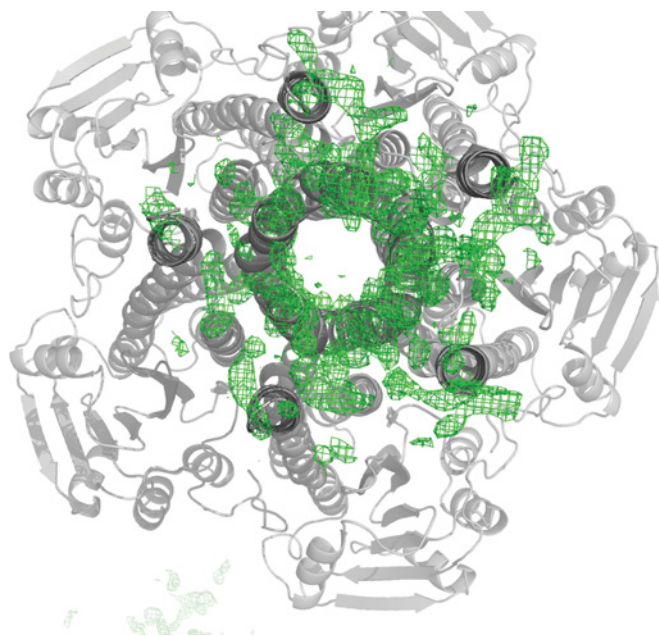


SUPPLEMENTARY ONLINE DATA

Exploring the structure and function of *Thermotoga maritima* CorA reveals the mechanism of gating and ion selectivity in $\text{Co}^{2+}/\text{Mg}^{2+}$ transportNurhuda NORDIN, Albert GUSKOV, Terri PHUA, Newsha SAHAF, Yu XIA, Siyan LU, Hojjat ESHAGHI and Said ESHAGHI¹

Division of Structural Biology and Biochemistry, School of Biological Sciences, Nanyang Technological University, Singapore 637551, Republic of Singapore

**Figure S1 Electron density of the periplasmic loop**

Example of electron density ($F_o - F_c$) in the area of periplasmic loop observed just after the molecular replacement and rigid body refinement before modelling of the loop. σ cut-off = 2.5

¹ To whom correspondence should be addressed (email said.eshaghi@ntu.edu.sg).

The structural co-ordinates reported for *Thermotoga maritima* CorA will appear in the PDB under accession code 4I0U.

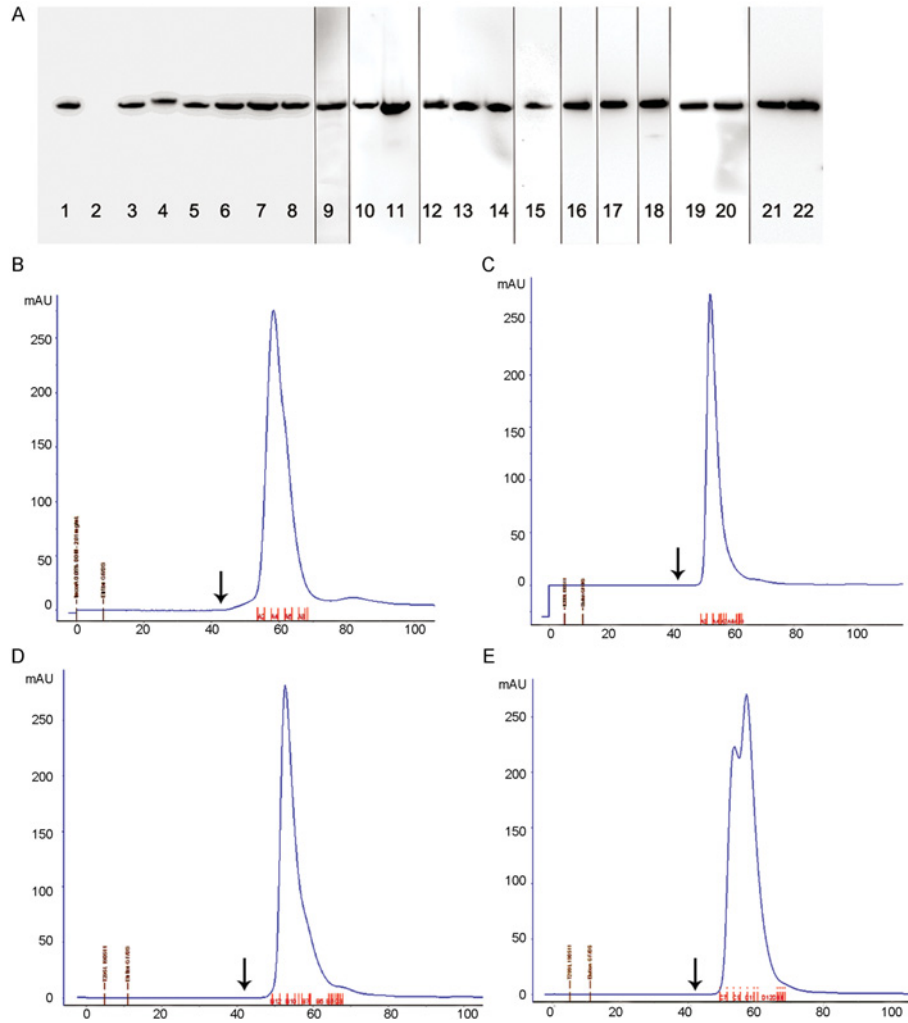


Figure S2 Quality control of the mutations produced

(A) Western blot of the whole cell protein expression of the different TmCorA mutants. Lane 1, wild-type TmCorA; lane 2, *corA*-less *E. coli*; lane 3, T295L; lane 4, T299L; lane 5, N288L; lane 6, N288L/T295L; lane 7, N288L/T299L; lane 8, N288L/T295L/T299L; lane 9, T305L; lane 10, T295M; lane 11, T299M; lane 12, T305S; lane 13, T295S; lane 14, T299S; lane 15, L294N; lane 16, D89K; lane 17, D89N; lane 18, H257A; lane 19, D253K; lane 20, D256A; lane 21, D253A/D256A; and lane 22, D253K/D256A. Black lines separate different Western blot membranes from each other. However, the expression level of the wild-type TmCorA was the same in all the experiments. (B–E) The gel-filtration profiles of (B) wild-type TmCorA, (C) N288L, (D) T295L and (E) T299L mutants. The void volume is indicated by an arrow.

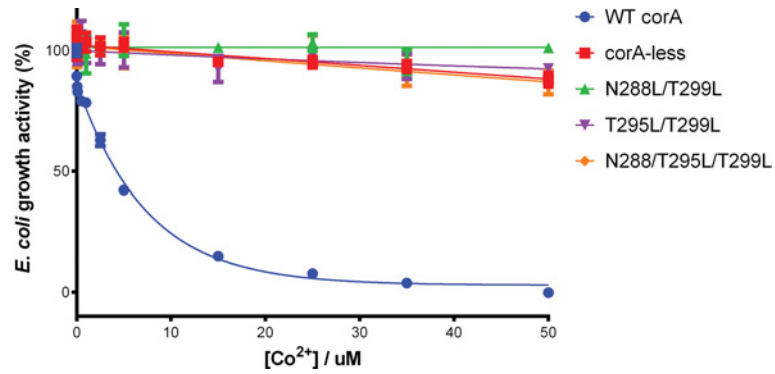


Figure S3 Co²⁺ transport assay of leucine double and triple mutants

The growth activity of TmCorA mutants was monitored in the presence of various Co²⁺ concentrations. A reduction in growth activity upon Co²⁺ concentration increase is indicative of the Co²⁺ transport activity of the TmCorA variant. The wild-type TmCorA (WT corA) and the empty CorA-less pBAD vector (corA-less) were used as positive and negative controls respectively. The results are the means \pm S.D. of at least three independent experiments.

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