Duke Human Vaccine Institute

Duke University School of Medicine

# DHVI Biomolecular Interaction Analysis (BIA) Core Facility

## Mission

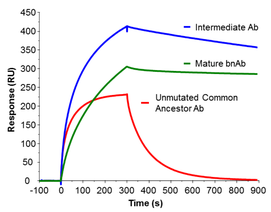
The DHVI Biomolecular Interaction Analysis (BIA) Core Facility offers state-of-the-art **Surface Plasmon Resonance (SPR)** and **Biolayer Interferometry (BLI)** instruments, expertise, training, and support for ***label-free, real-time*** analysis of biomolecular interactions to basic and clinical researchers within the Duke Community.

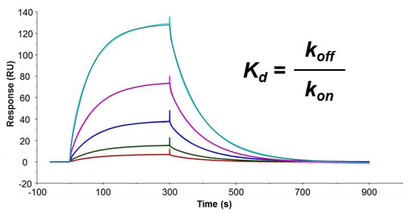
## Leadership / Experience

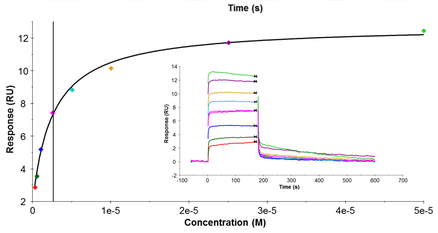
- **Director: S. Munir Alam, PhD**
  - Munir Alam is a Professor of Medicine and Pathology at Duke University, a DHVI Principal Investigator, and the Founding Director of the DHVI BIA Core Facility. Dr. Alam is a recognized expert in biochemical and biophysical analyses of receptor-ligand interactions, including TCR-ligand and antibody-antigen binding, and HIV-1 Envelope protein antigenicity characterization. He is Director of Protein Analytics at both the DHVI and its GMP facility, a Co-Investigator in the Duke CHAVD program, and was the Proteomics Director for the Duke CHAVI program.
- **Manager: Kenneth Cronin**
  - Ken Cronin has served as Lab Manager for Dr. Alam for 7 years and recently has taken on role of BIA Core Manager. He has extensive experience in assay development and biophysical characterization of molecular interactions by SPR, BLI, and FPLC.

## Services

- **Binding Specificity and Screening**
  - Yes/No Binding
  - Ranking of Binding Response
- **Kinetics and Affinity**
  - Direct Binding Kinetics
  - Steady State Affinity
  - In-solution Affinity
  - Avidity Assessment
- **Active Concentration**
  - Surface Competition
  - Solution Inhibition
- **Thermodynamics**
- **Epitope Mapping**
- **Conformation Changes**

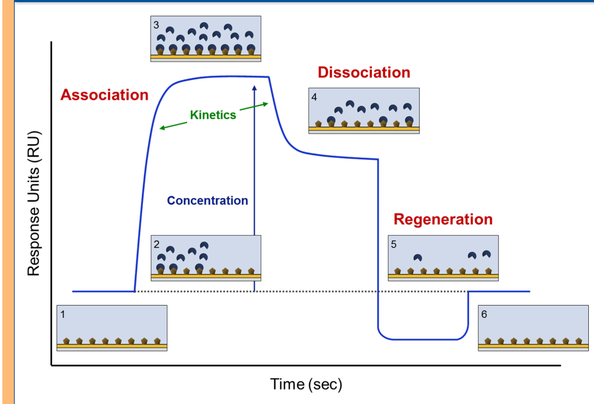



## Technology / Instruments / Resources

### Surface Plasmon Resonance



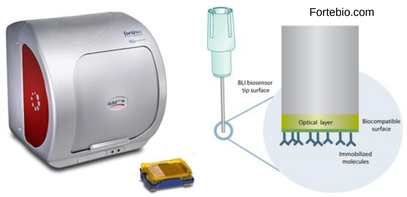

**SPR Sensorgram.** In a typical SPR experiment, a soluble analyte is injected over a ligand-immobilized sensor chip (**1**). Interaction between the biomolecular species results in surface site population and an increase in SPR response (**2, 3**). Upon injection completion, the bound analyte dissociates from the surface, reducing the SPR response (**4**). The surface can be regenerated to remove any residual analyte in preparation for the next injection (**5, 6**). The kinetics ($k_a$, $k_d$) and affinity ($K_d$) of the interaction and/or the active analyte concentration can be calculated.

- Biacore SPR systems are **low sample consumption** (typically < 25 µg) and are **compatible with a wide-range of biomolecules**
  - Proteins, peptides, DNA/RNA, small molecules, carbohydrates, polymers, lipid membranes, micelles, liposomes, polymers, whole viruses, and whole cells
  - Crude cell culture lysates or clinical sera/bodily fluids
- Biomolecules can be functionalized on sensor chips via:
  - **Covalent coupling**
  - **High-affinity capture** (with any affinity tag)
  - **Hydrophobic adsorption**

### DHVI BIA Core Facility Platforms

**Biacore 3000**
- Standard platform
- Simultaneously detects 4 interactions
- 10 RU Sensitivity

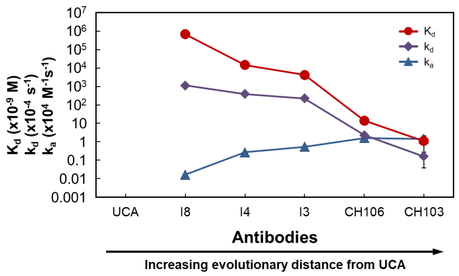
**Biacore T200/S200**
- LMW screening
- Simultaneously detects 4 interactions
- 1 RU Sensitivity
- T200 available for Independent Use




**ForteBio Octet-RED96**
- Biolayer Interferometry (BLI)
- Ligand-coated biosensors in 96-well plate
- Crude sample compatibility
- Simultaneously detects 8 interactions

**ForteBio Octet HTX**
- High-throughput Biolayer Interferometry (BLI) analysis Supports 96- and 384-well plate racks

## Recent Projects / Publications




**Affinity Maturation and Kinetic Trends in the CH103 bnAb Lineage.** Kinetic association rates ($k_a$, **blue**), dissociation rates ($k_d$, **purple**), and apparent affinities ($K_d$, **red**) of the CH103 lineage mAbs for HIV-1 heterologous gp120 Envelope antigen (B.63521). In this lineage, affinity maturation proceeded via two distinct kinetic regimes: early stage improvement in $k_a$ (I8 to I3) and late stage improvement in $k_d$ (I3 to mature bnAbs), highlighting the importance of kinetic characterization in the investigation of binding mechanisms and immunogen design.[1]

- **Kinetics/Affinity –** [1]Henderson R., Watts, B.E., et al., *Selection of immunoglobulin elbow region mutations impacts interdomain conformational flexibility in HIV-1 broadly neutralizing antibodies*. Nature Communications **2019**, 10 (654).; Enam, S.F., et al., *Enrichment of endogenous fractalkine and anti-inflammatory cells via aptamer-functionalized hydrogels*. Biomaterials **2017**, 142.
- **Vaccine-induced Avidity Screening –** Lynch, H.E., et al., *Surface plasmon resonance measurements of plasma antibody avidity during primary and secondary responses to anthrax protective antigen*. Journal of Immunological Methods **2014**, 404.
- **Competition –** Tomaras, G.D., et al., *Vaccine-induced plasma IgA specific for the C1 region of the HIV-1 envelope blocks binding and effector function of IgG*. PNAS-USA **2013**, 110 (22).
- **Epitope Mapping –** Liao, H.-X., et al., *Vaccine Induction of Antibodies against a Structurally Heterogeneous Site of Immune Pressure within HIV-1 Envelope Protein Variable Regions 1 and 2*. Immunity **2013**, 38 (1).
- **Nanoparticle/Liposome Antigenicity –** Alam, S.M., et al., *Role of HIV membrane in neutralization by two broadly neutralizing antibodies*. PNAS-USA **2009**, 106 (48).

## Reservations / Service Requests

- ***Independent Use*** – Self-Service, *T200 only*
  - Complete training in the independent operation of the Biacore T200
  - Available 24/7/365 through CoreResearch@Duke
- ***Sample Submission*** – Limited Full-Service, *All platforms*
  - All experimentation, optimization, and analysis will be performed by Core Staff.
- ***Consultation***
  - Assistance in experimental design and analysis/interpretation of data
- Contact Ken Cronin to schedule an initial consultation and to discuss sample submission goals and/or training

## Contact Us

- BIA Core Facility website:
  dhvi.duke.edu/programs-and-centers/shared-resources/cores/biomolecular-interaction-analysis-bia-core-facility
- Email: kenneth.cronin@duke.edu
- Phone: (919) 684-4380