Modeling of the open-channel structure of MscL using restrained simulations

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Mechanosensitive channels are membrane proteins that act as safety valves to protect bacterial cells from sudden osmotic shock. The gating is induced by tension in the surrounding lipid bilayer and the channel undergoes a large conformational change during the transition from closed to open. The structure of the mechanosensitive channel of large conductance (MscL) in the closed state has been solved by XRD (Chang *et al.*, 1998). The protein has been characterized using EPR (Perozo *et al.*, 2002) and FRET (Corry *et al.*, 2005) spectroscopy but a detailed structure of the channel in the open state is still unknown. Computational modelling of MscL is challenging as the gating transition spans several time and lengths scales.

In this study we present a method for incorporating structural data from EPR and FRET experiments into a coarse grained model of the MscL. The simulations system consisted of a solvated MscL protein, embedded in a lipid bilayer, and was modelled using the MARTINI force field (Marrink *et al.*, 2007). Restraints based on solvent accessibility from EPR data were implemented by altering the interactions of specific residues with water and lipid particles. Distance restraints between specific residues were implemented using harmonic potentials. A series of MD simulations of at least 1μs with different combinations of restraints and membrane tension were carried out. Restraints were slowly introduced to induce the opening of the channel. The simulations produced a set of open channel structures that were analysed using a range of structural features such as pore radius and helix tilt.

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