diamond

Drug Discovery

CASE STUDY

Pre-crystallisation screening for membrane protein crystallisation

Membrane proteins represent a large group of important drug targets with over 50% of approved drugs targeting these proteins within the body.

Understanding the 3D structure of membrane proteins is invaluable for target discovery, validation, and for hit optimisation. X-ray macromolecular crystallography (MX) is a principal research method for acquiring structural information on membrane proteins, but for this to be successful we require the production of good quality crystals from the target protein, or its complexes with potential drug candidates.

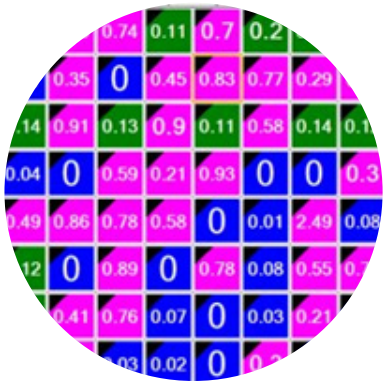
It is particularly difficult to produce and purify membrane proteins in sufficient yield and quality for crystallisation, and furthermore, membrane protein crystallisation itself represents a significant challenge.

The Challenge

Crystallisation of membrane proteins differs significantly from water-soluble proteins. To ensure successful handling of proteins outside the membrane, they must first be coated with detergent.

The crucial crystallisation approach used for preparation of membrane protein crystals is the *in meso* method using Lipidic Cubic Phase (LCP), the 3D network of a continuous lipid bilayer with water channels which mimics the membranes themselves. Membrane protein crystallisation requires screening of a very large number of potential crystallisation conditions, an exceptionally challenging and time-consuming process which is critically limited by the quantities of protein available.

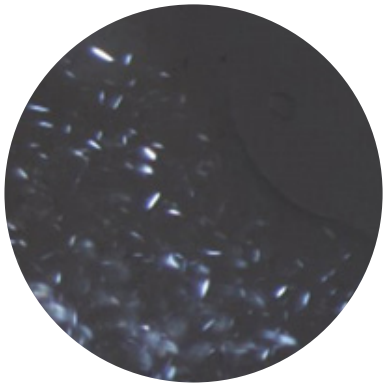
In order to obtain a membrane protein crystal in the LCP, the protein itself must be able to diffuse in LCP. Quantification of the protein diffusion is a critical parameter to monitor in order to determine formation of the LCP drop, protein aggregation and therefore chances for successful crystallisation.



The Solution

UCB used a technique called LCP fluorescence recovery after photobleaching (LCP-FRAP) at Diamond to help streamline their screening process. LCP-FRAP is an optical method designed to study the diffusion of labelled membrane proteins in LCP.

This high throughput procedure allows scientists to rapidly screen a wide range of LCP conditions, quantifying the diffusion of the protein in each condition in advance of *in meso* crystallisation.



The Benefits

The information acquired from using this research method demonstrates how easily a protein can diffuse within an LCP drop. It allows UCB to isolate any potential issues affecting crystallisation such as whether a protein is aggregated, or if the LCP structure has collapsed.

Proteins that do not diffuse will not crystallise, so the use of LCP-FRAP as a screening tool rapidly accelerates membrane protein structural projects by eliminating unsuitable conditions before conducting the lengthy process of crystallisation screening and optimisation.

"Since it is difficult to express and purify membrane proteins in high yields, it is crucial to carefully design the crystallisation experiments. Using the LCP-FRAP technique at Diamond, we are able to screen different membrane protein constructs and various host lipids and buffers to test protein mobility in LCP and select the most promising conditions for LCP crystallisation. As we don't perform new screens very often and don't have LCP-FRAP capabilities in-house it is very valuable for us to have access to Diamond's LCP-FRAP capabilities and expertise when needed."

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