MIDTERM EXAM

ANSWERS

EXPERIMENTAL BIOPHYSICS

FRIDAY, MAY 6TH, 2011

1400-1800

ROOM H221

DEPARTMENT OF PHYSICS, LUND

MIDTERM EXAM

EXPERIMENTAL BIOPHYSICS

FRIDAY, MAY 6TH, 2011

1400-1800

ROOM H221

DEPARTMENT OF PHYSICS, LUND

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 [16p]

Use a new sheet of paper for these questions.

[a] DNA is one of the longest molecules in biology. What are the relevant length scales to consider when studying the genomic and polymer-physical aspects of the molecule? [4p]

Total length of human DNA in one cell – 2 meters; $3x10^9$ bases long organized in 23 chromosomes

One gene – 1000bp ~ 350nm

Size of condensed chromosome ~ 1µm

Size of nucleus in human cell ~10µm

Size of basic genetic unit: base pair ~0.34nm

[b] How large are typical eukaryotic cells (cells with nuclei), bacteria, viruses? [3p]

Human cells ~10-100µm; nerve cells up to ~ a meter long

Bacteria ~ 1-10µm

Viruses ~ 50-500nm

[c] Explain three examples of tools for force measurements in biology. How do they work? [6p]

AFM - Cantilever equipped with a sharp needle. Angle of cantilever monitored by a laser.

Optical tweezers – mainly force

Magnetic tweezers – force and torque

[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. Basic Fluidics [20p]

Use a new sheet of paper for these questions.

[a] The behavior of fluids and objects in the fluid in our everyday life, for example when swimming or riding a boat differs dramatically from the case in microfluidics. Discuss three examples! [6p]

• *The Reynolds number for microchannels is typically < 1 which indicates laminar flow (no turbulence).*

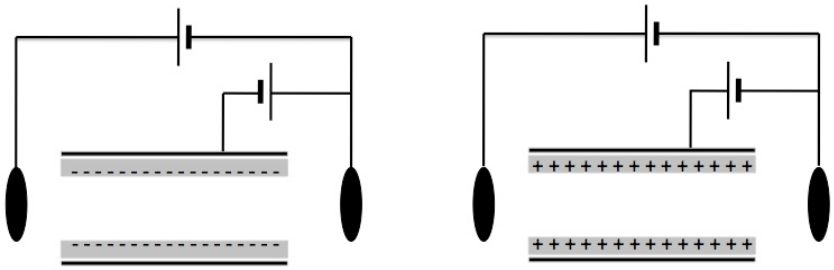
• *Diffusion: the average time it takes a particle to diffuse a given distance is proportional to the square of that distance:* $x^2=Dt$. *The small distances involved in micro- and nanochannels can thus be covered in very small time periods, whereas on the macro scale the effects of diffusion can often be neglected.*

• *The surface area to volume (SAV) ratio grows rapidly when we shrink dimensions. This affects, for example friction between liquid and channel walls, increasing the fluidic resistance drastically for smaller channels. Heat dissipation also depends on the SAV ratio.*

• *Surface tension forces become important for small channels. On the other hand the flow velocity scales with the radius of the channels so that the velocity decreases with radius of the channel.*

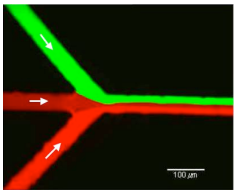
The main change in going down in size is that the surface-to-volume ratio increases. This makes effects that are associated with surfaces more important: Pressure driven fluid flow is slower due to viscous drag (friction) of the walls. Electroendosmotic flow is possible.

[b] With gate electrodes on the exterior of a microchannel it is known that the charge density of the inside of the channel under certain circumstances can be tuned from positive to neutral to negative. In the figures below, in what direction is the liquid moving the left and right case respectively? What is the underlying mechanism? [3p]



The charge of the inner walls of the nanochannel influences the electroosmotic flow. With a positively charged inner wall, the negative counter ions will carry with the fluid and with a negatively charged inner wall the positive counter ions will carry the fluid in the opposite direction.

[c] To understand diseases such as Alzheimers and Mad Cow Disease it is important to understand how proteins fold. These studies need to take place with time resolution of down to 1μs or at least 1ms. How can this be accomplished using microfluidics and a detector that has a time resolution of just 1 second? Explain how the device is designed and how the flow rates should be chosen. [6p]



Use a diffusive mixer as above.

Einstein Stokes relationship for the diffusion coefficient.

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

A typical ion: R~1nm

$\langle x^2 \rangle = 4Dt$

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.

[d] Estimate the velocity of the liquid in a microchannel that is filled by capillary action. Is the velocity constant with time? Assume a glass channel filled with water at normal room temperature. [5p]

Calculate the drag force of the liquid as a function of velocity

QR=P

R=L eta /a^4

The energy loss due to wetting of an additional area delta A is given by $\gamma_{LG} \cos$

θ_C.

3. Fluidics Applications [20p]

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

[a] Particle fractionation is a central tool in biology. Give examples of relevant performance parameters! [3p]

Throughput

Simplicity; ease of use

Precision; resolution

Purity

Specificity

[b] Describe the basic idea of the bumper array (also known as a device based on deterministic lateral displacement). How does it work? [3p] How may the flow velocity influence the separation? [2p]

[in total 5p]

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

High speed – Less dispersion due to diffusion and more deformation of the (soft) particles. [2p]

[c] 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 other microfluidic sorting devices? [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.

[d] Circulating tumor cells are important markers that can be used to stage cancers, to detect metastasizing cancers and to monitor treatment. The Toner group at Harvard came up with a simple device based on microfluidics to extract these cells with high efficiency. How is the device build up? [1p] What key features of microfluidics does it benefit from? [2p]

[in total 3p]

Post array with surfaces decorated with antibodies for the CTCs. [1p]

Key features: High surface to volume ratio. Precisely defined flow fields optimized for maximum contact of the cells with the posts. [2p]

[e] 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.*

4. Optics on the small scale [19p]

Use a new sheet of paper for these questions.

[a] What are the benefits of single-molecule studies as compared to bulk studies? [3p]

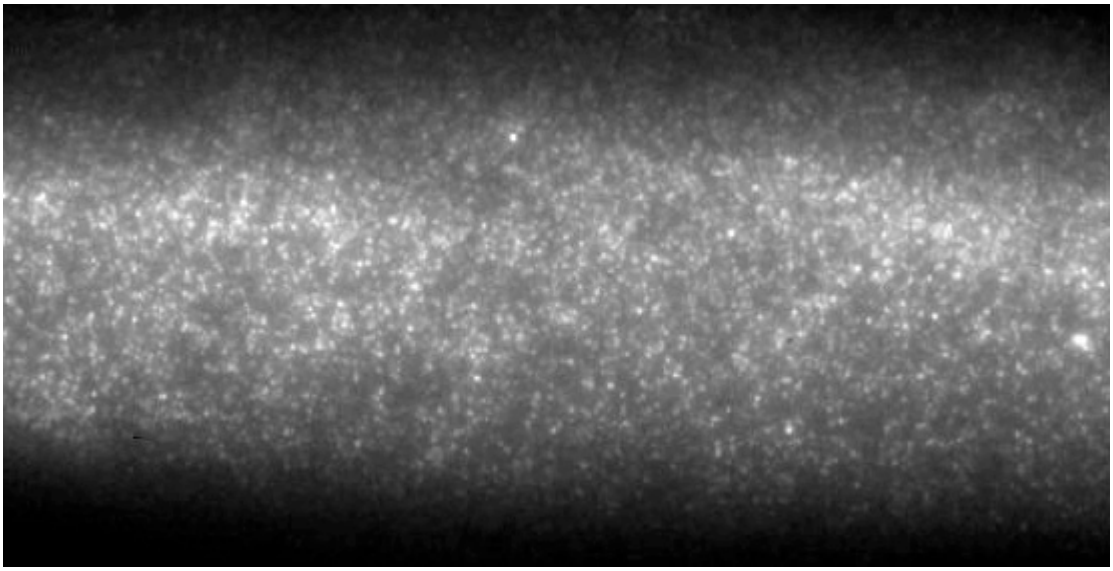
Heterogeneity

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] Localization enhancement can be used to determine the diffusion coefficient of single molecules. How is it implemented? [3p]

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.*

[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 using photo-switchable dye molecules?

Describe the basic idea and key features.

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

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

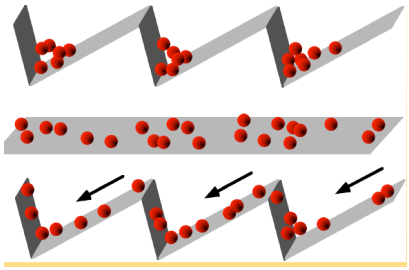
Performance: High-resolution, sub-diffraction limit imaging in the far-field. [4p]

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

5. Molecular Motors [17p]

Use a new sheet of paper for these questions.

[a] Describe a flashing ratchet. Draw a figure! 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 the thermal motion? [4p]

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.*
3. *There are no macroscopic forces, but turning on of the potential requires energy and adds energy to the system: it gives particles the energy to glide "down" the hills.*
4. *Without thermal motion, the diffusion during the off-phase would not take place, and the mechanism wouldn't work.*

[b] How can an optical trap be used to study a single molecular motor's performance? Give one example, and specify what is measured and how the optical trap helps with that. [3p]

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 bring 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, and its speed can be measured. With sufficiently high spatial and temporal resolution, individual steps can be observed.

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.

[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.

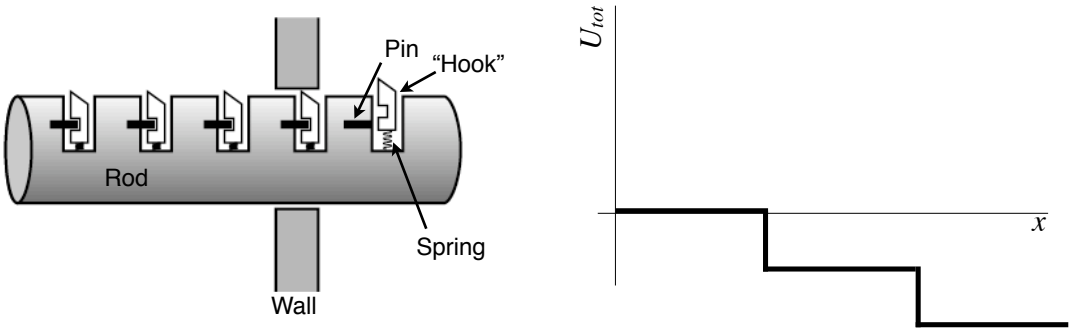
Other strategies can be:

- 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.

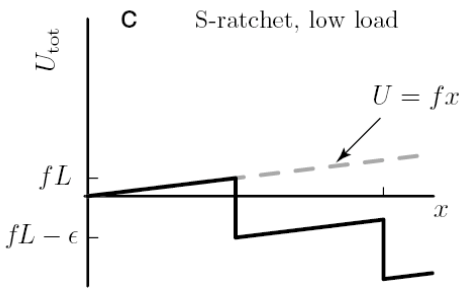
[in total 7p]



- 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 the role of thermal motion. [2p]

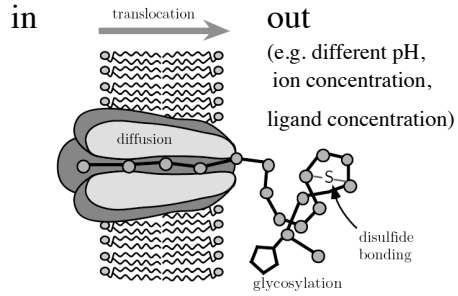
  Thermal fluctuations to the left are prevented by the hooks that get caught at the wall opening. Fluctuations to the right can take place, but will lead to the release of more hooks. Thus, an average motion to the right will occur. Without thermal motion, nothing happens. The rod just sits there.

- 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. [2p]
  Each time one of the springs is released, the system looses 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. [2p]



- Give an example for how this ratchet system may be realized in a biological system. [1p]

  Possible answer:

- 

6. NanoSafety [19p]

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

[a] Which are the principal cells involved in a foreign-body reaction in the brain? [4p]

- The microglial cells (ED1-positive cells), which are the resident macrophages of the brain and phagocytize any infectious agents and intruders. They are part of the immune response of the brain. Upon brain injury, there are also recruited macrophages (from outside the brain). [2p]

- The reactive astrocytes (GFAP-positive cells): these cells are involved in tissue repair. [2p]

[b] Why are cadmium nanoparticle toxic? [4p]

- *The small size increases the surface area to volume ratio and therefore increases the dissolution rate dramatically compared to bulk material. [2p]*
- *[2p] for two correct answers from the list below:*
- *Upon photo-oxidation of the NP, Cd^{2+} ions are produced, which bind to mitochondrial proteins, diminish cellular respiration and cause cell death.*
- *In general, Cd is a carcinogen (interference with DNA repair processes) and a neurotoxin (influencing lipid peroxidation).*
- *It has persistent effects in biological systems.*
- *Cd binds to metallothionein protein, disrupting zinc metabolic pathways in the liver and kidney.*

[c] What can be done in order to decrease the toxicity associated with a nanoparticle? [2p]

One can coat the nanoparticle with a non-toxic material such as BSA, ZnS, PEG-silane or phosphonate silane.

[d] Give five examples of nanoparticles properties of that should be known before investigating their toxicity. [5p]

Shape, Size, charge, zeta potential, hydrophobicity (including aggregation state in the medium that you are going to exposure the bio entity to), protein corona, particle core material, surface material, surface reactivity, radioactivity, crystalline structure (when applicable).

[e] Currently in Europe, the level of testing required is determined by the mass produced, with the lowest mass trigger currently set at 10kg per annum.

What is the volume of 10kg of GaP? How many nanowires (5 μm long, 50 nm in diameter) does 10 kg of GaP correspond to? How large would the surface area of the single GaP piece be (assuming a sphere)? How many times larger would the surface area of the nanowires be (same total mass as that of the sphere)? (GaP density=4.138 g/cm^3) [4p]

- *volume of 10 kg of GaP is V=2.417 dm3*
- *number of nanowires N= V/Vnw= 2.417 10-3/9.817 10-21= 2.46 10 17*
- *single sphere of GaP: R=8.3 10-2 m, Area= 8.66 10-2 m2*
- *surface area of 10 kg of nanowires: Surface (1nw) x N=1.94 105 m2*
- *Same mass, 2.2 million times more surface area for the nanowires!*