MIDTERM EXAM

ANSWERS

EXPERIMENTAL BIOPHYSICS

SATURDAY, AUGUST 27TH 2011

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 [19p]

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

[a] Biomolecules, viruses and cells come in a wide range of sizes. What are these sizes specifically and what microfabrication methods are suitable to use if one would like to create appropriate tools on the same length scales? [6p]

Proteins *~10nm; EBL, FIB, colloidal lithography, scanning probe lithography, NIL, injection molding*

DNA *– all sorts of length scales accessible using the above techniques, but also UV-lithography*

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

Viruses – ~100nm; nanofab tools as above

Bacteria *– 1-10µm; UV-lithography; soft lithography; injection molding*

Human cells *– 10-100µm; microfab as above*

[b] Viruses and bacteria typically float around in liquids. What is the expected mean square displacement of a typical virus and bacterium during 10 seconds in a water solution at room temperature? From a fundamental point of view, how do they differ? [6p]

$\langle x2\rangle=6Dt$ with $D=kT/f$ where $f=6\pi\eta R$ for spherical particles. Assume proteins, viruses and bacteria are not motile.

11µm; 36µm

In fact bacteria swim, which affects their apparent diffusivity.

[c] Force measurements on single molecules is a central type of experiment in modern biological research. Give three examples of commonly used tools for such measurements! [3p]

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

Optical tweezers – mainly force

Magnetic tweezers – force and torque

[d] The world wide web is an important source of up to date information. However, it is not always safe to take at face value what you read on the web. Give a few rules of conduct to be on the safe side! [4p]

Read multiple sources. Do they agree?

Find established web sites. A university website is typically good. A company website might give good specific information, but it might hide for example some key limitations of a technique that the company is manufacturing.

Compare information from websites that represents competing interests.

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. Draw figures!

[a] In microfluidics, the liquids can be moved in a wide variety of ways. Give three examples! [3p]

Pressure driven flow ($v \sim a^2$), capillary forces ($v \sim a$), electrophoresis (independent on size), electroendosmosis (works well for $a \sim < 100\mu m$), dielectrophoresis (typically $a \sim 1\mu m$-$10\mu m$). Centrifugal forces (www.gyros.com). Acoustic forces (Thomas Laurell group in Lund)

[b] Assume a plug of sample in a channel. With an applied pressure across the channel, the liquid flows in the channel and the plug is spread out. Why is it spread out? What mechanisms can be used to limit the effective spreading of the sample plug? [4p]

Spreading due to the parabolic flow profile $x \sim t$; figure!

Lateral diffusion limits the spreading to $x \sim \mathrm{sqrt}(t)$. This is known as Taylor diffusion!

[c] Mixing is a key tool for chemistry. In which ways can it be implemented in microfluidics? Describe three examples! [6p]

mixers: Mixing is a central topic in microfluidics. Due to the absence of turbulence (in most cases), the only way to mix two components is through diffusion. The key is then to decrease the diffusion length in an efficient manner so that the mixing takes place as quickly as possible.

Squires and Quake [3] describe three types of mixers:

T-sensor and H-filter – In the T-sensor, liquid from two channel are merged into a third channel mixing the liquids. In the H-filter, two U-shaped loops are merged together. Fast diffusing molecules (or particles) diffuse to the neighboring stream whereas slowly diffusing molecules (particles) stay in the original stream. Calculating the effective diffusion can be non-trivial. For example, near the boundaries diffusion scales as $(Dx)^2 \sim z^{2/3}$ instead of $(Dx)^2 \sim z$.

Rotary mixer – A liquid is pumped around a closed loop using for example an integrated peristaltic pump. Three domains can be identified:

a. diffusion-dominated mixing – at slow speeds ($Pe=uh/D<<1$) diffusion dominates. Mixing time is determined by the diffusion time around the loop.

b. Taylor-diffusion mediated mixing – for sufficient speeds so that $Pe>>1$, but molecules must diffuse across the channel before the sample plug folds onto itself, $Pe<<2pR/h$.

c. convectively dominated mixing – the sample plug folds onto itself before diffusion across the channel occurs, Pe>>2pR/h.

Chaotic advection – Using a device with a stripes along the walls, the fluid will rotate in two parallel vortices down the channel. If the symmetry of the stripes is varied, then the vortices span different fractions of the liquid. The effect is that the fluid is folded such that for each cycle (long stripes to the left – long stripes to the right) the width of one component is cut in half. The diffusion length along the channel thus decreases exponentially along the channel.

It can also be implemented in a structure consisting of a three-dimensional serpentine.

Other examples listed in Beebe's paper[1]:

Coanda effect – The Coanda effect is the propensity of a flow stream to stay attached to a convex surface rather than moving along the tangent of the surface. It is used in a device where the fluid stream is divided into two channels, one making a sharp turn.

Ultrasonic mixer – using a piezoelectric material (e.g. PZT) a sample solution can be excited by ultrasound. The high frequency results in speeds sufficient to create a local turbulence.

Electrokinetic and magnetic mixers – The idea is to create circulating flows folding the liquid, in turn decreasing the diffusion length. Using electrokinetics, the sample or the fluid is moved. Using magnetic fields, suspended magnetic particles move due to a rotating magnetic field, thereby stirring the fluid.

[d] Estimate the velocity of the liquid in the microchannel described in the figure below that is filled by capillary action. Assume that the liquid enters the channels from the left, i.e. from the wider channel. Is the velocity constant with time? Does the liquid slow down as it reaches the narrow channel? Assume a glass channel filled with water at normal room temperature. The total length of the channel is 200μm, the width of the wide part is 20μm and the width of the narrow part is 5μm. The depth of the channel is 2μm. The channel is made in glass. [7p]

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. ETC

3. Fluidics Applications [17p]

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

[a] The bumper array is based on deterministic lateral displacement. How can it be used to characterize the deformability of particles? [4p]

Principle of DLD

Particles deform and appear smaller

Run at different speeds

[b] In biology it is often important to expose cells to a sequence of different chemicals. In a standard laboratory one would use centrifugation of the cells followed by removal of the supernatant and subsequently addition of a different buffer solution. Using the basic idea behind the bumper array, how can this task be implemented in microfluidics? [2p]

Move the cells across flow streams. See Keith Morton's work!

[c] Inertial forces can be used for separation of particles. What is the general layout and underlying mechanism of a fractionation device based on inertial forces? [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] Two-phase fluidics can be used for single-cell biology. Describe how! [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.

Droplets are formed based on the competition between surface shear and viscous shear.

[e] How can microfluidics be useful for sorting of cells based on specific binding to antibodies? [2p]

Post array with surfaces decorated with antibodies for the CTCs.

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

OR

Some device for concentrating antibody-decorated microsphere, polystyrene or magnetic…

4. Optics on the small scale [18p]

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

[a] What are the experimental difficulties involved in realizing single-molecule detection with optical methods? [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

[b] Explain what additional information can be gained from studies on single molecules as compared to studies on a large number of molecules at once? [3p]

Heterogeneity

Local probe

Time trajectories

[c] How much light can typically be extracted from a single molecule? How can one maximize this number? [3p]

100 000 photons before bleaching.

Add mercaptoethanol to avoid photobleaching.

[d] 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 the basic idea and key features of one approach. What resolution is it possible to achieve? [4p]

STED, STORM etc

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]

[e] In super-resolution microscopy, high 3D resolution has been a challenge to achieve. Describe one example of a solution. [2p]

astigmatic lense in STORM

4Pi + STED

…

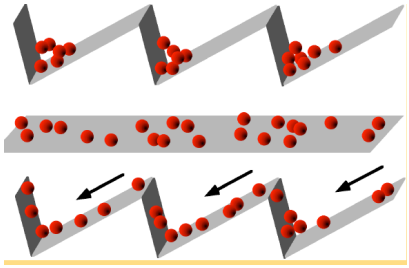
5. Molecular Motors [13p]

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

[a] What are the key differences between a car engine and a typical molecular motor? [2p]

A molecular motor uses Brownian motion to move forward. Power is not used to generate the motion but to rectify the random thermal motion.

[b] Draw a figure of a flashing ratchet! How does it work? 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.*

[c] What is the average speed of a molecule motor in a typical cell? Calculate the average diffusional speed of the molecular motor (assume a molecular weight of 500kDa ~ 8 10^{-22} kg). How do the two speeds compare? [3p]

Normal speeds for molecular motors are in the order of magnitude of 0.1-100µm/sec.

The speed during Brownian motion can be estimated by the following.

Center-of-mass speed:

$$\left.\begin{aligned} E_K &= \frac{1}{2}mv^2 \\ E_K &= \frac{1}{2}k_BT \end{aligned}\right\} v = \sqrt{\frac{k_BT}{m}} \Rightarrow \left[\begin{aligned} k_B &= 1.38\cdot 10^{-23} J/K \\ T &= 300K \\ m &= 500kDa = 8.3\cdot 10^{-22} kg \end{aligned}\right] \Rightarrow v = 2.2m/s$$

[d] The force developed by a muscle (per cross-sectional area) corresponds to approximately 200 kN/m^2, leading to a force e.g. from the gluteus maximus muscle (on which you are sitting) sufficient to lift a weight of about 1200 kg!

What is the average force per myosin head?

Assume that each myosin molecule has two force producing globular heads and that the heads, on average, spend 20 % of their ATPase cycle time in a force producing state. Furthermore, the molecular weight of myosin is approximately 500 kDa and the weight percent of myosin in muscle is 20%. [4p]

The average force per myosin head is on the order 2 pN.

Assume that you have a muscle of 1 m^2 cross-section. Then the force is 200 kN corresponding to 200 kN/2 pN/active head =10^{17} force producing heads. However, only 20 % of the heads are actually bound to actin at each given time so the total number of heads is 5 x 10^{17}. Since each myosin molecule has two heads this corresponds to 2.5 x 10^{17} myosin molecules.

The correct volume to consider in this connection is one half-sarcomere (1.25 µm in length) since the force production of each of the heads in this unit sum up, being equal to the force produced in all other half-sarcomeres over the cross-section of the muscle (otherwise the muscle will break). *Thus, calculate the volume of a*

cylinder of height 1.25 μm and a base of 1 m^2 (1.25 x 10^{-5} x 1 x 10^2 dm^3= 1.25 x 10^{-3} dm^3.

To get the weight of this cylinder assume a density of the muscle of 1.1 kg dm^{-3}. Since you know the number of myosin molecules in this cylinder (2.5 x 10^{17}) and the weight of each molecule (500 kDa = 500 000 x 1.67 x 10^{-27} kg = 8.35 x 10^{-22} kg) you can now easily calculate the weight percent of myosin in muscle. 2.5 x 8.35 x 10^{-5}/(1.25 x 10^{-3} x 1.1) ≈ 18.9 x 10^{-2} ≈ 20 %

6. NanoSafety [17p]

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

[a] Which are the principal two cell types 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 nanoparticles 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 types of effects may nanoparticles have on living matter? [5p]

- *Cytotoxicity (apoptosis, necrosis, cell survival rate compared to control)*
- *Cellular response (endocytosis, cell proliferation)*
- *Organ distribution*
- *Metabolic pathways (cellular respiration)*
- *Clearance*
- *Immuno-suppression / immuno-stimulation*
- *Formation of reactive oxygen species*

[d] 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? What is the area of a sphere of 10kg GaP? What is the total area of 10kg of nanowires (1 μm long, 30 nm in diameter) made of GaP? (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!*