Molecular Dynamics Study of Cl⁻ Permeation through Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

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Research Article

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**Molecular Dynamics Study of Cl⁻ Permeation through Cystic Fibrosis
Transmembrane Conductance Regulator (CFTR)**

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Abstract:

The recent elucidation of atomistic structures of Cl⁻ channel CFTR provides opportunities for understanding the molecular basis of cystic fibrosis. Despite having been activated through phosphorylation and provided with ATP ligands, several near-atomistic cryo-EM structures of CFTR are in a closed state, as inferred from the lack of a continuous passage through a hydrophobic bottleneck region located in the extracellular portion of the pore. Here, we present repeated, microsecond-long molecular dynamics simulations of human CFTR solvated in a lipid bilayer and aqueous NaCl. At equilibrium, Cl⁻ ions enter the channel through a lateral intracellular portal and bind to two distinct cationic sites inside the channel pore but do not traverse the narrow, de-wetted bottleneck. Simulations conducted in the presence of a strong hyperpolarizing electric field led to spontaneous chloride translocation events through the bottleneck region of the channel, suggesting that the protein relaxed to a functionally open state. Conformational changes of small magnitude involving transmembrane helices 1 and 6 preceded ion permeation through diverging exit routes at the extracellular end of the pore. Although permeating Cl⁻ ions remain mostly hydrated, partial dehydration occurs at the binding sites and in the bottleneck. This portion of the pore undergoes wetting prior to Cl⁻ translocation, suggesting that it acts as a hydrophobic gate. The observed Cl⁻ pathway is largely consistent with the loci of mutations that alter channel conductance, anion binding, and ion selectivity, supporting the model of the open state of CFTR obtained in the present study.

Keywords:

CFTR, Cystic fibrosis, Chloride channel, Ion permeation, ABC protein, Channel pore, Hydrophobic gate

Abbreviations:

MD: molecular dynamics

CF: cystic fibrosis

CFTR: cystic fibrosis transmembrane conductance regulator

ATP: adenosine triphosphate

ABC: ATP-binding cassette

TM: transmembrane (helix if followed by a number)

TMD: transmembrane domain

NBD: nucleotide-binding domain

SASA: solvent-accessible surface area

Introduction:

Cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel found in cells of epithelial tissues [1–4]. Vital for the secretory function of various tissues, this ion channel regulates the transport of ions and water onto the apical surface of epithelia lining the airways, sweat glands, and the lumen of exocrine organs [5–10]. Loss-of-function mutations in the gene encoding CFTR result in the lethal disease cystic fibrosis (CF). Because many organs rely on secretion for proper function, CF patients experience a wide range of symptoms such as malnutrition and infertility. The lethal consequence of CF is respiratory failure due to recurring infections, as CF patients' lungs have an impaired ability to secrete mucus needed to rid pathogens [11]. Due to its profound clinical significance, CFTR has attracted much research attention aiming to unravel the mechanism of its function and dysfunction.

CFTR is a member of the ATP-binding cassette (ABC) transporter superfamily, specifically the ABCC exporter subfamily [1, 12]. Like closely related ABC exporters, CFTR consists of two transmembrane domains (TMDs), which create a transport pathway across the membrane, and two nucleotide-binding domains (NBDs), which bind and hydrolyze ATP to facilitate substrate translocation through the TMDs [13]. CFTR also contains a disordered regulatory domain (R-domain) that can be phosphorylated. Remarkably, CFTR is the only member of the ABC proteins known to function as an ion channel. Whereas a typical ABC transporter utilizes energy from ATP-hydrolysis to actively transport substrates against a transmembrane concentration gradient, CFTR couples its gating cycles to ATP binding and hydrolysis, allowing controlled, rapid movement of ions along the electrochemical gradient [14, 15]. Gating is also regulated by protein kinase A (PKA), which is required to activate the CFTR channel by binding and/or phosphorylating its R-domain [16, 17].

In recent years, near-atomic resolution structures of full-length CFTR were determined by cryo-electron microscopy (cryo-EM) [18–22]. The structures were determined in two distinct functional states: the dephosphorylated, ATP-unbound state (inactive or inter-burst closed state); and the phosphorylated, ATP-bound state (putative open state). In the inter-burst closed state structure, the cavity of the channel is open to the intracellular

space but not to the extracellular space, comparable to the “inward-facing” (IF) conformation of closely related ABC transporters [14, 18]. The dephosphorylated R-domain is found between two TMD-NBD pairs, effectively preventing the two NBDs from dimerizing [18, 19]. ATP-binding-induced NBD dimerization is required for functional conformational changes to occur in the TMDs [23–25]. Indeed, in the putative open state structure, disengagement of the R-domain, likely due to phosphorylation, allows the NBD dimer to form with bound ATP; the TMDs also adopt a different conformation compared to the closed-state structure [20, 21]. In closely related ABC exporters, ATP binding induces the “outward-facing” (OF) conformation characterized by a wide opening on the extracellular end and closed-off intracellular access [14, 26]. In this IF-to-OF, alternating-access transport mechanism, the transporters ensure that the transmembrane cavity is never accessible from both sides of the membrane simultaneously [14, 27]. In contrast, the channel cavity of CFTR is still accessible from the intracellular space when the NBDs are dimerized. Because CFTR functions as an ion channel, its ion conducting state necessarily requires openings on both sides of the membrane. Due to this feature, CFTR has also been described as a “broken transporter” that bears a single gate at the extracellular end of the TMDs [20, 28].

In electrophysiological records, applying PKA and Mg-ATP to CFTR results in the opening of the channel with a single-channel conductance of 6-10 pS for Cl⁻ ions [19, 29]. However, none of the structures of phosphorylated CFTR in the ATP-bound, NBD-dimerized state show an open passage across the TM-region that is sufficiently wide to accommodate water or Cl⁻ ions [20, 21]. Therefore, these structures cannot represent the ion-conducting state observed under experimental and physiological conditions. It has been speculated that this NBD-dimerized but closed conformation represents the brief intra-burst closed state observed in electrophysiological records, which may be stabilized by the non-native environment in detergent micelles and requires additional transmembrane helical movements to reach the open state [20]. Molecular dynamics simulations of the phosphorylated, ATP-bound zebrafish CFTR structure revealed that this closed state with dimerized NBDs is stable over hundreds of nanoseconds [30, 31]. Enhanced sampling methods were used to derive putative structures of CFTR with an open pathway permissive of the passage of Cl⁻ ions [31, 32]. However, the molecular

mechanisms governing the spontaneous opening of the CFTR channel and Cl⁻ permeation events have yet to be described.

In this study, we present repeated MD simulations of ATP-bound, NBD-dimerized human CFTR in the microsecond time range. In the absence of transmembrane voltage, the extracellular portion of the channel remained largely de-wetted, and ion permeation was not observed. However, spontaneous Cl⁻ translocation events occurred in simulations where a hyperpolarizing transmembrane voltage was applied. The analysis of ion-protein interaction reveals at least two Cl⁻ binding sites in the portion of the channel lumen accessible from the intracellular medium ("inner vestibule") and diverging anion pathways through the narrow extracellular portion of the channel ("bottleneck"). Experimental measurements of ion selectivity are rationalized in light of observed Cl⁻ interactions with CFTR. Displacements of pore-lining helices occurring under transmembrane voltage are correlated with wetting of the bottleneck region prior to ion translocation, suggesting that the extracellular portion of the channel functions as a hydrophobic gate.

Results:

Overview of the channel vestibule

We performed MD simulations of human CFTR in its ATP-bound, NBD-dimerized state (PDB: 6MSM) embedded in a POPC bilayer and solvated in 150 mM NaCl aqueous solution. In all simulations, water filled the transmembrane channel cavity through a lateral intracellular opening formed by cytosolic extensions of transmembrane (TM) helices 4 and 6 and known as the “cytosolic portal” from structural and functional data (Fig. 1a) [18, 19, 33]. Chloride ions entered from the intracellular space through the same opening, whereas sodium ions did not enter the cavity (Fig. 1b). This observation suggests that the cytosolic portal imparts selectivity for anions over cations, possibly due to the abundance of positively charged residues at this location [20, 34].

Unlike most ion channels, in which the ion permeation pathway tends to be linear and symmetrically shaped, CFTR presents a curved passage to Cl^- ions. Two elbow-shaped turns create a short, laterally oriented segment at around $z = -25 \text{ \AA}$ (Fig. 1b). An apparent barrier opposing Cl^- occupancy and passage is found at the extracellular end of the transmembrane domains ($-5 \text{ \AA} < z < 5 \text{ \AA}$), suggesting the presence of an energetic or steric bottleneck (Fig. 1b; Fig. 2d). Lined by TM helices 1, 2, 6, 8, 11, and 12, this bottleneck region is rich in hydrophobic residues and is the most de-wetted region of the channel pore (Fig. 1c, d; Fig. 2b, c). In the absence of voltage, the ions reached as far as $z = -5 \text{ \AA}$ into the cavity, but Cl^- permeation did not occur. To induce Cl^- permeation, we simulated the same system in the presence of a uniform electric field along the membrane normal. This electric field creates an effective membrane potential of -500 mV without affecting the structural integrity of the TMDs (Fig. S1). The presence of the electric field increased the average ionic occupancy of the inner vestibule from 1.05 to 1.71 (Fig. 2a, e). Compared to simulations without an electric field, this extra Cl^- ion density occupies the region of the inner vestibule closest to the bottleneck (Fig. 2d, e: $-20 \text{ \AA} < z < -5 \text{ \AA}$). Despite the high transmembrane voltage, the apparent bottleneck for Cl^- persisted; however, Cl^- translocation events occurred in two out of ten 1- μs -long simulations, as discussed in detail below.

Cl⁻ binding sites in the inner vestibule

The analysis of the average Cl⁻ distribution in the pore (Fig. 2d) suggests the presence of multiple ion binding sites in the inner vestibule, where protein residues directly contacted Cl⁻ ions through the first solvation shell. In particular, two major binding sites were identified where Cl⁻ ions resided for long durations. The first site is located near the cytosolic portal and consists primarily of residues K190, R248, and R303 (Fig. 3a). The second binding site is located 10-15 Å above the elbow region and consists primarily of K95, R134, and Q98 (Fig. 3a). In the absence of an electric field, site 2 is the farthest location reached by Cl⁻ ions. The presence of an electric field increased the occupancy of site 2 from 0.14 to 0.50 (Fig. 2d, e). Between binding sites 1 and 2, Cl⁻ bound more transiently to two other sites: one involving R352 and W356, with some contribution from R303; and the other involving primarily R1097 (Fig. S4). Chloride binding resulted in time periods of hundreds of nanoseconds during which the Cl⁻ ion stayed relatively stationary along the channel pathway, even in the presence of voltage (Fig. S7; Fig. S8). Despite that multiple protein residues can bind Cl⁻ ion at either site, on average only one protein residue bound Cl⁻ ions in sites 1 and 2, respectively. Chloride ions at either site remained well hydrated regardless of whether the electric field is present (Fig. 3b).

Cl⁻ permeation through the bottleneck region

A total of 17 Cl⁻ translocation events occurred in runs #1 and #2 (Fig. 4; Table S1). These permeation events were closely spaced in time and occurred in three apparent bursts, suggesting interconversions between conductive and non-conductive states. Run #1 contained one such burst involving 3 Cl⁻ permeation events ($800 < t < 1000$ ns) and run #2 contained two bursts ($400 < t < 500$ ns and $600 < t < 800$ ns) involving 3 and 11 Cl⁻ permeation events, respectively.

The translocation of Cl⁻ through the bottleneck region begins at $z = -5$ Å. At this location, Cl⁻ ions bind S341 and N1138 from TM6 and TM12, respectively (Fig. 5d). These residues precede the bottleneck region and can be reached by intracellular Cl⁻ ions without leading to translocation, even in the presence of a strong transmembrane voltage (Fig. 4; Fig. S8). Beyond S341, translocating Cl⁻ ions traverse the bottleneck

region lined by a number of hydrophobic residues including L102, I106, F337, and M1137 (Fig. 5c). Halfway through the bottleneck ($z = 0$ Å), T338 and T1134 mark the narrowest region of the pore and occasionally coordinate permeating Cl^- ions (Fig. 5b). The extracellular end of the bottleneck is lined with several positively charged residues, including R104, R117, R334, and K335, which could facilitate the passage of permeating anions through the bottleneck (Fig. 5a). These cationic residues contribute to the partial dehydration of Cl^- by displacing on average about 1.2 ± 0.6 water molecules in the first solvation shell of Cl^- (Fig. 5e, f). Interestingly, the long sidechain of R334 was even observed to dip into the hydrophobic bottleneck and to bind permeating Cl^- ions before they reach T338 (Fig. S6), suggesting that R334 may serve as a chaperone for Cl^- translocation.

The Cl^- permeation pathway appears to diverge into different routes in the bottleneck region. The point of divergence is located shortly beyond S341. In the first route, Cl^- exited laterally between TM1 and TM6; this route will henceforth be referred to as the “1-6 pathway” (Fig. 6a, d). The 1-6 pathway is bounded by TM helices 1, 6, 8, and 12. In an alternative route, Cl^- exited between TM helices 1 and 12, which defines the “1-12 pathway” bounded by TM helices 1, 2, 6, 8, 11, and 12 (Fig. 6c, f). The third permeation route, which is intermediate between the other two, is lined by the same six TM helices in approximate hexagonal arrangement (Fig. 6b, e). The three different permeation routes also differ in which sets of extracellular residues coordinate translocating Cl^- ions: R334, K335, and R104 in the 1-6 pathway (Fig. 5a); R334, Y914, and Y917 in the intermediate pathway (Fig. 7c); R117 and polar groups of extracellular loops in the 1-12 pathway (Fig. 7b).

Structural features of the open pore

In the simulations in which Cl^- permeation events occurred, changes in the distances separating pore lining helices in the bottleneck region were correlated with transitions from the non-conductive state to conductive states (Fig. 8). In run #1, ion permeation may be facilitated by TM6 moving away from other pore lining helices (Fig. 8d). In particular, the distance d_{1-6} increased from ~ 9 Å to ~ 12 Å before the permeation burst

starting at 800 ns (Fig. 9a), creating a pore large enough for ion exit through the 1-6 pathway (Table S1).

In run #2, ion permeation was likely facilitated by TM1 moving away from other pore lining helices (Fig. 8e). The distances d_{1-8} and d_{1-12} increased at ~400 ns (Fig. 9e, h) even as d_{1-6} decreased before reverting to larger values during the second ion burst period (Fig. 9b). The decreased TM1-TM6 separation may explain that all three permeant Cl⁻ ions took the 1-12 pathway during the first ion burst (Table S1), during which d_{1-12} peaked (Fig. S3). The second ion burst featured a large d_{1-12} and a further increase in d_{1-8} (Fig. 9e, h). During this period, permeant Cl⁻ ions followed mostly the intermediate and 1-6 pathways (Table S1). After the second ion burst, d_{1-6} increased further while d_{1-12} decreased (Fig. 9b, h; Fig. S3), suggesting that the channel may be undergoing a transition towards a state in which the 1-6 pathway is the exclusive route, similar to that observed in run #1.

Hydration of the bottleneck region

The observation of partially hydrated Cl⁻ ions permeating through the CFTR channel suggests that a hydrated bottleneck region is a prerequisite for ion conduction. Overall, the average number of water molecules in the bottleneck increased slightly when the electric field was present (Fig. 10a), a phenomenon seen in hydrophobic gates and known as “electric field-induced wetting” [35]. The overall degree of hydration in run #1 was greater than that of all other simulations conducted with an electric field. All ion permeation events in this simulation run occurred when hydration was relatively high after d_{1-12} increased to permit water permeation through the 1-12 pathway (Fig. 4; Fig. 9g; Fig. 10b). Likewise, the time dependence of hydration in run #2 was strongly correlated with ion permeation events. The first and second permeation bursts occurred following major increases in hydration at $t \sim 400$ ns and $t \sim 600$ ns, respectively (Fig. 4; Fig. 10c). Permeation occurred during periods of wetting of the bottleneck region, which provided a hydrated pathway for ion translocation.

The coincidence of pore wetting and ion permeation events is reminiscent of hydrophobic gates in a large variety of other ion channels [36, 37]. To locate a putative hydrophobic gate in CFTR, we analyzed hydrophobicity along the permeation pathways.

A short but highly hydrophobic “gasket” consisting of residues L102, I106, and F337 constitutes the most hydrophobic and narrowest region within the 1-6 pathway (Fig. 11a, b). The hydrophobicity of this region decreases drastically when sustained hydration and permeation occur due at least in part to the penetration of the side chain of R334 (Fig. S6). Similarly, residues L102, I105, M1137 constitute a highly hydrophobic region within the intermediate pathway (Fig. 11e-f). This pathway is too narrow to be detected until it becomes available with a pore diameter of at least 4 Å throughout (Fig. 11f). In contrast, the 1-12 pathway does not have a distinct, sharply hydrophobic region; instead, the entire pathway is somewhat hydrophobic (Fig. 11c, d). During permeation, the entire pathway appears widened, with the narrowest region widening by about 1-2 Å in diameter.

Drastic changes in hydration around the gasket region occurred during transitions between non-conducting and conducting states (Fig. 10d-i). In run #1, P99 and L102 became substantially more and less hydrated, respectively, prior to the ion permeation burst (Fig. 10d, e). Since L102 was much more water accessible when water permeation occurred through the 1-12 pathway (Fig. 10e; $500 < t < 800$ ns), the decrease in water accessibility of L102 might coincide with the closure of the 1-12 pathway and the opening of the 1-6 pathway. These observations suggest that differential extent of wetting of these residues is correlated with the availability of different permeation pathways. Accordingly, L102 is more water exposed than P99 when Cl⁻ permeation occurs through the 1-12 and intermediate pathways in run #2 (Fig. 10g, h). Furthermore, I106 is more hydrated in run #2 than in run #1, especially during the second ion permeation burst (Fig. 10i), which may be a hallmark of a wide-open gate supporting all permeation routes. In summary, wetting of hydrophobic residues within the bottleneck region precedes permeation events regardless of permeation routes; however, which residues undergo wetting depends on the permeation pathway being activated.

Discussion:

Comparison with previous structural modelling studies

The elucidation of both open and closed state structures of the CFTR ion channel, as well as the transitions between the different states, are crucial for the understanding of CFTR function and dysfunction. Despite major progress made in structure determination, near-atomic resolution cryo-EM structures of this protein could not explain how it allows permeation of Cl⁻ ions [18–22]. In the present work, we performed atomistic MD simulation of human CFTR in a lipid bilayer. In the presence of a hyperpolarizing transmembrane voltage, spontaneous Cl⁻ translocation events occurred through divergent pathways in the extracellular portion of the pore.

Prior to this work, numerous *in silico* studies involving molecular simulations of homology models of CFTR were devoted to providing a picture of the channel in its open conducting state, both before and after the advent of high-resolution experimental structures (reviewed in [32]. A major limitation of these homology models is the use of homologous template ABC transporters sharing low sequence identity with CFTR [32, 38–40]. In addition, an unexpected feature revealed by the near-atomic resolution cryo-EM structures is that TM8 is partially unwound in the middle of the membrane and split into two helical segments [18]. As a result, the extracellular segment of TM8 participates in lining the pore, in stark contrast to typical, symmetric ABC transporters in which TM7 lines the translocation pathway. Recently, using substituted cysteine accessibility mutagenesis (SCAM) and cysteine crosslinking, Negoda and coworkers found that TM8 contains residues lining the narrow region of the pore in close proximity to TM1 and TM6 in an open CFTR channel [41]. This feature of TM8 is consistent with the near-atomic resolution experimental structures but is not captured by existing homology models. More recently, Farkas and coworkers performed equilibrium MD simulations on the cryo-EM structure of ATP-bound, NBD-dimerized zebrafish CFTR, which revealed a transient open pathway through the channel allowing water permeation [31]. Metadynamics was used to direct Cl⁻ through the bottleneck region, revealing two putative energetically favourable pathways [31]. Our simulation results on human CFTR, also performed on the ATP-bound, NBD-dimerized state, support the prediction of

multiple Cl⁻ exit pathways and reveal how conformational rearrangements of pore-lining helices give rise to different routes of permeation.

The peculiarity of the TM8 structure attracted attention due to its potential role in the gating of CFTR [18, 30]. It was speculated that movements of TM8 are key to transitions from a non-conducting state to a conducting state in the presence of ATP and dimerized NBDs [20]. In contrast, Fay and coworkers obtained a lower-resolution cryo-EM structure of engineered chicken CFTR in an ATP-bound form [42]. This structure, which was determined to have a high channel opening probability, does not have a “broken” TM8. In the current study, the conformation of TM8 as it appears in the near-atomic resolution cryo-EM structures is stable over microseconds of simulation time, with the RMSD of its extracellular segment from the PDB structure reaching up to ~2 Å after 1 μ s (Fig. S1). This observation is consistent with the findings from a previous MD simulation study of zebrafish CFTR supporting the conformational stability of TM8 [30]. Accordingly, spontaneous Cl⁻ permeation events occurred in the presence of an electric field without major conformational changes in TM8, such as the rotation of its extracellular segment or the restoration of its helical defect.

Chloride binding sites

The location and physico-chemical nature of Cl⁻ binding sites in CFTR have been somewhat contentious [43, 44]. Experimental studies conducted with negatively charged channel blockers point to the presence of anion binding sites inside the cavity [45, 46]. From our simulations, we identified two major binding sites that are consistent with the experimental findings: site 1, near the cytoplasmic portal, consisting of residues K190, R248, and R303; and site 2, deep inside the inner vestibule of the pore, consisting of K95, Q98 and R134 (Fig. 3a). Several of the residues that contribute to these two binding sites have previously been shown to interact with channel-blocking anions, which have been suggested either to occupy a superficial site close to the cytoplasmic portal [33, 46] or to enter deep into the cavity and interact with the positive charge of K95 [45]. Furthermore, the reduced conductance associated with mutations that remove positive charges in these binding sites suggests that Cl⁻ binding to these sites is an important feature of its permeation mechanism [34, 47, 48]. These two inner

binding sites are located in regions where Cl⁻ occupancy is likely to be important—namely, at the cytoplasmic entrance of the pore (site 1) and near the entrance of the bottleneck (site 2). Indeed, Cl⁻ ions are most likely to occupy site 1 regardless of electric field, while the presence of an electric field increases the affinity of the middle of the pore for anions, as seen by the increase in Cl⁻ occupancy at site 2 (Fig. 2d, e).

Even though simultaneous binding to multiple sidechains of site 2 does occur occasionally, usually only one residue binds Cl⁻ at a time, suggesting that Cl⁻-binding residues are loosely organized at the binding site. Accordingly, it was found by mutagenesis that the positive charge of K95 can be moved to a nearby location, such as I344, V345, or M348, without abrogating channel function [49, 50]. Similarly, the positive charges on K190 and R303 can be moved to nearby locations, such as N186 and L197, with minimal reduction in Cl⁻ conductance [51].

Ion permeation through the bottleneck region

Consistent with functional and SCAM studies, in the ion-conducting conformations of CFTR, the ionic pathway through the bottleneck is lined primarily by TM helices 1, 6, 8, and 12 (Fig. 1c) [41, 43, 52]. TM helices 2 and 11 also contribute to lining the permeation pathway, albeit to a lesser extent (Fig. 1c; Fig. 6). While there has been some evidence suggesting that TM11 lines the narrow region of the permeation pathway, evidence supporting the same role for TM2 is lacking [43]. Our simulations show two key residues by which TM2 could functionally contribute to the Cl⁻ permeation pathway: R134, which contributes to binding site 2; and R117, which binds Cl⁻ ions permeating through the 1-12 pathway. However, neither of these residues is located within the narrow bottleneck region. Furthermore, neither TM2 nor TM11 appear to make any contribution to the 1-6 pathway.

The simulations show that Cl⁻ entering the bottleneck region from the intracellular side first contact S341 (Fig. 5d, f). The hydroxyl group of S341 participates in Cl⁻ solvation, which might be important given that the S341A mutant is associated with a large decrease in Cl⁻ conductance [53]. Moreover, mutation S341K also causes greatly reduced conductance, highlighting the importance of this side chain to the permeation process [50]. Beyond S341, the narrow permeation pathway is lined by hydrophobic

sidechains including L102, I106, F337, and M1137 (Fig. 5c). Functional and SCAM data suggest that L102 and F337 contribute to the narrowest part of the open pore [54, 55]. Mutations of T338 show strongly size-dependent effects on conductance, consistent with the observation that this residue is located in the narrowest part of the bottleneck [56, 57]. At the extracellular end of the bottleneck, Cl⁻ ions interact with R104, R117, R334, and K335 (Fig. 5a; Fig. 7b, c). The positive charges at R104, R334, and K335 have each been shown to interact with extracellular anions, and charge-neutralizing mutations at these residues reduce Cl⁻ conductance [52]. In our simulations, R334 was seen to interact with Cl⁻ even before it reached the extracellular space. Finally, Cl⁻ ions exiting via the intermediate pathway interact with Y914 and Y917, mutations of which were shown to affect both Cl⁻ permeation and channel gating [55].

Conformational fluctuations leading to the open conformation of the channel involve relative movements of TM1 and TM6 (Fig. 8). Cysteine cross-linking studies suggested that the extracellular ends of TM1 and TM6 separate from each other when the channel opens [58], which is consistent with the 1-6 permeation pathway observed in this study. TM1 movements best explain gate wetting and ion translocation. These movements may be facilitated by a helical defect at residue P99. Consistent with this hypothesis, mutations that favour helicity, such as P99A and P99L, are both loss-of-function mutations [59]. TM6 movements may also facilitate ion translocation, as seen in run #1, albeit to a lesser degree. In contrast, we did not observe noticeable movements of TM8 and TM12 as proposed by Zhang *et al.* [20].

Putative explanations of lyotropic permeability selectivity

Like most anion channels, CFTR shows lyotropic permeability selectivity, meaning that lyotropic anions with a relatively low free energy of hydration have higher permeability than kosmotropic anions, which retain water in their hydration shell more strongly [60, 61]. One implication of lyotropic permeability selectivity is that partial dehydration is an important aspect of the anion permeation process. Consistent with this hypothesis, Cl⁻ ions lose water molecules from their first solvation shells as they pass through the bottleneck region (Fig. 5e). This finding suggests that the bottleneck might form the lyotropic “selectivity filter,” where the relative permeability of different anions is

predominantly determined. Amongst all the pore-lining residues studied, only mutagenesis of F337 and, to a lesser extent, L102, have been shown to disrupt the normal lyotropic selectivity pattern [55, 61]. These non-polar residues form a narrow, hydrophobic region of the open pore where Cl⁻ ions are partially dehydrated (Fig. 5e-f). It is conceivable that partial dehydration might favour the entry (and therefore permeation) of more lyotropic anions, thus explaining the lyotropic permeability selectivity pattern.

Diverging ion translocation pathways in the bottleneck region

Our simulations reveal multiple Cl⁻ exit pathways to the extracellular space (Fig. 6). In support of both 1-6 and 1-12 pathways, charge-neutralizing mutations at R104, R334, K335, and R117 lead to reduced channel conductance [62, 63]. In addition to these two permeation pathways, a less distinguishable, intermediate pathway (Fig. 6b, e; Fig. 11e) involving direct Cl⁻ coordination by tyrosine sidechains Y914 and Y917 was observed when the gate was most widely open (Fig. 7c). In support of the intermediate pathway, a number of mutations of these tyrosine residues lead to reduced channel conductance [41].

Given the small number of ion permeation events in the simulations, we cannot assess the relative stability and functional importance of each pathway. The existence of these different pathways may not correspond to observable changes in the functional properties unless specific blockage of individual putative pathways can be achieved. Mutagenesis of some residues in the outer mouth of the pore, including R117, had relatively minor effects on conductance, possibly because these residues contribute to the pathway taken by only a subset of all permeating Cl⁻ ions [63, 64]. Furthermore, rapid fluctuations in the conformation and relative arrangement of pore-lining helices governed the transitions between the different pathways over a 100-ns timescale (Table 1: simulation run #2: 600 < t < 800 ns). One implication of TM-helix movements is that the extracellular part of this pore is structurally dynamic, which might explain why we did not identify a clearly defined “outer vestibule” region as suggested in the classical description of the architecture of CFTR pore [52].

A hydrophobic gate with unusual features

We have uncovered evidence suggesting that the extracellular bottleneck region of the CFTR pore functions as a hydrophobic gate. Hydration of the hydrophobic bottleneck is greater in the presence of an electric field (Fig. 10a, j-l). Electric-field-induced permeation of water and ions was observed in simulations of hydrophobic nanopores [65]; more recently, wetting of hydrophobic gates was demonstrated in simulations of biological ion channels in the presence of a strong electric field [35, 66, 67]. It has been suggested that the presence of an electric field can alter the liquid-vapour equilibrium of water inside hydrophobic gate, resulting in an increased probability of wetting [66]. This effect might have contributed to water and ion permeation in our simulations. However, the wetting transition observed in the simulations can also be explained in terms of structural changes. Increasing both the polarity and the diameter of the gate are common features of hydrophobic gating [68]. In our simulations, both of these events occurred due to movements of helices and changes in sidechain conformations, both of which may be induced by the electric field.

Despite similarities to other hydrophobic gates, the proposed hydrophobic gate of CFTR also has some peculiarities. First, it is less distinctively shaped due to the presence of multiple permeation pathways. While the 1-6 pathway resembles a typical ion channel pore with a short hydrophobic segment, the 1-12 pathway does not seem to have a distinct gating region, yet it is overall lacking in polar residues and, as expected, it undergoes dilation prior to ion permeation. Second, partial dehydration of the permeant ion occurs as it passes through the hydrophobic bottleneck. The fact that sidechains such as R334 can participate in the solvation of permeant ions complicates its categorization as a hydrophobic gate. Curiously, size-reduction mutation F337A and hydrophobicity-reducing mutation L102T both result in reduced channel conductance [47, 55]. Along with many other intriguing effects of mutations in residues of the bottleneck region [44, 52], these are some of the transport properties of Cl⁻ in CFTR that remain to be unravelled.

Conclusions

Using MD simulations, we have characterized Cl⁻ permeation events through the human CFTR channel in the presence of transmembrane voltage. Traversing Cl⁻ ions interacted with pore lining residues that are critical for the ion conductance, supporting this model of open CFTR channel. The spontaneous opening of the channel from the cryo-EM-determined state involves conformational rearrangements of TM helices 1 and 6, which line the extracellular hydrophobic bottleneck region of the pore. The wetting/de-wetting properties of the bottleneck region evoke hydrophobic gating, adding CFTR to the list of ion channels that utilize this physico-chemical mechanism to modulate ion permeation.

Despite the high applied voltage, the small number and relatively short duration of conductive bursts observed in the present study resulted in a small number of ion permeation events. As such, our results do not permit a reliable estimate of the conductance of the open channel. In order to estimate Cl⁻ conductance and compare it to single-channel conductance measurements, longer simulations of the open state will be required to compute steady-state current-voltage relationships at lower, physiological voltages. These future studies will aim to clarify the relative stability of the diverging permeation pathways observed in the present study and provide a meaningful validation of our structural model of the conducting state of CFTR.

Methods:

Molecular System

The structural model of phosphorylated, ATP-bound human CFTR (PDB: 6MSM) was used for all simulations [21]. Residues that were missing in the PDB structure include the loops connecting each TMD-NBD pair (410-434, 1174-1201), the R-region (638-844), the extracellular loop between TM7 and TM8 (890-899; also known as the extracellular loop 4), and the segment at the C-terminal end of NBD2 (1452-1489). These missing segments were not modelled in this study, resulting in our structural model consisting of five peptide chains. All chains were acetylated at the N-termini and amidated at the C-termini into primary amides. Mg-ATP moieties from the original PDB structure were found at the NBD interface and were retained for the simulations. All other species present in the PDB structure were removed if they were not known regions of CFTR or Mg-ATP. These species include cholesterol, phospholipids, and the helix at the TMD-NBD interface believed to be part of the R-domain.

Before embedding the CFTR protein into lipids, the PPM server of Orientations of Proteins in Membranes (OPM) database was used to determine the starting position of the lipid bilayer [69]. The model for CFTR with Mg-ATP bound was then embedded in a POPC bilayer and solvated in water with 150 mM NaCl using the CHARMM-GUI server [70, 71], with no water or ions allowed inside the channel pore. The hexagonal periodic unit cell configuration was chosen with starting dimensions: $a = b = 11$ nm, $c = 18.5$ nm, $\alpha = \beta = 90^\circ$, and $\gamma = 120^\circ$. This procedure resulted in a total of 255 POPC molecules added around the protein within a periodic image.

Simulation setup and protocol

All MD simulations were conducted using GROMACS 2018 or 2019 [72]. The CHARMM36 forcefield was used for protein, lipids, ions, ATP, together with the TIP3P water model [73–76]. Simulations were run in the NpT ensemble ($T = 300$ K, $p = 1$ atm) at 2 fs integration timesteps. Constant temperature was maintained using the Nosé–Hoover thermostat ($\tau_T = 0.5$) [77, 78]; constant pressure was maintained using the Parrinello-Rahman barostat ($\tau_p = 2.0$) [79, 80]. Semi-isotropic pressure coupling was

used, with isothermal compressibility set to $4.5 \times 10^{-5} \text{ bar}^{-1}$ both in the xy-plane and along the z-axis. Nonbonded interactions were calculated using Verlet neighbor lists [81, 82]. Lennard-Jones interactions were cut off at 1.2 nm and a force-based switching function with a range of 1.0 nm was used. The particle-mesh Ewald (PME) method was used to compute electrostatic interactions with a real-space cut-off of 1.2 nm [83, 84]. The LINCS algorithm was used to constrain covalent bonds involving H atoms [85].

The simulation system was first subjected to steepest descent energy minimization until maximum force dropped below 1000 kJ/mol/nm. Random velocities were generated at the beginning of the *NpT* equilibration phase, which was conducted in three 10-ns stages, successively with protein heavy atoms, protein backbone atoms, and protein C α atoms restrained (with force constants of 1000 kJ/mol/nm² in x, y, and z directions). After the *NpT*-equilibration phase, the simulation was divided into two groups for the production run. In one group, the simulation was conducted in *NpT* ensemble at the same temperature and pressure without applied restraints. In the other group, a uniform electric field of magnitude 32 mV/nm was added along the z-axis, normal to the lipid bilayer and pointing towards the intracellular side (negative along z-axis). During the production runs, the z-dimension of the simulation box (i.e. parameter c of unit cell) equilibrated to around 16.5 nm, which gave rise to a transmembrane voltage of approximately -500 mV. This approach has been used to generate a membrane potential in MD simulations without having to maintain asymmetric ion concentration on both sides of the membrane [86, 87]. In both sets of simulations, new random velocities were generated at the beginning of production runs. Ten 1- μ s-long simulation runs were produced from each set of conditions.

Analysis of simulation data

All molecular snapshots of CFTR from production run trajectories were aligned to the first frame (i.e. the configuration at the end of the equilibration phase) through minimization of RMSD of C α atoms of TMDs.

All the visualizations and renderings of molecular representations of proteogenic components and ions were done with VMD [88]. The origin of the z-axis was chosen to be the position of the C α atom of T338. To identify ions located inside the channel pore,

a simple cylindrical method was used to define the boundaries. The C α atoms of pore-lining helices (TM1, 2, 6, 8, 11, and 12) located within $-40 < z < 20$ Å were selected. The centre-of-mass of these C α atoms in the xy -plane was used as the center of the cylinder. The average distance in the xy -plane from the center to these C α atoms was used as the radius.

Analyses of the ions inside the channel (axial position and solvation shell), the structure of pore-lining helices, the hydration of the bottleneck region (including solvent-accessible surface area) were done with homemade Tcl scripts aided by VMD and/or python scripts aided by MDTraj [88, 89]. In particular, to analyze ion binding and solvation, a molecule or residue is considered to form a direct contact with a Cl⁻ ion if it contains any atom within 3 Å of the ion. This cut-off criterion is based on radial pair distribution functions computed using VMD (Fig. S2). According to the distribution functions, the closest contacting atoms are most likely hydrogen atom. The hydrophathy and channel path radius analysis were aided by MOLE2 [90].

Figure Captions:

Fig. 1 Overview of the CFTR channel pore. **(A)** Simulation snapshot showing the water-filled channel cavity (inner vestibule). The inner vestibule is accessible to water from the intracellular space through the “cytosolic portal” (cyan arrow) formed by TM helices 4 and 6. Phosphorus atoms (cyan spheres) of POPC headgroups indicate membrane position. Bulk water and lipid tails are omitted for clarity. **(B)** Spatial view of occupancy of Cl^- ions (green spheres) inside the channel cavity from many timesteps overlaid. **(C)** Cartoon representation of TM helix segments lining the bottleneck region of the pore together with Cl^- ions drawn from multiple timesteps. **(D)** The bottleneck region of the pore is lined by many hydrophobic residues (grey), disfavours the presence of water (red surfaces). Selected hydrophilic/charged residues (cyan) are also shown.

Fig. 2 Effect of transmembrane voltage on water and Cl^- occupancy of the CFTR channel. **(A)** Probability distribution of the number of Cl^- ions occupying the inner vestibule (blue) without and (green) with applied voltage. Error bars represent bootstrapped 99% confidence intervals. **(B)** Snapshot of the CFTR channel filled with water molecules used as positional reference for (C)-(E). **(C)** Average number density of water molecules (N_{water}) in the channel vestibule computed along the z-axis. The bottleneck region is highlighted in yellow shading. **(D)** Average population density of Cl^- ions, $\rho(\text{Cl}^-)$, inside the channel pore (blue) without and (green) with -500 mV transmembrane voltage. The Cl^- occupancy of the region $-20 < z < 0 \text{ \AA}$ is enhanced by voltage. **(E)** Cumulative population densities of Cl^- ions, $n(\text{Cl}^-)$, inside the channel pore computed by integrating $\rho(\text{Cl}^-)$ from (D).

Fig. 3 Chloride solvation in the inner vestibule. **(A)** Left: overview of the Cl^- permeation pathway with overlaid ions (green spheres) from simulations in the presence of voltage with TM helices 1 (blue), 2 (red), 3 (cyan), 4 (orange), 6 (tan), 8 (lime), 11 (purple), and 12 (yellow). The locations of two Cl^- binding sites 1 and 2 are indicated in dashed boxes. Right: close-up views of binding sites 1 and 2. Amino acid sidechains in direct

contact with Cl⁻ ions (green spheres) are shown. The labels of positively charged residues, including K246 and K370 from the cytosolic portal, are shaded in indigo. **(B)** Coordination of Cl⁻ ions inside the channel cavity from simulations with voltage. The average numbers of protein residues (black) and water molecules (red) in the first solvation shell of Cl⁻ ions (n_{solv}) along the intracellular pathway are shown together with their sum (green). Shading represents 99% bootstrapped confidence intervals. Error bars are largest in the bottleneck region, which is least visited.

Fig. 4 Spontaneous permeation of Cl⁻ ions in the CFTR channel under applied voltage. Left: overview of the Cl⁻ permeation pathway with overlaid ions (green spheres) from simulations in presence of voltage highlighting TM helices 4 (orange) and 6 (tan). Right: time series of axial Cl⁻ ion positions from the two simulation runs in which permeation occurred (-500 mV). Ion permeation events are indicated by traces passing through the bottleneck region. Individual Cl⁻ ions are distinguished by color for clarity.

Fig. 5 Chloride translocation pathway through the bottleneck region. Top center: overview of the Cl⁻ permeation pathway with overlaid ions (green spheres) from simulations in the presence of voltage with TM helices 1 (blue), 2 (red), 3 (cyan), 4 (orange), 6 (tan), 8 (lime), 11 (purple), and 12 (yellow). **(A)-(D)** Close-up views showing the residues interacting with permeating Cl⁻ ions. The labels of positively charged residues are shaded in indigo. **(E)** Coordination of Cl⁻ ions inside the channel cavity from simulations with voltage. The average numbers of protein residues (black) and water molecules (red) in the first solvation shell of Cl⁻ ions (n_{solv}) along the intracellular pathway are shown together with their sum (green). Shading represents 99% bootstrapped confidence intervals. Chloride ions are less hydrated in the bottleneck region (yellow shading). **(F)** Significant amino-acid contributions (various colours) to the protein solvation number n_{solv} (black).

Fig. 6 The three different pathways of Cl⁻ permeation through the extracellular bottleneck. (Top row) Side views and (bottom row) top views from extracellular space. Pore-lining helices are colour-coded as indicated. Chloride ions (cyan) overlaid from timesteps during translocation. **(A), (D)** 1-6 pathway; **(B), (E)** intermediate pathway; **(C), (F)** 1-12 pathway.

Fig. 7 Outer Cl⁻ binding sites at the extracellular mouth. Boxed regions in the overview of the pore **(A)** are expanded in insets showing Cl⁻ exit binding sites for **(B)** the 1-12 pathway and **(C)** the intermediate pathway. TM helices 1 (blue), 2 (red), 3 (cyan), 4 (orange), 6 (tan), 8 (lime), 11 (purple), and 12 (yellow) are highlighted. Residue sidechains interacting with Cl⁻ ions are shown.

Fig. 8 Structural fluctuations of TM helices lining the extracellular bottleneck region. **(A)** Top view of the pore from the extracellular side highlighting amino acid residues of the pore-lining helices used to track TM helix movements. **(B)-(C)** Distributions of xy-positions of the extracellular ends of TM helices 1, 6, 8, and 12 in the absence and presence of transmembrane voltage. **(D)-(E)** Time evolution of TM helices 1, 6, 8, and 12 in conductive simulation runs #1 and 2 (-500 mV). In run #1, a brief departure of TM6 away from the other helices is seen towards 1000 ns. In run #2, TM1 shows significant fluctuations of up to ~5 Å from its average location.

Fig. 9 Time evolution of interhelical distances between TM 1 and other pore-lining helices at the extracellular end. Interhelical distances are computed as the distances between C α atom pairs of the following residues: d_{1-6} : I106-R334 (top row); d_{1-8} : I106-Y914 (middle row); and d_{1-12} : I106-I1131 (bottom row). **(A), (D), (G)** Time series of interhelical distances for run #1 (orange traces); **(B), (E), (H)** same for #2 (pink traces). Chloride translocation events (see Fig. 4) are indicated as vertical lines. **(C), (F), (I)** Distributions of interhelical distances in the absence (blue) and in the presence (green) of an electric field.

Fig. 10 Analysis of hydration of the extracellular bottleneck region. **(A)** Distributions of the number of water molecules in the bottleneck region, $N_{\text{water}}(\text{bottleneck})$, from non-conductive simulations in the presence (green) and absence (blue) of TM voltage. Results computed from conductive simulation runs #1 and #2 are shown separately. **(B)-(C)** Time evolution of $N_{\text{water}}(\text{bottleneck})$ for simulation runs #1 (orange) and 2 (pink). **(D)-(I)** Time series of solvent accessible surface areas (SASA) of residues P99, L102, and I106 found in the narrowest region of the bottleneck for simulation runs #1 [**(D)-(F)**] and 2 [**(G)-(I)**]. The time series suggest temporal correlation with ion permeation. **(J)-(L)** Distributions of SASA of these residues in the absence (blue) and in the presence (green) of TM voltage are shown on the rightmost column. Ion translocation events (see Fig. 4) are indicated as vertical lines.

Fig. 11 Structural and physico-chemical analysis of diverging permeation pathways through the extracellular bottleneck region. **(A)** 1-6, **(C)** 1-12, and **(E)** intermediate pathways are visualized as translucent closed surfaces. Estimates of width and hydrophobicity along the 1-6 **(B)**, 1-12 **(D)**, and intermediate pathways **(F)**; R , local radius and ζ , curvilinear distance along the pathway. The comparison of closed and open states shows reduced hydrophobicity and/or increased width of the permeation pathways. Note that the intermediate pathway in the “closed” state could not be detected and analyzed. The approximate ranges of pathway-lining residues are indicated for each of the three pathways.

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Availability of data and material: The MD simulation datasets are available from the corresponding author(s) upon reasonable request.

Code availability: Selected analysis scripts, parameter files used to reproduce the MD simulations, and parameter files used in MOLE2 analysis will be provided through Github upon publication or request during review.

Authors' contributions: Z.W.Z., R.P. designed and performed the MD simulation study; Z.W.Z. prepared the figures and performed analyses with R.P. and P.L.; all authors contributed to interpreting the results and writing the manuscript.

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Figures

Figure 1

Overview of the CFTR channel pore. (A) Simulation snapshot showing the waterfilled channel cavity (inner vestibule). The inner vestibule is accessible to water from the intracellular space through the “cytosolic portal” (cyan arrow) formed by TM helices 4 and 6. Phosphorus atoms (cyan spheres) of POPC headgroups indicate membrane position. Bulk water and lipid tails are omitted for clarity. (B) Spatial view of occupancy of Cl⁻ ions (green spheres) inside the channel cavity from many timesteps overlaid. (C) Cartoon representation of TM helix segments lining the bottleneck region of the pore together with Cl⁻ ions drawn from multiple timesteps. (D) The bottleneck region of the pore is lined by many hydrophobic residues (grey), disavouring the presence of water (red surfaces). Selected hydrophilic/charged residues (cyan) are also shown.

Figure 2

Effect of transmembrane voltage on water and Cl⁻ occupancy of the CFTR channel. (A) Probability distribution of the number of Cl⁻ ions occupying the inner vestibule (blue) without and (green) with applied voltage. Error bars represent bootstrapped 99% confidence intervals. (B) Snapshot of the CFTR channel filled with water molecules used as positional reference for (C)-(E). (C) Average number density of water molecules (N_{water}) in the channel vestibule computed along the z -axis. The bottleneck region is highlighted in yellow shading. (D) Average population density of Clions, $\rho(\text{Cl}^-)$, inside the channel pore (blue) without and (green) with -500 mV transmembrane voltage. The Cl⁻ occupancy of the region $-20 < z < 0$ Å is enhanced by voltage. (E) Cumulative population densities of Cl⁻ ions, $n(\text{Cl}^-)$, inside the channel pore computed by integrating $\rho(\text{Cl}^-)$ from (D).

Figure 3

Chloride solvation in the inner vestibule. (A) Left: overview of the Cl^- permeation pathway with overlaid ions (green spheres) from simulations in the presence of voltage with TM helices 1 (blue), 2 (red), 3 (cyan), 4 (orange), 6 (tan), 8 (lime), 11 (purple), and 12 (yellow). The locations of two Cl^- binding sites 1 and 2 are indicated in dashed boxes. Right: close-up views of binding sites 1 and 2. Amino acid sidechains in direct contact with Cl^- ions (green spheres) are shown. The labels of positively charged

residues, including K246 and K370 from the cytosolic portal, are shaded in indigo. (B) Coordination of Cl⁻ ions inside the channel cavity from simulations with voltage. The average numbers of protein residues (black) and water molecules (red) in the first solvation shell of Cl⁻ ions (nsolv) along the intracellular pathway are shown together with their sum (green). Shading represents 99% bootstrapped confidence intervals. Error bars are largest in the bottleneck region, which is least visited.

Figure 4

Spontaneous permeation of Cl⁻ ions in the CFTR channel under applied voltage. Left: overview of the Cl⁻ permeation pathway with overlaid ions (green spheres) from simulations in presence of voltage highlighting TM helices 4 (orange) and 6 (tan). Right: time series of axial Cl⁻ ion positions from the two simulation runs in which permeation occurred (-500 mV). Ion permeation events are indicated by traces passing through the bottleneck region. Individual Cl⁻ ions are distinguished by color for clarity.

Figure 5

Chloride translocation pathway through the bottleneck region. Top center: overview of the Cl⁻ permeation pathway with overlaid ions (green spheres) from simulations in the presence of voltage with TM helices 1 (blue), 2 (red), 3 (cyan), 4 (orange), 6 (tan), 8 (lime), 11 (purple), and 12 (yellow). (A)-(D) Close-up views showing the residues interacting with permeating Cl⁻ ions. The labels of positively charged residues are shaded in indigo. (E) Coordination of Cl⁻ ions inside the channel cavity from simulations with voltage.

The average numbers of protein residues (black) and water molecules (red) in the first solvation shell of Cl⁻ ions (nsolv) along the intracellular pathway are shown together with their sum (green). Shading represents 99% bootstrapped confidence intervals. Chloride ions are less hydrated in the bottleneck region (yellow shading). (F) Significant amino-acid contributions (various colours) to the protein solvation number nsolv (black).

Figure 6

The three different pathways of Cl⁻ permeation through the extracellular bottleneck. (Top row) Side views and (bottom row) top views from extracellular space. Pore-lining helices are colour-coded as indicated. Chloride ions (cyan) overlaid from timesteps during translocation. (A), (D) 1-6 pathway; (B), (E) intermediate pathway; (C), (F) 1-12 pathway.

Figure 7

Outer Cl⁻ binding sites at the extracellular mouth. Boxed regions in the overview of the pore (A) are expanded in insets showing Cl⁻ exit binding sites for (B) the 1-12 pathway and (C) the intermediate pathway. TM helices 1 (blue), 2 (red), 3 (cyan), 4 (orange), 6 (tan), 8 (lime), 11 (purple), and 12 (yellow) are highlighted. Residue sidechains interacting with Cl⁻ ions are shown.

Figure 8

Structural fluctuations of TM helices lining the extracellular bottleneck region. (A) Top view of the pore from the extracellular side highlighting amino acid residues of the pore-lining helices used to track TM helix movements. (B)-(C) Distributions of xypositions of the extracellular ends of TM helices 1, 6, 8, and 12 in the absence and presence of transmembrane voltage. (D)-(E) Time evolution of TM helices 1, 6, 8, and 12 in conductive simulation runs #1 and 2 (-500 mV). In run #1, a brief departure of TM6 away from the other helices is seen towards 1000 ns. In run #2, TM1 shows significant fluctuations of up to ~ 5 Å from its average location.

Figure 9

Time evolution of interhelical distances between TM 1 and other pore-lining helices at the extracellular end. Interhelical distances are computed as the distances between Ca atom pairs of the following residues: d_{1-6} : I106-R334 (top row); d_{1-8} : I106-Y914 (middle row); and d_{1-12} : I106-I1131 (bottom row). (A), (D), (G) Time series of interhelical distances for run #1 (orange traces); (B), (E), (H) same for #2 (pink traces). Chloride translocation events (see Fig. 4) are indicated as vertical lines. (C), (F), (I) Distributions of interhelical distances in the absence (blue) and in the presence (green) of an electric field.

Figure 10

Analysis of hydration of the extracellular bottleneck region. (A) Distributions of the number of water molecules in the bottleneck region, $N_{\text{water}}(\text{bottleneck})$, from nonconductive simulations in the presence (green) and absence (blue) of TM voltage. Results computed from conductive simulation runs #1 and #2 are shown separately. (B)-(C) Time evolution of $N_{\text{water}}(\text{bottleneck})$ for simulation runs #1 (orange) and 2 (pink). (D)-(I) Time series of solvent accessible surface areas (SASA) of residues P99, L102, and I106

found in the narrowest region of the bottleneck for simulation runs #1 [(D)-(F)] and 2 [(G)-(I)]. The time series suggest temporal correlation with ion permeation. (J)-(L) Distributions of SASA of these residues in the absence (blue) and in the presence (green) of TM voltage are shown on the rightmost column. Ion translocation events (see Fig. 4) are indicated as vertical lines.

Figure 11

Structural and physico-chemical analysis of diverging permeation pathways through the extracellular bottleneck region. (A) 1-6, (C) 1-12, and (E) intermediate pathways are visualized as translucent closed surfaces. Estimates of width and hydrophobicity along the 1-6 (B), 1-12 (D), and intermediate pathways (F); R, local radius and ζ, curvilinear distance along the pathway. The comparison of closed and open states shows reduced hydrophobicity and/or increased width of the permeation pathways. Note that the intermediate pathway in the “closed” state could not be detected and analyzed. The approximate ranges of pathway-lining residues are indicated for each of the three pathways.

Supplementary Files

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