

O-antigen Polymerase Adopts a Distributive Mechanism for Lipopolysaccharide Biosynthesis

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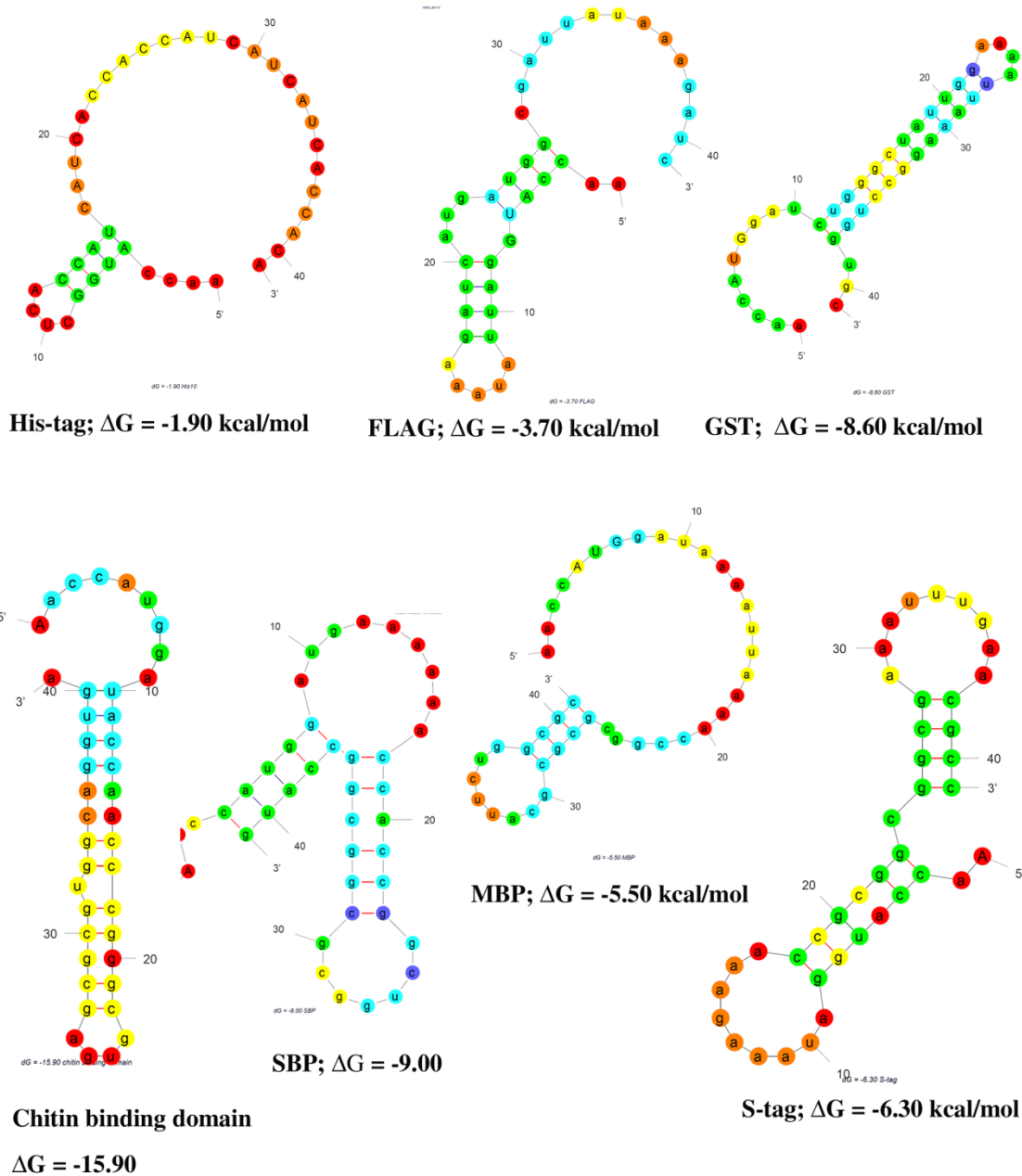


Figure S1. The mRNA structure of the nucleotides (-4 to +37) and their corresponding folding free energy which is calculated by mfold web server. All the genes are supported to be inserted into *Nco*I restriction site on pBAD/myc-his A plasmid.

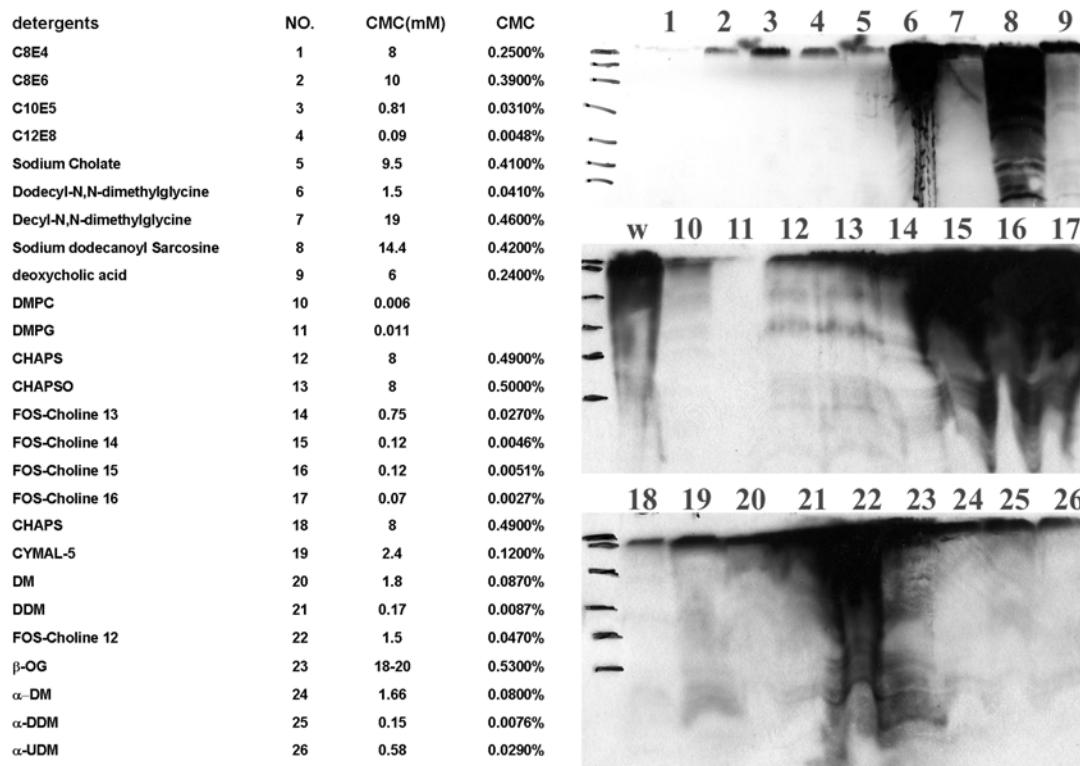


Figure S2. The initial screening using twenty-six detergents and the detection of His-tagged Wzy by western blot. In most cases, Wzy form the aggregates. The possible candidate detergents were selected on the basis of the western signal even the proteins may be aggregates.

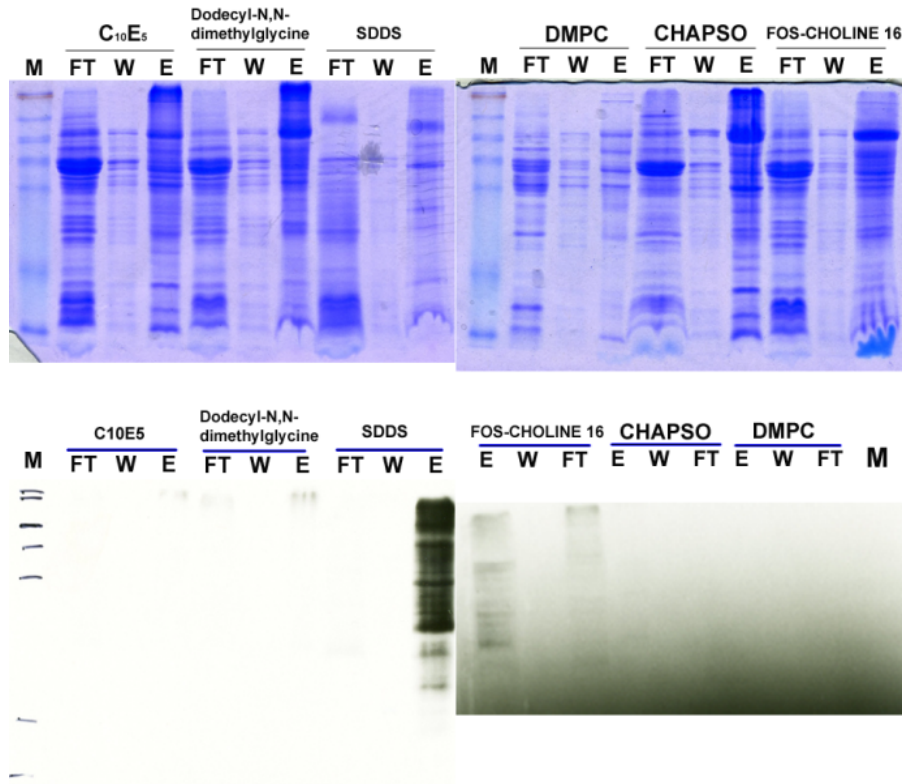


Figure S3. The 2nd detergents screening and visualization of Wzy by SDS-PAGE and Western blot.

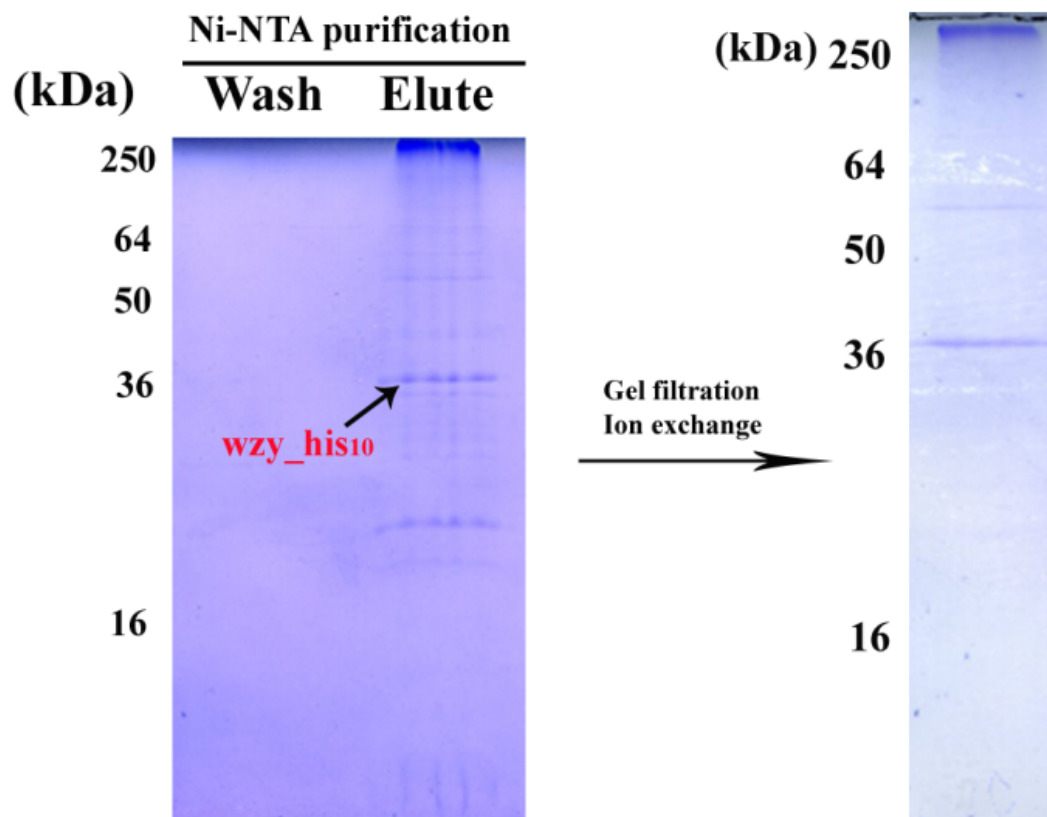
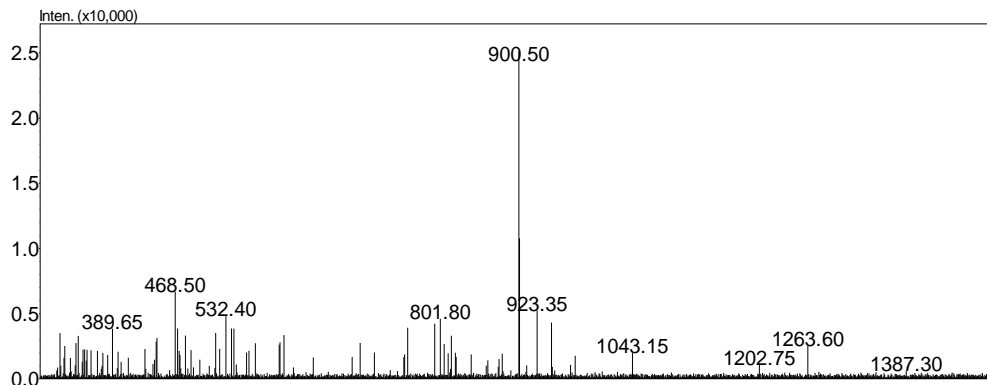
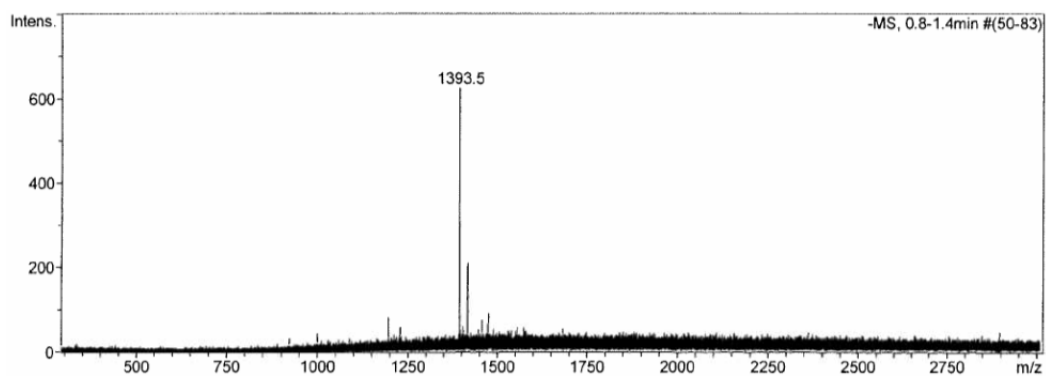


Figure S4. The expression and purification of Wzy with C-terminal histidine tag.

RU-PP-Undecaprenyl: LRMS (m/z): [M-2H]²⁻ calcd. for C₈₉H₁₄₆N₂O₃₁P₂, 900.5; found, 900.4.



RU-PP-cis-Pentaprenyl: LRMS (m/z): [M-H]⁻ calcd. for C₅₉H₉₉N₂O₃₁P₂, 1393.6; found, 1393.5.



RU-PP-MS-Pentaprenyl: LRMS (m/z): [M-2H]²⁻ calcd. for C₅₉H₁₀₀N₂O₃₁P₂, 697.3; found, 697.3.

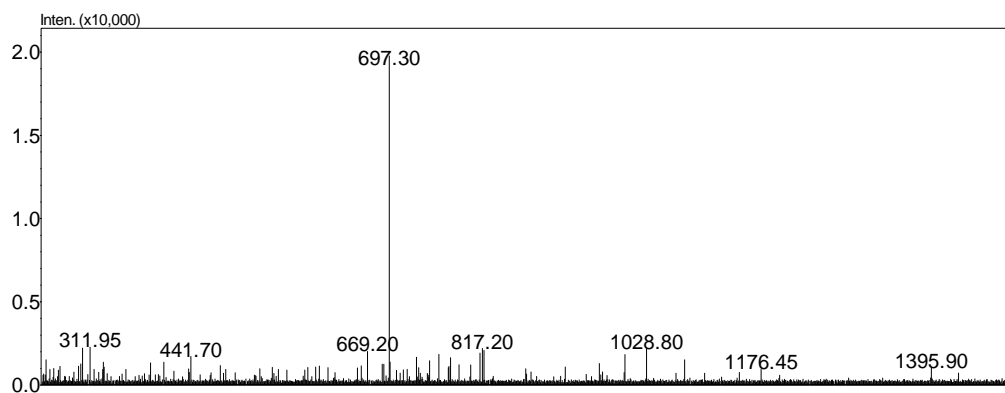


Figure S5. Determination of synthetic RU-PP-lipid with mass spec

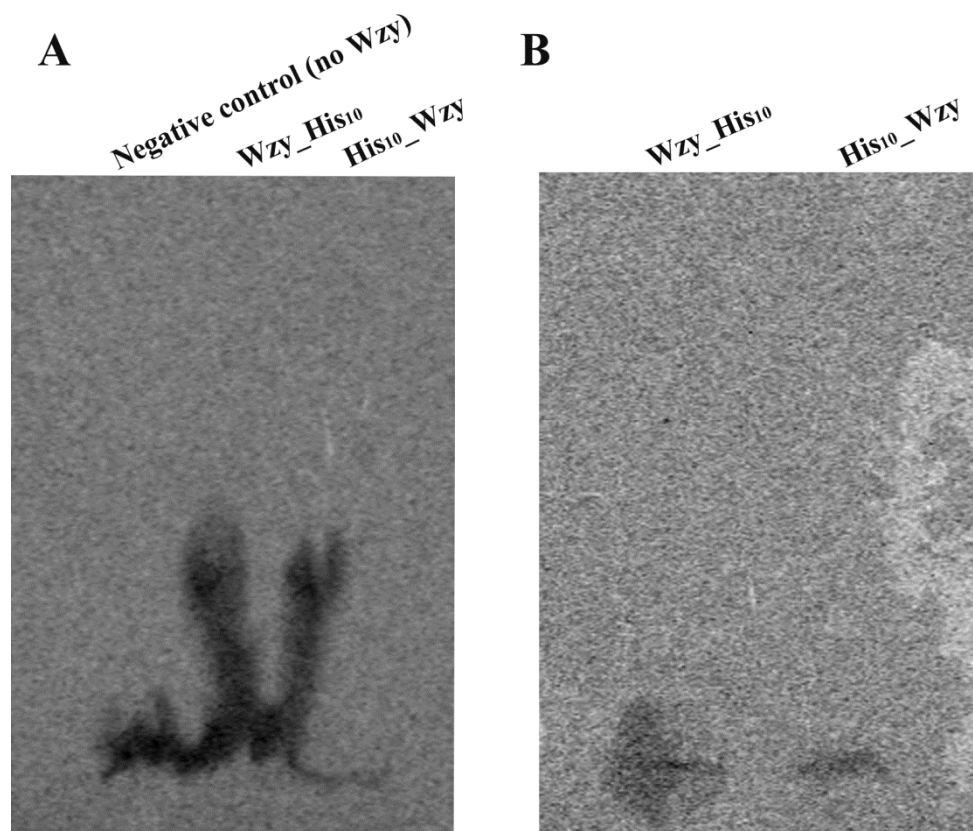
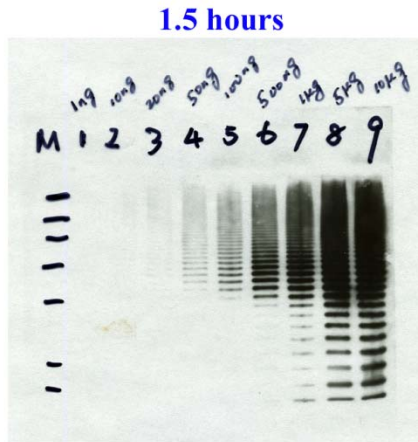
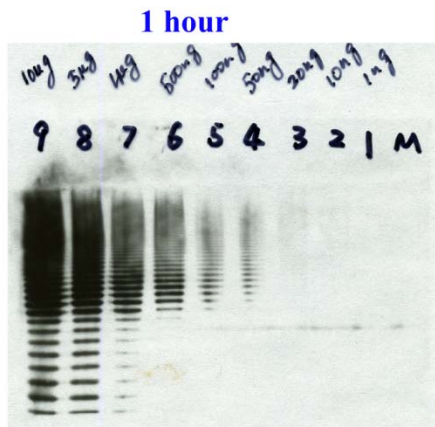


Figure S6. Determination of Wzy activity using radio-labelled substrate. A. cis-pentaprenol-PP linked RU can be polymerized by Wzy; (B) MS-pentaprenol-PP linked RU could not be polymerized by Wzy.

A Wet Membrane transferring System



B Dry Membrane transferring system

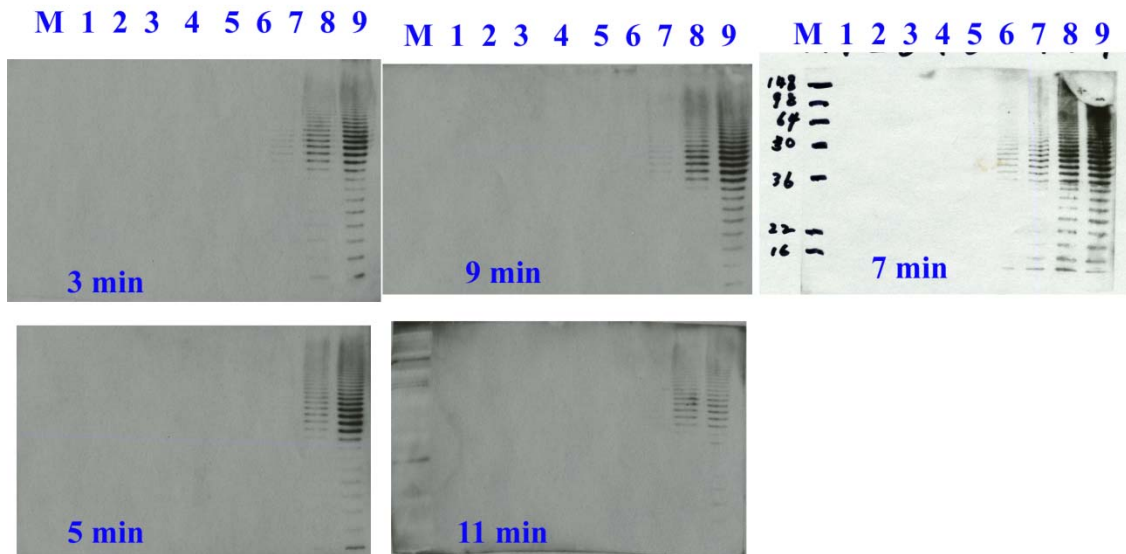


Figure S7. The detect limit comparison of two commonly used Western blot system. The results show that wet transfer is ten times higher on efficiency than semi-dry transfer. It further shows that transfer time has little effect on the results, especially for the dry transfer. However, longer time (1.5 hours vs 1 hour) can improve the wet transfer efficiency to some extent.

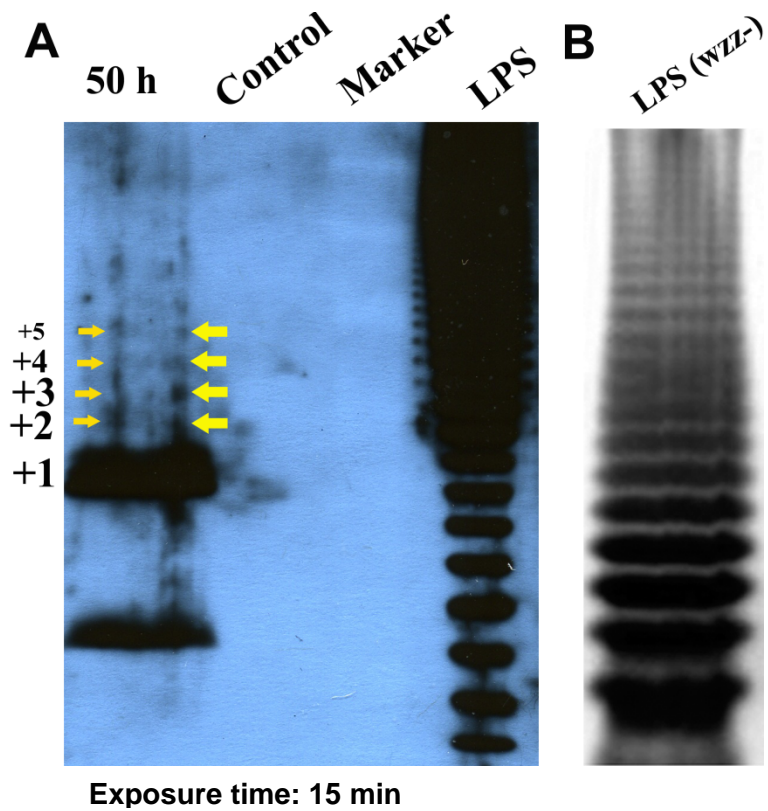


Figure S8. (A). The Wzy reaction after 50 hours. Some low Mw bands form with very low concentration; (B) LPS from *E. coli* O86 wzz KO strain. It seems that the product accumulated on the both sides of the lane as labeled by yellow arrows.

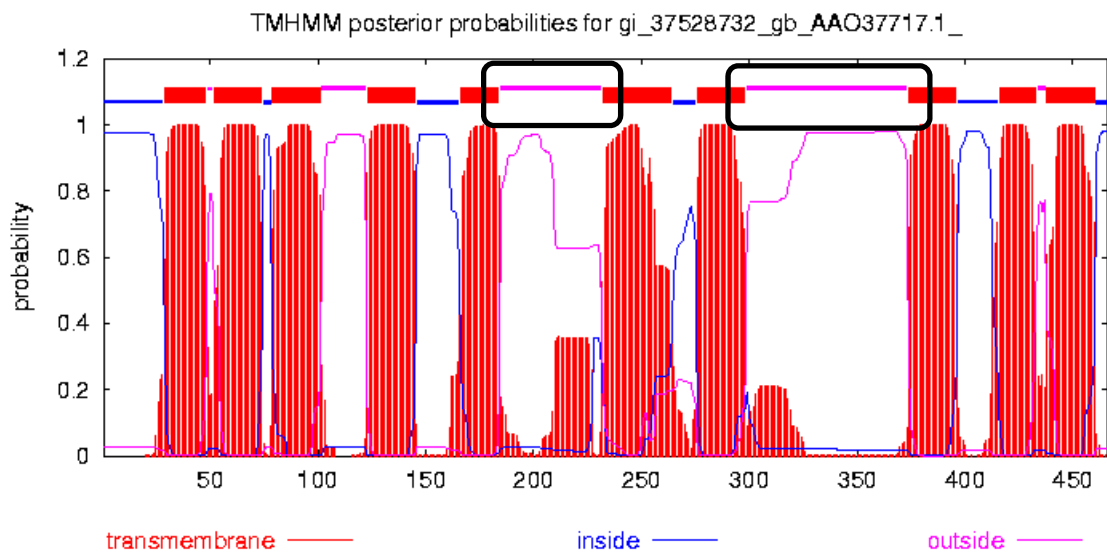


Figure S9 The Wzy secondary structure. The largest two loops are highlighted in black boxes, which are located in the periplasmic domain (shown as outside) and suggested to be essential in Wzy function.

Table S1. The detergents used in membrane protein solubilization screening

Ionic detergents	non-ionic detergetns	Zwitterionic detergents	synthetic lipids
Sodium cholate	DDM	FOS-CHOLINE-13	DMPC
n-Dodecyl-N,N-dimethylglycine	DM	FOS-CHOLINE-14	DMPG
n-Decyl-N,N-dimethylglycine	b-OG	FOS-CHOLINE-15	
Sodium dodecanoyl sarcosine	UM	FOS-CHOLINE-16	
Deoxycholic acid (sodium salt)	CYMAL-5	CHAPS	
	C ₈ E ₄	CHAPSO	
	C ₈ E ₆		
	C ₁₀ E ₅		
	C ₁₂ E ₈		