PERSPECTIVES

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And they're out of the gate…

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We have entered a golden age for the structural biology of gap junction-like proteins. The first high-resolution structure of a connexin was published in 2009 (Maeda *et al.* 2009). It took nearly 10 years for the next advance, which occurred only after cryoEM had matured to become the method of choice to determine the structures of membrane proteins. Recently, and in quick succession, high-resolution structures for Innexin6, Pannexin1, CalHM2, Cx46 and Cx50 (Myers *et al.* 2018) and Cx31.3 (Lee *et al.* 2020) have been published. To continue my horse-racing analogy, the most recent structures for Cx46/50 (Flores *et al.* 2020), at a resolution of 1.9Å, narrowly lead the field.

In the gap junction configuration, connexins form a dodecameric complex consisting of two hexameric channels docked end to end. Each connexin subunit has four transmembrane segments, and an intracellular C-terminus. The helical N-terminus resides within the pore. As there are six of these N-termini, a long-standing idea has been that they form a plug that gates the channel. The paper by Yue *et al* (2021) published in this issue of the *Journal of Physiology* has made substantive steps towards detailed mechanistic understanding of channel gating by this N-terminus in Cx46/50 at an atomic level.

CryoEM provides snapshots of structure. Although some dynamic information can be gleaned directly from cryoEM data, molecular dynamics (MD) simulations, by bringing life into what would otherwise be static images, help to reinforce the link between structure and function. MD takes into account the movements of residues within the constraints of the tertiary structure, and the energies of, and thus likelihood of, interactions between residues to produce a dynamic simulation of how they jockey for position. Clearly the better the quality of the input structure, the better the output from the MD. Fortunately, the residues in the N-terminus of Cx46/50 are well resolved even in the 3.4Å structures used for this study, and this provides the basis for the insights gained in the current paper. As the authors had two structures of the very closely related Cx46 and Cx50 at their disposal, they swapped the N-termini of Cx46 and Cx50 to make chimaeric structures and compared the sequences of the N-termini to identify differences and thus make the relevant mutations that could alter function.

While they simulated many different variants, their studies on the ninth residue (Asn in Cx50 and Arg in Cx46) are highly interesting. Their MD simulations revealed that in Cx46, Arg9 can form a salt bridge with Glu12 of the N-terminus of the neighbouring subunit and this was a predominant conformation in the simulations. An alternative conformation also existed (but less frequently), in which a pi-bond with Arg9 of the neighbouring subunit stabilized an interaction of Arg9 and Glu12 within the N-terminus of the same subunit. Obviously, these interactions are not present for the N-termini of Cx50 where the uncharged residue Asn9 is present instead. However, they are present to some degree for Cx50 where Asn9 has been mutated to Arginine. The net effect of these interactions is to move Arg9 to the side of the pore away from the permeation pathway, rather than sticking into the centre of the pore as modelled in the cryoEM structure. The free energy experienced by ions as they traverse the channel pore can also be calculated in MD simulations. The authors found that the free energy landscape for Cl^- is fairly constant (and similar) throughout the pore in both Cx46 and 50. However, the N-terminus of Cx46, but not Cx50, offers a significant barrier to K^+ permeation. Despite the fact that Arg9 is pressed against the side of the pore in the MD simulations, introduction of the Asn9Arg mutation in Cx50 gave a free energy barrier profile for K^+ very similar to that of Cx46. This suggests that Arg9 is an important residue for determining ion permeation and channel conductance. While this brief summary does not do full justice to the range of simulations they performed, it gives a flavour of their strategy and importantly the type of testable predictions that they made for the effect of these mutations on gap junction gating and single channel conductance.

In the second part of the paper, the authors set about testing these predictions with patch-clamp recording methods of gap junction variants formed between pairs of coupled cells. For me, the highlights are the recordings of single gap junction channel gating between cells. They show that mutating the ninth residue (from Arg to Asn in Cx46 and from Asn to Arg in Cx50) has an effect on single channel conductance that qualitatively matches the predictions from the MD simulations. They also quantified the dwell time in the fully open state and found that it was altered by either swapping the N-termini between channels or mutating the ninth residue. This key result shows that interactions between the N-termini influence the kinetics of channel gating. There will of course be other structural determinants of gating such as hydrophobic interactions between the N-termini and residues in TM2, also described by the authors. These other interactions probably explain why the effect on open state dwell time of putting the N-terminus of Cx46 into Cx50 is not the same as the Asn9Arg mutation.

It would be remiss not to mention that the MD simulations cannot explain all the empirical features observed by the authors. In their discussion of this, the authors give various possible explanations and point out the need for further experimentation. I for one eagerly look forward to their next outing. There are many fascinating questions to address, such as the basis of ion selectivity and the kinetics of physiologically relevant modes of channel gating.

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Additional information

Competing interests

No competing interests declared.

Author contributions

Sole author.

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