

Structure, Volume 29

Supplemental Information

**Structure-based modeling and dynamics of MurM,
a *Streptococcus pneumoniae* penicillin resistance
determinant present at the cytoplasmic membrane**

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Table S1. Summary of course grained simulations, Related to STAR Methods

System	Membrane size (nm)	Time (μ s)	Membrane % Composition		
			PE	PhG	CL
1	16.1x16.1	6x5	75	25	0
2	16.1x16.1	6x5	76	16	8
3	16.3x16.3	6x5	72	12	16

Table S2. Summary of atomistic simulations, Related to STAR Methods

CG label refers to the coarse grained system (Table S1) from which the simulations were constructed.

System	Time (μ s)	CG Label
4	2x0.25	1 r1
5	2x0.25	1 r2
6	2x0.25	2 r1
7	2x0.25	2 r6
8	2x0.25	3 r1
9	2x0.25	3 r4

W.vi_FemX	--MPVLNLNDPQAVERYEEFMRQSPYQVQTQDLGWAKVK--NNWEPVDVYLEDDQGAI IA	56
S.pn_MurM	MYRYQLG----IPLSEYDGFVKEHPMVNLLQSSAWEKV[red]--SD[red]NHERLGVYEGE-NLLA	53
S.au_FemA	--MKFTN----LTAKEFGAFTDSMPYSHFTQTQVGHYELKLAEGYETHLVGIKNN--NNEV	52
S.au_FemX	--MEKMH----ITNQEHDADFVKSHPNGDLLQLTKWAETKKL TGWYARRIAVGRDG-EVQG	53
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W.vi_FemX	AMSMLLGDTP--DKKFAYASKGPMVDVTDVLLDRLVDEAVKA-LDGRAYVLRFDPEVA	113
S.pn_MurM	VASILIKSLPL--GYKMFYIPRGPILDYRDTELLKFVQLSISYARSKRAVFTFDPSIC	111
S.au_FemA	IAACLLTAVPMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPYLP	112
S.au_FemX	VAQLLFKKVPKL-PYTLCYISRGFVVDYSNKEALNALLDSAKE IAKAEKAYAIKIDPDVE	112
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W.vi_FemX	YSDEFNT-----TLQDHGYVTRNRNVADAGMHATI QPRLNMVLDLTKFPDAKT	162
S.pn_MurM	LSQHLVNQDKREYPENLAIVEILGQLGVKWSGRTIEMDDTI QPRIQAKIYKENFEEDKL-	170
S.au_FemA	YQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKDKTADDI-	171
S.au_FemX	V-----DKGTD-ALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDEL-	159
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W.vi_FemX	LDLYPSKTKSKIKRPF RDGVEVHSGNSATELDEFFKTYTTMAERHGITHRPIEYFQRMQA	222
S.pn_MurM	----SKSTRQAIRTARNKGLEIQYGG-LELLDSFSELMKKTEKRKEIHL[red]RNEAY[red]YKLLD	225
S.au_FemA	IKNMDGLRKRNTKVKVKNVGRFSL-EEELPIFRSFMEDTSES KAFADRDDKFYFNRLK	230
S.au_FemX	LNSFERRNRSKVRLALKRGTTVERSD-REGLKTF AELMKITGERDGF LTRDISYFENIYD	218
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W.vi_FemX	AFDADTMRIF-----	232
S.pn_MurM	NFKEDSYITLTSL--DVSKRLRELEEQLEKNRVVAEKF-ND-----ATRSSKVQ	271
S.au_FemA	YYKDRVLVPLAYI--NFDEYIKELNEERDILNKDLNKALKDIEKR-PENKKAHNKRDNLQ	287
S.au_FemX	ALHEDGDAELFLVKLDPKENIAKVQELNELHAEIAKWQKMETSEKQAKKAQNMINDAQ	278
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W.vi_FemX	-----VAEREGKLLSTGIALKYGRKIWYMYAGSMDG-NTYYAPYA	271
S.pn_MurM	ENIKEKERLKEEIDFLQGYMMNGKSNIPLAATLSLEFGNTSVNLYAGMDDDFKRYNAPIL	331
S.au_FemA	QQLDANEQKIEEGKRLQ---EEHGNELPISAGFFFINPFVYVYAGGTSNAFRHFAGSYA	344
S.au_FemX	NKIAKNEDLKRDLLEALE---KEHPEGIYLSGALLMFAGSKSYLYGASSNEFRDFLPNHH	335
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W.vi_FemX	VQSEMIQWALDNTDLYDLGGIESESTD----DSL YVFKHVFVKDAPREYIGEIDKVLDP	327
S.pn_MurM	TWYETARYAFERGMVWQNLGGVENS----LNGGLYQFKEKFNPTIEEYLGEFTMPT-H	384
S.au_FemA	VQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKGYNA-EIIEYVGD FIKPINK	403
S.au_FemX	MQYTMK YAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGT-YLSEKIGEFDYILNQ	394
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W.vi_FemX	EVYAE LVK-----	336
S.pn_MurM	PLYPLRLALDFRKT LRRKHK	406
S.au_FemA	PVYAA YTALKKVKDRIF-----	420
S.au_FemX	PLYQLIEQ-----	402
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Figure S1. Sequence alignment showing putative MurM binding site residues, Related to STAR Methods and Figure 4. Alignment of *W. viridescens* FemX, *S. pneumoniae* MurM, *S. aureus* FemA and *S. aureus* FemX using CLUSTAL Omega (1.2.4). The sequence identity between MurM and *S. aureus* FemA, *S. aureus* FemX and *W. viridescens* FemX was 20.25 %, 26.93 % and 24.38 % respectively. Residues of the putative MurM binding site, proposed to interact with the Lipid II substrate are indicated by red boxes.

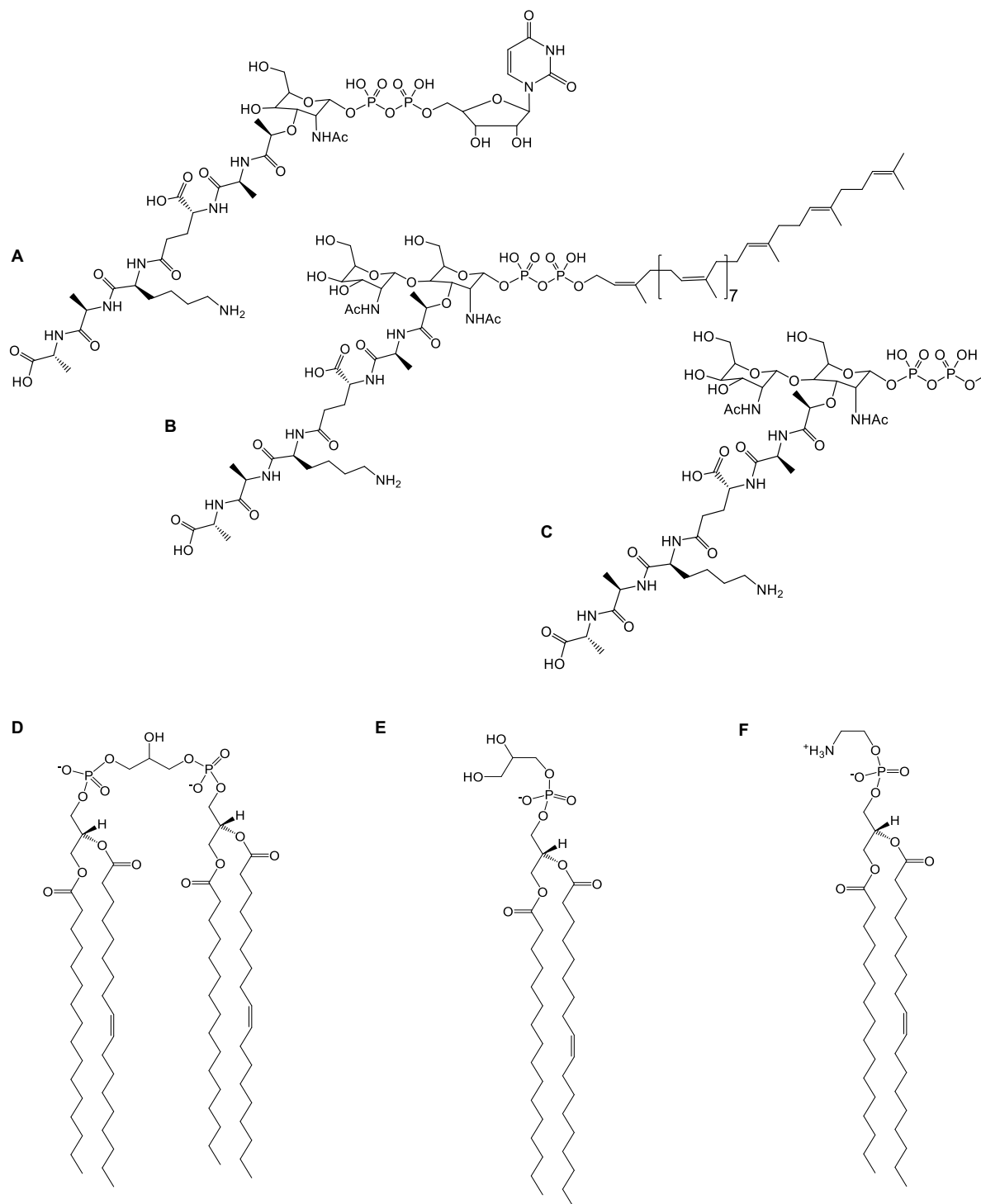


Figure S2. Structures of lipids investigated in these studies, Related to Figure 3 and Figure 6. A) UDP-MurNAc-pentapeptide (Lysine variant), B) Lipid II, C) Truncated Lipid II structure where the C55 prenyl chain has been replaced with a methyl group, D) cardiolipin, E) phosphatidyl-glycerol and F) phosphotidylethanolamine. All Lipid II precursors and variants contain L-Lysine at the third position of the pentapeptide chain. Structures produced in ChemDraw (Version 19.1).

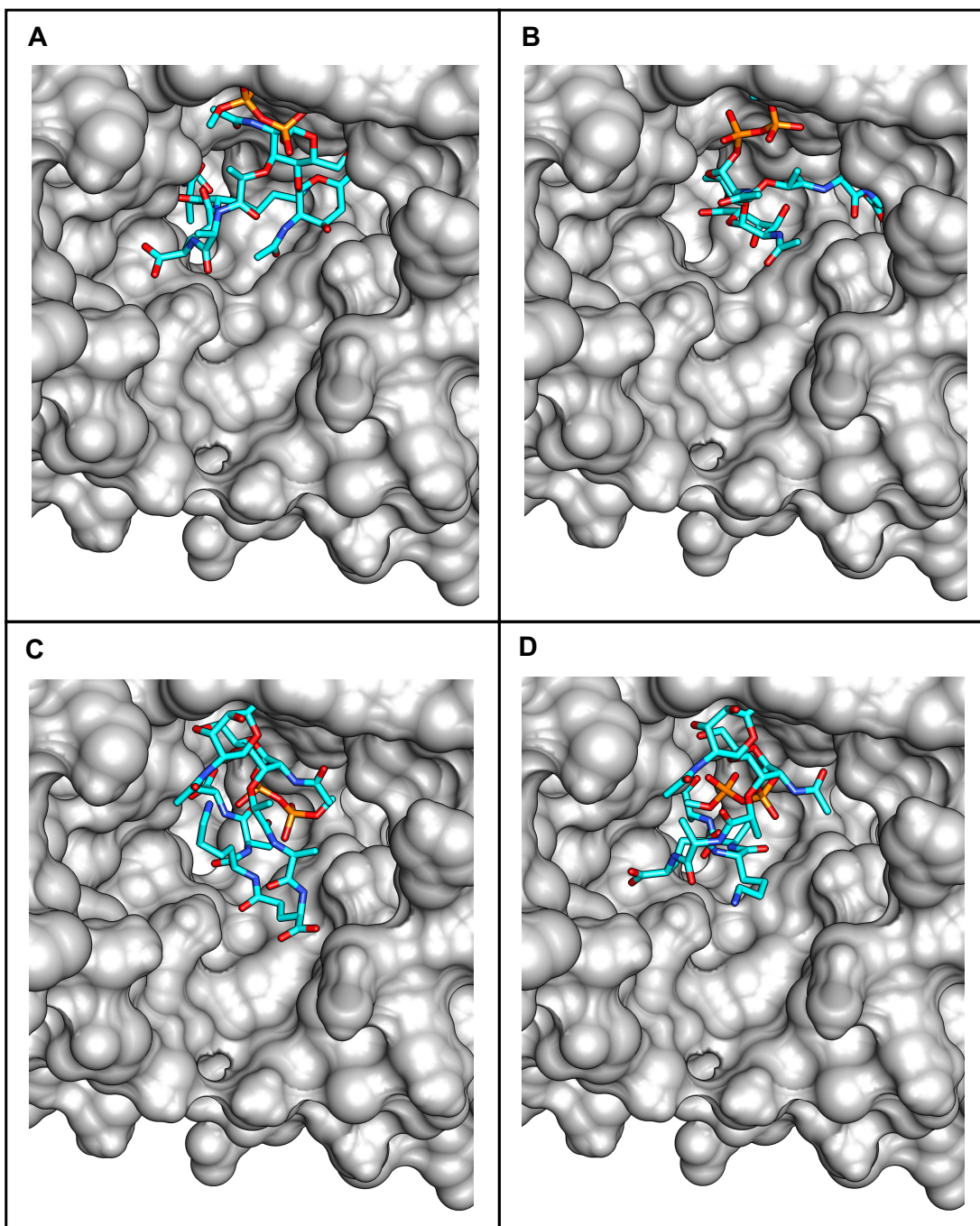


Figure S3. The remaining four highest scoring poses from molecular docking of truncated Lipid II to MurM using AutoDock Vina, Related to Figure 3. All possessed identical binding affinities A) and B) show the phosphate group located near the entrance of the cavity, with the pentapeptide located deeper into the pocket. C) and D) show orientations that are not considered possible, since the phosphate group would be linked to the membrane embedded Lipid II, and this would prevent the phosphate from being located deep in the binding site as shown.

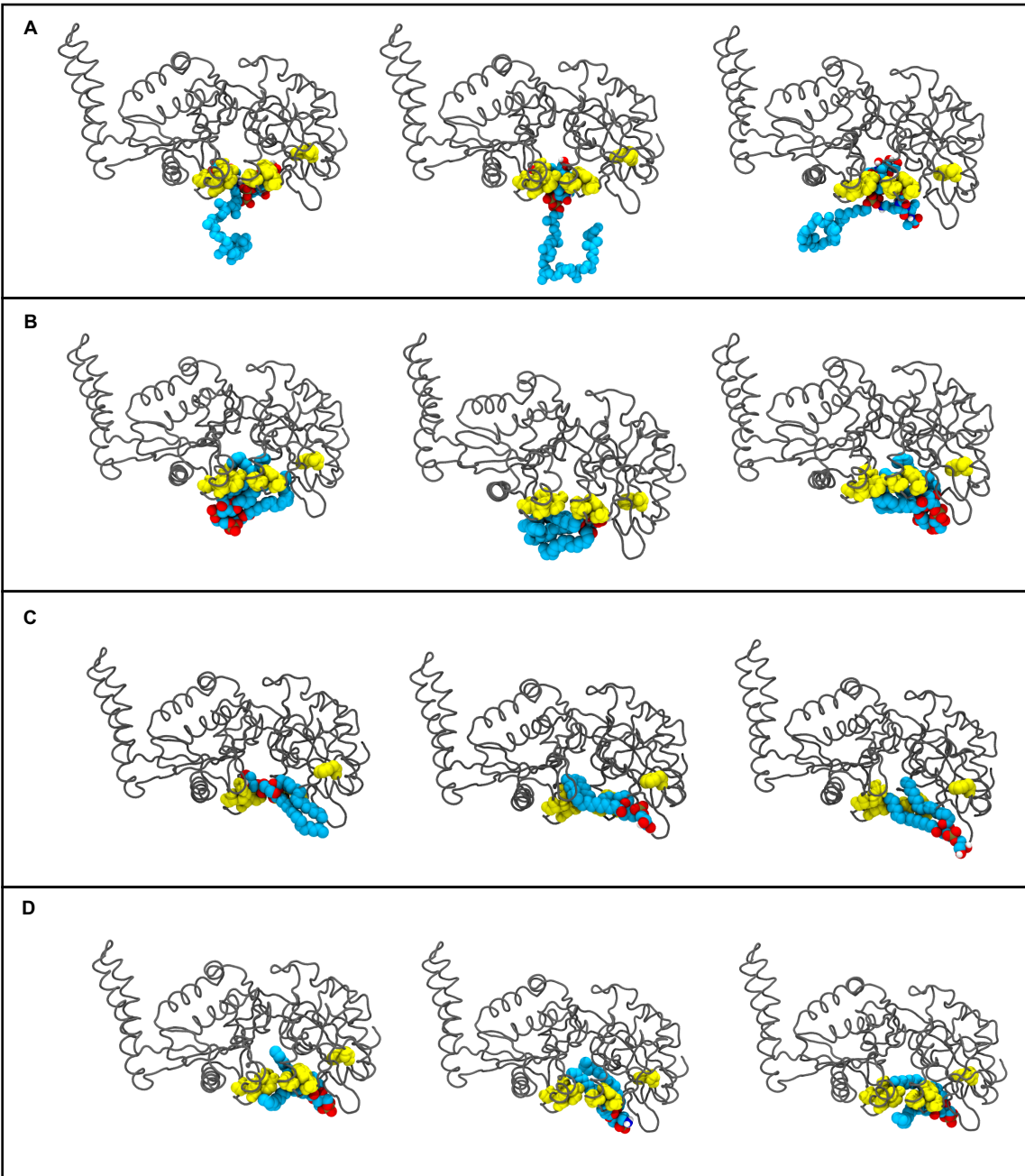


Figure S4. Top 3 binding poses for the docking of different lipids to the MurM binding site, Related to STAR Methods. Docking of A) Lipid II, B) cardiolipin, C) phosphatidylglycerol and D) phosphatidylethanolamine to the MurM binding site, where residues K35, W38, R215 and Y219 are shown in yellow.

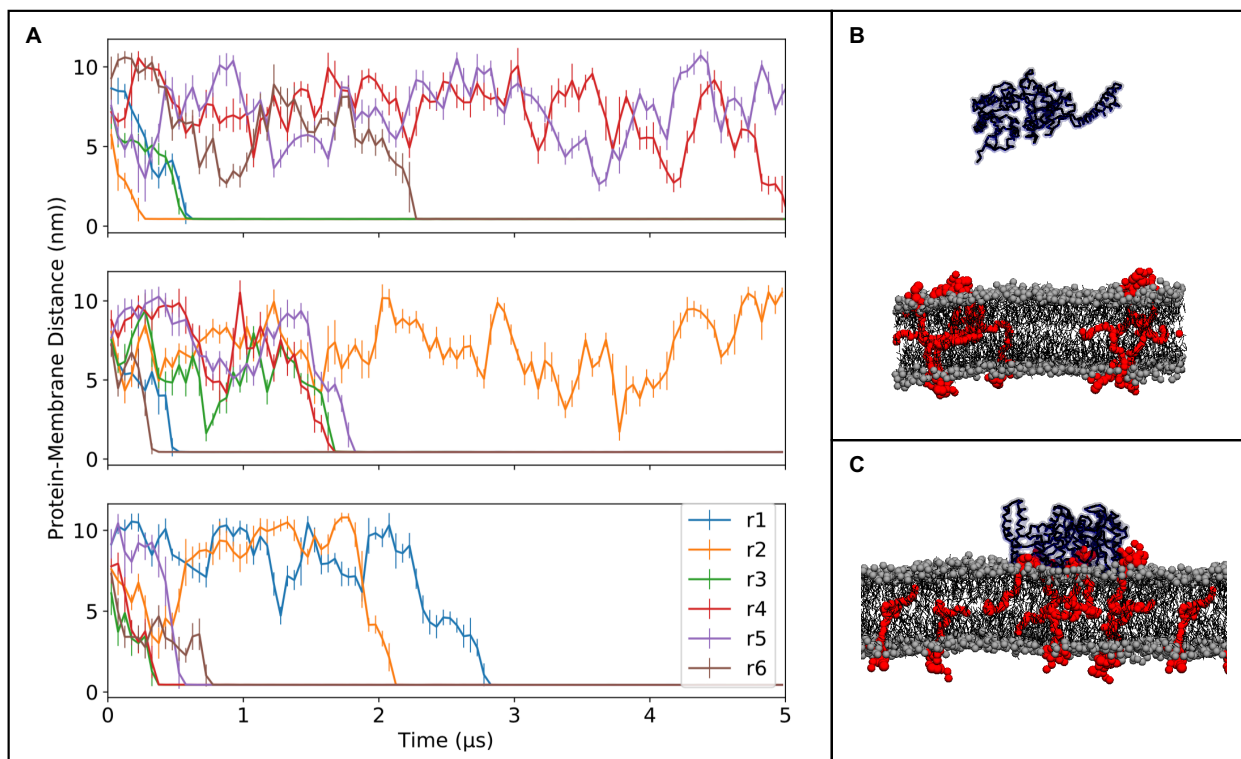


Figure S5. The association of coarse-grained MurM to the surface of the membrane, Related to STAR Methods. A) The minimum distance between MurM and the membrane surface for System 1 (top), System 2 (middle) and System 3 (bottom). Snapshots taken of the B) first and C) last frame of repeat 2 (r2), for System 1 (Supplemental Information: Table S1) Colour key: red = Lipid II, blue = protein, and grey = membrane.

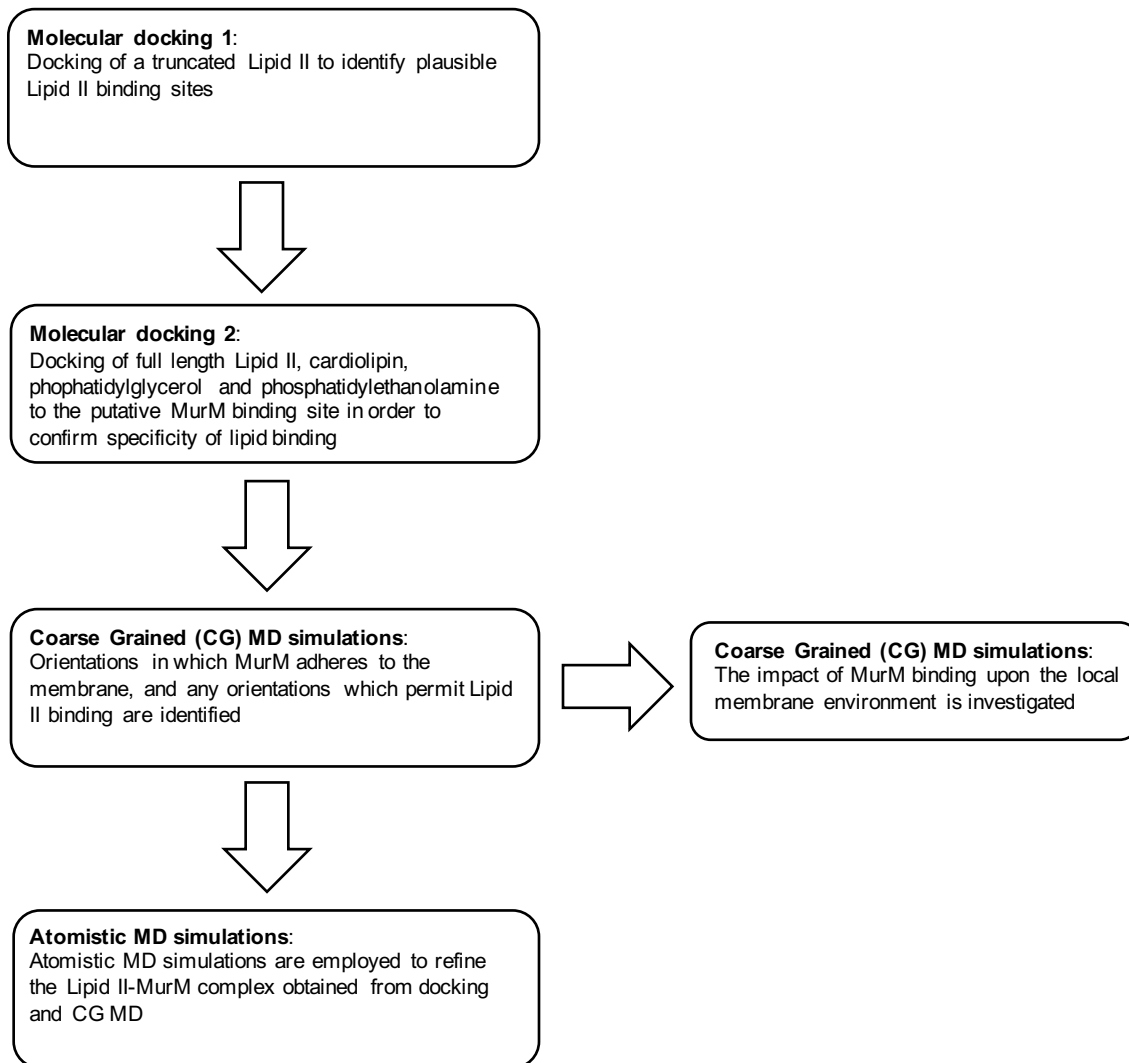


Figure S6. Flow chart showing the logistics of the computational studies, Related to STAR Methods.

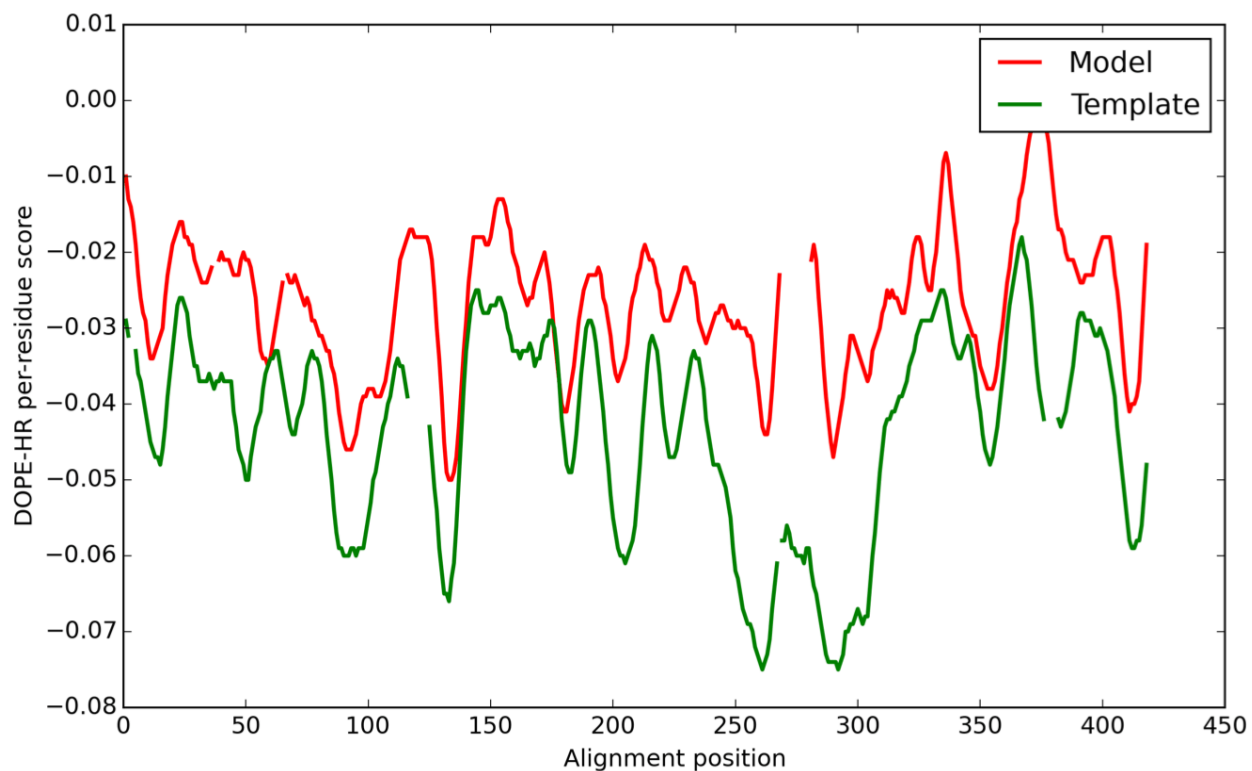


Figure S7. Discrete Optimized Protein Energy Profile for MurM and FemX, Related to STAR Methods. Comparison of DOPE-HR profiles for MurM model (red) and FemX template (green).