Engineering the activity and specificity of *Saccharomyces cerevisiae* Acetate Transporter Ady2/Ato1

Toni Rendulic[1,2], João Alves[1], Isabel Soares-Silva[1], Elke Nevoigt[2], Margarida Casal[1]

[1]Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, Braga, Portugal

[2]Department of Life Sciences and Chemistry, Jacobs University Bremen, Campus Ring 1, Bremen, Germany

Organic acids are industrially relevant chemicals with application in polymer, food, agricultural and pharmaceutical sectors. Yeasts commonly represent the organisms of choice for production of organic acids, namely due to their tolerance of low pH environments since such production conditions allow for direct formation of the desired protonated form of the acid and thus cut downstream processing costs. Since organic acid export over the plasma membrane represents one of the key steps in microbial production of these compounds, organic acid transporters started receiving greater attention in metabolic engineering strategies.

Ato1 is the main transporter responsible for uptake of acetic acid into the cytosol in *S. cerevisiae*, while also being able to mediate organic acid transport in the opposite direction, as it was shown to be involved in the export of lactic acid from *S. cerevisiae* cells engineered for lactic acid production. Ato1 is a member of the Acetate Uptake Transporter Family (AceTR), with several functionally characterized homologues in yeast, fungi, and bacteria. Recently solved crystal structure of its bacterial homologue, SatP, depicts a hexameric anion channel.

In this work, we studied the relationship between structure and function of Ato1 via rational mutagenesis and identified residues critical for Ato1 substrate specificity and transport activity. By utilizing computer-assisted three-dimensional modelling tools, we provide possible explanations of acquired features. Our final goal is to test applicability of these transporters in yeast cell factories that produce organic acids.