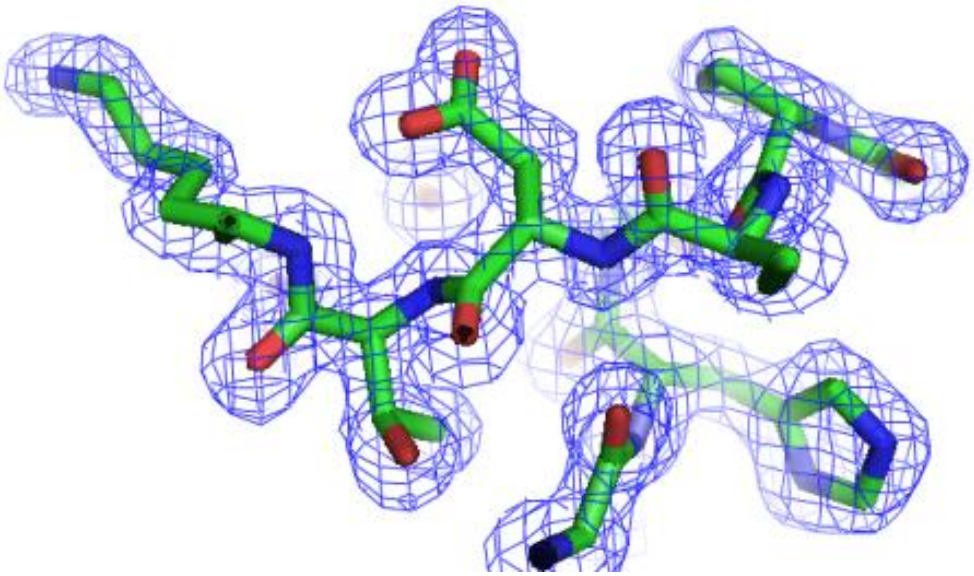
SUPPLEMENTARY MATERIAL

Supplementary Table S1. Seven, five and eight residues with two conformations in protomers A, B, and C are found in the crystal structure of PduB, respectively. These residues are listed below:

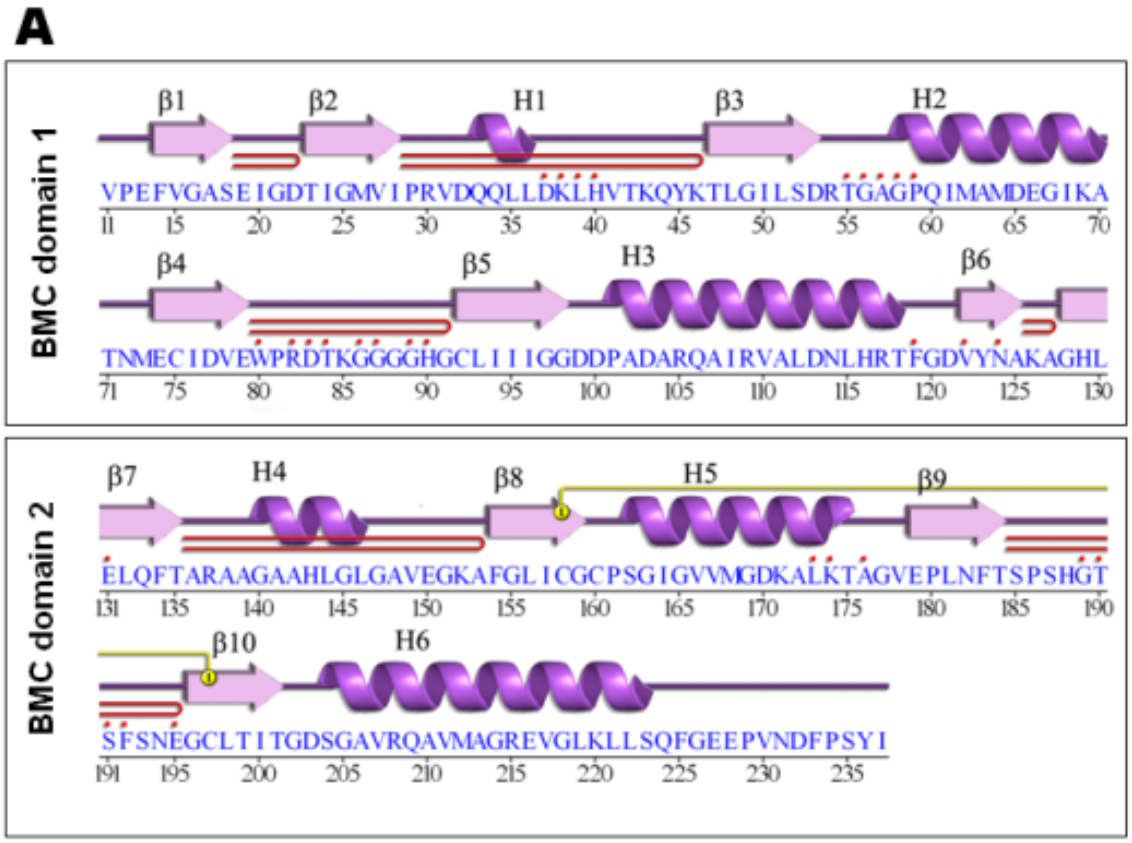
Chain A	Lys46, Asp99, Arg117, Lys152, Cys158, Cys197, Ile237
Chain B	Arg117, Cys158, Cys197, Ser223, Ile237
Chain C	Arg105, Arg109, Lys152, Leu156, Cys158, Cys197, Ser223, Ile237

Supplementary Table S2. Interaction of glycerol molecules with the pore-lining residues of PduB and other glycerol molecules.

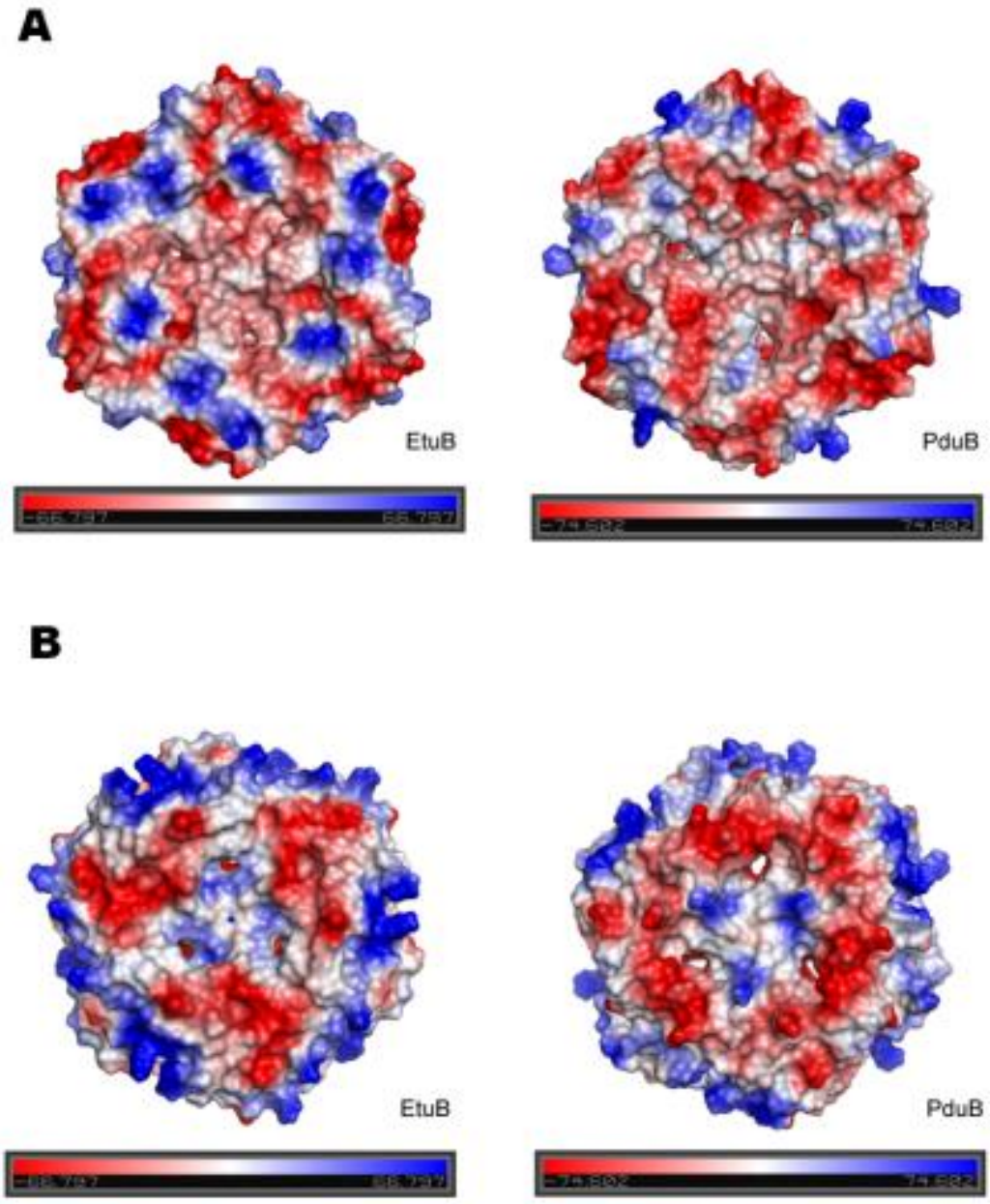
Glycerol in the subunit pore	**Glycerol Atom Number**			**Atom in Contact**	**Distances (Å)**		
	Chain A	Chain B	Chain C		Chain A	Chain B	Chain C
GOL1 / GOL5 / GOL9	O1	O1	O1	Ser 191 (OG)	2.79	2.71	2.55
GOL2 / GOL6 / GOL10	O1	O1	O1	Glu195 (OE1)	2.72	2.64	2.63
	O2	O2	O2	His90 (ND1)	2.86	2.85	2.95
	O2	O2	O2	GOL3 (O1) / GOL7 (O3) / GOL11 (O1)	2.78	3.00	2.91
	O3	O3	O3	Gly58 (N)	2.96	2.91	2.87
	O3	O3	O3	Glu131 (OE1)	2.54	2.66	2.67
GOL3 / GOL7 / GOL11	O1	O1	O1	His90 (N)	2.92	2.95	3.01
	O1	O1	O1	Glu195 (OE2)	2.72	2.73	2.73
	O1	O1	O1	GOL2 (O2) / GOL6 (O2) / GOL10 (O2)	2.78	3.03	2.86
	O2	O2	O3	Glu131 (OE1)	2.91	2.87	2.63
	O3	O3	O3	GOL4 (O3) / GOL8 (O3) / GOL10 (O3)	2.86	2.66	2.80
GOL4 / GOL8 / GOL12	O2	O2	O2	Asn124 (OD1)	3.14	4.45	2.82
	O3	O3	O3	GOL3 (O3) / GOL7 (O3) / GOL11 (O2)	2.86	2.66	2.95



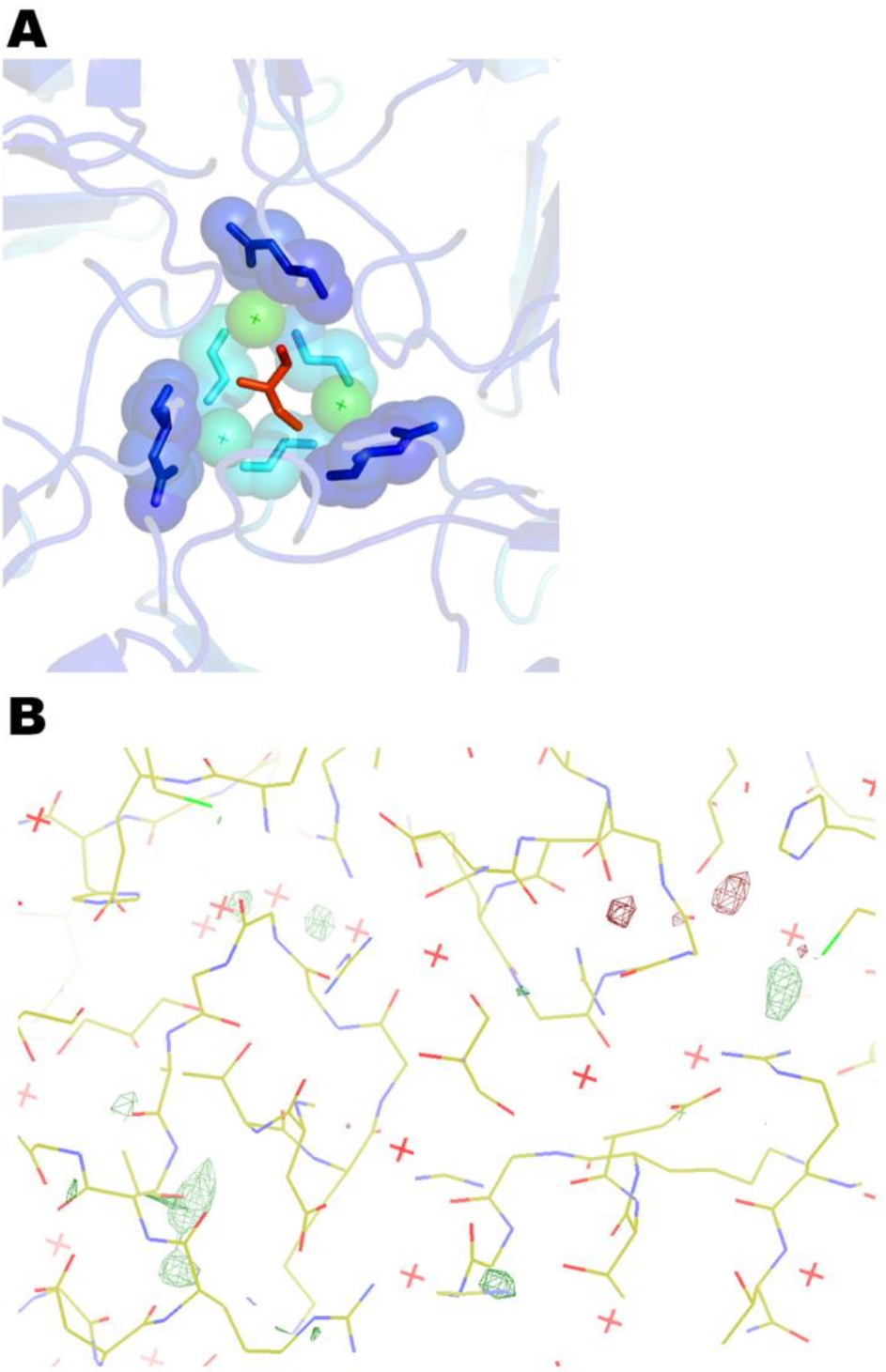
Supplementary Fig S1. σA-weighted 2Fobs-Fcalc Fourier synthesis contoured around Asp83 at 1 σ showing the quality of the electron density map (blue chicken wire mesh).



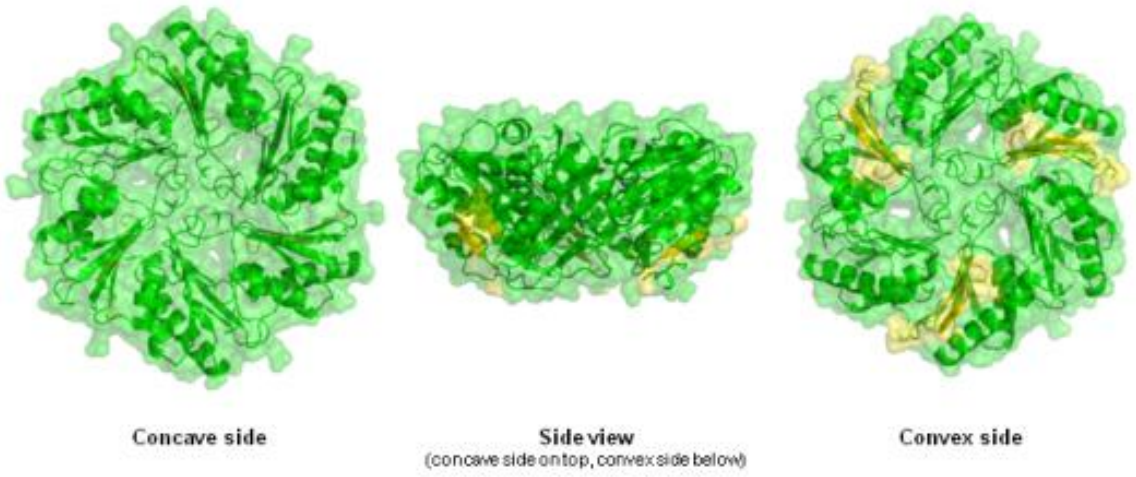
Supplementary Fig S2. Comparison of the first and second BMC repeats of the PduB subunit. **(A)** Amino acid sequence of the PduB with secondary structures marked. Red dots represent ligand binding residues. The first BMC domain spans from residue 1 to 109 while the second BMC domain spans from residue 110 to 235. A single disulphide bridge between Cys158 and Cys197 can be found in BMC domain 2. This figure was generated using PDBsum. (Laskowski *et al.*, 1997) **(B)** The sequences of the first and second BMC repeats aligned using ClustalW (Larkin *et al.*, 2007).



Supplementary Fig S3. Electrostatic difference between EtuB and PduB. **(A)** Concave and **(B)** Convex side of EtuB (left) and PduB (right). Red patches refer to the negatively charged region; blue patches refer to positively charged region. This Figure was produced using vacuum electrostatic by PyMOL (DeLano & Lam, 2005).



Supplementary Fig S4. Temperature factor and quality of electron density map of central region of PduB. **(A)** The central glycerol with surrounding water molecules and residues are coloured accordingly to their temperature factors, from dark blue for low B-factor to red for high B-factor. **(B)** σA-weighted Fobs-Fcalc Fourier synthesis at 3 σ.



Supplementary Fig S5. N-terminal region of PduB. PduB is known to be synthesised in two forms due to two translation start sites on the polycistronic message (Parsons *et al.*, 2008, Havemann & Bobik, 2003). The two N-terminal anti-parallel β-strands are deleted in PduB' (in yellow).