

A novel approach to detect electrogenic transporter activity in intact cells applied to investigate iPSC derived cardiomyocytes and neurons

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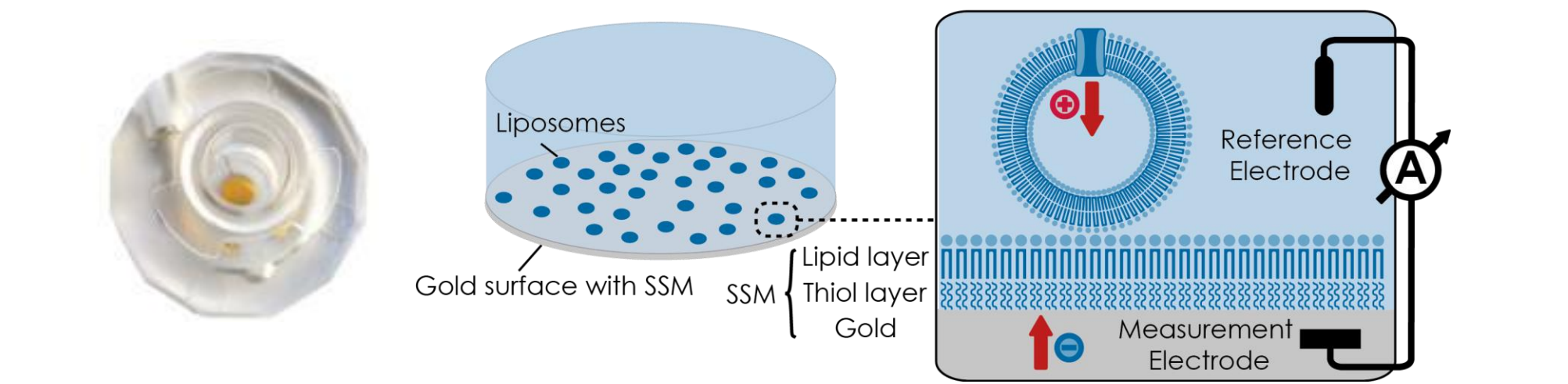
AXAM
INNOVATES, ENABLES, PERFORMS

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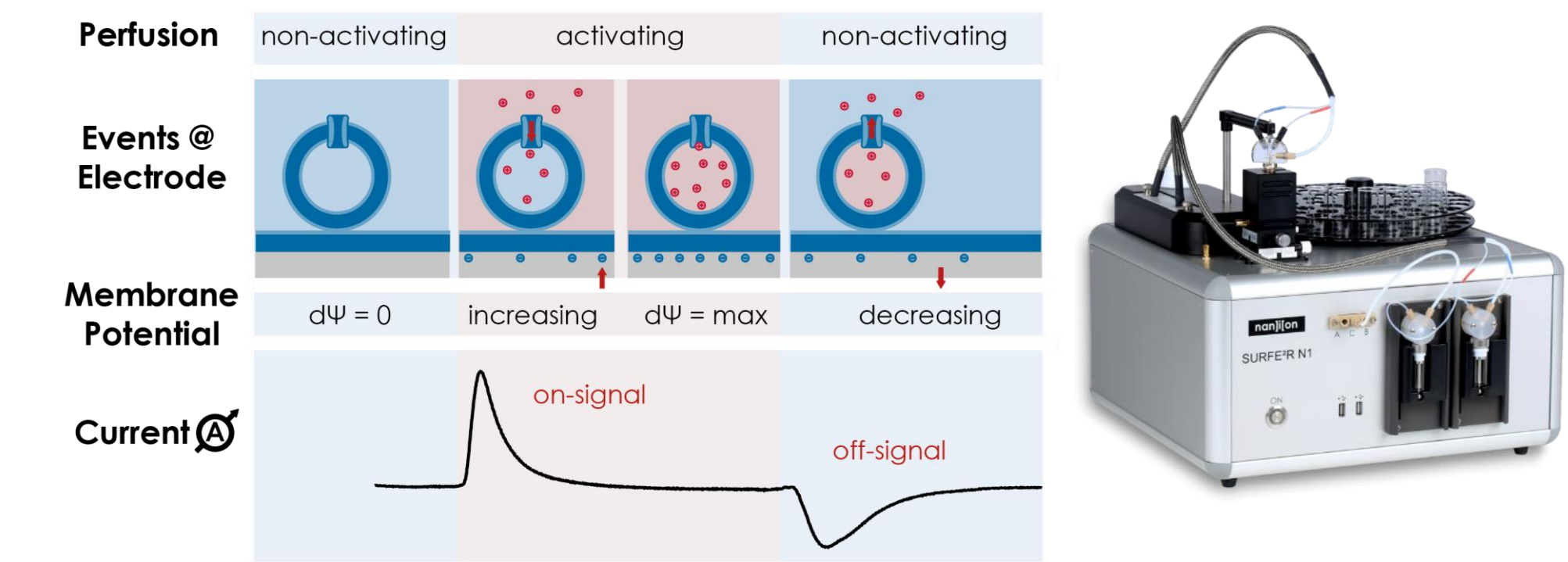
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Measuring capacitive currents enables resolution of small transporter currents

The measurement of capacitive induced currents enables the resolution of very small biological currents since it allows accumulation of the signal generated by multiple cells



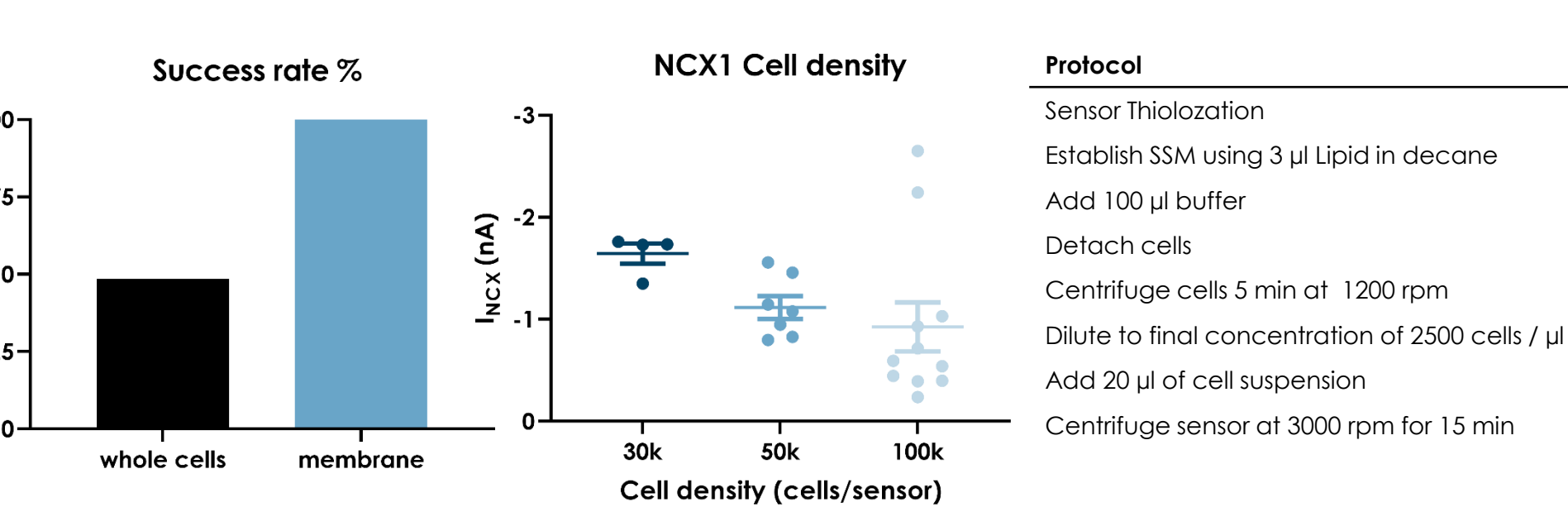
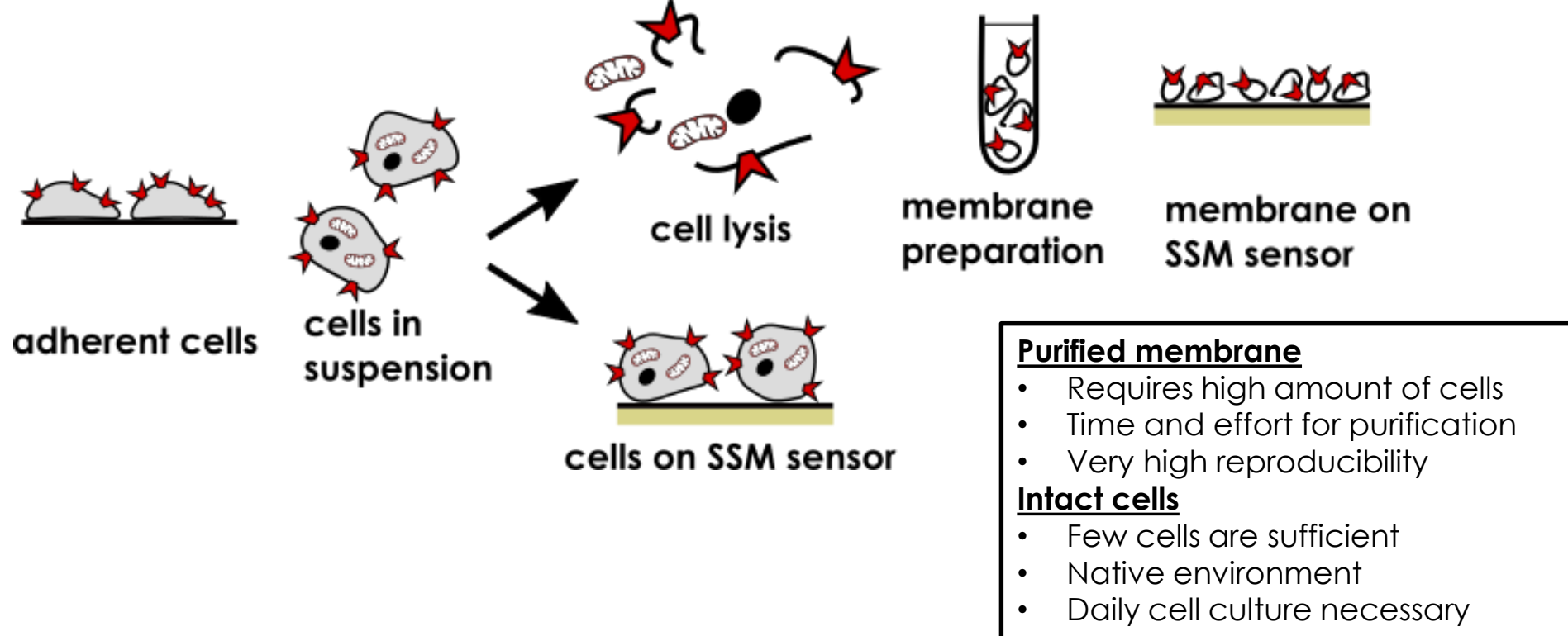
For a capacitive readout ("SSM-based electrophysiology") purified plasma membrane or artificial liposomes are immobilized on a gold sensor. Charging and un-charging of the vesicles caused by electrogenic transport can be detected.



To trigger transport activity a substrate is flushed in and out of the sensor well with a fast fluidic system. It is possible to apply one or two different substrate gradients.

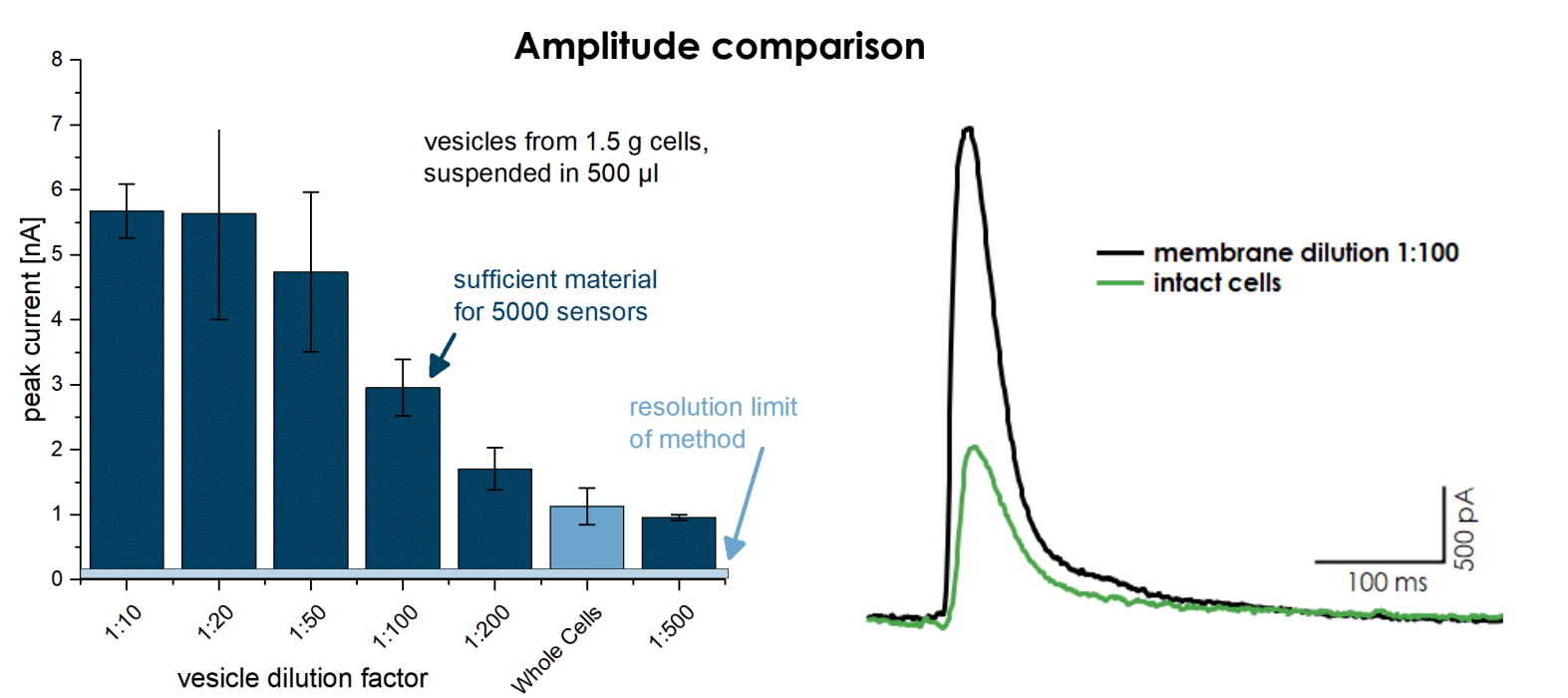
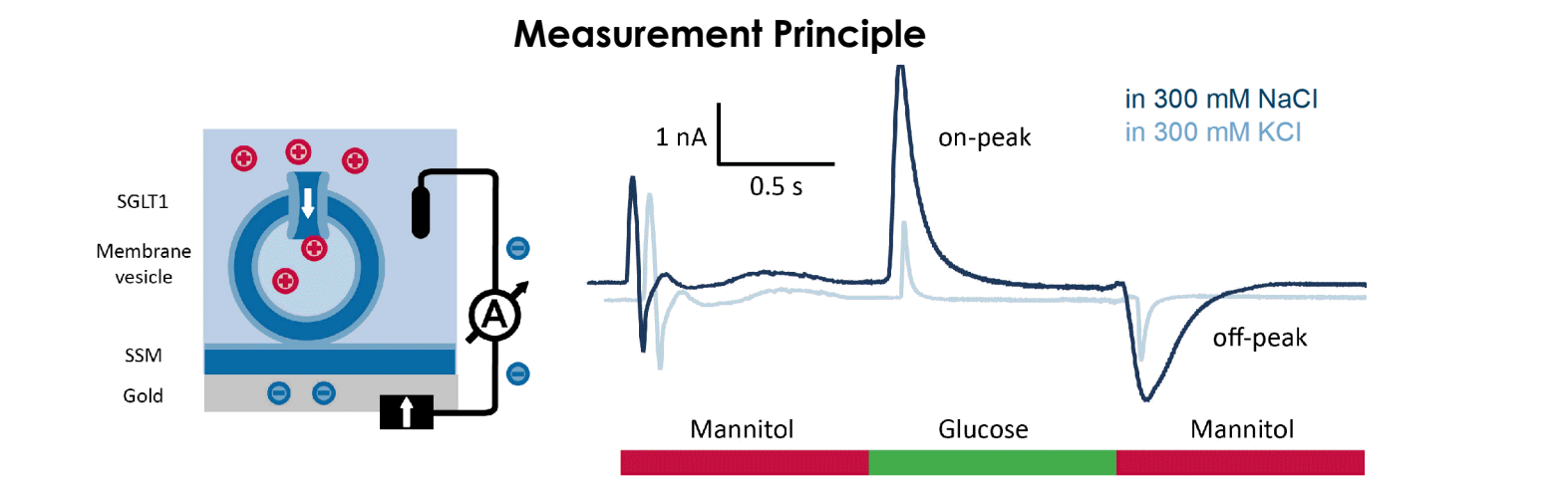
Establishing a new procedure to use intact cells as a new sample type

The use of purified plasma membrane vesicles (~200 nm) or artificial liposomes is established. We developed a protocol to use whole eukaryotic cells instead.



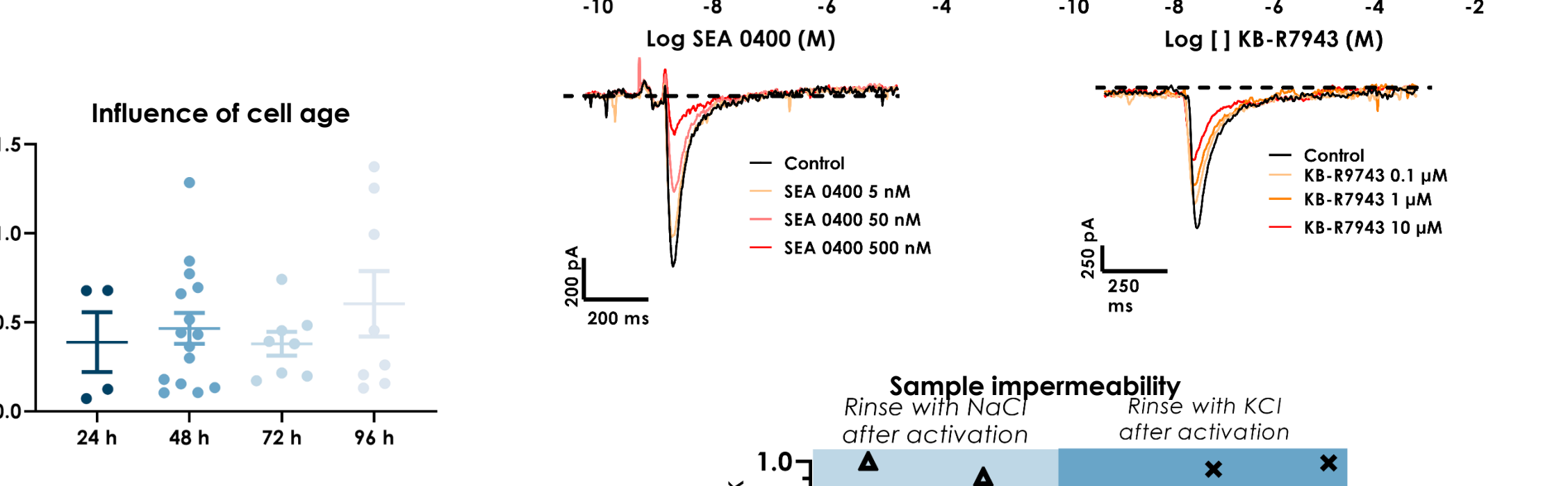
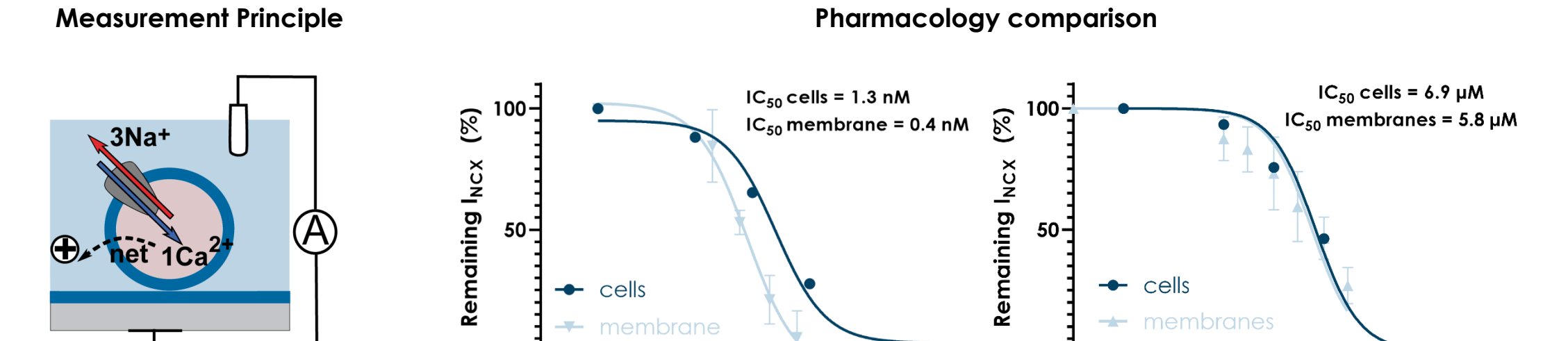
- After optimization it was possible to **immobilize whole cells to the sensor**
- **Success rate when using whole cells is decreased by 50% and the current amplitude is scattering** when comparing different sensors.

Validation of the method using a CHO-SGLT1 cell line



- An existing assay for the measurement of SGLT1 activity was **successfully transferred** to the new whole cell approach.
- However **signal amplitudes are smaller and less stable** compared to purified plasma membrane of the same cell line.

Validation of the method using a HEK-NCX1 cell line



- An existing NCX assay was **successfully transferred** using whole cells
- Sensors with intact cells and membrane fragments have **comparable pharmacological properties**
- The **time after seeding of the cells did not have a significant influence**.
- Enclosed salts cannot be washed out of the sensors with whole cells, **indicating that the cells are undamaged**.

Capacitive current measurement delivers functional evidence for transporters in iPSC derived neurons and cardiomyocytes

Conclusion

The use of whole cells enables the investigation of expensive or rare cell types due to much lower material consumption, enabling completely new applications.

- Primary cell cultures can be functionally tested for transporter activity
- Transporter function can be investigated in a native environment close to physiological conditions

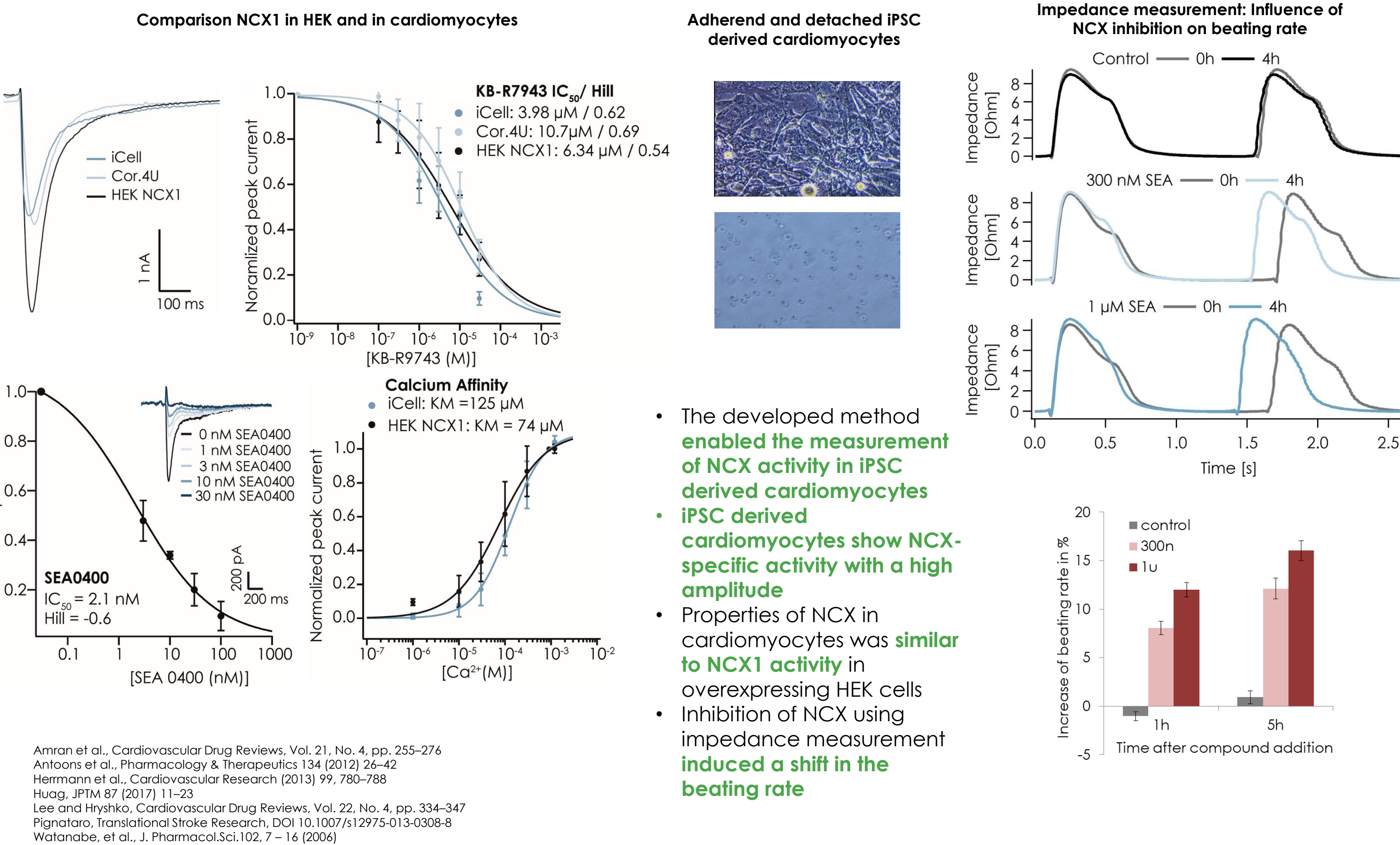
Using whole cells as a sample for SSM based electrophysiology delivers smaller and less reproducible signals compared to membrane preparations and results in a significantly lower success rate.

- The use of purified membrane fragments is still the gold standard when it comes to investigation of eukaryotic transporters in cell lines
- Using intact cells can serve as a quick method for proof-of-principle experiments or pre-tests

By applying the newly developed method it was possible to collect functional evidence for the expression of several transporters in iPSC derived cells.

- iPSC derived cardiomyocytes show NCX activity with high amplitude which has an influence on the cells beating behavior when inhibited
- iPSC derived neurons show glutamate and GABA transport activity
- This supports the suitability of these cells as models for human physiology

Application: Evidence for NCX in iPSC derived cardiomyocytes



Application: Evidence for GABA and Glutamate transport in iPSC derived Neurons

