Engineering Post Synaptic Architectures with Native Biomembranes

Alice Hattar[1,2], Janic Töx[1,2], Nevena Stajkovic [1,2] , Mehdi Ravadneh , Francesca Santoro[1,2]

[1] Institute of Biological Information Processing – Bioelectronics, IBI-3, Forschungszentrum Juelich, Germany

[2] Faculty of Electrical Engineering and IT, RWTH Aachen, Germany

a.hattar@fz-juelich.de

01| Motivation

Building on existing strategies and techniques that focus on optimizing cell-chip coupling in bio- and neuro electronics[3], my project focuses on using the neuron's own membrane to coat an organic electrode (*Figure 1*) by incorporating neuronal blebs into supported lipid bilayers (SLBs). These biohybrid SLBs present functional interfaces that mimic key aspects of living synapses. When coupled to electrochemical neuromorphic organic devices, these systems respond to neurotransmitters and neuromodulators, paving the way for engineering neurohybrid interfaces that connect biological and artificial systems, opening new directions for neuromorphic bioelectronics.

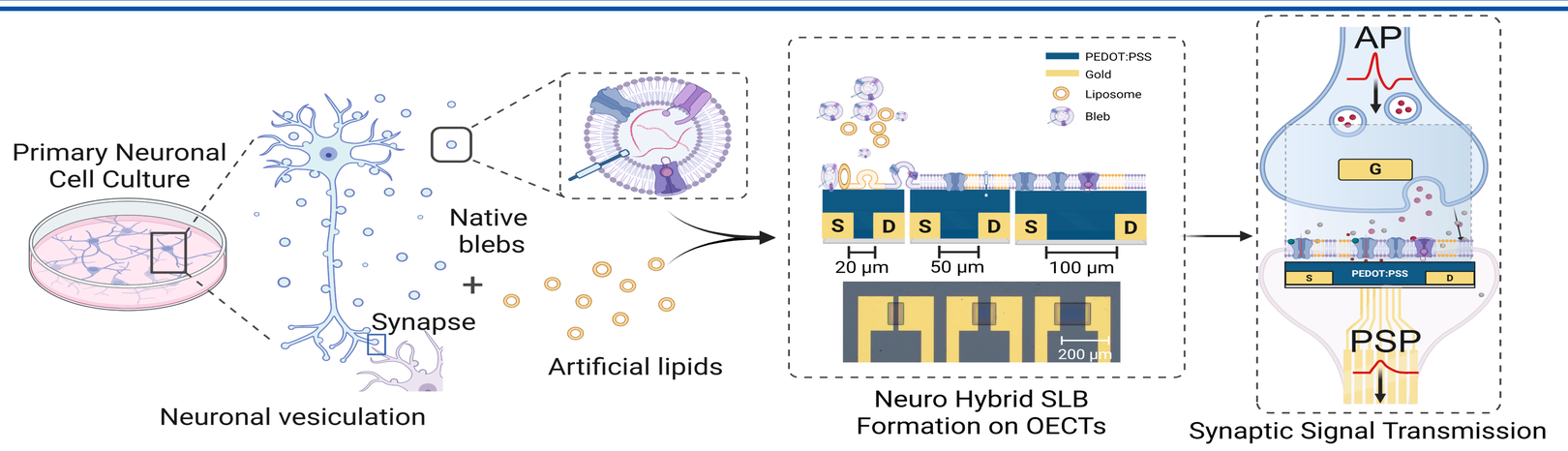


Figure 1: Graphical abstract of this project: chemically induced neuronal vesiculation (blebbing) from rat primary neurons and subsequent formation of a supported lipid bilayer with synaptic proteins on an organic electrochemical transistor (OECT) of different channel sizes, emulating a biological synapse. Ideal outcome: establishing "structural" and functional biorecognition or synaptic adhesion by growing neurons on top of bleb-SLBs enriched with synaptic proteins and coupled to OECTs that would allow not only the stimulation and recording of electrical signals but also monitoring of electrochemical activity of ions/neurotransmitters that would be reflected in the modulation of the OECT behaviour.

02| Material and Methods

Blebbing

Introducing components of a cell's membrane into an artificial lipid bilayer (Supported Lipid Bilayer, SLB) has been a promising strategy to mimic the cell membrane[5]. The formation of extracellular vesicles (blebs) was induced by the addition of Giant Plasma Membrane Vesiculation buffer[7] to a culture of adherent rat primary (R18) cortical (RCN) and hippocampal neurons (RHN) (*figure 2, A and B) respectively*. Where the nuclei were stained with HOECHST (i), cell membrane with DiO (ii) and dead cells with EthD (iii). Blebs were also visualized by staining neurons for live imaging *(figure 2 C (i), (ii)) , labeling suspended blebs in tube (figure 2 D) and* fixed for scanning electron microscopy (SEM, *figure 2, E*).

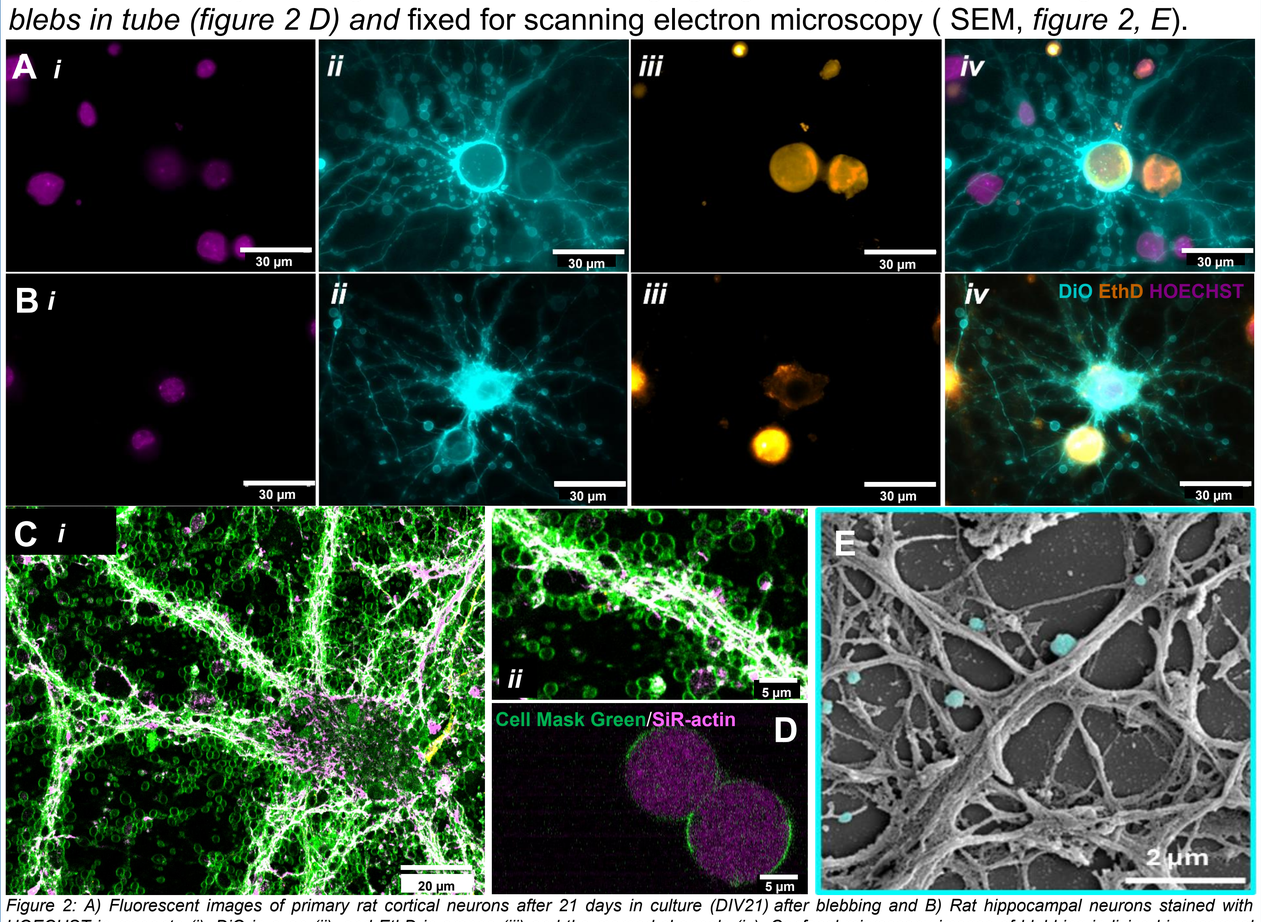


Figure 2: A) Fluorescent images of primary rat cortical neurons after 21 days in culture (DIV21) after blebbing and B) Rat hippocampal neurons stained with HOECHST in magenta (i), DiO in cyan (ii), and EthD in orange (iii) and the merged channels (iv). Confocal microscopy images of blebbing in living hippocampal neurons at DIV21, F-actin is labeled with SiR-actin in pink, the plasma membrane with CellMask in Green, and the axon initial segment with Nuerofascin (NF) –in orange (i). Magnified view of dendritic blebbing shown in (ii). D) Vesicles isolated from neurons labeled in suspension. E) SEM image of blebbing in a primary hippocampal neuron at DIV15 (blebs false colored in cyan).

Nano Particle Tracking Analysis and Dynamic Light Scattering

RCN and RHN were blebbed at different days *in vitro* (DIV) and using different PFA percentages in the GPMV buffers. Nano Particle Tracking analysis (NTA) was carried out to determine the mean size, mode size and concentration of the collected blebs, while dynamic light scattering (DLS) was used to measure their surface charge. *Figures 3, A, B and C* are plots representing the distribution of data among the different conditions and cell types.

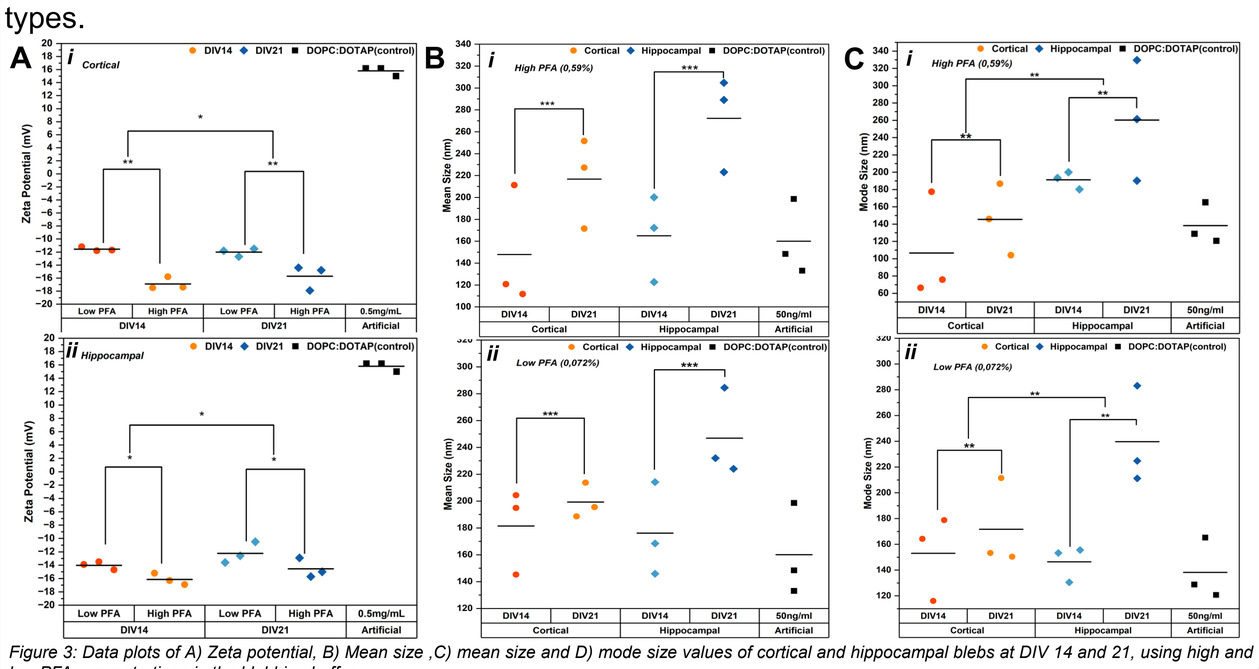


Figure 3: Data plots of A) Zeta potential, B) Mean size ,C) mean size and D) mode size values of cortical and hippocampal blebs at DIV 14 and 21, using high and low PFA concentrations in the blebbing buffer.

03| Results

Neurohybrid Supported lipid bilayers

Collected blebs were labelled with DiO and assembled with artificial lipids, DOPC (1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine) and DOTAP (4:1) to form a hybrid membrane on glass and PEDOT:PSS, through vesicle fusion[4] (*figure 4, A, C and E)*. Current experimental methods include introducing water to rupture vesicles by osmotic shock[6]. Fluorescence Photobleaching after recovery (FRAP) *(figure 4, B)* was performed to study the recovery of the Bleb-SLBs, results are represented in *(figure B, ii)*. Electrochemical Impedance Spectroscopy (EIS) was also performed for samples on PEDOT:PSS electrodes and OECTs *(figures 4, D, F and G)* show the different results obtained with the bare electrodes, only artificial SLB, and hybrid SLBs.

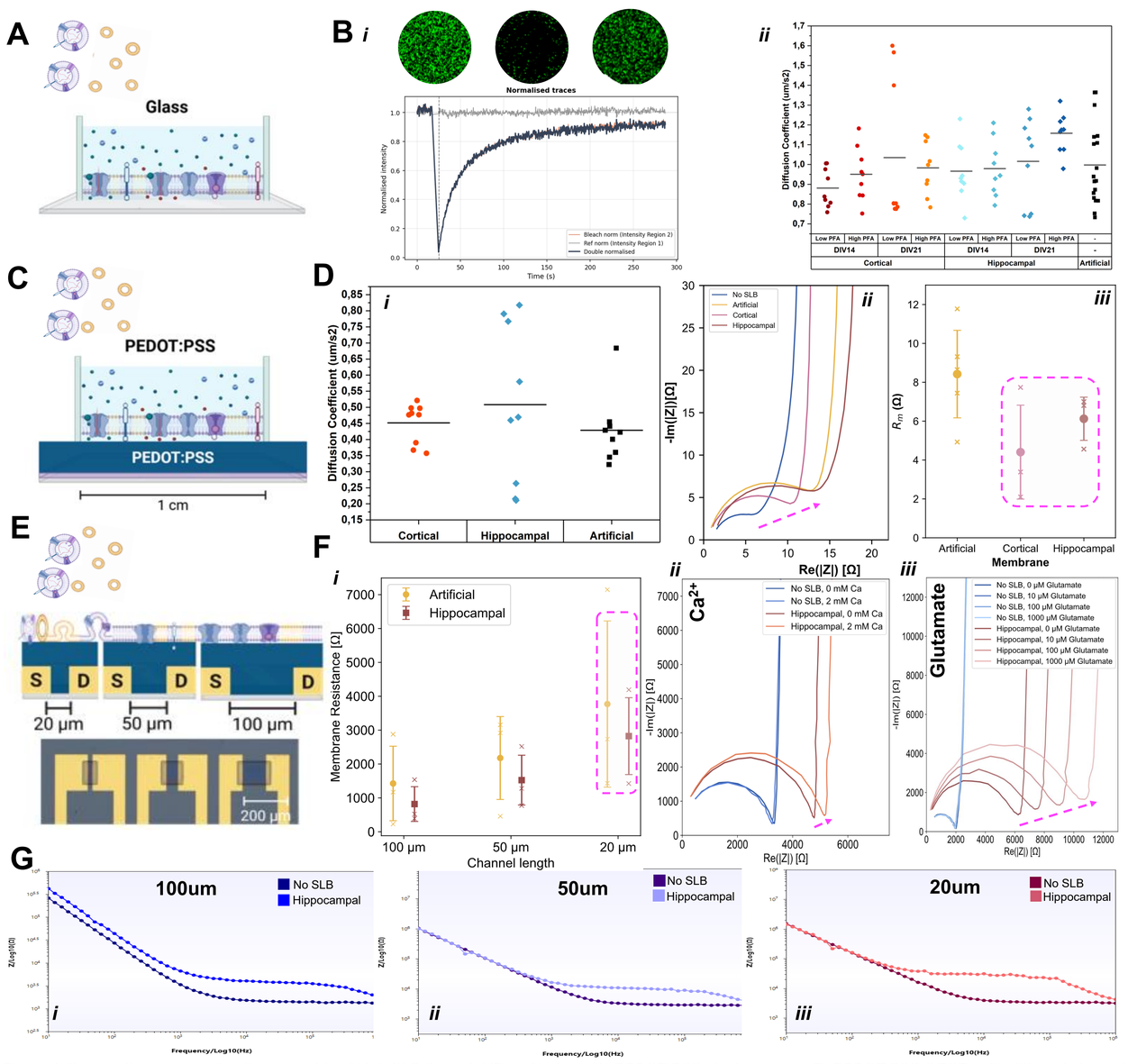


Figure 4: Schematics representing the assembly of bleb-SLB through VF on A) glass, C) PEDOT:PSS electrodes and E) PEDOT:PSS OECTs with different channel sizes and their corresponding characterization results: B) i) Representation of a bleb-SLB bleaching and recovery on glass and ii) graph of diffusion coefficients of SLBs across different conditions. D) i) graph of diffusion coefficients of SLBs on PEDOT:PSS, ii) EIS curves of different samples and iii) extracted membrane impedance. F) i) extracted membrane impedance of SLBs on channels of different sizes, EIS curves with ion-channel blocking experiments of ii) calcium and iii) glutamate. G) Formation of hybrid SLB of RHN DIV21 blebs on OECTs and corresponding EIS under sterile conditions on i)100um ii) 50um and iii) 20um channels.

04| Future outlook

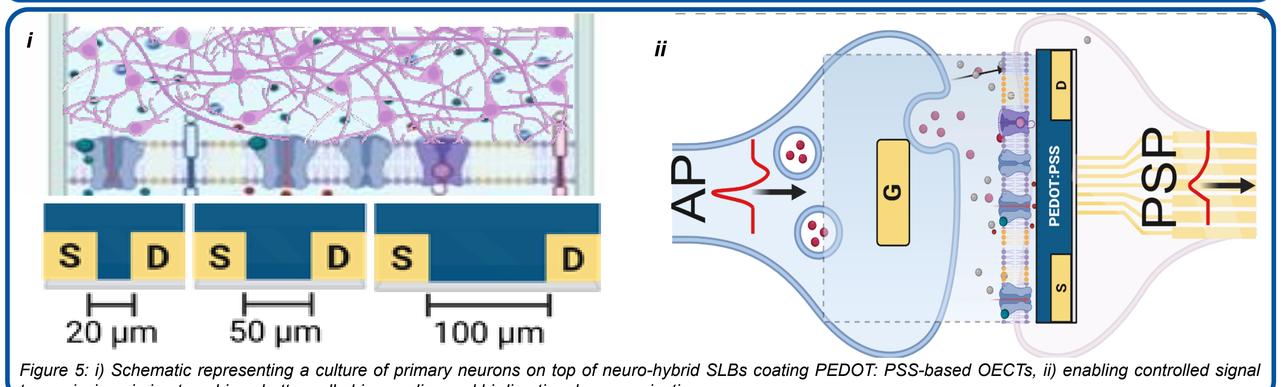


Figure 5: i) Schematic representing a culture of primary neurons on top of neuro-hybrid SLBs coating PEDOT: PSS-based OECTs, ii) enabling controlled signal transmission aiming to achieve better cell-chip coupling and bi-directional communication.

1.Hai, A., Kamber, D., Malkinson, G., Erez, H., Mazurski, N., Shappir, J. & Spira, M. E. Changing gears from chemical adhesion of cells to flat substrata toward engulfment of micro-protrusions by active mechanisms. J. Neural Eng. 6, 066009 (2009).
2.Hai, A., Dormann, A., Shappir, J., Yitzchaik, S., Bartic, C., Borghs, G., Langedijk, J. P. M. & Spira, M. E. Spine-shaped gold protrusions improve the adherence and electrical coupling of neurons with the surface of micro-electronic devices. J. R. Soc. Interface. 6, 1153–1165 (2009).
3.Lubrano, C., Matrone, G. M., Forro, C., Jahed, Z., Offenhaeusser, A., Salleo, A., Cui, B. & Santoro, F. Towards biomimetic electronics that emulate cells. MRS Communications 10, 398–412 (2020).
4.Richards, M. J., Hsia, C.-Y., Singh, R. R., Haider, H., Kumpf, J., Kawate, T. & Daniel, S. Membrane Protein Mobility and Orientation Preserved in Supported Bilayers Created Directly from Cell Plasma Membrane Blebs. Langmuir 32, 2963–2974 (2016).
5.Svetlova, A., Ellieroth, J., Milos, F., Maybeck, V. & Offenhäusser, A. Composite Lipid Bilayers from Cell Membrane Extracts and Artificial Mixes as a Cell Culture Platform. Langmuir 35, 8076–8084 (2019).
6.Zhang, Y., Inal, S., Hsia, C.-Y., Ferro, M., Ferro, M., Daniel, S. & Owens, R. M. Supported Lipid Bilayer Assembly on PEDOT:PSS Films and Transistors. Advanced Functional Materials 26, 7304–7313 (2016).
7. 2012-Blebbing paper-Levental et al.

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