Figure S1

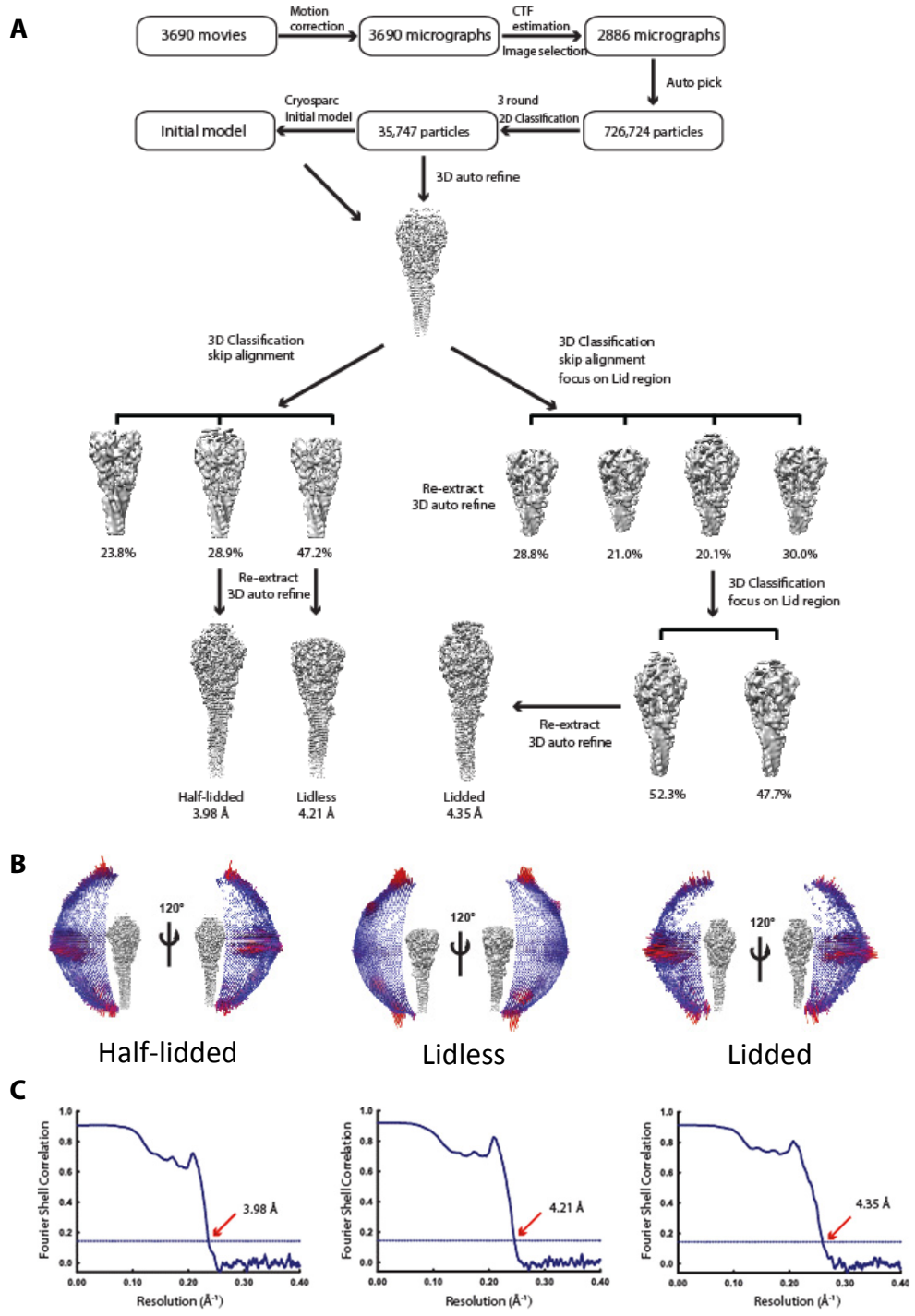

Figure S1. Related to Figure 1. Structure determination of the PdpA-VgrG complex

(A) Workflow of image processing, 3D reconstruction and structure refinement.

(B) Angular distribution of all particles used for reconstruction of each of the 3 maps of the PdpA-VgrG complex.

(C) Fourier shell correction coefficient as function of spatial frequency showing the resolutions for the final reconstructions of the PdpA-VgrG half-lidded, lidless and lidded complexes to be 3.98, 4.21 and 4.35 Å, respectively.

Figure S2

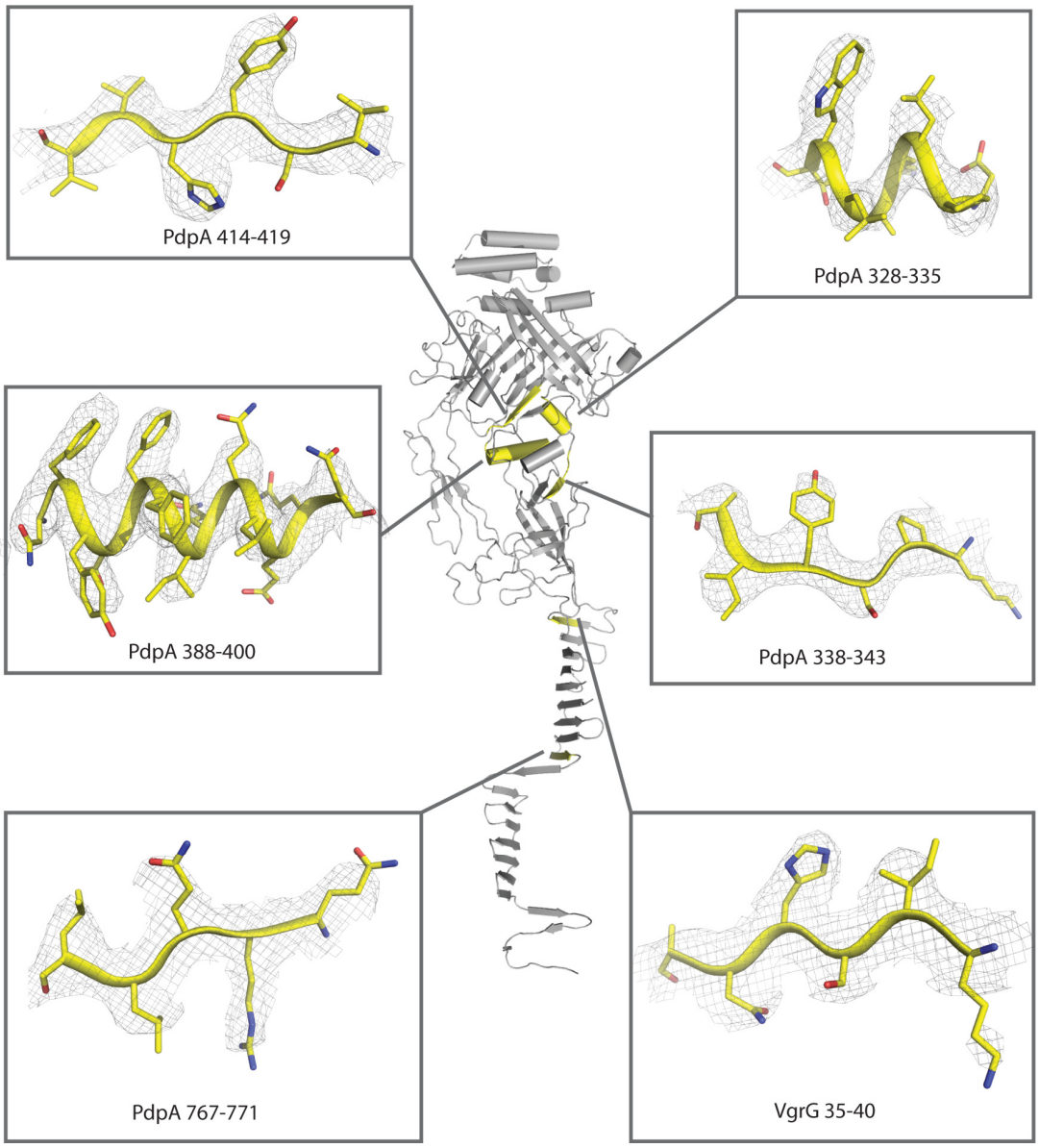

Figure S2. Related to Figure 1. Representative cryoEM density maps (grey mesh) superposed with our atomic model (ribbon and sticks) for various parts of the PdpA-VgrG complex. The atomic model is shown in the middle as a ribbon diagram.

Figure S3

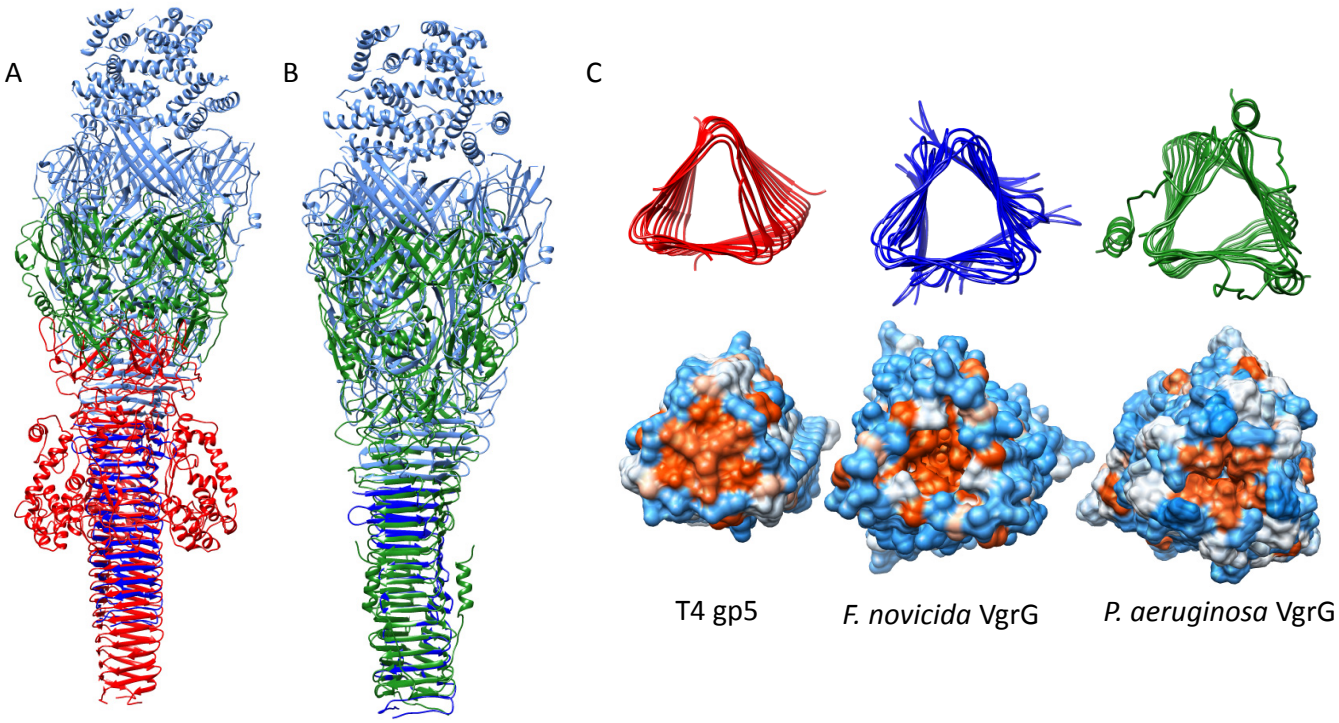

Figure S3. Related to Figure 1. Comparison of central spike structures.
(A) Ribbon diagrams of T4 gp27 (green) and gp5 (red) superposed on lidded *F. novicida* PdpA (light blue) and VgrG (dark blue).
(B) *P. aeruginosa* VgrG (green) superposed on lidded PdpA (light blue) and VgrG (dark blue).
(C) Ribbon (top) and hydrophobicity surface views (bottom) of the C-terminus of T4 gp5, *F. novicida* VgrG, and *P. aeruginosa* VgrG. Colors in the top ribbon diagrams are as in panels (A) and (B). In the hydrophobicity surface views, the most hydrophobic surfaces are shown in red and the most hydrophilic surface are shown in blue. T4 gp5 and gp27 structures are from pdb 2Z6B (Koshyiama *et. al*, 2008) and *P. aeruginosa* VgrG structure is from pdb 6H3L (Quentin *et al.*, 2018).

Figure S4

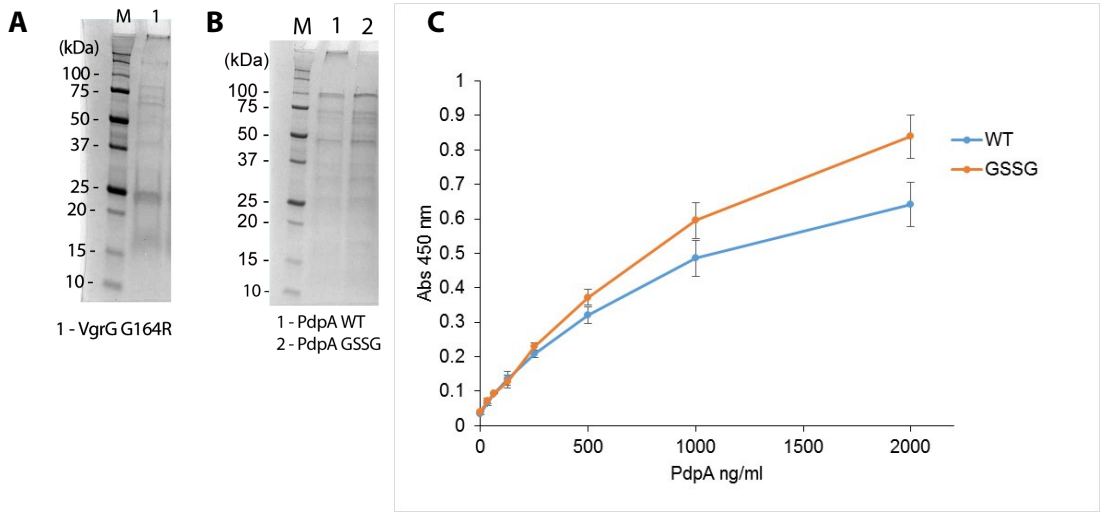

Figure S4. Related to Figure 6. Interactions of recombinant FLAG-VgrG G164R and PdpA.
(A) Histidine (His)- and FLAG-epitopes tagged VgrG G164R was expressed and purified from *E. coli* by Ni-NTA affinity chromatography and evaluated by SDS-PAGE and Coomassie protein staining.
(B) His-epitope tagged PdpA and PdpA::GSSG were expressed and purified from *E. coli* by Ni-NTA affinity chromatography and evaluated by Coomassie protein staining.
(C) Interaction of purified recombinant FLAG-VgrG G164R and His-tagged PdpA and PdpA::GSSG. ELISA plate wells were coated with His-tagged PdpA or PdpA::GSSG (0-2 µg/ml); blocked with BSA and gelatin; incubated with purified FLAG-VgrG (0.1 µg/ml); and binding evaluated by incubation with HRP-conjugated mouse anti-FLAG M2 monoclonal antibody and peroxidase substrate. Data shown are means ± s.e. of duplicates. The experiment was conducted twice with similar results.

Table S1. Related to Figure 1. Most abundant proteins identified by Mass Spectrometry

Gene Product Identified	Accession Number	Relative Abun-dance	Sequence Count	Spectrum Count	Cover-age	MW (Da)	NSAF *
VgrG FPI	A0Q7H3	1.000	20	874	68.9%	17472	0.300
PdpA	A0Q7H0	0.672	150	2935	82.0%	95345	0.202
VgrG of FNI	A0Q3Y4	0.301	5	151	81.9%	10412	0.091
Acetyl-CoA carboxylase	A0Q5E3	0.150	8	128	66.2%	16659	0.045
60 kDa chaperonin	A0Q838	0.048	33	139	49.1%	57317	0.014
30S ribosomal protein S10	A0Q4I2	0.036	11	20	53.3%	11924	0.011
Pyruvate dehydrogenase	A0Q7Z6	0.029	58	138	53.6%	100182	0.009
Major outer membrane protein	A0Q7V4	0.023	11	22	56.7%	19786	0.007
Uncharacterized Protein, FTN_0041	A0Q3Y7	0.021	33	82	41.5%	85456	0.006
IglC	A0Q7I3	0.020	9	22	44.0%	22133	0.006

*Normalized spectral abundance factor. The experiment was conducted twice on separate biological samples with similar results.

Table S2. Related to Figure 1.
CryoEM data collection and refinement statistics

Data Collection	**Half-lidded**	**Lidded**	**Lidless**
EM equipment		FEI Titan Krios	
Voltage (kV)		300	
Detector		Gatan K2	
Pixel size (Å)		1.07	
Electron Dose ($e^-/Å^2$)		46.65	
Defocus range (μm)		-2.5~-1.5	
Reconstruction			
Software		Relion 2.1	
Number of used particles	10343	3765	16648
Accuracy of rotation (°)	1.735	1.683	1.922
Accuracy of translation (pixels)	0.729	0.743	0.789
Map sharpening B-factors ($Å^2$)	-150	-150	-150
Final Resolution (Å)	3.98	4.35	4.21
Model Building			
Software		COOT	
Refinement			
Software		PHENIX real_space_refinement	
Resolution (Å)	3.98	4.35	4.21
Model composition			
Protein Residues	2355	2550	2148
Validation			
RMS deviation			
Bonds Length (Å)	0.005	0.009	0.009
Bonds angle (°)	1.0	1.1	1.3
Ramachandran plot statistics (%)			
Favorite	87.74	86.57	85.94
Allowed	12.26	13.43	14.06
Outlier	0	0	0
Rotamer outliers (%)	0	0.15	0
C-beta outliers (%)	0	0	0
Mean isotropic B	56.84	113.24	56.72
MolProbity score	1.91	2.05	1.96