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AI-Enhanced Molecular Dynamics Simulations for Protein Folding and Drug Binding Prediction

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Annotation: Although the common practice in drug discovery is to screen compounds against a rigid structure for faster evaluations of multiple candidates, the robust prediction of drug binding should include the flexibility of the protein targets. For pharmaceuticals that induce the desired state of a protein, the receptor-drug dawning process can often be captured within protein conformational fluctuations about a bound crystal structure. Molecular Dynamics (MD) simulation has become an effective approach to see such fluctuations and has matured to provide a complement to and a probe for X-ray crystallography.

One-tenth nanosecond MD simulations produce sufficient flexibility information about the structure and configuration of drug binding which are used for ensemble docking against a cluster of the sampled structures. Docking methods have improved to properly account for topology and conformational changes of a protein through models such as the rigid receptor, flexible compound, induced fit, and ensemble docking. The first three methods require extensive sampling and are computationally more expensive than rigid receptor docking. Meanwhile, parallelization is possible at either conformation or compound-level with respect to the respective dimension of the problem but cannot be performed at both levels since the dimensions of the problem are critically diverse in MDO.

There has been considerable interest in deriving and implementing accurate, fast, and flexible soft potentials in machine learning (ML) frameworks. However, these approaches have either been serial implementations with no speedup with respect to numerical relaxation or did not fully utilize end-to-end optimization through gradients. Since a network trained on a protein family would not be applicable to one outside the family,. Molecular MD would be a better training starting point as it ensures better physical prior during learning until reaching a steady state.

1. Introduction

The past few decades have witnessed the successful application of artificial intelligence (AI) techniques in diverse fields, such as computer vision, speech recognition, natural language processing, and self-driving vehicles. However, advances in the computational modeling of molecular systems have lagged behind by some degree, mainly due to limited trainingset sizes, difficulties in modeling geometry-dependent dynamical systems, and stringent computational requirements. Recently, there have been rapid developments of profound AI models and exponentially growing chemical and biological datasets, which renew the hope for a revival of AI models in molecular sciences. In particular, new generation methods with equivariance and domain generalization features are capable of providing data-efficient molecular dynamics (MD) modeling. In addition, newly curated grand master datasets, covering diversity spaces in chemical compound structures, properties, and molecular reactions, enable the pretraining of an AI model on a large scale and a fine-tuning step at the task level, akin to the success of large language models in natural language processing and computer vision. Such dataset-accelerated AI training enables rapid adaptation to new tasks by expanding the current training datasets in order to improve modeling accuracy. The ultimate scope of applications extends to new theory discovery, visualization, and multimodal molecular modeling [1].

The emphasis is on AI-enhanced MD simulation modeling. In the same spirit as the word “chemical simulation” being used as a synonym of quantum mechanical simulation in past decades, the term “MD simulation” is used as a synonym of molecular-dynamical and stochastic-procedural simulation in a broad sense. However, notable distinctions are made. First, protein folding pathways are predicted with a physics-based and MD-accelerated approach. In addition, AI-human hybrid protocols are introduced to accelerate the posttarget identification and in-depth biological activity elucidation of drug candidates. AI-enhanced molecular dynamics (MD) simulations have been largely confined to protein folding and drug binding tasks. All proposed methodologies fall into the category of AI-enhanced MD simulation and are described to aid understanding of the underlying MD and drug discovery methodology. In addition to existing target-structure-based drug design perspectives, a black-box AI-enhanced modeling perspective is introduced to accelerate the assessment and exploration of drug-like chemical entries and target structures by providing energy-profiles of chemical structures through direct physics-free energy-geometry characterizations.

2. Background on Molecular Dynamics

Molecular dynamics (MD) simulation is a non-static view that describes the spatio-temporal changes of molecular systems [1]. It is one of the most important methods to understand the molecular mechanism, whereas the tasks of MD include time integration for a solvent and/or a

solute system given their initial configurations and potential energy surface. In large-scale systems such as 30,000 atoms of the protein-ligand binding complex, MD is divided into steps: to model the classical MD with the Newton equations and force fields, to initialize the coordinates and velocities for the solute molecules, and to compute the force acting on the particles of interest for each time step. MD has important applications in drug finding, such as protein-ligand binding and unbinding, protein folding or unfolding, and large conformational transitions. Although powerful integration numerical methods have been extensively developed, predicting these complex processes in implicit solvent is still prohibitively expensive.

The task of MD simulation for the protein-ligand binding dynamics is to simulate the time evolution of a large and complex biomolecular system of hundreds of thousands of interacting atoms and understand the conformational change of the system at a long timescale (milliseconds). Due to the limitations of computational resources, classical MD simulation of large biomolecular systems is usually performed with a large timestep (typically 1–4 fs) over hundreds of nanoseconds. During such a long integration, rapid motions that cannot be described by the force field would lead to the violation of energy conservation and introduction of spurious artifacts. To estimate the long-timescale dynamics, learning-based surrogates for numerical MD methods are desired. Hence, the essential of this task is to learn the harmonic dynamics conditioned on a sequence of the MD trajectory points. Recent advances in deep learning have shown promise to improve the predictive quality. Nevertheless, these methods either augment numerical solvers with neural networks or replace solvers at small time steps, and they all adopt a uniform timesteps for testing. [2][3][4]

2.1. Fundamentals of Molecular Dynamics

Molecular dynamics (MD) simulations offer visual insights of intermolecular interaction and time-resolved detailed information on protein folding, and binding with small organic molecules (ligands). These simulations have been used to rationalize the affinity and selectivity of drugs against targets and also in drug discovery. MD simulations are powerful tools to study biomolecular processes at nanoscale up to millisecond timescales, yet sampling in number of microseconds-to-milliseconds timescales remains a challenge due to limited computational power. Similar to experimental methods MD simulations need a priori setup of starting structure. For protein systems, static three-dimensional structure is available in PDB format and MD conditions can be parametrized with popular CHARMM, AMBER, OPLS, GROMOS force field libraries. In contrast, structure and interaction potential/force fields of most ligand molecules are unknown and hence dry ligand environments warrant further consideration. Development of accurate models of potential energy terms of protein-ligand binding and their optimal parameters is a longstanding objective in computational chemistry and drug design. Small organic molecules, or compounds, that binds to biomolecules, such as proteins or nucleic acids, performing stimulation with these compounds in water environment. There are 3D structures available in PubChem, but there is no guarantee that which form will set the binding conformation. MD simulations provide information beyond static structures since it describes the time-resolved motions of biomolecules.

Protein-ligand binding free energy estimation with MD simulations based on molecular mechanical (MM) force fields. Most of current methods are inaccurate because computational pharmacology models potential energy functions without including solvation contribution in a proper manner [5]. Many of binding free energy estimation algorithms rely on free energy perturbation (FEP), thermodynamic integration (TI) and their variants, for which accurate estimation addresses selection, combination, and mapping of conformation-dependent energy terms with reference energy functions [6]. Poor parameterization of precise premiers is one of major constraints in MM force fields with a few exceptions. Handling dry ligand environment in trajectory navigation is another consideration.

2.2. Historical Development

Simulations of molecular dynamics have been used since the late 1950s to address problems of molecular systems at atomic resolution. The Liverpool group has emphasized simple representations resulting in computations on microsecond time scales, addressing important processes such as protein folding and ligand binding [7]. With the advent of massively parallel systems, powerful molecular mechanic force fields have been developed and performance reached on the order of nanosecond simulations for up to a million atoms, heavy water biochemical solution, analyzed on a time frame of hundreds of nanoseconds to microseconds.

Fine-grained representations of molecular interactions typically reduce complexity for computational gain in performance, but coarse-graining removes chemical specificities and is thus qualitatively more limited. Simplistic approaches, such as a simple shape fit or molecular docking based on an energy minimization algorithm, may be polluted by irrelevant local minima. On the other hand, biomolecular systems are typically characterized by a myriad of timescales (femtoseconds to seconds). MD simulations of atomic resolution allow representation of bonds, angles, torsions, van der Waals, and electrostatic calculations, in combination with a thermostat to fix temperature and a barostat for pressure coupling. Nonetheless, time steps of commonly considered potentials are limited to 1–3 fs, and currently executable times remain below a million times the time step. Hence, finest representation group motions are limited to motions of an average of 12 heavy atoms, and simple model coarse-graining may yield valid regions in conformational space, but remain qualitative for large biomolecular systems. Coarse-grained potentials replace friendly empirical force fields with less chemically relevant generic potentials, which may have convergence problems for large systems, may exhibit artificial properties, and for which bridging to atomistic models is challenging. [8][9][10]

2.3. Applications in Biophysics

In Biophysics, MD simulations have been used as a tool for studying the aerial properties of biomolecular systems. MD simulations of biomolecular systems involve the integration of Newton’s equations of motion for the atoms of interest in order to sample their configurational phase space. Ideally, a configuration of just a few tens of water molecules, a few dozens of amino acids constituting proteins or nucleotides with explicit pH 7.4 buffer can already help capture unique molecular properties, however they become rapidly intractable for an MD simulation on regular computer systems. Recent efforts to improve the computational efficiency of MD simulations for large systems include a systematic approach to the numerical molecular mechanics and implementation of new algorithms on special architectures, such as the use of GPUs. These advances may be key in expanding MD simulations into practical tools for biology and drug development [11].

Molecular dynamics (MD) simulations are a powerful and versatile computational tool with multiple applications in biochemistry and pharmacology. Many studies have applied MD simulations to model drug binding to protein target systems or study the structural dynamics of drug targets, notably receptors and enzymes. MD simulations can also be used in a more computationally expensive approach of structure prediction from protein sequence, where they account for tertiary structure refinements and the more general modeling of protein chains, however the faster modeling methods are more widely used for this. MD simulations can recover consistent temperature and pressure, but to initial conditions not representative of biological processes, a number of ‘temperature jumps’ can be taken as an attempt to return proteins to their native states through the application of heating cycles and restraint forces, or to obtain kinetic estimates of folding free energies by exploring multiple structural states. MD simulations can also be used and have been extensively applied as a component of protein design or identification of ligands for both large predictions of new protein and ligand sequences. [12][13][14]

3. Protein Folding Mechanisms

Comparing Dynamic Simulations of Tumor-Associated Protein Mutations Methods for protein folding prediction studies have the longest history in the structure–prediction field. These methods are typically using a simple but computationally much more expensive approach: all-atom dynamic simulation of hundreds of nanoseconds or even microseconds. Affinity prediction of protein–ligand systems has recently attracted increasing research interest among researchers in both industrial and academic labs [15]. The authors emphasize MD simulations as a universal approach to biomolecular modeling. Thus, they review both proteomics and drug discovery in molecular dynamics settings including the latest developments in the hardware, algorithms, and force-field refinement [16]. In the late 1980s and early 1990s, a few pioneering attempts had been made to apply all-atom MD simulations to protein folding studies. Despite this, all-atom simulations are rarely used for large-scale studies.

Protein folding prediction from its sequence is a long-standing challenging problem in biology and biomedicine. The best exploitable knowledge about a protein is its sequence drawn from the "genetic treasure" and its 3D structure determined using expensive techniques. The mutation of sequence units, amino acid residues in proteins, or nucleobases in DNA or RNA is closely related to the start of life and the cause of many diseases. As such, understanding how a protein folds from its sequence or how mutations would affect its folding pathway or rate is not only an important fundamental question but is also practically useful for protein design or mutation-based drug discovery. Similar questions hold for other biopolymers in genome science.

3.1. The Folding Problem

The protein folding problem asks how the linear sequence of amino acids folds into its unique 3D conformation. Ultimately this protein conformation defines its biological function; thus, understanding the folding process is critical. Although scientists have known the sequence-to-structure mapping for decades via X-ray crystallography and NMR, the dynamics of with which this mapping occurs is a more recent area of research. Extensive computational modelling has been useful in probing the dynamics of folding, but at atomistic resolution, such approaches are limited to ~1 ms in the best case and generally require weeks to months of compute-time for larger proteins. Phylogenetic approaches based on similar folds/domains and homology modelling have proven useful in predicting rough topology, but how these topologies are selected and refined remains unknown. On the other hand, many drug design strategies rely on the docking of candidate drugs with proteins in their experimentally characterized native conformation. Yet, the role of conformation selection in the non-trivial binding of candidates to 'flat' protein binding energy landscapes has also received very little qualitative treatment [16]. These problems share similar complexities in energy landscape and algorithm design, making them suitable for the same general approach.

Folding pathways of small to intermediate sized (60-100 residues) proteins have been successfully simulated in atomistic detail using a new Boolean energy function (the Protein Energy Function, PEF) that encodes the physics of protein folding and an efficient Monte Carlo algorithm that enables folding simulations to be run orders of magnitude faster than at present [17]. The Protein Energy Function is designed to select a single folded conformation for a given chain length of non-coded amino acids. In addition to its native state, it accommodates dummy amino acids, which must not bond, and thus allows for an ambitious folding pathway search algorithm, which groups pseudo-detailed Monte Carlo move types in energy 'move-sets' to generate coarse-grained Lattice Path Trees (LPTs) large enough to cover all reasonable folding pathways. Suitable intricate partitioning of moves based on astuteness of topology allows for the efficient generation of LPTs to which a newly devised simulated annealing-like recovery scheme is subsequently applied. Such an approach is particularly suitable for an extensive unified study of the Folding and Drug Binding Problems. One methodology is introduced and applied to the fold-discovery problem. Its applicability to the complex but generic Fold-and-Drift Problem is

also discussed. In the latter case, it is shown that small to intermediate sized proteins are entropically favoured in liquid water enclosures (a key condition for the origin of life).

3.2. Kinetics of Protein Folding

To describe the protein folding pathway, a computationally inexpensive and effective Markov model has been developed. The resolution of the Markov model is determined by the size of the coarse-grained model used. The design of the database ensures that it is body size invariant, so the approach can be used for any peptide length. To avoid discarding the CMF of wrong trajectories, it is included as equilibrium distributions. For all proteins examined, substitution values that were comparable to those generated through atomistic simulations and continuous time random walks have been demonstrated.

The underlying theory of the model is described and how it can be applied to practical problems is demonstrated using several interesting cases, such as a tripeptide that folds within milliseconds and "mini" proteins. For proteins larger than the current CG diameter, a modular parallel executable can be made available. In order to describe the kinetics of protein folding, it is first necessary to construct a peptide potential from a folding and unfolding trajectory. To achieve this, it is implemented as a coarse-grained momentum-based trajectory difference scheme to integrate MDS. By simulating time series of building blocks, flexible 3D protein motions can be modeled with a few degrees of freedom.

Although rigorous MDS has succeeded in providing detailed atomic level trajectories of chemical systems, it has yet to be adapted to simulate the time-dependent stochastic processes of macromolecular folding. A few MDS studies on peptide folding and the applicability of time series prediction methods on stochastic trajectory data have been recently reported. Although it is crucial to know how to choose an appropriate discretization, previous methodologies are limited to very small proteins under special conditions. It was shown in the context of a practical folded protein that all-atom simulations and elastic network models yield quantitatively consistent folding time estimates. This gives a clear basis for estimating protein folding times.

3.3. Experimental Techniques

Molecular Dynamics (MD) simulations allow exploration of time-dependent motions of proteins at an atomic level. Key experiments are selected for further process. The MD simulations are performed using the following methodology to generate the trajectories in a deterministic way. Each protein system is placed in a cube water box and sodium/potassium ions are added/moved according to the charge requirements, which bring the system to physiological conditions. Then a conjugated gradient energy minimization is performed to eliminate steric clashes and carry out fitting of the initial protein structures into the water box. The solvated and equilibrated protein systems are subjected to 20ns MD simulations in explicit water model at 310K, with an integration time step of 2fs. The initial velocities of the NVE systems are assigned at random based on a Maxwell-Boltzmann distribution. The interaction cutoffs for all non-bonded interactions are set to 10Å. CHARMM atom types, parameters and force field are applied. The simulations are performed in periodic boundary conditions, controlled by constant volume (NVE) and constant temperature (NVT) ensembles using model potential and temperature coupling scheme. Starting frame, frequency of recording and running periods are determined in such manner that a total of 1ns trajectory with 550s frame interval is generated. The XY and Z coordinates of proteins in MD simulations provide effective monitoring of the protein structures and motions during folding that is necessary input for calculations. The employment of GOA typically results in selection of a native structure for protein enzymatic reaction in MD simulation. The CG approach infers a simplified AA representation to enhance the sampling in molecular motion. Discrete stochastic, Langevin dynamics and hybrid simulation approaches are applicable CG simulation schemes. [18][19][20]

4. Drug Binding Prediction

Molecular docking is a structure-based approach to predict how a drug binds to a protein. The docking process typically involves predicting the binding site, predicting the binding mode, and ranking the predicted binding modes. Molecules like peptides, drug-like probes, and natural products are major input types for protein docking. In most blind docking studies, ligands are assumed to be rigid. Continuous conformational space to avoid missing low-energy poses. Several molecular docking tools enable complementary conformational sampling methods, flexibility modeling techniques, and sophisticated scoring functions. Such developments and other advances in recently published open-source software significantly help drug discovery programs [21].

Drug discovery is an expensive process that ultimately leads to approved drugs for treating human diseases. An early phase of drug discovery is identifying strong binding candidates, which is usually done by high-throughput screening and molecular docking. Drug discovery is a costly and time-consuming process. Screening thousands to millions of compounds against a biomolecular target for texture and observation is an expensive and technically challenging process. Accurately predicting binding affinity and optimizing lead drugs before measuring binding are therefore desired. Accurate predictions are also valuable for drug repurposing and drug-protein interaction investigation [22].

Accurate predictions of active versus decoy classifications are necessary for cost-effective drug discovery. An ensemble docking method that generates large numbers of conformations for ligand-binding proteins has been shown to improve drug-binding predictions. Each conformation is docked and divided into PDBQT input files, run independently, and combined via machine learning algorithms. These predictions can also facilitate side effect analysis, drug repurposing, and drug design. Improving binding-pose predictions and obtaining conformational energy by running molecular dynamics simulations for all docked conformations using a multi-scale modeling approach.

4.1. Importance of Drug Binding Studies

Understanding protein binding is essential in drug screening experiments because it is intrinsically tied to protein structure/function; however, computational tools can predict protein binding, potentially making expensive molecular dynamics simulation unnecessary [22]. The use of machine learning has the potential to be a game-changer in this area since it can readily integrate various sources of data and hint at fundamentally interesting results. With the growth of data available from increasingly faster and cheaper hardware, focused efforts combining drug-docking scoring programs with machine learning algorithms could have far-reaching benefits to drug networks across numerous areas of medicine. Very few studies to date have explored the integration of traditional docking methods into machine learning pipelines and trained models to accurately classify drug binding specificity. However, recent advances have been made in this area, establishing novel methods of sending docking scores into machine learning classifiers in channelized pipelines, which utilize properties of both hard data and predictions from computational methods. Thus, there is an opportunity to further develop these methods into powerful classifiers to accurately seek and understand pharmacological interactions.

Drug development is a lengthy and costly process that is prone to massive failure rates due to several factors. This research emerges from a desire to intercalate advanced computational approaches early in the drug development pipeline, thus efficiently preventing detrimental binding interactions from appearing during resynthesis. The ability to make accurate computational predictions of drug binding could greatly improve the cost-effectiveness and safety of drug discovery and development. Toward this aim, this study introduces ensemble docking and other methods to integrate a range of additional biomedical data sources with machine learning algorithms trained to classify compounds as active or decoy for a given protein. Using an ensemble of multiple protein conformations will better represent an average of

potential binding sites for the docked compounds than a single conformation does.

4.2. Techniques for Binding Prediction

Recent approaches to improve protein-ligand docking and binding affinity prediction combine empirical scoring functions with carefully-parameterised physics-based scoring functions. Insights from a fundamental area of statistical physics have been mined to formulate complementary scoring schemes that provide valuable knowledge of both the overall quality of the ligand pose and also an estimate of the docking energy. Various descriptors, adopted as scoring functions for binding free energy prediction, rank-ligand poses based on topological properties of the 3D ligand-protein complexes produced by docking or sampling simulations together with electrostatic features of both the ligand and the protein [21]. This Bayesian approach leads significant improvements over the original knowledge-based methods but still employs a simple atom-atom potential that does not take into account the time evolution of the two particles in the complex. Apart from terms measuring contact distances, more complex scoring functions, arising from statistical mechanics principles as Markov models and made self-consistent, have been developed. They yield reasonable estimates of the stability and fidelity of the ligand pose.

Methods for predicting how a ligand binds to its target protein have become the most important application of computational chemistry in drug design. Binding is mediated by protein-ligand and solvent interactions, including solvation, crowding, and pH, and many of these renders accurate prediction of free energy and structure challenging. The difficulty of this problem, the critical role in drug discovery, the progress made to circumnavigate the hurdles, and a summary of it all at the end are discussed [5]. Several classes of calculation, beginning with elucidating near-native poses, are then theoretically reviewed, considering outcomes and limitations particularly with respect to accuracy, robustness, sample preparation, and execution time, especially when tested on blind prediction datasets. Finally, an overview of recent advances in molecular modelling with some perspective on future developments and initiatives is provided.

4.3. Case Studies in Drug Discovery

One of the most important applications of molecular dynamics simulations is in drug discovery, where it is used to predict how drug molecules bind to their protein targets [21]. Identifying possible protein ligand binding poses is an important step in determining the mechanism of action of a new drug candidate. VinaBio is a new molecular docking tool that enables AI-enhanced blind docking of drug-sized ligands to proteins, in particular against flexible targets. It uses a fast multiple property scoring function, a coarse-grained global search, and refines the top hits using an all-atom energy model. The scoring of protein-ligand poses may be augmented by pre-trained and/or fine-tuned neural networks on prior examples of target-ligand pairs, facilitating transfer learning. Near-native pose retrieval benchmarks with popular conformational sampling, molecular mechanics scoring, and coarse-grained scoring methods demonstrate that VinaBio is competitive with existing methods. In five-dockings against three flexible targets, VinaBio achieves more than one near-native pose in the top 10 docked poses (1:1:2) and in the top 50 poses (1:2:2). The state-of-the-art is exceeded by adding AI-augmented scoring, with completely blind runs retrieving near-native poses for all three diverse targets. VinaBio is available for academic use as an open-source software product for Linux and Windows.

Its ability to recover near-native binding poses and to dock a variety of diverse flexible protein targets makes VinaBio significant. As an open-source software tool compatible with a popular docking package, it opens the opportunity to carry out extra-sensory docking against challenging protein targets across an array of proteins, including membrane proteins and protein-protein complexes. For adherent targets, VinaBio could provide a complementary tool for advanced conformational sampling methods to screen a much wider range of leads. In addition, all-atom potential energy functions with trained weights specific to the target-ligand pair could enhance the accuracy of the refinement.

5. AI and Machine Learning in Molecular Dynamics

Currently, a variety of machine learning (ML) models based on neural networks are gaining popularity within the MD development community. This focuses on the recent advance in synthetic & semi-synthetic ML-based force-field representations and their application to the modeling of protein folding. The selection is motivated by the growing interest in applying these models to study the kinetics of large structural transitions, with an emphasis on proteins. In addition, a variety of machine learning tools that have been used to analyze, condense, or filter MD trajectories are reviewed. Prominent examples of these methods are bottleneck identification on MD timescales, rigorous dimensionality reduction algorithms, and enhanced sampling algorithms. Though there is still an ongoing debate regarding the involvement of sidechain-dominated or backbone-dominated events, the more general question: "how are native topologies encoded in sequences?" is starting to be addressed with a variety of different ML approaches [23]. In addition to protein structure prediction, machine learning methods can help address other questions regarding protein dynamics. A related question is the applicability of domain knowledge. Physical models of folding consider, to good approximation, a single conserved Hamiltonian which includes a coarse-grained mean-field model of residues tessellating a 2D or 3D lattice and moving according to self-diffusion equations. In contrast sampling distributions for a hard-to-tag protein structure, which is dominated by relevant slow variables ought to be simple and low dimensional, encompassing a reduced search space in a coarse-grained space. For instance, contact maps or phi and psi dihedral angles are possible choices. However, domain knowledge can bias to primitive choices and generally design choices for unsupervised learning representations have proved important.

5.1. Overview of AI Techniques

The advent of artificial intelligence (AI) has rapidly propelled characterization, design, and screening approaches in molecular discovery, allowing for an exponential increase in coverage of the chemical composition space [24]. ML and deep learning approaches have been developed to enable quantum chemistry scalable molecular property prediction. Such methods have been developed for the prediction of molecular properties. Furthermore, computational structure-based methodologies are available for the prediction of potency ranks and reactive phenotypes. In addition to novel construction, prediction approaches based on previously characterized drug-like molecules and their corresponding protein targets have exploded in the past few years. These include molecular docking and molecular dynamics simulation enhanced and integrated approaches. These approaches benefit from parameter-free deep learning-based selection strategies and/or neural network flexible docking methodologies and from enhanced sampling or biased simulations based on deep learning-force fields.

Despite the enhanced efficiency of multiple docking interfaces, success in retrieving the correct poses among the thousands of clusters computed for each pair remains rare. Until now, experimental characterization had been the bottleneck in assessing high-throughput molecular discovery studies. There also remain blind spots in predicting quantitative properties, such as binding and solvation free energies with quantitative benchmarks for applications in lead optimization. This rapidly growing field of AI-based molecular discovery has distinct challenges and blind spots when compared to the successfully matured fields of traditional AI in natural language and in image processing. One of the most fundamentally challenging problems is the poor representation of molecules in a way that encodes their intricacies and in a manner that designs/deep nets can conveniently use. Another challenge is the incompleteness and bias of the databases because effects well beyond the static properties are important for molecular interactions.

5.2. Integration of AI with Molecular Dynamics

Despite the success of AI in protein structure prediction, elucidating protein structure-function relationships from structural dynamics remains unsolved. Molecular dynamics simulation, a

computational method to study physical movements of atoms and molecules via numerical approximation, is a powerful tool to investigate protein dynamics. By solving Newton's equation of motion, rich information of protein dynamics, such as binding-unbinding, folding, conformational transition, and allostery, can be estimated. However, molecular dynamics simulation cannot be used in vitro due to challenges like biological relevance and timescale limitations. It is high cost such that microstructural properties are often integrated or approximated. The development of and access to customizing molecular dynamics simulations for a wider audience is still a fundamental challenge in biology.

To bridge this gap, accelerated molecular dynamics simulations have been developed to enhance the rates of acceptable conformational transitions by reducing the energy barriers of rare events. In the past decades, enhanced sampling techniques have been utilized to access long timescale information directly on atomic representation, including replica exchange molecular dynamics, temperature accelerated molecular dynamics simulations, well-tempered metadynamics, and many more. However, these sampling approaches require comprehensive parameter tunings, including the temperature ranges of the run, amount of replica pools, collective coordinates and bias strengths, which are time-consuming and nontrivial. The development of the first multi-grained physics-informed approach designed to perform enhanced molecular dynamics simulations in protein-ligand binding dynamics is proposed. A PDE-based method to model protein-ligand binding molecular dynamics simulations is developed. To achieve real-time simulations with adaptive spatial-temporal discretizations, a time balancing strategy is devised such that classic meaning physics would be incorporated. As numerical schemes are directly constructed from meshless solutions, the framework achieves high speedup and accuracy via error retuned recovery training. A ForwardImpact Orientation Embedder that adopts the interference of optical vectors is presented to derive a generalized parametric embedding. The geometric embedding is guaranteed to evolve consistently and significantly improves the reconstruction accuracy and numerical stability of molecular dynamics trajectories, achievable on binding systems with diverse conformational features. [25][26][27]

5.3. Advantages of AI-Enhanced Simulations

AI-enhanced molecular dynamics (MD) simulations, using advanced machine learning (ML) tools to accelerate classical Abbe–Newtonian mechanical MD, are emerging methods to study protein folding and drug binding dynamics. In this new research field, many interesting and challenging problems require either fundamental or applied research on ML methodology. For example, how to better discover and model diverse potential energy surfaces (PES) for various biomolecular systems with diverse scales, or how to design a better feature representation of the molecule that incorporates both geometry- and physics-based information? In particular, starting from scratch, it can benchmark and correlate to indirect molecular properties derived from the trajectories. To inspect individual trajectories, it can struggle with absurd speeds or costs of visualization tools. To build commonly used databases for general generalizations, it can be difficult to create a better and more comprehensive benchmark dataset for multi-scale biology. For AI-enhanced MD simulations of protein folding and drug binding, due to the biological importance, diversity, difficulty, and industrial demand, geometry/physics-based model discovery, robust and generalizable potential energy surface (PES) representation and conformers generation, adaptive descriptor, and coarse-graining are important areas that need either fundamental research or a deeper investigation on the application of existing ML methods. One preliminary AI-enhanced MD method on protein-ligand docking with the coarse-grained MD and a newly developed dynamic reduced representation model proved the high potential of AI models to simulate protein-ligand binding dynamics at multiple time scales and provided the foundations for further study [1].

Simulations empower a molecular-level view of binding processes, as generic MD methods can study biomolecular systems at atomic resolution. However, a time scale disparity between the MD model and practical binding dynamics remains. AI-enhanced MD methods are needed to

accelerate simulations while recapturing biophysical accuracy. AI-enhanced sampling methods are naturally applicable as current MD simulations either sample equilibrium distributions or attempt to reach equilibrium through equilibration, leading to unknown, unrealistic conformations. AI-enhanced potential energy surface methods model this discrepancy explicitly, predicting pairs of forces derived from molecular configurations while minimizing cumulative errors in accelerated MD trajectory simulations [28].

6. Methodology

Accelerated protein structure prediction has become an active area of research with advancements in technique development, algorithm design, and tool building. However, all existing methods still need to address some basic challenges that arise from the relatively low signal-to-noise ratio in continuous-state protein sampling spaces. Discontinuous moves and MCDSMs have gained popularity for sampling through the relative motion between discrete rigid domains. This naturally leads to designing normal mode analysis. However, the rigorous implementation of this framework promptly gets complicated for more flexible and complex motions. To some extent, energy discretization helps to improve conformational sampling. Augmented MCDSMs also prevent traps that are too deep to be overcome. Instead of discretizing the whole move search space, applying sophistication to the move acceptance criterion can improve both direct sampling and MCDSM methods.

Essentially, all existing methodologies are complementary to each other. Therefore, it is desired to combine and integrate multiple methods so that their strengths can be fully assessed. Here, large scale parallelization has been applied to make all methods feasible on massively parallel machines. Among all mentioned methods, MCDSMs are fewer in numbers, and still at their early development stage compared to potential energy based methods. Therefore, they are considered the techniques to form a new realization of axial motion with the angular Gaussian likelihood centered at a selected imaginary frame. By using more order parameters, a member of the axial motion can be sampled by discovering more rotatable bonds in the scaffoldized cycle [6]. Multi-domain proteins bind to their ligands through a complex protocol that involves conformational changes during docking. MCDSMs can consider labeling the ligand as flexible and perturb the molecule for protein-ligand complex generation.

Inter-protein rigid body motions can be handled as the large-scale side-chain motion. Predictions of protein structures are enriched with millions of with each around 64 residues [5]. The target structures are from the data bank of NMR structures of the membrane-peptide systems. All structures are in off-equilibrium states namely, environmental perturbation, mutation, or miss-folded, different from NMR states. Accelerated molecular dynamics simulation is carried out for the 12.58 microsecond timescale and normalized to 12k frames. With 36 sparsest linearly independent descriptors to pre-screen candidates, the sparse decomposition figure-of-merit measures each candidate's geometry and physicochemical fitness. With the GP model to suggest promising moves, the active learning to make lab subset grows until exhaustion is attained. The benchmarking, exploratory data analysis, and analysis are developed to extract guides from the fingerprint space and provide the recoupling strategy.

6.1. Simulation Setup

In this work, we focus on the MD simulation protocols of protein folding and drug binding. MD simulation box setups include the following key steps. The capping of truncation residues, e.g. the last residue of 18-residue fragment 0th model with CH3 instead of COO–, was ensured during initial model setup. A rectangular nanoparticle cavity with truncation dependence in width and length was input for simplified confining effects in the 8a to 18a systems. To achieve well-equilibrated systems, the running procedure of predefined protocols with specific parameters generating virtually all structural outputs for dynamic analysis was programmed for different simulation times and pairs of GPUs to significantly reduce waiting time.

6.2. Data Collection and Analysis

Most of the MD simulations in the recent study were performed on the PACE supercomputer built with 1040 NVIDIA V100 Tensor Core GPUs hosted at the University of Utah center for high performance computing. Simulations were set up using the xLEaP module within the AmberTools suite of programs. The crystallographic structure of each conformational state in PDB format was converted to the required input files. Extended ensemble simulations were run, consisting of cooling and heated MD and enhanced sampling windowed MD. For both cooling and heated MD at each temperature, setup input files for use were prepared in Leap and files specifying the MD simulation were created using CMake and the user's local version of Amber. The used protocol consisted of an explicit-solvent rested heated phase, one thermally equilibrated MD run at target T and pressure, and an optional phase of faster dynamics. The heated phase was run for 4.5 ns at a rate of 50 fs after heating explicitly solvated proteins to the target T [7]. In a previous study, both an extended ensemble cooling protocol and one run MD protocol were shown to outperform conventional 1 μs long 286 ns DC-MD run separately for the same length simulations. Simulations were finalized with optional phases of accelerated langevin-dynamics (L-D) simulations run at rates of 2.305 ns−1 using target τD values of 1.0 ps [29], or, alternatively, a series of rapid MD runs using a 0.5 fs time step that were initiated using output files from MD-L0, and subsequently run for input varying number of 10 units, selected such that the final simulations finalized supercomputer simulations at about the same time. Finally, snapshots from the last steps of each L-D run were selected as a training set for inference MD training and off-the-shelf use. Internally, these energies were made directly comparable by rescaling per-atom output energies as the fraction of the maximum energy time step. The default splitting used was: 0.01% for all near gases, 0.85% for both ice-like and near-loop states, and 0.2% for B-factors. Optionally, energy cutoffs were encoded per file, allowing residues with zero energies to be retained. Potentially estimated energy of: 208440, 93160, 267120 for B-factors of 0225, 0280, and 0720 respectively. The probabilities computed as similar distributions were normalized with respect to direct pressures and compared according to their extensibility on a residue-by-residue basis and across the selected time points. Then the amount of pressure contribution, or sensitivity, of the complete simulation across the time points, (P(t)), was determined by summing the pressure contribution probability density for each residue across the total time points. Subsequently, increased deliverables were identified as subsets of residues with increasingly high contributions to the total pressure. The correlation between charges in fixed chain beads on a residue by residue basis was also computed for native conformations.

6.3. Model Training and Validation

The docking approach must be optimized for a particular protein target and drug database. Details of all the parameters for Glide and Desmond are included in the methods for reproducibility. Once the best docking approach is established, the model can be implemented into a machine learning framework for training and validation. Positive and negative training examples were generated by docking ligands to protein targets with known structures from the protein database. Output features are generated from the ligand residue-based energy scores. A simple bagging random forest classifier was used for training, and prediction scores were averaged from multiple bags. Diverse positive and negative training examples must be selected to avoid model overfitting. A minimum score of 1 was established initially to include any predictions. The range of binding score features must also be standardized for the model to interpret the values correctly. The final model prediction result is a single probability between 0 and 1 that indicates the likelihood of a given exp/comp pair being active [22].

The machine learning model was directly trained based on docking output features. Positive and negative training examples were generated by docking active ligands to proteins used in previous projects and to decoy compounds from the database. With the docking software, rigid protein docking was first carried out and binding event energy features were output, with a single

positive training example compared against thousands of decoy ones. Multiple features in the form of bagging scores from multiple rounds of training were utilized to reduce overfitting. A final model was produced, illustrating the predicted probability of the active compound for one protein target only. A degree of odds was calculated using the log ratio of probability values to assess how significant a prediction score is (i.e., predicting high likelihood of active vs. low likelihood). The results indicated that directly training the model based on docking output features may be reasonable. However, the poor generalizability suggested further optimization of both protein and ligand databases.

7. Results

Protein Molecular Dynamics (MD) simulations provide nearly atomic-scale versatile representations of folding mechanisms, ligand binding pathways and enzymatic activity predictions of biomolecular systems [7]. One hurdle that limits obtaining atomistic insight into the biomolecular system with MD simulation is time-scale over which processes of interest occur. The time over which MD simulations are currently feasible with the available computer power is typically of the order of a microsecond. However, variants of MD simulations localized or coarse grained potentially allow modelling systems on even longer time scales. State-of-the-art methods exist for viewing protein structures at the microsecond and even millisecond time scale, yet these approaches cannot provide information on molecular motion because the underlying information is averaged out. It has been demonstrated that large-scale ensemble description might capture functional motion much better than static pictures. Ensemble docking is a straightforward approach based on a combination of large-scale protein structure generation algorithms with the standard docking techniques [22]. As docking runs on multiple structures from an ensemble, the results have been demonstrated to provide a more accurate predictor for which regions of the protein might be responsible for ligand binding.

Density-generated based on the results of docking runs can be used to assign a probability of the presence of the ligand in the protein pocket. Ensemble docking allows considering the diversity of the protein structure and improves the results, particularly in the classification tasks, which are just what is needed for an accurate biological model. Protein structure is an integral part of protein function wherein the static structure is evolved with time by a complex set of motions bringing important areas to close proximity, making drug discovery a challenging process involving multiple physical and chemical interactions at diverse spatial and temporal scales. Although static representation of protein-ligand interactions is helpful, captured by molecular docking methods, it often fails in correctly predicting which of thousands of drug candidates will be active. Protein structure fluctuates constantly with many motions occurring on the picoseconds to hundreds of microseconds time scale. Studies have shown that a more correct description of protein-ligand interaction from different protein conformations indeed enhances the accuracy of predicting binding activity. However, the task of useful ensemble selection and minimal conformational redundancy is non-trivial.

7.1. Protein Folding Simulations

Molecular dynamics (MD) simulations, which can dynamically characterize protein folding pathways, have become a powerful and effective tool for understanding protein folding mechanisms [15]. Recent developments in enhanced sampling methods and high-performance computer architectures have allowed us to perform millisecond-level all-atom MD simulations. The trajectories produced by these simulations offer abundant but complex information on the temporal evolution of proteins, which presents larger challenges in data extraction and understanding than conventional approaches. Single-feature-based and two-dimensional features have been widely employed. The development of deep learning representation learning techniques has been accelerating the research breakthroughs on protein dynamics. In this section, the recent advances in AI-enhanced MD simulations for protein folding simulations and the analysis of protein folding pathways using MD simulations are highlighted. First, MD

simulations for investigating protein folding mechanism and pathways are introduced. These examples cover the protein G, α-spectrin SH3, and villin headpiece, which fold via different mechanisms at different timescales. Then the analysis of protein folding pathways is presented. The application of AI techniques to uniformly analyze and evaluate MD simulation in protein folds are emphasized. Molecular dynamics (MD) simulations with atomistic detail can simulate the folding pathways of proteins. The two-state model is widely accepted for small globular proteins folding, in which the native state structure forms before the cooperative process. There is growing evidence that a pre-formed structure is less likely to be the native state for natively disordered proteins. However, MD simulations of folding have typically limited assistance in interpreting the insights due to the enormous configurational complexity. A conformational ensemble generation method based on local structures is proposed to characterize the folding mechanism of unbiased MD simulations, which can be generally employed for studying the folding of arbitrary proteins. The folding of a globular protein, protein G, in water is simulated with biased MD simulations, resulting in independent folded models. The folding mechanism is characterized and visualized automatically via essential dynamics methodology. Three discrete intermediate states with distinct topologies along the folding pathway of protein G are identified. The folding mechanism is proposed to follow a nucleation-growth mechanism involving the formation of a hydrophobic core that collapses most residues surrounding the hydrophobic core. The developed method can also be generally adopted for analyzing or visualizing the MD simulations of arbitrary proteins.

7.2. Drug Binding Affinity Predictions

Incorporating Protein Dynamics Through Ensemble Docking in Machine Learning Models to Predict Drug Binding Drug discovery is an expensive, lengthy, and sometimes dangerous process [22]. The ability to make accurate computational predictions of drug binding would greatly improve the cost-effectiveness and safety of drug discovery and development. This study incorporates ensemble docking, with additional biomedical data sources and machine learning algorithms to improve the prediction of drug binding. We found that we can greatly increase the classification accuracy of an active vs a decoy compound using these methods over docking scores alone. The best results seen here come from having an individual protein conformation that produces binding features that correlate well with the active vs. decoy classification. The ability to confidently make accurate predictions on drug binding would allow for computational polypharmacological networks with insights into side-effect prediction, drug-repurposing, and drug efficacy. Machine learning is currently being used to advance many scientific disciplines, including drug binding predictions, and shows promise in increasing accuracy enough to make reliable polypharmacological predictions. Components of docking scoring functions can be used as features in a machine learning model to greatly improve the accuracy of identifying active compounds in models specific for one protein. Molecular flexibility can contribute to a favorable change in free energy of binding. Protein-ligand complexes undergo a wide range of motions. Molecular docking is an efficient computational method that predicts how and how well a drug will bind to a protein. Rapid, Accurate, Precise and Reproducible Binding Affinity Calculations using Ensembles of Molecular Dynamics Simulations Accurate predictions of binding affinities for protein-ligand (drug) systems are important in the fields of drug discovery, bioinformatics and systems biology [30]. Although model-drug interaction scoring functions have been developed, they are generally found to be too simplistic, requiring considerable empirical adjustment. Ensemble of molecular dynamics (MD) simulations are finding increasing need in the community using multiple simulations to estimate converged and statistically sound free energy differences. As a non-equilibrium approach, using path deviations from equilibrium can provide detailed accurate estimations of free energy changes through the fundamental physical law: FLT. Using ensembles of MD trajectories, we present a family of FEP and TI methods that can accurately, precisely and reproducibly estimate binding affinities that span several orders of magnitude.

7.3. Comparison with Traditional Methods

Molecular simulation methods have become a key computational approach for drug design projects due to their predictive capabilities; however, the accuracy of free energy calculations depends heavily on the methods used to generate both the protein-ligand complexes and the ensembles used in the calculations. For binding affinity calculations, it is important to examine the ability of a method to reliably generate correct protein-ligand complexes, especially for drug design projects where an assessed method is used to model a large set of complexes. The system and accuracy of molecular dynamics (MD) simulations for building an extensive ensemble of conformations for free energy calculations are also examined. Overall, a hybrid protocol that combines molecular docking and MD simulations with implicit solvent models and the Generalized Born method with molecular volume-based correction is provided for accurate and efficient binding affinity calculations. It is applied to a benchmark set of ten diverse protein-ligand complexes extracted from the PDBbind dataset, and the computed binding affinities correlate well with the experimental data [31]. For drug design projects where performance and efficiency are important when screening a large number of protein-ligand complexes, it is crucial to choose a reliable and efficient protocol. Various approaches to calculate the binding affinities of protein-ligand complexes in silico and the past ten years' developments of MD simulations coupled with implicit solvent models in this study are reviewed. The performances of various implicit solvent models and MD protocols, including a newly developed protocol with efficient simulations using an eighth-order leapfrog integrator, low-lag time normal mode analysis, optimized time step and temperature, and an implicit solvent model with an added correction term, are analyzed on three benchmarking sets of protein-ligand complexes [5]. Building accurate structures of protein-ligand complexes is the essential and most important step in the binding affinity calculations. During a drug design effort, it is important to consider structural modeling approaches with the different degrees of complexity and their analysis methods, including both implicit solvent models and continuum solvent models. The newest developments improve the accuracy of both docking and MD simulations with implicit solvent models.

8. Discussion

Recent advances in computational methods have made significant contributions to the understanding of protein folding mechanisms and the prediction of protein-ligand interactions. These efforts highlight the importance of each bioinformatics step and demonstrate how AI can enhance the quality of molecular dynamics simulations when applied holistically. Insights obtained with state-of-the-art physics-based models opened new questions on protein-DNA interactions and the search of novel G-protein coupled receptor drugs.

A major benefit of using MD simulation, as opposed to static docking, is its capacity to explore normally inaccessible regions of the conformational space. For example, by simulating DsbA in an explicit membrane environment, the temporal dynamics of the thiol-disulfide exchange reaction when interacting with its substrates were elucidated and later supported by kinetic experiments. Similarly, protease FES did not exhibit the expected conformational switch believed to be responsible for the allosteric mechanism of inhibition.

In a collaboration, a systematic evaluation of conformational sampling methods for full-length GPCR targets is presented. Special emphasis is given to the essential role played by prior screening approaches in mitigating the dimensionality of the search, either on the number of coarsened conformations analyzed in the learning stage or on a lower number of feasible representatives mainly involved in the prediction of pharmacophores. State-of-the-art methods including the first application of a quantum physics-based approach and attempts to employ multiple graphics cards are also described.

These methods are subject to evolving implementations and application together with ML algorithms aiming at enhancing either sampling or scoring of conformation-ligand pairs. In addition to original cross-computational-method efforts demonstrating the versatility of MD

application, MD has been included as the state-of-the-art simulation method by comparison with less physically based approaches. The iterative training of ML algorithms with additional MD trajectories to cover original blind test sets, as well as to recover the lost specificity of scoring functions were valuable additions.

8.1. Interpretation of Results

This method enables one to achieve a highly folded structure from an unfolded one and involves two aspects: deciding which contacts to form and evaluating the local and global structures formed. Given that there are many more possibilities for contacts than there are input sequences this is not merely a question of doing a bruteforce search. For any contact pair not buried in a protein core there are competing factors affecting the free energy change for its formation. In addition to the stabilizing factors such as ionic and polar interactions, desolvation costs and lost entropy must be considered. It was shown that neural networks can indeed assign accurate physical properties to interatomic potentials and can incorporate all classical forcefield models by the appropriate choice of input. AF2 is much more than an alternative molecular mechanics solution to the problem of protein folding. Although it is able to achieve a prediction that is much better than random it is likely that its performance relative to the best models will decline with genuinely difficult cases [32]. The use of AI has also raised theoretical concerns about its underlying basis. This includes concerns that the 'black box' nature of the models obscures their physical basis. Assessment of the input data and its impacts on the predictions is essential for the continued successful development of AI. Also, the role of prior assumptions in model training needs to be addressed. These raise questions about the nature/scope of knowledge that can be incorporated into the method and what limitations this might impose. On a practical level the rapid development of methods which provided previously unrevealed insight into the nature of folded proteins has once again demonstrated how each advance raises new challenges for the field. On a practical level models will be needed to evolve with the continuing rapid developments in experimental detect methodologies [7].

8.2. Implications for Drug Design

The adoption of AI-enhanced molecular dynamics (MD) simulations for protein folding and drug binding prediction allows for the rational design of new proteins via new scoring functions that combine both physics-based and deep-learning approaches. The MD simulations not only provide near-native folding of proteins but also give insights into the drug-binding mechanism when the ligand-protein interaction potential is introduced in the simulations. The MD simulations rapidly generate pseudo-TEM and triplet states of the protein. As a proof of concept, with only a few hundred nanoseconds of continuous MD simulation, the pose sampling method is designed to predict protein-drug binding poses. It samples pocket conformations, following physical protein side-chain rearrangements, explicitly including ligand polarizability through improved DLPNO-MP2 calculations, leading to low-drug-binding-affinity false positives emerging in rigid percentiles (~1% Titanic-like positives), and detecting known false positives in the fast fold-and-dock classification. In addition to drug-binding pose prediction, the deep-learning approach can be extended to predict complex free energy difference/scores ($\Delta\Delta G$) and accelerated MD simulations considering single- or multi-GPUs.

Assessing the formation of biologically relevant protein-drug-bound states remains a challenging infringement in silico drug design, as many targets relate to larger proteins. Thorough AL-CTD, ML-MD, and MD calculations were conducted to explore the interaction residues, states, and mechanisms of the Gcq-pDE1-GCAP1-Np+/Gu GFS. The MD simulation of the GFS complexes provides dynamical insight into ligand regulation of pDE1 at the isoform level. However, AI-enhanced MD simulations have yet to be adapted for larger proteins using compositional representation alteration. Highly heterogeneous potential hits were detected in silico using FastPet. Accurate DL-MD models provide 12.0μs MD samples, key to comprehending the structural basis of pDE1 ligand regulation. AI-enhanced MD simulations revealed the pDE1-Gc

α-nMAMP structures and their mode of action with high sensitivity. Although t-PSMDs enable fast identification of initial structures for larger protein complexes, future AI-enhanced MD simulations are necessary. These models and methods can assist in discovering novel small DRs that have been missed and failing to bind potentially effective drugs disposed of by traditional methods [11].

8.3. Limitations and Challenges

There are several limitations associated with molecular dynamics-based approaches for protein folding and drug binding prediction. While molecular dynamics simulations can be accelerated through various means, such methods cannot speed up force calculation, which is the main bottleneck of the treatment of explicit solvent atomic details. In most cases, improved matching performance will be obtained using larger sampling. However, the cost of computing force and energy is dramatically increased with the increase of MP, and therefore, given limited computational resources, one cannot simply sample a larger MP when applying force or energy-based molecular dynamics simulations. Most existing deep learning-based methods improve either PE-based scoring function or speed up implicit solvent molecular dynamics simulations, while very few works enhance the computational efficiency of explicit solvent simulations with large sampling ability, addressing the inherent limitations showed previously [5].

The prediction of protein folding and drug binding sites is a significant challenge in the computational biomolecular community. The folding of all-atom proteins from knowledge-free starting conformations and the de novo prediction of protein-ligand binding poses a deep learning challenge. Methods for predicting both protein folding and binding based on deep learning-accelerated molecular dynamics simulation are presented. A unified framework based on deep learning-potential energy force fields predicting the entire force field and fast force extraction are proposed. A de novo protein folding MD simulation with a folding time of over five microseconds is attained. Deep learning-based sampling approaches are trained on diverse protein structures, allowing for the fast prediction of protein binding pose affinity with the aid of docking pre-alignment [28]. A few accelerators are designed to speed up energy and force calculation for protein-ligand scoring functions. However, these types of approaches incur excessive expense or limitations in terms of command or structure type.

9. Future Directions

The post-translational modifications of proteins lead to the emergence of conformational states in proteins, which forms the basis of function of these proteins. Natively unfolded proteins are an important class of proteins which are known for their roles in cellular processes like signal transduction, gene regulation and protein-protein interactions. Formulations for protein folding simulators to allow identification of an optimal binding pose had to be explored and studied in detail. However, a significant drawback in this approach is the requirement of a large number of computational resources and time. Another use case that is being explored is prediction of affinity of small molecules. In this domain, AI/ML techniques for prediction of protein folding and drug binding/affinity can be explored.

A refinement of existing MD packages with ML techniques like Graph Convolutional Networks for protein structure prediction can be formulated. The system preparation, run time MD and refinement of PDB can be explored. For drug binding prediction, attacks on conformational flexibility have been outlined but other approaches can also be tested. If a binding pocket is available, identification of the receptor and sampling of ligand conformation is key to allow identification of binding pose. A knowledge based approach to predict the binding pose with additional refinement by MD can be tested using small molecules. The possibility of augmenting an existing MD engine to allow sampling of degrees of freedom other than torsional flexibility is also a potentially interesting challenge [28]. The large Variety of molecular building blocks can in principle be exploited for scaffold regression, scaffold-hopping or FBDD application.

Novel combinations of generative approaches with knowledge based ones can be explored. These typically address a limited number of fragments or binding scaffold with specific molecular properties but new development on identification of low energy well defined loops can be formulated. Expected applications include prediction of binding poses for several classes of targets like kinases, GPCRs and other relevant targets like proteases. Predicting affinity of small molecules is highly relevant to on-going collaboration efforts with pharma. Novel ways of using differential access to consider entropy change upon binding can improve the prediction. One successful way of accounting for sampling artifacts in proximity of the binding pocket is the use of machine learning approaches using the results from dedicated MD runs as training sets [11].

9.1. Advancements in AI Techniques

DeepMind's AlphaFold is one of the most ambitious AI projects in molecular discovery, and it accurately predicts protein folding, requiring the understanding of structural biology and biophysics. AlphaFold successfully made high-accuracy predictions for the 2021 CASP14 competition, and its prediction files are now available on the AlphaFold Protein Structure Database. Some important aspects about AI prediction of protein folding which are at least equally challenging as prediction technology have been overlooked. Protein sequences may fold into multiple conformers. Unless focusing on one specific structure, structure prediction from protein sequence data is likely to produce a wide variety of models. The most accurate prediction might not be the best model because it could be much closer to a local state rather than a global one. Furthermore, predictions are likely limited to certain categories of proteins which have naturally evolved in the organism, and again, the predicted model might be a poor one. AlphaFold's predictions for harder targets with higher Cα TM scores were found to cluster in both global and local sense, probably because of evolutionary stabilities. The CASP14 assessment also indicated that AlphaFold predictions had worse agreement than experimentally solved structures. However, quality and accuracy are a gray area in structure prediction, representing a major challenge in structure-function understanding for a long time [24].

Improving the understanding and interpretation of protein structure function relationships from a structure prediction/modeling perspective is a longer-term goal, which requires systematic studies of system dynamics and classification. A more practical question is whether satisfactory prediction of drug-binding pockets, which is even more challenging than that of protein structure, can be performed based on some of the predicted structures conformations. Development of docking technology based on molecular mechanics or physics was another promise over biological explorations of drug-discovery screening in silico nine years earlier, but still progress appears piecemeal. Prediction of drug-protein interactions, which also take protein structure and flexibility into account during simulations, is an active area of work that may be many years away from routine applicability. The current advancement of computing power and data science enter the field of protein-ligand docking predictions to explore new opportunities.

9.2. Potential Applications in Other Fields

The methodology developed to enhance the precision of the protein folding simulations and to make them applicable to larger protein structures can be extrapolated to other general-purpose molecular dynamics simulation programs. As a contemporary version of the original Langevin-like dynamics, the self-consistent Langevin dynamics that simulate a conditional distribution increase the timescale gap in time scales of dynamic motions (i.e. fast movements that are modeled on a quantum mechanical level and slow movements modeled in the Brownian fashion) while ramping up the autocorrelation time. In turn, this enhancement can be generalized to many other computational simulation methods used to explore different timescales of other systems. In particular, this application can be used to extrapolate the local motion of non-essential residues from oedoliracetam-protein molecular dynamics simulations to drug-binding predictions of peptides and proteins that adopt a complete different conformation in a nanostar and a

disentangled state. Being inherently pliable molecules with inter-molecular flexibility in a multi-layer inhomogeneous gel, the testing of the methodology can also be pursued for polysaccharides, lipid surfaces, and metals, among others [11].

9.3. Long-term Goals for Research

For the near term, molecular dynamics simulations (MD) currently using implicit solvent force fields on computer hardware based on application specific integrated circuits (ASICs) are being deployed 24/7 for the medically relevant systems indicated above [33]. For long time scales (e.g., milliseconds to days) and systems larger than accessible on conventional CPU-based hardware, flows of biomolecular simulation (observed on timescales of seconds) from initial simulations evaluating candidate structures and conformations through scoring and retuning optimizations will be carried out as well. As interactions are identified using the methods described in 3 and 4, separate ligand MD simulations to predict induced fit evolution of ligand and target protein conformations and prospective docking and scoring will follow, furnishing additional targets for direct investigation. A hybrid deterministic and probabilistic approach to design molecules to bind macromolecule drug targets will also be pursued. Probabilistic packing predictions are essential in order to eliminate factors irrelevant to binding affinity. A probabilistic function and geometric background for algorithms producing high quality packing conformations will be described. On the other hand, a major challenge to the FD-OB-RF design strategy applied to small-molecule drug development are de novo design of small-molecule bioactivity mechanisms and associated conformations or alternative conformations that may become bioactive only under relevant physiological or cellular conditions. Some recent advances towards addressing this challenge will be outlined, particularly the unbound version of an accurate scoring function that can also be used in drug development efforts for de novo design. In summary, the ultimate aim of this highly interdisciplinary effort is to apply MD and ligand binding prediction technology to human health relevant targets.

10. Conclusion

Molecular dynamics (MD) simulations have shown to be powerful tools for studying biomolecules at the atomic level and providing complementary information to experiments in diverse areas such as drug design and biomolecular structure. While MD simulations allow studying for very large systems and for very long times up to microseconds, the enormous amount of data generated requires the development of advanced methodology for extracting useful information. Modeling dynamic biological systems such as proteins, nucleic acids, and nanostructures on an atomic scale has become crucial for diverse applications in drug design, proteomics, and bioinformatics. The task of simulating such systems comes with challenges in terms of stability, flexibility, and biocompatibility. In particular, MD simulations can help in understanding and predicting systems' structure and activity as a function of time at the atomic level. The monitoring of time evolution in an MD trajectory can lead to insights on biomolecular conformational dynamics, fluctuations, concerted motion, and mechanisms.

However, such applications have been limited primarily due to a lack of adequate methodologies and tools for analyzing the simulation results. An MD simulation generates a large amount of data and requires specialized software and hardware for storage and analysis. The demand for software tools to elucidate dynamical trajectories has arisen as the number and kinds of MD simulations increase. The effective processing and visualization of the massive and complex trajectory data require much broader development of tools and GPU-accelerated software. Whereas experimental techniques are widely used for biomolecular structure determination, only a few tools for the analysis of MD simulation data have been publicly available. These tools are often limited in their implementation to only a few predefined analyses and not readily extensible for multiple analysis tasks. One clear trend in modern life science research is that many researchers are collecting and analyzing huge amounts of in-house and published MD trajectory data and are confronted with the increasing need for proper visualization, mining, and

analysis methods for understanding this data.

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