HTS and Lysopatch? New frontiers of Organellar Electrophysiology

Manuel Paina, Katja Blasi, Elena Sirtori, Fernanda Ricci, Mattia Saggioro, Anna Mondini, Jean-Francois Rolland

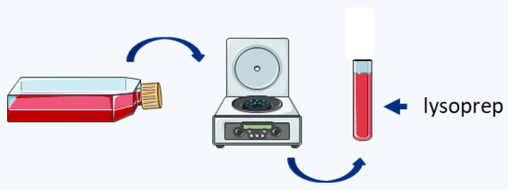
Axxam S.p.A., Via Meucci 3, 20091 Bresso (Ml, Italy)

Summary

- Intracellular ion channels are known to play an essential role in various signaling pathways for health and disease
- Targeting lysosomal dysfunction has emerged as a potential therapeutic approach for ALS with a particular interest in the study of function and pharmacological modulation of lysosomal ion channels
- Large interest in exploring intracellular ion channels as therapeutic targets has grown tremendously indicating a need for high-throughput electrophysiology assays for drug discovery, however so far lacking the possibility to collect data from native Lysosomes
- The present study shows for the first time data registered from isolated lysosomes on an HTS automated patch-clamp device

This study is part of AICoS project (CUP B79J23000340005) supported by Italian Ministry of Economic Development (MISE) – Innovation Agreements D.M. 31/12/2021

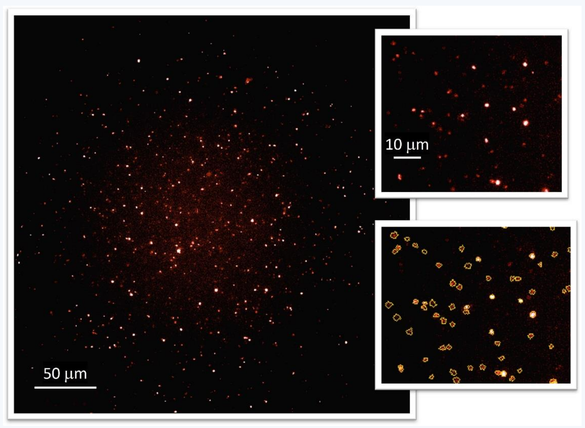
Automated LysoPatch: lysoprep validation



Lysoprep

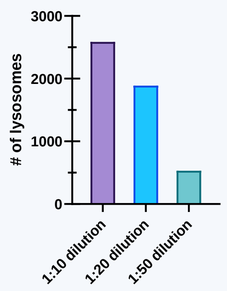
Lysosomes isolation protocol developed and customized in Axxam

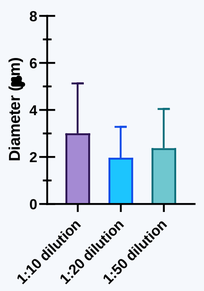
- High purified preparation
- Optimized lysosomal density



Evaluation of Lysoprep using Operetta system (Revvity)

- LysoTracker™ Red DND-99 (Invitrogen) is a cell-permeable red fluorescent dye that stains acidic compartments within a cell, such as lysosomes. Staining was carried-out before isolation protocol to allow the permeabilization of the dye inside the cells

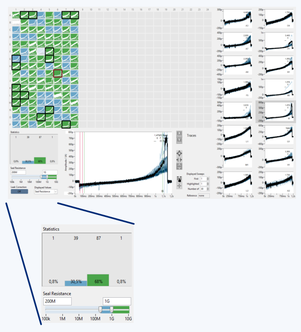




Automated LysoPatch: material & methods

After sealing

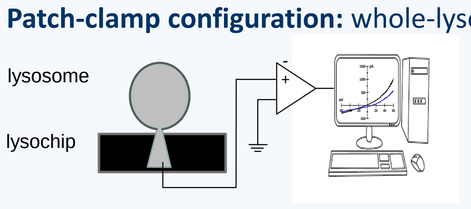
After wash



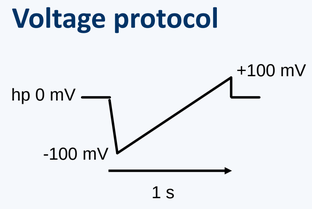
Statistics

- Capture: 121/128
- Sealing (Rseal > 200 MΩ): 114/128
- Exp started: **114/128**

Patch-clamp configuration: whole-lysosome



Voltage protocol

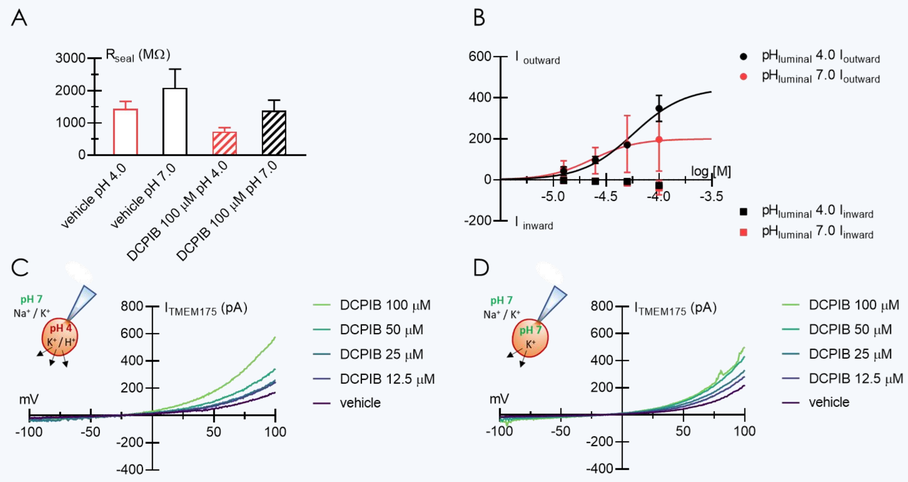


Application protocol (cumulative approach)

vehicle → C1 → C2 → C3 → C4 → ion exchange

Endogenous TMEM175 – DCPIB dose-response curve

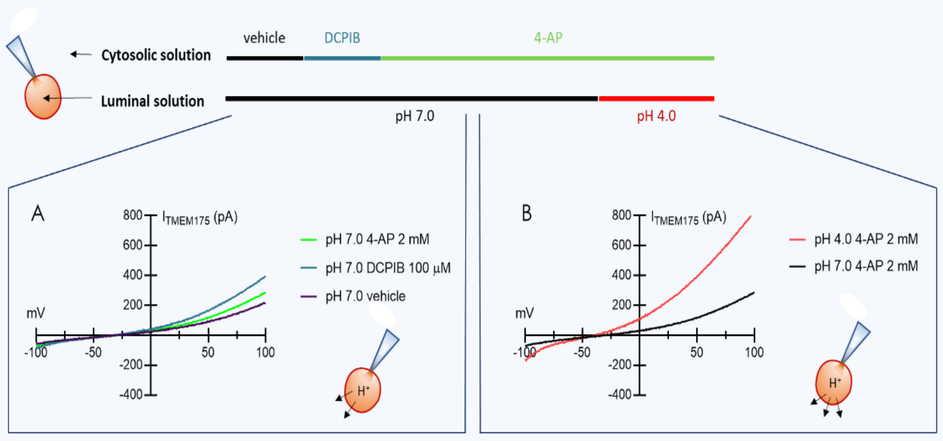
Cumulative dose-response curves obtained on lysosomes in the presence of increasing concentrations of DCPIB, a novel TMEM175 activator (Hu et al., 2023). Since TMEM175 channels release luminal H^+ into the cytosol, we developed assays using luminal solutions with different pH values, to enhance proton conductance, in addition to potassium flux.



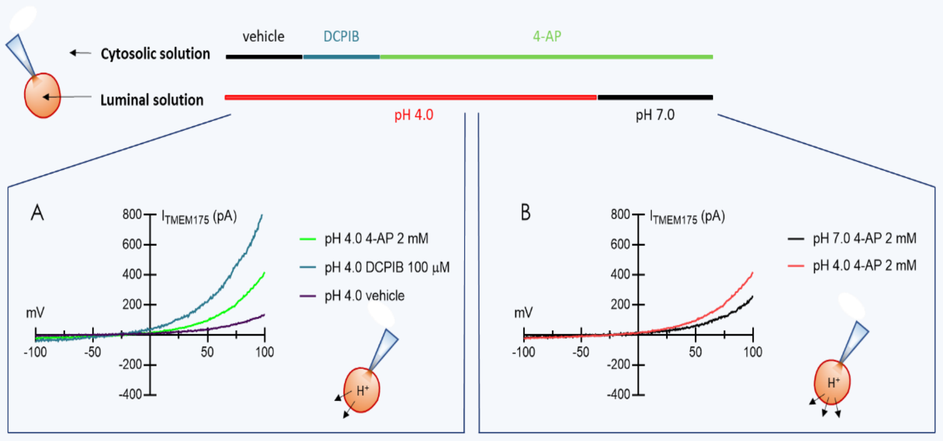
A) Bar graph of seal resistance calculated before and after DCPIB application. B) Dose-response curve of DCPIB application using different luminal solution, with pH 4.0 (red) and 7.0 (black); in both experiments, cytosolic solution has pH 7.0. C) Representative traces recorded in control and in the presence of increasing concentrations of DCPIB, using luminal solution with pH 4.0, and D) pH 7.0

Automated LysoPatch: intraluminal solution exchange

Given the known pH dependence of TMEM175 activity, we also employed intraluminal solution exchange for the first time where we observed a current modulation after changes in luminal pH. During experiment with luminal pH 7.0, TMEM175 current was first evoked by DCPIB application, then partially blocked by 4-AP. In the presence of 4-AP, acidification of the luminal solution, due to the internal exchange from luminal pH 7.0 to 4.0, increases TMEM175 current. A similar experiment was repeated by inverting the luminal pH, starting from 4.0 and moving to 7.0, using the internal perfusion feature of the SyncroPatch 384. In the presence of 4-AP, the reduction of H+ in the luminal solution induces a reduction in TMEM175 current due to a lower proton contribution.



A) Representative TMEM175 traces recorded in control and in the presence of 100 µM DCPIB (light green) and 2 mM 4-AP (dark 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



A) Representative TMEM175 traces recorded in control and in the presence of 100 µM DCPIB (light green) and 2 mM 4-AP (dark green); pH luminal 4.0 – pH cytosolic 7.0. B) Effect of luminal solution exchange (from pH 4.0 to pH 7.0) on TMEM175 current in the presence of 4-AP

Automated LysoPatch: conclusion

For the first time we report recordings from freshly isolated lysosomes on a high throughput automated patch clamp device.

We present a pharmacology of positive and negative reference modulators, data that corroborates all those previously obtained on manual patch-clamp. This technological and scientific advancement enables us to perform lysosomal patch-clamp investigations at a throughput level previously deemed unattainable, thereby allowing us to offer an unprecedented capability for compound profiling.

Intraluminal solution exchange was achieved by using the internal perfusion feature of the Syncropatch, enhancing the broad range of applications aimed at investigating the mode-of-action of compounds.

Axxam SpA · OpenZone

Via Meucci 3, 20091, Bresso · Milan(Italy) · phone +39 02 210561 · **www.axxam.com**

For further information: Dr. Sabrina Corazza · phone +39 02 2105634 · sabrina.corazza.sc@axxam.com