Proof of Concept

Channel:	TMEM175
Sample:	Lysosomes
Origin:	Cell line
Tools:	SURFE²R N1

TMEM 175 activity measurement in lysosomes on Nanion's SURFE²R N1

SURFE²R-Team at Nanion Technologies GmbH, Munich, Germany.

Summary

TMEM175 is a lysosomal cation leak channel, which impairment has been linked to Parkinson's disease and other neurodegenerative disorders; thereby making it an interesting drug target.

Using the SURFE²R N1, a SSM-based electrophysiology platform, TMEM175-currents from lysosomal membranes were recorded with high signal and temporal resolution (***Figure 1***).

The presented recordings illustrate SURFE²R's potential as means for target validation and compound screening against TMEM175 channels residing in lysosomal membranes.

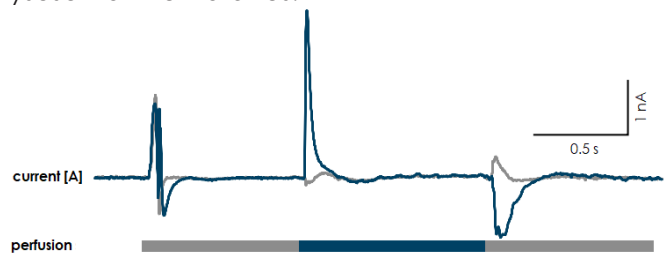

Figure 1: Representative recording from an individual SSM sensor holding lysosomal membranes from TMEM175-overexpressing cells (blue) and knockout cells (grey). TMEM175 current response is triggerd by the fast perfusion of conducted ions (blue).

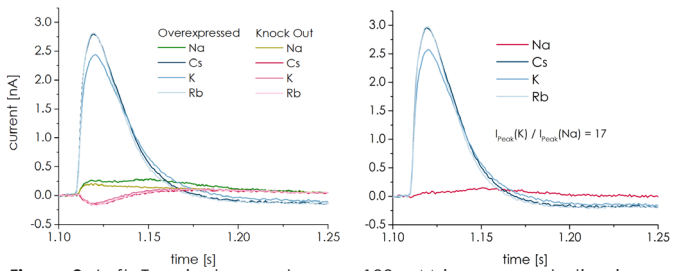

Figure 2: *Left:* Transient currents upon 100 mM ion concentration jumps against 100 mM choline chloride using lysosomal membranes obtained from TMEM175-overexpressing (blue and green) and knock-out cell lines (red and yellow). *Right:* Currents from the knock out sample were subtracted from the currents obtained for the sample containing TMEM175. Consistent with the expected ion conductivity, Na^+-containing solution generated no currents, whereas Cs^+, K^+, and Rb^+ yielded large current amplitudes.

Results

TMEM175 is cation-selective leak channel, with a high conductivity for K^+, Cs^+ and Rb^+, and low permeability for Na^+ and Ca^{2+}. TMEM175 is the major potassium conductance in lysosomes (Cang *et al.*, Cell, 162(5), 2015).

For this study two cell lines were used; one overexpessing TMEM175 in lysosomal membrane, the other a knock-out cell line for negative control. The lysosomal membranes were isolated by differential sedimentation (Jinn *et al.*, PNAS, 114(9), 2017).

As seen from ***Figures 2*** and ***3***, expected conductances were observed: *i.e.* Na^+ was not conducted, whereas convincing currents from the permeation of K^+, Cs^+, and Rb^+ were obtained.

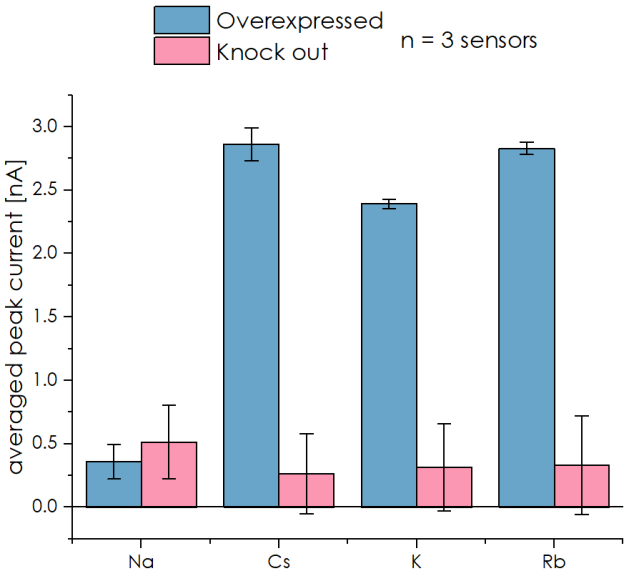

Figure 3: Average of peak current amplitudes across multiple sensors for lysosomal membranes obtained from TMEM175-overexpressing cells (blue) and from a TMEM175 knock-out cell line (pink). Four different cations were applied at 100 mM concentration.