



HAL
open science

Biochemical and structural organization of the unique phosphatase of Orf virus

Gwenaëlle André-Leroux

► **To cite this version:**

Gwenaëlle André-Leroux. Biochemical and structural organization of the unique phosphatase of Orf virus. 22. IUBMB Congress, Aug 2015, Foz do Iguacu, Brazil. , 2015. hal-02739714

HAL Id: hal-02739714

<https://hal.inrae.fr/hal-02739714>

Submitted on 2 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Segovia, D. ¹, Segovia, E. ¹, Porley, D. ², Olivero, N. ², André-Leroux, G. ³, Berois, M. ², Villarino, A. ¹

¹ Facultad de Ciencias, UdelaR, Sección Bioquímica y Biología Molecular (Montevideo, Uruguay), ² Facultad de Ciencias, UdelaR, Sección Virología (Montevideo, Uruguay), ³ INRA, Unité Mathématique, Informatique, Génome (Jouy en Josas, France)

BACKGROUND

The Orf virus is the causal agent of *Contagious Ecthyma*, a pustular dermatitis disease of sheep and goats that occasionally affects humans. Orf disease has a relevant significance in the ovine production because of its highly contagious nature and its elevated rate of reinfections. The understanding of viral mechanisms of immune suppression is of great interest for developing new methods to control the disease.

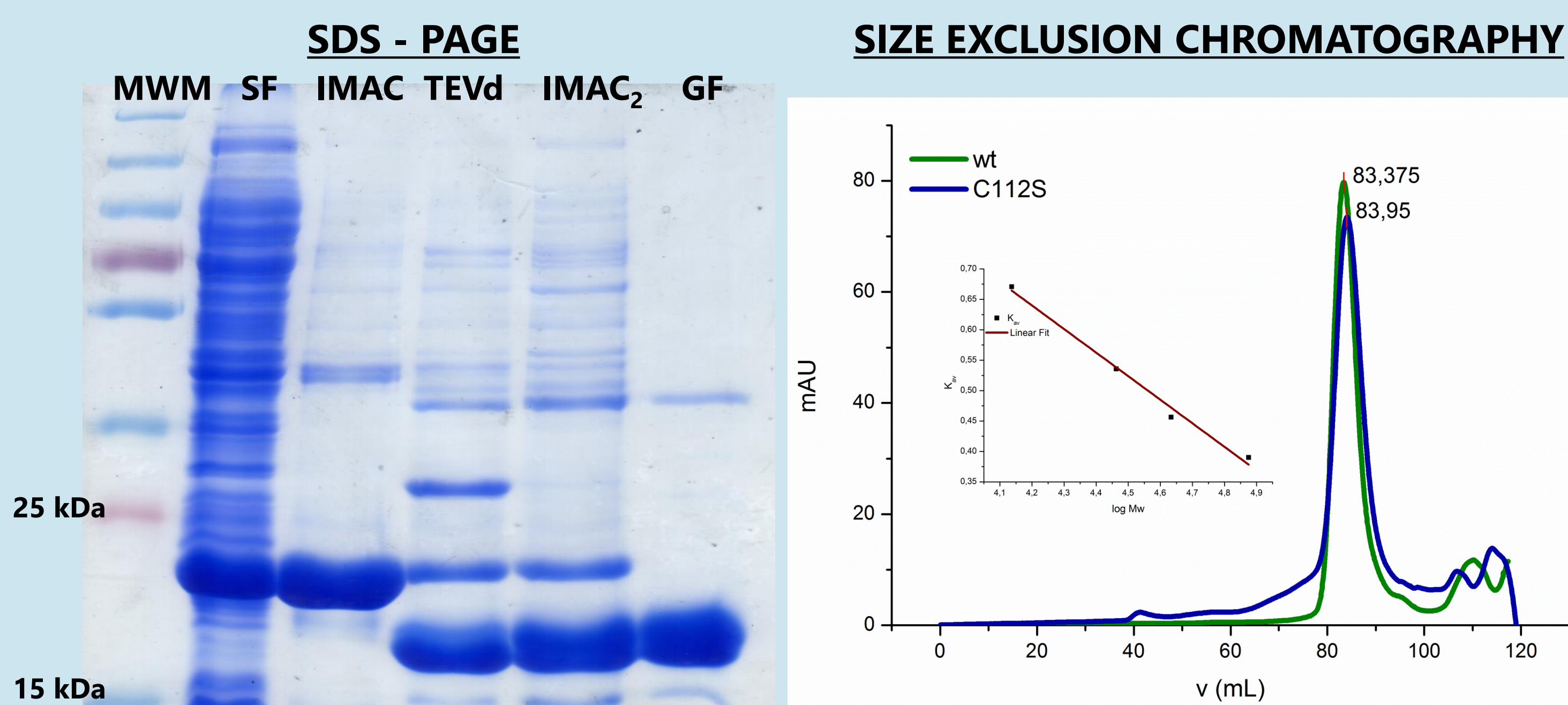
In the genome of Orf virus, there is an open reading frame coding for a tyrosine phosphatase, with a 40% sequence homology with the VH1 phosphatase of *Vaccinia virus*. VH1 phosphatase is a virulent factor, crucial for the viability and replication of the viral particle, and the blocking of interferon- γ signaling in the host. Recently, it has been shown that the Orf virus phosphatase is also capable to modulate the γ -interferon pathway.

REASERCH GOALS

- Achieve the recombinant production of the Orf virus phosphatase
- Perform an enzymatic characterization
- Modeling of the structure of the Orf virus phosphatase

RESULTS AND DISCUSSION

1. Expression and Purification of the Orf Virus Phosphatase

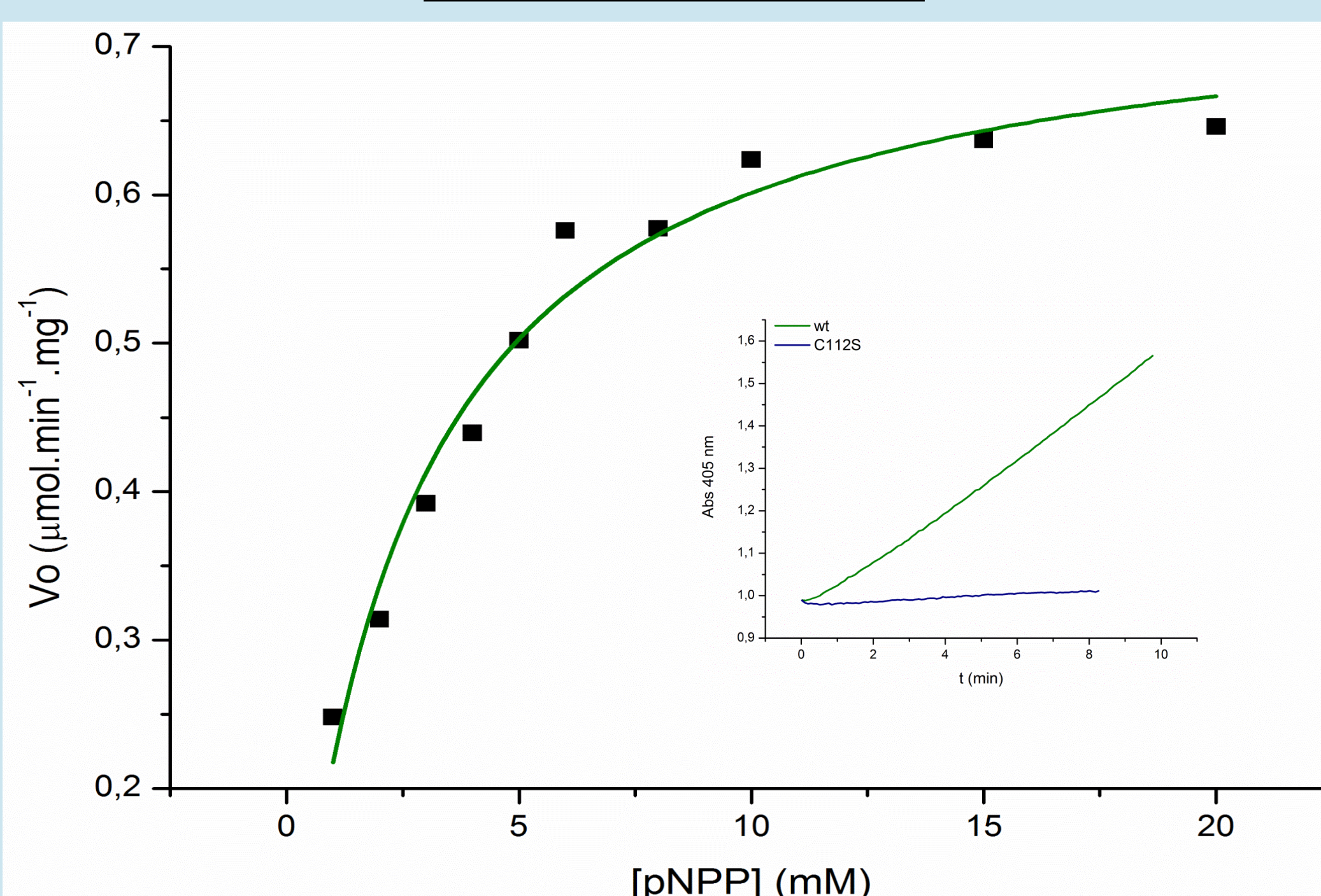


- The wt and the activity mutant C112S were purified from the soluble fraction. The $\Delta 20$ Nt mutant was insoluble. The His-tag was removed by digestion with TEV protease. After a second IMAC, the proteins were purified by size exclusion chromatography (Superdex 200 16/60).

- The wt and C112S eluted with an apparent molecular weight of 40 kDa, consistent with a dimeric protein.

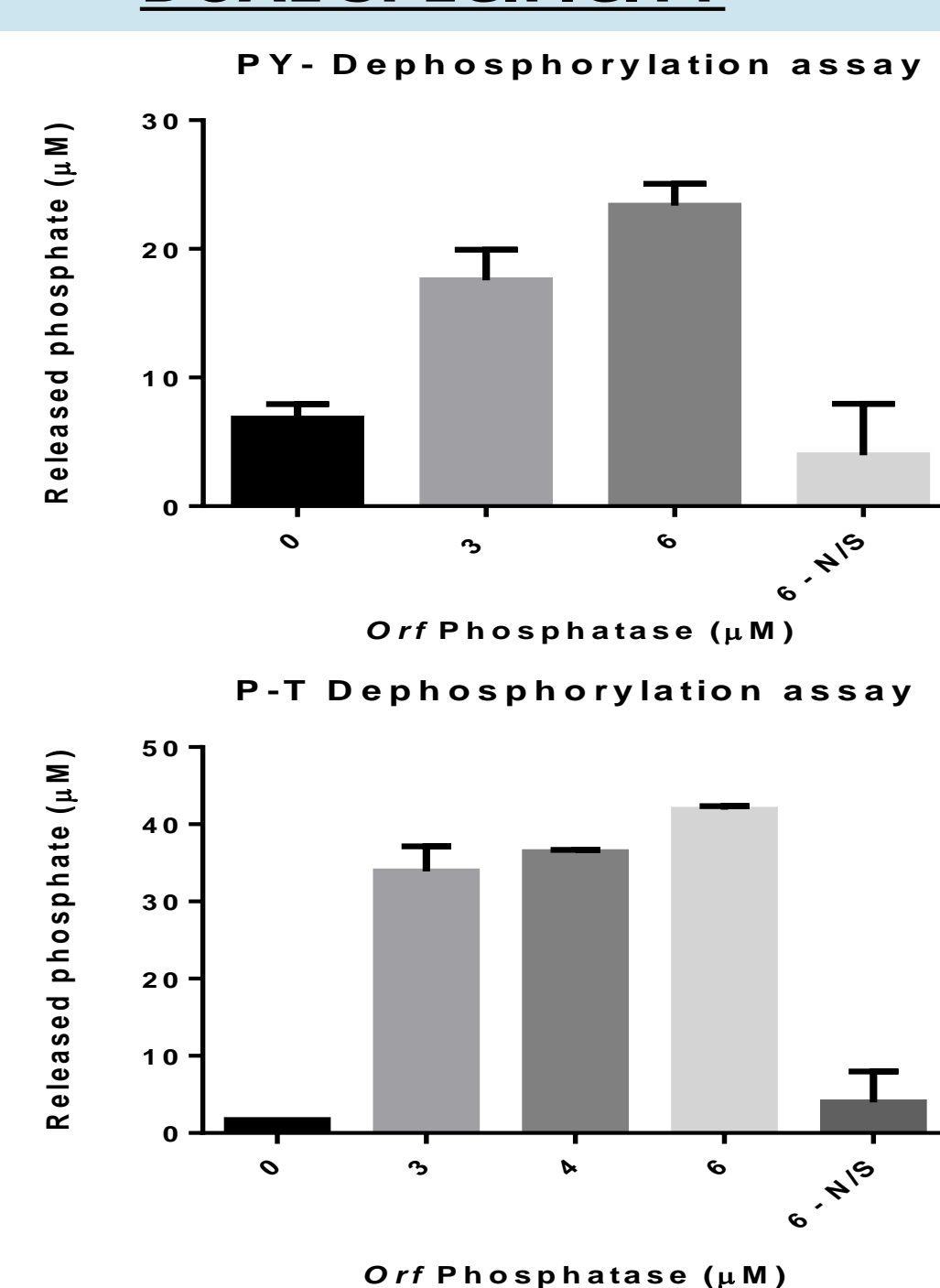
2. Enzymatic characterization

KINETIC PARAMETERS



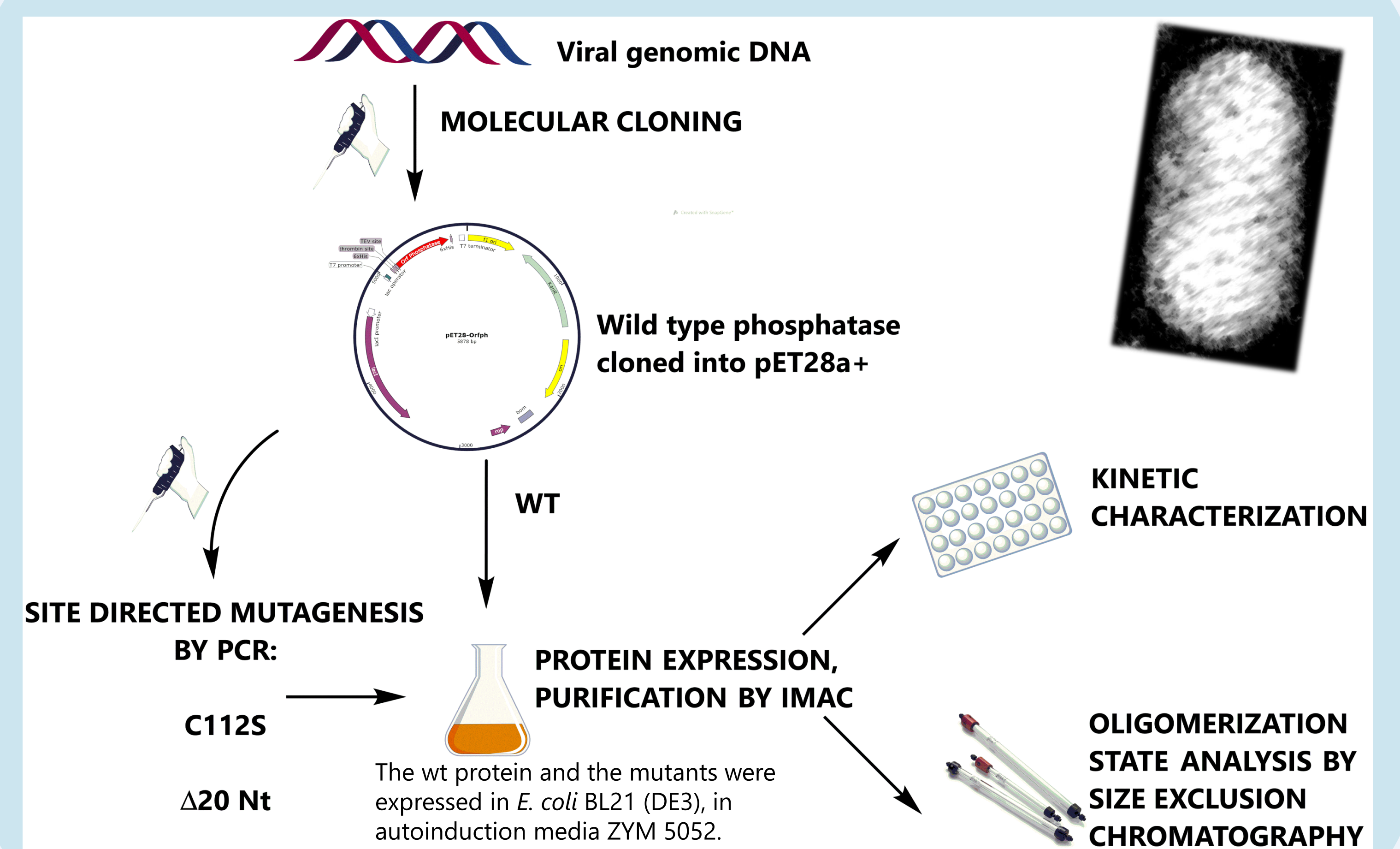
- The kinetic parameters were determined with the pNPP substrate.
- For the wt protein V_{max} : 0,74 μmol of pNP.min⁻¹.mg⁻¹; K_m : 2,43 mM
- The mutant C112S didn't shown any phosphatase activity.

DUAL SPECIFICITY



- PY/PThr peptides were used as substrates and the release of Pi was detected with Malachite Green.
- The wt phosphatase has a dual specificity

EXPERIMENTAL STRATEGY



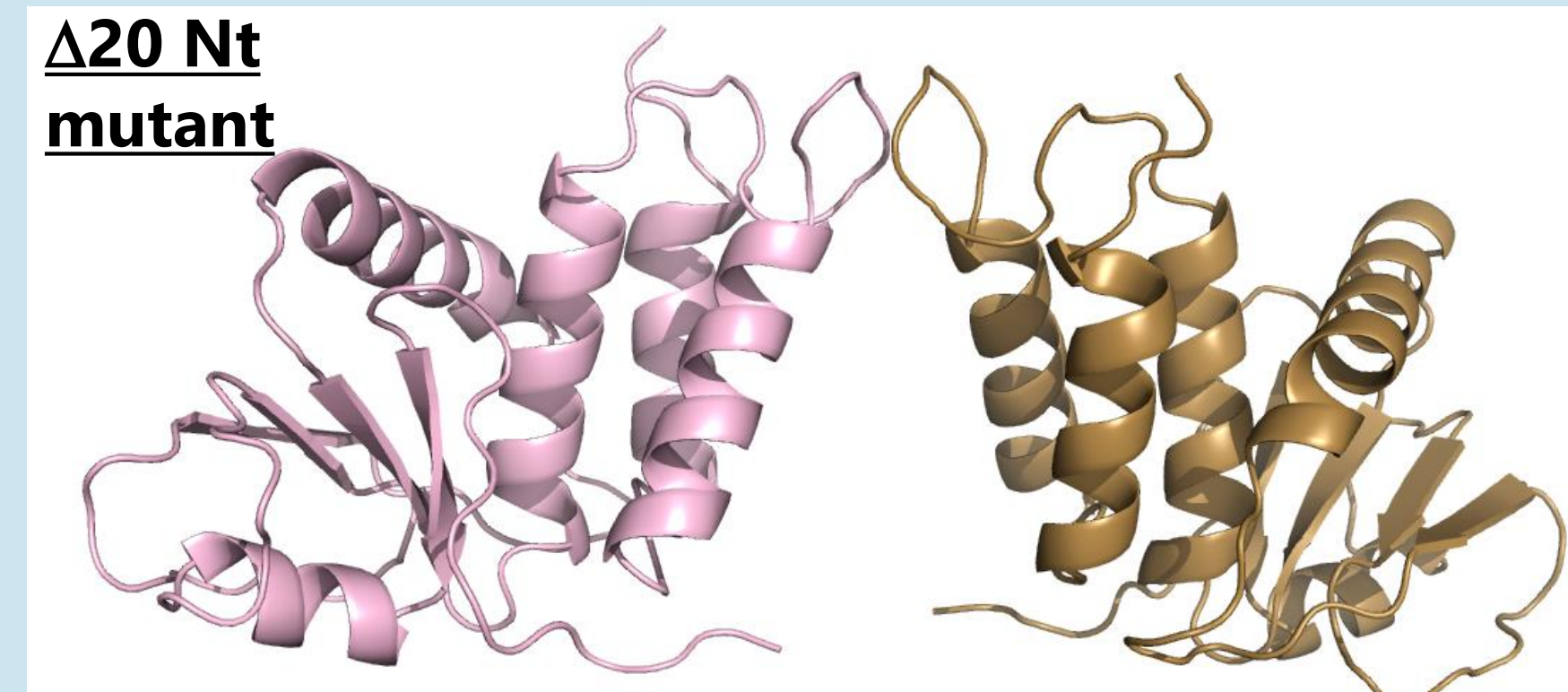
3. Molecular modeling

Wt Phosphatase



The Orf virus wt phosphatase and the $\Delta 20$ Nt mutant were homology-modeled, using the crystal structures of VH1 phosphatase from *Vaccinia virus* as a 3D template. The wt and the mutant C112S show a stable dimer *in silico*, with the N-terminal helix $\alpha 1$ of chain A that swapover C-terminal helices $\alpha 5$ and $\alpha 6$ of chain B and vice-versa.

$\Delta 20$ Nt mutant



The $\Delta 20$ Nt mutant encompasses a complete deletion of helix $\alpha 1$. We hypothesize that such deletion should preclude the dimer formation, because it does not display enough interface with helices $\alpha 5$ and $\alpha 6$ of the second protomer to stabilize the dimer.

CONCLUSIONS AND OUTLOOK

- The wild type Orf virus phosphatase and the mutant C112S are dimers in solution, as suggested by molecular modeling
- The Cys 112 is essential for the activity, a signature of PTPs
- The wild type protein is a dual specificity phosphatase, as VH1
- Even though the $\Delta 20$ Nt mutant was insoluble, molecular modeling strongly suggests it would be a monomer
- Currently, our team is working in obtaining the soluble form of the $\Delta 20$ Nt mutant, and in developing a cell cultured model to study the Orf virus phosphatase in a cellular context
- Crystallization assays were initiated with the wild type protein and the mutant C112S



Contact: Danilo Segovia – Facultad de Ciencias
dsegovia@fcien.edu.uy

Acknowledgements: CSIC, Ecos-Sud