Additional discussion of structure

As before[7] the open structure shows no interaction between D62 and R128 or R131, although a salt bridge between these residues has been suggested in the open state[8]. In the open structure, these residues are even further apart than in the closed structure, and a salt bridge does not seem realistic in the open state. Although the original study had not favored an interaction in the closed state structure[6], adjusting the side chain conformation of D62 and R128 would create a salt bridge between them in the published closed state structure[9]. It is important to point out that side chain conformations are often highly uncertain at the resolution reported for the closed structure[9].

Discussion of cross sectional area

This is a very complex issue and requires careful analysis. The literature has the figure 8.4 nm^2 as the increase in cross sectional area and gating energy of 11.4 kT; this is derived from fitting a two state Boltzmann model to the observed plot of tension against open probability[10]. The equation used was

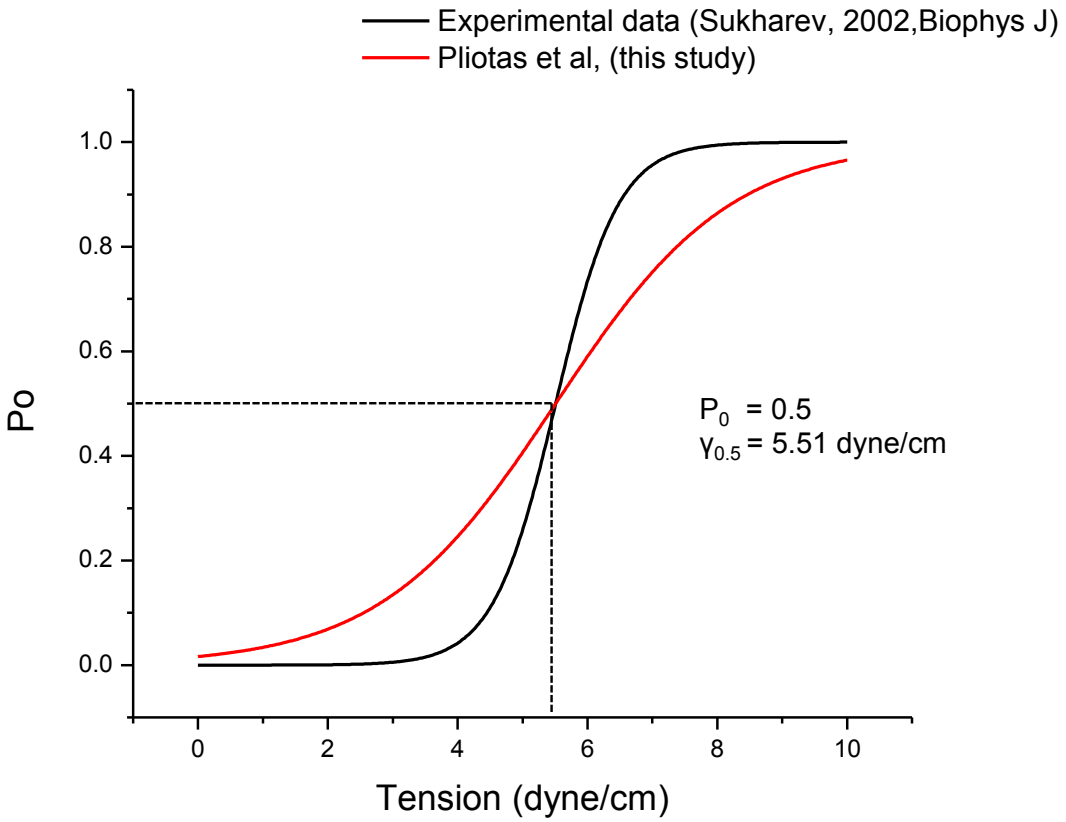
$$Po= (1 +\exp[(1/kT)(\Delta E- \gamma \Delta A)]^{-1}$$

- Po is the probability of the open state
- ΔE is the intrinsic free energy difference between two structures at zero tension in a bilayer
- γ is membrane tension (measured at 5.51 dyne/cm (5.51 x 10^{-3} N) at MscS gating threshold; Po=0.5)[13]
- ΔA is the change in area between the two structures

The approach assumes the change in surface area results from an expansion of a smooth walled regular cylinder in a uniform material, which itself has no re-organisation term.

This approach predates structural data, we now know MscS is not a regular cylinder (it is conical in the closed state and becomes less conical in the open state.) Critically the protein does not have smooth walls. Our calculations of course do not include residues 1-27 which are not located in the crystal structures.

Even the expansion of the central pore, the closest to a smooth wall, of MscS is complex. TM3a (pore helix) does not expand as a smooth wall; gaps appear between TM3a upon expansion[5]; meaning we are overestimating the area change. No simple tools exist for measuring change in cross sectional area of a protein. Thus, we aligned the central pore of the open and closed structures along the X-axis. 6Å slices of each were taken along the pore axis. The file SOI_radii.pdf has the slices. For each slice, the radius of the cα atoms was calculated over 30 Å (vertical height of TM3a) the average increase in area is 2.0 nm^2. Adding 6 Å to each radial section (to account for side chains and vdw radii), increases the area change to just less than 3.0 nm^2. Using a value of 3.0 nm^2 for the area change and relying on the fact the function must take a value of Po = 0.5 at 5.56 dye/cm (defined from experimental data[10]) results in a free energy change of 4.1 kT. If these values are then plotted using the same two state Boltzmann model based on simple cross sectional expansion in a fluid then the resulting plot profile clearly does not fit the experimental data, Figure S7. We propose that the discrepancy arises because there is a missing energy term, that arises from the re-organization of the lipids.



Supplementary Figure 7 The values derived from experimental observation when fed into a two state Boltzmann model result in a curve (red) that does not fit the experimental data (black taken from[10]). The red curve is defined to pass through coordinates Po = 0.5 and tension = 5.51 Dye/cm.

Instead of looking at the pore helix, which is surrounded by TM1/TM2, one can consider the overall circumference of the protein. This is the method proposed by Schulten *et al*. [11] and we believe was intent of the original Sukharev paper[10]. MscS has large gaps between the outer helices (TM1 and TM2), thus the circumference does not expand smoothly, rather the outer helices project into the bilayer. The energetics of this are complex, but in the simplest terms expanding 'fins' (helices) into a bilayer requires less energy than expanding a smooth cylinder to the same radial extent. Thus assuming a smooth walled expansion will overestimate the energetic significance of expansion in the bilayer.

In order to set an upper limit on the expansion area we have calculated the change in area of MscS, treating it as if indeed it was a smooth cylinder with diameter on the edge of the fins. Using this approach for the 36Å pore (approximate bilayer), the average change in area is around 130 A^2 (1.3 nm^2); over a 42 Å axis length (beyond the extent of the lipid bilayer) further exaggerates the area change, gives an average of 296 $Å^2$ (2.96 nm^2). (Soi_radii.pdf). Irrespective of how the area change is calculated it does not fit the simple two state model.

Schulten and colleagues[11] have pointed out simple average change in area is not a reliable measure of energy change for a protein that changes shape during gating. This is because the pressure profile changes across the bilayer and the change in conical nature of the protein is important. This group derived a new equation (which still assumes smooth walls and a simple fluid membrane) [11].

$$Po= (1 +\exp[(1/kT)(\Delta E- \gamma \Delta A + 2\pi^2(So^2-Sc^2)M_2)])^{-1}$$

The paper defines the following

- Po is the probability of the open state

- ΔE is the intrinsic free energy difference between two structures at zero tension in a bilayer
- γ is membrane tension (measured at 5.51 dyne/cm (5.51 x 10^{-3} N) at MscS gating threshold)[13]
- M_2 is the second moment of POPE membrane (tabulated in the paper, -11.2kT at 5.6 dyne/cm)[11],
- ΔA is the change in area between the two structures half way through the membrane ($\pi(Ro^2-Rc^2)$),
- So is the slope of the cone in open structure, Sc the slope in the closed
- ΔE is the intrinsic free energy between the states at zero pressure.
- T = 300, kT= 4.14 x 10^{-21} JK^{-1}

For the 36 Å pore, the values are

So = 0.173, Sc = 0.373 gives So^2-Sc^2= -0.109;

Ro =32.6, Rc = 32.55 ΔA =10A^2, 0.1nm^2, 0.1 x 10^{-18} m^2 ;

$\gamma\Delta A/kT$ = 0.136

For the 42 Å pore the values are

So = 0.122, Sc =0.3875, gives So^2-Sc^2 =-0.135;

Ro =32.05, Rc = 31.1, ΔA =-189$Å^2$, 1.9 x 10^{-18} m^2;

$\gamma\Delta A/kT$ = 2.57

At the gating threshold midpoint (5.51 dyne/cm) Po = 0.5

This means $\Delta E- \gamma \Delta A + 2\pi^2(So^2-Sc^2)M_2 = 0$; this is re-arranged to

$\Delta E = \gamma \Delta A - 2\pi^2(So^2-Sc^2)M_2$

ΔE for 36 Å pore = 0.136 – 19.74 (-0.109)-11.2 = -23.96 kT = -63 $kJmol^{-1}$

ΔE for 42 Å pore = 2.57 – 19.74 (-0.135)-11.2 = -27.3 kT = -72 $kJmol^{-1}$

These values would indicate MscS is more stable in the open form which we argue is nonsense and the result of a missing term, lipid re-organisation. We stress the thermodynamic models[10,11] are based on sound principles they fail only because at the time they were advanced the structural data were unknown and the models assumed no lipid re-organisation term.

An energy difference of -60$kJmol^{-1,}$ results in the proportion of the closed state at zero tension would be vanishingly small and the open probability would be > 0.9999999. This is totally different from the experimentally measured open probability of MscS reconstituted into liposomes as a function of increasing tension, the open probability at zero tension is 0[10].

Our own data in single channel experiments, detects as others have done, that the predominant form of the channel is closed at zero tension. The presence of the spontaneous openings indicates the equilibrium constant is not at extremes and thus the free energy gap may not large. A small energy gap is partially consistent with the fact that crystal structures have been reported for both closed and open forms for native protein. However, we stress that detergent solubilized protein is likely to have a different free energy from that embedded in the membrane and that crystallization, a kinetic process, does not select the lowest energy state.

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