# Multidisciplinary Molecular Interaction Core (MMIC) Facility

## Shelby Biomedical Research Building (SHEL) 420

## MMIC Information


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## Introduction

- The MMIC facility provides use of a GE Biacore T200 instrument (http://www.biacore.com) which employs surface plasmon resonance (SPR) technology for monitoring biomolecular binding interactions.
- The instrument has the capacity to provide comprehensive real-time information without the use of labels.

### Biacore T200 technology

*Key Features*

Integrated microFluidic Cartridge (IFC)

Sensor Chips

SPR detection

User-friendly Software

### Capable of analyzing a wide range of molecular interactions

- **Proteins**
- **Nucleic acids**
- **Lipid & membrane associated molecules**
- **Carbohydrates**
- **Low MW compounds (100-1000 Da)**
- **Whole cell cells**
- **Viruses/bacteria**

### Can be applied to understand biological functions

- **Specificity analysis**

Is the molecule of interest specific to its target?

- **Concentration analysis**

How much of the product of interest is in a sample?

- **Affinity**

How strong is the binding between molecules of interest?

- **Kinetic analysis**

How fast does binding association or dissociation occur?

- **Thermodynamic analysis**

Is the interaction of molecules temperature dependent?

## Advantages of the Biacore T200

- **Label–free**

Measures/defines binding of unlabeled molecules

- **Real-time**

Binding characteristics (on- and off-rates) observed in real-time

Weak and fast interactions can be studied

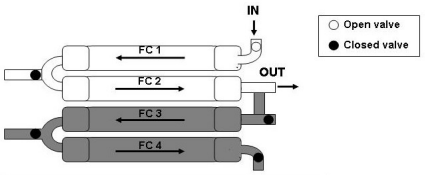
- **Non-invasive**

Directly measures opaque samples without compromise of sensitivity or accuracy

## Biacore T200 Components

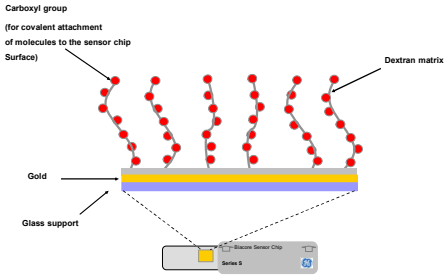
### Integrated fluidic cartridge (IFC)

- The Biacore T200 IFC is optimized for the highest quality kinetics
- The system has 4 flow cells connected in pairs (FC1-FC2, FC3-FC4)
- However, flow cells can be run single, pair-wise or serially
- Pair-wise runs give good reference subtraction
- The system requires low volume reagents



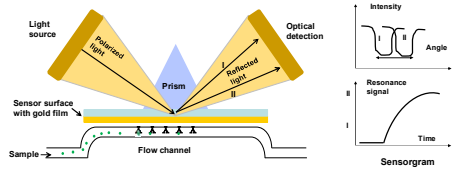

Schematic representation of Biacore fluidic system

### Biacore gold-dextran Sensor chip




## How the SPR System Works

- Measures changes in refractive index
- Measurements depend on concentration and temperature
- 1 Resonance unit (RU) is equivalent to a change in surface concentration of approximately 1 pg/mm$^2$ (proteins on a sensor ship)




Schematic representation of SPR

## Biacore Assay Steps

Surface preparation (immobilization of the ligand to the Sensor Chip)

Sample (analyte) injection

Regeneration

Data evaluation

### Terminology

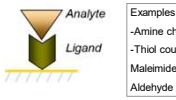
***Ligand:*** molecule to be immobilized on the sensor chip

***Analyte:*** sample to be injected over the chip surface for analysis

### Surface preparation-ligand immobilization

- **Direct ligand immobilization**

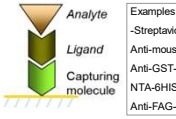
Covalent chemistry
Heterogeneous orientation
Requires high binding capacity




Examples:
-Amine chemistry
-Thiol coupling
Maleimide coupling
Aldehyde coupling

- **Capture approach**

Orientation specific
Selectively capture from crude samples
Low binding capacity required




Examples:
-Streptavidin-Biotin
Anti-mouse IgG-MAb
Anti-GST-GST
NTA-6HIS
Anti-FAG-FLAG

### Sample injection

- The sample is injected over the chip surface with immobilized ligand at a constant flow rate
- The analyte from the sample binds to the immobilized ligand resulting in a change in the mass on the chip surface, which is recorded
- Continued buffer flow allows monitoring of the analyte dissociation from the ligand

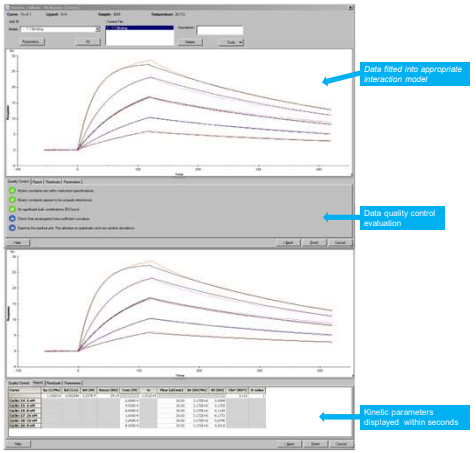
### Regeneration

- The bound analyte is completely removed from the ligand
- Can be achieved by use of buffers with changes in pH, salt, or detergents
- After regeneration the immobilized ligand is maintained on the chip surface, with full activity
- To achieve high quality data effective regeneration is essential

### Data evaluation

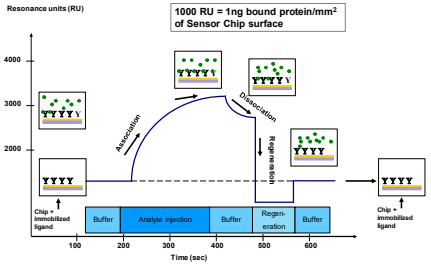
- Flexible evaluation software for data analysis
- Software has quality control tools for guidance on data quality and validity

*A few simple clicks to complete kinetic evaluation*




## Biacore Assay Steps (cont)

### Typical Interaction Sensorgram (RU vs. time)




## Conclusions

### Use of the Biacore T200 can provide comprehensive information from one system

Analyzes molecular interactions in real time and obtain a wide range of critical binding-related data.

**Detect** — Yes/No

**Identify** — Specificity, Binding partners

**Characterize** — Affinity/Kinetics, Concentration, Thermodynamics, Immunogenicity

### Biacore data is included in over 20,000 publications

Publications include basic and applied research in the following fields:

- Cancer
- Neurobiology
- Immunology
- Infectious diseases
- Functional proteomics
- Cell signaling
- Vaccines
- Drug discovery
- Selection and characterization of binding reagents

## Selected MMIC-related Publications(out of 23)


1. Shea LK, Honjo K, Redden DT, Tabengwa E, Li R, Li FJ, Shakhmatov M, Chiorazzi N, **Davis RS.** Fc receptor-like 2 (FCRL2) is a novel marker of low-risk CLL and refines prognostication based on IGHV mutation status. Blood Cancer J. 2019 May 15;9(6):47. PMID: PMID: 31092613; PMCID: PMC6520396
2. Harris BD, Schreiter J, Chevrier M, Jordan JL, **Walter MR.** Human interferon-ε and interferon-κ exhibit low potency and low affinity for cell-surface IFNAR and the poxvirus antagonist B18R. J Biol Chem. 2018 Oct 12;293(41):16057-16068. PMID: PMID: 30171073; PMCID: PMC6187621
3. Mitra A, Speer A, Lin K, Ehrt S, **Niederweis M.** PPE Surface Proteins Are Required for Heme Utilization by Mycobacterium tuberculosis. MBio. 2017 Jan 24;8(1). pii: e01720-16. PMID: PMID: 28119467; PMCID: PMC5263243
4. Pillai VG, Bao J, Zander CB, McDaniel JK, Chetty PS, Seeholzer SH, Bdeir K, Cines DB, **Zheng XL.** Human neutrophil peptides inhibit cleavage of von Willebrand factor by ADAMTS13: a potential link of inflammation to TTP. Blood. 2016 July7;128(1):110-9. PMID: 27207796; PMCID: PMC4937355
5. Sun J, Siroy A, Lokareddy RK, Speer A, Doornbos KS, Cingolani G, **Niederweis M.** The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD. Nat Struct Mol Biol. 2015 Sep;22(9):672-8. PMID: 26237511; PMCID: PMC4560639
6. Sharifov OF, Xu X, Gaggar A, Tabengwa EM, White CR, Palgunachari MN, Anantharamaiah GM, **Gupta H.** L-4F inhibits lipopolysaccharide-mediated activation of primary human neutrophils. Inflammation. 2014 Oct;37(5):1401-12. PMID: 24647607; PMCID: PMC5822683
7. Logsdon NJ, Deshpande A, Harris BD, Rajashankar KR, **Walter MR.** Structural basis for receptor sharing and activation by interleukin-20 receptor-2 (IL-20R2) binding cytokines. Proc Natl Acad Sci U S A. 2012 Jul 31;109(31):12704-9. PMID: 22802649; PMCID: PMC3412030
8. Logsdon NJ, Eberhardt MK, Allen CE, Barry PA, **Walter MR.** Design and analysis of rhesus cytomegalovirus IL-10 mutants as a model for novel vaccines against human cytomegalovirus. PLoS One. 2011;6(11):e28127. PMID: 22132227; PMCID: PMC3221699


### Figure from a MMIC-related publication

**IL19/IL-20 receptor interactions and complex stability**