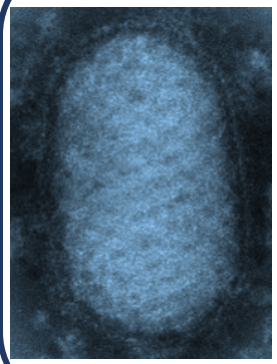
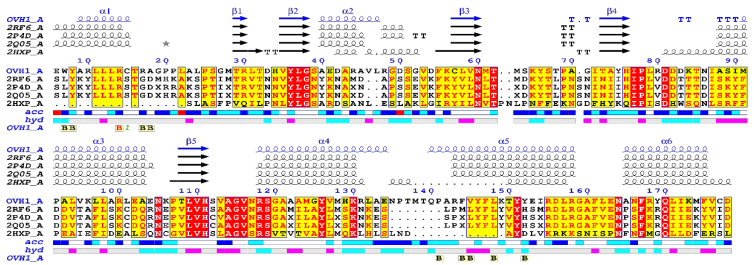
OH1 from Orf virus: a new tyrosine phosphatase that displays distinct structural features and triple substrate specificity

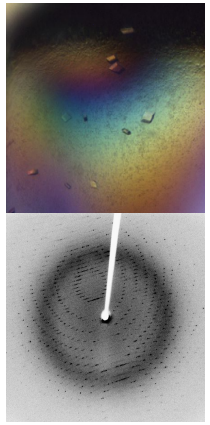
Gwenaëlle André-Leroux

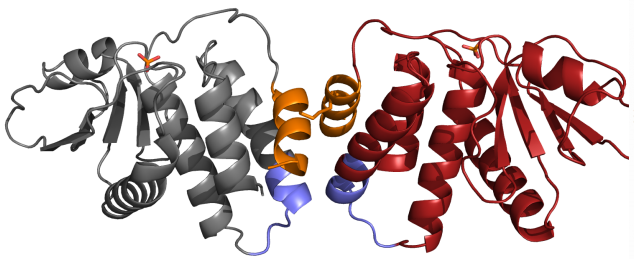
OH1 from Orf virus: a new tyrosine phosphatase

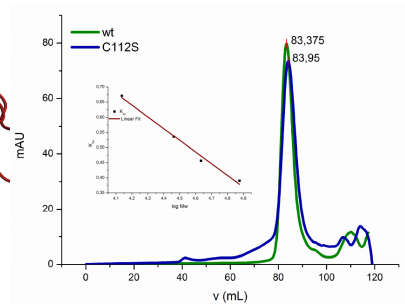
Distinct structural features & triple substrate specificity

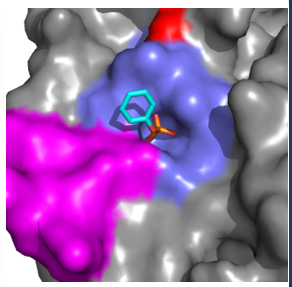








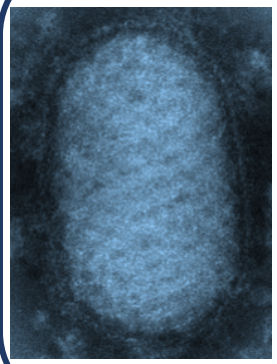


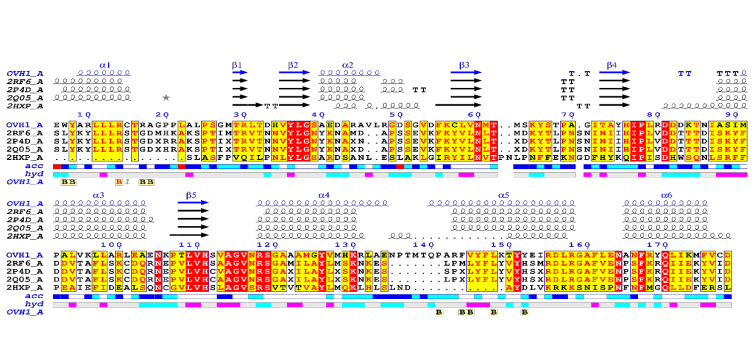


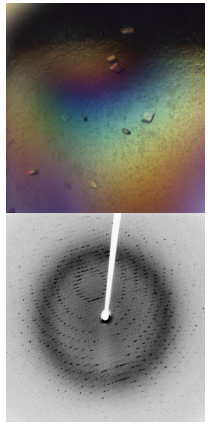
Gwenaëlle André-Leroux
MaIAGE, INRA Jouy-en-Josas
Mathématique et Informatique Appliqués du Génome à l'Environnement

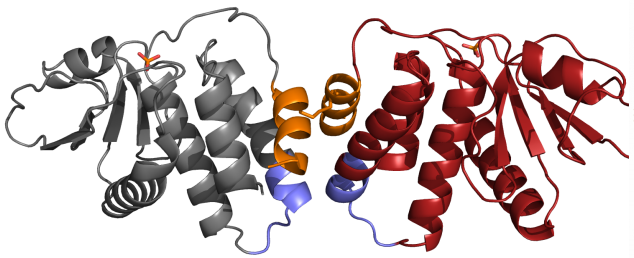
Workshop: « Modelling bio-molecular interactions: A multi-scale approach joining theory and experiments »

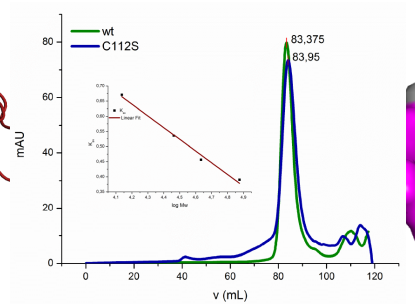
OH1 from Orf virus: a new tyrosine phosphatase

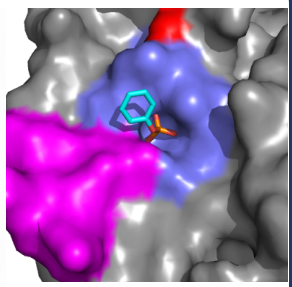












Outline

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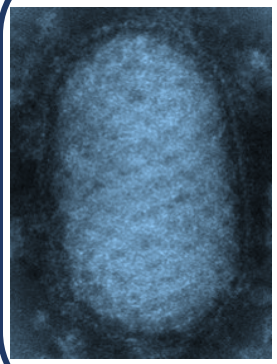
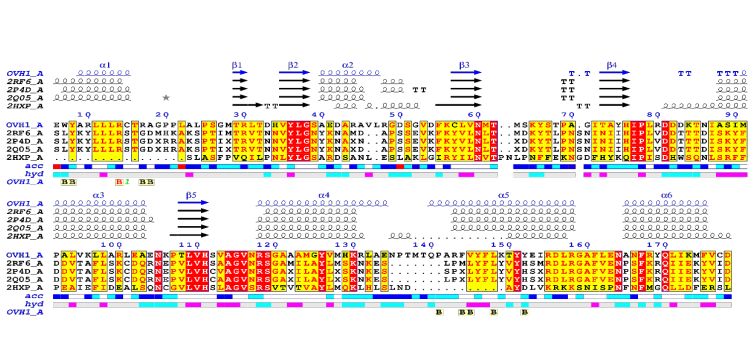
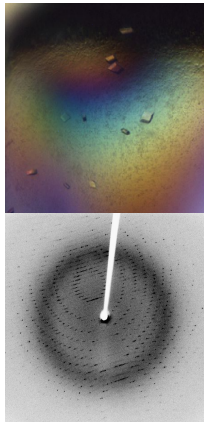
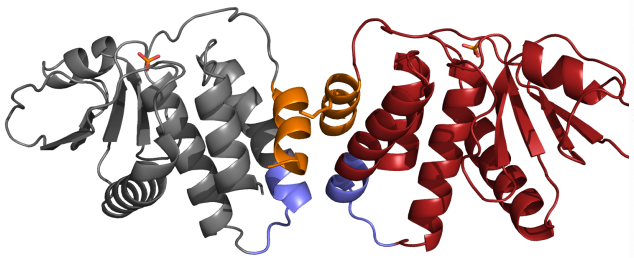
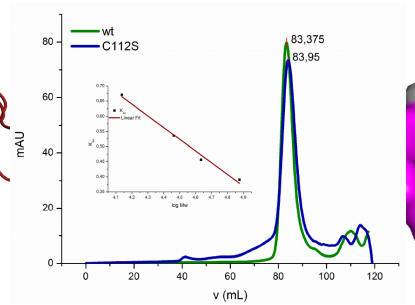
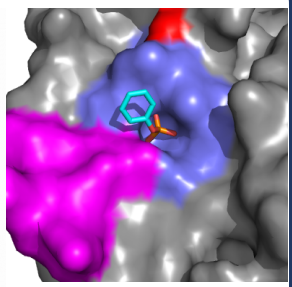
OH1 from Orf virus: a new tyrosine phosphatase



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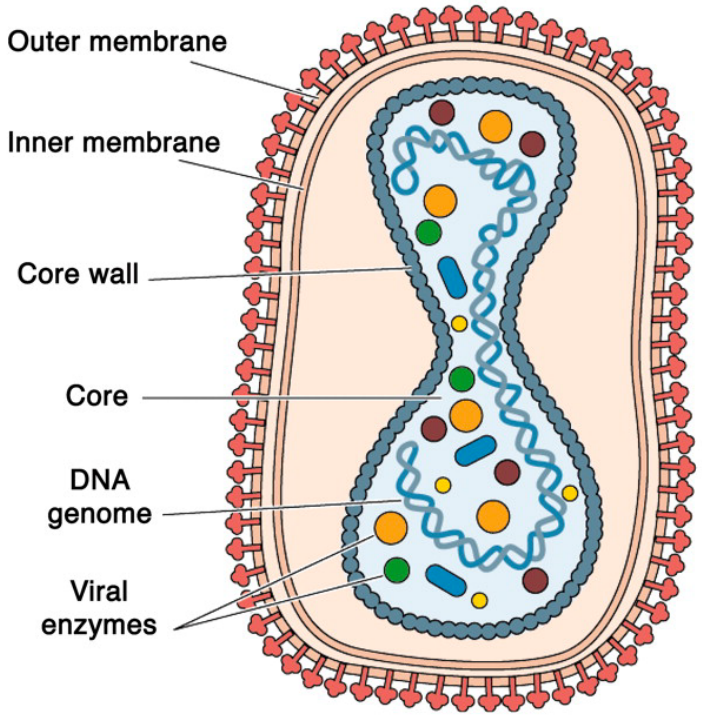


Orf Poxvirus family
Like Vaccinia & Variola virus

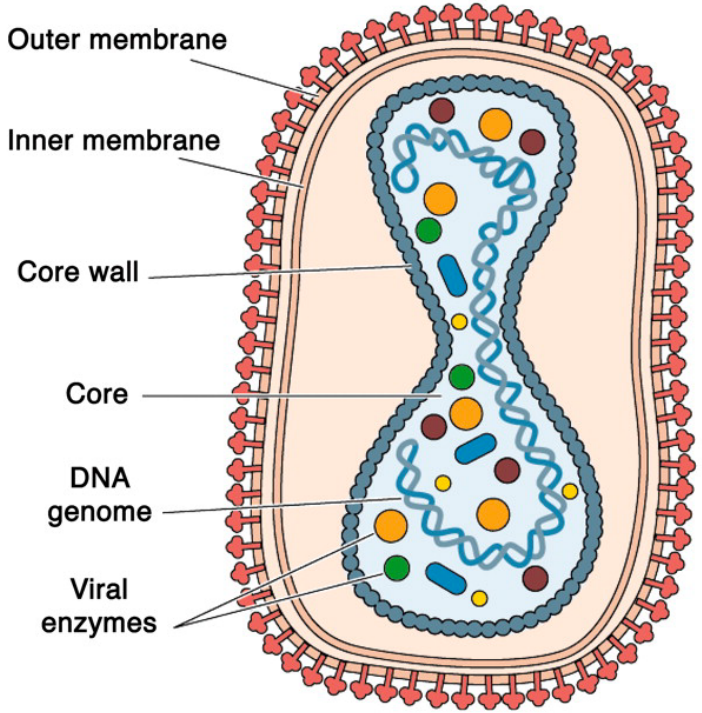
Orf = old english for « rough »

dsDNA virus

Large 200-300 nm

Envelope

Orf virus



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Poxvirus family

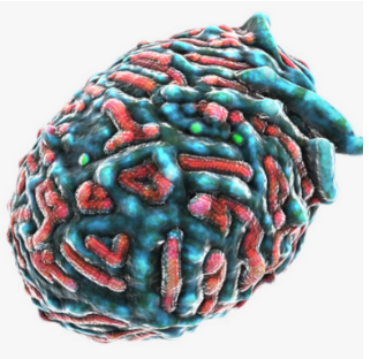
Chordopoxvirinae

Entomopoxvirinae

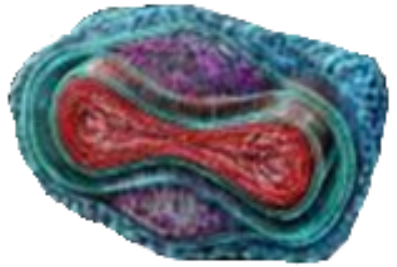
6 other genera

Orthopoxvirus genus

Parapoxvirus genus



Bovine papular stomatitis virus
Parapoxvuirus of red deer in New Zealand
Pseudocowpox virus



Vaccinia virus
Variola virus
Cowpox virus

Orf virus

- Is responsible for Echtyma disease primarily in sheep and goats
- Is contagious pustular dermatitis
- Replicates in keratinocytes and epithelial cells of the oral mucosa.
- Is reported worldwide
- Is purulent for 7 weeks
- Prevents lambs form sucking and cause weight loss
- Causes mastitis to mother ewes
- In its severe form on immune compromised animals can lead to death

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Highly contagious by nature

Zoonotic through direct contact

Live vaccine that can cause reinfection

Vaccine: immunity for 6-8 months

Secondary infections

Economical impact +++

Orf virus : new strain isolated in Uruguay in 2015

Strain UY1107 was fully sequenced : shows 90 essential and conserved genes

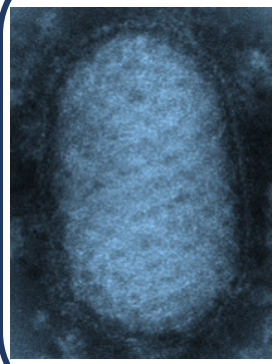
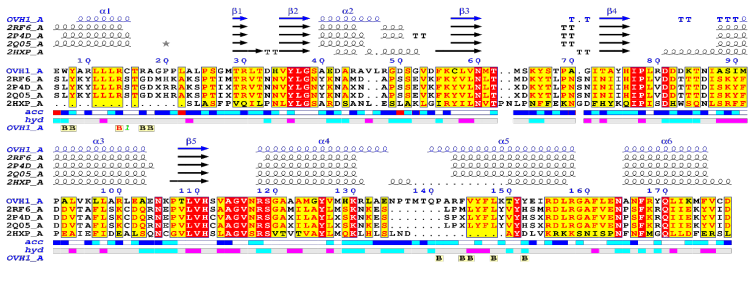
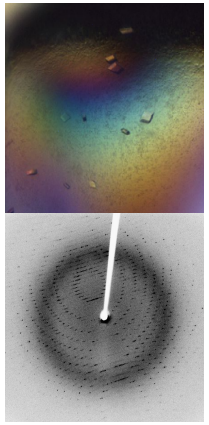
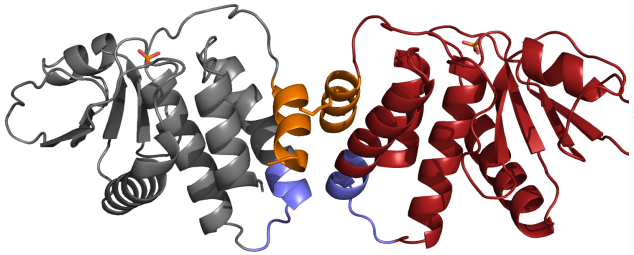
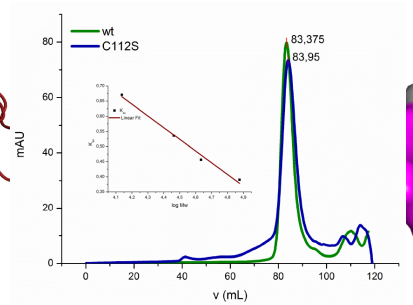
- Among them, a putative virulence factor was identified, by analogy with Vaccinia and Variola viruses
- Codes for a putative tyrosine phosphatase
- Deposited at genbank ID: KY651216

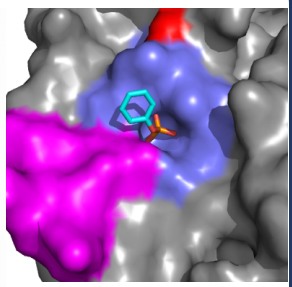
- Hypothesis: KY651216 is a Tyr-phosphatase responsible for dephosphorylation of phosphoylated substrates

Signaling mediators: Tyrosine phosphatase/kinase are molecular switches that turns on/off substrates

OH1 from Orf virus: a new tyrosine phosphatase

Distinct structural features & triple substrate specificity



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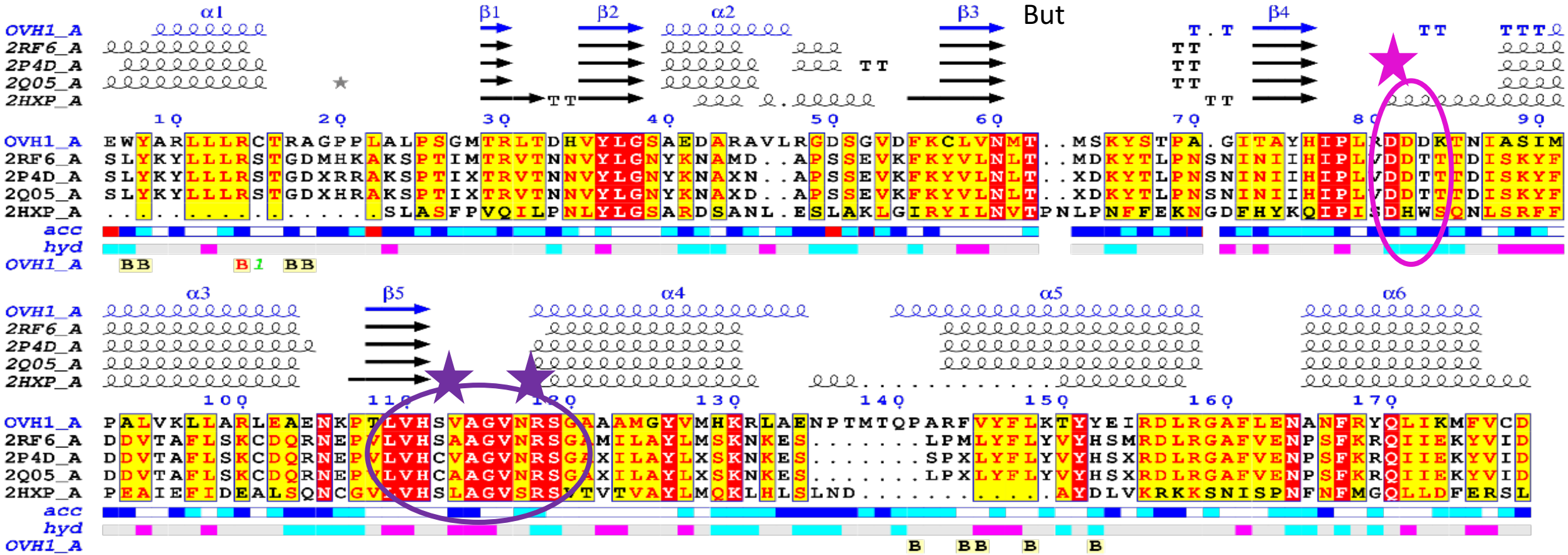
Putative tyrosine phosphatase

- High 40% similarity with Vaccinia and Variola Tyr-phosphatase
- Strict conservation of the active site of a Tyr-phosphatase

Active site Aspartate loop

But



Active site Phosphate loop

7 residue insertion

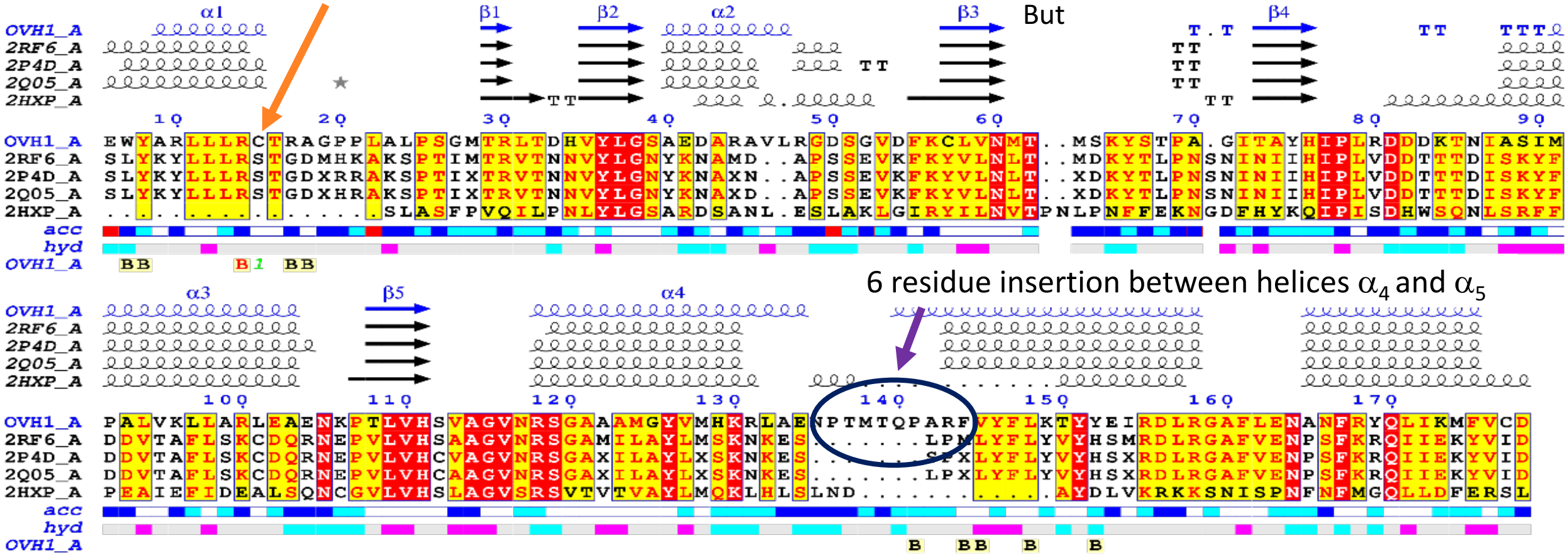
Putative tyrosine phosphatase

- High 40% similarity with Vaccinia and Variola Tyr-phosphatase
- Strict conservation of the active site of a Tyr-phosphatase

Ser to Cys substitution within helix α_1

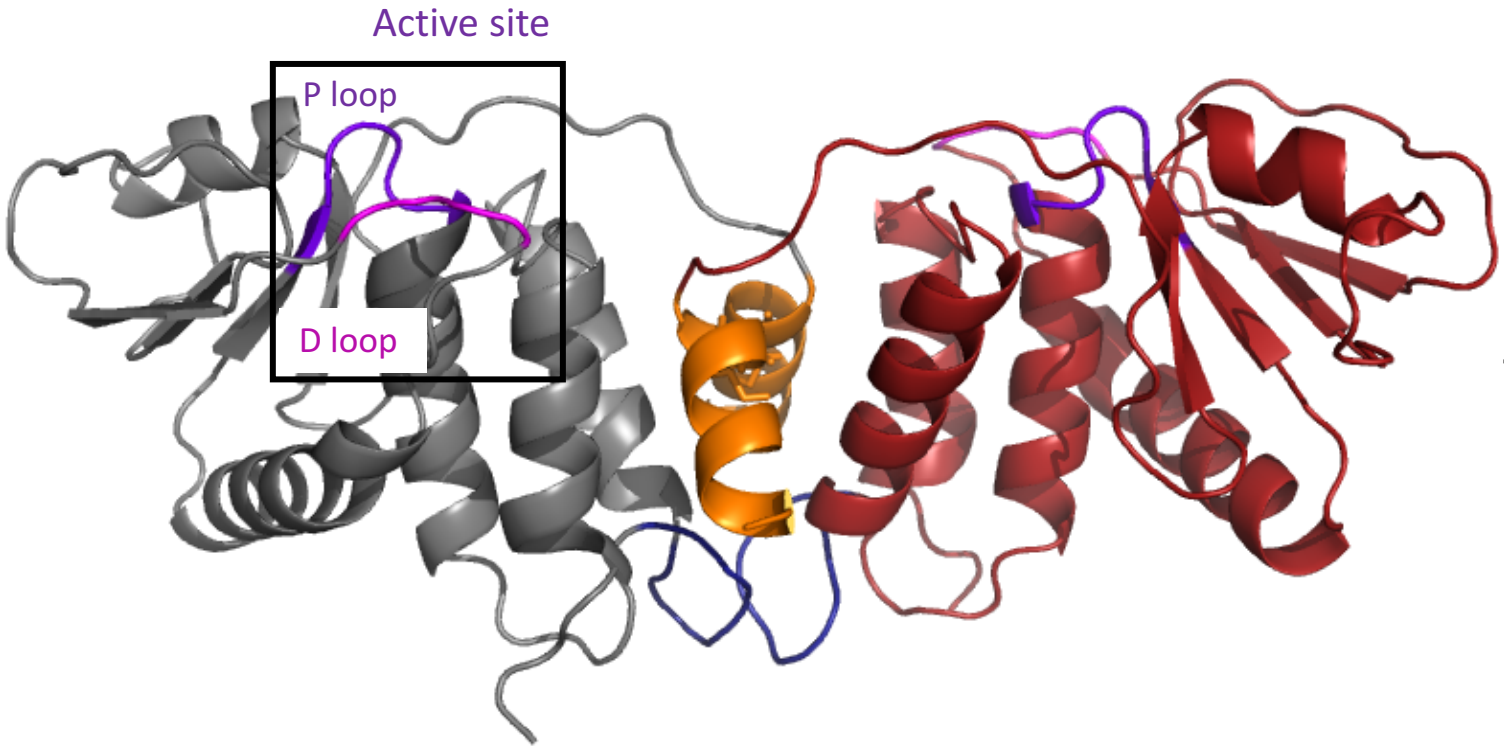
But

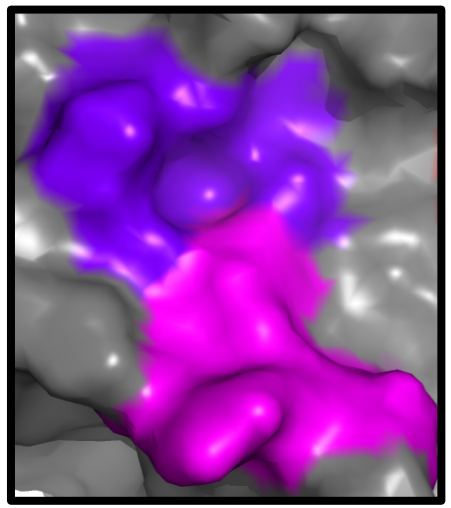
6 residue insertion between helices α_4 and α_5



Putative tyrosine phosphatase

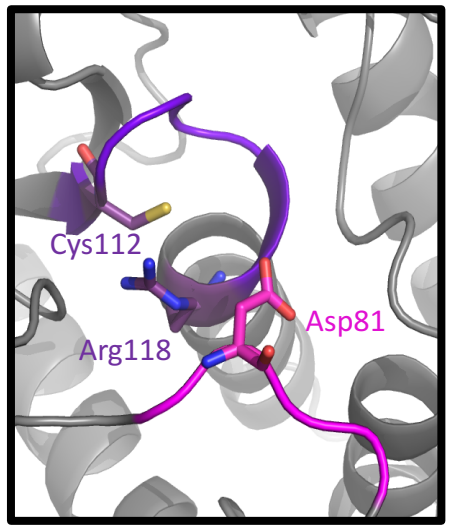
- Homology modeling of Tyrosine phosphatase with 3D Vaccinia and Variola VH1 as 3D templates: Chains A then B.
- Reconstruction of the dimer by superposition onto the dimer template.
- Minimisation with Charmm to relax the side chains





Phosphate group pocket

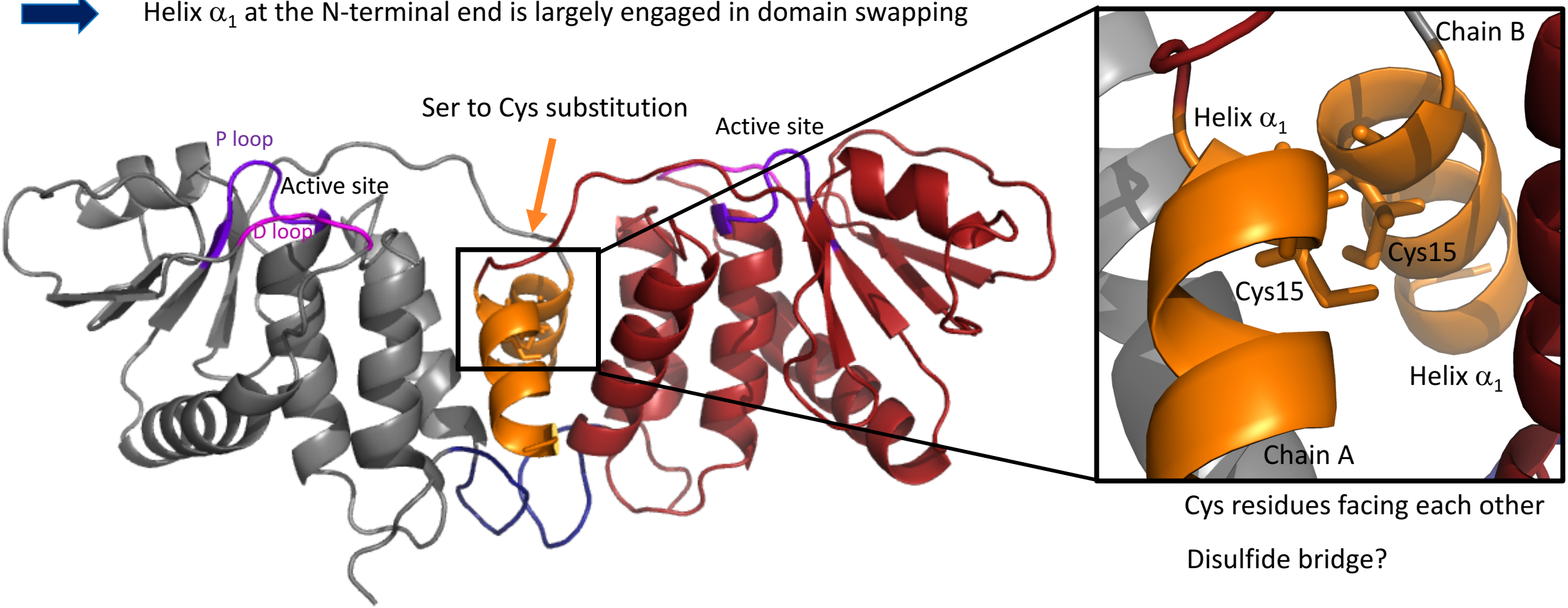
Active site conserved



Catalytic triad

Putative tyrosine phosphatase

- The main differences are located at the interface
- Helix α_1 at the N-terminal end is largely engaged in domain swapping



Cys residues facing each other

Disulfide bridge?

Putative tyrosine phosphatase

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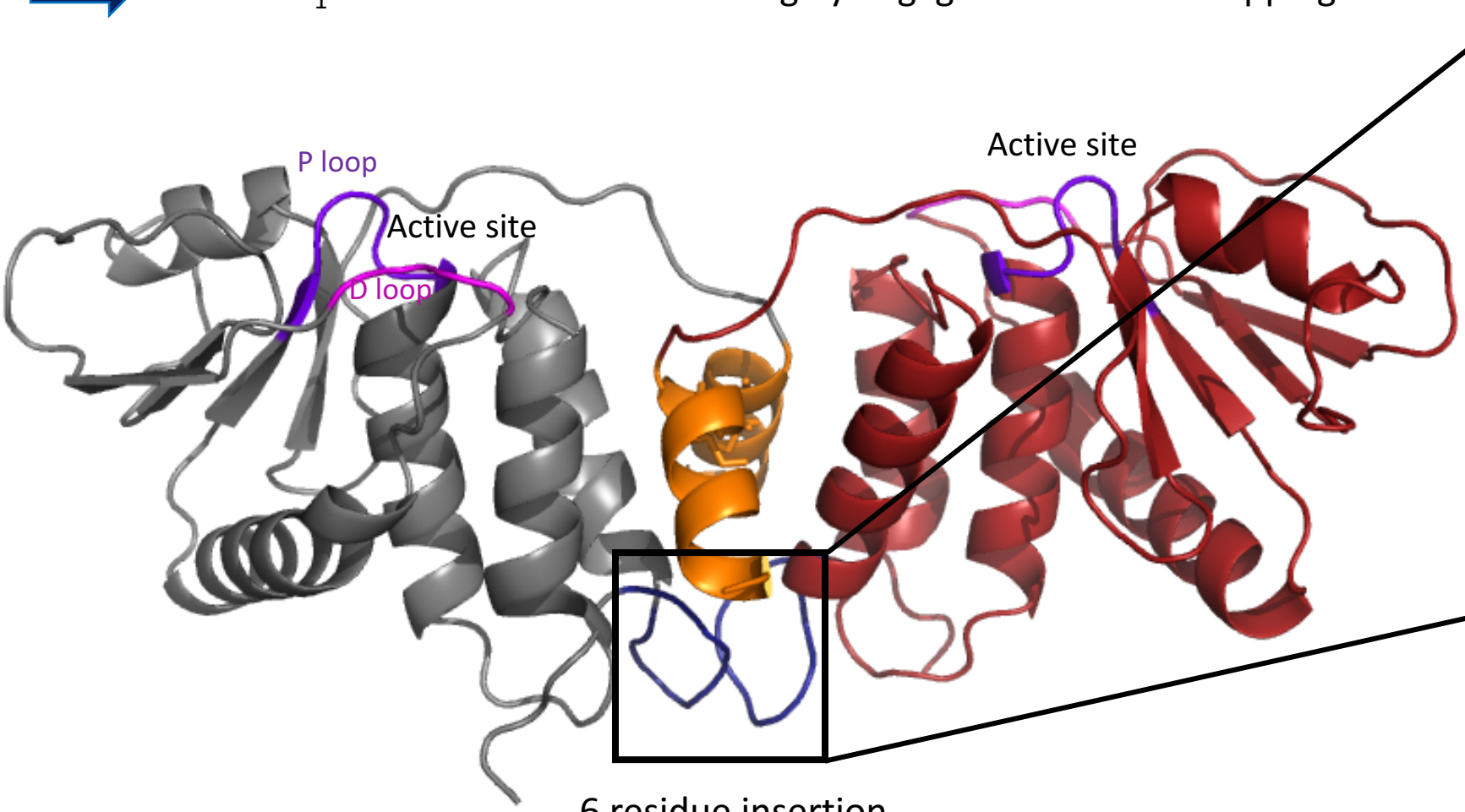
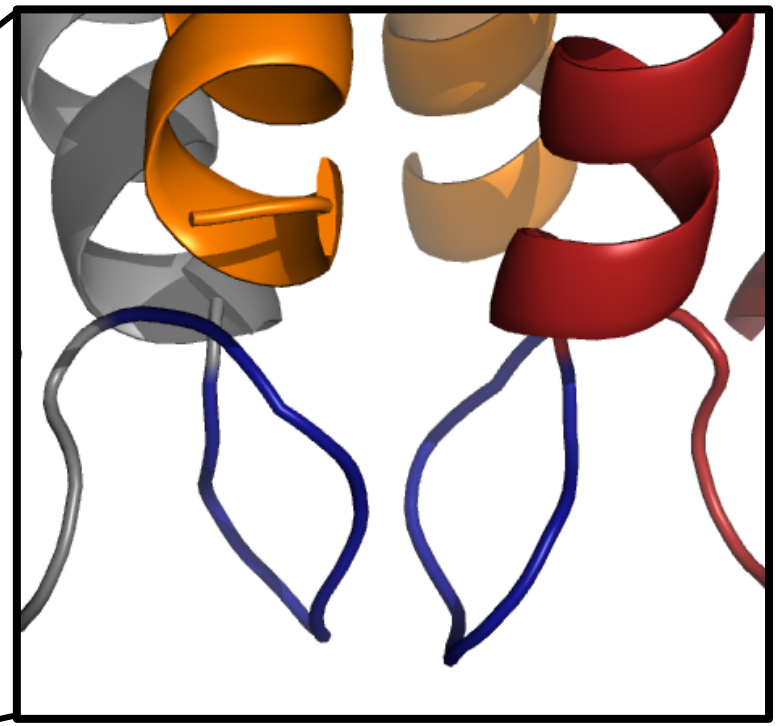


6 residue insertion

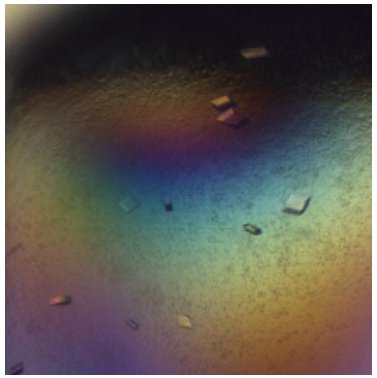


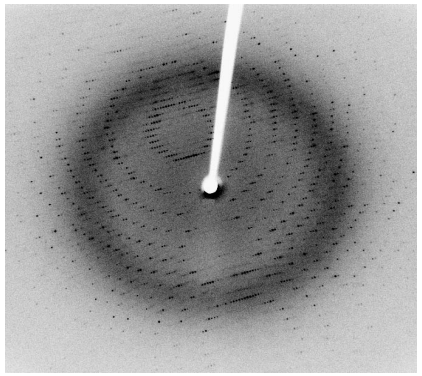
Modeled as a extended loop ?

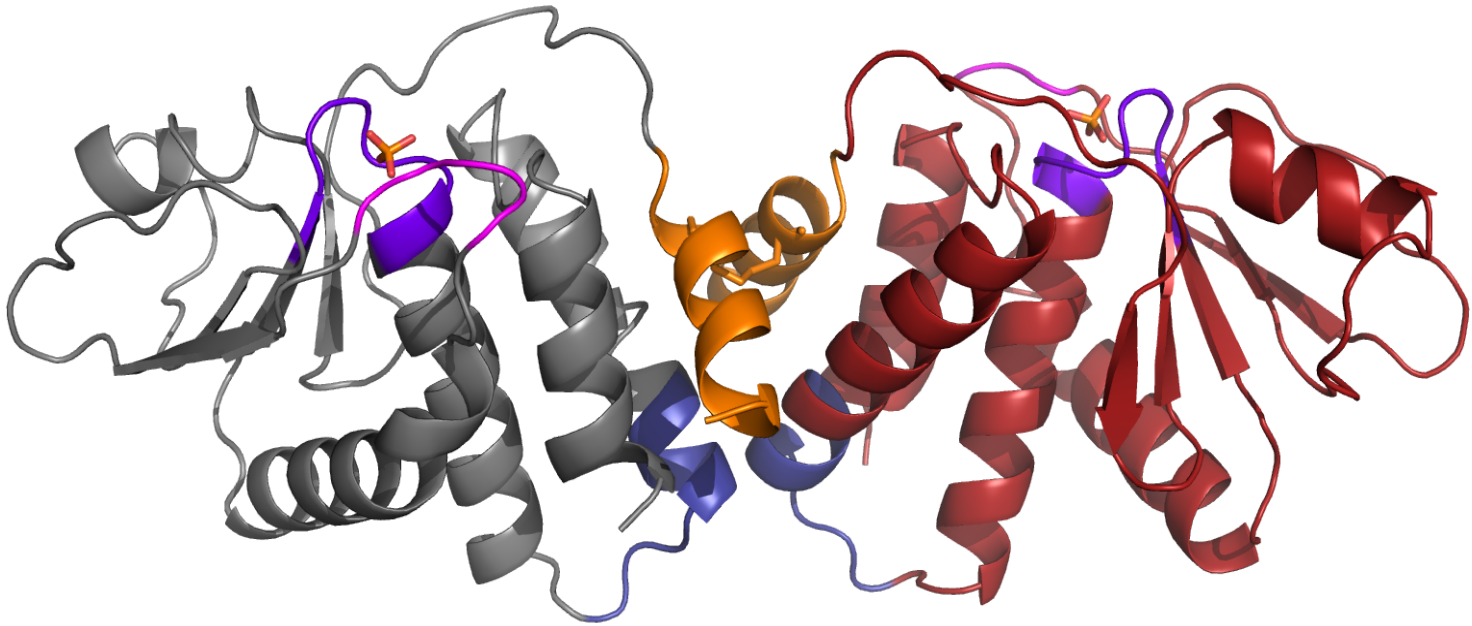
Really a similar dimer?

Orf virus : tyrosine phosphatase

- Protein purified to homogeneity Histrap and size-exclusion chromatography wt and C112S
- Crystallisation screening using sitting drop vapor diffusion for both







Cristal conditions
200 mM NH4SO4,
100mM NaAc, pH 4,6
PEG MME 2K 30%

Institut Pasteur

Beamline Proxima1
2 sets of data collection

SOLEIL SYNCHROTRON

Data statistics 5NCR
$P2_1$
a, b, c (Å) 49.56 63.55 55.39
α, β, γ (°) 90.00, 97.07 90.00

Refinement of the best at 1.89 Å resolution
Molecular replacement using Phaser
Model building with Coot
Refinement cycles within Refmac CCP4 package and Buster
Rwork/Rfree 18.72/21.69

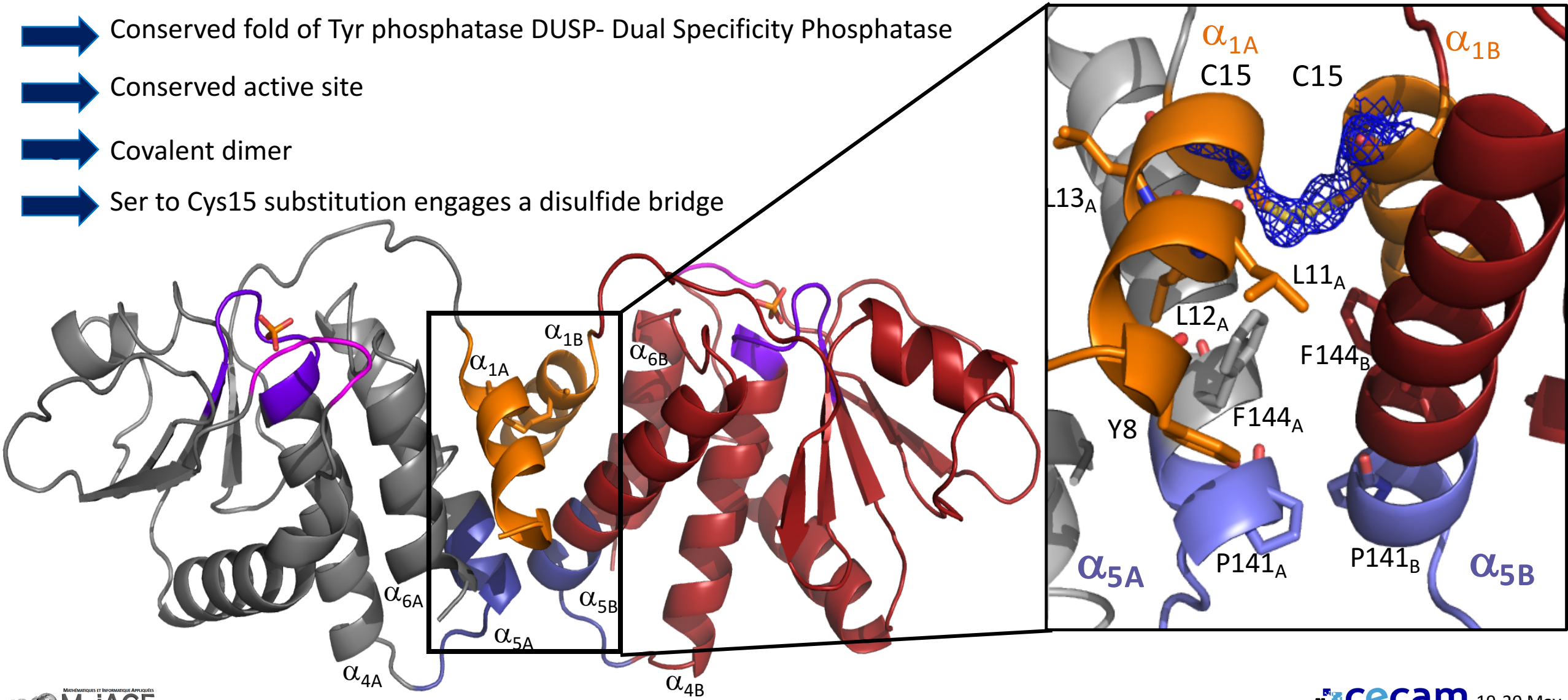
Crystal grew after 3 days for C112S
No crystal after 3 months for wt

Pdb deposition 5NCR

Orf virus : tyrosine phosphatase

Solved structure reveals:

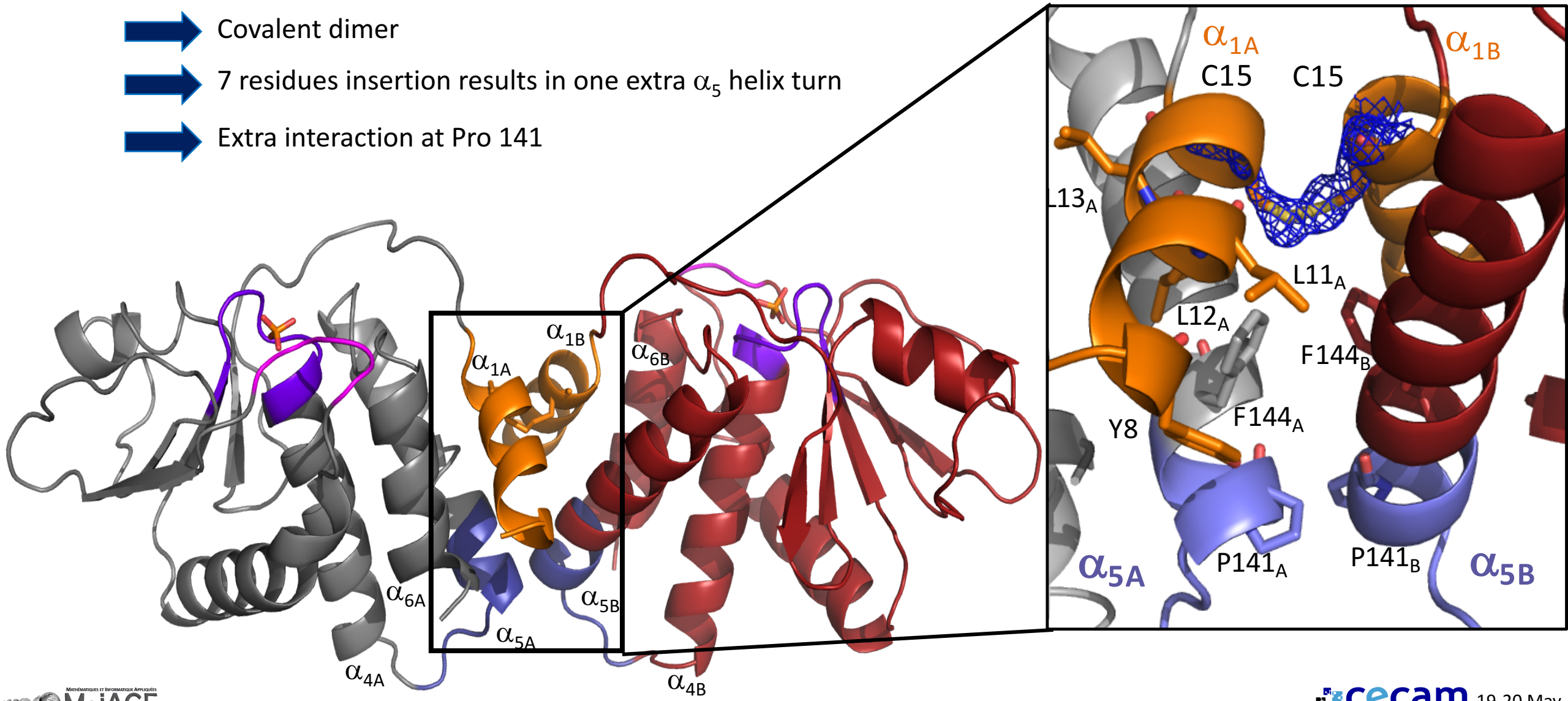
- Conserved fold of Tyr phosphatase DUSP- Dual Specificity Phosphatase
- Conserved active site
- Covalent dimer
- Ser to Cys15 substitution engages a disulfide bridge



Orf virus : tyrosine phosphatase

Solved structure reveals the conserved fold of DUSP –Dual Specificity Phosphatase

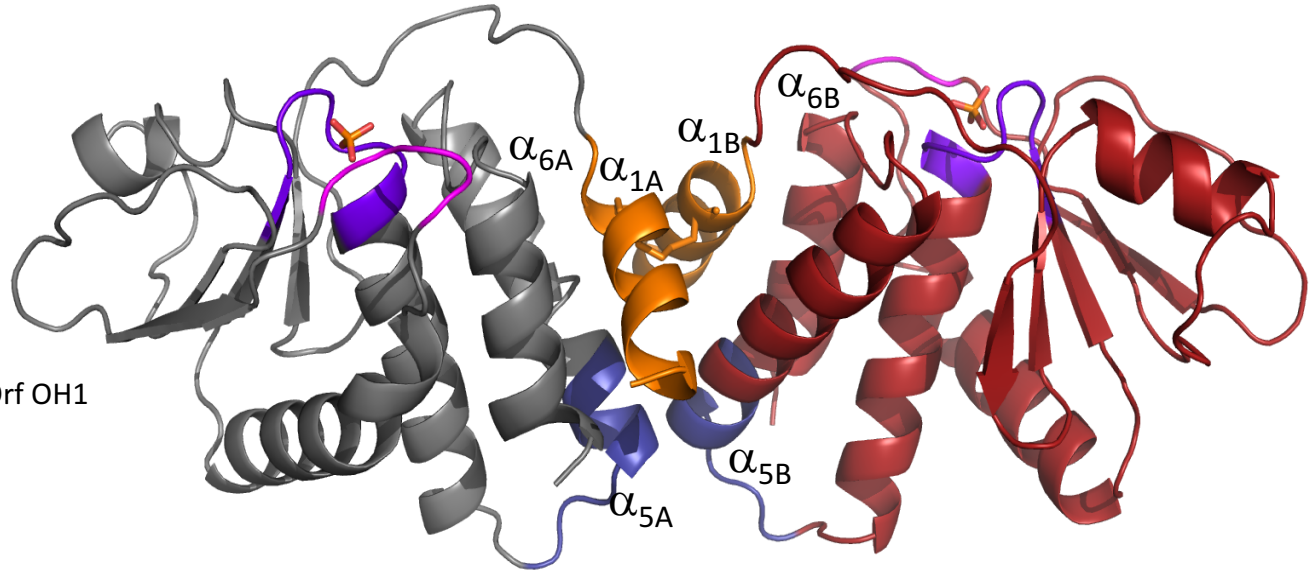
- Covalent dimer
- 7 residues insertion results in one extra α_5 helix turn
- Extra interaction at Pro 141



Orf virus : tyrosine phosphatase

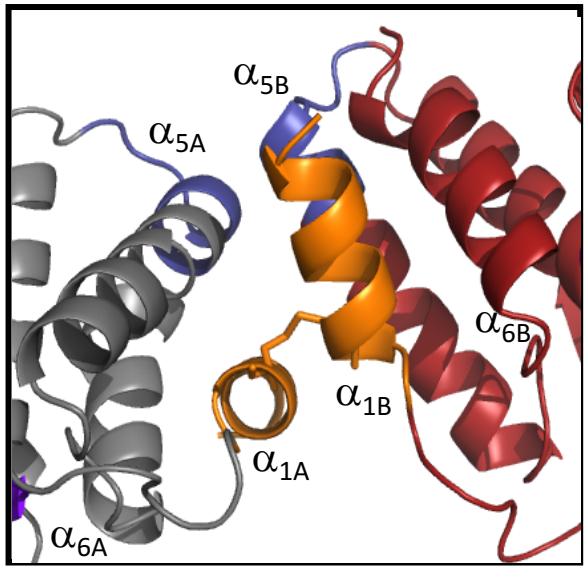
Solved structure reveals a dimer organization distinct from Vaccinia phosphatase

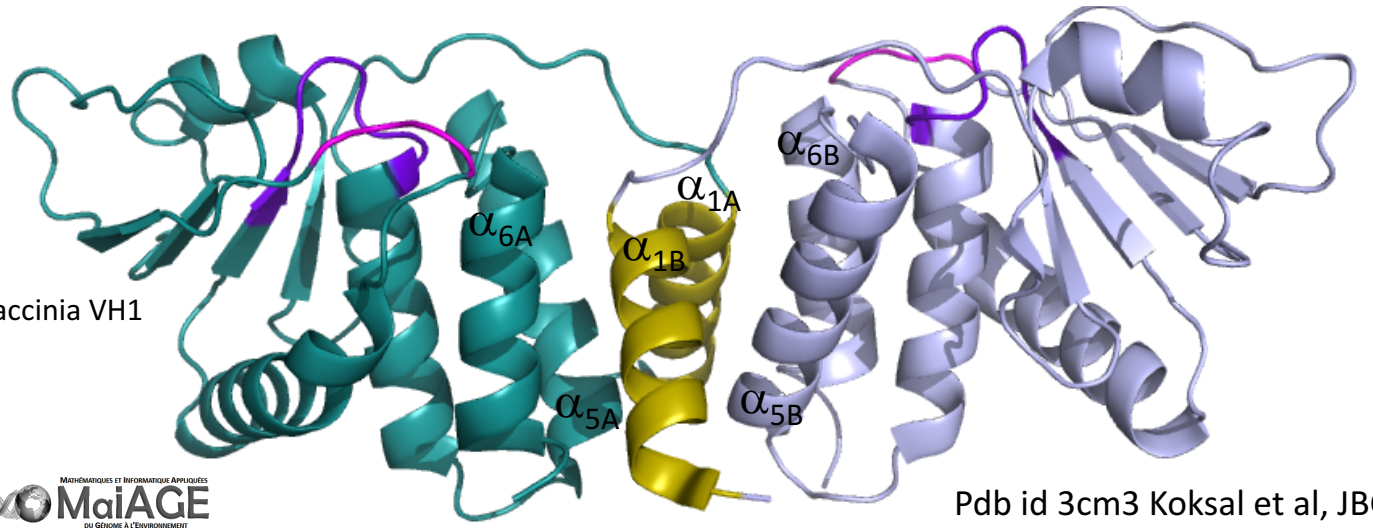
➡ The interface engages the same cluster of helices α_1, α_5 et α_6



Orf OH1

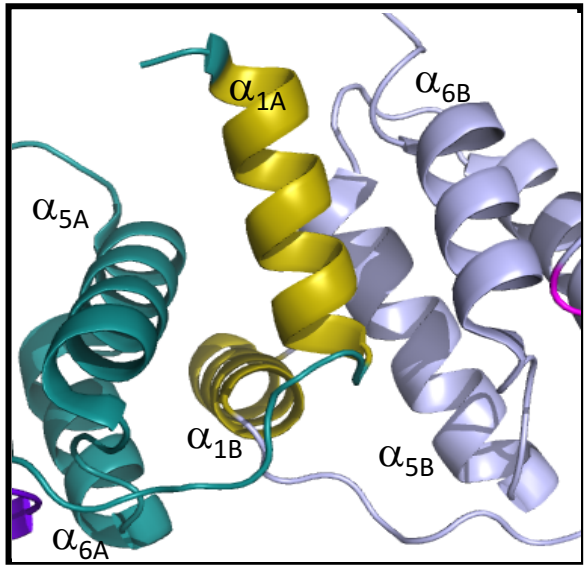
Interface of 678 Å²





Vaccinia VH1

Interface of 978 Å²



Pdb id 3cm3 Koksal et al, JBC 2009

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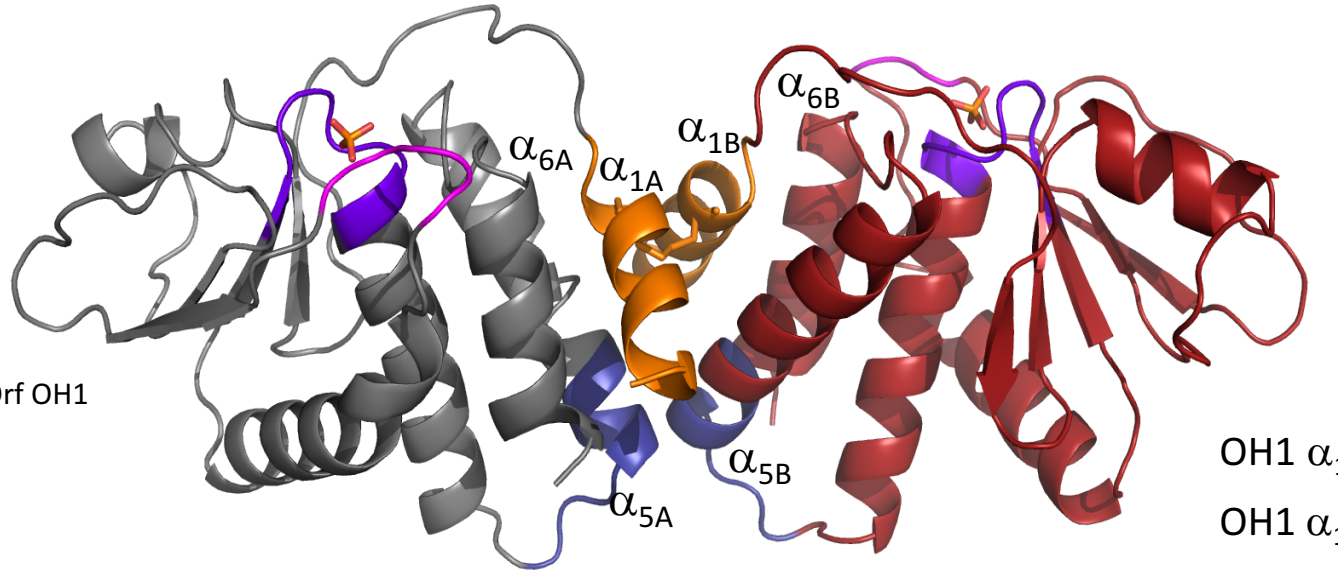
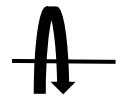
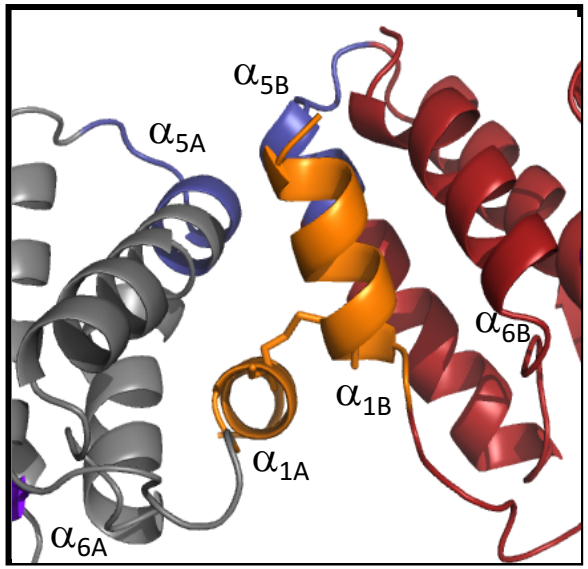


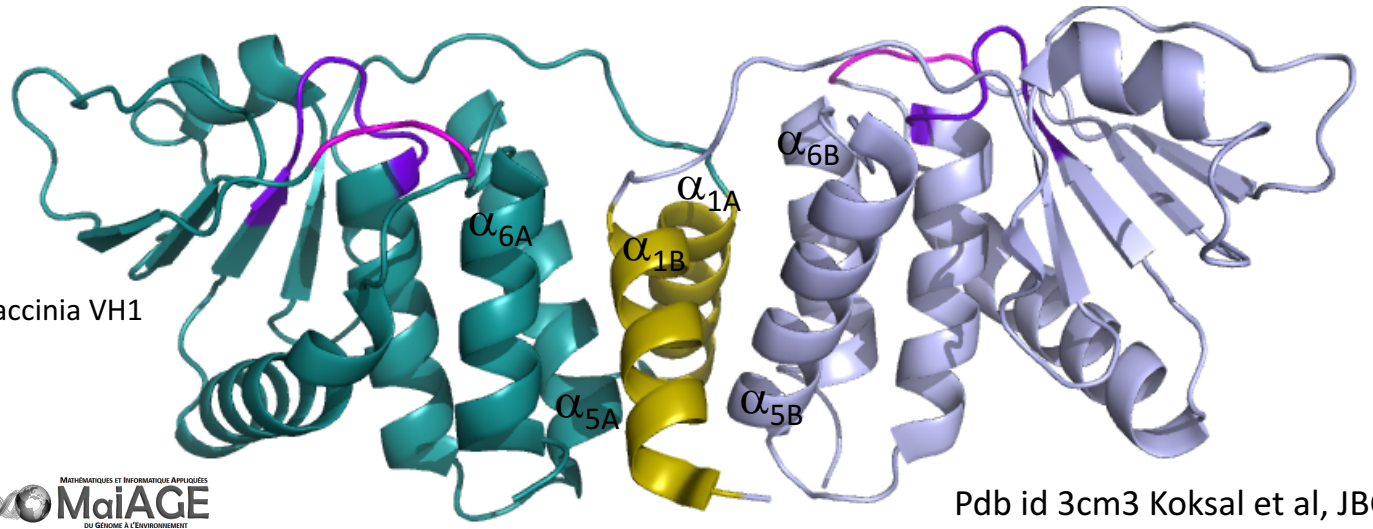
Orf OH1



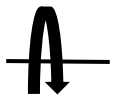
OH1 α_{1A} takes the position of VH1 α_{1B}

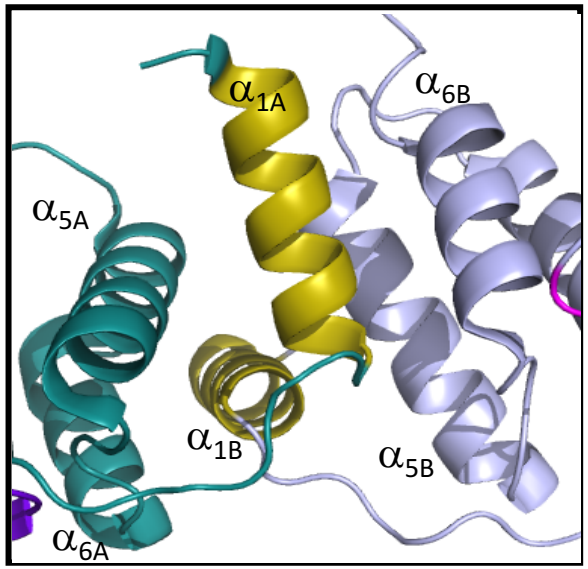
OH1 α_{1B} takes the position of VH1 α_{1A}





Vaccinia VH1



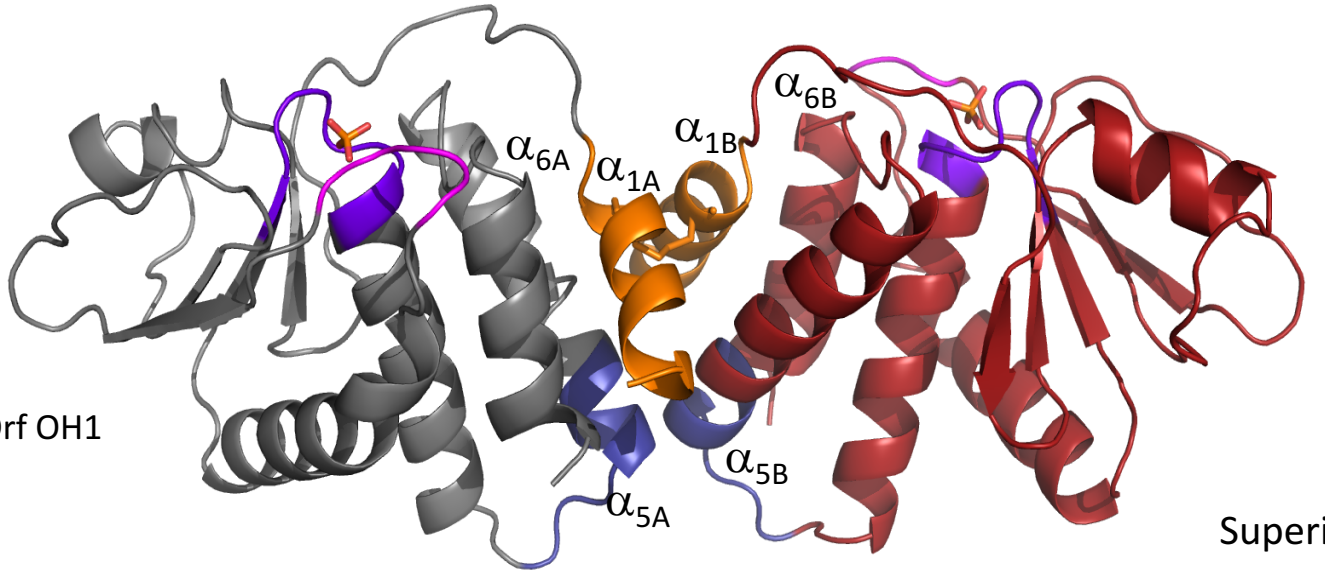


Pdb id 3cm3 Koksal et al, JBC 2009

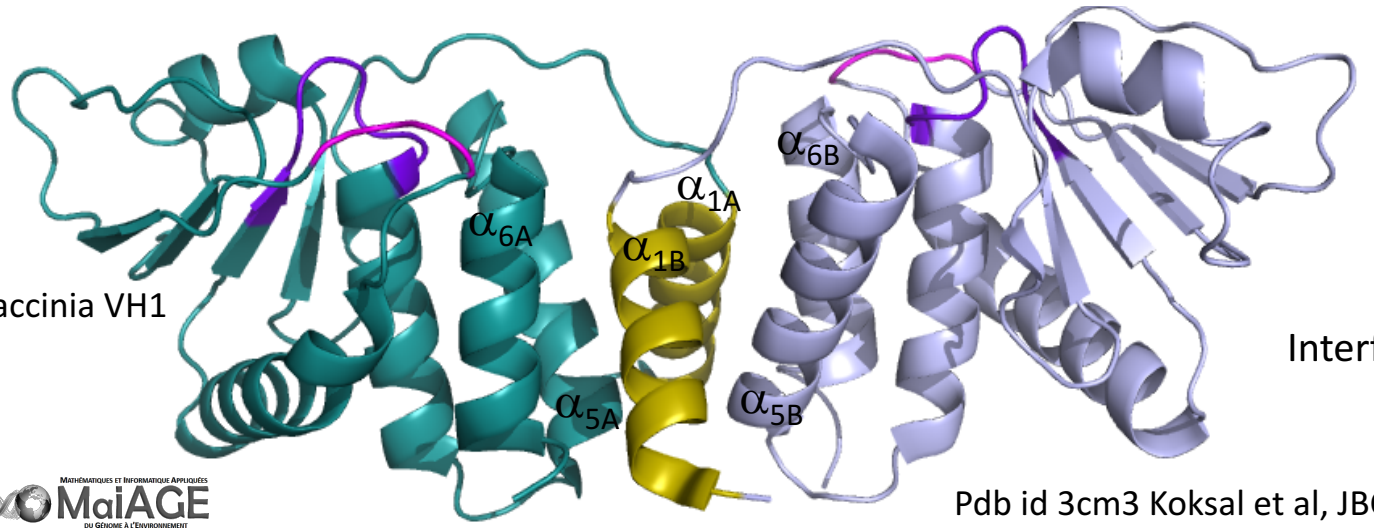
Orf virus : tyrosine phosphatase

Solved structure reveals a dimer organization distinct from Vaccinia phosphatase

➡ Incompatible with domain swapping

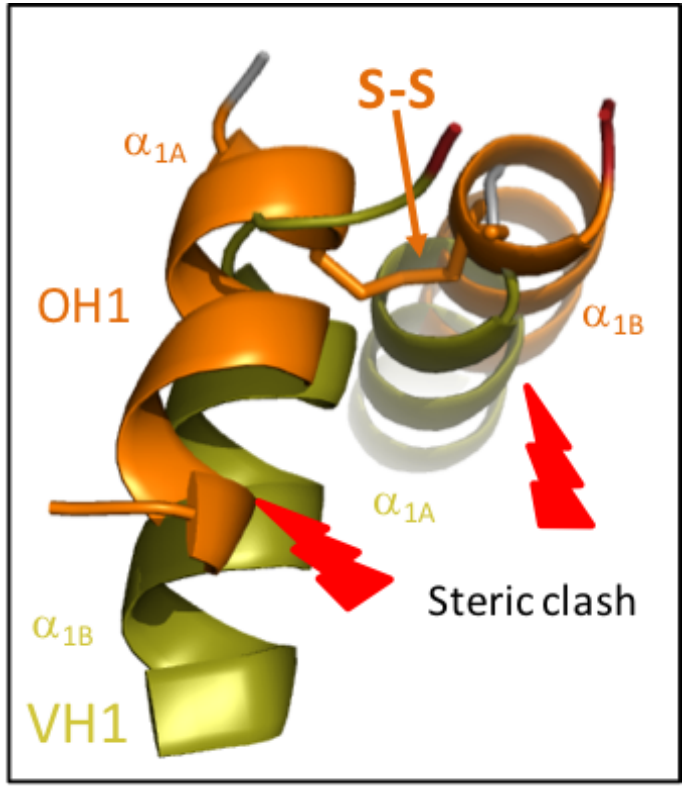


Orf OH1



Vaccinia VH1

Superimposition of the two dimers



Interface of 978 Å^2

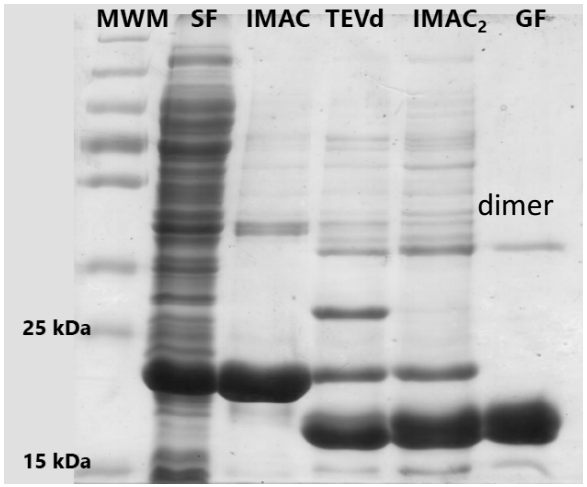
Pdb id 3cm3 Koksal et al, JBC 2009

Orf virus : tyrosine phosphatase

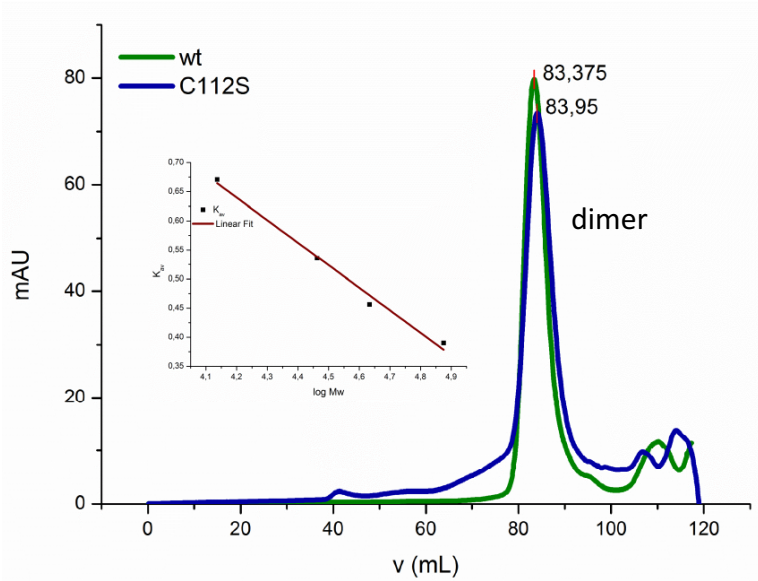
Solved structure reveals a dimer organization distinct from Vaccinia phosphatase

➡ Not a crystallization bias because

- Production of recombinant OH1



- Size exclusion chromatography

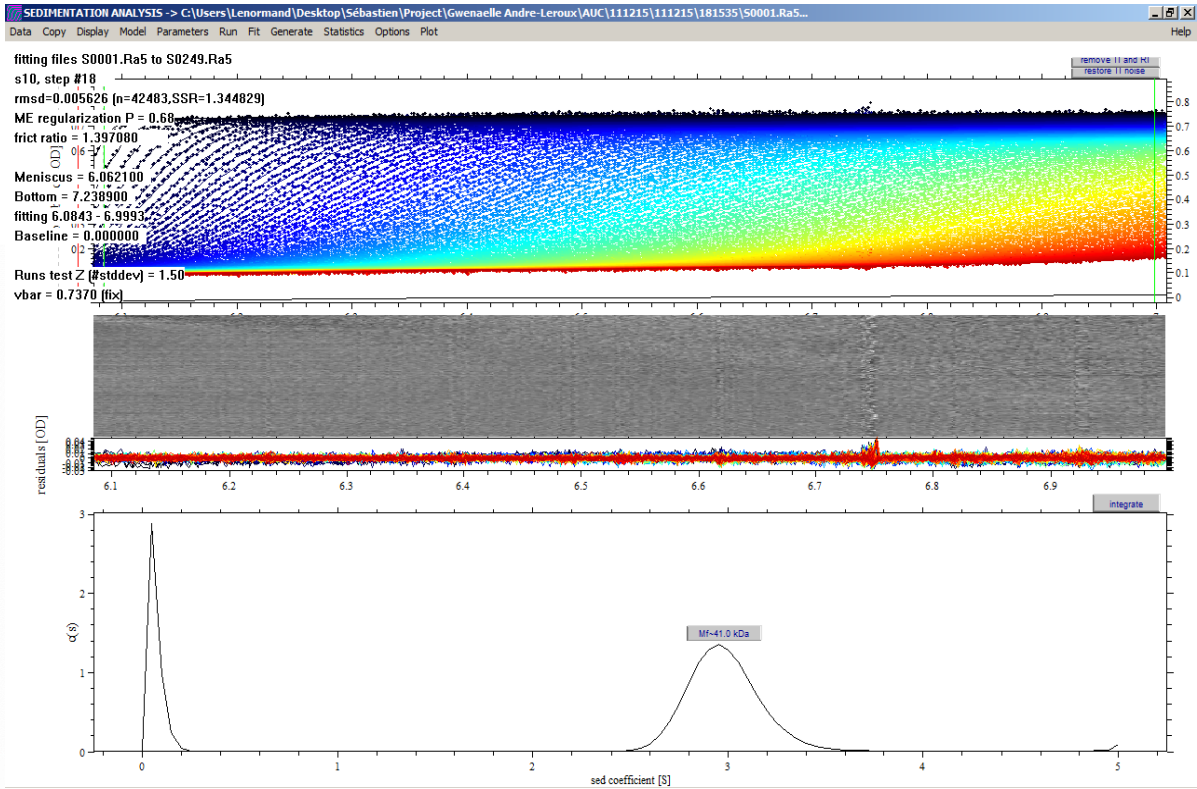


OH1 is a dimer in solution

C15S shows also a dimer (data not shown)

C15S is not mandatory for a dimerization

- Ultra Analytical Centrifugation

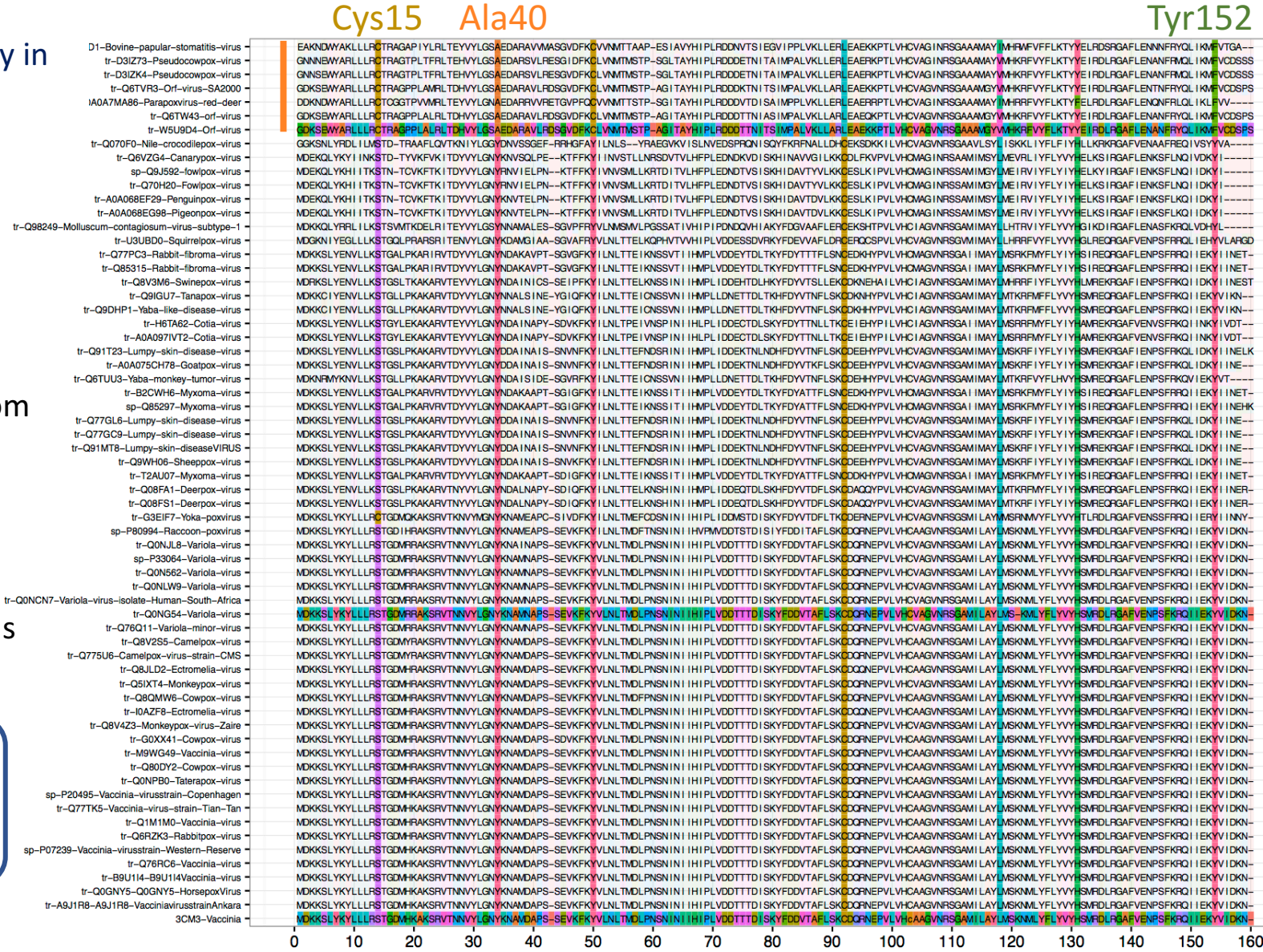


40 kDa = 2*20 kDa

Detection of Parapox specific features of OH1

- Cys 15 and 6 residues insertion conserved but only in Parapoxviruses
- Phylogenetic studies explore how parapoxvirus associates N-terminal Ser15 to Cys substitution

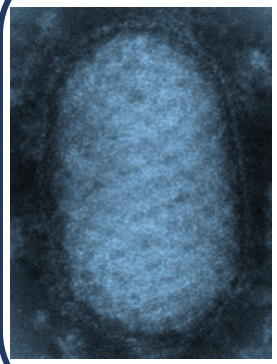
- Multiple sequence alignment of 64 phosphatases from Chordopox family
- Tcoffee
- Position with >50% gaps filtered
- Correlation techniques based on mutual informations

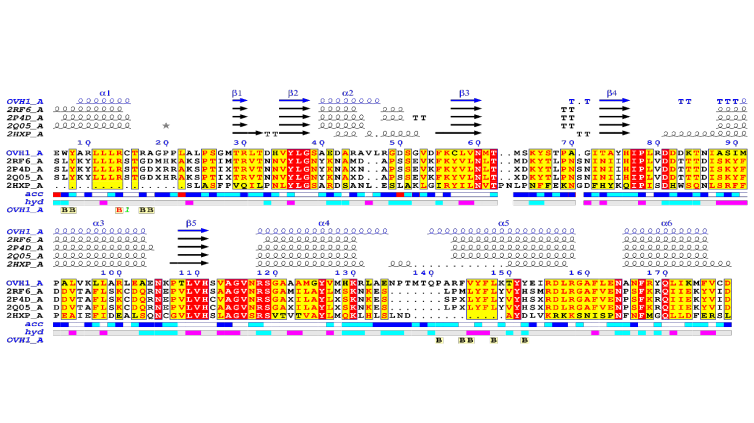


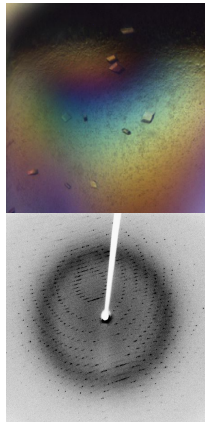
Ala40 locates in the vicinity of the active site

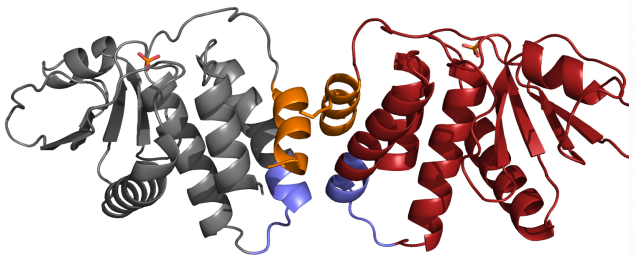
Tyr152 could stabilize helix α_1

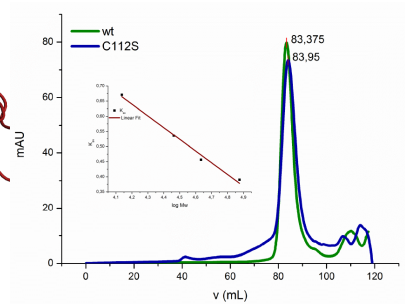
OH1 from Orf virus: a new tyrosine phosphatase

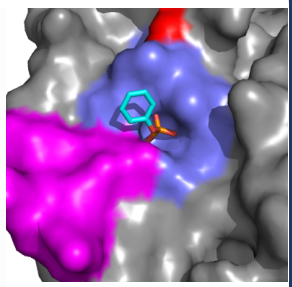








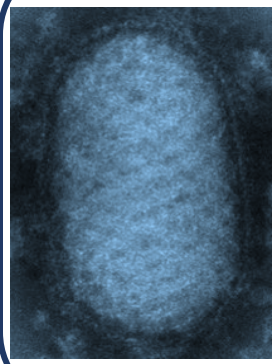
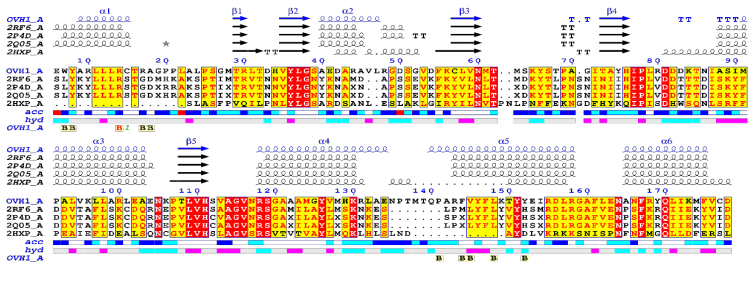
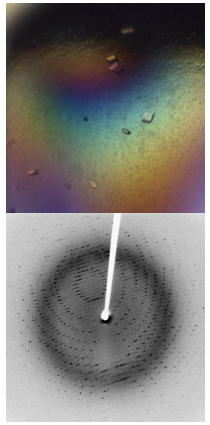
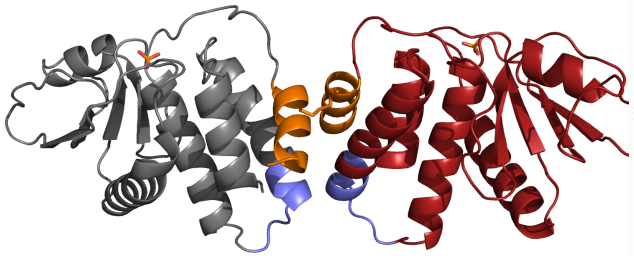
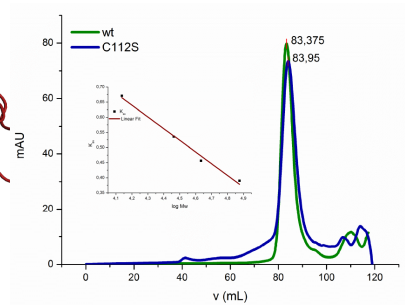


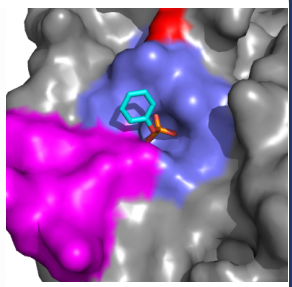


Highlights

- OH1 displays distinct structural features compared to VH1 phosphatases
- Orf virus OH1 is a covalent dimer involving the N-terminal Cys15
- Orf virus OH1 possibly depicts the structure of Parapoxvirus genus phosphatases

OH1 from Orf virus: a new tyrosine phosphatase

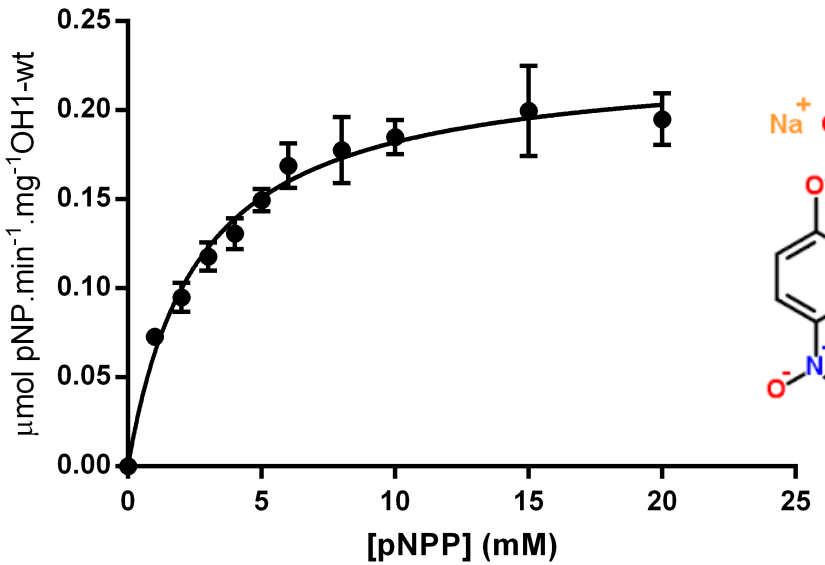


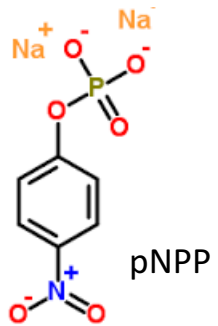
Outline

- Orf virus introduction
- OH1 structural characterization *in silico/in vitro*
- OH1 functional characterization *in silico/in vitro*
- Conclusions & perspectives

Tyrosine phosphatase

OH1 shows a phosphatase activity *in vitro*





pNPP

Monitoring of dephosphorylation by absorbance at 405nm of pNP

Phosphatase activity assayed at 37°C using the artificial substrate pNPP

Michaelis-Menten Kinetics constants

OH1 wt	OH1 C15S
K_m = 2.6 mM K_{cat} = 0.08 s^{-1}	K_m = 2.0 mM K_{cat} = 0.08 s^{-1}

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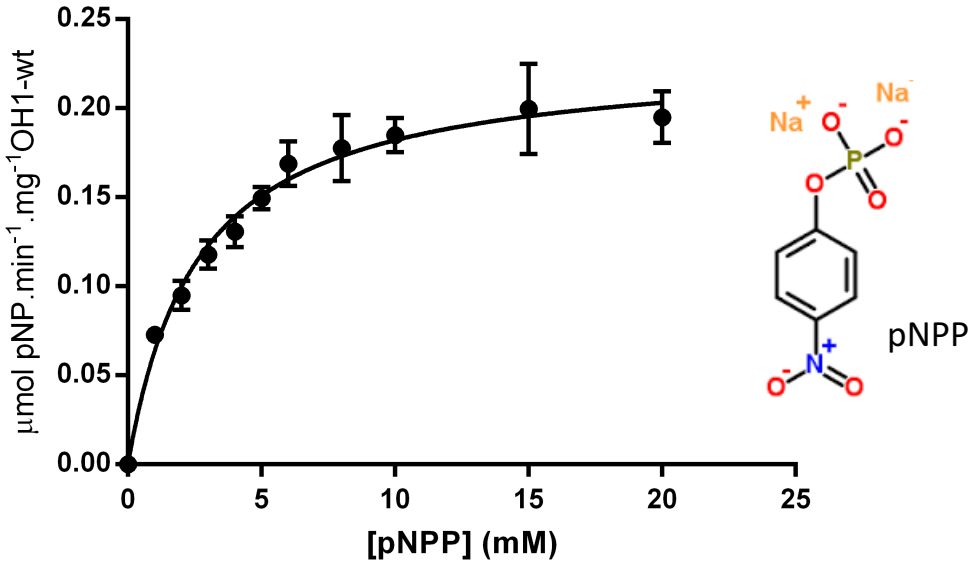

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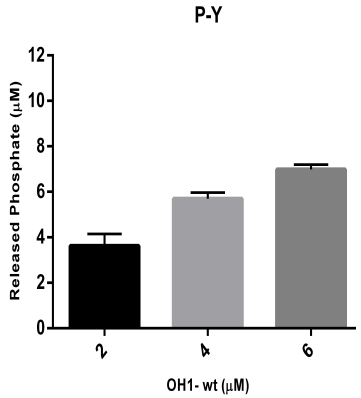
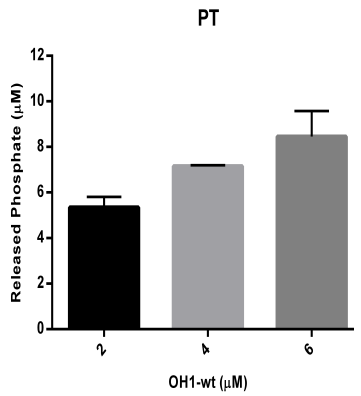
OH1 shows a DUSP –Dual Specificity Phosphatase *in vitro*

Active on p-Tyr containing protein



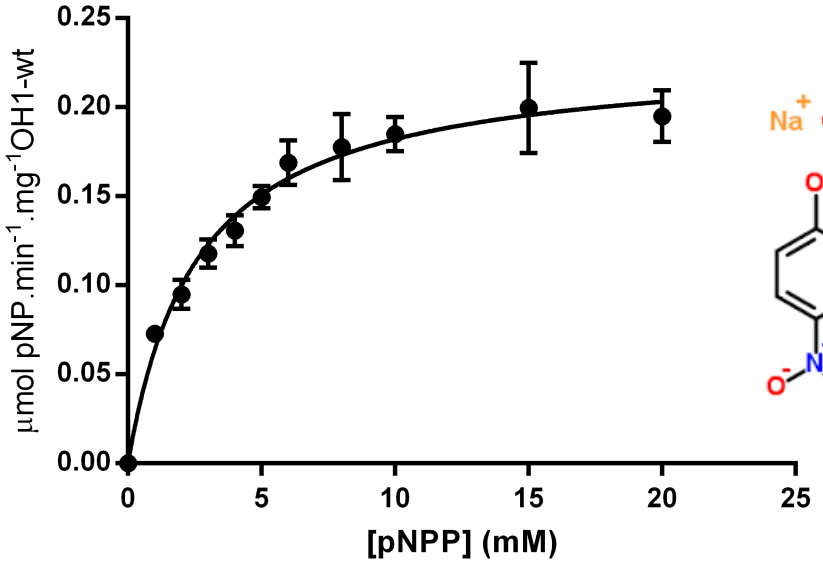
Active on p-Thr 17mer peptide

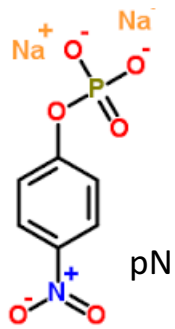


Dose dependent manner

Tyrosine phosphatase

OH1 shows a phosphatase activity *in vitro*





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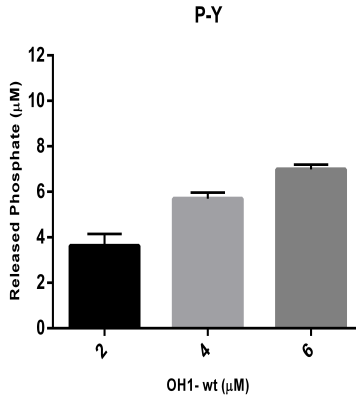
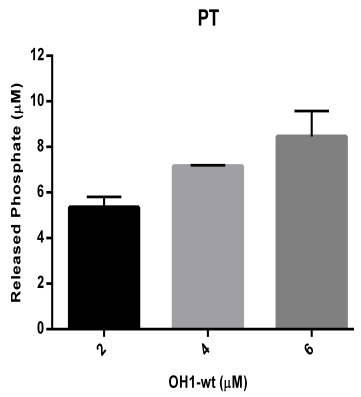
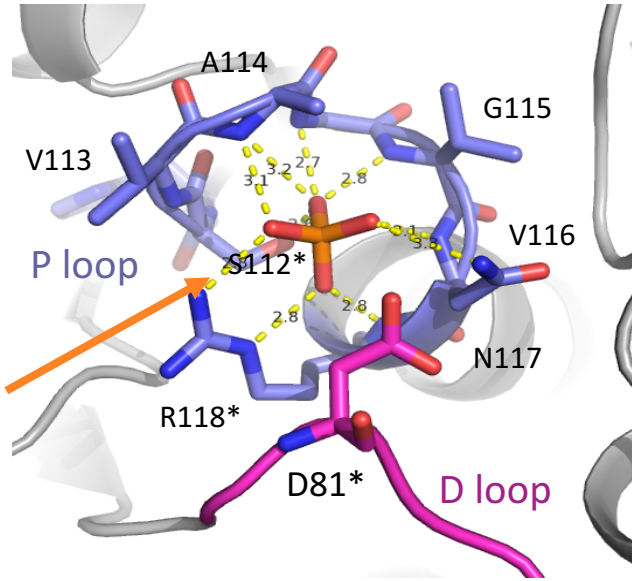


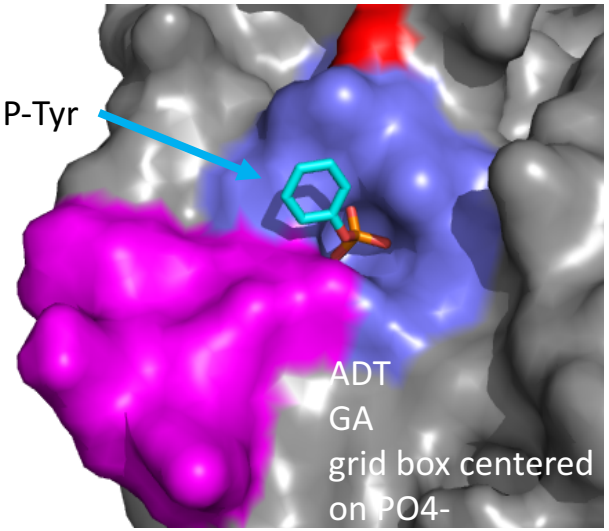
Active on p-Thr 17mer peptide



Dose dependent manner

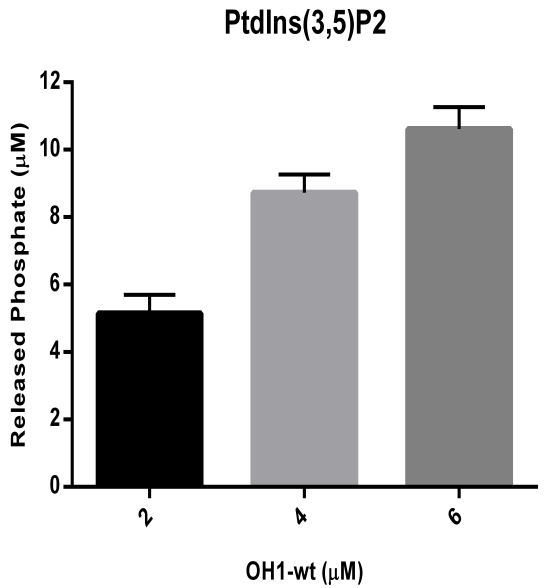
OH1 accommodates p-Tyr in the active site

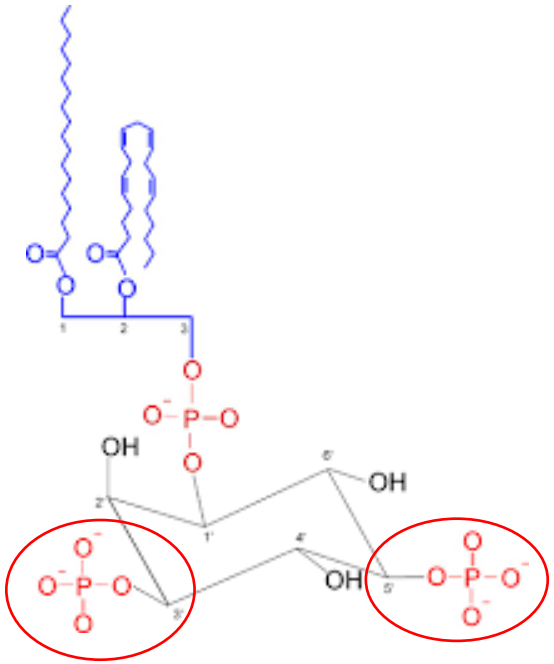




A new tyrosine phosphatase

OH1 highlights a new activity *in vitro: a* triple specificity

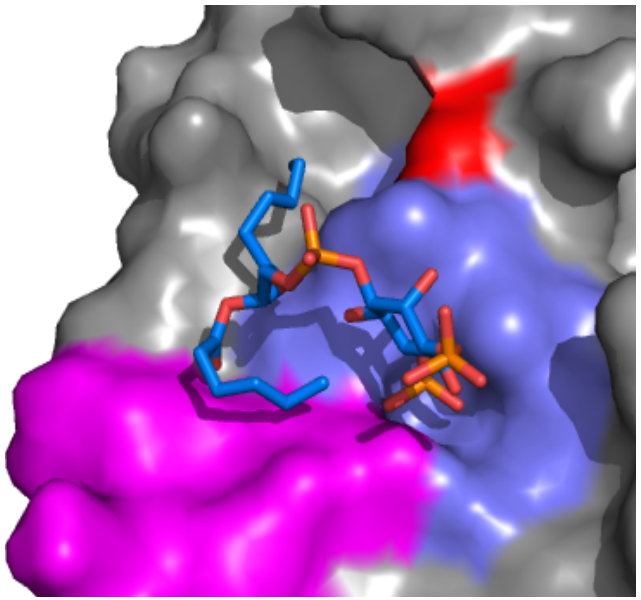


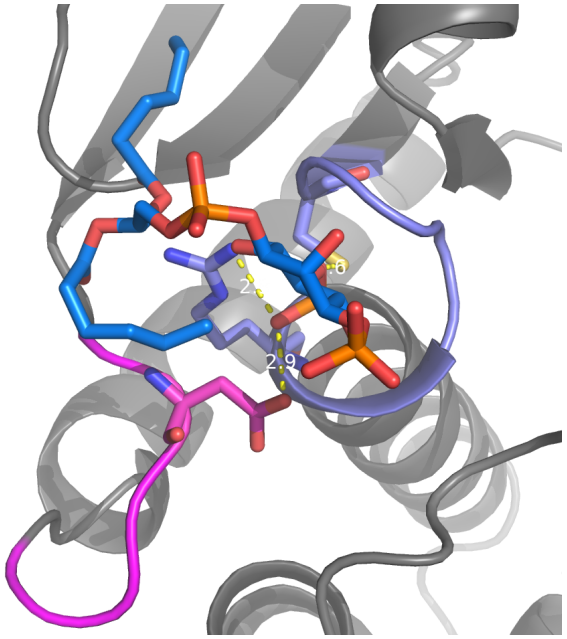


Phosphoinositides can be dephosphorylated

PtdIns3P and PtdIns5 (3,5)P2 can be dephosphorylated

Docking evidences PdtIns bind in the pocket





The phospho-sugar part can be accommodated

The phospho-group in 5’ binds into the active pocket

Relevant physiologically?

A new tyrosine phosphatase

Why this phospholipid specificity should be relevant?

PtdIns

- Belong to phosphatidyl glycerides
- Compose the cytosolic side of eukaryotic cell membrane
- Regulate membrane homeostasis
- Are signaling lipids

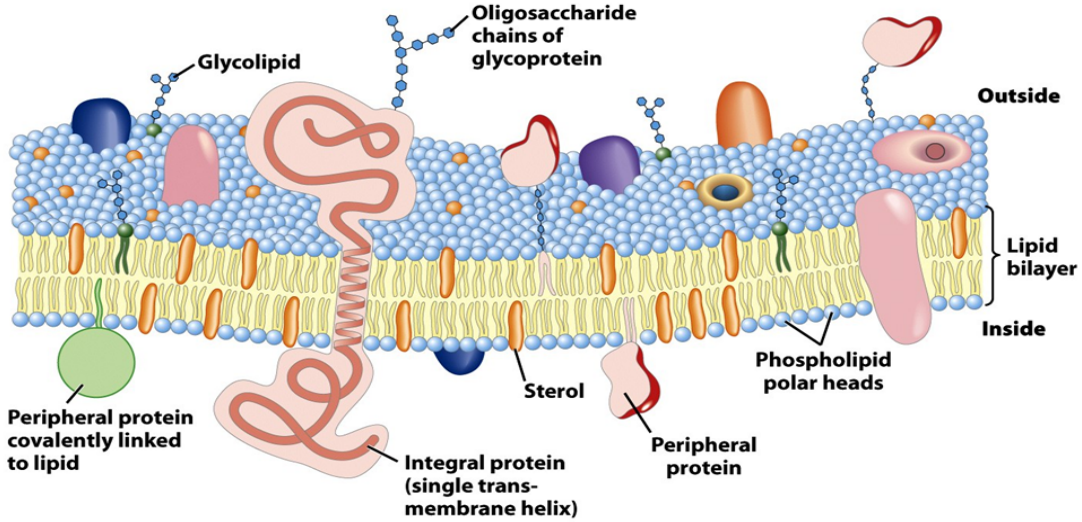
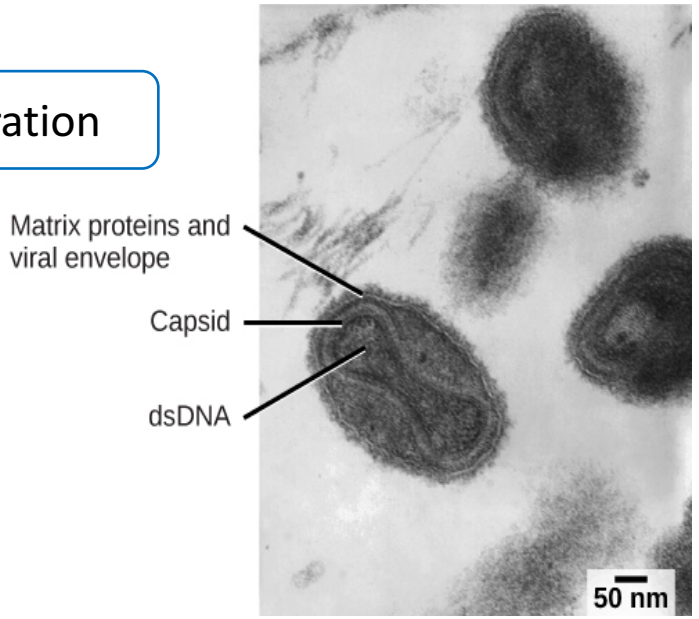


Figure 11-3
Lehninger Principles of Biochemistry, Fifth Edition

OH1 could extract and recycle host phospholipids to form viral envelop during virion maturation

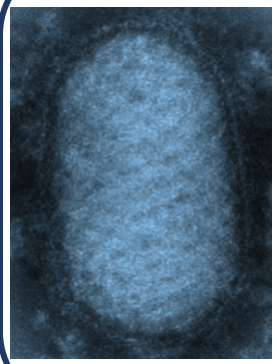
Viral envelop allows to:

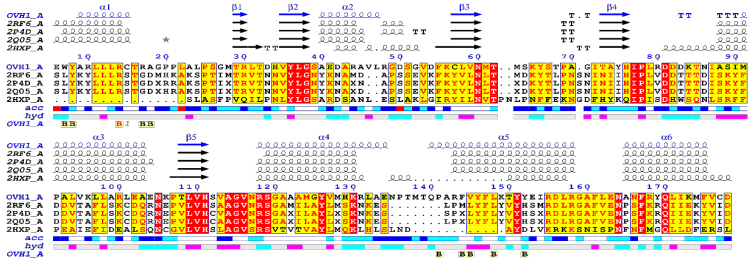
- Escape from the cell,
- Stay stable
- Get protected from immune system
- Reinfect cell

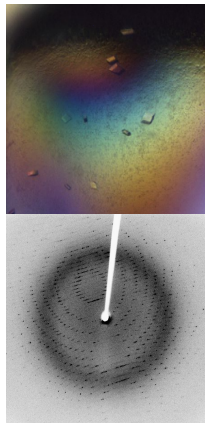


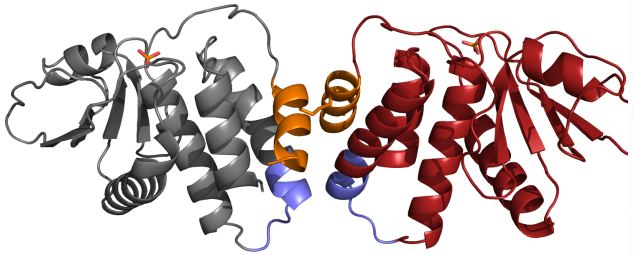
Evolution in favor of enveloped virus : Influenza, HIV, Ebola Virus

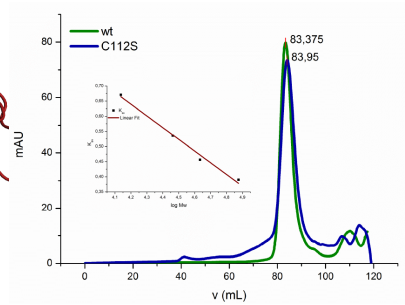
OH1 from Orf virus: a new tyrosine phosphatase

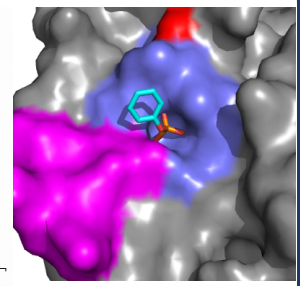












Highlights

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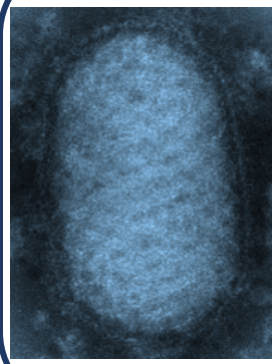
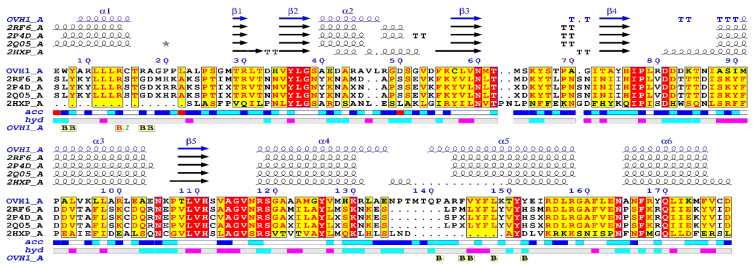
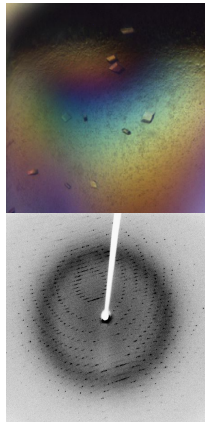
- Orf virus OH1 is a covalent dimer involving the N-terminal Cys15
- Orf virus OH1 possibly depicts the structure of Parapoxvirus genus phosphatases
- Orf virus OH1 is a dual specifity phosphatase that presents activity towards PInsP *in vitro*

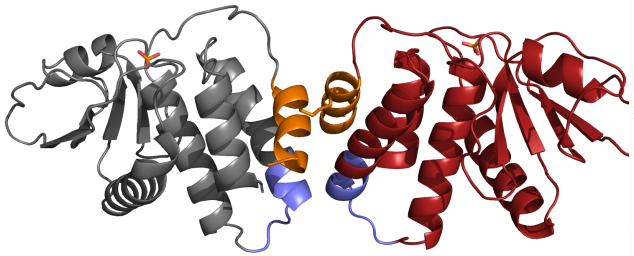
Segovia D, xxx, André-Leroux G & Villarino A, Submitted in JMB in may 2017

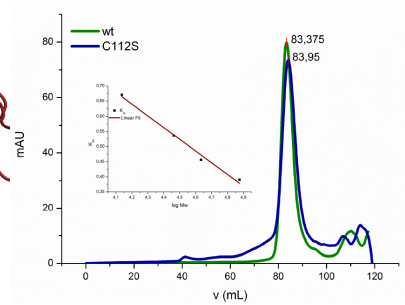
OH1 from Orf virus: a new tyrosine phosphatase

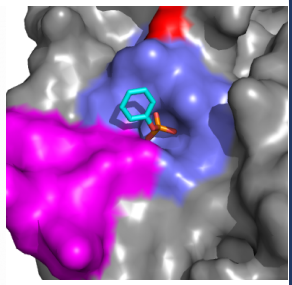












Outline

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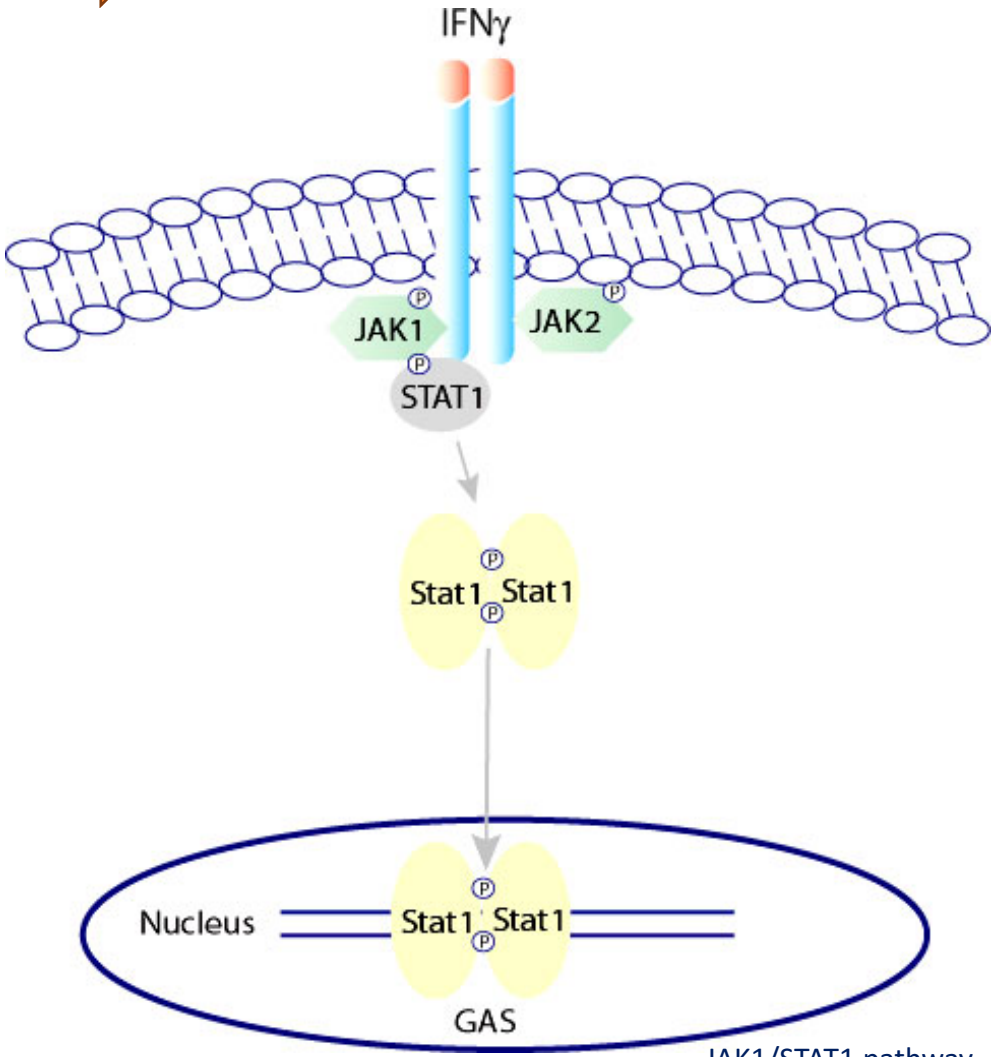
- OH1 structural characterization *in silico/in vitro*
- OH1 functional characterization *in silico/in vitro*
- Conclusions & perspectives

Interaction with STAT1

Hypothesis: by analogy with Vaccinia and Variola viruses, OH1 could dephosphorylate STAT1



JAK1/STAT1 pathway

STAT : Signal Transducers and Activators of Transcription

STAT1 is activated upon phosphorylation of its tyrosine Tyr701 -> STAT1 (P)

STAT1 (P) proteins homodimerize

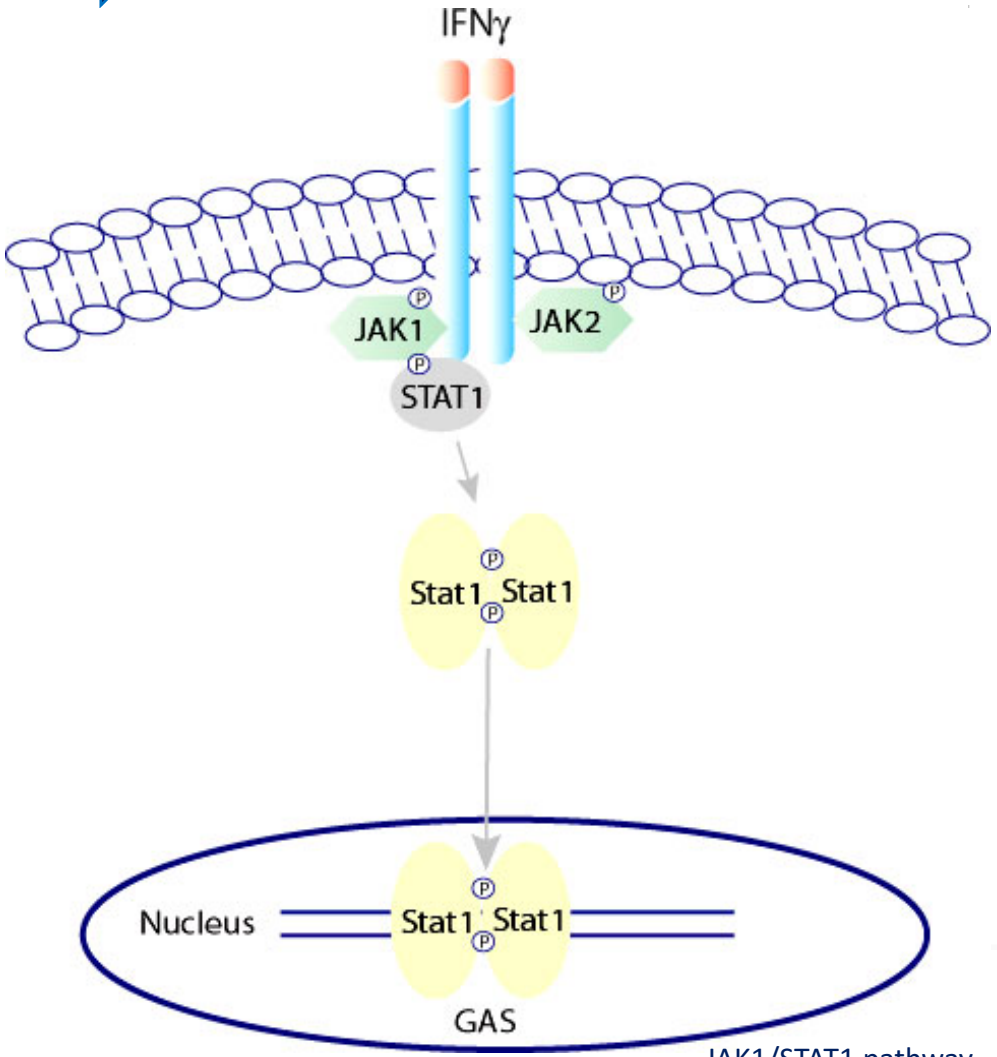
STAT1 (P) homodimer is now capable to translocate to the nucleus

STAT1 (P) transactivates Stat-responsive genes

STAT1 (P) is involved in cardiac hypertrophy, apoptosis, and inflammation.

Interaction with STAT1

OrfV dephosphorylates STAT1



JAK1/STAT1 pathway

Format: Abstract Send to

Virus Res. 2015 Oct 2;208:180-8. doi: 10.1016/j.virusres.2015.06.014. Epub 2015 Jun 22.

Orf virus inhibits interferon stimulated gene expression and modulates the JAK/STAT signalling pathway.

Harvey R[1], McCaughan C[2], Wise LM[3], Mercer AA[4], Fleming SB[5].

Author information

Abstract

Interferons (IFNs) play a critical role as a first line of defence against viral infection. Activation of the Janus kinase/signal transducer and activation of transcription (JAK/STAT) pathway by IFNs leads to the production of IFN stimulated genes (ISGs) that block viral replication. The Parapoxvirus, Orf virus (ORFV) induces acute pustular skin lesions of sheep and goats and is transmissible to man. The virus replicates in keratinocytes that are the immune sentinels of skin. We investigated whether or not ORFV could block the expression of ISGs. The human gene GBP1 is stimulated exclusively by type II IFN while MxA is stimulated exclusively in response to type I IFNs. We found that GBP1 and MxA were strongly inhibited in ORFV infected HeLa cells stimulated with IFN-γ or IFN-α respectively. Furthermore we showed that ORFV inhibition of ISG expression was not affected by cells pretreated with adenosine N1-oxide (ANO), a molecule that inhibits poxvirus mRNA translation. This suggested that new viral gene synthesis was not required and that a virion structural protein was involved. We next investigated whether ORFV infection affected STAT1 phosphorylation in IFN-γ or IFN-α treated HeLa cells. We found that ORFV reduced the levels of phosphorylated STAT1 in a dose-dependent manner and was specific for Tyr701 but not Ser727. Treatment of cells with sodium vanadate suggested that a tyrosine phosphatase was responsible for dephosphorylating STAT1-p. ORFV encodes a factor, ORFV057, with homology to the vaccinia virus structural protein VH1 that impairs the JAK/STAT pathway by dephosphorylating STAT1. Our findings show that ORFV has the capability to block ISG expression and modulate the JAK/STAT signalling pathway.

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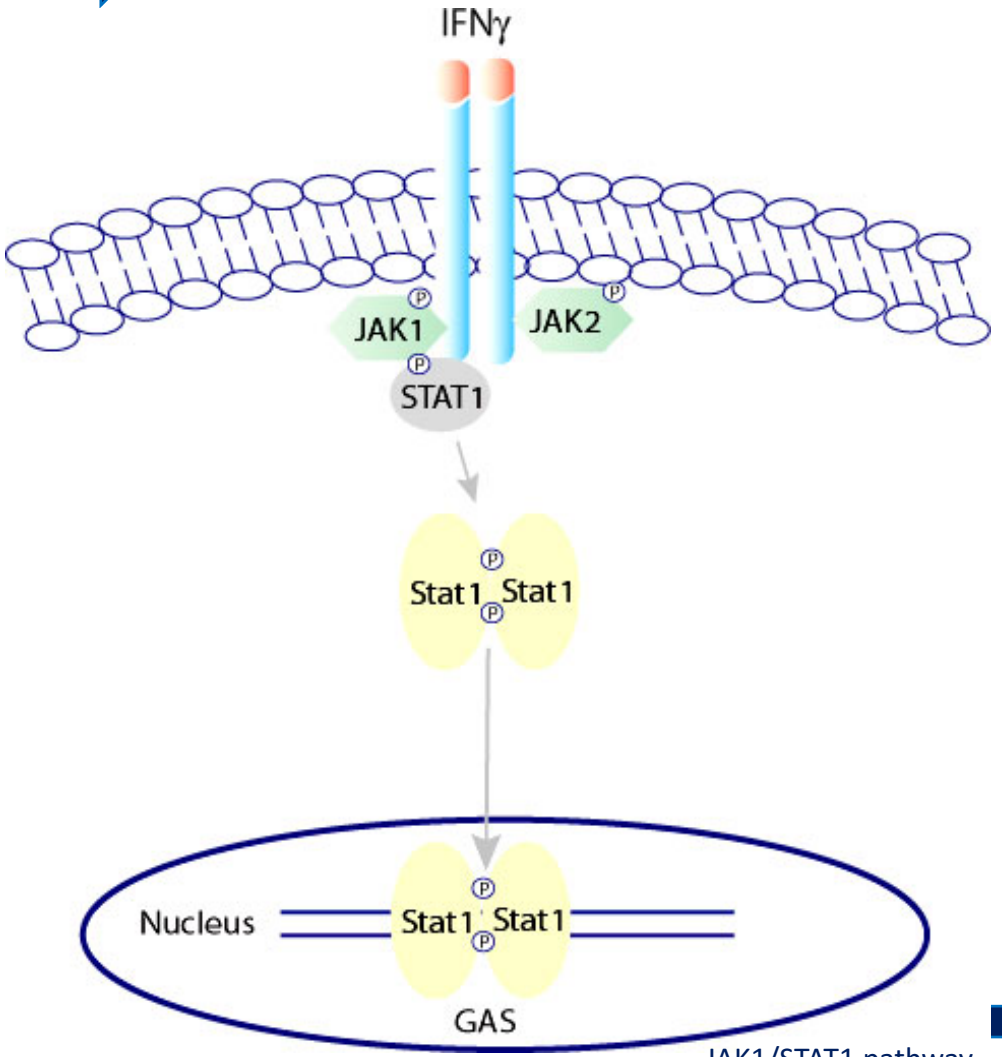
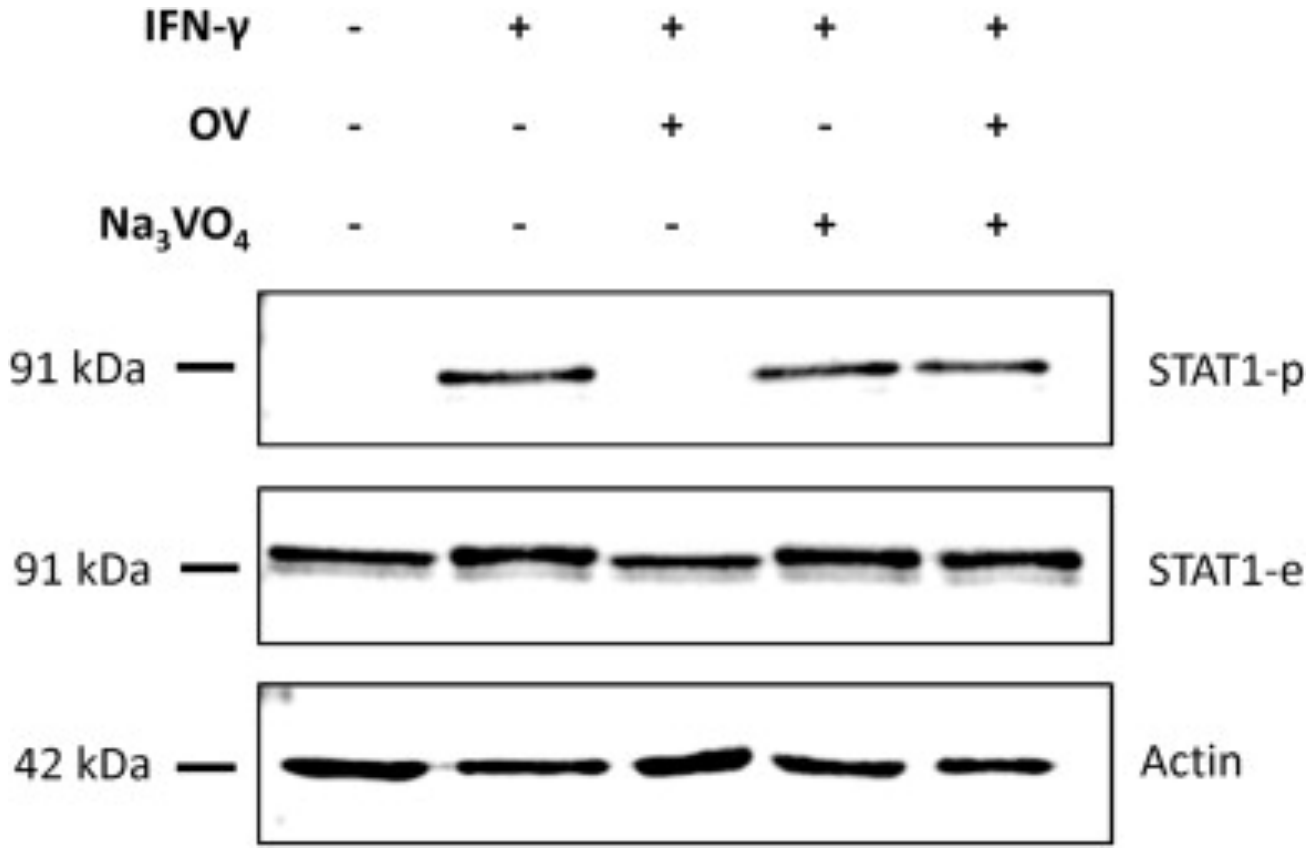


JAK1/STAT1 pathway



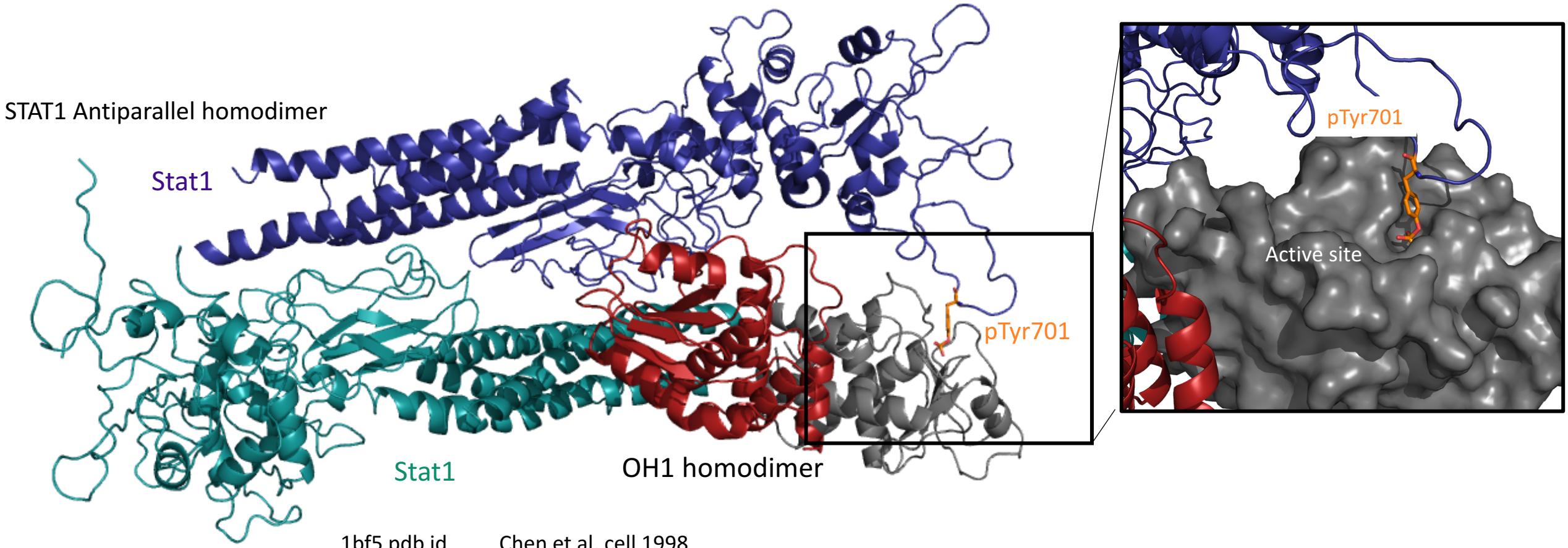
Harvey et al, 2015

Modeling of OH1/STAT1 direct interaction

Interaction with STAT1

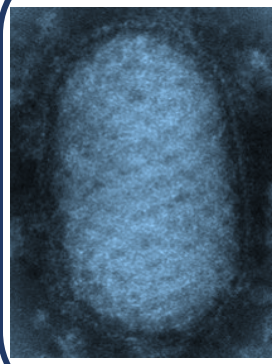
Running hypothesis: OH1 dephosphorylates STAT1 by a direct interaction

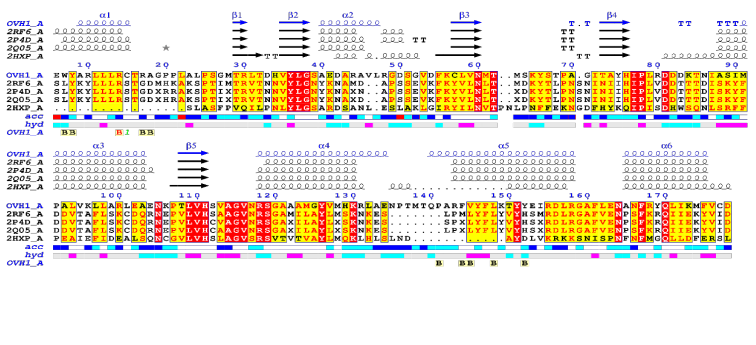
- Modeling of the missing loop and phosphorylation at Tyr 701
- Docking of Protein/Protein interaction using HADDOCK

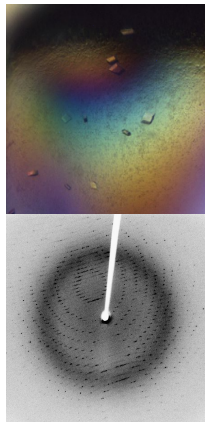


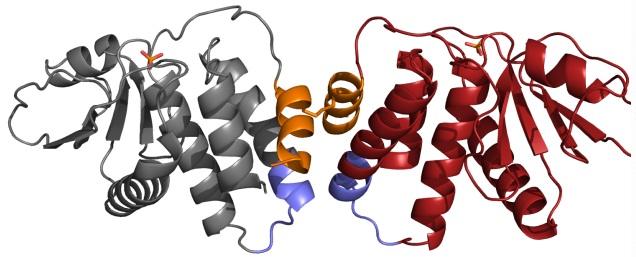
1bf5 pdb id Chen et al, cell 1998

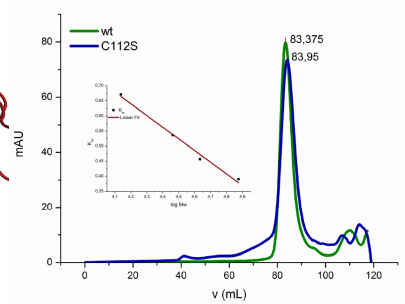
OH1 from Orf virus: a new tyrosine phosphatase

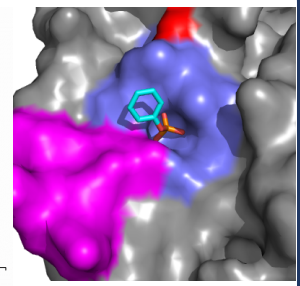










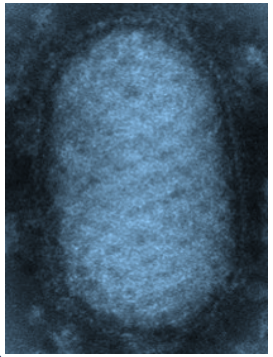


Perspectives

- OH1 /STAT1 needs further investigations *in silico*
- Pull-down *in vitro* experiments show that OH1 interacts directly with STAT1
- Pull-down *in vitro* experiments show that OH1 interacts with GAPDH - Glyceraldehyde-3-phosphate dehydrogenase
- GAPDH is a house-keeping protein involved in glycolysis and modulation of the organization of the cytoskeleton
- GAPDH could not only be a metabolic enzyme but in immunity also

Acknowledgements

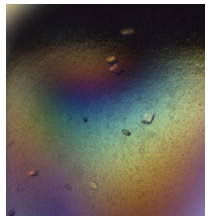
Virology

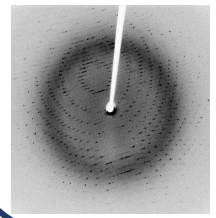


Dario Porley

Mabel Berois

Crystallization





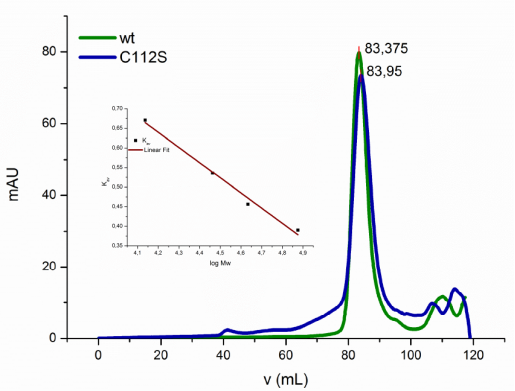
Danilo Segovia

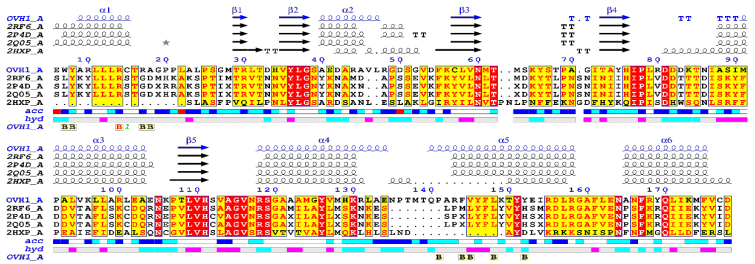
Frederik Saul

Patrick Weber

Ahmed Haouz

Biochemistry



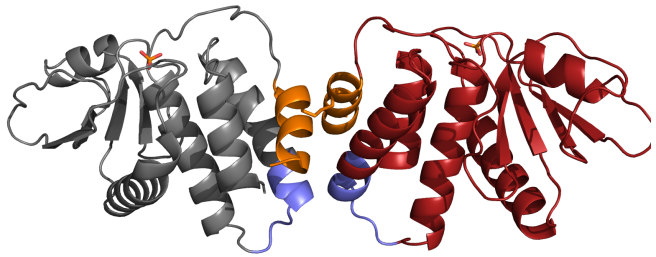


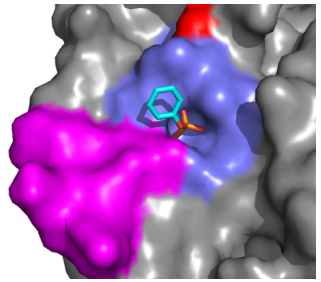
Danilo Segovia

Mariano Martinez

Andrea Villarino

Crystallography, modeling & phylogeny





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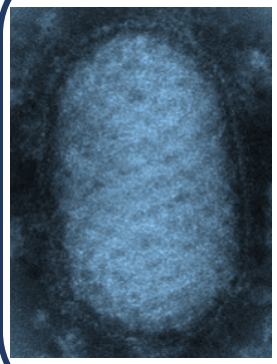
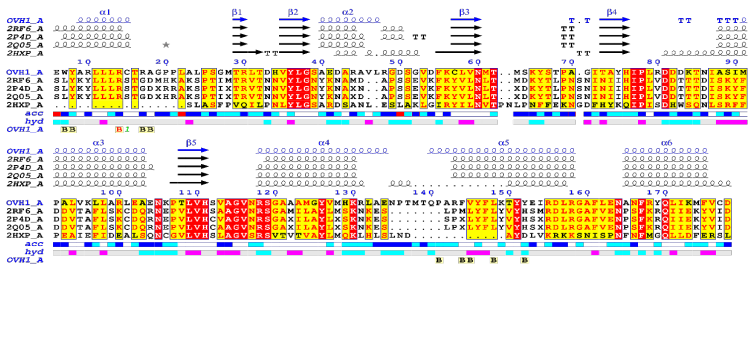
Dario Porley

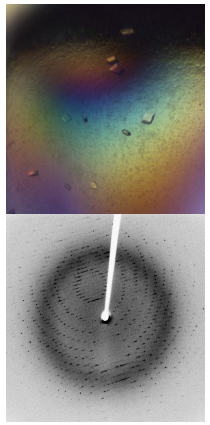
Mahendra Mariadassou

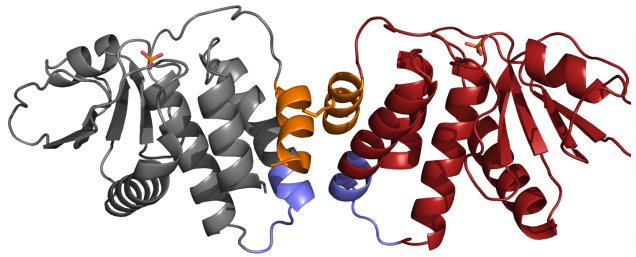
OH1 from Orf virus: a new tyrosine phosphatase

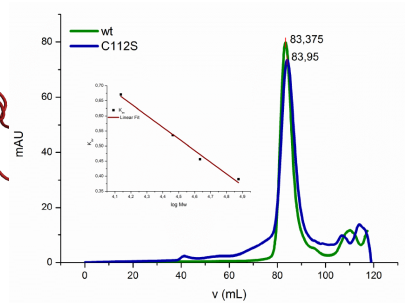
Distinct structural features & triple substrate specificity

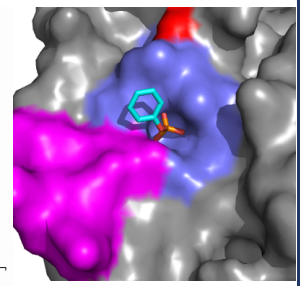












Thank you very much for your kind attention