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Supporting information for article:

***AlphaFold*-assisted structure determination of a bacterial protein of unknown function using X-ray and electron crystallography**

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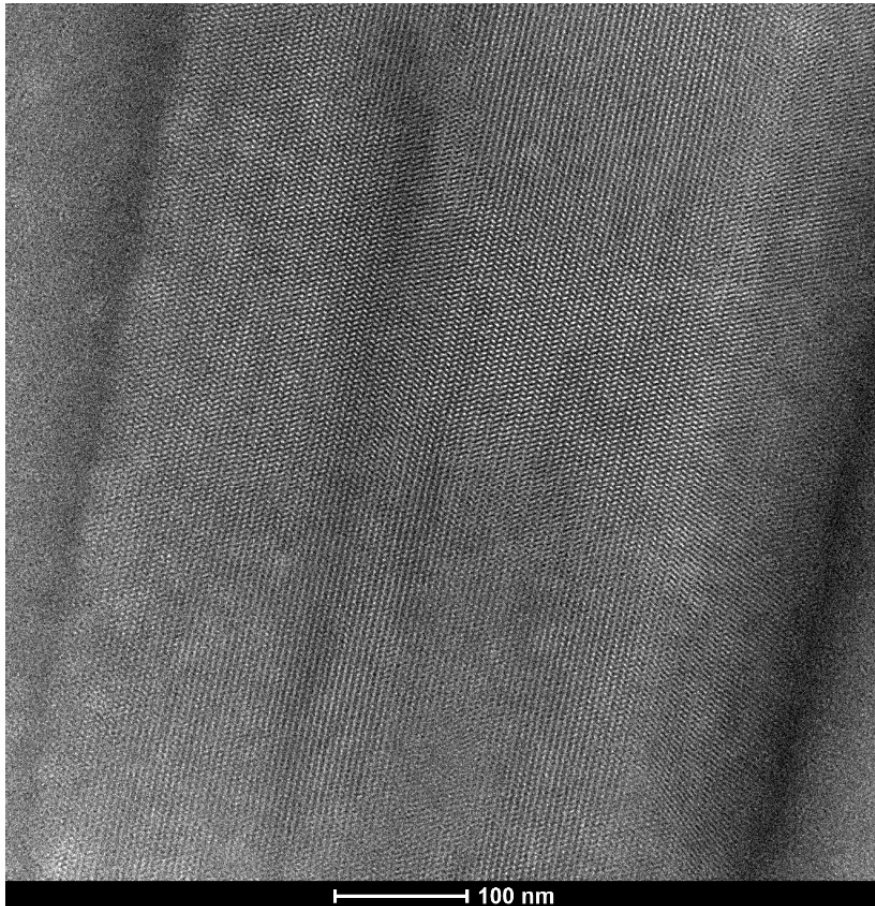


Figure S1 Negatively stained Q63NT7 crystal visualized on a Talos F200C electron microscope.

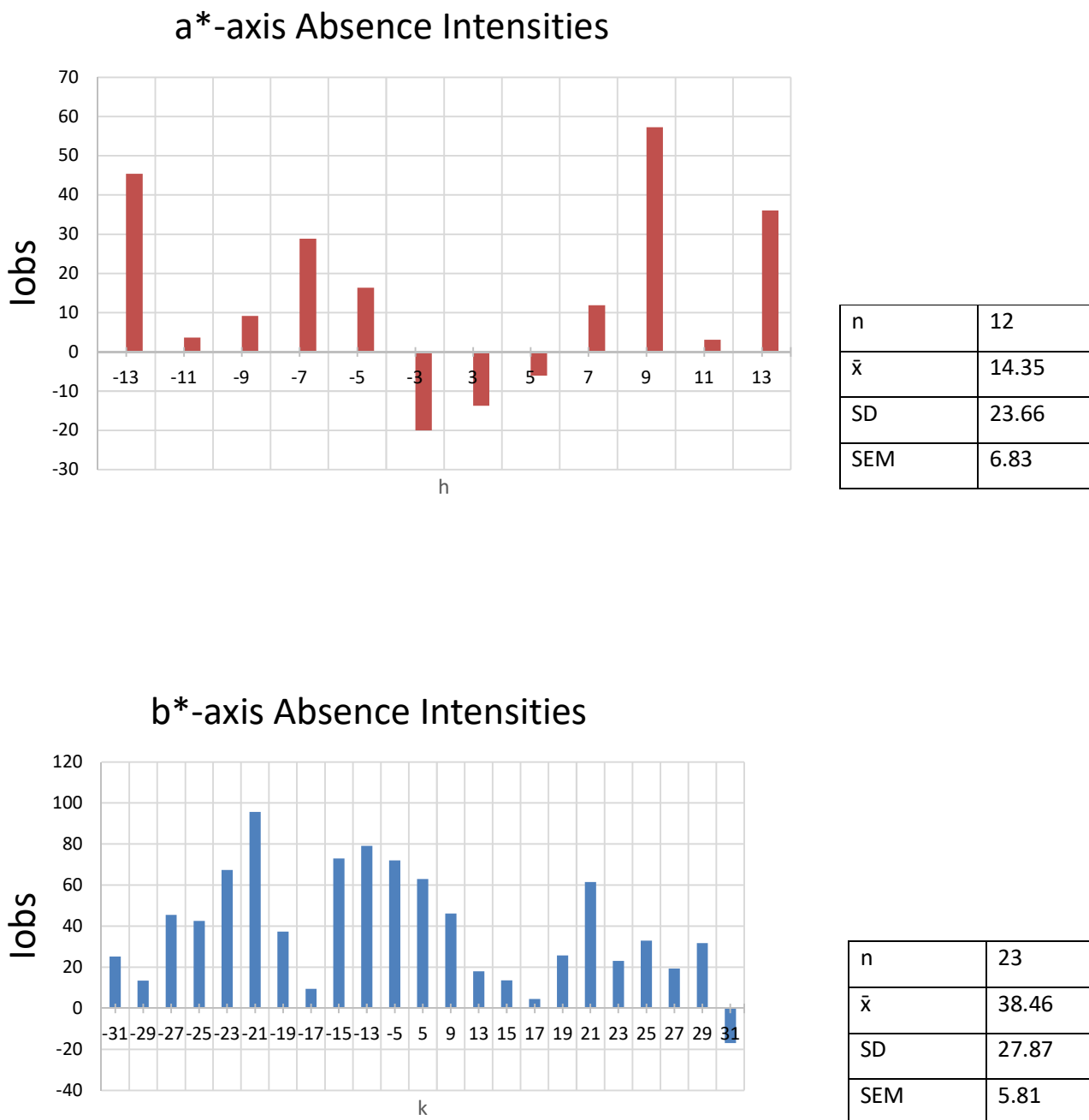


Figure S2 Histogram representations of intensities of systematic absences in microED data collected from form 2 crystals. The SEM value is the uncertainty in the mean value (equal to SD/\sqrt{n}). Along b^* , the average intensities of odd index reflections substantially exceed the uncertainty in the mean, which suggests the presence of dynamical scattering. For comparison, the mean intensities for $(2n, 0, 0)$ and $(0, 2n, 0)$ reflections were 94.7 and 107.3, respectively over the same index ranges as above.



Figure S3 Light microscope image of form 3 crystals.

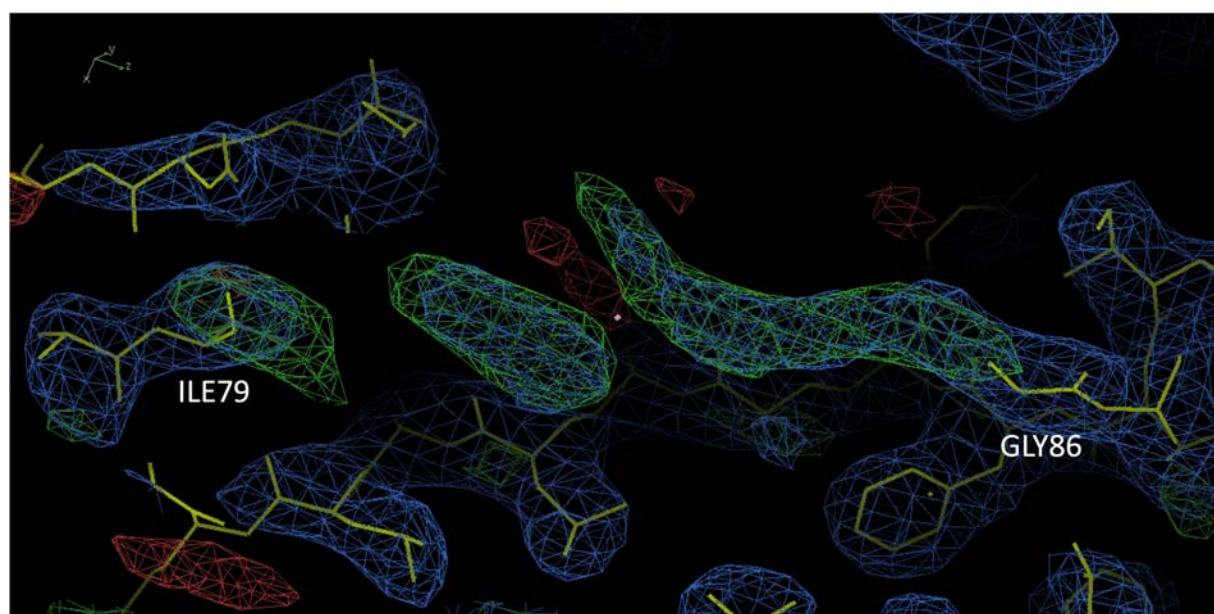


Figure S4 A Micro-ED omit-map confirms the correct molecular replacement solution when using the AlphaFold search model on form 2 diffraction data. Six residues from beta strand 5 were excluded from the search model, and density appears for this missing segment in an F_o-F_c map calculated using model

phases. Molecular replacement search model shown as atomic model in yellow, green density corresponds to positive density in $F_o - F_c$ map.

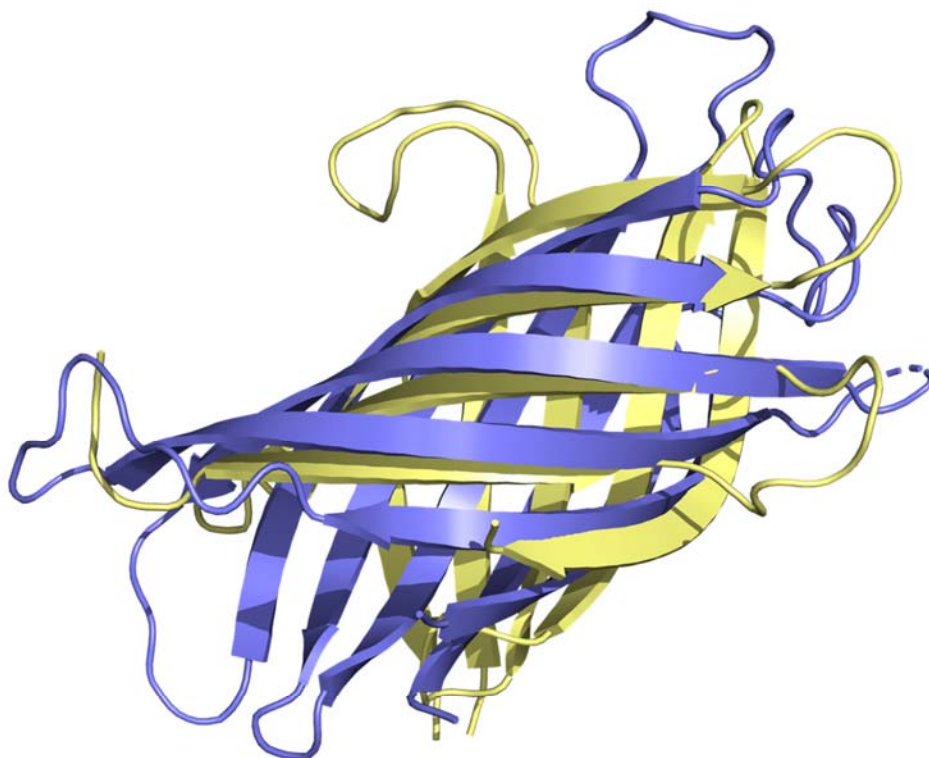


Figure S5 Comparison of the closest identifiable homolog of known structure (PDB 2erv) with the experimental structure of protein Q63NT7. The 2erv structure is shown as a cartoon in purple overlaid with the form 1 crystal structure of Q63NT7 (yellow).

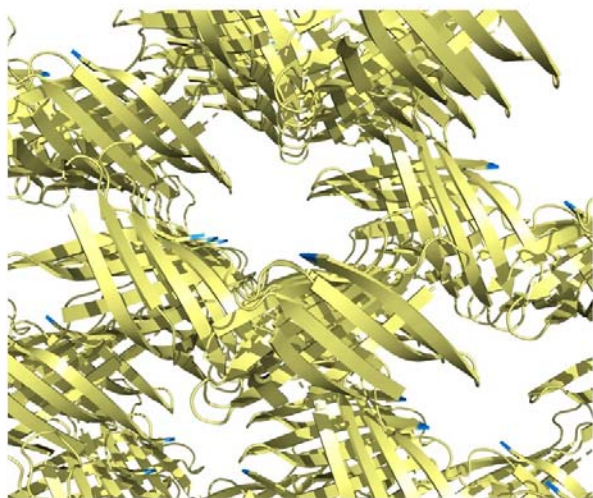


Figure S6 Crystal packing for the form 1 crystal of the Q63NT7 protein reveals solvent channels at the C-terminus of the β -barrel domain.

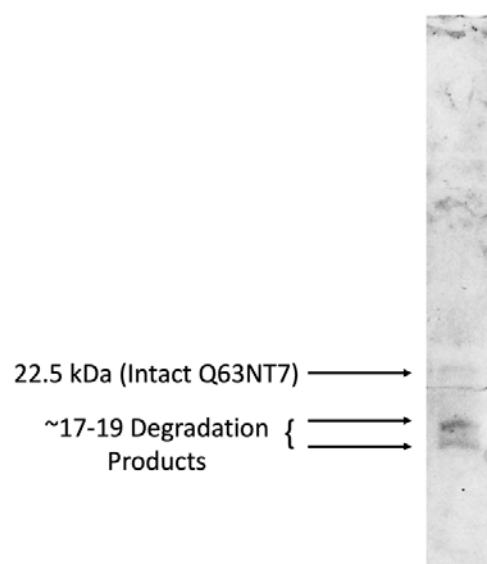


Figure S7 SDS-PAGE analysis of Form 3 crystals reveals prominent degradation products for the Q63NT7 protein.

Table S1 Macromolecule Production

Source Organism	<i>Burkholderia pseudomallei</i>
DNA Source	Synthetic
Expression vector	Pet 22b (+)
Plasmid Construction method	Gibson assembly
Expression host	<i>Escherichia coli</i> (BL21 (DE3))
Expression details	Autoinduction(Studier, 2005)
Complete amino-acid sequence of the protein produced: MSEDLRVGLFPVRYLVGTGLPGAPQLVLDLMVDTV DHSVVGRAAVSQAVSPPLNFHADVWG SYVFR LGPPRRD GSGAIVQISLQGNQGGPQSN SMITFYGELLLKGDGKTGVASYRYYSNGSW HEVENVPVKADPELVPIEPGPVIGQSSMSAIGSAAMYGVAIQSAAASGDLAHMRTL SAYARQQ LESRDEIAAALSELKAEIAKLESRQH HHHHHH	

Table S2 Crystallization Form 1 Crystals

Method	Hanging drop
Plate type	96 well
Temperature (°C)	20
Protein Concentration	20 mg/ml
Buffer composition of protein	100mM BisTris pH 5.5, 25% PEG 3350
Volume and ratio of drop	2:1
Drop setting	SPT LabTech Mosquito
Seeding	No

Table S3 Crystallization Form 2 Crystals

Method	Hanging drop
Plate type	96 well
Temperature (°C)	20
Protein Concentration	20 mg/ml
Buffer composition of protein	100mM BisTris pH 5.5, 100mM Ammonium Acetate 17% PEG 10,000
Volume and ratio of drop	2:1
Drop setting	SPT LabTech Mosquito
Seeding	No

Table S4 Crystallization Form 3 Crystals

Method	Hanging drop
Plate type	96 well
Temperature (°C)	20
Protein Concentration	100 mg/ml
Buffer composition of protein	100mM TRIS HCl pH 8.5, 150mM MgCl, 12.5% PEG 8000
Volume and ratio of drop	1:1
Drop setting	SPT LabTech Mosquito
Seeding	No