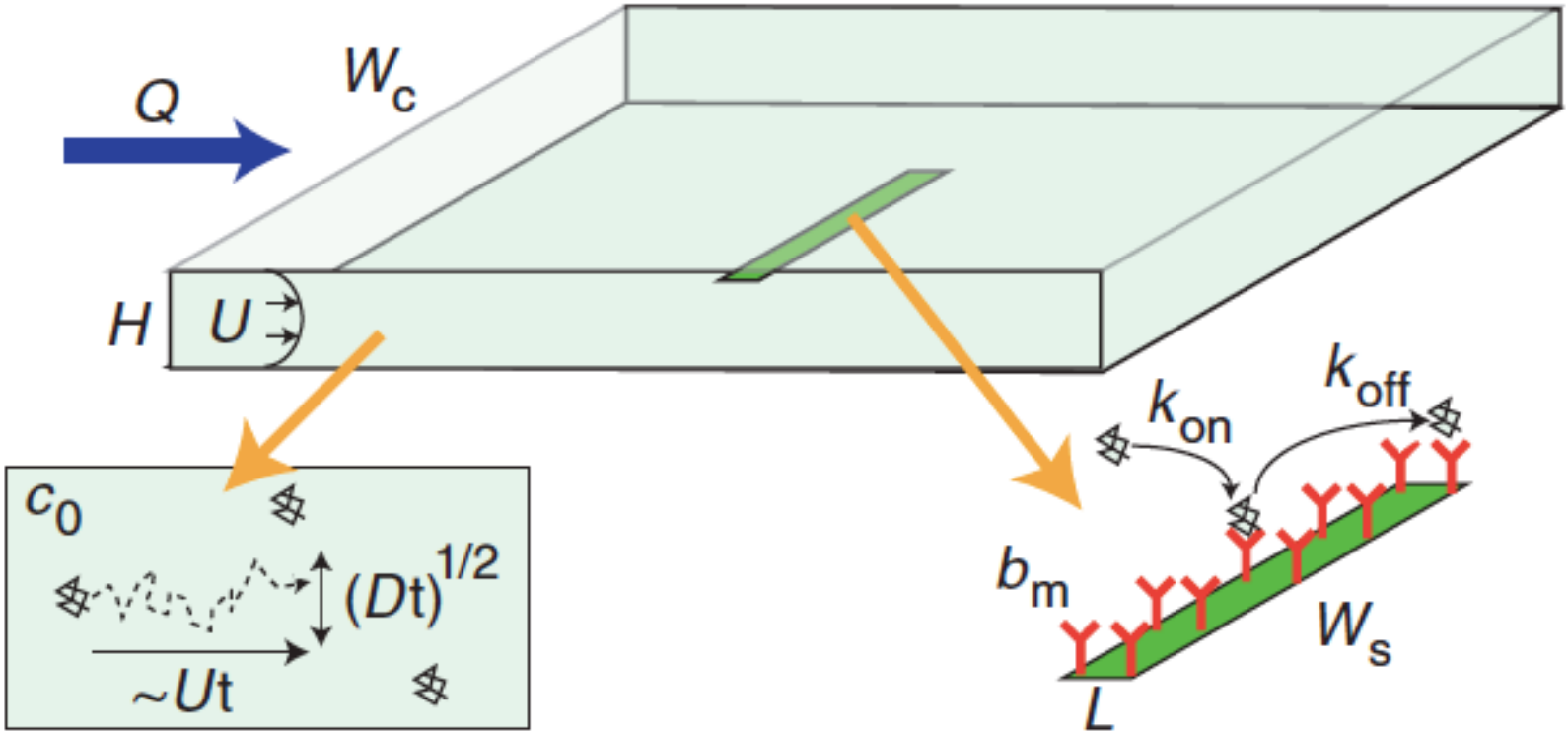
(5) SENSOR PERFORMANCE IN PRESENCE OF CONVECTION.

Heterogeneous assay. Study of the impact of Flow and mass transport phenomena on the binding kinetics

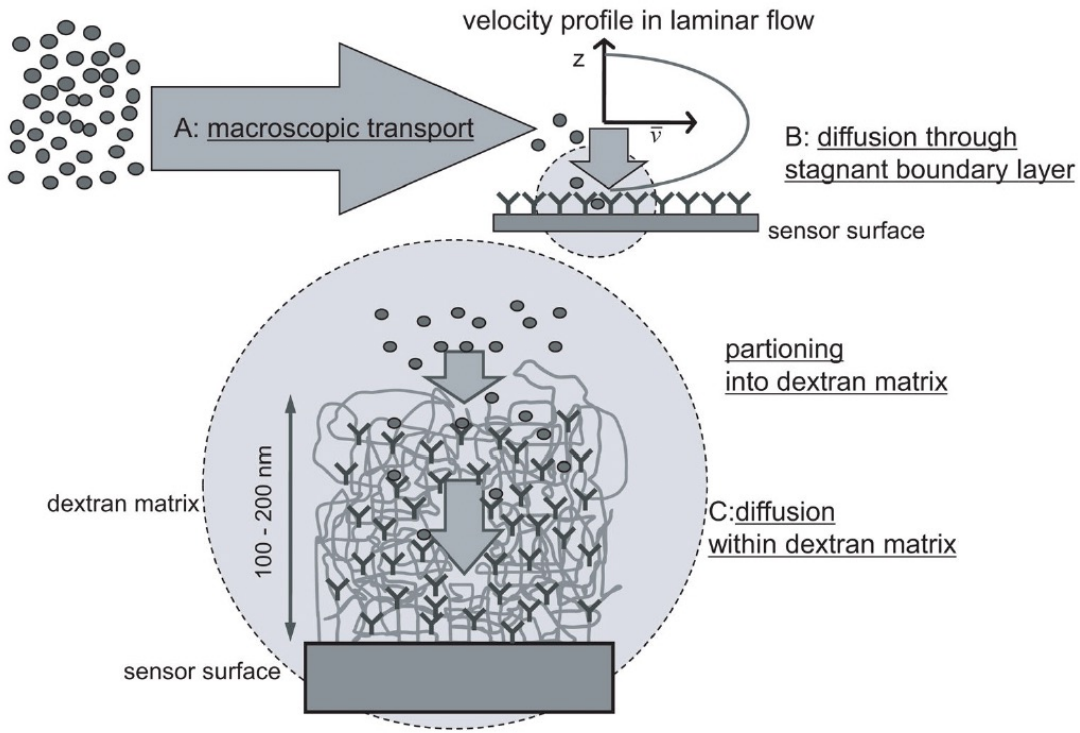


Making it stick: convection, reaction and diffusion in surface-based biosensors

Todd M Squires[1], Robert J Messinger[1] & Scott R Manalis[2]

Figure 1

Mass transport phenomena

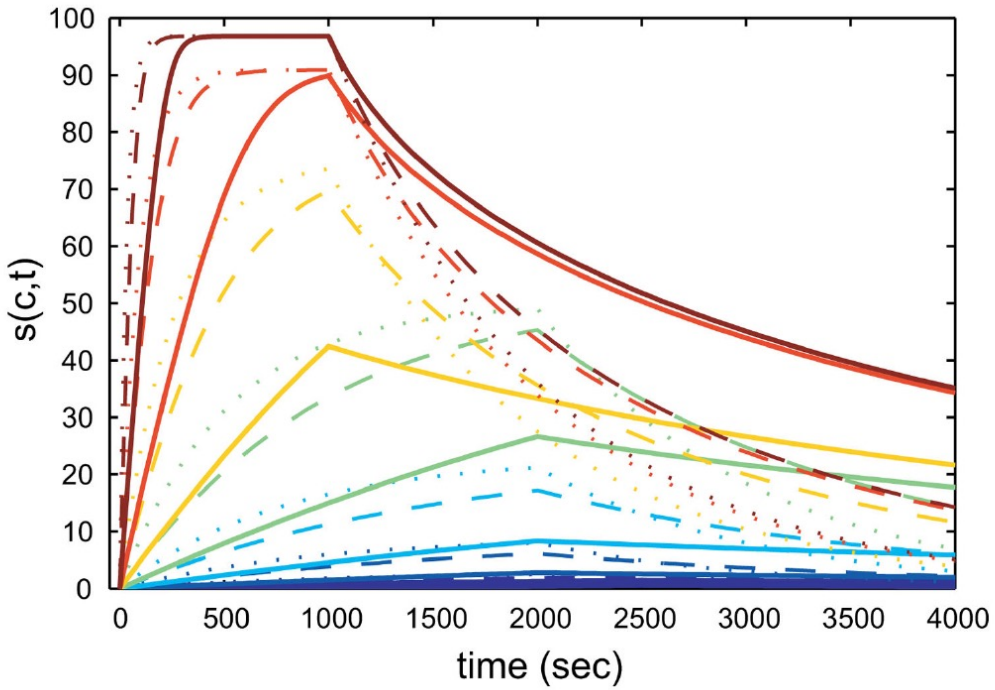


(A) transport via CONVECTION through the microfluidic system, depends on the bulk flow rate Q.

(B) Transport via DIFFUSION through the channel height and width, particularly relevant in the non-stirred boundary layer in laminar flow. Its impact depends on the bulk flow rate, flow cell geometry, and the diffusion coefficient of the analyte in the solution.

(C) The diffusion through the immobilization matrix. It depends on the size and charge of the analyte, thickness and density of the dextran matrix, the diffusion coefficient of the reactant in polymer solution and close to surfaces, the spatial distribution of surface binding sites, and on non-specific binding properties.

Effect of mass transport limtations on the surface binding kinetics



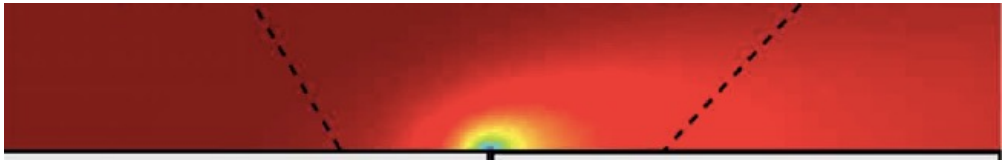
K_D = 10 nM
k_{on} = 1.0 X 10^5 $M^{-1}s^{-1}$
k_{off} = 1.0 X 10^{-3} s^{-1}

range of analyte concentrations:
0.3 nM navy, 1 nM blue,
3 nM cyan, 10 nM green,
30 nM orange, 100 nM red,
300 nM dark red

With increasing mass transport limitation (decreasing the collection rate J_D), both the association and dissociation phases exhibit slower kinetics. In the association phase, due to the local depletion zone at the surface, slower binding progress curves are expected. In the dissociation phase, when the rate of dissociation is higher than the transport rate, a non-vanishing concentration of analyte in the vicinity of the sensor surface allows rebinding to empty surface sites. The retention effect results in a slower overall dissociation from the surface and to apparent biphasic decays, in particular when the dissociation is started from close to saturation.

Depletion region in presence of strong convection

microsensor



nanosensor

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Figure 4

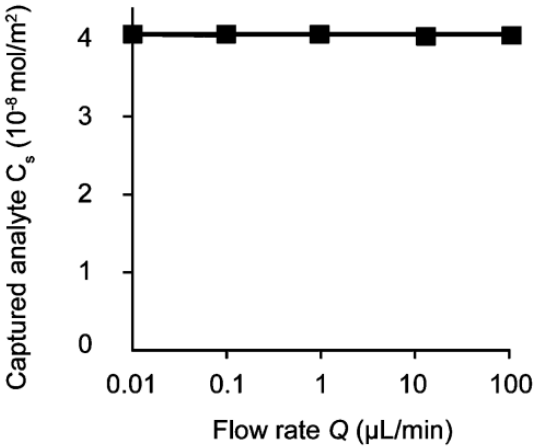
Microfluidics for bioanalytics. Convection.

- Effective mixing strategies and convection of fluid on sensors with benefit to transport of molecules to the surface
- Highly precise liquid metering and liquid handling
- Automation and portability/wearablility
- Microfluidic substrates should be inert against the expected sample and assay reagents (organic and inorganic solvents or extreme pH values)
- Sample must not be affected by the microfluidic substrate

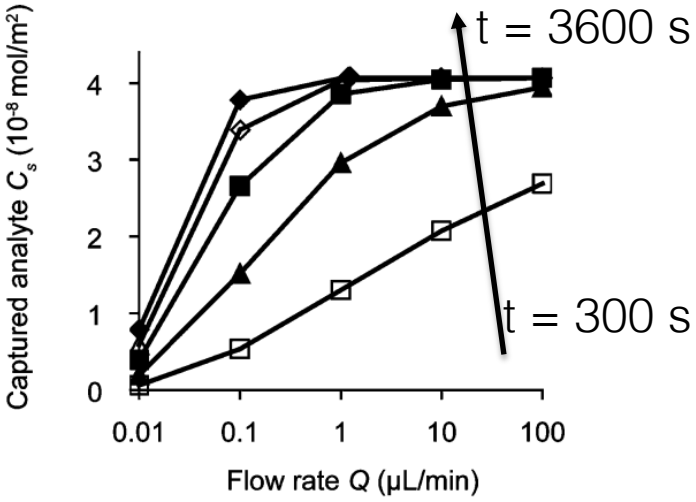
Captured analytes as a function of flow rate

C_{so} density of biding sites = 7 X 10^{-8} mol m^{-2}
C_0 analytes bulk concentration = 10 nM

k_{on} = 240 m^3 mol^{-1} s^{-1} ,
k_{off} = 3.5 10^{-3} s^{-1}
L= sensor length in the direction of the flow



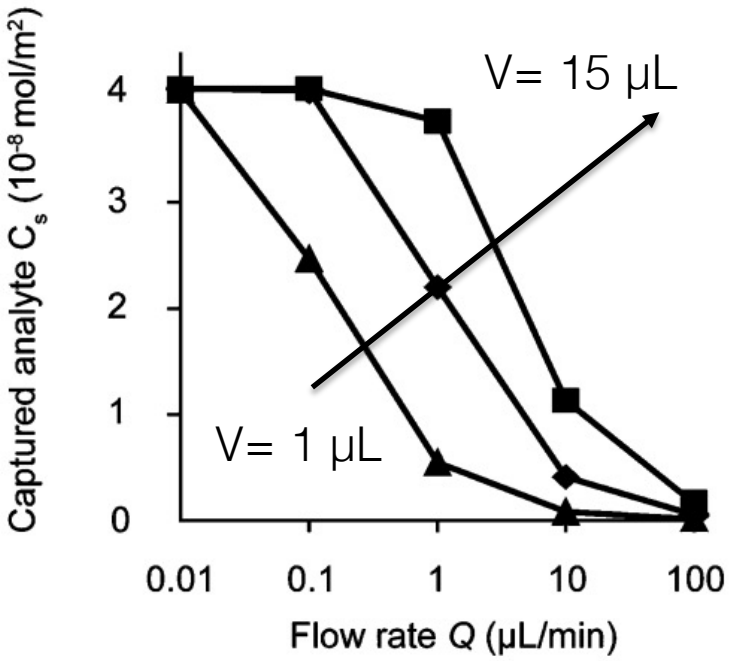
Unlimited sample and unlimited time.
L = 100 µm, t = 60 000 s (to approximate unlimited time),



Unlimited supply of sample within a fixed time interval and long capture zone.
L = 1500 µm

Factors limiting the maximum value of flow rate

- limited available volume (relevant for instance for Lateral Flow Assays)
- maximum pressure tolerable



Limited sample volume and unlimited time for the assay (t = up to 30 000 s, depending on the volume)

C_{so} density of biding sites = 7 X 10^{-8} mol m^{-2}
C_0 analytes bulk concentration = 10 nM

k_{on} = 240 m^3 mol^{-1} s^{-1} ,
k_{off} = 3.5 10^{-3} s^{-1}

Slow flow: increased residence time

Hydrodynamic pressure. Device tolerance to pressure determines a limitation to the maximum flow rate.

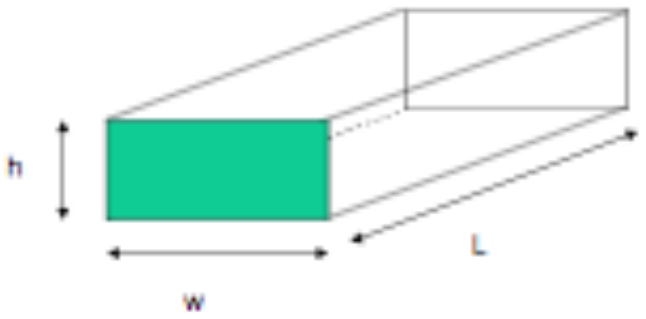
Poiseuille law $$Q = \frac{\Delta P}{R}$$

Q: flow rate (may impact the time to reach equilibrium on the surface)
ΔP: pressure difference in the channel (should be compatible with the channel material and assembly. In general, it should be kept **below** 100 psi)
R: Flow Resistance of the channel (depends only on its geometry and on the viscosity of the fluid η)

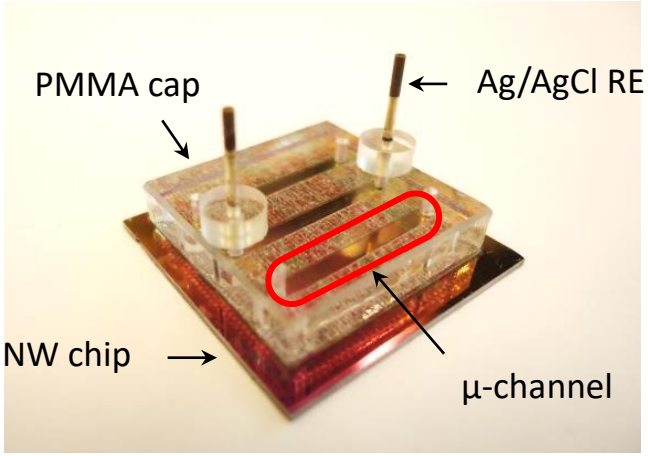
Calculate the pressure drop

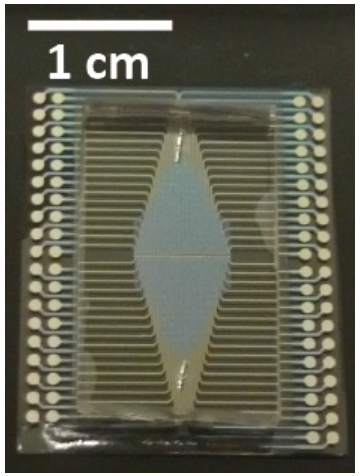
Q = 1µl/sec
w=100µm
h=10µm
L=1 mm
η = 10^{-3} Pa s

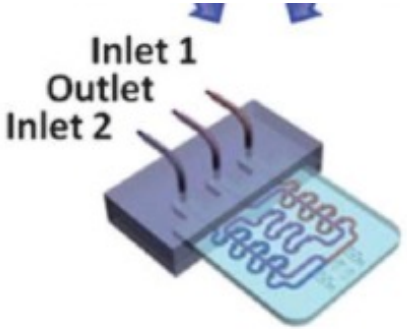
$$R = \frac{12\eta L}{(1 - 0.63\frac{h}{w})h^3 w}$$



Example of microfluidic channels and modules







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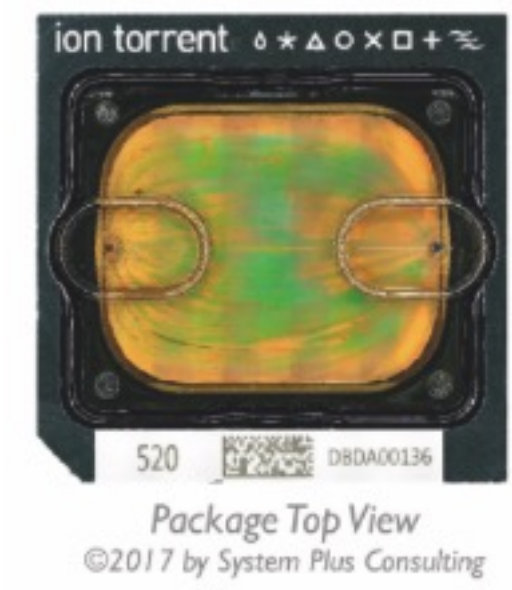

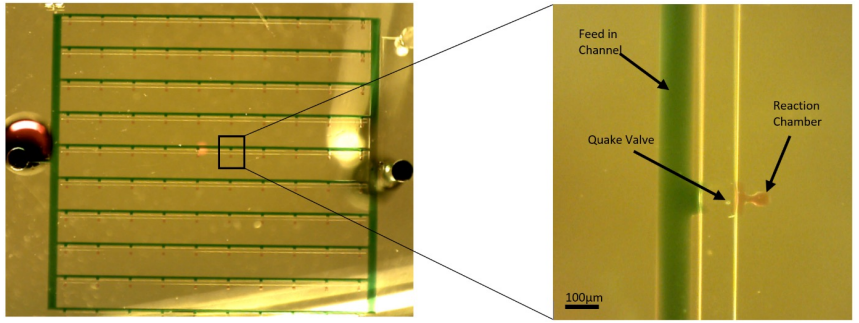

Issues related to microfluidic modules

- Possible adsorption onto the surfaces (charged molecules such as nucleotides and proteins)
- The catalytic activity of the enzymatic proteins can be reduced by interaction with the substrates
- Possible solution: block the surface with a suitable molecule added in excess (e.g.: BSA)

- The activity of proteins can be affected during processes such as thermal bonding or UV curing steps.
- Possible solution: approaches to post-functionalize the chip or avoid bonding (with the drawback that only low pressure in the channel can be sustained)
- The long-term stability of pre-stored dry reagents might be affected by microfluidic bonding.