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SECOND PUBLIC EXAMINATION

Honour School of Physics Part C: 4 Year Course

Honour School of Physics and Philosophy Part C

C7: BIOLOGICAL PHYSICS

TRINITY TERM 2011

Monday, 27 June, 9.30 am – 12.30 pm

Answer **four** *questions.*

Start the answer to each question in a fresh book.

A list of physical constants and conversion factors accompanies this paper.

The numbers in the margin indicate the weight that the Examiners anticipate assigning to each part of the question.

Do NOT turn over until told that you may do so.

1. List two of the main functions of RNA polymerase, as well as the two main differences between nucleotides in DNA and RNA. [4]

Using sketches, describe the basic principles behind using magnetic tweezers to study the formation of an open transcription complex between RNA polymerase and DNA. [8]

In a single molecule experiment, transcription elongation proceeds under the influence of an opposing force F applied between the RNA-polymerase and the template DNA strand. The experiment is performed using conditions that ensure that all nucleotides bind to the elongation complex with the same affinity. Assume that the reaction cycle comprises several reversible transitions (with a total mean time τ_1) involving nucleotide binding and hydrolysis but no RNA-polymerase movement on DNA, followed by one irreversible transition (with a mean time τ_2) that involves movement on DNA but no nucleotide binding. Sketch the reaction cycle and show that the reaction velocity is:

$$V = \frac{V_{\max}}{1 + A\mathrm{e}^{F\Delta/k_{\mathrm{B}}T}}$$

where $V_{\max}$ is the maximum velocity, Δ is a characteristic distance, A is a constant for a fixed nucleotide concentration, and $k_{\mathrm{B}}T$ is the thermal energy. [8]

Derive an expression for the force at half-maximum velocity, $F_{1/2}$. Sketch the velocity as a function of F, indicating $F_{1/2}$ and $V_{\max}$. For $\Delta = 0.3\,\mathrm{nm}$, estimate the change in $F_{1/2}$ due to a decrease in nucleotide concentration that doubles the average time spent in transitions involving nucleotides. [5]

2. Describe briefly the principles of fluorescence correlation spectroscopy (FCS) and sketch the essential elements of a confocal FCS instrument. Explain with sketches how FCS can be used to determine the diffusion coefficient and concentration of fluorescent molecules in solution. [7]

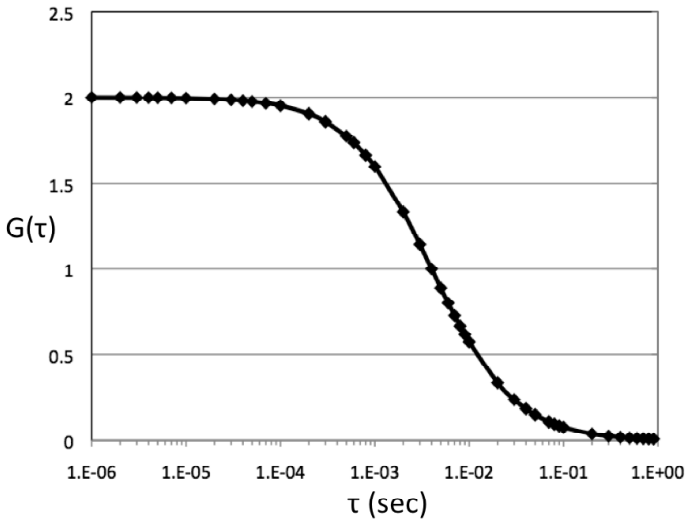
The mean fluorescence intensity $\langle F \rangle$ in FCS is proportional to the mean number of molecules $\langle N \rangle$ in the effective volume $V_{\text{eff}} = \pi^{3/2}\, r_0^2\, z_0$, where r_0 and z_0 are the radial and axial dimensions. For intensity fluctuations that occur solely due to random 3D diffusion of fluorescent particles that follow Poisson statistics, show that the fluorescence correlation function

$$G = \frac{\langle F^2 \rangle}{\langle F \rangle^2} \quad \text{equals} \quad 1 + \frac{1}{\langle N \rangle} \, . \tag{6}$$

The temporal fluorescence autocorrelation function $G(\tau)$ for 3D diffusion in confocal FCS is given by

$$G(\tau) = \frac{1}{\langle N \rangle} \left(1 + \frac{\tau}{\tau_{\text{D}}} \right)^{-1} \, ,$$

where τ_{D} is the mean time a particle spends in V_{eff}. Using the figure, which shows the results of an FCS experiment with an aqueous solution of fluorescent protein X and a V_{eff} with $z_0 = 1\,\mu\text{m}$ and $r_0 = 400\,\text{nm}$, estimate the mean concentration of X and the diffusion coefficient of X. [6]



Sketch how the autocorrelation function for X will change (a) upon increasing the excitation power by 10-fold, while molecules of X still perform 3D diffusion as above; (b) upon causing the molecules of X to "blink" on and off with a time constant of $10\,\mu\text{s}$ while they still perform 3D diffusion as above; and (c) upon immobilizing the molecules of X in a matrix. [6]

3. Explain the origin of the electrical potential $\Psi_{\rm m}$ that develops across a cell membrane. By considering the current that flows across the membrane, or otherwise, obtain an expression for the membrane potential that occurs in steady state conditions (i.e. no net electrical current) when only a single ionic species is present (Nernst potential). [7]

For nerve cells the electrical characteristics are determined principally by the flow of Na^+, K^+ and Cl^- ions across the membrane. Show that in steady state the potential is given by:

$$\Psi_{\rm m} = \frac{k_{\rm B}T}{q} \ln \left[\frac{P_{\rm Na} c^{\rm e}_{\rm Na} + P_{\rm K} c^{\rm e}_{\rm K} + P_{\rm Cl} c^{\rm i}_{\rm Cl}}{P_{\rm Na} c^{\rm i}_{\rm Na} + P_{\rm K} c^{\rm i}_{\rm K} + P_{\rm Cl} c^{\rm e}_{\rm Cl}} \right] ,$$

where P_j is the membrane permeability for ion species j, $c^{\rm e}_j$ and $c^{\rm i}_j$ are the external and internal ion concentrations respectively, and q is the magnitude of the charge on the electron. [8]

Estimate the membrane potential for a cell in which $c^{\rm e}_{\rm Na} = 142\,{\rm mM}$, $c^{\rm i}_{\rm Na} = 10\,{\rm mM}$, $c^{\rm e}_{\rm K} = 5\,{\rm mM}$, $c^{\rm i}_{\rm K} = 148\,{\rm mM}$, $c^{\rm e}_{\rm Cl} = 114\,{\rm mM}$, $c^{\rm i}_{\rm Cl} = 6\,{\rm mM}$, given that $P_{\rm K}/P_{\rm Na} \approx 50$. The role of Cl may be neglected: explain why this is justified. [4]

Sketch the temporal behaviour of the membrane potential during the course of an action potential "spike". Indicate on your diagram values of the potential which arise at important times during the spike, and justify them. [6]

4. A protein molecule is illuminated by a beam of collimated, monochromatic X-rays of wavevector $\mathbf{k}$. The amplitude of isotropic, elastic scattering from a volume element of the protein is $n(\mathbf{r})d^3r$, where $n(\mathbf{r})$ is a real function. Show, by means of a diagram or otherwise, that the scattered amplitude $F(\mathbf{\Delta k})$ in direction $\mathbf{k} + \mathbf{\Delta k}$ is given by

$$F(\mathbf{\Delta k}) = \int d^3r\, n(\mathbf{r})\, \exp(i\Delta\mathbf{k}.\mathbf{r}).$$

The intensity of scattering from a crystal of the protein is given by

$$I(\mathbf{\Delta k}) = \sum_{\mathbf{G}} \delta(\mathbf{\Delta k} - \mathbf{G})\, |F(\mathbf{\Delta k})|^2$$

where $\delta(\mathbf{k})$ is a function sharply peaked at $\mathbf{k} = 0$ with unit volume integral in $\mathbf{k}$-space (closely approximated by the three-dimensional Dirac delta function) and $\{\mathbf{G}\}$ is the set of reciprocal lattice vectors, defined such that $\mathbf{G}.\mathbf{R} = 2m\pi$ where $\mathbf{R}$ is any real lattice vector and m is an integer. The intensities of many thousands of reflections are measured. What is the most important additional information that is needed to calculate the structure of the protein? [4]

Scattering from two different crystals is measured: the only difference between them is that a single mercury ion is bound to the same site on each protein molecule in the second crystal. The integrated intensity $|F([hkl])|^2$ of the Bragg peak corresponding to a particular reciprocal lattice vector $\mathbf{G}_{hkl}$ is $2.0a^2$ for the first crystal and $5.0a^2$ for the second and is known to be $1.0a^2$ for a hypothetical crystal with the same lattice containing only the mercury ions (a is a real constant). The primitive unit cell contains one protein molecule, and the origin $\mathbf{r} = \mathbf{0}$ is defined to coincide with the position of a mercury ion. Deduce as much as you can about the amplitude and phase of $F([hkl])$. [8]

The inverse Fourier transform of the diffracted intensity is proportional to the Patterson function $P(\mathbf{r})$:

$$P(\mathbf{r}) = \int d^3r'\, n(\mathbf{r}')\, n(\mathbf{r}' - \mathbf{r})$$

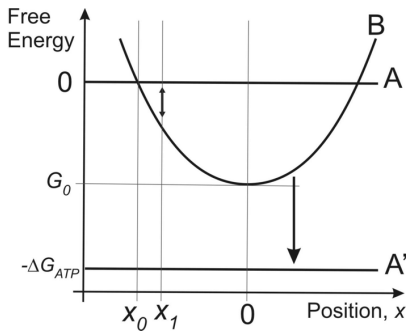
Given that $n(\mathbf{r})$ contains N sharp peaks per unit cell, at the positions of the atoms, describe the condition for there to be a peak in $P(\mathbf{r})$ and hence write down an expression for the number of such peaks per unit cell.

A third crystal is prepared whose structure is very closely similar to the second except that each unit cell contains two additional mercury ions. The positions of the ions, $\mathbf{0}, \mathbf{p}, \mathbf{q}$, are deduced by analysis of the Patterson function. Given that $\mathbf{G}_{hkl}.\mathbf{p} \simeq 2.5\pi$, $\mathbf{G}_{hkl}.\mathbf{q} \simeq 6.5\pi$ and, for the third crystal, $|F([hkl])|^2 \simeq 5.0a^2$, determine the phase of $F([hkl])$ for the first, protein-only, crystal. Why is mercury suitable as a reference ion? [9]

How is it possible to determine the phases of Bragg reflections using a single protein crystal and a tunable X-ray source? What is a suitable X-ray source? [4]

5. A 2-state system has first-order rate constants k_1 for the transition $A \to B$ and k_2 for the reverse transition, and a free energy difference $\Delta G_{AB} = G_A - G_B$. State the principle of detailed balance, and use it to derive a relation between k_1, k_2 and ΔG_{AB}. The system starts in state A at time $t = 0$. Show that the probability that the system will be in state B as a function of time is given by $B(t) = B^*(1 - e^{-at})$, and derive expressions for B^* and a in terms of k_1 and k_2. [7]

In muscle acto-myosin, myosin heads can bind actin filaments and exert force in a "powerstroke" at each of a series of widely-spaced binding sites. The free energies of bound and unbound states around one site in a simplified model of acto-myosin are sketched below as functions of the relative position of the myosin head and actin filament (x). The motor cycle couples each powerstroke to the hydrolysis of a single molecule of ATP: state A' is equivalent to state A except that it contains one fewer molecule of ATP and one more of ADP and P_i. Reversible binding to initiate a powerstroke, coupled to release of P_i, can occur in a narrow range $x_1 \leq x < (x_1 + \Delta x)$, ($\Delta x \ll |x_0|$), with forwards and reverse rate constants $k_1(x)$ and $k_2(x)$ respectively. For $(x_1 + \Delta x) \leq x < 0$ neither binding nor unbinding can occur. At the end of a powerstroke, actin un-binding coupled to ATP binding can occur for $x \geq 0$ with rate constant k_3, and the reverse process is negligible. When bound, the track exerts a force $F = -\kappa x$ on the motor. κ and k_3 are independent of x.



The muscle contracts at a constant velocity v due to the combined effect of a large array of myosin and actin filaments. Show that the average force in the steady-state is proportional to

$$\kappa B_1 \left(\frac{{x_1}^2}{2} - \frac{v^2}{{k_3}^2} \right)$$

and give an expression for B_1 in terms of $k_1(x_1)$, $k_2(x_1)$, Δx, and v. [8]

In the limit $v \to 0$, make labelled sketches of the probability $B_1(x_1)$ that a powerstroke occurs at a given binding site, and of the work $W(x_1)$ done by each powerstroke; both as functions of ${x_1}^2$ for $x_1 \leq 0$. Label the positions corresponding to $x_1 = x_0$ on both plots. For the value of x_1 that maximizes the stall force at fixed G_0, κ and temperature T, show that

$$\frac{k_1(x_1)}{k_2(x_1)} = \frac{W(x_1)}{k_B T} - 1$$

where k_B is Boltzmann's constant. [10]

6.

(a) Estimate the conductance through a pore of 0.15 nm diameter and 0.5 nm in length, where the ionic solution on both sides of the membrane has a resistivity of $\rho = 80\,\Omega\,\mathrm{cm}$ and unrestricted access to the mouth of the pore. [4]

(b) 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 $-50\,\mathrm{mV}$ is 9 nA. It is estimated that there are 3×10^3 of these channels in a cell and that their open probability (P_o) is 0.6. Calculate the individual conductance of these K^+ channels. [3]

(c) Assume a nerve cell to be a perfect cylinder of length $100\,\mu\mathrm{m}$ and diameter $2\,\mu\mathrm{m}$. The capacitance of the membrane is $0.01\,\mathrm{F\,m^{-2}}$. The intracellular concentration of Na^+ is 15 mM. During the upstroke of an action potential the cell depolarises by 85 mV. Calculate the change in intracellular Na^+ concentration required to achieve this depolarisation and explain the importance of the result you have calculated for the generation of nerve action potentials. [7]

(d) Voltage-sensitive ion channels are gated (i.e. switched on and off) by changes in transmembrane potential. Which parts of the protein structure are important for this process and what experimental evidence has been used to support our current understanding of this mechanism? [11]

7. Enzyme Q methylates substrate S with Michaelis-Menten enzyme kinetics. Q and S bind with a rate constant k_+^S, and the complex QS dissociates with the rate constant k_-^S. Methylated S is formed from the complex with a rate constant V_Q. The rate of production of methylated S is given by:

$$\frac{V_Q Q_T[S]}{K_Q + [S]},$$

where Q_T is the total amount of enzyme Q, and $[S]$ is the concentration of S. Derive the Michaelis-Menten coefficient K_Q. [4]

In bacterial chemotaxis, the number of active receptors determines the frequency of bacterial tumbling behaviour. Input ligands can bind to receptors to inactivate them. Receptors can also be methylated by CheR and demethylated by CheB. CheB works with Michaelis-Menten enzyme kinetics with a rate constant V_B and a Michaelis-Menten coefficient K_B, while CheR works with saturation kinetics with a rate constant V_R. In the Barkai-Leibler model for robust adaptation, only methylated receptors not bound to an attractant are active, and CheB only demethylates active receptors. Derive the concentration of active receptors. Explain why this model exhibits adaptation that is robust to ligand levels. [7]

Enzyme A combines with substrate S to produce product P_S. A and S bind with a rate constant k_+^S, and the complex AS dissociates with the rate constant k_-^S. The complex AS can also be modified in an irreversible reaction with rate constant m to form the modified complex AS^*. AS^*, in turn, can either dissociate to $A + S$ with an rate l_-^{S*} or it can form P_S with a rate constant V_A, leaving the enzyme A free to work again:

$$\begin{array}{ccccc} A + S & \underset{k_-^S}{\overset{k_+^S}{\rightleftharpoons}} & AS & \xrightarrow{m} AS^* & \xrightarrow{V_A} A + P_S \\ & & & \Big\downarrow l_-^{S*} & \\ & & & A + S. & \end{array}$$

A can also act on a different substrate R to produce product P_R. The reaction pathway has the same structure as that of A and S, with the same reaction rate constants m and V_A, but with modified binding and dissociation rate constants k_+^R, k_-^R and l_-^{R*}. What is the ratio F of the production rates of P_R to P_S? [6]

Assume that the concentrations of S and R are equal, that $k_+^S = k_+^R$, and that $k_-^S < k_-^R$ and that $l_-^{S*} < l_-^{R*}$. If the production of P_S is desirable, but P_R is not, what conditions on the rates allow for effective kinetic proofreading so that the ratio F is minimized? Explain your reasoning. [4]

Describe how an alternative kinetic proofreading scheme would work that doesn't include the irreversible reaction with rate m above, but instead introduces a time-lag τ between the formation of complex AS or complex AR, and the production of products P_S or P_R respectively. Assume that τ is the same for both complexes. [4]

8. Write short notes on **two** of the following:

(a) The interactions and flow of information involved in the production of a folded protein.

(b) The main forms of biological free energy and the ways in which they are utilised and inter-converted.

(c) How ion-selective membrane pores achieve such high flow-rates.

(d) Single-molecule experiments to investigate the mechanism of F1-ATPase. [25]