Typical Strategies for Common Problems

problem under study:	sedimentation equilibrium	sedimentation velocity
determine the molar mass of a tight protein complex (assume known partial-specific volume)	single gradient possible, but desirable are 2 – 3 rotor speeds, 2 – 3 concentrations; Model with a single exponential to extract Mw **advantage:** direct measurement, usually reliable estimate, error < 5% **disadvantage:** resolves contaminants only poorly, experiment takes up to a few days	single SV experiment is possible, but desirable is a dilution series of concentrations; Modeling with the Lamm equation is in theory possible but usually very sensitive to heterogeneity and only gives a lower limit of M; c(M) can be advantageous; optimal is a hybrid discrete continuous model describing impurities with continuous sections, floating Mw of main discrete component **advantage:** relatively tolerant of impurities outside the size-range of interest (they will be resolved), takes several hours **disadvantage:** frequently lower precision (~ 10 %)
determine the oligomeric state of a membrane protein in detergent solution	density compensation, or Edelstein-Schachman technique {Edelstein, 1967 #186}	
determine the purity of the sample, detect protein aggregates	results can be highly variable dependent on the nature of the sample	long-column, high-speed SV with c(s) analysis is the method of choice, very sensitive for detection of higher oligomers, stable complexes
determine the number of species	(variable resolution)	long-column SV with c(s) gives better resolution
presence of self-association	dilution series, powerful negative control: can all data be fit with a single species model?	dilution series, test: are c(s) peak position concentration dependent?
kinetics of association	no information possible	diagnostics: are c(s) peak positions concentration dependent, or shift only the peak heights with concentration? global modeling of sedimentation boundaries
determine the stoichiometry of a weak protein complex	dilution series, model SE globally with different stoichiometries, compare	dilution series With c(s) analysis, analyze global model isotherm of $s_w(c)$ with models of different stoichiometries **advantage:** may work better than SE when complexes cannot be populated well, shape of c(s) may give hint of complexes formed. Tolerant of some impurities. Alternative: global modeling of sedimentation boundaries; **advantage:** boundary shape can exhibit a characteristic shape for higher-order oligomerization, but is very dependent on association kinetics
determine the association constant of self-association	dilution series, model SE globally **advantage:** very direct, use prior molar mass information complication: need good estimate of partial-specific volume, in particular for weak self-association	dilution series with c(s) analysis, global model of isotherms sw(c) **disadvantage:** need to span a very large range of concentrations, since s(1) and s(n) are not known a priori (in contrast to M(1) and M(n) for a given association scheme)
determine association constant of heterogeneous and mixed protein interaction	sediment and completely characterize the sedimentation behavior of both components separately, then use dilution series of mixture, model SE globally **advantage:** very direct, use prior molar mass information; no need to know partial-specific volume	sediment and completely characterize the sedimentation behavior of both components separately, then use dilution series of mixture With c(s) analysis, global model of isotherm of sw(c) Alternative: global modeling of sedimentation boundaries; **disadvantage:** dependent on association kinetics
hydrodynamic shapes of complexes, ligand-induced conformational change	no information possible	populate complex near saturation, best in concentration series to verify limiting s-value of peak in c(s); followed by hydrodynamic modeling.