Binding - SPR or BIA

"The secret of life is molecular recognition"

"Binding is the first step necessary for a biological response"

Biacore's SPR technology: **label-free technology for *monitoring biomolecular interactions as they occur*.**

The detection principle relies on surface plasmon resonance (SPR), an electron charge density wave phenomenon that arises at the surface of a metallic film when light is reflected at the film under specific conditions.

The resonance is a result of energy and momentum being *transformed* from incident photons into surface plasmons, and is sensitive to the refractive index of the medium on the opposite side of the film from the reflected light.

Hackert – CH370

Note: Many of these figures/notes were taken from on-line resources from Biacore

BIACORE® 3000 & 2000 Series

- **Flexible research system**
  - Low sample consumption
  - Early screening – cell lines
  - Assay development
  - Screen validation

Objectives of the Biacore Experiment

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

Concentration Analysis:
How MUCH?
Active Concentration

Solution Equilibrium
Inhibition

Affinity Analysis:
HOW STRONG?

$K_{D,}$ K_A
Relative Ranking

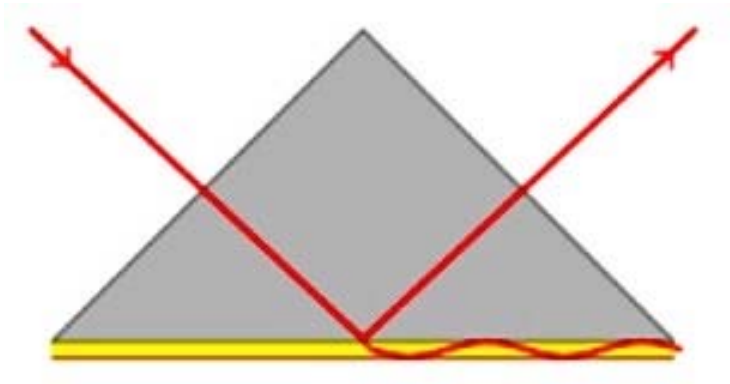
- **Kinetic Rate Analysis:**
- **How FAST?**
  - k_a, k_d
  - $K_D = k_d/k_a$, $K_A = k_a/k_d$

Biacore's proprietary SPR technology

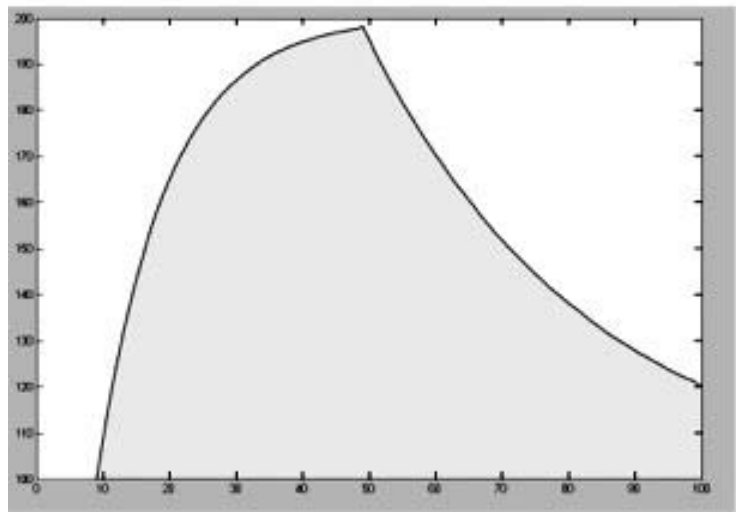
- Non-label
- **Real-time**
- **Unique, high quality data** on molecular interactions
- **Simple assay** design
- Robust and reproducible
- Walk-away automation
- **Small amount of sample** required

Binding constant determination

When the affinity of two molecules (ligand and receptor) has to be determined, the bonding constant can be found using the dynamical SPR parameters.



For this, a so-called **bait ligand** is coated to the gold surface of the SPR crystal. Through a microfludics system, a solution with the **prey ligand can flow over the bait layer and bind**. Binding will make the SPR signal change to a new equilibrium. After some time, a solution without the prey is applied, and a new equilibrium will be reached. From these association ('on rate', v_{on})and dissociation speeds ('off rate', v_{off}), the binding constant can be calculated.



Plasmons & SPR "angle"

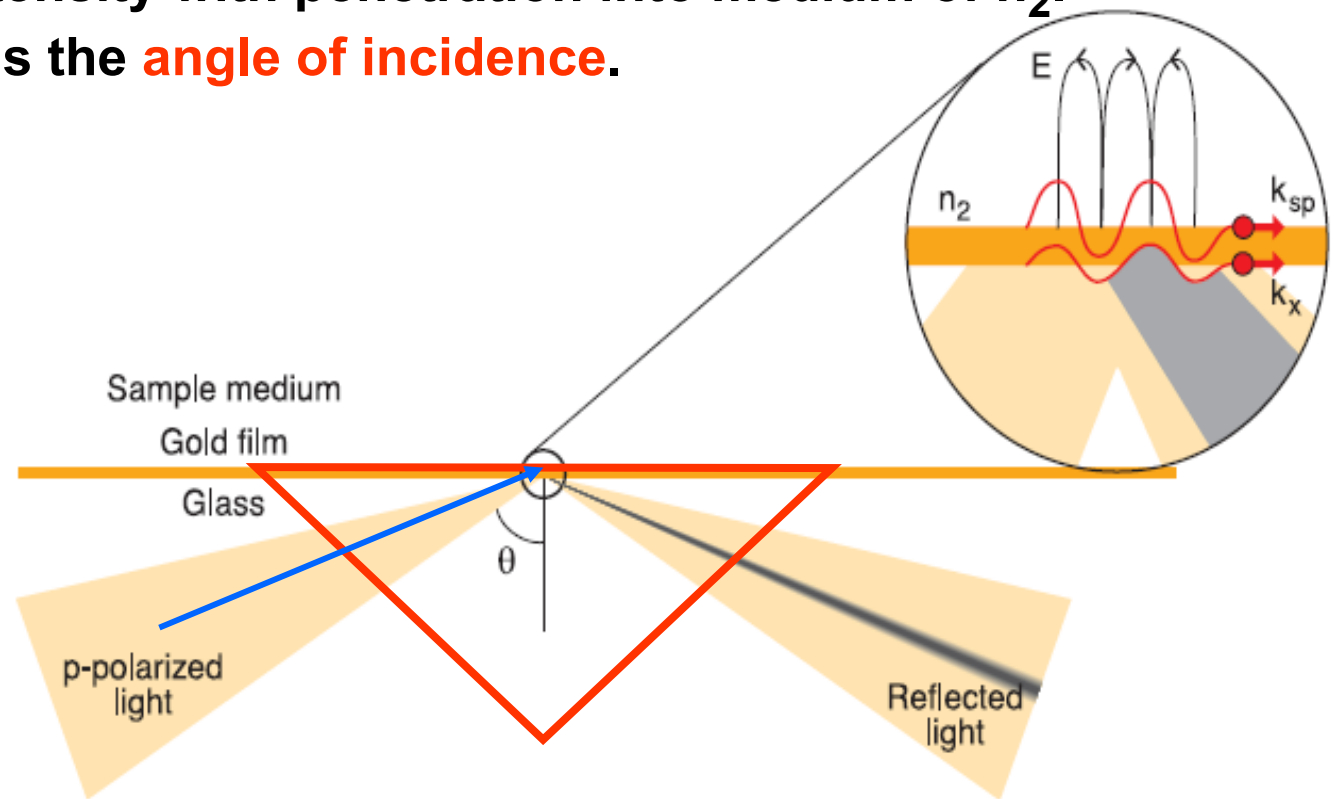
Plasmon – A plasmon is basically just an oscillation of the conduction electrons in a metal. The plasmon is a quasiparticle resulting from the quantization of plasma oscillations. A plasmon can be regarded as a hybrid of the conducting electrons and the photon – collective oscillation of the free electron gas at optical frequencies.

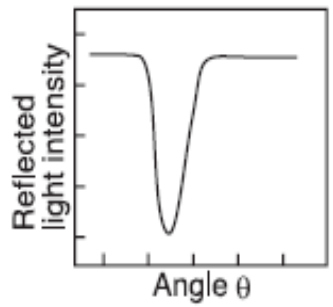
"Magic Angle" - Total Internal Reflection

Total Internal Reflection (TIR) for a non-absorbing media

Light propagating in a medium of refractive index n_1 undergoing total internal reflection at the interface with the medium of a lower refractive index n_2. The evanescent field, E, is a non-transverse wave having components in all spatial orientations, decreasing in field intensity with penetration into medium of n_2.

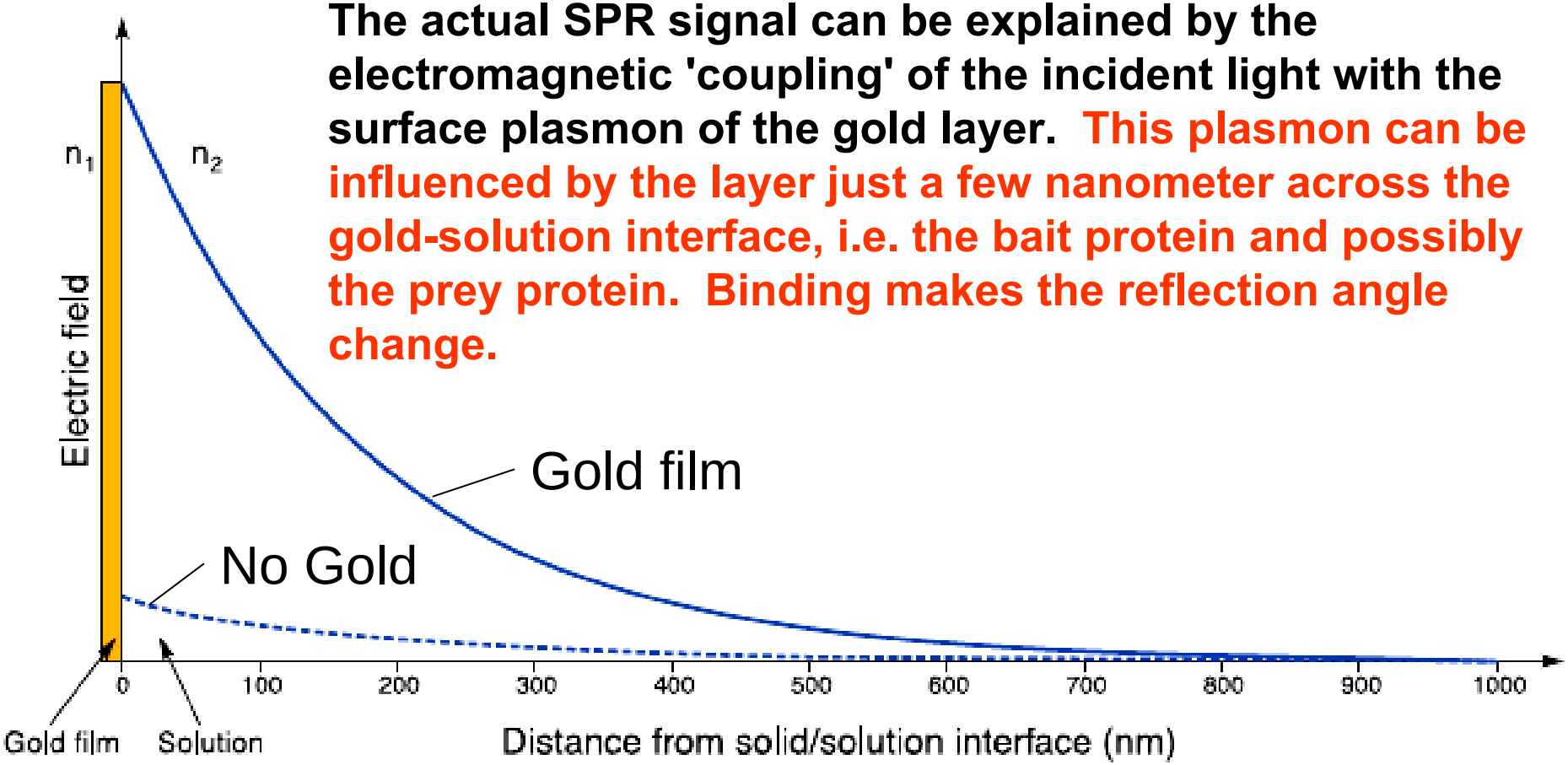
θ is the angle of incidence.



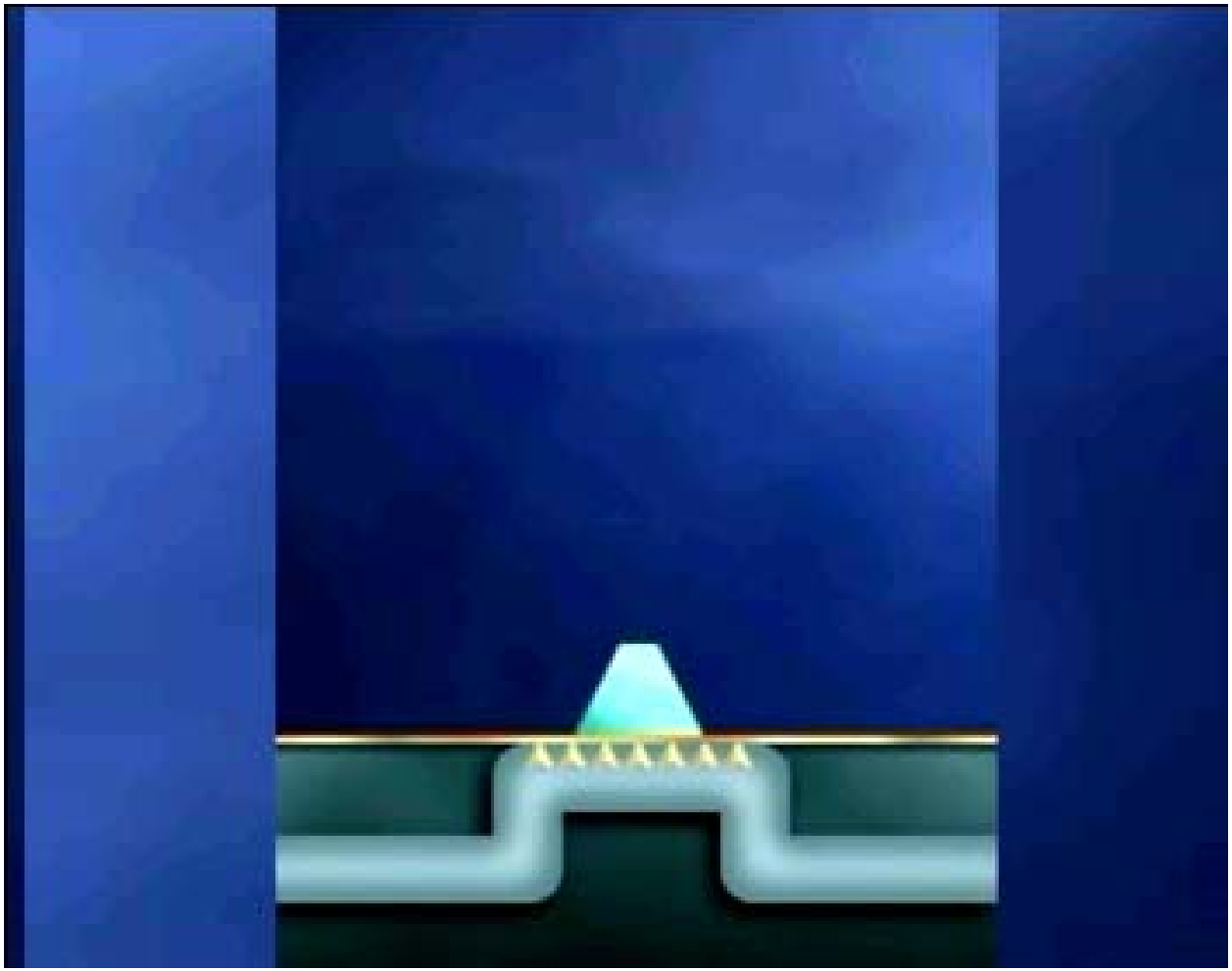


SPR - The need for Gold

The actual SPR signal can be explained by the electromagnetic 'coupling' of the incident light with the surface plasmon of the gold layer. **This plasmon can be influenced by the layer just a few nanometer across the gold-solution interface, i.e. the bait protein and possibly the prey protein. Binding makes the reflection angle change.**

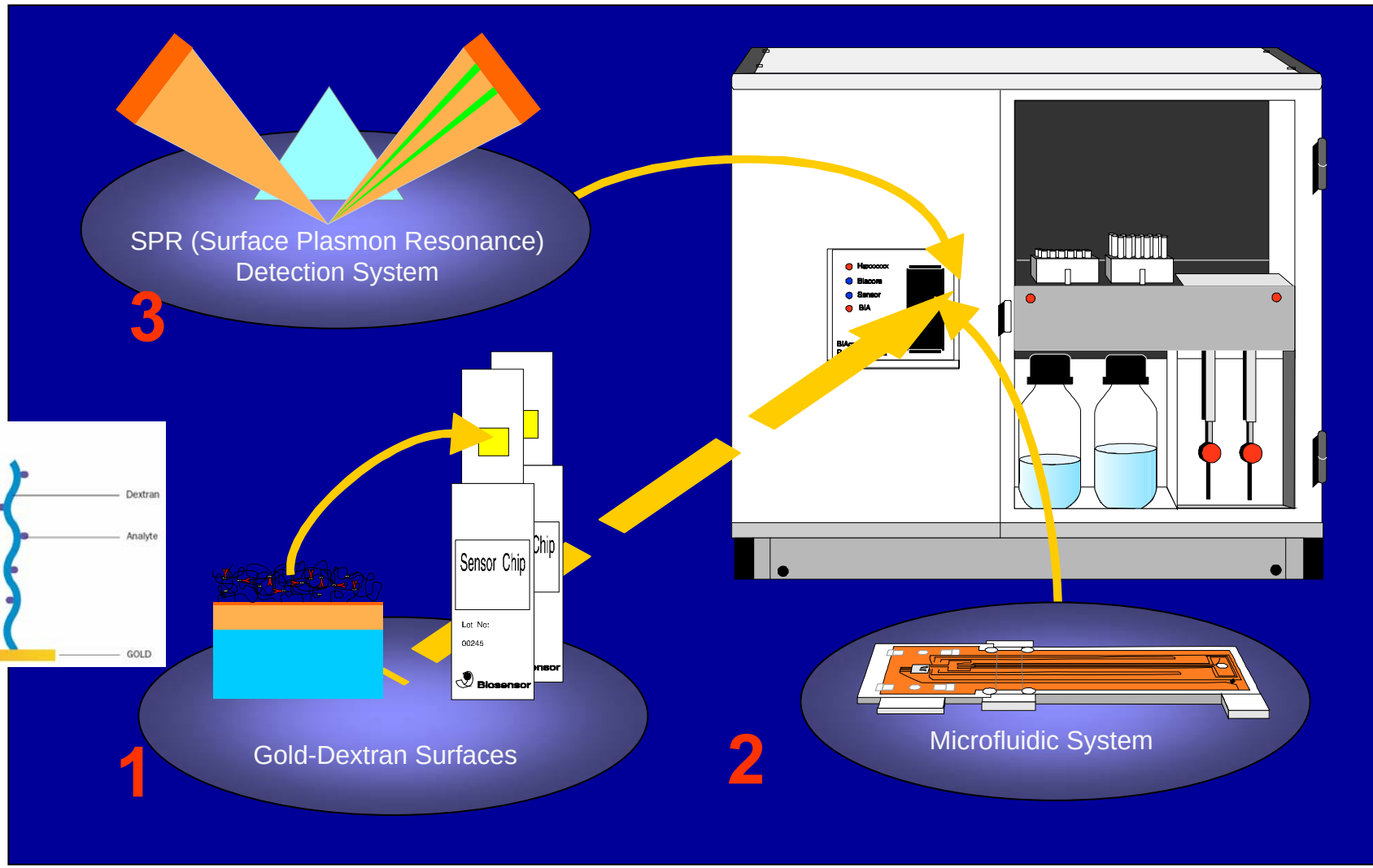


Biacore SPR:
binding event ==> real time sensorgram

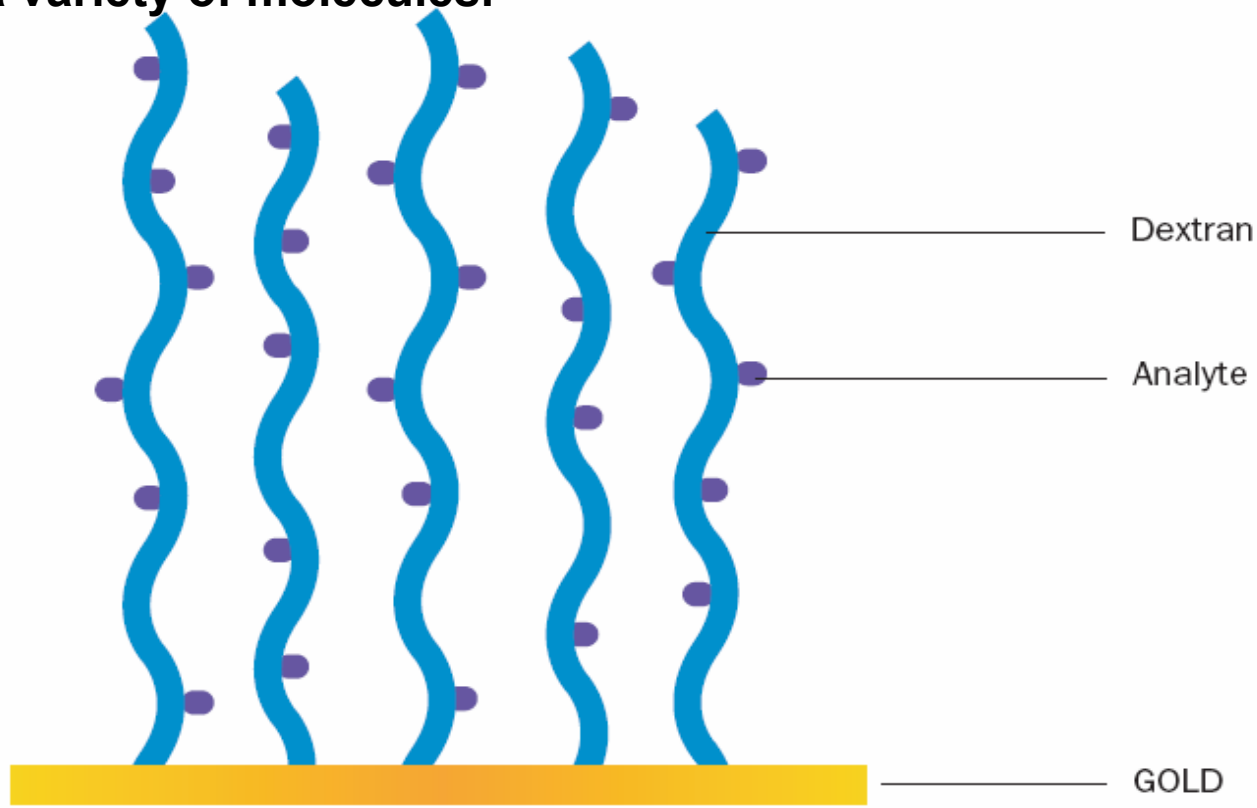


- SPR monitors Binding
- Response Proportional to Mass Bound
- Real Time

Three Corner Stones of Biacore Technology

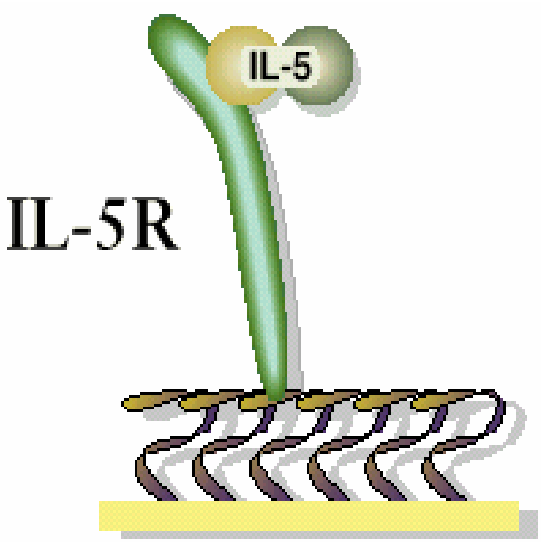


1. The Biacore sensor chip is at the heart of the technology. Quantitative measurements of the binding interaction between one or more molecules are dependent on the immobilization of a target molecule to the sensor chip surface. Binding partners to the target can be captured from a complex mixture, in most cases, without prior purification (for example, clinical material, cell culture media) as they pass over the chip. Interactions between proteins, nucleic acids, lipids, carbohydrates and even whole cells can be studied. The sensor chip consists of a glass surface, coated with a thin layer of gold. This forms the basis for a range of specialized surfaces designed to optimize the binding of a variety of molecules.

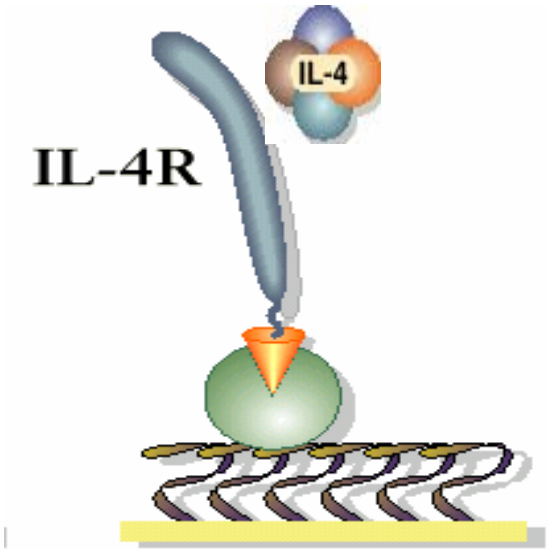


Flexibility to Create Your Biospecific Surfaces

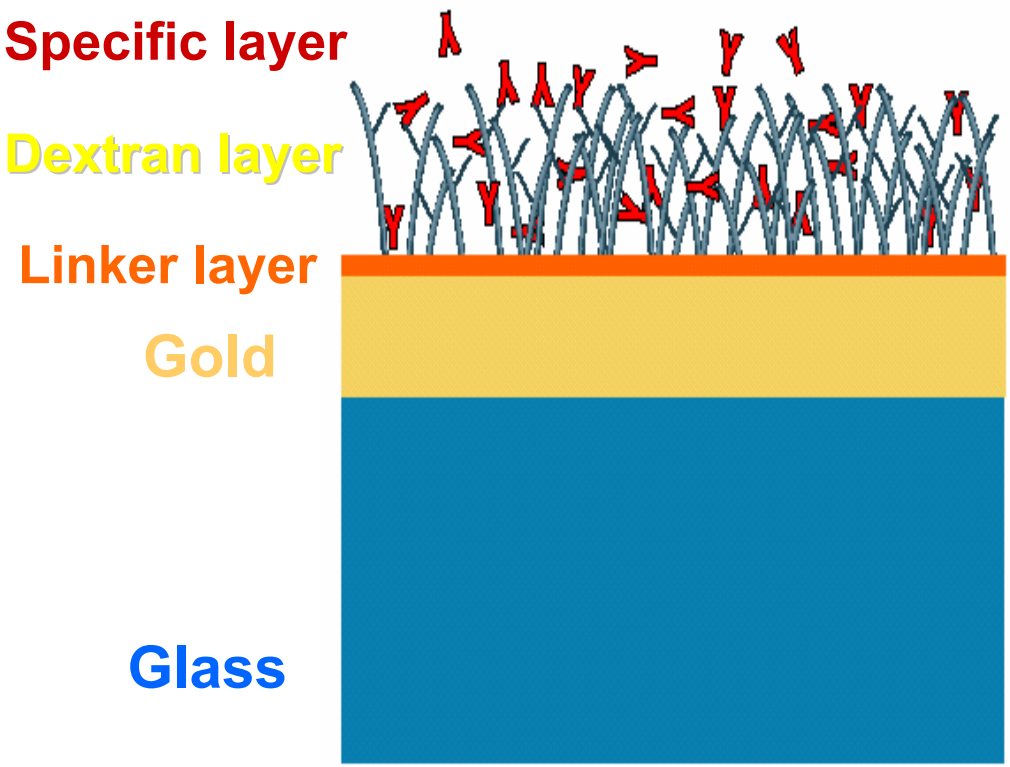
- **Direct: covalent coupling**
  - Amine
  - Thiol
  - Aldehyde
  - Carboxyl



- **Capture**
  - Streptavidin - Biotin
  - NTA- Ni2+-His
  - Anti- His-His
  - Anti-GST- GST



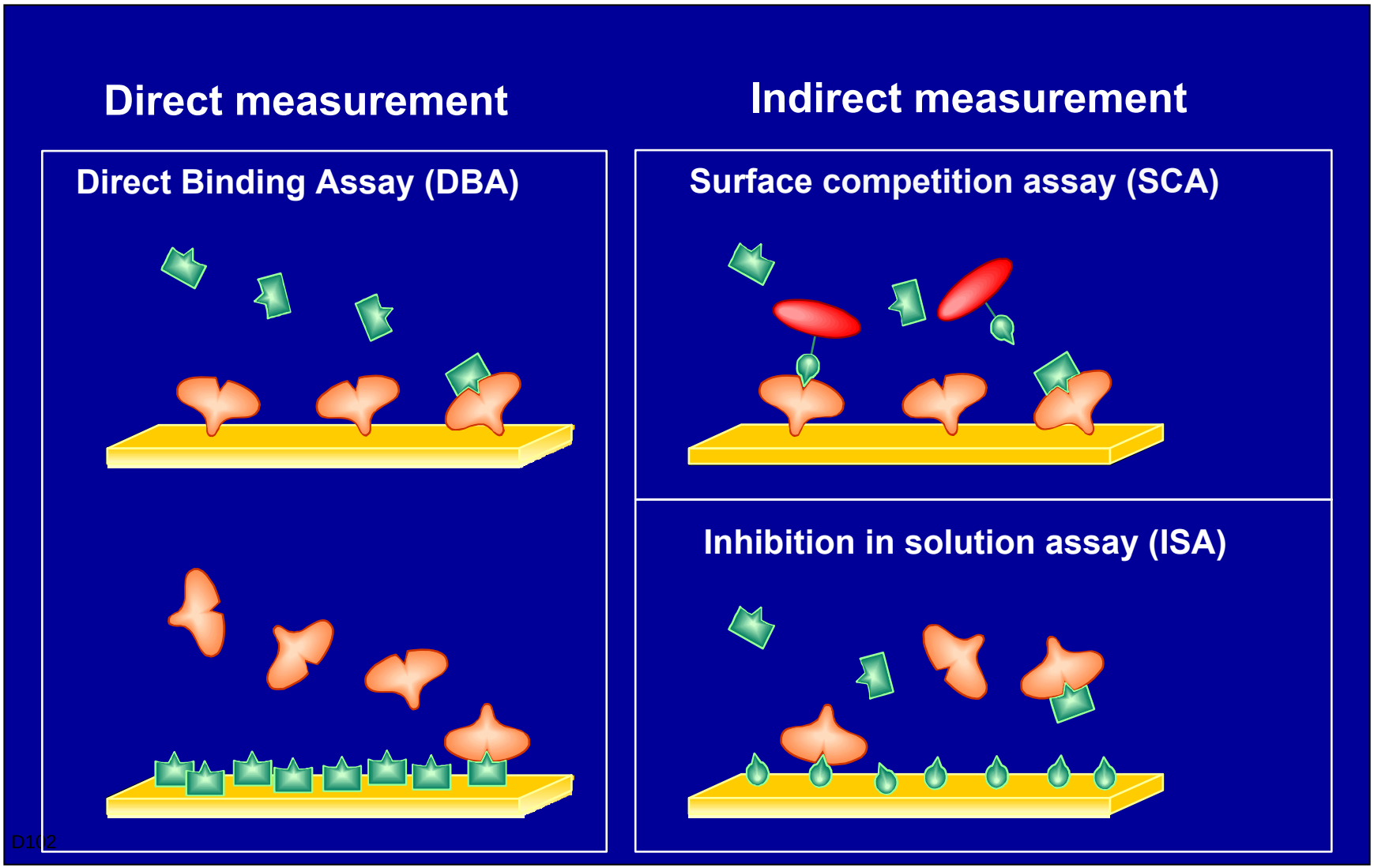
User-defined Biospecific Surface



- Biocompatible
- Low non-specific binding
- Robust
- 100 to 400 runs on the same surface

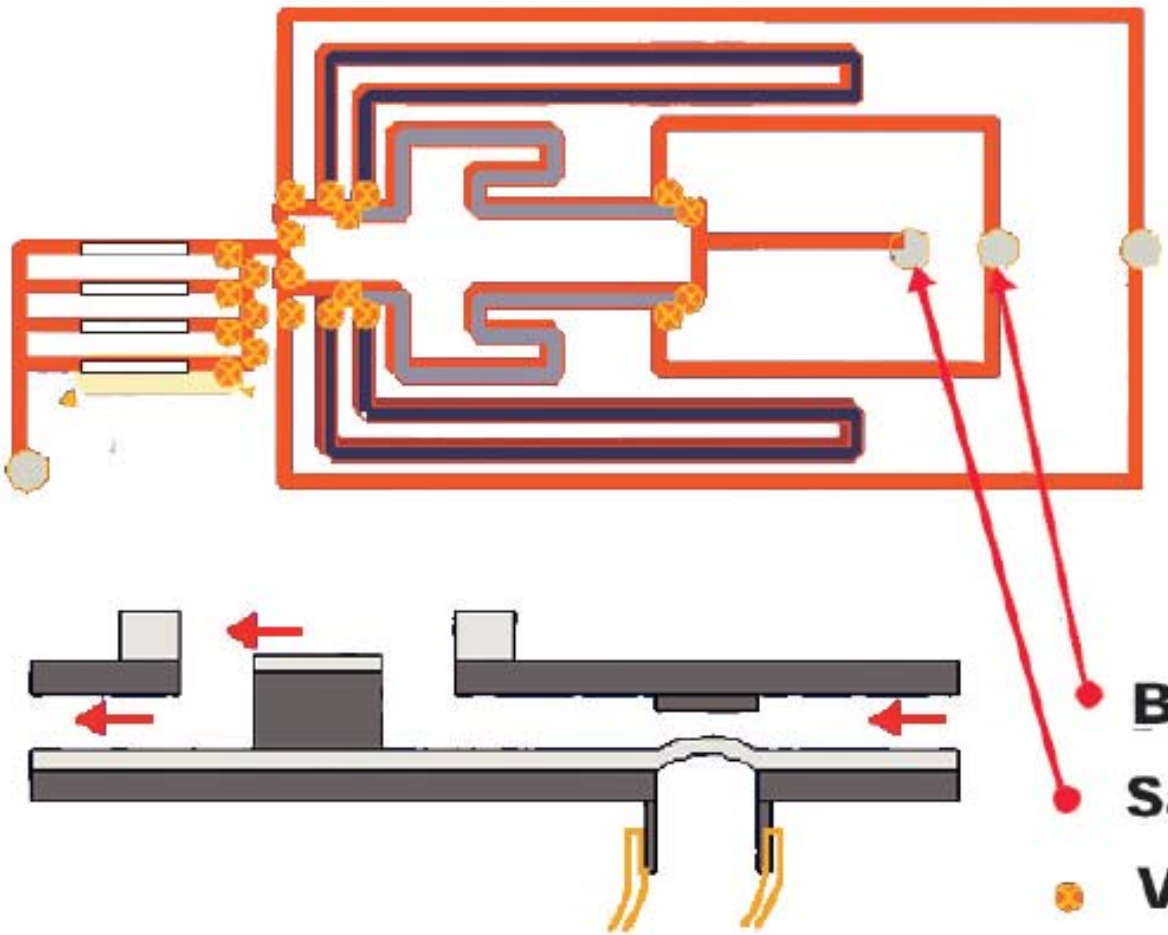
Flexibility in Assay Design

- **Multiple assay formats providing complementary data**



2. Integrated micro Fluidics Cartridges (IFC)

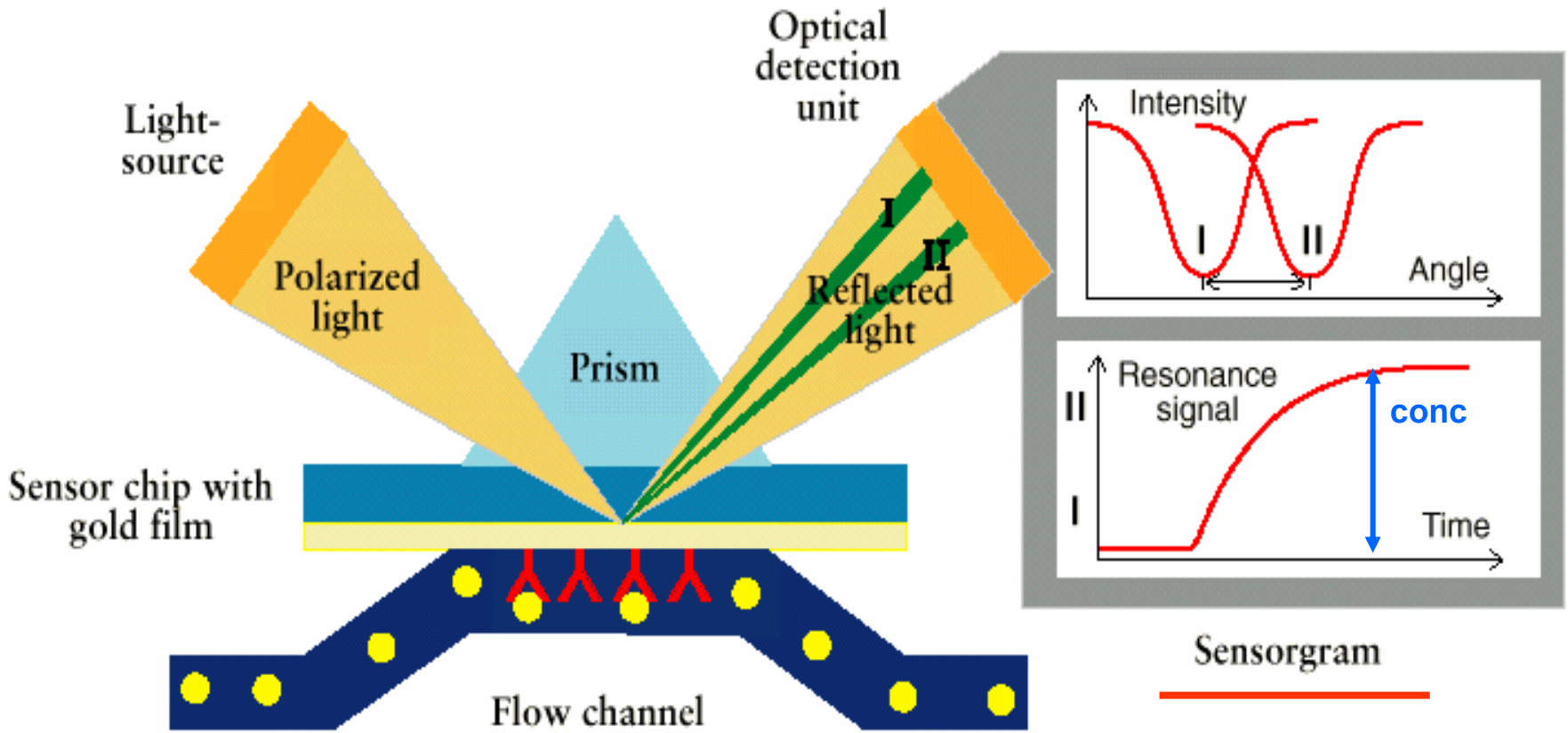
Liquid Handling



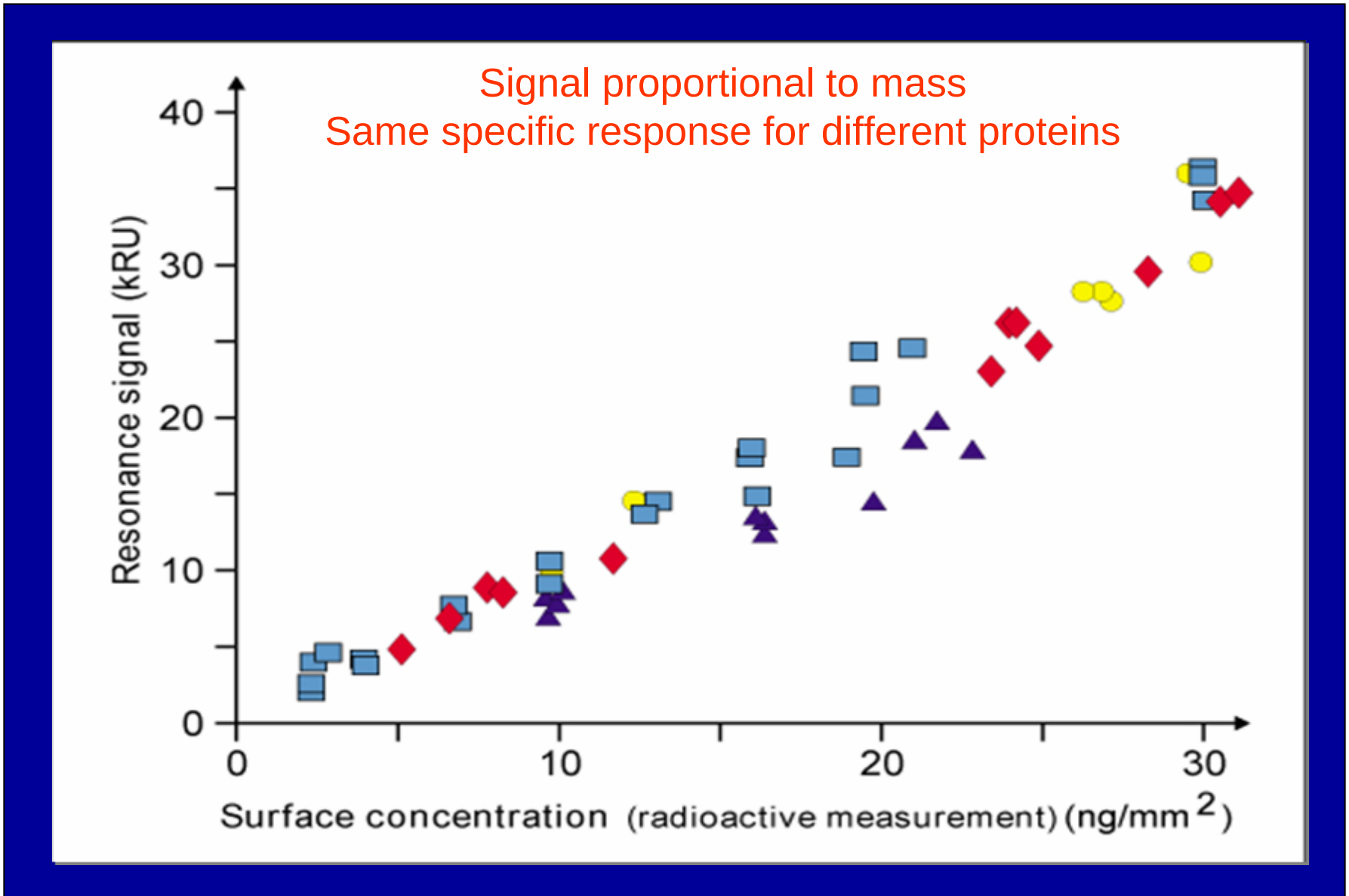
- Minaturized system
- Low volume of reagents
- Intergrated and automated liquid handling

3. Surface Plasmon Resonance Detection: Biomolecular Binding in Real Time

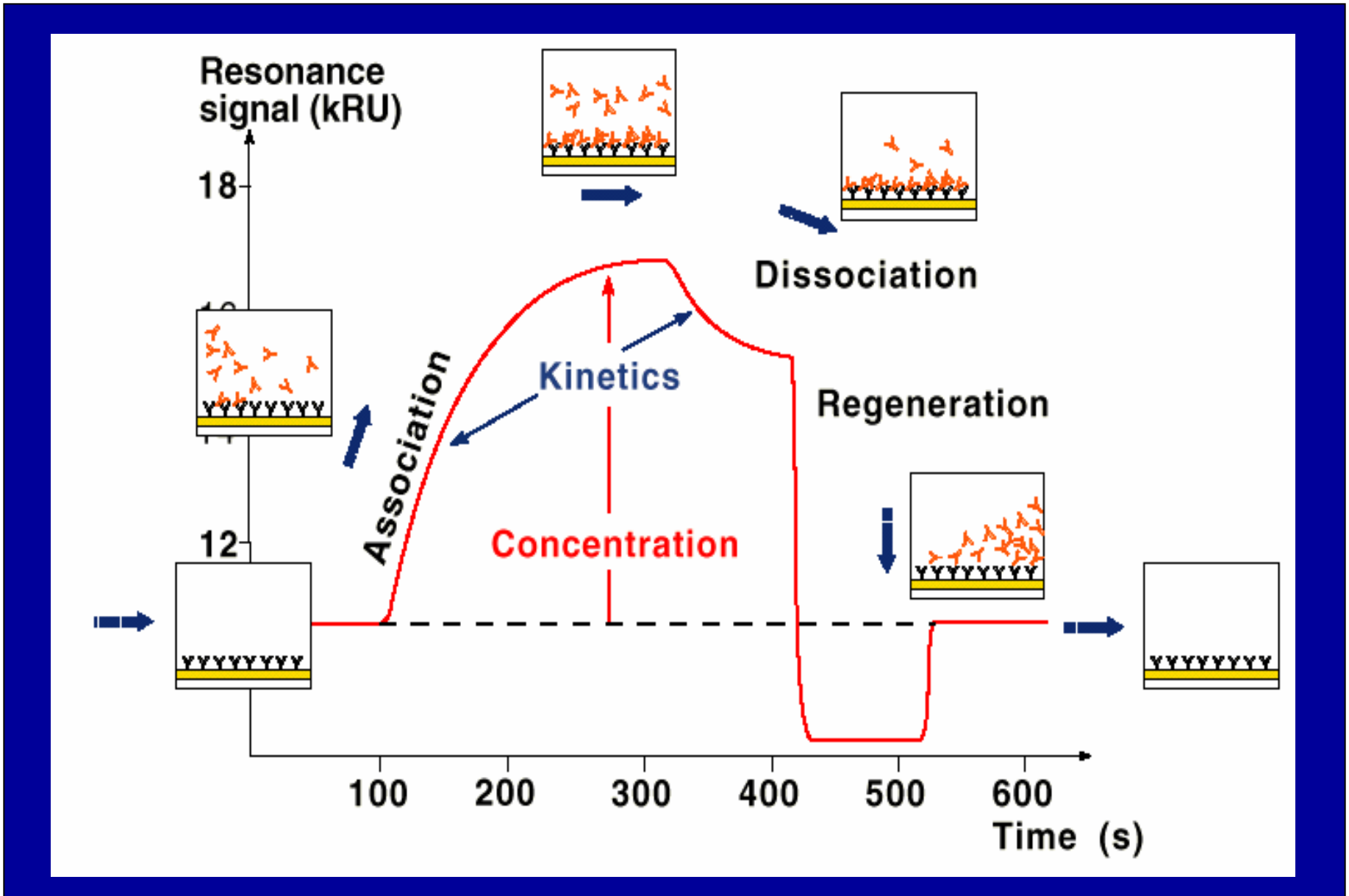
Principle of Detection



Correlation between SPR Response and Surface Concentration



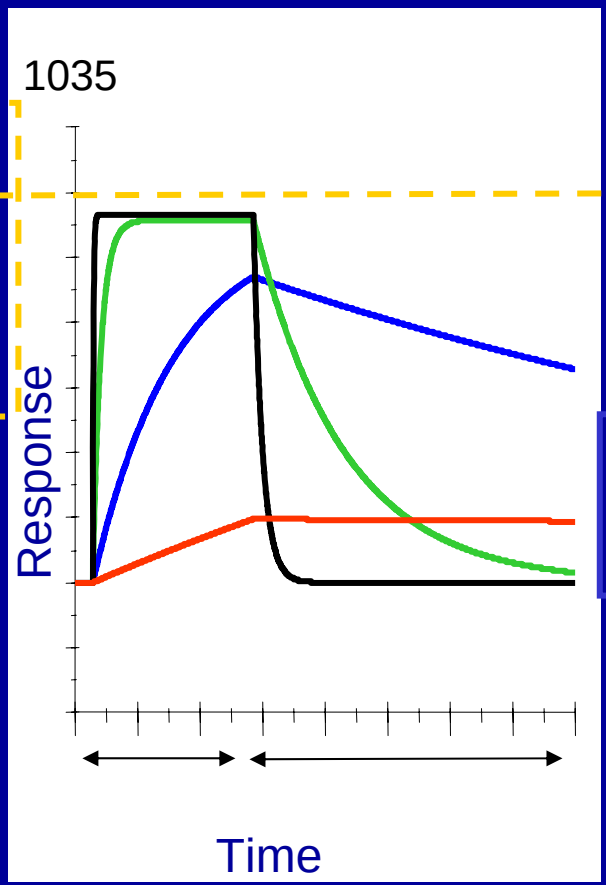
The Sensorgram is Information Rich



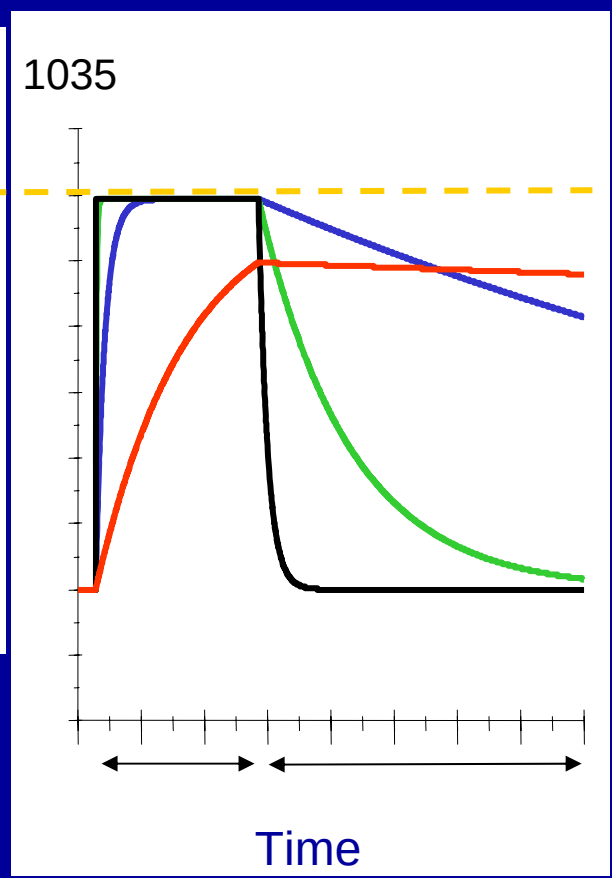
Same affinity but different kinetics

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

Concentration = 100 nM



Concentration = 1000 nM

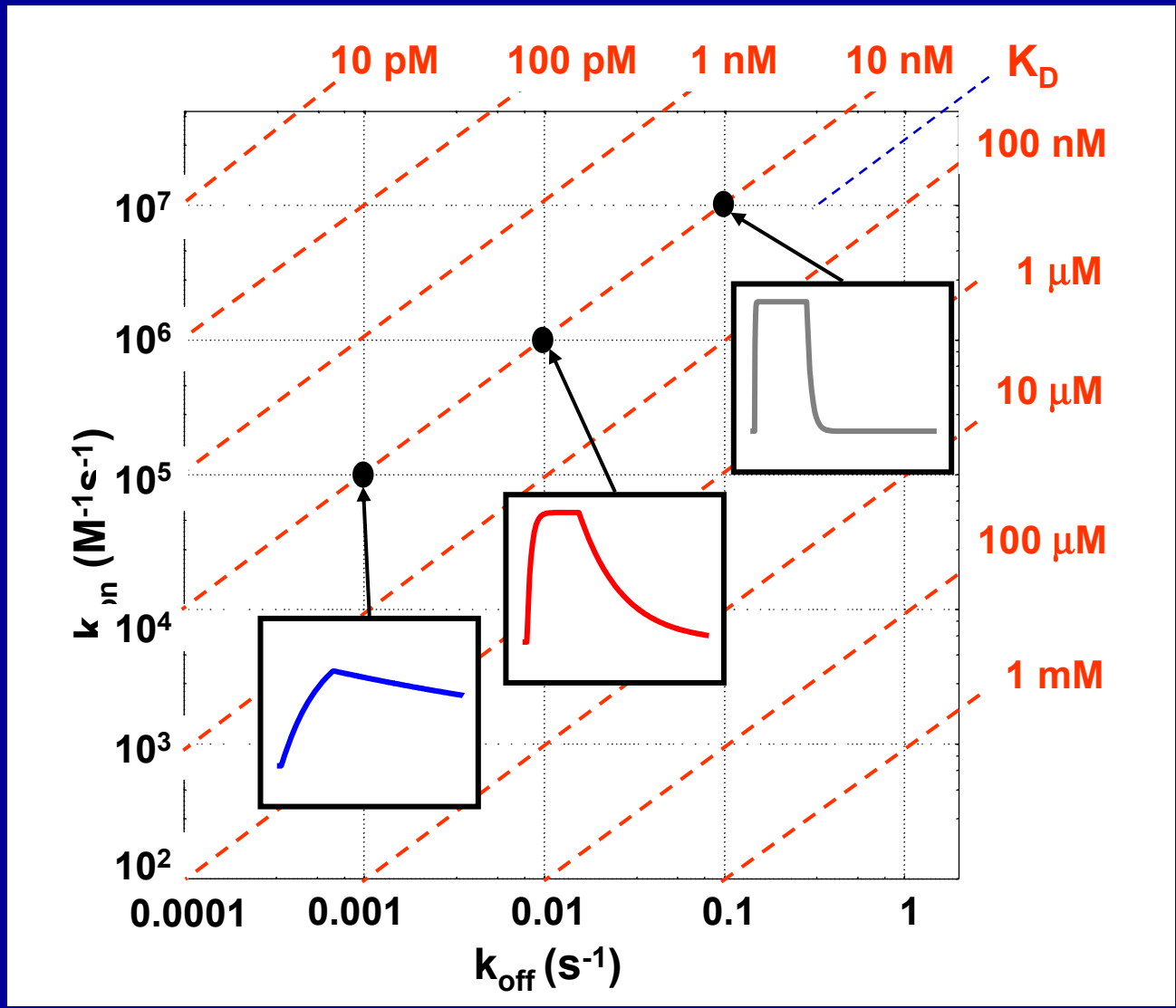


Completely blocked target - all target sites occupied

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

Compounds with slow off-rates occupy the target for a longer time

HIV-p inhibitors: on-off rate map



Areas where Kinetic Information is Needed

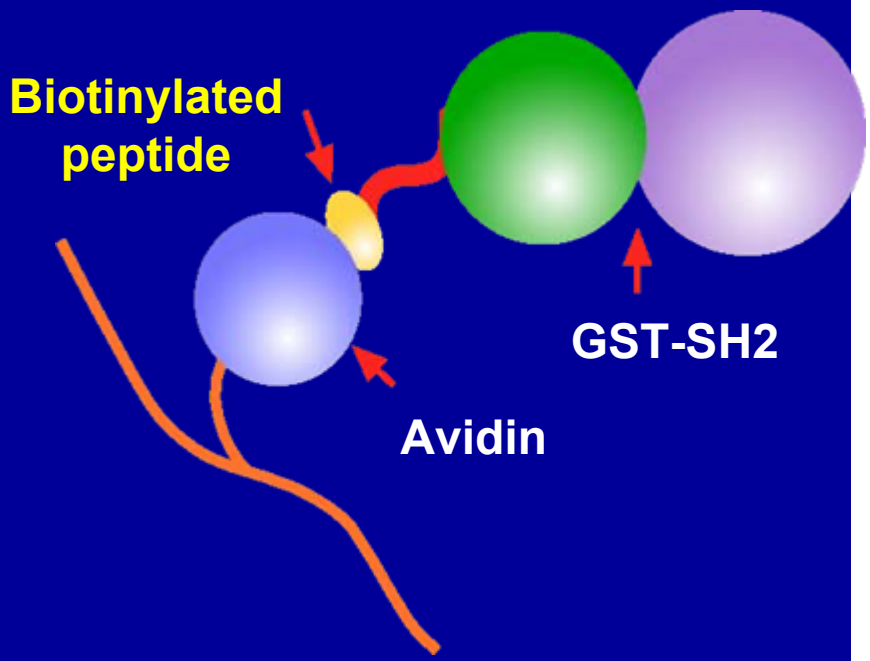
Quantification of effects of structural changes on interactions
- Understanding of structure-function relations
- Design of affinity pairs

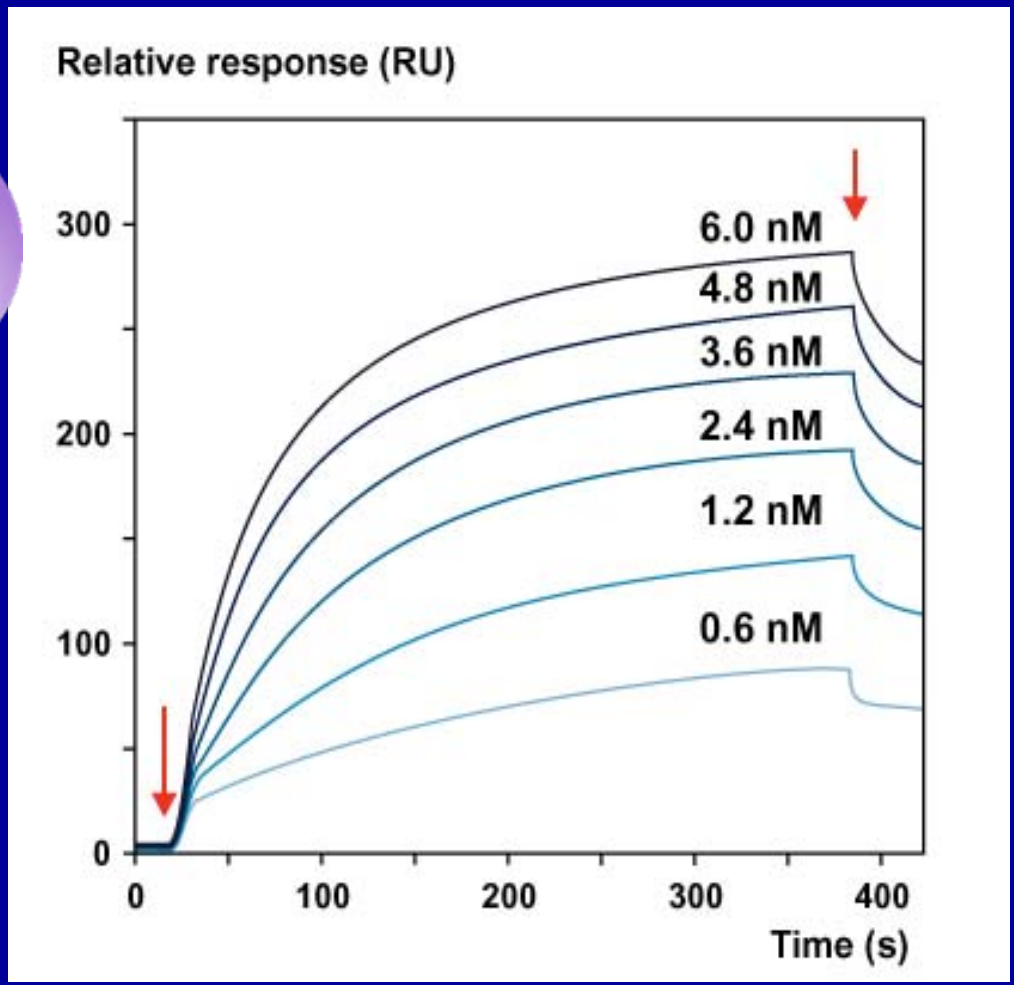
Characterization of biopharmaceutical products
- Recombinant proteins
- Characterization of the immune response in vaccine development

Development of assays based on affinity
- Selection of reagents

Development of purification schemes
- Selection of affinity ligands and conditions for use
- Study the effect on function of conditions used

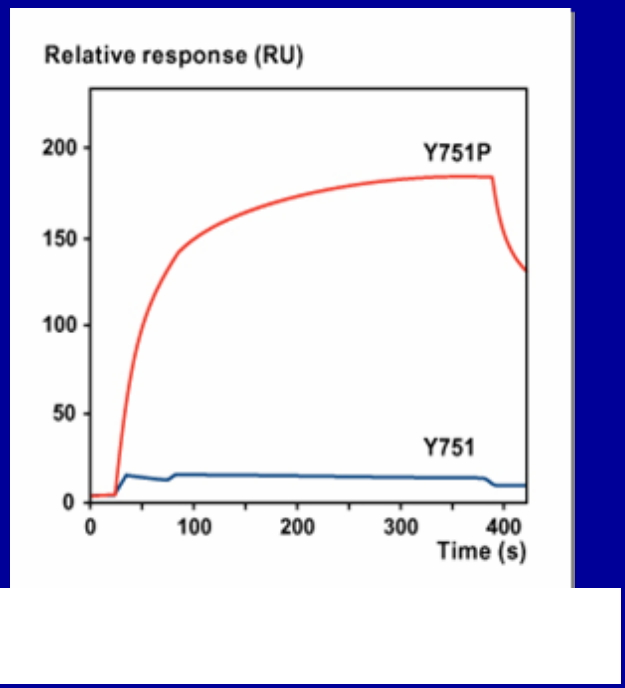
SH2 Domains Binds to Tyrosine Phosphorylated PDGF β-Receptor Sequences



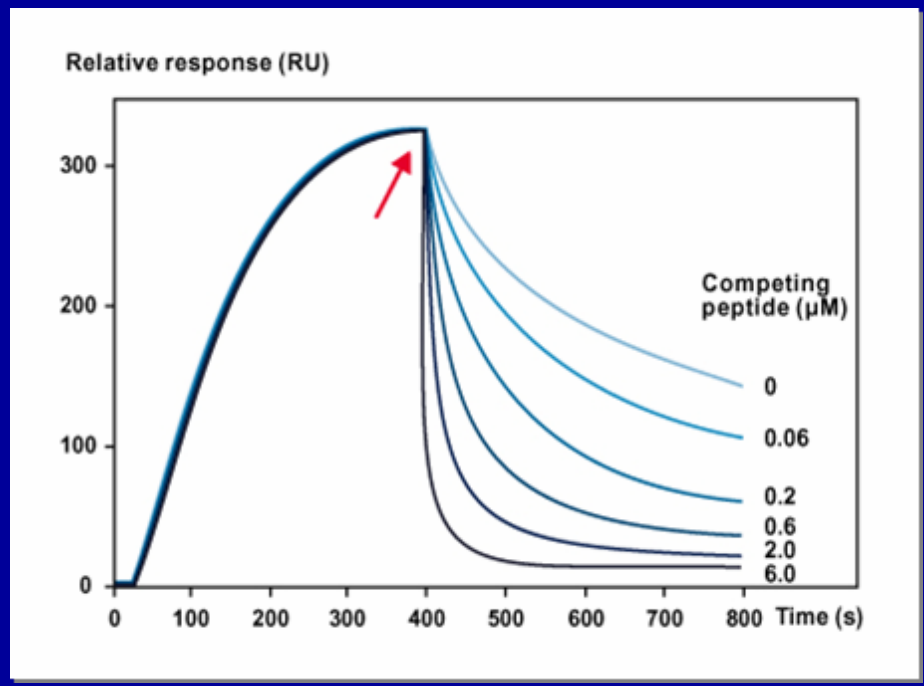


Panayotou, G. et al. (1993) Molecular and Cellular Biology 13:3567-3576.

SH2 Domains Binds to Tyrosine Phosphorylated PDGF β-Receptor Sequences



Specificity of binding to phosphorylated and non-phosphorylated immobilized peptide



Addition of competing peptide

Panayotou, G. et al. (1993) Molecular and Cellular Biology 13:3567-3576.

SH2 Domains Binds to Tyrosine Phosphorylated PDGF β-Receptor Sequences

Kinetic constants of the interactions between SH2-domain containing proteins and phosphopeptides

	Y740P			Y751P		
Peptide	k_{ass} (10^5 M^{-1} s^{-1})	k_{diss} (s^{-1})	K_D (M^{-1})	k_{ass} (10^5 M^{-1} s^{-1})	k_{diss} (s^{-1})	K_D (M^{-1})
p85	19.3 ± 5.8	0.100 ± 0.003	1.93×10^7	92.4 ± 2.7	0.127 ± 0.006	7.28×10^7
p85 N-SH2	0.14 ± 0.04	0.095 ± 0.010	1.47×10^5	33.4 ± 2.0	0.141 ± 0.006	2.37×10^7
p85 C-SH2	15.9 ± 4.3	0.102 ± 0.026	1.56×10^7	16.9 ± 3.3	0.098 ± 0.004	1.72×10^7
PLC C-SH2	1.16 ± 0.03	0.045 ± 0.006	2.58×10^6	16.4 ± 3.4	0.049 ± 0.009	3.35×10^7
PLC N+C	1.51 ± 0.36	0.034 ± 0.002	4.44×10^6	12.0 ± 3.3	0.045 ± 0.001	2.67×10^7
GAP N-SH2	2.06 ± 0.94	0.039 ± 0.008	5.28×10^6	0.40 ± 0.05	0.054 ± 0.007	7.41×10^5

Panayotou, G. et al. (1993) Molecular and Cellular Biology 13:3567-3576.

Antibody Characterization

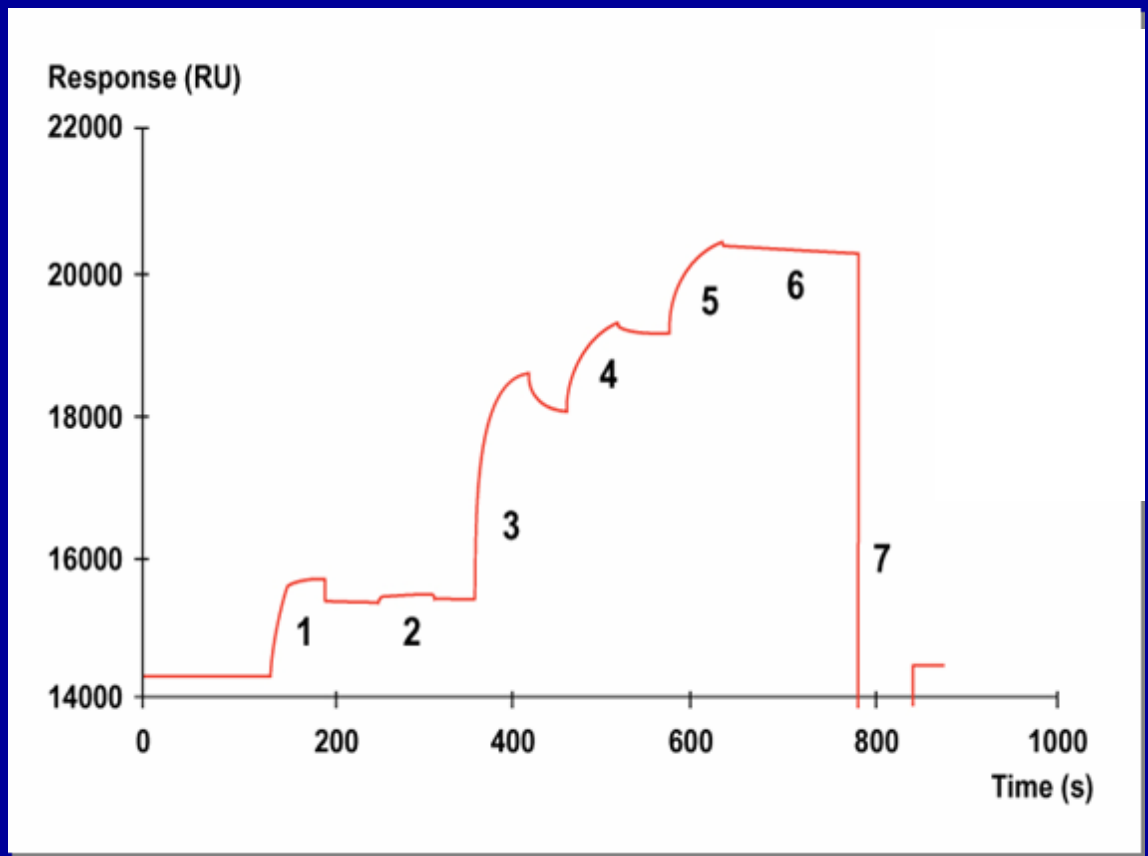
BIACORE®

- No purification
- No labelling
- Earlier characterization
- Kinetic information

	BIACORE®		Conventional
	Time	Method	Time
Isotyping	Day 1	ELISA	One Day
Affinity	Day 1 & 2	RIA	Weeks + labelling
Kinetics	Day 1 & 2	NA	NA
Epitope Map	Overnight	ELISA	Weeks + labelling
Assay	Day 2	Various EIA	Days - Weeks
Extended map	Day 3	ELISA	One day + labelling
TOTAL	2 - 3 days		Weeks - Months

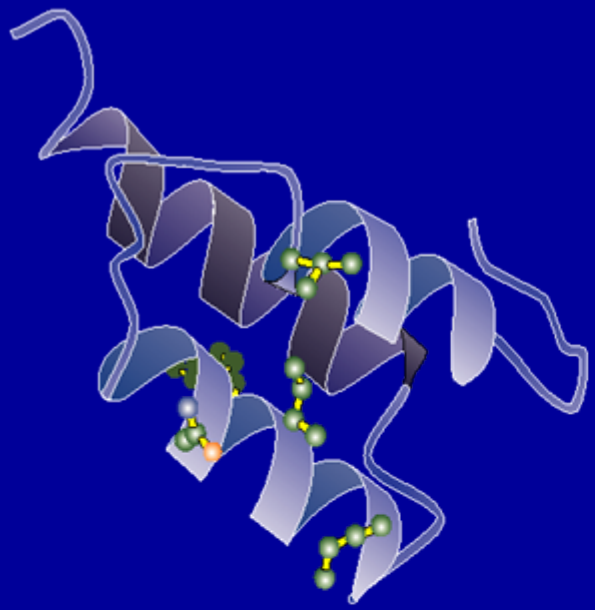
Johne, B. et al. (1993) Journal of Immunological Methods 160:191-198.

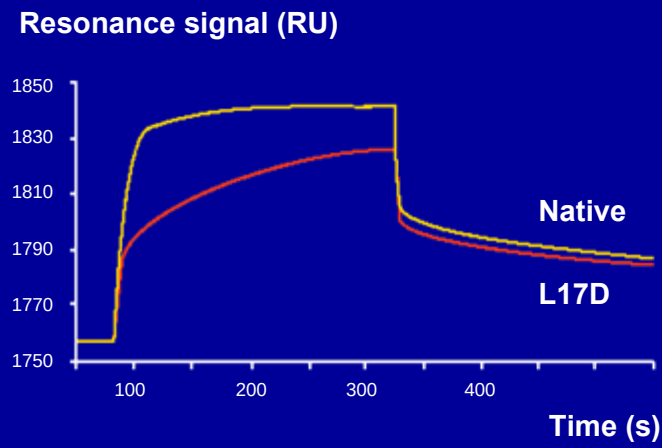
Multisite Binding Analysis of Troponin using BIACORE®

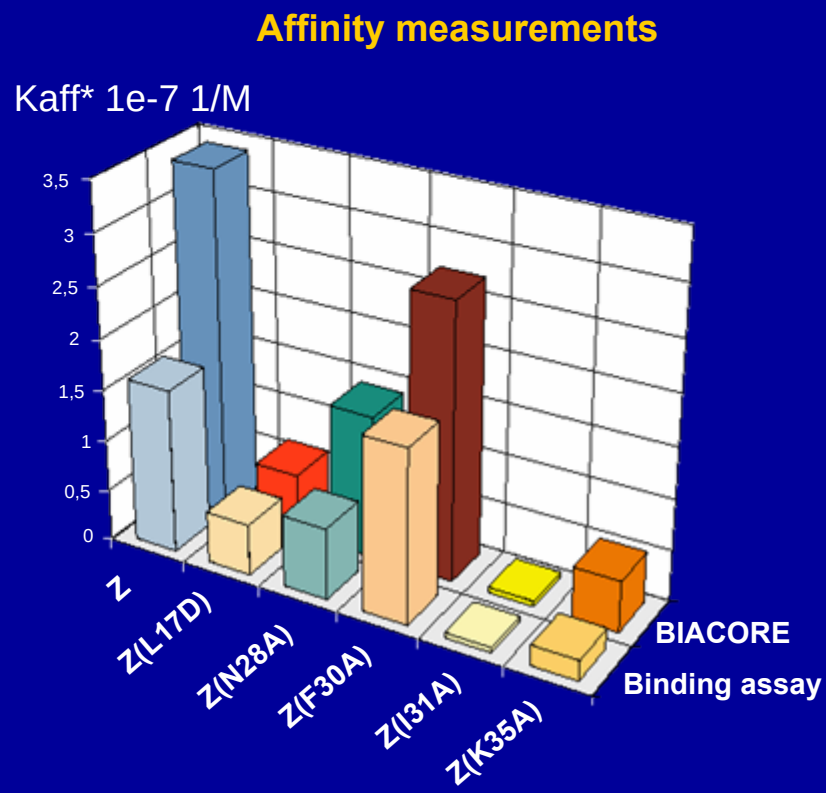


1. Troponin-I 5 µg/ml
2. MAb 1122 100 µg/ml
3. MAb 1240 100 µg/ml
4. MAb 1192 100 µg/ml
5. MAb 1190 100 µg/ml
6. MAb 535 100 µg/ml
7. HCl 10 mM

Kinetic Effects of Alterations in the Z-domain of Protein A



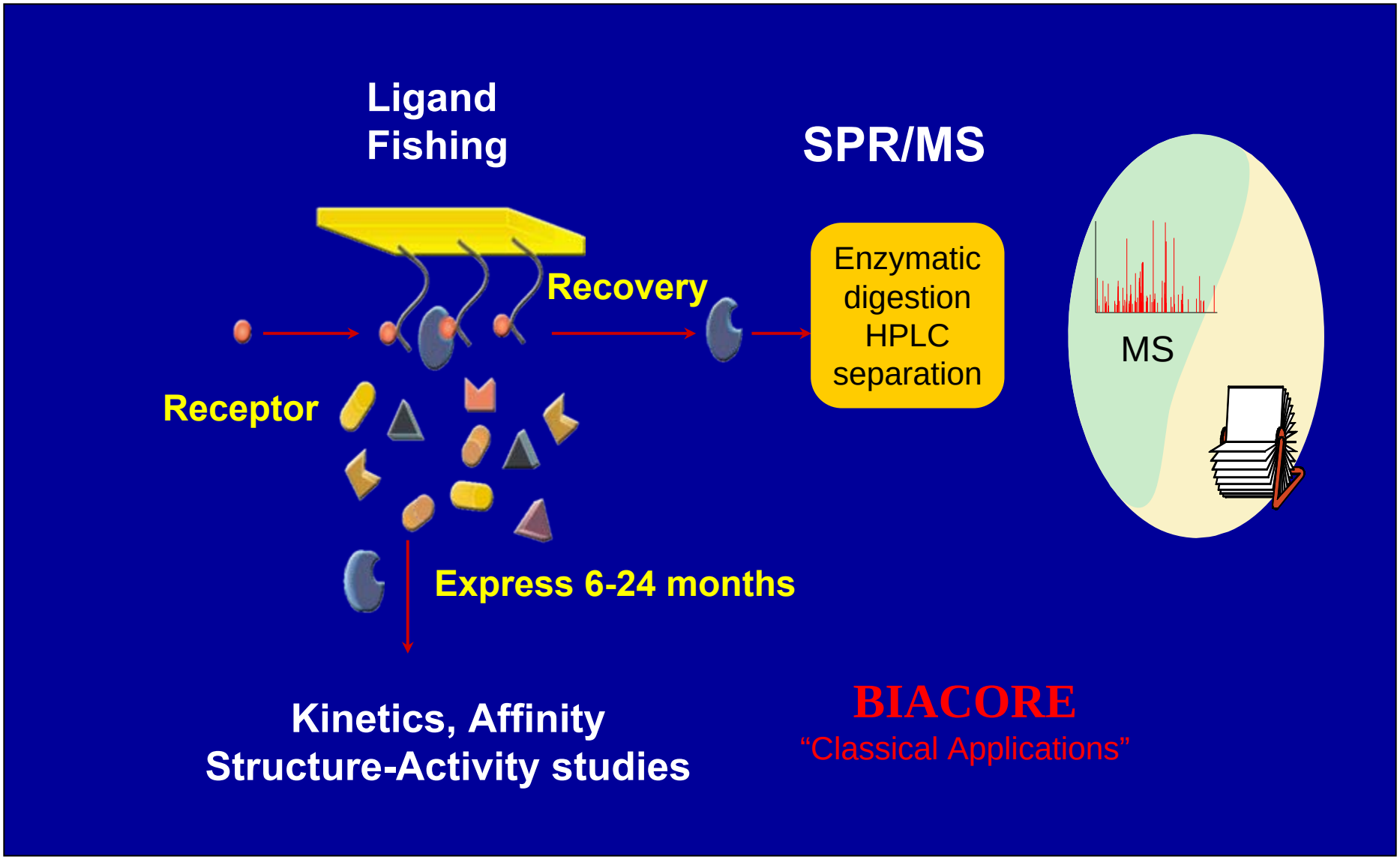




BIACORE® in Proteomics

- Fast, simple and compatible with any biological sample
- Monitors binding of native proteins from crude or purified samples
- Detects even low affinity binding events
- Recovers samples for MS analysis and identification
- Confirms results from other techniques
- Provides functional (interaction) data

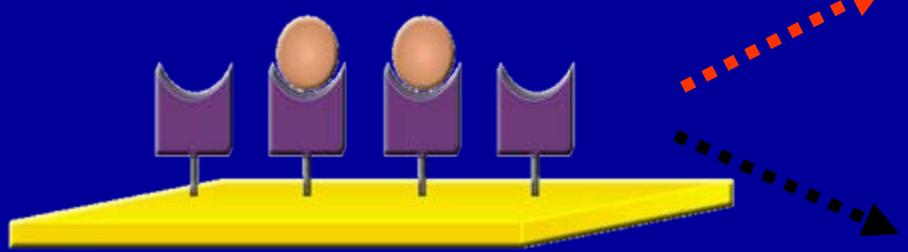
Biacore Proteomics Study



SPR / MS Approaches

- Direct On-Chip
- Elution/Microrecovery

BIA + MS



Direct detection of bound molecules on the surface (Krone et al 1997)

Recover bound molecules and analyze with MS (Fitz et al 1997)

Summary

- SPR detects binding events as changes in mass at the chip surface
- Real-time kinetic measurements
- Qualitative rankings
- Measurement of *active* concentration
- Information about structure-activity relationships
- Low volumes of precious samples needed

BUT !!! -

SPR is not a true solution method (vs. ITC)

Attaching receptor to surface can influence binding properties.