Major Option C7: Biological Physics

Problem set: Bio-energetics and molecular machines

Chemical reactions, free energy, diffusion and Brownian motion

1. a) Write down the diffusion equation in 1 dimension.

   b) Show by direct substitution that a solution exists of the form

   $C(x,t) = A\, t^{-½}\, exp\{ -x^2 / (4Dt) \}$,

   where $C(x,t)$ is the number density of particles at position x and time t, and

   determine the normalization constant, A.

   c) Verify that $\langle x^2 \rangle = 2Dt$ for this solution, and sketch the solution for several values of $t > 0$. By considering the solution at $t = 0$, explain the significance of this result in terms of diffusive transport.

   d) The longest cells in a human body are the motor neurons running from the spinal cord to the feet, consisting of a cell body in the spine and an axon ~1 m long and ~1 μm in diameter. Neurotransmitters are small molecules synthesized in the cell body but needed at the tip of the axon. If all the molecules arriving at the tip are removed by some unspecified continuous process, and the concentration in the cell body is maintained at 1 mM, estimate how many molecules arrive at the tip per second due to diffusion alone, if the diffusion coefficient is ~1000 $\mu m^2\, s^{-1}$.

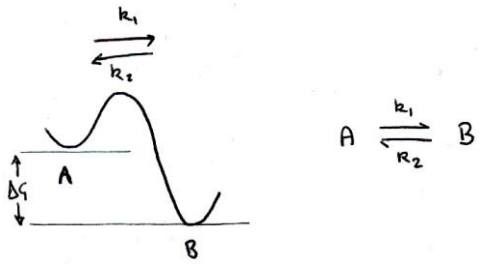
   e) Real neurons package neurotransmitters into synaptic vesicles containing about 10^4 molecules each. About 300 vesicles are required to send a signal to a muscle. What signal rate could be supported by diffusion alone? Is this a reasonable number?

2. Derive the Einstein Relation between the coefficients of diffusion and viscous drag by considering the 1-D diffusion equation with drift.

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

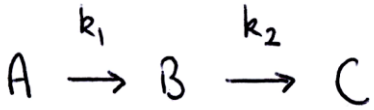
   b) An optical trap exerts a lateral force of 10 pN on a polystyrene bead of diameter 2 μm when the bead is displaced 100 nm from the trap centre. The trap laser power passing through the bead is 100 mW and the laser wavelength 1064 nm. By considering momentum exchange, make a rough "order of magnitude" estimate of 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 ?

4. a) Two representations of the interconversion between any two states of any system are shown below. State the principle of detailed balance as it applies to this system, and the relationship between k_1, k_2 and ΔG that follows from it.



b) If the system starts in state A at time $t = 0$, and A and B are the only possible states, derive an expression for the probability that the system will be in state A as a function of time. Sketch your solution.

c) Derive an expression for the probability that the conversion of a single molecule initially in state A to state C, by the pathway shown below, takes a time between t and $t+dt$: where k_1 and k_2 are unimolecular rate constants for the sequential, irreversible reactions $A \rightarrow B$ and $B \rightarrow C$ respectively.



Biological membranes

5. a) Make a labelled sketch of a phospholipid molecule and discuss the features that determine how phospholipids self-assemble when mixed with water.

b) Derive conditions on the parameter $\alpha = v/(l\ a)$ that determine whether amphiphilic molecules are able to form bilayers and spherical micelles; where v and l are the volume and extended length of the hydrophobic tail and a is the packing area of the hydrophilic head-group.

c) Find expressions for the free energy per unit area and the total free energy required to bend a symmetric planar bilayer of thickness $2t$ into a sphere of radius $R >> t$, assuming that the free energy required to change the packing area of a single lipid head by an amount Δa from its optimal packing area is equal to $\frac{1}{2}k\Delta a^2$, where k is a constant.

d) An experimentalist determines that the free energy required to change the area of a particular type of lipid bilayer by 1% is ~125 kT/(micron^2). The bilayer thickness is 5 nm and the head packing area is 0.7 nm^2. Estimate the total bending free energy of a vesicle made from a symmetric bilayer of the same type, and the bending free energy per head for vesicles of diameter 100 nm and 5 μm.

6. a) A lipid bilayer of thickness $t = 5\ nm$ and relative dielectric constant $\varepsilon_r = 2.5$, incorporating bacteriorhodopsin (bR) molecules and sodium channels, forms a vesicle of radius $r = 100\ nm$. Calculate the capacitance of the bilayer, neglecting the effect of the proteins. The vesicle is illuminated with the sodium channels closed. How many protons must be pumped across the bilayer by BR to establish a membrane voltage of 150 mV ?

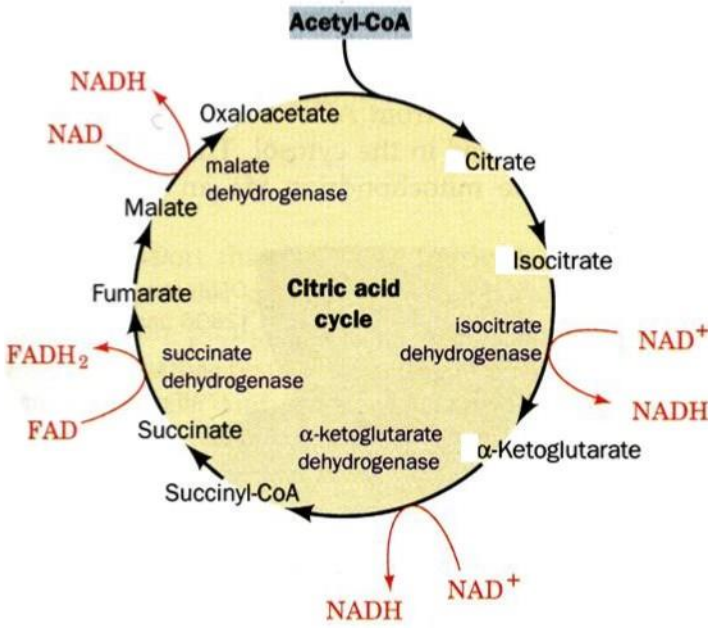
b) At time $t=0$ the light is removed and a total of 10 sodium channels open instantaneously with a single-channel conductance of *10 pS*. Initially the solutions are *100 mM* NaCl, both inside and outside the vesicle: how many sodium ions are there initially inside the vesicle? Obtain an expression for, and make a labelled sketch of, the membrane voltage as a function of time after the channels open, justifying any assumptions you make.

c) If the external medium were changed to *50 mM* NaCl, what would the membrane voltage and the excess osmotic pressure inside the vesicle be, after equilibration?

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Bioenergetics

7. a) The Citric Acid Cycle is shown below.



The half-reactions in the last reaction in the cycle and their standard redox potentials are…

$NAD^+ + H^+ + 2e^- \rightarrow NADH$: $\Delta E'_0 = -0.315$ V

$(oxaloacetate)^- + 2H^+ + 2e^- \rightarrow (malate)^-$: $\Delta E'_0 = -0.166$ V

Write down the full reaction and calculate the standard free energy change in units of kT per molecule. Under standard conditions, which way will the reaction go ?

b) In the mitochondrial matrix, the cycle is driven in the direction shown by a high concentration of Acetyl-CoA, which is in turn maintained by the reactions that break down glucose. The resulting concentrations of oxaloacetate and malate are 50 nM and 0.2 mM respectively, and the pH is 7.5. Assuming that there is negligible loss of free energy as heat during reaction in a) above, calculate the ratio of NADH to NAD^+ concentrations in mitochondria.

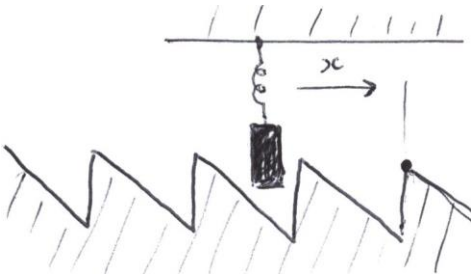
c) The free energy of oxidation of NADH is used to pump protons (H^+) across the mitochondrial inner membrane, generating a protonmotive force of -200 mV. The final resting place of the high-energy electrons is oxygen (hence the term "oxidation"). Given the half-reaction …

$$\tfrac{1}{2}\, O_2 + 2H^+ + 2e^- \rightarrow H_2O \qquad : \Delta E'_0 = +0.815\ V,$$

estimate how many protons can be pumped using the free energy stored in NADH in one turn of the citric acid cycle, under these conditions? Assume a partial oxygen pressure of 0.2 atmospheres.

Molecular motors I: linear motors

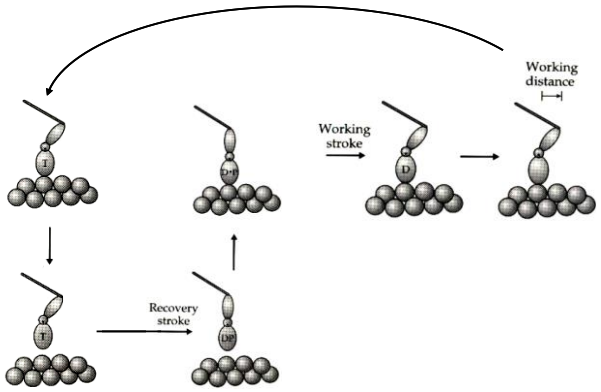
8. A Feynman thermal ratchet is shown below.



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.

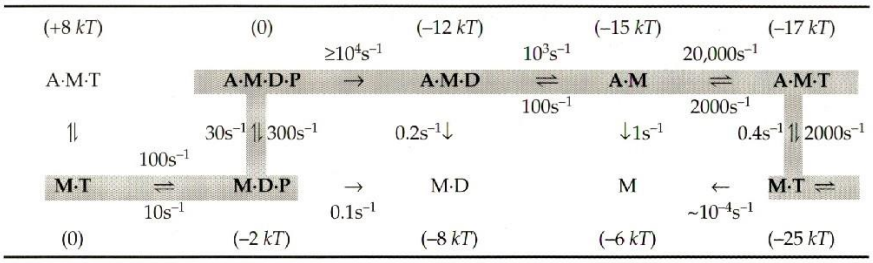
Explain why such a "perpetual motion" machine that rectifies thermal fluctuations would not work as described. What would be required to make it work?

9. a) The mechano-chemical cycle of the actin-myosin molecular motor in muscle is shown below.



Describe the states and transitions in the cycle, and explain how they couple the hydrolysis of ATP to active contraction of muscles.

b) Experimentally measured (or estimated) rate constants and relative free energies for reactions in the cycle are given below (the state top-left in the diagram above corresponds to A·M·T below. A = actin, M =myosin, T=ATP, D=ADP, P=phosphate).



The cycle can be modelled as a sequence of instantaneous chemical transitions between the states. Make a labelled sketch of the free energy profiles for each state in the cycle, as functions of the relative position of actin and myosin filaments. Indicate with arrows the transitions between states. Using the same position axis, sketch also the rate constants for these transitions.

c) The model can be reduced to a minimal form that includes one binding powerstroke with rate constant k_{on} (combining M·D·P → A·M·D·P → A·M·D) and one release step with rate constant k_{off} (combining A·M·D → A·M → A·M·T → M·T → M·D·P). Make similar sketches to those in b) above for the reduced model.

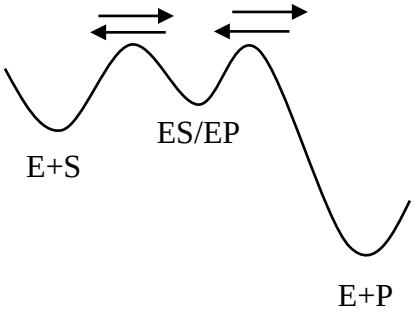
d) Use the minimal model to show that the average force per crossbridge is given by

$$\langle F \rangle = \{\kappa / D\} \{ 1 - \exp(- k_{on}\Delta x / v) \} \{ \tfrac{1}{2} x_o^2 - v^2 / k_{off}^2 \},$$

where D is the distance between accessible binding sites for a myosin head. Assume that the muscle fibre slides at a constant velocity v due to the combined action of millions of crossbridges, that the crossbridge can be described by a linear spring of stiffness κ in all bound states, that binding occurs only in a very small range of positions between x_o and $x_o+\Delta x$, where $x = 0$ is defined as the position where the post-powerstroke crossbridge is relaxed, and that release is possible only when $x > 0$.

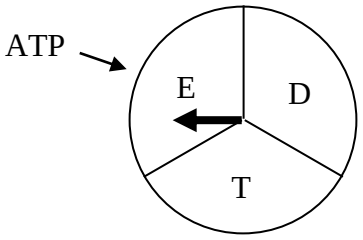
Molecular motors II: ATP synthase

10. A free-energy diagram for the conversion of substrate *S* to product *P*, catalysed by enzyme *E*, is shown below.



a) Derive the Michaelis-Menten equation for the rate of production of *P* in the limit of excess substrate concentration, as a function of the concentration of substrate, the total concentration of enzyme and the rate constants for the reactions indicated above by arrows. Give expressions for the maximum rate and the substrate concentration at half-maximum rate, and discuss the assumptions that are made in the derivation.

b) Describe the binding-change mechanism for the hydrolysis of ATP by the enzyme F_1-ATPase ("F_1"). Illustrate the mechanism with diagrams similar to the one shown below, which represents F_1 in the "ATP-waiting" state with one empty site (E) one site occupied by ATP (T) and one site occupied by ADP (D). The heavy arrow indicates the orientation of the gamma subunit.



c) The binding-change mechanism is "tightly coupled". Use your answers to a) and b) above to write an expression for the rate of rotation of the gamma subunit as a function of the rate of product release and the rate constant and dissociation constant for ATP binding. Discuss quantitatively how applying a load opposing rotation of gamma would affect this rate?

d) F_1 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.

Molecular Evolution

11. Paper C7, 2008, Q1b

Write (a) short (essay) on…

Molecular evolution, including a discussion of phylogenetic trees based on sequence information and their time calibration.

Diffusion revisited…

12. Paper C7, 2008, Question 4:

(Note: the bacterial flagellar motor is now off-syllabus)

Describe briefly the structure and function of the bacterial flagellar motor. *[8]*

By considering the 1-D diffusion equation with drift, derive the Einstein Relation between the rotational diffusion coefficient D_θ and the rotational viscous drag coefficient γ_θ of a tethered bacterial cell (in which a single flagellar filament is firmly attached to a solid surface). [5]

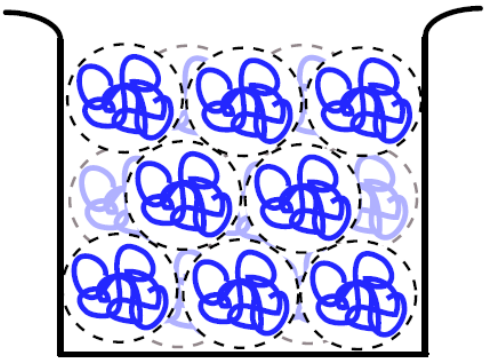
Measurements are made of the mean ($<\theta>$) and variance (σ^2_θ) of the angles rotated by a single tethered bacterial cell in a large number of independent intervals of 1 s, with the aim of distinguishing between two competing theories of the flagellar motor mechanism. In the first theory, the motor is a "fluid drive" which exerts a constant torque but sets no direct constraint on the rotation angle. In the second theory, Brownian fluctuations in motor angle are negligible, the motor always takes *m* steps per revolution, and steps are governed by a Poisson process such that the variance σ^2_N in the number of steps taken in a fixed time interval is equal to the mean number of steps $<N>$. For a cell with $\gamma_\theta = 8 \times 10^{-21}$ J radian^{-2} s the results are: $<\theta> = 20\pi$ radians, $\sigma^2_\theta = 0.1\ \pi^2$ radians^2. Do the measurements distinguish between the two theories? [7]

The experiment is repeated with the same cell and an additional constant external torque applied to the cell, and the results are $<\theta> = 40\pi$ radians, $\sigma^2_\theta = 0.19\ \pi^2$ radians^2. What further conclusions can be drawn with respect to the two theories of the motor mechanism? [5]

Problems. C7. Sonia Contera. 2021

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1. Explain why a freely jointed chain left to itself tries to coil itself. What is it trying to maximize or minimize by doing that?
2. Derive the exact relation of force and extension in a freely jointed chain. Discuss two extreme conditions: (i) the elasticity in the weak force limit, and (ii) the strong force limit. Explain if and how it deviates from a Hookean spring.
3. (a) A polymer, feely jointed chain, with a statistical segment length of 5 nm and containing 10,000 segments is stretched with a force of 0.25×10^{-12}N at room temperature (25ºC). What is the end-to end distance of the chain?
   (b) If the temperature is raised to 100ºC, what does the end-to-end distance become if you assume the polymer does not degrade? What if the statistical segment length is 2 instead of 5nm, but the contour length remains the same at room temperature?
4. How dilute must a polymer solution be in order that each polymer chain be non-intersecting?



Schematic of a container of ideal freely jointed polymer chains at a semi-dilute concentration, i.e., at the highest concentration at which the polymers are isolated single chains and do not interpenetrate each other. The size of an isolated, single chain is given by $R \sim a\sqrt{N}$, and the dotted lines identify spheres of this radius that circumscribe the ideal chains. Higher concentrations require that the chains interpenetrate, i.e. the circumscribing spheres would intersect.

5. **<u>Energy of assembly of a virus as a charged sphere</u>**

(a) Virus capsids are made up of proteins held together for contact forces (e.g. hydrophobic). An important contribution to the energy needed to assembly the capsid is the electrostatic cost for bringing the capsomers in close proximity to form the capsid. The energy cost for assembling a spherical shell of charge Q and radius *R* is *½ QV(R)* where *V(R)* is the potential of the surface of the sphere. Calculate the potential for a charge distribution that is spherically symmetric, and from the potential calculate the electrostatic energy of the spherical shell in a salty solution. (hint: derive the Debye-Hueckel equation for spherical symmetry) .

(b) A simple model for the energy needed for assembly of the capsid is:

$$\Delta G_{capsid} = \Delta G_{contact} + \Delta G_{electrostatic}$$

Calculate the energy for assembling a hepatitis B virus using the electrostatic energy calculated in 1.1 assuming the capsid is made of 120 capsomers, and that each of them has a charge ze. Assume a 100 mM monovalent ion concentration, and hence the Debye length is ~ 1 nm, which is much smaller of the radius of the virus (R= 70 nm). Compare the result to experimental data for the assembly of the hepatitis B virus shown in figure 1

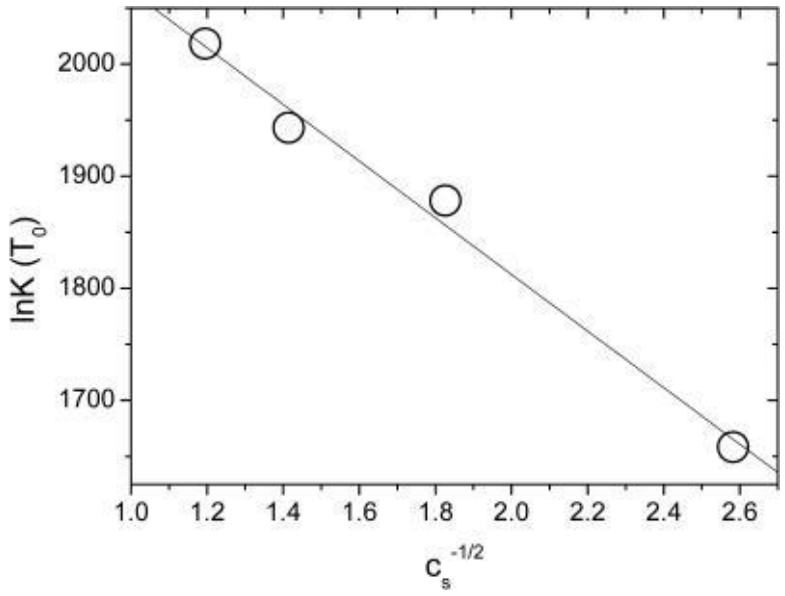


Figure 1.The logarithm of the equilibrium constant, ln **K**, *at the temperature* **T** = T_0 = *273 K as a function of the inverse square-root of the salt concentration* $1/\sqrt{c_S}$. *The slope of the fitted line is −(253 ± 19) and its intercept is +(2317 ± 35). From Biophys J. 2004 Jun; 86(6): 3905–3913. doi: 10.1529/biophysj.104.040055.*

6. Estimate the force to break a van der Waals bond between two methane molecules of molecular radius 0.2 nm using the sphere-on-sphere geometry a Hamaker constant A= 5 10^{-20} J and a cut off distance of 0.4 nm.

7. <u>Nernst potentials and the resting membrane potential.</u>

(a) Fill in the missing values in the following table:

Ion	Cytoplasmic Concentration (mM)	Extracellular Concentration (mM)	Equilibrium Potential (mV)
K^+	400	20	
Na^+	50	440	
Cl^-	40	560	

(b) Calculate the resting membrane potential given the following conductances:

$$g_{Na} = 0.5 \cdot 10^{-6}\ S,$$

$$g_{K} = 10 \cdot 10^{-6}\ S,$$

$$g_{Cl} = 2.5 \cdot 10^{-6}\ S.$$

(c) Fill in the missing information in the following table:

Conductance Change	Ion Action	Membrane Potential Change
large g_{K} increase		
	Na^{+} enters	
		V moves to V_{Cl}

Hint: Using the steady state equation (

$$V = \frac{k_B T}{q} ln\left[\frac{P_{Na}c^e_{Na} + P_K c^e_K + P_{Cl}c^i_{KCl}}{P_{Na}c^i_{Na} + P_K c^i_K + + P_{Cl}c^e_{Cl}}\right]$$

Where P_j =membrane permeability for ion species j, c^e_j, and c^i_j, are the external and internal ion concentrations respectively, and *q* is the charge on the electron)

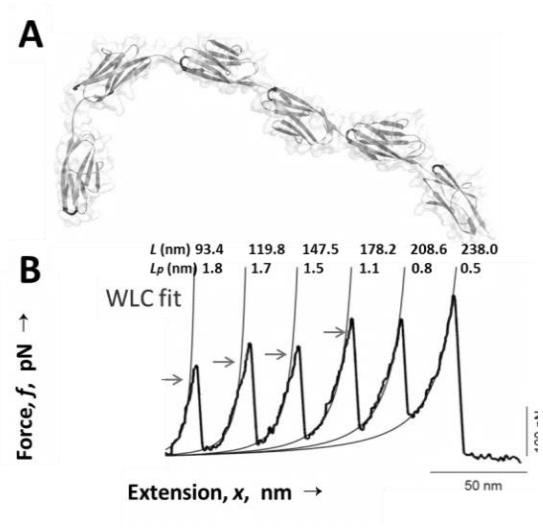
if we define the conductance as g= 1/Resistance, we can calculate the membrane potential of a cell from

$$V_m = \frac{\sum_i (V_i\ g_i)}{\sum_i g_i}$$

where *i* is the ionic species.

8. Assume that a proto-cell has a membrane capacitance of 1 μF/cm^2 and contains only K+ selective channels. The initial intracellular and extracellular concentrations for K^+ are given in the table above, and there are intracellular and extracellular anions A^- equal in concentration to K^+.
(a) the membrane K^+ channels are opened, what is the steady–state voltage across the membrane assuming that ion concentrations do not change?
(b) How many K^+ ions must move out of the cell per cm^2 to achieve a voltage difference equal to that in part (a)?
(c) Assuming that our proto-cell is perfectly round and that the extracellular K^+ concentration does not change, plot the final intracellular K+ concentration as a function of the diameter of the cell. Use a range of realistic diameter values and the number of ions transferred that you calculated in part (b).
(d) Assuming that the extracellular K^+ concentration remains constant, what would the reversal potential be for the range of intracellular K^+ concentrations calculated in part (c).
(e) How do you reconcile parts (a) and (d)?

9. Titin is a protein that functions as a molecular spring and is responsible for the passive elasticity of muscle. It is composed of individually folded protein domains connected by unstructured peptide sequences Fig. A These domains unfold when the protein is stretched and refold when the tension is removed. Fig. B shows a typical force vs. extension curve with a characteristic saw-tooth pattern, obtained from an experiment where a titin protein is unfolded using the cantilever of an atomic force microscope.



1. Qualitatively describe the meaning of the force (*f*) vs. extension (*x*) curve, the presence of peaks, and how this curve relates to the protein unfolding as is being stretched by the AFM cantilever during the experiment.

2. The Worm Like Chain (WLC) model can be used to fit the *f* vs. *x* curve peaks of unfolding proteins. Fig. B shows WLC fits for titin unfolding. It describes proteins as inextensible continuum chains, formed by isotropic, homogeneous elastic rods with contour length *L* (the polymer length at maximum extension), when x < *L*. WLC provides an estimate of entropic elasticity and does not take into account energetic elasticity. Using WLC, the force that resists stretching can be approximated as a function of *x/L* by

$$f = \frac{k_B T}{L_p}\left(\frac{1}{4\left(1-\frac{x}{L}\right)^2} - \frac{1}{4} + \frac{x}{L}\right)$$

where L_p is the persistence length (L_p is the arc length at which the rod bends on average by an angle θ of 68.4° away from a straight rod standard due to thermal fluctuations, it is a measure of the polymer chains bending rigidity). T is the temperature and k_B is the Boltzmann constant.

What physical information can be extracted from fitting each peak of the experimental curves with this model?

Explain why the WLC model is not a good fit for the last part for the first 4 peaks (indicated by arrows in Fig. B).

SJT Membrane Proteins Problem Set C7 MT 2021

The class will also be an opportunity to discuss how to approach some of these Q should they appear in an exam and to discuss the observations that you will make about the structures in Q2.

1. Ionic Currents

a) Briefly describe and illustrate the main intrinsic functional and biophysical properties of K+ and Na+ ion channels which allow them to generate a typical action potential *(No more than ~250 words).*

b) Assume a heart muscle cell (cardiomyocyte) is a cylinder 50μm long and 5μm in diameter (c_m: 0.02 Farads m^{-2}). How many Na^+ ions would be required to depolarize the cell by 100 mV during the upstroke of an action potential of 5 ms duration. Assume intracellular [Na^+] = 15mM.

What change would this produce in the intracellular concentration of sodium? What is significant about the value you have obtained?

c) Estimate the conductance through a pore of 0.15 nm diameter and 0.5nm in length, where the ionic solution on both sides of the membrane has a resistivity of ρ= 80Ω•cm and unrestricted access to the mouth of the pore.

d) In an electrical recording of a cell the macroscopic K^+ current recorded through all of the channels in the membrane at a clamped voltage of -50 mV is 9 nA. It is estimated that there are 3 x10^3 of these channels in a cell and that their open probability (*Po*) is 0.6. Calculate the individual conductance of these K^+ channels and briefly explain why channels like this are able to sustain high rates of conductance.

e) Long QT Syndrome is an inherited cardiac disease which increases the duration of a heart muscle cell action potential. Describe very briefly how this might be achieved by changing the biophysical properties of either the Na^+ or K^+ current which underlies the cardiac action potential *(No more than ~250 words).*

f) For an ion channel that exists in only two states (e.g. open or closed), illustrate how differences in the rates of transition between these two states can define the macroscopic behaviour of these channels. Illustrate your answer by comparing Channel A, where the opening and closing rates are both 0.5/ms with Channel B where both rates are 5/ms.

With reference to the answers you have calculated, describe simple kinetic schemes that might explain the macroscopic behaviour of a typical voltage-gate K+ channel and voltage-gated Na+ channel during an action potential. Briefly explain how these schemes might need to vary in a heart cell firing action potentials at 1 Hz versus a nerve cell firing at 100 Hz.

2. Hydropathy analysis on membrane proteins. Visualization of Structures

a) Using hydropathy analysis, determine the location of the transmembrane α helices in this human voltage-gated potassium channel (*Kv1.2)* which has the following amino acid sequence:

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MTVATGDPADEAAALPGHPQDTYDPEADHECCERVVINISGLRFETQLKTLAQFPETLLGDPKKRMRYFDPLRNEYF
FDRNRPSFDAILYYYQSGGRLRRPVNVPLDIFSEEIRFYELGEEAMEMFREDEGYIKEEERPLPENEFQRQVWLLFE
YPESSGPARIIAIVSVMVILISIVSFCLETLPIFRDENEDMHGSGVTFHTYSNSTIGYQQSTSFTDPFFIVETLCII
WFSFEFLVRFFACPSKAGFFTNIMNIIDIVAIIPYFITLGTELAEKPEDAQQGQQAMSLAILRVIRLVRVFRIFKLS
RHSKGLQILGQTLKASMRELGLLIFFLFIGVILFSSAVYFAEADERESQFPSIPDAFWWAVVSMTTV GYG DMVPTTI
GGKIVGSLCAIAGVLTIALPVPVIVSNFNYFYHRETEGEEQAQYLQVTSCPKIPSSPDLKKSRSASTISKSDYMEIQ
EGVNNSNEDFREENLKTANCTLANTNYVNITKMLTDV
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Sequence also available on this database:

(http://www.ncbi.nlm.nih.gov/protein/NP_004965.1)

(Hint: it takes about 20aa for a helix to extend across the membrane – **don't set window size too small** – gets noisy!). You could write a simple program for the analysis and use a hydropathy-related table of values from your notes or any text book for the energy values of each amino acid e.g.

http://gcat.davidson.edu/DGPB/kd/aminoacidscores.htm

Web based methods of analysis also exist (e.g. https://web.expasy.org/protscale/ and use Hphob Kyte-Doolittle analysis with window size >15) but basic form of the analysis should be explained if you use it.

Can you identify the selectivity filter region and the transmembrane domains? What is unusual about the 4th transmembrane segment (occurs at ~amino acid 300)?

b) Go to the protein data bank (http://www.rcsb.org/pdb/home/home.do) and download the pdb file containing coordinates for the structure of the Kv1.2 voltage-gated K+ channel (PDB ID: 2A79). Use a simple pdb viewer to view the structure. (e.g. http://www.geneinfinity.org/rastop/ or http://pymol.org/edu/ and educational version available for free with relatively easy drop-down menus) or you may even be able to use the online viewer options offered through the PDB website itself (though these are less flexible and you may not be able to select residues).

Questions to address:

i) What is unusual about the structure you have downloaded? Does it look like a K+ channel? Compare it to the fully assembled 'biological unit' which you can download from the Canvas site alongside this problem set. Or if using an online viewer select the 'bioassembly' or 'biological unit' option – not the 'asymmetric' unit determined in the crystal structure. (**Note that the PDB file provided on Canvas has been truncated and contains only the transmembrane helices and not the intracellular parts of the channel found in the PDB file**.)

ii) Highlight all the **arginine** (Arg/R) and **lysine** (Lys/K) amino acids within the protein. What is unusual about the distribution of arginine and lysine residues within the structure (what physical property do these two amino acid side chains have? What environment would they be exposed to? (**Hint**: see Roux, *J.Gen. Physiol.* (2007) **130** 233)).

iii) Work out which part of the structure is the pore i.e. permeation pathway (clue: pore looks like KcsA K+ channel), which sections are the voltage-sensor, which part sits in the membrane?

Create images of your findings (helps if you set the background to white) and annotate the structures with your answers.

Problem set for DNA packaging and gene expression module (+ fluorescence spectroscopy)

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Note: In some problems, you have to make reasonable assumptions concerning sizes, rates, timescales etc. State all assumptions clearly.

1. DNA constitutes ~1% of the mass of an *E. coli* cell.

a. What is the mass of the *E. coli* chromosome?

b. Assuming a molecular mass of 660 for the average basepair, estimate how many base pairs are in the *E. coli* genome. State all assumptions.

c. Compare your estimate with the known size of the *E. coli* chromosome and offer explanations for any discrepancies.

2. In the derivation of the diffusion-controlled association rate constant (see Lecture on Transcription) :

a. Finish the derivation of the expression for the rate of molecular collisions for single B molecule. Calculate the conversion factor needed to convert inward flux to association rate constant.

b. Estimate the mean time τ_3 needed in an *E. coli* cell for one lacR molecule (modelled as a sphere with 10 nm diameter) to find one chromosomal target site solely by 3D diffusion. Cytoplasm viscosity $\eta = 10^{-2}$ Pa·s .

3. DNA packing in bacteriophage capsids.

a. Work out the expressions for the elastic energy costs and the associated force for the packing configuration of stacked rings in a cylindrical capsid (see slide 14, lecture 2).

b. Solve the differential equation that describes the spacing dependence of the electrostatic interaction pair potential v_{ds} to obtain the electrostatic energy cost for the packing configuration of stacked rings in a cylindrical capsid (see slide 17).

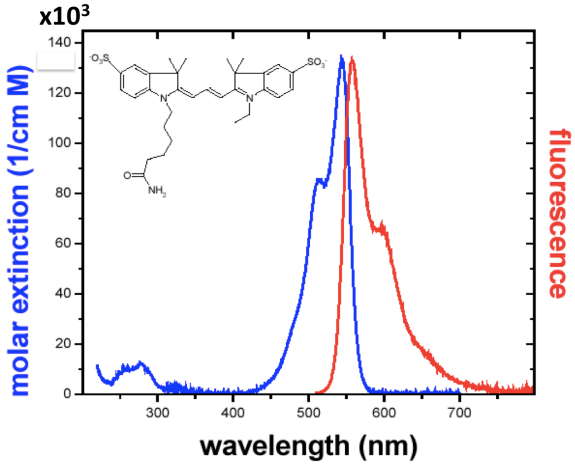
4. You perform single-molecule fluorescence studies using a diffraction-limited focused laser and confocal optics.

a. How does the volume of the focused laser beam compare to the volume of an *E. coli* cell?

b. If the focused beam is occupied by one molecule for 10% of the time, what is the concentration of the solution being examined?

c. Calculate the signal-to-noise ratio (SNR) for detecting single Cy3-labeled proteins that excites Cy3 (with fluorescence quantum yield Q_F ~ 20%) with 100 µW from a 532-nm laser. The setup has detection efficiency of 1%, background rate bV of 1 Hz/µW and dark current of 20 Hz. Assume that the fluorophores spend ~1 ms in the observation volume. Note: to convert molar extinction coefficients ε (in $M^{-1}cm^{-1}$) to absorbance cross sections (in cm^2), multiply ε by 3.8 x 10^{-21}.

d. In the above scenario, what will be the SNR if the observation volume V increases to 1 µL?



5. Using the theory of diffusion-controlled association reactions, calculate the time required to detect one molecule from a 1-pM solution of a fluorescently labeled nucleotide in an aqueous buffer (viscosity of 10^{-3} Pa·s). To simplify, assume that the confocal spot is a sphere with diameter of 0.5 µm.

6. A protein is labeled with a pair of one FRET donor and one FRET acceptor fluorophores with Förster radius R_o of 60 Å. The fluorescence lifetimes of donor and the acceptor fluorophore are 6 ns and 1 ns, respectively. You want to study conformational changes from state A to B.

a. From the definition of k_{FRET} (slide 9, single-molecule fluorescence lecture), show that

$$E = \left(1 + \left(R/R_o\right)^6\right)^{-1}$$

b. Considering that FRET creates an additional path for the non-radiative relaxation of the FRET donor, show that $E = 1 - \left(\tau_{DA}/\tau_D\right)$, where τ_{DA} and τ_D are the fluorescence lifetimes of the donor in the presence and absence of acceptor, respectively.

c. State A shows a donor lifetime of 60 ps. What is the FRET efficiency and the corresponding donor-acceptor distance?

d. In state B, the fluorophore separation decreases by 10 Å. What is the donor fluorescence lifetime? Would you be able to see the distance change if your error of your FRET efficiency measurement is ≥2%?

e. Suggest a strategy that will allow you to see the conformational change more clearly. What is the maximum FRET change you can possibly see for this conformational change?

7. The full equation that describes the lateral localization precision for a single fluorescence emitter is given by the equation:

$$\sigma = \sqrt{\left(\frac{s^2 + \frac{a^2}{12}}{N}\right) \cdot \left(\frac{16}{9} + \frac{8\pi(s^2 + \frac{a^2}{12})b^2}{a^2 N}\right)}$$

where s is the standard deviation of the PSF of the fluorophore used, N is photon count of the signal per frame, a is the pixel size of the camera, and b is the background count. Assume that the fluorophore is Cy3 (see problem 4), that your camera has pixels corresponding to 100 nm in the specimen plane, and that you have a background of 10 photons. What is your localisation precision for a high signal-to-noise measurement with a signal of 5000 photons, and for a low signal-to-noise with a signal of 200 photons?

8. What is the minimum length of time required for the synthesis by *E. coli* RNA polymerase of an mRNA encoding a 100-kDa protein? What is the average distance in base-pairs between two adjacent transcription bubbles (i.e., the single-stranded DNA portions around the sites of transcription) on *E. coli* genes being transcribed at a maximal rate?

9. Consider the zipper model of the helix-coil transition.

a. Show that the partition function for the zipper model is $q = 1 + \sum_{i=1}^{n} N_i \sigma s^i$ and that the number of possible ways to form a zipper-allowable state is $N_i = n - i + 1$ (for a chain with i coil amino acids out of n total amino acids).

b. Using spreadsheet software, plot the probability of having a chain with i coil amino acids out of n=20 total amino acids for s=0.5, s=1, and s=2 and nucleation constant $\sigma = 1 \times 10^{-3}$.

c. Using spreadsheet software, plot the degree of conversion vs the equilibrium constant s for nucleation constants $\sigma = 1 \times 10^{-4}$, 1×10^{-3}, and 1×10^{-2}.

10. Consider a bimolecular reaction of the type $A + B \rightleftharpoons AB$ (with on-rate k_1 and off-rate k_{-1}). Assume that the equilibrium binding constant for a protein-DNA interaction is $K_a = 10^9\ M^{-1}$, and that the total concentration of B (i.e., $[B]_t = [AB]+[B]$) is held constant at 10 pM. Answer the following:

a. if you increase the concentration of A in equilibrium by 50-fold (while keeping the *total* concentration of component B constant), how will K_a be affected at equilibrium? How will K_d be affected at equilibrium? How will the ratio {[AB]/[B]} be affected?

b. if the fraction of bound B can be measured for a wide range of [A] (while keeping the *total* concentration of component B constant), would you be able to measure a) the equilibrium constant K_a? b) the rate constant k_{-1}? c) the activation energy? If yes, how?

11. The following kinetic scheme is used to describe the formation of RNA polymerase-promoter open complex, RP_o (R: RNA polymerase; P: promoter DNA; RPc; RNAP-promoter closed complex):

$$R + P \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} RP_c \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} RP_o$$

Making steady-state approximations, show that the observed rate constant k_{obs} that controls the formation of RP_o is:

$$k_{obs} = \frac{k_{12}k_{23}[R]}{k_{12}[R] + k_{21} + k_{23}}$$ (where [R] is the concentration of the enzyme).

12. LacR finds its binding site through a combination of 1D and 3D diffusion with diffusion coefficients D_1 and D_3, respectively.

a. Go through the derivation of the search time that a DNA-binding protein needs to find a specific DNA-binding site in the cell (Lecture 6 of HT) and show that the total search time is $\tau_{search} = \frac{V}{D_3 l_{sl}} + \frac{L l_{sl}}{D_1}$

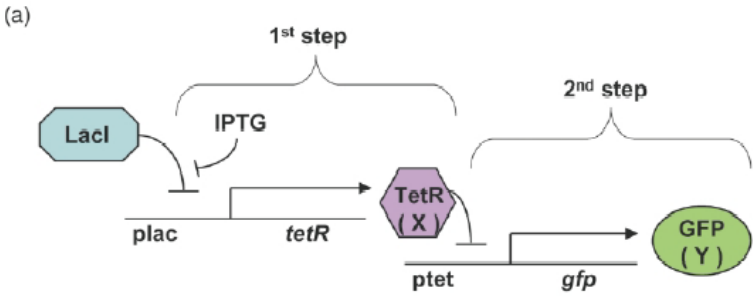
b. Sketch and discuss the dependence of τ_{search} upon l_{sl}, and explain the origin and the dependence of the two terms that contribute to τ_{search} as well as their functional significance for the combined search process. Find expressions for the optimum l_{sl} and the minimum τ_{search}; and derive an expression for τ_{search} as a function of τ_3, diffusion coefficients, V, L and l_{sl}.

c. Using the minimum search time and diffusion coefficients $D_1 = 2.1 \times 10^{-10}\ cm^2/s$ and $D_3 = 4 \times 10^{-7}\ cm^2/s$, calculate the average time needed for one *lac* repressor molecule to find one of its operators on the *E. coli* chromosome.

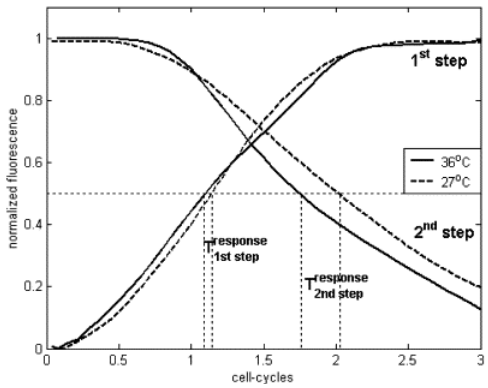
PROBLEM SET Biological Physics C7

Biological Networks and Systems Biology (2020)

1) Cascades. Consider a cascade of activators: X→Y→Z. Protein X is initially present in the cell in its inactive from. The input signal of X, S_x, appears at time t=0. As a result, X rapidly becomes active and binds the promoter of gene Y, so that protein Y starts to be produced at rate β. When Y levels exceed a threshold K_{yz}, gene Z begins to be transcribed. What is the concentration of gene product Z as a function of time? What is its response-time with respect to addition of S_x? What about a cascade of repressors: X—| Y—| Z? Assume $Y(0)=Y_{st}$, Z(0)=0. Briefly describe the experiment of N. Rosenfeld and U. Alon, Journal of Molecular Biology Volume 329, Issue 4, 13 June 2003, Pages 645-654, and compare your solutions to the experiments shown in the figures below, taken from the paper. (Assume that the degradation rate α is the same for all proteins.)



The transcription cascade (LacI—| TetR—| GFP) is made of two well-studied repressors, the lacI and tetR . TetR controls the green fluorescent protein gene.



Response time is about one cell generation per cascade step. The first step in the cascade rises in response to the inducer IPTG that inactivates the repressor LacI. This step encodes a repressor TetR that causes a decreases in the activity of the second-step promoter. Both steps were monitored using a green fluorescent protein (GFP) reporter, in two strains, one that reports for each step in the cascade. The experiment was carried out at two temperatures, which show different cell-generation times (about 2-fold slower at 27C compared to 36C). The x-axis shows time in units of the cell generation time in each condition.

2) ***Pulse of activation****:* Consider the positive feedback cascade of exercise **1**. The input signal S_x appears at time t=0 for a pulse of duration D, and then vanishes.
(a) What is the concentration Y(t)?
(b) What is the minimal pulse duration needed for activation of gene Z?
(c) Plot the maximal level reached by the gene product Z as a function of the pulse duration D.

3) ***Positive feedback***. What is the effect of *positive* auto-regulation on the response time? Use as a model the following linear equation:
$dX / dt = \beta + \beta_1 X - \alpha X$
Explain the biological origin of each term and solve for the response time. What happens when $\beta_1 > \alpha$? When might such a design be biologically useful?

4) ***Auto-repression with Hill input function***. What is the response time for a repressor that cooperatively represses its own promoter (described by a Hill-function with Hill coefficient *n*):

$$dX / dt = \beta / (1+(X / K)^n) - \alpha X$$

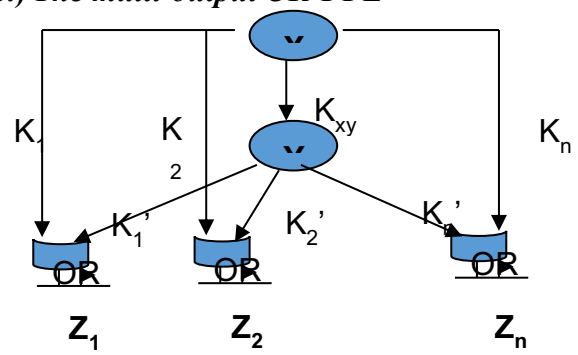
How much faster is the response than in non-auto-regulated circuits?
To solve this problem, use the approximation of strong auto-repression, that is $(X / K)^n >> 1$ (Show the conditions for which this approximation works)

$$dX/dt = \beta K^n / X^n - \alpha X$$

To solve this equation, multiply both sides by X^n and switch to the new variable $u=X^{n+1}$. Note that $d u/dt = (n+1) X^n \, dX /dt$.
Show that the response time becomes shorter for higher values of n.

5) ***The second input.*** What is the affect of steps of S_y on the expression dynamics of Z in the C1-FFL with AND logic? Are there delays in Z expression for ON or OFF steps of S_y? What is the response time of Z for such steps? Assume that S_x is present throughout.

6.) ***The multi-output OR-FFL***



In a multi-output C1-FFL with OR-gate logic at the Z promoters, transcription-factor X begins to be produced at a constant rate β at time t=0. At time t=T, the production rate β suddenly becomes equal to zero. Calculate the dynamics of the downstream genes Zi. What are the delays between genes? (use logic input functions).
How should the threshold be designed to achieve "First In First Out" (FIFO) behaviour?
Explain how this circuit filters out short OFF pulses of X. Why would this be biologically useful?

7.) Kinetic Proofreading

$$[tRNA_c] + [C] \underset{k_-^c}{\overset{k_+^c}{\rightleftharpoons}} [tRNA_c C] \xrightarrow{m} [tRNC_c^*\ C] \xrightarrow{V} tRNA_c + \textbf{correct amino acid}$$
$$\downarrow l_-^{c*}$$
$$tRNA_c + C.$$

The reaction equation above describes a kinetic proofreading scheme for translation of a codon C on mRNA to correct amino acid by the ribosome. $tRNA_c$ stands for the transfer-RNA that carries the correct amino acid. Assume that there is also a different $tRNA_d$ that carries incorrect amino acid. Derive the error rate produced by the proofreading process above, and compare it to a process that does not have the additional proofreading step (the step with $tRNA_c^*$). What conditions (inequalities) on the rates allow for the most effective kinetic proofreading?

8.) **Noise in gene expression**

(a) A gene is transcribed to mRNA molecules, with a concentration R, that are in turn translated to proteins, with a concentration P. The concentrations can be described by the Langevin equations:

$$\frac{dR}{dt} = k_R - \gamma_R R + \eta_R(t)$$
$$\frac{dP}{dt} = k_p R - \gamma_P P + \eta_P(t)$$
$$<\eta_i(t)>= 0; \quad <\eta_i(t)\eta_j(t-t')>= q_i\delta(t')\delta_{ij}$$

where the γ_R and γ_P are the degradation rates, and k_R and k_P are production rates and η_R and η_P are the noise terms. for the RNA and proteins respectively. What are the typical time-scales for the degradation rates of RNA and proteins and why are they different?

Derive the steady-state concentration of protein, P and comment on how it varies with degradation rates and production rates. The scientific literature on this problem often defines an average burst size $b = k_P/\gamma_R$. What does this dimensionless number describe?

(b) In a classic paper, E. M. Ozbudak, I. Kurtser, A.D. Grossman and A. van Oudenaarden, Nature Genet. **31**, 69 (2002), showed that the noise strength, measured by the variance σ_P^2 in protein concentration P, takes the form: $\sigma_P^2 = <P>(1 + b)$ where the Fano factor F= 1+b measures the deviation from Poissonian behaviour. In the Appendix of this paper, they sketch a derivation of this result. Go through their derivation equation by equation and explain what assumptions they make in each step (note that there is a small error – for you to spot ...). In

particular, show explicitly how to derive each step up to the equation for the steady-state value of the fluctuations in RNA concentration, and explain how the strength of the noise was fixed through a fluctuation-dissipation relation.

To increase your physical insight into the results, first briefly describe what a Poisson process is and how its fluctuations scale. Explain qualitatively why the fluctuations here are non-Poissonian and give a semi-quantitative argument for the origin of the factor b.

Describe the difference between intrinsic and extrinsic noise in gene expression. How would you measure this in a cell? What kind of noise are Ozbudak et al., describing?