Investigating lysosomal ion channels using high throughput APC and SSM electrophysiology

Alison Obergrussberger[1], Markus Rapedius[1], Andre Bazzone[1], Rocco Zerlotti[1], Manuel Paina[2], Riccardo Rizzetto[2], Anna Mondini[2], Maria Barthmes[1], Nina Brinkwirth[1], Cecilia George[1], Giovanna De Filippi[1], Jean-Francois Rolland[2], Niels Fertig[1]

[1]Nanion Technologies, Munich, Germany . Contact: ali@nanion.de
[2]Axxam SpA, Milan, Italy. Contact: Jeanfrancois.rolland.jr@axxam.com

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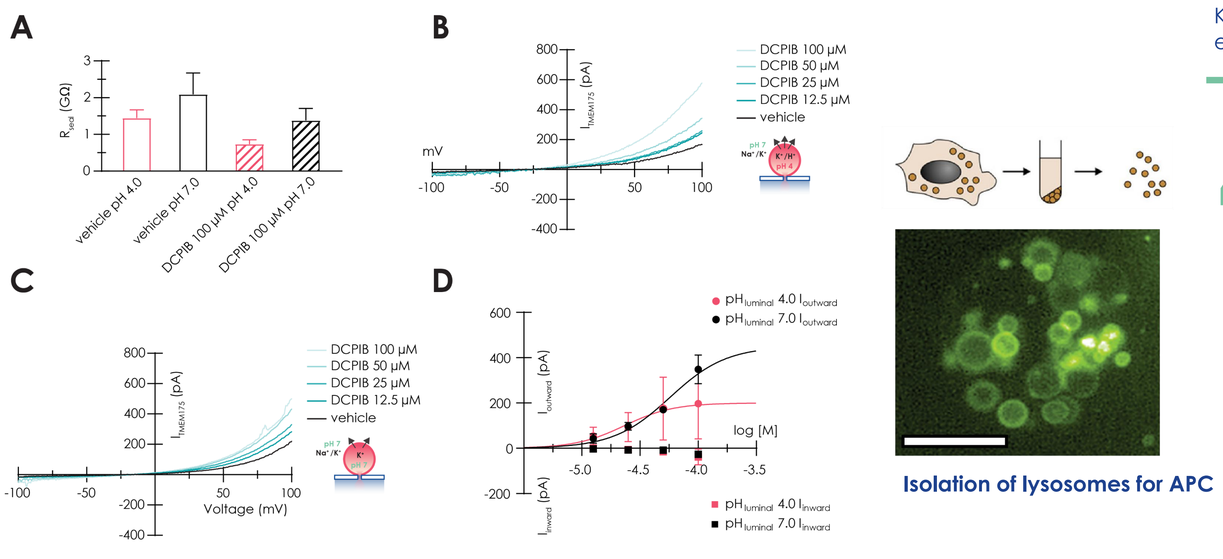
INNOVATES, ENABLES, PERFORMS

1 Introduction

Intracellular ion channels are known to play an essential role in various signaling pathways for health and disease, considering that over 80% of transport processes occur inside the cells. Among the variety of organellar channels and transporters, TMEM175, TRPML1 and TPC channels have received increasing attention in the field given their potential roles in connecting lysosomal homeostasis with pathophysiological conditions such as Parkinson's disease and cancer. Consequently, the interest in exploring intracellular ion channels as therapeutic targets has grown tremendously indicating a need for high-throughput electrophysiology including patch clamp. In addition, some progress has been made in alternative approaches such as solid supported membrane electrophysiology (SSME using the SURFE^{2}R instruments), however, until now, HTS patch clamp has lacked the possibility to collect data from native lysosomes.

2 Lysosomal TMEM175 recorded on high throughput automated patch clamp



Isolation of lysosomes for APC

Figure 1: TMEM175 in intact lysosomes recorded on the SyncroPatch 384 with specially developed NPC-384 chip. A Bar graph of seal resistance calculated before and after DCPIB application. **B** Representative traces recorded in control conditions and in the presence of increasing concentrations of DCPIB, using luminal solution with pH 4.0, and **C** pH 7.0. **D** Concentration response curve of DCPIB application using different luminal solution, with pH 4.0 (red) and 7.0 (black); in both experiments, cytosolic solution was pH 7.0.

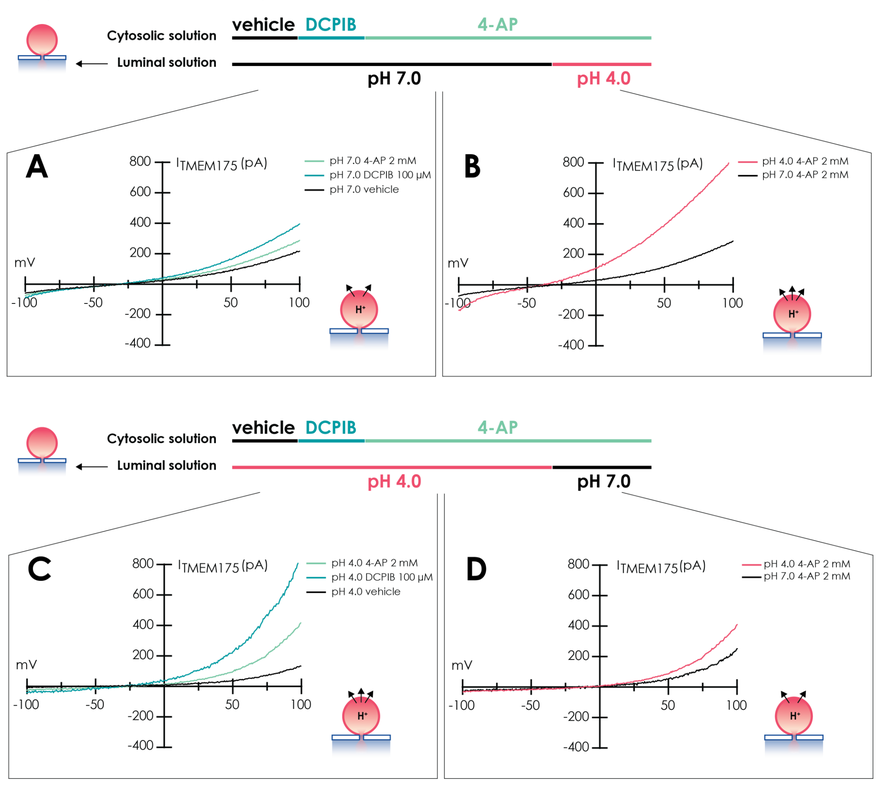


Figure 2: Effect of internal solution exchange of luminal pH on the SyncroPatch 384. Lysosomes isolated from HEK293 cells overexpressing TMEM175 were used on the SyncroPatch 384 and internal solution was exchanged during the experiment. **A** Representative TMEM175 traces recorded in control and in the presence of 100 µM DCPIB (dark green) and 2 mM 4-AP (light green); $pH_{luminal}$ 7.0 – $pH_{cytosolic}$ 7.0. **B** Effect of luminal solution exchange (from pH 7.0 to pH 4.0) on TMEM175 current in the presence of 4-AP. **C** Representative TMEM175 traces recorded in control and in the presence of 100 µM DCPIB (dark green) and 2 mM 4-AP (light green); $pH_{luminal}$ 4.0 – $pH_{cytosolic}$ 7.0. **D** Effect of luminal solution exchange (from pH 4.0 to pH 7.0) on TMEM175 current in the presence of 4-AP.

3 Activation of lysosomal TMEM175 and TRPML1 recorded using SSME

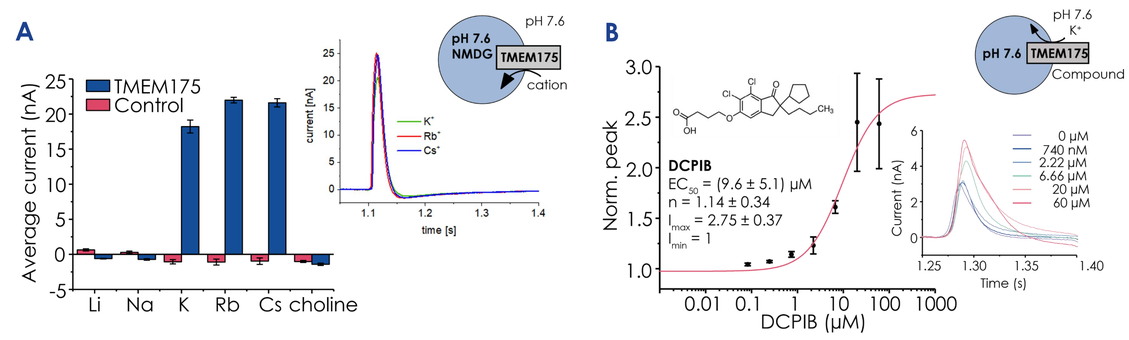
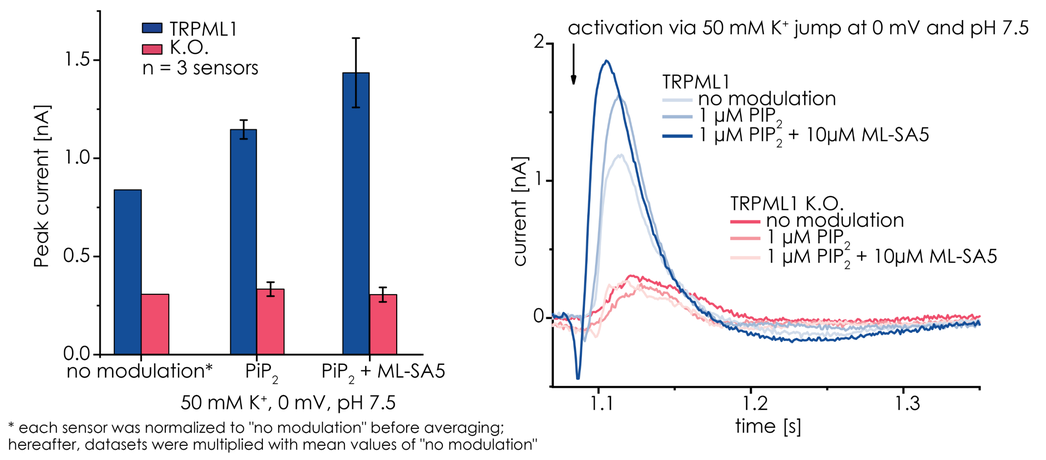


Figure 3: Lysosomes isolated from a TMEM175 overexpressing HEK cell line (kindly provided by SB Drug Discovery) were measured on SSME devices SURFE^{2}R N1 and SURFE^{2}R 96SE. A We measured different cations and found that TMEM175 conducts K^+, Rb^+, and Cs^+, but not Li^+, Na^+ or choline$^+$. **B** On the SURFE^{2}R 96SE, which records from 96 sensors simultaneously, DCPIB enhanced TMEM175 activity in a concentration-dependent manner and consistent with enhancement of activity seen on APC (Figure 1).



* each sensor was normalized to "no modulation" before averaging; hereafter, datasets were multiplied with mean values of "no modulation"

Figure 4: On the SURFE^{2}R N1, TRPML1 from lysosomes isolated from an overexpressing cell line was recorded. TRPML1 was activated by K^+ concentration jumps (no modulation), and enhanced by PiP_2 and ML-SA5. Example traces showing activation in the absence of enhancer, and the effect of PiP_2 or a combination of PiP_2 and ML-SA5 on the recorded currents are depicted. Example traces of a control K.O. cell line is also shown, where only background currents are recorded and there is no effect of the enhancers. The bar graph on the left shows a summary of the data for n = 3 sensors.

4 Lysosomal TPC2 recorded on high throughput automated patch clamp

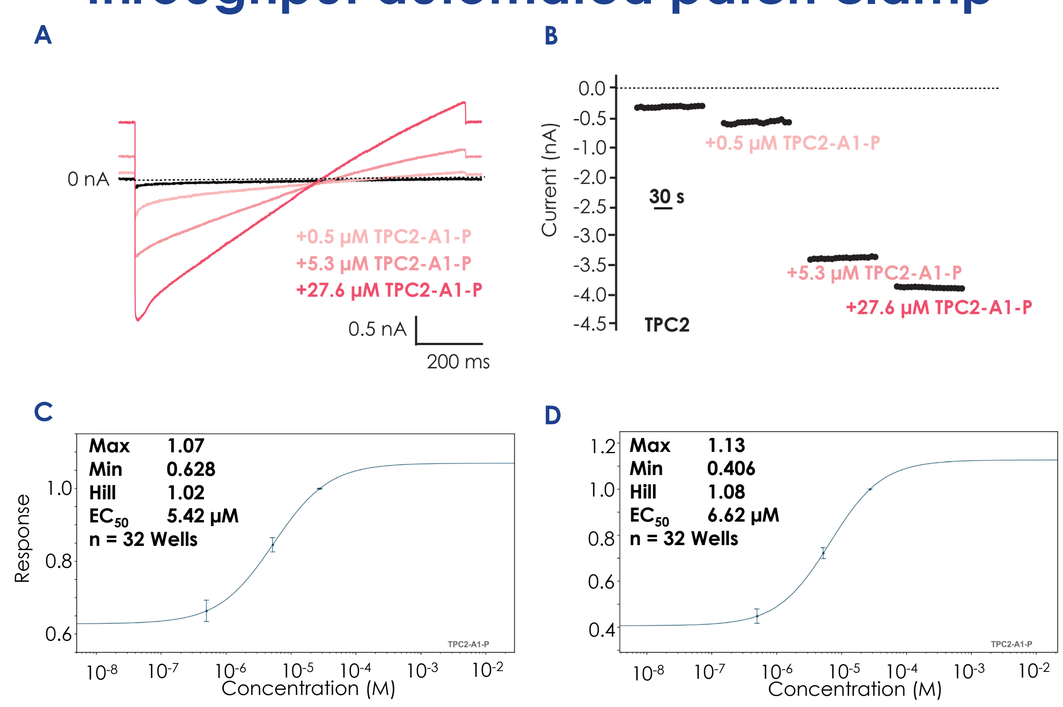


Figure 5: Lysosomes isolated from a HEK293 cell line overexpressing TPC2 (cell line kindly provided by Professor Christian Grimm, LMU) were used on the SyncroPatch 384 with specially developed NPC-384 chip. A TPC2 was activated by increasing concentrations of the specific activator TPC2-A1-P. Shown are traces from an example cell to a voltage ramp protocol. **B** Timecourse of the experiment. **C** Concentration response curve to TPC-A1-P at -100 mV and (**D**) +80 mV giving an EC_{50} 5.4 ± 1.6 µM (n=32) and 6.6 ± 2.7 µM (n=32), respectively.

5 Conclusions

- Recordings of TMEM175 and TPC2 from intact lysosomes are shown on a high throughput automated patch clamp system.
- A specialized NPC-384 chip has been developed for recordings of intact lysosomes for improved success rates. Combined with 32-well mode, this has made higher throughput, automated patch clamp recordings of intact lysosomes feasible.
- Recordings of lysosomal ion channels TMEM175 and TRPML are shown using SSM-based electrophysiology
- Two electrophysiological techniques are shown for higher throughput recordings of intact lysosomes.