Monitoring the conformational changes involved in MscL channel gating using FRET microscopy and simulation

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Large scale conformational changes often play an essential role in the functioning of proteins, yet they can be hard to probe with experimental methods and take place over timescales that are difficult to simulate. We are using a combination of low resolution experimental techniques in combination with a variety of computational methods to try to understand the large structural rearrangements taking place in the gating of the mechanosensitive channel MscL. These bacterial channels open large pores in response to membrane tension in order to rescue the cell under osmotic shock. In this case we gain structural data on the protein in a natural environment using patch-clamp studies and confocal FRET microscopy. Combining this with existing EPR data as restraints in molecular and coarse grain simulations has allowed us to determine the likely structure of the open state of the pore. While there are many challenges to be overcome in this methodology, including careful approaches to labelling; control of the protein state; careful analysis of the orientation, geometry and number of fluorescent probes; and rigorous sampling of the conformational space; we believe that it provides a useful tool for studying the structures of a range of membrane proteins in natural environments.