

Perspective

Colloidal Systems with both a Short-Range Attraction and a Long-Range Repulsion

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Abstract

Even though the Derjaguin-Landau-Verwey-Overbeek theory has been used to describe the interactions between charged colloidal particles for over half a century, research on systems with a weak short-range attraction and long-range repulsion (SALR) has begun receiving the intensive attention from the colloidal community only very recently. Systems with a SALR interaction include micrometer sized colloidal particles in nonpolar solvents and many protein solutions. It has been demonstrated that with the right balance between the short-range attraction and long-range repulsion, cluster phases can be formed in solution. This provides a way to control the structure of solutions and gels. Also, an understanding of protein clusters is important for many applications such as protein crystallization, and the formulation of pharmaceutical drugs. There have been many theoretical, computational, and experimental works with a focus on understanding the mechanisms of cluster formation and the structural/dynamical properties of clusters. In this mini-perspective article, some current works related to protein clusters in solution are briefly reviewed. And some personal thoughts are presented about the key questions/challenges we need to solve for a colloidal system with a SALR interaction in future.

Keywords

- Colloids
- Charge interaction
- Short-range attraction
- Long-range repulsion
- Protein

INTRODUCTION

The famous Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [1] has been the starting point to understand the stability of charged colloids and their solution properties even though more and more systems have been found that the DLVO potential is not sufficient to describe the observed properties [2-4]. A DLVO potential basically consists of two terms: a charge repulsion and a van der Waals attraction. The range of the repulsive interaction can be either longer or shorter than that of the attraction depending on many factors, such as ionic strength and colloidal particle size. The DLVO theory has been able to explain the stability of the charged colloids as a function of ionic strength in many systems. Recently, people have begun to focus on the cases where the range of the repulsion is much longer than that of attraction [5-14].

With an appropriate combination of the potential parameters of both a short-range attraction (SA) and a long-range repulsion (LR), computer simulations, [7,10,15,16] theoretical works [8,17-19] and experimental results [5,6,9,12-14,20,21] have conclusively demonstrated that rich phase behavior can be observed in a system with both a SA and LR (SALR) interaction. By controlling the potential parameters, a system can have different phases such as cluster crystals, lamellar phase, and Wigner glass.

The morphology of clusters is sensitive to the delicate balance between the SA and LR [6,14,15]. The addition of a LR to a system with a SA can affect the route of gelation transition [14,16]. Therefore, systems with a SALR interaction provide scientists great freedom to control the structure of systems by tuning the interaction potential so that the desired macroscopic properties can be obtained. In particular, controlling cluster formation is very important for many protein systems since protein clusters are important for the understanding of protein crystallization, protein solution phase diagrams, and the formulation stability of monoclonal antibody drugs in the pharmaceutical industry. Especially, the mechanisms of cluster formation in monoclonal antibody solutions are very important for the delivery of therapeutic drugs to patients through the subcutaneous injection [22,23].

Even though much progresses have been made in understanding the competitive effects of these two potential features, many questions remain unclear. In this min-perspective article, I will try to provide some personal understanding and thoughts of the current progress of this exciting field and what need to be addressed in future. This is not, by any means, a comprehensive review article of every aspect of the field. I will mainly focus on the experimental progress related to protein clustering effect and only touch upon experimental works on

micrometer sized colloidal systems, theoretical and simulation results when necessary. My comments and suggestions are certainly biased by my own research experience. But I still hope that this short perspective article could help generate some interest for readers who are not familiar with this field.

Current progresses in understanding protein clusters driven by a SALR interaction

The recent wave of interest in a colloidal system with a SALR interaction was initially triggered by the experimental works of studying the cluster formation in lysozyme protein solutions [5] and cytochrome C protein gels [24] in 2004. The authors of both groups observed that the scattering patterns of small angle neutron scattering (SANS) and small angle x-ray scattering (SAXS) show a peak at a much smaller Q value than that of its first diffraction peak due to the packing of nearest neighbor particles. Here, Q is the scattering wave vector during a scattering experiment. This is very interesting, especially for the case of lysozyme solutions, as people hadn't observed this kind of low-Q peaks in scattering patterns of colloidal systems with either purely a repulsive or an attractive interaction at equilibrium conditions. It is known that when a colloidal system experience a gel transition, a low-Q peak can appear in the structure factor due to the slow evolution of the system structure through the spinodal decomposition, which is a non-equilibrium process [25]. However, in protein solution, everything is at equilibrium. The appearance of this low-Q peak is thus very interesting. As a peak in a scattering pattern is an indication of the correlation of the structure with a certain length scale, this peak observed in lysozyme protein solutions was initially interpreted due to the correlation of the equilibrium clusters in solution [5,7,8,26]. This interpretation immediately triggered wide interest in the study of clustered phases of colloidal systems.

As protein molecules are too small for optical tools, larger colloidal particles at micrometer size are used to study the properties of systems with a SALR interaction. By using the confocal microscope to image the positions of large colloidal particles, it has been demonstrated that the LR has strong effect on the morphology of clusters formed in solution by favoring elongated clusters at high concentrations [6]. The elongated clusters can branch out, and eventually percolate to form gels. A special linear structure, a rod like cluster with a Bernal spiral structure, was observed experimentally [6]. A computer simulation later confirmed this observation showing that this is due to the fact that the range of the LR is comparable to the particle size [15]. When the repulsion strength is very strong a Wigner glass state, a cluster state, or a gel state can be observed by tuning the concentration and the attraction strength [13]. Interestingly, the transitions between different glassy states show different behaviors too. A recent experiment has further proposed that when the SA is strong enough, one-dimensional string like clusters can be formed due to the non-equilibrium effect [14]. It has been suspected that given sufficient time, the intra-cluster structure can rearrange to form more compact clusters. However, the speculated rearrangement was not observed at the experimental time scale. Further experiments are needed to investigate the possible rearrangements of the cluster structure.

Compared to the fast progress in understanding large colloidal systems at micrometer size, the understanding of protein cluster formation in concentrated solutions has been relatively slow. Even the existence of protein clusters in lysozyme solutions have been debated after the initial proposal to use the low-Q peak in SANS/SAXS patterns to determine the cluster formation. This is partly due to the small size of typical proteins so that a direct optical imaging technique is not very helpful. People have relied on indirect experimental results to infer the existence of cluster formation in protein solutions, such as SANS/SAXS. However, the interpretation of SANS/SAXS patterns from concentrated protein solutions is highly non-trivial [27]. The low-Q peak had been originally interpreted as being due to the correlation of formed clusters in lysozyme protein solutions [28]. In the same paper, it was shown that the peak position is independent of the protein concentration signaling that the density of clusters is a constant as a function of concentration. Based on these observations, the aggregation number of a cluster was estimated, which implied that the clusters have a preferred size in solution. This peak was then widely termed as a cluster peak in many studies [8,26,28]. However, this initial interpretation of the low-Q peak was later challenged by a group who studied lysozyme solutions at the identical conditions [9,29]. The authors used the two-Yukawa method [8] to model the interaction between lysozyme proteins where one Yukawa function term is used to simulate the SA and another Yukawa term is used to simulate the LR. By using the two-Yukawa model to calculate the inter-particle correlation, they were able to fit all SANS and SAXS patterns satisfactorily. And they noticed that the position of the low-Q peak has observable shifts as a function of concentration inconsistent with the previous observation. Therefore, they claimed that there is no cluster formation in lysozyme protein solutions.

The completely different interpretations from scattering patterns from the same kind of samples indicate that SANS/SAXS may not be an ideal tool to investigate the cluster formation. Actually, SANS/SAXS patterns only provide static structure information. As it is pointed out recently, a cluster in solution is more appropriately investigated based on dynamic measurements [12]. A cluster in solution is different from a cluster in gels where particles motions are frozen so that clusters have to be determined from the structure. In a solution, as all particles can move, particles can be claimed to belong to one cluster only if they attach to each other and move together for a certain time. Therefore, the measurement of particle motions is very important. Based on this concept, a recent study obtained the short-time self-diffusion coefficient to identify the formation of cluster in lysozyme protein solutions [12]. Different types of clusters are proposed based on their life time. The structural relaxation time, τ_s , is defined as the time for a particle to diffuse freely through its own diameter. If the life time of a cluster, τ_c , is far smaller than τ_s , the cluster is called a transient cluster. If τ_c is much longer than both τ_s and the probing time of a macroscopic measurement technique, the cluster is a permanent cluster. For a cluster whose life time is finite, but still larger than τ_s , a cluster is a dynamic cluster. The use of the neutron spin echo technique (NSE) made it possible to directly measure the self-diffusion coefficient of lysozyme proteins at a wide range of temperature and concentrations. It has been concluded that a protein

solution can be dominated by monomers even there is a low-Q peak in their corresponding SANS patterns. Only at very large concentrations, dynamic clusters are formed [12,21]. Hence, the combined SANS and NSE studies indicate that the low-Q peak is merely an indication of the formation of the intermediate range order (IRO) in solution [20]. The appearance of this low-Q peak does not necessarily mean that the dominating species in a solution are protein clusters. Therefore, this low-Q peak is more appropriately termed as an IRO peak[20].

It is worth to mention that for many monoclonal antibody (mAb) protein solutions, their interaction can be also approximated by a SALR interaction. It has long been speculated that the formation of reversible clusters can cause undesired increase of solution viscosity in many mAb solutions [23,30]. Therefore, it would be interesting to apply the knowledge of globular proteins to understand mAb solutions.

OUTLOOK

Even though there are many experimental and theoretical works investigating systems with a SALR interaction, much of the experimental understanding is made by studying systems with micrometer sized particles where the system structure can be visualized. However, as the dynamics of large colloidal particles are very slow comparing to that of protein systems, it is not yet clear if all the information/results obtained from systems with large particles can be applied to protein solutions. For example, it has been recently observed that the formation of linear structure of clusters could be due to non-equilibrium effect [14]. It was speculated that the structure can finally reach equilibrium after waiting long time even though this change of the cluster structure to a more compact packing was not observed in the experiments yet.

Because the size of many proteins is about a few nanometers, their dynamics in solutions are much faster than that in large colloidal systems. Hence, it is reasonable to believe that all formed protein clusters in solutions should not be affected by the non-equilibrium effect. Therefore, we need to be very careful to apply information obtained from large colloidal particles to protein systems. In order to understand protein clusters, it would be very useful to make progresses in the following aspects.

1) The non-equilibrium effect for systems with micrometer sized particles is in need of further careful investigations as this is important to understand the dynamics and structures of large colloidal particles at equilibrium conditions.

2) It will be useful to quantitatively estimate the interaction strength of both the SA and the LR for large colloidal systems. Most of the phase diagrams have only been presented as a function of experimental control parameters such as polymer concentrations used to control the strength of the SA in many systems. Because of the origin of a SA among proteins is not due to the polymer depletion attraction, this makes difficult to relate the phase diagram of the large colloidal particles to that of protein systems. In some cases, potential parameters are determined only at dilute solutions. However, the parameters of the effective pair wise potential can be different at different concentrations. In fact, it is known that the effective potential is a function of a concentration [30,31]. Therefore, it is still not clear

how the interaction parameters change at larger concentrations in those experimental systems. Therefore, the phase diagram of large colloidal particles as a function of potential strength and range is actually not available. This makes it difficult to use these phase diagrams to understand protein solutions.

3) The determination of the hydrodynamic radius of the protein clusters using the NSE is useful and informative. However, its accuracy is affected by the estimation of the hydrodynamic effect at different concentrations. Note that the self-diffusion coefficient decreases as a function of volume fractions due to the hydrodynamic effect even without the formation of clusters. New theoretical works and experimental results are in great need to estimate the hydrodynamic contribution to the self-diffusion coefficient when the interaction between colloidal particles is very complex. In fact, till now, scientists have only understood systems interacting either with a hard sphere interaction [32,33] or a hard sphere with a charge repulsion [34]. The theory for a spherical particles interacting with either a SA or a SALR interaction is not available yet to the colloidal community. The empirical results from large colloidal particles with a complex potential will be thus very useful. And for many protein systems, such as mAbs, they are not spherical. We would expect that the computer simulation can play important roles here to understand the hydrodynamic effect of anisotropic particles.

4) As it has been discussed, [12,21] the properties of different clusters in solution depend on their life time. However, there are currently no experimental/theoretical/computer simulation results to directly investigate the life time of protein clusters. It is still not clear yet how the bond life time between two colloidal particles affects the life time of a cluster. It is expected that the dynamic response of macroscopic properties, such as the viscosity, can be closely related to the average lifetime of clusters in a solution.

There are certainly many other aspects worth of careful investigations that are not covered by this mini-perspective article. However, I personally feel that the above unresolved questions are very important for the further understanding of clustering effect in protein solutions. Recently we noticed that protein clusters in mAb solutions are very important for the development of subcutaneous injection method for the pharmaceutical industry [30]. Therefore, the understanding of a colloidal system with a SALR interaction has an immediate impact on industrial vapplications too in addition to the interests in studying general phase diagram, biological effect in human diseases, and protein crystallization.

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