ACCOUNTS of chemical research

Article

pubs.acs.org/accounts

Ion Selectivity Strategies of Sodium Channel Selectivity Filters

Todor Dudev*,† and Carmay Lim*,‡,§

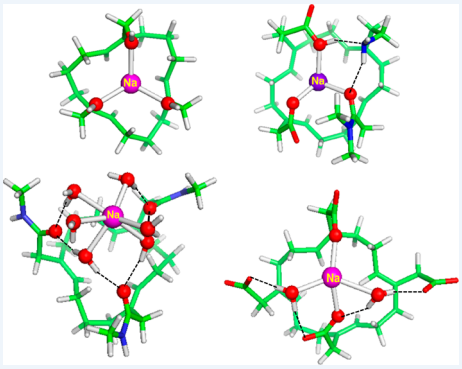
†Faculty of Chemistry and Pharmacy, Sofia University, Sofia 1164, Bulgaria

‡Institute of Biomedical Sciences, Academia Sinica, Taipei 115, Taiwan

§Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan

Supporting Information

CONSPECTUS: Sodium ion channels selectively transport Na^+ cations across the cell membrane. These integral parts of the cell machinery are implicated in regulating the cardiac, skeletal and smooth muscle contraction, nerve impulses, salt and water homeostasis, as well as pain and taste perception. Their malfunction often results in various channelopathies of the heart, brain, skeletal muscles, and lung; thus, sodium channels are key drug targets for various disorders including cardiac arrhythmias, heart attack, stroke, migraine, epilepsy, pain, cancer, and autoimmune disorders. The ability of sodium channels to discriminate the native Na^+ among other competing ions in the surrounding fluids is crucial for proper cellular functions. The selectivity filter (SF), the narrowest part of the channel's open pore, lined with amino acid residues that specifically interact with the permeating ion, plays a major role in determining Na^+ selectivity. Different sodium channels have different SFs, which vary in the symmetry, number, charge, arrangement, and chemical type of the metal-ligating groups and pore size: epithelial/degenerin/acid-sensing ion channels have generally trimeric SFs lined with three conserved neutral serines and/or backbone carbonyls; eukaryotic sodium channels have EKEE, EEKE, DKEA, and DEKA SFs with an invariant positively charged lysine from the second or third domain; and bacterial voltage-gated sodium (Na_v) channels exhibit symmetrical EEEE SFs, reminiscent of eukaryotic voltage-gated calcium channels. How do these different sodium channel SFs achieve high selectivity for Na^+ over its key rivals, K^+ and Ca^{2+}? What factors govern the metal competition in these SFs and which of these factors are exploited to achieve Na^+ selectivity in the different sodium channel SFs? The free energies for replacing K^+ or Ca^{2+} bound inside different model SFs with Na^+, evaluated by a combination of density functional theory and continuum dielectric calculations, have shed light on these questions. The SFs of epithelial and eukaryotic Na_v channels select Na^+ by providing an optimal number and ligating strength of metal ligands as well as a rigid pore whose size fits the cognate Na^+ ideally. On the other hand, the SFs of bacterial Na_v channels select Na^+, as the protein matrix attenuates ion−protein interactions relative to ion−solvent interactions by enlarging the pore and allowing water to enter, so the ion interacts indirectly with the conserved glutamates via bridging water molecules. This shows how these various SFs have adapted to the specific physicochemical properties of the native ion, using different strategies to select Na^+ among its contenders.



INTRODUCTION

Sodium ion channels are pore-forming transmembrane proteins that selectively transport Na^+ cations across the cell membrane. These integral parts of the cell machinery are implicated in regulating the cardiac, skeletal and smooth muscle contraction, nerve impulses, salt and water homeostasis, as well as pain and taste perception.[1,2] Because sodium channels play a critical role in fast processes, they are targets for deadly toxins, which block sodium channels.[3] Their malfunction often results in various channelopathies of the heart, brain, skeletal muscles, and lung;[4] thus, sodium channels are key drug targets for various disorders including cardiac arrhythmias, heart attack, stroke, migraine, epilepsy, pain, cancer, and autoimmune disorders.[3,5]

Central to the proper functioning of sodium channels is their ability to correctly select the native Na^+ from the mixture of ions in the surrounding fluids. These channels can discriminate Na^+ from K^+ with the same net charge or Ca^{2+} with nearly the same ionic radius. In the degenerin or epithelial sodium channel superfamily, the epithelial sodium channel is highly Na^+-selective with a Na^+/K^+ permeability ratio of 100−500,[6,7] whereas the acid-sensing ion channel (ASIC) is much less Na^+-selective with a Na^+/K^+ permeability ratio of 13.[8] Vertebrate voltage-gated sodium (Na_v) channels exhibit Na^+/K^+ and Na^+/Ca^{2+} selectivity ratios of 30[9] and >11,[10] respectively, whereas bacterial Na_v channels are more Na^+/K^+-selective with a permeability ratio of 170 but less Na^+/Ca^{2+}-selective with a permeability ratio of ∼7.[11] Sodium-selective isoforms of the sodium leak conductance channel are more permeable to Na^+ than Ca^{2+} by a factor of ∼3.[12]

Although different segments of the channel pore may contribute to Na^+ selectivity, the selectivity filter (SF), the

Received: August 6, 2014
Published: October 24, 2014

   dx.doi.org/10.1021/ar5002878 | Acc. Chem. Res. 2014, 47, 3580−3587

narrowest part of an open pore lined with protein ligands that specifically interact with the passing ion, plays the key role in controlling the channel selectivity, as altering its structure/composition results in substantial/total loss of selectivity. Crystallographic, site-directed mutagenesis, and channel-blocker binding studies have revealed the SF oligomeric structure, composition, and pore size of several sodium channels. The epithelial/degenerin sodium channels have trimeric SFs containing a conserved (G/S)XS tract with a narrow pore of radius < 2.5 Å.[13−15] The ASIC also has a trimeric SF lined by three carbonyl oxygens but a wider pore of radius 3.6 Å that matches the radius of hexahydrated Na^+.[16] Eukaryotic Na_v channels have constricted DEKA SFs where the conserved Asp, Glu, Lys, and Ala are donated by each of the four nonidentical protein domains.[9,17] The same four conserved residues but with Lys from the second domain and Glu from the third domain line the SF of the Na_v channels in cnidarians forming the DKEA motif.[12,18] The DKEA filter is not as Na^+-selective as its DEKA counterpart.[18,19] Like vertebrate Na_v channels, Na^+-selective isoforms of the sodium leak conductance channel in many eukaryotes have EKEE/EEKE SFs with a Lys from the second/third domain.[12] Unlike their eukaryotic counterparts, the bacterial Na_v channels are *homo*tetramers with a relatively wide, water-filled EEEE SF pore of radius 3.2 Å.[11,20]

The short survey outlined above shows that different types of sodium channels possess different SFs that vary in the overall symmetry, number, charge, arrangement, and chemical type of the metal-ligating groups as well as in the pore size. Yet, these SFs can efficiently discriminate the native Na^+ from other competing ions in the ambient solutions. Interestingly, such a diversity of Na^+-selective SFs appears to be specific for sodium channels since the potassium and calcium channel SFs are more uniform in composition: A four-layered tetrameric structure lined by eight backbone carbonyl ligands at each site is the signature SF of various potassium channels,[21,22] whereas a single-layered tetrameric ring of Asp/Glu carboxylates (EEEE/EEDD loci) constitutes the SF of voltage-gated calcium (Ca_v) channels.[23,24] The diversity of SFs found in sodium channels raises the following intriguing questions: (1) What factors govern the metal competition in these SFs? (2) Which of these factors are exploited in achieving Na^+ selectivity in the different types of sodium channel SFs?

Here, we endeavor to answer these questions based mainly on our studies, relying on the original publications for details about the models and computational approaches. We aim to elucidate how sodium channel SFs varying in oligomeric structure, ligand composition, local charge, and pore size can achieve the same goal of selecting the native Na^+ from competing ions. As we focus on the SF, we do not assess long-distance effects from other segments of the pore, kinetic barriers, or bulk properties (e.g., various ion concentrations in the baths surrounding the channel) on ion selectivity, which have been discussed in previous studies.[25−32] Since the open-state metal-bound structures of all the sodium channels (except ASIC[16] and a bacterial Na_v channel[33]) remain unsolved, we have used density functional theory to optimize the geometries of metal-bound model SFs resembling the various sodium channel SFs. The optimized geometries were then used to compute the free energy ΔG^x for replacing K^+ or Ca^{2+} bound inside a model SF, [K^+/Ca^{2+}-filter], characterized by an effective dielectric constant x with Na^+; that is,

$$[Na^+(H_2O)_6] + [K^+/Ca^{2+}\text{-filter}] + nH_2O \rightarrow [Na^+\text{-filter}] + [K^+/Ca^{2+}(H_2O)_{6+n}] \quad (1)$$

where $n = 0$ or 1. The ΔG^x for eq 1 was computed as a sum of the gas-phase free energy, ΔG^1 (electronic effects) and the solvation free energy difference between the products and reactants (solvation effects). A positive ΔG^x implies a K^+- or Ca^{2+}-selective filter, whereas a negative ΔG^x implies a Na^+-selective one. As the results herein are based on an equilibrium theory of ion selectivity, the scope of this Account is limited to equilibrium as opposed to nonequilibrium processes. A caveat to this approach is its limitations in dealing with the ionic composition and content of surrounding solutions. Nevertheless, our approach based on eq 1 has yielded trends in the free energy changes with varying parameters (e.g., the metal/ligand type, the metal hydration number, and SF pore size) that are consistent with experimental observations.[34−42] Notably, it has yielded results for an eclectic group of sodium channel SFs that are in accord with available experimental data, as described herein.

This Account is structured as follows: First, we outline the key factors governing the Na^+/K^+ and Na^+/Ca^{2+} competition in sodium channel SFs. We then reveal how the various SFs of epithelial, acid-sensing, eukaryotic Na_v, bacterial Na_v, and sodium leak conductance ion channels employ different factors to achieve Na^+ selectivity.

FACTORS GOVERNING THE Na^+ VERSUS K^+ OR Ca^{2+} COMPETITION IN SODIUM CHANNEL SFs

Electronic and solvation effects, which determine the net ΔG^x for eq 1, favor different metal ions: Increasing the magnitude of ion−protein interactions such that electronic effects dictate the ΔG^x favors the cation that is a better electron acceptor, that is, divalent Ca^{2+} over monovalent Na^+ and K^+. In contrast, diminishing electronic effects enhances the relative contribution of solvation effects and increases the competitiveness of the ion with the smaller dehydration penalty, that is, K^+ over Na^+ and Ca^{2+}. Hence, the sodium channel SFs have to achieve the right balance of ion−protein and ion−solvent interactions that would favor Na^+ over its rival cations. Several studies have revealed the factors that help to achieve this fine balance in sodium channels:[28,39,42−46] These factors involve the inherent properties of the metal ions, the metal-ligating residues, and the protein matrix, which could control the SF pore size, rigidity, and solvent accessibility.

Metal Coordination Number (CN)

Decreasing the metal CN favors Na^+ binding to the pore more than K^+ or Ca^{2+} binding, mainly because K^+ and Ca^{2+} generally prefer larger CNs in both protein and aqueous solution compared to Na^+.[38,39,47] The smaller CN in trimeric SFs enhances especially Na^+/K^+ selectivity, as it reduces the steric repulsion among the bulky protein ligands around Na^+ more than that around the larger K^+.

Ligand Ligating Strength

Increasing the charge and charge-donating ability (i.e., ligating strength) of a protein ligand favors the ion with the better electron-accepting ability.[47] The ligand's charge-donating ability increases in going from the Ser/Thr hydroxyl group to the Asn/Gln/backbone amide group to the Asp/Glu carboxylate, whereas the electron-accepting ability increases as $K^+ < Na^+ < Ca^{2+}$. Hence, Asp^-/Glu^- interacts more favorably with Na^+

dx.doi.org/10.1021/ar5002878 | Acc. Chem. Res. 2014, 47, 3580−3587

than with K^+, helping to offset the larger dehydration penalty of Na^+ (−98 kcal/mol) compared to that of K^+ (−81 kcal/mol),[38] but they interact more favorably with Ca^{2+} than with Na^+. As increasing the ligand's ligating strength has opposite effects on the Na^+/K^+ and Na^+/Ca^{2+} selectivity, a delicate balance should be maintained between the number and chemical type of the ligands lining the SF of sodium channels to achieve appreciable Na^+ selectivity over both K^+ and Ca^{2+}.

SF Pore Size, Rigidity, and Solvent Exposure

A narrow, rigid pore optimized to fit a bare Na^+ is Na^+-selective, as it cannot optimally fit a bulkier cation.[39] A wide, rigid pore that fits a partially/fully hydrated permeable ion with a CN > 3 can also be Na^+-selective: Ion hydration favors Na^+/Ca^{2+} selectivity, as the metal ion indirectly binds the SF ligands via its first-shell water molecules, resulting in longer metal−ligand distances, which disfavors Ca^{2+} binding more than Na^+ binding (see Dudev and Lim[46]). It also favors Na^+/K^+ selectivity partly because Na^+, being a stronger Lewis acid, polarizes the first-shell water molecules more than K^+, resulting in stronger metal−water−ligand interactions in the Na^+ complexes than in the respective K^+ clusters. Increasing the SF pore's solvent exposure (dielectric constant) also enhances Na^+ selectivity: In a high-dielectric pore, Na^+/Ca^{2+} selectivity is enhanced because the dehydration penalty for Na^+ is far less than that for Ca^{2+}.

■ ION SELECTIVITY IN SSS/BBB SFs OF EPITHELIAL Na CHANNELS OR ASIC

Epithelial/degenerin sodium channels consist of α, β, and γ subunits that form heterotrimers with SFs containing a conserved (G/S)XS tract with a pore radius <2.5 Å.[13,15,48,49] In this asymmetric SF, an invariant Ser from the α subunit plays a key role in conferring Na^+/K^+ selectivity,[14] but the conserved Gly and Ser residues from the β and γ subunits, respectively, are important in restricting K^+ permeation.[15] In line with experimental observations, the calculated free energies ΔG^x for replacing K^+ or Ca^{2+} bound to the Ser hydroxyl O (SSS SF, Figure 1a) or backbone carbonyl O (BBB SF, Figure 1b) with Na^+ are all negative, indicating a Na^+-selective pore. The key determinants of Na^+ selectivity in these SFs appear to be a narrow rigid pore[13−15] formed by the trimeric SF and undercoordination (CN of 3) of the passing ion, which suits Na^+ better than its contenders who favor larger CNs. Rigidifying the pore and maintaining its optimal size to fit dehydrated Na^+ imposes a huge energy penalty for binding larger cations (e.g., K^+), thus enhancing selectivity for the cognate metal (Figure 1a, numbers in parentheses). Increasing the CN from three to four in a model SF lined with four serines attenuates the Na^+ affinity for the pore, resulting in reduced Na^+/Ca^{2+} selectivity and reversed Na^+/K^+ selectivity.[39,45] Conformational changes in the pore that would free ≥1 backbone carbonyls to interact with permeant ions[7] instead of the Ser hydroxyl group(s) would enhance Na^+/K^+ selectivity (Figure 1b): Compared to the hydroxyl group, the carbonyl group has stronger ligating strength and stronger interactions with Na^+ than with K^+.

Indeed, the X-ray structure of an ASIC/snake toxin complex shows a BBB SF formed by the glycine of the GAS motif. Compared to epithelial channel SF, the ASIC SF has a wider pore of radius (~3.6 Å) similar to the Na^+ hydration radius (3.58 Å).[50] In this wide BBB SF, the protein matrix dictates Na^+ selectivity by enlarging the pore size to permit passage of fully hydrated ions. This favors the permeation of Na^+ over K^+

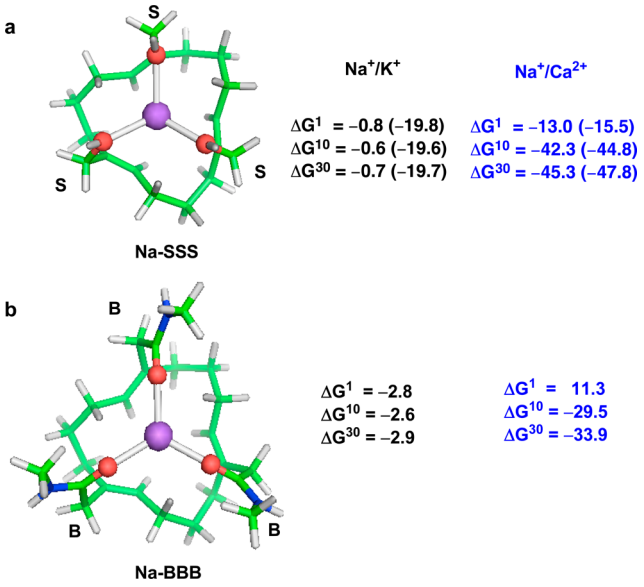


Figure 1. Free energies, ΔG^x (in kcal/mol), for replacing K^+ and Ca^{2+} (numbers in blue) bound to (a) three OH-ligating groups (representing Ser side chains) in the SSS filter and (b) three $-CONHCH_3$ ligating groups (representing backbone peptide groups denoted by B) in the BBB filter with Na^+ (eq 1). ΔG^1 refers to the metal exchange free energy in the gas phase, whereas ΔG^{10} and ΔG^{30} refer to the metal exchange free energies in an environment characterized by an effective dielectric constant of 10 and 30, respectively. The free energies for eq 1 in a rigid Na^+-optimized SSS filter prohibited from relaxing when K^+/Ca^{2+} is bound in the SF are in parentheses. The metal-ligating groups are coordinated to the permeating ion and attached to a carbon−hydrogen ring scaffold via methylene spacers. Shown are B3-LYP/6-31+G(3d,p) fully optimized structures of Na^+ bound to the model SFs.

because the hydrated Na^+ fits snugly in the SF pore and its hydration sphere is more polarizable than that of K^+, securing more favorable interactions with the SF walls.[51] The relatively high dielectric water-filled ASIC pore favors Na^+ over Ca^{2+} because of the smaller dehydration penalty for Na^+ compared to Ca^{2+}.[51] However, increasing the Na^+ CN from three in the epithelial channel SF to six in the wider ASIC pore diminishes Na^+ selectivity: the Na^+/K^+ permeability ratio for the ASIC (~13)[8] is an order of magnitude smaller than that for the epithelial channel (100−500).[6,7]

■ ION SELECTIVITY IN DEKA/DKEA SFs OF EUKARYOTIC Na_v CHANNELS

Eukaryotic Na_v channels and sodium leak conductance channels are heterotetramers with DEKA/DKEA and EEKE/EKEE SFs, respectively. In accord with experimental findings, these SFs are all predicted to be Na^+-selective (Figure 2): The ΔG^x for replacing K^+ with Na^+ in the SF (numbers in black) varies in a narrow range between −3 and −5 kcal/mol, whereas those for replacing Ca^{2+} with Na^+ exhibit larger variations ranging from −6 to −19 kcal/mol (numbers in blue). Also in agreement with experimental observations,[1,9,12,17−19,52] the DEKA SF is predicted to be more Na^+-selective than the DKEA and EEKE/EKEE SFs (most negative $\Delta G^{10}/\Delta G^{30}$ in Figure 2).

Role of Lys in Ion Selectivity

The presence of a Lys in the SF is necessary and sufficient to generate Na^+-selective channels: Mutation of the Lys in the

dx.doi.org/10.1021/ar5002878 | Acc. Chem. Res. 2014, 47, 3580−3587

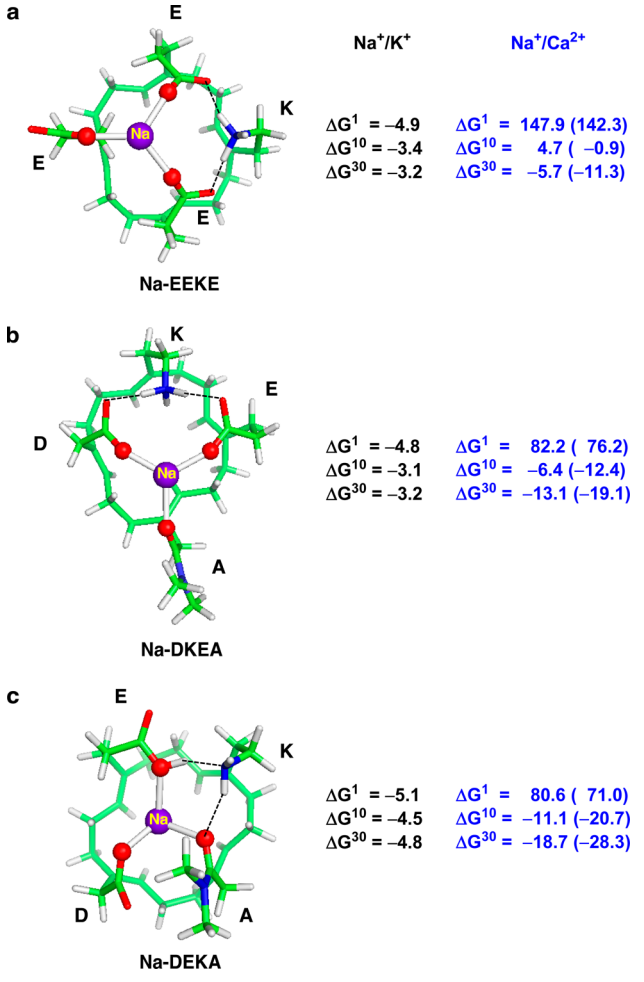


Figure 2. Free energies, ΔG^x (in kcal/mol), for replacing K^+ and Ca^{2+} (numbers in blue) in model (a) EEKE, (b) DKEA, and (c) DEKA SFs with Na^+. The free energies for eq 1 in a rigid Na^+-optimized pore prohibited from relaxing when Ca^{2+} is bound in the SF are in parentheses. Shown are B3-LYP/6-31+G(3d,p) fully optimized structures of Na^+-bound to (a) EEKE, (b) DKEA, and (c) DEKA model SFs with $-CH_2-COO^-$, $-CH_2-CH_2-COO^-$, and $-CH_2-CH_2-NH_3^+$ modeling the Asp, Glu, and Lys side chains, respectively, and $-CON(CH_3)_2$ representing the Ala backbone peptide group. The dashed lines denote hydrogen bonds.

DEKA SF to another residue, even to a positively charged arginine, drastically reduces (or even reverses) the channel's Na^+/K^+ selectivity,[9,10,19] whereas mutation to an aspartate/glutamate rendered the channel Ca^{2+}-selective.[19,52] Conversely, mutation of the third domain Glu in the EEEE SF of human cardiac Ca_v channel and in the DEEA SF of the Ca^{2+}-selective BSC1 channel to Lys made the channel more permeable to Na^+ than to Ba^{2+}.[53] Consequently, in the course of evolution, the presence of a Lys in the SF has played a key role in converting Ca^{2+}-selective EEEE/DEEA SFs to Na^+-selective EEKE/EKEE and DEKA/DKEA ones.[12,18,42] Lysine plays mainly three roles in the Na^+ selectivity process:

Lysine Decreases the Metal CN. Because Lys is not a metal ligand, only three residues lining the SF coordinate the metal cation (Figure 2). This increases the competitiveness of Na^+ over K^+ and Ca^{2+}, as the small CN of three favors Na^+ over K^+ or Ca^{2+} binding (see above).[39,45]

Lysine Reduces the SF's Negative Charge. This role is especially important in the competition between Na^+ and divalent Ca^{2+}.[43,45,54−57] Exchanging a glutamate in the ancestral EEEE/DEEA SF for Lys attenuates electronic effects in the $Na^+ \rightarrow Ca^{2+}$ exchange free energy: The gas-phase free energy for eq 1 decreases linearly with decreasing SF net charge.[42] On the other hand, increasing the pore's solvent accessibility enhances the contribution of solvation effects, which favor Na^+ over Ca^{2+} (see above). Thus, in high-dielectric Lys-containing pores, solvation effects become dominant, strongly favoring Na^+ over Ca^{2+} (negative ΔG^{30}, Figure 2), as Na^+ has a much smaller dehydration penalty than Ca^{2+}.

Lysine Constricts and Rigidifies the SF Pore. This is via hydrogen bonding interactions with its neighbors (Figure 2), consistent with the experimental finding that the DEKA SF is permeable only to ammonium (of radius 1.8 Å), but not to larger mono/di/tri/tetramethylammonium ions.[10] Rigidifying the pore that optimally fits the native Na^+ enhances Na^+ selectivity: In Figure 2, the computed metal exchange free energies in Na^+-optimized pores that were not allowed to relax upon Ca^{2+} binding (numbers in parentheses) are more negative (by ∼6−10 kcal/mol) than those in pores that could relax upon the passage of the rival cation (blue numbers without parentheses).

Importance of the Lys Position in the SF

The Lys position in the SF also affects ion selectivity, as the DEKA SF is more Na^+-selective than the DKEA one (free energies for DKEA in Figure 2b are less negative than those for DEKA in Figure 2c). This agrees with the finding that (i) the DEKA SF of rat brain Na_v channel is impermeable to Ca^{2+}, but the DKEA SF of the mutant channel allows Ca^{2+} through,[19] and (ii) replacing the Glu from the DEEA SF of the *Nematostella vectensis* $Na_v2.1$ channel with Lys resulted in a Ca^{2+}-impermeable DEKA SF but a Ca^{2+}-permeable DKEA SF.[12] *Why and how does the Lys position in the SF affect ion selectivity?*

The different Lys position in the DEKA and DKEA SFs changes neither the metal CN nor the SF net charge, but it rigidifies and constricts the DEKA pore more than the DKEA one, making the DEKA SF more Na^+-selective than the DKEA one: The Lys interacts with the Asp and Glu metal-*free* O atoms in the Na-DKEA SF (Figure 2b), but with the Glu and Ala metal-*bound* O atoms in the Na-DEKA SF (Figure 2c), making the latter more rigid than the former. Rigidifying the DEKA pore increases Na^+/Ca^{2+} selectivity more than rigidifying the DKEA pore (by 4 kcal/mol). The Lys also makes the DEKA pore narrower than the DKEA one, as the three metal-bound O atoms are closer to each other in the DEKA SF than those in the DKEA SF.[42] Hence, in the DKEA SF, Na^+ is nearly in the plane formed by the three metal-ligating O atoms, but in the DEKA SF Na^+ has sunk below this plane.

Factors Governing Na^+ Selectivity in DEKA SFs

The above results suggest that Na_v channels with DEKA SFs are highly Na^+-selective due to the favorable combination of several factors:

Metal "Undercoordination". As Lys does not bind the metal directly, the ion is coordinated to three rather than four protein ligands in the SF even though the channel is tetrameric.

Balanced SF Charge Density. Interactions with two high-field strength residues (D and E) favor Na^+ binding more than K^+ binding, thus enhancing Na^+/K^+ selectivity. Increasing further the number of anionic ligands in the SF would favor Na^+ in the Na^+ vs K^+ competition, but not in the Na^+ vs Ca^{2+} competition: SFs with three/four[45,46] acidic residues binding directly to the metal cation exhibit decreased/reversed $Na^+/$

dx.doi.org/10.1021/ar5002878 | Acc. Chem. Res. 2014, 47, 3580−3587

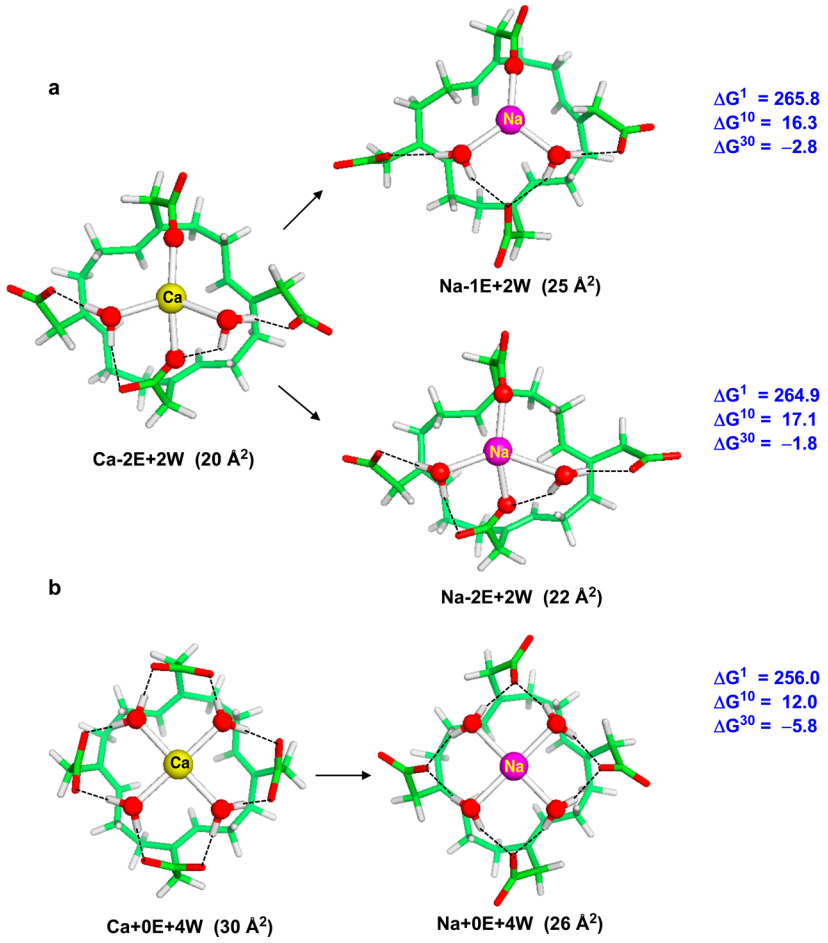


Figure 3. Free energy (in kcal/mol) for replacing Ca^{2+} in the model EEEE SF with Na^+ where the metal cation is bound to (a) two and (b) four water molecules. Fully optimized Na^+ and Ca^{2+}-bound B3LYP/6-31+G(3d,p) structures are shown. The pore aperture area is given in parentheses.

Ca^{2+} selectivity, respectively. Thus, two acidic residues and a basic Lys apparently provide the optimum field strength for the DEKA SF so that Na^+ could effectively outcompete K^+ and Ca^{2+} to bind to the SF.

Rigid and Constricted SF Pore. Due to the Lys interaction network inside the SF, the pore becomes rigid and constricted, thus fitting Na^+ better than rival cations.

■ ION SELECTIVITY IN EEEE SFs OF BACTERIAL Na_v CHANNELS

Whereas Asp, Glu, Lys, and Ala residues from four different domains line asymmetric DEKA/DKEA SFs in *vertebrate* Na_v channels,[9,12,17,18,52] Glu residues from four identical subunits comprise symmetrical EEEE SFs in *bacterial* Na_v channels.[11,20] In line with mutagenesis studies,[11] the 2.7 Å X-ray structure of the *Arcobacter butzleri* Na_v channel (PDB 3rvy) captured in a closed-pore, metal-free conformation reveals four Glu carboxylates lining a water-filled SF.[20] Unlike the Ca^{2+}-selective EEEE Ca_v channel SFs, bacterial Na_v channels exhibit a Na^+:Ca^{2+} permeability ratio of ∼15.[11] *Without the Lys to reduce the metal CN, the SF net charge, and the pore size and flexibility, how does the high-field strength EEEE filter bind preferentially* Na^+*?*

Consistent with the hypothesis of Payandeh et al.,[20] molecular dynamics simulations[26,27,29,31,32,58] and combined density functional theory and continuum dielectric calculations[45,46] show that the ion inside the large EEEE SF is partially hydrated and is coordinated to only one or two Glu side chains and indirectly to the other Glu carboxylates via its first-shell water molecules (Figure 3a). The Na^+ selectivity in bacterial Na_v channels can be attributed to the following factors:

Optimal Ligating Strength of the Glu Residues

The strong charge-donating ability of the Glu carboxylates has opposite effects on the Na^+/K^+ and Na^+/Ca^{2+} selectivity (see above): It enables Na^+ to outcompete the weaker electron-acceptor K^+,[27] but not Ca^{2+}. On the other hand, protonation of the Glu residues would have a favorable effect on the Na^+/Ca^{2+} selectivity,[45] as it attenuates the favorable electrostatic interactions with dicationic Ca^{2+} more than that with monocationic Na^+. Thus, fine-tuning of the metal−carboxylate electrostatic interactions via protonation of the Glu residues or bridging water molecules (see below) could help to achieve optimal selectivity for Na^+ over K^+ and Ca^{2+}.

Wide, Solvent-Accessible SF Pore

The bacterial Na_v channel X-ray structure shows a wide, water-filled pore (aperture area of ∼21 Å^2) that can fit a metal ion bound to two water molecules in the EEEE ring plane.[20] Such a pore aperture fits a partially hydrated Na^+, but is too small for the bulkier hydrated K^+ to fit in plane with water molecules bridging to the carboxylates. Hence, the electrostatic interactions between K^+ and the EEEE filter are suboptimal, so K^+ binding inside the filter is less favorable than Na^+ binding.[27,29] The electrostatic interactions between Ca^{2+} and the EEEE filter are also suboptimal due to the loss of ≥1 direct metal−

dx.doi.org/10.1021/ar5002878 | *Acc. Chem. Res.* 2014, 47, 3580−3587

carboxylate contacts and longer metal−O(carboxylate) distances, which disfavors Ca^{2+} binding more than Na^+ binding.[46] As shown in Figure 3, a wide, solvent-accessible pore that binds the metal cation via ≥2 water molecules confers moderate selectivity for Na^+ over Ca^{2+} (negative ΔG^{30}).

Rigid Pore

The crystal structure of the bacterial Na_v shows hydrogen bonds between the metal-free carboxylate O atoms of Glu residues in the SF and the backbone amide groups of neighboring residues.[20] These first-shell to second-shell hydrogen bonds help to rigidify the SF, thus enhancing the selectivity for Na^+ over K^+ and Ca^{2+}.

■ CONCLUDING REMARKS

Sodium channels work in concert with other highly discriminatory ion channels such as voltage-gated potassium and calcium channels, which also exhibit remarkable ion selectivity toward their cognate metal cation. Over billions of years of cell evolution, the SFs of these molecular devises have adapted to the specific physicochemical properties of the cognate ion, using various strategies to enable them to efficiently select the native ion among its contenders. Among the SFs of sodium, potassium, magnesium, and calcium channels, those of sodium channels seem to be the most diverse with respect to their oligomericity, composition, overall charge, pore size, and solvent accessibility. Accordingly, these SFs have adopted different strategies to achieve the desired Na^+ selectivity.

The SFs of epithelial and eukaryotic Na_v channels help to select Na^+ over both K^+ and Ca^{2+} using a well-balanced combination of several factors. These SFs provide three metal-ligating residues, which suits the coordination preference of the native Na^+ better than its rivals. Another factor that plays an important role in tailoring the Na^+ selectivity of these SFs is the well-balanced ligating strength of the residues lining the pore which, on one hand, is strong enough to favor Na^+ over K^+ but, on the other hand, not so strong as to favor dicationic Ca^{2+}. In addition, rigidifying and constricting the pore to geometrically fit the cognate Na^+ further enhances the Na^+ selectivity of these SFs, as binding of a bulky metal ion (e.g., K^+) in a narrow Na^+-optimized pore imposes a large energy penalty. This strategy is exemplified by the DEKA SF, where the conserved Lys rigidifies and constricts the pore via a tight network of hydrogen bonds.

A different strategy is used in the BBB SF of the ASIC and the EEEE SF of bacterial Na_v channels: By allowing water inside the pore and enlarging the pore to fit a fully/partially hydrated cation, the SF preferentially binds the cognate ion over its competitors. Relative to K^+, Na^+ polarizes its first-shell water molecule(s), resulting in more favorable interactions with the SF walls than its rival K^+. Furthermore, the water-mediated Ca^{2+}···O(carbonyl/carboxylate) interactions become more attenuated than the respective Na^+ interactions, thus diminishing the magnitude of the electronic effects (which favor Ca^{2+}) relative to that of solvation effects (which favor Na^+). This shows that the protein matrix controls ion selectivity in the ASIC and bacterial Na_v SFs by attenuating ion−protein interactions relative to ion−solvent interactions.[45,46]

When X-ray structures of epithelial and eukaryotic sodium channels and calcium channels bound to their cognate ions become available, they would enable all-atom molecular dynamics simulations using force fields that account for charge transfer and polarization effects[59] and umbrella sampling calculations to compute free energy profiles for the movement of ≥1 ions across the SF, as performed for bacterial Na_v channels based on their X-ray structures.[27,29,31,32] The free energy simulations could help to elucidate how rigidity effects are finely tuned so that the channel protein can not only select but also permeate its cognate ion. They could also help to determine to what extent the conserved residues lining the SF, the protein matrix, the coupling between ions and kinetic barriers contribute to Na^+/K^+ or Na^+/Ca^{2+} selectivity in the channel. In addition, nonequilibrium simulations using force fields that can account for electronic effects could help to determine how, and to what extent, ion selectivity depends on the composition and concentration of the ionic solutions in which sodium channels function.

■ ASSOCIATED CONTENT

Supporting Information

Methodology and tables showing a comparison between calculated and experimental molecular dipole moments of water, methanol, and formaldehyde, hydration free energies of metal cations and ligands, free energies of metal exchange, and areas of various sodium channel SF pores. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: 359 2 8161 323. Fax: 359 2 9625 438. E-mail: t.dudev@chem.uni-sofia.bg.
*Phone: 886 2 2652 3031. Fax: 886 2 2788 7641. E-mail: carmay@gate.sinica.edu.tw.

Notes

The authors declare no competing financial interest.

Biographies

Todor Dudev obtained his Ph.D. degree from the University of Sofia, Bulgaria in 1989, and specialized at the Tokyo Institute of Technology, Japan and the Instituto de Estructura de la Materia, CSIC, Spain. In 1997, he joined the Institute of Biomedical Sciences, Academia Sinica as a Senior Research Associate. In 2013, he returned to Bulgaria as a Professor at the Faculty of Chemistry and Pharmacy, University of Sofia. His current research interests are in applying computational methods to study the role of metals in biology, medicine, and ecology.

Carmay Lim obtained her Ph.D. from the University of Minnesota, Minneapolis in 1984. After postdoctoral studies at AT&T Bell Laboratories, Murray Hill and Harvard University, she joined the faculties of the Departments of Medical Genetics, Biochemistry, and Chemistry at the University of Toronto. In 1995, she moved to the Institute of Biomedical Sciences, Academia Sinica, where she is currently Distinguished Research Fellow. Her research interests include (1) unraveling the principles governing biological processes and applying them to guide drug design and identify new drug targets and (2) developing computational tools for studying macromolecular interactions.

■ ACKNOWLEDGMENTS

We thank Chih-Cheng Chen for helpful comments. This work is supported by Academia Sinica and MOST, Taiwan (Grant NSC-98- 2113-M-001-011). T.D. is supported by the Institute of Biomedical Sciences at Academia Sinica and EU Grant "Beyond Everest", FP7-REGPOT-2011-1.

■ ABBREVIATIONS

SF, selectivity filter; Na_v, voltage-gated sodium; Ca_v, voltage-gated calcium; CN, coordination number; ASIC, acid-sensing ion channel

■ REFERENCES

(1) Hille, B. *Ionic channels of excitable membranes*, Third ed.; Sinauer Associates: Sunderland, MA, 2001.
(2) Hall, J. E. *Guyton and Hall Textbook of Medical Physiology with Student Consult Online Access*, 12th ed.; Elsevier Saunders: Philadelphia, 2011.
(3) Zhorov, B. S.; Tikhonov, D. B. Ligand action on sodium, potassium, and calcium channels: Role of permeant ions. *Trends Pharmacol. Sci.* **2013**, *34*, 154−161.
(4) Kass, R. S. The channelopathies: Novel insights into molecular and genetic mechanisms of human disease. *J. Clin. Invest.* **2005**, *115*, 1986−1989.
(5) England, S.; de Groot, M. J. Subtype-selective targeting of voltage-gated sodium channels. *Br. J. Pharmacol.* **2009**, *158*, 1413−1425.
(6) Palmer, L. G. Ion selectivity of the apical membrane Na channel in the toad urinary bladder. *J. Membr. Biol.* **1982**, *67*, 91−98.
(7) Kashlan, O. B.; Kleyman, T. R. ENaC structure and function in the wake of a resolved structure of a family member. *Am. J. Physiol.: Renal Physiol.* **2011**, *301*, F684−F696.
(8) Waldmann, R.; Champigny, G.; Bassilana, F.; Heurteaux, C.; Lazdunski, M. A proton-gated cation channel involved in acid-sensing. *Nature* **1997**, *386*, 173−177.
(9) Favre, I.; Moczydlowski, E.; Schild, L. On the structural basis for ionic selectivity among Na, K and Ca in the voltage-gated sodium channel. *Biophys. J.* **1996**, *71*, 3110−3125.
(10) Sun, Y. M.; Favre, I.; Schild, L.; Moczydlowski, E. On the structural basis for size-selective permeation of organic cations through the voltage-gated sodium channel. *J. Gen. Physiol.* **1997**, *118*, 693−715.
(11) Yue, L.; Navarro, B.; Ren, D.; Ramos, A.; Clapham, D. E. The cation selectivity filter of the bacterial sodium channel, NaChBac. *J. Gen. Physiol.* **2002**, *120*, 845−853.
(12) Senatore, A.; Monteil, A.; van Minnen, J.; Smit, A. B.; Spafford, J. D. NALCN ion channels have alternative selectivity filters resembling calcium channels or sodium channels. *PLoS One* **2013**, *8*, e55088.
(13) Snyder, P. M.; Olson, D. R.; Bucher, D. B. A pore segment in DEG/ENaC Na^+ channels. *J. Biol. Chem.* **1999**, *274*, 28484−28490.
(14) Kellenberger, S.; Gautshi, I.; Schild, L. A single point mutation in the pore region of the epithelial Na^+ channel changes ion selectivity by modifying molecular sieving. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 4170−4175.
(15) Sheng, S.; Perry, C. J.; Kashlan, O. B.; Kleyman, T. R. Side chain orientation of residues lining the selectivity filter of epithelial Na^+ channels. *J. Biol. Chem.* **2005**, *280*, 8513−8522.
(16) Baconguis, I.; Bohlen, C. J.; Goehring, A.; Julius, D.; Gouaux, E. X-Ray structure of acid-sensing ion channel 1−snake toxin complex reveals open state of a Na^+-selective channel. *Cell* **2014**, *156*, 717−729.
(17) Backx, P. H.; Yue, D. T.; Lawrence, J. H.; Marban, E.; Tomaselli, G. F. Molecular localization of an ion-binding site within the pore of mammalian sodium channels. *Science* **1992**, *257*, 248−251.
(18) Gur Barzilai, M.; Reitzel, A. M.; Kraus, J. E. M.; Gordon, D.; Technau, U.; Gurevitz, M.; Moran, Y. Convergent evolution of sodium selectivity in metazoan neuronal signaling. *Cell Rep.* **2012**, *2*, 1−7.
(19) Schlief, T.; Schonherr, R.; Imoto, K.; Heinemann, S. H. Pore properties of rat brain II sodium channels mutated in the selectivity filter domain. *Eur. Biophys. J.* **1996**, *25*, 75−91.
(20) Payandeh, J.; Scheuer, T.; Zheng, N.; Catterall, W. A. The crystal structure of a voltage-gated sodium channel. *Nature* **2011**, *475*, 353−359.
(21) Gouaux, E.; MacKinnon, R. Principles of selective ion transport in channels and pumps. *Science* **2005**, *310*, 1461−1465.
(22) Long, S. B.; Tao, X.; Campbell, E. B.; MacKinnon, R. Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment. *Nature* **2007**, *450*, 376−382.
(23) Cibulski, S. M.; Sather, W. A. The EEEE locus is the sole high-affinity Ca^{2+} binding structure in the pore of a voltage-gated Ca^{2+} channel: Block by Ca^{2+} entering from the intracellular pore entrance. *J. Gen. Physiol.* **2000**, *116*, 349−362.
(24) Perez-Reyes, E. Molecular physiology of low-voltage-activated T-type calcium channel. *Physiol. Rev.* **2003**, *83*, 117−161.
(25) Boda, D.; Nonner, W.; Valisko, M.; Henderson, D.; Eisenberg, B.; Gillespie, D. Steric selectivity in Na channels arising from protein polarization and mobile side chains. *Biophys. J.* **2007**, *93*, 1960−1980.
(26) Carnevale, V.; Treptow, W.; Klein, M. L. Sodium ion binding sites and hydration in the lumen of a bacterial ion channel from molecular dynamics simulations. *J. Phys. Chem. Lett.* **2011**, *2*, 2504−2508.
(27) Corry, B.; Thomas, M. Mechanism of ion permeation and selectivity in a voltage gated sodium channel. *J. Am. Chem. Soc.* **2012**, *134*, 1840−1846.
(28) Csanyi, E.; Boda, D.; Gillespie, D.; Kristof, T. Current and selectivity in a model sodium channel under physiological conditions: Dynamic Monte Carlo simulations. *Biochim. Biophys. Acta* **2012**, *1818*, 592−600.
(29) Furini, S.; Domene, C. On conduction in a bacterial sodium channel. *PLoS Comput. Biol.* **2012**, *8*, e1002476.
(30) Kaufman, I.; Luchinsky, D. G.; Tindjong, R.; McClintock, P. V. E.; Eisenberg, R. S. Energetics of discrete selectivity bands and mutation-induced transitions in the calcium-sodium ion channels family. *Phys. Rev. E* **2013**, *88*, 052712.
(31) Finol-Urdaneta, R. K.; Wang, Y.; Al-Sabi, A.; Zhao, C.; Noskov, S. Y.; French, R. J. Sodium channel selectivity and conduction: Prokaryotes have devised their own molecular strategy. *J. Gen. Physiol.* **2014**, *157*−*171*, 804−818.
(32) Boiteux, C. l.; Vorobyov, I.; Allen, T. W. Ion conduction and conformational flexibility of a bacterial voltage-gated sodium channel. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 3454−3459.
(33) Bagnéris, C.; DeCaen, P. G.; Naylor, C. E.; Pryde, D. C.; Nobeli, I.; Clapham, D. E.; Wallace, B. A. Prokaryotic NavMs channel as a structural and functional model for eukaryotic sodium channel antagonism. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 8428−8433.
(34) Dudev, T.; Lim, C. Metal selectivity in metalloproteins: Zn^{2+} vs Mg^{2+}. *J. Phys. Chem. B* **2001**, *105*, 4446−4452.
(35) Babu, C. S.; Dudev, T.; Casareno, R.; Cowan, J. A.; Lim, C. A combined experimental and theoretical study of divalent metal ion selectivity and function in proteins: application to *E. coli* ribonuclease H1. *J. Am. Chem. Soc.* **2003**, *125*, 9318−9328.
(36) Dudev, T.; Lim, C. Bidentate vs monodentate carboxylate coordination modes in magnesium and calcium proteins: what are the basic principles? *J. Phys. Chem. B* **2004**, *108*, 4546−4557.
(37) Dudev, T.; Chang, L.-Y.; Lim, C. Factors governing the substitution of La^{3+} for Ca^{2+} and Mg^{2+} in metalloproteins: A DFT/CDM study. *J. Am. Chem. Soc.* **2005**, *127*, 4091−4103.
(38) Dudev, T.; Lim, C. Determinants of K^+ vs Na^+ selectivity in potassium channels. *J. Am. Chem. Soc.* **2009**, *131*, 8092−8101.
(39) Dudev, T.; Lim, C. Factors governing the Na^+ vs K^+ selectivity in sodium ion channels. *J. Am. Chem. Soc.* **2010**, *132*, 2321−2332.
(40) Dudev, T.; Lim, C. Competition between Li^+ and Mg^{2+} in metalloproteins. Implications for lithium therapy. *J. Am. Chem. Soc.* **2011**, *133*, 9506−9515.
(41) Dudev, T.; Lim, C. Importance of metal hydration on the selectivity of Mg^{2+} vs Ca^{2+} in magnesium ion channels. *J. Am. Chem. Soc.* **2013**, *135*, 17200−17208.
(42) Dudev, T.; Lim, C. Evolution of eukaryotic ion channels: Principles underlying the conversion of Ca^{2+}-selective to Na^+-selective channels. *J. Am. Chem. Soc.* **2014**, *136*, 3553−3559.
(43) Boda, D.; Valisko, M.; Eisenberg, B.; Nonner, W.; Henderson, D.; Gillespie, D. Combined effect of pore radius and protein dielectric coefficient on the selectivity of a calcium channel. *Phys. Rev. Lett.* **2007**, *98*, 168102.

(44) Lipkind, G. M.; Fozzard, H. A. Voltage-gated Na channel selectivity: The role of the conserved domain III lysine residue. *J. Gen. Physiol.* **2008**, *131*, 523−529.
(45) Dudev, T.; Lim, C. Competition among Ca^{2+}, Mg^{2+}, and Na^{+} for ion channel selectivity filters: Determinants of metal ion selectivity. *J. Phys. Chem. B* **2012**, *116*, 10703−10714.
(46) Dudev, T.; Lim, C. Why voltage-gated Ca^{2+} and bacterial Na^{+} channels with the same EEEE motif in their selectivity filters confer opposite metal selectivity. *Phys. Chem. Chem. Phys.* **2012**, *14*, 12451−12456.
(47) Dudev, T.; Lim, C. Competition among metal ions for protein binding sites: Determinants of metal ion selectivity in proteins. *Chem. Rev.* **2014**, *114*, 538−556.
(48) Kellenberger, S.; Auberson, M.; Gautschi, I.; Schneeberger, E.; Schild, L. Permeability properties of ENaC selectivity filter mutants. *J. Gen. Physiol.* **2001**, *118*, 679−692.
(49) Jasti, J.; Furukawa, H.; Gonzales, E. B.; Gouaux, E. Structure of acid-sensing ion channel 1 at 1.9 Å resolution and low pH. *Nature* **2007**, *449*, 316−324.
(50) Nightingale, E. R., Jr. Phenomenological theory of ion solvation. effective radii of hydrated ions. *J. Phys. Chem.* **1959**, *63*, 1381−1387.
(51) Dudev, T.; Lim, C. Ion selectivity in the selectivity filters of acid-sensing ion channels. *Sci. Rep.* **2014**, submitted.
(52) Heinemann, S. H.; Terlau, H.; Stuhmer, W.; Imoto, K.; Numa, S. Calcium channel characteristics conferred on the sodium channel by single mutations. *Nature* **1992**, *356*, 441−443.
(53) Tang, S.; Mikala, G.; Bahinski, A.; Yatani, A.; Varadi, G.; Schwartz, A. Molecular localization of ion selectivity sites within the pore of a human L-type cardiac calcium channel. *J. Biol. Chem.* **1993**, *268*, 13026−13029.
(54) Eisenberg, B. Proteins, channels and crowded ions. *Biophys. Chem.* **2003**, *100*, 507−517.
(55) Ramakrishnan, V.; Henderson, D.; Busath, D. D. Applied field nonequilibrium dynamics simulations of ion exit from a beta-barrel model of the L-type calcium channel. *Biochim. Biophys. Acta* **2004**, *1664*, 1−8.
(56) Corry, B.; Chung, S.-H. Mechanisms of valence selectivity in biological ion channels. *Cell. Mol. Life Sci.* **2006**, *63*, 301−315.
(57) Malasics, A.; Gillespie, D.; Nonner, W.; Henderson, D.; Eisenberg, B.; Boda, D. Protein structure and ionic selectivity in calcium channels: Selectivity filter size, not shape, matters. *Biochim. Biophys. Acta* **2009**, *1788*, 2471−2480.
(58) Qui, H.; Shen, R.; Guo, W. Ion solvation and structural stability in a sodium channel investigated by molecular dynamics calculations. *Biochim. Biophys. Acta* **2012**, *1818*, 2529−2535.
(59) Sakharov, D.; Lim, C. Force fields including charge transfer and local polarization effects: Application to proteins containing multi/heavy metal ions. *J. Comput. Chem.* **2009**, *30*, 191−202.