Figures and figure supplements

Single molecule FRET reveals pore size and opening mechanism of a mechano-sensitive ion channel

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Figure 1. Cartoon representation of the structure of MscL in the closed conformation in the (A) top view and (B) side view (PDB ID: 2OAR [Chang et al., 1998; Steinbacher et al., 2007]), and scheme of single molecule FRET setup. MscL is a homo-pentamer consisting of five identical subunits. Each subunit consists of one cytoplasmic α-helix (CP) and two transmembrane α-helices (TM1 and TM2), which extend through the cell membrane and are joined by a periplasmic loop (Chang et al., 1998). (C) Residues measured using smFRET. Three residues on each of the transmembrane helices (M42C, A27C and I25C on TM1; Y75C, Q80C and V82C on TM2) were chosen. Note that no residues on the CP were chosen because the complete deletion of the CP does not change the gating parameters substantially (Anishkin et al., 2003). (D) Labeled MscL proteins were reconstituted into liposomes, which were then immobilized on a coverslip and used for smFRET experiments. (E) The addition of LPC traps the protein in the open conformation (Perozo et al., 2002b).
Figure 2. The opening models for MscL. The MscL opens from (A and B) the closed state, to (C and D) the open state via the barrel-stave model or (E and F) the open state via the helix-tilt model. The top figures (A, C, E) are top views and the bottom figures (B, D, F) are the side views. TM1 helices are shown in red while TM2 in blue. In the barrel-stave model (C and D), TM1 swings away from the pore center but TM2 remains stationary upon channel activation, generating an open pore lined by both TM1 and TM2 and the helices are more parallel to the membrane normal than the membrane plane. In the helix-tilt model (E and F), both TM1 and TM2 swing away from the symmetry axis and both helices tilt toward the plane of membrane.

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Figure 3. Single molecule FRET results for MscL M42C. The distribution of FRET efficiency of M42C in the (A) absence and (B) presence of LPC were plotted and fitted with Gaussians. (C) The difference between the normalized FRET distributions under the two conditions (±LPC), $\Delta P$, emphasizes the diminishing of the third peak at $E \sim 0.6$ after adding LPC. (D) The variance between the normalized FRET distributions under the two conditions (±LPC), $\Delta P^2$, decreases as the fraction of BPE in the liposomes is increased from 2% to 16%. (E) Histograms of FRET efficiencies in the absence (top row, −LPC) and presence (bottom row, +LPC) of LPC for the other five residues (I25C, A27C, Y75C, Q80C, and V82C) measured in the current study.

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**Figure 3—figure supplement 1** Examples of FPLC traces and SDS-PAGE gel for purification of MscL. DOI: 10.7554/eLife.01834.006

**Figure 3—figure supplement 2** Examples of fluorescent intensity traces showing (A) a single photobleaching step, (B) multiple photobleaching steps, and (C) a single photobleaching step but the acceptor photobleached first. DOI: 10.7554/eLife.01834.007

**Figure 3—figure supplement 3** FRET efficiencies between non-neighboring subunits ($E_{i,j}$). DOI: 10.7554/eLife.01834.008
Figure 4. Movement of residues. (A) Each residue (highlighted in green) defines a circumscribed circle (dashed red circle) of radius \( r \) (or diameter \( D \), where \( D \), as shown, is \( D_{closed} \), although upon opening would be \( D_{open} \)), centered at the pore center (\( O \)). Upon channel activation, the protein expands (radius changes from \( r_{closed} \) to \( r_{open} \)), or equivalently, the residue moves by \( \Delta r = r_{open} - r_{closed} \), measured from the pore center (\( O \)). (B) Sketch of MscL from closed state (blue pentagon) to open state (purple pentagon). The residue of interest (vertices of the pentagons) moves \( \Delta r \) from the pore center. (C and D) Translational movements (\( \Delta r \)) of residues on TM1 and TM2 measured via smFRET. All the residues move away from the pore center, arguing in favor of the helix-tilt model. DOI: 10.7554/eLife.01834.010
**Figure 4—figure supplement 1.** Molecular structures of fluorophores used in the experiments.
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**Figure 4—figure supplement 2.** Geometric analysis of the distances of interest while taking into account the finite size of fluorescent probes and the breaking of fivefold symmetry of the protein due to attachment of probes.
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Figure 4—figure supplement 3. Effect of hexameric MscL in protein preparation. DOI: 10.7554/eLife.01834.013

Figure 5. Measurement of $R_0$. (A) Absorbance and fluorescence spectra of AF488-MscL and fluorescein (as a standard), used to determine the quantum yield of AF488 conjugated to MscL mutants. (B) Anisotropy of AF488 and AF568 conjugated to MscL mutant (M42C), corrected for the intrinsic polarization properties of the microscope, and for the high numerical aperture of the objective. DOI: 10.7554/eLife.01834.014
Figure 5—figure supplement 1. Anisotropy of AF488 and AF568 conjugated to MscL mutants, corrected for the intrinsic polarization properties of the microscope, and for the high numerical aperture of the objective.

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Figure 6. Activation thresholds, $P_a$, of MscL mutants at the proximity of the narrowest pore constriction. The activation thresholds were determined by electro-physiological recordings by patch-clamping without and with 10 mM DTT. Three recordings in the presence of DTT are shown as examples: (A) G22C, (B) I24C, and (C) I25C. (D) Comparison of the mutants with the wild type (WT) shows that the thresholds for mutants G22C and I24C are more than twice higher than the wild type, indicating the function of the channel was affected by the mutations. This was also observed via ensemble and single molecule FRET experiments. However, the mutation I25C does not affect the gating parameter substantially.

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Figure 6—figure supplement 1. Positions of MtMscL mutants at the proximity of the narrowest pore constriction.
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Figure 7. Model of the MscL structure in the open conformation. (A and C) The crystal structure of MscL in the closed state is shown for comparison (PDB: 2OAR [Chang et al., 1998; Steinbacher et al., 2007]). (B and D) The structure of MscL in the open state (Source Code 1 and 2) was developed based on the smFRET measurements, satisfying all the distance constraints measured from smFRET experiments. In the open conformation, the pore is mainly lined by TM1 (indicated by blue arrows), consistent with the helix-tilt model. In addition, both TM1 and TM2 tilt toward the membrane plane (horizontal) upon channel activation, which is emphasized by the green and yellow arrows in the side views. The green arrows show the orientation of TM1 in the closed state while the yellow arrow indicated the orientation of TM1 in the open state. The angle between the two arrows is 27°. (E) A sphere with a diameter of 2.7 nm (blue) is shown in the MscL channel in the top view. (F) The surfaces of water molecules (green) inside the tunnel of MscL (magenta) are drawn and the narrowest constriction is ∼2.7–2.8 nm.

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Figure 7—figure supplement 1. Developing a model for the open structure of MscL by inserting virtual springs.

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Figure 7—figure supplement 2  Side views of MscL open model (orange) in the POPC lipid bilayers.
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