

SUPPORTING INFORMATION

Engineering the PduT shell protein to modify the permeability of the 1,2-propanediol microcompartment of *Salmonella*.

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Supplementary Table S1. Bacterial Strains used in this study

Strains	Genotype/Variant	Source
WT	<i>Salmonella enterica</i> serovar Typhimurium strain LT2	Lab collection
BE293	LT2/pKD46	Lab collection
BE464	LT2, $\Delta pduA::frt$	Lab collection
BE885	LT2, $\Delta pduT::frt$	This study
BE1621	<i>E. coli</i> 10 beta/ pET41a ⁺ -PBlaP3- <i>sacB-cat</i>	Lab collection
BE97	<i>E. coli</i> BW25141/pKD4	Lab collection
BE201	LT2/pCP20	Lab collection
BE2377	LT2, PduA-S40L	This Study
BE2384	LT2, PduT-C38S	This Study
BE2389	LT2, PduA-S40L / $\Delta pduT::frt$	This Study
BE2390	LT2, PduA-S40L / PduT-C38S	This Study
BE2391	LT2, PduT-C38I	This Study
BE2392	LT2, PduA-S40L / PduT-C38I	This Study
BE2393	LT2, PduT-C38W	This Study
BE2394	LT2, PduA-S40L / PduT-C38W	This Study
BE2395	LT2, PduT-C38A	This study
BE2396	LT2, PduA-S40L / PduT-C38A	This study
BE2225	LT2, $\Delta pduQ::kan$	Lab collection
BE903	LT2, $\Delta pduQ::frt$	Lab collection
BE2397	LT2, $\Delta pduQ::frt$ / PduT-C38S	This study
BE2398	LT2, $\Delta pduQ::frt$ / PduT-C38I	This study
BE2399	LT2, $\Delta pduQ::frt$ / PduT-C38A	This study
BE2547	LT2, $\Delta pduQ::frt$ / PduT-C38W	This study
BE2568	LT2, $\Delta pduQ::kan$ / $\Delta pduT::frt$	This study

Supplementary Table S2. Growth of *Salmonella* PduT variants on 1,2-PD minimal medium

Strains	Doubling time (h)
WT	16.4 ± 1.1*
PduT-C38S	16.7 ± 0.5
PduT-C38A	17.1 ± 0.6
PduT-C38I	17.4 ± 0.6
PduT-C38W	17.9 ± 0.8
<i>ΔpduT::frt</i>	17.8 ± 1.1

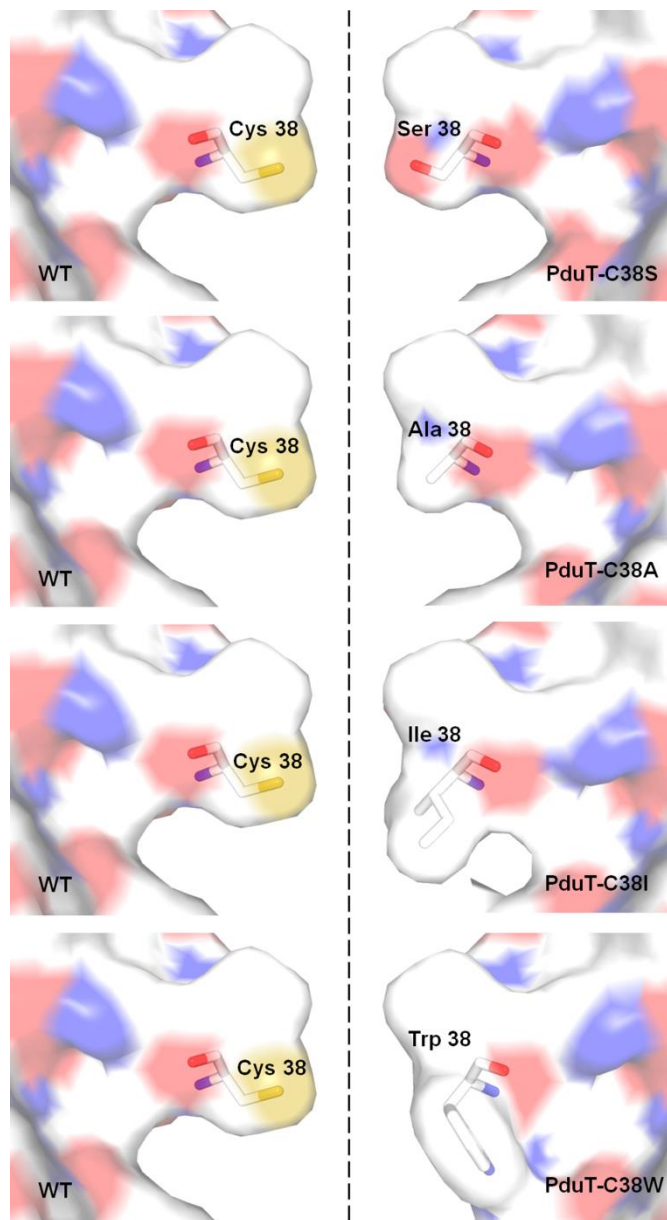
*Growth assays were performed on 1,2-PD minimal medium with limiting B₁₂ (25 nM).

Doubling times were calculated from at least three biological replicates. The error estimate shown is one standard deviation. The growth rates of the mutants are not significantly different from wild type (WT) as determined by two-tailed student's *t*-test.

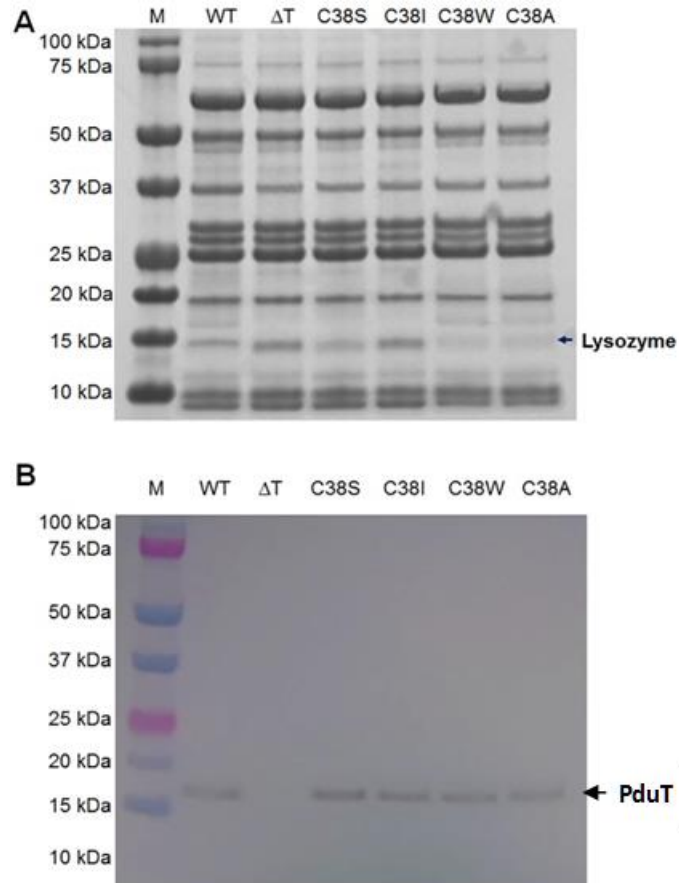
Supplementary Table S3. Diol dehydratase activities of MCPs purified from *pduT* mutants

MCPs from Pdu mutants	Specific Activity ($\mu\text{mol /min/mg}$)
WT	$27.8 \pm 1.2^*$
PduT-C38S	28.2 ± 0.9
PduT-C38A	27.2 ± 0.8
PduT-C38I	26.5 ± 0.7
PduT-C38W	24.9 ± 0.6
$\Delta pduT::frt$	26.8 ± 0.6

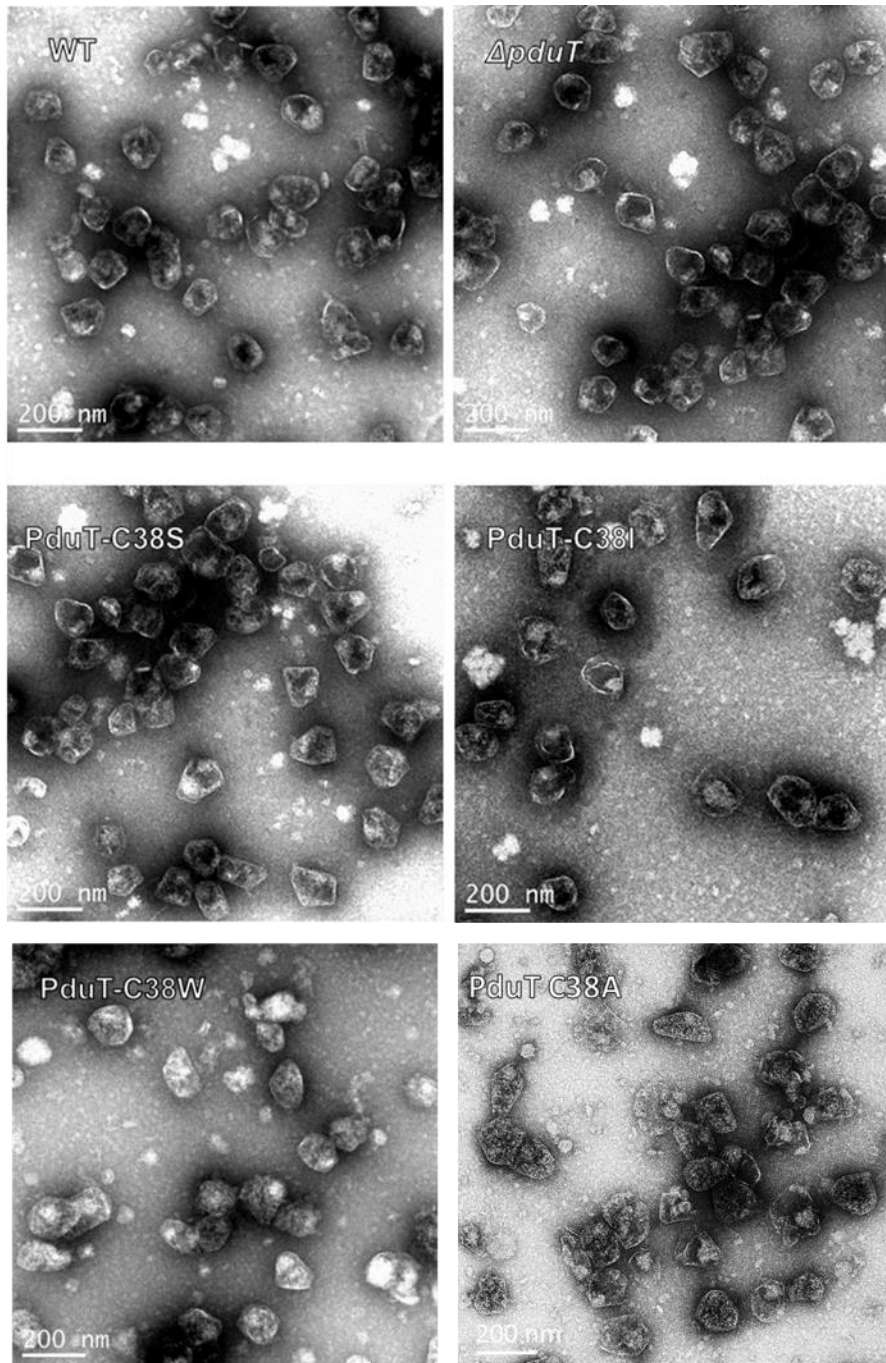
*Enzyme activities are based on at least three replicates. The error estimate shown is one standard deviation. The diol dehydratase activities of the mutants are not significantly different from wild type (WT) as determined by two-tailed student's *t*-test.



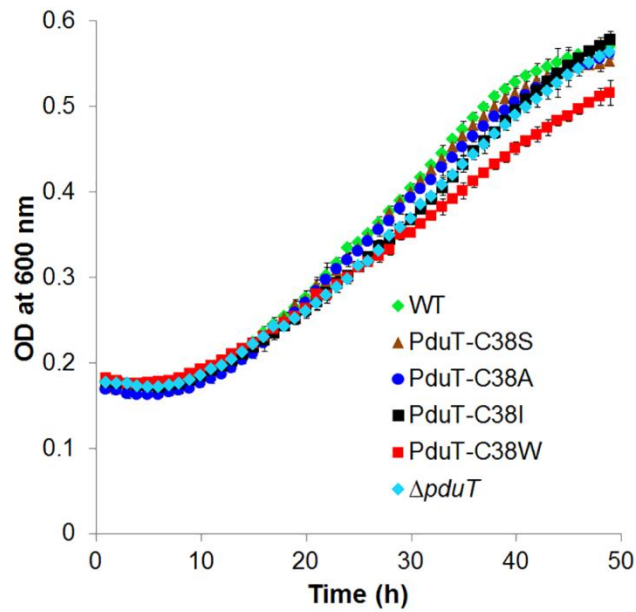
Supplementary Figure S1: Effects of PduT position 38 mutations on the PduT pore. The images show a side view through the point of constriction of PduT trimer. Atoms are colored by type - carbon: gray (non-polar), sulfur: yellow (less-polar), oxygen: red (polar), nitrogen: blue (polar). A change in Cys38 to Ser increases polarity at the pore region whereas changes to Ile and Trp decrease polarity.



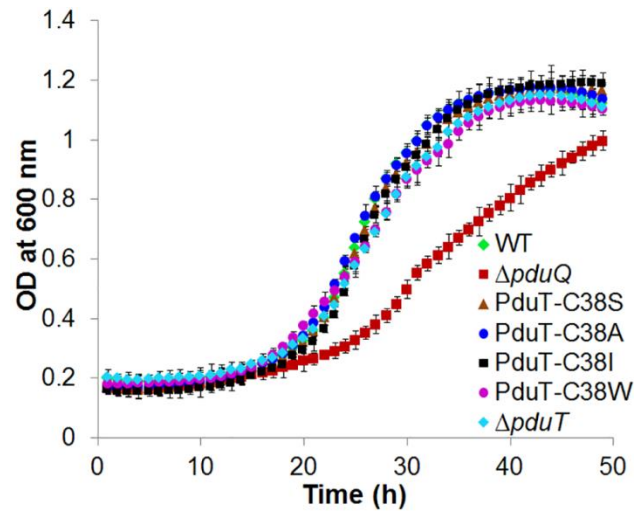
Supplementary Figure S2: SDS-PAGE and Western Blot analyses of the MCPs isolated from of wild-type (WT) and mutants used in this study. SDS PAGE (A) and Western Blot (B) analyses demonstrated proper recruitment and incorporation of the PduT mutants into the MCP shell.



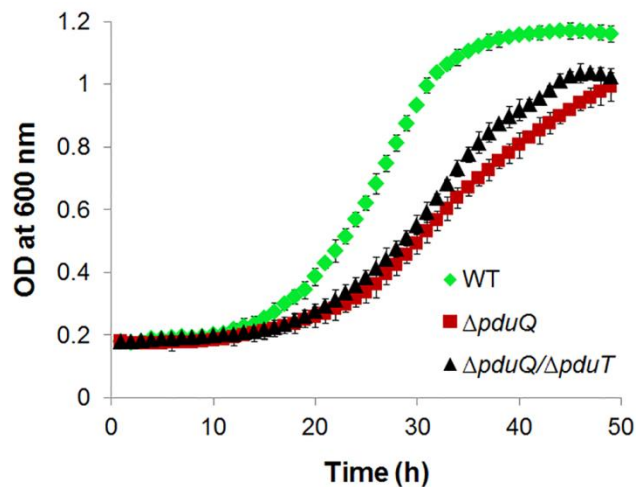
Supplementary Figure S3: Transmission electron micrographs of purified MCPs from wild-type, *ΔpduT* and PduT mutants. WT is wild-type *Salmonella enterica* serovar typhimurium LT2. There were no apparent morphological changes in the mutant MCPs compared to wild-type.



Supplementary Figure S4: Growth of wild-type and PduT mutants on 1,2-propanediol minimal medium at limiting B₁₂ (25 nM). WT= *Salmonella enterica* serovar Typhimurium LT2. Growth assays were performed three times with similar results in microplate reader. Error bars represent one standard deviation from three measurements.



Supplementary Figure S5: Growth of wild-type and selected PduT mutants on 1,2-propanediol. WT= *Salmonella enterica* serovar Typhimurium LT2. Growth assays were performed three or more times with similar results in microplate reader. Error bars represent one standard deviation from three measurements.



Supplementary Figure S6: Growth of wild-type, $\Delta pduQ$ and $\Delta pduQ$ - $\Delta pduT$ double mutants on 1,2-propanediol at saturating B₁₂ (100 nM). WT= *Salmonella enterica* serovar

Typhimurium LT2. A $\Delta pduQ$ mutant grows slowly due to impaired recycling of NAD/H inside the MCP lumen. A $\Delta pduT$ mutant does not correct slower growth phenotype of $\Delta pduQ$ significantly. Growth assays were performed three or more times with similar results in microplate reader. Error bars represent one standard deviation from three measurements.

Supplementary Figure S7: Transmission electron micrographs of purified MCPs from wild-type, and key double mutants. WT is wild-type *Salmonella enterica* serovar typhimurium LT2. There were no apparent morphological changes in the mutant MCPs compared to wild-type.

