Kinetic & Affinity Analysis

An introduction

What are kinetics and affinity?

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- Kinetics
  - » How fast do things happen? – Time-dependent
  - » Association – how fast molecules bind
  - » Dissociation – how fast complexes fall apart
  - » Kinetics determine whether a complex forms or dissociates within a given time span
- Affinity
  - » How strong is a complex? – Time-independent
  - » Affinity determines how much complex is formed at equilibrium (steady state where association balances dissociation)

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What is the relevance of binding kinetics?

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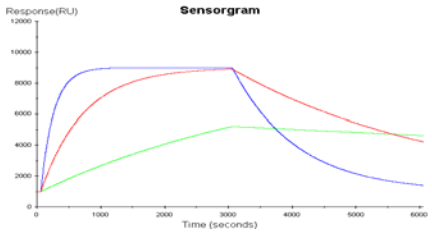
- The cell is a dynamic system – rarely in equilibrium
- The same affinity can be resolved into different on and off rates for different interactions
  - » Kinetic data reveal more information



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Same affinity but different kinetics

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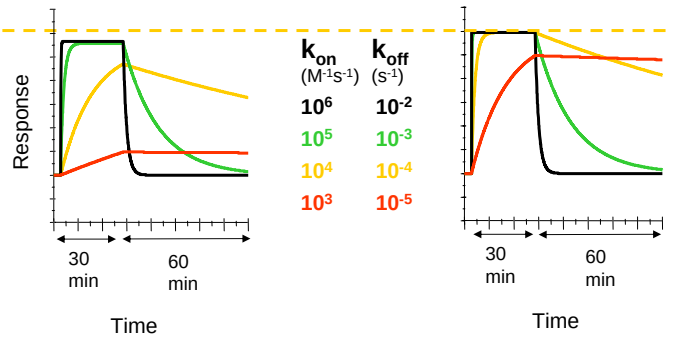
- All 4 compounds have the same affinity K_D = 10 nM = 10^{-8} M
- The binding kinetic constants vary by 4 orders of magnitude

All target sites occupied

Concentration = 100 nM

Concentration = 1000 nM



k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})
10^6	10^{-2}
10^5	10^{-3}
10^4	10^{-4}
10^3	10^{-5}

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Three ways to obtain kinetic and affinity data in Biacore

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- Monitor association and dissociation rates
  - » Affinity yes Kinetics yes
- Monitor steady state levels
  - » Affinity yes Kinetics NO
- Measure free analyte in solution
  - » Affinity yes Kinetics NO

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Rate equations for 1:1 kinetics

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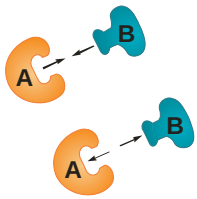
$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$



Association: $\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B]$

Dissociation: $\frac{-d[AB]}{dt} = k_d \cdot [AB]$

Net rate equation:

$$\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B] - k_d \cdot [AB]$$

M/s $M^{-1}s^{-1}$ M M s^{-1} M

where

k_a = association rate constant $[M^{-1}s^{-1}]$

k_d = dissociation rate constant $[s^{-1}]$

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Equilibrium constants

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At equilibrium:

Association = Dissociation

$k_a \cdot [A] \cdot [B] = k_d \cdot [AB]$

$M^{-1}s^{-1}$ M M s^{-1} M

The equilibrium constants:

$K_A = \frac{k_a}{k_d} = \frac{[AB]}{[A] \cdot [B]}$ the equilibrium association constant $[M^{-1}]$

$K_D = \frac{k_d}{k_a} = \frac{[A] \cdot [B]}{[AB]}$ the equilibrium dissociation constant [M]

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Equilibrium and kinetics in Biacore

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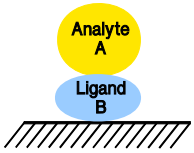
$$A + B \underset{k_d}{\overset{k_a}{\Leftrightarrow}} AB$$



- A is the analyte in solution
  - » Free concentration maintained constant by flow system
- AB is the complex
  - » Concentration of complex measured directly as R in RU
- B is the ligand on the surface
  - » Total concentration can be expressed in RU, as maximum binding capacity R_{max}
  - » Free concentration is R_{max}-R

We do not need to know the “real” concentration of ligand or complex

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Rate and affinity in Biacore terms

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$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

$$\frac{d[AB]}{dt} = k_a \cdot [A] \cdot [B] - k_d \cdot [AB]$$

$$\frac{dR}{dt} = k_a \cdot C \cdot [R_{max} - R] - k_d \cdot R$$

RU/s $M^{-1}s^{-1}$ M RU s^{-1} RU

A has one binding site and reacts with immobilized ligand
B has n identical and independent binding sites

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The net rate equation terms in a sensorgram

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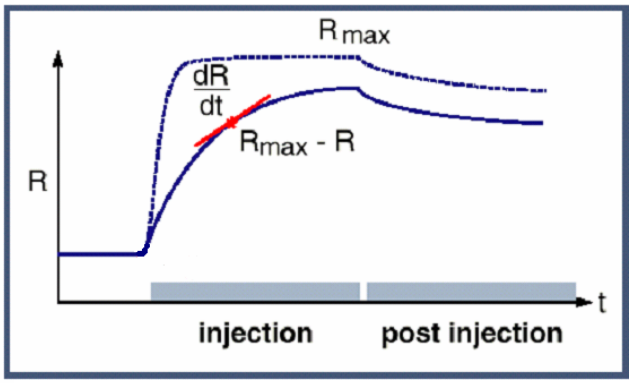

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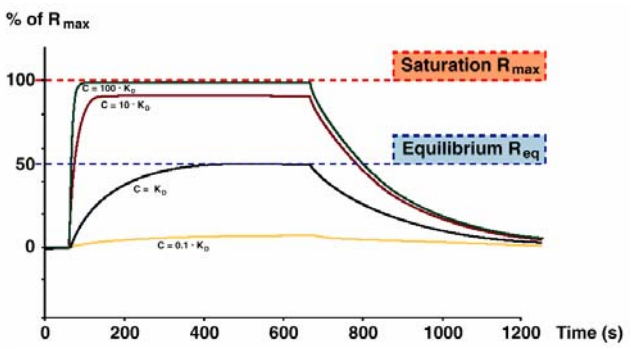
Information in a Sensorgram

- The relationship between R_{max}, R_{eq} and K_D



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Mass transport

- A phenomenon with relevance to kinetics measurements in Biacore
  - » Describes the movement of molecules from solution to a surface
  - » Is independent of biomolecular interaction processes
- Rates measured in Biacore depend on both mass transport and biomolecular binding
  - » The relative importance of mass transport effects can be largely controlled by the assay conditions used

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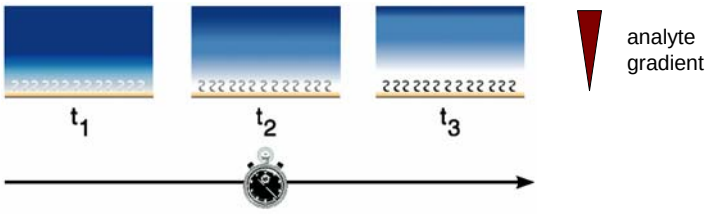
What is mass transport?

- Diffusive mass transport
  - » Simple example in a static system



- Over time, analyte concentration at the surface will be depleted and a gradient will be generated through the liquid layer

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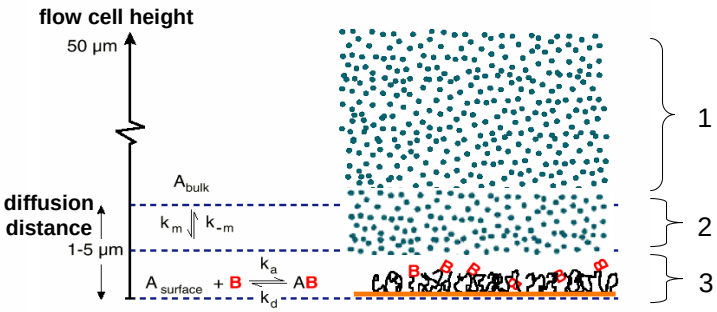
Analyte consumption & supply



1. Analyte supplied by convection (continuous flow)
2. Diffusion becomes increasingly important as the flow rate reduces closer to the surface
3. Biomolecular interaction processes at the ligand/analyte interface

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Dealing with mass transport limitations

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- Low R_{max} (ligand density)
- High flow rates
  - » High flow rates reduce diffusion distance
- Mass transport correction included in all kinetic models

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Experimental Design

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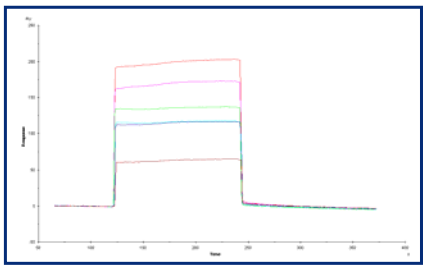
Experimental design

Affinity determination by steady-state analysis

- Determine steady state binding levels over a range of analyte concentrations
- High immobilization level
- Concentration range should cover at least 20-80% saturation of the surface
- Use reference surface
- Include at least one concentration in duplicate
- Include zero concentration sample



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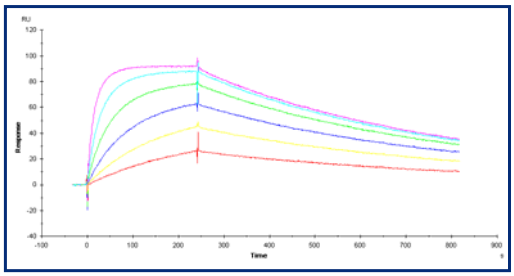
Important experimental parameters

Kinetic analysis

- The purity of the reagents
- Immobilization procedure
- Immobilization level
- Ligand activity
- Flow rate
- Analyte concentration range



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Analyte concentrations
Kinetic analysis

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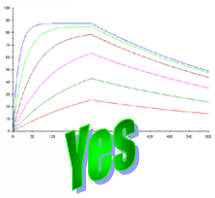
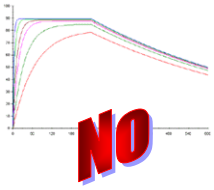
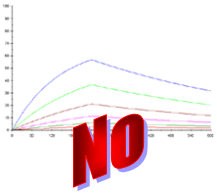
- Concentrations should cover a full range of binding curves
- Include at least one concentration in duplicate
- Include zero-concentration samples







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Summary

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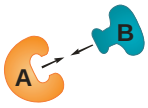

- Affinity analysis
  - » Derives the affinity constants
  - » For analysis of interactions with very fast on and off rates
- Kinetic analysis
  - » Derives the rate constants and the affinity constants
  - » For detailed characterization of a molecular interaction
  - » Interactions with the same affinity may have entirely different association and dissociation rate constants
  - » Rate constants may be more significant than affinity in understanding biological processes

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