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Supplemental information

Length matters: Functional flip of the short TatA transmembrane helix

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Table S 1 Template plasmids and primers for the construction of the TatA constructs

TatA mutant	plasmid	template plasmid	forward primer	reverse primer
$TatA_{2-45}$ ΔLIL	pET28 $TatA_{1-45}$ ΔLIL	pET28 $TatA_{1-45}$	5'-GA ATA CCG GGC ATC TTC GTC ATC GC-3'	5'-GC GAT GAC GAA GAT GCC CGG TAT TC-3'
$TatA_{2-45}$ LAL	pET28 $TatA_{1-45}$ LAL	pET28 $TatA_{1-45}$	5'-GGC TTG ATT CTC CTG GCG TTG ATC TTC GTC ATC-3'	5'-GAT GAC GAA GAT CAA CGC CAG GAG AAT CAA GCC-3'
$TatA_{2-45}$ LALAL	pET28 $TatA_{1-45}$ LALAL	pET28 $TatA_{1-45}$	5'-GGC TTG ATT CTC CTG GCG TTG GCC CTG ATC TTC GTC ATC-3'	5'-GAT GAC GAA GAT CAG GGC CAA CGC CAG GAG AAT CAA GCC-3'
$TatA_{2-45}$ LALALAL	pET28 $TatA_{1-45}$ LALALAL	pET28 $TatA_{1-45}$	5'-GGC TTG ATT CTC CTG GCG TTG GCC CTG GCG TTG ATC TTC GTC ATC-3'	5'-GAT GAC GAA GAT CAA CGC CAG GGC CAA CGC CAG GAG AAT CAA GCC-3'
$TatA_{2-45}$ F_2D	pET28 $TatA_{1-45}$ F_2D	pET28 $TatA_{1-45}$	5'-CGC GGC AGC CAT ATG GAT TCA AAC ATT GGA ATA CCG-3'	5'-CGG TAT TCC AAT GTT TGA ATC CAT ATG GCT GCC GCG-3'
$TatA_{2-45}$ F_2D I_7D	pET28 $TatA_{1-45}$ F_2D I_7D	pET28 $TatA_{1-45}$ F_2D	5'-GAT TCA AAC ATT GGA GAT CCG GGC TTG ATT CTC-3'	5'-GAG AAT CAA GCC CGG ATC TCC AAT GTT TGA ATC-3'
E. coli TatA LAL	pET22b+TatA LAL BCD	pET22b+TatABCD	5'-GT ATT TGG CAG TTA TTG ATT CTG GCG TTG ATT GCC GTC ATC GTT GTA C-3'	5'-G TAC AAC GAT GAC GGC AAT CAA CGC CAG AAT CAA TAA CTG CCA AAT AC-3'
E. coli TatA LALA	pET22b+TatA LALA BCD	pET22b+TatABCD	5'-GT ATT TGG CAG TTA TTG ATT CTG GCG TTG GCC ATT GCC GTC ATC GTT GTA C-3'	5'-G TAC AAC GAT GAC GGC AAT GGC CAA CGC CAG AAT CAA TAA CTG CCA AAT AC-3'
E. coli TatA LALALAL	pET22b+TatA LALALAL BCD	pET22b+TatABCD	5'-GT ATT TGG CAG TTA TTG ATT CTG GCG TTG GCC CTG GCG TTG ATT GCC GTC ATC GTT GTA C-3'	5'-G TAC AAC GAT GAC GGC AAT CAA CGC CAG GGC CAA CGC CAG AAT CAA TAA CTG CCA AAT AC-3'

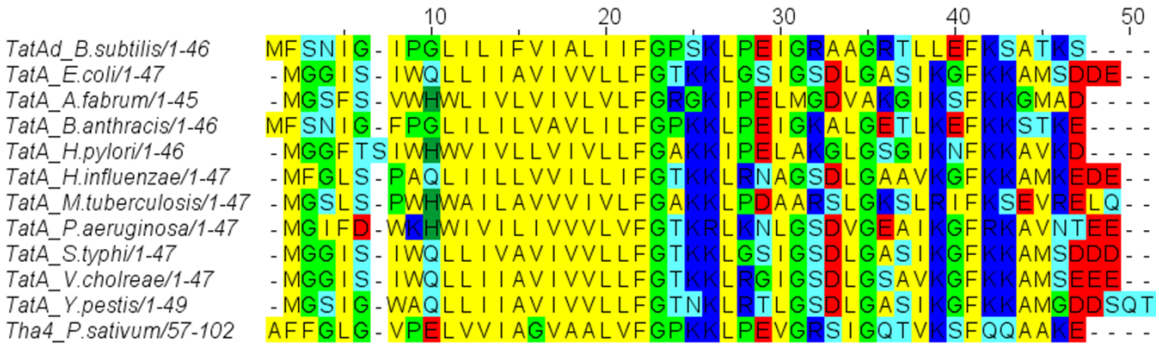
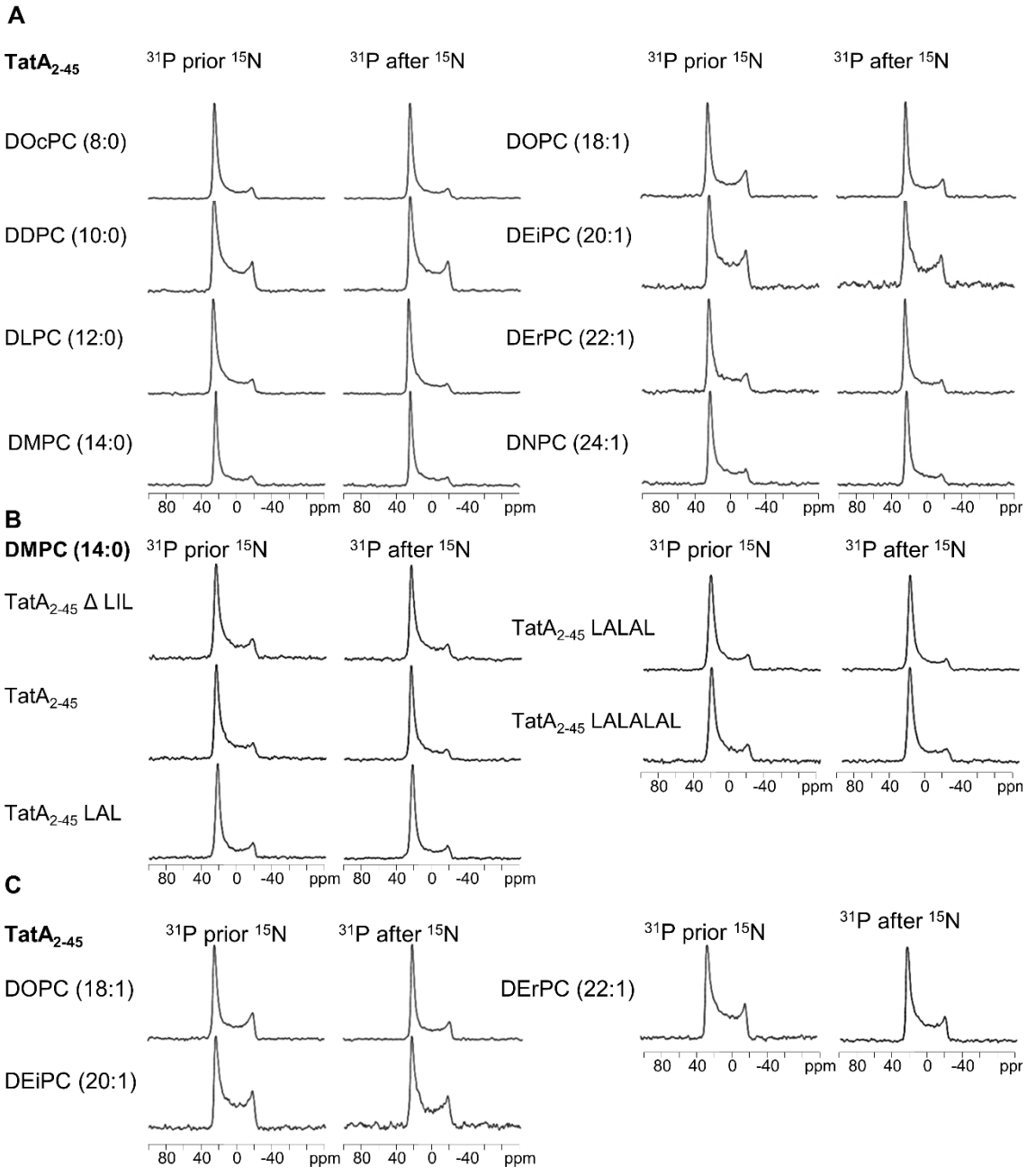


Figure S1: Sequence alignment of TatA from different organisms. Only the sequences of the TMH and APH are shown (the sequence of *P. sativum* is additionally N-terminally truncated). The sequence alignment proves that the unusual short length of the TatA TMH is highly conserved. The colors illustrate the different types of residues: yellow - hydrophobic (Leu, Ile, Val, Phe, Ala, Met, Trp); red - anionic (Asp, Glu); blue - cationic (Lys, Arg); light blue - polar (Ser, Thr, Gln, Asn, Tyr, Cys); light green - helix modifiers (Pro, Gly); dark green - ionizable (His). Data were created using the software Clustal [147] and Jalview [148].


A
TatA2-45
31P prior 15N
31P after 15N
31P prior 15N
31P after 15N
DOcPC (8:0)
DOPC (18:1)
DDPC (10:0)
DEiPC (20:1)
DLPC (12:0)
DErPC (22:1)
DMPC (14:0)
DNPC (24:1)
80 40 0 -40 ppm
B
DMPC (14:0)
31P prior 15N
31P after 15N
31P prior 15N
31P after 15N
TatA2-45 Δ LIL
TatA2-45 LALAL
TatA2-45
TatA2-45 LALALAL
TatA2-45 LAL
C
TatA2-45
31P prior 15N
31P after 15N
31P prior 15N
31P after 15N
DOPC (18:1)
DErPC (22:1)
DEiPC (20:1)

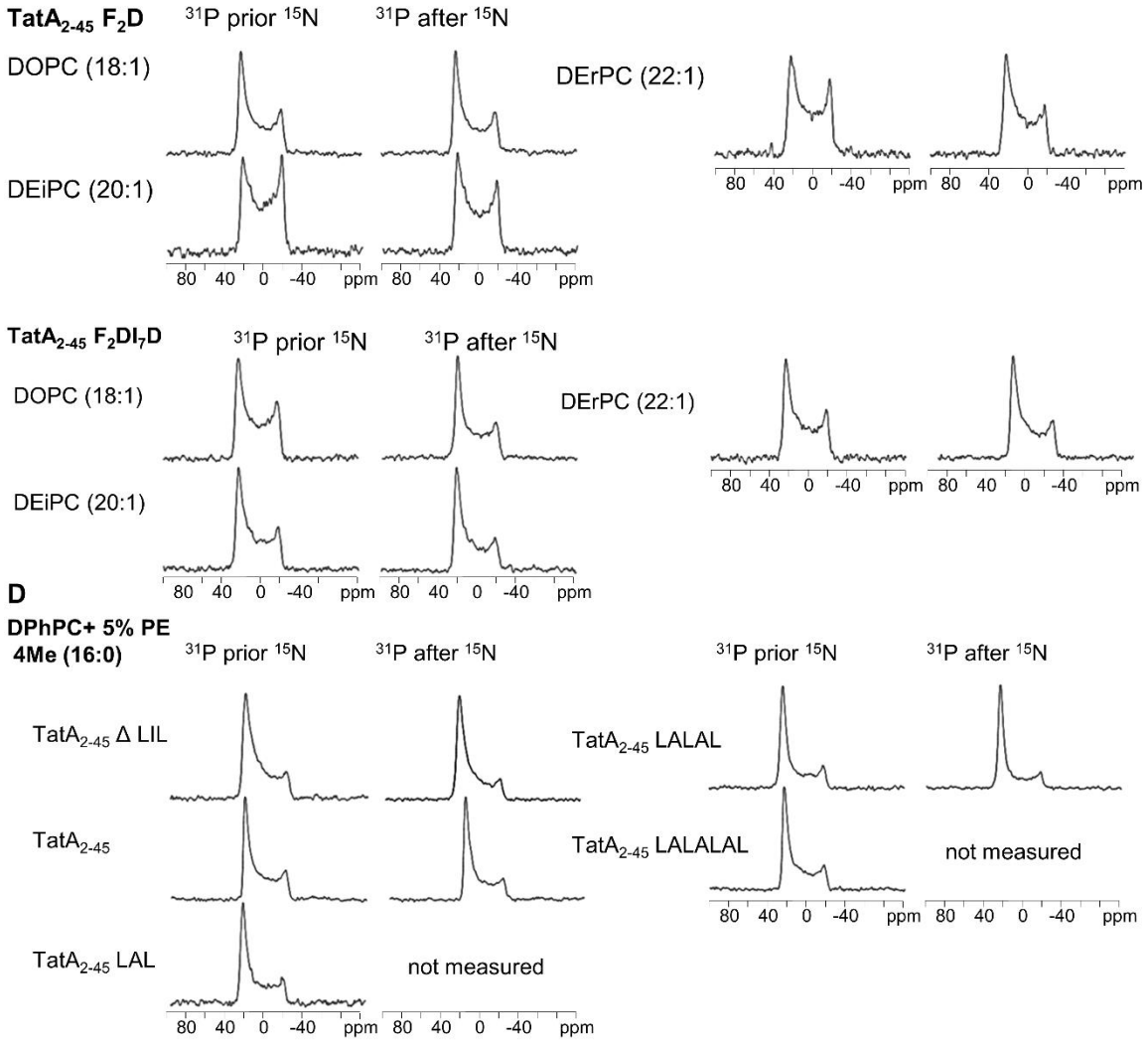


Figure S2: ^{31}P-NMR spectra before and after the ^{15}N-NMR measurements. One-dimensional solid-state ^{31}P-NMR spectra were recorded to assess the quality of the lipid alignment and any potential sample degradation. NMR measurements were performed using the standard sample orientation in which the bilayer normal is aligned parallel to the static magnetic field. **(A)** The uniformly ^{15}N-labelled $TatA_{2-45}$ wild type was reconstituted in macroscopically aligned phosphatidylcholine bilayers with varying membrane thickness, composed of DOcPC, DDPC, DLPC, DMPC, DOPC, DEiPC, DErPC, and DNPC (corresponding to Fig. 2). **(B)** $TatA_{2-45}$ variants with an extended or shortened TMH were reconstituted in macroscopically aligned lipid bilayers composed of DMPC (corresponding to Fig. 3). **(C)** $TatA_{2-45}$ variants with varying N-terminal charge density were reconstituted in macroscopically aligned lipid bilayers composed of DOPC, DEiPC and DErPC (corresponding to Fig. 4). **(D)** $TatA_{2-45}$ variants with an extended or shortened TMH were reconstituted in branched phytanoyl lipids composed of a mixture of DPhPC/DPhPE (95/5 mol/mol) (corresponding to Fig. 5).

The spectral lineshape is composed of a superposition of the aligned lamellar phase (narrow signal at around 30 ppm, which arises due to the parallel alignment of the phospholipids to the magnetic field) and an upfield powder content, which is exceptionally pronounced only for the N-terminally charged variants. The ^{31}P-NMR spectra show that the 14-22 hour ^{15}N-NMR experiments had no perturbing effect on the orientation of the macroscopically aligned membranes in general.

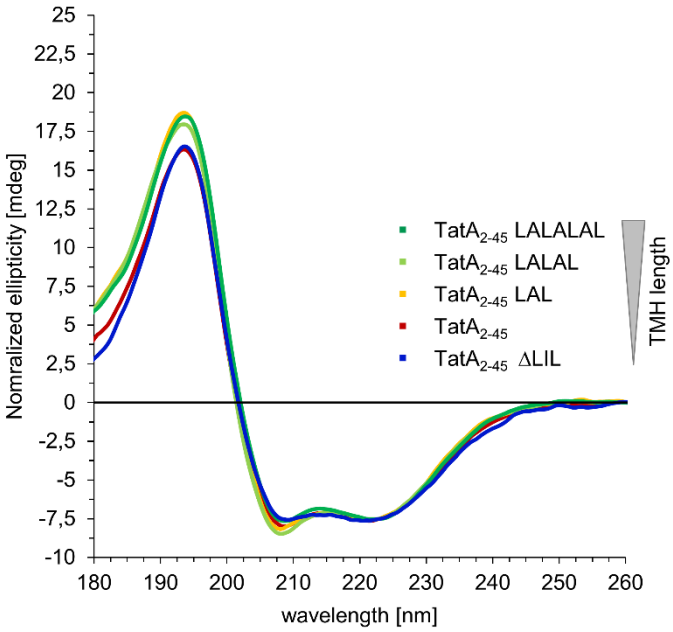


Figure S3: SRCD spectra of $TatA_{2-45}$ and $TatA_{2-45}$ variants with an extended or shortened TMH reconstituted in DMPC vesicles. All proteins were reconstituted in DMPC vesicles with a peptide to lipid ration of 1:50. For direct comparison, the spectra were normalized at 223 nm. All spectra show a typical α-helical line shape with characteristic bands at around 194 nm, 209 nm and 223 nm.

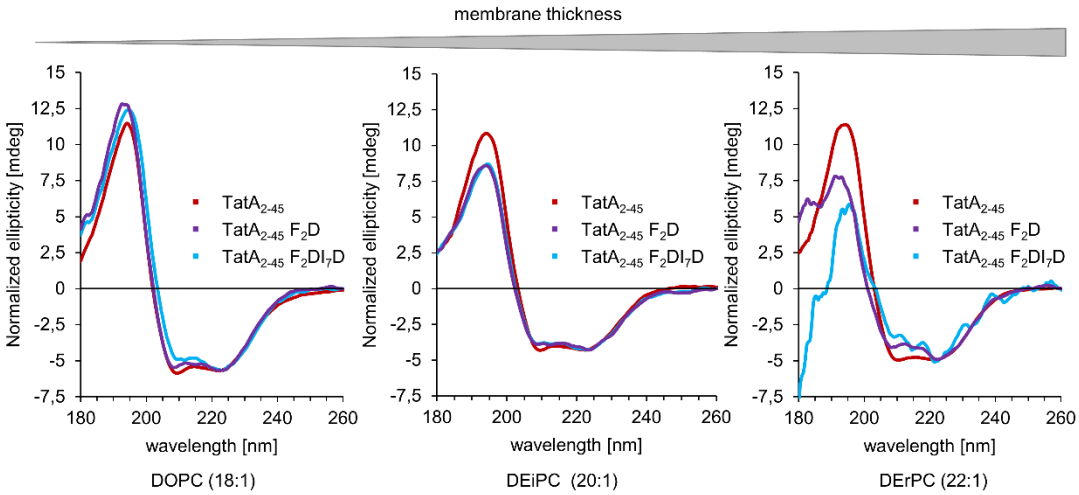


Figure S4: SRCD spectra of $TatA_{2-45}$ and $TatA_{2-45}$ variants with an N-terminal charge reconstituted DOPC, DEiPC and DErPC vesicles. All proteins were reconstituted with peptide to lipid ration of 1:50. For direct comparison, the spectra were normalized at 223 nm. All spectra show a typical α-helical line shape with characteristic bands at around 194 nm, 209 nm and 223 nm. In DEiPC $TatA_{2-45}$ F_2D and $TatA_{2-45}$ F_2DI_7D start to show some amount of absorption flattening, which is in DErPC strongly pronounced. This behaviour is consistent with the observed protein aggregation in ^{15}N-NMR.

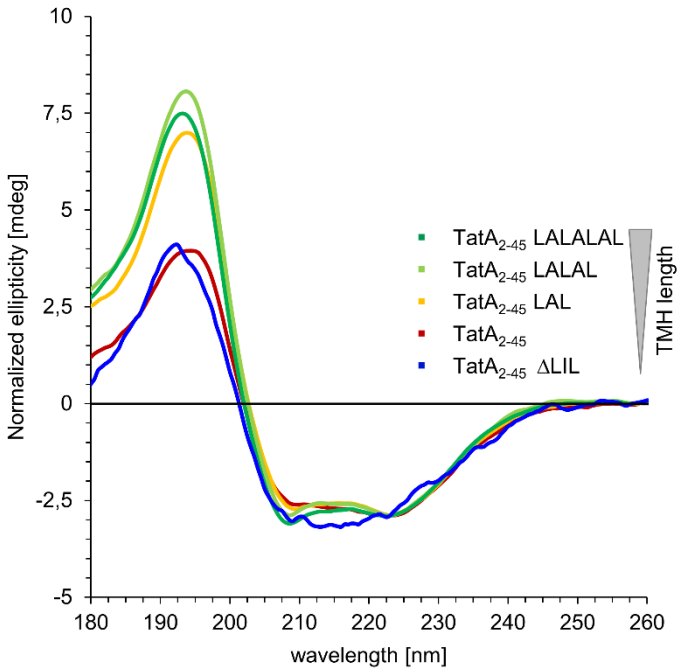


Figure S5: SRCD spectra of $TatA_{2-45}$ and $TatA_{2-45}$ variants with an extended or shortened TMH reconstituted in DPhPC/DPhPE (95/5 mol/mol) vesicles. All proteins were reconstituted in mixture of DPhPC/DPhPE (95/5 mol/mol) with a peptide to lipid ratio of 1:50. For direct comparison, the spectra were normalized at 223 nm. All spectra show a typical α-helical line shape with characteristic bands at around 194 nm, 209 nm and 223 nm. The variants with extended TMHs possess a larger α-helical content.

Supplemental References

[147] Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23 (21): 2947-2948.

[148] Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M. and Barton, G. J. 2009. Jalview Version 2--a multiple sequence alignment editor and analysis workbench. *Bioinformatics* 25 (9): 1189-1191.