# 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**
YES/NO **binding response**

**Concentration**
**How MUCH?**

**Affinity**
**How STRONG?** $K_D$, $K_A$

**Kinetics**
**How FAST?** Rate constants $k_a$, $k_d$

**Thermodynamics**
Affinity and kinetics *vs* temperature

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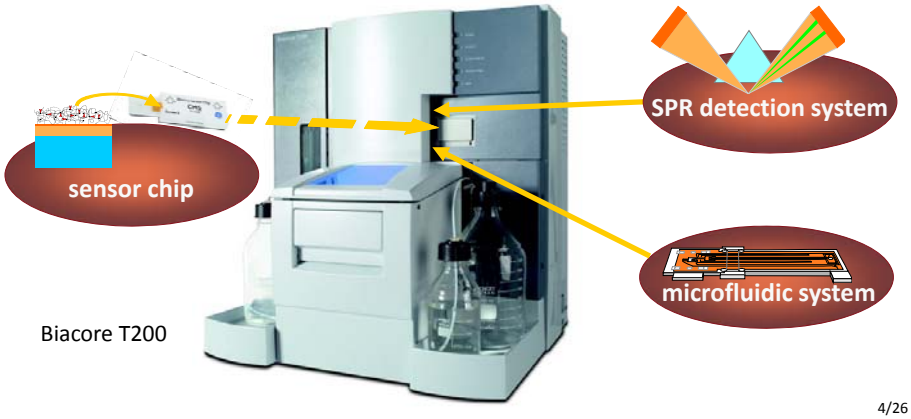
# Basic Principle of the Biacore™ technology

- 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




Biacore T200

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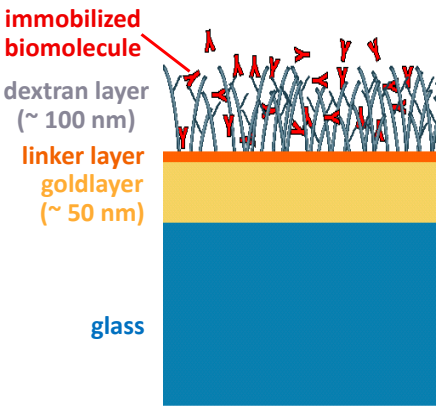
# The biospecific surface (sensor chip)




**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

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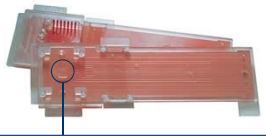
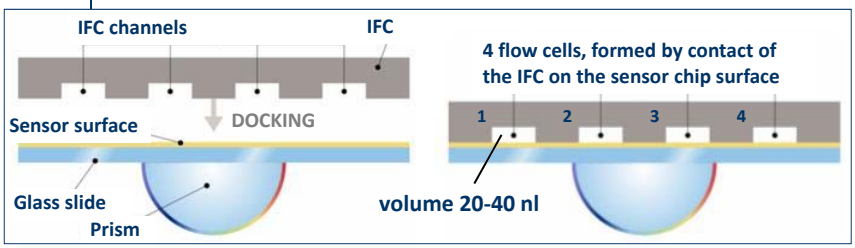
# Liquid handling

## Integrated micro Fluidics Cartridge (IFC)



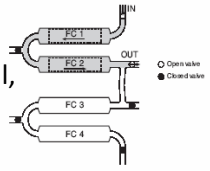
Miniaturized system

Integrated and automated liquid handling




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**

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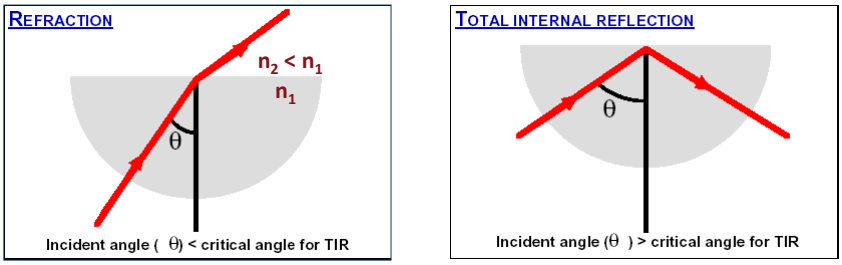
# How does SPR work?

## 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 (θ)

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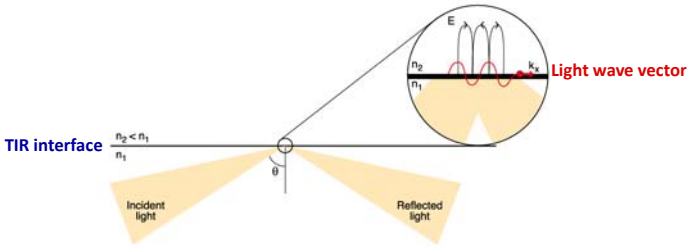
# Creation of the evanescent field wave




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.

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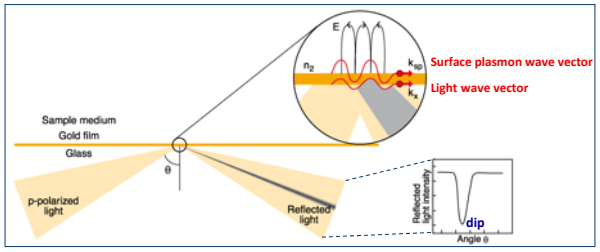
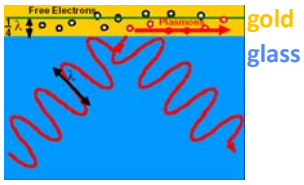
# Surface plasmon resonance (SPR)




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 (θ).

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

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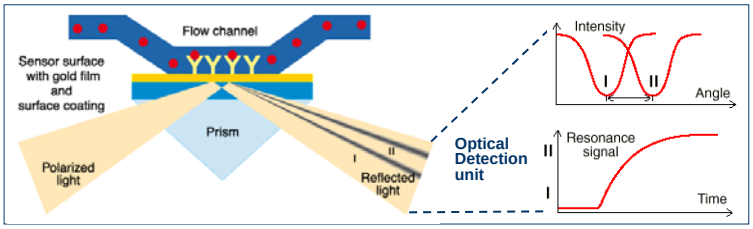
# How does BIAcore use SPR to detect biomolecular interactions?




- 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 ⇔ $10^{-4}$ deg deviation ⇔ 1 RU (Resonance Unit) ⇔ 1 pg bound/mm² surface**

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## 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)

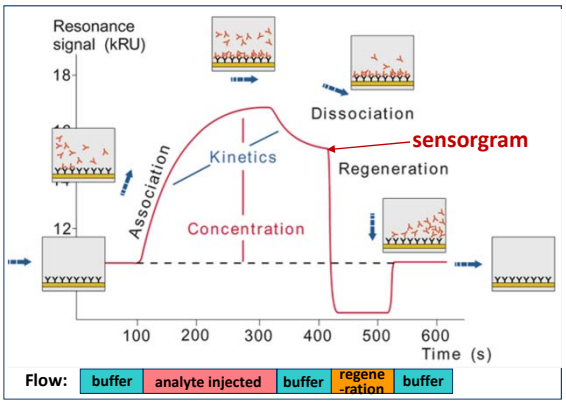


## A typical interaction study

- 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)

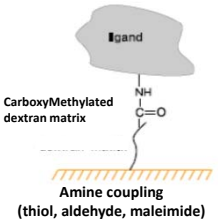
Flow rate : 1 - 100 µl/min
Sample volume : 5 - 750 µl
Temperature : 4 - 40°C



# Immobilization Strategies

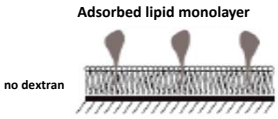
## Direct coupling

Covalent binding



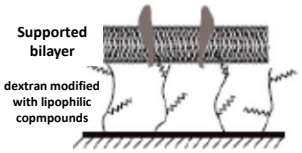

CM chip

Membrane-bound proteins




HPA-chip

Integral membrane proteins

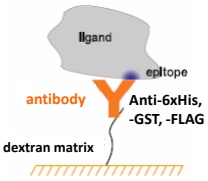



L1-chip

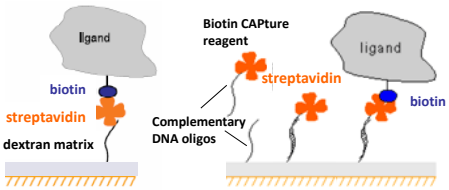
## Indirect coupling

Via a capture molecule covalently coupled to a CM chip

Antibodies



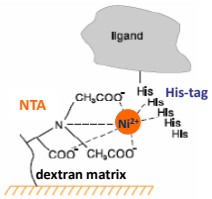

Biotinylated ligands




SA chip

CAP chip

His-tagged ligand




NTA chip

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# 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

| Strength | Acidic | Basic | Hydrophobic | ionic |
|---|---|---|---|---|
| **Weak** | pH > 2.5<br>formic acid, HCl<br>10 mM Gly/HCl | pH < 9<br>10 mM<br>Hepes/NaOH | pH < 9<br>50% ethylene glycol | 1 M NaCl |
| **Intermediate** | pH 2- 2.5<br>formic acid, HCl, $H_3PO_4$<br>10 mM Gly/HCl | pH 9-10<br>NaOH<br>10 mM Gly/NaOH | pH 9-10<br>50% ethylene glycol | 2 M $MgCl_2$ |
| **Strong** | pH < 2<br>formic acid, HCl, $H_3PO_4$<br>10 mM Gly/HCl | pH > 10<br>NaOH | pH > 10<br>25-50% ethylene glycol | 4 M $MgCl_2$<br>6 M GdnCl |

Nucleic acids, heparin: 0.2-0.5% SDS

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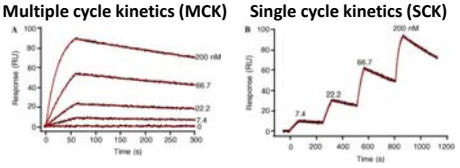
## Data evaluation (BIAeval software)

- Global fitting of experimental data from several concentrations to a predefined model

Multiple cycle kinetics (MCK) | Single cycle kinetics (SCK)



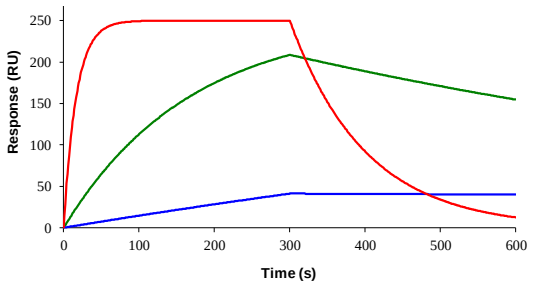
- Determination of **kinetic constants** (Langmuir 1:1)
  - association rate constant **($k_a$)** : $10^3$ - $10^7$ $M^{-1}s^{-1}$
  - dissociation rate constant **($k_d$)** : 5 x $10^{-6}$ – 0.5 x $10^{-1}$ $s^{-1}$
- Determination of **affinity constant ($K_D$)**
  - from the kinetic constants: **app** $K_D = k_d/k_a$ (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**




| $k_a$ ($M^{-1}.s^{-1}$) | $K_d$ ($s^{-1}$) |
|---|---|
| $10^5$ | $10^{-3}$ |
| $10^4$ | $10^{-4}$ |
| $10^6$ | $10^{-2}$ |

$K_D = k_d/k_a = 10^{-8}$ M

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 x $10^{-11}$ - $10^{-4}$ 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.

**Clustering** **Chelation** **Statistical rebinding (proximity)**


Adapted from Ordanini *et al* (2015) *Chem. Commun.* 51: 3816

**SPR** => importance of immobilized partner choice and of ligand surface density

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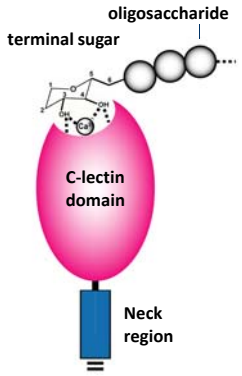
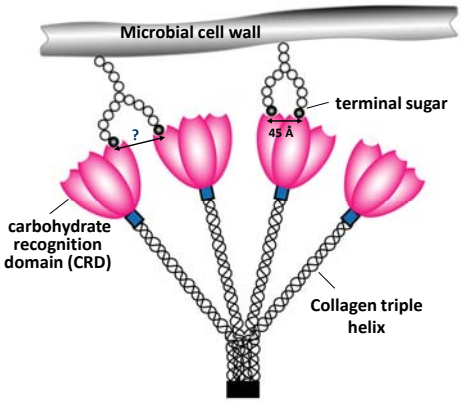
## Avidity in carbohydrate pattern recognition by the innate immune recognition protein mannan-binding lectin (MBL)

Lectin domain-**micropattern** interaction




MBL-**macropattern** multivalent interaction




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

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

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

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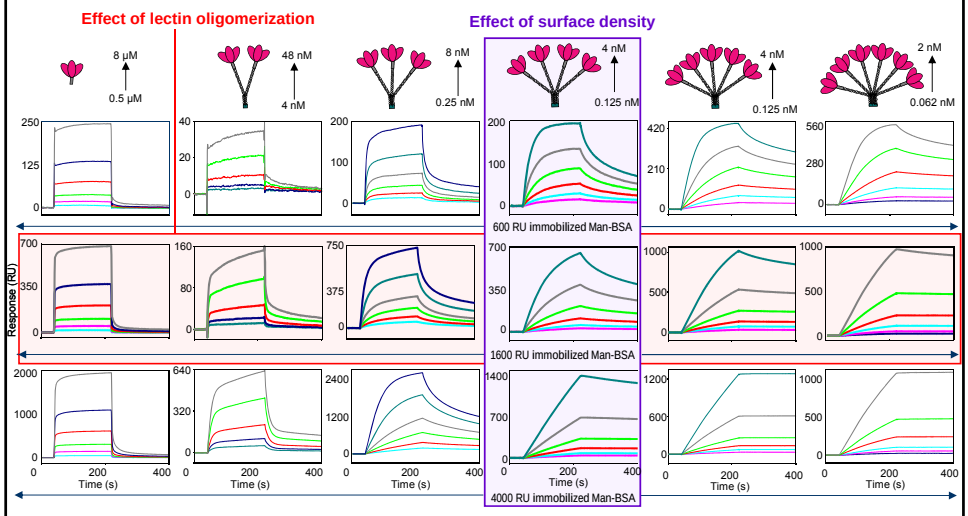
## Avidity in the context of SPR analysis MBL-neoglycocojugate (Man-BSA) interaction

Immobilized glycoconjugate - soluble lectin




SPR: sugar-lectin interactions

Gjelstrup et al (2012) J Immunol 188:1292

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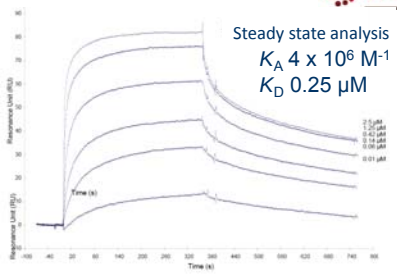
# Lectin-carbohydrate interactions

## Lectin immobilized - soluble glycoconjugate

- **Direct binding**

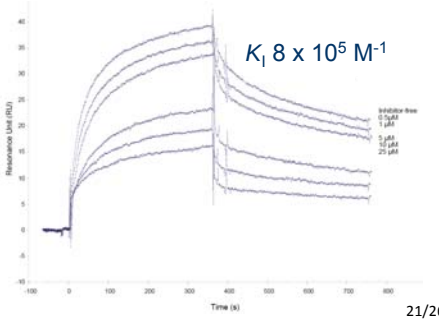
- Immobilized ectodomain of the macrophage mannose receptor
- Soluble glycoprotein (Man-BSA) (0.0125-2.5 µM)




Duverger *et al (2010) Methods Mol. Biol.* 627:157

- **Inhibition study**

- Immobilized ectodomain of the macrophage mannose receptor
- Soluble Man-BSA (0.125 µM) injected in the presence of (Manα2man)$_4$Lys$_3$ glycocluster (0.5-25 µM)
- Estimation of the lectin-glycocluster affinity from the inhibition of Man-BSA binding to the lectin

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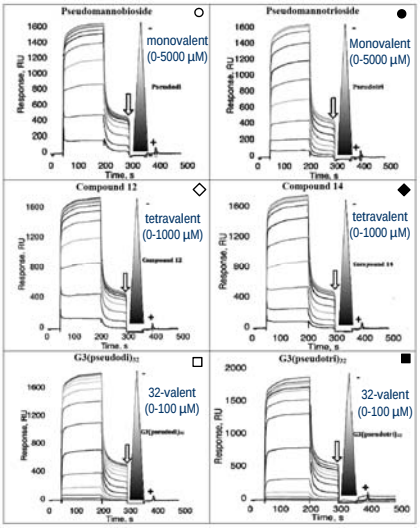
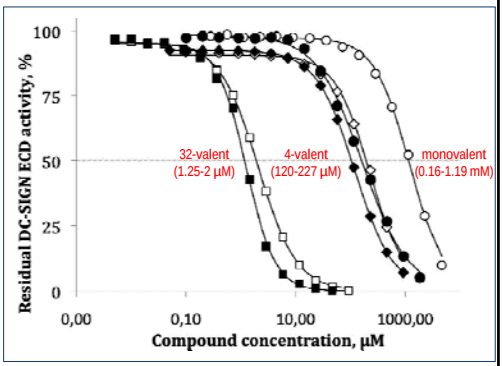
# Inhibitors of lectin-carbohydrate interactions

- 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 => **$IC_{50}$ determination**




Strong antiviral activity found for the higher valency compounds with $IC_{50}$ in the nM range => new compounds in anti-viral strategy

Luczkowiak et al (2011) **Bioconjugate Chem. 22, 1354**

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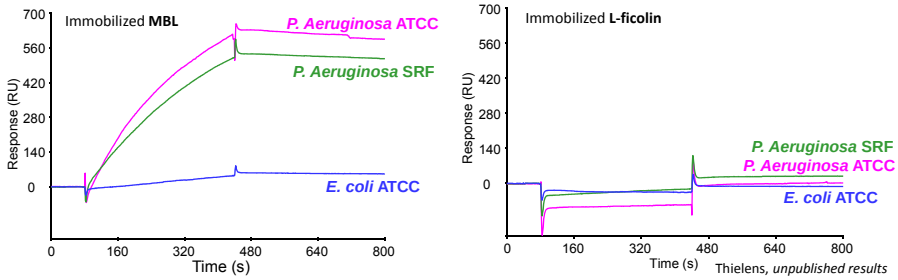
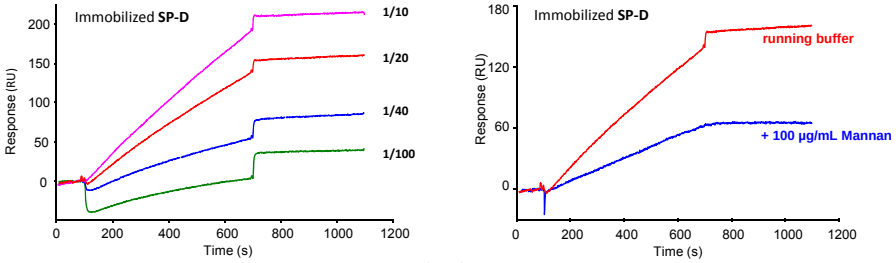
# Cell-lectin interaction

Injection of soluble bacteria ($10^8$ CFU/ml)




Thielens, *unpublished results*

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




Perino *et al* (2013) *Viruses*, 5, 928

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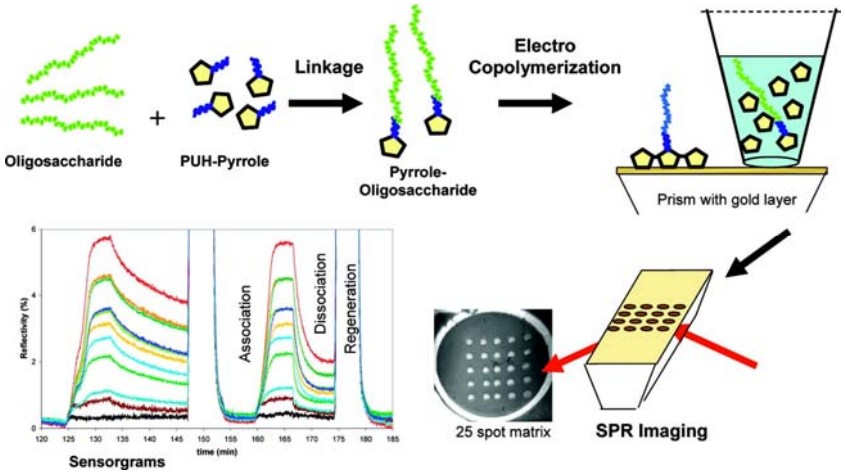
# Screening of lectin-carbohydrate interactions
## SPR imaging (SPRi)

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




Mercey et al (2008) *Anal. Chem.* 80, 3476

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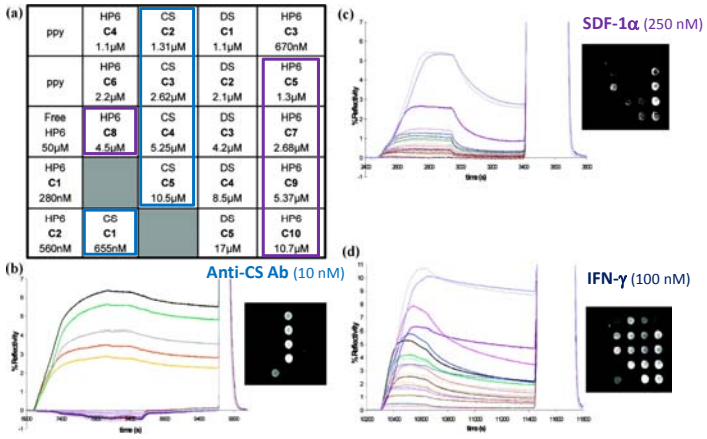
# SPRi of GAG-protein interactions

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)




Mercey *et al* (2008) *Anal. Chem.* 80, 3476



# Useful References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# Acknowledgements


**SPR platform:** **Partnership for Structural Biology (PSB)**
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**MP** (Membrane and Pathogens) team
**SAGAG** (Structure and Activity of GlycosAminoGlycans) team