Problem set 3: Thermal fluctuations

BIO-369

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1 Membrane ion channel

Gramicidin is a membrane protein with antibiotic activity. In lipid bilayer 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. This dimer is an integral protein which acts as an ion channel.

a) Estimate the binding energy of a gramicidin dimer, assuming that the binding energy of one hydrogen bond is $\Delta E_H = 3\, k_\mathrm{B}T$. Based on this, do you expect a gramicidin channel to be open or closed most of the time? Do you expect it to often open and close spontaneously?

b) While gramicidin monomers do not deform the membrane, the dimeric channel presents a hydrophobic thickness mismatch with the membrane, so that dimer formation involves a local deformation of the membrane, as shown in Fig. 1. Do you expect this deformation to favor the dimer or the momomer, i.e. the open or the closed channel? How does this affect your conclusion from the previous question?

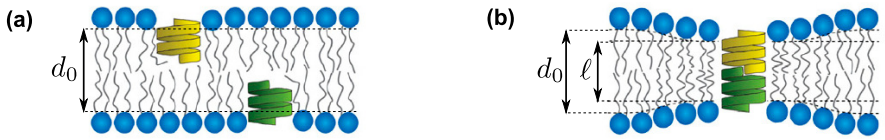

Figure 1: (a): Sketch representing two gramicidin monomers in a lipid bilayer. The unperturbed thickness of the membrane is denoted by d_0. (b): A gramicidin dimer in a lipid bilayer. The hydrophobic mismatch of the channel induces a deformation of the bilayer, which locally matches the hydrophobic thickness $\ell < d_0$ of the channel. *Original illustration from Ref. [1], adapted and modified.*

c) The gramicidin channel being large enough for the passage of monovalent cations, conductivity measurements can detect its formation and lifetime. An example of conductivity measurement in a vesicle containing a small number of gramicidin channels is shown in Fig. 2. What are the jumps in current across the membrane correspond to? What do you think happened in the inset? Based on this data, is the dimer or the monomer state more favorable? What does it mean for the energetic cost of the membrane deformation associated to the formation of a gramicidin channel?

d) Suppose that these measurements tell us that 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. What is the energy variation associated to the opening of the channel (dimerization)? Comment on the result, including the sign and magnitude obtained.

e) In a simple model, the membrane deformation can be modeled like that of a spring, and the energy cost of the deformation is $\Delta E_m = H(d_0 - \ell)^2$. Using your previous results and assuming that $d_0 - \ell = 1$ nm, calculate the value of the spring stiffness H. Recall that $k_\mathrm{B}T = 4.1 \times 10^{-21}$ J at usual temperatures ($T = 300$ K).

f) How do you expect the energy cost of the membrane deformation to change if different membranes (with different lipid compositions, but assuming that H is the same for all these membranes) are considered, such that their equilibrium thickness d_0 is smaller than on Fig. 1?

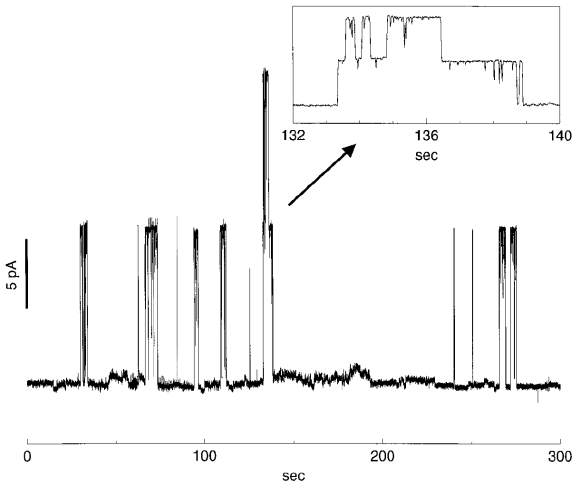

Figure 2: Time evolution of the current across a membrane vesicle adhering to a micropipette. The current is measured between one electrode in the micropipette and another one in the buffer which contains the vesicle. *Illustration reproduced from Ref. [2].*

2 Pulling on DNA

Single-molecule experiments can be performed to study the properties of biopolymers. In particular, by using "magnetic tweezers", it is possible to pull on a DNA molecule. For this, one end of the DNA molecule is fixed to a glass surface while the other one is attached to a magnetic bead. Using magnets, a controlled force can be applied to the bead, and thus to the DNA molecule (see Fig. 3).

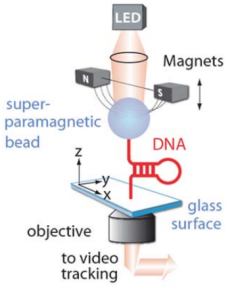

Figure 3: Single-molecule experiment setup using magnetic tweezers on a DNA hairpin. *Illustration reproduced from Ref. [3].*

Here we consider an experiment where this setup is employed to pull on a DNA hairpin molecule, which is a single-stranded DNA molecule with complementary sections that spontaneously forms a hairpin where a large region is double-stranded (see Fig. 3 and Fig. 4, right panel).

a) Imagine that the force applied by the magnetic tweezers to the DNA hairpin is gradually increased, so that the upper end of the molecule in Fig. 3 is pulled upwards with an increasing force. What do you think will happen to the hairpin (before it breaks)?

b) Consider Fig. 4, left panel, and specifically focus on the blue curve, which corresponds to experimental data obtained in the situation described in the previous question. Comment on the shape of the curve. What is the force necessary for the hairpin to start unzipping? What is the work necessary to unzip most of the hairpin? Recall that work is the force applied to a point times the variation of position of this point (here, the point is the bead, and thus, its variation of position is the extension of the molecule). Express this work in units of $k_{\mathrm{B}}T$, recalling that $k_{\mathrm{B}}T = 4.1\times10^{-21}$ J at usual temperatures ($T = 300$ K), and comment. Here and in the next questions until the very last one, please ignore the step that can be observed in the curve around 1 μm extension.

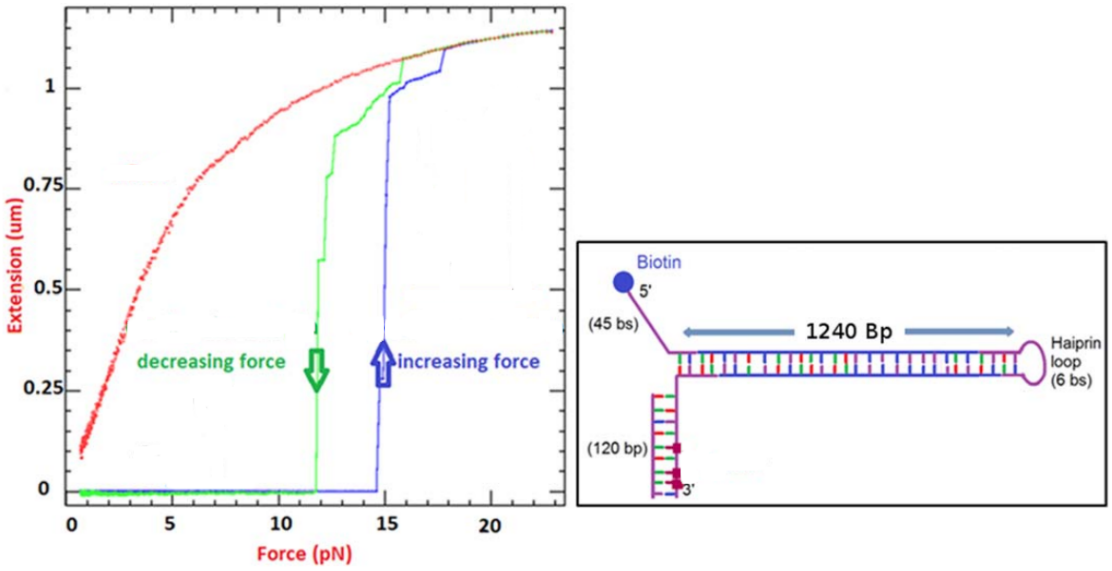

Figure 4: Pulling on a DNA hairpin: experimental data. Extension (μm) versus force (pN) curve (left) and schematic of the DNA hairpin employed (right). The extension of the molecule measured here is the variation of the distance between the glass surface and the bead. *Illustration reproduced and adapted from Ref. [3].*

c) Given that the hairpin double-stranded region is 1240 base pair long (see Fig. 4, right panel), estimate the average energy required to unbind one base pair of this hairpin, using the result from the previous question. Express this energy in units of $k_{\mathrm{B}}T$, recalling that $k_{\mathrm{B}}T = 4.1 \times 10^{-21}$ J at usual temperatures ($T = 300$ K), and comment on your result.

d) If we continue to pull on the molecule with an increasing force after the hairpin has fully unzipped, what elastic properties will we observe (before the molecule breaks)? The red curve in Fig. 4, left panel, corresponds to the case where the force is gradually decreased while the hairpin is prevented from rezipping (by using an oligonucleotide that binds to a portion of the region that is usually double-stranded). Comment on the differences between the blue and the red curves.

e) The green curve in Fig. 4, left panel, corresponds to the case where the force is gradually decreased while the hairpin is allowed to rezip. Compare the blue and the green curves.

f) The hairpin used in the experiment has a large majority of G-C bases in the region closest to the hairpin loop (see Fig. 4, right panel). Explain why a step is observed in the blue curve around 1 μm extension.

3 Additional problem: Adhesion between cells

This problem was previously given as part of the final exam of this class.

Data: The value of the Boltzmann constant is $k_{\mathrm{B}} = 1.38 \times 10^{-23}$ J/K.

a) The tension of the actin cortex underlying eukaryotic cell membranes is about 10^{-3} J/m^2. Meanwhile, the tension of the lipid bilayer membranes in these cells is about 10^{-6} J/m^2. When estimating the total tension of the system composed by the membrane and the underlying actin cortex, what dominates?

b) Cadherin is a protein that is involved in adhesion between cells. It goes through the lipid membrane, and one of its extremities binds to the actin cortex inside the cell, while the other one is outside the cell and can bind to another cadherin molecule protruding from another cell. A pair of two bound cadherin molecules provides an adhesion energy of about 10 $k_{\mathrm{B}}T$ at room temperature. On

an interface between two cells, there are about 10 pairs of cadherin molecules per μm^2. Calculate the energy per unit area that is associated to the cadherin molecules, for an interface between two cells, in J/m^2.

c) Why can the quantity computed in the previous question be compared to the total tension of the system composed by the membrane and the underlying actin cortex? Is it negligible or of the same order? In this light, do you expect cadherin molecules to be able to significantly deform cells?

d) Calculate the ratio $P(E_2)/P(E_1)$ of the probability of being unbound (state 2, with energy E_2) to being bound (state 1, with energy E_1) for one pair of cadherin molecules. What distribution did you use to perform this calculation?

e) Consider two cells that adhere through a surface of 1 μm^2 that comprises 10 pairs of cadherin molecules. What is the ratio of the probability of being unbound to being bound for these two cells? Comment.

References

[1] J. A. Lundbaek, R. E. Koeppe II, and O. S. Andersen. Amphiphile regulation of ion channel function by changes in the bilayer spring constant. *Proc. Natl. Acad. Sci. U.S.A.*, 107:15427–15430, 2010.

[2] M. Goulian, O. N. Mesquita, D. K. Fygenson, C. Nielsen, O. S. Andersen, and A. Libchaber. Gramicidin channel kinetics under tension. *Biophys. J.*, 74:328–337, 1998.

[3] 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.