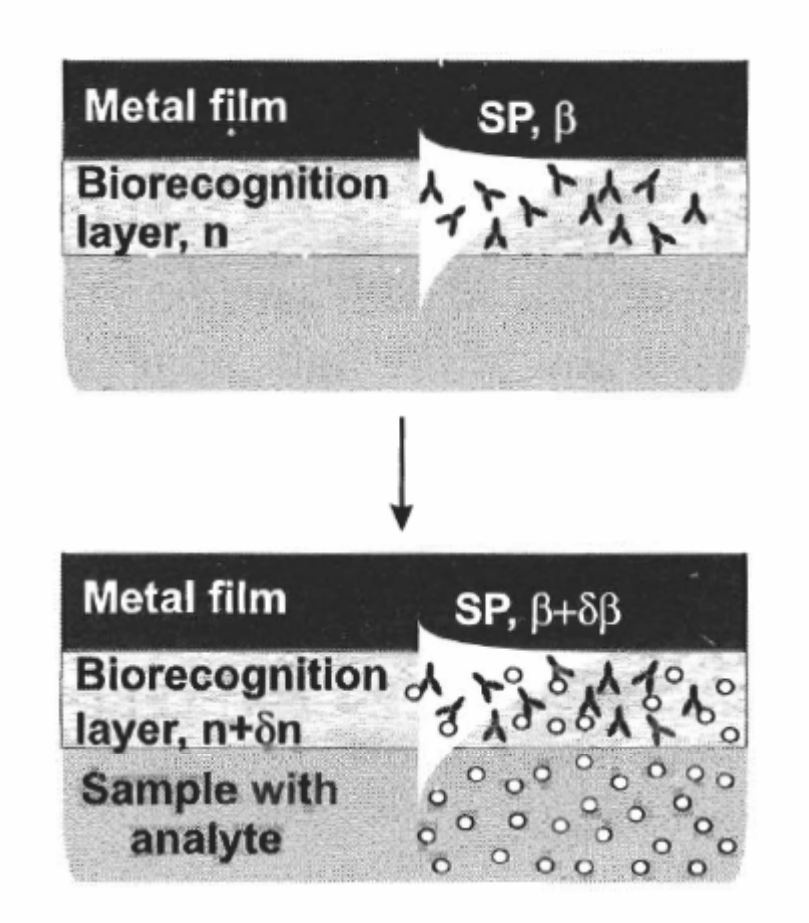
Lecture 7

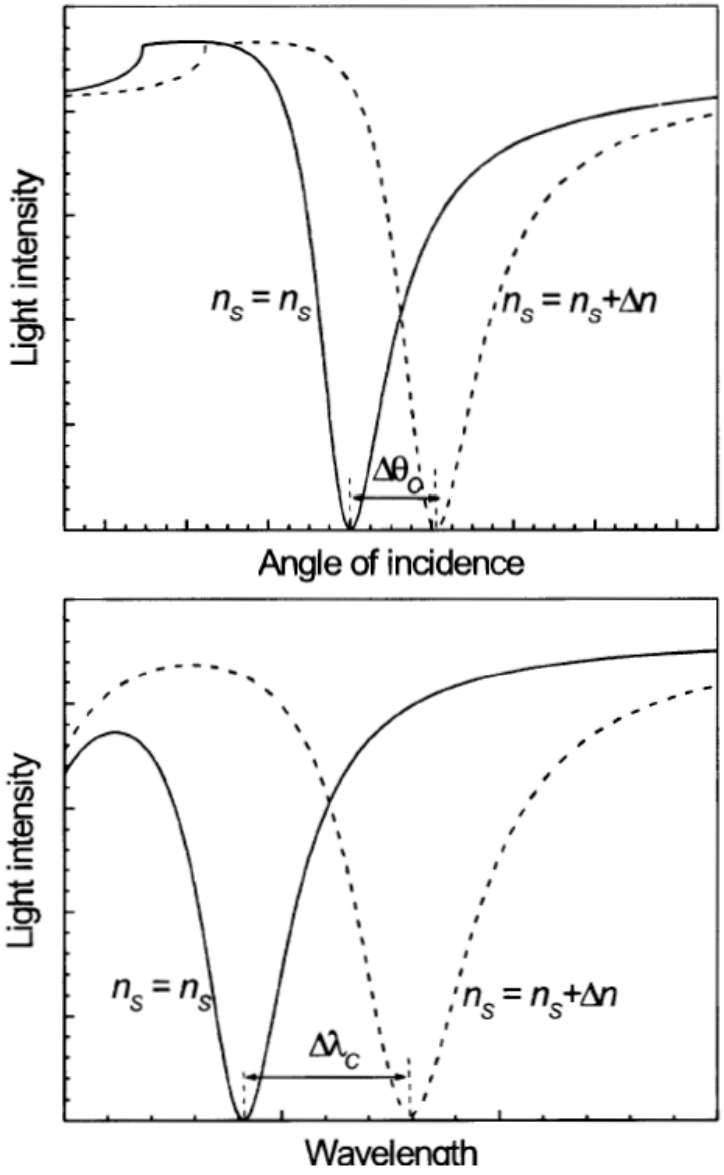
Data processing in SPR

Surface Reaction Kinetics on a Biochip

Surface plasmon sensor

- Principle of affinity SP biosensor



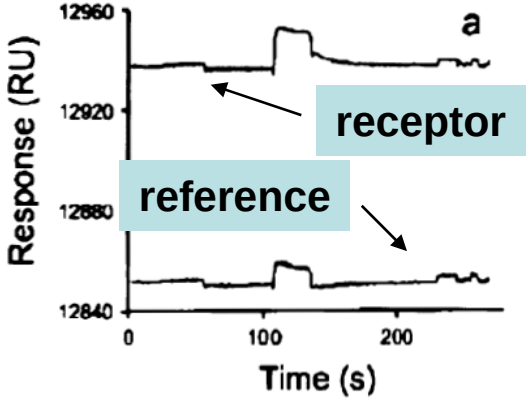


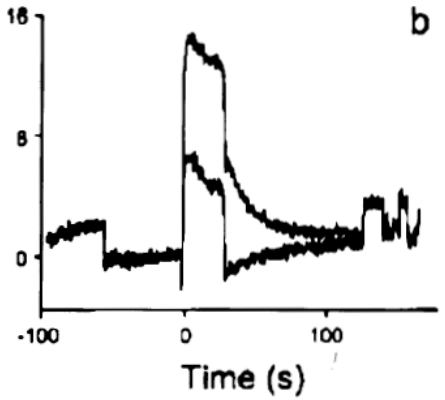
Data processing

- Processing steps:
  - zero response to the base line before analyte injection
  - align all responses so that injection starts at the same point
  - subtract the reference cell response
  - subtract the averaged response to buffer injection

Data processing

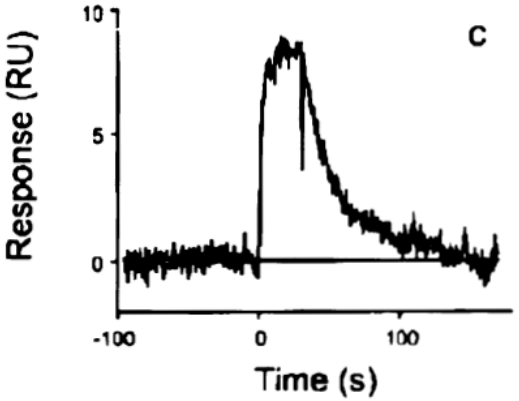
measured response:

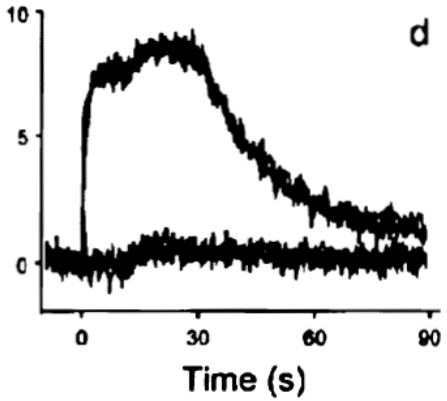




receptor and reference aligned, base line subtracted

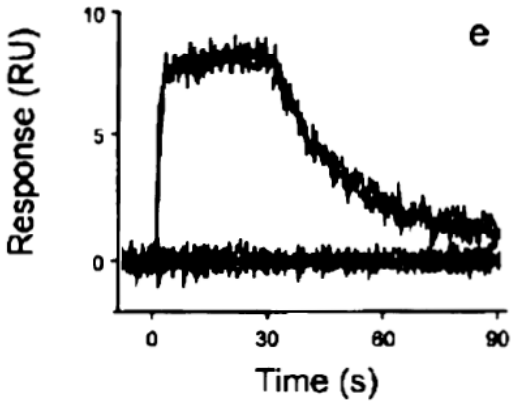
reference subtracted

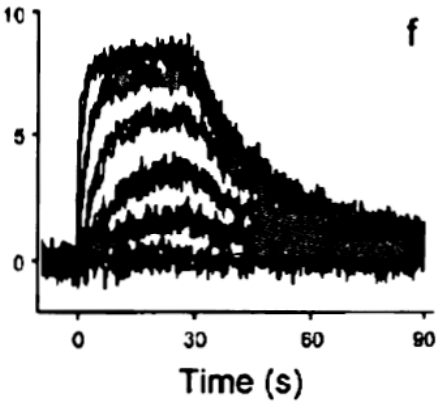




4 injections averaged + buffer injection

buffer injection subtracted

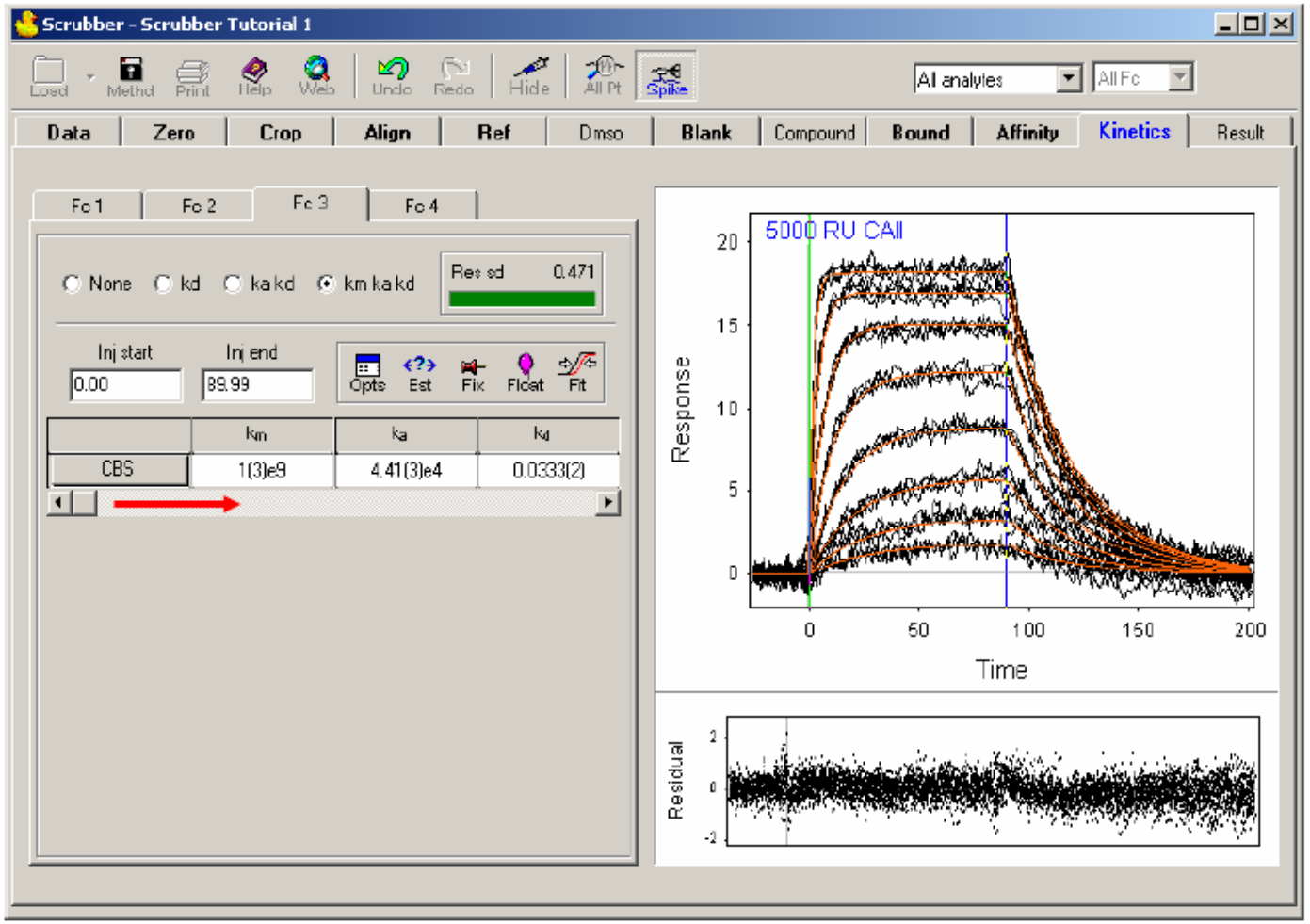




data, ready to be analyzed

Data processing

- Software:
  - Scrubber2, (Biosensor Tools http://www.cores.utah.edu/Interaction/scrubber.html)
  - *BiaEvaluation (Biacore AB, Sweden)*

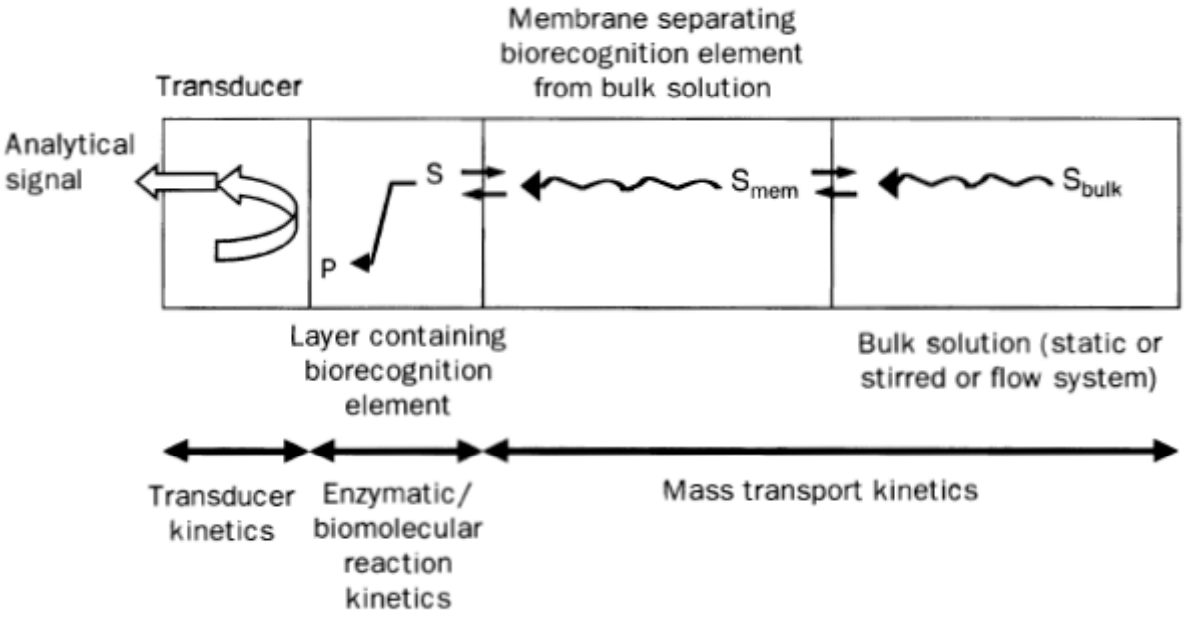


General biosensor model

Kinetics

Reaction kinetics

Mass transport



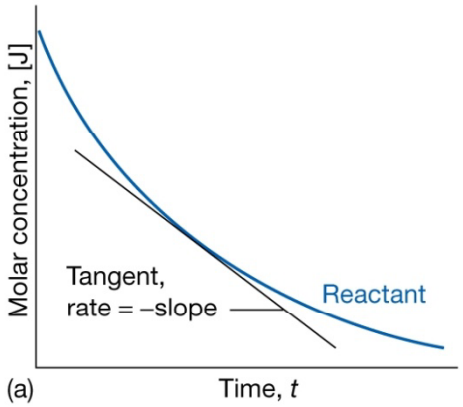
Rates of chemical reactions

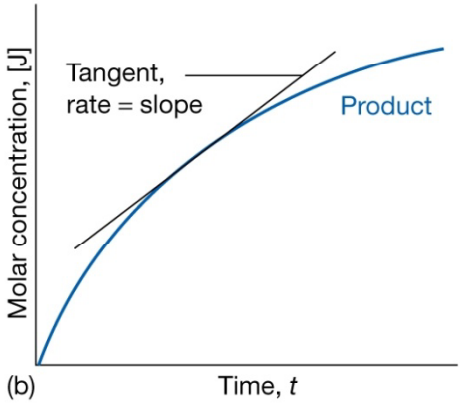
Consider reaction:

A+2B -> 3C+D

The rates are:

$$\frac{d[D]}{dt} = \frac{1}{3}\frac{d[C]}{dt} = -\frac{d[A]}{dt} = -\frac{1}{2}\frac{d[B]}{dt}$$





We can define rate of reaction:

$$v = \frac{d[\xi]}{dt}, dn_j = \nu_j d\xi$$

0 -> 3C+D-A-2B

ν_j

Rate laws

- Rate of reaction is often proportional to the concentration raised to some power, e.g.

$$v = k[A]^a[B]^b$$

- Overall order of the reaction: ***a+b+...***
  order of the reaction with respect to A: ***a***
- Reactions of <u>zero</u> order

$$v = k$$

First order reaction close to equilibrium

- Consider reactions:

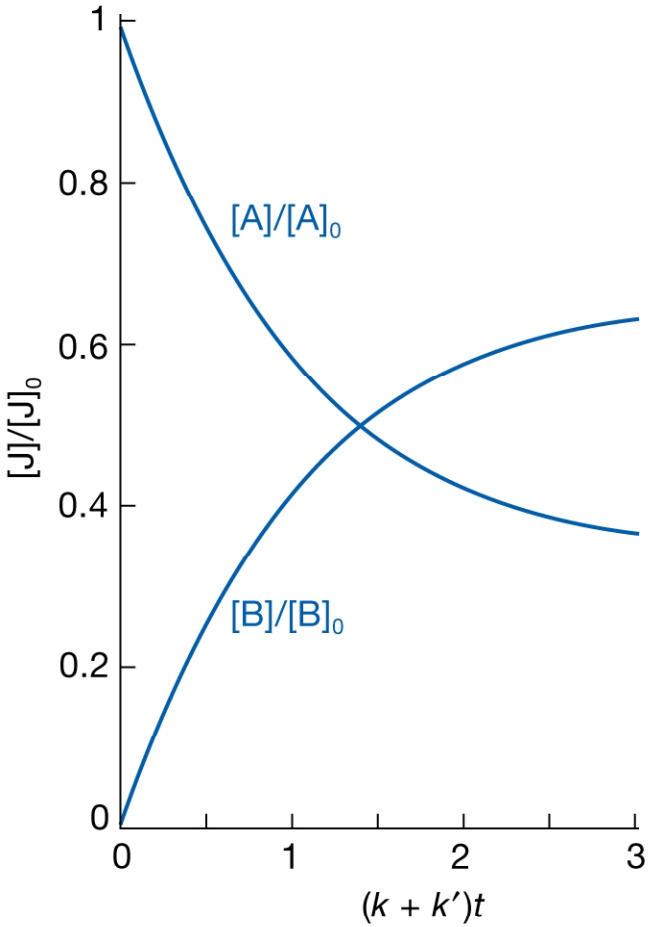
$$A \rightarrow B$$
$$B \rightarrow A$$

- At equilibrium forward and reverse rates are the same

$$\frac{d[A]}{dt} = \frac{d[B]}{dt},$$

$$k[A]_{eq} = k'[B]_{eq}, K = \frac{k}{k'} = \frac{[B]_{eq}}{[A]_{eq}}$$

Equilibrium constant



Modeling Molecular Interaction in SPR

- The aim: design a model kinetic equation that describes how amount of ligand-analyte depends on time, concentration of analyte and amount of free binding sites left.

The signal is proportional to the mass (per unit area) of bound analyte:

mass of the analyte

surface concentration of a complex

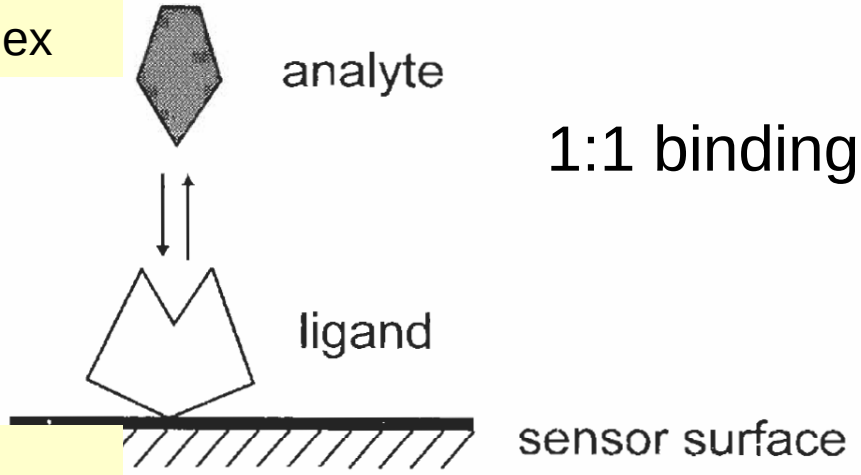
$$\xi = const \cdot M_A \cdot \gamma$$

The maximal signal:

$$\xi_S = const \cdot M_A \cdot \beta$$

$$\xi / \xi_S = \gamma / \beta$$

surface concentration of a ligand



1:1 binding

Pseudo-first order Kinetics

- For an analyte A (in solution) and a receptor R (immobilized)

$$A + R \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AR$$

- Association rate:

$$\frac{d\gamma_a}{dt} = k_a \alpha (\beta - \gamma) \qquad \alpha = [A]; \beta = [R]_0; \gamma = [RA]$$

- Dissociation rate:

$$\frac{d\gamma_a}{dt} = -k_d \gamma$$

- Summing:

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

Pseudo-first order Kinetics

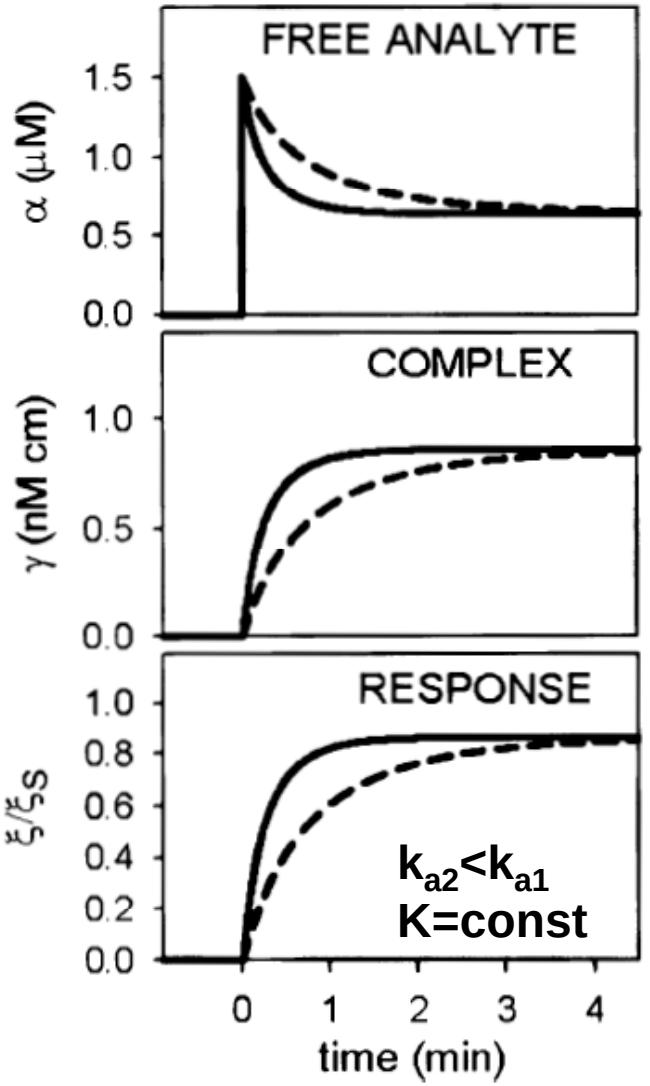
- Let's consider a situation when a concentration of analyte a_0 is injected into the volume V with the surface S.

$$\alpha V + \gamma S = \alpha_0 V = const$$

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

- At the equilibrium (when time passed)

$$\frac{d\gamma}{dt} = 0 \quad K = \frac{k_a}{k_d} = \frac{\gamma_{eq}}{\left(\alpha_0 - \frac{S}{V}\gamma_{eq}\right)(\beta - \gamma_{eq})}$$

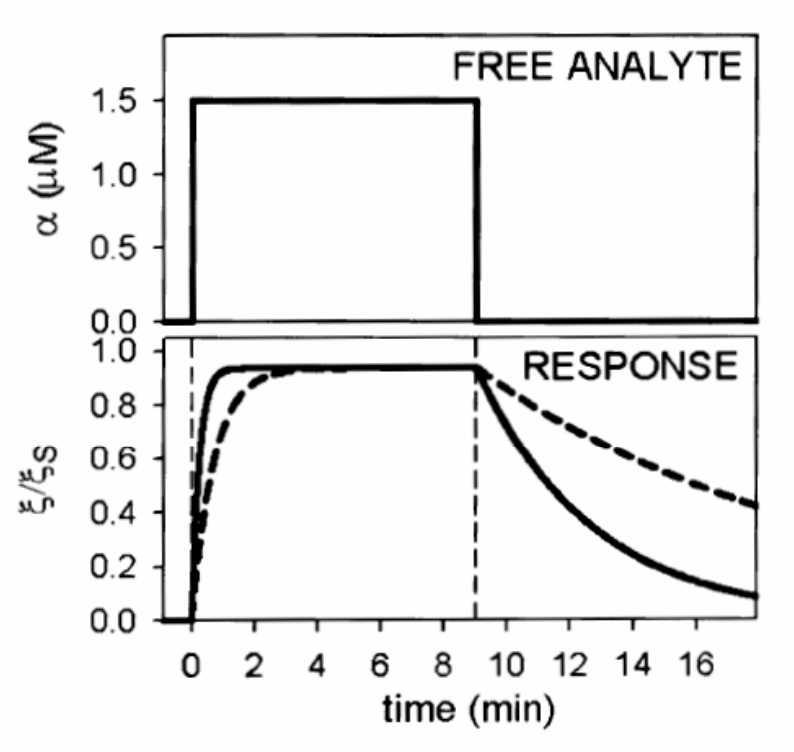


Pseudo-first order Kinetics

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

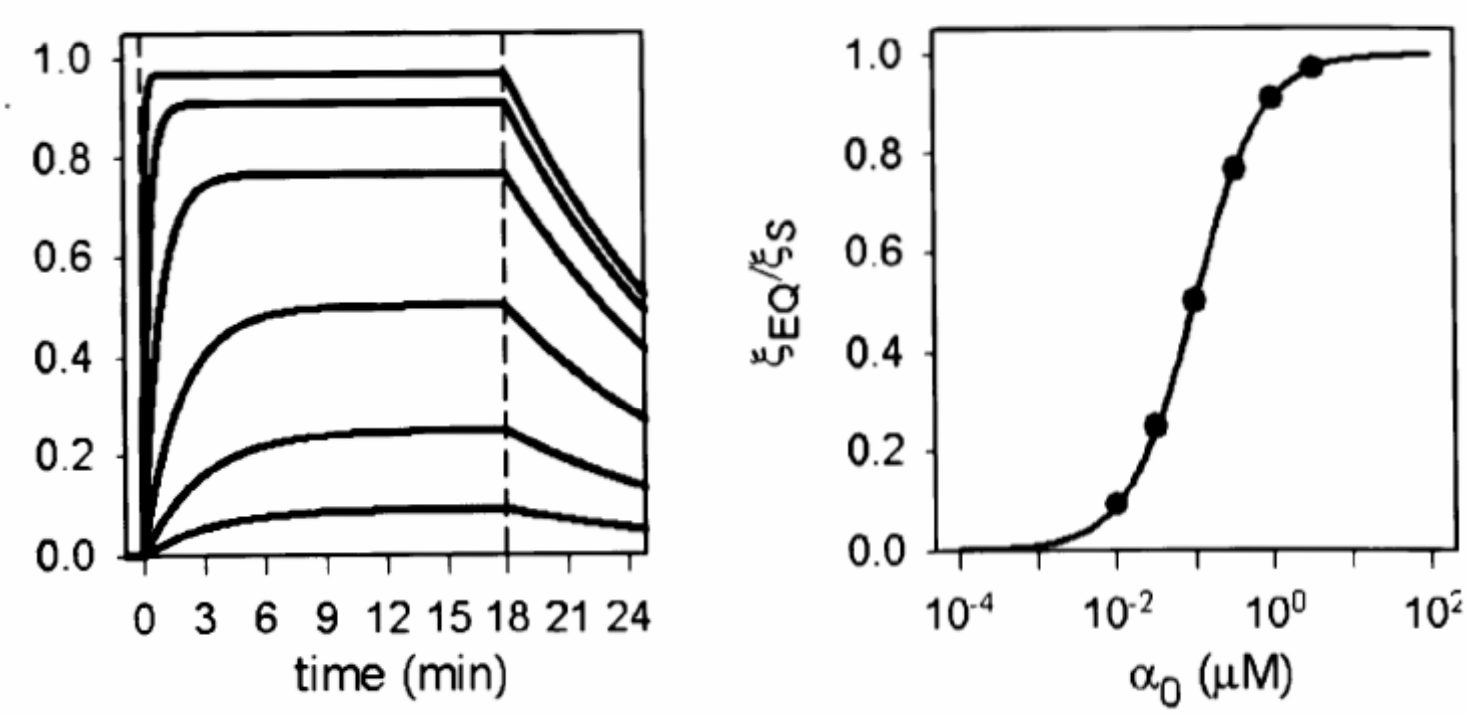
- if the concentration of analyte is high:

$$\frac{d\gamma}{dt} = k_a\alpha_0(\beta - \gamma) - k_d\gamma; \quad K = \frac{k_a}{k_d} = \frac{\gamma_{eq}}{\alpha_0(\beta - \gamma_{eq})}$$



Pseudo-first order Kinetics

- Equilibrium analysis (not affected by mass transport)



$$\frac{\xi_{EQ}}{\xi_S} = \frac{\gamma_{eq}}{\beta} = \frac{K\alpha_0}{(1+K\alpha_0)}$$

Other kinetic models

- Zero-order reaction following initial binding (conformational change in AR complex that blocks dissociation)

$$A + R \underset{k_{d1}}{\overset{k_{a1}}{\rightleftarrows}} AR \underset{k_{d2}}{\overset{k_{a2}}{\rightleftarrows}} AR^*$$

$$\frac{d\gamma_2}{dt} = k_{a2}\gamma_1 - k_{d2}\gamma_2$$

$$\frac{d\gamma_1}{dt} = k_{a1}\alpha_0(\beta - \gamma_1 - \gamma_2) - k_{d1}\gamma_1 - k_{a2}\gamma_1 + k_{d2}\gamma_2$$

- As the total mass is measured by the sensor

$$\xi / \xi_S = (\gamma_1 + \gamma_2) / \beta$$

Other kinetic models

- Parallel pseudo first-order reactions (e.g. two different receptors or two different analytes)

$$A + R_1 \underset{k_{d1}}{\overset{k_{a1}}{\rightleftarrows}} AR_1$$

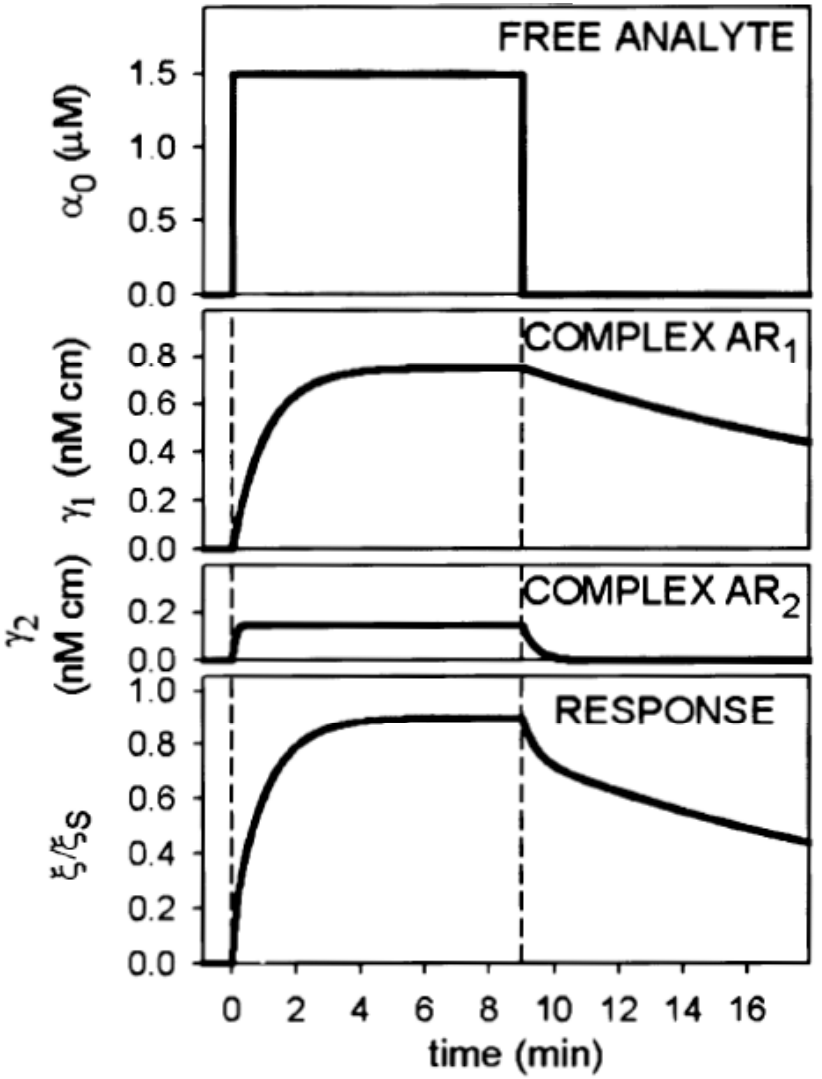
$$A + R_2 \underset{k_{d2}}{\overset{k_{a2}}{\rightleftarrows}} AR_2$$

$$\beta_1 = [R_1] = p\beta \qquad \beta_2 = [R_2] = (1-p)\beta$$

$$\frac{d\gamma_2}{dt} = k_{a1}\alpha_0(\beta_1 - \gamma_1) - k_{d1}\gamma_1$$

$$\frac{d\gamma_1}{dt} = k_{a2}\alpha_0(\beta_2 - \gamma_2) - k_{d2}\gamma_2$$

$$\xi / \xi_S = (\gamma_1 + \gamma_2) / \beta$$



Other kinetic models

- Multivalent receptor binding: single receptor binds more than one molecule (e.g. streptavidin, antibodies, triplex formation)

$$A + R \underset{k_{d1}}{\overset{k_{a1}}{\rightleftharpoons}} AR$$

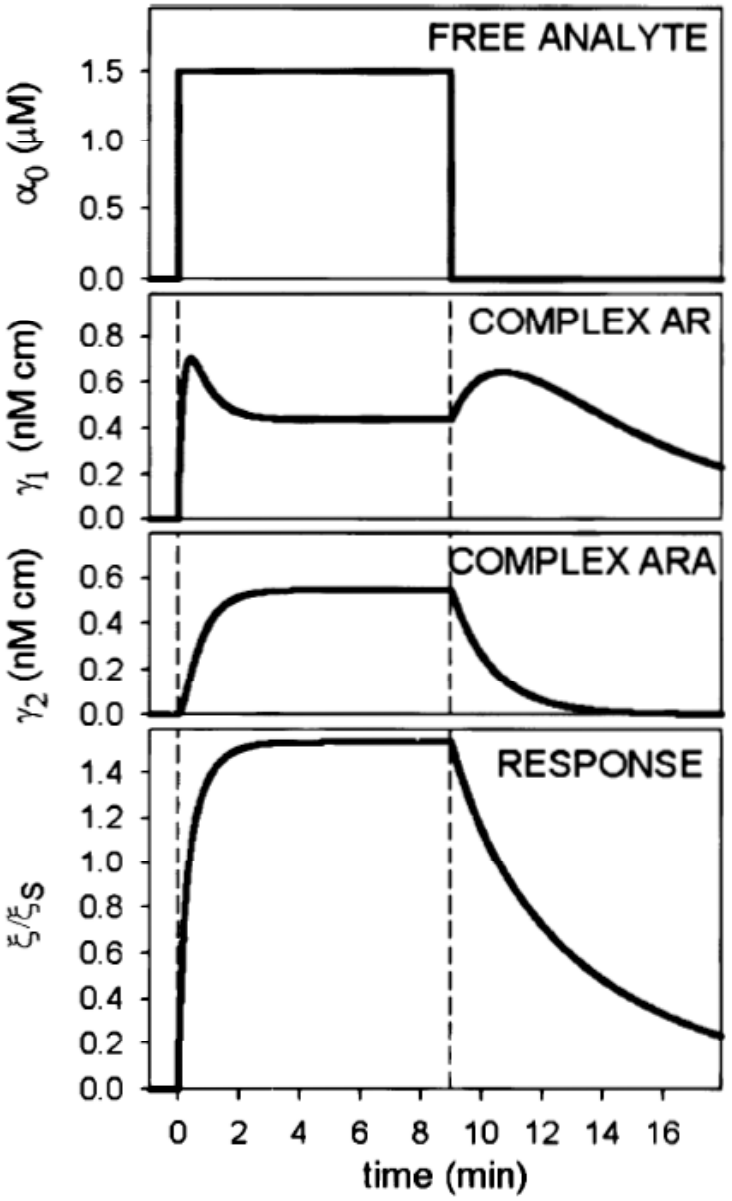
$$AR + A \underset{k_{d2}}{\overset{k_{a2}}{\rightleftharpoons}} ARA$$

$$\beta_1 = [R_1] = p\beta \qquad \beta_2 = [R_2] = (1-p)\beta$$

$$\frac{d\gamma_2}{dt} = k_{a2}\alpha_0\gamma_1 - k_{d2}\gamma_2$$

$$\frac{d\gamma_1}{dt} = k_{a1}\alpha_0(\beta - \gamma_1 - \gamma_2) - k_{d1}\gamma_1 - k_{a2}\alpha_0\gamma_1 + k_{d2}\gamma_2$$

$$\xi / \xi_S = (\gamma_1 + 2\gamma_2)/\beta$$



Thermodynamics in SPR

- change in Gibbs energy can be found from equilibrium constant:

$$\Delta G^0 = -RT \ln K$$

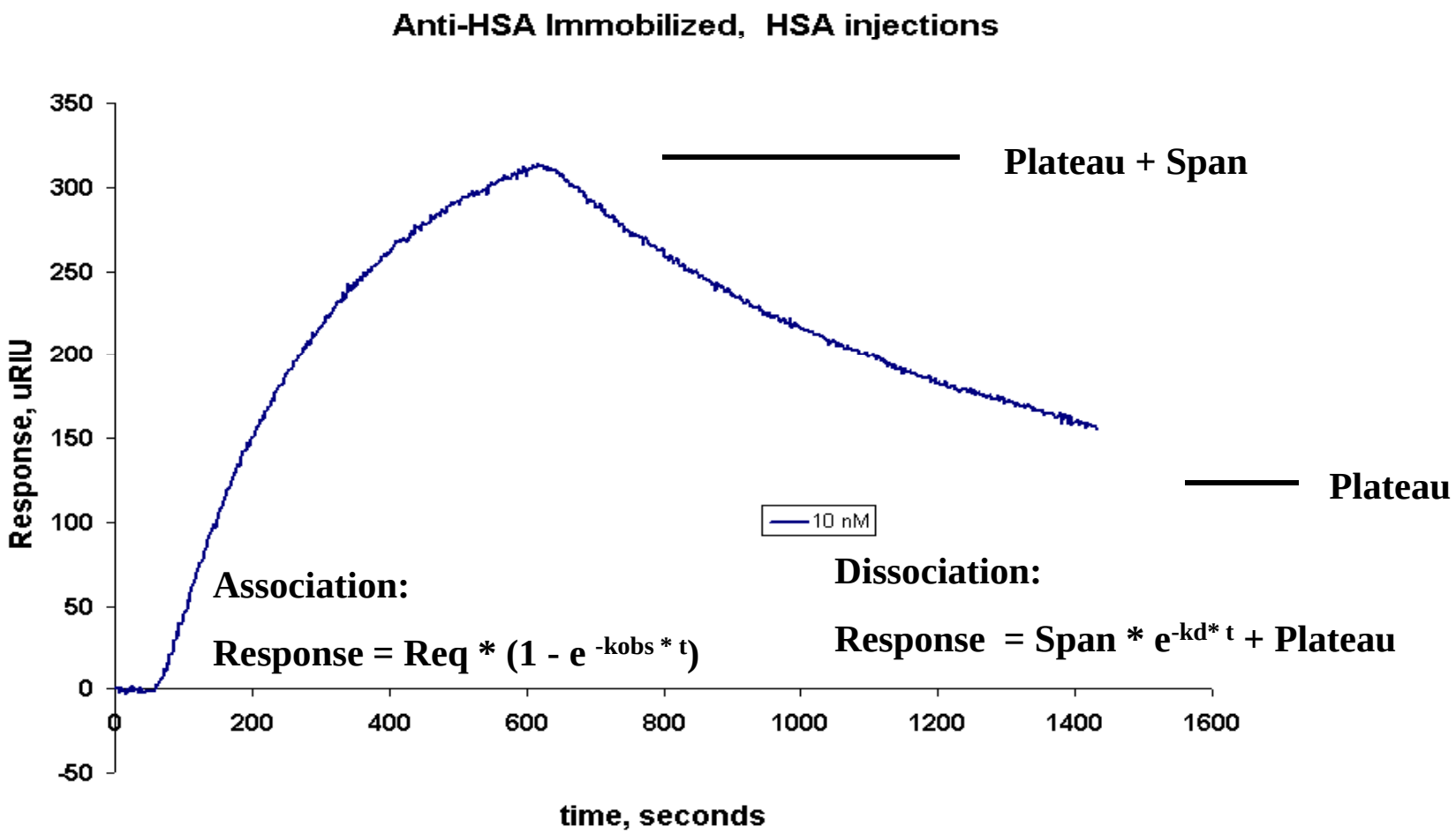
- Enthalpy and entropy of the reaction can be found from temperature dependence (van't Hoff equation)

$$\Delta G = \Delta H - T\Delta S$$

- Activation energy for association and dissociation can be found from Arrhenius equation:

$$k = P \exp(-E^{act} / RT)$$

Association/Dissociation in an experiment



Mass transport effects

- **In a flow cell:**
  - **flow is laminar**

volume flow rate

Reynolds number: $\mathrm{Re} = \frac{\rho \Phi}{\eta h}$

For water at 20ºC: $\mathrm{Re} = (\Phi / h) \cdot 0.998\, mm^2 / s$

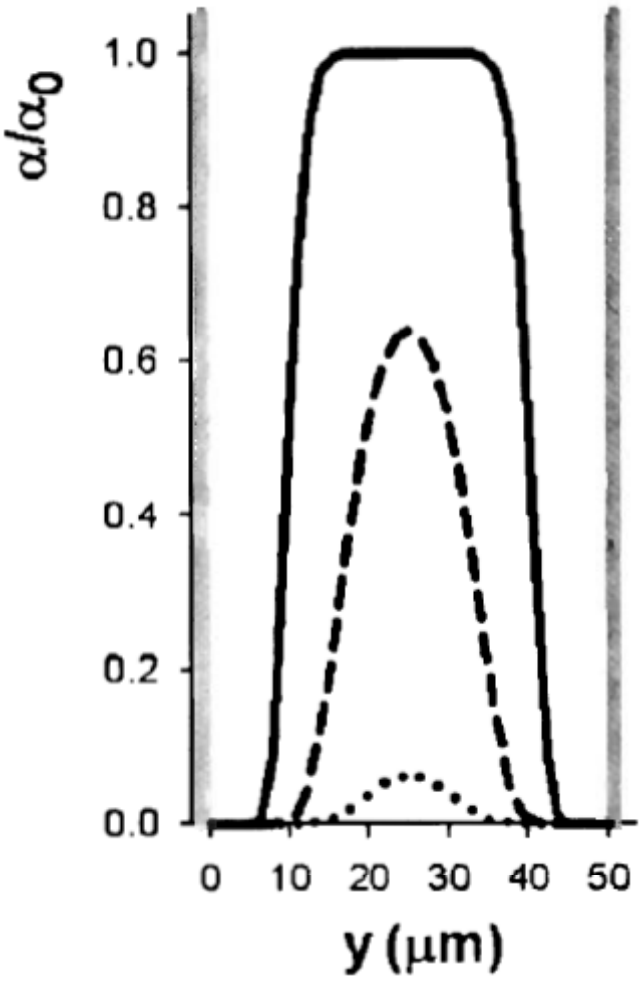
flow is laminar for Re<2100.

  - **velocity profile is parabolic**

$$v_{\max} = \frac{3}{2} \frac{\Phi}{hw}$$

  - **in a case of no diffusion the transport is by convection**

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



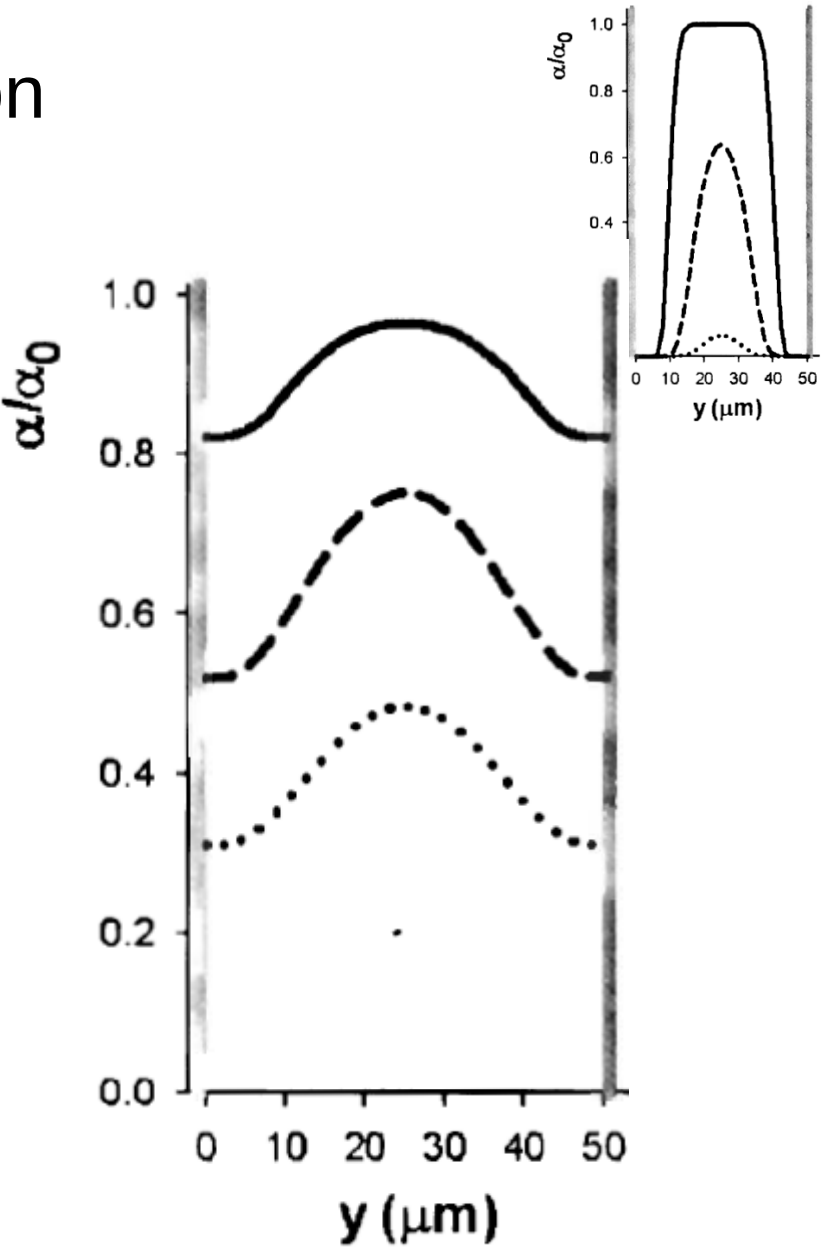
Mass transport effects

- Let's take into account diffusion

$$\frac{\partial \alpha(x,y,t)}{\partial t} =$$

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

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



Mass transport effects

- Complete model can be solved numerically like following
  - Navier-Stokes and Convection-Diffusion in the volume, e.g.

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

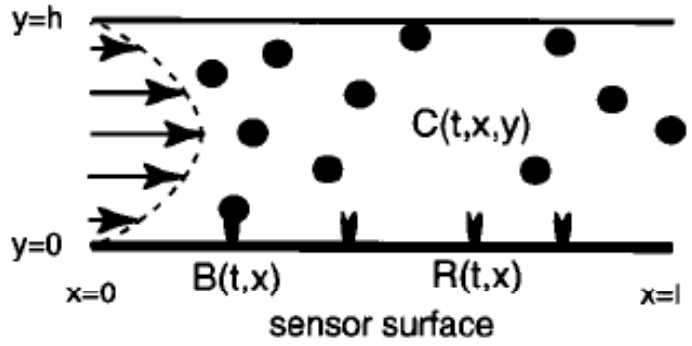
  - Reaction kinetics on a biochip

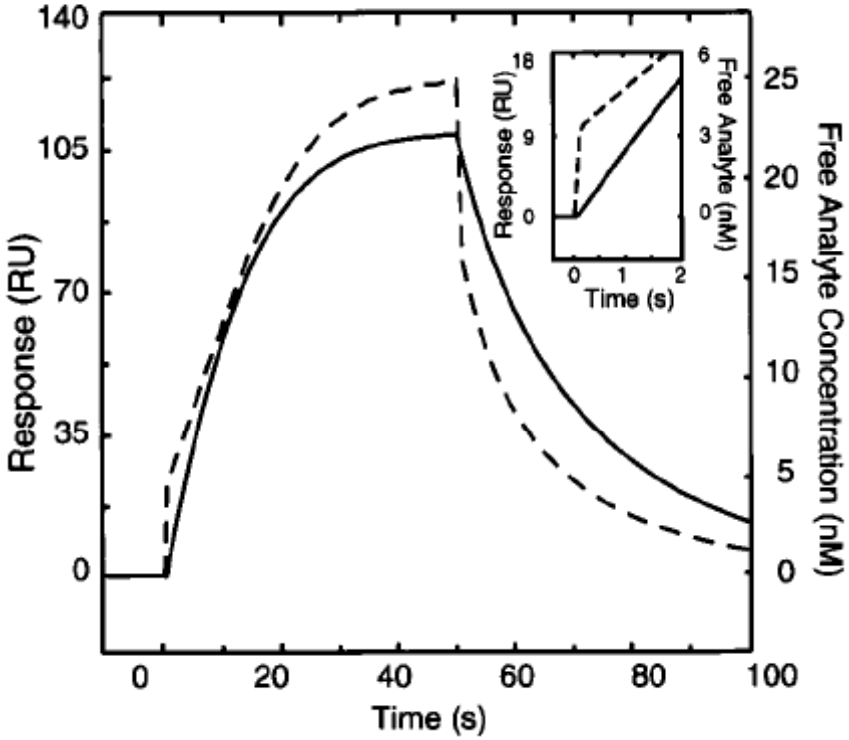
$$\frac{d\gamma(x,t)}{dt} = k_a \alpha(x,0,t)\left[\beta - \gamma(x,t)\right] - k_d \gamma(x,t)$$

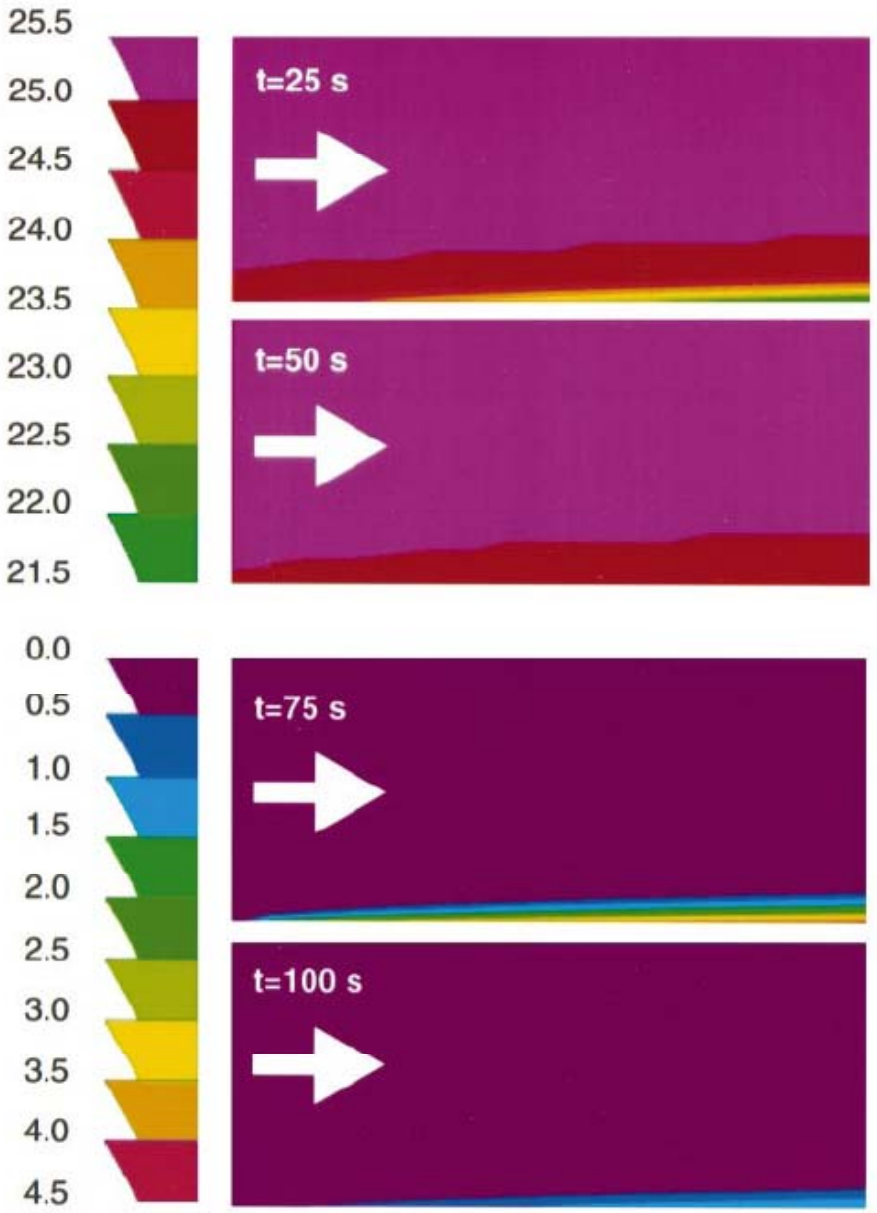
  - Boundary condition at the biochip

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

SPR case: Mass transfer+Reaction







Simplified model of mass transport

- Assumptions:
  - analyte transport in x direction is convective (no diffusion)

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

  - velocity dependence v(y) considered linear (as only area near surface is important)

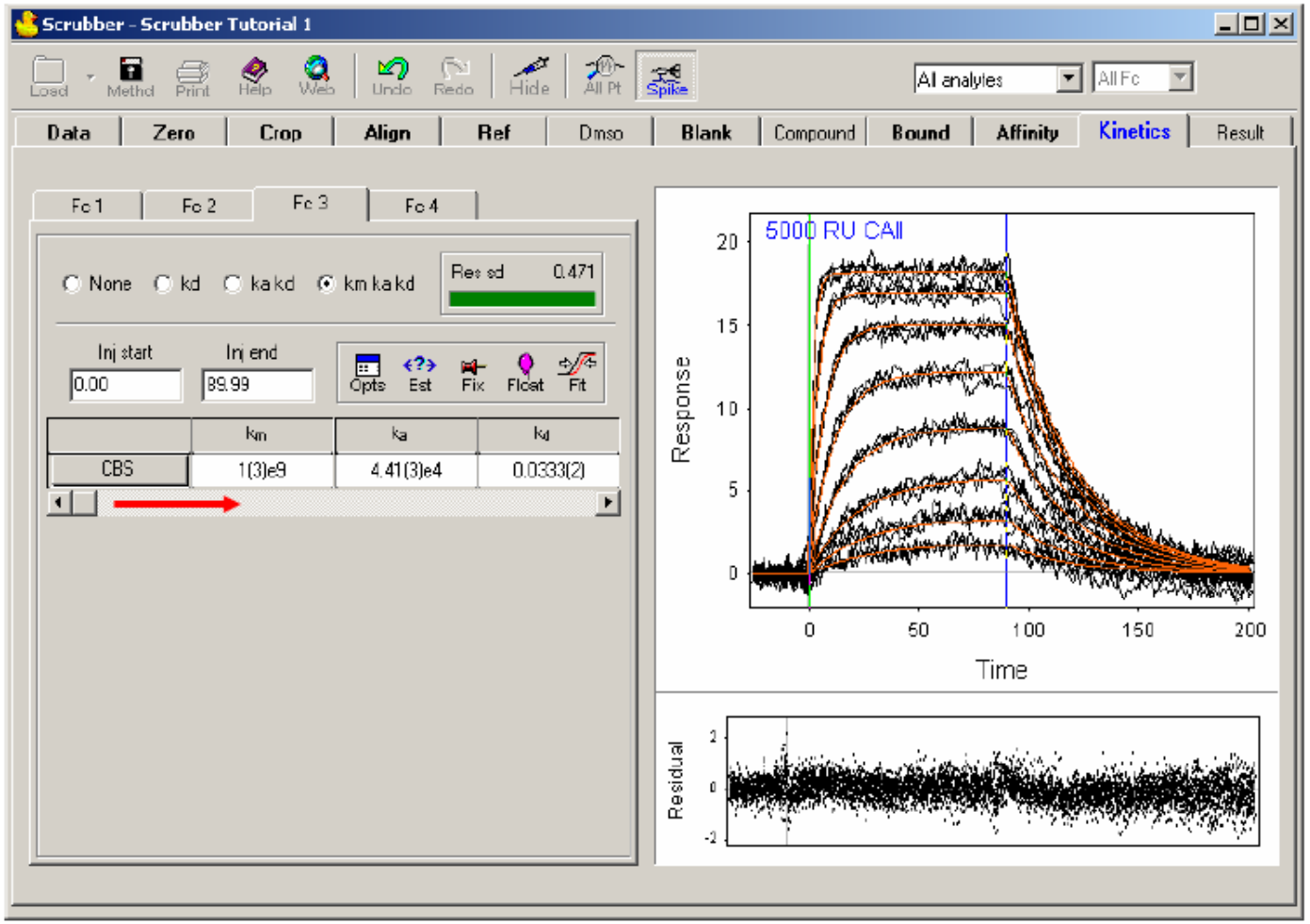
$$\frac{\partial \alpha(x,y,t)}{\partial t} = D\left(\frac{\partial^2 \alpha(x,y,t)}{\partial y^2}\right) - 4v_{\max}\frac{y}{h}\frac{\partial \alpha(x,y,t)}{\partial x}$$

  - “two compartment model”: bulk of the channel with convection transport and constant α and layer of thickness h_l next to the sensor.

$$\frac{d\alpha}{dt} = \frac{1}{h_l}\left[k_M\left(\alpha_0 - \alpha\right) - \frac{d\gamma}{dt}\right] \qquad k_M \approx 1.282\left(\frac{v_{\max} D^2}{hl}\right)^{1/3}$$

Data processing

- Software:
  - Scrubber2, (Biosensor Tools http://www.cores.utah.edu/Interaction/scrubber.html)
  - *BiaEvaluation (Biacore AB, Sweden)*



Data processing

- Global analysis:

$$A + B \underset{k_D}{\overset{k_A}{\rightleftarrows}} AB$$

  - All responses within the data set are fitted to the same values of k_A and k_D.
  - Chi-squared is calculated for all curves

mass transfer limited $$A \underset{k_m}{\overset{k_m}{\rightleftarrows}} A + B \underset{k_D}{\overset{k_A}{\rightleftarrows}} AB$$

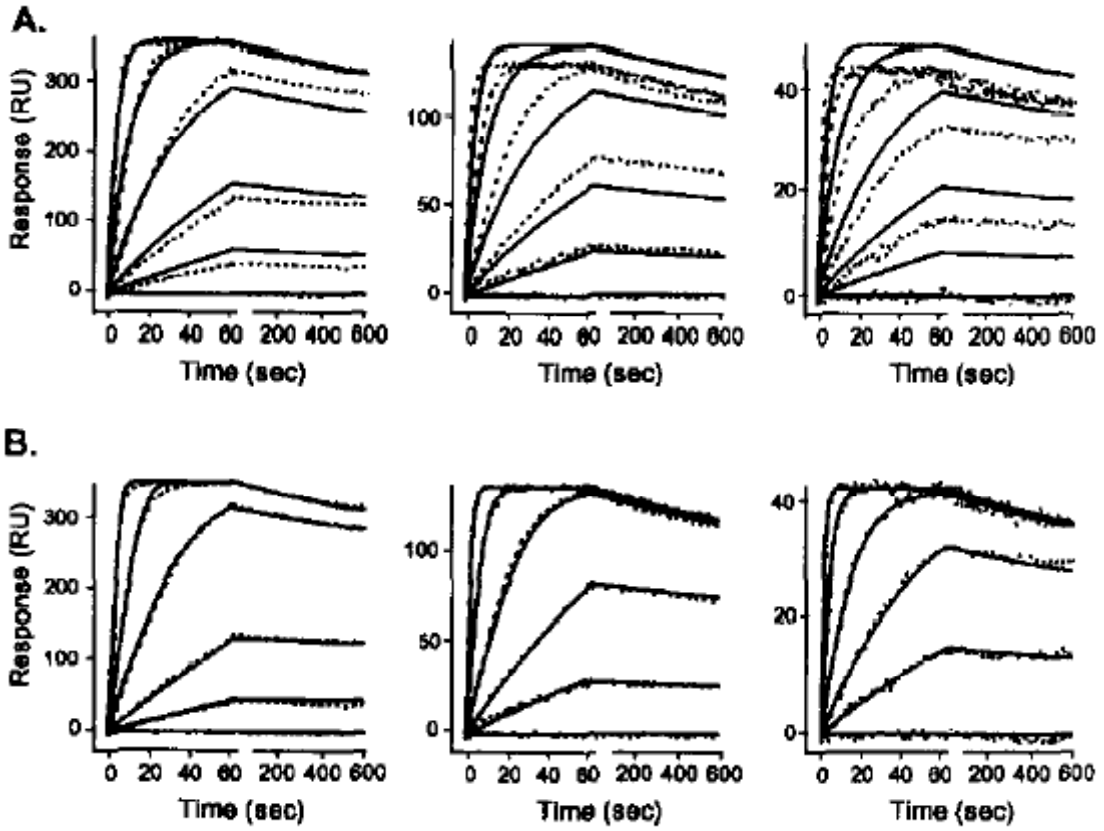
  - conditions to reduce mass transfer effect: low ligand density, high flow rate.

Data processing

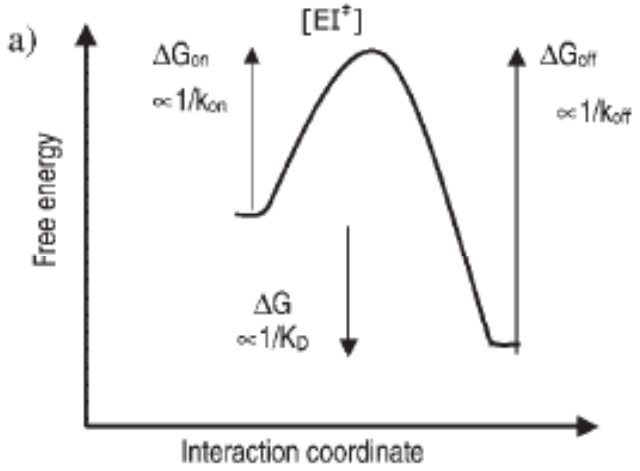
- Protein-antibody interaction

Best fit using bimolecular model

Best fit using mass-transport model



Thermodynamics for drug discovery

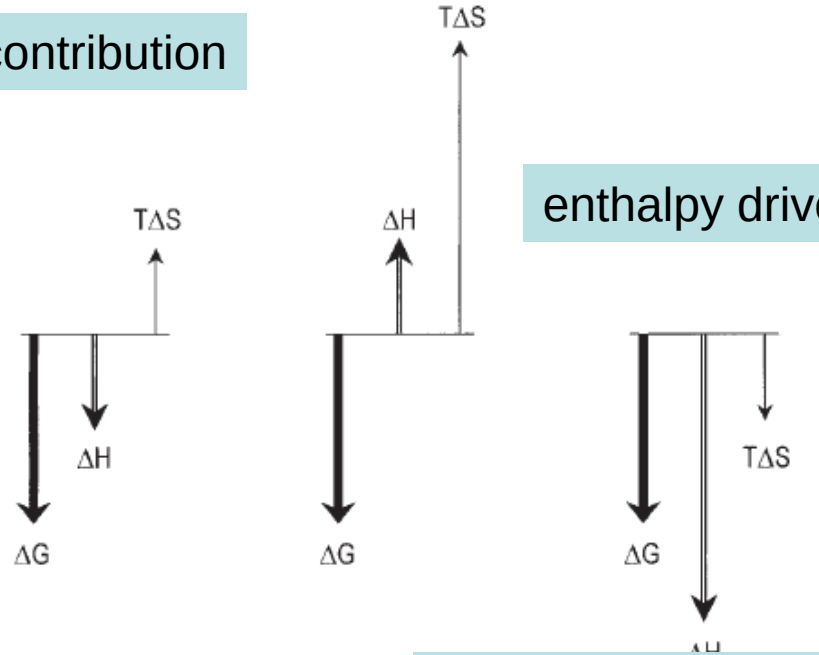


$$\Delta G = \Delta H - T\Delta S$$

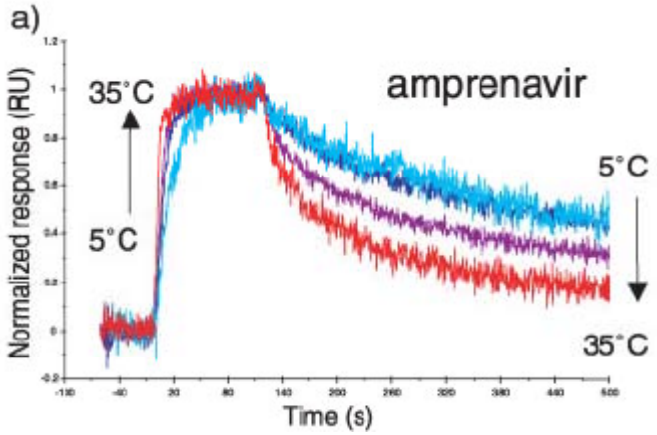
entropy driven

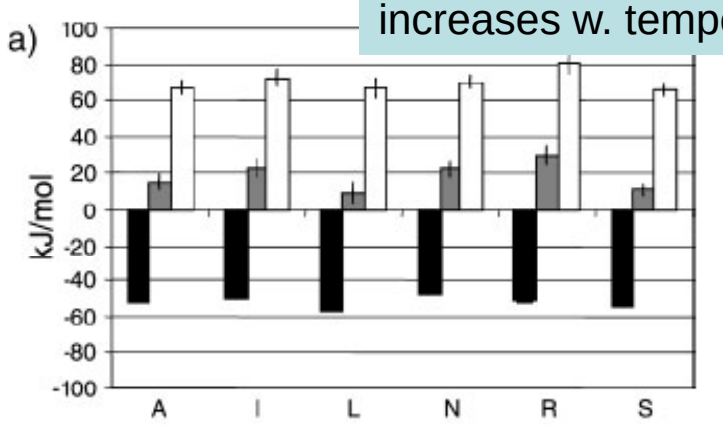
even contribution

enthalpy driven



large entropy: affinity increases w. temperature





Shuman et al, J.Biomol.Rec.17, p.106 (2004)

Problem

- Derive equations for parallel pseudo first-order reaction kinetics with a single receptor and two different analytes.