## Appendix for

# A DNA damage-activated kinase phosphorylates a transcriptional repressor to control bacterial immune pathway expression

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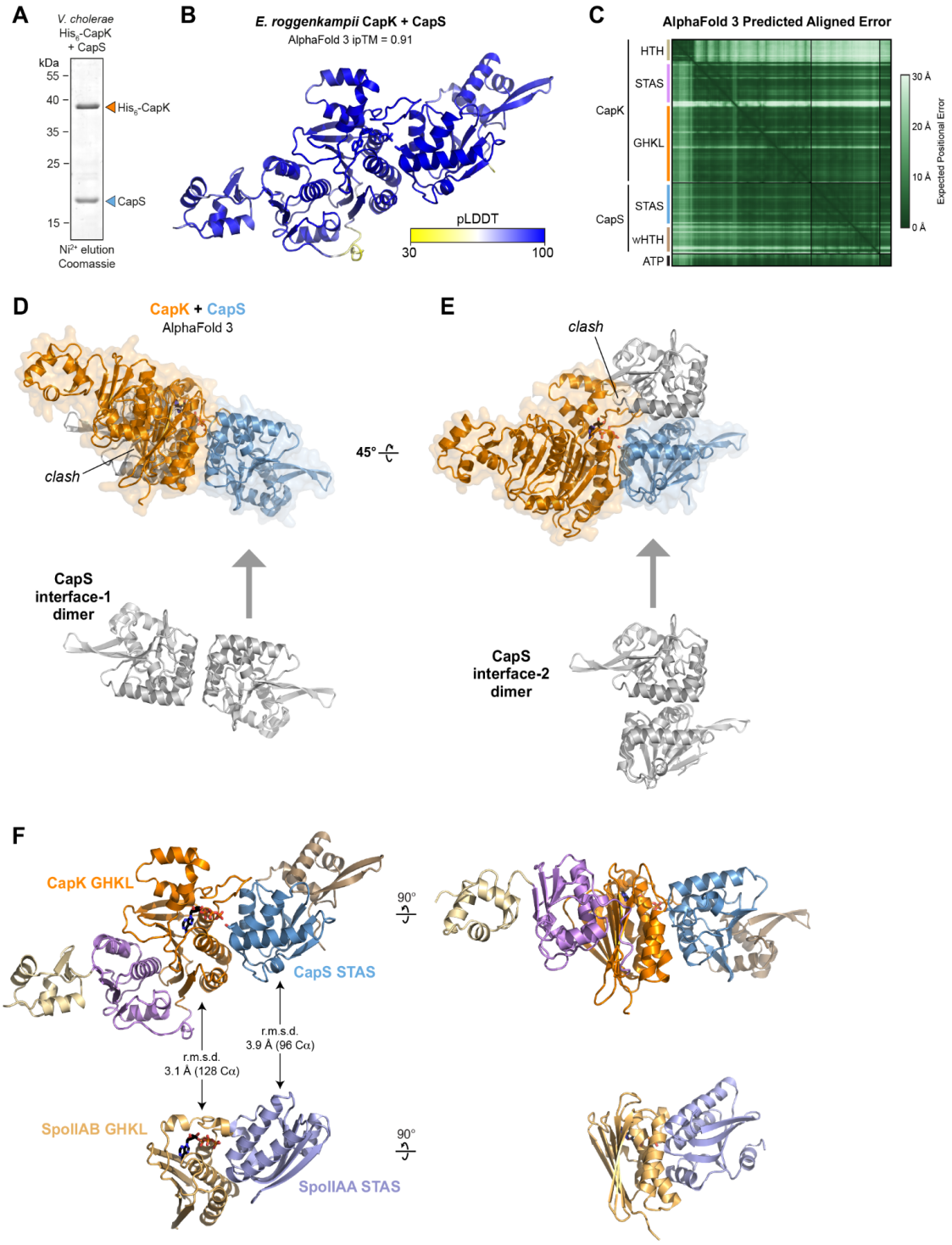
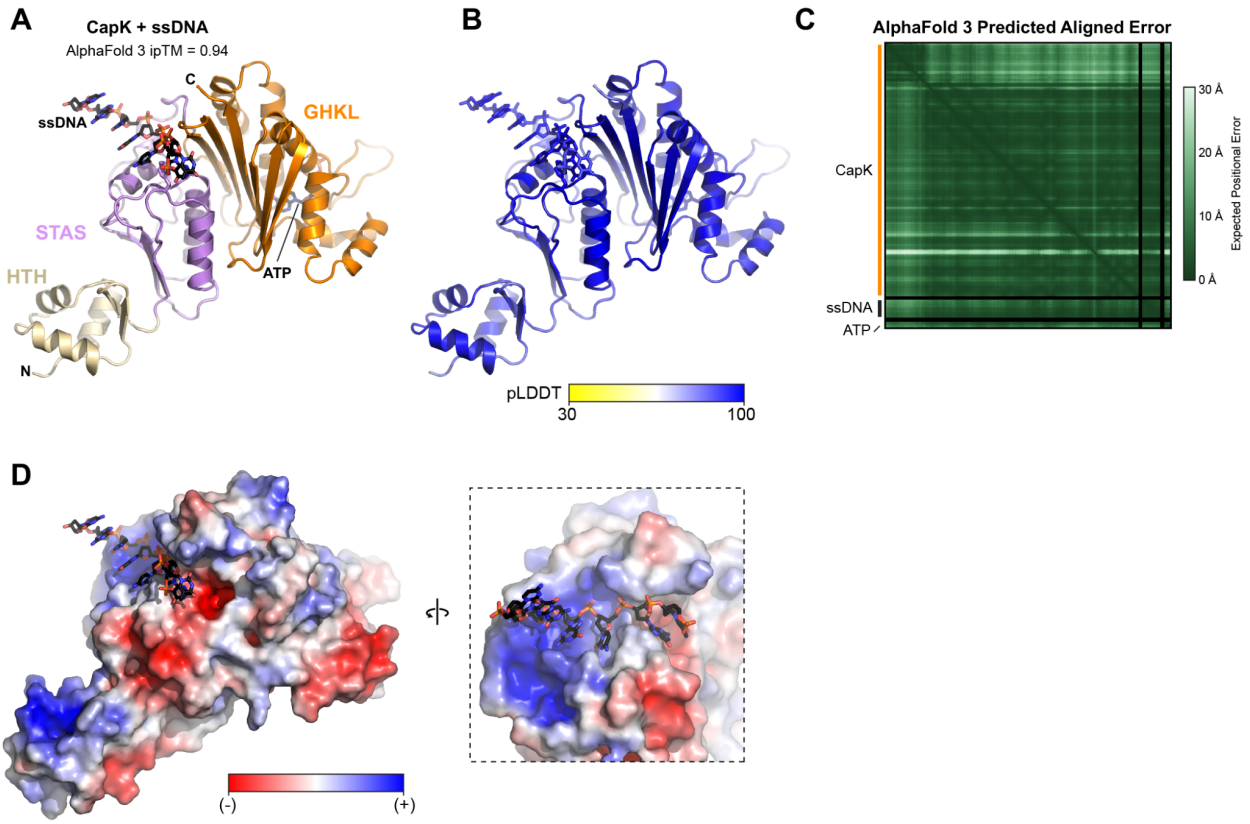


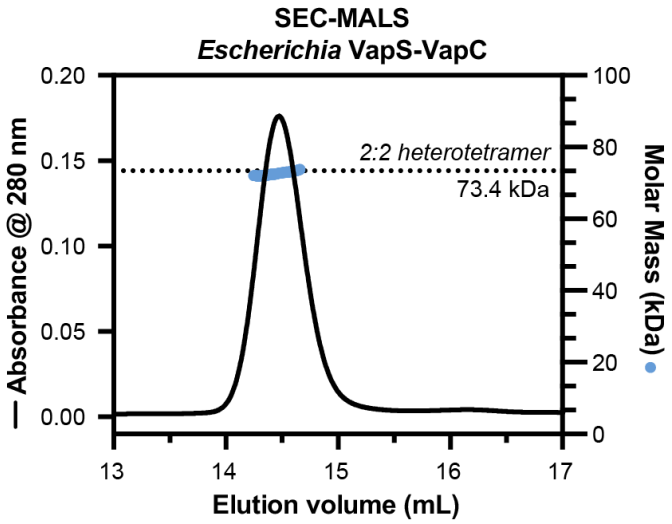

**Appendix Figure S1 - CapK and CapS resemble bacterial anti-sigma factors and their antagonists.**

**(A)** $Ni^{2+}$ elution of co-expressed *V. cholerae* $His_6$-CapK and untagged CapS. Data presented in panels (C)-(D) is representative of three independent trials. **(B)** AlphaFold 3 predicted structure of *E. roggenkampii* CapK bound to CapS, colored by confidence (pLDDT). **(C)** Predicted Aligned Error (PAE) plot for the model shown in panel (A). **(D)** *Top:* Two views of the AlphaFold 3 predicted structure of *E. roggenkampii* CapK bound to CapS, colored by domain as in **Figure 4A**. ATP bound to the CapK GHKL domain is shown in sticks. *Bottom:* Two views of the *Bacillus* SpoIIAB-SpoIIAA complex (PDB ID 1TID) (Masuda *et al*, 2004), aligned to CapK-CapS. ATP bound to the SpoIIAB GHKL domain is shown in sticks.



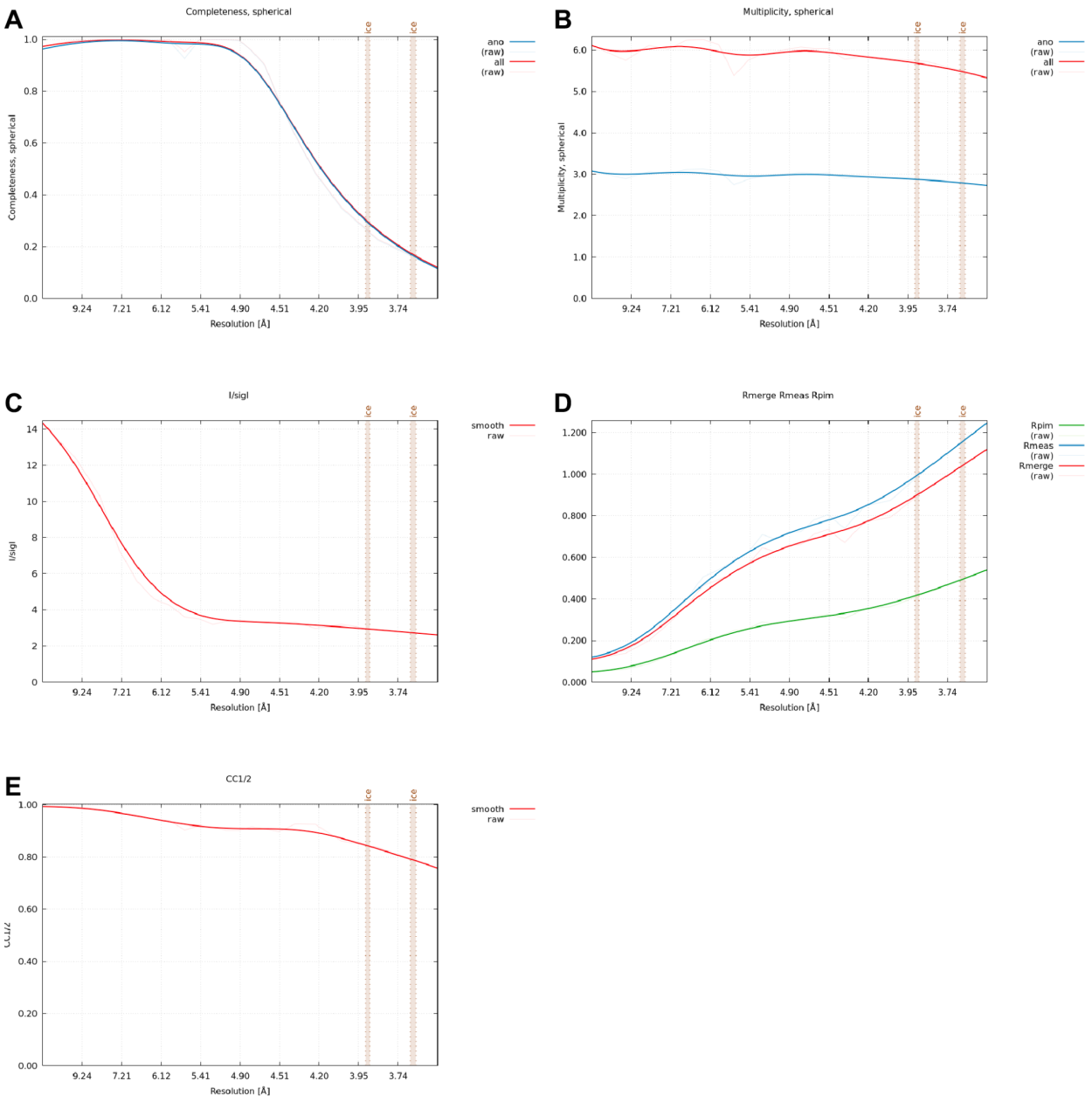

**Appendix Figure S2 - AlphaFold 3 predicted model of the *E. roggenkampii* CapK-ssDNA complex.**

**(A)** AlphaFold 3 predicted model of a complex of *E. roggenkampii* CapK (HTH domain yellow, STAS domain pink, GHKL domain orange), poly-T ssDNA (black), ATP (black), and $Mg^{2+}$ (gray; not visible). CapK Trp68 is shown as sticks. **(B)** View as in panel (A), colored by confidence (pLDDT). **(C)** Predicted Aligned Error (PAE) plot for the model shown in panel (A). **(D)** Two views the predicted CapK-ssDNA structure, with CapK shown as a molecular surface and colored by charge (negative charge red, positive charge blue).



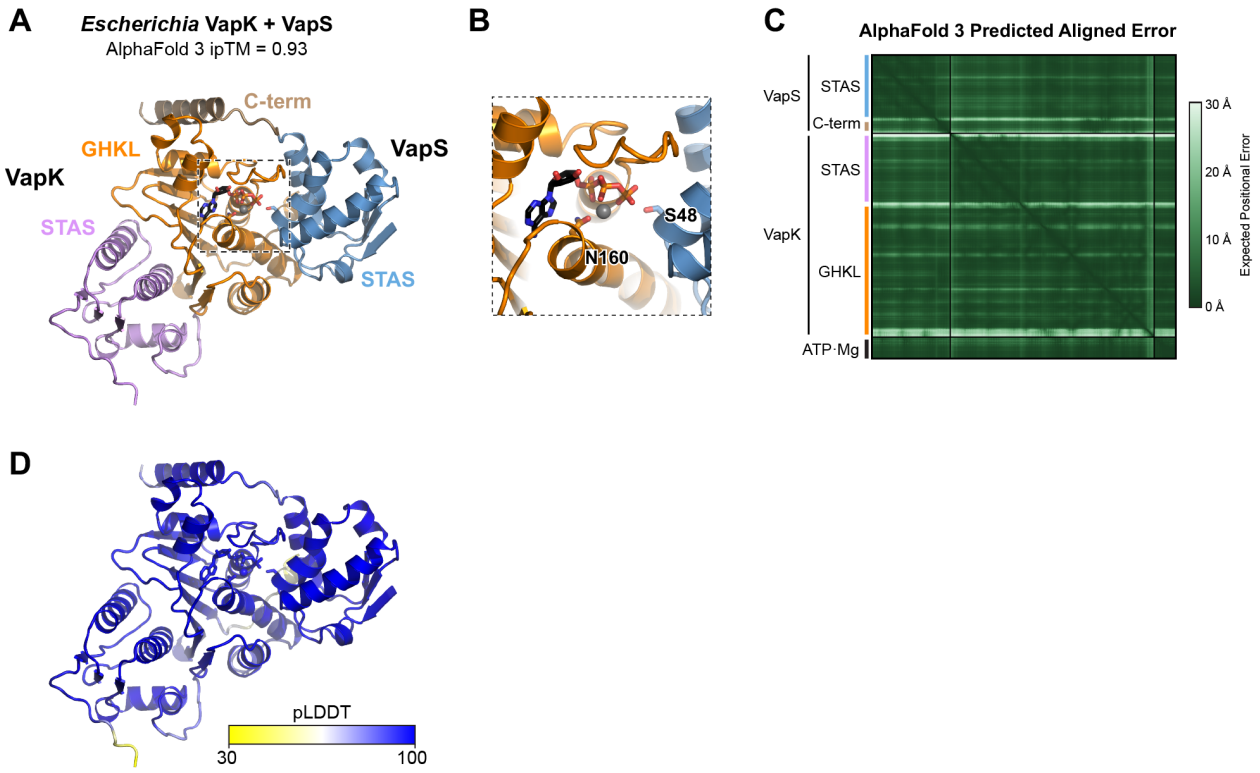

**Appendix Figure S3 - SEC-MALS of *Escherichia* VapS-VapC.**

Size exclusion chromatography coupled to multi-angle light scattering (SEC-MALS) of the Escherichia VapS-VapC complex. Black line indicates absorbance at 280 nm, and blue circles indicate measured molecular weight. A dotted line indicates the molecular weight of a 2:2 heterotetramer of VapS and VapC. This experiment was performed once.



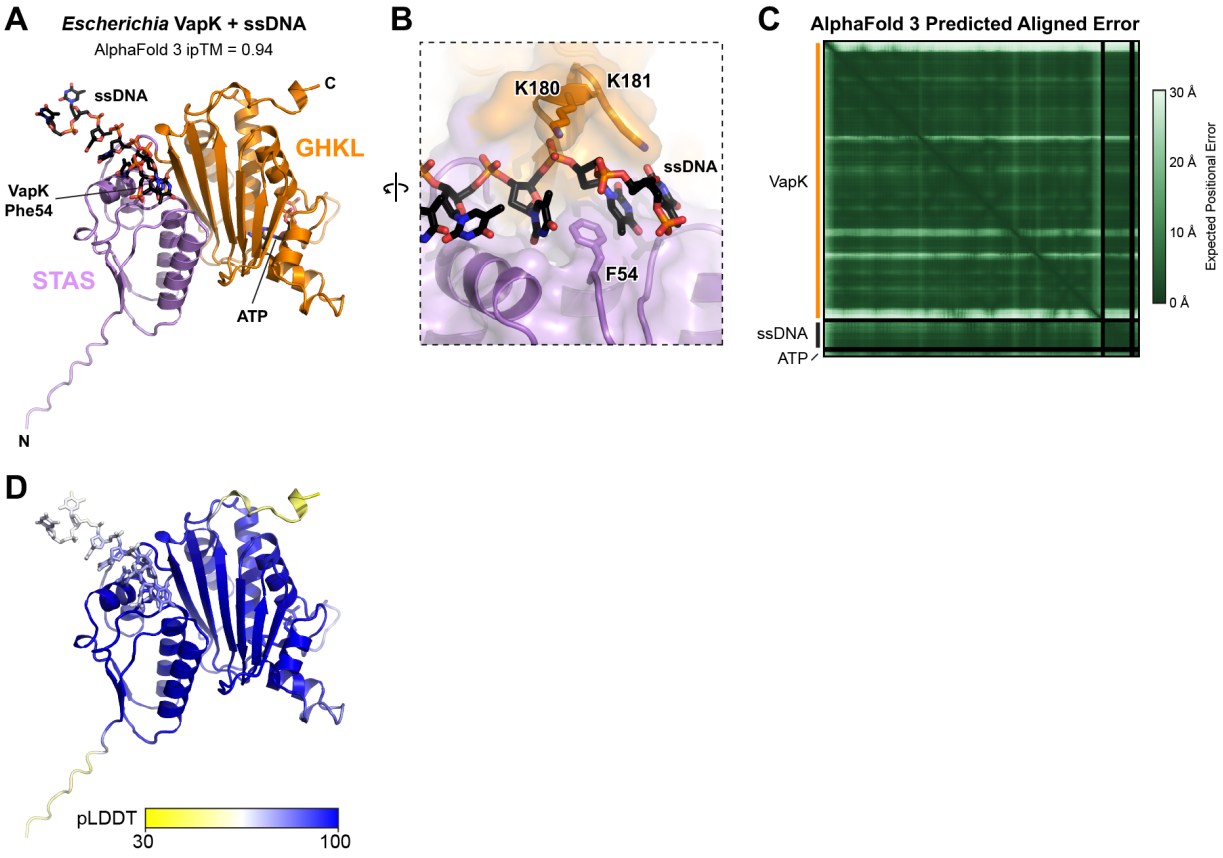

**Appendix Figure S4 - Anisotropic processing of *Escherichia* VapS-VapC crystallographic data.**

**(A)** Graph of completeness versus resolution for the STARANISO-processed X-ray diffraction dataset of *Escherichia* VapS-VapC. Red line indicates native completeness; blue line indicates anomalous completeness. Brown vertical bars indicate resolution ranges typical of ice crystal diffraction. **(B)** Graph of multiplicity versus resolution for the STARANISO-processed X-ray diffraction dataset of *Escherichia* VapS-VapC. **(C)** Graph of intensity (I/σI) versus resolution for the STARANISO-processed X-ray diffraction dataset of *Escherichia* VapS-VapC. **(D)** Graph of *R* values versus resolution for the STARANISO-processed X-ray diffraction dataset of *Escherichia* VapS-VapC. Red line indicates $R_{merge}$; blue line indicates $R_{meas}$, and green line indicates $R_{pim}$. **(E)** Graph of $CC_{1/2}$ versus resolution for the STARANISO-processed X-ray diffraction dataset of *Escherichia* VapS-VapC.




**Appendix Figure S5 - AlphaFold 3 predicted model of the *Escherichia* VapK-VapS complex.**

**(A)** AlphaFold 3 predicted model of a complex of *Escherichia* VapK (STAS domain pink, GHKL domain orange), VapS (STAS domain blue, C-terminal region brown), ATP (black), and $Mg^{2+}$ (gray). **(B)** Closeup of the VapK GHKL kinase active site, with VapK Asn160 and VapS Ser48 shown as sticks. **(C)** AlphaFold 3 predicted aligned error (PAE) plot for the prediction shown in panel (A). **(D)** View as in panel (A), colored by confidence (pLDDT).




**Appendix Figure S6 - AlphaFold 3 predicted model of the *Escherichia* VapK-ssDNA complex.**

**(A)** AlphaFold 3 predicted model of a complex of *Escherichia* VapK (STAS domain pink, GHKL domain orange), a 7mer poly-T ssDNA (black), ATP (black), and $Mg^{2+}$ (gray; not visible). VapK Phe54 is shown as sticks. **(B)** Closeup of ssDNA binding by VapK, with Phe54, Lys180, and Lys181 shown as sticks and labeled. **(C)** Predicted Aligned Error (PAE) plot for the model shown in panel (A). **(D)** View as in panel (A), colored by confidence (pLDDT).

**Appendix Table S1. Crystallographic data collection and refinement statistics**

| | *E. roggenkampii* CapS | *V. cholerae* CapS S58A form 1 | *V. cholerae* CapS S58A form 2 | *Escherichia* VapS-VapC |
|---|---|---|---|---|
| **Data Collection** | | | | |
| Synchrotron/Beamline | SSRL 12-1 | SSRL 9-2 | SSRL 9-2 | APS 24ID-C |
| Date collected | 5-29-2024 | 6-19-2024 | 6-19-2024 | 10-5-2025 |
| Wavelength (Å) | 0.97946 | 0.97946 | 0.97946 | 0.97905 |
| Space Group | $P6_522$ | $P3_121$ | $C222_1$ | H3 |
| Unit Cell Dimensions (a,b,c) Å | 55.83, 55.83, 184.51 | 59.17, 59.17, 426.20 | 79.28, 98.19, 279.00 | 159.08, 159.08, 176.20 |
| Unit cell Angles (α,β,γ)° | 90, 90, 120 | 90, 90, 120 | 90, 90, 90 | 90, 90, 120 |
| Resolution (Å)* | 38.01-1.55 (1.58-1.55) | 51.25-2.38 (2.41-2.38) | 61.68-1.84 (1.88-1.84) | 74.22-3.40 (3.73-3.40) |
| *I*/σ* | 17.6 (1.6) | 4.8 (1.6) | 12.3 (1.0) | 5.0 (2.6) |
| $R_{merge}$* | 0.101 (1.169) | 0.157 (1.654) | 0.092 (2.572) | 0.352 (1.119) |
| $R_{meas}$* | 0.107 (1.35) | 0.173 (1.724) | 0.099 (2.772) | 0.386 (1.246) |
| $CC_{1/2}$* | 0.999 (0.660) | 0.994 (0.606) | 0.995 (0.335) | 0.985 (0.757) |
| Completeness % | 99.9 (99.3) | 99.8 (100.0) | 99.9 (100.0) | 57.5 (11.9) |
| No. of unique reflections* | 25795 (1209) | 36315 (1731) | 93914 (4570) | 13126 (655) |
| Redundancy* | 17.1 (7.6) | 4.7 (4.8) | 7.0 (7.2) | 5.9 (5.3) |
| **Refinement** | | | | |
| Resolution (Å) | 29.33-1.55 | 51.25-2.38 | 41.38-1.84 | 74.22-3.40 |
| No. of reflections | 25,705 | 36,296 | 93,496 | 13,104 |
| *working* | 24,405 | 34,468 | 88,812 | 12,457 |
| *free* | 1,300 | 1,828 | 4,684 | 647 |
| $R_{work}$ (%) | 16.66 (28.22) | 21.10 (27.23) | 18.83 (34.67) | 23.97 (34.58) |
| $R_{free}$ (%) | 19.02 (30.53) | 26.93 (32.88) | 21.70 (35.51) | 26.41 (39.75) |
| No. of atoms | | | | |
| total | 3,017 | 14,256 | 14,794 | 10394 |
| solvent | 203 | 274 | 740 | 0 |
| hydrogen | 1,418 | 7,049 | 7,075 | 0 |
| ligand | 0 | 0 | 0 | 4 ($Mg^{2+}$) |
| r.m.s.d. bond lengths (Å) | 0.0063 | 0.0023 | 0.0016 | 0.0198 |
| r.m.s.d. bond angles (°) | 0.82 | 0.58 | 0.53 | 2.13 |
| Ramachandran | | | | |
| favored (%) | 98.33 | 97.32 | 97.30 | 98.70 |
| allowed (%) | 1.67 | 2.68 | 2.70 | 1.30 |
| disallowed (%) | 0 | 0 | 0 | 0 |
| MolProbity Score | 1.18 | 1.15 | 1.03 | 1.65 |
| MolProbity ClashScore | 3.91 | 2.43 | 1.57 | 13.78 |
| SBGrid Data Bank ID | 1238 | 1239 | 1240 | 1241 |
| Protein Data Bank ID | 9Z71 | 9Z72 | 9Z73 | 9Z7O |

*Values in parentheses are for highest-resolution shell.

**Appendix Table S2. Proteins used in this study**

| Protein | Database and accession number | Sequence |
|---|---|---|
| *E. roggenkampii* CapK | NCBI WP_001567866.1 | MLFDTEKIKELLRESPGLTGKQIAKTLGYADKSALNSFLYSHAEGLKRV EWKWYVDDEYVLVLDGDAWIDENIFEANLSASGCLLSASARRCSISF PESCSILLAAGARIIALANQAAYSGKSIELDFSKCPSAKNYLNRLGFFD HLHPDVLVKPERPTNSRAQRYQGNSDNLVEIASIDLDDFDNSIPVKLT KKFVVHAGQKYYMAVFTIFSELIGNVRDHSESPIPGFAALQLYKGKRR HIQTVISDSGLGIATTLKRNLKKHYPEIFEELESSGEDADLFLVTHALKN GGLSQFGSAPDEAARGLGLKRSQDLAAKYDAVVLVRQPDFELRITYK NGVITEITSKKRLALIKGTQVCFDFFLGYD |
| *E. roggenkampii* CapS | NCBI WP_001567865.1 | MKVKLLELTENNNHPFGNVLGREVFKRLQNVVDSNPGCKSFEISLEGI VATDSSFPRESVIALAKQLCGEKYFFITDVSSTDLIDNWDYAAIAKQQS MIVVLAGGVRVIGPEAKSSTKALLDVVLGRNGVSTANVAKSLNISVQN ASTRLKKLSSEGVIMRSEVSSPTGGIEFIYSGPNISA |
| *V. cholerae* CapK | NCBI WP_160230705.1 | MLKAISELLDESPGLKGRQIAKELGLDKSQVNSFLHKNQDTFVKNSNH EWCLIRAQHVEIDFATGWIDDKAFESAIGKLAYQKDANKITFRFGIDCK FLLIALARFLALANQLAANGKDVVMDTTACPNTRGFFSRNGFFDYLNQ SVTCLPERPVLSAAKTYRDNSDTLVELGEIAQPTQNKELVIRLGDRFV EHSSASYFLAAKTVFSELVGNVTDHSESKIPGLAGLQVYRPYNKPKHI QTVISDSGLGIAATLRTTLQSEHPKLYAQFSAETVENDIALVQKAFTSG EVSRFGKGRGLGFKSSREHASKEKVIIVIRQLTFSLALEYAKGQLINVS EDRGLVPITGTHICFDFYVDNF |
| *V. cholerae* CapS | NCBI WP_046127252.1 | MKQLTHKILLSEVVGSDHAFGNDEGSEAYVKIKKIVDGHPSCDIFAISL EGIRFTDASFPRESVISLAKALKGEKGFYLSNVPSRDLLDNWSYGATA KDQPLLVKSDSGYEVLGVKLSATVKELLDFVIAKKTVTSSHVSKHFDIS AQNASGRLKKLHATGLVLGQKEVAESGGLEFVYRSIL |
| *Escherichia* VapK | NCBI WP_135559716.1 | MFNTKTGLTIMIPTLNDGEGDFMRLFMIYKQVMETDAPQITFIFTHCRF LRPNAVAFLGGTIRSLQKKGVTVFVDWKSIPSAVMASLKQNTFCSKLG YSSHTNPGHAIPYREDPKEDANSILDYLTQNWIGKGWVKVSEPLRDAI AGKVWEIYANSFEHSKSKIGVFSCGQHFIKKNELVLSVVDFGVGIPHN VREFLSSDARAASLSAESCLKWACQSGNTTATANGVPRGLGLHLLKE LVRVNNGKLELYSHNGYVKMSSQGEEYKTQPFYFEGTILNITLLCDEK YYRLTSELG |
| *Escherichia* VapS | NCBI WP_135559717.1 | MKILIKDFIGTRCILKEDGQKLFEEISRHLESKDEVILDFSNVKMFASPF FNYSIGQLFNKFSENEIRNNLHLDNLEVVGHSIIERVVENASRFKSDLD YKKIVDEILEQQARESD |
| *Escherichia* VapC | NCBI WP_135559718.1 | MAFSGVILANVFNIGNYTPTKDDKFLVDTNVWYWMTYTKGIPSNRQY LNTYIKFISDCISKESQLFHSGLSLAELAHIIESTEREIHEATIKSRIMTKE FRYKYAKERQLAMKEVEVSWAQVKQIAPQVDLTICQDLTDSCASKMT SNTLDGYDLMIHETMLKHGITNIITDDGDYTSVPGINVFTINKNVIVSAA TQKKLMN |