MS8-P4 Unconventional Molecular Replacement approaches for ab initio phasing of transmembrane helical protein targets

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Transmembrane helical proteins are underrepresented in the PDB due to the experimental difficulties associated with their protein expression and crystallisation. This results in a small pool of structures being available for conventional homology-based Molecular Replacement (MR) and places a premium on the development of unconventional homology-independent approaches.

AMPLE is a pipeline for using unconventional models in automated MR. It has been particularly successful in its cluster and truncate approach to creating search ensembles from ab initio molecular models[1]. Recent work has focussed on exploring the viability of different approaches to the clustering and truncation. By exploring multiple clusters and using TM scoring, we have been able to double the success rate of AMPLE on a difficult set of test cases without an increase in processing time.

Using the new approach, and combining it with intermolecular contact prediction[2] to improve the modelling, we have been exploring the effectiveness of AMPLE on a selection of alpha-helical transmembrane proteins. Our work has demonstrated that AMPLE is a powerful method to use on this class of protein, outperforming a baseline approach based on short alpha-helices, and able to solve the majority of cases in our test set with an average runtime of less than two days on a single processor.

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2. Protein structure prediction from sequence variation. Marks, D. S., Hopf, T. A., & Sander, C. (2012). Nat. Biotechnol. 30, 1072–1080.

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MS8-P5 Structural basis for the regulation of the G protein coupled receptor Smoothened by its extracellular domains

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Developmental signals of the Hedgehog (Hh) and Wnt families are transduced across the membrane by Frizzled-class G-protein coupled receptors (GPCRs) composed of both a heptahelical transmembrane domain (TMD) and an extracellular cysteine-rich domain (CRD). How such large extracellular domains of GPCRs regulate signalling by the TMD is unknown. Here, I present crystal structures of the Hh signal transducer and oncoprotein Smoothened (SMO), which contains two distinct ligand-binding sites in its TMD and CRD. The CRD is stacked atop the TMD, separated by an intervening wedge-like linker domain. Structure-guided mutations show that the interface between the CRD, linker domain and TMD stabilises the inactive state of SMO. Unexpectedly, we find a cholesterol molecule bound to SMO in the CRD-binding site. Mutations predicted to prevent cholesterol binding impair the ability of SMO to transmit native Hh signals. Binding of a clinically used antagonist, vismodegib, to the TMD induces a conformational change that is propagated to the CRD, resulting in loss of cholesterol from the CRD-LD-TMD interface. Our work elucidates the structural mechanism by which the activity of a GPCR is controlled by ligand-regulated interactions between its extracellular and transmembrane domains.

Keywords: G protein coupled receptor, GPCR, Structural biology, Morphogen, Hedgehog signaling, Glycoprotein, Cancer mutations, X-ray crystallography, Lipidic cubic phae crystallization, Membrane protein SAXS