doi:10.1017/S1431927617004779
Microsc. Microanal. 23 (Suppl 1), 2017

Ion Channel in Lipid Nanodisc By Single Particle Cryo-EM – Pushing The Technology Limit

Daniel Asarnow[1], Eugene Palovcak[1], Yuan Gao[1], David Julius[2] and Yifan Cheng[1,3]

[1.] Department of Biochemistry and Biophysics, University of California San Francisco, CA, USA
[2.] Department of Physiology, University of California San Francisco, CA, USA
[3.] Howard Hughes Medical Institute, University of California San Francisco, CA, USA

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. In membrane protein structural biology, atomic structures of many 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 [1,2,3]. In most of these studies, membrane proteins were solubilized in detergent, or detergent-like amphipathic polymers ("amphipols"), with a few exceptions [4]. 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 [5]. This highly native-like system is the first choice for a general platform for single particle cryo-EM of membrane proteins. However, a major technical concern has been that the unstructured mass of the lipid bilayer could hinder accurate image alignment. Previous applications of lipid nanodiscs in single particle cryo-EM were thus limited to particles with relatively large soluble domains [4,6], and it was generally believed that such domains were necessary to drive accurate image alignments for high-resolution structure determinations.

Recently, we tested the feasibility of using lipid nanodisc in structural studies of relatively small integral membrane proteins, such as TRPV1 [7]. 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. These structures demonstrated that lipid nanodisc can be used to study membrane proteins with relatively small soluble domains.

The next question is if the nanodisc system can be used for single particle cryo-EM studies of integral membrane proteins that have no soluble domain, and are completely embedded in the lipid bilayer. We tested this possibility by determine a three-dimensional (3D) reconstruction from synthetic particle images generated from our cryo-EM dataset of nanodisc-embedded TRPV1 in complex with ligands. For every particle image, we computationally subtracted all soluble domainsof TRPV1, both intracelluar

and extracellular. All particle images in this synthetic dataset therefore contain only the transmembrane core of TRPV1 completely embedded in the lipid nanodisc. As a control, we calculated two-dimensional (2D) class averages using orientation parameters determined from the original dataset where all particles contain both soluble and transmembrane domains (Figure 1A). Indeed, there are no visible major soluble domains in all 2D class averages, demonstrating that there is no major residual density from soluble domains in the subtracted particles that could still facilitate image alignment. We then discarded the known orientation parameters of all particles, and determined a *de novo* 3D reconstruction from this synthetic dataset following the gold standard refinement procedure (Figure 1B and C). The resolution estimated from Fourier Shell Correlation (FSC) = 0.143 criterion is 3.3 Å, compared with the 2.9Å resolution of the original dataset. Notably, the total protein mass from TRPV1 in this synthetic dataset is merely ~90kDa, and the unstructured mass from MSP and lipids are estimated as about 200 kDa in total.

Although the dataset used in this study is not entirely experimental, our test does demonstrate that even for membrane proteins without soluble domains, it is possible to determine a high-resolution structure from images of particles entirely embedded in lipid nanodisc [8].

References:

[1] M Liao, E Cao, D Julius and Y Cheng, *Nature* **504** (2013) 107.
[2] E Cao, M Liao, Y Cheng and D Julius, *Nature* **504** (2013) 113.
[3] CE Paulsen, *et al,* Julius, *Nature* **520** (2015) 511.
[4] RG Efremov, *et al*, *Nature* 517 (2015) 39.
[5] TH Bayburt, YV Grinkova, and SG Sligar, *Nano Lett*. **2** (2002) 853.
[6] J Frauenfeld, J. *et al*. *Nature Struct. Mol. Biol.* **18** (2011) 614.
[7] Y Gao, E Cao, D Julius and Y Cheng, *Nature* **534** (2016) 347.
[8] The authors acknowledge the funding from National Institute of Health (R01NS047723, R37NS065071 and R01NS055299 to DJ, S10OD020054, R01GM098672, P01GM111126 and P50GM082250 to YC). YC is an Investigator of Howard Hughes Medical Institute.

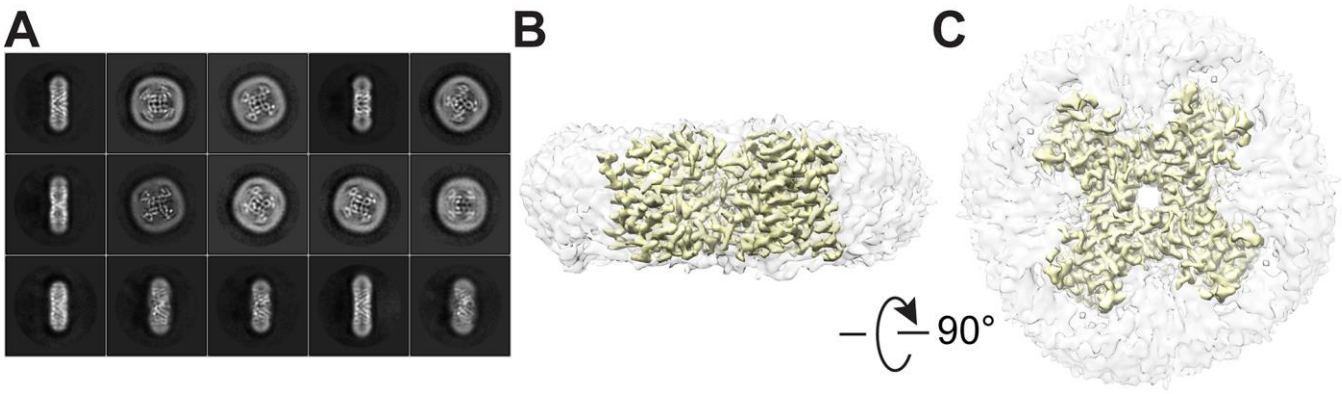

Figure 1. 3D reconstruction of TRPV1 transmembrane segment. 2D class averages (**A**) of nanodisc embedded TRPV1 particles with soluble domain subtracted from the original particle images were calculated by using the classification and alignment parameters determined from the original dataset. 3D reconstruction is shown in its side (B) and top (C) views. Transparent density shown with a low isosurface value corresponds to nanodisc. Solid density represents the transmembrane domain of TRPV1 ion channel.

https://doi.org/10.1017/S1431927617004779 Published online by Cambridge University Press