Solutions 3: Thermal fluctuations

BIO-369

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1 Membrane ion channel

a) In lipid membranes, two gramicidin monomers, one on each side of the bilayer, associate via the N-terminus to form a dimer, via six intermolecular hydrogen bonds. Assuming that for one hydrogen bond, $\Delta E_H = 3\,k_\mathrm{B}T$, the binding energy of our dimer is $\Delta E_{binding} = 6 \times \Delta E_H = 18\,k_\mathrm{B}T$. Based on this, one would expect a gramicidin channel to be open most of the time (because the bound state is energetically favored, and it is the one that corresponds to the open channel, since the dimer acts as an ion channel, see Fig. 1). It might be able to close and reopen spontaneously every now and then due to thermal fluctuations (because the binding energy is larger than $k_\mathrm{B}T$ but not by many orders of magnitude), but this is expected to be quite rare. Indeed, according to the Boltzmann distribution

$$\frac{P(E_2)}{P(E_1)} = \exp\left(-\frac{E_2 - E_1}{k_\mathrm{B}T}\right) = \exp\left(-\frac{\Delta E_{binding}}{k_\mathrm{B}T}\right), \tag{1}$$

where state 2 is the unbound (closed) state and state 1 is the bound (open) state. With $\Delta E_{binding} = 18\,k_\mathrm{B}T$, the probability of the unbound (closed) state is $\exp(-18) \approx 1.5\times10^{-8}$ fold smaller than that of the bound (open) state, and thus the bound (open) state is quite stable to thermal fluctuations.

b) Dimer formation involves a local deformation of the membrane compared to its usual state: this local binding of each monolayer costs some energy. This makes the dimer (bound, open) state less favorable than expected in the previous question, as formation of the dimer requires deforming the membrane. This deformation thus favors the unbound momomer, i.e. the closed channel. At this point, our conclusion from the previous question no longer holds, because the dimer is favored by the binding energy of the hydrogen bonds, but its formation also carries an energy cost as it involves deforming the membrane. Depending on how these two energies compare, the dimer or the monomer may end up being more favorable.

c) Each sudden rise of the current corresponds to the formation of a gramicidin channel in the membrane, and the subsequent sudden drop to the dissociation of this channel. In the inset, there is a phase where two channels are open simultaneously.

Given that the channels spend more time closed than open this data, the monomer state appears to be more favorable. It means that the energetic cost of the membrane deformation associated to the formation of a gramicidin channel is larger than the binding energy calculated at the first question.

d) If a gramicidin channel is open (and in the dimer form) 10% of the time, and closed (and in the monomer form) the rest of the time, it means that

$$\frac{P(E_1)}{P(E_1) + P(E_2)} = 0.1\,, \tag{2}$$

where state 1 is the open (bound dimer) state and state 2 is the closed (monomer) state, which yields $P(E_2) = 9 \times P(E_1)$. Using the Boltzmann distribution in Eq. 5 we can write

$$\frac{\Delta E}{k_\mathrm{B}T} = -\ln\left[\frac{P(E_2)}{P(E_1)}\right] = -\ln(9) = -2.2\,, \tag{3}$$

and thus $\Delta E = -2.2\, k_{\mathrm{B}}T$. This is the total binding energy, i.e. the total energy one should spend to unbind the dimer that makes the channel ($\Delta E = E_2 - E_1 = E_{unbound} - E_{bound}$), i.e. the total energy variation associated to channel closing. It comprises both the binding energy and the membrane deformation energy. We notice that it is negative, contrary to usual binding energies. This is consistent with the fact that opening the channel (involving binding and membrane deformation) is not favorable, and that it is more likely to find a closed channel than an open one. We also notice that it is of order a few times $k_{\mathrm{B}}T$, which is consistent with the fact that the unfavorable state is observed nevertheless.

e) We can write $\Delta E = \Delta E_{binding} - \Delta E_m$, where $\Delta E_{binding}$ was computed at the first question and ΔE was computed just above. If the membrane deformation is modeled like that of a spring, and the associated energy cost is $\Delta E_m = H(d_0 - \ell)^2$, then we obtain

$$H = \frac{\Delta E_{binding} - \Delta E}{(d_0 - \ell)^2}\,, \tag{4}$$

Using our previous results and assuming that $d_0 - \ell = 1$ nm, we obtain $H = 8.3 \times 10^{-2}$ J.m^{-2} = 8.3×10^{-2} N.m^{-1} for the value of the spring stiffness. Here we have used $k_{\mathrm{B}}T = 4.1 \times 10^{-21}$ J at usual temperatures ($T = 300$ K). Hence, by observing the behavior of the gramicidin channel via the current going through the vesicle, this experiment provides information about lipid bilayer membrane elasticity at the nanoscale.

f) If different membranes (with different lipid compositions) are considered, such that d_0 is smaller than on the figure, assuming that H is the same for all these membranes, the energy cost of the membrane deformation should decrease as long as d_0 remains larger than ℓ, cancel when d_0 is equal to ℓ, and then increase again if the membrane equilibrium thickness d_0 becomes smaller than ℓ, because then matching the hydrophobic thickness of the gramicidin channel will again require a local deformation of the lipid bilayer membrane, albeit in the opposite direction.

2 Pulling on DNA

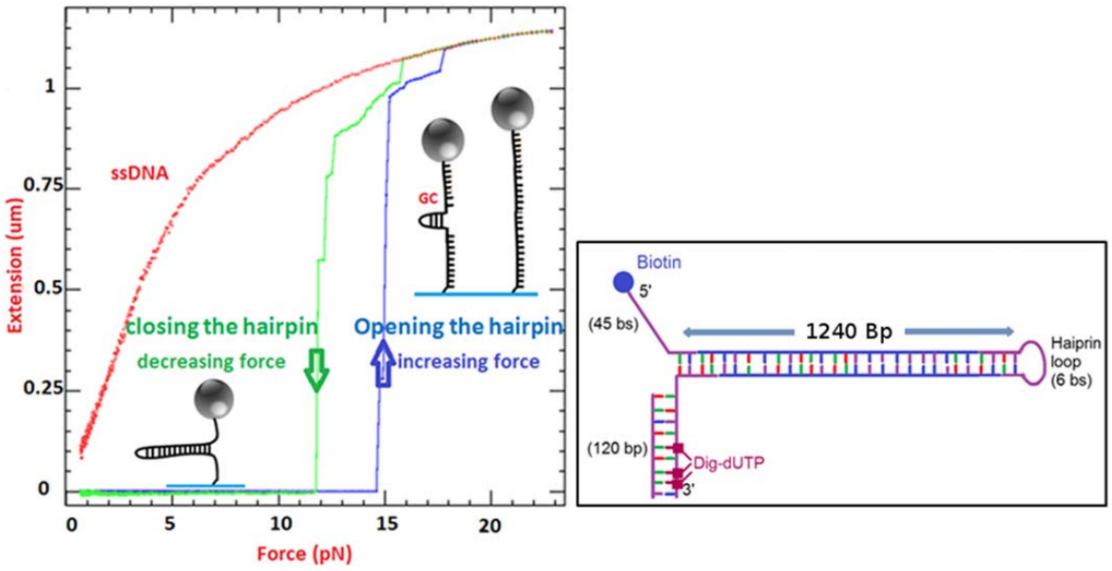

Figure 1: Pulling on a DNA hairpin: extension (μm) versus force (pN) curve (left) and schematic of the DNA hairpin employed (right). *Illustration reproduced from Ref. [1].*

a) As the force applied by the magnetic tweezers to the DNA hairpin is gradually increased, the single-stranded parts of the hairpins will first straighten and perhaps stretch a bit, but this will

result in a very minor extension of the molecule (negligible on Fig. 1). Then the hairpin will unzip, i.e. the hydrogen bonds between the two strands in the double-stranded region will break. Then we will be left with a single-stranded DNA molecule, with a length between the glass surface and the bead much larger than when the hairpin was closed (see inset schematics in Fig. 1, left panel). This single-stranded DNA molecule will then stretch (before breaking) if we keep increasing the force. Note that in Fig. 1, it does not break, and the last portion of the blue curve is the same as the red one: it confirms that this portion regards the elasticity of single-stranded DNA (ssDNA).

b) The blue curve features an abrupt increase of the molecule extension for a force of order 15 pN. This corresponds to the unzipping of the hairpin: all the hydrogen bonds break at this point. Thus, the force necessary for the hairpin to start unzipping is about 15 pN.

The work W necessary to unzip most of the hairpin is obtained by multiplying the force F applied to the bead when the hairpin unzips, namely 15 pN, by the variation of position ℓ of this point at this force value (here, the point is the bead, and thus, its variation of position is the extension of the molecule), which is the steep increase in the blue curve, i.e. about 1 μm. Thus, $W = F \times \ell = 15 \times 10^{-12} \times 10^{-6} = 1.5 \times 10^{-17}$ J. Given that $k_{\mathrm{B}}T = 4.1 \times 10^{-21}$ J at usual temperatures, this energy corresponds to $3.7 \times 10^3\, k_{\mathrm{B}}T$. This is much larger than the energy scale of thermal fluctuations, and thus the double-stranded region of the hairpin is stable (in the absence of a force pulling on it!).

c) Given that the hairpin double-stranded region is $N = 1240$ base pair long (see Fig. 1, right panel), and that an energy $W = 1.5 \times 10^{-17}$ J is required to unzip the hairpin, the average energy required to unbind one base pair of this hairpin is $\Delta E = W/N = 1.2 \times 10^{-20}$ J. Given that $k_{\mathrm{B}}T = 4.1 \times 10^{-21}$ J at usual temperatures, this energy corresponds to $3.0\, k_{\mathrm{B}}T$. It does correspond to the (free) energy associated to DNA Watson-Crick base pairs mentioned in the lectures. Recall that in the DNA double helix, each base pair involves either 2 (for A–T) or 3 (for C–G) hydrogen bonds, which in principle yields binding energies of order $\Delta E_{\mathrm{DNA}} \approx 10\, k_{\mathrm{B}}T$ to $20\, k_{\mathrm{B}}T$ for each base pair, but there is an entropic contribution that reduces the binding free energy to about $3\, k_{\mathrm{B}}T$. Here we measure the total free energy of binding. With this binding free energy, one single base-pairing interaction can resist thermal fluctuations, but not by a large margin, and can unbind spontaneously even though the bound state is favored. However many base-pairing interactions taken together are robust, as seen in the previous question.

d) After the hairpin has fully unzipped, we are left with single-stranded DNA. Thus, if we continue to pull on the molecule with an increasing force after the hairpin has fully unzipped, we will observe the elastic properties of single-stranded DNA (before the molecule breaks). The red curve in Fig. 1, left panel, corresponds to the single-stranded DNA case. For forces larger than about 17 pN, the blue curve completely matches the red curve, thus confirming that the properties of single-stranded DNA are then observed. In the red curve, in contrast to the blue one, there is no big step in the extension versus force curve, and instead, extension increases gradually with force. This corresponds to probing the elastic (and entropic) properties of single-stranded DNA, without the abrupt unzipping phenomenon.

e) The difference between the blue and the green curves in Fig. 1, left panel, is that in the blue curve the force is gradually increased (and the hairpin opens) and in the green one it is gradually decreased (and the hairpin closes). The overall shape of these two curves is similar, with a large step corresponding to zipping/unzipping. However, a striking difference is that this occurs at a different force value in the two cases, namely 15 pN when force is increased and 12 pN when it is decreased. Qualitatively, in the closing case, the molecule starts in a stretched state, and it is difficult for complementary bases to come close to one another, which is required for binding. This partially explains why the hairpin stays closed at forces smaller than 15 pN in that case. A more detailed explanation would involve studying the zipping/unzipping of the hairpin as a phase transition.

f) In the DNA double helix, each base pair involves either 2 (for A–T) or 3 (for C–G) hydrogen bonds. Hence, G-C pairs are more robust than A-T pairs. When increasing the force that pulls on the hairpin, the bounds that are closest to the hairpin loop are the last ones to unbind. Because the hairpin used in the experiment has a large majority of G-C bases in the region closest to the hairpin loop, this final step of unbinding requires a larger force than the rest (17 pN rather than

15 pN). Hence, the step observed in the blue curve around 1 μm extension is a direct consequence of the composition of the hairpin. In fact, experiments similar to this one but more sophisticated can actually allow DNA sequencing and even epigenetic sequencing.

3 Additional problem: Adhesion between cells

a) When estimating the total tension of the system composed by the membrane and the underlying actin cortex, the contribution of actin cortex strongly dominates over the one of the membrane. The latter is negligible.

b) For an interface between two cells, the energy per unit area that is associated to the cadherin molecules reads $\epsilon = 10 \times 10\, k_\mathrm{B}T$ per $\mu\mathrm{m}^2$, and thus $\epsilon = 100 \times 1.38 \times 10^{-23} \times 300 \times (10^6)^2 = 4.1 \times 10^{-7}$ J/m^2.

c) The quantity computed in the previous question can be compared to tension because it is a tension in terms of dimensions, it is expressed in J/m^2. It is much smaller than the total tension of the system composed by the membrane and the underlying actin cortex, which is itself dominated by the actin cortex. It is negligible. In this light, we do not expect cadherin molecules to be able to significantly deform cells.

d) Using the Boltzmann distribution, we obtain:

$$\frac{P(E_2)}{P(E_1)} = \exp\left(-\frac{E_2 - E_1}{k_\mathrm{B}T}\right) = \exp\left(-\frac{\Delta E}{k_\mathrm{B}T}\right) . \tag{5}$$

Here, we have $\Delta E = E_2 - E_1 = 10\, k_\mathrm{B}T$ for one pair of cadherin molecules. Thus, we obtain

$$\frac{P(E_2)}{P(E_1)} = \exp\left(-10\right) = 4.5 \times 10^{-5} . \tag{6}$$

e) For two cells that adhere through a surface of 1 μm^2 that comprises 10 pairs of cadherin molecules, the binding energy is 10 times the one above. Thus, the ratio of the probability of being unbound to being bound for these two cells is

$$\frac{P(E_2)}{P(E_1)} = \exp\left(-100\right) = 3.7 \times 10^{-44} . \tag{7}$$

This extremely small value means that cells adhering via cadherin molecules are very strongly bound together and cannot be unbound by thermal fluctuations.

References

[1] S. Hodeib, S. Raj, M. Manosas, W. Zhang, D. Bagchi, B. Ducos, F. Fiorini, J. Kanaan, H. Le Hir, J. F. Allemand, D. Bensimon, and V. Croquette. A mechanistic study of helicases with magnetic traps. *Protein Sci*, 26(7):1314–1336, Jul 2017.