Supplementary Materials and Methods

Analysis of the redox state of ClyA in the membrane-bound form

For analysis of the redox state in the membrane-bound form, 250 nM ClyA were first incubated with a suspension of 1% (v/v) horse erythrocytes for 30 min at 37 °C, then treated with IAEDANS and finally analyzed by SDS-PAGE as described in Supplementary Figure 1 for the soluble protein.

Cross-Linking

ClyA\textsubscript{red} (5 μM) was incubated in PBS, 0.1% DDM at 15 °C. After different incubation times (2 – 120 min) samples were mixed with DSP (final concentration 3 mM) and incubated for 5 min at 25 °C. The reaction was stopped by addition of TrisHCl, pH 8.0 (final concentration 100 mM). The samples were applied onto a 16% polyacrylamide-SDS gel. The areas of the monomeric ClyA\textsubscript{red} bands were quantified using densitometric analysis with the AlphaEase® Imaging System (AlphaInnotech).

Hemolysis Assay

Hemolysis of defibrinated horse erythrocytes (Oxoid AG) was measured according to Rowe and Welch (1994). Briefly, 800 μl of a 1% solution (2.19 x 10\textsuperscript{7} cells/ml) of erythrocytes were mixed with 200 μl of various dilutions of ClyA in PBS (final concentrations 0.01 – 1000 nM), and incubated for 120 min under shaking at 37 °C. After centrifugation, the absorbance of the supernatant was determined at 540 nm. The kinetics of hemolysis were measured by monitoring the decrease in turbidity of a 0.1% suspension of erythrocytes upon addition of ClyA (final concentration 250 nM). The optical density at 650 nm was followed in a stirred cuvette at 37 °C.
Scanning Transmission Electron Microscopy

A Vacuum Generators (East Grinstead) HB-5 STEM, interfaced to a modular computer system (Tietz Video and Image Processing Systems), was used. Samples were prepared on 200-mesh-per-inch, gold-plated copper grids (Müller, 1992). Grids were washed to remove detergent and either negatively stained (2% uranyl acetate) for structural examination or plunge frozen and freeze-dried for mass measurement. Grids were imaged at recording doses between 500 and 600 electrons/nm² for the mass measurements (pixel size 0.85 nm) and between 3450 and 10250 electrons/nm² for the negative stain microscopy (pixel size 0.33 nm). Beam-induced mass-loss was corrected as detailed in Müller (1992), and tobacco mosaic virus (kindly provided by R. Diaz Avalos) was used for absolute mass calibration. Mass analysis was achieved with the IMPSYS program package (Müller, 1992). Mass values were binned into a histogram and multiple Gauss curves were fitted. The overall experimental uncertainty of the results was estimated from the corresponding SE ($SE = SD/\sqrt{n}$) and the $\approx 5\%$ uncertainty in the calibration of the instrument.