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Prediction of thermodynamic instabilities of protein solutions from simple protein–protein interactions

Tommaso D'Agostino [a], José Ramón Solana [b], Antonio Emanuele [a,*]

[a] Dipartimento di Fisica e Chimica, Università di Palermo, Via Archirafi 36, 90123 Palermo, Italy
[b] Departamento de Física Aplicada, Universidad de Cantabria, 39005 Santander, Spain

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ABSTRACT

Statistical thermodynamics of protein solutions is often studied in terms of simple, microscopic models of particles interacting via pairwise potentials. Such modelling can reproduce the short range structure of protein solutions at equilibrium and predict thermodynamics instabilities of these systems. We introduce a square well model of effective protein–protein interaction that embeds the solvent's action. We modify an existing model [45] by considering a well depth having an explicit dependence on temperature, i.e. an explicit free energy character, thus encompassing the statistically relevant configurations of solvent molecules around proteins. We choose protein solutions exhibiting demixing upon temperature decrease (lysozyme, enthalpy driven) and upon temperature increase (haemoglobin, entropy driven). We obtain satisfactory fits of spinodal curves for both the two proteins without adding any mean field term, thus extending the validity of the original model. Our results underline the solvent role in modulating or stretching the interaction potential.

1. Introduction

An aqueous solution of a single protein species is a highly complex system. The basis of such complexity is the large number of microscopic configurations thermally available to each solute (protein) molecule alone and the even larger number of microscopic configurations available to liquid water alone [1]. When solutes are in solution, configurations of solute and solvent are modified by interactions among solutes and liquid water with corresponding entropy and enthalpy costs (or gains) of those modifications. These costs are highly nonadditive [1] and, in consequence, the observed effects can be exquisitely specific. On the grounds of such complexities, it is not a surprise if entropy plays a crucial role in the interaction of proteins with other proteins and solutes. This consideration makes the free energy of the entire system the appropriate quantity describing the thermodynamic behaviour of protein solutions, and not the simple energy landscape coming from energies of intra- and inter-protein interactions microscopically described by local pair potentials.

Studying statistical thermodynamics of protein solutions is a necessary task for understanding the macroscopic phenomena occurring in them, such as phase separation and formation of crystals [2–4] and aggregates. Besides, it is of high relevance for understanding mechanisms of protein activity [5]. These studies are also very important for nanophysics, clinical sciences, biotechnologies and food technologies, as well as for fundamental physics [6–18]. In addition, an accurate microscopic description of protein–protein and protein–solvent interactions can also be used to predict microscopic structural details of protein solutions.

The theoretical description of the thermodynamic and structural properties of protein solutions requires the accurate knowledge of the protein–protein interactions combined with appropriate statistical mechanical methods. This is a formidable challenge because of the complexity of the interaction themselves. As an example of such complexity we only mention the relevance of the solvent mediated protein–protein interactions included in the so called hydrophobic effect [19]. Some success has been achieved with the use of simple, short-ranged, potential models in combination with statistical mechanics theories, an approach that has proved to be useful to reproduce some of the properties of colloidal suspensions. Phase transitions, as well as other macroscopic phenomena, are on the contrary intrinsic long range effects of the microscopic interactions [19], and thus short-ranged interactions are hardly appropriate to describe the experimental data. Nevertheless, it is possible to describe correctly liquid–liquid phase transition by using a short range interaction potential and the modified Mean Spherical Approximation (mMSA)[1] [20]. Often, sim-

* Corresponding author.
E-mail address: antonio.emanuele@unipa.it (A. Emanuele).

[1] This feature is explained by noticing that the use of mMSA brings a mean field character to the model and thus accounts for averaged long range effects; conversely, short range properties of those solution are not correctly predicted by using mMSA.

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ple potential models are combined with simple theoretical approximations and it is very difficult to trace back the origin of the inaccuracies either to the chosen description of interactions or to the approximations in the method. To overcome this drawback, sometimes an extra long range term is added to a simple model with short-ranged interactions in order to reproduce phase transitions in protein solutions [21–23]. This long range term includes adjustable enthalpy and entropy parameters which, in the cited works, cannot be considered as being perturbative. A different though successful route used to describe liquid–liquid phase separation in protein solutions is the use of mean field models, such as van der Waals or Flory–Huggins ones, with an explicit entropic term in the interaction energy. These models are capable of predicting thermodynamic instabilities of protein solutions exhibiting demixing either upon cooling [3,24] or upon heating [25,26] (inverse temperature or entropy driven phase transitions). Other recent efforts [27–29] have been directed towards the development of more sophisticated potential models with anisotropic interactions, eventually in combination with more complex theoretical approaches. However, for practical applications it is desirable to use simpler models capable of capturing the essential features of phase stability of protein solutions.

In this paper we present an analytical theoretically-based approach for the liquid–liquid phase transition of protein solutions, based on a simple model of protein–protein interactions with solute–solvent interactions incorporated in an approximate way. We will show that our model is capable of predicting the thermodynamic instabilities of protein solutions for which the demixing transition is reached upon cooling as well as upon heating, in contrast with other simple models which fail in the latter situation.

2. The model

The first step, in order to apply statistical mechanics theories to a specific fluid, is to choose an appropriate potential model. For globular proteins in solution, experimental evidence reveal that the protein–protein interactions consists of a strongly repulsive core, plus a short-ranged attraction and longer-ranged interactions. The simplest potential model including the first two contributions is the sticky hard-sphere (SHS) potential

$$u(r)/k_BT = \begin{cases} \infty, & r < \sigma \\ \ln[12\tau(\lambda-1)/\lambda], & \sigma < r < \lambda\sigma \\ 0, & r > \lambda\sigma \end{cases}, \tag{1}$$

where τ, the sitickiness parameter, is a dimensionless measure of the temperature, and $\lambda - 1$ is infinitesimally small. This potential model has been successfully used [30] to reproduce the experimental data for the osmotic pressure of aqueous solutions of lysozyme using Baxter [31] analytical solution, based on integral equation theory, to obtain the equation of state of the system.

However, the SHS model was found to be unable of fitting the spinodal line of lysozyme solutions [23,22] because of the presence of long-range interactions not accounted for by such simple model. A way to improve the results is based on a mean field perturbation theory, obtained by adding to the isothermal compressibility of the reference SHS fluid a van der Waals mean-field term, in the form [23,22]:

$$\frac{1}{\kappa_T} = \frac{1}{\kappa_T^{(0)}} - 2a\eta^2 \tag{2}$$

where κ_T and $\kappa_T^{(0)}$ are the isothermal compressibilities of the actual and reference fluids, respectively, η is the volume fraction of the protein in the solution, and a is the perturbation energy in units of volume fraction, which may be considered as consisting of energetic (or enthalpic) and entropic contributions [32], namely:

$$a = h - Ts \tag{3}$$

Eq. (2) with a given by Eq. (3) and $\kappa_T^{(0)}$ obtained from the Baxter [31] solution for SHS, constitute the generalized van der Waals model used by Manno et al. [23,22] to fit the experimental data for the spinodal line of lysozyme.

Alternatively, the square-well (SW) potential

$$u(r) = \begin{cases} +\infty, & r < \sigma \\ -\varepsilon, & \sigma < r < \lambda\sigma \\ 0, & r > \lambda\sigma \end{cases} \tag{4}$$

where σ is the diameter of the particles, ε the potential depth, and $(\lambda - 1)\sigma$ the potential width, can be used to model protein–protein interactions in protein solutions. To this end, the potential depth ε is set to be dependent upon the salt type and concentration in the solution [33] and even temperature-dependent through them [34]. According to these authors the dependence of the potential depth on temperature is due to the entropic contribution arising from the specific effect of a particular salt on the structure and dynamics of water molecules around the protein surface. This entropic effect may be accounted for by replacing the potential depth ε with a free energy parameter $\varepsilon - Ts$.

Concerning the theory, mean-field perturbation theories are known not to be accurate for predicting phase transitions near the critical point. More advanced perturbation theories, like the second-order Barker–Henderson perturbation theory [35] or the first-order mean spherical approximation [36], are not accurate in the critical region of the SW fluid with variable width. Better accuracy is provided by some recently proposed perturbation theories [37–39], based on the expansion of the free energy in terms of a parameter coupling the reference and perturbation potentials, but this requires the use of an integral equation theory which must be solved numerically and this makes the procedure impractical in our context. Good performance may be achieved by combining perturbation theory with computer simulations. The perturbation expansion of the free energy F in terms of the inverse of the temperature has the form:

$$\frac{F}{Nk_BT} = \sum_{n=0}^{\infty} \frac{F_n}{Nk_BT} \left(\frac{\varepsilon}{k_BT}\right)^n \tag{5}$$

The zeroth-order term F_0 is the contribution of the reference fluid, the hard-sphere (HS) fluid in the case of the SW potential, which can be obtained from integration of any suitable equation of state, like the very accurate Carnahan–Starling (CS) equation [40]. Several of the higher-order terms can be obtained from computer simulations [41,42]. The first- and second-order terms have the form:

$$\frac{F_1}{Nk_BT} = \frac{1}{N}\sum_i \langle N_i\rangle_0 u_1^*(r_i) \tag{6}$$

$$\frac{F_2}{Nk_BT} = -\frac{1}{2}\frac{1}{N}\sum_{i,j}\left[\langle N_iN_j\rangle_0 - \langle N_i\rangle_0\langle N_j\rangle_0\right]u_1^*(r_i)u_1^*(r_j) \tag{7}$$

Higher-order terms can be calculated in a similar way. In the previous formulas, N is the number of particles in the simulation, N_i is the number of intermolecular distances in the range $r_i \pm \Delta r/2$, with $\Delta r \ll \sigma$ and $i = 0, 1, \ldots$, angular brackets mean ensemble averages, subscript 0 indicates that the averages are performed in the reference system, and $u_1^*(r) = u_1(r)/\varepsilon$, where $u_1(r)$ is the perturbation part of the potential. For the SW fluid the reference system is the HS fluid, as said before, and the perturbation potential is hence: