Two master thesis positions in Structural & Molecular Biology on the Na^+/Ca^{2+} exchanger (NCX)

Regulation of intracellular Ca^{2+} is crucial for sustained contraction and relaxation in the heart. In this process, Na^+/Ca^{2+} exchange is the dominant Ca^{2+}-efflux mechanism that rapidly ejects large amounts of Ca^{2+} from the cytosol after the muscle contraction phase (**Fig. 1**). Mishandling of cytosolic Ca^{2+} often causes contractile dysfunction and heart arrhythmias that can lead to heart disease, which is the leading cause of death in the western world.

Ion transport by NCX is mediated by two Ca^{2+} binding domains (CBDs) that sense the Ca^{2+} concentration in the cytosol. We determined the structures of the CBDs in different states and postulated a model for intact NCX [1,2]. To elucidate the molecular mechanism that links Ca^{2+} binding and release events to transport function we wish to determine the structure of intact NCX by either X-ray crystallography or electron microscopy.

To produce Ca^{2+} exchangers of mammalian and bacterial origin for structural studies, we have built an expression system in yeast on the basis of GFP fusion proteins. The GFP-fusion protein approach allows monitoring of the membrane proteins at any stage during overexpression, solubilization, purification and stabilization by conveniently measuring fluorescence.

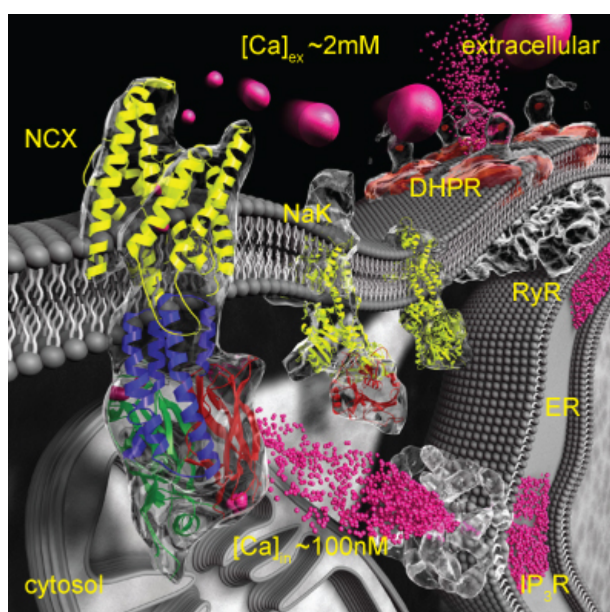

Figure 1. Ca^{2+} homeostasis: Ca^{2+} enters the cytosol through either plasma membrane L-type Ca^{2+} channels such as the DHPR and/or through Ca^{2+} release channels, the IP_3 and ryanodine receptors of the endo/sarcoplasmic reticulum. In order to maintain constant cytosolic Ca^{2+} concentrations over time, the equivalent amount of imported Ca^{2+} has to be removed again. This task is predominantly accomplished by the plasma membrane Na^+/Ca^{2+} exchanger (NCX) and the sarcoplasmic Ca^{2+} ATPase.

Until now, I established expression for ten different exchangers (five mammalian/five bacterial). Together with two master students I would like to characterize these and determine their suitability for crystallisation. In their projects master students will receive a first-class guidance and a solid introduction into the molecular biology and biochemistry of membrane proteins. This includes state-of-the-art cloning techniques, membrane protein expression in yeast and *E. coli*, their evalution using a new GFP-approach (in-gel fluorescence, confocal fluorescence microscopy) and their purification using affinity and fluorescence size exclusion chromatography. With the suitable candidates we will set up 2-D and 3-D crystallisation trials.

Highly motivated students are kindly invited to contact me and arrange a detailed discussion of the projects.

Dr. Mark Hilge
C-CINA, University of Basel,
Mattenstrasse 26
CH-4058 Basel
Phone: 061 387 32 14
e-mail: markhilge@me.com
http://www.mark-hilge.com

[1] **Hilge, M.***, Aelen, J. & Vuister, G.W. (2006) Ca^{2+} regulation in the Na^+/Ca^{2+} exchanger involves two markedly different Ca^{2+} sensors. *Mol Cell* **22** (1), 15-25.

[2] **Mark Hilge***, Jan Aelen, Alice Foarce, Anastassis Perrakis & Geerten W. Vuister (2009) Ca^{2+} regulation in the Na^+/Ca^{2+} exchanger features a dual electrostatic switch mechanism. *PNAS* **106**, 14333-14338.