ESRF	**Experiment title: Myelinated nerves – Structure and elemental composition of the nodes of Ranvier**	**Experiment number**: MD 352
Beamline: ID 21	**Date of experiment**: from: 11.02.2009 to: 17.02.2009	**Date of report**: 31.08.2009
Shifts: 18	**Local contact(s)**: Jean Susini and Margaret Rak	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Tanja Ducic*, Tim Salditt*, André Beerlink*, Matthias Bartels*, Joachim Herbst

Institute for x-ray physics
Friedrich-Hund-Platz 1
37077 Göttingen
Germany

Report:

All details about chemical and structural organisation of the multilamellar structure of myelin sheath which wraps around the axon in the central (CNS) and peripheral nervous systems (PNS) are still not known [1, 2]. This structure enables the high velocity of nerve signal conduction in higher animals. Myelin structure and respective structural changes are associated with a number of neural diseases. An understanding of molecular mechanisms in myelin assembly and dissassembly, differentiation, ect. necessitates a highly local probe with two separate length scales, the first length scale is given by the membrane distance d in the multilamellar architecture in the range of 15 to 20 nm, the second length scale is the resolution element which defines in which area (volume) this information is probed. Each myelin sheath segment or internode appears to be 150–200 μm in length; internodes are separated by spaces where myelin is lacking, the nodes of Ranvier. The nodes of Ranvier play a major role in nerve impulse conduction. However, to date components and structural information is won only at the expense of quite invasive sample preparation, requiring thin slicing and fixation of the sample which can easily introduce artefacts. Classical X-ray diffraction has also been used to a large extent to characterize the structure of myelin, but as an ensemble averaging technique, it can tell us more about the native state of this important organelle in CNS.

This work is part of the programme of the recently founded excellence cluster Molecular Physiology of the Brain, and is carried out in collaboration with Prof. K.-A. Nave, MPI Experimentelle Medizin, Göttingen.

Here we made full use of recent progress in synchrotron x-ray optics to map the orientation, and the elemental composition of myelin, using spectromicroscopy and infrared spectroscopy (FTIR), in particular close to the node of Ranvier.

Our preliminary results obtained from test measurements at ID21 showed high accumulation of phosphorus compounds (PC) in the node of Ranvier (Fig. 1.) It is known that these parts of neurons are rich in sodium channels, but high concentration and strong location of PC deserve further investigations. Adenintriphosphate (ATP) would be a possible candidate to explain this result. Here we performed experiment with taking fluorescence maps, complemented by absorption spectra at the Fe edge (spectromicroscopy) by using recently developed cryo-stage at ID 21 and FTIR spectra to specify the origin of this signal at the Ranvier node.

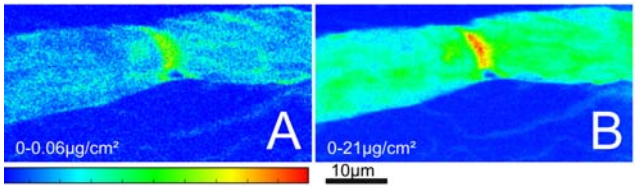

Fig.1. *Na (A) and P (B) distribution* and their concentration in µg/cm².Image 50 x 25 µm 246x125 pixels with a dwell time 180 ms per pixel. E=2.9 keV (ESRF ID21).

Neurophysiology/sample preparation: The experimental model- the sciatic neuron separated to the single fibres mechanically and shock freezes the samples in liquid ethane, and stored under liquid nitrogen before measurements.

Results

We examined the elemental distribution and composition of myelinated neuron in the vicinity of the node of Ranvier. In particular our goal was to clarify the phosphorous species which we detected recently in a test measurement, see Fig. 1. To identify which metabolites or macromolecules are responsible for this component, we used an additional synchrotron FTIR spectroscopy study at ID21. This method of vibrational spectroscopy provides direct information on the molecular level, is cost-effective, and can be universally applied for biomacromolecules and for whole cells under near-physiological conditions [4]. We used the synchrotron radiation-based Fourier transform infrared (SR-FTIR) spectromicroscopy at ID21, which is one of few instruments is a newly emerging bioanalytical and imaging tool. This unique technique provides mid-infrared (IR) spectra, hence chemical information, with high signal-to-noise at spatial resolutions as fine as about 5 µm. Thus it enables researchers to locate, identify, and track specific chemical events within an individual neural cell. It was presented several examples to demonstrating the application potentials of SR-FTIR spectromicroscopy in biomedical research [4]. We examined phosphorus compounds, but also protein and lipids composition in the surrounding area of node of Ranvier with both, high spatial and high spectral resolution (Fig. 2).

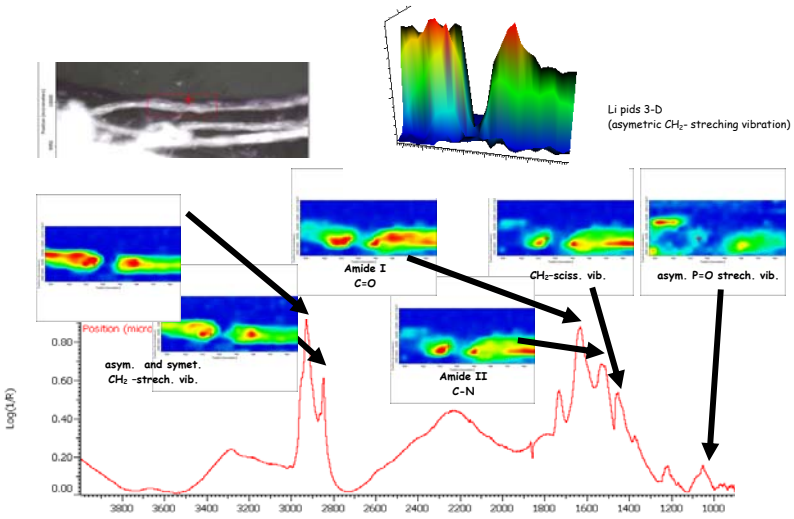

Fig. 2. *FTIR microscopy and summarized spectra of freeze-dried single isolated axon.*

Using different high resolution microscopical techniques combined with X-ray microscopy we were able to perform elemental and biomacromolecular composition of myelinated axons of sciatic neuron. Recently we showed element mapping on freeze dried olfactory neural cell carried out at room temperature [3]. Here we demonstrate measurements performed under the cryo conditions at ID21 at ESRF, what allowed us to perform measurements under the most physiological condition. Element mapping and summarized spectra of neuron in the vicinity of node of Ranvier is shown in Figure 3. Additionally we were able to show native *in situ* present of trace elements like Mn and Fe without any additional staining (data now shown).

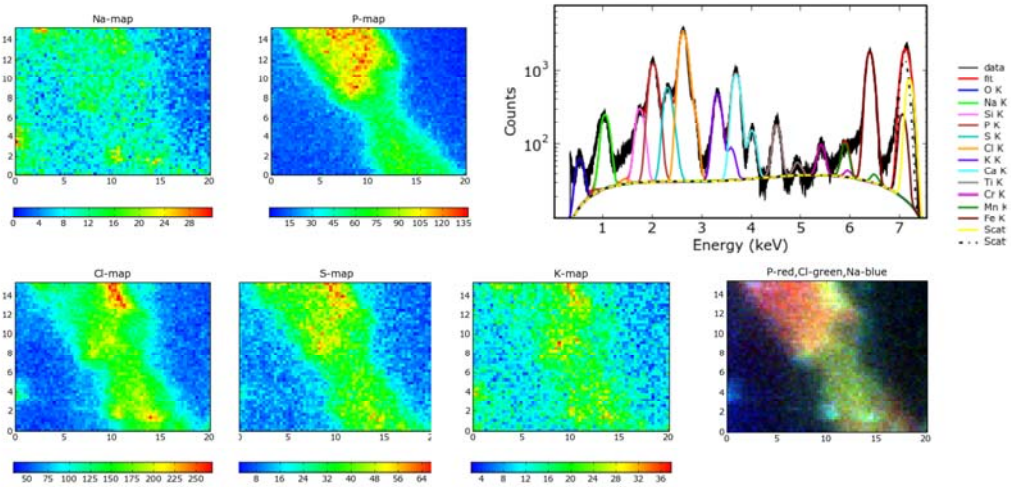

Fig. 3. *Native cryo-measurement in the single isolated myelinated axon in the vicinity of Ranvier node: Na, P, Cl, S and K maps, overlaying image of P, Cl and Na, and their corresponding spectra. Image size 20 x 15 µm, steps size 300nm with dwell time 300 ms per pixel. E=7.2 keV (ESRF ID21).*

References

[1] Peteres A. Observations on the connections between myelin sheaths and glial cells of the optic nerve of young rats. *J Anat* 98: 125–134, 1964.

[2] Boiko T. and Winckler B. Myelin under construction—teamwork required. *J. Cell Biol.* 172:799-801, 2006.

[3] Dučić T., Breunig E., Schild D., Herbst J., Novakova E., Susini J. , Tucoulu R., Salditt T. (2009) X-ray fluorescence microscopy of olfactory receptor neurons. Journal of Physics: Conference Series.

[4] Holman H-Y N, Martin MC, Blakely EA, Bjornstad K, Mckinney WR. IR spectroscopic characteristics of cell cycle and cell death probed by synchrotron radiation based Fourier transform IR spectromicroscopy. *Biospectroscopy* 6: 329-335, 2000.