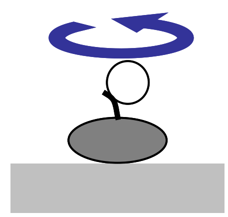
Short Option: Introduction to Biophysics. Trinity Term 2011

Problem set 2 (Lecture 12)

Molecular Motors

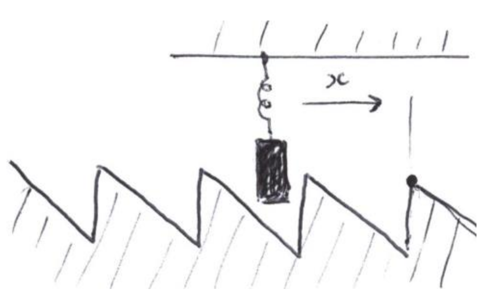
1. Describe with the aid of sketches the structure and function of the enzyme ATP-synthase, including in your description the "binding change" mechanism.
   F_1-ATPase isolated from ATP-synthase rotates in 120° steps, each corresponding to hydrolysis of a single molecule of ATP. Under certain conditions, each step can be resolved into sub-steps of 90° and 30°. Describe how such steps are observed experimentally, and the dependence of the time intervals between steps upon ATP concentration. What conclusions that can be drawn from this experiment about the mechano-chemical cycle of F_1-ATPase.

2. Describe the structure and function of the bacterial flagellar motor, and one technique that allows its rotation to be measured.
   Polystyrene beads of diameter 1 μm rotate at about 80 revolutions per second when attached to bacterial flagella as shown below.



   Given that the viscous drag coefficient of a sphere of radius a rotating around its diameter in water is $8\pi\eta a^3$, where the viscosity of water $\eta = 10^{-3}\ N\ m^{-2}\ s$, estimate the average torque generated by the flagellar motor under these conditions. The flux of protons through the flagellar motor is believed to be on the order of 1000 per revolution, and the pmf in bacteria around -150 mV. Estimate the mechanical efficiency of the motor under these conditions, and comment on your answer.

3. A Feynman thermal ratchet is shown below.



The saw-toothed "rotor" is free to move left and right (x) and the spring-loaded "pawl" is free to move up and down, compressing the spring. One might suppose that Brownian motion of the rotor would occasionally carry it far enough to the right to allow the pawl to pass over the left edge of a tooth, whereupon the spring-loaded pawl would prevent the rotor from moving back towards the left.
Explain why such a "perpetual motion" machine that rectifies thermal fluctuations would not work as described. What would be required to make it work?

Single-Molecule techniques

4. Describe the principle of operation of a single-beam optical trap or "optical tweezers".

   An optical trap exerts a lateral force of 10 pN on a polystyrene bead of diameter 2 μm when the bead is displaced 100 nm from the trap centre. If the trap laser power passing through the bead is 100 mW and the laser wavelength 1064 nm, estimate the average angle of deviation of laser photons. (You may assume that the lateral force arises mainly from photons travelling close to the optical axis.) How can this deviation be used to measure the displacement of the bead from trap centre?

   Make a labelled sketch of the frequency power-spectrum of the bead displacement.

5. Describe briefly two of the following experimental techniques, and give examples of their application in modern biophysics: optical tweezers, patch-clamp, atomic force microscopy (AFM), Forster resonant energy transfer (FRET).

6. Describe briefly the technique of Total Internal Reflection Fluorescence (TIRF) microscopy, and give an example of its application in modern biophysics.

   The wave equation has solutions of the form

   $$E = Eo\ exp\{\ i[(k \sin\theta)x + (k \cos\theta)y - \omega t]\ \}.$$

   For real angles θ, this represents a travelling wave in the (x,y) plane. Show that the same solution but with complex θ describes the electric field in water near a water/glass interface in the case where plane wave illumination is totally internally reflected within the glass. Obtain an expression in terms of the angle of incidence in glass, the frequency ω and the refractive indices of glass and water, for the electric field in water in the above case. Describe with the aid of a sketch the wave that this respesents.

   The water contains a dilute solution of fluorescent molecules which are excited by the incident light. How deep a layer of water, illuminated by an equally intense plane wave at normal incidence, would give the same fluorescence intensity as the above case?