Published in final edited form as:

Proteins. 2011 January ; 79(1): 126–141. doi:10.1002/prot.22866.

Computational studies of colicin insertion into membranes: The closed state

Lidia Prieto[†] and Themis Lazaridis[†]

Lidia Prieto: lidiaPF@sci.ccny.cuny.edu; Themis Lazaridis: tlazaridis@ccny.cuny.edu

[†]Department of Chemistry, The City College of CUNY, Convent Avenue & 138th Street, New York, NY 10031.

Abstract

Colicins are water-soluble toxins that, upon interaction with membranes, undergo a conformational change, insert, and form pores in them. Pore formation activity is localized in a bundle of 10 α -helices named the pore-forming domain (PFD). There is evidence that colicins attach to the membrane via a hydrophobic hairpin embedded in the core of the PFD. Two main models have been suggested for the membrane-bound state: penknife and umbrella, differing in regard to the orientation of the hydrophobic hairpin with respect to the membrane. The arrangement of the amphipathic helices has been described as either a compact three-dimensional structure or a two-dimensional array of loosely interacting helices on the membrane surface. Using molecular dynamics simulations with an implicit membrane model, we studied the structure and stability of the conformations proposed above for four colicins. We find that colicins are initially driven towards the membrane by electrostatic interactions between basic residues and the negatively charged membrane surface. They do not have a unique binding orientation, but in the predominant orientations the central hydrophobic hairpin is parallel to the membrane. In the inserted state, the estimated free energy tends to be lower for the compact arrangements of the amphipathic helix, but the more expanded ones are in better agreement with experimental distance distributions. The difference in energy between penknife and umbrella conformations is small enough for equilibrium to exist between them. Elongation of the hydrophobic hairpin helices and membrane thinning were found unable to produce stabilization of the transmembrane configuration of the hydrophobic hairpin.

Keywords

Colicin; implicit membrane; lipid-protein interaction; molecular dynamics simulations; closed state; pore forming toxins

Introduction

Colicins are bactericidal proteins produced by *E. coli* against related *E. coli*¹. They exert their toxicity in different ways, such as nuclease activity² and pore-formation³. Pore-forming colicins are monomeric water-soluble proteins that permeabilize cell membranes, leading to cell death. Toxicity has a first order relation to protein concentration, which implies that a single molecule is able to produce cell death¹. One possible explanation is the formation of monomeric channels. Ion channel activity has been found to be relatively non-selective and voltage-gated with a small pH-dependent conductance $1\cdot4^{-7}$. Colicins have become a model system for studying the processes of membrane insertion⁸, pore formation⁹

and, because of their solubility both in membranes and in water, membrane protein folding and unfolding¹⁰.

The structure of pore-forming colicins consists of three domains, each of which carries out a different function: translocation, receptor-binding and pore formation¹. The pore-forming domain (PFD) is at the C-terminus of the protein and has been shown to form pores in membranes when isolated from the rest of the protein *in vitro*^{11–14}. In all colicins it consists of 10 α -helices arranged in three layers^{15–21}. In the central layer, helices 8 and 9 form a hydrophobic hairpin embedded in the hydrophobic core of the domain. These two hydrophobic helices are thought to act as an anchor for colicin insertion into the lipid environment^{5,15,22–24}. This structural motif is present in other proteins (such as diphtheria toxin²⁵ or the Bcl-2 family^{9,26,27}) which are capable of undergoing a water-soluble to membrane-bound transition^{28–30}. According to the sequence similarity of the PFD, pore-forming colicins can be classified into two groups¹⁷: A-type (which includes colicins A and B, among others) and E-type (such as colicins Ia and E1), that have a shorter hydrophobic hairpin.

The mechanism of colicin pore formation consists of three steps. First, colicins approach the membrane and interact with it. We call this the adsorbed state, in which the PFD retains its soluble form¹⁷. As colicin action takes place on anionic membranes and is enhanced *in vitro* at low pH, it has been suggested that this interaction is mainly electrostatic^{1,31}. Once the PFD is electrostatically bound to the membrane, it unfolds and inserts into the membrane through the hydrophobic hairpin. The insertion of helices 8 and 9 into the membrane is a spontaneous^{5,32} process that forms the “closed state” of the pore. It may be accompanied by helix elongation^{33,34}. Finally, opening of the pore takes place in a voltage-dependent manner that requires translocation of a significant part of the PFD¹.

There have been different views on the mechanism of colicin insertion into membranes. Initially, Parker *et al.* proposed an “umbrella” model for the closed state of the channel, based on the structure of the PFD of colicin A¹⁵. In this model, the hydrophobic hairpin formed by helices 8 and 9 inserts in a transmembrane fashion while the rest of the helices remain on the membrane surface. However, fluorescence energy transfer distance measurements on colicin A showed that helices 8 and 9 remain in a similar packing arrangement when bound to the membrane as in the water-soluble structure³⁵, which is incompatible with the umbrella model. Based on these results and on disulfide bond engineering experiments³⁶, Duché *et al.* proposed the “penknife” model, in which the hydrophobic hairpin orients roughly parallel to the water-membrane interface. There is ample evidence supporting the umbrella model, such as circular dichroism³³, solid state NMR^{37–39} and site labeling in combination with EPR spectroscopy⁴⁰ experiments. However, there is also a significant amount of experimental data supporting the penknife model^{35,36,41,42}. It has been suggested that the hydrophobic hairpin may not be in a single conformation in the closed state. A mechanism has been proposed in which the hairpin is first on the water-membrane interface and then inserts in a transmembrane orientation, both conformations being in equilibrium with each other⁴³. This mechanism is supported by experiments with the streptavidin-biotin system and also by fluorescence quenching⁴⁴, red edge excitation⁴⁵ and solid state NMR⁴⁶. A similar mechanism has been proposed for diphtheria toxin^{47,48}, the translocation domain of which shares structural similarities to the PFD of colicins. Depending on the specific colicin under study and the experimental conditions, the balance between the two configurations may change. To reconcile experimental evidence supporting the two models, it has also been suggested that the penknife model may be an intermediate for the umbrella model conformation⁴⁹ or that colicins from different groups follow different mechanisms³⁷. What appears to be clear is

that the hydrophobic hairpin has to be oriented perpendicular to the membrane plane for the channel to open^{43,50}.

The disposition of the rest of the helices of the PFD in the closed state of the colicin pore is also in question. In the initial umbrella and penknife models in the literature, these eight amphipathic helices are depicted as a stack on the membrane surface^{15,36}. However, studies on colicin E1 by the Cramer group suggested that the amphipathic helices form a flexible extended two dimensional array of weakly or non interacting helices³³. Solid-state NMR experiments also support this model for colicin Ia^{39,51}. Site directed fluorescence labeling shows that the amphipathic helices are spread on the membrane surface^{34,52–55}. However, Differential Scanning Calorimetry (DSC) experiments suggest that the tertiary structure is retained at least to some degree in the inserted state⁵⁶.

Here we study the insertion of colicins into membranes using computational methods. The size of colicins and the complexity of the conformational changes involved preclude fully atomistic modeling. Instead we use an implicit model for the membrane to carry out molecular dynamics (MD) simulations of the adsorbed state of colicins and of the umbrella and penknife conformations using three different arrangements of the amphipathic helices. The main goal of this paper was to examine theoretically the thermodynamic stability of the various conformational states proposed, taking into account the possible effects of helix elongation and membrane thinning. All simulations were done on membranes containing 30% anionic lipids. To test the generality of the results, we studied four different colicins belonging to two classes. This report focuses on the closed state. Future work will address the voltage-dependent channel opening process.

Methods

IMPLICIT MEMBRANE

Obtaining estimates of the thermodynamic stability of very different protein conformations with explicit representation of the solvent is very difficult. Therefore, in this work we use implicit solvation, that is, the protein is represented in atomic detail while the effect of the solvent (water and lipids) is taken into account through an extra term in the energy function. We use the implicit membrane model IMM1⁵⁷, an extension of the EEF1 energy function for proteins in water⁵⁸ implemented in the CHARMM program⁵⁹. The IMM1 energy function can be written as:

$$W_{IMM1} = E + \Delta G^{slv} \quad (1)$$

where E is the intramolecular energy based on the CHARMM polar hydrogen force field⁶⁰ and ΔG^{slv} is the solvation free energy, which is computed as a sum of the solvation free energy of each atom. The latter is calculated by subtracting the solvation free energy lost due to the presence of surrounding atoms from the solvation free energy of the atom fully exposed to the solvent (ΔG_i^{ref}). ΔG_i^{ref} is a linear combination of experimental solvation free energy values in water and cyclohexane (which is the reference solvent to model the membrane environment). It varies depending on the position of the atom with respect to the membrane (z). The membrane is parallel to the xy -plane with its center in the coordinate origin. For our simulations the membrane thickness (T) is 26 Å (corresponding to DOPC/DOPG membranes)⁶¹ unless otherwise specified. A continuous, sigmoidal switching function is used to describe the transition from the membrane interior to the water phase⁵⁷. The dielectric constant for electrostatic interactions is distance dependent, so that it is a

function of the positions of the interacting atoms with respect to the membrane. This way, the strengthening of electrostatic interactions in the membrane is taken into account⁵⁷.

All simulations in this work were done on membranes containing 30% anionic lipids. The electrostatic interactions between the anionic lipid head groups and the protein are modeled using the Gouy-Chapman theory⁶². According to this theory, the charge due to the anionic head groups can be described as a uniform “smeared” charge on the membrane plane. The electrostatic interaction energy (W_{GC}) is calculated as the sum over all atoms of the product of the atom i partial charge (q_i) with the electrostatic potential at the position of this atom⁶³, $\psi(z_i)$. The mol fraction of anionic lipids (30% in this work), the area per lipid ($70 \text{ \AA}^2$), the salt concentration (0.1 M), the valence of the electrolyte (+1) and the position of the plane of smeared charge relative to the non-polar/polar interface (offset=3 Å) have to be specified.

To estimate membrane-binding affinity we use the average transfer effective energy:

$$\Delta W_{tr} = W_{eff}(water) - W_{eff}(membrane) \quad (2)$$

In Eq. 2, $W_{eff}(water)$ and $W_{eff}(membrane)$ correspond to the effective energy of the same conformation far (water conditions) and close to the membrane, respectively. The calculation in Eq. 2 was done for the minimized final conformation of each MD simulation, which was translated in the z direction 200 Å to place the structure in bulk water and calculate its energy. This approach for estimating membrane-binding affinity has the advantage of producing more precise values due to omission of noise from conformational fluctuations. It does, however, neglect any changes in the conformational distribution of the protein upon membrane binding.

The effective energy does not contain entropic contributions arising from protein degrees of freedom. Rotational and translational entropies were neglected, as they are likely to be similar in all the membrane-bound states compared. The conformational entropy of each state was estimated using the probability distribution of the side chain torsion angles, computed by rotating each torsion angle independently⁶⁴. The effective energy profiles obtained for the different side chain conformations sampled allow the calculation of their probabilities (p). The conformational entropy of a certain structure can be obtained by integrating $p \cdot \ln(p)$ for each torsion angle and by summing over all torsion angles. This calculation was done over 10 configurations of each production run and then averaged over all the simulations run for each case.

DEFORMATION FREE ENERGY

In examining membrane-thinning effects, it was necessary to estimate the membrane deformation free energy, which was done using the linear-spring description⁶⁵. According to this approximation, the deformation free energy can be calculated as $\Delta G_{def} = H \cdot (2u_0)^2$, where $2u_0$ stands for the deformation of the membrane and H is the spring constant that can be determined using the equation:

$$H = H^* \left(\frac{K_a}{K_a^*} \right)^\nu \left(\frac{K_c}{K_c^*} \right)^\mu \quad (3)$$

In Eq. 3, K_a^* , K_c^* and H^* are reference values for the expansion modulus (K_a), the splay-distortion modulus (K_c) and the spring constant (H), respectively⁶⁶. The values used for Eq. 3 are^{66,67}: $K_a^* = 142.5 \text{ pN/nm}$, $K_c^* = 28.5 \text{ pN}\cdot\text{nm}$, $K_a = 254 \text{ pN/nm}$, $K_c = 76 \text{ pN}\cdot\text{nm}$, $\nu = 0.667$ and $\mu = 0.334$.

To solve Eq. 3 it is necessary to estimate an appropriate value for H^* . This parameter is proportional to the radius of the inserted cylinder that causes the deformation, according to the relation $H^* \propto r^\delta$, $\delta=0.815$ [see reference 65]. When this radius is 1.0 nm, $H^*=63.6$ KJ/mol·nm² [see reference 66]. Once the radius of the inserted cylinder is estimated, the corresponding value for H^* can be calculated as:

$$H^*=63.6 \cdot \left(\frac{r}{1.0}\right)^{0.815} \quad (4)$$

COLICINS STUDIED AND SIMULATION PROTOCOL

In order to compare the results of our simulations with as much of the available experimental data as possible and to examine the variability of the results across the colicin family, we selected for study four molecules: colicins A and B (A-type) and colicins Ia and E1 (E-type). The structures of the PFD of the colicins were obtained from the Protein Data Bank68 (PDB) under the codes: 1COL (for Colicin A); 1RH1 (for Colicin B); 1CII (Colicin Ia) and 2I88 (Colicin E1). They are depicted in Fig. 1, where it can be appreciated that the A-type colicins have a longer hydrophobic hairpin than the E-type colicins selected for this study.

First we studied the adsorbed state. The PFDs of the four colicins were initially located with their center of mass at $z=33$ Å from the membrane center. Then, six MD simulations were run, each starting from a different initial orientation with respect to the membrane. The six orientations correspond to the six faces of a cube into which the protein is embedded. The simulations were 3 ns long and were run at pH=7 and low pH. The first 1.5 ns correspond to the equilibration phase. Data were collected during the last 1.5 ns. Low pH conditions were emulated by protonating all the residues in the protein. This part of the study gave us thermodynamic information about how the PFD interacts with the membrane at different pH conditions and also which is its most favorable orientation with respect to the membrane prior to membrane insertion.

We then simulated the inserted state. To do that we first created an umbrella model by applying a 500 pN force in the $-z$ direction on the middle residue of the loop between helices 8 and 9 (see Fig. 1). To preserve the secondary structure of these two helices, constraints were applied on their backbone. The starting conformation of this process was one of the resulting structures of the adsorbed state simulations. Since 500 pN is a very strong force, the resulting structure is strained (mostly in the loops between helices 7 and 8 and between helices 9 and 10). Therefore, once the hydrophobic hairpin was inserted perpendicularly into the membrane the system was equilibrated by applying a softer force (400 pN) on the same residue for 5 ns to prevent the system from recovering the PFD soluble structure. With this procedure we obtained an umbrella conformation with the eight amphipathic helices on the membrane surface forming a compact structure as shown in Fig. 2a. This is called the “Compact” model throughout this work. This Compact model was equilibrated by several 1 ns simulations with different random initial velocities without any constraints.

As mentioned in the Introduction, there is some evidence that supports an extended two-dimensional arrangement of the amphipathic helices for colicins A54, E133 and Ia39. In order to unfold the compact structure of the amphipathic helices, we carried out sequential rotations, starting from the Compact umbrella conformation, until the PFD was unfolded and the helices were located on the membrane surface. The resulting conformation was equilibrated by running 2 ns simulations with constraints on the center of mass of the eight amphipathic helices, so that it remained on the water-membrane interface plane. After this

step, we obtained the “Loose-Array” model, shown in Fig. 2b. This structure, in which the hydrophobic side of the amphipathic helices is facing the membrane, was equilibrated for 4 *ns* more (without constraints).

In this Loose-Array model, however, the amphipathic helices do not arrange circularly around the transmembrane hydrophobic helices as proposed by Cramer and coworkers [see Fig. 4 in reference 10 and Fig. 5 in reference 33]. Therefore, we created another model with the arrangement of the helices as proposed by Cramer’s group. To that end, we broke the PFD into pieces and manually arranged the helices as desired, making sure that the hydrophobic side of the amphipathic helices was facing the membrane. The pieces were joined together and 5 *ns* molecular dynamics simulations were run with the helices fixed at their positions and the side-chains and loops allowed to move freely. This way, we obtained the structure shown in Fig. 2c, which we call the “Circular” model. 1 *ns* equilibration molecular dynamics simulations without constraints were then run for this model.

The structures in Fig. 2 correspond to three initial configurations for the simulations run to study the inserted state of colicins. For each of them, after equilibration, six 4 *ns* simulations with different random initial velocities were carried out. In some of these simulations, the hydrophobic hairpin changed its orientation, getting parallel to the membrane plane as in the penknife model. For those colicins that this did not happen, a penknife model was created by rotating the hydrophobic hairpin. This model was equilibrated for 10 *ns* applying constraints on the position of the hairpin, followed by six 4 *ns* unconstrained simulations.

As mentioned, the E-type colicins of Fig. 1 have shorter hairpins than the A-type colicins. The analysis described in the last paragraphs was repeated for colicins Ia and E1 having a longer hairpin. To extend the hydrophobic hairpins, we isolated it and added to each helix 4–6 residues in a α -helical arrangement by defining the values of the ϕ and ψ angles for the necessary peptide bonds. These extended helices were placed in the membrane center perpendicularly to the surface. After that, the amphipathic helices were added to the hairpin. Then, a minimization was performed to relax the structure. This model (called here the “ ϕ - ψ ” model) was equilibrated for 1 *ns* without constraints and simulations were run as described before. To test the reliability of the results, we repeated these simulations by creating the extended hairpin model in a different way. In this case, we applied NOE constraints so that the distance between the oxygen of the carbonyl group of residue *i* and the nitrogen of the amino group of residue *i*+4 was fixed around a value of 2.8 Å. These constraints were applied to 4–6 residues flanking the hydrophobic hairpin. Extra constraints were applied to force the hairpin to remain in a transmembrane orientation. This model, called here the “NOE” model, was equilibrated for 10 *ns* with no constraints after which, six simulations with different random initial velocities were run as in the previous cases.

The length of the simulations was chosen based on the convergence of the total energy of the simulation. In most of the simulations, the total energy decreases during the equilibration phase and then acquires a stationary value. Additionally, a 100 *ns* simulation was run for the adsorbed state of colicin Ia, which yielded similar results to the ones reported here, further suggesting that the simulation length chosen is appropriate. We also carried out a 70 *ns* simulation for the inserted state with the Compact model, in order to check whether longer simulations would allow observing conformational changes of the amphipathic helices. However, the compact arrangement of the amphipathic helices remains stable and thus, to be able to study other conformational arrangements, we used the Loose-Array and Circular models previously described. It is worth keeping in mind that the real time scale of implicit solvent simulations is much longer than the nominal one, due to lack of solvent friction. All the simulations were run at constant temperature (298 K). Unless otherwise mentioned, all the results presented in this paper correspond to averages over the production-phases of the

simulations and over all the simulations run for each case. The number of configurations per simulation used to calculate these averages depends on the specific simulation and ranges from 750 to 2000.

Results

ADSORBED STATE

We first studied the process of association with the membrane prior to the conformational change that eventually leads to membrane insertion. Thus we ran several simulations for this adsorbed state as described in Methods. The average backbone root mean square deviation (*rmsd*) from the crystal structure is very similar at low pH and pH=7 (see Table S1 in Supplementary Material), suggesting that the solution conditions do not have a large impact on the stability of the PFD. These *rmsd* values are comparable to the ones obtained in simulations of the PFD in water, suggesting that the membrane does not cause significant structural changes.

Further results for the adsorbed state are summarized in Table 1. These results are averages over the six runs starting from different initial orientations with respect to the membrane. The small standard deviations show that the initial orientation makes little difference in the final calculated energies. Significant differences between pH=7 and low pH are observed. The first two columns of Table 1 show the values of the transfer effective energy (ΔW_{tr}), which corresponds to the energy required to unbind the PFD from the membrane (see Methods). These values show that the interaction with the membrane is stronger at low pH. This is confirmed by the average *z* coordinate of the center of mass of the PFD (Z_{CM}) being larger at pH=7, as can also be seen in Table 1. As the membrane is parallel to the *xy* plane and centered at (0,0,0), the *z* coordinate gives us an idea of the distance of the PFD from the membrane. For colicins A and B, the interaction with the membrane is almost negligible at pH=7 (very low ΔW_{tr} and high Z_{CM} values). The PFD of colicin A moves away from the membrane in all the simulations ($Z_{CM} > 46 \text{ \AA}$), while colicin B PFD remains relatively close to the membrane ($\sim 44 \text{ \AA}$) in three of them, resulting to a small residual interaction with it. For colicins Ia and E1, the interaction with the membrane (ΔW_{tr}) at pH=7 is more substantial.

Table 1 also lists the Gouy-Chapman transfer energy (ΔW_{GC}), which corresponds to the electrostatic interactions of the protein with the anionic smeared charge on the membrane (see Methods). The Gouy-Chapman transfer energy is slightly larger than the effective energy transfer, making clear that the initial approximation of the PFD to the membrane *in vitro* is dominated by electrostatic interactions as previously proposed³¹. The values in Table 1 are higher for low pH. This is because at low pH the acidic residues get protonated and their repulsive interaction with the negative surface charge is eliminated. In the sequences of colicins Ia and E1, there is a higher content of basic residues than acidic ones, while in colicins A and B, the opposite is observed. Therefore, at pH=7, the electrostatic repulsion with the anionic membrane for E-type colicins is lower, since the net charge is still positive. This explains why we observe a somewhat stronger interaction with the membrane for colicins Ia and E1 than for colicins A and B at pH=7. This does not necessarily mean that the E-type colicins should be more active at neutral pH because initial membrane binding is only one step in channel formation. Our predictions could be tested by experimental results for just membrane binding (see Discussion).

According to the umbrella model, the PFD should be oriented so that the hydrophobic hairpin is perpendicular to the membrane plane in order to favor its subsequent insertion in this same orientation [see Fig. 4 in reference 15]. To examine the preferred orientation in our simulations of the adsorbed state, we show in Fig. 3 the distributions of the angle formed by

the membrane plane and the hydrophobic hairpin axis (as indicated in the inset of the same figure). These distributions are averages over the six simulations run. For low pH simulations (upper graph) it can be seen that all the distributions have an intense narrow peak close to 0° . This value corresponds to an orientation of the hydrophobic hairpin parallel to the membrane plane (an example is shown also as an inset in Fig. 3). In the lower graph of Fig. 3 we show the angle distributions for colicins Ia and E1 PFD simulations at pH=7, as these are the only two colicins that interact significantly with the membrane in these conditions. In this case, the angle distributions spread over a wider range of values. Both at low and neutral pH the angle distributions have an essentially zero value at -90° , corresponding to the perpendicular orientation initially suggested based on colicin A structure¹⁵. This supports the hypothesis that the penknife model appears in the closed state formation mechanism at least as an intermediate for the umbrella conformation⁴⁹.

Analyzing the trajectories obtained in the six simulations run for each colicin, it can be observed that they do not all lead to the same orientation (this is why there is non-zero probability of angle values different from zero in Fig. 3). The preferred orientation is acquired in 3, 4, 4 and 5 simulations of colicins A, B, Ia and E1, respectively. Analyzing the snapshots corresponding to the most probable angle (-30 – 30°) formed by the hydrophobic hairpin and the membrane plane, it can be observed that this orientation is caused by interactions of a large number of basic residues with the membrane. Some, but not all, of these residues are conserved among the four sequences (as can be observed in Fig. S1 in the Supplementary Material). In conclusion, there is not a unique orientation that colicins adopt when they adsorb on the membrane, but there is strong preference for orientations where the hydrophobic hairpin is parallel to the membrane.

INSERTED STATE

It is experimentally known that the pore forming activity is enhanced at low pH¹. Therefore, the studies of the inserted state were done in these conditions.

a) Arrangement of the amphipathic helices—As explained in Methods, we used three different starting configurations for the simulations of the inserted state: the Compact (Fig. 2a), the Loose-Array (Fig. 2b) and the Circular (Fig. 2c) models. The structural characteristics of these models are quite different. In the Loose-Array and Circular configurations, the amphipathic helices are adsorbed on the membrane surface. The center of mass of the group formed by these helices is at $z \approx 14 \text{ \AA}$ and $z \approx 18 \text{ \AA}$, respectively. In the Compact model these helices are further from the membrane, with their center of mass at $z \geq 26 \text{ \AA}$ (up to $z = 30 \text{ \AA}$). Another difference between the models is in the degree of compaction of the amphipathic helices. In the Loose-Array and Circular models, they are spread in a two dimensional fashion, while in the Compact model, they pack together forming a globular structure. The radius of gyration of this group of helices is larger for the Loose-Array and Circular models (20–23 and 28–39 $\text{ \AA}$, respectively) than for the Compact model (15–18 $\text{ \AA}$).

In the simulations of the Compact model, the arrangement of the amphipathic helices is fairly stable. Conversely, during the equilibration phase of most of the simulations carried out with the Circular model, there is a significant rearrangement of the structure. The amphipathic helices come together to form a structure more compact than in the initial conformation. Furthermore, they move away from the membrane surface (the center of mass of the group getting as far as $z = 23$ – $27 \text{ \AA}$). In the simulations of the Loose-Array model there is not much change in the way the amphipathic helices are grouped together (here these helices are more compact than in the Circular model) but they also move away from the membrane (their center of mass getting to $z = 20$ – $30 \text{ \AA}$). For more details on the structural

changes that take place during the simulations, see Fig. S2A, S2B and S2C in the Supplementary Material.

The average structures obtained in the simulations with the different models are quite different, with an average *rmsd* of 10 ± 5 and 19 ± 2 Å within the models and between them, respectively. Nevertheless, a common characteristic to most of them is that the amphipathic helices tend to group together. In Fig. 4 we compare the distance distributions between specific residues of colicin A in our simulations with the experimental data available⁵⁴. These distance distributions have been thought to support a circular arrangement of the amphipathic helices in the inserted state, as suggested by Cramer and coworkers⁵⁶. The distance distributions for the Loose-Array and Circular models in Fig. 4 are broader than those of the Compact model, which gives distances somewhat too short and too narrowly distributed compared to experiment. The former are, thus, in better agreement with experiment.

In the structure of the PFD of colicin A, a cluster of aromatic residues has been reported to be resistant to denaturation by 6M urea⁷⁰, which led the authors to suggest that the structure of the layer formed by helices 3–7 in the soluble state remains conserved in the closed state of the channel. This characteristic is only represented in the Compact model of the amphipathic helices, while in the simulations with the Loose-Array and Circular models, the cluster of aromatic residues is broken. These aromatic residues have been reported to insert into the hydrophobic core of the membrane⁷¹. Only in the Loose-Array and Circular models of Figs. 2b and 2c are these residues close to the membrane surface or inserted into it. Moreover, Fluorescence Energy Transfer distance measurements showed that the distance of residues S16 and K39 in colicin A from the aromatic residue cluster also increases in the inserted state^{35,72}. Such an increase is observed in the Loose-Array and Circular models, but not in the Compact model. Overall, the latter models seem to be in better accord with the Pattus group FRET experiments.

For a thermodynamic comparison, Table 2 shows the effective and free energies of the different conformations observed in our simulations (averages over the production phase and over all simulations run). In this table, the lowest energy obtained for each conformation, penknife or umbrella, is highlighted in bold. The Compact model produces the lowest effective energy structures in most cases. However, side chain entropy favors the less compact structures. In the case of colicins B and Ia the Compact model remains the lowest free energy structure, but in the case of colicins A and E1 the Circular and Loose-Array ones, respectively, become more favorable when entropy is included (in the umbrella conformation only).

b) Umbrella vs. penknife models—The comparison of the relative stability of umbrella and penknife models is based on the Compact model, which provides the lowest energy conformations in most cases.

The umbrella conformation is completely stable in all the simulations run for colicins B and Ia. In the case of colicin A, the hydrophobic hairpin acquired a parallel orientation in two out of the six simulations. The same happens in four out of the six runs for colicin E1. For the former two colicins (B and Ia) a penknife model was created as explained in Methods and six more simulations were carried out.

Table 2 shows that the umbrella conformation is clearly favored in the case of colicin B ($\Delta W_{PU} < 0$). For colicins A and E1, the two hairpin orientations have approximately equal effective energy. The penknife conformation is more stable than the umbrella one for colicin Ia. Inclusion of the side chain entropy term does not change the picture substantially (see

ΔG_{PU} values). Fig. S3 in the Supplementary Material shows the energy distributions for each of the conformational states considered. In all cases, there is a significant overlap of the energy distributions of the penknife and umbrella conformations.

Table 3 shows the effective and free energy differences between the adsorbed and lowest energy inserted models. Comparing $\Delta G_{ADS-ins}$ with $\Delta W_{ADS-ins}$, it can be seen that the side-chain entropy term favors insertion into the membrane. However, it is not enough to make the transition spontaneous in all cases. The free energy change from adsorbed to inserted (penknife or umbrella) is clearly negative in one case (colicin B umbrella), close to zero in another (colicin Ia penknife), and positive in the remaining two cases. It is possible that our simulations overestimate the energy of the inserted state due to limited conformational sampling (see Discussion).

In Table 4, the free energy change from umbrella to penknife (ΔG_{PU} in Table 2) is split into four contributions: van der Waals and covalent, electrostatic, solvation, and side-chain entropy. The largest contributions are the electrostatic and the solvation free energy, which have opposite effects. The two contributions balance out in the case of colicins A and Ia and favor the umbrella model for colicins B and E1. The variation of these two contributions is mostly due to the difference in the self-energy of the hydrophobic hairpin (analysis shown in the Supplementary Material, tables S2 and S3).

The value of $\Delta W_{PU}^{VDW} + \Delta W_{PU}^{COV}$ is very small for A-type colicins, so that the equilibrium only depends on the self-energy of the hydrophobic hairpin. However, this term makes a substantial contribution for colicins Ia and E1, disfavoring the umbrella conformation. The van der Waals and covalent energy depends on the interactions of the hydrophobic hairpin with the rest of the helices and the interactions among these (analysis summarized in Table S4 of the Supplementary Material). In Fig. 5 we show representative snapshots of each conformation for the four colicins studied. For colicins A and B the arrangement of the amphipathic helices and the interaction of the two hydrophobic helices with the others is not significantly different in the two conformations. In contrast, for colicins Ia and E1, the penknife conformation is more compact than the umbrella one. Furthermore, in the umbrella conformation of colicin Ia, because of the shortness of the hairpin, one of the loops flanking it (marked with an arrow in Fig. 5) has to extend to minimize the electrostatic energy and solvation free energy. Consequently, the van der Waals and covalent energy increases with a perpendicular orientation of the hairpin, making it less stable than the penknife conformation. This analysis suggests that the length of the hydrophobic hairpin may have a significant influence on the arrangement of the amphipathic helices, which is apparently related to the penknife to umbrella conformational transition.

c) Helix elongation—Transmembrane segments of integral membrane proteins have an average length of approximately 20 residues⁷³. The hydrophobic hairpin helices of colicins A and B are of about that length, while those of colicins Ia and E1 have only 17 residues. It has thus been suggested that helix elongation should take place in the hydrophobic hairpin of the E-type colicins of Fig. 1 to allow it to span the membrane⁷⁴. For colicin E1, overall helix content increase from 59 to 78% upon membrane insertion has been reported based on circular dichroism experiments³³. In order to check whether helix elongation is a favorable process and how it affects the equilibrium between the umbrella and penknife conformations, we ran new simulations of the inserted state of the PFD of colicins Ia and E1 with a longer hydrophobic hairpin. As described in Methods, two modeling procedures were used to extend the hairpins. The results obtained with both models lead to the same conclusions. The results reported below correspond to the ones obtained with the phi-psi modeling process (see Methods) and the ones obtained with the NOE-modeling method are summarized in Table S5 of the Supplementary Material.

The perpendicular orientation of the hairpin is stable in four out of six simulations run for colicin Ia and in three out of six for colicin E1. In the remaining simulations the penknife conformation is acquired. The results obtained for these simulations are summarized in Table 5. Comparing tables 5 with tables 2 and 3, it can be seen that the effective energy of colicin Ia increases upon helix elongation, while that of colicin E1 decreases to an extent that the transition from the adsorbed state to the penknife conformation becomes favorable, as shown by the value of $\Delta G_{\text{ADS-P}}$ in Table 5. Regarding the equilibrium between the two inserted conformations, hairpin elongation seems to shift the equilibrium towards the umbrella conformation for colicin Ia, while it does not affect it for colicin E1 (compare ΔG_{PU} in tables 2 and 5).

In Table 6, the difference in energy between the two inserted conformations for colicins Ia and E1 with the elongated hairpin is split into its four main contributions. For colicin Ia the elongation of the hairpin reduces the difference in van der Waals and bonded energy between the two orientations, without affecting significantly the electrostatic interactions and the solvation free energy (compare tables 4 and 6). For colicin E1 all three contributions change. For both colicins a decrease is observed in $\Delta W_{\text{PU}}^{\text{VDW}} + \Delta W_{\text{PU}}^{\text{COV}}$ with respect to the system with the shorter hairpin (Table 4). In Fig. 6, we show representative snapshots of the two conformations with the elongated hydrophobic hairpins. With the elongated hairpin, the penknife model is not so compact as with the short hairpin (compare the penknife models of colicins Ia and E1 in Figs. 5 and 6) and looks more similar to those of colicins A and B in Fig. 5. Apparently, the longer hairpin does not allow such a compaction of the whole PFD in the penknife conformation and, thus, the difference in van der Waals and bonded energy in the umbrella orientation is reduced. These results confirm the hypothesis made in the previous section that the length of the hydrophobic hairpin affects the relative stability of the umbrella and the penknife conformations.

Another observation from Fig. 6 is that, despite the lengthening, the hairpin of colicin E1 in the umbrella conformation does not cross the membrane. Actually, the depth of the hydrophobic helices in the membrane is the same regardless of their length. This is caused by the presence of aromatic residues flanking helices 8 and 9 (TYR478 and TYR507, circled in the sequence alignment of Fig. S1 and highlighted in Figs. 5 and 6). These residues tend to stay on the membrane surface, preventing the hairpin from getting deeper into the membrane. Also, in this colicin, further insertion into the membrane would force residues ASP473 and ASP509 (also highlighted in Figs. 5 and 6) to get very close to the membrane surface. Even with these residues protonated at low pH, this is electrostatically unfavorable. These results contradict the hypothesis that hydrophobic helix elongation would be associated with better solvation of the hairpin in the transmembrane conformation. ASP473, TYR478 and TYR507 are not conserved in colicin Ia (see Fig. S1) and therefore do not prevent the hairpin from spanning the membrane, as observed in Fig. 6. In this case, deeper insertion of the hairpin allows aromatic residues in the middle of the hairpin (PHE588 and TYR601, see Figs. 5 and 6) to come closer to the membrane surface.

In summary, for colicin E1 helix elongation is favorable, but no stabilization of the umbrella conformation is observed and the hairpin cannot span the membrane. For colicin Ia, although there is some stabilization of the umbrella conformation with respect to the penknife, hairpin length increase is not a favorable process, even though it allows the hairpin to span the membrane. Thus, according to our results, hydrophobic helix elongation does not seem to be a mechanism for stabilization of the transmembrane orientation of the hydrophobic hairpin.

d) Membrane thinning—Membrane thinning could also facilitate the insertion of the shorter hydrophobic hairpins of colicins Ia and E1. In the context of implicit membrane

models we can study this possibility by giving different values to the membrane thickness. We studied the effect of membrane thinning by calculating the energy of 5 representative structures of the umbrella conformation in membranes whose hydrophobic thickness varies from 20 to 25 Å. Fig. 7 shows that the effective energy of the umbrella conformation (discontinuous line) decreases as the membrane becomes thinner.

However, membrane deformation has a free energy cost (ΔG_{def}), which can be estimated using the linear-spring approach in membrane elasticity theory⁶⁵, as explained in Methods. For that it is necessary to estimate the radius of the portion of the protein inserted in the membrane. The hydrophobic hairpin of colicins Ia and E1 can be roughly embedded in cylinders of radius 1.1 and 1.2 nm respectively. The corresponding spring constants are $34 \pm 9 \text{ kcal/mol}\cdot\text{nm}^2$ and $36 \pm 9 \text{ kcal/mol}\cdot\text{nm}^2$. When this term is added to the effective energy, it can be seen (continuous line in Fig. 7) that the thermodynamic cost of membrane deformation is larger than the stabilization of the umbrella conformation. Therefore, according to these calculations, membrane thinning does not contribute to the stabilization of the closed state of colicins.

Discussion and conclusions

To the best of our knowledge, this is the first computational study of colicin insertion into membranes. The lack of high-resolution experimental information on the structure of the membrane-bound state makes this a challenging task. As a result, there is considerable uncertainty in the structures generated and their energies. However, the work tests specific ideas proposed in the literature and, hopefully, could stimulate and guide further experimental work. The main findings are summarized below and compared to available experimental data.

The initial approach of the PFD to the membrane, leading to what we term the adsorbed state, is guided by electrostatic interactions, as evidenced by the fact that the main contribution to the binding energy is the Gouy-Chapman term. This explains why colicin activity is enhanced at low pH^{53,75–79}, under which acidic residues are protonated, so that their repulsion with the negatively charged membrane surface is reduced. The PFD at low pH does not have a unique binding orientation but in the predominant orientations the hydrophobic hairpin is parallel to the membrane plane, in contrast to expectations in the literature¹⁵. The tip of the hydrophobic hairpin in the soluble state of the PFD of colicin A is surrounded by eight positively charged side chains [see Fig. 3 in reference 15], which have been suggested to form an appropriate interface for initial electrostatic binding. However, only two of these residues are conserved in colicin Ia⁷⁸. In the structure of the colicin E1 PFD there is a ring of seven residues, out of nine positively charged side chains conserved among E-type colicins, on one face of the PFD¹⁷. Three of these residues are conserved among the four colicins studied here. In our simulations, the PFDs do not interact with the membrane through any of the two suggested sets of residues. The binding interfaces we observe are not equivalent among the four colicins. The only common feature is that all four PFDs orient preferentially with the hydrophobic hairpin parallel to the membrane plane, which is reminiscent of the penknife conformation.

For the membrane-inserted state a variety of experimental data are available, though of low resolution. There is evidence from site-directed fluorescence labeling experiments that the amphipathic helices in colicin E1 are adsorbed to the membrane surface to a higher or lower degree^{34,52}. Quenching experiments using brominated lipids on colicin A have shown that the tryptophans in this colicin penetrate the membrane, also pointing to a membrane surface location of the amphipathic helices⁷¹. Based on distances between different residues in the amphipathic helices of the PFD of colicin E1, obtained with Fluorescence Resonance

Energy Transfer (FRET) experiments, a circular arrangement of these helices around the transmembrane hydrophobic hairpin was proposed^{33,56}. 2D solid-state NMR experiments showed an increase in inter-helical distances in colicin Ia upon membrane binding³⁹, which was considered as evidence for a 2D circular arrangement of the amphipathic helices and for their location on the membrane surface. The same conclusion was reached in site-directed EPR-spectroscopy studies of colicin A⁵⁴. In this work, distances of residues in the amphipathic helices from helix 9 in the hydrophobic hairpin were found to have quite wide distributions and inter-helical distance increment has also been reported for this colicin by FRET experiments^{35,72}. However, DSC experiments for colicin E1 show that globular structure is partially retained in the inserted state^{33,56}.

Three different models were used as initial conformations for the inserted state: Compact, Loose-Array and Circular (see Fig. 2). From the MD simulations it became evident that highly expanded conformations like that in Fig. 2c are not likely. This is in agreement with the calorimetric data mentioned above and with the hypothesis that the aromatic-residue cluster remains formed in the in closed state⁷⁰. Among the structures resulting from the MD simulations, the Compact model seems to provide, in most cases, the ones with the lowest free energy. On the other hand, the distance distributions are in better agreement with the experimental data for the Circular and the Loose-Array models (Fig. 4). This suggests that the experimental evidence at hand may not be enough to confirm a circular arrangement for the amphipathic helices. The wide distributions of distances observed experimentally⁵⁴ are not compatible with a single well-defined structure but with a wide ensemble. Such an ensemble cannot be sampled in current feasible MD simulations. It is possible that our simulations sample different regions from this wide ensemble.

The transition from the adsorbed state to the membrane-inserted conformation in our simulations is not always spontaneous ($\Delta G < 0$), as it should. One possible explanation is that, due to sampling limitations, the models used for the inserted state may not be as low in energy as the real structures. That is, comparing the adsorbed state, which is based on X-ray structures, to the membrane-inserted states, which are a result of computer modeling, the former may have an advantage. Limitations of the implicit membrane model used in this work should also be kept in mind. Lipid packing effects are not included in the model, the lipid head group region is treated like water, and the electrostatic potential profile across the membrane is approximate. All these factors could affect the calculated effective energies substantially.

Once the PFD is inserted, the hydrophobic hairpin could stay on the water-membrane interface ("penknife") or orient perpendicularly to the membrane surface ("umbrella"). The computational results suggest that in the closed state of the colicin pore there may be equilibrium between the two conformations. According to our results and relevant experimental evidence^{43,44,46}, the exact position of this equilibrium may depend on the specific colicin. As the hydrophobic hairpin in colicins Ia and E1 is too short to span the membrane in the umbrella conformation, it has been suggested that helix elongation should take place³³. This idea was supported by an increase in helical content in colicin E1 upon membrane binding, measured by CD and IR analysis³³. However, there is no direct evidence for helix elongation at the hydrophobic hairpin. In fact, helix elongation has only been reported for the amphipathic helices of colicin E1^{34,53}. In our simulations, the hydrophobic helix elongation process is unfavorable for colicin Ia, while it is favorable for colicin E1. This may be related to the fact that the PFD of colicin Ia has higher helical content than colicin E1, which, based on their crystal structures, is 78 (refs. 18,51) and 68.5% (ref. 17), respectively. Furthermore, the results show that helix elongation does not necessarily lead to spanning of the membrane by the hydrophobic hairpin. In our simulations with the Loose-Array model of colicin E1, amphipathic helix elongation is observed (Fig. S4

in the Supplementary Material), further supporting that helix elongation is favorable for this colicin, although it may not necessarily occur in the hydrophobic hairpin. Membrane thinning could also be a means to achieve membrane spanning by the shorter hairpins in the umbrella conformation. Our results do not support this idea, as the cost of membrane deformation is larger than the improvement in protein solvation energy.

Taken together, the results presented here seem to support the model depicted in Fig. 8: the colicin is first oriented with the hydrophobic hairpin parallel to the membrane plane (adsorbed state) and then undergoes a conformational change to the penknife model. Following this transition, the orientation of the hydrophobic hairpin may change and become perpendicular to the membrane plane (umbrella conformation in Fig. 8). The actual balance between the two conformations may differ depending on the experimental conditions and the specific sequence at hand. Both computational and experimental results suggest that the states depicted in Fig. 8 likely consist in an ensemble of configurations in equilibrium with each other: from umbrella to penknife orientation of the hydrophobic hairpin and from a compact to a loosely packed arrangement of the amphipathic helices. A recent study of a voltage dependent ion channel suggested that the closed state comprises a variety of conformations⁸⁰, albeit more well-defined and narrowly distributed than the wide ensembles suggested for colicins.

Future progress in this field could come from further experimental studies. For example, to study the adsorbed state, mutants that do not insert into membranes could be created (e.g. via disulfide bonds)³⁶. Then the binding interface could be probed by mutations of surface residues, such as the cationic ones identified in this study, and membrane-binding energetics could be measured. Fluorescence labeling⁵⁵ and site-directed EPR-spectroscopy experiments⁵⁴ have already given valuable information on distances between specific residues and could easily be extended to more residue pairs and more colicin sequences. Solid-state NMR is a highly promising technique that is increasingly used on membrane proteins^{37–39,46,51,81–83}. Combined with site-directed mutagenesis, labeling specific residues in the hydrophobic hairpin, it has proven to be efficient in the determination of equilibrium between its two possible conformations⁴⁶ in colicin E1. Interesting information could also be obtained from studies of synthetic peptides corresponding to the hydrophobic hairpin. Comparison with the results for the entire PFD would test the role of the interactions between this hairpin and the rest of the molecule. If the residues flanking helices 8 and 9 are included, the possibility of hydrophobic helix elongation could also be tested. Finally, studying the amphipathic helices individually would show whether these helices, indeed, get adsorbed to the membrane surface (as proposed in the literature) or they need to interact with each other to some degree when interacting with the membrane (as the results presented in this work suggest).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

L. Prieto acknowledges a Fellowship from the Spanish Ramón Areces Foundation. Additional support was provided by the National Science Foundation (MCB-0316667). Infrastructure support was provided in part by RCMI grant RR03060 from NIH. Alan Finkelstein, Burkhard Bechinger, Michael D. Grabe, M.W. Parker, Ruth Stark, and Maja Mihajlovic provided valuable advice and suggestions.

REFERENCES

1. Lakey, JH.; Slatin, SL. Pore forming toxins. In: Goot, Gvd, editor. Pore-forming colicins and their relatives. Springer; 2001. 166 p.
2. James R, Kleanthous C, Moore GR. The biology of E colicins: Paradigms and paradoxes. *Microbiology-(UK)*. 1996; 142:1569–1580.
3. Cramer WA, Heymann JB, Schendel SL, Deriy BN, Cohen FS, Elkins PA, Stauffacher CV. Structure-function of the channel-forming colicins. *Annu Rev Biophys Biomol Struct*. 1995; 24:611–641. [PubMed: 7545041]
4. Schein SJ, Kagan BL, Finkelstein A. Colicin-K acts by forming voltage-dependent channels in phospholipid bilayer membranes. *Nature*. 1978; 276(5684):159–163. [PubMed: 740032]
5. Cleveland MV, Slatin S, Finkelstein A, Levinthal C. Structure-function-relationships for a voltage-dependent ion channel - Properties of COOH-terminal fragments of Colicin-E1. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences*. 1983; 80(12): 3706–3710.
6. Pattus F, Cavard D, Verger R, Lazdunski C, Schindler H, Rosenbusch J. Is the formation of voltage dependent pores in planar bilayer by Colicin A of physiological importance. *Toxicon*. 1982; 20(1): 205–206.
7. Nogueira RA, Varanda WA. Gating properties of channels formed by Colicin Ia in planar lipid bilayer-membranes. *Journal of Membrane Biology*. 1988; 105(2):143–153. [PubMed: 2464064]
8. Garcia-Saez AJ, Mingarro I, Perez-Paya E, Salgado J. Membrane-insertion fragments of Bcl-x(L), Bax, and Bid. *Biochemistry*. 2004; 43(34):10930–10943. [PubMed: 15323553]
9. Muchmore SW, Sattler M, Liang H, Meadows RP, Harlan JE, Yoon HS, Nettesheim D, Chang BS, Thompson CB, Wong SL, Ng SC, Fesik SW. X-ray and NMR structure of human Bcl-x(L), an inhibitor of programmed cell death. *Nature*. 1996; 381(6580):335–341. [PubMed: 8692274]
10. Lindeberg M, Zakharov SD, Cramer WA. Unfolding pathway of the colicin E1 channel protein on a membrane surface. *J Mol Biol*. 2000; 295(3):679–692. [PubMed: 10623556]
11. Dankert JR, Uratani Y, Grabau C, Cramer WA, Hermodson M. On a domain-structure of Colicin E1 - A COOH-terminal peptide fragment active in membrane depolarization. *Journal of Biological Chemistry*. 1982; 257(7):3857–3863. [PubMed: 7037787]
12. Savitsky A, Kuhn M, Duche D, Mobius K, Steinhoff HJ. Spontaneous refolding of the pore-forming colicin A toxin upon membrane association as studied by X-band and W-band high-field electron paramagnetic resonance spectroscopy. *Journal of Physical Chemistry B*. 2004; 108(27): 9541–9548.
13. Ohnoiwashita Y, Imahori K. Assignment of the functional loci in the Colicin-E1 molecule by characterization of its proteolytic fragments. *Journal of Biological Chemistry*. 1982; 257(11): 6446–6451. [PubMed: 7042712]
14. Martinez MC, Lazdunski C, Pattus F. Isolation, molecular and functional-properties of the C-terminal domain of Colicin-A. *Embo Journal*. 1983; 2(9):1501–1507. [PubMed: 11892802]
15. Parker MW, Pattus F, Tucker AD, Tsernoglou D. Structure of the membrane-pore-forming fragment of colicin A. *Nature*. 1989; 337(6202):93–96. [PubMed: 2909895]
16. Parker MW, Postma JP, Pattus F, Tucker AD, Tsernoglou D. Refined structure of the Pore-Forming Domain of Colicin A at 2.4 Å resolution. *J Mol Biol*. 1992; 224(3):639–657. [PubMed: 1373773]
17. Elkins P, Bunker A, Cramer WA, Stauffacher CV. A mechanism for toxin insertion into membranes is suggested by the crystal structure of the channel-forming domain of colicin E1. *Structure*. 1997; 5(3):443–458. [PubMed: 9083117]
18. Wiener M, Freymann D, Ghosh P, Stroud RM. Crystal structure of Colicin Ia. *Nature*. 1997; 385(6615):461–464. [PubMed: 9009197]
19. Vetter IR, Parker MW, Tucker AD, Lakey JH, Pattus F, Tsernoglou D. Crystal structure of a Colicin N fragment suggests a model for toxicity. *Structure*. 1998; 6(7):863–874. [PubMed: 9687368]
20. Soelaiman S, Jakes K, Wu N, Li CM, Shoham M. Crystal structure of Colicin E3: Implications for cell entry and ribosome inactivation. *Molecular Cell*. 2001; 8(5):1053–1062. [PubMed: 11741540]

21. Hilsenbeck JL, Park H, Chen G, Youn B, Postle K, Kang CH. Crystal structure of the cytotoxic bacterial protein colicin B at 2.5 angstrom resolution. *Mol Microbiol.* 2004; 51(3):711–720. [PubMed: 14731273]
22. Shirabe K, Cohen FS, Xu SH, Peterson AA, Shiver JW, Nakazawa A, Cramer WA. Decrease of anion selectivity caused by mutation of THR501 and GLY502 to GLU in the hydrophobic domain of the Colicin E1 channel. *Journal of Biological Chemistry.* 1989; 264(4):1951–1957. [PubMed: 2563369]
23. Shiver JW, Peterson AA, Widger WR, Furbacher PN, Cramer WA. Prediction of bilayer spanning domains of hydrophobic and amphipathic membrane-proteins - Application to the Cytochrome-b and Colicin families. *Methods in Enzymology.* 1989; 172:439–461. [PubMed: 2747538]
24. Zhang YL, Cramer WA. Constraints imposed by protease accessibility on the trans-membrane and surface topography of the colicin E1 ion channel. *Protein Sci.* 1992; 1(12):1666–1676. [PubMed: 1284805]
25. Choe S, Bennett MJ, Fujii G, Curmi PMG, Kantardjieff KA, Collier RJ, Eisenberg D. The crystal-structure of Diphtheria-Toxin. *Nature.* 1992; 357(6375):216–222. [PubMed: 1589020]
26. Suzuki M, Youle RJ, Tjandra N. Structure of Bax: Coregulation of dimer formation and intracellular localization. *Cell.* 2000; 103(4):645–654. [PubMed: 11106734]
27. Chou JJ, Li HL, Salvesen GS, Yuan JY, Wagner G. Solution structure of BID, an intracellular amplifier of apoptotic signaling. *Cell.* 1999; 96(5):615–624. [PubMed: 10089877]
28. Kagan BL, Finkelstein A, Colombini M. Diphtheria toxin fragment forms large pores in phospholipid bilayer membranes. *Proc Natl Acad Sci U S A.* 1981; 78(8):4950–4954. [PubMed: 6272284]
29. Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB. Bcl-x(L) forms an ion channel in synthetic lipid membranes. *Nature.* 1997; 385(6614):353–357. [PubMed: 9002522]
30. Schendel SL, Montal M, Reed JC. Bcl-2 family proteins as ion-channels. *Cell Death Differ.* 1998; 5(5):372–380. [PubMed: 10200486]
31. Pattus F, Martinez MC, Dargent B, Cavard D, Verger R, Lazdunski C. Interaction of Colicin-A with phospholipid monolayers and liposomes. *Biochemistry.* 1983; 22(25):5698–5703.
32. Gonzalez-Manas JM, Lakey JH, Pattus F. Brominated phospholipids as a tool for monitoring the membrane insertion of Colicin-A. *Biochemistry.* 1992; 31(32):7294–7300. [PubMed: 1510921]
33. Zakharov SD, Lindeberg M, Griko Y, Salamon Z, Tollin G, Prendergast FG, Cramer WA. Membrane-bound state of the Colicin E1 channel domain as an extended two-dimensional helical array. *Proc Natl Acad Sci U S A.* 1998; 95(8):4282–4287. [PubMed: 9539728]
34. Ho D, Merrill aR. Evidence for the amphipathic nature and tilted topology of helices 4 and 5 in the closed state of the Colicin E1 channel. *Biochemistry.* 2009; 48(6):1369–1380. [PubMed: 19159330]
35. Lakey JH, Duche D, Gonzalez-Manas JM, Baty D, Pattus F. Fluorescence energy transfer distance measurements. The hydrophobic helical hairpin of colicin A in the membrane bound state. *J Mol Biol.* 1993; 230(3):1055–1067. [PubMed: 7683055]
36. Duche D, Parker MW, Gonzalez-Manas JM, Pattus F, Baty D. Uncoupled steps of the colicin A pore formation demonstrated by disulfide bond engineering. *J Biol Chem.* 1994; 269(9):6332–6339. [PubMed: 8119982]
37. Lambotte S, Jasperse P, Bechinger B. Orientational distribution of alpha-helices in the colicin B and E1 channel domains: a one and two dimensional ¹⁵N solid-state NMR investigation in uniaxially aligned phospholipid bilayers. *Biochemistry.* 1998; 37(1):16–22. [PubMed: 9453746]
38. Huster D, Yao X, Hong M. Membrane protein topology probed by (1)H spin diffusion from lipids using solid-state NMR spectroscopy. *J Am Chem Soc.* 2002; 124(5):874–883. [PubMed: 11817963]
39. Luo WB, Yao XL, Hong M. Large structure rearrangement of colicin Ia channel domain after membrane binding from 2D C-13 spin diffusion NMR. *Journal of the American Chemical Society.* 2005; 127(17):6402–6408. [PubMed: 15853348]
40. Padmavathi PV, Steinhoff HJ. Conformation of the closed channel state of Colicin A in proteoliposomes: an umbrella model. *J Mol Biol.* 2008; 378(1):204–214. [PubMed: 18353363]

41. Massotte D, Yamamoto M, Scianimanico S, Sorokine O, van Dorsselaer A, Nakatani Y, Ourisson G, Pattus F. Structure of the membrane-bound form of the pore-forming domain of colicin A: a partial proteolysis and mass spectrometry study. *Biochemistry*. 1993; 32(50):13787–13794. [PubMed: 8268153]
42. Duche D, Izard J, GonzalezManas JM, Parker MW, Crest M, Chartier M, Baty D. Membrane topology of the colicin A pore-forming domain analyzed by disulfide bond engineering. *Journal of Biological Chemistry*. 1996; 271(26):15401–15406. [PubMed: 8663026]
43. Kienker PK, Qiu X, Slatin SL, Finkelstein A, Jakes KS. Transmembrane insertion of the colicin Ia hydrophobic hairpin. *J Membr Biol*. 1997; 157(1):27–37. [PubMed: 9141356]
44. Tory MC, Merrill AR. Adventures in membrane protein topology - A study of the membrane-bound state of colicin E1. *Journal of Biological Chemistry*. 1999; 274(35):24539–24549. [PubMed: 10455117]
45. Tory MC, Merrill AR. Determination of membrane protein topology by red-edge excitation shift analysis: application to the membrane-bound colicin E1 channel peptide. *Biochim Biophys Acta-Biomembr*. 2002; 1564(2):435–448.
46. Aisenbrey C, Cusan M, Lambotte S, Jasperse P, Georgescu J, Harzer U, Bechinger B. Specific isotope labeling of colicin E1 and B channel domains for membrane topological analysis by oriented solid-state NMR spectroscopy. *Chembiochem*. 2008; 9(6):944–951. [PubMed: 18338351]
47. Sobko AA, Kotova EA, Antonenko YN, Zakharov SD, Cramer WA. Effect of lipids with different spontaneous curvature on the channel activity of colicin E1: evidence in favor of a toroidal pore. *FEBS Lett*. 2004; 576(1–2):205–210. [PubMed: 15474038]
48. Wang Y, Malenbaum SE, Kachel K, Zhan HJ, Collier RJ, London E. Identification of shallow and deep membrane-penetrating forms of diphtheria toxin T domain that are regulated by protein concentration and bilayer width. *Journal of Biological Chemistry*. 1997; 272(40):25091–25098. [PubMed: 9312118]
49. Kim Y, Valentine K, Opella SJ, Schendel SL, Cramer WA. Solid-state NMR studies of the membrane-bound closed state of the colicin E1 channel domain in lipid bilayers. *Protein Sci*. 1998; 7(2):342–348. [PubMed: 9521110]
50. Song HY, Cohen FS, Cramer WA. Membrane topography of Cole1 gene-products - the hydrophobic anchor of the Colicin-E1 channel is a helical hairpin. *Journal of Bacteriology*. 1991; 173(9):2927–2934. [PubMed: 1708383]
51. Huster D, Yao YL, Jakes K, Hong M. Conformational changes of colicin Ia channel-forming domain upon membrane binding: a solid-state NMR study. *Biochim Biophys Acta-Biomembr*. 2002; 1561(2):159–170.
52. White D, Musse AA, Wang J, London E, Merrill AR. Toward elucidating the membrane topology of helix two of the Colicin E1 channel domain. *J Biol Chem*. 2006; 281(43):32375–32384. [PubMed: 16854987]
53. Wei ZK, White D, Wang J, Musse AA, Merrill AR. Tilted, extended, and lying in wait: The membrane-bound topology of residues Lys-381-Ser-405 of the colicin E1 channel domain. *Biochemistry*. 2007; 46(20):6074–6085. [PubMed: 17455912]
54. Bohme S, Padmavathi PVL, Holterhues J, Ouchni F, Klare JP, Steinhoff HJ. Topology of the amphipathic helices of the colicin A pore-forming domain in *E. coli* lipid membranes studied by pulse EPR. *Phys Chem Chem Phys*. 2009; 11(31):6770–6777. [PubMed: 19639151]
55. Musse AA, Wang J, deLeon GP, Prentice GA, London E, Merrill AR. Scanning the membrane-bound conformation of helix 1 in the Colicin E1 channel domain by site-directed fluorescence labeling. *Journal of Biological Chemistry*. 2006; 281(2):885–895. [PubMed: 16299381]
56. Zakharov SD, Cramer WA. Insertion intermediates of pore-forming colicins in membrane two-dimensional space. *Biochimie*. 2002; 84(5–6):465–475. [PubMed: 12423790]
57. Lazaridis T. Effective energy function for proteins in lipid membranes. *Proteins-Structure Function and Genetics*. 2003; 52(2):176–192.
58. Lazaridis T, Karplus M. Effective energy function for proteins in solution. *Proteins-Structure Function and Genetics*. 1999; 35(2):133–152.
59. Brooks BR, Brooks CL, Mackerell AD, Nilsson L, Petrella RJ, Roux B, Won Y, Archontis G, Bartels C, Boresch S, Caflisch A, Caves L, Cui Q, Dinner AR, Feig M, Fischer S, Gao J,

- Hodoscek M, Im W, Kuczera K, Lazaridis T, Ma J, Ovchinnikov V, Paci E, Pastor RW, Post CB, Pu JZ, Schaefer M, Tidor B, Venable RM, Woodcock HL, Wu X, Yang W, York DM, Karplus M. CHARMM: The Biomolecular Simulation Program. *J Comput Chem*. 2009; 30(10):1545–1614. [PubMed: 19444816]
60. Neria E, Fischer S, Karplus M. Simulation of activation free energies in molecular systems. *Journal of Chemical Physics*. 1996; 105(5):1902–1921.
 61. Hristova K, Wimley WC, Mishra VK, Anantharamiah GM, Segrest JP, White SH. An amphipathic alpha-helix at a membrane interface: A structural study using a novel X-ray diffraction method. *J Mol Biol*. 1999; 290(1):99–117. [PubMed: 10388560]
 62. Mclaughlin S. The electrostatic properties of membranes. *Annual Review of Biophysics and Biophysical Chemistry*. 1989; 18:113–136.
 63. Lazaridis T. Implicit solvent simulations of peptide interactions with anionic lipid membranes. *Proteins-Structure Function and Bioinformatics*. 2005; 58(3):518–527.
 64. Ramos J, Lazaridis T. Energetic determinants of oligomeric state specificity in coiled coils. *J Am Chem Soc*. 2006; 128(48):15499–15510. [PubMed: 17132017]
 65. Nielsen C, Goulian M, Andersen OS. Energetics of inclusion-induced bilayer deformations. *Biophys J*. 1998; 74(4):1966–1983. [PubMed: 9545056]
 66. Lundbaek JA, Andersen OS. Spring constants for channel-induced lipid bilayer deformations estimates using gramicidin channels. *Biophys J*. 1999; 76(2):889–895. [PubMed: 9929490]
 67. Pan J, Tristram-Nagle S, Kucerka N, Nagle JF. Temperature dependence of structure, bending rigidity, and bilayer interactions of dioleoylphosphatidylcholine bilayers. *Biophys J*. 2008; 94(1): 117–124. [PubMed: 17827241]
 68. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. *Nucleic Acids Research*. 2000; 28(1):235–242. [PubMed: 10592235]
 69. Voet, D.; Voet, JG.; Pratt, CW. *Fundamentals of biochemistry*. John Wiley & Sons I., editor. 1999.
 70. Lakey JH, Massotte D, Heitz F, Dasseux JL, Faucon JF, Parker MW, Pattus F. Membrane insertion of the Pore-Forming Domain of Colicin-A - A spectroscopic study. *Eur J Biochem*. 1991; 196(3): 599–607. [PubMed: 2013283]
 71. Gonzalez-Manas JM, Lakey JH, Pattus F. Brominated phospholipids as a tool for monitoring the membrane insertion of Colicin-A. *Biochemistry*. 1992; 31(32):7294–7300. [PubMed: 1510921]
 72. Lakey JH, Baty D, Pattus F. Fluorescence Energy-Transfer distance measurements using site-directed single cysteine mutants - The membrane insertion of Colicin-A. *J Mol Biol*. 1991; 218(3): 639–653. [PubMed: 2016750]
 73. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol*. 1982; 157(1):105–132. [PubMed: 7108955]
 74. Zakharov SD, Kotova EA, Antonenko YN, Cramer WA. On the role of lipid in colicin pore formation. *Biochim Biophys Acta*. 2004; 1666(1–2):239–249. [PubMed: 15519318]
 75. Davidson VL, Cramer WA, Bishop LJ, Brunden KR. Dependence of the activity of Colicin E1 in artificial membrane-vesicles on pH, membrane-potential, and vesicle size. *Journal of Biological Chemistry*. 1984; 259(1):594–600. [PubMed: 6706954]
 76. Davidson VL, Brunden KR, Cramer WA. Acidic pH requirement for insertion of Colicin-E1 into artificial membrane-vesicles - Relevance to the mechanism of action of colicins and certain toxins. *Proceedings of the National Academy of Sciences of the United States of America*. 1985; 82(5): 1386–1390. [PubMed: 2579396]
 77. Bullock JO, Armstrong SK, Shear JL, Lies DP, McIntosh MA. FORMATION OF ION CHANNELS BY COLICIN-B IN PLANAR LIPID BILAYERS. *Journal of Membrane Biology*. 1990; 114(1):79–95. [PubMed: 1690810]
 78. Mel SF, Stroud RM. Colicin Ia inserts into negatively charged membranes at low pH with a tertiary but little secondary structural change. *Biochemistry*. 1993; 32(8):2082–2089. [PubMed: 8448167]
 79. Merrill AR, Steer BA, Prentice GA, Weller MJ, Szabo AG. Identification of a chameleon-like pH-sensitive segment within the colicin E1 channel domain that may serve as the pH-activated trigger for membrane bilayer association. *Biochemistry*. 1997; 36(23):6874–6884. [PubMed: 9188682]

80. Tao X, Lee A, Limapichat W, Dougherty DA, MacKinnon R. A gating charge transfer center in voltage sensors. *Science*. 2010; 328(5974):67–73. [PubMed: 20360102]
81. Hong M. Solid-state NMR studies of the structure, dynamics, and assembly of beta-sheet membrane peptides and alpha-helical membrane proteins with antibiotic activities. *Acc Chem Res*. 2006; 39(3):176–183. [PubMed: 16548506]
82. Aisenbrey C, Sudheendra US, Ridley H, Bertani P, Marquette A, Nedelkina S, Lakey JH, Bechinger B. Helix orientations in membrane-associated Bcl-X-L determined by N-15-solid-state NMR spectroscopy. *European Biophysics Journal with Biophysics Letters*. 2007; 37:71–80. [PubMed: 17492281]
83. Yao XL, Hong M. Effects of anionic lipid and ion concentrations on the topology and segmental mobility of colicin Ia channel domain from solid-state NMR. *Biochemistry*. 2006; 45(1):289–295. [PubMed: 16388605]
84. Humphrey W, Dalke A, Schulten K. VMD: Visual molecular dynamics. *J Mol Graph*. 1996; 14(1): 33. &. [PubMed: 8744570]

Figure 1. Three-dimensional structures of the PFD of the colicins studied in this work (cartoon representations were made using VMD84). The groups to which they belong are also indicated. The hydrophobic hairpin in each case is highlighted. The arrows show the location and direction of the pulling force applied to get an umbrella model (see Methods Section).

Figure 2. Side and upper views the **a)** Compact, **b)** Loose-Array and **c)** Circular model for the umbrella conformation. (Cartoon representations were made using VMD84). The horizontal lines represent the membrane surface. These structures correspond to the initial structures of the simulations run for the inserted state of colicins.

Figure 3.

Distribution of the values of the angle formed by the hydrophobic hairpin and the membrane. In the upper graph the results at low pH are shown and in the lower part, those for pH=7. The direction of the axis of the hydrophobic hairpin (highlighted in black) is shown as an inset of the lower graph as well as the angle corresponding to the abscissa axis (the membrane surface is represented by a horizontal line). An example of the adsorbed state with the hydrophobic hairpin oriented parallel to the membrane plane is shown as an inset in the upper graph (cartoon representations were made using VMD84).

Figure 4.

Qualitative comparison of the inter-helical distance distributions of colicin A in our simulations with the EPR spectroscopy results reported by Böhme *et al.*⁵⁴. The green lines correspond to the experimental results, while the black, red and blue ones correspond to the simulation results obtained for the compact, loose-array and circular models, respectively.

Figure 5.

Representative snapshots for the penknife and umbrella conformations in the inserted state of the four colicins studied here. The arrows point to the loop that becomes more extended in the umbrella model of colicin Ia. The line representation highlights the aromatic and acidic residues representative in the interaction with the membrane of the umbrella conformations of colicins Ia and E1 (see discussion about the helix elongation process).

Figure 6.

Representative snapshots of the penknife and umbrella conformations in the inserted state of colicins Ia and E1 with a longer hydrophobic hairpin. The line representation highlights the aromatic and acidic residues representative in the interaction with the membrane of the umbrella conformations of colicins Ia and E1 (see text).

Figure 7. Variation (Δ) with respect to the system with $T=26 \text{ \AA}$ of the effective energy (discontinuous line) and the effective plus the deformation free energy (continuous line) with membrane thickness.

Figure 8.

Mechanism of colicin insertion proposed by Kienker and coworkers⁴³ and supported by the results presented in the present paper. Adapted from Fig. 7 in reference 43. “ADS” stands for the adsorbed state; $\Delta G_{\text{ADS-P}}$ is the difference in free energy between the penknife conformation and the adsorbed state, and $\Delta G_{\text{P-U}}$ is the difference in free energy between the umbrella and the penknife conformations.

Adsorbed state simulation results. These are averages and standard deviations from 6 runs starting from different initial orientation.

Table1

	ΔW_{tr}^* (kcal/mol)		$Z_{CM}^{\ddagger}$ (Å)		$\Delta W_{GC}^{\ddagger}$ (kcal/mol)	
	pH=7	low pH	pH=7	low pH	pH=7	low pH
Col. A	0.7 ± 0.4	7.8 ± 0.7	46.9 ± 0.8	36.2 ± 0.4	1.0 ± 0.7	10 ± 1
Col. B	0.8 ± 0.6	7 ± 1	48 ± 6	36.3 ± 0.3	1.0 ± 0.8	9 ± 1
Col. Ia	2.9 ± 0.8	8.6 ± 0.5	39 ± 1	34.8 ± 0.7	3.4 ± 0.9	10.7 ± 0.8
Col. E1	2.1 ± 0.4	10 ± 2	41 ± 2	35 ± 1	2.6 ± 0.6	11.1 ± 0.6

* ΔW_{tr} : Effective transfer energy (see Eq. 7)

$\ddagger Z_{CM}$: z coordinate of the PFD center of mass

$\ddagger \Delta W_{GC}$: Gouy-Chapman transfer energy calculated as $W_{GC}(\text{water}) - W_{GC}(\text{adsorbed})$

Table 2

Average free energies of models for the membrane-inserted state in kcal/mol.

	Compact	Loose-Array	Circular	Compact	Loose-Array	Circular	Compact	Loose-Array	Circular
	$W_{\text{eff}}^{\text{PK}*}$			$W_{\text{eff}}^{\text{UM}^\dagger}$			$\Delta W_{\text{PU}}^{\ddagger}$		
Colicin A	-4172 ± 19	-4138 ± 7	-4155 ± 18	-4175 ± 4	-4136 ± 2	-4138 ± 12	-3 ± 19	2 ± 7	17 ± 22
Colicin B	-4113 ± 10	-4071 ± 14	-4080 ± 13	-4133 ± 3	-4071 ± 11	-4062 ± 13	-20 ± 10	0 ± 17	18 ± 18
Colicin Ia	-3778 ± 8	-3741 ± 17	-3741 ± 18	-3763 ± 3	-3705 ± 5	-3727 ± 12	15 ± 8	36 ± 18	14 ± 22
Colicin E1	-3803 ± 5	-3799 ± 13	-3778 ± 2	-3797 ± 4	---	-3774 ± 1	6 ± 6	---	4 ± 2

	$\Delta G^{\text{PK}}_{\S}$			$\Delta G^{\text{UM}}_{\P}$			$\Delta G_{\text{PU}}_{\ }$		
Colicin A	-4453 ± 19	-4422 ± 12	-4458 ± 19	-4458 ± 8	-4417 ± 9	-4446 ± 14	-5 ± 21	5 ± 15	12 ± 24
Colicin B	-4385 ± 11	-4341 ± 12	-4363 ± 15	-4409 ± 4	-4348 ± 14	-4358 ± 14	-24 ± 16	-7 ± 18	5 ± 20
Colicin Ia	-4028 ± 11	-3994 ± 18	-4001 ± 19	-4012 ± 6	-3963 ± 6	-3988 ± 12	16 ± 12	31 ± 19	13 ± 22
Colicin E1	-4066 ± 9	-4073 ± 13	-4054 ± 9	-4061 ± 7	---	-4051 ± 7	5 ± 11	---	3 ± 11

* $W_{\text{eff}}^{\text{PK}}$: effective energy of the penknife model

$^\dagger W_{\text{eff}}^{\text{UM}}$: effective energy of the umbrella model

$^\ddagger \Delta W_{\text{PU}} = W_{\text{eff}}^{\text{UM}} - W_{\text{eff}}^{\text{PK}}$

$_{\S} \Delta G^{\text{PK}}$: free energy of the penknife model

$_{\P} \Delta G^{\text{UM}}$ effective energy of the umbrella model

$_{\|} \Delta G_{\text{PU}} = \Delta G^{\text{UM}} - \Delta G^{\text{PK}}$

$_I$ The umbrella model is not stable for colicin E1 with the Loose-Array model.

Table 3

Difference in effective and free energy (kcal/mol) between the adsorbed and inserted states (see Fig. 8).

	$\Delta W_{\text{ADS-ins}}^*$	$\Delta G_{\text{ADS-ins}}^\dagger$
Colicin A	35 ± 19	30 ± 21
Colicin B	-11 ± 4	-25 ± 15
Colicin Ia	3 ± 10	-7 ± 15
Colicin E1	28 ± 12	21 ± 16

* $\Delta W_{\text{ADS-ins}} = W_{\text{eff,ins}} - W_{\text{eff,ADS}}$, where “ins” stands for the minimum energy inserted conformation (umbrella or penknife) and $W_{\text{eff,ADS}}$ is the effective energy of the adsorbed state

$\dagger \Delta G_{\text{ADS-ins}}$: free energy required for the transition from the adsorbed state to the minimum energy inserted conformation

Table 4

Contributions to the free energy change from penknife to umbrella model (all values in kcal/mol).

	Colicin A	Colicin B	Colicin Ia	Colicin E1
$\Delta W_{PU}^{VDW} + \Delta W_{PU}^{COV*}$	-3 ± 15	1 ± 6	17 ± 14	21 ± 4
$\Delta W_{PU}^{ELEC}^{\dagger}$	-30 ± 11	-89 ± 15	-67 ± 17	-36 ± 14
$\Delta W_{PU}^{SLV}^{\ddagger}$	30 ± 34	68 ± 12	65 ± 10	21 ± 11
$-T\Delta S_{PU}^{\P}$	-2 ± 7	-4 ± 12	1 ± 9	-1 ± 11

* $\Delta W_{PU}^{VDW} + \Delta W_{PU}^{COV}$: difference in van der Waals and covalent effective energy between the umbrella and penknife conformations

$\dagger \Delta W_{PU}^{ELEC}$: difference in electrostatic effective energy between the umbrella and penknife conformations

$\ddagger \Delta W_{PU}^{SLV}$ difference in solvation free energy between the umbrella and penknife conformations (includes the Gouy-Chapman term)

$\P -T\Delta S_{PU}$ difference in conformational free energy between the umbrella and penknife conformations

Table 5

Effective and free energy of colicins Ia and E1 with the elongated hairpin (kcal/mol).

	$W_{\text{eff}}^{\text{PK}*}$	$W_{\text{eff}}^{\text{UM}^\dagger}$	$\Delta G_{\text{ADS-P}}^{\ddagger}$	$\Delta G_{\text{PU}}^{\P}$
Colicin Ia	-3747 ± 8	-3742 ± 2	47 ± 13	4 ± 9
Colicin E1	-3829 ± 3	-3819 ± 8	-2 ± 15	3 ± 11

* $W_{\text{eff}}^{\text{PK}}$: effective energy of the penknife model $^\dagger W_{\text{eff}}^{\text{UM}}$: effective energy of the umbrella model $^\ddagger \Delta G_{\text{ADS-P}}$: difference in free energy between the penknife model and the adsorbed state $^\P \Delta G_{\text{PU}}$: difference in free energy between the umbrella and penknife models

Table 6

Contributions to the free energy difference between the two possible conformations of the inserted state of colicins Ia and E1 with the elongated hydrophobic hairpin (all values in kcal/mol).

	Colicin Ia	Colicin E1
$\Delta W_{PU}^{VDW} + \Delta W_{PU}^{COV}^*$	0 ± 14	14 ± 8
$\Delta W_{PU}^{ELEC}^\dagger$	-62 ± 11	-50 ± 11
$\Delta W_{PU}^{SLV}^\ddagger$	67 ± 14	46 ± 14
$-T\Delta S_{PU}^\S$	-1 ± 4	-7 ± 6

* $\Delta W_{PU}^{VDW} + \Delta W_{PU}^{COV}$: difference in van der Waals and bonded effective energy between umbrella and penknife models

$^\dagger \Delta W_{PU}^{ELEC}$: difference in electrostatic effective energy between umbrella and penknife models

$^\ddagger \Delta W_{PU}^{SLV}$: difference in solvation free energy between umbrella and penknife models

$^\S -T\Delta S_{PU}$: variation in conformational free energy