Supplementary information for:

Structure of colicin I receptor bound to the R-domain of colicin Ia: implications for protein import

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MATERIALS AND METHODS

Bacterial strains and plasmids

A colicin Ia-bearing *E. coli* strain (BZB2114) (Feldgarden and Riley, 1998), provided by M. A. Riley (Yale University), was used to amplify the receptor binding domain of colicin Ia. Strain TG1 containing plasmid pKSJ101, which has the complete colicin Ia operon, including the promoter and immunity protein, cloned between the EcoRI and BamHI sites of pUC19 (Jakes et al., 1998), produces large amounts of full-length colicin Ia. Strain ASH102 (Buxton and Holland, 1973; Buxton and Holland, 1974) contains a *cirA* mutation and was obtained from the Yale *E. coli* Genetic Stock Center (CGSC). Strain A586 (Nagel de Zwaig and Luria, 1967) contains a *tolC* point mutation and strain EW1b (Whitney, 1971) contains a 5' *tolC* deletion; both strains were obtained from CGSC. R. Misra (Arizona State University) provided the following porin deletion strains for colicin Ia susceptibility assays: MC4100 (all porins present) (Casadaban, 1976), RAM725 (MC4100, OmpF-minus *[ompF::lacZ]*), RAM726 (MC4100, OmpC-minus *[ompC::lacZ]*, RAM105 (MC4100, OmpF-minus, LamB-minus, OmpC-plus) (Misra and Benson, 1988), RAM123 (RAM105, OmpF-minus, LamB-minus, OmpC-plus, OmpG-plus) (Misra and Benson, 1989), B2045 (RAM105, OmpF-minus, LamB-minus, OmpC-minus, OmpG-plus), and RAM1129 (MC4100, TolC-minus *[tolC::Km^r]* (Augustus et al., 2004). Strain TJB3 was provided by M. McIntosh (University of Missouri) (Barnard, 2003). BL21(DE3) cells used for expression and for colicin Ia susceptibility assays were obtained from EMD BioSciences.

Recombinant DNA techniques

The nucleotides encoding residues 26-663 of colicin I receptor (Cir) were amplified from *E. coli* genomic DNA and cloned into pET20b (EMD BioSciences) using EcoRV and EcoRI restriction sites, such that the expressed protein contained a 10-histidine tag followed by a tobacco etch virus (TEV) recognition site prior to residue 26, the first residue of the mature receptor. pET20b contains a *pelB* signal sequence which allows expression of Cir in the *E. coli* outer membrane. *E. coli* BL21(DE3) cells were transformed with resulting plasmid. Cells were grown in terrific broth (TB) supplemented with carbenicillin at 20°C without induction, for two days. To improve crystallographic phasing, we increased the number of methionines in Cir from 6 (naturally occurring) to 10 using site-directed mutagenesis (QuikChange, Stratagene). From multiple sequence alignments, we identified residues in putative transmembrane beta strands predicted to be exposed to the lipid bilayer as first demonstrated for BtuB (Chimento et al., 2003). The following mutations were made: W338M, L343M, F589M, V591M (Cir4M). The

pET20b-Cir4M plasmid was transformed into B834(DE3) (EMD BioSciences) and cells were grown in minimal media supplemented with selenomethionine and the other 19 amino acids.

The receptor binding domain of colicin Ia, corresponding to residues 282 to 385, was amplified from an *E. coli* strain (BZB2114) carrying the colicin Ia gene on a naturally occurring plasmid, and cloned into pET17b (EMD BioSciences) using NdeI and HindIII restriction sites. BL21(DE3) cells were transformed with the resulting plasmid. Cells were grown in LB media supplemented with ampicillin to OD_{600} = 0.6 at 37°C, with subsequent induction using 1 mM IPTG and continued growth for 3 hours.

Full-length colicin Ia was produced from TG1 cells carrying plasmid pKSJ101 (Qiu et al., 1994). Cells were grown in 2xTY media supplemented with ampicillin to OD_{600} = 0.6 at 37°C, and induced with 0.5 μg/ml mitomycin C (Sigma) for three hours. A TonB box mutant in colicin Ia (M25P) was created with QuikChange (Stratagene), and expressed in TG1 cells as described above, for use in killing assays.

To make a Cir-minus strain for expression of (Cir) TonB box mutants, the Wanner method (Datsenko and Wanner, 2000) was used to disrupt the *cirA* gene of strain RW193 (F^-, *thi trpE proC leuB lacY mtl xyl rpsL azi tsx supA entA403*). To make the disruption, RW193 containing pDK46 was electroporated with PCR product generated using the primers H1P1CIRA (AACGAAGGGGATAACCGTAAGGGCGTTAGTATTCGTGGTCTGGTGTAGGCT GGAGCTGCTTC) and H2P2CIRA (CCCAGCCCCCTTTCACCGTTACGGTGTCGGTGGCGTTATAAACCATATGAAT ATCCTCCTTAG) and the plasmid pDK3 (Datsenko and Wanner, 2000; Barnard, 2003). The *ΔcirA::cat* lesion of the resulting strain (TJB3) was transduced into BL21(DE3) using P1vir (Miller, 1972) and confirmed by resistance to colicin Ia and by PCR using the primers cirAforward (ACGGGTCGGGCTGTGTTTG) and cirAreverse (GTTGCTGACATCACGACCATCG). BL21(DE3) containing the *ΔcirA::cat* mutation was transduced to *recA*$^-$ using a P1vir lysate made on JC10284 (*srlR::Tn10 srlC srlD metB mtl gatC gatA malA xyl rpsL supA Δ(srlR-recA*). This strain, BL21 (DE3 *ΔcirA::cat Δ(srlR-recA*)), was used as the host for the pET20b plasmids expressing the Cir TonB box mutations. An M33C/V35P TonB box mutant was made in Cir (QuikChange; Stratagene) and expressed in the *cirA* deletion strain as described for wild-type Cir in BL21(DE3) cells.

Protein purification

Cir: Colicin I receptor was purified from *E .coli* outer membranes as follows. 30 g cells (corresponding to approximately 2 litres of cell culture) were resuspended in lysis buffer (50 mM Tris-Cl, pH 8.0, 200 mM NaCl, 10 mM $MgCl_2$, 100 μg/ml AEBSF, and 10 μg/ml DNaseI), to a final volume of 120 ml. Cells were disrupted by passage through a French pressure cell at 18000 psi. Membranes were collected by ultracentrifugation using a 45-Ti rotor (Beckman) at 160,000 x *g* for 1 hour. After centrifugation, the supernatants were discarded and the pelleted membranes were resuspended in solubilization buffer (50 mM potassium phosphate (KPi), pH 7.5, 200 mM NaCl, 30 mM imidazole, and 5%

Elugent (CalBiochem)), to a final volume of 100 ml. The solution was stirred overnight at 4°C.

Solubilized protein was collected by ultracentrifugation using a 70-Ti rotor (Beckman) at 250,000 x *g* for 1 hour. Cir was purified using a procedure involving three successive chromatography columns. The solubilized protein was applied to a 15 ml Ni-NTA column (Qiagen) equilibrated with 50 mM KPi, pH 8.0, 200 mM NaCl, 10% glycerol, and 0.1% dodecyl maltoside (DDM, Anatrace). Cir was eluted with an imidazole step gradient. Pooled fractions were analyzed for protein content. Purified TEV (Kapust et al., 2001; White et al., 2004) was added at a molar ratio of 1:100, along with 2 mM DTT and 1 mM EDTA, to remove the histidine tag. The mixture was dialyzed overnight at room temperature into 50 mM Tris-HCl, pH 7.5, 10% glycerol, 2 mM DTT, and 1 mM EDTA. The dialyzed sample was further purified on a 10 ml Q-sepharose high performance column (GE Healthcare) equilibrated with 50 mM Tris-HCl, pH 7.5, 10% glycerol, and 0.1% DDM. A linear NaCl gradient was used to elute the protein. Peak fractions were pooled and applied to a Sephacryl S300 16/60 column (GE Healthcare) equilibrated with 20 mM Tris-Cl, pH 7.5, 200 mM NaCl, 0.02% NaN_3, and 1.0 % 2-Hydroxyethyloctylsulfoxide (CHESO) or 0.05% LDAO plus 0.45% C_8E_4. Final protein yields ranged from 20 – 50 mg of purified Cir or Cir4M.

Colicin Ia R domain: The receptor binding domain of colicin Ia was expressed as cytoplasmic aggregates (inclusion bodies) and refolded in the presence of the receptor, as follows. Cells (3 g) were resuspended in 30 ml lysis buffer and disrupted using a French pressure cell as described above. Inclusion bodies were collected by centrifugation in an SS34 rotor (Sorvall) at 8000 x *g* for 10 minutes. The supernatant was discarded and the pelleted inclusion bodies were resuspended in 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 2% Triton X-100 (Sigma), and then stirred for 30 minutes at room temperature. The suspension was centrifuged as before, and the pellets were washed twice more without Triton X-100. The washed inclusion bodies were solubilized in 10 ml of 6M guanidinium HCl, and centrifuged as before to remove insoluble material. The solubilized inclusion bodies were rapidly diluted into 100 ml of 50 mM Tris-Cl, pH 8.0, 200 mM NaCl, and 550 mM sucrose. The solution was incubated at room temperature overnight. The following day, the protein concentration of the filtered sample was determined. LDAO was added to 0.1% (vol), and then a 5-fold excess of colicin Ia R domain was added to Cir4M in 0.05% LDAO plus 0.45% C_8E_4. The complex was purified by size exclusion chromatography in the LDAO/C_8E_4 detergent mixture.

Full length colicin Ia: To assess killing by colicin Ia, full-length colicin Ia was purified as follows. Cells (18 g) were resuspended in buffer containing 20 mM Tris-HCl, pH 8.0, 20 mM NaCl, and 2 mM EDTA, and the cells were disrupted by passage through a French pressure cell as described above. The cell lysate was clarified by ultracentrifugation using a 45-Ti rotor (Beckman) at 160,000 x *g* for 1 hour. The supernatant was filtered through a 0.22 μm filter and applied to a 10 ml SP-Sepharose column (GE Healthcare) equilibrated with 20 mM Tris-HCl, pH 8.0 and 10% glycerol at 4°C. Colicin Ia was eluted with a linear salt gradient. Pooled fractions were concentrated to 5 ml and applied to a Sephacryl

S-300 16/60 column equilibrated with phosphate buffered saline (PBS). Peak fractions were pooled and used for killing assays as described below.

***In vivo* cytotoxicity assays**

To probe possible contributions to colicin uptake from TolC, we carried out colicin Ia killing assays in suspension cultures. Overnight cultures of MC4100 (wild-type), EW1b (partial TolC deletion), A586 *(*TolC point mutant), and RAM1129 (complete TolC deletion) were used to inoculate shake flasks containing 50 ml LB. Cultures were grown at 37°C to OD_{600} 0.35 to 0.50, and these cultures were divided into additional flasks and incubated with 0.15 µg/ml purified colicin Ia or without colicin Ia (control). Bacterial growth was monitored for about 6 hours (Supplementary Figure 4).

Crystallization

We found that the additional four methionines in Cir4M yielded better crystals than wild-type Cir, so the four-methionine mutant (with or without selenomethionine substitution) was used in all crystallization experiments. For receptor crystals, Cir4M (chromatographed in 1% CHESO (Locher et al., 1998)) was concentrated to 8 mg/ml. Crystals grew from hanging drops by mixing 1 µl protein with 1 µl well solution containing 100 mM Tris-Cl, pH 7.5, 20 – 50 mM $SrCl_2$, 5% isopropanol, and 32-40% PEG 2000 MME, at 21°C. Crystals were cryoprotected with 5-10% PEG 400 plus 0.5% CHESO. The Cir-colicin complex, chromatographed in 0.05% LDAO plus 0.45% C_8E_4, was concentrated to 9 mg/ml. 3% heptanetriol was added to the concentrated protein. Crystals grew from hanging drops by mixing 1 µl protein with 1 µl well solution containing 100 mM MES, pH 6.2, 10% glycerol, 200 mM NaCl, and 22% PEG 2000 MME, at 21°C. No further cryoprotection was required. All crystals were flash frozen in propane cooled to -170°C.

Structure determination

Diffraction data were collected at the SER-CAT 22-ID beamline at the Advanced Photon Source (Argonne, IL). All data were collected at 100K. A three-wavelength MAD dataset was collected on a single (Cir4M) selenomethionine-containing crystal on a Mar225 CCD detector. The crystal had the symmetry of the monoclinic spacegroup C2, with unit cell dimensions a = 90.61 Å, b = 84.35 Å, c = 99.47 Å, β=109.2°, having one molecule in the asymmetric unit. For the Cir-colicin complex, a dataset was collected using a Mar300 CCD detector. The complex crystal also had the symmetry of spacegroup C2, with unit cell dimensions a = 132.64 Å, b = 130.49 Å, c = 56.27 Å, β=101.2°, having one (complex) molecule in the asymmetric unit. All data were integrated and scaled with HKL2000 (Otwinowski and Minor, 1997).

For Cir4M, the direct methods program SHELXD (Schneider and Sheldrick, 2002) located nine selenium atoms, which were subsequently used in autoSHARP (version 1.4.0) (Vonrhein et al., 2006) for phase determination. Phases were improved by solvent flipping in SOLOMON (Abrahams and Leslie, 1996) resulting in an overall correlation of 0.70 (based on E^2) assuming a solvent content of 39.5%. Even though previous attempts at molecular replacement had failed, a model of the related iron transporter FepA (1FEP; sequence identity to Cir about 40%) could be placed into the electron density using

MOLREP (Vagin and Isupov, 2001). Several cycles of manual rebuilding in O (Jones et al., 1991) and Coot (Emsley and Cowtan, 2004), and refinement in REFMAC (Winn et al., 2003), converged to the final model of Cir4M. Electron density was absent or insufficient for the following residues: 26-29 and 115-122 from the plug domain; 214-227, 266-267, 305-307, 348-354, 598-609, and 643-646 in the barrel.

The Cir4M-colicin Ia complex structure was solved by molecular replacement using the coordinates of Cir4M (2HDF) and the R-domain of colicin Ia (1CII, residues 282-385) in the program Phaser (McCoy et al., 2005). A single copy of each molecule was found resulting in a 1:1 complex, and the model was built and refined as described above. Electron density was absent or insufficient for the following residues: 26-30 from the plug domain; 215-229, 265-269, 600-609, and 644-648 in the barrel.

Determination of the screw axis describing movement of extracellular loops 7 and 8

We determined the screw axis describing movement of extracellular loops 7 and 8 using the procedure described below (based on an appendix to Gerstein *et al.*) (Gerstein et al., 1993). A rigid body transformation of a set of points from the coordinates $\{\mathbf{x}\}$ to the coordinates $\{\mathbf{x}'\}$ can be expressed in a non unique fashion as a rotation $\mathbf{R}_{\mathbf{n},\theta}$ of angle θ around the axis $A_{\mathbf{x}_0,\mathbf{n}}$ (of direction $\mathbf{n}$ and going through the point of coordinates $\mathbf{x}_0$) followed by a translation of vector $\mathbf{t}$:

$$\mathbf{x}' - \mathbf{x}_0 = \mathbf{R}_{\mathbf{n},\theta}(\mathbf{x} - \mathbf{x}_0) + \mathbf{t}.$$

There is a unique decomposition, called screw motion, for which the translation is along the axis of rotation. Given $\mathbf{n}$, θ, $\mathbf{x}_0$ and $\mathbf{t}$, the screw decomposition can be found as follows: The translation is decomposed into translation components that are parallel and perpendicular to $\mathbf{n}$:

$$\mathbf{t} = \mathbf{t}_\perp + \mathbf{t}_\parallel, \text{ where } \mathbf{t}_\parallel = \mathbf{n}(\mathbf{n}\cdot\mathbf{t}).$$

The rotation around the axis $A_{\mathbf{x}_0,\mathbf{n}}$ following the translation perpendicular to that axis is equal to a rotation of the same angle around a new axis $B_{\mathbf{x}_1,\mathbf{n}}$ parallel to the original axis. For instance, the point $\mathbf{x}$ is transformed into $\mathbf{x}''$ equivalently as follows:

$$\mathbf{x}'' - \mathbf{x}_0 = \mathbf{R}_{\mathbf{n},\theta}(\mathbf{x} - \mathbf{x}_0) + \mathbf{t}_\perp$$

and

$$\mathbf{x}'' - \mathbf{x}_1 = \mathbf{R}_{\mathbf{n},\theta}(\mathbf{x} - \mathbf{x}_1).$$

Since the entire transformation can now be expressed as a rotation around the new axis B followed by a translation $\mathbf{t}_\parallel$ parallel to B, it is a screw motion:

$$\mathbf{x}' - \mathbf{x}_1 = \mathbf{R}_{\mathbf{n},\theta}(\mathbf{x} - \mathbf{x}_1) + \mathbf{t}_\parallel.$$

The expression to determine x_1 is:

$$\mathbf{x}_1 - \mathbf{x}_0 = \frac{\left[\mathbf{t}_\perp + \mathbf{n} \times \mathbf{t}_\perp \cot(\theta/2)\right]}{2}.$$

The program LSQKAB (Kabsch, 1976) was used to determine $\mathbf{n}$, θ and $\mathbf{t}$, assuming $\mathbf{x_0} = \mathbf{0}$, following the transformation of the coordinates defining L7 and L8 of uncomplexed Cir onto equivalent atoms of the Cir-colicin complex. The calculated axis passes through two points, which in the coordinate system of the 2HDI.pdb file have the following x,y,z values: (35.782, -13.069, 34.884) and (26.757, 2.210, 4.716). The same screw axis also approximately connects the N atoms of residues V299 and K483.

Analytical Ultracentrifugation

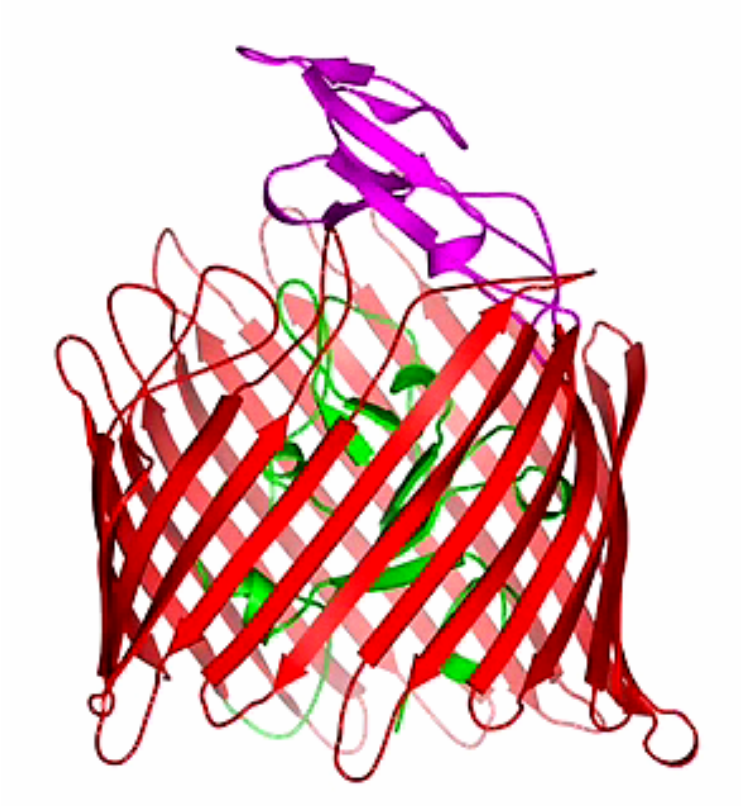
Sample preparation: All samples were purified by gel filtration in PBS containing 1% (w/v) octyl-POE and 0.02% (w/v) NaN_3 prior to use. Samples for analytical ultracentrifugation were prepared by dilution of the protein stocks into the same buffer.

Sedimentation equilibrium: Sedimentation equilibrium experiments were conducted at 4.0°C on a Beckman Optima XL-A analytical ultracentrifuge. Samples of the colicin I receptor and colicin Ia (loading volume of 130 µL) were studied at loadings (A_{280}) of approximately 0.4, 0.8 and 1.2, and rotor speeds of 6, 8, 10, 12 and 14 krpm. Data were acquired as an average of 6 absorbance measurements at 280 nm using a radial spacing of 0.001 cm. Sedimentation equilibrium was achieved within 48 hours. Data collected at different speeds and different loading concentrations were analyzed globally in terms of various species analysis models using SEDPHAT 4.1b (Schuck) to obtain the sample molecular mass. Solution densities ρ were measured at 20.0°C on a Mettler-Toledo DE51 density meter and corrected to values for ρ at 4.0°C. Values of the partial specific volume ν were calculated based on the amino acid composition using SEDNTERP (Philo).

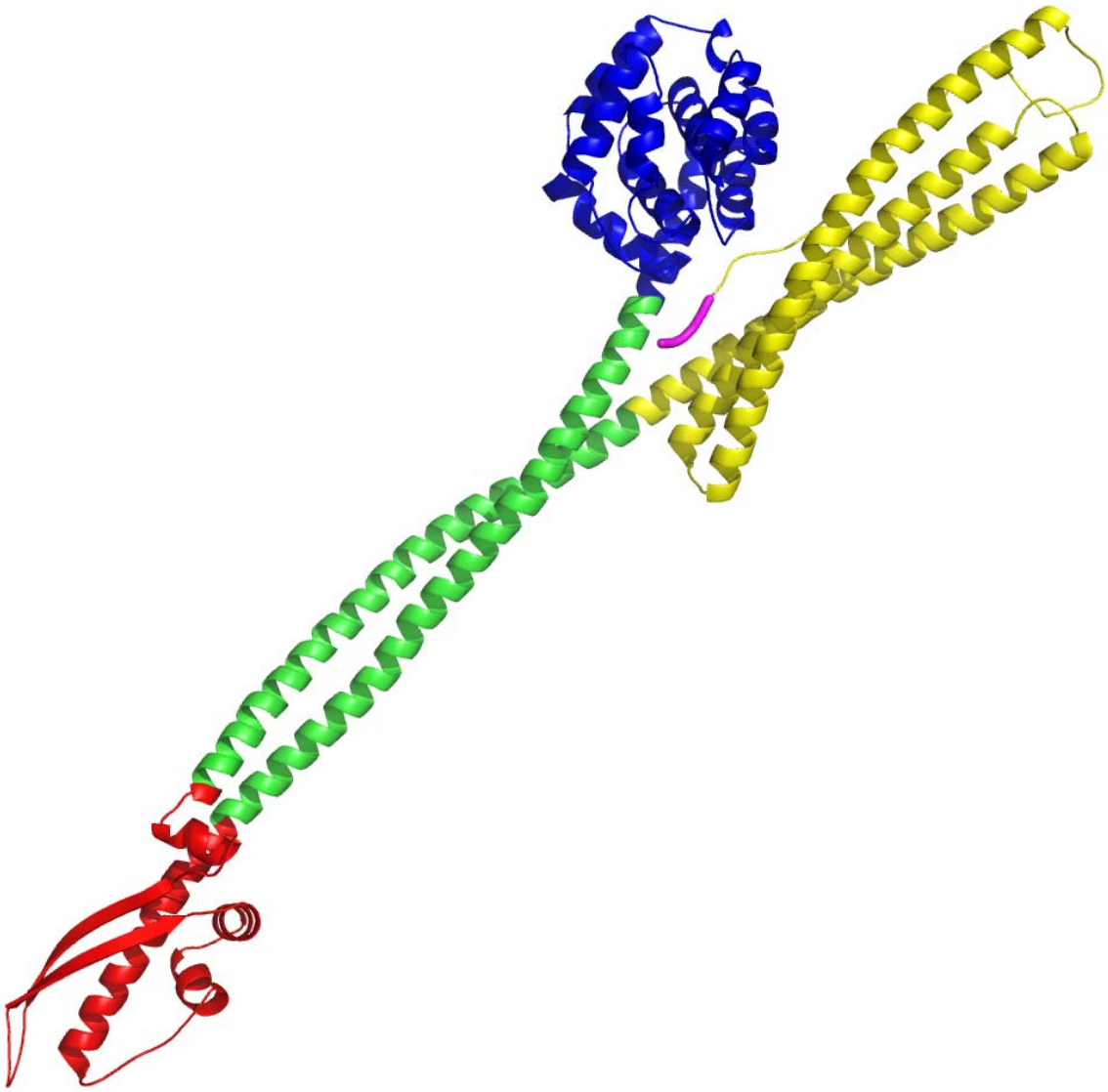
Octyl-POE, consisting of a mixture of octyl-tetraoxyethylene (C_8E_4) and octyl-pentaoxyethylene (C_8E_5), has a partial specific volume of 0.993 cm^3g^{-1} (le Maire et al., 2000). Under the buffer conditions used, the detergent is gravitationally transparent; in addition to being isotropically distributed, any bound detergent does not contribute to the molecular mass of either the colicin I receptor or colicin Ia.

To characterize the interaction between the colicin I receptor and colicin Ia, 2:1, 1:1 and 1:2 molar mixtures having a loading A_{280} of 0.65 and 1.2 were analyzed concurrently by sedimentation equilibrium. Data collected at different speeds and loading ratios were analyzed globally in terms of a reversible A + B interaction in SEDPHAT 4.1b (Schuck) to obtain an estimate of the interaction affinity. Conservation of mass was implemented and excellent data fits were observed (Supplementary Figures 6-8, Supplementary Table IV).

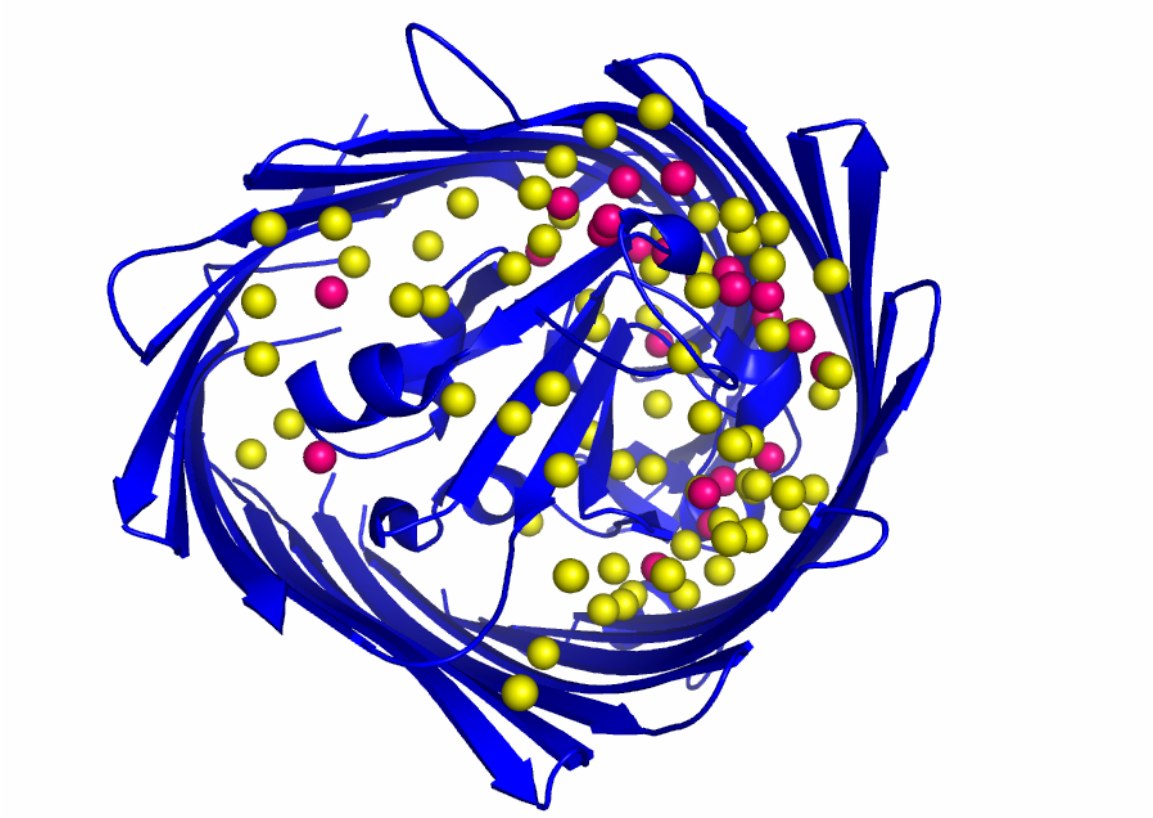
MOVIE, FIGURES, AND TABLES



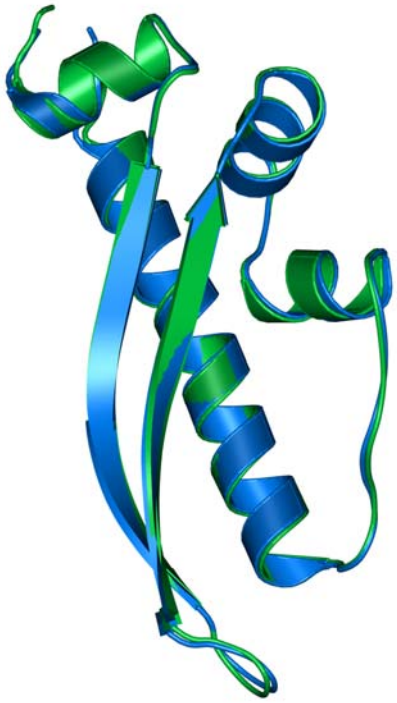
Supplementary Movie 1. Colicin Ia stabilizes a large and unusual ‘open’ conformation of Cir upon binding. The Cir beta barrel is colored red and the plug domain is colored green. Extracellular loops 7 and 8, which undergo large conformational changes upon colicin binding, are colored magenta. The R-domain of colicin Ia is colored blue (visible in the movie, but not in the starting image depicted above). The observed conformational changes are the largest ever observed in a TonB-dependent transporter, and in the opposite direction to changes induced by small molecules. The morphing movie was created from the two crystal structures determined, namely Cir alone and in complex with the R-domain of colicin Ia. The images were created with morphing software (Krebs and Gerstein, 2000) and drawn using Bobscript (Kraulis, 1991; Esnouf, 1997) and GLR (Esser). The movie can be viewed with QuickTime [Sup_2.mov].



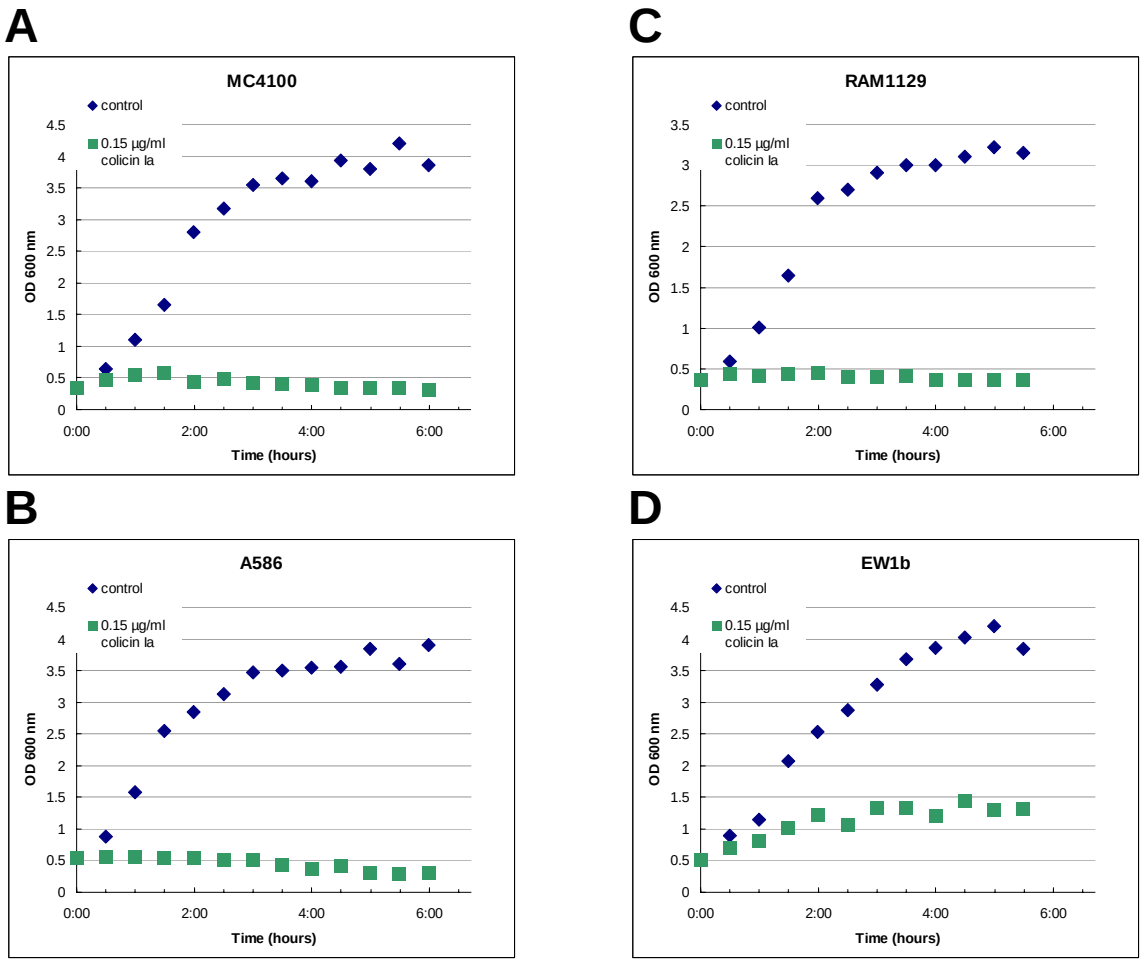
Supplementary Figure 1**.** Colicin Ia colored by domains. The translocation (T) domain consists of three alpha helices shown in yellow, and an unstructured region at the N-terminus containing the TonB box (magenta tube). The receptor binding (R) domain is shown in red and the channel forming (C) domain is colored blue. The T- and C-domains are close to one another in the crystal structure (Wiener et al., 1997) and are separated from the R-domain by a pair of very long alpha helices, shown in green. The full-length structure is about 210 Å long. Supplementary Figures 1 and 2 were prepared with PYMOL (DeLano).



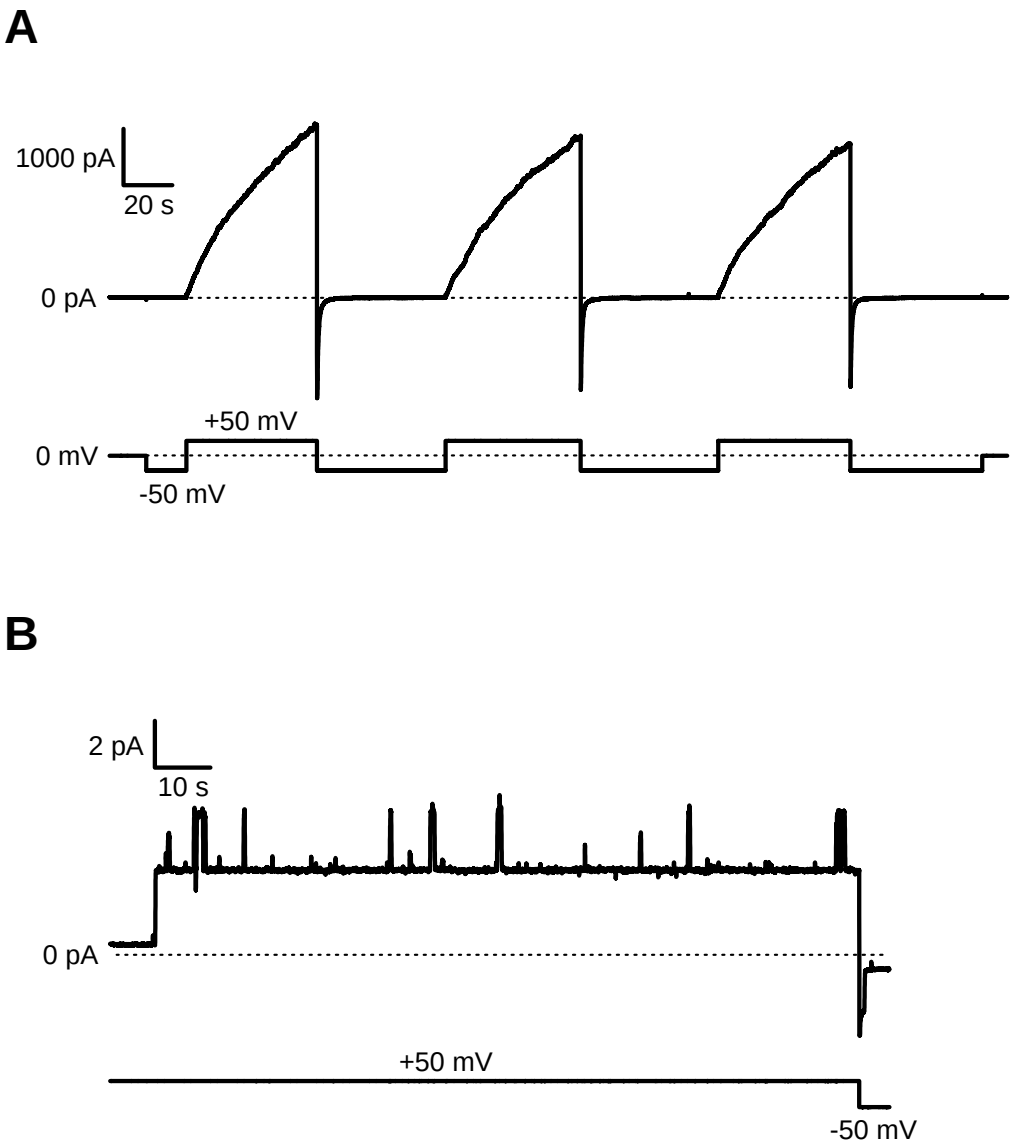
Supplementary Figure 2. Bridging and non-bridging water molecules in the plug-barrel interface. Twenty-three bridging waters (magenta) coordinate both the plug and the barrel. Approximately 70 non-bridging waters (gold) coordinate either the plug or the barrel, but not both. The large number of water molecules in the plug-barrel interface creates a highly solvated surface which has been suggested to facilitate partial or complete removal of the plug domain (Chimento et al., 2005). The view is from the extracellular surface, looking down toward the periplasm.



Supplementary Figure 3. The R-domain of colicin Ia does not change conformation upon binding cir. Superimposition of the colicin Ia receptor binding domain from the full length structure (colored green; PDB ID 1CII, residues 282-385) with the colicin Ia receptor binding domain from the Cir-colicin Ia complex (colored blue; PDB ID 2HDF). The two structures were superimposed with an r.m.s.d. of 0.65 Å for Cα positions and 1.15 Å for all atoms. This figure was prepared with Bobscript (Kraulis, 1991; Esnouf, 1997) and GLR (Esser).

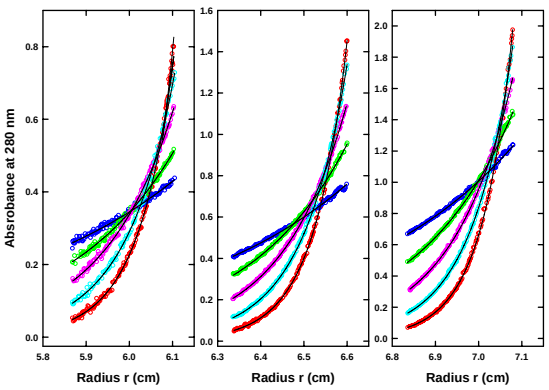


Supplementary Figure 4. Absence of TolC does not influence killing by colicin Ia. Strains MC4100 (wild-type), A586 (TolC point mutant), RAM1129 (TolC complete deletion), and EW1b (TolC partial deletion) were grown in LB to an OD_{600} of 0.35 – 0.50. Cultures were divided, and colicin Ia was added to one flask of each strain at a concentration of 0.15 µg/ml. The other portion of each culture was allowed to grow normally. All cultures were killed by the addition of colicin Ia, although EW1b is clearly more resistant in both solution and plate assays. Because RAM1129 is a complete TolC deletion and is killed as effectively as wild-type strains, we conclude that TolC does not influence killing by colicin Ia.

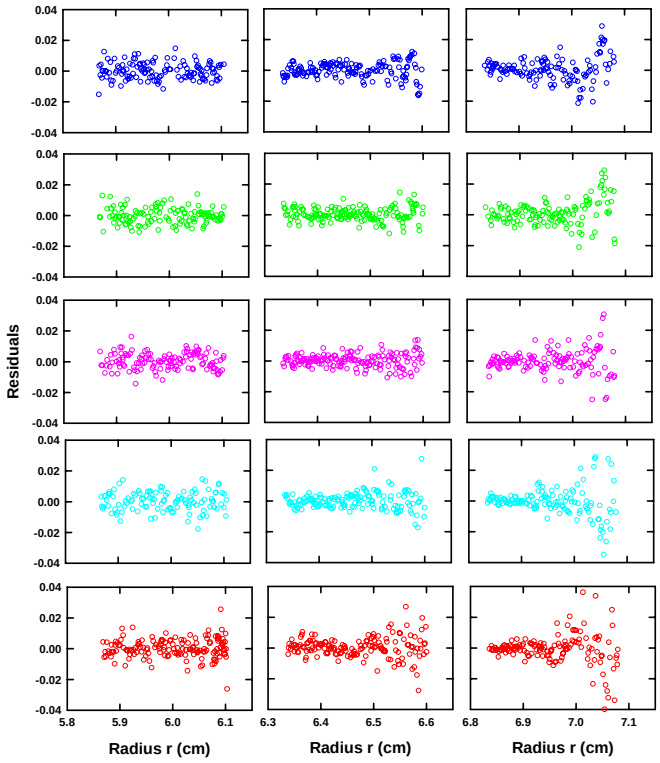


Supplementary Figure 5. Colicin Ia M25P retains normal ion channel activity. (A) Colicin Ia M25P many-channel record, showing the normal voltage-dependent gating of these mutant channels. 1.1 μg of M25P (mixed with 5 μg of octyl glucoside) was added to the *cis* compartment. Positive voltage means the potential is positive on the *cis* side, relative to the opposite, *trans*, side. Each compartment has a volume of 1 ml. The bathing solution was 1 M KCl, 5 mM $CaCl_2$, 1 mM EDTA, 20 mM HEPES, plus KOH to pH 7.2. Membranes were formed from asolectin with squalene. (B) Single-channel record for colicin Ia M25P. This channel has a conductance of 64 pS, at +50 mV; other channels were in the range of approximately 52 to 64 pS. This is typical for wild-type colicin Ia under these conditions (same bathing solution as above). 4.4 ng of M25P were added to the *cis* compartment.

A

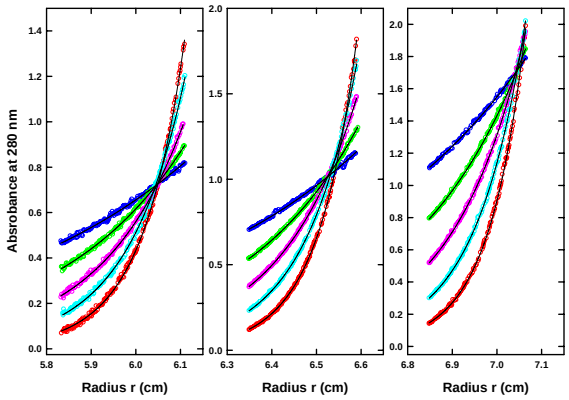


B

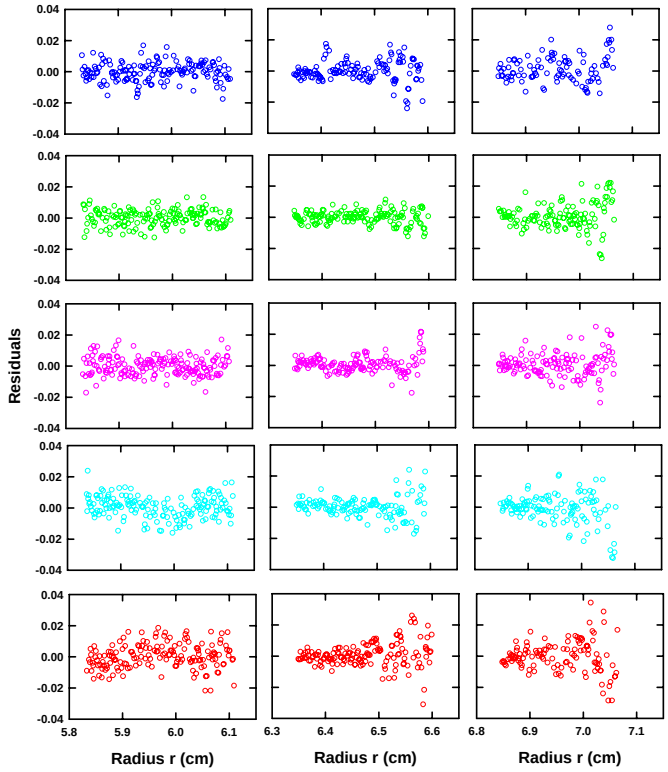


Supplementary Figure 6. The colicin I receptor is monodisperse and monomeric. (A) Sedimentation equilibrium profiles obtained for Cir shown in terms of A_{280} versus the radius r for data collected at a loading A_{280} of 0.36 (left), 0.75 (center) and 1.10 (right). Data were collected at 6 (blue), 8 (green), 10 (pink), 12 (cyan) and 14 (red) krpm at 4.0°C and analyzed in terms of a single ideal solute. Best fits, corresponding to a molecular mass of 75.0 kDa, are shown as black lines through the experimental points. (B) The corresponding distributions of the residuals are shown in the accompanying plot.

A

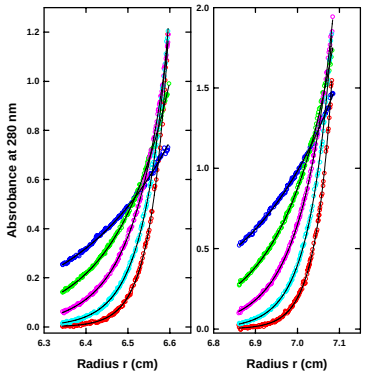


B

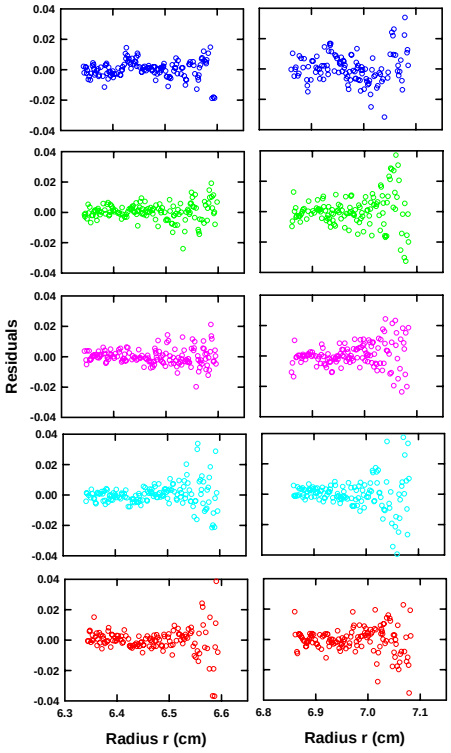


Supplementary Figure 7. Colicin Ia is monodisperse and monomeric. (A) Sedimentation equilibrium profiles obtained for colicin Ia shown in terms of A_{280} versus the radius r for data collected at a loading A_{280} of 0.44 (left), 0.86 (center) and 1.28 (right). Data were collected at 6 (blue), 8 (green), 10 (pink), 12 (cyan) and 14 (red) krpm at 4.0°C and analyzed in terms of a single ideal solute. Best fits, corresponding to a molecular mass of 68.8 kDa, are shown as black lines through the experimental points. (B) The corresponding distributions of the residuals are shown in the accompanying plot.

A



B



Supplementary Figure 8. The colicin I receptor and colicin Ia interact with high affinity to form a 1:1 complex. (A) Sedimentation equilibrium profiles obtained for a 1:1 mixture of colicin receptor and colicin Ia shown in terms of A_{280} versus the radius r for data collected at a loading A_{280} of 0.65 (left) and 1.20 (right). These correspond to loading concentrations of 2.6 and 4.9 μM, respectively, for each species. Data were collected at 6 (blue), 8 (green), 10 (pink), 12 (cyan) and 14 (red) krpm at 4.0°C and analyzed in terms of a single ideal solute. Best fits, corresponding to a molecular mass of 139.0 kDa, are shown as black lines through the experimental points. (B) The corresponding distributions of the residuals are shown in the accompanying plot.

Supplementary Table I. TonB-dependent transporters used by colicins. Colicins requiring the Ton translocation system use a single outer membrane transporter for cell entry, whereas Tol-dependent colicins that target TonB-dependent transporters (BtuB) require a co-transporter for uptake (Cascales et al., 2007).

TonB-dependent transporter	**Colicin**	**Co-transporter**	**Translocation system for colicin uptake**
Cir	Colicin Ia, Ib	none	TonB-ExbB-ExbD
FepA	Colicin B, D	none	TonB-ExbB-ExbD
FhuA	Colicin M	none	TonB-ExbB-ExbD
BtuB	Colicin E2, E3, E4,	OmpF	TolABQR
BtuB	E5, E6, E7, E8, E9	OmpF	TolABQR
BtuB	E1	TolC	TolAQ
BtuB	A	OmpF	TolABQR

Supplementary Table II. Hydrogen bonds between Cir and the colicin Ia R-domain. Cir apical loop 2 (plug domain) residue R116 contributes 1 interaction, extracellular loop 5 residue N348 contributes 1 hydrogen bond, loop 7 residues S434 and R346 make 7 interactions, and loop 8 residues I488, S489, R490, T491, and Y520 form 9 hydrogen bonds with colicin Ia.

Cir	**Distance (Å)**	**colicin Ia**
AP2: R116 NH2	2.99	E357 O
L5: N348 ND2	3.06	E357 OE1
L7: S434 OG	2.55	D358 OD2
L7: R436 N	2.86	D362 OD1
L7: R436 NE	2.96	P353 O
L7: R436 NH2	3.20	N352 O
L7: R436 NH1	2.70	D350 OD2
L7: R436 NH2	2.57	D350 OD1
L7: R436 NH1	2.91	E369 OE1
L8: I488 N	2.93	A315 O
L8: S489 OG	2.98	T317 N
L8: R490 N	3.04	T317 O
L8: R490 O	2.99	K319 N
L8: R490 NH1	2.85	F314 O
L8: R490 NH2	2.80	F314 O
L8: R490 NH2	3.06	D358 OD2
L8: T491 OG1	2.56	K319 O
L8: Y520 OH	2.84	R313 O

Supplementary Table III. TonB box sequences for wild-type Cir and colicin Ia, and for TonB box mutants discussed in the text. Mutations are shown in blue.

Protein	**TonB box sequence**
Cir (wt)	E T M V V
Cir mutant 1 (Bell et al., 1990)	E T M V G
Cir mutant 2 (this work)	E T C V P
Colicin Ia (wt)	E I M A V
Colicin Ia mutant 1	E I P A V

Supplementary Table IV. Analytical ultracentrifugation data for wild-type and mutant colicin I receptor, colicin Ia, and complexes.

Sample	**Experimental mass (kDa)**	**Monomer mass (Da)**	**Stoichiometry**
Cir (wt)	75.0 ± 2.4	71,215.5	1.05 ± 0.03
Cir M33C/V35P	71.5 ± 1.1	71,185.4	1.00 ± 0.02
colicin 1a (wt)	68.8 ± 4.8	69,429.0	0.99 ± 0.07
colicin Ia M25P	70.4 ± 1.4	69,394.9	1.01 ± 0.02
1:1 mixture (wt:wt)	139.0 ± 2.1	140,644.5	0.99 ± 0.02
Cir (wt) : col (mut)	124.0 ± 6.0	140,610.4	0.88 ± 0.04
Cir (mut): col (wt)	132.0 ± 4.0	140,614.4	0.94 ± 0.03

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