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Applications of Discrete Molecular Dynamics in biology and medicine

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Discrete Molecular Dynamics (DMD) is a physics-based simulation method using discrete energetic potentials rather than traditional continuous potentials, allowing microsecond time scale simulations of biomolecular systems to be performed on personal computers rather than supercomputers or specialized hardware. With the ongoing explosion in processing power even in personal computers, applications of DMD have similarly multiplied. In the past two years, researchers have used DMD to model structures of disease-implicated protein folding intermediates, study assembly of protein complexes, predict protein–protein binding conformations, engineer rescue mutations in disease-causative protein mutants, design a protein conformational switch to control cell signaling, and describe the behavior of polymeric dispersants for environmental cleanup of oil spills, among other innovative applications.

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Introduction

The Discrete Molecular Dynamics (DMD) simulation method utilizes the physics of ballistic motion to generate trajectories of particles in space over time according to discrete, distance-based energetic potentials [1–5]. Originally developed for hard-sphere fluid systems [6], in the past 20 years DMD has emerged as a key tool in the study of biomacromolecules and their assemblies, due primarily to the vastly increased time and length scales allowed by advanced event scheduling and search algorithms used to progress the simulation. In place of integrating continuous energetic potentials at set time steps to determine forces that will impact new velocities and position, DMD assumes ballistic motion and assigns time step as the time until the next occurring interaction ('event'), saving time and computational resources. Upon interaction, energy is assessed with a distance-based step function, and velocity and position change instantaneously upon collision according to the conservation of momentum [7]. The use of energetic step potentials also readily allows for incorporation of distance constraints derived from experimentally derived proximity and solvent exposure information [8–10]. Here, we review several applications in biology and medicine for which DMD has made a key impact in advancing understanding and accelerating technological innovation (Figure 1).

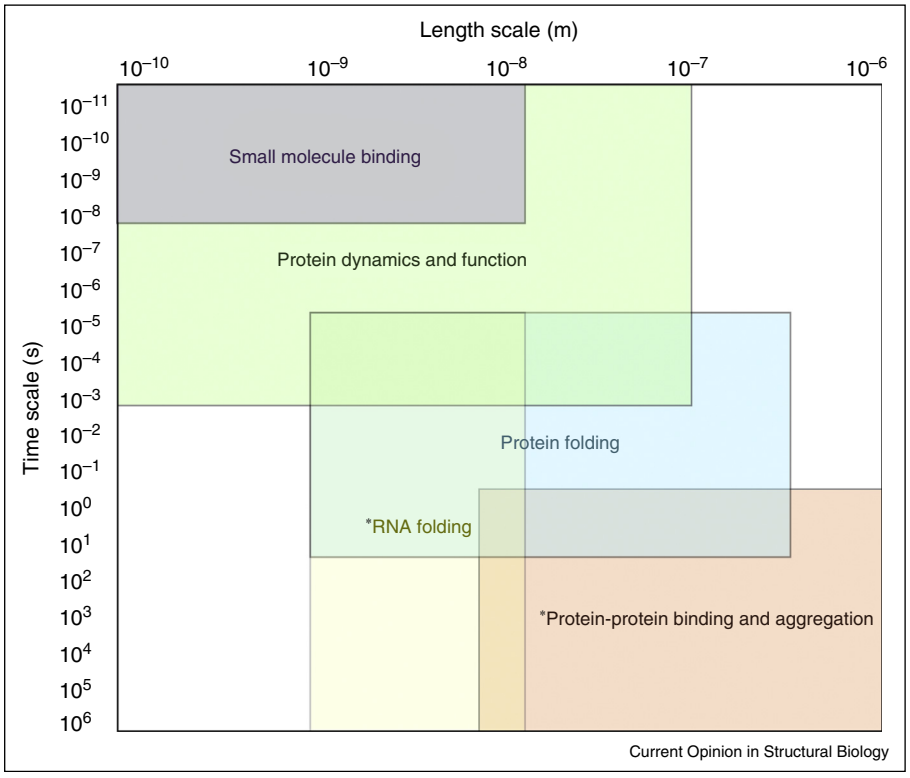
Protein folding and aggregation

The protein folding problem, determining the three-dimensional folded structure of a protein given its amino acid sequence, has been a challenge in the field of physics and computer science since Anfinsen's landmark paper in 1973 [11]. Because it allows for increased sampling of the folding landscape while retaining physically relevant dynamics [3,5], DMD is a tool well-suited for the study of aberrant folding intermediates relevant to protein misfolding diseases such as Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS). Recently, Williams *et al.* [12] utilized DMD simulations to identify a misfolded intermediate of the protein ApoE4, an isoform of the ApoE protein associated with dramatically increased risk of AD. Ding *et al.* [10] combined a coarse-grained protein model with experimentally derived structural information to determine that different ALS-associated SOD1 mutants display distinct patterns of misfolding, causing them to form differently shaped aggregates of 8 SOD1 monomers (153 residues per monomer). Distinct aggregate morphology suggests an explanation for differences in disease progression for patients with different SOD1 mutations. Meral and Urbanc [13] investigated oligomeric formation in four different peptides of amyloid-β, a protein that forms brain plaques in the vast majority of AD patients, finding that those peptides known to be more toxic feature more flexible and solvent-exposed N-termini. Kimura *et al.* [14•] performed DMD simulations to observe the folding of HIV-1 protease monomers and their assembly into active dimers, finding that the precursor to the mature protein can form non-native dimers of natively folded monomers.

Molecular modeling

Despite recent advances in experimental methods and technology, the number of high-resolution protein structures available is dwarfed by the number of known

Figure 1



Length and time scales of molecular phenomena studied with DMD. Asterisk (*) denotes that RNA folding and Protein aggregation can extend to time scales of seconds. Various molecular models (coarse-grained versus all-atom) are appropriate for reaching different length and time scales, with larger scales being better represented by coarse-grained models. The incorporation of experimental information can accelerate simulations by several orders of magnitude.

proteins and complexes. Computational molecular modeling is often faster and cheaper than experimental methods, and can also circumvent the technical issues encountered in solving structures of large, insoluble, and/or meta-stable proteins and aggregates. As discussed in the previous sections, the rapid sampling of the DMD simulation method, as well as the incorporation of experimental information to reduce the size of conformational space that must be explored, allows for accurate modeling of proteins and their assemblies for which experimentally obtained structures do not exist. For example, Konrad *et al.* [15] utilized DMD simulations to construct three-dimensional structures of deoxyribonucleoside kinases between arthropods and vertebrates from their reconstructed ancestral sequence information, in order to determine evolutionary differentiation in substrate specificity. Szöllősi *et al.* [16] performed simulations of intrinsically disordered proteins and found evidence of transient secondary structural elements called helical prestructured motifs, which can play an important role in the binding of these proteins. Emperador *et al.* [17] designed a method for the efficient modeling of protein–protein binding using DMD simulations to address the outstanding problem of conformational change and protein flexibility upon docking. Similarly, Freeman *et al.* [18] used DMD simulations to identify and model novel protein–protein binding interactions.

Protein dynamics and function

Molecular function is often reliant on dynamic behavior. Proteins are highly flexible structures whose function is enabled by a change in shape. This conformational change may be a subtle rearrangement of a binding pocket to accommodate a drug or binding partner, or a dramatic allosteric global shift in shape. Because in many cases we cannot know *a priori* whether conformational changes will be mostly local or necessitate global rearrangement, use of an unconstrained, physics-based atomistic force field is ideal. DMD allows for increased sampling with fewer computational resources, which is imperative in dynamics studies where multiple replicates of long trajectories are necessary for statistically distinguishing thermodynamic fluctuations from meaningful rearrangements in structure. For example, Sfriso *et al.* [19••] implemented coarse-grained DMD simulations to map protein conformational transitions. By taking advantage of the increased sampling

of conformational space made possible by DMD event search algorithms [3–5], the authors create a tool that can trace complex, non-linear transitions in agreement with experiment without distortions in protein structure. Kota *et al.* [20] performed DMD simulations to elucidate the structural and energetic basis for substrate recognition in the proteolytic regulation of the epithelial sodium channel (ENaC), a key player in salt balance, blood pressure, and organ function. Proctor *et al.* [21•] studied the dynamics of disease-causative mutants of the cystic fibrosis transmembrane conductance regulator (CFTR) using DMD. They discovered networks of correlated motion allowing transfer of aberrant dynamics from the mutation site to distal regions associated with ion channel function and stability. Stiffening of a 'hotspot' residue located along the determined path of propagation by mutation to proline abolished the aberrant correlated dynamics and rescued CFTR function. The increased time and length scales of DMD are especially evident in atomistic simulations uncovering mechanistic details. Using multiple replicate simulations of the integral membrane protein cytochrome b_6f, part of the electron transfer chain that generates energy from light in plants and is analogous to the mitochondrial protein cytochrome bc_1 in animals, Hasan *et al.* [22] determined that the presence of chlorophyll in the plant protein increased residence time of quinone redox reaction product in the binding pocket, explaining the order of magnitude greater production of superoxide in the plant protein as compared to the animal protein.

Protein engineering

An understanding of protein structure and dynamics allows for rational perturbation in order to inhibit, enhance, or alter protein function. DMD simulations are useful in the design of proteins as both a method of determining the structural effects of the designed perturbations and, conversely, to determine the best shape or energetic properties of a desired binding partner. As an example of these uses of DMD, Dagliyan *et al.* [23••] built upon previous work [24] to design a single-chain protein conformational switch controlled by binding of the drug rapamycin. Using DMD simulations, the authors built a structural model of the desired kinase-switch-rapamycin complex to test and refine conformational switching ability. Inserted into conserved kinase allosteric sites, the stabilization of the protein conformational switch upon binding of rapamycin activates the kinase, offering a powerful tool for the study of signaling pathways *in vivo*.

RNA tertiary structure

The recent discovery of the importance of non-coding RNA in catalysis, development, and gene regulation has led to an increased interest in the three-dimensional structure and dynamics of RNA molecules. However, the additional flexibility of RNA in comparison to proteins, with six backbone dihedrals as compared to two, exponentially increases the complexity of *ab initio* structural determination. As discussed in the above sections, DMD is an exceptionally appropriate tool for structural determination and description of dynamics in systems with high degrees of freedom, and sampling can be even further accelerated by use of a coarse-grained model. Ding *et al.* [25] addressed this problem by introducing a three-bead model (one bead each for the sugar, base, and phosphate) of RNA for use with DMD, which was used by Tsao *et al.* [26] to study differences in the dynamics of mRNA transcripts for variants of the protein catechol-O-methyltransferase (COMT), which plays a major role in the perception of pain. The authors determined that a silent mutation to the gene for COMT results in identical protein sequence but significantly influences the structural stability and dynamics of mRNA structure near the 5′ end, affecting translation efficiency and hence protein levels of COMT, influencing pain sensitivity. As in proteins, discussed above, the incorporation of experimental information as constraints can significantly decrease the size of conformational space necessary to be explored in simulation, and therefore increase the size of the system that can be evaluated. Ding *et al.* [27] incorporated base-pairing and solvent accessibility information at each nucleotide, derived from hydroxyl radical probing (HRP), as constraints in DMD simulations for the structural refinement of RNAs from 80 to 230 nucleotides in length. After rough tertiary structure determination using coarse-grained DMD simulations with base-pairing information, the authors applied a bias potential derived from the HRP data and constructed ensembles of low-energy, high-experimental agreement structures. Cole *et al.* [28] used this method of incorporating experimental data into DMD to model minimal telomerase RNA complex, revealing conformational changes that occur during telomerase assembly. Before this study, no high-resolution structural data on telomerase complexes had been available, due to the large size and high flexibility of the RNA molecules.

Surface chemistry

Surface chemistry of materials can play an important role in binding properties and outcomes. For example, the role of nanoparticles as potentially revolutionary drug delivery vehicles has attracted attention in recent years, but their binding properties must be carefully controlled in order to be safe and effective. The accelerated sampling of DMD is well-suited characterization of binding properties in simulations, which requires study of many interactions. Radic *et al.* [29•] utilized DMD simulations to illustrate the effects of surface chemistry on the binding of nanoparticles to their target proteins. They demonstrated that highly hydroxylated fullerene nanoparticles form hydrogen bonds with protein surfaces, stabilizing them without inducing structural changes, while less hydroxylated fullerene particles are hydrophobic and can cause proteins to misfold. Geitner *et al.* [30] conducted DMD simulations of poly(amidoamine) dendrimers (PMAMs), a class of

highly branched polymers, to determine differences in oil dispersion interactions between cationic, anionic, and neutral charged PMAMs with various hydrocarbons. Their results can be applied to inform design of polymers used in environmental cleanup, water purification, and drug delivery, among other industrial and biomedical applications.

Therapeutic development

Computational drug screening methods have long been employed to decrease the cost of drug development, but oversimplification of the docking process can result in high numbers of false negatives and false positives. Most docking methods match rigid bodies, while some generate ensembles of static conformations to imitate dynamic movement. Neither of these scenarios matches reality, where factors such as the entropy of binding, protein allostery, and synergistic dynamics between ligand and target can all affect drug binding. Proctor *et al.* [31] utilized many short replicate DMD simulations to obtain statistics of the residence time of a drug in the binding pocket in many different generated poses. The authors were able to identify the correct binding pose of the drug in 'difficult' targets: those for which traditional computational drug screening methods could not do so. In a similar study, Dagliyan *et al.* [32] used DMD simulations to correctly identify native binding sites of peptides on their protein targets, as well as recapitulating native-like poses of the protein–peptide complex. The authors stressed the importance of protein target flexibility and dynamics as well as electrostatic interactions in identification of binding site and pose, highlighting the crucial use of ultrafast DMD simulation for this application.

Conclusions

As advanced technology has allowed us to make discoveries on larger scales and in finer detail, both experimentally and computationally, the nearly overwhelming complexity of biological processes and human diseases has become increasingly evident. Despite the explosion of revolutionary, 'textbook-rewriting' discoveries in recent years, we are left with more questions, not fewer, regarding interactions between signaling pathways, drug resistance, the influence of the immune system on diverse tissues, and other phenomena beyond the molecular scale that nonetheless influence the environment in which molecules interact. While emerging fields such as systems biology and systems pharmacology are beginning to untangle signaling pathways on the cellular and tissue levels, the atomistic details of molecular interactions are critical to the elucidation of mechanism and the engineering of effective therapeutic strategies. The computational power of DMD is capable of providing these details on relevant time and length scales to real biological processes. The implicit solvent used with DMD provides an opportunity for parameterization to take into account the crowded cellular milieu, and the event-driven nature of the algorithms that advance the simulations are more appropriate to large, crowded systems than are continuous potentials with necessary integration over increasingly complex energetic potentials. In the near future, the combination of parallelization [7,33] and increasing processing power will likely make DMD the method of choice for the first atomistic cellular simulations and beyond.

Conflict of interest

Nothing declared.

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