Soutenance

THESE

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Visioconférence

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Optimization of crystal growth of membrane proteins for advanced diffraction techniques

Thèse de Doctorat de l'Université Grenoble Alpes

Membrane proteins are potential targets for drug design due to their vital biological functions. Crystallography is one of the techniques used to determine their 3D structure essential in understanding the protein function. However, obtaining high diffraction quality crystals of membrane proteins, whilst maintaining their native conformation during the production, purification and crystallization steps, is challenging.

Knowledge of the protein crystallization phase diagram allows the quality of the crystals to be controlled and optimized by adjusting physico-chemical parameters such as the temperature or the chemical composition of the crystallization solutions. Automated dialysis-based crystallization with variation of the temperature and composition of the crystallization solution, using a recently designed crystallization bench, enabled to screen different crystallization conditions using a single sample as well as to optimize the crystal growth in a reversible manner. The mass transport of detergents across semi-permeable membranes was also studied to establish effective strategies for optimizing the crystallization of membrane proteins using this technique.

This rational approach to crystallization was applied to the membrane proteins Acriflavine resistance protein B from *Escherichia coli* and TonB-dependent heme outer membrane transporter from *Shigella dysenteriae*. The purification of these proteins for crystallization was optimized. The purified proteins were then crystallized using the different crystallization techniques, identifying the dialysis method as the most successful and consistent although comparable crystals were obtained with almost all techniques, but not always reproducible. AcrB crystals co-crystallized with rifampicin were used for systematic X-ray diffraction studies and the best statistics for the AcrB-rifampicin crystal from each method were compared. By co-crystallization of ShuA with europium (III) chloride, the 3D structure of this protein was solved at 2.49 Å. The results also showed that the purity of the protein sample and well-chosen additives improve the crystal quality. Finally, preliminary studies using MicroED have been performed for our two model membrane proteins co-crystallized with additives.

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