Investigating lysosomal membrane proteins using SSM-based electrophysiology: Improving amplification and accessibility

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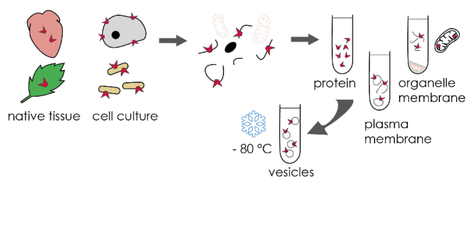
1 Introduction

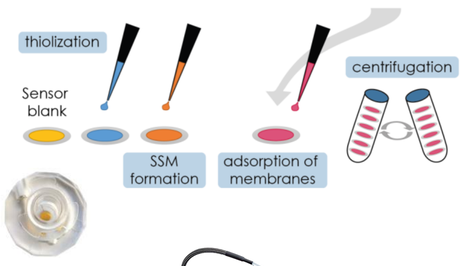
TMEM175 is a novel, constitutively active ion channel involved in regulating lysosomal pH and autophagy. Mutations in this gene impair normal lysosomal and mitochondrial function, thereby increasing aggregation of insoluble proteins such as phosphorylated a-synuclein, leading to symptoms typical of Parkinson's Disease (PD). Consequently, TMEM175 demonstrates significant potential as a key player in the treatment of PD. The lack of specific pharmacological tools has hampered further investigation into the exact role of TMEM175 in normal lysosomal function and pathological processes. Here, lysosomes from a TMEM175 stable cell line were measured on instruments employing solid supported membrane-based electrophysiology (SSME), the SURFE^{2}R N1 and SURFE^{2}R 96SE.

2 SSM-based electrophysiology – How it works

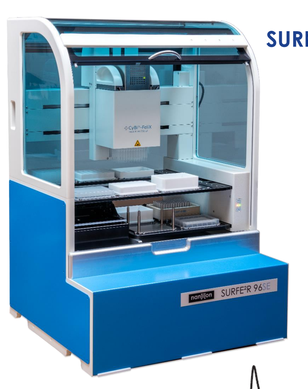
SSME relies on the adsorption of any membrane, native, cell culture-derived or organellar, to a lipid coated electrode, i.e the solid supported membrane, and the direct current read-out caused by the capacitive charging of the membranes when the substrate is applied via fast solution exchange.



The SSM itself consists of a lipid monolayer on top of a thiolated gold coated sensor chip. One important advantage compared to patch-clamp is the large sensor size of up to 3 mm. This allows the measurement of about 10^9 transporters at the same time and a >1000-fold amplification of the currents compared with conventional patch clamp, allowing for the measurements of low-conducting membrane proteins, such as transporters.

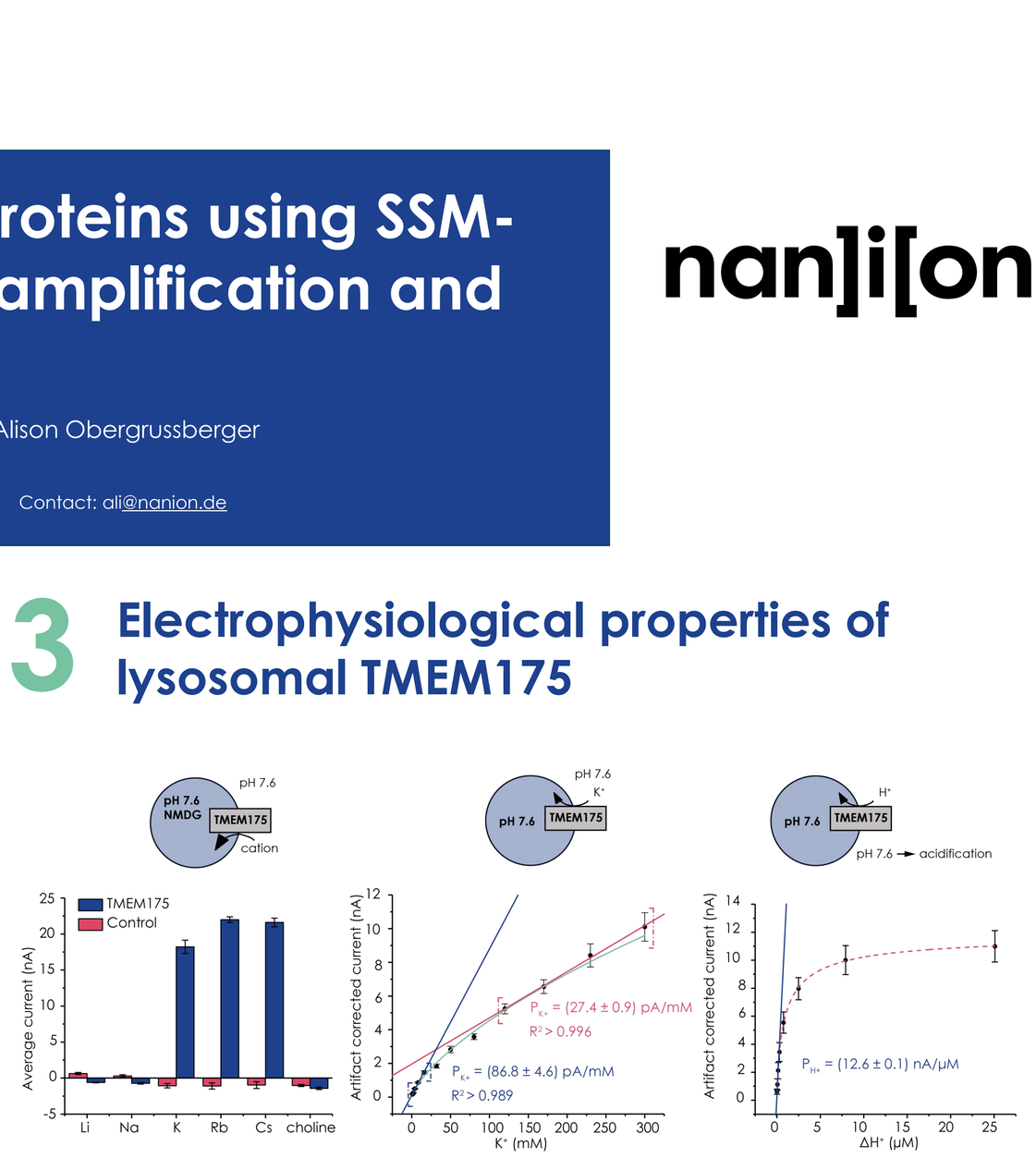


The fact that also intracellular membranes can be accessed by SSME means that ion channels and transporters located in these membranes can be characterized using a more native preparation.

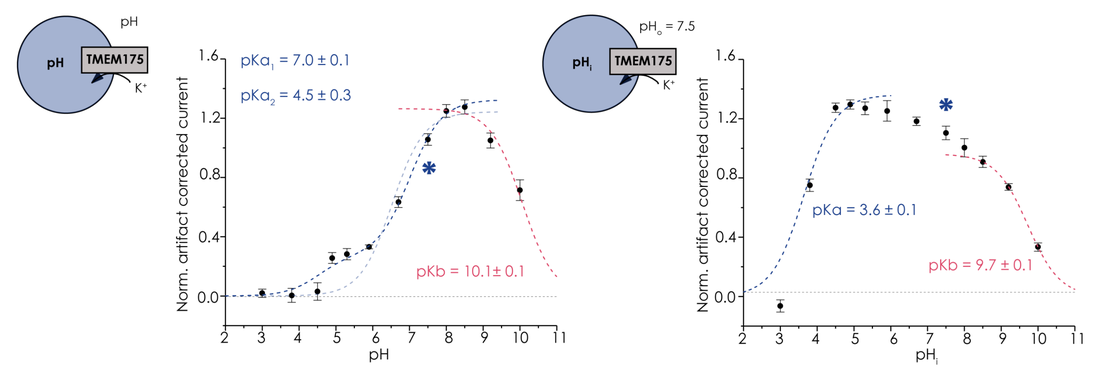


In SSM-based electrophysiology a substrate gradient established by a fast solution exchange is the main driving force. The transport of charged substrates or ions into the liposomes or vesicles generates a membrane potential. This potential can be detected via capacitive coupling between the sample membrane and the SSM on the gold layer of the sensor. In short: The change in membrane potential due to electrogenic transport is measured. At some point the membrane potential equals the chemical driving force and the transport process comes to a halt. This is why any current measured with SSM-based electrophysiology is transient. The peak current amplitude reflects the transporter activity under steady-state conditions. Since the current decay is fast, one measurement takes only one second. Due to the high stability of the SSM, multiple measurements can be performed using the same sensor and different buffer conditions to determine kinetic parameters such as EC_{50}, IC_{50} or even rate constants.

3 Electrophysiological properties of lysosomal TMEM175

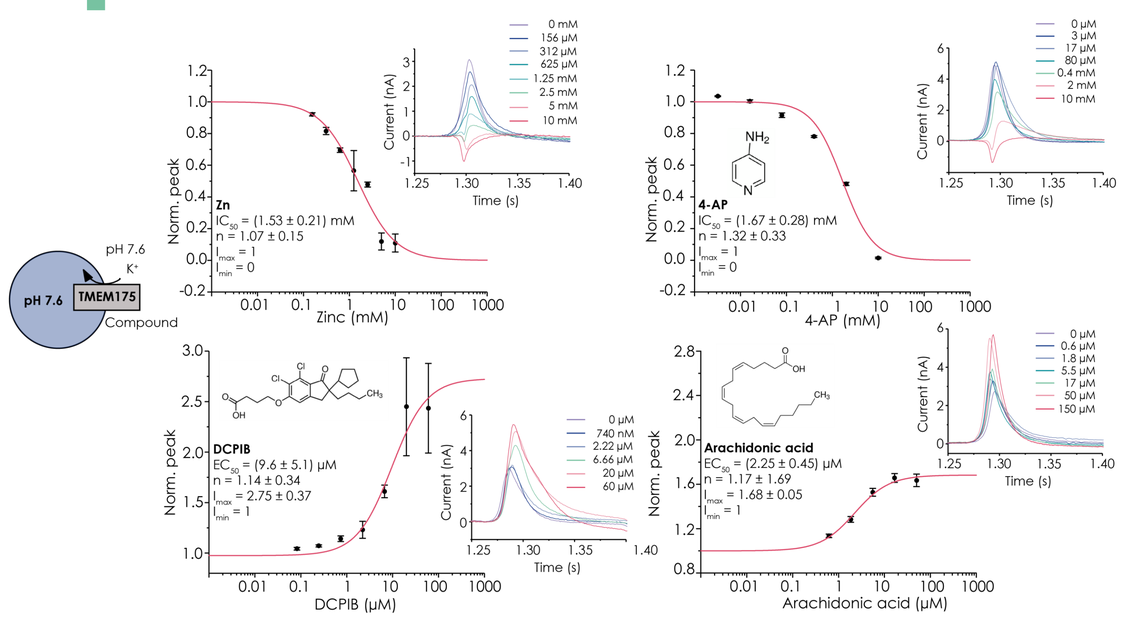


Left: We measured different cations and found that TMEM175 conducts K^+, Rb^+, and Cs^+, but not Li^+, Na^+ or choline$^+$. **Center**: A concentration sequence for K^+ reveals two permeabilities, depending on the K^+ concentration range. **Right**: H^+ concentration jumps reveal that TMEM175 conducts H^+ with high permeability. We found an average permeability ratio between protons and potassium of P_H/P_K=48.500.



Left: K^+ flux through TMEM175 is downregulated to 30% of I_{max} upon acidification on both sides of the membrane, with a pK of 7.0. Below pH 4.5, K^+ flux is completely blocked, likely due to competition with H^+ fluxes. **Right**: In the presence of a pH gradient, keeping the cytosolic pH constant at pH=7.5 and only acidifying the lysosomal pH, K^+ flux through TMEM175 is stable over a broad pH range. Consequently, downregulation with a pK of 7.0 is due to effects of the cytosolic pH.

4 Pharmacology of lysosomal TMEM175



We tested four tool compounds on their ability to modulate K^+ flux through TMEM175 in our SSME assay. We employed the HTS platform SURFE^{2}R 96SE, capable of recording up to 10,000 data points per day. Zinc and 4-AP blocked TMEM175 whereas DCPIB and arachidonic acid enhanced TMEM175. With SSME, also H^+ conductivity assays are possible and the effects of native lysosomal pH gradients on compound potency may be investigated.

5 Conclusions

- Functional and pharmacological investigations of TMEM175 were performed on lysosomes purified from HEK293 cells overexpressing TMEM175, kindly provided by SB Drug Discovery.
- SSM-based electrophysiology enables the characterization of cation and H^+ fluxes through TMEM175 and efficient compound screening, compatible with an HTS environment.
- This technology was developed for the characterization of intracellular target proteins which are barely accessible via patch-clamp, but also for transporters, that are too slow for detection via conventional electrophysiology.

Cells kindly provided by SB Drug Discovery.

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