MIDTERM EXAM

ANSWERS

EXPERIMENTAL BIOPHYSICS

FRIDAY, MARCH 9TH, 2012

1400-1800

ROOM H221

DEPARTMENT OF PHYSICS, LUND

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THE QUESTIONS ARE ORGANIZED ALONG THE CONTENTS OF THE LECTURES. IF YOU CANNOT ANSWER ONE QUESTION, DO NOT DWELL TOO LONG. TRY THE NEXT QUESTION INSTEAD!

FEEL FREE TO DRAW FIGURES IN YOUR ANSWERS. OFTEN IT IS EASIER AND MORE EFFICIENT TO CONVEY A MESSAGE USING A FIGURE THAN IN TEXT.

TYPICALLY, FOR A PASSING GRADE [G], YOU SHOULD HAVE AT LEAST 50% CORRECT, AND A PASSING WITH DISTINCTION GRADE [VG] REQUIRES AT LEAST 75%. THE FINAL GRADING SYSTEM USED WILL DEPEND ON IN WHAT CONTEXT YOU FOLLOW THE COURSE.

MAKE SURE TO WRITE YOUR NAME ON EACH SHEET OF PAPER. USE A NEW SHEET OF PAPER FOR EACH NEW FIELD, *I.E.* ONE NEW SHEET OF PAPER FOR *BIOLOGY AND SCALES*, ONE NEW SHEET FOR *OPTICS ON THE SMALL SCALE*, ETC ETC.

NOTE THAT THE NUMBER OF POINTS ASSIGNED TO EACH QUESTION GIVES YOU AN INDICATION ON HOW MUCH TO WRITE.

HAND IN YOUR ANSWERS TOGETHER WITH THE QUESTIONS.

A FEW USEFUL NUMBERS:

$1\ k_BT \approx 4$ pN nm $= 10^{-21}$ J AT ROOM TEMPERATURE

$N_A = 6\ 10^{23}$ MOLES^{-1}

$\eta_{water} = 1\ 10^{-3}$ kgm^{-1}s^{-1} AT ROOM TEMPERATURE

$\gamma_{LG} = 0.0728$ J m^{-1} TYPICAL SURFACE ENERGY OF THE WATER-AIR INTERFACE

$\theta_C = 20°$ TYPICAL CONTACT ANGLE OF A WATER DROPLET ON GLASS

GOOD LUCK!

JONAS T

1. Biology and Scales [17p]

Use a new sheet of paper for these questions.

[a] DNA is one of the longest molecules in biology. Estimate the total length of all DNA in the human body. [4p]

Calculate number of cells based on:

Size of eukaryotic cell ~ 100µm

Volume of a human ~ 0.25 m^2

Total length of DNA in each cell is about 2m

This gives us the length.

[b] How long time does it take on average for a typical protein and a typical virus to diffuse from one end to the other in a typical bacterium? [4p]

E coli – 1µm

Virus ~ 100nm

Assume spherical particles and use Einstein-Stokes relation to extract diff coeff.

$$D = \frac{k_B T}{f} = \frac{k_B T}{6\pi\eta a}$$

Use $\langle x^2 \rangle = 2Dt$ to calculate the expected distance.

[c] A setup for atomic force microscopy (AFM) is a very versatile tool. What different types of experiments can it be used for that have relevance for biology? Give a short explanation for each example. [6p]

Imaging - topography

Force measurements – attach to molecule and pull

Injection – inject molecules into cells

[d] In science critical thinking is crucial. One important question is whether the source of information is reliable. If you are reading an article in a reputable scientific journal, how would you assess the reliability of the contents of the article. Discuss in a few sentences. [3p]

Check for references. Do they make sense? Do they cover the field? Are any key references lacking?

Who has cited the paper? In which sense: positively or negatively?

Do the authors have a self-interest (scientific, economic, political, religious, career related etc?)?

Has key aspects of the work been reproduced?

2. Optics on the small scale [20p]

Use a new sheet of paper for these questions.

[a] What types of information can be obtained using single-molecule detection techniques that bulk-based methods cannot access? Give three examples. [3p]

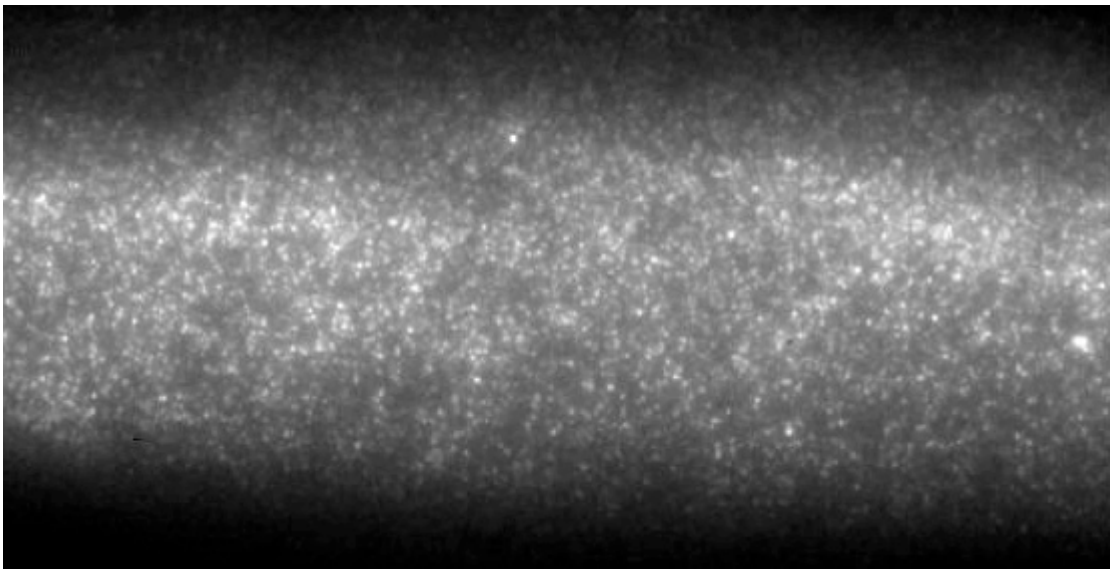
Heterogeneity

Local probe

Time trajectories

[b] The picture below is one frame from a movie of GFP molecules embedded in agarose gel. How do you tell the difference between aggregates of fluorescent molecules and single fluorescent molecules from these types of data? Note that in the real case you would have access to the entire movie.

In other words, how do you make sure that these spots indeed represent the fluorescent signal from individual GFP molecules? [3p]



Blinking, ON/OFF behavior – the intensity is either on or off, never in between

Reasonable photon count

Antibunching – the photons come one by one, not two at the same time.

[c] Give two examples of how the diffusion coefficient can be determined using single-molecule imaging. Explain how they work. [4p]

Localization enhancement – *A diffusive molecule will be too blurred to be detectable for long exposure times, whereas a molecule bound to one position will be clearly visible especially for long exposure times. The blurred spot size x is related to the exposure time t as* $\langle x^2 \rangle = 4D_{eff}t$*. Use stroboscopic illumination with equal number of excitation photons in each flash, but with flashes that differ in length.*

Measurement of trajectory – *follow the trajectory of a molecule and calculate how far it has moved from the origin. Calculate the diff coeff from* $\langle r^2 \rangle = 4D_{eff}t$*. Need stroboscopic illumination and sufficient S/N to localize the molecule at each point in time.*

[d] Discuss the experimental difficulties involved in realizing single-molecule detection [2p]. What are the solutions? [2p] What types of microscopy setups may be used? [2p]

[in total 6p]

Issues: Background fluorescence; shot noise; limited photon budget due to bleaching; one dye per diffraction limited spot size.

Solutions: Small excitation volume; excitation towards red; efficient dyes; antibleaching agents; diluted dye solution; fused silica slides

Techniques: TIRF, confocal, SNOM

[e] The resolution limit of standard microscopy is set by the Abbé limit to $r \sim \frac{0.66\lambda}{NA}$

How can this limit be circumvented?

Describe one commonly used technique for super-resolution microscopy and its key features.

What resolution is it possible to achieve typically in biological samples using the technique? [4p]

STORM…

Principle: FIONA $r \sim \frac{0.66\lambda}{NA}\frac{1}{\sqrt{N}}$

Performance: High-resolution, sub-diffraction limit imaging in the far-field. [4p]

In practice ~25nm resolution, but requires skill and expensive equipment.[1p]

STED…

SIM…

3. Basic Fluidics [17p]

Use a new sheet of paper for these questions.

[a] In our everyday lives, fluid is made to flow in pipes typically by an applied pressure difference. Why is this not suitable for microfluidics when the channels become very small? [2p] Explain three examples of alternative ways to move the sample in microfluidics! Explain for each one of them why they are more suitable. [3p]

Total number of points [5p].

In pressure driven flow velocity scales with r^2.

Electrophoresis

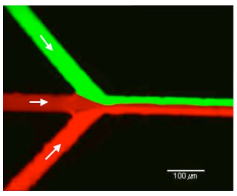
Electroosmosis

Dielectrophoresis

Capillary flow

[b] Mixing is an important tool in microfluidics. Explain how it can be used to study the time dependence of protein-folding. Assume that we want to study a folding process on the scale of 1ms. How would we set up the experiment? Give information on the device design and flow rates. [6p]

Use a diffusive mixer as below.



Einstein Stokes relationship for the diffusion coefficient.

$D=k_BT/(6\pi\eta R)$

A typical ion: R~1nm

$<x^2> = 4Dt$

relative width of the central stream in the exit channel ~ relative flow rate in the central input channel.

Time is mapped to space and time resolution is directly connected to spatial resolution, which in turn depends on the optical resolution of the microscope/camera system, i.e. the NA of the objective, the pixel size, the number of photons collected, noise levels etc.

[c] In microfluidics it is sometimes important to transport a well-defined amount of sample through a channel system without diluting the sample plug. How can this be realized? Give two examples and explain how they work. [6p]

Taylor diffusion **–** Ensure that the lateral diffusion is sufficient to counteract the spreading due to the parabolic flow profile.

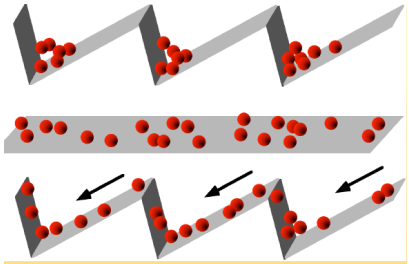
Electroosmosis **–** No parabolic flow profile. Straight profile.

Droplet – Complete enclosure.

4. Molecular Motors [19p]

Use a new sheet of paper for these questions.

[a] Describe a flashing ratchet. Does it provide a means to convert thermal energy to motion without any thermal gradient and without any additional energy input? What is the role of thermal motion? [5p]

1. 
2. *Asymmetric potential that is periodically (or stochastically) turned on and off. Diffusion takes place during the off-phase. Turning on the potential rectifies part of the diffusive motion. [3p]*
3. *There are no macroscopic forces, but turning on of the potential does add energy to the system: it gives particles the energy to glide "down" the hills. [1p]*
4. *Without thermal motion, the diffusion during the off-phase would not take place, and the mechanism wouldn't work. [1p]*

[b] How can an optical trap be used to study a single molecular motor's performance? Give one example, and specify what is to be measured and how the optical trap helps with that. [4p]

1. *An optical trap can be used to measure the force exerted by a motor, as well as its stepping behavior. For this purpose, the motor is attached to a small bead. The trap can then be used to brig the motor close to its track (for example an actin filament immobilized on a surface). When the motor moves, this can be observed as a displacement of the bead.*
   *Possible examples of things that can be measured: (1) speed. (2) With sufficiently high spatial and temporal resolution, individual steps can be observed. (3) As the bead moves away from the center of the trap, the restoring force increases and bead motion will eventually stop. In this way, the motor's stall force can be measured. (4) If, in addition, a feedback loop is used to keep the bead displacement in the trap constant, a "force clamp" is created, and motor speed can be measured as a function of load force. (**Only one or two examples are expected).***

[c] Many molecular motors move along a filament by cyclically binding and unbinding. A concern is that the motor can simply diffuse away from the track when it is not bound, for example during stepping. Describe, in some detail, one strategy that a motor can employ to make sure it does not fall off the track during stepping. [3p]

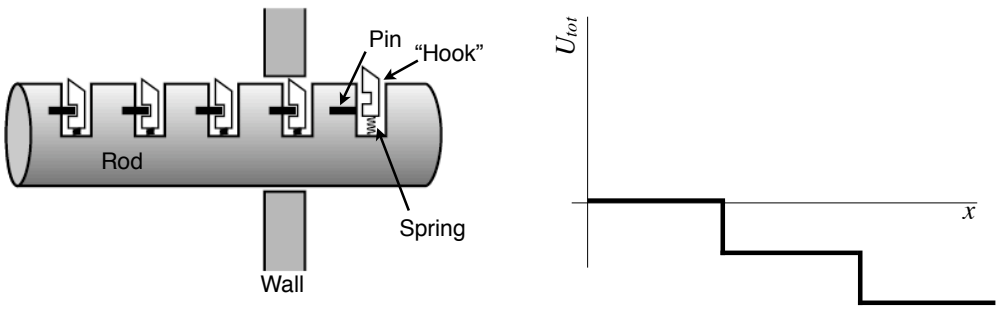
In bi-pedal motors, "gating" is used to make sure that one head remains bound when the other is unbound. For example, this is achieved by letting the binding state of one head influence the unbinding rate of the other head. An example is intramolecular strain. Binding of one head induces strain that is communicated to the other head, changes its conformation and thereby its binding strength.

Other strategies can be:

- strong non-specific binding that makes sure that even a detached head doesn't fully detach

- a ring-like structure that allows the motor to glide along the filament.

[d] The sketch to the left below shows a conceptual example of a ratchet-like device that can do work. It consists of a rod with little "hooks" that can be pushed up by loaded springs, but that are held in place by little pins. Each time a hook passes through the wall opening, its pin is released and the hook is pushed up by the spring. [7p]

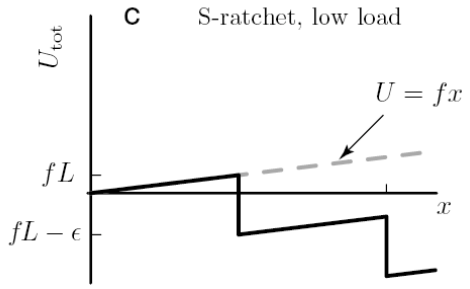


- In the presence of thermal motion, the rod will move in one direction on average. Explain how this works, specifying the direction in which the rod will move, and why thermal motion plays any role in this process.

  Thermal fluctuations to the left are prevented by the hooks that get caught at the wall opening. Fluctuations to the right can take place, but will lead to the release of more hooks. Thus, an average motion to the right will occur. Without thermal motion, nothing happens. The rod just sits there.

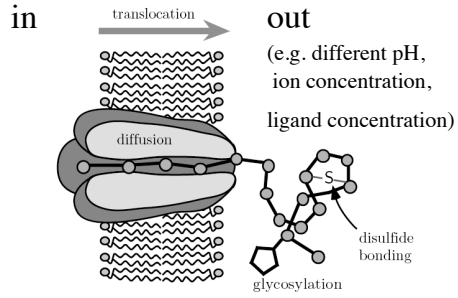
- The diagram to the right shows an energy diagram of the total potential energy of the rod system as a function of the position of the rod. Explain why the energy diagram looks like this.
  Each time one of the springs is released, the system loses stored potential energy, and the potential energy overall goes one step down, with the step height corresponding to the energy stored by one spring. Between pin-releases, the potential energy is independent of position. An object diffusing in this effective potential will clearly move to the right on average.

- A small force is now applied to the rod towards the left. How will this change the free-energy diagram? Sketch qualitatively and explain briefly.



- Give an example for how this ratchet system may be realized in a biological system.

  Possible answer:



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5. NanoSafety [16p]

Use a new sheet of paper for these questions. Draw figures!

[a] Please describe what might happen to you if you would inhale the following particles.

1- 40μm long, 50 nm diameter cylindrical gelatin-made particles, [2p]
2- 40μm long, 50 nm diameter cylindrical quartz particles [2p]

3- 400 μm long 200 μm diameter cylindrical quartz particles [2p]

In addition, compare 1 with 2 and 2 with 3 [2p].

Hint: Gelatin is made of collagen. It is in solid form at room temperature and in liquid form at temperatures above 35°C. [in total 8p]

- If you would inhale 40 µm long, 50 nm diameter cylindrical gelatin-made particles, they would either melt right away (since gelatin melts at body temperature) and leave a little bit of collagen in your lungs, or they would be taken up by macrophages and be degraded by the cells.

- If you would inhale 40µm long, 50 nm diameter cylindrical quartz particles, they would go deep into the lung and possibly to the pleural tissue (sac around the lung). There, they would be phagocytized by immune cells (such as macrophages). Since the length of the particle is bigger than the size of the cells, that would result in frustrated phagocytosis, chronic inflammation leading to lung fibrosis, granuloma formation and possibly cancer development after many years.

The difference between 1 and 2 is the biopersistence: gelatin is degradable whereas quartz is not. Quartz is biopersistent.

- If you would inhale 400 µm long 200 µm diameter cylindrical quartz particles, they would be stopped in the upper part of your respiratory system and expelled with the help of the mucus.

The difference between 2 and 3 is the particule size and therefore the location where the particles will end up. The smaller particules (number 2) would be able to reach the alveoli where there is no clearing mechanism, whereas the bigger particles (number 3) would be stopped in the upper respiratory system and cleared by the mucus.

[b]

(1) Define the general risks associated with nanoparticles. [2p]

(2) Specifically describe and evaluate the risk associated to the following nanoparticle applications:

1- CdSe quantum dots in biomedical samples for fluorescence microscopy applications [2p]

2- Silver nanoparticles on sale on internet (see picture below) [2p]

3- Water nanodroplets spread on fruits and vegetables in grocery stores. [2p]

[in total 8p]

(1) Risk= function of toxicity and exposure.
(2) 1- CdSe nanoparticles are very toxic but their use in biomedical samples is limited to technicians/researchers only (who have been informed about the risks by reading the Material Safety Data sheet). High toxicity- Little exposure= Average risk
2- Silver nanoparticles are toxic and the fact that they are available for sale on the internet makes the exposure potentially significant. High risk.
3- Water nanodropplets are not toxic. The exposure is very big (everyone visiting a grocery stores). Since the toxicity is 0, the risk is 0.

6. Fluidics Applications [20p]

Use a new sheet of paper for these questions. Draw figures!

[a] Describe the basic idea of deterministic lateral displacement. (The device is known as the bumper array.) How does it work? [3p] How can it be used to fractionate based on deformability? [2p] How can it be used to fractionate based on shape? [2p]

[in total 7p]

Tilted array of posts. Large particles move with the device. Small particles move with the flow. Critical size. Sharp transition. [3p]

High speed – more deformation; scan the speed and see how the particles are deformed. [2p]

Align the particles [2p]

[b] Normally microfluidics works at reasonably low speeds where the Reynolds number is less than one and thus viscous forces dominate over inertial forces. However, inertial forces on the liquid may sometimes be used to sort particles. What is the general layout and underlying mechanism of the device? [3p] What is the main advantage and drawback as compared to the bumper array? [2p] Note that we are not speaking about centrifugation and that the flows are not turbulent.

[in total 5p]

1<Re<2000; laminar flow inertial flow; wall effect; shear effect; Dean flow; asymmetric flow

High speed and throughput; low resolution; only one fraction is focused.

[c] Single-cell biology is a growing field that gives important information on the differences in properties between individual cells. Describe in detail two microfluidic-based (Lab on a Chip) techniques to study large numbers of cells one by one. Note that we are not referring to optical-tweezers-based approaches, nor fluorescence activated cell sorting (FACS). [4p]

***Droplets** – single cells are trapped in one droplet of water each. The droplets are formed e.g. in a T-junction where water is introduced into one channel and oil in the two other channels. The droplets are formed due to a balance between viscous shear tending to rip apart the droplets and surface forces tending to hold them together.*

***Small cups** – Small U-shaped cups are made in a channel. The structures leave a small spacing between to the ceiling of the fluidics channel. In this way cells can be*

pulled into the cup, but once the cell is in place the flow is deviated around the cup to ensure that only one cell is trapped in each cup.

[d] In molecular biology it is important to fractionate large DNA molecules. However using standard gel electrophoresis requires long running times on the order of 24 hours. How can this time be shortened several orders of magnitude using microfluidics? Describe the design of the device and the main principle. [4p]

HEX array – *The DNA is moved through an array of posts with an alternating field in two different directions separated by an angle of 120°. The short molecules move faster than the longer.*

Drawing of principle…