| | Technique | Sample Requirements | Key References | Advantages | Disadvantages |
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| ***De novo* interaction ID and validation** | **Y2H** | Cell based – requires a set of plasmids encoding all proteins to be screened | (Mehla, Caufield, and Uetz 2015) | * Identifies direct interactions proteome-wide | * Requires significant cloning to set up |
| | **AP-MS** | Coomassie staining: 20–50 ng, Sypro Ruby: 1–5 ng, Western blotting: 0.1 ng (per protein band) | (Keilhauer, Hein, and Mann 2015) | * Simple MS setup available in most facilities | * Bridging by, for example, DNA can be an issue<br>* Not quantitative<br>* Not good to ID weak, transient interactions |
| | **Bio-ID/APEX** | | (Roux et al. 2018) | * Can detect weak, transient interactions | * High background<br>* APEX requires $H_2O_2$, restricting its use in some cell types and *in vivo* contexts |
| | **Pulldowns** | 1–10 mg total input protein (0.01–1 µg total protein after affinity enrichment) | (Louche, Salcedo, and Bigot 2017) | * Simple set-up<br>* No specialist equipment required | * Difficult to validate weak, transient interactions<br>* Requires access to good antibodies or tagged protein |
| **Stoichiometry** | **SEC** | 20–100 µg purified protein at 5–20 µM | (Sahin and Roberts 2012; Gell, Grant, and Mackay 2012) | * Simple set-up | * Elution volume dependent on molecular shape |
| | **SEC-MALLS** | Size dependent: 10–50 µg for species > 40 kDa | (Folta-Stogniew 2006) | * Allows molecular weight determination | * Complexes with intermediate or low affinity will partially dissociate at typical concentrations, introducing ambiguity |
| | **DLS** | Size dependent: 10–50 µg for species > 40 kDa | (Stetefeld, McKenna, and Patel 2016) | * Quick method to assess sample homogeneity | * Molecular weight estimation inaccurate as relies on Stokes radius which is shape dependent |

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| | **SAXS** | 50–100 µL at ~5 mg/mL | (Hura et al. 2009) | * Gives information on molecular shape | * High concentrations required can lead to aggregation |
| | **Mass photometry** | <1 ng protein | (Soltermann et al. 2020; Young et al. 2018) | * Single molecule detection<br>* Can determine binding constants and molecular weights<br>* Low sample requirements | * Lower mass precision than MS techniques |
| | **Native MS** | 10–100 µL of 1–10 µM protein | (Snijder and Heck 2014; Donnelly et al. 2019) | * Very high mass precision | * Complex must be stable in MS-compatible buffers, such as ammonium bicarbonate<br>* Specialist MS setup required |
| | **Analytical ultracentrifugation** | 200–400 µL @ A280 = 1.0 (absorbance optics), 0.1 mg/mL (interference optics), 100 pM (fluorescence optics) | (Cole et al. 2008) | * Can use complex mixtures e.g. lipids | * Challenging for heterotypic interactions<br>* Not useful for complexes with small ligands |
| **Binding affinities** | **ITC** | 2–300 uL @ 10 µM P and 2 x ~60 µL @ 100 µM L for one titration and an L into buffer control | (Freyer and Lewis 2008) | * Widely applicable<br>* Can determine thermodynamic parameters | * Enthalpy change is limiting factor for signal to noise<br>* Sample components that cause a heat change interfere with the experiment<br>* Very sensitive to buffer mismatch<br>* High protein requirement |
| | **SPR** | 1–10 µg ligand and 30 µL analyte at each concentration (optimising ligand immobilisation level is critical) | (Nikolovska-Coleska 2015) | * Gives kinetic, as well as equilibrium, data<br>* Low protein requirements | * Disposable biosensors expensive<br>* Non-specific sensor binding can interfere<br>* Very sensitive to buffer mismatch<br>* Can be difficult to measure binding of small molecules to large immobilized species |

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| | **BLI** | Ligand loading 50–100 nM, 200 µL analyte per well (for 96 well plate) (optimising ligand immobilisation level is critical) | (Abdiche et al. 2008) | ∗ Gives kinetic, as well as equilibrium, data<br>∗ Sensitive to binding of small ligands (down to 150 Da) | ∗ Disposable biosensors tips relatively expensive<br>∗ Non-specific sensor binding can interfere |
| | **Intrinsic tryptophan fluorescence** | low µM quantities of protein, [ligand] 10-fold either side of $K_D$ (volume depends on assay setup, *i.e.*, cuvette vs plate) | (Lakowicz 2006) | ∗ Cheap and straightforward to perform<br>∗ Binding affinities are relative | ∗ Requires appropriately positioned tryptophan<br>∗ Analysis can be complicated if multiple tryptophan residues are present<br>∗ Fluorescent binding partners can interfere |
| | **MST** | 80 µL fluorescently labelled protein/ligand @ 10–50 nM | (Jerabek-Willemsen et al. 2011) | ∗ Wide range of compatible binding partners<br>∗ No significant size limits | ∗ Protein binding to capillary walls can interfere (different capillary coatings can ameliorate this) |
| | **FA/FP** | 1–100 nM labelled ligand, binding partner 10–100-fold either side of $K_D$ (volume depends on assay setup, *i.e.*, cuvette vs plate) | (Rossi and Taylor 2011) | ∗ Cheap and easy to perform<br>∗ Relatively easy to adapt to HTS | ∗ Fluorescent ligands can interfere<br>∗ Requires large size change on complex formation |
| | **FRET/BRET** | Partners must be at similar concentrations so that background fluorescence doesn't dominate the signal | (Kobayashi et al. 2019; Algar et al. 2019) | ∗ Can be performed in cells or *in vitro*<br>∗ Can measure exact distances | ∗ Requires dual labelling (fluorescent pairs must be carefully chosen) |
| | **AlphaScreen/LISA** | Small quantities of suitably tagged P and L for binding to acceptor and donor beads | (Yasgar et al. 2016) | ∗ Compatible with a wide range of complex media<br>∗ Easily adapted to high-throughput screening | ∗ Requires specialist fluorescence plate reader<br>∗ Signal is temperature sensitive<br>∗ Beads are light sensitive |
| | **DSF** | 10 µg protein per replicate, 10–20 molar excess ligand | (Gao, Oerlemans, and Groves 2020) | ∗ Binding affinities are usually relative unless performed in an isothermal setup<br>∗ Easy to screen large ligand sets<br>∗ Low sample requirements | ∗ Fluorescent ligands can interfere<br>∗ Difficult to interpret for multidomain proteins |

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| **Structural information** | **XL-MS** | 10–100 µg protein of interest | (Steigenberger et al. 2020; Klykov et al. 2018) | ∗ Can be applied to wide range of samples<br>∗ Relatively limited technical requirements | ∗ Effect of protein flexibility on XL-MS data not well understood |
| | **HDX-MS** | nanomole quantities @ <1 µM | (Hodge, Benhaim, and Lee 2020) | ∗ Can be used for large, multi-component complexes<br>∗ Low sample requirement compared to many biophysical methods | ∗ Peptide rather than residue level resolution<br>∗ Requires relatively sophisticated setup and expert analysis |


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