Structural investigation of the twin-arginine translocase

Formatiert: Schriftart: (Standard) Arial

Formatiert: Titel, Links

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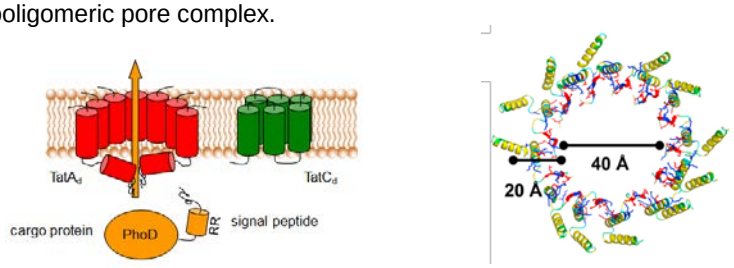
The “twin arginine translocase” (Tat) is a protein export machinery of bacteria, archaea, and plant thylakoids. In contrast to the ATP-dependent Sec-system, the Tat-system transports folded proteins across the plasma membrane, driven only by the proton electrochemical gradient. In *Bacillus subtilis* the minimally active translocation complex consists only of the two essential transmembrane proteins TatA and TatC. The TatC receptor recognizes the cargo protein via a “twin arginine” containing signal peptide, and TatA has been suggested to form a homooligomeric pore complex.



Twin arginine translocase of Bacillus subtilis, and our proposed model of the TatA translocation pore

We have determined the membrane orientation of monomeric TatA by solid-state NMR and OCD spectroscopy. TatA consists of a short α-helical transmembrane segment (TMS), an amphiphilic α-helix (APH), and a densely charged region (DCR) at the C-terminus.[1] Remarkably, the charges on the APH are perfectly complementary to those on the DCR. We thus proposed that the TatA pore assembles via these complementary charges, by forming ladders of intra- and intermolecular salt bridges. Experimental evidence for this “charge zipper” model was obtained by monitoring the monomer-oligomer equilibrium of specific charge mutants by BN-PAGE, and molecular dynamics simulations were used to assess the steric feasibility.[2] Recently, the TatC crystal structure was published by Rollauer *et al.*[3], suggesting how the TatA transmembrane segment could interact with TatC. To determine the role of this unusually short TatA segment in this interaction, we performed solid state NMR measurements of TatA in mechanically oriented lipid bilayers by varying the lipid chain length. Furthermore, we are investigating TatA mutants with a prolonged or shortened transmembrane helix. Very recently we were able to establish an *in vitro* translocation assay. To this aim, fluorescent labelled TatA and TatC were incorporated in giant unilamellar vesicles (GUVs), and GFP coupled to the signal peptide was externally added to monitor the transport into the vesicles. This assay will be used to examine various TatA and TatC mutants to obtain insights into the Tat dependent translocation mechanism.

References

[1] Walther T. H. et al. 2010. Membrane alignment of the pore-forming component TatA(d) of the twin-arginine translocase from Bacillus subtilis resolved by solid-state NMR spectroscopy. *J Am Chem Soc*. 132:15945-15956.

[2] Walther T. H. et al. 2013. Folding and self-assembly of the TatA translocation pore based on a charge zipper mechanism. *Cell.* 152:316-326.

[3] Rollauer, S. E. et al. 2012. Structure of the TatC core of the twin-arginine protein transport system. *Nature.* 492**:** 210-4.