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# Molecular modeling in cardiovascular pharmacology: current state of the art and perspectives

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Teaser: We present a comprehensive review of molecular modeling applications in cardiovascular medicine. Computer modeling constitutes a promising new tool to improve knowledge from molecular to personalized medicine.

## Author biographies

### Jennifer Lagoutte-Renosi

Jennifer Lagoutte-Renosi was awarded a PhD in pharmacology in 2017. She is currently a pharmacologist in the University Hospital of Besançon, France, where she works on anti-infectious and cardiovascular agents. Moreover, she also works in the University of Burgundy-Franche-Comté (UFC), France. Her research focuses on interactions of antiplatelet agents on the platelet plasma membrane, integrating computer simulation approaches. She is working to improve understanding of membranotropic drugs to contribute to the optimization and development of novel antiplatelet candidates.

### Christophe Ramseyer

Christophe Ramseyer studied physics and chemistry at UFC. His PhD (awarded in 1994) was devoted to adsorption phenomena and surface sciences under the supervision of C. Girardet. After postdoctoral training with P. Zeppenfeld and G. Comsa at IGV-KFA Julich, Germany, he moved to UFC to obtain his research oversight qualification in nanosciences and growth of adsorbate on surfaces containing defects. Since 2002, he has been a professor at UFC, where he develops simulation studies applied to nanosciences, with a current focus on biophysics and drug targeting in cancer therapies.

**Molecular modeling in pharmacology is a promising emerging tool for exploring drug interactions with cellular components. Recent advances in molecular simulations, big data analysis, and artificial intelligence (AI) have opened new opportunities for rationalizing drug interactions with their pharmacological targets. Despite the obvious utility and increasing impact of computational approaches, their development is not progressing at the same speed in different fields of pharmacology. Here, we review current *in silico* techniques used in cardiovascular diseases (CVDs), cardiological drug discovery, and assessment of cardiotoxicity. *In silico* techniques are paving the way to a new era in cardiovascular medicine, but their use somewhat lags behind that in other fields.**

## Introduction

In 2013, the Nobel Prize for Chemistry was awarded jointly to Martin Karplus, Michael Levitt, and Arieh Warshel 'for the development of multiscale models for complex chemical systems'.<sup>1</sup> Computer models mirroring real life are now crucial tools in modern chemistry, often predicting the outcome of traditional experiments. Computer-aided drug design (CADD) comprises a range of theoretical and computational approaches that form part of modern drug discovery. Among these methods, molecular modeling has made key contributions to the development of drugs. For instance, expansions in computational analysis, using new computer methodologies such as quantum chemistry, molecular docking, and molecular dynamics (MD), have opened new opportunities for rationalizing drug interactions with their pharmacological targets. For emerging diseases, these advances in computer modeling are particularly well suited.<sup>2</sup> They make it possible to save time during drug development and require more moderate budgets. In this regard, the current Coronavirus 2019 (COVID-19) pandemic is an illustrative example, requiring the rapid development of new antivirals.<sup>3</sup> For this purpose, computer modeling was used as an important tool to

accelerate drug design projects and validation of drug candidates.<sup>4</sup> As a consequence, molecular modeling is garnering increasing importance because of its potential application to all drug classes.

In recent years, molecular simulations have shown a promising synergy with emerging machine learning (ML) and AI techniques, which are expected to bring a revolutionary change to diagnostics, management and analysis of medical data, drug discovery, and optimization of clinical trials.<sup>5-7</sup> Traditional molecular simulations integrate well into AI pipelines, serving as solid references and providing data for model training and validation.<sup>8-12</sup>

CVDs remain the main cause of death worldwide, followed by cancer,<sup>13,14</sup> but data from computer modeling appear, at first glance, rather limited in the field of CVDs compared with oncology. In this review, we compare the development of computer modeling in three major drug classes (antineoplastic agents, cardiovascular agents, and antiviral agents) to understand the advances in computer simulations in the field. We provide a focused review of computer modeling in the field of cardiovascular drugs and cardiotoxicity induced by these drugs.

Molecular modeling can provide major mechanistic insights into drug–target<sup>15</sup> or drug–membrane<sup>16,17</sup> interactions, paving the way for the design of novel cardiovascular drug candidates. Instead of blindly searching through drug candidates, a mechanistic understanding of the interactions between drugs and their therapeutic targets enables the rational design of drug candidates that minimizes side effects and improves drug efficacy. Thus, computer simulations at the molecular scale enable a better understanding of the behavior of cardiovascular drugs or of the mechanisms of cardiotoxicity.<sup>18-20</sup> As molecular modeling continues to grow in the CVD field, it is important to demonstrate its principles, benefits, and limitations. Here, we highlight the prospects for computer simulation based on recent success stories.

### Literature trends

To find relevant publications, we searched publication databases using combinations of Medical Subject Heading (MeSH) keywords, the controlled vocabulary thesaurus used for indexing articles for PubMed. Combinations of keywords, such as ‘computer simulation’ with ‘cardiovascular agents’ or ‘antineoplastic agents’ or ‘antiviral agents’ yielded 1129, 5502, and 2706 results respectively from 1987 until April 15, 2021. The impact on pharmacology resulting from the development of multiscale models for complex chemical systems is shown in Figure 1. Indeed, the percentage of publications increased drastically from 2012 onward for each of the three fields, although the slopes of these increases differ. The proportion of publications related to simulations in the field of antineoplastic and antiviral agents increased continuously, whereas that related to simulation in the field of cardiovascular agents (Figure 1) remained stable and of a lower magnitude. Furthermore, peaks appearing in Figure 1 relate to an emerging disease and, thus, an urgent need to find a drug. For example, peaks related to computer simulation and antiviral agents around 1994 reflect the active development of drugs to against HIV. The crystal structure of the protease of HIV-1 was determined in 1989<sup>21</sup> and served as a new pharmacological target for the development of inhibitors of this specific protease with the help of computer-aided design.<sup>22,23</sup> Recently, the emergence of COVID-19 has also led to a marked increase in publications related to computer simulation in the antiviral literature (Figure 1). Thus, computer modeling has become increasingly important during the drug development process and the search for candidate drugs against COVID-19 and other emerging diseases.

Thus, molecular modeling is now a powerful tool for drug discovery. There are three main reasons for the differences in drug discovery observed between the cardiovascular field, and the antiviral and antineoplastic fields. The first concerns the reduction in pharmaceuticals investments in CVD research over the past few decades, reflected by Eroom’s Law, whereby a drastic decline in innovation combined with rising costs has been observed over the past few decades in drug development. Therapeutics for CVD appear to be more sensitive to this trend than in other fields. Over the past 5 years, drugs to treat CVD diseases comprised only 6% of all new drug launches.<sup>24</sup> However, *in silico* simulations, including molecular modeling, should be useful tools to reduce the cost of drug discovery for predicting, for instance, the cardiotoxicity of potential drugs early in their development and for high-throughput screening of new molecules. Second, drug discovery is usually linked to the discovery of new target or biochemical pathways. In terms of the number of 3D protein structures that have been elucidated so far, and recorded in the Protein Data Bank (PDB), there are fewer for CVD (448) compared with oncology (2768) and virology (2498).<sup>25</sup> Finally, it might be that there are unique limitations to using computer simulations to study cardiovascular agents compared with other fields. Therefore, here we review molecular modeling techniques and address their complementarity and limitations in the cardiovascular field.

### *In silico* methods available for pharmacologists

#### *Drug–target interaction modeling*

The search for a new drug candidate requires the identification of a pharmacological target at the molecular level, usually a protein.<sup>26</sup> This can be identified by a pharmacology network approach, by direct experiments or by AI-powered searches. This molecular target then serves as a template for rational drug design. The drug discovery pipeline is adjusted depending on the availability of the 3D structure of the target (Figure 2). Since the 1990s, the concept of polypharmacology has been gaining increasing attention in drug discovery, and focuses on multitarget drugs to achieve the desired therapeutic effect.<sup>27,28</sup> Indeed, polypharmacology has potential applications in repurposing existing approved drugs, or designing *de novo* multitarget drugs. *In silico* methods have demonstrated great potential for the application of polypharmacology.

The 3D structure of the target protein is determined ideally either directly, using crystallography, nuclear magnetic resonance (NMR), or cryo-electronic microscopy (cryo-EM); or indirectly, by homology modeling, using a protein with a similar primary sequence. However, the tertiary structure of the pharmacological target is sometimes unknown, because only the endogenous ligands have been described,<sup>26</sup> or because the target is a transmembrane protein, which is difficult to extract from its lipid environment and characterize accurately with sufficient resolution. When the tertiary structure of the pharmacological target is known, structure-based methods can be used to further investigate the interaction between the target and drug candidates. If the tertiary structure is unknown, ligand-based methods can be used to identify drugs with homologies with endogenous ligands. In both cases, virtual screening can be used to identify leads, but with different approaches<sup>29</sup>.

When structure-based methods are used, screening is performed by two major techniques: (i) by direct docking molecules from a database to the structure of the target and by evaluating this binding, with software such as AutoDock<sup>30</sup>, AutoDock Vina<sup>31</sup>, or GOLD<sup>32</sup>; and (ii) by drug–target interaction AI or regression models pretrained on available target–ligand pairs with known binding affinities or biological activities. Such models implicitly account for both quantitative structure–activity relationships (QSARs) between the tested ligand and known ligands from the training data set and intermolecular interactions between the ligands and the protein-binding pocket. Pharmacophore models are often used to predict physicochemical characteristics of the ligands and the protein-binding sites and to evaluate their interactions quickly at a coarse-grained level.

When ligand-based methods are used, screening is performed by matching the endogenous pharmacophores of tested compounds with those of previously known ligands, drugs, or drug candidates specific for given target protein, or by using various other QSAR models, which estimate the biological activities of tested compounds based on their structure similarities with known ligands. AI models often excel in the ligand-based approach because they are able to capture complex, multiparametric, or otherwise hidden structure–function relations, which are overlooked by humans.<sup>33,34</sup> Design of a *de novo* ligand constitutes an alternative to virtual screening<sup>29</sup> by building a ligand that binds to the target with a docking or pharmacophore approach.

Once a lead has been identified, it is necessary to optimize its pharmacokinetic (PK) and pharmacodynamic (PD) profiles. Screening methods, such as docking and QSAR, can be used to improve the binding of the lead to the target and its activity. These methods use the crystallized structure of the protein under different conditions of temperature, such as low temperature for cryo-EM, or room temperature for X-rays and NMR. Therefore, all the structure-based methods mentioned so far do not fully consider the natural flexibility of the ligand, especially of the target protein. Small movements around equilibrium positions can be taken into account with so-called flexible ligands and flexible protein docking software. However, large conformational changes in proteins, such as folding, or domain motions that occur over longer time and space scales are not considered. Such conformational changes should be taken into account because they might reflect a closed, open, or intermediate state<sup>35</sup> of the proteins of interest. Only MD makes it possible to simulate these conformational changes with a timescale ranging from nanoseconds to a few milliseconds.<sup>36</sup> MD is a computer simulation technique that makes it possible to predict how a system will evolve over time and, consequently, to predict the movement of the molecules in the system. Free software is available, such as GROMACS,<sup>37</sup> NAMD,<sup>38</sup> AMBER,<sup>39</sup> or CHARMM.<sup>40</sup> MD can also be used to investigate the time-dependent dynamical and structural properties of the systems at the atomic level. The effects of the environment can also be considered. For instance, solvent, pH, temperature, and pressure can be modified in accordance with experimental conditions. For example, lipid–protein interactions can affect protein conformation and activity,<sup>41</sup> and MD makes it possible to simulate systems including complex mixtures of lipids, sugars, and cholesterol molecules with transmembrane proteins. MD simulations usually determine energy values and are the primary means for determining ligand-binding free energies. For instance, free energies can be computed through free energy perturbation (FEP) methods, which are based on statistical mechanics and calculate free energy differences in protein–ligand affinities in a more rigorous way.<sup>42</sup> However, they can be time consuming. The site identification by ligand competitive saturation (SILCS) technique is a way of bridging the gap between accurate, yet expensive FEP methods and fast, but less accurate docking methods.<sup>43</sup> MD requires substantial computational resources, because it uses elementary time steps at the femtosecond timescale to describe motions of the lightest atoms and molecules (generally those of the solvent; i.e., ions and water molecules). The duration of the MD simulation, which is the accumulation of these elementary steps, must last long enough to account for the dynamics of the proteins (micro to milliseconds). Aside from this major advantage, MD cannot be used to perform virtual screening with dozens of ligands, unlike docking, pharmacophore, and QSAR methods. An interesting coupling between MD and docking methods, the ensemble docking method, was recently developed<sup>44</sup> to account for the protein dynamics and efficiency of the docking procedure. Ensemble docking involves simulating the dynamics of a target over several hundred nanoseconds. Then, dockings are performed on numerous conformations extracted from the MD trajectory. This makes it possible to study the interaction of a ligand in all conformations of the target. However, MD has some limitations. The force fields that are usually implemented in MD contain bonded and nonbonded interactions. The latter are only described by electrostatic and Van der Waals terms (i.e., interactions with no electron exchange or polarization). Thus, the creation of covalent bonds is forbidden in classical MD.

Polarization effects can be taken into account with the use of polarizable force fields.<sup>45</sup> In enzymes, when ligands chemically ‘react’ with the active site of the target, only quantum chemistry-based methods are suitable for studying reactivity at the molecular level. Software, such as Gaussian,<sup>46</sup> NWChem,<sup>47</sup> ORCA,<sup>48</sup> and TeraChem,<sup>49</sup> make it possible to determine binding energies, structural features such as bond lengths and angles, and

characteristic vibration energies. Quantum mechanics (QM) methods also make it possible to determine energy barriers along reaction pathways, and through reaction kinetics. Most docking programs are rooted in classical molecular mechanics. However, the use of a more accurate description of the chemical interactions can now be used in QM-based docking software.<sup>50,51</sup> For instance, Cavasotto and Aucar developed a new QM scoring function for AutoDock Vina for high-throughput docking.<sup>52</sup> MD can be used particularly to study interactions with the plasma membrane<sup>53</sup> and investigate any resulting cardiotoxicity.<sup>20</sup> A non-exhaustive list of computer tools and software for computer simulation is available at [www.click2drug.org/](http://www.click2drug.org/). Specialized vocabulary for nonexperts in computer simulation is summarized in Box 1.<sup>54–58</sup>

## **Applications and outcomes of computer simulations in the cardiovascular field**

Despite the limited number of publications reporting the use of computer simulations in the cardiovascular field, we describe here the main aims of such approaches in this research field.

### *Discovery of new ligands*

Computer simulation, through virtual screening of drug candidates, is a powerful tool to accelerate the discovery of new ligands of a known therapeutic target. In this way, 3D QSAR, and comparative molecular field analysis (CoMFA) combined with molecular docking studies and MD have been used to predict probable binding modes of cardiovascular drug candidates<sup>59–187</sup> (Table 1).

For example, Silva *et al.*<sup>188</sup> used 3D QSAR studies and MD simulations to elucidate electrostatic and steric requirements related to the action of angiotensin 1 (AT1) antagonists. These data, providing insights into the structural and chemical requirements, were useful for optimizing the pharmacological and biological activity of a new generation of AT1 antagonists.

Potential drug candidates extracted from natural products, such as plants, fungi, or animals, have also been investigated using such *in silico* tools. Several of these ‘natural’ drugs are a mix of molecular species, although the most active compound of this cocktail is usually unknown. The extraction of each compound can be done experimentally and characterized by mass spectroscopy. However, it is hard to identify the most active one, because the efficiency test assays are time consuming. In such cases, docking methods can predict how each compound revealed binds with the pharmacological target. For instance, Li *et al.*<sup>76</sup> estimated angiotensin-converting enzyme (ACE) inhibitory potential of different peptides extracted from adlays. Further molecular simulation screening and a series of optimization led to a potent peptide, GAAGGAF, which was synthesized and assessed *in vivo* in hypertensive rats, confirming the potent antihypertensive effect of this peptide. This illustrates how computer simulations make it possible to choose among several peptides from a natural product to select those with optimal pharmacological activity against a specific target. They also make it possible to optimize the peptide sequence of a drug candidate. Moreover, molecular modeling methods enable the discovery of multitarget drugs. Medina-Ruiz *et al.* identified by virtual screening a potent inhibitor of endothelial NO synthase (eNOS) and cystathionine  $\gamma$  lyase,<sup>86</sup> both of which could be targeted to treat hypertension in a polypharmacological approach.

### *Validation of a modeling target*

MD can be used to validate one receptor model among several computer models generated, such as the AT1 receptor. Indeed, a study compared three models generated for the AT1 receptor, in terms of stability, quality, and ligand binding using MD.<sup>189</sup> These results showed that the commonly used bovine rhodopsin-based AT1 model has limitations, with AT1 homology modeling with multiple templates, including bovine rhodopsin, squid rhodopsin, human  $\beta 2$  receptor, and turkey  $\beta 1$ -adrenergic receptor, showing higher predictive power. This study highlights how MD makes it possible to validate the computer model of the therapeutic target before using it for other computer calculations.

### *Target prediction*

Computer analysis has also been used to screen potential targets. Chen *et al.*<sup>166</sup> applied network pharmacology analysis for investigating targets related to genistein, a drug candidate that treats pulmonary hypertension. For this purpose, two types of target were identified: targets of genistein and genes related to pulmonary hypertension. The intersection of targets in the network indicated potential antipulmonary hypertension targets of genistein. Different analyses were combined to identify targets and biological pathways involved, namely: (i) phenotype correlation of pulmonary hypertension; (ii) protein–protein networks; and (iii) gene ontology. In addition, molecular docking was used to predict the interaction of genistein with the identified target, peroxisome proliferator-activated receptor  $\gamma$ . This illustrates how computational approaches can identify a single target of a possible ligand in a specific disease. However, such an approach remains poorly described in the literature relating to CVDs.

### *Drug-binding mechanisms with therapeutic target*

The molecular details of drug/target interactions are not readily determined by experiments. Consequently, numerous studies have been conducted in cardiovascular research using computer simulations to elucidate the mechanisms involved in the interaction between cardiovascular drugs and their targets.

For example, using mainly MD, Nguyen *et al.* provided new insights into interactions between the human  $\text{Na}_v1.5$  ( $\text{hNa}_v1.5$ ) channel and antiarrhythmic drugs.<sup>141</sup> This channel is responsible for the rapid upstroke of cardiac action potential and is thought to be a target for antiarrhythmic therapy. The authors identified several potential binding sites at the pore lumen of this target. They also defined the maximum number of antiarrhythmic and anaesthetic

drugs that the pore lumen can accommodate simultaneously. In addition, they were able to identify the pathways for accessing the binding sites. Two distinct pathways were revealed: (i) hydrophilic access through the intracellular gate; and (ii) hydrophobic access through a fenestration between homologous domains DIII and DIV of the hNav1.5 channel. Understanding this phenomenon would not have been possible with conventional *in vitro* experiments. These molecular details can be used for further design of novel therapeutics. The authors also performed docking analysis of the static molecular model of the hNav1.5 channel, but were unable to reveal how the drug accesses the binding sites. This clearly shows that the choice of the computational method is crucial.

Another striking example is the case of vitamin K antagonists (VKAs). Vitamin K epoxide reductase (VKOR) regenerates vitamin K hydroquinone by reducing the vitamin K epoxide. Vitamin K is a cofactor of a post-transcriptional gamma-carboxylation that activates vitamin-K dependent proteins, such as factors of the coagulation cascade II, VII, IX, and X. Thus, VKAs, such as warfarin, are well-known inhibitors of the regeneration cycle of vitamin K, leading to hypocoagulable blood. Warfarin is used to prevent venous and arterial thrombotic events.<sup>190</sup> However, one of the major drawbacks of VKAs is their narrow therapeutic range and that polymorphism of *VKOR* can influence the VKA response. Thus, it is vital to identify the details of VKA interactions with VKOR. However, the binding site of VKAs on VKOR has not been clearly defined. Experimentally it has been shown that mutations in *VKOR* decrease affinity for warfarin binding without decreasing vitamin K affinity. A computational study<sup>191</sup> clarified the process of warfarin binding on a three-transmembrane VKOR model by MD. When warfarin binds to VKOR, the overall three-transmembrane structure is preserved, albeit with a modest, reversible conformational change of the architecture of VKOR, involving several residues. One key residue, Y139, was found to have a major role in this binding. This residue is located in the hydrophobic binding pocket and interacts through a complex T-shaped  $\pi$ - $\pi$  stacking interaction. Mutations of the residues inside the pocket were also studied in this numerical approach. Mutation of L128 or Y139 was found to significantly affect warfarin binding without affecting vitamin K binding. Results obtained by MD were confirmed by cell-based activity assay, strengthening the relevance of MD simulations.

Computer simulations could also guide the choice among several enzyme mutants used in a therapeutic setting. For example, several mutants obtained from site-directed mutagenesis of nattokinase, a serine protease that showed cardiovascular properties, in particular for improving circulation and reducing the risk of thrombosis,<sup>192</sup> were assessed by MD.<sup>193</sup> These simulations showed that conformation of the nattokinase active site changed with mutation, resulting in a closer relationship between Asp<sup>32</sup> and His<sup>64</sup>, which induced a stronger attraction of the proton of Ser<sup>221</sup> for the nitrogen atom in the side chain of His<sup>64</sup> and promoted the reaction catalyzed by the enzyme.

Taken together, these three examples show that computational methods, particularly MD, are of useful in elucidating drug-binding mechanisms.

#### *Exploration of additional mechanisms of action*

The human Kv1.5 (hKv1.5) channel, involved in the electrophysiological mechanism of the heart, has been studied in relation to the development of antiarrhythmic drugs. Docking simulations identified binding sites on the hKv1.5 channel of verapamil, an antagonist of L-type Ca<sup>2+</sup> channels. Verapamil was found to bind in the pore region of the hKv1.5 channel, contributing, in part, to prolonging the effective refractory period of human atrium and, therefore, preventing re-entrant-based atrial arrhythmias.<sup>135</sup> This example shows that computer simulations can also be predictive and can contribute to elucidating polypharmacological effects. Using network pharmacology, Li *et al.* identified a potent lead that could be optimized as a multitarget drug to treat CVDs.<sup>194</sup> Moreover, computer simulation can support the potent inhibition of enzymes involved in the metabolism of drugs, such as cytochrome P450 (CYP), which might be responsible for an additional mechanism of action. Ikemura *et al.* confirmed that manidipine, an antihypertensive drug from the family of dihydropyridines, was a potent reversible inhibitor of CYP2J2, involved in the conversion of arachidonic acid to epoxyeicosatrienoic acids (EETs) with a major role in CVDs and tumor angiogenesis.<sup>163</sup> CYP2J2 is highly expressed in a variety of cancer cells. Thus, a reduction in production of EETs by inhibition of CYP2J2 could have antitumorigenesis effects. The discovery of these inhibitory effects of CYP2J2 suggests additional anticancer effects of manidipine and azelnidipine, although these require further investigation.

#### *Permeation of drug through the plasma membrane*

Most common drugs are small molecules acting on intracellular proteins. They usually need to cross the cell membrane in the absence of specific transporters. Although membrane permeation constitutes a key factor influencing PK outcomes, it has not been widely studied. MD simulations have become a well-established method for studying permeation since the early work of Marrink and Berendsen.<sup>195</sup> They offer atomistic details of drug permeation through the plasma membrane, although only transcellular passive diffusion is considered and paracellular diffusion is usually disregarded. However, there have been few investigations of the permeation of CVD drugs through the plasma membrane. The plasma membrane is usually modeled as a simple planar bilayer comprising a single phospholipid, mainly 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). Yue *et al.*<sup>168</sup> studied the membrane permeation of propranolol with MD on a POPC model membrane to optimize its PK profile. In conventional models, which consider that only neutral molecules can permeate membranes, the intrinsic permeability of propranolol has to be overestimated to explain its measured permeation. In a given solvent, this molecule is a mixture of the charged and neutral forms of molecules and the partition coefficient results from the pH of the molecular environment. The authors showed that charged molecules can also cross the barrier as a result

of a deprotonation process. Dynamic protonation of a weak base, such as propranolol, revealed that protonation state and conformation equilibria are dynamically coupled, influencing the membrane permeation of the drug. Propranolol migrates into the lipid bilayer in the charged form and deprotonates at the hydrophobic boundary. Thus, a combined pH-coupled partitioning and permeation model is essential for ionizable drugs to take account of this phenomenon. Such knowledge can only be obtained from computational analysis. This result completely changed our understanding of how dynamic changes in protonation state might have a crucial role in the permeation of ionizable molecules that dynamically neutralize at the lipid-tail interface, reducing the permeation free-energy.

Another interesting example concerns epigallocatechin-3-gallate (EGCG),<sup>167</sup> a polyphenol that shows protective effects against CVDs by multiple mechanisms of action, including inhibition of the cardiac Nav1.5 channels in a dose-dependent manner. In this study, MD was used to investigate the interaction of EGCG with the bilayer of POPC lipids. EGCG initially positioned at the center of the lipid bilayer was found to equilibrate at the membrane headgroup region. No spontaneous crossing through the plasma membrane was observed, even though three very long runs of MD simulations were conducted. These simulations indicated that EGCG probably reaches its binding site on the Nav1.5 channel through the cell membrane.

Models using a lipid bilayer of a single type of lipid are not realistic, mainly because the exact composition of the lipid membrane is often unknown and no lipidomics analyses are available. However, the lipid composition of cell membranes is more complex, including different types of lipid, all with specific physicochemical particularities, which influences drug partitioning and permeation. In addition, lipid composition varies according to the type of cell,<sup>196</sup> emphasizing the importance of enhancing our knowledge of the lipid composition of each cell type to better investigate the effects of drugs according to the target cells involved. Work is under way to overcome this issue. For instance, MD simulations with complex lipid membranes have been performed to be more predictive not only of drug permeation across the plasma membrane, but also of the direct impact of the lipid environment on the dynamics of embedded protein.<sup>18</sup>

#### *Modulation of the lipid environment of the therapeutic target*

The lipid composition of the plasma membrane could impact the properties of drugs (conformation, electrostatics, and dynamics).<sup>197,198</sup> Conversely, drugs can also impact the properties of the plasma membrane, either directly, by modification of the lipid composition,<sup>199,200</sup> or indirectly, by modification of the fluidity of the membrane.<sup>201</sup> Recently, computer simulations highlighted that the lipid environment could impact the dynamics of receptors as a function of lipid bilayer composition. Haghghi *et al.*<sup>18</sup> performed MD simulations and ensemble docking analysis of a new realistic model of the P2Y<sub>12</sub> receptor. This target is of interest because of its crucial role in platelet activation and arterial thrombosis therapy. In this study, P2Y<sub>12</sub> was embedded in different models of membranes containing either phosphatidylcholines, sphingomyelins, or a combination of sphingomyelins with cholesterol. This work showed that the lipid environment constrains the dynamics and internal flexibility of P2Y<sub>12</sub> and, hence, influences the binding of P2Y<sub>12</sub> with its antagonists. A lipid environment comprising ordered (i.e., enriched in cholesterol and sphingomyelins) improved the binding propensity of P2Y<sub>12</sub> with its antagonists such as ticagrelor. This computational analysis combining MD and ensemble docking is one of the first in the cardiovascular field to highlight the role of receptor dynamics modulated by the surrounding lipid microenvironment and their involvement in the binding of antiplatelet agents.

Another example from Kiriakidi *et al.*<sup>202</sup> showed that the membrane incorporation of candesartan, an antagonist of the AT<sub>1</sub> receptor, used mainly for the treatment of high blood pressure and congestive heart failure, impacts recognition by its G-protein-coupled receptor AT<sub>1</sub> receptor. By using MD, the authors showed that the neutral form of candesartan modifies the microenvironment of the receptor through lipid diffusion inside the membrane. In addition, the deprotonated form of candesartan failed to diffuse through the membrane and directly interact with the extracellular loop of the AT<sub>1</sub> receptor. These simulations highlight the importance of physicochemical properties of the membrane, particularly pH in this case.

#### *Understanding pharmacokinetics*

The PK of drug–drug interactions (DDIs) can be studied by using computer simulation. For example, combinations of drugs usually involving DDIs can be simulated by MD in a system involving CYP in the metabolism to predict the DDI. Yousefpour *et al.*<sup>19</sup> investigated the interaction between the PEGylated form of amlodipine (PEGAML) and paroxetine (PAR), both of which are metabolized by CYP2B<sub>4</sub>. Interference between these drugs was reported because of their aggregation as well as their competition in occupying the active site of CYP2B<sub>4</sub>. MD simulations predicted that the presence of these drugs with the specific ratio of 1PEGAML:5PAR induced more effective diffusivity and stability with respect to CYP2B<sub>4</sub>. This result can guide clinicians prescribing these drugs simultaneously to patients with hypertension and depression. Thus, computer simulation led to the prediction of the optimum ratio of drugs involving the same metabolizing enzyme, which is directly relevant for clinicians to assist in the simultaneous prescribing of drugs that induce PK DDIs.

Specific features of cardiovascular drug PK can be studied to better understand the molecular mechanisms involved in drug absorption, distribution, metabolism, or elimination. For example, absorption of bisoprolol (BSP), a  $\beta$ -adrenergic receptor-blocking agent, across the skin, was studied using MD to design a transdermal drug delivery system.<sup>173</sup> This approach showed that BSP ion-pair complexes remained stable in the stratum corneum rich in ceramide 2 but dissociated in the viable epidermis rich in water. These findings contribute to a better

understanding of the molecular mechanisms involved in the absorption of BSP, leading consideration of the development of new dosage forms, such as transdermal drug delivery systems. In addition, computer simulations pave the way for the design of new optimized dosage forms, as described below.

#### *Optimization of dose form*

To optimize the dose form of a drug in terms of bioavailability, solubility, or targeting, improvements could be made through, for instance, vectorization and functionalization of the drugs. Jadhav *et al.*<sup>179</sup> assessed a polymer, VA-64, as a carrier for olmesartan, an antihypertensive drug with poor solubility and poor bioavailability. Indeed, modern thermoforming techniques for amorphization, such as hot-melt extrusion (HME), which involves the formation of a stable homogenous dispersion of drugs with pharmacologically inert, polymeric ingredients at elevated temperature, are used to enhance the solubility of drugs. These authors conducted MD simulations to better understand the molecular interactions between olmesartan and VA-64, and to maximize the dissolution of the olmesartan/VA-64 complex. *In vitro* dissolution experiments and *in vivo* PK studies were in agreement with computer simulations, signifying their potential application in rationally developing the optimal dosage form of cardiovascular drugs.

Zhang *et al.*<sup>180</sup> studied 1-(4-isopropylphenyl)- $\beta$ -carboline-3-carboxylic acid (ICCA), a promising cardiovascular agent able to inhibit glycoprotein IIb/IIIa (GpIIb/IIIa), a P-selectin involved in arterial and venous thrombosis. ICCA was coupled with Trp-Phe-Phe to form a selective GpIIb/IIIa and P-selectin nanoparticle to release ICCA on the surface of platelets, which are involved in thrombosis formation. This process limits the potential toxicity of drugs by releasing the drug in direct proximity to the target cells. In this case, computer simulations not only predicted the three steps of the formation of the nanoparticles, but also estimated how many molecules were involved in the nanoparticle.

#### *Toxicity prediction*

In clinical development, the prediction of the cardiotoxicity of new drugs is necessary to render drug development safer and efficient. Disturbance of the human ether-à-go-go-related gene (hERG), a voltage-gated potassium channel, induces disorders in the rapid delayed rectifier  $K^+$  current ( $I_{Kr}$ ) in the heart, which leads to arrhythmias caused by prolonging the time between the Q-wave and the T-wave of the electrical cycle of the heart, named long QT syndrome.<sup>203</sup> Several drugs are responsible for blocking hERG potassium channels. Consequently, regulatory agencies, such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMA), require that the proarrhythmic risk of drugs prolonging the QT interval be systematically assessed by different models, including *in silico* models.<sup>204</sup> *In silico* models have been developed as a reliable and faster solution<sup>205</sup> compared with *in vitro* assays to assess hERG-mediated cardiotoxic drugs. A new computational model of potential hERG-binding affinity of drugs was presented by Anwar-Mohamed *et al.*<sup>20</sup> and provided a new alternative to predict cardiotoxicity. Indeed, despite preclinical evaluation, a hepatitis C virus polymerase inhibitor, BMS-986094, exhibited fatal cardiotoxicity in a clinical trial, which was consequently abruptly halted.<sup>206</sup> In retrospect, implementation of the computational model developed by Anwar-Mohamed *et al.* confirmed the hERG-binding affinity of BMS-986094. Another multiscale model framework has been described to predict proarrhythmic drug interactions with the hERG channel to explain arrhythmia mechanisms.<sup>169</sup> Computer modeling can help to predict hERG-mediated cardiotoxic drugs in a unified framework for *in silico* drug discovery, development, and safety assessment<sup>207</sup>. Moreover, prediction of adverse drug reactions is essential in drug development. Different machine learning algorithms for cardiovascular adverse drug reaction prediction have been used, including features such as biological data (transporters, enzymes, and target involved), chemical data (substructure fingerprints) and phenotypic data (other adverse drug reaction data and therapeutic indications).<sup>208</sup> To appreciate the accuracy of these predictive models, their ability to predict already reported adverse drug events of known cardiovascular drugs was assessed. Results of the generated model were in accordance with a freely accessible database of adverse effects. Additionally, some adverse effects, not reported by this database, were predicted by generated models and were in accordance with literature evidence. FEP calculations were done by Mousaei *et al.* using the SILCS technique to test a set of 55 blockers on the hERG1 channel. pIC<sub>50</sub> predictions from the SILCS approach for this set of blockers were found to be in a good agreement relative to experimental data. These calculations showed that FEP computation using the SILCS technique can be used for the rapid screening of small molecules for their cardiotoxic potential as well as for exploring alternative binding pockets in the hERG1 channel with applications to the rational design of activators.<sup>209</sup> These models showed effective prediction of adverse cardiovascular effects, which could be used during drug development. Another machine learning algorithm example is a learning engine using virtual screening and docking, which was developed to predict drug-induced cardiac contractility.<sup>210</sup> These models showed effective prediction of adverse cardiovascular effects, which could also be used during the drug development.

#### *Cardiovascular drug resistance with efflux proteins*

P-glycoprotein (Pgp), also known as ATP-binding cassette, subfamily B, member 1 (ABCB1), or multidrug resistance-1 (MDR1), is a multidrug efflux pump with an important role in cardiovascular medicine.<sup>211</sup> Overexpression of Pgp occurs in hypoxic conditions,<sup>212-214</sup> a situation frequently encountered in patients with CVDs. Thus, upregulation of Pgp can decrease the effectiveness of treatment for such patients by reducing cardiovascular drug concentrations at the intracellular level of target cells, such as cardiomyocytes. Understanding the mechanism of drug recruitment by Pgp has a pivotal role in limiting drug resistance properties during drug



development. Jagodinsky *et al.*<sup>215</sup> explored interactions between Pgp and eight cardiovascular drugs (amiodarone, bepridil, diltiazem, dipyridamole, nicardipine, nifedipine, propranolol, and quinidine) using MD. A binding belt with multiple residues, which provides favorable electrostatic interaction, was found to bind all eight drugs. Hydrogen bonding, hydrophobic packing, and formation of an aromatic cage give electrostatic stability for binding. When the drugs were stabilized within the binding belt, water molecules condensed around them, resulting in a water influx essential for their catalytic transition and expulsion. This MD study shed light on the molecular mechanisms of Pgp, which are difficult to explore with experimental procedures. It also paves the way toward a new approach to the design of drugs regulating the efflux of Pgp at the molecular level.

### Limitations and challenges of molecular modeling

*In silico* simulations could be used to efficiently identify and design drug candidates, to study their interactions with their targets<sup>141</sup> and/or plasma membrane,<sup>53</sup> or to investigate their cardiotoxicity.<sup>20</sup> However, several limits hamper their applications.

First, to perform docking or MD simulations involving a targeted protein, its 3D structure must be resolved and characterized by crystallography, NMR, or cryo-EM.<sup>216</sup> As discussed above, few 3D protein structures from the CVD field have been published. Although this lack of newly discovered protein structures can be compensated by homology modeling, such models are questionable. Research is currently focusing on predicting 3D models of proteins with only knowledge of their primary structure.<sup>217</sup> Recent advances in AI-based *de novo* protein structure prediction, which involve algorithms such as AlphaFold2,<sup>218</sup> are usually considered to be the ultimate solutions of the protein folding problem from a practical aspect. Thus, in the coming years, the number of targets that are considered 'not druggable' because of the absence of the 3D structure are likely to decrease dramatically.

Docking, QSAR, and MD are useful tools for screening drugs and identifying a lead with optimal affinity for the binding site of the protein target.<sup>188</sup> However, proteins are rigid and can undergo large conformational changes over time that might manifest in 'closed' or 'open' states.<sup>35</sup> Only MD can in principle simulate these large conformational changes. However, these calculations have been restricted to nanosecond–microsecond timescales,<sup>36</sup> whereas most conformational changes occur over much longer times. Although numerous enhanced sampling techniques<sup>219,220</sup> are now available in MD, designed to overcome these limits, none of them have been universally adopted to date.

The size of the systems that could be investigated by all-atom MD is also restricted to the nanometer scale. Thus, there is a significant magnitude gap between the largest all-atom simulations and the 'real' systems to be studied. Coarse-grained MD can help to gain up to three orders of magnitude in time and space, at the cost of a lower spatial resolution of the system and inability to simulate changes in secondary structure.<sup>221</sup> Currently, MD engines utilize the whole spectrum of available supercomputer facilities, including modern GPUs, high performance computational clusters, and decentralized computational infrastructures.

In the same way, it is notoriously hard and laborious to perform screening of ligands with MD. However, ensemble docking appear to be an approach to bridge this gap.<sup>44</sup> Although this technique is limited by the timescales of MD simulations, it is capable of screening large numbers of ligands or drug candidates, introducing details of large-scale protein flexibility into the usual docking pipeline. Other techniques, which are often considered game changers, are AI and machine learning, which can be used on all stages of the drug discovery pipeline, including target identification, hit identification, ADMET prediction, lead optimization, drug repurposing, and prediction of clinical trials results.<sup>6,7</sup>

Computational modeling at the molecular level and pharmacometrics have always been performed separately. The new challenge will be to combine molecular and computational modeling at the cellular, tissue, organ, whole-body, or even population levels.<sup>222</sup> Indeed, larger scale modeling has been employed in the setting of therapeutic and safety drug discovery. CVD studies have been performed using both human cardiac cells and heart computer models to investigate the functional effect of therapeutic drugs<sup>223</sup>. Moreover, pharmacometric modeling methods are already frequently used. These include population PK or population PK/PD, which were developed to predict how individuals from different populations might respond to the same drug. Population modeling helps to identify covariates that might be associated with sources of PK variability in the target patient population. In safety pharmacology, electrophysiology models are used to screen for potentially harmful compounds. The public-private Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative proposed an *in silico* human cardiomyocyte model that integrates multi-ion channel pharmacology data obtained *in vitro* for proarrhythmia risk assessment.<sup>224,225</sup> Such models are part of a multiscale approach.

Additionally, computational modeling at the molecular level and above has also always been performed separately. The integration of molecular simulation data into larger scale models will make it possible to achieve more realistic models of physiology. Such models will reveal novel pathophysiological knowledge and eventually predict the optimal treatment option for an individual patient.

Thus, molecular modeling could also pave the way to personalized medicine. Recently, several studies have investigated the impact of mutations in proteins considered therapeutic targets in predicting drug activity.<sup>134,226</sup> For example, resistance to AT1 blockers was described according to a variety of polymorphisms of AT1 receptors. These models demonstrate the potential of molecular modeling in achieving personalized prediction of drug resistance. Although this field remains underdeveloped at the molecular scale, it could be a first step toward creating a personalized medicine approach. Indeed, patient-specific computer models have been developed to represent the cardiovascular system and are starting to be incorporated into clinical practice.<sup>227</sup>

Finally, the better the description of the system studied, the better the computer simulations will be. The 3D structures of proteins are usually published with high resolution, which is sufficient as a starting point for the computations. We have already mentioned that the availability of membrane compositions according to type of cells is limited.<sup>200</sup> In this regard, ‘omics’ will be vital for future computational developments. For instance, precise lipidomics will be necessary to model the exact lipid composition of plasma membranes. Proteomics will help to investigate protein–protein complexes and their potential roles with respect to drugs. The concept of metabolomics, whereby individuals would have their own metabolic profiles that could be reflected in their biological fluids, such as blood, would be helpful in the field of cardiovascular research.

### Concluding remarks

Thus, the work done by Martin Karplus, Michael Levitt and Arieh Warshel ‘for the development of multiscale models for complex chemical systems’ has clearly impacted pharmacology, but needs to be more anchored in the cardiovascular field. However, there is no doubt that new breakthroughs in computer simulations are paving the way to a possible bridge from molecular understanding to personalized medicine in the field of CVD.

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### Box 1. Glossary of terms

**Homology modeling:** technique to build the 3D structure of a target for which the 3D structure of a homolog protein, with a close primary sequence, is known.

**Ligand design:** *de novo* approach to build a ligand with physicochemical characteristics fitting those of the target.

**Molecular docking:** numerical simulation technique to predict the optimal conformation and structure of a ligand to bind to a receptor; the binding affinity can be evaluated by a scoring function.<sup>54</sup>

**Molecular dynamics (MD):** numerical simulation technique based on Newton's laws to predict the evolution of a system over time for a large number of molecules coupled together to access their dynamics.<sup>36</sup>

**Network pharmacology:** technique to predict drug actions and interactions with multiple targets by cataloguing molecular interactions of the drug in living cells.<sup>55</sup>

**Pharmacophore:** a molecule containing specific physicochemical characteristics (electron donor, electron acceptor, aromatic ring, etc.).

**Quantitative structure–activity relationship (QSAR) models:** models that use correlations between structure of molecules and biological responses to predict biological responses of a new structure.<sup>56,57</sup>

**Quantum chemistry:** numerical simulation technique based on Born–Oppenheimer approximation to resolve a Schrödinger equation that allows access to reactivity between molecules and their transition state, Gibbs free energy of reaction (useful to elucidate a reaction mechanism).<sup>58</sup>

**Virtual screening:** uses a computer simulation method to identify ligands from a database that bind to the target.<sup>54</sup>

**Figure 1.** Publications related to computer simulation among all publications in the field of cardiovascular agents, antineoplastic agents, and antiviral agents. Results are expressed as a percentage of publications found using the Medical Subject Heading (MeSH) database of MedLine for 'computer simulation' with 'cardiovascular agents', or 'antineoplastic agents', or 'antiviral agents', on all publications concerning each drug classes as cardiovascular agents, or antineoplastic agents or antiviral agents, respectively.

**Figure 2.** Decision-making tree in computer simulations. Illustrations show adenosine diphosphate and its receptor P2Y12, as the ligand and pharmacological target, respectively. Abbreviation: QSAR, quantitative structure–activity relationship.



**Table 1. Overview of publications reporting on computer simulations used in the cardiovascular field, found using the MeSH database of MedLine with the keywords ‘computer simulation’ and ‘cardiovascular agents’**

Ligands	Targets	PBD ID (technique used)	Technique/methods	Main conclusions and impact	Refs
<b>Discovery of new ligands</b>					
Black-eyed pea trypsin/chymotrypsin inhibitor (BTCI) peptides	ACE	1O8A (by X-ray)	Docking	BTCI and its related peptides occlude catalytic site of ACE	59
Peptides from naked oat globulin hydrolysates	ACE	1O8A (by X-ray)	Docking	Peptide binds with active site S1 of ACE via short hydrogen bonds with high affinity	60
Peptides from pearl oyster	ACE	1O86 (by X-ray)	Docking	One peptide binds competitively and another noncompetitively to ACE	61
Peptides from <i>Ganoderma lucidum</i>	ACE	2YDM (by X-ray)	MD	Three peptides inhibit ACE: One is a mixed-type inhibitor, which binds to ACE at positions including active and nonactive sites; second is a competitive inhibitor; and the third is a noncompetitive inhibitor	62
Peptides from glutelin of vinegar-soaked black soybean	ACE	1O86, 4CA5, and 4BZR (by X-ray)	Pharmacophore/docking	Two of five peptides had higher ACE inhibitory potency than lisinopril, phosphinic tripeptide FI, and K-26. Docking identified peptides that bind to different crystal structures of ACE	63
Peptides from <i>Saccharomyces cerevisiae</i>	ACE	1O8A (by X-ray)	Docking	Peptides have ACE-inhibitory activity by binding through hydrogen bonds, hydrophobic, hydrophilic, and electrostatic interactions	64
Peptides from <i>Ulva intestinalis</i>	ACE	1O8A (by X-ray)	Docking	Hydrogen bonds and interaction with Zn <sup>2+</sup> of ACE contribute to ACE-inhibitory activities of two peptides	65
Proline derivates	ACE	1O8A (by X-ray)	Docking	Ligand was identified with higher affinity for ACE compared with fosinopril	66
Thiocarbamates from <i>Moringa oleifera</i>	ACE	1O8A (by X-ray)	Docking	Some inhibitors extracted from <i>Moringa oleifera</i> showed better binding energy compared with enalapril and captopril	67
Peptides from Kefir milk	ACE	1O86 (by X-ray)	Docking	Most peptides with most favorable ACE interaction had hydrophobic amino acids or positively charged amino acids	68
O-heterocyclic analogs from intertidal seaweed <i>Sargassum wightii</i>	ACE	1O8A (by X-ray)	QSAR/Docking	Ligands were comparable with enalapril in terms of antihypertensive potential, because of functional role of molecular polarizability	69
Peptides from <i>Gracilariopsis lemaneiformis</i>	ACE	1O8A (by X-ray)	Docking	Peptides inhibit ACE via hydrogen bonds with active pocket	70
Peptides from <i>Oncorhynchus mykiss</i>	ACE	1O86 (by X-ray)	QSAR/Docking	QSAR predicted potential activity, solubility, and ADMET properties of several tripeptides. Strong inhibition of ACE by tripeptides appears to result from hydrogen bond interactions	71
Peptides from <i>Chlorella vulgaris</i>	ACE	1O8A (by X-ray)	Docking	Two peptides formed six hydrogen bonds with active site pockets of ACE	72
Peptides from winged bean seeds	ACE	1UZF (by X-ray)	Docking	Docking score for several peptides correlated well with respective IC <sub>50</sub>	73

Di/tripeptides from milk	ACE	4APH (by X-ray)	3D-QSAR/docking	Most potent inhibitory peptides contained hydrophobic amino acids that enter deep into hydrophobic pocket of ACE active site and interact with its residue	<sup>74</sup>
Triazolones and bis-triazolone derivatives	ACE	1O8A (by X-ray)	Docking	Compounds compete with substrate by inhibiting substrate entry to catalytic pocket or directly binding to catalytic site, explaining their antihypertensive effects	<sup>75</sup>
Peptides derived from adlay ( <i>Coix lachryma-jobi</i> L. var. <i>ma-yuen</i> Stapf) glutelin	ACE	1O86 (by X-ray)	Pharmacophore/docking	Most potent peptide obtained by pharmacophore and docking simulation screening and series of divisions and optimizations	<sup>76</sup>
Peptides from lentils	ACE	4APH and 4APJ (by X-ray)	Docking/MD	ACE inhibition due to formation of hydrogen bonds between C-terminal residues of lentil peptides and residues of ACE catalytic site	<sup>77</sup>
Terpenes from essential oil from aerial parts of <i>Seseli pallasii</i> Besser	ACE	1O86 (by X-ray)	Docking	Spathulenol exhibited optimal binding affinity with ACE	<sup>78</sup>
Peptides from broken rice	ACE/Renin	4APH and 2V0Z (by X-ray)	Docking	One peptide showed greatest potential to inhibit ACE and renin; energy of interaction similar to bradykinin and aliskiren for ACE and renin, respectively	<sup>79</sup>
Saccharides from <i>Clerodendrum colebrookianum</i>	ACE/Rho-associated coiled-coil protein kinase (ROCK)/phosphodiesterase type 5 (PDE5)	1O86, 3D9V, 4L6Q, and 1UDT (by X-ray)	Docking/MD	Acteoside, martinoside, and osmanthuside $\beta$ 6 interact with three targets with good affinity; protein–ligand complexes between acteoside/osmanthuside $\beta$ 6 and ROCK, and acteoside and PDE5 were stable, enabling design of new antihypertensive drugs	<sup>80</sup>
Diterpenes from <i>Dictyota menstrualis</i>	Thrombin	1PPB (by X-ray)	QSAR/docking	Diterpenes bind to catalytic site of thrombin, enabling design of new antithrombotic drugs	<sup>81</sup>
Oxazalone/imidazolone derivatives	AT1/hERG potassium channel	4YAY (by X-ray)/ homology modeling, and 5VA1 by cryo-EM	Homology modeling/docking/MD	Compounds with high affinity for AT1 and low affinity for hERG identified to design new effective, safe antihypertensive drugs	<sup>82</sup>
Xanthon derivatives	$\beta$ 1-Adrenoreceptor	4BVN (by X-ray)	Docking	Common binding orientation of xanthon derivatives in catalytic binding pocket of $\beta$ 1 adrenoreceptor identified. Amino alkyl moiety has important role in binding at catalytic site. These compounds represent new antihypertensive drugs with greater activity than propranolol and atenolol	<sup>83</sup>
Imperatorin analogs	Cardiac L-type calcium channel	3G43 (by X-ray)	Docking	Decrease in vasodilation caused by replacement of ether function with amine because numbers and lengths of bonds differ	<sup>84</sup>
Nifedipine analogs	CaV 1.2 channel	4MS2 (by X-ray) and homology modeling	Homology modeling/docking	Two compounds showed higher affinity for channel compared with nifedipine	<sup>85</sup>
Isoxsuprine	eNOS/Cystathionine $\gamma$ -lyase (CSE)	3NOS and 3COG (by X-ray)	Pharmacophore/docking/MD	eNOS binding sites located both on substrate access channel and within catalytic site. For CSE, binding site is close to substrate access channel and catalytic site. Isoxsuprine involves various mechanisms and is a potent candidate for polypharmacology	<sup>86</sup>
1,3,4-Oxadiazole derivatives	Factor Xa	1NFY (by X-ray)	Docking/quantum chemistry	Antithrombotic activity predicted by computer simulation was superior to that	<sup>87</sup>

				of unfractionated heparin	
Molecules from SPEC database	Factor IXa	4Z0K (by X-ray)	Pharmacophore/3D-QSAR /docking	Six molecules out of 75 671 were screened as potential FIXa inhibitors	<sup>88</sup>
Various molecules from <i>Alstonia scholaris</i>	Factor Xa/tissue plasminogen activator	2BOK, 1A5H and 1RTF (by X-ray)	Docking	Some ligands bind t-PA and Factor Xa more optimally compared with streptokinase	<sup>89</sup>
Molecules from MolMall database	Liver X receptor	1UHL, 1UPW (by X-ray)	Docking/MD	Two agonist candidates were identified with interactions with key residues. Both complexes occupied similar spatial position of region of LXR and could be potent treatment for LXR-related CVDs or cancers	<sup>90</sup>
New N-(phenylmethyl)-benzoxazol-2-thiones	Macrophage migration inhibitory factor (MIF)	1CA7, 1GCZ, 1LJT (by X-ray)	QSAR/docking	Preferred binding mode for within MIF tautomerase active site predicted for identified potential inhibitor	<sup>91</sup>
Salvianolic acids from traditional Chinese medicine	P2Y1/P2Y12 receptors	4XNW, 4PXZ, 4NTJ (by X-ray)	Ensemble docking/MD	Salvianolic acid A and C antagonized P2Y1 and P2Y12 receptors, whereas salvianolic acid B antagonized P2Y12 only, with high affinity, explaining their potential antithrombotic mechanism	<sup>92</sup>
Ginsenoside isomers	P2Y12 receptor	4NTJ (by X-ray)	Docking	Differential protein–ligand interaction appears responsible for stereoselective efficacy of ginsenoside isomers, a potent substrate of P2Y12 receptor	<sup>93</sup>
Compounds from drug-like natural product library	Peroxisome proliferator-activated receptor-γ (PPARγ)	4EMA (by X-ray)	Docking	Four natural product compounds identified with high or moderate agonistic potency against human PPARγ	<sup>94</sup>
66 Novel furyl/thienyl β-carboline derivatives	Phosphodiesterase 5 (PDE5)	1XOZ (by X-ray)	QSAR/Docking	Optimized 5-ethylfuryl or 5-ethylthienyl moieties constituted most potent and selective PDE5 inhibitors; compounds occupied Q2 pocket of PDE5 and could be potent vasorelaxant drugs	<sup>95</sup>
269 Antihypertensive phytochemicals	Phosphodiesterase 5A (PDE5A)	1TBF (by X-ray)	Docking	Lead scaffolds identified with nitrogen-containing tetrahydro-pyridonaphthyridine and dihydro-pyridoquinazoline tricyclic as novel PDE5A inhibitors	<sup>96</sup>
12 Novel 2-cyclopropyl-3-ethynyl-4-(4-fluorophenyl) quinolines	Protease-activated receptor 1/PI3 Kinase	3VW7, 3T8N (by X-ray)	Quantum chemistry/docking	Four compounds out of 12 presented good physicochemical and pharmacological properties as anticancer drugs with thrombolytic effects	<sup>97</sup>
N-Substituted prolinamido indazoles	Rho-associated coiled-coil protein kinase 1 (ROCK1)	3NDM (by X-ray)	Docking	Compounds containing β-proline moiety had improved activity against ROCK1, relative to analogs with α-moiety. Benzyl substituent on these compounds induced improved inhibitory activity compared with benzoyl substituent	<sup>98</sup>
Arylsparteine derivatives	Sigma-1 receptor	5HK1 (by X-ray)	Docking	Relevant sparteine derivatives targeted sigma-1 receptor at its binding site and modulate calcium signaling in cardiac muscle	<sup>99</sup>

Inhibitors of soluble epoxide hydrolase found in National Cancer Institute and Maybridge databases	Soluble epoxide hydrolase (sEH)	4JNC (by X-ray)	Pharmacophore/docking	Two potent, safe, and novel sEH inhibitors discovered that could be candidates for hypertension treatment	100
Thrombin inhibitors from BindingDB	Thrombin	2CF9 (by X-ray)	Docking/MD	Cyclopentyl moiety in JJ1 involved in interacting with thrombin. JJ1 identified as potential inhibitor of thrombin by binding its active site	101
Inhibitors of With-No-Lysine Kinase (WNK)	WNK	5TF9 (by X-ray)	QSAR/Docking	Optimized potent and selective allosteric inhibitors with improved physicochemical properties as antihypertensive agents	102
<b>Drug-binding mechanisms with therapeutic targets</b>					
Benzoate derivative: brozopine (BZP)	12/15-lipoxygenase (12/15-LOX)	4NRE by X-ray/homology modeling; 2P0M (by X-ray)	Homology modeling/docking/MD	BZP had higher affinity for 12/15-LOX-2 than for 12/15-LOX-1; its bromopheny group penetrates hydrophobic pocket of 12/15-LOX-2 and could be a cardioprotective agent	103
Peptide from enzymatic hydrolysates of <i>Cyclina sinensis</i>	ACE	1O86 (by X-ray)	Docking	Interaction between peptide and ACE occurred mainly through hydrogen bonding, hydrophobic interactions, and coordination bonds between Zn <sup>2+</sup> and the peptide; could be incorporated in antihypertensive food or dietary supplements	104
Peptide AM-1 from <i>Radix Astragali</i>	ACE	4APH (by X-ray)	Docking	AM-1 binds ACE in main interactive site for angiotensin II, explaining antihypertensive action of traditional Chinese medicine	105
Peptide from <i>Styela clava</i>	ACE	1O86 (by X-ray)	Docking	Peptide binds to active site of ACE and explains antihypertensive action of South Korea seafood	106
Peptides from bovine bone gelatin hydrolysate	ACE	1O86 (by X-ray)	Docking	Similar to captopril, peptides decreasing stability of conserved zinc-binding motif in ACE could be incorporated as nutritional supplements with antihypertensive action	107
Peptides from quinoa bran albumin	ACE	1O8A (by X-ray)	Docking	Peptide appears to interact with ACE active site via hydrogen bonds with high binding power; thus, quinoa could be source of bioactive peptide against hypertension	108
Peptide from tilapia	ACE	1O8A and 1UZF (by X-ray)	Docking	Peptide from tilapia binds to ACE active site with lower binding energy than captopril; could be food ingredient with antihypertensive effects	109
Peptides from <i>Cassia obtusifolia</i>	ACE	1O8A (by X-ray)	Docking	Peptide interacts with ACE active site as a competitive inhibitor and acts as antihypertensive agent	110
Peptide from <i>Saurida elongata</i>	ACE	1O8A (by X-ray)	Docking	Peptide interacts via static quenching mechanism involving alteration of ACE conformation to realize antihypertensive effects	111
Microginins (MG) peptide	ACE	2X8Z (by X-ray)	Docking	MG peptide exhibited similar interactions with ACE as captopril	112
Phenolic acid/dipeptide/borneol hybrids	ACE	1O86 (by X-ray)	Docking	Binding position of most potent compound showed similar binding mode to that of lisinopril	113
Phe-Tyr dipeptide	AT1	4ZUD (by X-ray)	Quantum chemistry/MD/docking	Dipeptide structure remained stable and had binding affinity with AT1, thus could be potent antihypertensive agent	114
D-myo-inositol 3,4,5,6-	Antithrombin	2ANT, 1E03 (by X-ray)	MD	TMI modulated AT reactive center loop flexibility similarly to that observed	115

tetrakisphosphate (TMI)	(AT)			with heparin	
Esculin 7,3',4',5',6'-O-pentasulfate (EPS)	AT	1E05 (by X-ray)	Docking	Binding sites of EPS were away from heparin-binding site, indicating that EPS is not acting as heparin mimic that changes AT conformation	<sup>116</sup>
(-)-Epicatechin (EPI)/L-arginine	Arginase (Ar)	2EAB (by X-ray)	Docking	EPI could bind Ar together with its natural substrate L-arginine, suggesting a noncompetitive interaction	<sup>117</sup>
Diethyl-4,4'-dihydroxy-8,3'-neolign-7,7'-dien-9,9'-dionate (neolignan1)	BK <sub>Ca</sub> channel	3NAF (by X-ray) and homology modeling	Homology modeling/docking	Neolignan1 binds within binding site pocket of BK <sub>Ca</sub> channel, which could explain its antihypertensive activity	<sup>118</sup>
Sodium thiosulfate (STS)	Caspase-3	2H5I (by X-ray)	Docking	Effectiveness of STS as cardioprotective agent is attributed to reduction in apoptosis by binding to active site of caspase-3	<sup>119</sup>
Isoquinoline derivative H-89	Cav1.2 channel	Homology modeling	Homology modeling/docking	H-89 docked at same region as (S)-(-)-Bay K 8644 although in different binding pockets with similar binding energy	<sup>120</sup>
Ranolazine	Cytosolic-5'-nucleotidase	2JC9 (by X-ray)	Docking	Docking revealed possible binding site for ranolazine on cytosolic-5'-nucleotidase	<sup>121</sup>
Benzamidine	Factor XIIa (Hageman factor)	6B74 (by X-ray)	MD	Prominent alterations of original conformational posture of FXIIa revealed by increased flexibility, decreased compactness, and increased exposure to solvent upon binding of benzamidine	<sup>122</sup>
Nitric oxide (NO) donor	Free fatty acid receptor 1 (FFA1)	4PHU (by X-ray)	Docking	Terminal phenyl group of NO donor was exposed to outside of FFA1 binding pocket	<sup>123</sup>
<i>Trowaglerix</i> venom polypeptides	Glycoprotein VI (GPVI)		Docking	Hexapeptide binds GPVI at different site to collagen	<sup>124</sup>
Saccharide: Araloside C	Heat shock protein 90 (Hsp90)	1AMW	Docking	Araloside C stably docked into ATP/ADP-binding domain of Hsp90 via formation of hydrogen bonds	<sup>125</sup>
Dofetilide and a derivative: LUF7244	hERG	5VA1 (by cryo-EM)	Docking/MD	LUF7244 prevented dofetilide from binding to fenestration site, whereas there was almost no overlap when dofetilide bound to central cavity	<sup>126</sup>
Benzamide derivative: ICA-105574	hERG	5VA1 by cryo-EM	Docking/MD	ICA-105574 influenced stability of ion selective filter of hERG	<sup>127</sup>
R-roscovitine	hERG	Homology modeling	Homology modeling/Docking	Residues involved in hERG inhibition by R-roscovitine identified. Their mutations induced changes in inhibition	<sup>128</sup>
Triphenyl derivative: cavalli-2	hERG	5VA1 (by cryo-EM), 1LNQ (by X-ray), and homology modeling	Homology modeling/docking	Cavalli-2 extends into hydrophobic pockets of new open-pore hERG structure with low-energy-score poses	<sup>129</sup>
Quaternary ammonium: clofilium	hERG	5VA1 (by cryo-EM) and homology modeling	MD	Unexpected binding mode identified, in which quaternary nitrogen was placed in upper part of inner cavity, whereas chlorophenyl group was located in lower part	<sup>130</sup>
Triarylmethane-34, benzothizininone NS6180, senicapoc	KCa3.1 channel	2R9R, 3FB5 (by X-ray) and homology modeling	Homology modeling/docking	All three compounds blocked ion conduction directly by fully or partially occupying site that would normally be occupied by K <sup>+</sup>	<sup>131</sup>

Pentamidine-analog 6 (PA-6)	K <sub>IR</sub> 2.1 channel	Homology modeling	Homology modeling/docking	Mutations were >30 Å apart from Pa-6 binding site on K <sub>IR</sub> 2.1	132
Chloroquine	K <sub>IR</sub> 3.1 channel	1N9P (by X-ray)	Docking	Chloroquine blocked passage of hydrated K <sup>+</sup> through intracellular domain of K <sub>IR</sub> 3.1. Mutant channel suggested that binding of drug to pore was off center, reducing its ability to block hydrated K <sup>+</sup> , explaining why chloroquine failed to revert paroxysmal atrial fibrillation in some patients	133
Verapamil	Kv 1.3 channel	2A79 (by X-ray) and homology modeling	Homology modeling/docking	To reach its binding site to block channel, verapamil interferes in Kv 1.3 channels, mainly with position 420, and is partially hindered by position 417 and 418, whereas position 419 does not interfere. Mutant channels could indirectly influence ability of verapamil to reach its binding site	134
Verapamil	Kv1.5 channel	Homology modeling	Homology modeling/Docking	Verapamil binds in central cavity of channel pore and has contact with multiple amino acids that reside between base of ion selectivity filter; this blocking effect contributes to prolongation of atrial effective refractory period	135
5222 compounds from ZINC Database	Kv1.5 channel	Homology modeling	Homology modeling/docking/MD	Tested inhibitors inhibited Kv1.5 in similar pattern to known inhibitors, mainly through nonpolar interactions, and formed stable complexes. Channel in open conformation was stabilized quickly within DPPC membrane, whereas most secondary structure elements were lost in closed -conformation	136
5-(4-phenoxybutoxy)psoralen (PAP-1)/vernakalant/flecainide	Kv1.5	Homology modeling	Homology modeling/MD	All three molecules bind to cavity between Ile508 and Val512 residues from four subunits of channel, blocking Kv1.5 channel in same manner	137
αs1-casein-derived peptides	μ-type opioid receptor (OPRM)	4RWA (by X-ray) and homology modeling	Homology modeling/MD/docking	One peptide had favorable structure and interaction energy to bind OPRM	138
Cinobufagins, steroid derivative	Na <sup>+</sup> ,K <sup>+</sup> -ATPase	4RES (by X-ray)	Docking	Length of spacer arms between cinobufagin steroid core and nitroxide group determined position of reporting group (N-O) confined to binding site	139
Lidocaine/quinidine	Nav 1.4 channel	5BZB (by X-ray) and homology modeling	Homology modeling/docking/pharmacophore	Common pharmacophore proposed including cationic moiety and aromatic moiety, usually linked by four bonds	140
Lidocaine	Nav 1.5 channel	5XSY and homology modeling	Homology modeling/docking/MD	Pore lumen simultaneously accommodated up to two drug molecules. Hydrophilic access pathway through intracellular gate and hydrophobic access pathway through fenestration were identified	141
Saponin D39	Non-muscular myosin heavy chain IIA (NMMHC IIA)	Homology modeling	Homology modeling/docking	Scaffold triterpene of D39 extended deep into hydrophobic pocket of NMMHC IIA and could be potential antithrombotic drug	142
Lectin from <i>Dioeclea reflexa</i> hook seeds (DrfL)	Oligomannosides /N-glycans	5TG3 (by X-ray)	Docking/MD	DrfL interaction with N-glycans in glycoconjugates on surface of endothelial cells appeared responsible for relaxant effect promoted by lectin	143
Raspberry ketone	PPARα	3V18 (by X-ray)	Docking	Ketonic group of raspberry ketone essential for binding effectiveness to PPAR-α to prevent cardiotoxicity of isoproterenol	144
Fisetin	PPARγ	2XKW (by X-ray)	Docking/MD	Fisetin works as full agonist of PPARγ	145
Inhibitor A293, an aromatic	pTASK-1	6CQ8 (by X-ray) and	Homology	A293 binds three key residues, I118, L239, and N240, in inner pore of	146

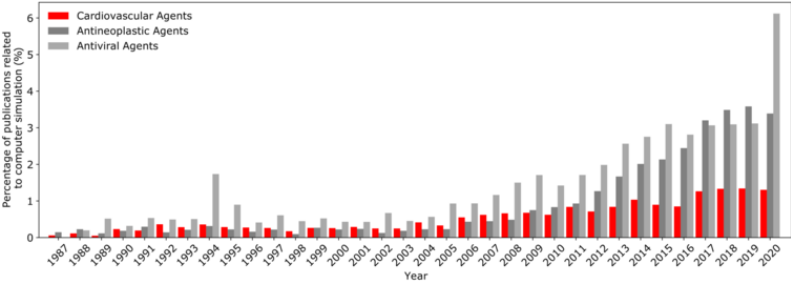
carbonamide	channels	homology modeling	modeling/docking	pTASK-1 channels	
Soyasaponin I	Renin	3VUC (by X-ray)	Docking/MD	Soyasaponin I binds two sites, one in active site of renin and another in region near active site. Inhibition effect results from competition on active site with substrate	147
Ca <sup>2+</sup>	Ryanodine receptor (calcium-activated calcium channel)	5TB4 (by cryo-EM)	MD	Revealed mechanism of Ca <sup>2+</sup> reversible binding	148
Carboxyl-terminal tail domains (CTT) of trimeric intracellular cation (TRIC) A and B	Ryanodine receptor 2 (RyR2)	5GOA, 5GO9 (by cryo-EM) and homology modeling	Homology modeling/docking/MD	Discovery of sites of interaction between RyR2 and CTT-A/CTT-B	149
Flavine adenine dinucleotide	Short-chain acyl-CoA dehydrogenase	2VIG (by X-ray)	MD	Flavine adenine dinucleotide stabilizes short-chain acyl-CoA dehydrogenase dimer structure and binding pocket	150
Neolignan1	Soluble guanylyl cyclase (sGC)/voltage dependent L-type calcium channel (VDCC)	1Y11, 3RDY (by X-ray) and homology modeling	Homology modeling/docking	Both sGC and vascular VDCC proteins could be targets of neolignan1	151
Astaxanthin (ASX)	Sphingomyelin phosphodiesterase 1 (SMPD1)	5FIB (by X-ray)	Docking	ASX produced tight fit in pocket of SMPD1	152
Hypericin, anthraquinone derivative	Thrombin	5AFY (by X-ray)	Docking	Hypericin could simultaneously bind on three distinct ligand-binding sites of thrombin; thus, hypericin could be novel thrombin inhibitor	153
Inhibitor of cardiac troponin I interacting kinase (TNNI3K), an aniline derivative	TNNI3K	4YFF (by X-ray)	Docking	Interactions between inhibitor and TNNI3K identified	154
Peptidomimetic 1-Benzyl-5-methyl-4-(n-octylamino)pyrimidin-2(1H)-one	Toll-like receptor 4 (TLR4)	1FYV, 2J67 (by X-ray), and homology modeling	Homology modeling/docking	One binding site located close to site with crucial role in association of TLR4 with adapter proteins and in TLR4 activation	155
Curcumin	Transient receptor potential vanilloid 4 (TRPV4)	4DX1 (by X-ray)	Docking	Amino acid sequence LYS340-LEU349 of TRPV4 implicated in binding site with curcumin	156
Inhibitors of TREK-1 Channel, benzohydrazide derivatives	TREK-1 channel	4XDL (by X-ray) and homology modeling	Homology modeling/MD	Intermediate state, between active and inactive state, identified with allosteric pocket for inhibitors	157
N-(4-chlorophenyl)-N-(2-(3,4-dihydroquinolin-2(1H)-yl)-2-oxoethyl)methanesulfonamide	TREK-1/TREK-2/TRAAK	4TWK, 4BW5, 4XDK, 4XDJ, 4XDL, 4I9W, 4WFFH, 4WFG, 4WFF, 4WFE,	Docking/MD	When TKDC binds to groove comprising E1 and E2 helices, E2 helix moves toward selectivity filter and directly interacts with pore region on different subunit to block extracellular ion pathway	158

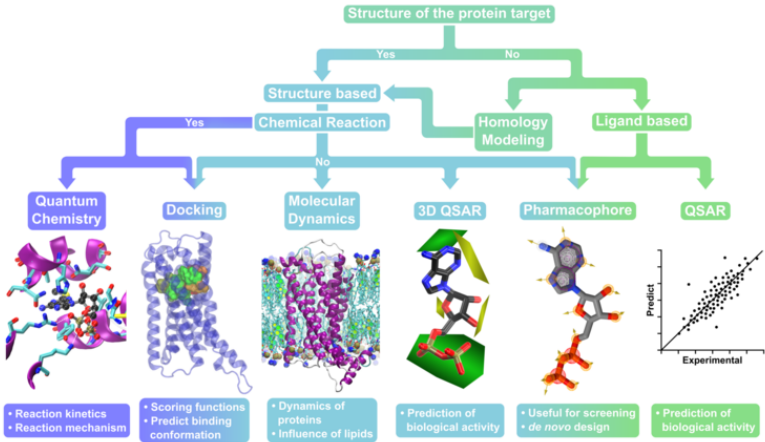
(TKDC)		4RUE, 4RUF (by X-ray)			
S,S'-dinitrosobucillamine	N/A	N/A	Quantum chemistry	Free energies for release of NO <sup>+</sup> entities computed by quantum chemistry. S,S'-dinitrosobucillamine could be potent NO donor	<sup>159</sup>
Dinitrobenzofuroxan aryl derivative	N/A	N/A	Quantum chemistry	High NO-donor activity of dinitrobenzofuroxan aryl derivative connected with its existence as mixture of 1- <i>N</i> -oxide and 3- <i>N</i> -oxide, where 3- <i>N</i> -oxide is more reactive toward SH <sup>+</sup>	<sup>160</sup>
<b>Exploration of additional mechanism of action</b>					
Captopril derivatives/dihydropyridine calcium channel blocker (DCCB) and losartan derivatives	Cytochrome P450 2J2	1OG5, 1PQ2, 2BDM, 1TQN (by X-ray) and homology modeling	Homology modeling/docking	Binding energies of DCCB significantly correlated with IC50 values for inhibition of CYP2J2. Among losartan derivatives, only telmisartan binds CYP2J2 with good affinity. For captopril derivatives, ester structure could have key role in inhibition of CYP2J2	<sup>163</sup>
<b><i>In silico</i> modeling of target</b>					
Losartan derivatives: azilsartan/valsartan/olmesartan/candesartan	AT1	4ZUD (by X-ray)	Docking	Binding between ligands and AT1 receptor compared to validate <i>in silico</i> immobilized AT1 receptor model	<sup>164</sup>
Nifedipine analogs	CaV 1.2 channel	4MS2 (by X-ray) and homology modeling	3D-QSAR/homology modeling	3D-QSAR model validation either internally or externally showed reliability and predictability of CaV 1.2 channel model	<sup>85</sup>
N/A	hERG	N/A	Pharmacophore/docking/MD	New picture for hERG1 channel activator field provided	<sup>165</sup>
<b>Target prediction</b>					
Genistein, a polyphenol	PPAR $\gamma$	N/A	Network pharmacology/docking	PPAR $\gamma$ predicted as target for genistein; binding between PPAR $\gamma$ and genistein was analyzed	<sup>166</sup>
<b>Permeation through plasma membrane</b>					
Epigallocatechin gallate (EGCG), a flavanol derivative	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) bilayer	N/A	MD	EGCG might not spontaneously permeate membrane but stays in headgroup region	<sup>167</sup>
Propranolol (PPL)	POPC bilayer	N/A	MD	PPL dynamically neutralizes at lipid-tail interface, which dramatically influences permeability	<sup>168</sup>
Dofetilide/Moxifloxacin	POPC/dimyristoyl phosphatidylcholine (DMPC) bilayer	N/A	MD	Diffusion in membrane interior slower compared with bulk aqueous solution, because of a more viscous space	<sup>169</sup>
<b>Modulation of lipid environment of therapeutic target</b>					
Ticagrelor and its metabolite, ATP analogues	POPC bilayer/SM	4NTJ and 4PXZ (by X-ray)	MD	Lipid environment of membrane modulates binding propensity of P2Y12 receptor of ticagrelor and its metabolite	<sup>18</sup>



		bilayer or SM/cholesterol bilayer				
<b>Drug binding with plasma membrane</b>						
Amlodipine/atenolol/lisinopril	DMPC bilayer	N/A	MD	Combined drugs penetrated deeper into DMPC lipid bilayer; lipid chains remained ordered		170
Irbesartan (IRB)/IRB-HP-β-CD	Dipalmitoylphosphatidylcholine (DPPC) bilayer	N/A	MD	Topological and orientational integration of irbesartan into DPPC lipid bilayer revealed that it is placed ~1 nm from membrane center		171
5-(4-phenoxybutoxy)psoralen (PAP-1)/vernakalant/flecainide	POPC bilayer	N/A	MD	All molecules readily partitioned into membrane		137
<b>PK understanding</b>						
Digoxin	Cgr2 protein of <i>Eggerthella lenta</i>	1QO8 (by X-ray) and homology modeling	Homology modeling/docking/MD	Digoxin binds Cgr2 at same binding site as fumarate (with a lower binding energy), which is proposed natural substrate		172
BSP	Ceramide 2/water	N/A	Docking	BSP ion-pair complexes remained stable in stratum corneum, rich in ceramide 2, but dissociated in viable epidermis, rich in water		173
PEGylated amlodipine (PEGAML)/paroxetine (PAR)	Cytochrome CYP2B4	4JLT (by X-ray)	MD	Amlodipine in PEGylated form associated with paroxetine promoted higher CYP2B4 stability. Optimum ratio with PAR (1PEGAML:5PAR) effectively reduced DDIs and stabilized interactions with CYP2B4		19
Flavonoids/dronedarone	Cytochrome P450 3A4	5TE8 (by X-ray)	QSAR/docking/pharmacophore	Flavonoids promoted stronger metabolism of dronedarone by binding to internal cavity of CYP P450 3A4		174
New coumarin derivatives	Human serum albumin (HSA)	1E71 (by X-ray)	Docking	New coumarin derivatives had single binding site with HAS but one compound suggested two binding sites, which would influence distribution and metabolism		175
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<b>Optimization of dose form</b>						
Olmesartan medoxomil (OLM)	N/A	N/A	MD	Interaction between OLM and polymer used as carrier was predicted		179
ICCA-WFF, a peptide	GPIIb/IIIa/P-selectin	Unspecified	Docking/MD	Modifying ICCA with WFF enhanced binding with GPIIb/IIIa and P-selectin. Simulation of nanoparticle of ICCA-WFF formation performed, which allowed access to several molecules per nanoparticle		180
Felodipine (FEL)	Hydroxypropylmethylcellulose (HPMC)	N/A	MD	Miscibility between HPMC and FEL and diffusion coefficients determined		181
Milrinone (MRN) nanoformulation	HSA	1HK4, 2BXD and 2BXG (by	Docking	MRN bound to hydrophobic pocket on subdomain IIA of HSA, which could be		182

		X-ray)		used as carrier	
Amlodipine besylate (AMB), a dihydropyridine	N/A	N/A	Quantum chemistry/MD	Structures of neat and binary AMB characterized and their physical stability evaluated	<sup>183</sup>
<b>Toxicity assessment</b>					
Oxazalone/imidazolone derivatives	AT1/hERG	4YAY (by X-ray)/homology modeling, and 5VA1 (by cryo-EM)	Homology modeling/docking/MD	Compounds with high affinity for AT1 and low affinity for hERG identified to design new effective and safe antihypertensive drugs	<sup>82</sup>
N/A	hERG	N/A	QSAR	Blockage of hERG channel by small molecules predicted	<sup>184</sup>
Dofetilide/moxifloxacin	hERG	Homology modeling	Homology modeling/MD	Neutral dofetilide and moxifloxacin forms could preferentially interact with open channel state	<sup>169</sup>
Diverse drugs	hERG	5VA2 (by cryo-EM)	Docking/MD	Calculated values of binding energies well correlated with experimental ones, thus affinity of drug for hERG channel can be accurately predicted	<sup>185</sup>
Vesnarinone	hERG	Homology modeling	Homology modeling/Docking/MD	Possible binding mode and stability of this drug at hERG channel models, both in open and open-inactivated states, elucidated	<sup>186</sup>
Dofetilide/estradiol	hERG	2A79 (by X-ray) and homology modeling	Homology modeling/docking/MD	Estradiol interacted with hERG mutations in pore loop containing G604 or in intracavity binding site, which constitutes a torsadogenic risk	<sup>187</sup>





## Physics / Chemistry



Chemical properties

## Physiology



*In vitro* experiments



*In vivo* experiments

