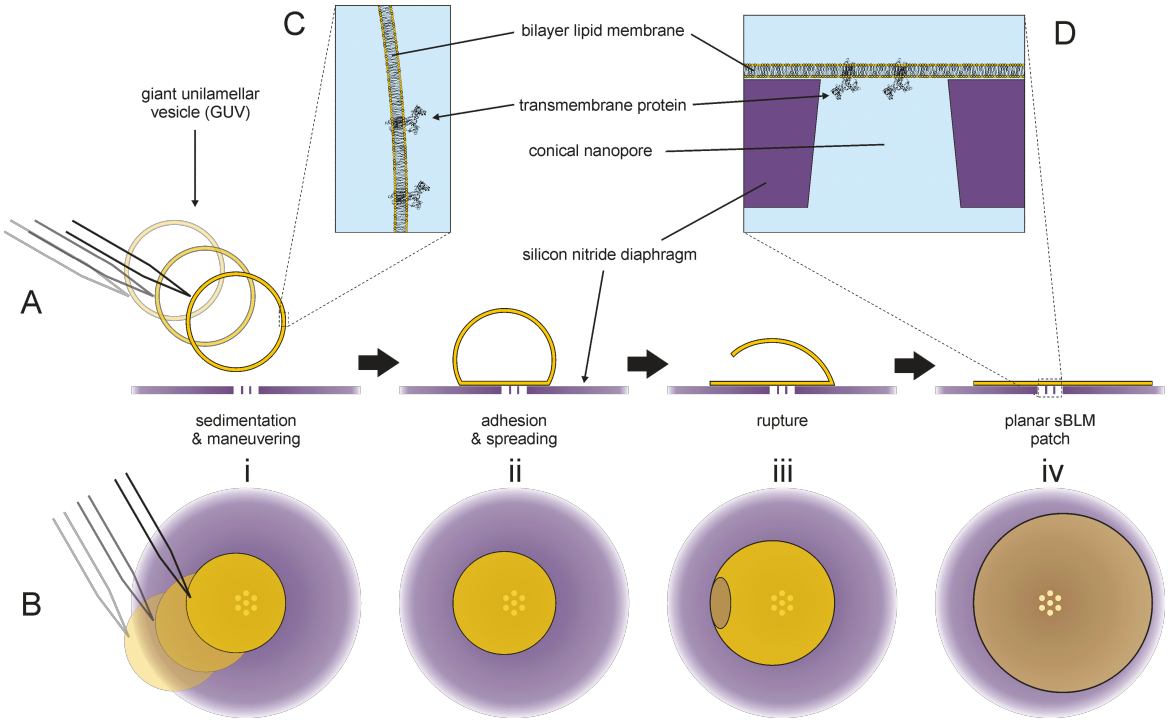
An Interdisciplinary Method of Studying Ion Channels

Ion channels are membrane proteins fundamental to cell signalling through the membrane. By regulating the flow of ions in and out of cells, they are key to a number of physiological processes. It is no surprise, therefore, that these membrane proteins are among the most important to study in the development of new pharmaceutical drugs.

The traditional method of studying ion channels makes it possible to directly measure transmembrane signalling, but this method – voltage clamp electrophysiology or the patch clamp technique – is costly and time-consuming to use. Based at the Max-Planck-Institute für Biophysik in Frankfurt, Germany, ***Slavoj Kresák*** is working on developing a new method of studying ion channels. Over the past few years, attempts have been made to form bilayer lipid membranes on substrates containing micrometer or nanometer sized pores. However, bilayer stability has been a problem, and in some cases organic solvents have been used in the process of forming the bilayer on the substrate. As organic solvents may affect the properties of the lipid environment and thus alter the behavior or proteins or peptides under study, finding ways to form the bilayer lipid membranes over substrates without any solvents involved is of great importance. Another problem with this new technique is being able to measure even very small currents.



Schematic of a bilayer lipid membrane formation by direct fusion of a giant vesicle to a diaphragm containing an array of pores. (A) and (B) are side and bird's eye views of the formation process, respectively. (C) A zoom-in at the giant unilamellar vesicle, displaying in detail the lipid bilayer with reconsituted transmembrane protein. (D) A zoom-in at a bilayer lipid membrane spanning a slightly conical nanopore. (i) Giant vesicle suspension is injected in the bathing electrolyte and giant vesicles sediment to the diaphragm. One of the giant vesicles sinking at the pore-bearing location is maneuvered to directly above the pore array by horizontal movements of a patch pipette tip attached to the micromanipulator. (ii) Upon contact with the diaphragm the giant vesicles adheres to the surface and spreads into a pancakelike shape, spanning over all pores of the array. (iii) Spontaneous rupture occurs at the rim of the giant vesicle as it flattens on the surface. (iv) The vesicle unfolds completely, forming a large bilayer lipid membrane patch spanning all pores of the array.

Slavoj is addressing all of these challenges. Using a sensor chip with many small nanopores, he uses directed fusion of giant liposomes to span these pores. Full bilayer coverage of the many pores on the chip is a prerequisite for electrical measurements at low noise and low background currents, since one ion channel is a very small pore, whereas the pores on the chip are, in comparison, very large. Spanning all pores of the chip can be a problem, but Slavoj was able to achieve full coverage with no use of organic solvents.

– Bilayers formed in this way may remain stable for several days, even with exchange of electrolytes and delivery of compounds. Since the bilayers cover all of the pores, we are able to detect even very low ionic currents through the ion channels, Slavoj says.

Slavoj Kresák at the Max-Planck-Institut für Biophysik in Frankfurt, Germany, has developed a platform for the development of pharmaceutical screening assays, biophysical research of membrane proteins, and biosensor devices based on the detection of low ionic currents through protein channels or pores.

Why giant liposomes?

– Liposomes are normally in the 50- to 100 nanometer range, and giant liposomes are about 100 times bigger. You can imagine them like bubbles, filled with an aqueous medium and surrounded by an aqueous medium as well. The nice thing about giant liposomes is their integrity: when they adhere to a surface, they unfold there, forming a layer. We have used direct fusion of these liposomes, controlling the spreading and rupture of them through optical and mechanical means. In this way, we are able to create a bilayer which fully covers arrays of nanopores, thus enabling electrical measurements at low noise and low background currents.

While still at the prototype stage, with several challenges left to meet, Slavoj and his colleagues have shown that this new platform can be applied for biophysical research of membrane proteins as well as be scaled up for pharmaceutical drug screening assays.

At present, Slavoj is working on solving some of the remaining challenges.

– I am working on checking the function of the proteins and then reconstituting them into liposomes again, making sure function of the proteins is not compromised. This is an important step, because when proteins exist within cells or bacteria, they have all the structural components they need, but when you take them out of there to insert them into an artificial lipid bilayer, they may no longer function, or they may no longer function in the same way.

A Perfect Fit

Slavoj says he was immediately attracted to the ASMENA project because of its interdisciplinary approach.

– It's a blend of biology, surface chemistry, and physics. I studied biomedical physics, which is already quite interdisciplinary, and this project added electrochemistry, for example. It fit my interest perfectly – I have had and I still have a lot of fun working on this project. I've learned a lot, scientifically of course, but also in terms of management skills. There is a lot that goes

into a project like this: planning, coordination, cooperation with other research groups and with companies, and so on. ASMENA has truly been a connecting project. I would like to follow up on this, working in a similarly interdisciplinary way once I reach the end of this project.

ASMENA *is part of the EU Seventh Research Framework Programme (FP7). Over three years, the consortium consisting of 15 partners in 7 countries aims to develop new platforms for drug screening and analytical profiling base on in vitro measurements of functional and conformational changes in membrane proteins. Such tools will allow standard profiling and screning also against membrane protein targets that can currently not be screened in these ways. They will shorten the time and cost involved in drug lead development by increasing predictability as well as contribute to fundamental understanding of structure-function relationships of membrane proteins.*

The partners of the consortium are world leading experts on surface functionaliztion, membrane self-assembly, biosensing, membrane protein functional measurements and commercialization of the same. Now, their complementary competences can be put together on the European level to create a timely breakthrough in the area.