Sufrace-based methods

S2004
Methods for characterization of biomolecular interactions – classical versus modern

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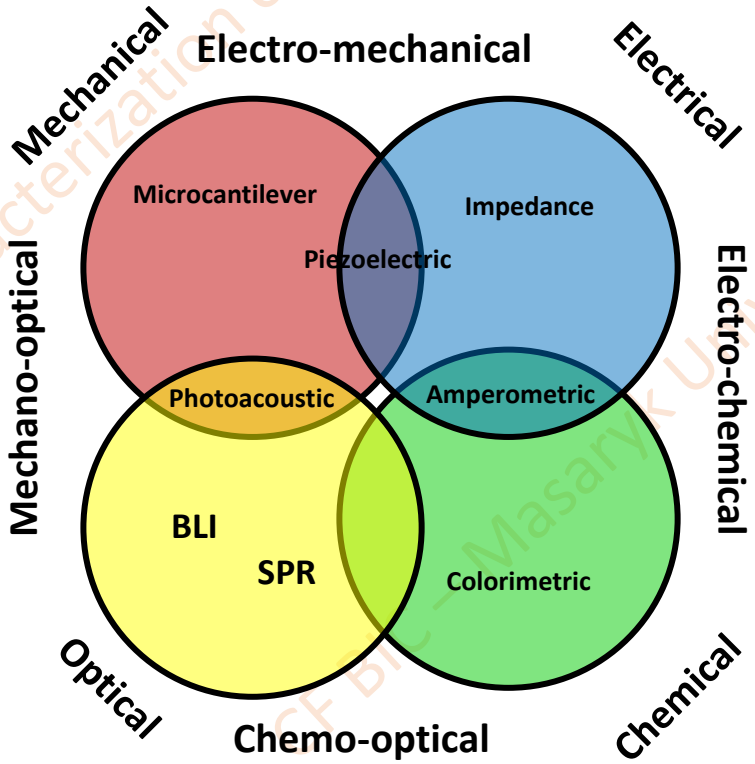
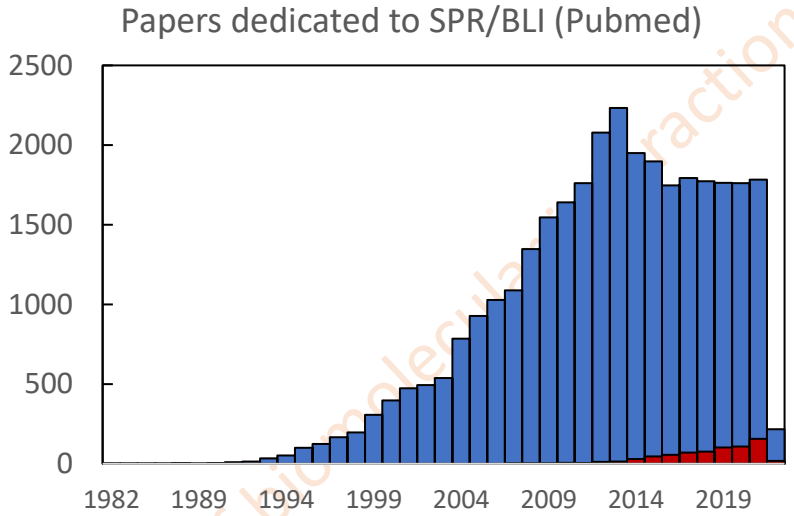
Interaction on surface

- Interaction with
  - One binding partner immobilized on surface – **ligand**
  - Second binding partner free in solution – **analyte**

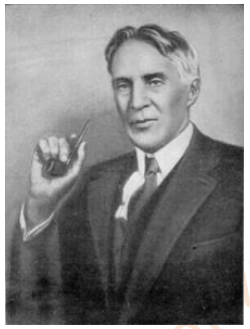
- **Biosensors** based on various **techniques**
  - **Surface plasmon resonance** (SPR)
  - **Biolayer interferometry** (BLI)
  - Grating-Coupled Interferometry (GCI)
  - Quartz-crystal microbalance (QCM)
  - Surface acoustic wave (SAW)
  - Switch-sense
  - …



History



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



1900's

Definition of plasmon

1950's

First SPR use in biomolecular interaction analysis

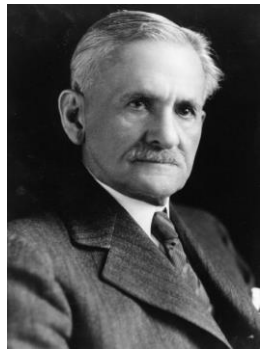
1980

First commercial **SPR** instrument (Biacore)

1990

1887

Invention of interferometry as a technique (A. Michelson)

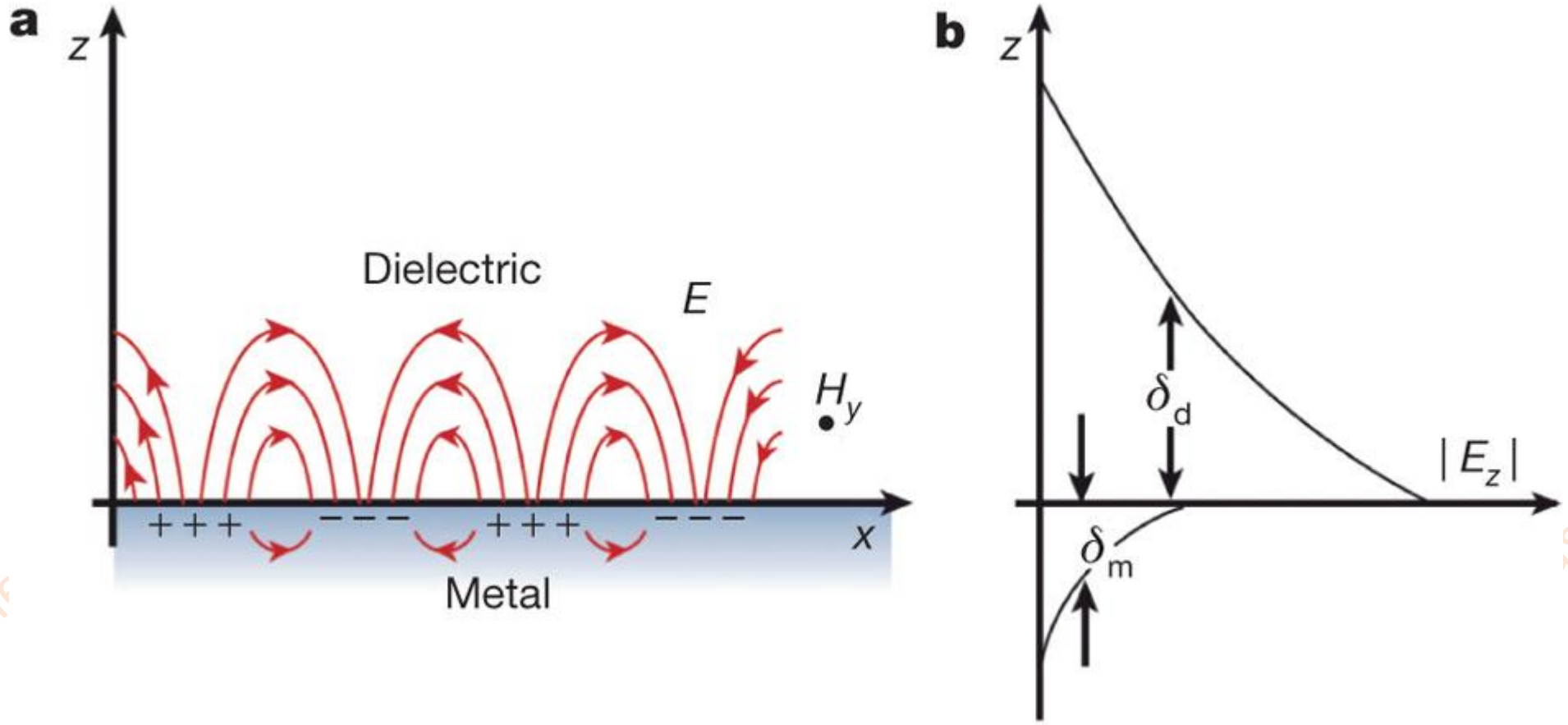


2008

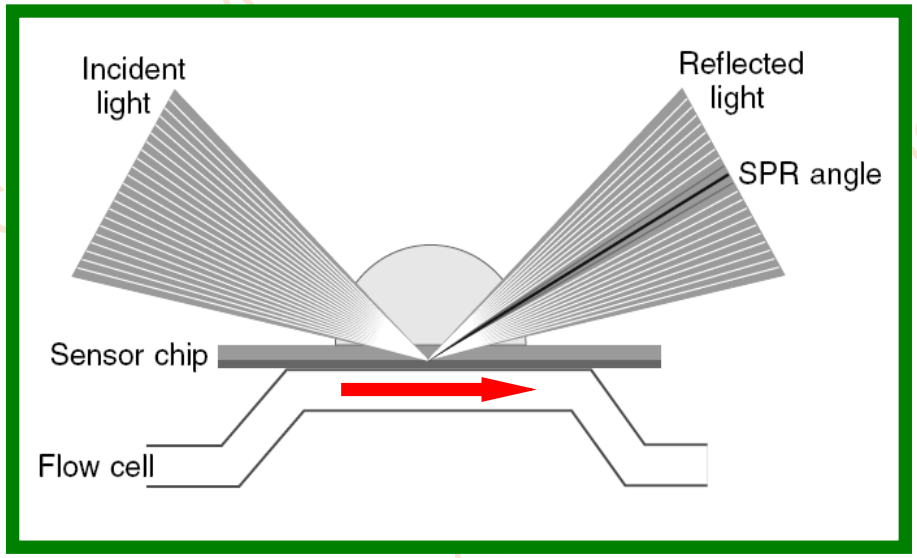
First commercial **BLI** instrument (ForteBio)

Surface plasmon resonance (SPR)

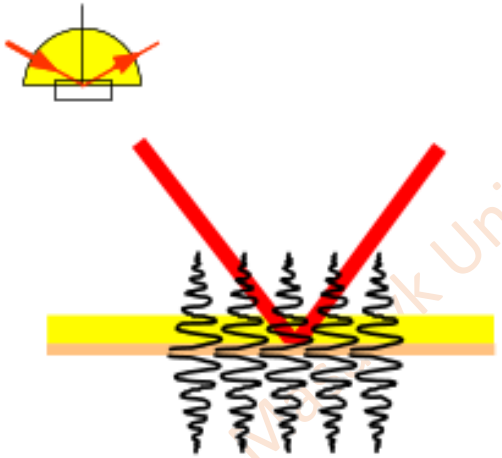
Collective oscillation of free electrons on metal-dielectric interface



SPR – Basic principles



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

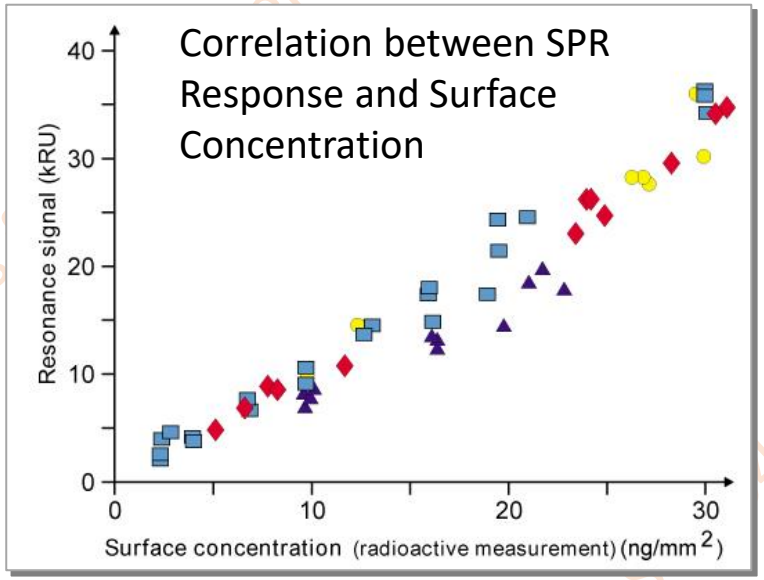


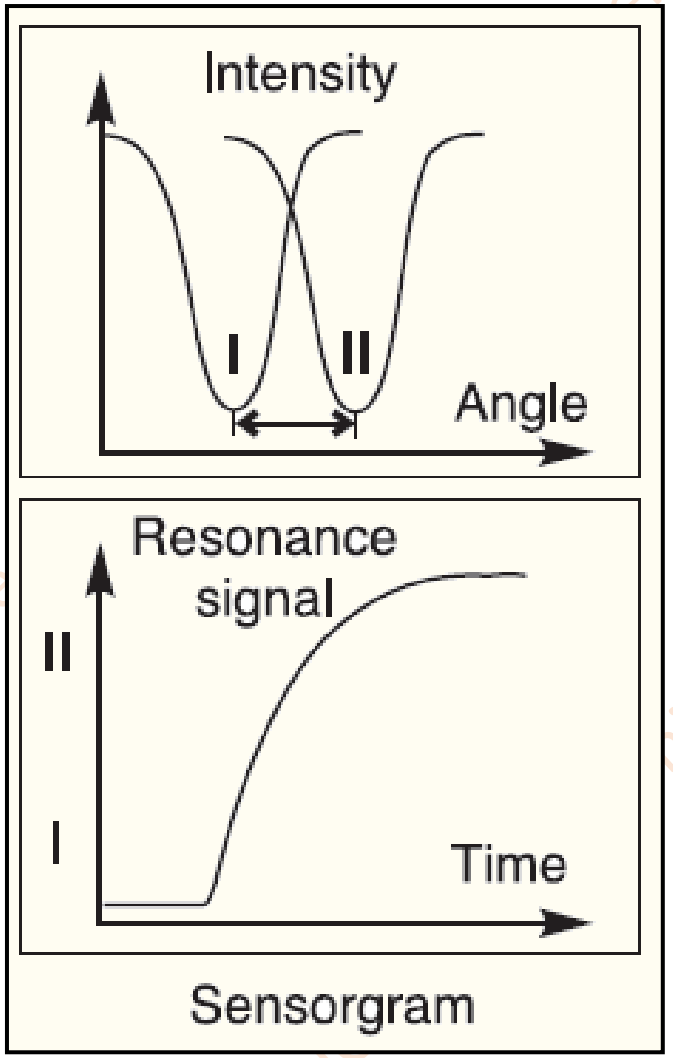
- At certain combination of incident angle and wavelength the free electrons on the metal surface are excited, what causes decrease in reflected light intensity.
- **This effect depends on refractive index that varies with the analyte binding to the surface-bound ligand.**

SPR – Basic principles

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

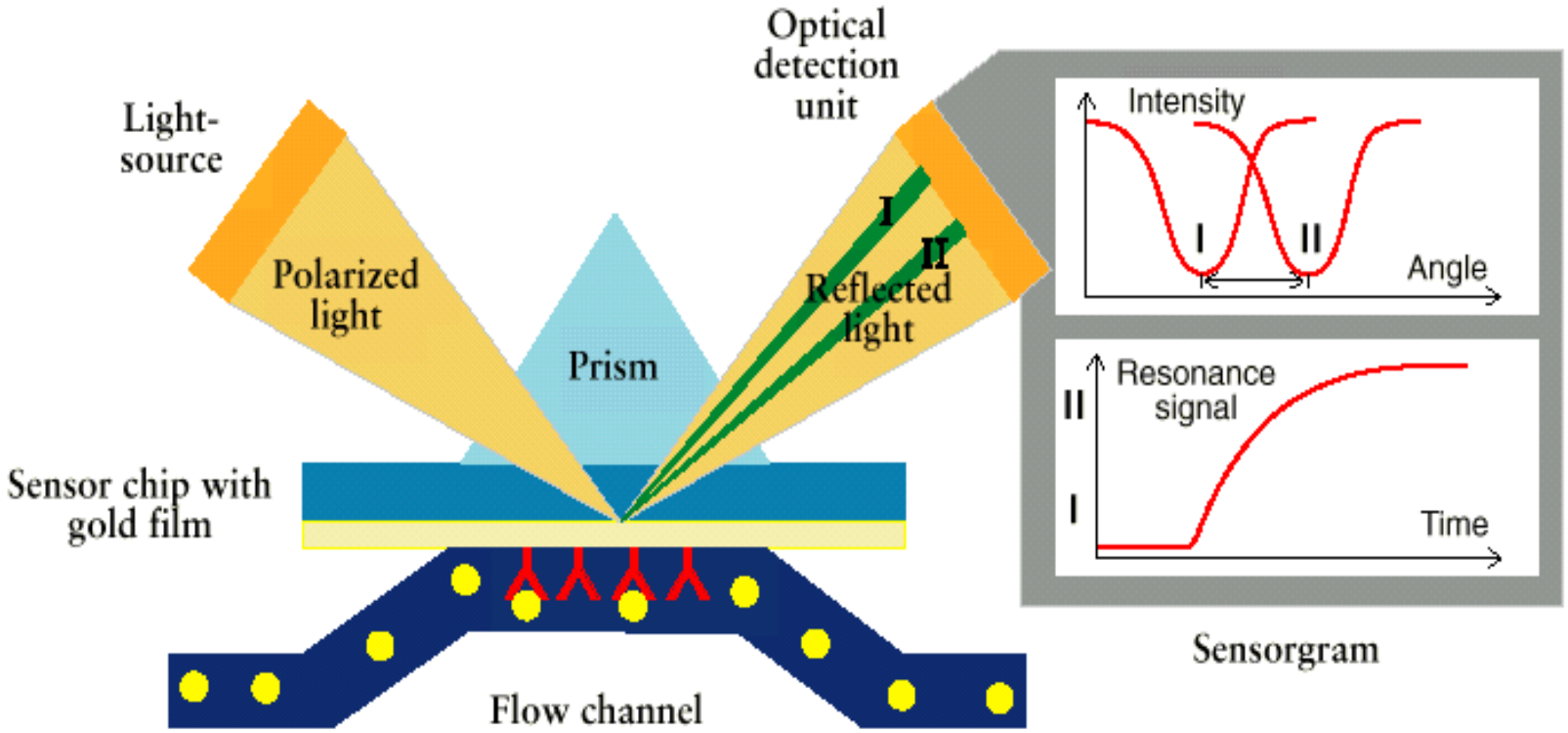
Corresponds also to change of mass on the chip surface = protein/ligand binding. (1 RU ~ 1 pg/mm^2)





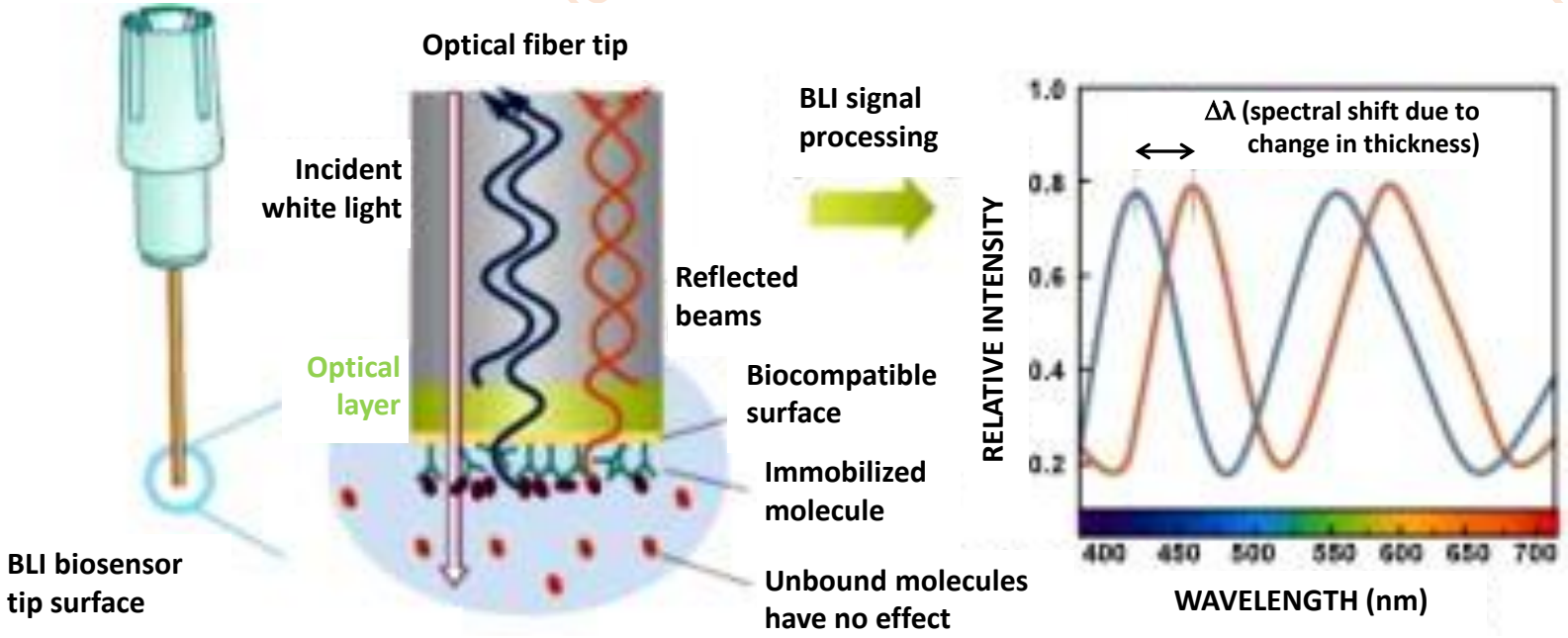
SPR – Basic principles

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



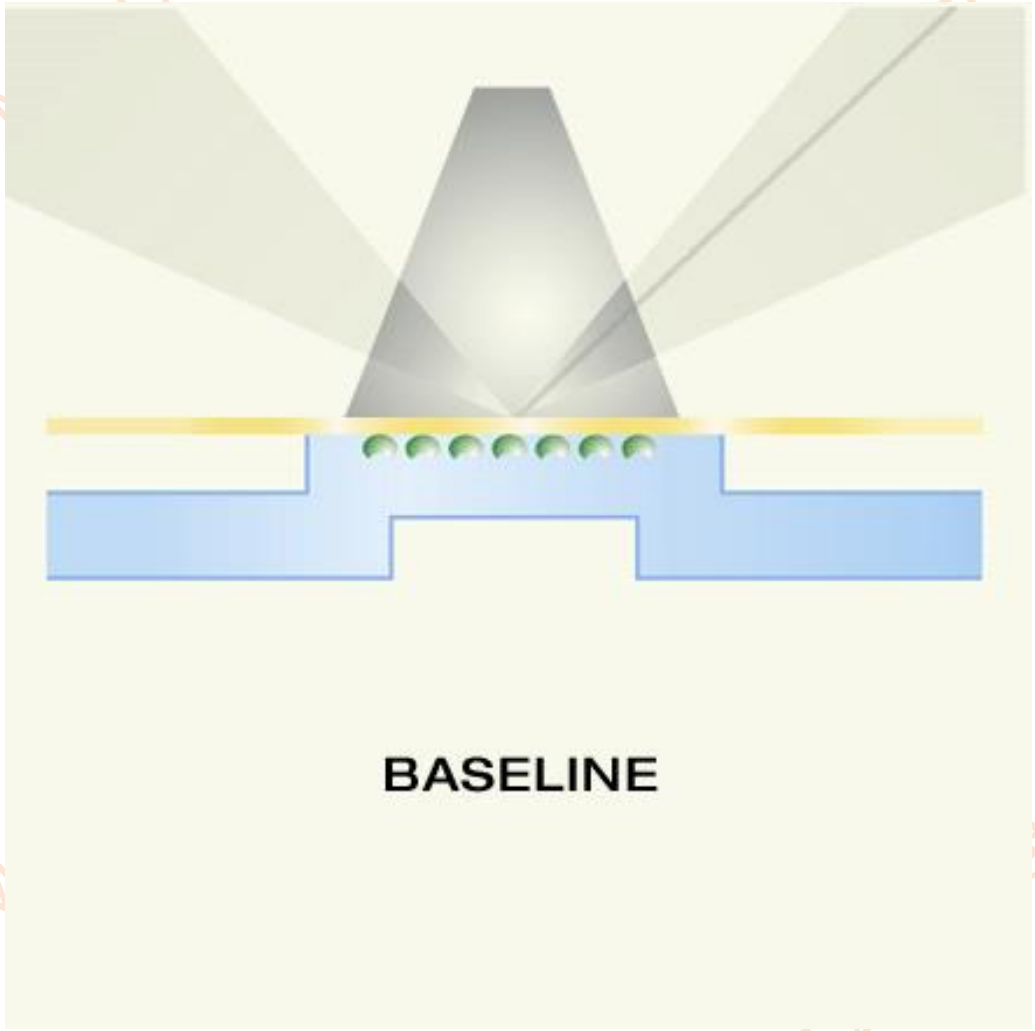
BLI – Basic principles

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

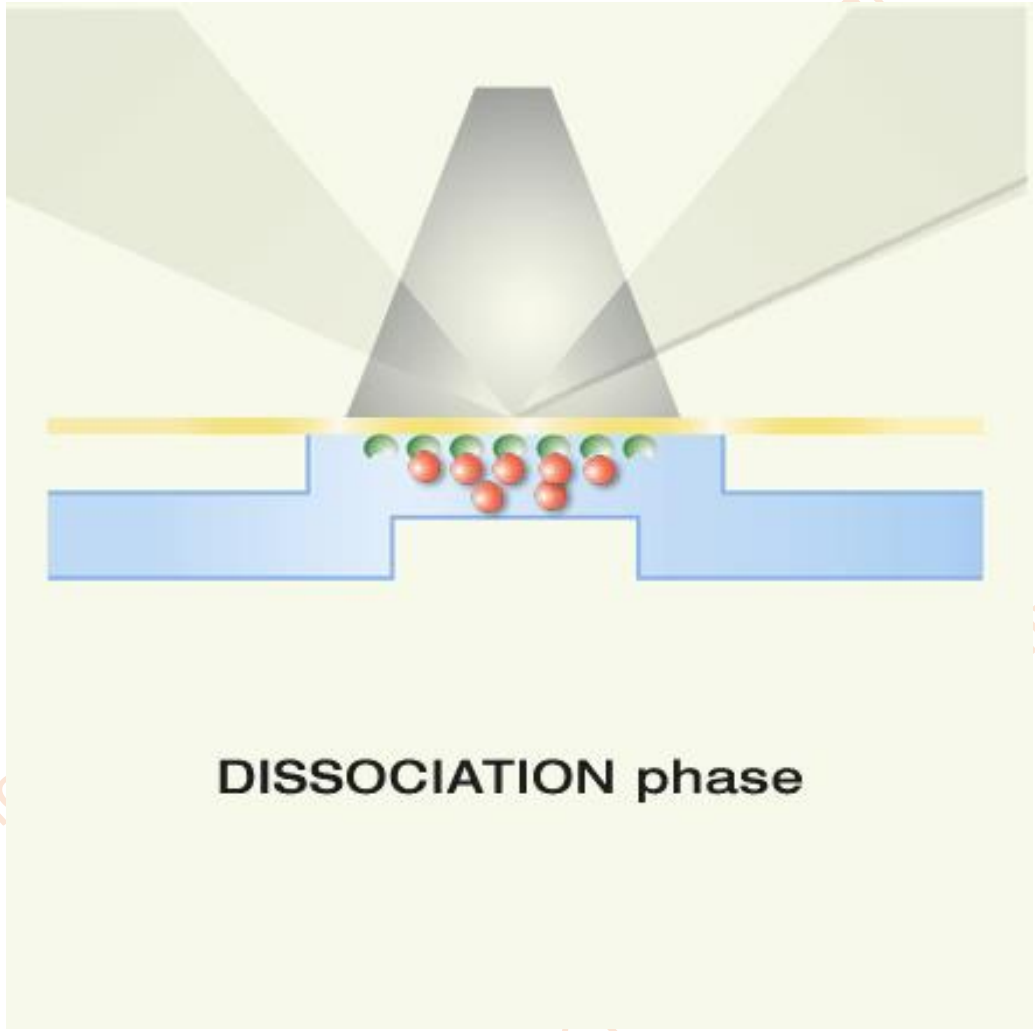


- Light reflects from the inner tip surface and outer tip surface resulting in formation of **interference pattern**.
- Binding of analyte on the sensor tip results in **change of the thickness** of the optical layer -> shift in the interference pattern.

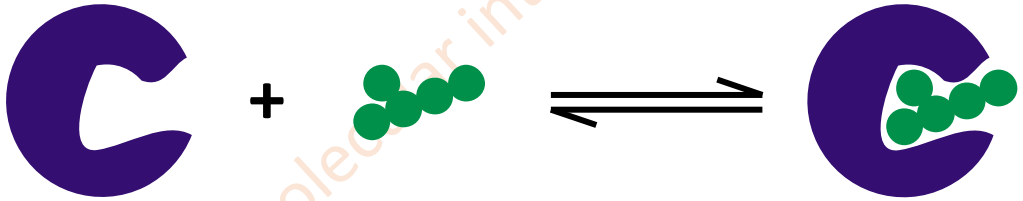
Association



Dissociation



Receptor ligand interaction



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

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

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

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

Binding experiment

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

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

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

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

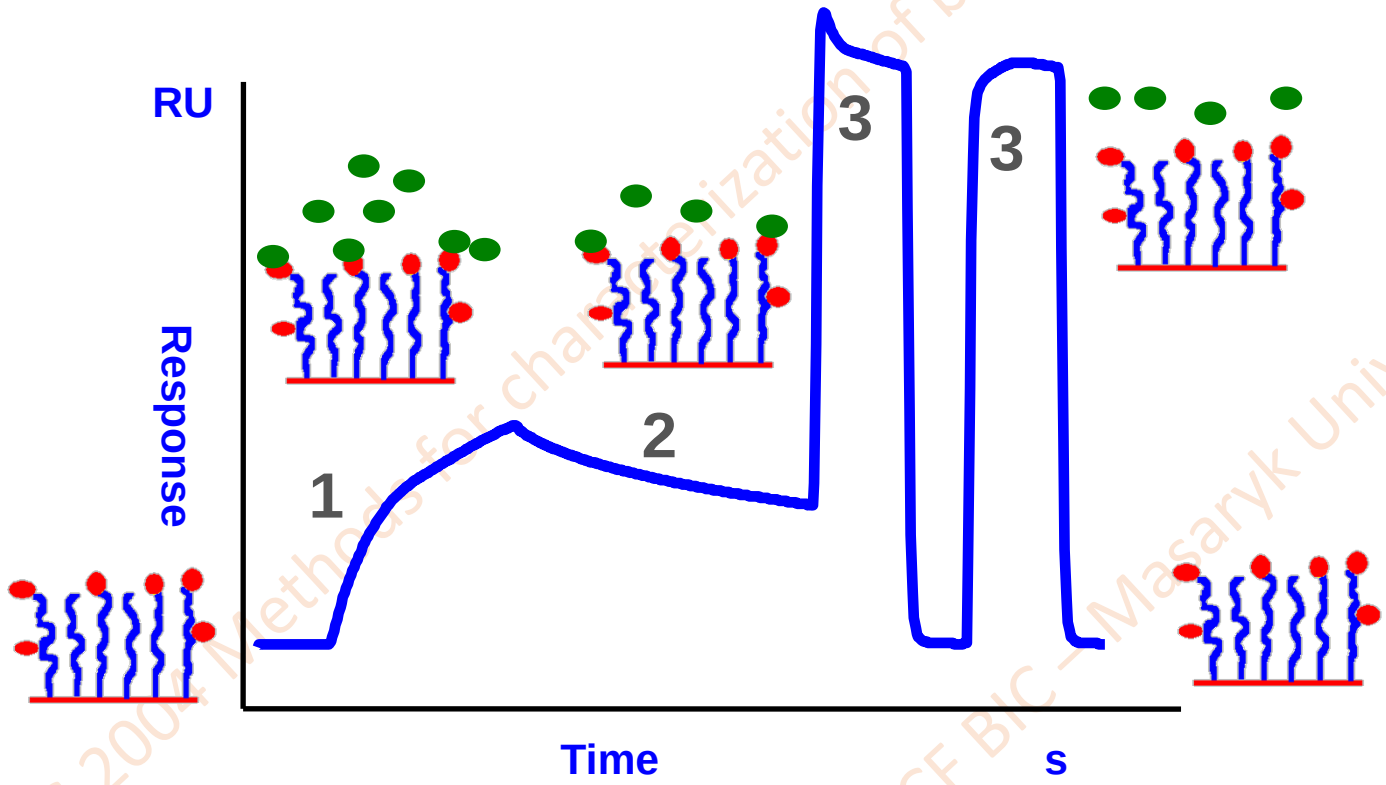
Simple binding - kinetics

Typical binding curve

1 - Association

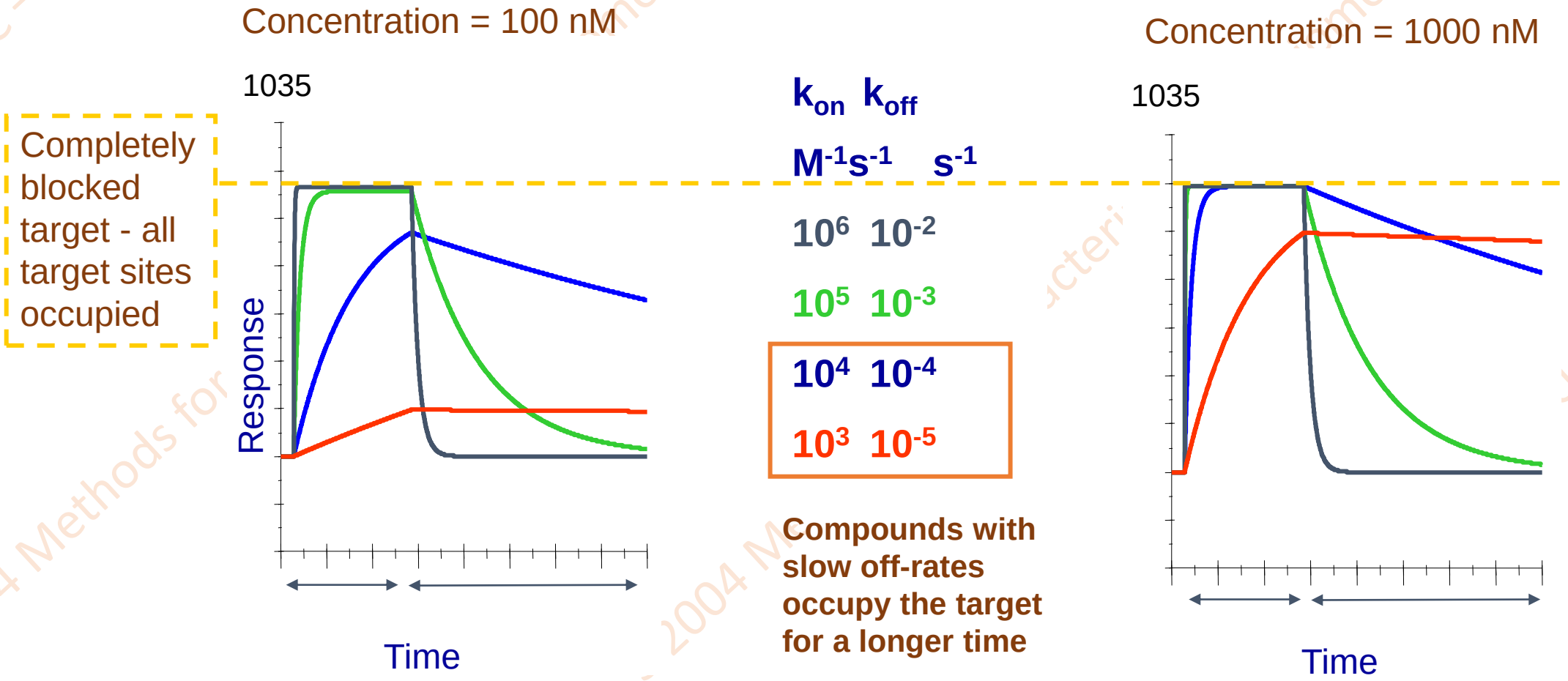
2 - Dissociation

3 - (Surface regeneration)



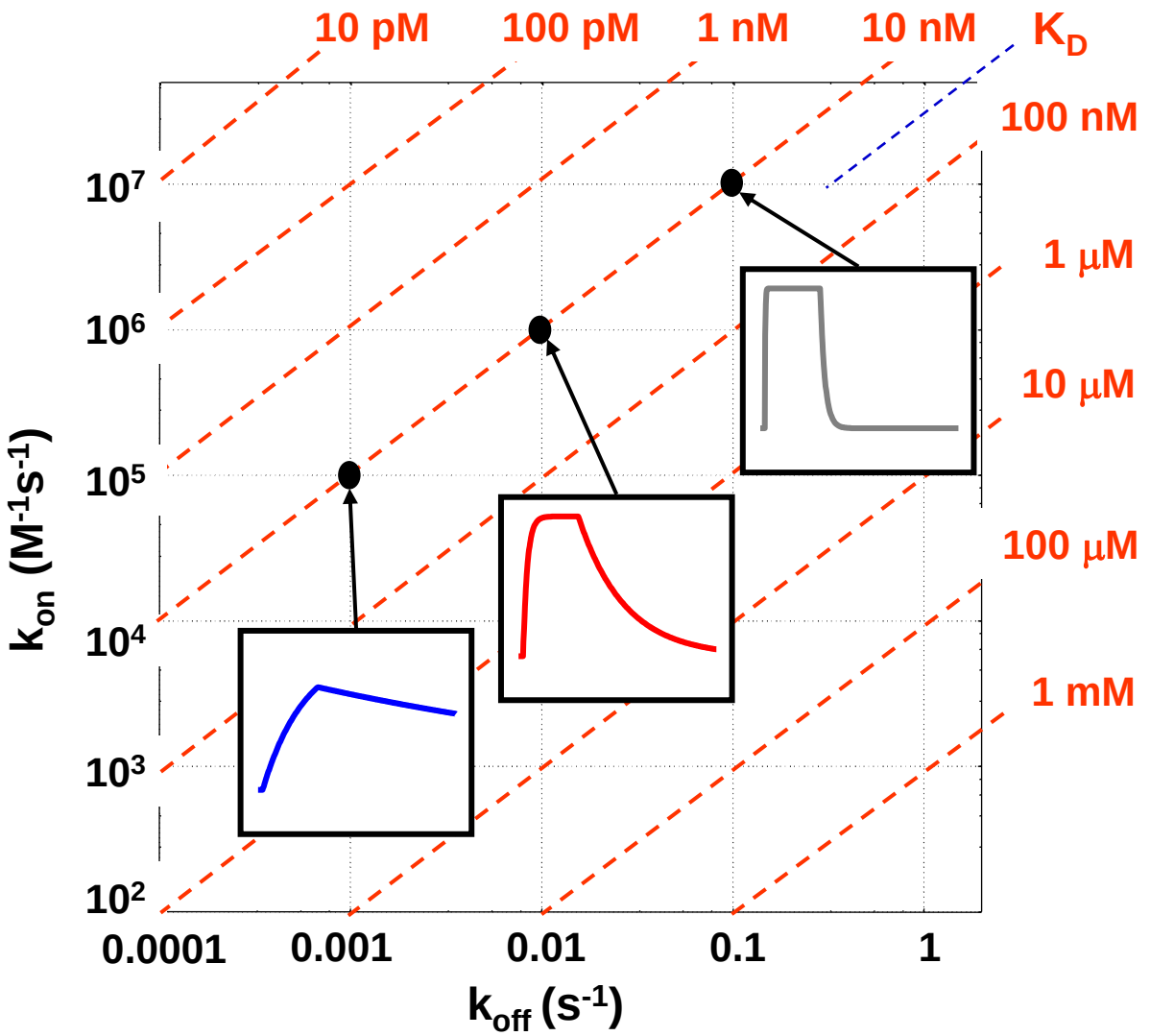
Same affinity but different kinetics

- 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

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 !

Experiment

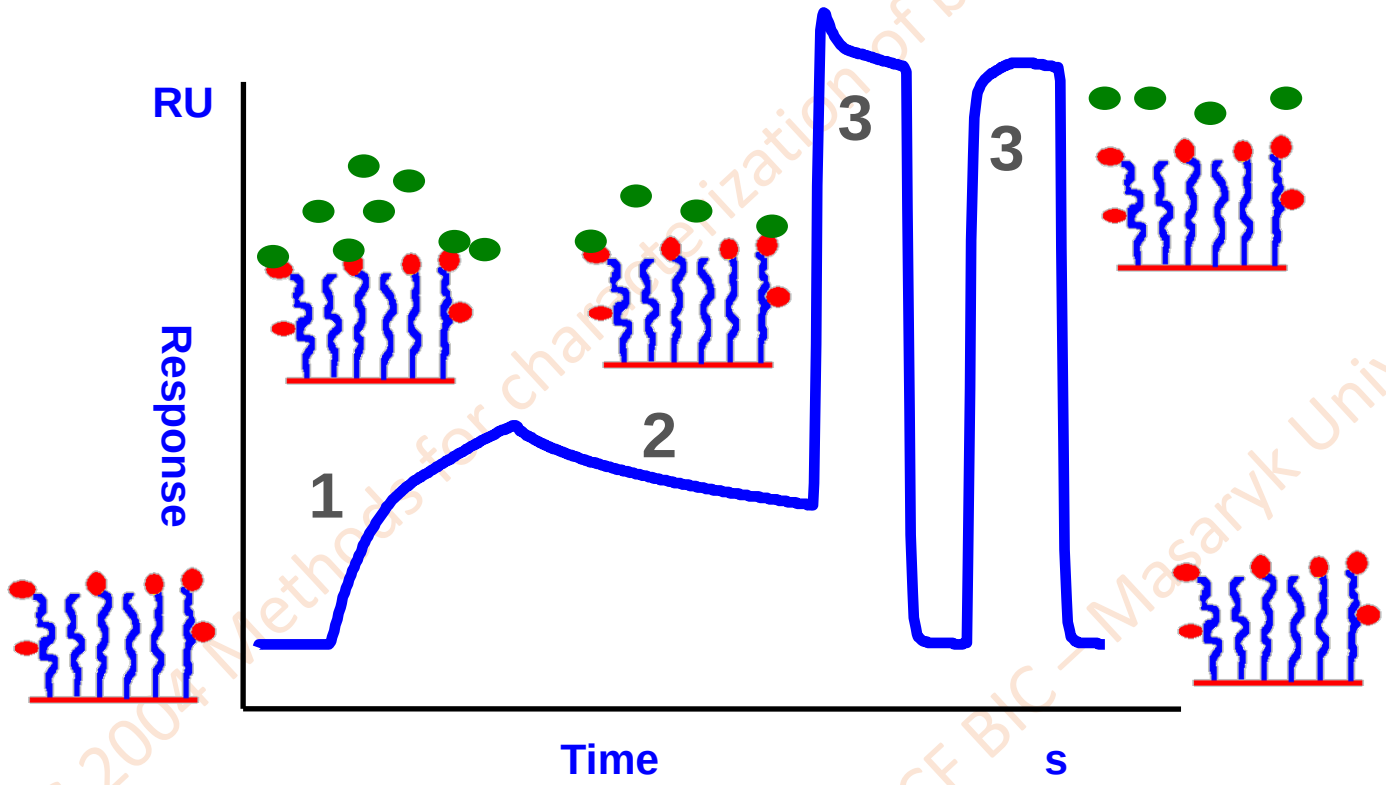
Simple binding - kinetics

Typical binding curve

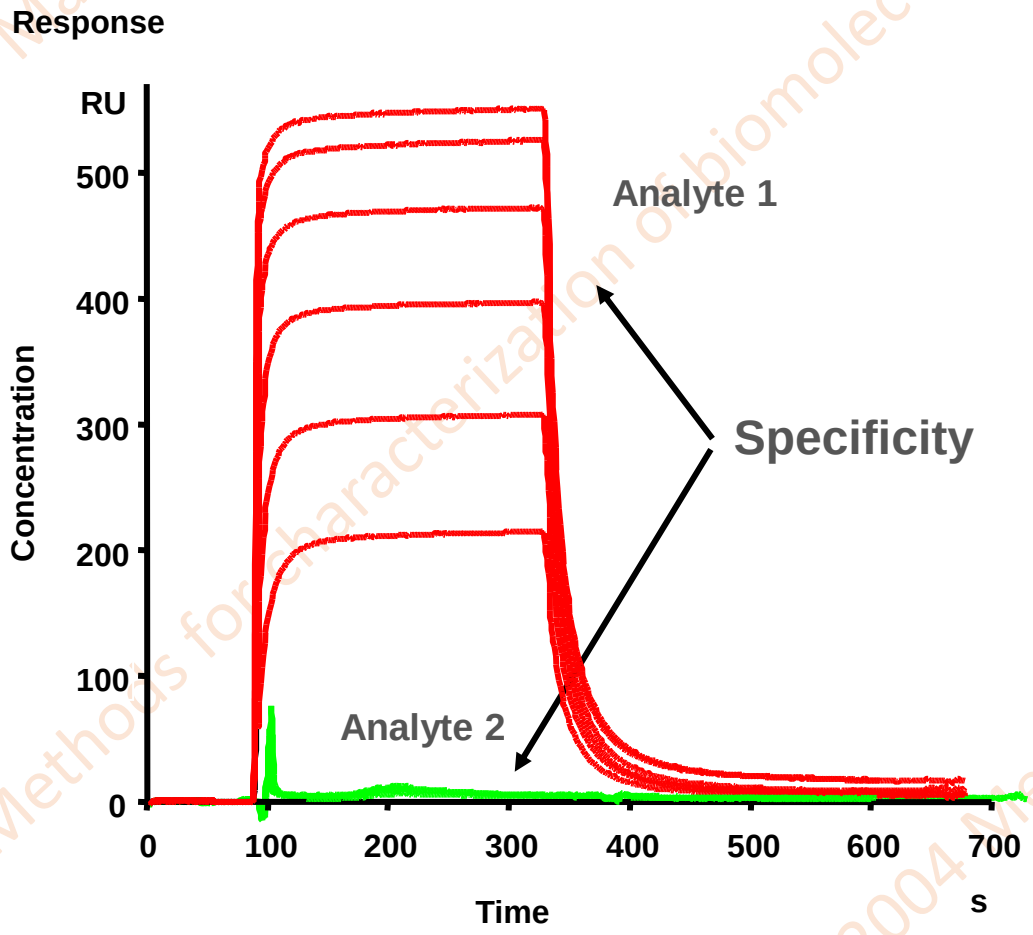
1 - Association

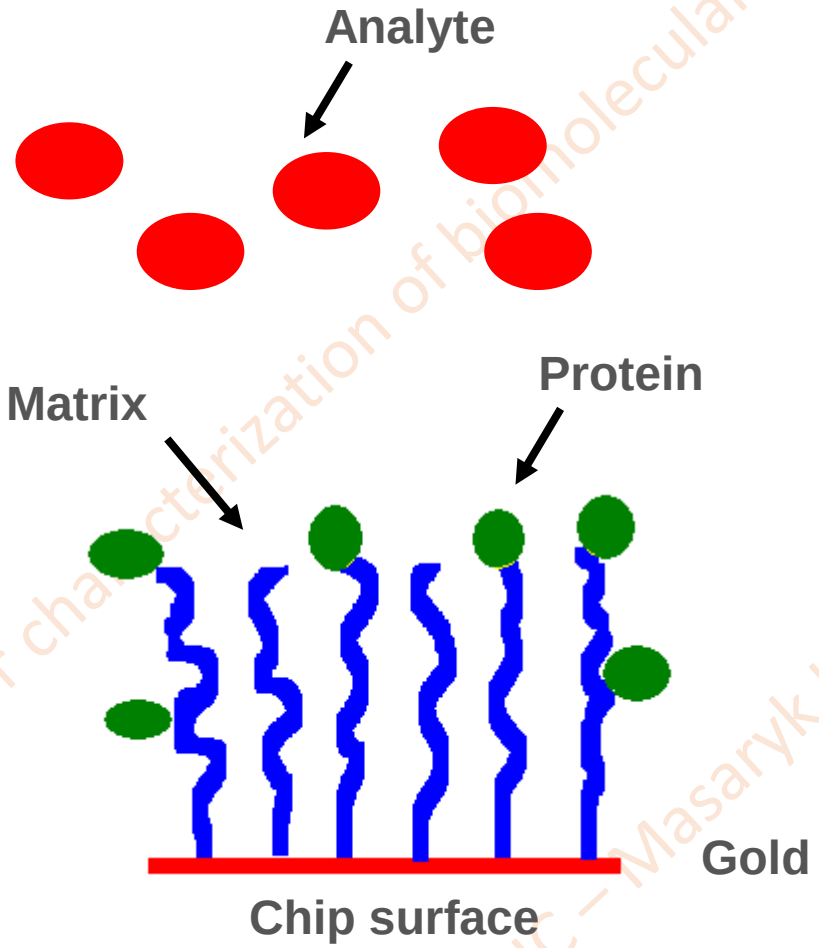
2 - Dissociation

3 - (Surface regeneration)



Simple binding – specificity

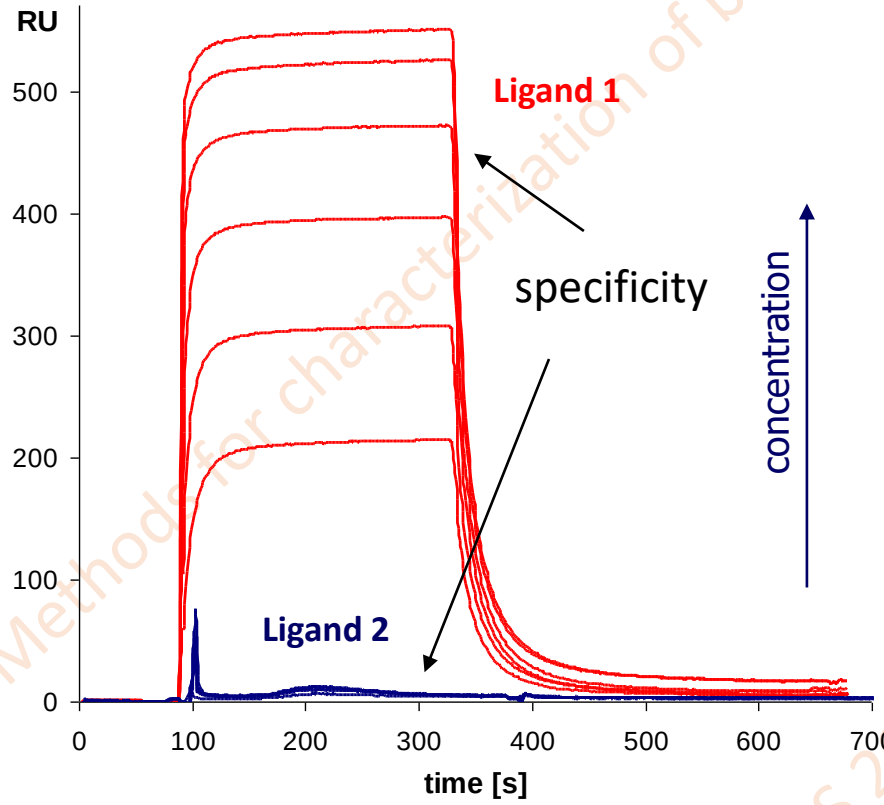




Simple binding – kinetics

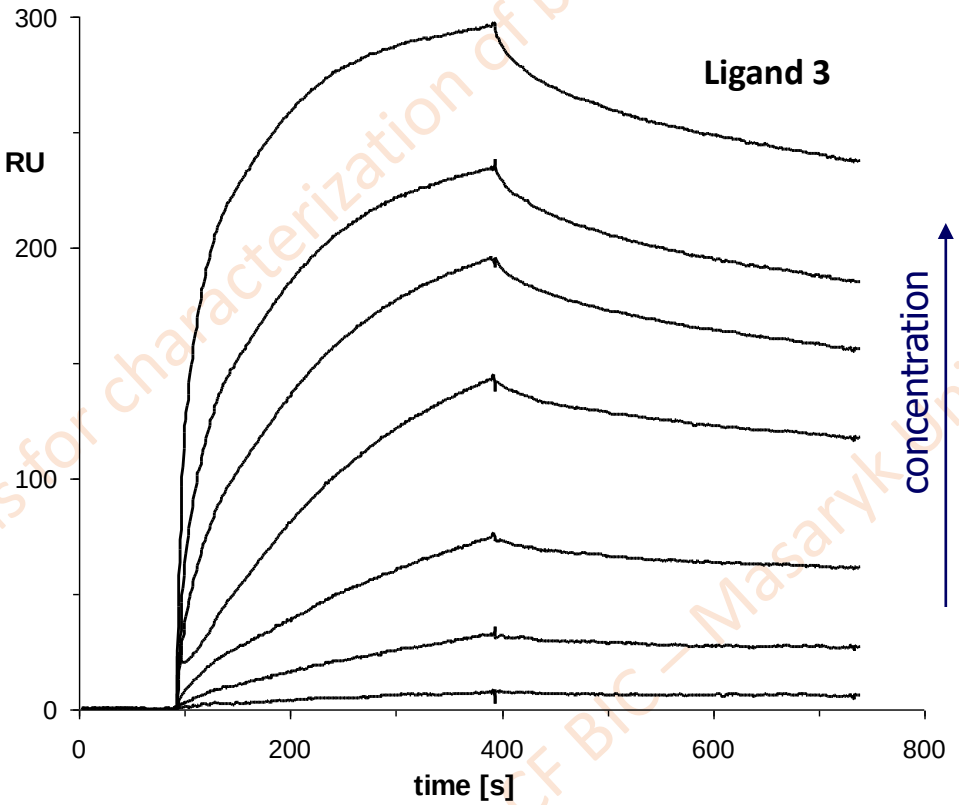
Fast complex association and dissociation

Fast equilibrium ⇨ K_A, K_D



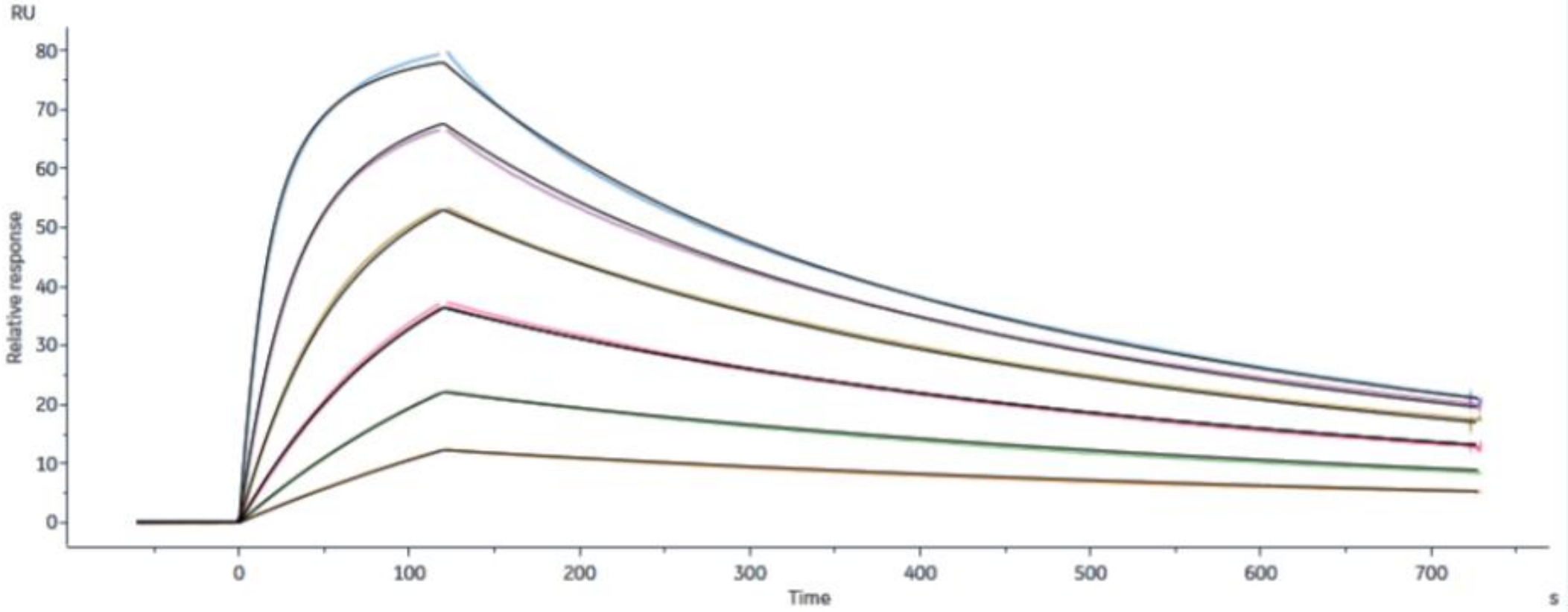
Slow complex association and dissociation

Kinetic constants k_a, k_d ⇨ K_A, K_D



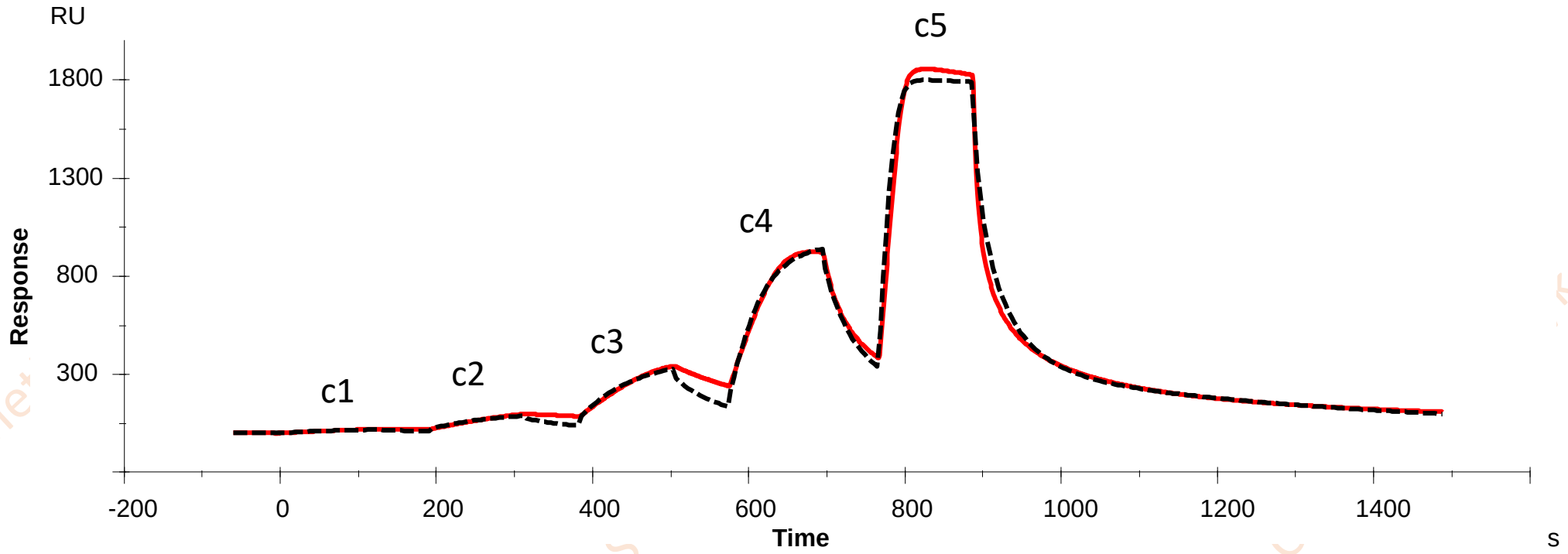
Simple binding – kinetics

- Kinetic evaluation – fitting of association and dissociation curves



Simple binding – single-cycle kinetics

- Association is concentration dependent
- Dissociation is concentration independent
- Multiple concentration followed by single dissociation – time effective



Simple binding – steady state

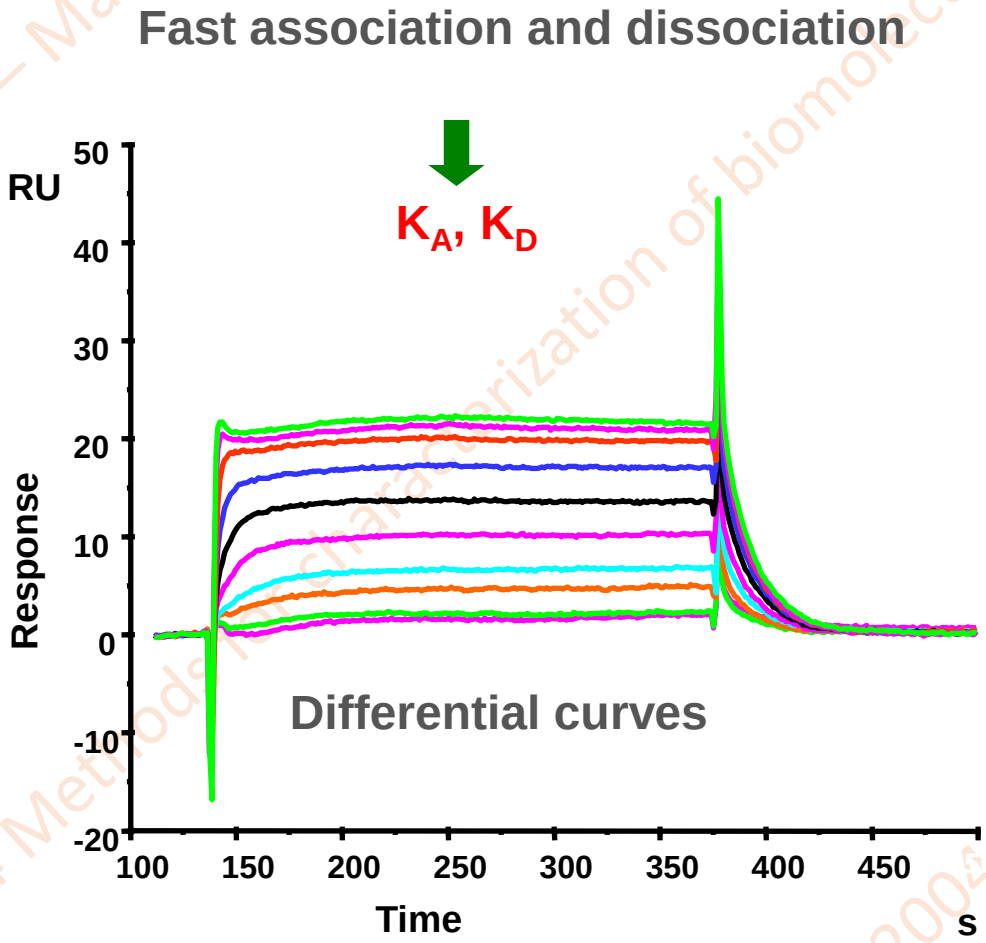
Fast association and dissociation data
are not easy to fit

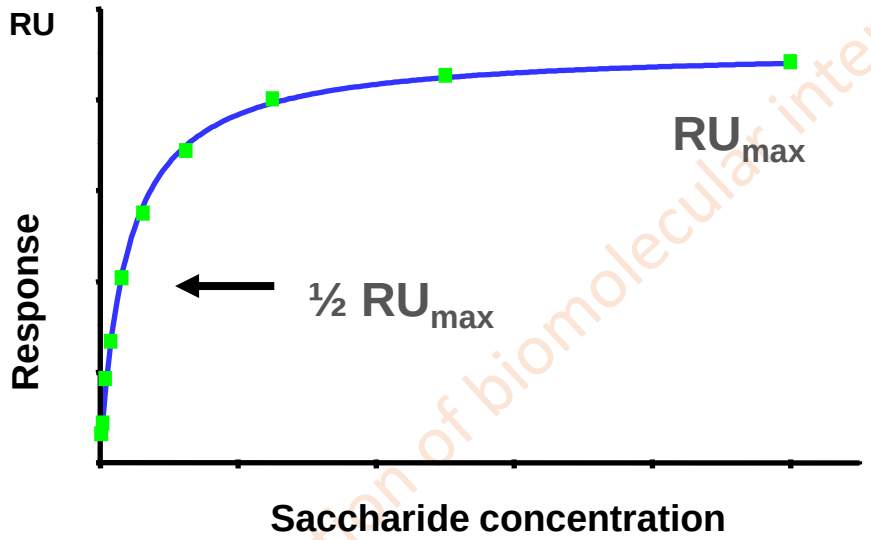
BUT

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

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

Direct binding assay





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

$RU = ½\ RU_{max}$

↓

[Protein] = [Protein-Analyte]

↓

K_D = [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** and/or **way of binding** of analyte
- Portion of **active molecules** present –
  proper sample characterization needed, changes upon immobilization – site accessibility restriction, conformational changes, intermolecular distance
- Conditions/**buffer** properties

Sensitivity

- **SPR**
  - Signal proportional to **mass** on the surface
  - High sensitive instruments – reliable analysis of **<100 Da** analytes (e.g. metal ions)
  - Suitable for both small molecules and proteins/nucleic acids

- **BLI**
  - Signal proportional to **thickness** of surface layer
  - High sensitive instruments – require **>1 kDa** analyte or structural change of immobilized molecule
  - Suitable mainly for proteins/nucleic acids

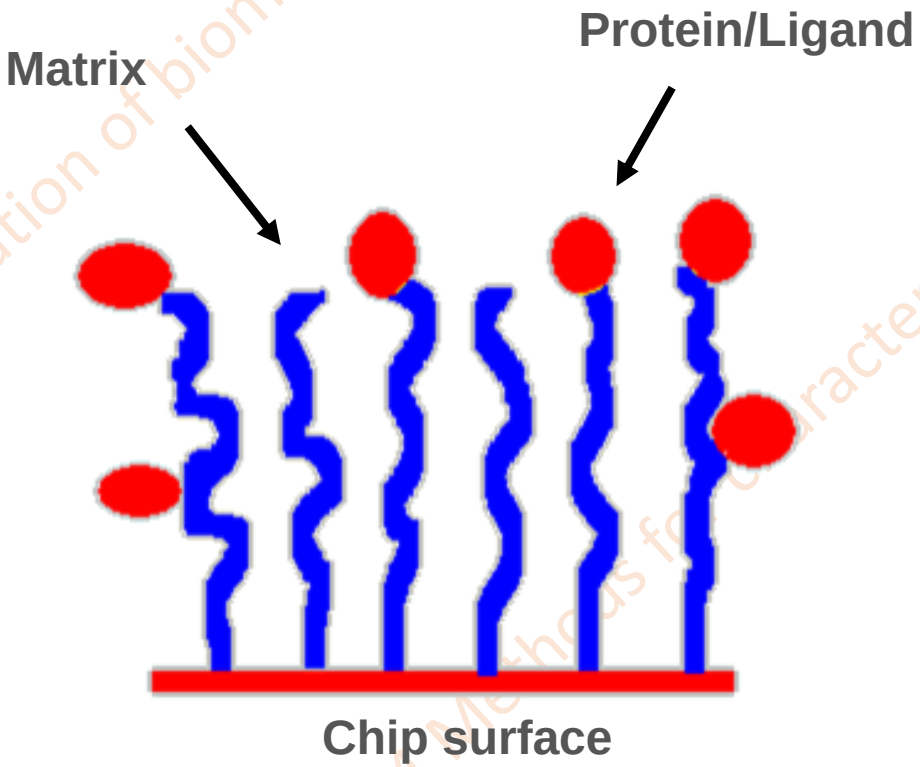
Which binding partner to immobilize?

- Stability
- Availability
- Molecular mass

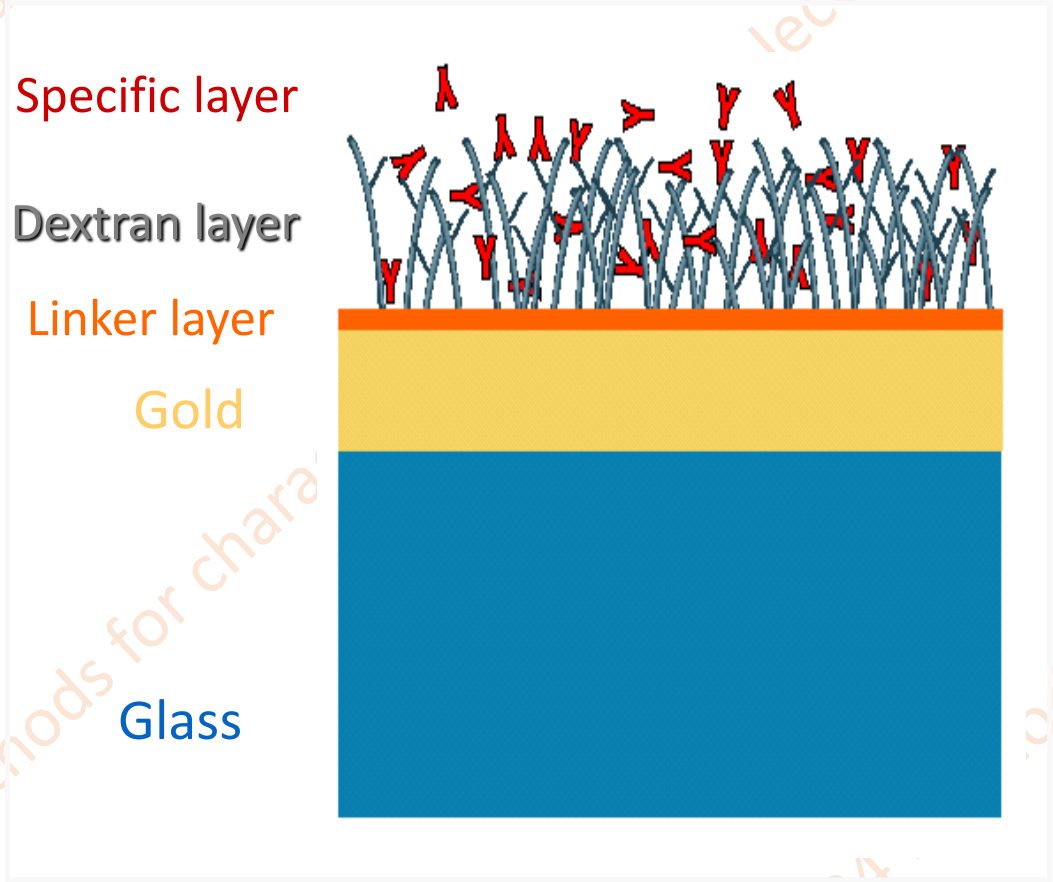
$$\text{Response}_{max} = \text{Response}_{ligand} \times \frac{Mr_{analyte}}{Mr_{ligand}} \times \frac{\left(\frac{\partial n}{\partial c}\right)_{analyte}}{\left(\frac{\partial n}{\partial c}\right)_{ligand}}$$

- Immobilization technique
- Multivalency

Sensor Chip – rough scheme



User-defined biospecific surface



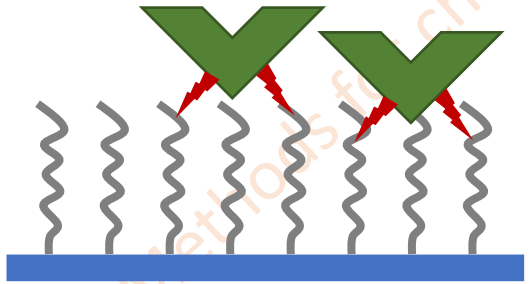
- Biocompatible
- Low non-specific binding
- Robust
- More than 100 runs on the same surface

Immobilization techniques

High flexibility in creating biospecific surfaces

Direct covalent coupling

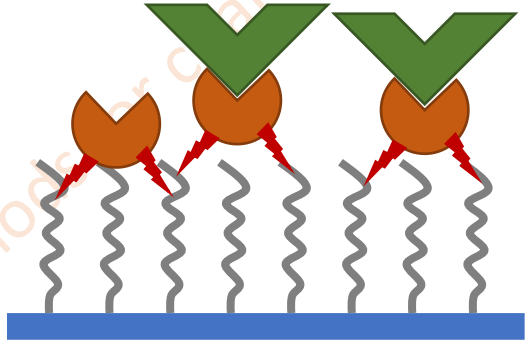
- Stable
- Suitable regeneration needed



- Amine (Lys, N-term)
- Thiol (Cys)
- Aldehyde
- Carboxyl

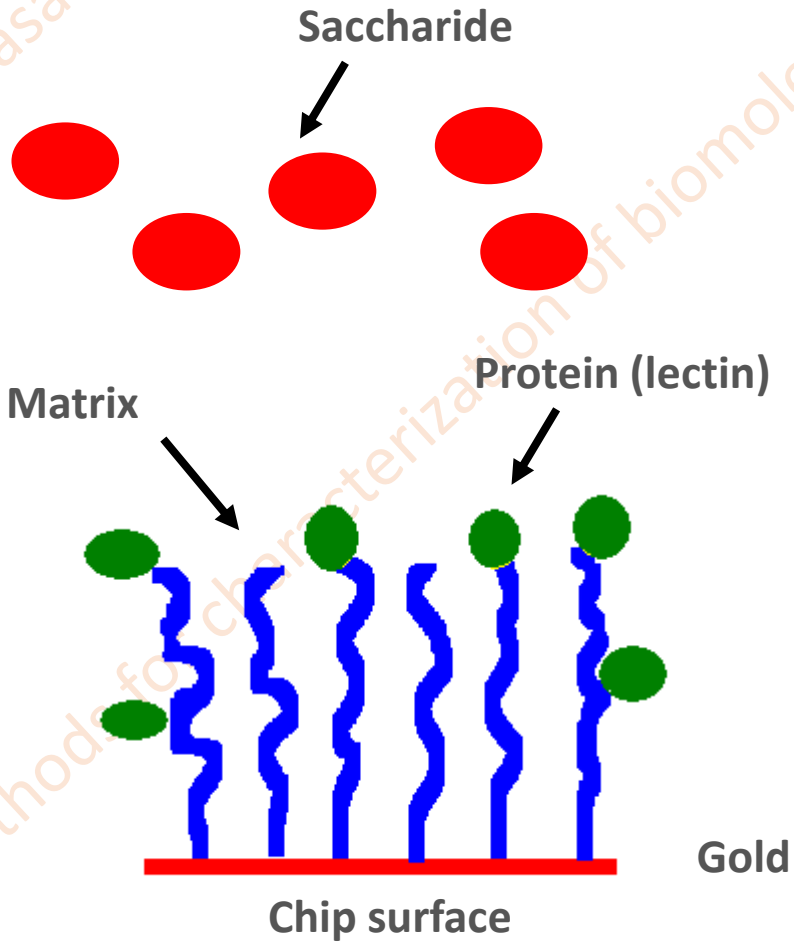
Capture

- Multi-step process
- Less stable binding
- Easier regeneration (not for SA)

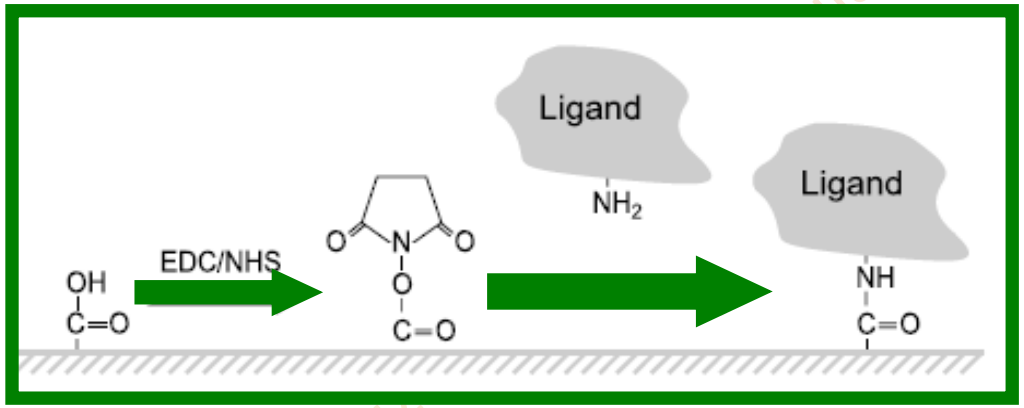


- Streptavidin – Biotin
- NTA-Ni^{2+} – His_6
- Anti-His – His_6
- ProteinA – mAb
- Anti-GST – GST

Protein immobilization



„Amine-coupling“

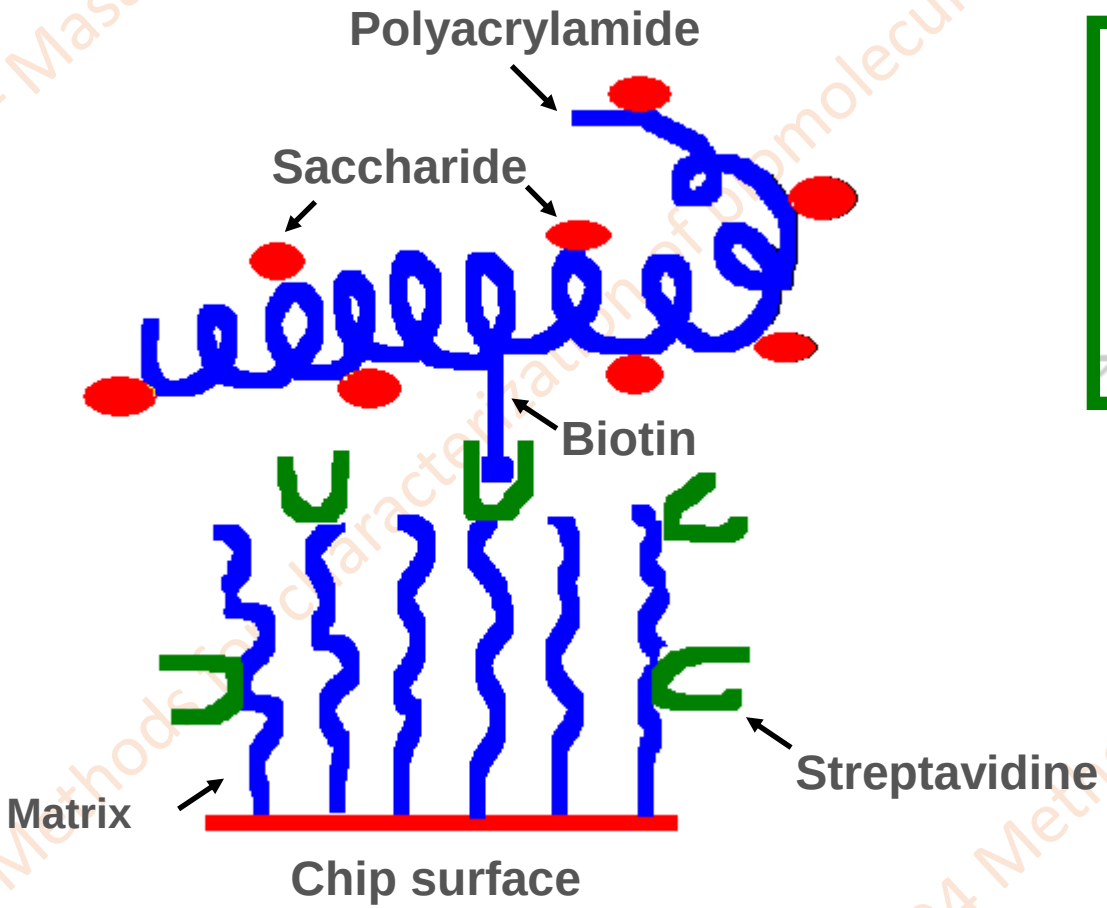


***N*-ethyl-*N'*-(3-diethylaminopropyl)karbodiimide**

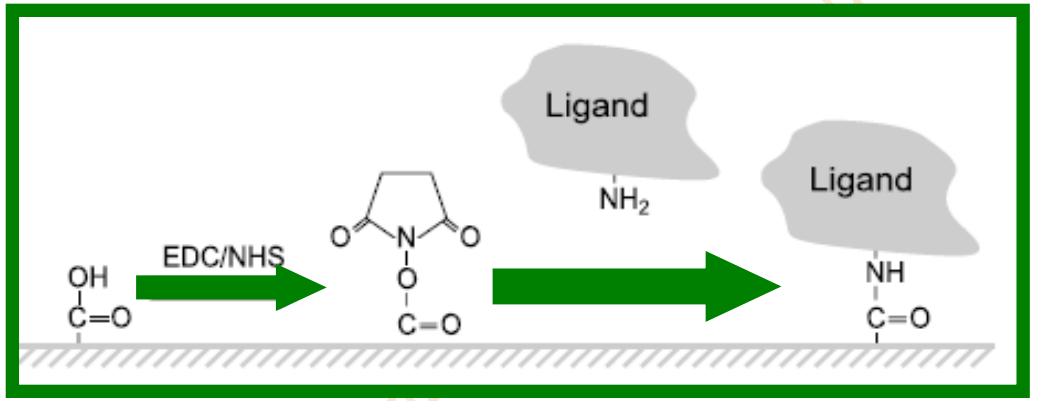
***N*-hydroxysuccinimide**

CM5 chip – surface modified by carboxymethylated dextran

Small molecule immobilization



„Amine-coupling“

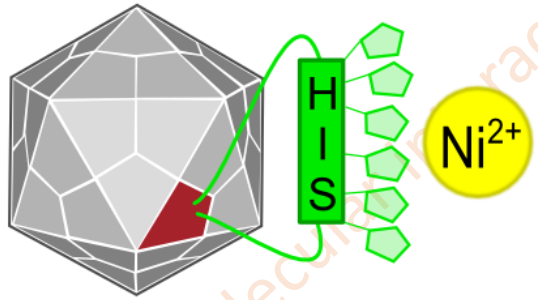


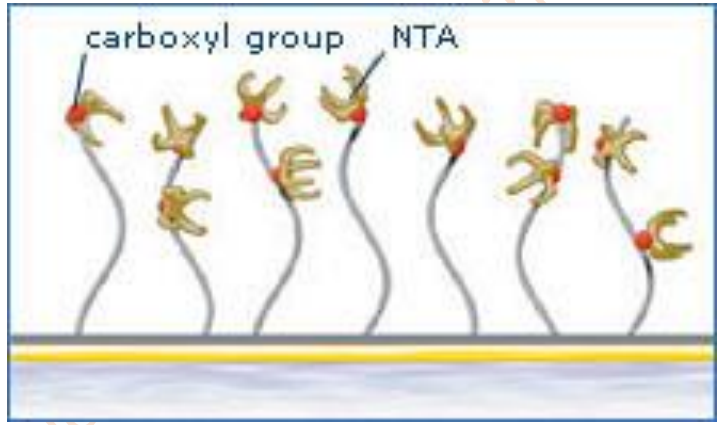
N-ethyl-*N'*-(3-diethylaminopropyl)karbodiimide

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

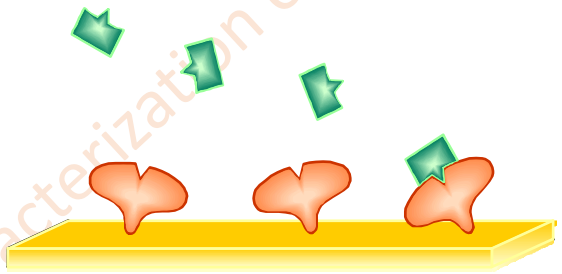
Regeneration

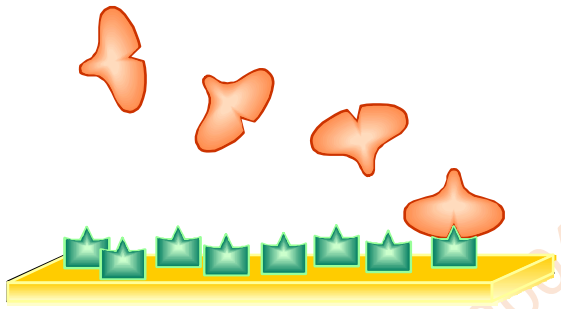
Flexibility in Assay Design

Multiple assay formats providing complementary data

Direct measurement

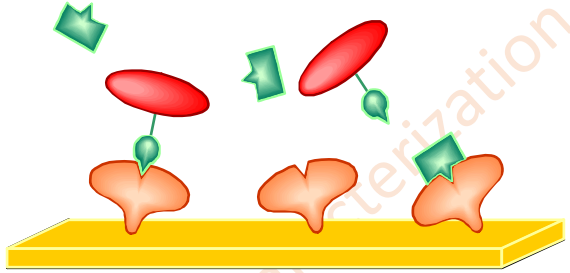
Direct Binding Assay (DBA)



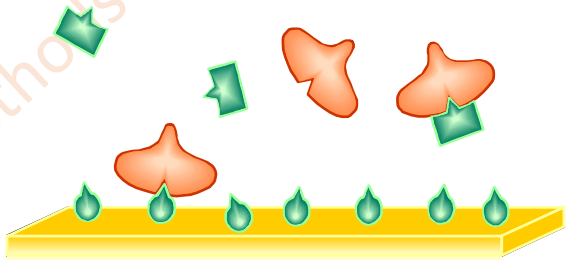


Indirect measurement

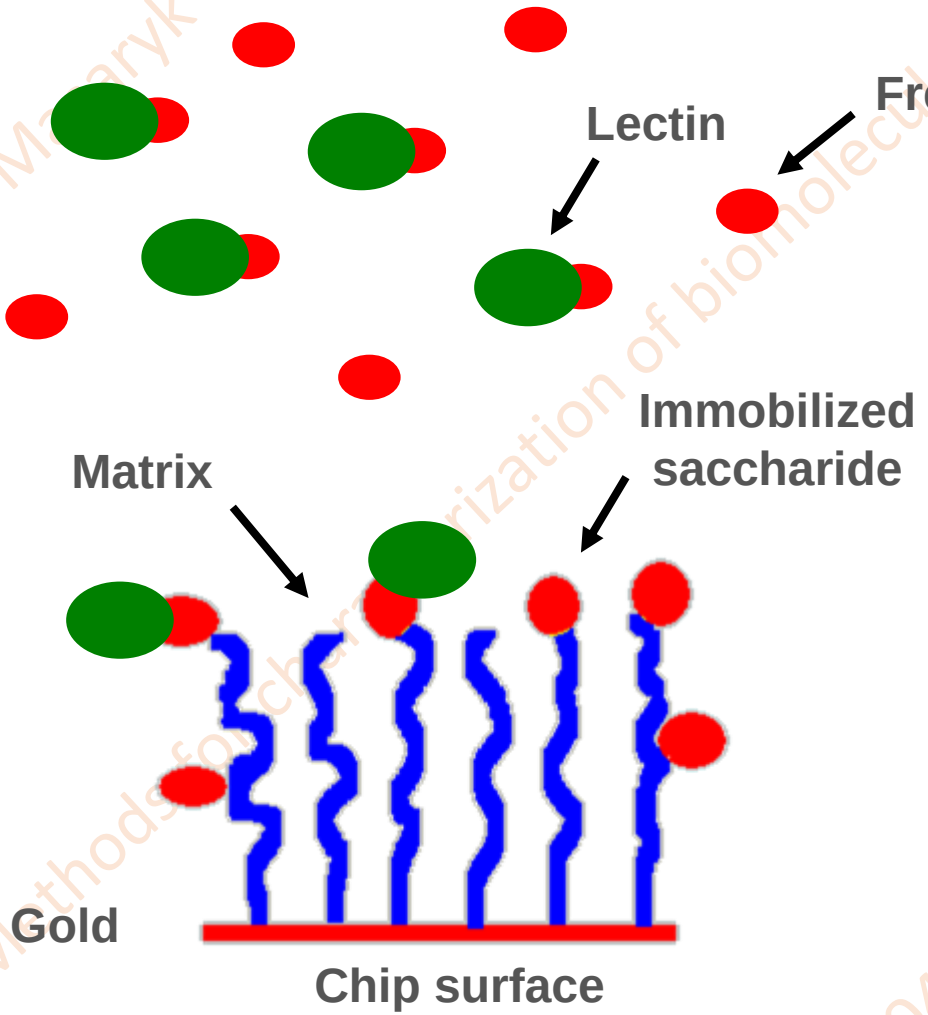
Surface competition assay (SCA)

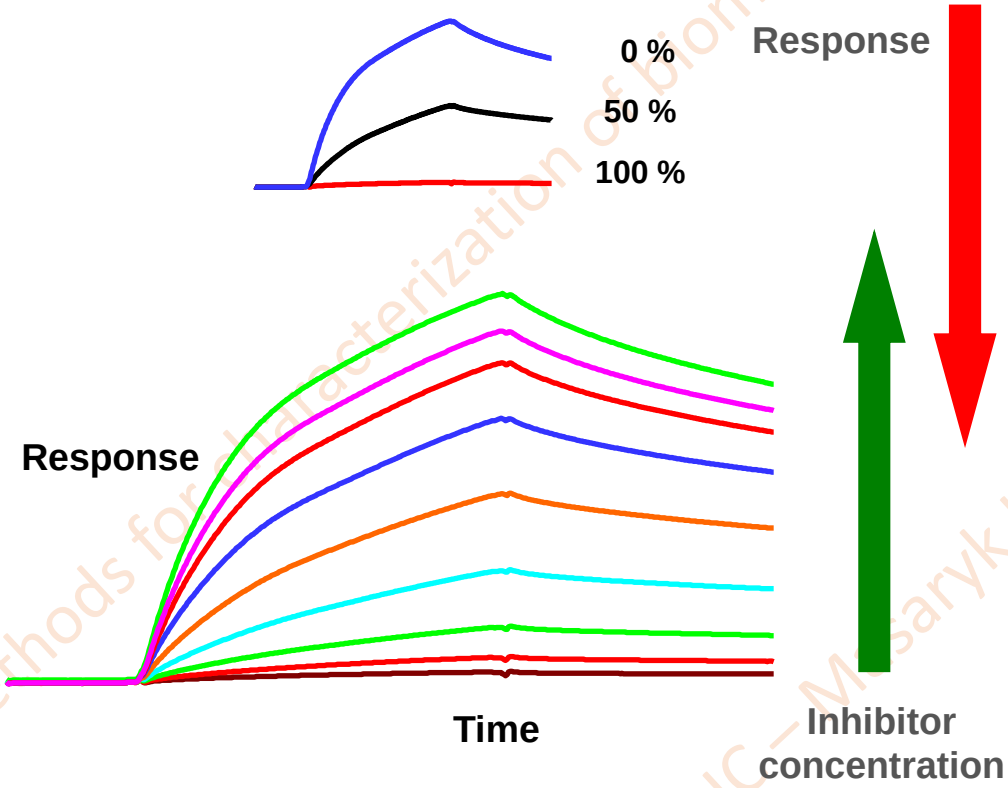


Inhibition in solution assay (ISA)

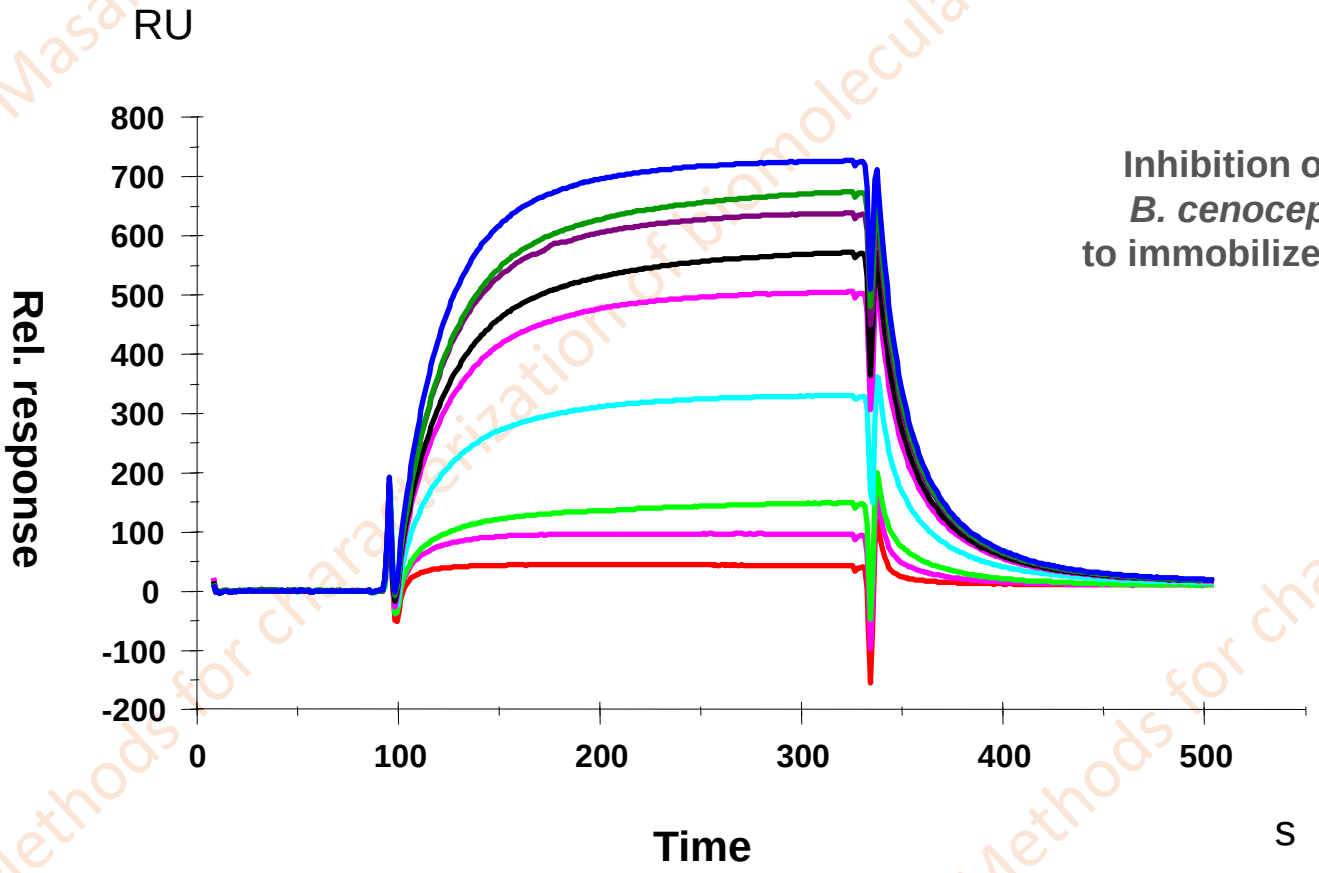


Inhibition in solution assay

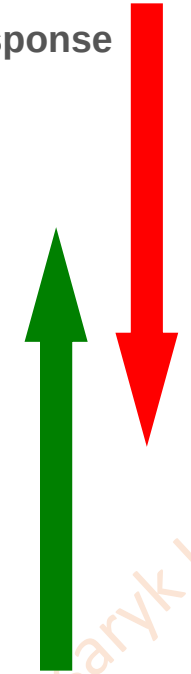




Inhibition in solution assay

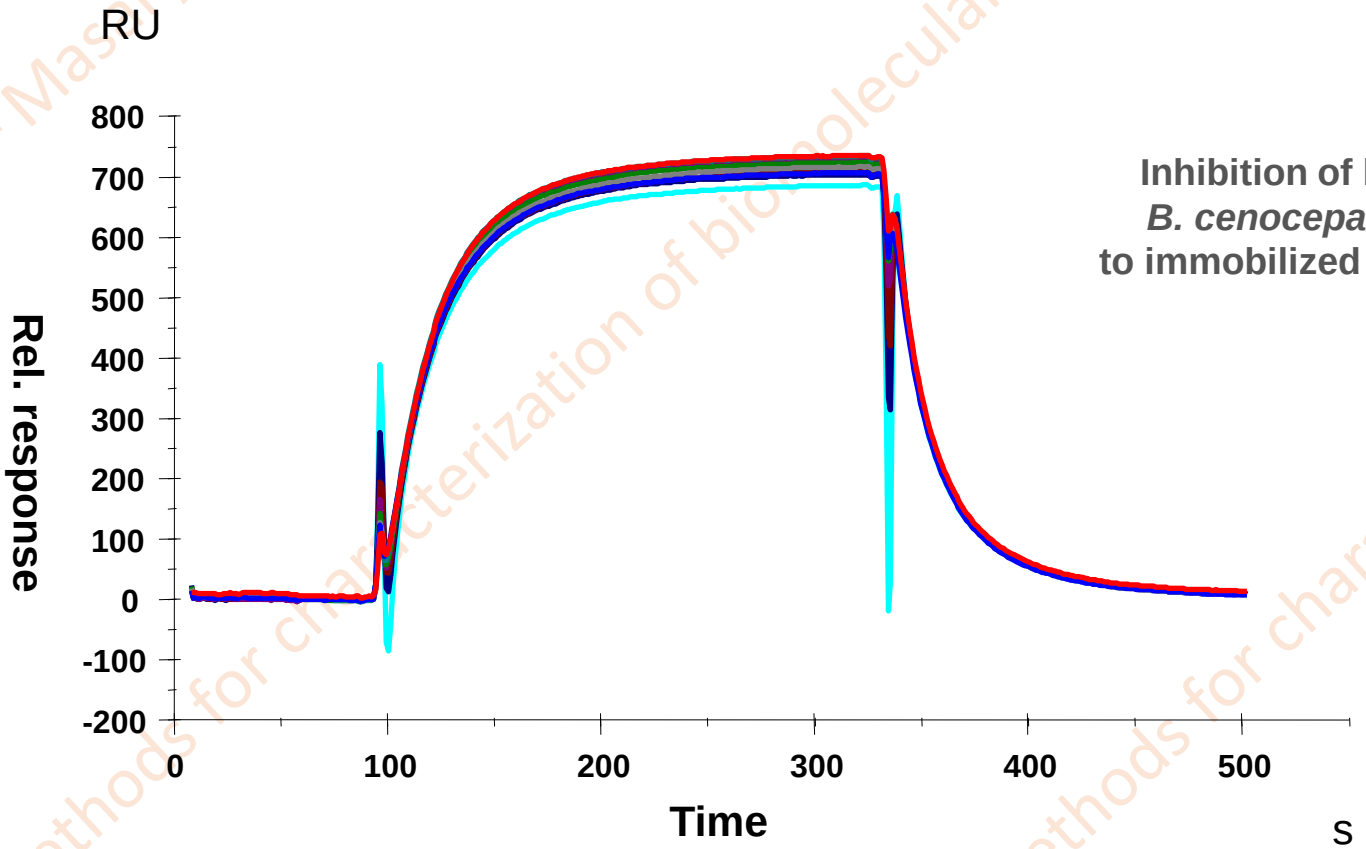


Inhibition of binding of *B. cenocepacia* lectin to immobilized D-mannose

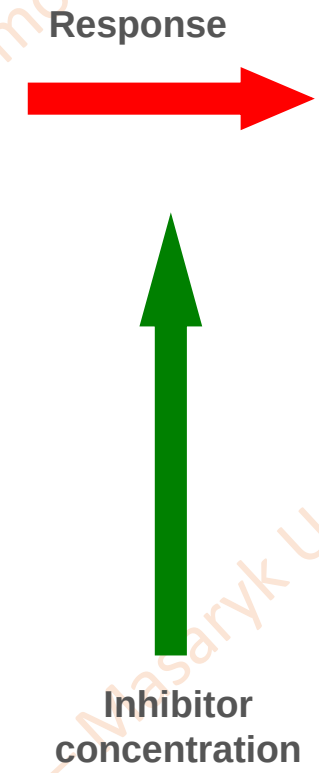


0-250 µM D-mannose

Inhibition in solution assay



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



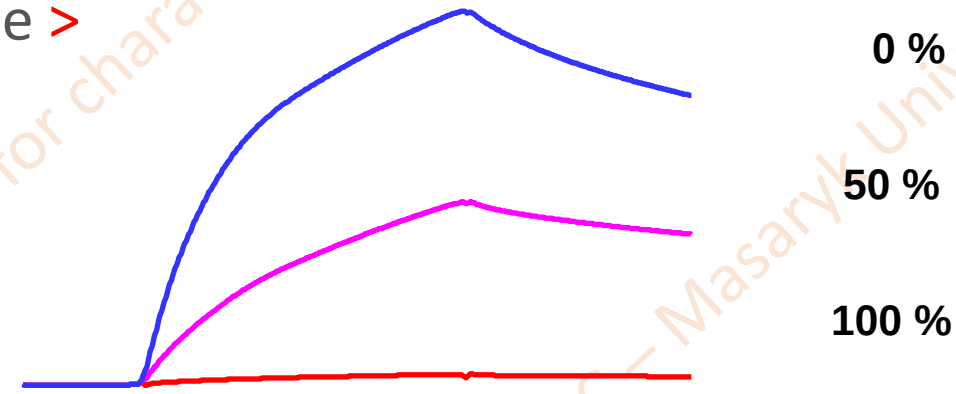
0-80 mM D-galactose

Inhibition in solution assay

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

Lectin from *B. cenocepacia*:

Benzyl-α-D-mannoside ≈ Methyl-α-D-mannoside ≈ D-mannose » L-fucose > D-arabinose > L-galactose > Methyl-α-L-fucoside » D-galactose

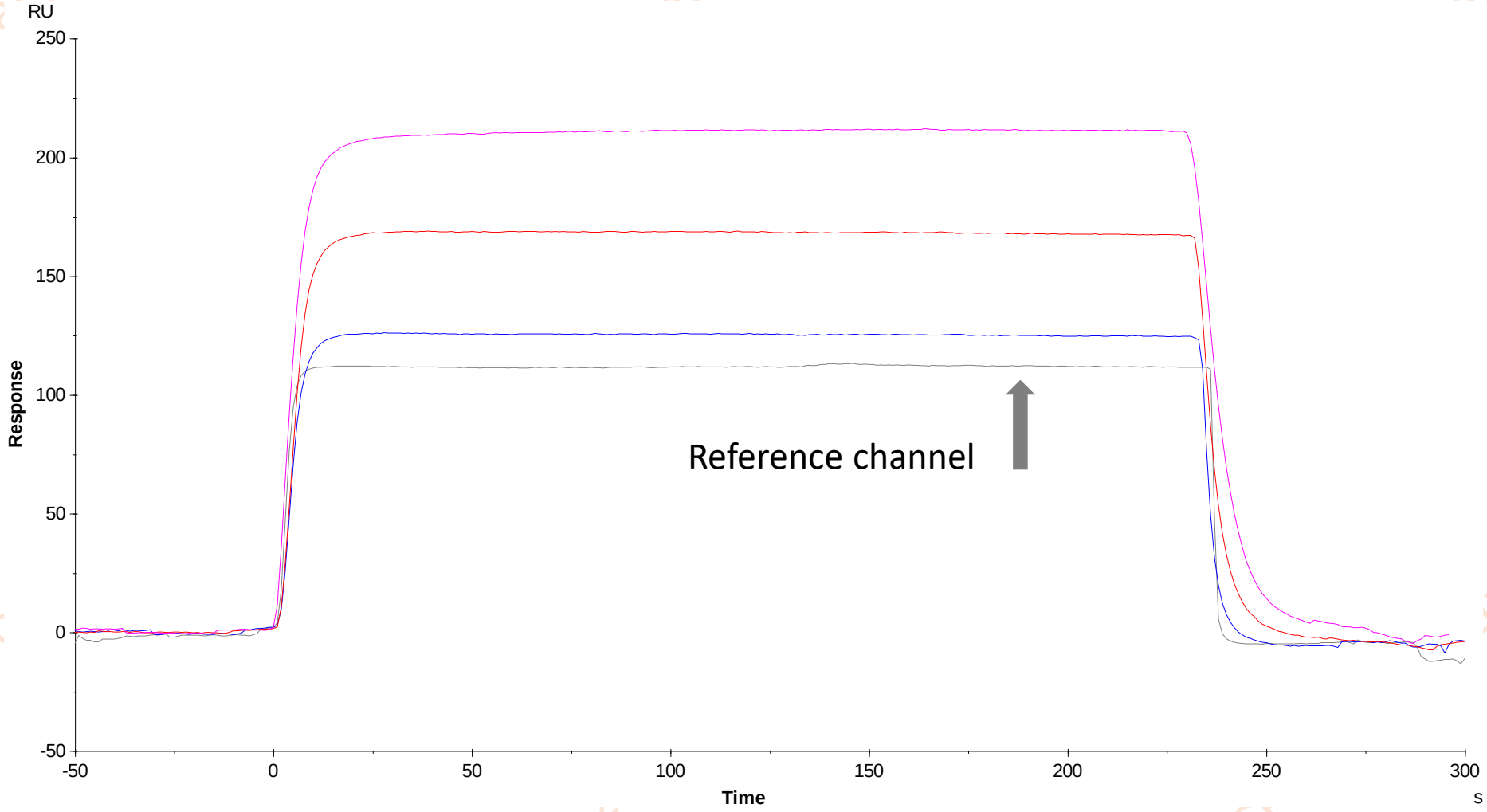


Two channels necessary - reference

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

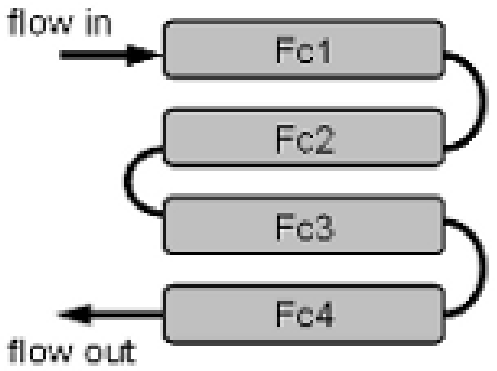
- **Possible reference surfaces**:
  - Unmodified surface – gold, dextran layer,…
  - Activated and blocked surface without immobilized ligand/protein
  - Inactivated/non-functional ligand/protein

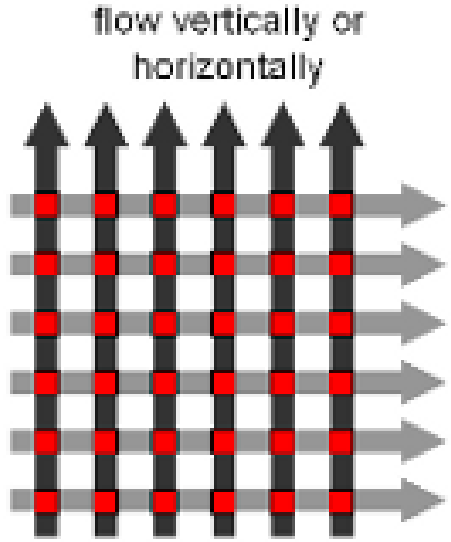
Two channels necessary - reference



Multichannel set-up

- One or more references
- Multiple channels – 2, 4, 6, 36,…
- Multiple detection spots





➢High throughput

➢Parallel reference

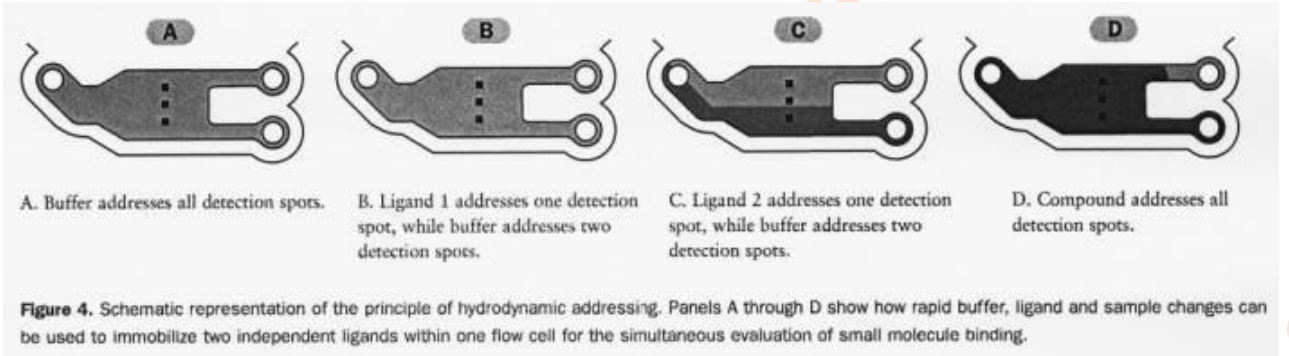


Figure 4. Schematic representation of the principle of hydrodynamic addressing. Panels A through D show how rapid buffer, ligand and sample changes can be used to immobilize two independent ligands within one flow cell for the simultaneous evaluation of small molecule binding.

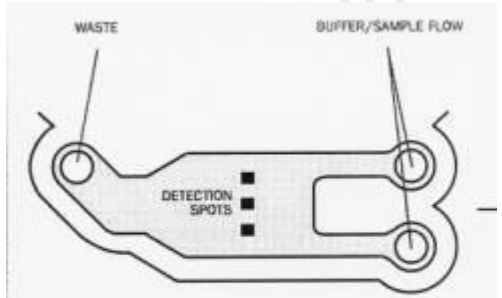


Figure 3. Assay flexibility of Biacore S51 flow cell and hydrodynamic addressing, illustrating three potential assay designs.

Specialized techniques

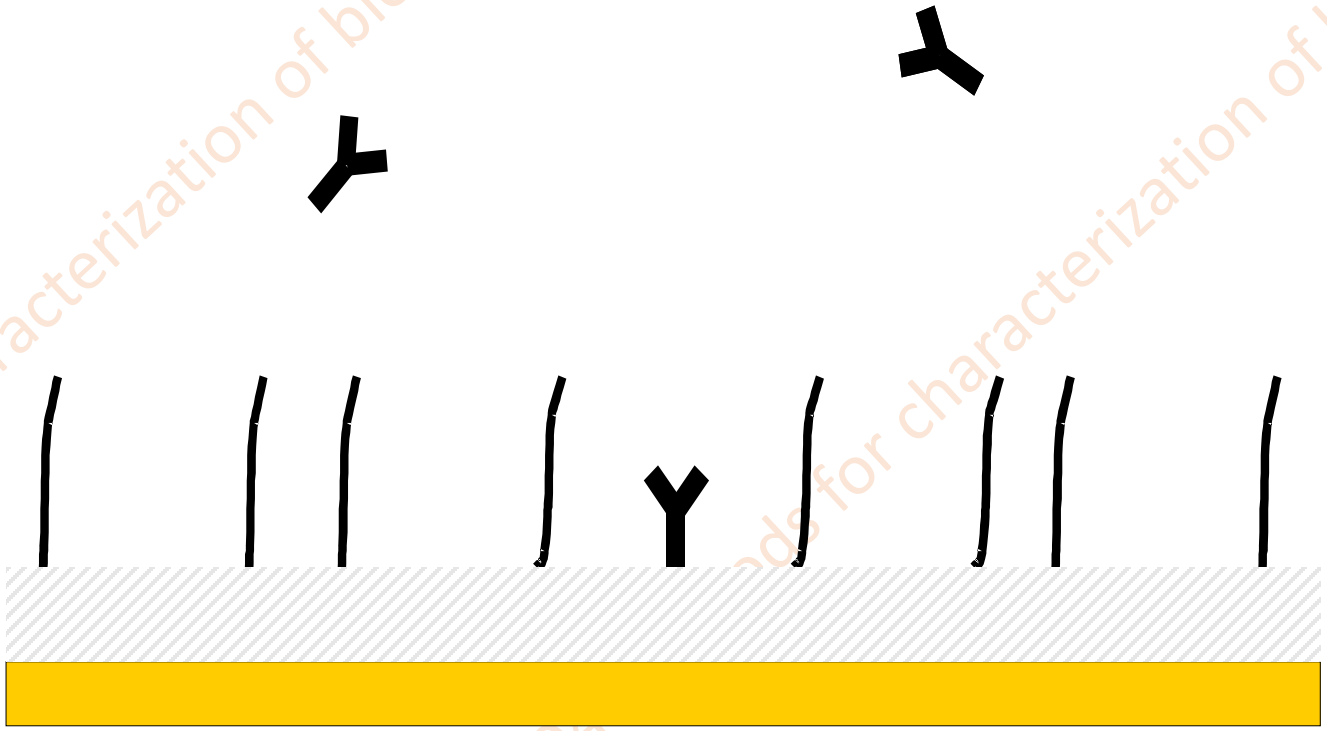
- **Membrane proteins**
- **Multi-layer** approaches – Ab's, protein complexes
- Whole **cell** immobilization
- **Thermodynamics** measured by SPR
- Ligand **recovery**

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).

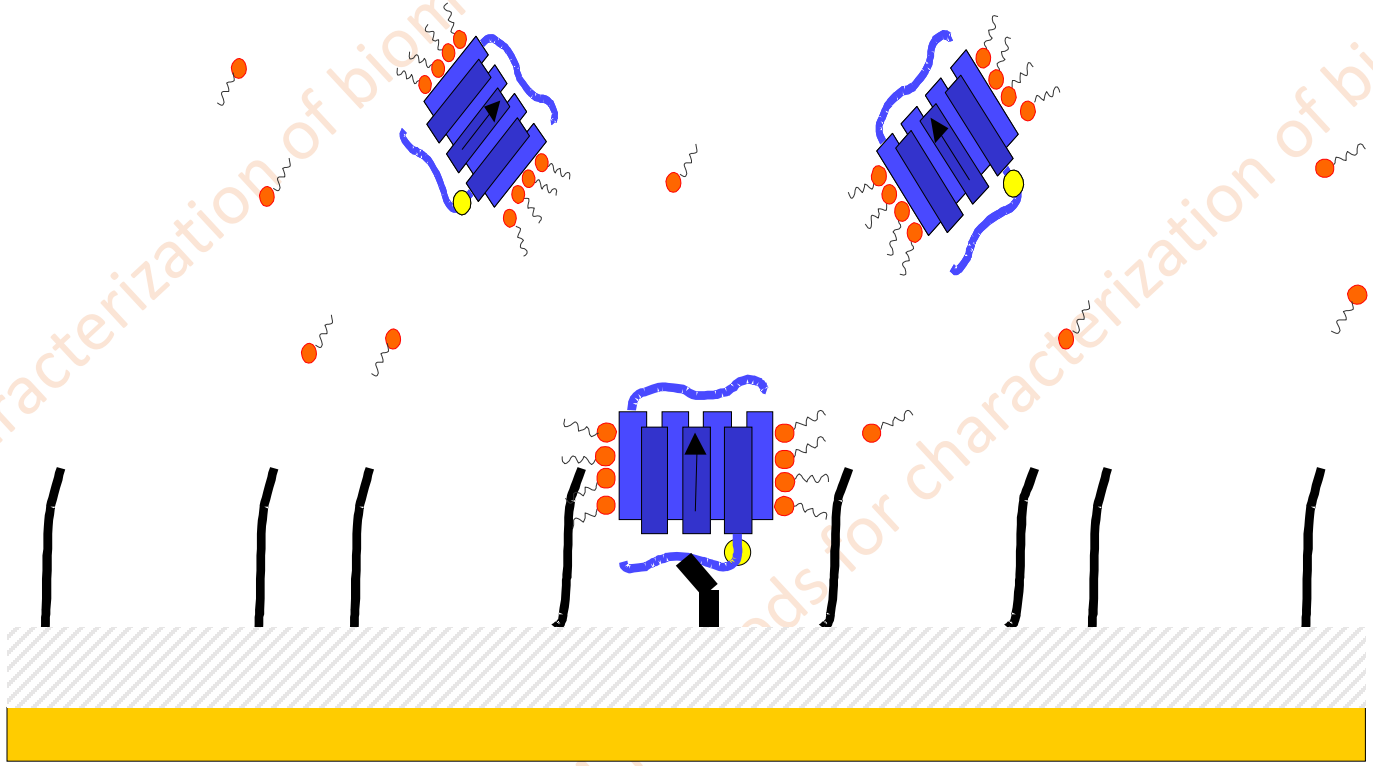
On-surface reconstitution approach

Immobilize a GPCR-specific mAb on a L1 chip.



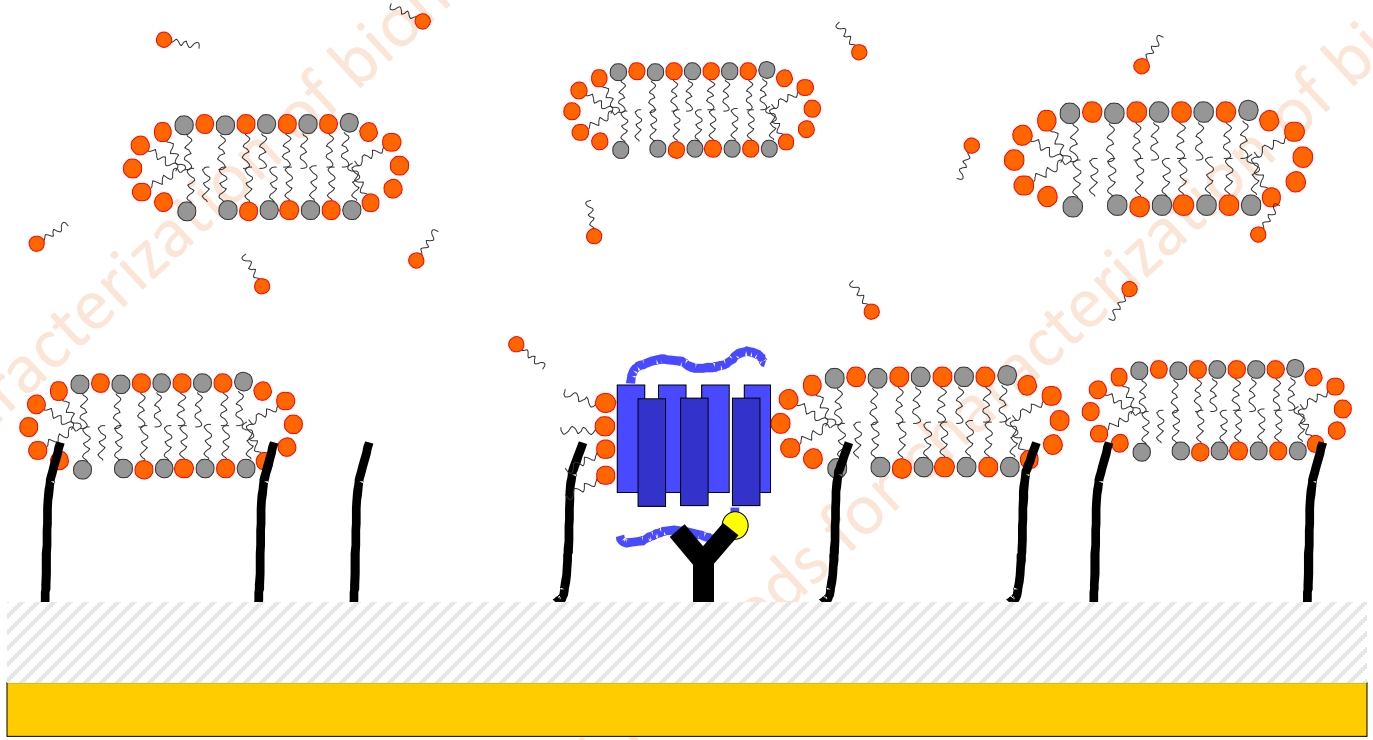
On-surface reconstitution approach

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



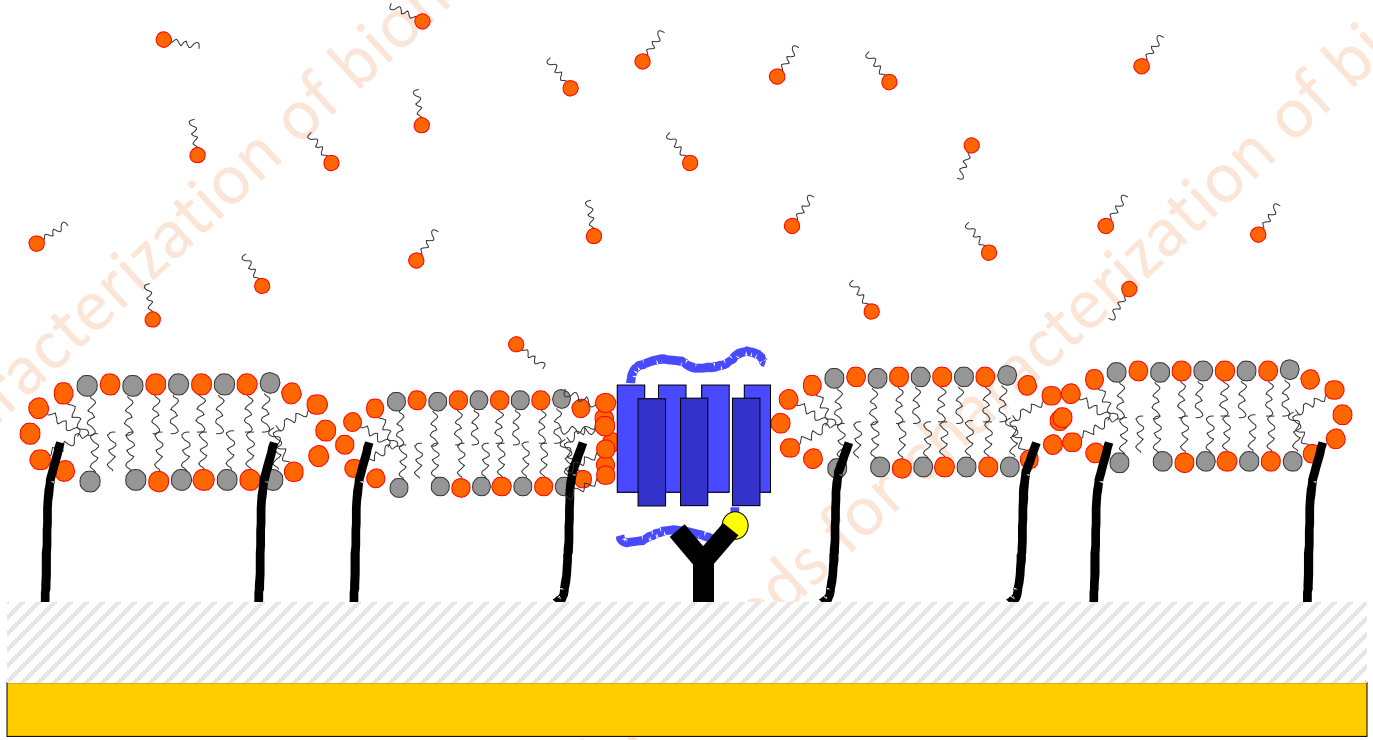
On-surface reconstitution approach

Reconstitute a lipid bilayer around the receptor



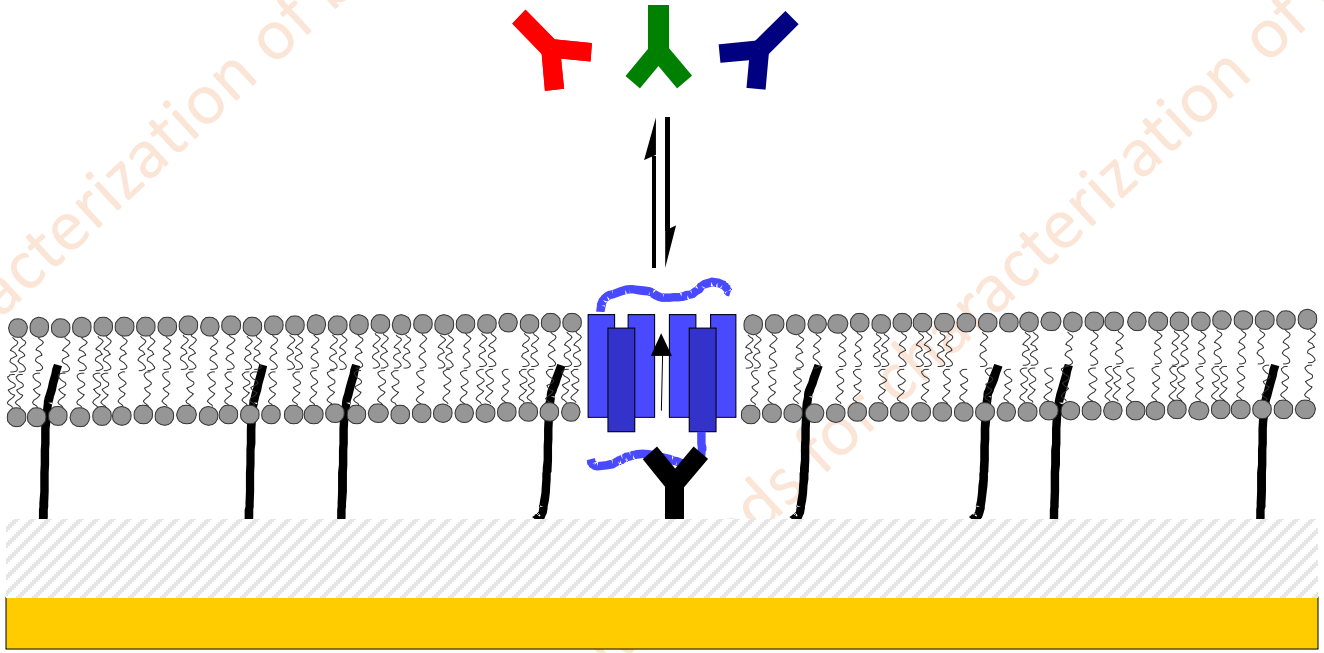
On-surface reconstitution approach

Wash the surface with buffer



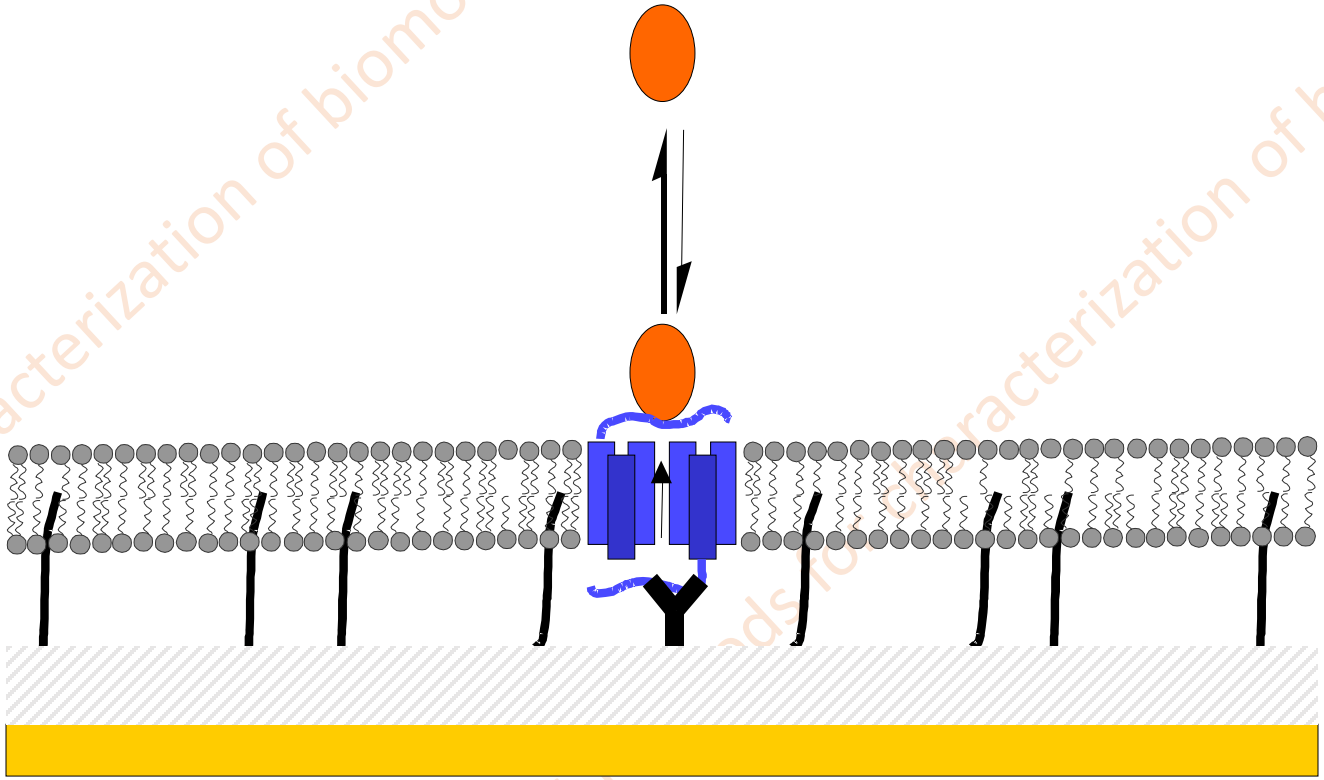
On-surface reconstitution approach

Establish the integrity of the reconstituted GPCR



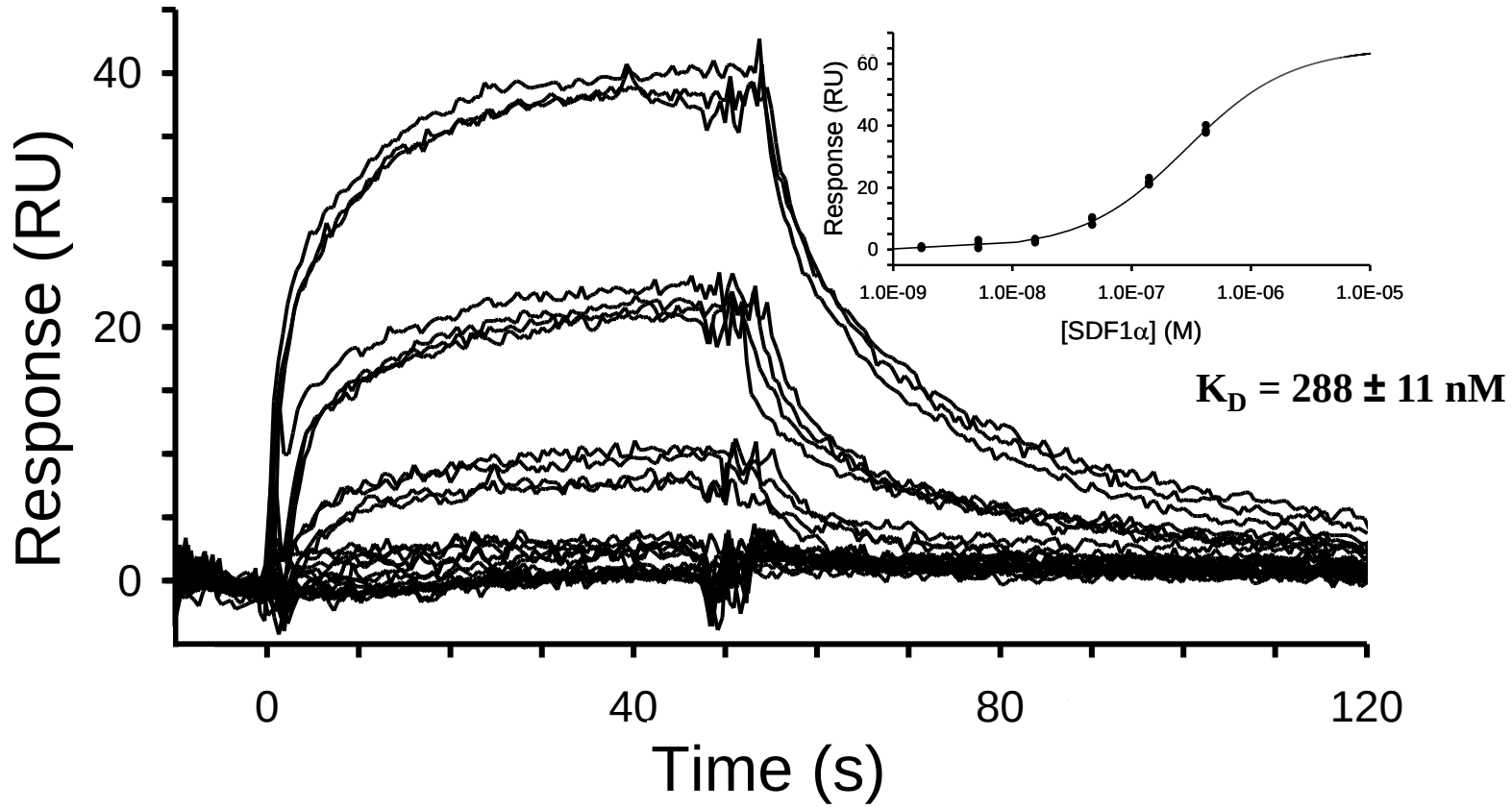
On-surface reconstitution approach

Study the kinetics of ligand/receptor interactions

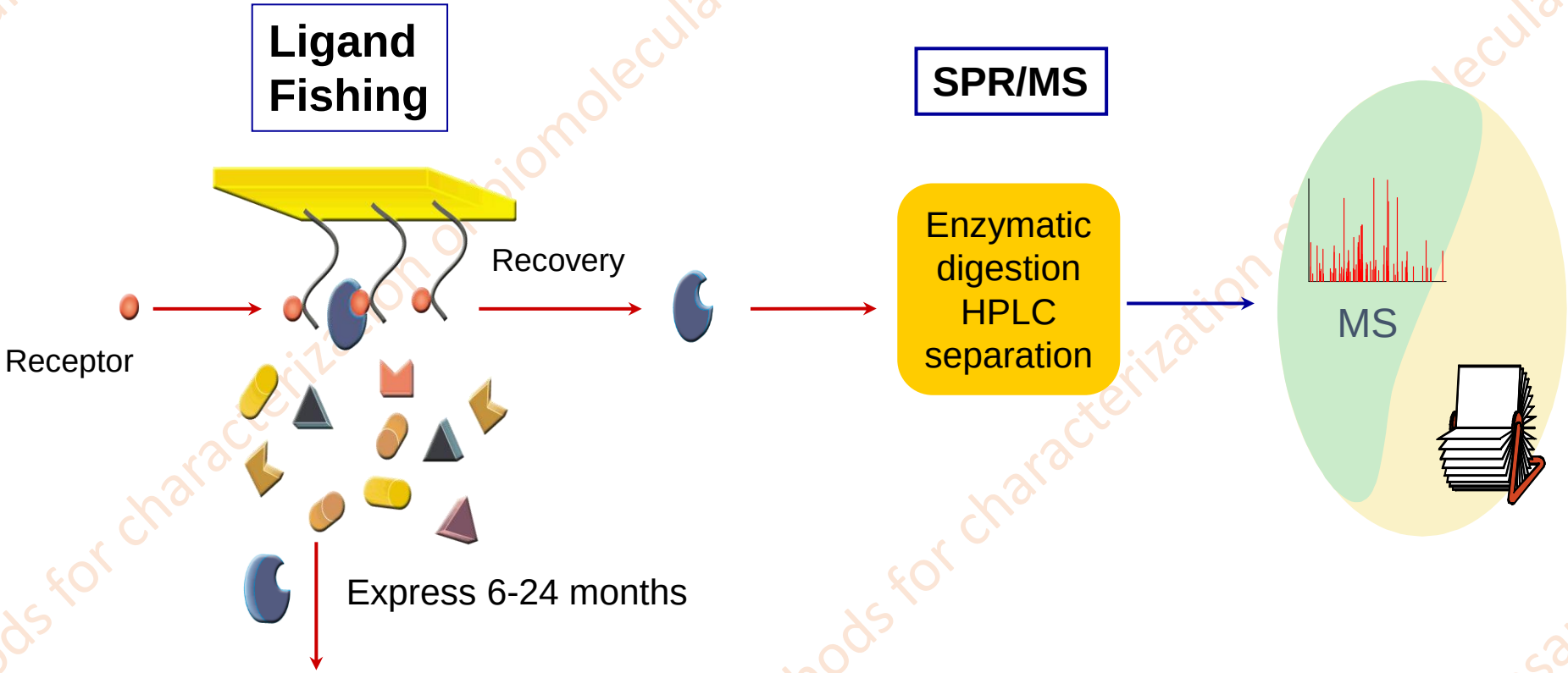


On-surface reconstitution approach

Binding of the chemokine SDF1α
to the reconstituted CXCR4 receptor



Proteomics Study



BIACORE
"Classical Applications"

Main SPR biosensors

- *GE Healtcare* – Biacore S200, Biacore T200, Biacore 4000, Biacore 3000, etc.
- *Reichert* – SR7000DC
- *BioRad* – ProteOn™ XPR36
- *Biosensing Instrument* – Bi4000, Bi3000, etc.
- *Nicoya* – Alto, OpenSPR

Alto

Biacore S200

ProteOn™ XPR36

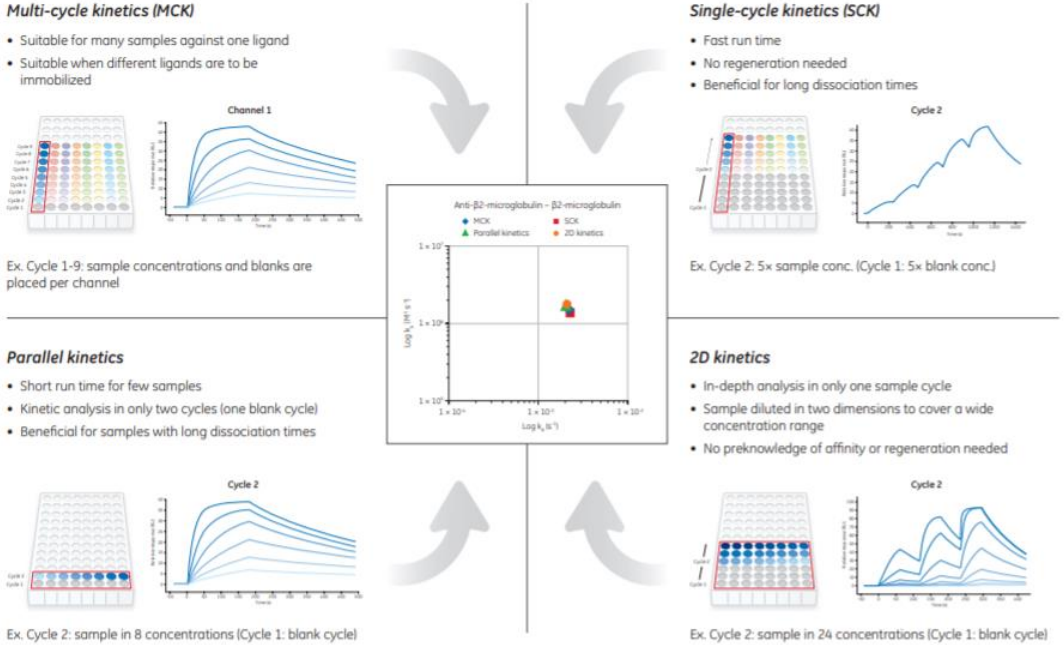
Bi4000

SR7000DC

High-throughput SPR

Biacore 8K (highest model)

- 16 channels
- **Up to 4x384 samples** in a run
- 2300 interacting molecules/day
- 64 kinetic characterizations/4 hrs



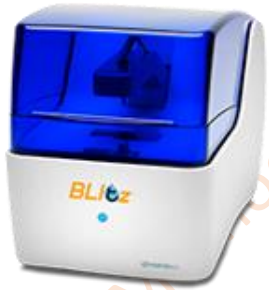
www.cytivalifesciences.com

Main BLI biosensors

- *Fortebio* – BLItz, Octeet R2, Octet R4, Octet RED96e, etc.
- *Gator Bio* – Gator

Octet RED96e

Octet R2



BLItz

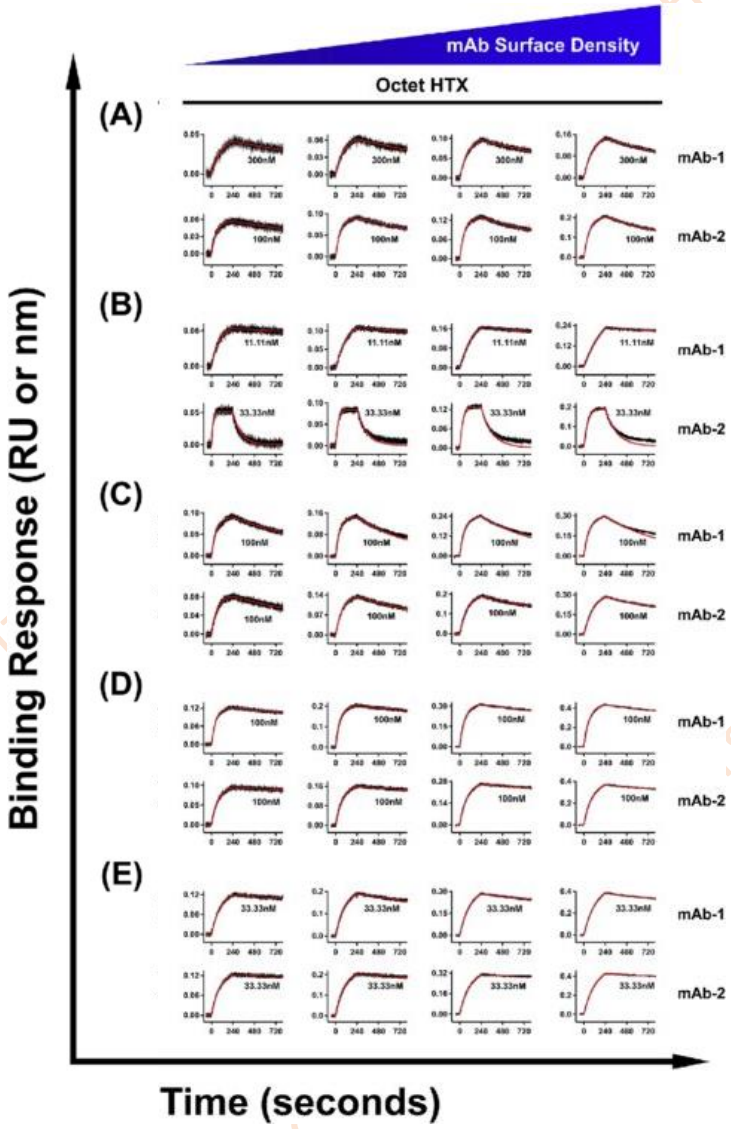
Gator

High-throughput BLI

Octet HTX

- **Up to 96 samples** simultaneously
- 96 samples quantitation/2 mins
- Up to 32x32 epitope binning/8 hrs

analytica-world.com



Kamat 2017

Objectives of SPR/BLI experiment

- Kinetic Rate Analysis:
  How **FAST**?
  - k_a, k_d
  - $K_D = k_d/k_a$, $K_A = k_a/k_d$

- Affinity Analysis:
  How **STRONG**?
  - K_D, K_A
  - Relative Ranking

- Concentration Analysis:
  How **MUCH**?
  - Active Concentration
  - Solution Equilibrium
  - Inhibition

- **Yes/No** Data
  - Screening
  - Ligand Fishing

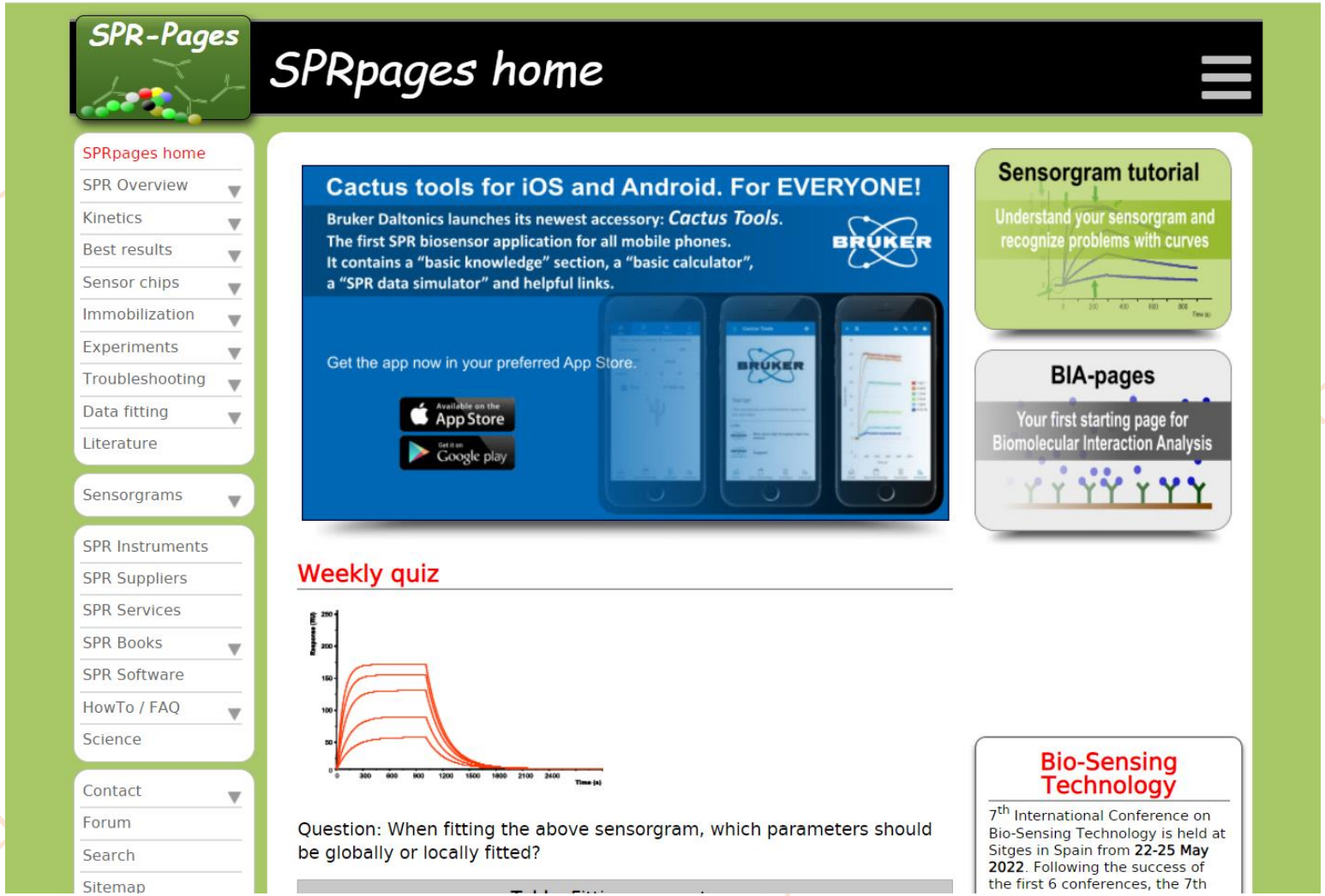
On-surface technology advantages

- No labeling
- Real-time
- Unique, high quality data on molecular interactions
- Simple assay design
- Robust and reproducible
- Walk-away automation
- Small amount of sample required

Method comparison

	SPR	BLI	MST	ITC	AUC
Parameters	K_D/K_A, k_{on}, k_{off}	K_D/K_A, k_{on}, k_{off}	K_D/K_A, N	K_D/K_A, N, ΔG, ΔH, ΔS	K_D/K_A, N
K_D range [M]	$10^{-13} – 10^{-3}$	$10^{-11} – 10^{-3}$	$10^{-11} – 10^{-1}$	$10^{-12} – 10^{-2}$	$10^{-8} – 10^{-4}$
Speed (per K_D)	15 – 120 min	15 – 60 min	15 – 30 min	30 – 120 min	4 – 72 hod
Sample modification	Immobilization	Immobilization	Labeling	None	None
Complex samples	✓	✓	✓	✗	✗
High throughput	✓	✓	✓	✓	✗

Materials for further study



http://www.sprpages.nl/

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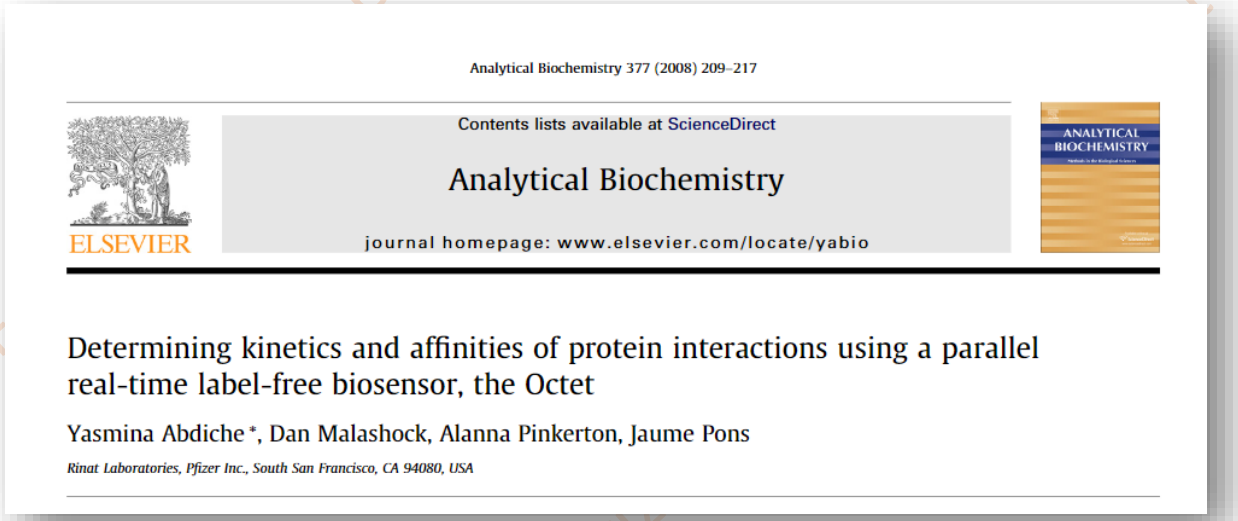
Materials for further study



Analytical Biochemistry 377 (2008) 209–217

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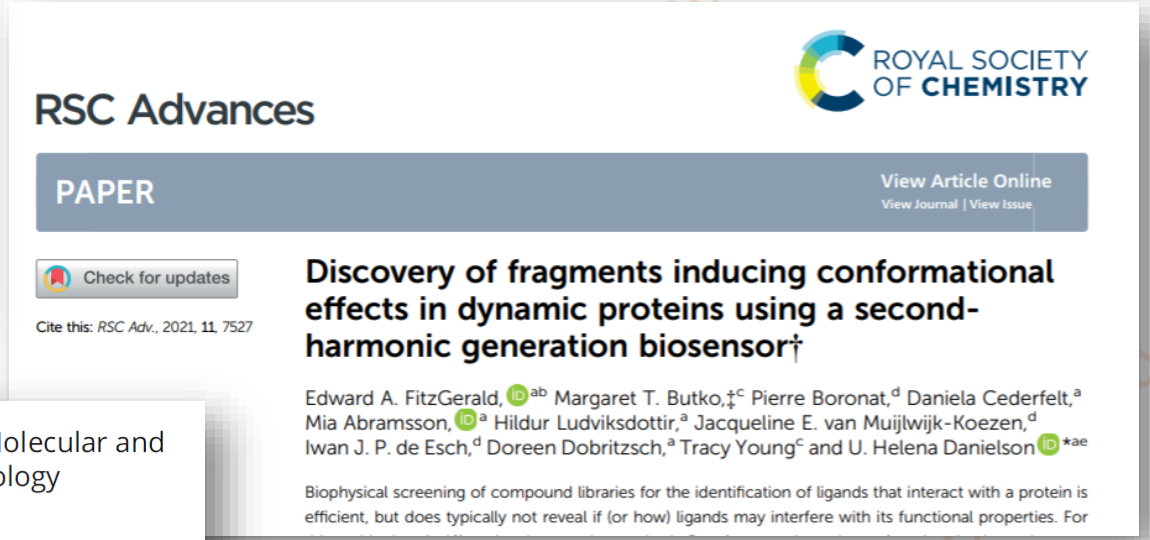
Determining kinetics and affinities of protein interactions using a parallel real-time label-free biosensor, the Octet

Yasmina Abdiche *, Dan Malashock, Alanna Pinkerton, Jaume Pons

Rinat Laboratories, Pfizer Inc., South San Francisco, CA 94080, USA



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Discovery of fragments inducing conformational effects in dynamic proteins using a second-harmonic generation biosensor†

Edward A. FitzGerald,[ab] Margaret T. Butko,‡[c] Pierre Boronat,[d] Daniela Cederfelt,[a] Mia Abramsson,[a] Hildur Ludviksdottir,[a] Jacqueline E. van Muijlwijk-Koezen,[d] Iwan J. P. de Esch,[d] Doreen Dobritzsch,[a] Tracy Young[c] and U. Helena Danielson *[ae]

Biophysical screening of compound libraries for the identification of ligands that interact with a protein is efficient, but does typically not reveal if (or how) ligands may interfere with its functional properties. For

Tiwari *et al. BMC Molecular and Cell Biology* (2021) 22:17
https://doi.org/10.1186/s12860-021-00354-w

BMC Molecular and Cell Biology

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SPRD: a surface plasmon resonance database of common factors for better experimental planning

Purushottam B. Tiwari[1*], Camelia Bencheqroun[2], Mario Lemus[1], Taryn Shaw[1], Marilyn Kouassi-Brou[1,3], Adil Alaoui[2] and Aykut Üren[1*]

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