CMI Technologies to Characterize Molecular Properties

technology	Light Scattering: Mass Photometry (MP)	Light Scattering: Size Exclusion Chromatography w/ Multi-Angle Light Scattering (SEC-MALS)	Light Scattering: Dynamic Light Scattering (DLS)	Differential Scanning Fluorimetry (DSF): Conventional DSF	Differential Scanning Fluorimetry (DSF): NanoDSF	Circular Dichroism (CD)
instrument	Refeyn TwoMP	Wyatt Dawn Heleos II	Wyatt Dynapro Plate Reader III	Life Technologies Quant Studio 6/7	NanoTemper Prometheus NT.Plex	Jasco J-1500
signal	Single particle light scattering intensity (ratiometric contrast)	Light scattering intensity during separation	Light scattering correlation times	Change in fluorescence of a protein-binding dye	Change in intrinsic fluorescence of protein residues	Differential absorbance of right and left circularly polarized light
measures	Molar Mass Oligomeric State Heterogeneity K_D (high affinity)	Molar Mass Oligomeric state Protein conjugate analysis	Radius of hydration Polydispersity Aggregation analysis	Thermal stability	Thermal stability Chemical Stability	Secondary structure thermal stability
sample limits	30 KDa to 5 Mda (ΔMW > 30%)	Depends on SEC capacity ~5KDa to 5MDa	1nm to 1000nm	protein sample ± non-protein analyte non-hydrophobic protein and solvent	protein sample ±non-protein analyte	protein sample ± non-protein analyte (typically)
sample amounts	10-20 nM (X10) $1x10^{12}$pp/ml (AAV) 2-20 µl per measurement	~ 50-200 µg (varies by MW) 5-100 µl per run	> 0.1 mg/ml 25-100 µl per well	~ 2-10 µg/well 20 µl per well	~1 mg/ml 20 µl per capillary	≥ 0.1 mg/ml 300 µl per measurement

CENTER FOR MACROMOLECULAR INTERACTIONS

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CMI Technologies to Measure Molecular Interactions

technology	Biolayer Interferometry (BLI)		Surface Plasmon Resonance (SPR)	Isothermal Titration Calorimetry (ITC)	MicroScale Thermophoresis (MST)
instrument	ForteBio Octet RED384	ForteBio BLItz	Cytiva Biacore T200	Microcal ITC200	NanoTemper Monolith NT.115$_{pico}$
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	ka, kd, K_D		ka, kd, K_D	ΔH, ΔS, n, K_D	K_D, EC50
KD range	<nM- mM		<nM- mM	nM-µM	pM-mM
sample limits	analyte >200 Da	load and analyte >10,000 Da	analyte >150 Da	Sample and analyte solvent must match	Target must be fluorescent
sample amounts	Load: 1-50 µg/ml Analyte: 0.1-10 K_D 80-200 µl each per measurement	Load: 1-50 µg/ml Analyte: 0.1-10 K_D ~5 x 4 µl each per measurement	Load: 1-50 µg/ml, ~200 µl per immobilization Analyte: 0.1-10 K_D, ~300 ul per measurement	cell: 10x K_D, >5µM, 300 µl syringe: ~100 K_D, 140 µl per experiment	Target: 0.5-50 nM, 200 µl Ligand: ~100x K_D, 20 µl per experiment

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