Eur Biophys J (2003) 32: 427–431
DOI 10.1007/s00249-003-0318-7

ARTICLE

Joachim Behlke · Otto Ristau

Sedimentation equilibrium: a valuable tool to study homologous and heterogeneous interactions of proteins or proteins and nucleic acids

Received: 19 July 2002 / Revised: 20 February 2003 / Accepted: 8 April 2003 / Published online: 29 May 2003

Abstract We present a short overview of our experience in analyzing the affinity and stoichiometry of self-associating and heterologous interactions by using the sedimentation equilibrium technique. Data acquisition and the fitting procedure employing the computer programs that we have developed, Polymole and Virial, are utilized for obtaining reliable results under ideal as well as non-ideal conditions. Such data derived from biologically important macromolecules find utility in understanding physiological events such as cell regulation.

Keywords Analytical ultracentrifugation · Binding constants · Heterogeneous association · Self-association · Virial coefficients

Introduction

Sedimentation equilibrium is a useful and powerful tool to determine the affinity and stoichiometry of interaction of numerous physiologically important molecules (for recent reviews, see: Minton 1990, 1997; Behlke and Ristau 1997; Laue and Stafford 1999; Rivas et al. 1999). Such interactions include the binding of two or more identical molecules (self-association) or different substances (heterogeneous association), e.g. complex formation between proteins or proteins and nucleic acids, and are frequently associated with alterations in the physiological properties of the reactants (Bär et al. 1988; Benndorf et al. 1994; Kisker et al. 1996; Behlke and Ristau 1997; Steinmetzer et al. 1998; Sossong et al. 1999). Thus, elucidation of the characteristics of these interactions can contribute to a better understanding of the in situ regulation of such vital cellular events as protein folding, and metabolic pathways.

Here we summarize our experience as well as the developed programs in analyzing these kinds of interactions under ideal and non-ideal conditions.

Presented at the conference for Advances in Analytical Ultracentrifugation and Hydrodynamics, 8–11 June 2002, Grenoble, France

J. Behlke (✉) · O. Ristau
Max Delbrück Center for Molecular Medicine,
Robert Rössle Strasse 10, 13092 Berlin, Germany
E-mail: behlke@mdc-berlin.de
Fax: +49-30-94062802

Materials and methods

Theoretical aspects

The estimation of binding constants, especially of heterogeneous systems, is limited by difficulties in data analysis (Philo 2000). Numerical constraints have been developed to overcome these difficulties (Lewis et al. 1994; Minton 1994; Behlke et al. 1997; Arkin and Lear 2001). One constraint is the use of mass conservation, while a second is statistically scaled binding constants that are especially useful for heterogeneous associations. Mass conservation employs the integration of the concentration distribution function (or parts) to determine the total amount of each component; the second method assumes equal binding sites for the ligand (see Wyman and Gill 1990).

Self-association

Ideal solutions. The radial concentration distribution (c_r) of solutions containing different non-interacting compounds can be described by Eq. (1) [from Tiselius (1926), Svedberg and Pedersen (1940) or Haschemeyer and Bowers (1970)]:

$$c_r = \sum_i c_{i,rm} \exp\left[F_i M_i \left(r^2 - r_m^2\right)\right] \quad (1)$$

with:

$$F_i = \left[(1 - \rho\bar{v}_i)\omega^2/2RT\right] \quad (2)$$

where is c the molar concentration, ρ the buffer density, v_i the partial specific volume of component i, ω the angular velocity, R the gas constant and T the absolute temperature. Proteins are usually solved in buffer or electrolytes. Thus, formally there is a three-component system, but at low physiological concentrations we can employ the usual buoyancy term for two-component systems. When the concentrations of these low molecular weight solutes are high (approximately >0.2 M), it is necessary to substitute the buoyancy term ($1-\rho v_i$ by the exact term ($\partial\rho/\partial c$) (Casassa and Eisenberg 1961, 1964).

For associating systems, which exist in an equilibrium between monomers and oligomers, Eq. (3) is obtained:

$$K_i = c_{i,\mathrm{rm}}/c^i_{1,\mathrm{rm}} \tag{3}$$

Substitution of $c_{i,\mathrm{rm}}$ in Eq. (1) leads to Eq. (4):

$$c_\mathrm{r} = \sum_i iK_i c^i_{1,\mathrm{rm}} \exp\left[FM_i\left(r^2 - r_m^2\right)\right], \quad K_1 = 1 \tag{4}$$

where c_r is the total concentration expressed in monomers. The binding constants K_i can be directly estimated by fitting the radial concentration distribution to Eq. (4). However, to obtain more reliable results it is important to use mass conservation as a numerical constraint. This requires knowledge of the loading concentration used in the experiment. For the fitting procedure, records of three different wavelengths are usually taken. We have developed a special computer program, Polymole, that additionally yields the partial concentrations (Behlke et al. 1997).

Non-ideal conditions. Solutions of charged macromolecules at higher concentration ($c > 1$ mg/mL) or dissolved in very dilute buffers far from the isoelectric point are considered to be non-ideal. As a consequence, it is necessary to consider the virial coefficients of the solute to describe the influence of charge and excluded volume on the association constants. The virial coefficients used in Eq. (5) are statistical-mechanical quantities defined by the solution theory of McMillan and Mayer (1945). Therefore, the radial concentration distribution, e.g. of a monomer–dimer equilibrium, is:

$$c_\mathrm{r} = z + 2(K_2 - B_{20})z^2 + \left(6B_{20}^2 - \frac{3}{2}B_{30} - 3B_{11}K_2\right)z^3 \ldots \tag{5}$$

where:

$$z = z_0 \exp\left(\frac{M(1 - \bar{v}\rho_0)\omega^2\left(r^2 - r_0^2\right)}{2RT}\right) \tag{6}$$

The introduction of the virial coefficients in this manner is of great advantage in convergence of the exponential power series by higher concentrations (activities) compared to the often used variant where the virial coefficients are placed in the exponents (Johnson et al. 1981; Wills et al. 1996; Behlke and Ristau 2000). The different virial coefficients (Bs) in Eq. (5) describe the interaction of two monomers (B_{20}), of three monomers (B_{30}) or of one monomer with one dimer (B_{11}). When the interaction can be reduced to the excluded volume, e.g. by addition of neutral salts and eliminating the charge part of non-ideality, all coefficients can be related to the second virial coefficient B_{20}, assuming suitable shapes for the individual complexes (Boublik and Nezbeda 1986).

Hetero-association (ideal systems)

Considering solutions of different reacting macromolecules designated receptor (R) or ligand (L), which are able to form a simple complex of a 1:1 or another stoichiometry, one obtains a radial absorbance distribution (A_r) consisting of a sum of at least three exponential functions according to Eq. (7):

$$A_\mathrm{r} = \varepsilon_\mathrm{R} c_\mathrm{R} \exp(DM_\mathrm{R}F) + \varepsilon_\mathrm{L} c_\mathrm{L} \exp(M_\mathrm{L}F) + c_\mathrm{R} \sum_{j=1}^{n} (\varepsilon_\mathrm{R} + j\varepsilon_\mathrm{L}) c_\mathrm{L}^j K_j \exp(DM_\mathrm{R} + jM_\mathrm{L})F \tag{7}$$

Here, F summarizes the parameters of Eq. (2) and D is the buoyancy ratio of receptor and ligand [$\mathrm{D} = (1-\rho v_\mathrm{R})/(1-\rho v_\mathrm{L})$]. Usually the extinction coefficients (ϵ_R, ϵ_L), the total concentrations of protein and ligand (c_R, c_L) and the molecular masses (M_R, M_L) of the reactants are known or can be determined in separate experiments. In order to increase the accuracy, it is desirable to collect the radial absorbancies at more than one wavelength. Furthermore, it is necessary to employ mass conservation as a numerical constraint, as mentioned above.

Practical approach

Ideal solutions

Sedimentation equilibrium runs were performed in a XL-A analytical ultracentrifuge (Beckman) equipped with UV absorbance scanner optics. Sedimentation equilibrium was analyzed using externally loaded six-channel centerpieces of 12 mm optical path, usually filled with 75 μL in each compartment. This type of cell allows the analysis of three solvent–solution pairs. Three or seven of these cells were used to study different samples in the same run.

Sedimentation equilibrium was reached after 2 h of an appropriate overspeed followed by an equilibrium speed for 24–36 h, usually at 10 °C. When substances were stable enough, equilibrium experiments were carried out at temperatures up to 37 °C to mirror more physiological conditions. The radial absorbancies of each compartment were recorded at three different wavelengths. Equilibrium constants of self-associating proteins were determined by simultaneous fitting of all radial absorbance distribution curves fitted to Eq. (4) using our computer program Polymole (Behlke et al. 1997). This program allows us to use mass conservation and also, when equal binding sites can be assumed, the option of statistically scaled binding constants as an additional constraint. For situations where such an option is not justified, either an analysis without statistically scaled binding constants but with mass conservation is recommended or, when possible, a separate study of binding domains should be done (Hohaus et al. 2002).

Non-ideal solutions (self-association)

In order to take into account the effect of thermodynamic non-ideality, more experimental work is necessary. Therefore, the sedimentation equilibrium experiments were carried out in standard double-sector cells filled with 300 µL of solute and solvent. When the association constants are small, it is not possible to estimate the second virial coefficient B_{20} with suitable accuracy. Nevertheless, in order to determine the association constant, the excluded volume as one contribution to non-ideality can be calculated from the size of the molecule and introduced in our program Virial. This requires preventing any charge effects by the addition of appropriate amounts of neutral salts. Seven cells with different loading concentrations were centrifuged in an eight-hole rotor. The concentration profiles at sedimentation equilibrium were fitted globally using the computer program Virial (Behlke and Ristau 2000). This program uses mass conservation as a numerical constraint.

Results

Self-association

Ideal solutions

Many proteins are able to form concentration-dependent monomer–oligomer equilibria. By using the sedimentation equilibrium technique and fitting the radial concentration distributions according to Eq. (4), the dissociation or association constants can be directly obtained. The correctness of these constants is supported when the sum of all calculated partial absorbancies (or concentrations) of monomers and oligomers at all radius positions corresponds to the recorded

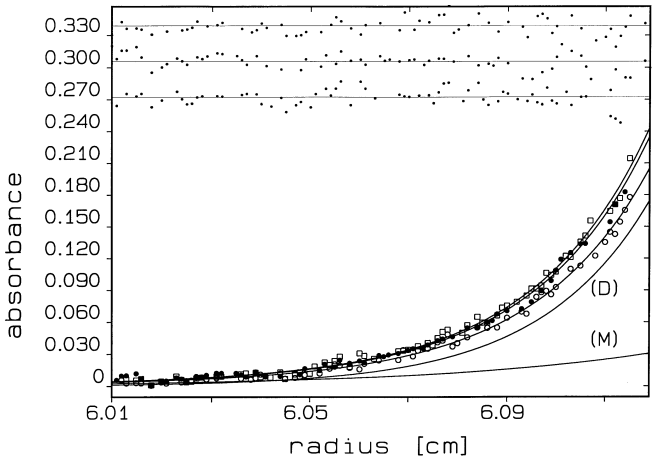

Fig. 1 Radial absorbance distribution of yeast hexokinase (0.081 mg/mL) at sedimentation equilibrium (*symbols*) recorded at 270 nm (*open circles*), 275 nm (*filled circles*) or 280 nm (*open squares*). The data were globally fitted to a monomer–dimer equilibrium using Eq. (4). The areas below the curves are equivalent to monomers (*M*) and dimers (*D*) corresponding to a $K_d = 250$ nM. The demonstrated partial concentrations correspond to the data set at 270 nm

values. Figure 1 demonstrates such radial concentration distributions of yeast hexokinase recorded at three different wavelengths. The curves of monomers and dimers for one wavelength are also shown. In order to obtain reliable data, the molar loading concentration expressed in monomers and the molar absorbancy coefficient for the wavelengths used must be known. Extensive experiments on yeast hexokinase at different loading concentrations have demonstrated a distinct dissociation of dimeric protein into monomers in very dilute solutions of about 2 μg/mL (Golbik et al. 2001). Such studies are of interest because, depending on the oligomeric state of the enzyme, substrate utilization differs and can be the basis of regulation of the metabolic pathway. Furthermore, the addition of effectors such as glucose-6-phosphate or the substrate glucose can considerably alter the dissociation of dimeric hexokinase (Golbik et al. 2001). Moreover, hexokinase dissociation can be increased by phosphorylation of Ser14 (Behlke et al. 1998; Golbik et al. 2001), changing K_d values by an average by three orders of magnitude.

Concentration-dependent monomer–dimer equilibria are evident over a large concentration range of several orders of magnitude. Often both components (parts) possess different enzymatic properties. From the average functional behavior and the knowledge of the partial concentrations, the activity of the monomers or dimers can be derived, as demonstrated for D-amino acid oxidase (Behlke and Ristau 1997).

Non-ideal solutions

The analysis of weak self-association reactions requires protein concentrations clearly higher than 1 mg/mL and therefore consideration of the virial coefficient(s) expressed by the excluded volume of the solute. Similar deviations from the ideal behavior of solutions were observed for charged macromolecules, especially under conditions of low ionic strength. The influence of virial coefficients on the association constants is complicated. Simultaneous consideration of association constants and virial coefficients necessary for protein self-association under non-ideal conditions were first discussed by Hill and Chen (1973), based on statistical thermodynamics to provide a thermodynamic precise description of associates in osmotic solution by a concentration power series. Since the direct application of the Hill–Chen theory to solutions of the centrifuge equation is difficult, Behlke and Ristau (2000) have expressed, analogous to Winzor et al. (1999), the thermodynamic non-ideality by an activity power series (Eqs. 5 and 6). The influence of virial coefficients on the association constants is additive to the ideal variant, as demonstrated in these equations. As checked on synthetic curves, the dependence of the apparent association constants on virial coefficients can be given graphically by use of the following parameter: $\delta = \left(K_2^w - B_{20}^w\right)c_w$. This expression (Behlke and Ristau 2000), with "w" for weight-related quantities, is suitable to describe conditions where the virial coefficients either scarcely ($\delta = 0.1$–1.0) or considerably (δ beyond these values) influence the dimerization constant (K_2^w). Experimental evidence could be given for the analysis of the Con A dimer–tetramer equilibrium using Eqs. (5) and (6). The calculated constant $K_2^w = 9.5 \pm 0.6$ g/L, obtained for $\delta = 2.0$, deviates only insignificantly when considering the virial coefficient (Behlke and Ristau 2000). Careful analysis of virial coefficients is also important for determining the state of oligomerization. Whereas Smalla et al. (1999) proposed a monomer–dimer–tetramer equilibrium for the receptor tyrosine kinase EphB2 SAM domain, we demonstrated that a monomer–dimer equilibrium exists only up to concentrations of 12 g/L when considering the thermodynamic non-ideality. The association constant for the dimerization $K_2 = 163$ M^{-1} at 10 °C is extremely low (Behlke et al. 2001).

Heterogeneous association

Interaction of different proteins or proteins and nucleic acids are of interest when such complexes fulfill a biological function. The charged initiator tRNA, fMet-$tRNA^{fMet}$ and the translation initiation factor IF2, together with GTP and 50S ribosomal subunits, are essential parts of protein biosynthesis. A short sequence of the C-terminal part of IF2 (IF2-C2) is crucial for the interaction with fMet-$tRNA^{fMet}$. The reactants (fMet-$tRNA^{fMet}$ and IF2-C2) in mixtures of different molar ratios were centrifuged until sedimentation equilibrium was reached and analyzed by fitting the data using Eq. (7) (Fig. 2). When analyzing protein–nucleic acid interactions it is desirable to measure the radial

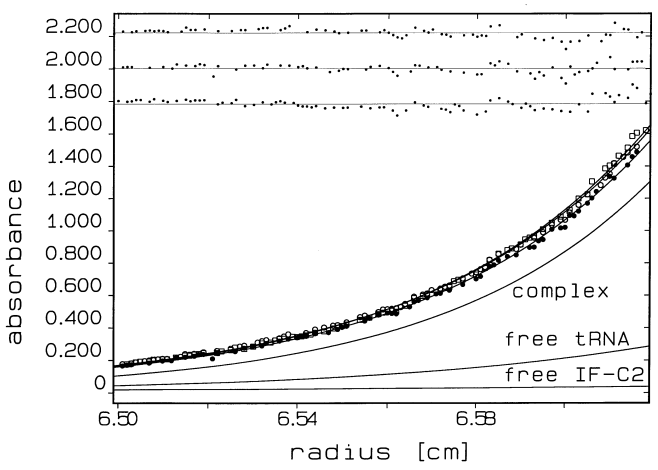

Fig. 2 Equilibrium sedimentation of a mixture of 1.5 μM fMet-tRNA^{fMet} ($M = 26.0$ kDa) and 1.5 μM IF-C2 ($M = 12.89$ kDa) at 24,000 rpm and 20 °C. The data recorded at 230 nm (*open circles*), 235 nm (*filled circles*) or 240 nm (*open squares*) were globally fitted. The *solid curves* represent the free reactants and a 1:1 complex resulting in a K_d of 135 nM

absorbance distributions at more than three different wavelengths. When the reactants are not temperature sensitive, it is useful to carry out the experiments at different temperatures; this allows determination of thermodynamic constants such as free energy, enthalpy or entropy (Krafft et al. 2000).

When receptor molecules have more than one ligand binding site, it is suitable to consider binding according to the statistical binding model (Wyman and Gill 1990). This is of advantage since only the binding constant for the first binding step has to be determined and can be done with high precision (Behlke et al. 1997). In the case of two identical binding sites, the affinity of the second binding step amounts to one quarter of the first one (Wyman and Gill 1990). This is only justified when assuming identical binding sites, for example for oligomeric proteins with defined quaternary structure or oligonucleotides with repeating sequences (Schade et al. 1999; Schwartz et al. 2001). The program Polymole (Behlke et al. 1997) allows analyzing radial concentration distributions with and without statistics but always with mass conservation. To evaluate the assumption of identical binding sites, the obtained statistically scaled *K*-values can be entered into the subprogram without this statistical constraint. When the start values are distinctly modified, it can be concluded that there are non-identical binding sites. In both subprograms, mass conservation should be considered. Such behavior of different binding sites was observed for the regulatory β2a protein subunit of the Ca channel that binds two molecules of ahnak with different affinity, as has been analyzed in an additional study of the separate β2a domains (Hohaus et al. 2002).

Discussion

In this short overview we present our approaches to the study of self- and heterogeneous association of macromolecules during the last five years. Detailed computer programs were developed to obtain reliable results on the stoichiometry and affinity of interacting macromolecules. Difficulties in the data analysis of sedimentation equilibrium experiments were treated by incorporating results from other groups (Boublik and Nezbeda 1986; Minton 1990, 1994, 1997; Lewis et al. 1994; Wills et al. 1996; Winzor et al. 1999; Philo 2000), as well as by developing novel strategies.

Binding constants were often derived from concentration-dependent weight-average molecular masses (M_w). Using the programs Polymole and Virial, our aim was the direct determination of equilibrium constants employing the constraints of "mass conservation" and, when possible, also of the statistical binding model. Support for the correct treatment of model-dependent data analysis is the inspection of the residuals, which should be distributed statistically. Generally, one can differentiate between wrong models and sudden deviations based on nonspecific aggregates near the cell base; omitting such a few wrong data points without strong alteration of the loading concentration leads to more accurate results. An accurate fit suggests that a model is correct, but a large concentration range should be considered in the data analysis.

Additional difficulties can appear in non-ideal systems. The evaluation of weak interactions between the macromolecules requires higher loading concentrations of clearly more than 1 mg/mL and therefore a consideration of the virial coefficients. To overcome this difficulty, our program Virial (Behlke and Ristau 2000) allows us to use seven cells filled with different loading concentrations (eight-hole rotor). The obtained seven radial distribution curves are simultaneously fitted. Neglecting the non-ideality can result in incorrect values for stoichiometry or binding constants (Smalla et al. 1999; Behlke and Ristau 2000, Behlke et al. 2001).

We suggest that the approaches presented here will be useful in understanding interactions in biological systems. We hope this will be of interest for AUC users as well as for biologists who are interested in combining physiological and structural results to better understand cell physiology.

Acknowledgements Many of the experiments presented here were carried out in fruitful collaboration with Thomas Kriegel, Dresden; Sabine Brantl, Jena; Heinz Welfle, Berlin; Udo Heinemann, Berlin; Hartmut Oschkinat, Berlin; Alexander Rich, Cambridge, Mass.; Hannelore Haase, Berlin. We are grateful to all these colleagues and thank Howard Etlinger for critical reading the manuscript. The programs Polymole and Virial are available on request.

References

Arkin M, Lear JD (2001) A new data analysis method to determine binding constants of small molecules to proteins using equilibrium analytical ultracentrifugation with absorption optics. Anal Biochem 299:98–107

Bär J, Behlke J, Huse K, Schulz W, Kopperschläger G (1988) Phosphofructokinase from bakers yeast: properties of a proteolytically modified active enzyme form. Int J Biol Macromol 10:99–105

Behlke J, Ristau O (1997) Analysis of interacting biopolymer systems by analytical ultracentrifugation. Eur Biophys J 25:325–332

Behlke J, Ristau O (2000) Analysis of protein self-association under conditions of the thermodynamic nonideality. Biophys Chem 87:1-13

Behlke J, Ristau O, Schönfeld H-J (1997) Nucleotide-dependent complex formation between the *Escherichia coli* chaperonins GroEL and GroES studied under equilibrium conditions. Biochemistry 36:5149–5156

Behlke J, Heidrich K, Naumann M, Müller EC, Otto A, Reuter R, Kriegel T (1998) Hexokinase 2 from *Saccharomyces cerevisiae*: regulation of oligomeric structure by *in-vivo* phosphorylation at serine-14. Biochemistry 37:11989–11995

Behlke J, Labudde D, Ristau O (2001) Self-association studies on the EphB2 receptor SAM domain using analytical ultracentrifugation. Eur Biophys J 30:411–415

Benndorf R, Hayess K, Ryazantsev S, Wieske M, Behlke J, Lutsch G (1994) Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. J Biol Chem 269:20780–20784

Boublik T, Nezbeda I (1986) P-V-T behaviour of hard body fluids. Theory and experiment. Collect Czech Chem Commun 51:2301–2432

Casassa EF, Eisenberg H (1961) Partial specific volumes and refractive index increments in multicomponent systems. J Phys Chem 65:427–435

Casassa EF, Eisenberg H (1964) Thermodynamic analysis of multicomponent solutions. Adv Protein Chem 19:287–395

Golbik R, Naumann M, Otto A, Müller EC, Behlke J, Reuter R, Hübner G, Kriegel TM (2001) Regulation of phosphotransferase activity of hexokinase 2 from *Sacchoromyces cerevisia* by modification at serine-14. Biochemistry 40:1083–1090

Haschemeyer RH, Bowers WH (1970) Exponential analysis of concentration or concentration difference for discrete molecular weight distributions in sedimentation equilibrium. Biochemistry 9:435–445

Hill TL, Chen YD (1973) Theory of aggregation in solution. I. General equations and application to the stacking of bases, nucleotides, etc. Biopolymers 12:1285–1312

Hohaus A, Person V, Behlke J, Schaper J, Morano I, Haase H (2002) The C-terminal region of ahnak provides a link between cardiac L-type Ca^{2+} channels and the actin-based cytoskeleton. FASEB J 16:1205–1216

Johnson ML, Coreira JJ, Yphantis DA, Halvorson HR (1981) Analysis of data from the analytical ultracentrifuge by nonlinear least-squares techniques. Biophys J 36:575–588

Kisker C, Schindelin H, Alber BE, Ferry JG, Rees DC (1996) A left-handed β-helix revealed by the crystal structure of a carbonic anhydrase from the archeon *Methanosarcina thermophila*. EMBO J 15:2323–2330

Krafft C, Diehl A, Lättig S, Behlke J, Heinemann U, Pon CL, Gualerzi C, Welfle H (2000) Interaction of fMet-$tRNA^{Met}$ with the C-terminal domain of translational initiation factor IF2 from *Bacillus stearothermophilus*. FEBS Lett 471:128–132

Laue TM, Stafford WF (1999) Modern applications of analytical ultracentrifugation. Annu Rev Biophys Biomol Struct 28:75–100

Lewis MS, Shrager RI, Kim S-J (1994) Analysis of protein-nucleic acid and protein-protein interactions using multi-wavelength scans from the XL-A analytical ultracentrifuge. In: Schuster TM, Laue TM (eds) Modern analytical ultracentrifugation. Birkhauser, Boston, Mass., pp 94–115

McMillan WG, Mayer JE (1945) The statistical thermodynamics of multicomponent systems. J Chem Phys 13:276–305

Minton AP (1990) Quantitative characterization of reversible molecular associations via analytical centrifugation. Anal Biochem190:1-6

Minton AP (1994) Conservation of signal: a new algorithm for the elimination of the reference concentration as an independently variable parameter in the analysis of sedimentation equilibrium. In: Schuster TM, Laue TM (eds) Modern analytical ultracentrifugation. Birkhauser, Boston, Mass., pp 81–93

Minton AP (1997) Alternative strategies for the characterization of associations in multicomponent solutions via measurement of sedimentation equilibrium. Prog Colloid Polym Sci 107:11–19

Philo JS (2000) Sedimentation equilibrium analysis of mixed associations using numerical constraints to impose mass or signal conservation. Methods Enzymol 321:100–120

Rivas R, Stafford W, Minton AP (1999) Characterization of heterologous protein-protein interactions using analytical ultracentrifugation. Methods 19:194–212

Schade M, Behlke J, Löwenhaupt K, Herbert A, Rich A, Oschkinat H (1999) A 6 bp Z-DNA hairpin binds two Zα domains from the human RNA editing enzyme ADAR1. FEBS Lett 458:27–31

Schwartz T, Behlke J, Löwenhaupt K, Heinemann U, Rich A (2001) Structure of the DLM-1:Z-DNA complex reveals common recognition features within a conversed family of Z-DNA-binding proteins. Nat Struct Biol 8:761–765

Smalla M, Schmieder P, Kelly M, Ter Laak A, Krause G, Ball L, Wahl M, Bork P, Oschkinat H (1999) Solution structure of the receptor tyrosine kinase EphB2 SAM domain and identification of two distinct homotypic interaction sites. Protein Sci 8:1954–1961

Sossong TM Jr, Brigham-Burke MR, Hensley P, Pearce KH Jr (1999) Self-activation of guanosine triphosphatase activity by oligomerization of the bacterial cell division protein FtsZ. Biochemistry 38:14843–14850

Steinmetzer K, Behlke J, Brantl S (1998) Plasmid pIP501 encoded transcriptional repressor CopR binds to its target DNA a dimer. J Mol Biol 283: 595–603

Svedberg T, Pedersen KO (1940) Die Ultrazentrifuge. Steinkopff, Dresden

Tiselius A (1926) Über die berechnung thermodynamischer eigenschaften von kolloidalen lösungen mit der ultrazentrifuge. Z Physik Chem (T) 124:449–463

Wills PR, Jacobsen MP, Winzor DJ (1996) Direct analysis of solute self-association by sedimentation equilibrium. Biopolymers 38:119–130

Winzor DJ, Jacobsen MP, Wills PR (1999) Allowance for the thermodynamic nonideality in the analysis of sedimentation equilibrium distributions reflecting complex formation between dissimilar reactants. Prog Colloid Polym Sci 113:69–75

Wyman J, Gill SJ (1990) Binding and linkage. University Science Books, Mill Valley, Calif