Biophysical Journal, Volume *110*

Supplemental Information

PIP Water Transport and Its pH Dependence Are Regulated by Tetramer Stoichiometry

Cintia Jozefkowicz, Lorena Sigaut, Florencia Scochera, Gabriela Soto, Nicolás Ayub, Lía Isabel Pietrasanta, Gabriela Amodeo, F. Luis González Flecha, and Karina Alleva

Supporting Material

PIP Water Transport and its pH Dependence are regulated by Tetramer Stoichiometry

Cintia Jozefkowicz,[1] Lorena Sigaut,[2,3] Florencia Scochera,[1,4] Gabriela Soto,[5] Nicolás Ayub,[5] Lía Isabel Pietrasanta,[2,3,6], Gabriela Amodeo,[7] F. Luis González Flecha,[1] Karina Alleva[1,4,*]

[1] Instituto de Química y Fisicoquímica Biológica "Alejandro C. Paladini" (IQUIFIB, UBA-CONICET), Buenos Aires, Argentina; [2] Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires; [3]Instituto de Física de Buenos Aires (IFIBA), CONICET, Ciudad Universitaria, Buenos Aires, Argentina; [4]Departamento de Fisicomatemática, Facultad de Farmacia y Bioquímica, UBA, Buenos Aires, Argentina; [5]Instituto de Genética "Ewald A. Favret", CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Argentina; [6]Centro de Microscopías Avanzadas, Facultad de Ciencias Exactas y Naturales-UBA, CONICET, [7]Departamento de Biodiversidad y Biología experimental, Instituto de Biodiversidad y Biología Experimental y Aplicada, Facultad de Ciencias Exactas y Naturales, UBA-CONICET, Buenos Aires, Argentina.

*Correspondence: kalleva@ffyb.uba.ar

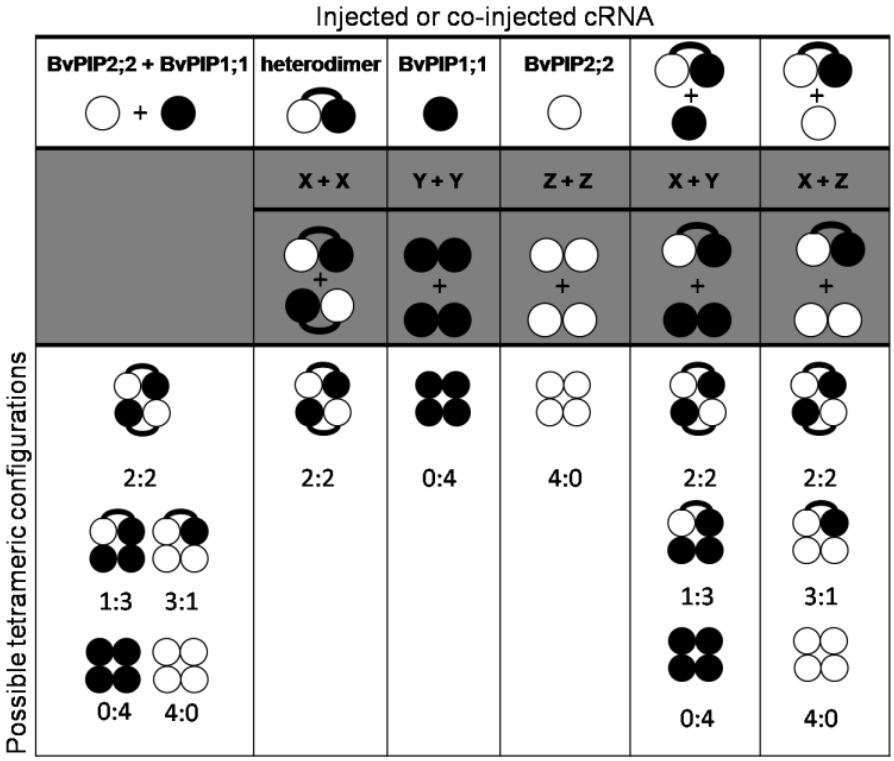

Figure S1. Schematic representation of possible tetramer configurations generated by the injection of heterodimer, BvPIP1;1, BvPIP2;2 cRNA or by the co-injection of combinations of cRNA. Upon coexpression of BvPIP1;1 plus BvPIP2;2 monomers several tetrameric configurations are possible; but when heterodimer or BvPIP2;2 or BvPIP1;1 cRNA are injected alone in oocytes, single tetrameric species are expressed. By co-injecting the heterodimer with either BvPIP1;1 or BvPIP2;2 monomers, only three different tetrameric configurations are plausible. This strategy is based on the assumption that the injection of single cRNA species alone (PIP1, PIP2 or dimeric constructs), or co-injected, will result in the assembly of tetramers with different configurations. Our mathematical model consider that: i- PIP1 and PIP2 monomers associate as dimers in a first step and that tetramerization occurs by dimerization of dimers (shown in gray background); ii- the dimerization step is given randomly allowing different stoichiometries to be ensemble. Then, in our model, we considered the following types of dimers: X=PIP2-PIP1; Y=PIP1-PIP1, Z=PIP2-PIP2.

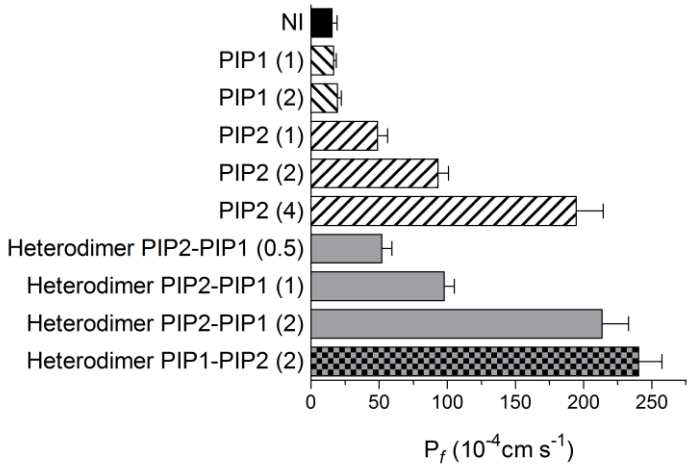

Figure S2. Functionality assay of single PIP1 (BvPIP1;1), PIP2 (BvPIP2;2), heterodimer PIP2-PIP1 (BvPIP2;2-BvPIP1;1) and heterodimer PIP1-PIP2 (BvPIP1;1-BvPIP2;2) injected at different cRNA masses. Different amounts of cRNA of BvPIP1;1, BvPIP2;2, or different tandem constructs made of covalently link BvPIP1;1 and BvPIP2;2 (BvPIP2;2-BvPIP1;1 and BvPIP1;1-BvPIP2;2 heterodimers) were injected in Xenopus oocytes and the osmotic water permeability coefficient (P_f) was determined after three days. We found that the osmotic water permeability coefficient (P_f) for oocytes expressing heterodimers or single BvPIPs were proportional to the mass of cRNA injected. Both heterodimers (BvPIP1;1-BvPIP2;2 and BvPIP2;2-BvPIP1;1) were able to transport water, independently of which monomer was linked by the C-terminal end to the other monomer in the fusion polypeptide. The relative quantity of cRNA injected in each oocyte is shown in parentheses, being (0.5) equal to 1.25 ng cRNA/oocyte, (1) equal to 2.5 ng cRNA/oocyte, (2) equal to 5 ng cRNA/oocyte and (4) equal to 10 ng cRNA/oocyte. NI: non-injected oocytes. Data are expressed as mean values (mean P_f ± SEM, n= 5).

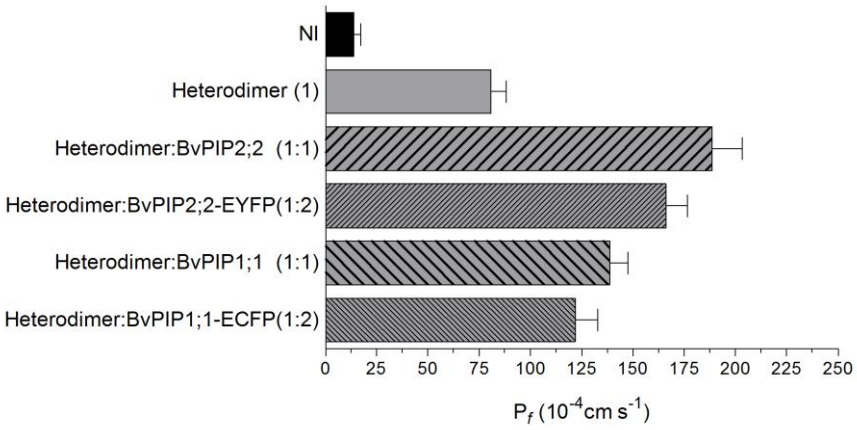

Figure S3. P_f of oocyte membranes expressing fluorescent tagged-BvPIP. The coexpression of both BvPIP2;2-EYFP and BvPIP1;1-ECFP + heterodimer showed water transport activity similar to that of their corresponding coexpressions of the heterodimer with wild-type BvPIP2;2

and BvPIP1;1 monomers, indicating that the fluorescent tag does not modify protein activity or functional interaction. The relative quantity of cRNA injected in each oocyte is shown in parentheses. NI, non-injected oocytes used as negative controls. Values are representative of four independent experiments using different oocyte batches. For each condition, mean values are shown as mean $P_f \pm$ SEM, n = 7−10.

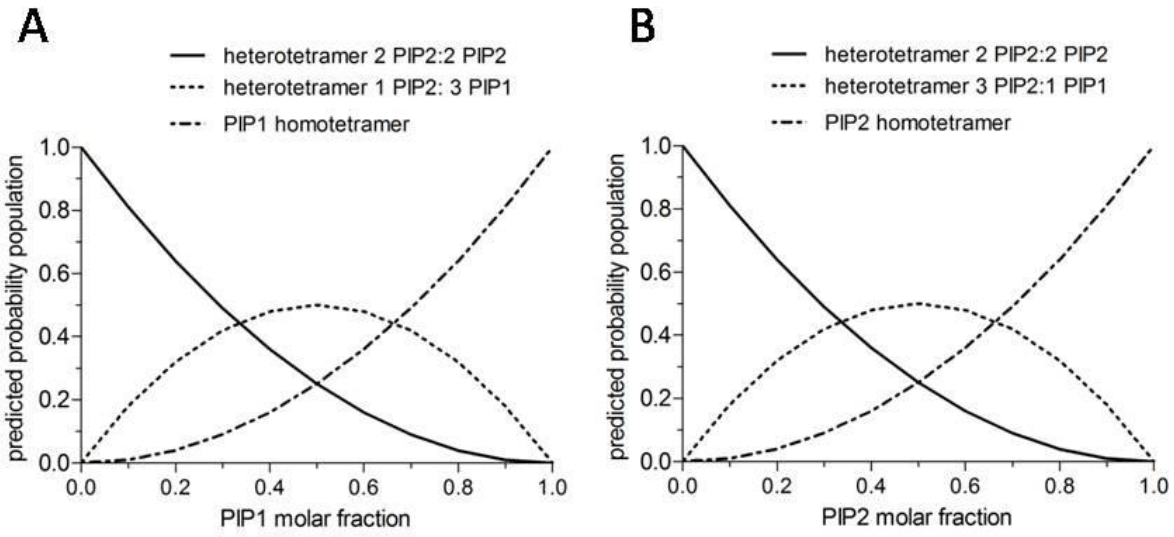

Figure S4. Predicted fraction of each tetrameric species as function of the molar fraction of PIP1 (A) or PIP2 (B) subunits. Curves were calculated using Eq. 2.