CHEM-E8135 Microfluidics and BioMEMS

Microfluidics 3: Diffusion and Adsorption

27.1.2021

Ville Jokinen

Microfluidics 1-3 lectures, key phenomena:

Lecture 1:

-**Viscous forces** are dominant at microscale -> Laminar flow

Lecture 2:

-**Surface forces** are dominant at microscale -> Capillary pressure

Lecture 3:

-**Diffusion is fast at microscale** … but residence times are short as well.

-There is **a lot of surface are per volume** at microscale

Intended learning outcomes for lecture 3:

ILO1: The student understands fluid flow at the microscale, scaling laws and can analyse microfluidic circuits. Laminar flow, **diffusion**, Reynolds number.

ILO2: The student is familiar with liquid and **solute interactions with surfaces**. The student understands surface energy, wetting and capillary filling. **The student is familiar with mechanisms for biomolecular adsorption on surfaces.**

Part 1: Diffusion

Diffusion, Brownian motion

- Diffusion is stochastic random drift of molecules based on thermal fluctuations, collisions, etc.

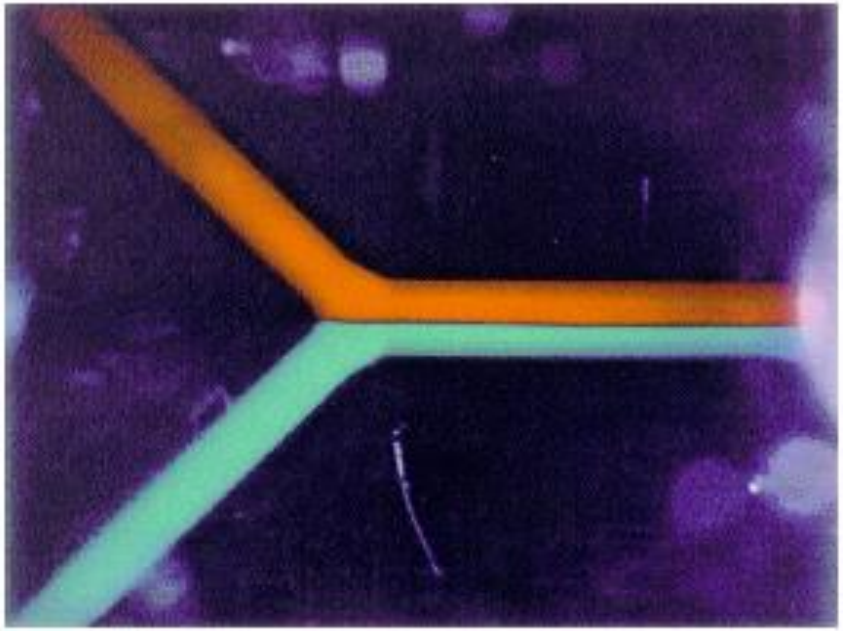
1D: $\langle x^2 \rangle = 2Dt$ 3D: $\langle x^2 \rangle = 6Dt$

D = diffusion coefficient, t = time and $\langle x^2 \rangle$ is the expectation for the square of the distance traveled.

D depends on the size o the molecule, the temperature and the viscosity.

1D diffusion in a microfluidic channel: only x direction considered

Mixing by diffusion



Microfluidic parallel laminar flows
(2 dyes in water)

Danube (left) joins another river

- Low reynolds number: mixing only by diffusion, no turbulent mixing
- Diffusion distances compared to flow speed / resident time

http://www.ifh.uni-karlsruhe.de/lehre/envflu_i/related_resources/photos.htm

Diffusion in microfluidics

Typical diffusion constants (in water, room temperature):

Small molecules: >500 μm^2 /s
Small proteins: 100 μm^2 /s
Large proteins: 10 μm^2 /s
Large DNA molecules: <1 μm^2 /s

1D: $\langle x^2 \rangle = 2Dt$
From which we can approximate:
$x \approx \sqrt{2Dt}$

We see that the distance traveled in 1s timescale is in the order of 1µm – 10 µm.

Depending on the dimensions of the microfluidic channel and the flow rate, diffusion can either be very slow of very fast compared to distances and residence times inside the channel!

Case 1: diffusion is fast -> Mixing, mixers
Case 2: diffusion is slow -> No mixing, parallel laminar flows
Case 3: diffusion is moderate -> Gradient generators, diffusive filters

Example calculation

Microchannel is 100 µm wide, 100 µm high and 1 cm long. From inlet 1 water containing green dye is pumped at 0.05 µl / min, and from inlet 2 plain water is pumped at 0.05 µl / min. The diffusion constant of the dye is 600 $\mu m^2/s$. Will the dye mix or stay confined to one side of the channel?

Residence time:

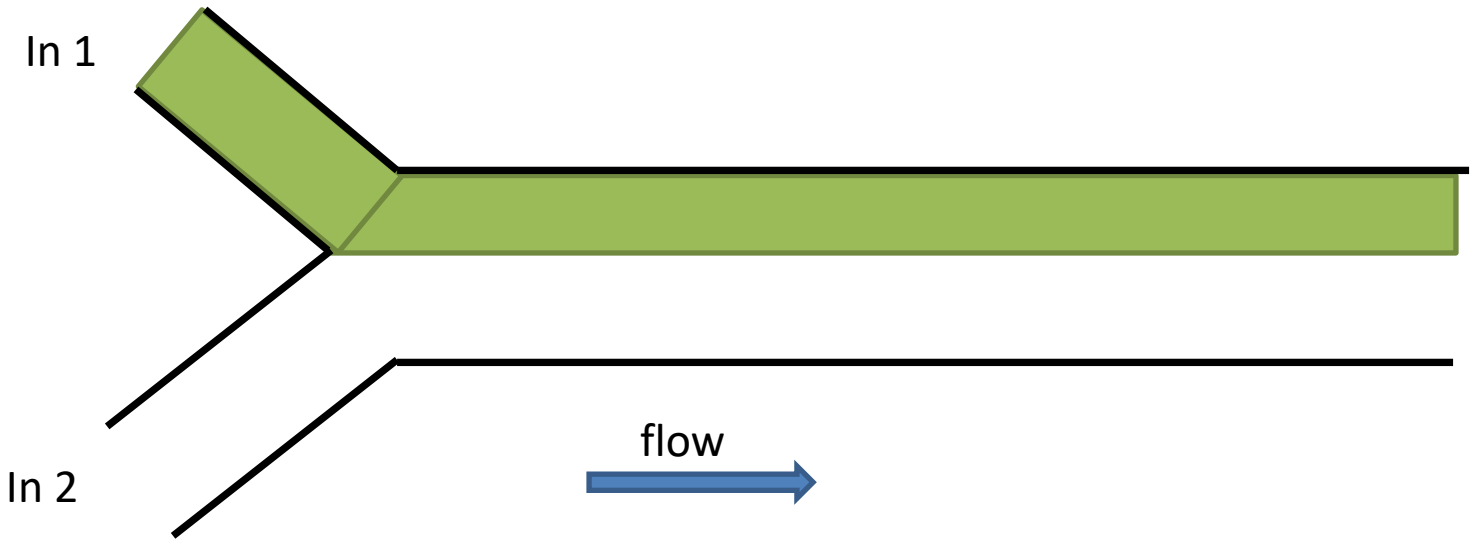
v_{ave} = Q/A = $0.1mm^3/(60s*0.1mm*01mm) \approx 0.17mm/s$.

Residence time = 10mm/0.17mm/s ≈ 60s

Diffusion distance (1D): $\langle x^2 \rangle = 2Dt = 2* 600\ \mu m^2/s *60s = 72\ 000\ \mu m^2$

By taking the square root we get a rough estimation for the lateral diffusion distance

$\sqrt{72000 \mu m} \approx 270\ \mu m$



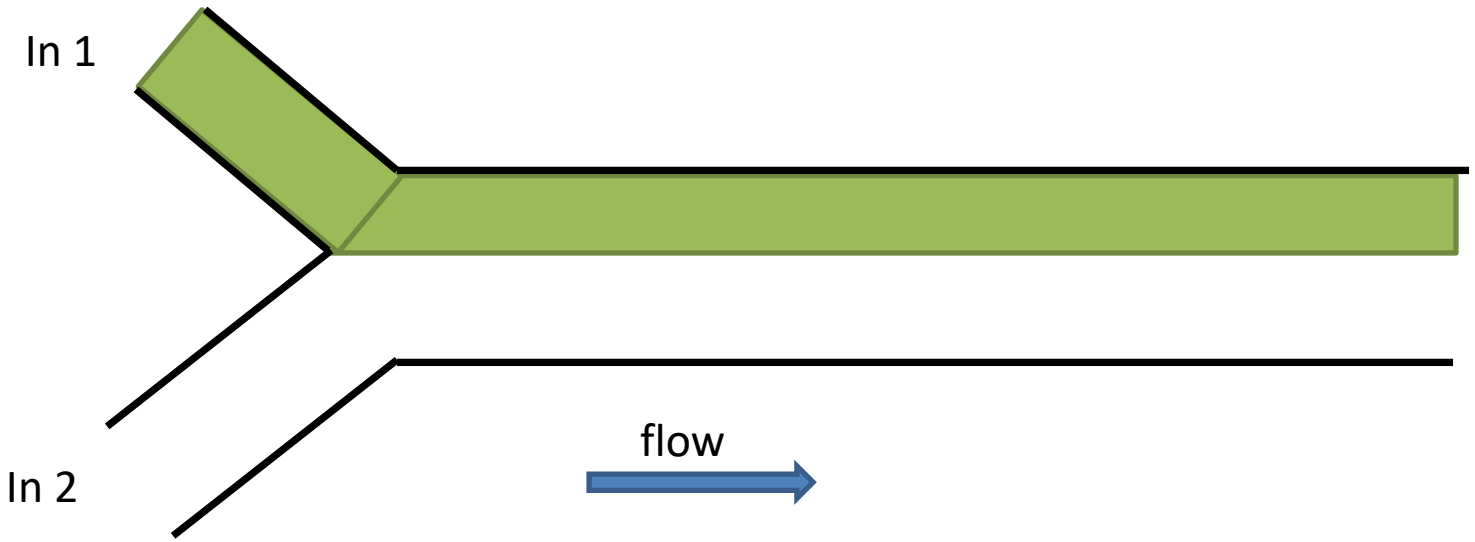
Example calculation, cont

Microchannel is 100 µm wide, 100 µm high and 1 cm long. From inlet 1 water containing green dye is pumped at 0.05 µl / min, and from inlet 2 plain water is pumped at 0.05 µl / min. The diffusion constant of the dye is 600 $\mu m^2/s$. Will the dye mix or stay confined to one side of the channel?

Since the channel width is 100 µm and the lateral diffusion distance is 270 µm, we would expect a decent amount of mixing to occur.

By changing the channel dimensions and the flow rate, things could different…

(an upcoming chip design task maybe?)



Peclet number

Dimensionless number showing the relative magnitudes of convective and diffusive mass transport.

$$Pe = \frac{Lv}{D}$$

, where L is the characteristic dimension, v is characteristic velocity and D the diffusion coefficient.

The lower the Peclet number, the more mixing occurs.

Previous example: L ≈ 100 µm, v ≈ 0.1 mm/s, D = 600 $\mu m^2/s$, Pe ≈ 17.

This means that the flows remain unmixed until the lateral dimension traveled is approximately 17 times the width of the channel.

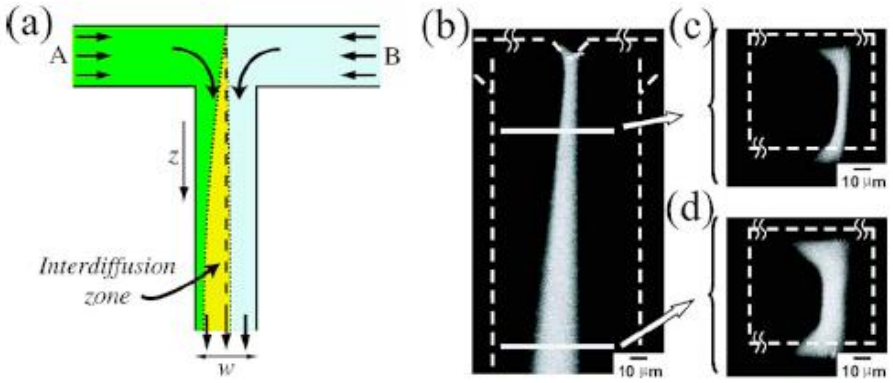
But in the example given, the lateral dimension traveled was 1 cm and the width of the channel was 100 µm, so the distance traveled was 100 times the width, so mixing would be expected,

Case 1: Microfluidic mixing

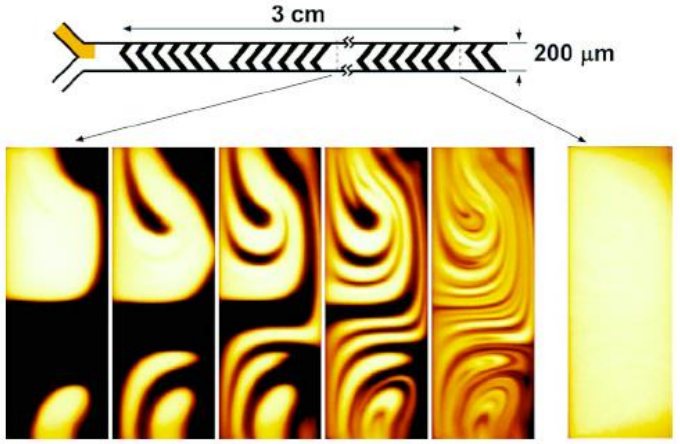
Small dimensions, high diffusion coefficients (small analytes) and slow flow rates lead to diffusive mixing.

Drawback: Flow needs to be quite slow.

To enhance: There are microstructures that enhance mixing folding layers of liquid among eachother to reduce diffusion distances needed.



Diffusive mixing



Enhanced mixing by "herringbone" structures

Ismagilov *et al.*, Appl. Phys. Lett. **76**, 2376. 2000

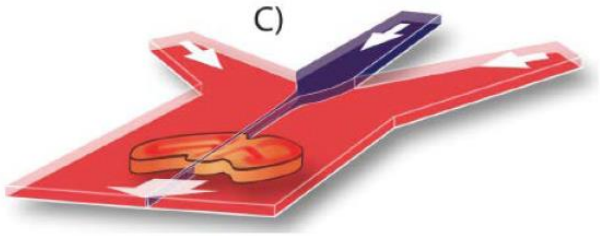
Stroock *et al.*, 2002, Science **295**, 647 2002.

Case 2: Parallel laminar flows

The opposite of mixing

Large dimensions, low diffusion coefficients (big analytes) and high flow rates lead to (almost) no mixing.

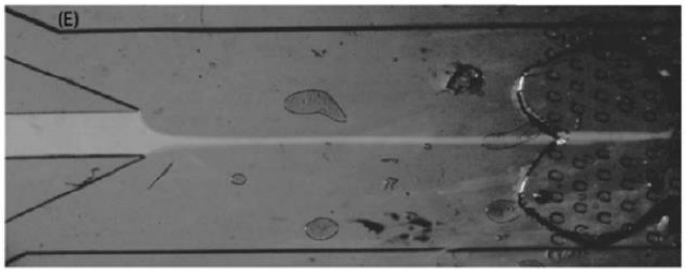
This can be used in some applications to e.g. do a reaction at the phase boundary or to target a treatment to a specific spot.



Pump 1 Inlet 1

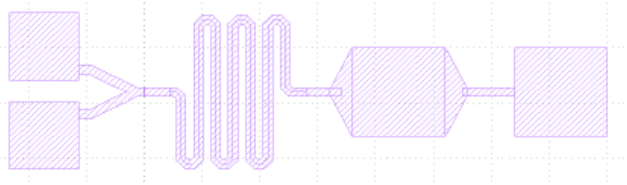
Pump 2 Inlet 2

Pump 3 Inlet 3



Lab Chip, 2007, **7**, 842–849

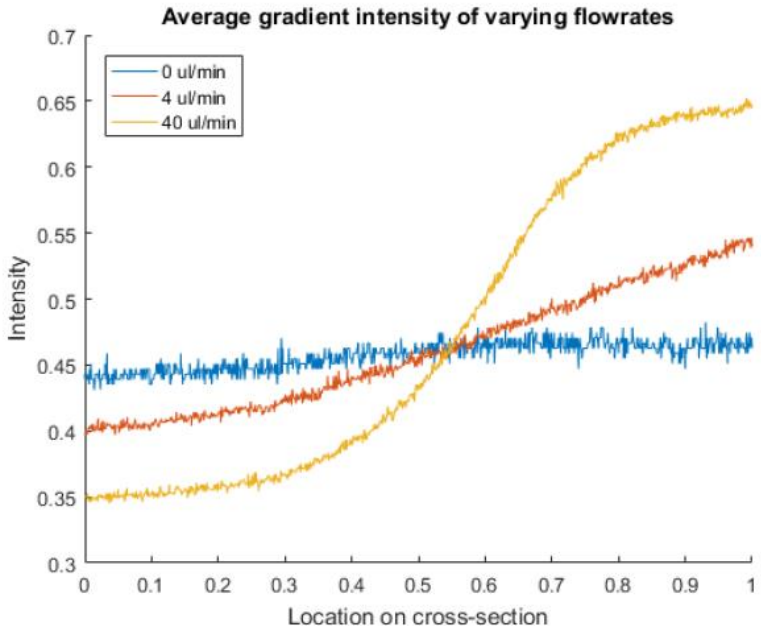
Case 3: Gradient generators

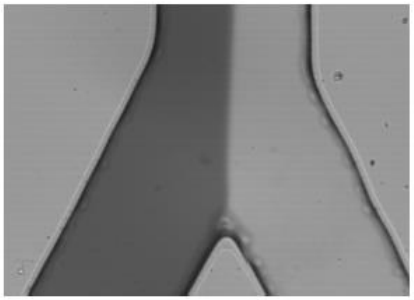


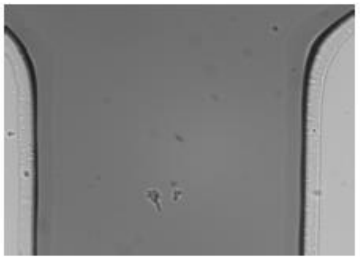
Channel width 400 µm
Length 46 mm

Mask designed in Klayout

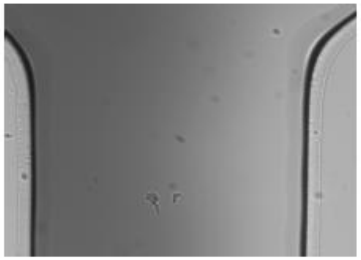
COMSOL-simulation using 4 µl/min channel flow







0 µl per minute



12 µl per minute

100 µl per minute

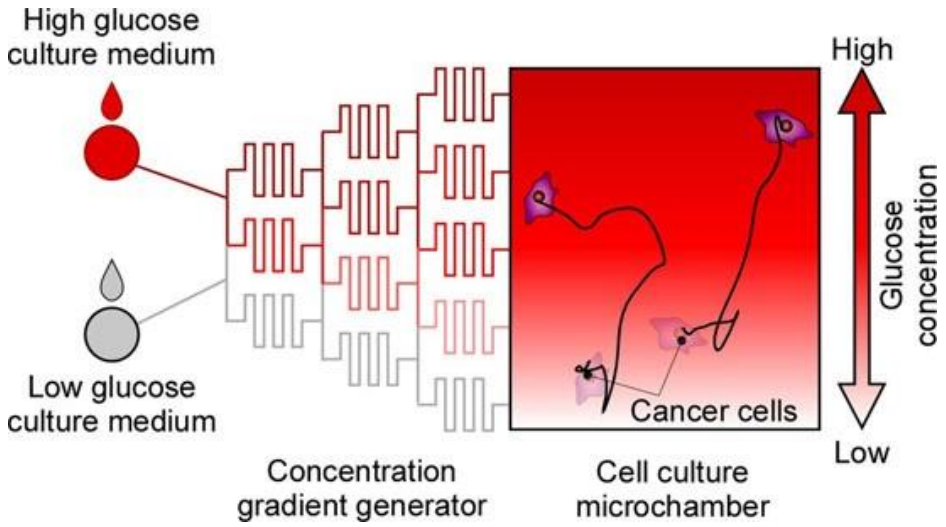
Henri Honkanen, PRA 2018

Case 3: Gradient generators 2

You can also do a gradient by mixing.

Two inlets and one or more sets of parallel mixing channels.

Laminar flow and symmetry enable relatively easy designs.

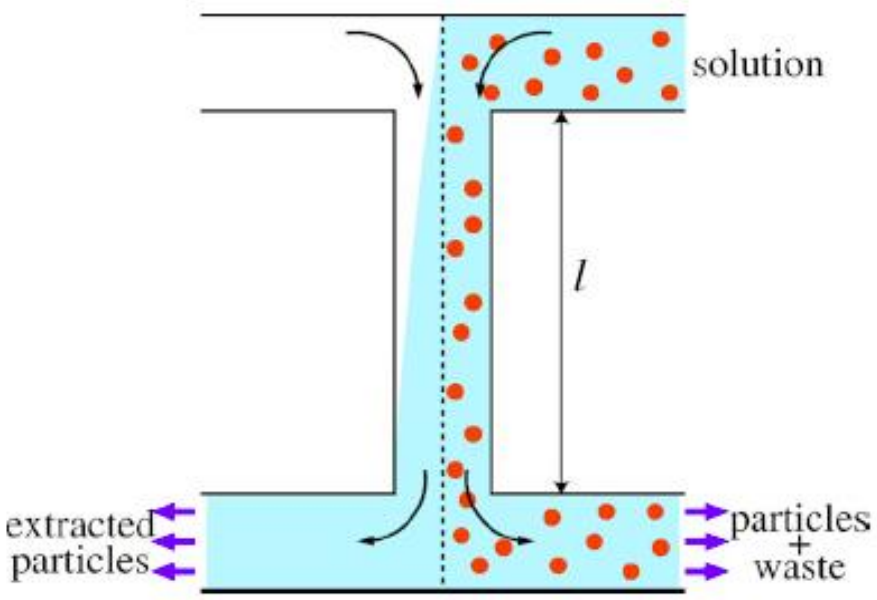


https://doi.org/10.3390/mi7090155

Case 3: H filter

If there are more than one analyte then due to differences in diffusion constants, diffusion can be fast for one analyte and slow for the other

-> H Filter



Red circles = bigger particles (smaller diffusion constant)
Blue liquid = contains smaller particles (higher diffusion constant)

Brody, J. P., and P. Yager, 1997, Sens. Actuators, A **58**, 13.1997

Static gradient

It is also possible to use microfluidics to create a gradient over a static system. If one side of a system is kept at concentration C_A, and other side at concentration C_B, diffusion creates a linear gradient from C_A to C_B.

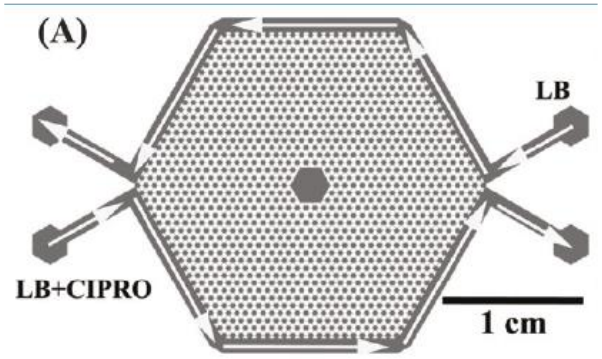
An example: creating an antibiotic gradient to study the emergence of antibiotic resistance.

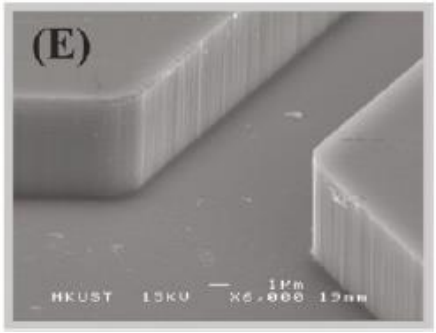
Top channel: nutrients only

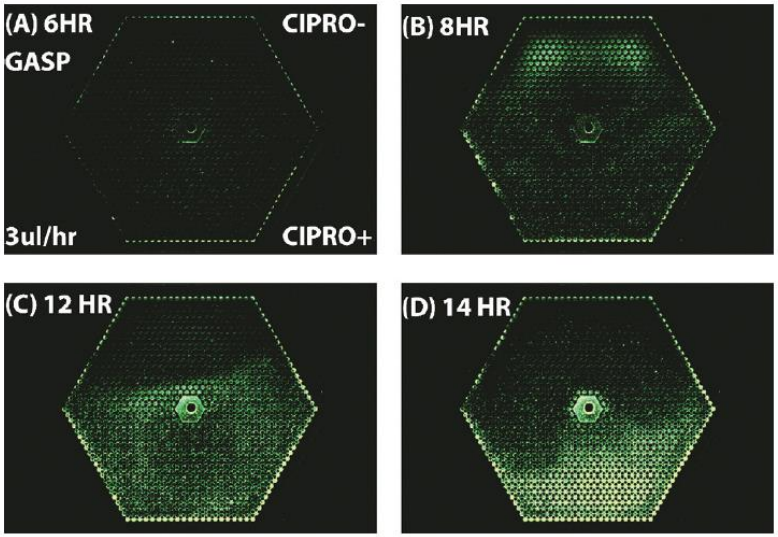
Bottom channel: nutrients + antibiotics

Seeding bacteria at center

Green fluorescent bacteria over time adapt to higher antibiotic concentrations.



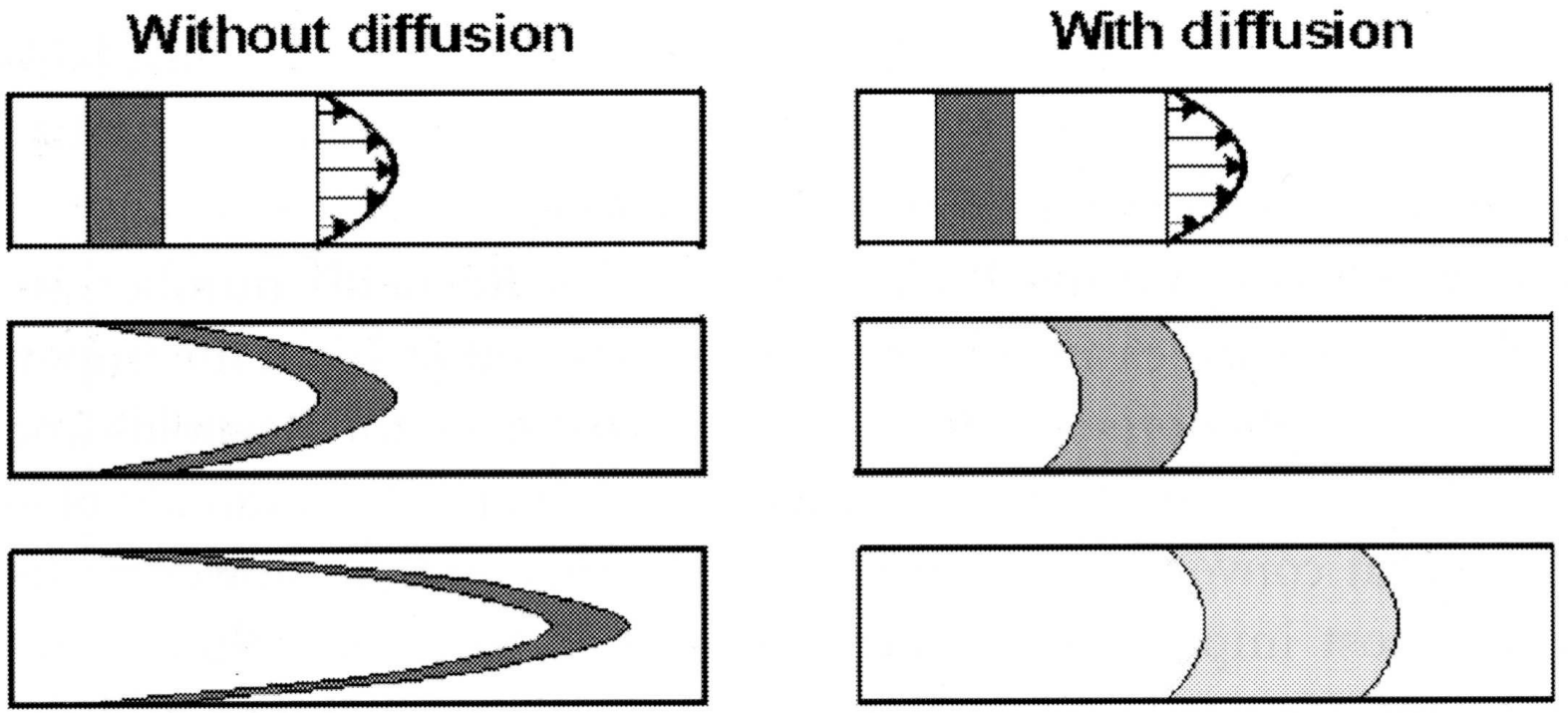




dx.doi.org/10.1021/mp200274r |*Mol. Pharmaceutics* 2011, 8, 2063–2068

Taylor dispersion

What happens to a liquid element under pressure driven flow with also diffusion taken into account?



Distortion of a plug profile

Increased diffusivity

Part 2: adsorption

- This part deals with solute (biomolecules, drugs etc.) interactions with the surface, mainly adsorption.
- Adsorption happens also from the gas phase but this lecture presents the topic only from the adsorption from liquid phase point of view.

Adsorption Basics

Adsorption = molecular attachment to a surface (≠ absorption)

Adsorption highly significant in microfluidics because:

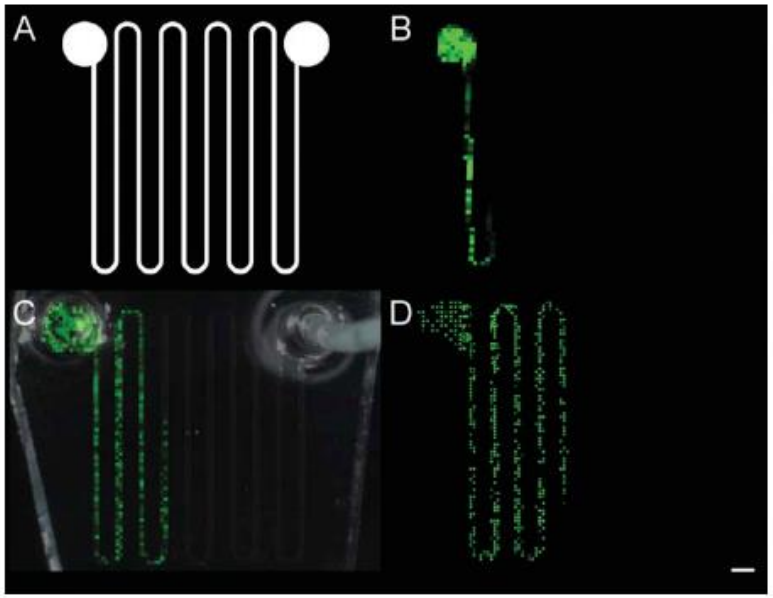
- Surface area to volume ratio scales as d^{-1}
- Diffusion time scales as d^{2}

- Many applications benefit from adsorption, e.g. affinity capture and chromatographic methods.
- On the other hand, unwanted adsorption can cause analyte depletion, surface property changes and biofouling in microsystems.

Analyte depletion

Due to fast diffusion and high surface to volume ratio**, it is possible to lose all of the analytes by adsorption in microfluidics**!

Example: a peptide monolayer on a surface with 100% coverage.
Microchannel width 100 µm and height 100 µm, length 10 mm and monolayer height 1 nm. Lets also assume that density of the peptide is similar to water 1000kg/m^3. With these parameters, 4 ng of analyte will be lost per 10 mm of the channel. (the volume is 0.1 µl)



Depletion of peptide angiotensin II in a microchannel

B: 1 ng
C: 2.5 ng
D: 4 ng

Jo et al, Lab Chip 2007, 1454-1460

Bonus Slide: Microfluidics vs macrosystems for low abundance analytes

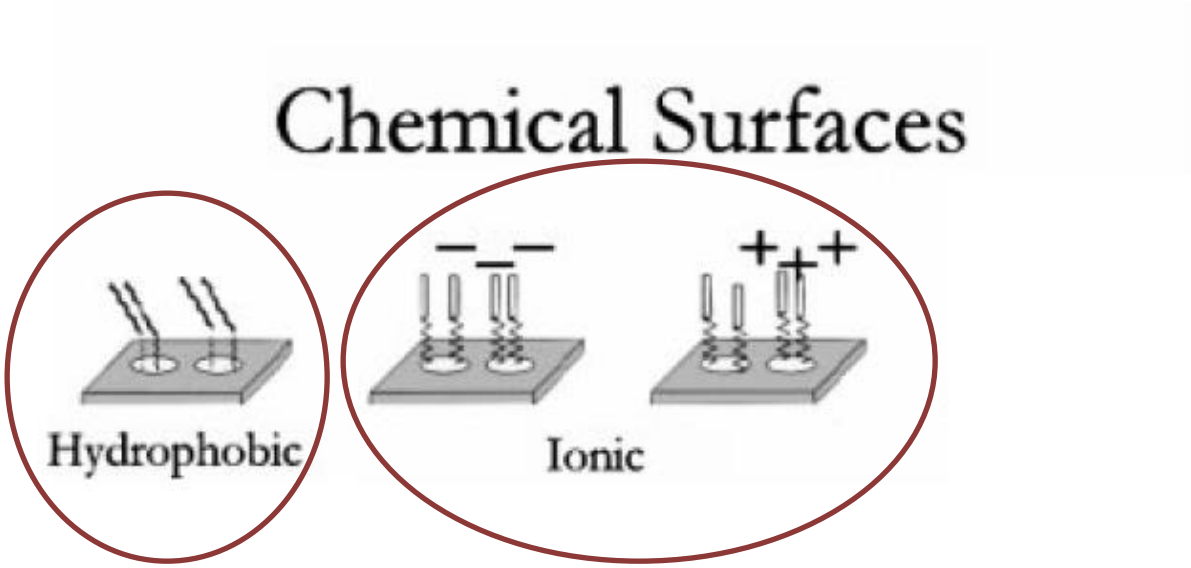
Since microsystems have high A/V ratio, as well as short distances for diffusion, adsorption can be significant. On the other hand, the absolute surface area of a microsystem is still smaller by a big margin compared to a macrosystem (d^2 scaling). Because of this, if a rare or expensive analyte needs to be analysed, the use of microfluidics can be advantageous.

The typical volume of a microfluidic chip is <1 µl. However the amount of liquid pumped throug the chip is commonly much larger than this. So even if initially the walls adsorb a lot, they will very quickly saturate.

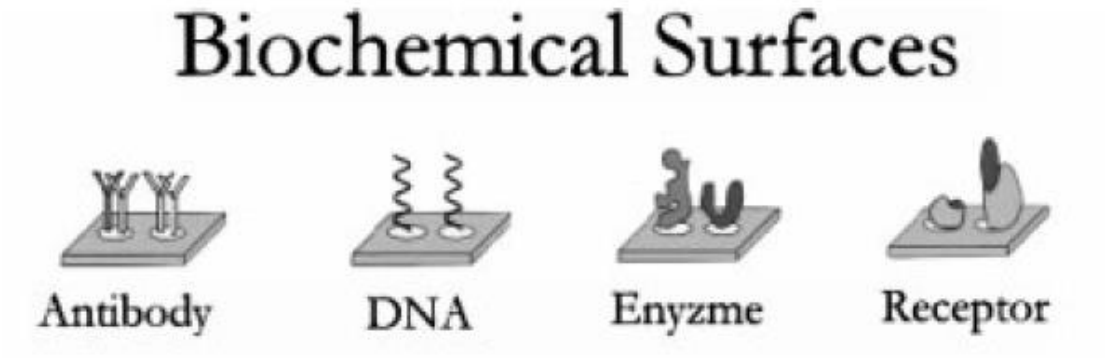
Thus, a microsystem is a solution to an adsorption problem if the same amount of total analyte is available since the absolute surface area of a microsystem is small.

Commonly materials or coatings that minimize the adsorbtion area utilized.

Adsorption types:



-<u>Nonspecific</u> binding
-Based on physico-chemical properties of the surfaces



Later lectures
-<u>Specific</u> binding
-Based on shape recognition (shape matching).

Hydrophobic interaction 1: Hydrophobic surfaces and analytes

Hydrophilic surfaces contain one or more of:
- charged groups (e.g. -COO-, -CO-)
- highly polar groups (e.g. –C-OH, -Si-OH)
- high potential for forming hydrogen bonds

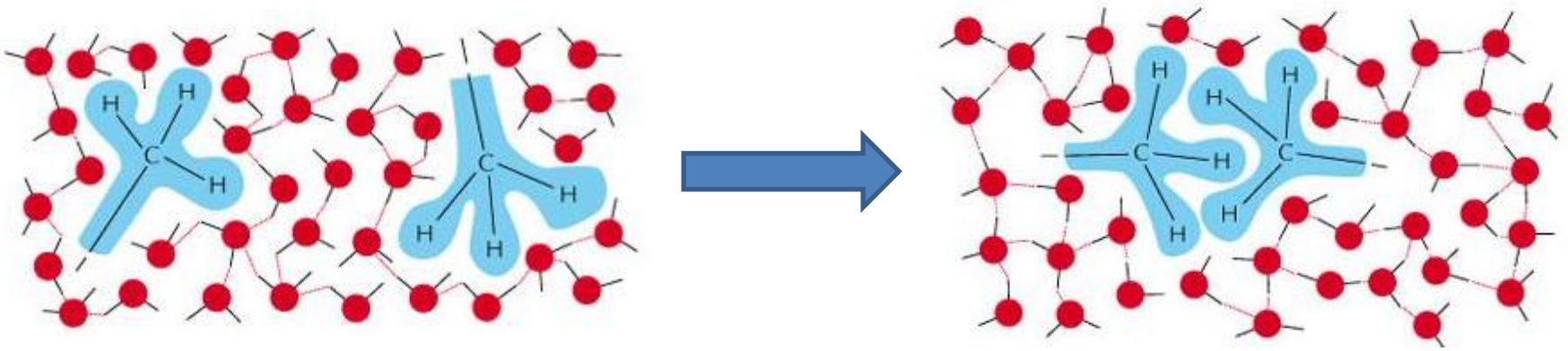
Surfaces that lack any of these characteristics, are hydrophobic. Examples:
- Methyl terminated surfaces $–CH_3$
- Fluorine terminated surfaces $-CF_3$
- Many polymers, including PDMS (the most common polymer in microfluidics).

Examples of analytes and biomolecules with hydrophobic characteristics:
- Proteins and peptides (partially)
- Lipids (one end)
- Drugs (some)

Hydrophobic interaction 2

It is well known that under water, hydrophobic surfaces stick to each other.
What is the physical basis for hydrophobic interaction?

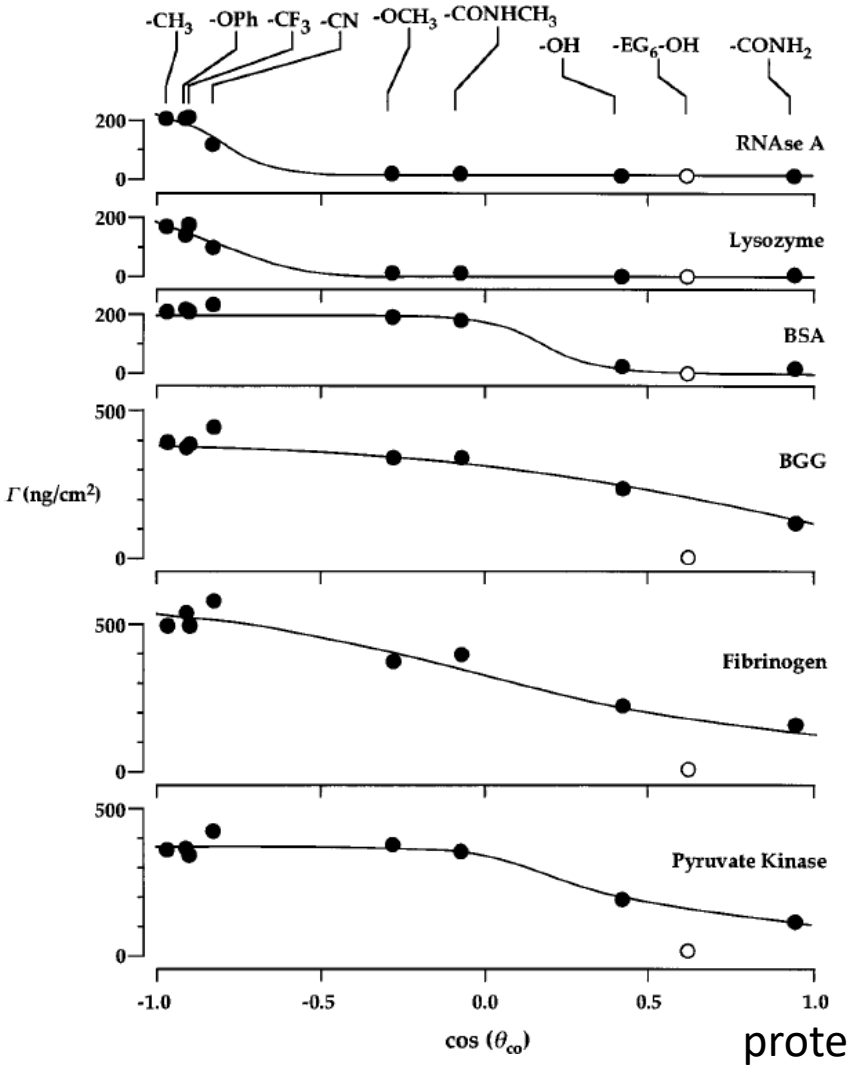
More CH_3-CH_3 interaction? Contributes (Van-der-Waals), but not the main reason
Less CH_3 – H_20 interaction? No, weaker but attractive interaction
More H_20- H_20 interaction? YES!! Water-water bonds are strong.



• Range ≈ 2 – 10 nm , mediated by local ordering of water molecules.

Hydrophobic interaction 3

All proteins adsorb on hydrophobic surfaces by hydrophobic interaction!



Small Proteins

Medium

Large

protein size increases

Water contact angles:

$-CH_3$	$\theta \approx 112°$
-OH	$\theta \approx 15°$
$-EG_6$-OH (PEG)	$\theta \approx 38°$

Sigal et al. J. Am. Chem. Soc. 120, p.3464, 1998

Charged surfaces/molecules

Charged surfaces can be either permanently charged or pH dependent

pH dependent negatively charged groups (e.g. COOH):

- at high pH: negatively charged, at low pH: uncharged

pH dependent positively charged groups (e.g. amino groups):

- at high pH: uncharged, at low pH: positively charged

Example: Silanol groups on silicon surface: Si-O-H, $pK_a \approx 5$

→ negatively charged at neutral pH

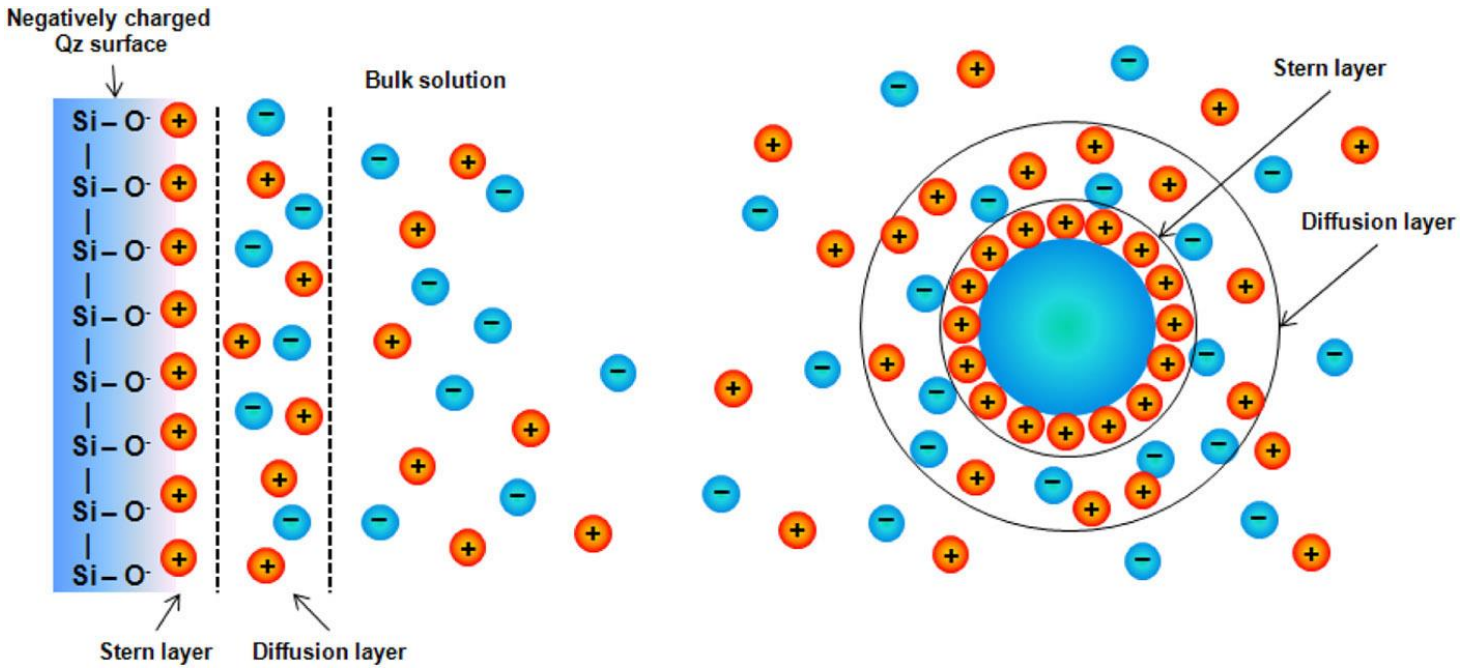
Note: Si surfaces are not always silanol group terminated

Charged molecules: proteins and peptides (+ and -), drugs (+ and -), DNA (-), RNA (-).

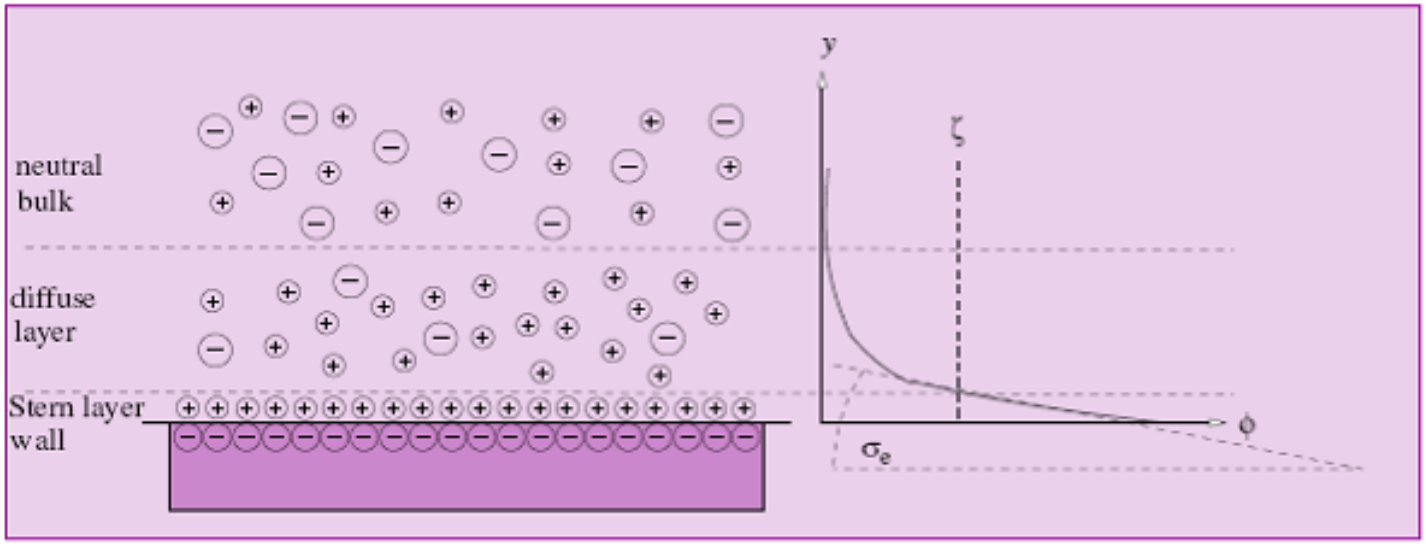
Ionic adsorption

- Analytes adsorb on surfaces with the opposite charge.
- However, there are many complications to electrostatic attraction in a dielectric, ion containing medium.
- Electric double layer (Stern model) has elements of:

1. An immobile layer of counterions (as proposed by Helmholtz)
2. A more loosely bound diffuse layer (as proposed by Gouy and Chapman)



Zeta Potential



• The electric field of a charged surface is screened by the solvent and dissolved ions

• Potential at the interface between the immobile Stern layer and the diffuse layer is called zeta-potential ζ. (This is also the plane for the no-slip condition for laminar flow.)

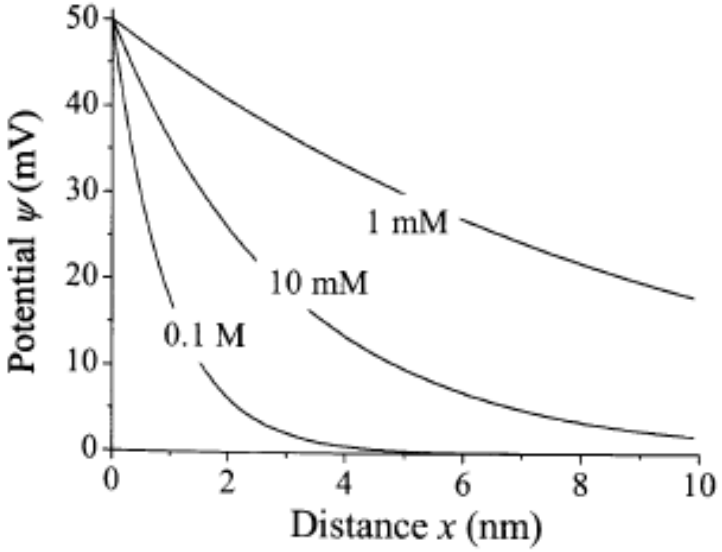
• Zeta potential can be measured and it describes how the particle interacts with its surroundings.

Screening of electrostatic potential

- Counterions screen the electric potential of a surface: $\psi \sim e^{-x/\lambda}$
- Debye length: $\lambda_D = \left(\frac{e^2}{\varepsilon \varepsilon_0 k_B T} \sum c_i Z_i^2 \right)^{-0.5}$

  c = concentration in ions/m^3

  Z = valency
- Example: human plasma, $\lambda_D \approx 0.8$ nm
- Debye length is a characteristic thickness of the double layer.
- *Nanofluidics:* Debye length and channel dimensions are close to each other.



Potential for 3 concentrations of monovalent salt

Non-adsorbing surfaces

• A non-adsorbing surface important for many applications
• Especially crucial when working with small concentrations (depletion) or with implantable devices (biofouling)

• On aqueous solvents, the least adsorbing surfaces tend to be hydrophilic and electrically neutral, in order to minimize hydrophobic and electric interaction respectively.

• Non-adsorbing surfaces are quite often hydrated polymer brushes, leading to a steric component of the adsorption resistance

• The most commonly used coating is poly-ethylene glycol (PEG)
• Other candidates: Poly(vinylalcohol) (PVA), Poly(acrylamide) (PAA)

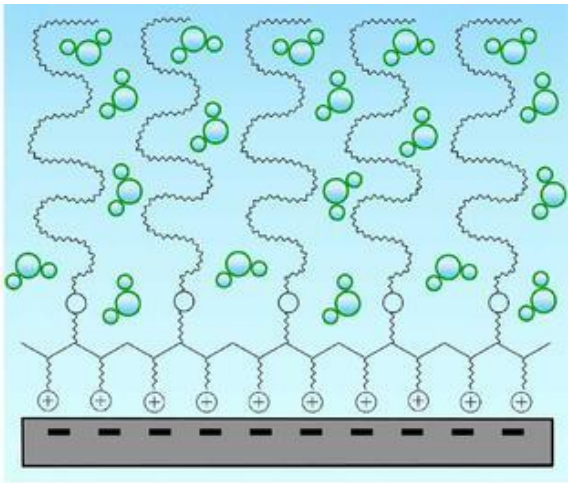
• A study by Whitesides: PEG surfaces resist protein adsorption and bacterial adhesion, but equally good non PEG alternatives were found utilizing a varied set of self-assembled monolayers.
• The key characteristics of non-adsorbing surface were found to be: i) hydrophilic (ii) hydrogen bond acceptors but (iii) *not* hydrogen bond donors.

Langmuir **2001,** *17,* 1225-1233

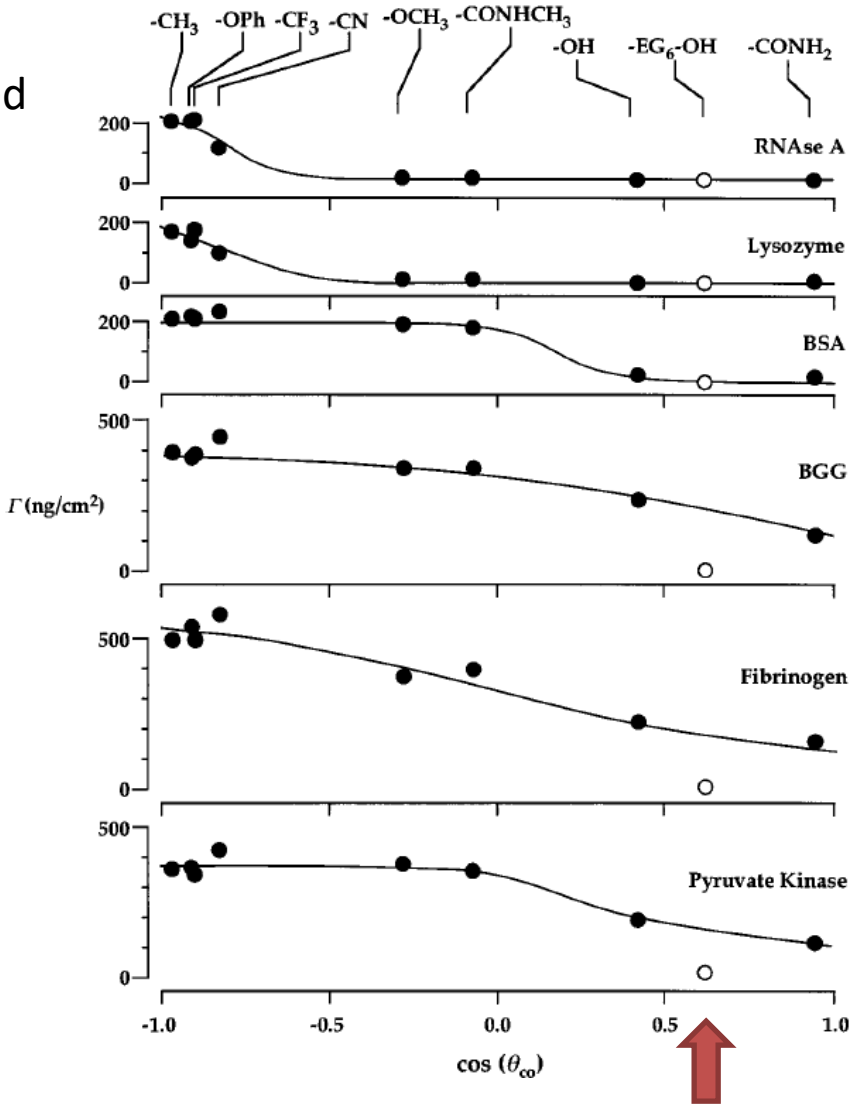
PEG

- Poly(ethylene glycol) PEG, alternatively called Poly(ethylene oxide) PEO

- Generally resistant to protein adsorption and cell adhesion. (exemptions do exist)

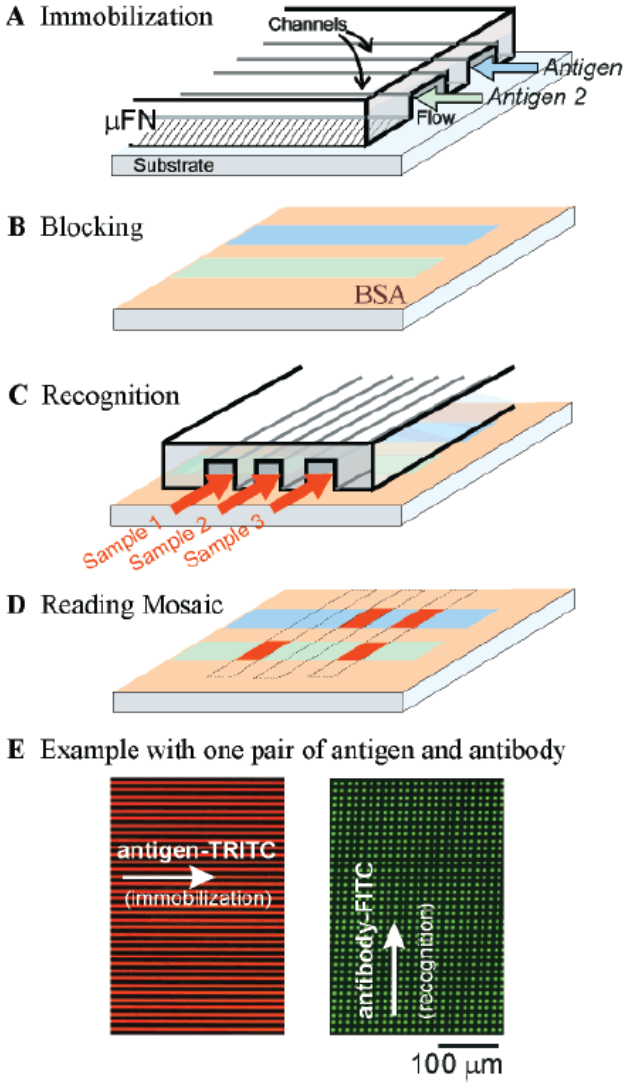


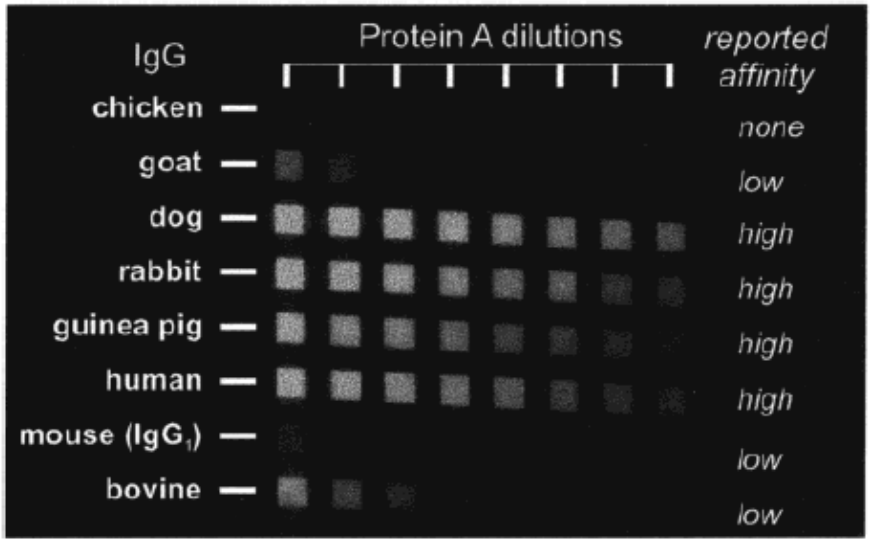
PEG brush, steric hindrance.

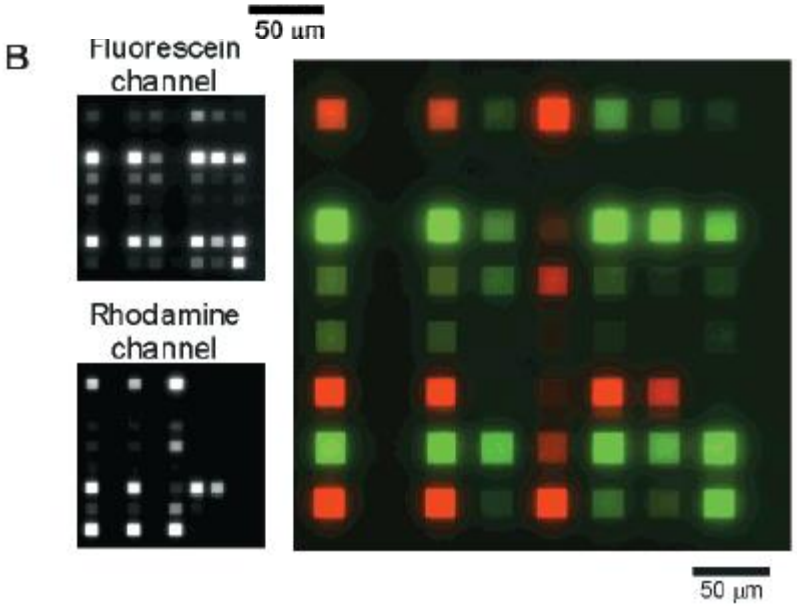


PEG

Microfluidic networks







Bernard et al. Anal. Chem. 2001

Microfluidic network mosaic immunoassay

Detachable PDMS microchannels used to pattern a line of antibodies. Antibodies attach through hydrophobic interaction, but retain their activity.

The surface is incubated with BSA to coat all parts that would nonspecifically adsorb proteins (blocking).

The channel network is turned 90 degrees and different antigens are introduced.

Fluorescent readout shows cross species recognition.

(for images, see previous slide)

These are some questions you can find answers to from this lecture:

Diffusion in microscale:
What effects are possible when diffusion is slow (for the systems timescales and dimensions)?
What effects are possible when diffusion is fast?
How about intermediate?

Adsorption:
Why is it significant in microfluidics?
Disadvantages and … advantages?
Overview of nonspecific adsorption

Next week:
Exercise session from 10-11. Design task 2 DL. Lecture 11-12.
The course now shifts from basic physics to more engineering aspects.