Protein-sugar interaction: 
Surface Plasmon Resonance (SPR) biosensor analysis 
(Biacore™ technology)

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Overview

- Presentation of the Biacore™ technology
- Basic principles of SPR
- SPR detection and interaction analysis
- A typical interaction study
- SPR and lectin-carbohydrate interactions
- Examples
The Biacore™ technology

- Label-free, real time detection and monitoring of biomolecular interactions, based on SPR
- Information on a wide range of interaction parameters:
  - Specificity
  - Concentration
    - YES/NO binding response
    - How MUCH?
  - Affinity
    - How STRONG? $K_D$, $K_A$
  - Kinetics
    - How FAST? Rate constants $k_a$, $k_d$
  - Thermodynamics
    - Affinity and kinetics vs temperature

Basic Principle of the Biacore™ technology

- A binding molecule (ligand) is immobilized on a sensor surface
- The target molecule (analyte) is passed over the surface in a continuous buffer flow through a microfluidic system
- Analyte binding to the immobilized ligand is detected using SPR
The biospecific surface (sensor chip)

- Glass slide
- Prism
- Glass
- dextran layer (~100 nm)
- linker layer (goldlayer (~50 nm))
- immobilized biomolecule

The carboxymethylated (CM) dextran matrix (Biacore)
- Hydrophilic
- Easy use for covalent coupling
- High binding capacity
- Low non-specific binding
- Flexible => immobilized ligands can move to a certain extent on the surface
- High chemical resistance

Other matrices
- BioRad: alginate
- Xantec 3D hydrogel: agarose, alginate, PEG, cellulose, pectin

Liquid handling

Integrated micro Fluidics Cartridge (IFC)

Miniaturized system
- Integrated and automated liquid handling

Sensor surface
- Glass slide
- Prism
- IFC channels
- IFC
- Volume 20-40 nl

4 flow cells, formed by contact of the IFC on the sensor chip surface

Individual or serial use of the flow cells
- Automatic reference signal subtraction from sample signal, using flow cells 1 or 3 as references

Constant analyte concentrations at the sensor surface
How does SPR work?

Total internal reflection (TIR)

Light entering the glass semi-circular prism (refractive index $n_1 = 1.5$) undergoes total internal reflection at the interface with the medium of a lower refractive index (buffer, $n_2 = 1.33$) at an angle of incidence above a critical angle ($\theta$).

Creation of the evanescent field wave

A light electromagnetic component, called evanescent wave, enters the low refractive index medium over a short distance from the TIR interface (evanescent field).

The amplitude of this wave decreases exponentially with distance, decaying over a distance of about one wavelength from the surface.
Surface plasmon resonance (SPR)

The interposition of a gold film at the interface generates a resonance phenomenon between free electrons at the metal surface and incident photons, yielding a loss of energy in the reflected light => SPR is seen as a dip in the intensity of reflected light at a specific angle of incidence ($\theta$).

The conditions for SPR are sensitive to the refractive index (RI) of the medium in which the evanescent wave propagates.

How does BIAcore use SPR to detect biomolecular interactions?

- The detector continuously records the position of reduced light intensity and calculates the SPR angle
- Biomolecular interactions at the sensor surface change the RI within the evanescent wave penetration range
  => the angle of incidence required to create SPR is altered
  => this change is measured as a response signal

$10^6$ RI change $\Leftrightarrow 10^{-4}$ deg deviation $\Leftrightarrow 1$ RU (Resonance Unit) $\Leftrightarrow 1$ pg bound/mm$^2$ surface
SPR detection and interaction analysis

- Importance of the buffer refraction index (DMSO, glycerol)
- RI values for glycoproteins, lipoproteins and nucleic acids of the same order of magnitude => mass detector essentially independent of the nature of the interactants
- SPR observed within a short distance from the gold interface: no quantitative analysis possible for analytes of big size (supramolecular assemblies, microorganisms, cells)
- Soluble analyte not penetrated by incident light => measurements possible on turbid or opaque samples
- Real-time measurement => kinetics (not necessary to reach equilibrium)
- Detection limit: 100-180 Da (Biacore T100-3000)

A typical interaction study

- Immobilization of the ligand on the sensor chip (+ reference surface)
- Injection of the analyte on immobilized ligand and reference surfaces
- Regeneration of the surface
- Data evaluation

Reference surface:
- mock surface (activated/blocked, capture surface without ligand)
- similar but non-interacting ligand (BSA, scrambled peptide)

Flow rate: 1 - 100 µl/min
Sample volume: 5 - 750 µl
Temperature: 4 - 40°C
**Immobilization Strategies**

**Direct coupling**
- Membrane-bound proteins
- HPA-chip
  - Adsorbed lipid monolayer
- Integral membrane proteins
  - Supported bilayer
  - Dextran-modified with lipophilic compounds

**Indirect coupling**
- Via a capture molecule covalently coupled to a CM chip
- Antibodies
- Biotinylated ligands
- His-tagged ligand

**Regeneration strategies**
- The activity of the immobilized ligand must remain unaffected
- Regeneration solution selected according the nature of the interaction (if known) => specific regeneration (interaction competitor (sugar), EDTA)
- Mild to more stringent conditions applied

<table>
<thead>
<tr>
<th>Strength</th>
<th>Acidic</th>
<th>Basic</th>
<th>Hydrophobic</th>
<th>Ionic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weak</strong></td>
<td>pH &gt; 2.5 formic acid, HCl</td>
<td>pH &lt; 9 HEPES/NaOH</td>
<td>pH &lt; 9 50% ethylene glycol</td>
<td>1 M NaCl</td>
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<tr>
<td></td>
<td>10 mM Gly/HCl</td>
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<tr>
<td><strong>Intermediate</strong></td>
<td>pH 2-2.5 formic acid, HCl, H3PO4</td>
<td>pH 9-10 NaOH</td>
<td>pH 9-10 50% ethylene glycol</td>
<td>2 M MgCl2</td>
</tr>
<tr>
<td></td>
<td>10 mM Gly/HCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Strong</strong></td>
<td>pH &lt; 2 formic acid, HCl, H3PO4</td>
<td>pH &gt; 10 NaOH</td>
<td>pH &gt; 10 25-50% ethylene glycol</td>
<td>4 M MgCl2 6 M GdnHCl</td>
</tr>
<tr>
<td></td>
<td>10 mM Gly/HCl</td>
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Nucleic acids, heparin: 0.2-0.5% SDS
Data evaluation (BIAeval software)

- Global fitting of experimental data from several concentrations to a predefined model
- Determination of kinetic constants (Langmuir 1:1)
  - association rate constant ($k_a$) : $10^3 - 10^7$ M$^{-1}$s$^{-1}$
  - dissociation rate constant ($k_d$) : $5 \times 10^{-6} - 0.5 \times 10^{-4}$ s$^{-1}$
- Determination of affinity constant ($K_D$)
  - from the kinetic constants: \[ K_D = \frac{k_d}{k_a} \]
  - (50pM -100 µM)
  - from equilibrium analysis (steady state model)
- Evaluation of fitting quality (acceptable statistics: residuals, Chi$^2$)
- Biological and experimental relevance of the calculated parameters ($R_{max}$)

Relevance of binding kinetics

The same affinity (identical $K_D$) can be resolved into different kinetic rate constants for different interactions

$k_a$ : driven by molecular recognition ≠ $k_d$ : driven by complex stability

Kinetic properties are critical to the therapeutic performance of drugs and affect multiple functional aspects (pharmacokinetics, dosing)

Rapid kinetics => frequent administration of low dose required to occupy target
Slower kinetics => administration of high dose occupies target for long time
Some limitations of Biacore™ SPR

- **Kinetics**
  - typical $k_a$ values range: $10^3$ to $10^7$ M$^{-1}$s$^{-1}$
  - typical $k_d$ values range: $10^{-5}$ to 0.5-1 s$^{-1}$

  To avoid mass transport (diffusion of the analyte from the bulk to the surface vicinity) limitation: use high flow rate and low immobilized ligand level

- **Affinity**
  
  typical $K_D$ range: $5 \times 10^{-11} - 10^{-4}$ M

  Equilibrium measurements: time to reach equilibrium determined primarily by $k_d$

  => high affinity interactions ($K_D < 10$ nM) with very slow $k_d$ values unsuitable for equilibrium analysis

- **Small molecules**
  
  <100 Da: difficult to detect

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SPR and lectin-carbohydrate interactions

Carbohydrate-protein interactions in biological systems mostly occur among **multivalent partners**

**Affinity**: describes the binding of a monovalent ligand to its partner

**Avidity**: takes into account multivalent interactions between partners

=> apparent enhanced functional affinity

Potency enhancement in multivalent ligands can result from different mechanisms, including clustering, chelation and statistical rebinding effects

=> may be exploited for generation of lectin inhibitors.

<table>
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<tr>
<th>Clustering</th>
<th>Chelation</th>
<th>Statistical rebinding (proximity)</th>
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<td><img src="image" alt="Clustering" /></td>
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<td><img src="image" alt="Statistical rebinding" /></td>
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**SPR** => importance of immobilized partner choice and of ligand surface density
Avidity in carbohydrate pattern recognition by the innate immune recognition protein mannan-binding lectin (MBL)

Lectin domain-micropattern interaction
- C-lectin domain
- Neck region
- Terminal sugar
- Oligosaccharide

Low affinity ($K_D > 10^{-3}$ M)
- 1:1 interaction

MBL-macropattern multivalent interaction
- MBL recognition domain (CRD)
- Carbohydrate recognition domain (CRD)
- Terminal sugar
- Microbial cell wall
- Collagen triple helix

High affinity ($K_D \sim 10^{-9}$ M)
- Multivalent interaction

Adapted from Hoffmann et al. (1999) Science 284:1313

Avidity in the context of SPR analysis
MBL-neoglycoconjugate (Man-BSA) interaction

Immobilized glycoconjugate - soluble lectin

Effect of lectin oligomerization
- 8 µM
- 0.4 µM
- 0.2 µM
- 0.125 µM

Effect of surface density
- 4000 RU immobilized Man-BSA
- 1600 RU immobilized Man-BSA
- 600 RU immobilized Man-BSA

SPR: sugar-lectin interactions
Gjelstrup et al. (2012) J Immunol 188:1292
Lectin-carbohydrate interactions

Lectin immobilized - soluble glycoconjugate

- Direct binding
  - Immobilized ectodomain of the macrophage mannose receptor
  - Soluble glycoprotein (Man-BSA) (0.0125-2.5 µM)

- Inhibition study
  - Immobilized ectodomain of the macrophage mannose receptor
  - Soluble Man-BSA (0.125 µM) injected in the presence of (Manα2man)₄Lys₃ glycocluster (0.5-25 µM)
  - Estimation of the lectin-glycocluster affinity from the inhibition of Man-BSA binding to the lectin

Inhibitors of lectin-carbohydrate interactions

- Glycoconjugate (BSA-mannotriose) immobilized
- Soluble ectodomain of DC-SIGN receptor (20 µM) injected in the presence of increasing concentrations of multivalent glycomimetics (pseudomanno-bioside and -trioside compounds)
- Steady state response => conversion to lectin residual activity => IC₅₀ determination

Strong antiviral activity found for the higher valency compounds with IC₅₀ in the nM range => new compounds in anti-viral strategy

Cell-lectin interaction

Injection of soluble bacteria [10^8 CFU/ml]

Injection of soluble inactivated vaccinia virus (stock: eq 2 x 10^{10} CFU/ml)

Screening of lectin-carbohydrate interactions
SPR imaging (SPRI)

Oligosaccharide array for the measurement of glycosaminoglycan (GAG)-protein interactions
SPRi of GAG-protein interactions

GAG: negatively charged glycans => regulate the activity of growth factors and cytokines
- Pyrrole-GAG spotted: 6 kDa heparin (HP6), chondroitin sulfate (CS), dermatan sulfate (DS)
- Proteins used: stromal derived factor(SDF)-1α, interferon(IFN)-γ, anti-CS IgM (control)

Useful References
[Several figures in this presentation were modified from those found in various Biacore® T100 Manuals, Handbooks and Brochures from www.biacore.com. © 2001-2007, Biacore AB]

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