SURFACE PLASMON RESONANCE-BASED SYSTEMS

ADVANCED METHODS IN BIOENGINEERING LABORATORY

Schedule

- Week 1:
  - Introduction
  - Reagents preparation
  - Ligand immobilization of Protocol 1
- Week 2:
  - Kinetics of Protocol 2
  - Data analysis

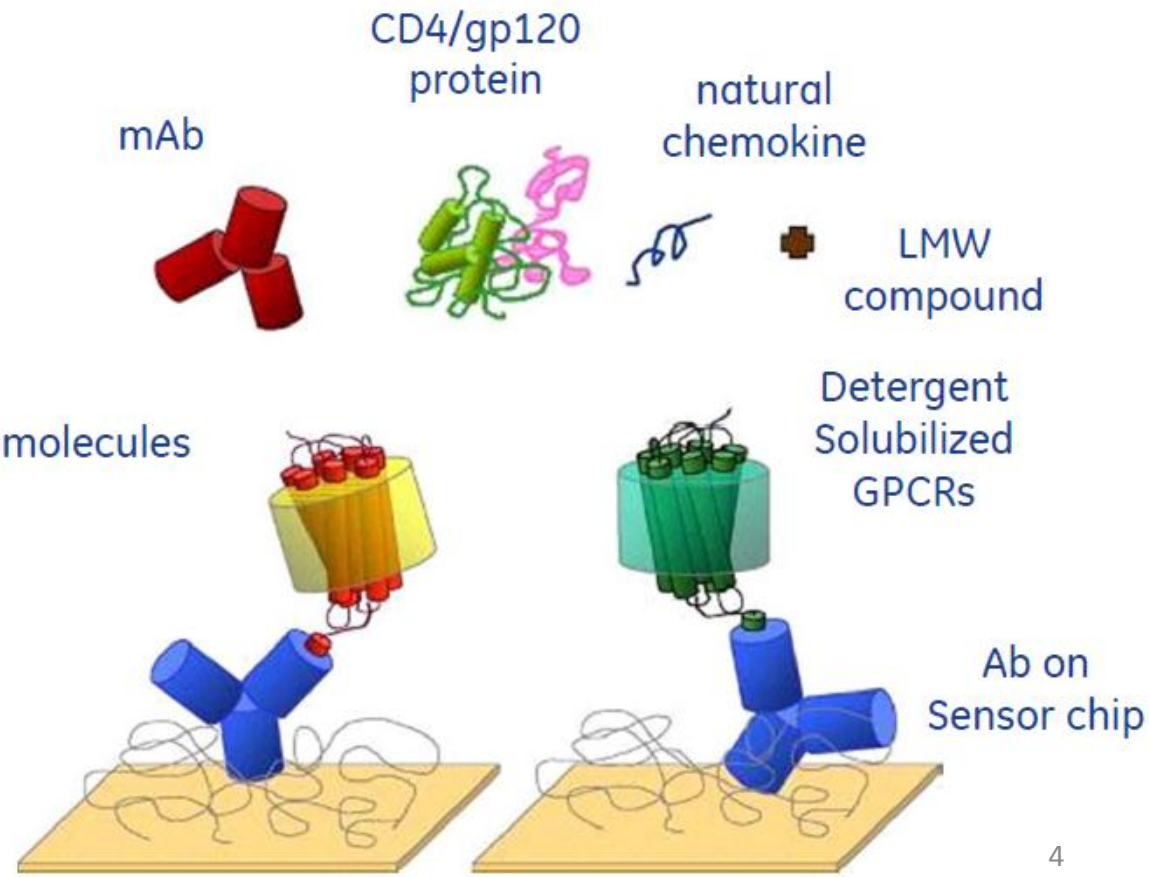
Introduction Outline

- Idea and Objectives
- Biacore components
- Experiment description

Idea

- We want to see binding between any biomolecules

- Proteins
- Small molecules
- Oligonucleotides
- Carbohydrates
- Lipids & membrane-associated molecules
- Viruses & cells



Idea

- Specificity: how selective?
- Concentration: how much active sample?
- Kinetics: speed of the interaction.
- Affinity: how strong?
- Thermodynamics: what drives the interaction?

label-free system with real-time detection

Advantages of label-free detection

Label free

Study binding of unmodified substances

Reduce time and workload

Contact free

Measure opaque or coloured samples

No loss of sensitivity or accuracy

Real time

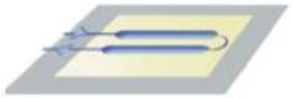
Fast results

On- and off-rates

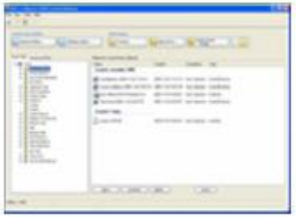
Study weak and fast interactions

Biacore X100

In-line referencing with dual flow cell design

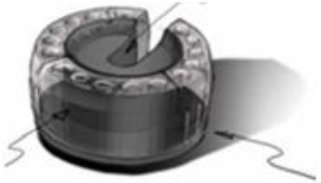


Software with built-in guidance



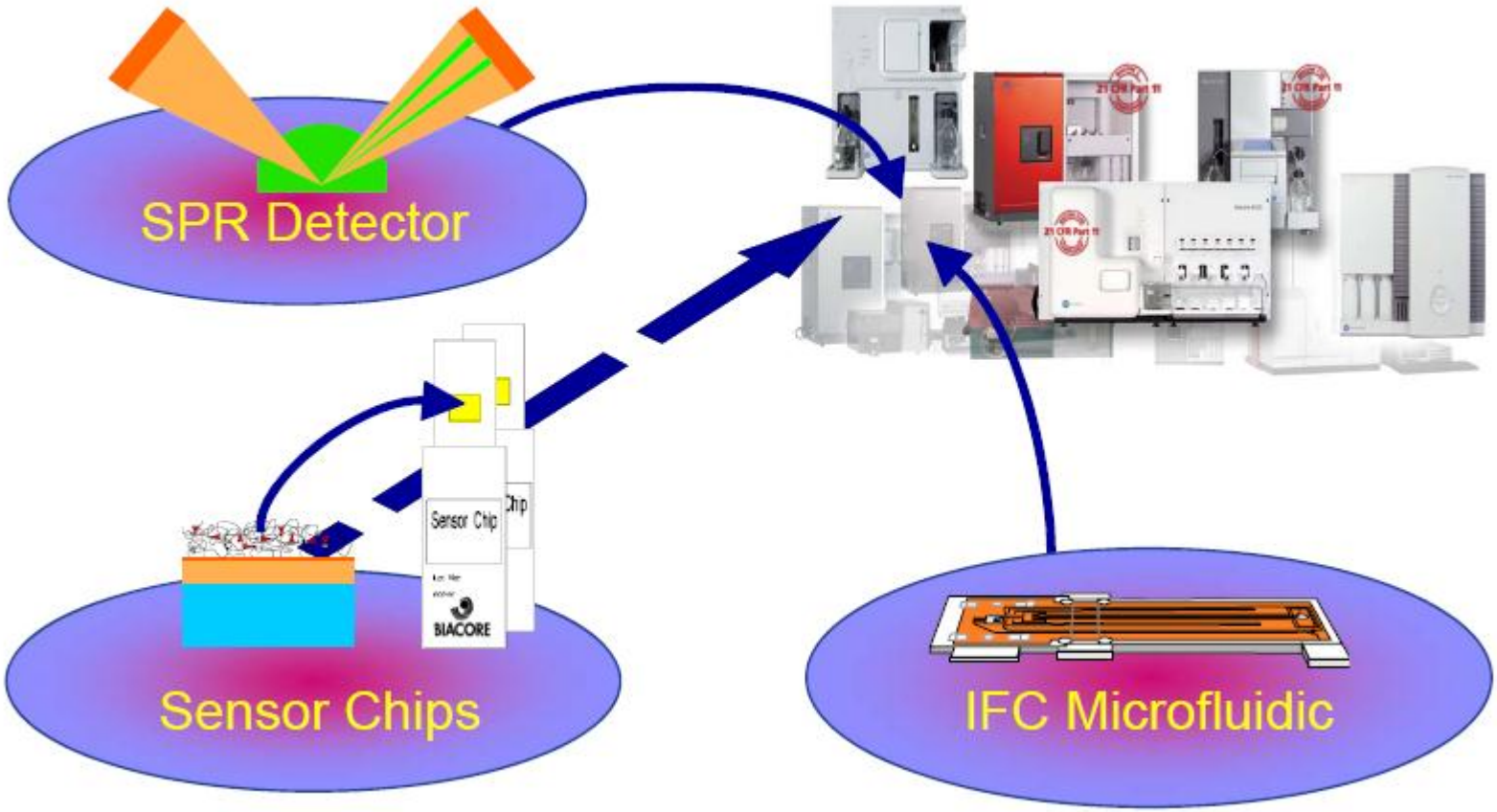
Integrated methodology and evaluation support

Automation for 15 samples (vials)

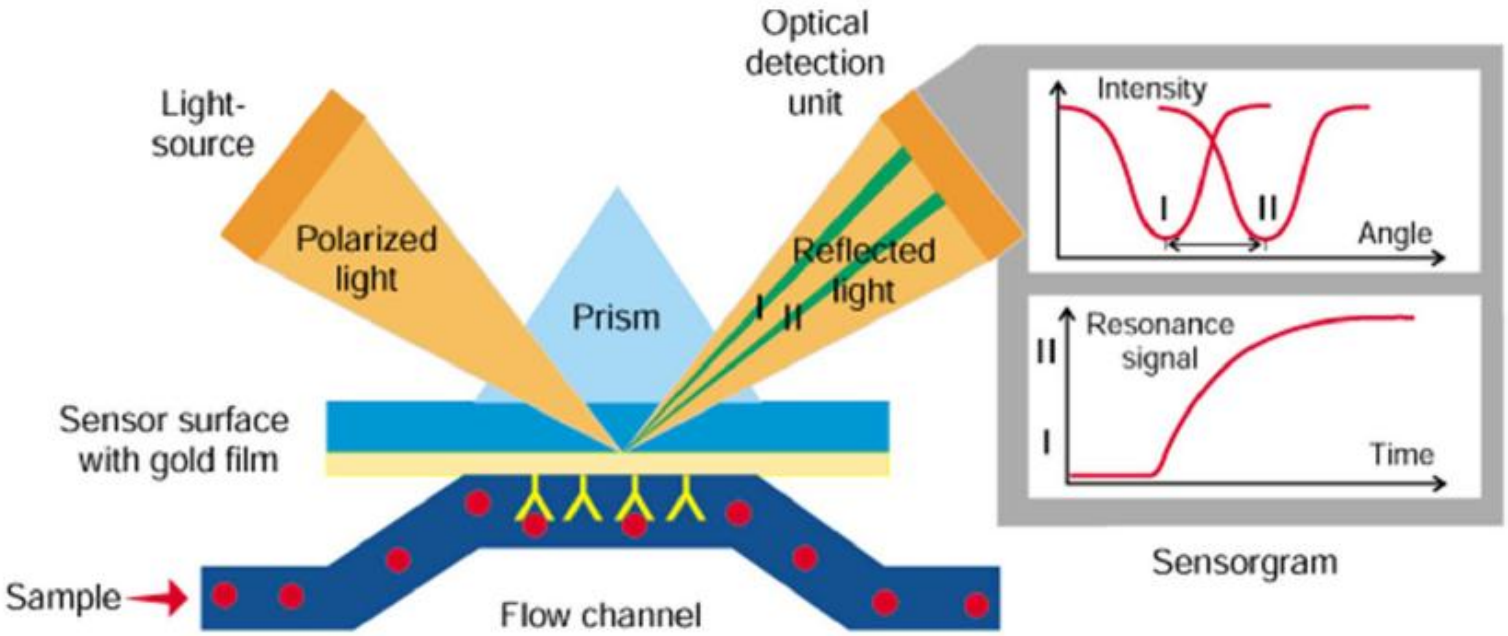


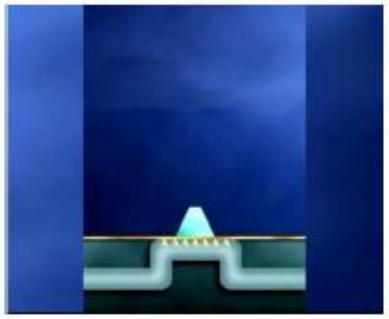
Capture kits & surfaces for easier assay development

Components


SPR Detector
Sensor Chip
BIACORE
Sensor Chips
IFC Microfluidic

SPR detection

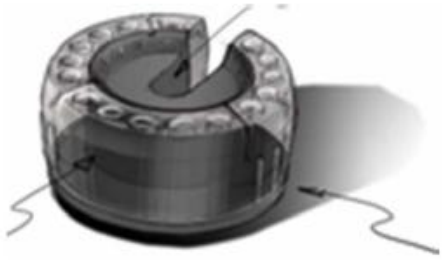


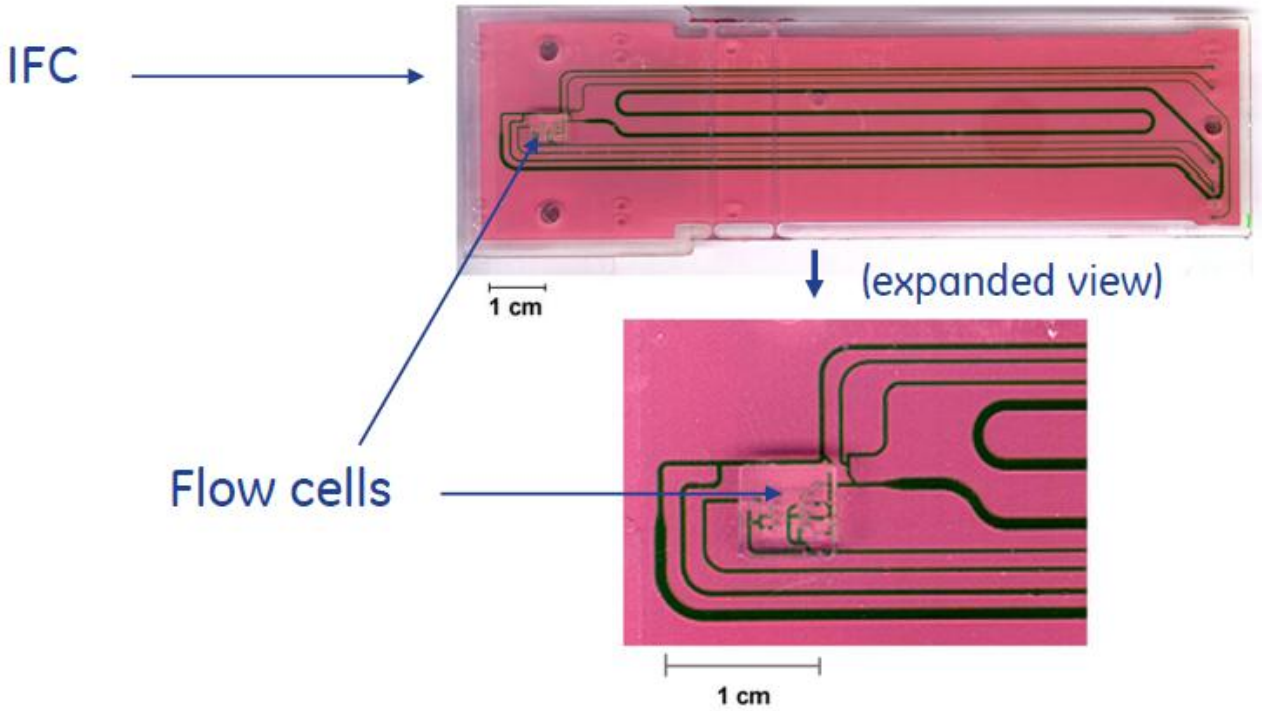


- Refractive index sensor
- Change in the resonance angle
- 1 RU ~ 1 pg/mm²
- (in reality slightly more complex)

Microfluidics

- Inject 2 to 90 µl at 1 to 100 µl/min
- Concentration maintained constant during injection
- Integrated and automated liquid handling





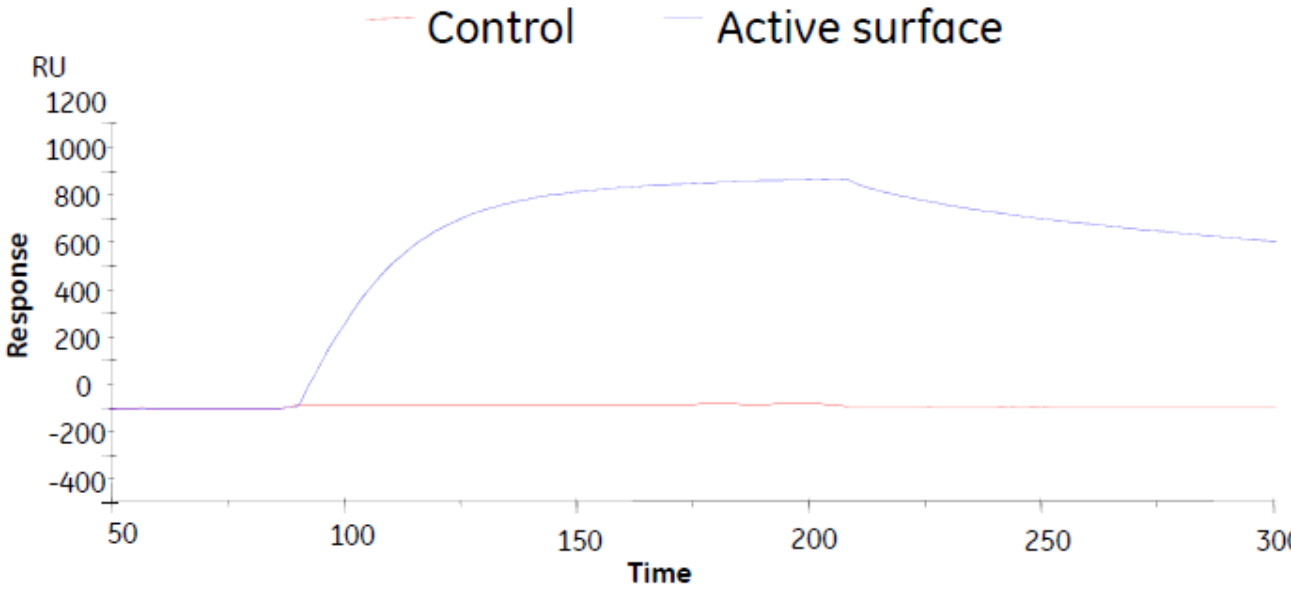
Flow cells

During surface preparation or immobilization

One flow cell at the time

During assay or analysis

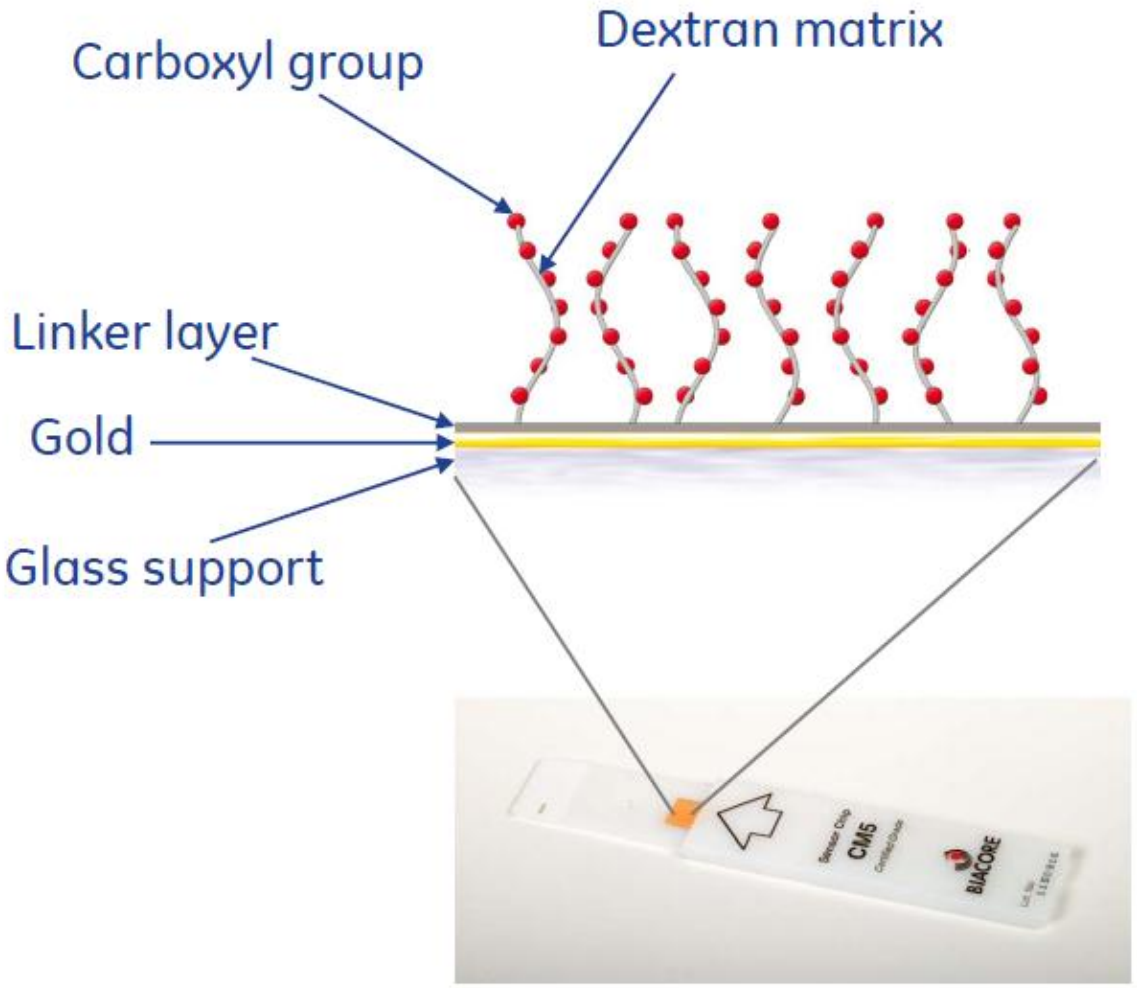
Serial flow, same analyte two sensorgrams



Sensorchips

- 50 nm uniform gold layer
- Well defined reflectance minimum
- Suitable for covalent attachment
- Inert in physiological buffer conditions

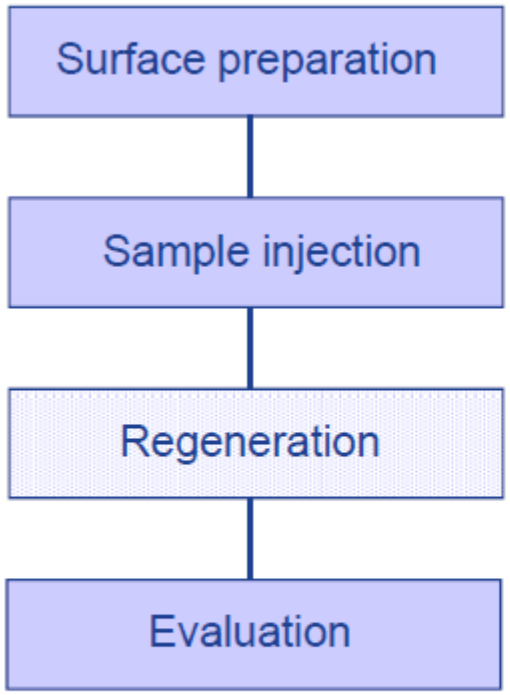
CM5 sensorchip

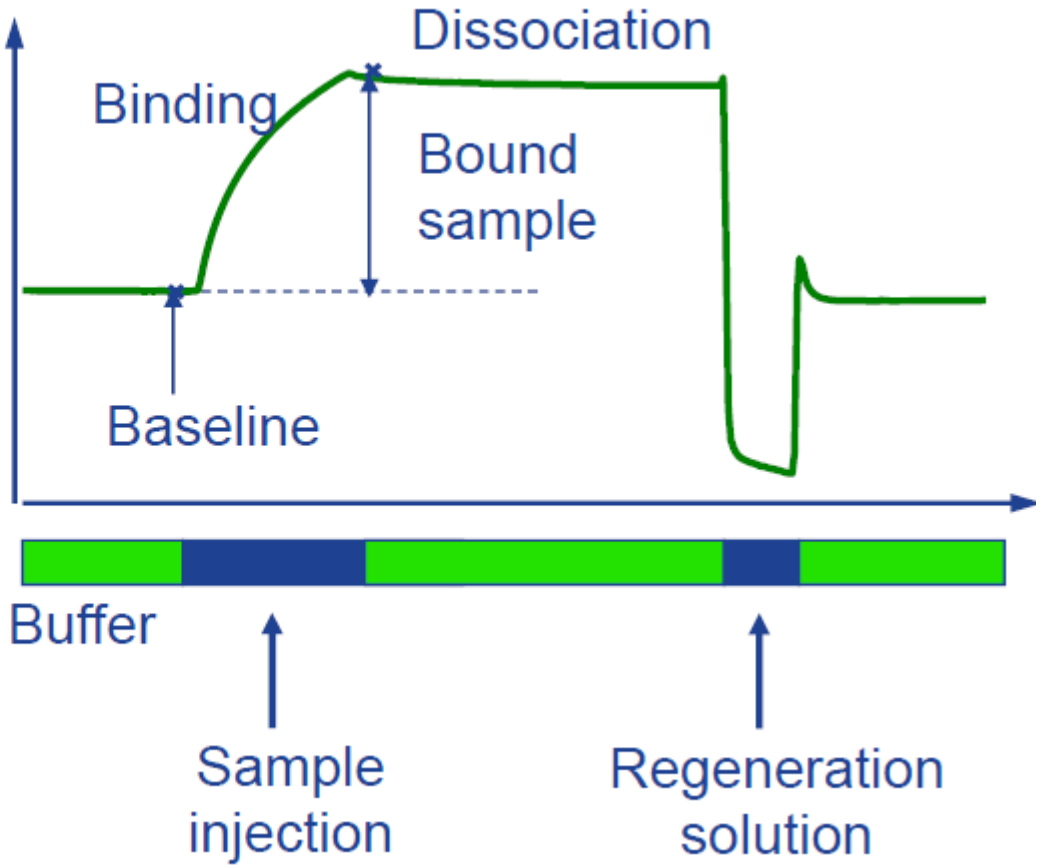


CM5 sensorchip

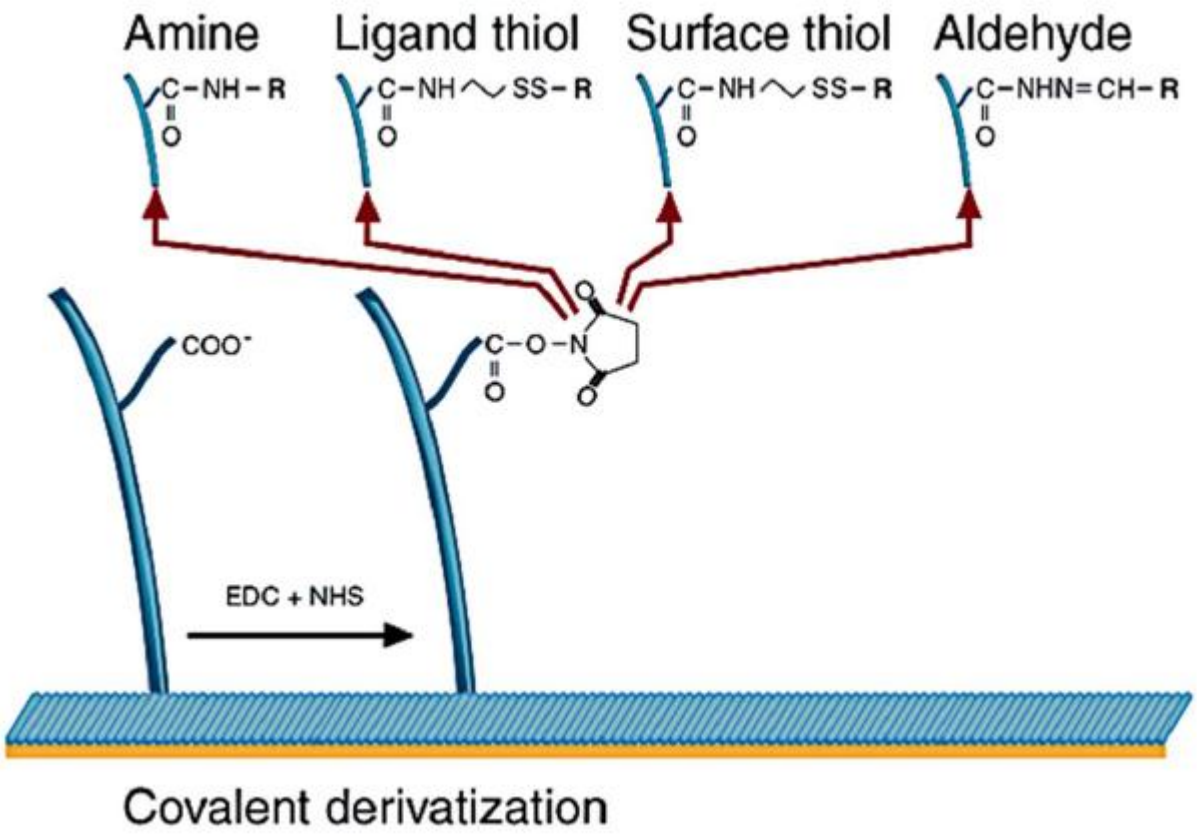
- Advantages of the dextran matrix:
  - Hydrofilic and flexible
  - Low non-specific binding
  - Matrix increases surface and allows high immobilization
  - Easy to activate and use for covalent coupling
  - Withstands extensive regenerations

Experiment

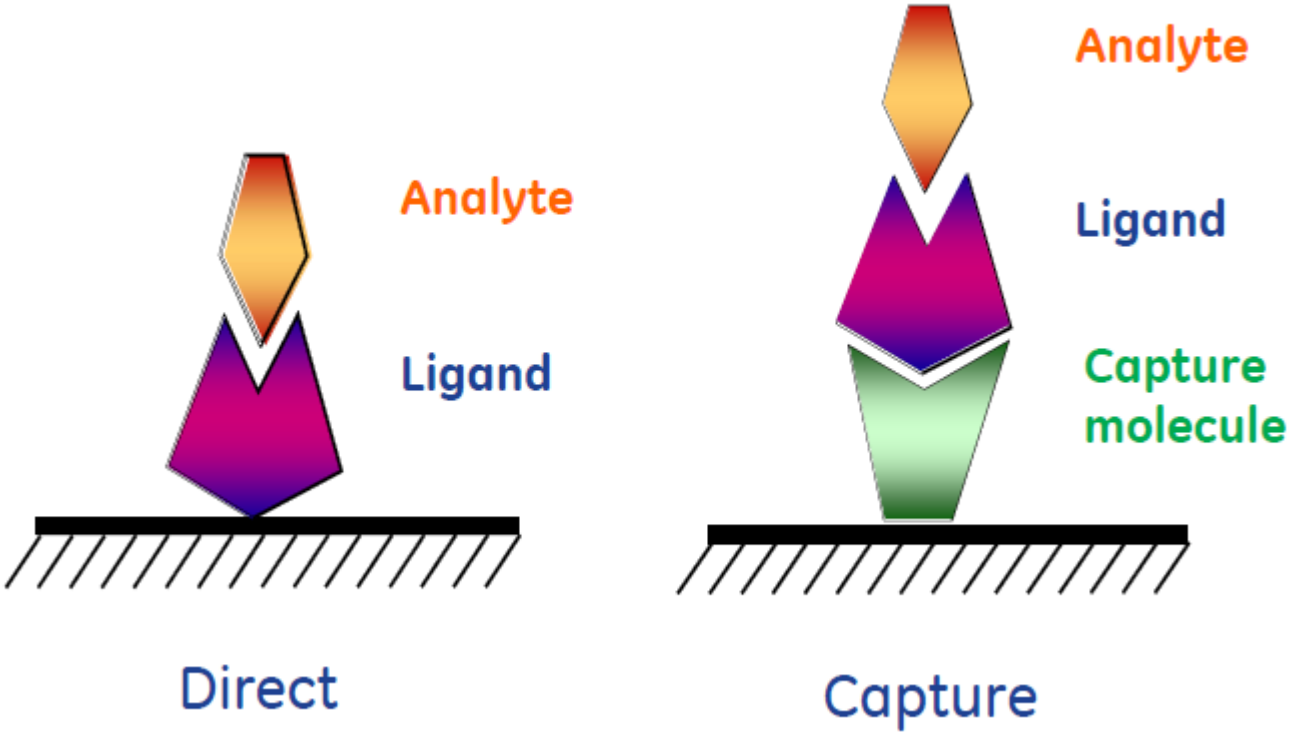

Surface preparation
Sample injection
Regeneration
Evaluation


Dissociation
Binding
Bound sample
Baseline
Buffer
Sample injection
Regeneration solution

Surface preparation

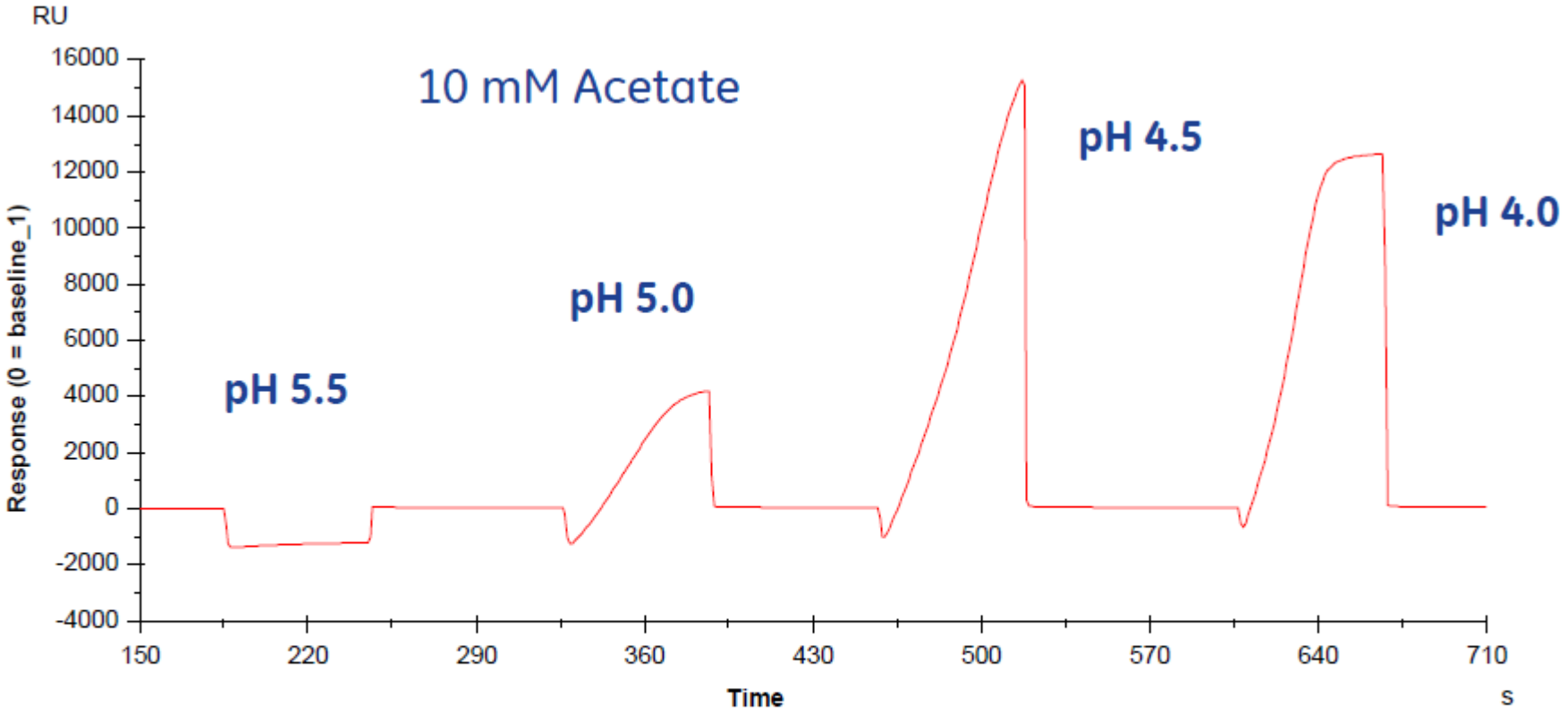


Direct or Capture immobilization



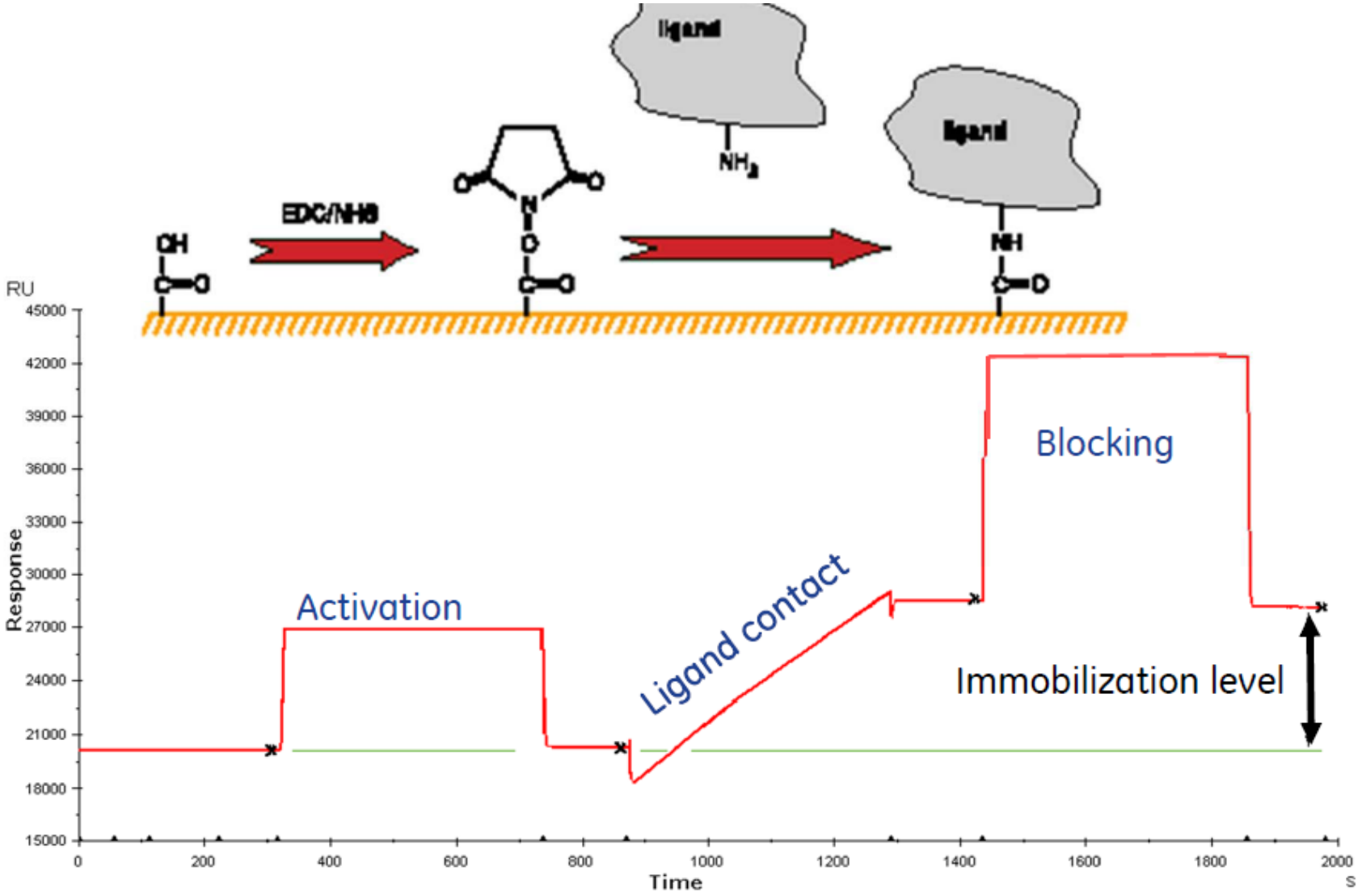
Find optimal coupling conditions

pH scouting/ concentration scouting



- Ligand concentration between 10 and 100 µg/ml.
- Low ionic strength buffer with pH >3.5 and pI of ligand.

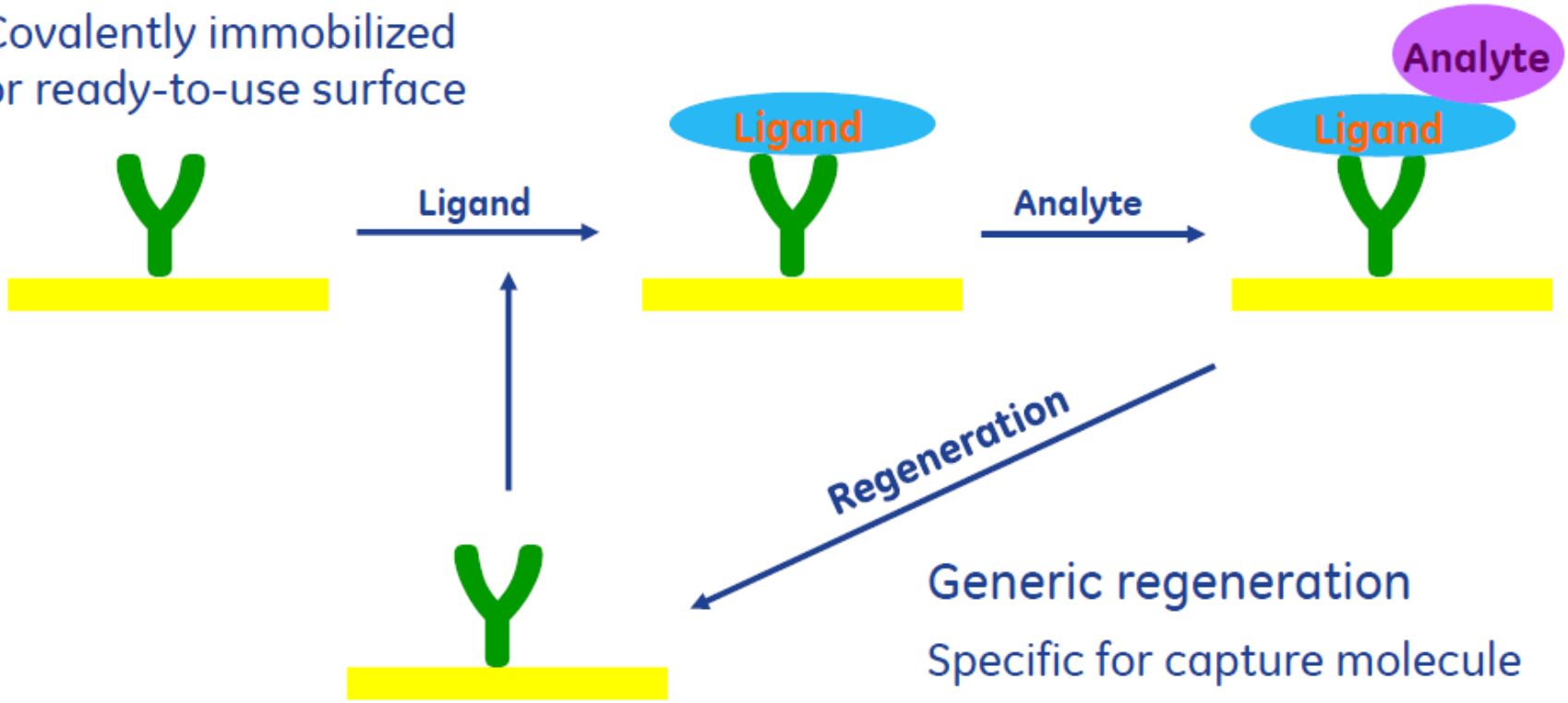
Amine coupling



Capture analyses cycle

Capture molecule

Covalently immobilized
or ready-to-use surface



Generic regeneration

Specific for capture molecule

Independent on ligand-analyte interaction

Pro's and con's in capturing

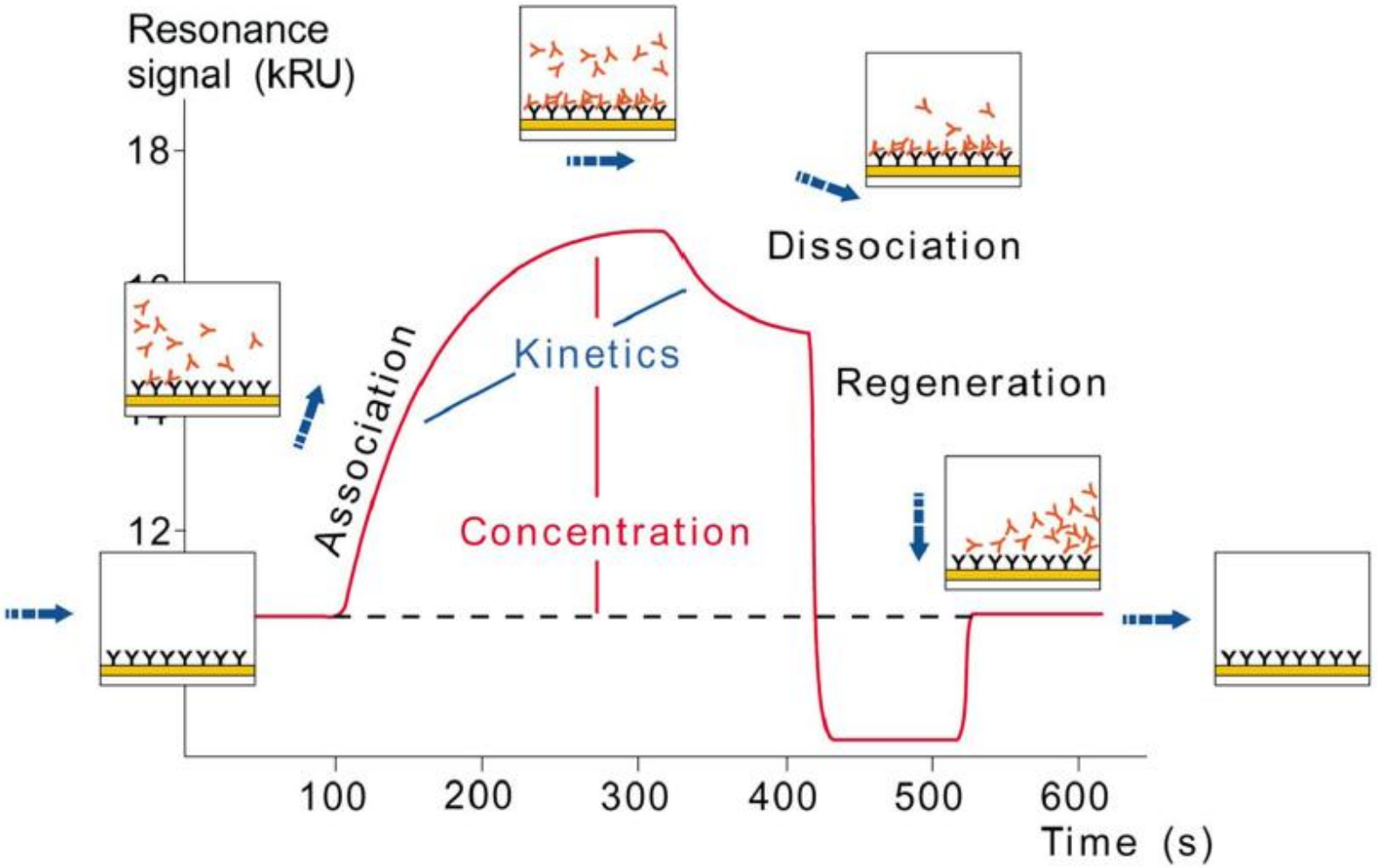
Pro's

- Less assay development time
- Ligand is oriented
- Kept in physiological conditions
- Out of complex solution
- Generic regeneration
- Unstable ligands can be investigated
- Same surface – many ligands

Con's

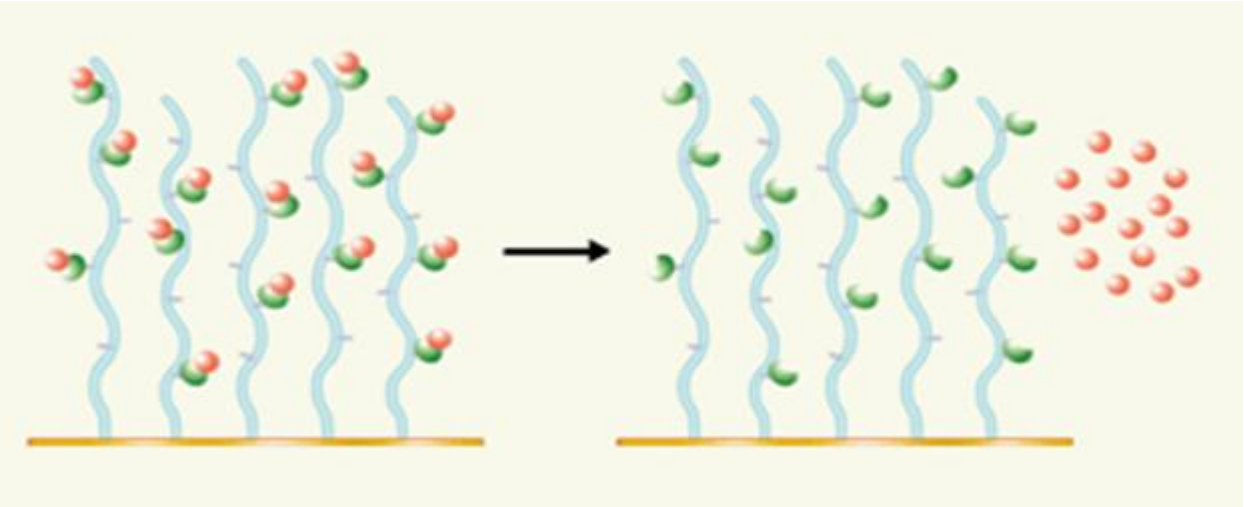
- Increased ligand consumption
- Extra time per sensorgram
- Dissociation between capture molecule and ligand
- Lower surface capacity

Binding kinetics result: the sensorgram



Regeneration

- Removes bound analyte completely from the surface
- The activity of the surface must remain unaffected
- Efficient regeneration is crucial for high-quality data

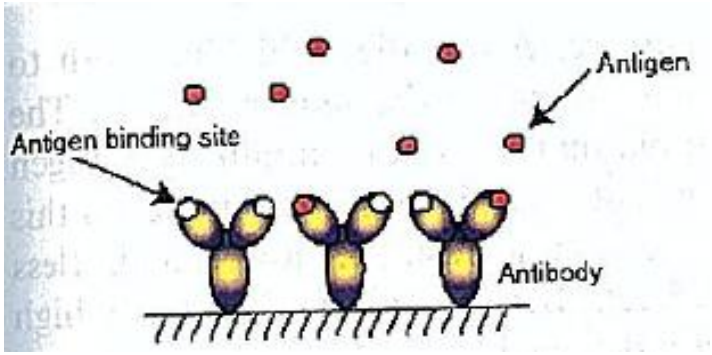


Assay formats

- Direct
- Sandwich
- Competitive
- Inibition

Direct assay

Suitable for high molecular weight molecules



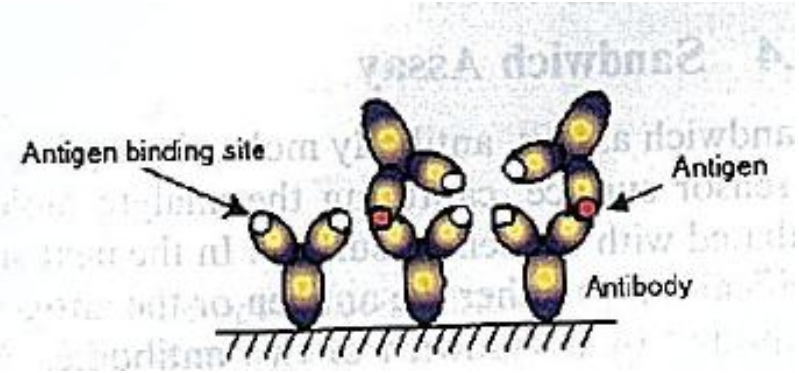
- Sensor preparation:
  Antibodies directed against the antigen are immobilized on the sensor surface.
- Detection:

  Sample solution containing the antigen is then incubated with the sensitized sensors surface.
- Signal-measurand relationship:

  The signal increase correlates with the amount of antigen in the sample.

Sandwich Assay

To be selected fro relatively high molecular weight antigens and when high affinity antibodies are available.



- Sensor preparation: Antibodies are immobilized on the surface
- Detection:

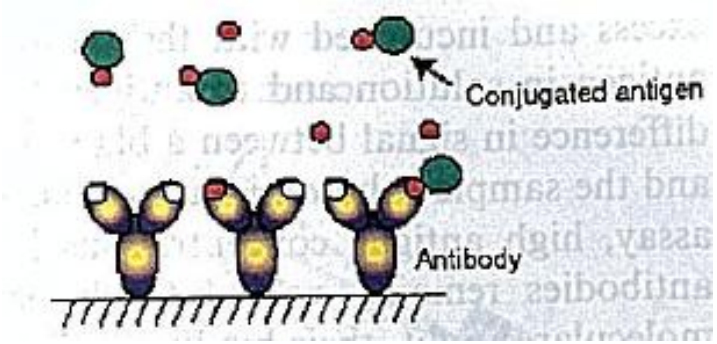
Sample solution containing the antigen is then incubated with the sensitized sensors surface. In a second step, a secondary antibody binds specifically with the antigen.

Signal-measurand relationship:

The increase in signal is proportional to the amount of antigen in the sample. The high molecular weight of the secondary antibody is usually sufficient to monitor the binding process. Conjugated antibodies can be used.

Competition Assay

Designed for low molecular weight antigens that do not generate sufficient signal when they accumulate on the surface (Direct assay) and are too small for a sandwich assay.



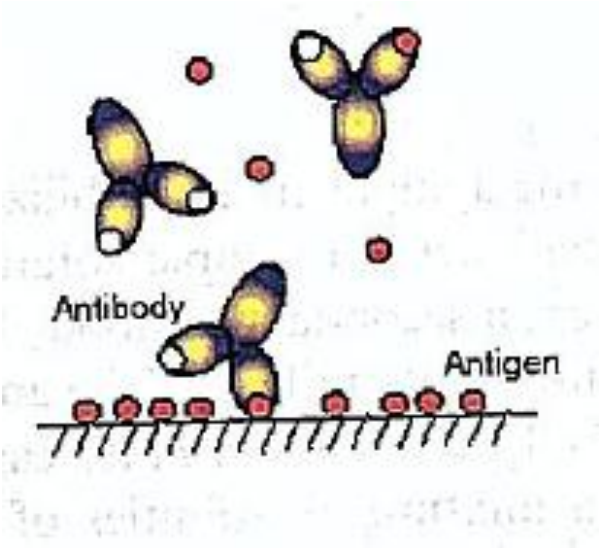
- Sensor preparation: Antibodies are immobilized on the surface
- Detection:

  Sample solution that contains the antigen is mixed with an antigen conjugate
- Signal-measurand relationship:

  The difference in signal between a reference sample containing only conjugated antigen and the sample solution indicates the amount of antigen in the sample. High antigen concentration in the sample will result in low signals (less conjugated antigen can be bound).

Inhibition Assay

Designed for low molecular weight antigens that do not generate sufficient signal when they accumulate on the surface (Direct assay) and are too small for a sandwich assay.



- Sensor preparation: The target antigen is immobilized on the sensor surface.
- Detection:

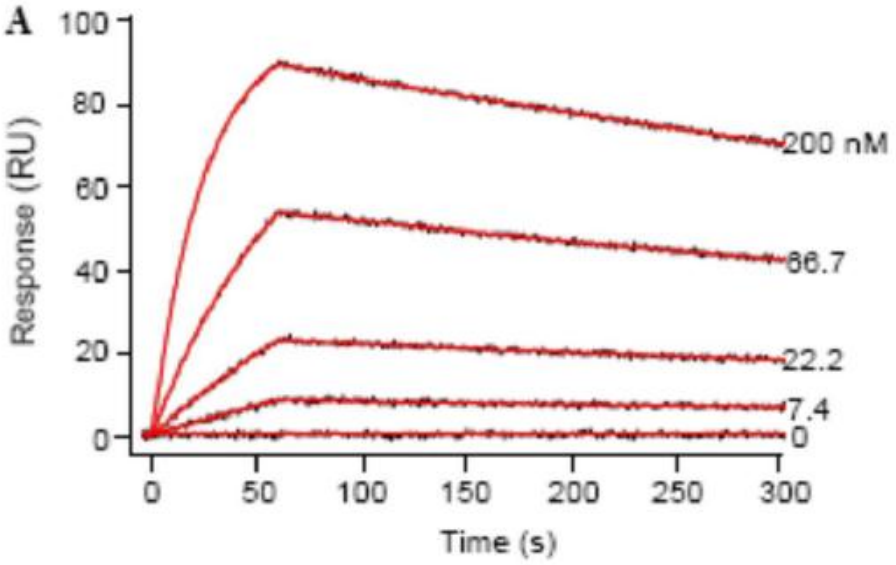
Sample solution that contains the antigen is mixed with specific antibodies in excess. Antibodies bind both to the antigen in solution and to the antigen bound previously on the sensor surface.

- Signal-measurand relationship:

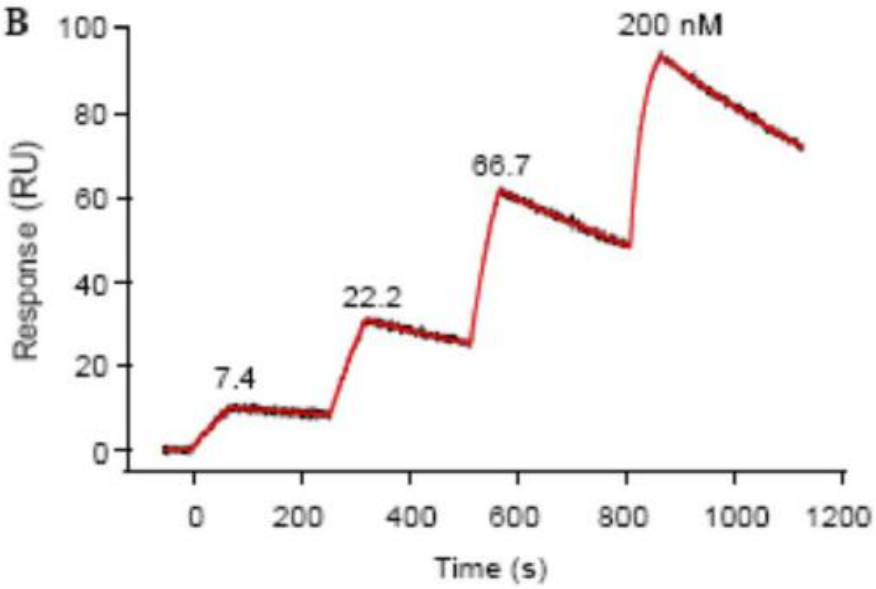
The difference in signal between a blank sample that does not contain the antigen and the sample solution indicates the amount of antigen in the sample. High antigen concentration result in low signals. Antibodies have high molecules weight and can be directly detected.

Multi-cycle and Single-cycle kinetics

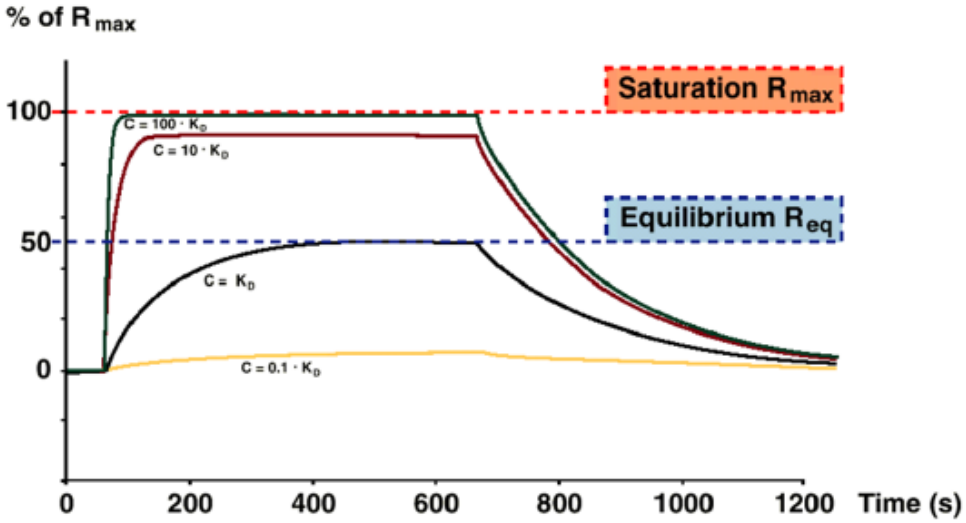
analyte injection followed by regeneration
=
amount of free ligand identical

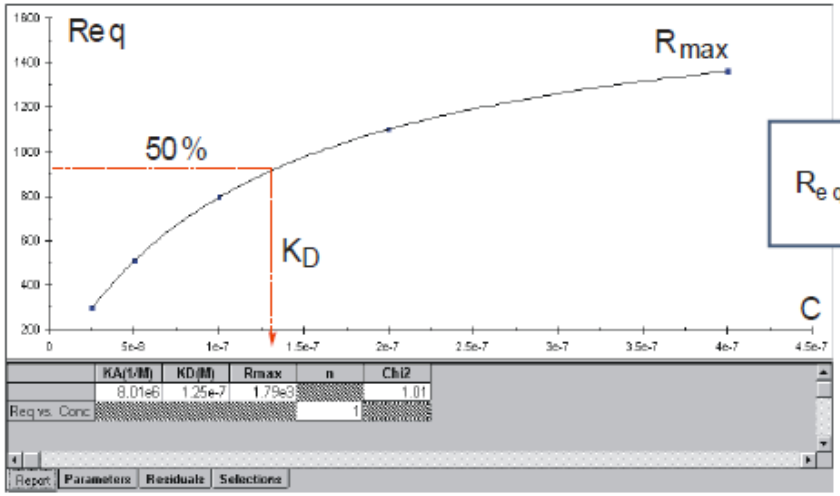


sequential analyte injection without regeneration
=
amount of free ligand decrease



Affinity constants





$$R_{eq} = \frac{K_A \cdot C \cdot R_{max}}{(K_A \cdot C \cdot n + 1)}$$

Fit to model $A + B \rightleftharpoons AB$ at steady state

Biacore X100

In-line referencing with dual flow cell design

Autosampler Needle Ports

Ventilation openings

Running buffer, Bottle and cap Tray

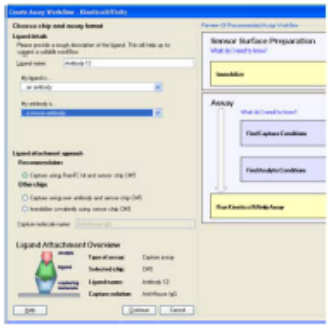
Syringe pump (runs)
Behind door: peristaltic pump (sample handling) and degasser

Waste Bottle with cap Tray

Set-up to results

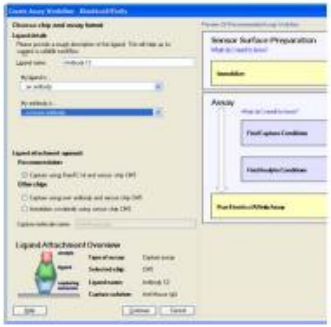
Start up your Biacore X100

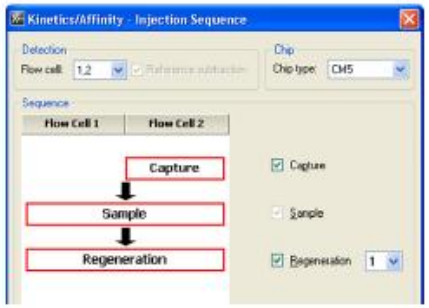
Set-up to results



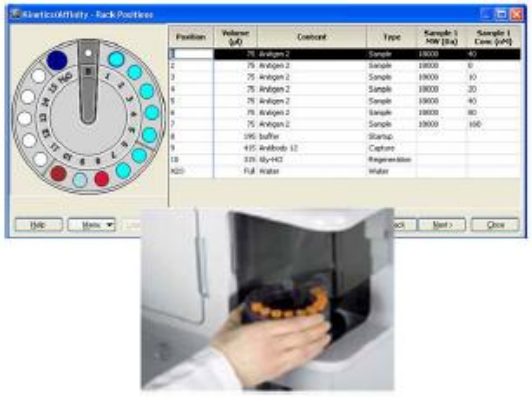
Software guides your assay design

Set-up to results

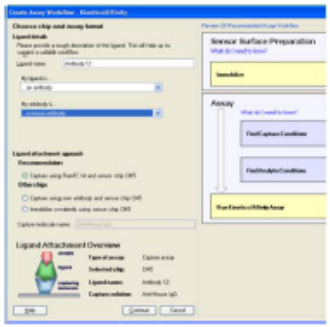


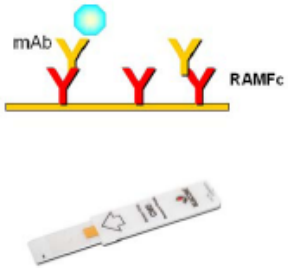


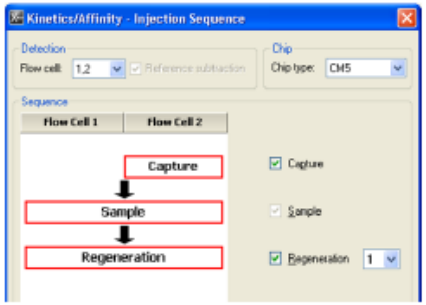
Prepare sensor surface and load samples



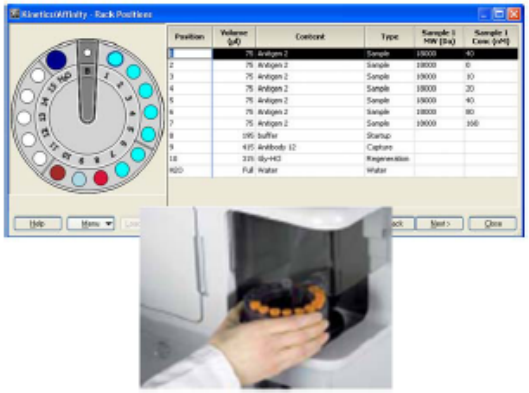
Set-up to results

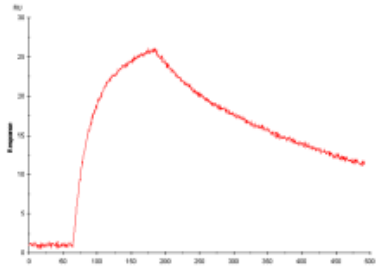




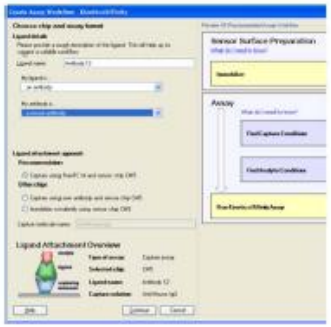


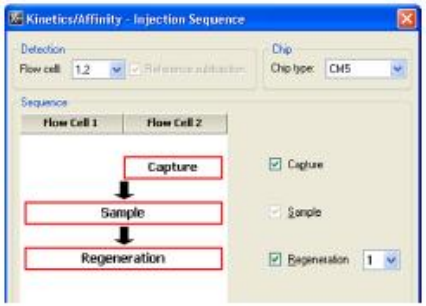
Run assay – see interactions as they happen



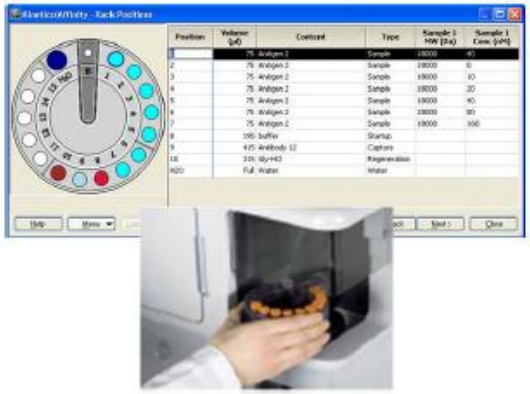


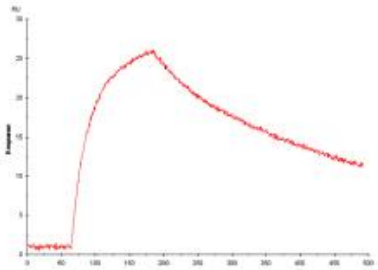
Set-up to results

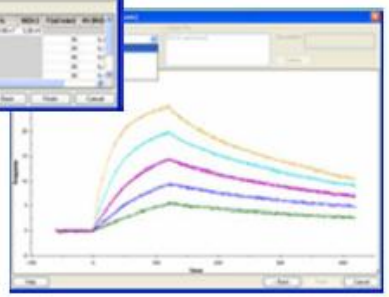




Evaluate data & see the results







it work