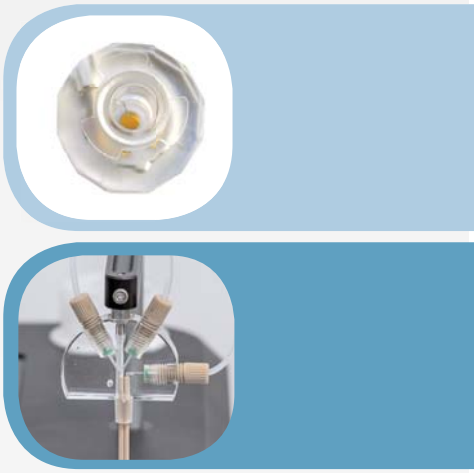
SURFE²R N1.

Real-time measurements of electrogenic transport.



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SURFE²R N1. Based on proven SSM-technology.

Quality Data

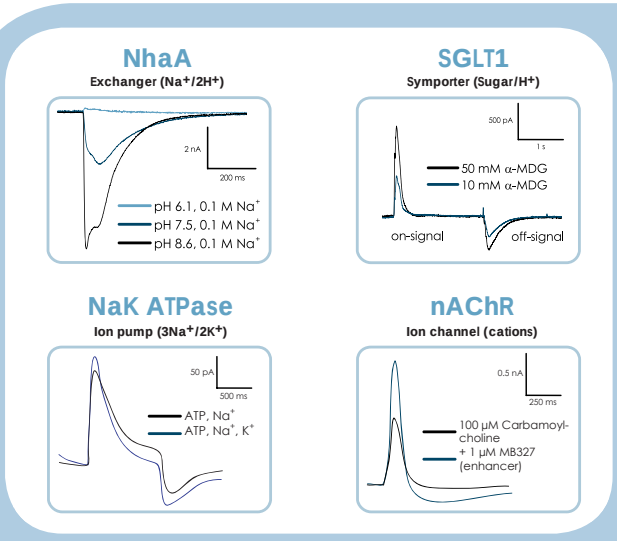
- Real-time data, not single point read-out
- High signal amplification compared to patch-clamp
- Fast binding kinetics can be resolved

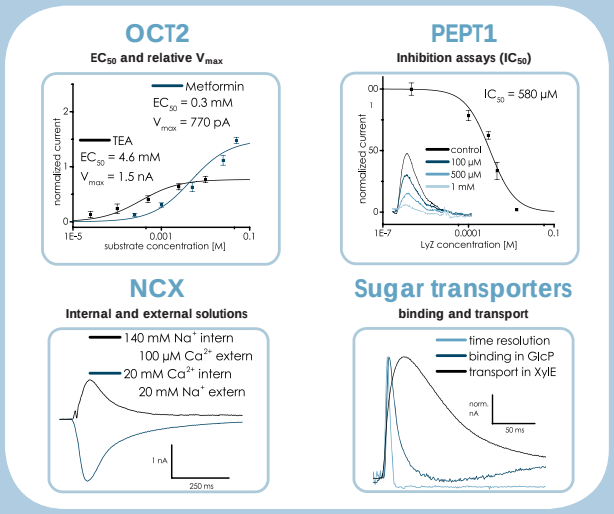
Fast & Easy

- Label-free electrical measurements
- Automated recording and analysis
- Requires only 0.1 - 1 µg protein/sensor
- 150 Datapoints per day!

Large Scope

- > 100 targets tested
- ~ 100 peer reviewed papers
- Transporters, pumps and channels
- Native membranes or proteoliposomes
- Flexible experimental design





Which targets can be measured?

The SURFE²R technology allows the investigation of electrogenic membrane proteins with high sensitivity. So far more than 100 targets have been studied with SSM based electrophysiology, including symporters, exchangers, uniporters, ion pumps and ion channels. In addition, light-sensitive rhodopsins have been characterized using this method and electroneutral transporters can be analyzed providing partial reactions are electrogenic

What information can be obtained?

The electrophysiological characterization of a transporter using the SURFE²R technology involves testing different substrates for their relative V_{max} and apparent K_M values. Different inhibition assays can be performed, either as control experiment or for determination of IC_{50} values. In the case of proton-coupled transporters, pH profiles are determined. Many parameters can be investigated including the influence of concentration gradients or membrane potential; determination of transport versus binding signals; calculation of rate constants; stoichiometry and overall kinetic transport models. Furthermore SURFE²R technology allows the comparison of isoforms, homologues, mutations or variation of the lipid environment.

Our Service: Assay development support by experienced Nanion scientists.

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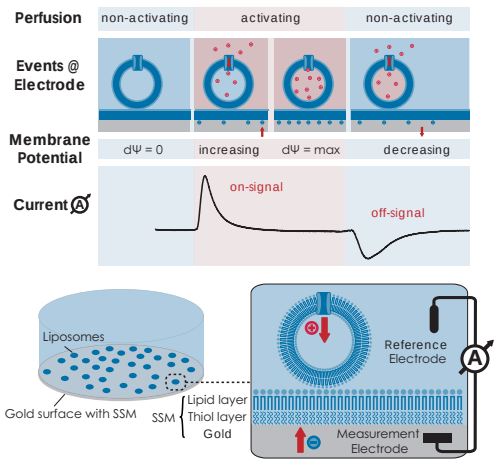
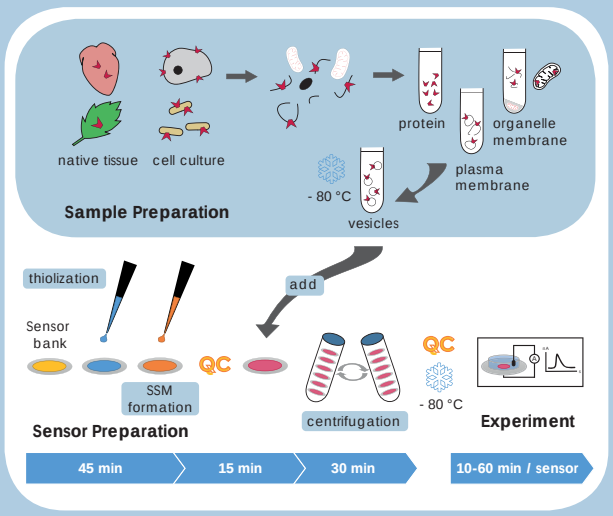


How does it work?

A membrane preparation containing the protein of interest is adsorbed to a solid supported membrane (SSM) on a gold coated sensor chip. The protein from up to 1000 cells can be added to the 3 mm electrode, thereby generating increased signals. The protein of interest is activated by a fast solution exchange. In electrogenic transporters this generates a membrane potential, which can be measured at the electrode via capacitve coupling. Size and shape of this transient current is characterized by the type of transporter, substrate concentration and other assay conditions. The peak current is proportional to the turnover rate and is essential for analysis.



How is it done?

The SURFE²R technology allows the use of isolated membrane vesicles from native tissues or cultured cells, proteins in reconstituted proteoliposomes and even organelles or whole cells. Small quantities of membranes are required: about 1 µg protein per sensor. The daily workflow starts with the preparation of the sensor chip. Typically multiple sensors are prepared simultaneously, starting with the thiolization of the gold sensor followed by the application of the SSM and the adsorption of the membrane/liposomes. Each single measurement takes only 2 min, allowing different assay conditions on multiple sensors to be tested each day. Each sensor lasts for up to 100 measurements.

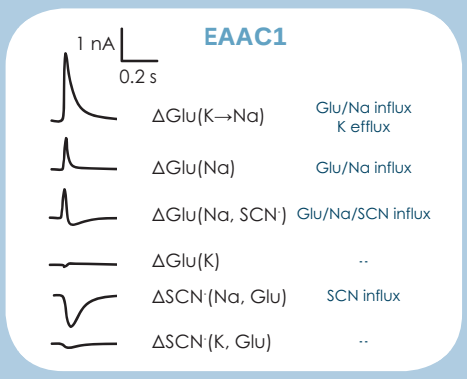
Learn More: Schedule your SURFE²R demo today!

Specifications.

Data output	signal amplitude: 0.1 – 10 nA, detection limit: 50 pA, noise: 20 pA
Low noise amplifier	gain: 10^8 – 10^{11} V/A, resolution: - 10 V to + 10 V (1.2 mV steps)
	RMS noise: 0.5 – 22 pA, low pass: 480 Hz, rise time: 0.7 ms
Measuring electrode	re-usable gold coated sensor chip with 3 mm diameter
Reference electrode	steel capillary of IonJet
Integrated computer	Win10, SURFE²R N1 Control (data recording and analysis software)
Fast solution exchange	200 µl/s, continuous flow: 0.5 s – 6 s, time resolution: 5 – 30 ms
Fluidic components	2 pumps, fast switching valve, tubing cassette, IonJet with Y-fluidic
Autosampler	53 positions, each ~10 ml
Solution consumption	volume per measurement/substrate: ~ 300 µl (inhibitor: 1 – 2 ml)

Turn page for more validated targets

SSM-based electrophysiology: Research highlights



Respiratory Chain Complexes →

Using mitochondrial inner membrane fragments all respiratory chain complexes could be analyzed using the same sensor and different assay conditions. In addition the ATP/ADP exchanger was characterized.

Watzke et al.

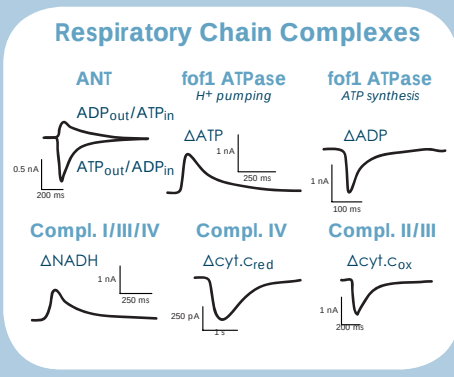
Biochemistry. 2010; 49(48):10308-18

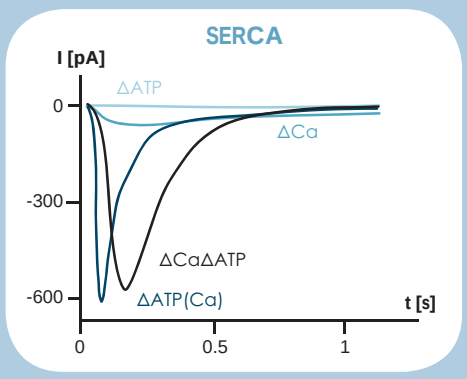
← Transport assay variations

Different assays were performed by using concentration jumps, Δx with various compounds in background, (x) or compound gradients, (x→y). The transient currents show distinct kinetics depending on the reaction observed.

Krause et al.

J Neurosci Methods. 2009; 177(1):131-41





pH profile of H+/sugar symport →

The pH profile reflects the pH dependence of H⁺/sugar symport activity. The down regulation in the acidic and alkaline pH range is due to reduced proton release and proton binding rates, respectively. Each transporter, therefore, shows at least two distinct pK values which are shifted between transporter species.

Bazzone et al.

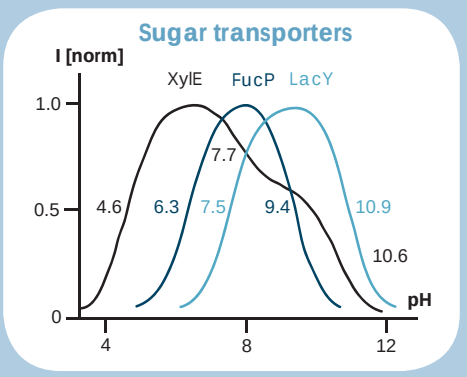
PLoS One. 2016; 11(5):e0156392

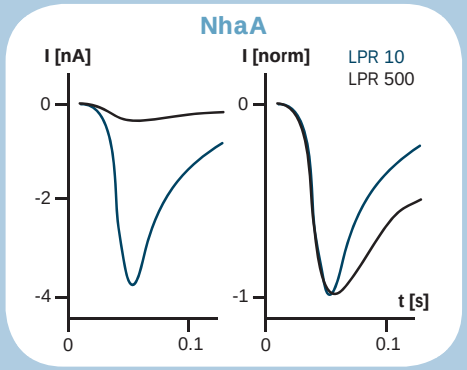
← Kinetics of co-substrate binding

Depending on the performed assay either Ca^{2+} binding, Ca^{2+} release or both can be observed. ATP cannot bind before Ca^{2+}, since no ATP signal is observed without Ca^{2+} background. Ca^{2+} binding without ATP background is slow. Therefore pre-bound Ca^{2+} speeds up the transport reaction.

Tadini et al.

J Biol Chem. 2006; 281(49):37720-7



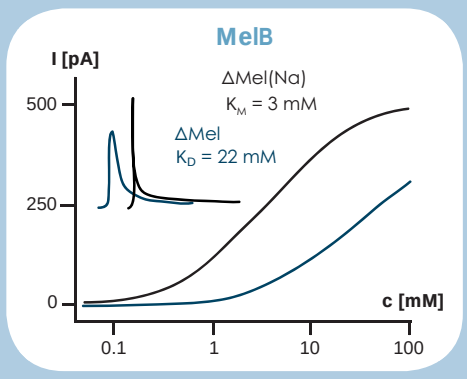


← Transport or binding?

The Na^+/H^+ exchanger NhaA was tested using proteoliposomes with different protein densities. Higher LPR yield lower signal amplitudes. But the decay time of the transient currents only depends on the LPR for steady-state-like transporter currents. For binding reactions the signal shape would be independent of the LPR.

Zuber et al.

Biochim Biophys Acta. 2005; 1709(3):240-50

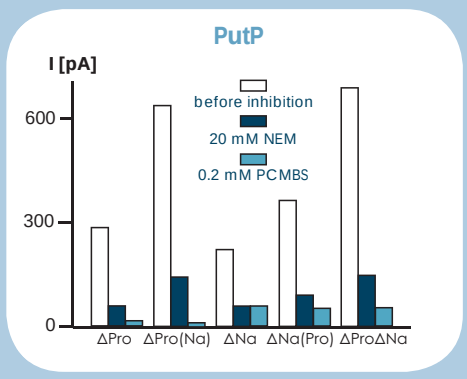


↑ EC50 of transport & binding currents

Sugar concentration jumps with Na^+ background yield transient currents representing Na^+/sugar symport activity with low EC_{50}. Without Na^+ background the same concentration jumps yield fast transient currents with much higher decay times representing electrogenic binding events with a much higher EC_{50} value.

Ganea et al.

Biochemistry. 2001; 40(45):13744-52



↑ Inhibition of Na⁺/proline symport

Different transport assays were tested followed by inhibition with a reversible inhibitor and an irreversible inhibitor which binds covalently to the transporter. The inhibition efficiency is viewed by the decrease in peak current for each assay.

Zhou et al.

J Mol Biol. 2004; 343(4):931-42

Selection of validated targets

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Target	Substrate	Preparation & Source	Literature
		Ion pumps	
NaK-ATPase	Na^+/K^+	Plasma membrane fragments (pig kidney)	Pintschovius 1999 (+6)
HK-ATPase	H^+/K^+	Plasma membrane fragments (pig gastric mucosa)	Kelety 2006
SERCA	Ca^{2+}	Sarcoplasmatic reticulum vesicles (rabbit muscle tissue)	Tadini-Buoninsegni 2004 (+9)
v-ATPase	H^+	Synaptic vesicles (rat brain tissue)	Obrdlik 2010
fof1-ATPase *(I. Tart.)*	Na^+	Proteoliposomes (be)	Burzik 2003
fof1-ATPase	H^+	Inner mitochondrial membranes (pig heart)	Watzke 2010
ATP7B	Cu^{2+}	Microsomes (oe COS-1 cells)	Tadini-Buoninsegni 2010 (+1)
CopA	Cu^{2+}	Plasma membrane fragments (be)	Mattle 2015
VrPPase	H^+	Proteoliposomes (yeast expression)	Li 2016
Resp. chain compl.	H^+	Inner mitochondrial membranes (pig heart)	Watzke 2010 (+1)
Compl. I *(Y. Lipol.)*	H^+	Inner mitochondrial membranes (oe in CHO cells)	Siebels 2016
Bacteriorhodopsine	H^+	Purple membrane fragments (be)	Seifert 1993 (+1)
Rhodops. (O. Mar.)	H^+	Synaptic vesicles (rat brain tissue)	Janke 2013
		Symporters	
PEPT1	H^+/peptide	Membrane vesicles (oe in CHO cells)	Kelety 2006
YdgR	H^+/peptide	Proteoliposomes (be)	Weitz 2007
Yhip	H^+/peptide	Proteoliposomes (be)	Harder 2008
PutP	Na^+/pro	Proteoliposomes (be)	Zhou 2004
GltP	Na^+/glu	Proteoliposomes (be)	Raunser 2006
PAT1	H^+/ser	Membrane vesicles (oe in CHO cells)	-
CAT2b	Arg	Lysosomes and lysosomal membrane fragments (oe)	-
EAAC1	$3Na^+$/glu	Membrane vesicles (oe in CHO cells)	Krause 2009
SGLT1	Na^+/glucose	Membrane vesicles (oe in CHO cells)	Weinglass 2008
LacY	H^+/lactose	Proteoliposomes (be)	Garcia-Celma 2009 (+3)
MelB	Na^+/melbiose	Proteoliposomes (be)	Ganea 2001 (+3)
XylE & FucP	H^+/xyl & H^+/fuc	Proteoliposomes (be)	Bazzone 2016
GlcP	H^+/glucose	Proteoliposomes (be)	-
BetP	Na^+/betaine	Proteoliposomes (be)	Khafizov 2012 (+1)
SulP	Bicarbonate	Proteoliposomes (be)	Srinivasan 2016
CNT1	Nucleosides	Membrane vesicles (oe in CHO cells)	-
GAT1	GABA	Membrane vesicles (oe in CHO cells)	-
		Exchangers	
NhaA (Ec, Hp, St)	$Na^+/2H^+$	Proteoliposomes & membrane fragments (be)	Zuber 2005 (+5)
NhaP1	Na^+/H^+	Proteoliposomes (be)	Calinescu 2014 (+1)
NCX	$3Na^+/Ca^{2+}$	Giant unilamellar vesicles (be)	Barthmes 2016 (+1)
Clc-7 & EcClc	H^+/Cl^-	Proteoliposomes (be) & membrane vesicles (oe in CHO cells)	Schulz 2010 (+1)
NirC	NO_2^-	Proteoliposomes & everted membrane vesicles (be)	Rycovska 2012 (+1)
ArcD	Arg/Orn	Proteoliposomes (be)	Wimmer 2008
AAC/ANT	ADP/ATP	Proteoliposomes (bovine heart mitochondria)	Gropp 1999 (+1)
		Ion channels	
P2X2	Cations	Membrane fragments (oe in HEK cells)	Schulz 2009
nAChR	Cations	Membrane vesicles (electric organs, *T. Californica*)	Niessen 2016 (+1)
AM2	H^+	Membrane fragments (oe in CHO cells & cell-free expr.)	Balannik 2009 (+1)
UCP1	H^+	Proteoliposomes (mouse brown adiopose tissue mitochondria)	Blesneac 2012
TRPC5	Ca^{2+}	Membrane vesicles (oe in HEK cells)	Kendall 2011
		Other targets	
OCT2 (uniporter)	Organic cations	Membrane vesicles (oe in CHO cells)	Gaiko 2011
Amt3 (mode unknown)	NH_4^+	Proteoliposomes (be)	Wacker 2014
AQP6	Cations	Proteoliposomes (cell-free expression)	Bock 2014

Abbreviations: be = bacterial expression, oe = overexpression

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"Using the SURFE2R N1 we have recently obtained high quality data in a very short period of time. We have developed an assay to measure the activity of ammonium transporters from the Amt protein family. There has been considerable controversy over the mechanism of ammonium transport by Amt proteins and the controversy was due to the lack of quantitative kinetic data characterizing the activity of the proteins at the single channel level. The SSM technologies allows to overcome this hurdle and we are now capable of answering very challenging functional questions concerning the mechanisms and the energetics of these transporters."

Dr. Arnaud Javelle,
Strathclyde Institute of Pharmacy and Biomedical Sciences

"The SURFE²R makes extremely challenging electrophysiological targets accessible to robust routine analyses. For example, we have developed an assay for investigating nicotinic acetylcholine receptor a7 ion channels, using membranes from the Pacific electric ray eel, Torpedo California. In this way, we overcome many of the well-known difficulties associated with this ion channel, which allows us to efficiently obtain information regarding compound pharmacology. Obtained pharmacology values match those of patch clamp recordings exceptionally well. We find that the SURFE²R is an excellent platform for characterization of electrogenic processes in isolated membranes."

Karin V. Niessen, Dr. Thomas Seeger,
Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany

SSM-based electrophysiology is a well-established technique developed in our lab in the early 1990s for the investigation of electrogenic membrane transporters. It works with a wide range of samples from mammalian cell membranes over microbial membrane vesicles to proteoliposomes. SSM-based electrophysiology is especially useful in cases where conventional electrophysiology cannot be applied, e.g. for transporters residing in intracellular membranes or plasma membranes of small microbial cells. The SURFE²R N1 is a commercial instrument for SSM based electrophysiology which facilitates basic research in the field of membrane transport."

Prof. Dr. Klaus Fendler
Max-Planck Institute of Biophysics, Frankfurt, Germany

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