MS8-P3 **SAXS analysis of membrane proteins reconstituted into nanodiscs.** Michael Järvå,[a] Pie Huda,[b] Nicholas Skar-Gislinge,[b] Lise Arleth,[b] Susanna Törnroth-Horsefield,[a] *[a] University of Gothenburg, Dept. Of Chemistry and Molecular Biology, Sweden, [b]University of Copenhagen, Dept. of Basic Sciences and Environment, Denmark*
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Phospholipid bilayers host and support the function of membrane proteins. Today the standard process of isolation and purification of membrane proteins is to solubilize the membranes in detergent, replacing the lipid environment with detergent micelles. Not only does this raise the question of whether or not the structure and function remains the same in this micelle; the detergent more often than not interferes and hinders many analytical tools and techniques. For functional studies reconstitution of membrane proteins into liposomes is the most common technique. It is a system that is hard to control, reproduce and are prone to large variations due to small changes in experimental variables. The development of self-assembled discoidal phospholipid bilayer nanoparticles (Nanodiscs) is therefore an intriguing new tool for structural and functional studies.[1] Membrane scaffold proteins (MSP) are mixed lipids and detergent. Upon removal of detergent two MSPs fold together and form a disc containing a lipid bilayer. The size of the disc is narrowly defined by the choice of MSP (8-15 nm).[2] This uniformity and reproducibility together with the small size makes this system ideal for reconstitution of membrane proteins. Here a reconstitution protocol using a spinach aquaporin (SoPIP2;1) has been developed. SoPIP2;1 was chosen as a model protein to evaluate the nanodisc system using SAXS. Initial crystallization trials have also been started.

[1] Ritchie et al, Methods in Enzymology, **464**, 211-31
[2] Skar-Gislinge et al, Journal of the American Chemical Society, **132**(39), 137 13-22

Keywords: membrane proteins; saxs; nanodiscs

MS8-P4 **Cardiotonic Steroids and the Na/K-ATPase.** Jonas Lindholt,[ab]Linda Reinhard,[ab]Poul Nissen,[ab][a]*Centre for Membrane Pumps in Cells and Disease, Danish National Research Foundation, Denmark [b]Department of Molecular Biology and Genetics, Aarhus University, Denmark*
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The Sodium Potassium Adenosine Triphosphatase (Na/K-ATPase) is a plasma membrane protein present in all animal cells and its action is fundamental for many processes: from energizing secondary transporters to sustain the resting potential. The Na/K-ATPase consists of two subunits; the catalytic á subunit and the regulatory β subunit.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 one ATP being hydrolysed. Cardiotonic steroids, such as ouabain, digoxin and digitoxin, are well known to bind to the á subunit of the Na/K-ATPase. Upon binding, the steroids inhibit the pumps functional activity. A 4.6 Å resolution structure of the pig Na/K-ATPase in complex with ouabain has previously been reported [1]. We aim to expand the structural studies on the Na/K-ATPase in complex with other cardiotonic steroids using protein purified from bovine kidneys. A purification protocol for this has been established. Crystallization conditions of the Na/K-ATPase in complex with six different cardiotonic steroids have been identified. A complete X-ray diffraction data set of the bovine Na/K-ATPase in complex with ouabain is available to a resolution of 4.8 Å, whereas anisotropic diffraction to a resolution of 4.3 Ĺwas observed. The space group $P222_1$ with unit cell parameters of 65.0, 300.6, 241.2 Å – which presents a space group not obtained for our Na/K-ATPase.Optimization of the crystallization conditions is currently in progress.

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

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