DEVELOPMENT OF A NEW FLUORESCENT PROBE FOR DETERMINATION OF WATER TRANSPORT IN SUBCELLULAR ORGANELLE

Adriana Maria Chaurra A.

Nanociencia 2010

Octubre 22 de 2010

Barranquilla, Colombia

WATER IN LIFE

Most abundant substance in cells.
Solvent for ions and molecules.
Bathing medium for organelles.

Haussinger, D.; Lang, F.; Gerok, W. *Am. J. Physiol.* 1994, 267, E343- 355.

WATER IN LIFE

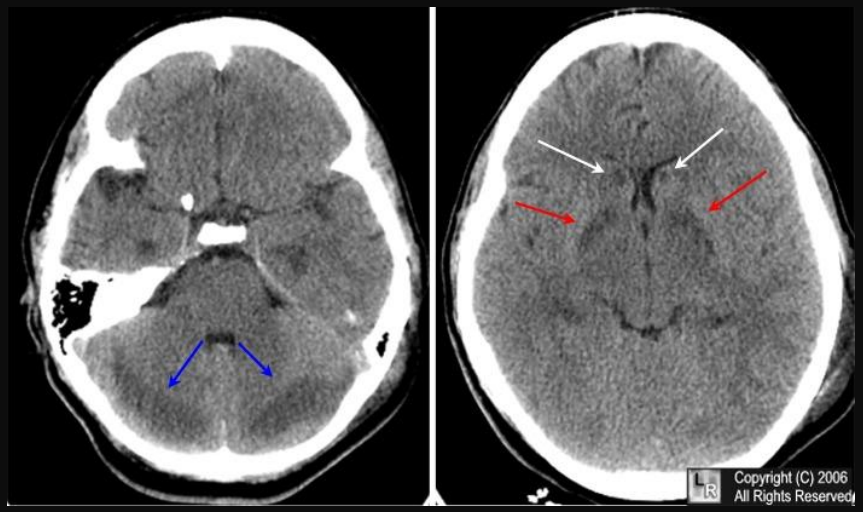
Water content in Red Blood Cells from different animals

Species	Water Content (%)
Man	(63.9 - 66.1) ± 1.4 65 ± 10
Rabbit	74.0 ± 1.1
Fox	(58.6 – 71.2) ± 1.4
Sheep	(60.5 - 70.9) ± 1.1
Pig	62.6
Horse	61.3
Dog	66. 66 ± 0.9
Cat	62.4
Goat	60.9

Ling, G. Physiol. Chem. Phys. & Med. NMR 2004, 36, 1-19.
Afifi, A. Tohoku J. exp. Med., 1985, 145, 7-14
Kogawa, H.; Yabushita, N.; Nakajima, T.; Kageyama, K. Life Sciences 1998, 62, 823-828

DEFICIENCIES IN WATER FLUX

Cerebral Edema



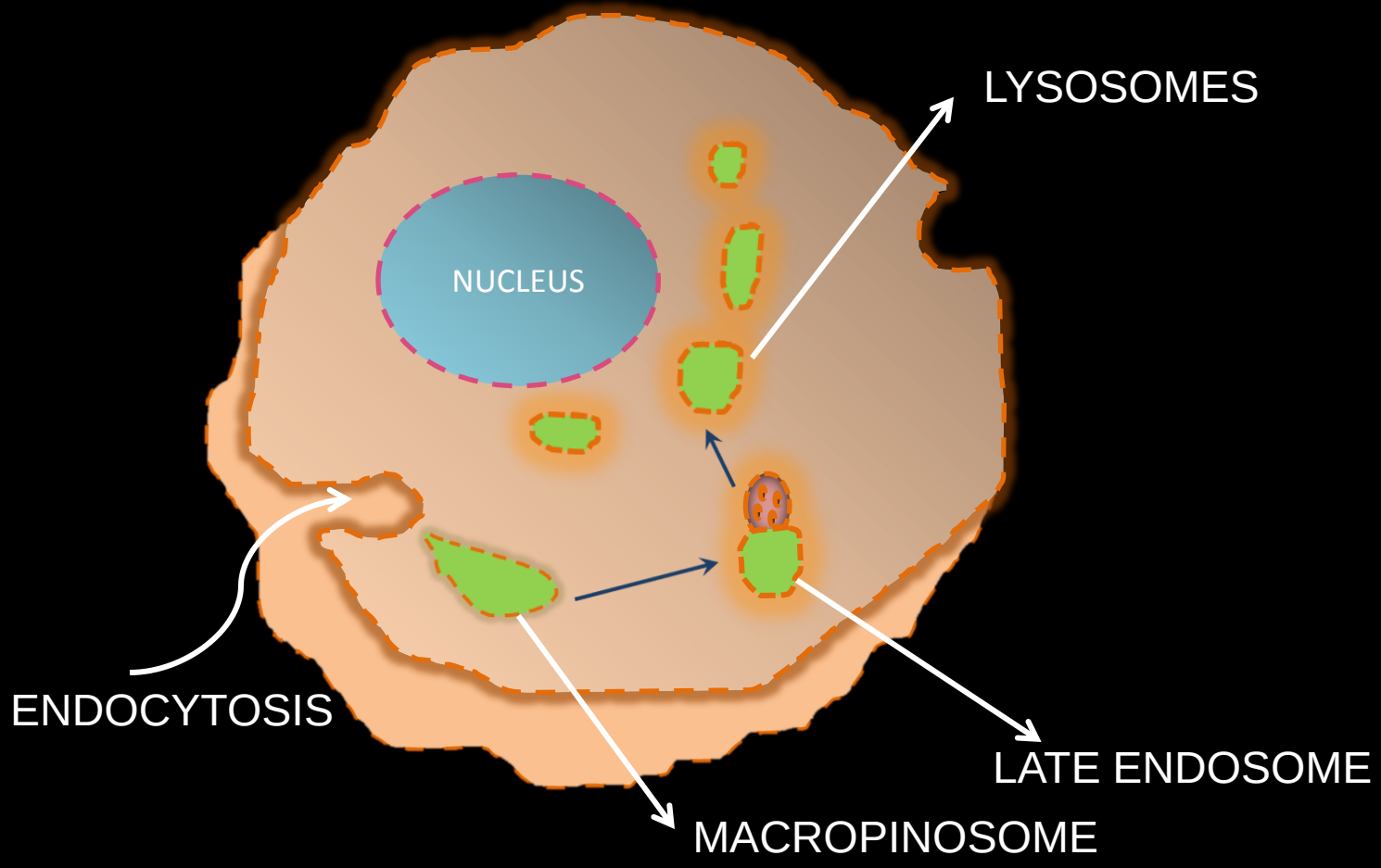
Cerebral edema is an excess accumulation of water in the intracellular and/or extracellular spaces of the brain.

Diabetes insipidus

Diabetes insipidus is an uncommon condition that occurs when the kidneys are unable to conserve water as they perform their function of filtering blood.

Jousuf, F.; Atif, F.; Ahmad, M.; Hoda, N.; Ishrat, T. Khan, B.; Islam. F. *Brain Research* 2009, in Press.
P . Hadjizacharia, P.; Beale, E.; Inaba, K.; Chan, L.; Demetriades, D. *J. Am. Coll. Surg*, *207* , 477 - 484

WATER TRANSPORT AND ENDOCYTOCIS



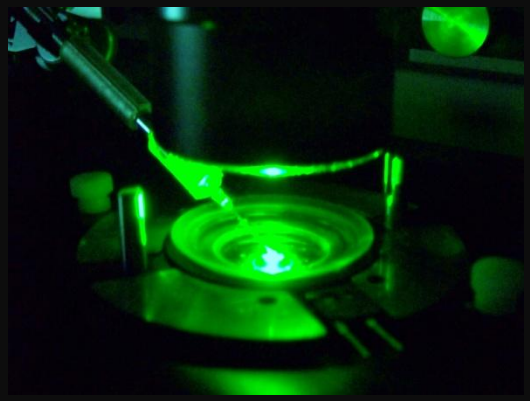
Mellman, I.; Fuchs, R.; Helenius, A. *Annu Rev Biochem* 1986, *55*, 663-700.

METHODS FOR MEASURING WATER TRANSPORT IN CELLS

- Light Scattering
- Fluorescence microcopy
- NMR

DEVELOPMENT OF THE PROBE

EXPERIMENTAL DESIGN

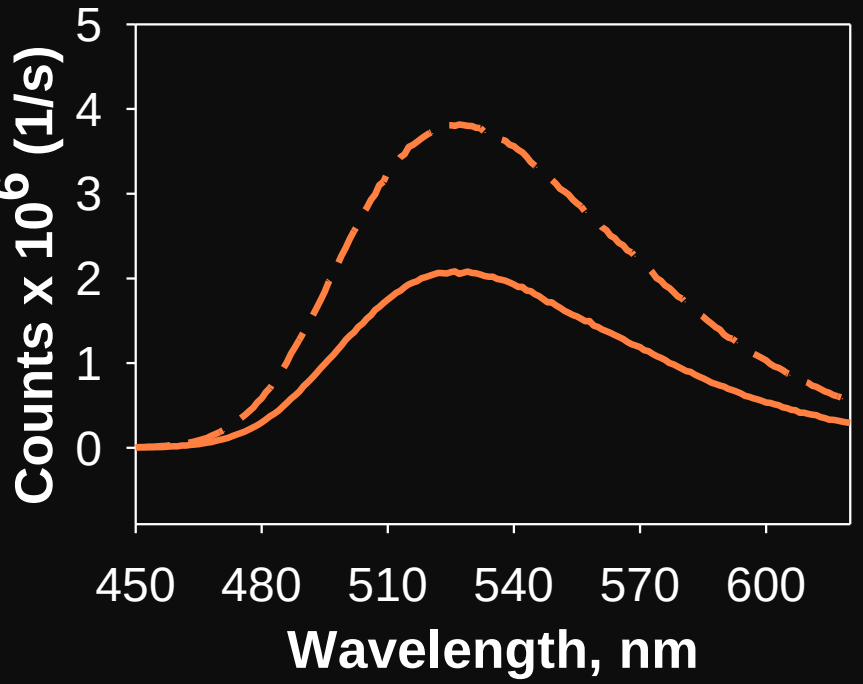


Incubation with dyes

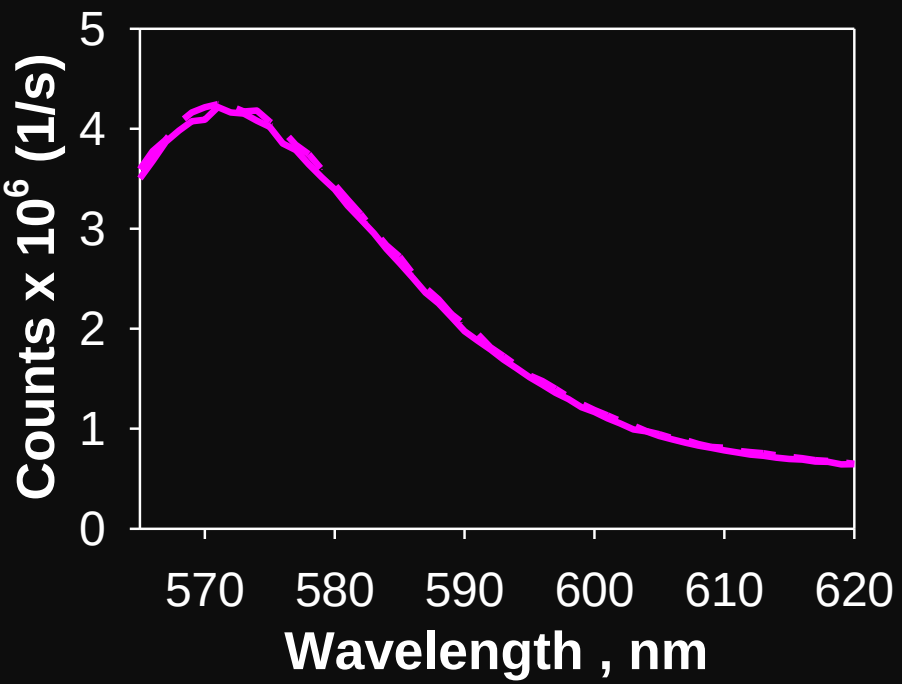
EXPERIMENTAL DESIGN

DEVELOPMENT OF THE PROBE

Lucifer Yellow Dextran (LY)
D_2O sensitivity 100%



Alexa Fluor Dextran (ALF)
D_2O sensitivity 2- 4%

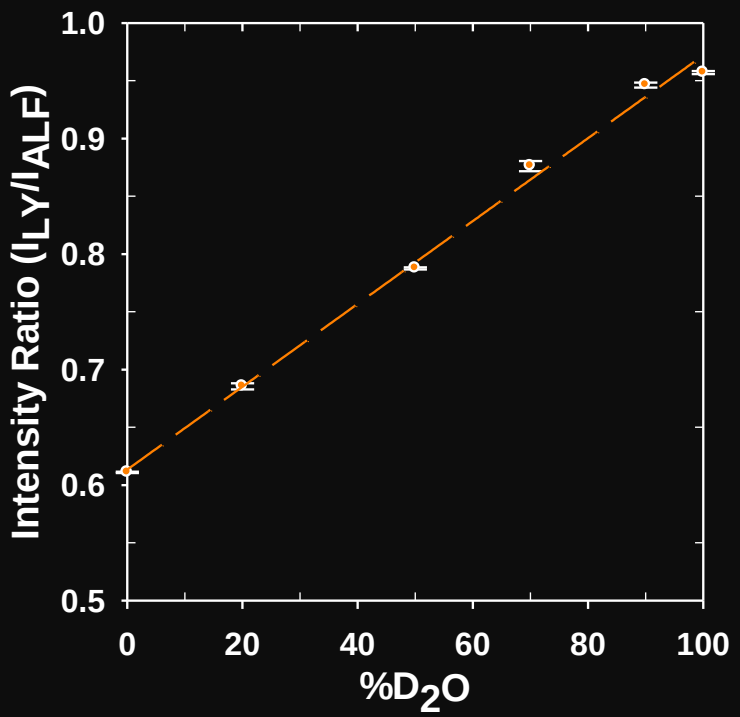


Chaurra, A.; Gutzman. B.; Ackroy. C.; Christensen. K.; Accepted for publication: *Applied Spectroscopy.* 2010

FLUORESCENCE INTENSITY RATIO *vs.* D_2O CONCENTRATION

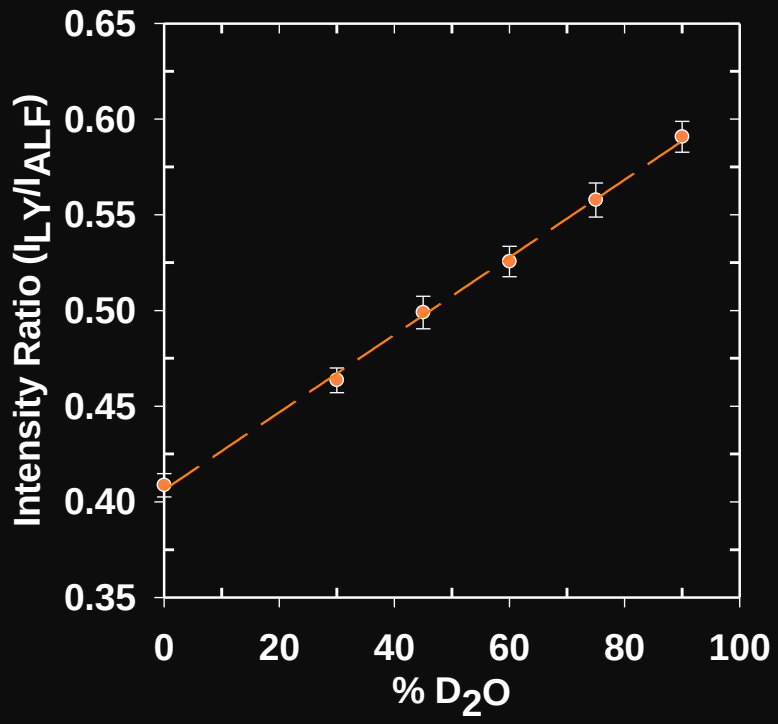
LY / ALF

In vitro



slope= 0.004 ± 0.00004

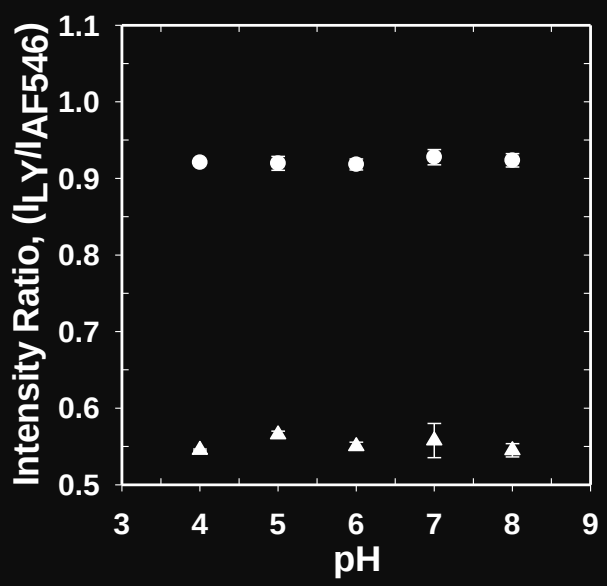
Ex vivo

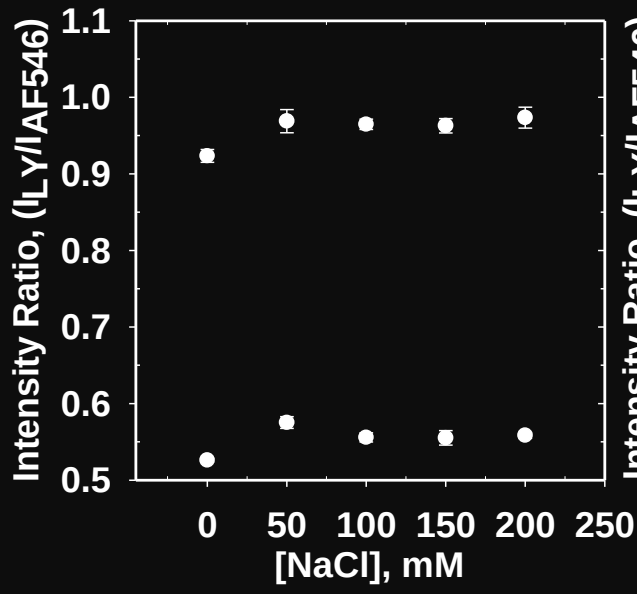


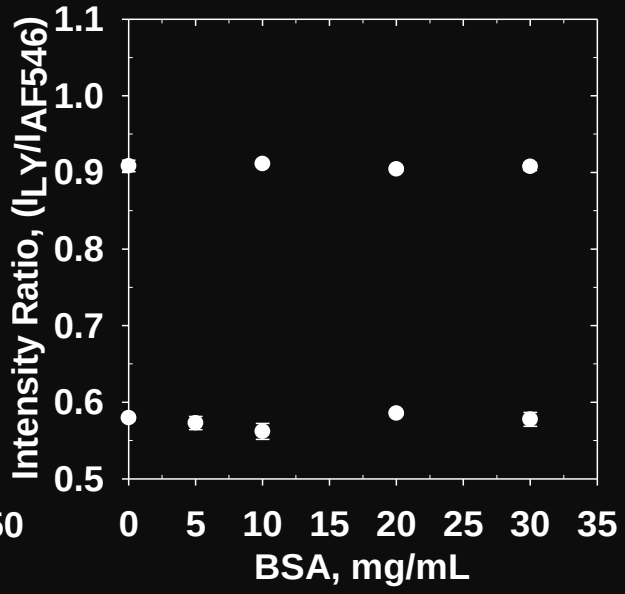
slope = 0.002 ±0.00006

CHO-K1 cells

EVALUATION OF REPONSE DEPENDENCE ON pH, SALT AND PROTEIN CONC.

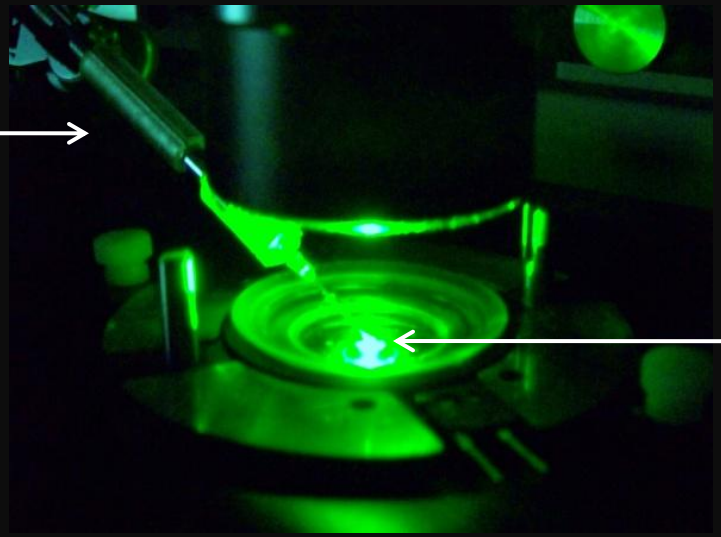






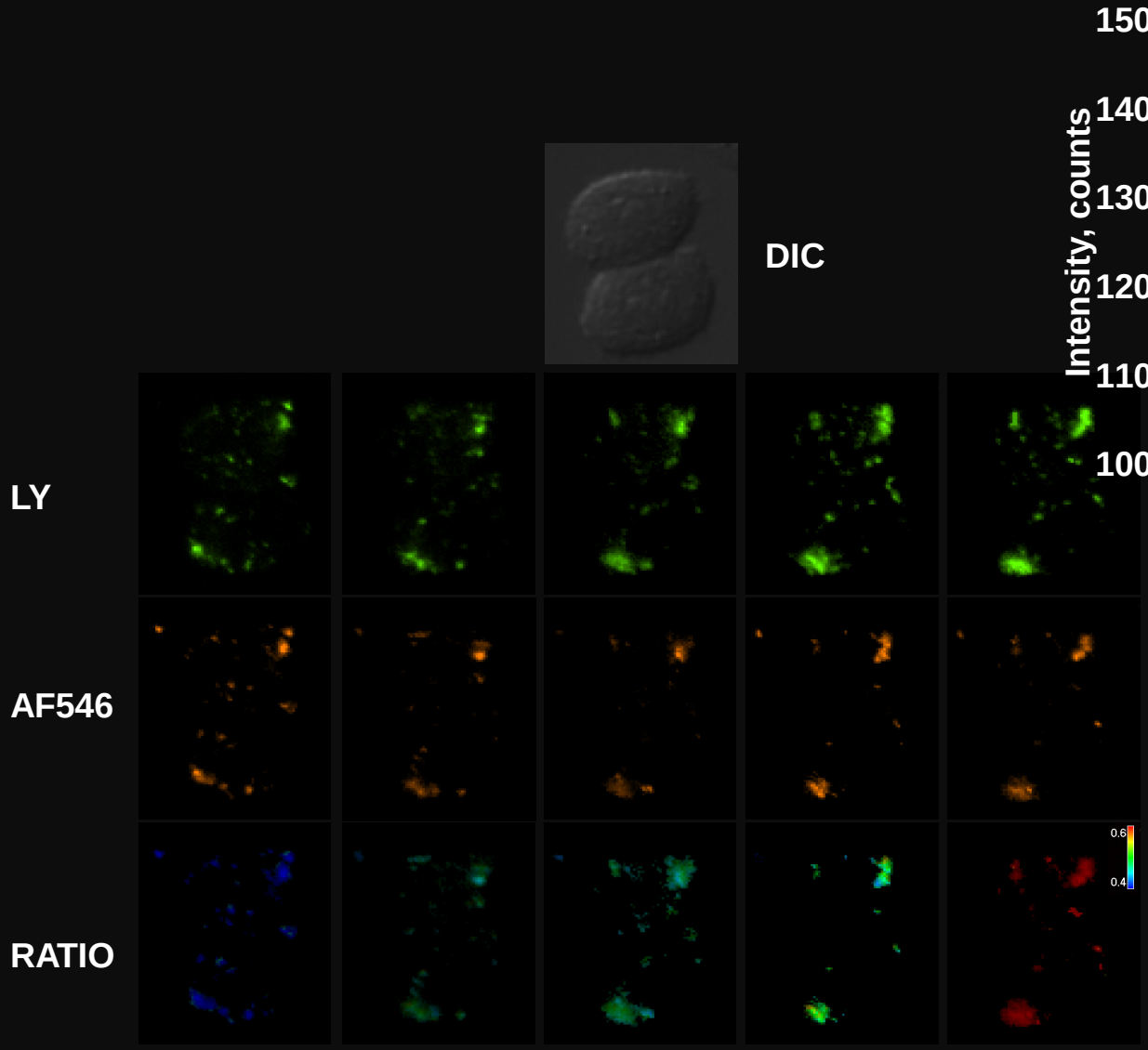
Geisow, M. J.; Hart, P. D'Arcy; Young, R.; J. Cell Biol. 1981, 89, 645-652
Christensen, K. A.; Myers, J.T.; Swanson, J. A. J. Cell Sci. 1982, 115, 599-607

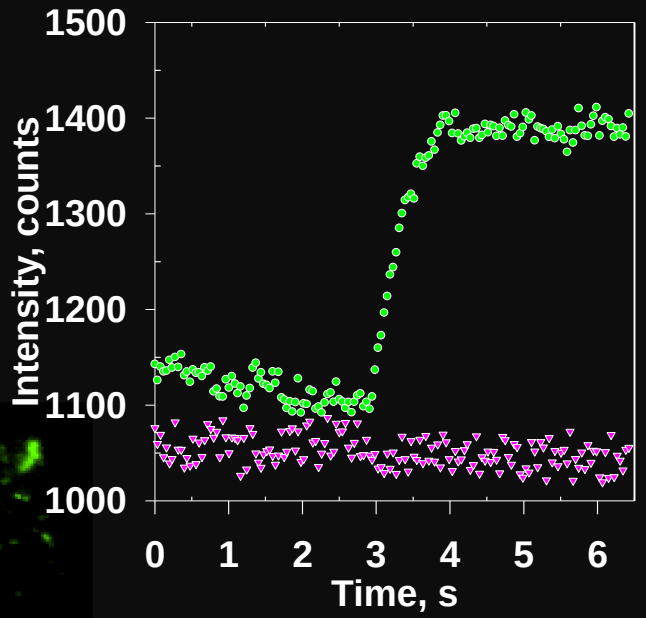
EXPERIMENTAL DESIGN



D_2O sensitive dyes encapsulated inside the organelles

LY AND ALF IMAGING IN CHO LYSOSOMES



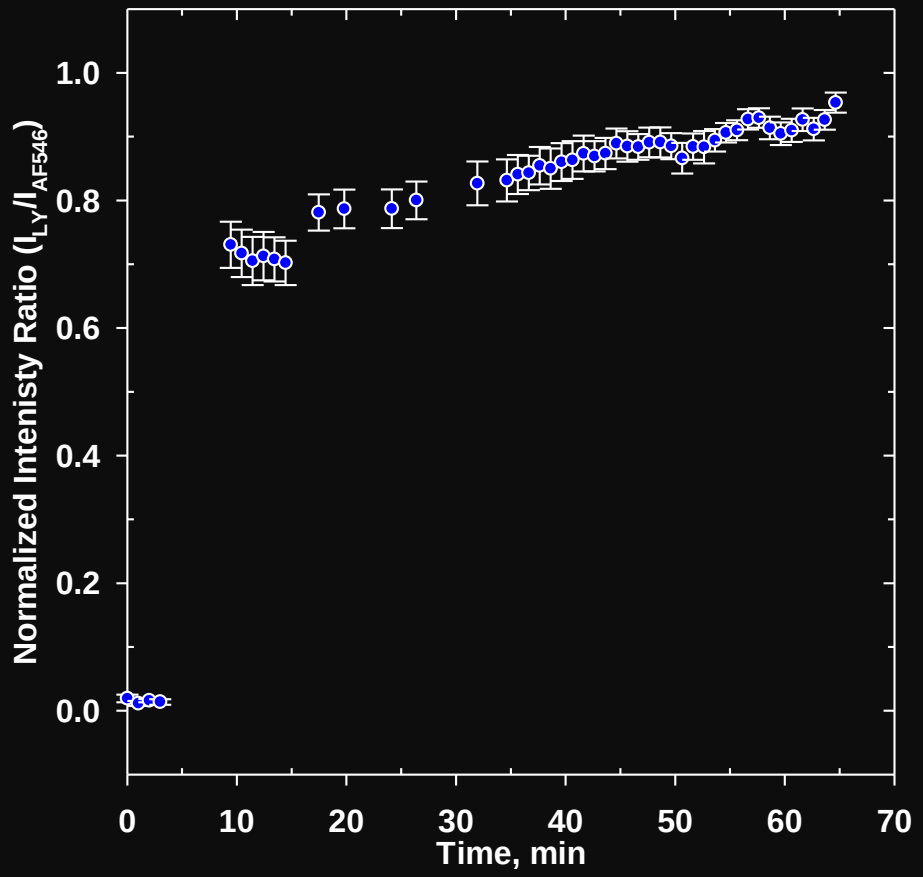


$$P_o = \frac{V}{\tau S}$$

$P_o \times 10^{-3}$ (n= 31 lysosomes)
mean ± sem, cm/s
5.1 ± 0.2

DIRECT QUANTIFICATION OF WATER TRANSPORT IN SUBCELLULAR ORGANELLES

DIRECT QUANTIFICATION OF WATER TRANSPORT IN SUBCELLULAR ORGANELLES



Permeabilization experiment n =13 lysosomes

Permeabilization experiment

RB in H_2O

↓

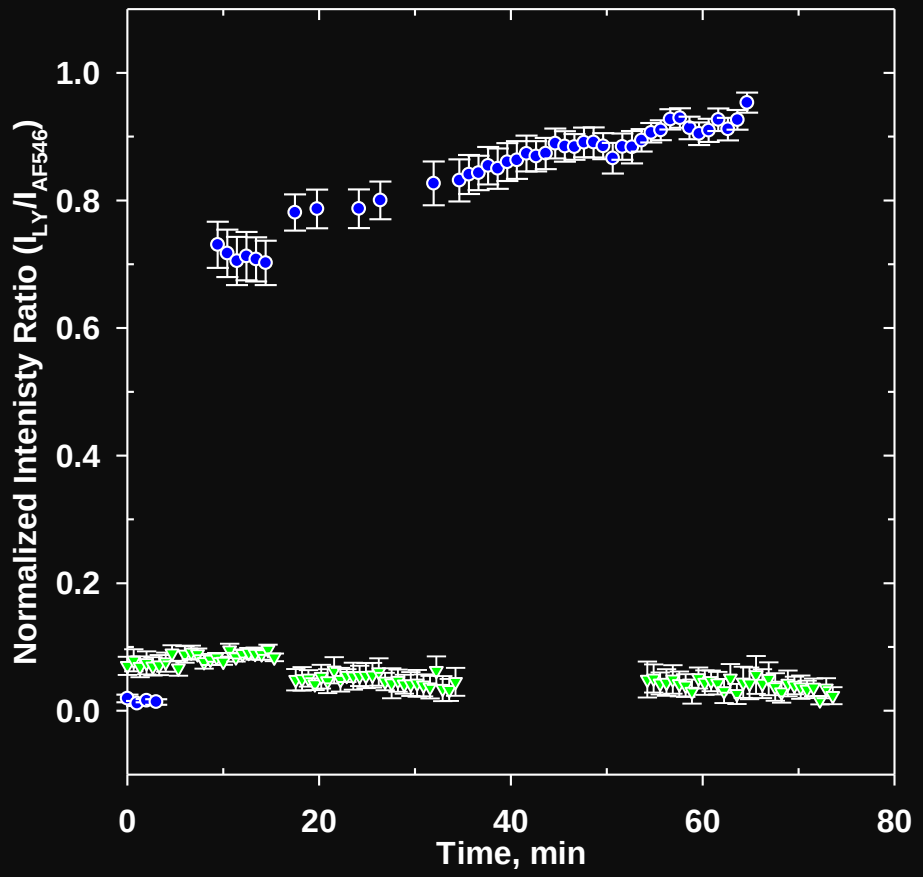
RB in D_2O

↓

RB in D_2O with 40 μg/ml Digitonin

Chaurra, A.; Gutzman. B.; Ackroy. C.; Christensen. K.; submited for publication to Journal of Membrane Biology 2010

DIRECT QUANTIFICATION OF WATER TRANSPORT IN SUBCELLULAR ORGANELLES



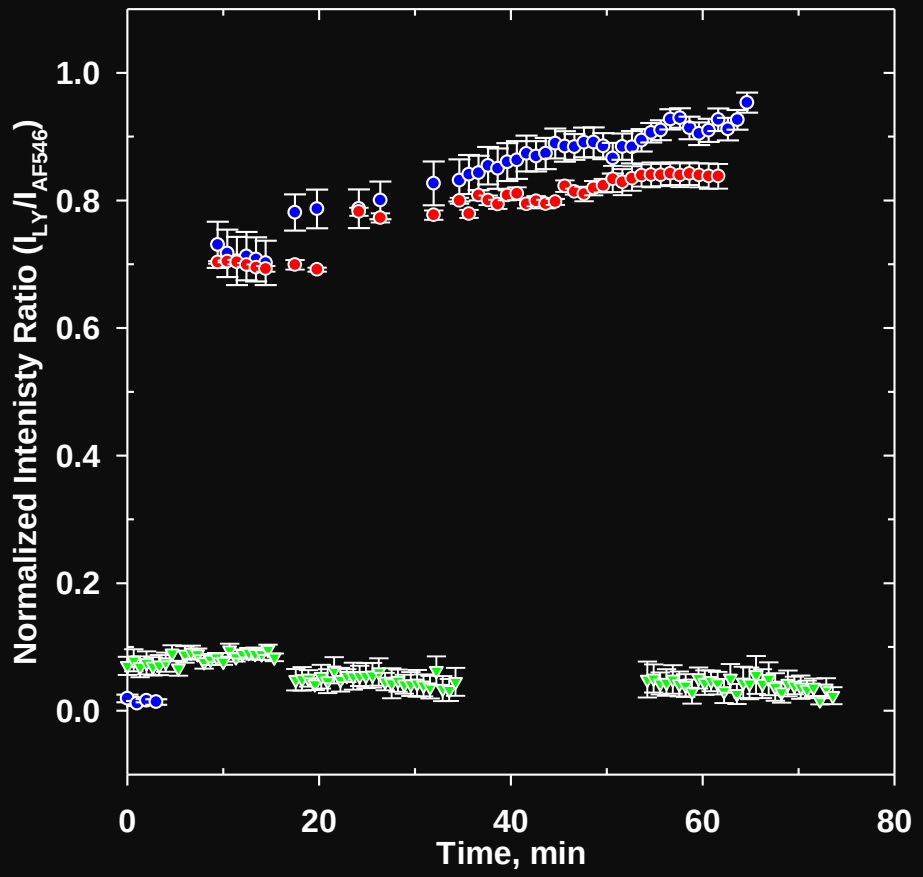
Permeabilization experiment n =13 lysosomes

Control experiment 2 n = 5 lysosomes

RB in H_2O

RB in D_2O with 40 μg/ml Digitonin

DIRECT QUANTIFICATION OF WATER TRANSPORT IN SUBCELLULAR ORGANELLES



- Permeabilization experiment — n =13 lysosomes
- Control experiment 1 — n = 8 lysosomes
- Control experiment 2 — n = 5 lysosomes

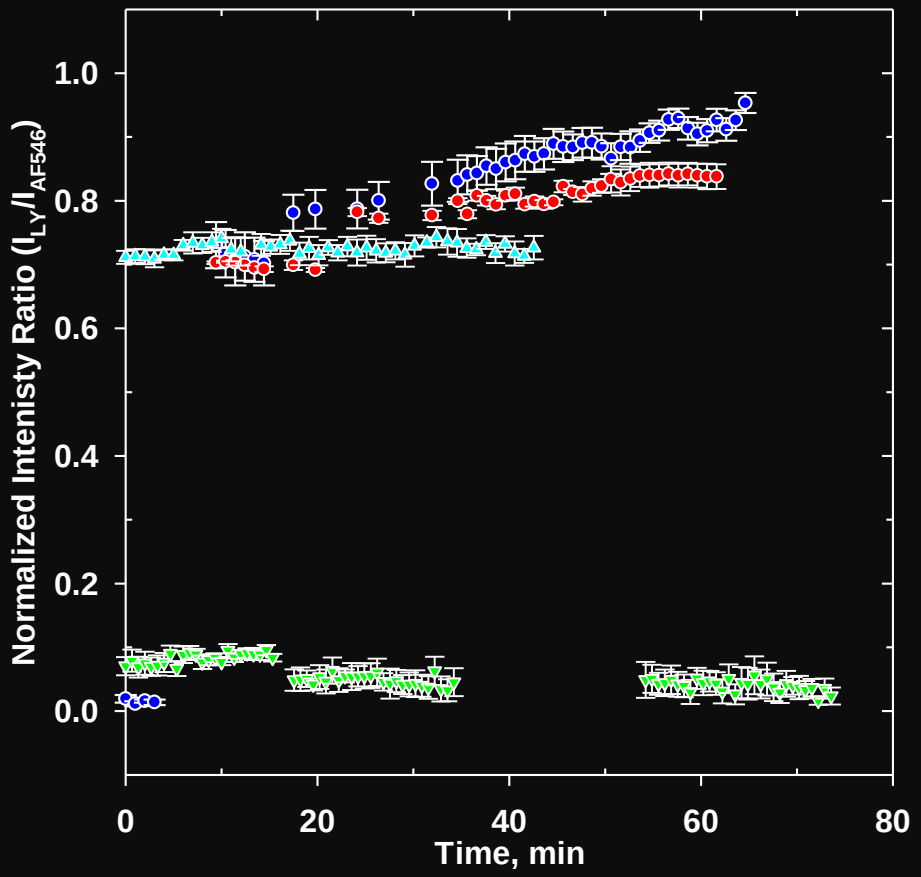
Permeabilization experiment

RB in D_2O

↓

RB in D_2O with 40 μg/ml Digitonin

DIRECT QUANTIFICATION OF WATER TRANSPORT IN SUBCELLULAR ORGANELLES



No Permeabilization

RB in D_2O

↓

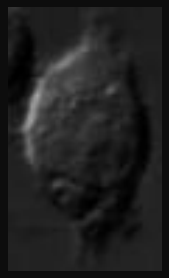
↓

RB in D_2O

Under equilibrium conditions, the exchange of water in lysosomes: 72± 2%.

In order to influx the remaining water into lysosomes, it is necessary to induce open equilibrium across the membranes.

LY AND ALF IMAGING IN J774 LYSOSOMES

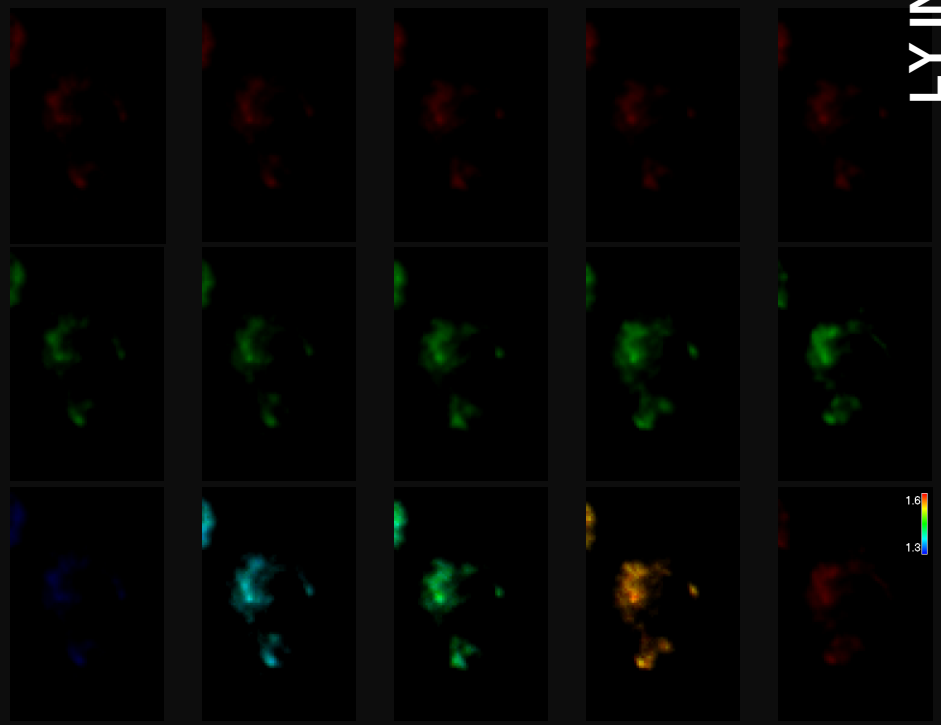


DIC

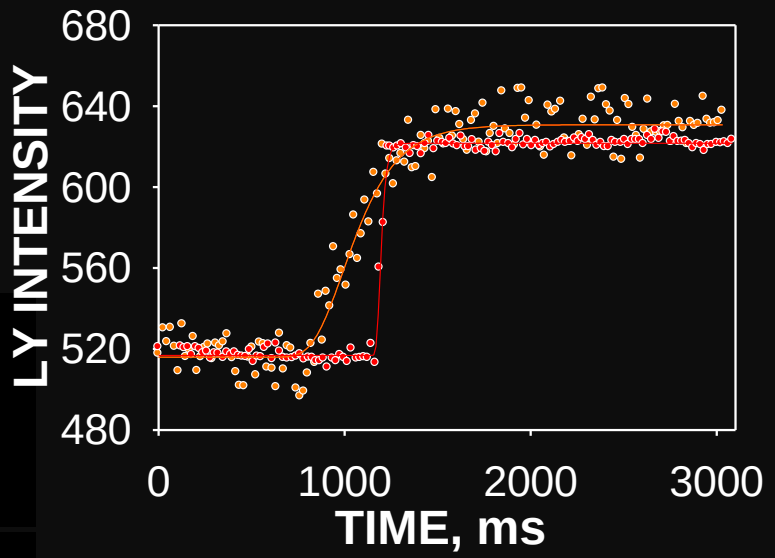
AF546

LY

RATIO



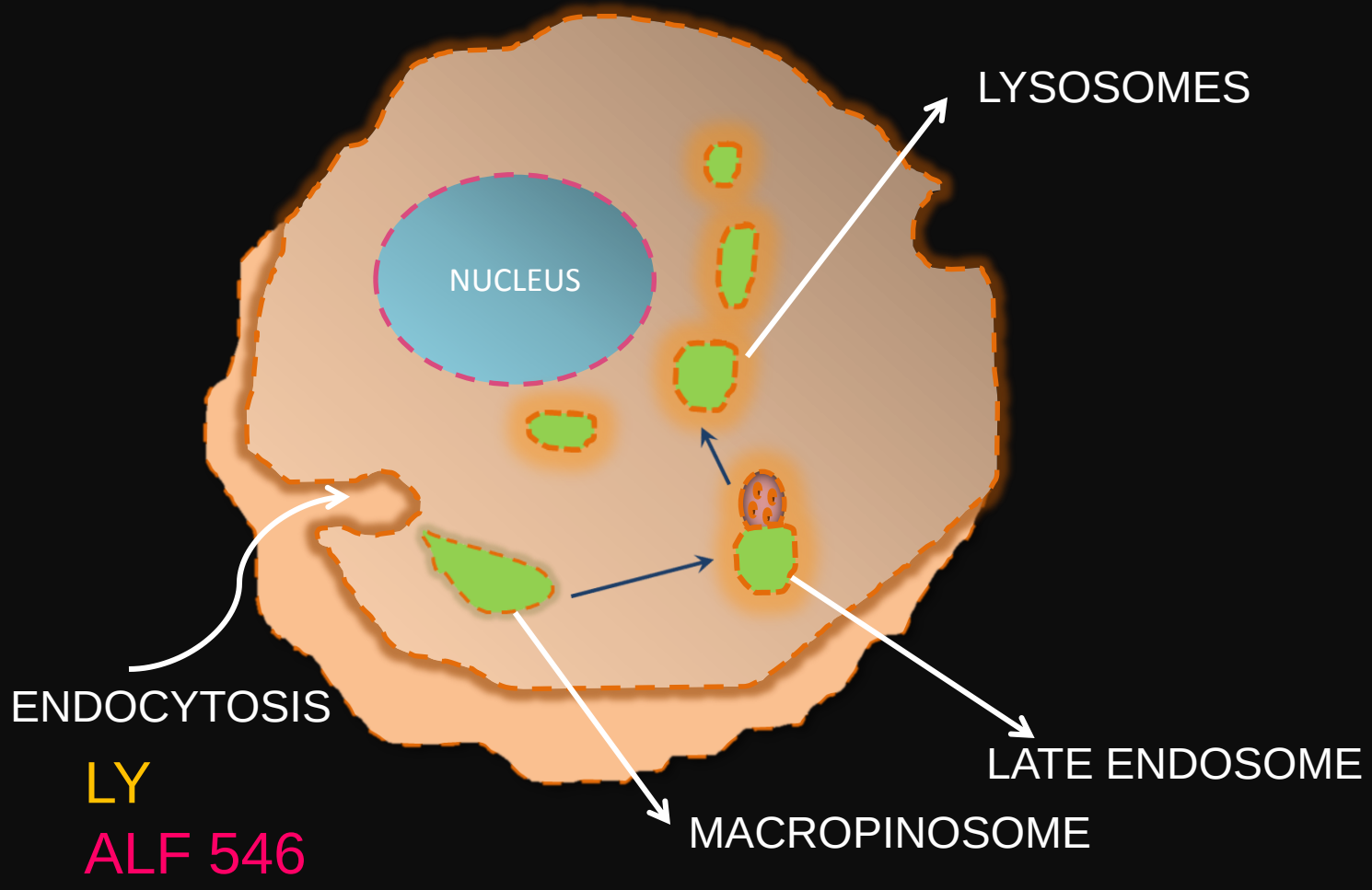
% D_2O 0 30 50 70 90



$$P_o = \frac{V}{\tau S}$$

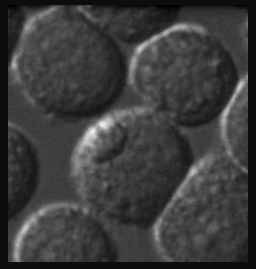
$P_o \times 10^{-3}$ (n= 62 lysosomes)
mean ± sem, cm/s
6.8 ± 0.2
RT

LY / ALF ENDOCYTOCIS

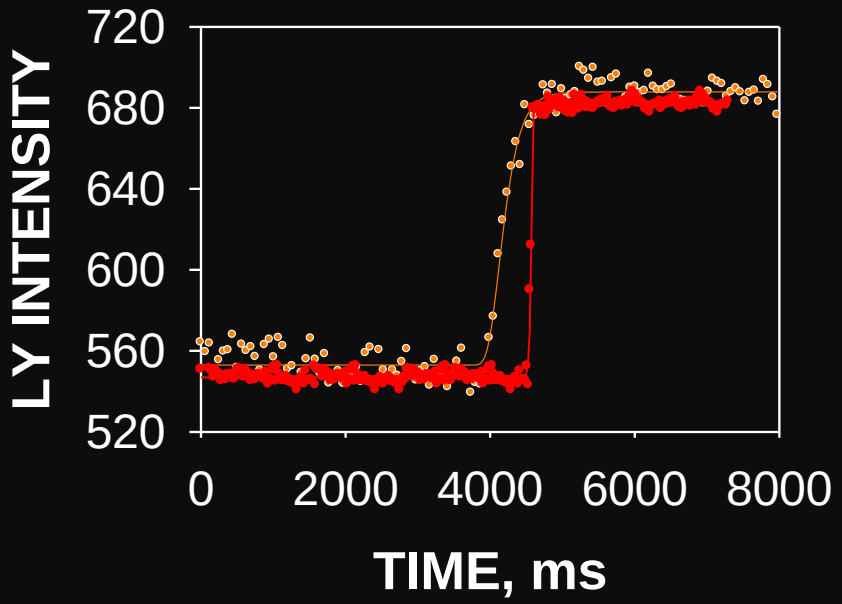


Mellman, I.; Fuchs, R.; Helenius, A. *Annu Rev Biochem* 1986, *55*, 663-700.

LY AND ALF IMAGING IN MACROPINOSOMES

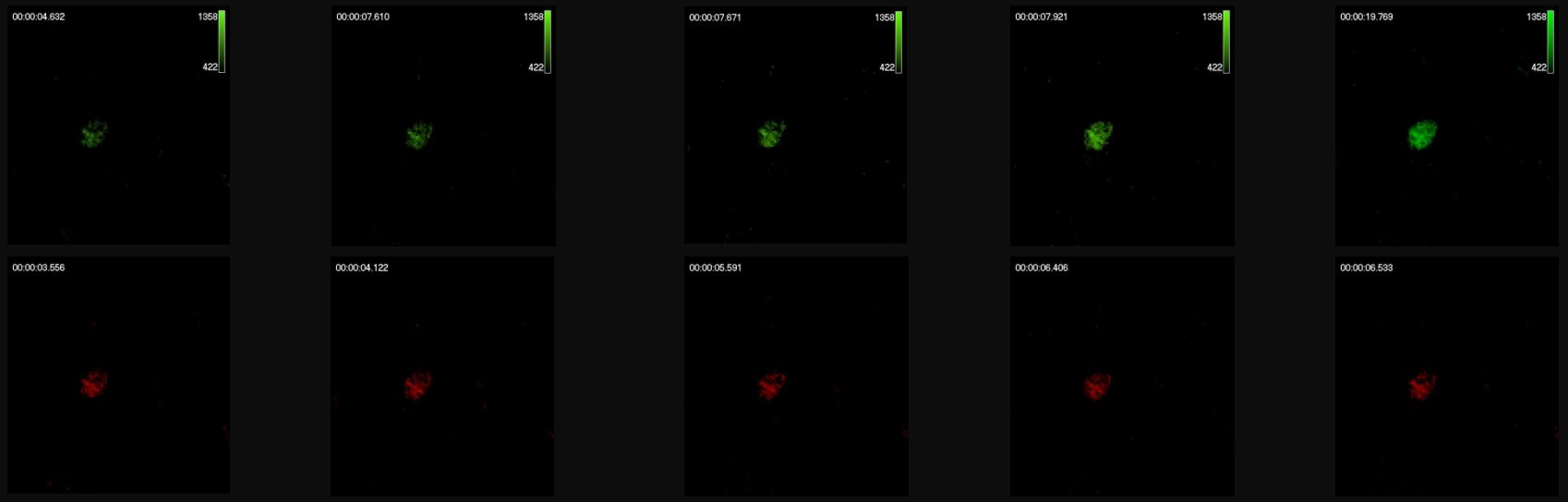


$$P_o = \frac{V}{\tau S}$$



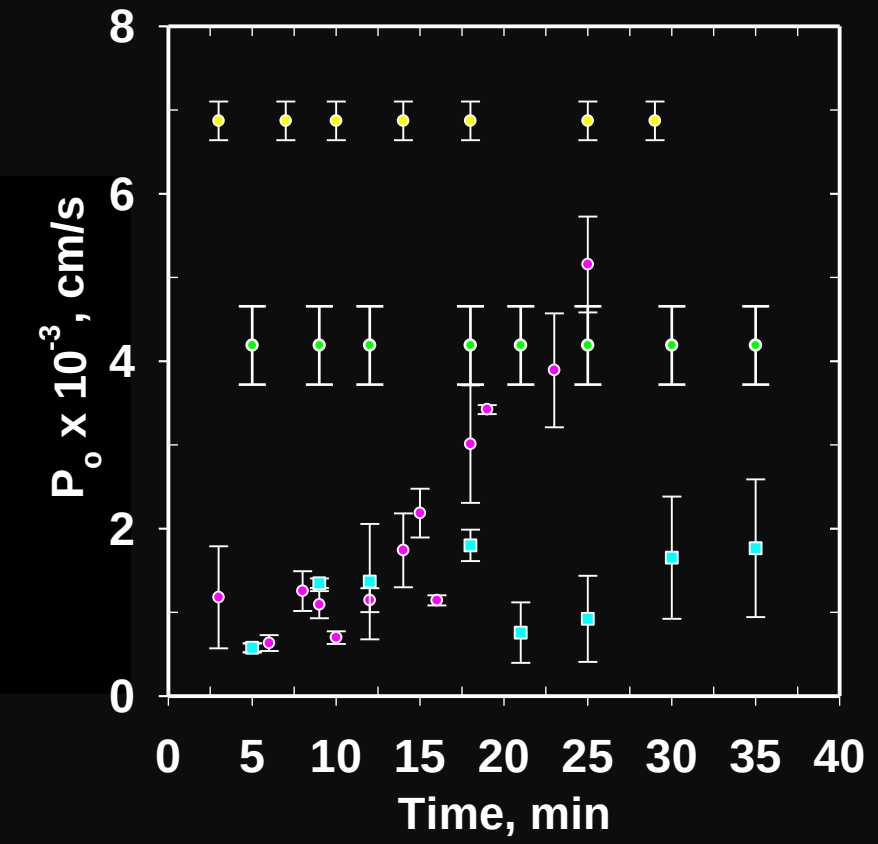
LY

ALF



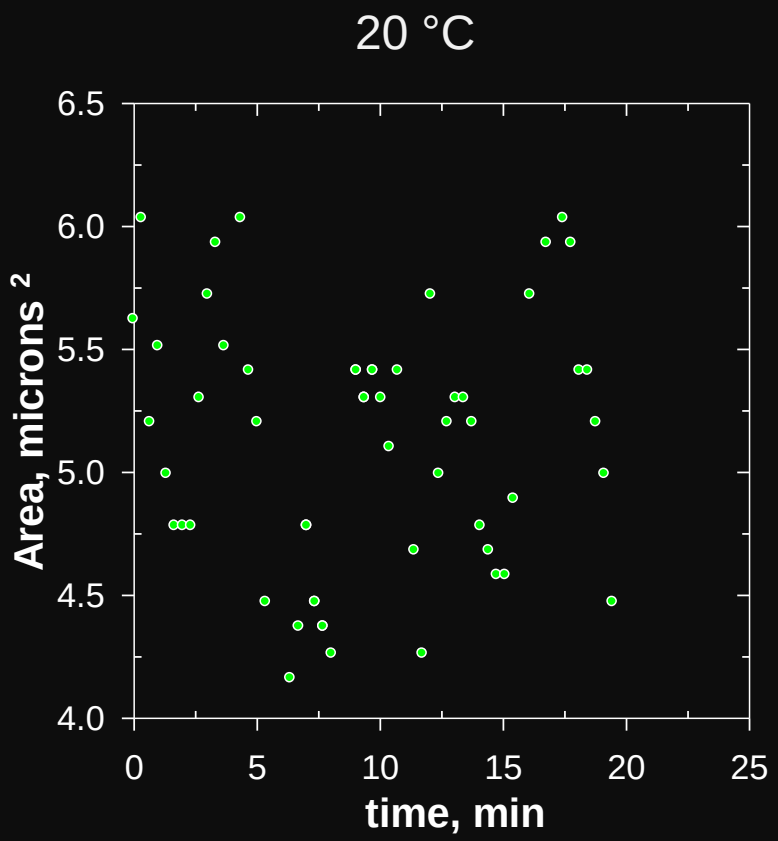
100%H_2O H_2O/D_2O H_2O/D_2O H_2O/D_2O 90%D_2O

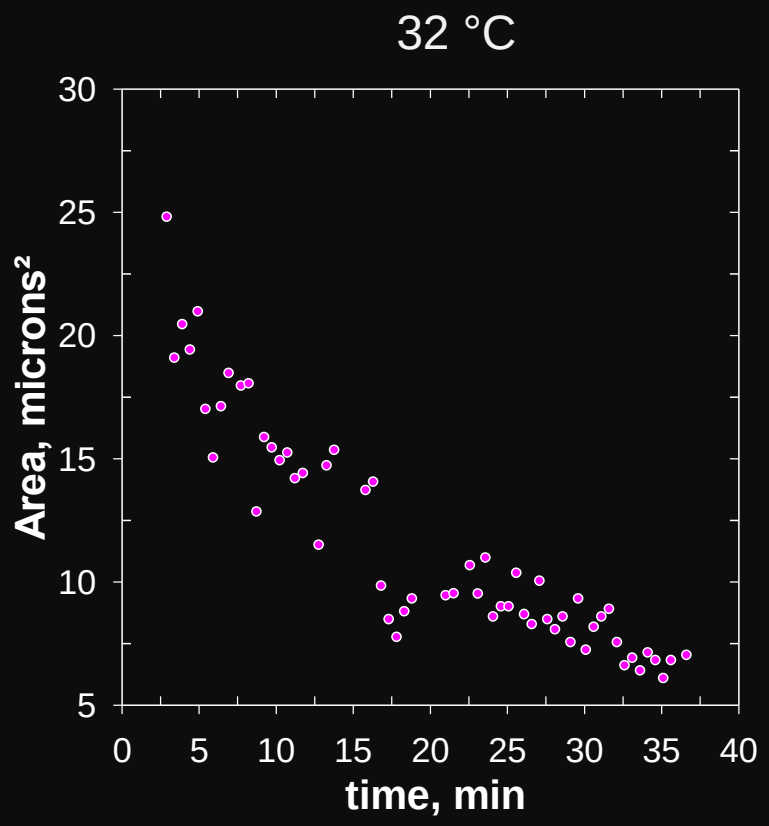
P_o THROUGH ENDOCYTIC PATHWAY



- macropinosomes 30 C n= 12
- lysosomes n= 64
- pinosomes room temperature n=10
- lysosomes 32 ^{0}C n=10

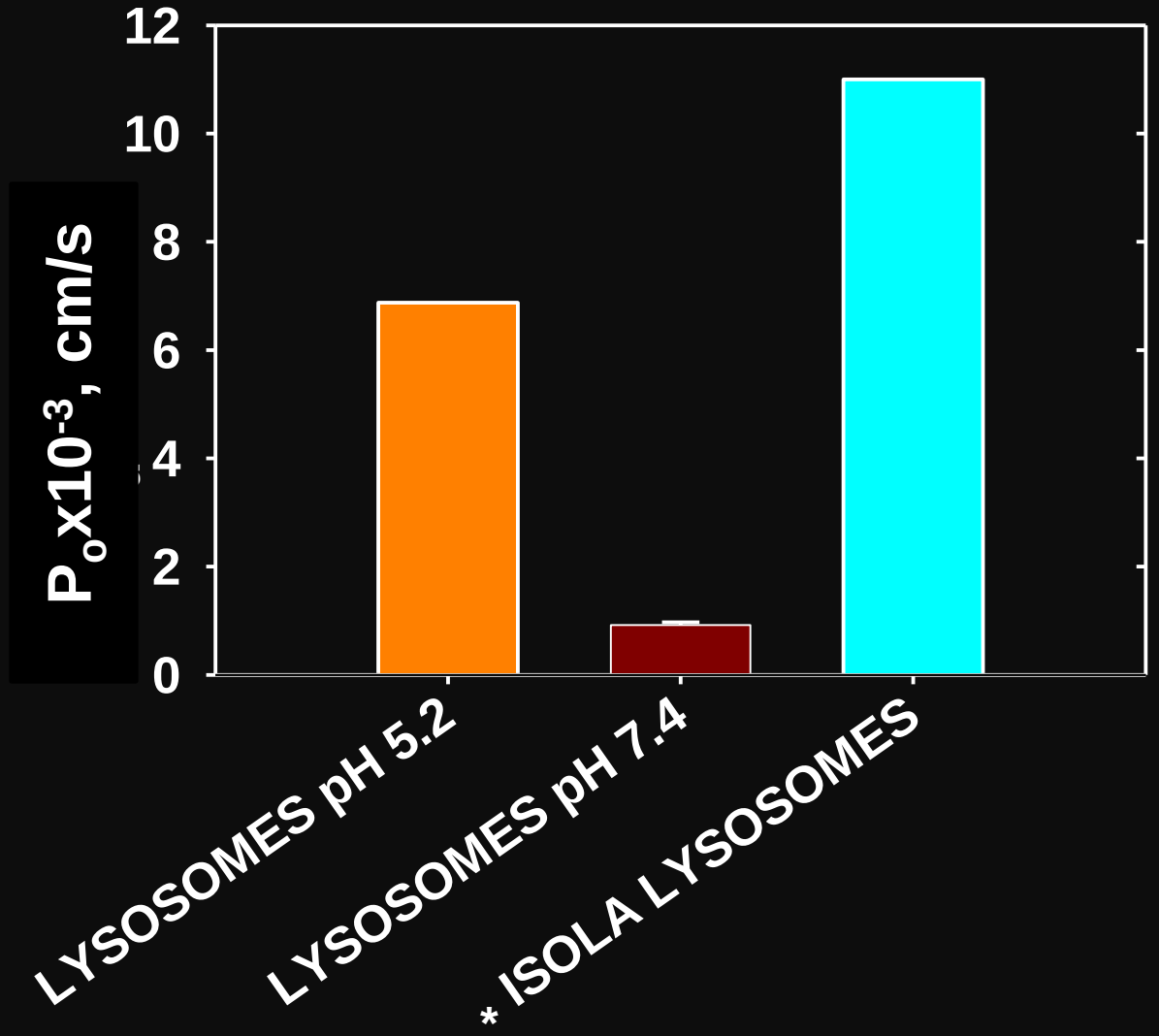
CHANGES IN MACROPINOSOME VOLUMEN





Racoosin, E. L; Swanson, J. A. ***J. Cell Bio.*** **1993, 121, 1011-1020**

WATER FLUX IN LYSOSOMES



At higher pH, water exchange across the lysosome membrane is slower than at natural pH.

*** Piqueras, A. I.; Somers, M.; Hammond, T. G.; Strange, K.; JR., H. W. H.; Gawry, M.; Zeidel, M. L. *Am. J. Physiol. Cell Physiol.* 1994, *266*, C121-C133.**

WATER TRANSPORT MECHANISM IN ENDOCYTIC ORGANELLES

Two possible explanations for our results are:

1. **Water transport in lysosomes is regulated through some unknown mechanism present in live cells. (AQP?)**
2. **In turn, in macropinosomes, water flux follows a diffusional mechanism or the flux of water is preferential from inside to outside.**
2. **Temperature plays an important role in macropinosome maturation and maturation increases water exchange in endocytic organelles.**

Nemeth-Cahalan, K.; Hall, J. *J. Biol. Chem.* 2000, *275*, 6777-6782.
Yasui, M.; Kwon, T.; Knepper, M.; Nielsen, S.; Agre, P. *PNAS* 1999, *96*, 5808-5813.

AQP DETECTION

AQP DETECTION

m-RNA isolation

↓

First strand DNA

↓

DNA amplification (PCR)

↓

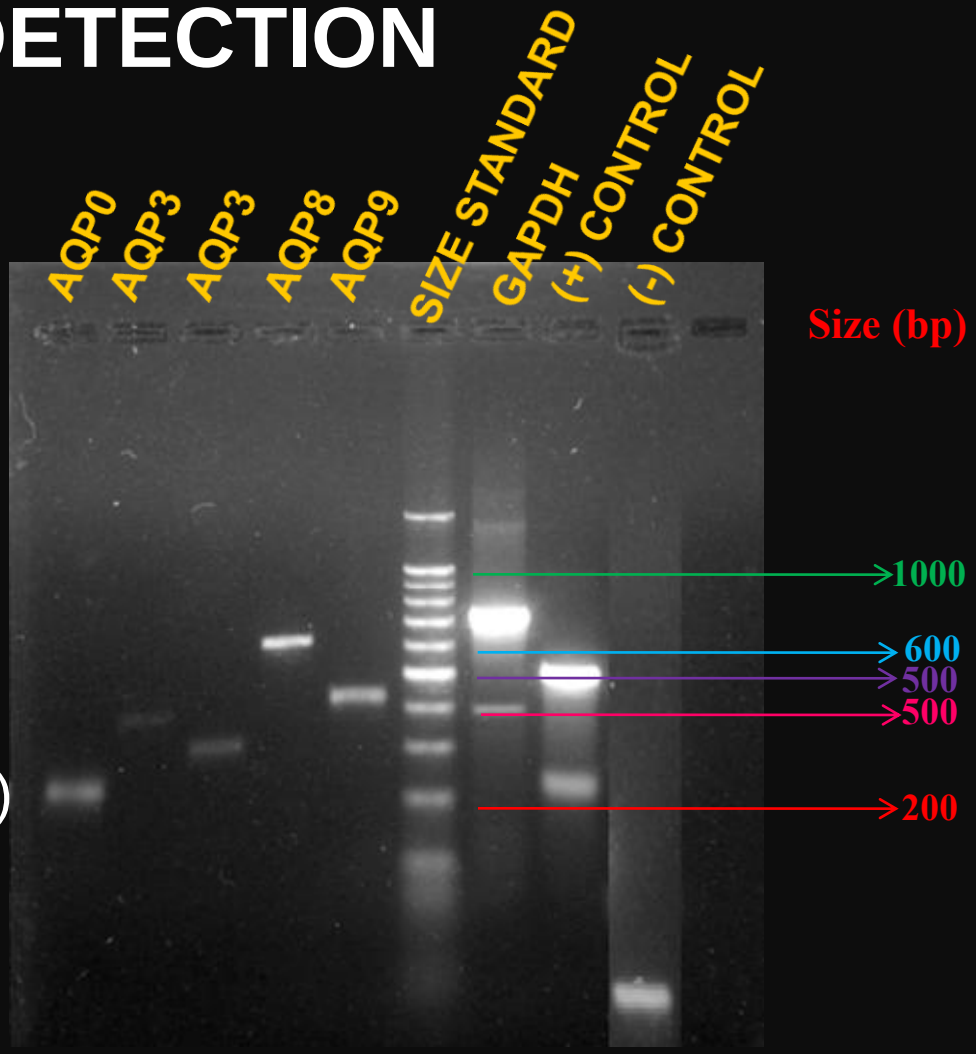
DNA identification (agarose gel)

↓

DNA sequencing

↓

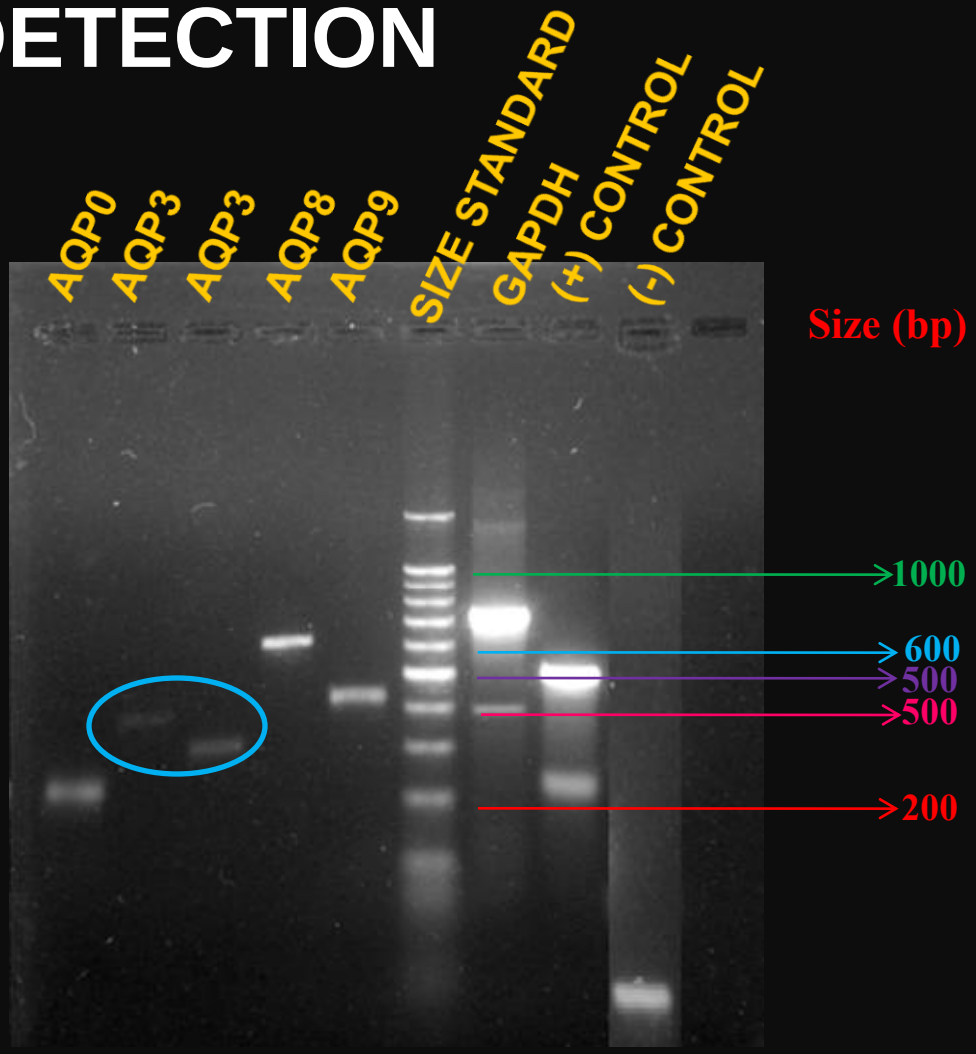
Identity confirmation (BLAST)
98 - 99.7% identity



AQP0 - AQP9 were tested

AQP DETECTION

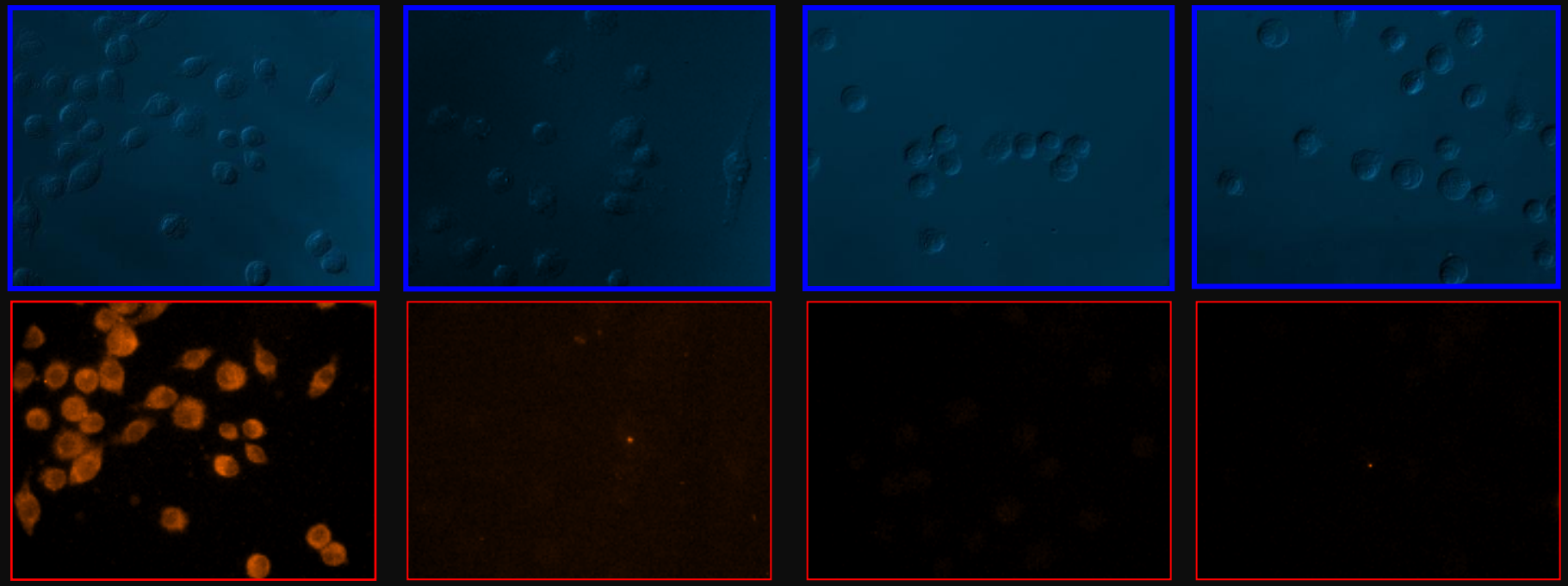
AQP	Amplified size, bp	m-RNA
0	245	+
3	398	+
8	610	+
9	432	+



AQP0 - AQP9 were tested

AQP9 INMUNOFLUORESCENCE IN J774 CELLS

PRIMARY ANTIBODY : mouse monoclonal anti- AQP9
SECONDARY ANTIBODY: Rabbit anti-mouse Texas Red

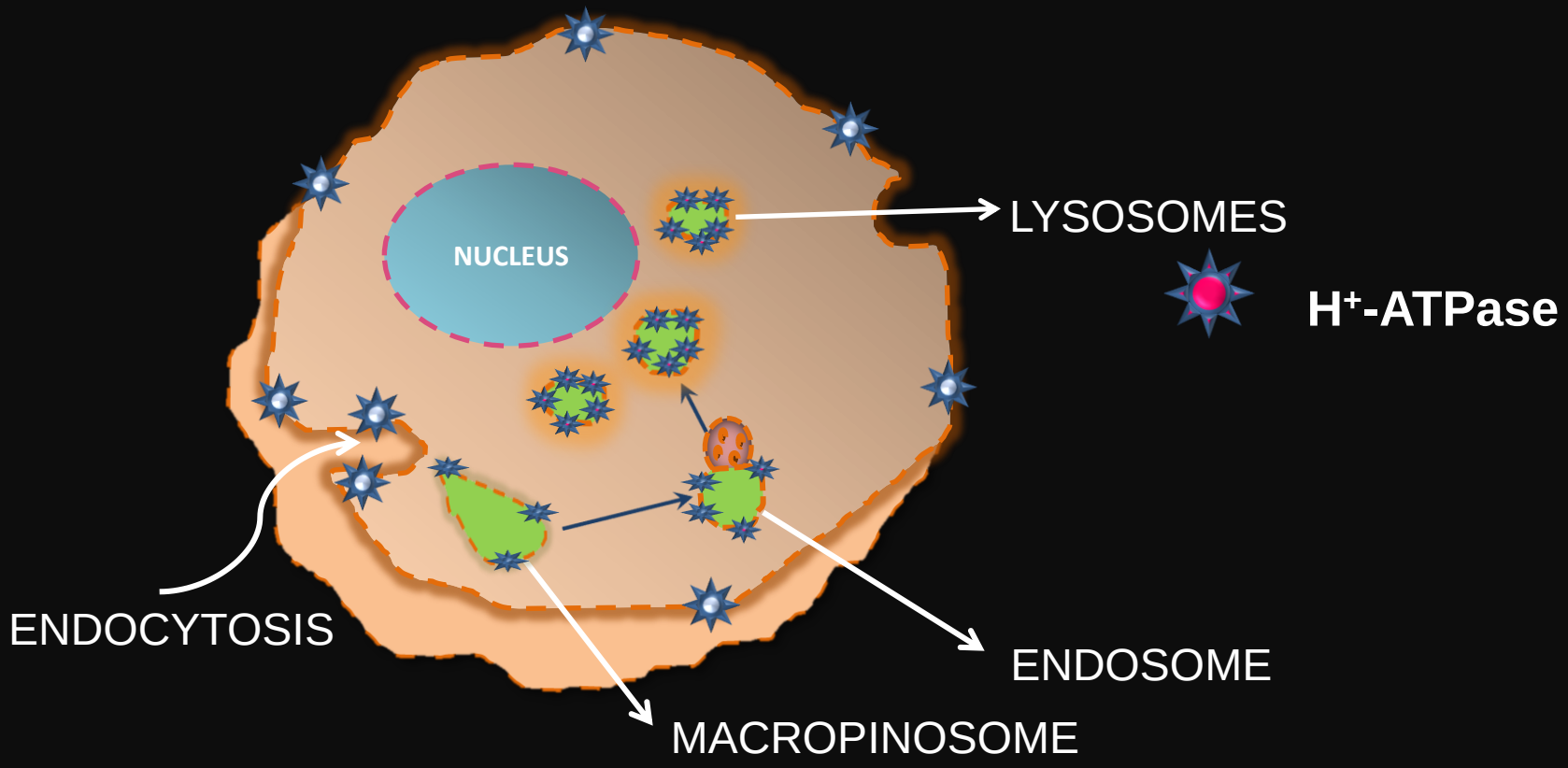


1:10 Primary and 1:50 Second antibody	CONTROL 1:10 Primary antibody	CONTROL 1:50 Secondary antibody	CONTROL CELLS

Gradilone, S. A.; Tietz, P. S.; Marinelli, N. F.; LaRusso, R. A. *Biomedical Central Physiology* 2005, *5*, 2-8

PROPOSED MODEL

Proton pump H^+-ATPase may play an important role in water transport in organelles of the endocytic pathway.



Clarke, M.; Kohler, J.; Arana, Q.; Liu, T.; Heuser, J.; Gerisch, G. *Journal of Cell Science*, 2002, 115, 2893-2905