Supporting Information to

Quaternary Structure of the Small Amino Acid Transporter OprG of *Pseudomonas aeruginosa*

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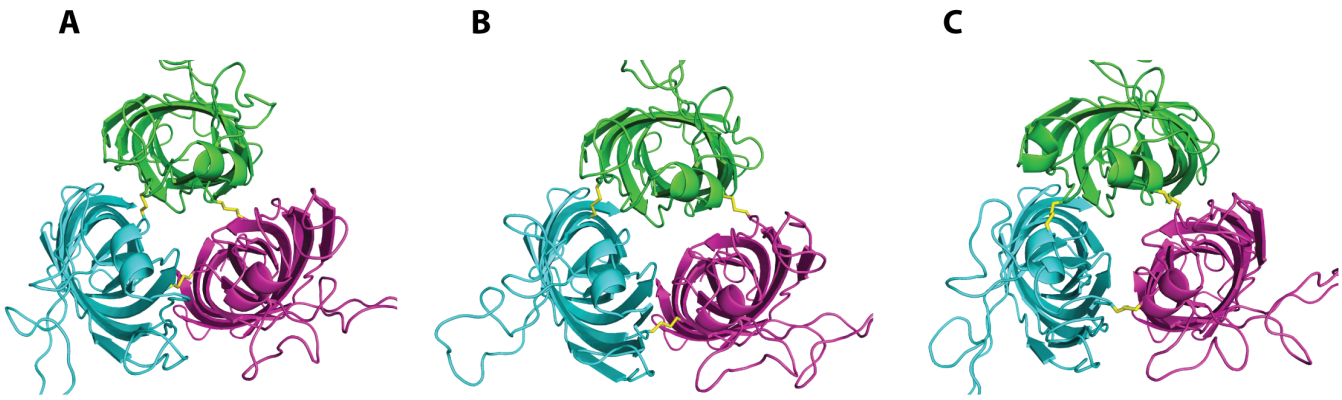
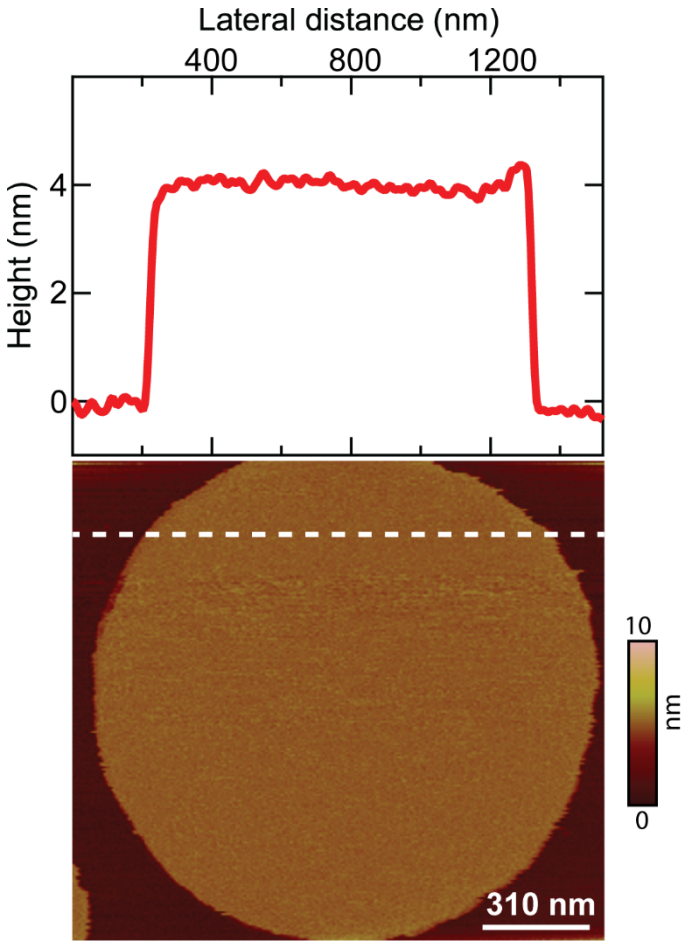
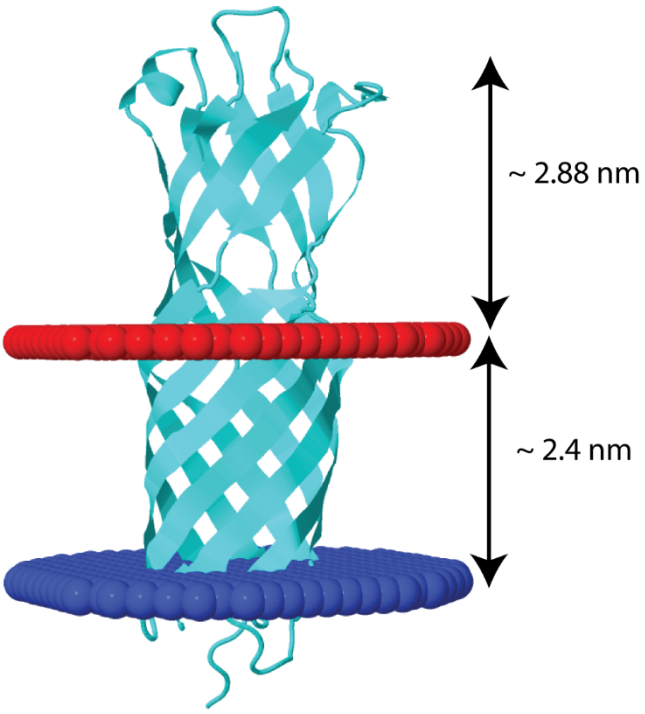


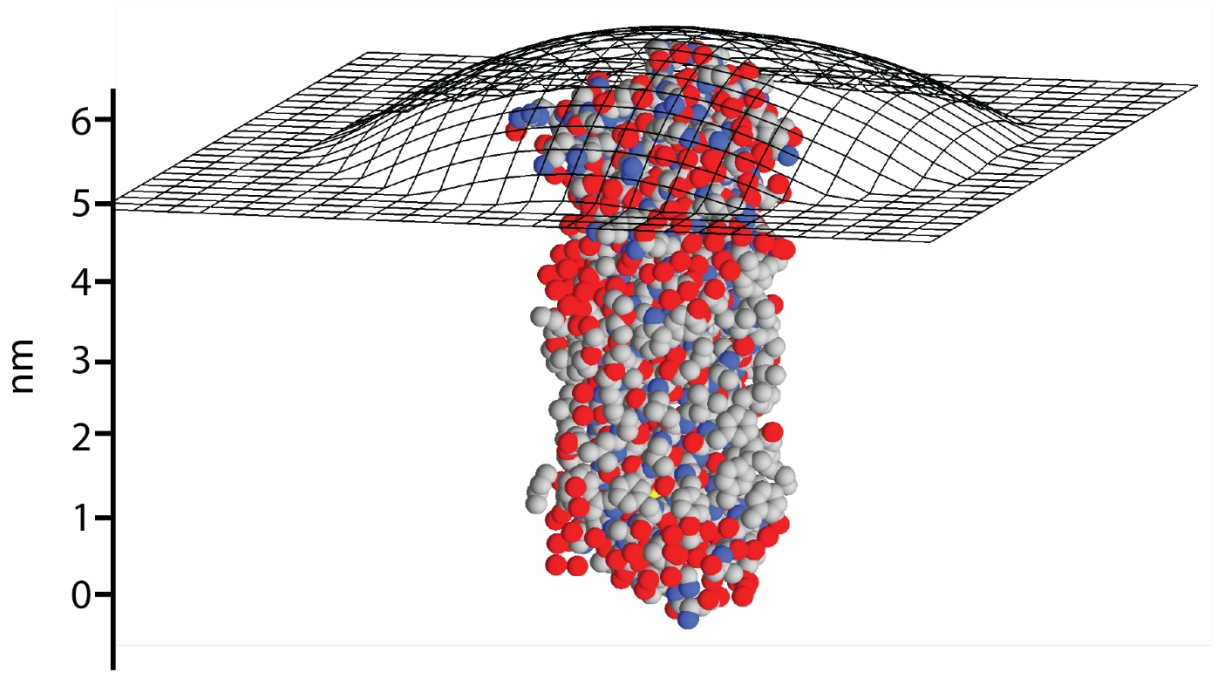
Supplementary Figure 1: A. Disulfide-linked trimer of T65C-L120C OprG based on the homology model of Figure 1B. **B**. Disulfide-linked trimer of Q44C-D142C OprG based on the homology model of Figure1B. **C**. Disulfide-linked trimer of S128C-S137C OprG based on the homology model of Figure 1B.



Supplementary Figure 2: AFM imaging of pure supported lipid bilayers of POPE:POPG (80:20) in the absence of OprG proteins. The cross-section profile (red line at the top) shows that the lipid bilayer has a height of ~ 4 nm above the mica substrate. The location of the cross-section is shown as a white dashed line in the image below. These membranes show no protrusions as expected for a protein-free bilayer.

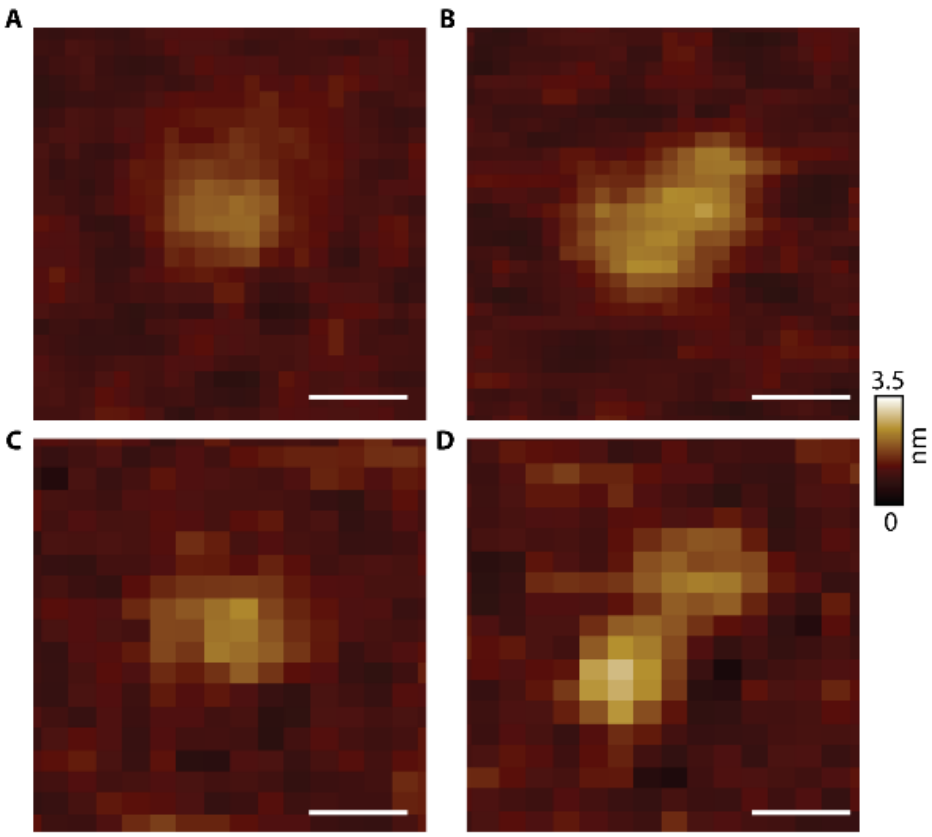


Supplementary Figure 3: Crystal structure of OprG (PDB entry 2X27): The hydrophobic thickness of the lipid bilayer is ~ 2.4 nm as provided by the Orientations of Proteins in Membranes (OPM) database (1). The protein height above this lipid bilayer is ~2.88 nm. However, the typical height of the lipid bilayer observed by AFM ~4 nm. Therefore, the height of the extracellular protrusion may be estimated as 1.28 nm (2.4 nm + 2.88 nm – 4 nm). The expected height of the periplasmic protrusion is smaller than 1 nm.

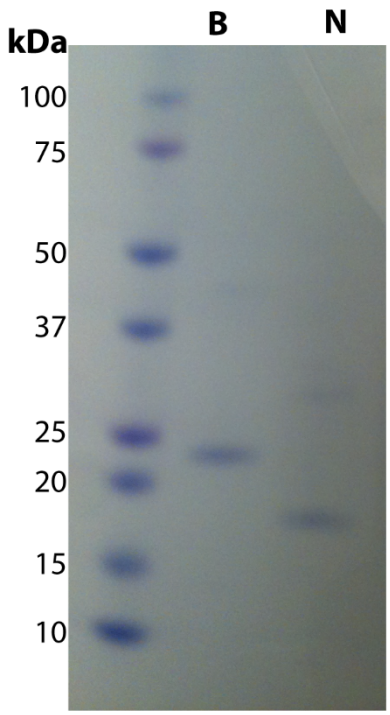


Supplementary Figure 4: Simulated AFM image using PDB coordinates (2X27) of OprG. The volume of the extracellular protrusion is 0.8 X 10^5 $Å^3$.

Method to construct a simulated AFM image from a crystal structure: To construct a simulated AFM image of OprG, we computed the morphological dilation between the crystal structure of OprG and a standard AFM tip geometry. OprG was modelled from its crystal structure (PDB entry 2X27(2)) where each atom was represented by a sphere with its van der Waals radius. The AFM tip was modelled as an isotropic paraboloid, with an effective radius of Rx=Ry=10 nm (i.e., the standard specification from the manufacturer, BL-AC40TS, Olympus). A simulated AFM image (black wireframe) was then computed as a dilation of the OprG model by the AFM tip model using custom software (WaveMetrics, Inc., Lake Oswego, OR 97035, USA).



Supplementary Figure 5: Representative protrusions of monomeric and dimeric OprG. **A, C**, Monomers with heights of ~1 nm and ~1.2 nm and volumes ~1.5 X 10^5 $Å^3$ and ~1.3 X 10^5 $Å^3$, respectively. **B, D,** Dimers with heights of ~1.3 and ~1.4 nm and volumes of ~2.5 X 10^5 $Å^3$, respectively.



Supplementary Figure 6: Refolding of Alexa-647 labelled G134C OprG in β-octylglucoside detergent. SDS-PAGE gel showing boiled (B) and refolded (N) G134C OprG. G134C OprG exhibited a shift from about 29 kDa to 24 kDa upon refolding in β-octylglucoside.

Supplementary Table 1:

Primers used

#	Name	Sequence (5'->3')
1	oprG_HERD30T_fw	GTTTAACTTTAAGAAGGAGATATACATACCCATGCGTAAGTCCTGGCTTACC
2	oprG_HERD30T_rv	GCCTGCAGGTCGACTCTAGAGGATCCCCGGGTCAGAACTTGTAGCCGAAACC
3	ST2_into_oprG_fw	**CAGTTCGAGAAGGGCTCG**GCGGATATTCAAGGACACAAGGCCG
4	ST2_into_oprG_rv	**CGGGTGCGACCAGCTTGC**AGCGGCGAACGGGGAGGC
5	Q44C_fw	GACTGTCGACAGCGACACC**TGT**CTCGGCCTGACCTTC
6	Q44C_rv	GAAGGTCAGGCCGAG**ACA**GGTGTCGCTGTCGACAGTC
7	L90C_fw	CTGGCCGACATCAAGCAA**TGT**CCGCCGACCCTGCTG
8	L90C_rv	CAGCAGGGTCGGCGG**ACA**TTGCTTGATGTCGGCCAG
9	T120C_fw	CTGGGCGTGAACTACACC**TG**CTTCTTCGACGAAGACC
10	T120C_rv	GGTCTTCGTCGAAGAAG**CA**GGTGTAGTTCACGCCCAG
11	S128C_rv	CTGCGCCTTGCGGTTGC**A**GGCGAGGTCTTCGTCG
12	S136C_fw	CGCAAGGCGCAGGGTTTC**T**GCAGCATGAAGCTGC
13	S137C_fw	CGCAAGGCGCAGGGTTTCAGC**T**GCATGAAGCTGC
14	D142C_fw	GCAGCATGAAGCTGCAG**TG**CTCCTGGGGCCTGG
15	D142C_rv	CCAGGCCCCAGGAG**CA**CTGCAGCTTCATGCTGC
16	ΔL1_fw	GGCTTCGCCACCGTCGATCCCGACACCCAGCTCGGCCTG
17	ΔL1_rw	CAGGCCGAGCTGGGTGTCGGGATCGACGGTGGCGAAGCC
18	ΔL2_fw	GCCGCCACTCCGTTCAACCAACTGCCGCCGACCCTGC
19	ΔL2_rw	GCAGGGTCGGCGGCAGTTGGTTGAACGGAGTGGCGGC
20	ΔL3_fw	GGCCTGGGCGTGAACTACACCGACTCCTGGGGCCTGGCCGGC
21	ΔL3_rw	GCCGGCCAGGCCCCAGGAGTCGGTGTAGTTCACGCCCAGGCC
22	ΔL4_fw	GCCGTCTGGTACATGGACGATCCGTGGGTCTACATGATCGG
23	ΔL4_rw	CCGATCATGTAGACCCACGGATCGTCCATGTACCAGACGGC

Base changes and insertions are highlighted in bold.

References

1. Lomize MA, Pogozheva ID, Joo H, Mosberg HI, Lomize AL. OPM database and PPM web server: Resources for positioning of proteins in membranes. *Nucleic Acids Res.* 2012; 40(D1): D370-D376.

2. Touw DS, Patel DR, Van Den Berg B. The crystal structure of OprG from pseudomonas aeruginosa, a potential channel for transport of hydrophobic molecules across the outer membrane. *PloS one.* 2010; 5(11): e15016.