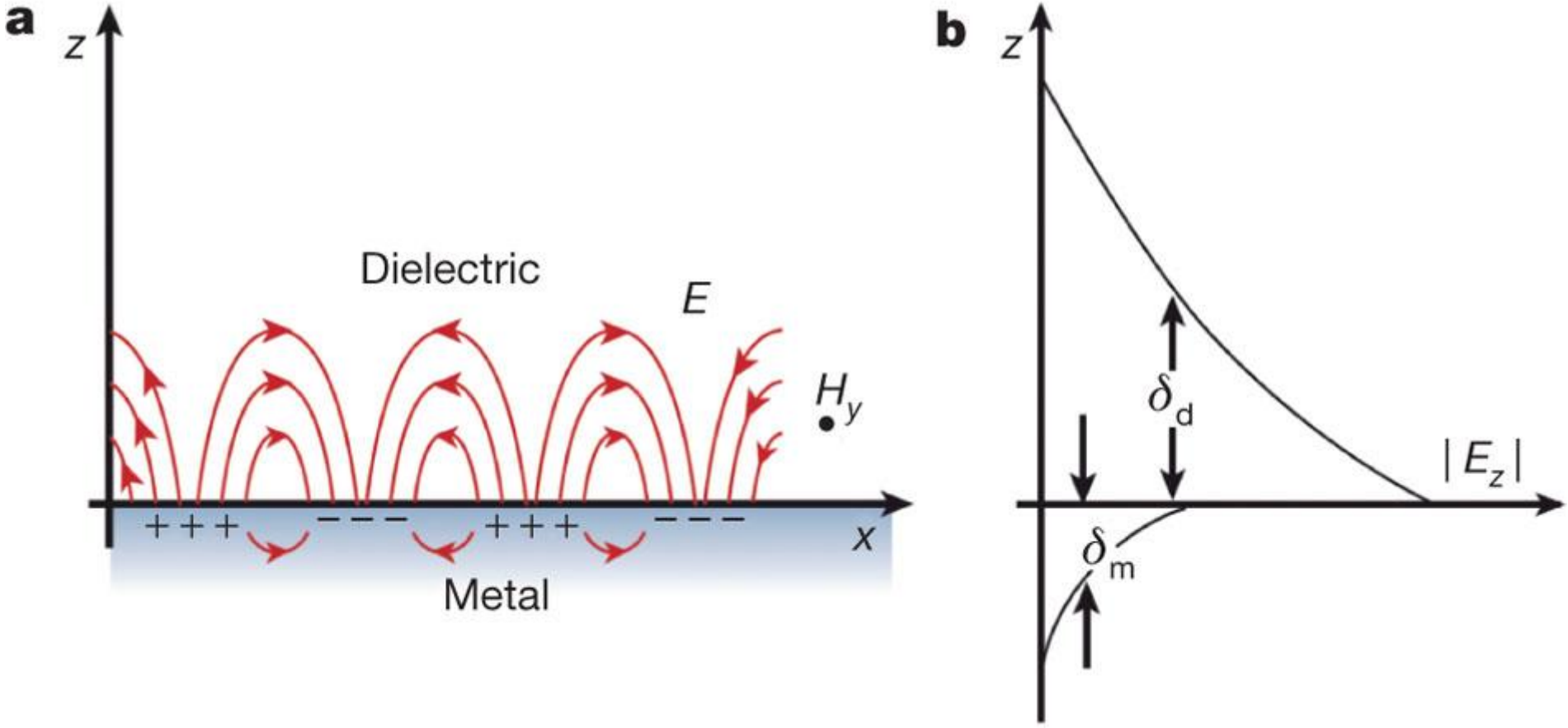
Surface plasmon resonance

S2004
Methods for characterization of biomolecular interactions – classical versus modern

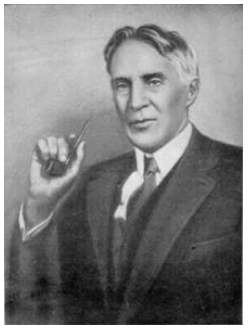
Mgr. Josef Houser, Ph.D.
houser@mail.muni.cz

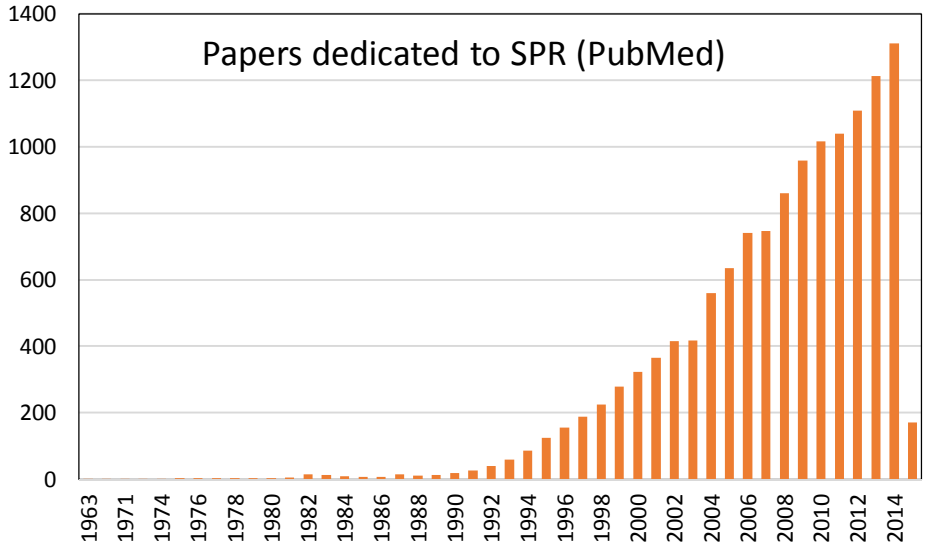
Surface plasmon resonance (SPR)

(Rezonance povrchového plasmonu) – collective oscillation of free electrons on metal-dielectric interface



History





1900's

Anomalous light reflection on metal grating (R. W. Woods)

1950's

Definition of plasmon

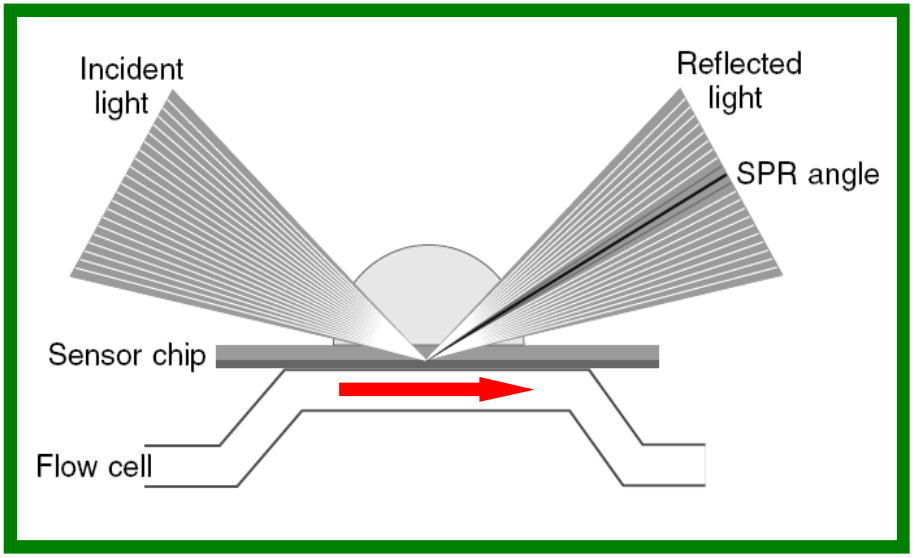
1980

First trials to use SPR in biomolecular interaction analysis

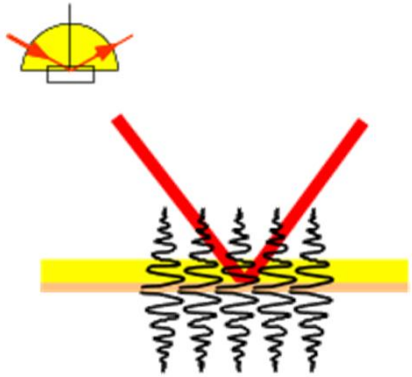
1990

First commercial instrument (Biacore)

SPR – Basic principles



- At the conditions of total internal reflexion (angle, wavelength) the incoming beam evokes exponential wave spread in opticaly less dense environment.

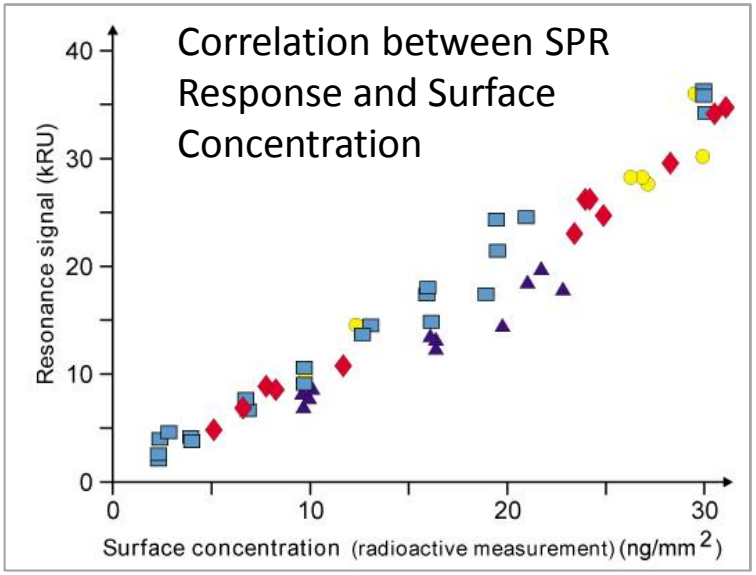


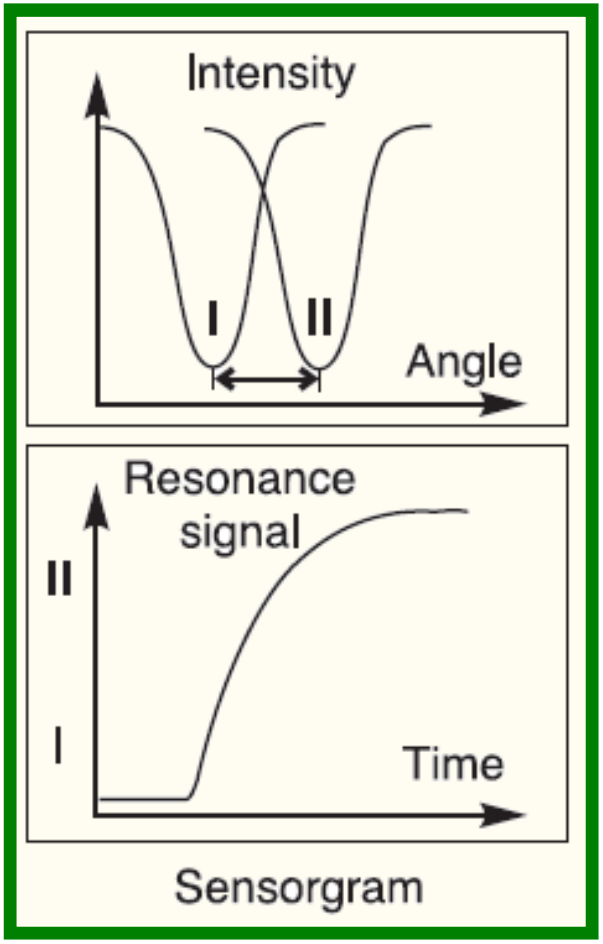
- At certain combination of incident angle and wavelength the free electrons on the metal surface are excited, what causes decrease in reflected light intensity.
- **This effect depends on refractive index that varies with the analyte binding to the surface-bound ligand.**

SPR – Basic principles

Refractive index change = change in light intensity at certain wavelength.

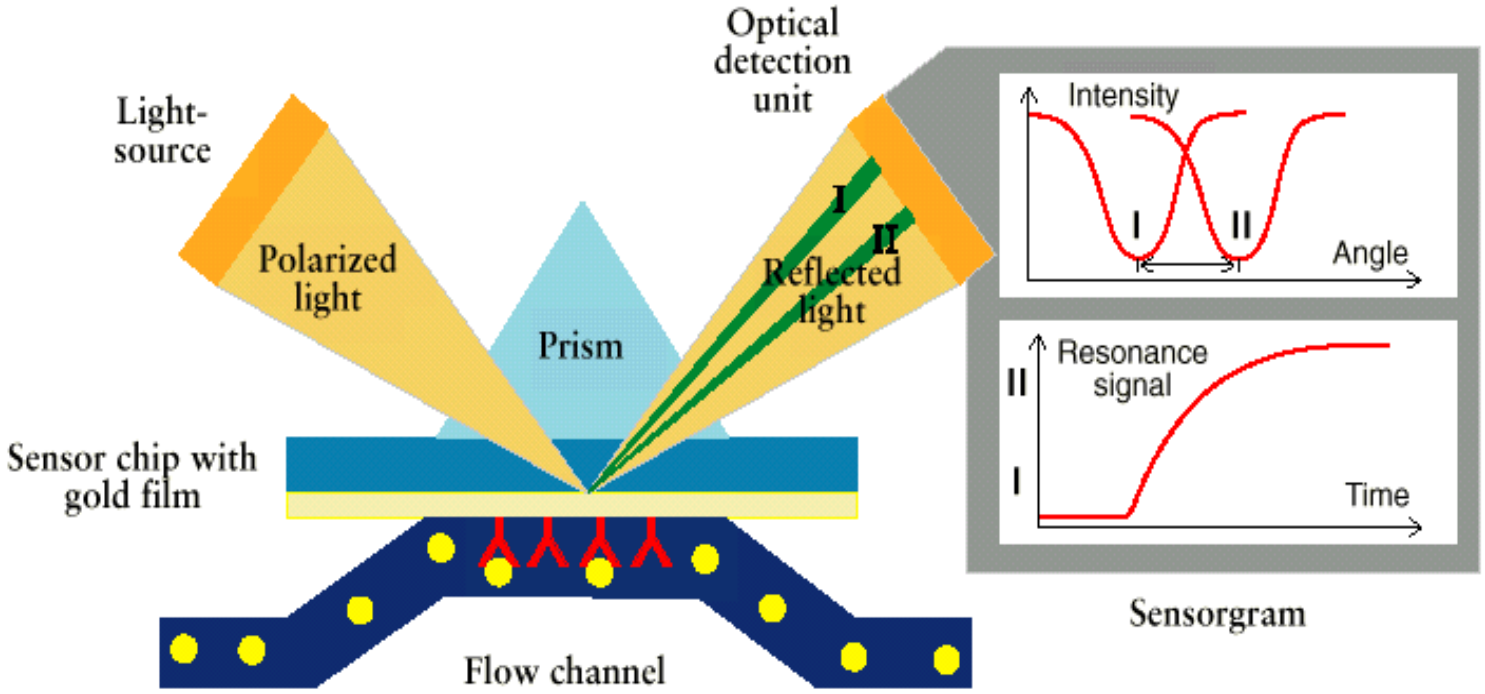
Corresponds also to change of mass on the chip surface = protein/ligand binding. (1 RU ~ 1 pg/mm^2)





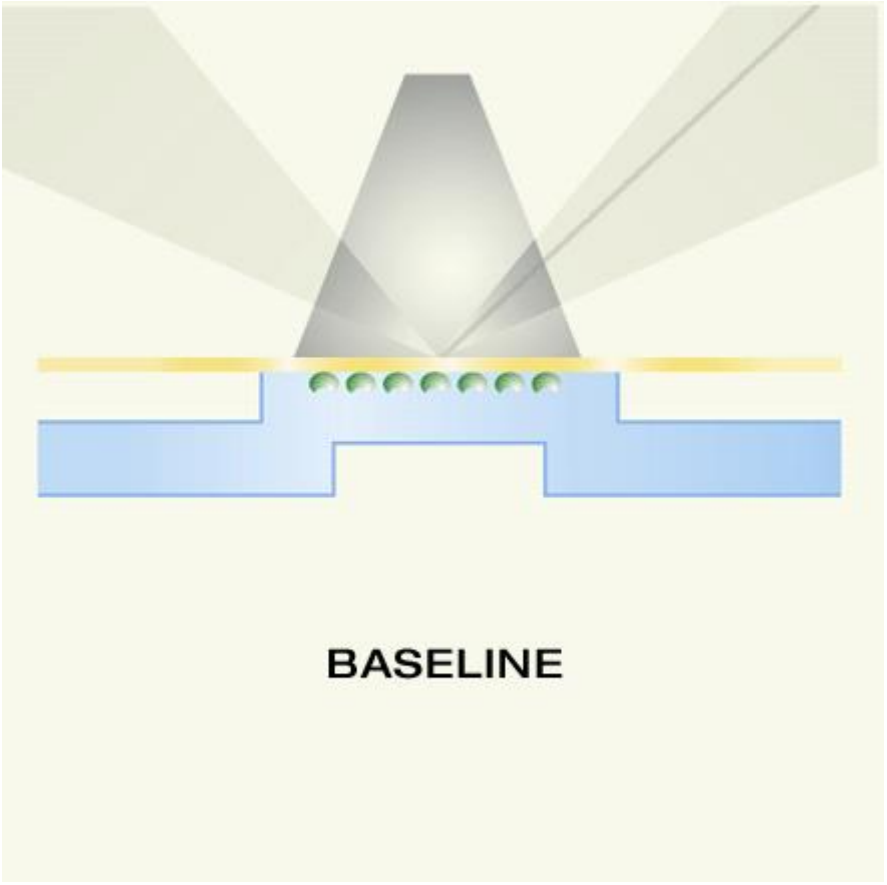
SPR – Basic principles

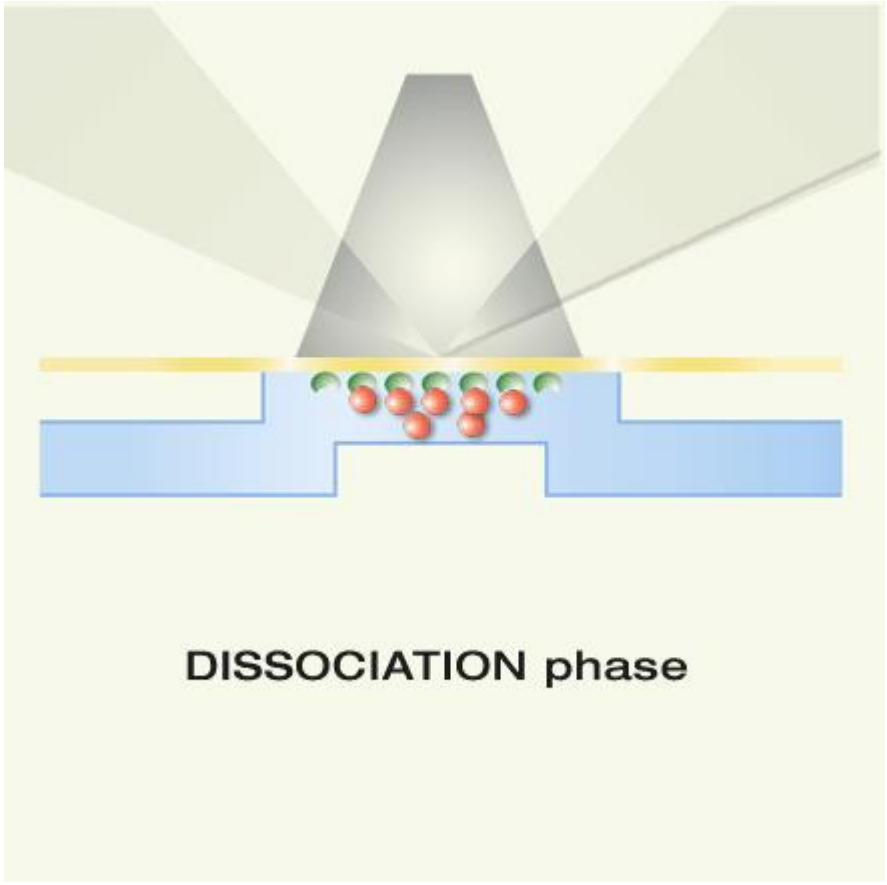
One binding partner immobilized on the chip surface (ligand), second is free in solution (analyte).



Association

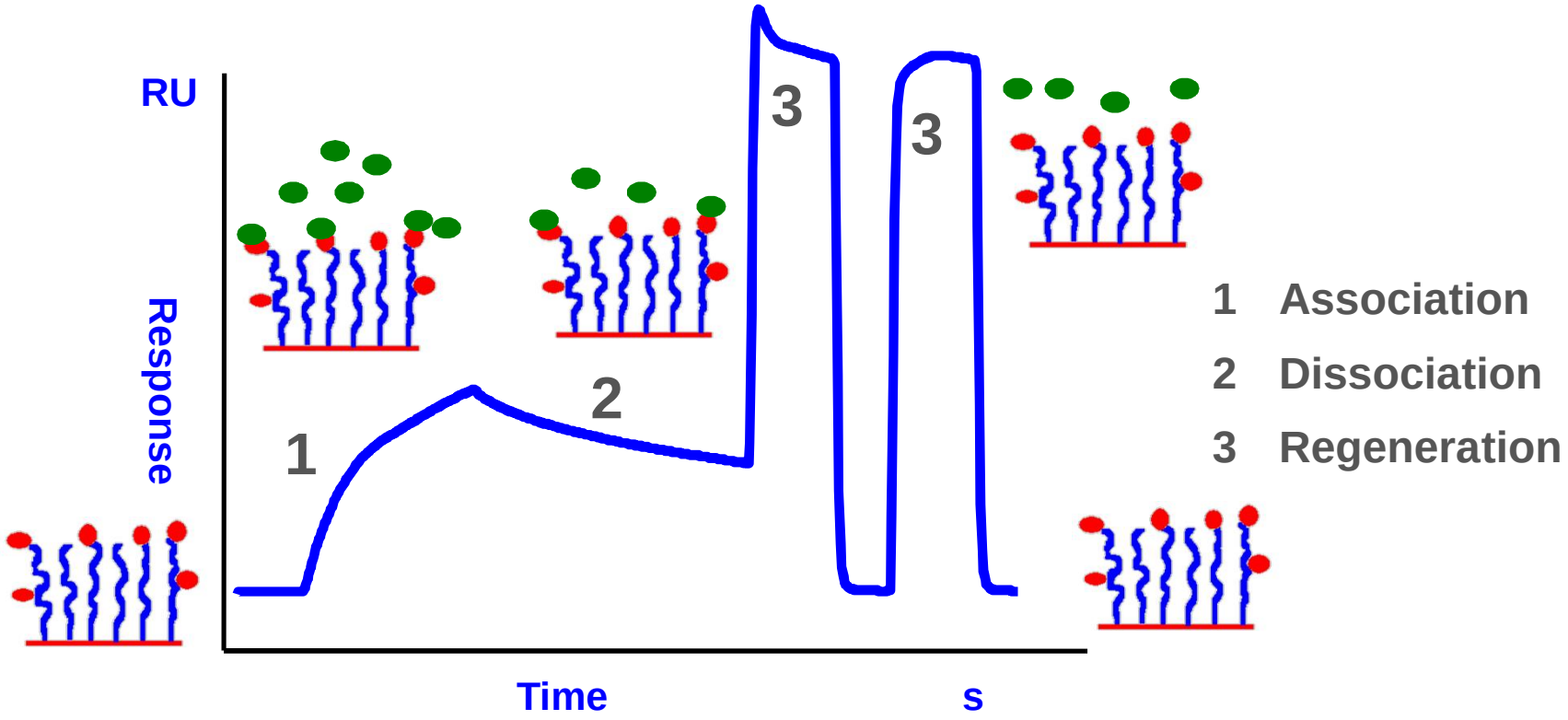
Dissociation





Simple binding - kinetics

ÅTypical binding curve – association and dissociation phase, (surface regeneration)



Binding experiment – steady state

$$v_{(association)} = k_a * [analyte]_{(solution)}$$
$$v_{(dissociation)} = k_d * [analyte]_{(bound)}$$

$$[analyte]_{(solution)} >> [analyte]_{(bound)}$$

$v_{(association)} >> v_{(dissociation)}$ **association phase**

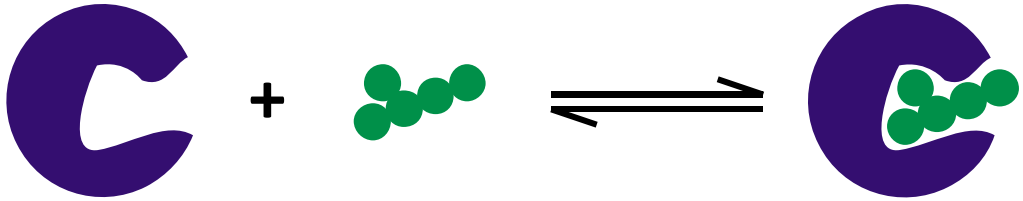
$v_{(association)} = v_{(dissociation)}$ **steady state**

-> response is proportional to K_D and R_{max}

$$[analyte]_{(solution)} << [analyte]_{(bound)}$$

$v_{(association)} << v_{(dissociation)}$ **dissociation phase**

Receptor ligand interaction



$$\frac{d[\mathrm{MX}]}{dt} = k_a[\mathrm{M}][\mathrm{X}] - k_d[\mathrm{MX}]$$

$$equilibrium: \frac{d[\mathrm{MX}]}{dt} = 0$$

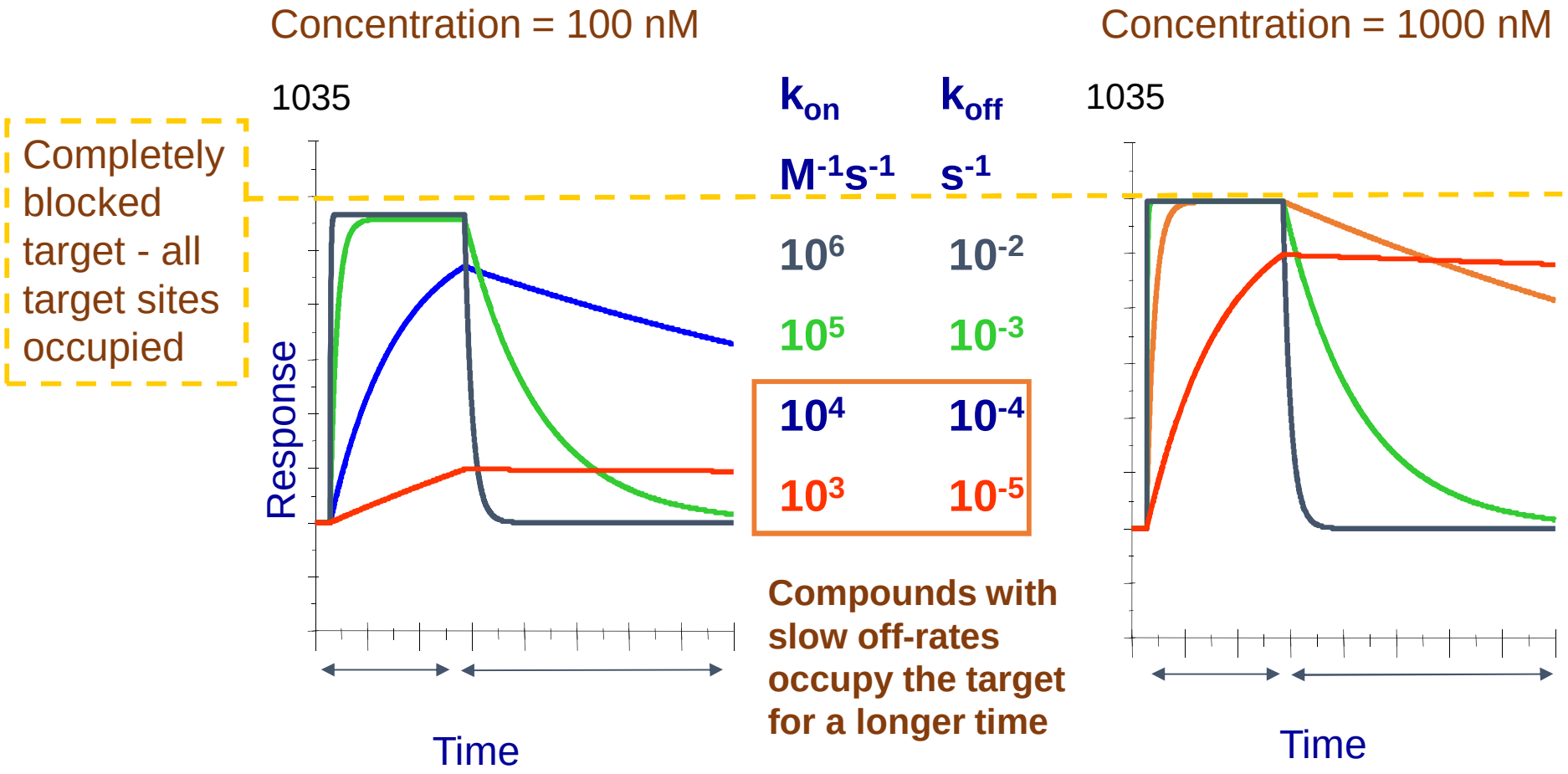
$$\mathrm{K_D} = \frac{1}{\mathrm{K_A}} = \frac{k_d}{k_a} = \frac{[\mathrm{M}][\mathrm{X}]}{[\mathrm{MX}]}$$

Surface plasmon resonance (SPR)

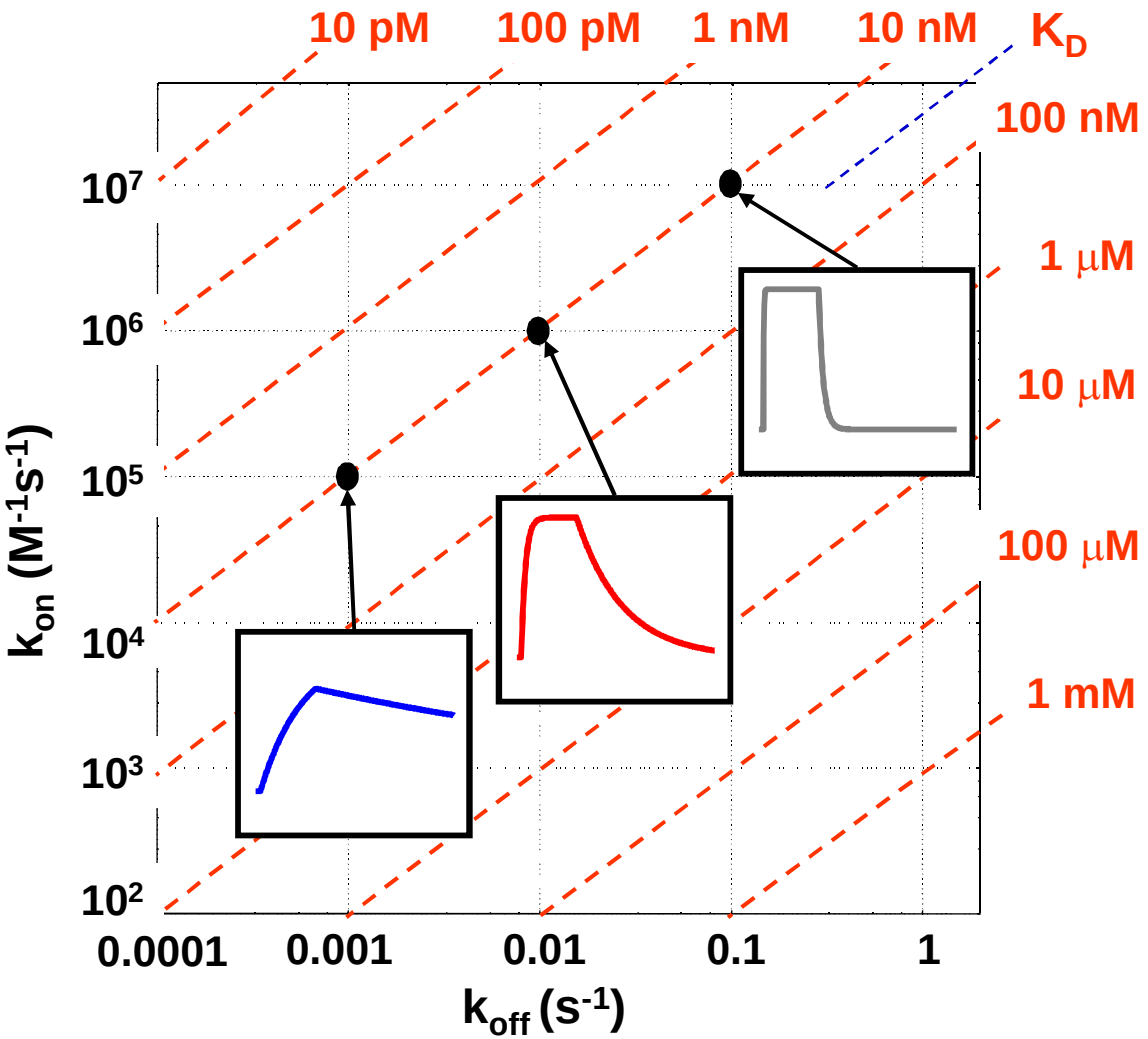
- **Kinetics of interaction**
- **Steady state**

Same affinity but different kinetics

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



Same affinity but different kinetics



HIV-p inhibitors: on-off rate map

Kinetics vs. affinity in Drug design

High affinity – first aim in drug discovery

BUT

May be caused by high k_a and k_d = fast dissociation (!)

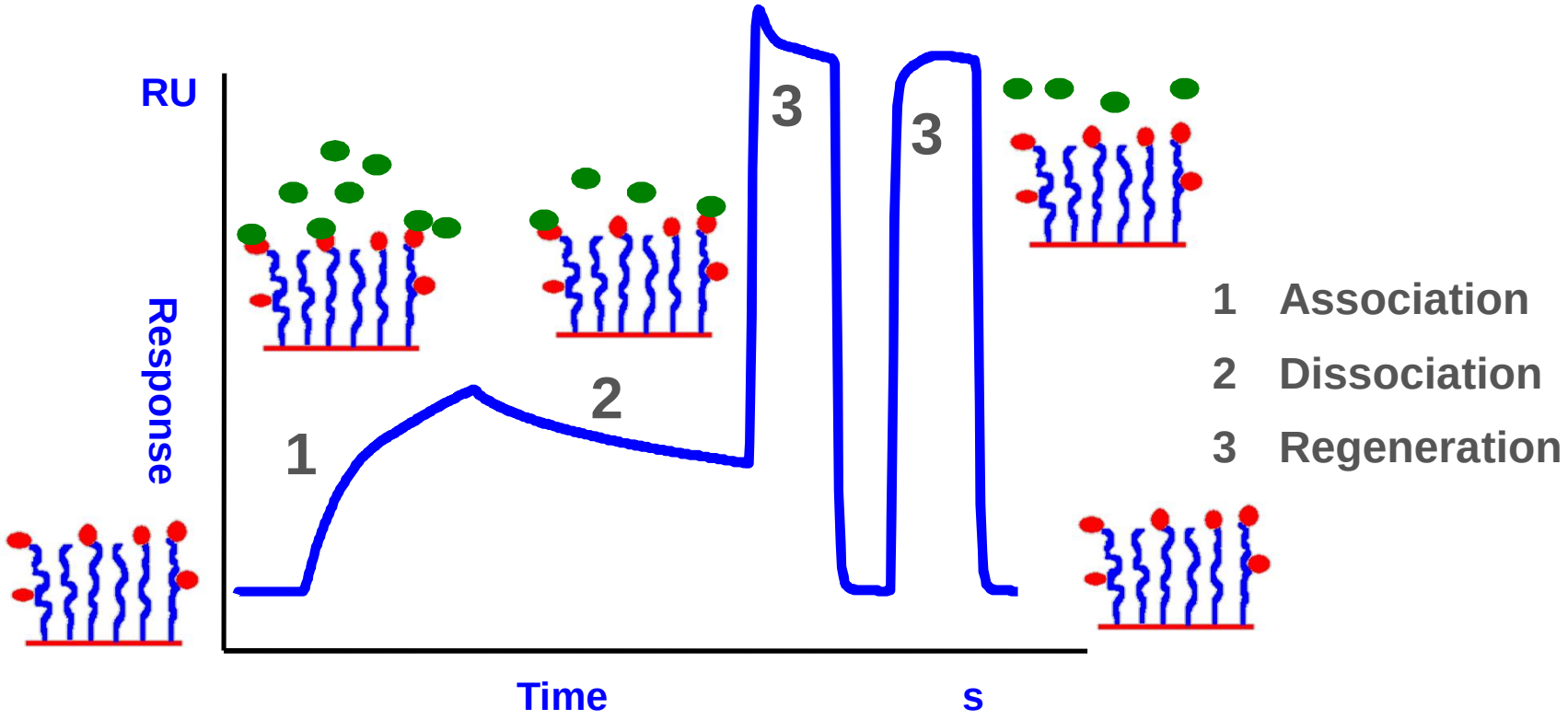
Kinetics – lower k_a AND k_d may mean longer effect

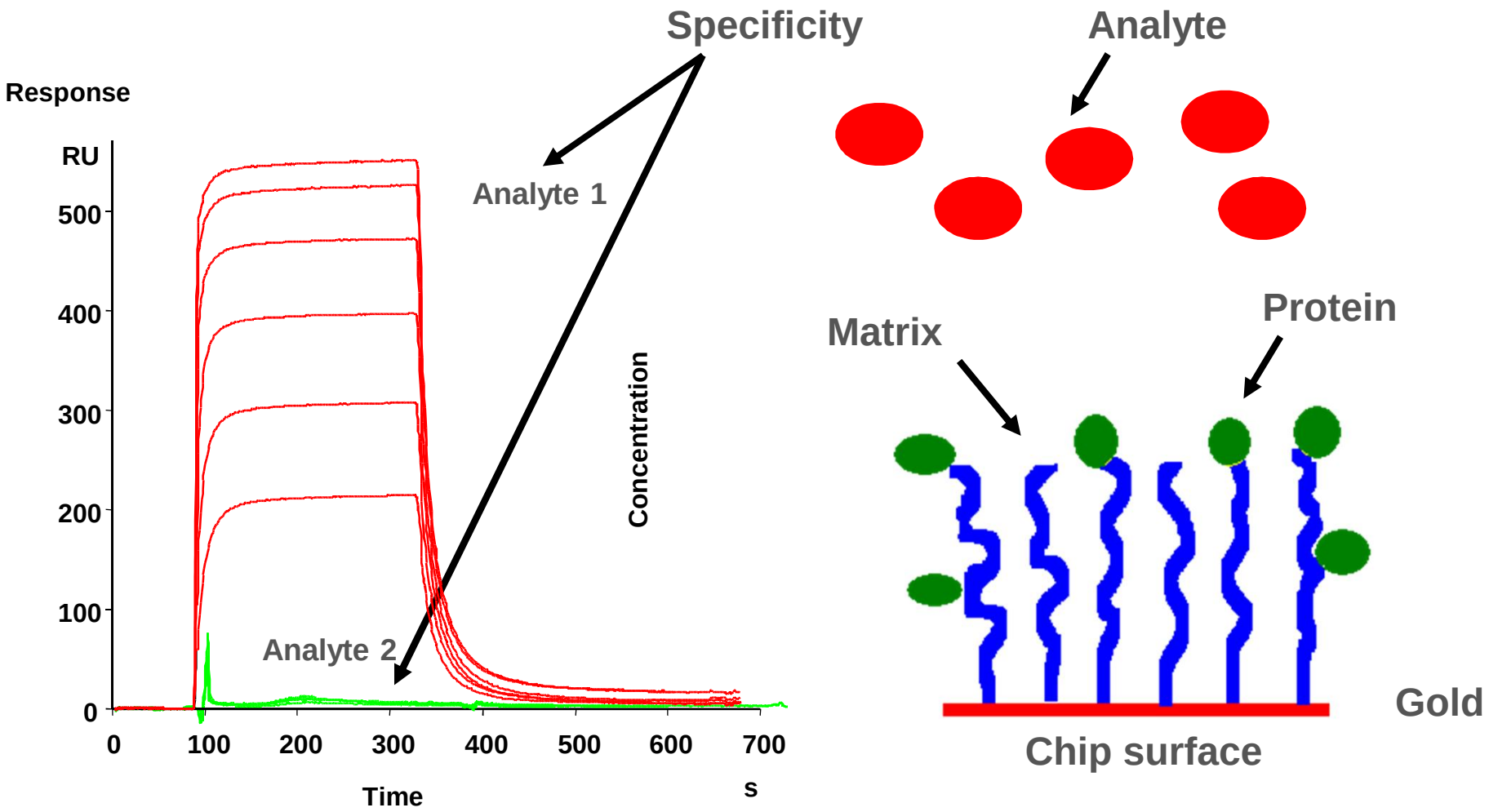
This fact is known but usually not considered !

Experimental results

Simple binding - kinetics

ÅTypical binding curve – association and dissociation phase, (surface regeneration)



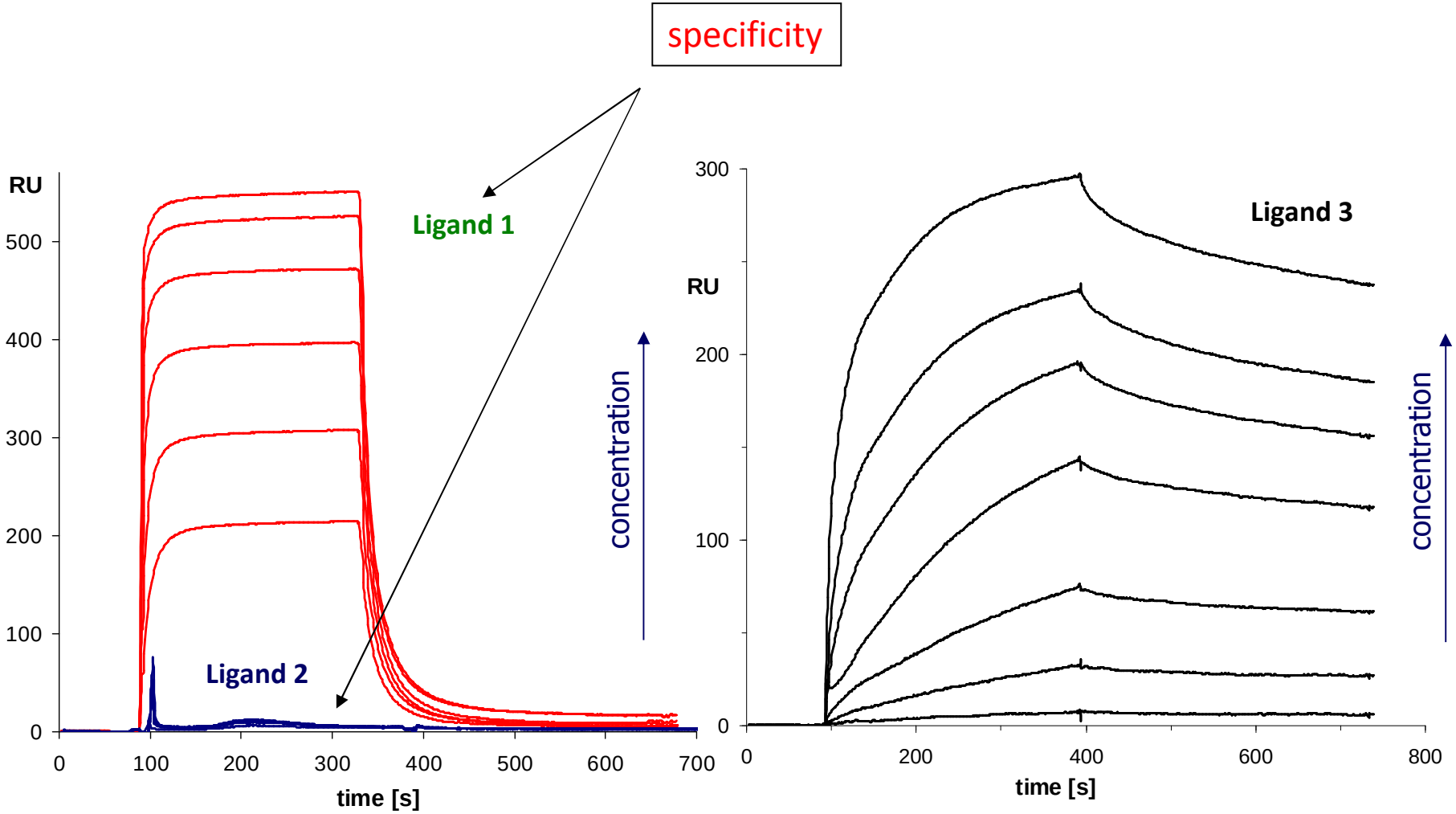

Specificity
Analyte
Response
RU
500
400
300
200
100
0
Analyte 1
Concentration
Analyte 2
0
100
200
300
400
500
600
700
Time
s
Matrix
Protein
Gold
Chip surface

Fast complex association and dissociation

Fast equilibrium ⇨ K_A, K_D

Slow complex association and dissociation

Kinetic constants k_a, k_d ⇨ K_A, K_D



Simple binding – steady state

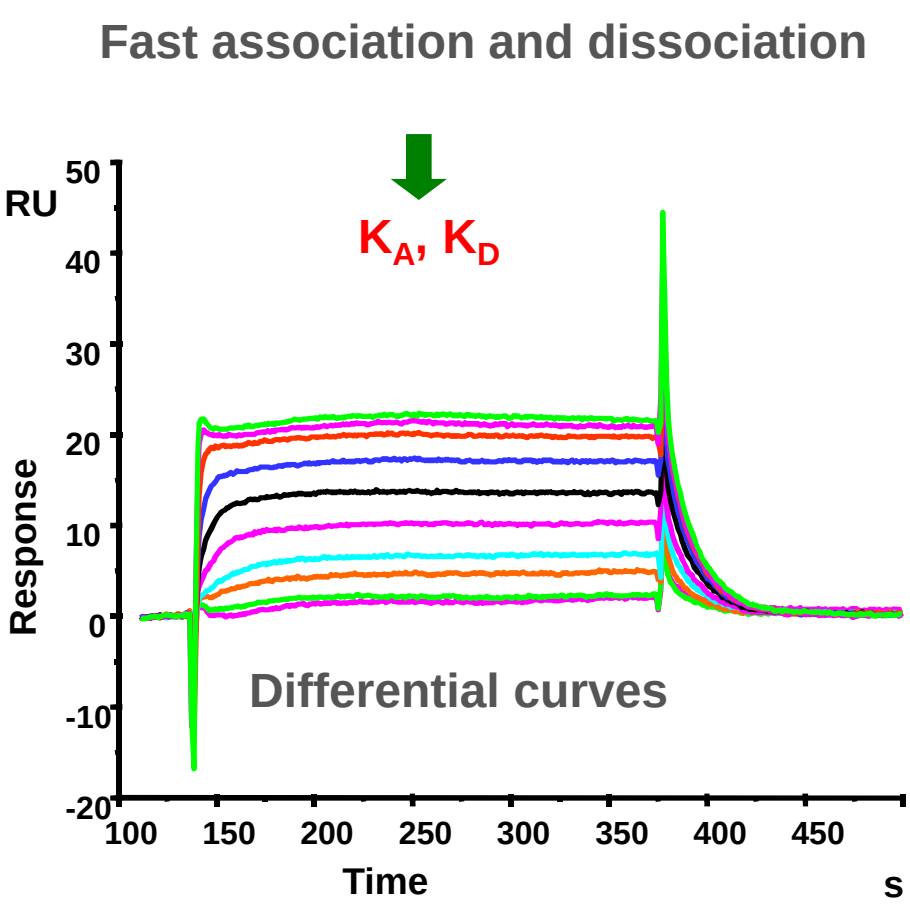
Fast association and dissociation data
are not easy to fit

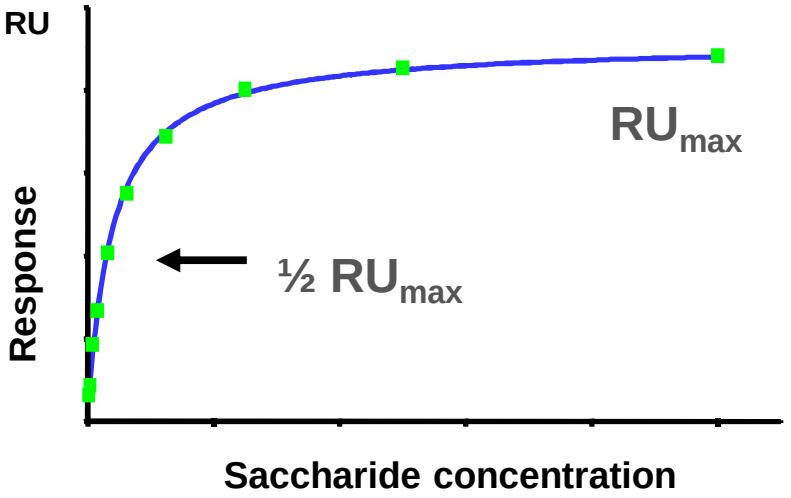
BUT

$v_{(association)} = v_{(dissociation)}$ **steady state**

-> response is proportional to K_D and R_{max}

Direct binding assay





$$K_D = \frac{[\text{Protein}]\ [\text{Analyte}]}{[\text{Protein-Analyte}]}$$

$RU = ½\ RU_{max}$

↓

$[\text{Protein}] = [\text{Protein-Analyte}]$

↓

$K_D = [\text{Analyte}]$

Factors influencing binding and response

- **Density** of the molecules **on chip**
- **Concentration** of molecules **in solution**
- **Strength of interaction** between both molecules
- Total **mass** of interacting partner
- Portion of **active molecules** present –
  proper sample characterization needed, changes upon immobilization – site accessibility restriction, conformational changes, intermolecular distance

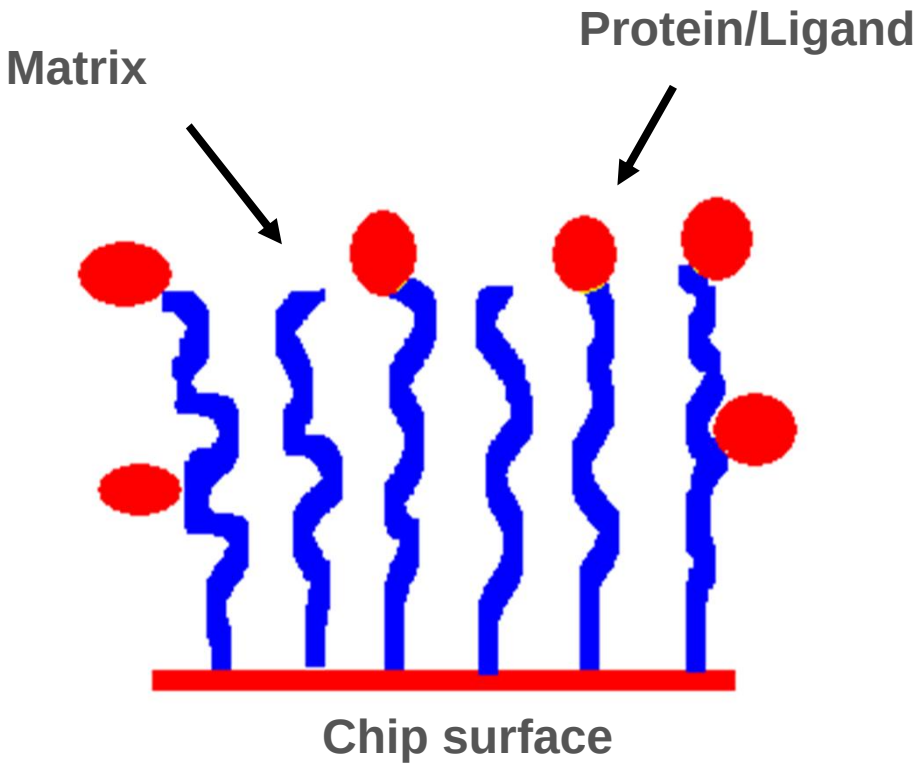
Which binding partner to immobilize?

- Stability
- Availability
- Molecular mass

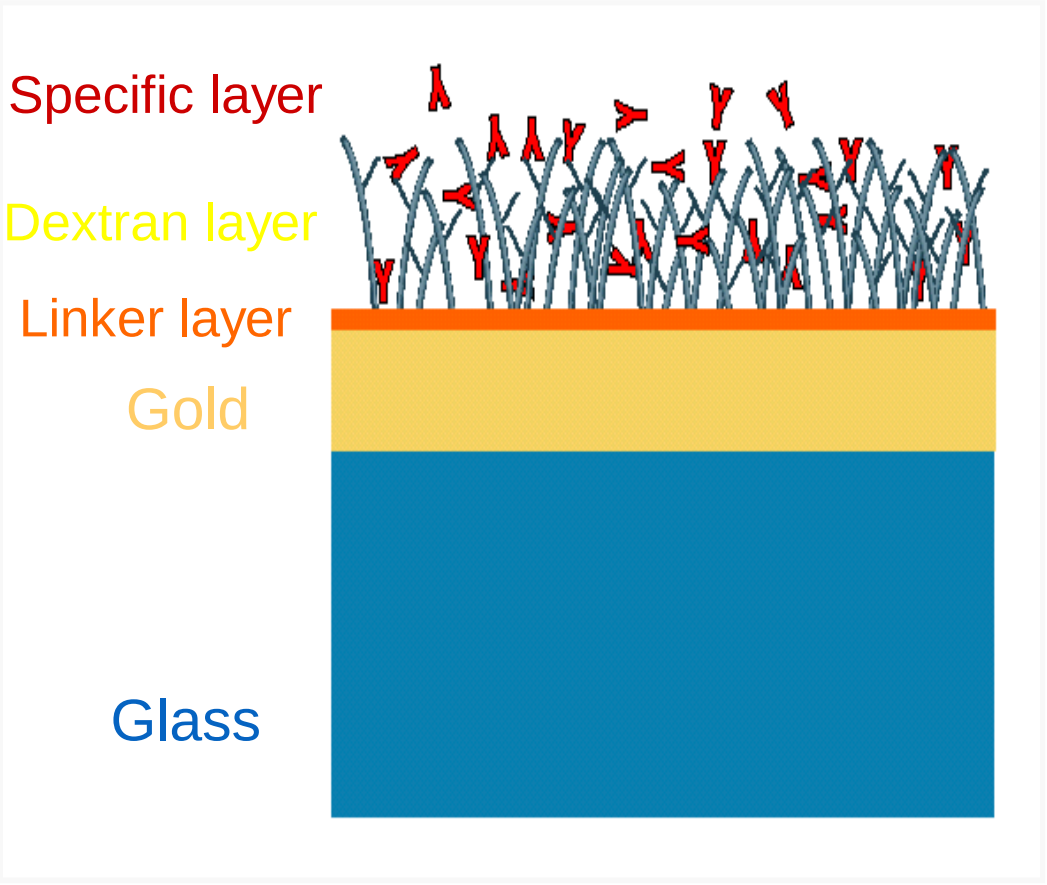
$$\text{Response}_{max} = \text{Response}_{ligand} \times \frac{Mr_{analyte}}{Mr_{ligand}} \times \frac{\left(\frac{\partial n}{\partial c}\right)_{analyte}}{\left(\frac{\partial n}{\partial c}\right)_{ligand}}$$

- Immobilization technique
- Multivalency

SPR Chip – rough scheme



User-defined biospecific surface

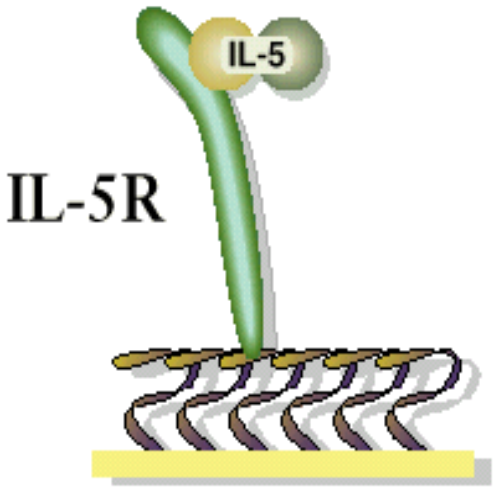


- ÅBiocompatible
- ÅLow non-specific binding
- ÅRobust
- ÅMore than 100 runs on the same surface

Various immobilization techniques

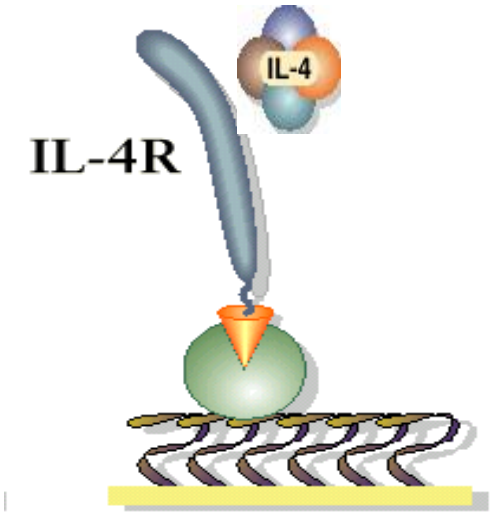
High flexibility in creating biospecific surfaces

Å Direct: covalent coupling



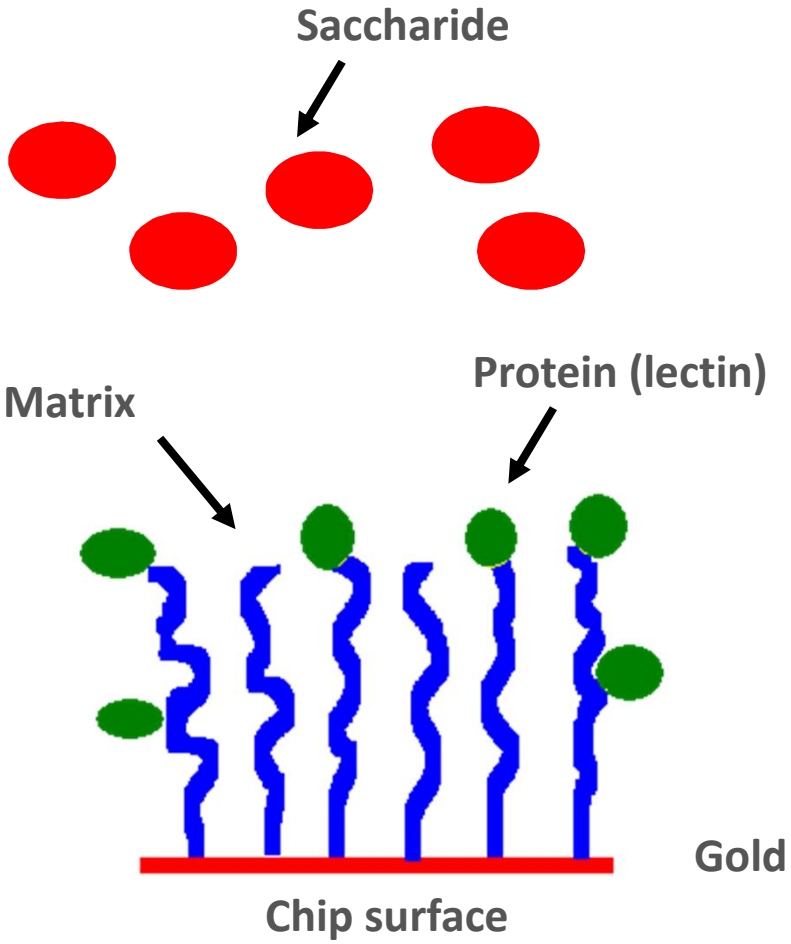
Å **Amine**
Å **Thiol**
Å **Aldehyde**
Å **Carboxyl**

Å Capture

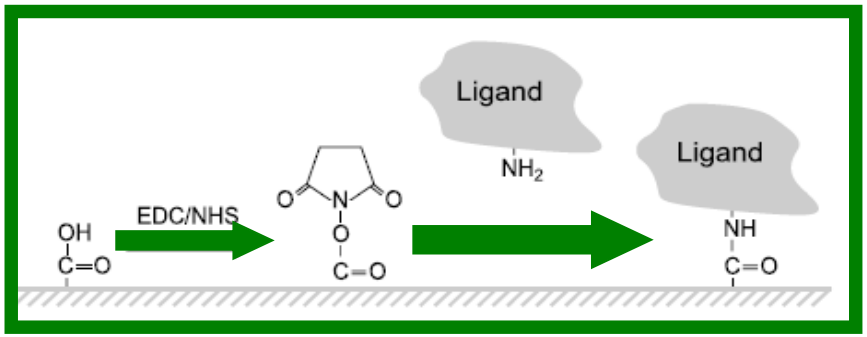


Å **Streptavidin - Biotin**
Å **NTA-Ni2+-His**
Å **Anti-his-His**
Å **RaM Fc - MAb**
Å **Anti-GST- GST**

Protein immobilization



„Amine-coupling“

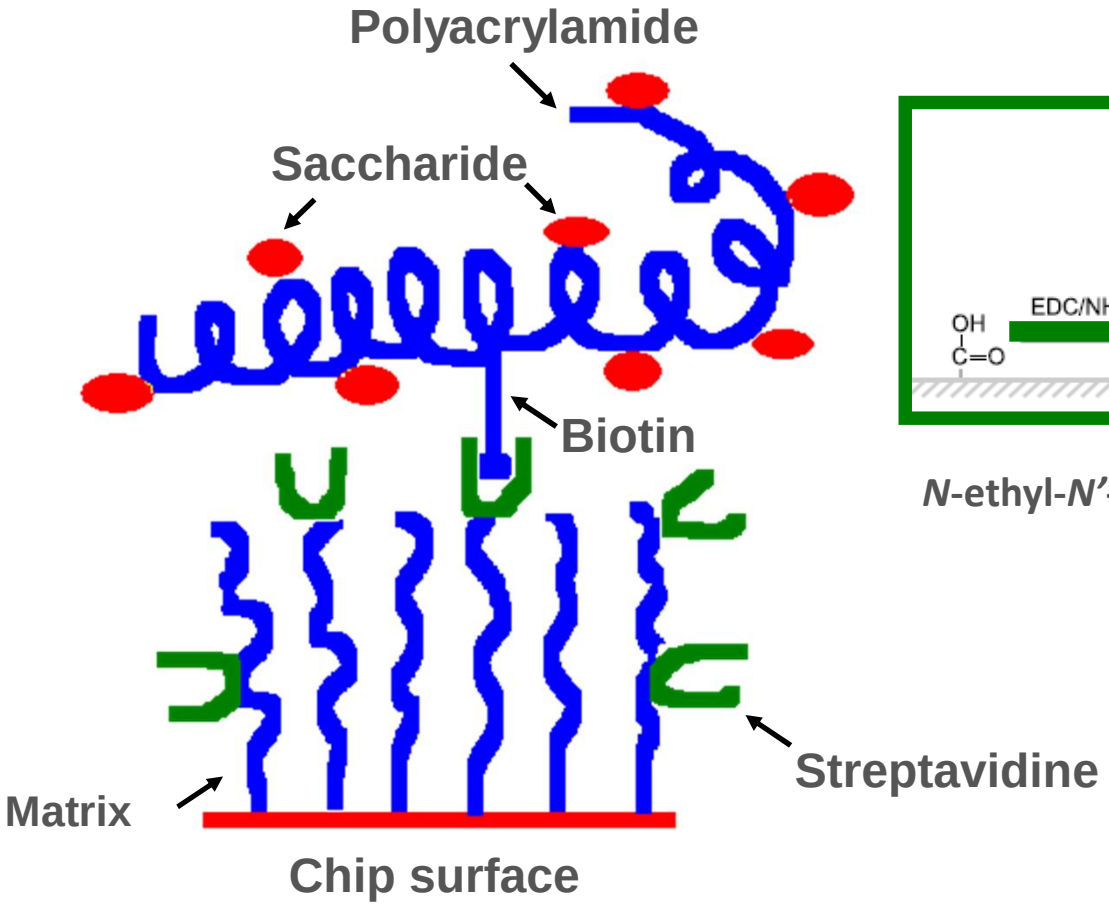


***N*-ethyl-*N'*-(3-diethylaminopropyl)karbodiimide**

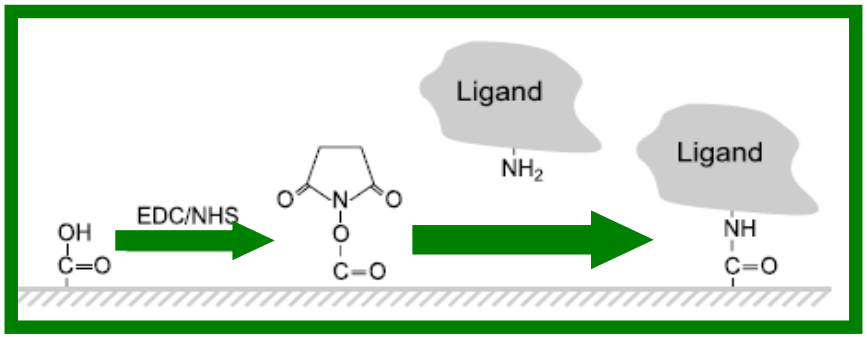
N-hydroxysuccinimide

CM5 chip – surface modified by carboxymethylated dextran

Saccharide immobilization



„Amine-coupling“

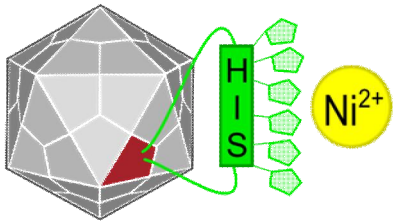


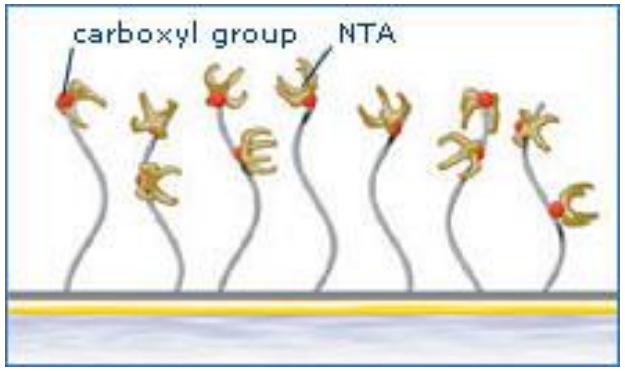
N-ethyl-*N'*-(3-diethylaminopropyl)karbodiimide

N-hydroxysuccinimide

Typical spacer for saccharides is $-(CH_2)_3-$, for biotin $-(CH_2)_6-$

Ni-NTA utilization





+Ni^{2+}

Activation

Sample application

Protein binding

+EDTA

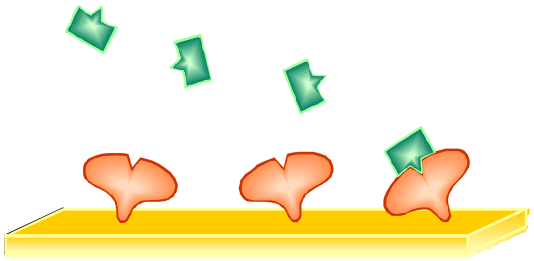
Regeneration

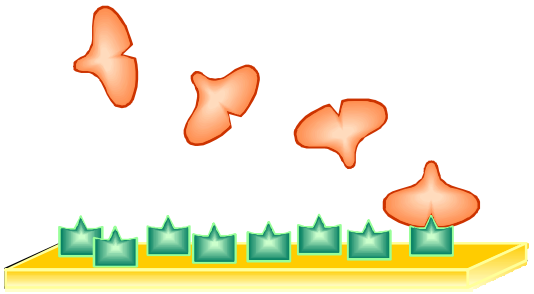
Flexibility in Assay Design

Multiple assay formats providing complementary data

Direct measurement

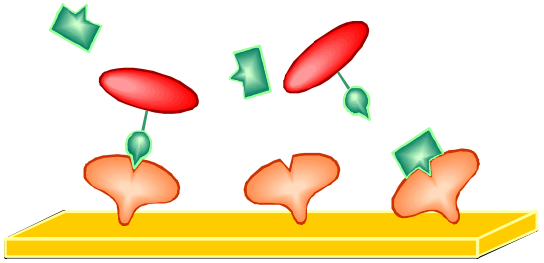
Direct Binding Assay (DBA)



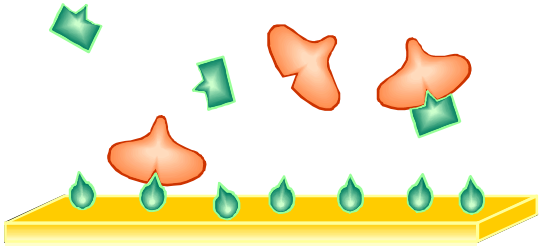


Indirect measurement

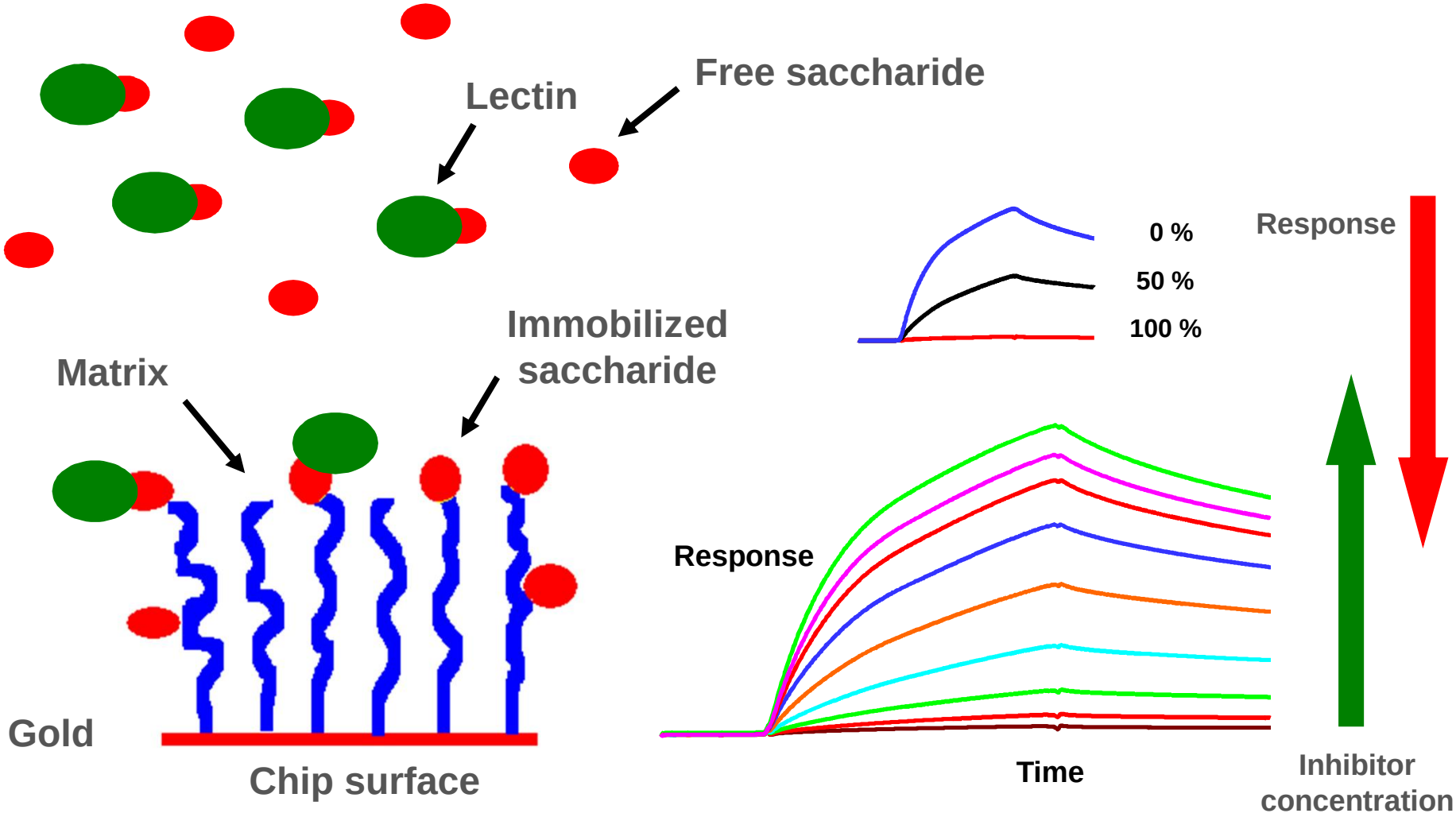
Surface competition assay (SCA)



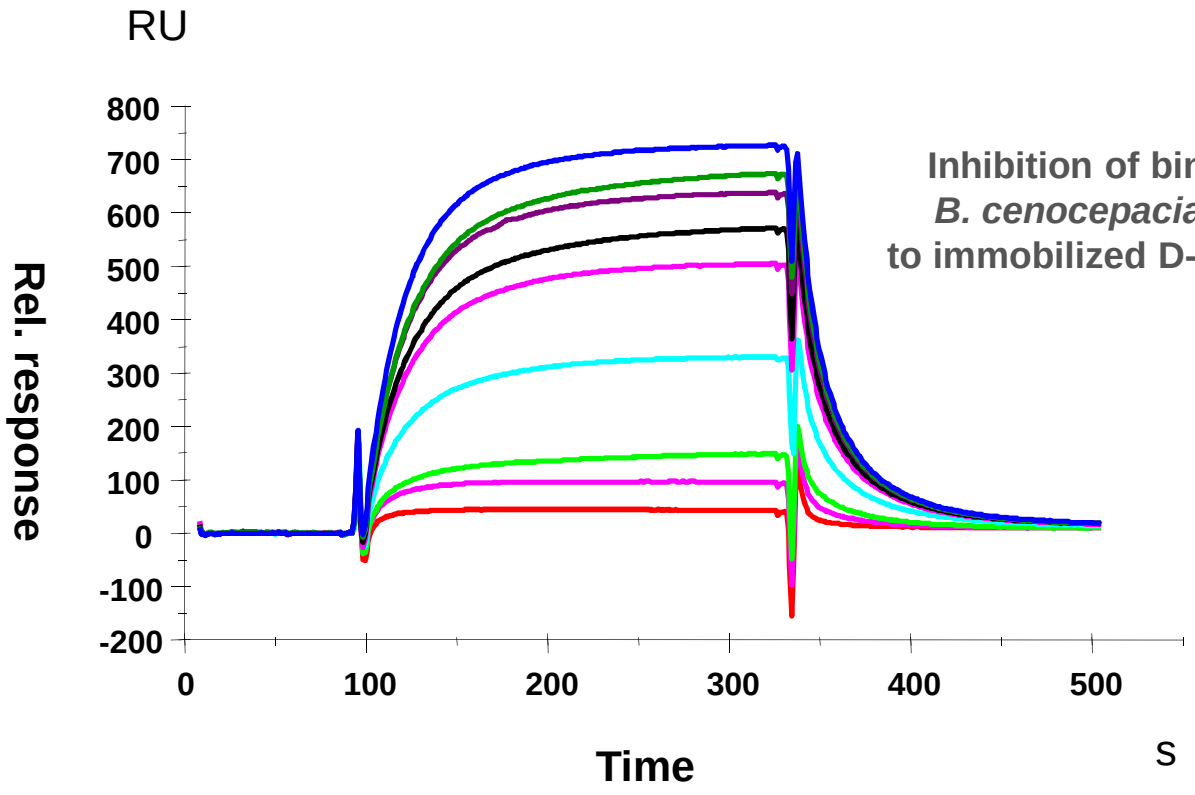
Inhibition in solution assay (ISA)



Inhibition in solution assay

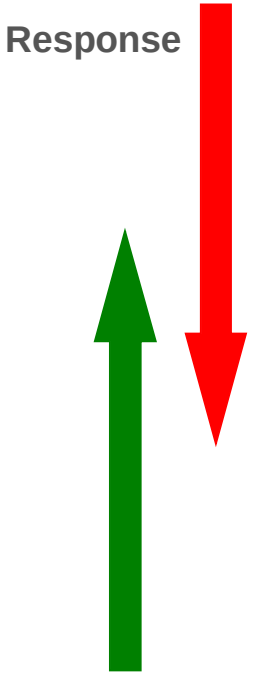


Inhibition in solution assay

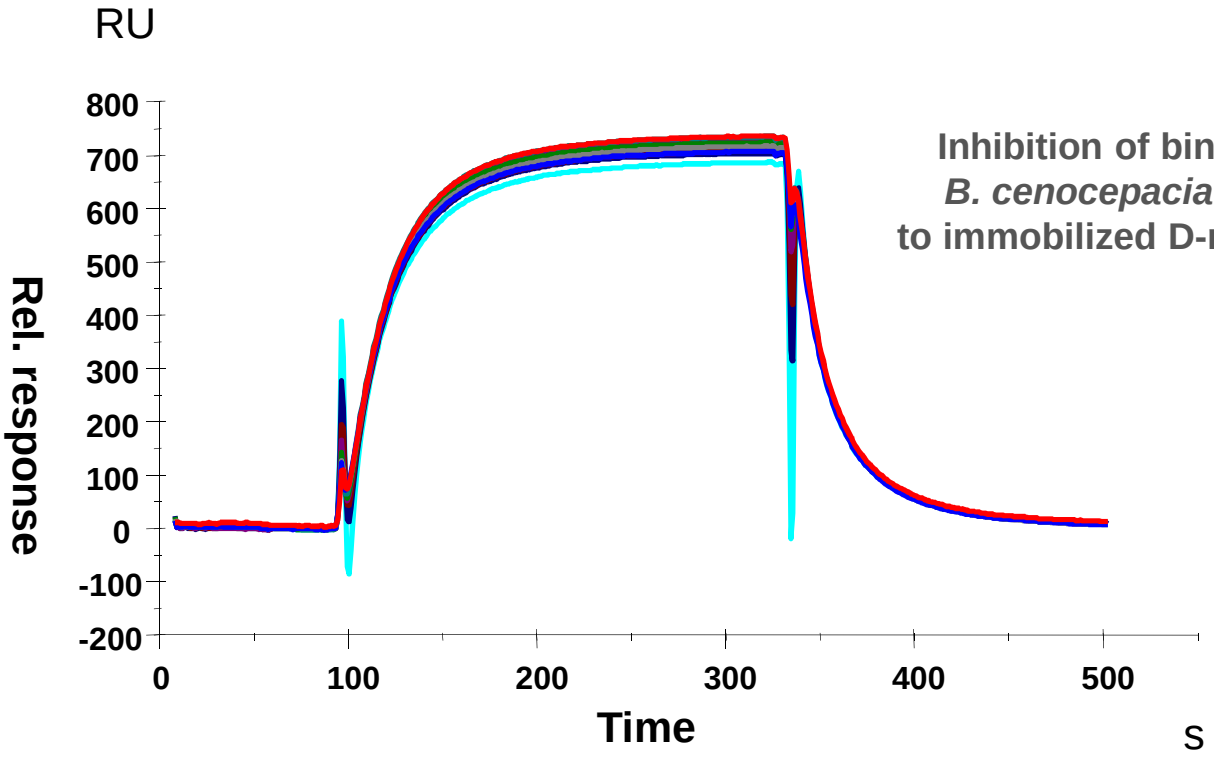


Inhibition of binding of
B. cenocepacia lectin
to immobilized D-mannose

0-250 εM D-mannose



Inhibition in solution assay



Inhibition of binding of
B. cenocepacia lectin
to immobilized D-mannose

Response

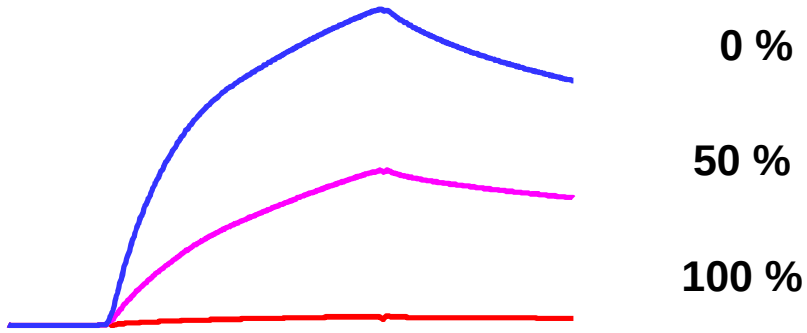
Inhibitor
concentration

0-80 mM D-galactose

Inhibition in solution assay

$$\text{Effectivity} = \frac{IC_{50\text{D-mannose}}}{IC_{50\text{saccharide}}}$$

Lectin from *B. cenocepacia*:
Benzyl-Ŭ-D-mannoside å Methyl-Ŭ-D-mannoside å D-mannose » L-fucose > D-arabinose > L-galactose > Methyl-Ŭ-L-fucoside » D-galactose

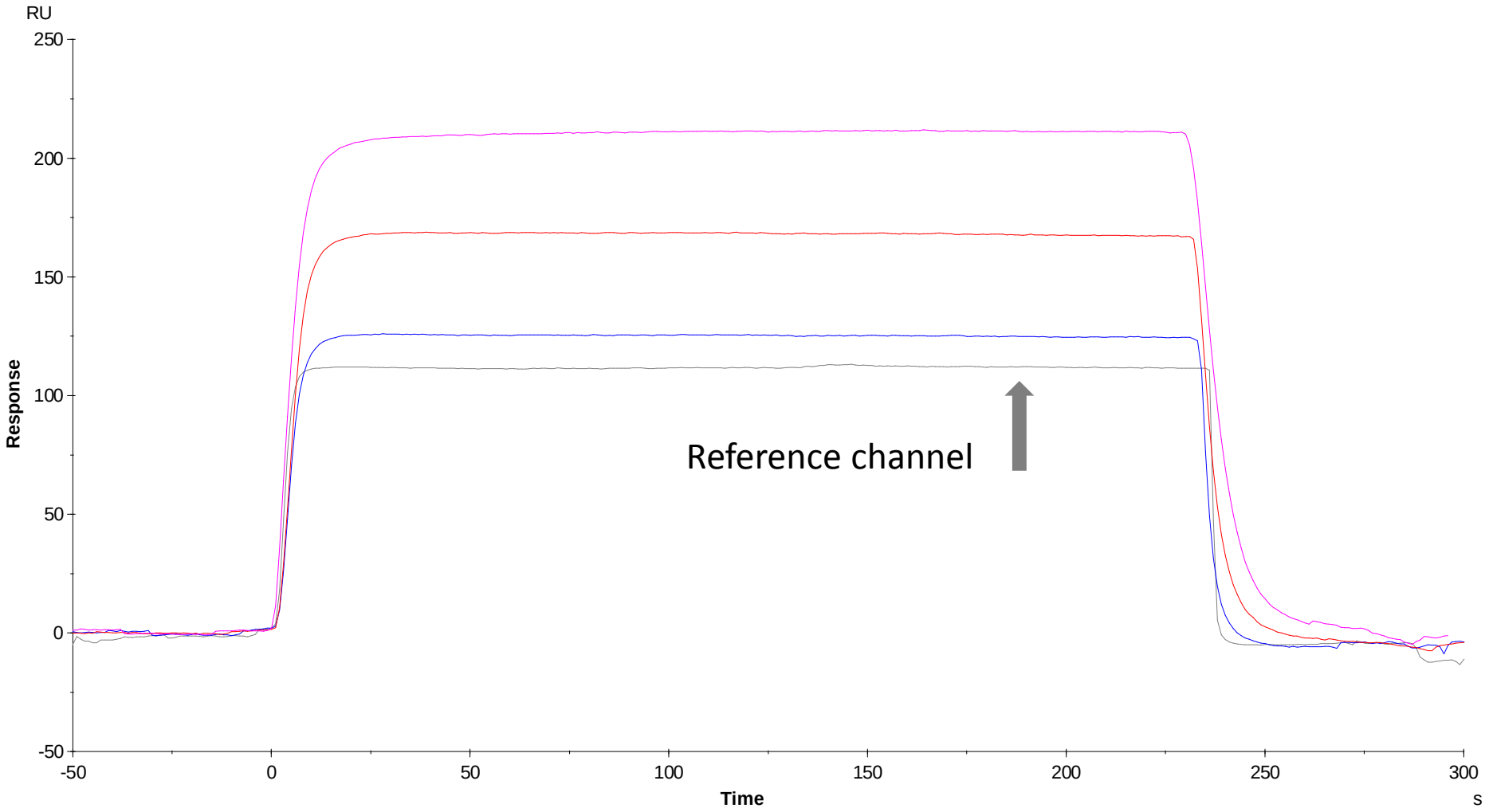


Two channels necessary - reference

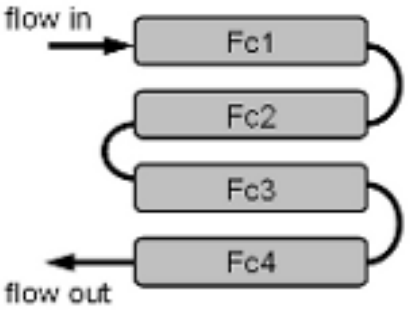
- “Non-interacting” surface serves as a blank
- Elimination of non-specific interactions
- Enhancement of weak interaction resolution

- **Possible reference surfaces**:
  - Unmodified surface – gold, dextran layer,…
  - Activated and blocked surface without immobilized ligand/protein
  - Inactivated/non-functional protein

Two channels necessary - reference



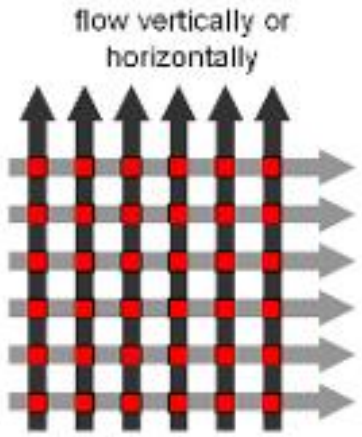
Multichannel set-up



ÅOne or more references

ÅMultiple channels – 2, 4, 6, 36,…

ÅMultiple detection spots



➢High throughput

➢Parallel reference

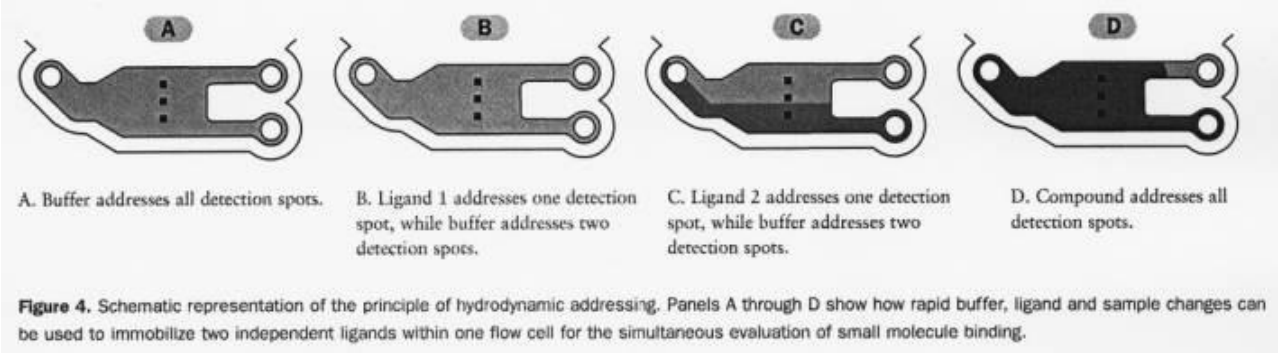


Figure 4. Schematic representation of the principle of hydrodynamic addressing. Panels A through D show how rapid buffer, ligand and sample changes can be used to immobilize two independent ligands within one flow cell for the simultaneous evaluation of small molecule binding.

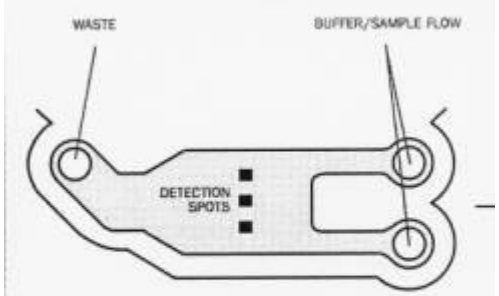


Figure 3. Assay flexibility of Biacore S51 flow cell and hydrodynamic addressing, illustrating three potential assay designs.

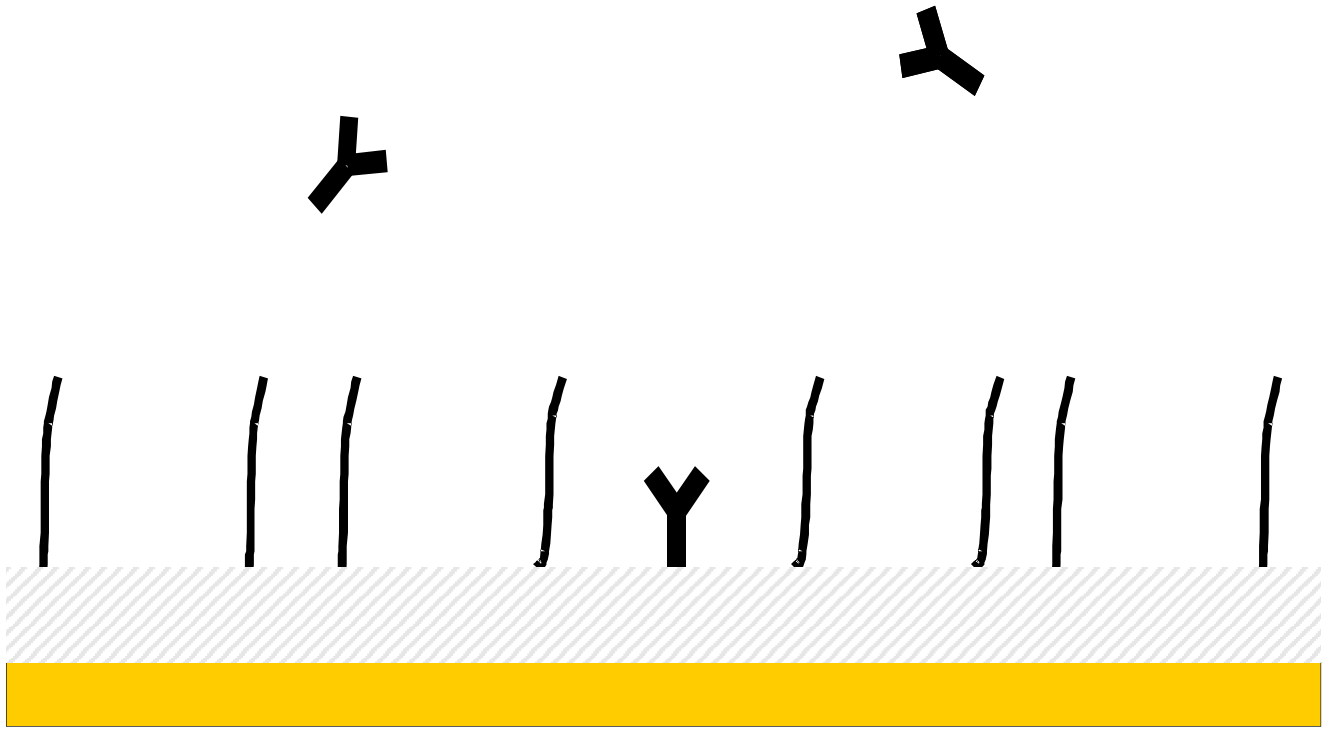
Specialized techniques

- Membrane proteins
- Multi-layer approaches – antibodies, protein complexes
- Whole cell immobilization
- Thermodynamics measured by SPR
- Ligand recovery

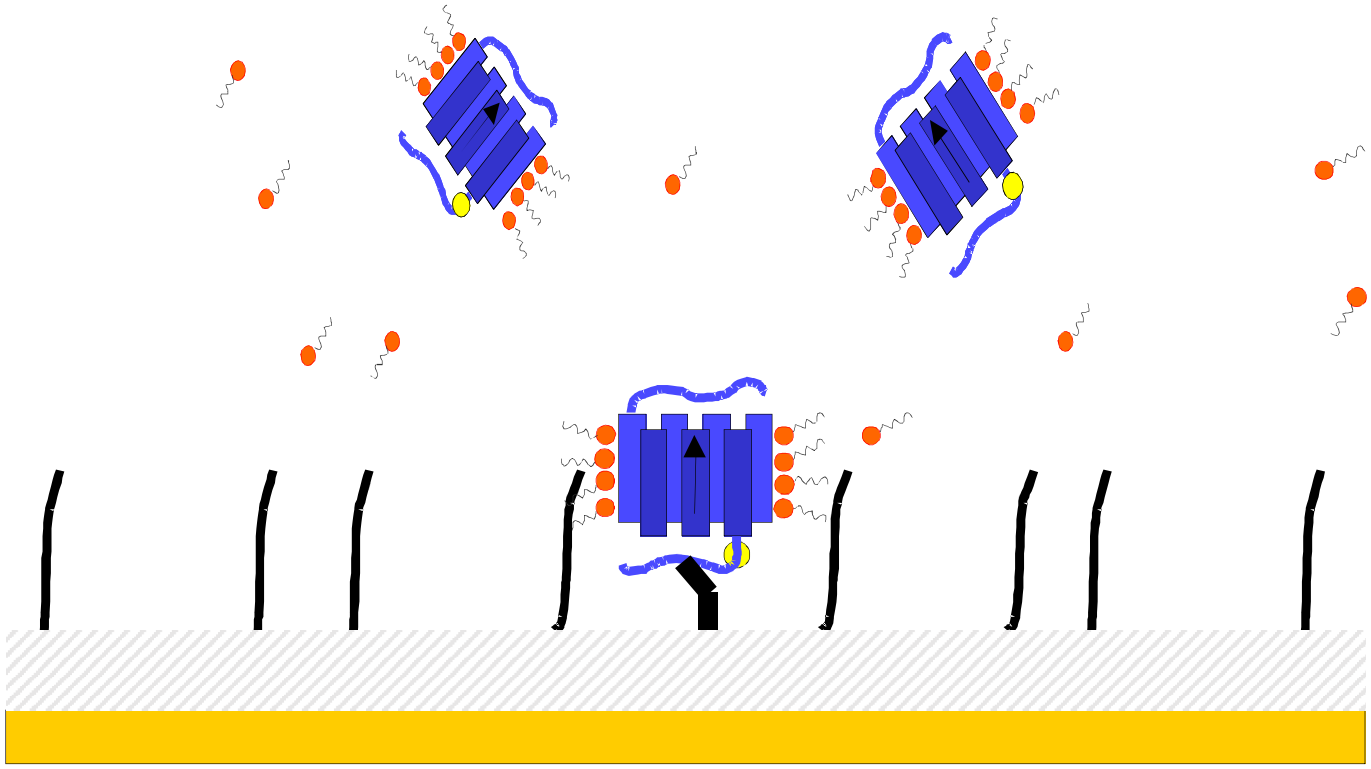
On-surface reconstitution approach

- ✓ A very quick and easy method for functional reconstitution of immobilized membrane proteins with lipids.
- ✓ Conventional immobilization techniques are applicable on membrane proteins.
- ✓ Surfaces with high density of receptor can be prepared.
- ✓ The lipid matrix can be renewed after every cycle.
- ✓ “Lipid bilayers” can be very rapidly and easily built and rebuilt on Pioneer Chip L1 (Biacore).

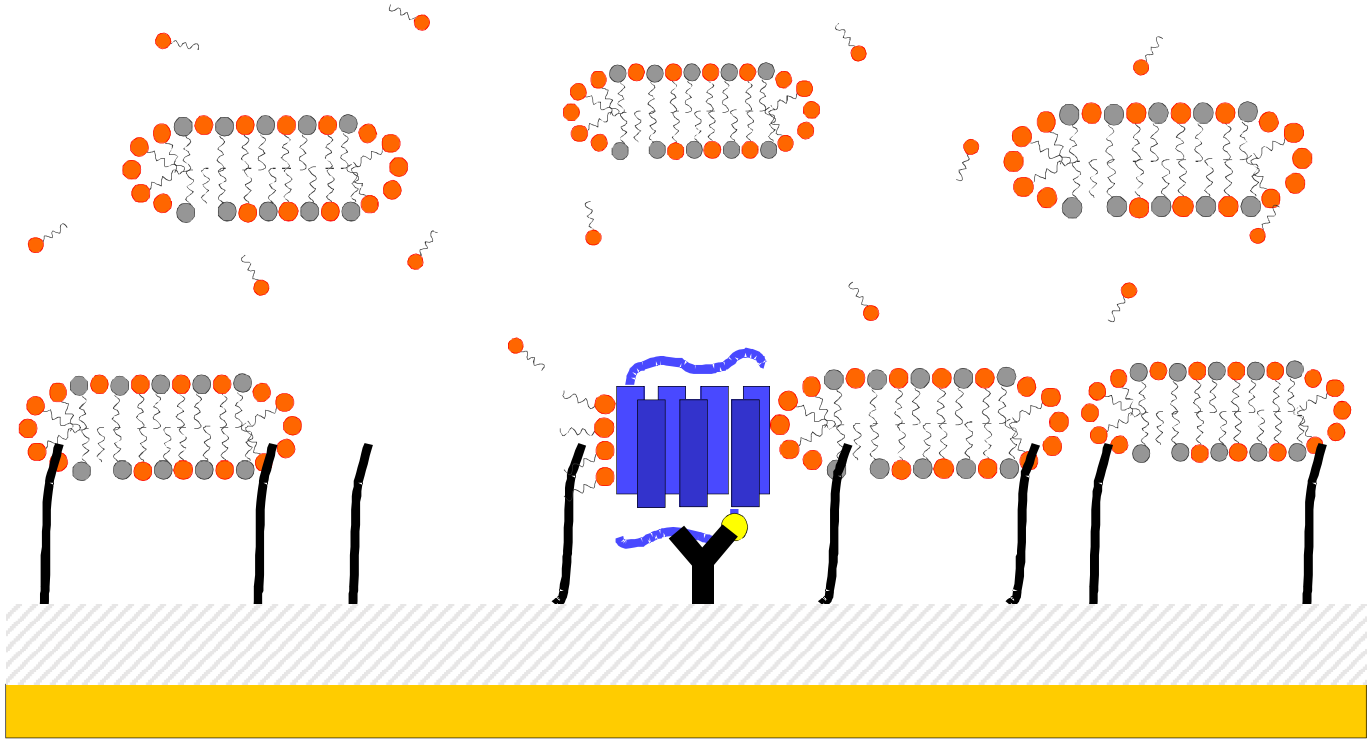
Immobilize a GPCR-specific mAb on a L1 chip.



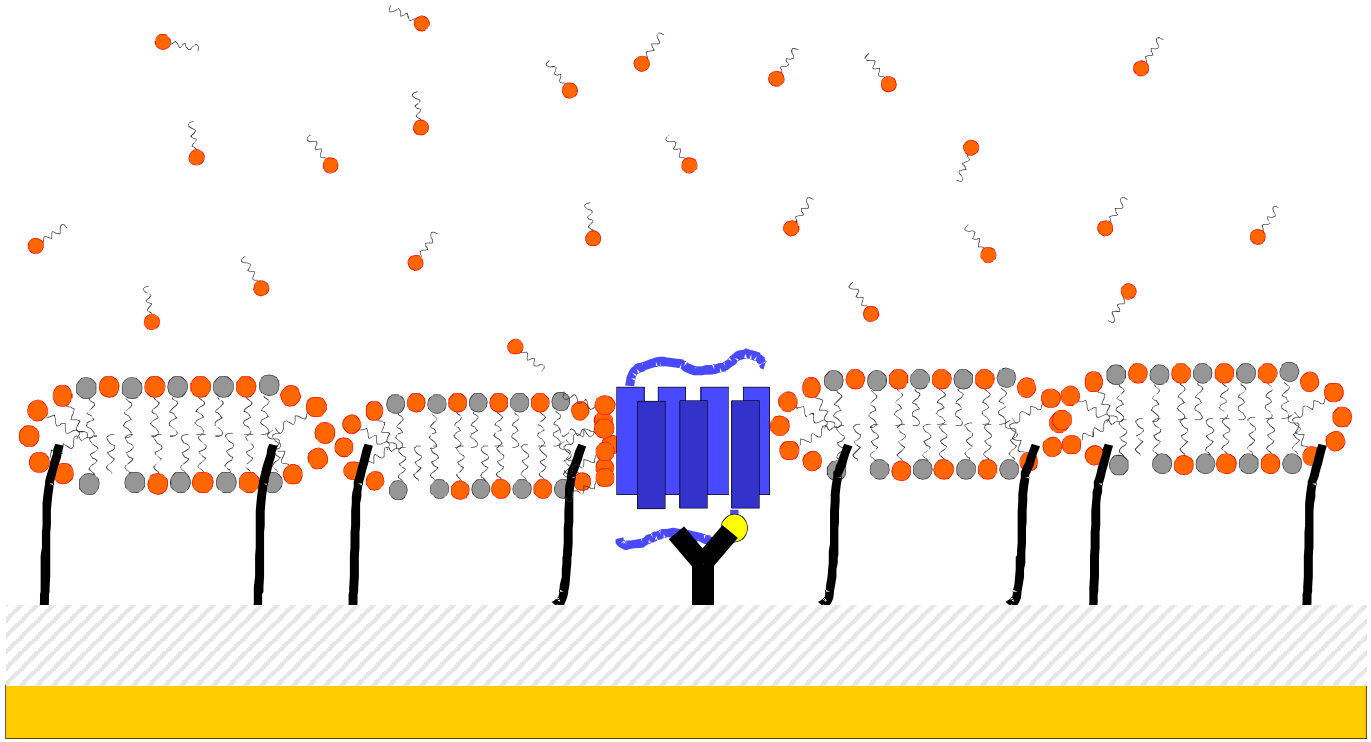
Capture a detergent-solublized GPCR on the immobilized mAb surface.



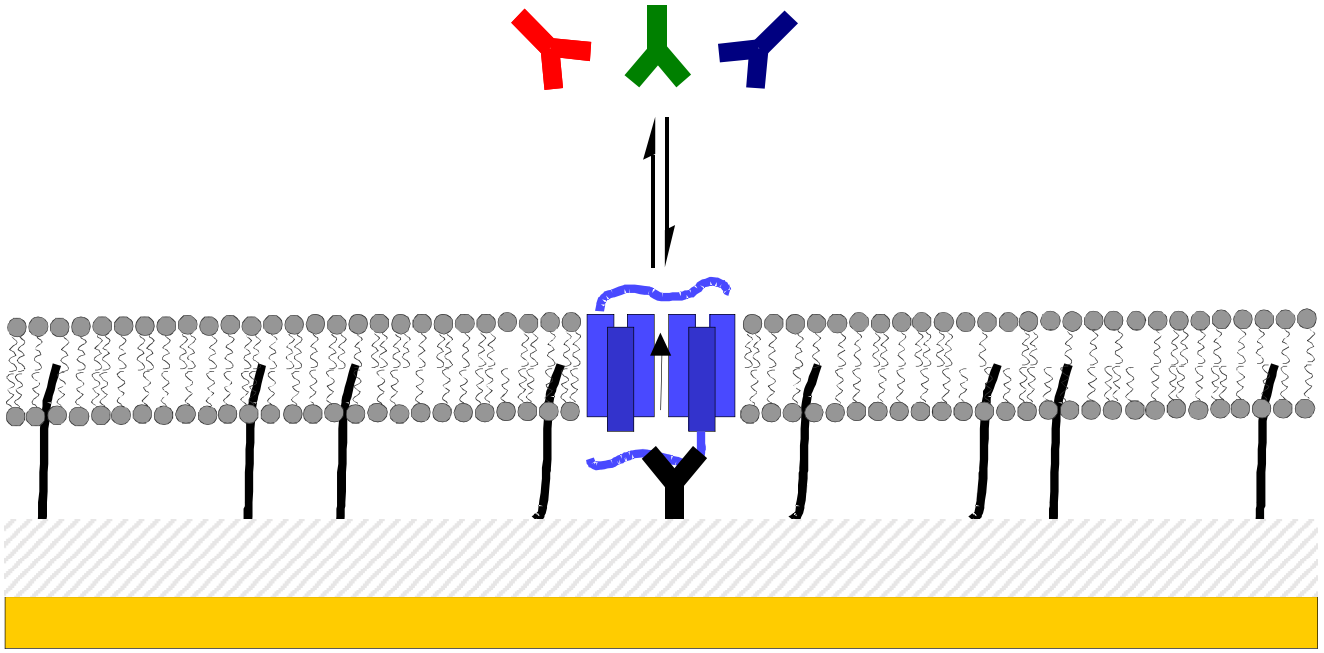
Reconstitute a lipid bilayer around the receptor



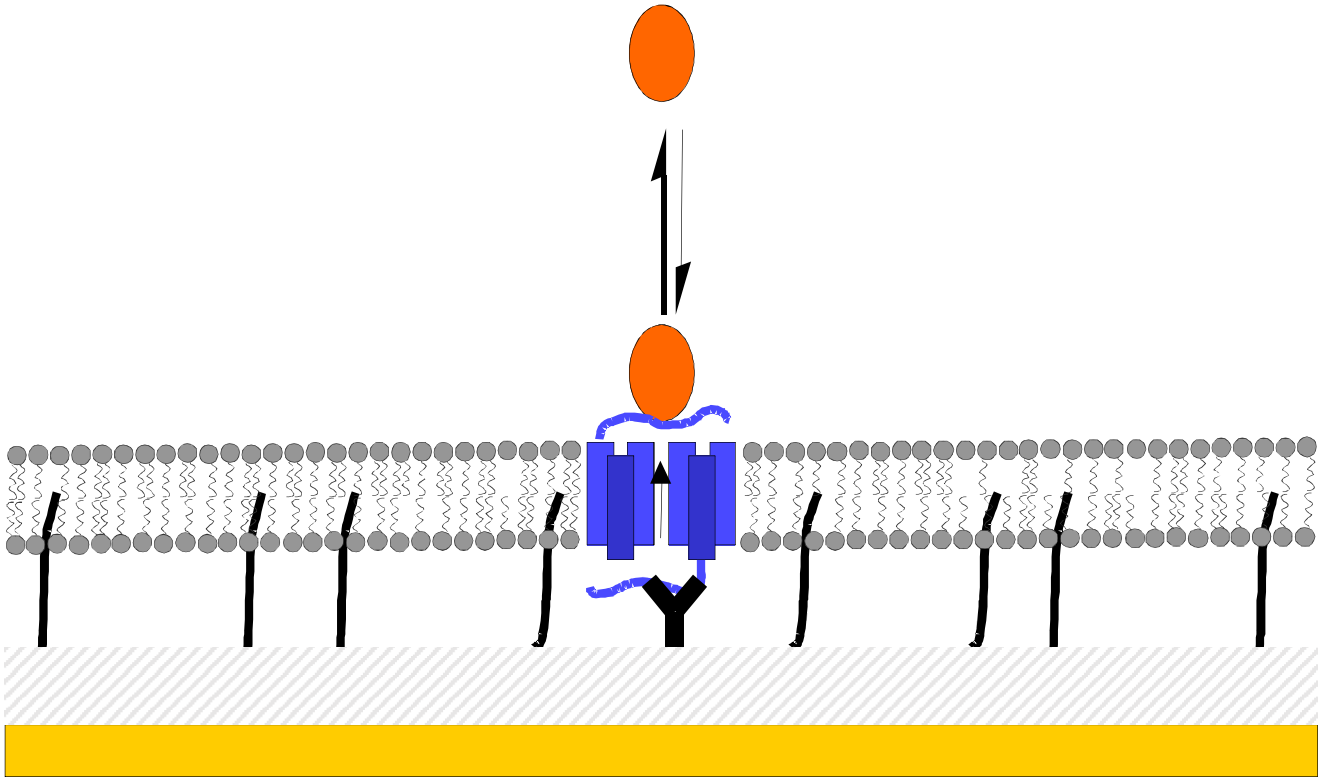
Wash the surface with buffer



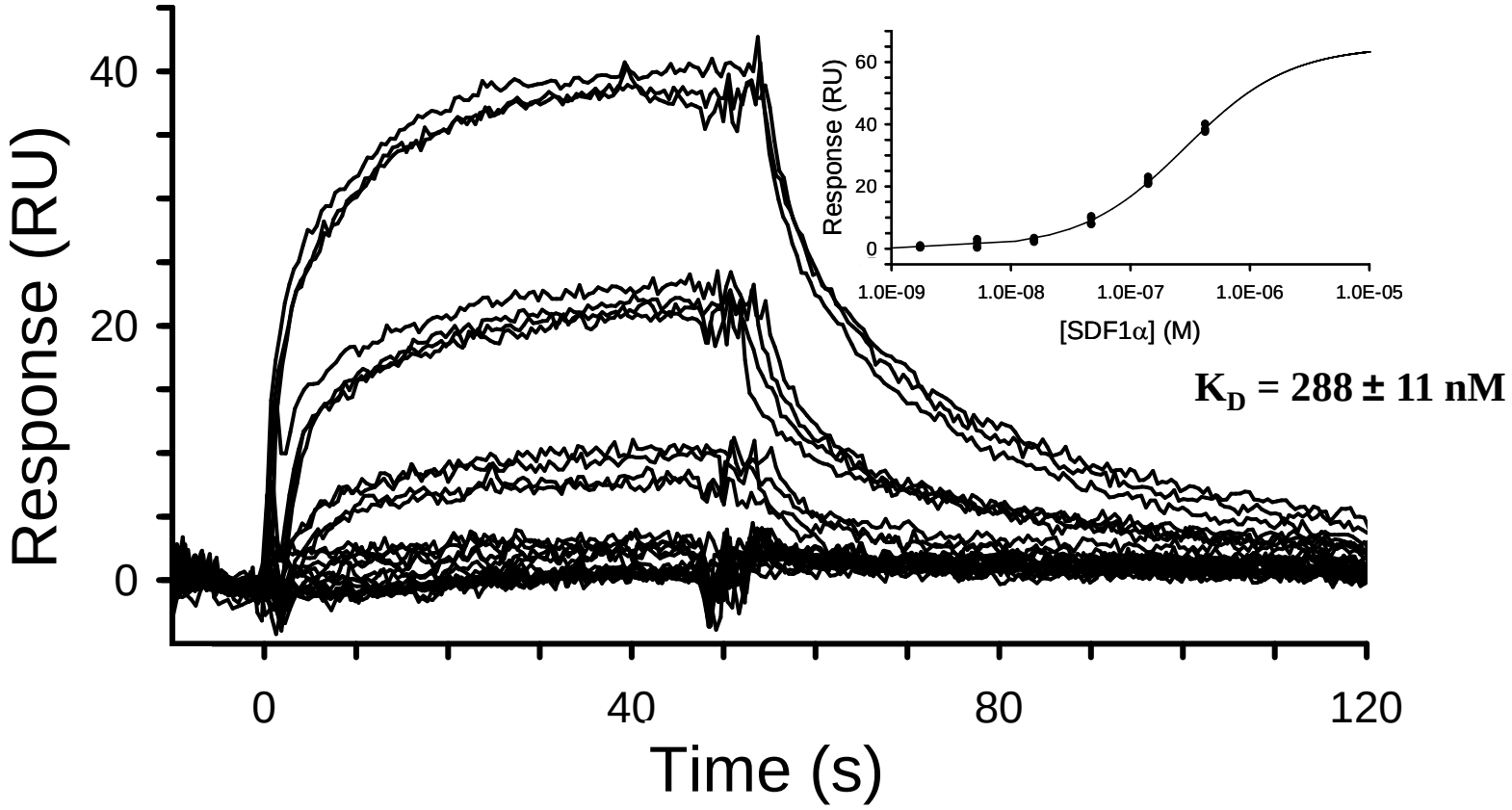
Establish the integrity of the reconstituted GPCR



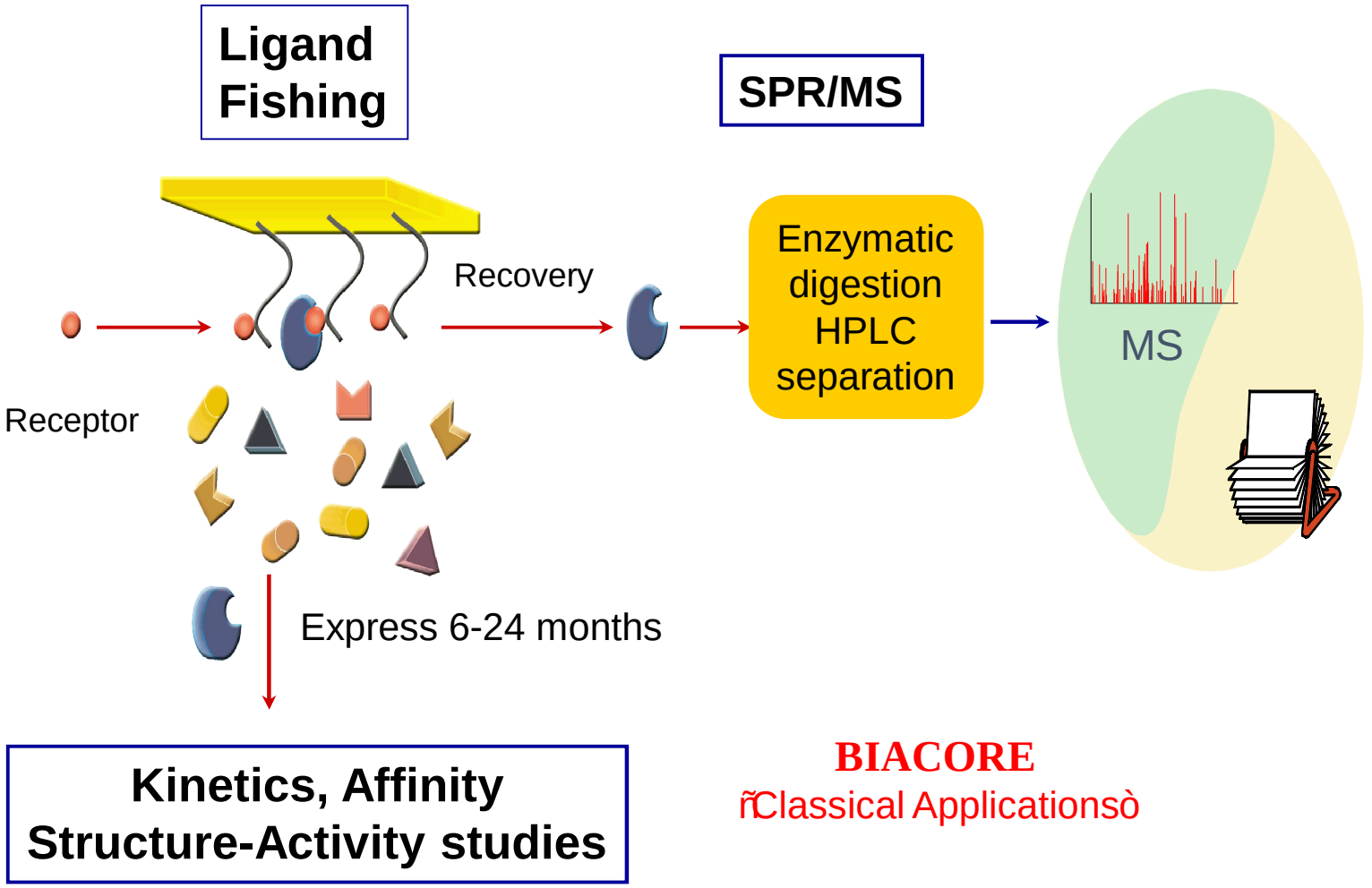
Study the kinetics of ligand/receptor interactions



Binding of the chemokine SDF1α to the reconstituted CXCR4 receptor



Proteomics Study



BIACORE

ñClassical Applicationsò

Main SPR biosensors

- *GE Healtcare* – Biacore T200, Biacore 4000, Biacore 3000, etc.
- *Reichert* – SR7000DC
- *BioRad* – ProteOn™ XPR36
- *Biosensing Instrument* – Bi4000, Bi3000, etc.

Biacore T200

SR7000DC

ProteOn™ XPR36

Bi4000

Biacore 3000 (GE Healthcare)

Simultaneous 4-channel system

Study of small molecules (200 Da), proteins, complex mixtures, lipids, viruses, prokaryotic and eukaryotic cells

Possibility to isolate binding partners for subsequent MS analysis

Objectives of the SPR experiment

- Kinetic Rate Analysis: How **FAST**?
  - k_a, k_d
  - $K_D = k_d/k_a$, $K_A = k_a/k_d$
- Concentration Analysis: How **MUCH**?
  - Active Concentration
  - Solution Equilibrium
  - Inhibition
- Affinity Analysis: How **STRONG**?
  - K_D, K_A
  - Relative Ranking
- **Yes/No** Data
  - Ligand Fishing

SPR technology advantages

- Non-label
- Real-time
- Unique, high quality data on molecular interactions
- Simple assay design
- Robust and reproducible
- Walk-away automation
- Small amount of sample required

SPR & ITC combination

SPR

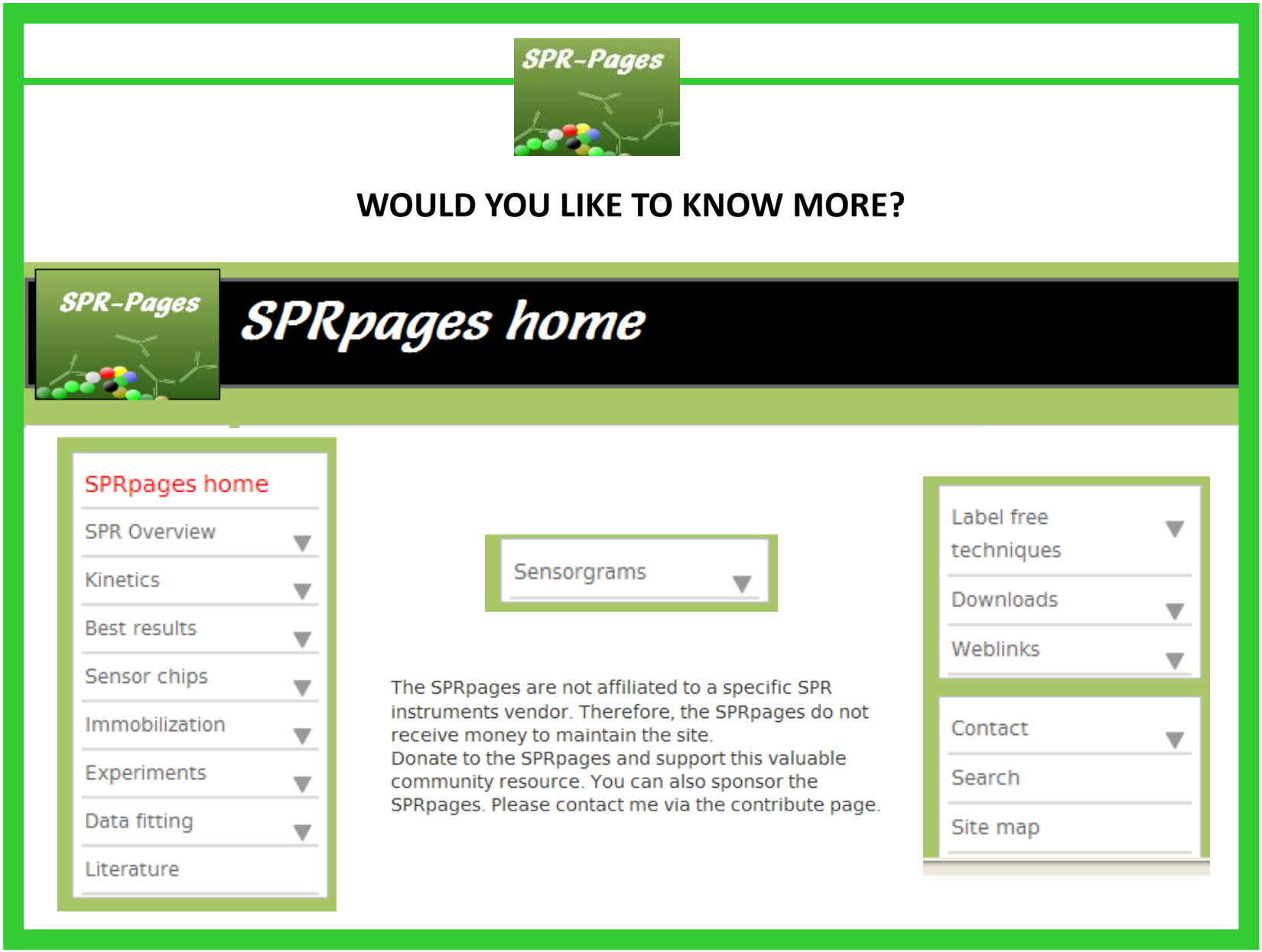
- Real time measurement
- Fast
- No labeling, no additional detection needed
- Low sample consumption
- Robust
- Automatization possible
- High sensitivity

ITC

- No labeling, no immobilization
- In solution
- ñEliminationò of non-specific interactions
- Thermodynamic and affinity parameters within one measurement

Materials for further study

http://www.sprpages.nl/



Core Facility: Biomolecular Interaction and Crystallization

Josef Houser

Å +420 549 492 527

Å josef.houser@ceitec.cz

Michaela Wimmerová

Å +420 549 498 166

Å michaela.wimmerova@ceitec.cz

bic@ceitec.cz

www.ceitec.cz/z4