

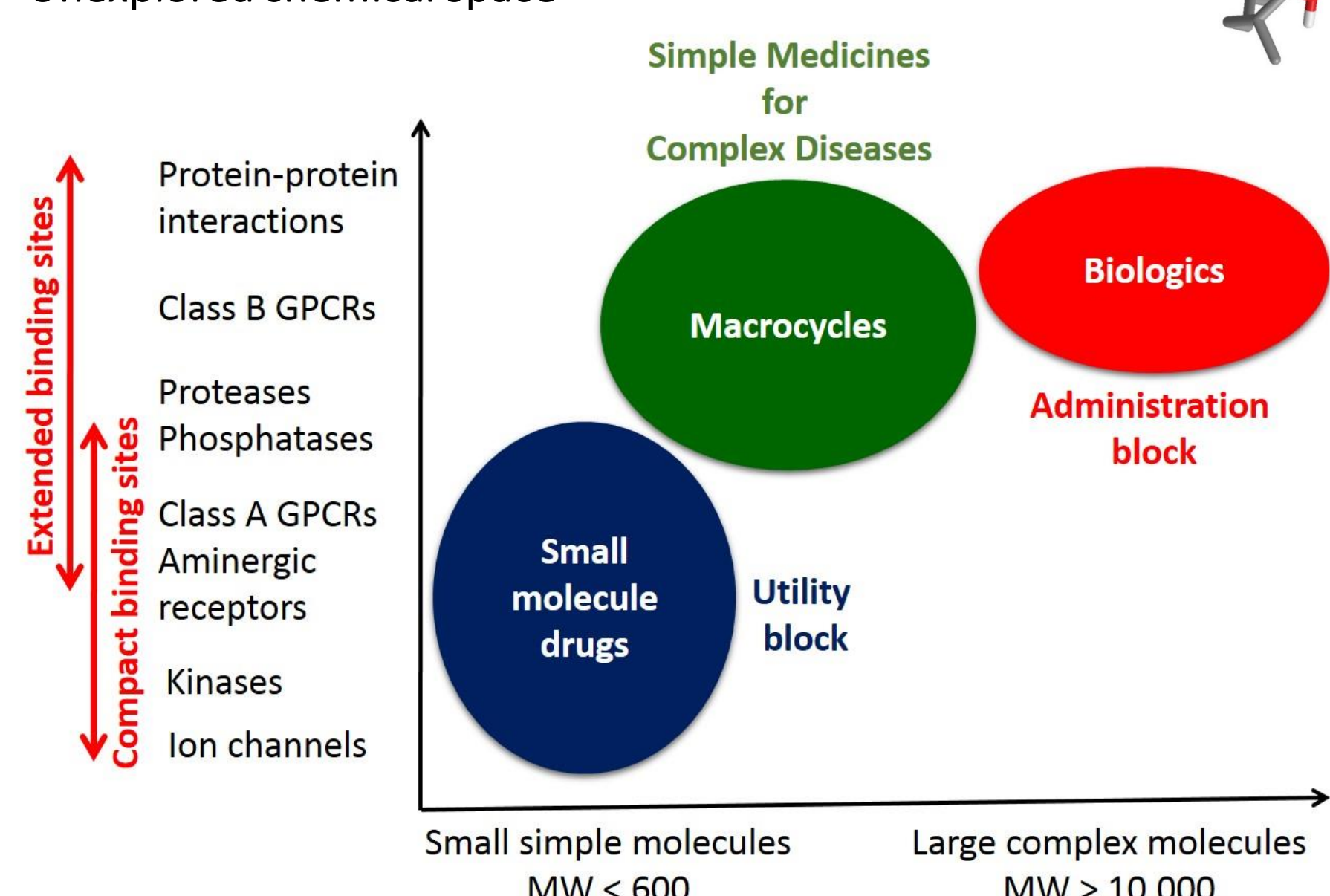
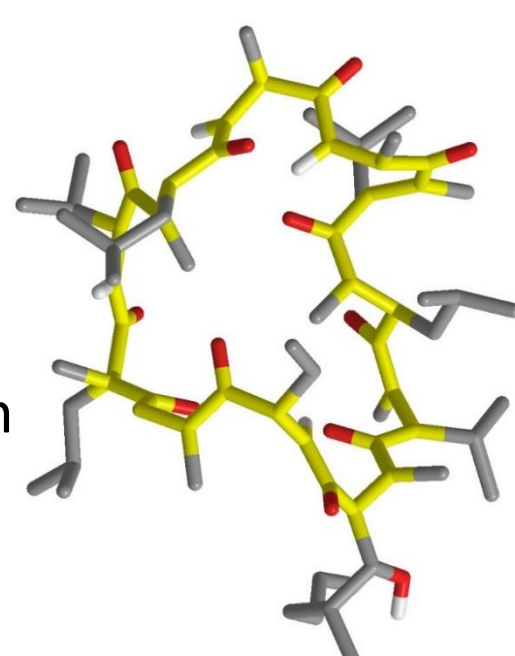
A Chemoenzymatic Solution for Cell-Permeable Therapeutic Macrocyces

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1. The Opportunity for Macrocyces

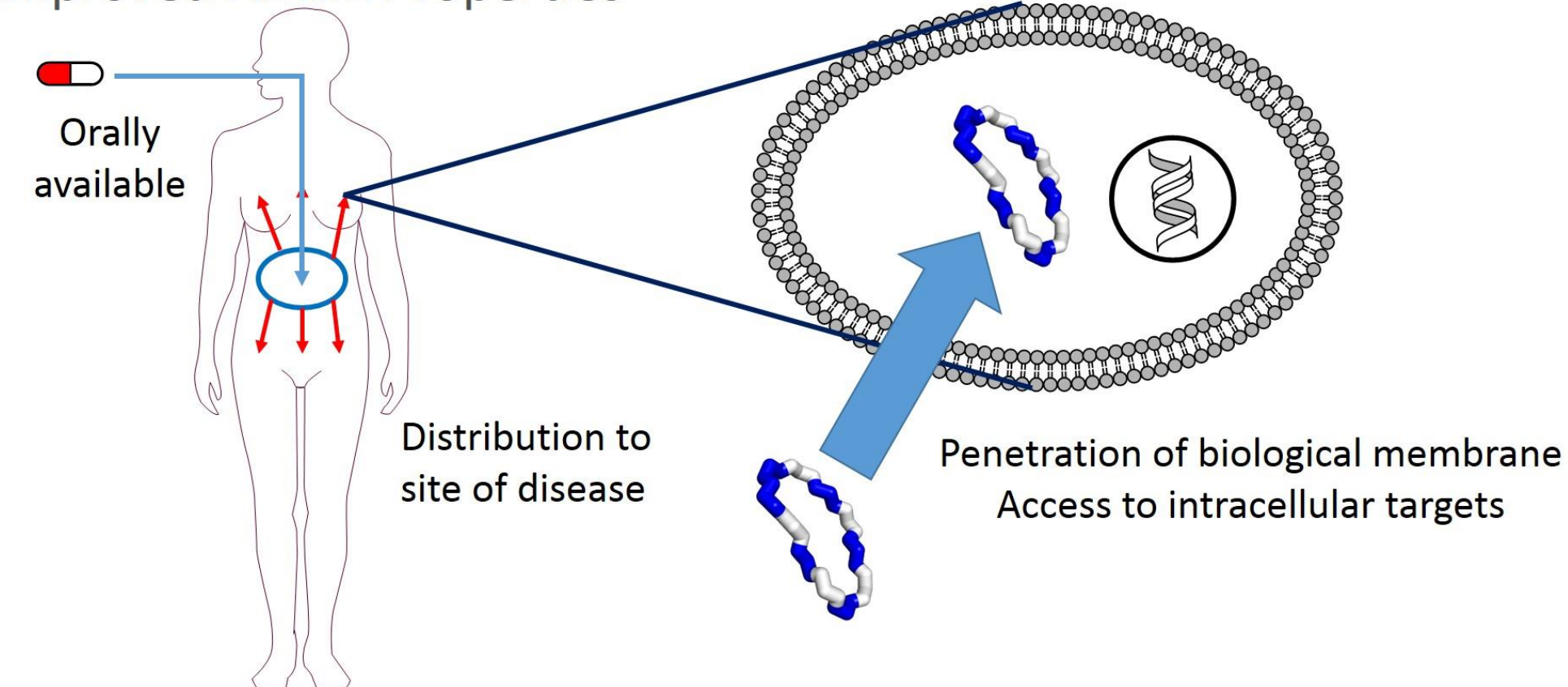
- Large cyclic molecules (600 – 1200 Da)
- Can be highly modified cyclic peptides
- Greater utility than classical small molecule drugs
- Able to address complex targets
- Alternative to biologics but with oral administration
- Development and formulation like small molecules
- Unexplored chemical space



2. The Advantages of Macrocyces

- Ability to cross biological membranes
- Access intracellular targets
- Potential for oral bioavailability

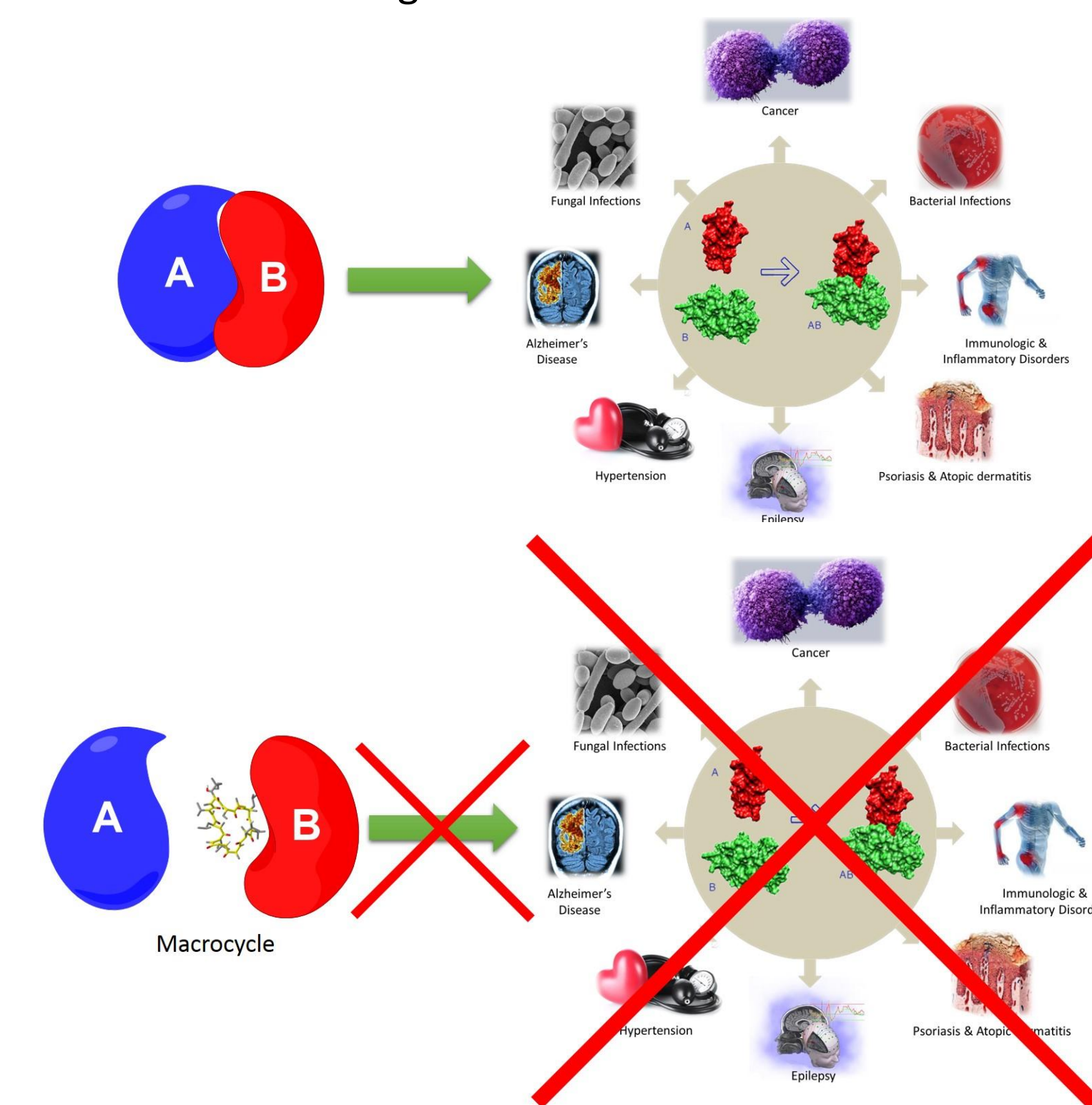
Improved ADME Properties



- More stable to metabolism than simple peptides
- Conformational tailoring allows better target affinity / specificity

3. Protein – Protein Interactions as Drug Targets

- Protein-Protein Interactions (PPIs) are present in all diseases
- Interfaces are broad and shallow
- Small molecule drugs will not inhibit these interactions

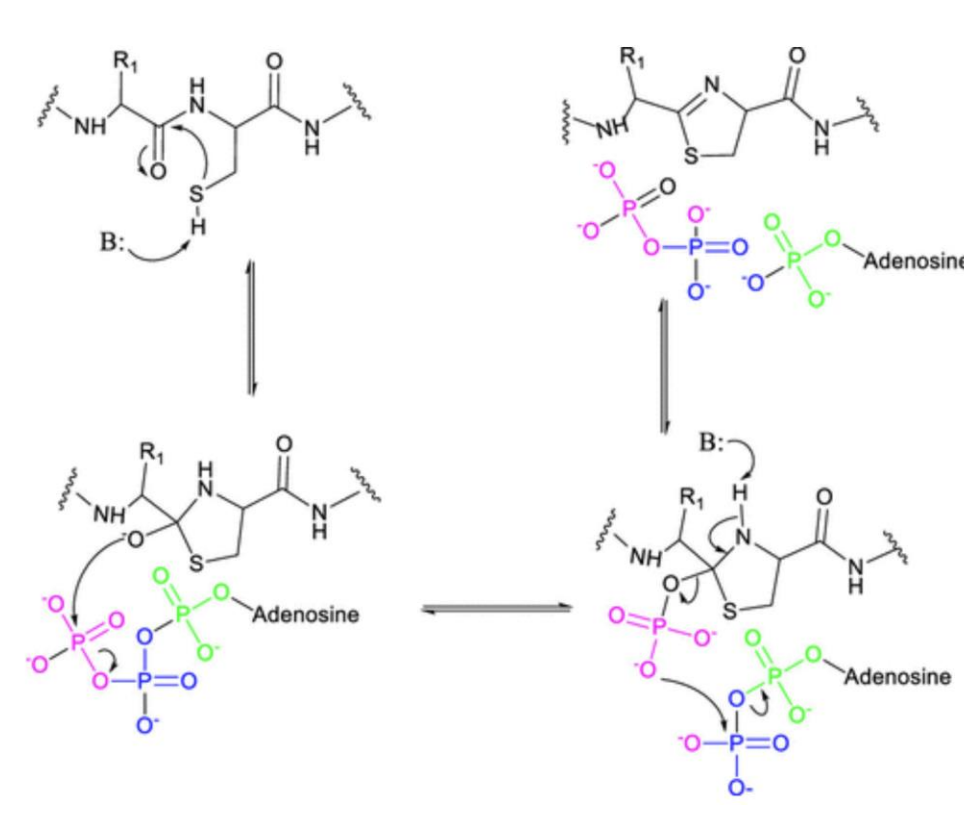


4. GyreOx Technology

Technology originally developed within the groups of Professors Jim Naismith and Marcel Jaspers. Comprehensive understanding of the structures and mechanisms of enzymes of the cyanobactin biosynthesis pathway allowed specific engineering to provide unique new reagents for the creation of complex and novel macrocycles.

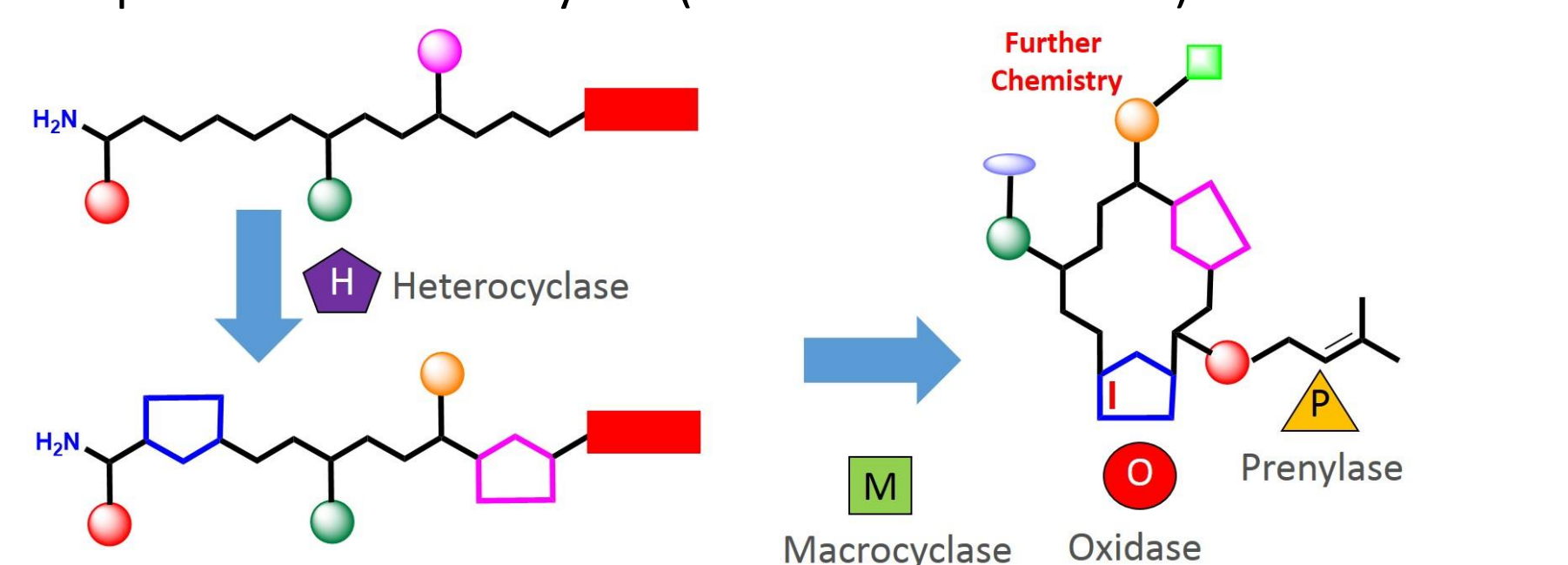
GyreOx was funded in 2020 to exploit and transform this technology into an automated platform for the development of cell permeable macrocyclic leads.

Creation of heterocycles by engineered enzyme through ATP-dependent cyclodehydration reaction¹



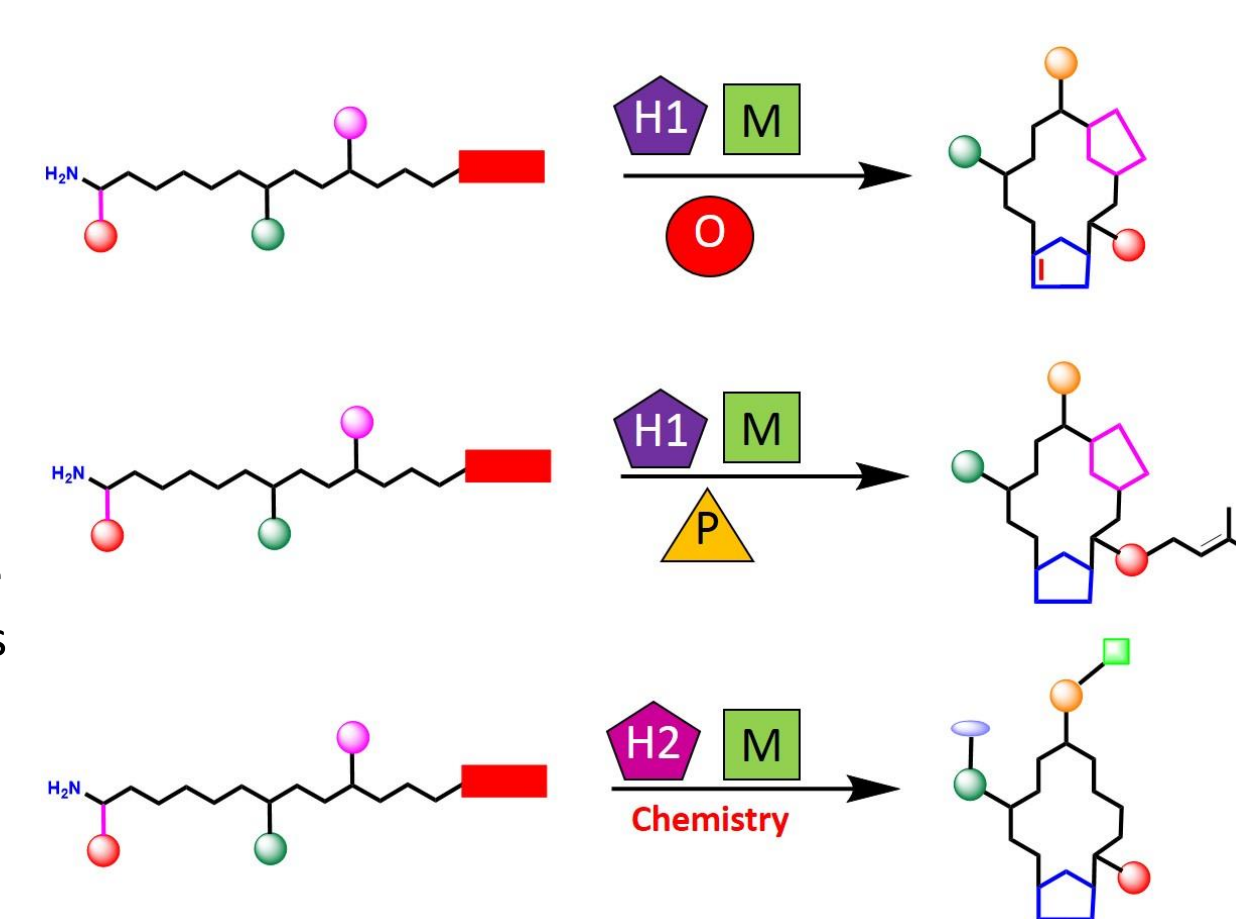
5. Technology Development

- One pot biotransformations creating discrete macrocycles
- Macrocycle ring sizes from 4 – 12 residues
- Incorporation of heterocycles (reduced and oxidised)



- Diversity is introduced by:
- Varying the substrate
 - Use of different tailoring enzymes
 - Additional chemical modification

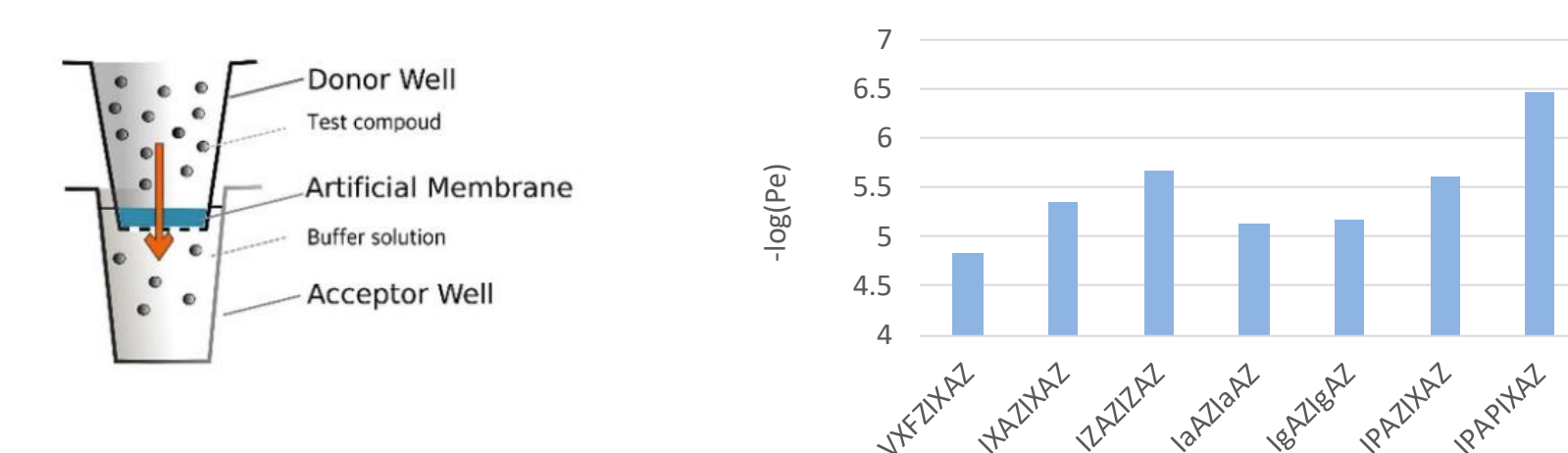
Multiple products from a single substrate allows subtle changes to be made around an initial compound and makes the technology suitable for hit-to-lead optimisation



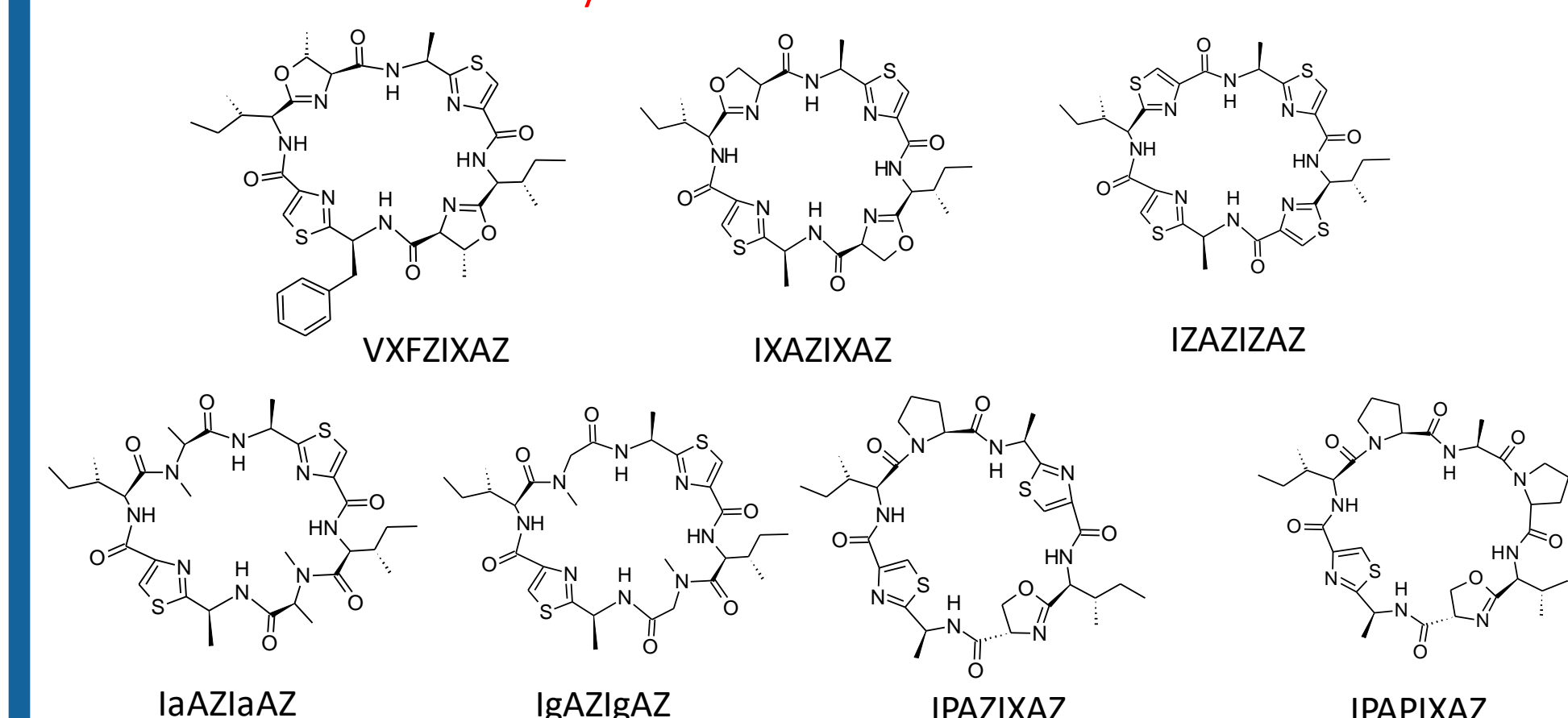
6. Results: Permeability

Parallel Artificial Membrane Permeability Assay (PAMPA)

PAMPA is used to measure permeability across an artificial membrane. $-\log(P_e)$ values below 5.8 are likely to be cell permeable

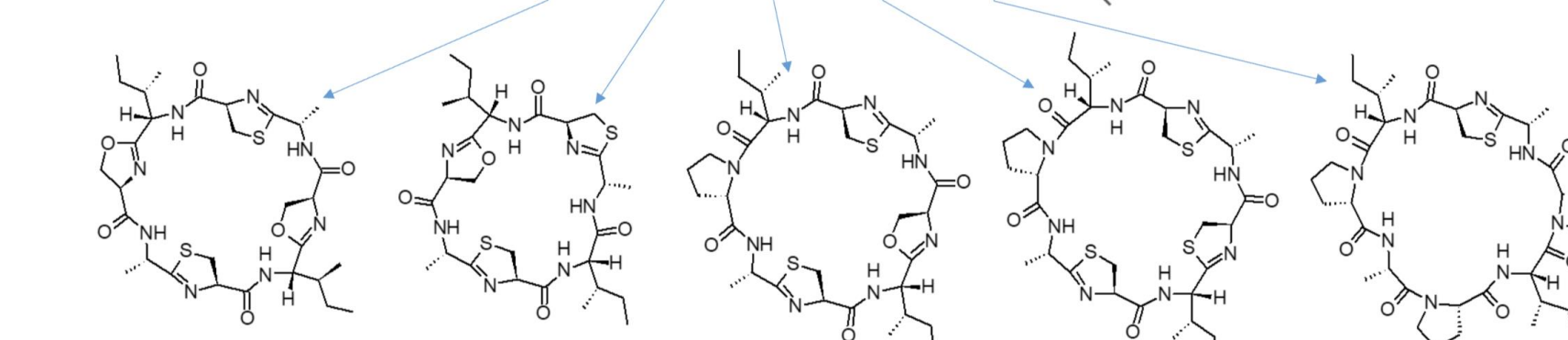
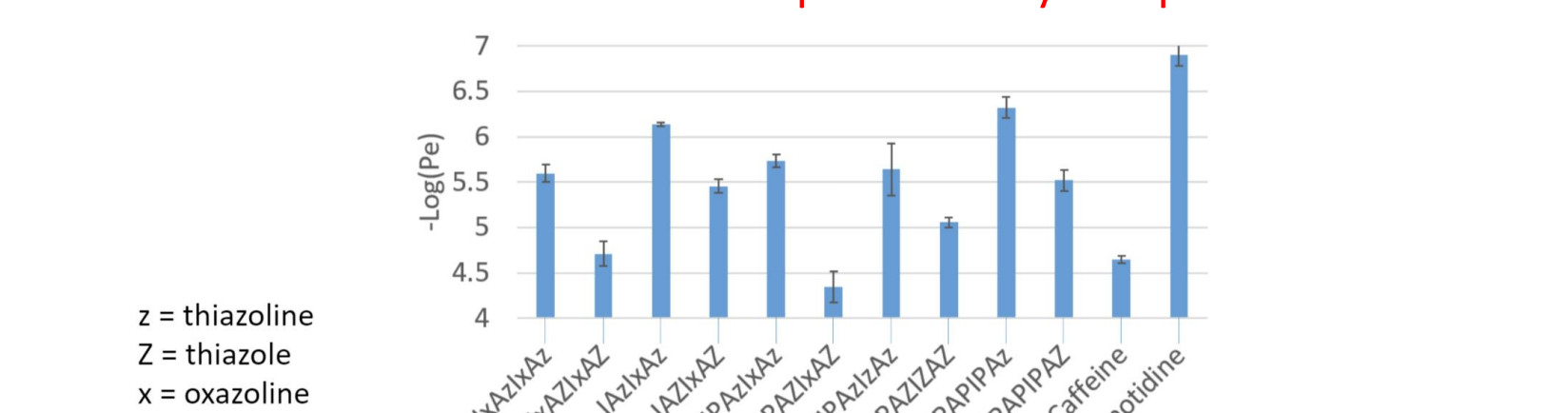


Oxazole and thiazole have similar beneficial effect on membrane permeability as N-Me-Ala or N-Me-Gly

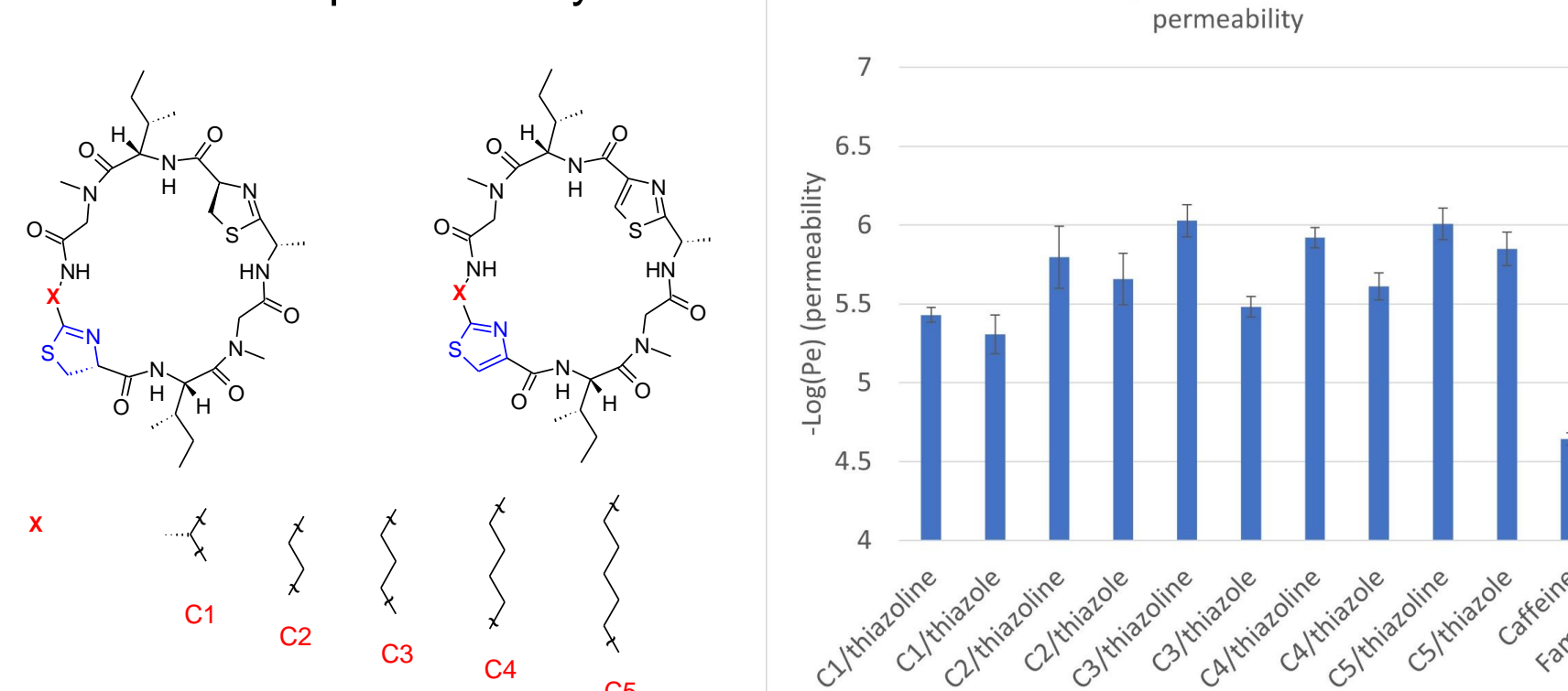


7. Results: Permeability

Thiazoles have increased membrane permeability compared to thiazolines



- Introducing linkers in Patellamide-like macrocycles reduces membrane permeability



8. Results: Chemical Macrocyclization

- A variety of electrophiles were used to macrocyclise different linear peptides by linking two thiols (SS), or a thiol and amine (SN)
- Alkyl stapling is compatible with the engineered heterocyclase enzyme MicD fusion
- Diversity is introduced by using different stapling moieties for macrocyclisation

Entry	Linear peptide sequence	Staple type	Macrocyces generated
1	Ac-C-G-A-A-I-G-W-C	SS	4
2	V-P-A-P-A-P-W-C	SN	4
3	G-P-A-P-A-P-W-C	SN	3
4	I-S-A-TH-I-S-A-C	SN	4
5	V-G-A-TH-I-G-W-C	SN	4
6	TS-I-G-A-TH-I-G-A-C	SS	4

TS = 2-thioacetamide, TH = Thiazoline

9. Conclusions

- Diverse libraries of macrocycles are routinely assessed for membrane permeability using PAMPA
- Thiazole and oxazoles have similar permeability as N-methylated macrocycles (backbone N-methylation of macrocycles is a known modulator of cell permeability in nature)
- The introduction of alkyl linkers of varying length causes a slight reduction in permeability
- Each series contains macrocycles that show desirable pharmacokinetic properties that would allow them to penetrate biological membranes
- Enzymatic cyclodehydration has been successfully combined with chemical stapling to generate a range of novel compounds

Reference

1. Ge,Y *et al* *Biochemistry* 2019, 58, 16, 2125–2132

GyreOx's chemoenzymatic technology is ideally suited for creating novel macrocycles as part of a hit-to-lead optimisation programme, particularly for developing inhibitors targeting intracellular protein-protein interactions. Compounds of interest will be scaled-up for further analysis.