Biomolecular Interaction Analysis (BIA) Core Facility

Applications in SPR Binding Analysis

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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**
Surface Plasmon Resonance

\[ K_D = \frac{k_{off}}{k_{on}} \]

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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

- Samples can be purified or complex
  - For affinity/kinetics – SEC purified (>95%)
  - Cell culture medium
  - Clinical sera or bodily fluids
Experimental Considerations

1. **Immobilization**
   - How to functionalize a binding partner to the sensor chip?

2. **Binding**
   - What type of assay/design?

3. **Regeneration**
   - How to remove bound analyte without damaging ligand?

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**SPR Sensor Chips**

- **Carboxymethylated Dextran**
  - Biocompatible and Robust
  - Low non-specific binding
  - Available in varying dextran lengths and CM density
  - 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

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**Direct vs. Capture**

- **Direct Immobilization**
  - Immob. → Binding → Regeneration

- **Ligand Capture**
  - Immob. → Capture → Binding → Regeneration
Direct vs. Capture

- Direct Immobilization
  - Simple and Robust
  - Immob. level less flexible
  - 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
  - Chaotrope: 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
     - Low (Kinetics) vs. High (Screening or LMW Affinity)

2. Binding
   - Desired experimental outcome
   - Assay design
   - Protein purity and concentration
   - Protein quantity (~100 µg)

3. Regeneration
   - Mildest conditions necessary
   - Complex and surface ligand stability
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SPR Experiments

• Specificity and Screening
  • Yes/No Binding
  • Ranking of binding response
• Kinetics and Affinity
  • Multi-cycle or single-cycle
  • Direct binding
  • Steady-State Affinity
  • Solution-based Affinity
• Concentration
  • Surface Competition
  • Solution Inhibition
• Epitope Mapping
• Conformational Changes
• Thermodynamics

\[ K_D = \frac{k_{off}}{k_{on}} \]

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Biacore 3000  Biacore 4000  Biacore T200

Biacore S200  ForteBio Octet-RED96

gihilcare.com/biacore, fortebio.com
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Biacore 3000

- Two (2) 3000s available
- Four (4) serial flow cells
- Simultaneous detection of up to 4 interactions per injection cycle
- Autosampler
- Sensitivity down to 10 RU

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Biacore 4000

- Four (4) independent flow cells each equipped with 5 detection spots
- Simultaneous detection of up to 16 interactions per injection cycle
- Autosampler supports 96- and 384-well plates in 10-plate hotel
- Designed for high-throughput analyses
- Approved for clinically derived and/or infectious samples
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**Biacore T200**
- Four (4) serial flow cells
- Simultaneous detection of up to 4 interactions per injection cycle
- Autosampler supports 96- and 384-well plates
- Integrated degasser
- Sensitivity down to 1 RU allows detection of small molecule analytes

**Biacore S200**
- Four (4) serial flow cells
- Autosampler supports 96- and 384-well plates
- Integrated degasser
- Increased sensitivity below 1 RU allows for improved detection of small molecule analytes
- Specifically designed for high throughput screening of LMW analytes
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**ForteBio Octet-RED96**
- Biolayer Interferometry (BLI)
- Generates similar results as SPR
- Ligand-coated biosensor tips submerged in a 96-well plate
- Parallel processing of up to 96 interactions
- No need for regeneration
- Crude sample compatibility

How to Utilize the BIA Core
- **Sample Submission**
  - Available for the 3000, 4000, S200, and BLI
  - Trained technicians will optimize experiments and complete data analysis.
- **Independent Use**
  - Available for the T200
  - Specialized training and support
  - Reserve T200 through CoreResearch@Duke
  - Available to researchers 24/7