Objectives

During this session you will get introduced to

- Biacore data
  - What type of analyses can be performed
  - The sensorgram

- Biacore technology
  - Surface Plasmon Resonance (SPR)
  - Microfluidics
  - Sensor Chips

- General steps in a Biacore assay
  - Immobilization
  - Sample injection
  - Regeneration

Biacore systems monitor molecular binding events in real-time
What are ligand and analyte?

The ligand is the interaction partner attached to the surface.
The analyte is the interaction partner that passes in solution over the surface.

Data output from label-free interaction analysis

Sensorgram

Yes/no binding?
Specificity of binding?
How strong?
How fast?
How much?

Comprehensive information from one system

Analyze molecular interactions in real time and obtain a wide range of critical, binding-related data:

- Yes/No
- Specificity
- Binding partners
- Affinity
- Kinetics
- Concentration
- Thermodynamics
Analysis of a wide range of biomolecular interactions

• Proteins
• Nucleic acids
• Lipids & membrane-associated molecules
• Carbohydrates
• LMW compounds (>200 Da)
• Whole cells
• Viruses/bacteria

Biacore™ data in almost 10,000 scientific publications

Basic and applied research in the fields of

• Cancer
• Neurobiology
• Immunology
• Infectious diseases
• Functional proteomics
• Cell signaling
• Vaccines
• Selection and characterization of binding reagents
• Drug discovery
• and many more...

Cornerstones of the system

• SPR detection system
• Microfluidic system
• User-friendly software
• Sensor chip
**SPR detection**

[Diagram showing SPR detection process]

**Total Internal Reflection & SPR**

[Diagram illustrating total internal reflection and SPR]

**SPR Sensing**

Measuring at the Surface

[Diagram showing SPR sensitivity measurements]

- **High refractive index medium**: Prism
- **Low refractive index medium**: Water
- **Gold layer**: Incident Light
- **Total Internal Reflected light (TIR)**
- **Critical angle for TIR**
- **Evanescent field**

**Distance [nm]**

Glass

Metal film

Dextran + immobilized protein
SPR detection

- SPR is a refractive index sensor
- Measurements are dependent on surface concentration & temperature
- SPR response is a measure of changes in the resonance angle
- As a rough approximation, a response of 1 RU is equivalent to change in a surface concentration of about 1 pg/mm² (for proteins on Sensor Chip CM5)

Microfluidics

Liquid handling

- Miniaturized system
- Low volume of reagents
- Integrated and automated liquid handling

Biacore technology: Microfluidics

Flow cells

- The number of flow cells and the type of flow varies between different Biacore systems
- Serial flow (4 flow cells): Biacore 2000, Biacore 3000, Biacore T100
- Parallel flow (4 flow cells): Biacore 1000, Biacore C, Biacore Q
- Serial flow (2 flow cells): Biacore X, Biacore X100, Biacore J
Microfluidics – Flow cells

4 flow cells – single, pairwise and serial run

Flow cells optimized for use in pairs (FC1+FC2, FC3+FC4)

Buffer Selector

Buffer selector valve
• Assay development
• Study binding in different environments (e.g. pH, salt)
• Controlled from the software
• Up to 4 different buffers can be used

Integrated buffer degasser

Integrated buffer degasser
• Eliminates precipitation of air in the flow system
• Buffers do not need to be degassed externally

Improves robustness when studying interactions at temperatures > 25 °C
Liquid handling system

The sensor chip

Dextran matrix

- Hydrophilic
- Flexible
- Resembles a 2% aqueous dextran solution environment
- Low non-specific binding
- High binding capacity
- Easy to activate and use for covalent coupling
- Withstands extensive regeneration
- Present on all sensor chips except Sensor Chip C1, Sensor Chip HPA and Sensor Chip Au
Sensor Chip CM5

For covalent attachment of ligand or capturing molecule using:
- Amine coupling
- Ligand thiol coupling
- Surface thiol coupling
- Maleimide coupling
- Aldehyde coupling

- The most versatile chip
- Carboxylated dextran matrix
- Low non-specific binding
- High binding capacity

A wide range of sensor chips

General Steps in a Biacore Assay

Surface preparation
Sample injection
Regeneration
Evaluation
Surface Preparation - Immobilization

- What is immobilization?
  - Coupling of ligand or capture molecule to the sensor surface

- Direct immobilization
  - Covalent coupling of ligand to surface

- Capture approach
  - Covalent coupling of capture molecule
  - Capture of ligand during each cycle via a high-affinity interaction

Sample injection

- The sample is injected over the surface with a constant flow and concentration
- Analyte in the sample binds to the immobilized ligand on the surface, the mass on the surface changes and a response is recorded
- After sample injection, buffer flows over the surface to allow monitoring of the dissociation of analyte from ligand

Regeneration

- Removes bound analyte completely from the surface
- The activity of the surface must remain unaffected
Summary

- Biacore technology
  - SPR
  - Sensor Chip Surfaces
  - Microfluidics

- Important Biacore Assay Steps
  - Immobilization
  - Sample injection
  - Regeneration

- Biacore data
  - Includes specificity, kinetics, affinity
  and concentration

**The Sensorgram**

Gives information-rich real-time data.

Allows flexible assay design and analysis of a wide range of molecular interactions.

Results in detailed characterization and analysis of biomolecular interactions.