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Chapter 1

Advances in molecular dynamics simulations and free energy calculations relevant for drug design

Nadine Homeyer & Holger Gohlke

Drug compounds and their biomolecular targets are no rigid objects, but exhibit, due to motions on the atomic level, dynamic changes in their structure. Molecular dynamics (MD) simulations describe these motions using the laws of classical mechanics. Simulations considering the dynamics of ligand–target systems provide information that is not accessible from static structures. As such, MD simulations can reveal transiently accessible binding sites, give insights into the process of ligand binding and release, and provide information about dynamic receptor–ligand contacts. Furthermore, MD-based free energy calculations allow computing binding energies for ranking ligands according to their binding affinity to a biomolecular target. The applications of MD simulations and free energy calculations in drug design and the types of methods used are manifold. Here, we focus on recent studies and methodological advances that are likely to play a role in future drug design.

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Molecular dynamics simulations: general principles & applications to drug design

In MD simulations, motions in (bio)molecular structures are simulated employing atomistic models and the laws of classical mechanics. Structures solved by x-ray crystallography or nuclear magnetic resonance experiments, or generated by homology modeling, are used to set up model systems in which atoms are treated as balls connected by springs; the latter represent covalent bonds between atoms. The forces acting on the atoms and the new atom positions resulting from these forces are repeatedly calculated based on Newton's equations of motion employing a potential energy function and a set of parameters describing the properties of the atoms and their interactions [1]. By conducting these calculations multiple times, a trajectory is generated that shows the atomic motions with respect to the simulation time.

As drug candidates and biomolecular drug targets (e.g., enzymes and receptors) are, in general, inherently mobile, MD simulations can reveal changes in the structural features of ligand–target systems that are not observable in static structures. These changes include transiently formed ('cryptic') binding pockets and binding-related structural changes due to induced fit processes or shifts in the relative population of conformations. In addition, the thermodynamics and kinetics of ligand binding can be studied by MD simulations **(Table 1)**. In summary, taking into account the dynamics of ligand–target systems results in a more detailed picture and understanding of ligand–target interactions and, hence, the mechanism underlying a ligand's action. This knowledge is invaluable for the design of new drugs.

Identification of binding pockets by MD simulations

Multiple strategies to detect potential binding pockets for ligands on the surface of biomolecules have been developed, including shape complementarity-based, energy-based, knowledge-based, and sequence similarity-based approaches. However, to identify binding pockets that are only transiently present or only form upon interaction with a binding partner, for example, as observed in protein–protein interfaces targeted by small-molecule modulators, it is essential to take into account the dynamics of the biomolecular target [2,3]. Binding site searches conducted using representative conformational ensembles from MD simulations have led to the successful identification of previously unknown binding sites [2,4]. By applying a pocket detection algorithm in combination with principal component analysis and clustering, it is possible to select a set of representative structures with distinct binding pockets from MD ensembles and to obtain information about the population of binding

Table 1. Main areas of application of molecular dynamics simulations in the context of drug design.

Information derived from MD simulations	Application	Knowledge gained/benefit for drug design
Conformational sampling	▪ Binding pocket detection ▪ Analysis of binding paths ▪ Rearrangement processes	▪ Identification of transient binding sites that can be targeted in drug design ▪ Insights into the contacts and dynamic features essential for ligand binding ▪ Detection of structural changes associated with induced fit or shifts in the relative populations of conformations by ligand binding
Energetics	▪ Simulation of (un-)binding ▪ Free energy calculations	▪ Direct insight into the thermodynamics of binding ▪ Absolute or relative ligand binding affinity
Kinetics	▪ Sampling of (un-)binding events	▪ Ligand on/off rates; valuable for the design of drugs with optimal kinetic properties

MD: Molecular dynamics.

pocket conformations in these ensembles [4]. Furthermore, in a recent innovative study, it has been demonstrated that binding pockets on protein surfaces can also be detected by MD simulations of protein systems in a water–organic solvent mixture [5]. Small organic solvent molecules, such as isopropyl alcohol, have the tendency to bind to such pockets, and therefore assemble at the respective sites. In this study, known binding sites could successfully be reconfirmed as those regions where the organic solvent molecules were over-represented. To quantify the binding potency of the detected sites, the free energy associated with the over-representation of the organic solvent in a certain region was determined. The maximum binding affinity of a binding site was then estimated by summing up the free energies of all such regions of a high affinity area using cut-off criteria, which ensure that the size of the area resembles the size of a drug-like molecule. The determined maximum binding affinity can be used as a measure for the druggability of a binding site. Thus, the procedure allows probing the druggability of biomolecular targets with surface-exposed nonionic binding sites and can be valuable for the identification of new targets for drug design.

Detection of pathways to & from deeply buried binding sites

MD simulations have successfully been used to identify pathways on which ligands, for example, agonists, antagonists or educts and products of an enzymatic reaction, reach the binding site, or the bulk solvent. They

Random acceleration MD: MD simulations in which a force pointing into a random direction is applied to the center of mass of the ligand. If the ligand does not shift by a threshold distance in a predefined number of steps, the ligand is dragged into another, randomly chosen direction; or else the direction of the force is maintained. This allows simulating release processes that take place on time scales that cannot be covered by classical MD simulations.

are especially valuable for the identification of pathways that usually cannot be deduced from crystal structures such as tunnels that are only transiently open for a short period of time, exchange routes through the biomolecular matrix and allosterically controlled systems, where the accessibility to the binding site is regulated by an effector molecule [6,7]. Pathways to binding sites can either be detected by analysis of cavities/tunnels in structures from MD simulations (similar to the identification of binding pockets, see above) or by tracing the binding/unbinding process of a ligand molecule. As for the latter, it has been shown that the pathway of a typical ligand from the bulk solvent into the binding pocket can be observed in MD simulations without any guiding forces or preassumptions on microsecond time scales [8]. However, as the release of bound common-size ligands would require even longer simulations, which are currently not feasible without enormous computational effort, a common approach to simulate ligand unbinding is to apply a force on the ligand that 'drags' it out of the binding pocket [9]. A promising method in this context is the random acceleration MD simulation method, which has successfully been used to reveal pathways of ligands from deeply buried binding sites [6].

Knowledge of the pathways on which ligands access and are released from binding sites can help to identify structural features that are critical for ligand binding. These critical points can then be taken into account in the development of drug candidates against the respective target. Besides designing drug candidates that can easily reach a binding site, it is also possible to modulate the accessibility of binding pathways, thereby affecting the exchange of natural ligands and thus the activity of the biomolecular target.

Information about the kinetics & thermodynamics of ligand binding from MD simulations

Both the thermodynamics and the kinetics of ligand binding are important for the design of drugs with optimal binding properties.

Long, unbiased MD simulations can provide direct access to these quantities.

Steered, that is, nonequilibrium MD simulations allow, retrieving information about the energetics of binding.

Besides the thermodynamics of binding, the binding kinetics of drug candidates is also of great interest because such information can be used to design drugs that faster access a binding site or longer reside in it resulting in higher therapeutic efficacy [8]. If multiple binding/unbinding events are observed in

free ligand-binding MD simulations conducted without any biasing forces or restraints, on and off rates and association/dissociation constants can directly be extracted from the simulations [8,10]. As a large number of binding/unbinding events need to be sampled to obtain statistically significant values, the computational demand for such calculations is high. Nevertheless, it can be worth the effort to conduct free ligand-binding MD simulations for promising drug candidates or fragments because they yield extensive additional information, including structural data in atomic detail, of the binding process.

Aa Steered MD: MD simulations in which an external force with a defined direction is applied to a system. The application of the force induces and/or accelerates processes, for example, conformational changes or the movement of a ligand out of the binding pocket, so that events not observable by classical MD simulations can be studied.

Another less computationally expensive approach by which information about the thermodynamics of ligand binding can be obtained is based on the so-called constant velocity steered MD (SMD) simulations. In this type of simulation, a harmonic potential – a spring – is attached to the ligand, and the free end of the spring is moved with constant velocity along a predefined reaction coordinate, for example, determined with random acceleration MD. The force that acts on the ligand can be measured from the extension of the spring and is recorded in the course of the simulation [11]. The obtained force profile can be used to calculate the mechanical irreversible work that is required for undocking of the ligand. Furthermore, by sampling the unbinding trajectory several times and employing the Jarzynski relationship, the potential of mean force can be derived from the force profile. Thus, free energy profiles and binding affinities of ligands can be obtained from SMD. Recently, in a combined docking and SMD study, it has been demonstrated that it is possible to identify thermodynamically favored binding poses and to distinguish active from inactive compounds based on force/energy profiles derived from SMD [12]. Furthermore, by analyzing the unbinding events observed in MD simulations with respect to the force profiles, it is possible to identify anchoring points that are critical for strong ligand binding. This information is valuable for designing ligands with improved binding properties.

MD simulations for studying ligand-binding affinity by docking/scoring approaches

In addition to detecting a ligand's binding pose, docking/scoring approaches are capable to semi-quantitatively estimate the affinity of ligand compounds to a biomolecular target, thus enabling enrichment of actives among large sets of nonactive compounds. However, the accuracy of binding affinity predictions by these approaches commonly suffers from the neglect of the

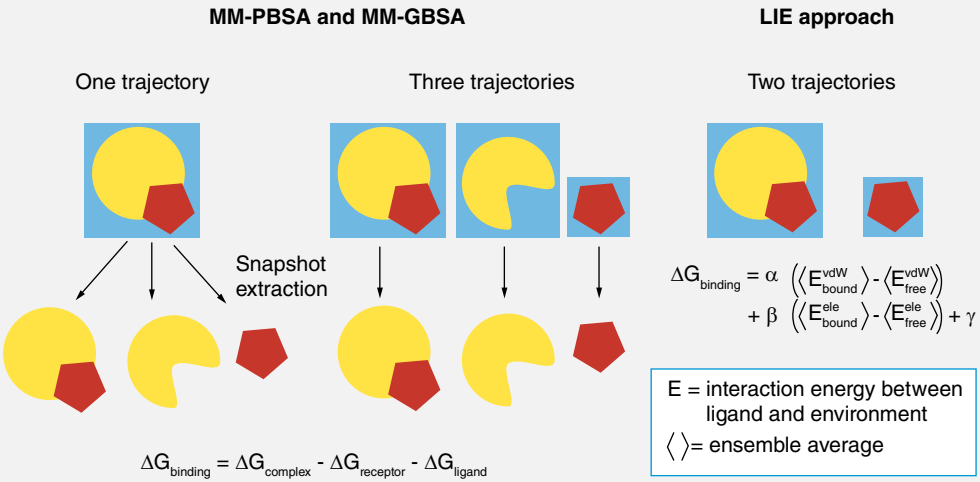
dynamics of the receptor and the usage of simplified energy functions optimized for high-throughput virtual screening. The first drawback can be overcome by using conformational ensembles of receptor structures generated by MD simulations for docking, as in the relaxed complex scheme approach [13]. The second problem is often treated by postprocessing of docking results employing one of the more rigorous binding free energy estimation approaches described in the following section.

Binding affinity estimation by end point free energy calculations

End point free energy calculation methods take into account only the end states between which the difference in free energy shall be determined – that is, in the case of binding free energy calculations, the unbound and the bound molecules [14].

Among the methods that belong to this type of calculations are the widely used continuum solvent approaches: molecular mechanics Poisson–Boltzmann surface area and molecular mechanics generalized Born surface area (MM-PB(GB)SA). They estimate the free energy of a molecule as the sum of its gas phase energy, solvation free energy, and configurational entropy. The solvation free energy is approximated by a continuum model describing the polar solvent properties and a term accounting for the nonpolar effects of the solvent. Free energy estimates computed by this approach for complex, receptor, and ligand molecules can be used to calculate the binding free energy as the difference in free energy between the complex and its unbound components **(Figure 1)**. To take into account the dynamic features of the molecules, the free energy is usually computed by averaging over conformational ensembles extracted from explicit solvent MD simulations. The ensembles can either be obtained from simulations of complex, receptor, and ligand (three-trajectory approach) or from a single simulation of the complex (one-trajectory approach). In the latter case, energetic contributions arising from the difference in the conformations between the bound and unbound state are neglected; however, as an advantage, noise from intramolecular energetic contributions is removed, and the computational expense is reduced by at least a half. Therefore, this approach is often used if the receptor and ligand do not undergo large conformational changes upon binding. Although MM-PB(GB)SA has been shown to provide good estimates of ligand-binding free energies, its importance for lead optimization will probably decrease because more accurate methods become amenable due to the continuous growth in computational power. In turn, future applications of MM-PB(GB)SA in drug design will most probably focus on the rescoring of docking results in virtual screening and on the investigation

Figure 1. Binding free energy determination by end point free energy calculation methods molecular mechanics Poisson–Boltzmann (generalized Born) surface area and linear interaction energy.



LIE: Linear interaction energy; MM-GBSA: Molecular mechanics generalized Born surface area; MM-PBSA: Molecular mechanics Poisson–Boltzmann surface area.

of individual energetic contributions to ligand binding by structural decomposition of the free energy [15,16].

Another popular end point method that is often used for binding free energy estimation in the context of drug development is the linear interaction energy approach developed by Åqvist and colleagues [17]. In this approach, conformational simulations are run for the solvated ligand and the solvated complex. Conformational ensembles extracted from these simulations are then used to compute average van der Waals and electrostatic interaction energies between the ligand and its environment **(Figure 1)**. In contrast to MM-PB(GB)SA, interactions with water molecules are explicitly taken into account. The binding free energy is assumed to be proportional to the sum of the van der Waals and electrostatic differences in the average interaction energy between the bound and the unbound state. The differences in interaction energy are scaled by weighting factors, that is, coefficients for which standard values have been established. However, it is under debate as

MD-based end point free energy methods consider dynamical properties of the ligand–receptor systems.

Due to approximations in the calculations end point free energy methods allow to estimate binding free energies at relatively low computational cost. However, the accuracy of the binding free energy predictions is limited due to these approximations.

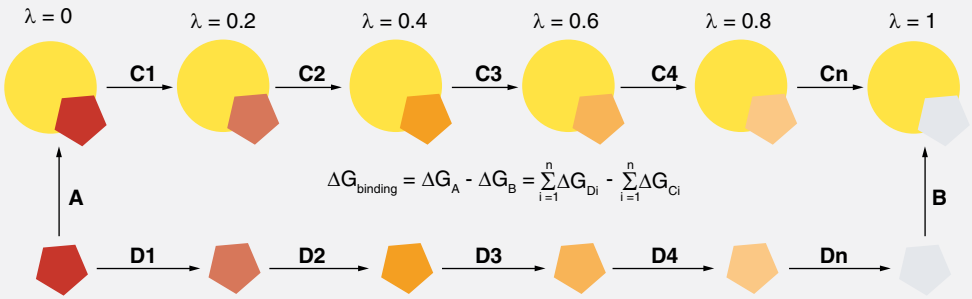
to what extent standard coefficients are transferable among different systems so that it may be necessary to determine coefficients based on a sample dataset of experimental values for each system for obtaining good binding free energy estimates [14].

Binding free energy determination by rigorous free energy calculations

Rigorous free energy calculation methods referred to as pathway methods determine the difference in free energy between two states by a gradual 'alchemical' transformation of one state into another. Absolute binding free energies can be computed by this approach via a thermodynamic cycle by decoupling a ligand molecule from its surrounding through a pathway of multiple unphysical states, both when bound to the receptor and when free in solution **(Figure 2)**. An order parameter (λ) is assigned to the transformation path and gradually changed from 0 to 1. This parameter describes the progress in the transformation relative to the end states. Simulations are conducted at a series of λ states to determine ensemble averages from which free energies are calculated. As large differences between the sampled λ states can lead to inaccuracies and slow convergence of ensemble averages, the annihilation of a ligand can require extensive sampling at several λs. Relative binding free energy calculations between similar ligands merit from converging relatively rapidly [14]. Such calculations in which a ligand is converted via a series of small 'alchemical' changes into another one are of special interest for the lead optimization phase of drug design in which binding affinities of structurally related compounds are determined.

The classical, well-established pathway methods comprise the free energy perturbation (FEP), the thermodynamic integration (TI) and the

Figure 2. Thermodynamic cycle used to determine the absolute binding free energy of a ligand by rigorous pathway free energy calculation methods.

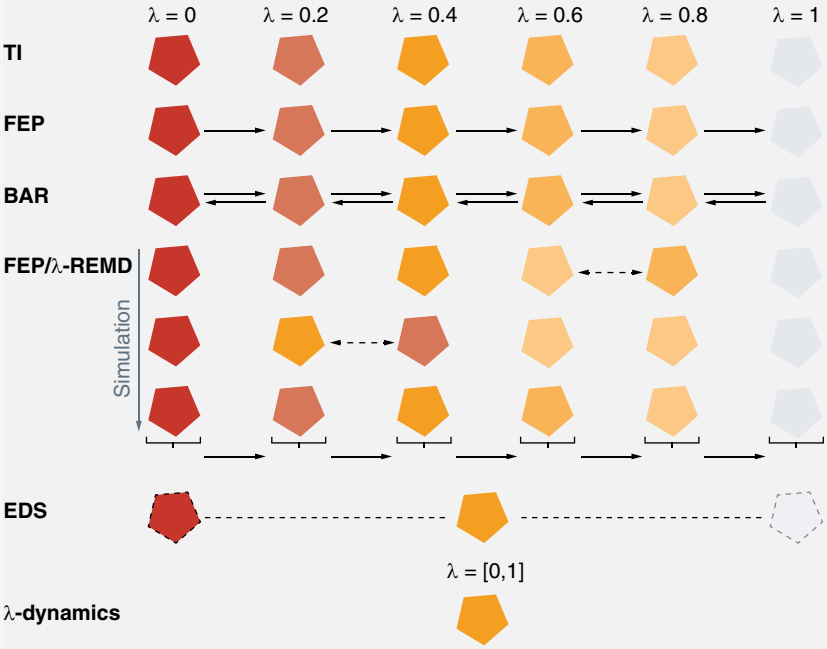


λ: Order parameter; Ci: Step i of complex transformation; Di: Step i of ligand transformation; G: Free energy.

Aa Free energy perturbation: this method, which was originally termed 'free energy perturbation', is now often referred to as the exponential formula or exponential averaging method to differentiate it from other perturbation approaches.

Bennett's acceptance ratio approach. In FEP, the difference in free energy between two end states, that is, the terminal states of one horizontal arm of the thermodynamic cycle in **Figure 2**, is computed as the sum of the changes in free energy between adjacent λ states **(Figure 3)**. TI commonly uses the derivatives of the free energy with respect to λ determined at discrete λ states to compute the total change in free energy by integration of these derivatives along the path from λ = 0 to λ = 1. Bennett's acceptance ratio considers FEP averages from both forward and reverse transformation steps in the calculation of the total free energy difference. As information from two states is taken into account in this approach, it is more efficient, that is, requires sampling at a smaller number of λs than one-directional FEP and TI [18].

Figure 3. Principles of rigorous pathway free energy calculation methods.



Transformation along one horizontal arm of the thermodynamic cycle shown in **Figure 2**. λ-REMD: Replica exchange MD in λ space; BAR: Bennett's acceptance ratio; EDS: Enveloping distribution sampling; FEP: Free energy perturbation; TI: Thermodynamic integration.

Aa Replica exchange MD: a simulation approach in which several simulations of a system, that is replicas, are run in parallel, for example at different temperatures or at different λs. Exchanges of conformations, called swaps, are attempted in regular intervals along the trajectory. The successful swaps ensure that conformations, for example obtained by enhanced sampling in simulations at high temperatures, are propagated into the other simulations.

All conventional free energy calculations suffer from three major drawbacks: a large computational burden; a complex setup; and a requirement for extensive sampling. The increase in computational power and the development of tools that facilitate calculation setup [19] will soon render the first two issues less important, whereas sufficient sampling of the conformational space remains a challenge. MD simulations on the nanosecond time scale cannot overcome large energy barriers and thus may be trapped in metastable states [20]. Consequently, even small reorganization events, for example, the rotation of an amino acid side chain, may not be adequately sampled, which can result in inaccurate free energy estimates.

Several methods have been developed in the last years with the goal to overcome the sampling problem. One possibility to enhance sampling is to run multiple independent simulations starting from different conformations at each λ state. An example is the independent trajectories thermodynamic integration approach where the derivatives in free energy obtained from independent simulations are considered in the calculation of the total change in free energy [21]. Another ansatz to improve sampling is to combine the free energy calculations with the replica exchange MD (REMD) approach such that random swaps between adjacent λ states can take place **(Figure 3)**, which leads to a faster convergence of the free energy calculations. To further improve sampling the FEP/λ-REMD method has been combined with an accelerated MD approach based on Hamiltonian REMD that allows to overcome energetic barriers between metastable states more frequently [22]; for example, this combined approach is capable of enhancing the interconversion between side chain rotamers of amino acids flanking the binding site. However, it requires a large number of parallel replica simulations. A recently developed elegant method called FEP/replica exchange with solute tempering reduces the number of required replicas by introducing a 'hot region'. In the hot region, usually consisting of the ligand and binding site residues, the temperature is first increased and then decreased along the alchemical transformation path by scaling the potential energy terms. This allows enhanced sampling in the 'hot region' at intermediate λ states. Through replica exchange between neighboring λs it is ensured that the conformations sampled at intermediate λs can also reach the end states [23].

Rigorous, pathway-based free energy calculations allow determining relative and absolute free energies.

Special care must be taken so that all relevant conformations are sampled for the free energy calculations.

As intermediate λ states do not describe chemically meaningful molecules, it is desirable to decrease the computational demand of free energy calculations by reducing the number of simulations that need to be carried out at these intermediate states [24]. In enveloping distribution sampling (EDS), a single simulation is run for a reference state connecting the end states. However, the gain in computational efficiency achieved that way can be lowered by a requirement for extensive sampling [25]. In the λ-dynamics approach all λ values are covered in a single simulation by treating λ as a dynamic variable that can adopt values between 0 and 1. A great advantage of this approach is that multiple ligands can be considered in one simulation by assigning each of them a specific value of λ [26]. More advanced applications of this promising approach are currently under development so that it is likely that λ-dynamics will be more widely applied in drug design in the future.

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Summary

- Molecular dynamics (MD) simulations provide information about the mechanism, kinetics, and thermodynamics of ligand binding.
- Conformational ensembles generated by MD simulations give insights into structural features important for ligand binding, for example, transient binding pockets or pathways, which cannot be gained from static structures.
- Accelerated/steered MD simulation approaches can be used to study ligand unbinding.
- End point free energy calculation methods provide fast estimates of ligand-binding affinities.
- Rigorous pathway free energy calculations allow an accurate determination of absolute and relative binding affinities, and several approaches to reduce the large computational demand of these calculations have been proposed.

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