MIDTERM EXAM

ANSWERS

EXPERIMENTAL BIOPHYSICS

FFFN20 – FYST23 – FAF010F

WEDNESDAY, MARCH 14, 2018

0800-1300

MA10E

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HJÄLPMEDEL: INGEN FORMELSAMLING TILLÅTEN

NO FORMULA COLLECTIONS ALLOWED

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.

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.

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

HAND IN YOUR ANSWERS TOGETHER WITH THE QUESTIONS.

A FEW USEFUL NUMBERS:

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

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

$\eta_{WATER} = 1\ 10^{-3}$ KGM^{-1}S^{-1} AT ROOM TEMPERATURE

GOOD LUCK!

JONAS T

1. Biology, Scales and Critical Thinking [16p]

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

[a] News paper articles are typically not written by trained scientists. The same is true for so called alternative media. There are therefore good reasons to be vigilant when reading!

Explain three examples of mistakes or deliberate strategies of deception that may be found in these types of texts. [6p]

- see bad science!

- sensational headlines

- …

[b] DNA is one of the longest molecules in biology. Assuming a typical human cell, for example a skin cell, what would be the radius of a sphere of the DNA in that cell if the DNA would be condensed into a solid particle? Show your calculations. [5p]

size of a cell; size of a liver --> number of cells; 2m DNA in each cell; --> total length of DNA

number of cells in the liver $n_{cells} = \frac{V_{liver}}{V_{cell}} = \left(\frac{r_{liver}}{r_{cell}} \right)^3 = \left(\frac{0.1m}{100 \cdot 10^{-6} m} \right)^3 = 10^9$

number of bases per cell 2 x 3 10^9

total number of bases in the liver 6 10^18

speed of enzyme ~1000 bases per sec

total time required with one enzyme: 6 10^15 sec ~ 190 million years

one could also consider running the PacBio machine in parallel

[c] Phage therapy is an approach with potential to treat infectious disease. If a virus is formed inside a bacterium, how long time would it take on average to diffuse from one end of the bacterium to the other? If the bacterium is replaced by a typical human cell, what would the diffusion time be? [5p]

1D diffusion: $\langle r^2 \rangle = \langle x^2 \rangle = 2Dt$ *; the diffusion takes place from one end to the other end; only one dimension can be considered*

Stokes-Einstein relation gives (with f = the viscous drag; $F_{drag} = f v$*)*

$$D = \frac{k_B T}{f} = \frac{k_B T}{6\pi\eta a}$$
$$\Rightarrow t = \frac{\langle r^2 \rangle}{2D} = \frac{\langle r^2 \rangle 6\pi\eta a}{2k_B T} = 3\pi \frac{\langle r^2 \rangle \eta a}{k_B T}$$

With typical sizes: protein - 10nm; bacterium 1µm; eukaryotic cell 30µm

With typical numbers for water at 25°C we have (37°C more accurate for a reaction in vivo)

$$k_B T = 4 \cdot 10^{-21} J [= kgm^2 s^{-2}]$$
$$\eta = 1 \cdot 10^{-3} kgm^{-1} s^{-1}$$
$$\Rightarrow t = 3\pi \frac{\langle r^2 \rangle \eta a}{k_B T} = 3\pi \frac{1 \cdot 10^{-3} kgm^{-1} s^{-1} 10^{-8} m 10^{-12} m^2}{4 \cdot 10^{-21} kgm^2 s^{-2}} \left(\frac{\langle r^2 \rangle}{1\mu m^2} \right) \left(\frac{a}{10nm} \right)$$
$$\Rightarrow t = 2.35 \cdot 10^{-2} s \left(\frac{\langle r^2 \rangle}{1\mu m^2} \right) \left(\frac{a}{10nm} \right)$$

Answers: diffusion time in a bacterium: 24msec; in a eukaryotic cell: 21 sec. This is a rough estimate. Values may differ if numerical prefactors are ignored. Viscosity in a cell is significantly higher than that of pure water.

A typical molecular motor has a speed of 1µm/sec giving a transport time of 1 sec and 30 sec for the bacterium and the liver cell respectively. Diffusion is therefore much faster at shorter length scales.

One can also make an argument based on the needs of the different organisms. The diffusion time is adequate for the bacterium. The speed of the molecular motor can then be estimated by the speed necessary to achieve the same transport time for the larger cell.

2. Optics on the small scale [19p]

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

[a] DNA origami is an exciting technology to build small structures. For some applications (e.g. for drug delivery) there is an interest in building small boxes containing small molecules that can be released, for example when the box is exposed to a certain chemical environment.

To develop the technique it is necessary to optimize the opening and closing of the box. Describe a technique that can be used to monitor the opening and closing of the box!

Assume that the movements of the parts of the box during opening are on a length scale sufficient to release small molecules. [5p]

Use FRET, fluorescence resonance energy transfer! One donor and one acceptor are attached to the biomolecules at well-defined locations. The two dyes have overlapping

emission and excitation spectra. If located in close proximity (Förster distance ~5nm) energy transfer takes place and an excitation of the donor causes an emission of the acceptor. If located far away only the donor emits. The distance between the donor and the acceptor can be calculated using an expression for the energy transfer efficiency:

$$\frac{I_A}{I_A + I_D} = \frac{1}{1 + \left(\frac{R}{R_{Förster}} \right)^6}$$

Note that the energy transfer efficiency is affected by the orientation of the transition dipole moments as well. For dye molecules with fixed orientation this is highly relevant. In most cases the dye molecules are attached such that they can rotate quite freely. Therefore, the distance is the most important variable under study.

Length scale probed by FRET is governed by the Förster distance ~5nm. Distances 1-10nm are typically probed by the technique.

Single-molecule (referring to the protein, not to the individual fluorophores) detection of the FRET-signal is necessary to calculate the individual distances and

[b] Noise is a key limiting factor in single-molecule detection. Give four examples of sources of noise in a single-molecule measurement. Explain how they can be minimized. [8p]

- *Background fluorescence is the key challenge when doing single-molecule detection.*
- *Minimize excitation or detection volume. Longer wavelengths also cut background.*
- *Confocal, 2-photon microscopy, TIRF – all minimizing the excitation or detection volume.*
- *Note that FIONA and STORM rather are techniques that require single-molecule detection. STED is also not considered a technique that can be used in order to enable single-molecule imaging.*

[c] How does STED microscopy work? Draw a figure and give the basic mechanisms. What optical resolution can be attained? [6p]

- *Donut, two lasers, stimulated emission* ***depletion***
- *25nm in XY and 50nm in Z?*

3. Basic Fluidics [18 p]

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

[a] The device below consists of a section with a large channel to the left and a section with many narrow channels to the right. (1) How many narrow channels to the right are needed so that the average flow velocity is the same in the left and right sections? In the drawing the number of output channels is N=5. How many do you need to have? (2) What value of N is needed so that the flow resistance is the same in the left and right sections? [4p]

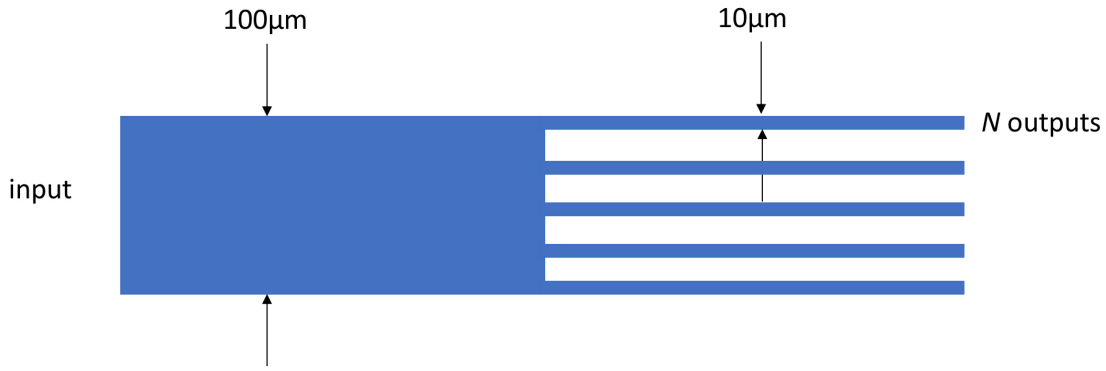

Figure 1. Schematic of fluidic device consisting of two sections. One large area with the input to the left (left section) and N=5 outputs to the right (right section). Device dimensions are given in the schematic. Device depth is 10µm. There is a pressure difference applied across the device of 1 atm.

Assume that the liquid is incompressible.

(1) To have same velocity, we need same total cross section at the beginning and at the end.

(2) To have the same flow resistance, once needs to take into account that the flow resistance is different for a wide channel and a narrow channel.

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[b] There are several techniques to drive liquid in microfluidics channels. Give two examples that are well-adapted to small channels. Explain how they work. [4p]

- *Electrokinetics*
- *Capillary force*

[c] Capillary flow is useful for single-use devices. For the two cases described in the figure below, give the flow velocity of the liquid in the small channels. [6p]

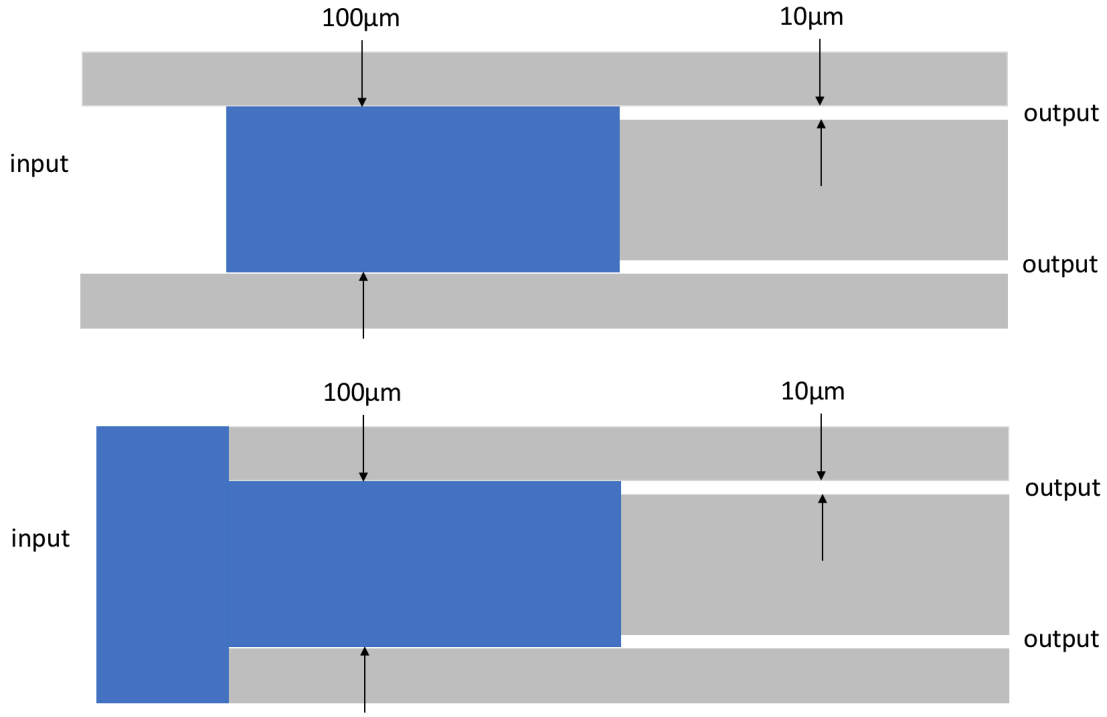

Figure 2. Schematic of fluidic devices with one input to the left and two outputs to the right. Device dimensions are given in the schematic. Device depth is 10µm. Inputs and outputs are all at atmospheric pressure. (TOP) Case 1: The input contains an isolated volume of water. (BOTTOM) Case 2: The input is connected to a large open reservoir of water.

Viscous-drag forces balance forces due to surface energy (assume low Reynolds number, i.e. no inertial effects).

$$F_{drag} = F_{surface}$$

Viscous forces are given by the expression for flow and the fluidic resistance (in our case for a circular cylinder, radius a).

For the first wide section *we have a width* $w=10a_0=100\mu m$ *and depth* $d=a_0=10\mu m$

$$Q=\frac{\Delta P}{R} \Leftrightarrow vA=\frac{F_{drag}}{AR} \Rightarrow F_{drag}=vA^2R=vw^2d^2\frac{12\eta x}{wd^3}=12\eta v\frac{w}{d}x=120\eta v x$$

with surface forces simply given by the circumference (2w+2d) times the surface energy.

$$F_{surface}=2\left(w+d\right)\gamma_{LG}\cos\theta=22a_0\gamma_{LG}\cos\theta$$

For the second quadratic section *we have a width* $a_0=10\mu m$ *and depth* $a_0=10\mu m$

$$Q=\frac{\Delta P}{R} \Leftrightarrow vA=\frac{F_{drag}}{AR} \Rightarrow F_{drag}=vA^2R=va^4\frac{29\eta x}{a^4}=29\eta v x$$

with surface forces simply given by the circumference ($4a_0$) times the surface energy.

$$F_{surface} = 4a_0\gamma_{LG}\cos\theta$$

Note that we do not ask for the position and velocity as a function of time.

We understand that the transport starts in ***the wide section.*** *We now have*

$$F_{viscous} = F_{surface}$$
$$\Rightarrow 120\eta v x = 22a_0\gamma_{LG}\cos\theta$$
$$v = \frac{22a_0\gamma_{LG}\cos\theta}{120\eta x} = \frac{22a_0\,0.0728 Nm^{-1}\cos 20^\circ}{120\;10^{-3} kgm^{-1}s^{-1}\,x} = 12.5 ms^{-1}\frac{a_0}{x}$$

Subsequently the transport continues in ***the quadratic section.*** *We now have*

$$F_{viscous} = F_{surface}$$
$$\Rightarrow 29\eta v x + 120\eta v L = 4a_0\gamma_{LG}\cos\theta$$
$$v = \frac{4a_0\gamma_{LG}\cos\theta}{\eta\left(29\,x + 120L\right)} = \frac{4a_0\,0.0728 Nm^{-1}\cos 20^\circ}{10^{-3} kgm^{-1}s^{-1}\left(29x + 120\cdot 10^{-3} m\right)} = 274 ms^{-1}\frac{a_0}{\left(29x + 120\cdot 10^{-3} m\right)}$$

$$= 9.4 ms^{-1}\frac{a_0}{\left(x + 4.1\cdot 10^{-3} m\right)}$$

To summarize:
- at the beginning of section one the fluid moves with an infinite velocity if the entrance resistance is zero. In practice it is not, so that the velocity will be finite.
- at the end of section one the fluid moves with a velocity of 0.125m/sec
- at the beginning of section two the fluid moves with a velocity of 0.022m/sec
- at the end of section two the fluid moves with a velocity of 0.018m/sec

[d] Assume instantaneous mixing of the compounds A, B and C at the mixing point in the device as outlined in the figure below. Once the mixing has occurred, the time evolution of any chemical or physical reactions between A, B and C can be monitored. What defines the temporal resolution of this measurement? How can it be improved? [4p]

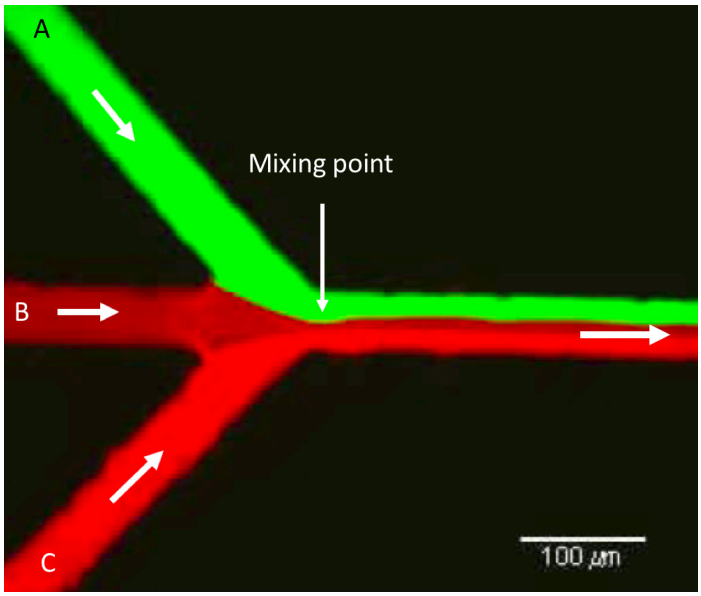

Figure 3. Device schematic. Device for mixing of compounds A, B and C. The mixing takes place in the mixing point as indicated. Flow directions are indicated with arrows.

Taylor diffusion. Lateral diffusion decreases the effect of the stick boundary condition and the parabolic flow profile.

4. Fluidics Applications [16p]

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

[a] High precision sorting can take place by allowing the sample to pass through an array of posts in a channel based on deterministic lateral displacement (DLD). Explain how it works. How can it be designed to be sensitive to (1) shape, (2) deformability of the particles? [8p]

- DLD device

- orientation – shape sensitivity

- deformability – shear deforms particles.

[b] DLD is based on an array of posts in a channel. This may cause clogging. There are other methods that use plain channels without any obstacles. Explain how one such method works that does not rely on diffusion for the sorting! [4p]

Inertial focusing

Margination

Acoustophoresis

Focusing of the streams is the result of three interacting components: inertial lift due to the "wall effect" from the wall; inertial lift due to the shear gradient from the center towards the wall; Dean vortices. An asymmetricly curved channel will result in the focusing of one single stream. The focusing is strongly dependent on the size of the

particles so that by careful extraction of the focused stream size-based sorting can be implemented.

A Dean vortex is formed in a curved channel (radius of curvature R) where the inertial forces act on the fluid. Since we have a parabolic flow profile, the centrifugal force will not be equal for each part of the fluid. The fluid at the center will move at the highest speed and so will experience the greatest centrifugal force. See fig 4 in Squires' and Quakes' review paper[9].

Dean vortices are formed because of the centrifugal force on the fluid. Remember that the centrifugal acceleration is ~v2/r , where r is the radius of curvature of the tube in this case. The inertial effects refer to effects of the fluid, not on the particles. This was shown by running particles in buffer solutions of different densities where the result was the same independent on whether the particles were denser or less dense than the surrounding fluid.

Centrifugation on the other hand relies on the sedimentation in a (artificial) gravitational field. Denser particles fall down whereas particles lighter than the buffer solution move up. This obviously happens in the curved channels, but the net motion due to sedimentation will be zero on average since the curves are going both left and right.

In comparison to DLD, inertial sorting is

- much faster

- requires dilute samples

- selective only to the large particles; the large particles are focused; the small particles remain everywhere in the liquid

[c] Single-cell experiments are challenging, but they give some important benefits. How can they be implemented? Give two examples that can be used for large (>> 1) number of cells and explain how they work. [4p]

- *Droplets*
- *Traps*

5. Interaction of nanostructures and biological matter [15p]

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

[a] The usefulness of nanoparticles in medicine and their toxicity derive from similar mechanisms. What are nanoparticles capable of doing that makes them so useful and potentially so toxic? [3p]

- *Better access to different parts of the body, access into cells, higher surface area to volume ratio.*

[b] Give two examples of potential medical uses of nanoparticles? Explain! [4p]

- *Drug delivery*
- *Mechanical destruction of cells; energy harvester*

[c] Nanowires can be made so that they exhibit a barcode pattern. What could these types of barcoded nanowires be useful for? Explain! [4p]

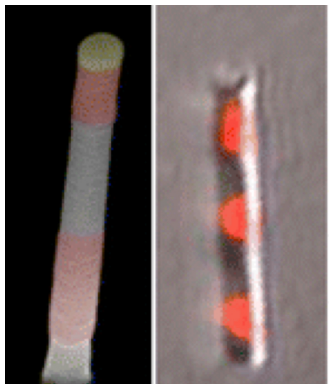

Figure 4. Barcoded nanowires. The red areas are fluorescent and made of GaInP and and the gray sections are made of non-fluorescent GaP. Left image shows an artificially colored SEM image and the right image shows a fluorescent image.

- *MUX*
- *Means of entering the body*
- *Chemistry*
- *Ability of the body to clear itself of the nanoparticles.*

[d] Typically the safety of a compound is expressed in acceptable concentration levels or total dose in terms of a total ingested mass of the material. For example, in Europe if more than 10kg of a new chemical is handled per year, detailed testing needs to be done of the toxicity of the compound.
For nanoparticles the surface area is more relevant than the total mass. Estimate the increase in the total surface area if a solid sphere of some material is divided into nanoscale particles. Explain in detail how to arrive at a simple expression for the increase in total surface area. [4p]

The surface area to volume of a sphere scales as 3/r where r is the radius of the particle. In a typical case the particle size differs by a million times. Thus the surface area to volume ratio of the nanopowder would then be million times greater than that of the "normal" powder.

6. Membrane Biophysics and the Immune System [12p]

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

[a] Describe the main steps in the kinetic segregation model? Explain why the gap size between contacting T cells and antigen presenting cells (of the order of 15 nm) is important. [5p]

TCR triggering is caused by exclusion of the phosphatase CD45 from contacts between T cells and APCs. CD45 keeps TCR unphosphorylated in resting T cells. The exclusion of CD45 results in a shift in the balance between phosphatases (CD45) and kinases (Lck) in the contact → TCR phosphorylation → TCR triggering. [4p]

The height of CD45 is larger than 15 nm (>20 nm) and since the cell-cell gap is smaller this drives exclusion of CD45 based on its length. [1p]

[b] Mention two methods that can be used to measure 2D binding across contacting cells. [2p]

Fluorescence-based methods and mechanical assays (e.g. biomembrane force probe). [2p]

[c] The Zhu-Golan expression can be used to estimate the fraction of bound receptors in the contact between two immune cells:

$$\frac{B}{F} = \frac{N_{\mathrm{t}} \times f}{K_{\mathrm{d}} \times S_{\mathrm{cell}}} - \frac{B \times p}{K_{\mathrm{d}}}$$

where B = receptor-ligand density, F = free ligand density, Nt×f = number of mobile receptors per cell, Kd = 2D dissociation constant, Scell = cell area, p = Scontact/Scell. Use this expression to estimate how many TCR molecules that are binding MHC in the contact between a T cell and an SLB containing MHC. Assume the following values: Kd = 100 molecules/μm2, Nt = 10 000 molecules, f = 1, Scell = 200 μm2, F = 50 molecules/μm2 and p = 10%. What is the fraction of TCR molecules in the contact that are bound to MHC (bound TCR in the contact / total number of TCR in the contact)? [5p]

The Zhu-Golan expression can be rewritten as:

$$B = \frac{N_t \times f}{S_{cell}(p + K_d/F)}$$

The number of bound receptors in the contact is given by:

$$N_{bound} = B \times p \times S_{cell}$$

which inserted into the previous expression gives:

$$N_{bound} = \frac{N_t \times f \times p}{(p + K_d/F)} = \frac{10000 \times 1 \times 0.1}{(0.1 + 100/50)} molecules \approx 476\, molecules$$

[3p]

The concentration of unbound receptors on the cell surface is:

$$c_{unbound} = \frac{(N_t \times f) - N_{bound}}{S_{cell}}$$

which gives that the number of unbound receptors in the contact to the SLB is:

$$N_{unbound} = c_{unbound} \times p \times S_{cell} = \big((N_t \times f) - N_{bound}\big) \times p$$
$$N_{unbound} = \big((10000 \times 1) - 196\big) \times 0.1 \approx 952\, molecules$$

$$\frac{N_{\text{bound}}}{N_{\text{unbound}} + N_{\text{bound}}} = \frac{476}{952 + 476} \approx 0.33$$

[2p]