Recovery of Equilibrium Free Energy from Nonequilibrium Thermodynamics with Mechanosensitive Ion Channels in E. coli

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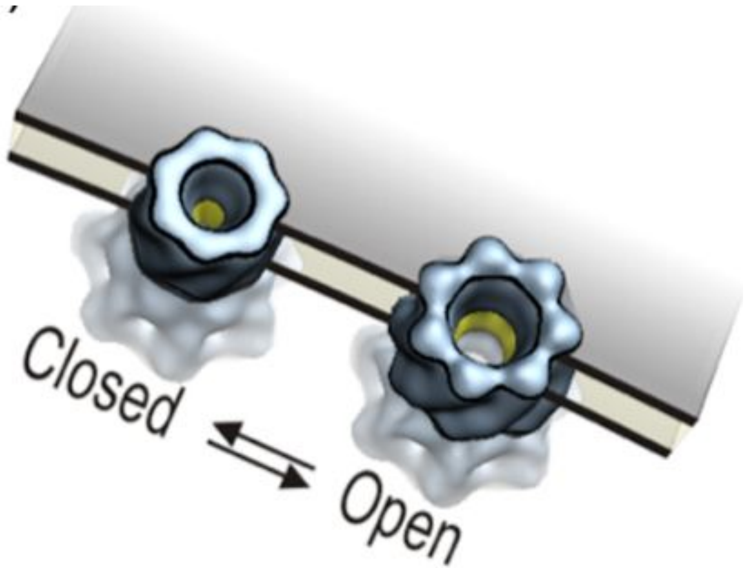


Introduction

Physical Review Letters, 2 June of 2020 by Uğur Çetiner, Oren Raz, Sergei Sukharev and Christopher Jarzynski

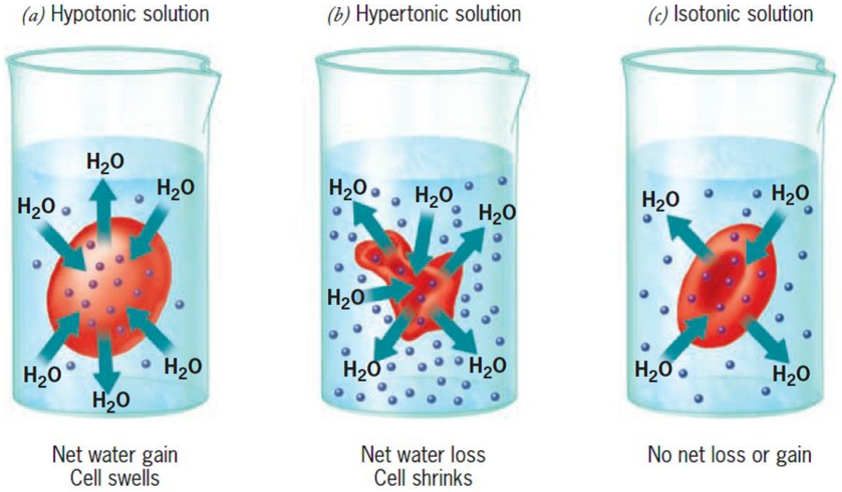
Problem: determining the difference of free energy of an open/closed ion channel is complicated because they are not in equilibrium.

Solution: using non equilibrium formalism in order to obtain the differences in free energy.

Theoretical introduction: Osmotic pressure

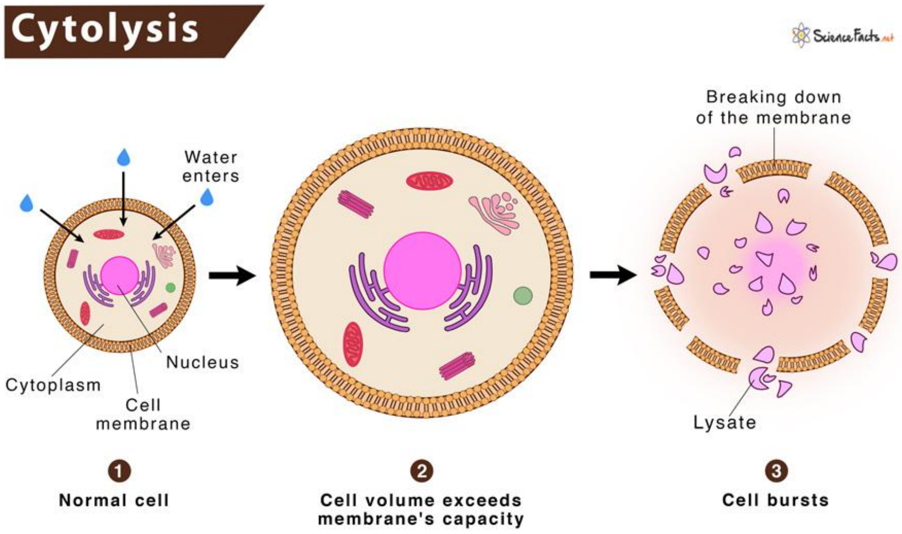
Osmotic pressure: entropic force that originates from the difference of concentration in the inside and the outside of the cell. $\Delta p = k_B T \Delta c$

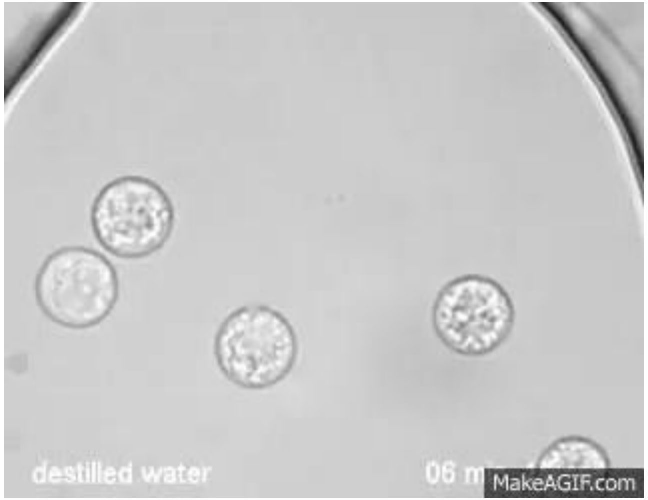
The osmotic pressure entropic behavior suggests that cells naturally gravitate towards a state where the concentrations inside and outside are balanced.



Theoretical introduction: Osmotic pressure

What happens when suddenly the outside concentration dilutes?

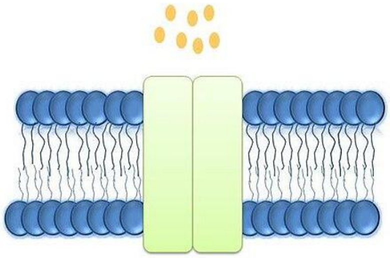




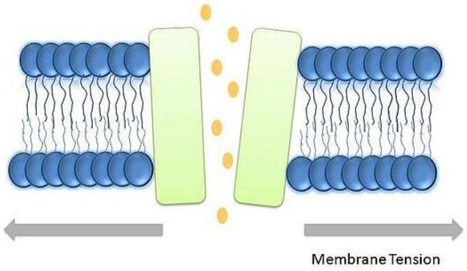
Theoretical introduction: Mechanosensitive ion channels

Mechanosensitive ion channels or MscS: safety valves of cells.

CLOSED C



OPEN O



<u>Two types:</u>

-**Mechanosensitive channels of small conductance**: 1 nS

-**Large conductance**: 3 nS LAST RESORT

Path independent thermodynamic variable:

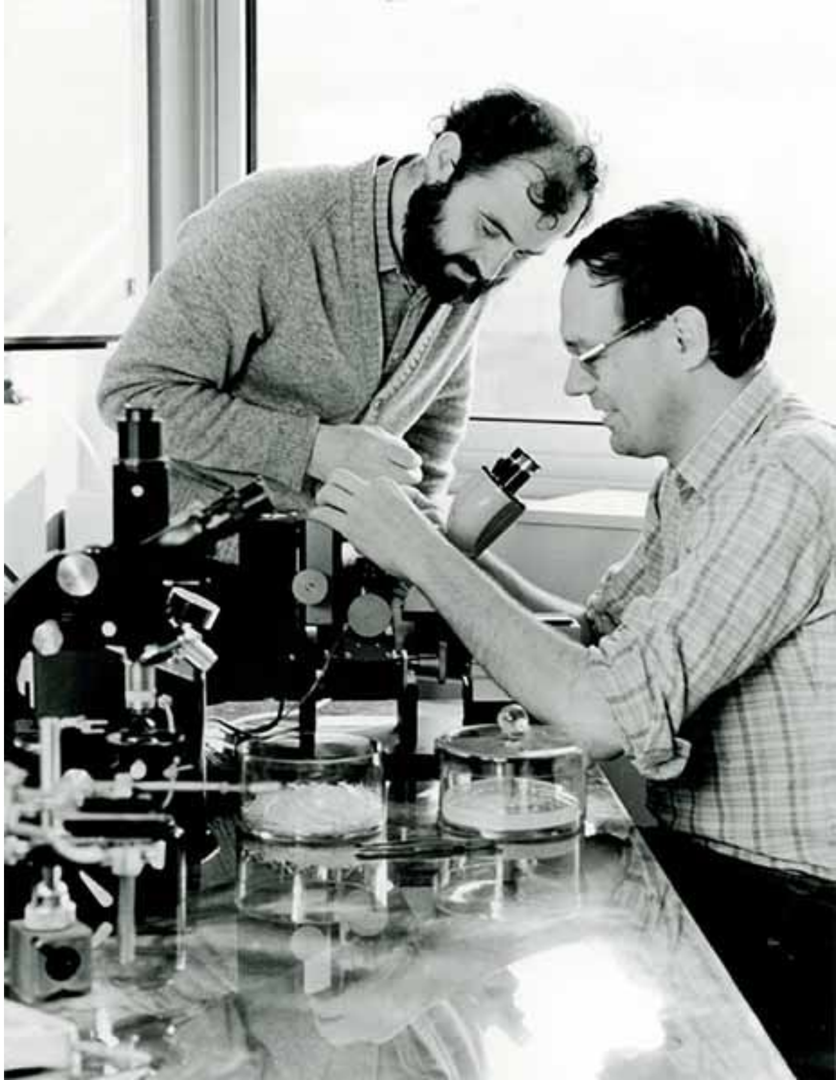
$$\Delta F_{O \to C}$$

Patch clamp

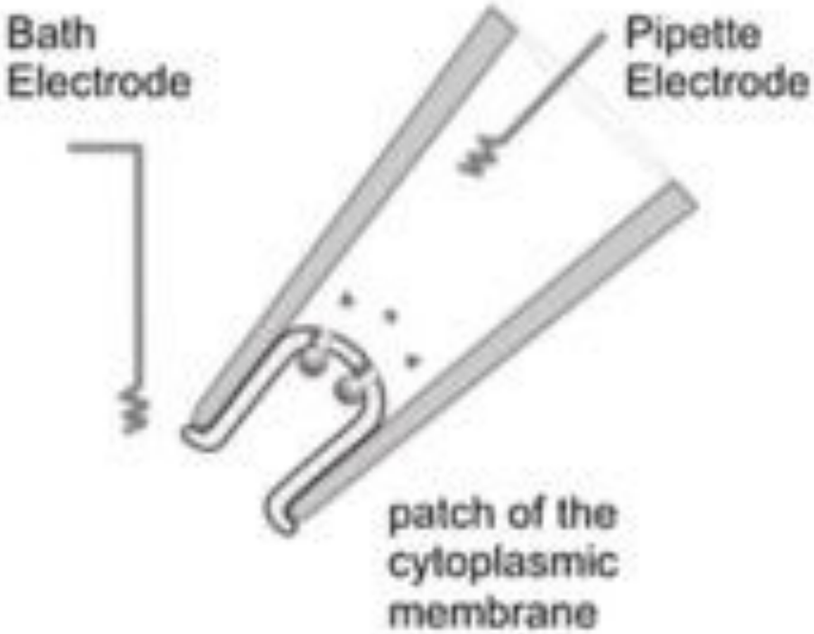
Developed in late 1970s and early 1980s by Neher and Sakmann →

Nobel prize in Physiology and medicine in 1991

Widely used in neuroscience



Channel recording

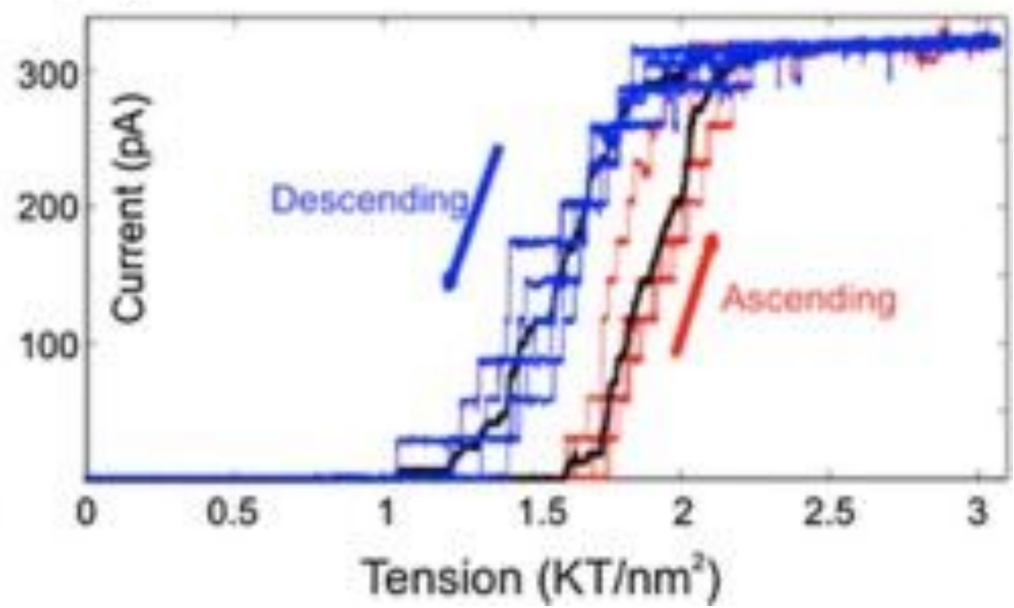


Common methods for measuring ΔF

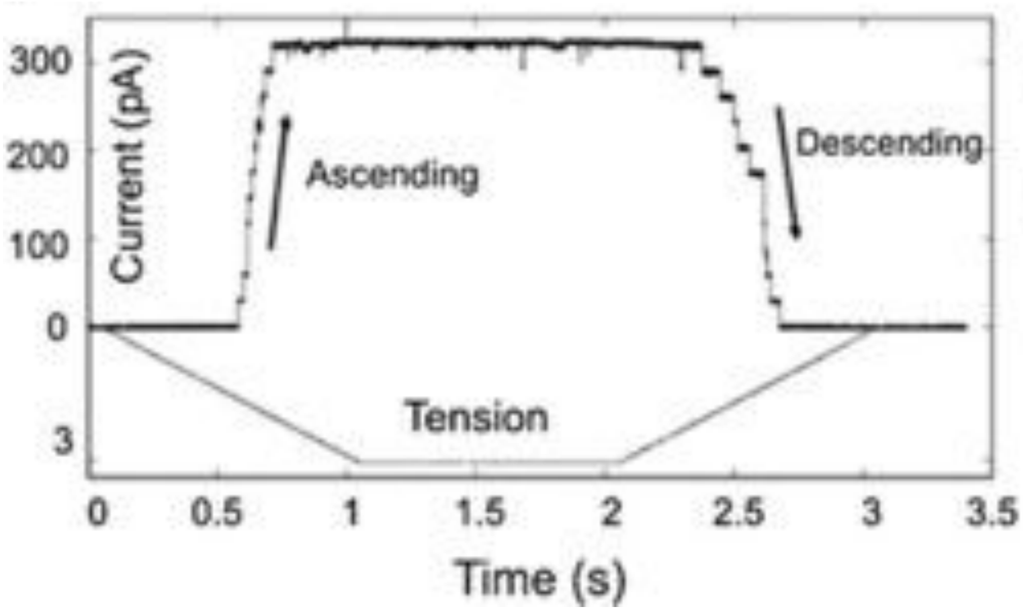
- Equilibrium assumption
- Fitting 2-state Boltzmann distribution

Literature values range from 5-28 kT

Hysteresis → non-equilibrium behaviour!



Method assuming non-equilibrium behaviour



Longer exposure to high tension → higher chance of rupturing

Two-state model of MscS gating

Energy of the system

$$H(\sigma, \gamma) = H_0(\sigma) - \gamma A(\sigma)$$

where

$$H_0(\sigma) = (1 - \sigma)\epsilon_{\text{closed}} + \sigma\epsilon_{\text{open}}$$

$$\gamma A(\sigma) = \gamma\sigma\Delta A$$

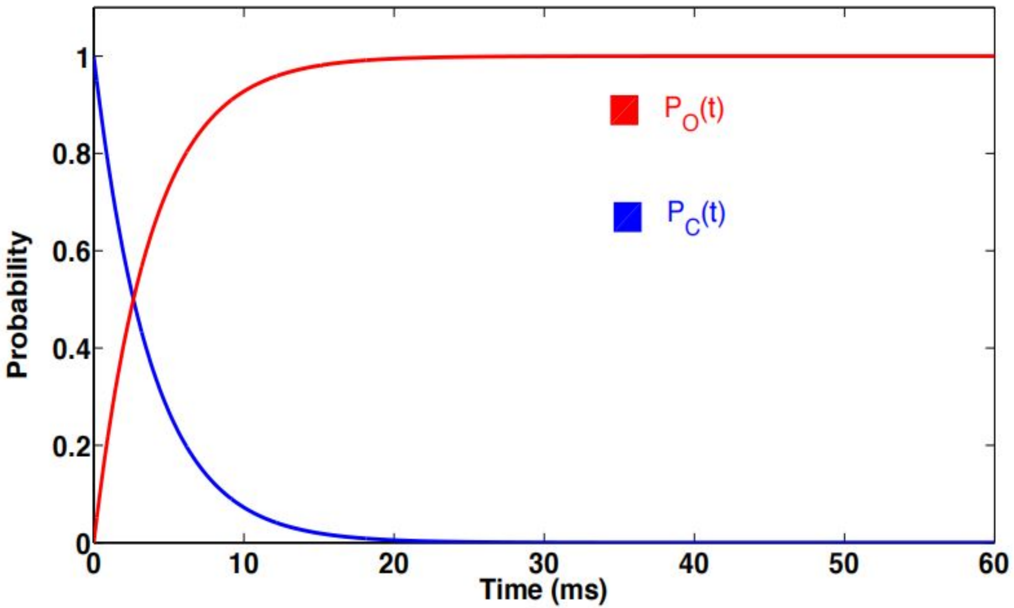
Method determining the work

The work performed on the system

$$W \equiv \int_0^\tau \dot{\gamma} \frac{\partial H}{\partial \gamma} dt = -\Delta A \int_0^\tau \dot{\gamma} \sigma dt$$

which can be rewrite as

$$W = -\Delta A \sum_{k=0}^{M-1} (\gamma_{k+1} - \gamma_k) \sigma_{k+1}$$



Method determining the work

The work performed during the opening:

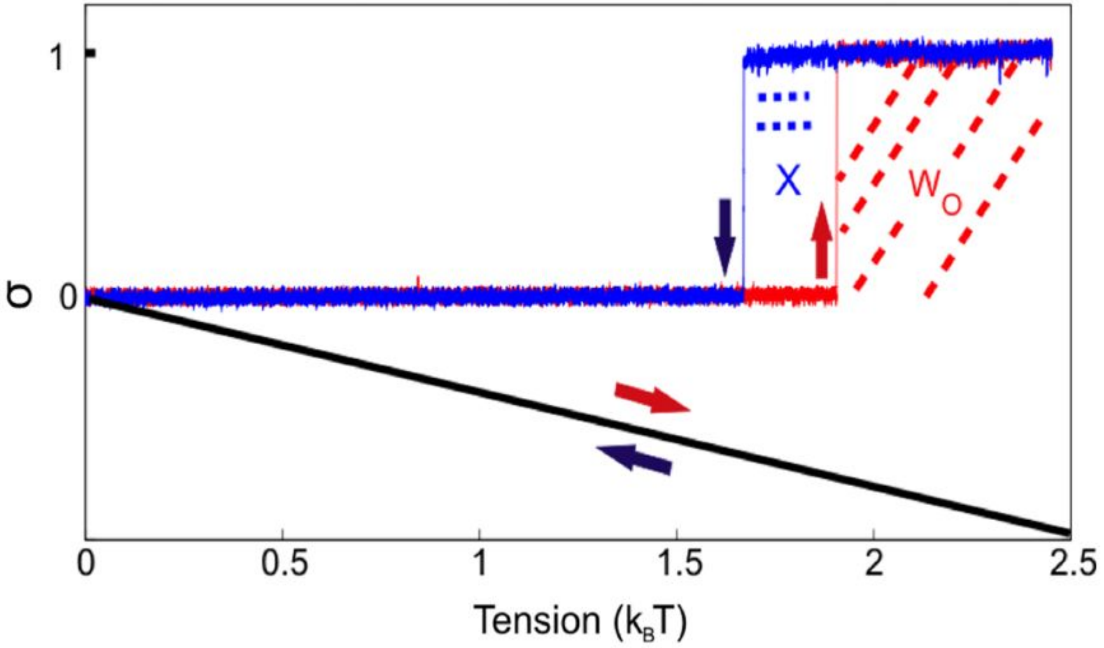
$$W_{C\to O} = -\Delta A \times W_0$$

The work performed during the closing:

$$W_{O\to C} = \Delta A \times (W_O + X)$$

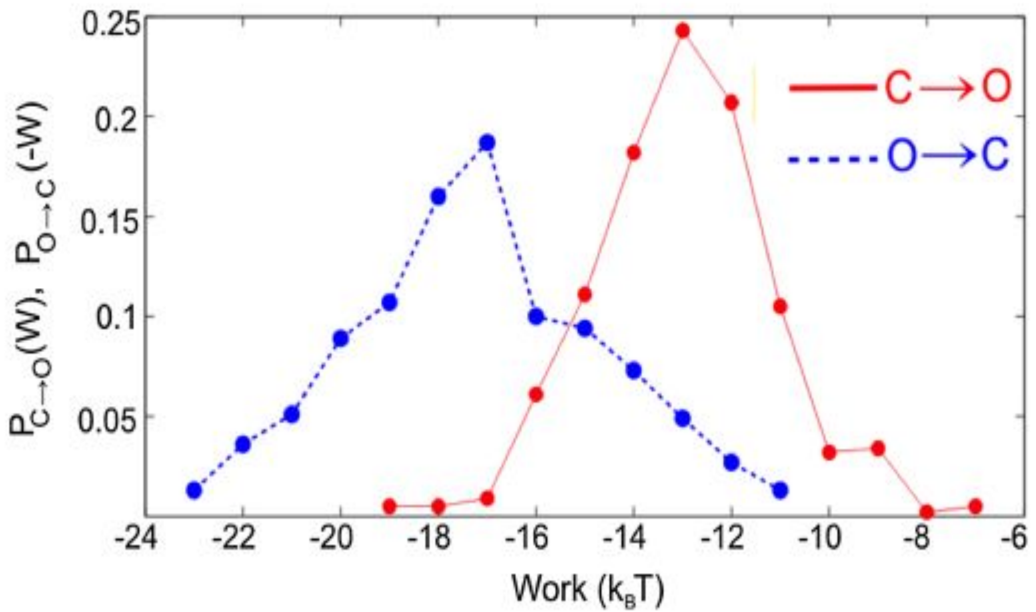
The total dissipation during this thermodynamic cycle:

$$W_{\text{diss}} = W_{C\to O} + W_{O\to C} = \Delta A \times X$$



Results

The figure shows the work distribution measured in many repetitions (440 opening events. and 449 closing events).



They used Crook’s and Jarzynski’s theorems to find ΔF. There are many methods:

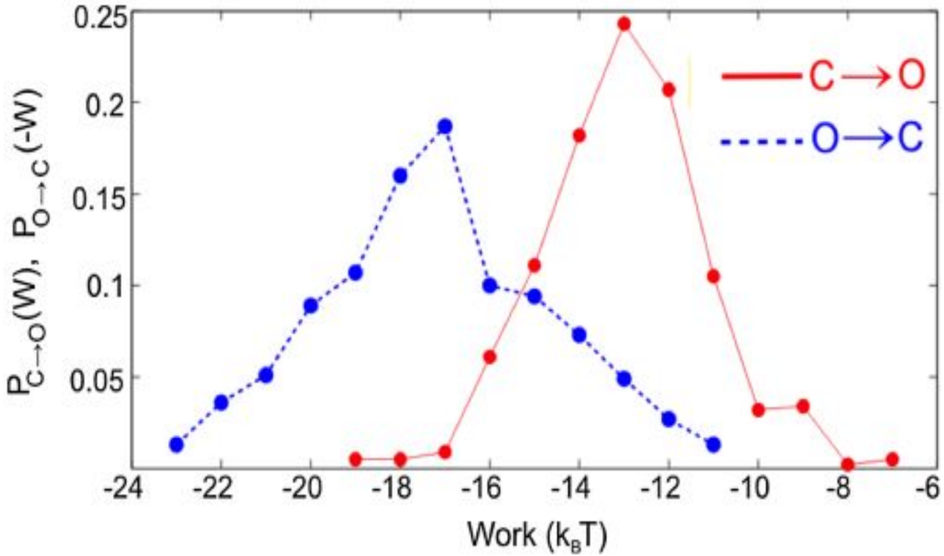
1. **Intersection Method:**

From Crook’s theorem

$$\frac{P_{C\to O}(W)}{P_{O\to C}(-W)} = e^{\beta(W-\Delta F)}$$

the distributions intersect when $W = \Delta F$.

ΔF_X = - 15.3 k_BT.



2. Line fitting:

Taking the log of Crook's theorem, we get a linear function.

$$\ln\left(\frac{P_{C\to O}(W)}{P_{O\to C}(-W)}\right) = \beta W - \beta \Delta F$$

By fitting it to a straight line and locating the interception, we can find ΔF_C = - 14.7 $k_B T$.

3. Jarzynski:

From Jarzynski's theorem:

$$\langle e^{-\beta W} \rangle_{A \to B} = e^{-\beta \Delta F}$$

We can get an estimate of ΔF for the closed to open transition and for the open to closed. Averaging both, we get ΔF_J = - 14.5 $k_B T$.

4. Bennett's Acceptance Ratio Method

Introduce an arbitrary function $f_\mu(W)$ and a parameter μ to Crooks theorem:

$$\beta \Delta F = \ln \langle f(W) \rangle_{O \to C} - \ln \langle f(W) e^{-\beta W} \rangle_{C \to O}$$

It is shown that the result has the least variance when $\mu = \Delta F$ and

$$f(W) = \frac{1}{1 + \frac{N_O}{N_C} e^{\beta(W - \Delta F)}}$$

If we put this into the first equation, we get an equation for ΔF. $\Delta F_{BAR} = -15.0\ k_B T$.

The authors consider this method as the best, since it doesn't depend on the binning of the histograms.

ΔF_x	ΔF_C	ΔF_J	ΔF_{BAR}
-15.3 ± 0.6	-14.7 ± 0.2	-14.5 ± 0.3	-15.0 ± 0.5

Correction for free energy in absence of tension.

The states measured were: closed and without tension and open with tension $3k_BT/\ nm^2$.

However, we want ΔF between closed and open states (both without tension).

The open state with tension has a difference in free energy of $\gamma\sigma\Delta A = 36\ k_BT$.

Therefore, the corrected free energy difference is $\Delta F = -15.0\ k_BT + 36\ k_BT = 21\ k_BT$

Conclusions

- Found the nonequilibrium work distributions between the open and closed states of the ion channel in E. coli's membrane.

- Evaluated ΔF with many different methods and got similar results.

- Control experiment the method with another protocol (tension was increased in 250ms to a total of 3.6 $k_B T$ /nm^2) and got a similar result (22 $k_B T$).

- Change the way of perceiving the non equilibrium theorems from verifying results to obtaining them.

- Paves the work toward studies of other channels.

Future work

1. Determine the difference in free energy for <u>other type of cells</u>. Is it similar?

2. Maybe now we can study <u>large conductance ion channels</u> by carefully choosing the applied tension. (so it does not break)

3. *Now that we have used non equilibrium formalism, can we use it to determine the <u>free gibbs energy in other type of ion channels</u>? ex: Na-K pump

4. *Can we use it for <u>other biological systems</u>? ex: protein folding with more than 2 states?

Thanks for your attention! Questions?