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UNIVERSITY LINZ

Master/ Bachelor Thesis Project
Protein Engineering Group
Department of Molecular Biophysics and Membrane Biophysics

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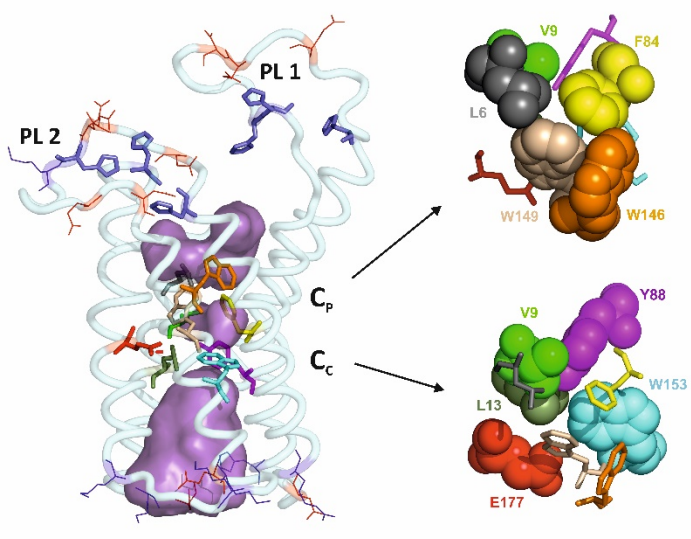
PROTEIN CHARACTERIZATION
In vitro vesicle assays

More than 50% of the world's population is infected with *Helicobactor pylori*, a pathogenic bacterium responsible for numerous gastroduodenal disorders such as chronic gastritis, peptic ulcer disease and gastric cancer. Standard treatment comprises the use of a proton-pump-inhibitor in combination with two antibiotics but struggles with increasing therapy failures. A different therapeutic toehold might pose *H. pylori*'s life insurance, a small proton-gated inner membrane channel, *Hp*UreI. It ensures survival in the acidic gastric juice, by means of urea transport from the periplasm to the cytoplasm, where urea is hydrolyzed by urease. The hydrolysis products, ammonia and carbon dioxide, in turn presumably buffer the cytoplasm to a neutral pH and the periplasm to pH 6.1.



HpUreI monomer; Inner surface representation of HpUreI (pdb code: 3UX4) with its two constriction sites (Cp and Cc). Periplasmic loops PL1 and PL2 constiute HpUreIs pH sensor (protonatable His residues are depicted in blue sticks).

The channel manages to conduct and select urea-like molecules, water and potentially ammonia, while it is assumed to exclude protons from the gastric juice. Facilitation of proton transport would be lethal to H.pylori since the acidification of the cytoplasm would result in the breakdown of the proton-motive-force across the inner membrane of HpUreI.

We plan to investigate *Hp*UreIs selectivity mechanism using protein mutants and *in vitro* assays after protein reconstitution into lipid vesicles.

We are looking for highly motivated students, with a background in biochemistry, biophysics or molecular biology to join our group.

Tasks to be performed by the student:

- *Literature Research*
- *Site-directed mutagenesis*
- *Overexpression & Purification (E. Coli)*
- *Reconstitution*
- *Functional assays*
- *Data analysis & interpretation*