Utilizing Solid Supported Membrane Electrophysiology (SSME) to Accelerate Discovery of TRPML1 Modulators

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SYGNATURE DISCOVERY

Neurodegenerative disorders such as Alzheimer's and Parkinson's disease remain conditions with major unmet clinical needs. Abnormalities in the endosomal-autophagic-lysosomal system, progressive neurological dysfunction and regional neuronal loss constitute their common characteristics. Transient receptor potential mucolipin 1 (TRPML1) is a well-known, non-selective cation channel of the endolysosomal system that can transport Ca^{2+}, K^+, Na^+, Fe^{2+} and Zn^{2+}. There is a strong connection between endolysosomal TRPML1 dysfunction and neurodegenerative disorders, thought to be a result of its importance in controlling calcium signalling and homeostasis of lysosomes, autophagy, and modulation of oxidative stress. Therefore, modulation of TRPML1 represents a promising strategy to improve the function of neurons impacted by neurodegenerative disorders by increasing autophagy and promoting the clearance of protein aggregates and reactive oxygen species (ROS) build-up. Numerous platforms offer insights into TRPML1 function and pharmacology, however, lack of specific tools allowing for investigation of TRPML1's role in pathological processes is still an obstacle for drug discovery. Solid Supported Membrane Electrophysiology (SSME) present a novel high throughput method to resolve this challenge. Using enriched lysosomal fractions from recombinant HEK cell lines expressing TRMPL1 we have successfully developed SSME assays, using both SURFE²R N1 (single sensor) and SURFE²R 96SE (high throughput) platforms to investigate TRPML1's cation selectivity and pharmacology. Both platforms show excellent reproducibility and platform-to-platform correlation.

1 Methods

HEK cells expressing recombinant TRPML1 were produced by Sygnature Discovery. Parental, untransfected cells served as a negative control. Cells were lysed using nitrogen decompression in a cell disruption buffer (10 mM Tris, pH 7.5, 250 mM sucrose, Complete™ protease inhibitor cocktail). Membranes were prepared through centrifugation and sucrose gradient ultracentrifugation and diluted in storage buffer (5% glycerol, 0.2 mM DTT). Samples were stored at -80°C until ready for assessment on SURFE²R. Electrogenic events were measured using SURFE²R N1 and SURFE²R 96SE SSM-based electrophysiology platforms (Nanion Technologies). Charge translocation is triggered by rapidly perfusing the sensor with an activating solution resulting in a gradient across the membrane. The current size and profile provide information about speed of transport, coupling ratio and substrate affinity. Data analysis was performed using Data Control 96 V2.3 and GraphPad Prism V10.1.

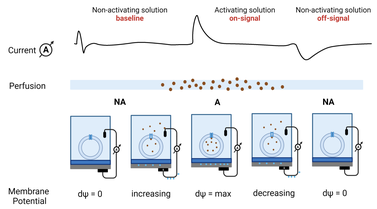

Figure 1: Channel activity is triggered by perfusion of the membrane-coated sensor with a substrate-containing solution (activating solution).

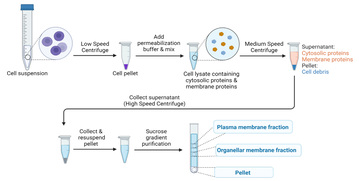

Figure 2: Lysosome extraction for SURFE²R SSM-based electrophysiology assays. Typically, 0.01-1 µg of protein is used per well/sensor.

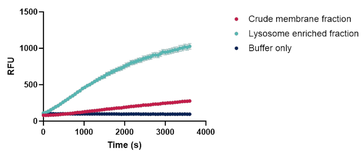

Figure 3: Cathepsin B assay. Conversion of the substrate Ac-RR-AFC was monitored for 1h at 37°C (λex = 400 nm, λem = 505 nm) [n=3]. The figure shows the change in 505 nm emission as the Ac-RR-AFC substrate is cleaved by Cathepsin B.

Cathepsin B was used as a lysosomal marker to confirm enrichment of organellar fraction. Cathepsin B activity was compared in crude membrane fraction (pre-sucrose gradient sample) and in lysosome-enriched organellar fraction. Cathepsin B activity in organelle fraction was distinctively higher (~4x) in comparison to crude sample, confirming that sucrose purification/separation had successfully enriched lysosomes.

2 SURFE²R N1 Characterization

The single sensor SURFE²R N1 was used to perform initial characterization of TRPML1. Lysosome-enriched membranes expressing TRPML1 were assessed for activity using a range of ions, with clear selectivity shown for Ca^{2+} over K^+ and Na^+. The SURFE²R N1 was also used to confirm concentration-dependent modulation of TRPML1 activity by the small molecule activator ML-SA5 and inhibition of activity by Lanthanum Chloride.

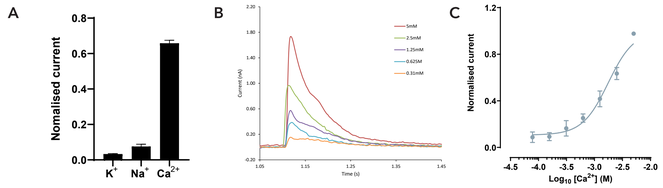

Figure 4: Ion selectivity profile of TRPML1. A) Net TRPML1 currents upon 50 mM cation (KCl, NaCl, $CaCl_2$) concentration jumps. TRPML1 net currents were obtained by subtracting currents recorded with control lysosomes (UT HEK) from currents recorded with TRPML1 lysosomes (n=3). B) Raw traces of TRPML1 dose response to $CaCl_2$ from a single sensor. C) Concentration response curve for activation of TRPML1 by $CaCl_2$. EC_{50} value was 1.7 mM.

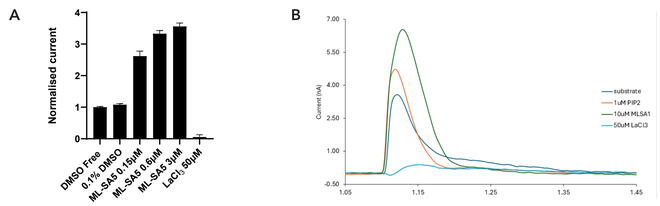

Figure 5: A) TRPML1 activation induced by increasing concentration of ML-SA5 in presence of 5 mM $CaCl_2$, followed by a block with $LaCl_3$. B) Raw traces of TRPML1 translocating 5 mM $CaCl_2$ (blue), stimulated with 1 µM PIP2 (orange), 10 µM MLSA1 (green) and inhibited with 50 µM Lanthanum chloride (light blue).

3 SURFE²R 96SE

Following validation of TRPML1 activity using SURFE²R N1, the assay was transitioned to the high-throughput SURFE²R 96SE platform. Similar to the N1 platform, TRPML1 was activated by calcium in a concentration-dependent manner with an EC_{50} value of approximately 1.3 mM which correlates well with that on the single sensor N1 platform.

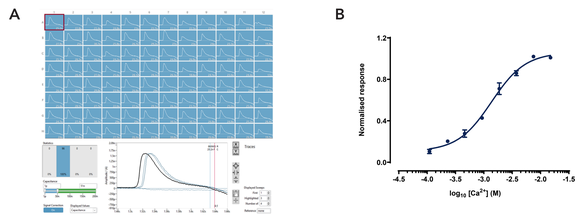

Figure 6: Example screenshot from SURFE²R 96SE experiment. A) Example signal generated upon repetitive perfusion with 5mM $CaCl_2$ B) Concentration response curve showing activation of TRPML1 by $CaCl_2$. EC_{50} values was 1.3 mM.

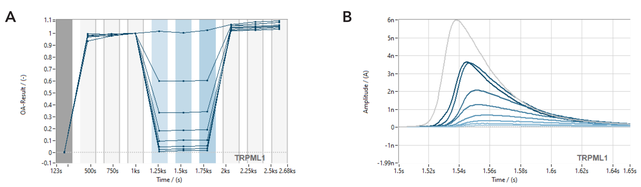

Figure 7: Calcium dose response A) Online Analysis (OA) depicting repeat activations (x3) with 5 mM $CaCl_2$ (liquid period in grey), followed by additional activation steps with reducing concentrations of $CaCl_2$ (5 mM 1:2 serial dilution - liquid periods in blue), and back to 5 mM $CaCl_2$ (liquid period in grey) to show current stability. B) overlayed traces of peaks generated in response to increasing $CaCl_2$ concentrations.

4 Reference Validation

SURFE²R 96SE was used to characterize the effect of known reference modulator of TRPML1 (ML-SA5) and reference inhibitors ML-SI1 (GW405833) and ML-SI3. All compounds performed as expected and generated XC_{50} values in line with data generated using both fluorescence-based calcium flux assays and automated patch clamp electrophysiology.

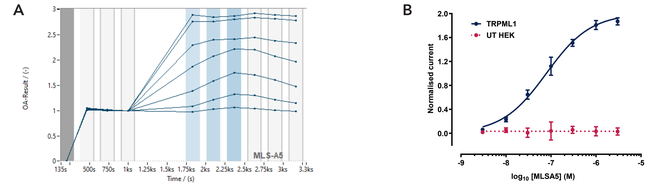

Figure 8: Potentiation of TRPML1 by ML-SA5. A) Online Analysis (OA) showing TRPML1 potentiation by increasing concentrations of ML-SA5. B) Concentration response curve showing activation of TRPML1 by ML-SA5. The EC_{50} value was 80 nM.

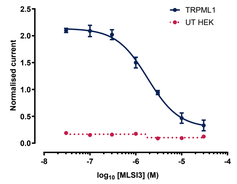

Figure 9: Inhibition of TRPML1 activity by ML-SI3. The IC_{50} value was 1.8 µM

Table 1: Platform to platform correlation of tool compounds.

Reference Compound	Platform	XC_{50} (µM)
MLSA5	Fluorescence	0.6
	Electrophysiology	0.6
	SURFE²R 96SE	0.08
GW405833	Fluorescence	1.3
	Electrophysiology	1.9
	SURFE²R 96SE	5.6
MLSI3	Fluorescence	1.3
	Electrophysiology	0.8
	SURFE²R 96SE	1.8

5 Summary

We have successfully established reliable TRPML1 assays on both SURFE²R N1 and SURFE²R 96SE platforms to allow investigation of TRPML1 activity in lysosomes and response to specific modulators. In conjunction with fluorescence-based and electrophysiology technologies, SSME enhances compound screening cascades, enabling the discovery of novel TRPML1 modulators and advancing knowledge of TRPML1 and its role in normal physiology and disease.

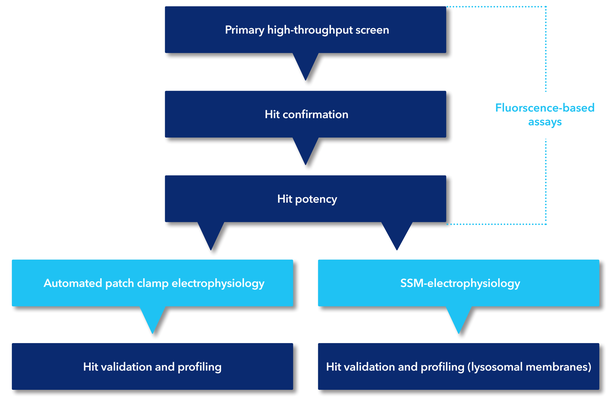

Figure 10: Example screening cascade utilizing high-throughput fluorescence, automated electrophysiology and lysosomal SSME to discover novel TRPML1 modulators.