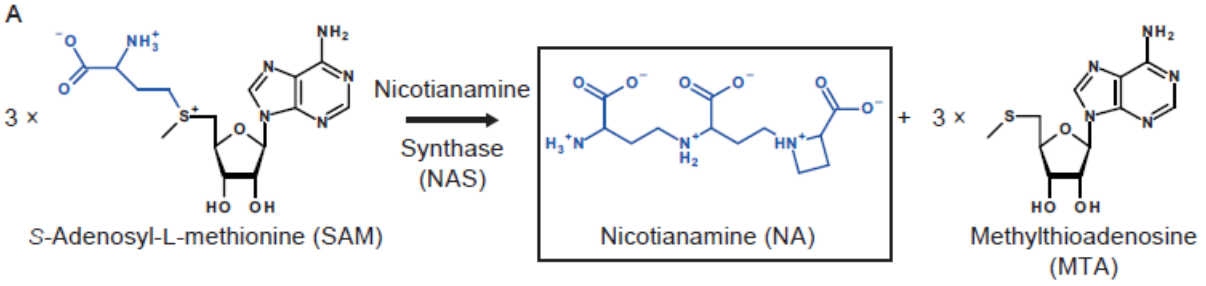
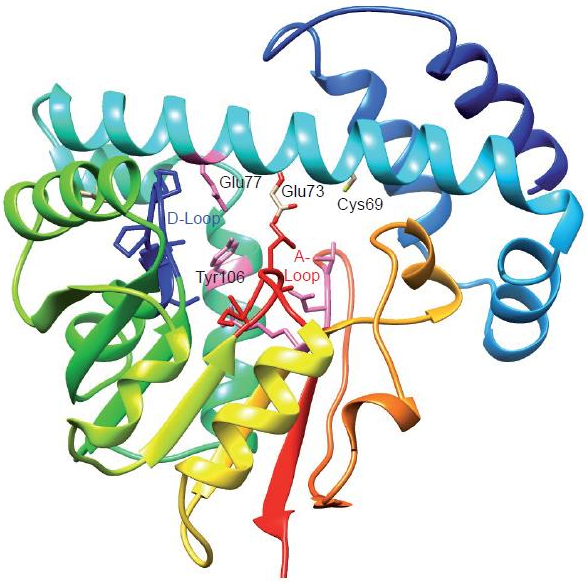
Project Ute Krämer

**Biosynthesis of the metal chelator molecule nicotianamine**

**Background and preliminary work:** The Krämer group has discovered that a plant membrane transport protein, *Arabidopsis thaliana* Zinc-Induced Facilitator 1 (ZIF1), mediates the vacuolar sequestration of nicotianamine (NA). NA is a non-proteinogenic amino acid that acts as a high-affinity metal chelator molecule in metal homeostasis of plants and filamentous fungi. Furthermore, NA has promising anti-hypertensive pharmacological activity. Roots of *Arabidopsis thaliana* overexpressing *AtZIF1* accumulate ~30-fold enhanced NA concentrations. Nicotianamine synthases (NAS) of plants and filamentous fungi convert three S-adenosylmethionine (SAM) molecules to one NA molecule. NAS catalyses several consecutive reactions in a single active site, as concluded based on crystal structures of an NAS-like archaeal thermoNAS. Its reaction product, thermoNA, contains a glutamate instead of the terminal SAM-derived unique azetidine-2-carboxylate moiety that is characteristic of NA. NAS proteins were first identified in barley and other plant species, and only later in filamentous fungi. More recently, *NAS*-like genes were found to be part of a *Pseudomonas aeruginosa* operon essential for survival on human airway mucus secretions, and to act in the synthesis of an NA-related compound of *Staphylococcus aureus* that utilizes only a single SAM molecule. Interestingly, no proteins acting in the catabolism of NA are known. A possible NA breakdown product – the toxic proline analogue and allelopathic chemical azetidine-2-carboxylate – was detected in only a few plants to date.




**Work planned:** The proposed project will address **structure-function relationships of fungal and plant NAS proteins** and **explore avenues for enhancing cellular NA production**. To identify residues acting in intermediate formation and azetidine ring closure, a combination of site-directed mutagenesis and biochemical *in vitro* activity assays will be conducted based on protein structural modelling (cooperation with Eckhard Hofmann; see Figure on the right). Furthermore, the respective roles in biosynthetic efficacy of a possible membrane association of some NAS proteins and of product compartmentalization will be compared between plant and fungal NA production systems (cooperation with Ulrich Kück). In an international collaboration (Pascal Arnoux, France), we will address the unknown physiological functions of archael and bacterial NA-like metabolites. In preparation for this project, the Krämer group has established heterologous expression of NAS proteins in *E. coli*, their purification and *in vitro* enzyme assays, as well as site-directed mutagenesis of NAS proteins.




**Selected references:**

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