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Molecular Dynamics as a Tool in Rational Drug Design: Current Status and Some Major Applications

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Abstract: Rational Drug Design has become a well-established discipline in pharmaceutical research. It uses computational chemistry with the aim to discover or study drugs and their related biologically active molecules. The purpose is to reduce the number of targets for a good drug that have to be subjected to expensive and time-consuming synthesis. The advanced methods developed in this field united with the increased potency of the new computer generation are the tools for the scientist to explore the conformational variability and properties of a large number of potentially active molecules and their interaction with each other or with their biological target (i.e. enzyme or receptor). Among these methodologies, Molecular Dynamics (MD) is one of the most useful tools in this process now routinely used to simulate complex dynamic processes that occur in biological systems such as molecular recognition in drug-receptor complexes. This paper reviews the current status of Molecular Dynamics methods, and some of its most recent and interesting applications in the field of Drug Design and Discovery.

Keywords: Molecular dynamics, drug design, docking, drug-receptor complexes.

1. INTRODUCTION

Molecular Dynamics (MD) is a powerful theoretical method routinely used to simulate the dynamics of complex physical, chemical and biochemical systems. Its success stems from the development of a battery of algorithms and the avaibility of powerful computers. A great deal of modern theoretical research is based on the results of such simulations and in the development of new algorithms to extend the range of such simulation methods to large systems and to longer times [1]. At present, systems currently investigated may contain up to 10^{10} atoms and simulation times may be as long as μs.

The dynamic properties and processes of molecules can thus be investigated by researchers in a high number of fields such as structural biochemistry, biophysics, molecular biology and pharmaceutical industry. Using MD simulations the thermodynamic properties and time-dependent phenomena (i.e. kinetic) can be studied and this allows an understanding of various dynamic aspects of biomolecular structure, recognition and function. The major strengths of MD simulations are the possibility of its combination with statistical mechanics which connects microscopic simulation with macroscopic observables. Statistical mechanics can provide a rigorous framework of mathematical expressions that can relate the distribution and motion of atoms with macroscopic observables such as temperature, pressure, heat capacity and free energies.

In this way we are able to predict, for instance, changes in the binding free energy of a particular drug candidate or the mechanisms and energetic consequences of conformational changes in a protein. Other aspects which can be studied by the aim of MD are macromolecular stability [2], the role of dynamics in enzyme activity [3, 4], molecular recognition and the properties of complexes [5] and small molecule transport [6].

The aim of this review is to focus on the potential applications of MD on studying biochemically relevant systems finalized to the rational design of new potential drugs for various target diseases.

2. MOLECULAR DYNAMICS METHODS

Given the structure of a biomolecular system i.e. the coordinates of the constituent atoms, there are various computational methods able to investigate the dynamics of the molecular system.

All the dynamics methodologies employed are, however, highly dependent upon the description of a suitable potential energy function to describe the energy of the system with respect to the molecular degrees of freedom.

In particular, the choice of an appropriate energy function for describing the intermolecular and intramolecular interactions is critical for a successful MD simulation.

In conventional MD, the energy function is calculated by means of molecular mechanics methods thus considering the atomic motion only from a nuclear point of view according to the Born-Oppheneimer approximation [7].

For applications in studying biologically relevant molecular systems such as proteins, drug-receptor interactions, many well parameterized molecular mechanics force fields (FF) have been developed. In this area one widely applied FF is the CHARMM22 [8, 9] which is a typical class I FF. The CHARMM ffIds have been separately parameterized for proteins [8], nucleic acids [10], lipids [11] and carbohydrates [12, 13].

In the next paragraphs, a brief survey of the currently used approaches based on MD which find application in Computer Aided Drug Design (CADD), is given.

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2.1. Classical MD

In a simplified concept, MD simulations consist in an iterative calculation of instantaneous forces present in a molecular system and the consequential movements in that system. This molecular system is a set of particles that move in response to their interactions according to equations of motion of classical (Newtonian) mechanics. The energetics of the system are calculated by molecular mechanics theory, thus each atom is considered to be a point mass. This approach is justified by the Born-Oppheneimer approximation [7] which states the separability of the electron and nuclear motion and allows the MD-MM to consider only the nuclear displacements.

A range of experimental conditions can be simulated by MD. The earliest macromolecular simulations considered the molecules in a vacuum. Later on, MD simulations included both water and neighboring macromolecules such as proteins as in a crystal environment. The most typical approach is the periodic boundary conditions which ensure that all simulated atoms are surrounded by neighboring atoms in a cubic box. In the absence of periodicity, the stochastic boundary conditions are used instead [14].

This approach is usually useful when only a particular region is considered such as the binding site in a ligand-macromolecule binding study. Thus, the region considered is enclosed within a spherical shell and the atoms found there are subjected to stochastic dynamics evaluated by Langevin dynamics. Furthermore, the stochastic shell is enclosed in a region that maintains the overall structure of the system, while the shell region accommodates any local fluctuations in conformation, density and energy typical of any MD simulation. The length of a typical MD run is determined by a number of factors including, (1) number of interactions that need to be calculated each time step, (2) the period of this time step (ts) and of course (3) the number of degrees of freedom considered in the system.

The number of interactions evaluated [point (1)] can be reduced using the implicit solvent models or by reduced representation of the biomolecular structure. Improvement in efficiency is often obtained by freezing the fastest modes of vibrations by constraining the bonds to hydrogen atoms to fixed lengths using algorithms such as SHAKE [15, 16], RATTLE [17], LINCS [18].

The simulations of accurately reproduced experimental conditions are also important. Various states for physical state variables such as pressure and temperature may be considered in the simulations (i.e. ensembles).

An ensemble is a collection of all possible systems that have different microscopic states but belong to a single macroscopic thermodynamic state [19].

The most widely simulated ones are (1) the canonical ensemble (NVT) which corresponds to a thermodynamic state with fixed number of atoms N, fixed volume V and fixed temperature T, (2) the isobaric-isoenthalpic ensemble (NPH) with fixed number of atoms N, fixed pressure P and fixed enthalpy H; (3) the isobaric-isothermal ensemble (NPT) which keeps fixed the number of atoms N, the pressure P and the temperature T, (4) the gran canonical ensemble (μVT) with a fixed chemical potential μ, fixed volume V and fixed temperature T; (5) the micro canonical ensembles (NVE) with a fixed number of atoms N, fixed volume V and fixed energy E, corresponding to a closed (i.e. isolated) system where the total energy is conserved.

Some words must also be spent on the problem of including solvation in order to reproduce realistic thermodynamic data. In fact, for accurate MD simulations of biological systems, as already introduced in this review, the correct choice of the environment is important. Since the full physiological environment of the systems cannot be included, it can be simulated according to some approximations. For simulating *in vitro* systems, the aqueous solvent is used in the majority of cases. Furthermore implicit solvation can be considered. In fact it is common to assume that a protein or other macromolecule is fully solvated in pure or ion-containing water during simulations. However, a considerable portion of the computation time is spent on evaluating solvent solvent interactions. To avoid this, solvent implicit models are chosen and *ad hoc* developed [20-22]. One of the best known implicit solvent models is the generalized Born (GB) [23]. Like all implicit models, GB cannot reproduce certain microscopic solvent features [20] and to avoid this, a hybrid method was developed which considers explicitly the solvent molecules around a well defined region of the system (binding site, or a channel) called generalized solvent potential method [24]. Instead, apolar solvation models use a cavity potential together with dispersing potential decomposition. One of these models is the generalized Born and non polar (AGBNP) solvent model [25] and is very effective since the apolar component seems to be necessary to reproduce large conformational changes [26].

Explicit water models are used only in all those cases when the specific molecular interactions between the solute and the solvent molecules are important. There are a lot of models available such as TIP3P, TIP4P [27], TIP5P [28], SPC and SPC/E [29].

2.2. Other Non-Classical Dynamics Methods

2.2.1. Langevin Dynamics

This dynamic method includes stochastic terms to approximate the effects of degrees of freedom neglected in dynamics simulations. Its basis is the Langevin equation in place of Newton's second law of the classical MD. The Langevin equation has two additional terms: the first is a function used to represent the fictional drag experienced by solute molecules in a solvent not explicitly considered. The second consists in a random force applied in order to mimic the random impulses which are expected from both the solvent and any other solute molecule.

2.2.2. Brownian Dynamics

Brownian dynamics (BD) is a diffusional analogue of MD [30-32] which is carried out through integration of Langevin's equation. This is applied when the solvent surrounding the molecules has high viscosity; in fact in this case the motion of that molecule can be described in terms of random walk and this reproduces a representative diffusional trajectory. Among the biological processes which can be accurately described by BD simulations there are diffusion–controlled reactions, diffusional encounters, ionic diffusion

under the influence of an electrostatic field. The choice among classical MD, LD or BD depends upon which contributions are thought to dominate in the system of interest.

2.2.3 Monte Carlo

Monte Carlo simulations (MC) are a significant alternative to classical MD. These simulations have a stochastic approach and have the great advantage that no forces need to be evaluated because only the potential energy is normally calculated during the steps of the simulation, thus resulting in less time consuming calculations. In its simplest form it uses the MC algorithm for numerical integration. Later on, Metropolis *et al*. [33] introduced a technique known as Metropolis Monte Carlo simulation. In this approach the problem is described in terms of a thermodynamic system with potential energy V and temperature T. Actually, MC methods are inefficient to explore the conformational space of large biomolecules compared to classical MD [34]. Furthermore, MC gives no information on the time evolution of structural events. These problematics are solved by Hybrid MC/MD methods now developed and well described in the literature [35-37].

2.2.4. Simulated Annealing

The simulated annealing uses an algorithm [38] very similar to the Monte Carlo one but on the contrary it is an efficient method to find the lowest energy minimum conformation of a molecular system, since it uses most of the approaches of classical MD.

2.2.5. QM/MD: The Search for Accuracy

Long MD simulations often generate cumulative errors in numerical integration that can be minimized with proper selection of algorithms and parameters, but not eliminated entirely. Furthermore, currently developed potential functions are, in some cases, not sufficiently accurate to reproduce the dynamics of the represented molecular systems. To avoid this, the more computationally demanding Ab Initio Molecular Dynamics method (AIMD) has been developed. This methodological approach is particularly important since, as previously pointed out in this review, in classical molecular dynamics, a single potential energy surface (usually the ground state PES) can be reproduced by the chosen force field as a consequence of the Born-Oppenheimer approximation. If excited states, chemical reactions or a more accurate representation is needed, electronic behaviour can be obtained from first principles by using a quantum mechanical method, such as Density Functional Theory (DFT). In this way, *ab initio* quantum-mechanical methods may be used to calculate the potential energy of a system *on the fly*, as needed for conformations in a trajectory. Although various approximations may be used, these are based on theoretical considerations, not on empirical fitting. *Ab Initio* calculations produce a vast amount of information that is not available from empirical methods, such as density of electronic states or other electronic properties. A significant advantage of using *ab initio* methods is the ability to study reactions that involve breaking or formation of covalent bonds, which correspond to multiple electronic states. On beside, due to the cost of treating the electronic degrees of freedom, the computational cost of such simulations is much higher than classical molecular dynamics. This implies that AIMD is limited to smaller systems and shorter periods of time. Among these QM/MD methods, the Carr-Parrinello Molecular Dynamics, better known as CPMD, must be mentioned. The Car–Parrinello method is a type of *ab initio* molecular dynamics, which usually employs periodic boundary conditions, planewave basis sets, and density functional theory. The CPMD explicitly introduces the electronic degrees of freedom as (fictitious) dynamical variables, writing an extended Lagrangian for the system which leads to a system of coupled equations of motion for both nuclei and electrons. In this way an explicit electronic minimization at each iteration is not needed: after an initial standard electronic minimization, the fictitious dynamics of the electrons keep them on the electronic ground state corresponding to each new nuclear configuration found out along the dynamics. In order to maintain this adiabaticity condition, it is necessary that the fictitious mass of the electrons is chosen small enough to avoid a significant energy transfer from the nuclei to the electronic degrees of freedom. This small fictitious mass in turn requires that the equations of motion are integrated using a smaller time step than the ones (1–10 fs) commonly used in Born–Oppenheimer molecular dynamics, but it is possible to extend the Car–Parrinello formalism in order to overcome this limitation [39].

3. APPLICATION OF MD IN RATIONAL DRUG DESIGN

In rational drug design one of the most important problems to be solved is the description of the molecular aspects and the related energetics which are at the basis of the interactions between proteins or more general macromolecular receptors, and molecules which could be either endogenous natural ligands or a drug. These interactions are responsible for most biological processes such as signal transduction, metabolic regulations, physiological responses and many others which are all dependent upon non-covalent binding. Thus, the prediction and design of ligands that can reversibly bind to pharmaceutical targets (enzyme inhibitors, receptor agonists and antagonists etc) is at the heart of Structure Based Drug Design (SBDD). The prediction of the strength of non-covalent associations, as well as the structures of the bimolecular association complexes, has therefore been an important objective in computational chemistry which can be investigated through many modeling techniques all based on MD simulations. In the next sections some of these aspects will be discussed.

3.1. Free Energy of Binding

Many approaches can be chosen in the calculations of the free energy of binding, and they cover a broad range of accuracies and computational requirements. Free energy perturbation (FEP) and Thermodynamic integration (TI) methods are computationally expensive but well-known to be high in accuracy since they have been applied successfully in the prediction of the binding strengths of many complexes [40, 41]. Other methods have been developed subsequently which gain in computational speed but loss in accuracy. These are the linear interaction energy (LIE) method [42], the molecular mechanics/Poisson-

Boltzmann surface area (MM/PB SA) method [43, 44], the chemical Monte Carlo/Molecular Dynamics method (CMC/MD) [45, 46], the pictorial representation of the free energy components (PROFEC) method [47], the one-window free-energy grid (OWFEG) method [48, 49], the λ-dynamics method [50, 51], the 4D-PMF method [52, 53].

3.2. Activated MD

Conventional MD makes use of simulation time scales in the order of nanoseconds while biological processes might take milliseconds or longer since most of them are a so-called activated process.

An activated process is one in which a high energy barrier exists between the initial and final states and it must be overcome. Even if the barrier crossing in itself is relatively fast, the time required for the molecular system to arrange the constituent atoms in a suitable way by random thermal fluctuations, can be very long. An example of such a process is local conformational changes which occur in proximity of the active site of the protein associated with ligand binding [54].

Activated MD consists in a two stage process: in the first stage a series of simulations are performed and each one is constrained to a successive portion of the transition pathway. The purpose of this scan is to locate the free-energy barrier peak. The second stage consists in running conventional MD simulations from the region of the free-energy barrier crossing events and their full analysis gives useful information on the mechanism of the activated process itself [55].

3.3. Steered MD

Steered molecular dynamics (SMD) introduces a time-dependent or position-dependent force. This force steers the system along certain degrees of freedom, allowing to focus only onto a dynamic event of interest, minimizing the computational efforts [56, 57]. An example is the force driving to a particular binding or unbinding event.

3.4. MD in Ligand Docking and Molecular Design

As already pointed out, the non covalent interactions between proteins and substrates are critical to many biological processes such as signal transduction, metabolic regulations and physiological responses. Through MD the binding modes and the corresponding free energy may be estimated for many kinds of complexes such as protein-ligand [58], protein-protein and protein-DNA. As a definition, ligand docking (or more in general molecular docking) is the prediction of the stable minimum energy geometry of the intermolecular complex which is generally calculated by means of MD [59, 60].

The most typical case is protein-ligand docking which has the final goal to find out the biological activities of a given ligand [61].

4. MAJOR CASE STUDIES

In this section some application studies of methods based on MD will be reported focusing on the target of the research studies instead of in the specific methodological approach.

From the recent literature it arises that one of the most challenging is the development of antiviral drugs targeting HIV. Thus, in the following sections the state of art of the studies relative to three HIV related enzymes, HIV-1 reverse transcriptase, HIV-1 protease and HIV-1 integrase, is reported. Furthermore, other interesting recent examples with different macromolecular target are also pointed out.

4.1. HIV-1 Reverse Transcriptase Inhibitors

HIV-1 reverse transcriptase is a key enzyme playing an important role in the HIV-1 life cycle for the replication of the RNA genome into DNA form [62]. This enzyme (HIV-RT) catalyses a series of reactions to convert the single-stranded RNA genome of HIV into double-stranded DNA for host-cell integration. This task requires the reverse transcriptase to discriminate a variety of nucleic-acid substrates [63], thus showing selectivity for backbone compositions or base sequences. The mechanism by which substrates regulate RT activities is still unclear. For all these reasons, this enzyme is an important target in developing anti-AIDS pharmaceuticals. In fact the investigations of new inhibitors are continuously being reported [64-66].

In general, inhibitors of HIV-1 RT can be divided into two classes: nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs).

NRTIs are substrate analogs that act at the catalytic site of HIV-RT by terminating DNA synthesis, whereas NNRTIs are compounds that non-competitively bind to the hydrophobic pocket located approximately 10 Å away from the catalytic site and force the HIV-RT subunit to change into an inactive conformation [67]. Because of their high potency, low toxicity and high selectivity many investigations on NNRTIs have been reported [68]. Moreover, since the resistance mechanism is widely common and related to the presence of residue mutations (Lys103Asn mutant is observed frequently in HIV-1 RT), many research studies focus on the design of new inhibitor compounds which could overcome these phenomena.

Therefore, a series of novel non-nucleoside reverse transcriptase inhibitors have been developed [69]. Diaryltriazine analogs (DATA) as one series of NNRTIs, are a class of compounds which are highly effective against wild-type and various mutant strains of HIV-1 [70]. Thus, a model of their binding interaction with the RT enzyme has been found out and this can be useful in the activity prediction of new lead compounds.

In this study, Li *et al.* [69] used the CoMFA and CoMSIA methods in order to determine the active conformer and the alignment rules of HIV-1 NNRTIs. But since the 3D-QSAR alone cannot fully predict the binding mechanism between a ligand and HIV-RT, MD simulations were used in conjunction. This methodological approach aims not only to describe the binding but also to construct an activity prediction model useful for further drug design.

Thus, the MD simulations of the complexes formed by HIV-RT (pdb code 1S6Q) and two inhibitors 8E and 9H (Fig. **1**) were conducted at 300 K for 2 ns monitoring the RSMD of the backbone during the full simulation.

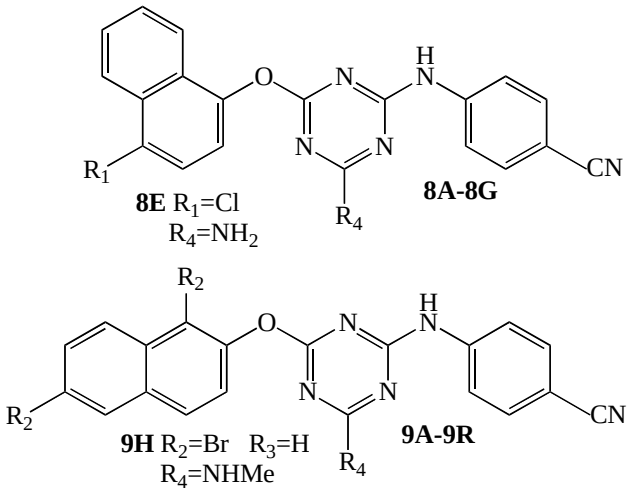


Fig. (1). Structure of diaryltriazine analogs 8E, 9H –for further details see table 1 in [69].

This work clearly demonstrates the superiority of MD simulations against 3D-QSAR on directly exploring the exact binding mechanism between ligand and HIV-RT. As a result, it arises that for compound 9H, hydrogen donor N is the major factor in the formation of the hydrogen bond with Glu(B)138, which is a potent hydrogen acceptor positioned in a strong donor favor area. At this site, the presence of substituents NH_2 and NHMe seems to have the best binding effect. Moreover, 9H also induces strong hydrophobic potentials due to the presence of the benzonitrile ring and the diarylic ring. Four are the emerging hydrophobic interactions formed with residues Val(A)106, Tyr(A)181, Tyr(A)188 and Phe(A)227, and the presence of these widespread specific non-covalent bindings take account of their high activity (Fig. **2**).

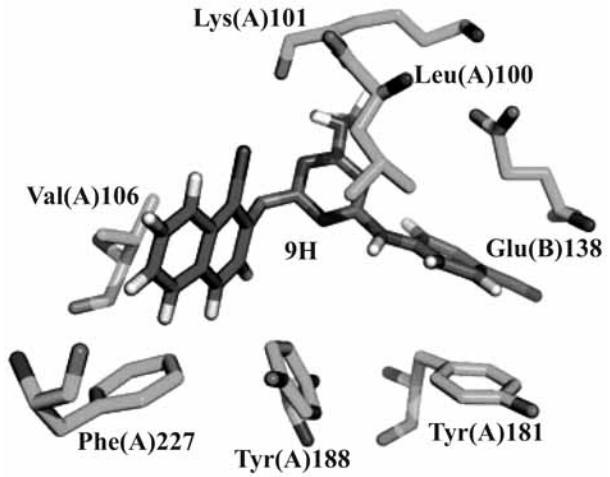


Fig. (2). The interactions between ligand (9H depicted) and the surrounding residues of HIV-RT [69].

Beside the research studies on developing new NNRTIs inhibitors, the mechanism of the selectivity of NRTIs drugs are also currently investigated. In general, nucleoside analogs are a family of biological molecules (ddI, d4T, ddC, dTC) which mimics the classical nucleoside substrates but which generally lacks the 3'-OH group, acting consequentially as chain terminators when incorporated into DNA by RT. As already mentioned, they show high activity but also elevated toxicity which prevents the long-term use of these drugs. Other problems are related to resistance and mutations. Thus, in order to solve these limitations and thus develop a new drug belonging to this class, is important to better understand the exact mechanism of interaction and resistance in the active site.

Amongst all the published results, we must cite out Taft *et al.* [71] which studied the interactions of ddI (didanosine), d4T (stavudine), ddC (zalcitabine) and 3TC (lamivudine) inhibitors with their receptor using molecular docking, DFT and MD simulations (Fig. **3**). They also proposed a novel HIV-1 RT inhibitor which seems to show a higher affinity of the previous developed inhibitors since it forms additional interactions at the RT active site.

Thus, as a protocol, the molecules studied were docked into the active site by the automated docking software GOLD and the complexes with highest docking score were submitted to MD stabilization (discover/CVFF force field) by 1.5 ns simulation at 298 K with an equilibration phase of 80 ns. Those MD simulations were necessary to better asses the molecular interactions which occur at the active site and that are responsible of the ligand binding affinity.

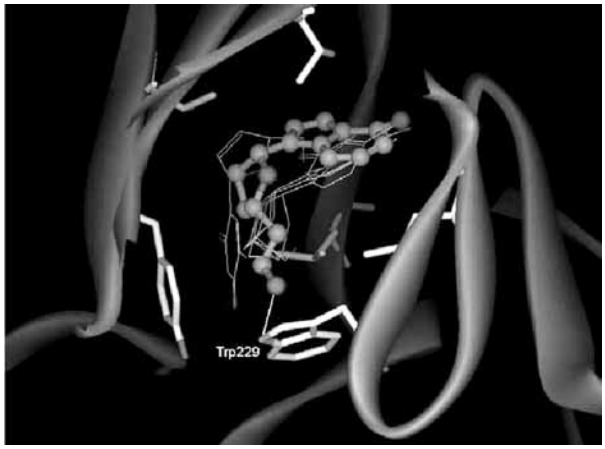


Fig. (3). The superposition of the four NRTIs (ddI, D4T, ddC, 3TC) with novel designed ligand (ball-and-stick) in the orientation after the docking simulations and into the active site of HIV-1 RT [71].

The enzyme during the MD runs is left free to relax and adjust the positions of the residues involved at the catalytic site. In addition, it must also pointed out that the ligands were previously minimized and their charges calculated at DFT level prior the docking simulations into the active site. This step is important since accurate calculated charges assure an efficient description of the electrostatic interactions in the ligand-receptor complex. Of course, the substrate binding site (dNTP) of HIV-1 RT was already identified likewise the fact that these NRTIs mimics act as competitive inhibitors binding at the same active site. From the docking studies, it emerges that the ligands occupy a similar region (the crystallographic binding site). The residues which are in closest contact with the ligands are Tyr183, Met184, Asp185, Asp186, all included in the omega loop of the active RT area. The docking scores obtained well represent the

scale of bioactivity of the ligands. The novel inhibitors have a chain extension compared to ddI, particularly a methylene group between the hydroxyl group which contains the oxygen that participates in the important process of phosphorylation, and the furane ring allows the molecule to bind to the receptor *via* Trp229. This new interaction suggests a stronger binding interaction since it adds to the other maintained hydrophobic interactions observed for all the other NRTIs drugs. Furthermore this ligand is in agreement with the Rule of Five [72] and the GolScores and ADMET properties combined with the high stability of the MD for the complex suggest that it could be a promising potential drug for anti-HIV chemotherapy.

Moreover, as already mentioned CALD (Computer Aided Ligand Design), i.e. accurate prediction of the correct binding free energies, is strictly related to the achievement of a good 3D model of the receptorial target in the complex with the ligand of interest, and this fundamental for high accuracy predictions. To this purpose, in their research study, Aqvist *et al.* carried out free energy calculations to predict the binding modes of HIV-RT and some NNRTIs inhibitors [73]. They used two different receptor structures and assessed the importance of the chosen protein model in a docking scoring approach. They divided the problem into two levels: the first is the choice of a proper scoring function for the docking, the second the correct conformational sampling of the ligands, which can be achieved through molecular dynamics (MD) simulations. The aim has been to generate an ensemble of thermodynamically accessible conformations of the ligand and from this ensemble calculate averages of the affinities. These two levels must be deeply correlated. This study is significant since it attempts to find a suitable method which can help to choose between binding modes not only for HIV-RT (the test case) but for all kinds of receptorial targets.

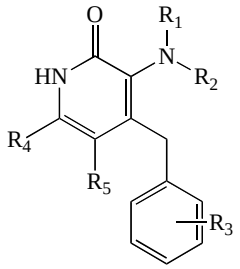
The NNRTIs inhibitors were chosen as ligands because of their structure. In fact they are mainly rigid and very fast to dock. Hence a set of 43 HIV-RT inhibitors were docked using GOLD 3.0 [74] using two different target structures (PDB codes 2BAN, 1RT1). These molecules correspond to a series of benzylpyridinone derivatives (Fig. **4**) [75].

The docking results in two distinct clusters of possible conformations. One of these clusters is compatible with an existing crystal structure, whereas the other displayed a flipped heterocyclic group.

Binding free energies calculated with many scoring functions were simulated for the two clusters. To discriminate between the conformations and localize the bound one, a LIE (linear interaction method) in combination with MD simulation was used and performed with software package Q. In fact this approach leads to the prediction of binding free energies in agreement with experimental data. Finally, it is well known that common TR mutations that confer resistance to NNRTIs are L100I, K103N, V106A/I/L, Y181C, G190A/T/V. Therefore is important to recognize the origin of this resistance at the atomic level in order use this knowledge in the design of new NNRTIs which overcome the HIV-RT resistance.

Other studies have been accomplished with the aim to assess the effect of mutation for HIV-RT on the binding of NNRTIs [76]. Different mutations have different effects on distinct inhibitors. In this paper, Monte Carlo/Free Energy Perturbation (MC/FEP) calculations were used to evaluate the binding free change for HIV-RT complexes upon L100I mutation. Five inhibitors were considered in complex with RT mutated, nevirapine (Viramune), MKC-442 (emivirine), 9-Cl TIBO, efavirenz (Sustiva) and UC-781. Fundamentally, this research work suggests that anti-viral resistance can arise from mutations that reduce favorable protein-drug interactions (type I), and that increase or enhance unfavorable protein-drug interactions, or rigidify the complex (type II). As a result, it emerges that for HIV-RT, the Y181C and V106A mutations are type I, while L100I is mainly type II.

(**A**)



(**B**)

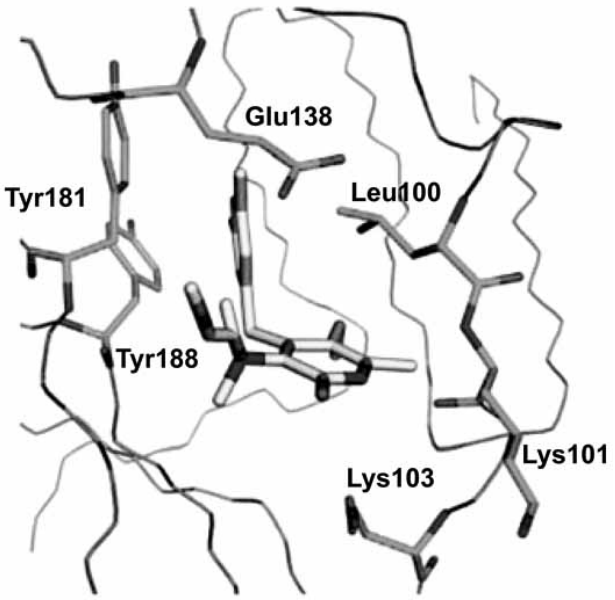


Fig. (4). (**A**) Base structure of the 43 NNRTIs benzylpiridinones inhibitors. For further details see table 1 in ref. [73-75]; (**B**) Six RT residues that make the three largest van der Waals and electrostatic contributions to binding for the inhibitors. The binding mode of inhibitor **40** is also shown (R_1=CH_3, R2=$CH(CH_3)CH_2OCH_3$, R_3=3,5-diCH_3, R_4=CH_3, R_5=C_2H_5)

4.2. HIV-1 PR Inhibitors

The Human immunodeficiency virus type 1 aspartic protease (HIV-1 PR) is an important enzyme due to its key role in viral maturation. It cleaves the viral poly-proteins during the replication of the HIV virus. Because of the importance of this enzyme, inhibitors of HIV-1 PR are still widely used in the treatment of AIDS [77]. However, as

already found out for HIV-RT, due to the rapid development of drug resistance of the virus and side effects encountered during treatment, new inhibitors of this enzyme are currently needed.

HIV-1 PR is a homodimer composed of 198 residues per chain, and its active site is covered by two flexible β-hairpins, called flaps, controlling the entry of the polypeptidic substrate [78]. The flaps seem to sterically restrict access to a polypeptide into the binding cavity. The initial step consists in the encounter of the substrate with the target enzyme, the second is the entry and the correct positioning of the ligand inside the active site. The flap opening has already been studied computationally through MD simulations [79]. In particular, the HIV-1 PR was subjected to unrestrained all-atom MD simulations (activated MD at 300 K length 42 ns) with the sampling of large conformational changes of the active site flaps. The data collected suggest that the unliganded protease predominantly populates the semiopen conformation, with closed and fully open structures being a minor component of the overall ensemble. In addition, McCammons *et al.* [80] studied the influence of mutations on the equilibrium between the semi open and closed conformations of HIV-1 PR. This aspect is particularly interesting since it could be one of the mechanisms of drug resistance for the V82F/I84V mutant. All these molecular aspects are important and a deeper understanding of all the events associated with the ligand binding can be precious for the design of new potent and more selective HIV-1 PR inhibitors.

During their research work, McCammon *et al.* [80] studied the encounter of a peptide substrate with the native HIV-1 protease together with its incorporation into the active cleft and the dissociation of products after substrate hydrolysis by means of activated MD. Molecular and Langevin dynamics pointed out that the flaps need to open to let the substrate bind and that the deep interaction between the protease with the ligand influences the flap opening frequency and interval. Instead, the release of the final products does not require the flap opening since they can easily slide out from the cleft. The results obtained suggest that the presence of the substrate modifies the protease's internal mobility in order to favor its capture into the binding site itself.

Furthermore, a lot of efforts have been made to focus the effect of binding various inhibitors on the protease structure [81]. For this purpose, the authors pinpointed the role and the importance of dynamics and considered a set of twenty-five HIV-1-PR inhibitors in complex with the target enzyme whose structure was experimentally determined and published in the Protein Databank. The goal was to accurately reproduce the binding energies of the association complexes. As a result of the study, they clearly showed that only if relaxation of the protein-ligand complexes was enabled by MD simulation, the structure is accurate and thus the calculated binding energies are in agreement with the experimentally determined values.

Other studies aimed at the same purpose have been carried out. In particular, Ringhofer *et al.* studied the conformational dynamics of HIV-1-PR in complex with the inhibitor SDZ283-910 [82] by means of the same activated MD protocol. More recently, Stoica *et al.* tested the accurate prediction of the binding modes and energy for the complex between the HIV-1 protease and its inhibitor Saquinavir [83], a first generation transition state analogue which blocks the maturation step of HIV-1 life cycle (Fig. **5**).

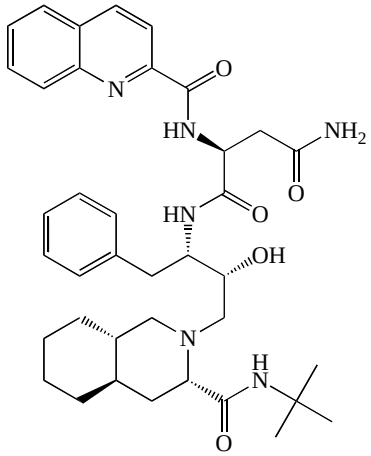


Fig. (5). Structure of Saquinavir, a protease inhibitor.

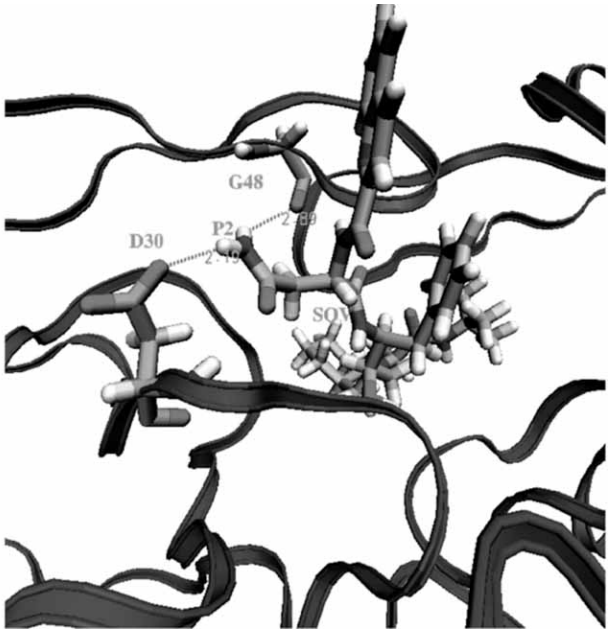
Other efforts are being made in order to explain exactly and with accuracy the molecular basis of drug resistance. In order to develop a computational methodology suitable to this purpose, Stoica *et al.* carried out a molecular modeling study applying the molecular mechanics Poisson Bolzmann technique (MM/PBSA) to rank the binding affinities of Saquinavir with respect to both the wild type of the HIV-1 protease and three mutants of this enzyme L90M, G48V and G48V/L90M (Fig. **6**). The accuracy of this computational approach is also confirmed in another recent research work aimed to evaluate the potency of HIV-1 PR drugs in affecting resistance [84].

In this study, the authors aimed to quantify resistance in terms of decrease in binding affinities of the inhibitor and furthermore to compute changes in binding affinity upon mutation as changes in calculated ligand binding energies of protein-inhibitor complexes. For each ligand-enzyme complex, they performed a fully unrestrained 10 ns molecular dynamics simulation with explicit solvent. The entropy and enthalpy estimated in this way were compared with the obtained experimental binding affinities resulting in an average error of 1.5 Kcal/mol which is a good and remarkable level of correlation with the observed ranking of resistance. Furthermore, a detailed analysis of the entropic/enthalpic ratio of drug-protease complex helps to explain resistance in mutant L90M in terms of different vibrational entropy which is higher in the Wild Type (WT) complex and lower in the mutant enzyme. On the contrary, in mutant G48V resistance can be ascribed to the disruption of critical hydrogen bonds in the inhibitor's binding site.

As a result, this computational investigation pointed out that on the one hand a higher flexibility of the inhibitor is desirable in developing and designing new resistance-evading drugs in order to lower the entropy-based susceptibility to mutations but on the other hand the enthalpic compensation must also occur.

Another way of bypassing the resistance phenomena against HIV-1 PR inhibitors consists in design of new non-

(**A**)



(**B**)

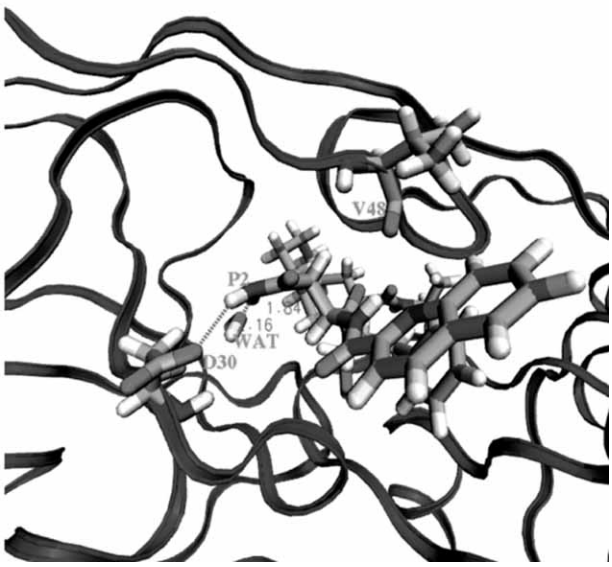


Fig. (6). Representative snapshots of the L90M-saquinavir complex (**A**) and the G48V-saquinavir complex (**B**) after 4 ns of post equilibration MD [83].

peptidic based drugs [85-87]. Mono-adducts and bis-adducts of [60] fullerene analogues belong to this emerging inhibitors class and they have been studied in order to analyze their binding interactions with HIV-1 PR by molecular docking. As already pointed out, the catalytic site of PR consists in a cylindrical hydrophobic cavity with 10 Å diameter composed of two catalytic aspartic acid residues Asp25 and Asp25'. The high complementarily spatial relationships between this active site and [60] fullerene derivatives led to the suggestion that these molecules are good candidates as potential drugs against HIV-1 PR. Thus many derivatives have been synthesized but only a limited number of them were subjected to bioactivity tests against

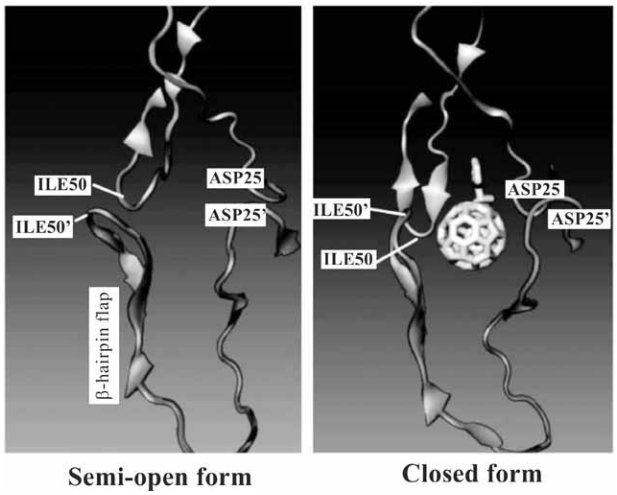


Fig. (7). Side-view of the binding cavity of HIV-1 PR (free (left) and inhibitor bound (right) systems) in the semi-open and closed forms [See ref. 85 for further details].

the target enzyme. For this reason, it is useful to use suitable computational methodologies in order to predict such activity, saving and orienting the synthetic effort. Therefore MD simulations (2ns at 300K) of ligand-free and inhibitor bound HIV-PR systems have been carried out and the computational results showed a different orientation of the β-hairpin flaps in these two different systems (bound-unbound). In fact in the inhibitor bound system, the enzyme flaps are pulled in toward the bottom of the active site of the enzyme (the closed form) while, in the ligand-free system, the flaps shifted away from the dual Asp25 catalytic site thus adopting a semi-open form (Fig. **7**).

Hence many molecules (i.e. 53 compounds) belonging to the same class have been studied using the 3D QSAR/ CoMSIA approach in order to predict novel compounds with improved inhibition effect. In this way, the binding energies have been calculated by MD/molecular docking calculations whereas the correlation between structures and binding affinities has been studied with CoMSIA method. High relative contributions of steric fields from derived contour maps of CoMSIA models confirm the importance of Van der Waals interactions with non-polar HIV-1 PR surface in the activity of fullerenes. All these results were later on used by the same authors in order to design novel [60] fullerenes based inhibitors with optimal binding affinity for the HIV-1 PR enzyme.

Moreover, in the quest for HIV-1 protease inhibitors not affected by resistance mutation, Clemente *et al.* [88] have synthesized and tested a second generation of C2-symmetric PR inhibitors containing a cyclohexyl side chain group (Cha) which are active against the V82A enzyme mutation. The binding affinity results show an improved activity of this second generation compounds with respect to the first one. The X-ray analysis together with MD simulations helped the rational understanding of the resistance profile. In fact it seems that the cyclohexane ring of the Cha side chain might be in a boat instead of chair conformation.

4.3. HIV-1 Integrase Inhibitors

In order to overcome the big problem of drug resistance, beside the HIV-RT and HIV-PR ligand design (for which

many drugs have already been made available and mainly used in cocktails), it is very important to explore other aspects of the HIV life cycle to escape resistance mutations. One of the most recent targets is HIV-integrase whose 3D structure was obtained and refined [PDB codes 1ITG and 2ITG] [89, 90]. The HIV-integrase is an essential enzyme in HIV life cycle responsible for inserting the reverse-transcribed viral genome into the host DNA [91].

This enzyme is composed of a single polypeptide chain that folds into three functional domains (Fig. **8**). 3D structures have been determined for all three domains separately and for all dimers.

The N-terminal domain (residues 1-50) has a zinc binding motif that is different from the typical zinc finger fold and is important for protein-protein multimerization. The C-terminal domain (residues 212-288) has an SH3-type fold and binds DNA strongly but non specifically. The catalytic domain is composed of residues 51-211 and has an RnaseH-type fold. It belongs to the super family of polynucleotidyl transferases. All three domains are required for full catalytic activity.

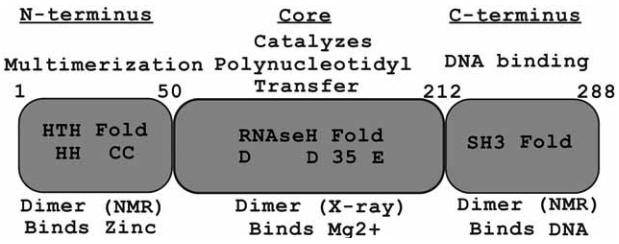


Fig. (8). Structure and function of the various domains of the HIV-1 integrase [See ref. 91 for further details].

The active site comprises two Asp residues and one Glu. Since the catalytic domain is missing in the published structures, McCammons *et al.* [92] crystallized this domain. The missing residues were added and the model refined according to the principle of homology modeling. Then they carried out 1ns MD simulations on completely hydrated models of HIV-IN catalytic domain, one with one magnesium atom and one with no metal ions in the catalytic site. The simulations predict that the region of the active site that is missing in the published crystal structure has a defined secondary structure. The flexibility of this region was thus compared with the mechanistic function of the enzyme. In particular, they pointed out the importance of one metal ion in the active site. This study gives useful information for inhibitor design projects with target this enzyme.

Recently, EBR28, a 12mer peptide has been synthesized and it showed to strongly bind to HIV-IN. It is one of the most potential small peptide leading compounds inhibiting IN binding to DNA. In their research work, Hu *et al.* [93] studied the binding mechanism between this peptide and the HIV-IN by using molecular docking and molecular dynamics simulations. The results showed that EBR28 bound to the interfaces of the monomer core domain (IN1) and the dimmer core domain (IN2) mainly realizing hydrophobic interactions.

The binding free energy for IN1 with a series of mutated peptides based on EBR28 were calculated using MM/GBSA model (correlation with experimental, good r=0.88) thus confirming the predicted binding mode of EBR28 with IN1. Therefore, based on these binding modes, the inhibition of EBR28 was explored by analyzing the essential dynamics (ED). Energy decomposition and the mobility of EBR28 in the two docked complexes (EPR29 with IN1, and EPR28 with IN2) using RosettaDock 1.0 package were studied.

Four independent MD simulations were carried out for IN1, IN2, IN1-EBR28, IN2-EBR28 using amber 8.0 suite programs [94, 95], AMBER FF and a TIP3P solvation model. At the end of calculations, the binding modes between the peptide inhibitor EBR28 with IN1 and IN2 were proposed. They show that EBR28 interacts with the interfaces of IN1 and IN2, thus excluding the previous proposed mechanism which considers the inhibitor interaction at the catalytic region characterized by the conserved DD-35-E motif.

In the proposed inhibition mechanism, EBR28 binds to the interface of IN1 to form the complex and thus prevent the formation of the IN dimer, preventing the IN binding to DNA. All the simulations were compared with experimental data with good agreement. Thus it is of significance to design anti-HIV small peptide drugs.

From all these studies it arises that HIV-1 integrase can actually be a good and promising new rational target in developing new anti–HIV drugs. However, the clinical research is still at the beginning. It will take up to 12 years to develop clinically usable inhibitors of integrase, since Phase I clinical trials have just begun. The main classes of lead compounds are reported [96] and the current Computational methodologies could improve the development of new usable drugs.

Finally, more recently other macromolecular targets involved in HIV life cycle are now object of research studies such as glycoprotein gp120 [97-99] and gp41 [100].

In particular, a small molecule inhibitor (BMS-378806) targeting gp120 has been discovered and is now undergoing preclinical testing as it seems to block viral entrance into cells. This molecule is an indole based compound (Fig. **9**) and represents a new class of HIV-1 inhibitors since they are small and potently effective in binding to the HIV surface protein gp120. In this study, a binding mode analysis was performed by means of MD, docking simulations of the complex between BMS-378806 and its receptor (PDB code 1G9N). It arises that the indole inhibitor inserts the azaindole ring deeply into the Phe43 cavity and makes contact with a number of residues in the cavity and near the cavity. All this information could be used in developing new lead compounds belonging to the same inhibitor family.

4.4. Other Applications

Many are the targets of combined molecular docking-MD simulation studies, most of all aimed both to explore the molecular basis of the drug/ligand binding to the macromolecular receptor and to calculate the affinity of different compounds. In this section some of them will be briefly introduced.

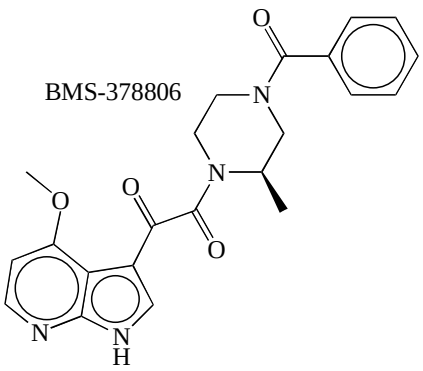


Fig. (9). Structure of BMS-378806.

Lavecchia *et al.* computationally studied the enantioselective binding of penicillin G acylase (PGA) with a series of 2-aryloxyalkanoic acids trying to rationalize the molecular aspects of the interactions [101].

Molecular dynamics simulations of h5-HT4 receptor bound to a non-peptidic antagonist ligand was carried out in a realistic membrane environment. This receptor is a G-protein coupled receptor known to be involved in many pathological disorders such as cardiac atrial arrhythmia and memory deficit. The GPCR-ligand complex already built by homology modeling in vacuum [102] was relaxed by means of a 10 ns MD run carried out with Pressure and Temperature constants. The membrane used for this study was an equilibrated fully hydrated palmitoyl-oleyl-phosphatidylcholine POPC bilayer. This simulation revealed a high stability of the association and allowed to identify the key interactions at the basis of the complexation. Furthermore, it suggested that the water molecules migrate from the extra cellular milieu towards the putative hydrophobic pocket accommodating the ligand thus forming a network of interactions with the receptor, stating the limitations of the vacuum model. This refined verisimilar model can be used for further docking studying for the quest of new potential inhibitors [103].

The inhibitory mechanism of coumarins (CM) toward aldose reductase (ALR2) was investigated by Wang *et al.* [104]. This enzyme plays a central role in glucose metabolism *via* the polyol pathway, which seems to be implicated in long-term diabetic complications. Under a normal state the ALR2 affinity for glucose is low but in a hyperglycemic environment this enzyme is highly activated increasing the glucose metabolism rate by 2-4 times. Coumarins are a class of inhibitors of aldose reductase, and are bicyclic lactone compounds with a rigid main structure. Fourteen CM compounds were considered in this study and they were docked to ALR2 (pdb code 2fzd) using the empirical free energy function and the Lamarckian genetic algorithm. Then the docked complex was stabilized by 3 ns MD simulation at 300K using the NPT ensemble and the results confirm that coumarins can steadily anchor to the enzyme to exert a strong inhibitory effect. Furthermore, they clearly demonstrated that only conformations with the CM aromatic backbone buried in the hydrophobic pocket are dynamically stable.

Some other interesting examples oriented towards the elucidation of enzymatic reaction mechanisms are herein reported.

Xiao *et al.* studied computationally the human phoshomannose isomerase [105]. Beside, another interesting and very recent study aimed to clarify some molecular binding details of ligand-enzyme complexes is the MD simulation and docking of human microsomal prostaglandin E synthase-1 (mPGES-1) with natural substrates and inhibitors [106]. In the biological conversion pathway which leads arachidonic acid to prostaglandin H2 (PGH2) followed by conversion of PGH2 to PGE2 several enzymes are involved namely COX (COX-1 and COX-2) for the first step and three types of prostaglandin E synthase (PGES) (mPGES-1, mPGES-2, cPGES). Amongst them three mPGES-1 has been identified as the most promising target for a new class of anti-inflammatory drugs. This enzyme is an inducible enzyme and is localized in the perinuclear membrane. Moreover mPGES-1 seems to be over expressed in many types of cancers [106-108].

In this research study, the 3D structure of mPGES-1 was modeled and refined by homology modeling-MD simulations methodology in its trimer quaternary structure. Then a molecular docking study combined with MD simulations was performed for different inhibitors-enzyme complexes. This lead to identify the key amino acids involved in the binding and to compare their calculated binding free energies with the experimental available data which support the model validity and accuracy. All the data collected gave new structural insights valuable for rational design of a new generation of anti-inflammatory drugs.

Another enzymatic binding computationally studied is that involving an essential RNA-editing ligase in *Trypanosoma brucei* and some inhibitors [109]. This enzyme appears in trypanosomatid RNA editing pathway, a unique process vital for these organisms, and thus is a potential drug target for a group of protozoa that include the causative agents for African sleeping sickness and other devastating tropical and sub-tropical diseases. Thus a set of inhibitors-enzyme complexes were computationally simulated (RNA-editing ligase 1, REL1). Some 30 top compounds were identified by means of a MD strategy based to account for protein flexibility. These molecules were predicted to bind into REL1's ATP-binding pocket and after the identification of the bound initial geometry the inhibitors were redocked considering an explicit solvent medium (Fig. **10**). This procedure also screened 8 compounds, two of which were predicted to have micro molar activity towards REL1. A subsequent search over the full National Cancer database confirms the findings and helped to design promising compounds against the most closely related bacteriophage T4 RNA ligase 2, together with human DNAligase IIb, as promising scaffolds for future drug discovery efforts against this class of important pathogens.

The molecular associations was also studied by Van Gunsteren *et al.* [110] In particular the research identified computationally the molecular modes and the free energy involved in the process of binding between antitumoral antibiotics such as netropsin and distamycin and DNA in the specific sequence site AAAAA. In particular, MD simulations were performed on netropsin under two different charge states and on distamycin forming the association complex in the minor groove of a DNA duplex. The relative free energy of binding of these two compounds was

calculated using the thermodynamic integration method (TI) and is in agreement with experimental findings. The MD simulations were performed for 2ns at 300K in solutions for the ligand-free and bound to DNA. From the collected results, it arises that distamycin is less hydrated then netropsin but the loss of ligand-solvent interactions involved in the binding process is similar for the two molecules. The relative mobilities of the ligands in their bound and free forms underline a larger entropic penalty for distamycin compared with netropsin, thus partially explaining the lower binding affinity of the first drug. This work showed that both the energetic and structural information can be obtained from MD simulations of two antitumoral compounds bound to the AAAAA site of a duplex oligonucleotide by using a thermodynamically calibrated biomolecular force field (GROMOS96). The explicit treatment of solvent molecules ensures adequate solvation reproduction of the changes which occur for the ligands. This provides an improved basis for the design of DNA-binding drugs.

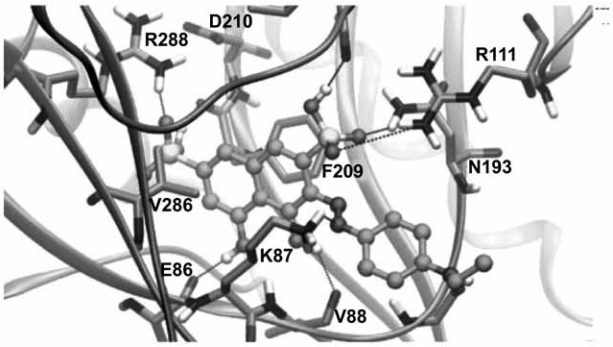


Fig. (10). Predicted binding mode of S5, one of the selected inhibitors (see ref. [109]). The most populated and lowest energy pose is shown for S5 docked into the *Tb*REL1 crystal structure.

Besides all these works on enzyme targeting, there are other studies oriented instead to elucidation of the signal transducing mechanism peculiar to certain kinds of intracellular receptors.

Amongst these, it is worth mentioning even if it not too recent, the study carried out on ionotropic glutamate receptors (iGluRs) [111]. This class of glutamate receptors are involved in excitatory synaptic transmission through the ligand induced transient opening of transmembrane ion channels. The flux of the monovalent and divalent cations through the postsynaptic membrane depolarizes the cell and propagates the electrical impulse. The iGluRs are classified according to their sensitivity to different Glutamate agonists: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainic acid (KA), and N-methyl-D-aspartic acid (NMDA). The three-dimensional structure of the extra cellular ligand binding core of iGluRs shares the overall features of bacterial periplasmic binding proteins (PBPs). In this family of proteins, the ligand binding site is arranged in two separate domains (S1, S2) divided by a cleft and connected by two peptide stretches. During ligand binding these two domains undergo a typical hinge motion that leads to a conformational change from an open to a closed form. The common architecture suggests that the same activation mechanism must occur also in iGluRs binding core after binding of specific agonists. The study started from the experimentally determined S1-S2-iGluR2 with kainate bound structure [pdb codes: 1gr2 (with glutamine bound), 1ggg (with kainate bound), 1wdn (free form)] and by means of MD simulations (activated MD protocol) reproduced the opening motion of this receptor domain in the presence and the absence of both glutamate and kainate. The results clearly showed that the opening/closing interdomain motions are associated with the conformational changes which occur at the insertion region of the transmembrane segment and are triggered by the molecular interactions which involve the agonist itself and an essential Glu209 residue.

Another research worth of notice is the computational study of the ligand dissociation from estrogen receptor. Estrogen receptor (ER) is a well known important target for pharmaceutical research [111]. In fact this nuclear receptor together with its hormone 17β-estradiol (E2, Fig. **11**) are important for female health and abnormal ER activity is associated with breast cancer, often treated with drugs which are competitive inhibitors of E2 binding (faslodex, tamoxifen, raloxifen (RAL)). Amongst these, RAL is one of the most interesting compounds since it is a selective ER modulator (SERM) and functions as an antagonist in breast but as agonist in other tissues, including also bones, thus it is used for its potential applications not only in breast cancer but even in osteoporosis therapy [112].

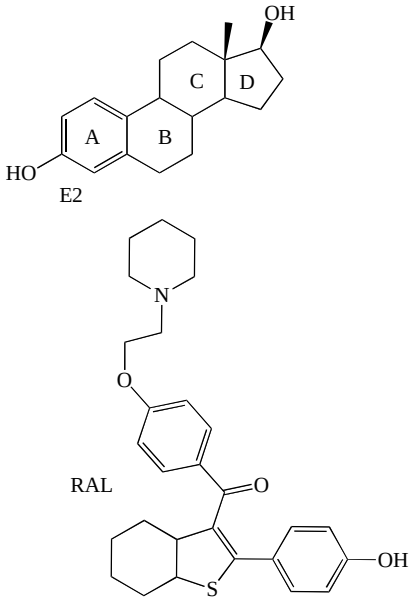


Fig. (11). Structure of ER ligands, E2 and raloxifene (RAL).

NRs are a family of receptors which have modular structures consisting in an N-terminal domain, the DNA-binding domain and the C-terminal ligand-binding domain (LBD). The latter have also cofactors binding sites, including surfaces that mediate dimerization, fundamental for optimal transcriptional activation. Hormone binding alters LBD conformation thus activating ER. Agonists trigger multiple conformational rearrangements needed for dimerization, nuclear translocation or DNA binding. Among these changes, one of the best encoded events is the enhanced packing of LBC C-terminal helix 12 (H-12) against H3 and H5 to form activation function-2, a hydrophobic cleft that interacts with NR coactivators. Antagonists or SERMs contain a molecular extension which prevents docking of H-

12 into the active conformation, thus inhibiting coactivators binding. On the other hand, the ligand determines H12 conformational changes which functions as a molecular switch for NR activity.

From X-ray structures of NR bound LBDs, it can be seen that ligands are buried into the hydrophobic pocket of the protein with no entry or exit route open. Hence, it is clear that significant LBD conformational rearrangements must occur to let the ligand came in or escape from its binding site. The exact knowledge of these mechanisms may open new potentialities in pharmaceutical drug design [112-116]. Some important research studies in this area involve MD simulations of the conformational movements of H12 considering the retinoic release from retinoic acid receptors (RARs) [114], and also the thyroid hormone receptor (TR) [115, 116]. All of them suggest a mouse trap mechanism during ligand association. This mechanism has also been confirmed in the locally enhanced sampling (LES) MD simulations of the ligand binding-unbinding pathways of E2 and RAL upon ER LBD [112] (Fig. **12**).

In particular, the authors found four possible pathways and that the dissociation depends both on the ligand and on the ER quaternary state. From the results collected, the researchers suggested that it may be possible to develop new active ligands that could interact preferentially with specific oligomeric states of NRs that could represent a new class of selective modulators.

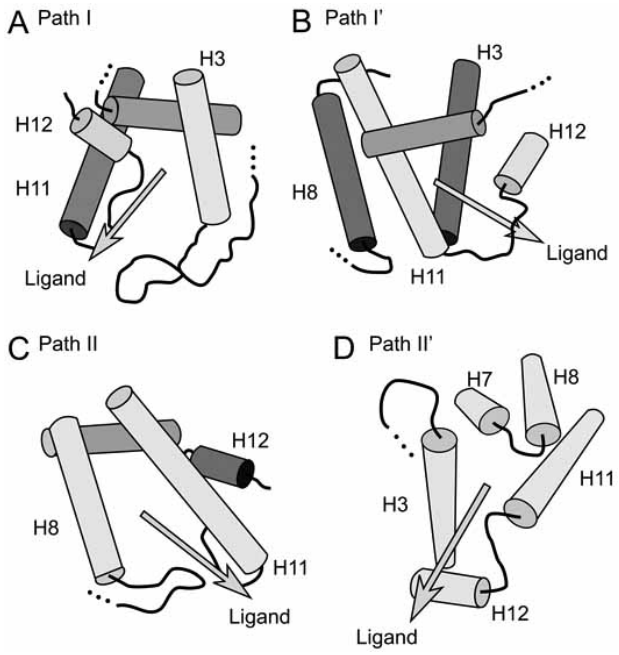


Fig. (12). Schematic representation of the four possible pathways for ER ligands dissociation (for further details see ref. [112]).

Often, receptorial conformational changes are associated not only with ligand binding but with the reveal of alternative binding sites especially in protein receptors which become apparent only after inhibitor binding. This aspect is interesting in drug design since it opens new potential sites for targeting the receptor itself, thus potentiating or modulating the original inhibitor activity.

These binding cavities which appear only after the ligand association are the so-called cryptic binding sites [117].

A good example aimed to identify and exploit a cryptic binding site is represented by the study of diaryl urea inhibitors binding to p38 MAP kinase, a target in the treatment of inflammatory disease [118]. Binding of the inhibitors causes structural rearrangements of the kinase conserved Asp-Phe-Gly (DFG) motif, shifting the Phe169 side chain from its original buried location to a new position 10 Å away from the initial one. This shift leaves a vacant hydrophobic pocket (the cryptic binding site) that is filled by the t-butyl of the inhibitors, such as BIRB79, which shows selectivity for p38 MAPkinase and is in phase III clinical trials for the treatment of rheumatoid arthritis. Thus p38 MAP kinase seems to be a good test case for testing the computational methodologies aimed to identify cryptic ligand binding sites formed by local rearrangements ligand induced into the receptor. The authors confirmed that long time (up to 60 ns) and conventional high temperature MD simulations in the presence of explicit solvent can sample all these rearrangements, and thus they may be of surprising utility in the predictions of these cryptic sites pior to their experimental discovery.

CONCLUSIONS

Over the last decades, Molecular Dynamics has became the method of choice for studying the behaviour of complex molecular systems and found lots of application in many fields such as chemistry and biology. Thanks particularly to the powerful new algorithms developed, MD simulations can now reproduce with high accuracy the dynamics of many biochemical phenomena such as the binding association between a ligand and its target receptor or the conformational changes which occur in many activated macromolecular receptors. The knowledge of the molecular basis of such events helps the process of drug design (CADD) since a potential active compound can be “seen” in action interacting in its receptorial site and its activity can be predicted on the basis of the strenght of such interactions (i.e. its binding energy).

APPENDIX

List of most common freely available softwares for MD simulations.

MM/MD Softwares

1. AMMP: fully featured molecular modelling program extensively vectorized. The program and a full description on its use are available at the URL http://www.cs.gsu.edu/~cscrwh/progs/progs.html

2. Desmond is a software package to perform high-speed molecular dynamics simulations of biological systems available at: http://www.deshawresearch.com/download_desmond.html

3. GROMACS is a set of molecular dynamics code and analysis tools which is available from the GROMACS website: http://www.gromacs.org/

4. LAMMPS (Large-scale Atomic/Molecular Massively Parallel Simulator) is an open source program for molecular mechanics and dynamics on a variety of

molecular systems. LAMMPS is distributed by Sandia National Laboratories at http://lammps.sandia.gov/

5. MDScope is an integrated set of computational tools which functions as an interactive visual computing environment for the simulation and study of biopolymers. The project implements standard visualization and simulation methods and offers a foundation for testing new algorithms and capabilities. It has three major software components which can be used together or independently 1) VMD (Visual Molecular Dynamics) http://www.ks.uiuc.edu/ Research/vmd/), 2) NAMD (Not (just) Another Molecular Dynamics program) is a MD program designed for the simulation of large biomolecular systems on distributed memory machines, http://www.ks.uiuc.edu/Research/namd/; 3) MDComm is a set of communications routines and programs which exchanges simulation data and interactive forces between NAMD and VMD:http://www.ks.uiuc.edu/ Research/mdcomm/.

6. TINKER molecular modeling software is a complete package for molecular mechanics and dynamics of molecules, especially polypeptides. http://dasher.wustl.edu/ tinker/

QM/MD Softwares: 1. A popular software for *ab-initio* molecular dynamics is the Car-Parrinello Molecular Dynamics (CPMD) package based on the density functional theory. Carr-Parrinello Software for QM/MD: http://www. cpmd.org/

2. DACAPO is an ab-initio quantum mechanical molecular dynamics (MD) code using pseudopotentials and a plane wave basis set. It has been developed at the institute of physics at the Technical University of Denmark. It is part of The CAMP Open Software project (CAMPOS). http://dcww w.camd.dtu.dk/campos/Dacapo/

REFERENCES

[1] Adcock, S.A.; McCammon, J.A. Molecular dynamics: a survey of methods for simulating the activity of proteins. *Chem. Rev.*, **2006**, *106*, 1589-1615.

[2] Tiana, G.; Simona, F.; De Mori, G.M.S.; Broglia, R.A.; Colombo, G. Understanding the determinants of stability and folding of small globular proteins from their energetic. *Protein Sci.*, **2004**, *13*, 113-123.

[3] Wang, A.; Donini, O.; Reyes, C.M.; Kollman, P.A. Biomolecular simulations: recent developments in force fields, simulations of enzyme catalysis, protein-ligand, protein-protein, and protein-nucleic acid noncovalent interactions. *Ann. Rev. Biophys. Biomol. Struct.*, **2001**, *30*, 211-220.

[4] Warshel A. Computer simulations of enzyme catalysis: methods, progress, and insights. *Ann. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 425-443.

[5] Brooijmans, N.; Kuntz, I.D. Molecular recognition and docking algorithms. *Ann. Rev. Biophys. Biomol. Struct.*, **2003**, *32*, 355-373.

[6] a) Roux, B. Computational studies of the gramicidin channel. *Acc. Chem. Res*., **2002**, *35*, 366-375. b) Bond, P.J.; Sansom, M.S.P. The simulation approach to bacterial outer membrane proteins. *Mol. Membr. Biol.*, **2004**, *21*, 151-161.

[7] Born, M.; Oppenheimer, J.R. Zur Quantentheorie der Molekeln. *Ann. Phys*,, **1927**, *84*, 457-484.

[8] a) MacKerell, A.D.; Bashford, D.; Bellott, M.; Dunbrack, R.L.; Evanseck, J.D.; Field, M.J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F.T.K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D.T.; Prodhom, B.; Reiher, W.E.; Roux, B.; Schlenkrich, M.; Smith, J.C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B,* **1998**, *102*, 3586-3616; b)MacKerell, A.D. Jr.; Brooks, B.; Brooks, C.L., III; Nilsson, L.; Roux, B.; Won, Y.; Karplus, M. CHARMM: The energy function and its parameterization with an overview of the program. In *The Encyclopedia of Computational Chemistry;* Schleyer, P.v.R.; Schreiner, R.R.; Allinger, N.L.; Clark, T.; Gasteiger, J.; Kollman, P.; Schaefer, III H.F.; Eds.; John Wiley & Sons: Chichester, **1998**; vol. *1*, pp. 271-277.

[9] Brooks, B.R.; Bruccoleri, R.E.; Olafson, B.D.; States, D.J.; Swaminathan, S.; Karplus, M. CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.*, **1983**, *4*, 187-217.

[10] MacKerell, A.D.; Wiorkiewiczkuczera, J.; Karplus, M.; All-atom empirical energy function for the simulation of nucleic acid. *J. Am. Chem. Soc.*, **1995**, *117*, 11946-11975.

[11] Feller, S.E.; MacKerell, A.D. An Improved empirical potential energy function for molecular simulations of phospholipids. *J. Phys. Chem. B*, **2000**, *104*, 7510-7515.

[12] Kuttel, M.; Brady, J.W.; Naidoo, K.J. Carbohydrate solution simulations: producing a force field with experimentally-consistent hydroxyl rotational frequencies and populations. *J. Comput. Chem.*, **2002**, *23*, 1236-1243.

[13] Reiling, S.; Schlenkrich, M.; Brickmann, J. Force field parameters for carbohydrates. *J. Comput. Chem.*, **1996**, *17*, 450-468.

[14] Berkowitz, M.; McCammon, J.A. Molecular dynamics with stochastic boundary conditions. *Chem. Phys. Lett.* **1982**, *90*, 215-217.

[15] Van Gunsteren, W.F.; Berendsen, H.J.C. Algorithms for macromolecular dynamics and constraint dynamics. *Mol. Phys.*, **1977**, *34*, 1311- 1327.

[16] Ryckaert, J.P.; Ciccotti, G.; Berendsen, H.J.C. Numerical integration of the cartesian equations of motion of a system with constraints: Molecular dynamics of n-alkanes. *J. Comput. Phys.*, **1977**, *23*, 327-341.

[17] Andersen, H.C. Rattle: a "velocity" version of the Shake algorithm for molecular dynamics calculations, *J. Comput. Phys.*, **1983**, *52*, 24-34.

[18] Hess, B.; Bekker, H.; Berendsen, H.J.C.; Fraaije, J.G.E.M. LINCS: A linear constraint solver for molecular simulations. *J. Comput. Chem.*, **1997**, *18*, 1463-1472.

[19] McQuarrie, D.A. *Statistical Mechanics;* Harper and Row: New York, **1976**.

[20] Simonson, T. Macromolecular electrostatics: continuum models and their growing pains. *Curr. Opin. Struct. Biol.*, **2001**, *11*, 243-252.

[21] Feig, M.; Brooks, C.L. Recent advances in the development and application of implicit solvent models in biomolecule simulations. *Curr. Opin. Struct. Biol.*, **2004**, *14*, 217-224.

[22] Baker, N.A. Improving implicit solvent simulations: a Poisson-centric view. *Curr. Opin. Struct. Biol.*, **2005**, *15*, 137-149.

[23] Bashford, D.; Case, D.A. Generalized born models of macromolecular solvation effects. *Ann. Rev. Phys. Chem.*, **2000**, *51*, 129-152.

[24] Im, W.; Berneche, S.; Roux, B., Generalized solvent boundary potentials for computer simulations. *J. Chem. Phys.*, **2001**, *114*, 2924-2937.

[25] Gallicchio, E.; Levy, R.M. AGBNP: An analytic implicit solvent model suitable for molecular dynamics simulations and high-resolution modeling. *J. Comput. Chem.*, **2004**, *25*, 479-499.

[26] Felts, A.K.; Harano, Y.; Gallicchio, E.; Levy, R.M. Free energy surfaces of beta-hairpin and alpha-helical peptides generated by replica exchange molecular dynamics with the AGNP implicit solvent model. *Proteins: Struct. Funct. Bioinform.*, **2004**, *56*, 310-321.

[27] Jorgensen, W.L.; Chandrasekhar, J.; Madura, J.; Impey, R.W.; Klein, M.L. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.*, **1983**, *79*, 926-935.

[28] Mahoney, M.W.; Jorgensen, W.L. A five-site model for liquid water and the reproduction of the density anomaly by rigid, nonpolarizable potential functions. *J. Chem. Phys.*, **2000**, *112*, 8910-8922.

[29] Berendsen, H.J.C.; Grigera, J.R.; Straatsma, T.P. The missing term in effective pair potentials. *J. Phys. Chem.*, **1987**, *91*, 6269-6271.

[30] Elcock, A.H. Molecular simulations of diffusion and association in multimacromolecular systems. *Numerical Comput. Methods D: Methods Enzymol.*, **2004**, *383*, 166-198.

[31] Ermak, D.L.; McCammon, J.A. Brownian dynamics with hydrodynamic interactions. *J. Chem. Phys.*, **1978**, *69*, 1352- 1360.

[32] Gabdoulline, R.R.; Wade, R.C. Biomolecular diffusional association. *Curr. Opin. Struct. Biol*., **2002**, *12*, 204-213.

[33] Metropolis, N.; Rosenbluth, A.; Rosenbluth, R.; Teller, A.; Teller, E. Equation of state calculations by fast computing machines. *J. Chem. Phys.*, **1953**, *21*, 1087-1092.

[34] Northrup, S.H.; McCammon, J.A. Simulation methods for protein structure fluctuations. *Biopolymers*, **1980**, *19*, 1001-1016.

[35] La Berge, L.J.; Tully, J.C. A rigorous procedure for combining molecular dynamics and Monte Carlo simulation algorithms. *Chem. Phys.*, **2000**, *260*, 183- 191.

[36] Faller, R.; de Pablo, J.J. Constant pressure hybrid molecular dynamics-Monte Carlo simulations. *J. Chem. Phys.*, **2002**, *116*, 55-59.

[37] Hampton, S.S.; Izaguirre, J.A. Shadow hybrid Monte Carlo: an efficient propagator in phase space of macromolecules, *J. Comput. Phys.*, **2004**, *200*, 581-604.

[38] Kirkpatrick, S.; Gelatt, C.D.; Vecchi, M.P. Optimization by simulated annealing. *Science*, **1983**, *220*, 671-680.

[39] a) Car, R.; Parrinello, M. Unified Approach for molecular dynamics and density-functional theory. *Phys. Rev. Lett.*, **1985**, *55*, 2471-2474; b) Kuhne, T.D.; Krack, M.; Mohamed, F.R.; Parrinello, M. Efficient and accurate Car-Parrinello-like approach to born-oppenheimer molecular dynamics. *Phys. Rev. Lett.*, **2007**, *98*, 066401-5. c) Marx, D.; Hutter, J. Ab initio molecular dynamics: Theory and Implementation, *Modern Methods and Algorithms of Quantum Chemistry*). Grotendorst, J., Ed. Jonh von Neumann Institute for Computing, Julich, NIC Series, **2000**, vol. *1*, ISBN 3-00-005618-1, pp. 301-449.

[40] Straatsma, T.P.; McCammon, J.A. Computational alchemy. *Ann. Rev. Phys. Chem.*, **1992**, *43*, 407-435.

[41] Kollman, P. Free energy calculations: applications to chemical and biochemical phenomena. *Chem. Rev*., **1993**, *93*, 2395-2417.

[42] Almlof, M.; Brandsdal, B.O.; Aqvist, J. Binding affinity prediction with different force fields: evaluation of the linear interaction energy method. *J. Comput. Chem.*, **2004**, *25*, 1242-1254.

[43] Gilson, M.K.; Given, J.A.; Bush, B.L.; McCammon, J.A.., Statistical-thermodynamic basis for computation of binding affinities: a critical review. *Biophys. J.*, **1997**, *72*, 1047-1069.

[44] Srinivasan, J.; Cheatham, T.E.; Cieplak, P.; Kollman, P.A.; Case, D.A. Continuum solvent studies of the stability of DNA, RNA and phosphoramidate-DNA helices. *J. Am. Chem. Soc.*, **1998**, *120*, 9401-9409.

[45] Eriksson, M.A.; Pitera, J.; Kollman, P.A. Prediction of the binding free energies of new TIBO-like HIV-1 reverse transcriptase inhibitors using a combination of PROFEC, PB/SA, CMC/MD, and free energy calculations. *J. Med. Chem.*, **1999**, *42*, 868- 881.

[46] Pitera, J.W.; Kollman, P.A. Exhaustive mutagenesis *in silico*: multicoordinate free energy calculations on proteins and peptides. *Proteins: Struct. Funct. Genet*., **2000**, *41*, 385-397.

[47] Radmer, R.J.; Kollman, P.A. The application of three approximate free energy calculations methods to structure based ligand design: Trypsin and its complex with inhibitors, *J. Comput-Aided Mol. Des.*, **1998**, *12*, 215-227.

[48] Pearlman, D.A. Free energy grids: a practical qualitative application of free energy perturbation to ligand design using the OWFEG method. *J. Med. Chem.*, **1999**, *42*, 4313-4324.

[49] Pearlman, D.A.; Charifson, P.S. Improved scoring of ligand-protein interactions using OWFEG free energy grids. *J. Med. Chem.*, **2001**, *44*, 502-511.

[50] Banba, S.; Guo, Z.Y.; Brooks, C.L. III. Efficient sampling of ligand orientations and conformations in free energy calculations using the λ-dynamics method. *J. Phys. Chem. B*, **2000**, *104*, 6903-6910.

[51] Damodaran, K.V.; Banba, S.; Brooks, C.L. III. Application of multiple topology l-dynamics to a host-guest system: β-cyclodextrin with substituted benzenes. *J. Phys. Chem. B*, **2001**, *105*, 9316-9322.

[52] Rodiger, T.; Howell, P.L.; Pomes, R. *J. Chem. Phys.*, **2005**, *123*, 034104.

[53] Brandsdal, B.O.; Osherberg, F.; Almof, M.; Feirberg, I.; Luzhkov, V.B.; '*Free Energy calculations and ligand binding*' in *Advances in protein Chemistry*, Amsterdam: Elsevier, **2003**, vol. *66*, 123-158.

[54] Debrunner, P.G.; Frauenfelder, H. Dynamics of proteins. *Ann. Rev. Phys. Chem.*, **1982**, *33*, 283-299.

[55] McCammon, J.A.; Lee, C.Y.; Northrup, S.H. Side-chain rotational isomerization in proteins: a mechanism involving gating and transient packing defects. *J. Am. Chem. Soc*., **1983**, *105*, 2232-2237.

[56] Israelewitz, B.; Gao, M.; Schulten, K. Steered molecular dynamics and mechanical functions of proteins. *Curr. Opin. Struct. Biol.*, **2001**, *11*, 224-230.

[57] Isralewitz, B.; Baudry, J.; Gullingsrud, J.; Kosztin, D.; Schulten, K. Steered molecular dynamics investigations of protein function. *J. Mol. Graphics Modelling* **2001**, *19*, 13-25.

[58] Wong, C.F.; McCammon, J.A. Protein simulation and drug design. *Adv. Protein Chem.*, **2003**, *66*, 87-121.

[59] Brooijmans, N.; Kuntz, I. D., Molecular recognition and docking algorithms. *Ann. Re*V. *Biophys. Biomol. Struct.*, **2003**, *32*, 335-373.

[60] Halperin, I.; Ma, B. Y.; Wolfson, H.; Nussinov, R., Principles of docking: an overview of search algorithms and a guide to scoring functions. *Proteins: Struct., Funct. Genet.* **2002**, *47*, 409-443.

[61] Pospisil, P.; Folkers, G. Making the best account of molecular docking in drug design. *FABAD J. Pharm. Sci*., **2004**, *29*, 81-92.

[62] Jonckheere, H.; Anne, J.; De Clercq, E. The HIV-1 reverse transcription (RT) process as target for RT inhibitors. *Med. Res. Rev*., **2000**, *20*, 129-154.

[63] Abbondanzieri, E.A.; Bokinsky, G.; Raush, J.W.; Zhang, J.X.; Le Grice, S.F.J.; Zhuang, X. Dynamic binding orientations direct activity of HIV reverse transcriptase. *Nature*, **2008**, *453*,184-190.

[64] Boojamra, C.G.; Mackman, R.L.; Markevitch, D.Y.; Prasad, V.; Ray, A.S.; Douglas, J.; Grant, D.; Kim, C.U.; Cihlar, T. Synthesis and anti-HIV activity of GS-9148 (2′-Fd4AP), a novel nucleoside phosphonate HIV reverse transcriptase inhibitor. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 1120-23.

[65] Wainberg, M.A.; Cahn, P.; Bethel, R.C.; Sawyer, J.; Cox, S., Apricitabine: a novel deoxycytidine analogue nucleoside reverse transcriptase inhibitor for the treatment of nucleoside-resistant HIV infection. *Antivir. Chem. Chemother*., **2007**, *18*, 61-70.

[66] Raval, K.J.; Tripathi, H.E.; Katti, S.B.; Pannecocque, C.; De Clercq, E. Synthesis and biological evaluation of 2,3-diaryl substituted-1,3-thiazolidin-4-ones as anti-HIV agents. *Med. Chem*., **2007**, *3*, 355-363.

[67] Tantillo, C.; Ding, J.; Jacobo-Molina, A.; Nanni, R.G.; Boyer, P.L.; Huges, S.H.; Pauwels, R.; Andries, K.; Janssen, Arnold E. Locations of anti-AIDS drug binding sites and resistance mutations in the three-dimensional structure of HIV-1 reverse transcriptase. Implications for mechanisms of drug inhibition and resistance, *J. Mol. Biol.*, **1994**, *243*, 369-387.

[68] Gulick, R.M. New antiretroviral drugs. *Clin. Microbiol. Infect*., **2003**, *9*, 186-193.

[69] Li, Z.; Han, J.; Chen, H.F. Revealing interaction mode between HIV-1 reverse transcriptase and diaryltriazine analog inhibitor, *Chem. Biol. Drug Des*., **2008**, *72*, 350-359.

[70] Das, K.; Clark, A.D. Jr.; Lewis, P.J.; Heeres, J.; De Jonge, M.R.; Koymans, L.M.; Vinkers, H.M.; Daeyaert, F.; Ludovici, D.W.; Kukla, M.J.; De Corte, B.; Kavash, R.W.; Ho, C.Y.; Ye, H.; Lichtenstein, M.A.; Andries, K.; Pauwels, R.; De Béthune, M.P.; Boyer, P.L.; Clark, P.; Hughes, S.H.; Janssen, P.A.; Arnold, E. Roles of conformational and positional adaptability in structure-based design of TMC125-R165335 (etravirine) and related non-nucleoside reverse transcriptase inhibitors that are highly potent and effective against wild-type and drug-resistant HIV-1 variants. *J. Med. Chem*., **2004**, *47*, 2550-2560.

[71] Da Silva, C.H.T.P.; Carvalho, I.; Taft, C.A. Molecular dynamics, docking, density functional and ADMET studies of HIV-1 reverse transcriptase inhibitors. *J. Theor. Comput. Chem.*, **2006**, *5*, 579-586.

[72] Lipinski, C.A.; Lombardo F., Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug. Deliv. Rev*., **2001**, *46*, 3-26.

[73] a) Nervall, M.; Hanspers, P.; Carlsson, J.; Boukharta, L.; Aqvist, J. Predicting binding modes from free energy calculations. *J. Med. Chem*., **2008**, *51*, 2657-2667; b) Carlsson, J.; Boukharta, L.; Aqvist, J. Predicting binding modes from free energy calculations. *J. Med. Chem*., **2008**, *51*, 2648-2656.

[74] Jones, G.; Willet, P.; Glen, R.C.; Leach, A.R.; Taylor, R.D. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol*., **1997**, *267*, 727-748; b) Verdonk, M.L.; Cole, J.C.; Hartshorn, M.J.; Murray, C.W.; Taylor, R.D. Improved

protein−ligand docking using GOLD. *Proteins: Struct. Funct. Genet.*, **2003**, *52*, 609-623.

[75] Marelius, J.; Kolmodin, K.; Feirberg, I.; Aqvist, J.Q. A molecular dynamics program for free energy calculations and empirical valence bond simulations in biomolecular systems. *J. Mol. Graph*., **1998**, *16*, 213-225.

[76] Wang, D.P.; Rizzo, R.C.; Tirado-Rives, J.; Jorgensen, W.L. Antiviral drug design: co**m**putational analyses of the effects of the L100I mutation for HIV-RT on the binding of NNRTIs. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2799-2802.

[77] DeClerq, E. New anti-HIV agents and targets. *Med. Res. Rev*., **2002**, *22*, 531-565.

[78] Tryska, J.; Tozzini, V.; Chang, C.A.; McCammon, J.A. HIV-1 protease substrate binding and product release pathways explored with coarse-grained molecular dynamics. *Biophys. J*., **2007**, *92*, 4179-4187.

[79] Hornak, V.; Okur, A.; Rizzo, R.C.; Simerling, C. HIV-1 protease flaps spontaneously open and reclose in molecular dynamics simulations. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 915-920.

[80] Perryman, A.L.; Lin, J.H.; McCammon, J.A. The role of hydrogen bonding in the enzymatic reaction catalyzed by HIV-1 protease. *Protein Sci*., **2004**, *13*, 1108-1123.

[81] Jenwitheesuk, E.; Samudrala, R. Improved prediction of HIV-1 protease-inhibitor binding energies by molecular dynamics simulations, *BMC Struct. Biol.*, **2003**, *3*, 1-9.

[82] Ringhofer, S.; Kallen, J.; Dutzel, R.; Billich, A.; Visser, A.J.W.G.; Scholz, D.; Steinhauser, O.; Schreiber, H.; Auer, M.; Kungl, A.J. X-ray structure and conformational dynamics of the HIV-1 protease in complex with the inhibitor SDZ283-910: agreement of time-resolved spectroscopy and molecular dynamics simulations. *J. Mol. Biol.*, **1999**, *286*, 1147-1159.

[83] Stoica, I.; Sadiq, S.K.; Coveney, P.V. Automated molecular simulation based binding affinity calculator for ligand-bound HIV-1 proteases. *J. Am. Chem. Soc.*, **2008**, *130*, 2639-2648.

[84] Wittayanarakul, K.; Hannongbua, S.; Feig, M. Accurate prediction of protonation state as a prerequisite for reliable MM-PB(GB)SA binding free energy calculations of HIV-1 protease inhibitors. *J. Comput. Chem*., **2008**, *29*, 673-685; b) Hou, T.; McLaughlin, W.A.; Wang, W. Evaluating the potency of HIV-1 protease drugs to combat resistance, *Proteins: Struct. Funct. Bioinform.*, **2008**, *71*, 1163-1174.

[85] Durdagi, S.; Mavromoustakos, T.; Chronakis, N.; Papadopoulos, M.G. Computational design of novel fullerene analogues as potential HIV-1 PR inhibitors: Analysis of the binding interactions between fullerene inhibitors and HIV-1 PR residues using 3D QSAR, molecular docking and molecular dynamics simulations. *Bioorg. Med. Chem.*, **2008**, *16*, 9957-9974 and references therein.

[86] Durdagi, S.; Mavromoustakos, T.; Papadopoulos, M.G. 3D QSAR CoMFA/CoMSIA, molecular docking and molecular dynamics studies of fullerene-based HIV-1 PR inhibitors. *Bioorg. Med. Chem*., **2008**, *18*, 6283-6289.

[87] Zhang, D.W.; Huang, P.L.; Lee-Huang, S.; Zhang, J.Z.H. Design of hybrid inhibitors to HIV-1 protease. *J. Theor. Comput. Chem. (JTCC)*, **2008**, *7*, 485-503.

[88] Clemente, J.C.; Robbins, A.; Grafia, P.; Paleo, M.R.; Correa, J.F.; Villaverde, M.C.; Sardina, F.J.; Govindasamy, L.; Agbandje-McKenna, M.; McKenna, R.; Dunn, B.M.; Sussman, F. Design, synthesis, evaluation and crystallographic based structural studies of HIV-1 protease inhihbitors with reduced Response to the V82A mutation. *J. Med. Chem.*, **2008**, *51*, 852-860.

[89] Dyda, F.; Hickman, T.M.; Jenkins, A.; Engelman, R.C.; Davies, D.R. Crystal structure of the catalytic domain of HIV-1 integrase reveals similarity with other polynucleotidyl transferases. *Science*, **1994**, *266*, 1981-1986;

[90] a) Bujacz, G.; Jastolski, M.; Alexandratos, J.; Wlodawer, A.; Merkel, G.; Katz, R.A.; Skalka, A.M. The catalytic domain of avian sarcoma virus integrase: conformation of the active-site residues in the presence of divalent cations. *Structure*,**1996**, *4*, 89-96; b) Lubkowski, J.; Yang, F.; Alexandratos, J.; Wlodawer, A.; Zhao, H.; Burke, T.R. Jr.; Neamati, N.; Pommier, Y.; Merkel, G.; Skalka, A.M. Structure of the catalytic domain of avian sarcoma virus integrase with a bound HIV-1 integrase-targeted inhibitor. *Proc. Natl. Acad. Sci. USA*, **1998**, *9*, 4831-4836. http://www.pnas.org/content/95/9/4831.full?ck=nck - aff-1#aff-1

[91] Pommier, Y.; Johonson, A.A.; Marchand, C. Integrase inhibitors to treat HIV/AIDS. *Nat. Rev. Drug Discov*., **2005**, *3*, 236-248.

[92] Lins, R.D.; Briggs, J.M.; Straatsma, T.P.; Carlson, H.A.; Greenwald, J.; Choe, S.; McCammon, J.A. Molecular dynamics studies on the HIV-1 integrase catalytic domain. *Biophys. J*., **1999**, *76*, 2999-3011.

[93] Gray, J.J.; Moughon, C.; Wang, O.; Schouler-Furman, B.; Kuhlman, B.; Rohl, C.A.; Baker, D. Protein–protein docking with simultaneous optimization of rigid-body displacement and side-chain conformations. *J. Mol. Biol.*, **2003**, *331*, 281-299.

[94] Wang, J.M.; Cieplak, P.; Kolmann, P.A. How well does a renstrained electrostatic potential (RESP) model perform in calculating conformational energies of organic and biological molecules? *J. Comput. Chem*., **2000**, *21*, 1049-1074.

[95] Wang, J.M.; Wolf, R.M.; Caldwell, J.W.; Kolmann, P.A.; Case, D.A. Development and testing of a general amber force field. *J. Comput. Chem*., **2004**, *25*, 1157-1174.

[96] a) Cotelle, P. Patented HIV-1 integrase inhibitors (1998-2005), *Rec. Pat. Antiinfect. Drug Discov.*, **2006**, *1(1)*, 1-15; b) Jegede, O.; Babu, J.; Di Santo, R.; McColl, D.J.; Weber, J.; Quiñones-Mateu, M. HIV type 1 integrase inhibitors: from basic research to clinical implications. *AIDS Rev*., **2008**, *10*, 172-189.

[97] Kong, R.; Tan, J.J.; Ma, H.; Chen, W.Z.; Wang, C.X. Prediction of the binding mode between BMS-378806 and HIV-1 gp120 by docking and molecular dynamics simulation. *Biochem. Biphys. Acta Sec. Proteins Proteom.*, **2006**, 766-772; and references therein.

[98] Manetti, F.; Tintori, C.; Armand-Ugo´n, M.; Clotet-Codina, I.; Massa, S.; Ragno, R.A.; Este´, J.; Botta, A. Combination of molecular dynamics and docking calculations to explore the binding mode of ADS-J1, a polyanionic compound endowed with anti-HIV-1 activity. *J. Chem. Inf. Model*., **2006**, *46*, 1344-1351.

[99] Fano, A.; Ritchie, D.W.; Carrieri, A. Modeling the structural basis of human CCR5 chemokine receptor function: from homology model building and molecular dynamics validation to agonist and antagonist docking. *J. Chem. Inf. Model*., **2006**, *46*, 1223-1235.

[100] Martins Do Canto Antonio, M.T.; Palace Carvalho, A.J.; Prates Ramalho, J.P.; Loura, L.M.S. T-20 and T-1249 HIV fusion inhibitors' structure and conformation in solution: a molecular dynamics study. *J. Peptide Sci.*, **2008**, *14*, 442-447.

[101] Lavecchia, A.; Cosconati, S.; Novellino, E.; Calleri, E.; Temporini, C.; Massolini, G.; Carbonare, G.; Fracchiolla, G.; Loiodice, F. Exploring the molecular basis of the enantioselective binding of penicillin G acylase towards a series of 2-aryloxyalkanoic acids: A docking and molecular dynamics study. *J. Mol. Graph. Model*., **2007**, *25*, 773-783.

[102] Rivail, L.; Giner, M.; Gastineau, M.; Soulier, J.L. New insights into the human 5-HT4 receptor binding site: exploration of a hydrophobic pocket. *Br. J. Pharmacol*., **2004**, *143*, 361-370.

[103] Rivail, L.; Chipo, C.; Maigret, B.; Bestel, I.; Sicsis, S.; Tarek, M. Large-scale molecular dynamics of a G protein-coupled receptor, the human 5-HT_4 serotonin receptor, in a lipid bilayer. *J. Mol. Struct. (TEOCHEM)*, **2007**, *817*, 19-26.

[104] Wang, Z.; Ling, B.; Zhang, R.; Liu, Y. Docking and molecular dynamics study on the inhibitory activity of coumarins on aldose reductase. *J. Phys. Chem. B*., **2008**, *112*, 33-40.

[105] Xiao, J.; Guo, Z.; Guo, Y.; Chu, F.; Sun, P. Computational study of human phosphomannose isomerase: Insights from homology modeling and molecular dynamics simulation of enzyme bound substrate, *J. Mol. Graph. Mod*., **2006**, *25*, 289-295.

[106] Hamza, A.; AbdulHamees, M.D.M.; Zhan, C. Understanding microscopic binding of human microsomal prostaglandin E synthase-1 (mPGES-1) with substrates and inhibitors by molecular modeling and dynamics simulation. *J. Phys. Chem. B*, **2008**, *112*, 7320-7329.

[107] Yoshimatsu, K.; Altorki, N.K.; Golijanin, D.; Zhang, F.; Jakobsson, P.J.; Danneberg, A.J.; Subbaramaiah, K. Inducible prostaglandin E synthase is overexpressed in non-small cell lung cancer. *Clin. Cancer Res*., **2001**, *7*, 2669-2674.

[108] Mehrotra, S.; Morimiya, A.; Argarwal, B.; Konger, R.; Badve, S. Microsomal prostaglandin E2 synthase-1 in breast cancer: a potential target for therapy. *J. Pathol*., **2006**, *208*, 356-363.

[109] Amaro, R.E.; Schnaufer, A.; Interthal, H.; Hol, W.; Stuart, K.D.; McCammon, J.A. Discovery of drug-like inhibitors of an essential RNA-editing ligase in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. USA*, **2008**, *105*, 17278-17283.

[110] Dolenc, J.; Oostenbrink, C.; Koller, J.; van Gunsteren, W.F. Molecular dynamics simulations and free energy calculations of

netropsin and distamycin binding to an AAAAA DNA binding site. *Nucleic Acids Res.*, **2005**, *33*, 2, 725-733.

[111] Mendieta, J.; Ramirez, G.; Gago, F. Molecular dynamics simulations of the conformational changes of the glutamate receptor ligand-binding core in the presence of glutamate and kainate, *Proteins: Struct. Funct. Genomics*, **2001**, *44*, 460-469.

[112] Sonoda, M.T.; Martinez, L.; Webb, P.; Skaf, M.S.; Polikarpov, I. Ligand dissociation from estrogen receptor is mediated by receptor dimerization: evidence from molecular dynamics simulations. *Mol. Endocrinol.*, **2008**, *22*, 1565-1578.

[113] Jordan, V.C. Antiestrogens and selective estrogen receptor modulators as multifunctional medicines. 2. Clinical considerations and new agents. *J. Med. Chem.*, **2003**, *46*, 1081-1111.

[114] Blondel, A.; Renaud, J.P.; Fisher, S.; Moras, D.; Karplus, M. Retinoic acid receptor: a simulation analysis of retinoic acid binding and the resulting conformational changes. *J. Mol. Biol.*, **1999**, *291*, 101-115.

[115] Carlsson, P.; Burendhal, S.; Nilsson, L. Unbinding of retinoic acid from the retinoic acid receptor by random expulsion molecular dynamics. *Biophys. J.*, **2006**, *91*, 3151-61.

[116] Kosztin, D.; Izrailev, S.; Shulten. K. Unbinding of retinoic acid from its receptor studied by steered molecular dynamics. *Biophys. J.*, **1999**, *76*, 188-197.

[117] Frembeng-Kesner, T.; Elcock, A.H. Computational sampling of a cryptic drug binding site in a protein receptor: explicit solvent molecular dynamics and inhibitor docking to p38 MAP kinase. *J. Mol. Biol.*, **2006**, *359*, 202-214.

[118] Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P.F.; Gilmore, T.; Graham, A.G. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat. Struct. Biol.*, **2002**, *9*, 268-272.

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