[MS7-P03] Cardiotonic steroids and the Na^+/K^+-ATPase. Jonas Lindholt[ab], Mette Laursen[ac], Linda Reinhard[ab], Natalya Fedosova[ac], Poul Nissen[ab]

[a]*Centre for Membrane Pumps in Cells and Disease – PUMPKIN, Danish National Research Foundation, Denmark*, [b]*Department of Molecular Biology and Genetics, Aarhus University, Denmark*, [c]*Department of Biomedicine, Aarhus University, Denmark*
E-mail: lindholt@post.au.dk

The Sodium Potassium Adenosine Triphosphatase (Na^+/K^+-ATPase) is a plasma membrane protein present in all animal cells. Its function is vital for the cell - from sustaining the resting potential to regulation of the cell volume. In a cyclic manner the Na^+/K^+-ATPase pumps three sodium ions out and two potassium ions in across the membrane at the expense of hydrolysis of one ATP molecule. Hereby, the positively charged ions are net transported against the gradient creating the basis for the secondary active transport. The Na^+/K^+-ATPase consists of two subunits, the catalytic α subunit and the chaperone β subunit. A representative of the FXYD family is often involved in the regulation. Cardiotonic steroids (CTS) of natural origin are used in treatment of arrhythmia and congestive heart failure. CTS, such as ouabain, digoxin and digitoxin, bind to the α subunit of the Na^+/K^+-ATPase with high affinity and inhibit the enzymatic activity. The structure of the Na^+/K^+-ATPase in complex with ouabain was first reported to a resolution of 4.6 Å [1], and has later been improved to 3.4 Å [2]. This project addresses specifically the role of the glycosylation of the cardiotonic steroids for their affinity towards the Na^+/K^+-ATPase. Therefore, the complexes of the Na^+/K^+-ATPase with two other cardiotonic steroids (digoxin and digitoxin) are crystallized. These CTS have additional sugar moieties attached to the steroid core compared to ouabain. Currently, a complete data set has been collected to 5.5 Å for the digoxin complex. The electron density reveals that it is positioned between the transmembrane helices 1 through 6 in the α subunit, in the same binding pocket as ouabain. Crystal optimization is on-going. Improvements of these crystallisation experiments will serve as a template for crystallisation of the Na^+/K^+-ATPase in complex with other cardiotonic steroids.

[1] Yatime, L., Laursen, M., Morth, J. P., Esmann, M., Nissen, P. & Fedosova, N. U. (2011 *). J. Struct. Biol.*174, 296-306. [2] Laursen, M., Yatime, L., Nissen, P. & Fedosova, N. U. (2013) *PNAS* (in press).

Keywords: sodium potassium atpase; membrane protein crystallization; cardiotonic steroids