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Modeling hERG and its Interactions with Drugs: Recent Advances in Light of Current Potassium Channel Simulations

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The hERG K^+ channel is responsible for the rapid delayed rectifier current in cardiac myocytes, and a block of its functioning may be related with the (inherited or drug-induced) long QT syndrome. For this reason, in recent times, some interest has arisen around computational studies aimed at developing hERG/drug models for the prediction of drug binding (docking) modes, in view of the assessment of the hERG blocking potential. On the other hand, voltage-gated K^+ channels have been the subject of molecular simulations for several years, and rigorous protocols for studying the main aspects of their functions (permeation, gating, voltage sensing) have been published. In this article, we briefly introduce these classical computational works on K^+ channels, and then review in depth the reports on the latest advanced modeling studies on hERG. The aim is to put the hERG modeling work in the more general context of the ion channel simulations field, to show the peculiarity of hERG on the one side, and also to indicate some possible new avenues in the use of modeling techniques to increase our knowledge of this important channel.

Introduction

The hERG (human Ether-à-go-go-Related Gene) K^+ channel (IUPHAR gene name: K_V11.1; other names: KCNH2, erg1) was isolated in 1994.[1] The main expression and localization of hERG is in the heart, even though it has also been shown to play, a still not fully understood, role in several other organs and cells such as gut, neurons, and cancer.[2] The function (and dysfunction) of hERG in the myocardium has to do with the repolarization of cardiomyocytes, where this channel is responsible for the rapid delayed rectifier current (I_{Kr}), the most important component of phase III of the repolarization. When the electrical gradients in the heart are detected by the body surface electrocardiogram, the time interval between the onset of the QRS complex and the end of the T wave is defined as the QT interval, and represents the time between initial depolarization and final repolarization of the ventricles. The emphasis on hERG started to increase when it was found that the block of its activity originated by inherited mutations or by high doses of some common drugs could be involved in the prolongation of the QT interval, which, in turn, could give rise to the so-called long QT syndrome (LQTS), a cardiac disorder that predisposes individuals to a potentially lethal form of arrhythmia (torsades de pointes, TdP).[3]

In the last years, drug-induced-LQTS has become a major concern of drug safety, as evidence started to accumulate of the proarrhythmic effects elicited by widely used noncardiac medications. Although for the wide majority of pharmacological agents the incidence of TdP is an absolutely rare event, nevertheless, in some cases, the occurrence of serious adverse effects related to QT prolongation led to the withdrawal of drugs from the market (Figure 1).[4] In this context, it is evident how relevant the implementation of the right strategies for the early detection of the QT prolonging potential during the drug development process can be.[5] At the present, different (in vitro and in vivo) preclinical models are available to investigate on the risk of QT interval prolongation by drugs, but none per se is sufficiently predictive, and costs are high.

Biological studies carried out in the last decade have greatly advanced our knowledge on the molecular basis of LQTS, and nowadays a great amount of evidence exists concerning the central role played by the hERG blockage in the development of this disorder.[3,6] Moreover, the mechanisms by which some drugs are able to bind to the hERG channel and to impair the ion flow are being increasingly understood both from the electrophysiological and the molecular points of view. Amino acid residues of the interior cavity of hERG responsible for the binding of drugs have been identified through the combination of several experimental techniques (such as, for example, voltage-clamp and amino acid scanning) that allow researchers to evaluate the degree of involvement of single residues in the current block caused by the drug molecules.[7–10]

Based on the assumption of the role played by the hERG blockage in the drug-induced LQTS, in recent times, a great interest has arisen around computational ligand- and target-based studies aimed at developing effective models for the assessment of the hERG blocking potential of drugs or candidate drugs. Such studies have been recently reviewed,[11–13] and con-

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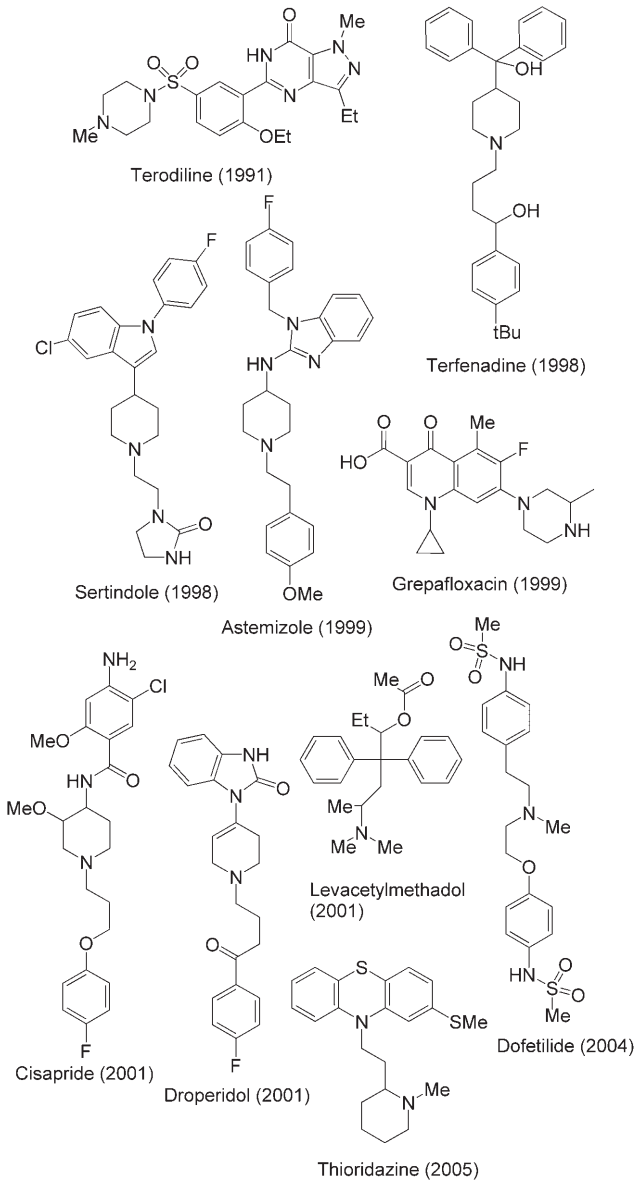

Figure 1. Drugs withdrawn from the market because of QT prolongation and TdP risk. In parentheses, the year of withdrawal is reported.

stitute an initial body of knowledge starting to outline the structural features of hERG blockers and the general characteristics of the drug/hERG interactions. In this paper, we will review the last reported attempts to model the hERG 3D structure, function, and ability to bind drugs. The aims of this paper are to present the state-of-the-art of the simulations of hERG and hERG-related issues, and to place these studies in the context of the general advancements in the simulation of potassium channels. This should help to highlight the future directions of hERG modeling efforts, in the belief that simulations (eventually in combination with experimental data) can increase the understanding of biological phenomena at the molecular level and contribute to the design and development of safer drugs.

Potassium channels structure and modeling

Potassium-selective channels represent the most populated group within the superfamily of voltage-gated-like ion channels, and comprise several families, among which the voltage-gated K_v channels form the largest one, and one of the best known.[14] In the last years, the main aspects of their general structural organization regarding ion selectivity, channel gating, and voltage sensing have been increasingly disclosed, such that nowadays we have a consistent picture of the structure and functioning of potassium channels.[15] Indeed, most of our knowledge about the structural features of these protein complexes derives from the X-ray crystallographic studies carried out by MacKinnon on bacterial K^+ channels.[16]

In the case of the K_v channels family (to which hERG belongs), the overall architecture shows a tetrameric organization composed of four six-transmembrane (6TM) monomeric fragments (S1–S6) that form the pore-containing (α-)subunit. This tetrameric structure leads to the formation of the ion permeation pore comprising both the selectivity filter (SF, in the loop between S5 and S6), and the inner cavity (also named inner channel vestibule, made by helices S5 and S6). The channel gate (allowing for opening and closing) involves the C-terminal parts of the S6 helices that are supposed to undergo an appropriate conformational change at a hinge point. Helices S1–S4 form the voltage sensor (S4 bearing four arginine residues is a key component of it). The main structural features of K_v channels are schematically depicted in Figure 2.

As soon as the crystallographic studies started to provide sets of coordinates permitting the construction of 3D models of reliable conformations of the potassium channels, simulation work was undertaken to achieve a more in depth comprehension of the key functions, that is, ion permeation and selectivity, gating, and voltage sensing. Most of the simulations studies have been carried out by using as a starting atom configuration the crystallographic snapshots obtained for two bacterial K^+ channels, namely, KcsA[17] from *Streptomyces lividans* and KvAP[18] from *Aeropyrum pernix*. Actually, it is generally recognized that the basic structural characteristics of the pore region are quite similar for all ion channels: differences in key features provide the functional peculiarities that characterize each family and subfamily, that is, mostly, ion selectivity and gating control mechanism. In this context, KcsA (showing a 2TM motif instead of the 6TM one typical of K_v channels, that is, lacking for the voltage sensor) was mainly used in modeling studies aimed at understanding ion selectivity, permeation through the channel, and opening–closing movements, whereas KvAP (showing the complete 6TM architecture) was (and still is) employed to investigate the complex mechanism that links the change in membrane potential to the channel gating through the voltage sensing fragment.

Ions permeation and selectivity

Studies on channel permeation were mostly focused on the passage of ions through the selectivity filter, and aimed at describing the dynamics and energies of the process. Several dif-

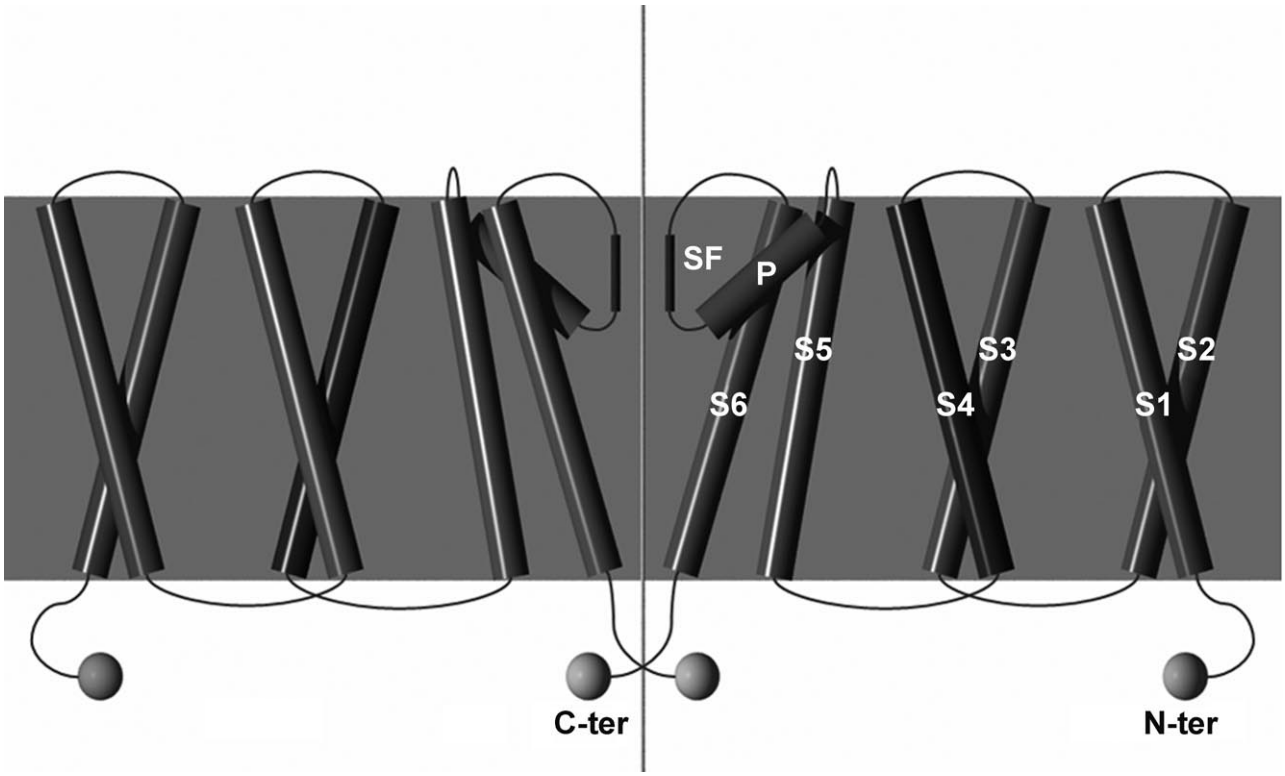

Figure 2. Schematic view of the transmembrane part of K_v channels: two of the four α-subunits are shown separated by the pore axis. The pore domain is formed by the pore loop comprising the selectivity filter (SF) and the pore helix (P), and by the helices S6 and S5. The voltage sensing domain comprises the helices S1–S4, with S4 carrying the four positively charged residues. C- and N-terminal domains are also indicated.

ferent computational approaches have been applied, that is, mainly molecular dynamics[19–21] (MD), but also Brownian dynamics,[22] and Monte Carlo simulations[23] to study the conformational behavior of the filter, and free energy perturbation[24,25] (FEP), potential of mean force[26,27] (PMF), and quantum mechanical (QM) calculations[28,29] to capture free energy and electronic aspects of ion conduction and selectivity. From these studies, a coherent picture of the selectivity filter occupancy emerges consisting of a single line of two K^+ ions (plus one at the external mouth) separated by two water molecules located in the five S_0 (external) through S_4 crystallographic sites (Figure 3). Each potassium ion is coordinated by eight oxygen atoms, and the ion flux through the filter can be depicted as the switch from the site occupancy configuration S_0-S_2-S_4 to the S_1-S_3. A certain degree of flexibility of the filter structure has been deemed necessary to allow a fast ion translocation. As regards selectivity, the early view that the filter prefers K^+ over Na^+ ions because of an almost perfect compensation of the potassium ions dehydration is still the prevailing one, even though more in depth investigations by means of ab initio calculations have pointed out a nonmarginal role played by polarization and charge transfer effects among oxygen atoms and ions.[30]

Channel gating

The conformational aspects of the gating mechanism have been studied mainly through the use of MD simulations (see references [31–34] for examples), albeit a recent work has addressed the same process from an electronic point of view by means of QM calculations.[35] Here, the point is to understand how the lowest part of the channel cavity (the bundle formed by the four N-terminal fragments of helices S5 and the four C-terminal fragments of helices S6) can switch from a structure that does not allow the passage of K^+ ions (because of the restricted volume of the gate) to one that allows it, and vice versa. Static pictures of "closed" (KcsA[17] and KirBac1.1[36]) and "open" (MthK[37] and KvAP[18]) potassium channels obtained by X-ray crystallography led to the hypothesis that the gating is due to a conformational change of the helices that results in a backward-outward movement (with respect to the pore axis) of the lowest part of them leading to a widening of the pore and a consequent opening of the ion pathway (Figure 4). In 2TM channels (such as KcsA, MthK, KirBac1.1) this conformational transition is made possible by the presence of a conserved glycine residue located halfway in the M2 helix (corresponding to S6 in K_v), whereas the situation for K_v channels (6TM, such as KvAP) is a bit more complex. In fact, in this case, the possibility of a further hinge point was found corresponding to a highly conserved PXP motif downstream the Gly. MD simulations were largely employed to investigate K_v channel gating, and although the time-scale of gating movement is not accessible to these simulations, useful insights have been obtained from such studies. In particular, Bright and Sansom studying models of the KvAP gate region of increasing complexity (from single S6 helix to the tetramer of S5-P loop-S6) ar-

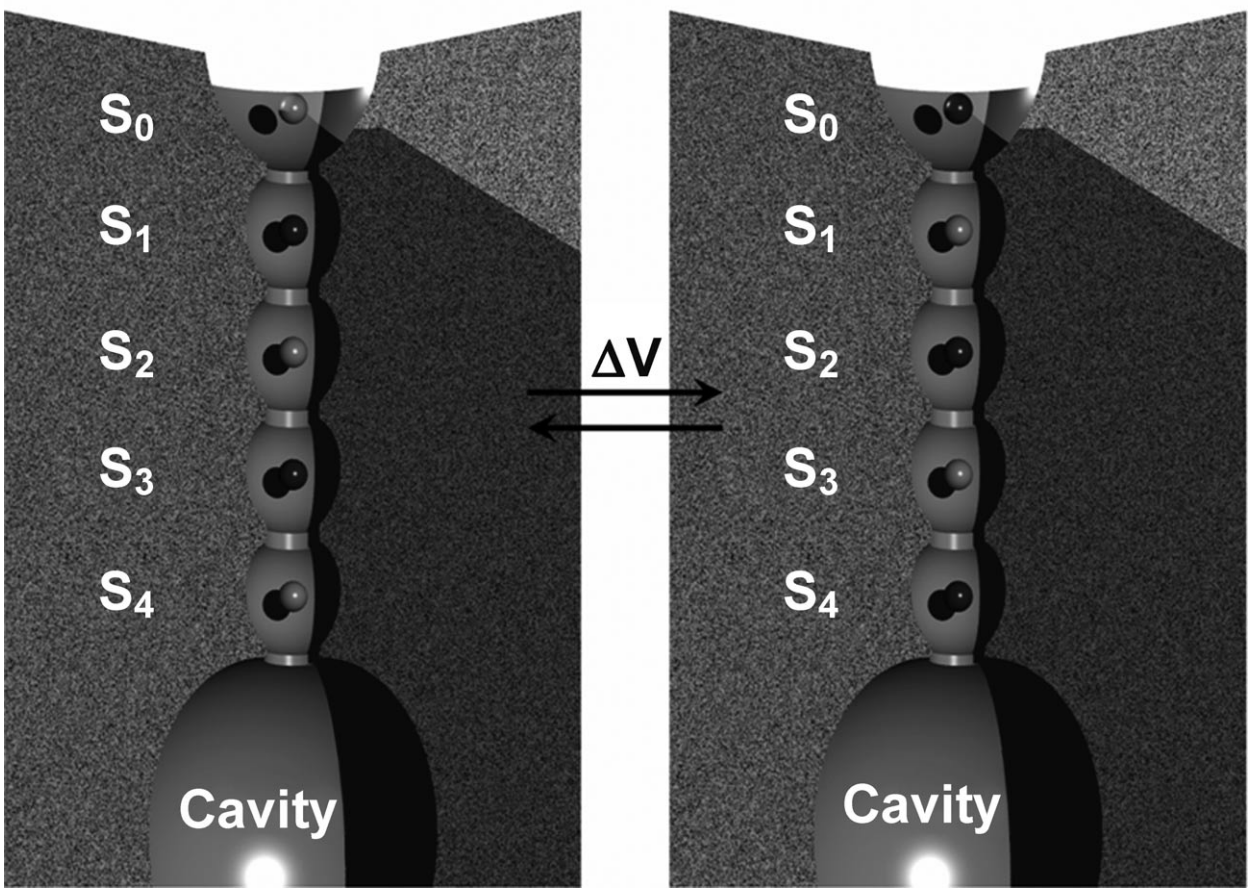

Figure 3. Scheme of the selectivity filter occupancy by K^+ ions (light grey little spheres) and water molecules (dark grey little spheres). The left part of the picture shows the "crystallographic" situation with sites S_0, S_2, and S_4 occupied by potassium ions, and the two parts together illustrate the SF permeation as the result of a shift of K^+ ions from S_0-S_2-S_4 to S_1-S_3.

rived at the conclusion that in the case of K_v channels it cannot be excluded that the PXP motif (here PVP) plays a relevant role in the gating conformational transitions giving rise to a second hinge point and participating in a hinge-swivel movement of the S6 helices that might be typical of K_v channels.[33]

Voltage sensing

The third main field of simulation studies on voltage-gated ion channels regards the voltage-sensing mechanism, through which the conformational gating movements described above are coupled to the membrane depolarization or hyperpolarization. It has long been known that a specific domain of the protein α-subunits (helices S1–S4) is devoted to this role, particularly centered on helix S4 carrying the four positively charged residues deemed to move in response to transmembrane voltage variations and to transmit such a movement to the gating apparatus. The conventional model to describe the voltage sensing mechanism depicted the helices S1–S4 as a typical transmembrane domain spanning the membrane perpendicular to its plane, and the S4 motion as a "helical screw" movement, whereby the positive charges shifted upwards upon depolarization.[38] However, surprisingly enough, the first X-ray resolved structure of a full-length voltage-gated K^+ channel (KvAP[18]) appeared in striking contrast with the previous models and with several other experimental evidences, depicting the critical part of the voltage sensor as formed by a "paddle", that is, a hairpin structure made by part of helix S3 (S3b) and S4, lying parallel to the plane of lipid bilayer. In Figure 5, schematic pictures of the two main voltage-sensing models are shown. In this situation, simulation studies have been carried out in the attempt to unravel the issue and reconcile the diverging experimental conclusions, but despite the performance of extensive and thoughtful computational works, the formidable problem of understanding at the molecular level the voltage-sensing mechanism remains still open. With regard to this, two papers are worth mentioning by the groups of Robert Guy and Mounir Tarek. In the first one,[39] the modeling of a K_v channel is carried out by using the structure of the KvAP voltage sensor "reinterpreted" following the classical transmembrane topology and docked to the pore domain, in a way to obtain a reliable model consistent with the "helical screw" hypothesis (and able to account for experimental data[40]). On the other hand, Treptow et al.[41] modeled the Shaker B channel by docking the 4×S1–S4 helical segments

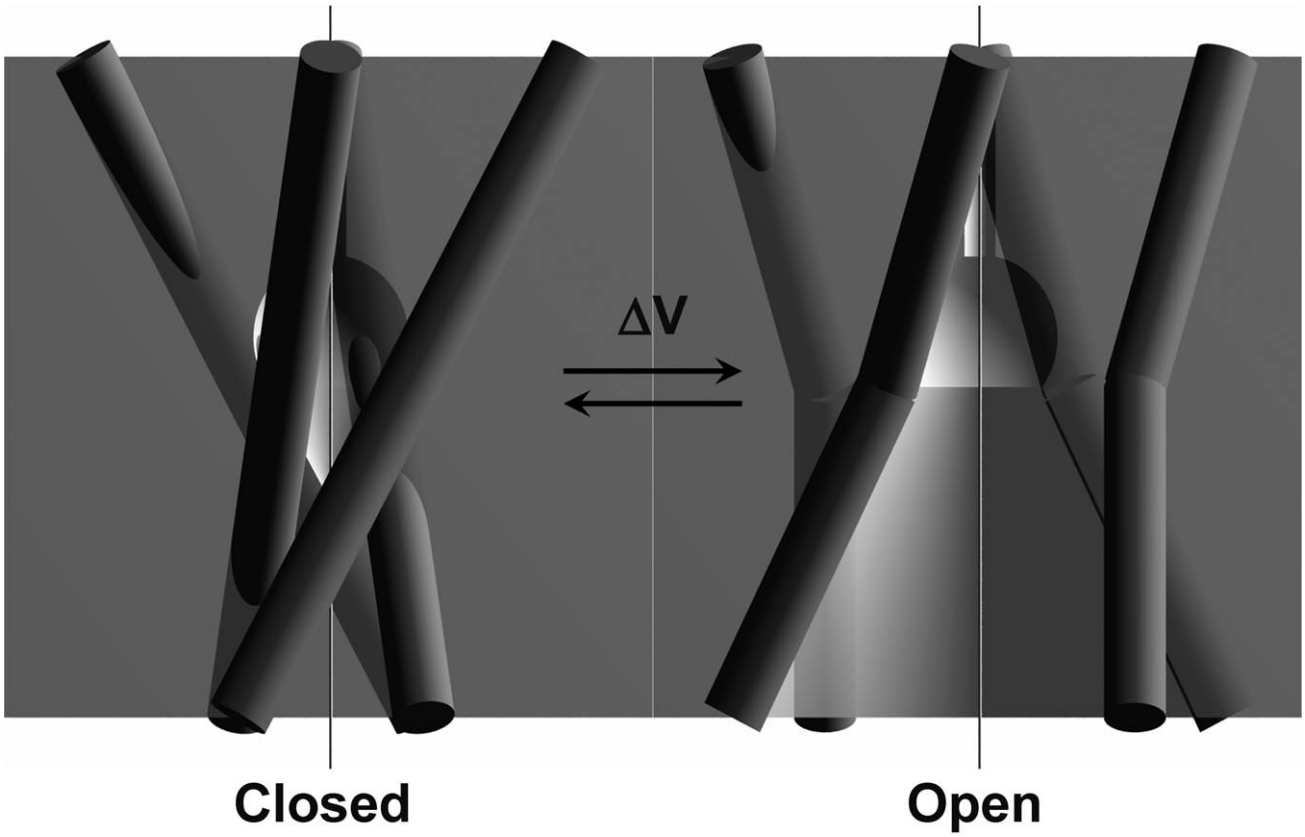

Figure 4. Schematic view of the conformational changes of helices S6 assumed to be at the base of the gating mechanism of K_v channels. In the open state, a hinge point (corresponding to a Gly residue) is supposed to allow the kinking of S6 and consequently the widening of the intracellular part of the pore cavity.

around the pore domain inferred from the KcsA channel, and, after an in-depth analysis of extended MD simulations, concluded that their results (mainly that a specific hydration of S4 buried in the protein transmembrane environment justifies its limited conformational motion upon activating voltage) rationalized a variant of the conventional activating model, that is, the transporter-like model proposed by Starace and Bezanilla.[42] Despite these computational studies which are in contrast with the "paddle" model of voltage sensing, the investigations on this intriguing structural hypothesis are still continuing.[43–45]

Ligand binding

In addition to the above-mentioned simulation studies concerning potassium channel function, a parallel line of research was carried out by several groups on the binding of ligands to the channels. Studies were mainly devoted to the understanding of the binding mode (often already hypothesized on the basis of experimental data) of cationic blockers and toxins, again more in a mechanistic perspective than with drug discovery aims. Representative of the first case are some reports on the simulation of tetraethylammonium (TEA) binding to the KcsA channel.[46–48] In two of these studies, a standard docking program was used to provide initial positions of the cation at the outer,[48] or at both outer and inner[46] mouth of the selectivity filter, and in all of them, MD simulations and electrostatic calculations were carried out to investigate different possible TEA-channel binding modes and to reveal atomistic details thereof. Interestingly, in this regard, all the simulations ruled out a cation–π interaction that was previously advanced between TEA and Tyr82 residues on the basis of crystallographic and mutagenesis studies. However, a recent combined experimental and computational study by Ahern et al.[49] reaffirms the possibility of such an interaction confirmed by QM calculations on a truncated model of the complex.

Peptide toxins produced by poisonous animals often have the ability to selectively block ion channels or impair their gating, and in the case of voltage-gated potassium channels, they have also been studied in the hope of obtaining information on the gating and voltage-sensing mechanisms.[50] These ligands bearing from few to several tens of amino acid residues cannot usually be treated as small organic molecules as regards docking, but may require different approaches to be docked to the channel surface. BD simulations assisted by electrostatic potential calculations proved efficient in building such protein–protein complexes, and MD simulations in explicit membranes provided data for investigating the stability of the complexes and the role of the flexibility of selected residues in the conformational adjustments that are at the basis of the toxin–channel recognition.[51,52]

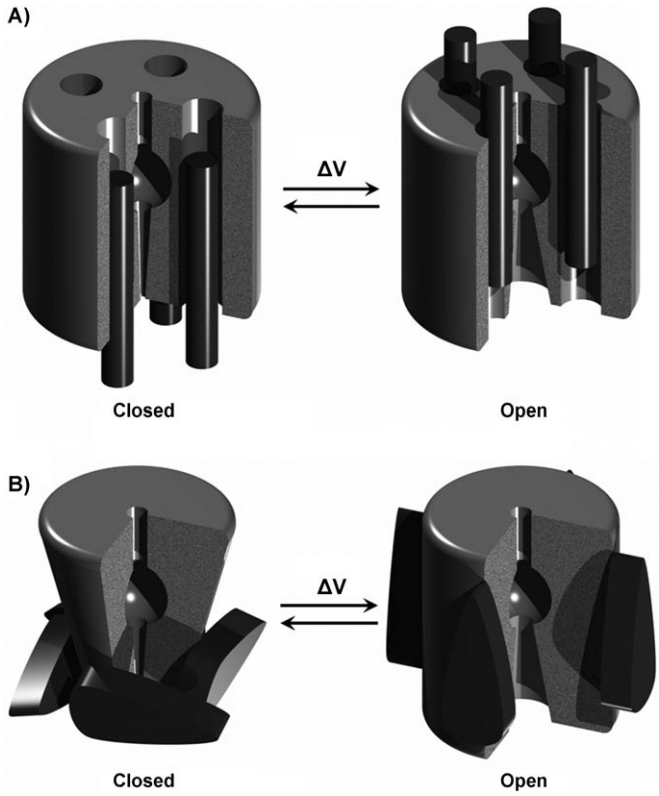

Figure 5. A. Schematic representation of the conventional model of voltage sensing in K_v channels: upon depolarization, the positively charged S4 helices (dark gray cylinders) translate/rotate upward and cause channel opening. B. In the model based on the crystallographic structures of KvAP[18] and Kv1.2,[69] the gating mechanism is driven by the movement of the "paddles" (dark gray) formed by helices S3 and S4.

Computational models of hERG

Since the seminal paper by Mitcheson and Sanguinetti[7] on the molecular basis of hERG blockage by drugs, models of the hERG channel and of its interactions with drugs have been used to illustrate and help the understanding of experimental results (see for examples references [53–57]). Actually, those early models had the merit of allowing a clear (qualitative) visualization of some aspects of the drug–channel interactions that might have remained hindered behind numbers and plots obtained by the electrophysiological experiments, but, on the other hand, from the computational point of view, several approximations had to be introduced to obtain 3D models of the complex tetrameric structure. Such limitations not only prevented the performance of studies such as those cited in the sections above regarding the functioning of hERG, but even made it impossible to model the channel in its entirety. These problems were brought to light at a Novartis Foundation Symposium devoted to hERG,[58] and, in summary, concerned: 1) the availability of adequate X-ray crystallographic structures of K^+ channels to be used as templates for the comparative modeling of the channel structure, 2) the consideration of the environment (membrane and external water layers) in the refinement of the model, and, 3) the use of the model to simulate the ligand–channel binding interactions with a view to prediction of drugs' affinity to hERG.[12] Coincidentally, this situation started to change in the last two years, when a number of papers appeared proposing accurate solutions to some of these issues. Second generation hERG models were described that can now be considered as suitable for undertaking more in-depth investigations on the channel functioning, and for attempting drug safety predictions.[59–64] Indeed, several further recent papers[65–68] dealt with the docking problem, indicating that this aspect of hERG modeling is not only quite intriguing, particularly for the drug research community, but also one of the most controversial. In the following, we will examine in some detail these models trying to highlight how some of the above mentioned problems have been addressed and eventually solved.

Building 3D models of hERG

In the case of hERG, differently from the other Kv channels presented above, the lack of an experimentally determined 3D structure of the protein complex implies the need to resort to comparative modeling techniques to obtain a starting configuration of the atomic ensemble. In such a context, the aspect that probably influences most in depth the possibility of obtaining fully reliable models of hERG is the lack of a proper template structure, on which to fold the primary sequences of the subunits and build the tetramer. In fact, the potassium channels whose 3D structure has been resolved to date number only five, that is, KcsA,[17] MthK,[37] KirBac1.1,[36] KvAP,[18] and Kv1.2,[69] and of them, only two have the 6TM topology (KvAP and Kv1.2), only one (Kv1.2) is mammalian, and none possesses a fragment characteristic of hERG, that is the long (approximately 40 amino acid residues) chain linking helix S5 to the selectivity filter (the so-called S5-P linker, or the "turret"). This moiety is crucial for the functioning of hERG,[70] and its modeling has been undertaken only quite recently (see below).

However, given that the main interest in hERG simulations regards the prediction of drug binding modes, all the published models of hERG were limited to the pore region formed by the segments S5-P-S6. Even in such a "simplified" case, things are not straightforward, because, although the identification of secondary structure motifs might be achieved in a relatively easy way through the multiple alignment analysis,[71] crucial details are sometimes hard to solve. To illustrate this point, we show in Figure 6 the alignments (used in some of the advanced models mentioned above) of the sequences of the pore-forming fragments of hERG on the corresponding ones of KvAP or KcsA, which are the most popular templates for modeling the "open" and "closed" conformations of hERG, respectively. In Figure 6B, the alignment of the sequences of the P-loop (including the selectivity filter) and S6 are shown, indicating that the presence of some highly conserved equally spaced residues (such as the "signature sequence" T(S)VGY(F)G and the glycine at the hinge point corresponding to G648 of hERG) leads to a rather univocal alignment. In contrast, in Figure 6A, it is shown how the lack of certain reference residues makes the alignment of helix 5 of hERG onto the correspond-

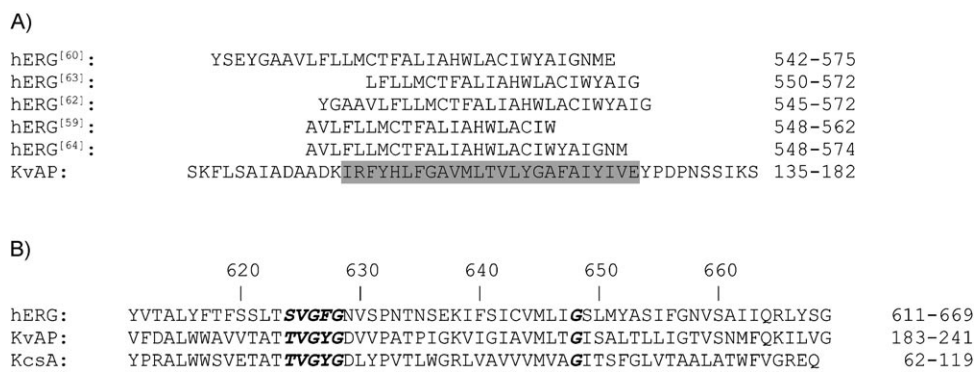

Figure 6. A. Sequences of S5 and alignment on the S5 helix of KvAP as reported in some recently published models of hERG; in the 135-182 fragment of KvAP, the S5 sequence as defined in the paper by Jiang et al.[18] is highlighted. B. Alignment of the P-loop-S6 sequence of hERG on the corresponding fragments of KvAP and KcsA; the "signature sequences" and the glycine residues supposed to make the hinge point are bold italics.

ing one of KvAP much more variable, such that in all of the considered models different solutions were adopted.

In this regard, a very thoughtful work on the homology modeling of hERG has been carried out by Stansfeld et al.,[62] who described in detail the procedure followed to obtain a reliable 3D model to use in their subsequent docking studies. In the case of helix S5, for example, they reasoned that the alignment of E575 with a conserved anionic residue of potassium channels (E in KvAP, see Figure 6 A) was not a mandatory choice, as the role of an acidic residue in that region of the protein could be played by a glutamate residue of S6. Moreover, these authors suggested that, to obtain a model of the closed channel able to host drug molecules in a way consistent with experimental mutagenesis data, a one-residue rotation of helix S6 (inserted at N635) was required. This modeling hypothesis was based on previous experimental hints,[72] and was supported by challenging it with mutagenesis results and docking experiments (see below) that gave consistent results and confirmed the validity of this rather unusual modeling solution.

As mentioned above, the modeling of the S5-P linker at the external mouth of hERG is another key issue in the simulation of this channel that only recently has been addressed. Actually, in building models of hERG, the "turret" was simply omitted, because of the lack of enough experimental structural information on which to base the modeling work, even though it has to be reminded that the presence of a helical segment in the linker was earlier identified and its structure modeled on the basis of NMR spectroscopy data.[73] In Table 1, the sequences of the S5-P linkers of the K^+ channels whose 3D structures were resolved are reported and compared to that of hERG, from which the wide difference in extension of the fragments and the consequent unsuitability for comparative modeling purposes immediately appear. In a paper published in 2007,[63] Tseng et al. reported an extensive work combining mutant cycle analysis and molecular simulations on hERG and a peptide toxin (BeKm-1), at the end of which they were able to obtain consistent models of both the pore-forming region of the channel including the "turret" and the complex between hERG and bound BeKm-1. The modeling was carried out in the laboratory of Robert Guy by adopting a protocol centered on an iterative approach to the selection of the "best" model. In practice, the structures of the transmembrane region of the hERG's pore domain (modeled on KvAP) and of the S5-P linker (modeled using secondary structure and NMR information) were docked by applying constraints based on experimental information (mostly, Cys scanning of selected hERG residues). Then, an iterative MD protocol was applied that, making use of numerous starting models, allowed, 1) identification of residues changing their conformation in most of them, 2) remodeling of the structures accordingly, and 3) proceeding until no consistent changes in the models were detected. The model thus obtained was further validated through docking experiments with BeKm-1 (see below), and provided the first sound representation of the external mouth of hERG comprising an essential fragment excluded from the modeling until then. In an almost contemporaneous paper,[68] Yi et al. described a model of hERG including the S5-P linker obtained by separately building the pore domain (on KcsA) and the "turret" (on the NMR structure). The conformations of the linker to be docked to the external mouth for building the entire model were sampled from a 5 ns MD simulation, and, again, an extended MD simulation was carried out on the whole structure to equilibrate it. In contrast to the work of Tseng et al., here it was observed that the four S5-P linker domains tended to lose their secondary structure conformations and to "open" like petals of a flower after the simulations. Actually, these characteristics of the model will have an impact on the structure of the hERG/BeKm-1 complex (see below). After all, as claimed in the Tseng's paper,[63] modeling loop domains in the absence of an homologous template structure can lead at best to approximate models that have to be rigorously validated by experimental evidence.

Table 1. Sequence of the S5-P linker ("the turret") of hERG compared to the same fragment of the potassium channels, whose 3D structure has been crystallographically resolved.

Potassium channel	S5-P linker sequence
hERG	NMEQPHMDSRIGWLHNLGDQIGKPYNSSGLGGPSIKDKY
KcsA	ERGAPGAQLITY
MthK	EGESW
KirBac1.1	YQLGDAPIANQSPPGF
KvAP	EYPDPNSSIKSV
Kv1.2	ARSQP

In this paragraph devoted to the building of hERG 3D models, a further observation is required on the issue of modeling closed and open states of hERG, as it has been stated above that they were modeled on the basis of crystallographic templates, namely KcsA and KvAP (or MthK in the earlier models), respectively. Actually, in recent works, some authors have attempted to simulate the opening–closure changes by modifying the conformation of helix S6. In two cases,[60,65] this was obtained by manually changing the torsion angles at the G648 hinge point, obtaining a number of intermediate states between those resembling the closed (matching KcsA) and the open (matching MthK) ones. On the other hand, Stansfeld et al.[62] found it convenient to use intermediate structures generated by a morph server[74] that on the basis of normal mode analysis produced a set of intermediate states between a starting (closed) and a final (open) one. It is remarkable to observe that all of these attempts to simulate the conformational modifications occurring upon opening and closing of the channel were in no way intended to study the gating mechanism of hERG, but rather were considered important to define the volume of the pore cavity (or the degree of pore opening) in view of the docking of drug molecules.

Simulations on hERG models

In the previous section, we showed the relevant role played by simulations in the study of such processes as ion channel permeation and selectivity, gating, and voltage sensing. The same does not happen in the field of hERG modeling, even though some recent works highlight the growing impact of simulation studies in addressing both basic modeling aspects, and some functional issues particularly linked to hERG. Among the former, it has to be noted that in some papers published in 2007, the use of MD simulations to equilibrate the structures and investigate the stability of the models was reported.[61,63,64,68]

The use of MD in the hERG modeling protocols developed by Tseng et al.[63] and Yi et al.[68] was already illustrated, and it can be considered as inherent to the construction of the protein complex as an assembly of different domains. The actual MD simulations extended to the whole system possibly including the membrane and the water environment come next, and (hopefully) result in the overall stabilization of the system that allows one to obtain a final model in a putative low-energy state. However, the data generated by MD simulations regarding trajectories of the single atoms and forces acting on them can be analyzed to retrieve further information on the dynamic aspects of the system. In this sense, Masetti et al.[64] carried out a detailed analysis of their MD simulations on models of hERG embedded in a phospholipid bilayer, after applying a protocol reminiscent of those used for the classical simulations of potassium channels.[19–21] The results were consistent with those obtained starting from crystallographic K^+ channel structures[19–21] (thus indirectly validating the hERG model), particularly in regard to the simulation of the ion permeation through the selectivity filter and the flexibility of this critical domain. On the other hand, for the pore cavity that in hERG bears some crucially different residues with respect to other Kv channels, the MD simulations brought to light an aspect that might be influential on the use of hERG models in docking experiments. By analyzing the dynamic changes of the cavity volume in the open channel, it appeared that such a volume drastically decreased during the simulation, due to some kind of "hydrophobic collapse" of F656 side chains that partially occupied the inner pore. Interestingly, however, during the MD simulations, the backbones of helices S6 in both closed and open models did not significantly change their conformations, thus confirming that a time scale of ns is too short to simulate the gating event.

MD simulations can be used at a further level, that is to estimate free energy-related quantities to be then employed to address the study of certain phenomena from the thermodynamic point of view. In this context, in a paper published by the group of Åqvist,[59] the authors applied the linear interaction energy (LIE) method (developed by the same group[75]) to estimate binding free energies on the basis of averaged energy terms calculated from MD simulations. Dealing with the docking problem, this paper will be reviewed in the following section.

At this point, it is worth mentioning the first relevant example of a simulation study regarding the functioning of hERG recently reported by Kutteh et al.[61] In this work, the authors, using the model including the S5-P linker developed by Robert Guy,[63] first examined the K^+ selectivity of the channel as a means to check the reliability of the model, and then investigated the mechanism of the hERG inactivation (a process involving the "closure" of the channel at the outer mouth, while keeping the intracellular gate open[15,70]). To determine the selectivity between Na^+ and K^+ ions at the selectivity filter, the FEP[76] method was employed, whereby the relative free energies of binding of the ions to the pore were estimated. Interestingly, in contrast to results obtained in a similar investigation on KcsA, the S1 site of hERG (more external, see Figure 3) was nonselective for either ion, whereas site S2 was definitely K^+-selective. This result was considered consistent with the experimental evidence of the ability of extracellular Na^+ ions to block hERG.[77] As regards the inactivation, after carrying out electrostatic and structural calculations on models of both open and inactivated hERG, the authors reached the conclusion that a conformational change leading the S5-P linker close to the pore might cause inactivation both for steric (narrowing the pore opening) and for electrostatic (causing an increase of positive potential) reasons. To carry out the latter part of the work, the program DelPhi[78] was used to calculate the electrostatic potential profiles along the axis, the potential maps of the systems, and the electrostatic potential energy profiles of a K^+ probe approaching the channel mouth.

Ligand docking to hERG

The construction of models of drug molecules bound to hERG has been the main goal of simulations on this potassium channel since the beginning.[7] The reasons for that reside in the possibility of getting a deeper understanding of the molecular

basis of hERG blockade by drugs from the atomic level representation of the complex, and ultimately in the hope of obtaining a predictive tool able to estimate the binding affinity of ligands to the channel. More recently, the study of hERG–toxin complexes has been undertaken in line with similar simulations performed on other K^+ channels to help the comprehension of certain functional mechanisms of hERG. A short account of the most recent studies will be given here.

A thoughtful and rigorous study on the docking of several drugs to a model of the open state of hERG was published by Farid et al. in 2006.[60] In this work, the authors applied a docking protocol aimed at simulating the induced fit of the drug molecules upon binding to the channel, by first identifying and refining each drugs' binding site at the inner cavity of the channel, and then redocking the compounds in the such fitted sites. The binding modes of the racemic drugs cisapride, terfenadine, ibutilide (both enantiomers were considered for all three compounds), and of MK499 (one enantiomer), clofilium, and sertindole (plus five sertindole analogues) were then analyzed in terms of both number and type of contacting residues, and interactions with hydrophobic and hydrophilic potentials mapped on the inner pore. It resulted that for all drugs two to four Y652 and one to two F656 residues were simultaneously involved in the binding, in agreement with previous inferences from experimental[9] and computational ligand-based[13] studies, pointing to the hydrophobic effect as one of the important driving forces for the small ligands' affinity to hERG. In apparent contradiction with the current knowledge about the physicochemical basis of hERG blockade by drugs, no role was found for the cation–π interaction between the charged nitrogen of hERG blockers and Y652 supposed to be a key determinant for the binding of most drugs.[9] In support of their conclusion, Farid et al.[60] speculated that the overall negative electrostatic field inside the hERG cavity might favor the binding of cationic species rather than a localized effect due to specific residues. Also in this context, the same authors observed that the organization of the residues lining the pore walls (deriving from the tetrameric assembling of the S6 helices) favored the possibility of multiple binding modes giving rise to a rather unspecific host–guest-like binding site different from that of an enzyme or a receptor. Finally, they pointed out that the blockers' conformations predicted by their simulations were generally U-shaped and adapted to the cylindrical symmetry of the pore, in contrast with the extended conformations predicted by several ligand-based models. Notably, a seemingly similar result was obtained by Choe et al.[66] for the docking of clozapine to hERG: the molecule bound at the base of the selectivity filter, almost perpendicularly to the pore axis, engaging in a H bond between its protonated nitrogen and the carbonyl oxygen of T623.

In their paper,[62] Stansfeld et al. described an exhaustive work where they mainly used their closed hERG model (presented above) to study the docking of many channel blockers, for which mutagenesis studies had been previously produced. When using a model of the open state of hERG, these authors obtained docking poses comparable to those reported by Farid et al.[60] (U-shaped or curled), whereas the binding modes to the so-called rotated-hinged model of (closed) hERG involved extended conformations of the molecules. It is remarkable that the rotation introduced in the S6 helix to get the latter model led to an almost perfect rationalization of the results of the experiments on the sensitivity of mutants to the hERG blocking activity of drugs. Accordingly, Y652 and F656 were found to undertake hydrophobic and π-stacking interactions, as well as, in a few cases, cation–π ones; T623 and S624 were two residues often involved in polar and H-bond interactions. A further validation of the hERG model and of the docking protocol came from the comparison of the physicochemical features of the hERG cavity binding site with the pharmacophoric scheme earlier developed by Cavalli et al.[79] The two models (target- and ligand-based, respectively) matched consistently allowing the identification of possible hydrophobic or π-stacking contacts between pharmacophoric features C0 and C1 and Y652 residues, whereas the protonated nitrogen and the C2 feature could be in positions suitable for cation-π or hydrophobic contacts, respectively, with F656 side chains.

The two above outstanding works of docking simulations, point out two complementary pictures of how small ligands may bind at the inner pore of hERG, as the open and the closed states of the channel seem to show different requirements for binding, at least in terms of conformation. This was noted by Stansfeld et al.,[62] and it also reminiscent of the idea of Farid et al.[60] that the "binding site" of hERG blockers cannot be simply and statically defined as one made by few key contact points, but it involves redundant multiconfigured residues. In the same context, Rajamani et al.[65] made it explicit and (computationally) probed the hypothesis of considering the preference of some drugs for either gating state. After building models for a sequence of intermediate gating states of hERG (from closed, based on KcsA, to open, based on MthK), they picked two of them corresponding to partially and fully open, and docked a set of 32 ligands at both. By comparing the estimated interaction energy for each drug in both models, the preference was established for a given state: 21 compounds showed higher (estimated) affinity for the open state of hERG, 11 for the closed one. To statistically validate this hypothesis, a regression model was derived correlating the experimental pIC_{50} values with the calculated van der Waals and electrostatic energy differences between the bound and free states of the systems.

From the considerations above, it might be inferred that simulations of the docking of drugs to hERG should also carefully take into account aspects such as the nontrivial (with respect to a simple pocket) cylinder-like shape of the pore, the tetrameric symmetry, and also the dynamics of the channel structure (for example, of the gating movements as in Rajamani et al.,[65] or, at least, of the side chains protruding inside the cavity). A step in this direction was attempted by Masetti et al.[64] who developed a docking protocol for the drug astemizole taking into account the conformational mobility of the hERG cavity side chains projecting inside the pore. Actually, the docking was carried out on snapshots of the MD trajectory of the open state model, thus putatively simulating the induced-fit effect of the drug onto the target binding site resi-

dues. This resulted in the identification of several (mostly degenerate) docking poses for the drug, one of which reproduced the extended conformation able to contact the canonical S624, Y652, and F656 residues through H bonds, π-stacking, and cation–π interactions, respectively. However, intriguingly, in all of the other docking configurations, cation–π interactions were seldom detected, even though the two aromatic residues were always involved in the binding mode. Finally, this result is in line with those reported in the two basic papers discussed before[60,62] and again points out the need of addressing the problem of docking to the inner walls of a channel in a perhaps different way with respect to the usual static manner.

Given that almost all of the simulation works on hERG have been carried out to help the understanding of how drugs bind to this channel (to learn how to avoid it), one may say that in this context the ultimate information required is the value of the binding affinity of such drugs to hERG. This problem was first tackled by Österberg and Åqvist[59] and Rajamani et al.[65] by estimating the energetics of drug binding, whereas more recently, Du et al.[67] used docking scores to the same aim. In all cases, relative affinities were calculated in a series of compounds and subsequently compared to the experimental values to validate the models. The approach followed by Österberg and Åqvist was the calculation of relative free energies of binding (through the LIE method[75]) that were obtained from the differences of the average (from MD sampling) ligand intermolecular electrostatic and van der Waals energies in the complex and in aqueous solution. By applying this method to a small set of sertindole analogues docked at a model of open hERG (KvAP-based), the calculated relative binding free energies obtained from simulations and from experiments gave a good correlation as seen from the published plot (we calculated an r^2 value of 0.972 for the correlation between reported ΔG_{LIE} values and pIC_{50}). Similarly, for their two-states model[65] described above, Rajamani et al. reported an r^2 value of 0.82 for the correlation of the pIC_{50} data and electrostatic and van der Waals energy values, even though in this case five data points out of 32 had to be omitted as outliers. Finally, Du et al. docked 56 compounds to a closed hERG model (based on KcsA) and tried to correlate the values of the scoring function (GoldScore) used by the docking program with the experimental pIC_{50} values. The statistics of the model ($s = 0.92$, $r^2 = 0.60$, $q^2 = 0.56$) seem to indicate in this case a lower precision of the parameter used to estimate the binding affinity, even though other factors limiting the accuracy of the prediction cannot be excluded. However, overall, it must be underlined that estimating free energies of binding is a quite difficult challenge, and that, as far as hERG is concerned, several preliminary problems need to be resolved before fully reliable results in this field can be obtained.

To conclude this section on docking simulations with hERG, the papers by Tseng et al.[63] and by Yi et al.[68] have to be mentioned again, as they report the building of models of the complex between hERG and a peptide toxin (BeKm-1). To carry out this task, both groups used the hERG models they developed including the S5-P linker, which is recognized to play a role in the binding of the toxin.[80] The approach followed by Tseng et al. to build the complex implied using the NMR-determined structure of BeKm-1 in the search for docking complexes that could be consistent with experimental data (Ala scanning of the toxin,[81] and mutant cycle analysis showing the combined effects of Cys mutations on 32 residues of the hERG outer region and five residues on BeKm-1), and carrying out MD simulations on candidate models. This work resulted in a model of the complex consistent with the experimental information and showing the toxin bound above the hERG pore entrance with the aromatic residues Y11 and F14 contacting the S5-P linker helices, and the charged K18 and R20 making strong interactions with two S631 residues at the entrance of the pore; no toxin side chain was detected to protrude inside the channel pore. It can be observed that this model, still with the limitations inherent to the absence of a firm structural basis (mentioned above), was carefully built strongly restraining it with experimental data. On the other hand, Yi et al.[68] built their model of the complex by using a protein–protein docking program (two candidate models were selected), and carrying out extended MD simulations (seemingly unrestrained in the hERG–BeKm-1 contact region), after which they chose the most favorable and stable complex. To validate the model, a computational Ala scanning simulation was performed, whereby all the residues of the toxin were mutated to Ala and the differences in binding free energy between each mutant- and wild type-bearing complex calculated. The resulting binding mode of the toxin at the outer region of hERG was somewhat different from that proposed by Tseng et al.[63] (also because of the difference in the modeled structure of the outer vestibule of the channel), the main differences being that the S5-P linker was not found to contact the toxin and the R20 side chain was predicted to penetrate inside the pore thus plugging it; the main interactions of the latter residue were with N629 of hERG.

Outlook

During the present decade, the structural and biophysical discoveries on potassium channels on the one hand, and the advancements of the computational modeling techniques on the other have provided a strong impetus for simulation studies regarding this important family of protein complexes. Initially, the directions of the research were mainly dictated by the need to understand the basic molecular features of the functioning of these ion channels and by the availability of crystallographically resolved structures, on which to carry out the simulation work. This is illustrated by the studies briefly reviewed in the first part of this article, which take into consideration issues such as ion permeation and selectivity, channel gating, and voltage sensing. In contrast, in the case of hERG the emphasis was on the interaction with ligands from the beginning, even though direct crystallographic information were lacking (and still are, at the time of publication of this paper). Docking simulations aimed at predicting the binding mode(s) of hERG blockers were already performed in the early studies, and this may have led to a rather "unbalanced" development

of the modeling efforts that only recently has been realized. Remarkably, the task of modeling hERG is made even harder by the presence of peculiar structural (and functional) characteristics, such as, for example, the presence of the unusually long S5-P linker, and the presence and position in the inner pore of the aromatic residues Y652 and F656. However, the recent papers illustrated in the second part of this review account for studies accurately addressing critical basic issues, and witness the progresses made in building hERG models[62,63] and the first attempts to simulate channel functions.[61]

The simulation of the docking of drugs at the inner cavity is certainly the most frequently addressed aspect when dealing with hERG modeling. However, instead of leading closer to a definitive solution (one might expect to find "the" binding mode of single blockers), it seems that more accurate studies have brought to light new problems to be tackled. This should not be surprising, at least because the binding site on hERG is rather uncommon, and presents characteristics, which the currently applied docking procedures may not be able to treat in a fully appropriate manner. In fact, such factors have to be taken into account as the fourfold symmetry and the cylindrical shape of the pore (different from the pocket-like shape of "usual" binding sites), or the different volumes of the inner cavity in the open and closed states and their dynamic fluctuations. Furthermore, different sequence alignments on the template(s) can lead to different exposure of amino acid side chains into the pore lumen with consequent variation of binding possibilities. All this can lead to results such as those illustrated in Figure 7, where the docking poses of sertindole are shown as reported in the papers by Farid et al.[60] (top), Stansfeld et al.[62] (middle), and Österberg and Åqvist[59] (bottom). All these poses appear different as a consequence of the aspects outlined above, and in our opinion represent a clear indication of the need of thinking carefully about the definition of docking at a channel cavity, in terms of both procedures and expected results. With regard to the latter, for instance, one might wonder how realistic it may be to keep pursuing "the"

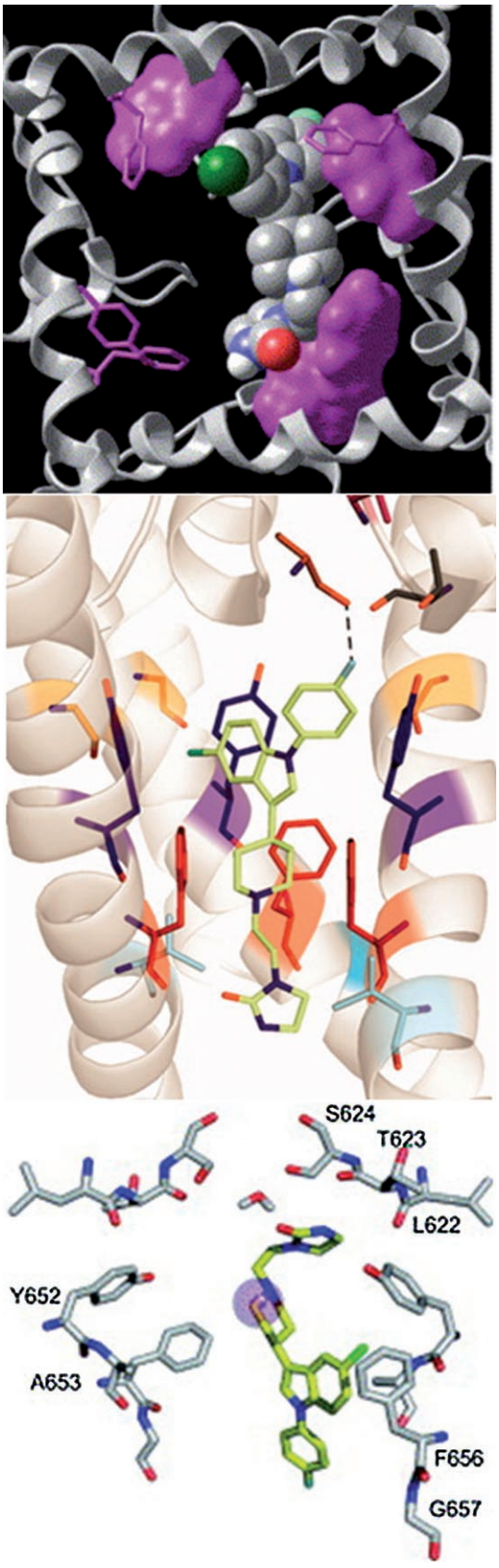

Figure 7. Top: a view of the channel pore (open state) from the outer mouth is shown, where sertindole binds perpendicularly to the pore axis (in a "curled" conformation) making four contacts with the aromatic side chains of Y652 and F656; reprinted from Bioorg. Med. Chem., 14, R. Farid et al., New insights about hERG blockade obtained from protein modeling potential energy mapping and docking studies, pp 3160–3173, 2006, with permission from Elsevier (ref. [60]). Middle: the same molecule is shown bound to a model of the closed state in an extended conformation parallel to the pore axis that allows interactions of the fluorophenyl moiety with S624 (H-bond) and Y652 (π-stacking), and of the protonated piperidine nitrogen with F656 (cation-π); taken from Drug block of the hERG potassium channel: Insight from modeling, P. J. Stansfeld, P. Gedeck, M. Gosling, B. Cox, J. S. Mitcheson, M. J. Sutcliffe, © 2007 Wiley-Liss Inc., reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley & Sons, Inc. Bottom: sertindole docked to an open channel model appears again in an extended conformation (but turned upside down) with the imidazolidinone ring interacting with T623 and S624, while the aromatic portion of the molecule makes hydrophobic contacts with F656 residues; the protonated nitrogen occupies the crystallographic position of the cavity K^+ in KvAP; reprinted from FEBS Lett., 579, F. Osterberg and J. Åqvist, Exploring blocker binding to a homology model of the open hERG K^+ channel using docking and molecular dynamics methods, pp 2939–2944, 2005, with permission from Elsevier (ref. [59]).

ligand binding mode inside the pore instead of trying to define a ligand binding space.

As regards future developments in the field of hERG simulations, the achievement of sound modeling protocols has opened the way to advanced studies aimed at studying basic mechanisms of channel functions.[61] Further work could be done, even considering the limitations imposed by the need of using a homology model as the starting structure. For instance, simulations of the gating mechanism may be feasible, in the line of analogous works carried out on other potassium channels, and, in this context, a reappraisal of the lipid models used to simulate the membrane environment might also be appropriate.

However, given the strong pharmacological implications of the hERG functions, the most wanted modeling applications will be those regarding the study of the interactions with drugs. In this context, as already stated, the prediction of ligand affinity is a goal, and reports of early attempts to achieve it have been reviewed in the previous section. However, it should be observed that, although the quantitative assessment of the capacity to bind to hERG can be an interesting result (mainly from the drug designer point of view), not less important might be the possibility of interpreting through simulations the effects of ligands on some channel functions, such as, for example, ion permeation, gating, or inactivation. An effort in this sense should be based on the acquired ability to simulate the same functions in other potassium channels, and proceed a step forward including the presence of a drug molecule as a perturbant of the system. After all, also from information coming from this kind of studies hints might be obtained on how to optimize a hit or design a channel activator.

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Keywords: drug safety · ion channels · long QT · molecular dynamics · molecular modeling

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