This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Potential and limits of a colloid approach to protein solutions

Anna Stradner,*a,b and Peter Schurtenberger*a,b

Looking at globular proteins with the eyes of a colloid scientist has a long tradition, in fact a significant part of the early colloid literature was focused on protein solutions. However, it has also been recognized that proteins are much more complex than the typical hard sphere-like synthetic model colloids. Proteins are not perfect spheres, their interaction potentials are in general not isotropic, and using theories developed for such particles are thus clearly inadequate in many cases. In this perspective article, we now take a closer look at the field. In particular, we reflect on the fact that modern colloid science has been undergoing a tremendous development, where a multitude of novel systems have been developed in the lab and in-silico. During the last decade we have seen a rapidly increasing number of reports on the synthesis of anisotropic, patchy and/or responsive synthetic colloids, that start to resemble their complex biological counterparts. This experimental development is also reflected in a corresponding theoretical and simulation effort. The experimental and theoretical toolbox of colloid science has thus rapidly expanded, and there is obviously an enormous potential for an application of these new concepts to protein solutions, which has already been realized and harvested in recent years. In this perspective article we make an attempt to critically discuss the exploitation of colloid science concepts to better understand protein solutions. We not only consider classical applications such as the attempt to understand and predict solution stability and phase behaviour, but also discuss new challenges related to the dynamics, flow behaviour and liquid-solid transitions found in concentrated or crowded protein solutions. It not only aims to provide an overview on the progress in experimental and theoretical (bio)colloid science, but also discusses current shortcomings in our ability to correctly reproduce and predict the structural and dynamic properties of protein solutions based on such a colloid approach.

1 Introduction

The use of a colloid approach to understand protein solution behavior is not a very recent development. In fact, a number of the early founders of colloid science have treated synthetic and bio-colloids such as proteins alike, adhering thereby to the early definition of colloids solely based on the size of the dispersed or dissolved colloidal matter.1–6 The subsequent enormous advance-
and a generic phase diagram for globular proteins was proposed that provided general rules for finding optimal crystallization conditions. Particularly important was also the development of an extended law of corresponding states, which further supported the universality of this generic phase diagram and provided a simple link between the location of the binodal and a reduced second virial coefficient $B_2^* = B_2/B_{2,HS}$, where $B_{2,HS}$ is the corresponding hard sphere second virial coefficient, as an effective temperature irrespective of the detailed shape of the interaction potential.

Since these pioneering developments in the mid 90’s, we have seen a renaissance in the use of the experimental and theoretical toolbox of colloid science in order to understand various solution properties of globular proteins. While protein phase diagrams and crystallization was initially in the focus, this colloid approach to proteins was subsequently extended to a much wider range of topics that included protein self-assembly and aggregation, the stability of crowded protein solutions and mixtures, and the dynamics of strongly interacting protein solutions. However, this approach has recently been questioned in an article by Sarangapani et al. where the authors concluded that the proteins’ capacity to adjust their shape and thus the nature of the interprotein interactions in response to variations in pH and concentration severely limits the usefulness of colloid models to describe the structural and dynamic properties of protein solutions. They also pointed out that proteins are macromolecular entities rather than rigid colloidal particles, and suggested that one should try to consider links to synthetic polymer and polyelectrolyte solutions. The article was enthusiastically received in a “new and notable” article in the same journal by Prausnitz, who wrote that “now, after the report of Sarangapani et al., the colloidalike theory is dead; the Sarangapani group have delivered a coup de grace. We can take comfort in the remark of Sarangapani et al. that, while scientifically erroneous, the colloidalike theory may nevertheless be useful for some purposes in biotechnology. Thank you! That’s like saying even a placebo can sometimes cure an illness.”

Does this mean that we no longer should try to use colloid models when attempting to understand and predict various properties of protein solutions? We believe that this is not the case, and that this rather reflects a too narrow and outdated view of what colloids and colloid models are, and in particular neglects the many exciting developments that have happened in the area of synthetic colloids in recent years. While colloidal hard spheres have been very important in creating the foundation of modern colloid science, we have seen a multitude of anisotropic, patchy and/or responsive colloids emerging that we believe are highly relevant as model systems for proteins. It is common knowledge that protein interactions depend on solution parameters such as pH, ionic strength or temperature, but so do colloid interactions. Proteins are indeed biological macromolecules, but one would expect polymer- or polyelectrolyte behavior only for completely denatured proteins, which we do not consider here. While conformational changes in proteins can obviously happen as a function of pH or other external variables, the globular nature of the overall protein (tertiary) structure remains, and will require models that are colloid rather than polymer/polyelectrolyte based. The question of polymer vs colloid nature will certainly become much more important in the current focus on intrinsically disordered proteins (IDPs).

However, it is also clear that much more emphasis will have to be given to the anisotropic and patchy nature of protein interactions and its influence on the various structural and dynamic properties of protein solutions. In this article we try to give a brief overview of recent developments in colloid science that may be of importance for protein solutions, and point out a number of topics where such improved colloid models could have a considerable impact in our strive for understanding and predicting protein solutions. We restrict our discussion to a small number of selected topics where we feel a particular need for improvement, and a large potential for profiting from parallel developments in colloid and protein science: i) improved models for interparticle interactions and the resulting phase behavior; ii) characterizing and understanding colloid and protein dynamics in dense systems, and the importance of appropriate characterization and simulation tools; iii) self-association and its consequences on viscosity and dynamical arrest. These topics are chosen for their importance in understanding complex biological systems and processes as well as in view of the needs of pharmaceutical industry for the development of future high concentration drug formulations.

### 2 Current trends in colloid science - from hard spheres to anisotropic and patchy particles

Much of the progress made in understanding the structural and dynamic properties of colloidal suspensions is a result of their frequent use as ideal model systems to address fundamental issues in condensed matter physics such as liquid ordering, crystallization and glass formation, and the corresponding structural and dynamic properties of the various systems as a function of the interaction potential. The initial work had primarily focused on the use of hard sphere-like colloids, which was instrumental in experimentally demonstrating the existence of an entropically driven crystallization in a purely hard sphere-like system, and in developing a new research thrust focusing on glass transition, dynamic arrest and jamming. Subsequently the field has seen a dramatic increase in the complexity of colloids and colloid interactions, and there were in particular four developments that are especially important for the topic of this article: the importance of short range attractions on colloid phase behavior; equilibrium cluster formation in systems with mixed potentials, combining a long range soft repulsion and a short range attraction; the development of anisotropic particles, and finally the dramatic consequences of patchy interactions on phase behavior, self-assembly and colloid dynamics. As we will see below, these recent developments are also of prime importance for our understanding of protein solutions, and we therefore briefly summarize the most important features.

The investigation of non-equilibrium phenomena in colloidal suspensions has clearly emerged as one of the most important...
Fig. 1 A schematic state diagram for colloids interacting via short-range attractions and long-range (screened Coulomb) repulsions. Shown in the volume fraction/attraction strength plane at zero electrostatic repulsion are the freezing or crystallization line (dashed black line) and the arrest line (solid light blue line), which in the case of no attractions occurs at a volume fraction of about \( \phi_{c}(HS) \approx 0.58 \), where \( \phi_{c}(HS) \) corresponds to the glass transition for hard sphere suspensions. Highlighted in red is also the binodal line denoting the metastable liquid-liquid phase separation in short range attractive systems, which can lead to interesting new arrested states when in competition with the arrest or gel-line. The dark blue line in the volume fraction/range of repulsion plane at zero attractions (blue shaded) denotes the arrest line that moves to lower volume fractions as the electrostatic repulsion is turned on. In the volume fraction/attraction strength plane at long-range electrostatic repulsions, where the range of the repulsion is significantly larger than the attraction range, we find the formation of equilibrium clusters, with a monomer-cluster transition (red dotted line) moving to lower volume fractions as the attraction strength increases, and a cluster glass transition at higher volume fractions (black line). The phase behaviour of systems where the mixed potential consists of attractions and repulsions with comparable range or longer-ranged attractions is even more complex and a recent attempt to describe this in a generic state diagram can be found in ref. 59. Adapted and modified from ref. 59 with permission from The Royal Society of Chemistry.

fields of soft matter research. Topics such as dynamical arrest or jamming in suspensions of (weakly) short-range attractive colloids, the interplay between spinodal decomposition and glass formation or the existence of a metastable liquid-liquid binodal have had a considerable attention from the experimental and theoretical soft matter community.\(^{39,54,55}\) A schematic view of our current understanding is given in Fig. 1.

For ideal hard sphere particles, we first observe a transition from a liquid to a crystal phase, followed by a disordered solid phase, a glass, at volume fractions of approximately \( \phi \approx 0.58 \).\(^{7,35,37}\) If a weak and short-ranged attraction is now turned on, this leads to the astonishing observation of a melting of the glass, followed by a so-called re-entrant glass or solid formation at even stronger attractions.\(^{39,54}\) For very strong interparticle attractions, we reach the regime of so-called irreversible aggregation, where soft fractal gels form already at very low volume fractions.\(^{56-58}\) At intermediate strength of the attraction, the situation is even more complicated due to the fact that phase separation into a dilute (gas-like) and a concentrated (liquid-like) suspension can occur, and this can subsequently lead to the formation of a long-lived ‘interaction network’ of particles.\(^{59}\)

Colloids frequently carry charges, and thus interact via a screened Coulomb repulsion. When combined with a short range attraction, new states appear, the metastable liquid-liquid phase separation is suppressed and we observe the formation of (transient) equilibrium clusters.\(^{25,40-42}\) Particularly interesting in this context is the fact that such clusters were found to undergo an arrest transition from a cluster fluid to a cluster glass that occurs at a significantly lower volume fraction when compared to the hard sphere glass at \( \phi_{c} \approx 0.58 \).\(^{41,60,61}\) Phase behavior and the occurrence of dynamical arrest upon an increase in concentration is indeed of prime importance in protein solutions in a wide range of areas, and the contents of Fig. 1 will thus reappear again below.

Another very important aspect in this context is shape anisotropy, as proteins in general are far from being perfect spheres. In recent years we have seen a wealth of colloid synthesis strategies, leading to a spectacular array of colloids of different shapes, compositions and functionalities (see Fig. 2 for some key examples).\(^{43,46,62,65-69}\) Together with similar advances in computer simulations of anisotropic particles, they have improved our understanding of the influence of anisotropy in both shape and interactions on aggregation, self-assembly and phase behaviour in colloidal suspensions.\(^{43,65,69-72}\) However, most of the work has been devoted to phase behavior and self assembly, and there is still a lack of knowledge on the dynamics of anisotropic particles, and the influence of shape anisotropy on dynamical arrest. Particles with moderate shape anisotropy such as ellipsoids with axial ratios of order 1-4 are particularly relevant for comparisons with globular proteins. While there are some simulation as well as experimental studies focusing on various aspects of the dynamics of ellipsoids at high concentrations, most of the experimental work is on (quasi-) 2D systems, with only a few notable exceptions on 3D bulk behavior.\(^{73-79}\) Progress on the experimental side is primarily hindered by the fact that most existing model systems for such ellipsoidal particles are highly turbid at high concentrations, thus making an investigation of dynamic properties such as the collective or self diffusion coefficient or the particle mean square displacement very difficult or impossible with standard optical tools. It is only through the use of recently introduced techniques such as differential dynamic microscopy or X-ray photon correlation spectroscopy that these properties have become accessible at high concentrations close to the glass transition for these model ellipsoids also.\(^{63,79}\)

During the last 2 decades, enormous experimental and theoretical effort has also been devoted to the design and synthesis and to the understanding of the phase behaviour of so-called patchy colloidal particles, i.e. particles with site-specifically engineered surfaces (see Fig. 2 for some key examples).\(^{43,51,82-85}\) As a consequence of their surface patchiness these particles can interact via directional and specific interactions. Both experiments and simulations demonstrate that this can result in a considerably more complex phase and self-organization behaviour than in colloids exhibiting purely isotropic interactions. This has recently been
are either simulation studies of their self-assembly behavior, descriptions of new synthesis routes or of the self-assembly patterns found with these particles. There is a clear lack of studies providing an experimental verification of the many predictions on the phase behavior of patchy colloid, and virtual no work at all is devoted to the consequences of patchy interactions on the dynamics of these suspensions. This is particularly important for an extension of the colloid approach to protein solutions beyond classical spherical particles with centrosymmetric potentials, as this currently limits our ability to use less coarse-grained models that are based on the molecular structure of proteins for a calculation of key structural and dynamic properties and their dependence on solution parameters such as the ionic strength, temperature, pH or protein concentration.

With these recent developments in colloid science in mind, we will now survey the current trends in the application of concepts and models from colloid science to proteins, and point out the current limits and future prospects of such an approach. This discussion basically centers on the question of the optimal coarse-graining strategy necessarily required to allow for a quantitative and predictive understanding of the various aspects of protein solutions. As schematically described in Fig. 3, this requires a strategy where sufficient molecular details are retained in order to allow for a quantitative reproduction of the particular quantity aimed for, while limiting the computational costs to make the necessary calculations or simulations feasible.

3 Protein phase behavior

The stability of protein solutions is vital for biological functionality or drug formulation using so-called biologics (or biopharmaceuticals), and globular proteins are generally well optimized against non-functional interactions. Although there is recent and exciting evidence that phase transitions in living cells may in fact represent an important mechanism in cellular organization, protein phase separation has often been related to the occurrence of so-called protein condensation diseases. The connection between liquid-liquid phase separation in solutions of eye lens proteins and the formation of cataract, still one of the leading causes of blindness worldwide, has initially spurred a systematic study of the phase diagrams of various representatives of the \( \gamma \)-crystallin family by Benedek and collaborators. At the same time, this group also extended the investigation of protein liquid-liquid phase separation to another key model protein, lysozyme, for which early work had already indicated the existence of critical phenomena, and found very similar behavior. In addition to providing a first systematic study of liquid-liquid phase separation in globular proteins, these studies also provided evidence that the resulting binodal is actually metastable with respect to the liquid-crystal transition.

In parallel, work on the phase behavior of colloid-polymer mixtures indicated that the gas-liquid transition could become metastable with respect to the liquid-solid transition, provided that the size ratio polymer/colloid was sufficiently small to render the depletion interaction between the colloids short range. The analogy between the phase behavior of colloids with short range attractions and globular proteins exhibiting

![Fig. 2 Examples for the recent developments in the synthesis of anisotropic particles. Top: Examples of anisotropic colloids. Ellipsoidal core shell and cubic hematite particles (reprinted and adapted with permission from ref. \textsuperscript{62}; Copyright (2008) Schweizerische Chemische Gesellschaft); peanut-shaped core-shell particles (reprinted and adapted with permission from ref. \textsuperscript{63}; Copyright (2018) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim); faceted and bowl-shaped core-shell microgel particles (reprinted and adapted from ref. \textsuperscript{46} with permission from The Royal Society of Chemistry). Bottom: Patchy colloids and colloidal molecules. Bottom left: Colloidal clusters made of different thermoresponsive particles that form structures similar to multi-patch particles, where the different patches can change from overall repulsive to overall attractive as a function of temperature (reprinted with permission from Peng et al. \textsuperscript{64}). Bottom right: Examples of so-called reconfigurable colloidal molecules formed by thermoresponsive particles with complementary shapes that can reversibly assemble into molecule-like clusters, and change configuration as a function of temperature. Shown are confocal laser scanning micrographs (CLSM) and schematic representations illustrating the specific self-assembly in colloidal molecules with a valency of 4 at 20°C (methane, CH\textsubscript{4}; top). Increasing the temperature to 40°C, the valency of the assembly decreases to 2 (carbon dioxide, CO\textsubscript{2}; middle). At 48°C, the assembly resembles a dihydrogen, H\textsubscript{2}, configuration (bottom) (reprinted from ref. \textsuperscript{48}).](image-url)
metastable liquid-liquid phase separation was quickly realized, and immediately explored to advance the difficult and tedious search for solution conditions favorable for growing protein crystals.\cite{15,16,20,21,29}

This development was motivated by the pressing need for high quality protein crystals for determining protein structures through X-ray crystallography, and the notorious difficulties in finding appropriate solution conditions for the growth of such crystals. The striking similarity of the known phase diagrams of different globular proteins, and the discovery of George and Wilson\cite{11} that the second virial coefficient $B_2$ of proteins that could successfully be crystallized were all found in a narrow range of slightly negative values, provided the initial scientific basis.\cite{18,101}

Another key ingredient was the "extended law of corresponding states" proposed by Noro and Frenkel,\cite{19} which indicated that the reduced second virial coefficient $B_2^*$ of proteins that could successfully be crystallized were all found in a narrow range of slightly negative values, provided the initial scientific basis.\cite{18,101}

This scenario provides a rational approach to protein crystallization, where a measurement of $B_2^*$ and its dependence upon various solution conditions would allow for a relatively rapid screening to find optimal nucleation and growth conditions. However, has this advancement in understanding of the physics of protein phase behavior and crystal formation indeed changed the picture? Unfortunately, growing high quality protein crystals still largely happens via a trial-and-error approach, and we lack predictive understanding of the link between the protein composition and the choice of the optimal solution composition in order to control the kinetics of nucleation and growth. Reality is obviously much more complex, and a lower degree of coarse-graining in the models used to describe protein solutions is clearly required. While fully atomistic simulations of a few hundred proteins have been performed,\cite{103,104}, determining phase diagrams requires hundreds of simulations, which is not yet feasible. We therefore need to find an appropriate coarse-graining strategy that is optimized for the problem at hand.

At this point it is thus helpful to step back and look again more carefully at the existing data. While the available data on experimental phase diagrams is limited to few proteins such as lysozyme and different variants of $\gamma$-crystallin only, it covers a variety of different solution conditions. Overall, the similarities between binodals of short-range attractive colloid models such as a square-well (SW) fluid are striking.\cite{106} However, a closer look has revealed distinct differences between the measured and calculated binodals for a SW model, and required the use of patchy colloid models with directional interactions to achieve much more

---

**Fig. 3** Schematic view of potential steps in the coarse-graining procedure for modeling globular proteins. The bunny figures (top row, sketch 2, 3 and 4 from left to right) are taken from the website: Gareth Bradshaw, February 2003, "Sphere-Tree Construction Toolkit", http://isg.cs.tcd.ie/spheretree/. Bottom row, first sketch from left: Image of the bovine eye lens protein $\gamma$-crystallin from the RCSB PDB (rcsb.org) of PDB ID 1AMM from ref\cite{80}. Bottom row, second sketch from left: Reprinted with permission from ref.\cite{81}. Copyright (2012) American Chemical Society.
Fig. 4 Generic state diagram for globular proteins postulated by Muschol and Rosenberger in their pioneering work from 1997 [16]. The coloured zones below the solubility curve are metastable with respect to crystallization. Below the (metastable) binodal line, protein solutions phase separate into a concentrated and dilute solution (zone II, blue shaded area). The optimal region for protein crystallization is the yellow shaded area in zone I, while zone III is prone to gelation. Redrawn after ref [252] 16.

This is illustrated with data for lysozyme shown in Fig. 5(top), which clearly shows that the isotropic SW potential cannot reproduce the relatively wide binodal, which is much better approximated by the patchy colloid model. [105] Given the complex surface of globular proteins, this is not really surprising. Similarly, while colloids with isotropic potential form dense crystal structures such as face-centered cubic (fcc), body-centered cubic (bcc) or hexagonal close-packed (hcp), protein crystal structures are generally much more open. [109] Again, these type of structures can indeed be reproduced using patchy models. [110] On the other hand, almost quantitative agreement with the ELCS was found when using an effective protein size \( \sigma_{\text{eff}} \) that includes contributions from repulsive interactions such as screened charges in the normalization of the measured \( B_2 \) values, as shown in Fig. 5(bottom). [106] Where does that leave us in our attempt to design a coarse-graining strategy?

Given the large effects that patch number, size and strength can have on the phase diagram of patchy particles, [83] one would not expect that the corresponding binodals for different proteins and solution conditions would follow an ELCS even remotely. However, dramatic changes occur for low numbers of strongly attractive and small patches. [83, 112] In proteins, this primarily corresponds to conditions where strong specific interactions lead to the formation of dimers, larger oligomers or other molecular complexes, which may in fact suppress phase separation that would otherwise proceed via weaker non-specific attractions. [113, 114] On the other hand, simulations for patchy particles with different patch interactions show that the binodals of particles with the
same patch distribution, but different types of patches, indeed collapse when plotted as a function of the normalized second virial coefficient $B_2^*$. This indicates that we may indeed be able to use a simple measurement of $B_2^*$ as a quite robust parameter to estimate the location of a critical point and binodal for globular proteins, and their dependence upon variations in solution conditions. This is of course good news for example for formulation science. On the other hand, however, when looking at the behavior of patchy particles, the situation of crystallographers is much less fortunate. We obviously need much more detail in the anisotropic interaction potential and the underlying coarse-grained structural model of the protein to successfully reproduce or predict protein crystal formation. There were a number of attempts to develop a detailed angularly dependent interaction potential based on known crystal structures in order to calculate $B_2^*$, investigate protein assembly, or gain insight into protein crystallization. This requires much more more detail in the anisotropic interaction potential and the underlying coarse-grained structural model of the protein to successfully reproduce or predict protein crystal formation. There were a number of attempts to develop a detailed angularly dependent interaction potential based on known crystal structures in order to calculate $B_2^*$, investigate protein assembly, or gain insight into protein crystallization. This is in contrast to both the experimental work in ref. and as Brownian and Newtonian computer simulations in ref. In both cases, the particles chosen were purely short-range attractive, whereas lysozyme under the conditions chosen to look at an arrested spinodal decomposition has a mixed potential, with a short-range attraction as well as Brownian and Newtonian computer simulations. This indicates that for interactions typical for proteins in aqueous solutions we may not be able to construct and use a generic state diagram that captures both equilibrium phase behavior as well as non-equilibrium arrest transitions.

Fig. 6 Effect of interaction potential details on the liquid-liquid phase separation and dynamical arrest in lysozyme solutions. The lysozyme state diagram with the metastable binodal (open symbols) and the dynamical arrest lines (full symbols) for different ionic strengths (from 200 mM (inverse triangles) to 500 mM (squares)) is represented in two different ways. Middle: Plotted as temperature versus protein volume fraction the dynamical arrest lines for different ionic strengths all overlap, indicating that they are primarily determined by the contact potential (indicated in red in the potential on the left) and rather insensitive to the longer-ranged repulsive part of the potential. Right: In agreement with the ELCS, the binodal lines all overlap when plotted using the normalized second virial coefficient as an effective temperature and measure for the integral features of the interaction potential.

This indicates that for interactions typical for proteins in aqueous solutions we may not be able to construct and use a generic state diagram that captures both equilibrium phase behavior as well as non-equilibrium arrest transitions. Until now we have concentrated on globular proteins only. However, other classes of proteins have gained enormous attention from the community, primarily for reasons connected to the formulation of novel biopharmaceuticals. Prime examples are monoclonal antibodies (mAbs) such as immunoglobulin gamma (IgG), which are considered as a major platform for potential drug candidates. However, successful mAb applications require stable and low viscosity high concentration formulations, which are often difficult to achieve as mAbs are prone to exhibit reversible self-association at high concentrations that result in enhanced viscosity, and are also known to exhibit high turbidity due to the presence of liquid-liquid phase separation.
A number of studies have made attempts to characterize cluster formation in mAb solutions, and to interpret antibody solution properties through analogies with colloids.\textsuperscript{129–137} This is by no means straightforward due to the non-spherical shape and internal flexibility of mAbs, since interactions between proteins are frequently treated based on spherical approximations, and in particular the enormous effect that specific, directional interactions can have are often not considered.

The binodals observed for mAbs differ substantially from those observed from globular proteins. Their critical concentrations ($C_c$) and temperatures ($T_c$) typically are lower than those of globular proteins, and the binodal and spinodal is wider, resembling those of low valency patchy particles. This is illustrated in Fig. 7(top), which shows a comparison between the measured binodals for a mAb, two different $\gamma$-crystallins and lysozyme.\textsuperscript{138} All binodals are rescaled with their values of $T_c$ and $C_c$, respectively. There are indeed a number of quite successful attempts to use patchy particle theory to model protein interactions and the resulting self-assembly and phase behavior.\textsuperscript{129,130,135} This is demonstrated in Fig. 7(bottom), which shows a comparison between calculated and measured binodals for two different mAbs.\textsuperscript{129} However, there is still need for significant improvements in relating model parameters to the actual composition and structure of the mAb and the solution, and we also need more work on establishing the impact of the high flexibility of the mAb structure.

4 Protein dynamics and crowding effects in cells and pharmaceutical formulations

While analogies to colloids have primarily been used in order to understand protein interactions and characterize and predict structural properties and phase behavior of protein solutions, we have recently seen an increasing amount of work also devoted to the dynamics of protein solutions. The internal motion, the diffusion of proteins in crowded solutions and their macroscopic flow properties are key issues related to drug design, drug delivery formulations and our understanding of the cellular machinery, yet we lack both adequate theoretical models with predictive power as well as the necessary experimental data needed to validate these models on the required broad range of length and time scales. We therefore also briefly discuss the current status of investigating protein dynamics in highly concentrated or crowded environments, and how analogies to colloids can help in this field.

Life sciences today are at a crossroads. Current approaches towards understanding the cellular machinery predominantly focus on the molecular level.\textsuperscript{199} However, creating an inventory of detailed molecular structures of and interactions between biological macromolecules is simply not enough to fully understand complex systems such as living cells. While the molecules of biology do remarkable and surprising things, they still obey the laws of physics. It is important to realize that collective properties such as the entropy and free energy represent driving forces for chemical reactions, self-assembly, and phase transitions within cells. A quantitative understanding of a cellular system using an integrative systems biology approach will thus have to start from collecting information about molecular interactions, and then devise a statistical physics-based model to arrive at a mathematical/numerical modelling with predictive power. This also requires a sound understanding of dynamic processes such as internal motion and diffusion of proteins, and their dependence on the various interactions present in a crowded environment. This not only poses enormous theoretical challenges, but their experimental characterization on the relevant time and length scales

![Fig. 7 Phase behavior of monoclonal antibodies. Top: Rescaled coexistence curves for four different proteins where the phase separation temperature $T_{ph}$ is scaled with the critical temperature $T_c$, and the protein concentration $c$ is scaled with the critical concentration $c_c$. The short-dashed line shows the theoretical coexistence curve of spherical particles using a mean-field approximation for the attraction and the Carnahan-Starling expression for the hard sphere entropy contribution. The long-dashed line shows the theoretical fit for the data of bovine $\gamma$-crystallin (open squares) while the solid line is a guide to the eye for the coexistence curve of a monoclonal antibody (mAb; solid circles). Data points on the coexistence curves of human $\gamma$-crystallin (open diamonds) and chicken egg white lysozyme (open triangles) are also shown. Reprinted with permission from ref.\textsuperscript{138} Bottom: Coexistence curves as a function of temperature (in K) versus protein concentration for two monoclonal antibodies, the one shown in the top figure\textsuperscript{138} and another monoclonal antibody. The symbols denote experimental data, while the full lines are calculations. The two-phase region is indicated by the shaded area. Reprinted with permission from ref.\textsuperscript{128} Copyright (2018) American Chemical Society.](image-url)
requires novel approaches. It is clear that significant progress in this area can only be achieved on the basis of a concerted long-term effort that combines traditionally disparate intellectual disciplines. It is here where analogies to colloids and the use of the experimental and theoretical toolbox developed for colloids can help.

The interior of living cells is a highly concentrated or crowded medium that can contain thousands of proteins up to very high volume fractions of 40% or more. Diffusion of proteins in cells is essential, as it strongly influences the cellular machinery through numerous processes such as signal transmission or reactions between proteins. In a dense and crowded environment such as the cell, an individual protein will feel the presence and interaction potential of all the surrounding proteins. Direct and hydrodynamic interactions strongly alter diffusion already on length scales comparable to the protein size, and we thus expect that they significantly influence reactions between different proteins compared to the situation encountered in typical in-vitro model experiments. It is thus vital to measure, understand and predict the diffusion of proteins in crowded media.\textsuperscript{23,140-143}

There is an increasing number of studies devoted to this topic, but experimentally these studies primarily rely on the use of potentially disturbing labelling combined with techniques such as fluorescence recovery after photobleaching (FRAP) or fluorescence correlation spectroscopy (FCS). These techniques measure long time tracer diffusion over distances much larger than the protein size.\textsuperscript{142,143} Other popular techniques to characterize the self and collective diffusion of proteins at high concentration are NMR diffusion measurements\textsuperscript{145} and dynamic light scattering (DLS)\textsuperscript{28,144,146-148}. Once again, these techniques characterize self and collective diffusion over distances much larger than the protein size. Short and long time diffusion over distances of order the protein size that are highly important for cellular processes, however, are very difficult to investigate. Here neutron and x-ray based methods such as neutron spin echo (NSE) measurements or x-ray photon correlation spectroscopy (XPCS) in principle are ideally suited as they are capable of characterizing diffusive processes on these short characteristic length scales.

The importance of the appropriate choice of length scales is demonstrated in Fig. 8, which shows a comparison of DLS and NSE results for the concentration and temperature dependence of the short time collective diffusion coefficient of \( \gamma\) -crystallin. This globular protein is known to exhibit liquid-liquid phase separation, with a critical concentration around \( c_t \approx 220 - 240 \) mg/ml, or a critical volume fraction of \( \phi_t \approx 0.15 - 0.17 \).\textsuperscript{91,92,144} The DLS data shows a dramatic effect of both temperature and concentration on the collective short time diffusion coefficient \( D_s(q) \) (Fig. 8 top). DLS measures the decay of large length scale density or concentration fluctuations, and for globular proteins not too far away from a critical point these are entirely dominated by critical fluctuations. What we thus observe in DLS is critical slowing down when approaching the critical point or a spinodal.\textsuperscript{146,147} Due to the universal nature of critical phenomena, the only thing that matters here is the reduced temperature or distance away from the critical point or the spinodal, and simple coarse grained colloid models of particles with short range attractive potentials that can reproduce the binodal and spinodal will also yield the concentration and temperature dependence of \( D_s(q) \). This changes only when approaching the arrest line at very high concentrations close to the arrest line, where the measured correlation functions show an unusual almost logarithmic decay due to the competition between critical slowing down and dynamical arrest, and where more work that also takes into account the effects of patchy interactions on diffusion will be needed.\textsuperscript{144}

The situation is very different for NSE, where collective diffusion is characterized at much larger \( q \)-values or smaller characteristic length scales. The NSE data shown in Fig. 8 has been obtained at a \( q \)-value corresponding to \( q^* \), the nearest neighbor peak of the structure factor, or a length scale of the nearest neighbor distance or protein diameter.\textsuperscript{144} At this large \( q \)-value, critical contributions to the structure factor have decayed completely,
and $S(q)$ is now dominated by the local structure, i.e., by the nearest neighbor cage formed by the surrounding proteins. On these length scales, collective dynamics characterized by $D_c^0(q^*)$ corresponds to the relaxation of structural correlations between neighboring proteins or cage diffusion.\textsuperscript{31,149} As the interaction potential is only weakly dependent or independent on temperature, the resulting rescaled cage diffusion coefficient $D_c^0(q^*)/D_0$, where $D_0$ is the free diffusion coefficient of $\gamma_h$-crystallin in the absence of interaction effects, shows no detectable temperature dependence (Fig. 8 bottom). However, the NSE data also shows a dramatic decrease of cage diffusion with increasing concentration, with $D_c^0(q^*)/D_0$ decreasing by one order of magnitude already for a volume fraction of around 15%. This slowing down of collective short time diffusion is much more pronounced than what one would expect for example for excluded volume effects, where $D_c^0(q^*)/D_0$ should decrease by only about 30%.\textsuperscript{31}

There are indeed a number of attempts in the literature to use colloid theory in order to interpret protein diffusion in crowded solutions measured by neutron-based quasi-elastic scattering methods such as NSE or neutron backscattering.\textsuperscript{27,31,60,150,151} While for proteins with dominating repulsive interactions such as described by screened Coulomb and effective hard sphere-like potentials agreement is very good,\textsuperscript{27,31} this is clearly not the case for proteins with weak short-range attractive potentials such as $\gamma_h$-crystallin. Attempts to use a coarse-grained colloid model with an isotropic short-ranged attractive potential completely failed in particular at low volume fractions, dramatically underestimating the slowing down of collective diffusion due to crowding effects. Using a patchy model where the overall strength of the effective pair potential as characterized by $B_1^r$ is comparable, demonstrated that weak attractive patches can indeed strongly influence $D_c^0(q^*)/D_0$ due to the formation of transient clusters and networks (Fig. 9).\textsuperscript{31} The importance of transient cluster formation on short time dynamics for short range attractive particles has also been pointed out in other simulation studies.\textsuperscript{152,153} With the extreme sensitivity of the collective short time diffusion to the exact interaction potential in patchy systems, it is clear that understanding and predicting protein dynamics in crowded environments on these short length scales requires a considerable future effort for conditions where the interactions are not primarily repulsive, but influenced by directional attractive contributions.

There is however a word of caution needed. The characteristic length scales over which motion is measured in quasi-elastic neutron scattering (QENS) experiments such as NSE or backscattering is not only relevant because of the underlying mechanisms that determine collective diffusion in strongly interacting particle systems. For proteins, this also has other consequences, related to their internal structure and flexibility. At short length scales, smaller than the protein size, QENS also becomes sensitive to rotational diffusion and internal motion.\textsuperscript{27,155–159} Analysing protein diffusion at $q$-values beyond the nearest neighbor peak is thus often more complex than for synthetic hard colloids due to these additional contributions that need to be taken into account. However, protein conformational dynamics as measured by QENS at larger $q$-values is interesting in its own right, as it is essential for their biological function: A protein must adopt specific active conformations in order to properly function. In particular large-scale domain motions, which can be induced by ligand binding or are intrinsically related to the protein domain structure, can for example modulate the entry of substrates into active sites and control enzymatic reactions. Neutron spin echo (NSE) spectroscopy is ideally suited for probing domain motions on the required length ($\text{nm}$) and time ($\text{ns} - \mu\text{s}$) scales without the need of any perturbing labelling, and has indeed been used successfully in a few cases already.\textsuperscript{155–159} It is however also important to point out that this requires an analysis where contributions from translational and rotational diffusion and local internal domain motions can be decoupled. This can be achieved in principle using molecular dynamics simulations (MD) based on the known protein structure.\textsuperscript{158} However, this becomes much more difficult at higher concentrations, where direct and hydrodynamic interactions between proteins complicate the resulting dynamics.\textsuperscript{27}

There is another aspect of proteins that has frequently been neglected when using a colloid analogy to understand and predict diffusion of proteins in crowded environments. Globular proteins are rarely perfect spheres, but rather possess an anisotropic shape. $\gamma_h$-crystallin for example has a shape that resembles a triaxial ellipsoid, with axial ratios of about 1.7 and 2.\textsuperscript{144} The importance of shape anisotropy in the interpretation and simulation of short
time diffusion in crowded solutions has been discussed before, but the prevailing assumption has been that for small axial ratios such as those in γB-crystallin an effective sphere approximation should be adequate to treat interaction effects for these systems. However, a recent simulation study using molecular dynamics simulations that includes hydrodynamic interactions based on a mesoscale hybrid approach has clearly shown a strong effect from shape anisotropy for shapes such as those resembling γB-crystallin, which is particularly pronounced for weakly attractive interactions. Moreover, not only are these effects quite strong, but they also contribute in a non-monotonic way as a function of concentration, as shown in Fig. 9. In order to understand and predict the dynamics of crowded protein solutions, it is thus not sufficient to perform in vitro experiments under dilute conditions and use estimates of the overall strength of interparticle interactions together with standard effective sphere colloid models. There is a clear need for an extension of the often used simple colloid models and to incorporate more molecular features into such coarse-grained models when attempting to describe local short-time diffusion in crowded solutions.

The ability to understand and predict dynamic properties of concentrated protein solutions and mixtures is not only vital for an understanding of cellular mechanisms, but also important for the formulation of future protein drugs. The development and use of mAb-based human therapeutics has for example rapidly evolved during the last 20 years and led to an almost exponential growth in market value. Together with the need for high concentration formulations that allow for facile home administration, this creates the need for an advanced predictive understanding of the stability and viscosity of concentrated protein solutions. In particular the frequently observed propensity of mAbs to reversibly aggregate, and the consequences of this concentration dependent cluster formation on the resulting sample viscosity significantly adds to the effects of particle interactions on the flow properties of concentrated formulations. There is thus a need to improve our analytical capability to investigate concentrated formulations, and to disentangle the effects of interprotein interactions, concentration dependent reversible aggregation, and degradation on the long-term stability and viscosity. The question is whether we can use the different routes towards dynamical arrest discussed in connection with short-range attractive colloids and summarized in Fig. 1 for this purpose?

We can look at this by comparing the available information for three proteins that have been used as well-defined model proteins: lysozyme, γB-crystallin and α-crystallin. Given the detrimental nature of an increased viscosity in high concentration biologics formulations due to the existence of a dynamical arrest transition, this is a question that is not only of interest for basic research on protein solutions, but of enormous practical importance for pharmaceutical industry. Similar to attempts made to use the ELCS in order to link easily measurable experimental parameters such as B* with the thermodynamic stability with respect to a possible liquid-liquid phase separation, B* or its dynamic analogue kD, obtained from DLS experiments, could in principle allow for an early prediction of the location of such an arrest line. This would be particularly attractive as such measurements require small amounts of sample only, which is another key concern in the early stages of drug development. However, when looking at the B* values for these three proteins, the picture looks as follows: Lysozyme is strongly repulsive (B* ≫ 1) at these low ionic strengths; γB-crystallin is overall weakly attractive (B* ≈ −1); and α-crystallin shows hard sphere behavior, i.e. B* ≈ 1. On the other hand, as shown in Fig. 10, the arrest transition occurs at φc (Lysozyme) ≈ 0.28 ≤ φc (γB-crystallin) ≈ 0.35 ≪ φc (α-crystallin) ≈ 0.58. As discussed previously, there is good evidence that for these more complex mixed and/or patchy potentials found for proteins, the ELCS does not hold for the location of the arrest line, and we need to consider the actual arrest mechanisms that act for these three proteins.

Lysozyme at low ionic strength at a pH value where it carries a net positive charge interacts via a combination of a short-range attraction and a long-range screened Coulomb repulsion, which leads to the formation of equilibrium clusters. These charged clusters increase in size with increasing protein concentration and form a cluster glass. The relative open structure and
the long-range repulsion between the clusters, the corresponding arrest transition occurs already at volume fractions as low as \( \phi_0 \approx 0.28 \). Increasing the temperature reduces the cluster growth slightly, and thus also shifts the arrest transition to somewhat larger volume fractions. It is interesting to note that the concentration dependence of the relative (zero-shear) viscosity \( \eta_r = \eta / \eta_0 \) where \( \eta_r \) is the zero shear viscosity of the sample and \( \eta_0 \) that of the solvent, is well described by the Quemada relation \( \eta_r = (1 - \phi / \phi_c) \) also found for hard sphere systems, indicating that the clusters act as effective hard spheres, with a sphere size given by the combination of the cluster radius and the Debye length. The onset of dynamical arrest is also reflected in the rescaled local short-time collective diffusion coefficient measured at the cluster-cluster peak in the structure factor, and we see that the cluster growth at higher concentrations results in values of \( D^*_c(q^*)/D_0 \) that strongly decrease at higher \( \phi \)-values, where cluster growth becomes important (Fig. 10).

At the solution conditions discussed here, the bovine eye lens protein \( \gamma \)-crystallin exhibits short time collective diffusion indicative of weak patchy attractions, where \( D^*_c(q^*)/D_0 \) decreases dramatically with increasing concentration (Fig. 10). Computer simulations of patchy colloids have indicated that this unusually strong decrease is linked to the formation of transient clusters, which become much more pronounced for patchy than for isotropic potentials. These open transient clusters are also responsible for the macroscopic arrest that occurs already at low volume fractions \( \phi_0 \approx 0.35 \), where these networks become space spanning, and where the network then is load-bearing despite the transient nature of the individual bond.

Finally, the bovine eye lens protein \( \alpha \)-crystallin has been shown to interact via a hard-sphere-like potential, resulting in structural and dynamic properties that are quantitatively described by a colloid model of polydisperse hard spheres (Fig. 10). This hard sphere nature of \( \alpha \)-crystallin is also reflected in an arrest transition that occurs at the expected value for hard sphere colloids, \( \phi_0 \approx 0.58 \). Here the mechanism is due to the formation of cages by the nearest neighbor particles, resulting in a two step decay of the intermediate scattering function. While the long-time diffusion coefficient, which is related to the escape of particles from the transient cages is not visible with NSE, the short-time \( D^*_c(q^*)/D_0 \) is well characterized by the weaker concentration dependence of pure hard spheres.

The three examples shown in Fig. 10 not only document the dramatically different arrest lines and arrest scenarios for these globular proteins that differ in their effective interaction potential. The figure moreover shows that the measured collective short-time diffusion on length scales of the nearest neighbor distance is also strongly influenced by the potential, and that \( D^*_c(q^*)/D_0 \) is extremely sensitive to the actual potential details. While the structural features of these protein solutions can be well reproduced by a strongly coarse-grained model with an isotropic potential (mixed potential for lysozyme, short range attractive for \( \gamma \)-crystallin, hard sphere for \( \alpha \)-crystallin), this does completely fail for the calculation of short time diffusion in the case of the patchy potential of \( \gamma \)-crystallin. There are two lessons to be learned, one utterly inconvenient and one rather convenient from the viewpoint of those who are interested in describing and predicting properties of protein solutions. On the one hand, there is unfortunately no easy way of predicting the existence and location of an arrest transition for concentrated solutions of globular proteins from simple low-concentration measurements such as a measurement of a second virial coefficient. On the other hand, however, measuring short-time collective diffusion on length scales of the nearest neighbor distance (or the protein diameter) offers an exquisitely sensitive test for specific interaction potentials through a comparison of the concentration dependence of the measured and simulated \( D^*_c(q^*)/D_0 \). At the same time, \( D^*_c(q^*)/D_0 \) is providing unique insight into the underlying mechanisms of a given arrest transition. We believe that in combination with appropriate simulation schemes, the method has enormous potential for evaluating interactions between proteins and their dependence on small variations in composition such as for example induced by point mutations. The drawback is, however, that the field currently suffers from the very limited availability of the technique, with only a few neutron spin echo instruments existing worldwide.

At first sight, these findings also seem quite discouraging when considering the current challenge to develop stable high concentration and low viscosity biologics formulations. As described before, a key problem in antibody formulation is that mAbs can exhibit enhanced self-association, causing too high viscosities for administering the drug. There have again been numerous studies where researchers tried to determine the molecular origins of self-association, and to predict enhanced self-association and viscosity based on simple low concentration parameters such as \( B^*_c \) and \( k_0 \), albeit mostly with limited success.

In this study, mAb self-association and viscosity as a function of concentration was characterized using a combination of experiments (static and dynamic light scattering and micro rheology), theory and simulations using analogies to patchy colloids. In a first step, self-association and cluster formation due to attractive electrostatic interactions between oppositely charged points on the arms (antigen binding or FAB domains) and on the tail (constant or FC domain) was identified as the driving force for the ionic strength dependence of the concentration-induced increase of the relative viscosity. A model that takes into account the anisotropic...
character of both the mAb shape (Y-shaped particle) and of the mAb-mAb interactions (patchy attraction) was then constructed, based on electrostatic calculations of a single antibody molecule in buffer, and MC simulations and analytical calculations were performed, the latter using an analytic solution based on Wertheim\textsuperscript{173} theory. A comparison between calculated and measured values of the concentration dependence of the isothermal compressibility or apparent molecular weight provided quantitative values for the strength of the attractive patches. In combination with so-called hyperbranched polymer theory\textsuperscript{174} this directly provided the cluster size distribution as a function of concentration.

To further predict the dynamic properties, an additional coarse-graining step was made, by considering mAb clusters rather than single mAb molecules as effective hard or sticky hard spheres. Here the cluster size distribution from the initial fit to the static light scattering data was used together with the corresponding theories for the concentration dependence of the apparent collective diffusion coefficient or hydrodynamic radius for these two models. The only additional free parameters in this step were a constant required for the rescaling of the volume fraction from the one based on individual mAbs to the one describing the cluster fluid, and the stickiness parameter for the sticky hard sphere model. In a final step, the relative viscosity \( \eta_r \) was calculated for each concentration without any additional free parameter using the Mooney relation \( \eta_r = \exp \left[ \frac{2.5}{\eta_{HS} - \eta_{HC} / \eta_{HS}} \right] \) often applied for polydisperse hard spheres,\textsuperscript{175} and assuming \( \phi_c \approx 0.71 \) based on literature values for hard spheres with comparable polydispersity. Remarkably, the measured data - the apparent molecular weight as obtained by static light scattering, the apparent hydrodynamic radius from dynamic light scattering and the zero-shear viscosity from microviscosity measurements - were in good agreement with the model predictions, showing that excluded volume interactions between the assembled clusters are indeed at the origin of the strong increase of the viscosity at high mAb concentrations. Moreover, the attraction strength of the patch-patch interactions calculated from the Wertheim fit to the static light scattering data agreed remarkably well with an estimate based on screened Coulomb interactions and a charge distribution on the IgG based on electrostatic calculations for the specific type of immunoglobulin.

This is indeed quite encouraging, and indicates that this could be a suitable approach to investigate and quantitatively assess the effects of additional excipients or chemical modifications on the antibody interaction. Such a model could then be used to estimate their effect on antibody stability and the resulting viscosity from molecular information, which would be vital for an advanced formulation strategy where unpromising candidates could be discarded at an early stage. Another interesting feature of such a successive coarse-graining procedure with appropriate patchy models is that it allows to determine the interaction strength and the cluster size distribution as a function of concentration for a given mAb system, which can subsequently be tested with DLS and (micro)rheology measurements without additional free parameters other than a rescaling of the volume fraction. The development of theoretical models to describe mAb self-association into clusters, and its consequences on other easily measurable structural and dynamic properties, has been in the focus of the community. With this approach, such models can be submitted to a critical assessment. Obviously this type of approach could easily be extended to other proteins that also display patchy interactions, and would allow for example to investigate various arrest scenarios for such systems as a function of key solution parameters.

5 Conclusions

This perspective article was initially largely motivated by the notion described in ref.\textsuperscript{33}, questioning altogether the usefulness of applying colloid-like theory to protein solutions. Having spent many years in trying to gain understanding of the structural and dynamic properties of protein solutions with such an approach, this asked for a re-evaluation of the situation. With the discussion presented above, we hope that it becomes clear that the statements made in ref.\textsuperscript{33} rather reflect a too narrow view of what colloids are than a serious game stopper. Colloids are more than simple hard spheres, and in particular the recent trends in synthesizing, characterizing and simulating patchy, responsive and anisotropic particles have provided a wealth of information and new models that can be harnessed to learn much more about protein solutions. However, we have also seen that while the currently existing models indeed allow us to make fairly precise predictions about the phase behavior and the static properties of proteins under well-defined solution compositions (keeping in mind that quantities such as the interaction potential, the size and the shape of synthetic colloids also do not necessarily remain invariant as a function of pH, temperature or concentration), the situation already changes unfavourably when aiming at processes such as crystallization where angularly resolved interaction potentials and local dynamics matter. We clearly lack well-defined procedures to extract the relevant details from a 3D structure of a particular protein and then create a maximally coarse-grained patchy model that retains just the necessary anisotropic interaction features for understanding or predicting a specific property.

This becomes even worse when interested in dynamic properties such as length-scale dependent collective and self diffusion and viscosity. Here we suffer primarily from two deficiencies: First of all, the existing work on patchy colloids has primarily focused on self-assembly and phase behavior. Very little attention has however been given to dynamic properties, and we thus find a lack of suitable theoretical models that could help us to understand the dynamics of proteins at higher concentrations. While there have been isolated attempts to harvest the significant progress made in the understanding of diffusion in concentrated colloidal suspensions and apply the same concepts and theoretical models to protein solutions, we are still unable to predict local and global dynamic properties of dense protein solutions based on the known molecular structure of the individual components, or to assess the effect of single point mutations on them. The existing theoretical models for (spherical and isotropically interacting) colloids cannot be directly transferred to protein solutions due to their complex structure and internal flexibility, and there is correspondingly a considerable lack of quantitative characterization.
and understanding of protein diffusion in crowded environments on the required molecular level. While for example our understanding of polymer dynamics has advanced tremendously since the early 1980's by the availability of neutron spin echo and other quasielastic neutron techniques and the simultaneous progress in polymer theory and multi-scale modelling techniques,\textsuperscript{174,176} the corresponding field of protein dynamics has clearly lacked similar progress. There is thus an urgent need for improving our ability to characterize, interpret and predict the dynamic properties of individual proteins as well as of their concentrated solutions and mixtures.

Moreover, the experimental toolbox of colloid physics has primarily been developed for investigating larger colloids with sizes of several hundred nanometers, and the wealth of existing data on colloid dynamics comes mainly from techniques such as DLS or confocal laser scanning microscopy (CLSM). Proteins are much smaller, and there are only a few techniques that can for example deliver measurements of short and long time collective and self diffusion on length scales of the protein size. Neutron and X-ray based scattering methods have the potential to deliver the required experimental data; however, we lack a theoretical and simulation toolbox similar to what already exists for structural properties and the interpretation of SAXS and SANS data. While detailed near-atomic simulations and a comparison with neutron and X-ray based spectroscopic data is feasible for reproducing the internal dynamics of individual proteins, a similar approach is not viable for crowded solutions and mixtures. We need significant methodological development, including developing both advanced coarse-graining approaches in simulation and a combination of several experimental methods such as NSE and X-ray photon correlation spectroscopy (XPCS).\textsuperscript{177} This needs to go hand in hand with the development of new sample environments to account for the very limited quantities of proteins typically available for such investigations, and the design of new measurement and data analysis procedures to overcome the notorious beam damage problem when working with intense synchrotron radiation.

However, having discussed all what is still missing, we should nevertheless not end without pointing out the enormous progress already made. We believe that a colloid approach to protein solutions has indeed an enormous potential in many areas as diverse as cell and systems biology, pharmaceutical formulation and materials sciences. The essential point in applications of concepts from colloid physics to proteins, however, has to be a case by case reflection on the required level of coarse graining needed for a given problem, and a critical choice of the experimental techniques and data chosen for a meaningful test of model predictions.

## Acknowledgments

We gratefully acknowledge financial support from the Knut and Alice Wallenberg Foundation (Project Grant KAW 2014.0052), the Swedish Research Council (VR Grants 2016-03301 and 2018-04627), the European Research Council (ERC-339678-COMPASS), and the Faculty of Science at Lund University.

## References

1. T. Graham, *Philosophical Transactions of the Royal Society of London*, 1861, 151, 183–224.
2. W. Pauli, *Pflüger’s Archiv für die Gesamte Physiologie des Menschen und der Tiere*, 1910, 136, 483–501.
3. W. Pauli, *Kolloid-Zeitschrift*, 1922, 31, 252–256.
4. J. Loeb, *Science*, 1920, 52, 449–456.
5. J. Loeb, *The Journal of General Physiology*, 1918, 1, 237–254.
6. H. S. Wells, J. B. Youmans and D. G. Miller, *Journal of Clinical Investigation*, 1933, 12, 1103–1117.
7. P. N. Pusey and W. Van Megen, *Nature*, 1986, 320, 340–342.
8. V. J. Anderson and H. N. Lekkerkerker, *Nature*, 2002, 416, 811–815.
9. D. Frenkel, *Science*, 2006, 314, 768–769.
10. Y. Xia, B. Gates, Y. Yin and Y. Lu, *Advanced Materials*, 2000, 12, 693–713.
11. A. George and W. W. Wilson, *Acta Crystallographica Section D Biological Crystallography*, 1994, 50, 361–365.
12. M. Muschol and F. Rosenberger, *J. Chem. Phys.*, 1995, 103, 10424–10432.
13. M. H. Hagen and D. Frenkel, *The Journal of Chemical Physics*, 1994, 101, 4093–4097.
14. H. N. W. Lekkerkerker, W. C.-K. Poon, P. N. Pusey, A. Stroobants and P. B. Warren, *Europhysics Letters (EPL)*, 1992, 20, 559–564.
15. N. Asherie, A. Lomakin and G. B. Benedek, *Physical Review Letters*, 1996, 77, 4832–4835.
16. M. Muschol and F. Rosenberger, *Journal of Chemical Physics*, 1997, 107, 1953–1962.
17. D. Rosenbaum, P. C. Zamora and C. F. Zukoski, *Physical Review Letters*, 1996, 76, 150–153.
18. P. R. Ten Wolde and D. Frenkel, *Science*, 1997, 277, 1975–1978.
19. M. G. Noro and D. Frenkel, *J. Chem. Phys.*, 2000, 113, 2941–2944.
20. N. Asherie, *Methods*, 2004, 34, 266–272.
21. R. Piazza, *Current Opinion in Colloid and Interface Science*, 2000, 5, 38–43.
22. R. Piazza, *Current Opinion in Colloid & Interface Science*, 2004, 8, 515–522.
23. R. P. Sear, *Journal of Physics: Condensed Matter*, 2005, 17, S3587–S3595.
24. R. Sear, *Current Opinion in Colloid & Interface Science*, 2006, 11, 35–39.
25. A. Stradner, H. Sedgwick, F. Cardinaux, W. C. K. Poon, S. U. Egelhaaf and P. Schurtenberger, *Nature*, 2004, 432, 492–495.
26. T. Ando and J. Skolnick, *Proc. Natl. Acad. Sci. USA*, 2010, 107, 18457–18462.
27. F. Roosen-Runge, M. Hennig, F. Zhang, R. M. J. Jacobs, M. Szucki, H. Schober, T. Seydel and F. Schreiber, *Proceedings of the National Academy of Sciences*, 2011, 108, 11815–11820.
28. G. Foffi, G. Savin, S. Bucciarelli, N. Dorsaz, G. M. Thurston,
A. Stradner and P. Schurtenberger, *Proceedings of the National Academy of Sciences*, 2014, 111, 16748–16753.

29 D. Fusco and P. Charbonneau, *Colloids and Surfaces B: Biointerfaces*, 2016, 137, 22–31.

30 J. J. McManus, P. Charbonneau, E. Zaccarelli and N. Asherie, *Current Opinion in Colloid & Interface Science*, 2016, 22, 73–79.

31 S. Bucciarelli, J. S. Myung, B. Farago, S. Das, G. A. Vliegenthart, O. Holderer, R. G. Winkler, P. Schurtenberger, G. Gompper and A. Stradner, *Science Advances*, 2016, 2, e1601432.

32 P. S. Sarangapani, S. D. Hudson, R. L. Jones, J. F. Douglas and J. A. Pathak, *Biophysical Journal*, 2015, 108, 724–737.

33 J. Prausnitz, *Biophysical Journal*, 2015, 108, 453–454.

34 H. J. Dyson and P. E. Wright, *Nature Reviews Molecular Cell Biology*, 2005, 6, 197–208.

35 P. N. Pusey and W. van Megen, *Physical Review Letters*, 1987, 59, 2083–2086.

36 W. van Megen, *Journal of Physics: Condensed Matter*, 2002, 14, 310.

37 G. L. Hunter and E. R. Weeks, *Reports on Progress in Physics*, 2012, 75, 066501.

38 A. J. Liu and S. R. Nagel, *Annual Review of Condensed Matter Physics*, 2010, 1, 347–369.

39 K. A. Dawson, *Current Opinion in Colloid & Interface Science*, 2002, 7, 218–227.

40 A. I. Campbell, V. J. Anderson, J. S. Van Duijneveldt and P. Bartlett, *Physical Review Letters*, 2005, 94, 1–4.

41 J. C. F. Toledano, F. Sciortino and E. Zaccarelli, *Soft Matter*, 2009, 5, 2390.

42 B. Ruzicka and E. Zaccarelli, *Soft Matter*, 2011, 7, 1268.

43 S. C. Glotzer and M. J. Solomon, *Nature Materials*, 2007, 6, 557–562.

44 J. J. Crassous, H. Dietsch, P. Pfleiderer, V. Malik, A. Diaz, L. A. Hirshi, M. Drechsler and P. Schurtenberger, *Soft Matter*, 2012, 8, 3538.

45 Y. Wang, Y. Wang, D. R. Breed, V. N. Manoharan, L. Feng, A. D. Hollingsworth, M. Weck and D. J. Pine, *Nature*, 2012, 491, 51–55.

46 J. J. Crassous, A. M. Mihut, L. K. Månsson and P. Schurtenberger, *Nanoscale*, 2015, 7, 15971–15982.

47 P. Schurtenberger, *SOFT MATTER SELF-ASSEMBLY*, IOS PRESS, NIEUWE HEMWEG 6B, 1013 BG AMSTERDAM, NETHERLANDS, 2016, pp. 81–136.

48 A. M. Mihut, B. Stenqvist, M. Lund, P. Schurtenberger and J. J. Crassous, *Science Advances*, 2017, 3, e1700321.

49 E. Bianchi, J. Largo, P. Tartaglia, E. Zaccarelli and F. Sciortino, *Phys. Rev. Lett.*, 2006, 97, 168301.

50 E. Bianchi, P. Tartaglia, E. Zaccarelli and F. Sciortino, *J. Chem. Phys.*, 2008, 128, 144504.

51 F. Romano and F. Sciortino, *Nature Communications*, 2012, 3, year.

52 K. van Gruijthuijsen, M. Obiols-Rabasa, P. Schurtenberger, W. G. Bowman and A. Stradner, *Soft Matter*, 2018, 14, 3704–3715.

53 T. Gibaud, N. Mahmoudi, J. Oberdisse, P. Lindner, J. S. Pedersen, C. L. P. Oliveira, A. Stradner and P. Schurtenberger, *Faraday Discussions*, 2012, 158, 267.

54 K. N. Pham, A. Puertas, J. . Bergenholtz, S. Egelhaaf, A. Moussaid, P. . Pusey, A. Schofield, M. Cates, M. Fuchs and W. Poon, *Science*, 2002, 296, 104–106.

55 E. Zaccarelli, *Journal of Physics: Condensed Matter*, 2007, 19, 323101.

56 V. Trappe and P. Sandkühler, *Current Opinion in Colloid & Interface Science*, 2004, 8, 494–500.

57 S. Romer, F. Scheffold and P. Schurtenberger, *Physical Review Letters*, 2000, 85, 4980–4983.

58 S. Romer, H. Bissig, P. Schurtenberger and F. Scheffold, *EPL (Europhysics Letters)*, 2014, 108, 48006.

59 F. Cardinaux, T. Gibaud, A. Stradner and P. Schurtenberger, *Physical Review Letters*, 2007, 99, 1–4.

60 F. Cardinaux, E. Zaccarelli, A. Stradner, S. Bucciarelli, B. Farago, S. U. Egelhaaf, F. Sciortino and P. Schurtenberger, *Journal of Physical Chemistry B*, 2011, 115, 7227–7237.

61 M. J. Bergman, T. Gartner, P. Schurtenberger and A. Stradner, *The Journal of Physical Chemistry B*, 2019, 123, 2432–2438.

62 H. Dietsch, V. Malik, M. Reuffer, C. Dagallier, A. Shalkevich, M. Saric, T. Gibaud, F. Cardinaux, F. Scheffold, A. Stradner and P. Schurtenberger, *CHIMIA International Journal for Chemistry*, 2008, 62, 805–814.

63 A. Pal, T. Zinn, M. A. Kamal, T. Narayanan and P. Schurtenberger, *Small*, 2018, 14, 1802233.

64 F. Peng, PhD thesis, Lund University, 2019.

65 M. J. Solomon and P. T. Spicer, *Soft Matter*, 2010, 6, 1391.

66 S. Sacanna, W. T. M. Irvine, P. M. Chaikin and D. J. Pine, *Nature*, 2010, 464, 575–578.

67 S. Sacanna and D. J. Pine, *Current Opinion in Colloid & Interface Science*, 2011, 16, 96–105.

68 L. Rossi, S. Sacanna, W. T. M. Irvine, P. M. Chaikin, D. J. Pine and A. P. Philipse, *Soft Matter*, 2011, 7, 4139–4142.

69 K. Zhao and T. G. Mason, *Reports on Progress in Physics*, 2018, 81, 126601.

70 P. Bolhuis and D. Frenkel, *The Journal of Chemical Physics*, 1997.

71 A. Haji-Akbari, M. Engel, A. S. Keys, X. Zheng, R. G. Petschek, P. Palfy-Muhoray and S. C. Glotzer, *Nature*, 2009, 462, 773–777.

72 G. Bautista-Carbajal, A. Moncho-Jordá and G. Odriozola, *The Journal of Chemical Physics*, 2013, 138, 064501.

73 R. Schilling and T. Scheidsteger, *Physical Review E*, 1997, 56, 2932–2949.

74 M. J. Solomon and D. V. Boger, *Journal of Rheology*, 1998, 42, 929–949.

75 M. Letz, R. Schilling and A. Latz, *Physical Review E*, 2000, 62, 5173–5178.

76 C. De Michele, R. Schilling and F. Sciortino, *Physical Review Letters*, 2007, 98, 265702.
77 Z. Zheng, F. Wang and Y. Han, Physical Review Letters, 2011, 107, 065702.
78 Z. Zheng, R. Ni, F. Wang, M. Dijkstra, Y. Wang and Y. Han, Nature Communications, 2014, 5, 3829.
79 A. Pal, V. Martinez, T. Ito, J. Arlt, J. Crassous, W. C. Poon and P. Schurtenberger, Science Advances, 2019.
80 V. S. Kumaraswamy, P. F. Lindley, C. Slingsby and I. D. Glover, Acta Crystallographica Section D Biological Crystallography, 1996, 52, 611–622.
81 A. Kurut, B. A. Persson, T. Åkesson, J. Forsman and M. Lund, The Journal of Physical Chemistry Letters, 2012, 3, 731–734.
82 N. Kern, D. Frenkel and I. Introduction, Journal of Chemical Physics, 2003, 118, 9882–9889.
83 E. Bianchi, R. Blak and C. N. Likos, Physical Chemistry Chemical Physics, 2011, 13, 6397–6410.
84 É. Duguet, C. Hubert, C. Chomette, A. Perro and S. Ravaine, Comptes Rendus Chimie, 2016, 19, 173–182.
85 E. Bianchi, B. Capone, I. Coluzza, L. Rovigatti and P. D. Van Oostrum, Limiting the valence: Advancements and new perspectives on patchy colloids, soft functionalized nanoparticles and biomolecules, 2017.
86 J. Gsponer and M. M. Babu, Cell Reports, 2012, 2, 1425–1437.
87 J. Berry, C. P. Brangwynne and M. Haataja, Reports on Progress in Physics, 2018, 81, 046601.
88 G. B. Benedek, Investigative Ophthalmology & Visual Science, 1997, 38, 1911 – 1921.
89 J. D. Gunton, A. Shiriyayev and D. L. Pagan, Protein condensation: Kinetic pathways to crystallization and disease, 2007.
90 R. J. Siezen, M. R. Fisch, C. Slingsby, G. B. Benedek, C. Slingsby and G. B. Benedek, 1985, 82, 1701–1705.
91 J. A. Thomson, P. Schurtenberger, G. M. Thurston and G. B. Benedek, Proceedings of the National Academy of Sciences, 1987, 84, 7079–7083.
92 P. Schurtenberger, R. A. Chamberlin, G. M. Thurston, J. A. Thomson and G. B. Benedek, Physical Review Letters, 1989, 63, 2064–2067.
93 M. L. Broide, C. R. Berland, J. Pande, O. O. Ogun and G. B. Benedek, Proceedings of the National Academy of Sciences, 1991, 88, 5660–5664.
94 J. Pande, C. Berland, M. Broide, O. Ogun, J. Melhuish and G. Benedek, Proceedings of the National Academy of Sciences, 1991, 88, 4916–4920.
95 C. Liu, A. Lomakin, G. M. Thurston, D. Hayden, A. Pande, J. Pande, O. Ogun, N. Asherie and G. B. Benedek, The Journal of Physical Chemistry, 1995, 99, 454–461.
96 G. B. Benedek, J. Pande, G. M. Thurston and J. I. Clark, Progress in Retinal and Eye Research, 1999, 18, 391–402.
97 C. Ishimoto and T. Tanaka, Physical Review Letters, 1977, 39, 474–477.
98 V. G. Taratuta, A. Holschbach, G. M. Thurston, D. Blankschtein and G. B. Benedek, The Journal of Physical Chemistry, 1990, 94, 2140–2144.
99 C. R. Berland, G. M. Thurston, M. Kondo, M. L. Broide, J. Pande, O. Ogun and G. B. Benedek, Proceedings of the National Academy of Sciences, 1992, 89, 1214–1218.
100 A. Gast, C. Hall and W. Russel, Journal of Colloid and Interface Science, 1983, 96, 251–267.
101 F. R. ten Wolde and D. Frenkel, Theoretical Chemistry Accounts: Theory, Computation, and Modeling (Theoretica Chimica Acta), 1999, 101, 205–208.
102 G. A. Vliegenthart and H. N. W. Lekkerkerker, The Journal of Chemical Physics, 2000, 112, 5364–5369.
103 S. R. McGuffee and A. H. Elcock, Journal of the American Chemical Society, 2006, 128, 12098–12110.
104 P. Mereghetti, R. R. Gabdoulline and R. C. Wade, Biophysical Journal, 2010, 99, 3782–3791.
105 C. Gogelein, G. Nagele, R. Tuinier, T. Gibaud, A. Stradner and P. Schurtenberger, Journal of Chemical Physics, 2008, 129, 085102.
106 F. Platten, N. E. Valadez-Perez, R. Castañeda-Priego and S. U. Engelhaaf, Journal of Chemical Physics, 2015, 142, year.
107 A. Lomakin, N. Asherie and G. B. Benedek, Proceedings of the National Academy of Sciences, 1999, 96, 9465–9468.
108 M. Kastelic, Y. V. Kalyuzhnyi, B. Hribar-Lee, K. A. Dil and V. Vlachy, Proceedings of the National Academy of Sciences of the United States of America, 2015, 112, 6766–6770.
109 S. W. Wukovitz and T. O. Yeates, Nature structural biology, 1995, 2, 1062–7.
110 N. Dorsaz, L. Filion, F. Smallenburg and D. Frenkel, Faraday Discussions, 2012, 159, 9.
111 T. Gibaud, F. Cardinaux, J. Bergenholtz, A. Stradner and P. Schurtenberger, Soft Matter, 2011, 7, 857–860.
112 A. Giacometti, F. Lado, J. Largo, G. Pastore and F. Sciortino, Journal of Chemical Physics, 2010, 132, 1–15.
113 C. J. Roberts and M. A. Blanco, Journal of Physical Chemistry B, 2014, 118, 12599–12611.
114 W. M. Jacobs, D. W. Oxtoby and D. Frenkel, The Journal of Chemical Physics, 2014, 140, 204109.
115 G. Foffi and F. Sciortino, Journal of Physical Chemistry B, 2007, 111, 9702–9705.
116 I. Staneva and D. Frenkel, The Journal of Chemical Physics, 2015, 143, 194511.
117 B. Neal, D. Asthagiri, O. Velev, A. Lenhoff and E. Kaler, Journal of Crystal Growth, 1999, 196, 377–387.
118 M. Hloucha, J. Lodge, A. Lenhoff and S. Sandler, Journal of Crystal Growth, 2001, 232, 195–203.
119 A. Kurut and M. Lund, Faraday discussion., 2013, 160, 271–278.
120 G. Pellicane, G. Smith and L. Sarkisov, Physical Review Letters, 2008, 101, 248102.
121 A. Taudt, A. Arnold and J. Pleiss, Physical Review E, 2015, 91, 033311.
122 A. P. R. Eberle, R. Castañeda-Priego, J. M. Kim and N. J. Wagner, Langmuir, 2012, 28, 1866–1878.
123 G. Foffi, C. De Michele, F. Sciortino and P. Tartaglia, Physical Review Letters, 2005, 94, 1–4.
124 A. L. Nelson, E. Dhimolea, and J. M. Reichert, Nat. Rev. Drug
125 J. M. Reichert, Mabs, 2012, 4, 413–415.
126 C. Narasimhan, H. Mach and M. Shameem, Ther. Deliv., 2012, 3, 889–900.
127 S. J. Shire, Curr. Opin. Biotechnol., 2009, 20, 708–714.
128 B. D. Mason, J. Zhang-van Enk, L. Zhang, R. L. Remmele and J. Zhang, Biophysical Journal, 2010, 99, 3792–3800.
129 M. Kastelic and V. Vlachy, The Journal of Physical Chemistry B, 2018, 122, 5400–5408.
130 N. Skar-Gislinge, M. Ronti, T. Garting, C. Rischel, P. Schurtenberger, E. Zaccarelli and A. Stradner, Molecular Pharmaceutics, 2019, 16, 2394–2404.
131 E. J. Yearley, I. E. Zarraga, S. J. Shire, T. M. Scherer, Y. Gokarn, N. J. Wagner and Y. Liu, Biophysical Journal, 2013, 105, 720–731.
132 E. J. Yearley, P. D. Godfrin, T. Perevozchikova, H. Zhang, P. Falus, L. Porcar, M. Nagao, J. E. Curtis, P. Gawande, R. Taing, I. E. Zarraga, N. J. Wagner and Y. Liu, Biophysical Journal, 2014, 106, 1763–1770.
133 M. M. Castellanos, J. A. Pathak, W. Leach, S. M. Bishop and R. H. Colby, Biophys. J., 2014, 107, 469–476.
134 P. D. Godfrin, I. E. Zarraga, J. Zarzar, L. Porcar, P. Falus, N. J. Wagner and Y. Liu, The Journal of Physical Chemistry B, 2016, 120, 278–291.
135 C. Calero-Rubio, A. Saluja and C. J. Roberts, The Journal of Physical Chemistry B, 2016, 120, 6592–6605.
136 D. Corbett, M. Hebditch, R. Keeling, P. Ke, S. Eki zigouli, P. Sarangapani, J. Pathak, C. F. V. D. Walle, S. Uddin, C. Baldock, C. Avendaño and R. A. Curtis, J. Phys. Chem. B, 2017, 121, 8276–8290.
137 C. Calero-Rubio, A. Saluja, E. Sahin and C. J. Roberts, The Journal of Physical Chemistry B, 2019, 123, 5709–5720.
138 Y. Wang, A. Lomakin, R. F. Latypov and G. B. Benedek, Proceedings of the National Academy of Sciences, 2011, 108, 16606–16611.
139 D. Plewczynski and K. Ginalska, The interactome: Predicting the protein–protein interactions in cells, 2009.
140 R. J. Ellis, Current opinion in structural biology, 2001, 11, 114–9.
141 R. Ellis, Trends in Biochemical Sciences, 2001, 26, 597–604.
142 J. A. Dix and A. Verkman, Annual Review of Biophysics, 2008, 37, 247–263.
143 M. Weiss, International Review of Cell and Molecular Biology, Elsevier Inc., 1st edn, 2014, vol. 307, pp. 383–417.
144 S. Bucciarelli, L. Casal-Dujat, C. De Michele, F. Sciortino, J. Dhont, J. Bergenholtz, B. Farago, P. Schurtenberger and A. Stradner, Journal of Physical Chemistry Letters, 2015, 6, 4470–4474.
145 W. S. Price, F. Tsuchiya and Y. Arata, Journal of the American Chemical Society, 1999, 121, 11503–11512.
146 B. M. Fine, J. Pande, A. Lomakin, O. O. Ogun and G. B. Benedek, Physical Review Letters, 1995, 74, 198–201.
147 B. M. Fine, A. Lomakin, O. O. Ogun and G. B. Benedek, Journal of Chemical Physics, 1996, 104, 326–335.
148 M. Heinen, F. Zanini, F. Roosen-Runge, D. Fedunová, F. Zhang, M. Hennig, T. Seydel, R. Schweins, M. Sztucki, M. Antalik, F. Schreiber and G. Nägele, Soft Matter, 2012, 8, 1404–1419.
149 A. J. Banchio and G. Nägele, The Journal of Chemical Physics, 2008, 128, 104903.
150 F. Roosen-Runge, M. Hennig, T. Seydel, F. Zhang, M. W. Skoda, S. Zorn, R. M. Jacobs, M. Maccarrini, P. Fouquet and F. Schreiber, Biochimica et Biophysica Acta - Proteins and Proteomics, 2010, 1804, 68–75.
151 M. Grimaldo, F. Roosen-Runge, M. Hennig, F. Zanini, F. Zhang, M. Zamponi, N. Jalarvo, F. Schreiber and T. Seydel, Journal of Physical Chemistry Letters, 2015, 6, 2577–2582.
152 S. von Bülow, M. Siggel, M. Linke and G. Hummer, Proceedings of the National Academy of Sciences, 2019, 116, 201817564.
153 S. Das, J. Riest, R. G. Winkler, G. Gompper, J. K. G. Dhont and G. Nägele, Soft Matter, 2018, 14, 92–103.
154 J. S. Myung, F. Roosen-Runge, R. G. Winkler, G. Gompper, P. Schurtenberger and A. Stradner, The Journal of Physical Chemistry B, 2018, 122, 12396–12402.
155 M. Monkenbusch, D. Richter and R. Biehl, ChemPhysChem, 2010, 11, 1188–1194.
156 R. Biehl, M. Monkenbusch and D. Richter, Soft Matter, 2011, 7, 1299–1307.
157 R. Biehl and D. Richter, Journal of Physics: Condensed Matter, 2014, 26, 503103.
158 N. Smolin, R. Biehl, G. Kneller, D. Richter and J. Smith, Biophysical Journal, 2012, 102, 1108–1117.
159 D. J. E. Callaway, B. Farago and Z. Bu, The European Physical Journal E, 2013, 36, 76.
160 B. R. Jennings and K. Parslow, Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences, 1988, 419, 137–149.
161 A. Beck, T. Wurch, C. Bailly and N. Corvaia, Nature Reviews Immunology, 2010, 10, 345–352.
162 S. J. Shire, Z. Shahrokh and J. Liu, Journal of Pharmaceutical Sciences, 2004, 93, 1390–1402.
163 C. J. Roberts, Current Opinion in Biotechnology, 2014, 30, 211–217.
164 T. Garting and A. Stradner, Small, 2018, 14, 1801548.
165 S. Bucciarelli, N. Mahmoudi, L. Casal-Dujat, M. Jéhannin, C. Jud and A. Stradner, Journal of Physical Chemistry Letters, 2016, 7, 1610–1615.
166 M. Kastelic, K. A. Dill, Y. V. Kalyuzhnaya and V. Vlachy, Journal of Molecular Liquids, 2018, 270, 234–242.
167 M. S. Neegaard, D. S. Kalonia, H. Parshad, A. D. Nielsen, E. H. Møller and M. Van De Weert, European Journal of Pharmaceutical Sciences, 2013, 49, 400–410.
168 B. D. Connolly, C. Petry, S. Yadav, B. Demeneule, N. Ciaccio, J. M. R. Moore, S. J. Shire and Y. R. Gokarn, Biophysical Journal, 2012, 103, 69–78.
169 S. Saito, J. Hasegawa, N. Kobayashi, N. Kishi, S. Uchiyama
170 P. M. Buck, A. Chaudhri, S. Kumar and S. K. Singh, *Mol. Pharmaceutics*, 2014, **12**, 127–139.

171 D. S. Tomar, S. Kumar, S. K. Singh, S. Goswami and L. Li, *mAbs*, 2016, **8**, 216–228.

172 M. A. Woldeyes, W. Qi, V. I. Razinkov, E. M. Furst and C. J. Roberts, *Journal of Pharmaceutical Sciences*, 2019, **108**, 142–154.

173 M. S. Wertheim, *J. Stat. Phys.*, 1984, **35**, 19.

174 M. Rubinstein and R. H. Colby, *Polymer Physics*, Oxford University Press, 2003.

175 M. Mooney, *J. Colloid and Interface Science*, 1951, **6**, 162–170.

176 D. Richter, M. Monkenbusch, A. Arbe and J. Colmenero, *Neutron Spin Echo in Polymer Systems*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2005.

177 J. Möller, M. Sprung, A. Madsen and C. Gutt, *IUCrJ*, 2019, **6**, 794–803.
We critically discuss the application of colloid science concepts to better understand protein solution properties in the entire concentration range.