

Directory	Sites	Ligands	Method	Notes
Cyp_CsA+	1	1	fluorescence intensity	<p>Basic protein-ligand Kd determination</p> <p>Cyp = recombinant human cyclophilin CsA+ = Cyclosporin A labeled with a fluorescent probe (+)</p> <p>This is the control experiment, a prerequisite for analysis of competitive displacement assay data using the unlabeled CsA (molecule of interest)</p>
Cyp_CsA_CsA+	1	2	fluorescence intensity	<p>Competitive ligand displacement assay</p> <p>Cyp = recombinant human cyclophilin CsA = Cyclosporin A CsA+ = Cyclosporin A labeled with a fluorescent probe (+)</p> <p>Kd for the spectroscopically "invisible" ligand is determined by a competing out the "visible" ligand from the protein-ligand complex. Compare two methods of analysis: (1) determine Kd for CsA+ separately. or (2) determine both Kd's simultaneously.</p>
RIZ1_H3pep	1	1	NMR	<p>NMR chemical shift titration to determine weak protein-protein Kd</p> <p>RIZ1 = tumor suppressor protein H3pep = synthetic peptide representing histone H3</p> <p>Combine chemical shifts for multiple nuclei (¹H and/or ¹⁵H) to increase overall sensitivity of Kd measurement.</p>
TDG_16U11+	2	1	fluorescence polarization	<p>Two binding sites: one strong ("specific") and one weak ("nonspecific")</p> <p>TDG = thymine DNA glycosylase 16U11+ = Texas Red (+) labeled 28 bp DNA fragment, U as target nucleotide</p> <p>Two-site vs. one-site model discrimination analysis.</p>
ThT_22AG	4	1	ESI-MS	<p>Mass spectrometry titration: Four cooperative binding sites</p> <p>ThT = Thioflavin T 22AG = human telomeric G-quadruplex sequence dAGGG(TTAGGG)₃</p> <p>"Progressive cooperativity": Each added ligand is causing the subsequent ligand to bind ever more strongly (up to four binding sites).</p>
WIN61651_p56lck	2	2	³² P liquid scintillation counting	<p>Initial rate enzyme kinetics: determine mechanism / mode of inhibition</p> <p>WIN61651 = small molecule kinase inhibitor (Sterling Winthrop) p56lck = p56^{lck} kinase</p> <p>Determining the mode of inhibition (competitive, non-competitive, mixed type) is strongly affected by substrate inhibition.</p>
Lig_Prot_BDom	1	2	(unspecified)	<p>Full-length protein and just the binding domain compete for a ligand of interest</p> <p>Lig = some type of ligand (undisclosed) Prot = some type of protein (undisclosed) BDom = a binding domain taken out of this protein and expressed separately</p> <p>The purpose of this experiment is to determine the affinity (Kd) between the binding domain and the ligand. One big problem is that only the binding to the full-length protein can be observed, and -- most importantly -- the binding to the full length protein is approximately 50 times weaker than binding to the binding domain. This very large difference in binding affinities presents a significant challenge for experiment design.</p>