Poster

Optimising GOLD pipelines for *in silico* PROTAC screening

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PROTACs (Proteolysis Targeting Chimeras) are bi-functional molecules which use the body's own protein degradation system to remove unwanted proteins. They bind an E3 ligase with the “anchor” end and the target protein with the “warhead” end with a linker joining the ends, thereby bringing the target protein into close proximity to an E3 ligase. The E3 ligase then (poly-)ubiquitinates the target protein, flagging it to be degraded by a proteasome. Although research is comparably limited [1], linker design is incredibly important to functional PROTAC development. Linker length, content, and attachment point can affect binding affinity, and even selectivity [1,2]. Therefore, this project aims to develop computational methods to help direct PROTAC linker design by improving computational protein dynamics predictions.

We are using the Cambridge Crystallographic Data Centre’s (CCDC) Genetic Optimisation for Ligand Docking (GOLD) software [3] for visualising and ranking PROTAC binding interactions with the target protein and ligase. Docking poses are scored by using many different methods (scoring functions) which use the ability to form hydrogen bonds, van der Waals interactions, and hydrophobic interactions among others to determine docking ability. We will evaluate and optimise these methods for use in understanding and predicting PROTAC ternary structure.

At the ECM34 conference, we will present how GOLD scoring functions among other methods could help to predict how different PROTAC linker designs could affect the PROTAC overall viability.

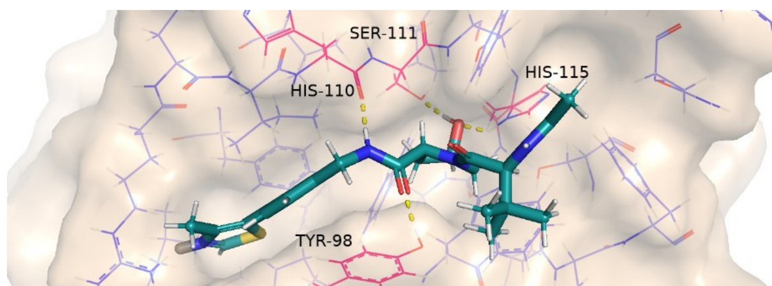

Figure 1. Docking pose of the experimental ligand, named VH032, binding to Von Hippel-Lindau tumour suppressor (pVHL, taken from PDB 4W9C [4]) predicted by GOLD software. The binding pose shows potential hydrogen bonds between VH032 and His110, Ser111, His115 and Tyr98 residues of pVHL (yellow dashed lines).

Our current method is to take a known protein-ligand structure, separate the protein from the ligand, dock the ligand back onto the protein in the given binding pocket, and compare the difference in pose (calculated by root mean square deviation, RMSD) between the docked ligand and the native pose. This has been done for many proteins including protein-DNA complexes. Although this is done via scripts, RMSD can also change depending on the native ligand and therefore user discretion is also necessary. Figure 1 shows the ligase we are currently using for PROTAC development. We have used this method to compare the shown ligand with other potential ligands to determine the most suitable anchor.

We hope that this will help improve the reliability of PROTACs, reducing the time taken validating each individual PROTAC's effectiveness *in vitro*.

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[2] Smith, B. E., Wang, S. L., Jaime-Figueroa, S., Harbin, A., Wang, J., Hamman, B. D. & Crews, C. M. (2019). *Nat Commun.*, **10**, 131.

[3] GOLD 2023.3.1. (2023). CCDC

[4] Galdeano, C., Gadd, M. S., Soares, P., Scaffidi, S., Van Molle, I., Birced, I., Hewitt, S., Dias, D. M. & Ciulli, A. (2014). *J. Med. Chem.*, **57(20)**, 8657-8663

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