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How computational chemistry helps to investigate membrane transporters

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Abstract

Membranes transporters and ion channels are transmembrane proteins responsible of the exchange of a wide variety of substances between the extra and intracellular sides of the membrane. Ion channel malfunctions, also referred to as channelopathies, have been associated with many human diseases such as cardiac, muscular, and neuronal disorders. Channelopathies could be broadly classified as acquired or congenital. The acquired ones may result from drug interaction with ion channels such as the voltage-gated potassium channels, hERG. Indeed, an acquired loss of function of hERG channel may increase the risk of sudden cardiac death. Thus, it becomes important for pharmaceutical industries to test potential drug interactions with ion channels as early as possible in drug discovery process. Moreover, *in silico* methods were developed to reduce the failure rate of drug candidates as well as their high development costs. This mini review presents an overview of the main computational chemistry methods that are used for the investigation of ion channel proteins.

1. Introduction

Membranes transporters and ion channels are transmembrane proteins that allow the passage of a wide variety of substances such as ions across cell membranes [1]. Several human diseases such as hypertension, cancer, cardiac arrhythmias, and neurodegenerative disorders have been linked to dysfunction of these proteins [2-6]. Thus, membranes transporters and ion channels become one of the most important molecular targets for several classes of drugs.

Ion channels facilitate the passive movement of ions according to the direction of their electrochemical gradients. These proteins are pore-forming membrane proteins whose normal function is critical for several homeostatic processes in cells. Indeed, in non-excitable cells, such as secretory and epithelial cells, ion channels control the flow of electrolytes and water allowing the regulation of cellular volume. However, in excitable cells such as cardiovascular, neuronal, and skeletal cells, the activity of these proteins maintains the resting membrane potential and generates action potentials.

Ion channels are classified into different groups based on three properties: gating, selectivity, and number of gates (pores). First, *gating*, as a mechanism of opening and closing of ion channels, allows to sub-classify them as voltage-dependent, ligand-dependent, and mechano-sensitive gating. Second, *ion selectivity* reflects the channels ability to discriminate between specific ions regarding their size, valency, and hydration

energy. Third**,** *number of gates (pores)* gives two main classes; single-pore channel (the majority of ion channels), and two-pore channels such as TRP channels.

Ion channel malfunctions (channelopathies) are associated with many human disorders such as cardiovascular, muscular, and neuronal diseases [3, 4, 7-10]. Channelopathies result either from genetic mutations or acquired dysfunctions of ion channels [11]. Acquired channelopathies is attributed to non-desired effect of drugs on cardiac ion channels such as the voltage-gated potassium channels (K_v11.1/hERG). Indeed, an acquired loss of function of this channel can result in lengthening the QT interval and increasing the risk of sudden cardiac death [12, 13]. Subsequently, this triggers pharmaceutical industries to test potential drug interactions with ion channels as early as possible in the screening process. Moreover, *in silico* methods were developed to reduce the failure rate of drug candidates as well as their high development costs.

Computational approaches become a pivotal step in early drug discovery process. Indeed, it provides time-saving and cost-effective procedures for pharmaceutical companies [14]. The application of these approaches aims to improve the selected lead compounds. Two approaches are mainly used in computer-assisted drug design: i) Structure-based drug design, where the structure of the drug target is used to guide drug discovery. The structural information can be obtained using X-ray crystallography or nuclear magnetic resonance spectroscopy (NMR). Structure-based drug design includes docking, virtual screening, and molecular dynamics. ii) Ligand-based drug design approach is used in the absence of the three-dimensional structure of the target. It represents a powerful method based on only small-molecule information using a series of known active compounds. This approach includes quantitative structure-affinity relationship (QSAR) and pharmacophore modeling based on ligand properties.

This short review presents an overview of some *in silico* methods that are widely utilized in structural studies of ion channel as well as in drugs discovery and development.

2. Computational approaches for ion channels

2.1 Molecular Dynamics (MD)

Molecular Dynamics (MD) technique is a powerful theoretical method for the investigation of dynamical processes in biological systems. It provides accurate descriptions of the structure and dynamics of these systems.

The time evolution of the interacting particles in MD simulations is followed via the solution of Newton's law of motion. The equation is numerically solved using algorithms such as the Verlet [15] or velocity Verlet [16] algorithms.

For ion channel studies, MD simulations can provide an atomic picture aiming to investigate the fundamental properties, specificity, and ion conduction mechanisms for a given channel [17]. In addition, this approach allows to evaluate the contribution of external factors such as pH and voltage to the ion permeation control. Moreover, this technique may help to understand the biophysical and structural bases of the alterations induced by genetic mutation that are associated with aberrant cardiac excitability phenotypes.

An example of MD contribution is the identification of functional interaction between S1 and S4 segments in $Na_{v1.4}$ and $Na_{v1.5}$ sodium channels in the sitting of human channelopathies [18]. In this line, molecular dynamics simulations were performed in order to understand the structural bases of the biophysical alterations observed in $Na_{v1.4}$-I141V and $Na_{v1.5}$-I141V mutants. The initial molecular model of $Na_{v1.4}$ was built using homology modeling and the crystal structure of the bacterial Na_{vAb} channel [19]. The selected structure of $Na_{v1.4}$ was embedded in a palmitoyl-oleyl-phosphatidylcholine (POPC) hydrated bilayer, surrounded by a KCl salt solution at a physiological concentration of 150 mM. Then, molecular dynamics simulations were run. Simulation data suggested that the p.I141V mutation brings closer the S1-Y168 amino acid to the S4-R225 one allowing the formation of a hydrogen bond between these two residues (Figure 1). Thus, the author suggested that the observed hydrogen bond could be responsible for the stabilization of the activated state of the mutant channel explaining the channel gain of function and the cardiac hyperexcitability that is observed in the presence of p.I141V mutation [18].

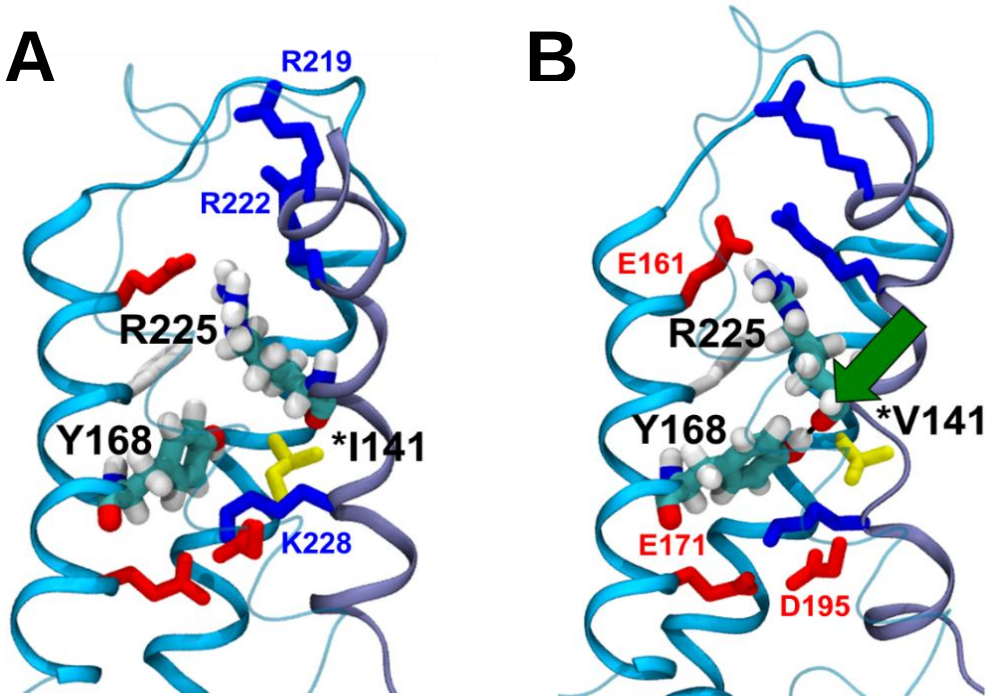

Figure 1, Voltage sensing domain configurations of the WT (a) and the p.I141V mutants (b) of $Na_{v1,4}$ channel. In the presence of the p.I141V mutation, MD simulation predicted the formation of a hydrogen bond (green arrow) between the S2-Y168 and S4-R225 residues (From Amarouch *et al*., [18]).

2.2 Molecular Docking approach

Molecular docking is a computational approach that has become one of the most frequently used methods in structure-based drug design. This approach aims to characterize ligand-protein or protein-protein interactions [20], and allows to predict the optimal orientation between molecules and target proteins [20]. Indeed, depending of the ligand conformations and orientations, several docking modes (also referred to a 'pose') could be tested. Then, based on ligand-target fitting, each pose receive a score. A high score for a given molecule indicates it as a potentially good binder. Finally, the molecules that are predicted to be most active are selected for synthesis and biological investigation.

Depending on the degrees of freedom of ligands and proteins, the docking technique is classified into three categories: (1) flexible docking where ligands and proteins are flexible; (2) semi-flexible docking where ligand is considered as a flexible body and rotation around bonds is allowed, while the protein is considered as a rigid body; and (3) rigid docking where ligands and proteins are rigid bodies and rotation around bonds is not allowed.

Zhang's group has recently published an interesting study using molecular modeling methods to elucidate molecular determinants of amiodarone binding to hERG channel. In combination with the patch-clamp and Alanine mutagenesis techniques, molecular docking approach was used to investigate the roles of pore cavity amino-acid residues in amiodarone binding. In the absence of a crystal structure for the hERG channel pore, the authors performed molecular docking using an homology model based on the crystal structure template of the *Methanobacterium thermoautotrophicum* Ca^{2+}-activated K^+ channel (MthK). Alanine-mutagenesis identified multiple residues implicated in amiodarone binding. Then, computational docking differentiated residues likely to interact directly with drug and those whose Alanine substitution may affect drug block allosterically. Thus, based on the measured IC_{50} values, the relative importance for amiodarone binding of the residues investigated being:

S624A ~ Y652A > F656A > V659A > G648A > T623A [21].

2. 3 Quantitative structure-activity relationship and pharmacophore modeling

Quantitative structure-activity relationship (QSAR) modeling refers to the construction of predictive models of biological or chemical activities of compound candidates based on the activity of related known compounds. This technique represents a valuable tool that is widely used in drug design and medicinal chemistry [22]. QSAR approach has consistently been used for drug discovery and development; its various applications allow correlating molecular information not only with biological activities but also with other physical and chemical properties, known as Quantitative Structure-Property Relationship (QSPR).

QSAR modeling is based on physico-chemical properties as theoretical molecular descriptors such as geometrical, hydrophobic, lipophilicity, solubility. These descriptors are tabulated along with their activity in table of two entries, descriptors and observations. Then after, a regression analysis is performed. Once a reliable QSAR model is established, the activities of untested molecules can be predicted, and structural features involved in the biological process may be determined as well.

Molecular descriptors could be classified into three types, (1) 1D descriptors (1D-QSAR) including basic molecular properties such as molecular weight and molar refractivity; (2) 2D descriptors (2D-QSAR) predicting the physicochemical properties of the studied molecules, and quantifying their functional effects based in their topology; and (3) 3D descriptors (3D-QSAR) involving the analysis of the quantitative relationship between the biological activity of molecules and their three-dimensional properties using statistical correlation methods.

Since its first utilization in biological analysis of molecule activity, QSAR approaches have been extensively used to understand the structure-activity relationship of ion channel blockers [23-25]. One of the earliest structure-activity was performed on a set of class III antiarrhythmic drugs that prolongs the QT interval [26]. Regarding the pharmacophore model, the authors suggested a general structure including a *para*-substituted phenyl ring linked to basic nitrogen through a chain of 1-4 atoms. Moreover, the charged nitrogen is linked by 1-3 atoms to two features which might be an aromatic ring or an alkyl moiety [26].

As described above, depending of the used molecular descriptor, 1D, 2D or 3D-QSAR models can be constructed. An example of 1D-QSAR model was developed based on the analysis of a set of molecules collected from the literature [27]. For the generation of the hERG model, 6 atom descriptors were used to generate the model. The first descriptor was the size (number of heavy atoms). The remainder of the atom descriptors is based on the E-state keys separated according to five atom types: C-Aliphatic (non aromatic)-Estate, C-Aromatic-Estate, N-Acceptor-Estate, N-Donor-Estate, and Oxygen-Estate. The statistical analysis of the model showed a correlation coefficient of 0.68 for the training set compounds, and of 0.76 for the test compounds set. The descriptors that mainly contribute to hERG inhibition were the N-Acceptor-E-state and the C-Aromatic-Estate [27].

For two dimensional models, several models have been developed for pharmacological and toxicological investigation of ion channels. One of these models was developed by Garg *et al* [28]. The authors have used similar approach as Diller *et al*. Indeed, a set of 68 molecules from literature source was used to perform a 2 Dimensional Quantitative Structure-Toxicity Relationships (2D-QSTR) analysis for hERG channel. Seven following descriptors were used to generate the model: E-state indices (S_sNH2 defined as sum descriptor for nitrogen bonded to two hydrogens and one single bond), topological descriptors (Balaban index JX, and the third order of Kier shape index Kappa-3), ADMET_PPB (tendency to bind to plasma protein), and thermodynamic descriptors Atype_O_57 (atom type Oin phenol, enol, carboxyl OH), Atype_O_59 (atom type O in Al-O-Al), and Atype_H_46 (atom type H attached to Csp3 0). The obtained result shows a good prediction ability reflected by a correlation coefficient (r^2) of 0.84 [28].

Finally, one of the earliest 3D-QSAR model developed for prediction of hERG blockers was published by Ekins *et al*. [29]. The authors use a set of 15 compounds to generate 3D-QSAR model. The result shows a high correlation with an r^2 of 0.90, and also a high ability to predict the activity of the external test set, with r^2 of 0.83.

More recently, Wiśniowska *et al*. has developed predictive models for the drug-triggered inhibition of the main ion channels responsible for cardiac action potential generation. Models include the fast sodium current (INa), late calcium current (ICaL), rapid delayed rectifying potassium current (IKr), slow delayed

rectifying potassium current (IKs). The aim was to develop models able to correlate the chemical structure of the studied compound and the *in vitro* test settings with the half-maximal inhibitory concentration (IC_{50} value). For hERG channel, the obtained models were challenged and validated through comparison of simulated and experimental results obtained with the PatchXpress technique [25].

2.4 Virtual Screening

Virtual Screening (VS) is a commonly used method in drug discovery; it was developed as an alternative to high-throughput screening. This technique aims to virtually select lead compounds by predicting their binding mode to a given target protein. Thus, this approach allows to reduce chemical libraries for further synthesis and biological *in vitro* testing [30]. VS methods could be divided into three categories: Structure Based Virtual Screening (SBVS), Ligand-Based Virtual Screening (LBVS), and hybrid approach.

The SBVS applies different structure-based modeling methods, such as docking, to mimic the binding interaction of ligands to a target protein. This approach requires structural information about target protein, otherwise homology models can be used instead [31]. However, LBVS is usually used in the absence of structural information about the target protein. LBVS permits to hierarchize the investigated chemical compounds since they are subjected to structure-activity relationship properties [32]. Finally, these two approaches could be combined in one referred as hybrid approach [33].

2.5 Homology modeling

As described above, molecular dynamics simulation of ion channels can be performed only when the 3D structure of the channel is available. In the absence of a crystal structure, the homology modeling strategy can be used to generate an all-atom model. In this light, an alignment between the required sequence and a homologous one with defined crystal structure should be performed. Then, based on the homologous sequences, the secondary structures of the sequence of interest are built. Finally, the whole structure is refined.

The success of homology modeling approach was variable depending of the studied ion channel. Indeed, based on the high resolution structure of KcsA channels [34], reliable homology models were constructed for the other potassium channels [35].

Concerning voltage gated sodium channels, several models have been generated since the recent determination of the crystal structures of bacterial sodium channels (Na_{VAb}) [36-39]. Most of them were constructed for the $Na_{v1.4}$ channel due to the availability of functional data for binding of micro-conotoxins to this channel. These data were used to constrain and validate the generated model.

3. Conclusions

Computer modeling becomes an essential component for the investigation of human channelopathies as well as for drug discovery in the ion channel field. Indeed, the utilization of these techniques becomes essential for pharmaceutical industries to test potential drug interactions with ion channels as early as possible in drug discovery process. Thus, the failure rate of drug candidates and their high development costs are reduced.

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