MFBM, Class Exercise 3, 3.2.2021

1. Diffusion

a) The dye Brilliant Blue FCF (E133) has a diffusion coefficient about $5* 10^{-10}$ m^2 s^{-1}. This unit is not very useful for understanding microsystems. Write the diffusion coefficient instead in units of cm^2 s^{-1} and μm^2 s^{-1}.

b) In exercise 1 we were looking at a microchannel which had width and height 100 µm and length 2 cm. We calculated that with volumetric flow rate of 0.25 µl/min, the average linear velocity was 400 µm/s. What is the average residence time of a liquid element in the channel? (t = L/v, length of the channel divided by the average linear velocity).

c) In the same amount of time, estimate how far the dye travels in one dimension by diffusion. For 1D diffusion: $<x^2> = 2Dt$.

d) Based on your result and the channel dimensions, estimate whether the channel will be a diffusive mixer, or whether there are two parallel laminar flows that largely do not mix, or whether a gradient will form.

Bonus: change the dimensions or flow rate to get full mixing or (practically) no mixing

2. Adsorption

a) You are designing a chip to analyze a medium sized protein. Your chip is made from a polymer that is slightly hydrophobic (Advancing contact angle 95°, receding contact angle 79°). The chip will adsorb a monolayer of proteins with a thickness of 2 nm. You can assume that the density of the adsorbed protein layer is $1 g/cm^3$.

If the channel dimensions are once again 100µm width and height and 2 cm length, how big problem is adsorption if the total amount of protein in the sample is 1 ng, 100 ng or 10 µg.

b) What would be possible solutions to the problem of adsorption in that kind of chip?

Bonus:

c) Single molecule analysis. You want to analyze very low concentrations of the above protein, up to single molecule sensitivity. To limit adsorption, an anti-adsorption PEG (polyethyleneglycol) coating is used and the chip is made as small as possible which in this case is 10 µm width and height and 2 mm length. If we assume that the coating prevents 99.999% of adsorption, will you be able to reliably do single molecule analysis? The size of the protein is 50 kDa.

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a)

$5* 10^{-10}$ m^2 s^{-1} = $5* 10^{-6}$ cm^2 s^{-1} = 500 μm^2 s^{-1}

b)

The linear velocity is about 0.4 mm/s. The length of the channel is 2cm so the average residence time would be 20mm / 0.4mm/s = 50 s.

c)

For 1d diffusion, $\langle x^2 \rangle = 2Dt = 2*500$ μm^2 s^{-1} * 50s = 50 000 μm^2.

The notation $\langle x^2 \rangle$ means the expected value of the square of the distance traveled. The expected value of the square has a simple mathematical form. But it is not very intuitive.

We can take the square root and get a rough **estimate ≈ 220 µm**.

This is just an estimate, some molecules travel more, some less. (The average distance traveled is somewhat less than the 220 µm since molecules that travel more than this contribute heavily to the $\langle x^2 \rangle$ term.).

d) The average distance the molecule travels is few times the width of the channel. Therefore, definitely we do not have parallel laminar flows. Most likely a gradient will form, as the diffusion might not be enough for complete mixing.

2. Adsorption

a) You are designing a chip to analyze a medium sized protein. Your chip is made from a polymer that is slightly hydrophobic (Advancing contact angle 95°, receding contact angle 79°). The chip will adsorb a monolayer of proteins with a thickness of 2 nm. You can assume that the density of the adsorbed protein layer is 1g/cm^3.

If the channel dimensions are once again 100µm width and height and 2 cm length, how big problem is adsorption if the total amount of protein in the sample is 1 ng, 100 ng or 10 µg.

The total surface area of the chip is:

$$A = 2 * (w + h) * L = 2 * (0.1mm + 0.1mm) * 20mm = 8\ mm^2$$

and the total volume of the 2 nm adsorbed layer is then:

$$V = A * d = 8mm^2 * 2nm = 1.6 * 10^{-5}mm^3 = 1.6 * 10^{-8}cm^3$$

The total mass of the protein is then:

$$m = V * \rho = 1.6 * 10^{-8}cm^3 * \frac{1g}{cm^3} = 1.6 * 10^{-8}g = 16\ ng$$

So: A huge problem for 1 ng, as all the protein would be lost to adsorption. A noteworthy issue for 100 ng as up to 16% of the protein would be lost, but this might not be catastrophic. For the 10 µg case, loss of protein by adsorption would not be an issue (although other issues related to adsorption e.g. fouling of a sensor surface could still be noteworthy).

b) What would be possible solutions to the problem of adsorption in that kind of chip?

Use smaller chip if possible.

Use higher concentrations of protein OR bigger volumes, if possible. In practice the concentration and available/preferred sample amounts are not free parameters to change but depend on the application,

Make the chip out of more hydrophilic material to reduce hydrophobic interaction.

Use a coating to reduce adsorption. This could be simply a hydrophilic coating to reduce hydrophobic interaction or a dedicated anti-adsorption coating.

Blocking with some other protein before the analysis.

Bonus:

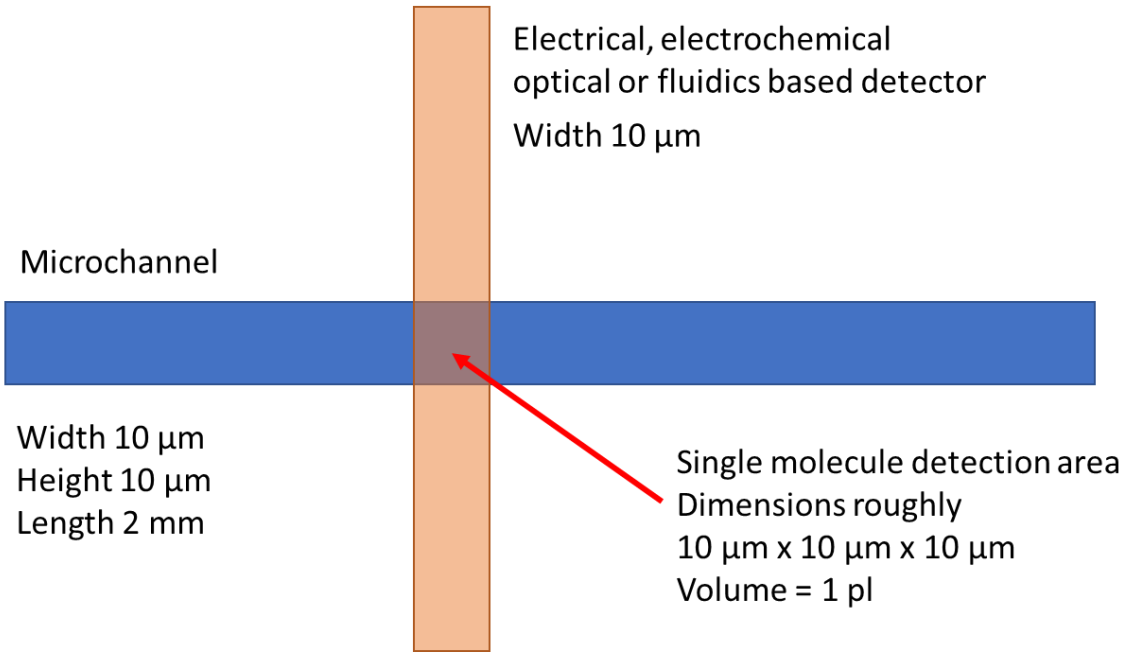
c) Single molecule analysis. You want to analyze very low concentrations of the above protein, up to single molecule sensitivity. You are planning a straightforward setup where the sample is diluted to the desired protein amount and then the analysis is performed by running the solution through the microfluidic chip. To limit adsorption, an anti-adsorption PEG (polyethylene-glycol) coating is used and the chip is made as small as possible which in this case is 10 µm width and height and 2 mm length. If we assume that the coating prevents 99.999% of adsorption, will you be able to reliably do single molecule analysis? The size of the protein is 50 kDa.

The above calculation can be repeated to find out that without the PEG coating this smaller chip would adsorb 0.16 ng of protein. If the antiadhesion coating eliminates 99.999%, that leaves $1.6 * 10^{-15}$ g of protein that adsorbs.

For 50 kDa protein, this is $3.2 * 10^{-20}$ moles of protein. Multiplying by Avogadros number, we finally get that the adsorbed amount is about 20 000 molecules.

In order for single molecule analysis to work like this, the coating would have to be 99.99999999% effective, which clearly is unrealistic.

Real microfluidic single molecule analysis doesn’t mean that there is only one molecule on the entire chip. Rather, a certain small area, where the analysis is performed, only has a single molecule either by statistical dilution or by some physical effect.



Single molecule detection strategy based on defining a suitably small volume by the "intersection" of the detector (=where the molecules would be detected) with the channel (=where the molecules are). (with the dimensions given in this calculation there would still be some work to do).