LBNL-44258
Preprint

ERNEST ORLANDO LAWRENCE
BERKELEY NATIONAL LABORATORY

Diffusivities of Lysozyme in Aqueous-$MgCl_2$ Solutions from Dynamic Light-Scattering Data: Effect of Protein and Salt Concentrations

J.J. Grigsby, H.W. Blanch, and J.M. Prausnitz

Chemical Sciences Division

September 1999

Submitted to
Journal of
Physical Chemistry

REFERENCE COPY
Does Not
Circulate
Bldg. 50Ø Library - Ref.
Lawrence Berkeley National Laboratory

LBNL-44258
Copy 1

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor The Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or The Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof, or The Regents of the University of California.

Ernest Orlando Lawrence Berkeley National Laboratory
is an equal opportunity employer.

LBNL-44258

Diffusivities of Lysozyme in Aqueous-$MgCl_2$ Solutions from Dynamic Light-Scattering Data: Effect of Protein and Salt Concentrations

J. J. Grigsby, H. W. Blanch and J. M. Prausnitz
Department of Chemical Engineering
University of California
and
Chemical Sciences Division
Lawrence Berkeley National Laboratory
University of California
Berkeley, CA 94720, U.S.A.
September 1999

This work was supported by the Director, Office of Science, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract Number DE-AC03-76SF00098.

recycled paper

Diffusivities of Lysozyme in Aqueous-$MgCl_2$ Solutions from Dynamic Light-Scattering Data: Effect of Protein and Salt Concentrations

J. J. Grigsby, H. W. Blanch, J. M. Prausnitz

Chemical Engineering Department, University of California, Berkeley, CA 94720

Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720

Dynamic light-scattering (DLS) studies are reported for lysozyme in aqueous magnesium chloride solutions at ionic strengths 0.6, 0.8, and 1.0 M for a temperature range10 to 30°C at pH 4.0. The diffusion coefficient of lysozyme was calculated as a function of protein concentration, salt concentration, temperature, and scattering angle. A Zimm-plot analysis provided the infinitely-dilute diffusion coefficient and the protein-concentration dependence of the diffusion coefficient. The hydrodynamic radius of a lysozyme monomer was obtained from the Stokes-Einstein equation; it is 18.6 ± 0.5 Å. The difference (1.4Å) between the hydrodynamic and the crystal-structure radius is attributed to binding of Mg^{2+} ions to the protein surface and subsequent water structuring. The effect of protein concentration on the diffusion coefficient indicates that attractive interactions increase as the temperature falls at fixed salt concentration. However, when plotted against ionic strength, attractive interactions exhibit a maximum at ionic strength 0.84 M, probably because Mg^{2+}-protein binding and water structuring become increasingly important as the concentration of magnesium ion rises. The present work suggests that inclusion of ion binding and water structuring at the protein surface in a pair-potential model is needed to achieve accurate predictions of protein-solution phase behavior.

Introduction

Salt-induced protein precipitation from aqueous solution is often the sole step in protein purification (Rothstein, 1994; Scopes, 1994) when crude fractionations of protein are sufficient, or provides a first step when the protein is subsequently further purified by other methods such as chromatography or electrophoresis. No useful theoretical model is now available to predict solution conditions that induce the selective precipitation of a target protein. The initial step for developing a predictive molecular-thermodynamic model is to quantify the effect of solution conditions on intermolecular forces between protein molecules.

Understanding intermolecular forces between protein molecules is also vital for protein-crystallization science and technology. High-quality crystals are necessary for determining three-dimensional protein structure by x-ray crystallography. Finding conditions that yield high quality crystals is often the most time-consuming step in the structure-determination process.

Recent work has focused on correlating precipitation and crystallization conditions with the osmotic second virial coefficient and the potential of mean force (PMF) (Vlachy *et al.*, 1993; George and Wilson 1994; *Chiew et al.*, 1995; Rosenbaum *et al.*, 1996; Guo *et al.*, 1999; Neal *et al.*, 1999). Dynamic-Light-Scattering (DLS) data give the dependence of the protein diffusion coefficient on the concentration of protein and solution conditions. This dependence can be related to the PMF to obtain model parameters (e.g.

Kuehner et al., 1997; Eberstein et al., 1994). Once a model for the PMF is available, the second osmotic virial coefficient can be calculated (McMillan and Mayer 1945). George and Wilson (1994) have shown that the crystallization of lysozyme occurs over a narrow range of second virial coefficient values. For positive coefficients (i.e., repulsive interactions) lysozyme remains stable in solution, but when the coefficient is large and negative, lysozyme forms an amorphous precipitate.

Hen-egg-white lysozyme has been extensively studied due to its compact globular structure and stability over a wide range of solution conditions. Sophianopoulos and Van Holde (1961, 1962) were the first to report the pH-dependent self-association of lysozyme. Equilibrium-sedimentation studies revealed lysozyme aggregation at 20°C in 0.15M KCl solutions for protein concentrations up to 15 g/L. Later studies (Sophianopoulos, 1969) showed that the pH-dependent dimerization mechanism involved the deprotonated form of Glu-35 in the active site (Glu-35 has an unusually high pK_a of 6.3). Below pH 4.5, there is no dimerization because the Glu-35 carboxylic acid remains protonated. Lysozyme aggregation is a result of attractive interaction between the deprotonated Glu-35 carboxylic acid and a protonated nitrogen on the Trp-62 side chain of a neighboring lysozyme molecule. This leads to a head-to-tail association (Blake *et al.*, 1967; *Repley et al.*, 1967; Sophianopoulos, 1969; Banerjee *et al.*, 1975; Norton and Allerhand, 1977).

In this work, DLS measurements are reported for hen-egg-white lysozyme at concentrations to 30 g/L in aqueous-$MgCl_2$ solutions for the temperature range 10 to

30°C. Solution pH was maintained at pH 4.0 to assure that any lysozyme association is not due to head-to-tail association. Kuehner *et al.* (1997) have performed DLS experiments with lysozyme in aqueous-ammonium-chloride solutions for an ionic strength range 0.05 to 5 M and pH range 4.0 to 7.0. Ammonium chloride preferentially hydrates the lysozyme surface (Arakawa, 1990). $MgCl_2$ was chosen for this study because Mg^{2+} is a highly kosmotropic ion that is able to bind to protein surfaces and to structure water molecules (Collins, 1997).

Materials and Methods

Lysozyme Solution Preparation

Lysozyme was purchased from Boehringer Mannheim (Germany). Gel electrophoresis showed less than 1% contamination by other proteins, and therefore no further purification was performed. Reagent-grade $MgCl_2$ was purchased from Fischer Scientific (Pittsburgh, PA). Syringe-tip 0.2-μm pore-size filters were purchased from Millipore (Bedford, MA). Deionized water was obtained from a Barnstead-Nanopure II filtration unit.

A 4L stock solution of salt was prepared by weighing the appropriate amount of salt for a given ionic strength and dissolving it in deionized water. The pH of the solution was adjusted to 4.0 using aqueous HCl of the same ionic strength as that of the salt solution.

Lysozyme was dissolved in 20 mL of the salt-stock solution. Although gel electrophoresis showed minimal protein contaminants, small amounts of salts are present in the lyophilized lysozyme. While these salts are not significant scattering species compared to lysozyme, they may influence protein-protein interactions. Therefore, the protein-salt solution was dialyzed against the salt solution to minimize the amount of salt contaminants. Dialysis tubing with a molecular-weight cut-off of 6000 to 8000 was purchased from Spectrum Medical Industries (Los Angeles, CA). After dialysis, the pH of the protein-salt solution was adjusted with HCl of the same ionic strength as that of the salt-stock solution. Since the protein solution has a small natural buffering capacity, the pH was stable over the duration of the light-scattering measurements. The pH of the solution was measured after each series of measurements to check pH stability.

Precision-ground Pyrex NMR tubes with 12-mm O.D., 0.5-mm wall thickness (Wilmad Glass, Buena, NJ), and 5-ml volume were thoroughly cleaned before sample loading. The tubes were stored in concentrated H_2SO_4 until needed. They were first rinsed with filtered deionized water and placed in a 2 M NaOH solution containing 3.5g/L $KMnO_4$ for two hours. The tubes were again rinsed with filtered deionized water and stored in 1 M HCl until needed. Before sample loading, the tubes were again rinsed with filtered deionized water and allowed to dry. All transfers were performed in a laminar-flow hood to minimize dust contamination.

A tube-cap assembly permitted the closed-loop filtration of the protein-salt solution. This design was essential for the removal of small aggregates of protein and dust which are

significant light scatterers. The tubing was connected to a syringe containing the sample solution and rinsed with the solution. The tube-cap assembly was next connected to the sample tube and filled with the protein-salt solution. The closed-loop system contained a 0.2-μm filter to facilitate the continuous filtration of the sample. Samples were filtered using a peristaltic pump for a minimum of 30 minutes at the lowest setting of the pump to prevent shear denaturation of the protein.

Dynamic Light-Scattering Measurements

Figure 1 shows a schematic of the dynamic light-scattering (DLS) apparatus. The DLS system contains an Innova-90 argon-ion laser (Coherent Inc., Santa Clara, CA), tuned to a wavelength of 488 nm, a BI-240SM multi-angle goniometer, a BI-EMI-9865 photomultiplier and a BI-9000 digital autocorrelator. The autocorrelator is able to measure the electric-field autocorrelation function, $g^E(\tau)$, in real time allowing for the calculation of z-average diffusion coefficients. Decalin (Aldrich 29477-2, CAS [91-17-8], refractive index $n = 1.47$) was used as an index-matching liquid to reduce flare at the glass-liquid interface. The decalin was recirculated through a 47-mm OD 0.1 μm pore-size hydrophobic membrane filter (GSEP 047A0, Millipore) until all visible dust was removed. The sample in the light-scattering apparatus was allowed to equilibrate thermally for at least one hour. Constant temperature was achieved with a VWR Model 1160 recirculating water bath.

Dynamic light-scattering (DLS) measurements were made for a range of scattering angles at ten-degree increments from 30 to 90°. At least three measurements were made at each angle. Time for data collection ranged from ten minutes to one hour depended on the concentration of protein and the angle of detection. A minimum of 5×10^8 photons was collected for statistically meaningful data. Data were rejected if the difference between the calculated and measured baselines was greater than 0.02%. To assure that thermal equilibrium was maintained throughout the duration of the experiment, an additional measurement at 30° was taken and compared to the 30° measurements at the start of the experiment.

The temperature was lowered by 5°C after each series of measurements. Again the sample was allowed to equilibrate for at least one hour before measurements were made. The temperature of the sample was not lowered below 10°C due to fogging of the windows on the light-scattering apparatus. Light-scattering measurements were made for a specific ionic strength and temperature at three protein concentrations.

The pH of the sample solution was checked after each series of measurements. For any experiment, the change in pH was less than 0.1 units. The filtration process removed aggregates of lysozyme. The concentration of lysozyme generally decreased no more than 5 % from the initial concentration. Protein concentrations were determined by measuring absorbance at 280 nm and 25°C using a Beckman DU-6 spectrophotometer with an extinction coefficient for lysozyme of 2.365 L/g-cm (Sophianopolos and Van Holde, 1964). The refractive index of the protein solution was measured at the

appropriate temperature using a Zeiss refractometer and white light. Viscosity of $MgCl_2$ solutions as a function of ionic strength and temperature were measured using an Ubbelohde viscometer with water as a reference.

Data Reduction

DLS measurements were made at three protein concentrations for the ionic strengths (IS) and temperatures given in Table 1. Measurements could not be obtained for IS 1.0 at 15 M and 10°C due to rapid precipitation of the lysozyme solution.

The signal generated by the light scattered from diffusing particles can be analyzed by its intensity autocorrelation function, $G^I(\tau)$:

$$G^I(\tau) = \langle I(t) \cdot I(t+\tau) \rangle \qquad (1)$$

where $I(t)$ is the scattered-light intensity at time t and $I(t + \tau)$ is the scattered-light intensity at some later time $(t + \tau)$. The normalized intensity autocorrelation function $g^I(\tau)$ is

$$g^I(\tau) = \frac{G^I}{\langle I(t) \rangle^2} \qquad (2)$$

The electric-field autocorrelation function $g^E(\tau)$, is related to the normalized intensity autocorrelation function by

$$g^I(\tau) = 1 + B[g^E(\tau)]^2 \qquad (3)$$

where B is an equipment parameter.

For a monodisperse system of particles, $g^E(\tau)$ follows a simple exponential decay with decay constant Γ.

$$g^E(\tau) = \exp[-\Gamma\tau] \tag{4}$$

For a system containing a distribution of different-sized particles, $g^E(\tau)$ becomes the sum of the electric-field autocorrelation functions of each of the species weighted by the concentration of the species in solution. The quadratic-cumulant-expansion analysis (Koppel, 1972) was used to obtain an apparent decay constant, Γ_{ap}, which represents an average decay constant for all species in solution (monomers, dimers, ect.). The second cumulant in the analysis, Q, provides an indication of the polydispersity of the system. For most experiments, Q was less than 0.02 indicating a narrow protein-size distribution with few aggregates. For the few cases when Q was greater than 0.02, inversion of the autocorrelation function was performed with a Brookhaven Instruments Corp. version of CONTIN program (Provencher, 1982a and 1982b). CONTIN results showed a narrow unimodal diffusion-coefficient distribution near the value given by the quadratic-cumulant result. This analysis showed that lysozyme is a monomer at solution conditions studied here.

The apparent diffusion coefficient, D_{ap}, for lysozyme molecules was calculated from

$$D_{ap} = \frac{\Gamma_{ap}}{q^2} \tag{5}$$

where q is the magnitude of the scattering vector. An apparent diffusion coefficient is used here since Γ_{ap} is an intensity-weighted concentration average of all species (monomers, dimers, ect) in the aqueous protein solution. The magnitude of the scattering vector, q, was calculated from

$$q = \frac{4\pi n}{\lambda_{laser}} \sin(\theta/2) \qquad (6)$$

where n is the refractive index of the protein salt solution, λ_{laser} is the wavelength of the laser light, and θ is the scattering angle of detection.

A Zimm plot was constructed for each ionic strength and temperature. Diffusion coefficients are plotted as a function of both concentration and angle. D_{ap} was plotted for each protein concentration as a function of the q^2+kc_p which depends both on the scattering angle [$q \approx \sin(\theta/2)$] and protein concentration c_p. The parameter k is chosen to facilitate graphical inspection of the Zimm plot; the value of k does not affect the results. Figure 2 shows a representative Zimm plot for $MgCl_2$ ionic strength 0.80 M at 25°C. At constant protein concentration, the open symbols represent the diffusion coefficient extrapolated to zero angle. At constant angle, the open symbols represent the diffusion coefficient extrapolated to zero protein concentration. The two extrapolated lines intersect at a point D_o, that represents the diffusion coefficient of a lysozyme molecule in an infinitely-dilute protein solution.

Infinite-Dilution Hydrodynamic Radii

To calculate the hydrodynamic radius, of a lysozyme molecule at different solution conditions, the viscosities of $MgCl_2$ solutions were measured. Results are given in Table 2.

The infinite-dilution hydrodynamic radius, r_H, is calculated from the Stokes-Einstein equation for infinitely-dilute monodisperse spheres:

$$D_o = \frac{k_B T}{f_o} = \frac{k_B T}{6\pi\eta_o r_H} \qquad (7)$$

where f_o is the hydrodynamic friction factor, k_B is Boltzmann's constant, T is absolute temperature, and η_o is the viscosity of the solvent. Calculated hydrated radii of lysozyme in $MgCl_2$ solutions are given in Table 3 and a comparison plot is given in Figure 3. There does not appear to be a clear trend for the hydrodynamic radius as a function of ionic strength or temperature. The average hydrodynamic radius from the DLS data is 18.6 ± 0.5Å.

The crystal structure of hen-egg-white lysozyme determined by x-ray crystallography (Brookhaven protein database structure 2LYZ) is a prolate ellipsoid of revolution with major semiaxis α_x=22.5Å and symmetric minor semiaxes β_x=15Å. The equivalent spherical radius of a lysozyme monomer is 17.2Å. The difference between the calculated hydrodynamic radius and the crystal structure radius is 1.4Å.

Arkawa *et al.* (1990) performed densimetry experiments with lysozyme in $MgCl_2$ solutions at pH 3.0 and 4.5 and found preferential binding of Mg^{2+} to the surface of the protein. Based on their results, 5 to 6 Mg^{2+} ions are estimated to bind to the lysozyme surface for conditions used in this study. Mg^{2+} is a highly kosmotropic ion that structures water around the ion (Collins 1997). Therefore, the binding of Mg^{2+} to the lysozyme surface structures water around the protein molecule, adding a hydration layer of 1.4Å. Kuehner *et al.* (1997) performed similar DLS studies with lysozyme in ammonium sulfate solution and calculated a hydration-layer thickness of 0.8Å. The difference between the two studies is due to the difference in ion binding. The concentration of ammonium sulfate immediately adjacent to the lysozyme surface is lower than that in bulk solution and therefore preferentially hydrates the protein molecule. Mg^{2+} binds to the lysozyme surface and structures water around the ion due to its kosmotropic nature.

Concentration-Dependent Diffusivities

Figure 4(a-c) shows the normalized apparent diffusion coefficient D^o_{ap}/D_o plotted as a function of protein volume fraction at fixed ionic strength, where D^o_{ap} is the zero-angle extrapolated value at each protein concentration. Protein concentration is expressed in terms of protein volume fraction given by $\phi = c_p M v/1000$ where M is the molecular weight of lysozyme (14,400 g/mol) and v is the partial specific volume of lysozyme (0.703 mL/g) (Sophianopoulos *et al.*, 1962). The slope of this line, λ, contains information about interactions between protein molecules. Relationships between λ and the potential of mean force (PMF) have been derived (Felderhof, 1978: Batchelor, 1983;

Batchelor, 1976; Phillies, 1995a; Phillies *et al.*, 1995b) for monodisperse systems. Negative values of λ indicate a net attractive interaction and positive values indicate a net repulsive interaction. Figure 4(a-c) shows that λ becomes more negative as temperature decreases at fixed ionic strength, indicating increasing attractive interactions, as expected.

Figure 5 shows λ as a function of volume fraction of protein at fixed temperature. The interaction parameter attains a minimum near ionic strength 0.80 M indicating maximum attraction. This observation is unexpected because previous studies with lysozyme in other salt-solution systems have shown λ to decrease monotonically as salt concentration rises (Kuehner *et al.*, 1997: Muschol and Rosenberger, 1995: Eberstein *et al.*, 1994). The previous studies contained salts that preferentially hydrate the lysozyme surface.

Arakawa et al. (1990) determined the solubility of lysozyme in $MgCl_2$ solutions. The solubility near zero salt concentration is very high (>250 g/L) and sharply decreases to a minimum solubility at ionic strength 0.9 M. Above this salt concentration, the solubility of lysozyme in $MgCl_2$ solutions increases. Our observed minimum in λ at 0.84 M is in very good agreement with Arakawa's solubility studies.

Cloud-point-temperature (CPT) studies have been reported for lysozyme at a fixed protein concentration 87 g/L in $MgCl_2$ solution at pH 7.0 (Broide, 1996). The CPT is the temperature for liquid-liquid phase separation. The CPT is an indication of the net attractive interactions between protein molecules; the higher the CPT, the greater the net attractive interactions. Broide reported a maximum in the CPT for lysozyme in $MgCl_2$

solutions at ionic strength 0.9 M. Above $MgCl_2$ ionic strength 0.9 M, the net attractive interactions decrease. Broide's observation is in good agreement with the present study.

Discussion

Numerous authors (e.g. Retailleau *et al.*, 1999; Piazza, 1999; Rosenbaum and Zukoski, 1996) have described phase behavior of simple colloidal systems using simple pair potentials. Several forms of the pair potential have been proposed to represent the strength and range of interactions between protein molecules. Examples of such pair potentials include charge-charge, van der Waals, osmotic, solvation/hydration, and specific interactions. Protein molecules are often represented as hard spheres with uniform charge distribution contained in a continuous saline medium, i.e. the discrete nature of ions is not taken into account when the presence of salt is represented by point charges.

Regression of model parameters for pair potentials has been performed for DLS measurements of lysozyme. Kuehner *et al.* (1997) reported DLS measurements of lysozyme in ammonium sulfate solutions as a function of ionic strength, protein concentration, and pH. The attractive interaction parameter decreases as ionic strength rises but becomes nearly constant at 1.0 M ionic strength due to screening of electrostatic repulsion between proteins molecules. The attractive interaction parameter increases at higher ionic strength due to osmotic attraction of protein molecules at higher salt

concentrations. The Hamaker constant and net charge on the protein molecule regressed from the DLS were $8.9k_BT$ and 5.5, respectively. A similar analysis was performed by Eberstein *et al.* (1994) for lysozyme in acetate-buffered (pH 4.2) solutions of sodium chloride at 25°C. Muschol and Rosenberger (1995) performed static and dynamic-light-scattering measurements on lysozyme in solutions of sodium chloride and sodium acetate at pH 4.7. The Hamaker constant and protein charge were 7.2 k_BT and 5.4, respectively.

Pair-potential models are able to describe the salting-out of proteins at high salt concentrations. However, at present, no pair potential model is able to account for the present DLS observations, solubility measurements, and CPT results. The models proposed by previous authors cannot explain the salting-in of lysozyme at high $MgCl_2$ concentrations.

Arakawa *et al.* (1990) have shown that, as the concentration of $MgCl_2$ increases, there is a greater extent of Mg^{2+} binding to the surface of lysozyme, bovine serum albumin, and β-lactoglobulin. The extent of Mg^{2+} binding increases as the pH of the solution approaches the isoelectric point because the net positive charge on the protein surface approaches zero. The highly kosmotropic Mg^{2+} ion structures water around itself and consequently produces water structuring around the protein suface.

The Cl^- ion may also bind to the protein surface. Cl^- is a slightly chaotropic ion (Collins, 1997). Addition of Cl^- to a solution decreases the water structure of the solution. Although Cl^- may bind to the positively charged sites of the protein surface, this

interaction is relatively weak compared to the Mg^{2+} protein interaction. Therefore, it is likely that the minimum in λ is a consequence of Mg^{2+} binding, not Cl^- binding, to the protein surface and not of Cl^- binding.

These qualitative comments are supported by our recent CPT data (not published) for lysozyme in several magnesium salt solutions. In these studies, the CPT maximum decreases for magnesium salts as the anion becomes more kosmotropic. The highest CPT maximum was observed for magnesium nitrate. The NO_3^- ion is highly chaotropic and therefore breaks the water structuring around the protein surface. The lowest CPT maximum was observed for magnesium sulfate. The SO_4^{2-} ion is kosmotropic and further increases water structuring around the protein surface. It appears that structuring water around the protein surface plays a critical role in the net attractive force between protein molecules in aqueous solution.

In a similar manner, the minimum observed for λ can be explained by ion binding. At $MgCl_2$ concentrations below the minimum, the addition of salt screens the repulsive interactions between lysozyme molecules and therefore, there is a greater net attraction between the protein molecules. However, as the concentration of $MgCl_2$ increases, there is a greater extent of Mg^{2+} binding to the protein surface producing more water structuring around the protein molecules. Structuring of water around the protein surface provides a repulsive barrier. For protein molecules to come to close approach, the water structure around the protein must be broken. Therefore, as Mg^{2+} concentration increases,

structuring of water increases, leading to a greater repulsive barrier and decreased net attractive interaction.

The current DLS study coupled with solubility and CPT data suggests that the pair-potential models must be modified. A model describing phase behavior must account for interactions beyond Coulombic, van der Waals, and osmotic forces. The extent of ion binding and water structuring at the protein surface and solution must be taken into account. Inclusion of ion binding may explain the effect of different salts on the observed phase behavior of aqueous protein solutions. Also, the pH dependence of the phase behavior may be better understood if the extent of ion binding is known. The pH of the solution determines the net charge on the protein surface, and therefore the extent of ion binding. The pH dependence of protein solubility may also be better understood at high salt concentrations. By taking into account ion binding and water structuring around the protein surface, a better pair-potential model is more likely to predict accurately the qualitative and quantitative nature of protein-solution phase behavior.

Acknowledgments

This work was supported by the National Science Foundation and by the Director, Office of Science, Office of Basic Energy Sciences, Chemical Sciences Division of the U.S. Department of Energy under Contract Number DE-AC0376SF0009.

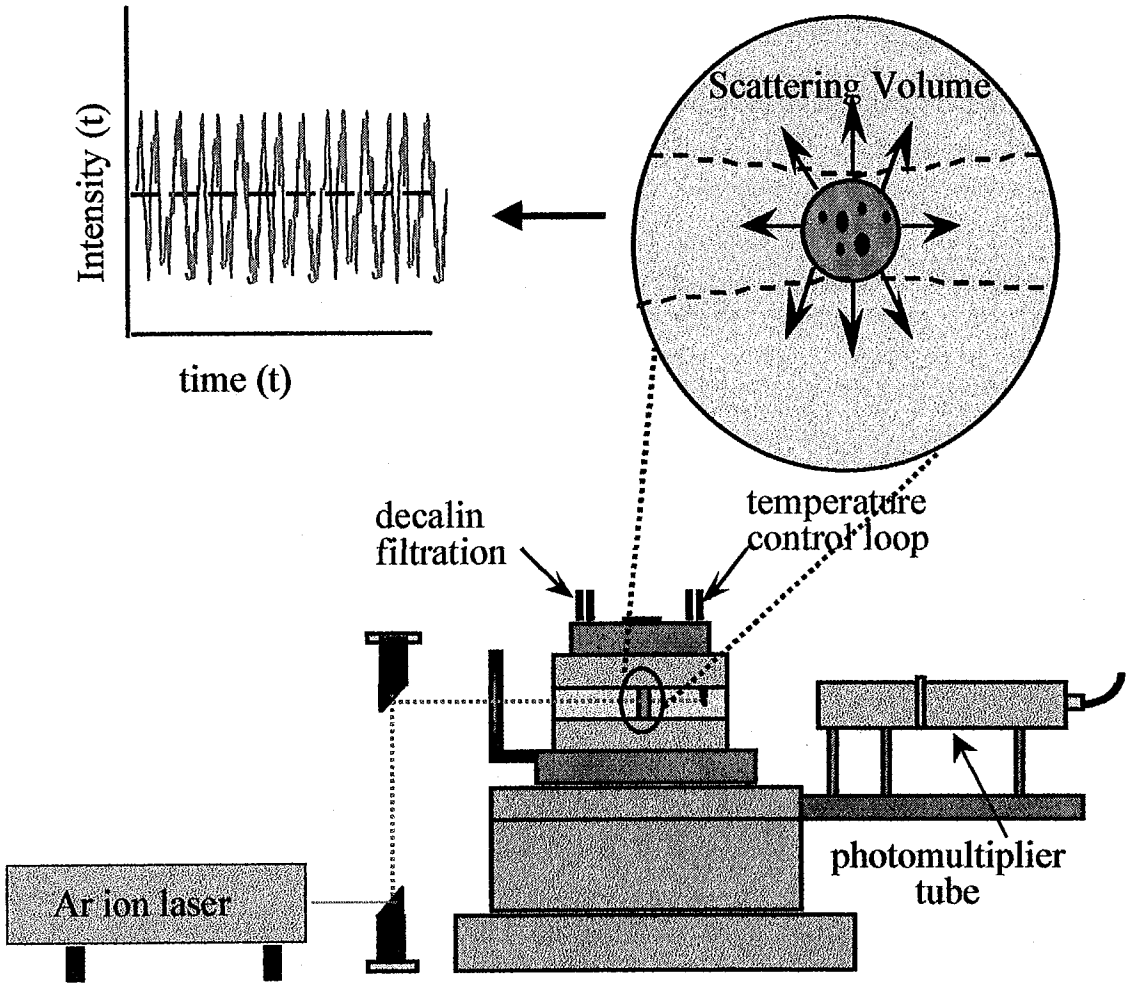

Figure 1. Schematic of the dynamic light-scattering apparatus

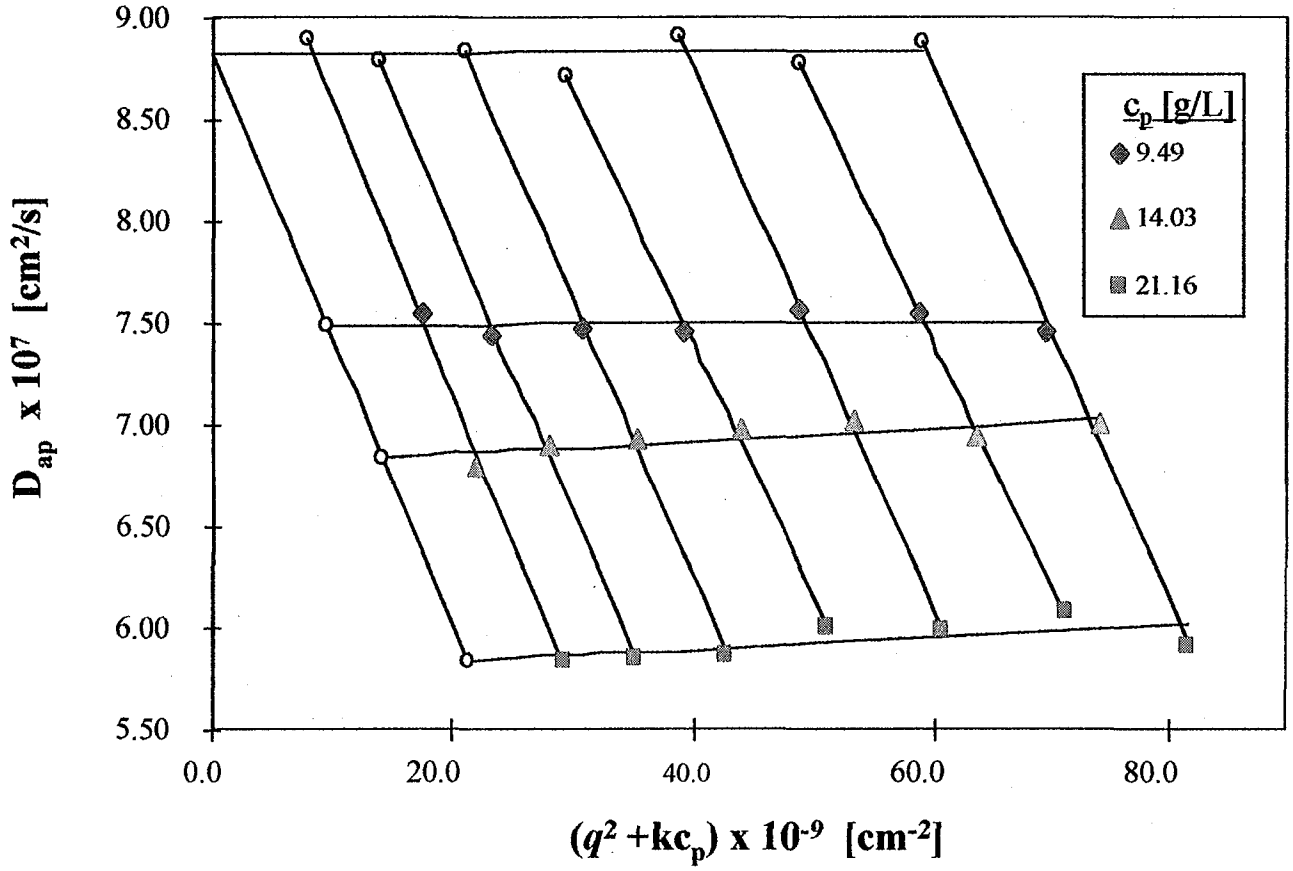

Figure 2. Zimm Plot. Dependence of the apparent diffusion coefficient of lysozyme on protein concentration and angle of detection for $MgCl_2$ solution at of IS 0.80 M, pH 4.0, and 10°C

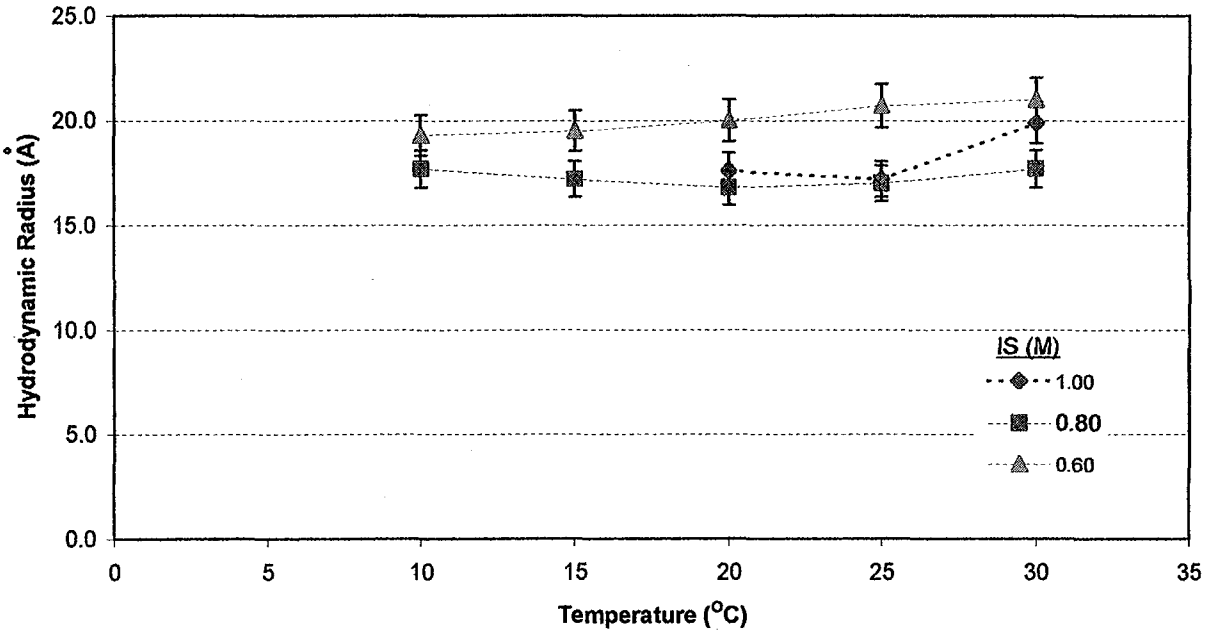

Figure 3. Hydrodynamic radius of lysozyme in $MgCl_2$ solutions at various ionic strengths

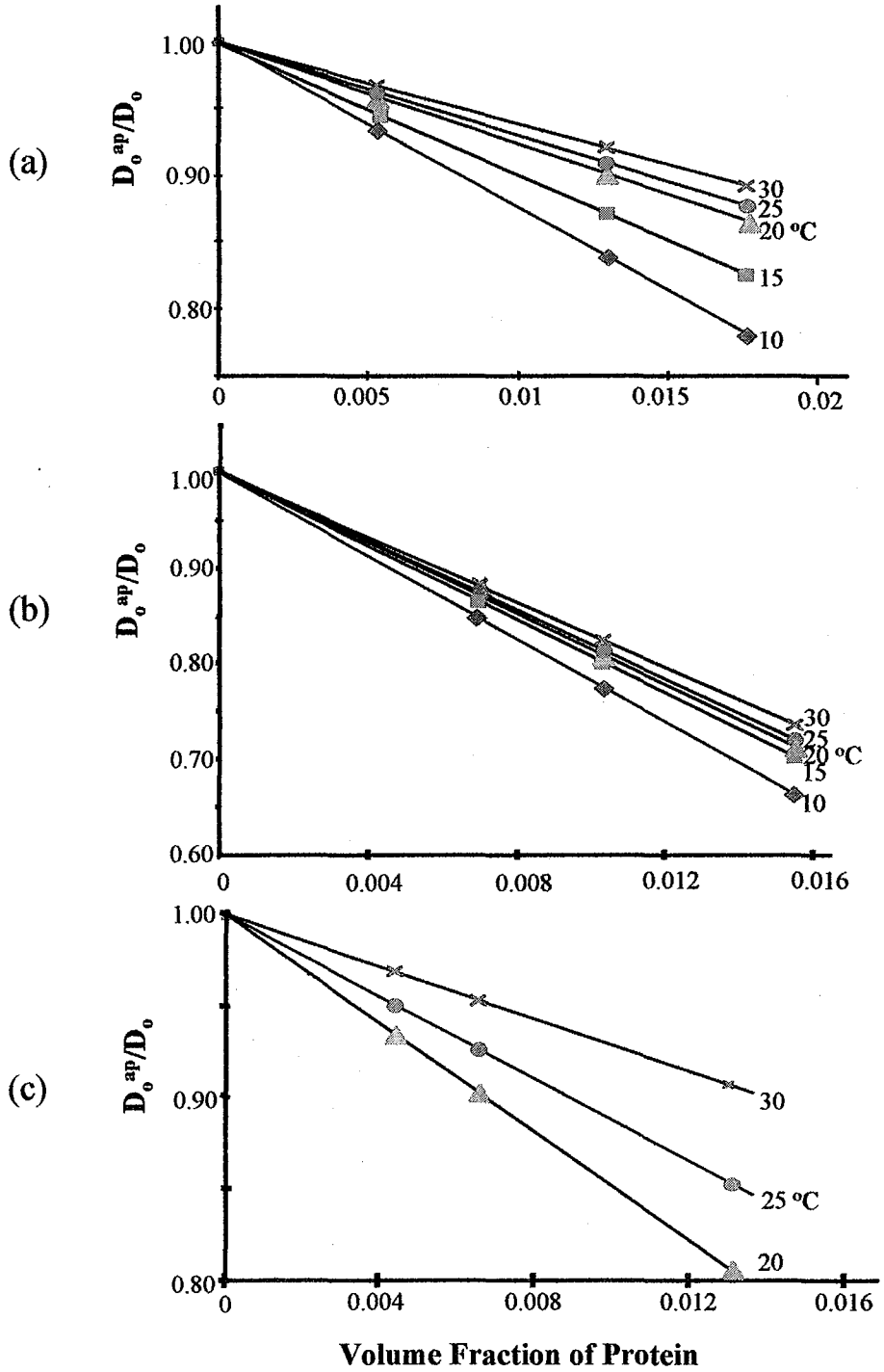

Figure 4. Determination of the interaction parameter, λ for aqueous lysozyme in $MgCl_2$ solutions at pH 4.0 and ionic strength (a) 0.60 (b) 0.80 (c) 1.00 M. λ is the slope of the lines shown here.

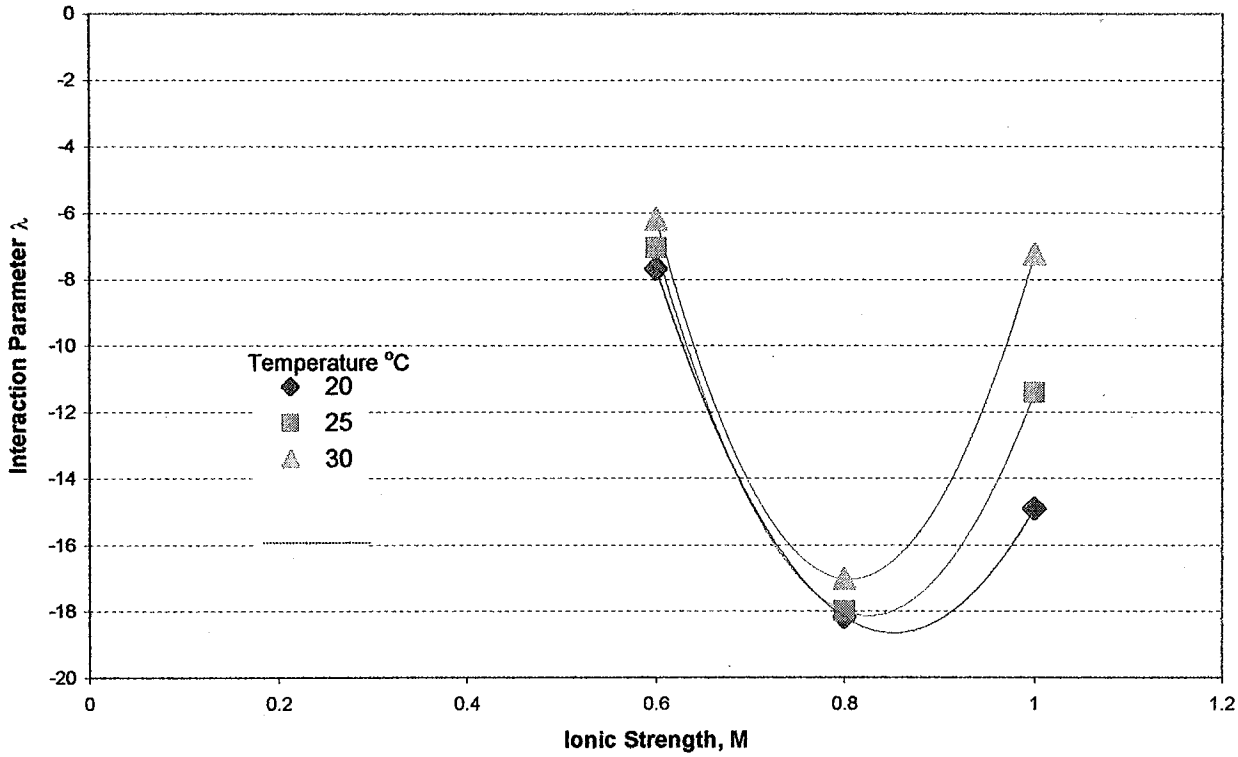

Figure 5. Interaction parameter λ as a function of $MgCl_2$ ionic strength at different temperatures

IS (M)	0.6	0.8	1.0
Temperature (^{o}C)	30	30	30
	25	25	25
	20	20	20
	15	15	
	10	10	

Table 1. Temperatures and ionic strengths of $MgCl_2$ solutions studied in this work

Viscosity (centipoise)

	Ionic Strength, M		
T(°C)	**0.60**	**0.80**	**1.0**
30	0.865	0.887	0.913
25	0.971	0.995	1.019
20	1.097	1.124	1.156
15	1.230	1.268	
10	1.374	1.402	

Table 2. Viscosities of aqueous $MgCl_2$ solutions as a function of ionic strength and temperature

Ionic Strength 1.00 M		
T(°C)	10^6 D^o_{ap} (cm^2/sec)	r_H (Å)
20	1.06	17.6
25	1.15	17.2

Ionic Strength 0.80 M		
T(°C)	10^6 D^o_{ap} (cm^2/sec)	r_H (Å)
10	0.879	17.7
15	0.997	17.2
20	1.14	16.8
25	1.29	17.0
30	1.41	17.7

Ionic Strength 0.60 M		
T(°C)	10^6 D^o_{ap} (cm^2/sec)	r_H (Å)
10	0.832	19.3
15	0.940	19.5
20	1.05	20.0
25	1.16	20.7
30	1.30	21.0

Table 3 Zero-angle apparent diffusion coefficient and hydrodynamic radius of lysozyme as a function of temperature and ionic strength of $MgCl_2$

NOMENCLATURE

B	equipment parameter relating $g^I(\tau)$ to $g^E(\tau)$
c_p	protein concentration (g/L)
D_{ap}	apparent diffusion coefficient (cm^2/sec)
D^o_{ap}	apparent diffusion coefficient extrapolated to zero angle (cm^2/sec)
D_o	infinitely-dilute diffusion coefficient (cm^2/sec)
f_o	infinite-dilution friction factor
$g^E(\tau)$	electric-field autocorrelation function
$g^I(\tau)$	normalized intensity autocorrelation function
$G^I(\tau)$	Intensity autocorrelation function
$I(t)$	intensity of scattered light at time t (photons)
k	graphical constant
k_B	Boltzmann's constant (1.38×10^{-23} J/K)
M	molecular weight of protein (g/mol)
n	refractive index of the protein salt solution
q	magnitude of the scattering vector (cm^{-1})
Q	second central moment of diffusion-coefficient distribution
r_H	infinite-dilution hydrodynamic radius (Å)
T	absolute temperature (K)
t	time (sec)

Greek Symbols

α_x, β_x	crystallographic monomer ellipsoidal major, minor semiaxis (Å)
Γ	decay constant for the electric-field autocorrelation function (sec)
Γ_{ap}	apparent decay constant (sec)
η_o	viscosity of the salt solution (centipoise)
λ	interaction parameter
λ_{laser}	wavelength of laser light (488×10^{-7} cm)
θ	scattering angle
ϕ	protein volume fraction
τ	time increment (sec)
ν	partial specific volume of lysozyme (0.703 mL/g)

LITERATURE CITED

Arakawa, T., Bhat, R., Timasheff, S. N., "Preferential interactions determine protein solubility for three-component solutions: the $MgCl_2$ system" Biochemistry (1990) **29**(7): 1914-1923

Banerjee. S. K., A Pogolotti and J. A. Rupley, "Self-association of lysozyme," J. Biol. Chem. (1975) **250**(20): 8260-8266

Batchelor, G. K., "Brownian diffusion of particles with hydrodynamic interaction," J. Fluid Mech. (1976) 74(1): 1-29

Batchelor, G. K., "Diffusion in a dilute polydisperse system of interacting spheres," J. Fluid Mech. (1983) **131**: 155-175

Blake, C. C. F., G. A. Mair, A. C. T. North, D. C. Phillips and V. R. Sarma, "On the conformation of the hen egg-white lysozyme molecule," Proc. Roy. Soc. Ser. B. Biol. Sci. (1967) **167**: 365-377

Broide, M. L., Tominc, T. M. and Saxowsky, M. D.; "Using phase transitions to investigate the effect of salts on protein interactions," Physical Review E (1996) **53** (6b): 6325-6335

Chiew, Y. C., D. E. Kuehner, H. W. Blanch and J. M. Prausnitz, "Molecular thermodynamics of salt-induced protein precipitation," AIChE J. (1995) **41**(9):2150-2159

Collins K. D., "Charge density-dependent strength of hydration and biological structure," Biophys. J. (1997) **72**:65-76

Eberstein W, Y. Georgalis, W. Saenger, "Molecular interactions in crystallizing lysozyme solutions studied by photon correlation spectroscopy," J. Crystal Growth (1994) **143**: 71-78

Felderhof, B. U., "Diffusion of interacting Brownian particles," J. Phys. A: Math. Gen. (1978) **11**: 929-937

George, A. and W. W. Wilson, "Predicting protein crystallization from a dilute solution property," Acta Cryst. D (1994) **50**: 361-365

Guo B., S. Kao, H. McDonald, A. Asanov, L. L. Combs, W. W Wilson, "Correlation of second virial coefficients and solubilities useful in protein crystal growth," J. Crystal Growth (1999) **196**: 424-433

Koppel, D. E., "Analysis of macromolecular polydispersity in intensity correlation spectroscopy: The method of cumulants," J. Chem. Phys. (1972) **57**(11): 4814-4820

Kuehner, D. E., C. Hayer, C. Ramsch, U. M. Fornefeld, H. W. Blanch and J. M. Prausnitz, "Interactions of lysozyme in concentrated electrolyte solutions form dynamic light-scattering measurements," Biophys. J. (1997) **73**(6): 32111-3224

McMillan W. G. and J. E. Mayer," The statistical thermodynamics of multicomponent systems," J. Chem. Phys. (1945) **13**: 276

Muschol, M. and F. Rosenberger, "Interactions in undersaturated and supersaturated lysozyme solutions: static and dynamic light scattering results," J. Chem. Phys. (1995) **103**(24): 10424-10432

Neal B. L., D. Asthagiri, O. D. Velev, A. M. Lenhoff, E. W. Kaler, "Why is the osmotic second virial coefficient related to protein crystallization?," J. Crystal Growth (1999) **196**: 377-387

Norton, R. S. and A. Allerhand, "Participation of tryptophan 62 in the self-association of hen egg-white lysozyme," J. Biol. Chem. (1977) **252**(5): 1795-1798

Phillies, G. D. J., "Dynamics of Brownian probes in the presence of mobile or static obstacles," J. Phys. Chem. (1995a) **99**: 4265-4272

Phillies, G. C. J., R. H. Hunt, K. Strang and N. Sushkin, "Aggregation number and hydrodynamic hydration levels of Brij-35 micelles from optical probe studies," Langmuir (1995b) **11**: 3408-3416

Piazza, R., "Interactions in protein solutions near crystallization: a colloid physics approach," J. Crystal Growth. (1999) **196**: 415-23

Provencher, S. W., "A constrained regularization method for inverting data represented by linear algebraic or integral equation," Comput. Phys. Commun. (1982a) **27**: 213-227

Provencher, S. W., "CONTIN: A general purpose constrained regularization program for inverting noisy linear algebraic and integral equations," Comput. Phys. Commun (1982b) **27**: 229-242

Retailleau, P., Ries-Kautt, M., Ducruix, A., Belloni, L., and others, "Coulombic interaction and ion-protein macroion coupled diffusion evidenced by light scattering," Europhys. Lett. (1999) **46**(2): 154-159

Rosenbaum, D., P. C. Zamora and C. F. Zukoski, "Phase behavior of small attractive colloidal particles," Phys. Rev. Lett. (1996) **76**(1): 150-153

Rothstein, F. (1994). Protein Purification Process Engineering. R.G. Harrison ed., Dekker, New York

Rupley, J. A., L. Butler, M. Gerring. F. J. Hartdegen and R. Pecoraro, "Studies on the enzymatic activity of lysozyme, III. The binding of saccharides," Proc. Nat. Acad. Sci. U.S.A (1967) **57**: 1088-1095

Scopes, R. K. (1994). Protein Purification: Principles and Practice. New York, Springer-Verlag

Sophianopoulos, A. J. and K. E. Van Holde, "Evidence for dimerization of lysozyme in alkaline solution," J. Biol. Chem. (1961) **236**(12):PC82-PC83

Sophianopoulos, C. K.Rhodes, D. N. Holcomb and D. E. Van Holde, "Physical studies of lysozyme. I. Characterization," J. Biol. Chem. (1962) **237**(4): 1107-1112

Sophianopoulos, A. J. and K. E. Van Holde, "Physical studies of muramidase (lysozyme) II. pH-dependent dimerization," J. Biol. Chem. (1964) **239**(8):2516-2524.

Sophianopoulos, A. J., "Association sites of lysozyme in solution," J. Biol. Chem. (1969) **244**(12):3188-3193

Vlachey, V., H. W. Blanch and J. M. Prausnitz, "Liquid-liquid phase separations in aqueous solutions of globular proteins," AIChEJ. (1993) **39**(2):215-223