Simulation and experimental analysis of the structure and antimicrobial activity of linear peptoids

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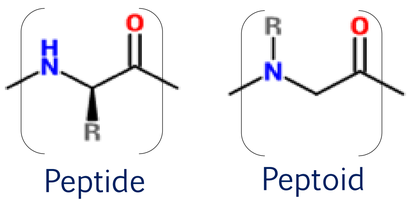
1. Introduction

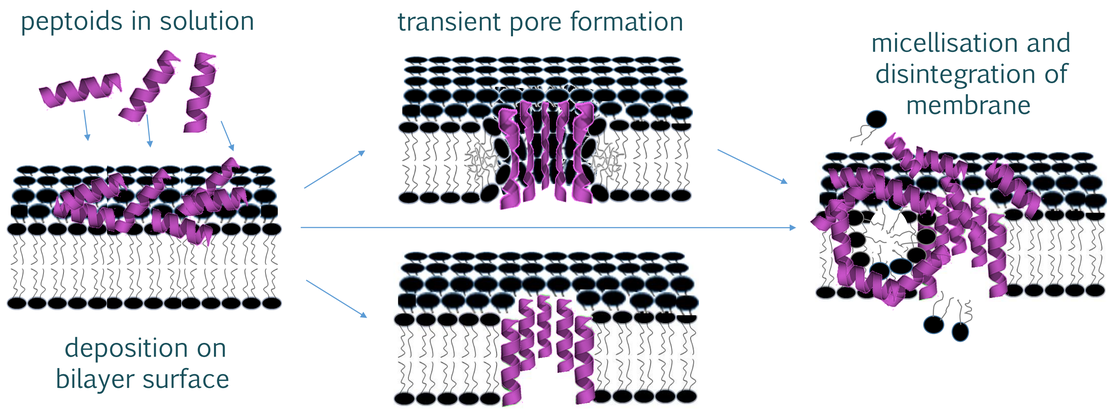
We use a range of approaches both experimental and computational to link the primary structure of linear peptoids to their biological activity by investigating their interactions with lipid membranes.

- Peptoids are poly-*N*-substituted glycines: a class of synthetic peptide mimics with side chains substituted onto the amide nitrogen atom,



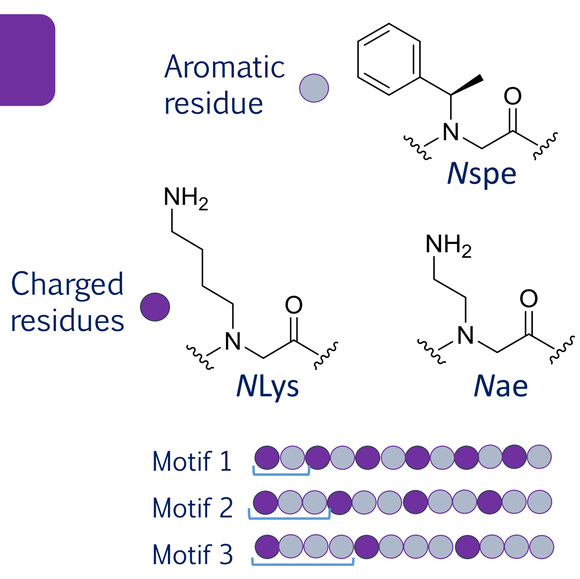
- Substitution of the side chain (R) onto the amide nitrogen prevents hydrogen bonding in peptoid backbone, affecting secondary structure stabilisation,
- Peptoids can be designed to have structural similarities to natural antimicrobial peptides (AMPs) with antimicrobial activity and selectivity believed to originate from their ability to form cationic, amphipathic secondary structures,[1]
- Small structural differences afford peptoids better in vivo properties than AMPs and therefore greater potential for clinical use in the future. [2]

Membrane disruption mechanisms



2. Peptoid library

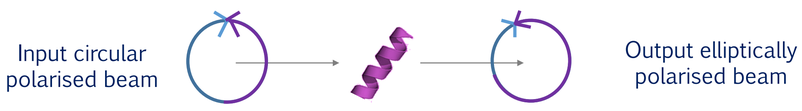
- 3 different structural motifs, each a combination of residues with charged and aromatic side chains,
- Each motif is repeated until the peptoid is 12 residues in total *e.g.* $(N\text{Lys}N\text{spe})_6$ (motif 1),
- Peptoids active against range of bacterial and parasitic pathogens.[3]



3. Methods

Experimental

- Peptoid secondary structure characterised by circular dichroism spectroscopy (CD) in different solvent environments and the presence of small unilamellar vesicles (SUVs) which act as model biomembranes,
- Here we use far UV CD to probe electronic transitions in the peptoid backbone.



Differential absorption of right and left handed polarised light by optically active sample

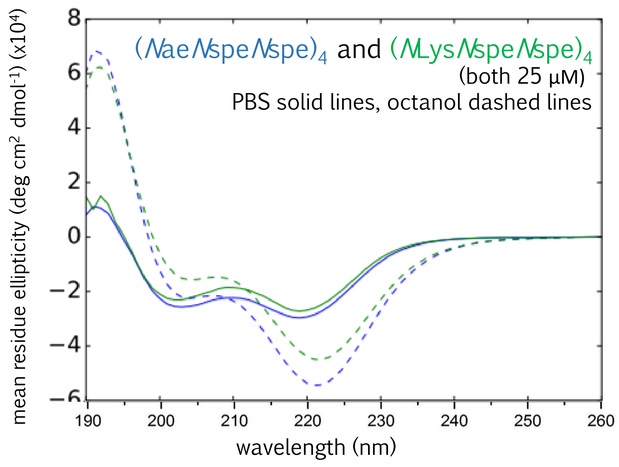
Computational

- Molecular dynamics simulations are used here to predict and analyse peptoid secondary structure,
- Peptoids are simulated in GROMACS, using modified AMBER forcefields with parameters obtained through ANTECHAMBER software.

4. Results

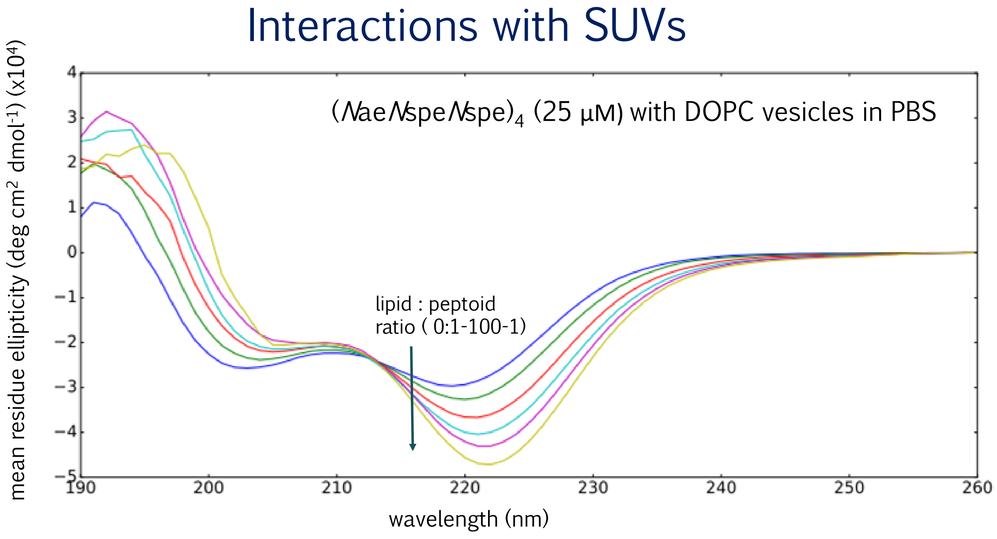
CD in PBS and octanol

- Phosphate buffered saline and octanol act as human isotonic conditions and bilayer interior mimics respectively,
- Spectral differences indicate peptoid structural differences and are qualitatively similar for all peptoids in library,



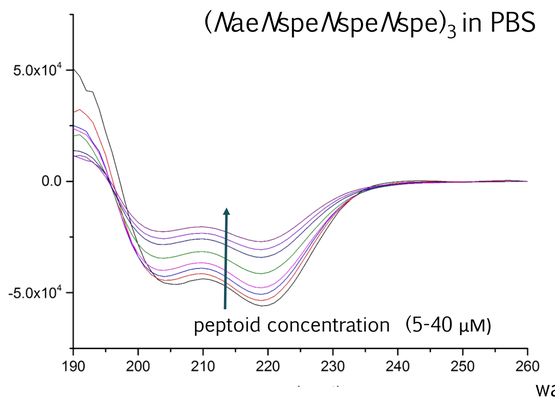
- Spectra indicate helical structures, similar but not identical to the alpha-helix seen in peptides.

Interactions with SUVs

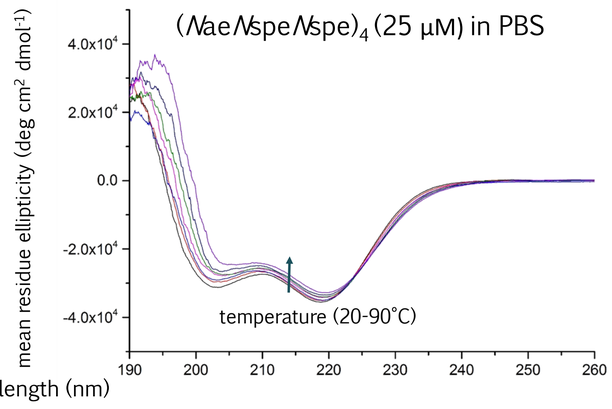


- Spectral changes indicate that interactions with SUVs induce structural changes in peptoids and possible insertion into bilayer interior,
- Presence of isodichoric point indicates that the system could be described by a two state model with spectra in PBS and octanol representative of the free in solution and fully membrane bound states.

Concentration dependent CD

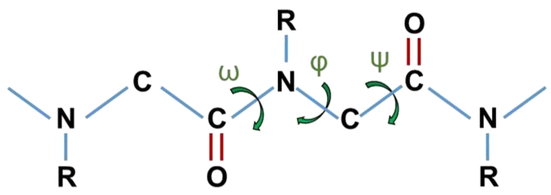


Thermal stability

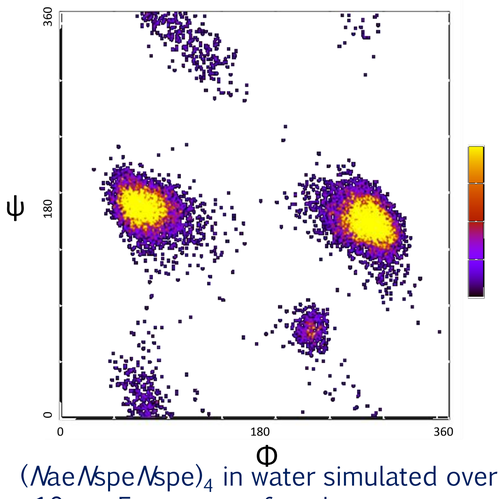


- CD indicates peptoids in library have good thermal stability,
- One peptoid, $(N\text{ae}N\text{spe}N\text{spe}N\text{spe})_3$ shows concentration dependent CD, indicating the occurrence of some aggregation or self-assembly,
- Further work is currently being carried out to characterise this.

Simulations in water and octanol



- Secondary structure defined by the backbone dihedral angles ω, Φ and ψ,
- Simulations in water and octanol can be used to map structural differences with Ramachandran plots of Φ *vs* ψ,



$(N\text{ae}N\text{spe}N\text{spe})_4$ in water simulated over 10 ns. Frequency of angle occurrence increases from purple to yellow

- Further computational work will involve simulating peptoids with an atomistic lipid bilayer in order to understand the mechanism of insertion and analyse peptoid structural changes during this process.

References

[1] Fowler, S. a & Blackwell, H. E. Structure-function relationships in peptoids: recent advances toward deciphering the structural requirements for biological function. *Org. Biomol. Chem.***7,**1508–1524 (2009).
[2] Czyzewski, A. M. *et al.*In Vivo, In Vitro, and In Silico Characterization of Peptoids as Antimicrobial Agents. *PLoSOne***11,**e0135961 (2016).
[3]Eggiman, G. A. *et al.* Inverstigating the Anti-Leishmanial Effects of Linear Peptoids. *ChemMedChem Communication.* 10, 233-237 (2015).

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SOFI CDT EPSRC Centre for Doctoral Training in Soft Matter and Functional Interfaces

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