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Supporting Material

Mechanosensitive closed-closed transitions in large membrane proteins: osmoprotection and tension damping

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Appendix A

Model including the stiffness of the proteins

Protein states can be viewed as oscillating about a minimum energy configuration. In this model, each protein can exist in either a contracted (CN) or an expanded (EX) state, and to each state corresponds a particular stiffness which is the curvature of the energy well. The stiffness is a property of the state and is independent of any external factor.

The minima of energy (i.e. the contracted and expanded states) are displaced on the area axis towards larger areas as tension increases (Fig. S1), because of the curved shape of the energy landscape around the energy minima (assumed to be parabolic). The same reasoning applies to the barrier except that since it is a maximum, the displacement upon application of tension is towards smaller areas. In this section, the work by Markin and Sachs [38] is followed.

Sukharev and Markin [35] assume that the transition occurs at the point where the energy parabolas for each state intersect. The position of the barrier thereby becomes linked to the stiffness of the closed and opened states. Although it can be valid as a first approximation, there is no a *priori* reason why the transition should occur at the intersection of the two wells. A barrier state is hence added to the model as a parameter, just like in the model without the stiffness of the states. The stiffness of the barrier, B_*, is also not a function of the stiffness of the contracted state, B_{CN}, nor of the expanded state, B_{EX}.

Modeling the precise energy landscape is not necessary. What are required are the initial positions of the minima and the maximum and the curvature around these points. These values enable the elaboration of a model for a spandex protein incorporating stiffness. Only the difference in energy between the initial state and the barrier for the transition (expansion or contraction) are relevant to the kinetics.

Generalizing Eq. 1, the rate equations are:

$$k_{EX} = k_{0EX} exp\left(-\frac{(E_* - E_{EX})}{k_B T_r}\right)$$
$$k_{CN} = k_{0CN} \exp\left(-\frac{E_* - E_{CN}}{k_B T_r}\right) \quad \text{(S1)}$$

where k_{0EX} and k_{0CN} are taken to be the same and equal to k_0.

Assuming the energy is parabolic around the minima and maximum, the energies of the states and of the barrier are now given by:

$$E_{CN} = \frac{1}{2} B_{CN}(A - A_{CN}^0)^2 - \gamma A$$
$$E_{EX} = E_0 + \frac{1}{2} B_{EX}(A - A_{EX}^0)^2 - \gamma A$$
$$E_* = E_0 + \frac{1}{2} B_{EX}(A - A_{EX}^0)^2 - \gamma A \quad \text{(S2)}$$

where ${A_{CN}}^0$, ${A_{EX}}^0$ and ${A_*}^0$ are the areas associated with each state and E_0 and ${E_*}^0$ are the energies of the expanded and barrier states at zero tension. The states are displaced by the application of tension. A is the area of the protein when oscillating around the energy extrema ${A_{CN}}^0$, ${A_{EX}}^0$ and ${A_*}^0$.

Taking the derivative of these energies as a function of the area, A, to find the area where the energy is a minimum (or a maximum in the case of the barrier) for each state:

$$A_{EX}^{min} = A_{EX}^{0} + \frac{\gamma}{B_{EX}}$$
$$A_{CN}^{min} = A_{CN}^{0} + \frac{\gamma}{B_{CN}}$$
$$A_{*} = A_{*}^{0} - \frac{\gamma}{B_{*}} \quad \text{(S3)}$$

These areas (with the superscript 'min') correspond to the areas of each state when the bilayer is under tension. The change in area between the contracted and expanded states is now given by:

$$\Delta A = A_{EX}^{0} - A_{CN}^{0} - (\frac{1}{B_{CN}} - \frac{1}{B_{EX}})\gamma \quad \text{(S4)}$$

Since the states do not necessarily have the same stiffness, the area change upon expansion is no longer a constant but a function of the bilayer tension, γ. For MscL this could allow for reconciliation of the x-ray structure which predicts an area change of about *20* nm^2 and kinetic models that give an estimate of *6* nm^2 [36].

Replacing the values from Eq. S3 into Eq. S2 to get the energies of the minima and the maximum as a function of tension only, which can then be re-written in terms of energy difference between each state and the barrier:

$$E_{*} - E_{CN} = E_{*}^{0} - (A_{*}^{0} - A_{CN}^{0})\gamma + \frac{\gamma^2}{2}(\frac{1}{B_{*}} + \frac{1}{B_{CN}})$$
$$E_{*} - E_{EX} = E_{*}^{0} - (A_{*}^{0} - A_{EX}^{0})\gamma + \frac{\gamma^2}{2}(\frac{1}{B_{*}} + \frac{1}{B_{EX}}) \quad \text{(S5)}$$

Interestingly, since the energies are second-order functions of the tension, there are mathematically two values for the midpoint, $\gamma_{0.5}$. The midpoint occurs at the tension which allows an equal occupation of the two possible states, that is, when both states have the same energy. However the larger value must be considered unphysical because it occurs when the value of A_{CN} has become larger than the value of A_{EX}. Remember that the minima not only decrease in energy when there is no bilayer tension, but are also displaced towards larger areas and the softer states are displaced towards larger areas faster than the stiffer states. The midpoint is then given by:

$$\gamma_{0.5} = \frac{(A_{CN}^{0} - A_{EX}^{0}) + \sqrt{(A_{EX}^{0} - A_{CN}^{0})^2 - 2\,E_0\left(\frac{1}{B_{EX}} - \frac{1}{B_{CN}}\right)}}{\frac{1}{B_{EX}} - \frac{1}{B_{CN}}} \quad \text{(S6)}$$

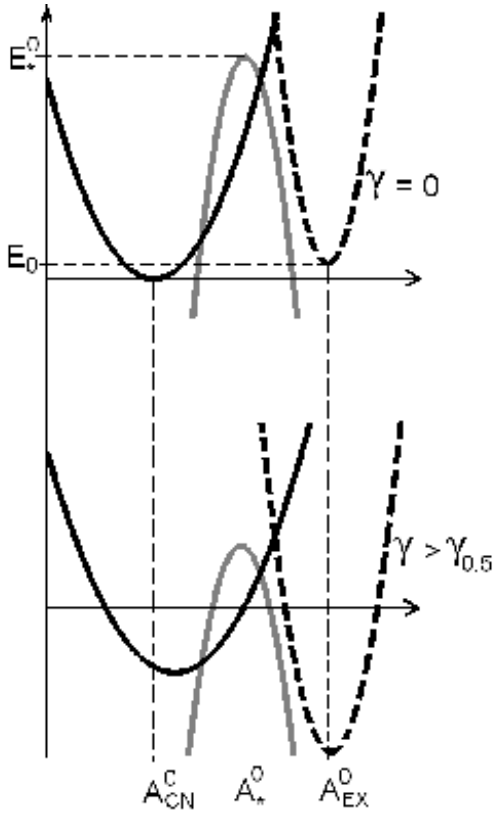

Figure S1 : Energy of the spandex protein as a function of its area, including the stiffness of the states. A: Energy of each state separately: the full line corresponds to the contracted state, the dashed line to the expanded state, the grey line to the barrier. The actual energy landscape is a composition of these three states, but the precise shape of the energy landscape is not important, only the position and height of the maximum and minima as well as the curvature around them are important for the kinetics of transition of the spandex protein. B: Energy of the three states at a tension larger than the midpoint tension. The area of the contracted and expanded states increase and the area of the barrier decreases. However the expanded state moves towards larger areas slower than the contracted state because it is stiffer (i.e. its parabolic shape is more pronounced). E_*^0 is the barrier height, E_0 the energy of the expanded state when there is no bilayer tension, A_{CN}^0, A_*^0 and A_{EX}^0 are the areas of the contracted, expanded and barrier states at zero tension. The zero of energy is set at the energy of the contracted state at zero tension.

By increasing their size, even protein that remain in the contracted state partially relieve bilayer tension. Taking this into account, the expected average tension is:

$$\gamma = K\frac{\Delta a_m}{a_m} + \frac{K\,C_{prot}}{A_{CN}}\left(\left(A_{CN}^0 - A_{EX}^{min}\right)P_{EX} + \left(A_{CN}^0 - A_{CN}^{min}\right)(1 - P_{CN})\right) \quad \text{(S7)}$$

Taking the derivative with respect to time and noting that A_{EX}^{min} and A_{CN}^{min} are functions of the tension γ from Eq. S3, Eq. S7 becomes:

$$\frac{d\gamma}{dt} = K\,\frac{d}{dt}\frac{\Delta a_m}{a_m} + \frac{K\,C_{prot}}{A_{CN}^0}\frac{\left(A_{CN}^0 - A_{EX}^{min}\right)\frac{dP_{EX}}{dt} + \left(A_{CN}^0 - A_{CN}^{min}\right)\frac{dP_{CN}}{dt}}{1 + \frac{K\,C_{prot}}{A_{CN}^0}\left(\frac{P_{EX}}{B_{EX}} + \frac{P_{CN}}{B_{CN}}\right)} \quad \text{(S8)}$$

For clarity, the values of A_{CN}^{min} and A_{EX}^{min} have not been substituted with their values from Eq. S3 (even though they have been taken into account in the derivative). Also, like in the previous section, $\Delta a_m/a_m$ is the strain imposed on the bilayer plus protein system and is independent of any protein properties.

The tension where the time constant is maximum cannot be found analytically in this model, but it can be readily obtained through simulation. Once this $\gamma_{\tau max}$ is obtained the value of τ_{max} can be set by adjusting the values of k_0 and E_*^0 (in Eq. 7).

The model including the stiffness of the protein states is solved numerically in a similar way as the model without the stiffness of the protein states. The parameters used are the same adding just the parameters: B_{CN}, B_{EX} and B_*.

Appendix B

Protein under tension including the stiffness of the proteins

Including the stiffness of the proteins, the probability of being in the contracted (CN) and expanded (EX) states and the time constant are now not only functions of tension but of the stiffness of the states and of the barrier.

The value of B_* was taken to be $5\ k_B T_r$. There are no values in the literature, but the barrier should be stiff so that when it moves to smaller area under the action of tension it does not go below the contracted state area which increases. The stiffness values given by Sukharev and Markin [35] were taken as reference values for B_{CN} and B_{EX}. As above, the value of C_{prot} was set to zero to avoid the effect of protein expansion on tension.

Our model imposes a lower limit on the stiffness of the contracted state. Under biologically relevant tensions, the contracted state must not cross the barrier nor the expanded state. Hence it must be stiff enough to avoid this.

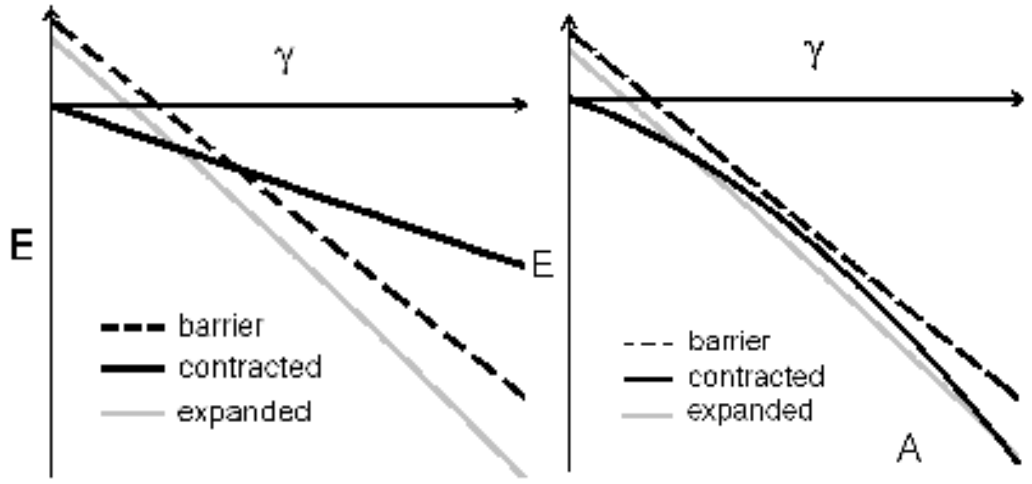

Figure S2 : Varying the stiffness of the contracted state, B_{CN}, and keeping the stiffness of the expanded state, B_{EX}, and of the barrier state, B_*, constant, the energy of the protein is shown as a function of tension, γ for the different protein states. A: The energy of the expanded and contracted states cross twice when the expanded state is stiffer than the contracted state. There are two midpoints, two tensions where the occupancy of the states is *0.5*. B: When the contracted state is stiffer than the expanded state, the energies only intersect once, at the midpoint.

Fig. S2 shows that the energies of the contracted and expanded states will intersect (on the tension scale) once or twice depending on which state is stiffer. Having the energies intersect twice means that there will be two midpoints. This situation is probably unphysical as it results from the assumption that the energy well of a state is parabolic at any tension.

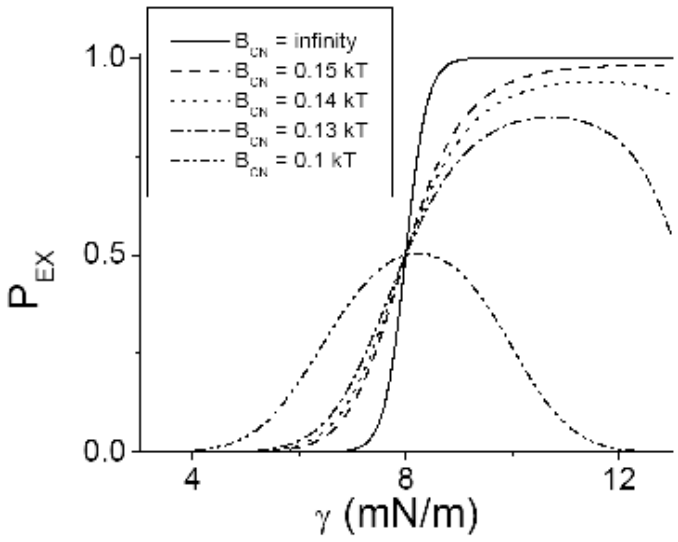

Figure S3 : Changing the stiffness of the contracted state, B_{CN}, changes the probability of being in the expanded state, P_{EX} as a function of bilayer tension,γ. The curve becoming shallower with a smaller B_{CN}, the protein appears to have a smaller expansion upon transition (ΔA). If the contracted state is softer than the expanded state, then it may, at sufficiently high tension, become bigger than the expanded state. This is shown by the curve for P_{EX} curving down with increasing tension. B_{EX} was set to infinity.

As can be seen from Fig. S3 a stiffer contracted state makes the transition less shallow (more abrupt) because as tension increases the expanded states moves to larger areas faster than the contracted state, thus increasing the effective change in area upon expansion (ΔA).

If all the states are very stiff, the model is essentially the same as the model without stiffness as the area difference between the states remains practically constant.

Now if the contracted state is made very stiff and the expanded state soft, as in Fig. S4, the results show a slope at the midpoint giving a larger area change upon expansion than that at zero tension (i.e. the curve in Fig. S4 is steeper for smaller values of B_{EX}). This is because the soft expanded state, upon tension increase, will move towards larger areas faster than the stiff contracted state thus giving a larger spandex area change.

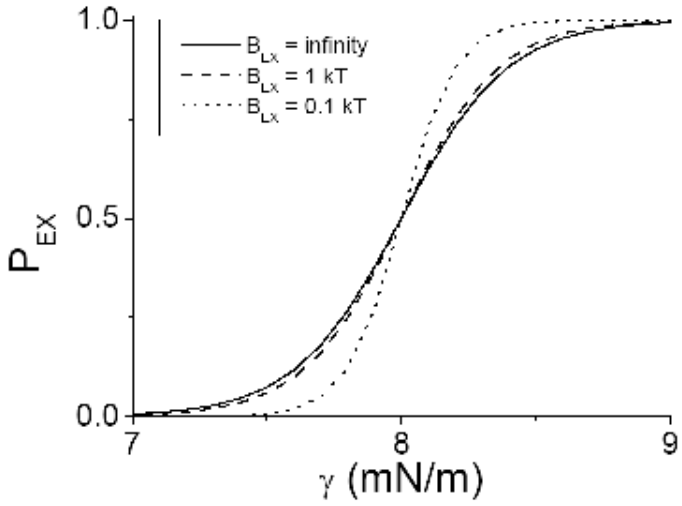

Figure S4: Changing the stiffness of the expanded state, B_{EX}, the probability of being in the expanded state, P_{EX}, varies as a function of bilayer tension. The area of the expanded state increases faster with tension than the area of the contracted state, thus making the curve for P_{EX} steeper, giving the impression of a larger change in area between states than the actual value. B_{EX} was set to infinity.

However, this picture of protein stiffness is not complete. A protein cannot expand indefinitely in the plane of the membrane, there is a maximum beyond which the protein structure would be destroyed or the chemical bonds would break. To correct this, the energies of the states should be calculated in more detail: although the parabolic approximation is accurate close to the zero-tension states, it is probably not valid at larger tensions.