The intracellular Na^+/H^+ exchanger NHE7 effects a novel Na^+ but not K^+-coupled proton-loading mechanism in endocytosis



Université Nice Sophia Antipolis

LΦ2m

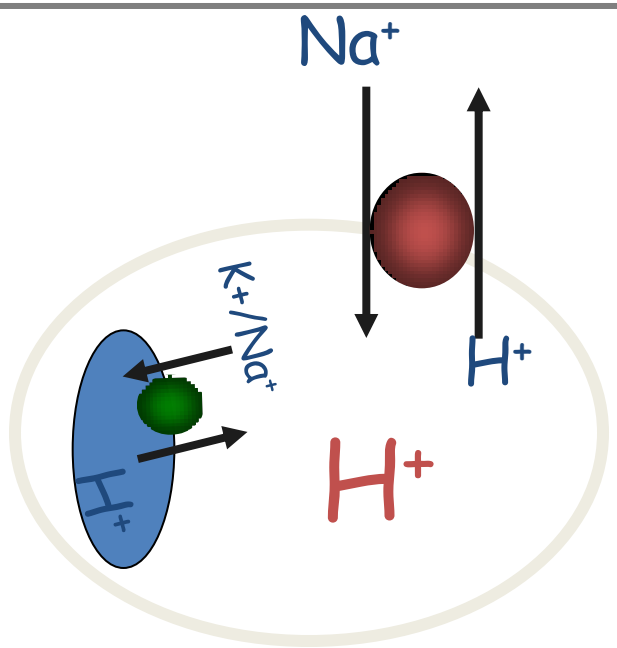
cnrs

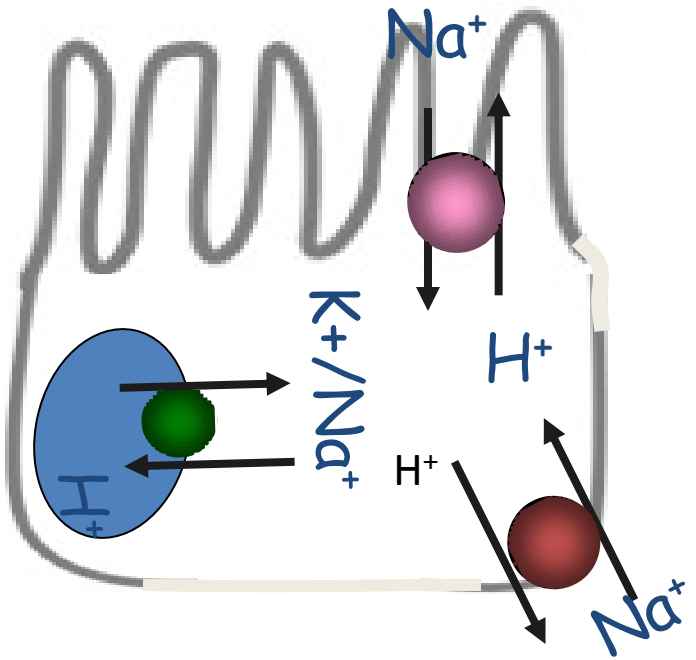
Laurent Counillon

The Na^+/H^+ exchangers,key effectors in pH regulation

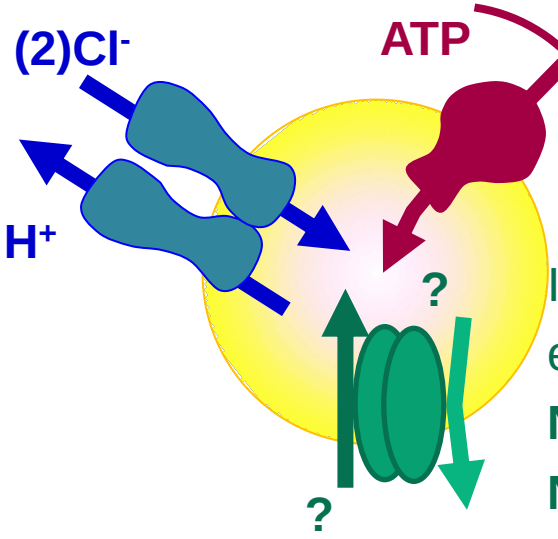
10 Genes

NHE-1 : Ubiquitous
NHE-2 et 4 : Stomach Intestine kidney
NHE-3 : Intestine and kidney
NHE-8 : Kidney
NHE-5 : Brain
NHE-6, 7 and 9 : Intracellular compartments
NHE-10 : Acrosome





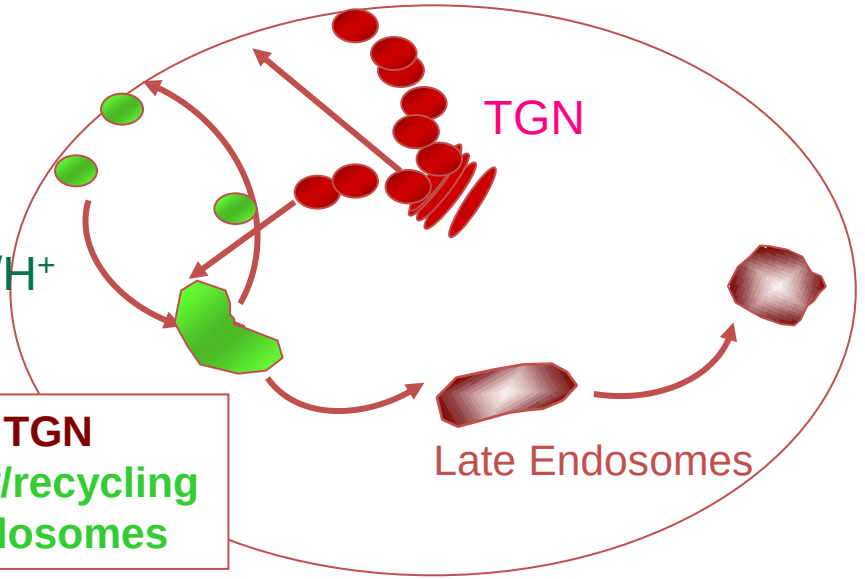
iNHEs Function and link with pathological states

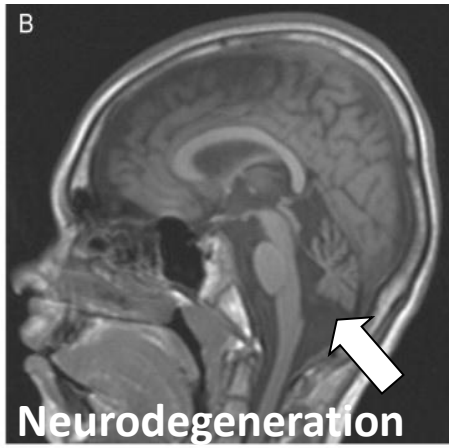


Intracellular Na^+/H^+ exchangers

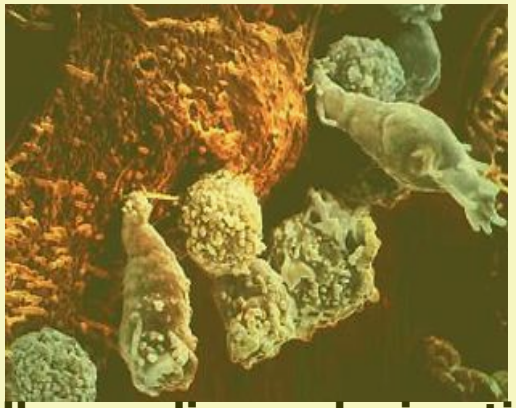
- NHE-6
- NHE-7
- NHE-9

TGN
Early/recycling Endosomes

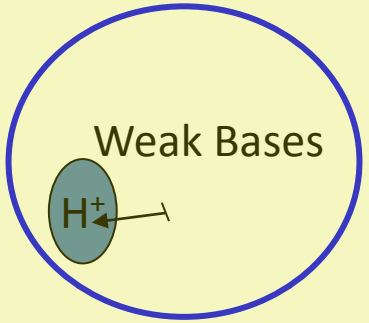






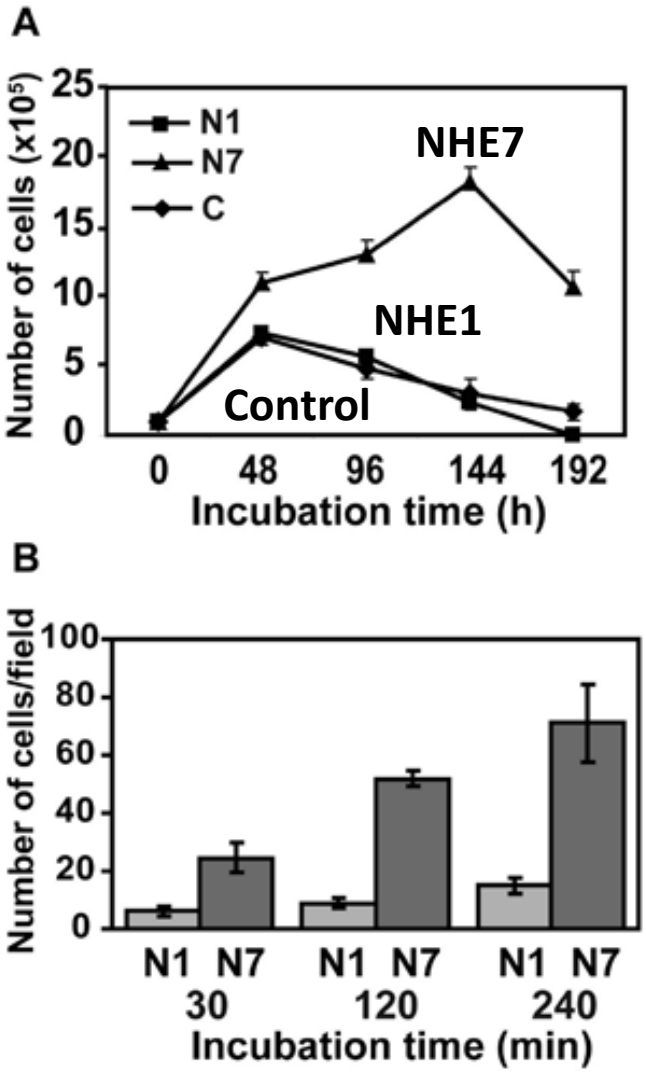


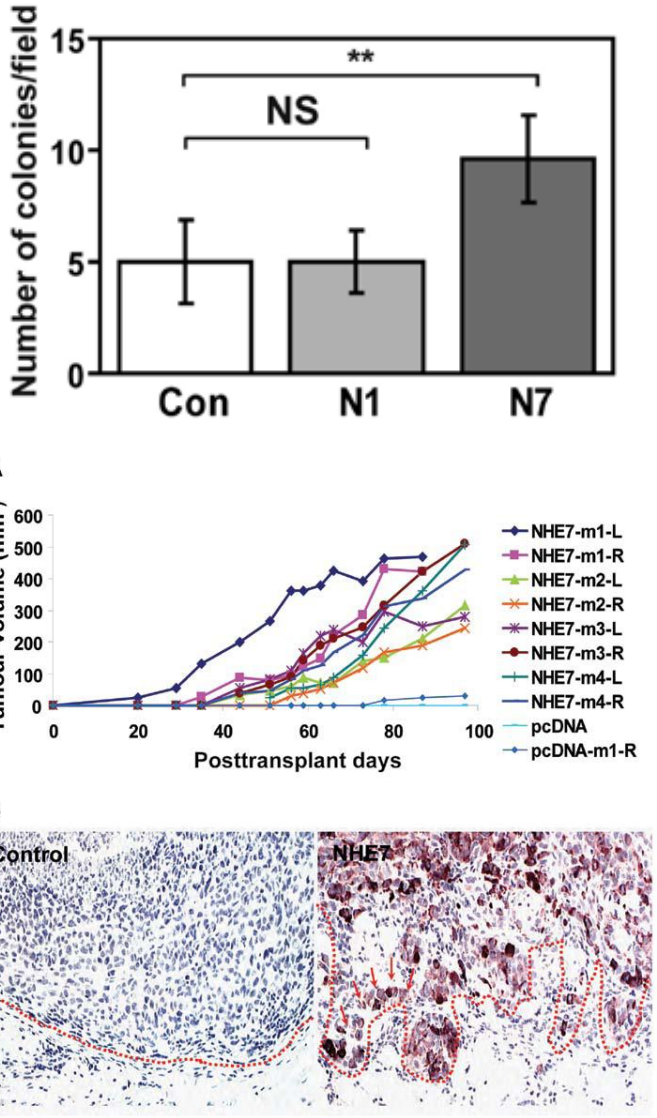
Cell spreading and migration



Drug Resistance

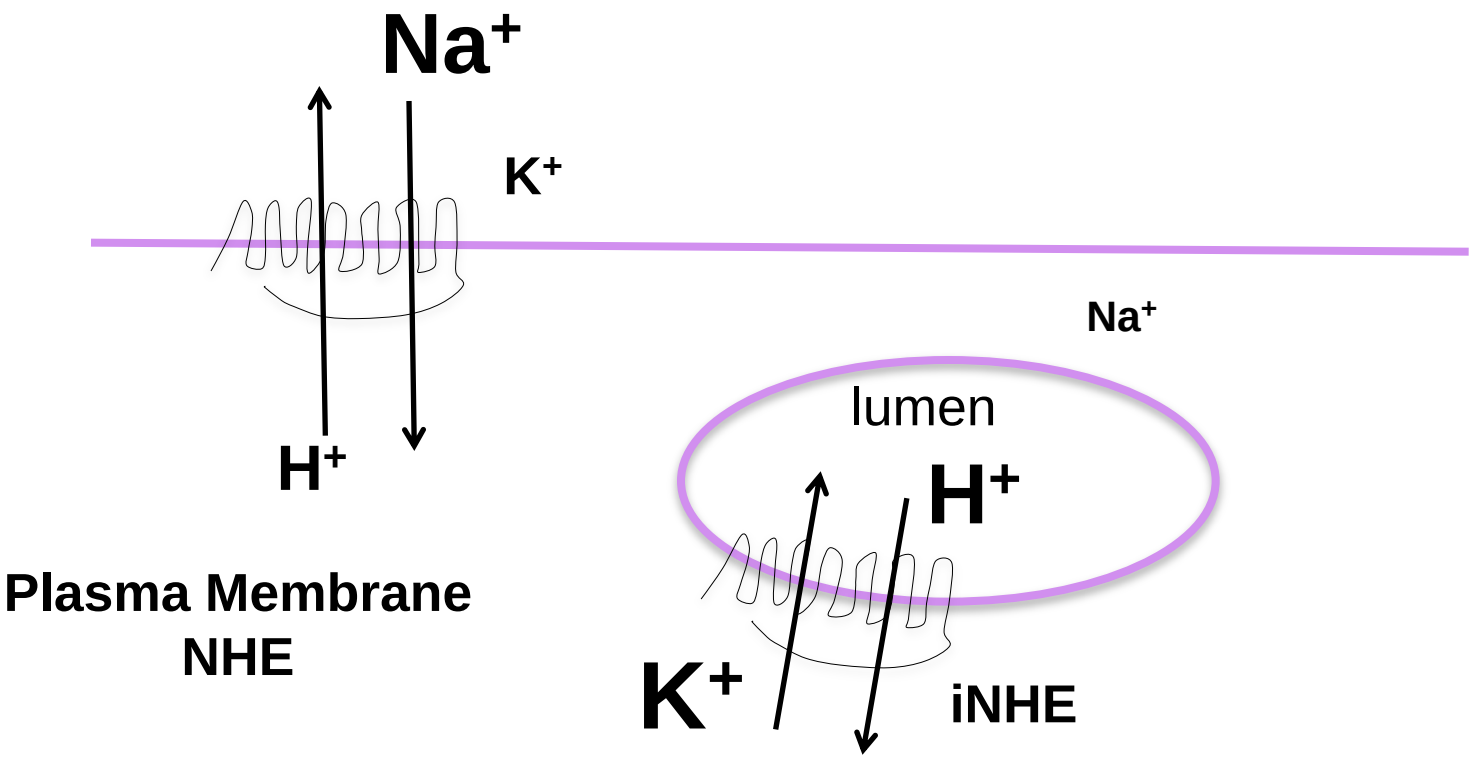
NHE7 overexpression enhances invasion of breast cancer-derived cells





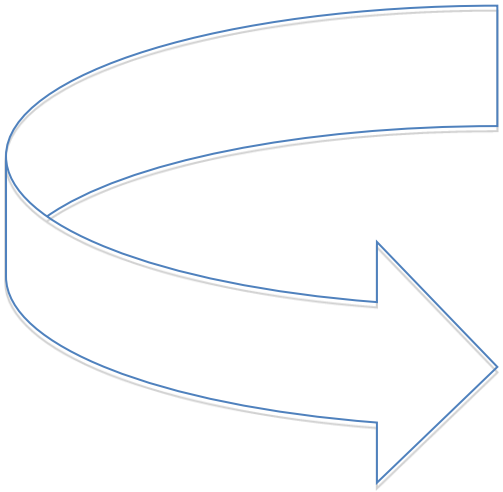
Roberge et al. Oncology Reports 2011

What is the function of these iNHEs ?



They are believed to work in reverse mode and be K+ coupled proton leaks, like their homologues in Plants and Yeast

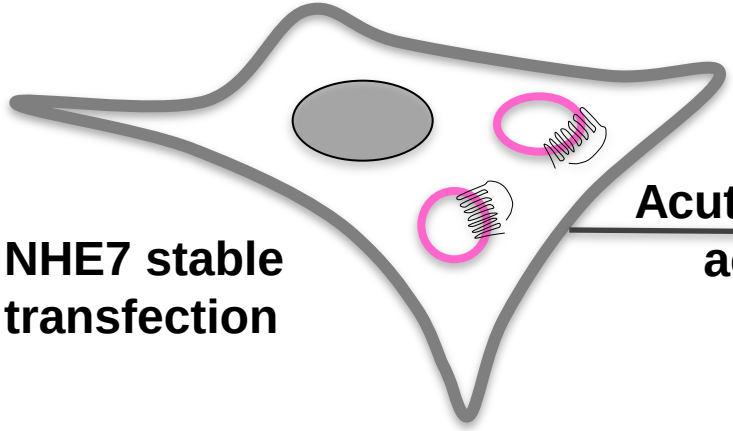
As these exchangers are intracellular it was not possible to precisely_measure their functional parameters and therefore to understand their function



Design a strategy to express a non-mutated non-tagged NHE7 at the plasma membrane

Screen for NHE7 plasma membrane expression

NHE-deficient cells



NHE7 stable transfection

Acute intracellular acidification

120mM Na+

No survival

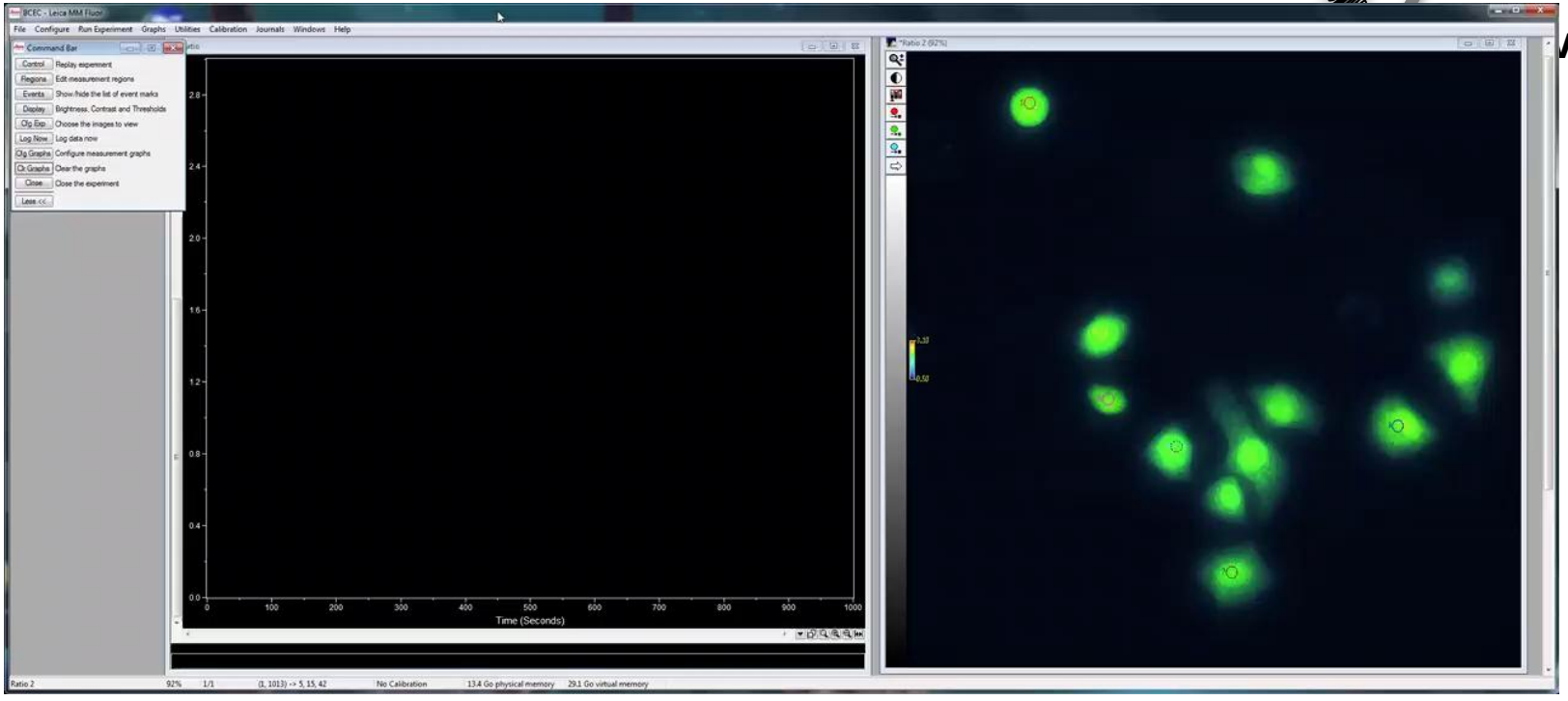
WT-NHE7 cells

H^+

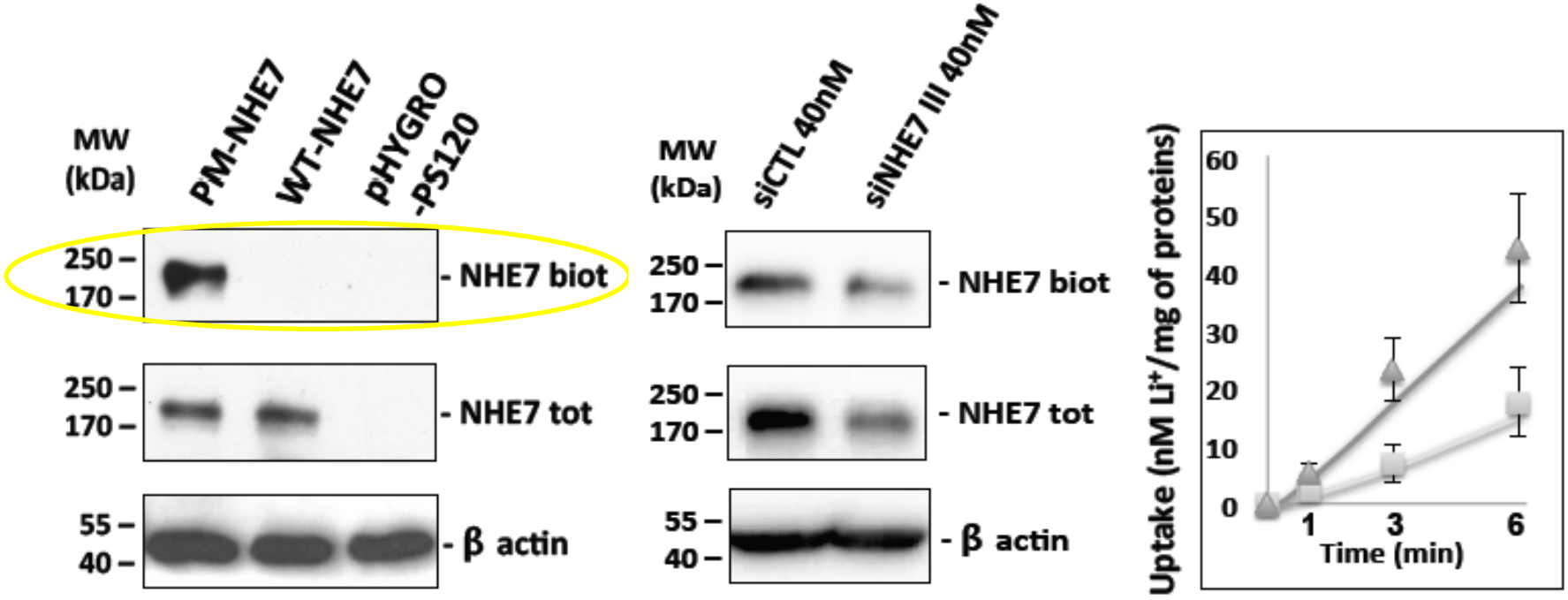
PM-NHE7 cells

H^+

val



Is it really NHE7 at the plasma membrane?

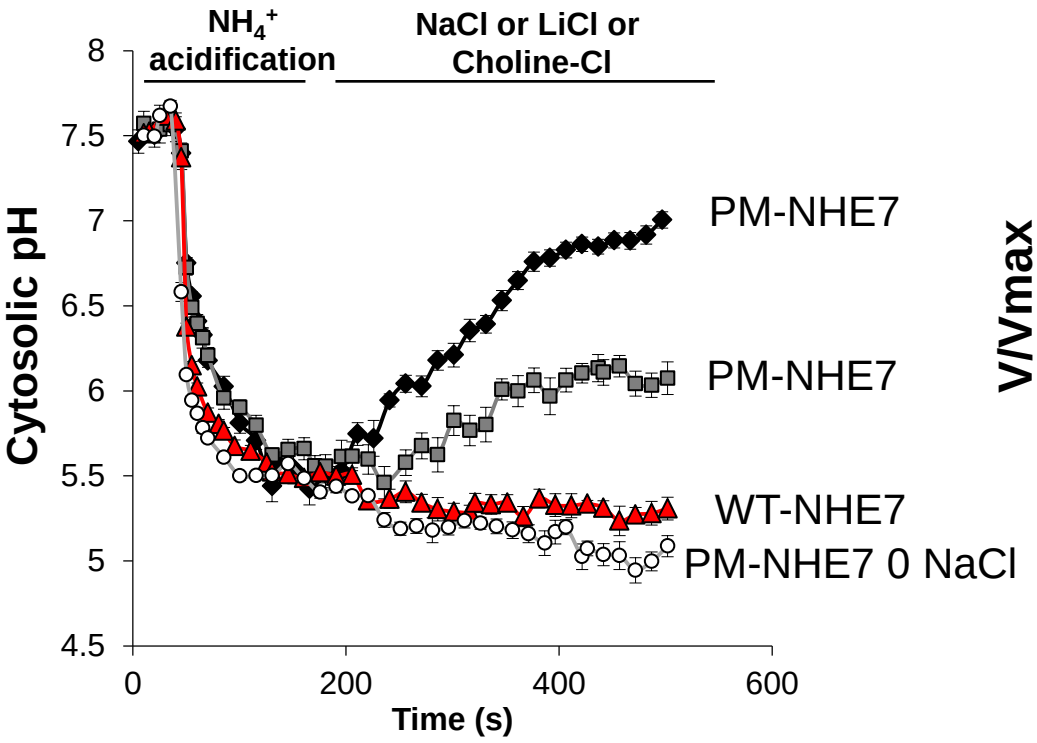


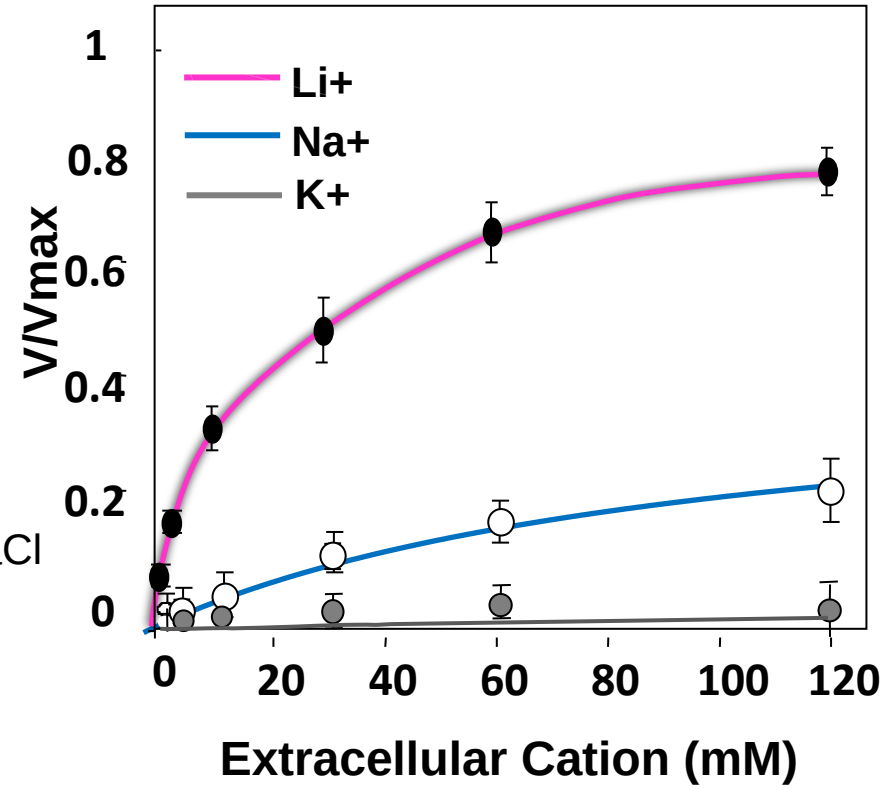
cDNA Sequencing

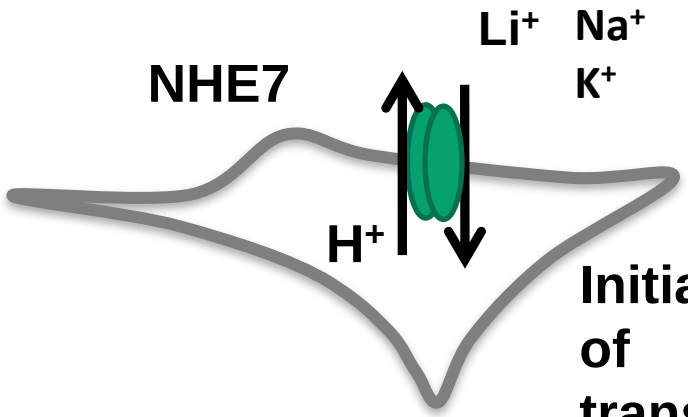


NO mutations were found

Transport of External/Vesicular Cations







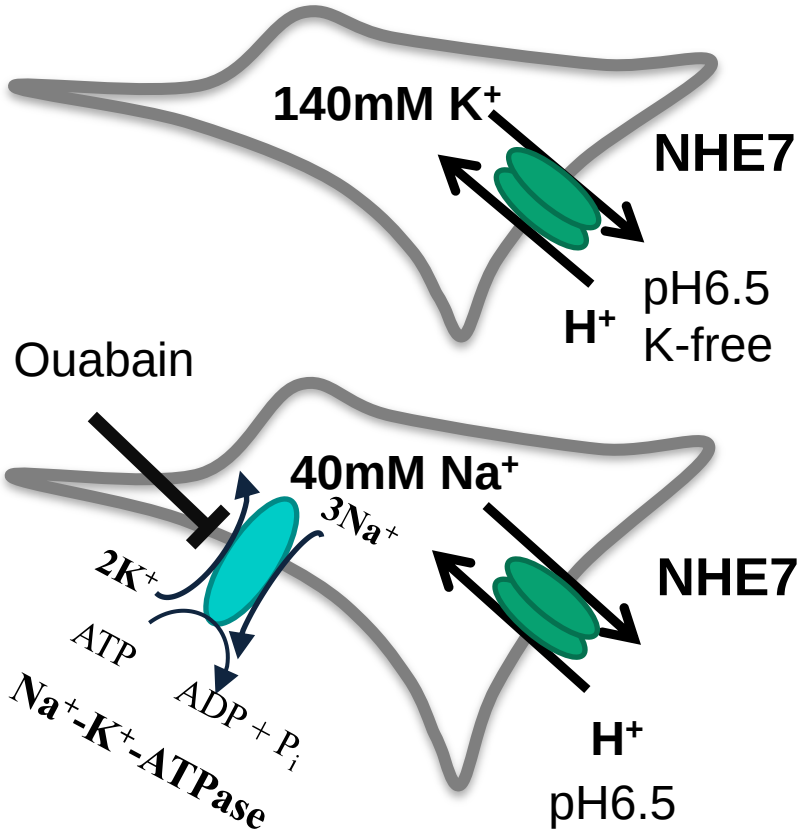
Initial rates of transport

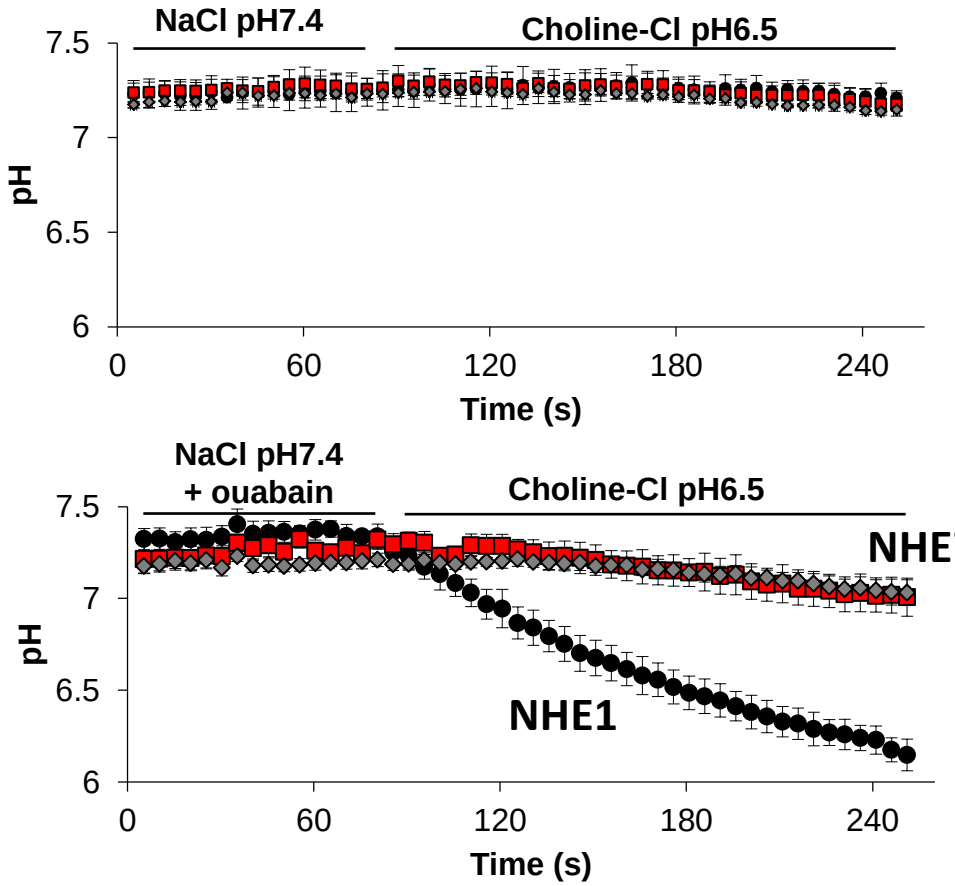
Li+: Km=16.6mM

Na+: Km=240mm

K+: NO

Reversibility

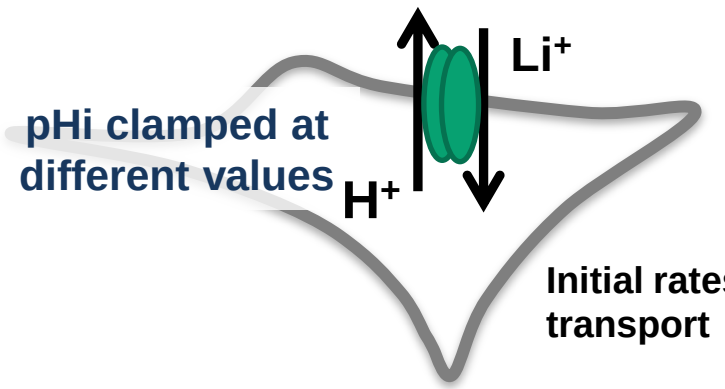




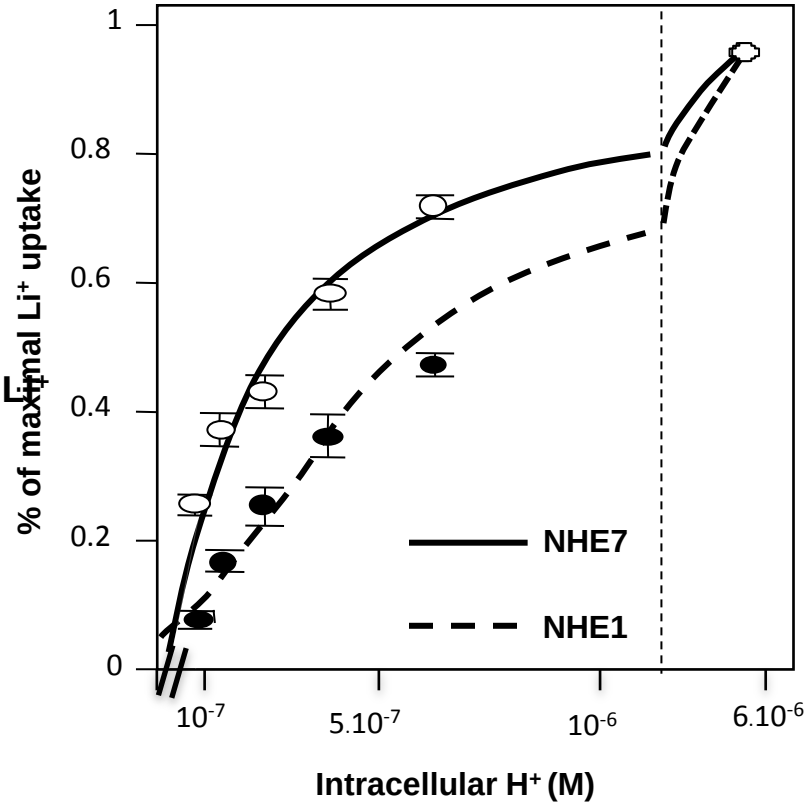
Cytosolic Na^+, K^+ and Li^+ cannot be transported outside when the gradients are inverted.

Unlike NHE1, NHE7 does not transport reversibly in the tested conditions

Intracellular H+



Initial rates of Li+ transport



NHE7: Michaelis Menten

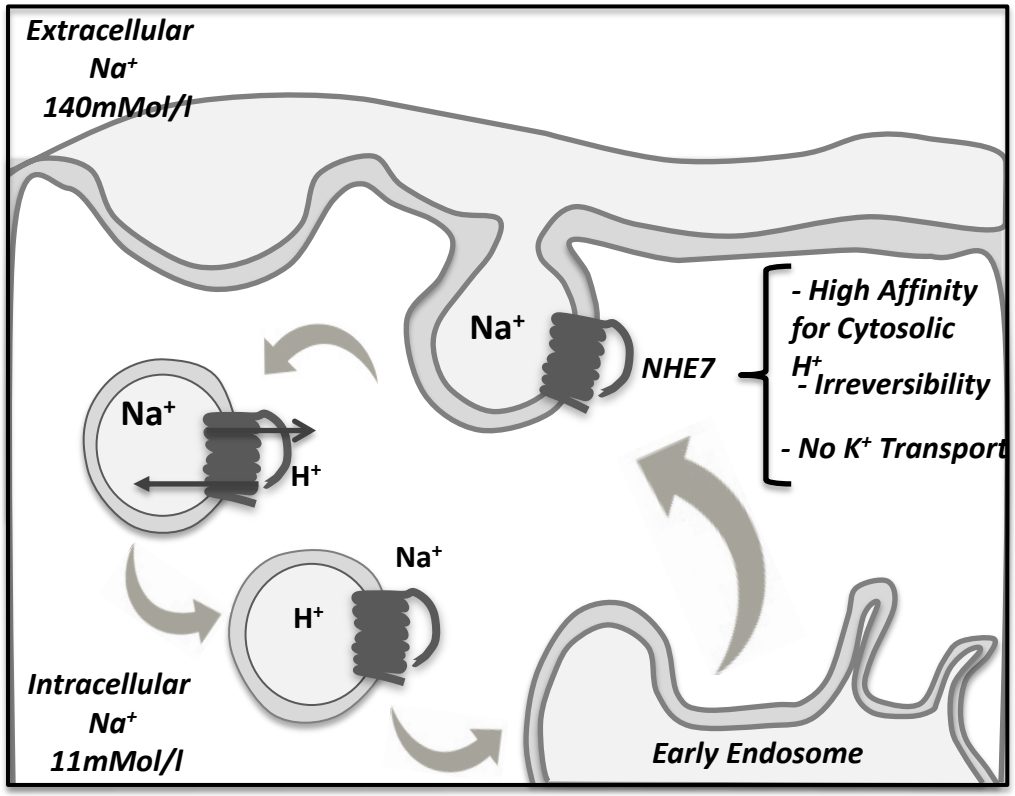
NHE1: Cooperative (MWC)

NHE7 is the first member of the NHE family with such a high affinity for intracellular proton !

Working Model

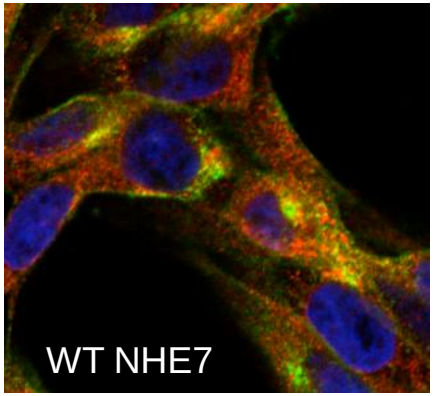
- Low affinity for vesicular Na^+
- High affinity for cytosolic H^+
- Inhibited by vesicular H+

- It Passes by the Plasma Membrane to go into Early and Recycling Endosomes

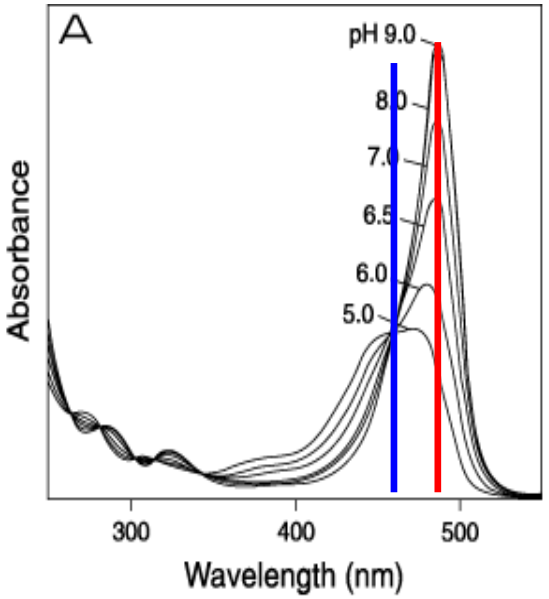


NHE7 is programmed to acidify vesicles until the Na^+ gradient dissipates and then it stops!

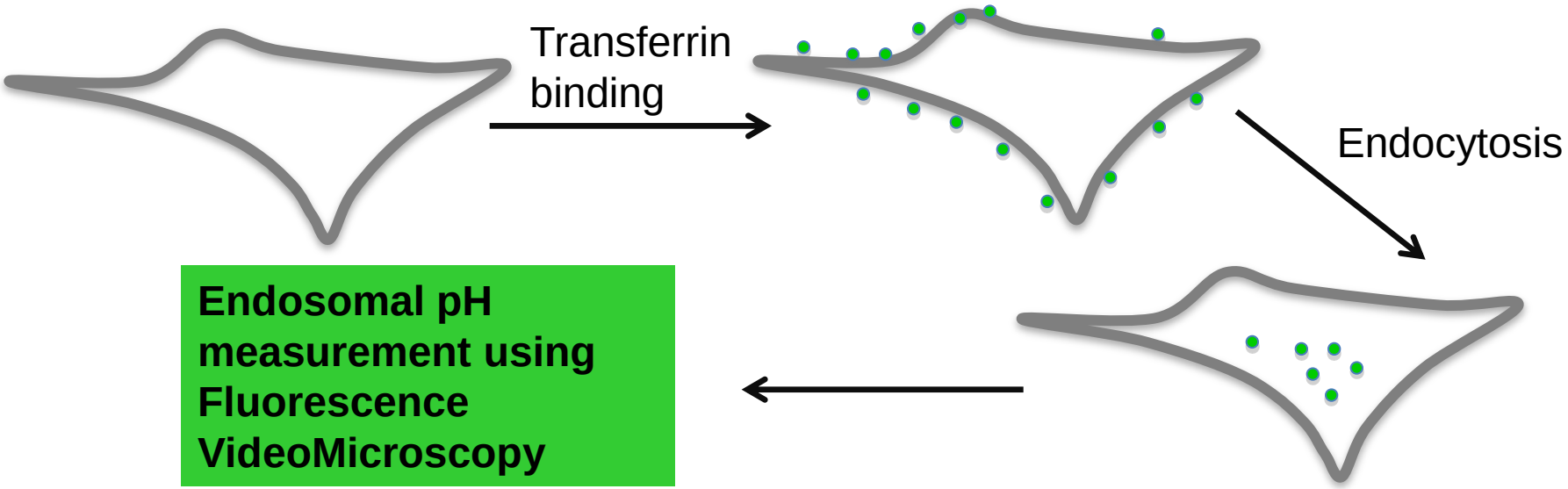
How to measure pH in NHE7 positive compartments in WT-NHE7 cells?



TF-R (Green)
NHE7 (Red)



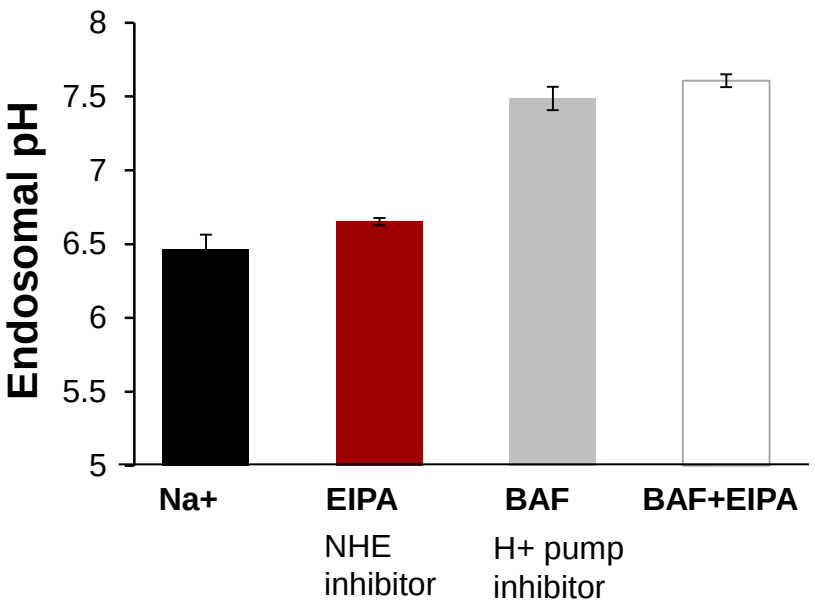
NHE7 colocalizes with the TF receptor



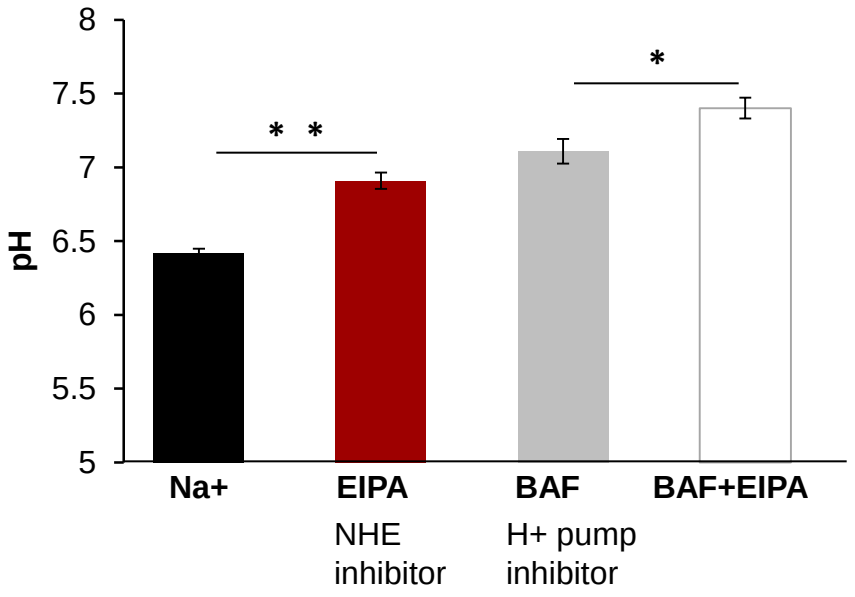
Endosomal pH measurement using Fluorescence VideoMicroscopy

What are the results ?

Control cells



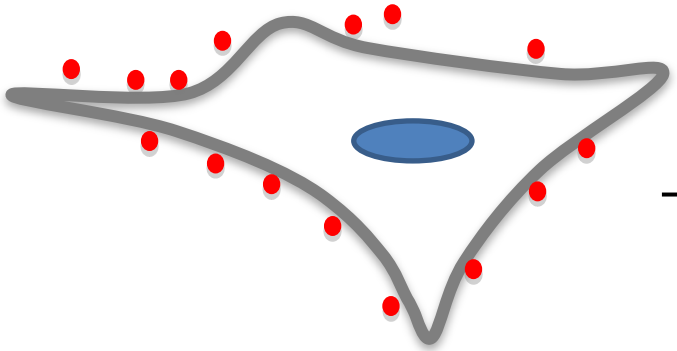
WT-NHE7 cells



The same results obtained without Na^+ (NMDG)

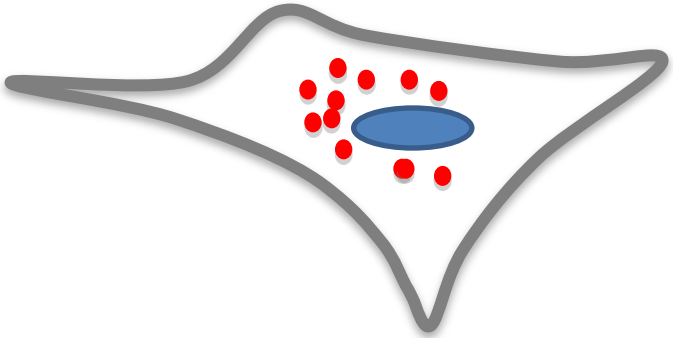
NHE7 is an acidifying mechanism additive to the H^+ pump

What is the effect on Vesicular trafficking ?



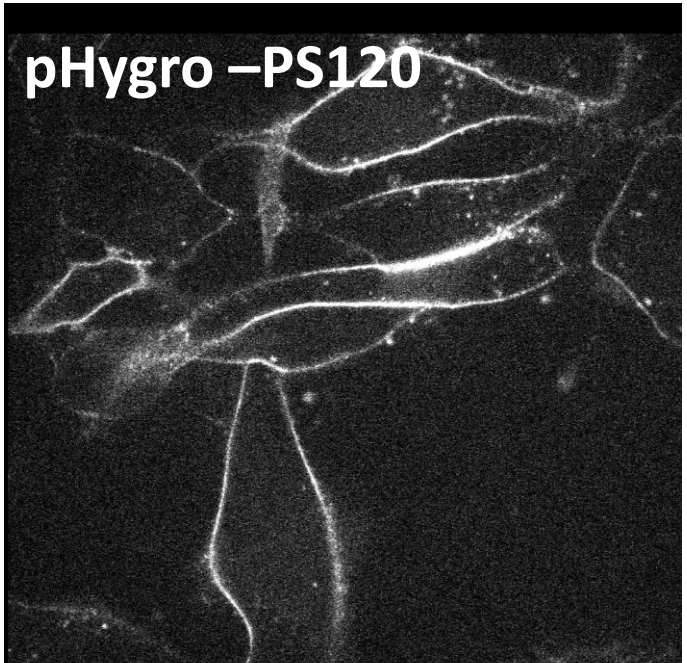
Spinning disk confocal microscopy

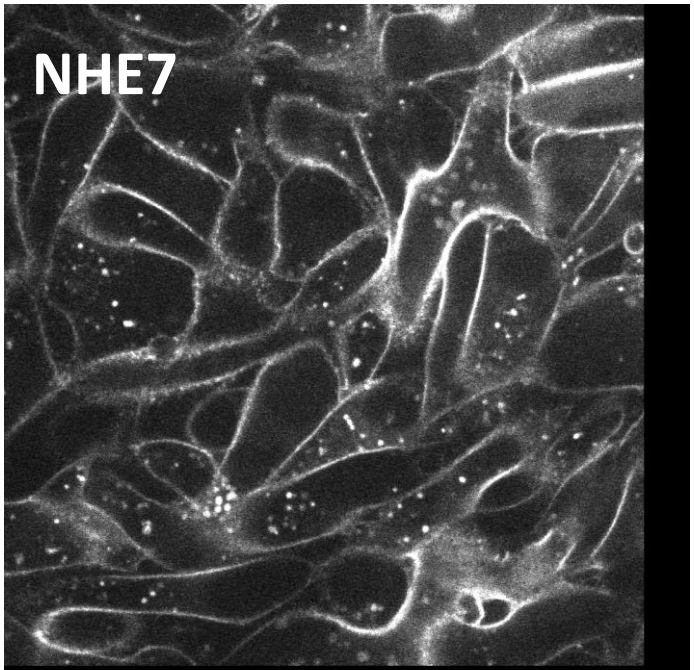
37°C



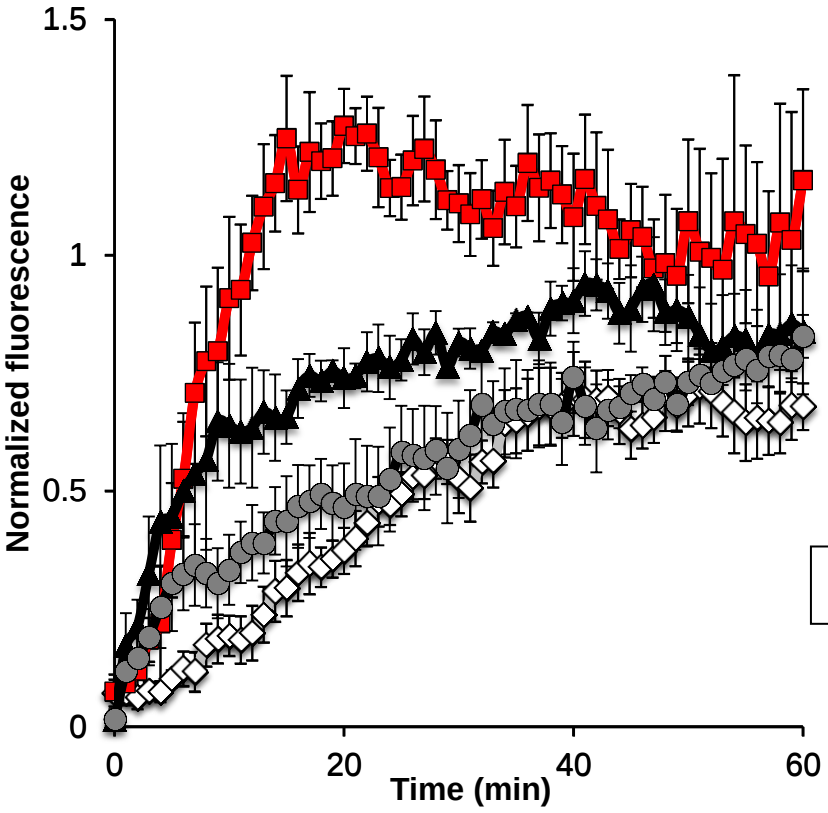
Untransfected control cells or WT NHE7 cells
Binding at 0° C

Quantification of cluster formation





Quantification



WT-NHE7 pH7.4

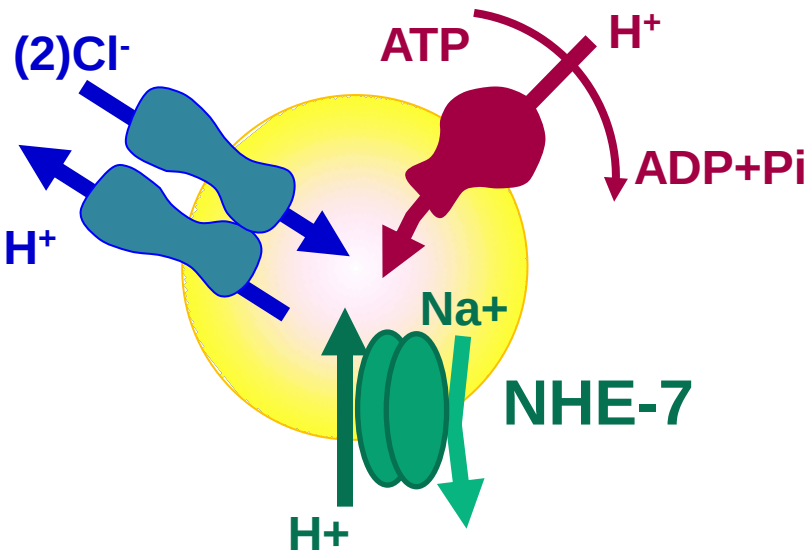
Control PS120 pH7.4

Control PS120 pH7

WT-NHE7 pH8

3.3-fold acceleration

Concluding remarks



Na^+/H^+ exchanger 7 was believed to alkalinize vesicles
In fact it is a new acidifying mechanism additive to V-ATPases and ClCs

This acidification results in enhanced vesicular trafficking.

This has important implications for tumor cells and neurological disorders.

Milosavljevic et al. 2014 Cell Reports

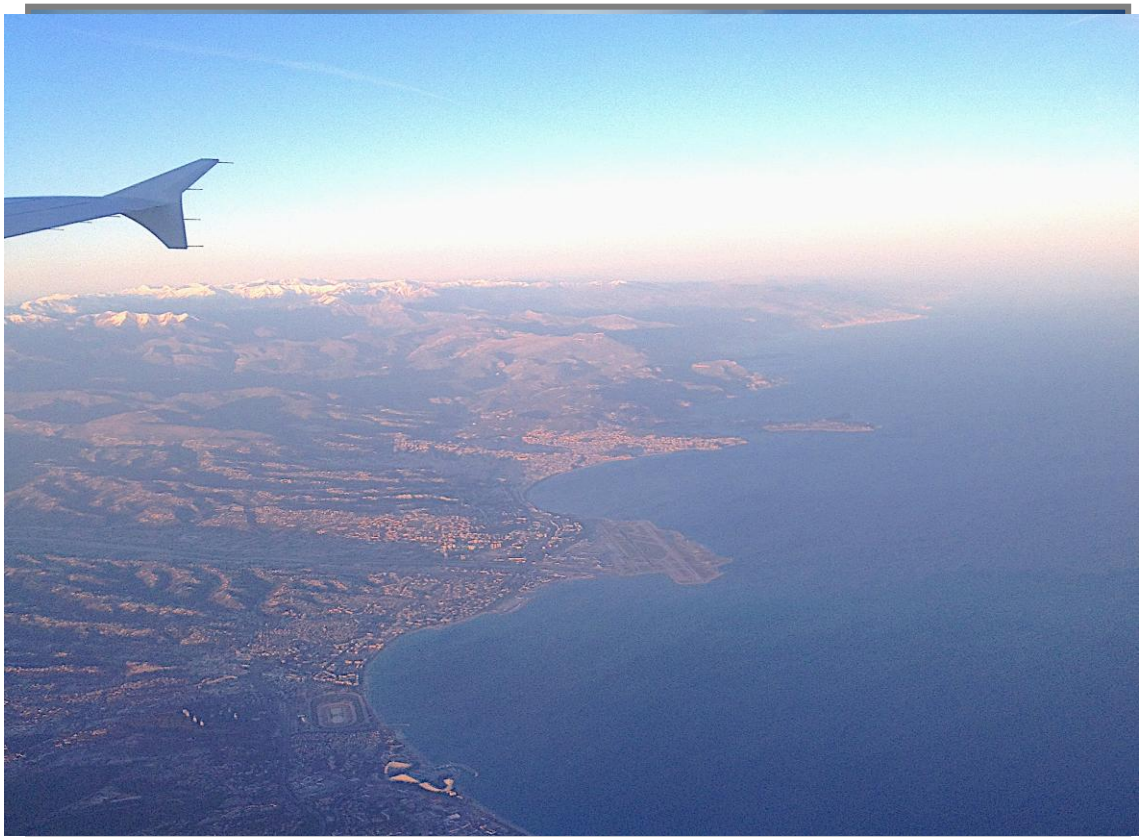
Members of the Team:

Eléonore Birgy
Auréa Cophignon
Laurent Counillon
Nina Milosavljevic,
Michaël Monet
Mallorie Poët
Isabelle Léna

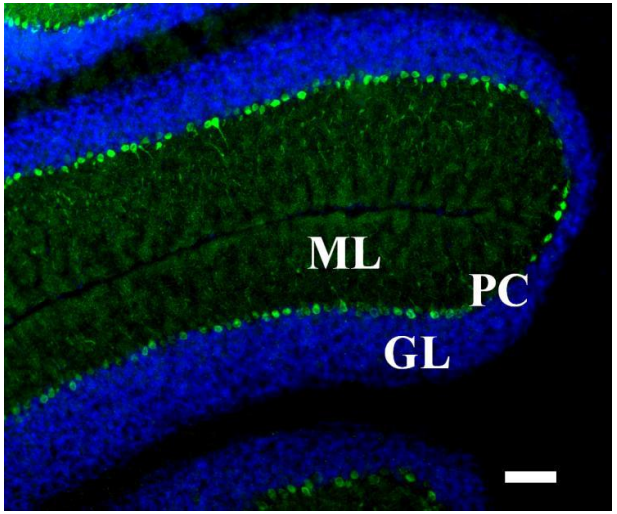
Our main Collaborators

Pierre Gounon (CCMA)
Frédéric Brau (IPMC)
Yann Bouret, Mederic Argentina (LJAD)

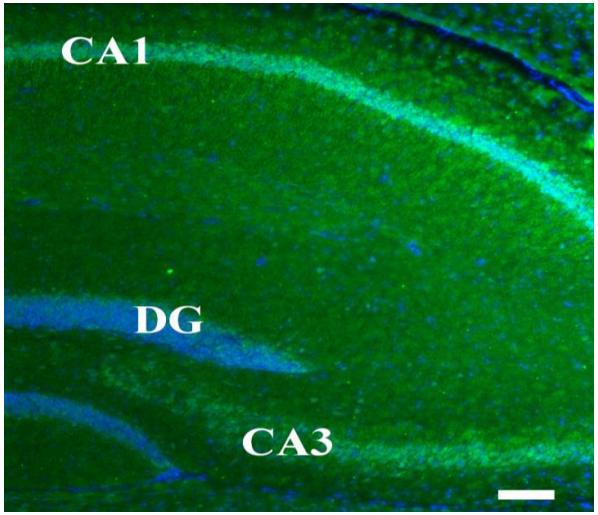
Pierre Henri Puech (Marseille)
Dominique Lagadic & Patrick Legembre (Rennes)
Sébastien Roger (Tours)
Cyril Rauch (Nottingham)
Nils Gautier, Marie-Véronique Clément (Singapore)



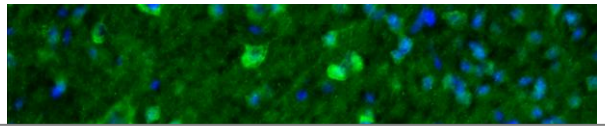
NHE7 expression seems restricted to large integrative neurons

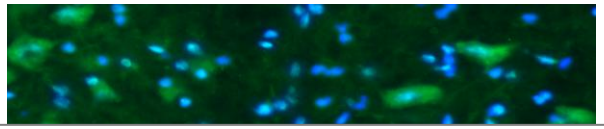


Cerebellum

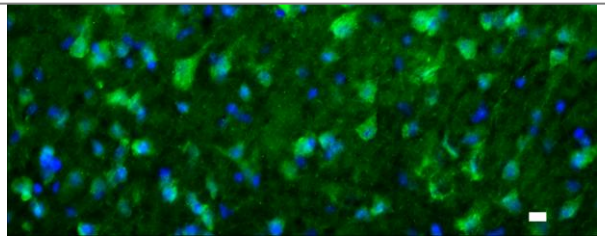


Hippocampus

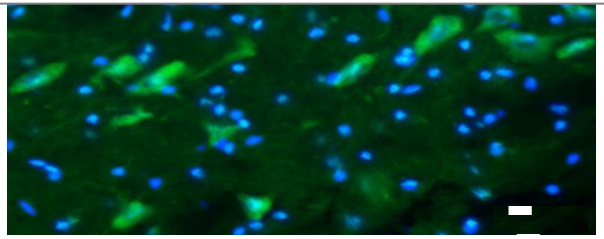




A similar organization is observed for NHE6



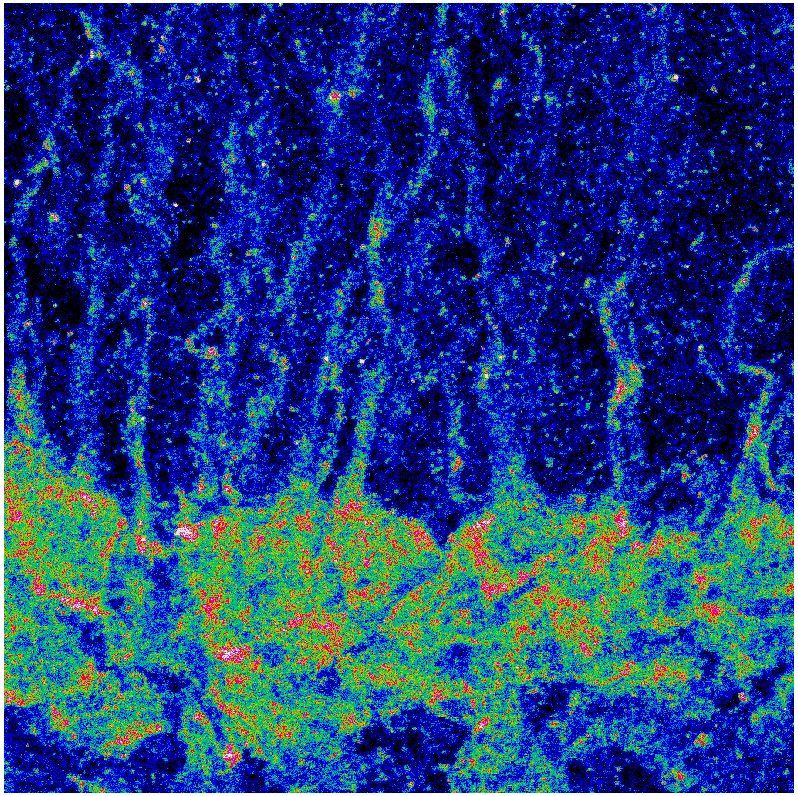
Cortex



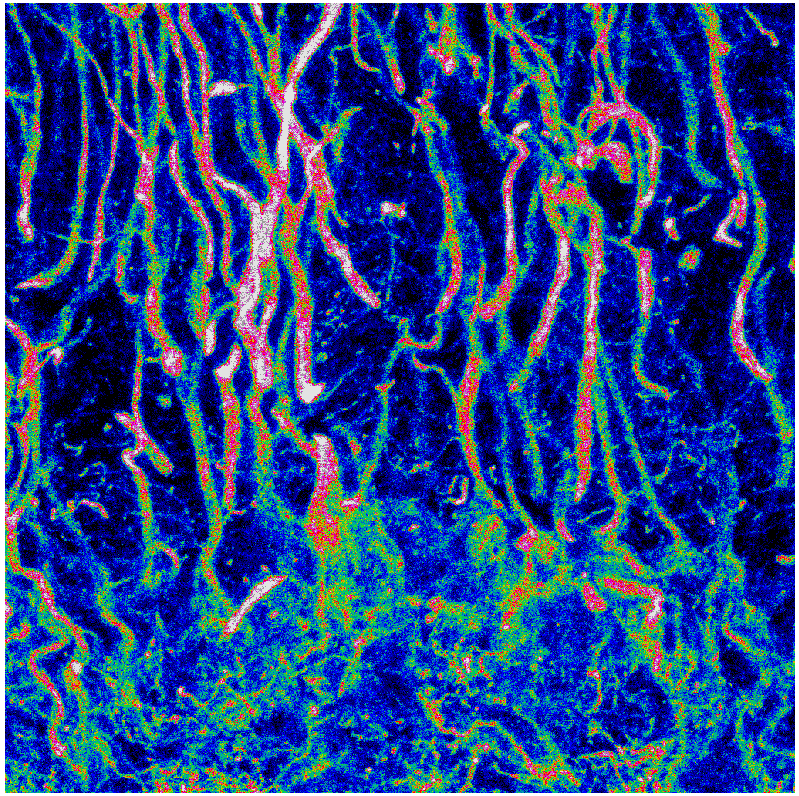
Brainstem

HIPPOCAMPUS
Pyramidal Cells (CA1)

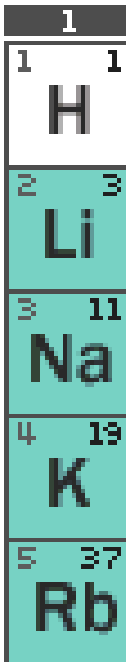
NHE-7

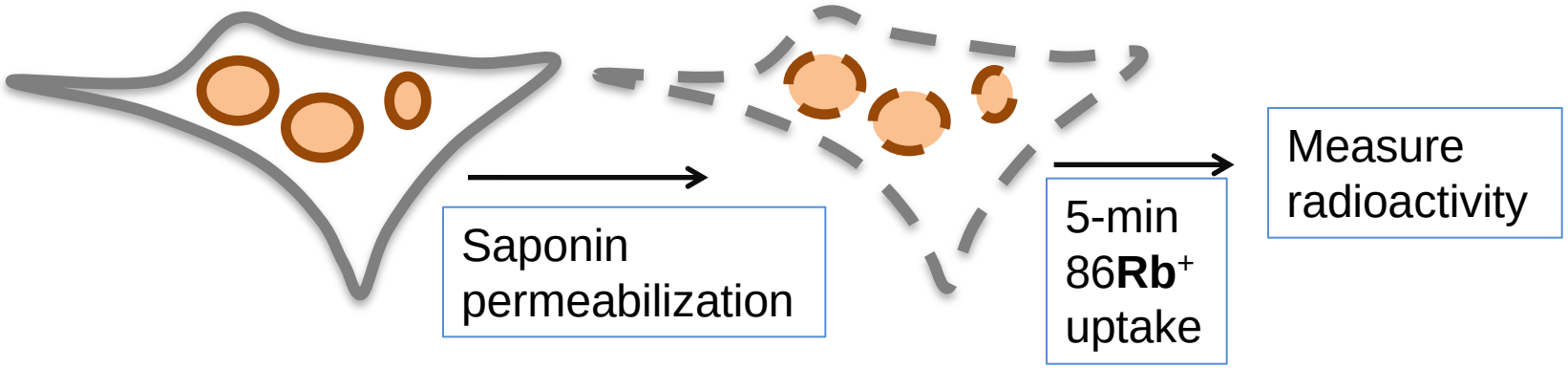


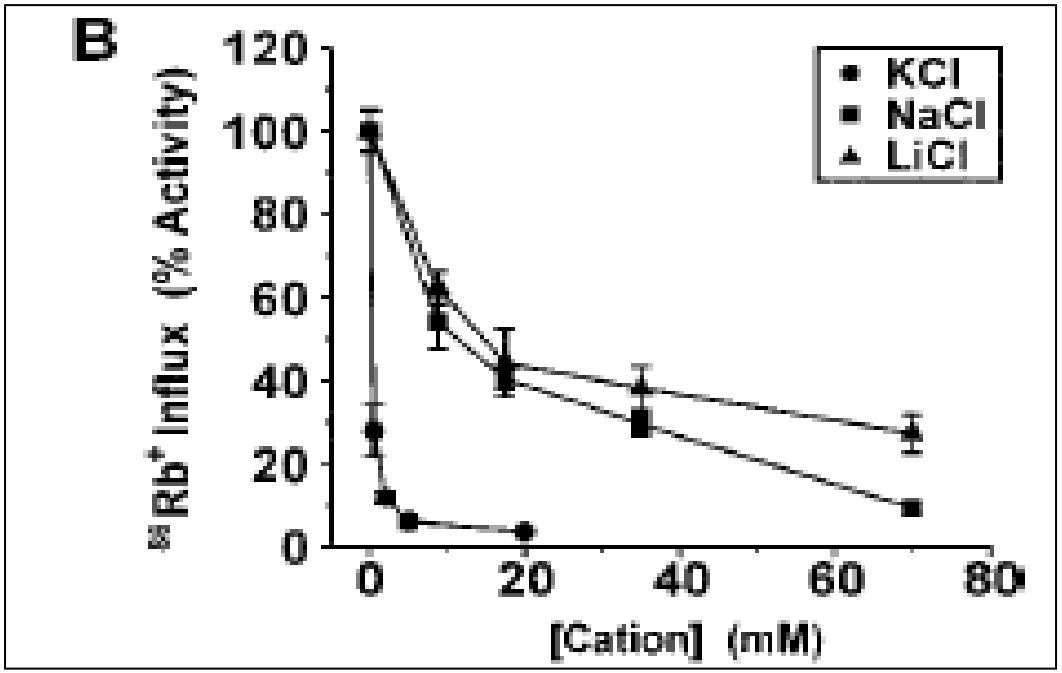
NHE-9



Experimental evidences for an alkalinizing role of NHE7

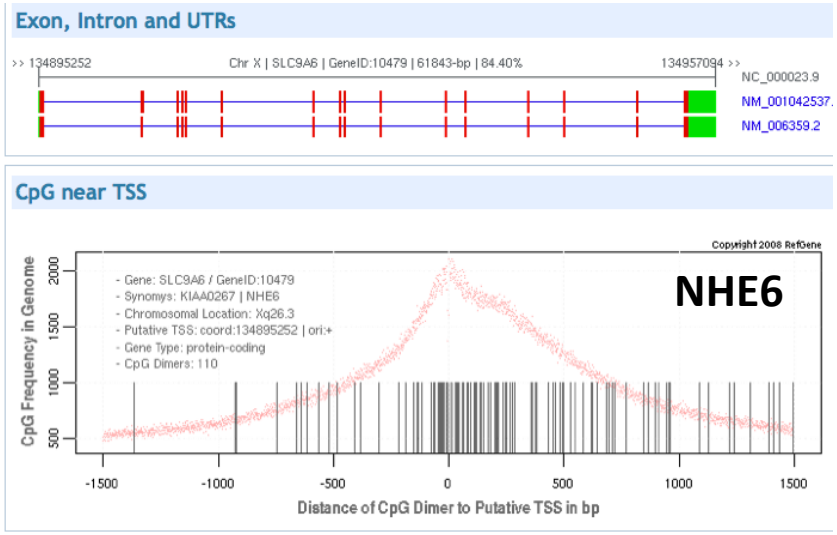


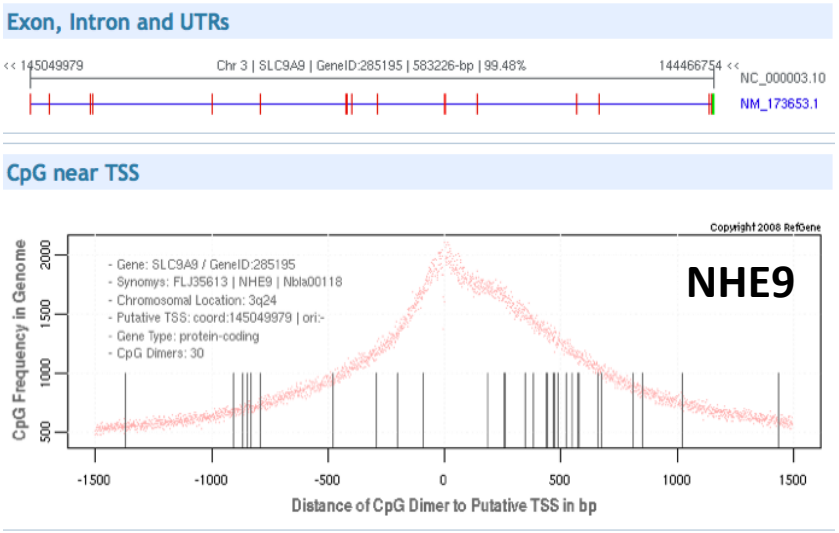


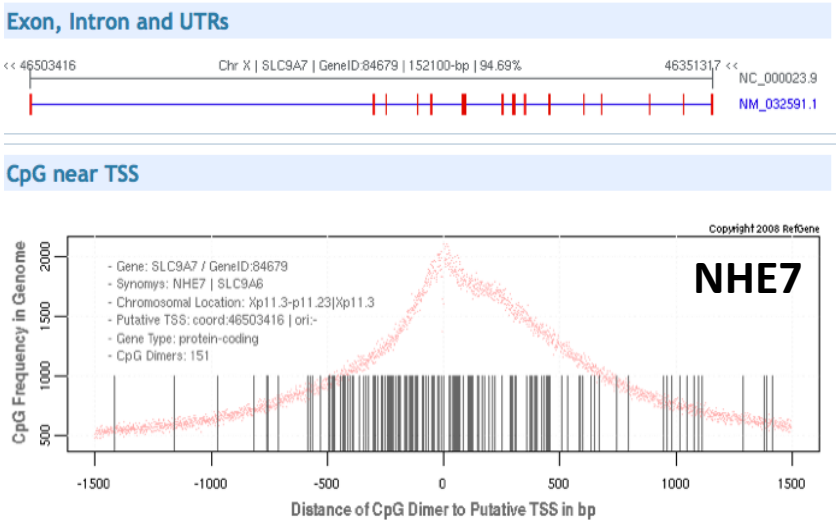


Numata et al. 2001

NHE6, 7 and 9 transcription initiation regions are enriched in CpG-dimers







NHE6 and NHE7 genes are on the X chromosome and are subjected to X epigenetic inactivation