

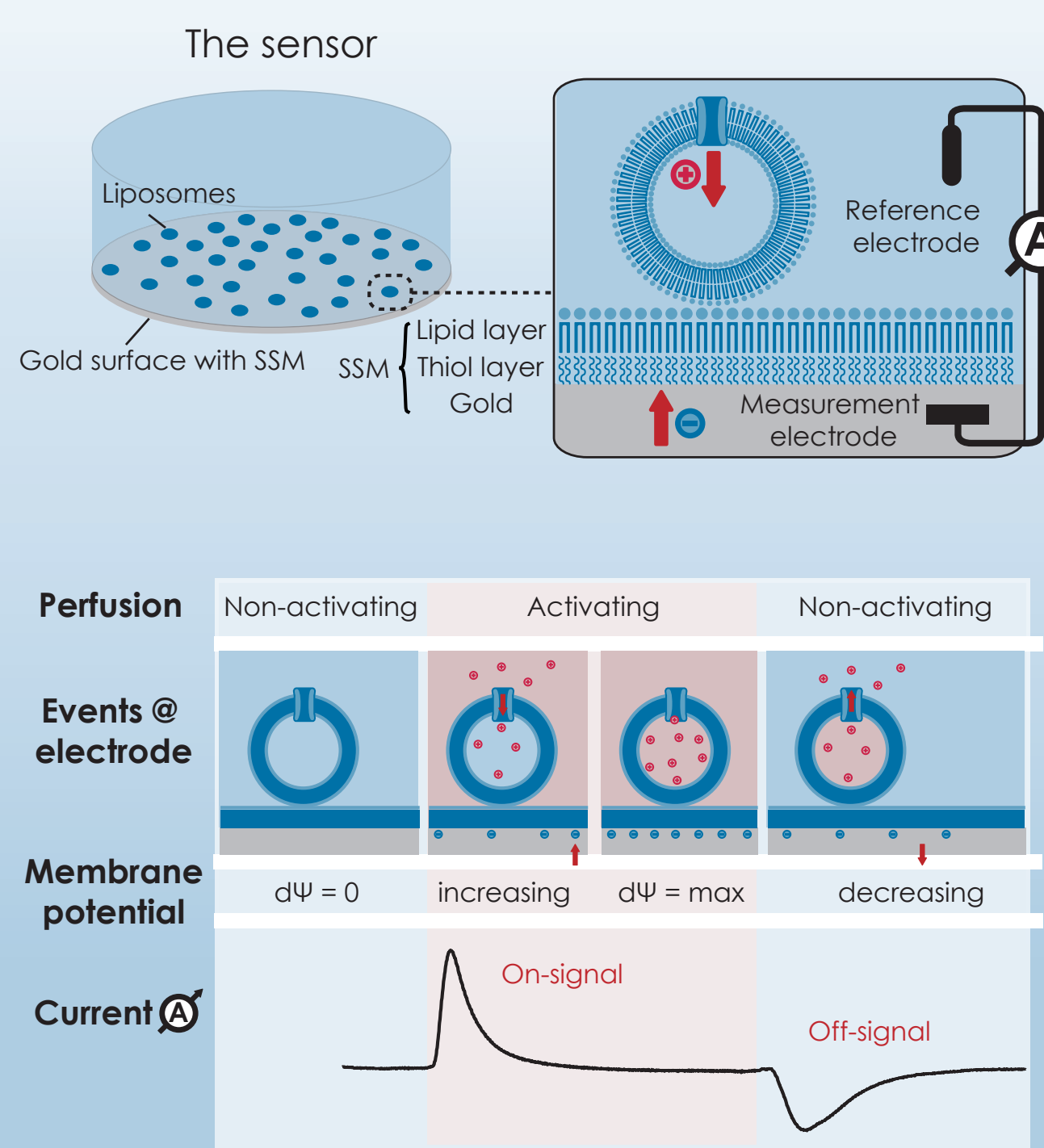
An emerging technique for the characterization of transport proteins: SSM-based electrophysiology



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About the technique: SSM-based electrophysiology



In SSM-based electrophysiology any membranes containing the protein of interest are adsorbed onto a solid supported membrane (SSM). The SSM is assembled on a gold coated sensor chip and consists of a thiol layer with a lipid monolayer on top. The large sensor of 3 mm in diameter allows the simultaneous analysis of up to 10^9 transporters. The possibility to use purified samples further increases the signal to noise ratio which allows the measurement of targets with lower turnovers compared to ion channels, such as uniporters, symproters, exchangers and ion pumps.

During the experiment a fast solution exchange provides the substrate and activates the electrogenic transport reaction. This is accompanied by the charging of the membrane which is detected as a transient current. The high sensor stability allows for different solution exchange experiments using the same sensor. Both transport and binding can be resolved and kinetic parameters like rate constants, KD, KM or IC50 can be determined in a fast and easy workflow. One single measurement takes only seconds.

About the instrumentation: SURFE²R N1 and SURFE²R 96SE



SURFE²R N1: compact solution for research purposes

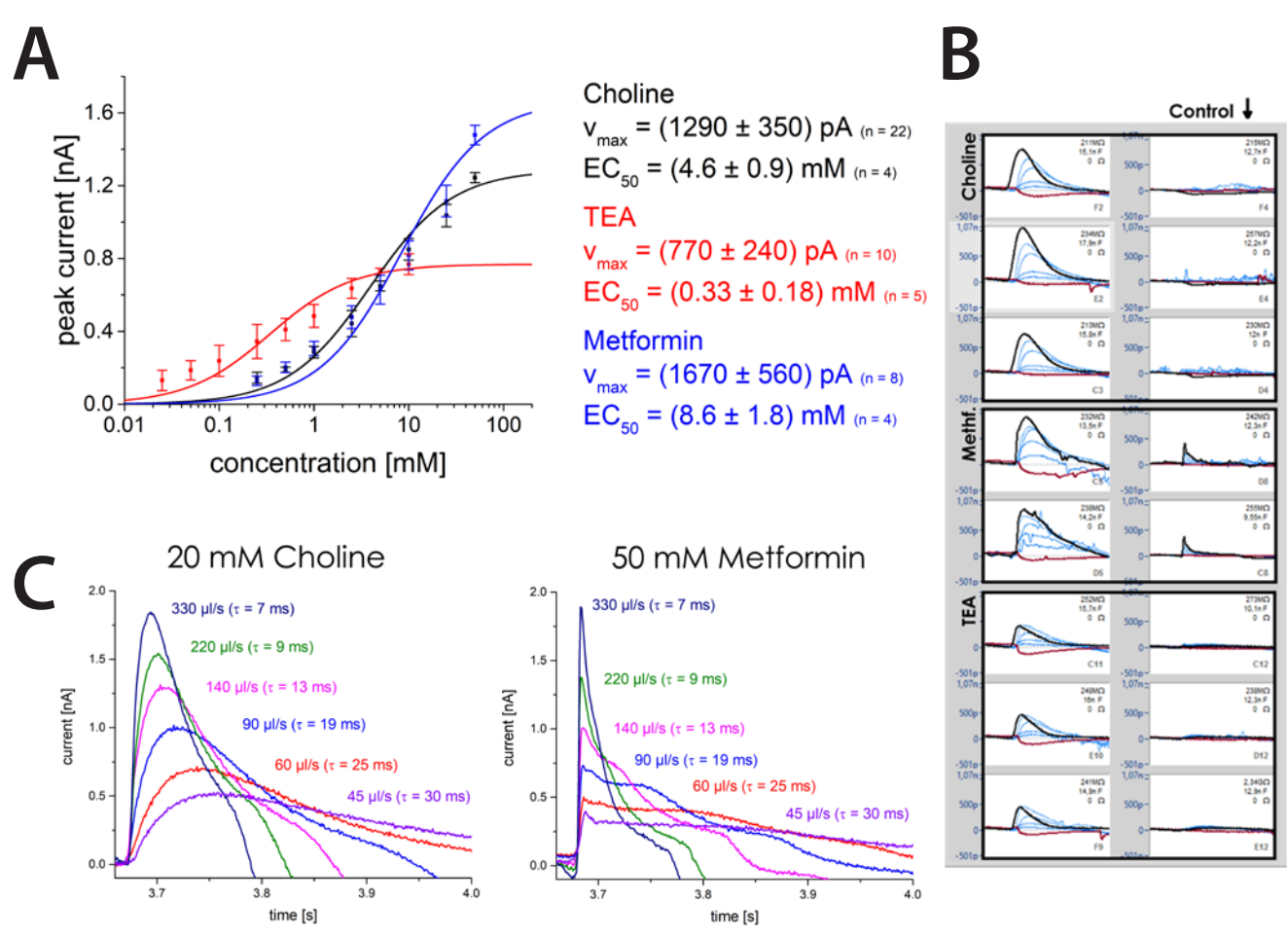
The SURFE²R N1 device contains electrophysiological hardware, liquid handling components and the computer running the data recording and analysis software SurfControl. It's an easy-to-learn, all-in-one robotic workstation which can measure 150 data points a day in a fully automated manner. The solution exchange is controlled by the lonjet which loads solutions from storage containers and injects them to the sensor well enclosed by a Faraday cage. An autosampler on top of the device allows placing up to 53 solutions for automated sequential measurements.

SURFE²R 96SE: high throughput instrumentation

The SURFE²R 96SE was released this year to address the requirements of high throughput for industrial drug screening purposes. It allows the simultaneous measurement of 96 sensors in a 96-well plate format. The electrophysiological hardware from Nanion Technologies is integrated into the robotic liquid handling platform CyBio Felix.

OCT2

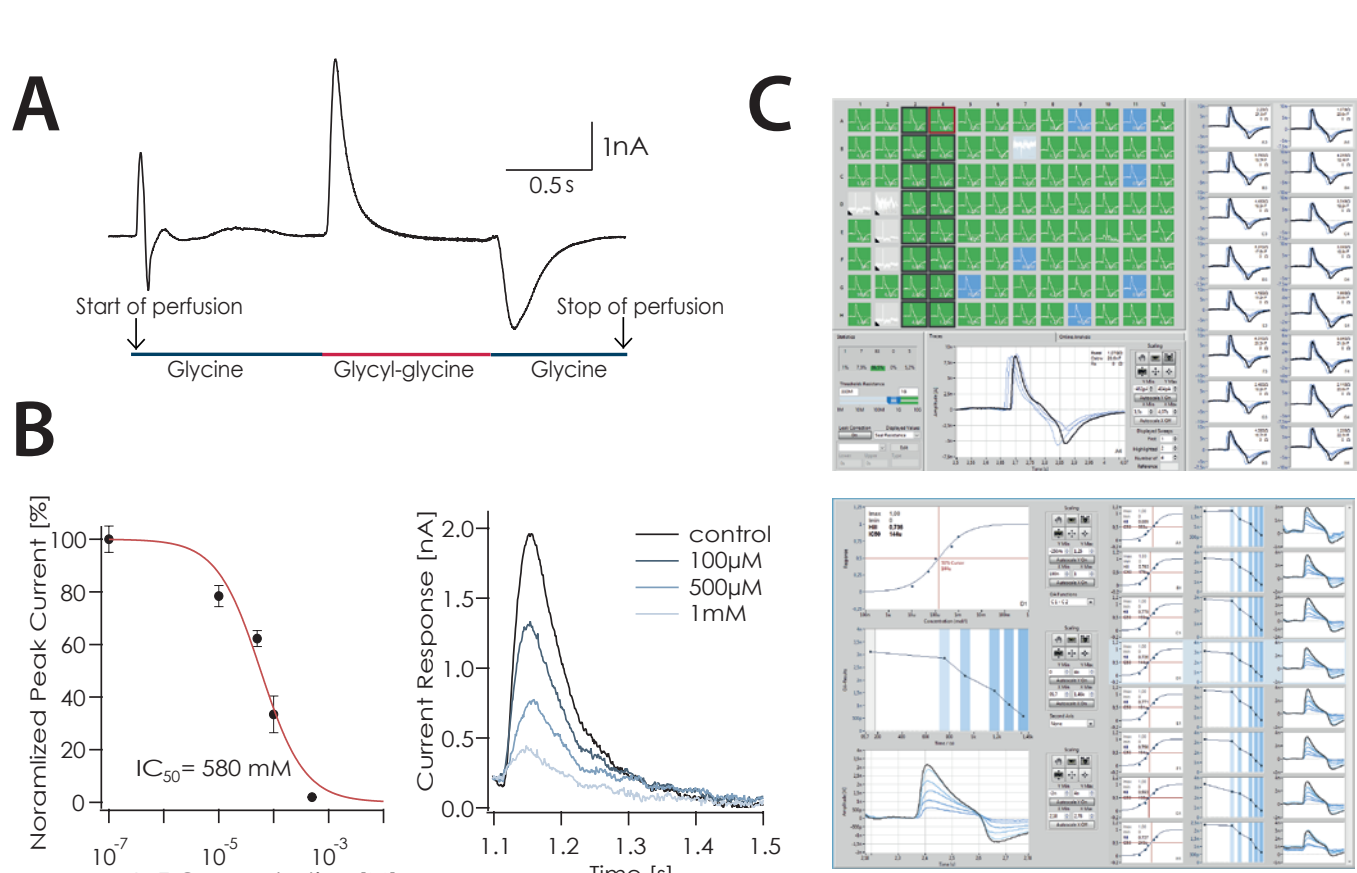
Vmax and EC50 for different organic cations



A EC₅₀ values and relative Vmax for three different substrates using the SURFE²R N1. TEA is the highest affinity substrate with lowest Vmax, while Metformin shows the lowest affinity and the highest Vmax.
B Example of transient currents recorded on two sensor columns using the SURFE²R 96SE. Left: Incubated with OCT2 sample; Right: Control membrane without OCT2.
C Using the SURFE²R 96SE we performed measurements with different speeds of solution flow. The faster the solution flow, the higher the time resolution of solution exchange. The time resolution affects the signal shape of fast transient currents. The possibility to switch between different time resolutions allows to resolve fast binding kinetics or to reduce the impact of fast artifacts on the signal shape.

PEPT1

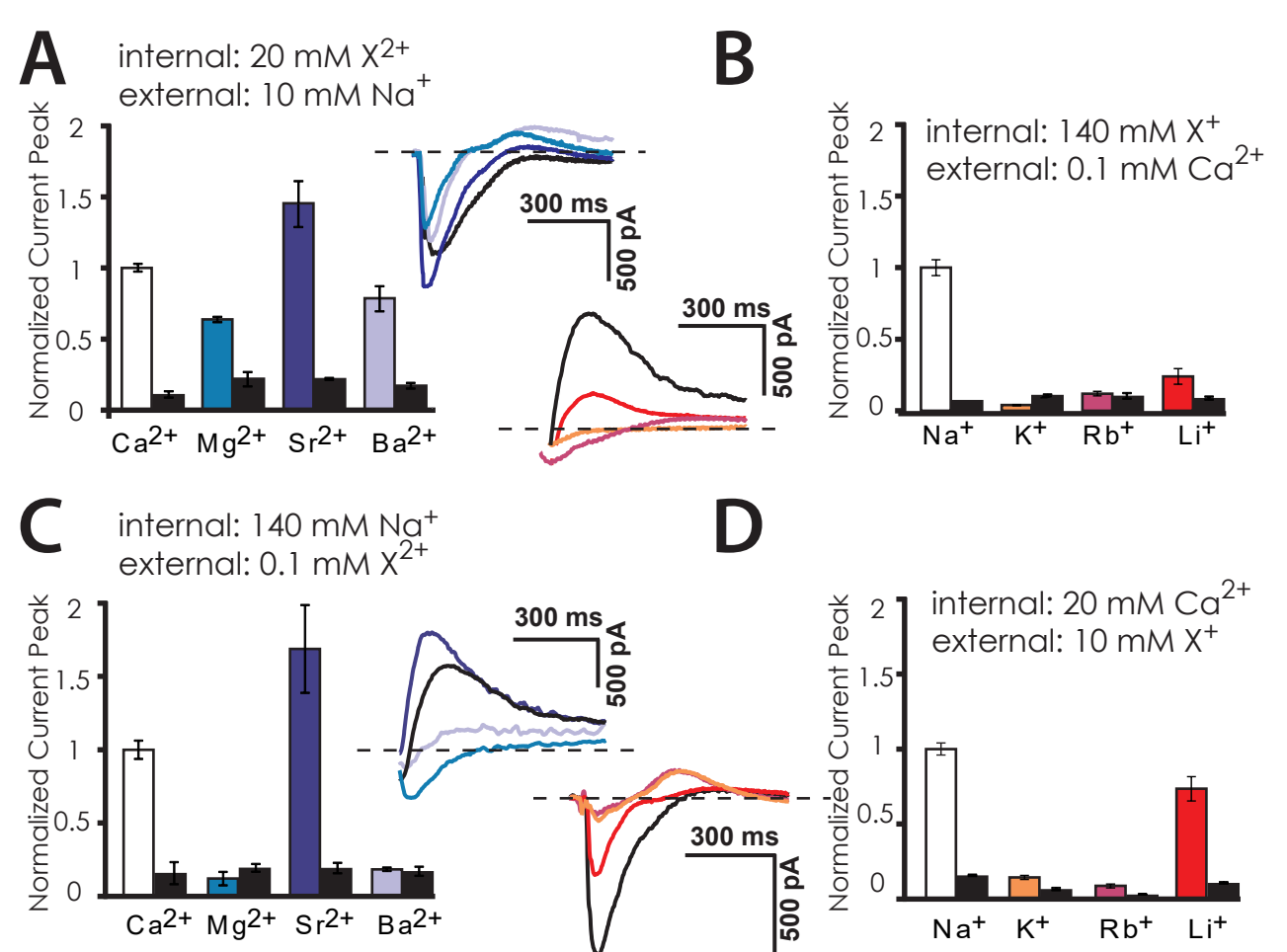
Inhibition assays for proton-coupled peptide transport



A Whole trace of a standard solution exchange protocol on the SURFE²R N1 exchanging from 30 mM glycine containing buffer to a 30 mM Gly-Gly containing buffer. Gly-Gly is the substrate and activates the transporter.
B The same solution exchange was repeated with different background concentrations of the inhibitor Lyz to determine IC₅₀ value.
C The concentration response experiment was repeated using the SURFE²R 96SE to measure 96 sensors simultaneously. The screenshots show the measurement software SurfControl (top) and the data analysis software DataControl (bottom).

NCX

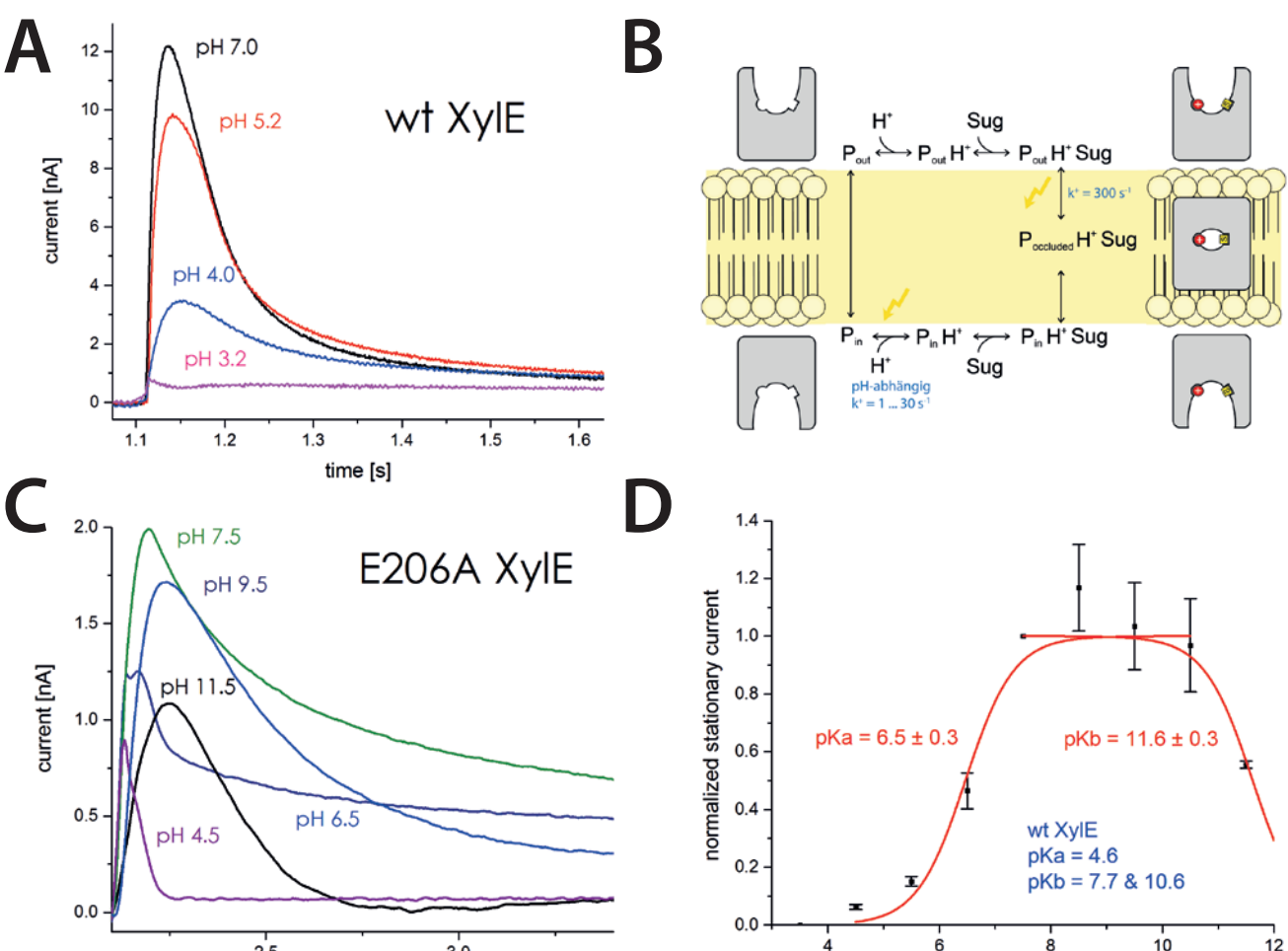
Ion selectivity and modes of Na⁺/Ca²⁺ exchange



The SURFE²R N1 allows preloading of the vesicle with Ca²⁺ or Na⁺ using the double solution exchange protocol. This allows the observation of different transport directions with positive or negative current amplitudes. Different ions were used to test the ion selectivity of the transporter. Black bars represent measurements using negative control membranes without NCX.
A: Selectivity for internal divalent cations against an inward directed Na⁺ flow.
B: Selectivity for internal monovalent cations against an inward directed Ca²⁺ gradient.
C: Selectivity for external divalent cations against an outward directed Na⁺ flow.
D: Selectivity for external monovalent cations against an outward directed Ca²⁺ gradient.

XyIE

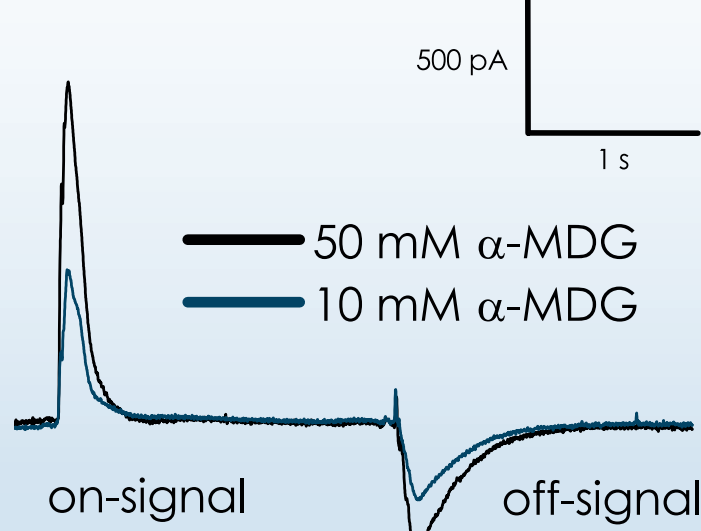
pH dependence of sugar transport and binding



A Sugar induced transient currents at different pH for wild type XyIE (H⁺/xylose symport) using the SURFE²R N1.
B Kinetic model of H⁺/sugar symport in XyIE. There are two electrogenic reactions: one sugar binding induced conformational transition and one conformational transition following proton release reflecting the steady-state electrogenicity.
C The E206A mutation inside the sugar binding pocket decreases Vmax of sugar transport. At acidic pH proton release is blocked and a fast sugar binding current becomes visible, which is also observed in wild type XyIE.
D The mutation also yields a pK shift in transport activity compared to wild type XyIE. E206 therefore plays a crucial role in coupling between proton and sugar transport.

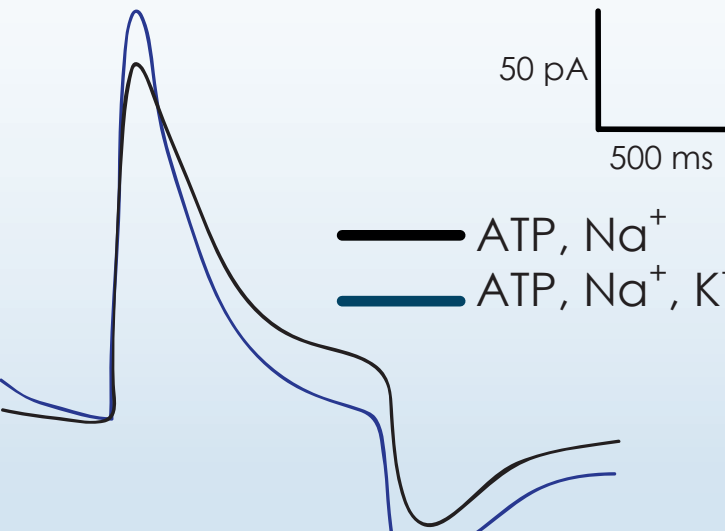
SGLT1

Symporter (sugar/H⁺)



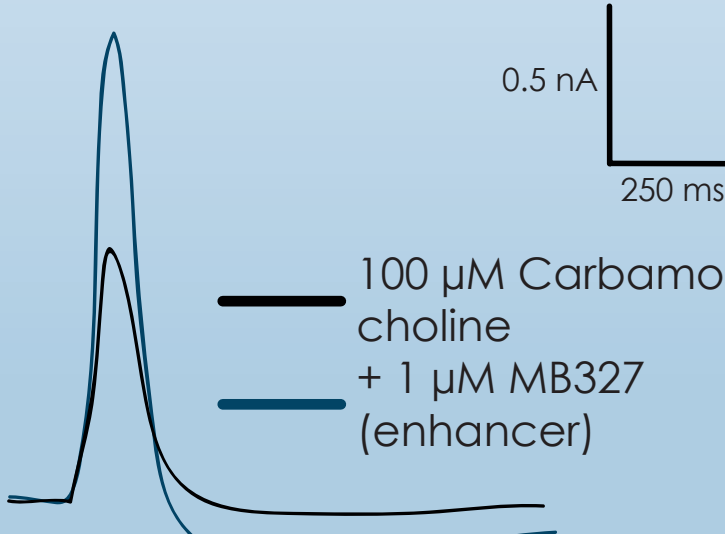
Na⁺/K⁺ ATPase

Ion pump (3Na⁺/2K⁺)



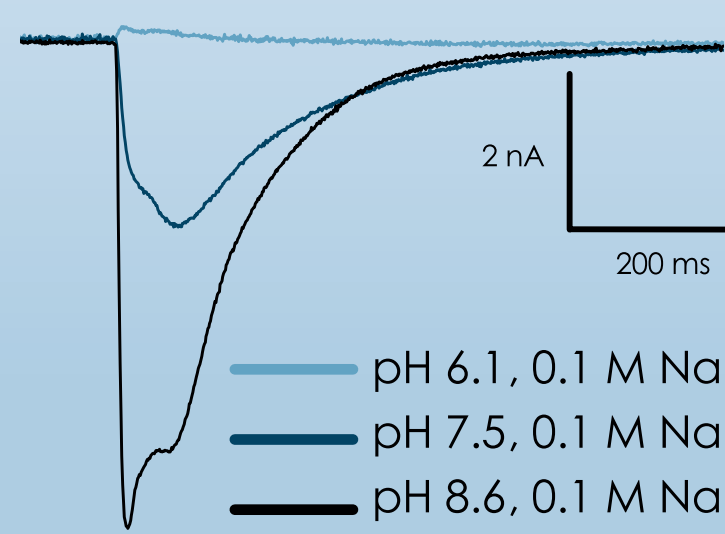
nAChR

Ion channel (cations)



NhaA

Exchanger (Na⁺/2H⁺)



Verified targets

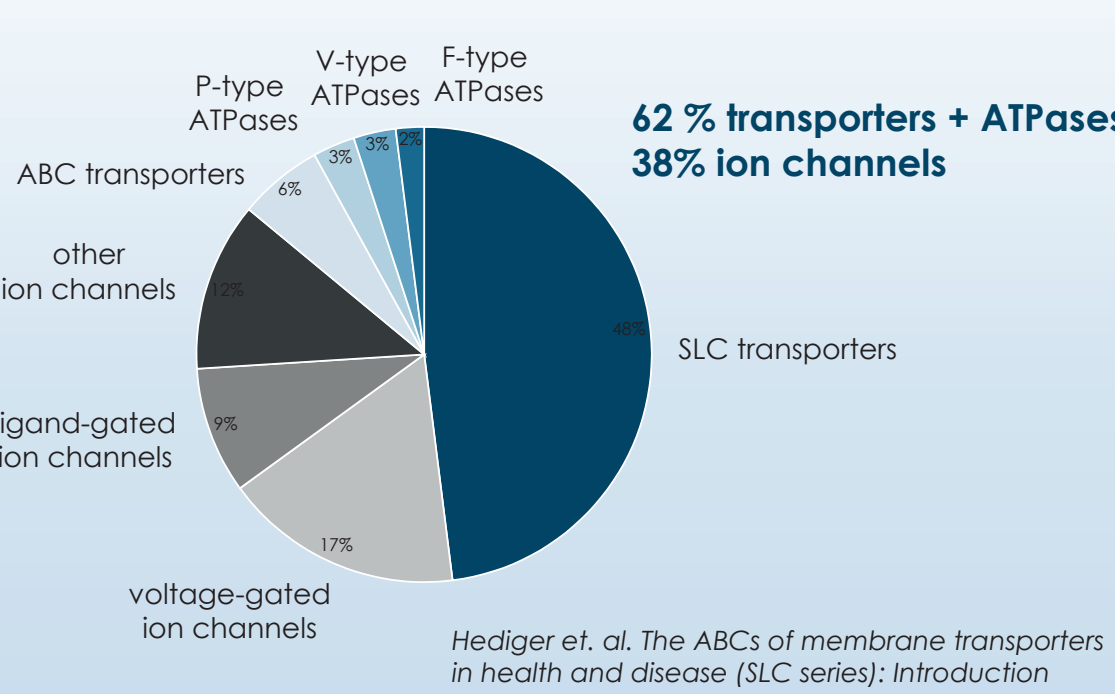
ATPases: NaK-ATPase, HK-ATPase, SERCA, v-ATPase, fof1-ATPase, Kdp-ATPase, CopA, ATP7, PPase. **Redox driven pumps:** Complex I, I/III, II/III, COX. **Light driven pumps:** BR, KR2, HR, ChR, Acrodopsin. **Ion channels:** P2X2, nAChR, AM2, UCPI, TRPC. **Inorganic ion transporters:** NhaA, NhaP, NCX, Clc, NirC, Amt, SulP, NIS, NaPi. **Amino acid / peptide transporters:** PEPT1, YdgR, YhiP, PutP, GltP, EAAC1, PAT1, ArcD, Slc6a15, CAT2B, GlyT. **Sugar transporters:** SGLT1/2, MelB, LacY, FucP, XyIE, GlcP. **Organic ion transporters:** OCT2, CNT1, ANT, GAT1, BetP, CHT.

Verified samples

Proteoliposomes, membrane vesicles, plasma membrane fragments, synaptic vesicles, lysosomes, microsomes, ER/SR vesicles, mitochondrial membranes, nanodiscs, whole cells.

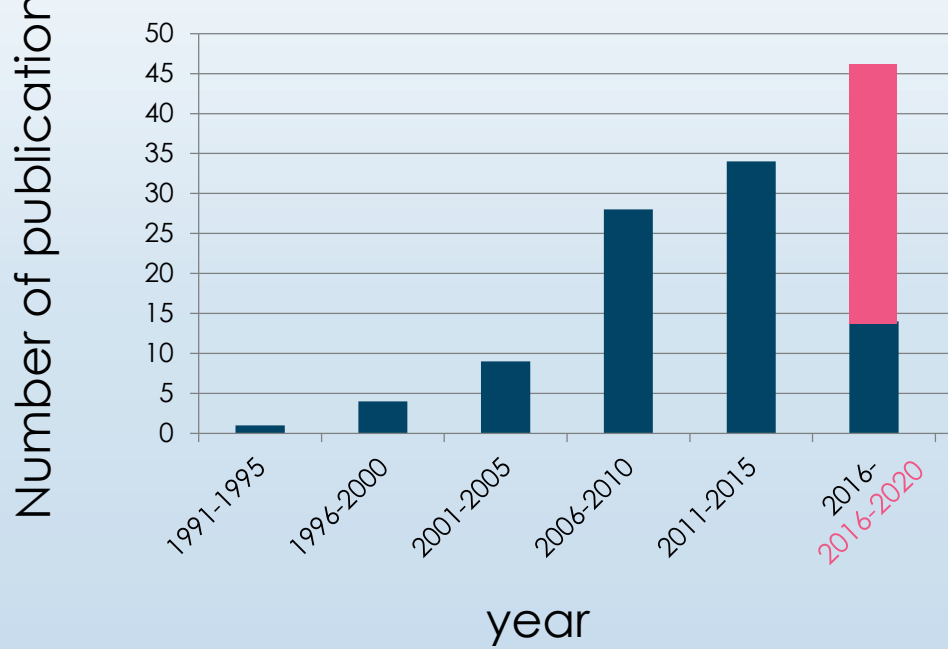
The future of transporter research

Classification of human transport proteins



Analysis of the human transport proteins shows that 62% are potential targets for SSM-based electrophysiology and only 38% are ion channels which are analyzed mostly using conventional electrophysiology. It is known that SLC transporters play a crucial role in human health and disease. But the tools for functional characterization of transporters are rare. SSM-based electrophysiology may be the missing tool to push transporter research to the next level.

SSM-based electrophysiology on the fast track



SSM-based electrophysiology has already been proven as an efficient tool for the characterization of electrogenic membrane proteins. Until now more than 100 different proteins have been tested; almost 100 peer reviewed papers have been published. The number of publications using this technique steadily increased over time proving the acceptance of this technique in the scientific community.

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