Functional characterization of lysosomal channels TMEM175, TPC2 and TRPML1 using solid supported membrane-based electrophysiology

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Rocco Zerlotti[1], Maria Barthmes[1], Nina Brinkwirth[1], Cecilia George[1], Niels Fertig[1], Andre Bazzone[1]

1 Nanion Technologies, GmbH, Germany

Contact: Rocco.Zerlotti@nanion.de

1 Introduction

Solid-supported membrane-based electrophysiology (SSME) is a method for measuring transport and electrogenic activities in transporters, pumps, and ion channels. In addition to plasma membrane proteins, transporters or channels localized in inner membranes, can also be characterized. In this work, we focused on the lysosomal channels **TMEM175**, **TPC2** and **TRPML1**. For **TMEM175**, we assessed K^+ and H^+ permeabilities, finding that their ratio closely matches literature results. We also studied the effect of pH on K^+ conductivity.

TPC2, studied with SSME for the first time here, exhibited a saturable conductance towards Na^+ ions, with an EC_{50} of ~40 mM. The maximum Na^+ currents were enhanced by the potentiator TPC2-A1P in a dose-dependent manner.

TRPML1 activity was assessed by inhibiting or enhancing the currents induced by the perfusion of 2 mM Ca^{2+}. We found a IC_{50} of ~700 nM for the inhibitor ML-SI3 and a EC_{50} of ~20 nM for the activator ML-SA5.

2 SSME – an overview

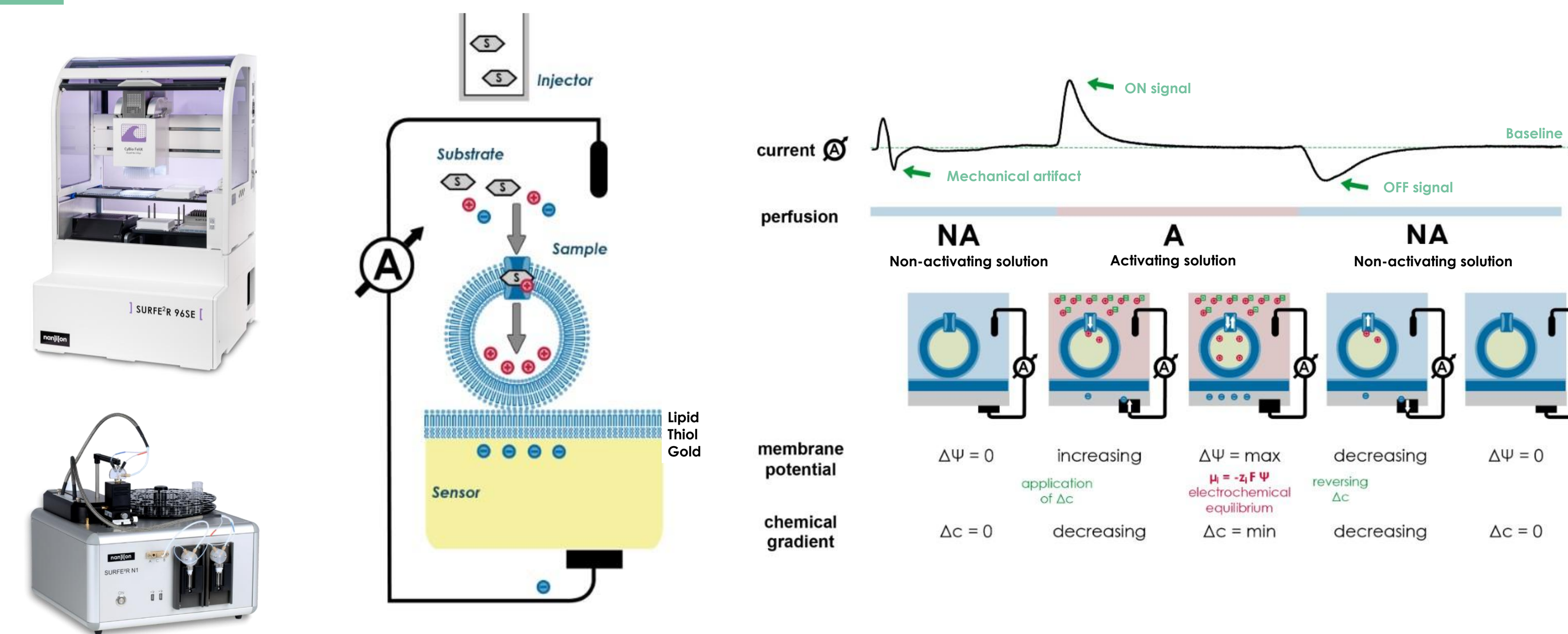
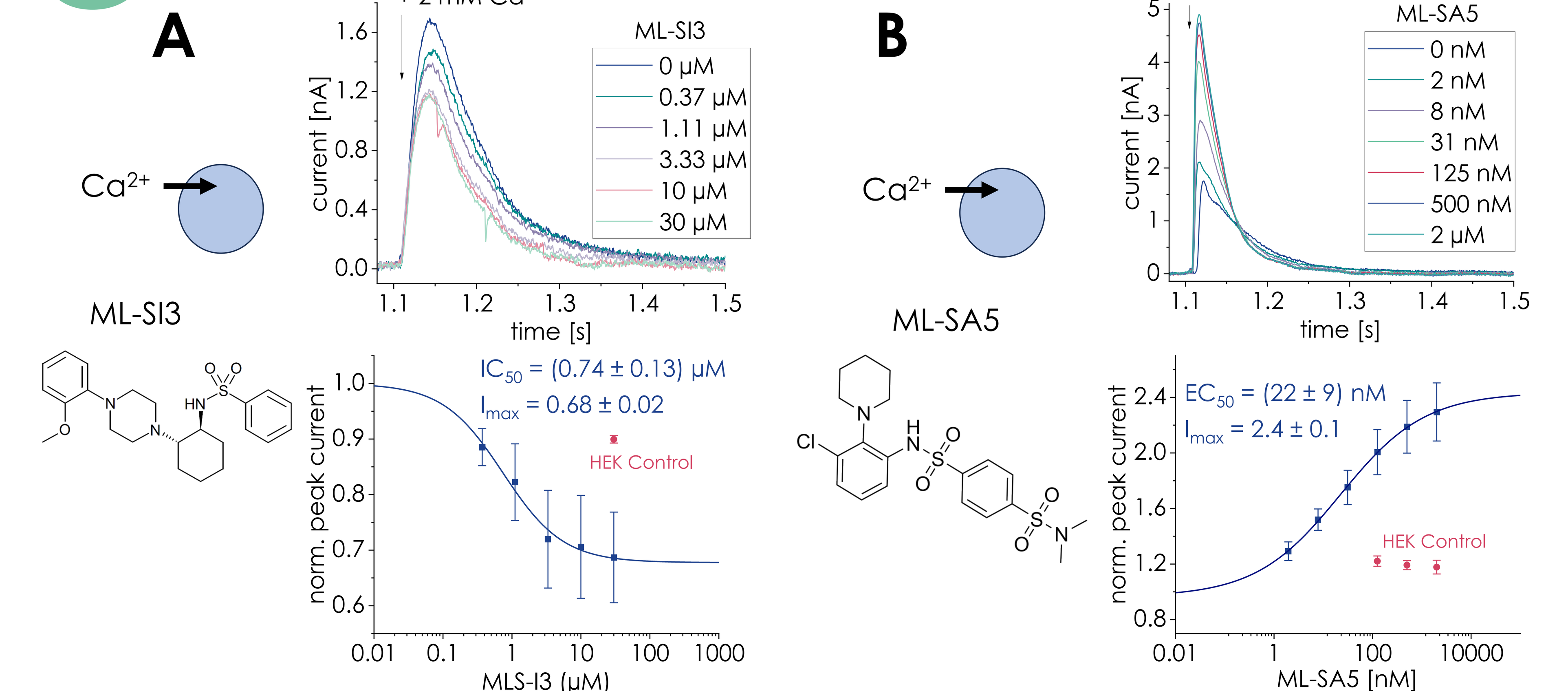


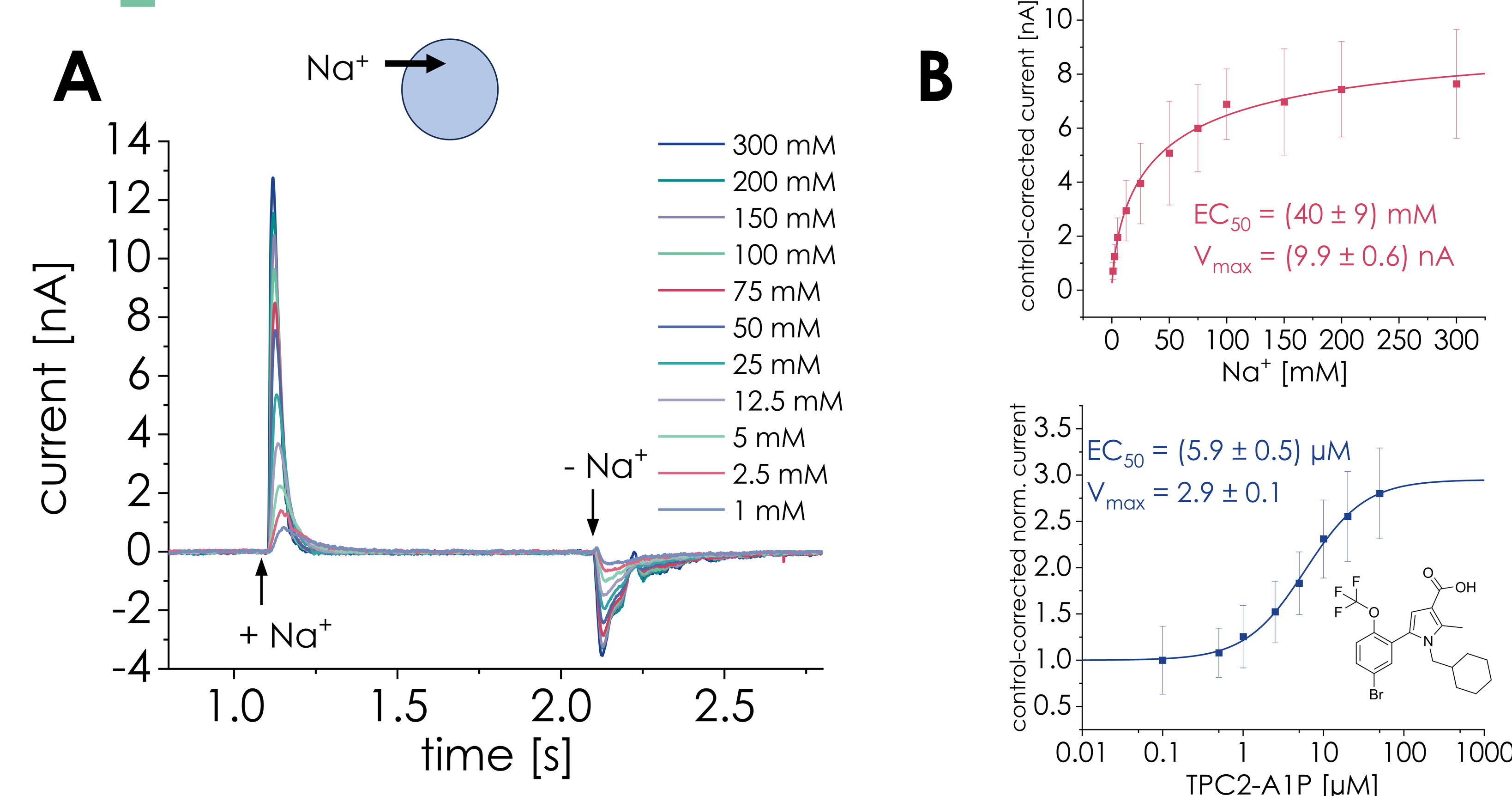
Diagram of the measurement chamber in a SURFE2R N1 and the measurement principle of SSME. The protein of interest (expressed in membrane vesicles or reconstituted in proteoliposomes) adheres to an artificial bilayer on top of a gold-coated sensor. The measurement occurs at 0 mV, and the introduction of substrate creates a concentration gradient that propels the transport reaction. In a typical SSME workflow, a non-activating solution is perfused to establish the electrical signal baseline and, if necessary, to generate a co-substrate gradient for protein activation. Subsequently, the activating solution with the primary substrate is perfused, initiating charge transfer. Due to the capacitive-coupled nature of the system, only transient currents are recorded. Finally, a second flow of non-activating solution restores the system to its initial state.

3 TRPML1 responds to MLSA5/MLSI3



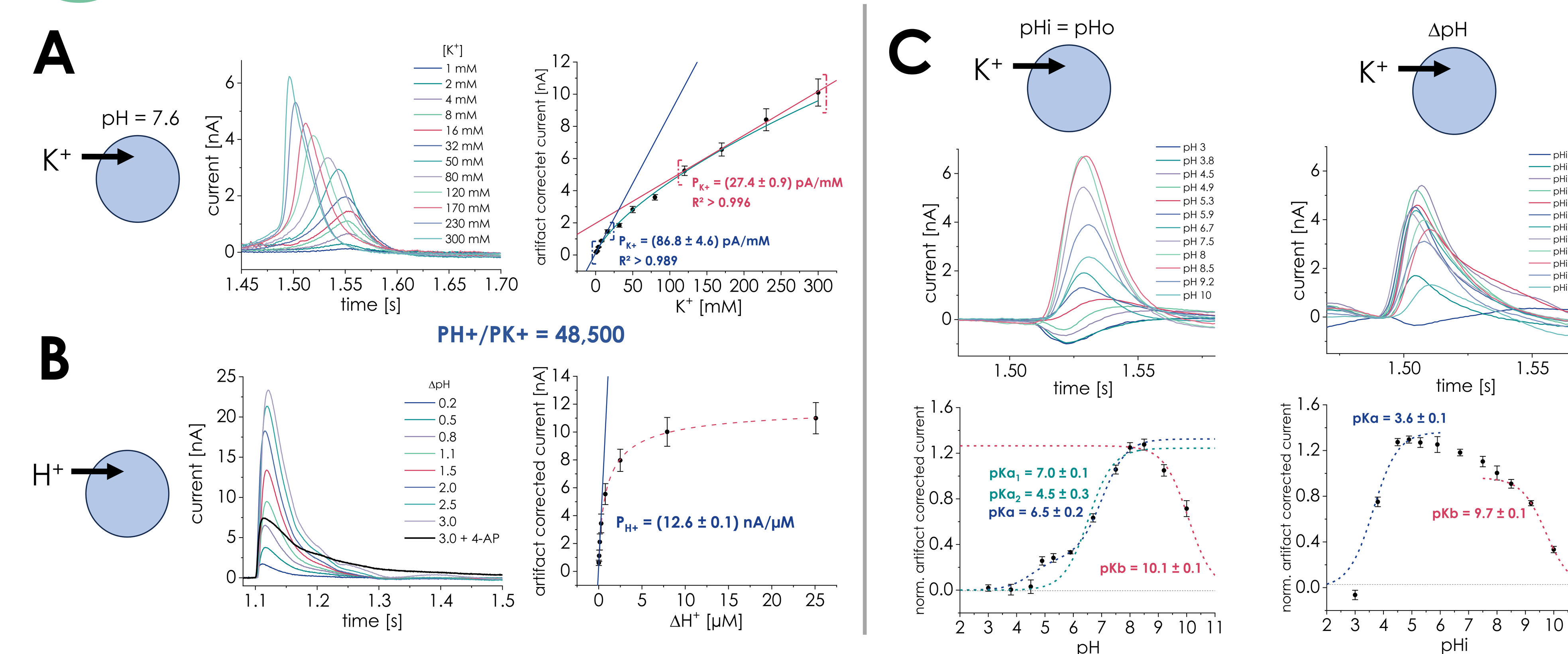
A) Scheme, traces and IC_{50} of ML-SI3 inhibition effect on 2 mM Ca^{2+} induced TRPML1 inward-directed currents in SSME. A single-point ML-SI3 control at 30 µM shows that empty HEK membranes do not react to the inhibitor. **B)** Scheme, traces and EC_{50} of ML-SA5 activation effect on 2 mM Ca^{2+} induced TRPML1 inward-directed currents in SSME. A three-points ML-SA5 control at 2, 0.5 and 0.125 µM shows that empty HEK membranes do not react to the activator.

4 TPC2 Na^+ EC_{50} and A1P effect



A) Na^+ induced currents through TPC2 overexpressed in lysosomal membranes. **B)** Above: Perfusion with increasing Na^+ concentrations show a saturable behaviour of Na^+ conductivity through TPC2, with a EC_{50} for Na^+ of ~40-50 mM. Below: Perfusion of 5 mM NaCl in presence of different concentrations of the activator TPC2-A1P. The half-maximal enhancement is obtained at ~6 µM TPC2-A1P.

5 TMEM175 conducts K^+ and H^+



A) Scheme, traces and permeability plot of K^+ influx experiments in SSME. The pH has been kept constant and two distinct permeabilities have been found. **B)** Scheme, traces and permeability plot of H^+ influx experiments in SSME. The H^+ influx permeability is close to the H^+ efflux permeability, suggesting absence of H^+ gating. The ratio between P_{H+} and average P_{K+} is 48,500 – a value very close to literature results.
C) Scheme, traces and pK_a/pK_b plots of K^+ influx measured at different symmetric pH (left) and different pH gradients (right).

6 Summary

SSME is a suitable technique for studying lysosomal targets, which do not need enlargement treatment to be studied. The results obtained nicely match literature results in most cases. Lysosomal targets TMEM175, TPC2 and TRPML1 have been successfully characterized.

Acknowledgements

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