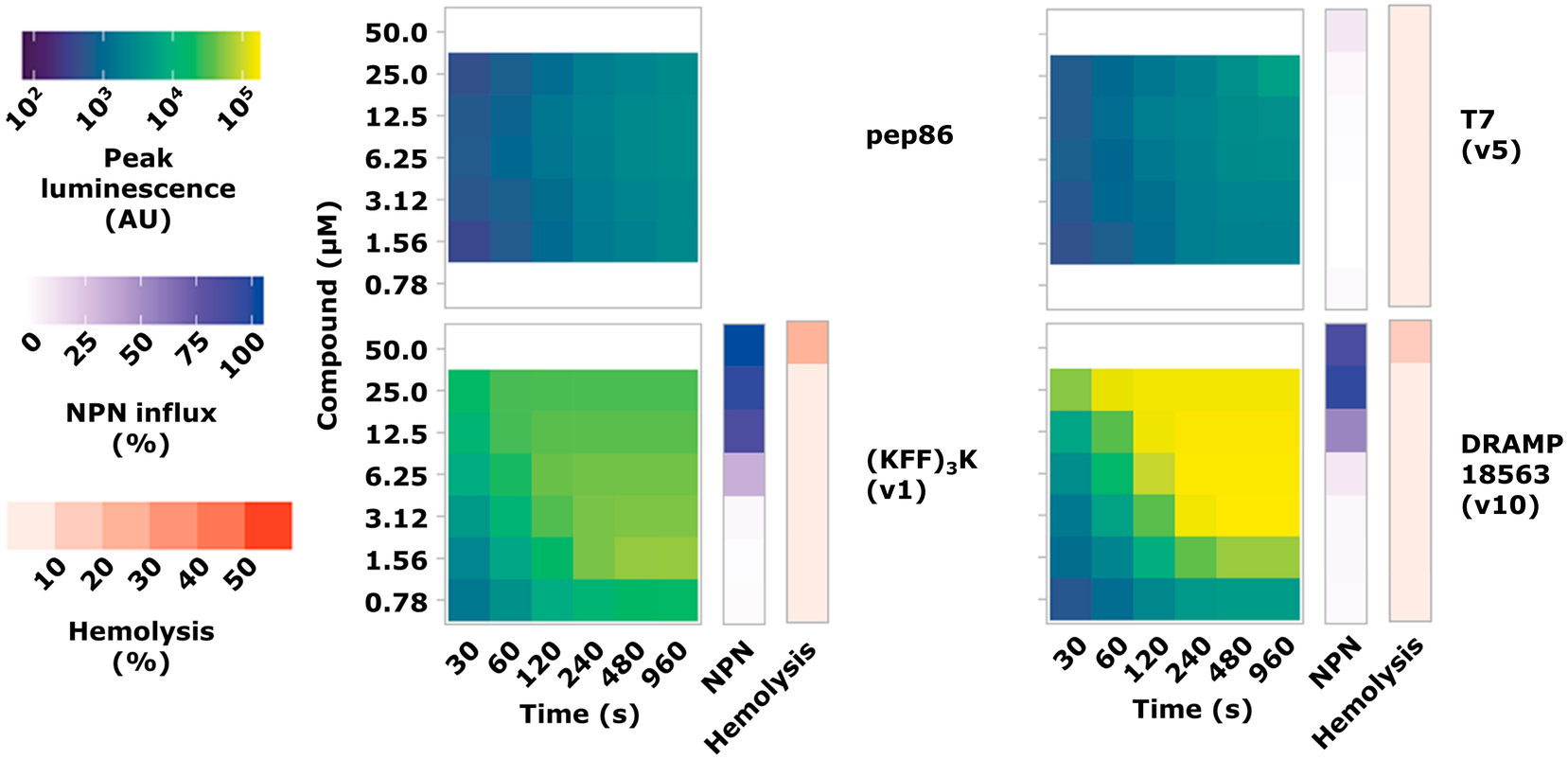
Model using periplasm entry assay (**SLALOM** – **S**plit **L**uciferase **A**ssay for **L**ive monitoring of **O**uter **M**embrane transit)



Antimicrobial peptides (AMPs) offer a starting point for vector sequences

- **Potential vector peptides chosen from AMPs in the DRAMP database** (Shi *et al.* (2022) Nucleic Acids Res.)
- **Conjugates made via click chemistry and screened for uptake, MIC and hemolysis** (Wagstaff *et al.* (2020) ACS Inf Dis)

SLALOM assay identifies potential vector peptides for *Bicycles*



$(KFF)_3K$	K F F K F F K F F K	Good *et al.* (2001) Nature Biotechnology
T7	N A G S L L S G W G	Li *et al.* (2018) Appl Environ Microbiol.
DRAMP18563	K S L R R V W R S W W	Shi *et al.* (2022) Nucleic Acids Research

Encouraging data for DRAMP18563, derived from a tick AMP

Wagstaff *et al.* (2020) ACS Inf Dis

Whole-cell activity of conjugates is driven by the *Bicycle®*

	Bicycle®	Vector	MIC (µg/ml) *E. coli* GKCW101 (WT)	*E. coli* GKCW102 (EcPore)	*E. coli* ATCC 25922	*E. coli* ATCC BAA-2469	*K. pneumoniae* ATCC 43816	Hemolysis
1	Parent *Bicycle®*	none	>128	0.5 - 2	>16	NT	NT	NT
2	Parent *Bicycle®*	Vector RI	0.5	0.5	1-2	1-2	4	>50uM
3	All-D *Bicycle®*	Vector RI	NT	>256	64	≥64	>64	>100uM

Vector RI = retroinverse DRAMP18563

1. EcPBP3-binding *Bicycle®* is potent in hyperporinated strain, but lacks activity in WT *E. coli*
2. Conjugation to an AMP vector conferred activity in WT *E. coli*
3. Activity of the conjugate was lost when the *Bicycle®* was switched to all-D amino acids

Vector drives periplasm access of an EcPBP3-targeting *Bicycle®*

Whole-cell activity of conjugates is driven by the *Bicycle®*

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Bicycle

Whole-cell activity of conjugates is driven by the *Bicycle*®

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Vector drives periplasm access of an EcPBP3-targeting *Bicycle*®

Promising spectrum of activity against Enterobacterales

	Organism	% sequence homology of ftsI* with *E. coli*	Conjugate MIC (µg/mL)
Enterobacterales	*Escherichia coli*	100	4-32 (n=4 including one NDM-1 producer)
	Citrobacter freundii	96	4-8 (n=2 including one meropenem resistant)
	Klebsiella pneumoniae	94	4-8 (n=4 including one KPC producer)
	Enterobacter cloacae	94	2-4 (n=4 including 2 meropenem resistant)
	Proteus mirabilis	76	>128 (n=2)
	Pseudomonas aeruginosa	45	>128 (n=2)

** PBP3 is the gene product of ftsI*

- Spectrum correlates with sequence homology
- Encouraging activity against resistant strains
- Encouraging stability in biological matrices (Blood (m) $T_{1/2}$ = 8h); not highly protein bound Fu (m) = 23%

PBP inhibitors discovered using a modified phage display platform

- The Bicycle discovery platform offers unique opportunities for novel antimicrobials, and is readily applicable to other targets
- *E. coli* PBP3 is highly tractable to the Bicycle platform, with low nanomolar binders identified in phage selections
- *Bicycle*-AMP conjugates show promising activity against WT bacteria – novel non-covalent PBP inhibitors
- *Bicycle*-AMP conjugates are being progressed towards a potential clinical candidate under a project funded by a BMC

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WARWICK
THE UNIVERSITY OF WARWICK

ANTIMICROBIAL ADS DISCOVERY SOLUTIONS

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UKRI UK Research and Innovation

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