Nanopore Cavities on Silicon-on-Insulator Chips for High Throughput and Multiplexed Single-Transporter Recordings

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Membrane proteins involved in transport processes are key targets in pharmaceutical research. Multiple applications for the characterization of transport kinetics of ion channels and pores with high flux rates, *i.e.* patch clamp, are available. However, integral membrane proteins with low transport rates or for non-ionic substrates like the human transporter associated with antigen processing (TAP) are rarely available. Recently, silicon chips containing nanopore cavity arrays with femtoliter compartments have become an ideal platform for the massively parallel recording of transport events across lipid membranes. Here, we report the realization of chips with nanopore microcavities based on silicon-on-insulator (SOI) substrates coated with silicon nitride, containing more than 14,000 inverted-pyramidal cavities of ~50 femtoliter volumes and 80 nm diameter openings. The ultraflat surfaces with highly defined structure dimensions and pore edges provide optimal spreading conditions for artificial membranes like large unilamellar vesicles (LUV), with spreading efficiencies up to 95%, stable for more than 24 hours. Fluorescence recovery after photobleaching (FRAP) experiments of the supported lipid bilayer revealed high lipid mobility. This new chip design features transparent cavity bottoms which provides parallel fluorescence readout of both, the cavities and the buffer reservoir for unbiased single-transporter recordings. Single cavities were visualized and reconstructed to 3D images. As proof of concept, the flux kinetics of pore proteins were characterized. The high parallelism and throughput, as well as the single-protein resolution represent an ideal platform for pharmaceutical research.

References

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