CENTER FOR
MACROMOLECULAR
INTERACTIONS

HARVARD
MEDICAL SCHOOL
DEPARTMENT OF
Biological Chemistry and
Molecular Pharmacology

Technologies Available at the CMI

- ***Surface Plasmon Resonance (SPR): Biacore T200***
  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).

- ***Bio-Layer Interferometry (BLI): ForteBio Octet RED384 and BLItz***
  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).

- ***Isothermal Titration Calorimetry (ITC): Microcal ITC200***
  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 (Ka), enthalpy (ΔH), entropy (ΔS), and stoichiometry (n).

- ***MicroScale Thermophoresis (MST): NanoTemper Technologies Monolith NT.115$_{pico}$***
  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.

- ***Differential Scanning Fluorimetry (DSF): Life Technologies QuantStudio 6***
  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.

- ***Circular Dichroism (CD): Jasco J-815***
  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.

- ***Size-exclusion Chromatography with Multi-angle Light Scattering (SEC-MALS): Wyatt Dawn Helleos II MALS detector, Ootilab TrEX RI detector, Agilent chromatography***
  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.

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