Studying Electrogenic Transporters of the SLC Family in a Parallel Way Utilizing Solid Supported Membrane Technology

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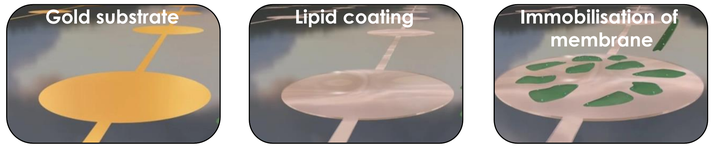
Abstract

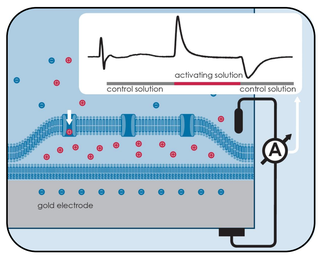
The solute carrier (SCL) family of membrane transport proteins includes over 300 members. Many of them are related to conditions and diseases, or are involved in the transport of pharmacological agents. Therefore, there is a strong need for efficient and flexible assays to study properties and interactions of those transporters.

We utilized solid supported membrane based electrophysiology to study real-time SLC-transporter activity in a 96-well based format, introducing a method also suitable for drug screenings and safety tests. Here applications to study activation and inhibition properties of the SLC transporters PepT1, SGLT1, OCT2 and ANT are presented.

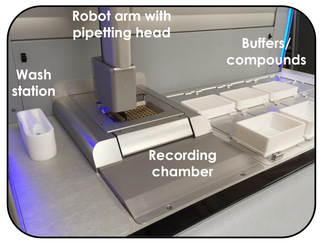
Method



SSM based biosensors
Membrane fragments or liposomes containing the target are immobilized on a functionalized gold electrode of 3 mm diameter. One sensor contains the protein of about 10000 cells.



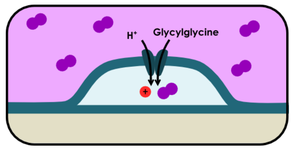
Accumulated transport current
The protein on the sensor is synchronously activated by a fast perfusion system. The transport of charged substrates over the membrane generates an electrical current. It's measured as a sum signal of all the protein on one sensor, thereby enabling a high amplification.



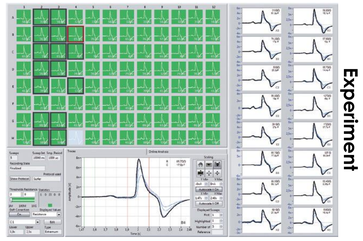
Platform
The experiments are performed by a 96-well based robotic platform, integrating liquid handling, recording and a matching software for experiment setup and data analysis.

PepT1/ SLC15A1

Function: Absorption of dietary oligopeptides in the small intestines
Mechanism: Proton/Peptide cotransport
Clinical significance: Bioavailability of drugs



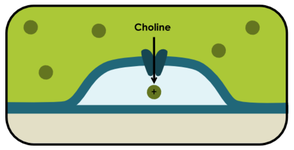
Assay: Activation by application of Glycyl-glycine as substrate. Gly-Gly gradient is sufficient to generate a driving force. Electric current is induced by proton cotransport.



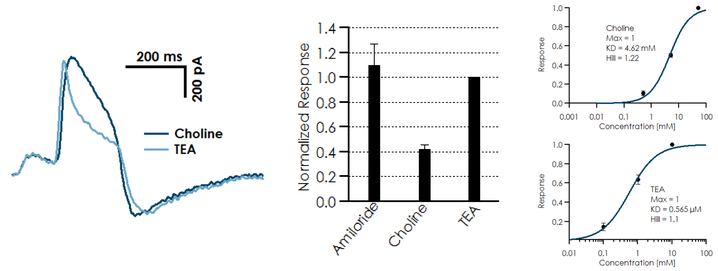
Application: Inhibitor characterization
Glibenclamide was used as an inhibitor of PepT1 activity. In each well an individual IC50 was determined by cumulative application of increasing inhibitor concentrations.

OCT2/ SLC22A1

Function: Renal secretion of organic cations
Mechanism: Polyspecific cation transport
Clinical significance: Renal clearance, drug interactions



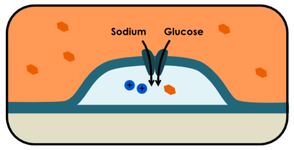
Assay: Activation by fast application of a cationic substrate, e.g. choline. The substrate gradient also generates the driving force.



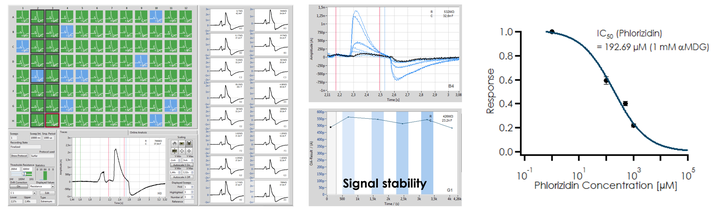
Application: Substrate characterization
TEA, choline and amiloride were compared as cationic substrates. Induced current amplitude and shape differ. The evoked amplitude at 1mM was compared, and EC50 of TEA and Choline were determined.

SGLT1/ SLC5A1

Function: Sugar uptake in intestines and kidney
Mechanism: Sodium coupled cotransport
Clinical significance: Glucose-galactose malabsorption, Oral rehydration therapy



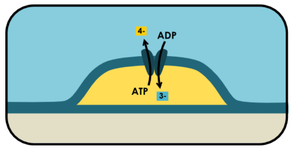
Assay: Activation by fast application of a-MDG. Optional a sodium gradient can be generated in advance. Sodium and/or sugar gradient generate a driving force.



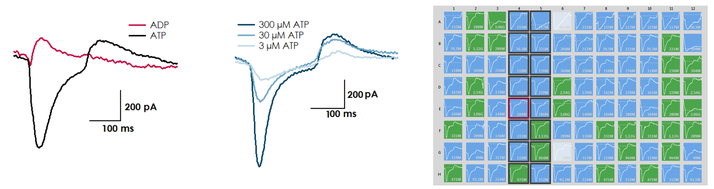
Application: Inhibitor characterization
Phlorizidine is used as a competitive inhibitor of SGLT1 activity. After signal stability is ensured, different compound concentrations are applied sequentially.

ANT/ SLC25A4

Function: Mitochondrial ATP export/ADP import, part of mPTP
Mechanism: Several modes of action
Clinical significance: Associated with several hereditary and autoimmune related diseases, e.g. dilated cardiomyopathy



Assay: ATP and/or ADP supply. Native mitochondrial membranes as source material



Application: Transport modes
Different modes of action were compared. ANT is able to transport only ATP or ADP in an bidirectional way over the membrane, but also to exchange ATP against ADP. The current characteristic and amplitude change depending on the mode of action.