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Investigation of the membrane orientation of the TatA transmembrane segment using SROCD-spectroscopy

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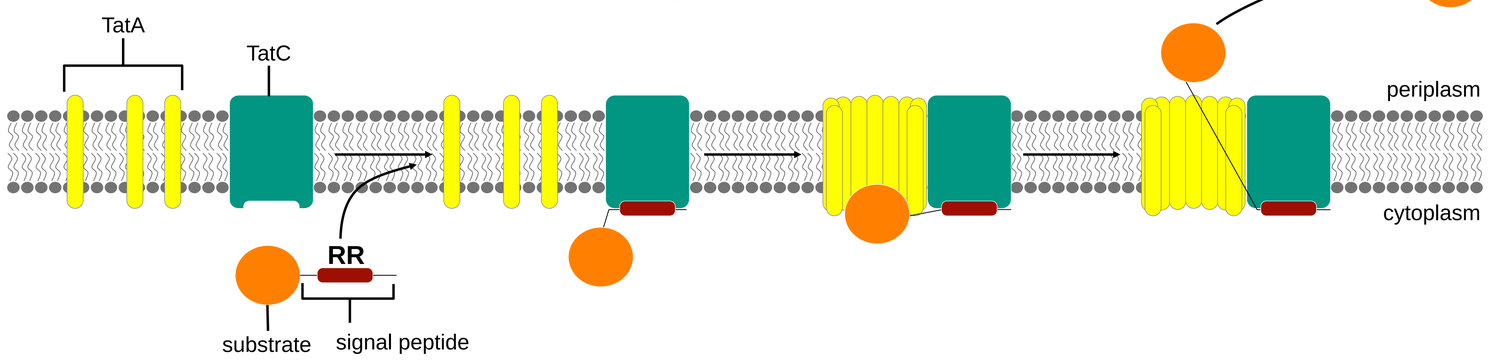
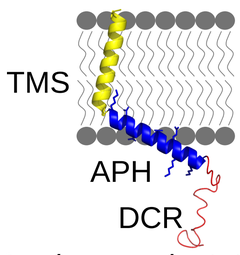
Introduction: Protein export via the twin-arginine translocase (Tat)

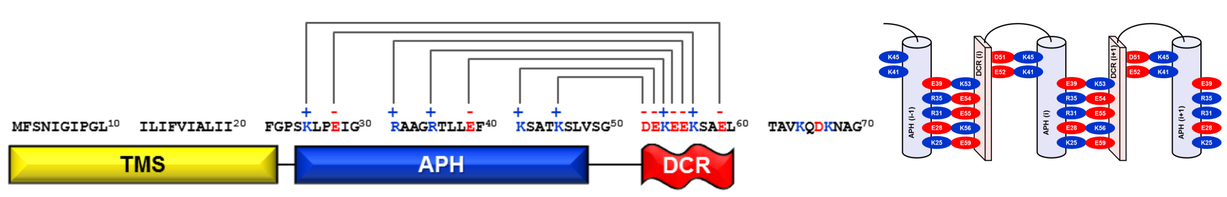


The “twin-arginine translocase” (Tat) has the unique ability of transporting fully folded proteins across the membrane using the proton-electrochemical gradient as its energy source. A minimal Tat-system found in Gram positive bacteria is composed only of the two membrane proteins TatA and TatC.
TatC functions as a receptor, recognizing cargo proteins by their “twin-arginine” containing signal peptide, while a homooligomeric TatA complex forms the actual translocation pore.

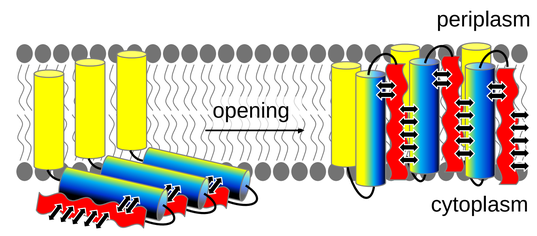
Background: Proposed structure and self-assembly of TatA based on electrostatic “charge zippers”



Membrane orientation of monomeric TatA



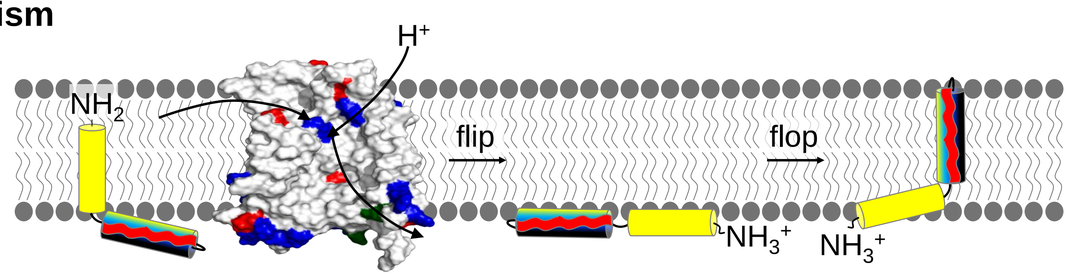
Postulated “charge zipper” between APH and DCR due to a ladder of inter- and intramolecular salt-bridges



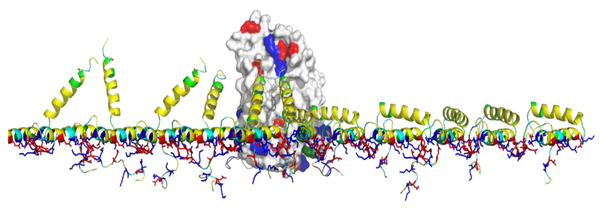
Overview of the TatA pore formation based on “charge zippers”

Hypothesis: Tat transport mechanism

Our postulated translocation mechanism is based on a pH-induced flip of the unusually short TatA transmembrane segment (TMS, only 14 hydrophobic amino acids), which is triggered by protonation of its N-terminus. We hope to prove this mechanism using oriented SRCD and solid state NMR-spectroscopy.



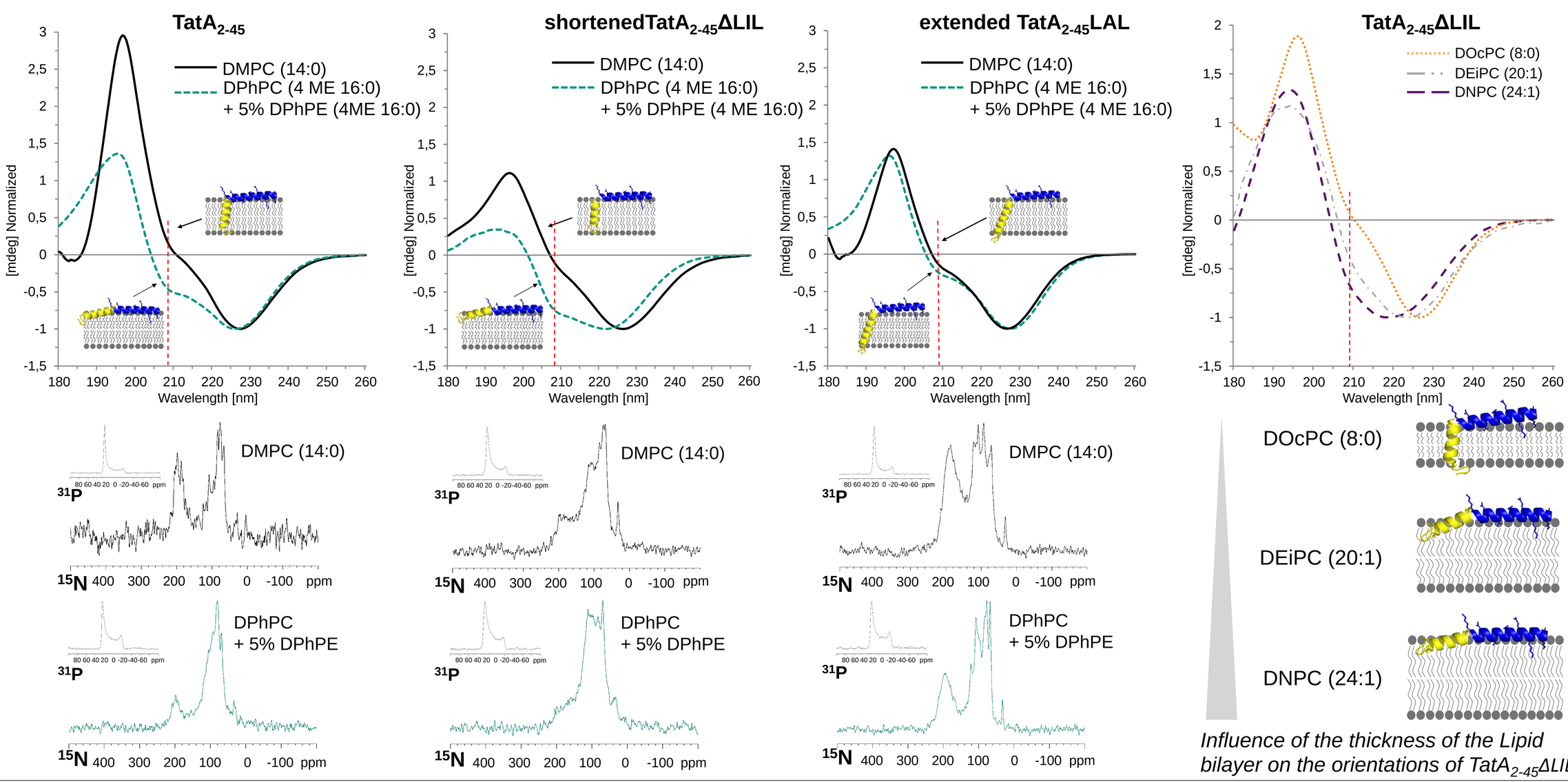
Flipping of the TatA transmembrane segment by the proton driven flippase TatC



MD simulation of the TatC-driven flip of the TatA transmembrane segment

Results: Membrane orientation the TatA transmembrane segment (TMS) in different lipid environments

To find evidence of a “flipped” state of the TatA TMS we determined the membrane alignment of the two helical segments of $TatA_{2-45}$ in different membranes using solid state NMR and oriented SRCD in oriented samples. We varied the lipid acyl chain-lengths and also combined voluminous phytanoyl chains with small headgroups. In a complementary approach, we changed the length of the transmembrane segment by using mutants with an extended ($TatA_{2-45}$LAL) or a shortened ($TatA_{2-45}$ΔLIL) TMS.



Influence of the thickness of the Lipid bilayer on the orientations of $TatA_{2-45}$ΔLIL

Conclusions: Consistent with our hypothesis, both solid state NMR as well as SRCD showed a flipping of $TatA_{2-45}$ upon going from thin DMPC to thick phytanoyl bilayers. A shortening of the TatA TMS promotes this effect, whereas an extension of the TMS holds this helix inside the lipid bilayer in a transmembrane state. Only in very thin membranes an inserted state of the shortened $TatA_{2-45}$ΔLIL TMS could be monitored, while increasing membrane thickness leads to a surface orientation of the protein.

References
Walther T. H., Grage S. L., Roth N., Ulrich A. S., J Am Chem Soc **132**, 15945-15956 (2010)
Lange C., Müller S. D., Walther T. H., Bürck J., Ulrich A. S., BBA **1768**, 2627-2634 (2007)
Walther T. H., Gottselig C, Grage S. L., Wolf M., Vargiu A. V., Klein M. J., Vollmer S., Prock S., Hartmann M., Afonin S., Stockwald E., Heinzmann H., Nolandt O. V., Wenzel W., Ruggerone P., Ulrich A. S., Cell **152**, 316-326 (2013)