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WEBINAR Q&A

Q: Are the MECA 4 chip consumables a single use item?

A: No, the chips can thoroughly be cleaned (e.g. with water, ethanol and *iso*-propanol) and reused several times. The overall lifetime of a single chip depends on the conditions like ionic strength and height of applied potentials used in the experiments but one MECA 4 chip will be stable at least for a whole day of experiments.

Q: How does the temperature control work? And how is it connected to the device?

A: The temperature can be controlled via a Peltier element that is located right beneath the MECA 4 recording chip. The optional temperature control add on would read out this temperature and automatically keep it at a given set temperature between 5-50°C for hours by means of active liquid cooling.

Q: Does the temperature control introduce additional noise?

A: The overall noise will inevitably rise with elevated temperature due to additionally introduced thermal motion. But no artifacts or disturbances in the signal will be added due to the temperature control as such.

Q: Do you have the temperature sensor in the buffer?

A: No, this would not be feasible, the temperature is read out at the herein before mentioned Peltier element. The desired temperature for an experiment would have to be determined with a thermometer (included in the temperature control add-on) and the set temperature would be chosen accordingly in the software. This would have to be done only once before the actual experiment(s) to determine the temperature at the membranes for a certain set value.

Q: How do I actually get my protein of choice inside the bilayer?

A: The artificial lipid membranes generated on the Orbit mini are essentially the same as on any other bilayer setup so the approach would be the same as well. In general there are three different scenarios:

- hydrophilic species like toxins could directly be added to the buffer
- partly hydrophobic species like membrane proteins would be added in detergent micelles
- proteo-liposomes are fused to the membranes after membrane generation

A variety of methods and protocols covering all mentioned scenarios can be found throughout literature – even for a plethora of concrete examples.

WEBINAR Q&A

Q: Can so-called almost solvent free lipid bilayers be generated using squalene as solvent?

A: This is feasible in principle but we would not recommend it as the bilayers might not thin out correctly.

Q: What about membrane proteins that must be pre-integrated into membranes before recording? As in proteoliposomes containing ion channels.

A: Small vesicles (not GUVs) bearing membrane proteins can be fused to the bilayers (vide supra).

Q: Do you recommend insertion from nanodiscs?

A: Yes, absolutely! We, for example, investigated several different viral potassium channels in cooperation with the group of Professor Gerhard Thiel from the TU Darmstadt. We received the proteins in nanodiscs, could incorporate them into the membranes at outstanding success rates and found them to be in excellent shape during the successive experiments.

Additional Publications of Interest

Pseudo painting/air bubble technique for planar lipid bilayers – Read More

Voltage-controlled insertion of single α-hemolysin and *Mycobacterium smegmatis* nanopores into lipid bilayer membranes – Read More

High-Resolution Size-Discrimination of Single Nonionic Synthetic Polymers with a Highly Charged Biological Nanopore – Read More

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