

Dynamic light scattering as a relative tool for assessing the molecular integrity and stability of monoclonal antibodies

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Introduction

Proteins consist of polypeptide chains that are sensitive to various treatment conditions such as preparation, storage, buffer, etc. This short review describes examples of how light scattering may be used as a complementary tool for characterization of proteins in general, working alongside other procedures such as HPLC (high performance liquid chromatography) and AUC (analytical ultracentrifugation). To do this we focus on three sets of therapeutic antibodies, all of similar origin, but different in their pre-treatment and levels of purification. Although several commercial instruments are now available we also focus on one particular instrument - the Zetasizer Nano-S system produced by Malvern instruments (Mavern, UK).

Dynamic light scattering (DLS) is a relatively fast method of characterizing the size of biomolecules in solution, taking only minutes for a measurement. DLS may be used to distinguish between a homogenous monodisperse and an aggregated sample.

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Abbreviations used: AUC: analytical ultracentrifugation, DLS: dynamic light scattering, HPLC: high performance liquid chromatography, PCS: photon correlation spectroscopy, QELS: quasi elastic light scattering, PDI: polydispersity index, kDa: kiloDalton, SLS: static light scattering, M_w : weight average molar mass, S: Svedbergs, IgG4: immunoglobulin G subclass 4, Fab: fragment antigen binding, T_m : melting point temperature.

When measured at different temperatures, the size and intensity can provide information about the denaturation or melting point, which is related to the integrity and thermal stability of a material. Quite frequently in nature, oligomeric states exist in equilibrium in solutions of biomolecules. So in our review we compare samples which are assumed to be a mixture of monomer and dimer as confirmed by previous results from analytical ultracentrifugation data. The two different methods can be used to find the contribution from monomer in samples where another oligomeric species is present in equilibrium with the monomer.

DLS is a much more rapid technique compared to analytical ultracentrifugation although has a more limited resolution. Finally in our review of its performance we show that DLS when combined on-line with the high separation power of HPLC chromatography it is possible to rapidly identify separate species in solution: a heterogeneous material is separated by the material of the column and the elution peaks can be analysed separately downstream by the DLS.

Light scattering and dynamic light scattering

When light passes through a solution containing molecules, depending on the optical parameters of the system, part of the light will be scattered. This scattered light may be analysed either in terms of its intensity or in terms of its fluctuations. The former is called static light scattering and may be used to find the molar mass and radius of gyration (see, for example Harding, Sattelle and Bloomfield 1992 and references therein). However, for many typical biomolecules (< 20 nm), the radius of gyration is not easily measurable since only a minor angular variation in the intensity is exhibited. This means that measurements may be performed at a single angle, provided the concentration and the refractive index are known. We leave the details to references (Chu, 1991, Jumel *et al.*, 1992), but state here that an average molar mass may be determined from the average scattered intensity. A cumbersome detail is the preparation of several known concentrations of the sample, and the requirement for relatively clean, dust-free samples. The concentration dependence of the scattering intensity has been used for the investigation of solubility and crystallizability of macromolecules (George *et al.*, 1994, Wilson *et al.*, 1997, Bonneté *et al.*, 2002 – but see also Deszczynski *et al.*, 2006).

Dynamic light scattering, on the other hand (Berne, 2000, Pecora, 1985), detects the fluctuations of the scattering intensity due to the Brownian motion of molecules in solution. The statistics of the scattering signal are analysed with a correlator, the resulting correlation function may be inverted to find a size distribution for the molecules in solution. This technique works without exact knowledge of the sample concentration and has been used with success in structural biology (see, for example, Bergfors, 1999, D'Arcy, 1994, Ferre D'Amare, 1994). The only requirement is that enough light must be scattered to achieve sufficient statistical accuracy of the correlation function. The technique is also known as photon correlation spectroscopy (PCS) and quasi elastic light scattering (QELS). The wave vector determines the length scale over which molecular motions are detected and it is given by

$$q = 2\pi n \sin(\theta/2)/\lambda \quad (1)$$

where n is the refractive index of the buffer, λ is the wavelength of the radiation, and θ the scattering angle. The resulting correlation function

$$G(\tau) = \int \frac{I(t)I(t+\tau)}{\langle I(t) \rangle^2} dt \quad (2)$$

is accumulated over the duration of the measurement of the intensity $I(t)$ as a function of time, and is expected to show — under ideal conditions — a single exponential decay

$$G(\tau) = 1 + \beta \exp(-Dq^2\tau) \quad (3)$$

where the decay rate Dq^2 includes the diffusion coefficient D of the molecules, and the fitting parameter β is related to the ratio of coherent signal to incoherent noise. The Stokes-Einstein relation may be used to convert the measured diffusion coefficient

$$D = \frac{k_B T}{3\pi\eta d_H} \quad (4)$$

into a hydrodynamic diameter d_H which is the size of a sphere that has the same diffusion behaviour. The only parameters required in this equation 4 are the absolute temperature T , the universal Boltzmann constant k_B , and the viscosity η of the buffer. In practice, the main parameters of interest, when performing DLS experiments, are the temperature, the refractive index and the viscosity.

The simple exponential model indicated in equation 3 yields an overall average, often called the z-average or cumulant average, particle diameter. In practice, a polynomial fit to the logarithm of the function leads to the size (the first cumulant) and its deviation (the second cumulant), where the polydispersity index PDI corresponds to the square of the normalised standard deviation of an underlying Gaussian size distribution. A more complicated regularization fitting scheme may be used to find the distribution of particle sizes (Provencher, 1982, Braginskaya *et al.*, 1984). This is similar to an inverse Laplace transformation, and therefore requires mathematical and physical constraints to solve. In practice, the most severe one is the limit of resolution: The measured scattering function from a distribution containing two close peaks can only be inverted successfully (that is resolve into separate peaks) if the peaks are separated by at least a factor two. If the peaks are closer together, the inversion algorithm will yield only a single, albeit broader, peak located between the two distributions. This downside may be partly overcome by *a priori* knowledge: if the single peak is known to consist of two peaks of known size, for example a monomer and dimer or trimer, then it is possible to back-calculate how much of each species are consistent with the measured ‘average peak’ in between the two expected species.

Antibodies

An absolute requirement of dynamic light scattering is that all protein solutions to be analysed need to be in the appropriate form. In the examples we quote here the antibody samples (all from the human immunoglobulin subclass IgG4 of molar mass

~ 146 kDa) consisted of three different sets. The first set, Ab1, consisted of two different preparations: a native (Ab1-a) and a treated (Ab1-b) form. Ab2 was a purified antibody product and consisted of six different manufacturing lots. Ab3 was highly purified and concentrated. All samples were prepared in concentrations between 0.1 to 7 mg/mL. The solution buffer was 0.1M ammonium hydrocarbonate buffer (pH not adjusted) and no filtration was used during the preparation.

We present data from static and dynamic light scattering for the antibody Ab1. Comparisons with results from analytical ultracentrifugation are shown for Ab2. The highly purified Ab3 is investigated with chromatography coupled on-line to DLS.

Methodology employed

MOLECULAR WEIGHT BY STATIC LIGHT SCATTERING (SLS)

Besides DLS measurements the Zetasizer Nano-S system also facilitates static light scattering measurements (SLS) and this can be used to quantify the molar mass of the protein being analysed prior to DLS measurements. The average scattering intensity from five different concentrations each of Ab1-a and Ab1-b was thus recorded. Samples were analysed using the Malvern supplied 'molecular weight' operating procedure, the light being detected at an angle of 173 ° and a temperature of 25.0 °C in automatic duration mode. Scattering from a toluene reference (Malvern Instruments Ltd., Malvern, UK) and the buffer alone were recorded as part of the procedure. Between measurements the cuvettes had to be cleaned meticulously with ample clean water, and force-dried using compressed air. The resulting data were then analysed using the commercial manufacturers "DTS (Version 4.2)" software (Malvern Instruments Ltd., Malvern, UK). In this way the weight average molecular weights M_w (weight average molar masses) of the two samples Ab1-a (native) and Ab1-b (treated) were assessed by the Zetasizer Nano-S prior to the investigation of its performance as a DLS instrument. The expected molecular weight for the antibody is about 145 kDa. However, due to the production process, some antibody fragments might have been present which had not completely been removed during the purification. As shown in *Table 1*, the pure antibody samples exhibited a molecular weight slightly less than the expected value. It is quite likely that this discrepancy originated from the presence of smaller Fab fragments. The molecular weight of the treated sample was significantly larger than the pure antibodies. The treated sample appears to contain a large amount of aggregates and this gave rise to the significantly larger weight average molecular weight.

Table 1. Molecular weight (weight average, M_w) measured by static light scattering. The error is the fit error given by the linear regression to the data from different concentration data. Duplicate measurements were performed to check repeatability. Sample Ab1-b is a treated form of Ab1-a.

Sample	Molecular weight [kDa]	Error [kDa]
Ab1-a	129	+/- 21
Ab1-a	139	+/- 8
Ab1-b	1210	+/- 125
Ab1-b	1270	+/- 65

DYNAMIC LIGHT SCATTERING (DLS)

Dynamic light scattering measurements were made on the fixed scattering angle Zetasizer Nano-S system (Malvern Instruments Ltd., Malvern, UK). Samples were measured in a 12 μL quartz cuvette. The instrument was used for sizing (dynamic light scattering, DLS) to determine the z-average molecular “size” in terms of the hydrodynamic diameter d_H in solution, a parameter inversely related to the z-average translational diffusion coefficient, D (equation 4) in solution. Samples were measured at 25.0 °C and the light scattering was detected at 173 ° and collected in automatic mode, typically requiring a measurement duration of 150 seconds. The resulting data were analysed using the “DTS (Version 4.2)” software (Malvern Instruments Ltd., Malvern, UK). The viscosity of the buffer used was calculated using a solvent builder interface and takes the effects of buffer salts into account.

SAMPLE HOMOGENEITY BY DYNAMIC LIGHT SCATTERING

Utilizing the ‘general purpose’ regularization mode of the DTS software we compared the size distributions obtained from the correlation functions. In addition, we looked at the polydispersity index or PDI, indicating how overall homogenous the sample appeared, at least from a light scattering perspective.

MELTING POINT BY DYNAMIC LIGHT SCATTERING

The Zetasizer Nano has built-in Peltier temperature control. For this set of experiments we performed an automated temperature trend measurement to find the so-called “melting point”. This is the temperature where significant thermal denaturation and aggregation have set in so as to cause a noticeable increase in both the measured intensity and the measured cumulant size. The viscosity of the buffer was automatically adjusted for the temperature.

MONOMER-DIMER EQUILIBRIA AS STUDIED BY DYNAMIC LIGHT SCATTERING

Light scattering measures the diffusion of molecules in solutions. Just as the sedimentation coefficient may be used to find the molecular “size”, as we have noted above (eq. 4) so can the diffusion coefficient. The result may be obtained from fitting the decay of the correlation function $G(\tau)$. Assuming a Gaussian distribution, a simple exponential fit produces the z-average (or cumulant) size. As the measured property is the intensity of scattered light (rather than the %mass as in UV absorption), it is necessary to take the high sensitivity to larger sizes (intensity \sim size⁶ \sim volume²) into account when converting to the volume contributions. It is possible to calculate the contribution from the monomer when a monomer-dimer equilibrium is assumed to contribute to the measured light scattering result. The result can be expressed in terms of %intensity, %mass and %number of each of the two species. For the example of the antibody Ab2 used in this comparison of different preparations or “lots” the monomer is expected to show a hydrodynamic diameter of 10.1 nm: the dimer would then be 13.5 nm, as predicted from a globular protein assumption.

SEDIMENTATION VELOCITY BY ANALYTICAL ULTRACENTRIFUGATION

Analytical ultracentrifugation (AUC) data had been taken from the Optima XL-A system (Beckman Instruments, Palo Alto, USA) with solute concentration distributions recorded as ultraviolet absorbance at 290 nm (Garcia de la Torre, 1992, Lebowitz *et al.*, 2002) and ready for comparison with the DLS data. As the performance of the AUC is not the principal aim of the current review we will not go into any great detail, but sedimentation velocity runs were performed at high rotor speed (typically 42000 rpm) for at least 7 hours at 20°C.

The distribution function of apparent sedimentation values $g^*(s)$ had been modelled as a collection of Gaussian distributions for individual species (Dam and Schuck, 2004). All samples exhibited various amounts of monomer, with typical sedimentation coefficients of the main component at about $s_{20,w} \sim 6.7$ S (Svedbergs, $1\text{S} = 10^{-13}\text{sec}$). The suffix 20,w indicates that the parameter had been corrected to standard solvent conditions – the density and viscosity of water at 20.0°C. This $s_{20,w}$ value is precisely what would be expected for these antibodies at a molecular weight of ~146 kDa (Carrasco *et al.*, 2001).

SIZE EXCLUSION CHROMATOGRAPHY COUPLED WITH DYNAMIC LIGHT SCATTERING

The final combination in our review of the performance of the Zetasizer Nano system was DLS with size exclusion chromatography. To do this a custom supplied flow cell (Malvern Instruments, UK) was connected as the last detector in a commercial chromatography setup (Äkta system, GE Healthcare, Uppsala, Sweden). The flow cell is inserted into the Zetasizer Nano we described above. A special flow mode operating procedure of the DTS software allows continuous data acquisition of the flowing sample eluting from the column (Superdex 200). The eluent (0.1M ammonium hydrocarbonate buffer, pH not adjusted) was pumped at 0.5 mL/min and 100 μL (7.0 mg/mL) of Ab3 were injected. Correlation functions and scattering intensities are recorded every 3 seconds. The software analyses the chromatogram automatically, and produces the size distribution function directly from the measured sizes, i.e. there is no column calibration involved with this method. The hydrodynamic size of the molecule is measured directly after elution from the column (see, e.g., Claes *et al.*, 1994).

Comparing the performance of the instrument

SAMPLE HOMOGENEITY BY DYNAMIC LIGHT SCATTERING

The DLS measurements showed a significant difference between the treated and the pure samples. While the untreated sample Ab1-a showed a typical z-average size (hydrodynamic diameter d_H) of 11 nm (PDI=0.14), the treated sample had a z-average size of 50 nm (PDI=0.49). For a near-monodisperse sample, a PDI of 0.1 or lower is expected. Here, however, both show a polydispersity index larger than 0.1 indicating that the simple cumulant fitting is not a complete representation and that more than a single species are present. This was confirmed by the size distribution analysis shown in *Figure 1*. The more homogenous sample Ab1-a showed a single peak near the

expected size, however the peak is broader than for a single species, indicating the presence of some fragments and oligomeric assemblies. The treated sample Ab1-b was clearly aggregated and significantly larger in size.

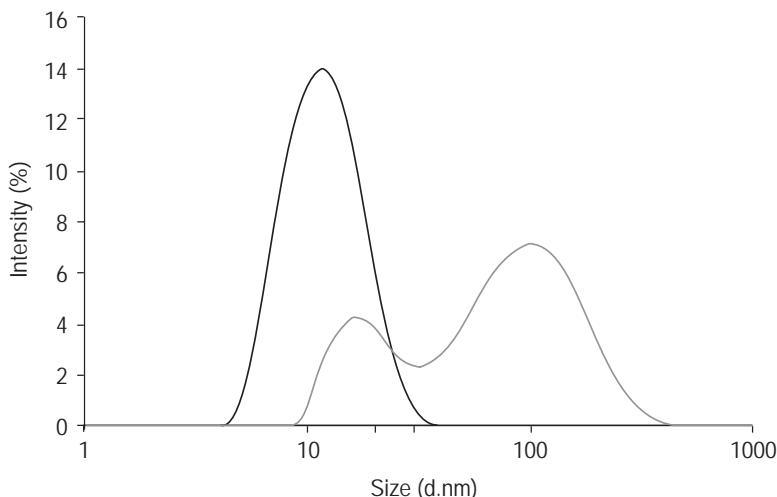


Figure 1. Size distribution (by intensity) from two antibody samples, Ab1-a (black) and its treated form Ab1-b (grey). Ab1-b is aggregated, while Ab1-a shows no evidence of larger components.

MELTING POINT DETERMINATION

An automated thermal scan of the sample chamber allows observation of both the size and the scattering intensity as a function of temperature. The marked point, where both the size and the intensity start to increase significantly, is called the melting point. Here, denaturation of the molecules leads to massive aggregation, which manifests itself as both a larger size and a larger scattering intensity.

Figure 2a shows the melting point measurement for the untreated antibody sample Ab1-a. There is a clear transition in the intensity plot at about 56°C. The treated sample Ab1-b, on the other hand, shows no transition at all: It appears as if the treatment has had a denaturing effect on the antibody.

MONOMER-DIMER EQUILIBRIA

Dynamic light scattering

As already indicated in the discussion concerning *Figure 1*, the width of the size distribution indicates that more than a single oligomeric species is present in solution. We tested this assumption using analytical ultracentrifugation as an independent probe and compared the results from this with the light scattering records.

With the results from a DLS experiment it is possible to calculate the contribution from the monomer alone, when a monomer-dimer equilibrium is assumed to contribute to the measured light scattering result. *Figure 3* shows the expected monomer fraction both in terms of %intensity and %mass, for the example of the antibody used in this

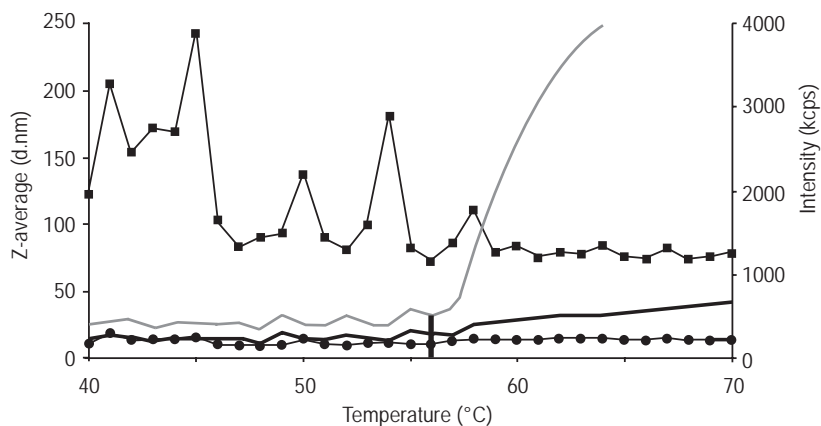


Figure 2. Melting point determination of two different antibodies. For sample Ab1-a (solid lines, no symbols, size in black, intensity in grey) a clear transition is visible and automatically determined as $T_M = 56^\circ\text{C}$. The treated form Ab1-b (symbols, size as squares, intensity as circles) shows no melting point transition.

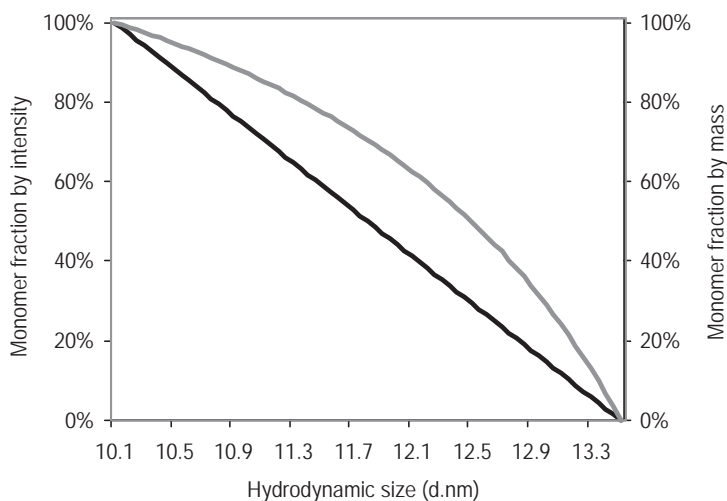


Figure 3. Monomer contribution predicted from DLS, versus measured hydrodynamic size, by intensity (black) and by mass (grey) for antibodies. The monomer is modelled as 10.1 nm the dimer as 13.5 nm diameter. As an example, a measured size of 11.7 nm would correspond to 75% monomer by mass in solution.

comparison of different sample lots. The monomer is expected to show a hydrodynamic diameter of 10.1 nm, the dimer would then be 13.5 nm. *Figure 3* can be used to find the amount of monomer for any measured hydrodynamic size. The predicted monomer contributions in terms of %mass are shown in *Table 2*.

Table 2. Comparison of DLS results with AUC. Antibody series Ab2 contained both monomer and dimer. The monomer fraction predicted from DLS closely matches the measured fraction from AUC. The deviations within this lot series are small.

Sample	Size (hydrodynamic diameter) d_H (nm)	DLS %monomer (by mass)	Concentration (mg/mL)	Main species $s_{20,w}$ (S)	AUC %monomer (by mass)
Ab2-Lot1	11.3	81.4	0.8	6.71	84.4
Ab2-Lot2	11.4	79.4	1.3	6.67	86.4
Ab2-Lot3	11.4	79.4	0.6	6.73	82.5
Ab2-Lot4	11.3	81.4	1.3	6.54	80.4
Ab2-Lot5	11.1	85.1	1.0	6.63	82.0
Ab2-Lot6	11.3	81.4	1.7	6.56	81.1

Analytical ultracentrifugation

Detailed sedimentation velocity experiments and modelling of the concomitant sedimentation coefficient distributions following the procedure of Lu *et al.* (2007) revealed that the six samples of the series Ab2 all contained mostly monomer, with some dimer contribution. The measured sedimentation coefficients of the different sample lots are shown in *Table 2*, with the resulting monomer contribution. The numbers compared very well with the monomer contributions predicted by DLS.

ABSOLUTE SIZE EXCLUSION CHROMATOGRAPHY

The high separative power of size exclusion chromatography provides the most commonly used method to separate oligomeric species of antibodies in solution. Here, we combine the chromatography with DLS in flow, where the light scattering is used to quickly identify the different peaks of the chromatogram. *Figure 4* shows the elution profile of Ab3, a highly purified antibody, expected to consist mostly of monomer. The analysis results for the different peaks by intensity and by mass are summarised in *Table 3*.

Conclusion

Dynamic light scattering appears to be a highly useful probe for monitoring the structural integrity of monoclonal antibody preparations. The integrity and stability of these macromolecules can be investigated by (a) using dynamic light scattering alone; (b) combining results from DLS with analytical ultracentrifugation; (c) temperature dependence studies; or (d) combining with size exclusion chromatography. The advantages of the technique are its speed, low volume requirement, and ease of use. Molecules are “seen” in their native buffer environment which means that their behaviour is not compromised by external influences. The

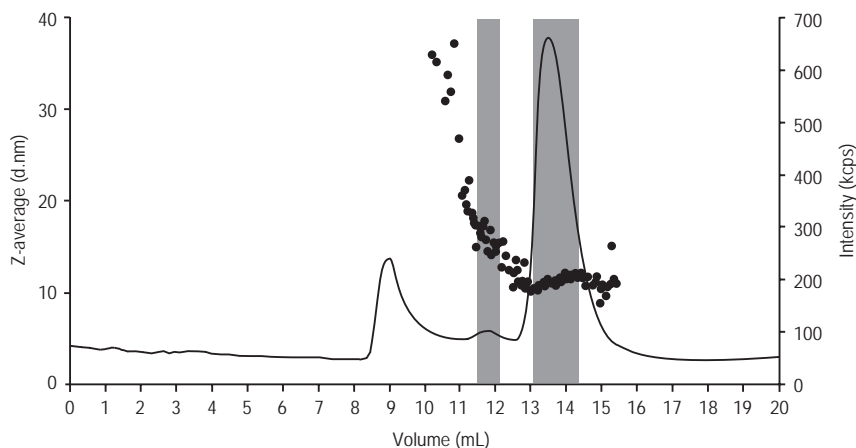


Figure 4. Chromatogram of the purified antibody Ab3. Intensity (grey line) and hydrodynamic diameter (black symbols) versus elution volume. The main peak is the monomer, with some aggregation visible in the void volume and a minor component from dimer. The monomer shows a high intensity with a relatively constant size during the elution of the peak.

Table 3. Results obtained from coupling DLS to SEC. The monomer of Ab3 is clearly identified (11.6 nm diameter) and makes up more than 95% by mass of the antibody sample. The contributions are calculated directly from the eluted size measurements, no calibration was involved.

DLS Chromatogram	Results	Size d_{H^*} (nm)	Relative contribution
Intensity	Peak1	11.6	78.6 %
	Peak2	15.9	7.2 %
Mass	Peak1	11.5	95.8 %
	Peak2	15.9	3.9 %

non-invasive nature of DLS makes it an ideal tool for routine characterisation of biomolecules in any laboratory.

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References

BERGFORS, T. (1999). Dynamic Light Scattering. In *Protein Crystallization: Strategies, Techniques, and Tips*. Ed. T. Bergfors, pp. 29-38. La Jolla, CA: International

University Line.

- BERNE, B.J. AND PECORA, R. (2000). *Dynamic Light Scattering with Applications to Biology, Chemistry, and Physics*. Mineola, NY: Dover Publications.
- BONNETÉ, F. AND VIVARÈS, D. (2002). Interest in the normalized second virial coefficient and interaction potentials for crystallizing large macromolecules. *Acta Cryst.* **D58**, 1571-1575.
- BRAGINSKAYA, T.G., DOBITCHIN, P.D., IVANOVA, M.A., KLYUBIN, V.V., LOMAKIN, A.V., NOSKIN, V.A., SHMELE, G.E. AND TOLPINA, S.P. (1983). Analysis of the polydispersity by photon correlation spectroscopy. Regularization procedure. *Physica Scripta*, **28**, 73-79.
- CARRASCO, B., GARCIA DE LA TORRE, J., DAVIS, K.G., JONES, S., ATHWAL, D., WALTERS, C., BURTON, D.R. AND HARDING, S.E. (2001) Crystallohydrodynamics for solving the hydration problem for multi-domain proteins: open physiological conformations for human IgG1. *Biophysical Chem.* **93**, 181-196.
- CHU, B. (1991). *Laser Light Scattering, Basic Principles and Practice*. Second Edition. San Diego, CA: Academic Press.
- CLAES, P., DUNFORD, M., KENNEY AND VARDY, P. (1992). An on-line dynamic light scattering instrument for macromolecular characterisation. In *Laser Light Scattering in Biochemistry*. Eds. S.E. Harding, D.B. Sattelle, V.A. Bloomfield, pp. 66-76. Cambridge, UK: Royal Society of Chemistry.
- D'ARCY A. (1994). Crystallizing proteins: a rational approach? *Acta Cryst.* **D50**, 469-471.
- DAM, J. AND SCHUCK, P. (2004). Determination of sedimentation coefficient distributions by direct modeling of the sedimentation boundary with Lamm equation solutions *Meth. Enzymol.* **384**, 185-121.
- DESZCZYNSKI, M., HARDING, S.E. AND WINZOR, D.J. (2006) Negative second virial coefficients as predictors of protein crystal growth: evidence from sedimentation equilibrium studies that refutes the designation of those light scattering parameters as osmotic virial coefficients. *Biophysical Chemistry* **120**, 106-113.
- FERRE-D'AMARE, A. AND BURLEY, S. (1994). Use of dynamic light scattering to assess crystallizability of macromolecules and macromolecular assemblies. *Structure* **2**, 357-359.
- GARCIA DE LA TORRE, J. (1992). Sedimentation Coefficients of Complex Biological Particles. In *Analytical Ultracentrifugation in Biochemistry and Polymer Science*. Eds. S.E. Harding, A.J. Rowe, J.C. Horton., pp. 333-358. Cambridge, UK: Royal Society of Chemistry.
- GEORGE, A., CHIANG, Y., GUO, B., ARABSHAH, A., CAI, Z. AND WILSON, W.W. (1997). Second virial coefficient as predictor in protein crystal growth. *Meth Enzymol* **276**, 100-110.
- GEORGE, A. AND WILSON, W.W. (1994). Predicting protein crystallization from a dilute solution property. *Acta Cryst.* **D50**, 361-365.
- HARDING, S.E., SATTELLE, D.B. AND BLOOMFIELD V.A. (1992) eds. *Laser Light Scattering in Biochemistry*. Cambridge, UK: Royal Society of Chemistry
- JUMEL, K., BROWNE, P. AND KENNEDY, J.F. (1992). The use of low angle laser light scattering with gel permeation chromatography for the molecular weight determination of biomolecules. In *Laser Light Scattering in Biochemistry*. Eds. S.E. Harding, D.B. Sattelle, V.A. Bloomfield, pp. 23-34. Cambridge, UK: Royal Society of Chemistry.
- LEBOWITZ, J., LEWIS, M. AND SCHUCK, P. (2002). Modern Analytical Ultracentrifugation

- in Protein Science: A Tutorial Review. *Protein Science* **11**, 2067-2079.
- LU, Y., HARDING, S.E., ROWE, A.J., DAVIS, K.G., FISH, B., VARLEY, P., GEE, C. AND MULOT, S. (2007) The effect of a point mutation on the stability of IgG4 as monitored by analytical ultracentrifugation. *J. Pharm. Sci.* (in press)
- PECORA, R. (1985). *Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy*. New York, NY: Plenum Press.
- PROVENCHE, S.W. (1982). A constrained regularization method for inverting data represented by linear algebraic or integral equations. *Comput. Phys. Commun.* **27**, 213-227.