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

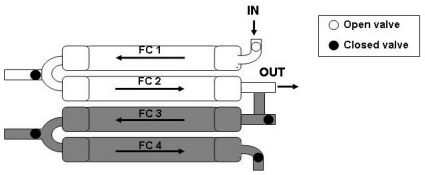
- **Non-invasive**

Directly measures opaque samples without compromise of sensitivity or accuracy

Biacore T200 Components

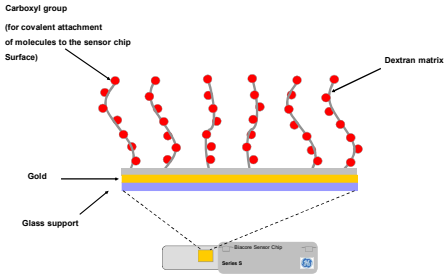
Integrated fluidic cartridge (IFC)

- 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



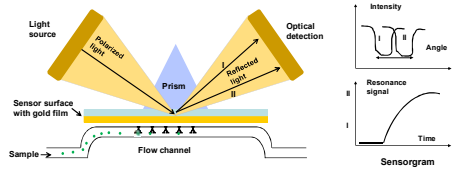
Schematic representation of Biacore fluidic system

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

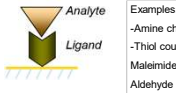
Ligand: molecule to be immobilized on the sensor chip

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

Surface preparation-ligand immobilization

- **Direct ligand immobilization**

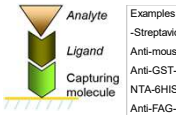
Covalent chemistry
Heterogeneous orientation
Requires high binding capacity



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

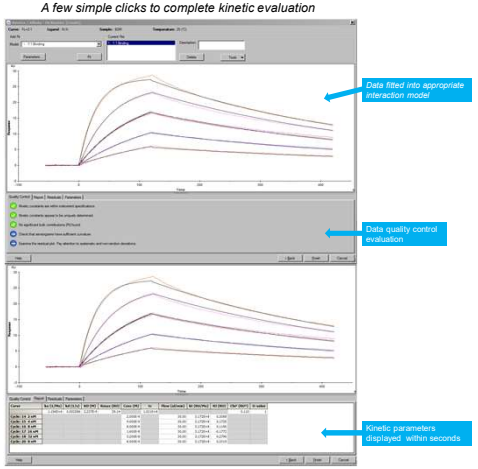
Regeneration

- 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

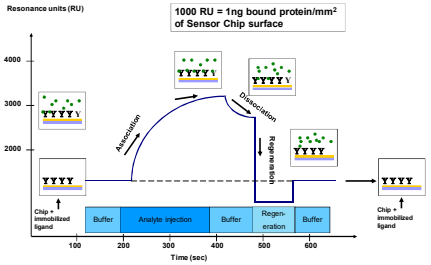
- Flexible evaluation software for data analysis
- Software has quality control tools for guidance on data quality and validity

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

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: 31092813; 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: 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