Protein Assembly and Crowding Simulations

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Running Title

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Abstract

Proteins encounter frequent molecular interactions in biological environments. Computer simulations have become an increasingly important tool in providing mechanistic insights into how such interactions *in vivo* relate to their biological function. The review here focuses on simulations describing protein assembly and molecular crowding effects as two important aspects that are distinguished mainly by how specific and long-lived protein contacts are. On the topic of crowding, recent simulations have provided new insights into how crowding affects protein folding and stability, modulates enzyme activity, and affects diffusive properties. Recent studies of assembly processes focus on assembly pathways, especially for virus capsids, amyloid aggregation pathways, and the role of multivalent interactions leading to phase separation. Also discussed are technical challenges in achieving increasingly realistic simulations of interactions in cellular environments.

Highlights

- Crowding effects on protein structure, dynamics, and function are investigated via simulations.
- Protein assembly pathways are predicted from multi-scale simulations
- There are still many technical challenges in simulations of protein interactions in cellular environments

Keywords

crowding, protein folding, enzyme activity, protein clustering, diffusion, protein complex, virus capsid, amyloid, phase separation, molecular dynamics simulation, coarse-graining, force field, high-performance computing, enhanced sampling

Introduction

The dense interior of cells gives rise to extensive interactions between biological macromolecules. Some of those interactions lead to the assembly of long-lived complexes and aggregates, whereas crowding effects mainly result from non-specific interactions as well as the volume exclusion effects. Assembly mechanisms tend to be discussed in the specific context of different systems, such as protein complexes [1], amyloids [2], virus capsids [3], or bio-inspired nanomaterials [4]. Another consequence of dense environments is macromolecular crowding that may have more universal effects on protein stability, dynamics, and functions [5]. However, assembly and crowding can be viewed as different aspects of the same kind of biomolecular interactions under highly concentrated conditions. The main distinctions are whether interactions between molecules are mainly attractive or repulsive and, if attractive, how specific, *i.e.,* long-lived, a certain interaction is. While specific interactions are perhaps easier to understand and well-characterized for many systems, recent experimental and computational research has focused on weak, non-specific [6-10] in relation to repulsive interactions that give rise to volume exclusion effects [5]. Experimental characterization of such interactions, e.g., via nuclear magnetic resonance (NMR) spectroscopy [6,11] is possible but difficult, especially under *in vivo* conditions, but much atomistically detailed insight has come from computer simulations at increasing levels of detail as computers have become more powerful and computational methods have improved. Atomistic molecular dynamics (MD) simulations are now routinely performed on the systems containing multiple proteins and other biomolecules in explicit solvent water [8-10,12-17] enabled by technical advances with current MD software optimized for graphical processing units (GPUs) [18] and large-scale supercomputers [19,20], but also special computing platforms such as Anton 2 [21]. This has enabled further progress in understanding assembly and crowding processes at the molecular level. This review focuses on the most recent advances from computer simulation studies.

Crowding and non-specific interactions

We begin by discussing crowding effects that result from frequent encounters of molecules in dense molecular environments. The focus here is on non-specific interactions and their effect on protein structure, dynamics and function (see example of altered ligand binding due to crowding in Figure 1).

Crowding effects on protein folding and stability

Protein stability is essential for function, and crowding effects on structure have been studied extensively in the past. A central topic is the balance between stabilizing excluded volume effects expected for any crowder and potentially destabilizing non-specific interactions that vary between protein crowders. As in the past, much focus has been on the protein folding transition in the presence of crowders, such as a detailed analysis of crowder interactions with unfolded intermediates of SOD1 in the presence of BSA [22], followed up by a mutational analysis that correlated altered crowder interactions in simulations with *in vivo* experiments [23]. Another example is an all-atom folding simulation study of a WW domain in a small cytoplasmic model where the protein remained trapped in unfolded states due to extensive interactions with crowder proteins rather than folding rapidly without crowders [24]. Beyond single-domain folders, crowding effects were investigated via coarse-grained (CG) simulations in a multi-domain protein, where the presence of crowders reduced cooperativity of domain folding, thereby significantly altering folding pathways and kinetics [25]. Recent simulation studies have also looked at

more detailed aspects such as how crowding effects on protein stability may vary by temperature from REST2 (Replica Exchange with Solute Tempering 2) simulations [26] after the extensvie sampling via lattice-Boltzmann MD [27], the balance between energy and entropy for interacting dipeptide crowders in atomistic simulations [28], the effect of crowder plasticity to reduce volume exclusion effects [29], and the ability of rod-like crowders to stabilize elongated unfolded states studied by CG MD simulations [30]. Finally, towards more functional relevance, polyethyleneglycol crowders were found to induce partially helical conformations in a disordered protein segment of a protein complex as a proposed first step of membrane insertion [17].

Crowding and enzyme activity

Crowding has also been found to directly impact enzyme activities at the heart of how cellular environments may modulate biological function. One important aspect is that crowding may shift conformational equilibria between active and inactive states and thereby affects catalytic rates as documented in detail for a number of enzymes via CG simulations [31,32] and atomistic MD simulations [15], where reactions were accelerated or inhibited in the presence of crowders. Another aspect is the impact of crowding on the ability of metabolites to reach enzyme active sites. A recent atomistic simulation study showed not just competing non-specific binding of a kinase inhibitor to crowder proteins that reduced effective inhibitor concentrations, but also an altered binding pathway in the presence of crowding, illustrating that crowding may have a variety of effects on enzyme function *in vivo* [16]. This point was examined further via 2D lattice Monte Carlo simulation to suggest that substrate channeling resulting from co-localization of enzymes may be necessary to overcome crowding effects on enzymes [33].

Protein clustering and diffusion

One of the well-known effects of crowding on proteins is reduced diffusion. A long-established explanation is that crowders simply present obstacles to free diffusion as volume is excluded. While this topic has been well explored in the past, a new aspect is the effect of different crowder shapes that was studied via simulation [34]. However, more recent studies have suggested that protein cluster formation is perhaps the more important determinant of retarded protein diffusion upon crowding. This topic has been explored further in recent large-scale atomistic simulations of concentrated protein solutions [13,35] and a small model of an *E. coli* cytoplasm [14]. These studies provide further evidence of transient protein clusters due to non-specific contacts generally lasting less than 1 μs and could explain a greater slow-down for rotational vs. translational diffusion that cannot be explained by hard-sphere volume-exclusion crowder models.

Molecular crowding effects in different environments

Many studies have focused on crowding effects on proteins in the presence of other cellular components in biological environments such as membranes and chromatin. Diffusion in the periplasm of Gram-negative bacteria. In an ambitious study, the diffusion of the antibiotic PMB1 in the periplasm of Gram-negative bacteria was examined [36]. This work showcases the degree of realism that can be achieved today with atomistic simulations with the main observation of a variety of contacts with periplasmic components resulting in slow diffusion [36]. From another set of atomistic simulations, crowding effects of soluble proteins near membranes were found to be enhanced due to preferential exclusions of proteins from the membrane surface while at the same time allowing faster diffusion at the membrane interface

due to protein depletion [37]. The interplay of crowding and membranes has also been studied in a more specific biological context for the Piezo1 channel. Here atomistic simulations were used to analyze how crowding-induced membrane flattening led to pore opening and subsequent selective cation transport [38]. Other studies have analyzed the diffusion of proteins inside membranes, both in terms of general determinants such as protein size effects on rotational diffusion via MARTINI CG simulations [39] and by exploring translational diffusion in the presence of specific interactions with scaffolding molecules in the context of synapse function via Monte Carlo simulations [40]. Finally, in a different direction, protein dynamics in the presence of chromatin was studied via CG MD simulations [41] to find that, even although proteins diffused slowly at high nucleosome densities, the DNA target sequence search accelerated based on a faster protein-DNA contact rate.

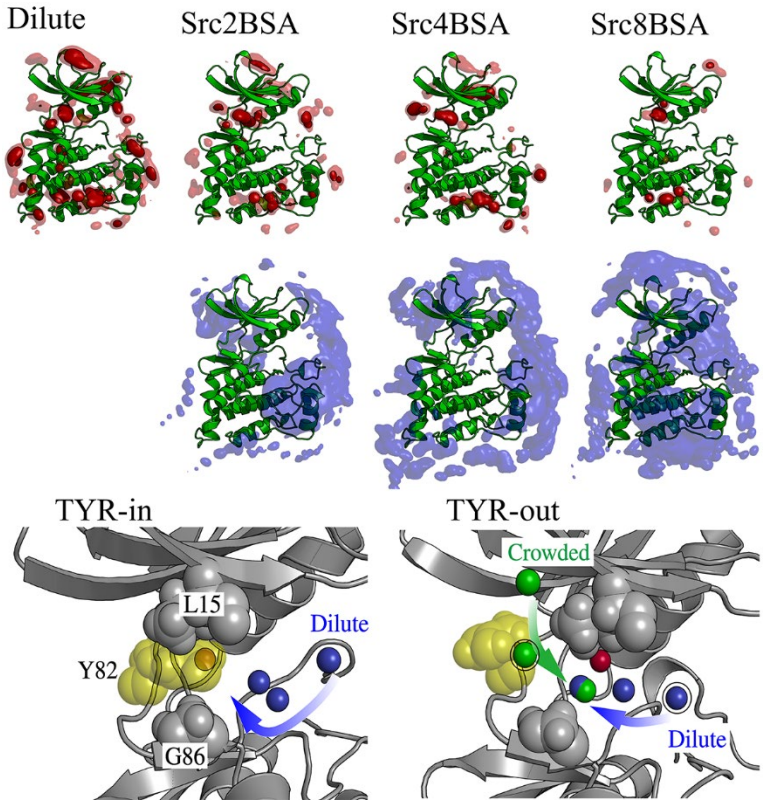

Figure 1. Crowding effects on ligand binding. Distribution of small molecule inhibitor PP1 (red densities) and bovine serum albumin crowder (blue densities) around c-Src kinase (green) at dilute conditions and at different crowder concentrations (adapted from Fig. 1 in Kasahara et al. [16]; alternate PP1 ligand binding pathways to c-Src kinase in TYR-in and TYR-out conformations favored by crowding (adapted from Fig. 5 in Kasahara et al. [16]).

Protein assembly into complexes and aggregates

Specific interactions between proteins lead to the formation of long-lived complexes and irreversible aggregates that are important for understanding function and disease pathology. Multivalent specific interactions are also relevant for liquid-liquid phase separation. The main role of simulation studies is to identify the mechanisms of association and determinants of specificity (see amyloid assembly pathway example in Figure 2). Recent studies of relevance are discussed in the following.

Assembly of protein complexes

The main focus of recent simulation studies on protein complexes is the mechanism of self-assembly for systems ranging from oligomeric complexes to heterogeneous assemblies and protein filaments. Two atomistic simulation studies have examined the coupling between oligomerization and functional activation. In one of them, the lipid-induced dimerization of Bruton's tyrosine kinase at the cell membrane is described as a key activation step [42]. In another study, the coupling between folding, tetramerization, and membrane insertion of a potassium channel in the presence of other proteins in the membrane is studied to understand the mechanism of its activation [43]. Two other studies focus on the kinetic mechanisms of complex assembly at larger scales via multi-scale and CG simulations. In the first example, the assembly of FtsZ filaments was examined to suggest nucleation mechanisms based on switching between alternative dimer species [44]. A second example describes assembly pathways for inflammasome assembly [45].

Virus capsid assembly

Orchestrated self-assembly is especially important for virus capsid formation and many simulation studies have examined this topic with a view on identifying new avenues for developing antiviral therapies. Several studies have focused on the energetics of various binding modes of capsomers, the basic assembly units. The studies analyzed details of heterotetrameric capsomer formation for dengue virus [46] and the trimer of dimer capsomers in giant viruses [47]. A common theme was an emphasis on electrostatic interactions, often captured via continuum electrostatics frameworks and the identification of key residues relevant for driving oligomerization. Another study focused on Hepatitis B virus capsomer formation as a function of conformational changes induced by mutation to explain altered assembly kinetics [48]. Other studies targeted assembly of entire virus capsids relying on CG models to access the larger scales. In one example, Hepatitis B capsid assembly pathways were studied to understand how dimer association energies determine trapping in intermediate states [49]. A different study looked at how HIV-1 capsid assembly depends on the dynamic distribution of intermediate units [50].

Amyloid aggregation

Protein aggregation into amyloids is primarily important in the context of disease. This continues to be a topic of significant research interest and here we only mention simulation studies that focus on specific aspects of amyloid aggregation in the context of protein self-assembly. Studies reviewed here used atomistic simulations and most revolved around the topic of oligomer formation for amyloid forming peptides under different conditions and as a function of sequence variations. Aβ dimerization was compared between water and membrane environments in one study [51] and as a function of an external electric field in another work [52]. Aβ aggregation was further discussed in the context of kinetic traps

formed by misregistered β sheets [53]. Aggregation as a function of sequence was investigated in human calcitonin [54] while another study ascribed peptide flexibility as a destabilizing factor for assembly [55].

Multivalent interactions and phase separation

Liquid-liquid phase separation (LLPS) is a topic of broad biological interest. We only focus here on simulation studies that analyze molecular interactions that may drive phase separation. A common theme is a focus on interactions between intrinsically disordered proteins (IDPs) as a function of amino acid patterning, mostly via CG or multi-scale simulations to address the larger scales and highly dynamic nature of IDPs. Two studies examine LLPS propensity as a function of sequence more generally [56] and attempt to delineate between propensities for aggregation and phase separation [57]. Other studies more specifically examine the sticker-spacer model for multivalency underlying phase separation propensity in the context of assembly kinetics [58], as a function of ligand binding [59], and for constructs with heterogeneous sequence patterns arranged in blocks that may give rise to more complex condensate morphologies [60].

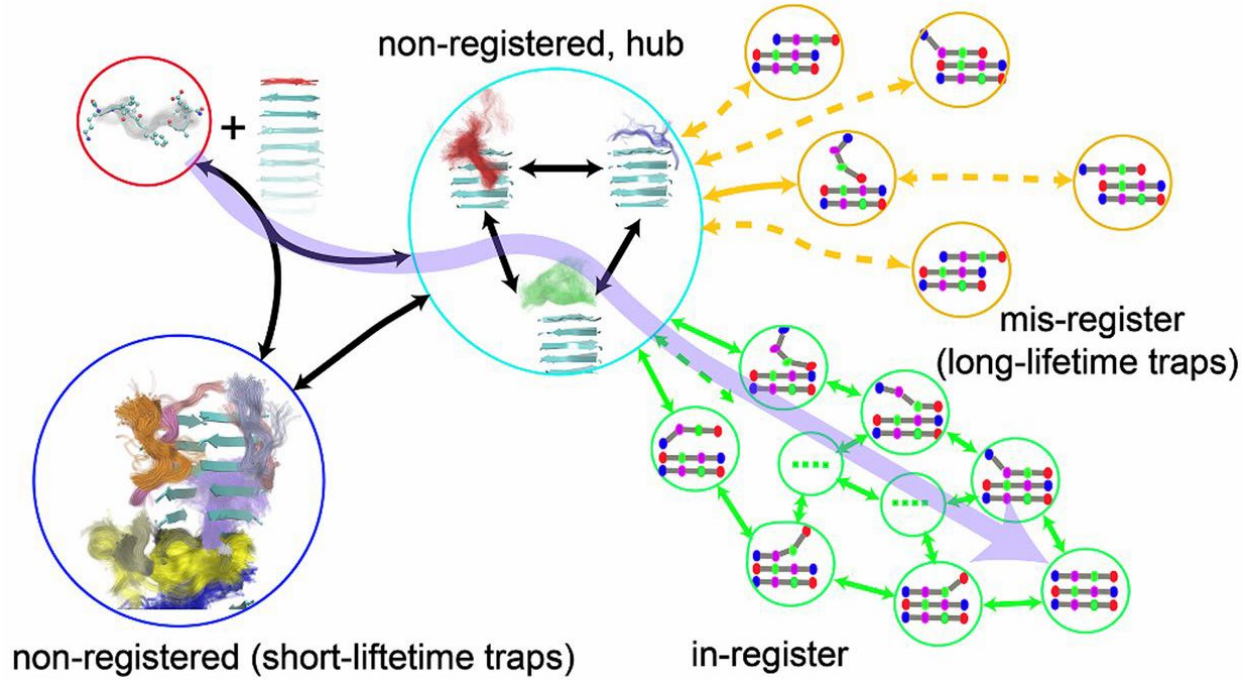

Figure 2. Amyloid assembly pathways. Schematic diagram of $A\beta_{16\text{-}22}$ fibril elongation mechanism with kinetic traps due to β-sheet misregistration (adapted from Fig. 6 in *Proc. Natl. Acad. Sci. U.S.A.* 2020, **117**:10322; Copyright (2020) National Academy of Sciences).

Technical challenges

Despite much progress, technical challenges remain in capturing protein interactions in diverse biological environments via simulations (see example of a complex periplasmic model in Figure 3). Briefly, the main issues are energy functions that need to accurately balance intra- and intermolecular interactions between different molecules and time-scale limitations in being able to cover μs-ms time scales for system sizes of 10 nm – 1 μm in order to adequately describe assembly and crowding phenomena.

Interaction parameters

Classical force fields for biomolecules are widely available and well-optimized for simulations of single macromolecules in solution or in a membrane [61,62], but their transferability to multi-component systems with heterogeneous compositions has been tested only recently. One issue is that many force fields appear to be imbalanced between protein-protein and protein-water interactions as they favor the stability of natively folded proteins, at the expense of overcompaction in intrinsically disordered regions (IDRs) [63] and aggregation when multiple proteins are present [10,64]. Specific insight into force field deficiencies for amyloid aggregation is available from two recent studies [65,66]. Corrections to the Lennard-Jones pair-wise potential [10,63,67] as well as recent force field reparameterizations [68] may address the issues [10,14]. Interactions between different classes of molecules, such as between proteins and nucleic acids, are another issue as correction factors have been proposed [69]. Further work is likely needed on this front, and it remains to be seen whether different approaches such as Kirkwood-Buff based force fields [70,71] and polarizable force fields [72] are ultimately needed for finding interaction potentials that are optimal for heterogeneous biological environments. However, a larger question may be what level of physical detail is needed in the end to describe phenomena on cellular scales and whether effective multi-scale approaches where models at coarser levels are calibrated against accurate atomistic potentials or against empirical data, e.g., from experiment, are a more effective approach.

Time scales

Relevant time scales for biomolecular association and crowding processes are at least on the order of microseconds and much longer if folding or condensation processes are considered. Recent studies based on atomistic MD simulations typically reach 1-10 μs MD simulations [10,13,14,16]. Some of them used the MD-special supercomputer Anton2, while multi-μs simulations are also attainable with the latest GPU platforms. These simulations provide good statistics on diffusive internal and intermolecular motions. However, processes with significant kinetic barriers, such as internal conformational rearrangements, specific ligand binding, and protein complex assembly, remain difficult to study. Enhanced sampling methods such as REST2 methods [26] that target part of a given system can address such challenges with reasonable computational resources as shown in recent studies of crowded systems focused on internal conformations [22,27]. REST2 likely remains the most effective strategy for extending the time scales that can be reached via simulation, while the enhancement of conformational sampling is limited to the target.

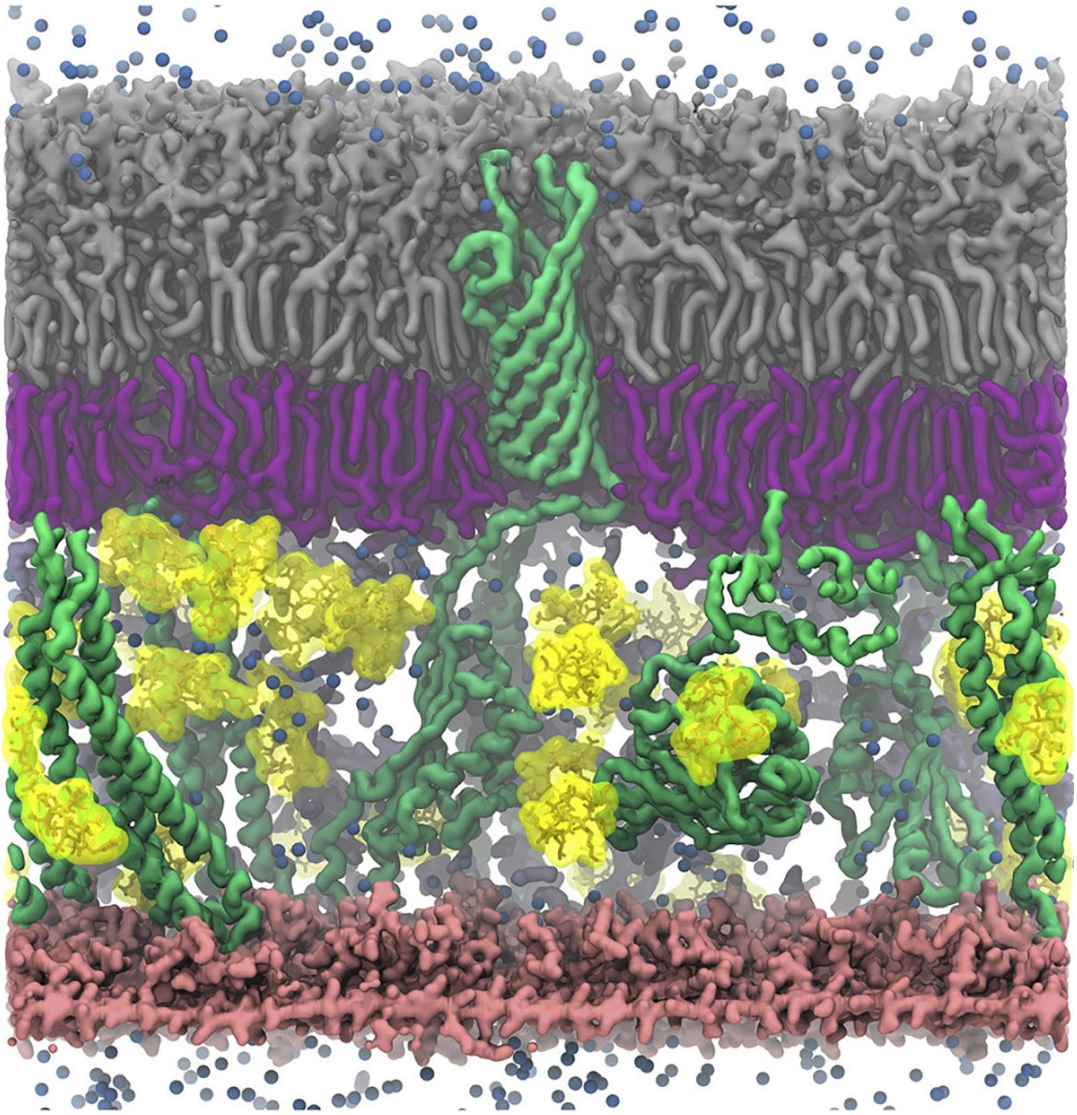

Figure 3. Molecular model of a bacterial periplasm. Snapshot of a crowded double-membrane system with proteins (green) and antibiotic polymyxin (yellow) (Reprinted from *Structure* 2021, **29**:444-456.e442 with permission from Elsevier).

System size

Systems of interacting proteins need to be large enough to reach experimental concentrations, provide sufficient statistics, and reduce finite-size effects. Some model crowded systems may contain only a few proteins when high concentrations are simulated, but in many cases, hundreds or thousands of interacting proteins are simulated [12,13] resulting in very large systems that are challenging for typical computational platforms. Continued developments in scaling MD software such as NAMD and GENESIS to take advantage of massively parallel supercomputers are one avenue for simulating very large cellular systems up to a billion atoms [19]. Another strategy is to reduce model resolutions via CG so that larger systems can be simulated with fewer particles [73,74]. There are many examples of recent CG simulations that focus on assembly and crowding processes [25,30,44,50,57,59,75]. CG models may be

even more limited by the quality of interaction parameters. On the other hand, the lower resolution may be inherently more appropriate to describe interactions at larger scales and to match with relevant experimental data of biomolecular behavior under *in vivo* conditions. However, as a practical issue, the advantage of coarse-graining may be lost because larger-scale CG simulations are often not optimized to the same extent as atomistic simulations for high-performance computing environments. The reduced optimization in larger-scale CG simulations mainly originated from difficulties in balancing computational load between different CPUs. Clearly these are technical challenges that remain to be addressed to increase the impact of CG simulations of cellular environments in the future.

Outlook

What are the next big challenges of atomistic and CG MD simulations of the biological systems in cellular environments? One of the most interesting challenges would be to investigate the relationship between LLPS in the cells and macromolecular crowding effects under *in vivo* and *in vitro* conditions. One significant issue is that the simulation of interactions between disordered proteins or highly dynamic globular proteins and enzymes remains a formidable sampling challenge. At the same time, dynamics is likely a critical component in understanding biological function in the context of crowding and phase separation but also protein assembly. Another issue is that even although the complexity of simulations has greatly increased in recent years, many key aspects of cellular environments are still lacking. For example, LLPS is not just observed in the cytoplasm but also in cellular nuclei, suggesting that interactions with nucleic acids may need to be considered more extensively. The eventual goal may be integrated simulations that fully reflect the entire cellular environments driven by available experimental data. To that end, new tools for building structural models of entire cells that are emerging now [76], will become critical.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- ● of special interest
- ●● of outstanding interest

1. Jones S, Thornton JM: **Principles of protein-protein interactions**. *Proc. Natl. Acad. Sci. U.S.A.* 1996, **93**:13-20.
2. Harrison R, Sharpe P, Singh Y, Fairlie D: **Amyloid peptides and proteins in review**. *Rev. Physiol. Biochem. Pharmacol.* 2007:1-77.
3. Zlotnick A: **Theoretical aspects of virus capsid assembly**. *J. Mol. Recognit.* 2005, **18**:479-490.
4. Luo Q, Hou C, Bai Y, Wang R, Liu J: **Protein assembly: versatile approaches to construct highly ordered nanostructures**. *Chem. Rev.* 2016, **116**:13571-13632.
5. Zimmerman SB, Minton AP: **Macromolecular crowding: biochemical, biophysical, and physiological consequences**. *Annu. Rev. Biophys. Biomol. Struct.* 1993, **22**:27-65.
6. Miklos AC, Sarkar M, Wang Y, Pielak GJ: **Protein crowding tunes protein stability**. *J. Am. Chem. Soc.* 2011, **133**:7116-7120.
7. Inomata K, Ohno A, Tochio H, Isogai S, Tenno T, Nakase I, Takeuchi T, Futaki S, Ito Y, Hiroaki H: **High-resolution multi-dimensional NMR spectroscopy of proteins in human cells**. *Nature* 2009, **458**:106-109.
8. 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.
9. Harada R, Tochio N, Kigawa T, Sugita Y, Feig M: **Reduced native state stability in crowded cellular environment due to protein–protein interactions**. *J. Am. Chem. Soc.* 2013, **135**:3696-3701.
10. Nawrocki G, Wang P-h, Yu I, Sugita Y, Feig M: **Slow-down in diffusion in crowded protein solutions correlates with transient cluster formation**. *J. Phys. Chem. B* 2017, **121**:11072-11084.
11. Ikeya T, Güntert P, Ito Y: **Protein structure determination in living cells**. *Int. J. Mol. Sci.* 2019, **20**:2442.
12. Yu I, Mori T, Ando T, Harada R, Jung J, Sugita Y, Feig M: **Biomolecular interactions modulate macromolecular structure and dynamics in atomistic model of a bacterial cytoplasm**. *eLife* 2016, **5**:e19274.
13. von Bülow S, Siggel M, Linke M, Hummer G: **Dynamic cluster formation determines viscosity and diffusion in dense protein solutions**. *Proc. Natl. Acad. Sci. U.S.A.* 2019, **116**:9843.

● Extensive atomistic MD simulation studies of transient protein cluster formations explain a slow-down of diffusion in crowded protein solutions.

14. Rickard MM, Zhang Y, Gruebele M, Pogorelov TV: **In-Cell Protein–Protein Contacts: Transient Interactions in the Crowd**. *J. Phys. Chem. Lett.* 2019, **10**:5667-5673.
15. Popielec A, Ostrowska N, Wojciechowska M, Feig M, Trylska J: **Crowded environment affects the activity and inhibition of the NS3/4A protease**. *Biochimie* 2020, **176**:169-180.
16. Kasahara K, Re S, Nawrocki G, Oshima H, Mishima-Tsumagari C, Miyata-Yabuki Y, Kukimoto-Niino M, Yu I, Shirouzu M, Feig M, et al.: **Reduced efficacy of a Src kinase inhibitor in crowded protein solution**. *Nat. Commun.* 2021, **12**:4099.

●● Atomistic MD simulations as well as experimental inhibition assays showed molecular mechanisms underlying the reduced efficacy of an inhibitor molecule on protein kinase.

17. 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.

● Extensive simulations together with experiments suggest that crowding may induce helical conformations in an intrinsically disordered region to facilitate membrane anchoring of a viral protease.

18. Stone JE, Hardy DJ, Ufimtsev IS, Schulten K: **GPU-accelerated molecular modeling coming of age**. *J. Mol. Graph. Modell.* 2010, **29**:116-125.
19. Jung J, Kobayashi C, Kasahara K, Tan C, Kuroda A, Minami K, Ishiduki S, Nishiki T, Inoue H, Ishikawa Y, et al.: **New parallel computing algorithm of molecular dynamics for extremely huge scale biological systems**. *J. Comput. Chem.* 2021, **42**:231-241.
20. Kalé L, Skeel R, Bhandarkar M, Brunner R, Gursoy A, Krawetz N, Phillips J, Shinozaki A, Varadarajan K, Schulten K: **NAMD2: greater scalability for parallel molecular dynamics**. *J. Comput. Phys.* 1999, **151**:283-312.
21. Shaw DE, Grossman J, Bank JA, Batson B, Butts JA, Chao JC, Deneroff MM, Dror RO, Even A, Fenton CH: **Anton 2: raising the bar for performance and programmability in a special-purpose molecular dynamics supercomputer**. In *SC'14: Proceedings of the International Conference for High Performance Computing, Networking, Storage and Analysis*: IEEE: 2014:41-53.
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. Gnutt D, Timr S, Ahlers J, König B, Manderfeld E, Heyden M, Sterpone F, Ebbinghaus S: **Stability Effect of Quinary Interactions Reversed by Single Point Mutations**. *J. Am. Chem. Soc.* 2019, **141**:4660-4669.
24. Rickard MM, Zhang Y, Pogorelov TV, Gruebele M: **Crowding, Sticking, and Partial Folding of GTT WW Domain in a Small Cytoplasm Model**. *J. Phys. Chem. B* 2020, **124**:4732-4740.

● Protein folding of a WW domain protein in a small cytoplasmic model highlights kinetic trapping due to non-specific interactions with crowder molecules. This study also discusses the influence of different force fields on crowding simulations.

25. Chu X, Suo Z, Wang J: **Confinement and Crowding Effects on Folding of a Multidomain Y-Family DNA Polymerase**. *J. Chem. Theory Comput.* 2020, **16**:1319-1332.

● Crowding effects on protein folding are analyzed for a multidomain protein finding that cooperativity of domain folding is reduced upon crowding.

26. Wang L, Friesner RA, Berne B: **Replica exchange with solute scaling: a more efficient version of replica exchange with solute tempering (REST2)**. *J. Phys. Chem. B* 2011, **115**:9431-9438.
27. Timr S, Sterpone F: **Stabilizing or Destabilizing: Simulations of Chymotrypsin Inhibitor 2 under Crowding Reveal Existence of a Crossover Temperature**. *J. Phys. Chem. Lett.* 2021, **12**:1741-1746.

●● Limitations in atomistic simulations of protein crowding systems were overcome by using lattice-Boltzmann MD combined with enhanced sampling to understand protein stability upon crowding at different temperatures.

28. Sahu R, Nayar D: **Crowding effects on water-mediated hydrophobic interactions**. *J. Chem. Phys.* 2021, **155**:024903.

29. 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.
30. Zegarra FC, Homouz D, Gasic AG, Babel L, Kovermann M, Wittung-Stafshede P, Cheung MS: **Crowding-Induced Elongated Conformation of Urea-Unfolded Apoazurin: Investigating the Role of Crowder Shape in Silico**. *J. Phys. Chem. B* 2019, **123**:3607-3617.
31. Skóra T, Popescu MN, Kondrat S: **Conformation-changing enzymes and macromolecular crowding**. *Phys. Chem. Chem. Phys.* 2021, **23**:9065-9069.
32. Alshareedah I, Moosa MM, Raju M, Potoyan DA, Banerjee PR: **Phase transition of RNA−protein complexes into ordered hollow condensates**. *Proc. Natl. Acad. Sci. U.S.A.* 2020, **117**:15650.
33. Anand R, Sanjeev BS: **Insights into the effect of molecular crowding on the structure, interactions and functions of siRNA–PAZ complex through molecular dynamics studies**. *J. Biomol. Struct. Dyn.* 2019, **37**:2745-2752.
34. Skóra T, Vaghefikia F, Fitter J, Kondrat S: **Macromolecular Crowding: How Shape and Interactions Affect Diffusion**. *J. Phys. Chem. B* 2020, **124**:7537-7543.
35. Bashardanesh Z, Elf J, Zhang H, van der Spoel D: **Rotational and Translational Diffusion of Proteins as a Function of Concentration**. *ACS Omega* 2019, **4**:20654-20664.
36. Pedebos C, Smith IPS, Boags A, Khalid S: **The hitchhiker's guide to the periplasm: Unexpected molecular interactions of polymyxin B1 in *E. coli***. *Structure* 2021, **29**:444-456.e442.

● Realistic model of a bacterial periplasm describes reduced diffusion of an antibiotic drug because of non-specific off-target interactions with a variety of different periplasmic molecules.

37. Nawrocki G, Im W, Sugita Y, Feig M: **Clustering and dynamics of crowded proteins near membranes and their influence on membrane bending**. *Proc. Natl. Acad. Sci. U.S.A.* 2019, **116**:24562.

● The interplay between protein crowding and membrane surfaces is studied from extensive simulations describing enhanced crowding effects, accelerated diffusion near the membrane, and induced membrane curvature due to crowding.

38. Jiang W, Del Rosario JS, Botello-Smith W, Zhao S, Lin Y-c, Zhang H, Lacroix J, Rohacs T, Luo YL: **Crowding-induced opening of the mechanosensitive Piezo1 channel in silico**. *Commun. Biol.* 2021, **4**:84.
39. Javanainen M, Ollila OHS, Martinez-Seara H: **Rotational Diffusion of Membrane Proteins in Crowded Membranes**. *J. Phys. Chem. B* 2020, **124**:2994-3001.
40. Kokolaki ML, Fauquier A, Renner M: **Molecular Crowding and Diffusion-Capture in Synapses**. *iScience* 2020, **23**.
41. Kanada R, Terakawa T, Kenzaki H, Takada S: **Nucleosome Crowding in Chromatin Slows the Diffusion but Can Promote Target Search of Proteins**. *Biophys. J.* 2019, **116**:2285-2295.
42. Wang Q, Pechersky Y, Sagawa S, Pan AC, Shaw DE: **Structural mechanism for Bruton's tyrosine kinase activation at the cell membrane**. *Proc. Natl. Acad. Sci. U.S.A.* 2019, **116**:9390.
43. Song KC, Molina AV, Chen R, Gagnon IA, Koh YH, Roux B, Sosnick TR: **Folding and misfolding of potassium channel monomers during assembly and tetramerization**. *Proc. Natl. Acad. Sci. U.S.A.* 2021, **118**:e2103674118.
44. Lv D, Li J, Ye S: **The Assembly Switch Mechanism of FtsZ Filament Revealed by All-Atom Molecular Dynamics Simulations and Coarse-Grained Models**. *Front. Microbiol.* 2021, **12**.
45. Su Z, Wu Y: **Multiscale simulation unravel the kinetic mechanisms of inflammasome assembly**. *Biochim. Biophys. Acta, Mol. Cell. Res.* 2020, **1867**:118612.

46. Salas GGS, Hernandez AEL, He J, Karki C, Xie Y, Sun S, Xian Y, Li L: **Using computational approaches to study dengue virus capsid assembly**. *Comput. Math. Biophys.* 2019, **7**:64-72.
47. Xian Y, Karki CB, Silva SM, Li L, Xiao C: **The Roles of Electrostatic Interactions in Capsid Assembly Mechanisms of Giant Viruses**. *Int. J. Mol. Sci.* 2019, **20**:1876.
48. Zhao Z, Wang JC-Y, Segura CP, Hadden-Perilla JA, Zlotnick A: **The Integrity of the Intradimer Interface of the Hepatitis B Virus Capsid Protein Dimer Regulates Capsid Self-Assembly**. *ACS Chem. Biol.* 2020, **15**:3124-3132.
49. Asor R, Selzer L, Schlicksup CJ, Zhao Z, Zlotnick A, Raviv U: **Assembly Reactions of Hepatitis B Capsid Protein into Capsid Nanoparticles Follow a Narrow Path through a Complex Reaction Landscape**. *ACS Nano* 2019, **13**:7610-7626.
50. Pak AJ, Grime JMA, Yu A, Voth GA: **Off-Pathway Assembly: A Broad-Spectrum Mechanism of Action for Drugs That Undermine Controlled HIV-1 Viral Capsid Formation**. *J. Am. Chem. Soc.* 2019, **141**:10214-10224.

● The hierarchy of HIV-1 virus capsid assembly is investigated via extensive CG MD simulations to distinguish successful from unsuccessful kinetic assembly pathways depending on the evolution of intermediate capsid units.

51. Fatafta H, Khaled M, Owen MC, Sayyed-Ahmad A, Strodel B: **Amyloid-β peptide dimers undergo a random coil to β-sheet transition in the aqueous phase but not at the neuronal membrane**. *Proc. Natl. Acad. Sci. U.S.A.* 2021, **118**:e2106210118.
52. Muscat S, Stojceski F, Danani A: **Elucidating the Effect of Static Electric Field on Amyloid Beta 1–42 Supramolecular Assembly**. *J. Mol. Graph. Modell.* 2020, **96**:107535.
53. Jia Z, Schmit JD, Chen J: **Amyloid assembly is dominated by misregistered kinetic traps on an unbiased energy landscape**. *Proc. Natl. Acad. Sci. U.S.A.* 2020, **117**:10322.

●● Kinetic processes of amyloid assembly were studied using atomistic MD simulations and interpreted via Markov State modeling to find that misregistered β-sheets with long life-times are a major source of kinetic traps during aggregation.

54. Paul S, Paul S: **Controlling the self-assembly of human calcitonin: a theoretical approach using molecular dynamics simulations**. *Phys. Chem. Chem. Phys.* 2021, **23**:14496-14510.
55. La Rosa C, Condorelli M, Compagnini G, Lolicato F, Milardi D, Do TN, Karttunen M, Pannuzzo M, Ramamoorthy A, Fraternali F, et al.: **Symmetry-breaking transitions in the early steps of protein self-assembly**. *Eur. Biophys. J.* 2020, **49**:175-191.

● The role of peptide flexibility during self-assembly of peptides as a destabilizing factor is described from extensive simulations with experimental validation.

56. Schuster BS, Dignon GL, Tang WS, Kelley FM, Ranganath AK, Jahnke CN, Simpkins AG, Regy RM, Hammer DA, Good MC, et al.: **Identifying sequence perturbations to an intrinsically disordered protein that determine its phase-separation behavior**. *Proc. Natl. Acad. Sci. U.S.A.* 2020, **117**:11421.
57. Rana U, Brangwynne CP, Panagiotopoulos AZ: **Phase separation vs aggregation behavior for model disordered proteins**. *J. Chem. Phys.* 2021, **155**:125101.
58. Ranganathan S, Shakhnovich EI: **Dynamic metastable long-living droplets formed by sticker-spacer proteins**. *eLife* 2020, **9**:e56159.
59. Ruff KM, Dar F, Pappu RV: **Ligand effects on phase separation of multivalent macromolecules**. *Proc. Natl. Acad. Sci. U.S.A.* 2021, **118**:e2017184118.

● Mechanisms of ligand-mediated control of phase separation for multivalent peptides are discussed from CG simulations to understand the impact of ligand binding location and affinity.

60. Batys P, Fedorov D, Mohammadi P, Lemetti L, Linder MB, Sammalkorpi M: **Self-Assembly of Silk-like Protein into Nanoscale Bicontinuous Networks under Phase-Separation Conditions**. *Biomacromolecules* 2021, **22**:690-700.
61. Best RB: **Atomistic Force Fields for Proteins**. In *Biomolecular Simulations: Methods and Protocols*. Edited by Bonomi M, Camilloni C: Springer New York; 2019:3-19.
62. Vanommeslaeghe K, MacKerell Jr A: **CHARMM additive and polarizable force fields for biophysics and computer-aided drug design**. *Biochimica et Biophysica Acta (BBA)-General Subjects* 2015, **1850**:861-871.
63. Best RB, Zheng W, Mittal J: **Balanced protein–water interactions improve properties of disordered proteins and non-specific protein association**. *J. Chem. Theory Comput.* 2014, **10**:5113-5124.
64. Petrov D, Zagrovic B: **Are current atomistic force fields accurate enough to study proteins in crowded environments?** *Plos Comp. Biol.* 2014, **10**:e1003638.
65. Man VH, He X, Derreumaux P, Ji B, Xie X-Q, Nguyen PH, Wang J: **Effects of All-Atom Molecular Mechanics Force Fields on Amyloid Peptide Assembly: The Case of Aβ16–22 Dimer**. *J. Chem. Theory Comput.* 2019, **15**:1440-1452.
66. Man VH, He X, Gao J, Wang J: **Effects of All-Atom Molecular Mechanics Force Fields on Amyloid Peptide Assembly: The Case of PHF6 Peptide of Tau Protein**. *J. Chem. Theory Comput.* 2021, **17**:6458-6471.
67. Yoo J, Aksimentiev A: **New tricks for old dogs: improving the accuracy of biomolecular force fields by pair-specific corrections to non-bonded interactions**. *Phys. Chem. Chem. Phys.* 2018, **20**:8432-8449.
68. Huang J, Rauscher S, Nawrocki G, Ran T, Feig M, De Groot BL, Grubmüller H, MacKerell AD: **CHARMM36m: an improved force field for folded and intrinsically disordered proteins**. *Nat. Methods* 2017, **14**:71-73.
69. 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.
70. Ploetz EA, Karunaweera S, Smith PE: **Kirkwood–Buff-Derived Force Field for Peptides and Proteins: Applications of KBFF20**. *J. Chem. Theory Comput.* 2021, **17**:2991-3009.
71. Ploetz EA, Karunaweera S, Bentenitis N, Chen F, Dai S, Gee MB, Jiao Y, Kang M, Kariyawasam NL, Naleem N: **Kirkwood–Buff-Derived Force Field for Peptides and Proteins: Philosophy and Development of KBFF20**. *J. Chem. Theory Comput.* 2021, **17**:2964-2990.
72. Lin F-Y, Huang J, Pandey P, Rupakheti C, Li J, Roux Bt, MacKerell Jr AD: **Further optimization and validation of the classical Drude polarizable protein force field**. *J. Chem. Theory Comput.* 2020, **16**:3221-3239.
73. McGuffee SR, Elcock AH: **Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm**. *Plos Comp. Biol.* 2010, **6**:e1000694.
74. Ando T, Skolnick J: **Crowding and hydrodynamic interactions likely dominate in vivo macromolecular motion**. *Proc. Natl. Acad. Sci. U.S.A.* 2010, **107**:18457-18462.
75. Matić M, Saurabh S, Hamacek J, Piazza F: **Crowding-Induced Uncompetitive Inhibition of Lactate Dehydrogenase: Role of Entropic Pushing**. *J. Phys. Chem. B* 2020, **124**:727-734.
76. Maritan M, Autin, L, Karr J, Covert MW, Olson AJ, Goodsell DS: **Building Structural Models of a Whole Mycoplasma Cell**. *J. Mol. Biol.* 2022, **434**:167351.