Comparison of methods

	Advantages	Disadvantages	Information obtained and Range
Affinity Selection Mass Spectrometry (AS-MS)	High-throughput Can be applied to solubilized membrane proteins Ligand mass detection allows verification of compound structure	Low-affinity binders are hard to detect	< 10 µM
Differential Scanning Fluorimetry (DSF)	Estimates the effect of the ligand on the thermal stability of a protein Fast and robust assay development	Requires a fluorescent dye Artefacts occur owing to fluorescence quenching or aggregation	1 nM–100µM
Dynamic Light Scattering (DLS)	Measures particle assize across the range ~0.1 nm to 10 µm Low probe consumption	Low resolution Large particles even when present in small quantities may impact the measurement	Translational diffusion coefficient (D_t), R_h, d_h, B_{22}, k_D, viscosity
Fluorescence Polarization (FP)	Homogenous assay	Narrow measurement window Sensitive to fluorescence interference	K_d 1 nM – 1 mM
Homogeneous Time Resolved Fluorescence (HTRF)	Homogenous assay Highly sensitive and robust	Requires two labels	K_d, $EC_{50,}$ k_{on}, k_{off} 1 pM – 1 mM
Isothermal Titration Calorimetry (ITC)	Direct determination of thermodynamic parameters for a binding event	Very high protein consumption Requires high solubility of titrated component	K_d, ΔH, ΔS, ΔG, stoichiometry 1 nM – 100 µM
Microscale Thermophoresis (MST)	In-solution measurements Applicable also for challenging targets (e.g., IDPs, solubilized membrane proteins) Low probe consumption	Requires labeling of the target with a fluorophore or strong intrinsic fluorescence Low protein consumption	K_d 1 pM – 1 mM
Nano-Differential Scanning Fluorimetry (nanoDSF)	Estimates the effect of the ligand on the thermal stability of a protein Fast and robust assay development Relies on intrinsic fluorescence of a protein Low protein consumption	No measurements possible when protein lacks tryptophan or tyrosine residues	T_m, C_m, ΔG
Surface Plasmon Resonance (SPR)	Time-resolved quantification of interactions	Requires immobilization of the probe to the surface Requires highly stable protein Signals affected by solvent effect	k_{on}, k_{off}, stoichiometry 1 pM – 500 µM
SwitchSense	Molecular dynamics Conformational change	Immobilization to DNA required	k_{on}, k_{off}, K_d, d_h, stoichiometry
Time-Resolved Fluorescence (TR-FRET)	Homogenous assay Highly sensitive and robust	Requires two labels	K_d, $EC_{50,}$ k_{on}, k_{off} 1 pM – 1 mM