In eukaryotic cells, amino acids serve as the building blocks of proteins as well as substrates for energy production in mitochondria and other biosynthetic pathways. Metabolism can only to a limited extent satisfy the need for amino acids, so uptake through the cell membrane from the environment is indispensable in order to ensure homeostasis. Amino acid uptake depends on a complicated network of transporters located in the cell membrane. These proteins are characterized by their specificity for individual amino acids, as well as by their requirements for ions necessary for proper function. Some transporters are capable of concentrating amino acids inside the cell, whereas others act as exchangers, i.e. uptake of the amino acid is accompanied by the export of another amino acid. The first group drives an increase in intracellular amino acid concentration, while the second balances the intracellular pool to satisfy the specific demand for specific amino acids. This is best illustrated by the uptake of leucine and isoleucine, which are synthesized by cells in a very limited amount, and only by exchanging for glutamine can they be present in cells at a high level concentration. Transporters are also characterized by the presence of many hydrophobic transmembrane domains, and as proteins localized to the cell membrane, they are inserted into the membrane of the endoplasmic reticulum cotranslationally. After this insertion step, the maturation of many transporters diverges. Some, whose activity is independent of post-translational N-glycosylation, can directly reach the cell membrane, while others must first go through the process of adding complex sugar structures in the endoplasmic reticulum. Before these ER-glycosylated transporters are ready to deliver to the cell membrane, this latter group requires further modification of the glycan structure in the Golgi apparatus. The purpose of this application is to identify which amino acid transporters are subject to modification in the Golgi apparatus, and to define the functional meaning of the addition of N-acetylglucosamine, galactose and sialic acid residues in the *trans*-Golgi compartment. We aim to identify enzymes and other proteins which direct the modification of these transporters in the *trans*-Golgi compartment, as well as driving their delivery to the plasma membrane. An additional aspect that will be examined is how various types of stress responses modify the process of amino acid transporter modification in the *trans*-Golgi compartment.

In order to examine the contribution of the *trans*-Golgi compartment, we will use genetic tools to inactivate proteins responsible for providing the donor sugar nucleotides during glycan synthesis in this compartment. As the expression of many transporters is limited to certain cell types, but absent in fibroblasts and myocytes. We will exogenously express transporters in cells with dysfunctional *trans*-Golgi compartment, and investigate whether they require *trans*-Golgi modification for normal function. In order to identify proteins involved in the modification of transporters, we will use BioID technology. This experiment will require the fusion of the transporter with a biotin ligase, which can then interact with and biotinylate nearby proteins in the cell. These biotinylated proteins can be isolated using the affinity of biotin to immobilized streptavidin, and identified using mass spectrometry.

The importance of amino acid transporters arises from their role in maintaining amino acid homeostasis as well as their involvement in diverse pathological processes, including cancer. Many cancer cells overexpress amino acid transporters to ensure continuous unlimited growth. Our goal is to understand how modification that occurs in the *trans*-Golgi compartment affects the activity of these transporters in tumor cells. In addition, we want to understand how changes in the glycosylation activity in the *trans*-Golgi relates to the decrease in the activity of amino acid transporters contributing to loss of muscle mass (sarcopenia) over the course of liver diseases associated with an increase of ammonium in the bloodstream.