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Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

Jakub Dostalek

AIT - Austrian Institute of Technology GmbH
Biosensor Technologies Unit
Konrad-Lorenz-Strasse 24 | 3430 Tulln | Austria
T +43(0) 664 2351773

FZU – Institute of Physics of the Czech Academy of Sciences,
Na Slovance 1 | Prague 182 00 | Czech Republic
T+420 776767927

jakub.dostalek@ait.ac.at | http://www.ait.ac.at | http://www.jakubdostalek.cz

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Tutorial 2: Evaluation of SPR Binding Kinetics for Affinity Interaction Analysis

Content

- Mass transfer and affinity – driven molecular binding kinetics.
- Design of the experiment to suppress the impact of diffusion limited binding kinetics.
- Tutorial on the fitting of equilibrium sensor response, fitting of the kinetics that are affinity driven, global analysis and taking into account the mass transfer.

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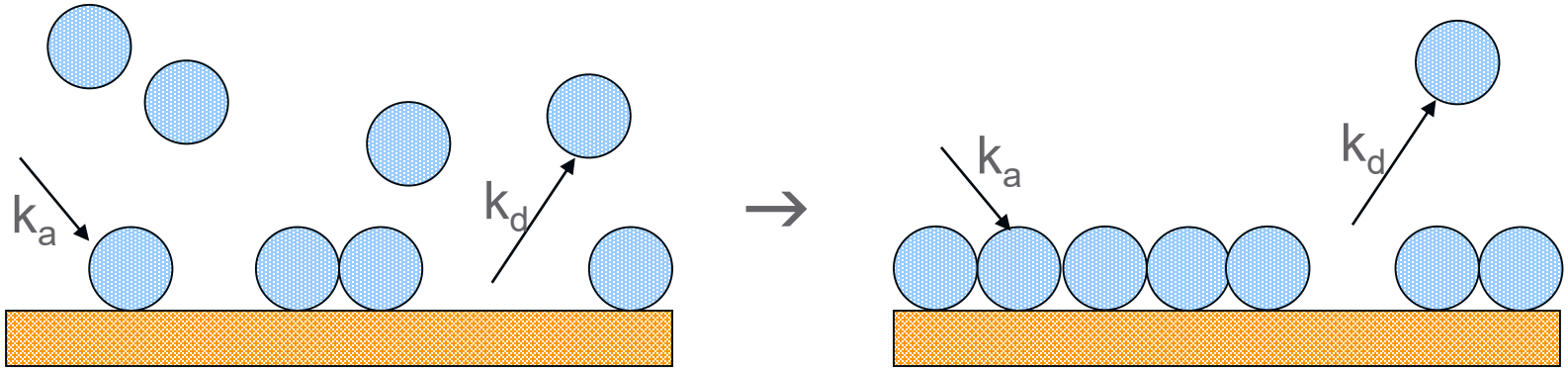
Langmuir Adsorption Isotherm: Assumptions

- All adsorption sites identical
- Adsorbed species interact only with adsorptions sites, not with each other
- Adsorption limited to a monolayer



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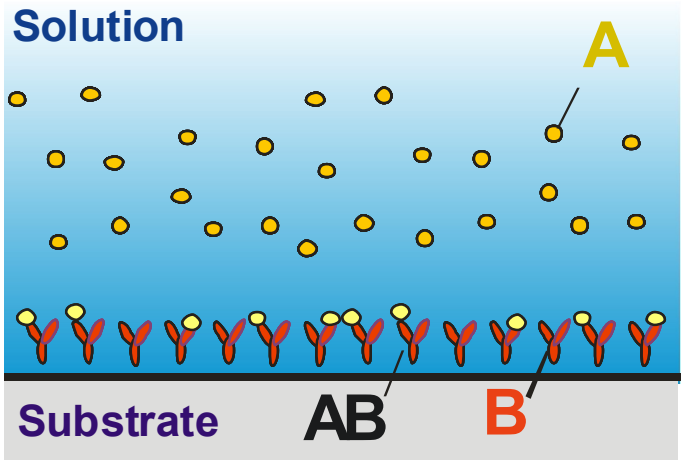
Langmuir Adsorption Isotherm

Equilibrium of a reaction:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftarrows}}$$

$$K = \frac{k_a}{k_d}$$



Kinetics of the reaction on a surface:

$$\frac{d\gamma}{dt} = k_a \alpha_0 (\beta - \gamma) - k_d \gamma$$

γ Concentration of [AB]

α_0 Concentration [A]

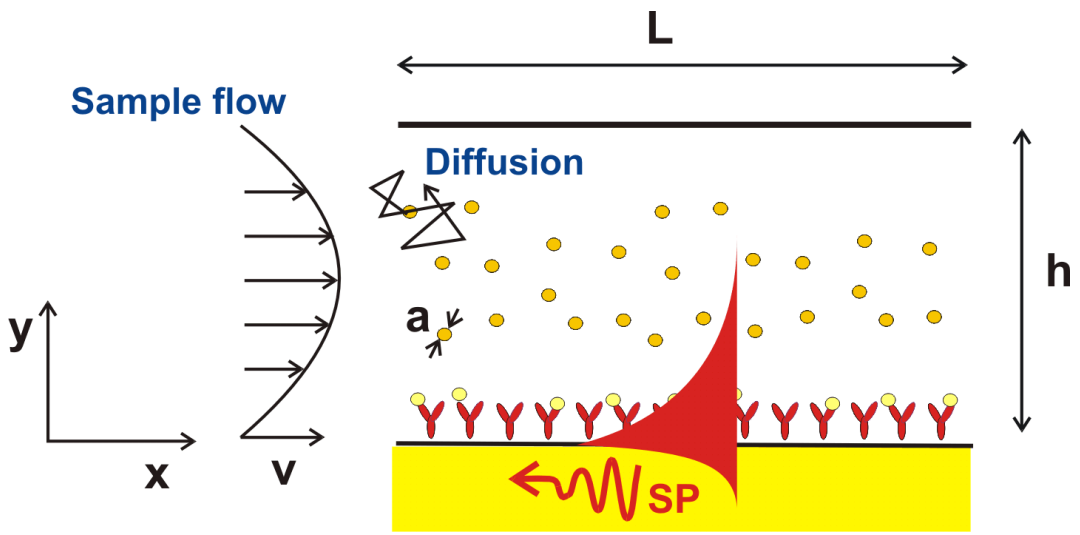
β Concentration [B]

Describes the interaction for:

a) Identical monovalent receptors B
b) Constant concentration of A in the solution ([A]>>[B])

(Possible to describe more complicated interactions e.g. multivalent receptors)

Two Compartment Model



D – diffusion coefficient

$$D \approx \frac{k_B T}{6\pi a \eta}$$

a – molecule A hydrodynamic radius

η - solution viscosity

In SPR biosensors, analyte molecules A in a liquid samples are flowed over the sensor surface.

Due to the friction, at the surface the flow velocity is *v*=0. Approximation that the analyte mass transfer rate occurs across an unstirred layer through diffusion:

$$k_m = \xi \left(\frac{v_{\max} D^2}{hL} \right)^{1/3}$$

Full Model

A) Description of laminar flow and diffusion in a flow-cell

$$\frac{\partial \alpha(x,y,t)}{\partial t} = D\left(\frac{\partial^2 \alpha(x,y,t)}{\partial^2 x} + \frac{\partial^2 \alpha(x,y,t)}{\partial^2 y}\right) - 4v_{\max}\frac{y}{h}\left(1-\frac{y}{h}\right)\frac{\partial \alpha(x,y,t)}{\partial x}$$

B) Binding to receptors on the flow-cell bottom

$$\frac{\partial \gamma(x,t)}{\partial t} = k_a \alpha(x,0,t)\left[\beta - \gamma(x,t)\right] - k_d \gamma(x,t)$$

C) Boundary conditions :

$$D\frac{\partial \alpha(x,h,t)}{\partial y} = 0 \qquad D\frac{\partial \alpha(x,0,t)}{\partial y} = \frac{\partial \gamma(x,t)}{\partial t}$$

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Mass Transport Limited Kinetics

„Corrected Langmuir equation“:

$$\frac{d\langle\gamma\rangle}{dt} = k_a^{eff}\,\alpha_0\left(\beta - \langle\gamma\rangle\right) - k_d^{eff}\,\langle\gamma\rangle$$

Valid when the diffusion parallel the sensor surface can be omitted (diffusion is much slower than the flow through the flow-cell)

$$k_a^{eff} = \frac{k_a}{1 + k_a\left[\beta - \langle\gamma\rangle(t)\right]/k_M}$$

$$k_d^{eff} = \frac{k_d}{1 + k_a\left[\beta - \langle\gamma\rangle(t)\right]/k_M}$$

$$\mathrm{Pe} = \frac{v_{\max} h^2}{DL} >> 1$$

$$k_M \approx 1.378\left(\frac{v_{\max} D^2}{hL}\right)^{1/3}$$

Mass transport can be omitted when Damköhler number Da<<1:

$$\mathrm{Da} = k_a \beta \left(\frac{v_{\max} D^2}{hL}\right)^{-1/3} << 1 \quad \Rightarrow \quad k_a^{eff} \approx k_a \quad k_d^{eff} \approx k_d$$

J. Stepanek et al. in J. Homola (Ed.), Surface Plasmon Resonance Based Sensors, Springer (2006) 45-69.

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Surface Reaction with Mass Transfer

Reaction kinetics become a function of mass transfer rate k_m.

$$\frac{d\gamma}{dt} = k_{on}\alpha(\beta - \gamma) - k_{off}\gamma$$

$$k_{on} = \frac{k_a}{1 + k_a\left[\beta - \gamma(t)\right]/k_m}$$

$$k_{off} = \frac{k_d}{1 + k_a\left[\beta - \gamma(t)\right]/k_m}$$

Fast diffusion

$k_m >> k_a\beta$

Reaction is affinity-controlled and $k_{on} \approx k_a$, $k_{off} \approx k_d$

Slow diffusion

$k_m << k_a\beta$

Reaction is diffusion-controlled, $k_{on} \approx k_m\beta^{-1}$ and $k_{off} \approx k_m k_d (k_a\beta)^{-1}$

(low probe / ligand density, high flow rate)

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Typical Characteristics

Properties of biomolecules:

Affinity constants: $k_a=10^{3}$-10^{7} $M^{-1}s^{-1}$ and $k_d=10^{-4}$-0.1 s^{-1} for majority of protein-protein interactions.

Diffusion constant: $D=2.4\times10^{-7}$ cm^2s^{-1} for water, T=20 ºC and a molecule with the diameter a=10 nm.

Fluidic system parameters:

Flow-rate: θ=100 µL/min

Flow-cell parameters: width *w*=5mm, height *h*=0.5mm, length *L*=10mm

Peclé number: Pe~400 (>>1 needed)

Damköhler number: Da~10^{-3} (<<1 needed) for β=10 ng mm^2, MW=160 kDa, $k_a=10^{7}$ M^{-1}s-1, $D=2.4\times10^{-5}$ mm^2s^{-1}, h=0.5 mm, Pe=400

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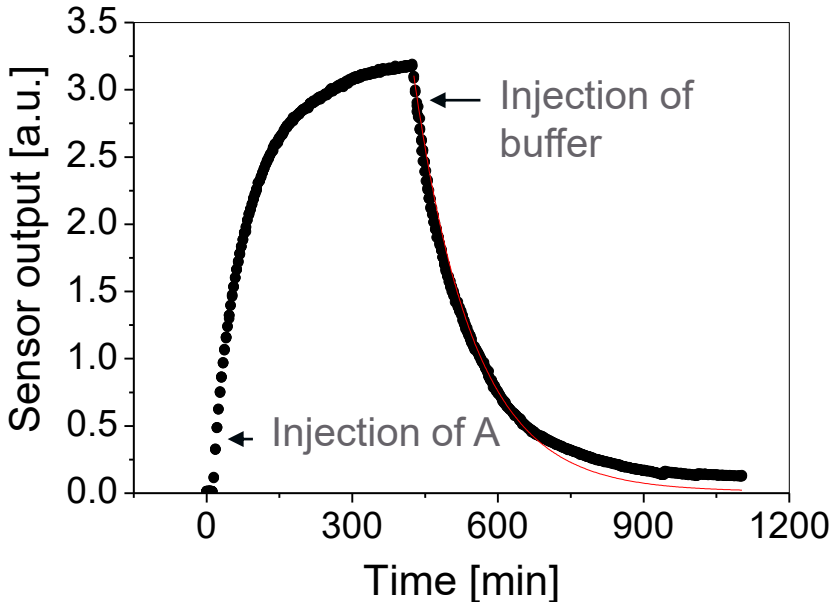
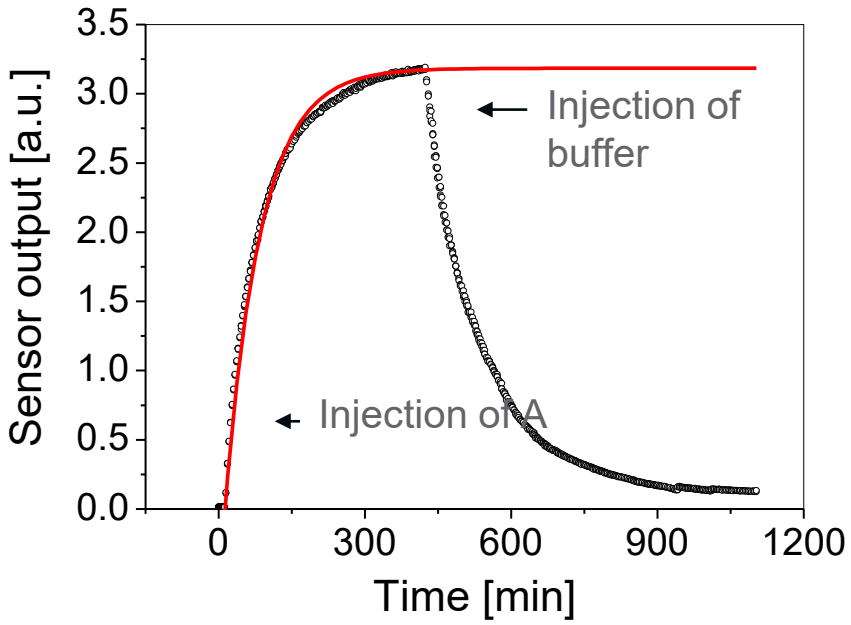
Fitting of the Sensor Kinetics



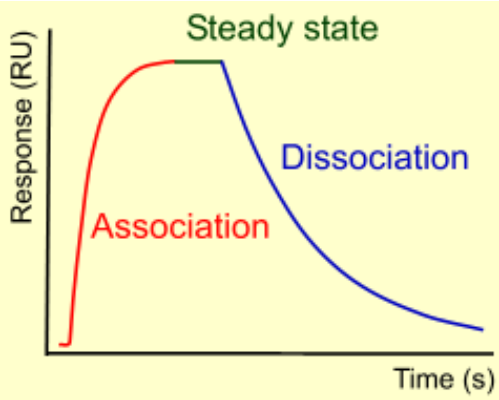


SPR biosensor output R(t) is proportional to $\gamma(t)$, one can fit k_a and k_d as:

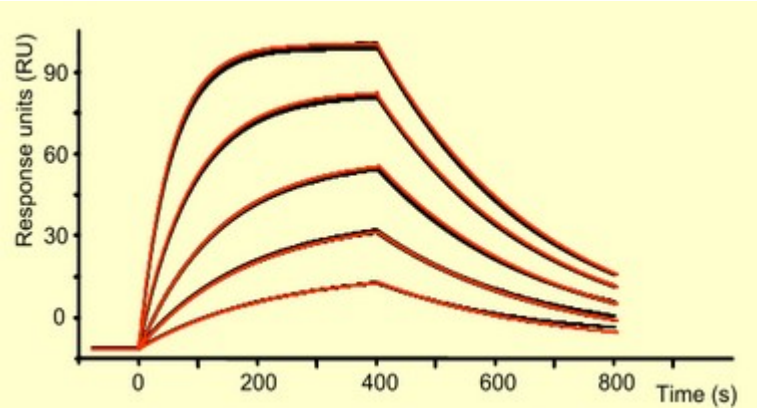
$$R_d(t) = (R_{max} - R_0)e^{-k_d(t-t_a)} + R_0 \qquad R_a(t) = (R_{max} - R_0)(1 - e^{-(k_a c_0 - k_d)(t-t_0)}) + R_0$$

k_d! k_a!

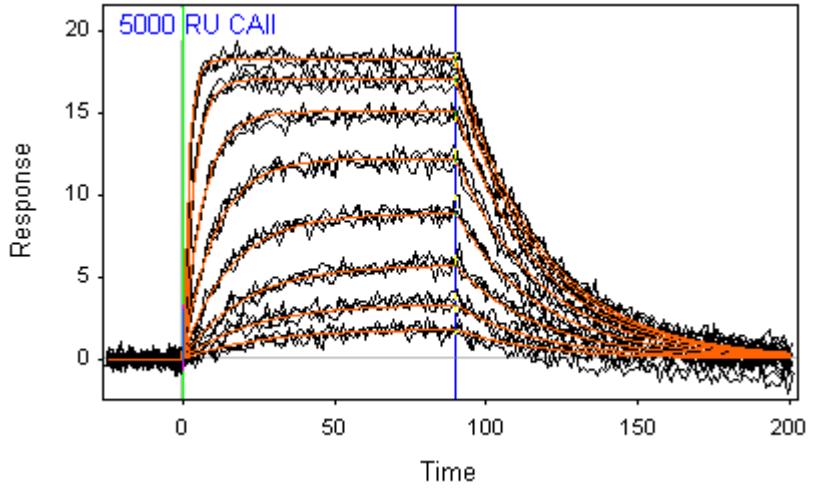
Global Analysis



Fitting ⬇ kinetics



- Association and dissociation binding rates (k_a and k_d, respectively) or more complex parameters can be determined by fitting with a model.
- Analysis can be performed through specialized software (*e.g.* from BIAcore, Scrubber) or by another tools allowing fitting with non-linear functions (*e.g.* Origin).



http://www.biologic.com.au/scrubber.html