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Mini-Review

In Silico View of Crowding: Biomolecular Processes to Nanomaterial Design

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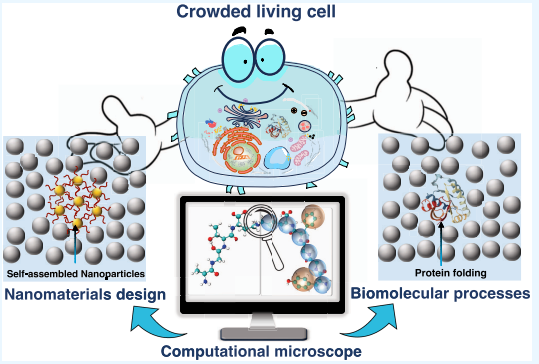
ABSTRACT: It is widely accepted that deciphering biomolecular structure and function requires going beyond the single-molecule or single-complex paradigm. The densely packed macromolecules, cosolutes, and metabolites in the living cell impose crowding effects on the biomolecular structure and dynamics that need to be accounted for. Molecular simulations have proven to be a powerful tool to advance the current molecular-level understanding of such a highly concentrated, complex milieu. This Mini-Review focuses on summarizing the understanding achieved so far for the effects of crowding on biomolecular processes using computational methods, along with highlighting a new direction in employing crowding as a tool for tunable nanomaterial design. The two schools of thought that form the pillars of the current understanding of crowding effects are discussed. The investigation of crowded solutions using physics-based models that encompass different time and length scales to mimic the intracellular environment are described. The limitations and challenges faced by the current models and simulation methods are addressed, highlighting the gaps to be filled for better agreement with experiments. Crowding can also act as an effective tool to modulate the structure−property−function relationships of nanomaterials, leading to the development of novel functional materials. A few recent studies, mostly experimental, have been summarized in this direction. The Mini-Review concludes with an outlook for future developments in this field in order to enable accurate mimicking of the intracellular environment using simulations and to bridge the gap between biological processes and nanomaterial design.



1. INTRODUCTION

Biomolecules attain their characteristic three-dimensional functional structure in the intracellular environment, which is tightly packed with high concentrations of macromolecules (200−300 g l^{-1}) and small cosolutes.[1] Such a distinctive feature of the crowded cellular interior results in a significant fraction of the cellular volume (≈20−30%) being physically occupied, which becomes unavailable for other molecules. This steric exclusion is expected to generate consequent effects on biomolecular self-assemblies, biochemical reaction rates, and equilibria. These effects induced by large macromolecules are popularly known as *macromolecular crowding effects*. Often, the effects induced by high concentrations of small metabolites and cosolutes are also termed *molecular crowding effects*. In addition to the volume exclusion effects,[2,3] the dense intracellular milieu results in extensive nonspecific interactions[4−6] between the biological macromolecules that can play a crucial role in determining the self-assembly or aggregation of these macromolecules. Despite the significance of these ubiquitous effects, crowding effects have remained unacknowledged over several years.[1c]

The single-molecule or single-complex view has hitherto provided a wealth of insights into biological structure and dynamics with limited or no consideration of cellular crowding. Recently, the significance of accounting for these effects has attracted various studies, both *in vitro* and *in silico*, to investigate their influence on biomolecular self-assembly, dynamics, and function. Some of the recent studies are summarized comprehensively in the latest reviews.[7]

One of the most recognized effects of the crowded environment has been to induce collapse in polypeptides to transform them into functional proteins, to promote the self-assembly of oligomers into larger ordered protein aggregates, and to promote rates of enzymatic reactions that involve protein−ligand binding.[1b] One of the earliest theories that originated the idea of crowding was given by Asakura and Oosawa, explaining the physics of colloidal aggregation in a solution of macromolecules. The theory attributed aggregation primarily to the excluded volume effects of the macromolecules (crowders) that induced entropically favorable *depletion*

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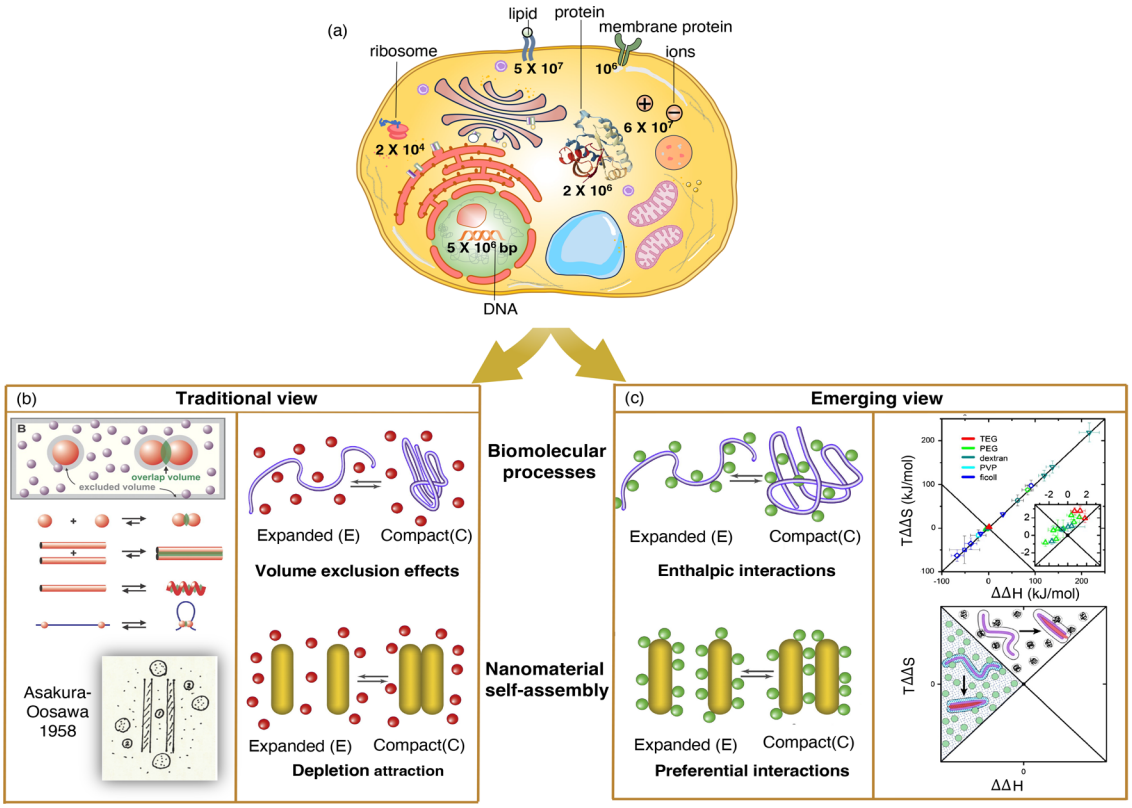

Figure 1. (a) Cartoon representation of a living cell indicating the crowded cytoplasm and the number of molecules involved in constituting the heterogeneous crowded milieu. Adapted from ref 10. Copyright Garland Science 2012. (b) The right panel shows a schematic of the traditional view of macromolecular folding-unfolding equilibria (top) and nanomaterial self-assembly (bottom) indicating the volume exclusion effects and depletion attraction. Reprinted with permission from ref 11. Copyright 2016 Elsevier Ltd. The left panel shows the schematic of overlap in excluded volumes when there is biomolecular collapse, aggregation, helix formation, or chromatin folding. Reprinted with permission from ref 1c. Copyright 2006 The Rockefeller University Press. The lower-most figure describes the Asakura−Oosawa theory schematic to explain colloidal aggregation in a solution of polymers. Reprinted with permission from ref 12. Copyright 2021 AIP Publishing. (c) Emerging view that explains crowding effects by including the enthalpic interactions via preferential adsorption of the crowders. The left panel indicates direct preferential interactions of crowders with biomolecules or nanorods. Reprinted with permission from ref 11. Copyright 2016 Elsevier Ltd. The extreme right panel summarizes the thermodynamic forces in an enthalpy−entropy plot for various crowders. The regions indicate both enthalpically and entropically favorable driven protein folding. The schematic at the bottom indicates the solvent-mediated enthalpic mechanism and volume exclusion entropically stabilizing mechanism. Reprinted with permission from ref 13. Copyright 2013 Elsevier Ltd.

attraction among the test colloidal particles.[2] This theory relies on the assumption that the crowders are "intert" to the reaction of interest and steric repulsions of the crowders or, in other words, that their size effects are the most crucial. The energetic consequences generated by these steric effects are assumed to have an insignificant influence on biomolecular compaction or self-assembly. Since such an assumption may not provide a complete view in the case of charged, polar biomolecules, recent advances have been made to reassess the classical view. The new focus has been on quantifying the effects of the soft, attractive energetic interactions on biomolecular structure, dynamics, and function.[7]

Considering that the most crucial effect of macromolecular crowding is controlling the intermolecular interactions, chemical reactions, and diffusion of molecules, this has attracted materials scientists to explore the employability of crowding as a versatile tool for controlling molecular or colloidal self-assembly.[8] It can act as an effective tool to modulate the structure−property−function relationships of the materials, leading to the development of novel functional materials. Moreover, recent advances have been made to employ nanostructured materials as substitutes for the synthetic polymers that are usually used to generate crowded environments in experiments.[8a]

Even though crowding effects have gained recognition, they have remained underappreciated.[1c] One of the reasons is that the investigation of the impact of a crowded environment on biomolecular processes *in vitro* is generally limited due to the high concentrations of the macromolecules that have to be dealt with. However, with increasing computational resources, it has been possible to investigate these systems *in silico*. Molecular simulations can be an effective tool to microscopically elaborate the influence of intermolecular interactions between biological macromolecules and unravel the thermodynamic driving forces underlying these biomolecular processes.[9] However, the field of molecular simulations is faced with several challenges, such as the lack of accurate models with an intricate description of the cellular environment (see Figure 1(a)). Several theoretical studies have provided quantitative descriptions of the macroscopic phenomena at the cellular level with mathematically complex but relatively physically simplified models that do not capture the detailed intermolecular interactions and their consequences.[9a] The other challenge lies with the spatial and temporal modeling scales of these systems and the cellular phenomena, which

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form the bottleneck for the simulation studies.[9b] Another issue is whether the generally used crowding agents such as ficoll, dextran, or sythetic polymers like polyethylene glycol are sufficient to mimic the true crowded cellular environment.

This Mini-Review aims to provide an overview of the role of macromolecular crowding on biomolecular processes and its role in designing controlled self-assemblies of nanomaterials from the perspective of computational investigation. The review focuses on summarizing the understanding achieved so far from molecular simulations, addressing the challenges associated with the simulation models and methods, and highlighting the gaps to be filled for better agreement with experiments, along with future prospects of this field that aim to bridge the gap between biological processes and nanomaterial design.

2. TWO SIDES OF THE SAME COIN

This section provides an overview of the basic theories that have formed the foundation for explaining the effects of crowding on biomolecular collapse or association. There are two views, the classical entropic view and the emerging enthalpic view, that have generally been successful in explaining the elementary mechanisms. While the entropy-centric classical view is well-established, the role of the enthalpic interactions in crowded systems still remains elusive and needs to be further quantified.

2.1. Traditional View: Entropic Origin. The current understanding of macromolecular crowding effects dates back to 1954 when Asakura and Oosawa proposed that the nonadsorbing polymers mediate attraction between the colloidal particles by exerting steric effects on the colloidal particles when the colloids are dispersed in a high concentration of polymers.[2] They proposed a two-phase hypothesis. The colloidal particles were modeled as extended interfaces (plates), representing the surfaces of large colloidal particles separated by a certain distance in a solution of polymers (see Figure 1(b)). The polymer−colloid interaction was assumed to be a hard-core repulsive interaction that defined the excluded volume of the colloids such that the polymers were not allowed to penetrate. The polymers were modeled as permeable spheres that were excluded from the volume between the surfaces of the approaching colloids once the distance between the surfaces decreased to less than the diameter of the macromolecules. This space could be assumed to be phase 1 (the local domain), and the macromolecules in solution outside the colloidal interface could be assumed to be phase 2 (the bulk domain). These two phases would have an osmotic pressure difference, with the second phase having higher osmotic pressure. Such a difference was proposed to exert an attractive force inward between the two plates. Two other cases were also examined: the case of the parallel plates in rod-like macromolecules and the case of two spherical bodies in a solution of spherical macromolecules. In each case, the attractive inward force was found to be proportional to the osmotic pressure difference. Such a volume exclusion effect inducing the depletion of macromolecules from the intermediate volume between the approaching colloids was entirely entropic in origin, which primarily regulated the colloidal attraction.[1b]

One of the first studies by Minton and co-workers in 1981 introduced the concept of cellular crowding and its impact on biomolecular structure and function.[3] It was proposed that the excluded volume effects of the crowders could be accounted for in the nonideal component of the chemical potential of the reactants by correcting for their thermodynamic activities. Due to the volume exclusion effects of the inert crowders, there would be a reduction in the available free volume accessible to the reactants, thereby increasing their thermodynamic activity accordingly. The activity coefficients could be computed by using the scaled particle theory of hard spheres. This theory was applied to explain the addition of globular proteins, altering the catalytic activity of the dehydrogenase enzyme by promoting the self-association of the enzyme to form a tetramer. Later, this theory was applied to explain various other biomolecular processes in crowded solutions. Crowding effects were attributed to the nonspecific steric repulsions of the crowders with the biomolecule of interest that led to compaction of the biomolecule. In the "effective hard sphere model",[1b] these interactions were shown not to strongly depend on the local details of the chemical nature of the macromolecule but rather to depend on the global features such as the size, net charge, dipole or multipole moment, and shape. Later, this effective attraction between the test biomolecules was coined as *depletion attraction* since it was induced by the depletion of the nonadsorbing crowders from the solvation shells of the biomolecules. The release of the crowders into the bulk led to an entropically favorable state of the system that could drive the process and is, therefore, sometimes also referred to as *entropic depletion attraction* (see Figure 1(b)). Such a theory is based on assuming that the enthalpic soft interactions of the crowders with the test biomolecule have negligible consequences. Even the effective hard-sphere models that are parametrized from experiments for single-protein solutions have worked well for a mixture of proteins that have similar pI or are electrostatically repulsive. However, for proteins having attractive interactions, this model may be inadequate to describe the true behavior of biomolecules.[14] In 1970, Ogston presented the view that an incompatible phase transition between solutes of different sizes can occur if the size difference between the solutes is large. He took the case of a mixture of spheres and rods, where the molecular covolume (overlap in excluded volume) of the pair of solutes interacting via purely entropic forces could be identified using molar second virial coefficients between like molecules or molar interaction coefficients (molar excluded volume between unlike molecules). These molar covolumes were expressed in terms of the volume exclusion due to rod−rod, rod−sphere, and sphere−sphere interactions. This view was analogous to the crowded environment inside the cell where macromolecules of different sizes and shapes interact via volume exclusion.[15]. In 1994, Meijer and Frenkel reported a simulation study on colloids dispersed in a polymer solution.[5] They presented a phase diagram based on polymer-induced interactions with the colloidal particles (Figure 1). The partition function of the three-segment lattice polymer was computed by assuming that the sites occupied by the spherical colloidal molecule were not accessible to the polymer. They tested the accuracy of scaled particle theory and thermodynamic perturbation theory to conclude that the many-body additivity of polymer-induced attraction between the colloidal particles cannot be neglected and has a pronounced effect on the phase behavior of the colloid−polymer mixture. This study was among the first ones to indicate that the many-body intermolecular attractions in such complex solutions could play an important role.

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2.2. Emerging View: Role of Soft Interactions. In addition to the well-established classical view, an emerging view of crowding is shaping up that is based on the significant nonideal solution behavior of crowded milieu due to the high concentrations of macromolecules, metabolites, and ions. The nonspecific effects of physical occupation of the volume by crowders and strong intermolecular interactions between the solution components, both attractive and repulsive (distinct from van der Waals repulsions due to steric effects), lead to the nonideality. Such a feature can lead to counterintuitive effects of crowding, namely, destabilizing the native biomolecular structure or promoting unfolding of biomolecules instead of compaction. One of the first studies to indicate that crowding can destabilize the proteins was by Lee and Lee. They showed that poly(ethylene glycol) (PEG) as a crowder destabilizes different kinds of proteins. The decrease in the melting point of the proteins was directly correlated to the hydrophobic content of the proteins.[16] In 1982, McConkey introduced the term *quinary* structure of proteins beyond the primary, secondary, and tertiary structure of proteins, which arises as a consequence of numerous macromolecular interactions with the protein given the complexities of the intracellular environment.[4]

In 2012, Pielak and co-workers investigated the debatable contributions of entropy and enthalpy in crowded solutions.[6] They pointed out that protein stability can be affected by either the hard-core repulsions (entropic excluded volume effect) or the weak, nonspecific chemical interactions (enthalpic effect) in the crowded solutions. The interactions of the direct cosolute with the proteins can destabilize the native state, such as that found for the effect of urea on protein native structure (see Figure 1(c)).[17] Harries and co-workers demonstrated that the depletion force must have contributions from both enthalpic and entropic components.[7a,17c] There can also be enthalpically mitigated depletion forces, where the driving force is still entropic but stabilization is decreased due to the unfavorable interaction energy. This could be the case for protein stability where the crowder has steric interactions (entropic) and there is an enthalpic penalty from the sticky crowder that has attractive interactions with the protein. In contrast, the enthalpically dominated depletion forces involve enthalpy as the major driving force being opposed to the destabilizing entropy. The authors also proposed the role of cosolutes or crowders in inducing changes in the hydration water ordering at the biomolecular interface, leading to loss in entropic degrees of freedom and resulting in concomitant gain in enthalpy for the folding process (Figure 1(c)). Attractive interactions with the native state can also lead to stabilization of the native state. It was found that the synthetic polymers such as PEG, dextran, and glucose could stabilize the structure of a β-hairpin peptide, but the origin of this effect was enthalpic rather than entropic with increasing crowder concentrations.[17d] Senske and co-workers found that dextran and glucose as crowders enthalpically stabilize but entropically destabilize the native state of ubiquitin.[17e] Unlike the hard-core repulsions that always have a stabilizing effect, the soft attractive interactions can lead to either a stabilizing or destabilizing effect. Aside form macromolecular crowders, the role of the interaction energy of crowders has been found to be significant even for small-sized *molecular* crowders. Our group has shown that for simplified hydrophobic and hydrophilic polymers the strength of the crowder−polymer interaction can determine the weakening and strengthening of the hydrophobic collapse. The results indicated that the stronger dispersion interactions of the molecular crowders (trialanine peptide) with the polymer could unfold the polymer via preferential interactions of the crowders with the polymer.[18] Moreover, charged model molecular crowders have been shown to induce collapse in polymers via *preferential interaction* with the polymer interface rather than *preferential depletion*.[18b] There are several other studies that have reassessed the entropic-centric view of crowding for protein folding, aggregation, and association.[19]

3. MODELING THE CROWDED ENVIRONMENT FOR BIOMOLECULAR PROCESSES

Although the single-molecule view has tremendously progressed the understanding of biomolecular processes, accounting for the influence of multicomponent intracellular environment has been computationally challenging. The development of realistic models for the cellular environment is conceivable if the wide range of length and time scales associated with this complex environment can be accounted for. Cellular dimensions range between 300 nm and 1 μm, whereas the atomistic detailed molecular processes occur at the scale of a few nanometers. The dynamics of molecules in the living cell at the biologically relevant time scales range between nanoseconds to microseconds and even seconds to hours for complete biological processes. Developing all-atom models for such time and length scales is computationally demanding. Therefore, several studies (as discussed below) have resorted to multiscale modeling as one of the viable strategies where low resolution and less computationally demanding, yet physically accurate, models of such complex environment have been developed. However, it is critical to investigate these systems with an accurate representation of the chemical details and intermolecular interactions in order to obtain a comprehensive and microscopic view of the crowding effects.

3.1. All-Atom Models. First, we summarize a few studies that have adopted all-atom models to investigate biomolecular structure in crowded solutions. The study by Feig et al. investigated the structure and dynamics of chymotrypsin (CI) protein in the presence of a crowded solution of lysozyme or bovine serum albumin (BSA) proteins using all-atom models.[20] BSA was found to interact weakly with CI and therefore only affect the diffusion of the protein, whereas lysozyme was found to interact strongly with CI, which induced structural fluctuations, destabilized the native structure and led to a significant reduction in diffusion rate of CI due to strong association between the two proteins, as shown in Figure 2(a). The study highlighted the role of enthalpic interactions, as well as their dependence on the local environment of the protein. In a recent study by Sterpone and co-workers, the thermal stability of chicken egg white lysozyme was investigated in an extremely crowded solution with a 60% packing fraction in water and glycerol.[21] Using all-atom models and an enhanced sampling method of replica exchange with solute scaling (REST2), they showed that the flexibility of the protein crowders plays a crucial role in determining the stability of the lysozyme. At a high temperature of 350 K, the flexibility of the protein crowders increases, as reflected in the RMSDs relative to the crystal structure. It renders the excluded volume effects no longer tunable, which has no effect on the melting point of the lysozyme protein, as shown in Figure 2(b). The simulation results agreed with the in-house calorimetric experiments where the specific heat of unfolding for lysozyme protein was

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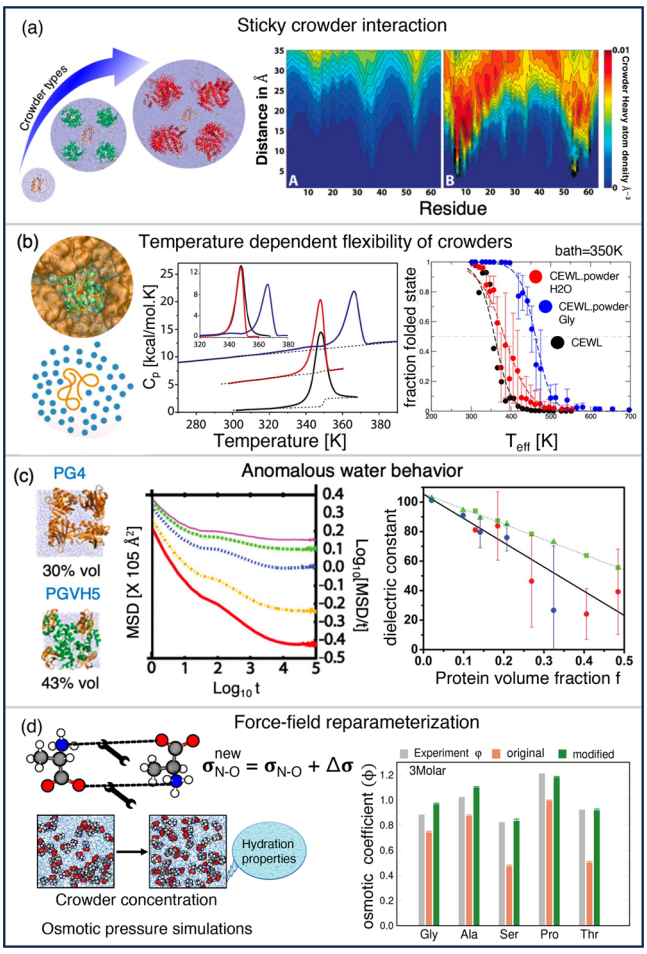

Figure 2. Investigations of crowding effects using all-atom models. (a) Snapshots of the simulated CI-lysozyme and CI-BSA systems along with the radial density of the heavy atoms of crowders (BSA, left and lysozyme, right) around C_α of CI. Reprinted with permission from ref 20. Copyright 2012 American Chemical Society. (b) Snapshots of lysozyme in a self-crowded solution (left), specific heat of unfolding of lysozyme in aqueous and glycerol solutions (center), and the fractions of the folded state of lysozyme as a function of temperature (right). Reprinted with permission from ref 21. Copyright 2021 American Chemical Society. (c) Snapshots of the crowded protein-G system (PG4) and protein-G/villin headpiece proteins system (PGVH5), anomalous mean squared displacement of water as a function of time for PGVH5 system, and decrease in the dielectric constant of water with protein volume fractions. Reprinted with permission from ref 30. (d) Reparameterization of AMBER ff99SB-ILDN dispersion interactions to reproduce the experimental osmotic coefficients of amino acids at high packing fractions. Reprinted with permission from ref 34b. Copyright 2023 Royal Society of Chemistry.

similar in pure water and in self-crowded aqueous solution at 350 K. Atomistic simulations have also been successful in identifying the role of intermediate unfolded conformations of superoxide dismutase protein in determining the thermal stability of the protein in a crowded solution of BSA proteins with different packing states.[22] At a given temperature, the fully or partially unfolded intermediate states were found to interact more with the crowder proteins than the folded structures. This implies the crucial role of the weak, transient (quinary) interactions in these solutions that lead to the unfolding of the protein. These quinary interactions represent the fifth level organization in protein structure, beyond the quarternary structure, that arises due to the interactions of proteins with other biomolecules in the crowded cellular environment.

3.2. Coarse-Grained Models. Another approach for investigating these complex solutions is to simplify the internal degrees of freedom of the macromolecules and make use of coarse-grained models. Crowders are generally modeled as hard spheres with hard-core nonspecific repulsive interactions. The proteins are also modeled using coarse-grained resolution, such as with the side-chain C_α (SCM) model.[23] It is a two-bead model per amino acid of the protein, where one bead is the C_α atom and the other one is the center of mass of the side chain. In one of the studies, the structure of an aspherical VlsE protein (using SCM model) was examined in the presence of Ficoll as the crowder, modeled using hard spheres, and the denaturant urea. The free energy landscape showed that with increasing denaturant concentration, the crowders trigger a shape change in the protein such as a football, a sphere, a bean, or the unfolded state, as shown in Figure 3(e). The effect of the shape of the crowders on the protein structure has also been examined using low-resolution models. Cheung and co-workers used spherical and rod-like coarse-grained crowders to model dextran and examined their influence on the structure of the native state of apoazurin protein in 6 M urea solution.[24] The rod-like crowders altered the urea-unfolded ensemble of apoazurin to an extended or unfolded ensemble, underscoring the counterintuitive effect of the crowded environment. The spherical model of crowders did not produce elongated protein ensembles, whereas the rod-like crowders stabilized the unfolded protein conformations via effective attractions. Authors explained that the elongated shape of the crowder could break the symmetry of the isotropic depletion force, which could create an anisotropic force to sandwich the protein backbone. At high crowder packing fractions, the compaction of apoazurin was observed. An increase in the excluded volume effects of crowders disintegrated the bundles because of the hard excluded volume interactions between the crowder particles, which increasingly suppressed protein−crowder interactions (see Figure 3(d)). Klumpp and co-workers investigated the effects of spherical and polymeric crowders on the folding of a 89-residue HigA protein using a structure-based coarse-grained model and molecular dynamics simulations.[25] It was found that the protein folding temperature decreased with increasing size of spherical crowders and increased with the chain length of polymeric crowders, with the latter trend being in line with qualitative predictions by the Flory−Huggins theory. Hence, crowders may enhance the stabilization of the folded protein state either by lowering the size of the spherical crowders or by elongating the length of the polymeric crowders. Hall and co-workers examined the effect of hydrophobic crowders on the aggregation of a heptamer of the $A\beta_{40}$ protein using discontinuous molecular dynamics simulations with an intermediate resolution PRIME20 model for the protein.[26] The crowders were modeled using a hard sphere potential, and the atoms or pseudo atoms of the polypeptide were modeled as hard spheres with softer square-well or square-shoulder potentials. The results indicated that beyond excluded volume effects, introducing crowder−peptide interactions and tuning the hydrophobicity of the polypeptide results in the formation of different types of aggregates of the protein, such as β-sheet oligomers, disordered oligomers, and fibrils as shown in Figure 3(c). Besides the crowder size and polypeptide hydrophobicity, the volume fraction of the nanoparticle crowder has also been found to influence the

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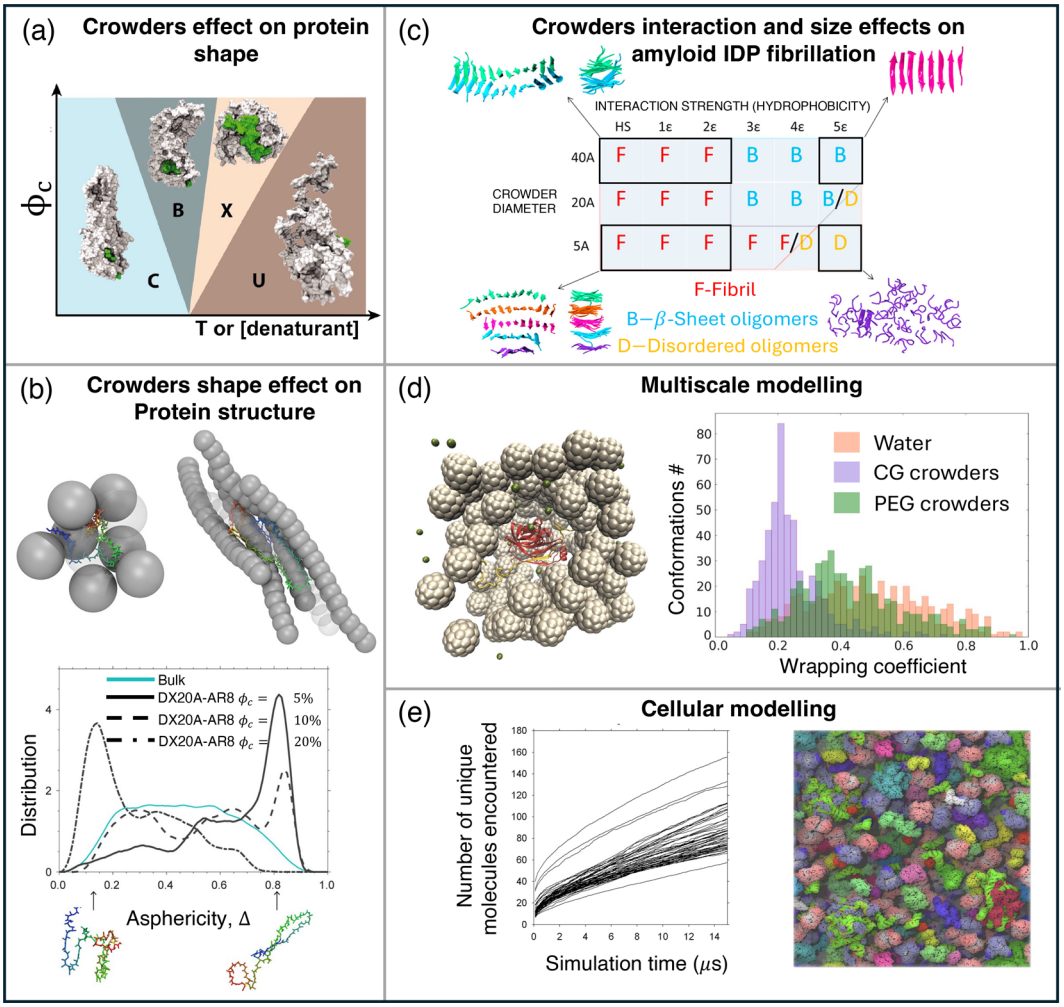

Figure 3. Investigations of crowded systems using coarse-grained modeling. (a) Conformational phase diagram of VlsE in the ϕ_C-T plane indicating different shapes: football (C), bean (B), spherical (X), and unfolded (U) states. Reprinted with permission from ref 23. Copyright 2008 The National Academy of Sciences of the USA. (b) (Top) Snapshots of the apoazurin in spherical and rod-like crowder solutions. (Bottom) The decreasing asphericity of apoazurin with increasing volume-fraction of rod-like crowder in 6 M urea solution. Reprinted with permission from ref 24. Copyright 2019 American Chemical Society. (c) Crowder size and interaction effects on the global conformational phase diagram of the types of aggregates formed by the heptamer of the Aβ protein. Reprinted with permission from ref 26. Copyright 2015 Biophysical Society. (d) (Left) Snapshots of enzyme NS3/4A surrounded by CG crowders. (Right) Number of conformations of NS4A with the wrapping coefficient of N-terminal tail wrapped around NS3 in crowded solutions. Reprinted with permissions from ref 29. Copyright 2021 Biophysical Society. (e) Average number of unique neighbors encountered by each molecule (left) during the simulation of the model cytoplasm with 50 macromolecules (right). Reprinted under the terms of the Creative Commons Attribution License from ref 31.

oligomerization of the amyloidogenic peptide. Thirumalai and co-workers demonstrated that the spherocylindrical crowders destabilized the peptide oligomers to a greater extent than the spherical crowders.[27] Using the Asakura−Oosawa theory, they computed the excluded volume of the crowders and peptide oligomers in ordered and disordered states. They found that the spherical crowders destabilize the small oligomers of the peptide. When the peptide concentration was increased, the spherical crowders stabilized the large-sized peptide oligomers due to the lower volume excluded by the ordered oligomer compared to that in the disordered state. The work highlighted the role of nanoparticle-induced complexity in the conformational preferences of the amyloid oligomers. Other than proteins, coarse-grained models of chromosomal material have also been employed to understand the collapse−swelling transitions of these biopolymers in a crowded environment. Slater and co-workers treated the supercoiled DNA structural monomers and small protein crowders as coarse-grained models with truncated Lennard-Jones interactions.[28] The simulations quantified the effective excluded volume of each monomer using the WCA potential. The change in the radius of gyration prediction with increasing depletant volume fraction indicated an entropy driven depletion-induced attraction between the monomers that was sufficient to cause a coil−globule transition, similar to that observed for a freely joined polymer chain in good to poor solvent conditions. The authors concluded that in order to observe the first-order phase transition, other effects such as enthalpic interactions and confinement effects must be included.[28a] In another study, Kang et al. investigated the reason for the coil−globule transition of DNA that is observed at very low crowder concentrations.[28b] Using self-avoiding walk polymer in explicit crowders showed the collapse of the polymer with increasing crowder volume fraction. They proposed that the crowder-induced polymer collapse can be defined by a parameter, which is the ratio of the radius of gyration of the polymer and the

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crowder volume fraction-dependent distance between crowders. Based on this parameter, a phase diagram was proposed that could explain the collapse of DNA in a small amount of PEG, whereas an IDP with large number of monomers would not collapse even at high crowder volume fraction. In the work by Chaboche et al., a mean field approach was adopted to model the two-body effective interactions between the monomers in a bath of crowders.[28c] The square-well potential with a range equal to crowder size was employed. Such an approach smoothly interpolated between the inelastic (globule) state of the polymer, where the short-range attractive energy was constant with the crowder density, and the elastic state (coil) of the polymer, where the energy linearly increased with the crowder density. Their model was able to account for solvent concentrations where jamming of the solvent (polymer collapse) could be achieved.

3.3. Multiscale models. Another commonly used strategy for the accurate representation of crowded environment is adopting multiscale modeling of the crowded systems. The modeling involves reduced chemical details, increased computational speed and can cover higher length and time scales for biological processes. For instance, in the study by Ostrowska et al., the internal dynamics and diffusion of hepatitis C virus proteases NS3/4A were examined in a crowded solution of PEG. The enzyme and water were modeled at atomistic resolution, whereas the PEG crowders were modeled using atomistic and coarse-grained (CG) models.[29] The all-atom PEG accounted for the protein−crowder interactions. The folded PEG chains were modeled using a bead−shell CG model representing mainly the solvent excluded volume effect. The model comprised of 42 carbon-sized beads or pseudoatoms on the surface of a large central sphere that stabilized the spherical structure and prevented the collapse of the surface pseudoatoms. The mass of this crowder was made equivalent to the mass of 28-mer PEG. The crowder−crowder interactions were kept purely repulsive, and protein−crowder interactions were modeled via Lennard-Jones potential. The CG crowders led to the formation of more compact conformations such that the NS4A tails could interact more with NS3 (see Figure 3(b)). This facilitated the formation of the NS3/4A TM helix precursor and its positioning near the membrane surface to enhance membrane anchoring and thereby viral replication.

3.4. Modeling the Solvation. An alternative method to reduce the computational expense in these complex environments is to model water as an implicit solvent. Water is known to affect the conformational preferences of the biomolecules. In addition, the diffusion and hydrodynamic properties of water also play critical role. The implicit representation of the solvent using a dielectric continuum is well within the framework of Poisson−Boltzmann theory. However, using explicit water models comprising representations of dipole interactions, hydrogen-bonding, and other nonspecific intermolecular interactions can help improve the understanding of solvation in the complex intracellular environment. Harada and co-workers investigated the effects of crowding on the hydration shell structure and dynamics of two globular proteins, *i.e.*, protein GB1 and a villin headpiece, using all-atom molecular dynamics simulations.[30] The tetrahedral arrangement of water molecules was shown to be disrupted in crowded solutions, as indicated by the low tetrahedral order values, implying random orientations of water molecules in the hydration shells. They also found that the dielectric constant was substantially reduced to 30−50 for high crowder packing fractions, as shown in Figure 2(c). Such a low dielectric response is expected to affect the hydrophobic interactions and therefore the protein structure stability. The self-diffusivity of water is significantly reduced, with an anomalous diffusion regime observed at short times scales (<10 ns) and a normal diffusion regime observed at larger time scales (>10 ns) due to constrained water motion (see Figure 2(c)).

3.5. Cellular Model. One of the ambitious goals has been to model a complete living cell using physics-based models. This involves correctly modeling the excluded volume effects, nonspecific interactions, and hydrodynamic interactions. Elcock and group presented a cytoplasm model of *Escherichia coli* comprised of 50 macromolecules, out of which 45 were proteins and 5 were RNAs or RNA−protein complexes, as shown in Figure 3(a).[31] The model was comparatively sophisticated since it included the representation of intermolecular interactions such as electrostatic and hydrophobic interactions, unlike the previous cellular models. First, the authors performed Brownian dynamics simulations and computed the translational diffusion coefficient of green fluorescent protein (GFP) in the cytoplasm "steric model" in which only steric interactions operated. It was found to be 3−6times higher than the experimental estimates. When the electrostatic interactions were included, it was found to be 2−5 times higher than the experimental value. To make the model more realistic, denoted as "full model", short-range attractions were added via the Lennard-Jones potential with an adjustable well-depth parameter (ϵ). With a value of ϵ = 0.285 kcal/mol, the translational diffusion coefficient was found to be within the experimental error. The folding free energies of several proteins and the protein−protein association free energies, such as those for RNase and SH3 proteins, were also found to be within the experimental estimates in this cytoplasm model.

3.6. Computationally Efficient Methods to Investigate Crowded Systems. Several attempts have been made to reduce the computational cost associated with investigating such complex systems. One possibility is to perform postprocessing of the trajectories using the particle-insertion method.[32] Zhou and co-workers developed an FFT-based method that allows the computation of the chemical potential change for proteins from conformational ensembles in the two end states in the crowded solution.[32a] It was denoted as FMAP (FFT-based method for modeling protein-crowder interactions) algorithm. Theoretically, the change in chemical potential due to interactions of the protein with crowders can be written in terms of the protein−crowder interaction energy. The protein−crowder interactions are assumed to be hard-core repulsions such that the protein−crowder interaction energy will be either infinite when the protein clashes with the crowder or zero otherwise. The simulation cell is treated with grid points, and the crowders are mapped onto the grid. From another simulation of the protein alone, the protein is mapped to the center of the grid. The mapping of the grid points and clashes are taken into account by the correlation functions that finally contribute to the chemical potential change. Another approach to ease the computational cost of investigating crowded systems was developed by the groups of Feig and Sugita, who developed a new MD package called the Generalized-Ensemble Simulation System (GENESIS). This package was designed to perform MD simulations of large-scale macromolecular systems efficiently on general-purpose supercomputers.[32b] It has two MD simulators: first ATDYN

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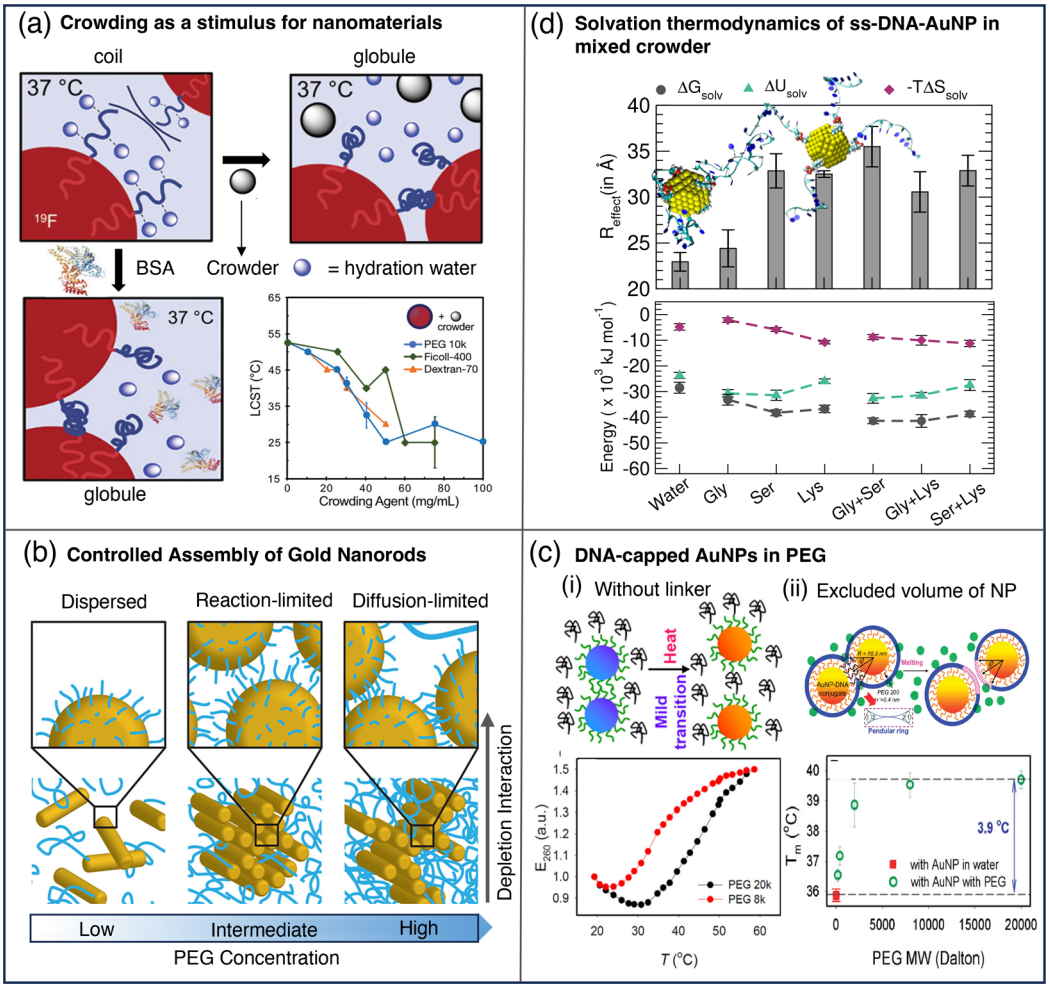

Figure 4. Intracellular stimulus for nanomaterial self-assembly. (a) Crowding effects of synthetic and biomolecular crowders on the coil−globule transition temperature (LCST) of the thermoresponsive nanoemulsions. Reprinted with permission from ref 8b. Copyright 2022 American Chemical Society. (b) Schematic illustrating the self-assembly of polymer-grafted gold nanorods in a crowded solution of PEG via different mechanisms. Reprinted with permission from ref 35a. Copyright 2018 American Chemical Society. (c) (i) Broadened melting curves of the non-base-paired DNA-capped AuNP in crowded solutions of PEG, indicating mild melting transition. Reprinted with permission from ref 35b. Copyright 2012 American Chemical Society. (ii) Schematic of the melting of DNA-linked AuNPs in the presence of PEG crowders, increasing the excluded volume of AuNPs. This is reflected in the increase in the melting point (bottom) of the AuNPs as a function of crowder molecular weight. Reprinted with permission from ref 35c. Copyright 2011 American Chemical Society. (d) (Top) Variation in effective radius of ssDNA-capped gold nanoparticles (AuNPs) in crowded solutions of single and mixed amino acids. (Bottom) The variation of the solvation free energy, solute−solvent energy, and entropy of ssDNA-AuNP in different crowded solutions. Reprinted with permission from ref 35d. Copyright 2024 AIP Publishing.

(atomic decomposition dynamics), which is parallelized on the atomic decomposition algorithm for atomistic and coarse-grained Go models, and second SPDYN (Spatial decomposition dyanamics), which is parallelized on the domain decomposition algorithm to perform large-scale MD simulations. In 2015, on the K computer, the simulation of crowded solution with 103.7 million atoms showed scalability up to 260 000 cores, resulting in a simulation time of 6.5 ns per day. With the currently increasing computational power, the scalability has been seen to be enhanced for larger-sized systems. A recent study from the same group implemented coarse-grained molecular dynamics (CGDYN) in GENESIS and showed scalability on 4096 nodes on Fugaku supercomputer with a performance of 50 million steps per day, showing a promising efficiency to simulate large biological systems.[32c]

4. LIMITATIONS OF THE EXISTING COMPUTATIONAL METHODS

The previous sections have highlighted the effective capability of computational methods to provide insights into the molecular behavior of biomolecules in a crowded environment. However, given the complexity of the crowded environment, there are still several challenges faced by computational methods that must be addressed in order to accurately mimic the cellular environment. One of the concerns has been that the current generation of biomolecular force-fields overrepresent the strength of protein−protein interactions in dense solutions, which can induce artificial aggregation or compact disordered states of proteins. A recent study by Gruebele and co-workers investigated the folding of a 33-residue GTT WW domain protein in an all-atom model of the *E. coli* bacterial cytoplasm using more than 200 μs long molecular dynamics simulations and three force-fields for the

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protein.[33] All force-field models showed inhibition of the folding of the protein due to sticking of the unfolded protein with the other macromolecular crowders in the solution. This implies the limitation of the current force-field models for proteins, which can underestimate the in-cell folding of proteins. Recent attempts have been made to calibrate the strengths of solute−solute nonbonded interactions in concentrated solutions of amino acids.[34] It was proposed to increment the size parameter of the mixed Lennard-Jones interaction parameter of the interaction between the zwitterionic amine and the carboxyl group of the amino acid termini. This reparameterization was systematically compared for different force-fields for reproducing osmotic coefficients of the solutions close to experimental values. We assessed this strategy in our recent work for the AMBER ff99SB-ILDN force-field for crowded concentrations (>2 M) of a few amino acids.[34b] The modified force-field improved the agreement of the simulated values of osmotic coefficients, density, viscosity, and self-diffusivity of amino acids with the experimental values (see Figure 2(d)). The reparameterization also improved the solute solvation shell structure, solute interaction energy, and radial distribution function tail convergence, which would aid in further accurate computation of the thermodynamic quantities. In the other biomolecular processes such as of protein−protein binding, crowding is expected to influence the protein structure and diffusion, which could play a crucial role in the binding process. However, Grassmann et al. summarize in their review that once the proteins are close in contact, they bind as if they were isolated. This raises an interesting open question as to whether the current force-field models for proteins would be sufficient to predict the binding affinities of the protein complexes.[34c] A limitation in the simulation methods is the relatively short time scale of the atomistic simulations of crowded systems, which has limited the investigation of dynamics to submicrosecond scales.[9] The choice of water models, such as the TIPnP family and SPC/E, being used in the simulations also affects the dynamics and sampling of the conformational landscape of biomolecules. These water models are known to differ in reproducing the experimental viscosity, diffusivity, and dielectric constant and therefore would be expected to alter the accurate modeling of the crowded environment. Such a sensitivity of the properties of intracellular water to the choice of water model could limit the accurate modeling of the cellular environment.

5. CROWDING-ASSISTED NANOMATERIAL SELF-ASSEMBLY: A NEW DIRECTION

Recent advances in the understanding of macromolecular crowding have inspired several applications in nanomaterial design. Particularly, the frontier of nanomedicine and materials chemistry has inspired the design of advanced smart materials that can selectively respond to the conditions of the intracellular environment.[8] Eastbrook et al. designed oil-in-water nanoemulsions that could be stabilized by thermoresponsive poly(2-oxazoline) amphiphiles.[8b] They are known to stabilize the nanoemulsions below the LCST but are ineffective at temperatures higher than the physiological temperature. The dynamic light scattering experiments indicated that nanoemulsions fused and aggregated above the LCST. The authors found that in the presence of synthetic polymers and sugars at crowded concentrations, such transformations observed in the nanoemulsion above the LCST could be induced at physiological temperatures, as shown in Figure 4(a). A similar "crowder-responsive" fusion of nanoemulsions was observed in crowded solutions of proteins such as of bovine serum albumin (BSA), as summarized in Figure 4(a).

Macromolecular crowding can be a useful tool to control the self-assembly and colloidal behavior of nanomaterials and their functional properties.[35] Decreased diffusive transport and enhanced depletion attraction have been shown to control the self-assembly of gold nanorods grafted with PEO in crowded solution of polyethylene glycol (PEG).[35a] PEG was shown to guide the self-assembly of polymer-grafted gold nanorods through two kinetic processes. Small-angle X-ray scattering (SAXS) experiments revealed that at low polymer concentrations the gold nanrods exhibited reaction-limited aggregation, where the strong depletion attraction and fast nanorod dynamics were the primary driving forces. At high polymer concentrations, slower dynamics and stronger depletion forces led to a transition to a diffusion-limited aggregation process, as shown in Figure 4(b). This interplay of the dynamics and cohesive forces was highlighted to be crucial in determining the reversibility of these aggregation processes.

In another work by Shin et al, the self-assembly of citrate-capped and DNA-capped gold nanoparticles (AuNPs) was examined in crowded solutions of PEG.[35b] The experiments showed that the citrate-capped AuNPs were stable in a crowded PEG solution, where they did not aggregate. The DNA-capped AuNPs were, however, found to aggregate in the crowded polymer solution. The authors attributed the differences in the stability of the AuNPs to depletion repulsion in the case of citrate-capped AuNPs and depletion attraction in the case of DNA-capped AuNPs. In the crowded PEG solutions, a sharp melting transition with an increase in the melting point was observed for the DNA-capped AuNPs where linker DNA was present, implying melting of the DNA. In contrast, in the case where no linker DNA was present, a broad melting transition with an increase in the melting point was observed in the crowded PEG solutions (see Figure 4(c)(i)). The authors concluded that the melting transition was not due to the denaturation of DNA but rather due to depletion attraction between the AuNPs induced by the polymeric crowders. On heating, the overall entropy favors the disassembly of the nanoparticle aggregates.

Self-assembly of DNA-functionalized gold nanoparticles through 24-mer linker DNA was also examined by Zaki et al. in the presence of high-molecular-weight PEG to mimic crowded solutions.[35c] Interestingly, heating the functionalized AuNPs melted the DNA and enhanced the excluded volume effects from AuNPs in addition to the excluded volume effects exerted by the PEG chains. This resulted in a further increase in the melting point of the AuNP aggregates, as shown in Figure 4(c)(ii). The results showed that the increase in the excluded volume change upon melting of the AuNPs amplified the stabilization effect of the PEG crowders. They also showed that with the increasing size of the AuNP there was an increase in the excluded volume change and therefore the change in the melting point.

As summarized so far, there are several experimental studies that have focused on modulating the self-assembly or aggregation of nanomaterials in the presence of macromolecular crowding. Very few *simulation* studies have been performed in this field that focused on elucidating the molecular driving forces for these effects. Moreover, there have been simulation studies explaining the collapse−swelling transitions of polymers in poor and good cosolvents using the

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view of entropic origin either due to depletion forces[36] or due to the direct cosolvent interactions with the polymer surface.[36b] For instance, one of the studies by Mukherji et al. explained the collapse−swelling−collapse transition in polymers in a mixture of poor (repulsive) solvents due to the interplay of reduced depletion forces and the bulk solution properties.[36][36a]Other studies by van der Vegt and co-workers have explained that cosolvent effects on coil−globule polymer transitions were determined by the entropic effects arising due to the preferential binding of cosolvents on the polymer surface leading to fluctuations in the attractive polymer−cosolvent interaction energy.[36b] However, in particular, very few simulation studies have focused on understanding the interaction of nanoparticles with biological fluids considering the crowding effects, which is crucial for applications in drug delivery and biodiagonostics. In an attempt to understand this, we recently investigated the structure and solvation of ss-DNA (T_{10}) capped AuNPs in crowded solutions of amino acids (Gly, Ser, Lys) and their mixtures (Gly + Ser, Ser + Lys, Gly + Lys) using molecular simulations.[35d] Our results showed that Gly exhibited the highest preference to adsorb on the surface of AuNP, followed by Ser and Lys. Such differential adsorption of the crowders on the Au surface resulted in the increase in the effective size of the AuNP in the following order: Gly < Ser < Lys (*i.e.*, increasing crowder size), as shown in Figure 4(d). Gly bends the ss-DNA chains on the Au surface through cohesive interactions with both Au and ss-DNA, reflecting a "glue" effect that decreases the overall size of AuNP. Interestingly, in the mixtures of Gly + Ser at crowded concentrations, the effective size of the AuNP additively increased more than that in the single amino acid solutions. Since both Gly and Ser showed a good tendency to adsorb on the Au surface, steric effects of the crowders on the Au surface prevented the bending of ss-DNA chains. However, in the Gly + Lys and Ser + Lys mixtures, the effective size of the AuNP decreased, implying a nonadditive effect of the crowders. Since Lys adsorbed poorly on the Au surface and preferentially on ss-DNA chains, the Gly could induce the "glue" effect to decrease the effective size of the AuNP. Such differential crowding effects were also reflected in the solvation thermodynamics, where the free energy of solvating the AuNP decreased with the increase in the effective size of the AuNP. Using the structural estimators of entropy, *i.e.*, the pair correlation functions, the entropy of solvent reorganization was computed. The solvation process was found to be entropically (including ligand conformational entropy and solvent reorganization entropy) favorable in both the single and mixed crowded solutions, where the solvent reorganization entropy was found to play the dominant role. Therefore, amino acids that can be considered as small molecular crowders (or cosolvents) are shown to induce structural changes in the ss-DNA-capped AuNP, highlighting the role of intermolecular interactions rather than the excluded volume effects in such a crowded environment.

The self-assembly of synthetic dyestuffs under the influence of a crowded environment has recently attracted investigation. These organic dyes tend to form long-range fibrils (J-aggregates) similar to those observed in amyloidogenesis and exhibit a sharp fluorescence peak at 575 nm close to the absorption peak. Huber and co-workers investigated the effects of crowded solutions of PEG, triethylene glycol (TEG), sucrose, and Ficoll-400 on the aggregation of the cationic pseudoisocyanine chloride or 1,10-diethyl-2,20-cyanine chloride (PIC) dye molecules.[37] It was found that Ficoll-400 increased the threshold temperature and promoted the formation of J-aggregates, unlike the crowded environment of PEG, TEG, and sucrose that inhibited J-aggregation of PIC dye by lowering the threshold temperature. These results indicated the role of excluded volume effects of the Ficoll-400 crowders in inducing aggregation. A recent simulation study supported these observations and highlighted that the oligomerization free energy of PIC dyes becomes less favorable in crowded solution of ethylene glycol relative to that in pure water. The ethylene glycol molecules were found to interact favorably with the dye molecules, reducing the favorable dye−water interaction energy and thus disfavoring the dye oligomerization.[37c]

6. FUTURE OUTLOOK AND CONCLUSIONS

Much of the understanding of biomolecular structure and function has been derived from dilute solutions, and even if dense solutions with high packing have been investigated, they lack the heterogeneity of the crowded solutions. This raises an intriguing question as to how the other components of the crowded cytoplasm contribute to the macromolecular crowding effects. Therefore, modeling and examining the role of *mixed* crowding on the stability and protein dynamics of the protein native structure is gaining attention, but a lot remains unexplored. The role of the presence of different types of charged and neutral proteins of varying sizes and strengths of specific or nonspecific interactions needs to be further explored. It is well-established that the physical behavior of interfacial water is different from that of bulk water. It is, therefore, expected that the behavior of water as a solvent would significantly differ in the presence of the macromolecular crowders. The effect of crowders on the hydration structure and dynamics, the expected decrease in the dielectric constant, and the increase in viscosity can alter the thermodynamics and kinetics of the biomolecular process in question. Moreover, whether the current force-field models of water are sufficient to mimic intracellular water needs to be investigated. Another feature of a crowded environment is that it also comprises cosolvents such as urea, trimethylamine oxide (TMAO), or metabolites at high concentrations. It is intriguing to ask how these small-sized cosolvents interact with the densely packed macromolecules in the living cell, where there would be possibilities for direct, specific intermolecular interactions. Such interactions can modulate the effect of macromolecular crowders on a given biomolecular process such as protein association or aggregation. The combined effects of confinement induced by the membranes and crowding macromolecules need to be investigated further in biomolecular processes. In particular, protein aggregation of certain intrinsically disordered proteins involves the interaction of the protein with the membrane. The physical origin of the confined space effects of different shapes such as cylindrical or two-dimensional slit-like spaces has been explored for flexible polymer chain collapse in the presence of spherical crowders of different sizes.[38] The results showed that molecular crowding effects become effective when the crowder size is equivalent to the monomer size and that these effects are insensitive to the shape of confinement. Another crucial phenomenon for which the crowding effects are unknown, is that of liquid−liquid phase separation (LLPS), which mediates several processes, such as signaling and RNA metabolism. Certain proteins such as IDPs are known to associate with each other via weak

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noncovalent interactions resulting in formation of gel-like or liquid-like droplets that phase separate. The role of the crowded environment in inhibiting or promoting such phase separation remains elusive. The use of machine learning methods to improve the parametrization of current force-fields such that they are not overly "sticky" in terms of intermolecular interactions at high concentrations of biomolecules could be another route to make the efficient computational methods to explore such complex solutions. Other ways to reparameterize force-fields for these complex solutions could be scaling the partial atomic charges of the ionized groups of biomolecules,[39] as implemented in the derivative of CHARMM36 force-field called prosECCo,[39b] targeting to balance the protein−protein and protein−water interactions,[39c] or mapping the diffusion and dynamics of biomolecules in crowded media accurately.[39d,e] The existing solvation theory such as the Kirkwood−Buff theory, which connects the microscopic correlations in the system with the macroscopic thermodynamic quantities, needs to be reassessed for the accurate computation of thermodynamic quantities for the dense, crowded solutions. The lack of computational studies in the literature specifically for using a crowded environment to design self-assembled nanomaterials indicates a dire need to explore this direction to motivate new experimental design and synthesis.

To conclude, this Mini-Review summarizes the recent developments in understanding the role of intracellular crowded environment on various biomolecular processes, with a view of computational strategies employed in examining such systems. It is evident from the recent findings that these crowding effects play a crucial role in determining biomolecular structure and function. Computer simulations can be an effective tool to probe molecular-level phenomena associated with crowding effects and thus lead to a comprehensive understanding of these densely packed systems. The challenge associated with the long simulation times and computational cost of the modeling these systems has been tackled by several studies by adopting low-resolution coarse-grained models or multiscale modeling. Several other studies have focused on using all-atom description to retain the chemical details of the macromolecules and therefore to obtain a microscopic view of these effects. The interplay of size effects (accurately described by coarse-grained models) and the interaction energy effects (accurately modeled by the all-atom models) in modulating the biomolecular processes has yet to be understood completely. The emerging view of crowding supports the role of both crowder size and attractive intermolecular interactions in determining the conformational equilibria of the biomolecules. There are several limitations associated with the current models and methods for investigating crowded solutions, which are discussed in this review. Finally, a new direction that is attracting several applications in materials science is the crowding-assisted self-assembly of nanomaterials. Crowding can act as a stimulus for nanomaterial self-assembly, which is discussed in this review. The future directions that have yet to be explored for crowding effects are also summarized.

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REFERENCES

(1) (a) Ellis, R.; Minton, A. P. Cell biology: join the crowd. *Nature* **2003**, *425*, 27−28. (b) Zhou, H. X.; Rivas, G.; Minton, A. P. Macromolecular crowding and confinement: Biochemical, biophys-

https://doi.org/10.1021/acsomega.4c03344
ACS Omega 2024, 9, 29953−29965

ical, and potential physiological consequences. *Annu. Rev. Biophys.* **2008**, *37*, 375−397. (c) Marenduzzo, D.; Finan, K.; Cook, P. R. The depletion attraction: An underappreciated force driving cellular organization. *J. Cell. Biol.* **2006**, *175*, 681−686.
(2) (a) Asakura, S.; Oosawa, F. On Interaction between Two Bodies Immersed in a Solution of Macromolecules. *J. Chem. Phys.* **1954**, *22*, 1255−1256. (b) Asakura, S.; Oosawa, F. Interaction between particles suspended in solutions of macromolecules. *J. Polym. Sci.* **1958**, *33*, 183−192.
(3) Minton, A. P. Excluded volume as a determinant of macromolecular structure and reactivity. *Biopolymers* **1981**, *20*, 2093−2120.
(4) McConkey, E. H. Molecular evolution, intracellular organization, and the quinary structure of proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 3236−3240.
(5) Meijer, E. J.; Frenkel, D. Colloids dispersed in polymer solutions. A computer simulation study. *J. Chem. Phys.* **1994**, *100*, 6873−6887.
(6) Wang, Y.; Sarkar, M.; Smith, A. E.; Krois, A. S.; Pielak, G. J. Macromolecular crowding and protein stability. *J. Am. Chem. Soc.* **2012**, *134*, 16614−16618.
(7) (a) Speer, S. L.; Stewart, C. J.; Sapir, L.; Harries, D.; Pielak, G. J. Macromolecular Crowding Is More than Hard-Core Repulsions. *Annu. Rev. Biophys.* **2022**, *51*, 267−300. (b) Rivas, G.; Minton, A. P. Influence of Nonspecific Interactions on Protein Associations: Implications for Biochemistry In Vivo. *Annu. Rev. Biochem.* **2022**, *91*, 321−351. (c) Alfano, C.; Fichou, Y.; Huber, K.; Weiss, M.; Spruijt, E.; Ebbinghaus, S.; De Luca, G.; Morando, M. A.; Vetri, V.; Temussi, P. A.; Pastore, A. Molecular Crowding: The History and Development of a Scientific Paradigm. *Chem. Rev.* **2024**, *124*, 3186−3219.
(8) (a) Hata, Y.; Sawada, T.; Serizawa, T. Macromolecular crowding for materials-directed controlled self-assembly. *J. Mater. Chem. B* **2018**, *6*, 6344−6359. (b) Estabrook, D. A.; Chapman, J. O.; Yen, S. T.; Lin, H. H.; Ng, E. T.; Zhu, L.; Van De Wouw, H. L.; Campás, O.; Sletten, E. M. Macromolecular Crowding as an Intracellular Stimulus for Responsive Nanomaterials. *J. Am. Chem. Soc.* **2022**, *144*, 16792−16798.
(9) (a) Feig, M.; Yu, I.; Wang, P.-h.; Nawrocki, G.; Sugita, Y. Crowding in Cellular Environments at an Atomistic Level from Computer Simulations. *J. Phys. Chem. B* **2017**, *121*, 8009−8025. (b) Feig, M.; Sugita, Y. Reaching new levels of realism in modeling biological macromolecules in cellular environments. *J. Mol. Graph.* **2013**, *45*, 144−156.
(10) *Physical Biology of the Cell*, 2nd ed.; Phillips, R.; Kondev, K.; Theriot, J.; Garcia, H., Eds.; Garland Science, 2012.
(11) Sapir, L.; Harries, D. Macromolecular compaction by mixed solutions: Bridging versus depletion attraction. *Curr. Opin. Colloid Interface Sci.* **2016**, *22*, 80−87.
(12) Oosawa, F. The history of the birth of the Asakura−Oosawa theory. *J. Chem. Phys.* **2021**, *155*, No. 084104.
(13) Sukenik, S.; Sapir, L.; Harries, D. Balance of enthalpy and entropy in depletion forces. *Curr. Opin. Colloid Interface Sci.* **2013**, *18*, 495−501.
(14) Fernandez, C.; Minton, A. Static light scattering from concentrated protein solutions II: experimental test of theory for protein mixtures and weakly self-associating proteins. *Biophys. J.* **2009**, *96*, 1992−38.
(15) Ogston, A. G. On the Interaction of Solute Molecules with Porous Networks. *J. Phys. Chem.* **1970**, *74*, 668−669.
(16) Lee, L. L. Y.; Lee, J. C. Thermal stability of proteins in the presence of poly(ethylene glycols). *Biochemistry* **1987**, *26*, 7813−7819.
(17) (a) Makhatadze, G. I.; Privalov, P. L. Protein interactions with urea and guanidinium chloride. A calorimetric study. *J. Mol. Biol.* **1992**, *226*, 491−505. (b) Sarkar, M.; Li, C.; Pielak, G. J. Soft interactions and crowding. *Biophys. Rev.* **2013**, *5*, 187−194. (c) Sapir, L.; Harries, D. Origin of Enthalpic Depletion Forces. *J. Phys. Chem. Lett.* **2014**, *5*, 1061−1065. (d) Sukenik, S.; Sapir, L.; Gilman-Politi, R.; Harries, D. Diversity in the mechanisms of cosolute action on biomolecular processes. *Farad. Disc.* **2013**, *160*, 225−237. (e) Senske, M.; Törk, L.; Born, B.; Havenith, M.; Herrmann, C.; Ebbinghaus, S. Protein stabilization by macromolecular crowding through enthalpy rather than entropy. *J. Am. Chem. Soc.* **2014**, *136*, 9036−9041.
(18) (a) Nayar, D. Small crowder interactions can drive hydrophobic polymer collapse as well as unfolding. *Phys. Chem. Chem. Phys.* **2020**, *22*, 18091−18101. (b) Nayar, D. Molecular Crowders Can Induce Collapse in Hydrophilic Polymers via Soft Attractive Interactions. *J. Phys. Chem. B* **2023**, *127*, 6265−6276.
(19) (a) Mukherjee, S. K.; Gautam, S.; Biswas, S.; Kundu, J.; Chowdhury, P. K. Do Macromolecular Crowding Agents Exert Only an Excluded Volume Effect? A Protein Solvation Study. *J. Phys. Chem. B* **2015**, *119*, 14145−14156. (b) Benton, L. A.; Smith, A. E.; Young, G. B.; Pielak, G. J. Unexpected Effects of Macromolecular Crowding on Protein Stability. *Biochemistry* **2012**, *51*, 9773−9775. (c) Kuznetsova, I. M.; Zaslavsky, B. Y.; Breydo, L.; Turoverov, K. K.; Uversky, V. N. Beyond the excluded volume effects: Mechanistic complexity of the crowded milieu. *Molecules* **2015**, *20*, 1377−1409.
(20) Feig, M.; Sugita, Y. Variable interactions between protein crowders and biomolecular solutes are important in understanding cellular crowding. *J. Phys. Chem. B* **2012**, *116*, 599−605.
(21) Katava, M.; Stirnemann, G.; Pachetti, M.; Capaccioli, S.; Paciaroni, A.; Sterpone, F. Specific Interactions and Environment Flexibility Tune Protein Stability under Extreme Crowding. *J. Phys. Chem. B* **2021**, *125*, 6103−6111.
(22) Timr, S.; Gnutt, D.; Ebbinghaus, S.; Sterpone, F. The Unfolding Journey of Superoxide Dismutase 1 Barrels under Crowding: Atomistic Simulations Shed Light on Intermediate States and Their Interactions with Crowders. *J. Phys. Chem. Lett.* **2020**, *11*, 4206−4212.
(23) Homouz, D.; Perham, M.; Samiotakis, A.; Cheung, M. S.; Wittung-Stafshede, P. Crowded, cell-like environment induces shape changes in aspherical protein. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11754−11759.
(24) Zegarra, F. C.; Homouz, D.; Gasic, A. G.; Babel, L.; Kovermann, M.; Wittung-Stafshede, P.; Cheung, M. S. Crowding-Induced Elongated Conformation of Urea-Unfolded Apoazurin: Investigating the Role of Crowder Shape in Silico. *J. Phys. Chem. B* **2019**, *123*, 3607−3617.
(25) Gomez, D.; Huber, K.; Klumpp, S. On Protein Folding in Crowded Conditions. *J. Phys. Chem. Lett.* **2019**, *10*, 7650−7656.
(26) Latshaw, D. C.; Hall, C. K. Effects of hydrophobic macromolecular crowders on amyloid β (16−22) aggregation. *Biophys. J.* **2015**, *109*, 124−134.
(27) O'Brien, E. P.; Straub, J. E.; Brooks, B. R.; Thirumalai, D. Influence of nanoparticle size and shape on oligomer formation of an amyloidogenic peptide. *J. Phys. Chem. Lett.* **2011**, *2*, 1171−1177.
(28) (a) Shendruk, T. N.; Bertrand, M.; De Haan, H. W.; Harden, J. L.; Slater, G. W. Simulating the entropic collapse of coarse-grained chromosomes. *Biophys. J.* **2015**, *108*, 810−820. (b) Kang, H.; Pincus, P. A.; Hyeon, C.; Thirumalai, D. Effects of macromolecular crowding on the collapse of biopolymers. *Phys. Rev. Lett.* **2015**, *114*, 068303. (c) Chaboche, Q.; Campos-Villalobos, G.; Giunta, G.; Dijkstra, M.; Cosentino Lagomarsino, M.; Scolari, V. F. A mean−field theory for predicting single polymer collapse induced by neutral crowders. *Soft Matter* **2024**, *20*, 3271−3282.
(29) Ostrowska, N.; Feig, M.; Trylska, J. Crowding affects structural dynamics and contributes to membrane association of the NS3/4A complex. *Biophys. J.* **2021**, *120*, 3795−3806.
(30) Harada, R.; Sugita, Y.; Feig, M. Protein crowding affects hydration structure and dynamics. *J. Am. Chem. Soc.* **2012**, *134*, 4842−4849.
(31) McGuffee, S. R.; Elcock, A. H. Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm. *PLoS Comput. Biol.* **2010**, *6*, No. e1000694.
(32) (a) Qin, S.; Zhou, H.-X. FFT-Based Method for Modeling Protein Folding and Binding under Crowding: Benchmarking on Ellipsoidal and All-Atom Crowders. *J. Chem. Theory Comput.* **2013**, *9*, 4633−4643. (b) Jung, J.; Mori, T.; Kobayashi, C.; Matsunaga, Y.;

https://doi.org/10.1021/acsomega.4c03344
ACS Omega 2024, 9, 29953−29965

Yoda, T.; Feig, M.; Sugita, Y. GENESIS: a hybrid−parallel and multi−scale molecular dynamics simulator with enhanced sampling algorithms for biomolecular and cellular simulations. *Wiley Interdiscip. Rev. Comput. Mol. Sci.* **2015**, *5*, 310−323. (c) Jung, J.; Tan, C.; Sugita, Y. GENESIS CGDYN: large−scale coarse−grained MD simulation with dynamic load balancing for heterogeneous biomolecular systems Amplifying the Macromolecular Crowding Effect Using Nanoparticles. *Nat. Commun.* **2024**, *15*, 3370.

(33) Rickard, M. M.; Zhang, Y.; Pogorelov, T. V.; Gruebele, M. Crowding, Sticking, and Partial Folding of GTT WW Domain in a Small Cytoplasm Model. *J. Phys. Chem. B* **2020**, *124*, 4732−4740.

(34) (a) Yoo, J.; Aksimentiev, A. Improved Parameterization of Amine-Carboxylate and Amine-Phosphate Interactions for Molecular Dynamics Simulations Using the CHARMM and AMBER Force Fields. *J. Chem. Theory Comput.* **2016**, *12*, 430−443. (b) Panigrahy, S.; Sahu, R.; Reddy, S. K.; Nayar, D. Structure, energetics and dynamics in crowded amino acid solutions: a molecular dynamics study. *Phys. Chem. Chem. Phys.* **2023**, *25*, 5430−5442. (c) Grassmann, G.; Miotto, M.; Desantis, F.; Di Rienzo, L.; Tartaglia, G. G.; Pastore, A.; Ruocco, G.; Monti, M.; Milanetti, E. Computational Approaches to Predict Protein−Protein Interactions in Crowded Cellular Environments. *Chem. Rev.* **2024**, *124*, 3932−3977.

(35) (a) Poling-Skutvik, R.; Lee, J.; Narayanan, S.; Krishnamoorti, R.; Conrad, J. C. Tunable Assembly of Gold Nanorods in Polymer Solutions To Generate Controlled Nanostructured Materials. *ACS Appl. Nano Mater.* **2018**, *1*, 877−885. (b) Shin, J.; Zhang, X.; Liu, J. DNA−functionalized gold nanoparticles in macromolecularly crowded polymer solutions. *J. Phys. Chem. B* **2012**, *116*, 13396−13402. (c) Zaki, A.; Dave, N.; Liu, J. Amplifying the macromolecular crowding effect using nanoparticles. *J. Am. Chem. Soc.* **2012**, *134*, 35−38. (d) Panigrahy, S.; Nayar, D. Differential local ordering of mixed crowders determines effective size and stability of ss−DNA capped gold nanoparticle. *J. Chem. Phys.* **2024**, *160*, 014901.

(36) (a) Mukherji, D.; Marques, C. M.; Stuehn, T.; Kremer, K. Depleted depletion drives polymer swelling in poor solvent mixtures. *Nat. Commun.* **2017**, *8*, 1374. (b) van der Vegt, N. F. A.; Nayar, D. The Hydrophobic Effect and the Role of Cosolvents. *J. Phys. Chem. B* **2017**, *121*, 9986−9998.

(37) (a) Hämisch, B.; Pollak, R.; Ebbinghaus, S.; Huber, K. Self-Assembly of Pseudo-Isocyanine Chloride as a Sensor for Macromolecular Crowding In Vitro and In Vivo. *Chem.-Eur. J.* **2020**, *26*, 7041−7050. (b) Hämisch, B.; Pollak, R.; Ebbinghaus, S.; Huber, K. Thermodynamic Analysis of the Self−Assembly of Pseudo Isocyanine Chloride in the Presence of Crowding Agents. *ChemSystemsChem* **2021**, *3*, No. e2000051. (c) Rajput, S.; Pollak, R.; Huber, K.; Ebbinghaus, S.; Nayar, D. Ethylene glycol energetically disfavours oligomerization of pseudoisocyanine dyestuffs at crowded concentrations. *Soft Matter* **2023**, *19*, 6399−6413.

(38) Jeon, C.; Jung, Y.; Ha, B. Y. Effects of molecular crowding and confinement on the spatial organization of a biopolymer. *Soft Matter* **2016**, *12*, 9436−9450.

(39) (a) Leontyev, I.; Stuchebrukhov, A. Accounting for electronic polarization in non-polarizable force fields. *Phys. Chem. Chem. Phys.* **2011**, *13*, 2613−2626. (b) Nencini, R.; Tempra, C.; Biriukov, D.; Polák, J.; Ondo, D.; Heyda, J.; Ollila, S. O.; Javanainen, M.; Martinez-Seara, H. Prosecco: polarization reintroduced by optimal scaling of electronic continuum correction origin in MD simulations. *Biophys. J.* **2022**, *121*, 157a. (c) Best, R. B.; Zheng, W.; Mittal, J. Balanced Protein−Water Interactions Improve Properties of Disordered Proteins and Non−Specific Protein Association. *J. Chem. Theory and Comput.* **2014**, *10*, 5113−5124. (d) Reiser, M.; et al. Resolving molecular diffusion and aggregation of antibody proteins with megahertz X−ray free−electron laser pulses. *Nat. Commun.* **2022**, *13*, 5528. (e) Roosen-Runge, F.; Hennig, M.; Zhang, F.; Jacobs, R. M. J.; Sztucki, M.; Schober, H.; Seydel, T.; Schreiber, F. Protein self−diffusion in crowded solutions. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 11815−11820.

https://doi.org/10.1021/acsomega.4c03344
ACS Omega 2024, 9, 29953−29965