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Single particle cryo-EM of membrane proteins in lipid nanodisc

Yifan Cheng[1]
[1]*HHMI, Biochemistry & Biophysics, UCSF, San Francisco, United States*
E-mail: ycheng@ucsf.edu

In the last few years, major technological breakthroughs, particularly the development of new direct electron detection cameras and associated technologies, have enabled single particle cryo-electron microscopy (cryo-EM) to become the technique of choice for high-resolution structure determination of many challenging biological macromolecules. Atomic structures of many membrane proteins, particularly ion channels, that are refractory to crystallization have now determined by this method, including our previous work of determining the atomic structures of TRPV1 and TRPA1. In most of these studies, membrane proteins were solubilized in detergent, or detergent-like amphipathic polymers ("amphipols"), with a few exceptions. However, for many ion channels, and integral membrane proteins in general, maintaining purified proteins in a near-native lipid bilayer environment is crucial for visualizing specific and functionally important lipid–protein interactions, and more importantly, for maintaining protein functionality. The next technical challenge for single particle cryo-EM studies of membrane proteins is therefore to enable atomic structure determination of integral membrane proteins in a native or native-like lipid bilayer environment.

Lipid nanodisc technology uses membrane-scaffolding proteins (MSP) to reconstitute integral membrane proteins into lipid nanoparticles. This highly native-like system is the first choice for a general platform for single particle cryo-EM of membrane proteins. Recently, we tested the feasibility of using lipid nanodisc in atomic structure determination of relatively small integral membrane proteins, such as TRPV1. We reconstituted TRPV1 ion channel in lipid nanodiscs, and determined atomic structures of nanodisc-embedded TRPV1 in three different conformations. These structures revealed locations of some annular and regulatory lipids that form specific interactions with the channel. Such specific phospholipid interactions enhance binding of a spider toxin to TRPV1 through formation of a tripartite complex. Our structures also reveal that, in the absence of vanilloid agonist, a phosphatidylinositol lipid occupies the capsaicin-binding site of TRPV1, providing important clues about physiological mechanisms of channel regulation.

Keywords: Single particle cryo-EM, Membrane protein