Recent advances in preparation of perdeuterated crystals for neutron diffraction and detectors have significantly decreased the required volume for measurement of useful data. This has opened the way to study biological systems with crystal volumes around 0.1-0.2 mm^3, as shown by our studies of type III antifreeze protein (AFP, 7 kDa). The highly homologous AFP sub-family shares the capability to inhibit ice growth in vivo at subzero temperatures. X-Ray crystallography studies have shown an Ice Binding Surface (IBS) through which the AFP binds the ice nuclei. The details of the antifreeze mechanism remain still unclear due to the known difficulty in identifying hydrogen atoms and disordered water molecules with X-ray diffraction data alone. Therefore, neutron studies were started. Perdeuterated crystals were produced and their qualities, together with the structural identity vs. the hydrogenated protein form, were checked by synchrotron X-ray data collection up to 1.05Å resolution at 293K. Neutron Laue diffraction data were collected up to a resolution of 1.85Å at 293K on the new Laue Diffractometer LADI-III at ILL in Grenoble with a “radically small” crystal of volume 0.13 mm^3 [1]. The structure has been refined using a joint X+N algorithm, the water molecules facing the IBS have been identified and a model of the IBS-ice interface has been built. Experimental details and current status of the project will be described. This example highlights the capability of Neutron Protein Crystallography to study biological systems at both the protonation and hydration levels with “tiny” perdeuterated crystals. This overcomes the major bottleneck of the large crystal volume needed so far for neutron diffraction, opening new perspectives to the structural biology community.

[1] I. Petit-Haertlein, M. Blakeley, E. Howard, I. Hazemann, A. Mitschler, M. Haertlein and A. Podjarny *Acta Cryst.*. F**65**, 406–409, 2009

Keywords: neutron crystallography, antifreeze proteins, macromolecular interactions

FA1-MS06-T05

Characterizing subtype specificity in nAChRs : hints to smoking cessation. Prakash Rucktooa[a], Titia K. Sixma[a]. [a]*Netherlands Cancer Institute, The Netherlands.* E-mail: p.rucktooa@nki.nl

Tobacco smoking is a major cause of mortality and accounts for around 30% of cancer related deaths in developed countries. Tobacco dependence arises from inhaled nicotine, an alkaloid which activates dopaminergic reward pathways by targeting nicotinic acetylcholine receptors (nAChRs) in the central nervous system. These nAChRs are members of the Cys-loop receptor family of ligand-gated ion channels, and form functional transmembrane protein assemblies composed of homo- or heteropentamers. Nicotine can target different nAChR subtypes but displays high affinity binding for the α4β2 nAChR subtype. Various nicotine replacement therapeutics have been devised to target the α4β2 nAChR subtype in order to help in smoking cessation. However, the variety of existing nAChR subtypes, together with the lack of high resolution structures for these proteins contribute to an as yet unresolved ligand selectivity issue.

Our goal resides in the structural and biophysical characterization of nicotinic acetylcholine receptor ligand binding sites and aims at defining those residues key to receptor subtype selectivity. We have used molluscan acetylcholine binding protein (AChBP), homologous to the extracellular ligand binding domain of nAChRs, as a surrogate to investigate the binding mode of different α4β2 selective compounds. We have solved structures of *Aplysia californica* AChBP in complex with cytisine and with varenicline, two anti-smoking compounds, and have compared the respective binding modes to that observed for an AChBP-nicotine complex. We have further investigated the importance of different binding site residues likely to account for subtype selective ligand binding in nAChRs.

Structural and biophysical data extracted from this study will allow us to better apprehend the nature of different nicotinic acetylcholine receptor binding sites, and particularly aspects that bring about ligand binding specificity. These data will be invaluable in terms of future drug design prospects.

Keywords: nicotinic acetylcholine receptors, ligand binding, x-ray structure