# Crystal structure and functional mechanism of a human antimicrobial membrane channel

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Multicellular organisms fight bacterial and fungal infections by producing peptide-derived broad-spectrum antibiotics. These host-defense peptides compromise the integrity of microbial cell membranes and thus evade pathways by which bacteria develop rapid antibiotic resistance. Although more than 1,700 host-defense peptides have been identified, the structural and mechanistic basis of their action remains speculative. This impedes the desired rational development of these agents into next-generation antibiotics. We present the X-ray crystal structure as well as solid-state NMR spectroscopy, electrophysiology, and MD simulations of human dermcidin in membranes that reveal the antibiotic mechanism of this major human antimicrobial, found to suppress *Staphylococcus aureus* growth on the epidermal surface. Dermcidin forms an architecture of high-conductance transmembrane channels, composed of zinc-connected trimers of antiparallel helix pairs. Molecular dynamics simulations elucidate the unusual membrane permeation pathway for ions and show adjustment of the pore to various membranes. Our study unravels the comprehensive mechanism for the membrane-disruptive action of this mammalian host-defense peptide at atomistic level. The results may form a foundation for the structure-based design of peptide antibiotics.

crystallography | electrophysiology | ion conduction | molecular dynamics

Host-defense peptides actively control a wide range of microbes across most tissues of the animal and plant kingdoms, which signifies their importance during the evolution of multicellular organisms (1–3). In comparison with traditional small-molecule antibiotics, host-defense or antimicrobial peptides (AMPs) are often considered to have a distinctly superior property, as they target the microbial Achilles heel, i.e., the unique but essential features of all microbial cellular membranes (albeit at somewhat lower efficacy) (1–5). Thus, microbes have not been able to develop efficient resistance mechanisms against AMPs within the time frame of their parallel evolution (3, 5).

In recent years, a steep global rise in infections by multi-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) has been recorded (6, 7). These often affect the patients' skin and epithelial injuries, and are particularly hard to treat with conventional small-molecule antibiotics (8). The development of high-efficiency antibiotic agents, less prone to evoking resistance, is thus essential (4–7). However, the rational design of AMPs requires a detailed understanding of their structural and mechanistic determinants of antimicrobial action, which has not been achieved to date (4, 9, 10). The lack of molecular-based understanding has been named as the main obstacle hampering progress in this field (11).

The human epithelium exposes a large external surface for the growth of microbes (12). Among the major AMPs detected on human skin is the negatively charged peptide dermcidin (DCD; refs. 13–15), which is constitutively produced in sweat glands as

a precursor protein, further processed and finally secreted into human sweat (refs. 13 and 16; Fig. S1 *A–C*). DCD is active against a broad spectrum of bacteria including MRSA and rifampin- and isoniazid-resistant *Mycobacterium tuberculosis* at concentrations of ∼1 μg/mL (16). Its antimicrobial activity is particularly robust against changes in pH and ionic strength (13, 16). When isolated from sweat or after recombinant expression, DCD forms an equilibrium mixture of oligomers of varying size, both in solution and in membrane mimetics (16, 17). Human sweat is enriched in divalent ions, among which $Zn^{2+}$ is of particular importance and has previously been demonstrated to be essential for AMP action on some microbes (18, 19).

AMPs are classified according to their overall charge, secondary structure, and more specifically the presence of certain amino acid combinations such as cysteines or prolines (1, 9). Many AMPs carry an excess of positive charges to interact favorably with the negatively charged surface of bacterial membranes (1–3). Although a number of models for the membrane-disrupting action of AMPs have been proposed, detailed and compelling structural and mechanistic evidence for any of these models involving mammalian (or human) AMPs has so far been elusive (4, 9, 10). To elucidate the antibiotic mechanism of DCD and reveal the underlying structural determinants, including the level of oligomerization, we crystallized the 48-residue DCD peptide (Fig. S1*A*), determined its channel-forming structure, and looked at membrane interaction with solid-state (ss) NMR spectroscopy. We then conducted electrophysiology experiments in which we characterized the activity of DCD in membranes under various conditions. The experimental results are in excellent agreement with data we obtained from extended computational electrophysiology and molecular dynamics (MD) simulations of the channel assembly, which further reveal the ion-transfer mechanism of DCD in atomic detail.

## Results

**Structural Architecture of the Hexameric AMP Channel.** The structure of assembled DCD solved at 2.5-Å resolution exhibits a channel architecture comprising a hexameric bundle formed by

elongated α-helices, which adopts overall dimensions of ~8 × 4 nm (Fig. 1A). Channel formation involves trimerization of antiparallel peptide dimers resulting in a firmly enclosed channel structure. Each monomer presents two distinct interfaces to neighboring subunits (Fig. 1A). The extended interface, displaying a contact surface of 930 Å$^2$, is mainly formed by salt bridges, and the second interface covers 520 Å$^2$ (IF1) and is primarily stabilized by Zn$^{2+}$ ions that intercalate between the two helices (Fig. 1A and Fig. S1D). The zinc ions in DCD are coordinated by N- and C- terminal residues of dimerizing peptides (residues involved: Glu5, Glu9, His38′, and Asp42′) (Fig. 1 A and B). They are attached to the inner wall of the channel and substantially change the overall charge of the assembly (from −12e to neutral). In addition, they modify the local charge distribution especially at the entrance of the channel.

DCD shows a unique distribution of charges, reflecting peptide oligomers that occur both in soluble and membrane-bound forms. The entire hexameric channel comprises 96 ionizable residues, which are all oriented toward the channel interior (Fig. 1 C, F, and G). This enormous charge density, which is not completely shielded to the exterior, is likely to contribute to the relatively high aqueous solubility of DCD. The inner space of the peptide channel has an apparent separation into five radially symmetric charge girdles I/II/III/II/I (Fig. 1C). Residues facing

the acyl chain region of the membrane are exclusively hydrophobic (Ala, Val, Leu) (Fig. 1D and Fig. S1A). However, no aromatic residues are found that are commonly considered to be important for the lateral adjustment of proteins in membranes (20, 21). The channel diameter is not homogeneous and varies along the y axis with two rather narrow entry sites, followed by a widened interior with windowlike eyelets in the IF1 interface (Fig. 1 A, E, and H). The six lateral openings have a diameter of ~1 nm and are surrounded by small amino acids as well as positively charged residues that may have an influence on the selection of ion entry. The distance between two adjacent eyelets is 2.5 (same plane) and 3 nm (opposite plane), respectively, roughly corresponding to the width of the membrane hydrophobic core.

**Interaction of DCD with Lipid Bilayers.** To investigate the interaction of DCD with bilayers, we conducted ss-NMR spectroscopy experiments. The structural preferences of DCD were investigated after labeling of the peptide with $^{15}$N at the Gly22 position and $^2$H$_3$-Ala at position 25, its reconstitution into oriented lipid bilayers and investigation by proton-decoupled $^{15}$N ss-NMR spectroscopy. DCD labeled with $^{15}$N at Gly22 exhibits a $^{15}$N chemical shift of (62 ± 2) ppm in oriented 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine/1-palmitoyl-2-oleoyl-
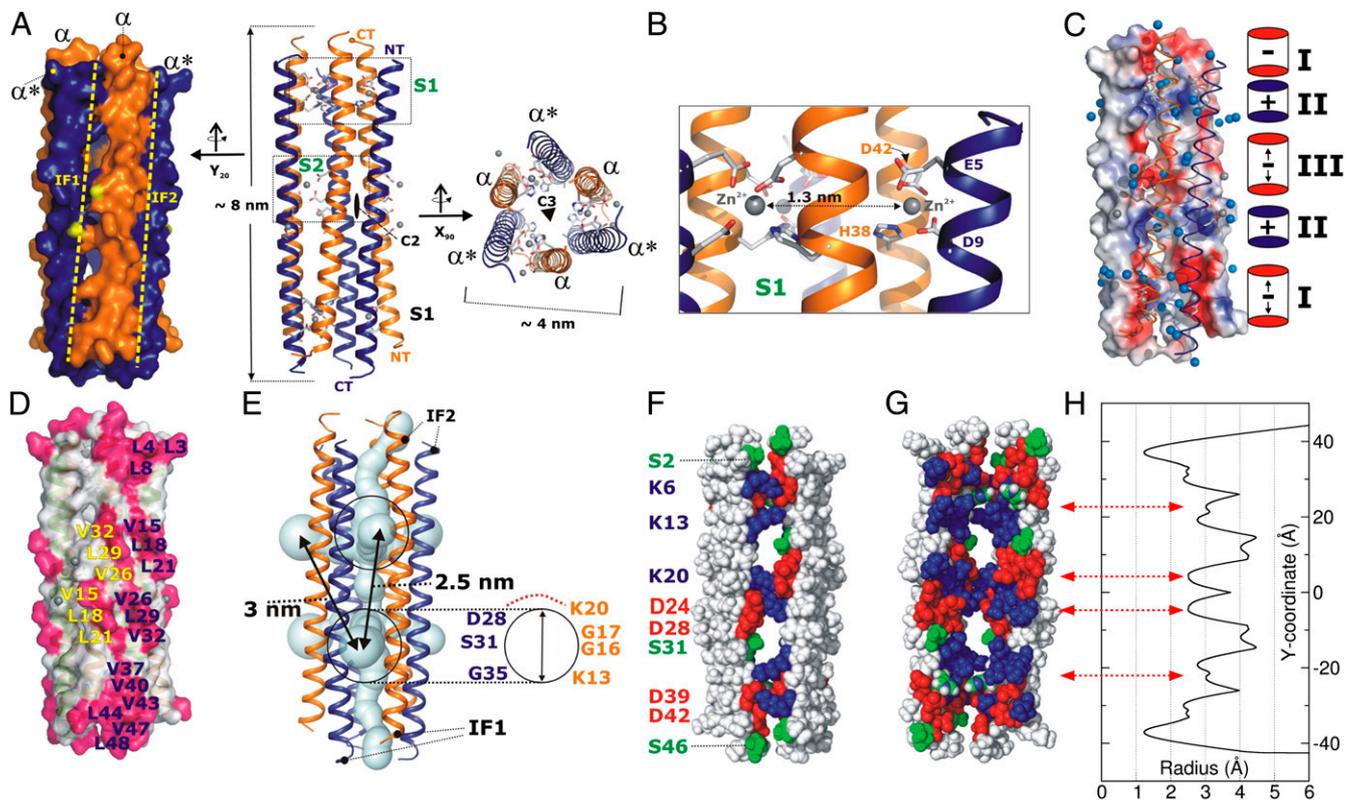


**Fig. 1.** Crystal structure and surface characteristics of the human dermcidin channel. (A) X-ray structure of the hexameric DCD channel shown in cartoon representation from the side and top (*Middle* and *Right*), and as surface representation (*Left*). The different orientations of the individual peptides relative to the membrane normal are marked in orange and dark blue, and termini are marked (NT, N terminus; CT, C terminus). Arrows combined with tilt angle and axes give the relative orientation. Residues involved in Zn binding are shown in stick representation and Zn ions are marked in gray. The symmetry axis of the channel is marked with C2 (for the side view) and C3 (for the top view). Two interfaces of different surface area are formed after trimerization and named IF1 and IF2. (B) Close-up into the Zn-binding site S1. Four residues (Glu5 and Asp11 from one peptide and Asp41 and His38 from the second) form each Zn-binding site. The distance between the Zn ions is marked by arrows. (C) Electrostatic surface representation of the channel with two monomers marked in ribbon representation. The channel comprises five alternating patches of elongated negative (red) and ring-like positive (blue) charge. (D) Side view of DCD (hydrophobic residues in magenta). (E) Ribbon model of DCD. The pore diameter is represented by spheres. The lateral entry points are marked with circles. (F) Hydrophilic residues on the trimeric interface (negatively charged residues in red, positively charged residues in blue, polar residues in green). Nonpolar residues are shown in white. (G) The hydrophilic channel interior. For clarity, the front dimer is omitted; colors as in F. (H) Channel radius along the pore axis.

*sn*-glycero-3-phospho(1'-rac-glycerol) (POPE/POPG, 3:1) membranes both in the absence or presence of a 10-fold excess of $Zn^{2+}$ (Fig. 2 *A* and *B*). This indicates an in-planar alignment of the major population of the labeled peptide domain (22), in agreement with previous oriented circular dichroism data (16). The chemical shifts are within experimental error identical in POPE/POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, and DPhPC/cholesterol 9:1 membranes and also independent of zinc ions ($ZnCl_2$ salt) and the sample preparation protocol. The $^2H$ ss-NMR spectrum of the $^2H_3$-Ala25 labeled site exhibits an $^2H$ quadrupolar splitting of $(45 \pm 3)$ kHz (Fig. 2*C*) and further restricts the tilt and pitch angle (23). A detailed topological analysis is shown by the red and black traces in Fig. S2 for the $^{15}N$ chemical shift and the $^2H$ quadrupolar splitting, respectively (see ref. 23). Both NMR parameters agree when their corresponding traces intersect leading to a set of tilt/pitch angular pairs in the proximity of (90°/90°), which would represent an ideal alignment of the amphipathic helix with the membrane interface (Fig. 2*B*). In agreement, the $^2H$ spectra of the fatty acyl chains show pronounced membrane disordering by the presence of DCD (Fig. S3).

### Functional Properties of the Channel Highlight the Importance of Zinc as Cofactor.

The solid-state NMR data represent probably the lowest energy state after extensive equilibration of the major population of the peptide and, in particular, in the absence of a membrane potential. In contrast, electrophysiological recordings monitor a population that may occur as a minor, but functionally relevant, species under the application of a transmembrane voltage. To obtain functional evidence for the activity of DCD channels in the membrane, we collected conductivity data in planar lipid bilayers. First, membranes were prepared in 1 M NaCl, 5 mM Hepes, pH 7.1 in the absence of $Zn^{2+}$. After the addition of DCD

in micromolar concentrations, two out of the six membranes showed weak activity of the peptide (Fig. 2*D*). Altogether, only 20 current steps were observed, from which a mean conductance of $G = (31 \pm 8)$ pS was evaluated.

By contrast, in the presence of $Zn^{2+}$, the addition of DCD at concentrations of 850 nM or higher resulted in current fluctuations for every membrane preparation, which eventually led to rupture of the membrane (Fig. 2 *E* and *F*). These concentrations are similar to DCD concentrations used to efficiently remove bacteria in antimicrobial assays. Evaluation of 1,009 events revealed a mean conductance of $G = (81 \pm 14)$ pS (Fig. 2*G*) and the mean open lifetime was determined to be $\tau = (4.4 \pm 0.2)$ ms (Fig. 2*H*). This shows that, in the presence of zinc ions, specific and individual channels with a defined conductance are formed in the membrane bilayer. Neither the membrane with DCD alone nor the addition of only $Zn^{2+}$ gave rise to the occurrence of individual channels in the electrophysiology experiments. However, we found that the polarity of the electric field had a significant impact on the efficiency of DCD-$Zn^{2+}$ insertion.

To further investigate the importance of the specific $Zn^{2+}$ interactions and their impact on the active membrane-bound form of DCD, we mutated His38, one of the main interaction partners of DCD with $Zn^{2+}$, to alanine and studied this mutant through electrophysiology. The single mutation was sufficient to abolish channel formation in membranes (Fig. S4). This finding is most compatible with the notion that the assembled structure, which is characterized by specific $Zn^{2+}$-conferred peptide–peptide interactions, is the most prominent membrane-disruptive form. It is important to note that the current fluctuations seen for DCD-$Zn^{2+}$ in electrophysiology are representative of a small number of channels efficiently conducting ions across membranes,
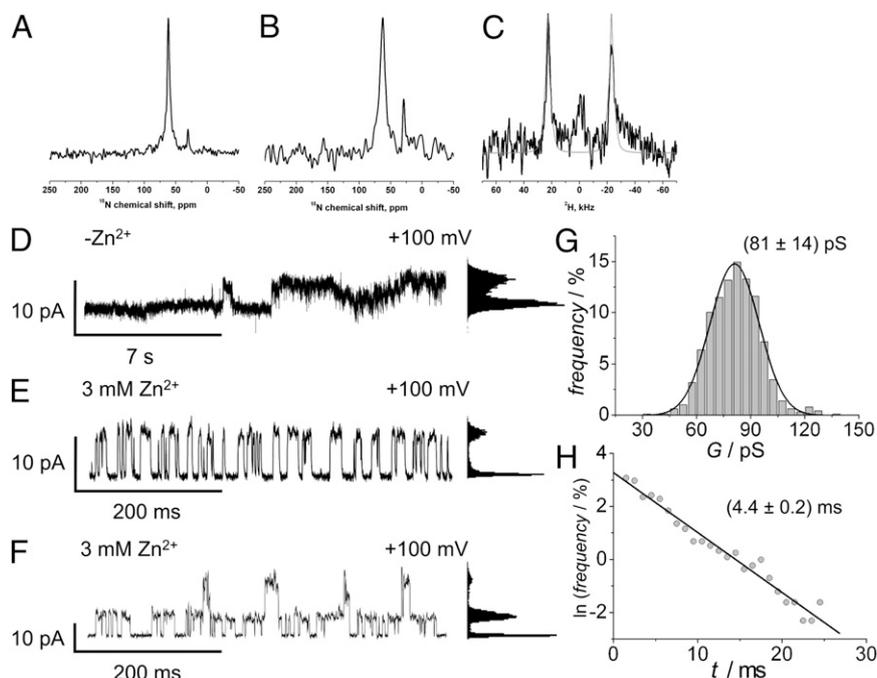


**Fig. 2.** Electrophysiology and ss-NMR of DCD in the presence and absence of zinc ions. (*A* and *B*) Proton-decoupled $^{15}N$ and (*C*) $^2H$ ss-NMR spectra of 2 mol % [$^{15}N$-Gly22,$^2H_3$-Ala25]-DCD in oriented POPE/POPG without $ZnCl_2$ (*A*) and with fivefold molar excess of $ZnCl_2$ relative to the peptide concentration (*B* and *C*). $^{15}NH_4Cl$ (40.0 ppm) and $^2H_2O$ (0 Hz) were used as external references and the spectra processed with an exponential line broadening of 50 (*A* and *B*) and 500 Hz (*C*), respectively. The gray line in *C* shows a simulated spectrum arising from a 3° Gaussian distribution for the peptide alignment and a spectral line width of 2 kHz. (*D*) Current trace and point amplitude histogram representing DCD activity in 1 M NaCl, 5 mM Hepes, pH 7.1 recorded at a holding potential of +100 mV. Protein activity was only rarely observed under these conditions. (*E* and *F*) Current traces and point amplitude histograms of DCD-1L in 1 M NaCl, 3 mM $ZnCl_2$, 5 mM Hepes, pH 7.1 recorded at a holding potential of +100 mV; (*E*) 3.3 μM DCD-1L was added resulting in one defined conductance state. In (*F*) 7.9 μM DCD-1L was used and two distinct conductance levels each of about 80 pS were monitored, suggesting the insertion of two channels. (*G*) An event histogram of the conductance levels (*n* = 1,009) shows a mean conductance of $G = (81 \pm 14)$ pS. (*H*) Open lifetime analysis with $\tau = (4.4 \pm 0.2)$ ms.

whereas the ss-NMR measurements observe the major peptide fraction only.

**Molecular Dynamics Simulations Reveal Unexpected Ion-Transfer Pathways.** To obtain a detailed mechanistic picture of DCD acting on membranes, we performed extensive atomistic MD simulations of the DCD channel in negatively charged, bacterial-like phosphatidyl ethanolamine/ phosphatidyl glycerol (POPE/POPG, 3:1) bilayers (24), and carried out computational electrophysiology simulations to monitor its ion permeation properties (Fig. S5). The DCD assembly including $Zn^{2+}$ exhibited a high level of structural stability in membranes (C-α root-mean-square deviation ~2 Å; Fig. S6). In aqueous solution, the aggregate was found to be stable, but showed a markedly raised structural variability (Fig. S6B). Consistent with our experimental data, removal of the $Zn^{2+}$ ions from the membrane-inserted assembly structure resulted in a substantial distortion of the oligomer, compromising its membrane-channel character (Fig. S6D). These findings agree well with previous NMR studies, in which both a membrane-mimetic solvent (TFE-$^2H_3$) and $Zn^{2+}$ were shown to induce oligomerization of DCD (16).

After initially positioning the complex normal to the bilayer plane, the assembly adopted tilted configurations relative to the membrane normal within 250 ns simulated time (γ~30°; Fig. 3A). The inclination was found to compensate for the hydrophobic mismatch between the bilayer and the hydrophobic region on the outer surface of DCD, and its magnitude depended on the length of the surrounding lipids (Fig. S7A).

During the simulations, the interior of the oligomer rapidly filled with water and formed a permanent water channel across the membrane (Fig. 3 B and C). Water also partitioned into the three hydrophilic crevices at the trimer interfaces, such that a high

osmotic water permeability coefficient of $(327 \pm 13) \times 10^{-14} \; cm^3 \cdot s^{-1}$ was determined that exceeds that of aquaporin channels by up to 50-fold (25). To investigate the conductivity of DCD for ions, we used computational electrophysiology to model biologically realistic electrochemical gradients across membranes (26). At 1 M NaCl concentration, we obtained a DCD channel conductance of $(108 \pm 11)$ pS, in excellent agreement with our experimental data (Figs. 2G and 3D). In simulations at a salt concentration of 150 mM, a total single-channel conductance of $(50 \pm 7)$ pS was obtained (Fig. 3D). In all of our simulations, DCD showed pronounced anion selectivity (Fig. S7B).

The remarkable agreement between computational and experimental data strongly suggests that the hexameric crystal structure is identical to the functional state in the membranes used for electrophysiology. Any higher or lower oligomerization state of DCD would very likely lead to markedly different conductance values (SI Materials and Methods and Fig. S8). This interpretation is corroborated by the strong dependence of channel current on zinc, which we observed in both the electrophysiology experiments and MD simulations in membranes, and which corresponds with the abundance and function of the $Zn^{2+}$ binding sites, linking the subunits in the crystal structure.

It is notable that DCD exhibited a unique ion-permeation pathway in the simulations, which offers an explanation for this unexpectedly high conductance (Movies S1 and S2), despite its limited channel cross-section. Through channel tilt, ions are capable of entering sideways into the pore across the eyelets that occur at the trimeric interfaces. This not only shortens the pathway across the channel, but importantly, exploits the increased ion concentration observed at the lipid head groups by enabling these ions to enter the channel directly, and to rapidly traverse the inner pore (Fig. 3C and Movies S1 and S2). Also within the channel, DCD shows an unusual anion traversal mechanism. Most anion transfer steps across the inner section of the pore consist of single ion "hopping" transitions. Near the channel termini, however, anions accumulate to form clusters of three or four ions, most clearly seen at the channel exit. Productive ion translocations exiting the channel usually involve multiion "knock-on" effects, through which individual anions are expelled from this cluster to the bulk solution (Movie S1).

The stabilization of DCD oligomers by a membrane mimetic, seen in earlier NMR studies (16), is also corroborated by molecular dynamics simulations, in which we tested the long-term stability of the DCD assembly in a membrane environment vs. aqueous solution. Fig. S6B shows that the assembled structure with $Zn^{2+}$ shows a substantially raised stability in bilayers relative to solution, which indicates a preference of the hexameric assembly for the membrane environment.

## Discussion

The existence of antimicrobial agents in various secretions of multicellular organisms has been discovered over 100 y ago (9), and to date, more than 1,700 known and putative antimicrobial peptides have been identified (4). Although there has been little doubt that the antimicrobial mechanism of AMPs includes interactions with microbial cell membranes, compelling structural and functional evidence for any of these mechanisms has not yet been achieved, and it is possible that there are a variety of different modes of action (4, 9). High-resolution structural information on the functionally active states of AMPs has so far been limited to agents from microbes, insects, and on β-sheet forming defensins from higher organisms, which form a distinct structural class (14, 27–32). In terms of function, electrophysiological measurements have suggested the formation of wide channels with varying conductance around 2.5 nS for the moth AMP cecropin (33), and of very large-conductance pores for defensin from rabbit (34), however without providing structural information for the assemblies.



**Fig. 3.** Computational electrophysiology and molecular dynamics simulations of the channel. (A) Evolution of DCD tilt in three 250-ns single-bilayer MD simulations in POPE/POPG(3:1). (B) Water distribution (red/white spheres) in and around the DCD channel in the bilayer (gray); view along the channel axis. (C) Pathway of ion permeation. One complete Cl⁻ permeation event is shown, with red spheres representing snapshots of Cl⁻ position. The brown arrows indicate the direction of permeation. (D) The I–V relationships from computational electrophysiology simulations. Each data point was obtained from a 100-ns MD trajectory by monitoring the number of permeation events (and thus the current) and the overall transmembrane potential during this time window. Blue and green circles are from simulations with 0.15 M and 1 M NaCl in solution, respectively. Dashed lines: linear fit of the conductance data, with the slope (and the asymptotic SE) showing overall conductance.

Here, we present a comprehensive functional mechanism of a human antimicrobial peptide in its active form, including its high-resolution structure, single-channel functional measurements, and atomistic simulations of permeation. In the absence of transmembrane voltage (TMV), in-plane alignments have been observed for DCD and other helical antimicrobial peptides such as magainins or cecropins (Fig. 2 *A–C*; reviewed in ref. 35). However, important differences exist between the latter peptides and DCD, already when their size and charge are compared. Of note is that when TMV is applied, the channel recordings reveal mostly membrane lysis in the case of magainins or cecropins, and only rarely are the stepwise conduction increases clearly observed with DCD. The electrophysiological recordings of lysis or stochastic membrane deformations, however, are erratic, highly variable, and characterized by considerable fluctuations. Therefore, it is likely that the tilted hexamer of DCD provides an example for a hitherto undescribed mechanism for the membrane interactions of antimicrobial peptides (for reviews of other mechanisms, see ref. 36). The ensemble of data from the herein and previously presented studies (16) suggest that DCD occurs in a number of states that are interconnected by sensitive equilibria as illustrated in Fig. 4, including oligomers and monomers in solution, as well as monomers or small oligomers lying parallel to the membrane surface (Fig. 2 *A–C*). Furthermore, transmembrane potentials and the presence of $Zn^{2+}$, by neutralizing the anionic charges of the DCD polypeptides, favor the formation of oligomeric structures spanning the membrane (Fig. 2 *D–H*). These channels display a conductance of about 80 to 110 pS.

Although the suggested mechanisms of AMP action have so far remained largely speculative (4, 9, 10), our study on DCD provides a structurally detailed and experimentally validated mechanism for membrane perturbation by an AMP. It is striking that none of the currently debated models explicitly predict this channel. Our X-ray, electrophysiology, and simulation data show a barrel-stave–like channel (10), which is held together by divalent

cations and displays a high permeability for water and ions, as ions can enter and exit through side eyelets of a tilted pore. DCD thereby forms a site of severe membrane disruption. As recently noted, a single ion channel with a conductance near 100 pS can dissipate the bacterial transmembrane potential on a time scale of as little as $10^{-4}$ s (37). The DCD channel characterized here thus represents highly efficient channels, and even a few of which are capable of rapidly abrogating the bacterial transmembrane potential that is essential for cell survival. Its structure and functional mechanism may form a foundation for the rational design of antibacterial, peptide-derived drugs that are less liable to evoke resistance.

## Material and Methods

**Crystallization and Structure Determination.** The DCD peptide sequence (Fig. S1) was synthesized by the company Peptide2.0 (www.peptide2.com) to yield a product of >97% purity. For crystallization, the peptide was solved in 10 mM Hepes at a concentration of 50 mg/mL (~10 mM final concentration). Crystallization drops were prepared by mixing 400 nL of the peptide solution with the same amount of reservoir, and drops were incubated by vapor diffusion (sitting drops) under a variety of different conditions (800 conditions from Qiagen screens). Two conditions, both of which contained Zn-acetate, yielded crystals suitable for X-ray diffraction analysis. Crystals for X-ray diffraction were taken from a drop containing 0.2 M Zn(ac)$_2$, 0.1 M Na-cacodylate, 18% (wt/vol) PEG8000 at pH 6.5. The channel has been crystallized in the absence of lipophilic molecules such as detergents or lipids. Crystals were taken from the mother liquor and flash-frozen in liquid nitrogen without adding cryoprotectant. Data were collected at the Swiss Light Source, beamline PXII at 100 K and 20 eV above the theoretical Zn-edge (9.658 keV). Three hundred sixty 1° images were collected at 2% beam intensity to generate a highly redundant dataset for single anomalous dispersion data processing. Diffraction data were processed with XDS/XSCALE (38). The structure was solved with the Phenix program package (39) with Phaser (38) for heavy atom detection and Resolve (40) for initial model building. Solvent flattening was repeated by Pirate of the CCP4 suite (41) and a model 85% complete was built with Buccaneer (42). The final model was obtained after several rounds of manual rebuilding and refinement with Coot (43), REFMAC (44), and PHENIX (39). For more details see *SI Materials and Methods* and Table S1.

**Solid-State NMR Spectroscopy.** Samples were prepared by dissolving 4 mg of peptide and ~30 mg of lipid in TFE/water 50/50 vol/vol, dried in air and under high vacuum, applied onto 20 ultra-thin cover glasses, and equilibrated at 93% relative humidity, as described previously (45). Where appropriate, ZnCl$_2$ was added in five- to ten-fold molar excess over the peptide. As an alternate, peptide and ZnCl$_2$ were added to preformed vesicles, and the resulting mixture was applied onto the glass plates (45). The ss-NMR spectra were recorded on a Bruker Avance wide-bore NMR spectrometer operating at 9.4 T with flat-coil probes as described previously (46). Orientational restraints were calculated from the solid-state NMR spectra as described in (46) by using the chemical shift tensor (44, 65, 211) ppm, a maximum quadrupolar splitting of 74 kHz for the alanine $^2H_3C$ group and DCD coordinates from the crystal structure. For further details of ss-NMR methods and data analysis see *SI Materials and Methods*.

**Electrophysiology.** Giant unilamellar vesicles (GUVs) composed of DPhPC/cholesterol (9:1) were prepared by the electroformation method with a 3-V peak-to-peak AC voltage at a frequency of 5 Hz for 2 h at 20 °C in the presence of 1 M sorbitol (47–49). A freestanding membrane was prepared by spreading a GUV in 1 M NaCl, 5 mM Hepes, pH 7.1 on an aperture in a borosilicate chip (Port-a-Patch; Nanion Technologies) by applying 15–40-mbar negative pressure resulting in a solvent-free membrane with a resistance in the GΩ range. After successful membrane formation, varying amounts of DCD stock solution (100 μM) were added to 50-μL buffer solution, while applying a DC potential of +100 mV. Current traces were recorded at a sampling rate of 50 kHz using an Axopatch 200B amplifier (Axon Instruments). They were filtered with a low-pass four-pole Bessel filter of 1 kHz and digitized by an A/D converter (Digidata 1322; Axon Instruments). Data evaluation was performed with the pClamp 9 software package (Axon Instruments). For additional details see *SI Materials and Methods*.

**Molecular Dynamics and Computational Electrophysiology Simulations.** All MD simulations were performed with the GROMACS package, version 4.5 (50) in combination with the CHARMM36 force field (51). Unless otherwise stated, the simulation temperature was 310 K. The protein, lipids, and water/ions



**Fig. 4.** Model of DCD interactions with membranes. Peptide monomers of DCD are in equilibrium with the hexameric channel form, which is stabilized by the presence of zinc. The channel and DCD monomers can interact with membrane surfaces through lateral association as shown by our NMR studies. The channel can be translocated into the membrane upon application of a TMV, as shown in planar lipid membrane conductance measurements, and forms a representative of the barrel-stave model. Once the channel is integrated into a membrane, channel conductance can be recorded by planar lipid membrane experiments and estimated by molecular dynamics studies.

were coupled separately to a temperature bath with the v-rescale method with a time constant of 0.1 ps (52). Short-range electrostatics were calculated with a cutoff of 1.3 nm. Long-range electrostatics were treated with the particle-mesh Ewald method (53). Short-range Van der Waals (VdW) interactions were calculated explicitly up to a distance of 0.8 nm, beyond which a switch function was used to smoothly switch off the VdW interactions to reach 0 at 1.2 nm. All bonds were constrained using the LINCS method (54). The time step was 2 fs for all-atom MD simulations and 4 fs for simulations with the virtual site model for hydrogen atoms (55), respectively. To study whether the oligomer is ion conductive, we used the computational electrophysiology method (26). For additional details see *SI Materials and Methods*.

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BIOPHYSICS AND
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# Supporting Information

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### SI Materials and Methods

**Crystallization and Structure Solution of the Dermcidin Peptide.** The dermcidin peptide sequence (Fig. S1) was synthesized by the company Peptide2.0 (www.peptide2.com) to yield a product of >97% purity. For crystallization, the peptide was dissolved in 10 mM Hepes at a concentration of 50 mg/mL (~10 mM final concentration). Crystallization drops were prepared by mixing 400 nL of the peptide solution with the same amount of reservoir, and drops were incubated by vapor diffusion (sitting drops) under a variety of different conditions (800 conditions from Qiagen screens). Two conditions, both of which contained Zn-acetate, yielded crystals suitable for X-ray diffraction analysis. Crystals for X-ray diffraction were taken from a drop containing 0.2 M Zn(ac)$_2$, 0.1 M Na-cacodylate, 18% (wt/vol) PEG8000 at pH 6.5. Crystals were taken from the mother liquor and flash-frozen in liquid nitrogen without adding cryoprotectant. Data were collected at the Swiss Light Source, beamline PXII at 100 K and 20 eV above the theoretical Zn-edge (9.658 keV). Three-hundred sixty 1° images were collected at 2% beam intensity to generate a highly redundant dataset for single anomalous dispersion data processing. Diffraction data were processed with XDS/XSCALE (1). The structure was solved with the PHENIX program package (2) with Phaser (3) for heavy atom detection and Resolve (4) for initial solvent flattening. Solvent flattening was repeated by Pirate of the CCP4 suite (5) and a model with 85% completeness was built with Buccaneer (6). The final model was obtained after several rounds of manual rebuilding and refinement with Coot (7), REFMAC (8), and PHENIX (2). The structural geometry of all ligand structures was verified with Whatcheck (9); values are given in Table S1. Crystal structure images were generated with Pymol (Schrodinger). Sequence alignment of human dermcidin (DCD) (Fig. S1) was performed against sequences deposited to the current EXPASY (10) sequence database. Sequences similar to DCD were extracted with a PSI-BLAST (11) search. In addition, the secondary structure was predicted with PSIPRED (12).

**Electrophysiology.** Giant unilamellar vesicles (GUVs) were prepared by the electroformation method (13–15). A lipid mixture composed of diphytanoyl phosphatidylcholine (DPhPC)/cholesterol (9:1) dissolved in chloroform at a concentration of 10 mM (6.25 μL) was deposited on indium-tin-oxide (ITO) coated cover slides and allowed to dry. Two slides were connected to form a sealed chamber electrically connected to a voltage generator (33210A; Agilent Technologies). The lipid was rehydrated in approximately 2 mL 1 M sorbitol and GUV formation was carried out by application of a 3-V peak-to-peak AC voltage at a frequency of 5 Hz for 2 h at 20 °C.

A freestanding membrane was prepared by spreading a GUV on an aperture with a size of a few micrometers in diameter in a borosilicate chip (Port-a-Patch; Nanion Technologies). Two microliters of the GUV-containing solution were added to 6-μL buffer (1 M NaCl, 5 mM Hepes, pH 7.1) and a GUV was gently sucked onto the aperture by application of 15–40-mbar negative pressure. Spontaneous spreading of the GUV on the surface gave rise to a solvent-free membrane with resistances in the GΩ range.

After successful membrane formation, 50 μL of buffer were added and varying amounts of DCD-1L stock solution (100 μM) were added at a DC bias of 100 mV. Current traces were recorded with an Axopatch 200B amplifier (Axon Instruments). The analog output signals were filtered with a low-pass four-pole Bessel filter of 1 kHz and subsequently digitized by an A/D converter (Digidata 1322; Axon Instruments). The sampling rate was 50 kHz. Data evaluation was performed with the pClamp 9 software package (Axon Instruments).

**Solid-State NMR Spectroscopy.** Samples for solid-state NMR spectroscopy were prepared by dissolving 4 mg of peptide and ~30 mg of lipid in TFE/water 50/50 vol/vol. Where appropriate ZnCl$_2$ was added in five- to ten-fold molar excess over the peptide. The solutions were mixed and carefully applied onto 20 ultra-thin cover glasses (6 × 11 mm; Paul Marienfeld) as described previously (16). Care was taken to remove all organic solvent in high vacuum. Thereafter, the samples were equilibrated at 93% relative humidity for several days.

An alternative preparation method consisted in mixing only the lipid in organic solvent and forming a dry film by evaporating the solvent and exposure to high vacuum (16, 17). Then 0.2 mL of water was added and a transparent vesicular suspension was prepared (five freeze/thaw cycles between liquid nitrogen and 310 K water bath). Thereafter 2 mg of peptide and 62 μg of ZnCl$_2$ were codissolved in 100 μL of water and added to the membrane vesicles, vortexed, and incubated for 4 h. The mixed lipid/peptide vesicles were applied onto glass plates, dried in air overnight, and equilibrated at 93% relative humidity.

Solid-state NMR spectra were recorded on a Bruker Avance wide-bore NMR spectrometer operating at 9.4 T. A commercial double-resonance solid-state NMR probe modified with flattened coils of dimensions 15 × 4 × 9 mm was used. Proton-decoupled $^{15}$N solid-state NMR spectra were acquired using a cross-polarization sequence and processed as described previously (18). The temperature was 310 K for 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE)/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho(1′-*rac*-glycerol) (POPG) and 295 K for 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and DPhPC/cholesterol 9:1 membranes. NH$_4$Cl (40.0 ppm) was used as an external reference corresponding to 0 ppm for liquid NH$_3$. An exponential apodization function corresponding to a line broadening of 50 Hz was applied before Fourier transformation.

Deuterium solid-state NMR spectra were recorded using a quadrupolar echo pulse sequence (19). The spectra were referenced relative to $^2$H$_2$O (0 Hz). An exponential apodization function corresponding to a line broadening of 500 Hz (for the $^2$H$_3$-peptide) or 30 Hz (for chain deuterated lipids) was applied before Fourier transformation. The deuterium order parameters are analyzed following reference (20).

To verify the alignment of the lipids, proton-decoupled $^{31}$P solid-state NMR spectra were recorded using a Hahn-echo pulse sequence as described previously (18) and referenced relative to 85% phosphoric acid (0 ppm).

**Calculation of Orientational Restraints from the Solid-State NMR Spectra.** To evaluate the peptide orientations that agree with the experimental spectra, a coordinate system was defined, with the tilt angle being the angle between the long axis of the helix and the membrane normal, and a pitch angle between membrane normal and the line within the arbitrary plane of peptide helical wheel projection (see Fig. S2B for angle definition). The calculations were performed using the $^{15}$N chemical shift main tensor elements for glycine (44, 65, 211) ppm (21) to agree with an isotropic chemical shift of 106.7 ppm and 74 kHz for the maximum quadrupolar splitting for the alanine $^2$H$_3$C-group at room temperature (22). The coordinates of DCD were from pdb:2ymk. By successively changing the tilt and pitch angles in (50 × 50 steps), the 3D topological space was systematically

screened and the corresponding $^{15}N$ chemical shift and quadrupolar splitting calculated (19). The restriction plot was calculated using azimuthal fluctuations of 18° Gaussian distribution and wobbling motions of 10°, similar to those which have been observed for other in-plane amphipathic helical peptides (e.g., refs. 23, 24). Contour plots mark the angular restrictions that agree with the experimental results.

**Molecular Dynamics Simulations.** All molecular dynamics (MD) simulations were performed with the GROMACS package, version 4.5 (25, 26) in combination with the CHARMM36 force field (27). Unless otherwise stated, the simulation temperature was 310 K. The protein, lipids, and water/ions were coupled separately to a temperature bath with the v-rescale method with a time constant of 0.1 ps (28). Short-range electrostatics were calculated with a cutoff of 1.3 nm. Long-range electrostatics were treated with the particle-mesh Ewald method (29, 30). Short-range Van der Waals (VdW) interactions were calculated explicitly up to a distance of 0.8 nm, beyond which a switch function was used to smoothly switch off the VdW interactions to reach zero at 1.2 nm. All bonds were constrained with the LINCS method (31). The time step was 2 fs for all-atom MD simulations and 4 fs for simulations with the virtual site model for hydrogen atoms (32), respectively.

The crystal structure of the DCD hexamer, as described in this study, was used to generate the initial configuration for our MD simulations. Specifically, the protein and six $Zn^{2+}$ ions at the monomeric interfaces of the DCD oligomer were taken from the crystal structure, which was then embedded into a mixed bilayer of palmitoyloleoyl-phosphatidylethanolamine and palmitoyloleoylphosphatidylglycerol (POPE/POPG; 3:1) lipids, which resembles the composition of the bacterial cytoplasmic membrane (33). To test the tilting of DCD in various bilayer environments, additional simulations were carried out in dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC), and diarachidonoyl phosphatidylcholine (DAPC) lipid bilayers. All membranes were generated with CHARMM-GUI (34).

The POPE/POPG bilayer consisted of 72 POPE and 24 POPG molecules in each bilayer leaflet, solvated by 13,938 TIP3P water molecules (35). Also, 82 $Na^+$ and 38 $Cl^-$ ions were added to the simulation system resulting in an ionic strength of about 150 mM. The POPE/POPG lipids were first energy-minimized for 5,000 steps with the steepest descent algorithm, then equilibrated in an NVT ensemble at 310 K for 500 ps, while position restraints were applied (1,000 kJ·mol$^{-1}$·nm$^{-2}$) on the lipid heavy atoms. Subsequently, the system was further equilibrated without any restraints under semi-isotropic Berendsen pressure coupling (36). The reference pressure was set to 1 bar and the pressure coupling time constant was 1.0 ps. After ~1 ns of simulation in the NPT ensemble, the area per lipid reached about 0.615 nm$^2$. This configuration was adopted for additional 15 ns of NPAT simulation to further equilibrate the system. The GROMACS utility g_membed (37) was used to insert DCD into the lipid bilayer. By comparing the lateral pressures to those of pure lipids, we found that by removing nine lipid molecules, the differences between the lateral pressures of DCD-embedded and pure systems are smallest. Therefore, approximately nine lipid molecules were removed in each leaflet before insertion of the DCD oligomer. To test convergence, we used three different initial configurations of the lipid bilayer in our MD simulations by taking frames at various points of the equilibration simulation (taken at 13, 14, and 15 ns from the above lipids equilibration simulation). The antimicrobial peptide (AMP) assembled structure was thus inserted into three different membrane

microenvironments. The adoption of three independent initial simulation systems can reduce possible bias on the simulation results arising from the initial system setup. One of the resulting single-bilayer simulation systems is shown in Fig. S5 *A* and *B*.

Starting from the three independent systems described above, we first performed 5,000 steps of steepest descent energy minimization, then 10 ns of NPAT equilibration simulations with the heavy atoms of the protein and $Zn^{2+}$ position restrained (1,000 kJ·mol$^{-1}$·nm$^{-2}$). These equilibration simulations allowed water molecules to fill the interior of the DCD oligomer. Monitoring of the number of water molecules showed that it no longer increased after 7.5 ns. Subsequently, 250-ns simulations were performed for all of the three systems under NPAT conditions. In addition, we performed three corresponding simulations without any $Zn^{2+}$ in the system to investigate the importance of $Zn^{2+}$ for the stability of the X-ray structure.

To study whether the oligomer is ion conductive, we used the computational electrophysiology method (38). Structures were taken from the three single-patch simulations at 100 ns and the systems were duplicated in the Y direction. The virtual-site model was used for peptides and lipids (32). A transmembrane electric field was generated by sustaining a slight imbalance of ions in the two compartments thus formed during the simulation by ion exchanges. This ionic imbalance evoked an electrostatic potential gradient across the two bilayers of ± 200–800 mV in multiple simulations, as determined by double integration of Poisson's equation (38). Under these conditions, six independent 200-ns production simulations were performed with a time step of 4 fs in the NPAT ensemble. The simulation system of the double-bilayer setup is shown in Fig. S5C.

To run simulations under conditions similar to our electrophysiological experiments, we also carried out computational electrophysiology simulations, in which 506 $Cl^-$ and 594 $Na^+$ ions were included in the double-patch system, which resulted in an ion concentration of ~1 M. Here again, an equal number of $Cl^-$ ions were included in the two compartments, and a small difference of $Na^+$ ions between compartment I and II was sustained during the simulation, which generated transmembrane potentials fluctuating in the range from ~20 to 300 mV. Subsequently, five independent simulations were performed to monitor ion permeation.

Altogether, we performed five independent double-patch simulations with 1M NaCl (200 ns each), six independent double-patch simulations with 150 mM NaCl (200 ns each), four single-patch simulations with various lipids (>250 ns each), three independent single-patch simulations (250 ns each), and three independent single-patch simulations in the absence of $Zn^{2+}$ (200 ns each). Simulation images were generated with Pymol (Schrodinger).

**Pore Size Analysis.** To good approximation, the conductance of membrane channels is proportional to their channel cross-sectional area; i.e., it varies with the square of the difference of the pore radii (39). Due to the fact that the channel is composed of units of antiparallel dermcidin dimers, it is reasonable to assume that channel-forming oligomers would consist of an even number of 4, 6, 8, etc., peptides. By using the peptide–peptide docking software m-Zdock (40), which is specialized for generating symmetrical pores, we find that (*i*) tetrameric assemblies are not capable of forming a pore, and (*ii*) peptide octamers with their hydrophobic face toward the membrane would have a pore diameter of about ~11 Å. According to our calculations, this pore size would lead to a conductance above 0.5 nS. This value is more than five times higher than that found in our experiments. Higher-order oligomers could be expected to have a further increased conductance. Hence, lower- or higher-order dermcidin channels are not a likely explanation for our experimentally measured channel currents.

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**Fig. S1.** Sequence and structural analysis of human DCD. (*A*) Sequence alignment of human DCD (isoform 1) and sequences deposited to the current EXPASY sequence database (www.expasy.org). Four sequences similar to DCD were extracted with a PSI-BLAST search, one of which is a second human isoform, and the remaining three sequences are derived from monkey genomes. Hydrophobic residues are colored in green, positively charged residues are blue, and negatively charged residues are red. Residues involved in Zn binding are marked and the similarity between the sequences chosen is represented by (*) for identical residues and (:) for similar residues. In addition, the secondary structure as predicted by PSIPRED is shown. (*B*) Analysis of the sequence for repetitive elements. The peptides show three of those elements (residue numbers are provided). (*C*) Amphipathic plot of the DCD peptide with Kyte–Doolittle values. (*D*) Analysis of the two peptide interfaces. Each peptide in the hexameric DCD structure faces two differently oriented peptide neighbors. Therefore, two different interfaces are formed, which are analyzed here with the PISA server software (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html). The size of the interfaces is given in Ångstrom and the residues important for interface stabilization are mentioned together with the residue numbers and distances.



**Fig. S2.** ss-NMR helix tilt/pitch angles. (*A*) The tilt/pitch angular pairs that agree with the $^{15}$N chemical shift and the $^2$H quadrupolar splitting observed in oriented POPE/POPG 3/1 (including error bars) are shown by the red and black traces, respectively. (*B*) The energetically most probable alignment is schematically illustrated. Neutral Leu, Val are colored in green, charged Lys, Glu and Asp in red, and Ala25 is in magenta.

**Fig. S3.** $^2$H ss-NMR spectra and order parameters of $^2$H-chain deuterated lipid membranes. (*A* and *C*) $^2$H solid-state NMR spectra of $^2$H-chain deuterated lipid membranes and (*B* and *D*) the corresponding deuterium order parameters. The spectra are recorded at 310 K from nonoriented and fully hydrated POPE/POPG 3:1 mol/mole membranes where the palmitoyl chains of either POPG (*A* and *C*) or POPE (*B* and *D*) are fully deuterated. In gray (squares) are shown the lipids alone, in black (inverted triangles) the pure lipids in the presence of ZnCl$_2$. The data in the presence of 2 mol % DCD are shown in blue and red (circles) in the absence and presence of zinc, respectively. The data with and without ZnCl$_2$ overlap in *B* as well as in *D* for the DCD/POPE/POPG mixtures.



**Fig. S4.** Electrophysiological measurements on the DCD mutant H38A. Even under addition of zinc ions, no specific current steps are observed.

**Fig. S5.** Model system used in the MD simulations. (*A*) The initial simulation system, which contains the X-ray structure of DCD (orange and deep blue cartoon helices), the POPE/POPG (3:1) lipids bilayer (gray sticks), water box (light blue surface), Na$^+$ (blue spheres), and Cl$^-$ (red spheres) ions. (*B*) The MD conformation after 100-ns simulation, which was then duplicated in the membrane normal direction to form the double-patch simulation system for the computational electrophysiology simulations (*C*).
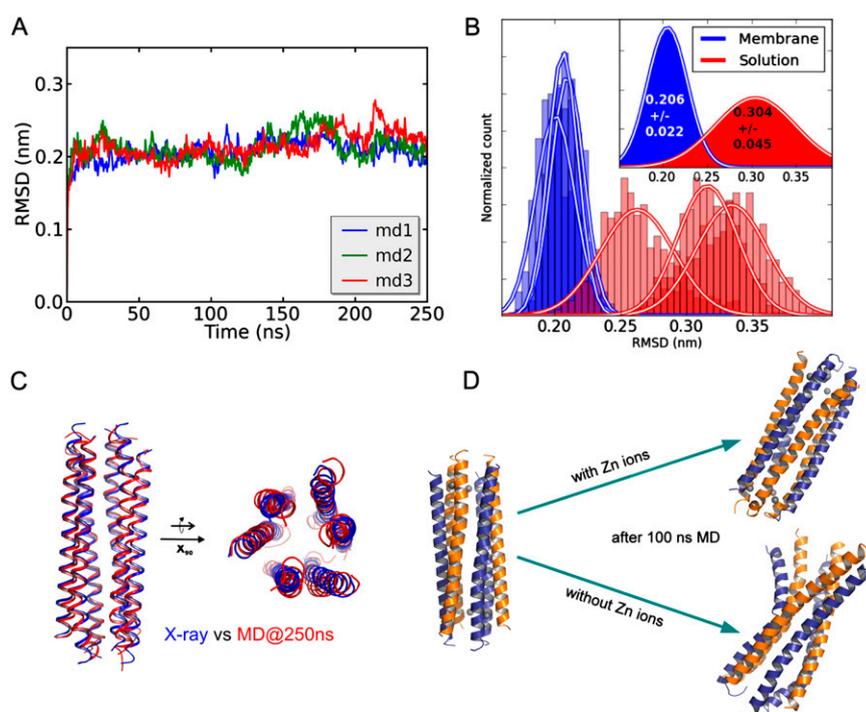


**Fig. S6.** Stability of the X-ray structure in MD simulations. (*A*) Evolution of the RMSD of the DCD oligomer (alpha carbon atoms) in the POPE/POPG bilayer during the 250-ns MD simulations. Results are calculated from three independent simulations. (*B*) Comparison of the stability of the DCD X-ray structure in membrane (blue) and solution (red). On average, the oligomer structure deviates from the X-ray structure by about 1 Å more in solution than in the membrane. Therefore, the X-ray channel structure is relatively more stable in a membrane environment. (*C*) Side and top views of the comparison between the X-ray structure (in blue) and the MD conformation at 250 ns (in red), respectively. The MD conformation was fitted onto the X-ray structure, which was then used as the starting structure for additional computational electrophysiology simulations. (*D*) Importance of Zn$^{2+}$ ions for the stability of the oligomer channel. Starting with the initial oligomer structure in the single-patch MD simulations, the channel structure is preserved in the MD simulations in the presence of six Zn$^{2+}$ ions. By contrast the channel structure evolves strong distortions in the absence of Zn$^{2+}$.

**Fig. S7.** Additional simulation results. (*A*) Evolution of the tilt angle of the DCD channel in various lipid bilayers in MD simulations. The simulations were performed in an NPT ensemble at a temperature of 345 K to keep all lipids in their fluid phase. As can be seen with the increase of the lipid tail length, i.e., the thickness of the bilayer, the tilt angle of the DCD channel decreases. In DPPC, the tilting process occurs on a much longer time scale than in the other lipid bilayers. Therefore, the type of the lipid bilayer and its composition may have a significant effect on the channel properties, which we reserve for a future study. (*B*) Exemplary ion permeation counts during the 200-ns computational electrophysiology simulations (averages from six independent simulations with SEs), with 0.15 M NaCl in solution.



**Fig. S8.** Helical wheel analysis of a monomer of DCD. Top view of a monomeric helical wheel, showing the distribution of residues and their types. Angular partitions of hydrophobic/hydrophilic regions are distinctly centered around 240°/120°, favoring hexamer formation when being embedded in a lipid bilayer, as shown (*Lower Left*) where the gray shaded areas indicate the hydrophilic surfaces and interfaces.

**Table S1.  Data collection and refinement statistics**

|  | Dermcidin* |
|---|---|
| Data collection | |
| Space group | C2 |
| Cell dimensions | |
| $a$, $b$, $c$ (Å) | 76.35, 50.72, 45.73 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90, 117.74, 90 |
| Resolution (Å) | 33–2.5 (2.66–2.5) |
| $R_{merge}$ | 0.02 (0.51) |
| $I/\sigma I$ | 17 (2.95) |
| Completeness (%) | 98.8 (96.8) |
| Redundancy | 7.2 (7.1) |
| Wavelength | 1.2795 |
| Refinement | |
| Resolution (Å) | 33–2.5 (2.56–2.5) |
| No. reflections | 5189 |
| $R_{work}/R_{free}$ | 0.21/0.27 (0.23/0.27) |
| No. atoms (all) | |
| Protein | 980 |
| Ions ($Zn^{2+}$) | 6 |
| Water | 20 |
| $B$ factors | |
| Protein | 27.7 |
| Ions ($Zn^{2+}$) | 70.5 |
| Water | 31.7 |
| Root-mean square.    deviations | |
| Bond lengths (Å) | 0.02 |
| Bond angles (°) | 2.0 |

*Values in parentheses are for highest-resolution shell.
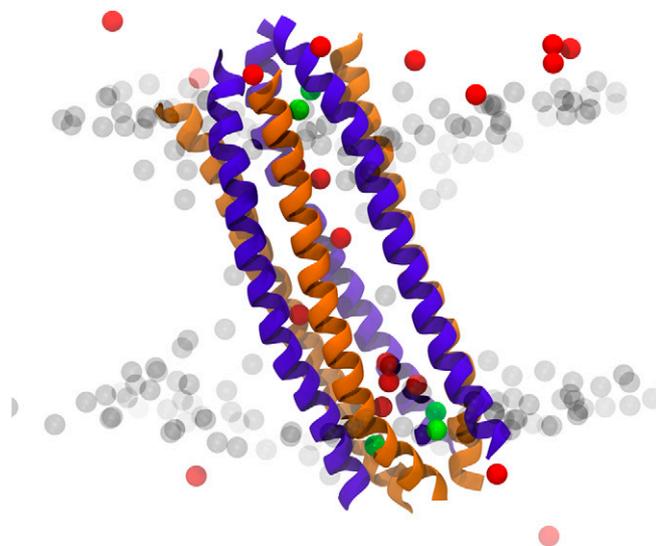


**Movie S1.**  Multiion permeation mechanism across DCD driven by transmembrane electric fields present across bacterial membranes. Ions enter sideways into the pore across the lateral openings that occur at the trimeric interfaces. Anion transfer across the inner pore usually involves single ion "hopping" steps. Near the channel exit, anions accumulate to form a cluster of three to four ions. Ions exiting the channel are often observed to be translocated by multiion "knock-on" effects, by which anions are transferred to the bulk solution.
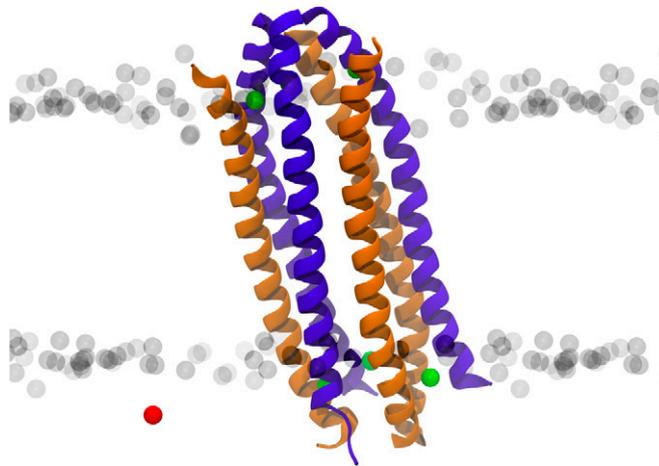
[Movie S1](#)

**Movie S2.** Single anion followed on its pathway through the DCD channel, driven by a transmembrane electric field. Simulations settings are identical to Movie S1, but only one permeating ion is shown to clearly illustrate the permeation pathway.

Movie S2