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## Applications of Cryo-Electron Microscopy in Structural Biology

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For many years, cryo-electron microscopy (Cryo-EM) was used only to determine low-resolution density maps of large proteins structure. However, thanks to recent technological developments, the resolution achievable with Cryo-EM reaches the same values as those obtained with X-Ray crystallography. Moreover, Cryo-EM can study flexible and functionally active macromolecules, which are impossible to study using X-Ray crystallography.

Below are presented the structures of four different protein complexes which, due to their flexible and heterogeneous nature, could not be solved without the aid of cryo-EM

Campylobacter jejuni serine protease HtrACj is implicated in bacterial virulence, stress response, proliferation, migration, and cell fate. We took a cryo-EM approach to understand how its unique architecture is responsible for a remarkable structural and functional plasticity that allows cells to respond to misfolded or mislocalized polypeptides rapidly. The 3D reconstruction pictures HtrACj as a dodecamer built of four trimers. The contact between the trimers is loose, which explains the dodecameric assembly's flexibility and mobility. Such flexibility allows the opening of the dodecamer to expose the proteolytically active site of the protease.

Tetanus neurotoxin (TeNT) is the bacterial protein toxin that causes tetanus. Although a vaccine exists for tetanus, it remains a health concern owing to the observations of decreasing anti-TeNT antibodies-load in human serum after the vaccination, implying the need to look for other therapeutics. Therefore, we explored the possibility of neutralizing TeNT using human-derived fabs. The structure of the TeNT-Fabs complex shows the relative movement of the toxin domains and the Fab bounding. Identifying new TeNT epitopes will help in antibody design to block the insurgents of tetanus.

Photosystem II-LHCII is a protein complex of the thylakoid membrane involved in photosynthesis and is formed by one or more LHCII trimers that bind in a stable (S) or moderate (M) manner to the PSII dimeric core (C2). To study the PSII-LHCII light acclimation, we collected two different datasets of PSII-LHCII grown in low and high-light conditions, which led respectively to a map of the stacked (C2S2M2)2, (C2S2M)2, and (C2S2)2 conformation, confirming that M trimers decrease with the increasing of light exposure. Furthermore, all these maps show the presence of flexible connections between the two PSII-LHCII and a different rotational offset between the two PSII-LHCII of the stacked conformation around the membrane plane's normal vector, responsible for modifying their interaction and energetic connectivity.

Potato virus X (PVX) is a flexible filamentous plant virus from the Alphaflexviridae family that infects herbaceous plants. While PVX has a detrimental impact on the global potato economy, PVX can be genetically engineered to be used as a scaffold for nanotechnology or nanomedicine applications. The 2.2 Å resolution map, obtained using helical reconstruction, provides near-atomic details of the interaction between its coat-protein and the genomic RNA, thereby opening the avenue to the design of antiviral compounds. Furthermore, the structure of the virus particle shows that the N-terminal region is flexible and exposed to the solvent, suitable for the transport and delivery of epitopes.

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