Research Week 2020

Visualization of Intercellular Communication Channels in a Dual-Lipid Bilayer at Near-Atomic Resolution by cryoEM

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Keywords

CryoEM, Gap Junction, Lipid, Nanodisc

Abstract

Intercellular communication by the gap junctions is facilitated by a unique macromolecular architecture, in which transmembrane channels directly couple the plasma membranes of neighboring cells. In each membrane, six connexin (Cx) subunits oligomerize to form a "hemichannel". Docking between extracellular (EC) domains of two hemichannels forms the complete gap junction intercellular channel. Our current understanding of gap junction structure lacks crucial information about how these channels interact with their native phospholipid environment. To address this gap in knowledge, we used lipid nanodisc technology to incorporate native connexin-46/50 (Cx46/50) intercellular channels into a dual-lipid bilayer—closely mimicking a native cell-to-cell junction. Structural characterization of lipid-embedded Cx46/50 revealed a dramatic lipid-induced stabilization to the overall channel architecture. The cryoEM images were refined to 1.9 Å resolution, providing an unprecedented level of detail for this class of protein. The connexin subunit interactions within each hemichannel are supported by clusters of hexagonally-packed lipid acyl chains. Remarkably, the lipid stabilization is exclusive to the EC leaflet and extends well beyond the annular lipid shell, indicating that Cx46/50 binds to and stabilizes lipids specifically at the EC leaflet. In addition, nearly 400 water molecules are resolved throughout the channel, which appear to cement the rigid extracellular docking interactions and stabilize the architectural integrity of these channels. 3D heterogeneity analysis of the cryoEM images identified three distinct, yet overlapping, classes of annular phospholipid headgroup configuration. We found by all-atom equilibrium MD-simulation that many states, including those captured by cryoEM, are in rapid-exchange, indicating that Cx46/50 is stabilized by a non-specific and dynamic lipid environment. These findings demonstrate the dramatic influence of the phospholipid membrane on the structure of Cx46/50, and establish a model system with which to investigate the influence of lipid composition on gap junction structure.