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Duration of training: 3 Months

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During my stay at Milosevic’s lab at European Neuroscience Institute (ENI) in Göttingen, Germany, I focused on phosphatidylinositols (PtdIns; PI), an important lipid that is especially abundant in brain tissue. PtdIns are the precursor of phosphoinositides (PIP) and its phosphorylation leads to several products as phosphatidylinositol-4,5-biphosphate (PtdIns(4,5)P$_2$ or PIP$_2$), on which I based my research. PI(4,5)P$_2$ represents only 1% of the lipids in the plasma membrane. It is a precursor of three second messenger: a) inositol-1,4,5-triphosphate (Ins(1,4,5)P$_3$); b) diacylglycerol (DAG) and c) PtdIns(3,4,5)P$_3$. PI(4,5)P$_2$ has diverse functions, as playing a role in endocytosis, exocytosis, actin-binding proteins, ion-channel activation, etc.$^{1,2}$.

PI(4,5)P$_2$ can regulate fusion via syntaxin-1 and synaptotagmins as a fusion restraint, or as an essential cofactor with a role in recruiting proteins to facilitate SNARE-dependent fusion.$^3$ Yet, syntaxin-1 clusters are facilitated by electrostatic interactions with PI(4,5)P$_2$. This high accumulation of PI(4,5)P$_2$ was required for syntaxin-1 sequestering, as destruction of PI(4,5)P$_2$ by the phosphatase, synaptotan-in-1, reduced syntaxin-1 clustering.$^6$ Munc-18, a cytosolic SM protein universally involved in all intracellular membrane fusion reactions, binds tightly to syntaxin-1 in the “closed” conformation, preventing the formation of the SNARE complex.$^5$ $^6$ Munc-18 can also bind to syntaxin-1 in its “open” conformation via the N-peptide, when syntaxin-1 is part of the SNARE complex.$^7$

Hence, we proposed to test the hypothesis that absence of Munc18-1 in neurons changes PI(4,5)P$_2$/syntaxin-1 clusters by disturbing syntaxin-1 levels and localization.

At Milosevic’s lab I studied, using 2-color STED (Stimulated Emission Depletion) super resolution microscopy available at the nearby Max Planck Institute for Biophysical Chemistry in the laboratory of Prof S. Hell, PI(4,5)P$_2$/syntaxin-1 clusters in Munc18-1 null and WT (wild-type) neurons. This experiment was performed by lentiviral overexpression of GFP-PH-PLCδ1 construct, that binds PI(4,5)P$_2$, allowing a direct detection of PI(4,5)P$_2$ clusters. We were also interested in syntaxin-1 co-localization with PI(4,5)P$_2$, thus a syntaxin-1 antibody was used to
stained for this endogenous protein. Performing this experiment allowed us to compare between WT and Munc18-1 null neurons at DIV3. As an outcome of this experiment, we expected to observe differences between Munc18-1 null and WT PI(4,5)P$_2$/syntaxin-1 clusters. Conversely, the outcome showed no differences between WT and Munc18-1 null PI(4,5)P$_2$ clusters. A plausible explanation relies on technical issues, the 2-color STED microscope used for this experiment does not have a 488nm channel and our construct is a GFP-fusion protein. Consequently, we used anti-GFP antibody followed by a secondary probe to visualize our PH-PLCδ1 probe, in addition to anti-syntaxin-1 antibody. Thus, the distribution of PI(4,5)P$_2$ and syntaxin-1, detected by our antibodies could be affected by the size of our probes (≈15nm per Antibody plus 4.2nm for GFP).

We were also interested in quantifying the levels of PI(4,5)P$_2$. Therefore, I learnt how to prepare membranes sheets from chromaaffin cells and neuronal cultures, and have used purified PH-PLCδ1-GFP to quantify the levels of PI(4,5)P$_2$ via immunocytochemistry.

Now that I am back at my home institution I will apply the knowledge acquired at Milosevic’s lab by: using our microscope to perform super resolution by stochastical optical reconstruction microscopy (STORM) and try to clarify whether there is no difference in PI(4,5)P$_2$/syntaxin-1 clusters or if it is an antibody size issue. Also, PI(4,5)P$_2$ levels will be measured between WT and Munc18-1 null cells by neuronal membrane sheets method. Moreover, I will teach this method to my colleague in order to measure PI(4,5)P$_2$ levels from chromaaffin cells, important for her project.

I recognize that getting the NENS stipend gave me the opportunity to join one of the most renowned scientific communities in Europe, a great achievement for my career. Yet, my staying at Milovevic’s lab was really positive, I had a chance to expand my knowledge and learn new methodology that I can now apply at my home institution.


7) Dulubova I, et al., Munc18-1 binds directly to the neuronal SNARE complex. PNAS 104(8), 2697-2702 (2006)