Structural investigation of the twin-arginine translocase (Tat)

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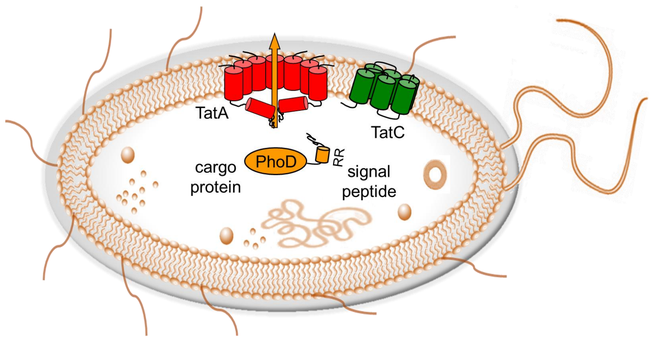
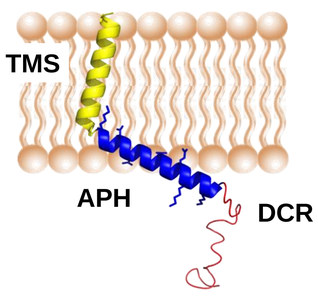
Tat dependent translocation

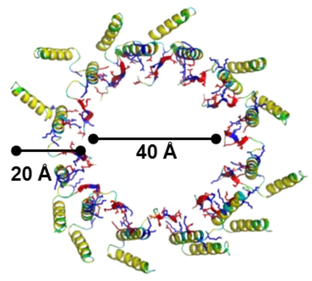


Twin arginine translocase (Tat) of Bacillus subtilis transports folded proteins across the plasma membrane, driven by the proton electrochemical gradient.

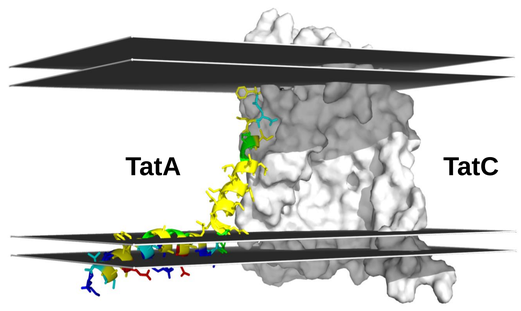
Structural analysis of TatA and TatC



Membrane orientation of monomeric TatA (Walther et al., 2010)

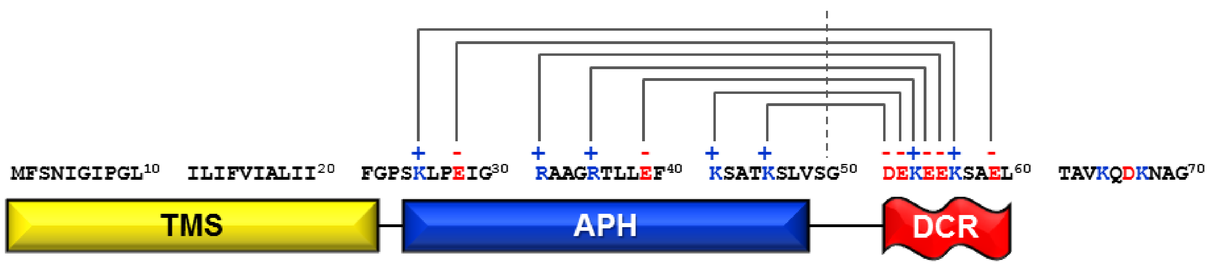


MD simulation of the TatA translocation pore based on "charge zipper" formation (Walther et al., 2013)

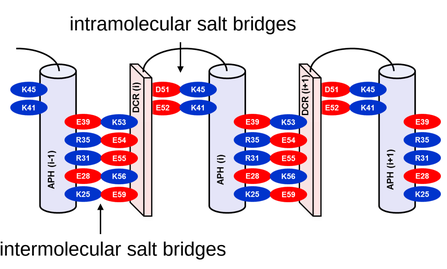


Docking of TatA against the TatC receptor in the membrane (TatC structure from Rollauer et al., 2012)

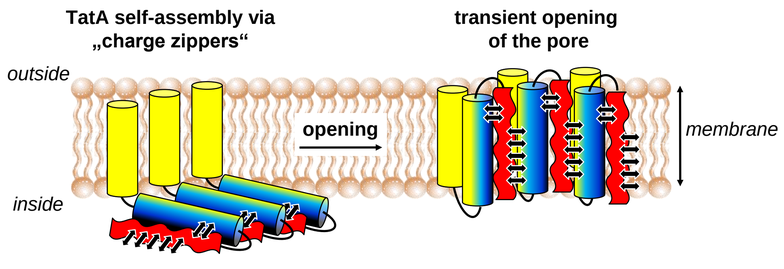
Self-assembly of TatA based on electrostatic "charge zippers"



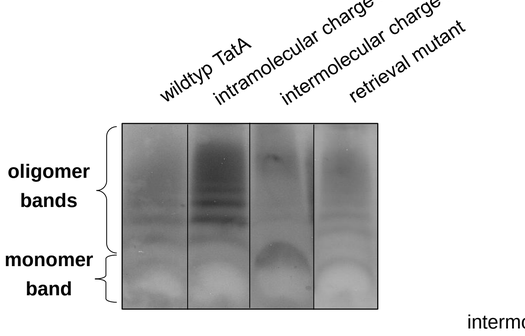
Charge pattern and predicted salt bridges along the primary sequence of TatA



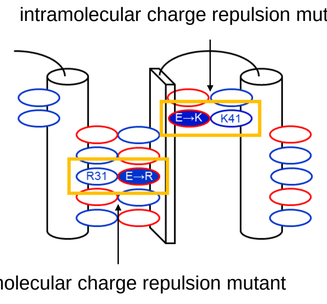
Postulated "charge zipper" between APH and DCR



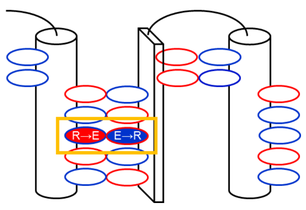
Schematical overview of the TatA pore mechanism based on "charge zippers" opening



Single repulsion mutants
Inhibition of one salt bridge by charge repulsion

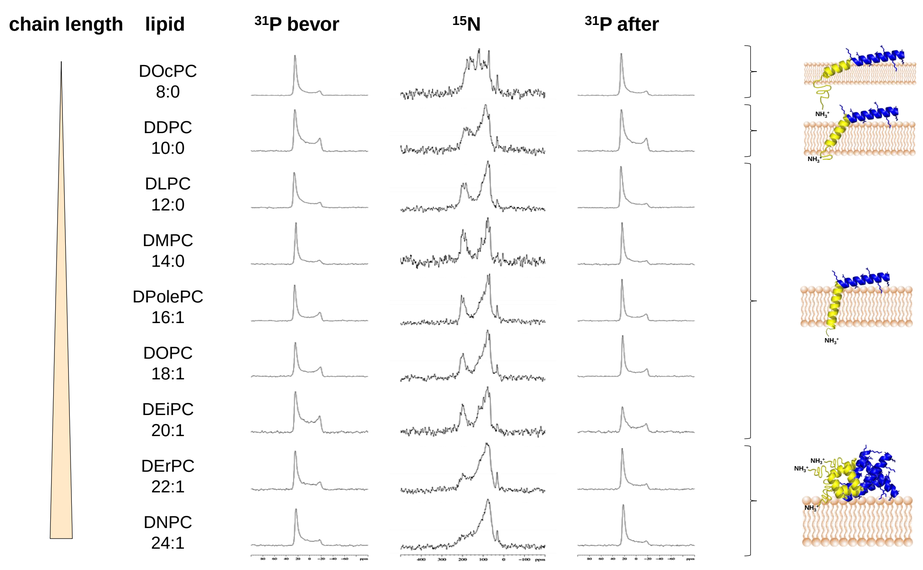


Retrieval mutants
Pairwise charge-inversion within one salt bridge to retrieve the salt-bridge



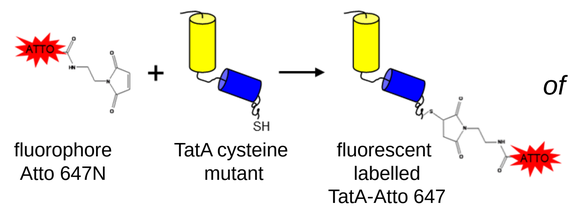
Experimental evidence for the "charge zippers" by monitoring the monomer-oligomer equilibrium of site-specific charge mutants of TatA in BN-PAGE

Influence of the lipid chain length on the reconstitution of TatA

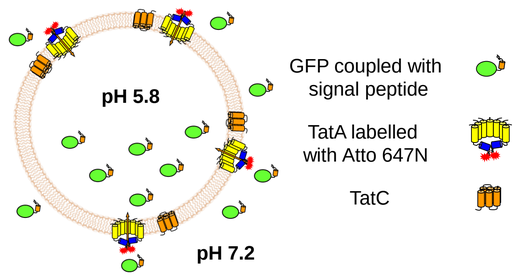


Solid-state NMR measurements of $TatA_{2-45}$ in mechanically oriented lipid bilayers with varying lipid chain lengths

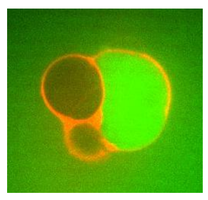
In vitro twin arginine translocation assay



Labelling reaction of TatA G70C and Atto 647N



Schematical overview of the in vitro translocation assay in giant unilamellar vesicles (GUVs)



GUVs imaged by spinning disc microscope. SP-GFP shown in green, TatA-Atto 647N in red

Conclusions

- A conserved pattern of complementary charges is present in the TatA primary sequence
- TatA can assemble via these complementary charges by forming intra- and intermolecular salt-bridges
- Experimental evidence for these "charge zippers" was obtained by monitoring the monomer-oligomer equilibrium of site-specific charge mutants of TatA in BN-PAGE
- MD simulations confirmed the steric feasibility of the TatA pore formation based on a "charge zipper" mechanism
- The reconstitution of TatA in different lipids was monitored by solid-state NMR spectroscopy
- Fluorescence measurements provide first hints that an *in vitro* assay with reconstituted $TatA_dC_d$ and GFP coupled with a Tat signal peptide is possible

References

- Walther T.H., C. Gottselig, S.L. Grage, M. Wolf, A.V. Vargiu, M.J. Klein, S. Vollmer, S. Prock, M. Hartmann, S. Afonin, E. Stockwald, H. Heinzmann, O. Nolandt, W. Wenzel, P. Ruggerone, A. S. Ulrich (2013) Folding and self-assembly of the TatA translocation pore based on a novel charge zipper mechanism, *Cell*, 152, 316–326
- Walther T.H., S.L. Grage, N. Roth, A.S. Ulrich (2010) Membrane alignment of the pore-forming component TatA(d) of the twin-arginine translocase from *B. subtilis* resolved by solid-state NMR spectroscopy, *J. Am. Chem. Soc.* 132, 15945-15956
- Rollauer S.E., M.J. Tarry, J.E. Graham, M. Jaaskelainen, F. Jager, S. Johnson, M. Krehenbrink, S.M. Liu, M.J. Lukey, J. Marcoux, M.A. McDowell, F. Rodriguez, P. Roversi, P.J. Stansfeld, C.V. Robinson, M.S. Sansom, T. Palmer, M. Hogbom, B.C. Berks & S.M. Lea (2012) Structure of the TatC core of the twin-arginine protein transport system. *Nature,* 492**,** 210-4.