DANDRITE Topical Seminar
by Laura Marchetti

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From 10:15-11:00

Aud. 6, 3rd floor, building 1170, room 347
Aarhus University, Ole Worms Allé 3, 8000 Aarhus C

Laura Marchetti
Postdoc at The Scuola Normale Superiore, Pisa, Italy

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Single molecule imaging– NGF – TrkA – p75NTR – proNGF

"Single molecule imaging and tracking of neurotrophins and their receptors in living neuronal cells"

We currently lack a satisfactory understanding of the membrane complexes and internalization routes underpinning the pleiotropic biological outcomes of neurotrophins (NTs), which exert their functions via interlaced binding of three different families of neurotrophin receptors (NRs). We are working to answer several open questions in this field: are NRs membrane movements linked to ligand-specific activation processes? Are different receptor functions linked to different movements at the cell membrane? How does p75NTR enhance NGF-TrkA signalling? Are NGF and its precursor proNGF different signalling molecules as far as NRs binding and internalization is concerned?
To address these issues, we developed non-invasive means to covalently fluorolabel with 1:1 stoichiometry both neurotrophins and their receptors. This toolbox was exploited to perform single molecule imaging and tracking (SMIT) at the plasma membrane and inside axons of living neuronal cells using wide-field and TIRF microscopy.
We have so far obtained results in three different directions. First, we gained clues about TrkA membrane mobility and oligomerization state upon binding of four different NTs. We proved that ligand binding shifts TrkA monomer-dimer equilibrium towards the dimer form, and causes the appearance of immobile clustered forms; however the extent of such changes in dynamics is strictly ligand-dependent. We also generated different TrkA mutants allowing for the dissection of three different receptor functions: kinase activity, recruitment of intracellular effectors, ubiquitination and further degradation. Our data point to kinase activity as a master regulator of TrkA membrane dynamics and hint at possible mechanisms by which the cell handles the trafficking of kinase-inactive TrkA receptors. Second, we developed a system for the stable inducible expression of TrkA and p75NTR constructs in living cells, which can be exploited for a dual-color labelling procedure and subsequent simultaneous SMIT of the two receptors. Last, we undertook a comparative study about the axonal transport displayed by "homologue" fluorescent proNGF and NGF in compartmented DRG neurons. We demonstrated that proNGF is internalized and retrogradely transported across axons like mature NGF, but the two NTs display remarkable differences both in terms of NTs flux and number of molecules carried per vesicle. Furthermore, we unveiled a competition mechanism favoring NGF transport upon coadministration of the two NTs.
SMIT analysis is a powerful method to study NTs-NRs membrane dynamics and internalization routes. We are currently optimizing our TIRF setup to get the quantitative description of the kinetics, dynamics and stoichiometry of any molecular complex formed upon proNTs or NTs binding to NRs.