HARVARD MEDICAL SCHOOL
DEPARTMENT OF Biological Chemistry and Molecular Pharmacology

Center for Macromolecular Interactions

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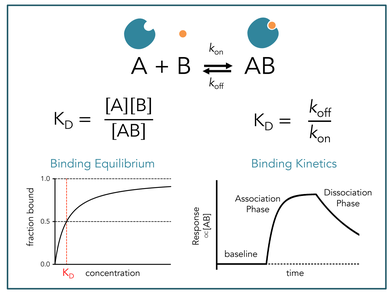
CENTER FOR MACROMOLECULAR INTERACTIONS

What is the CMI?

The BCMP Center for Macromolecular Interactions (CMI), is a biophysical instrumentation facility for the characterization of macromolecules and their interactions. The CMI mission is to enhance basic research in the HMS community by providing scientific consultation, training and access to shared biophysical equipment. The facility currently includes instruments for Isothermal Titration Calorimetry (ITC), Surface Plasmon Resonance (SPR), Biolayer Interferometry (BLI), MicroScale Thermophoresis (MST), Differential Scanning Fluorimetry (DSF), Circular Dichroism (CD), and Analytical Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS).

What can you do at the CMI?

- **Measure Molecular Interactions**
  - Proteins
  - Nucleic Acids
  - Small molecules
- **Characterize Protein Properties**
  - Secondary Structure
  - Thermal Stability (T_m)
  - Mass and Oligomeric State

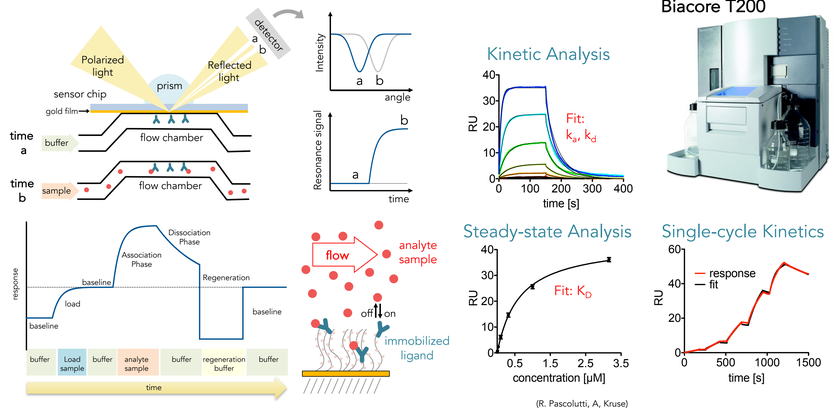


CMI Technologies for measuring binding

technology	Biolayer Interferometry (BLI)		Surface Plasmon Resonance (SPR)	Isothermal Titration Calorimetry (ITC)	MicroScale Thermophoresis (MST)	Differential Scanning Fluorimetry (DSF)	Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS)
instrument	ForteBio Octet RED384	ForteBio BLItz	GE Biacore T200	Microcal ITC200	NanoTemper Technologies Monolith NT.115$_{pico}$	Life Technologies Quant Studio 6	Wyatt Dawn Heleos II
signal	Change of interference pattern of white light due to size of bound molecule		Change of refractive index due to mass	Enthalpy of binding	Thermophoresis induced change in fluorescence	Change in protein-binding dye fluorescence intensity	Scattered light intensity during separation
measures	ka, kd, K_D		ka, kd, K_D	ΔH, ΔS, n, K_D	K_D, EC50	Tm, yes/no binding, pseudo-KD	MW, n, yes/no binding
KD range	<nM- mM		<nM- mM	nM-µM	pM-mM	<µM	<µM
sample/analyte limits	analyte >200 Da	sample and analyte >10,000 Da	analyte >150 Da	-	-	protein sample, non-protein analyte	Depends on SEC capacity (>5,000 Da)
sample volume per experiment	80-220 µl per measurement (up to 16 at once)	~5 x 4 µl per measurement	~200 µl per immobilization	~300 µl per titration	10 µl per capillary (16/experiment)	20 µl per well (perform 2-4 replicates)	5-100 µl per run
sample conc.	10-50 µg/ml	10-50 µg/ml	5-50 µg/ml	10x K_D, >5µM	50 pM-µM	0.05-5 µg/well	~ 100-200 µg (varies by MW)
analyte volume	80-220 µl for each of ~5 concentrations	4 µl for each of ~5 concentrations	~300 µl for each of ~5 concentrations	70 µl for each titration (~140 µl/expt)	20 µl per experiment	(mixed with sample)	(mixed with sample)
analyte conc.	0.1-10 K_D	0.1-10 K_D	0.1-10 K_D	~100x K_D	≥ 40x K_D	≥ sample, >10x K_D	≥ sample, >10x K_D

Surface Plasmon Resonance (SPR)

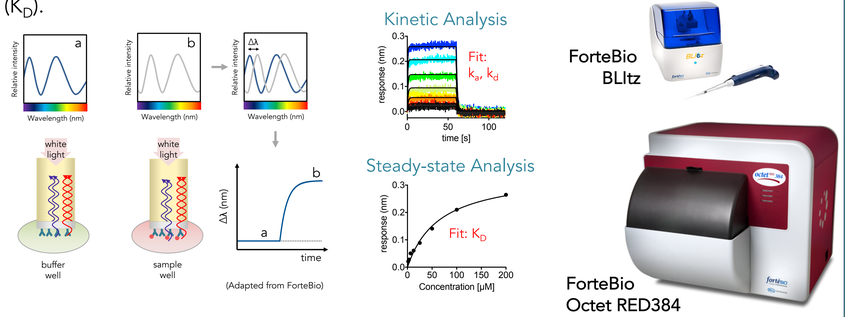
SPR is a technique to measure biomolecular interactions between an immobilized ligand and an analyte in solution. SPR can occur when plane-polarized light hits a metal film under total internal reflection conditions. The SPR signal is directly dependent on the refractive index of the medium on the sensor chip. The binding of biomolecules results in changes in the refractive index on the sensor surface. Real-time measurements of binding allow determination of association and disociation rate constants (k_a and k_d) and equilibrium binding constants (K_D).



(R. Pascolutti, A, Kruse)

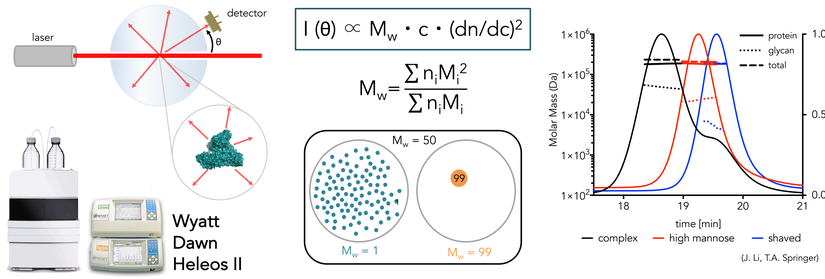
Bio-Layer Interferometry (BLI)

BLI is an optical technique for measuring macromolecular interactions by analyzing interference patterns of white light reflected from the surface of a biosensor tip. A change in the number of macromolecules bound to the end of the biosensor tip causes a shift in the interference pattern that can be measured in real-time to determine association and dissociation rate constants (k_a and k_d) and equilibrium binding constants (K_D).



Size-exclusion Chromatography with Multi-angle Light Scattering (SEC-MALS)

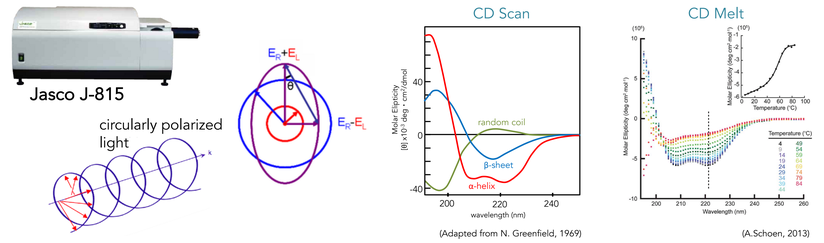
Using a multi-angle static light scattering (MALS) detector, the intensity of scattered light can be used to measure a weight-average molar mass in solution. Size-exclusion chromatography (SEC) separates molecules based on hydrodynamic volume. Combining SEC and MALS in an SEC-MALS experiment allows for more accurate mass measurements that either method alone. Combining two concentration detection modes (UV and RI), conjugate analysis can be performed to determine the mass contribution of modifiers such as carbohydrate or detergents.



(J. Li, T.A. Springer)

Circular Dichroism (CD)

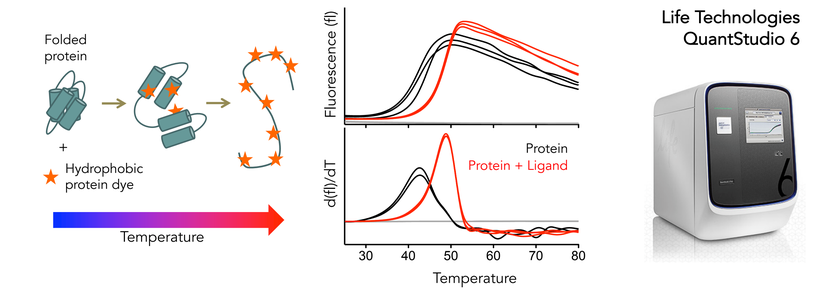
CD is a spectroscopic method for determining the optical isomerism of molecules. Circular dichroism (measured in molar ellipticity) is the difference in absorption of left-handed and right-handed circularly polarized light and can be observed in molecules with chiral centers. CD spectra in the "far UV" region (185-250 nm) can be used to determine protein secondary structure. Thermal stability (Tm) can be measured by following changes in molar ellipticity with increasing temperature.



(Adapted from N. Greenfield, 1969) (A.Schoen, 2013)

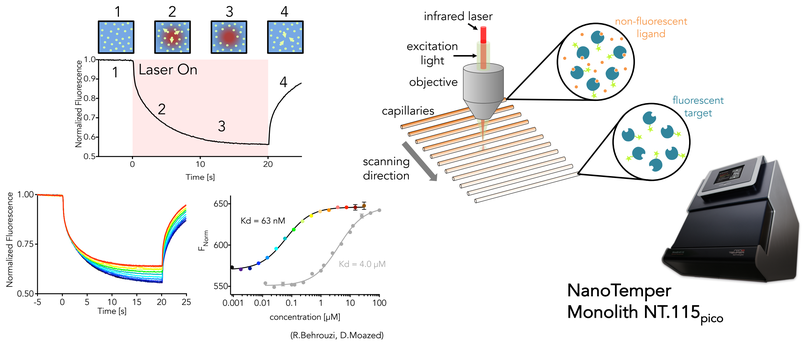
Differential Scanning Fluorimetry (DSF)

DSF uses a real-time PCR instrument to monitor thermally induced protein denaturation by measuring changes in fluorescence of a dye that binds preferentially to unfolded protein (such as Sypro Orange, which binds to hydrophobic regions of proteins exposed by unfolding). This experiment is also known as a Protein Thermal Shift Assay, because shifts in the apparent melting temperature can be measured upon the addition of stabilizing or destabilizing binding partners or buffer components.



MicroScale Thermophoresis (MST)

MST is an immobilization-free technology for measuring biomolecular interactions with a wide range of affinities (pM-mM). The MST instrument detects the motion of fluorescent molecules along a microscopic temperature gradient, which reflects changes in the molecular hydration shell, charge or size. Since one or all of these parameters changes with virtually every binding event, a wide range of biomolecules can be measured from ions and small molecule fragments to large macromolecular complexes in very small volumes (<10 ul) in a wide range of standard buffers and complex mixtures including liposomes, detergent, serum, and cell lysates.

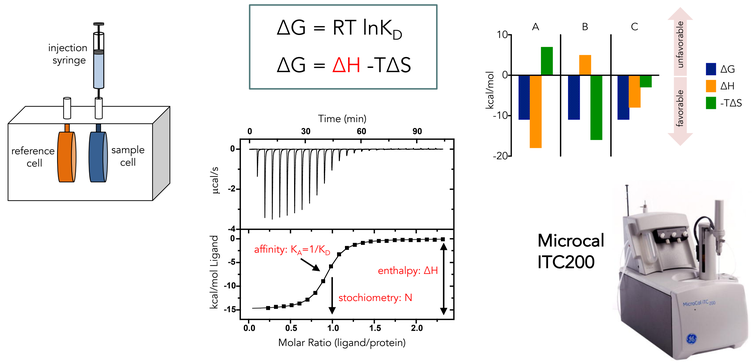


(R.Behrouzi, D.Moazed)

Isothermal Titration Calorimetry (ITC)

ITC is a label-free method for measuring binding of any two molecules that release or absorb heat upon binding. ITC monitors heat changes by measuring the differential power, applied to the cell heaters, required to maintain zero temperature difference between a reference and a sample cell as the binding partners are mixed. ITC can be used to measure the thermodynamic parameters of biomolecular interactions, including affinity (K_A), enthalpy (ΔH), entropy (ΔS), and stoichiometry (n).

$$\Delta G = RT \ln K_D$$
$$\Delta G = \Delta H - T\Delta S$$



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