AI/ML-ASSISTED REDESIGN OF NATIVE PROTEINS

De Novo Design Of Alpha-conotoxin Binding Proteins

Leonie Windeln Univeraity of Southampton **Jack Sawdon** University of Southampton **Jesko Koehnke** Unversity of Hannover **Andrew Jaimieson** University of Glasgow **Jeremy Frey** University of Southampton **Jonathan Essex** University of Southampton

α-Conotoxins are a class of disulfide-rich cyclic peptides produced by marine cone snails that target human nicotinic acetylcholine receptors (nAChRs). The specificity of these toxins against different isoforms of nAChRs make them attractive pharmacophore candidates but might impose a risk for their use as a neurotoxin and it is essential to develop biological countermeasures.

There has been a massive increase in AI/ML methods for de novo protein design in recent years. The development of diffusion model workflows for protein-protein interactions (PPIs) inspired us to generate novel protein scaffolds that bind to alpha conotoxins. For this we generated new backbones conditioned only on the conotoxin, aGI. In a subsequent design approach, we created a library of partially diffused backbones from known proteins binding to peptides or proteins in the presence of aGI using RFDiffusion. Afterwards we used ProteinMPNN to generate possible primary sequences for those backbones and filtered the resulting proteins using alphafold2 and in house computational methods. Preliminary analysis suggested that partially diffused proteins derived from known scaffolds might be more successful in binding conotoxins than the ab initio designs. This is likely due to the small size of the toxin compared to a protein and the relatively compact shape.

We are currently working on improving our filtering for successful candidates and are planning to generate a large library of our top protein designs, that we can then test for conotoxin binding in vitro. Upon identification of successful candidates, we will apply both machine learning and medicinal chemistry methods to improve their specificity through optimization of key residues. Further, we aim to use the initial hits to design a more general (set of) protein(s) that can bind to different alpha conotoxins and will be effective as a more general countermeasure.