Activity and Crystal Structure of *Arabidopsis thaliana* UDP-*N*-acetylglucosamine Acyltransferase

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Supporting Information

Figure S1. Sequence alignment of AtLpxA and EcLpxA.

Panel A: AtLpxA and EcLpxA showed 38% identity (red) and 53% similarity according to the alignment result by clustalW[9]. Highlighted regions in blue or green represent predicted extra inserts in AtLpxA. The N-terminal region of AtLpxA (underlined) is predicted to be a signal peptide by Mitoprot software[12]. Amino acids 1-32 were removed and replaced by a methionine residue during cloning of SJD-01.
Panel B: Alignment of AtLpxA and EcLpxA considering the AtLpxA crystal structure. The residues with insufficient electron densities to build into the model are indicated in grey. Abbreviations: PB, parallel beta strand; T, turn.

A.

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                PB1   T1    PB2   T2    PB3    T3

     AtLpxA                                      MISLLKAREKLLSPLVSSTIRRLSSSLSYSRED 33
     EcLpxA                                      --------------------------------

C1   AtLpxA      SRD   SE   VLIH   PS   AVVH   PN 50
     EcLpxA     -MID   KS   AFVH   PT   AIVE   EG 17

C2   AtLpxA     AVIG   KG   VSVG   PY   CTIG   SS 68
     EcLpxA     ASIG   AN   AHIG   PF   CIVG   PH 35

C3   AtLpxA     VKLG                            NGCKLYPSSHVFGNTELG 90 (extra insert 1)
     EcLpxA     VEIG                            ------------------ 21

C3(continued)
     AtLpxA           ES   CVLM   TG   AVVG   D      ELPG 107              (extra insert 2)
     EcLpxA           EG   TVLK   SH   VVVN   G      ----  52

C4   AtLpxA     TFIG   CN   NIIG   HH   AVV          GVKCQDLKYKHGDECF 139  (loop 1)
     EcKpxA     TKIG   RD   NEIY   QF   ASI          GEVNQDLKY-AGEPTR  83

C5   AtLpxA     LCIG   NN   NEIR   EF   CSI          HR-SSKPSDK 163        (loop 2)
     EcLpxA     VEIG   DR   NRIR   ES   VTI          REGTVQGGGL 108

C6   AtLpxA     TVIG   DN   NLIM   GS   CHIA   HD 181
     EcLpxA     TKVG   SD   NLLM   IN   AHIA   HD 126

C7   AtLpxA     CKIG   DR   NIFA   NN   TLLA   GH 199
     EcLpxA     CTVG   NR   CILA   NN   ATLA   GH 144

C8   AtLpxA     VVVE   DN   THTA   GA   SVVH   QF 217
     EcLpxA     VSVD   DF   AIIG   GM   TAVH   QF 162

C9   AtLpxA     CHIG   SF   AFIG   GG   SVV    SQ 234
     EcLpxA     CIIG   AH   VMVG   GC   SGV    AQ 179

C10  AtLpxA     DVP    KY   MM 241
     EcLpxA     DVP    PY   VI 186

C-terminal domain
     AtLpxA     VAGERAELRGLNLEGLRRNGFTMSEMKSLRAAYRKIFMSTETVSLSFEERLTELEQDQEL 301
     EcLpxA     AQGNHATPFGVNIEGLKRRGFSREAITAIRNAYKLIYRSGK----TLDEVKPEIAELAET 242

     AtLpxA     YSVPAVSAMLQSIRDSFTESRRGICKFRQWLDSTT 336
     EcLpxA     Y--PEVKAFT----DFFARSTRGLIR--------- 262
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B.

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                PB1    T1     PB2    T2     PB3    T3

C0   AtLpxA     DSRD   SE     VLIH   PS     AVVH   PN 50
     EcLpxA     ----   --     ----   --     ----   --

C1   AtLpxA     AVIG   KG     VSVG   PY     CTIG   SS 68
     EcLpxA     -MID   KS     AFVH   PT     AIVE   EG 17

C2   AtLpxA     VKLG   NG     CKLY   PS     SHVF   GN 86
     EcLpxA     ASIG   AN     AHIG   PF     CIVG   PH 35

C3   AtLpxA     TELG   ES     CVLM   TG     AVVG   D      ELPG 107            (loop 0)
     EcLpxA     VEIG   EG     TVLK   SH     VVVN   G      ----  52

C4   AtLpxA     TFIG   CN     NIIG   HH     AVV           GVKCQDLKYKHGDECF 139 (loop 1)
     EcKpxA     TKIG   RD     NEIY   QF     ASI           GEVNQDLKY-AGEPTR  83

C5   AtLpxA     LCIG   NN     NEIR   EF     CSI           HR-SSKPSDK 163       (loop 2)
     EcLpxA     VEIG   DR     NRIR   ES     VTI           HRGTVQGGGL 108

C6   AtLpxA     TVIG   DN     NLIM   GS     CHIA   HD 181
     EcLpxA     TKVG   SD     NLLM   IN     AHIA   HD 126

C7   AtLpxA     CKIG   DR     NIFA   NN     TLLA   GH 199
     EcLpxA     CTVG   NR     CILA   NN     ATLA   GH 144

C8   AtLpxA     VVVE   DN     THTA   GA     SVVH   QF 217
     EcLpxA     VSVD   DF     AIIG   GM     TAVH   QF 162

C9   AtLpxA     CHIG   SF     AFIG   GG     SVV    SQ 234
     EcLpxA     CIIG   AH     VMVG   GC     SGV    AQ 179

C10  AtLpxA     DVP    KY     MM 241
     EcLpxA     DVP    PY     VI 186

C-terminal domain
     AtLpxA     VAGERAELRGLNLEGLRRNGFTMSEMKSLRAAYRKIFMSTETVSLSFEERLTELEQDQEL 301
     EcLpxA     AQGNHATPFGVNIEGLKRRGFSREAITAIRNAYKLIYRSGKTL----DEVKPEIAELAET 242

     AtLpxA     YSVPAVSAMLQSIRDSFTESRRGICKFRQWLDSTT 336
     EcLpxA     Y--PEVKAFTDFFARST----RGLIR--------- 262
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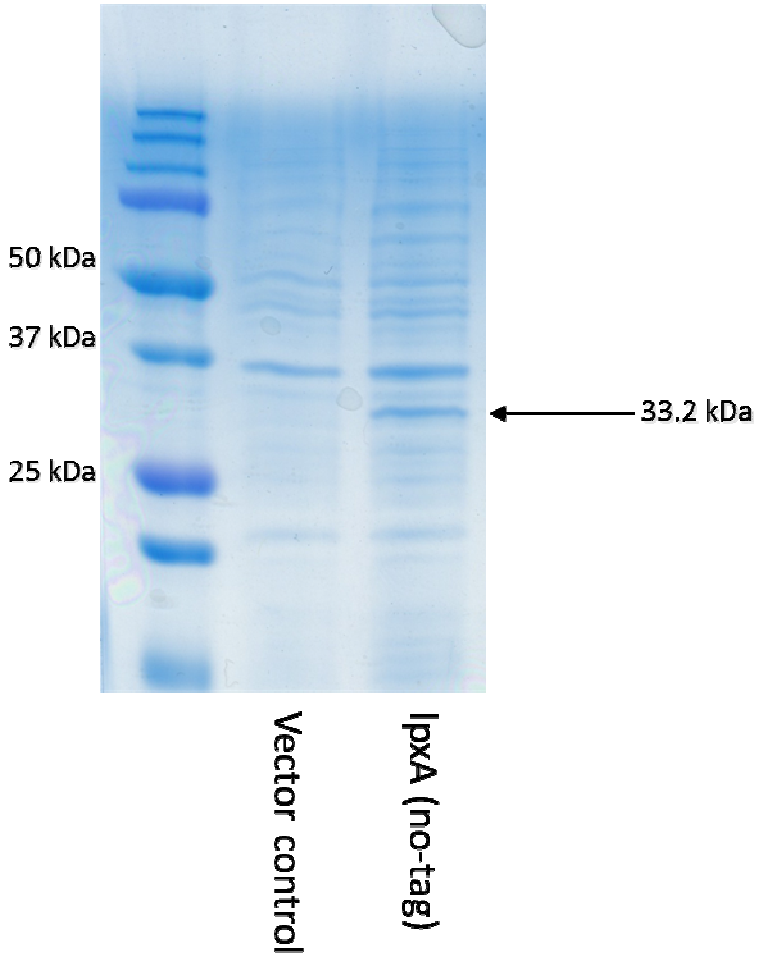
Figure S2. Overexpression and Purification of AtLpxA from *E. coli* C41 (DE3) strains

A. Expression of AtLpxA in *E. coli* C41 (DE3) strain. Cell lysate was loaded into each lane after boiling for 5 min in Laemmli sample buffer. The predicted molecular weight for AtLpxA is 33,241 Da.



B. Purification of AtLpxA with Green-19 dye column and size exclusion chromatography. Left side, About 10 mg of protein was purified from 1 liter culture of *E. coli* C41 (DE3) harboring pET-21b(+)-AtLpxA. Lane 1: cell lysate, 2: green column flow through, 3: wash with 0 M KCl, 4: 100 mM KCl, 5: 200 mM KCl, 6: 400 mM KCl, 7: 1 M KCl. **Right side**, chromatogram of AtLpxA in size exclusion gel filtration. The largest peak corresponds to the AtLpxA trimer, and the preceding peaks seem to be aggregates.

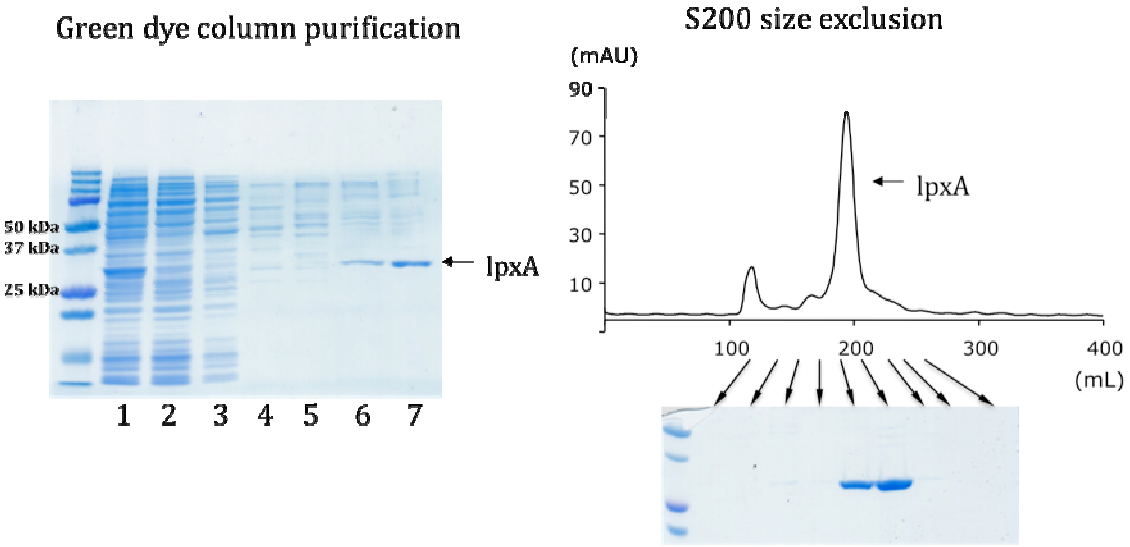
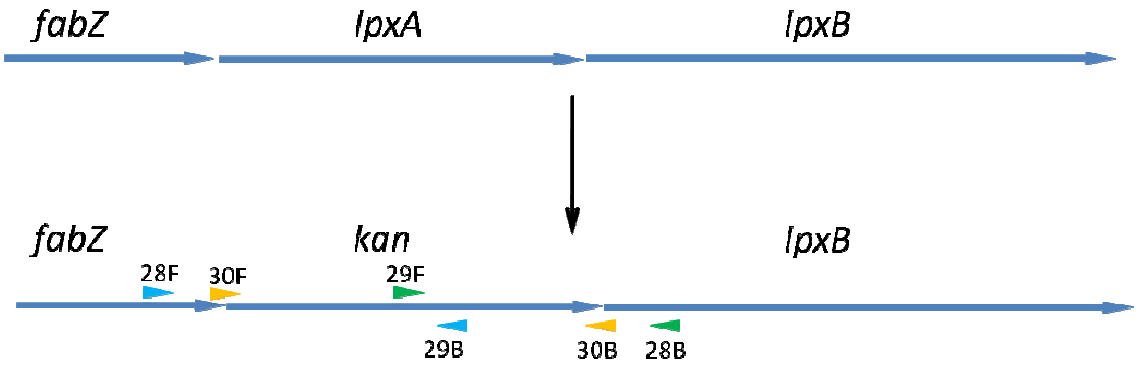


Figure S3. Construction of *E. coli* mutants lacking chromosomal *lpxA* gene.

A. Scheme for the replacement of *lpxA* by a *kan* cassette.



B. DNA sequence of the region flanking the *kan* cassette in *lpxA* knockout mutants. Nucleotides for *kan* are capitalized and numbered. DNA sequences corresponding to the PCR primers (SJP-030F, SJP-030B) for *kan* amplification are highlighted in yellow, and underlined are the start and stop codons in the region. The presence of *kan* inserted between *fabZ* and *lpxB* was first screened by amplifying the 3′ region of *fabZ* through 5′ region of *kan* (primers SJP-028F and SJP029B, highlighted in blue) and the 3′ region of *kan* through 5′ region of *lpxB* (primers SJP-029F and SJP-028B, highlighted in green). The larger piece of DNA was prepared for DNA sequencing by amplifying the 3′ region of *fabZ* through 5′ *lpxB* with primers SJP-028F and SJP-028B. Boxed is the ribosome-binding site for *lpxB* expression.

ggtgtgctgattctggaagcaatggcacaggcaacaggt
attctggcgtttaaaagcgtaggaaaactggaaccgggt
gagctgtactacttcgctggtattgacgaagcgcgcttc
aagcgcccggtcgtgcctggcgatcaaatgatcatggaa
gtcactttcgaaaaaacgcgccgcggcctgaccgtttt
aaaggggttgctctggtcgatggtaaagtagtttgcgaa
gcaacgatgatgtgtgctcgtagccgggaggcctgatac

1 ATGAGCCATA TTCAACGGGA AACGTCTTGC TCTAGGCCGC GATTAAATTC
51 CAACATGGAT GCTGATTTAT ATGGGTATAA ATGGGCTCGC GATAATGTCG
101 GGCAATCAGG TGCGACAATC TATCGATTGT ATGGGAAGCC CGATGCGCCA
151 GAGTTGTTTC TGAAACATGG CAAAGGTAGC GTTGCCAATG ATGTTACAGA
201 TGAGATGGTC AGACTAAACT GGCTGACGGA ATTTATGCCT CTTCCGACCA
251 TCAAGCATTT TATCCGTACT CCTGATGATG CATGGTTACT CACCACTGCG
301 ATCCCCGGGA AAACAGCATT CCAGGTATTA GAAGAATATC CTGATTCAGG
351 TGAAAATATT GTTGATGCGC TGGCAGTGTT CCTGCGCCGG TTGCATTCGA
401 TTCCTGTTTG TAATTGTCCT TTTAACAGCG ATCGCGTATT TCGTCTCGCT
451 CAGGCGCAAT CACGAATGAA TAACGGTTTG GTTGATGCGA GTGATTTTGA
501 TGACGAGCGT AATGGCTGGC CTGTTGAACA AGTCTGGAAA GAAATGCATA
551 AACTTTTGCC ATTCTCACCG GATTCAGTCG TCACTCATGG TGATTTCTCA
601 CTTGATAACC TTATTTTTGA CGAGGGGAAA TTAATAGGTT GTATTGATGT
651 TGGACGAGTC GGAATCGCAG ACCGATACCA GGATCTTGCC ATCCTATGGA
701 ACTGCCTCGG TGAGTTTTCT CCTTCATTAC AGAAACGGCT TTTTCAAAAA
751 TATGGTATTG ATAATCCTGA TATGAATAAA TTGCAGTTTC ATTTGATGCT
801 CGATGAGTTT TTCTAA

aaggag atatacc
atgactgaacagcgtccattaacgattgccctggtcgccg
gagaaacctccggcgatatcctgggggccggtttaatcc
gcgctctgaaagaacatgtgcccaacgcccgctttgttg
gtgttgccgggccacgaatgcaggctgaaggctgcgaag
cctggtacgaaatggaagaactggcggtgatgggcattg
ttgaagtgctcggtcgtctgcgtcgcttactgcatattc
gtgccgatctgacaaagcgttttggcgaactgaagccag

Figure S4. A bent rod-shape of LβH domain of AtLpxA.

An asymmetric unit of AtLpxA (green) is aligned to a monomer of the trimeric EcLpxA(1LXA, grey colored). Top: top down view from amino terminal side of rotational axis. Bottom: side view. Left Bottom: enlarged view of the coils C0 through C7 in the LβH domain. The angle measured between Cα carbons of coil C2 (Pro71 of AtLpxA, Pro28 of EcLpxA) and coil C6 (Gly174 of AtLpxA, Ile119 of EcLpxA) at turn T2 regions is ~7° (the angle between blue lines), and the distance between Cα carbons of coil C6 is 2.4 Å.