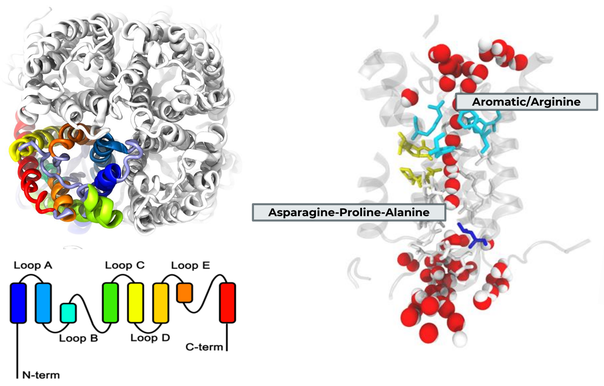
Role of prolines in PIP2 aquaporin gating mechanism

Canessa Fortuna, A[1,2], Zerbetto De Palma. G[1,2,3], Vitali, V[1,2], Zeida, A[4], Alleva, K[1,2]

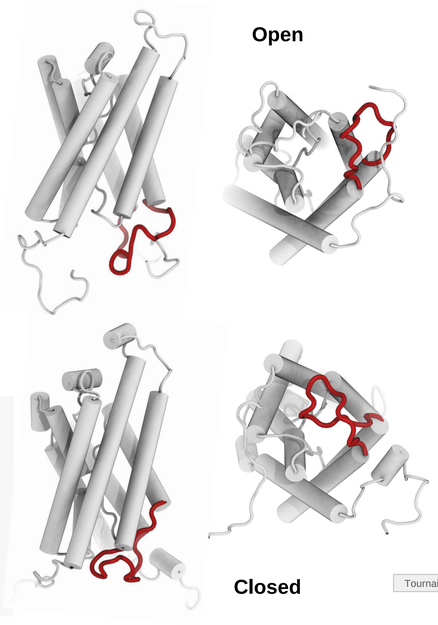
[1]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Cátedra de Física, Buenos Aires, Argentina. [2]Universidad de Buenos Aires-CONICET, Instituto de Química y Fisicoquímica Biológicas "Prof. Alejandro C. Paladini", Buenos Aires, Argentina. [3]Universidad Nacional de Hurlingham, Instituto de Biotecnología, Villa Tesei, Argentina. [4]Departamento de Bioquímica, Facultad de Medicina y Centro de Investigaciones Biomédicas (Ceinbio), Universidad de la República, Montevideo, Uruguay

PIP structure



- AQP quaternary structure is tetrameric.
- Each monomer is composed of six transmembrane alpha-helices and five connecting loops with N and C-terminal domains in the cytoplasmic side
- Each monomer forms a functional pore for water transport
- AQP present two conserved regions that regulate the specificity of transport: the Asn-Pro-Ala (NPA) motives and the aromatic/arginine (Ar/R) selectivity filter

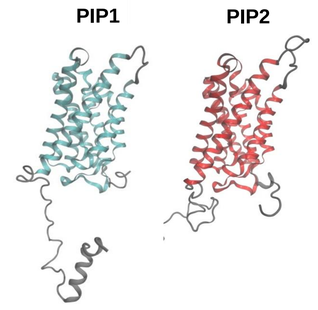
pH gaiting in PIP

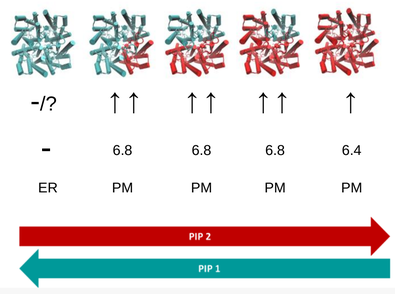


- PIP aquaporins are inhibited after intracellular acidification
- pH gating in PIP aquaporins involves N-term, loopB, loopD and C-term:
  - The protonation of a conserved histidine stabilize the closed conformation by interacting with two conserved residues of N-term
  - The insertion in the pore of a conserved leucine of loopD is crucial for pore blockage
  - The length and charge of C-term regulate the pH sensing by modifying the pKa of the histidine

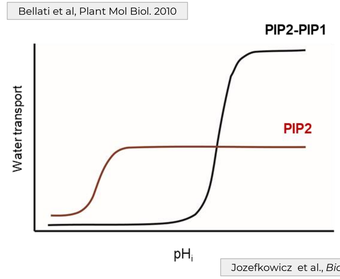
Tournaire-Roux et al, Nature 2003 | Canessa Fortuna et al., *FEBS J* 2019 | Scochera et al., *FEBS J* 2021

PIP1 and PIP2 paralogs





Bellati et al, Plant Mol Biol. 2010

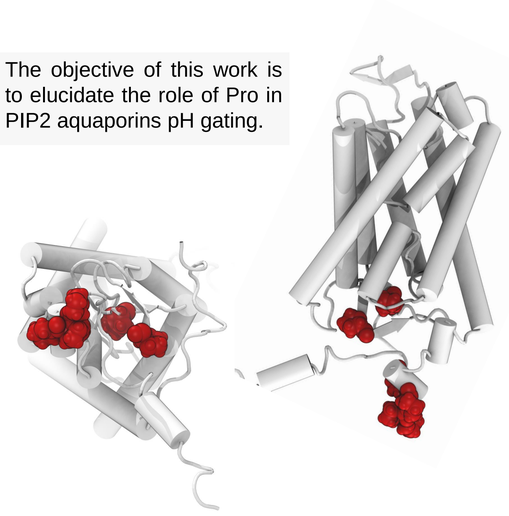


Jozefkowicz et al., *Biophys J* 2016

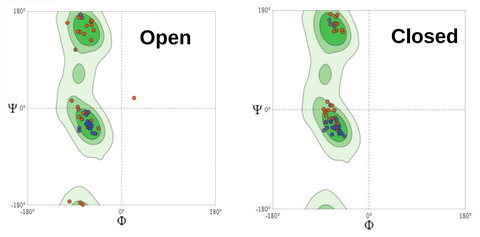
- PIP subfamily is composed by two paralogues: PIP1 and PIP2
- PIP2 localizes in the plasma membrane forming homotetramers
- PIP2 translocates PIP1 to the plasma membrane by forming heterotetramers
- In some PIP1-PIP2 pairs, the permeability to H_2O is modified if PIP2 alone is expressed in the membrane or a PIP1-PIP2 pair
- The response to changes in intracellular pH is modified if PIP2 alone or the PIP1-PIP2 pair are expressed in the plasma membrane

Prolines in PIP2

The objective of this work is to elucidate the role of Pro in PIP2 aquaporins pH gating.



N-term



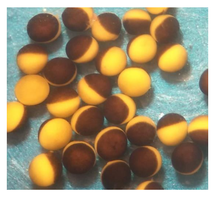
LoopD

There are 6 conserved Pro in PIP2: 4 in N-terminal forming a PPPAP motive and 2 in loopD in hinge positions for the loop movement.

Cis-Pro	
Asp 23 – Pro 24 Chain A	Pro 25 – Pro 26 Chain A, B, C, D

Methodology

Heterologous expression in *Xenopus laevis* oocytes

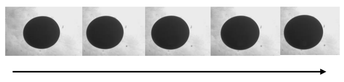


Xenopus laevis oocyte injection with cRNA

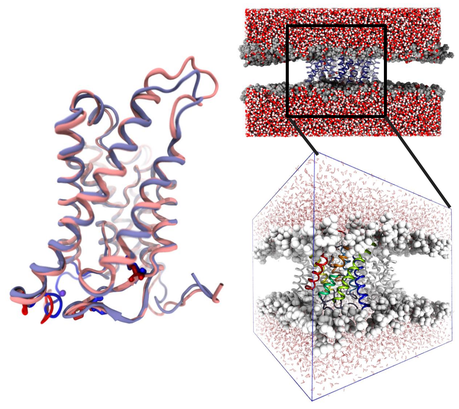
↓ 72 hours

Protein expression

Swelling experiment

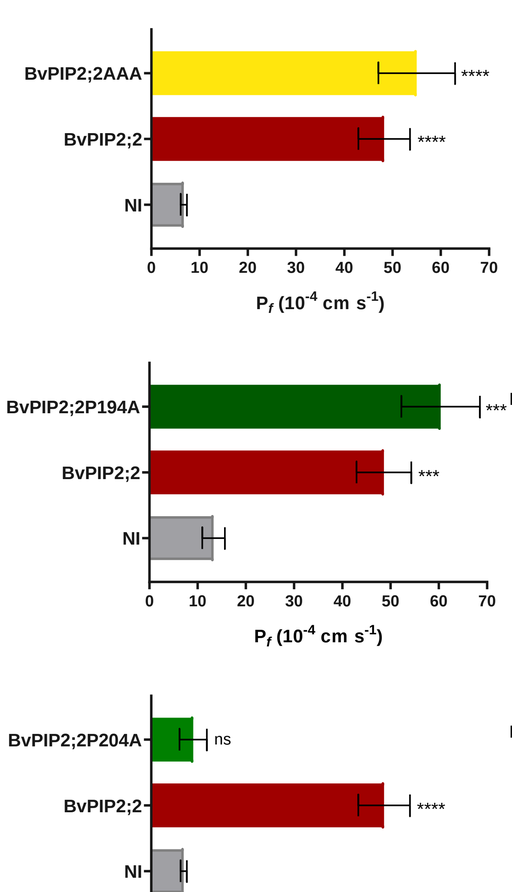


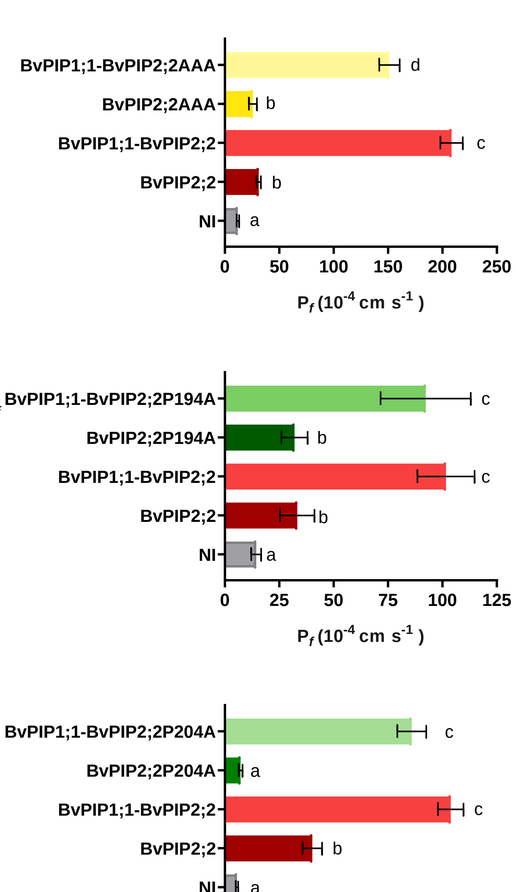
Atomistic Molecular Dynamics Simulations

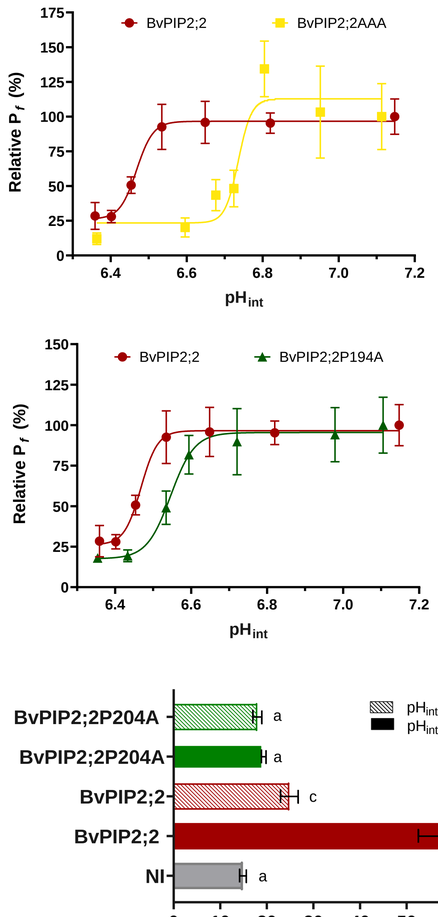


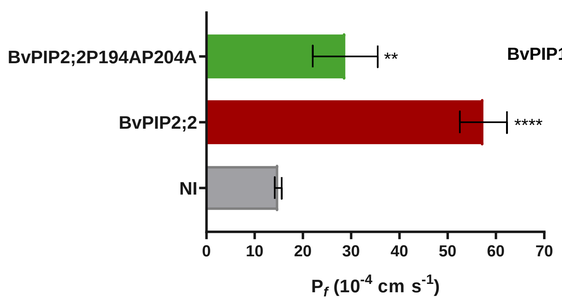
Time of simulation: 400 ns – 1 μs

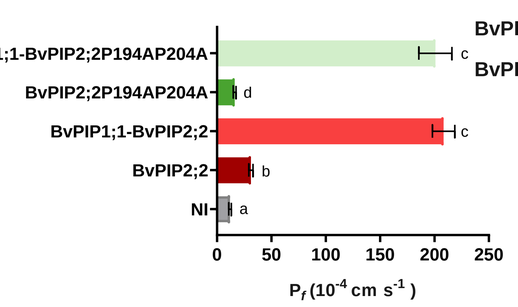
In vitro

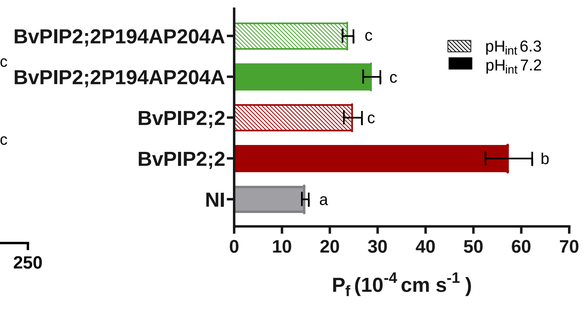










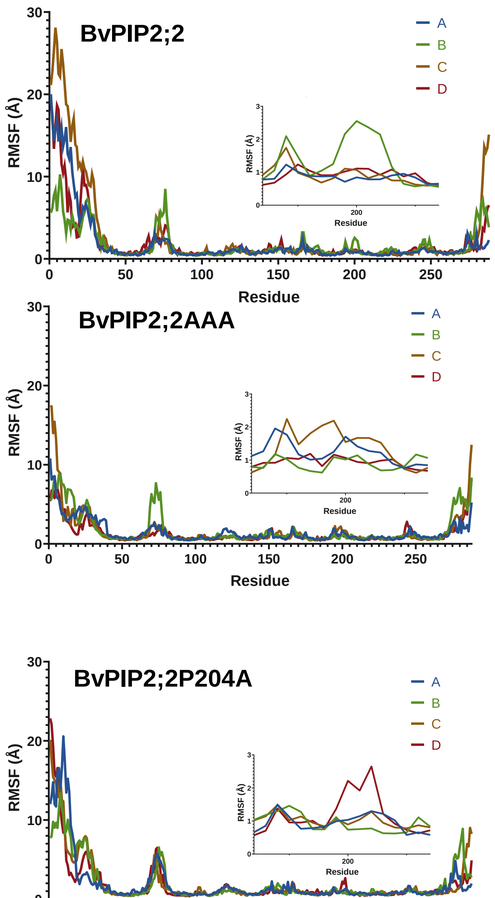


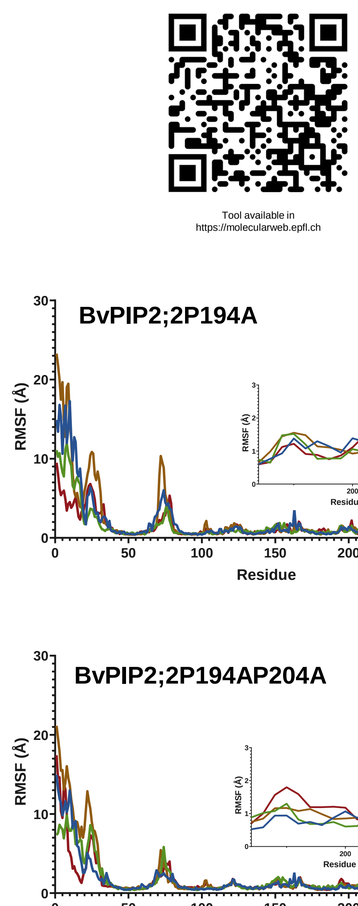
- BvPIP2;2AAA, BvPIP2;2P194A water activity dose not differ from BvPIP2;2 (WT)
- In oocytes experiments BvPIP2;2P204A do not render increased P_f in comparison with non injected oocytes. This could be because is in plasma membrane but closed or because is retain in endoplasmic reticule.
- BvPIP2;2P194AP204A is a functional protein that renders decreased P_f in oocytes experiments compared with WT.

- All mutants interact with BvPIP1;1.
- Oocytes co-injected with BvPIP2;2AAA and BvPIP1;1 show less P_f than oocytes co-injected with BvPIP2;2 and BvPIP1;1

- The curve of activity vs pH for BvPIP2;2AAA is shifted to more alkaline values ($pH_{0,5}$ = 6.43±0.01 n=3 for WT, 6.725±0.005 n=2 for the mutant channel)
- BvPIP2;2P194A present a $pH_{0,5}$ more alkaline than WT (6.43±0.01 n=3 for WT, 6.56±0.01 n=2 for P194A)
- BvPIP2;2P194AP204A seems to not be able to open or close under pH modification

In silico





Tool available in https://molecularweb.epfl.ch

- Mutant BvPIP2;2AAA presents lower RMSF values in N-term. That indicates that this zone is more rigid in this mutant.
- No significant differences in mutant loopDs flexibility were observed.

Conclusions

Our results show that proline residues located in the flexible domains of PIP2 aquaporins are key elements for pH gating but with different contributions:

- regulating pH sensing
- facilitating the conformational changes of the loop responsible for blocking the pore.

Acknowledgement

PICT-2017 0244, PICT-2019 00387, UBA2018 20020170100178BA