

BIACORE T100:

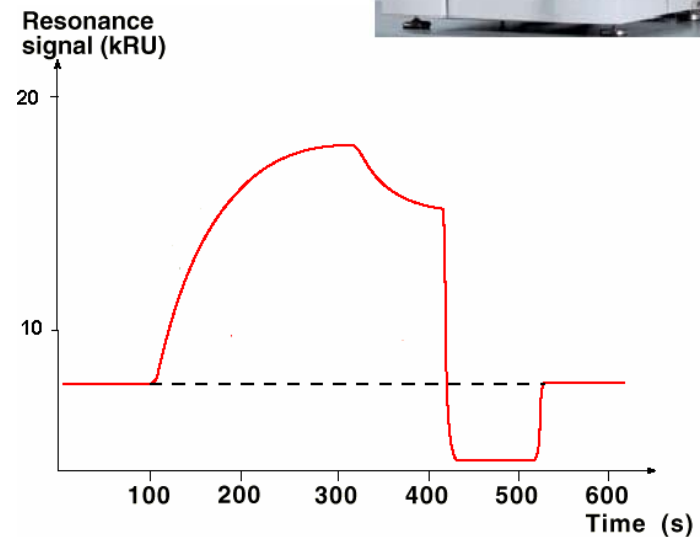
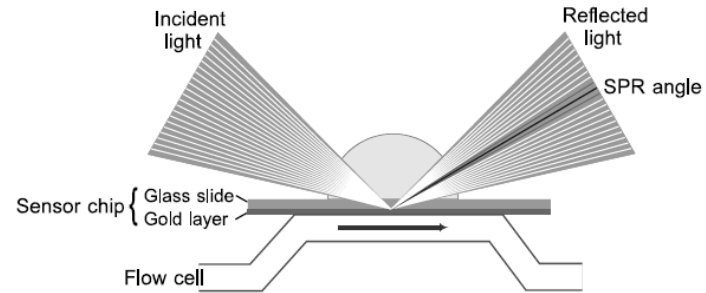
An overview of underlying concepts,
basic operation, experimental
techniques and potential applications

João Paulo

7/11/06

Outline

- **General Background**
General Relevance/SPR
- **Biacore® T100**
Basic Anatomy
General Operation
- **Sensor Chips**
- **Preparing chip surface**
Immobilization of Ligand
Pre-concentration scouting
Coupling chemistries
- **Interaction Assay**
- **Data Interpretation**
- **Application**
- **Potential projects**



General Information

- The Biacore protein interaction analysis system characterizes proteins in terms of **specificity**, **kinetics**, and **affinity**.
- Biacore exploits the phenomena of surface plasmon resonance (**SPR**).
- One interacting partner (“**ligand**”) is attached to the surface of a chip and the passing of a sample containing the second interaction partner (“**analyte**”) over the surface of the chip.
- Binding of molecules to the sensor surface generates a response that is proportional to the bound mass.

Data Obtained from Biacore Experiments

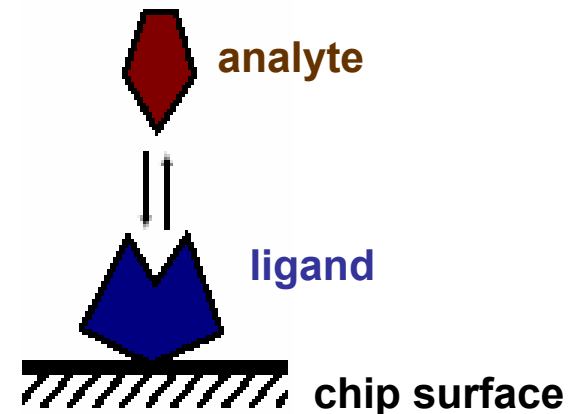
- **Specificity (yes/no binding response)**
 - Search for binding partners
 - Screen for inhibitors
 - Look for activity after protein purification
 - Test cell culture lines for expression of a given protein
- **Concentration**
 - Nanomolar concentrations can be measured in both purified molecules and complex mixtures (with a calibration curve).
- **Kinetics (rates of reactions)**
 - Complex formation
 - Complex dissociation
- **Affinity (strength of binding)**

Advantages of Biacore in Proteomics

- Monitor binding of **native proteins** from crude or purified samples.
- Detects **low affinity** binding events.
- Provides **real-time** kinetic measurements.
- **Recover** samples of MS analysis and identification.
- **Confirmation** of other techniques (e.g., MUDPIT MS, yeast-2-hybrid, IPs).
- Wide range of **temperature control** 4°C - 45°C. (Can easily perform assays at physiological temperature or investigate thermodynamic effects of protein/binding.)

Some Limitations of Biacore SPR

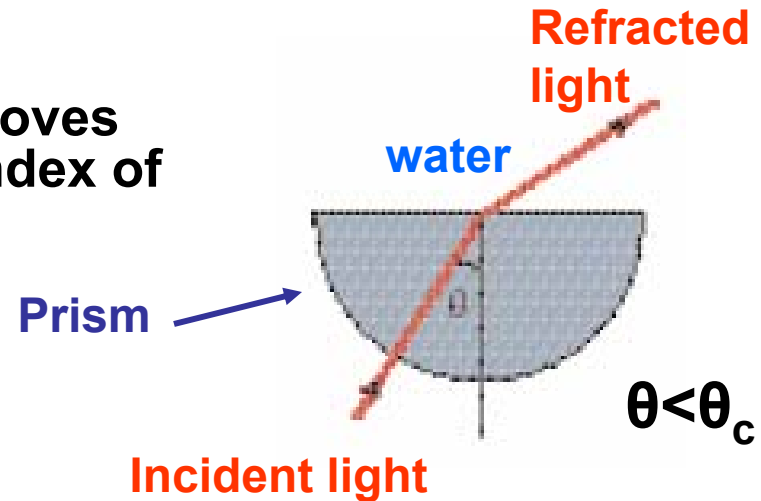
- **Kinetics**
 - k_{on} values are limited to a range of $10^3 \text{ M}^{-1}\text{s}^{-1}$ to $10^7 \text{ M}^{-1}\text{s}^{-1}$.
 - k_{off} values are limited to a range of 10^{-5} s^{-1} to 1 s^{-1} .
- **Affinity**
 - High affinity interactions where $K_D < 10\text{nM}$ have a slow k_{off} and are unsuitable for kinetic analysis.
 - (weak interactions, however, $K_D > 100 \mu\text{M}$ are easily studied)
- Quantitative analyses require that the **analyte** binds in a monovalent manner (one binding site).
- Very small molecules (<100 Da) are difficult to detect.



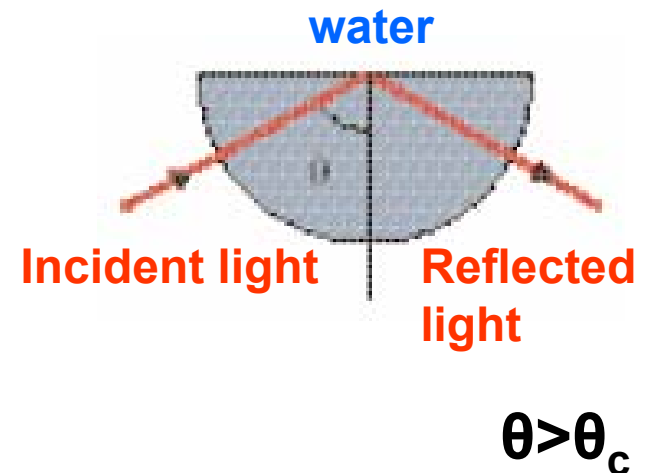
Principles of Biacore SPR technology

Optics 101

Refraction is the **bending** of light it moves from one material of a particular index of refraction to another.



Total internal reflection (**TIR**) occurs when light travels from a optically dense medium (e.g. glass prism) to a less dense (low index of refraction) medium (e.g. water).

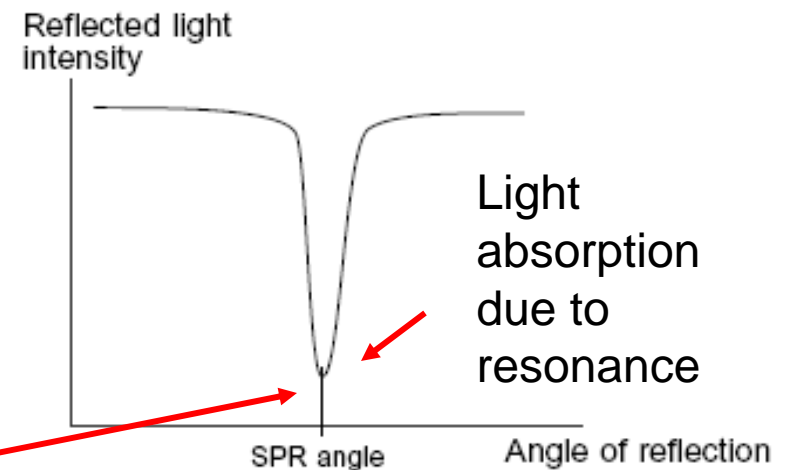
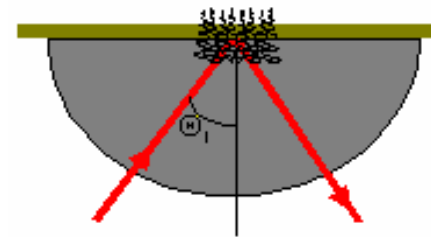
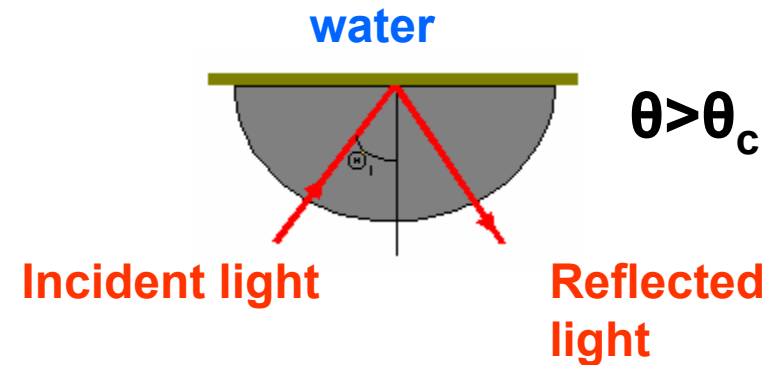


This phenomena occurs only beyond a critical angle (θ_c) and results in all light being reflected within a prism, thus no light will pass though.

Principles of Biacore SPR technology

Optics 201

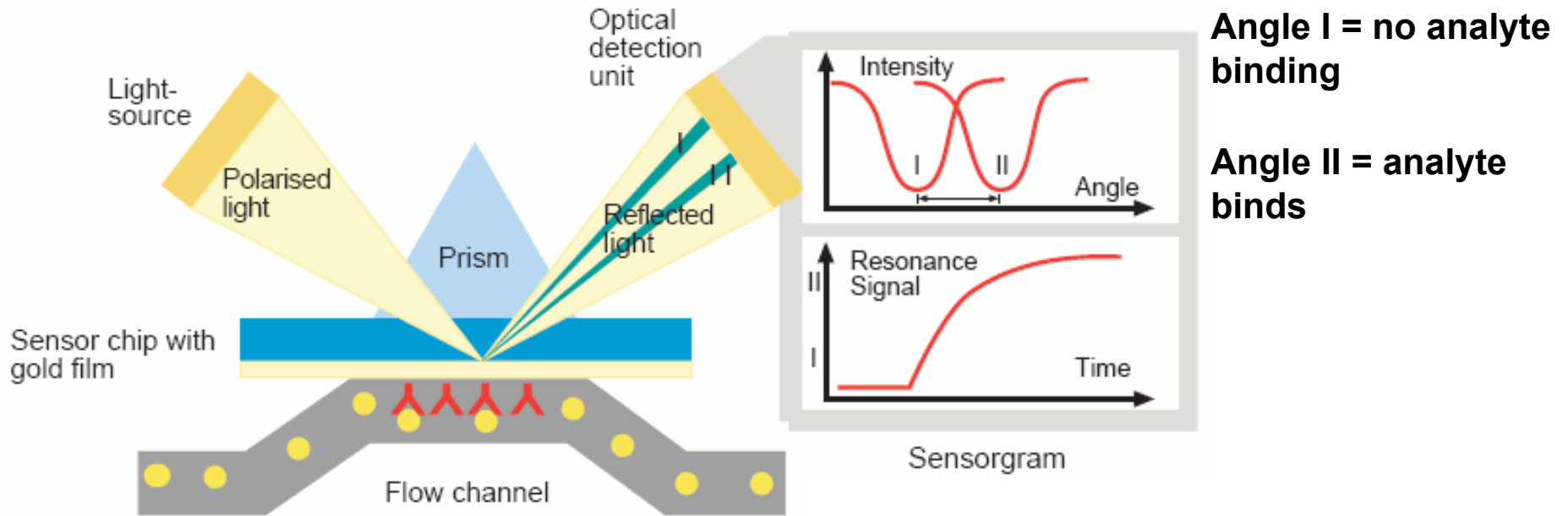
- In SPR a gold layer is inserted between the “glass” and the “water.”
- Light energy is **lost** to the gold electrons which oscillate to form what is known as **surface plasmons**
- **Resonance** occurs when the momentum of the incoming photons is equal to the momentum of the plasmons. In other words at a certain angle (θ_{SPR}) there is a minimum intensity of light



$$\theta = \theta_{spr}$$

Principles of Biacore SPR technology

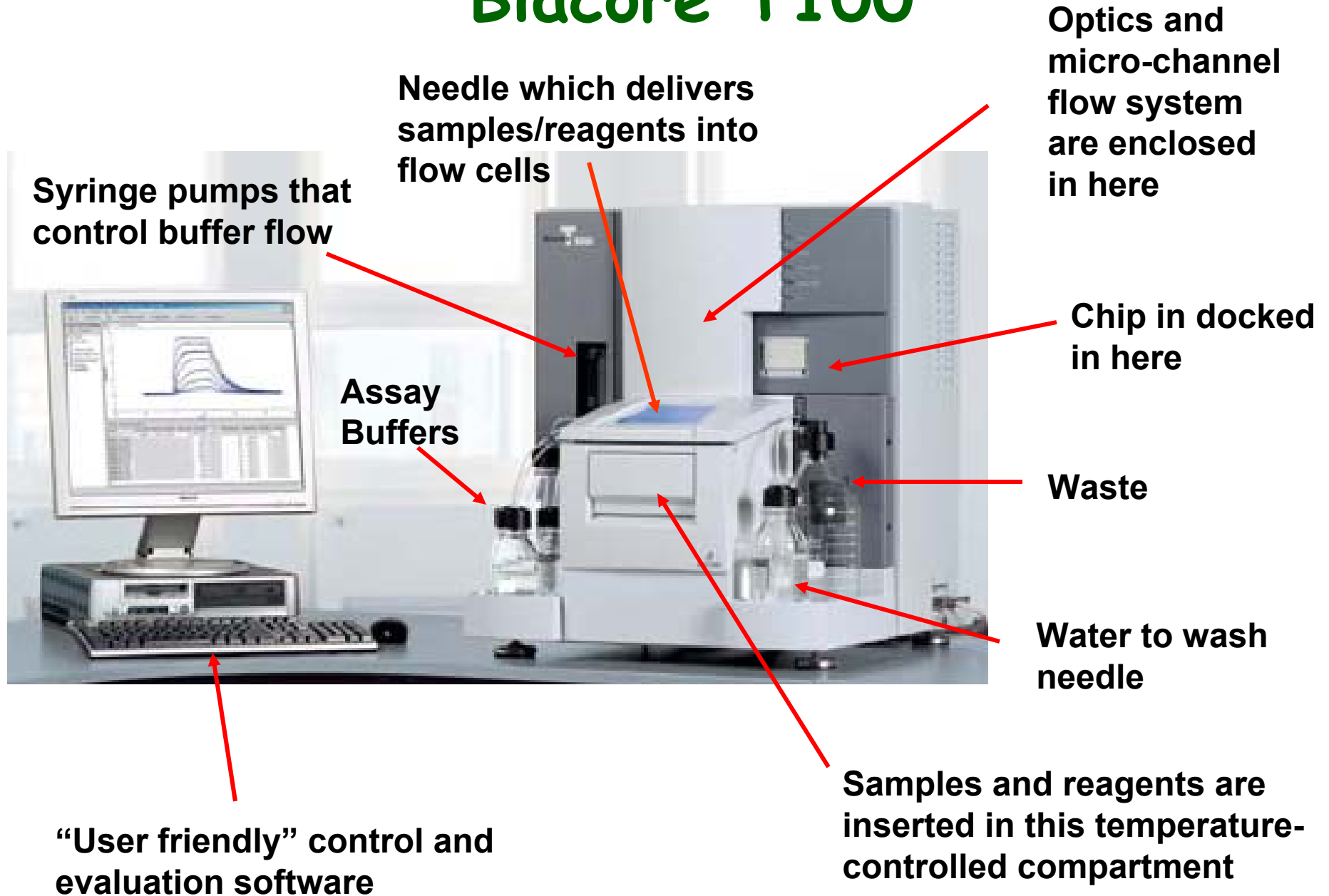
Optics 301



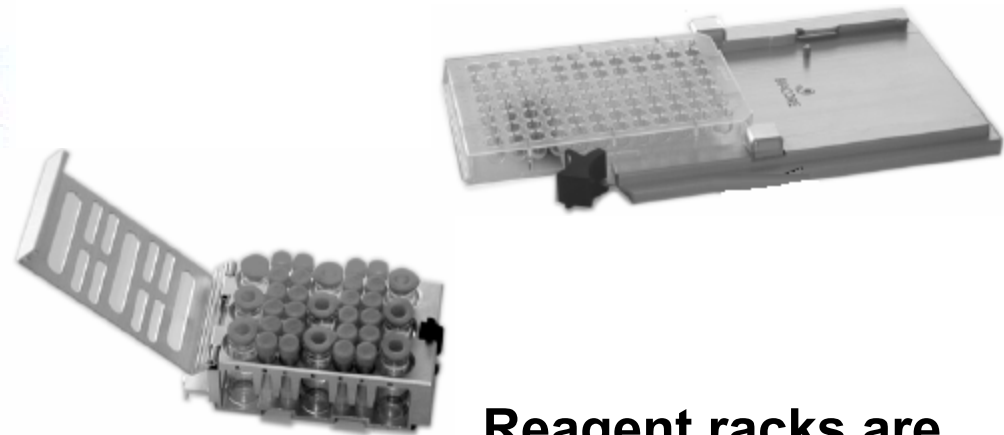
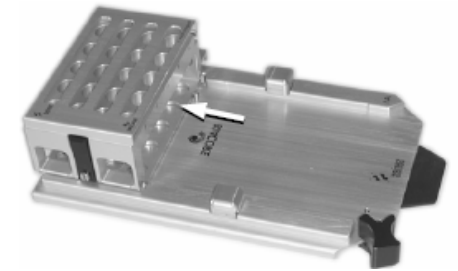
SPR measures **changes** in the **resonance angle** in real time.

The change in the SPR is directly related to the amount of protein near the surface of the chip.

Biacore T100



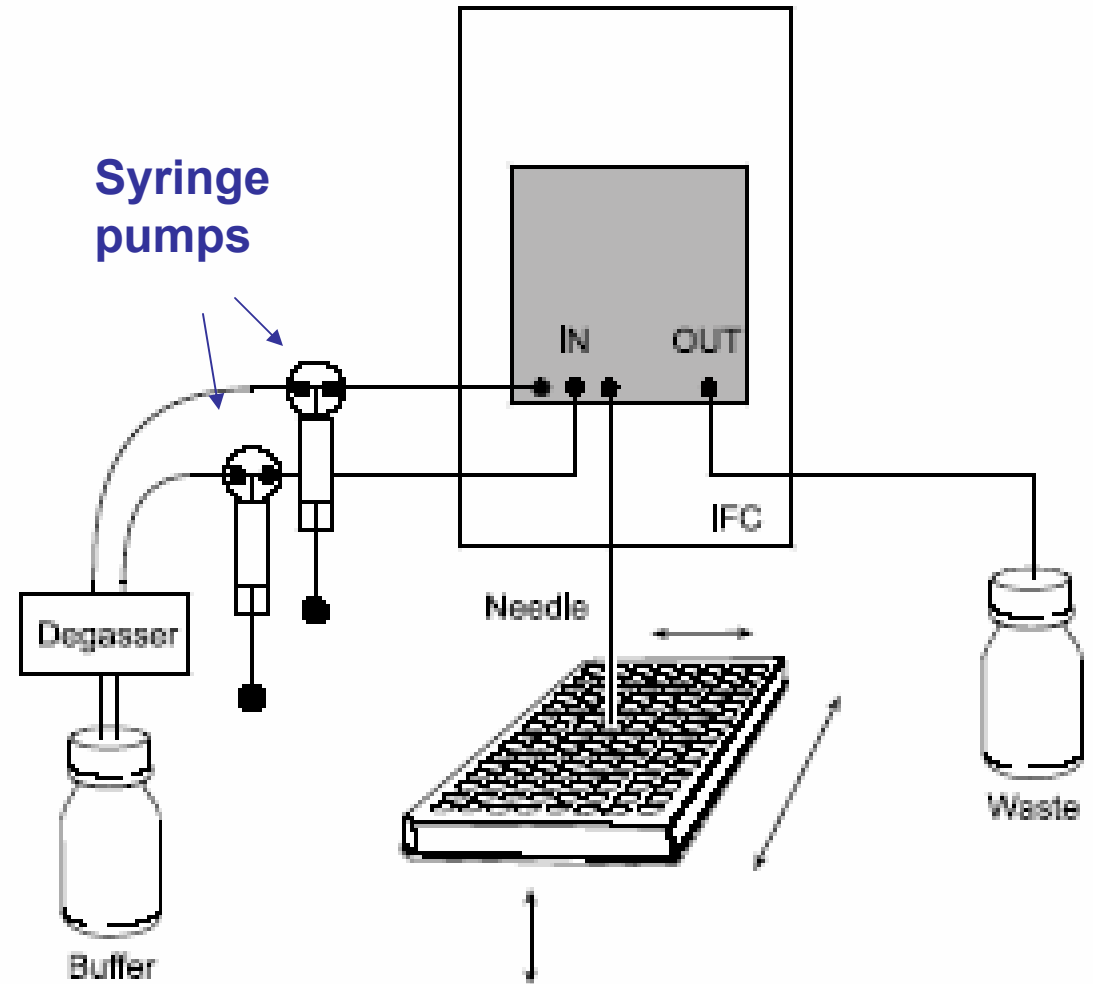
Reagent Rack



Reagent rack can hold ligands to be immobilized, analytes to be assayed and necessary reagents for immobilization procedures and system maintenance.

Reagent racks are interchangeable and can hold a 96 or even a 384 well plate.

Buffer System

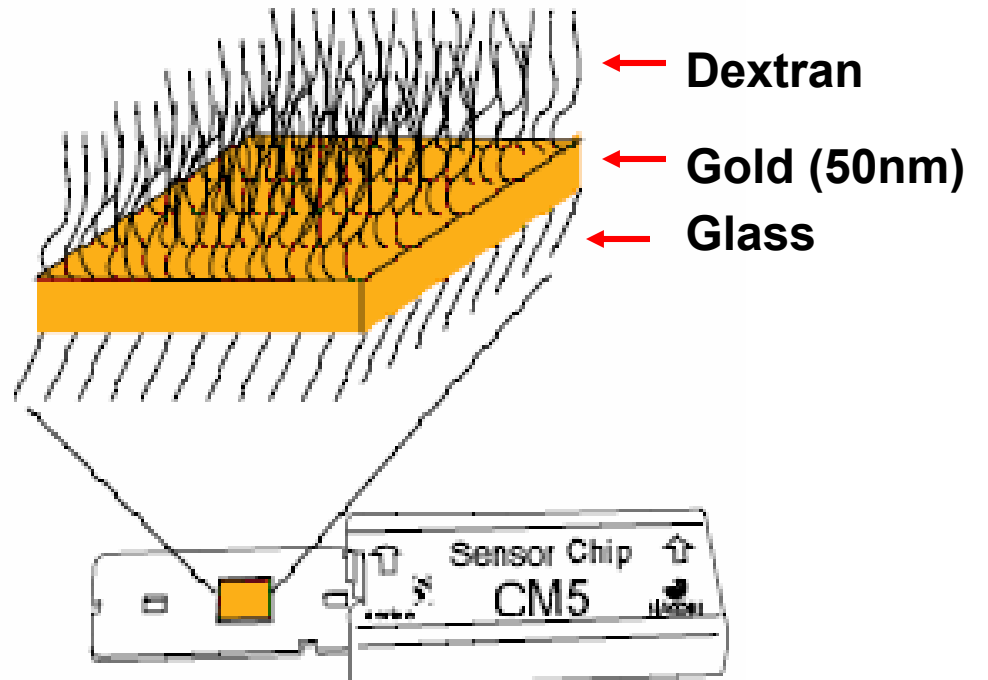
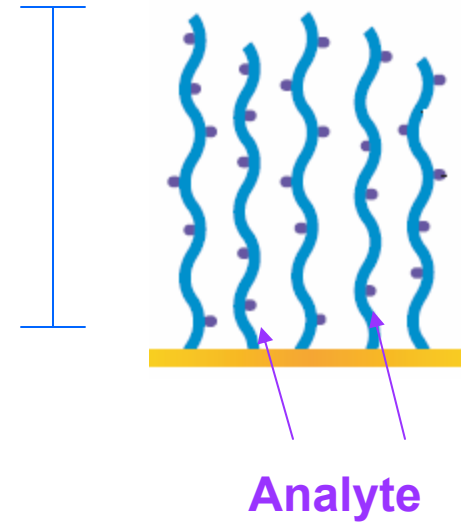


Four different buffers can be used

The needle, which takes samples from the reagent rack to the flow cell system is mechanically washed with water after dispensing each sample.

The Sensor Chip

100nm
carboxy
methyl
dextran on
CM5 Chip



A chip:



Sensor Chip Type

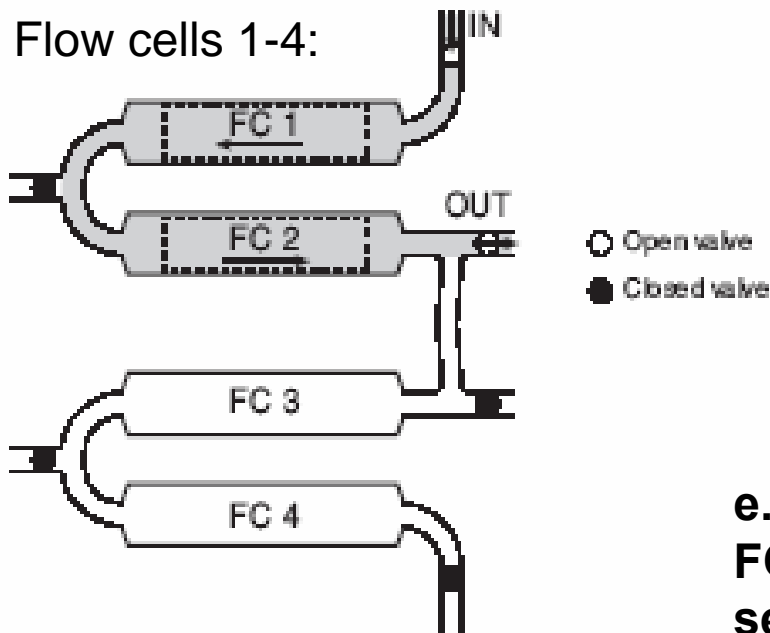
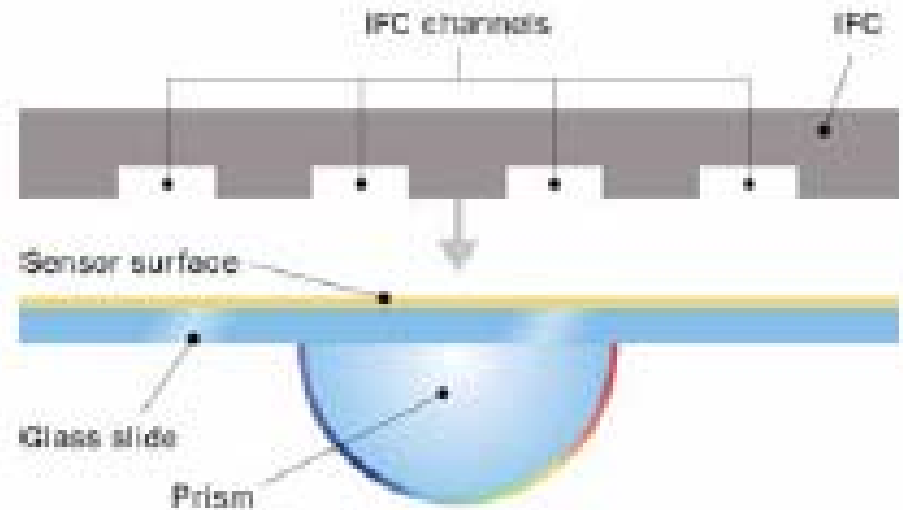
Chip name	Dextran	Modification	Purpose
CM5	100 nm	100% carboxylation	general
CM4 / B1	100 nm	30% carboxylation	cell extracts / serum and membranes
CM3 / F1	30 nm	100% carboxylation	cell extracts / serum and membranes
C1	none	100% carboxylation	phage binding
NTA	100 nm	nitrilotriacetic acid	capturing poly HIS-groups
HPA	none	hydrophobic	lipid capturing
L1	100 nm	lipophilic substances	bilayers to mimic membranes
SA	100 nm	streptavidin	capturing of biotin
AU / J1	none	none	user defined surface chemistry
SIA kit AU	none	none	surface interaction analysis in material science

A variety of chips are available for different applications

The Flow Cells

(where buffer/sample and sensor chip meet)

IFC: Integrated μ-fluidic cartridges are an integrated series of micro-channels and membrane valves that deliver liquid to the sensor surface.



Four flow cell channels are formed when the sensor chip is docked against the IFC.

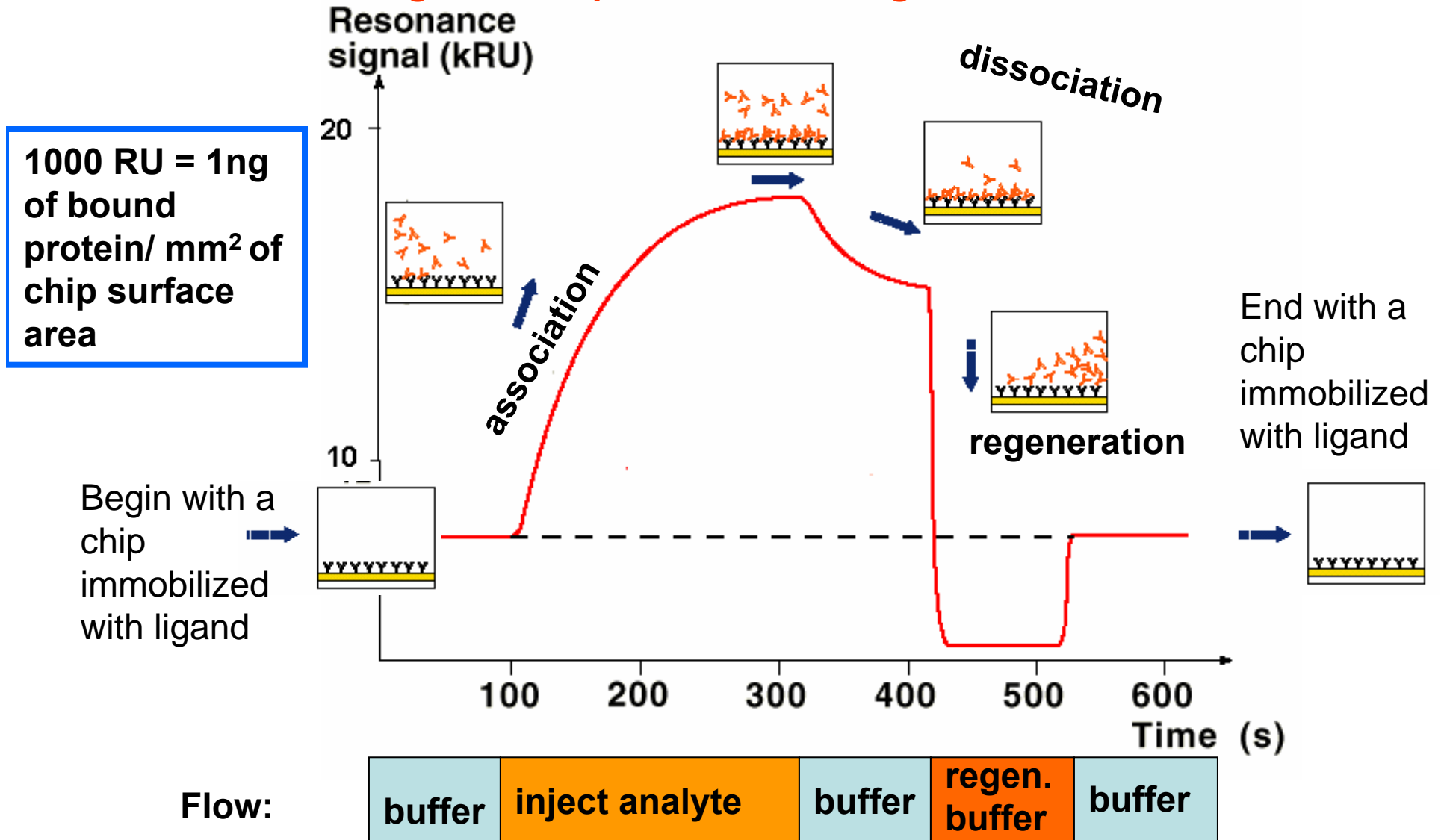
e.g. FC1 can serve as a reference for FC2 or both FC2 and FC3 or FC2, FC3 and FC4. Also FC3 can serve as a reference to FC4.

Basics Steps to a Biacore Experiment

- 1) **Decide on chip to use**
- 2) **Decide on which molecule will be the ligand and which will be the analyte**
- 3) **Prepare the ligand and analyte**
- 4) **Choose immobilization strategy**
- 5) **Pre-concentrate ligand on chip prior to activation (“pH scouting”)**
- 6) **Immobilize ligand onto sensor chip**
- 7) **Inject analyte and record response**
- 8) **Regenerate surface**
- 9) **Analyze data**

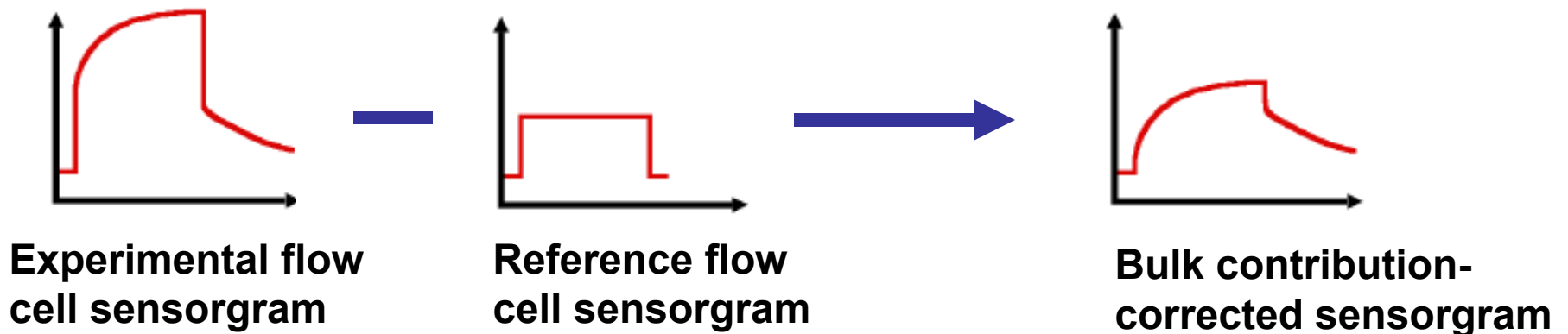
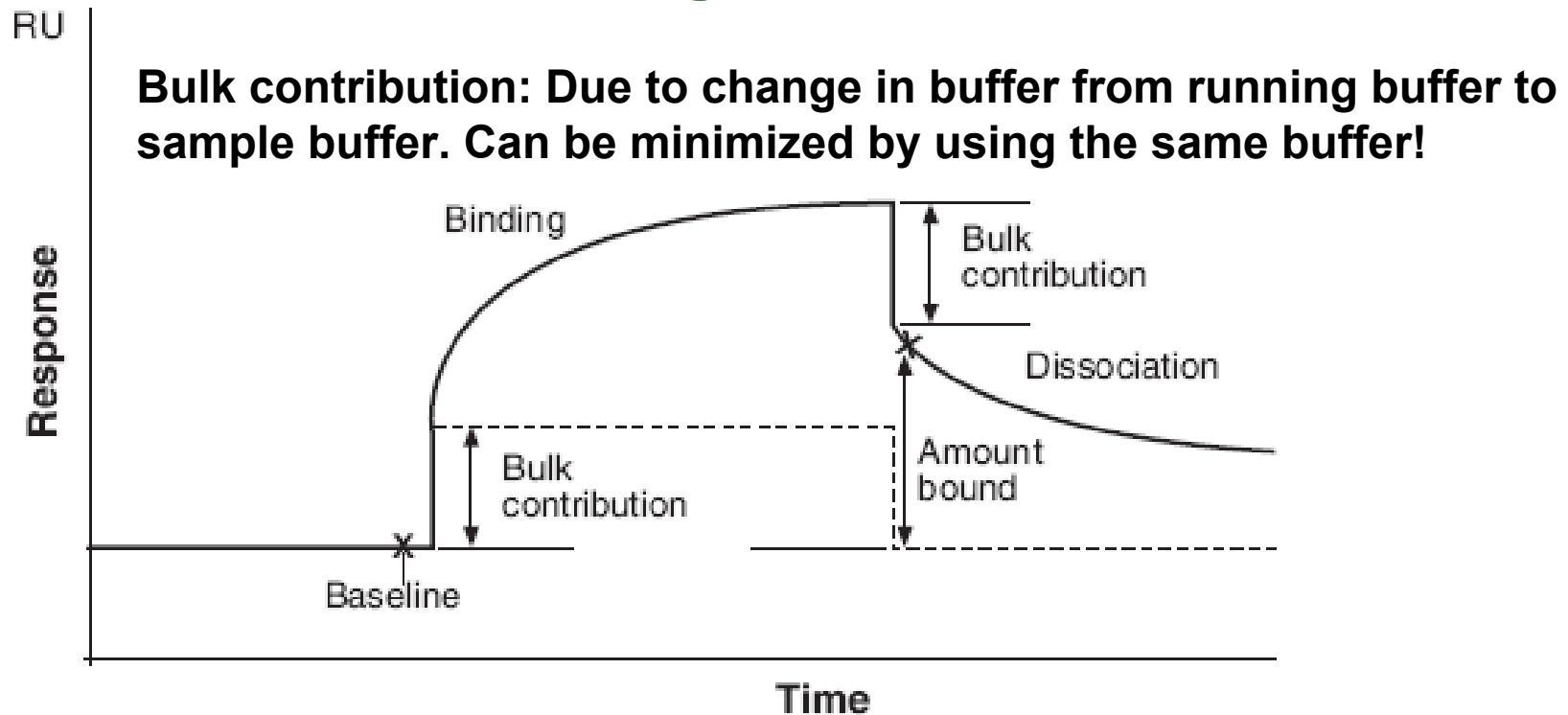
Typical Interaction Assay Sensorgram

A "sensorgram" is a plot of the SPR signal vs. time.



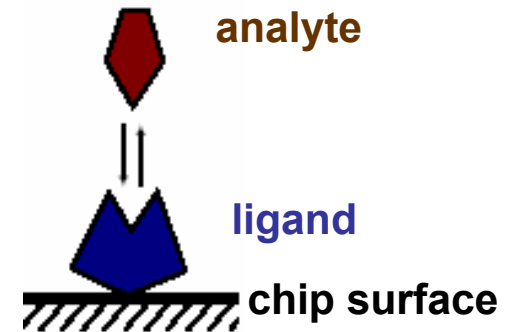
1000 RU is equivalent to the change in 0.1° in the resonance angle.

Automatic correction of buffer effect using reference cell

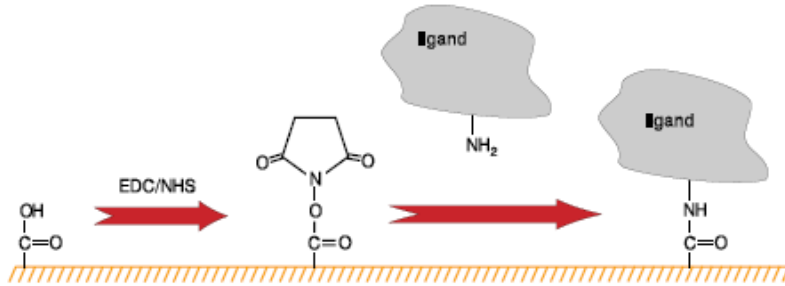


Sample and Buffer Preparation

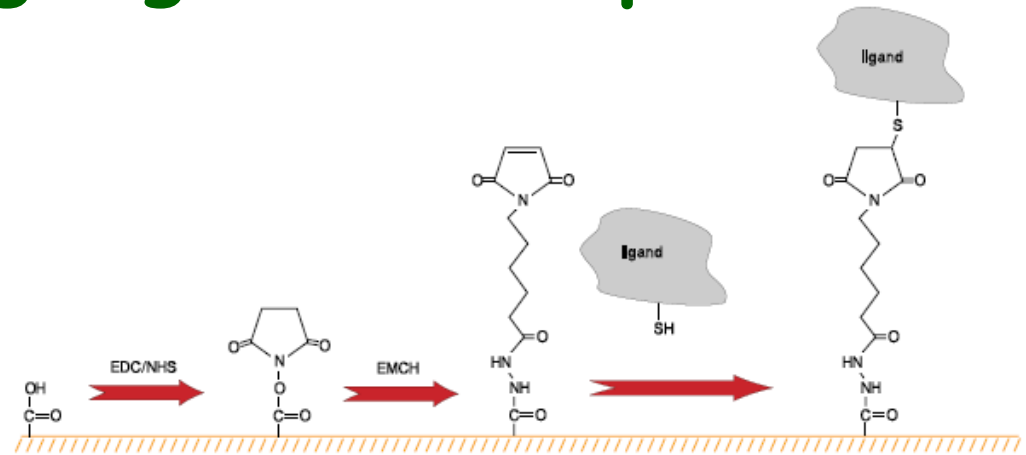
- Preparation of Ligand
 - Purity >90% on silver stain gel
 - Concentration 20-200µg/mL (1-2 µg)
 - Smaller → more change in SPR angle
- Preparation of Analyte
 - Concentrations in the mg/mL range for dilutions near K_D values
 - 10-100µg per injection (depending on K_D)
 - Buffer should match running buffer.
- Preparation of Buffer
 - Unlike older systems, the T100 has a built in degasser.
 - Buffers should be filtered with a 0.22 µm filter.
 - Detergent P20 can be added to reduce nonspecific binding.
 - For immobilization
 - Avoid Tris buffer or Sodium azide (primary amines) when performing amine coupling.
 - Buffer pH of buffer should be at least 0.5-1 unit below the pI of the ligand.
 - Ionic strength should be low 10-15 mM monovalent cation.
 - Buffer pH of buffer should be at least 0.5-1 unit below the pI of the ligand.



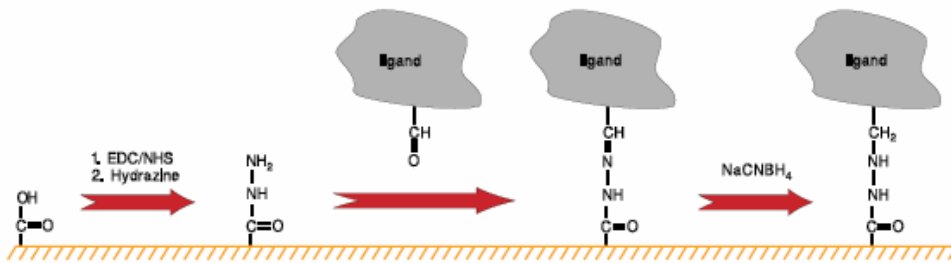
Several Chemistries Exist for Immobilizing Ligand to Chip



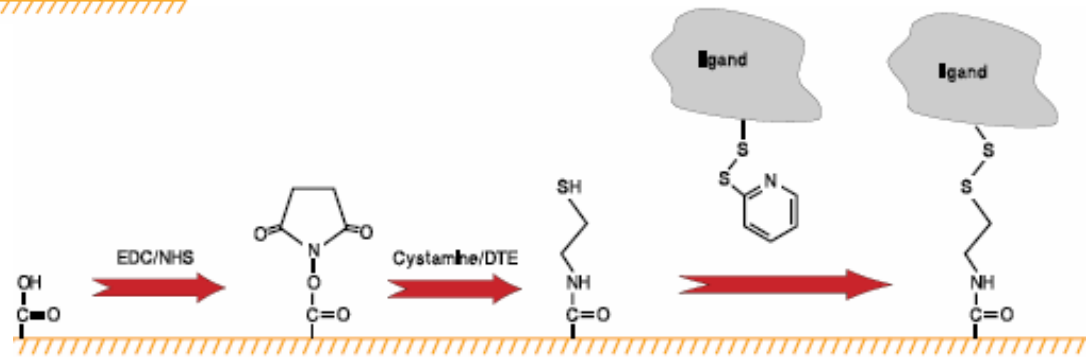
Amine coupling



Thiol Coupling



Aldehyde coupling



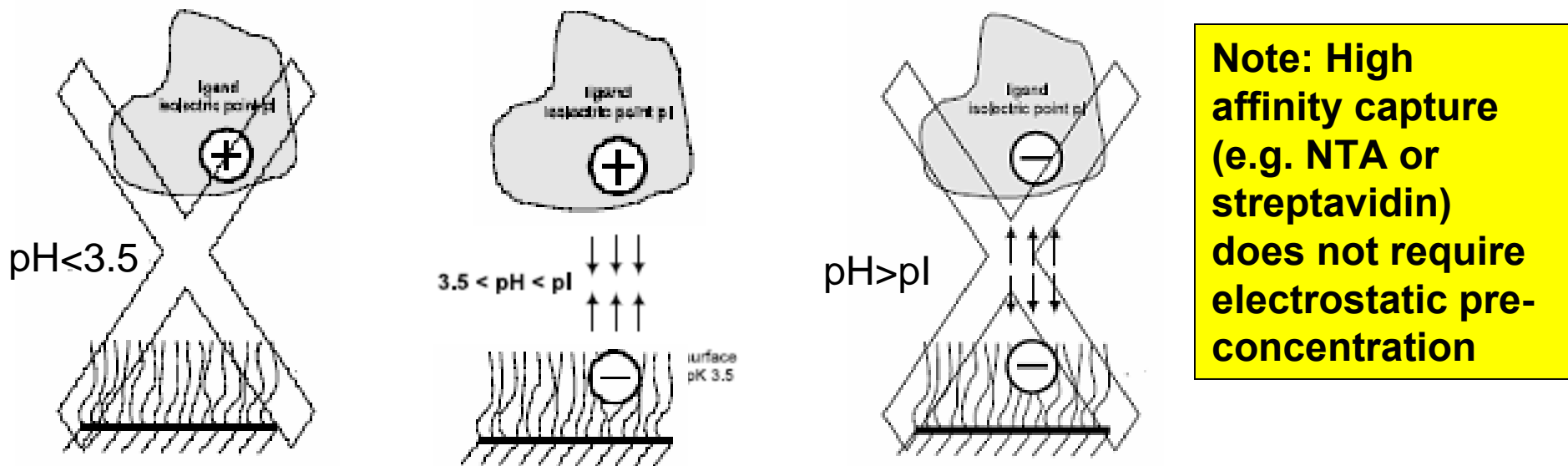
Maleimide coupling

Find Pre-Concentration Conditions before Immobilizing

Pre-concentration is required for efficient immobilization of ligand on surface of the chip

Pre-concentration entails the **electrostatic attraction** of ligand to the sensor surface via change in **buffer pH**.

Carboxymethyl dextran is negative at buffer pH > 3.5



Pre-concentration is successful when the net charge on a ligand is positive due to pH of buffer > pI of protein and pH > 3.5

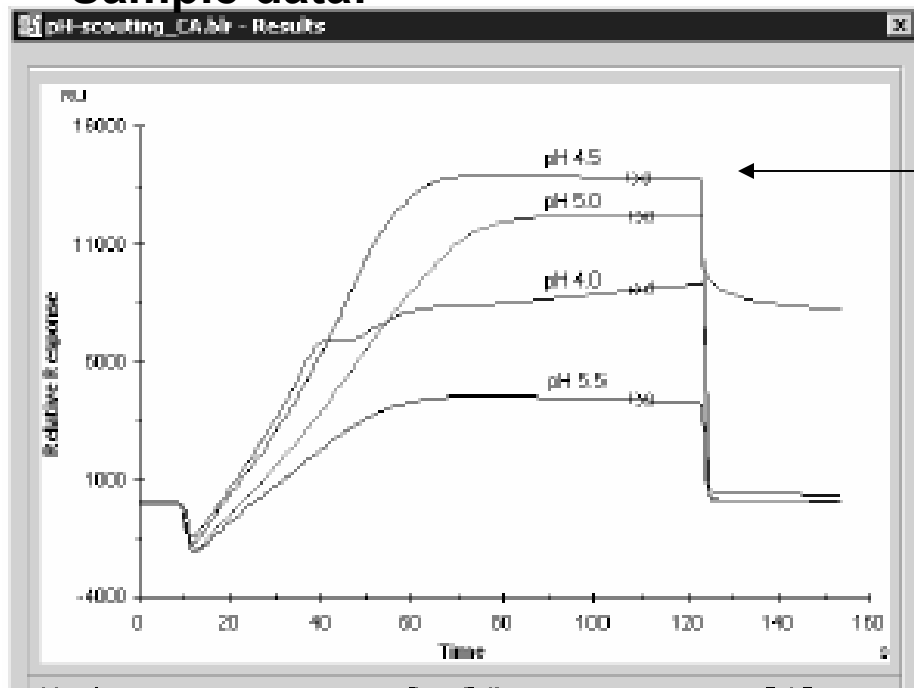
Pre-concentration "pH Scouting" Summary

Test a series of pHs and find which condition concentrates more ligand at the surface of the chip.

Remember: Surface is NOT activated, so there is not covalent immobilization!

There's only electrostatic attraction.

Sample data:

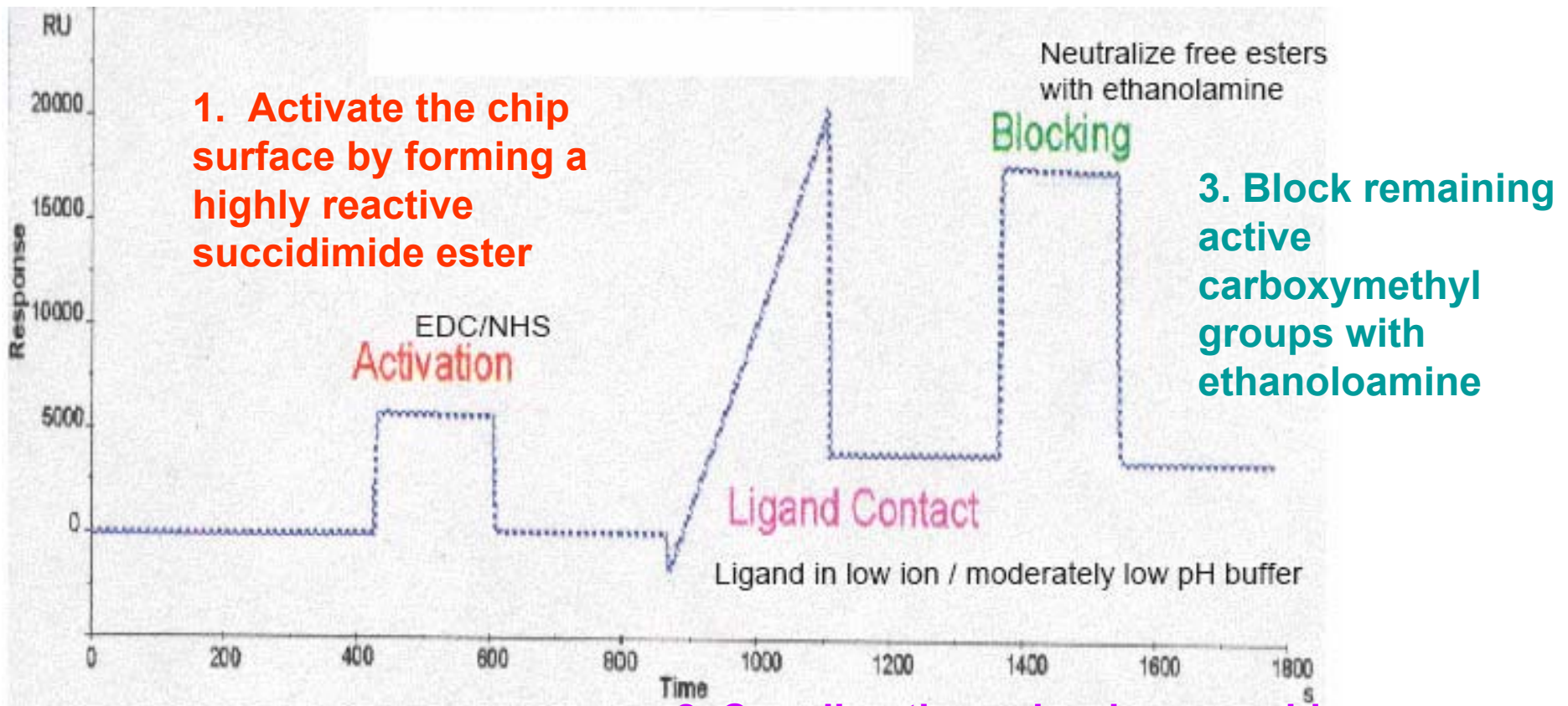


Here pH 4.5 immobilizes most ligand

Once pre-concentration conditions have been established, immobilization can be performed

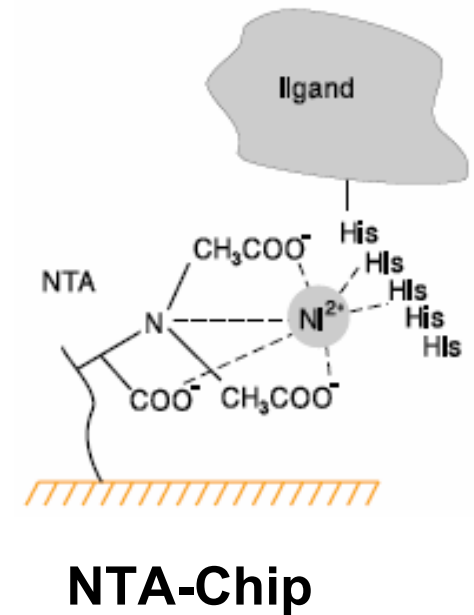
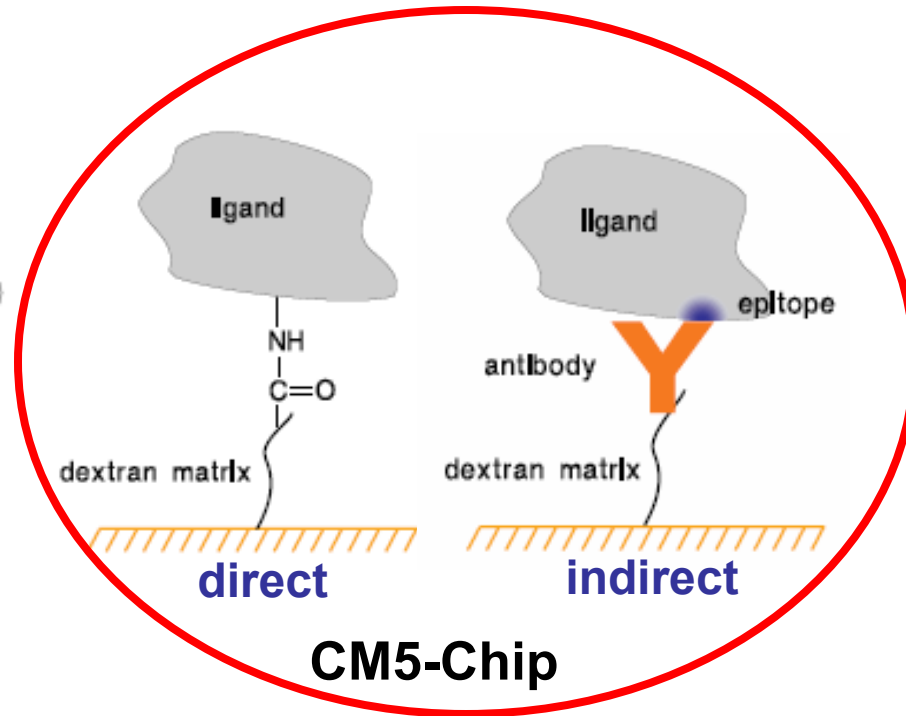
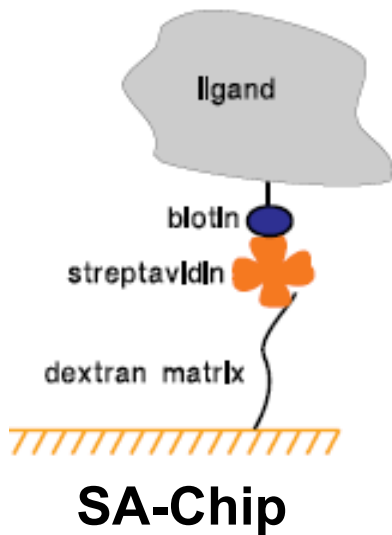
General Scheme for Immobilizing Ligand on CM Chip (amine coupling)

Activation/Coupling/Blocking

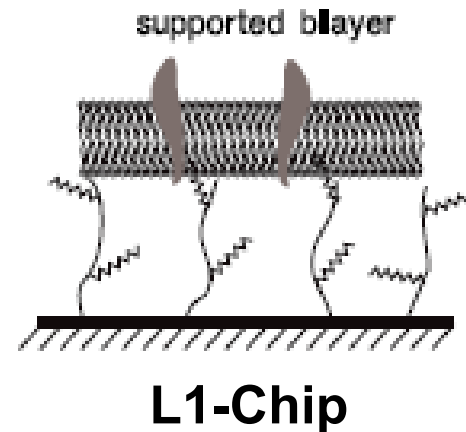
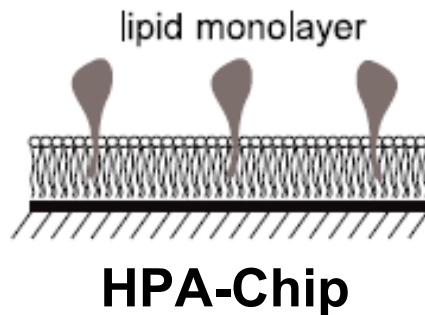


2. Coupling through primary amide group using best buffer from pre-concentration step

Alternative Immobilization Strategies



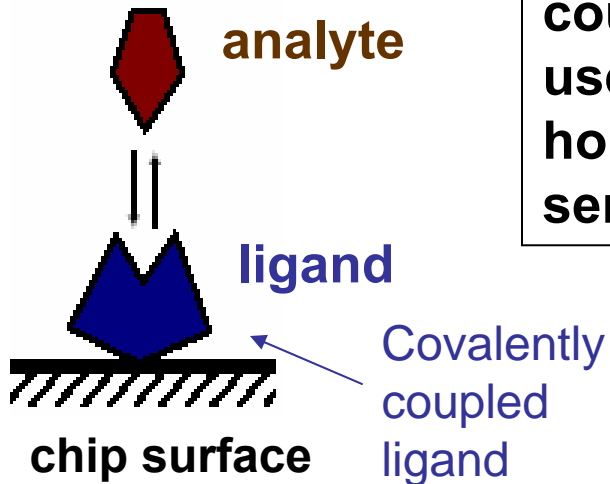
For membrane-bound proteins



For integral membrane proteins

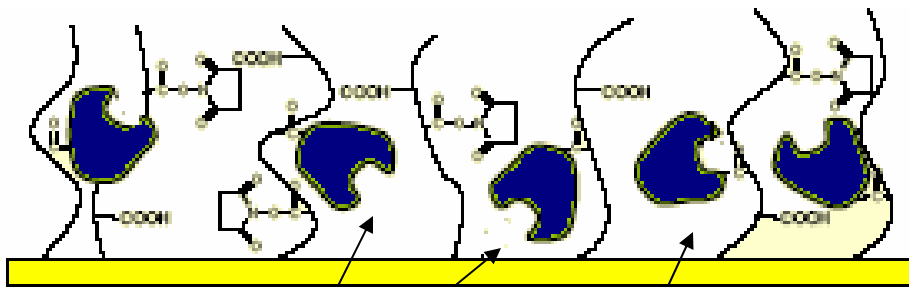
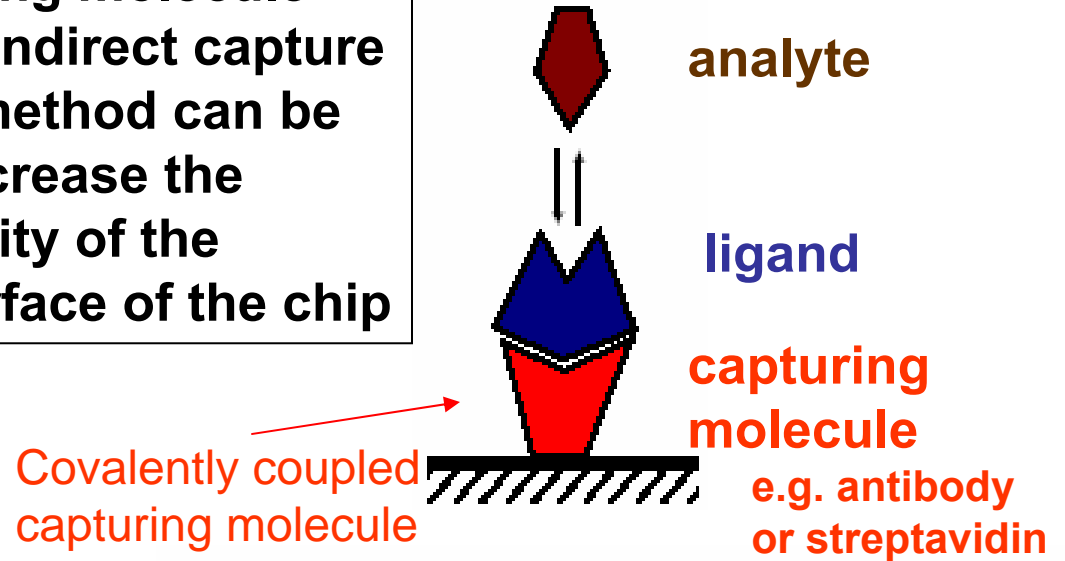
Direct vs. Indirect Capture of Ligand

Direct Coupling:



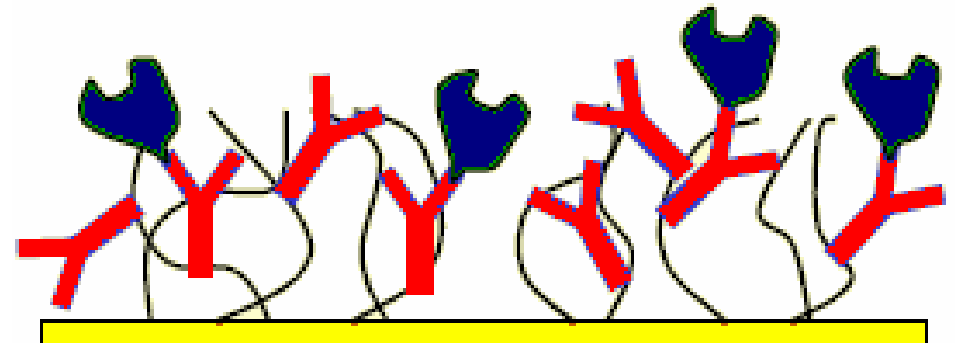
If a capturing molecule exists, an indirect capture coupling method can be used to increase the homogeneity of the sensor surface of the chip

Indirect Capture:



Obstructed binding sites

Binding site modified by coupling

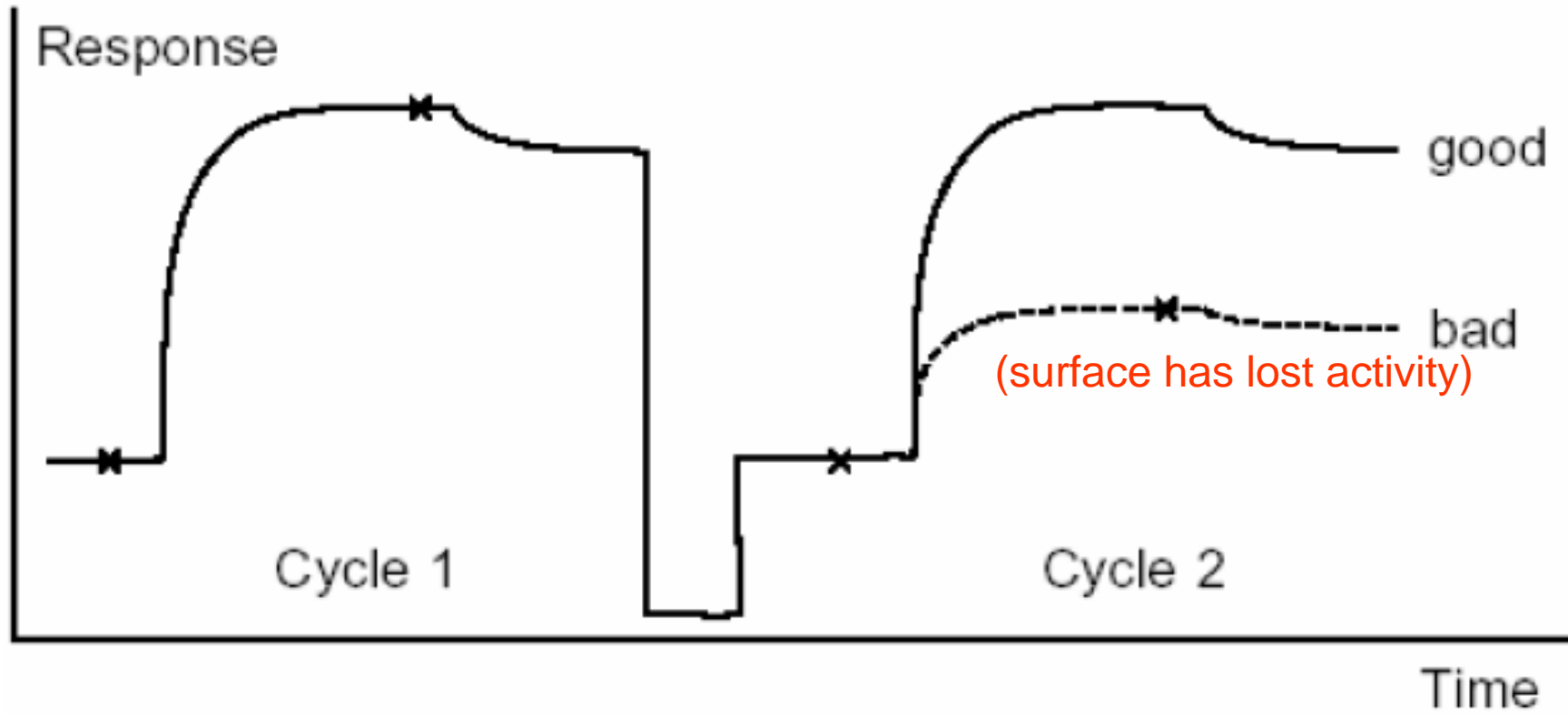


Also, ligand purity is no longer much of an issue

Regeneration

After experiment, attempt to “regenerate” the original ligand-coupled surface by **removal of bound analyte**.

Conditions must be harsh enough to remove bound analyte, but retain activity of ligand.



Regeneration Solutions

Milder conditions are initially used, but more stringent conditions are applied as needed.

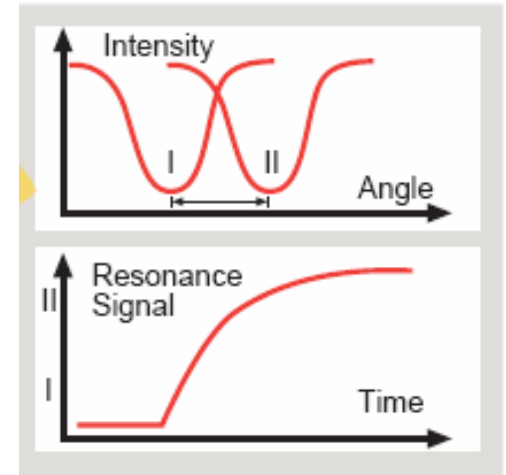
Type of bond	Acidic	Basic	Hydrophobic	Ionic
Strength				
Weak	pH > 2.5 formic acid HCl 10 mM Glycine/HCl	pH < 9 10 mM HEPES/NaOH	pH < 9 50 % ethylene glycol	1 M NaCl
Intermediate	pH 2-2.5 formic acid HCl 10 mM Glycine/HCl H ₃ PO ₄	pH 9-10 NaOH 10 mM Glycine/NaOH	pH 9-10 50 % ethylene glycol	2 M MgCl ₂
Strong	pH < 2 formic acid HCl 10 mM Glycine/HCl H ₃ PO ₄	pH > 10 NaOH	pH > 10 25-50% ethylene glycol	4 M MgCl ₂ 6 M guanidinechloride

A regeneration solution database for regeneration of various proteins is available on the Biacore website.

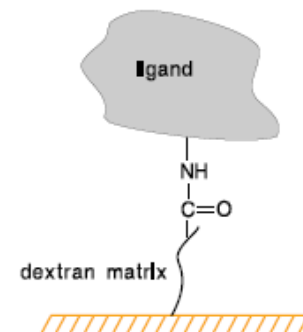
In general, however, different solutions should be tried to optimize regeneration, but remember once the ligand loses activity, good luck in getting it back...

Summary

- SPR measures changes in the **resonance angle** (which are directly related to the **mass concentration** of dissolved material close to the chip surface) in real time.
- The Biacore T100 can be used to investigate **specificity**, **kinetics** and **affinity** of even **low affinity** binding, with some limitations.
- Variety of **chips** and **immobilization** strategies exist that can be used for a wide-range of experiments.



Sensorgram



Useful References

Biacore T100 instrument handbook

Chatelier, RC et al. (1995) A general method to recondition and reuse BIACore Sensor Chips Fouled by Covalently Immobilized Protein/Peptide. Anal. Biochem. 229: 112-118.

Karlson, R and Falt, A. (1997) Experimental design for kinetic analysis of protein-protein interactions with surface plasmon resonance biosensors. J. Immun. Methods. 200: 121-133.

Myszka, DG (1999) Improving biosensor analysis. J. Mol Recognit. 12: 279-284.

“The SPR Pages” <http://home.hccnet.nl/ja.marquart/>

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