MS42- New approaches to structure solution by crystallography and CryoEM: computational features and new algorithms

Chairs: Prof. Isabel Usón, Dr. Tom Burnley

MS42-P01

A modeling study for cholesterol binding proteins, NPC family protein

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Understanding of the detailed dietary cholesterol exchange in cellular environment still remains as an open subject. The mutation of one of NPC1 (Niemann-Pick type C1) and NPC2 (Niemann-Pick type C2), which are main players of cholesterol control in lysosome, leads to disease, called Niemann-Pick disease type C (NPC) disease. Meanwhile, the transmembrane protein NPC1L1 (Niemann-Pick type C1 like 1), which is related to the dietary cholesterol absorption process in small intestine, shares sequence homology with NPC1. However, unlike NPC1, NPC2 is not involved in cholesterol internalization with NPC1L1. The structure of NTD of NPC1 in complex with cholesterol (PDB id: 3GKH and 3GKI) was known while only cholesterol free NTD (PDB id: 3QNT) is known for NPC1L1. It is noted that the whole cryo-EM structure of NPC1 is determined (PDB id: 3JD8).

We compared the cholesterol complex of NPC1L1 and NPC1 with molecular docking followed by molecular dynamics study for better understanding of these underlying behavior. The NTD molecular dynamics simulation of NPC1 and NPC1L1 complex with cholesterol shows different structural and dynamical features. The difference in cholesterol internalization mechanism between NPC1 and NPC1L1 must be closely related to these structural and dynamical behaviors. We believe the current study can contribute understanding of cholesterol absorption/re-absorption process via NPC1 and NPC1L1 and their difference with atomic detail.

References:

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MS42-P02

Expanding partial structures by assembling most probable side chain composition

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Knowledge of biological structures and their interaction plays an essential role in the comprehension of biological mechanisms. Crystallography and recently CryoEM provide macromolecular models in atomic detail. CryoEM requires the interpretaion of a map of heterogeneous resolution, while crystallography requires to overcome the bottleneck of phasing. Phasing through multisolution placement of ubiquitous small fragments, such as alpha-helices and beta-strands, with PHASER [1] followed by density modification and tracing with SHELXE is now established as an effective method to solve the phase problem, provided the structure is not too large and data to high resolution are available [2]. When the resolution limit of the diffraction data does not reach 2 Å, and if the helical content is low, fragments may still be correctly placed, but density modification and autotracing often fails to reveal the rest of the structure. The process stalls as no additional features develop in the map, whether in the extension of the polypeptide chain or in side chain electron density. SEQUENCE SLIDER is being developed to extend the initial and intermediate partial models by assembling multiple models from the most probable side chains into the current fragments. As secondary structure prediction of residues from sequence is reliable, this information is used to restrict possibilities matching the secondary structure of residues of the partial model and traces. Moreover, additional filters are applied based on free energy calculation using crystal contacts and assignment of hydrophobic and hydrophilic residues to protein cores and surface, respectively. Models with different side chain assignment are assembled with SCWRL4 [3] and may be modified with refinement. Extended partial structures with the best indicators are pushed on to density modification and tracing with SHELXE. Specific strategies of SLIDER have been designed within ARCIMBOLDO_SHREDDER and in the coil coiled mode. Three novel structures and other test cases determined with SLIDER are presented.

References:

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