A PYTHON TOOLKIT FOR EXPERIMENTAL AND COMPUTATIONAL STUDIES OF WATER TRANSPORT THROUGH CHANNELS

Gerardo Zerbetto De Palma[1,2,3], Agustina Canessa Fortuna[1,2], Úrsula Ramoa[1,2], María Victoria Espelt[2], Karina Alleva[1,2]

[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Cátedra de Física, Buenos Aires, Argentina.
[2]Universidad de Buenos Aires-CONICET, Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini", Buenos Aires, Argentina.
[3]Universidad Nacional de Hurlingham, Instituto de Biotecnología, Villa Tesei, Argentina.

INTRODUCTION: PROBLEM

EXPERIMENTAL APPROACH

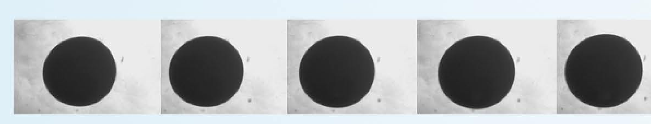
Experimental approaches on water transport through channels involve the measurement of a signal that is related to a volume change of an spherical compartment over time.

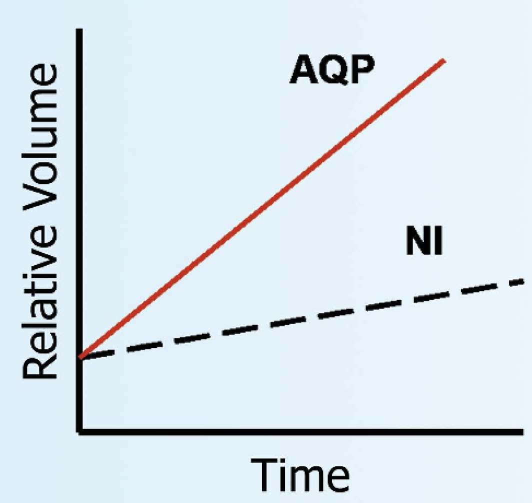
Volume changes are often calculated from projected areas by manually processing stacks of still images of the compartment.

Available open source alternatives cannot perform real time processing.

Computation are frequently done after the experiment, preventing decision making during the experiment.

Multiple-sphere image stacks consume even more time to be processed because extra manual processing is needed.

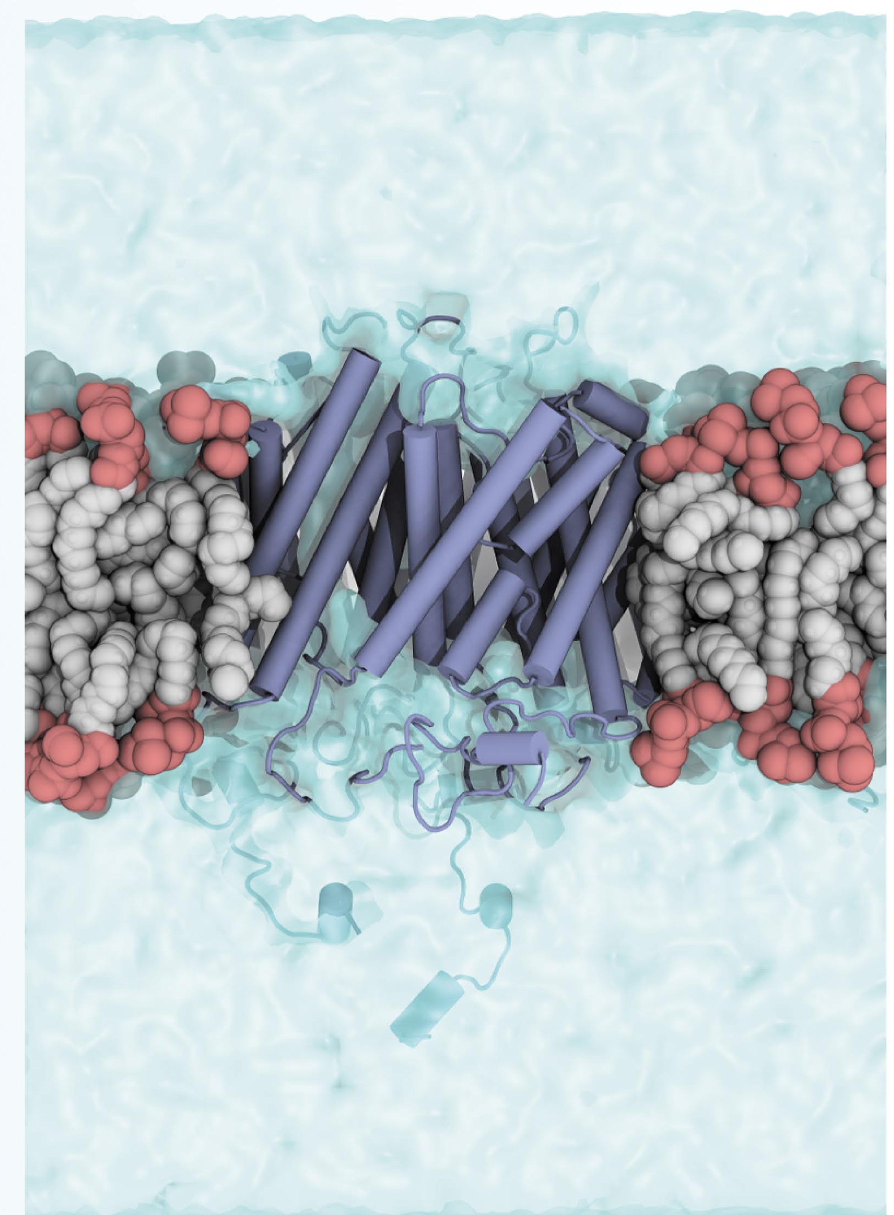




$$P_f = \frac{\frac{\Delta V}{\Delta t}}{V_w \cdot S \cdot \Delta\Pi}$$

Oocyte swelling experiment workflow. Heterologous expression of water transport protein is achieved by microinjecting Xenopus laevis oocytes with coding RNA. 72 hours after injection, water transport capabilities are tested with a swelling experiment. In this experiment, oocytes are challenged to a hipoosmotic shock and image stacks are recorded during the swelling. After the experiment, images are processed to calculate the relative volume over time and, finally, to compute the osmotic permeability coefficient of the membrane.

COMPUTATIONAL APPROACH



Computational methods include analysis of trajectories obtained from equilibrium molecular dynamics simulations (MD).

Useful data, such as intrinsic osmotic permeability coefficient (pf), potential of mean force (PMF) and permeation events, can be extracted from MDS.

Theory behind this calculations is well documented but implementations are not widely distributed.

Available MDS analysis do not perform these calculations by default.

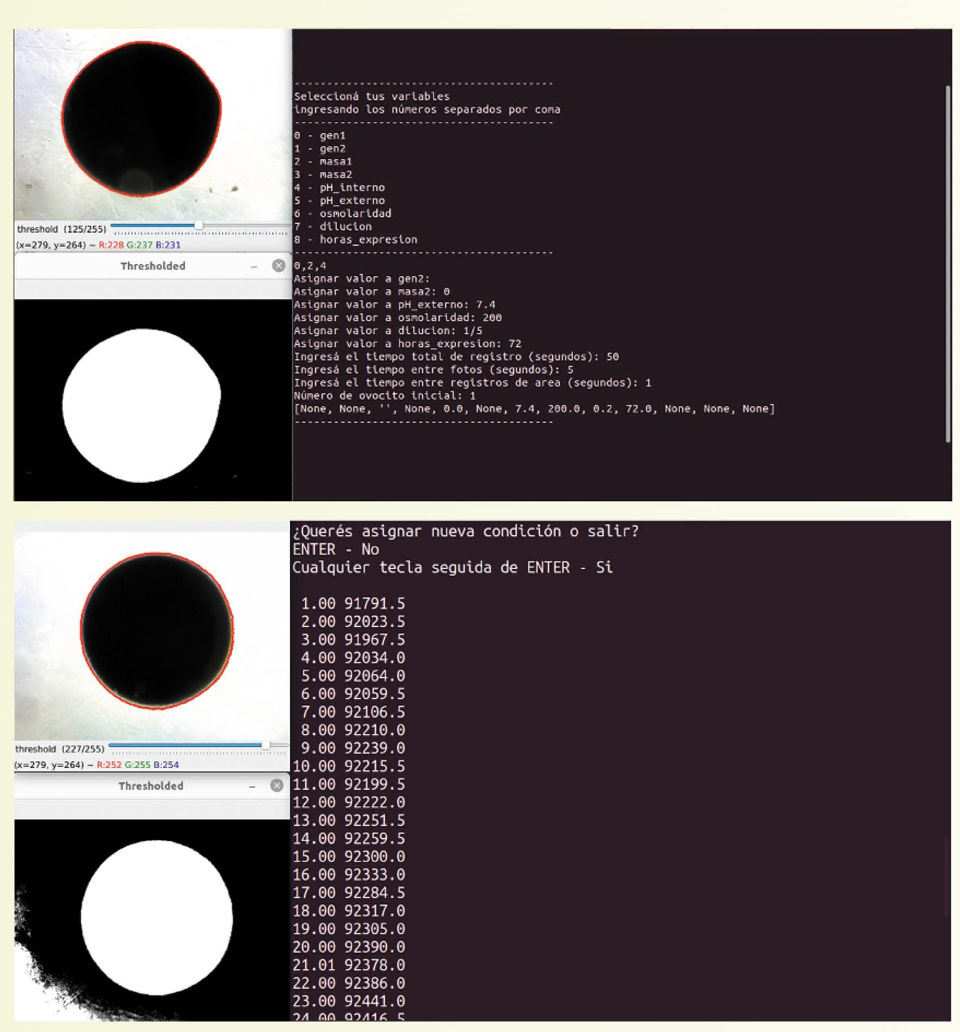
METHODS: TACKLING THE PROBLEM

A solution was implemented in three main tools: a stream/acquisition tool, an area record analysis tool and an image stack analysis tol. These are mainly intended for use in oocyte swelling experiments, but can be modified for other applications.

Stream/acquisition tool provides live camera streaming and real time image processing (thresholding and projected area change calculation), capable of recording both raw images and projected area data.

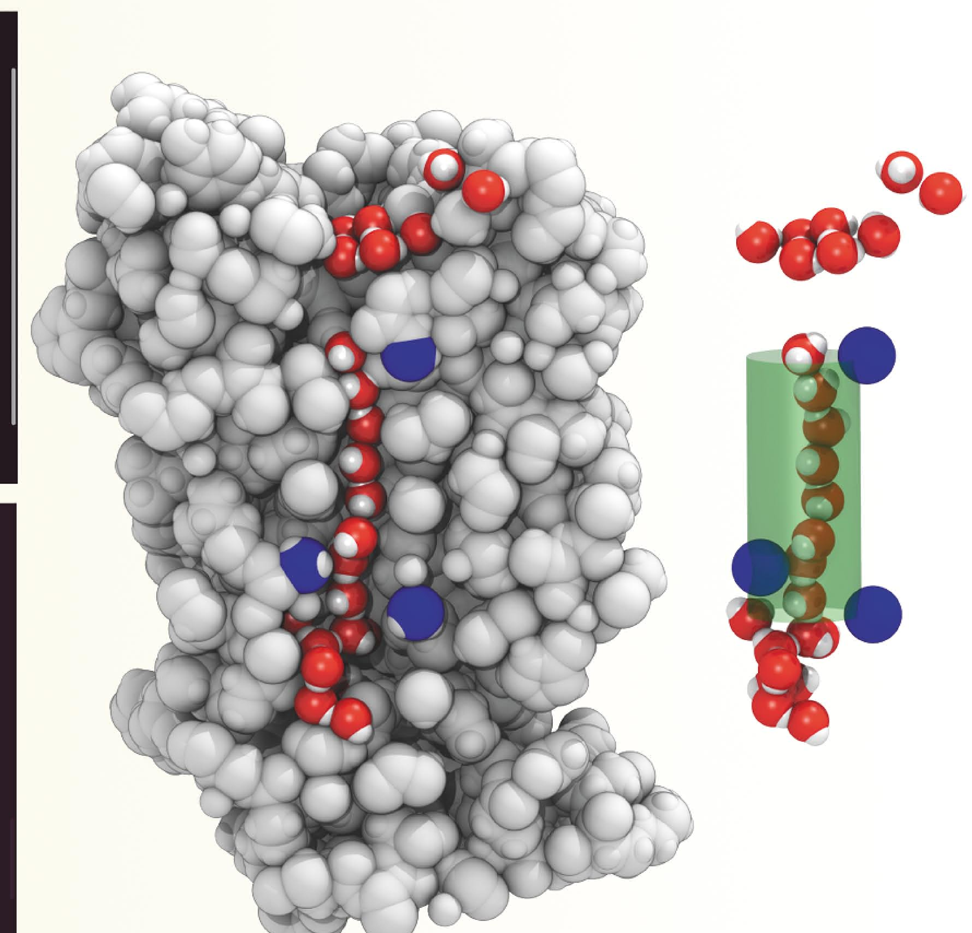
Area record analysis tool can use the area record as an input to compute osmotic permeability (Pf) during the experiment.

Image stack analysis tool was built for *a posteriori* image processing (thresholding and area calculation), including multiple cell swelling stacks.



Upper panel. Stream/acquisition tool interface. Two windows show the camera live stream: one to show the actual image and the other to show the result of the real time thresholding. A terminal window provides user interface to set variables and parameters that are recorded alng with the area record.

Lower panel. A red contour is overimposed in the actual image to provide a guide with the thresholding adjustment. A slider is provided to adjust this thresholding setting. Areas and times are shown in the terminal window while program is recording data.



Particle tracking inside the pore is carried out by discarding particles outside the pore in each frame. The pore boundaries are modeled from 3 carefully selected reference atoms (blue). One pair of reference atoms is used to obtain the pore xy-center and other pair defines the height of the cylinder. Radius is a user-defined parameter.

PMF, pf and permeation events calculations were implemented in NETCDF trajectories but can be adapted to use other formats.

All three calculations are based on particle tracking inside a cylinder, which allows to obtain particle density and displacements inside the cylinder. PMF, pf and permeation events are computed from this data.

Input trajectory requirement: 3 reference atoms (defining the cylinder) and water molecules.

TESTS & RESULTS

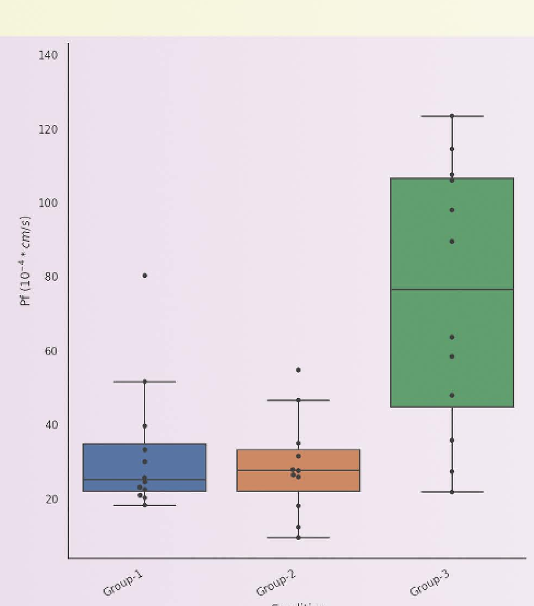
Immediate follow up of the experiment achieved by combining stream/acquisition and analysis tool.

Decreased image stack processing timings after the experiment by using the image processing tool.

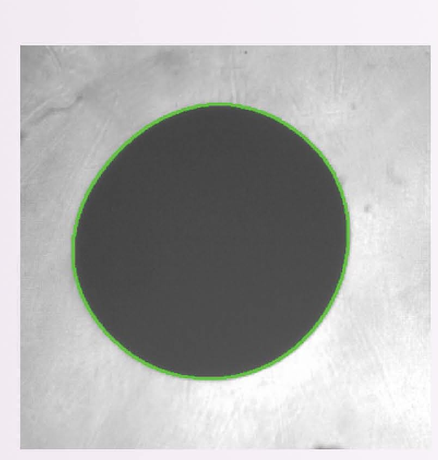
Automatic cell tracking in single and multi-cell image stacks.

Increased time availability to clean up and filter anomalous images.

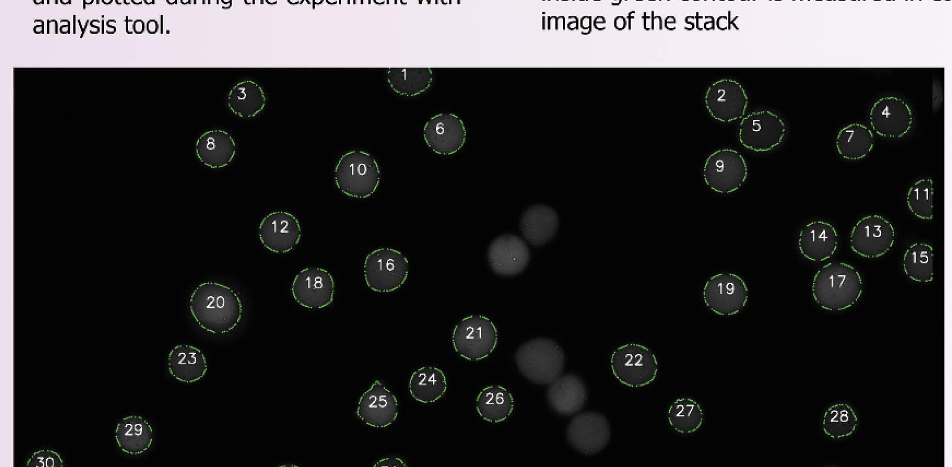
Shared code at Github



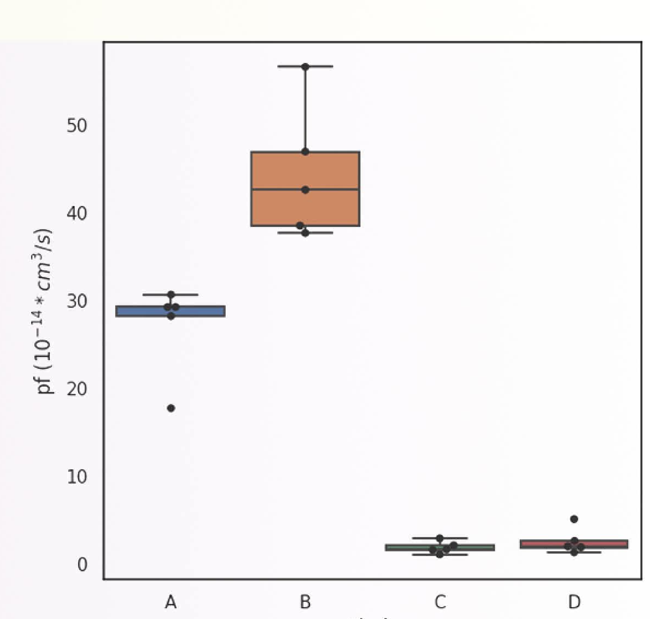
Experimental data obtained with the stream/acquisition tool and processed and plotted during the experiment with analysis tool.

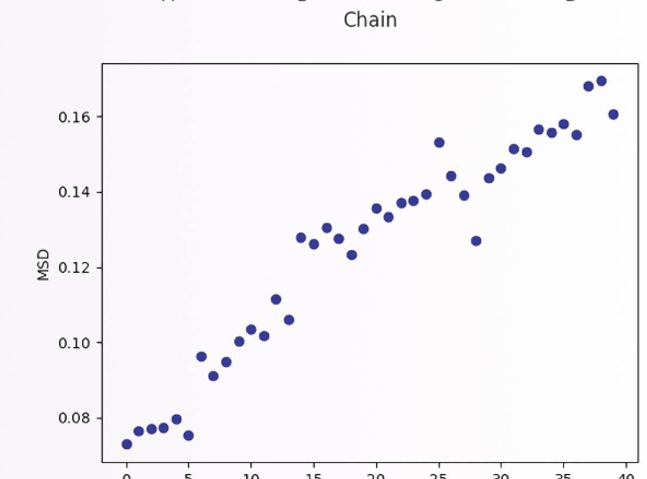


Thresholded image processed by automatic image processing tool. Area inside green contour is measured in each image of the stack



Thresholded multi-cell image processed by automatic processing tool. Each cell is tracked over time in each frame of the stack. Cells are filtered by an adjustable circularity criterion.





Upper panel. Example of *pf* calculation results. A MD was carried out in an aquaporin tetramer which has two protomers in open state and two in closed state. *pf* calculation was executed in all four chains, resulting in an increased *pf* in the open chains.
Lower panel. Mean squared displacements (MSD) of water file inside the pore is obtained during the process of *pf* calculation. As linear regression can be performed, *pf* calculation is validated.

PMF, *pf* and permeation events calculations were effectively implemented in a sharable code. *pf* calculation was tested in open and closed channels models, resulting in a clear difference beetween those.

Shared code at Github

Acknowledgements:
Funding: PICTO-UNAHUR-2019-00023
PICT-2017 0244
PICT-2019 00387
UBA2018 20020170100178BA

Contact: