Group: Membrane Enzymology

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Project title: Structure of eukaryotic amino acid transporters.

Description

Cells need the capacity to acquire nutrients from the outside. These nutrients therefore have to cross the lipophilic cell membrane. To facilitate this, organisms have evolved a multitude of dedicated transport proteins, which can transport almost anything from small ions to big peptides.

One of the most important groups of nutrients are amino acids. As there are 20 proteogenic amino acids, one organism typically produces many transporters that each has its own substrate spectrum. In yeast and other fungi, these transporters are mainly from the YAT family: appropriately named Yeast Amino acid Transporters. These proteins are secondary active transporters; they use the natural proton gradient and membrane potential to accumulate amino acids to high concentrations inside the cell.

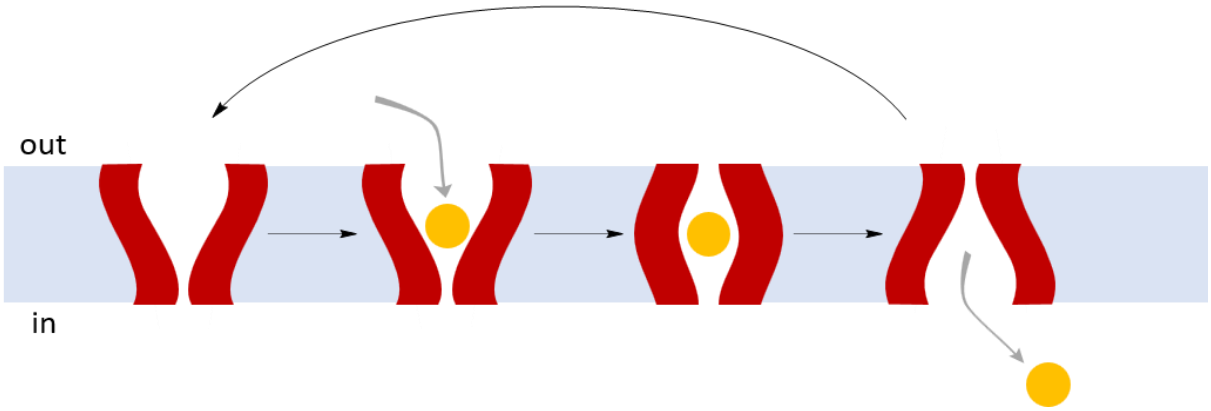

Figure 1. General reaction cycle of the alternating access mechanism of many transporters.

The YAT family is part of the APC (Amino acid-Polyamine-Organocation) superfamily. As such, it adopts a structure that is composed of 12 transmembrane helices that are composed of an inverted repeat of five helices plus two additional ones. This is the same general fold as, e.g., the human neurotransmitter transporters and the bacterial leucine transporter LeuT. However, there is no experimentally determined high-resolution structure for any of the YAT members. Such a structure would be very helpful to determine some interesting aspects of amino acid transport: What determines the substrate specificity of these transporters? How is it that some YAT members transport their substrate preferably in one direction, even if the membrane potential is reversed?

In this project, you will work towards the goal of obtaining a high-resolution structure of the lysine transporter Lyp1 from the yeast *Saccharomyces cerevisiae*. We have established an expression and purification protocol for the transporter and are now in the process of raising nanobodies to stabilize the protein. The structure will be determined by cryo-electron microscopy (cryo-EM) at our in-house facilities. You can participate in all aspects of the process, and your exact role will be tailored to your interests and background.

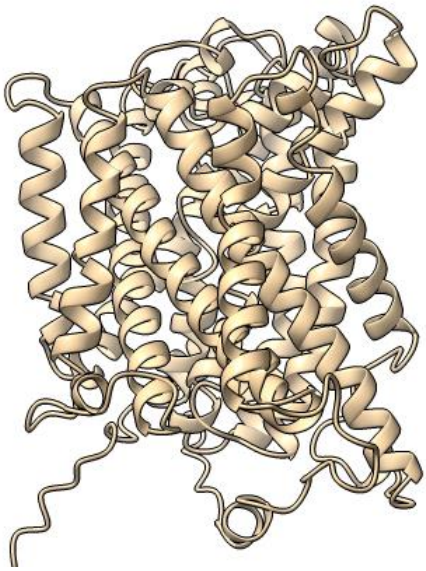

Figure 2. Predicted structure of the yeast lysine transporter Lyp1 (AlphaFold).

Techniques you can learn

- *Pichia pastoris* and *Saccharomyces cerevisiae* culture
- Membrane protein expression and purification
- Selection of nanobodies from a synthetic library
- Preparation of samples for cryo-EM
- Liposome reconstitution and radiolabeled uptake assays

References

Bianchi, Frans, Joury S. van't Klooster, Stephanie J. Ruiz, and Bert Poolman. "Regulation of Amino Acid Transport in Saccharomyces Cerevisiae." *Microbiology and Molecular Biology Reviews* 83, no. 4 (November 20, 2019). https://doi.org/10.1128/MMBR.00024-19.