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NEUTRONS FOR SCIENCE ®

Neutron Scattering analysis of the bacterial holotranslocon

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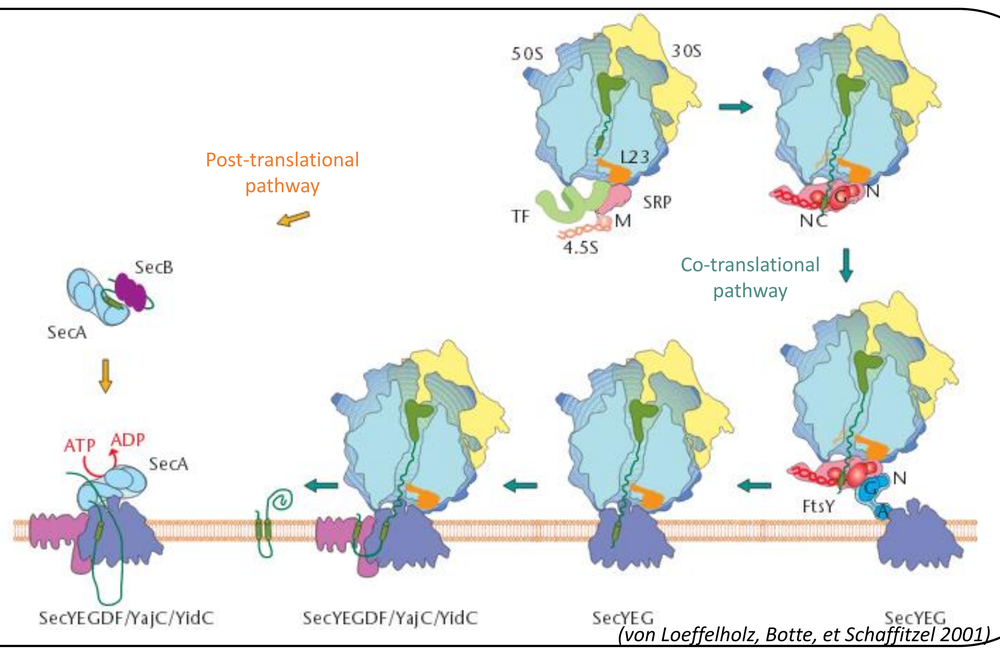
Background

In bacteria, up to 30% of all proteins are translocated into or through the bacterial membrane. This mechanism relies on the trimeric SecYEG (translocon) complex, which forms a pore through the membrane and can open laterally in order to integrate trans-membrane protein into the lipid bilayer[1]. The energy required to perform the translocation is provided either by the ribosome (co-translational pathway) or the secA ATPase (post-translational pathway)[2].

Four additional subunits can join the core translocon :

- Sec D and Sec F favor the translocation by using the proton-motive force[3]
- YidC, a conserved membrane protein insertase
- YajC, a small protein whose function remains unknown.

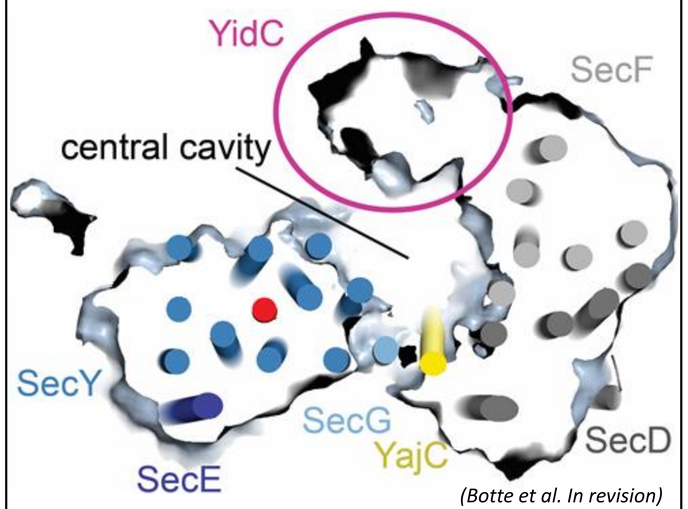
This seven-subunit membrane complex is called the **holotranslocon** (HTL). It is thought to interact with the ribosome and secA and is more efficient for membrane protein integration[3].



(von Loeffelholz, Botte, et Schaffitzel 2001)

PROJECT

A flexible lipid cavity has been described at the center of the holotranslocon *(Botte et al., in revision)*. We are now investigating its function and dynamic.



(Botte et al. In revision)

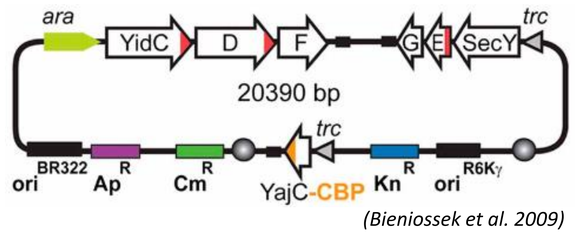
- Interactions with cytoplasmic partners
- Post-translational model
- Co-translational model
- Membrane protein integration

METHODS

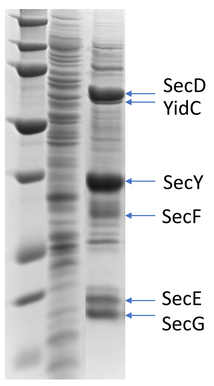
Biochemical preparation

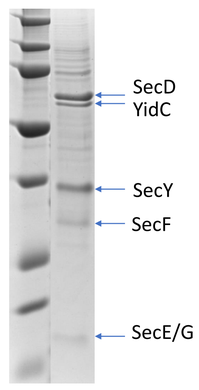
- Complex extracted from the membrane with a mild detergent : DoDecyl Maltoside (DDM)
- Two successive purification steps
- Detergent exchanged with synthetic amphiphatic polymers (Amphipol A8-35)
- Size exclusion chromatography

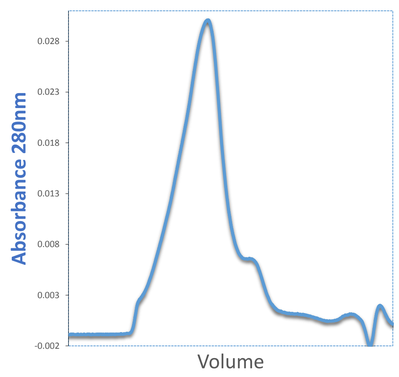
HTL overexpression → Cell lysis → Membrane solubilization (DDM) → Nickel purification → CBP purification → Amphipol exchange → Size exclusion



(Bieniossek et al. 2009)



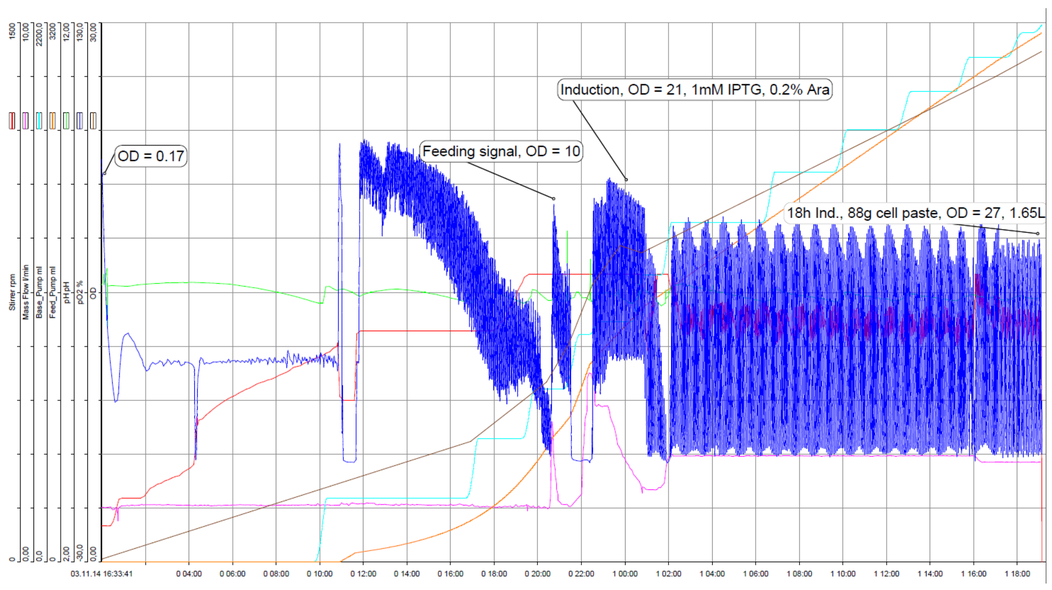




Small angle Neutron scattering

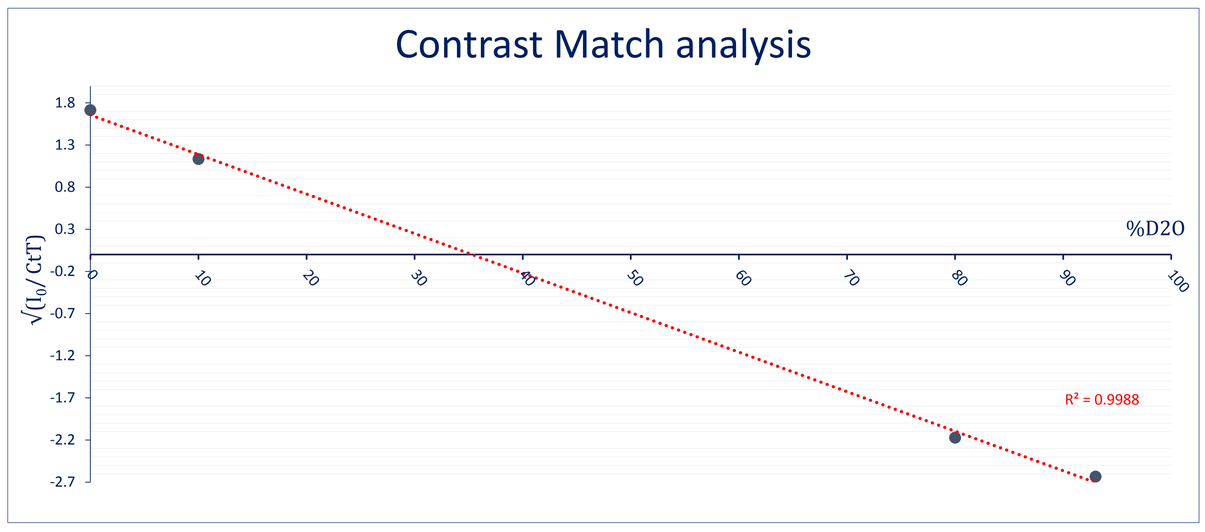
- Contrast match analysis uses the natural scattering length density contrast to distinguish protein, lipids and detergent/polymers.

PRELIMINARY WORK



Bacteria are cultivated in fermentors under controlled conditions :

- pH, temperature, pO_2, carbon source
- Optimization of the yield for deuteration



Theoretical scattering length density :

- Protein : 40.7%
- Lipid : 7%
- Amphipol : 23.5%

Estimated composition of the particle

- Protein : 82%
- Lipid : 14.1%
- Amphipol : 3.9%

References:

1. Berg, B. van den *et al. Nature* **427,** 36–44 (2003).
2. Von Loeffelholz, O., Botte, M. & Schaffitzel, C. in *eLS* (John Wiley & Sons, Ltd, 2001).
3. Schulze, R. J. *et al*. *PNAS* **111,** 4844–4849 (2014).

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