Mathematical and computational aspects of quaternary liquid mixing free energy measurement using light scattering

Chris W. Wahle,¹ David S. Ross,¹ and George M. Thurston²

¹*School of Mathematical Sciences, Rochester Institute of Technology, Rochester, New York 14623, USA*

²*Department of Physics, Rochester Institute of Technology, Rochester, New York 14623, USA*

(Received 12 January 2012; accepted 27 June 2012; published online 20 July 2012)

We provide a mathematical and computational analysis of light scattering measurement of mixing free energies of quaternary isotropic liquids. In previous work, we analyzed mathematical and experimental design considerations for the ternary mixture case [D. Ross, G. Thurston, and C. Lutzer, *J. Chem. Phys.* **129**, 064106 (2008); C. Wahle, D. Ross, and G. Thurston, *J. Chem. Phys.* **137**, 034201 (2012)]. Here, we review and introduce dimension-free general formulations of the fully nonlinear partial differential equation (PDE) and its linearization, a basis for applying the method to composition spaces of any dimension, in principle. With numerical analysis of the PDE as applied to the light scattering implied by a test free energy and dielectric gradient combination, we show that values of the Rayleigh ratio within the quaternary composition tetrahedron can be used to correctly reconstruct the composition dependence of the free energy. We then extend the analysis to the case of a finite number of data points, measured with noise. In this context the linearized PDE describes the relevant diffusion of information from light scattering noise to the free energy. The fully nonlinear PDE creates a special set of curves in the composition tetrahedron, collections of which form characteristics of the nonlinear and linear PDEs, and we show that the information diffusion has a time-like direction along the positive normals to these curves. With use of Monte Carlo simulations of light scattering experiments, we find that for a modest laboratory light scattering setup, about 100–200 samples and 100 s of measurement time are enough to be able to measure the mixing free energy over the entire quaternary composition tetrahedron, to within an $\mathcal{L}_2$ error norm of 10^{-3} . The present method can help quantify thermodynamics of quaternary isotropic liquid mixtures. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4736837>]

I. INTRODUCTION

This work examines the practicality of static light scattering as a tool for quantifying mixing free energies of isotropic liquids of four molecular components, extending analyses of the ternary mixture case.^{1,2} Quaternary molecular mixtures are important in a broad variety of scientific, physiological, engineering, and industrial contexts. Directly measuring free energies of quaternary mixtures can help in developing sound statistical thermodynamics models that relate molecular to supramolecular and macroscopic properties.

Since the scattered light intensity is a single, measurable quantity that depends on the second composition derivatives of the free energy, in combination with the dielectric response of the medium, it is not clear without further analysis that it can be used to reconstruct the mixing free energy of a multi-component solution. However, our previous work shows that the composition dependence of the light scattering intensity can be used to reconstruct the mixing free energy of ternary mixtures,¹ and has analyzed the corresponding experimental design problem.² Here, we show that quaternary mixing free energies can also be reconstructed from light scattering, and consider the quaternary experimental design problem. To introduce the basic ideas, here we outline the procedure and some underlying intuition.

As described previously,¹ we use the relationship between the second composition derivatives of the free energy and the light scattering not as a formula for obtaining the light scattering from the free energy, as one would in testing free energy models, but instead as a nonlinear, second-order partial differential equation enabling one to obtain the free energy of mixing itself from a set of light scattering measurements. This can be done provided that enough measurements are taken at a suitable variety of compositions.² In this view, the measured light scattering vs. composition is regarded as the source term for a light scattering partial differential equation (LSPDE). The boundary conditions needed to solve the LSPDE are the free energies of reduced mixtures, each of which is obtained from the full mixture by removing one of the original components. The needed boundary mixture free energies, in turn, can be determined by analyzing light scattering measurements using an appropriate, lower dimensional LSPDE, with its boundary conditions provided by the free energies of mixtures that have been simplified yet again. Such stepping stones continue until the boundaries are binary mixtures, for which the free energy of mixing and the light scattering are related by a linear, second-order ODE that can be solved more directly. The actual experimental and analytical process naturally proceeds from the binary

mixtures, to their parent ternary mixtures, to the full quaternary mixture.

Intuitively, the fact that the light scattering intensity involves a combination of second composition derivatives of the free energy¹ means that it gives *partial* information about the free energy in a *local neighborhood*, or patch, near a given sample composition. More precisely, each sample of the scattered intensity measures a specific local second directional derivative of the free energy of mixing of the components, but the relevant directions are at first unknown in a mixture that has more than two components. Sufficient samples,² suitably spaced in composition, permit the numerical solution of the light scattering partial differential equation to line the directions up into threads that traverse the composition space from one vertex to another, as depicted in Fig. 5. The threads stitch the patches together consistent with the boundary conditions, much like working a jigsaw puzzle starting with the edges. In the case of quaternary mixtures, examples of the patches can be visualized in Fig. 6. Effectively, the LSPDE carries the information that each sample of the second directional derivative stems from the same underlying free energy function, as probed here by light scattering with use of a given dielectric response function.

In some cases, four components could prove to be a minimal set for understanding some basic system properties. One motivation and purpose of the present work is to provide tools for studying the light scattering and phase boundaries of quaternary mixtures of the α -, β -, and γ -crystallin eye lens proteins with an aqueous buffer.^{3–5} These concentrated protein-water mixtures are found in all mammalian lenses, where their high indices of refraction and clarity enable the lens to help form images on the retina. The underlying protein-water mixture free energy not only determines the intensity of scattered light that can disrupt eye lens transparency, but also governs the chemical potentials that determine driving forces for phase separation,^{3,6} as well as aggregation that can lead to cataract.³ In addition, the free energy determines the osmotic pressure that is thought to help determine the refractive index gradient within the lens.⁷ Thus, rapid means of directly measuring eye lens protein mixture free energies can aid in understanding multiple aspects of normal and abnormal lens physiology.

Another essentially quaternary mixture of great physiological importance is bile, which primarily consists of water, bile salts, cholesterol, and phosphatidylcholine (lecithin).⁸ Bile is crucial for digestion of essential fats and oils, is the final route for cholesterol clearance from the body, and is the liquid within which gallstones form. Understanding the molecular origins of the intricate phase diagram of bile,^{8–11} and its sensitivity to bile salt and phosphatidylcholine type, can be greatly aided by directly measuring the underlying free energy and chemical potentials. The chemical potentials and phase boundaries, in turn, together with those of added components, contribute to governing certain equilibrium aspects of digestion, cholesterol clearance, and gallstone formation. Bile is also a physiological example of a microemulsion,¹² important in many scientific, food, drug delivery, household, personal care, and industrial contexts. Quaternary micromulsions can exhibit very interesting phase behavior, including four-phase coexistence.¹³

Mixtures of protein, ligand, solvent, and protein-ligand complexes are quaternary and higher order systems of fundamental importance in cell biology and biochemistry, absolutely basic to enzyme kinetics, and a multitude of other cellular processes. At the high macromolecule concentrations inside cells, typically 7%–40% by volume,¹⁴ dilute solution thermodynamics typically does not apply, phase transitions occur more readily, and profound changes in solution properties, changes that even mediate transitions between health and disease, can hinge on molecular interaction changes as small as $0.1 k_B T$ or $(1/400)$ eV.⁶ Because of this extreme sensitivity and the difficulty of the statistical physics involved,^{15–20} for given systems of biological macromolecules detailed experimentation is needed to work from formation free energies and molecular properties in dilute solution, to help infer their high concentration consequences for the free energy that are relevant in living cells. Understanding these connections poses exciting challenges to both liquid-state physics and molecular interaction theory and measurement. Light scattering at high concentrations,^{21,22} including light scattering free energy measurement^{1,2} can complement current means of characterizing concentrated biological macromolecule and ligand solutions, and thereby aid both in understanding specific systems and in guiding theoretical and computational advances. Similar considerations apply in the realm of gene regulation, for the manifold complexes of DNA, RNA, and proteins in the highly concentrated nucleus.

Spontaneous composition fluctuations at equilibrium, depicted in the quaternary composition space in Fig. 1, form the fundamental physical connection between light scattering and free energy.^{23,24} These fluctuations create local dielectric response variations that scatter light,^{25–28} or analogous fluctuations that scatter other probes such as x-rays or neutrons.^{29,30} In Fig. 1, the colored spheres represent distinct molecular components, and fluctuations are shown in snapshots from Metropolis Monte Carlo simulations³¹ of a lattice model of solutions,³² at different quaternary compositions. Near critical points (lower right cube in Fig. 1) dramatic fluctuations of nearly separating components occur, which scatter light intensely in the phenomenon known as critical opalescence, the original subject of work on the connection between thermodynamics and light scattering.²⁵

We first formulated the mathematical and computational aspects of the light scattering free energy determination problem for the ternary mixture case in terms of a fully nonlinear PDE, and showed that we could reconstruct mixing free energies by solving it numerically.¹ We also showed that when given equilibrium cloud-point compositions at the boundary between a single liquid phase and two or three liquid phases, the method can also reconstruct the tie lines and tie triangles joining the compositions of the coexisting phases.¹

We then extended our analysis to consider how the accuracy of the inferred free energy is affected by noise in light scattering measured at a finite number of compositions, and by numerical approximations resulting from interpolating the data onto a grid used for solving the PDE.² The central element for describing the effects of noise is a linearized, degenerate elliptic PDE. The characteristic curves of the linearized PDE govern a diffusive flow of information from

FIG. 1. Light scattering free energy determination relies on composition dependence of spontaneous local composition fluctuations in equilibrium,^{23,24} which create local dielectric response variations that scatter light.^{25–28} x (blue), y (green), z (red), and w (black) are volume fractions of molecular components. Fluctuations are shown in snapshots (cubes) from Monte Carlo simulations of a lattice model of solutions, at different quaternary compositions (arrows). Dilute components have Poisson spatial probability distributions (e.g., red, black, and green molecules in the lower left cube), resulting in linear light scattering composition dependence near vertices (see Fig. 4). Near critical points dramatic fluctuations of nearly separating components occur (blue and green molecules in lower right cube). These scatter light intensely, depending on composition dependence of dielectric response through $\nabla\epsilon$ (see Fig. 4, along binary axes). Each binary axis is labeled by regular solution test model molecular interaction parameters C_{ij} (see text).

light scattering data to the inferred free energy. We identified free energy error-norm scaling laws that can be combined to identify optimal sample numbers for a given total measurement time, and showed how to calculate or measure the instrumental time constant that connects actual time with the dimensionless measurement time used for the analysis. The analysis indicated that light scattering measurement times of minutes, seconds, or even less would, in principle, be sufficient to acquire data to reconstruct a ternary mixing free energy over the entire ternary composition triangle.²

Of course other practical matters affect measurement accuracy, and the summarized work did not include effects of sample impurities such as dust, or of systematic errors that can easily result, for instance, from composition-dependent sample evaporation. The work also used an approximation¹ to the exact expression for the scattering efficiency (see, e.g., Ref. 27), namely, that the molecular volumes are essentially additive, an approximation whose practical validity depends on the particular mixture and conditions of interest (see, e.g., Refs. 33 and 34). For mixtures that are not well-modeled by this assumption, we anticipate that additional data about mass density vs. composition will enable a suitably modified mathematical and computational formulation to reconstruct the mixing free energy from the light scattering intensity.

It is important to note that the present work, as it stands, is strictly applicable only to mixtures that form single phase,

isotropic liquids throughout the quaternary composition tetrahedron. Many small organic molecules will indeed form such mixtures, but the important biological examples given above will not. Nevertheless, this work is the first step towards establishing the mathematical, computational, and experimental basis for using light scattering to directly determine mixing free energies of quaternary systems that only form isotropic liquids in a portion of the phase diagram. We anticipate that such methods can be devised with use of scattering probes that have effective material cross sections with sufficiently different composition dependences, such as might be obtained with use of multiple light wavelengths. The present analysis is prerequisite to that generalization.

An outline of the paper is as follows. In Sec. II we review the previous mathematical background, and review and introduce dimension-free general formulations of the full nonlinear PDE and its linearization. These equations form the basis for applying the present method to composition spaces of any dimension. In Sec. III we describe the computational algorithms we used to apply this general formulation to quaternary mixtures. In Sec. IV we show that values of the Rayleigh ratio within the quaternary composition tetrahedron can be used to correctly reconstruct the composition dependence of a free energy model for liquid mixtures, the regular solution model.^{35,36} In Sec. V we discuss general aspects, including characteristics, of the linearized, degenerate elliptic PDE that

is basic to analyzing aspects of the experimental noise problem for quaternary mixtures. In Sec. VI we use simulations of light scattering noise to make estimates of the time required to determine mixing free energies of quaternary isotropic liquids throughout the composition tetrahedron. We leave the question of determining phase boundaries in quaternary mixtures with use of the present method, similar to that which can be done for ternary mixtures,¹ for future work.

II. MATHEMATICAL FORMULATION OF THE LIGHT SCATTERING PDE FOR COMPOSITION SPACES OF ARBITRARY DIMENSION

We introduce the mathematical forms of the key governing equations of the present analysis, before stating the precise connections with physically measured quantities. First, we have cast the relationship between the composition dependence of the Gibbs free energy of a mixture and the light scattering intensity as a fully nonlinear partial differential equation,¹

$$\min_{\text{all directions}} \frac{\frac{\partial^2 g}{\partial s^2}}{\left(\frac{\partial \varepsilon}{\partial s}\right)^2} = \frac{1}{R}. \quad (1)$$

The dimensionless free energy g is the unknown, R is a dimensionless light scattering efficiency, or Rayleigh ratio, ε is the known, dimensionless mixture dielectric coefficient, and directional derivatives are taken with respect to dimensionless composition directions s that are described in more detail below.

Second, the basis for our analysis of the influence of light scattering noise and interpolation approximation on the free energy is a linearized, degenerate elliptic PDE obtained from Eq. (1).² This linearized equation states that perturbations to the Rayleigh ratio ρ produce free energy perturbations f that satisfy

$$(H_g^{-1} \nabla \varepsilon)^T H_f (H_g^{-1} \nabla \varepsilon) = -\rho. \quad (2)$$

In Eq. (2), H_g and H_f denote compositional Hessian matrices of g and f , respectively, that are described in more detail below. Equation (2) states that ρ is proportional to the second directional derivative of f in the minimizing direction for Eq. (1), along $H_g^{-1} \nabla \varepsilon$. By quantifying the free energy perturbations that result from perturbations in the measured Rayleigh ratio, Eq. (2), together with Eq. (1), form the basis for practical determination of the quaternary and higher order mixing free energies of isotropic liquids. We now summarize the origin and derivation of Eqs. (1) and (2) and specify the physical quantities used in detail.

The detailed steps in the derivation of Eqs. (1) and (2) may be found in Refs. 1 and 2. The analysis in Ref. 1 starts from the general expression for the Rayleigh ratio for multi-component mixtures presented as Eq. (12) of Kirkwood and Goldberg,²⁷ which is cast in terms of a matrix of concentration derivatives of each of the chemical potentials of the components of the mixture. Specifically, each matrix entry is the derivative of one of the chemical potentials with respect to the average concentration of a specific component, with the average concentrations of all of the other components held fixed,

along with temperature and pressure. Further, each concentration in Ref. 27 is expressed in terms of the mass of the given component divided by the mass of the solvent. As Kirkwood and Goldberg show, this matrix of derivatives of the chemical potentials with respect to *average* concentrations is one way to parametrize the magnitudes of the *local* concentration fluctuations, which in turn produce local fluctuations in the dielectric response that scatter incident light, as indicated above.

The analysis in Ref. 1 makes use of the fact that the chemical potential derivatives in the matrix of Kirkwood and Goldberg may be re-expressed in terms of second concentration derivatives of an intensive free energy, which then provide an algebraic route for recasting the Rayleigh ratio in terms of the derivatives of a single function. It is important to note that while the chemical potentials can always be re-expressed in such a manner, the specific analysis in Ref. 1 applies to the restricted case of liquids that can be modeled as having partial molecular volumes that do not vary with composition. With use of that assumption, the Appendix of Ref. 1 details the steps needed to re-express the Kirkwood and Goldberg expression for the light scattering turbidity and forward scattering angle Rayleigh ratio, in excess of the Rayleigh ratio associated with the compressibility of the system, in terms of the Hessian matrix of second derivatives of the free energy per unit volume with respect to the average number densities of the components. Included in Ref. 1 are the needed relationships between the chemical potentials and the free energy per unit volume, and the relevant change of concentration variables from those used by Kirkwood and Goldberg, to number densities. In each successive first derivative implicit within the second derivatives in Eqs. (1) and (2), the average number densities of all of the other components, temperature, and pressure are held fixed. As detailed in the derivation resulting in Eq. (A3) in the Appendix of Ref. 1, the *overall* volume of the liquid mixture does change along such a path in number density at fixed temperature and pressure; this overall volume is to be distinguished from the *scattering* volume, which is instead a fixed volume that is effectively selected from within the liquid by the optics. Thus, the Hessian matrix provides an alternative way to parametrize the local concentration fluctuation magnitudes within local volumes. We term the resulting light scattering cross section the excess Rayleigh ratio. The resulting expression for the excess Rayleigh ratio reduces to zero for each of the pure components, as it must since for each of the pure components the light scattering intensity can be expressed solely in terms of its compressibility, which is not incorporated into the present analysis. Clearly, removing the approximations mentioned above is an opportunity for generalization of the present work.

Having outlined the origin of Eqs. (1) and (2), we now state more specifically the relationships between the dimensionless quantities used in Eqs. (1) and (2) and physical quantities.^{1,2} First, g denotes the intensive, dimensionless Gibbs free energy $\Omega_0 G / (V k_B T)$, where G is the extensive Gibbs free energy, V is the volume, k_B is Boltzmann's constant, T is absolute temperature, $\Omega_0 = \partial V / \partial N_0$ is the volume associated with each molecule of component 0, and $x = \Omega_1 N_1 / V$ and $y = \Omega_2 N_2 / V$ are the volume fractions of

components 1 and 2. As noted above, we take each Ω_i to be constant. $\varepsilon(x, y)$ is the real part of the dimensionless dielectric coefficient at the optical frequency used for measuring $R(x, y)$, $\varepsilon_x = \partial\varepsilon/\partial x$, and $\varepsilon_y = \partial\varepsilon/\partial y$. $R(x, y)$ is a convenient, dimensionless form of the excess Rayleigh ratio, $\Delta R(0)$, the latter having dimensions of inverse length. Specifically, $R(x, y) = \lambda^4 \Delta R(0)/(\pi^2 \Omega_0)$, where λ is the vacuum wavelength of the incident and the scattered light.

For testing the ability of light scattering to reconstruct quaternary mixing free energies, we have made use of a regular solution model free energy^{32,35–39} given by the sum of the ideal solution free energy

$$x \log x + y \log y + z \log z + w \log w, \quad (3)$$

plus the excess free energy $\phi(x, y, z)$,

$$g(x, y, z) = x \log x + y \log y + z \log z + w \log w + \phi(x, y, z),$$

where $w = 1 - x - y - z$. In the case of the regular solution model the excess free energy takes the simple form

$$\phi(x, y, z) = C_{xy}xy + C_{xz}xz + C_{xw}xw + C_{yz}yz + C_{yw}yw + C_{zw}zw.$$

In this model, phase separation occurs on each binary axis for $C_{ij} \geq 2$, while inside ternary composition triangles (Fig. 1) and the tetrahedral composition space, phase separation can occur for many other parameter combinations.^{35,36,38}

We note that to construct the Monte Carlo simulations shown in Fig. 1, we made use of the fact that in a lattice model for which the regular solution model is a high-temperature approximation (see, e.g., Ref. 32), near-neighbor adjacency potential energies E_{ij} may be assigned such that $C_{ij} = (q/k_B T)(E_{ij} - (1/2)(E_{ii} + E_{jj}))$, where q (6 here) is the lattice coordination number. Thus, each C_{ij} measures the difference between unlike and average like-like interaction energies, in units of thermal energy $k_B T$. For simulation, E_{ij} were taken to correspond to the C_{ij} of Fig. 3.

III. COMPUTATIONAL METHODS

The physics behind Eq. (1) does not equip it with conventional boundary conditions. Rather, the solution g must be asymptotic to the ideal solution form near the boundaries. Consistent with Eq. (3), this condition is imposed explicitly by setting

$$g(x, y, z) = x \log x + y \log y + z \log z + w \log w + \phi(x, y, z),$$

where ϕ is assumed to be a twice differentiable function throughout the domain. We then solve for ϕ instead of g . In this way, g will demonstrate the required asymptotic behavior near the boundaries.

To compute solutions numerically, we first compute the solution on the boundary of the tetrahedron, thus leaving us with a Dirichlet problem which determines the solution in the interior. The boundaries of the tetrahedron correspond to binary and ternary compositions. The six legs of the tetrahedron

are binary axes, along which exactly two of the components have zero concentration, while the four faces of the tetrahedron are ternary triangles. The solution along each binary axis may be computed independently from the rest of the domain. Once the solution is computed on the binary axes, we have a Dirichlet problem on each face. We may compute the solution on each ternary triangle, independent of the solution within the interior, using the known solution on the associated binary axes as boundary conditions. Knowing the solution everywhere on the boundary of the tetrahedron reduces our problem to a conventional Dirichlet problem in that we must solve Eq. (1) in the interior of the tetrahedron subject to prescribed boundary values.

Along each binary axis, Eq. (1) reduces to an ordinary differential equation, with the s -direction being along the binary axis itself. For example, along the x -axis, we solve the one-dimensional problem given by

$$g_{xx}(x, 0, 0) = \frac{\varepsilon_x^2(x, 0, 0)}{R(x, 0, 0)}, \quad g(0, 0, 0) = g(1, 0, 0) = 0,$$

or equivalently,

$$\phi_{xx}(x, 0, 0) = \frac{\varepsilon_x^2(x, 0, 0)}{R(x, 0, 0)} - \frac{1}{x(1-x)},$$

$$\phi(0, 0, 0) = \phi(1, 0, 0) = 0.$$

The boundary conditions are homogeneous since the endpoints of the binary axes coincide with the corners of the tetrahedral domain, at which g is assumed to be zero; a linear dependence of the actual intensive free energy on composition can be computed from its values on the vertices of the tetrahedron, and will not affect the second derivatives entering into the scattered light intensity. The solution of such a boundary value problem can be readily computed by elementary finite differencing methods. A similar boundary value problem is solved on each of the six binary axes, which make up the edges of the tetrahedral domain.

To compute solutions by way of finite differencing methods, we lay out a uniform grid over the tetrahedral domain, with a grid size of $\Delta = 1/n$. We define $x_i = i\Delta$, $y_j = j\Delta$, and $z_k = k\Delta$, with i, j , and k taking on values between 0 and n . The tetrahedral domain requires that $i + j + k \leq n$. Relaxation schemes are a common approach for computing solutions of Dirichlet problems, such as those encountered in the present work.^{40,41} Our iterative method is a relaxation scheme, adapted to our nonlinear problem.

We use second-order approximations of the second derivatives of g ,

$$g_{xx}(x_i, y_j, z_k) = \frac{1}{x_i} + \frac{1}{w_{ijk}} + \frac{\phi_{i-1,j,k} - 2\phi_{i,j,k} + \phi_{i+1,j,k}}{\Delta^2} + O(\Delta^2),$$

$$g_{yy}(x_i, y_j, z_k) = \frac{1}{y_j} + \frac{1}{w_{ijk}} + \frac{\phi_{i,j-1,k} - 2\phi_{i,j,k} + \phi_{i,j+1,k}}{\Delta^2} + O(\Delta^2),$$

$$\begin{aligned}
g_{zz}(x_i, y_j, z_k) &= \frac{1}{z_k} + \frac{1}{w_{ijk}} + \frac{\phi_{i,j,k-1} - 2\phi_{i,j,k} + \phi_{i,j,k+1}}{\Delta^2} + O(\Delta^2), \\
g_{xy}(x_i, y_j, z_k) &= \frac{1}{w_{ijk}} + \frac{\phi_{i-1,j,k} + \phi_{i+1,j,k} + \phi_{i,j-1,k} + \phi_{i,j+1,k} - 2\phi_{i,j,k} - \phi_{i+1,j-1,k} - \phi_{i-1,j+1,k}}{2\Delta^2} + O(\Delta^2), \\
g_{xz}(x_i, y_j, z_k) &= \frac{1}{w_{ijk}} + \frac{\phi_{i-1,j,k} + \phi_{i+1,j,k} + \phi_{i,j,k-1} + \phi_{i,j,k+1} - 2\phi_{i,j,k} - \phi_{i+1,j,k-1} - \phi_{i-1,j,k+1}}{2\Delta^2} + O(\Delta^2), \\
g_{yz}(x_i, y_j, z_k) &= \frac{1}{w_{ijk}} + \frac{\phi_{i,j-1,k} + \phi_{i,j+1,k} + \phi_{i,j,k-1} + \phi_{i,j,k+1} - 2\phi_{i,j,k} - \phi_{i,j+1,k-1} - \phi_{i,j-1,k+1}}{2\Delta^2} + O(\Delta^2),
\end{aligned}$$

where $w_{ijk} = 1 - x_i - y_j - z_k$ and $\phi_{ijk} = \phi(x_i, y_j, z_k)$. We do not use the conventional symmetric formulas for the mixed derivatives since the necessary points, e.g., $\phi_{i+1,j+1,k}$, are not available near the skew face of the tetrahedron.

On the faces of the tetrahedron, Eq. (1) reduces to that which describes ternary compositions. The solutions along the binary axes, having been computed by this point, serve as the boundary conditions for computing the free energy on each of the ternary triangles. We may compute the solution on the ternary faces using Eq. (1), as we do for the interior, but it is computationally more efficient to use a method similar to that described in Ref. 1. As we will see, the use of Eq. (1) has the additional expense of computing eigenvectors. For computational efficiency, we opt for a method similar to that introduced in Ref. 1. While the method of Ref. 1 is efficient and convenient for ternary mixtures, it is difficult to apply the same technique to quaternary mixtures. Thus, we use existing methods for the binary axes and ternary faces, and we employ Eq. (1) to compute solutions in the interior, corresponding to quaternary mixtures. We summarize our adaptation of the method in Ref. 1 by considering the xy -plane as an example. On the xy -plane, Eq. (1) reduces to that of a ternary mixture, and it is known¹ that the equation can be recast into the form

$$\frac{\varepsilon_y^2 g_{xx} - 2\varepsilon_x \varepsilon_y g_{xy} + \varepsilon_x^2 g_{yy}}{g_{xx} g_{yy} - g_{xy}^2} = R(x, y, 0), \quad (4)$$

where $g(x, y, 0) = x \log x + y \log y + w \log w + \phi(x, y, 0)$ and $w = 1 - x - y$. The associated finite difference equation at a grid point $(x_i, y_j, 0)$ is given by

$$\begin{aligned}
&\frac{\varepsilon_y^2 \hat{g}_{xx}(x_i, y_j, 0) - 2\varepsilon_x \varepsilon_y \hat{g}_{xy}(x_i, y_j, 0) + \varepsilon_x^2 \hat{g}_{yy}(x_i, y_j, 0)}{\hat{g}_{xx}(x_i, y_j, 0) \hat{g}_{yy}(x_i, y_j, 0) - \hat{g}_{xy}^2(x_i, y_j, 0)} \\
&= R(x_i, y_j, 0), \quad (5)
\end{aligned}$$

where $\hat{g}_{xx}(x_i, y_j, 0)$ denotes the finite difference approximation of g_{xx} at $(x_i, y_j, 0)$. This notational convention applies to the other second derivatives of g as well.

We use a relaxation scheme to solve the set of equation given by (5). We sweep through the grid points repeatedly, updating the value of each ϕ_{ij0} , in turn, until the desired accuracy is achieved. We begin with an initial guess function $\phi^1(x, y, 0)$, and let ϕ_{ij0}^l be the approximation of ϕ_{ij0} as we begin the l th sweep of the grid. Suppose we are at a grid point $(x_i, y_j, 0)$ during the l th sweep of the grid. At this grid point, Eq. (5) is satisfied only approximately. We make a correction C_{ij0}^l to ϕ_{ij0}^l so that the approximation becomes an equality. That is, we replace ϕ_{ij0}^l in Eq. (5) by $\phi_{ij0}^{l+1} = \phi_{ij0}^l + C_{ij0}^l$, and ideally solve for C_{ij0}^l . However, the expression that determines C_{ij0}^l is nonlinear and not readily solved. If the iterations are in fact converging, we expect C_{ij0}^l to be relatively small compared to ϕ_{ij0}^l . Employing this notion, we linearize the equation that determines C_{ij0}^l , which then allows us to solve for C_{ij0}^l ,

$$C_{ij0}^l = \frac{\Delta^2}{2} \cdot \frac{\left(\hat{g}_{xx}^l \hat{g}_{yy}^l - (\hat{g}_{xy}^l)^2 \right) R - (\varepsilon_y^2 \hat{g}_{xx}^l - 2\varepsilon_x \varepsilon_y \hat{g}_{xy}^l + \varepsilon_x^2 \hat{g}_{yy}^l)}{(\hat{g}_{xx}^l + \hat{g}_{yy}^l - \hat{g}_{xy}^l) R - (\varepsilon_x^2 - \varepsilon_x \varepsilon_y + \varepsilon_y^2)} \Bigg|_{(x_i, y_j, 0)}.$$

We continue to make updates in this way as we repeatedly sweep through the grid points until the solution converges to within a specified tolerance. It should be noted that once ϕ_{ijk}^l is updated in the l th sweep, the updated value is used for the remainder of the sweep. Once the prescribed degree of

accuracy is achieved, we construct the approximation of the solution g ,

$$\begin{aligned}
g(x_i, y_j, 0) &\approx x_i \log x_i + y_j \log y_j \\
&\quad + (1 - x_i - y_j) \log(1 - x_i - y_j) + \hat{\phi}_{ij0},
\end{aligned}$$

where $\hat{\phi}_{ij0}$ is the approximation of ϕ_{ij0} computed in the above iterations. The solution can be computed on the xz -plane and the yz -plane in the same manner.

Computing the solution on the skew boundary plane, where $w = 0$, requires special treatment because it does not align squarely with the grid. Our quaternary problem reduces to a ternary problem on the skew face, but only in an appropriate coordinate system. We need two of the coordinates, say u and v , to run parallel to the plane, while the third coordinate, w , runs perpendicular to the plane. In such a coordinate system, our problem reduces appropriately to that of the ternary mixture as $w \rightarrow 0$. We introduce the coordinate transformation given by

$$u = \frac{1}{\sqrt{2}}(x - y), \quad v = \frac{1}{\sqrt{6}}(x + y - 2z), \\ w = 1 - (x + y + z).$$

The uv -plane corresponds to the skew face of the tetrahedron. The coefficients in the definitions of u and v are chosen so as to preserve distances in the uv -plane. That is, on the uv -plane, distances in the uvw -coordinate system agree with distances in the xyz -coordinate system. In the limit as $w \rightarrow 0$,

our PDE reduces to

$$\frac{\varepsilon_v^2 g_{uu} - 2\varepsilon_u \varepsilon_v g_{uv} + \varepsilon_u^2 g_{vv}}{g_{uu} g_{vv} - g_{uv}^2} \\ = R(x(u, v, 0), y(u, v, 0), z(u, v, 0)), \quad (6)$$

where

$$\varepsilon_u = \varepsilon_x x_u + \varepsilon_y y_u + \varepsilon_z z_u = (\varepsilon_x - \varepsilon_y)/\sqrt{2}$$

and

$$\varepsilon_v = \varepsilon_x x_v + \varepsilon_y y_v + \varepsilon_z z_v = (\varepsilon_x + \varepsilon_y - 2\varepsilon_z)/\sqrt{6}.$$

We solve this PDE in the same manner as that described above, while making the necessary adjustments to account for the new coordinate system.

The second derivatives of g with respect to u and v are approximated by second-order finite difference formulas. Note that the grid is not rectangular in the uv -plane, and therefore, the usual finite differencing formulas do not apply there. To derive appropriate formulas at an arbitrary grid point, (x_i, y_j, z_k) , in the uv -plane, we refer to Fig. 2, which illustrates the available neighboring grid points in the uv -plane. At the ijk th grid point, we find that

$$g_{uu} = \frac{1}{2} \left(\frac{1}{x_i} + \frac{1}{y_j} \right) + \frac{\phi_{i-1,j+1,k} - 2\phi_{i,j,k} + \phi_{i+1,j-1,k}}{2\Delta^2} + O(\Delta^2), \\ g_{vv} = \frac{1}{6} \left(\frac{1}{x_i} + \frac{1}{y_j} + \frac{4}{z_k} \right) + \frac{2(\phi_{i-1,j,k+1} + \phi_{i,j-1,k+1} + \phi_{i,j+1,k-1} + \phi_{i+1,j,k-1}) - (6\phi_{i,j,k} + \phi_{i-1,j+1,k} + \phi_{i+1,j-1,k})}{6\Delta^2} + O(\Delta^2), \\ g_{uv} = \frac{1}{2\sqrt{3}} \left(\frac{1}{x_i} - \frac{1}{y_j} \right) + \frac{(\phi_{i-1,j,k+1} + \phi_{i+1,j,k-1}) - (\phi_{i,j-1,k+1} + \phi_{i,j+1,k-1})}{2\sqrt{3}\Delta^2} + O(\Delta^2).$$

To compute the solution on the $w = 0$ face of the tetrahedron, we employ the same iterative technique as that used on the coordinate planes, as described above. That is, we repeatedly sweep through the grid points on the uv -plane, while making updates to the solution at each point, in turn. The updates to ϕ_{ijk}^l differ here since we use different finite differencing formulas for the second derivatives of g . The correction to ϕ_{ijk}^l at the ijk th grid point on the uv -plane in the l th iteration is given by

$$C_{ijk}^l = \Delta^2 \cdot \frac{\left(\hat{g}_{uu}^l \hat{g}_{vv}^l - (\hat{g}_{uv}^l)^2 \right) R - (\varepsilon_v^2 \hat{g}_{uu}^l - 2\varepsilon_u \varepsilon_v \hat{g}_{uv}^l + \varepsilon_u^2 \hat{g}_{vv}^l)}{\left(\hat{g}_{uu}^l + \hat{g}_{vv}^l \right) R - (\varepsilon_u^2 + \varepsilon_v^2)} \Bigg|_{(x_i, y_j, 0)}.$$

Again, we continue to sweep through the grid points on the uv -plane, while making updates to ϕ_{ijk}^l in this way, until the desired accuracy is achieved.

In the interior of the tetrahedral domain, we use an iteration scheme similar to that described above, but here we employ Eq. (1) to determine the updates to the ϕ_{ijk} 's while sweeping the grid. The minimum of the ratio on the left-hand side of Eq. (1) occurs² in the direction of the eigenvector corresponding to the minimum eigenvalue, $\lambda_{\min}$, of $H_g - \nabla \varepsilon \nabla \varepsilon^T / R$. We compute H_g using the appropriate finite difference approximations of the second derivatives of g .

That is, we have

$$g_{ss} = \frac{\varepsilon_s^2}{R},$$

where s denotes the direction parallel to the unit eigenvector $\mathbf{v} = \langle v_1, v_2, v_3 \rangle^T$ corresponding to $\lambda_{\min}$. The eigenvector $\mathbf{v}$ is computed using the Lapack subroutine DSTEBZ,⁴² which is suited to real symmetric matrices. The directional derivative of the dielectric coefficient is given by

$$\varepsilon_s = \nabla \varepsilon \cdot \mathbf{v} = v_1 \varepsilon_x + v_2 \varepsilon_y + v_3 \varepsilon_z,$$

FIG. 2. *Left*: Grid points appearing on the uv -plane used to compute the second partial derivatives of g in the uv -coordinate system. *Right*: u and v axes.

and the second directional derivative g_{ss} is given by

$$g_{ss} = \mathbf{v}^T H_g \mathbf{v} = v_1^2 g_{xx} + v_2^2 g_{yy} + v_3^2 g_{zz} + 2(v_1 v_2 g_{xy} + v_1 v_3 g_{xz} + v_2 v_3 g_{yz}). \quad (7)$$

A second-order approximation of g_{ss} , denoted by $\hat{g}_{ss}$, is obtained by substituting into Eq. (7) the finite difference approximations for the second derivatives of g , thus yielding a finite difference equation at each grid point,

$$\hat{g}_{ss}(x_i, y_j, z_k) = \frac{(\varepsilon_s(x_i, y_j, z_k))^2}{R(x_i, y_j, z_k)}. \quad (8)$$

Equation (8) is satisfied only approximately at the ijk grid point during the l th sweep of the grid. Again, we make a correction C_{ijk}^l to ϕ_{ijk}^l so that Eq. (8) is satisfied exactly at (x_i, y_j, z_k) . That is, we replace ϕ_{ijk}^l in Eq. (8) by $\phi_{ijk}^{l+1} = \phi_{ijk}^l + C_{ijk}^l$ and solve for C_{ijk}^l . Since the differential equation is linear, we can readily solve for C_{ijk}^l without having to first linearize the equation that determines C_{ijk}^l , as we had to do for the ternary faces of the tetrahedron. We find that the appropriate update to ϕ_{ijk}^l is given by

$$C_{ijk}^l = \frac{\Delta^2}{2} \cdot \frac{\hat{g}_{ss}(x_i, y_j, z_k) - \frac{(\varepsilon_s(x_i, y_j, z_k))^2}{R(x_i, y_j, z_k)}}{1 + v_1 v_2 + v_1 v_3 + v_2 v_3}.$$

We sweep through the grid points, making updates to the ϕ_{ijk} 's in this way, until the solution converges to within a specified tolerance. It should be noted that once ϕ_{ijk}^l is updated in the l th sweep, the updated value is used for the remainder of the sweep. Once the prescribed degree of accuracy is achieved, we construct the approximation of the solution g ,

$$g(x_i, y_j, z_k) \approx x_i \log x_i + y_j \log y_j + z_k \log z_k + (1 - x_i - y_j - z_k) \log(1 - x_i - y_j - z_k)$$

FIG. 3. Light scattering measurement of quaternary mixing free energy, extending that for ternary mixtures,^{1,2} is illustrated by Rayleigh ratio contour surfaces (*left*), used in solving the PDE of Eq. (1) to obtain the mixing free energy g (contours, *center*). In the test regular solution model, quaternary excess free energy contours $g - g_{\text{ideal}}$ (*right*) show sensitivity to molecular interaction strengths $\{C_{ij}\}$ more easily than do contours of g .

$$+ \hat{\phi}_{ijk},$$

where $\hat{\phi}_{ijk}$ is the approximation of ϕ_{ijk} , computed in the above iterations.

IV. RECONSTRUCTION OF QUATERNARY FREE ENERGIES FROM RAYLEIGH RATIO MEASUREMENTS

We found that the numerical methods described in Sec. III were able to reconstruct quaternary mixing free energies from calculated light scattering data. This is clearly a needed first test of the numerical scheme presented above, before using it on simulated experimental data with noise, or on real experimental data. For this test we evaluated the $\mathcal{L}_\infty$ -norm, the maximum deviation of the reconstructed from the true free energy. We found that it was indeed possible to iterate according to the scheme above to achieve the desired levels of accuracy that were tested, down to $\mathcal{L}_\infty(\delta g) = 10^{-8}$.

An example of the results is shown in Figs. 3 and 4. Figure 3 (*left*) shows Rayleigh ratio contour surfaces, used in solving the PDE of Eq. (1) to obtain the mixing free energy g (contours, *center*). The excess free energy contours, which show structure more easily, are shown at *right*. To reconstruct the free energy, perfect light scattering data were used on the entire PDE solution grid; the effects of experimental noise are considered in Sec. VI. The same Rayleigh ratio shown in Fig. 3 (*left*) is plotted for the four ternary mixtures on triangular faces of the quaternary composition tetrahedron in Fig. 4 (*left*). This figure also illustrates the sensitivity of the light scattering to the direction of $\nabla \varepsilon$ (compare Fig. 4 *right* with *left*.)

V. LINEARIZED LIGHT SCATTERING PDE FOR QUATERNARY AND HIGHER ORDER MIXTURES

In this section we analyze the linearized light scattering PDE, Eq. (2), to investigate the nature of the diffusion of information from the light scattering to the free energy, as well as to help establish the formal basis for estimating time requirements for measuring the free energy throughout the quaternary composition tetrahedron. Although we demonstrate the results for the case of quaternary mixtures, the present analysis extends that given previously for ternary mixtures in Ref. 2 to apply to systems having any number of components.

FIG. 4. *Left*: Dimensionless Rayleigh ratio R for the four ternary mixtures on triangular faces of the quaternary composition tetrahedron, with both unit $\nabla\epsilon = (\sqrt{2}/3, \sqrt{2}/3, 1/3)$ and C_{ij} as in Fig. 3 (left). Tetrahedron faces were folded down to make the graph: *Blue*: $z = 0$, *Purple*: $w = 0$, *Green*: $y = 0$, *Red*: $x = 0$. Note R curves on triangle edges (binary mixtures) match pairwise. *Right*: Scattering sensitivity to dielectric gradient is illustrated by the contrasting R vs. composition for unit $\nabla\epsilon = (1/3, \sqrt{2}/3, \sqrt{2}/3)$, for the same C_{ij} .

We first give a dimension-independent derivation, showing that Eq. (2) can be recast into the form of a diffusion equation. The time-like direction is identified with the local unit normal $\hat{n}$ to the characteristic min- s directions, and the space-like direction is along the local unit tangent, $\hat{t}$. Directions perpendicular to both $\hat{n}$ and $\hat{t}$ do not enter into the linearized PDE. We identify the source terms and show that the effective, local free energy information diffusivity is given by the inverse of the signed curvature $\kappa(s)$ of the min- s directions.

We then set up the dynamical system for the min- s directions, and give examples of the resulting min- s curves for a quaternary composition space. We show by numerical computation that the effects of a perturbation do spread along the normal in a quaternary composition space, consistent with the recast, diffusion form of Eq. (2).

As shown in detail in Ref. 2, Eq. (1) is equivalent to the following form, derived from Ref. 27 and used in Refs. 1 and 2:

$$\nabla\epsilon^T (H_g)^{-1} \nabla\epsilon = R. \quad (9)$$

It is instructive to insert the identity matrix $(H_g)^{-1}(H_g)$ into Eq. (9),

$$[\nabla\epsilon^T (H_g)^{-1}](H_g)[(H_g)^{-1} \nabla\epsilon] = R. \quad (10)$$

Equation (10) states that the dimensionless Rayleigh ratio R specifies the second directional derivative of g along the vector $(H_g)^{-1} \nabla\epsilon$, multiplied by $|(H_g)^{-1} \nabla\epsilon|^2$. Thus, as also indicated above, directions parallel to $(H_g)^{-1} \nabla\epsilon$ are characteristic for the light scattering PDE, since g cannot in general be specified along such a direction in advance unless it precisely satisfies Eq. (10).

The form of Eq. (10) is also closely related to the first-order perturbation equation, Eq. (2), repeated here for convenience,

$$[\nabla\epsilon^T (H_g)^{-1}](H_f)[(H_g)^{-1} \nabla\epsilon] = -\rho. \quad (11)$$

We denote the composition coordinates by $x_1, x_2, \dots, x_N$, and use s to denote lengths along the local tangent lines to the characteristic, min- s directions,

$$\frac{d}{ds}(x_1, x_2, \dots, x_N) = \frac{(H_g)^{-1} \nabla\epsilon}{|(H_g)^{-1} \nabla\epsilon|}. \quad (12)$$

In terms of s , Eq. (11) becomes

$$\frac{\partial^2 f}{\partial s^2} = -\frac{\rho}{|\nabla\epsilon^T (H_g)^{-1}|^2}. \quad (13)$$

Equation (13) shows explicitly that in a composition space having an arbitrary number of dimensions, the light scattering perturbation PDE states that a positive change ρ to the measured light scattering efficiency reduces the inferred the second directional derivative of g along the tangent to the characteristic, min- s curves.

The min- s curves themselves satisfy the system of ODEs,

$$\frac{d\mathbf{x}}{d\sigma} = \left(\frac{dx_1}{d\sigma}, \frac{dx_2}{d\sigma}, \dots, \frac{dx_N}{d\sigma} \right) = (H_g)^{-1} \nabla\epsilon, \quad (14)$$

in which σ parametrizes a given characteristic. We use Eq. (14) below in demonstrating the nature of free energy information flow within the composition tetrahedron. However, we note here that it follows directly from Eq. (14) that ϵ is a Lyapunov function for the characteristic ODE system in the single-phase and the metastable regions of an isotropic liquid mixture of any number of components, in which $(H_g)^{-1}$ is positive definite. This generalizes the finding that ϵ is a Lyapunov function for the characteristic ODE in ternary mixtures, which we proved in Ref. 2.

To show the diffusion nature of the perturbation equation explicitly, we now re-express Eq. (13) in terms of the second

derivative of f along the characteristic curve, the first derivative normal to the curve, and the light scattering perturbation. To set the stage for this transformation, we first let ℓ denote arc length along the characteristic curve. In the usual fashion we then have

$$\sum_j \left(\frac{dx_j}{d\ell} \right)^2 = 1, \quad (15)$$

so that

$$\sum_j \left(\frac{dx_j}{d\ell} \right) \left(\frac{d^2x_j}{d\ell^2} \right) = 0. \quad (16)$$

Here, the unit normal vector $\hat{n}$ to the characteristic curve is given by

$$\kappa(\ell)\hat{n} = \left(\frac{d^2x_1}{d\ell^2}, \frac{d^2x_2}{d\ell^2}, \dots, \frac{d^2x_N}{d\ell^2} \right), \quad (17)$$

where $\kappa(\ell)$ is a curvature with sign, whose magnitude is given by

$$|\kappa(\ell)| = \sqrt{\sum_j \left(\frac{d^2x_j}{d\ell^2} \right)^2}. \quad (18)$$

We now take the first and second derivatives of the perturbation free energy f with respect to arc length along a characteristic curve. We denote the derivatives along the curve as partial since the transformed equation also involves the normal derivative of f . We find

$$\left(\frac{\partial f}{\partial \ell} \right) = \sum_j \left(\frac{dx_j}{d\ell} \right) \left(\frac{\partial f}{\partial x_j} \right), \quad (19)$$

from which

$$\begin{aligned} \left(\frac{\partial^2 f}{\partial \ell^2} \right) &= \sum_{j,k} \left(\frac{dx_j}{d\ell} \right) \left(\frac{dx_k}{d\ell} \right) \left(\frac{\partial^2 f}{\partial x_j \partial x_k} \right) \\ &+ \sum_j \left(\frac{d^2x_j}{d\ell^2} \right) \left(\frac{\partial f}{\partial x_j} \right). \end{aligned} \quad (20)$$

Using Eq. (11) we can identify

$$\sum_{j,k} \left(\frac{dx_j}{d\ell} \right) \left(\frac{dx_k}{d\ell} \right) \left(\frac{\partial^2 f}{\partial x_j \partial x_k} \right) = \frac{-\rho}{|(H_g)^{-1} \nabla \varepsilon|^2}, \quad (21)$$

and by substituting Eq. (21) into Eq. (20) we obtain the perturbation PDE in the form of a diffusion equation,

$$\frac{\partial f}{\partial n} = \frac{1}{\kappa(\ell)} \frac{\partial^2 f}{\partial \ell^2} + \frac{\rho}{\kappa(\ell) |(H_g)^{-1} \nabla \varepsilon|^2}. \quad (22)$$

This PDE shows that perturbations diffuse locally along characteristics with diffusivity $1/\kappa(\ell)$, with a time-like direction $\hat{n}$ along the positive normal to a given characteristic. The second term on the right-hand side of Eq. (22) is the effective source term for this information diffusion. Note that locally, Eq. (22) does not explicitly involve directions in the composition space other than those along $\hat{n}$ and $\hat{t}$.

For the purpose of demonstrating the character of the information diffusion numerically, we now specialize the char-

FIG. 5. Dynamical system flows that govern how light scattering perturbations, such as those from noise in measurements, affect inferred quaternary free energies; see also Fig. 6. *Left*: Arrows show binary directions governing min-s curves of Eqs. (1) and (2) (*Right*): binary (magenta), ternary (green, dark blue, light blue, orange) and quaternary (red) min-s curves are shown, each as calculated from Eqs. (23).

acteristic ODE system Eq. (14) to the case of the ideal solution free energy,

$$\begin{aligned} \frac{dx}{d\sigma} &= x(\varepsilon_x - \mathbf{x} \cdot \nabla \varepsilon), \\ \frac{dy}{d\sigma} &= y(\varepsilon_y - \mathbf{x} \cdot \nabla \varepsilon), \\ \frac{dz}{d\sigma} &= z(\varepsilon_z - \mathbf{x} \cdot \nabla \varepsilon). \end{aligned} \quad (23)$$

It is interesting to note that Eqs. (23) have the form of a competing species model, see, e.g., Refs. 43 and 44. We have plotted solutions of Eqs. (23) in Fig. 5 (*right*). For this purpose we chose the unit vector along $\nabla \varepsilon$ to be $(\sqrt{2/3}, \sqrt{2/3}, 1/3)$.

A given choice of $\nabla \varepsilon$ determines the source and sink of the characteristics, in this case $(0, 0, 0)$ and $(1, 0, 0)$, respectively, as well as the flow directions along the binary axes, as shown in Fig. 5 (*left*). In the following analysis, we show that a similar situation always results when $\nabla \varepsilon$ is constant.

First, it can be shown that to second-order at equilibria, which occur at the vertices of the composition space, the dynamical system for the min-s curves reduces to that for an ideal solution free energy. Second, the constant vector $\nabla \varepsilon$, which by assumption does not lie in any of the planes of the simplex, must point into the simplex at exactly one of the vertices. We index the components so that it points into the simplex at the origin, just to simplify the notation. This means that for all j , $\varepsilon_{x_j} > 0$.

In N dimensions, if we take x_j , $j = 1, 2, \dots, N$ to be the coordinates, the dynamical system for the characteristics for the ideal solution free energy has the form

$$x'_j = x_j \left(\varepsilon_{x_j} - \sum_k x_k \varepsilon_{x_k} \right), \quad (24)$$

for $j = 1, 2, \dots, N$. This dynamical system has $N + 1$ equilibria, one with $x_1 = x_2 = \dots = x_N = 0$, the others with $x_s = 1$ for some s and $x_j \neq 0$ for $j \neq s$. The linearized system at the origin is

$$x'_j = x_j \varepsilon_{x_j}. \quad (25)$$

FIG. 6. Light scattering free energy information spreads locally along normal vectors to min- s curves of Eq. (1); see also Fig. 5. *Left*: Tangent (blue), normal (red), and binormal (green) vectors along a min- s curve. *Center*: Local light-scattering perturbations within the sphere produce free-energy perturbations (colored three-dimensional contours) that spread along a min- s curve (black) and its normal (red), as for ternary mixtures.² *Right*: More complicated perturbations from interpolation basis functions also spread primarily along normals, here for a quadratic interpolation⁴⁵ tent function² that is 1 at a central measurement point, nonzero in its neighborhood, and 0 at other measurement points.

The linearized system at the equilibrium with $x_s = 1$ is

$$x'_j = x_j(\varepsilon_{x_j} - \varepsilon_{x_s}), \quad (26)$$

for $j \neq s$, and

$$x'_s = (1 - x_s)\varepsilon_{x_s}. \quad (27)$$

The origin, then, is clearly a source of this dynamical system; the eigenvalues are exactly ε_{x_j} for $j = 1, 2, \dots, N$.

At the equilibrium with $x_s = 1$, one eigenvalue is $-\varepsilon_{x_s} < 0$. The other eigenvalues are $\varepsilon_{x_j} - \varepsilon_{x_s}$, for $j = 1, 2, \dots, N$ except $j \neq s$. This means, among other things, that if $j = l$ is the index with the maximum value of ε_{x_j} , that is, if $\varepsilon_{x_l} > \varepsilon_{x_j}$ for $j \neq l$, then the vertex with $x_l = 1$ is a sink. The other vertices are all generalized saddles. The vertex with index c , where ε_{x_c} is second in magnitude, that is, it is bigger than all the other components of the gradient except for ε_{x_l} , has one negative and $N - 1$ positive eigenvalues. The next ε_{x_j} in magnitude has an index that corresponds to a vertex that has two negative and $N - 2$ positive eigenvalues.

To summarize, for constant $\nabla\varepsilon$ and $\varepsilon_{x_j} > 0$ for all j , the origin is a source of the characteristic dynamical system, the vertex whose index corresponds to the maximum component of $\nabla\varepsilon$ is a sink, and all the other vertices are saddles.

Having introduced the dynamical system that governs the characteristics, we now turn to demonstrating the influence of the characteristics on the spread of free energy information. Figure 6 illustrates the geometry of the spread of light scattering free energy information within the quaternary composition tetrahedron. First, Fig. 6 (left) shows the tangent, normal and binormal vectors along a given min- s curve. Note that the normal directions in general will not all be co-planar. Figure 6 (center) shows the free energy perturbation induced by a light scattering perturbation that is chosen to have a paraboloidal intensity profile within the sphere. Consistent with Eq. (22), the illustration indicates that perturbations spread along the normal vector, which is shown in red.

Finally, Fig. 6 (right) shows the free energy consequences of a more complicated free energy perturbation tent function² $T_i(\mathbf{x})$ directly relevant to the experimental design problem in the case of quadratic interpolation,⁴⁵ as used in the present

work and described in Sec. VI. The free energy effects of the perturbation also spread along the normal vector, as shown. In this context, the interpolation method is used to assign numerical values to R on the finely spaced grid used to solve Eq. (1), based on local measured values of R on a coarser sample composition grid presumed to be practical for light scattering experiments. The interpolation unit basis or tent functions² $T_i(\mathbf{x})$ each have the value 1 at a central measurement point $\mathbf{x}_i$, nonzero values in a specified, interpolation method- and grid-dependent neighborhood of $\mathbf{x}_i$, and are 0 at other measurement points $\mathbf{x}_j \neq \mathbf{x}_i$.

We note that the importance of the $T_i(\mathbf{x})$ stems from the fact that, as shown in Ref. 2, in the case of uncorrelated light scattering noise at neighboring measurement points, the expected value of the square of the $\mathcal{L}_2$ -norm of the free energy error function f is a sum over the variances of the local, experimental light scattering efficiency determinations, each multiplied by the integral of the square of the appropriate function $\phi_i(\mathbf{x})$ that satisfies Eq. (22), with $\rho(\mathbf{x}) = T_i(\mathbf{x})$.

VI. DIMENSIONLESS TIME REQUIREMENTS FOR LIGHT SCATTERING QUATERNARY MIXING FREE ENERGY MEASUREMENT

In this section, we present the results of simulations that provide estimates of the time and sample numbers required to determine quaternary mixing free energy measurement to desired accuracy. In Fig. 7 (left) we plot the expected value of the quaternary free energy error norm $\log_{10} \langle \mathcal{L}_2(\delta g) \rangle$ vs. sample index m , for three dimensionless total measurement times, $T = 10^8, 10^{10}$, and 10^{12} . The actual sample numbers N on the assumed, evenly spaced grid for quaternary mixtures are related to m by $N = (m + 1)(m + 2)(m + 3)/3$.

As for binary and ternary mixtures,² Fig. 7 shows that quaternary mixture constant total measurement time (T) curves follow interpolation-dominated error curves at low m , then branch off near optimal values m_{opt} and adopt T -dependent values dominated by light-scattering noise. Time is wasted for $m < m_{opt}$ because sample numbers are not sufficient to make full use of the available time. Sample is wasted

FIG. 7. Experimental design considerations for light scattering quaternary mixing free energy measurement to desired accuracy. *Left*: Average quaternary free energy error norm $\text{Log}_{10} \langle \mathcal{L}_2(\delta g) \rangle$ vs. sample index m , for three dimensionless total measurement times, $T = 10^8$, 10^{10} , and 10^{12} , from simulations; with $C_{xy} = 0$, $C_{xz} = .2$, $C_{xw} = .6$, $C_{yz} = .4$, $C_{yw} = 0$, and $C_{zw} = 0$. Sample numbers $N = (m + 1)(m + 2)(m + 3)/3$. As for binary and ternary mixtures,² constant T curves follow interpolation-dominated error curves at low m , then branch off near optimal values m_{opt} and adopt T -dependent values dominated by light-scattering noise. Time is wasted below m_{opt} ; sample is wasted above. *Right*: Relationship (black) between T , m_{opt} , and $\langle \mathcal{L}_2(\delta g) \rangle$, with projections in color. Actual measurement times for a specific instrument are given by $\tau_{inst}T$ (see text), and also depend on free energy.²

for $m > m_{opt}$ because the allotted time limits the accuracy, and one might as well have made fewer samples. The relationship among T , m_{opt} , and $\langle \mathcal{L}_2(\delta g) \rangle$ is shown on the right-hand side of Fig. 7, together with its projections onto the planes corresponding to relationships between any two of these quantities.

The actual time τ of measurement is given by $\tau = \tau_{inst}T$, where τ_{inst} is an instrumental time constant, the details of which are given in Ref. 2. For a modest laboratory light scattering setup, $\tau_{inst} = 10^{-6}$ seconds is reasonable, with improvements readily achieved by changes in source, geometry, and detector properties in accordance with the defining relation given in Ref. 2.

The present simulations were carried out under the assumptions that (1) quadratic interpolation⁴⁵ is used to fill the finely spaced PDE solution grid from more coarsely spaced, light scattering efficiency determinations, (2) samples are evenly spaced on a cubic lattice as described in detail in Sec. III, and (3) individual measurement times at each point are apportioned according to a convenient scattering intensity-based scaling that corresponds to the optimum for linear interpolation in the case of binary solutions,² namely,

$$\tau_j = T \frac{R_j^{-1/2}}{\sum_{k=1}^N R_k^{-1/2}},$$

where N is the total number of measurements. With this choice of τ_j , more time is allocated to concentrations that have smaller Rayleigh ratios, and therefore (at fixed intensity and scattering geometry) require a longer time to produce a given number of detected, scattered photons. In principle, each of these assumptions can be refined for greater efficiency. We have found for binary and ternary mixtures that dramatic reductions in time and sample number requirements can result from use of higher order interpolation methods and/or uneven, optimized sample spacing.² However, for quaternary mixtures, efficient refinement of assumptions (1)–(3) is expected to be quite complex and remains to be devised. For convenience we have included explicit expressions for the ex-

cess Rayleigh ratio for a quaternary regular solution model as an Appendix.

As an example of the use of Fig. 7, the $T = 10^8$ curve given there shows that an expected value of the error-norm of close to 10^{-3} can be achieved for the tested free energy using $m = 5$ – 7 , which corresponds to $N = 112$ – 240 samples. With an instrument having $\tau_{inst} = 10^{-6}$ seconds, 100 s of total measurement time is needed. To achieve a factor of 10 improvement in error-norm, the $T = 10^{10}$ curve shows that by spending 100 times as much light scattering measurement time, an expected free energy error-norm of close to 10^{-4} could be obtained using $m = 16$, which corresponds to $N = 1938$ samples, or roughly 10 times as many samples. For $\tau_{inst} = 10^{-6}$ seconds one then has about 3 h of light scattering measurement time. The question of how to refine assumptions (1)–(3) to reduce these numbers is one we have yet to investigate. We note that this measurement time estimate includes the time taken to measure light scattering for each of the binary and ternary sub-systems, and that, as mentioned above, the solution method proceeds from binary, to ternary, to quaternary mixtures.

It is important to note that the measurement times indicated above do not include the time associated with preparing or changing samples, nor do they include the time that is often needed to deal effectively with confounding factors such as light scattering by suspended dust. Each of these considerations will vary markedly with the specific physical systems and light scattering setups. Taken together, the facts that the needed sample numbers can be large, that overall measurement times are not necessarily extremely long, that τ_{inst} can be tailored by the optics and the source intensity as described in detail in Ref. 2, and that efficiency of free energy determination can be increased by customized selection of sample concentrations, as shown previously,² clearly suggest that multiplexing and automation of the measurement, preparation, and analysis process could be very useful. Along these lines, the present results effectively imply that if suitably diverse composition samples were to be assembled simultaneously, a

“photograph” of the free energy of mixing of multicomponent mixtures would be within reach, the light scattering from each sample being analogous to the pixels of a more usual image.

It should also be noted that particular physical liquid mixture systems will have somewhat different measurement time requirements, as was demonstrated for binary systems in Ref. 2. Thus, the results presented in Fig. 7 should be regarded as giving an approximate guide to the expected error norms achievable with the parameters given.

VII. DISCUSSION

Other examples of quaternary systems of importance for free energy measurement include microemulsion and micellar systems important in separations technology,^{46,47} and the high-concentration mixtures inside living cells.^{14,48,49} With use of nearly identical mathematics, scattering probes of suitable wavelength and detection geometry, such as those that can be obtained with x-rays and neutrons^{29,30,50} could extend the present method to measure mixing free energies of diverse systems such as molten metals³⁹ and cell membrane preparations showing phase separation.⁵¹

Model-independent measurement of free energies can also help in developing more accurate statistical physics paradigms to link molecular properties to macroscopic physical chemistry. While there is substantial theory linking molecular correlation functions to macroscopic properties,^{15,20,50,52–54} there are extremely important contexts for which further development of statistical physics paradigms is essential, notably for the difficult problem of short-range, orientation-dependent interactions that are ubiquitous within living cells.^{16–19,55} In this context, tiny changes in molecular interaction strengths, at the level of single amino acid substitutions, can completely change the phase diagram and possibly make the difference between health and disease.^{5,6,49,56} Given the stupendous number of components and their combinations, very rapid measurement of mixing free energies can be anticipated as crucial to developing the physical biology needed for medicine.

The present work is intended as part of the creation a new kind of imaging technology, namely, experimental imaging of key functions within the conceptual spaces that underly observed molecular processes. The free energies studied here are one example, being a well-established nexus between more basic statistical-mechanical constructs such as the partition function and molecular potential energies, on the one hand, and macroscopic behaviors of interest and importance, on the other. Another important example would be the mutual orientation-dependent effective molecular potentials of mean force between adjacent biological macromolecules. Mixture free energies are most naturally expressed in spaces whose dimensions are comparable to the number of molecular components. Potentials of mean force are also most naturally expressed in spaces of large dimensionality that reflect the relevant degrees of freedom. The fact that functions within spaces of such large dimension are hard to visualize, however, does not change the fact that such free energies and potentials govern numerous important thermodynamic properties including the magnitude of fluctuations near equilibrium, the chemical

potential driving forces for diffusion and chemical reactions, and the nature of phase boundaries. To give a more familiar example of a multidimensional object of importance, the conceptual difficulties of learning thermodynamics do not change the fact that molecular structures by themselves do not tell the full story; the equation of state is crucial.

VIII. SUMMARY AND CONCLUSIONS

We have provided a mathematical and computational analysis of light scattering measurement of mixing free energies of quaternary isotropic liquids, using the regular solution model as a test case. We introduced dimension-free general formulations of the full nonlinear PDE and its linearization, a basis for applying the method to composition spaces of any dimension. We analyzed the dynamical system of characteristics, showed that for constant dielectric gradient it has one source and one sink at vertices of the composition space, and found that the dielectric coefficient is in general a Lyapunov function for this system. The differential geometry of the characteristic curves governs information flow within the composition space, in the sense that information about the free energy diffuses along characteristic curves and in the direction of their positive normals. This generalizes the previous finding for ternary mixtures² to the case of an arbitrary number of dimensions. We then showed that values of the Rayleigh ratio within the quaternary composition tetrahedron can be used to correctly reconstruct the composition dependence of the free energy, and extended the analysis to the case of a finite number of data points, measured with noise. In so doing, we estimated optimal sample numbers and dimensionless measurement times needed to obtain quaternary mixing free energies to a desired accuracy, with use of static light scattering.

ACKNOWLEDGMENTS

This work was supported by National of Institutes of Health (NIH) Grant No. EY018249.

APPENDIX: QUATERNARY REGULAR SOLUTION FREE ENERGY LIGHT SCATTERING

As described in the text, the simulations in the present work make use of a quaternary regular solution model as a test case, which can be written as

$$g(x, y, z) = x \ln x + y \ln y + z \ln z + w \ln w + C_{xy}xy + C_{xz}xz + C_{yz}yz + C_{xw}xw + C_{yw}yw + C_{zw}zw.$$

Here, we give an expression for the forward light scattering $R(x, y, z)$ corresponding to this model. Aside from being interesting in its own right, this expression could be useful in the process of helping to test software for making use of light scattering measurements to determine quaternary mixture free energies.

It is known¹ that Eq. (1) can be written for quaternary mixtures as

$$\nabla \varepsilon^T H_g^{-1} \nabla \varepsilon = R(x, y, z).$$

By substituting into this equation the above expression for g , we find that

$$R(x, y, z) = 3 \frac{\varepsilon_x^2 x B_{11} + \varepsilon_y^2 y B_{22} + \varepsilon_z^2 z B_{33} - 2(\varepsilon_x \varepsilon_y x y B_{12} + \varepsilon_x \varepsilon_z x z B_{13} + \varepsilon_y \varepsilon_z y z B_{23})}{1 - w + (1 - 2C_{xw}x)B_{11} + xA_{11} + (1 - 2C_{yw}y)B_{22} + yA_{22} + (1 - 2C_{zw}z)B_{33} + zA_{33} - 2[xy(D_{xy}B_{12} + A_{12}) + xz(D_{xz}B_{12} + A_{13}) + yz(D_{yz}B_{23} + A_{23})]},$$

where

$$A_{11} = -2(C_{yw}y + C_{zw}z) + (4C_{yw}C_{zw} - D_{yz}^2)yz,$$

$$A_{22} = -2(C_{xw}x + C_{zw}z) + (4C_{xw}C_{zw} - D_{xz}^2)xz,$$

$$A_{33} = -2(C_{xw}x + C_{yw}y) + (4C_{xw}C_{yw} - D_{xy}^2)xy,$$

$$A_{12} = D_{xy} - (2C_{zw}D_{xy} + D_{xz}D_{yz})z,$$

$$A_{13} = D_{xz} - (2C_{yw}D_{xz} + D_{xy}D_{yz})y,$$

$$A_{23} = D_{yz} - (2C_{xw}D_{yz} + D_{xy}D_{xz})x,$$

$$B_{11} = 1 - x - 2C_{yz}yz + wA_{11},$$

$$B_{22} = 1 - y - 2C_{xz}xz + wA_{22},$$

$$B_{33} = 1 - z - 2C_{xy}xy + wA_{33},$$

$$B_{12} = 1 + (C_{xy} - C_{xz} - C_{yz})z + wA_{12},$$

$$B_{13} = 1 + (C_{xz} - C_{xy} - C_{yz})y + wA_{13},$$

$$B_{23} = 1 + (C_{yz} - C_{xy} - C_{xz})x + wA_{23},$$

$$D_{xy} = C_{xy} - C_{xw} - C_{yw},$$

$$D_{xz} = C_{xz} - C_{xw} - C_{zw},$$

$$D_{yz} = C_{yz} - C_{yw} - C_{zw}.$$

We use this expression in two contexts. First, we use it to test our numerical algorithm. We generate a light scattering function using the regular solution model, input this function into our algorithm, compute the corresponding solution to the light scattering partial differential equation, and compare it to the regular solution model. In this way, we find that our relaxation scheme converges to the exact solution over a wide range of the coefficient space. Second, we use the above expression for R as the exact light scattering to which we introduce measurement errors in the computations of Sec. VI.

¹D. Ross, G. Thurston, and C. Lutzer, *J. Chem. Phys.* **129**, 064106 (2008).

²C. Wahle, D. Ross, and G. Thurston, *J. Chem. Phys.* **137**, 034201 (2012).

³G. Benedek, J. Pande, G. Thurston, and J. Clark, *Prog. Retin. Eye Res.* **18**, 391 (1999).

⁴A. Stradner, G. Thurston, and P. Schurtenberger, *J. Phys.: Condens. Matter* **17**, 1 (2005).

⁵N. Dorsaz, G. Thurston, A. Stradner, P. Schurtenberger, and G. Foffi, *J. Phys. Chem. B* **113**, 1693 (2009).

⁶P. Banerjee, A. Pande, J. Patrosz, G. Thurston, and J. Pande, *Proc. Natl. Acad. Sci. U.S.A.* **108**, 574 (2011).

⁷F. Veretout and A. Tardieu, *Eur. Biophys. J.* **17**, 61 (1989).

⁸D. Q.-H. Wang, D. Cohen, and M. Carey, *J. Lipid Res.* **50**, S406 (2009).

⁹D. Small, *The Physical Chemistry of Lipids: From Alkanes to Phospholipids*, Vol. 4 (Springer, 1986), Vol. 4.

¹⁰D. Cohen, G. Thurston, R. Chamberlin, G. Benedek, and M. Carey, *Biochemistry* **37**, 14798 (1998).

¹¹J. Leng, S. Egelhaaf, and M. Cates, *Biophys. J.* **85**, 162401646 (2003).

¹²J. Sjöblom, R. Lindberg, and S. Friberg, *Adv. Colloid Interface Sci.* **65**, 125 (1996).

¹³P. Firman and M. Kahlweit, *J. Phys. Chem.* **100**, 1216 (1996).

¹⁴D. Hall and A. Minton, *Biochim. Biophys. Acta* **1649**, 127 (2003).

¹⁵C. Gray and K. Gubbins, *Theory of Molecular Fluids: Fundamentals Volume I*, International Series of Monographs on Chemistry (Oxford, 1985).

¹⁶M. Wertheim, *J. Stat. Phys.* **35**, 19 (1984).

¹⁷M. Wertheim, *J. Stat. Phys.* **35**, 35 (1984).

¹⁸M. Wertheim, *J. Stat. Phys.* **42**, 459 (1986).

¹⁹M. Wertheim, *J. Stat. Phys.* **42**, 477 (1986).

²⁰J. Hansen and I. McDonald, *Theory of Simple Liquids*, 3rd ed. (Academic, 2006).

²¹G. Thurston, *J. Chem. Phys.* **124**, 134909 (2006).

²²A. Minton, *Biophys. J.* **93**, 1321 (2007).

²³T. Hill, *Statistical Mechanics* (Dover, 1987).

²⁴E. Matteoli and G. Mansoori, *Fluctuation Theory of Mixtures* (Taylor and Francis, 1990).

²⁵A. Einstein, *Ann. Phys.* **33**, 1275 (1910), reprinted in *The Collected Papers of Albert Einstein*, Vol. 3, The Swiss Years: Writings, 1909–1911 (English translation supplement) (Princeton University Press, 1994).

²⁶F. Zernike, *L'opalescence Critique, Theorie et Experiments* (Thesis), Arch. Neerland. IIIA (Amsterdam, 1915), Vol. 4.

²⁷J. Kirkwood and R. Goldberg, *J. Chem. Phys.* **18**, 54 (1950).

²⁸W. Stockmayer, *J. Chem. Phys.* **18**, 58 (1950).

²⁹S.-H. Chen and M. Kotlarchyk, *Interaction of Photons and Neutrons With Matter: An Introduction*, 2nd ed. (World Scientific, 2007).

³⁰J. Als-Nielsen and D. McMorrow, *Elements of Modern X-ray Physics*, 2nd ed. (Wiley, 2011).

³¹D. Frenkel and B. Smit, *Understanding Molecular Simulation: From Algorithms to Applications*, 2nd ed. (Academic, 2001).

³²G. Rushbrooke, *Introduction to Statistical Mechanics* (Clarendon, 1964).

³³I. Prigogine and R. Defay, *Chemical Thermodynamics* (Longmans, Green, 1954).

³⁴J. Rowlinson, *Liquids and Liquid Mixtures* (Springer, 1995).

³⁵J. Meijering, *Philips Res. Rep.* **5**, 333 (1950).

³⁶J. Meijering, *Philips Res. Rep.* **6**, 183 (1951).

³⁷B. Widom, *J. Chem. Phys.* **39**, 2808 (1963).

³⁸D. Furman, S. Dattagupta, and R. Griffiths, *Phys. Rev. B* **15**, 441 (1977).

³⁹C. Lupis, *Chemical Thermodynamics of Materials* (Elsevier, 1989).

⁴⁰J. Stoer and R. Bulirsch, *Introduction to Numerical Analysis* (Springer, 2002).

⁴¹R. LeVeque, *Finite Difference Methods for Ordinary and Partial Differential Equations* (SIAM, 2007).

⁴²E. Anderson, Z. Bai, C. Bischof, S. Blackford, J. Demmel, J. Dongarra, J. Du Croz, A. Greenbaum, S. Hammarling, A. McKenney, and D. Sorensen, *LAPACK Users' Guide*, 3rd ed. (SIAM, 1987).

⁴³M. Hirsch, *Nonlinearity* **1**, 51 (1988).

⁴⁴S. Smale, M. Hirsch, and R. Devaney, *Differential Equations, Dynamical Systems, and an Introduction to Chaos*, 2nd ed. (Academic, 2003).

⁴⁵W. Thacker, J. Zhang, L. Watson, J. Birch, and M. Iyer, *ACM Trans. Math. Softw.* **37**, 1 (2010).

⁴⁶R. Strey and M. Kahlweit, *Prog. Colloid Polym. Sci.* **81**, 215 (1990).

- ⁴⁷E. Zambrano, M. Kotlarchyk, A. Langner, and A. Faraone, *J. Phys.: Condens. Matter* **18**, S2451 (2006).
- ⁴⁸S. Zimmerman, *J. Struct. Biol.* **156**, 255ð261 (2006).
- ⁴⁹N. Dorsaz, G. Thurston, A. Stradner, P. Schurtenberger, and G. Foffi, *Soft Matter* **7**, 1763 (2011).
- ⁵⁰P. Egelstaff, *An Introduction to the Liquid State*, 2nd ed., Oxford Series on Neutron Scattering in Condensed Matter (Oxford University Press, 1994).
- ⁵¹S. Veatch, O. Soubias, S. Keller, and K. Gawrisch, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 17650 (2007).
- ⁵²J. Barker and D. Henderson, *Rev. Mod. Phys.* **48**, 587 (1976).
- ⁵³H. Davis, *Statistical Mechanics of Phases, Interfaces and Thin Films*, Advances in Interfacial Engineering (Wiley VCH, 1995).
- ⁵⁴A. Grosberg, *Statistical Physics of Macromolecules* (American Institute of Physics, 2002).
- ⁵⁵A. Lomakin, N. Asherie, and G. Benedek, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9465 (1999).
- ⁵⁶A. Pande, J. Pande, N. Asherie, A. Lomakin, O. Ogun, J. King, N. Lubsen, D. Walton, and G. Benedek, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1993 (2000).