

Highly Reproducible Physiological Asymmetric Membrane with Freely Diffusing Embedded Proteins in a 3D-Printed Microfluidic Setup

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Experimental setups to produce and to monitor model membranes have been successfully used for decades and brought invaluable insights into many areas of biology. However, they all have limitations that prevent the full in vitro mimicking and monitoring of most biological processes. Here, a suspended physiological bilayer-forming chip is designed from 3D-printing techniques. This chip can be simultaneously integrated to a confocal microscope and a patch-clamp amplifier. It is composed of poly(dimethylsiloxane) and consists of a ≈ 100 μm hole, where the horizontal planar bilayer is formed, connecting two open crossed-channels, which allows for altering of each lipid monolayer separately. The bilayer, formed by the zipping of two lipid leaflets, is free-standing, horizontal, stable, fluid, solvent-free, and flat with the 14 types of physiologically relevant lipids, and the bilayer formation process is highly reproducible. Because of the two channels, asymmetric bilayers can be formed by making the two lipid leaflets of different composition. Furthermore, proteins, such as transmembrane, peripheral, and pore-forming proteins, can be added to the bilayer in controlled orientation and keep their native mobility and activity. These features allow in vitro recapitulation of membrane process close to physiological conditions. [1]

[1] Heo, P., S. Ramakrishnan, J. Coleman, J. E. Rothman, J.-B. Fleury, and F. Pincet, "Highly Reproducible Physiological Asymmetric Membrane with Freely Diffusing Embedded Proteins in a 3D-Printed Microfluidic Setup", *Small*, 2019.