MS8-O4 Architecture and regulation of a redox-driven Na^+-pump from the pathogen *Vibrio cholerae*

Guenter Fritz[1]

1. University of Freiburg, Institute of Neuropathology

email: guenter.fritz@uniklinik-freiburg.de

The human pathogen *Vibrio cholerae* maintains a Na^+ gradient across the cytoplasmic membrane. The generated sodium motive force is essential for substrate uptake, motility, pathogenicity, or efflux of antibiotics. This gradient is generated by an integral membrane protein complex, the NADH:ubiquinone oxidoreductase (NQR). The NQR complex is conserved in many other bacterial pathogens and might therefore represent a novel and promising target in antibacterial therapy.

In order to get insights into the mechanism of redox driven Na^+-transport we have isolated and crystallized the NQR of *Vibrio cholerae* [1]. The crystals of the entire membrane complex diffract to 3.5 Å. Moreover, we determined independently the structures of the major soluble domains of subunits NqrA, C and F at 1.9 Å, 1.6 Å and 1.7 Å, respectively, completing large parts of the structure of the respiratory complex at high resolution [1]. The structural information allows the detailed analysis of the ion translocation pathway across the membrane and of the coupling between redox and translocation reactions. Moreover, we recently determined two additional structures of the complex in different states representing two further snapshots of the pumping cycle including a regulatory K^+ binding site that facilitates large conformational changes required for Na^+ pumping.

References

[1] Steuber, J., Vohl, G., Casutt, M.S., Vorburger, T., Diederichs, K., Fritz, G. (2014) *Nature,* 516,62-67

[2] Steuber J., Vohl G., Muras V., Toulouse C., Claußen B., Vorburger T., Fritz G.(2015). *Biol Chem*., 396, 1015-30

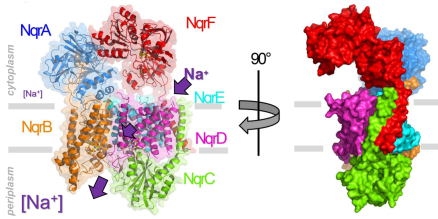

Figure 1. Overall structure of Na^+-NQR. The Na^+-NQR complex is composed of six subunits NqrA-F. The membrane plane is indicated by grey lines.

Keywords: respiratory complex, Vibrio cholerae

MS8-O5 Molecular basis of secondary multidrug transport by X-ray crystallography

Vincent Debruycker[1]

1. Université libre de Bruxelles - Université d'Europe, Brussels, Belgium

email: vdebruyc@ulb.ac.be

The Major Facilitator Superfamily groups a vast number of secondary transporters that import or export distinct substrates. Among these, multidrug antiporters constitute a peculiar class, not only because of their multispecificity, recognizing structurally very diverse substrates, but also due to their transport mechanism, that relies on bilayer-mediated extrusion of cytotoxic compounds. Using X-ray crystallography, we investigate the molecular basis of secondary multidrug transport of *LmrP*, a Major Facilitator Superfamily multidrug transporter from *Lactococcus lactis*. Through extensive screening and optimization of the lipidation state of *LmrP* we were able to produce crystals of LmrP-ligand complex diffracting at resolution up to 3Å. The protein has been co-crystalized with Hoechst 33342 in the outward open conformation. The structure unveils the presence of a lipid molecule in the substrate binding site and suggest a functional role of this lipid. In parallel to this, preliminary result show that point mutations and nanobodies targeting defined conformational state of LmrP are promising tools to determine the high resolution structure of distinct conformations of the transporter.

Keywords: multidrug resistance, MFS, membrane, transporter