Duke Human Vaccine Institute
Biomolecular Interaction Analysis Core Facility

Biomolecular Interaction Analysis (BIA) Core Facility

T200 Training

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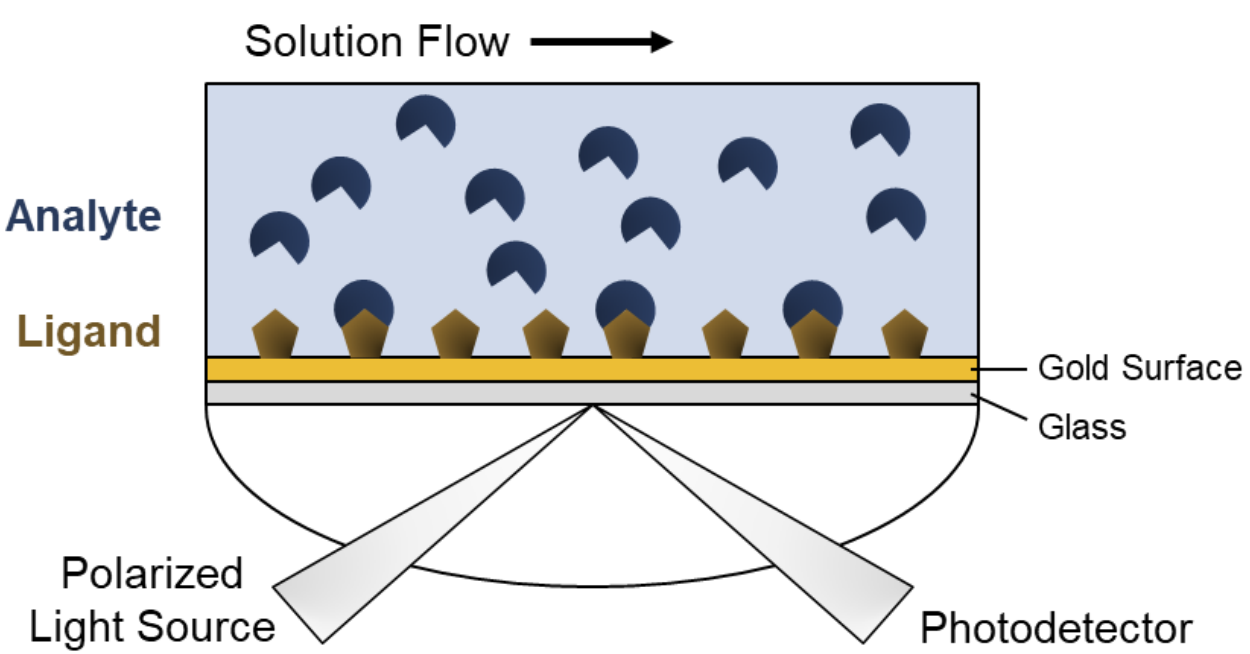
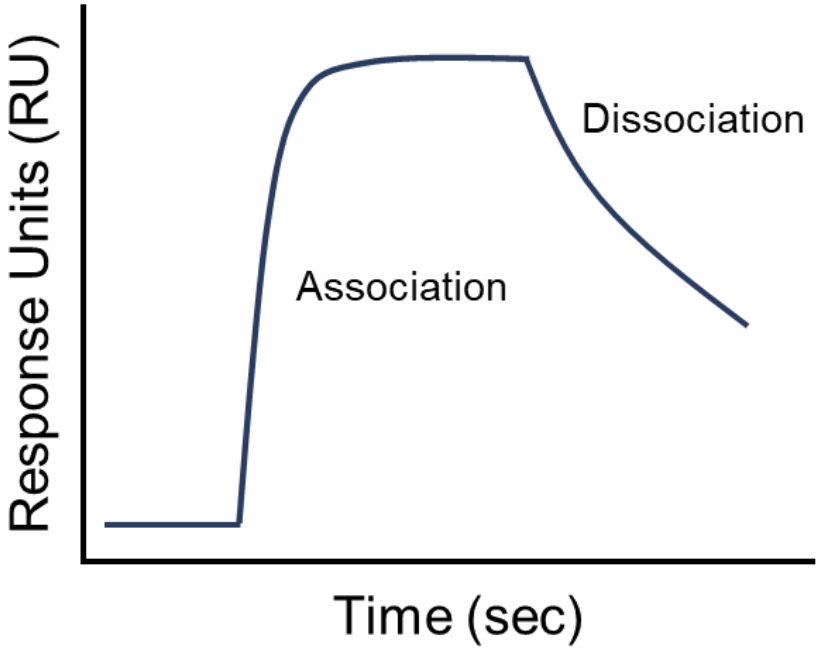
Facility Manager: Kenneth Cronin

Surface Plasmon Resonance





- SPR measures changes in refractive index at the surface
- In practice, measures changes in **mass** at the surface

Label-Free, Real-Time Detection

Relation of RU to measured quantity

If interested:

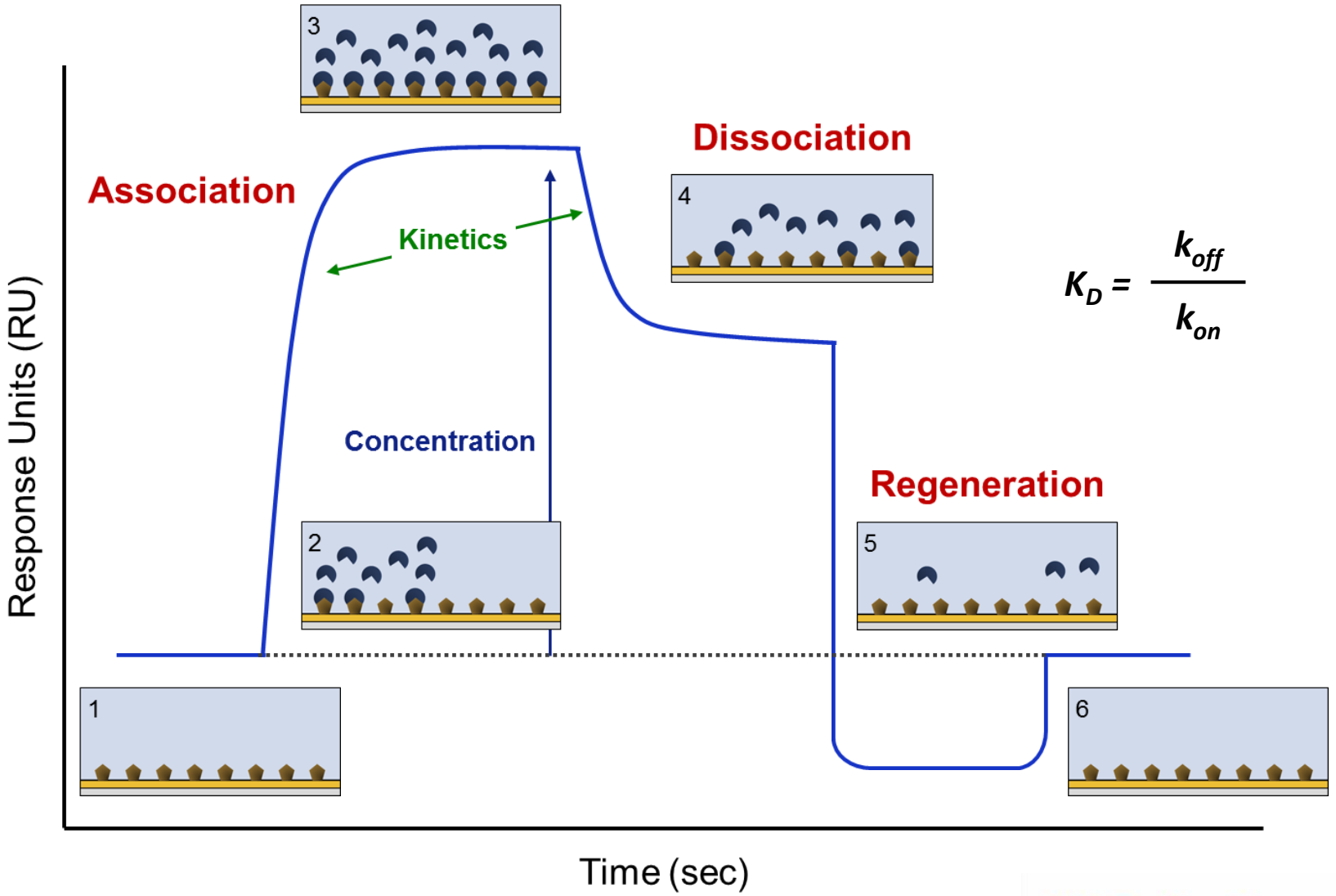
When the angle of incidence (θ) of the light is greater than the critical angle (θ_c), total internal reflection occurs. When the glass is coated with a thin film of gold, the light is not totally reflected but some is lost to the gold due the conductive nature of the metal. There is a second angle, greater than the critical angle, at which the loss in the intensity of the reflected light is greatest, this is the SPR angle (θ_{SPR}).

The light lost to the metal excites the electrons in the conduction band which causes oscillation of these mobile electrons (plasma) creating a surface plasmon wave. When the wave vector of this incident light matches the wavelength of the surface plasmons, surface plasmon resonance occurs.

An evanescent electrical field associated with the plasmon wave travels for a short distance into the medium (water) from the gold surface (~150-300 nm). The resonance frequency of the surface plasmon wave depends on the refractive index of the medium. When the refractive index changes, the SPR angle shifts.

SPR instruments measure small shifts in the θ_{SPR} and report them as Response Units (RUs) where 1 RU is equivalent to a 10^{-4} degree shift. This shift is directly related to the surface concentration which is generally referenced as 1 RU is equivalent to 1 pg/mm^2.

Surface Plasmon Resonance



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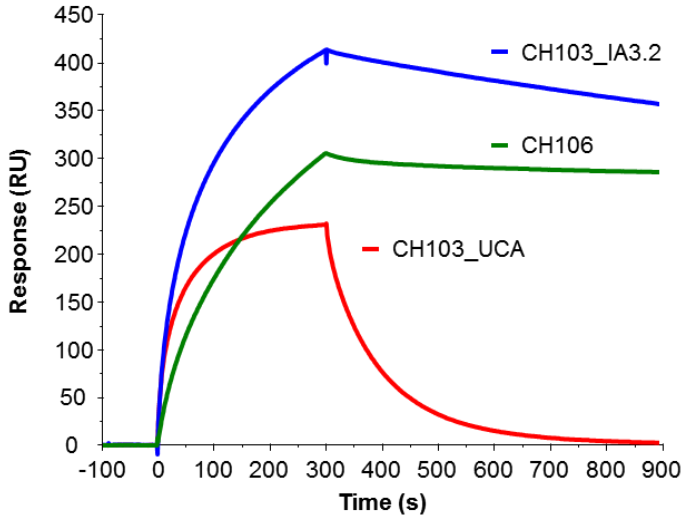
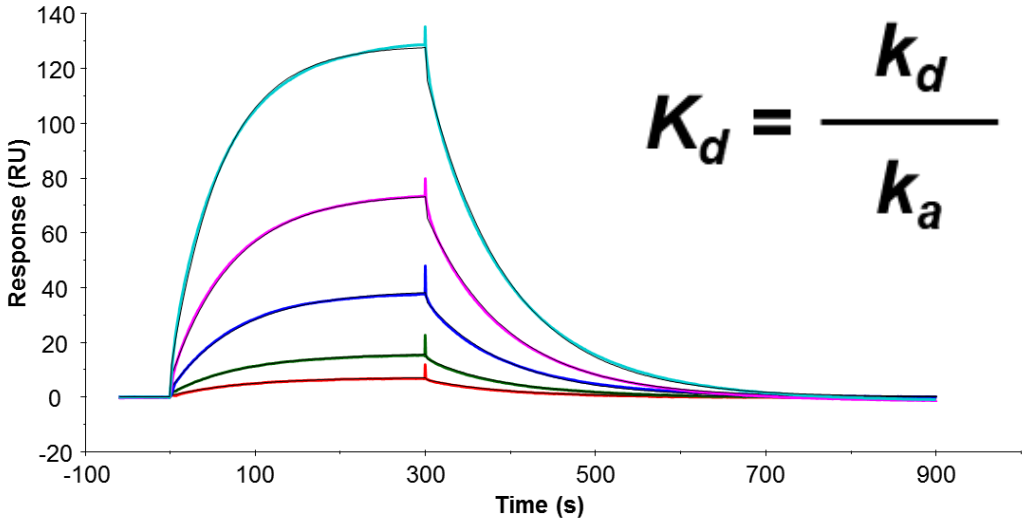
SPR Applications

- SPR is a technology that can be used to design multiple types of assays
- Specificity and Screening
  - Yes/No Binding
  - Ranking of binding response
- Kinetics and Affinity
  - Direct binding
  - Steady-State Affinity
  - Solution-based Affinity
- Concentration
  - Surface Competition
  - Solution Inhibition
- Epitope Mapping
- Conformational Changes
- Thermodynamics

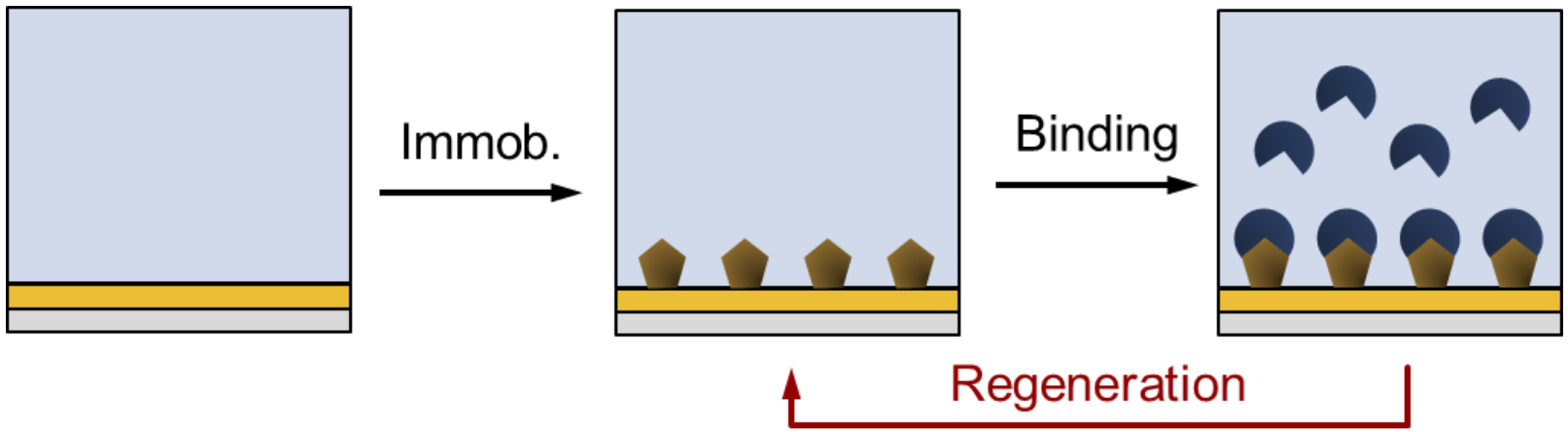




$$K_d = \frac{k_d}{k_a}$$

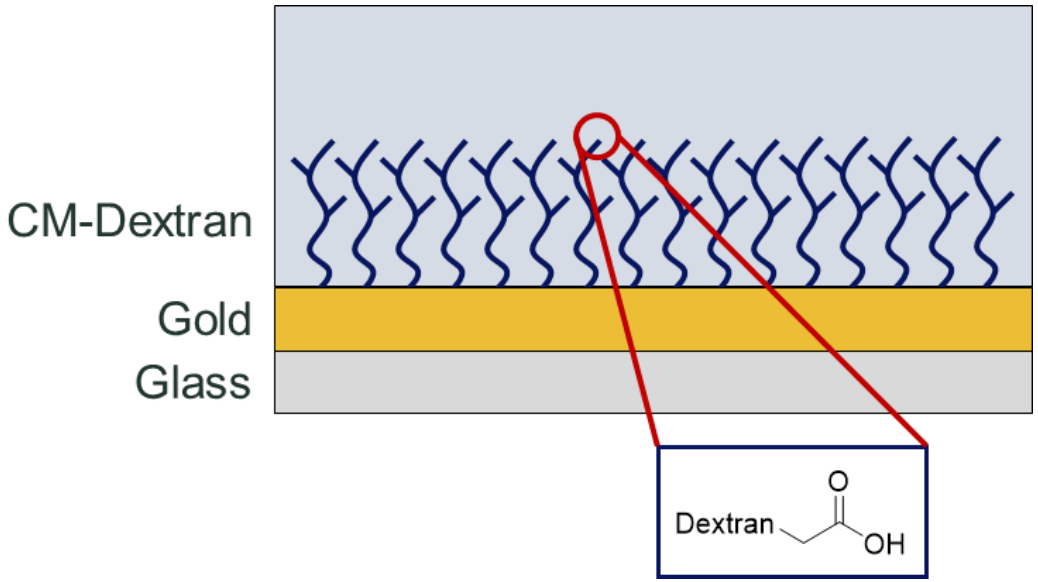
Experimental Considerations

1. **Immobilization**
   - How to functionalize one binding partner to the sensor chip
2. **Binding**
   - How to design the injection parameters to create a specific assay
3. **Regeneration**
   - How to remove the bound analyte without damaging the ligand

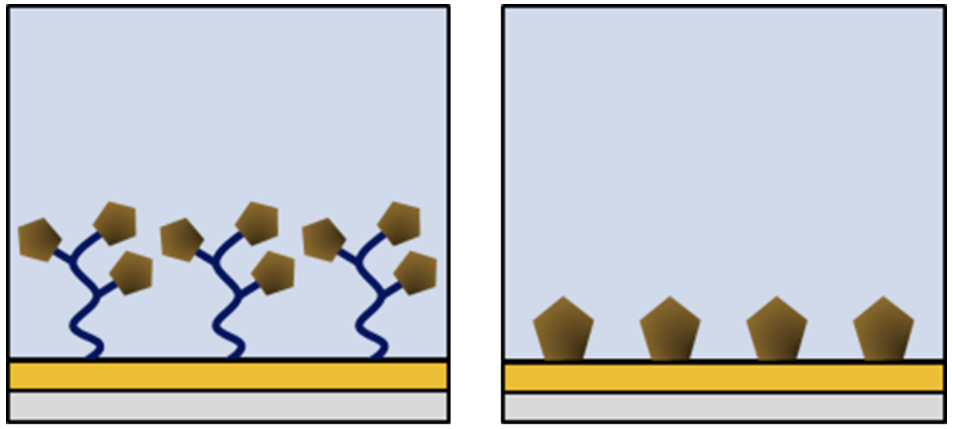


SPR Sensor Chips

- Carboxymethylated Dextran
  - Biocompatible and Robust
  - Low non-specific binding
  - Available in varying dextran lengths and CM densities (CM3, CM4, CM5, CM7)
- C1 – Carboxylated gold (no dextran)

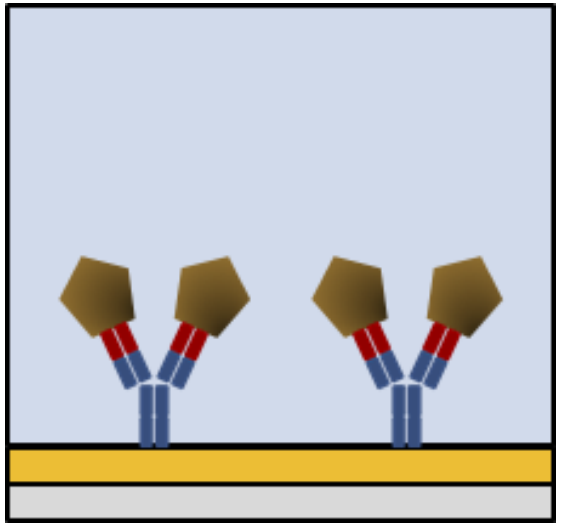


- Direct Immobilization
  - Reactive carboxyl handle
    - Amine Coupling – EDC/NHS
    - Thiol Coupling – Disulfide
    - Thiol Coupling – Maleimide
    - Aldehyde Coupling

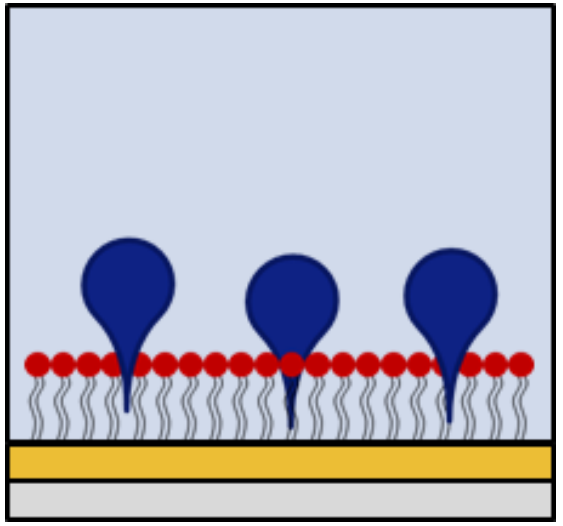


SPR Sensor Chips

- High-Affinity Ligand Capture
  - Modify CM dextran w/ capture molecule
    - Streptavidin-Biotin (SA chip)
    - α-Fc or Protein A (Prot A chip)
    - α-His or NTA (NTA chip)
    - α-GST
    - α-FLAG
    - Applicable to any high affinity tag

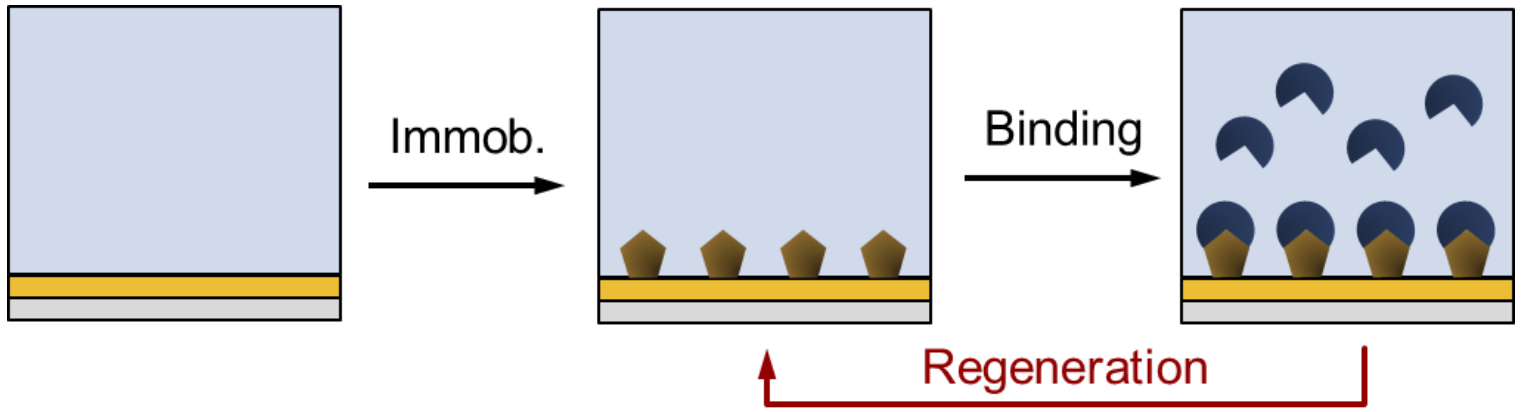


- Hydrophobic Adsorption
  - Sensor Chip HPA – Alkanethiol gold
    - Lipid bilayers or monolayers
  - Sensor Chip L1 - Lipophilic residues on CM5
    - Captured vesicles or micelles

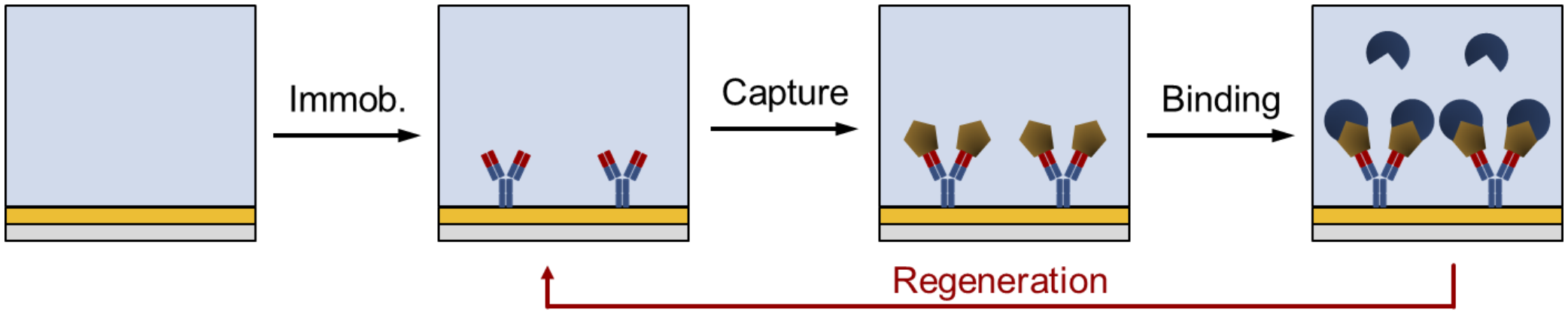


Direct Immobilization v. Capture

Direct Immobilization

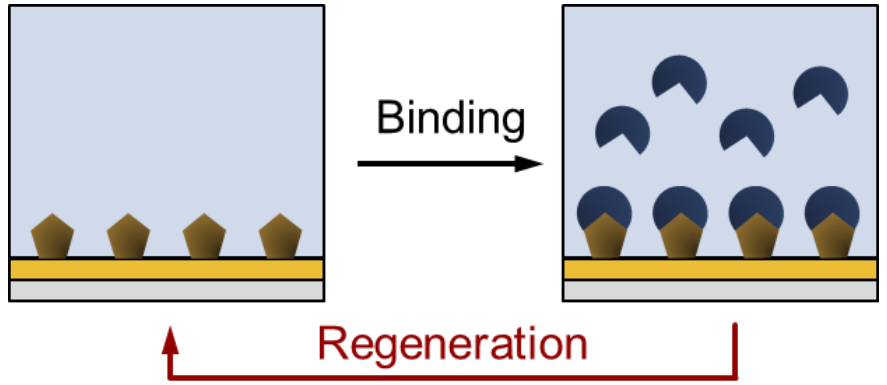


Ligand Capture

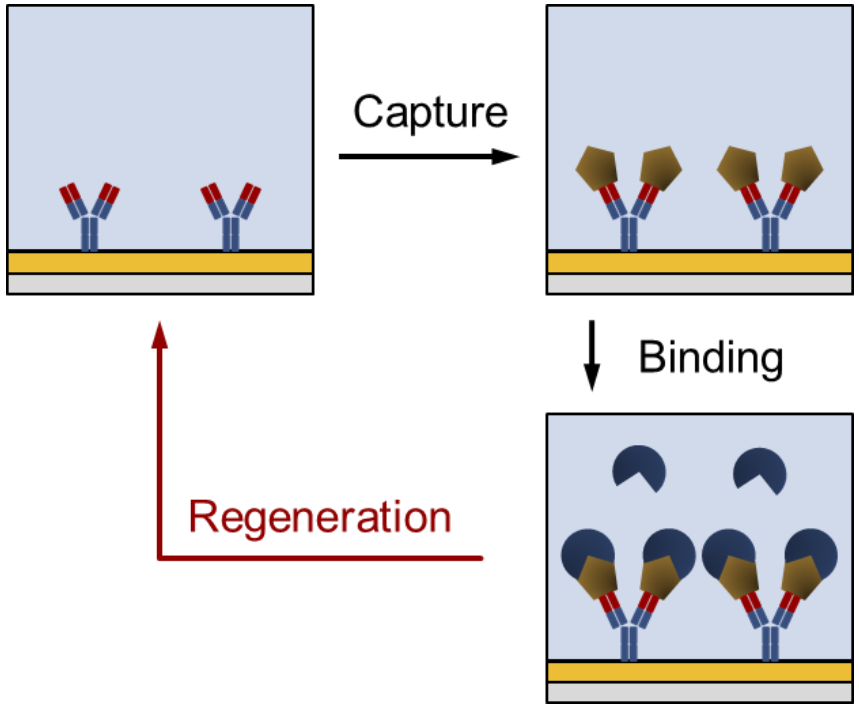


Direct Immobilization v. Capture

- Direct Immobilization
  - Simple and Robust
  - Fixed immobilization level
  - Requires less ligand
  - Requires regeneration scouting

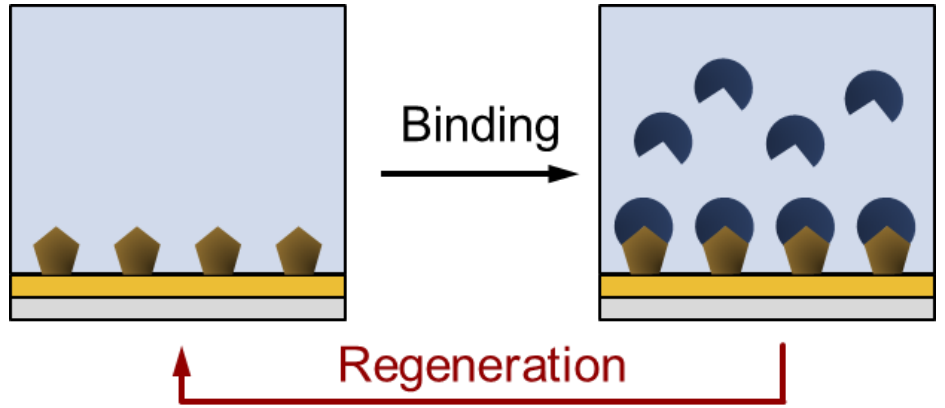


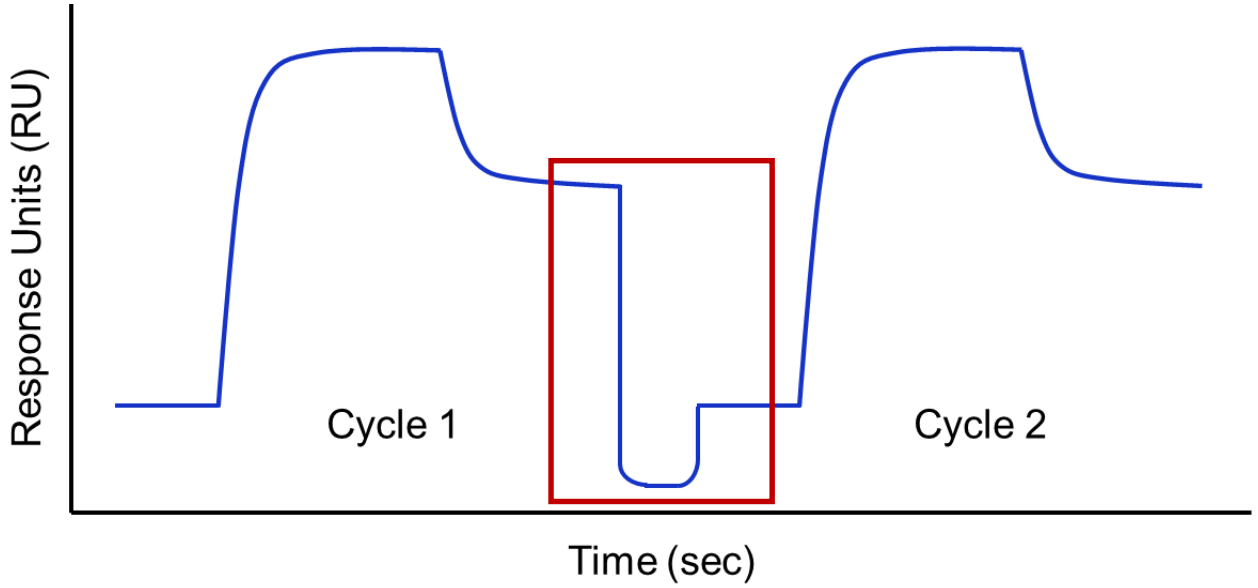
- Ligand Capture
  - More complex design
  - Can fine-tune ligand immob. level
  - Takes advantage of affinity tags
  - Regen. conditions may be known
  - Each cycle requires fresh ligand



Regeneration

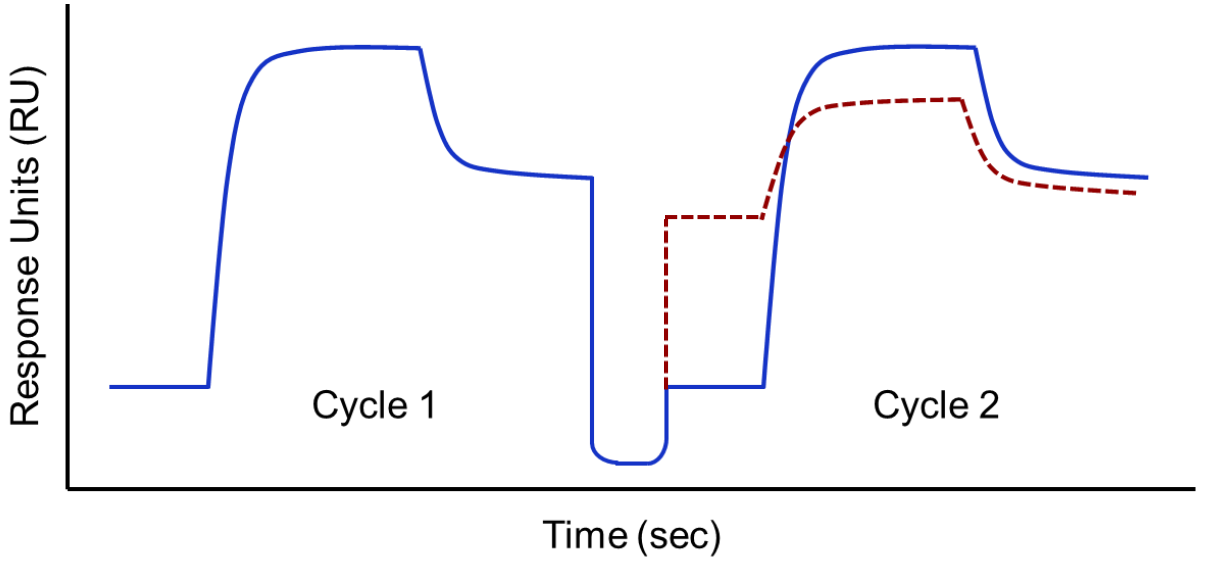
- Injection of a buffer designed to disrupt bound complex
- Often a trial and error process to identify ideal conditions
  - **Acidic**: 10 mM Glycine-HCl (pH 1.5-3.0)
  - **Basic**: 1-100 mM NaOH
  - **Ionic**: 4 M MgCl2, 5 M NaCl
  - **Chaotropic**: 2 M Guanidine-HCl, 1 M Urea
  - **Detergent**: ≤ 0.5% SDS, ≤ 0.5% Tween 20
  - **Chelating**: 20 mM EDTA



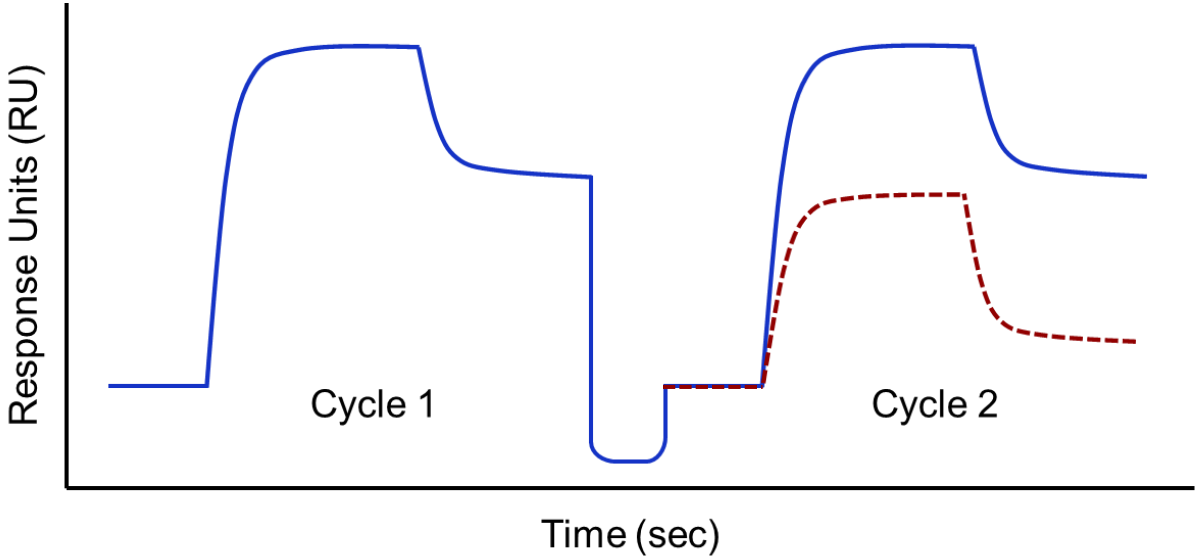


Regeneration

- Regeneration **too weak**
  - Incomplete analyte removal
  - Increasing baseline
  - Reduced binding response



- Regeneration **too strong**
  - Complete analyte removal
  - Loss of ligand activity
  - Reduced binding capacity

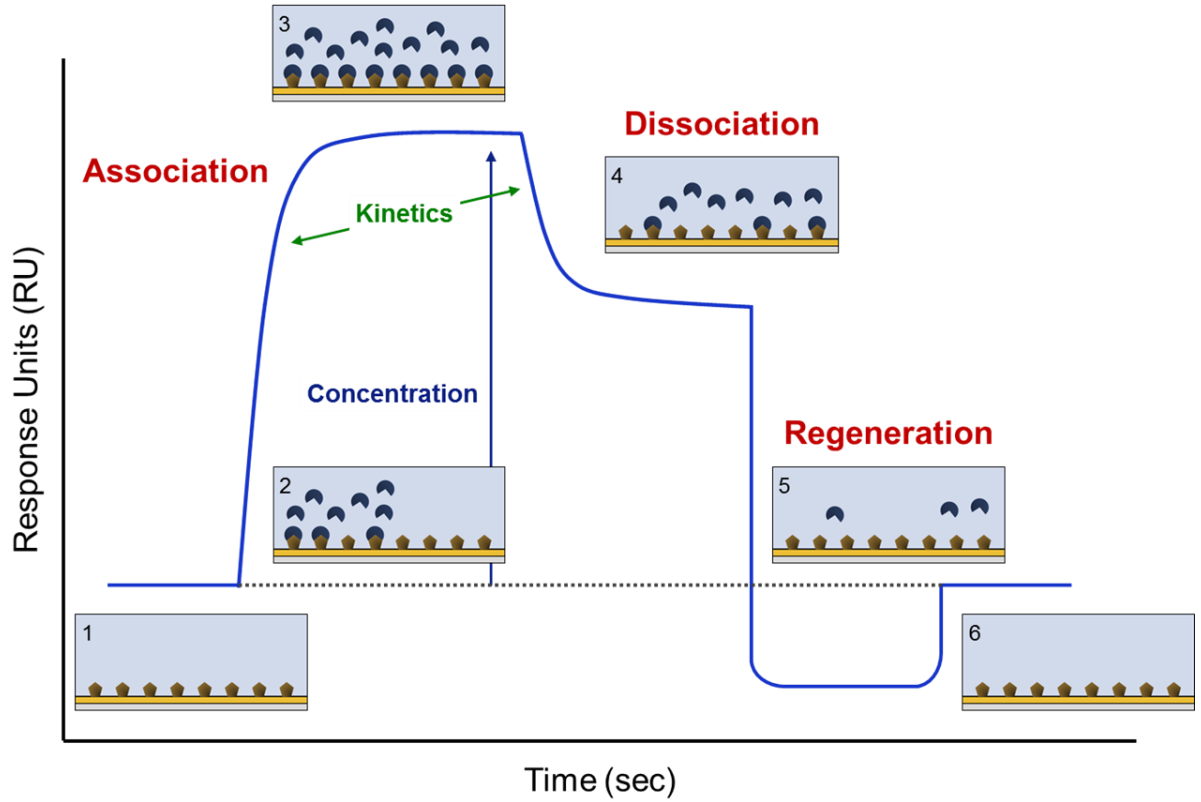


Experimental Considerations

1. **Immobilization**
   - Direct Immobilization vs. High Affinity Capture
   - Immobilization level
     - High Density (Screening or LMW Affinity)
     - Low Density (Kinetics)

2. **Binding**
   - Injection parameters depend on assay design
   - Protein purity and concentration
   - Protein quantity (<100 µg)

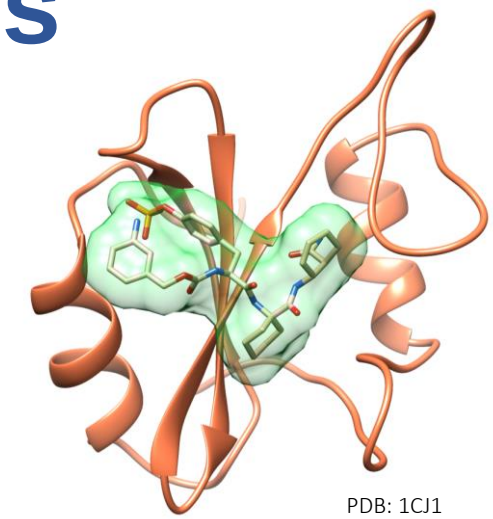
3. **Regeneration**
   - Use mildest conditions possible
   - Complex and surface ligand stability



Biomolecular Systems

Can measure protein interactions with

- Proteins and peptides
- DNA and RNA
- Small molecules
- Lipid membranes, micelles, and vesicles
- Carbohydrates
- Synthetic polymers
- Viruses
- Whole cells



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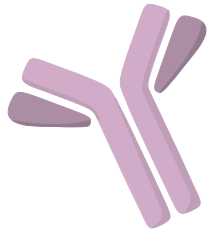
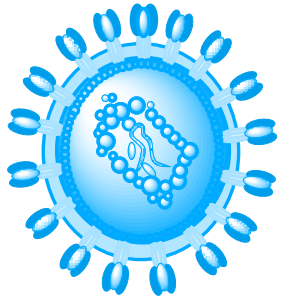
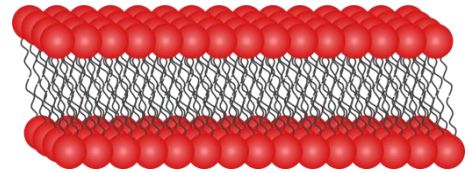




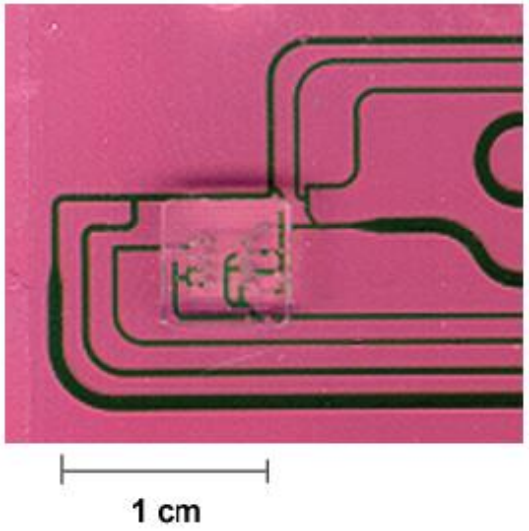


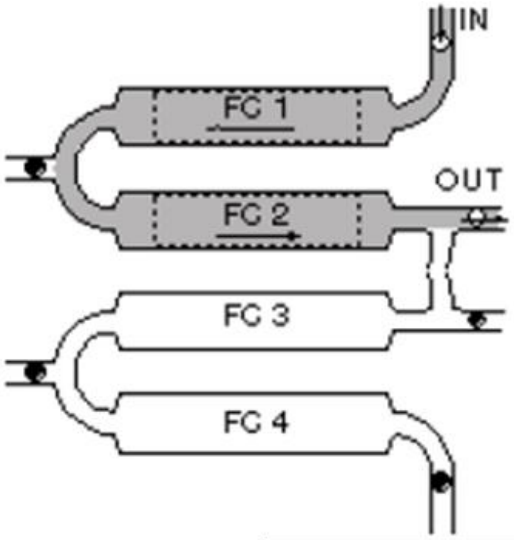
Samples can be purified or complex

- For affinity/kinetics – SEC purified (>95%)
- Cell culture medium
- Clinical sera or bodily fluids

Biacore T200

- Four (4) serial flow cells
- Simultaneous detection of up to 4 interactions per injection cycle (1 reference, 3 experimental)
- Autosampler supports 96- and 384-well plates
- Integrated degasser
- Sensitivity down to 1 RU allows detection of small molecule analytes





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