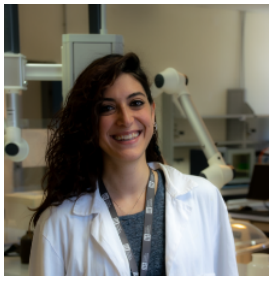
# INTERACTIVE BIOHYBRID SYNAPSES



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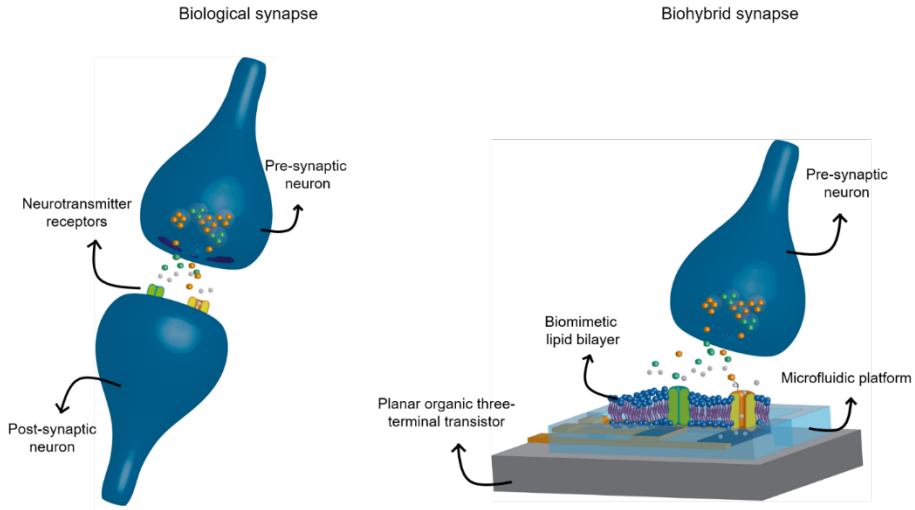
Nowadays neurodegenerative diseases affect millions of people worldwide: they result in progressive degeneration and / or death of nerve cells and currently there are no therapies able to stop the progression of the disease permanently. Examples of neurodegenerative diseases include Parkinson's, Alzheimer's, and Huntington's diseases: they are characterized by problems with movement (called ataxias), or mental functioning (called dementias). Among existing therapies, the use of implantable electronic devices is able to reduce some of the symptoms through direct stimulation of the damaged brain area: however, they do not provide a permanent cure. Several works have identified a strong connection between neurodegenerative pathologies[1–3] and synaptic plasticity (*i.e.* the property of the brain to adapt to environmental changes)[4], identifying a possible target for the development of new therapies. This PhD project focuses on engineering a biohybrid *in*-vitro system able to replicate the main functionalities of healthy synapses to counterbalance inadequate neuronal functionalities in neurodegenerative diseases such as Alzheimer's and Parkinson's'[5]. We aim at mimicking the same functionalities of neurons (*i.e.* synaptic plasticity) using an organic neuromorphic device directly coupled with pre-synaptic terminals of a biological neuronal culture. In detail we used an organic electrochemical transistors (OECTs) as learning core of our platform: lately, such devices has shown the ability to modify their conductive state upon an external stimulation (*i.e.* electrical pulses) and retain this altered conditioning over time, mimicking the short term plasticity of the brain.[6] The first part of the project was focused on the validation of the short and long term plasticity of a planar three terminal OECT (microfabrication provided by Stanford University) where both gate and channel were made of PEDOT:PSS, and the device was coupled with a microfluidic system to establish the electrolyte connection between gate and channel. To validate the plasticity of the system, dopamine solution in electrolyte at different concentrations (10-80 µM) has been used. The oxidation of the neurotransmitter was achieved applying a pulsed bias at the gate electrode (the voltage was determined first performing cyclic-voltammetry measurements). Through the application of a negative bias at the channel, protons produced from the reaction are pushed into the PEDOT:PSS channel causing the de-doping of the polymer and consequent decrease in conductance. Interestingly, we observed that the decrease in channel conductance is proportional to the dopamine concentration and is also retained over long time. Furthermore, both short and long term plasticity of biological synapses were emulated: the STP is emulated by simply pulsing the gate in absence of dopamine, while the additional protons injection in the neuromorphic channel in presence of the neurotransmitter, causes a long term modulation of the neuromorphic channel that can be used to emulate the LTP. Once demonstrated the plasticity of the device, the biohybrid interface was engineered by establishing a direct coupling between the neuromorphic device and a dopaminergic cell line. In detail, PC-12 cells (rat pheochromocytoma cells) have been chosen as they spontaneously secrete dopamine and can be differentiated into neuronal cells. First PC-12 cells were seeded on PEDOT:PSS film to confirm the physiological dopamine secretion through immunohistochemistry. Then, cells were cultured (770,000 cells $cm^{-2}$) through the microfluidic channel on the neuromorphic prior surface functionalization with collagen to promote cell adhesion. The biocompatibility of the substrate was validated through a live-dead essay, labelling live cells with calcein-AM and dead cells with propidium iodide. Here, fluorescence imaging revealed that PC-12 cells survived on neuromorphic devices with no alteration in cell viability after electrical measurement. Additionally, the plasticity of the biohybrid synapse was evaluated

oxidizing dopamine directly secreted from cells. Here, oxidizing dopamine released within 2 hours induced a memory effect in the OECT and after 4 hours in culture, the device keeps retaining memory of the previous oxidation and does not recover the initial conductance, confirming the long-term plasticity behavior. Since the PC-12 cell monolayer collectively constitutes the presynaptic domain, different cell densities influence the overall dopamine accumulation. We found that an increase of the cell density to 90% confluent monolayer creates a diffusion barrier, preventing the efficient circulation and oxidation of dopamine at the gate electrode. Furthermore, the long-term viability of cells extends to at least 24 hours, confirming the stability of the biohybrid synapse. In biological synapses, once the information has been transmitted from the pre to the post-synaptic neuron, the neurotransmitters are cleared from the synapse through enzymatic degradation or re-uptake by specific transporters. In order to emulate the neurotransmitter recycling process, fresh cell medium was delivered to refresh the cell-device interface: using low flow rates dopamine is continuously build-up and oxidized, thus the postsynaptic conductance decreases steadily. In contrast, at high flow rates dopamine is washed away from the surface before it can accumulate, inducing an increase of the conductance because dopamine is cleared from the synaptic cleft before the oxidation, thus emulating endocytosis.




*References.*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