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Review article

# Structural and functional insights into the cardiac $Na^+/H^+$ exchanger

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ABSTRACT

The NHE1 isoform of the $Na^+/H^+$ exchanger is present in the plasma membrane of the mammalian myocardium where it functions to regulate intracellular pH by exchanging one external $Na^+$ for an internal proton. The protein is involved in myocardial ischemia/reperfusion damage and in heart hypertrophy. Topology models and experimental evidence suggest that of the 815 amino acids of the protein, approximately 500 are embedded or closely associated with the lipid bilayer while the balance form a cytosolic, regulatory carboxyl-terminal tail. The precise structure of NHE1 is not known although that of an *Escherichia coli* homolog, NhaA, has been determined. The structures of fragments of the NHE1 membrane domain have been examined by nuclear magnetic resonance. Several transmembrane segments have a general structure of an extended central region flanked by helical segments. The extended regions often contain amino acids that are important in protein function and possibly in cation coordination and transport. The *E. coli* $Na^+/H^+$ exchanger NhaA has a novel fold consisting in part of two helical transmembrane segments with interrupted regions crossing amid the lipid bilayer. The similarity between the crystal structure of NhaA and partial structures of NHE1 suggests that there may be similarities in the mechanism of $Na^+/H^+$ exchange. This article is part of a Special Issue entitled "$Na^+$ Regulation in Cardiac Myocytes".



**Contents**



*Abbreviations:* DPC, dodecylphosphocholine; NHE1, $Na^+/H^+$ exchanger type 1 isoform; NMR, nuclear magnetic resonance; TM, transmembrane.

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## 1. Introduction

Mammalian $Na^+/H^+$ exchange was first demonstrated in rat intestinal and renal microvilli by Murer et al. in 1976 [1], and the first isoform, NHE1, was cloned and sequenced by Sardet et al. in 1989 [2]. In humans, nine isoforms, numbered NHE1 through NHE9, have since been identified, along with three more distantly related isoforms, NHA1, NHA2 and a sperm-specific NHE [3]. NHE 1–5 are located primarily on the plasma membrane. NHE1 is ubiquitous, and is sometimes considered the "housekeeping" isoform. NHE 2–4 are found primarily in the kidney and gastrointestinal tract. NHE5 is found in the brain. NHE 6–9 are found in intracellular membranes and regulate organellar pH.

NHE1 is the primary isoform found in the plasma membrane of cardiomyocytes [4]. Human NHE1 is 815 amino acids in size, with a 500 amino acid N-terminal membrane domain responsible for ion transport and pH sensing, and a 315 amino acid C-terminal regulatory domain. The membrane domain functions by removing intracellular protons in exchange for extracellular sodium in a 1:1 ratio to regulate the intracellular pH. NHE1 shows little activity under physiological resting conditions. However, it is activated allosterically by a decrease in intracellular pH, and its activation is also modulated by the regulatory domain through its phosphorylation and interactions with various regulatory proteins [5]. NHE1 has also been shown to be involved in cell volume regulation [6], cell motility [7], and is involved in cell growth and differentiation [8].

NHE1 has several pathological roles in the heart [9]. The build up of intracellular acid under hypoxic conditions leads to activation of NHE1 and an influx of sodium. This influx, coupled with reverse mode activity of the $Na^+/Ca^{2+}$ exchanger, leads to a detrimental influx of calcium which affects various cell signaling pathways, and promotes cell death. The activation of NHE1 has also been shown to be involved in cardiac hypertrophy [9]. NHE1 has been shown to be involved in cancer metastasis and invasiveness, by promoting the abnormal pH microenvironments and cell migration characteristic of cancers [10]. The increased activity of NHE1 in these various diseases suggests that NHE1 could present a target for drugs in the treatment of heart disease and cancer. However, clinical trials in humans, have proven inconclusive or disappointing [11]. A better understanding of the detailed structure and function of NHE1 could help in the design of better drugs and treatment strategies targeting NHE1.

The structure of NHE1 is currently not known; however, progress has been made in elucidating the structure of related proteins and NHE1 fragments. This review will describe developments in the topological and three dimensional models of NHE1, including initial topological predictions, biochemical evidence, and a 3D homology model based on a bacterial homologue. The second part of the review covers current direct structural and functional studies on mammalian NHE1.

## 2. NHE1 topology

### 2.1. Early topological assignments

The sequence of human NHE1 was first determined by Sardet et al. [2], who predicted that the membrane domain consisted of 500 amino acids with 10 TM helices using hydropathy analysis [12]. They predicted two potential N-linked glycosylation sites; it was later shown that only N75 between the first two TM helices acts as a N-linked glycosylation site [13]. The hydrophilic C-terminal tail of about 300 amino acids contained a net positive charge and putative phosphorylation sites, and thus was placed in an intracellular orientation. Phosphorylation of the C-terminal tail [14], and detection of a C-terminal domain isotope only in permeabilized cells [15] later confirmed the intracellular placement. Orlowski et al. [16] cloned rat NHE 1–4 and using hydropathy analysis [17] and sequence alignment also suggested 10 TM helices in NHE1. However, the first two helices proposed by Sardet et al. [2] were discarded and two additional helices were added, which while not as hydrophobic as the surrounding helices, contained highly conserved polar or charged residues that were likely important for function. Differences in predictions seem to result from differences in the hydrophobicity scales used and from window sizes used to predict the TM regions.

### 2.2. Experimental topology assignment

Detailed analysis of the topology of NHE1 was performed by Wakabayashi et al. [18], using cysteine scanning accessibility method [19]. The method involves introducing an individual cysteine mutation into a cysteineless mutant of the protein and testing the accessibility of that cysteine to the sulfhydryl reactive reagents. The use of whole vs. permeablized cells allowed them to test for extracellular and intracellularly accessible cysteines in NHE1. They also confirmed the intracellular localization of the N-terminus. They proposed a 12 TM model based on the accessibility of the residues tested (Fig. 1) with the N- and C-terminus in the cytoplasm and the glycosylation site at N75 between TM I and II. The tenth TM in the hydrophobicity model contained residues on either end accessible extracellularly and was proposed to be a re-entrant loop rather than a TM segment, entering and exiting on the same side of the membrane. Two intracellular loops, between TMs IV–V and VIII–XI, were also suggested to be re-entrant loops, as they had sequential residues that were accessible from opposite sides of the membrane, which might be explained by a pore lining location. Experiments by Sato et al. [20,21] using a cell-free protein expression and membrane insertion system support the cytosolic localization of the N-terminus and the unusual topology of the re-entrant segment. They also showed that a plant NHE, Nhx1, has a similar topology. Finally, the extracellular loop between the last two TM helices was found to be inaccessible and reassigned as TM XI.

### 2.3. Modeling and evolutionary relationships

Brett et al. [3] analyzed the evolutionary relationship between a large sample of proteins in the cation/proton antiporter superfamily, which includes NHE1. The family of proteins appear to have a common fold in the transmembrane domain corresponding to TM III–XII of the topology model of Wakabayashi [18] as suggested by the similar hydropathy profiles of the exchangers. TM I–II appeared to be poorly conserved, and in the mammalian NHEs, TM I often appeared to be missing in sequence alignments. The C-terminal regulatory region varied in size and has low sequence similarity, which could reflect the differences in regulation, localization, and function of the different isoforms.

## 3. NhaA and three dimensional modeling

### 3.1. NhaA structure

NhaA is a distant bacterial homologue of NHE1 which functions in salt tolerance. Unlike NHE1, it removes sodium using the proton gradient

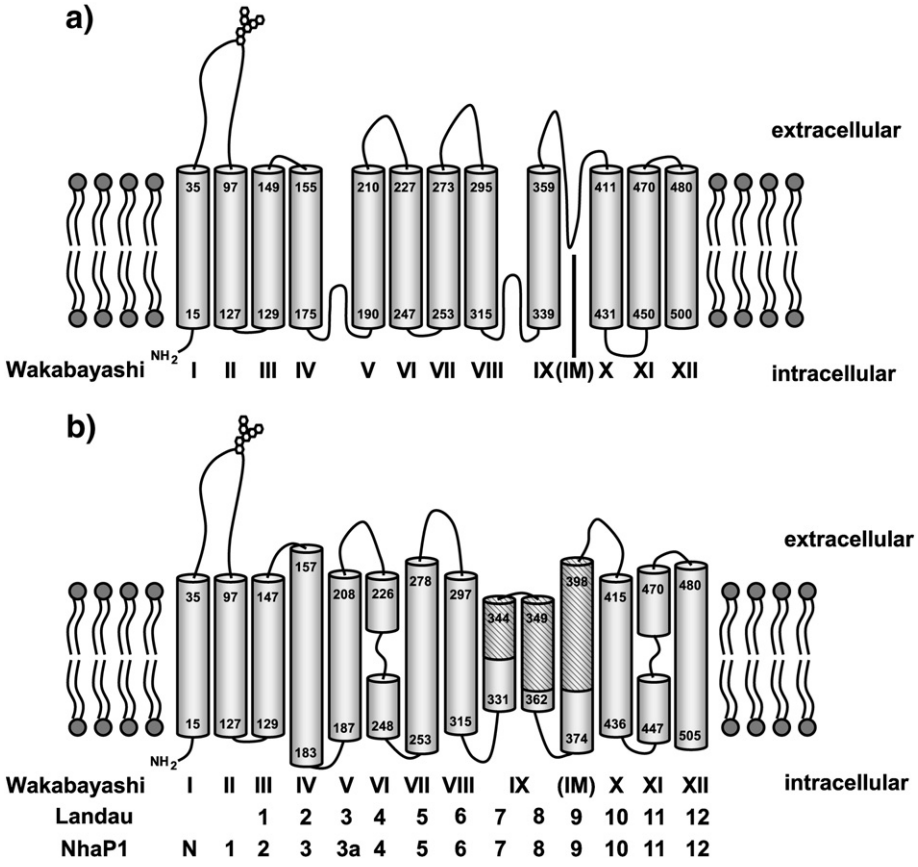


**Fig. 1.** Topology models of NHE1. (a) The topology model of NHE1 as predicted by Wakabayashi et al. [18]. The residue numbers corresponding to the ends of the TM helices are shown and TM helices are numbered using roman numerals. The large re-entrant loop between TM IX and X is labeled (IM). (b) Topology of NHE1 as predicted by Landau et al. [33]. The ends of the TM helices are labeled. Comparative number of the TM segments is indicated underneath. Wakabayashi (roman numerals), Landau (Arabic numerals). A third numbering system is indicated and is based on the observations recently reported with *Methanococcus janasschii* NhaP1 [82] with two additional helices (N, 3a) suggested to be present. The original TM IX and re-entrant loop IM reassigned in the Landau model are hatched.

in a 1:2 ratio, and is active at basic pH. The membrane domain is smaller, with 388 amino acids, and NhaA does not contain a C-terminal regulatory domain. NhaA is also insensitive to most eukaryotic NHE inhibitors [22]. The ability to overexpress and purify active NhaA in milligram quantities was crucial to the eventual structure determination and in functional studies of the protein [23].

A structure of NhaA was solved at a high resolution by X-ray crystallography [24], which gave the first detailed insights into the mechanism of transport of a NHE protein. NhaA consists of 12 TM helices and had a novel fold. There were several interesting parts of the structure. The structure showed a long loop between TM 1 and 2 and contained a surface associated helix and a 2 strand beta sheet that was important for dimerization [25]. When the beta-sheet was removed, monomers of NhaA were functional though with altered kinetics, suggesting that monomers were the principal functional unit. NhaA also contains a group of 6 TMs which contains two, three TM bundles, TM 3–5 and TM 10–12, which are related by a pseudo two fold symmetry. Two helices, TM 4 and TM 11 contained extended regions in the center of each helix and which crossed each other (Fig. 2a). These are a critical part of the novel fold of NhaA. Many transporter membrane proteins appear to contain similar internal structural repeats and extended regions in their structures, and recent studies have suggested this type of extended region is important for substrate binding and transport [26]. This novel fold includes a negatively charged D133 on TM 4 and positively charged K300 on nearby TM 10 which could compensate for the introduction of helix dipole partial charges of TM 4 and 11 in the center of the membrane. Two highly conserved aspartate residues, D163 and D164 on TM 5 are believed to be important in cation binding.

The structure of NhaA was solved at pH 4, where it is in an inactive, "acid-locked" state. Studies using electron microscopy [27], computational simulations [28,29], and various biochemical analyses suggest a mechanism for the activation and transport mechanism of NhaA. Charged residues around the cytoplasmic end of TM 9 (Fig. 2a) and the N-terminus act as a pH sensor, which undergoes a conformational change [27,30] at basic pH which in turn triggers a change in conformation of TMs 4,11, and 10 to fully expose the ion binding site and ready the protein for transport [27,31]. Ion transport can then proceed by an "alternating access" mechanism. Sodium binds in the pore near D163 and D164 [29,32]. Binding causes a disruption of the delicate electrostatic balance of the charged residues and helix dipoles, which triggers conformational changes in the transporter that release the cations on the opposite site of the membrane and allow binding and transport of protons back across the membrane.

### 3.2. Modeling NHE1 after NhaA (Fig. 1b)

A 3D model of NHE1 was proposed using the bacterial crystal structure of NhaA as a template [33] (Fig. 1b). This topology model also contains 12 TM helices; however, it does not include the first two helices of the model of Wakabayashi [18]. Landau et al. [33] argued that these helices do not appear to be important for the function of the protein and are poorly conserved. Enzymatic cleavage of NHE1 between TM II and III did not affect NHE1 function [34]. However, both fully and partially glycosylated NHE1 are often found in western blots of NHE1, and Wakabayashi et al. [18] locate the N-terminus in the cytoplasm [18], which suggests that these two helices and the

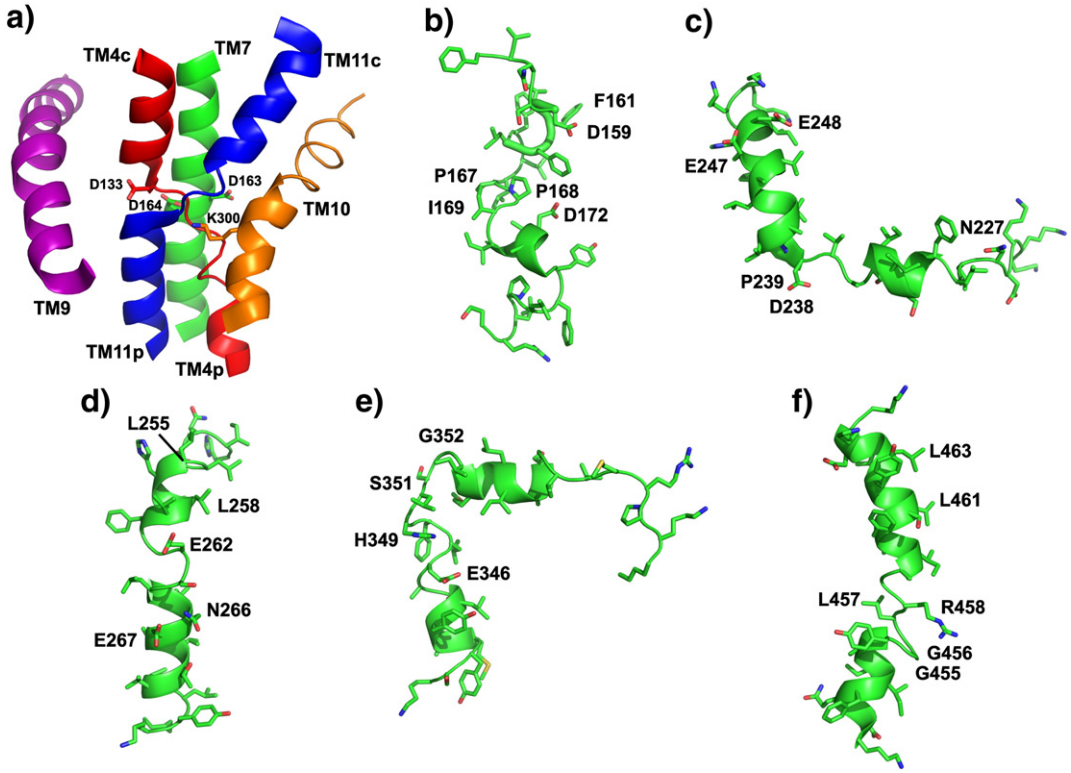


**Fig. 2.** Structures of segments of the NHE protein. a, TM's 4, 7, 11, 9 and 10 of NhaA taken from the crystal structure of *E. coli* NhaA [24]. b–f, representative NMR structures of; (b) TM IV, (c) TM VI, (d) TM VII, (e) TM IX, (f) TM XI in organic solvent (b) or DPC micelles (c–f). NMR structures are calculated as ensembles of structures consistent with the data. In all of the structures heterogeneity was observed resulting from flexibility: at the N- and C-termini and often within the TM helix around an extended or kinked section. The representative structures presented are the ones with the lowest energy in structure calculations, and the first structure of the ensemble from the deposited PDB files. The backbone is shown in a cartoon representation, with side chains shown as sticks. Functionally important and pore lining residues are labeled and discussed in the text.

intervening loop are present. This suggests that there could be 14 TMs in NHE1. The assignment of the next 6 TMs (TM III–VIII, now numbered TM 1–6) is the same. TM IX is split into two short helices (TM 7–8) and the re-entrant segment between TM IX–X is reassigned as TM9 (Fig. 1b). This rearrangement of the re-entrant loop places EL5 with numerous extracellularly accessible residues [18] on the inside of the membrane however they suggest that this loop could be near the pore of the protein, similar to the re-entrant loops suggested in the model of Wakabayashi [18]. The remaining three TMs (TM 10–12) are the same in both models. They also predict that TM 4 (TM VI) and 11 (TM XI) contain unwound central regions, like the TM 4/11 assembly observed in NhaA. The model also contains charged residues that are evolutionarily conserved among NHE family members in the core of the protein and are proposed to play a role similar to the corresponding residues in NhaA. D238 is thought to be similar to D133 in NhaA, compensating for the helix dipole charges. D163/D164 in NhaA is replaced with N266/D267 in eukaryotic exchangers, which may play a role in ion binding. Evidence which supports this model includes the presence of the evolutionarily conserved residues found in the core of the protein, and clustering in regions on the extracellular face of amino acid residues important in pH regulation and in inhibition of NHE1 activity.

## 4. Structural studies of NHE1

### 4.1. Dividing and conquering NHE1

There is currently only a limited amount of structural information on NHE1. Membrane proteins are difficult to overexpress as well as being difficult to crystallize for X-ray structure determination. This is evident in the low number of membrane protein structures relative to globular proteins in the PDB database [35]. Currently, direct evidence is limited to structures of individual TM helices of NHE1 determined using NMR and to a low resolution dimer structure determined by single particle electron microscopy. There is also some structure data on fragments of the C-terminal tail and associated regulatory proteins. Because of the difficulties in 3D structure determination of full length membrane proteins, our laboratory has adopted a "divide and conquer" [36,37] approach to studying the structure of NHE1. The divide and conquer approach is based on the two-stage folding model proposed by Popot and Engelman [38], which states that transmembrane helices can fold independently on interaction with the membrane, which then interact with each other to form the final MP structure. Isolated TM helices, therefore, would adopt conformations similar to their conformations in the full protein. This approach has been successfully shown earlier in NMR studies of the fragments of bacteriorhodopsin [39]. The short peptides needed for these studies are generally easier to produce either synthetically or in *Escherichia coli*, and can be studied by various biophysical methods including NMR. NMR can provide atomic resolution information about the secondary and tertiary structure of proteins, and is particularly suited to small proteins or peptides. We have found that the individual TM helices of NHE1 (described in the subsequent subsections) generally do not adopt simple alpha-helical structures, but rather have kinks or extended regions which correlate with biochemical evidence that these structures are important in the function of the protein.

### 4.2. TM IV[2] (Fig. 2b)

The NMR structure of a peptide representing TM IV (residues 155–177) of NHE1 was determined in an organic solvent mixture of 4:4:1 chloroform/methanol/water [40] (Fig. 2b).[3] Counter to

[2] The transmembrane helix and extracellular loop numbering scheme used in Sections 4.2–4.6 is from the Wakabayashi model.

[3] This segment was done in an organic solvent since a stable sample and/or high resolution NMR spectrum was not obtained in DPC micelles or other membrane mimetics.

expectations, TM IV did not adopt a canonical transmembrane alpha-helical structure. TM IV has an irregular structure, containing three structurally distinct regions. The N-terminal region (residues 159–163) contains overlapping beta turns. The central region (residues 165–168) contains two prolines and is extended. The C-terminal region (residues 169–176) contains a short helical segment at residues 170–174. While the individual regions can be superimposed in the NMR structural ensemble, the three regions rotate freely with respect to each other, so the entire structure cannot be superimposed. This could represent flexibility in the TM segment that could be important for function or could be a result of a lack of interactions between the isolated TM helix and the balance of the protein.

TM IV has been found to be important in modulating inhibitor efficacy towards NHE1. Mutations to F162, L163, F167, F165, and G174 [41–44] were all found to confer inhibitor resistance upon NHE1. Slepkov et al. [40,45,46] tested many mutants of TM IV for transport activity and found it very sensitive to mutation. Of 23 cysteine mutants of TM IV, 11 resulted in an exchanger with less than 20% of wild-type activity. P167/P168 was particularly important for the folding and activity of NHE1. Labeling of a F161C mutant NHE1 protein with either extracellularly applied, positively charged MTSET or negatively charged MTSES, resulted in inhibition of transport activity. This suggested that this residue is near the ion translocation pore, which would also be consistent with changes in inhibitor sensitivity with mutations in this region.

The residues important for inhibitor sensitivity are primarily on the N-terminal beta-turn region and the extended segment in the NMR structure, which corresponds to the extracellular half of TM IV (residues 159–168). The Landau et al. [33] topology model of NHE1 suggests TM IV is equivalent to TM 2 of NhaA (Fig. 2b). TM 2 of NhaA is kinked at its center rather than extended as TM IV is in the NMR structure. P167 and P168 in NHE1 TM IV maintain the extended structure of TM IV, and interactions with other TMs might be required for it to more resemble TM 2 of NhaA. TM 2 in NhaA also lines both the cytoplasmic and periplasmic channels which would be in agreement with the accessibility of F161 in NHE1 TM IV. Aspartates 159 and 172 in TM IV are on the structured helical or turn regions in the NMR structure, not near the center of the TM, making them unlikely to be directly involved in ion translocation. Studies on NhaA suggest that aspartates of TM 2 could play a role in pH induced activation of the exchanger [47], which could suggest a similar role for the TM IV aspartates of NHE1.

### 4.3. TM VI (Fig. 2c)

The structure of TMVI (residues 226–250) in DPC detergent micelles consists of a short, extended segment flanked by two helical segments at residues 229–236 and 239–252 [48] (Fig. 2c). Residues D238-P239 cap the end of the C-terminal helix where they may play a role in maintaining an unwound structure in the protein. The isolated structure has the two helices oriented at right angles to each other, however this is unlikely in the full length protein, where TM VI would interact with other TMs. Tzeng et al. [48] found that mutation of D328C, P239C and E247C drastically reduced the activity of the protein. Murtazina et al. [49] found that D238N and P239A did not affect activity of NHE1. An Asn residue could possibly cap the helix and provide charge compensation similar to Asp, while both Pro and Ala can adopt helical conformation. Mutation of N227 and E247-248 also affected activity, with different effects depending on the charge and size of the mutation [50]. Pore accessibility studies find that residues on both sides of the TM are MTSES accessible and inhibit the activity of the protein. Since both sides of the TM are accessible to MTSET, TM VI is likely a critical region of the protein, and is pore lining along its length. The importance of this region is reflected in the central positioning of this TM in the Landau topology model [33], where it is equivalent to TM 4 of NhaA. TM 4 of NhaA contains a discontinuous helix with a conserved D133 equivalent to D238 in NHE1, that may be important in balancing the partial dipole charges from the half-helices of TM 4 and 11 [24] and in promoting conformational changes during transport [29].

### 4.4. TM VII (Fig. 2d)

The structure of TM VII (residues 250–275) was solved in DPC micelles. It is primarily helical, with a kink at residues G261/E262/S263 in the center of the helix [51]. The peptide appears to show two conformations in solution: one where the TM helix is relatively straight, and one where the two halves are folded together around the kink. Further NMR based dynamic studies on this system suggest slow interconversion between these two states [52] that may be important for conformational changes necessary in ion transport. Mutations in the kink to residues with more helical propensity such as alanine or isoleucine inhibited activity, possibility by limiting this flexibility.

Mutagenesis of amino acid residues in TM VII often affected the activity of NHE1, with mutation of several residues influencing inhibitor efficacy [49,51,53]. TM VII was less sensitive to mutation than TM IV, with only a few key residues showing significant effects on NHE1 activity when mutated. N266, and D267 were critical as with their mutation to cysteine or alanine, NHE1 was nearly inactive. In contrast, retention of the charge with a D267E mutation maintains activity. In addition, inserting an additional amino acid into TM VII inhibited activity of NHE1, showing that the positioning of D267 is also important. These results together suggested a putative role in ion binding. When E262 was mutated and NHE1 was expressed in mammalian cells, there was generally increased intracellular protein and a moderate decrease in activity, suggesting that E262 may be important in proper protein folding or in attracting cations. Cysteine accessibility studies also suggest that L255 and L258 are pore lining [53].

Landau et al. [33] suggest that this TM is equivalent to TM 5 in NhaA (Fig. 1b). NhaA has a DD motif rather than a ND motif that is found in NHE1 and related exchangers. These aspartates in NhaA are implicated in direct ion binding [32]. NhaA does not have an additional residue equivalent to the more distal E262 in NHE1, however its importance in NHE1 could suggest some role in ion binding or activation or alternatively, could reflect differences in the functions of NHE1 and NhaA such as their differing stoichiometry. Interestingly, the two pore lining leucines identified in NHE1 (L255 and L258) are on the intracellular side of the membrane in both the models of NHE1 topology, yet were found to be accessible to extracellularly applied reagents. This could be similar to the intramembrane loops that are thought to be positioned inside the cell, but are accessible from the outside of the cell [18]. Their accessibility is also consistent with a role similar to NhaA TM 5 which is critical for the activity of the exchanger. The critical nature of the charged residues and pore lining nature of L255/258 suggest they all lie along the ion translocation pathway. This is supported in the location of TM 5 in the Landau [33] model, and these residues lie on one face of the TM in some structures in the NMR ensemble.

### 4.5. TM IX (Fig. 2e)

In DPC micelles the structure of TM IX (residues 338–365) shows two helical regions bent at a right angle to each other around a kink at residue S351 [54]. Mutations to E346, G352, H349, have been found to decrease drug sensitivity [55–57]. Mutations to H349 showed little effect on activity. E346 and G352 mutations, however, decreased activity. Reddy et al. [54] found that E346 and S351 are pore lining. Mutations to either of these residues also affected extracellular sodium and intracellular proton affinities. Most of the important residues lie in the center of the TM helix, near the kink in the NMR structure where they could play some role in ion transport and inhibitor binding. In the model of Landau [33], the sequence of TM IX forms two TM helices, TM 7–8, with the central kinked region forming an extracellular loop, and with TM 8 lining the extracellular pore. This could also be consistent with the accessibility and inhibitor

binding studies, and activity could be affected by changes to the pH sensitivity of the protein. In NhaA, TM 8 lines the pore and is involved in pH regulation [58]. It is not clear from current data whether TM IX represents a single helix as predicted by Wakabayashi [18], or if it is two TMs as predicted in the Landau topology model [33].

### 4.6. TM XI (Fig. 2f)

The structure of TM XI (residues 447–472) was determined in DPC micelles. It contains two helical segments with an extended, conformationally flexible segment (residues 455–459) in between [59]. A positively charged arginine is in the center of the extended region. Wakabayashi et al. [18] found that mutation of two glycines G455 and G456 to larger residues shifted the pH dependence of the exchanger to more basic levels [60]. In the NMR structure, these two glycines are located at the end of the N-terminal helix and the start of the extended region where the helix starts to unwind. This region could be important for helix–helix interactions and or conformational changes, which would be affected by introducing larger residues. Glycines in the extended region in NhaA (G336, G338) also appear to be important for the activity or pH sensitivity of the exchanger [29,61,62]. Lee et al. [59] found that many residues appear to be sensitive to mutation to cysteine. Mutations of residues around the extended region, which contains three glycines and an arginine particularly, affected the expression, localization and activity. The L465C mutant was extracellularly accessible and pore lining by reaction with MTSET. I461C and L457C mutants were partially inhibited by MTSET, which could be a result of their deeper location in the pore. TM 11 in NhaA has a structure similar to NHE1 TM XI, however it does not contain arginine. TM 10 on both proteins contains a lysine or arginine, which has been shown to be important for activity in NHE1 [63] and in NhaA [31] where it compensates for the helix dipoles and may affect ligand binding. The arginine on TM XI of NHE1 may play a similar role.

### 4.7. Intrahelical loops

There is less structural and biochemical information on the intracellular and extracellular loops of NHE1. The extracellular loops of NhaA contain residues important for pH induced activation [64] and contain a beta-sheet essential for dimerization [24]. The loop regions of NHE1 could similarly be important for pH induced activation, inhibitor binding and interacting with the C-terminal tail and associated regulatory proteins. Many charged residues on the intracellular loops were found to be important for the pH sensitivity of NHE1 and mutation of these residues can shift the intracellular pH dependence of the exchanger [65–67]. Murtazina et al. [49] found E391 important for activity, which is located in the intracellular loop between TM IX and X, however in the Landau model [33], this is near the center of TM 9. The structure of EL2 (residues 150–155) was examined by NMR and it was suggested to adopt a beta-turn structure in solution and contained residues important in inhibitor binding [56,68]. This could be related to its proximity to TM IV, which also has many inhibitor sensitive residues. EL4 (residues 274–296) appeared unstructured by NMR [69], although it could still be structured in the context of the full protein. Many residues in EL4, when mutated to cysteine, showed decreased activity. F277 is near the N-terminal end of the loop and a F277C mutant protein reacted strongly with MTSET and also inhibited the exchanger, suggesting it is near the pore [69]. Other residues more central to the loop were more weakly reactive with MTSET. EL4 is attached to TM VII, which is pore lining. Wang et al. [70] identify a cysteine (C477) in IL5 which also appears to be important for activity.

### 4.8. C-terminus

The C-terminus of NHE1 spans residues 500–815 and is found on the intracellular side of the membrane. Circular dichroism analysis suggests the domain contains 35% helix, 16% β-turn, and 49% random coil [71]. Circular dichroism analysis of the last 180 amino acids suggests mostly sheet, turn, and coil structure, with very little helical content [72]. The proximal membrane domain region (~500–600) appears to be involved in the dimerization of NHE1 [73]. The distal region (~600–815) may be intrinsically disordered based on NMR studies [74]. Structures of CHP1, CHP2 and calmodulin bound to the C-terminal tail have been solved. Residues 503–545 of the C-terminal tail form an amphipathic helix on binding to CHP1 [75] or CHP2 [76]. Calmodulin binding [77] to residues 622–690 of the C-terminus have also been determined. The C-terminal has been suggested to undergo $Ca^{2+}$ dependent conformational changes possibly as a result of calmodulin binding, which could affect the auto inhibition of NHE1 through disruption if interaction of the calmodulin binding region to an acidic D/E rich patch (residues 753–759) [78]. The complete structure of the C-terminal tail, its interactions with other proteins, and how it regulates the membrane domain activity remain to be elucidated.

### 4.9. Electron microscopy

A low resolution envelope of full length NHE1 has also been obtained using single particle electron microscopy [79]. NHE1 was overexpressed and purified in *Saccharomyces cerevisiae*. The envelope of the protein was determined by single particle reconstruction of negatively stained, purified NHE1 protein. The size of the envelope corresponds to a dimer of NHE1 and like NhaA, NHE1 has been shown to exist as dimers, although the functional unit is a monomer [80]. The envelope of NHE1 dimers contains two compact, higher density regions which are likely the two NHE1 monomers. These structures can fit the size and overall shape of the NhaA crystal structure. The NHE1 structure observed also contains a “ridge” which was interpreted to be density from the combined cytoplasmic domains of the NHE1 dimer. Recently a low resolution envelope of a plant NHE, SOS1, has also been solved [81]. This structure also appeared to be a dimer and two conformations of the C-terminal domain could be observed.

### 4.10. Other related NHE structures

A related NHE is that of *Methanococcus janasschii* NhaP1. A three-dimensional EM structure of this exchanger has been solved [82]. The organization of the helices of NhaP1 is similar to NhaA, with a linear arrangement of helices forming a dimer interface, and a 6 TM bundle. NhaP1 shows 13 TM helices, in contrast to the 12 in NhaA. The additional helix is likely at the N-terminus and interacts with the second monomer. This helix is important for activity, however it does not appear to be necessary for dimerization. The Landau model [33] of NHE1 does not model the first two predicted TM helices TM I–II, however multiple sequence alignments by Goswami et al. [82] suggest that NHE1 may have 14 TM regions, including TM I–II, with 13 TMs equivalent to NhaP1 and an additional helix corresponding to TM V. This would suggest the Landau model is accurate for the region containing the TM VI–XII region, while the helices in the first half of the model may be assigned differently. Their alignment also suggests also that human NHE6 has 15 TMs, although the first TM is cleaved as a signal sequence [83]. Additionally, a crystal structure of a sodium/bile salt symporter (ASBT) was recently found to have an almost identical fold to NhaA [84]. It contains 10 TMs, with the 6 TM bundle and 4 TM panel superimposable onto the NhaA structure.

## 5. Conclusion

While there are numerous studies looking at the function of wild-type and mutant NHE1, there is only a limited amount of structural information currently available for NHE1. Modeling of NHE1 suggests that the protein contains 12–14 TM helices, with 10–12 helices within residues 150–500 that may form a common motif among

NHE family members. Structural studies of NhaA provided the first insight into the three-dimensional structure and mechanism of a sodium/proton exchange. They provide a wealth of information which may be applicable to NHE1. NMR studies on fragments of the transmembrane domain provided some of the first structural insights into the protein and electron microscopy provided an envelope for full-length NHE1. Further NMR studies on larger fragments of NHE1 could provide more information on the structures of the helices, and overexpression of NHE1 may eventually allow for structure determination of the entire protein. Until a complete structure is solved, methods such as NMR and cysteine accessibility studies can be used to help clear up some of the details of the two topology models, such as the topology of TM I–II and VIII–XI and the re-entrant loops. Studies of NhaP1 and ABST have shown that NHE proteins likely have a common fold, and that unrelated proteins may also share this fold. Structural studies of NHE1 and related proteins will help towards understanding the function of the NHE1, and allow for the understanding and treatment of heart disease and cancer.

## Disclosures

None

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