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Free energy analysis of selective cation transport in the channelrhodopsin chimera, C1C2

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Cation-conducting channelrhodopsins (ChRs) are a popular tool used in optogenetics to control the activity of excitable cells and tissues using light. ChRs with altered ion selectivity are in high demand for use in different cell types and other specialized applications. Nevertheless, a detailed mechanism of ion permeation in ChRs is not fully resolved. Here, we use computational methods to uncover the mechanisms of cation transport and valence selectivity through the channelrhodopsin chimera, C1C2. Electrophysiology measurements identified a single-residue substitution within the central gate, N297D, that increased Ca^{2+} permeability vs. Na^{+} by nearly two-fold at peak current. We calculated free energy profiles for Na^{+} and Ca^{2+} permeation through each protein using well-tempered/multiple-walker metadynamics. Results of these studies agree well with experimental measurements and demonstrate that the pore entrance on the extracellular side differs from original predictions and occupies a gap between helices I and II. Cation transport occurs via a relay mechanism where cations are passed between flexible carboxylate sidechains lining the full length of the pore by sidechain swinging, like a monkey swinging on vines. In the mutant channel, residue D297 enhances Ca^{2+} permeability by mediating the handoff between the central and cytosolic binding sites via direct coordination and sidechain swinging. This work advances our understanding of ion selectivity and permeation in cation channelrhodopsins and provides insights important to developing new ion-selective optogenetic tools.

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