

Measuring Molecular Properties

technology	Size Exclusion Chromatography with Multi-Angle Light Scattering (SEC-MALS)	Dynamic Light Scattering (DLS)	Circular Dichroism (CD)	Differential Scanning Fluorimetry (DSF)	
instrument	Wyatt Dawn Heleos II	Wyatt Dynapro Plate Reader III	Jasco J-815	Life Technologies Quant Studio 6/7	NanoTemper Prometheus NT.Plex
signal	Scattered light intensity during separation	Light scattering correlation times	Differential absorbance of right and left circularly polarized light	Change in fluorescence of a protein-binding dye	Change in intrinsic fluorescence of protein residues
measures	Molar Mass Oligomeric state Protein conjugate analysis	Radius of hydration Polydispersity Aggregation analysis	Secondary structure thermal stability	Thermal stability	Thermal stability Chemical Stability
sample limits	Depends on SEC capacity (5KDa < M_w < 5MDa)	$1 \text{ nm} < R_h < 1000 \text{ nm}$	protein sample \pm non-protein analyte (typically)	protein sample \pm non-protein analyte non-hydrophobic protein and solvent	protein sample \pm non-protein analyte
sample volume per experiment	5-100 μl per run	25-100 μl per well	300 μl per titration (or per measurement)	20 μl per well (for each analyte concentration)	20 μl per capillary
sample conc.	~ 50-200 μg (varies by MW)	> 0.1 mg/ml	\geq 0.2 mg/ml	~ 2-10 μg /well	~1 mg/ml



Measuring Molecular Interactions

technology	Biolayer Interferometry (BLI)		Surface Plasmon Resonance (SPR)	Isothermal Titration Calorimetry (ITC)	MicroScale Thermophoresis (MST)
instrument	ForteBio Octet RED384	ForteBio BLitz	GE Biacore T200	Microcal ITC200	NanoTemper Monolith NT.115 _{pmc}
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
measures	k_a, k_d, K_D		k_a, k_d, K_D	$\Delta H, \Delta S, n, K_D$	K_D, EC_{50}
KD range	<nM- mM		<nM- mM	nM- μ M	pM-mM
sample limits	analyte >200 Da	load and analyte >10,000 Da	analyte >150 Da	-	-
sample volume per experiment	80-200 μ l per measurement of load and analyte (up to 16 at once)	~5 x 4 μ l per measurement of each	~200 μ l per immobilization and ~300 μ l for each analyte concentration	~300 μ l in cell, ~140 μ l titrant	200 μ l target and 20 μ l ligand per experiment
sample conc.	Load: 1-50 μ g/ml Analyte: 0.1-10 K_D		Load: 1-50 μ g/ml Analyte: 0.1-10 K_D	cell: 10x K_D , >5 μ M syringe: ~100 K_D	Target: 0.5-50 nM Ligand: ~100 K_D

