



	Experiment title: The transduction compartments of the olfactory system: structure and ion distribution	Experiment number: MD306
Beamline: ID22Ni ID21	Date of experiment: from: 07.11.2007 to: 12.11.2007 from: 21.11.2007 to: 27.11.2007	Date of report: 28.02.2008
Shifts: 15 and 18	Local contact(s): Rémi Tucoulu and Jean Susini	<i>Received at ESRF:</i>
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Report:

The aim of this experiment was the structural and functional characterization of the olfactory system of *Xenopus laevis*. The transduction compartments of olfactory signal processing are either stereocilia or microvilli issuing from the dendrite of an olfactory sensory cell into the mucus [1]. In our experimental model both kinds of transduction compartments were present. As both stereocilia and microvilli have diameters between 100 and 200 nm, conventional optical microscopy is not sufficient to elucidate their fine structure, but together with another analytical techniques we would be able to elicit the structure and ion concentrations in these important organelles of the sensory neurons.

Our experiment was divided into two parts- the elemental distribution and elemental speciation of isolated neural-receptor cells with intact ciliae by scanning x-ray microscopy range using the combined capabilities of the scanning transmission microscope (STXM) at ID21, as well as the nanoprobe imaging system at ID22NI. On the last beam line we probed a broad range of elemental distributions in the multi-keV photon energy range, as well as the structure in phase contrast mode using the holographic cone beam microscopy setup based on the adaptive Kirkpatrick-Baez (KB) mirror optics system.

For the neural receptor cells, measured here, the following ions are of the special importance: Cl, K, Ca, P, S, Na as well as tracing elements as Zn, Fe and Os.

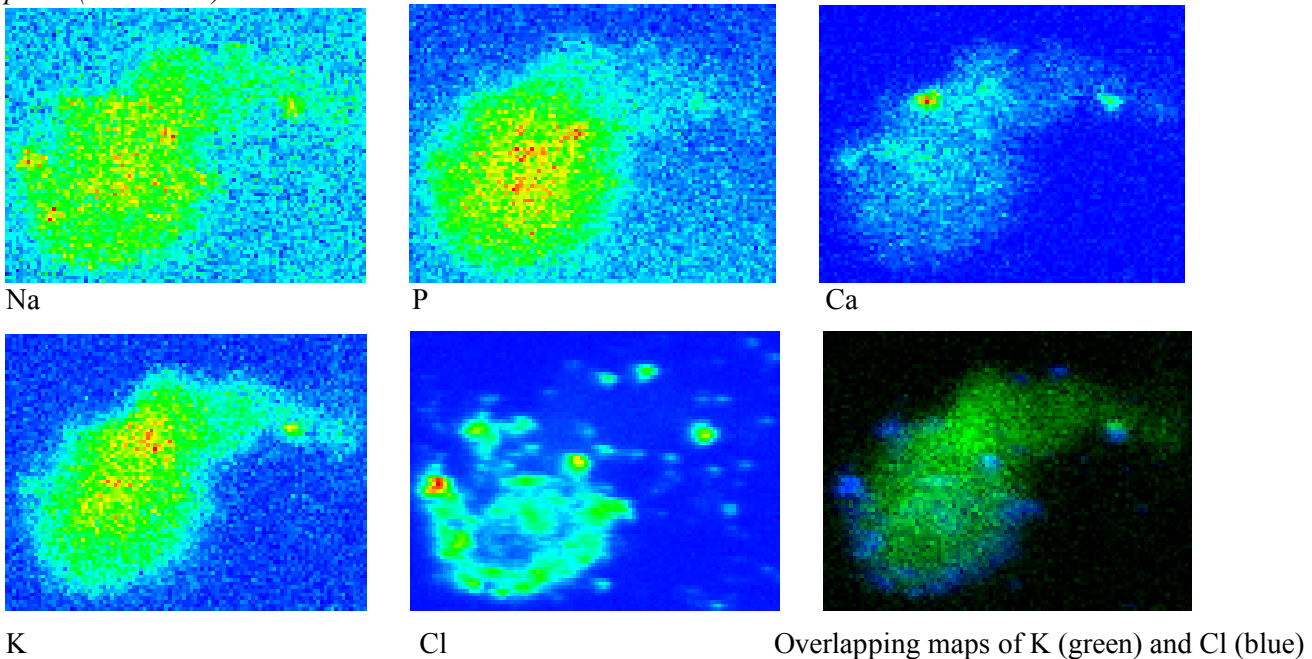
The sample of the olfactory receptor neurons (ORNs) were freshly isolated from the nasal epithelium of larvae *Xenopus laevis* stadium 54-56. The epithelial sheets from two animals were isolated according standard procedure [1, 2]. Isolated cells are gently transferred into mixed Ringer solution and 0.1M ammonium acetate buffer pH 7.8 (1:1 w:w). Suspended cells in 5 µl solution were placed on 200 nm thick Si-nitride membrane, which had been coated previously with 0.1% laminin and 0.01% poly-L-lysine. All cells used in this study were clearly identifiable before measurements as ORNs by their characteristic morphology, having a single thick dendrite with a knob-like swelling from which emanated 3–10 cilia. Samples are let for the naturally attaching on the substrate and after approximately 3 min prepared for the cryo- plunching procedure in liquid ethane. Samples are stored under liquid nitrogen until starting freeze drying at -70°C for two days and stored at room temperature in a desiccator over silica gel. Typical fluorescence mapping in the single cell is shown on Fig. 1.

Measurements on cross section of the embedded ORNs are shown on Fig. 2. These samples isolated from nasal epithelium of larvae *Xenopus laevis* were rapidly frozen in a mixture of propane:isopentane (2:1) cooled with liquid nitrogen to -196°C in an aluminium mesh. Samples were freeze-dried at -70°C for three days and stored at room temperature in a desiccator over silica gel. Freeze-dried samples were infiltrated with ether in a vacuum-pressure chamber and embedded in styrene-metacrylate using a technique specifically developed for analysis of diffusible elements. 1 µm thick sections were cut dry by glass knives, mounted on adhesive-coated 100-mesh hexagonal Cu- grits, coated with carbon, and stored over silica gel.

We used these preparations methods to scan the structurally intact organelle by a nanoprobe x-ray beam and image the ciliae in fluorescence x-ray microscopy and in phase contrast at ID21 and ID22NI, using the KB-setup. We are interesting in ions concentration and elemental mappings in the whole cell and in the ciliae organelles.

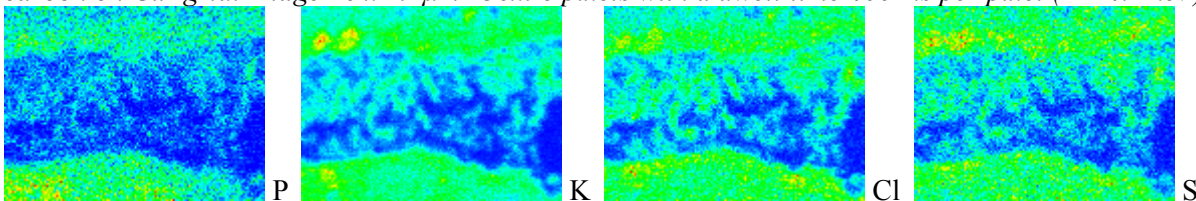
For the fluorescence analysis we used the program PyMCA (Python multichannel analyzer) for the spectrum fitting for X-ray fluorescence measurements.

Fig. 1. Sodium (Na), Phosphorus (P), Calcium (Ca), Potassium (K), Chloride (Cl) distribution and overlapped map of K and Cl. in one single isolated neural-receptor cell. Image $25 \times 15 \mu\text{m}$ 100×70 pixels with a dwell time 900 ms per pixel ($E=4.1 \text{ keV}$).



Different elemental distribution is shown on the Figure 1. On the special interest is distribution of Cl in this system because of the signal transduction. The main result of these measurements was localization of Cl in the cell. According earlier literature Cl was expected to be located on the ciliae part of the cell. We found out that Cl is mostly localized in the cell soma. This can be deduced in particular from isolated cell (Fig. 1.) Recently first time immunocytochemistry methods indicate that $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ cotransporter is located on the somata and dendrites of ORNs rather than the cilia, where transduction occurs [4]. In our analysis Na, K or Ca and Cl were distributed on different locations in the cell and do not overlap (Fig.1).

Fig. 2. Phosphorus (P), Potassium (K), Chloride (Cl) and Sulfur (S) distribution in the tissue of neural-receptor cells. Zoom in the small part ($26 \times 19 \mu\text{m}$) of the sample embedded in styrol/methacrilate and cut $1 \mu\text{m}$ thick, coated with carbon on Cu- grid. Image $26 \times 19 \mu\text{m}$ 130×96 pixels with a dwell time 400 ms per pixel ($E=4.1 \text{ keV}$).



Different ions topography is remarkably in the ORNs cell. Thus, a single functional architecture and chlorine specific distributions in the cell soma connections with membrane $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ or Ca- transporters deserve another (also molecular biology) investigation. The data analysis is in progress [5].

References

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