

- Organellar Transporters and Ion Channels - How to access their electrophysiology by using the SURFE²R technology and Planar Patch Clamp

Maria Barthmes¹, Tom A. Goetze¹, Stephan Holzhauser¹, Melanie Giesler¹, Christian Patzig¹, Raphael Bondes¹, Timo Stengel¹, Marius Vogel¹, Johannes Stiehler¹, Andrea Brüggemann¹, Michael George¹, Niels Fertig¹

nanjion

¹Nanion Technologies GmbH,
Gabrielenstrasse 9, Munich
Germany
Info@nanion.de

References

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Abstract

Electrophysiological characterization of ion channels from organellar membranes is vastly restricted due to limited accessibility. Even more so the direct measurement of transporter activity in these membranes is extremely challenging, because noise usually exceeds signal size due to comparably low transport rates.

Here we present electrophysiological data of the ADP-ATP exchanger (ANT) from purified inner mitochondrial membranes (IMM) acquired with the SURFE²R technology which is based on a solid supported

membrane approach. By measuring the total current of a large number of transporter molecules triggered simultaneously, the signal becomes available for pharmacological studies.

Also we show data from mitochondrial BK_{Ca} channel to demonstrate how planar patch clamp technology facilitates the study of organellar membrane ion channels.

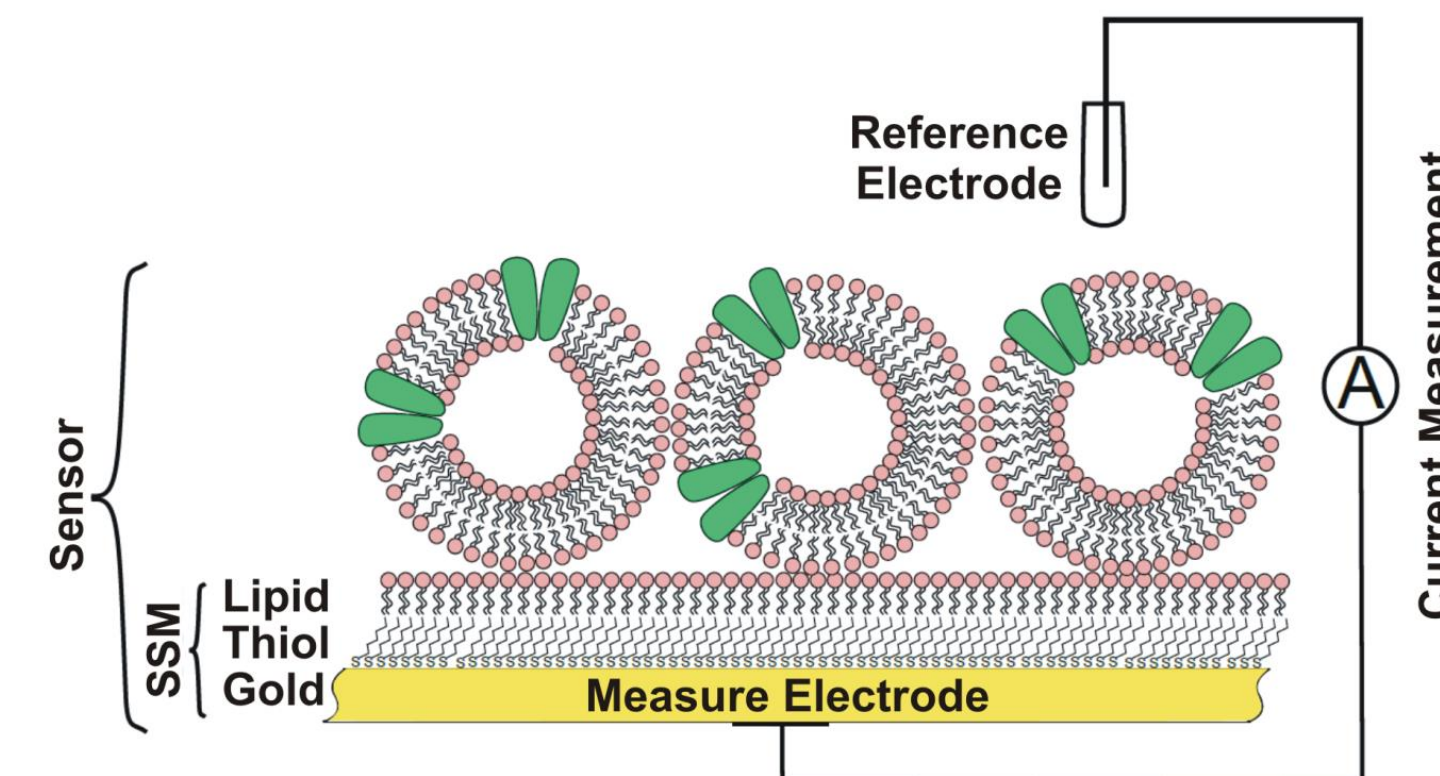
The SURFE²R Technology

Surface Electrogenic Event Reader (formerly IonGate)

Solid Supported Membrane (SSM) based biosensors A gold electrode of 3 mm diameter is functionalized by a thiol- and a lipid layer. This allows to immobilize membrane fragments or liposomes containing the target transporter on the sensor.

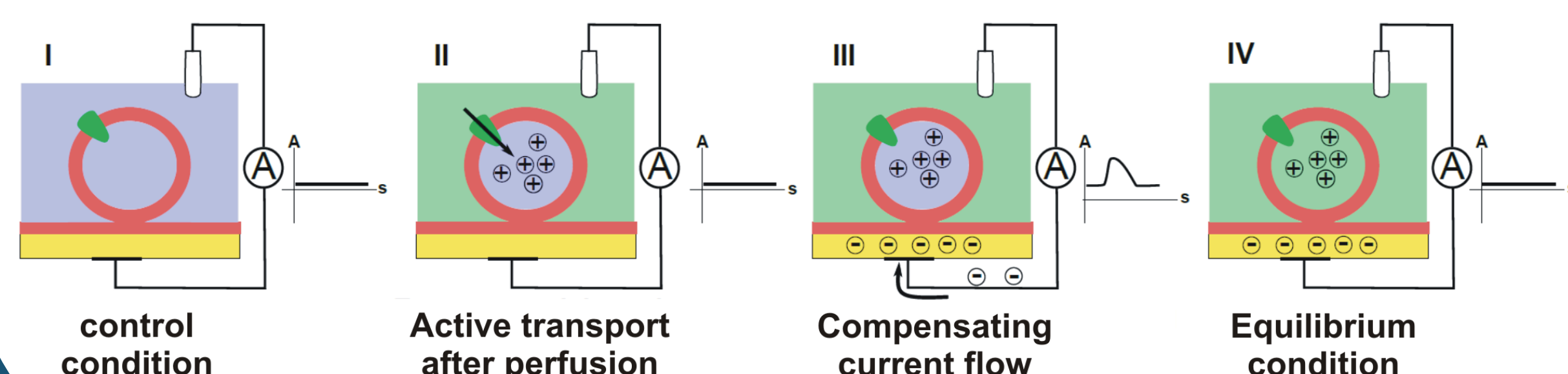
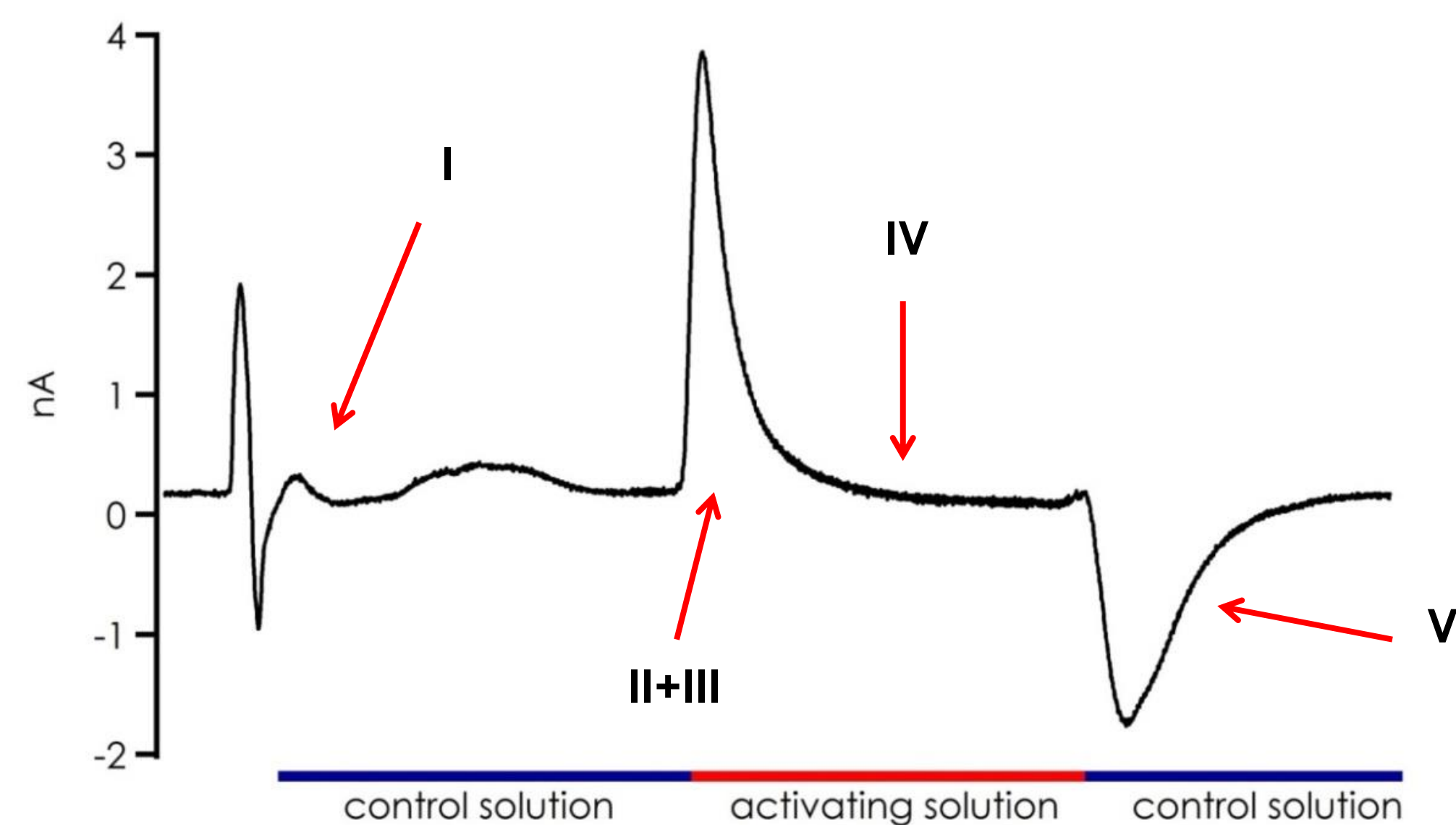
Sensor preparation

- 3 mm gold surface
- 30 min 0.5 M 1-octadecane-thiol
- 1 µl 7.5 µg/µl DPHPC
- loading buffer added
- 8 µl sonicated liposomes
- centrifugation 1 h, 2200 g



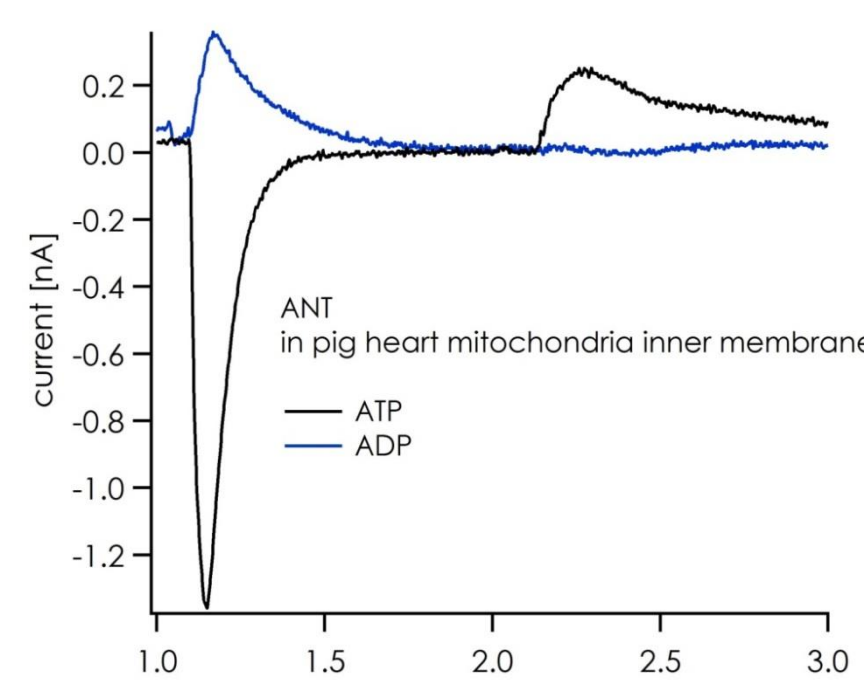
Accumulated transport current

The protein on the sensor is synchronously activated by a fast perfusion system. The transport of charged substrates over the membrane generates an electrical current (like capacitor plates). It's measured as a sum signal of all the protein on one sensor, thereby enabling a high amplification.



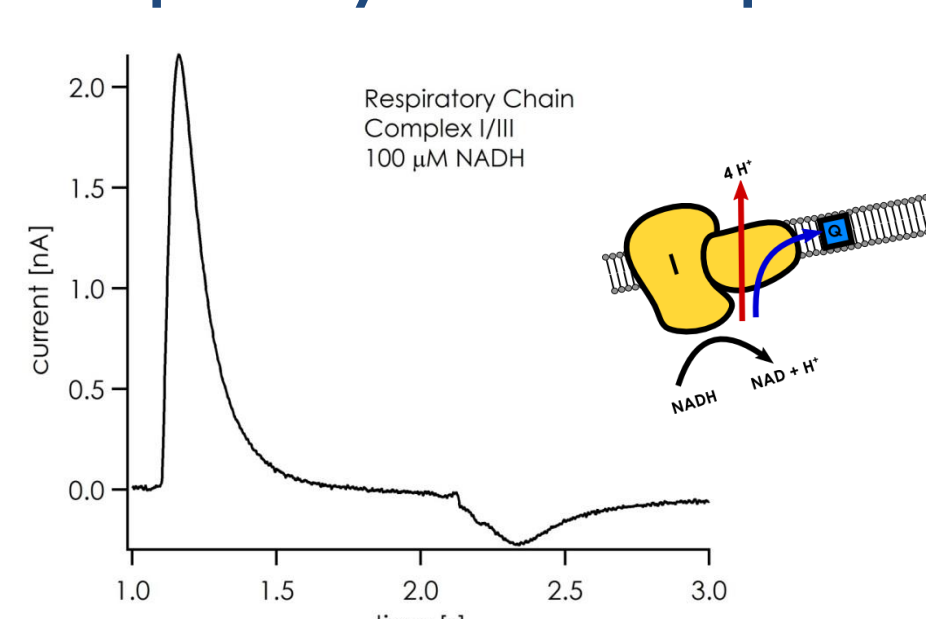
ANT and Respiratory Chain Complexes from IMM preparations

Adenine Nucleotide Translocator (ANT)

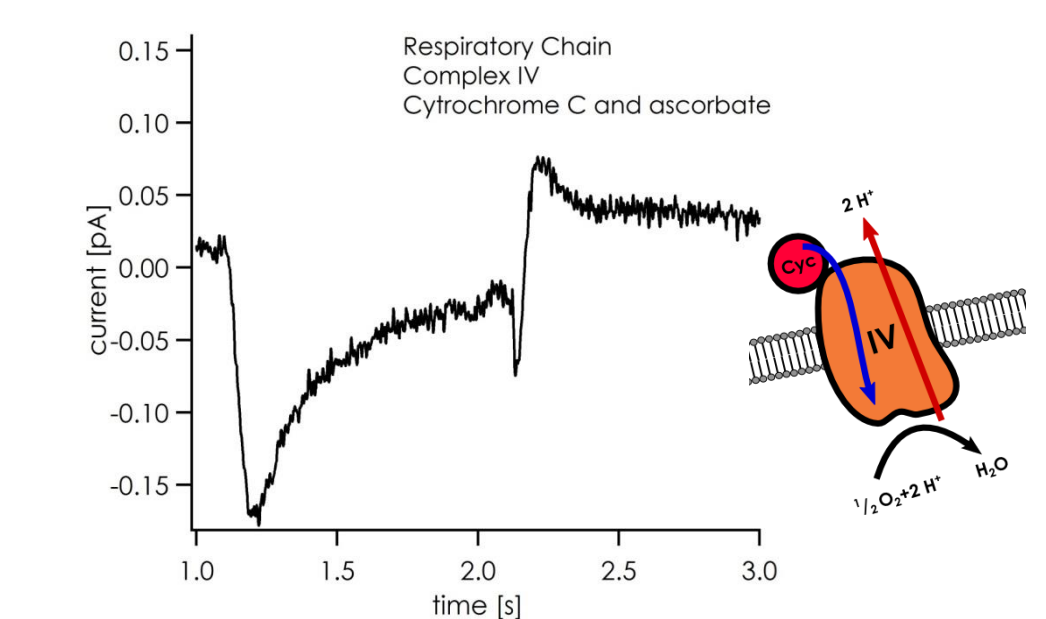


Two modes: ADP-ATP exchange and transport separately. Preparations from pig heart mitochondria inner membrane. Example traces: activation of ANT in the presence of ATP or ADP.

Respiratory chain complexes (I, II+III, IV, V)

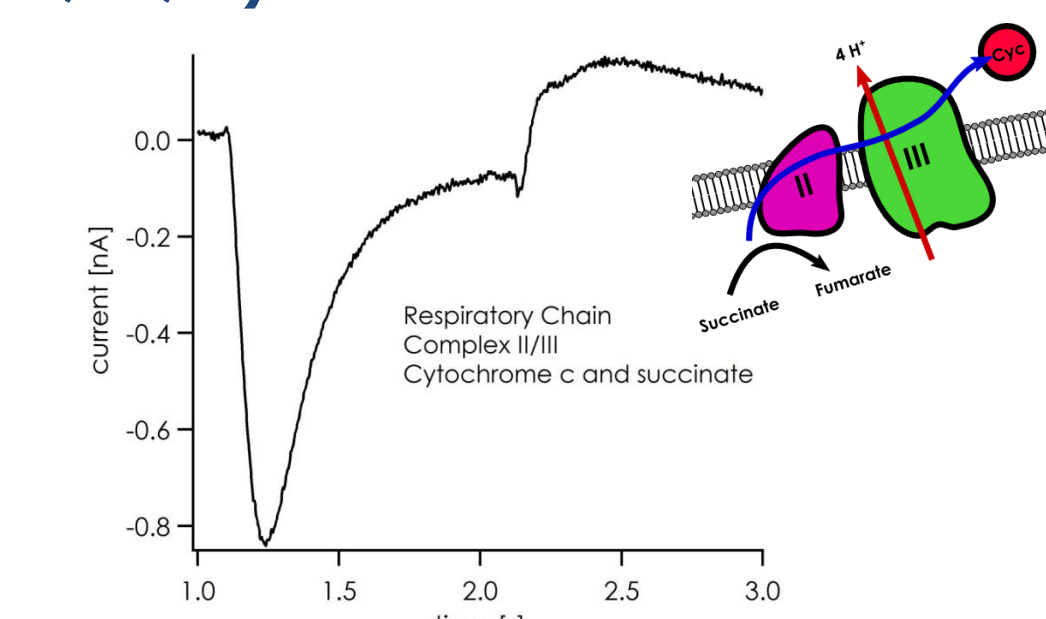


Oxidation of NADH leads to transport of four protons across the membrane. The electrons are transferred to ubiquinone. The signal is generated by addition of NADH.

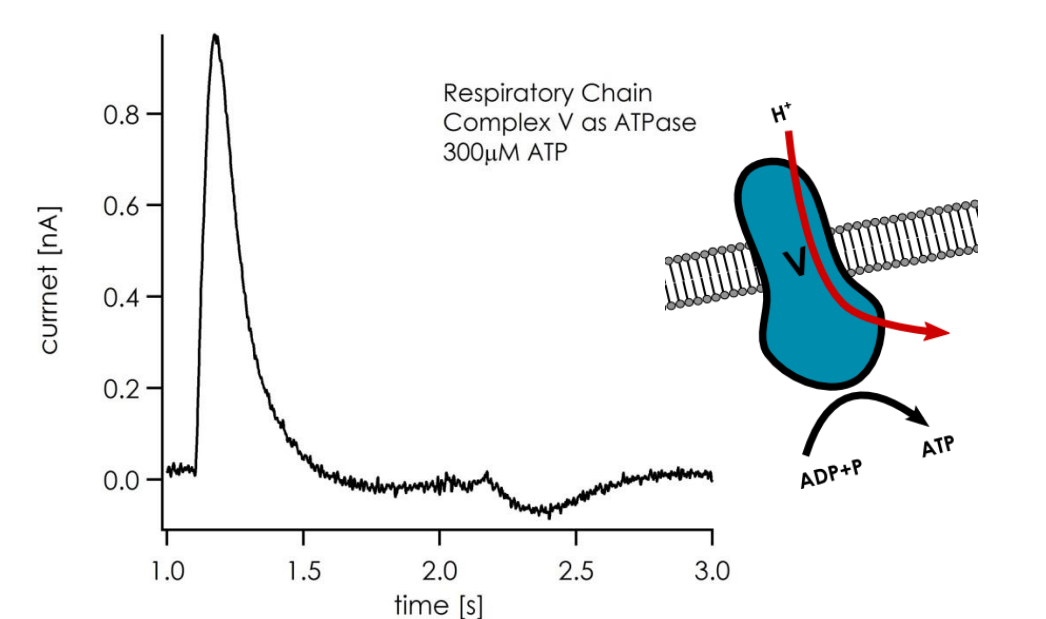


Reduction of oxygen by consumption of electrons from cytochrome c and transport of two protons to the intermembrane space. Signal is evoked by reduced (ascorbate) cytochrome c.

SURFE²R N1

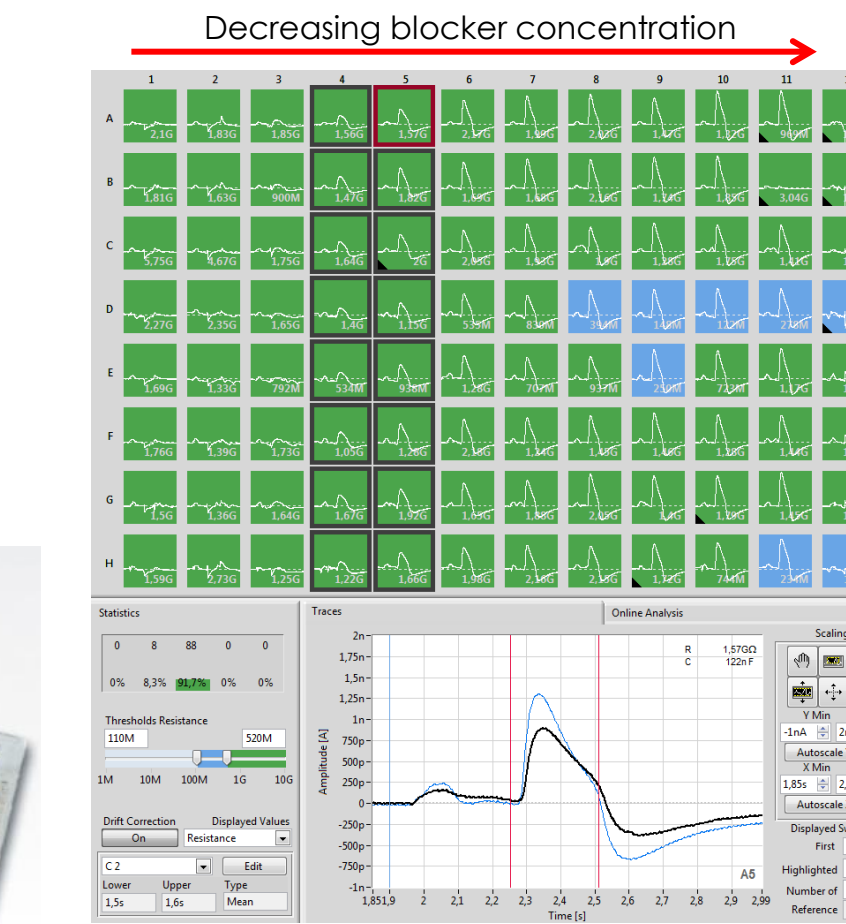
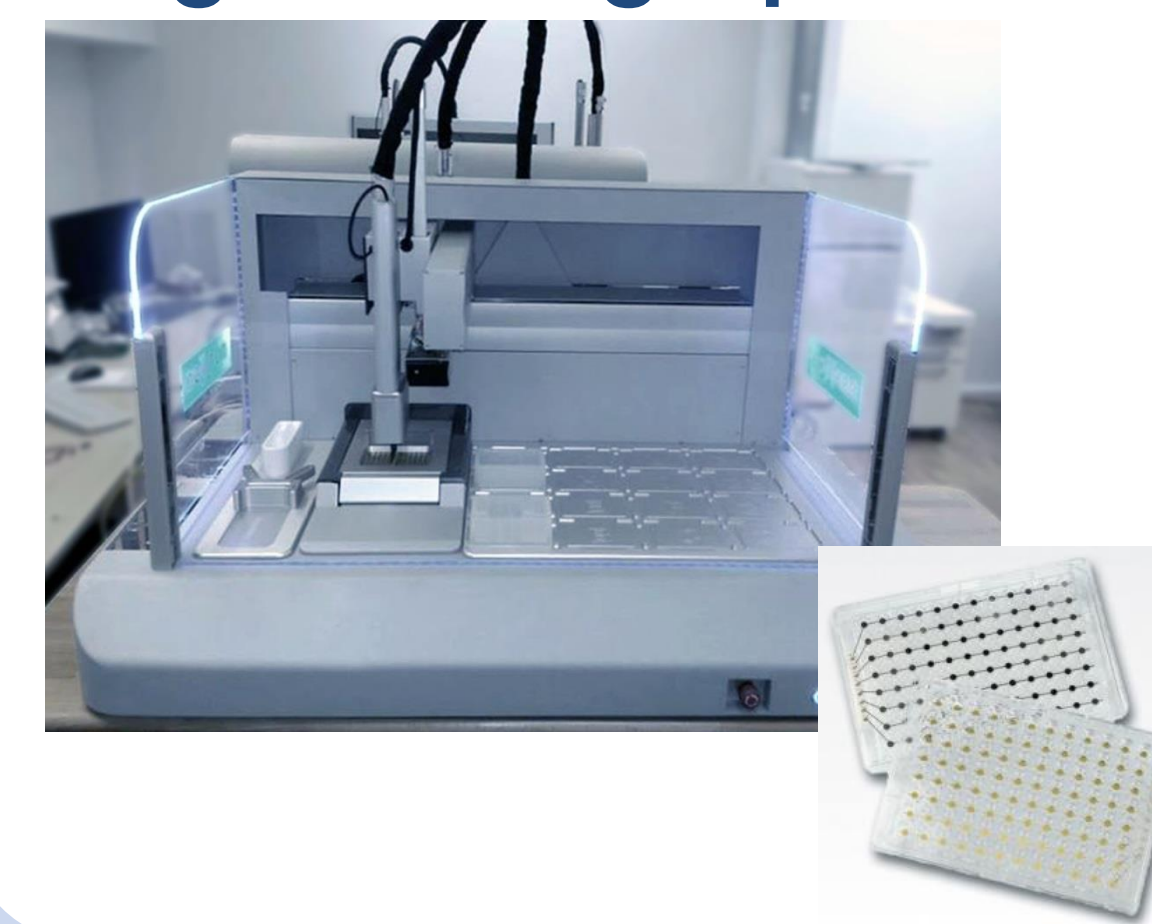


Oxidation of succinate to fumarate. The electrons are transferred to cytochrome c via complex III while four protons are transported to the intermembrane space. Signal is evoked by oxidized cytochrome c, succinate must be provided.

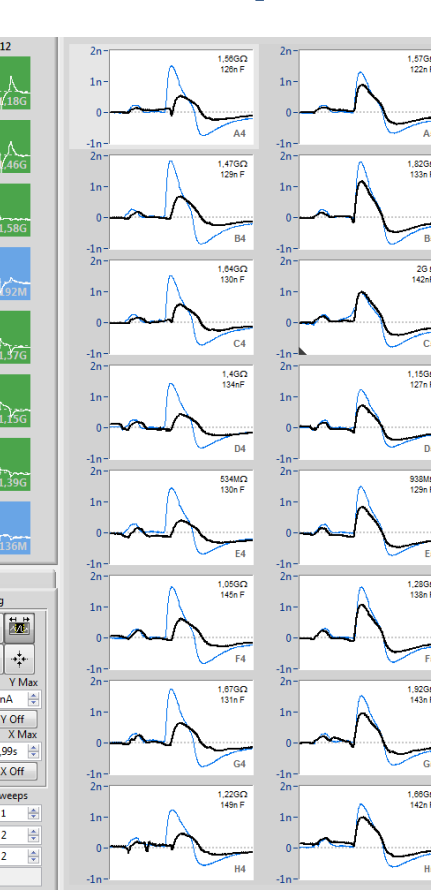


Complex V generates ATP from ADP plus phosphate. Here it works reverse as ATPase. Therefore signal is generated by addition of ATP. Membranes were treated with 5µM BKA (Bongkreic acid = specific ANT inhibitor)

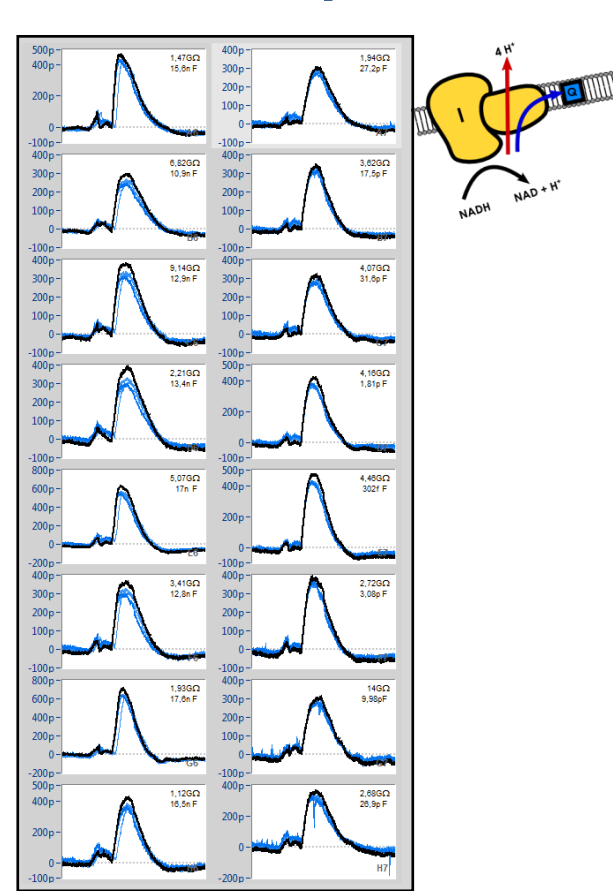
Higher Trough-put SURFE²R N96



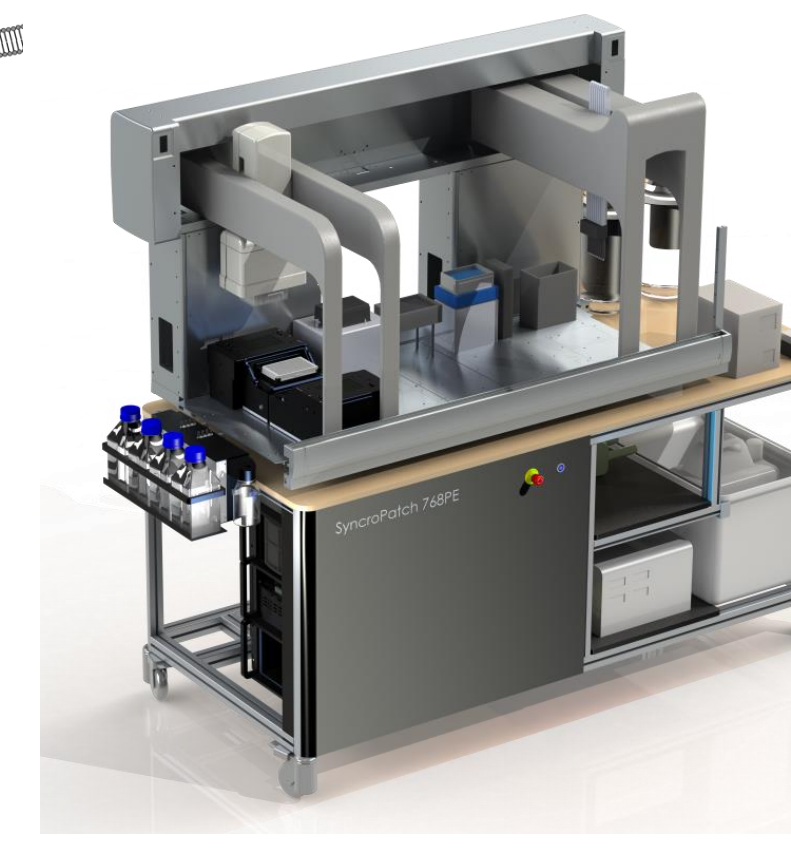
PepT1



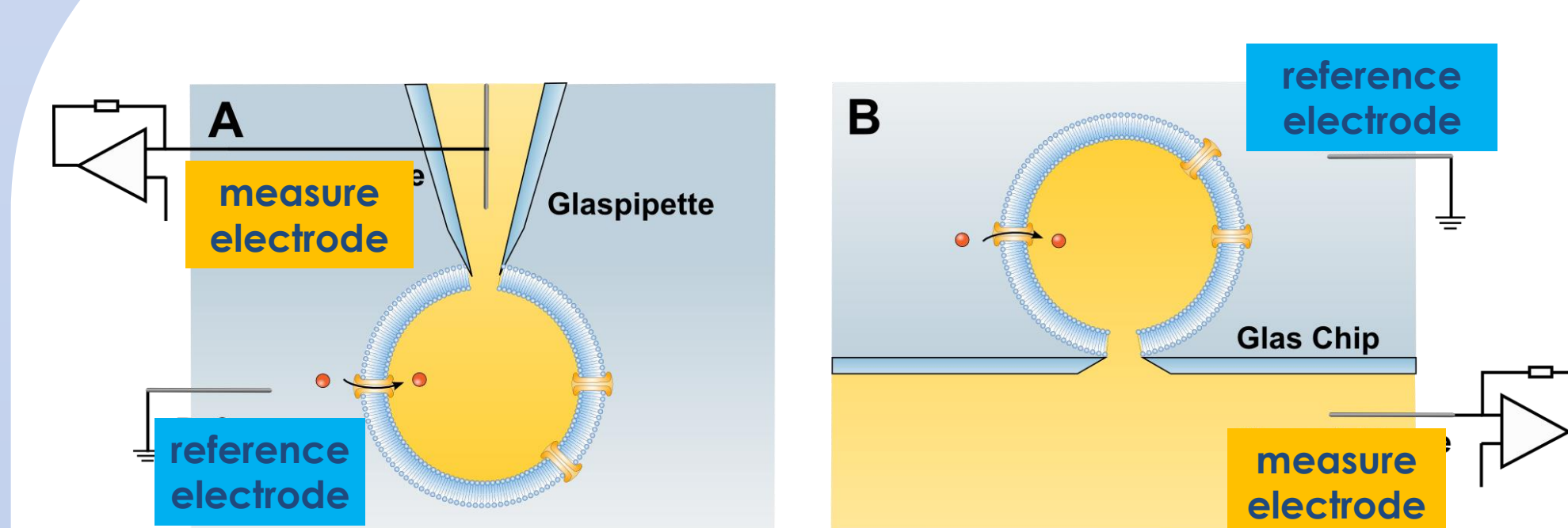
RC Complex I



the future... SURFE²R 96 SE



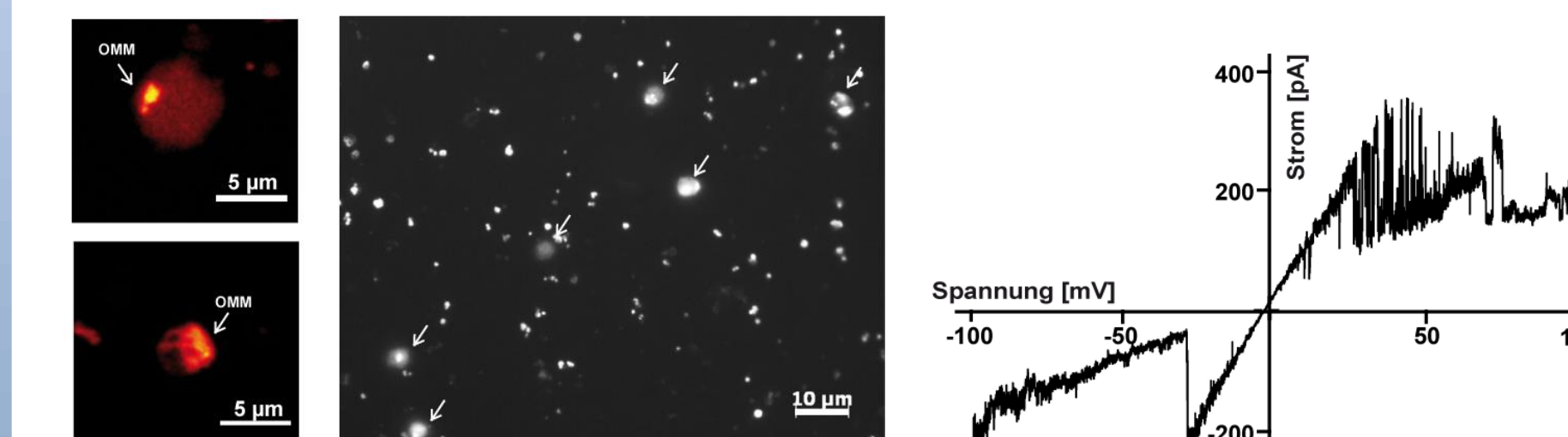
Planar Patch Clamp - from Port-A-Patch to SyncroPatch 384PE -



preparation of agar salt bridge (3M KCl)



The Search of the Putative Mitochondrial BK_{Ca} channel

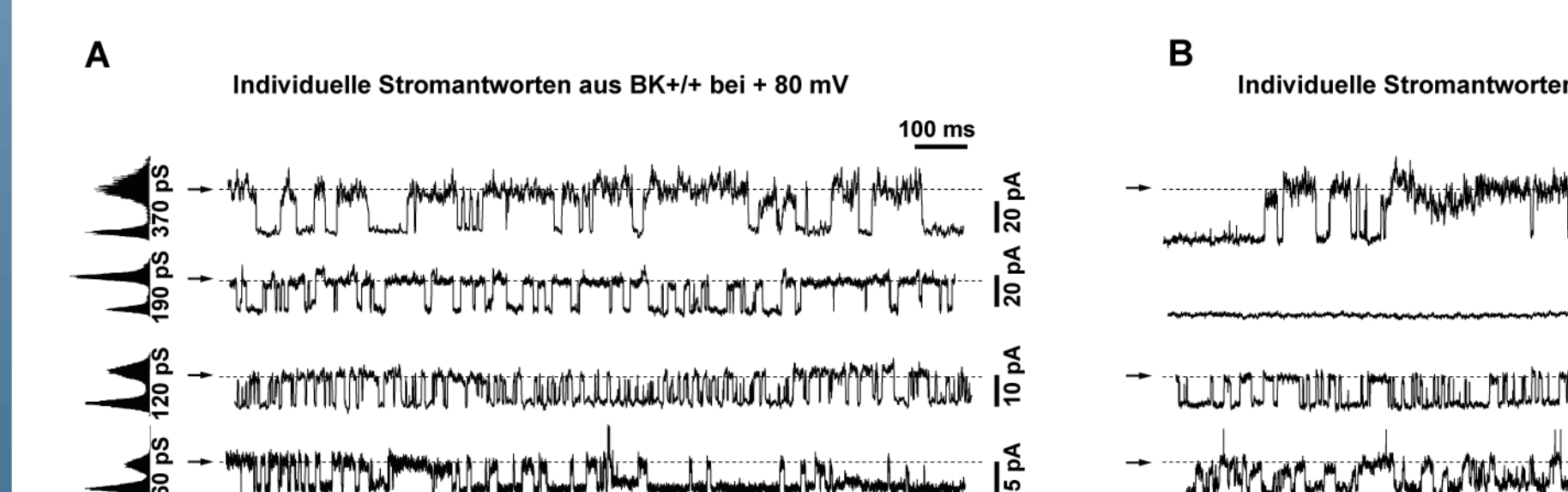
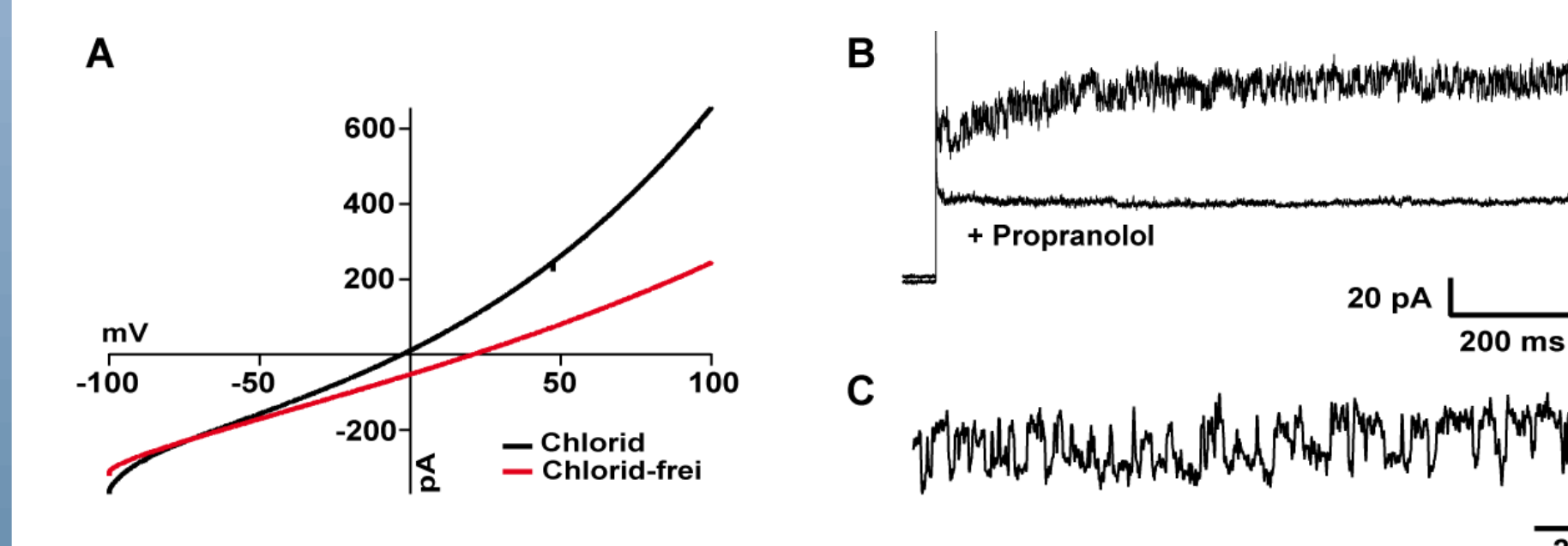


Does KCNMA1 gene not only code for KCa1.1 (plasma membrane) or also mitochondrial BK_{Ca}?

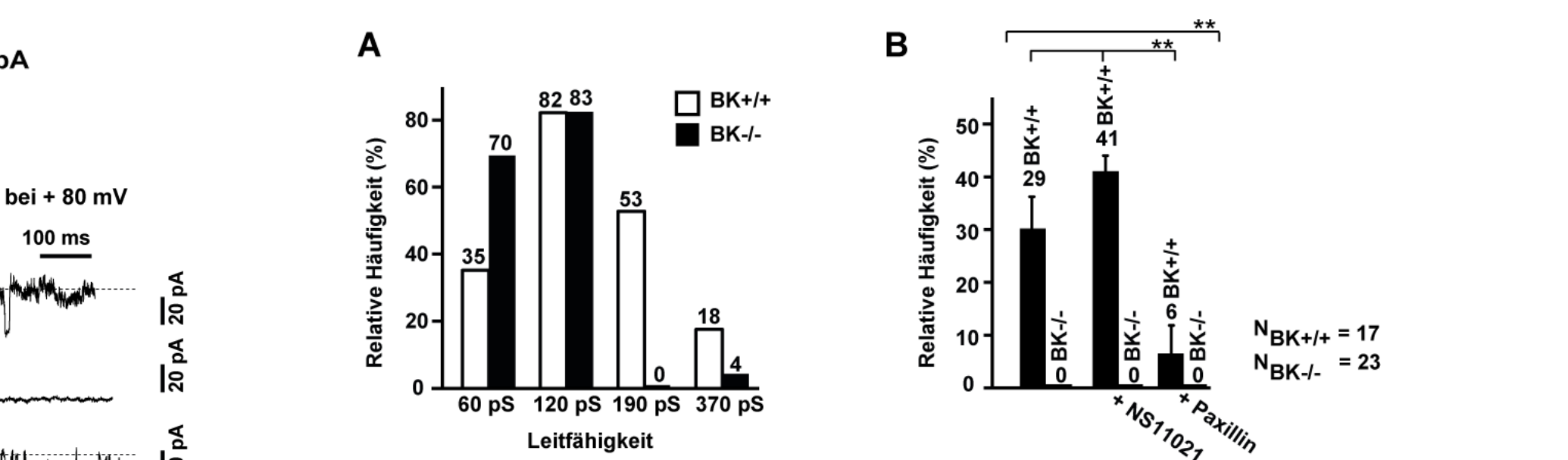
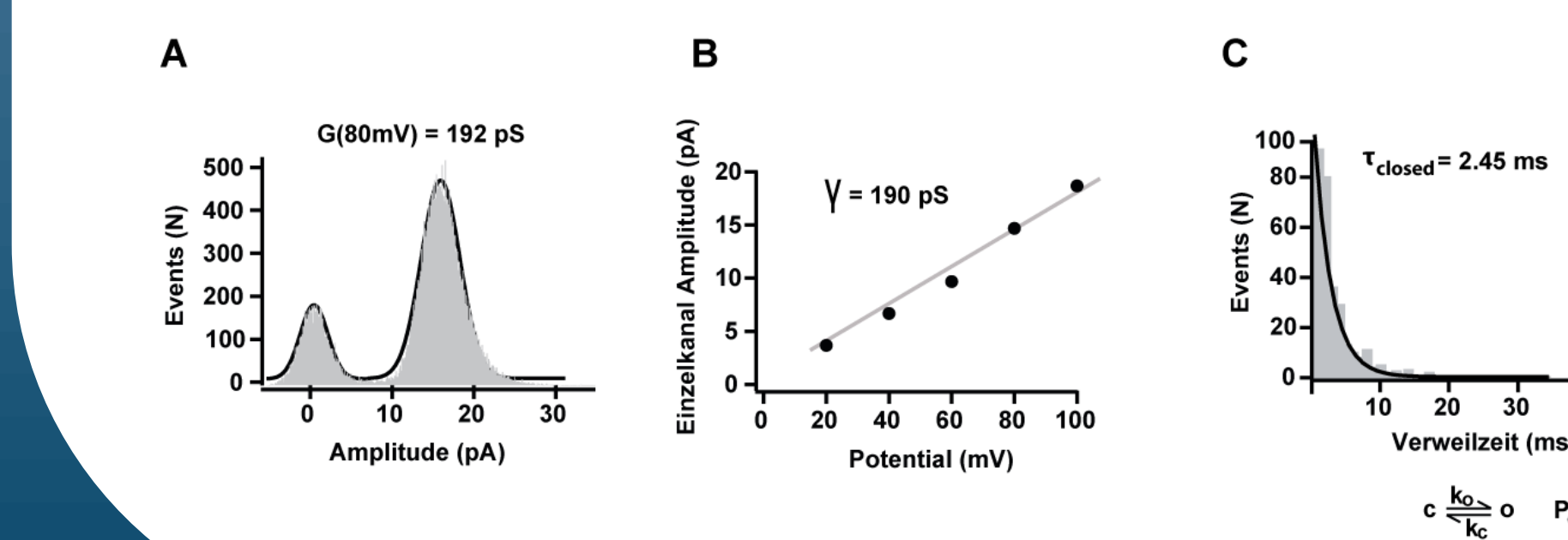
- Preparation of mitoplasts (2-5 µm) and mitochondria from HEK293
- mito-tracker red stained, OMM as „cap“ structure [Kirichok et al.]
- control for OMM, electroformation of liposomes + intact mitochondria bilayer measurements, large activities, VDAC

A-C macroscopic current + single channel events largely attributed to Cl⁻ channel IMAC ~100pS (Boreckyes et al.)

- Propranolol: IMAC inhibitor, Cl⁻ replaced by MSA- methane sulfonic acid
- NPC-chip: <1 µm aperture, R: 10-15MΩ



A-B Four different categories of conductance: 60 pS, 120 pS, 190 pS, 370 pS mitoplasts from wt BK^{+/+} and mutant BK^{-/-} mice, ionic conditions allowed potassium currents to flow only



Statistical analysis
A 4 channel appearances in wt BK^{+/+} and mutant BK^{-/-} mitoplasts
B Conductance of 190pS in different conditions, NS11021: increased appearance by >10%. Paxillin reduced by >20%. n-experiments: 27 (BK^{+/+}), 26 (BK^{-/-})

Analysis of single channel characteristics
A current histogram at 80mV
B linear IV at positive voltages, conductance of 190pS
C-D dwell time of closed and open state