Molecular transport during reactions with surface bound reagents

Jean-Francois Masson

Department of Chemistry, University of Montreal

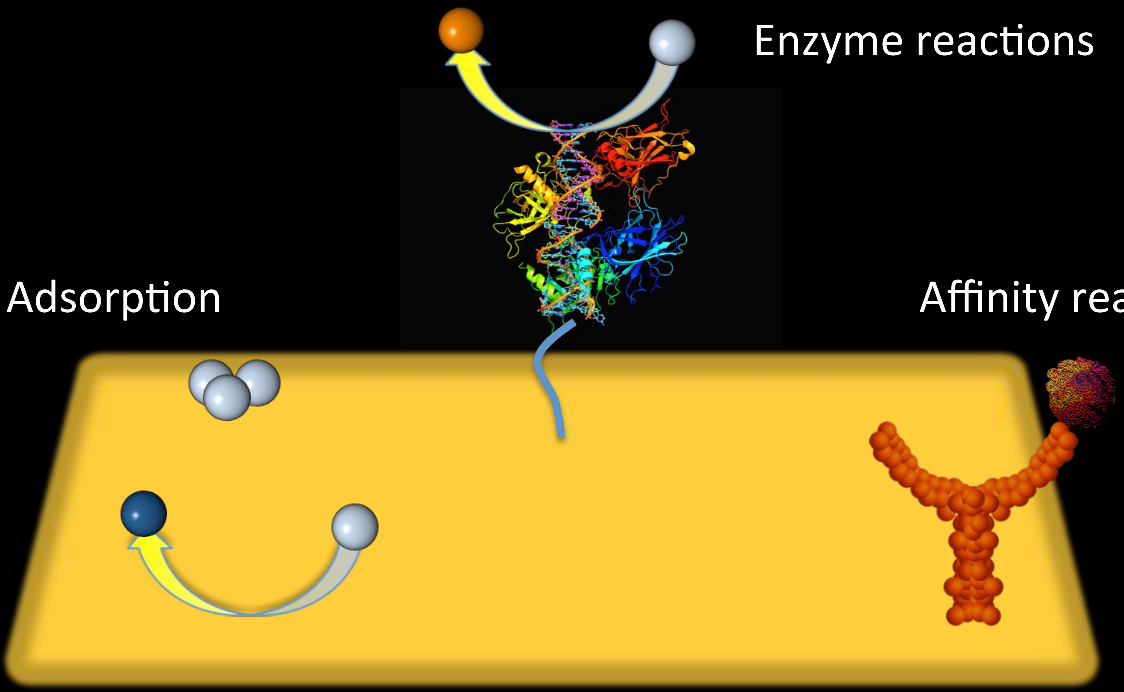
CREATE in Flow Chemistry

Université de Montréal

Topics covered

- Adsorption to surfaces (purification, catalysis)
- Langmuir isotherm
- Enzymatic reactions
- Affinity reactions
- Sensors
- Molecular transport in sensing

Surface reactions



Enzyme reactions

Adsorption

Affinity reactions

Catalysis

Impacts of the adsorption of molecules

- Specific
  - Preconcentrates a molecule on a surface (sensing)
  - Extracts molecules from a complex mixture (sensing)
  - Locates the molecule on a highly reactive surface (catalysis)

- Nonspecific
  - Reduces the concentration of molecules in solution (flow reactions)
  - Contaminates the reaction vessel or the sensing surface
  - Clogging if large deposits are created

Université de Montréal

Why molecules adsorb to surfaces

- Specific interactions
  - Molecular recognition

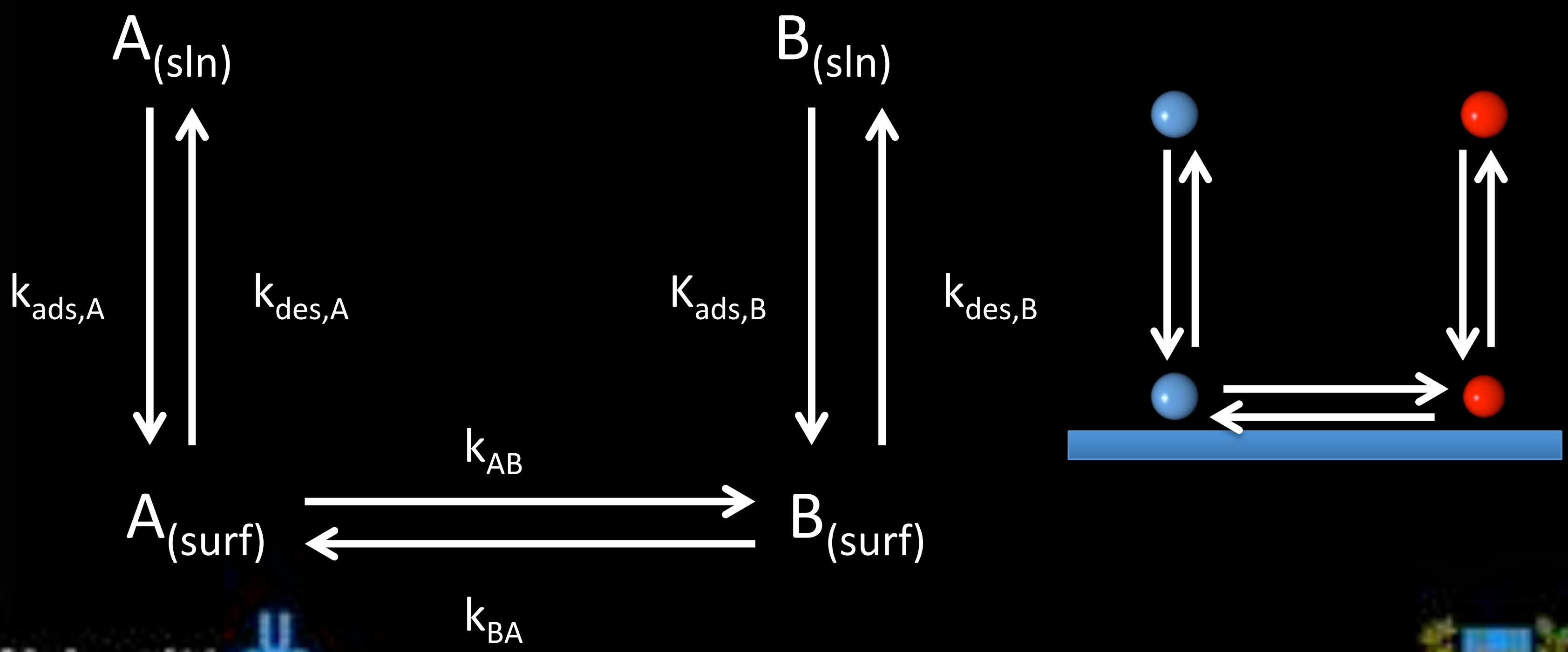
- Nonspecific
  - Electrostatic interactions
  - Dipolar interactions
  - Van der Waals forces

- Chemisorption = formation of a new chemical bond (catalysis)
- Physisorption = due to the contact of the molecule with a surface

Université de Montréal

The lifecycle of a surface reaction

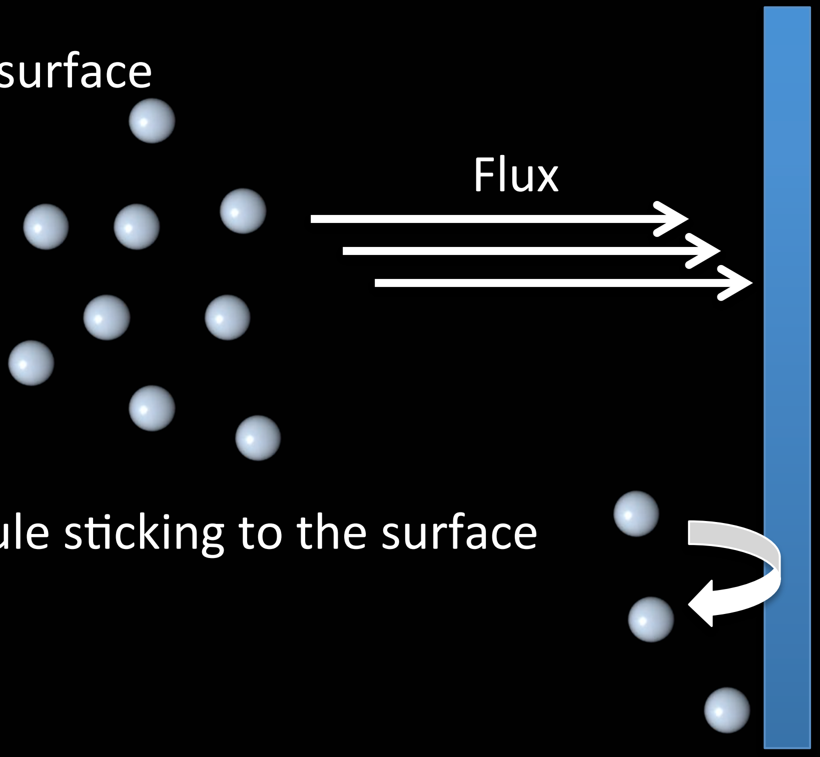
- Molecules can adsorb and desorb on surfaces. During the adsorption, a chemical reaction can occur transforming molecule A in molecule B



$$\begin{tikzcd}
A_{(sln)} \arrow[d, shift right, "k_{ads,A}"'] & B_{(sln)} \arrow[d, shift right, "K_{ads,B}"'] \\
A_{(surf)} \arrow[u, shift right, "k_{des,A}"'] \arrow[r, shift left, "k_{AB}"] & B_{(surf)} \arrow[u, shift right, "k_{des,B}"'] \arrow[l, shift left, "k_{BA}"]
\end{tikzcd}$$

Adsorption kinetics

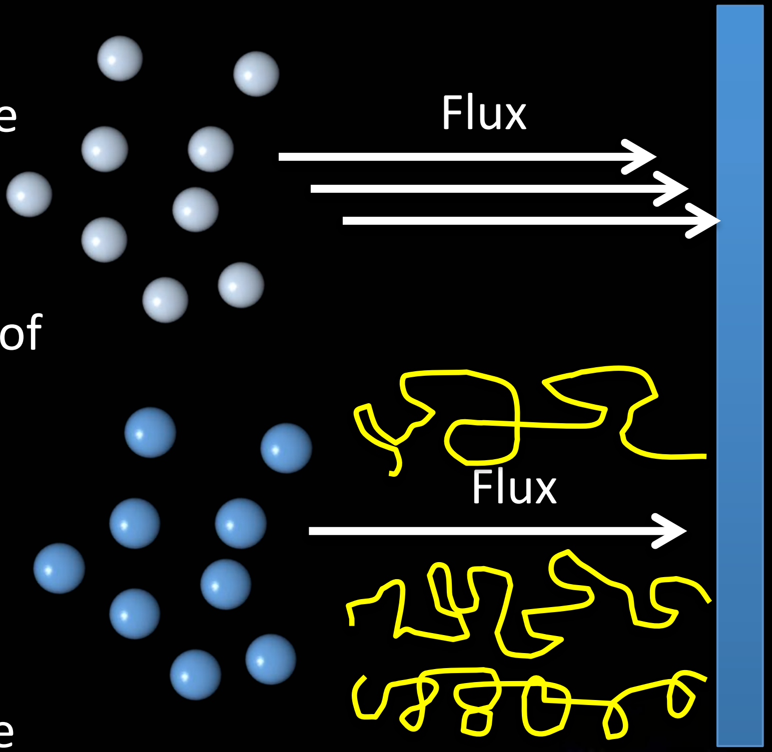
- Rate of adsorption
  - Unrestricted number of molecules that can bind to the surface
  - $R = k_{ads,A}\, C_A$
- Flux of molecules to the surface
  - Diffusion
  - Concentration



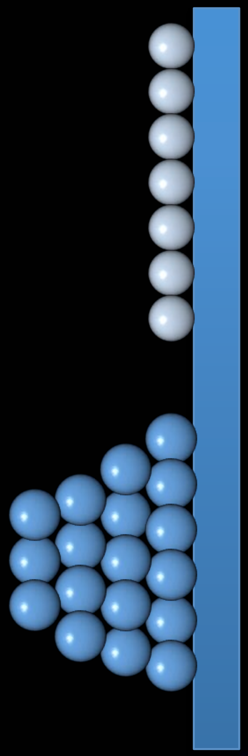
- Probability of the molecule sticking to the surface

Diffusion of molecules to surfaces

- Unimpeded diffusion
  - The diffusion constant of the molecule remains identical up to the contact with the surface
- Hindered diffusion
  - The presence of a chemical coating of finite thickness (larger than a monolayer) on a surface may limit the diffusion of molecules to a surface
  - Typically, this slows down the diffusion of molecules to the surface



Formation of monolayer or multilayers

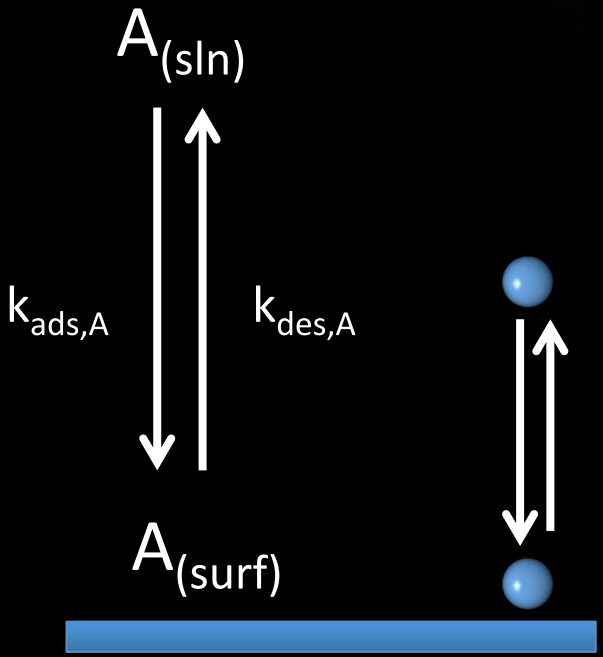


- Monolayer
  - Ordered (2D crystal) layer of a thickness of a single molecule
  - No adsorbate to adsorbate interactions
- Multilayers
  - Aggregation of multiple layers of the adsorbate
  - Significant adsorbate-adsorbate interactions
- The number of reaction sites is limited by the surface area

Université de Montréal

Langmuir isotherm

- The Langmuir isotherm describes the adsorption processes occuring on a surface
- Assumptions
    - Formation of a monolayer
        - First order reaction
        - 1 molecule per reaction site
    - Instantaneous reaction on the surface
    - No interaction between molecules
    - All sites are equivalent
    - Unhindered diffusion to the surface



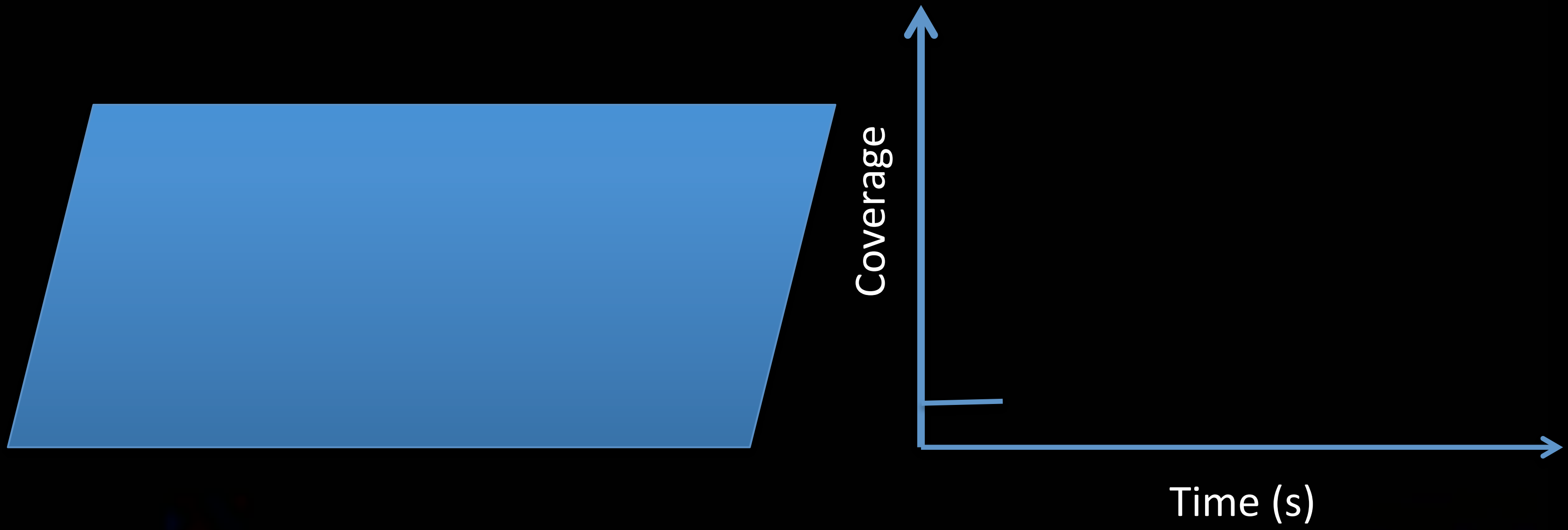
$A_{(sln)}$

$k_{ads,A}$ $k_{des,A}$

$A_{(surf)}$

Kinetics of the Langmuir isotherm

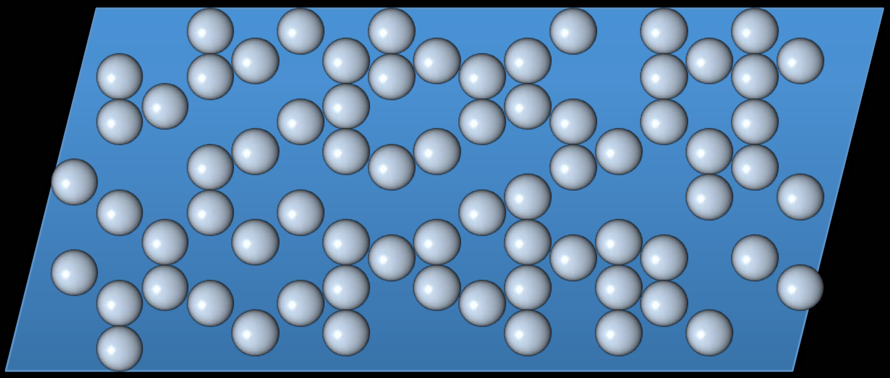
- Continuous exposure of the surface to a molecule will lead to an increase in concentration if the rate of adsorption exceeds the rate of desorption

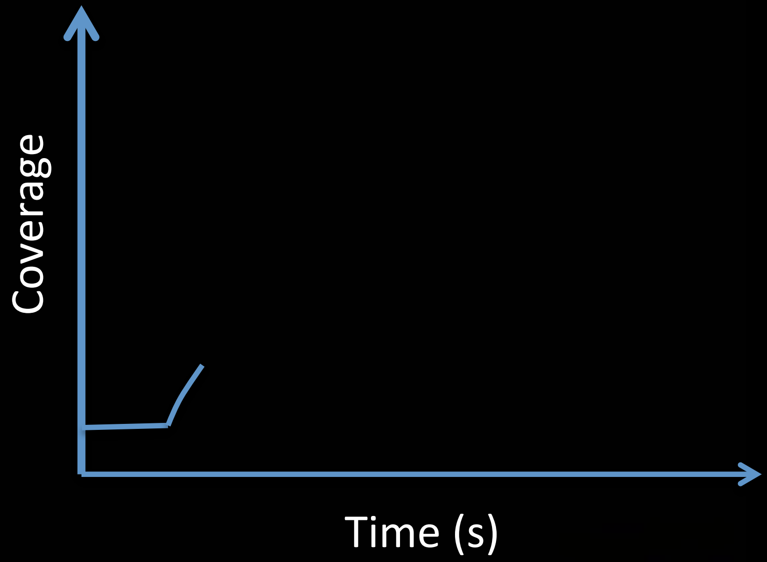


Université de Montréal

Kinetics of the Langmuir isotherm

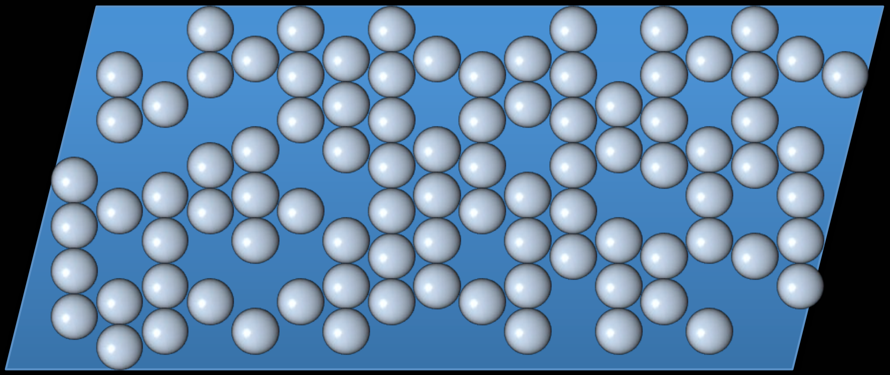
- Continuous exposure of the surface to a molecule will lead to an increase in concentration if the rate of adsorption exceeds the rate of desorption

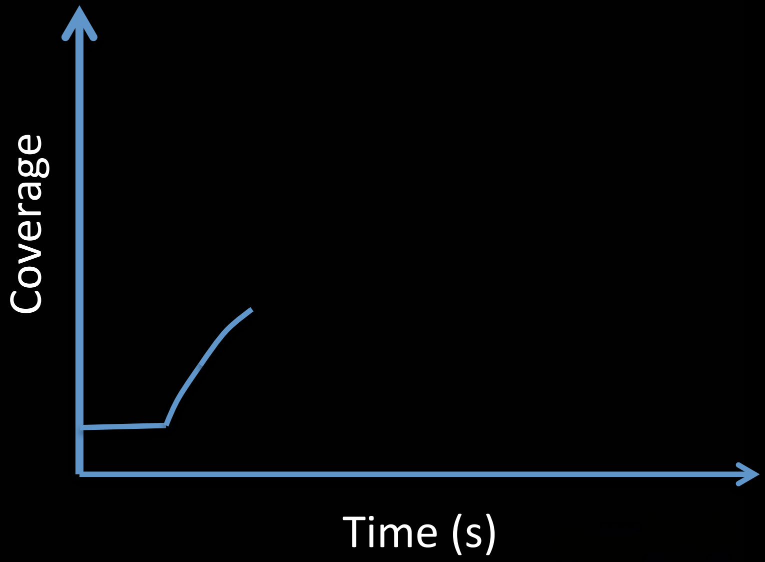




Kinetics of the Langmuir isotherm

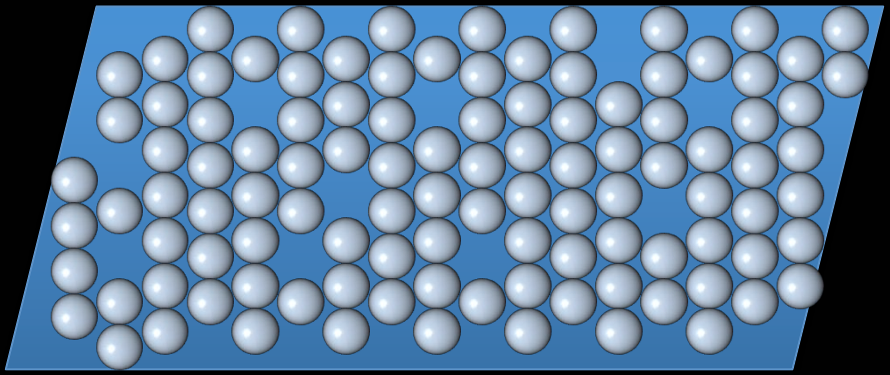
- Continuous exposure of the surface to a molecule will lead to an increase in concentration if the rate of adsorption exceeds the rate of desorption

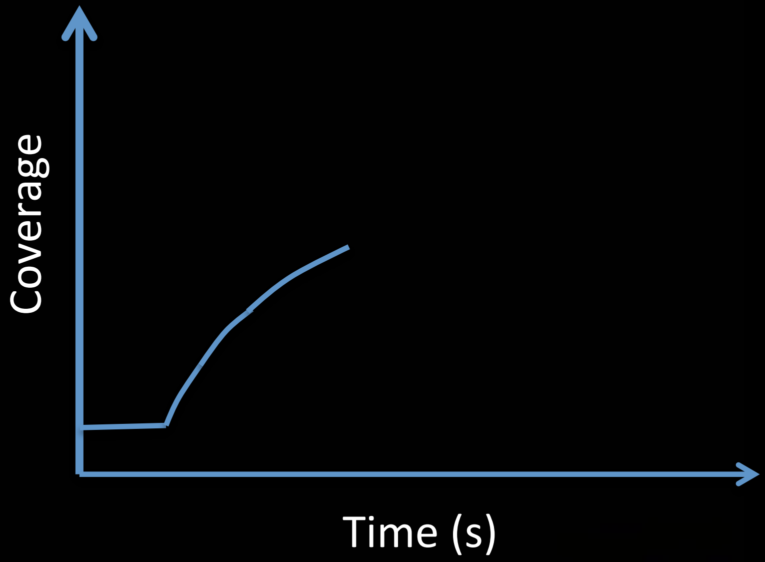




Kinetics of the Langmuir isotherm

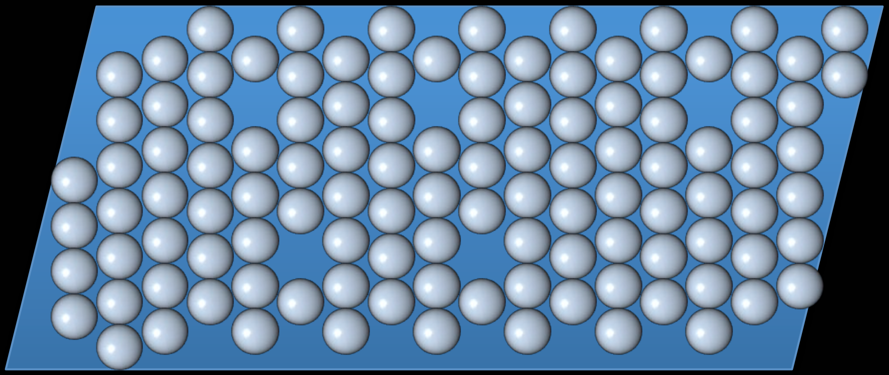
- Continuous exposure of the surface to a molecule will lead to an increase in concentration if the rate of adsorption exceeds the rate of desorption

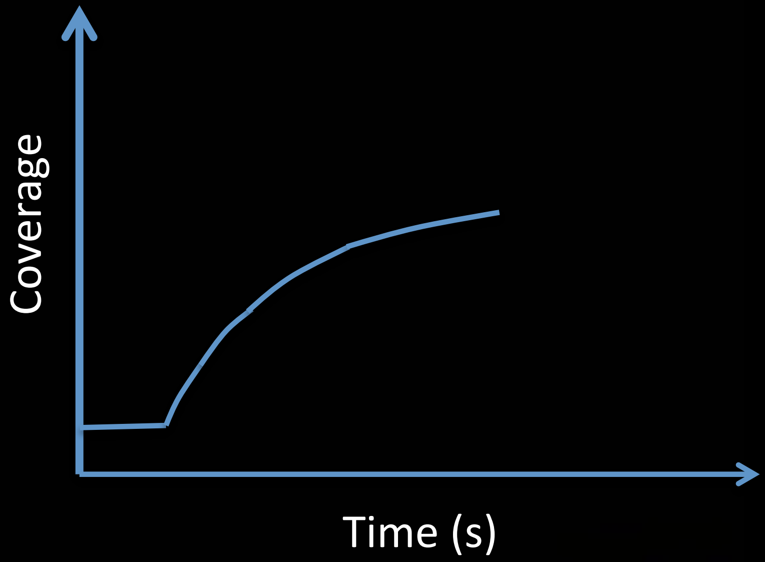




Kinetics of the Langmuir isotherm

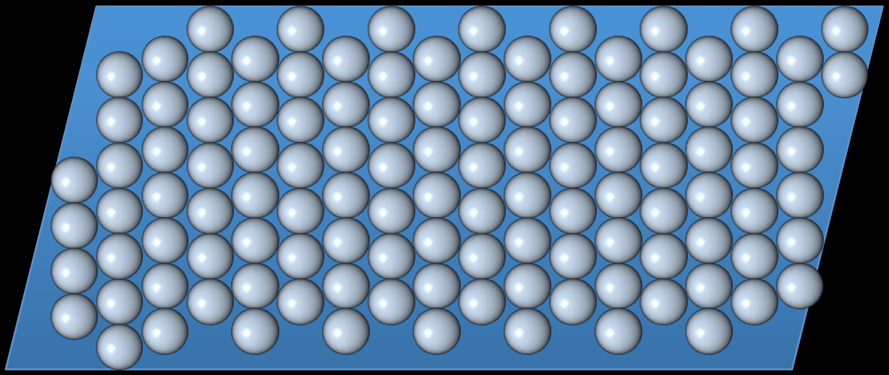
- Continuous exposure of the surface to a molecule will lead to an increase in concentration if the rate of adsorption exceeds the rate of desorption

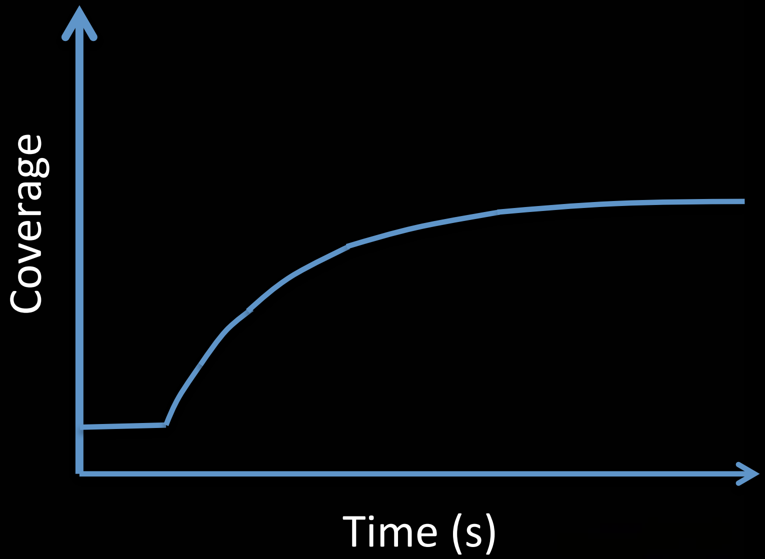




Kinetics of the Langmuir isotherm

- Continuous exposure of the surface to a molecule will lead to an increase in concentration if the rate of adsorption exceeds the rate of desorption



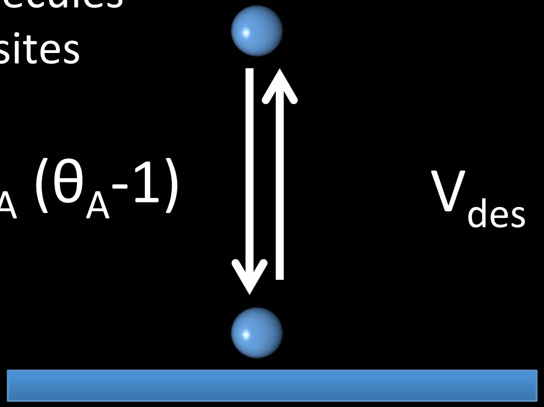


Equations: Langmuir isotherm

- The rates depend on the concentration of molecules in solution and on the surface, in addition to the constants

V_{ads} : adsorption rate
V_{des} : desorption rate
k_{ads} : adsorption constant
k_{des} : desorption constant
C_A : concentration of molecules
θ_A : fraction of occupied sites

$$\theta_A = \frac{\text{sites occupied}}{\text{total \# sites}}$$

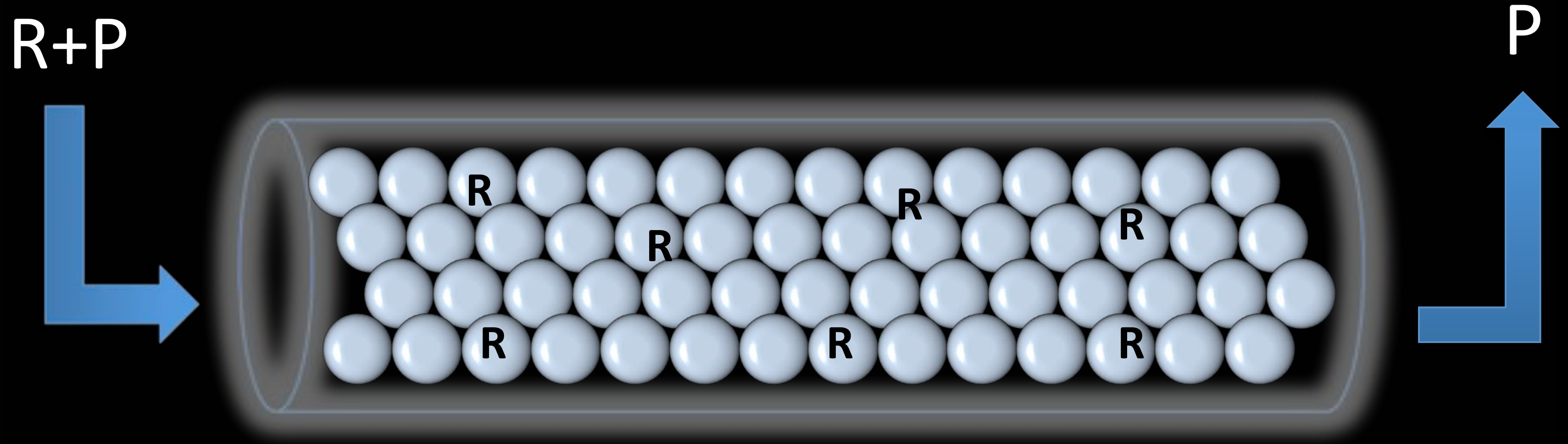


$$V_{ads} = k_{ads} C_A (\theta_A\text{-}1)$$

$$V_{des} = k_{des} \theta_A$$

Application: Online purification

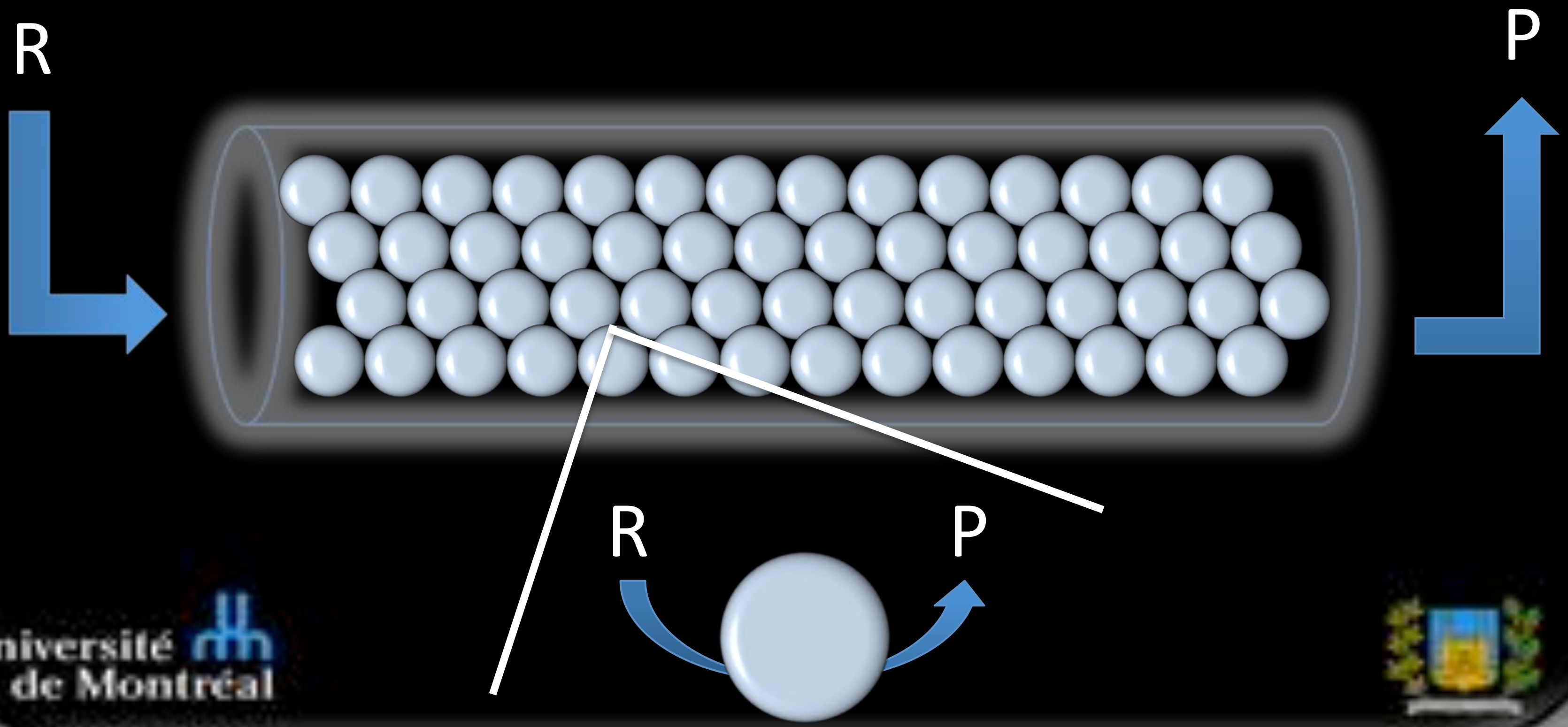
- A packed column with an adsorbant (stationary phase) can be placed online to remove reactants and flow pure product for a second reaction



R = reactants
P = products

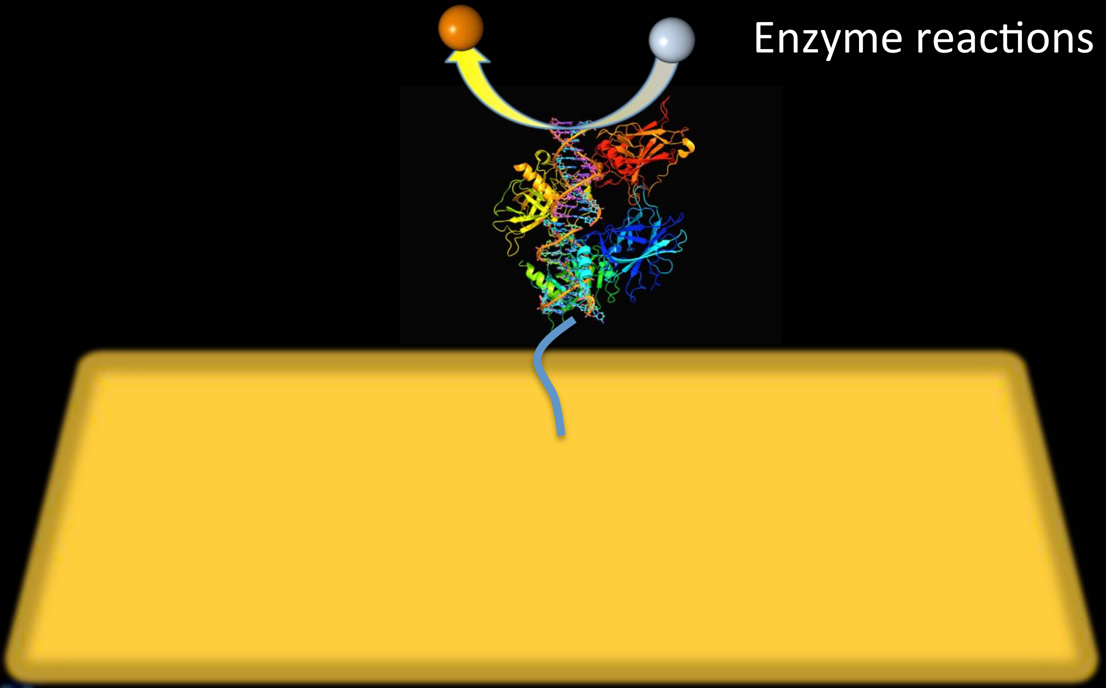
Application: Catalysis

- A packed column with a catalyst can serve to convert reagents to products. The reagent must adsorb rapidly on the catalyst, while the product must desorb rapidly



Enzyme reactions

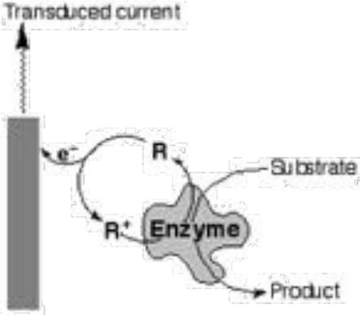
- Enzyme reactions depends on the number of molecules available on the surface and on enzyme kinetics



Enzyme reactions

Glucometer

- Glucose levels are on the order of mg/mL
  - A drop of blood contains glucose
  - Enzymatic conversion of glucose



  - Potentiometric reading of the glucose level

http://people.clarkson.edu/~ekatz/electrochemical_bio_tutorials.htm

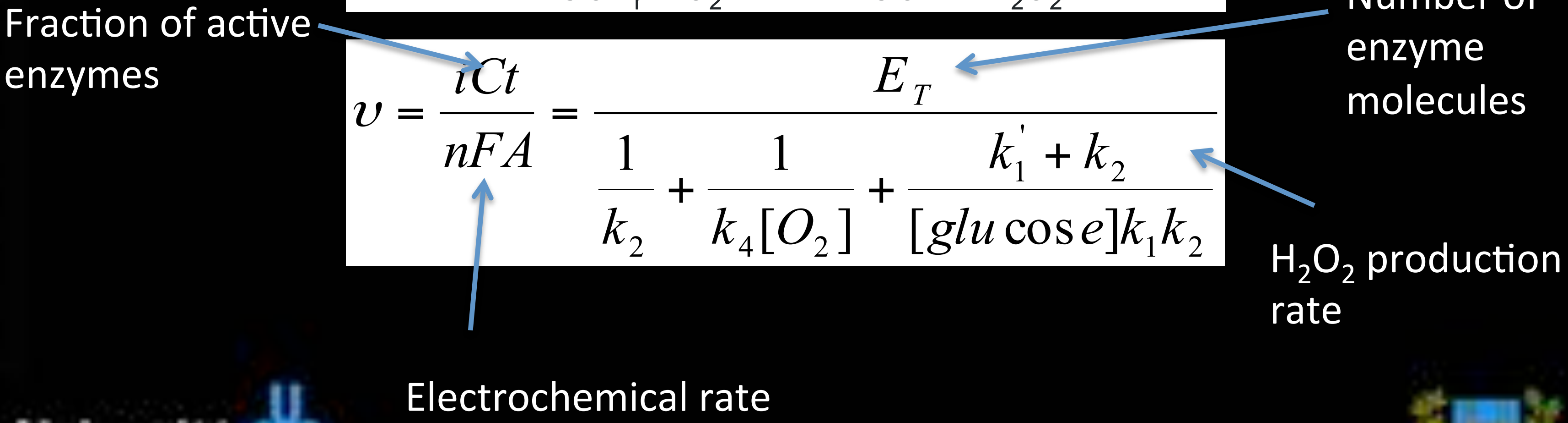
Enzyme reactions: glucose oxidase

- The response of the glucometer depends on the kinetic constants of glucose oxidase

Enzymatic reactions

$$\mathrm{GOD} + \mathrm{glucose} \underset{k_{-11}}{\overset{k_{11}}{\rightleftarrows}} \mathrm{GOD\text{-}glucose} \xrightarrow{k_{12}} \mathrm{GOD_r} + \mathrm{P}$$

$$\mathrm{GOD_r} + \mathrm{O_2} \xrightarrow{k_{14}} \mathrm{GOD} + \mathrm{H_2O_2}$$



$$\upsilon = \frac{iCt}{nFA} = \frac{E_T}{\frac{1}{k_2} + \frac{1}{k_4[O_2]} + \frac{k_1' + k_2}{[glucose]k_1 k_2}}$$

Fraction of active enzymes

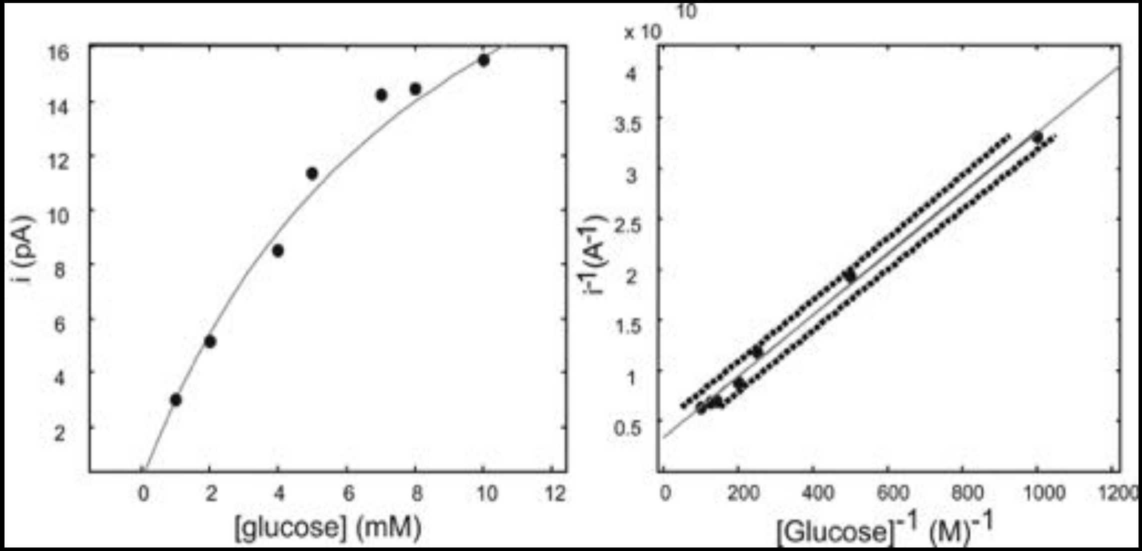
Number of enzyme molecules

H_2O_2 production rate

Electrochemical rate

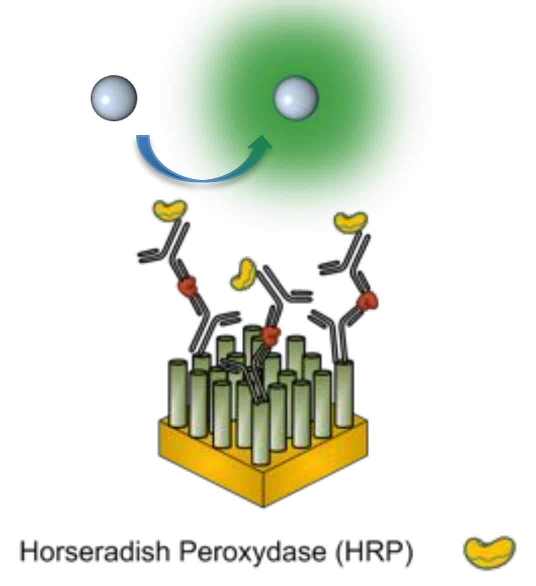
Calibration of the glucose meter

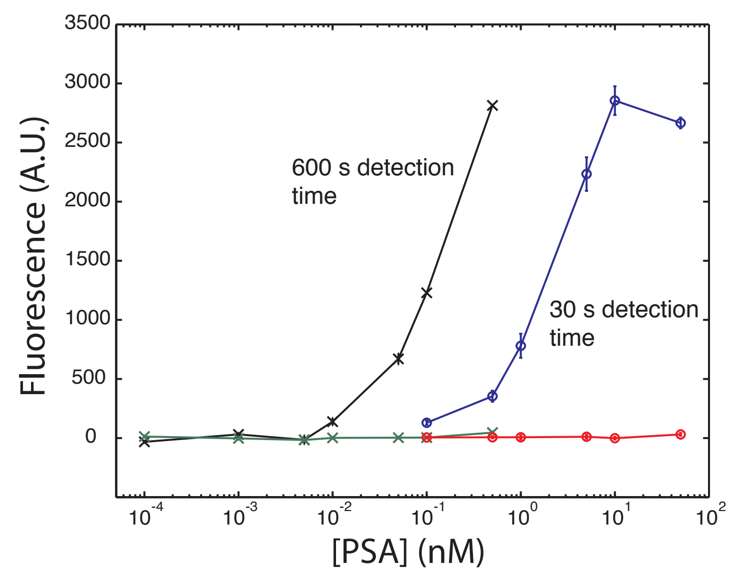
- The predicted current (solid line) correlates well with the experimental data (points)
- Can be used to predict the production rate of a reaction



Enzyme reaction to reveal the response of a sensor

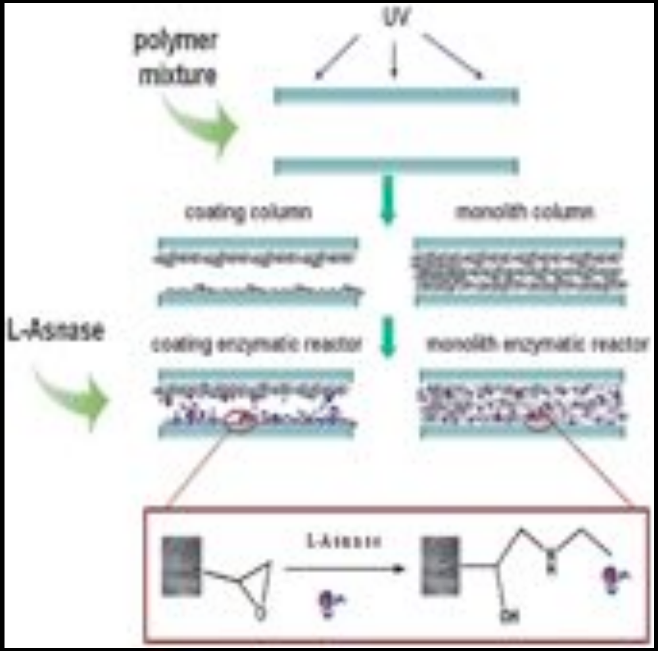
- Anti-PSA labelled with HRP secondary antibody enhanced the SPR response (mass increase) and was used to turnover the enzymatic reaction of AmpliFlu^{TM} Red

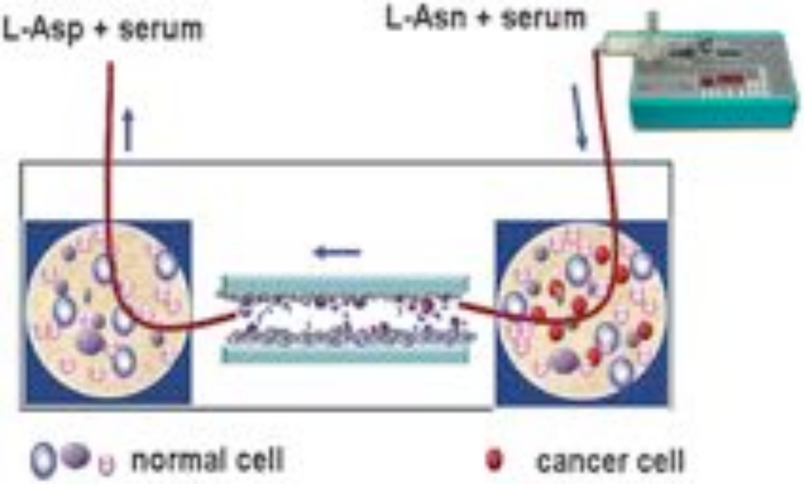




Enzymatic reactor

- Coated capillary or packed beads modified with enzymes can convert a reagent into a product



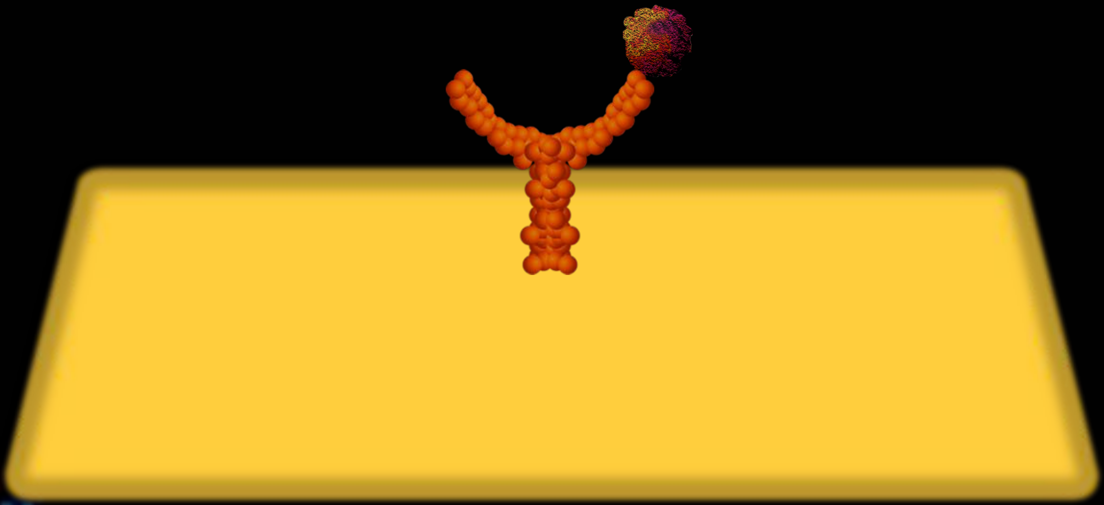


Analyst 2011, 136, 2077-2083

Affinity reactions

- Affinity reactions depends on the number of molecules available on the surface and on the association and dossiciation contants of the reaction
- Can be used to screen for the affinity of a product for the targeted receptor

Affinity reactions



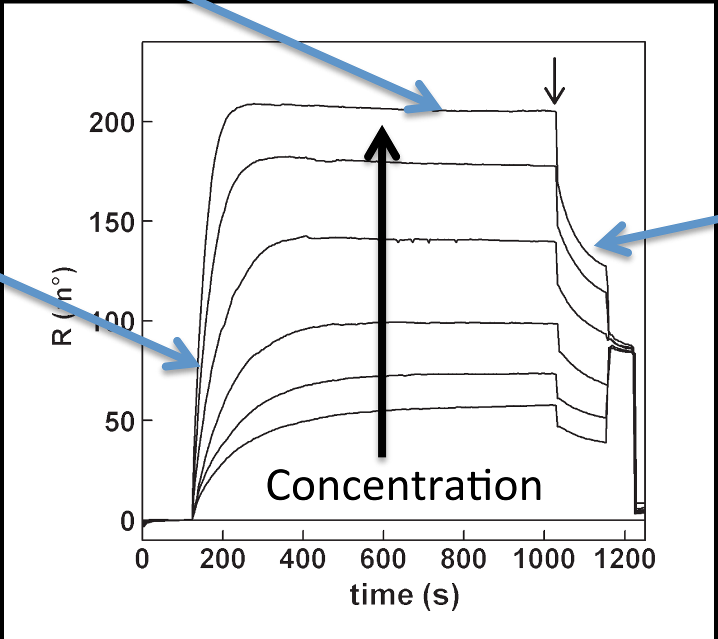
Université de Montréal

Association and dissociation

- A time-course of an experiment will undergo three phases

Equilibrium between association and dissociation

Mainly association

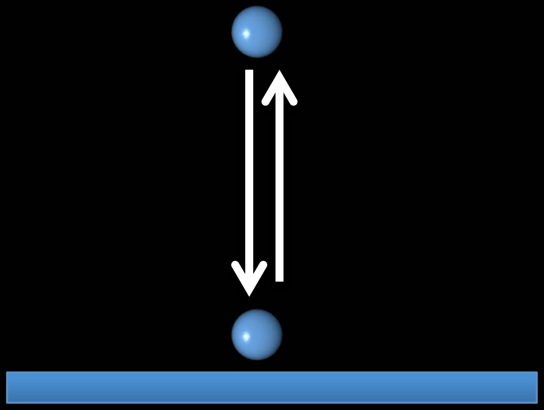


Concentration

Exclusively dissociation

Equilibrium conditions

- At equilibrium, the adsorption rate equals the desorption rates



$$V_{ads} = V_{des}$$

$$k_{ads}\ C_A\ (\theta_A\text{-}1) = k_{des}\ \theta_A$$

$$\theta_A = KC_A\ /\ (1 + KC_A)$$

$$K = k_{ads}\ /\ k_{des}$$

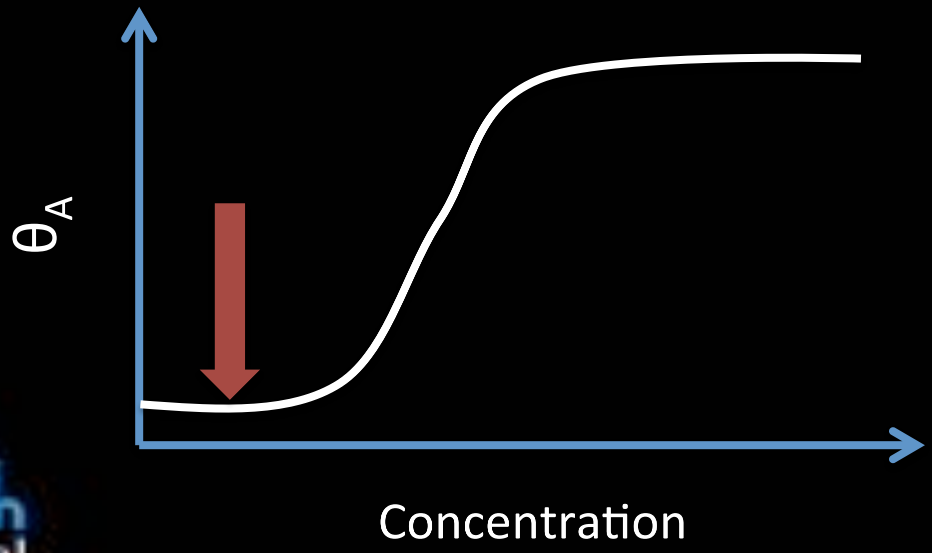
Thermodynamic constant

Case 1: Low concentration

- The term KC_A becomes negligible in the term $1 + KC_A$

$$\theta_A = KC_A$$

- The surface concentration is directly related to the solution concentration

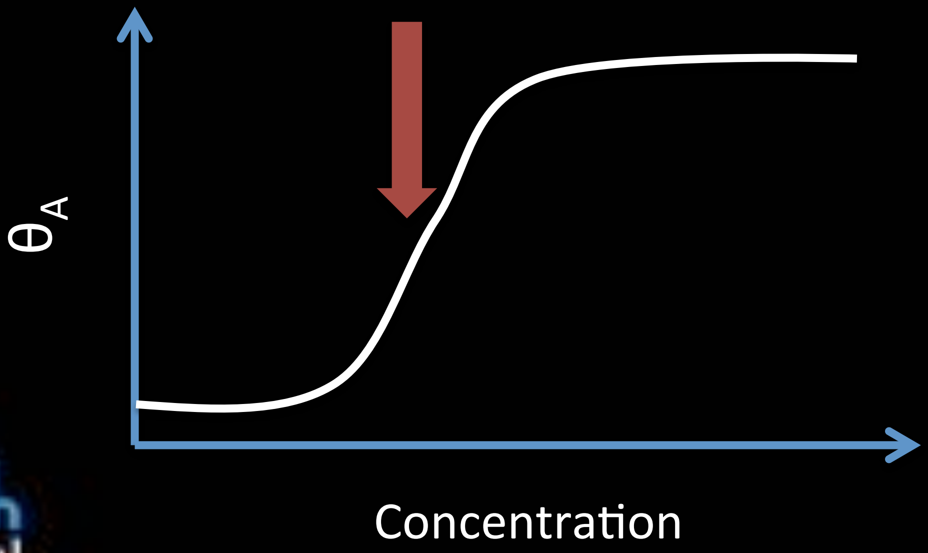


Case 2: Intermediate concentration

- The term KC_A is about equal to 1

$$\theta_A = KC_A / (1 + KC_A)$$

- Region of high loading of the surface, response moderately linear

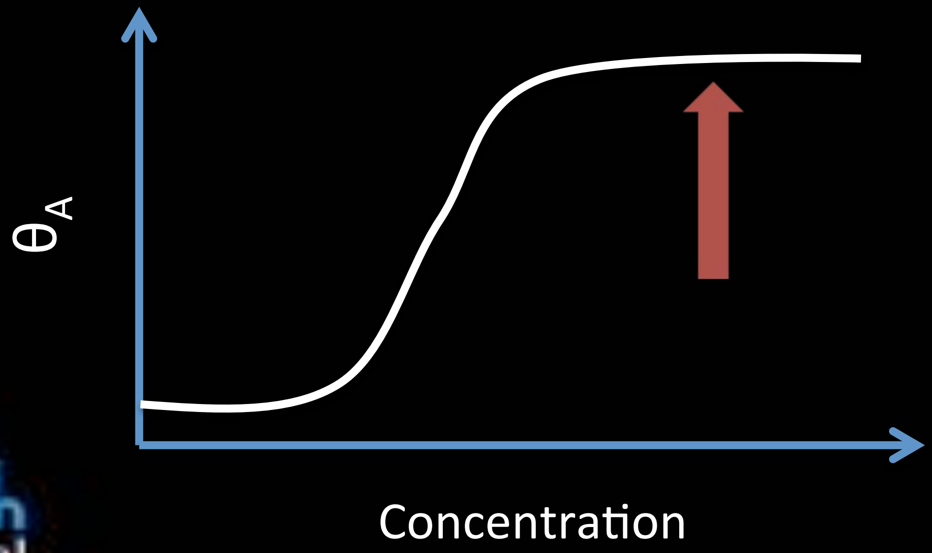


Case 3: High concentration

- The term KC_A far exceeds 1 in the term $1 + KC_A$, so $1 + KC_A = KC_A$

$$\theta_A = 1$$

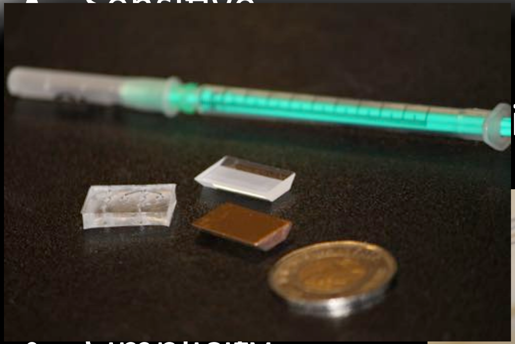
- The surface is saturated, the response reaches a plateau



Portable 4-channel SPR (P4SPR) instrument

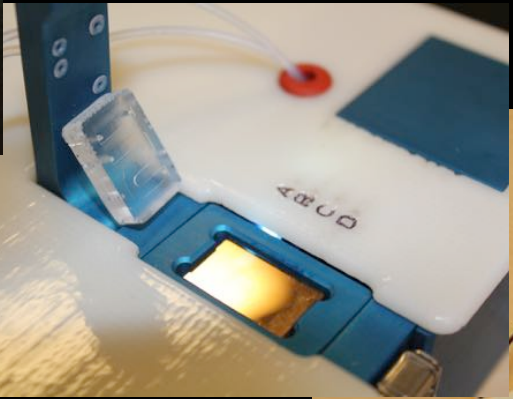
Affinité Biosensing

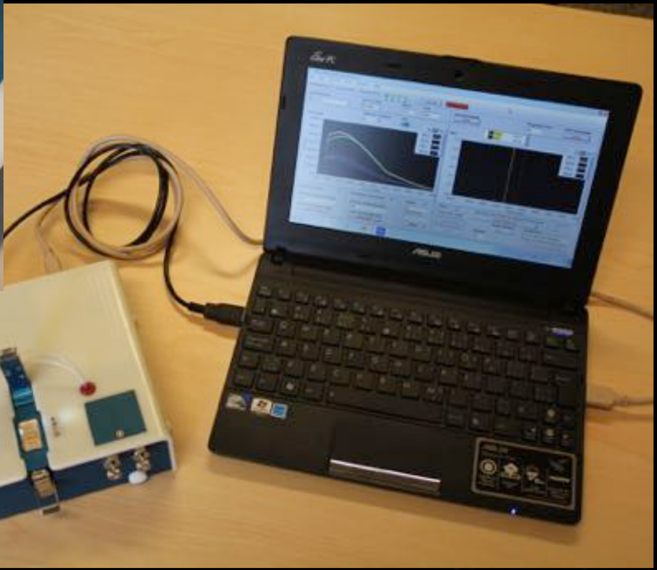
- Sensitive



ith USB – no external power cord

www.affiniteinstruments.com



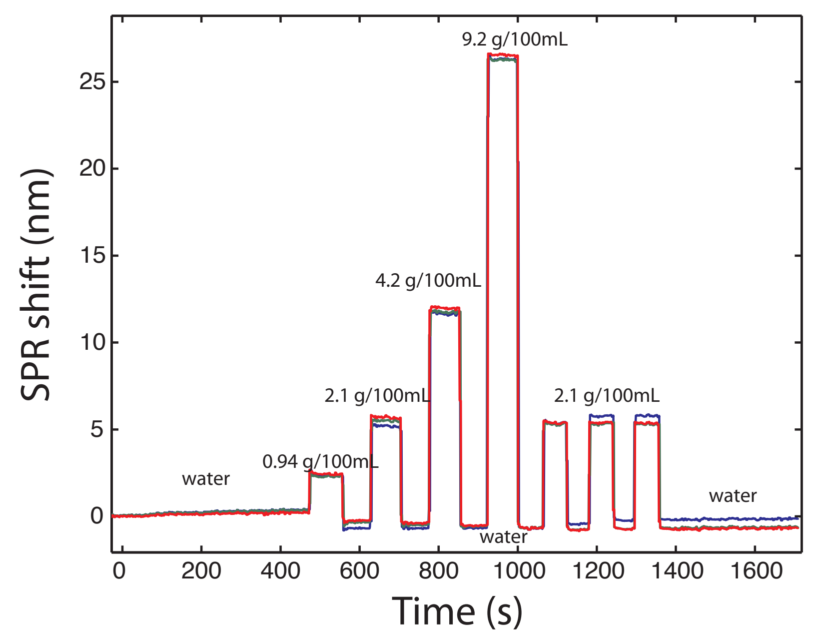


- Simplicity
- Versatility
- Performance
- Portability
- Customizable
- Low maintenance
- Low cost
- Low weight (less than 1.3 kg)
- Quiet operation

Biosensors & Bioelectronics, 2015, 64, 664-670

Refractive index sensor

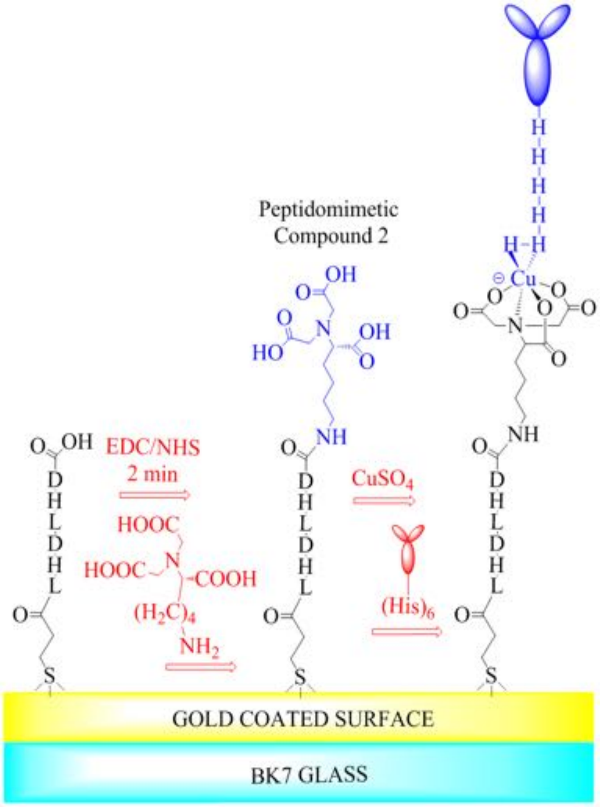
- Change in response due to the refractive index change of the bulk solution
  - Can be used as a detector (HPLC was coupled to SPR)



Université de Montréal

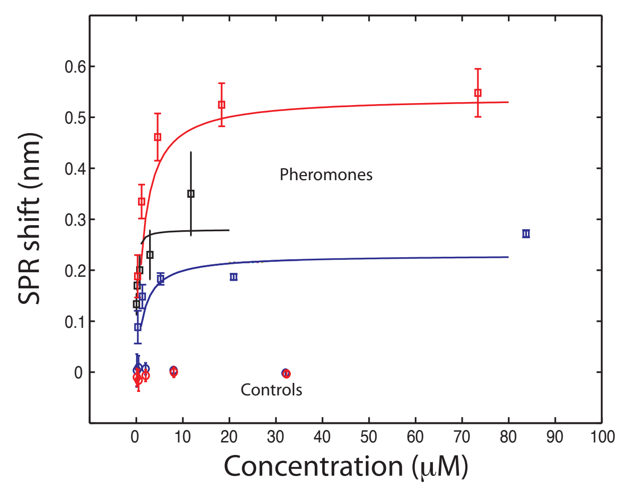
Sensors for screening small molecules

- Type B scavenger receptor CD36 (CD36: cluster of differenciation 36)
- EP80317: anti-atherosclerotic property mediated by CD36
    - interfering with the binding of oxLDL to the scavenger receptor expressed on macrophages



Analysis of pheromones - drosophilia

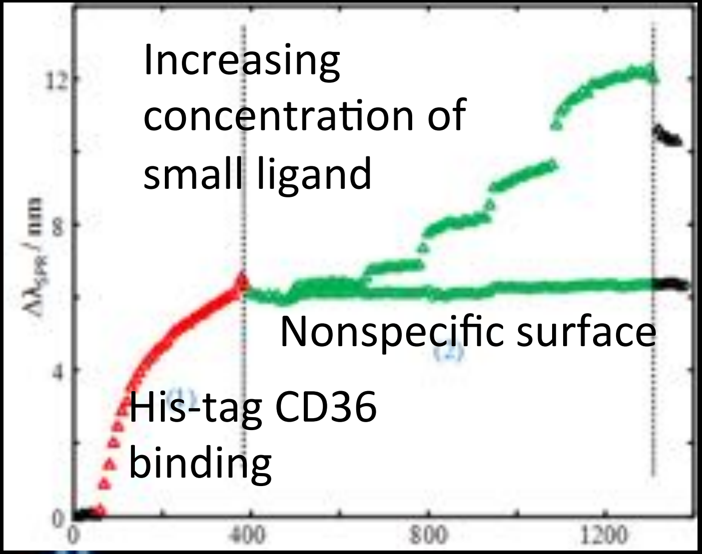
- CD36 is also a model receptor for monitoring the interaction of pheromones of drosophilia (fruit fly) with olfactory receptors
- There is no biochemical technique to measure the interaction of pheromones (small organic volatile compounds) with olfactory receptors

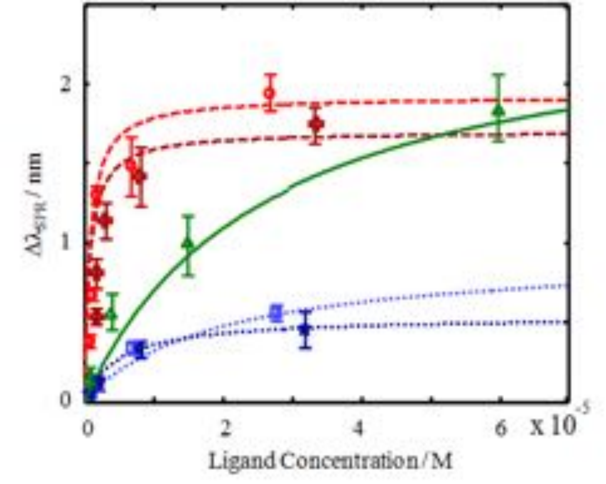


Ligands	***SPR K_d (10^{-6}M)***	***SPR $\Delta\lambda_{SPR}$ (nm)***
P1	**0.64**	**0.23**
P2	**0.64**	**0.54**
P3	**8.7**	**0.28**
P4	**20**	**0.71**
P5	**106**	**0.62**
C1	**No response**	
C2	**No response**	
C3	**No response**	
C4	**No response**	

Screening growth hormone-releasing peptides (GHRPs)

- Type B scavenger receptor CD36 (CD36: cluster of differenciation 36)
- EP80317: anti-atherosclerotic property mediated by CD36
  - interfering with the binding of oxLDL to the scavenger receptor expressed on macrophages

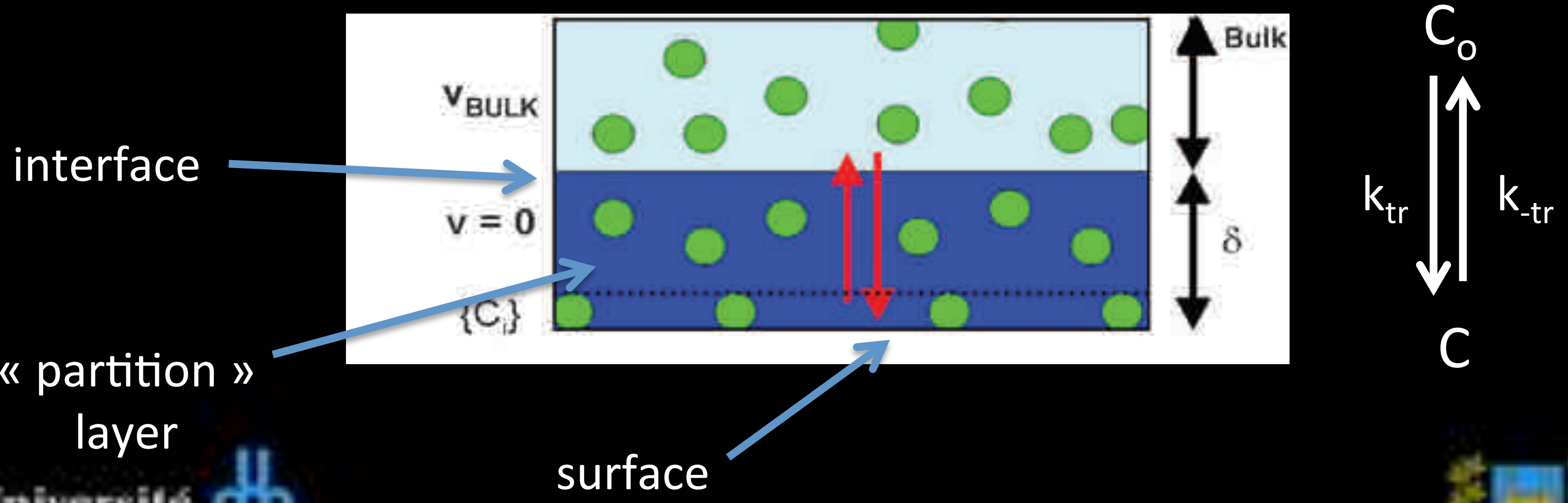




Université de Montréal

Mass transport to the surface

- In some cases, the concentration of the analyte in the bulk solution differs from the active concentration at the surface due to mass transport and partitioning of the molecules between the solid and liquid phases
  - Examples: coated inner walls, chromatographic surface, polymer layer on sensors



interface

« partition » layer

surface

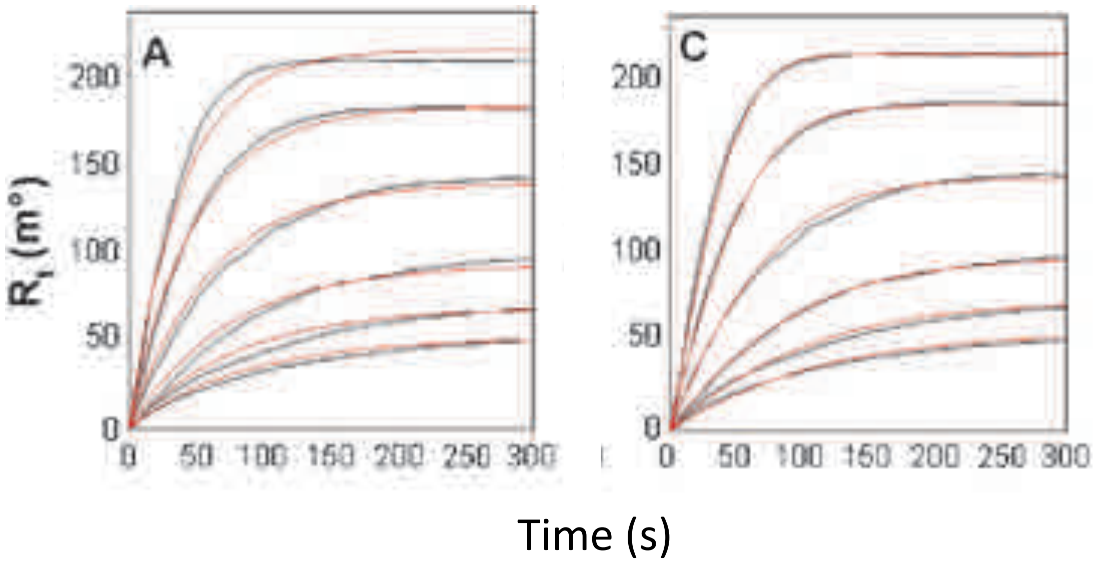
C_o

k_{tr} k_{-tr}

C

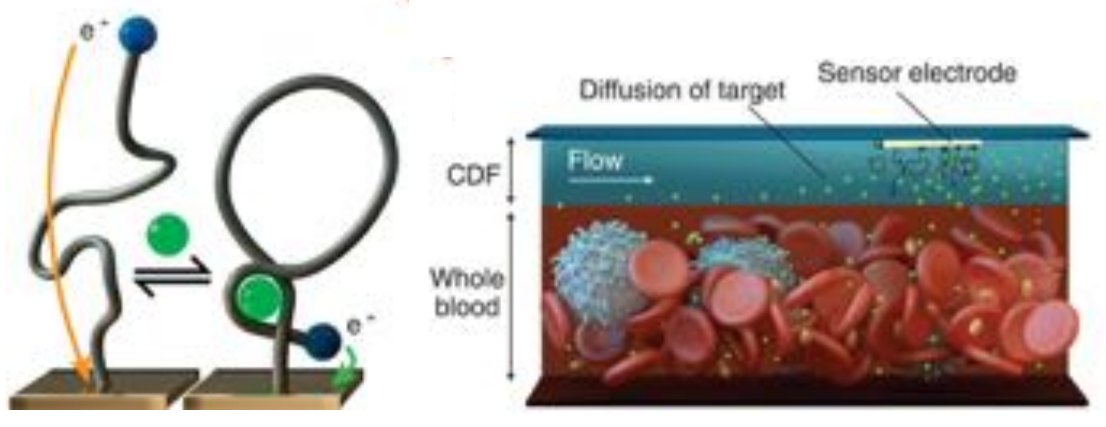
Mass transport to the surface

- Taking into account mass transport better predicts (red) experimental data (black)



Microdialysis in electrochemistry

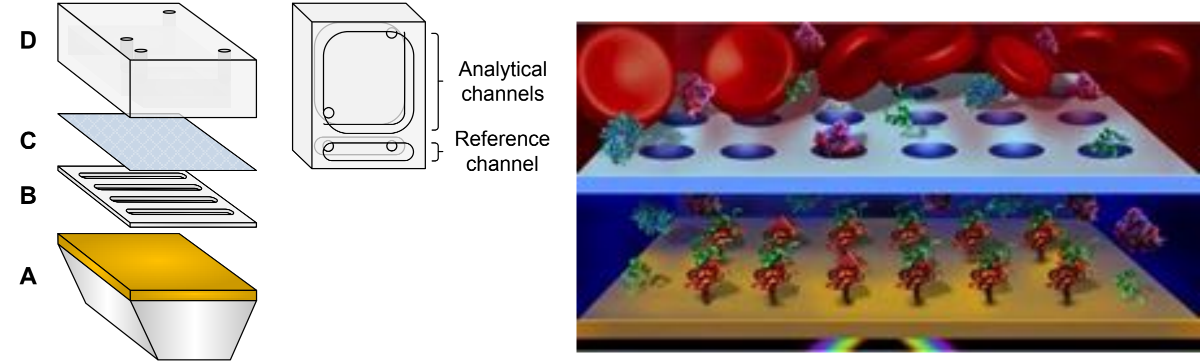
- Used in whole blood sensing for monitoring small molecules (example below is for continuous therapeutic drug monitoring)
- The fast diffusion of small molecules through a porous membrane effectively sort the molecule from a complex biofluid



Science Translational Medicine **2013**, *5,* 213ra165

Microdialysis SPR for sensing in blood

- Biosensing in blood is currently limited from background absorption of proteins and cells on the SPR surface
- Locating the SPR sensor in a chamber separated from the biofluid by a porous membrane create a microdialysis chamber



Chemical Science, 2015, **6**, 4247 - 4254

Patent application US 62/132,107

Université de Montréal

Diffusion of molecules through pores

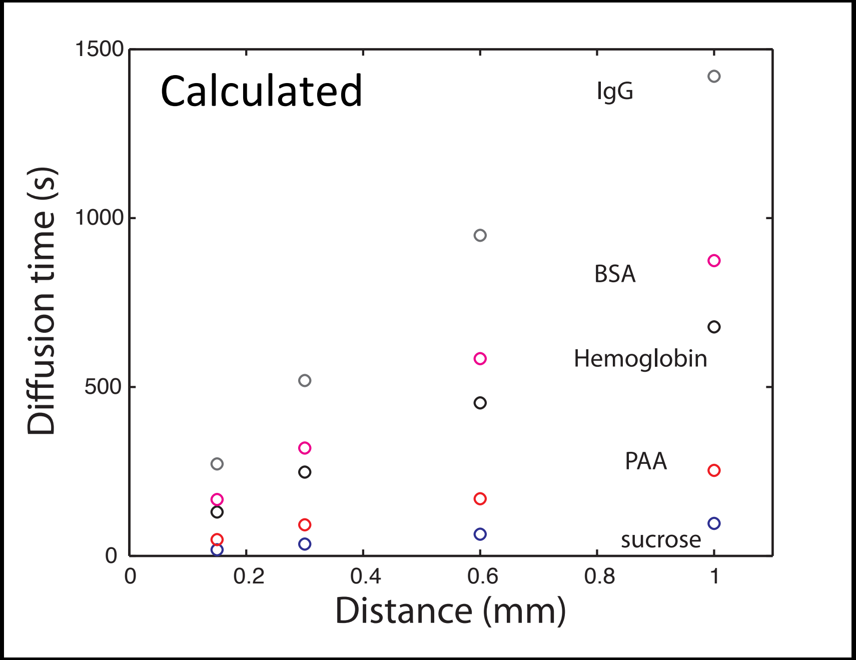
- Larger molecules have a lower effective diffusion coefficient in pores and a lower absolute diffusion coefficient

$D_{eff}/D_0 = (1 - R_H/R_P)^2\,(1 - 2.1R_H/R_P + 2.1R_H/R_P{}^3 - 0.95R_H/R_P{}^5)$

$A = L/[N_p \pi R_P{}^2(1/V_1 + 1/V_2)]$

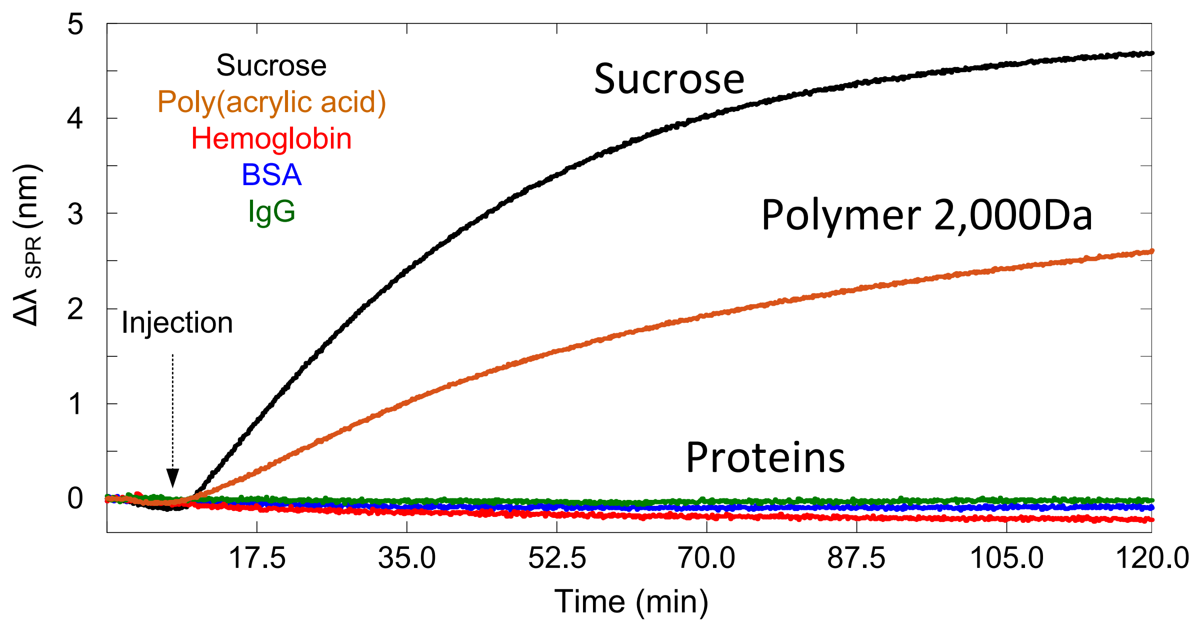
$D_{eff} = A/t_{eff}$

$c = c_{eq}\,(1 - e^{-t/teff})$



Experimental diffusion of molecules

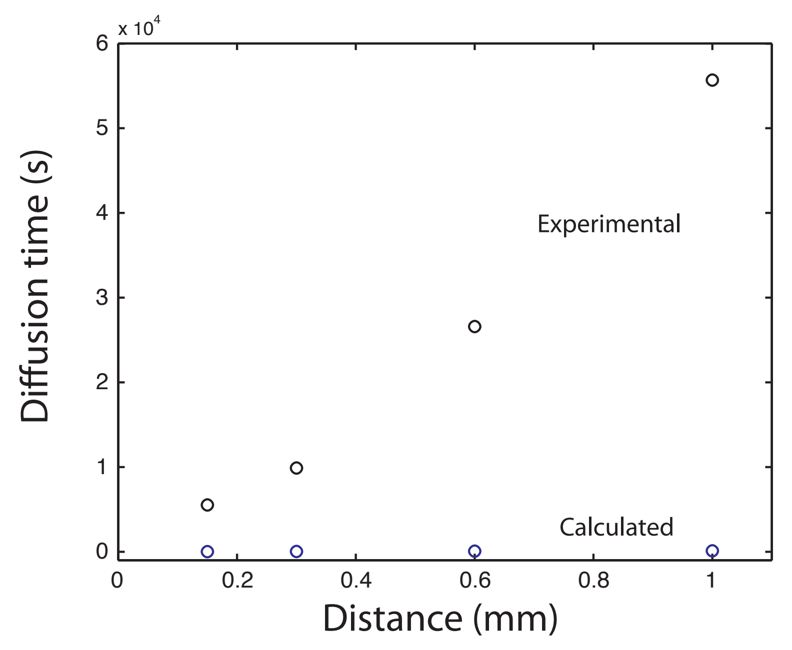
- The diffusion of proteins is slower than small analytes, such that the sensor will be exposed to a matrix of reduced complexity during a certain period of time



Chem. Sci., 2015, DOI: 10.1039/C5SC00716J

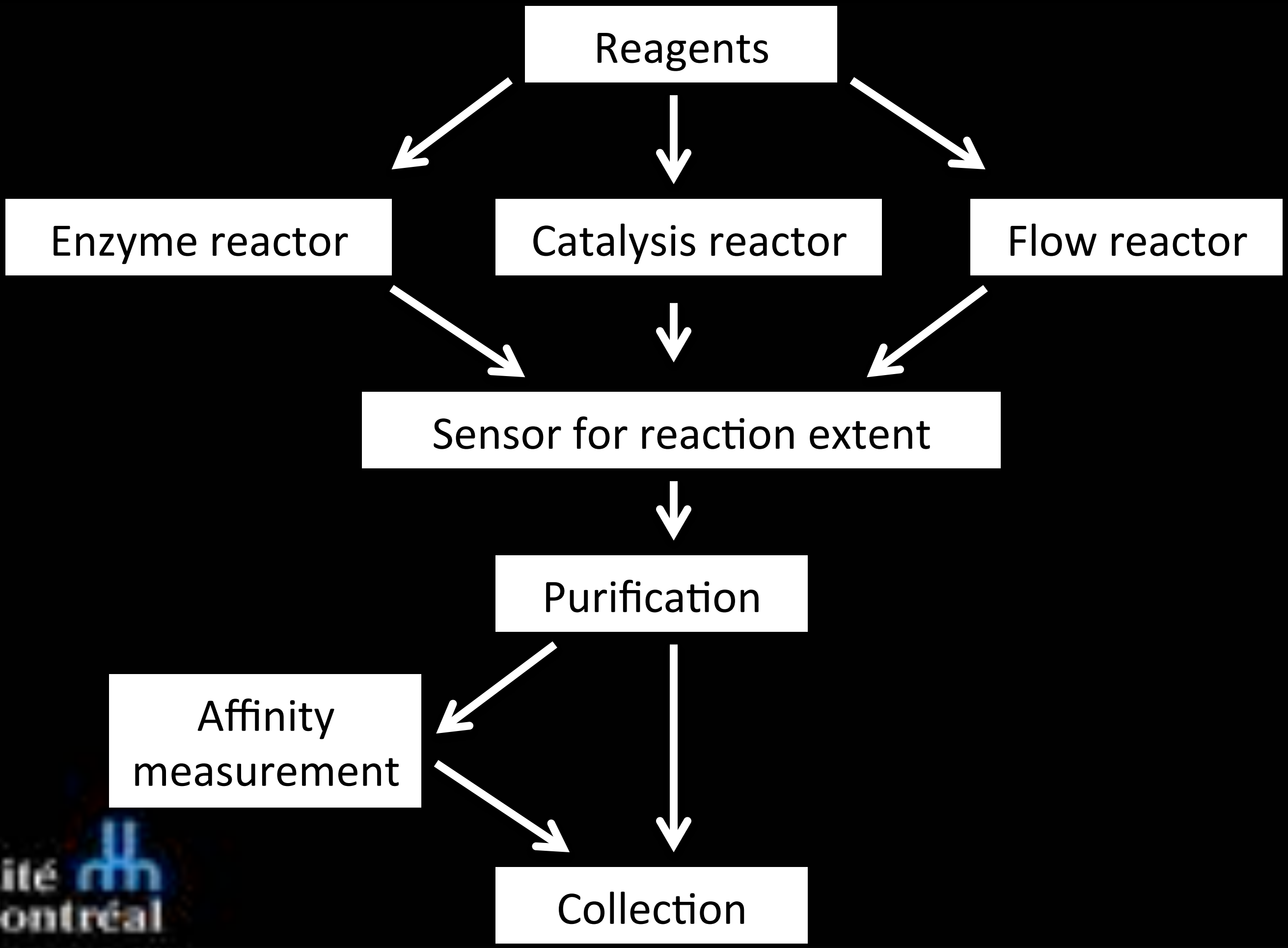
Influence of the absence of mixing on diffusion

- The experimental diffusion times are at least an order of magnitude longer than predicted, due to the absence of mixing



Université de Montréal

Integrated flow chemistry system



Reagents

Enzyme reactor

Catalysis reactor

Flow reactor

Sensor for reaction extent

Purification

Affinity measurement

Collection

Université de Montréal