Review Report – "Connexin-46/50 in a dynamic lipid environment resolved by CryoEM at 1.9 Å", Flores et al.

Summary

In the article (*1*), the authors wanted to determine how the local dynamic environment influences the gap junction structure and function. A gap junction is an assembly of proteins belonging to the family of Connexin and are particularly known for their key role in the metabolic or electrical coupling of 2 adjacent cells.

In this study, the researchers solved in particular the structure of gap junctions made out of Connexin-46/50 (Cx46/50) in an artificial environment designed to mimic the native one. To achieve this, they constructed an artificial environment using self-assembling lipid nanodiscs into which the isolated and purified channels were reconstituted. Having prepared the reconstructed channels, the researchers performed a high resolution single-particle electron cryo-microscopy (CryoEM). The measurements resulted in a high-quality CryoEM map allowing for a reconstruction of the structure with a resolution of 1.9 Å. Along with the found structure, molecular dynamics (MD) simulations to build systems for Cx46/50 subject to various solvent has been performed. The principle of MD is to first start from an initial system containing the structure and the solvent, then the forces acting on each atom is determined and from physics principle the velocity and acceleration are computed and used to update the position of the atoms until equilibration and energy minimization (*2*).

Thanks to CryoEM, the researchers were able to solve not only the structure of the Cx46/50, but also the interacting non-protein components. Along with the found CryoEM map, they confirmed the interactions observed by looking at the MD simulations. They observed that there were water molecules stabilized with H-bonds in specific key regions of the Cx46/50 contributing to the selectivity and gating mechanism. The suggestion made is that these water molecules may be part of the stabilization and function of these regions. They also found out that there may be specific interactions of the Cx46/50 with lipids at the extracellular leaflet and non-specific lipid interactions at the intracellular leaflet. Finally, the researchers observed that although the acyl-chains of the lipids are ordered and stabilized, this is not the case for their phosphatidylcholine (PC) heads which suggest these groups to be either dynamic or non-specifically interacting with the environment or the protein.

Limitations

One thing that is not clear to me is why the researchers specifically decided to study the Cx46/50 junctions. Maybe they were already studying this specific junctions before and had more data on these junctions before doing the research or maybe there was more papers on these specific junctions.

The fact that the PC groups as well as the non-stable domains are not resolved in the CryoEM map and in the MD map illustrates the limits of those techniques relying on stable and non dynamic molecules. Indeed, being able to also resolve these structures

could also lead to some crucial information as they may have important functional or stabilizing features. However, and they used this reasoning in the paper, the fact that these molecules are not resolved despite the sufficient resolution of the used technique is also an information about these molecules. It means that these molecules are either dynamics, non stable or have multiple conformations.

CryoEM captured the Cx46/50 junction in open-state conformation then all the analysis of the junction interactions with their environment made in this paper may not be the same if the CryoEM was done in another conformational state, because the stabilization of the molecules may have been different. Moreover, the researchers did not specify why the open-state conformation was captured and not another state. Was it by choice or on the contrary was it the only state they successfully reconstructed ?

This technique may not be reproducible with other transmembrane proteins because making an artificial environment mimicking the native one might be more difficult depending on the chosen protein. The critical step of self-assembly could also fail maybe due to a strong denaturation of the protein during the purification step. The environment for the same transmembrane protein may also be various and then the conclusions drawn in one type of cell located at a specific place may be very specific and not generalizable.

Strengths

In my opinion, one of the main strength of this study is the reconstruction of an artificial environment mimicking the native one. Even if the reconstruction is probably not perfectly representative of the native one, it allows the researchers to observe the interactions of a protein with a specific environment with a high resolution which is often not possible with purified proteins observed *in vitro*. Additionally, the fact that the researchers assessed the reconstruction by size-exclusion chromatography (SEC) and negative-stained electron microscopy allowed to confirm that their technique yields to a viable reconstruction of the junctions which is an important and necessary control.

Moreover, the use of CryoEM not only for protein but also for non-protein components seems to be less used. This paper seems then to remind that the possibility to use of CryoEM not only for conventional *in vitro* protein structure determination or protein-protein interactions but also to study other types of interaction such as protein-lipid interaction can be a very useful tool.

Conclusion and future applications

Most of the method and analysis of this article seems appropriate to me. Although the method they used was very specific, it is also very promising. Reconstruction of native environment *in vitro* allowed to use high resolution imaging technique to study the protein interaction with its surrounding molecules. However, the main limitations are the ability to reconstruct an environment sufficiently representative of the native one *in vitro* and also the ability to characterize the dynamic or non-stable molecules.

References

(*1*) Flores, J. A., Haddad, B. G., Dolan, K. A., Myers, J. B., Yoshioka, C. C., Copperman, J., Zuckerman, D. M., and Reichow, S. L. (2020). Connexin-46/50 in a dynamic lipid environment resolved by CryoEM at 1.9 Å. *Nature Communications 11*, 4331.

(*2*) Hospital, A., Goñi, J. R., Orozco, M., and Gelpi, J. L. (2015). Molecular dynamics simulations: advances and applications. *Advances and Applications in Bioinformatics and Chemistry 8*, Publisher: Dove Press, 37–47.