Universität Basel

Research Project

High-Resolution Membrane Protein Structures by Cryo-EM

Third-party funded project

Project title High-Resolution Membrane Protein Structures by Cryo-EM
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Membrane proteins are central to health and disease. Membrane proteins operate within the lipid membranes of cells, where they are responsible for a plethora of functions. They facilitate or regulate transport of molecules across the membrane, sense ligands or membrane potential or buffer gradients, and nourish and protect the cell. Cryo-transmission electron microscopy (cryo-EM) classically allows studying the high-resolution structure of membrane proteins, if these are lipid membrane-reconstituted and two-dimensionally crystallized. This method is called electron crystallography of membrane proteins. My group has developed a software package for the analysis of 2D crystal images, called the *2dx* package. *2dx* has become the leading software tool for electron crystallography.

Cryo-EM of isolated single particles has seen a major breakthrough recently from the introduction of direct electron detector (DED) cameras and the development of improved single particle image processing algorithms. Single-particle cryo-EM allows to rapidly determine the atomic resolution structure of detergent-solubilized membrane proteins without the need for crystallization. However, single particle cryo-EM is limited to larger particles and does not allow the direct study of membrane proteins in a full lipid membrane, as electron crystallography provides.

In this project, we will extend the *2dx* software suite to accelerate processing, to automate processing, to increase resolution of the 3D reconstructions, and to make this method largely independent of highly ordered 2D crystals, so that also membrane-reconstituted membrane proteins that are not –ăor only partly –ăin a crystalline arrangement can be studied at high resolution.

This will be achieved by developing novel image processing algorithms and tools to apply iterative refinement procedures to 3D structure reconstructions, and by adopting some of the recent algorithmic developments in the single particle field such as gold-standard FSC for reliable resolution estimation or drift-correction for dose-fractionation into the *2dx* electron crystallography software suite. Finally, we will implement an interface of the *2dx* package to leading single particle software suites such as RELION and/or FREALIGN, and adapt the latter to the specific settings of cryo-EM data of membrane-embedded membrane proteins, because these programs so far are not suited to deal with membrane-embedded particles.

For development purposes, a large high-resolution dataset of images of a potassium channel membrane protein system is available. These images are recorded of small 2D crystals that diffract only to about 12 Å resolution. Preliminary application of novel algorithms has already shown significant resolution improvements, so that the helical pitch of alpha helices and some side-chain densities are recognizable in the 3D reconstruction.

The *2dx* software will continue being available open-source under the GPL at 2dx.org, and we will organize regular workshops for knowledge transfer and planning of further development targets.

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