Supplementary Information

Visualizing Conformation Transitions of the Lipid II Flippase MurJ

Kuk et al.

12 Supplementary figures

4 Supplementary tables



Supplementary Figure 1. Crystal packing and electron density in the MurJ_{TA} structures. a. Crystals were obtained in lipidic cubic phase doped with Lipid II. Crystal contacts are mostly formed by end-to-end stacking, as well as lateral interactions mediated by the host lipid monoolein. TM 1 was not involved in crystal packing and thus its bending is unlikely to be a crystallographic artifact. The asymmetric unit is colored, while symmetry mates are shown in gray. b. 2Fo – Fc composite omit electron density maps (contoured to 1 σ), omitting 5% of the model at a time. Resolutions are (from left) 3.2 Å, 3.0 Å, 2.6 Å, and 1.8 Å.

				R18	G21 R24	D	39	R52 E57 G5	58 S61	
			Т	51/**				* *•		
Mar 7 (03)			MATTRACT							
MurJ-TA MurJ-EC	1		-MSILFSSIL	VSSMTMFSRV	LGFARDATVA	RIFGAGMATD	AFFVAFKLPN	LLRRIFAEGA	FSOAFVPLIS	69 69
MurJ-PF	1		-MNLLKSLAA	VSSITML <mark>SR</mark> V	LGFVRDTLIA	RIFGAGMATD	AFFIAFKLPN	LL <mark>R</mark> RIFA <mark>EG</mark> A	FSQAFVPILA	69
MurJ-VC	1	MFEVTV	SKRLLKSGII	VSAMTLI <mark>SR</mark> V	L <mark>GLVR</mark> DVVVA	NLM <mark>G</mark> AGASAD	VFFF <mark>A</mark> NRIPN	FL <mark>R</mark> RLFA <mark>EG</mark> A	FSQA <mark>FVP</mark> VLT	76
MurJ-LI	1		MSNAASRSIA	LSFYTFL <mark>SR</mark> I	L <mark>GLLR</mark> DHFMA	VSF <mark>G</mark> TGMVAS	AFSV <mark>A</mark> YRLPN	MF <mark>RNLLAEG</mark> T	LSQS <mark>FLPLY</mark> A	70
MurJ-RS	1	MK	PISLARGFLT	VGGWTLLSRG	AGFARDVMMA	AYLGAGPVAE	AFLVAFSLPN	MERREFAEGA	FNMAFVPMFA	72
NorM-NG	1	-LDRFSFSVF	LKEIRLLTAL	ALPMILLAOVA	OVGIGEVDTV	MAGGAGKEDL	AAVGLEEPVE	ATVYITFMGI	MAALNPMIAO	79
				-	-				-	
			TI	M 3a		1 3b		TM 4a		
MurJ-TA	70	EKSGEE	KDKFLSSVIN	GFSLIILALV	ILSYFFPELI	INLFGAG	SSHE	TKILAKKLLL	ITSPSIYFIF	136
MurJ-EC	70	EYKSKQGEDA	TRVFVSYVSG	LLTLALAVVT	VAGMLAAPWV	IMVTAPG	FADTAD	KFALTSQLLK	ITFPYILLIS	142
MurJ-PF	70	EYKSQKGDEA	TRTFIAYVTG	LLTLVLALVT	AAGMLAAPWV	IWATAPG	FTDTPE	KFQLTSDLLR	VTFPYILLIS	142
MurJ-VC	77	EYHASGDINK	TRDLIARASG	TLGVLVTIVT	LIGVLGSGAV	TALFGAGWFL	DWLNGGPAAG	KFELASLLLK	ITFPYLWFIT	156
MurJ-RS	73	KKLEGHED	AKAFARDAFS	GLAGILVVFT	LIGTLIMPWI.	VLAMASG	FAGDA	REDLAVEEGR	TAFSYTFFTS	142
PfMATE	80	RRIGARDKEG	ADNVAVHSLI	LSLILGVTIT	ITMLPAIDSL	FR	SMGAE	AVELAIEYAR	VLLAGAFIIV	146
NorM-NG	80	LYGAGKT-GE	AGETGRQGIW	FGLILGIFGM	ILMWA-AITP	FRNWLTL	SDYVE	GTMAQYMLFT	SLAMPAAMVH	149
		L145 N	146 F151 F	P158 N162						
		TM 4h		TM 5			TM	6		
Mun T ma	107	TWATEVET		TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	TOWEL OWNY	CT 707	TOPT TOPT	MERCTINGT	FURYY	0.05
MurJ-EC	143/ 143	LASLVGATIN	TWNRESTPAF	APTILNISMI	GFALFAAPYF	-NPDVLAL	AWAVTVGCVL	OLVYOLPHLK	KIGMLVLP	∠05 217
MurJ-PF	143	LSSLAGAILN	TWNRESVPAF	VPTLLNVSMI	VFAVELTPYE	-DPPVMAL	GWAVLVGGLA	QLLYQLPHLK	KIGMLVLP	217
MurJ-VC	157	FVALSGAI <mark>LN</mark>	TLG <mark>KF</mark> AVSSF	TPVFL <mark>N</mark> VMMI	LCAWYLSPNL	-EQPEVGL	AIGVFLG <mark>G</mark> LV	Q FLFQLPFLI	KAGVLVRP	231
MurJ-LI	140	lsaifmai <mark>sn</mark>	SKNRFFVPSL	SPIIL <mark>N</mark> LCYL	FVFICLFPFV	DDLHDRVIVL	CFAIITG <mark>G</mark> FL	Ö LAAÖIMÄAM	KNKDMPKI	217
MurJ-RS	143	LVALLSGVLN	AFGRETEASF	VPVLMNLMFI	AAMLIADRQG	-WDMGLTL	AWTVPVTGVA	QFLFTWFAAR	RLGFTLWP	217
NOTM-NG	14/	PATHAYASSI.	-NRPRI.TMLV	SFAAFVI.NVP	LNYIEVYCKE	GMPALGGAGC	GVATMAVEWE	SALALWIYIA	KEKFFRPFGI.	223
NOTH NO	100	Tumminoon	Mid Idlind,	brinn vinivi	LIGHT VIOL	ommoonioo	Q251	SILLIUWIIII	iditi fiti fob	220
					D235	G24	S248 R2	55 F256 ** •P260)	
			TM	7a –	TM 7b		- TM 8		TM8c	
Mur T_ 17 A	205		UEDUET KI PE		TNEWNOMNW	SEVDK	CSTEVIOVAS	PEVII DVCIE	AVEVENUUT	274
MULD-IN	205	FIIK	HEFHE BABEE	FIFMINV	TIVI A ADDIMA A	BEIDK	GOTOIDGINO	KETEDE IGHE	TAPASTA TP	2/4
MurJ-EC	217	-RINFHDA	GAMRVVKOMG	PAILGVSVSO	ISLIINTIFA	SFLAS	GSVSWMYYAD	RLMEFPSGVL	GVALGTILL P	289
MurJ-EC MurJ-PF	217 217	-RINFHDA -RLNLRDS	GAMRVVKQMG GVWRVMKQML	PAILGVSVS <mark>Q</mark> PAILGVSVS <mark>Q</mark>	ISLIINTIFA ISLIINTIFA	SFLAS SFLVA	GSVSWMYYAD GSVSWMYYAD	RLMEFPSGVL RLMELPSGVL	GVALGTILL <mark>P</mark> GVALGTILL <mark>P</mark>	289 289
MurJ-EC MurJ-PF MurJ-VC	217 217 231	-RINFHDA -RLNLRDS -KWGWKDP	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI	PAILGVSVS <mark>Q</mark> PAILGVSVSQ PALFGVSVS <mark>Q</mark>	ISLIINTIFA ISLIINTIFA INLLFDSFVA	SFLAS SFLVA SFLQT	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF	GVALGTILL <mark>P</mark> GVALGTILL <mark>P</mark> GIAIATVIL <mark>P</mark>	289 289 303
MurJ-EC MurJ-PF MurJ-VC MurJ-LI	217 217 231 217	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML	PAILGVSVSQ PAILGVSVSQ PALFGVSVSQ PAALGGGFYQ	ISLIINTIFA ISLIINTIFA INLLFDSFVA LSLLVDIFLA	SFLAS SFLVA SFLQT NWVQNQNPGL	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF RLVQLPTGII	GVALGTILLP GVALGTILLP GIAIATVILP GVALATTILP	289 289 303 294
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE	217 217 231 217 217 217	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSBEI	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP	PAILGVSVSQ PAILGVSVSQ PALFGVSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI	ISLIINTIFA ISLIINTIFA INLLFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAT	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV BITMLGI-VP	GVALGTILLP GVALGTILLP GIAIATVILP GVALATTILP GIAIGTVLLP ILCMAAATTS	289 289 303 294 288 297
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG	217 217 231 217 217 224 229	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLMI DLKRLAVIAA LKDILRVGLP VFKQIWKIGA	PAILGVSVSQ PAILGVSVSQ PALFGVSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA	ISLIINTIFA ISLIINTIFA INLLFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE APFGE	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI	GVALGTILLP GVALGTILLP GIAIATVILP GVALATTILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV	289 289 303 294 288 297 302
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG	217 217 231 217 217 224 229	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA	PAILGVSVSO PAILGVSVSO PALFGVSVSO PAALGGGFYO PAVLAGGVVO SSLSQLSMSI PIGLSYFLEA	ISLIINTIFA ISLIINTIFA INLLFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE APFGE	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI	GVALGTILLP GVALGTILLP GIAIATVILP GVALATTILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340	289 289 303 294 288 297 302
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG	217 217 231 217 217 224 229	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLMI DLKRLAVIAA LKDILRVGLP VFKQIWKIGA	PAILGVSVSQ PAILGVSVSQ PALGVSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA TM 9a	ISLIINTIFA ISLIINTIFA INILFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300	SFLAS SFLVA SFLQT NWQNQNPGL SFTE TAGGE APFGE	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF RLVQLPTGII RLVQLPLGVV RITMLGI-VP SLSGILY-MI	GVALGTILLP GVALGTILLP GIAIATVILP GIAIATVILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b	289 289 303 294 288 297 302
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA	217 217 231 217 217 224 229 275	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLMI DLKRLAVIAA LKDILRVGLP VFKQIWKIGA	PAILGVSVSQ PAILGVSVSQ PALGVSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA TM 9a ALKTTLFFTI	ISLIINTIFA ISLIINTIFA INLLFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 ESMVGLIFLS	SFLAS SFLVA SFLQT NWQNQNPGL SFTE TAGGE APFGE TM 9D TPIIRFFYEH	GSVSWMYYAD GSVSWMYYAD GSISWLYSD GAVVSLDYSQ GAVAVISYAD NGVAVFTSAW DYVAAQQVGI	RLMEFPSGVL RLMELPSGVL RLLEFPLGLF RLVQLPTGII RLVQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL	GVALGTILLP GVALGTILLP GIAIATVILP GIAIATVILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST	289 289 303 294 288 297 302 349
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC	217 217 231 217 217 224 229 275 290	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA	PAILGVSVSQ PAILGVSVSQ PALGGSVSQ PAALGGGFYQ PAVLAGGVYQ SSLSQLSMSI PIGLSYFLEA TM 9a ALKTTLFFTI GLRLCFLLAL	ISLIINTIFA ISLIINTIFA INILFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 PSMVGLIFLS PSAVALGILS	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE APFGE APFGE TM 9D TPIIRFFYEH GPLTVSLFQY	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD DYVAAQQVGI CAFTSKDTLI GAFTSKDTLI GRFTAFDALM	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV	GVALGTILLP GVALGTILLP GIAIATVILP GVALATTILP GIAIGTVLLP PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV	289 289 303 294 288 297 302 349 369
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-TA MurJ-EC MurJ-FZ	217 217 231 217 217 224 229 275 290 290	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA	PAILGVSVSQ PAILGVSVSQ PALGGSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA TM 9a ALKTTLFFTI GLRLCFLAL GLRLCFVLVL	ISLIINTIFA ISLIINTIFA INILFOSFVA LSLLVDIFLA VNILVGRQVA AMFFLNSVAI \$AF\$FIVFLI •P300 PSMVGLIFLS PSAVALGILS PCALALGILA	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE APFGE APFGE TPTIRFFYEH GPLTVSLFQY EPLTVSLFQY	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV TQRALIAYSV TQRALIAYSV	GVALGTILLP GVALGTILLP GTAIATVILP GVALATTILP GTAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLGIIVIKV	289 289 303 294 288 297 302 349 369 369
MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC MurJ-PF MurJ-VC MurJ-LI	217 217 231 217 217 224 229 275 290 290 304 295	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP IRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLOSLKKEE	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSTHOELAG	PAILGVSVSQ PAILGVSVSQ PALGCSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA TM 9a ALKTTLFFTI GLRLCFLIAL GLRLCFVLVI GLRMVTFLGI ALEFALFLTV	ISLIINTIFA ISLIINTIFA INILFOSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 •P30 •P3	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE APFGE TPTIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY KPMLMVLFMR CPTLDSTYFG	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI GATTSKDTLI GRFTAFDALM GQFNAFDALM GEFTPSDVG GKUDHIATHT	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI TSKILIAYTL TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATOPLVFYST	GVALGTILLP GVALGTILLP GVALATTILP GVALATTILP GTAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLSFMLIKV ALPFFSINKI	289 289 303 294 288 297 302 349 369 369 383 374
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS	217 217 231 217 224 229 275 290 290 304 295 289	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DLSRRLRAGD	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR	PAILGVSVSQ PAILGVSVSQ PALGCSVSQ PALGCGVSQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA MLKTTLFFTI GLRLCFLIAL GLRLCFLIAL GLRLCFVLVL GIRMVTFLGI ALEFALFLTV GTEFALMLTV	ISLIINTIFA ISLIINTIFA INLLFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 PSNVGLIFLS PSAVALGILS PCALALGILA PAALGMVIA PAALGMTFLA PAAVALVVIA	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE APFGE TPIIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY KPMLMVLFMR GPILDSIYFG LPLTQVLFQR	GSVSWMYYAD GSVSWMYYAD GSISWIYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI GAFTSKDTLI GRFTAFDALM GQFNAFDALM GQFNAFDALM GEFTPSDVEQ GKWDHIATHT GAFGPEDAWN	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI TSKILIAYTL TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATQPLVFYSI TALALGAYGL	GVALGTILLP GVALGTILLP GUALATTILP GUALATTILP GUALGTVLLP ILGMAAATTS POSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLSFMLIKV AIPFFSINKI GLPAFVLHKV	289 289 294 288 297 302 349 369 369 383 374 368
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC MurJ-PF MurJ-VC MurJ-RS PfMATE	217 217 231 217 224 229 275 290 290 304 295 289 298	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DLSRRLRAGD VTGAAYGERN	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR VEKLETAYLY	PAILGVSVSQ PAILGVSVSQ PALGGGVSQ PALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA ALKTTLFFTI GLRLCFLIAL GIRLCFLIAL GIRLCFVLVL GIRMVTFLGI ALEFALFLTV GTEFALMLTV AIKIAFMIEL	ISLIINTIFA ISLIINTIFA INLIFOSFVA LSLIVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 PSAVGLIFLS PSAVALGILS PCALALGILA PAALGMTFLA PAAVALVVIA AVVAFIMLFA	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE APFGE TPIIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY KPMLMVLFMR GPILDSIYFQ LPLTQVLFQR PQVAYLFTAQ	GSVSWMYYAD GSISWLYYAD GAIVSLDYSQ GAVVSLDYSQ GAVVSLDYSQ DYVAAQQVGI GATSKDTLI GKFTAFDALM GQFNAFDALM GETPSDVEQ GKWDHIATHT GAFGPEDAWN VIKGD	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI TSKILIAYTE TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATQPLVFYSI TALALGAYGL LISALRTLPV	GVALGTILLP GVALGTILLP GVALATTILP GVALATTILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLICLIVVKV GLLGIIVIKV GLLGIIVIKV GLLSFMLIKV FLVLTPFGMM	289 289 303 294 288 297 302 349 369 369 383 374 368 372
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC MurJ-PF MurJ-VC MurJ-RS PfMATE NorM-NG	217 217 231 217 224 229 275 290 290 304 295 289 298 303	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DLSRRLRAGD VTGAAYGERN RIGFSLGRRE	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI STRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR VEKLETAYLY FSRARYISGV	PAILGVSVSQ PAILGVSVSQ PALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA ALKTTLFFTI GLRLCFLIAL GLRLCFLIAL GLRLCFLIAL GLRLCFLIAL GLRLCFLIAL GLRMVTFLGI ALEFALFLTV GTEFALFLTV AIKIAFMIEL SLVSGWVLAV	ISLIINTIFA ISLIINTIFA INLIFDSFVA LSLIVDIFLA VNLLVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 PSAVGLIFLS PSAVALGILS PCALALGILA PAALGMTFLA PAALGMTFLA AVVAFIMLFA ITVLSLVLFR	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE TAGGE APFGE TPIIRFFYEH GPLTVSLFQY EPLTVSLFQY KPMI,MVLFMR GPILDSIYFG LPLTQVLFQR PQVAYLFTAQ SPLASMYNDD	GSVSWMYYAD GSISWLYYAD GAIVSLDYSQ GAVVSLDYSQ GAVVSLDYSQ DYVAAQQVGI GATSKDTLI GKFTAFDALM GQFNAFDALM GETPSDVEQ GKWDHIATHT GAFGPEDAWN VIKGD PAVLSI	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATQPLVFYSI TALALGAYGL LISALRTLPV ASTVLLFAGL	GVALGTILLP GVALGTILLP GIAIATVILP GVALATTILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 CLIPYGIYST GLICLIVVKV GLLGIIVIKV GLLGIIVIKV GLLSFMLIKV FLVLFFFSMM FQPADFTQCI	289 289 303 294 288 297 302 349 369 369 369 383 374 368 372 378
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-EC MurJ-EC MurJ-PF MurJ-CR PfMATE NorM-NG (K3	217 217 231 217 224 229 275 290 290 304 295 289 298 303 668 in Me	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DLSRRLRAGD VTGAAYGERN RIGFSLGRRE µJ-EC) 2352 H355	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR VEKLETAYLY FSRARYISGV	PAILGVSVSQ PAILGVSVSQ PALGGGVSVSQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA ALKTTLFFTI GLRLCFLIAL GIRLCFLIAL GIRLCFVLVL GIRMVTFLGI ALEFALFILTV GTEFALMLTV AIKIAFMIEL SLVSGWVLAV N374 D3	ISLIINTIFA ISLIINTIFA INILFDSFVA LSLLVDIFLA VNLLVGRQVA AMFFLNSVAI •P300 ESAVGLIFLS ESAVALGILS PCALALGILA PAALGMVIA PAALGMVIA AVVAFIMLFA ITVLSLVLFR	SFLAS SFLVA SFLQT NWVQNQNFGL SFTE TAGGE APFGE TPIIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY GPILDSIYFG LPLTQVLFQR PQVAYLFTAQ SPLASMYNDD (S413) V390 T39	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI GAFTSKDTLI GRFTAFDALM GQFNAFDALM GETPSDVEQ GKWDHIATHT GAFCPEDAWN VIKGD PAVLSI Bin MurJ-EC)	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATQPLVFYSI TALALGAYGL LISALRTLPV ASTVLLFAGL	GVALGTILLP GVALGTILLP GUALATTILP GUALATTILP GUALGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLGIIVIKV GLLSFMLIKV FLVLSFMLIKV FLVLTPFGMM FQPADFTQCI	289 289 294 288 297 302 349 369 369 369 369 374 368 372 378
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG (K3	217 217 217 217 224 229 275 290 290 304 295 289 295 289 298 303 68 in Mu	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP IRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DLSRRLRAGD VTGAAYGERN RIGFSLGRRE #J-EC) 352 H355	GAMRVVKQMG GVWRVMKQMI GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR VEKLETAYLY FSRARYISGV	PAILGVSVSQ PAILGVSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA MIKTTLFFTI GLRLCFLIAL GLRLCFVLVI GIRMVTFLGI ALEFALFLTV GTEFALMLTV AIKIAFMIEL SLVSGWVLAV N374 D3	ISLIINTIFA ISLIINTIFA INILFDSFVA LSLLVDIFLA VNILVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 SMVGLIFLS PSAVALGILS PCALALGILS PCALALGILS PCALALGILS PAALGMVLA PAALGMFLA PAALGMFLA AVVAFIMLFA ITVLSLVLFR	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE APFGE APFGE TDTIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY SPLASMYNDD (S413 V390 T39 A A	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI GATTSKDTLI GKFTAFDALM GQFNAFDALM GQFNAFDALM GEFTPSDVG GKNDHIATHT GAFGPEDAWN VIKGD PAVLSI 3 in MurJ-EC) 4	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATQPLVFYSI TALALGAYGL LISALRTLPV ASTVLLFAGL	GVALGTILLP GVALGTILLP GVALATTILP GVALATTILP GIAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLSFMLIKV GLLSFMLIKV GLPFYSINKI GLPAFYLHKV FLVLTPFGMM FQPADFTQCI	289 289 303 294 288 297 302 369 369 369 383 374 368 372 378
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-EC MurJ-PF MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG (K3	217 217 217 217 217 224 229 290 290 304 295 289 303 68 in M	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DISBRLRAGD VTGAAYGERN RIGFSLGRRE #J-EC) 352 H355	GAMRVVKQMG GVWRVMKQML GVVKIRTLMI SIRKIFKLML DLKRLAVIAA LKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR VEKLETAYLY FSRARYISGV	PAILGVSVSQ PAILGVSVSQ PAILGGSVSQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA ALKTTLFFTI GLRLCFVLVL GIRMVTFLGI ALEFALMLTV AIKIAFMIEL SLVSGWVLAV N374 D3 A A TM 11	ISLIINTIFA ISLIINTIFA INILFDSFVA LSLLVDIFLA VNILVGRQVA AMFFLNSVAI SAFSFIVFLI •P300 PSMVGLIFLS PSAVALGILS PCALALGILA PAMLGMVLA PAALGMTFLA PAAVALVVIA AVVAFIMLFA ITVLSLVLFR	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE APFGE TAGGE TPTIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY KPMLMVLFMR GPTLDSLYFG LPLTQVLFQR PQVAYLFTAQ SPLASMYNDD V390 T39 A A	GSVSWMYYAD GSVSWMYYAD GSISWLYYSD GAVVSLDYSQ GAVAWLSYAD DYVAAQQVGI CATSKDTLI GKFTAFDALM GQFNAFDALM GQFNAFDALM GQFNAFDALM GPFYSDVEQ GKNDHIATHT GAFGPEDAWN VIKGD PAVLSI 3 in MurJ-EC) 4 TM 12	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV TQRALIAYSV ASYSLLAYSS ATQPLVFYSI TALALGAYGL LISALRTLPV ASTVLLFAGL	GVALGTILLP GVALGTILLP GVALATTILP GVALATTILP GTAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLSFMLIKV AIPFFSINKI GLPAFVLHKV FLVLTPFGMM FQPADFTQCI	289 289 294 288 294 288 302 369 369 369 383 374 368 372 378
MurJ-EC MurJ-PC MurJ-VC MurJ-LI MurJ-RS PfMATE NorM-NG MurJ-TA MurJ-CC MurJ-CC MurJ-LI MurJ-RS PfMATE NorM-NG (K3 MurJ-TA	217 217 211 217 224 229 275 290 304 295 289 298 304 295 289 298 304 508 in Mt F	-RINFHDA -RLNLRDS -KWGWKDP -NWNWKHP -HLPRLTP LRDFSPSREI TAKFGKPDWA KISNDRKN SLSKSFASGN TLAKTYASQD ALSRKHVDAH ALLQSLKKEE DLSRRLRAGD VTCAAYGERN RIGFSLGRRE #J-EC) 352 H355	GAMRVVKQMG GVWRVMKQMI GVVKIRTLMI SIRKIFKLML DLKRLAVIAA IKDILRVGLP VFKQIWKIGA FNYHLND HDEYNRLMDW RHEYSRILDW SDGFAHTMDW SDGFAHTMDW WSSIHQELAG EAGSRASFNR VEKLETAYLY FSRARYISGV	PAILGVSVSQ PAILGVSVSQ PAILGVSVSQ PAALGGGFYQ PAVLAGGVVQ SSLSQLSMSI PIGLSYFLEA TM 9a ALKTTLFFTI GLRLCFVLVL GIRMVTFLGI ALEFALKTV AIKIAFMIEL SLVSGWVLAV N374 D3 A TM 11 VSLSNIILDI	ISLIINTIFA ISLIINTIFA INILFDSFVA LSLLVDIFLA VNILVGRQVA AMFFLNSVAI •P300 SMVGLIFLS PSAVALGILS PCALALGILS PCALALGILS PCALALGITA PAALGMTFLA PAALGMTFLA PAALGMTFLA 78 TFCLKYG	SFLAS SFLVA SFLQT NWVQNQNPGL SFTE APFGE APFGE TDIRFFYEH GPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY EPLTVSLFQY SPLASMYNDD SPLASMYNDD V390 T39 A A PIGVALATSI	GSVSWMYYAD GSVSWMYYAD GSISWIYYSD GAVVSLDYSQ GAVAWLSYAD NGVAVFTSAW DYVAAQQVGI CATTSKDTLI GKFTAFDALM GQFNAFDALM GQFNAFDALM GQFNAFDALM GQFNAFDALM GAFGPEDAWN VIKGD PAVLSI 3 in MurJ-EC) 4 CM 12 AGIIGVLYIL	RLMEFPSGVL RLMEFPSGVL RLLEFPLGLF RLVQLPTGII RLYQLPLGVV RITMLGI-VP SLSGILY-MI M 10a TSKILIAYTL TQRALIAYSV TQRALIAYSV TQRALIAYSV ATQPLVFYSI TALALGAYGL LISALRTLPV ASTVLLFAGL	GVALGTILLP GVALGTILLP GVALGTILLP GVALATTILP GTAIGTVLLP ILGMAAATTS PQSVGSAGTV •G340 TM 10b GLPFYGIYST GLIGLIVVKV GLLGIIVIKV GLLSFMLIKV GLASFMLIKV FLVLTPFGMM FQPADFTQCI	289 289 303 294 288 297 302 302 302 302 302 369 369 383 374 368 372 378
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Supplementary Figure 2. Alignment of MurJ sequences. Positions shaded in red are identical in >90% of Gram-negative MurJ sequences in an alignment with 36 sequences, while those shaded gray are similar in >70% of the sequences. Asterisks (*) denote essential residues in MurJ_{TA} of which alanine mutants fail to complement *E. coli* MurJ, as determined previously ²⁵. Carets (^) denote residues participating in Na⁺ coordination. Bullets (•) denote other residues discussed in this manuscript. MurJ_{TA} from *Thermosipho africanus* (UniProt ID: B7IE18) was aligned with 36 Gram-negative MurJ sequences from the UniRef50 library (no sequence is more than 50% identical to another). Drug exporters PfMATE and NorM-NG were also included in the alignment for contrast.

List of sequences shown: MurJ-TA, *Thermosipho africanus* (UniProt ID: B7IE18); MurJ-EC, *Escherichia coli* (P0AF16); MurJ-PF, *Pseudomonas fluorescens* (G8PWV6); MurJ-VC, *Vibrio cholerae* (O34238); MurJ-LI, *Leptospira interrogans* (A0A0C5X9A5); MurJ-RS, *Rhodobacter sphaeroides* (Q3J5H7); PfMATE (PDB ID: 3VVN); NorM-NG (4HUK).



Supplementary Figure 3. MurJ_{TA} assumes a predominantly inward-facing conformation in DDM micelles. a, Single cysteine mutants of MurJ_{TA} (no endogenous cysteines) were expressed, purified, delipidated, and probed for accessibility in DDM micelles by a direct labeling strategy in potassium acetate (absence of sodium). Accessible cysteines would be labeled by PEG5000-maleimide (PEG-Mal), resulting in a mass shift up the gel. Experiments were performed at either 20°C or 60°C. Source data are provided as a Source Data File. b, Mapping of these cysteine positions (for the 20°C data) to the inward-open and outward-facing crystal structures of MurJ_{TA}. Positions were considered inaccessible if only the lower band was observed, and accessible if the top band was observed and of higher intensity than the lower band. Remaining positions with both bands visible were considered partially accessible.



Supplementary Figure 4. Docking and MD simulation of Lipid II to the inward-occluded structure. a, Lipid II (yellow spheres) was docked to the inward-occluded structure by Autodock Vina. **b,** The docking result was used as a starting point for molecular dynamics simulation by NAMD in a hydrated and ionized (150 mM NaCl) POPE membrane bilayer system. The equilibrated system is shown, with lipids visualized as spheres and waters denoted by red dots. **c,** Cytoplasmic view of the docked complex (Lipid II shown as yellow sticks, charged residues in the cavity shown as gray sticks), showing how the Glu57-Arg352 thin gate could help occlude the substrate. **d,** Rotation of MurJ sidechain and Lipid II torsions in a molecular dynamics simulation starting from the docked model, visualizing the picosecond dynamics of the docked MurJ-Lipid II complex (snapshots at each 10 ps overlaid, total 100 ps). Surrounding lipids and waters are hidden for clarity.



Supplementary Figure 5. Complementation assay of MurJ_{TA} Asp25 and Arg352 mutants in *E. coli* NR1154. Cells transformed with plasmids encoding MurJ_{TA} (wild-type or mutant) or without insert (pEXT21) were depleted of endogenous *E. coli* MurJ by serial dilution on plates containing the anti-inducer D-fucose. MurJ_{TA} expression was induced by addition of IPTG. Data shown are representative of three biological replicates. Source data are provided as a Source Data File.



Supplementary Figure 6. Western blot analysis of MurJ_{TA} mutants. MurJ_{TA} proteins carrying a C-terminal FLAG tag were expressed by the addition of 0.1 mM IPTG in the presence of arabinose. Total membrane fractions were isolated by ultracentrifugation and probed by mouse anti-FLAG primary antibody and horseradish peroxidase-conjugated anti-mouse secondary antibody. Source data are provided as a Source Data File.



Supplementary Figure 7. Structural features of the putative lipid-binding site in the outward state. **a**, Putative lipid-binding site. Yellow sticks denote a model of the undecaprenyl tail of Lipid II, which was never used for structure refinement or map calculation. **b**, The lipid is restrained by the hydrophobic groove and a short tunnel near the periplasmic side. **c-d**, Unmodeled 2Fo - Fc electron density peaks (purple mesh, contoured to 0.7 σ) were observed in the hydrophobic groove (c), or in the tunnel (d) which is formed by hydrophobic residues Ile19, Leu22, Phe256, Leu259, and Phe316.



Supplementary Figure 8. Mapping of MurJ_{EC} cysteine accessibility data (Butler *et al*, 2013) to MurJ_{TA} structures. Positions accessible to the membrane impermeant reagent MTSES are shown as red spheres, while positions that are partially accessible are shown as yellow spheres. The structures of MurJ_{TA} are consistent with the cysteine accessibility data suggesting a central cavity that is accessible to the periplasm (in the outward-facing conformation).



Supplementary Figure 9. The Na⁺ site of MurJ_{TA} is conserved with that of Na⁺ coupled MATE transporters in the same superfamily. MurJ_{TA} is shown on the left, while *Pyrococcus furiosus* MATE (PfMATE) is shown on the right (PDB ID: 3VVO, Tanaka *et al.*, 2013; Ficici *et al.*, 2018), both with a DNDXT composition where X is a backbone carbonyl oxygen.



Supplementary Figure 10. The Na⁺ site of MurJ_{TA} is similar with that of MurJ_{EC}. MurJ_{TA} is shown on the left, while MurJ_{EC} is shown on the right (PDB ID: 6CC4, Zheng *et al.*, 2017). The putative sodium-coordinating oxygen atoms (black circles) are conserved.





Supplementary Figure 11. Mass spectrometry assay of cellular Lipid II accumulation. a, Growth curve of *E. coli* MurJ-depletion strain NR1154 expressing MurJ_{TA} wild-type (WT), mutants (R24A/R255A), or empty vector (pEXT21). Cultures were supplemented with D-fucose to deplete endogenous *E. coli* MurJ. At 3 hours post-inoculation, MurJ_{TA} expression was induced with IPTG, and growth was monitored by optical density (OD₆₀₀). **b**, At 6 hours post-inoculation, cells were harvested and cellular Lipid II levels were analyzed by mass spectrometry. Cellular Lipid II levels were normalized against either Lipid X, phosphatidylethanolamine (PE), or cell density (OD₆₀₀). Lipid X is an intermediate in lipopolysaccharide biosynthesis pathway that is separate from the peptidoglycan synthesis pathway, but has similar chemical properties as Lipid II. Normalized Lipid II level of each replicate was divided by that of the wild-type to obtain the fold change. Data are mean \pm s.d., n=3 biological replicates. Source data are provided as a Source Data File.



Supplementary Figure 12. Charged and polar residues in the central cavity of $MurJ_{TA}$ that were subjected to mutagenesis studies. Equivalent position in *E.coli* is in listed in parenthesis.

	Inward closed ^a	Inward open ^b	Inward occluded ^c	Outward ^d
	(6NC6)	(6NC7)	(6NC8)	(6NC9)
Data collection				
Space group	P 2 2 2 ₁	C 2	P 2 2 ₁ 2 ₁	C 2
Cell dimensions				
a, b, c (Å)	71.1, 101.8, 158.5	129.7, 105.5, 111.5	68.4, 80.3, 100.7	128.6, 57.4, 86.4
α, β, γ (°)	90, 90, 90	90, 125.3, 90	90, 90, 90	90, 100.7, 90
Resolution (Å)	85.6-3.2 (3.3-3.2) ^e	74.7-3.0 (3.1-3.0) ^e	68.4–2.6 (2.7–2.6) ^e	^e 84.9–1.8 (1.9–1.8) ^e
$R_{\rm pim}$	0.15 (0.94)	0.10 (0.83)	0.11 (>1.0)	0.076 (0.71)
$I/\sigma(I)$	9.48 (1.14)	11.41 (1.00)	13.29 (1.13)	22.71 (1.09)
Completeness (%)	99.4 (99.3)	99.0 (98.0)	99.8 (99.7)	99.9 (99.6)
Redundancy	20.7 (21.3)	5.5 (5.1)	48.8 (38.3)	23.4 (23.5)
Refinement				
Resolution (Å)	$85.6-3.2(3.3-3.2)^{e}$	74.7-3.0 (3.1-3.0) ^e	$68.4-2.6(2.7-2.6)^{e}$	^e 84.9–1.8 (1.9–1.8) ^e
No. reflections	17624 (1292)	20395 (1238)	17459 (1608)	51025 (3345)
$R_{\rm work}/R_{\rm free}$ (%)	25.5 / 28.0	25.4 / 27.8	22.9 / 25.8	17.9 / 19.9
No. atoms				
Protein	7292	7241	3672	3775
Ions/monoolein/PEG	27	126	125	294
Water	0	0	0	156
<i>B</i> factors ($Å^2$)				
Protein	31.18	46.54	48.19	20.55
Ions/monoolein/PEG	32.77	45.10	58.48	48.24
Water	N/A	N/A	N/A	28.35
R.m.s. deviations				
Bond lengths (Å)	0.005	0.005	0.003	0.003
Bond angles (°)	1.02	0.67	0.62	0.66

Supplementary Table 1. Data collection and refinement statistics (molecular replacement)

^a Merged from 3 crystals.
^b Merged from 2 crystals.
^c Merged from 7 crystals (2 collected at wavelength of 0.98 Å and 5 at 1.65 Å).
^d Merged from 6 crystals.
^e Values in parentheses are for highest-resolution shell.

		MurJ _{TA}			MurJ _{EC}		
Residues in TA	Residues in EC	Kuk et al., 2017, and this study	Butler et al., 2013, 2014		Zheng e	t al., 2018	Location
S17	S17	Α			L W	РТА	TM1
R18	R18	Α	ACEK	Р	C S L	GH	TM1
R24	R24	Α	ACEK	G	LQ	Р	TM1
D25	D25	A N	С	H Y	Ν	G V A E	TM1
R52	R52	Α	ACEK	С	S L P	н	TM2
E57	E57	Α	С		K	Q A V G D	TM2
N162	N168	Α		K*	I	D H Y S T	TM5
Q229	Q244	Α	С		L	EKPRH	TM7
N231	S246	Α			С	APTFY	TM7
D235	N250	A N L	С	K		DYHTIS	TM7
R255	R270	Α	ACEK	Р	C S L	н	TM8
R352	K368	A Q	С		ΕI	QTKN	TM10
N374	T394	A L		RK		PSAM	TM11
D378	N398	NLA				D H Y T S I K	TM11
T394	S413	C V A			Y	APTFC	TM12

Supplementary Table 2. Comparison of mutagenesis data for $MurJ_{TA}$ and $MurJ_{EC}$

*Nucleotide 504 C to G and to A mutations, although resulting in synonymous N168K mutation, one led to a fold change of 13 and the other 2. In this table, we choose 13 for categorization.

Table Legend:

Kuk <i>et al.</i> , 2017 and this study,	Butler et al., 2013 2014	Zheng <i>et al.</i> , 2018
No complementation	No complementation	Mutation frequency fold change >5
Partial complementation	Complementation but sensitive to low osmolarity	Mutation frequency fold change >2.5
Complementation	Complementation	Mutation frequency fold change <2.5

Structure	Na⁺ Fo — Fc omit peak height	\mathbf{Na}^+ coordination distance (in Ångstrom)					
		Asp235 OD1	Asn374 OD1	Asp378 OD1	Val390 O	Thr394 OG1	
Inward closed	7 sigma	2.49	2.32	2.45	2.39	2.35	
Inward occluded	9 sigma	2.44	2.43	2.40	2.40	2.41	
Outward	19 sigma	2.45	2.26	2.42	2.33	2.33	

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Supplementary Table 3. Sodium Fo – Fc omit peak heights and coordination distances

Primer name	Sequence (5' to 3')				
Sodium site					
MurJ_D235L_F	agccagatcaacactgtggtgctaatgaacgtggtttctttc				
MurJ_D235L_R	gtagaaagaaaccacgttcattagcaccacagtgttgatctggct				
MurJ_D235N_F	cagatcaacactgtggtgaacatgaacgtggtttctt				
MurJ_D235N_R	aagaaaccacgttcatgttcaccacagtgttgatctg				
MurJ_D378A_F	gcetgagcaatatcattetggctattatetttggcetgaaata				
MurJ_D378A_R	tatttcaggccaaagataatagccagaatgatattgctcaggc				
MurJ_D378L_F	gttagcctgagcaatatcattctgctaattatctttggcctgaaatacggc				
MurJ_D378L_R	gccgtatttcaggccaaagataattagcagaatgatattgctcaggctaac				
MurJ_D378N_F	ttagcctgagcaatatcattctgaatattatctttggcctgaaatac				
MurJ_D378N_R	gtatttcaggccaaagataatattcagaatgatattgctcaggctaa				
MurJ_N374L_F	cgcgaccattgttagcctgagcctaatcattctggatattatctttgg				
MurJ_N374L_R	ccaaagataatatccagaatgattaggctcaggctaacaatggtcgcg				
MurJ_T394A_F	gttgcgctggcggccagcattgcgg				
MurJ_T394A_R	ccgcaatgctggccgccagcgcaac				
MurJ_T394C_F	cgttgcgctggcgtgcagcattgcgggc				
MurJ_T394C_R	gcccgcaatgctgcacgccagcgcaacg				
MurJ_T394V_F	cgttgcgctggcggtcagcattgcgggc				
MurJ_T394V_R	gcccgcaatgctgaccgccagcgcaacg				
Other mutagenesis					
MurJ_D25A_F	cctgggtctgtttcgtgctgtactgttcgcaaaat				
MurJ_D25A_R	attttgcgaacagtacagcacgaaacagacccagg				
MurJ_D25N_F	gtatectgggtetgtttegtaatgtaetgttegeaaaatae				
MurJ_D25N_R	gtattttgcgaacagtacattacgaaacagacccaggatac				
MurJ_R352A_F	gcatctatagcaccattagcgctagctatcatgccatcaaaa				
MurJ_R352A_R	ttttgatggcatgatagctagcgctaatggtgctatagatgc				
MurJ_R352Q_F	ggcatctatagcaccattagccagagctatcatgccatcaaaaata				
MurJ_R352Q_R	tatttttgatggcatgatagctctggctaatggtgctatagatgcc				
Cysteine accessibility					
MurJ_A29C_F	gtctgtttcgtgatgtactgttctgtaaatacttcggtgtctcttacga				
MurJ_A29C_R	tcgtaagagacaccgaagtatttacagaacagtacatcacgaaacagac				
MurJ_I247C_F	etttetacgacaaaggtteetgetettatetgeaataegegt				
MurJ_I247C_R	acgcgtattgcagataagagcaggaacctttgtcgtagaaag				
MurJ_S113C_F	cctgttcggtgctggttgttctcatgaaactaag				
MurJ_S113C_R	ettagttteatgagaacaaceageaeegaacagg				
MurJ_S61C_F	cggtgagggtgcaatgtgttctgcatttgtacctc				
MurJ_S61C_R	gaggtacaaatgcagaacacattgcaccctcaccg				
MurJ_S69C_F	ctgcatttgtacctctgtactgtgaaaaatccggtg				
MurJ_S69C_R	caccggatttttcacagtacagaggtacaaatgcag				
MurJ_S161C_F	ggctctgactccgtctatctgtaacattaccatcattattg				
MurJ_S161C_R	caataatgatggtaatgttacagatagacggagtcagagcc				

Supplementary Table 4. List of primers used in this study

MurJ_S240C_F	gtggacatgaacgtggtttgtttctacgacaaaggttc
MurJ_S240C_R	gaacetttgtegtagaaacaaaceaegtteatgteeae
MurJ_S248C_F	cgacaaaggttccatctgttatctgcaatacgcgt
MurJ_S248C_R	acgcgtattgcagataacagatggaacctttgtcg
MurJ_S254C_F	tettatetgcaatacgcgtgccgtttetacetgc
MurJ_S254C_R	gcaggtagaaacggcacgcgtattgcagataaga
MurJ_S274C_F	tatccactgttgttctgtgcaaaatctccaacgatcg
MurJ_S274C_R	cgatcgttggagattttgcacagaacaacagtggata
MurJ_S277C_F	ctgttgttctgtccaaaatctgcaacgatcgtaaaaacttcaa
MurJ_S277C_R	ttgaagtttttacgatcgttgcagattttggacagaacaacag
MurJ_T232C_F	tggtcagccagatcaactgtgtgggggacatgaacg
MurJ_T232C_R	cgttcatgtccaccacacgttgatctggctgacca
MurJ_T270C_F	ctgtttgctgtatccgtatcctgtgttgttctgtccaaaatctc
MurJ_T270C_R	gagattttggacagaacaacacaggatacggatacagcaaacag
MurJ_V34C_F	gttcgcaaaatacttcggttgctcttacgagctggatgca
MurJ_V34C_R	tgcatccagctcgtaagagcaaccgaagtattttgcgaac