Master ICFP 2017-2018 - PHYSICS FOR BIOLOGY

Examination, December 20th, 2017, duration: 3 hours

based on the two articles:

A. Probing polymerization forces by using actin-propelled lipid vesicles, A. Upadhyaya *et al., PNAS* 2003, and **B.** Soft *Listeria*: Actin-based propulsion of liquid drops, H. Boukellal *et al., Phys. Rev. E* 2003

I. Experiments

1. Describe the systems studied in articles A and B. You will put particular emphasis on the differences between the two systems. Why are they interesting from a biological point of view? From a physical point of view? What are the main experimental findings reported in these works?
Please answer to this first question in less than 1 page.

2. In Figure 2.a of article A, why does the rear of the vesicle appear in yellow?

3. What are the typical speeds of vesicles (in article A) and oil droplets (in article B) propelled by actin comets? Comment.

4. In article A, one reads "For larger (50%) or smaller (2%) concentration (*of ActA*), no motility was observed". Give possible explanations for these observations.

5. How is the "actin pressure" depicted on Fig.4.b and 4.c of paper A inferred? Why is this "pressure" compressive on the sides of the vesicles (or droplets in article B) and dilative at the rear?

6. In article B, the Young modulus E of the actin tail is introduced. E has been measured in the range $10^3 - 10^4$ Pa. Propose an experiment that would allow to measure E.

7. In article A the medium is supplemented with Arp2/3. Draw a schematic diagram of an actin network cross-linked by Arp2/3 complexes. Is the Arp2/3 complex needed for the formation of the actin comet? Which properties of the comet does Arp2/3 influence?

8. The authors of article A write that they measured the values of the bending modulus k_c and the stretching modulus K_a of the vesicles membrane. Describe carefully how they could measure these parameters.

II. Theory

Preliminary question. There are no inertia terms in the proposed models. Discuss this approximation.

II.A. Polymerization force of an actin filament

1. Give the value of the diameter of an actin filament. Briefly define the persistence length ℓ_p of a semi-flexible polymer. What is the typical value of ℓ_p for an actin filament?

2. Briefly explain how the polymerization of an actin filament close to a membrane can apply a force.

   We write the polymerization reaction of actin as: $A_N + A \rightleftharpoons A_{N+1}$, A being an actin monomer, A_N an actin filament with polymerization degree N. We denote c_N the concentration in filaments of size N. For this precise reaction, we write: $\frac{dc_{N+1}}{dt} = k_{on}\, c_N\, c_1 - k_{off}\, c_{N+1}$.

3. We assume that the values of k_{on} and k_{off} do not depend on N. Discuss this approximation.

4. We consider a population of actin filaments. We denote $\langle L \rangle$ the mean value of the contour length of the filaments. Show that $d\langle L \rangle /dt$ is proportional to $(k_{on}c_1 - k_{off})$. Deduce the value c_1^* of the concentration in G-actin for which $\langle L \rangle$ is constant.

5. At polymerization equilibrium:
$$\frac{c_N\ c_1}{c_{N+1}} = K_d$$
   Relate the value of K_d on the one hand to k_{on} and k_{off} and on the other hand to the difference in Gibbs free energy ΔG^0 between the state "$A_N + A$" and the state "A_{N+1}". When the addition of an actin monomer to a filament applies a force f on a wall, how does f enter in the value of ΔG^0? Deduce that $c_1^*(f) = c_1^*(f = 0)\ \exp(fb/k_BT)$, b being the size of an actin monomer.

6. The experimental value of c_1^* *in vitro* is about $200nM$, and in a lamellipodium $c_1 \approx 20\mu M$. Explain under which assumptions these data can be used to deduce an order of magnitude for f and give that order of magnitude.

7. In a naive model, the force F applied by an actin comet is written as the sum of the polymerization forces applied by each actin filament. With the orders of magnitude for the density of actin filament extremities at the droplet surface to be found in articles A and B, what is the expected order of magnitude for F? Compare the predictions of this naive model with the measurements and models of articles A and B and comment.

II.B. Calculations on the vesicle membranes

We now seek to derive the equation (5) of paper A, namely the relationship between the area dilation $\alpha = (A_0 - A)/A_0$ and the tension τ of a fluctuating, fluid membrane.

1. Explain the presence of two terms in the right hand side of equation (5) and justify why these two terms contribute in an additive manner. In the following, we will concentrate on the first (temperature dependent) term.

2. We consider a fluctuating membrane which is weakly deformed compare to its ground state (a flat membrane), and we use the so-called Monge representation, where the position of the membrane is characterized by the function $u(\vec{r})$, $\vec{r}$ being the position vector in the reference plane. In this representation, the membrane deformation energy reads:

$$E = \int d\vec{r} \left(\frac{\tau}{2} (\nabla u)^2 + \frac{k_c}{2} (\Delta u)^2 \right)$$

   Explain the two terms appearing in the energy, and write the energy as a function of the Fourier transform of the membrane position, defined as $\tilde{u}(\vec{q}) = \int d\vec{r} e^{-i\vec{q}.\vec{r}} u(\vec{r})$. You may write the energy as a sum over discrete Fourier modes, or as an integral over (approximately) continuous Fourier modes, using the correspondence $\sum_{\vec{q}} \longleftrightarrow A \int d\vec{q}/(2\pi)^2$

3. Use the equipartition theorem to calculate the average of the square of the mode amplitude of the different modes $\langle |\tilde{u}(q)^2| \rangle$.[1] Use this expression to express the average value of the area difference ΔA between the undulating membrane and its flat reference ("projected") shape as an integral over all modes of a function which depends on the tension τ.

4. Use this expression to derive equation (5) of the paper. According to your calculation, what is $a_{\min}$ supposed to be? Does this correspond of the interpretation of the paper? Comment.

II.C. Model for actin-based propulsion

We discuss here the models proposed in articles A and B for the propulsion of soft objects by an actin comet.

1. Discuss equations (1) and (2) of article A. Discuss the sentence "The total stress is the sum of the osmotic pressure and the stress induced by stretching".

2. Derive equation (1) in article B. Reproduce Fig. 3 and add on it all the quantities you need to answer the present question and the next one.

3. Prove equation (2) in article B.

4. Compare equation (3) in article B and equation (7) in article A and comment.

5. Justify the expression in paper B for the size of the blunted region of the drop: $\ell \equiv \gamma a^2 \delta / k_B T$.

6. Prove equation (4) in article B and plot σ_{nn} as a function of h. Is this consistent with the experimental observations?

[1] We can drop the vector notation for $\vec{q}$ here since the membrane is isotropic and the energy only depends on the norm $|\vec{q}|$.