Improved Generation of Symmetric Cyclic Peptide Complexes with RFDiffusion

Rationale: Cyclic peptides *(Figure 1)* have demonstrated promising potential for their structural stability, membrane and cell permeability, and high binding affinity in the field of pharmaceuticals and drug design.[1] However, the *de novo* design of such molecules has been (A) mostly limited to classical, energy-based design techniques and (B) focused on design of cyclic peptides as monomers (that then associate with targets of interest), rather than as homo-oligomeric species. Recently, a generative neural network, RFdiffusion, have demonstrated successful generation of protein oligomers with high experimental success rates.[2] Furthermore, recent findings have shown that manipulating the relative positional encoding of amino acids can generate cyclized peptide predictions using RFDiffusion.[3] Leveraging these findings, we aim to combine RFDiffusion's symmetric oligomer generation capability with that of producing cyclic peptides and explore their experimental feasibility.

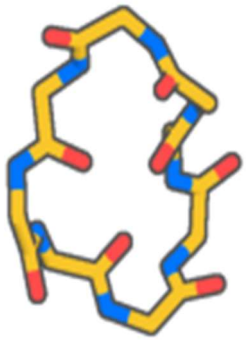

Figure 1. PyMOL Implementation of a cyclic peptide.

Then, we will validate the robustness of those generated symmetric oligomers through comparative analysis with experimentally determined structures. This will be a cornerstone for efficient generation of symmetric conformation of macrocyclic polypeptides[3]

Hypotheses:

- We can make symmetric oligomers of cyclic peptides, and they maintain cyclic peptide properties such as cell permeability, increased binding affinity, and structural stability.
- We can gain more insights and controls over the designing process for *de novo* protein binders and other targets.

Acknowledgement: First and foremost, I would deliver my deepest gratitude to David C. Juergens for providing insightful guidance and professional mentorship to proceed with this research. In addition to him, I appreciate Dr. Gaurav for initiating cyclic peptide prediction research which is a main source of initiating this project.

Objective: Robust generation process of symmetric oligomers made of cyclic peptides.

General Approach: Our main approach for the robust generation and testing of symmetric oligomers of cyclic peptides will include following key steps.

- We will integrate the protocols in RFDiffusion for generating symmetric oligomers with its capacity to produce cyclized peptides *(Figure 2)*. This combination aims to harness the structural stability and high binding affinity of cyclic peptides.
- We can use cyclized peptides as the asymmetric unit. Then, we can generate complexes with diverse point group symmetries as shown in *Figure 2*.
- To design the sequences for these RFDiffusion backbone structures, we will use ProteinMPNN.[4] This model will ensure that our generated structures have sequences with high performance and structural compatibility.
- We will use cyclic AlphaFold2 (AF2) structure prediction protocol to filter designs in silico and select designs for experimental validations.[1]
- We will perform comparative analysis using *r.m.s.d* and confidence metrics for validating robustness of generated structures.

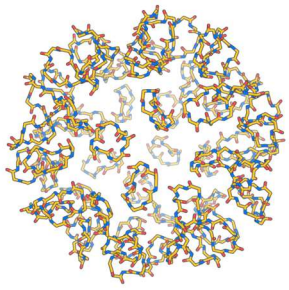

Figure 2. RF Symmetrized Inference. From "Motif Scaffolding in oligomers and repeat proteins" by David C. Juergens (Slide p. 5)

References

[1] Rettie, Stephen A, et al. *Cyclic Peptide Structure Prediction and Design Using AlphaFold*. 26 Feb. 2023, https://doi.org/10.1101/2023.02.25.529956.

[2] Watson, Joseph L., et al. *Broadly Applicable and Accurate Protein Design by Integrating Structure Prediction Networks and Diffusion Generative Models*. 10 Dec. 2022, https://doi.org/10.1101/2022.12.09.519842.

[3] Gaurav, Bhardwaj and Sergay Ovchinnikov (2023). *Unknown*.

[4] Juergens, David. (2023 June 12). Motif Scaffolding in oligomers and repeat proteins. Seattle, WA, United States.

[5] Dauparas, J., et al. *Robust Deep Learning Based Protein Sequence Design Using Proteinmpnn*, 2022, https://doi.org/10.1101/2022.06.03.494563.