Conformational changes involved in MscL channel gating measured using FRET spectroscopy

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Transmembrane channels facilitate the movement of small molecules and ions across cell membranes. Obtaining detailed structural information about such proteins has been difficult, due not only to the complications of crystallisation, but also because they adopt multiple conformational states that are not easy to probe with static x-ray images. We demonstrate that fluorescence resonance energy transfer spectroscopy (FRET) is a powerful tool for *in situ* structural analysis of multimeric membrane proteins by measuring the conformational changes involved in gating the mechanosensitive ion channel MscL.

MscL channels act as safety valves in bacterial cells, opening wide pores to prevent cell death during hypo-osmotic stress. The MscL protein monomers are fluorescently labelled by randomly attaching AlexaFluor 488 (AF488) and AlexaFluor 568 (AF568) to a single cysteine residue introduced *via* site directed mutagenesis. As the channel protein is a pentamer, this mutation introduces five identical cysteine sites each equally likely to be occupied by AF488 or AF568. The protein is reconstituted into artificial phosphatidylcholine liposomes and imaged in a laser scanning confocal microscope. The channels are normally closed in their resting state, but may be forced into the open conformational state with the addition of lysophosphatidylcholine (LPC) which inserts in the outer layer of the liposome membrane bilayer promoting membrane curvature and/or a change in the transbilayer pressure profile, thus leading to channel opening.

The liposomes are imaged in both AF488 (donor) and AF568 (acceptor) emission bands before and after bleaching of the acceptor AF568. The intensity of the AF488 donor emission increases after bleaching indicating FRET is taking place.

The proportion of energy being transferred from the donor to the acceptor is then related to the pentamer radius in both the closed and open channels using a Monte-Carlo ensemble analysis program. This accounts for each channel protein containing a random mix of five donors and acceptors and the fact that energy transfer could arise between fluorophores attached to different proteins.

As illustrated schematically in the figure, we find that the diameter of the fluorescently labelled MscL channel increases by 16Å upon activation, creating a large pore and representing one of the largest known conformational changes in membrane proteins.