CHAPTER 15

Analysis of Tubulin Oligomers by Analytical Ultracentrifugation

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Abstract

This chapter describes the use of analytical ultracentrifugation in a Beckman XLA to study the self-association properties of tubulin and the interaction of tubulin with antimitotic drugs. Procedures for sample preparation, operation of the ultracentrifuge, and collection of data conform to standard modern methods. Analysis of sedimentation velocity data initially includes generation of $g(s)$ sedimentation coefficient distributions with DCDT+2 and determination of weight average sedimentation coefficients $S_w$. $S_w$ versus concentration data are then fit to isodesmic or indefinite assembly models to extract $K_{iso}$ values, the association constant for each successive assembly step. Alternatively the raw data can also be analyzed by direct boundary analysis methods using the program Sedanal. Direct boundary analysis also extracts the $K_{iso}$ value by fitting to the shape of the sedimentation boundary as a function of total concentration. While the fitting of weight average data as a function of protein or drug concentration to indefinite assembly models has been shown to be equivalent to direct boundary fitting of multiple data sets with Sedanal, direct
boundary fitting is preferred because it robustly identifies the presence of irreversible aggregation or mechanisms that are more complex.

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**I. Introduction**

Analytical ultracentrifugation (AUC) has been a central technique in the development of our understanding of cytoskeletal components. Beginning in the 1970s with the discovery of isolation methods for tubulin and microtubules, numerous labs investigated the self-assembly properties of mammalian tubulin and its interaction with microtubule-associated proteins (MAPs). While many studies focused on assembly of microtubules by electron microscopy and turbidity approaches, investigations into the mode of association, stoichiometry, energetics, and thermodynamics of tubulin self-association were also explored in the Timasheff lab. These studies revealed the ability of tubulin to form indefinite spiral polymers and rings as a function of Mg\(^{2+}\) (Frigon and Timasheff, 1975a,b; Howard and Timasheff, 1986) and vinca alkaloid concentrations (Na and Timasheff, 1980, 1986). Detailed energetic and thermodynamic studies on families of vinca alkaloid derivatives have now also been performed (reviewed in Lobert and Correia, 2000; Lobert et al., 1995, 1996, 1997, 1998a,b, 2000, 2007) and correlated with IC\(_{50}\) and clinical drug doses (Correia and Lobert, 2001, 2008; Lobert et al., 2000, 2007). MAPs are also able to interact with tubulin heterodimers to make rings as demonstrated for both tau (Devred et al., 2004; Dönges et al., 1976) and MAP2 (Kirschner et al., 1974; Marcum and Borisy, 1978a,b; Scheele and Borisy, 1978; Valle and Borisy, 1978). More recently the hetero-interaction of tubulin with stathmin to make a 2:1 complex has been investigated by AUC approaches (Alday, 2009; Alday and Correia, 2009; Jourdain et al., 1997, 2004; Chapter 23 by Devred et al., this volume), while a surprisingly similar system involving interactions between actin and spire has also been studied (Bosch et al., 2007). Finally, molecular motors that interact with microtubules have been extensively characterized by AUC methods (Cochran et al., 2004; Correia et al., 1995; Foster et al., 1998; Mackey et al., 2004; Rosenfeld et al., 1996a,b, 2009). Here I present the methods we use to study tubulin self-association into small oligomers, focusing on experimental design, modeling, and data analysis.

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**II. Materials and Methods**

**A. Sample Equilibration, Buffer Composition, and Concerns for AUC Work**

We will assume you have tubulin available in sufficient quantity and will only comment briefly where appropriate on isolation from brain (see Chapter 1 by Miller and Wilson, this volume, although we use glycerol preps), purification, or handling.

AUC data are typically collected in double beam mode with a reference sector (exceptions are the new FDS system (MacGregor et al., 2004) and the pseudo absorbance mode (Kar et al., 2000)). Thus, samples must be equilibrated so the reference blanks any buffer component signals. For rigorous thermodynamic work this means in the exhaustive dialysis tradition (Casassa and Eisenberg, 1964). However, tubulin is relatively unstable and prone to aggregation and must be
prepared quickly for AUC work. Thus, we prefer a G-50 fine spun column method (Penefsky, 1979; we refer to this as a Penefsky) that equilibrates the sample in a few minutes. Our typical buffers are 10–100 mM Pipes or Mes, 1–10 mM MgSO$_4$, 2 mM EGTA, 50 µM GXP, pH 6.5–7.5. In addition, while tubulin stability requires the presence of GXP, absorbance measurements in an XLA cannot be performed above ~100 µM GXP or AXP because of degraded signal to noise. Similar concerns apply to the use of mM concentrations of DTT or DTE, but this is mostly a problem with oxidized reducing agent and thus old buffer. Keeping reductant concentrations at 0.1 mM is typically sufficient for activity while avoiding baseline problems. (We prefer to use 1 mM TCEP since it has much less absorbance, is not oxidized by air, and demonstrates an absence of pH dependence, although reaction with protein can produce small baseline drifts that are most evident in equilibrium runs.)

1. Spun columns are prepared from pre-hydrated G-50 fine (stored cold in 0.01% NaAzide) and 5-ml syringes with a polyethylene frit placed in 15-ml polypropylene conical tubes. The resin typically is allowed to settle at the 5-ml line and then the column is gravity washed with at least three column volumes of the desired equilibration buffer. Columns can be made in advance and stored at 4°C as long as you keep the surfaced covered with buffer to avoid resin drying out. Just before use, allow all buffers to gravity flow through the column and pour off the flow through.

2. We store our PC-tubulin (~4–10 mg/ml) dropwise frozen in liquid N$_2$ and then keep at −80°C in plastic bottles. A sufficient weight of tubulin beads (often 2–3 beads ~0.2 g) is carefully poured into a 10-ml glass beaker and weighed. The beaker is placed in a shallow pool of room temperature water (in a plastic petri dish) and 0.3 ml of buffer is added to the beads to speed up thawing. When the beads thaw the beaker is moved to an ice bucket.

3. While the beads are thawing centrifuge the spun column and the 15-ml polypropylene conical tube in a table top clinical centrifuge at speed 5 (max speed 7) for 2 min at 4°C. (Older clinical centrifuges have a rheostat design and we set approximately half speed—the appropriate speed needs to be checked to ensure complete recovery of material without drying out the resin in the process (see Penefsky, 1979)).

4. Discard the flow through buffer and place the spun column in a dry 15-ml conical tube.

5. Slowly dropwise load the thawed PC-tubulin (or MAP or stathmin) onto the dried column, being sure not to squirt material down the sides before the gel re-swells. Centrifuge at speed 5 for 2 min. The column flow-through tubulin will be equilibrated in the chosen buffer. Repeat this step for buffers containing glycerol. (We recycle the G-50 by dumping the used resin in a 500-ml flask with water and 0.01% NaAzide until a sufficient quantity accumulates for refining and extensive washing with deionized water.)

6. Mix the flow-through tubulin well, dilute 10-fold (60/600 µl) and measure the OD at 278 nm blanking with the appropriate buffer. The tubulin concentration is 10× OD/1.2 in mg/ml or 10 times larger in µM (tubulin is a 100-KD heterodimer). The concentration recovered is typically >90% of the loaded material and will thus depend upon the weight of the original frozen beads thawed and the volume of the dilution buffer (Application of this technique to
other proteins (tau or MAP2) should work equally well, although you may need to use G-10 for proteins the size of stathmin to achieve recovery. For details see Lobert and Correia, 2007).

7. A typical XLA 1.2-cm Beckman centerpiece holds ~450 µl so we make up three samples of 500–600 µl at the desired OD in 1.5-ml Eppendorf tubes. Label them A, B, C, lowest to highest concentration, to indicate which XLA cell to fill corresponding to which rotor hole the cell goes in. Mix each sample well by slight vortexing, followed by a brief 10-s spin in an Eppendorf bench top centrifuge (5415 R) to recover the full volume for cell filling. Note Beckman centerpieces come in 1.2- and 0.3-cm path lengths, so tubulin samples prepared at 0.1–5 mg/ml can be readily spun in an XLA (ε278 nm = 1.2) (see Stafford (2009) for a discussion of concerns for operation with interference optics).

B. Filling Cells, Setting Up the Instrument, and Performing a Run

1. Assemble three clean analytical cells being sure to torque at least three times to assure the desired tightness (125–150 depending upon the condition of your centerpieces). We use BD 1-ml tuberculin slip tip syringes fitted with a BD 24-gauge blunt tip syringe needle for cell filling. You can use the 27 G pointed needles that often come with these syringes if you can avoid scratching the surface of the centerpiece (can cause leaks) or the walls of the sector (can cause convection). With the filling holes up and screw ring facing toward you, fill the left side of each cell with reference buffer. While holding the cell in one hand with backlight to improve vision, fill to the very top leaving a small air bubble. (An alternative is to fill with a predetermined volume in both sectors, ~450 µl.) Now fill each sample side going from lowest to highest concentrations, again filling to the top but not exceeding the height of the reference sector. Filling in this order allows you to fill all reference and sample sectors with a single syringe.

a. While new-generation centerpieces are being produced, current Beckman velocity centerpieces are rated at 42 K. Most old timers ignore these limits since we all use to spin them up to 60 K. But with time they will fail and crack, usually at the center rib. Camus (Durham, NH) produces SEDVEL50K and SEDVEL60K centerpieces that have a wider rib, thus providing more stability, while using less sample volume. The filling holes are also sculpted to not trap air and are thus easier to fill to the very top.

b. It has become more common to use a centerpiece with a channel between the reference and sample at the bottom of the sectors (also sold by Camus as meniscus matching cells, SedVel60-MM). At low speed (<10 K) this allows reference buffer to flow into the sample side causing perfect matching of the menisci. The run must be stopped and the cell shaken to mix the sample with this dilution buffer. The purpose of this is to avoid signal due to radial mismatch of buffer components. This is more critical with interference optics and not typically an issue with the XLA.

c. It has become common for users to under fill cells and thus have a meniscus at 6.1 cm rather than at 5.9 cm or less. While you save sample volume you also lose resolution because separation of zones is enhanced by longer sedimentation columns (recall the adage of a long, thin column in chromatographic separation). We strongly recommend maximum filling to
achieve the longest sedimentation distance and thus the highest resolution possible (Stafford, 2009).

2. Seal the cells (we use red polyethylene plastic sheets as gasket plugs, cut with an old style Beckman punchers on a block of wood) and align them in the rotor (sample A in hole 1, sample B in hole 2, etc.). Place the rotor and monochromator in the chamber, push the vacuum button, and allow the rotor to come to temperature equilibrium before starting the run (We leave the rotor and the monochromator in the chamber under vacuum all the time, and pre-equilibrate temperature to speed up the process. We also calibrate the temperature by the method of Liu and Stafford, 1995; a 20°C setting gives an actual temperature of 19.7°C in our XLA.)

3. The current Beckman software (ProteomeLab XL-A/XL-I) requires you to setup wavelength and radial data for each cell. We typically scan at 278 nm (the peak in a tubulin absorbance scan) from 5.8 to 7.2 cm collecting a single flash of the lamp at a spacing of 0.002 cm. The speed should be chosen to collect at least 50–75 useable scans prior to pelleting of the boundary. Select at least 99 scans per cell (for proper c(s) analysis you want to pellet the smallest material) and the option stop XLA after last scan. Set up parameters and click on Start Methods Scan before the vacuum gets below 50 µm (The prohibition of running above 50 µm only applies to runs over 3 K and only with an analytical rotor. If you mistakenly click on Start Method Scan when the vacuum is below 50 µm the run will start regardless of temperature. You cannot just click on the vacuum button on the front panel and expect to achieve temperature and full vacuum in a reasonable time; the Start Method Scan button also turns on the diffusion pump, which is required to achieve full vacuum quickly).

a. We strongly recommend buying the Analytical Ultracentrifuge Service Manual for your XLA/XLI—this was part #679045 but may have changed with new software/firm ware versions. It is currently sold as a pdf and provides numerous tests and procedures useful for proper maintenance of your instrument, including how to calibrate wavelength and radial position, how to check lamp intensity, and how to calibrate delay times.

4. At temperature click on the start button on the front of the machine and watch the first few scans to be sure data are on scale and that there are no leaks. At the end of the run the data are compressed into a zip file to save disk space on the controller. These files should be transferred to another computer and stored in the folder format required for Sedanal analysis (Sedanal/User_Data/ProjectName/data/time) where date and time are in a six digit format (012010/123000).

a. A proper signal-to-noise ratio requires a clean lamp. Cleaning the flash lamp should be something each lab knows how to do. Air or vacuum oil oxidizes on the lamp surface leaving a dark brown spot. This diminishes lamp intensity, especially in the lower UV (The newer monochromometer mount has threads that allow a window to screw into place to prevent vacuum oil deposits). On our machine a clean lamp provides 20,000 intensity at 230 nm, air versus air, while a drop in intensity below 3000–5000 significantly begins to degrade data quality. Depending upon your system you may need to clean the lamp a few times a week. We use toothpaste and a soft brush with a final series of rinsing with methanol.
b. Manual stopping of the run (rather than the stop XLA after last scan) often causes the wavelength drive to be fully extended making it difficult to mount the monochromator. To retract the wavelength drive piston you need to go to Service, Absorbance, Hardware Parameters, and reset the wavelength to 800 nm. This requires a “password” which you should get from your service rep.

C. Data Analysis

Here we present typical analysis of sedimentation velocity data from tubulin self-association studies. The wide size distributions along with the instability of tubulin make sedimentation velocity the preferred experimental method (see Correia, 2000; Sontag et al., 2004). Data should be initially processed by DCDT$^+$ (Philo, 2006) to generate $g(s)$ distributions and weight average sedimentation coefficients, $S_w$. The Philo version of DCDT (Stafford, 1992) called DCDT$^+$, is user friendly, if you wish an automated program that will select the number of scans, often from the middle of the run, to construct a $g(s)$. We prefer to do it manually and select scans as late in the run as possible to maximize resolution (Fig. 1A). There is a tutorial on the Stafford

![Fig. 1](image_url)

DCDT$^+$ analysis of sedimentation velocity data collected with PC-tubulin. (A) Plot of 9/18 scans typically used to generate a $g(s)$ from this type of data. (B) Plot of the $g(s)$ curves generated from nine samples run between 2 and 30 µM tubulin. The three samples above 1 mg/ml are corrected for path length to properly scale with the data collected in 1.2-cm path length centerpieces. (C) The data in panel B were analyzed to generate $S_w$ values. These $S_w$ data were then fit to an isodesmic model (…) corresponding to a $K_{iso}$ values of $2.98 \times 10^4$ M$^{-1}$. (D) The $g(s)$ data in panel B were normalized $g(s)/co$ to show the utility of plotting normalized $g(s)$ curves. Small shifts in size distributions are more apparent in this representation.
Web site that may be of use (http://www.bbri.org/faculty/stafford/dcdt/dcdt.html). Sedfit (Schuck, 2000) can also be used to generate $c(s)$ distributions and the $S_w$ values produced with the integration function are equivalent to $g(s)$ derived values, that is, the information content is in the data not the analysis method (see Correia et al. (2005) for a detailed comparison of $g(s)$ and $c(s)$ analysis for isodesmic or indefinite assembly data. See the Sedfit Web site for information of the use of $c(s)$ at http://www.analyticalultracentrifugation.com) The central issue is making a plot of $S_w$ versus protein (or drug) concentration to verify the trend of the data. A superposition of $g(s)$ plots will also shift to the right, to larger $s$ values, as the concentration is increased. It is useful to plot the data as normalized $g(s)$ curves to enhance resolution (Stafford, 2009). (In DCDT$^{+2}$ this is done by pushing the Normalize button.) These representations ($g(s)$, $S_w$, and normalized $g(s)$) are shown in Fig. 1. Note we attempt to maintain a relatively constant “Peak broadening limit (kDa)” between data sets collected at different speeds because they have dramatically different size distributions. This helps to maintain a similar peak height to width character of the $g(s)$ plots and insure smoother appearing transitions as a function of protein or drug concentrations (Correia, 2000). A reasonable target value is 100–150 kDa, but appreciate this is not being used to extract molecular weights because it is an indefinite assembly mechanism; the goal is consistency of appearance and reasonable signal to noise in the family of $g(s)$ curves.

Direct boundary fitting is done with Sedanal (Correia et al., 2005; Sontag et al., 2004; Stafford and Sherwood, 2004). Data sets are preprocessed into bitmaps within the preprocess centrifuge data window (see the Sedanal Manual 5.03 for details, available at http://rasmb.bbri.org/rasmb/.sedanal/). Within the Fit preprocessed data window, data sets are selected along with the isodesmic model. Isodesmic data often require a large number of data points in the fitting, ~2400, and we need to vary the spacing so more points are located in the meniscus and the base regions that display the highest gradients (Fig. 2). The isodesmic fitting function must be instructed on how to relate $N_i$ to $s_i$ (Fig. 3; as described in Sontag et al. (2004)). These relationships were generated by Hydro calculations that built spiral polymers using a 42-bead model for the αβ-tubulin heretodimer (see Sontag et al., 2004 for details). To our knowledge this is the only software package that currently performs direct boundary fitting for isodesmic or indefinite association models as demonstrated by tubulin.

A final analysis issue concerns the importance of converting data to $S_{20,W}$ values. The data fitted in Fig. 1 is all expressed as $S_{app}$ data. It does not matter that you convert; what matters is the ability to assign $s_i$ values for each oligomer or each species. Those polynomials in Fig. 3 are expressed as $s_i/s_1$ so it works either way. However, Sedanal requires a density increment, which is typically entered as $(1–\nu_p)$, where many investigators estimate $\nu_p$ within Sednterp using amino acid composition and buffer composition. Our lab measures density in an Anton Paar DMA 5000 at the experimental temperature, while the tubulin $\nu$ has been measured experimentally as well. The issue is Sedanal fits the experimental data without converting it to $S_{20,W}$ values but rather uses the actual experimental conditions. It is recommended you have $\nu_p$ and extinction coefficient information (typically also from Sednterp) available at the start of the fitting sessions.
III. Results and Discussion

A. Tubulin Oligomers

Self-association reactions require collecting data as a function of concentration. Figure 1 presents typical data collected on tubulin at 19.7°C in 80 mM Pipes, 1 mM MgSO$_4$, 2 mM EGTA, 0.1 mM TCEP, 50 µM GDP at pH 6.9 with 0.5% DMSO (data adapted from Alday and Correia, 2009). Figure 1A shows a subset of the 18 scans used to generate a typical $g(s)$ (range of Peak Broadening Limit values 117–129 kDa). Note the meniscus position at ~5.85 cm reveals a slight mismatch between sample and reference that for absorbance data does not impact the results; as discussed above, this longer column length maximizes resolution. A family of $g(s)$ curves is presented in Fig. 1B. Note the shift to higher $s$ values and larger polymer sizes. Figure 1D displays the normalized $g(s)$ curves ($g(s)/co$) for the same data (Stafford, 2009). The shift in the distribution is more apparent even for the data below 1 mg/ml. This emphasizes the utility of using normalized plots. These $g(s)$ data were analyzed by DCDT$^+$ and the weight average values $S_w$ are plotted versus protein concentrations in Fig. 1C. (Note the data above 1 mg/ml corresponds to 1.7–3.5 OD and thus had to be collected in 3-mm centerpieces.) $S_w$ data are fitted to an isodesmic model (see dotted line) where the $S_i$ values correspond to a spiral
polymer model derived from a 42-bead hydro model (Sontag et al., 2004). The resulting $K_{iso}$ value is $2.98 \times 10^4 \text{M}^{-1}$ corresponding to a relatively weak indefinite association reaction under these conditions. $K_{iso}$ is a strong function of Mg concentration, varying by an order of magnitude between 0 and 10 mM Mg (Frigon and Timasheff, 1975a,b; Sontag et al., 2004). Figure 4 shows what this means in terms of polymer size distributions. Note the mass action dependence upon both Mg and protein concentration that shifts the distributions to larger oligomers.

The analysis of isodesmic sedimentation velocity data by direct boundary fitting requires the program Sedanal (Correia et al., 2005; Sontag et al., 2004). Data are preprocessed and then globally fit to an isodesmic model (selected from the “model to be fit” pull down list). Pairs of scans must be selected as with DCDT$^{+2}$, to in part remove the systematic noise. However, for direct boundary fitting a wider range of scans can be chosen since the fitter is simulating data throughout the run, as dictated by the Claverie method; so comparisons to earlier data scans are convenient to do as well. The number of points required for these simulations tends to be large, >2400, to avoid convergence errors, and it is best to use variable spacing of those points (see Fig. 2) with more point density at the base of the cell to deal with the steeper gradients. For an isodesmic model the user must also select the $S_i$ versus $N_i$ relationship (Fig. 3). The coefficients entered into the polynomial in Fig. 3 correspond to a 42-bead helical model as described by Sontag et al. (2004). An example of a direct
boundary fit of tubulin self-association data is shown in Fig. 5. This global fit of six data sets does a reasonable job of describing the shape of the sedimenting boundaries, although a few of the cells reveal systematic noise at the base. This is consistent with the presence of irreversible aggregates that are not involved in the isodesmic mechanism in some samples, but not all. This demonstrates the robust power of direct boundary fitting over $S_w$ analysis (for a recent comparison of these approaches see Alday and Correia (2009)).

The presence of tubulin oligomers is generally not appreciated in the field with most investigators assuming 100% of free tubulin in solution is heterodimers. The nomenclature of the field is nonpolymerized tubulin, meaning the fraction not in microtubules, that is, the fraction that does not pellet in a preparative centrifuge. However, at the scale of small oligomers this is clearly not the case (Fig. 1). It has been known for some time that microtubule disassembly rates increase at higher Mg concentrations (O’Brien et al., 1990). The molecular understanding of this is that oligomers are stabilized allowing dissociation in large steps. Stabilization of oligomers may also be related to the mechanism of action of microtubule destabilizing motors like MCAK (Desai et al., 1999). Recently Hunt and coworkers used nm-resolution optical tweezers to study the step size during steady state microtubule dynamics and concluded only heterodimers were involved in assembly/disassembly excursions (see Chapter 12 by Charlebois et al., this volume). These experiments were only done in 1-mM Mg conditions (the squares in Fig. 4), so a direct role for small oligomers in microtubule growth or shortening under other solution conditions may still be an open question. In the presence of XMAP215, microtubule growth has been shown to involve large excursions or steps consistent with MAPs nucleating or facilitating growth through interactions with oligomers (Kerssemakers et al., 2006).

Fig. 4 Size distributions were simulated for various $K_{iso}$ values corresponding to 0 and 10 mM Mg (Sontag et al., 2004). The curves were generated at two protein concentrations corresponding to 2 and 14 µM total tubulin.
B. Vinca Alkaloid-Induced Spirals

Similar methods have been used to study the induction of spirals by vinca alkaloids. Weight average analysis approaches have been extensively described (Lobert and Correia, 2000; Lobert et al., 1997). The major difference is that the fitter input requires $S_{sw}$, protein concentration in mg/ml and drug concentrations in $\mu$M for each data point. The extent of reaction is both protein and drug concentration dependent. Sample equilibration is again done by Penefsky at each drug concentration with the additional step of diluting the protein to the desired concentration prior to the drug equilibration step. Hence this requires two Penefsky steps. The ligand-induced indefinite $K_{2,app}$ value that applies to each sample can be expressed as

$$K_{2,app} = \frac{K_2}{\left(1 + \frac{1}{K_1[\text{Drug}]}ight)^2}$$

where $K_1$ describes the affinity for drug for tubulin heterodimers and $K_2$ is the indefinite association constant. Correia et al. (2005) describes the procedures required to fit these data with Sedanal. When collecting data at multiple drug concentrations, we fit each individual data set to extract the local $K_{2,app}$ value, that

Fig. 5 An example of an isodesmic fit of tubulin self-association data with Sedanal. The data sets shown in this screen dump are a subset of the data shown in Fig. 1. Note data are analyzed as $\Delta C$, or differences between scans, versus $r$ to remove systematic optical noise. The systematic residuals observed near the base of some cells, but not all, indicates the presence of irreversible aggregates not accounted for in the indefinite assembly model.
is, an isodesmic model. Assumptions about the shape of the oligomers and the relationship between $N_i$ and $s_i$ still must be applied. These $K_{2,\text{app}}$ data are then plotted versus drug concentration and fit to the $K_{2,\text{app}}$ equation shown above to extract global $K_1$, $K_2$ values. Recently, we (Alday and Correia, 2009) applied these methods to a new class of Halichondrin B analogs that only weakly induce oligomers. In this case runs were done as a function of protein concentration alone with a fixed excess drug concentration. These data were then globally analyzed by weight average and Sedanal approaches to extract a $K_{\text{iso}}$ value under those conditions without extraction of $K_1$ or $K_2$ values. As described here, the direct boundary fitting method is more robust than the $S_w$ fitting approach, although they generally agree within error (Alday and Correia, 2009).

### IV. Summary

This chapter outlines a detailed description of how to prepare samples and set up the instrument to study tubulin self-association by AUC. The studies apply to tubulin alone or in the presence of antimitotic drugs. These same procedures also apply to hetero-interactions where tubulin is mixed with a regulatory protein, that is, a motor domain, stathmin, or tau. The major difference in approaches is the equilibration of both components prior to mixing, mixing over a reasonable stoichiometry, at least 1:4 to 4:1, and possibly collecting data at multiple wavelengths, especially if one component is labeled with a colored probe, that is, GFP (see Alday and Correia, 2009 for an example). Otherwise the analysis approach is similar and only differs in the model used to directly fit the boundary shapes. We have recently published tutorials on the use of Sedanal for self- and hetero-associating systems (see Correia and Stafford, 2009; Correia et al., 2009).

### References


15. Analysis of Tubulin Oligomers by AUC


