

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

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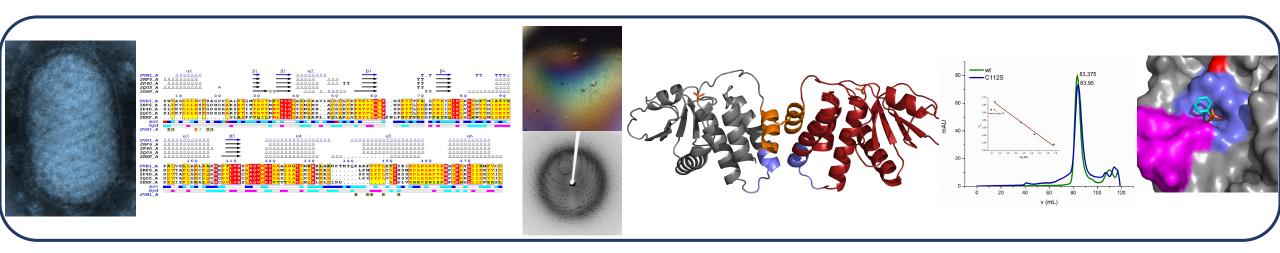
Submitted on 4 Jun2020

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Distinct structural features & triple substrate specificity



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

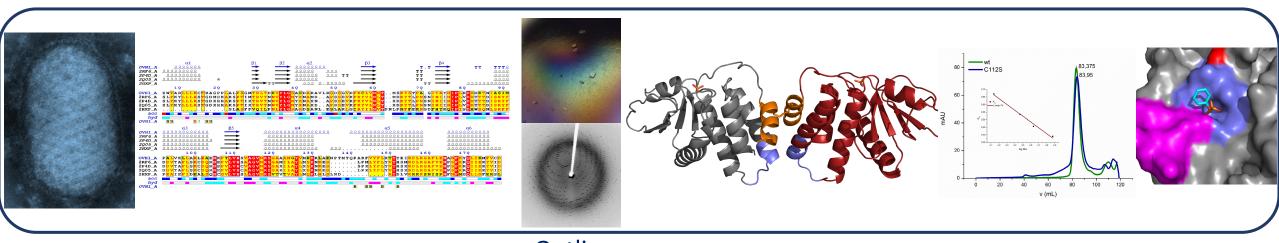
Workshop: « Modelling bio-molecular interactions: A multi-scale approach joining theory and experiments »











#### Outline

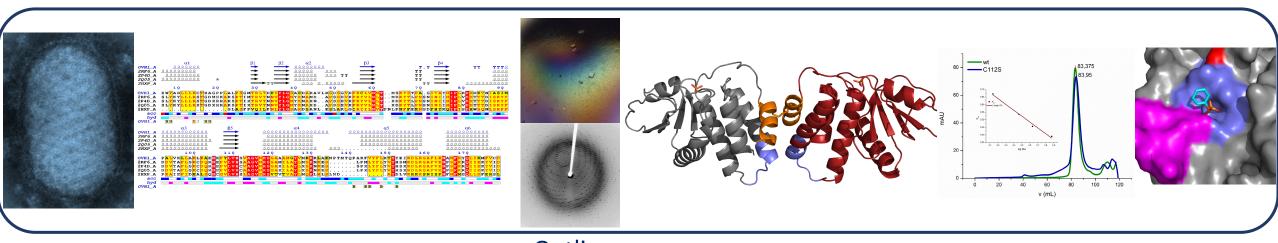
- Orf virus introduction
- OH1 structural characterization *in silico/in vitro*
- OH1 functional characterization in silico/in vitro
- Conclusions & perspectives











#### Outline

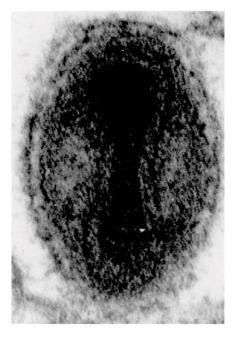
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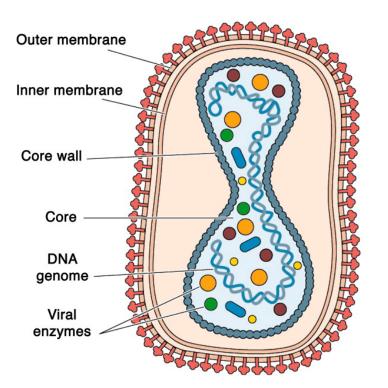




#### Introduction







Orf Poxvirus family Like Vaccinia & Variola virus

Orf = old english for « rough »

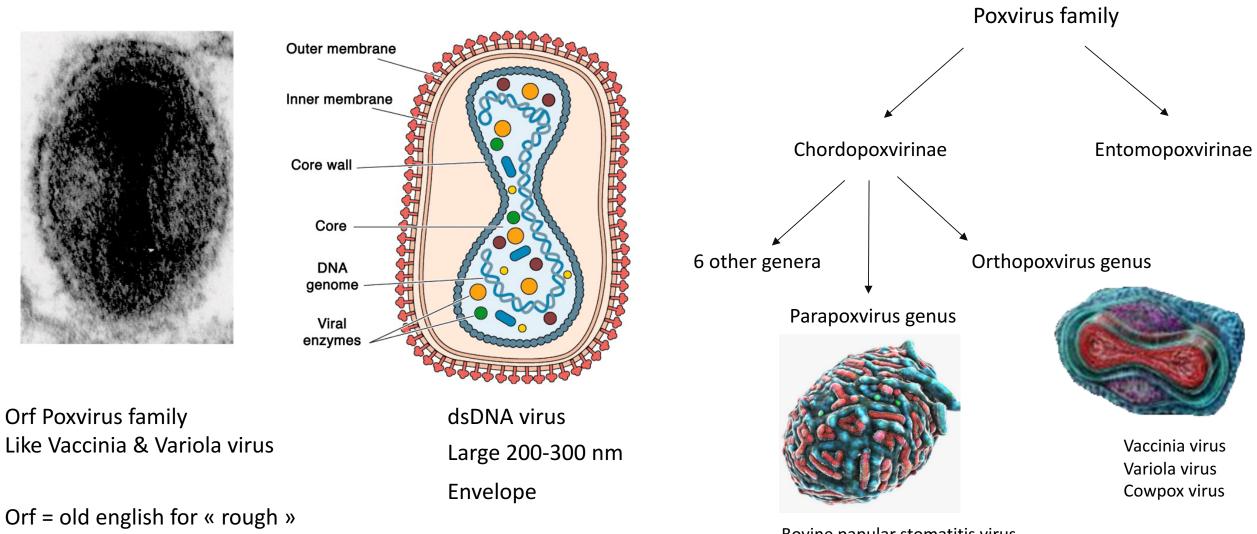
dsDNA virus Large 200-300 nm Envelope





#### Introduction

**Orf virus** 



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



**Orf Poxvirus family** 



### 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





### Orf virus



- Is responsible for Echtyma disease primarily in sheep and goats
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- In its severe form on immune compromised animals can lead to death

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

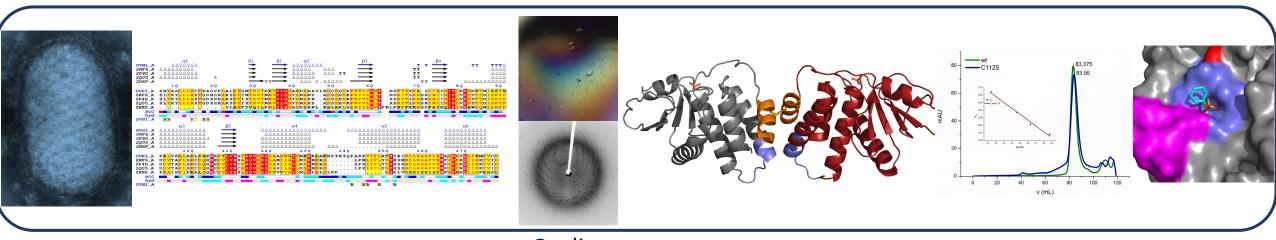








Distinct structural features & triple substrate specificity



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- Orf virus introduction
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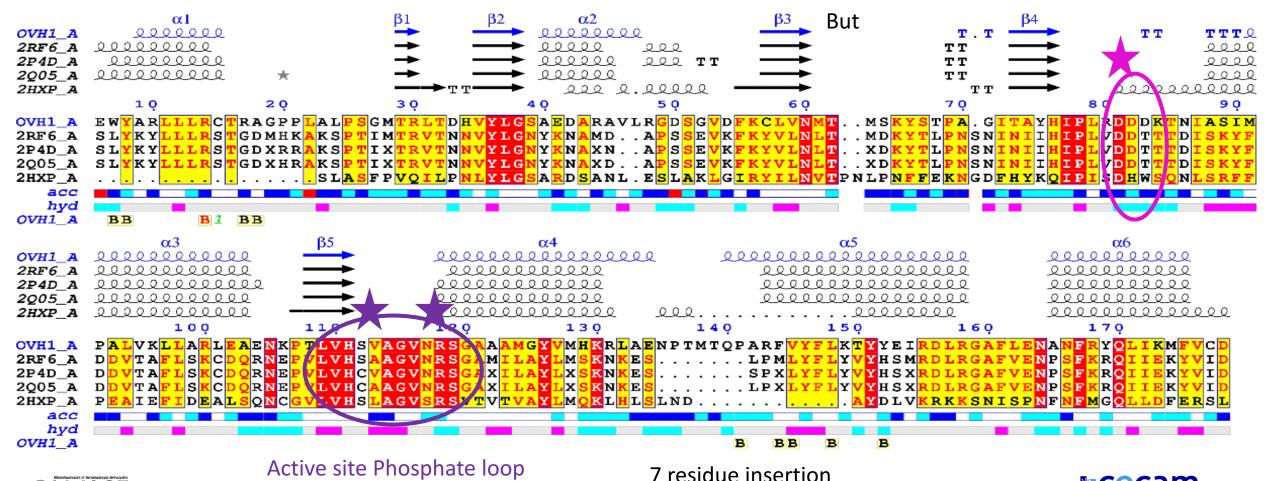


High 40% similarity with Vaccinia and Variola Tyr-phosphatase

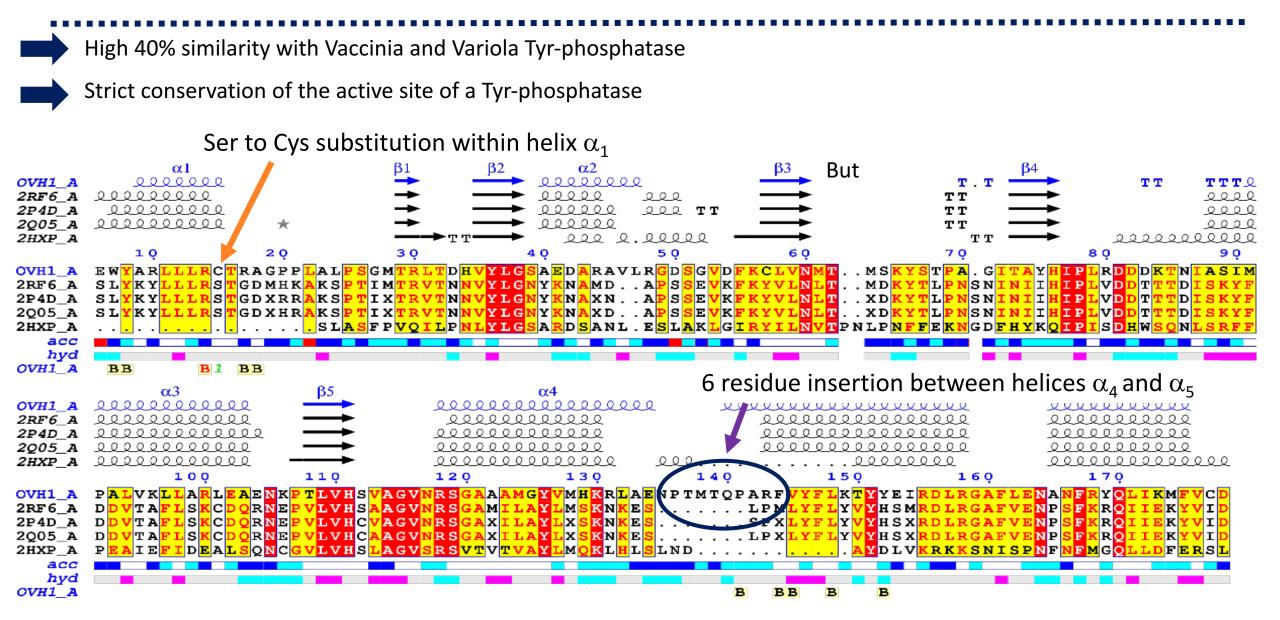
Strict conservation of the active site of a Tyr-phosphatase



**Cecam** 19-20 May



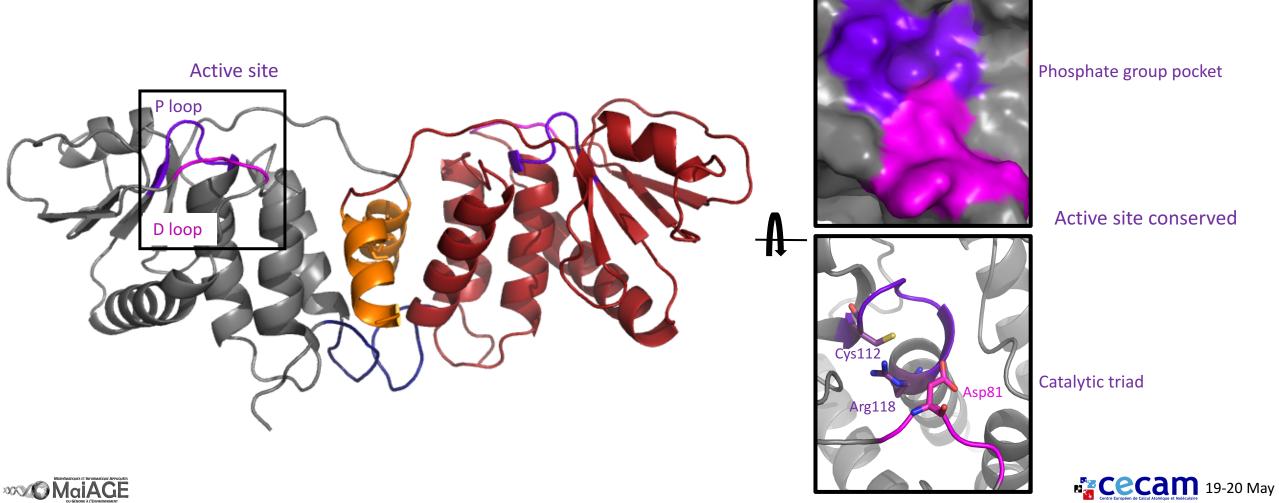


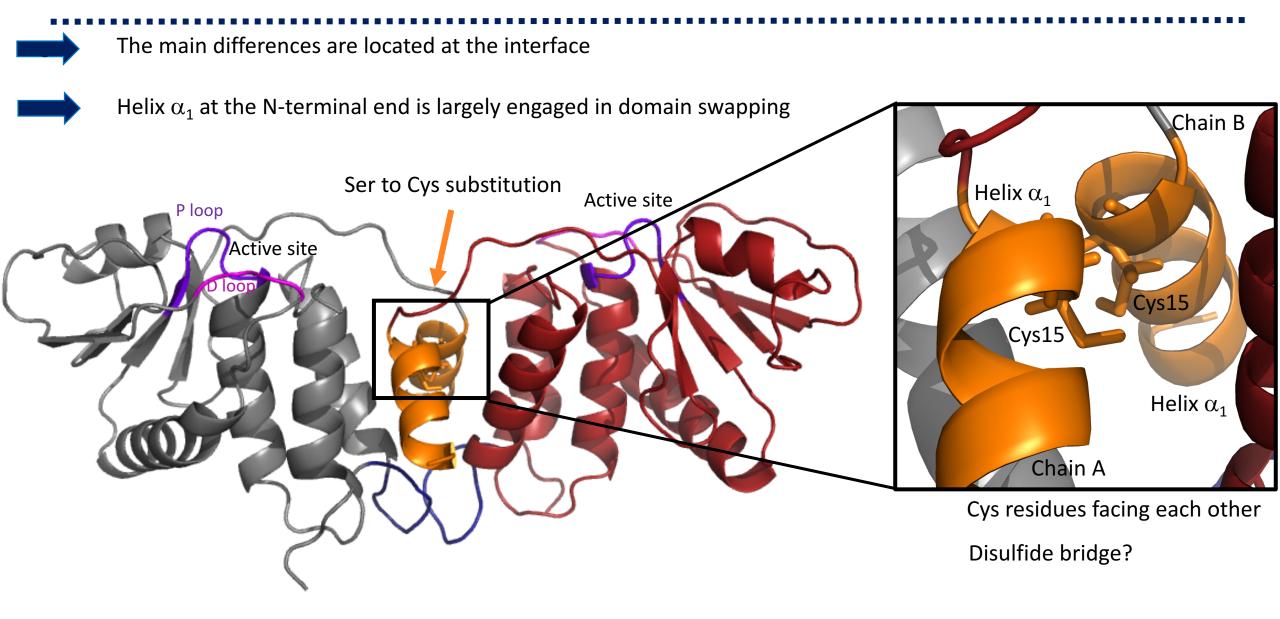






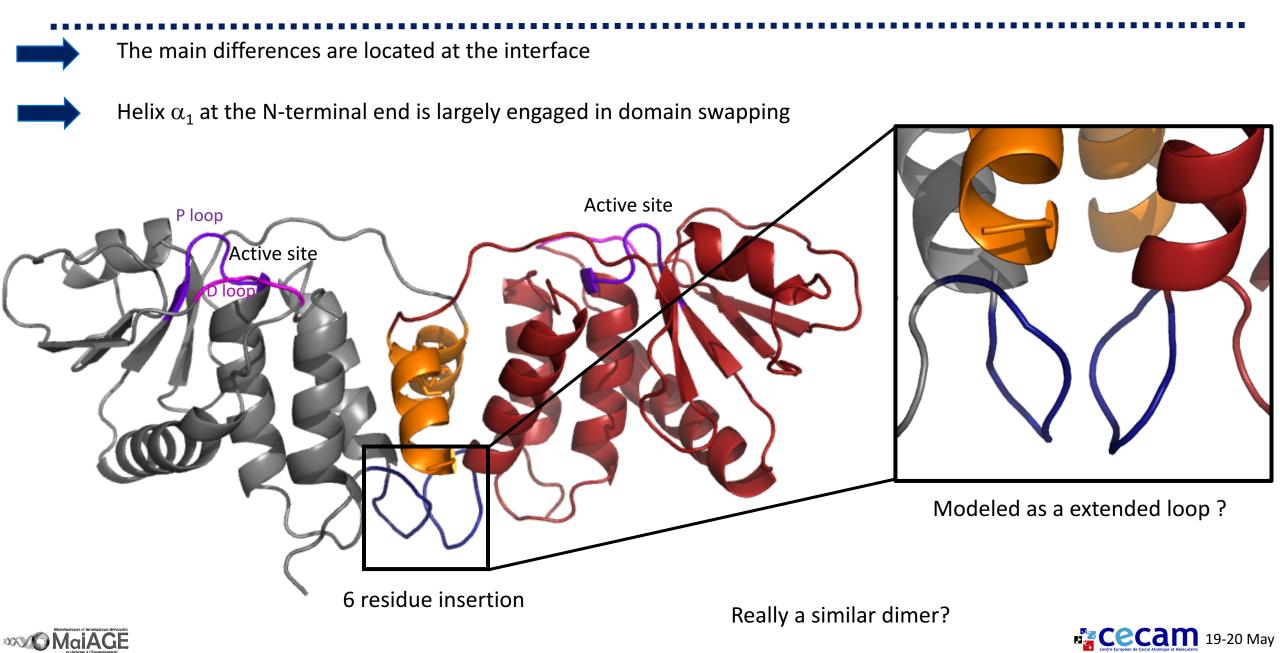
- Homology modeling of Tyrosine phosphatase with 3D Vaccinia and Variola VH1 as 3D templates: Chains A then B.
- Reconstruction of the dimer by superposition onto the dimer template.
- Minimisation with Charmm to relax the side chains







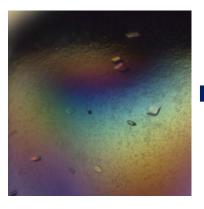


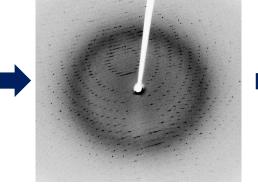


#### Structural characterization

# 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



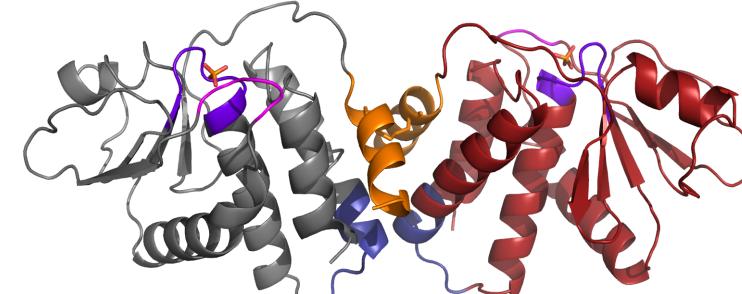


<u>Cristal conditions</u> 200 mM NH4SO4, 100mM NaAc, pH 4,6 PEG MME 2K 30%



Beamline Proxima1 2 sets of data collection

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



Data statistics 5NCR

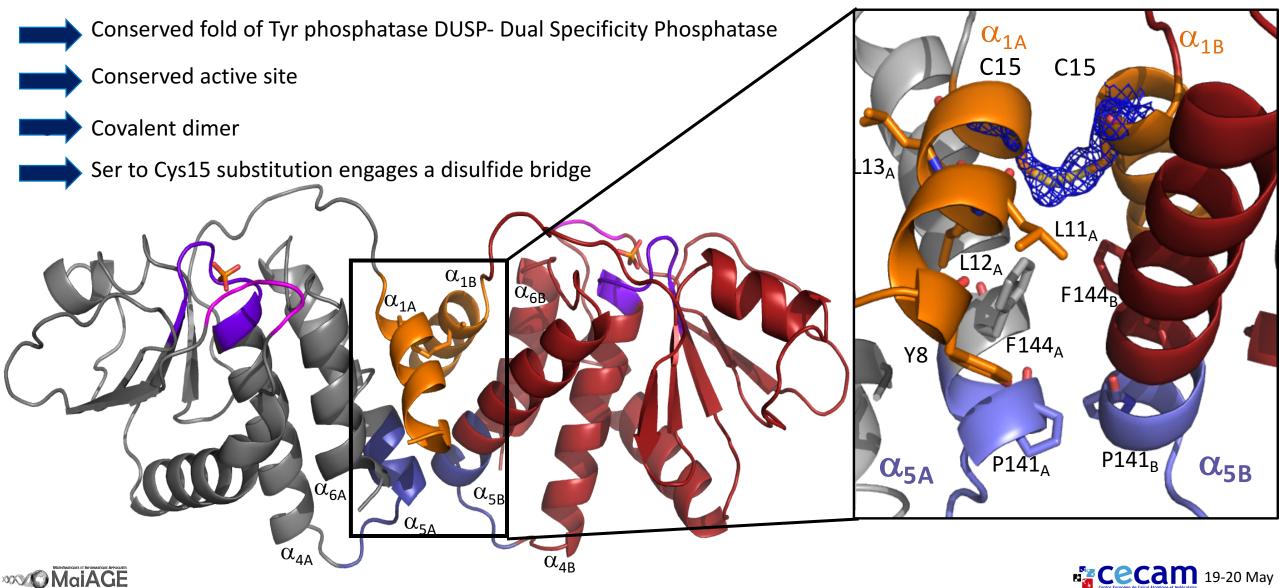
P2<sub>1</sub> a, b, c (Å) 49.56 63.55 55.39 α, β, γ (°) 90.00, 97.07 90.00 <u>Refinement of the best at 1.89 Å resolution</u> Molecular replacement using Phaser Model building with Coot Refinement cycles within Refmac CCP4 package and Buster Rwork/Rfree 18.72/21.69

Pdb deposition 5NCR

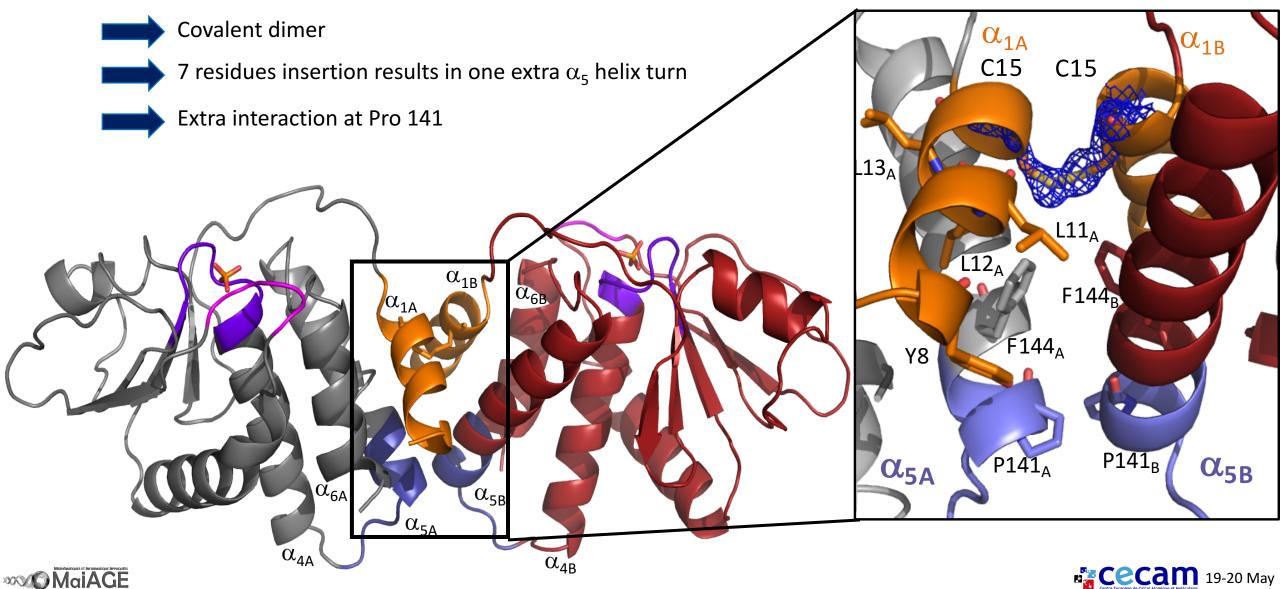


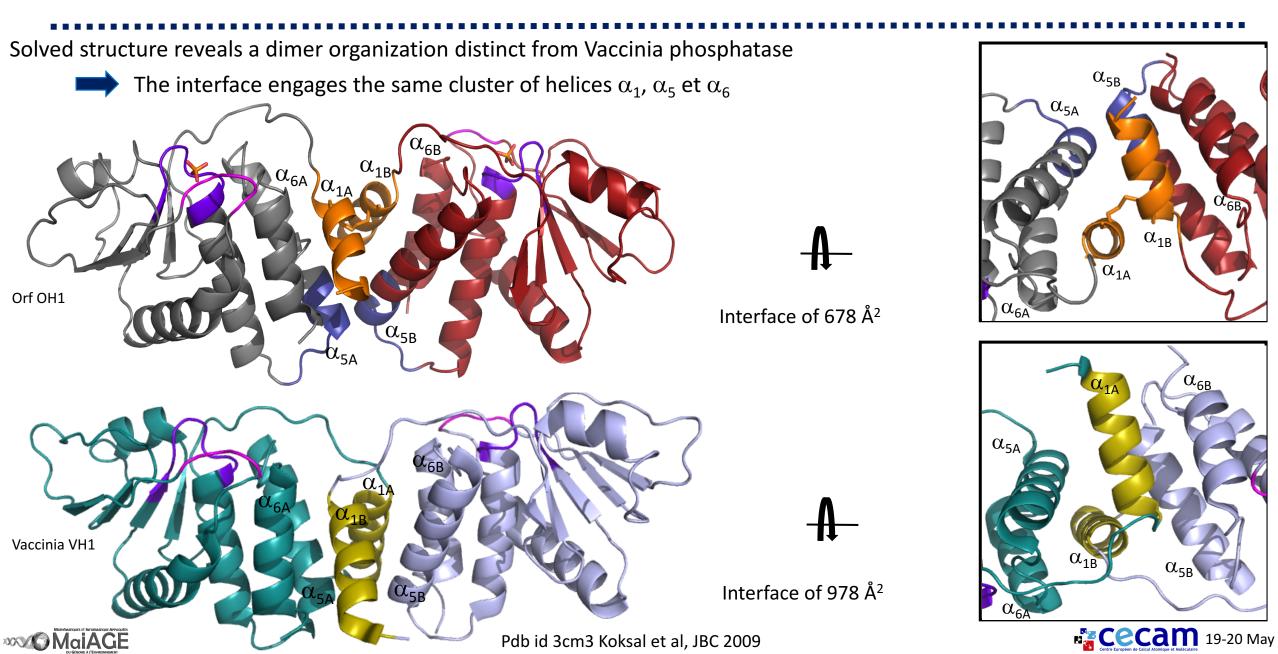


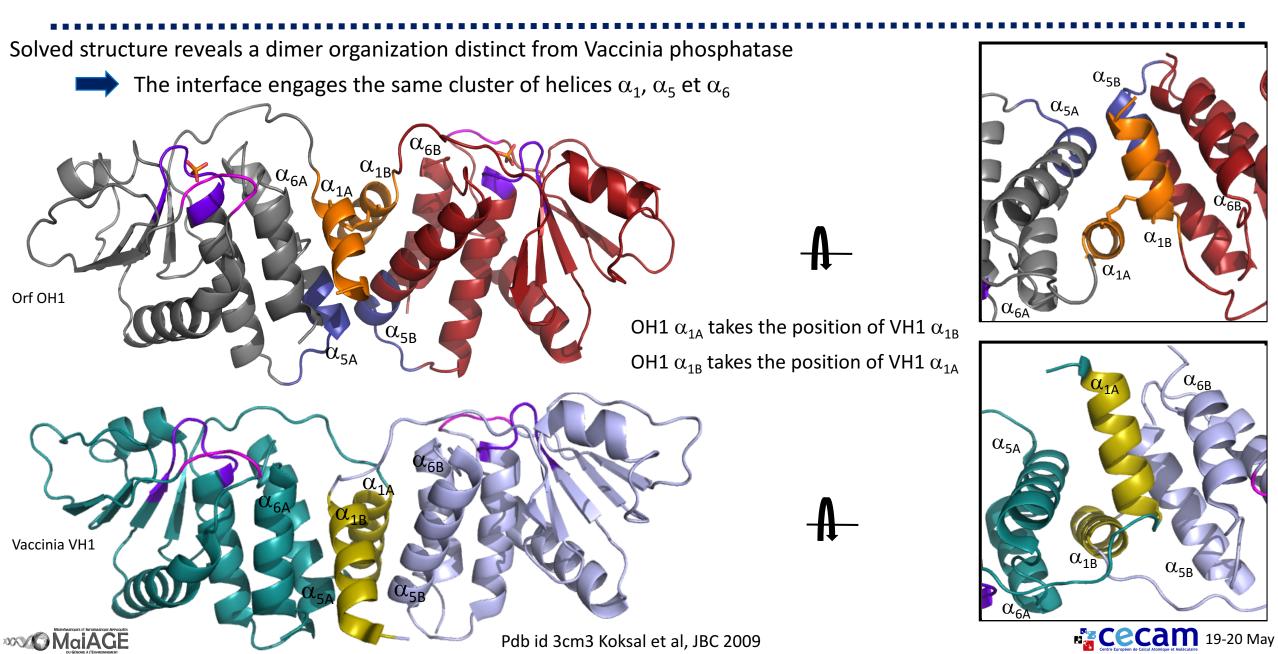
Solved structure reveals:

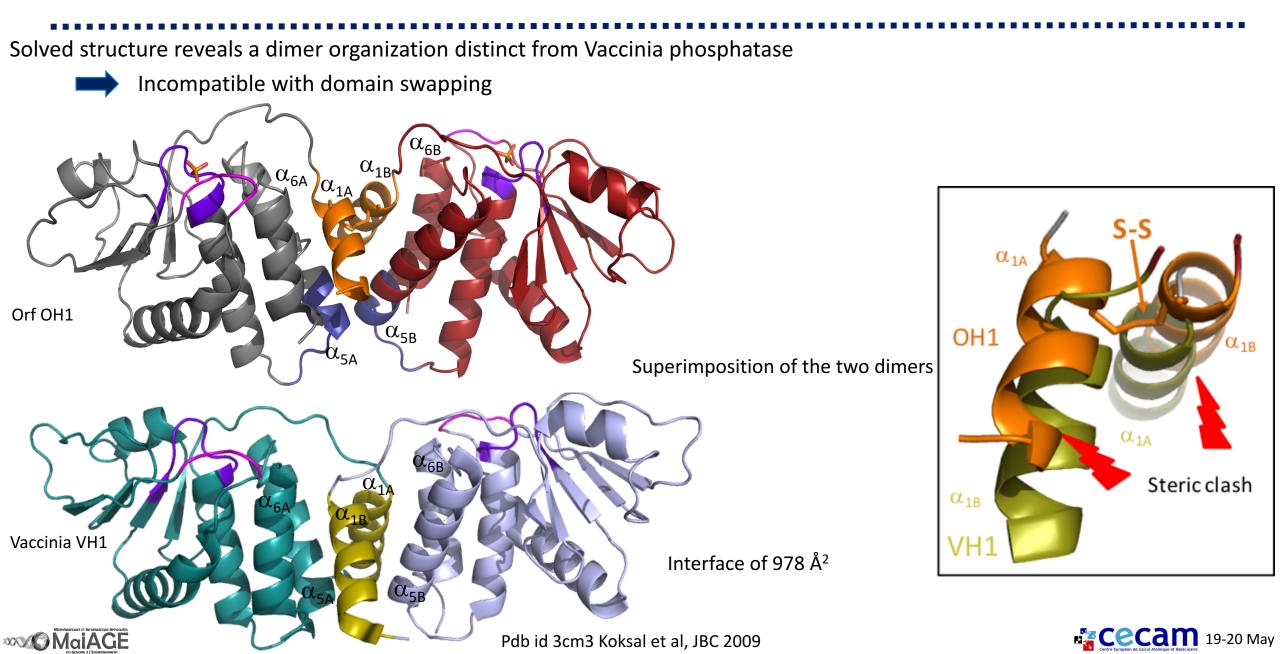


Solved structure reveals the conserved fold of DUSP – Dual Specificity Phosphatase







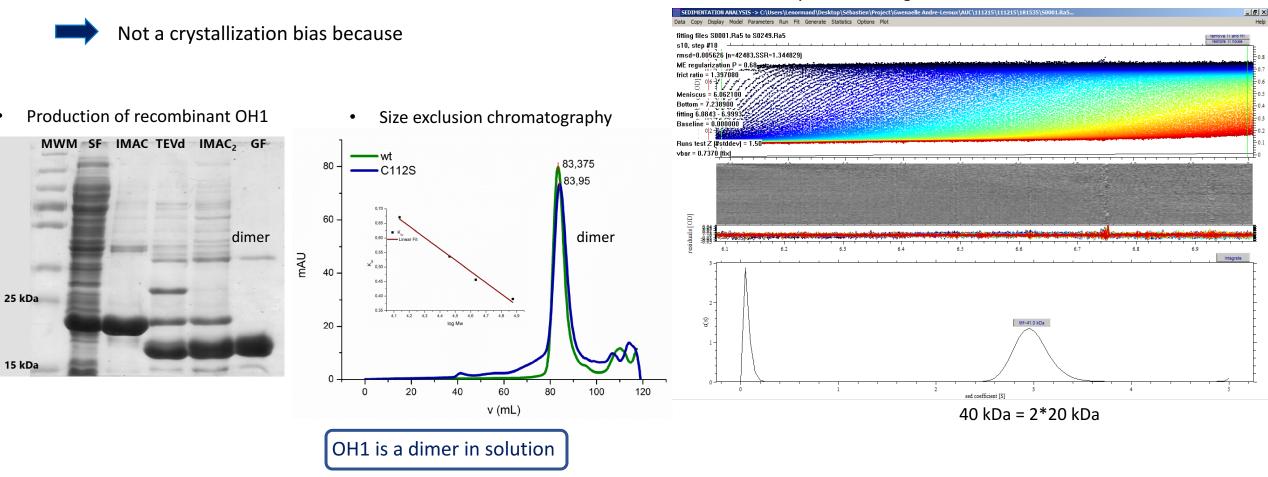


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# Orf virus : tyrosine phosphatase

Solved structure reveals a dimer organization distinct from Vaccinia phosphatase

Ultra Analytical Centrifugation



C15S shows also a dimer (data not shown)

C15S is not mandatory for a dimerization





### 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 ٠ 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  $\alpha_1$ 

D1-Bovine-papular-stomatitis-virus tr-D3IZ73-Pseudocowpox-virus · tr-D3IZK4-Pseudocowpox-virus tr-Q6TVR3-Orf-virus-SA2000 -A0A7MA86-Parapoxvirus-red-deer · tr-Q6TW43-orf-virus tr-W5U9D4-Orf-virus · tr-Q070E0-Nile-crocodilepox-virus tr-Q6VZG4-Canarypox-virus sp-Q9J592-fowlpox-virus · tr-Q70H20-Fowlpox-virus tr-A0A068EF29-Penguinpox-virus tr-A0A068EG98-Pigeonpox-virus tr-Q98249-Molluscum-contagiosum-virus-subtype-1 · tr-U3UBD0-Squirrelpox-virus tr-Q77PC3-Babbit-fibroma-virus tr-Q85315-Rabbit-fibroma-virus · tr-O8V3M6-Swinenox-virus = tr-Q9IGU7-Tanapox-virus tr-Q9DHP1-Yaba-like-disease-virus · tr-H6TA62-Cotia-virus tr-A0A097IVT2-Cotia-virus · tr-O91T23-Lumny-skin-disease-virus tr-A0A075CH78-Goatpox-virus = tr-Q6TUU3-Yaba-monkey-tumor-virus · tr-B2CWH6-Myxoma-virus sp-Q85297-Myxoma-virus tr-Q77GL6-Lumpy-skin-disease-virus tr-Q77GC9-Lumpy-skin-disease-virus tr-Q91MT8-Lumpy-skin-diseaseVIRUS tr-Q9WH06-Sheeppox-virus tr-T2AU07-Myxoma-virus tr-Q08FA1-Deerpox-virus tr-Q08FS1-Deerpox-virus · tr-G3EIF7-Yoka-poxvirus n-P80994-Raccoon-poxvirus tr-Q0NJL8-Variola-virus sp-P33064-Variola-virus tr-Q0N562-Variola-virus tr-Q0NLW9-Variola-virus tr-OONCN7-Variola-virus-isolate-Human-South-Africa tr-Q0NG54-Variola-virus tr-Q76Q11-Variola-minor-virus tr-Q8V2S5-Camelpox-virus tr-Q775U6-Camelpox-virus-strain-CMS tr-Q8JLD2-Ectromelia-virus tr-Q5IXT4-Monkeypox-virus tr-O8OMW6-Cowpox-virus = tr-I0AZE8-Ectromelia-virus tr-Q8V4Z3-Monkeypox-virus-Zaire · tr-G0XX41-Cowpox-virus · tr-M9WG49-Vaccinia-virus tr-Q80DY2-Cowpox-virus = tr-Q0NPB0-Taterapox-virus sp-P20495-Vaccinia-virusstrain-Copenhagen tr-Q77TK5-Vaccinia-virus-strain-Tian-Tan · tr-Q1M1M0-Vaccinia-virus tr-Q6RZK3-Rabbitpox-virus sp-P07239-Vaccinia-virusstrain-Western-Reserve tr-Q76RC6-Vaccinia-virus tr-B9U1I4-B9U1I4Vaccinia-virus tr-Q0GNY5-Q0GNY5-HorsepoxVirus tr-A9J188-A9J188-VacciniavirusstrainAnkara -3CM3-Vaccinia

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ŀ					LVNMTMSTP-SGLTAY									
ŀ					LVNMTMSTP-AGITAY									
ŀ					VVNMTTSTP-SGITAY									
ľ					LVNMTMSTP-AGITAY									
1					LVNMTMSTP-AGI TAY									
					ILNLSYRAEGVKVI									
					IVNVSTLLINHSDVTVL									
					IVNVSMLLKRTDITVL									
					IVNVSMLLKRTDITVL									
					IVNVSMLLKRTDITVL									
					VLNMSMVLPGSSATIV									
					VLNLTTELKOPHVTVV									
					ILNUTTEIKNSSVTII									
					ILNUTTEIKNSSVTII									
	MORKSLYENVLLKSTG	SLTKAKARVTEY	VYLGNYN	DAINICS-SEIPFKY	ILNLTTELKNSSINI	MPL I DDEHTDLHKY	FDYVTSLLEK	DKNEHAILV	HCLAGVNRSGAMIMAY		LMREKRGAF	ENPSFRKQI	DKYI	INES
	MDKKC I YENVLLKSTG	<b>SLLPKAKARVTDY</b>	VYLGNYN	NALSINE-YGIQFKY	I LNLTTE I CNSSVN I II	MPLLONETTOLTKH	FDYVTNFLSK	DKNHYPVLV	HCIAGVNRSGAMIMAY		SMREQRGAFL	ENPSFRKQI	EKYV	IKN-
	MDKKCIYENVLLKSTG	<b>SLLPKAKARVTDY</b>	VYLGNYN	NALSINE-YGIQFKY	ILNLTTEICNSSVNIII		FDYVTNFLSK	DKHHYPVLV	HC I AGVNRSGAMIMAY		SMREQRGAFL	ENPSFRKQI	EKYV	IKN-
	MOKKSLYENVLLKSTG	<b>SYLEKAKARVTEY</b>	VYLGNYN	DA I NAPY-SDVKFKY	ILNLTPEIVNSPINII	HLPLIDDECTDLSKY	FDYTTNLLTK	EIEHYPILV	HC I AGVNRSGA I IMAY	MSRRFMYFLYIY	AMREKRGAF	ENVSFRKQI	NKY I	VDT-
	MDKKSLYENVLLKSTG	<b>SYLEKAKARVTEY</b>	VYLGNYN	DA I NAPY-SDVKFKY	I LNLTPE I VNSP IN I II	HLPL I DDECTDLSKY	FDYTTNLLTK	EIEHYPILV	HC I AGVNRSGA I IMAY	MSRRFMYFLYIY	AMREKRGAF	ENVSFRKQI	NKY I	VDT-
	MDKKSLYENVLLKSTG	SLPKAKARVTDY	VYLGNYDI	DAINAIS-SNVNFKY	ILNLTTEFNDSRINII	HMPL I DDEKTNLNDH	IFDYVTNFLSK	DEEHYPVLV	HCVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAFI	ENPSFRKQL	DK <mark>Y</mark> I	INEL
	MDKKSLYENVLLKSTG	SLPKAKARVTDY	VYLGNYDI	DAINAIS-SNVNFKY	ILNLTTEFNDSRINII	HMPL I DDEKTNLNDH	IFDYVTKFLSK	DEEHYPVLV	HCVAGVNRSGAMIMAY	MSKRFIYFLYIY	SMREKRGAFI	ENPSFRKQL	DK <mark>Y</mark> I	INE-
					I LNLTTE I CNSSVN I II									
					ILNLTTEIKNSSITIII									
					ILNLTTEIKNSSITIII									
					I LNLTTEFNDSRINI II									
					I LNLTTEFNDSRINI II									
					ILNLTTEFNDSRINII									
					ILNLTTEFNDSRINII									
					ILNLTTEIKNSSITII									
					ILNLTTELKNSHINII									
					ILNLTTELKNSHINII									
					I LNLTMEFCDSN IN I II									
					VLNLTMDLPNSNINI									
	WURKSLIKTLLLHSIG	ALIVIAINAN SHV I NN	VILGINYKI	WWWWAPS-SEVKEKY	V LINE TWOLPINSINTNTT	TIFLYDDITIDISKY	FUDVIALLSK	LUGHINEPVLV	ICVAGVINHSGAWITLAY		OWHULHGAF	ENPSENHUL	ENTV	TUNN

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MDKKSLYKYLLLRSTGDMRRAKSRVTNNVYLGNYKNAMNAPS-SEVKFKYVLNLTMDLPNSN IN I HI PLVDDTTTD I SKYFDDVTAFLSKGDQRNEPVLVHCVAGVNRSGAM I LAYLMSKMMLYFLYVY SMRDLRGAFVENPSFKRQ I I EKYV I DKN

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80

VDKKSLYKYLLLRSTODWRAKSRVTNNYYLONYKNAWAPS-SEVKFKYVLNLTVDLPNSNINI HIPLVDDTTTDISKYFDDVTAFLSKODORNEPVLVHCVAGVNRSGAMILAYLMS-KMLYFLYVYHSWRDLRGAFVENPSFKRO

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MOKKSLYKYLLLBSTGDMHRAKSRYTNNYYLGNYKNAMDAPS-SEVKEKYVLNLTMOLPNSNINTLHLPLVDDTTTDLSKYEDDVTAFLSKODOONEPVLVHCAAGVNRSGAMILAY MSKNMLYELYV

MDKKSLYKYLLLRSTGDMHKAKSRVTNNVYLGNYKNAMDAPS-SEVKFKYVLNLTMDLPNSNINIIH PLVDDTTTDISKYFDDVTAFLSKODGNEPVLVHCAAGVNRSGAMILAYLMSKNMLYFLYVY

70

MDKKSLYKYLLLRSTGDMYRAKSRVTNNVYLGNYKNAMDAPS-SEVKFKYVLNLTMDLPNSNINIIHPLVDDTTTDISKYFDDVTAFLSKOOGNEPVLVHCAAGVNRSGAMILAY

MDKKSLYKYLLLBSTGDMBBAKSBYTNNYYLGN<mark>Y</mark>KNAMDAPS-SEVKEK<mark>Y</mark>YLNLTMDLPNSNINIIH PLVDDTTTDISKYEDDYTAFLSK<mark>O</mark>DG9NEPYLVHCAAGVNBSGAMILAY

MDKKSLYKYLLLRSTGDMHRAKSRVTNNVYLGN<mark>YKNAMDA</mark>PS-SEVKFK<mark>Y</mark>VLNLTMDLPNSNINIIH PLVDDTTTDISKYFDDVTAFLSK<mark>C</mark>OORNEPVLVHCAAGVNRSGAMILAY

MDKKSLYKYLLLRSTGDMHRAKSRVTNNVYLGNYKNAMDAPS-SEVKFKYVLNLTMDLPNSNINIIH PLVDDTTTDISKYFDDVTAFLSKCOORNEPVLVHCAAGVNRSGAMILAY

60

»»»
MaiAGE

90 100 110 Position **CCCAM** 19-20 May

HSMRDLRGAFVENPSFKRQ11EKYV1DKN

SMRDLRGAFVENPSFKRQ11EKYV1DKN

SMBDL BGAEVENPSEKBOLLEKYVLDKN

SMRDLRGAFVENPSFKRQ11EKYV1DKN

SWRDLRGAFVENPSFKRQ11EKYV1DKN

160

150

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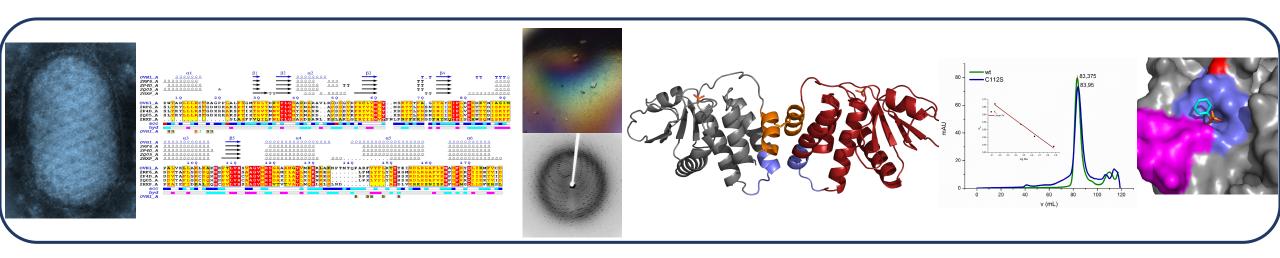
140

MSKNMLYFLYVY

MSKNMLYFLYVY

120

130

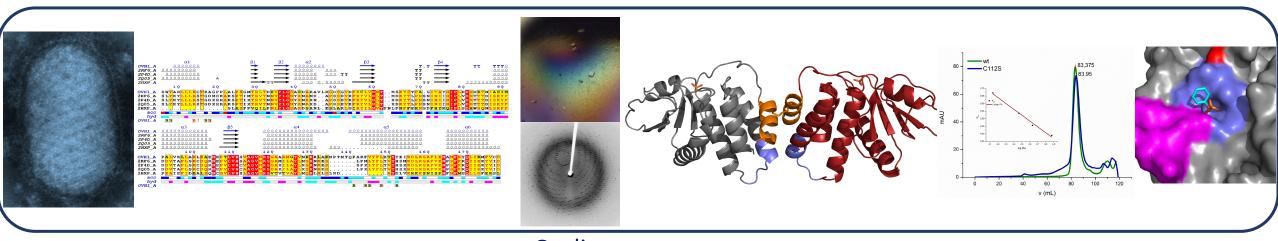


### Highlights

- 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







#### Outline

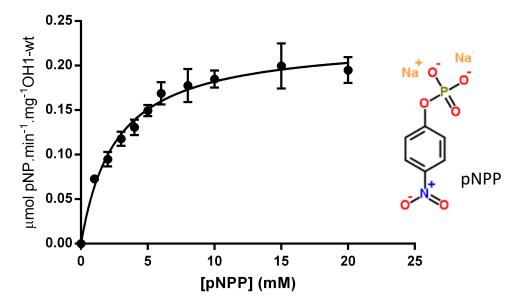
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- Conclusions & perspectives





## Tyrosine phosphatase

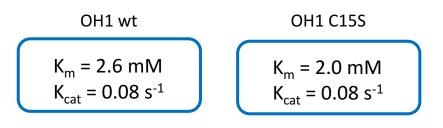
• OH1 shows a phosphatase activity *in vitro* 



Monitoring of dephosphorylation by absorbance at 405nm of pNP

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

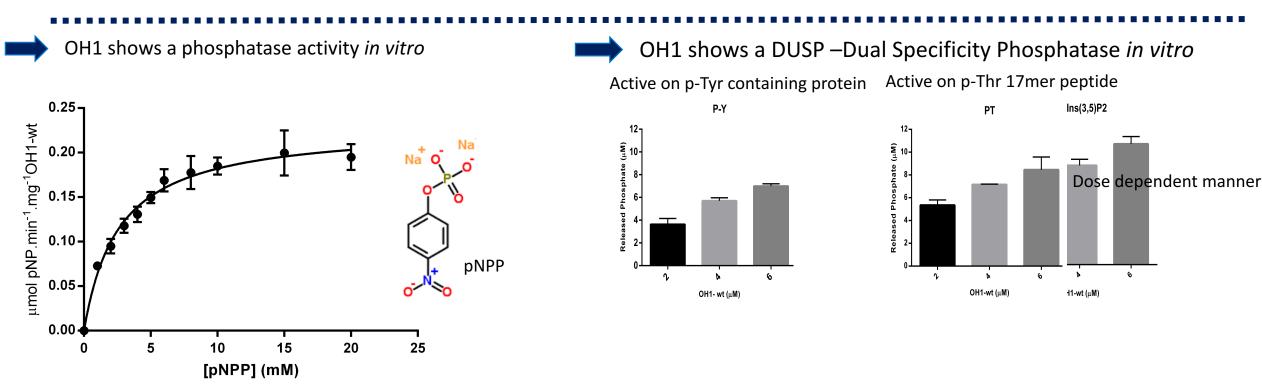
#### Michaelis-Menten Kinetics constants





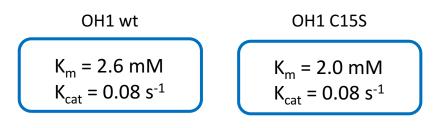


## Tyrosine phosphatase



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

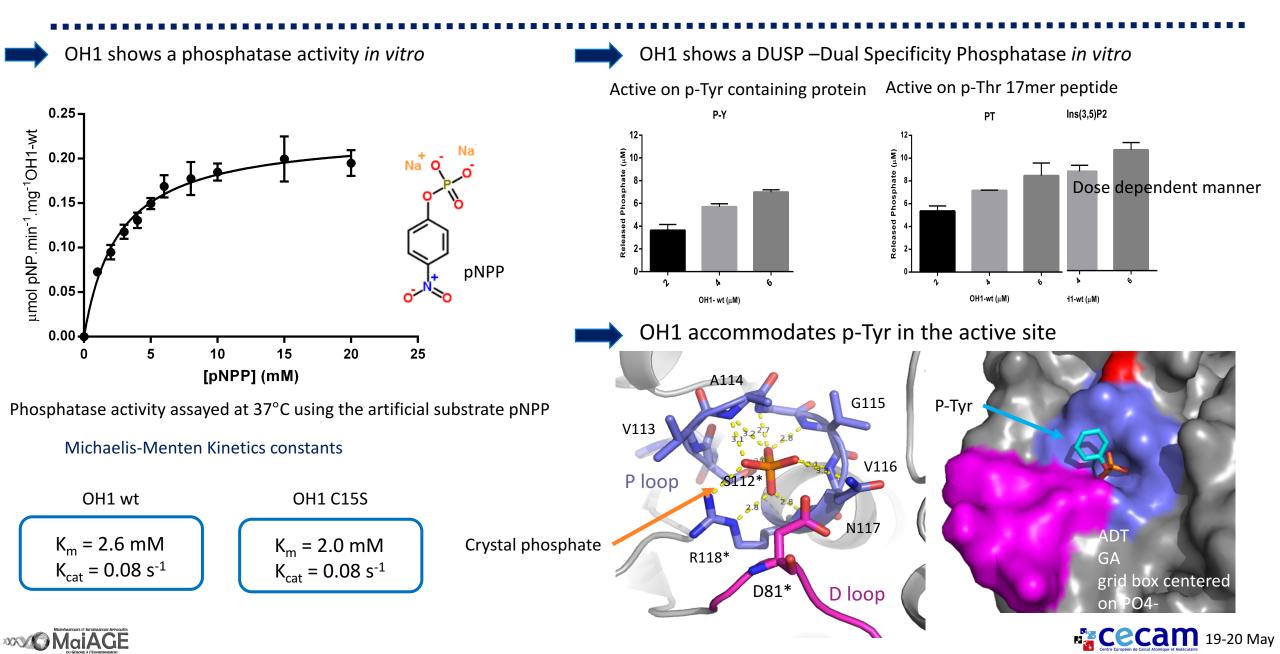
#### Michaelis-Menten Kinetics constants



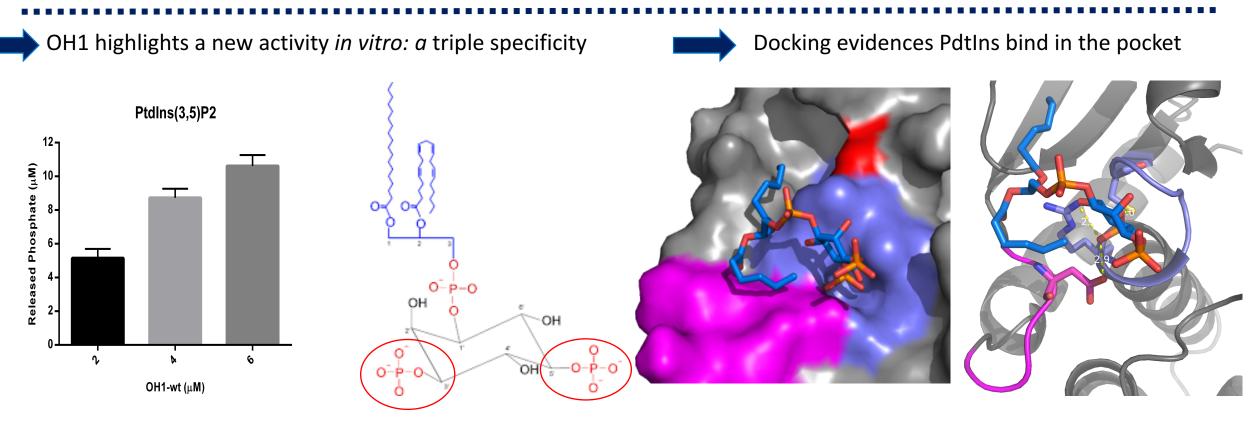




## Tyrosine phosphatase



# A new tyrosine phosphatase



Phosphoinositides can be dephosphorylated

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

The phospho-sugar part can be accommodated

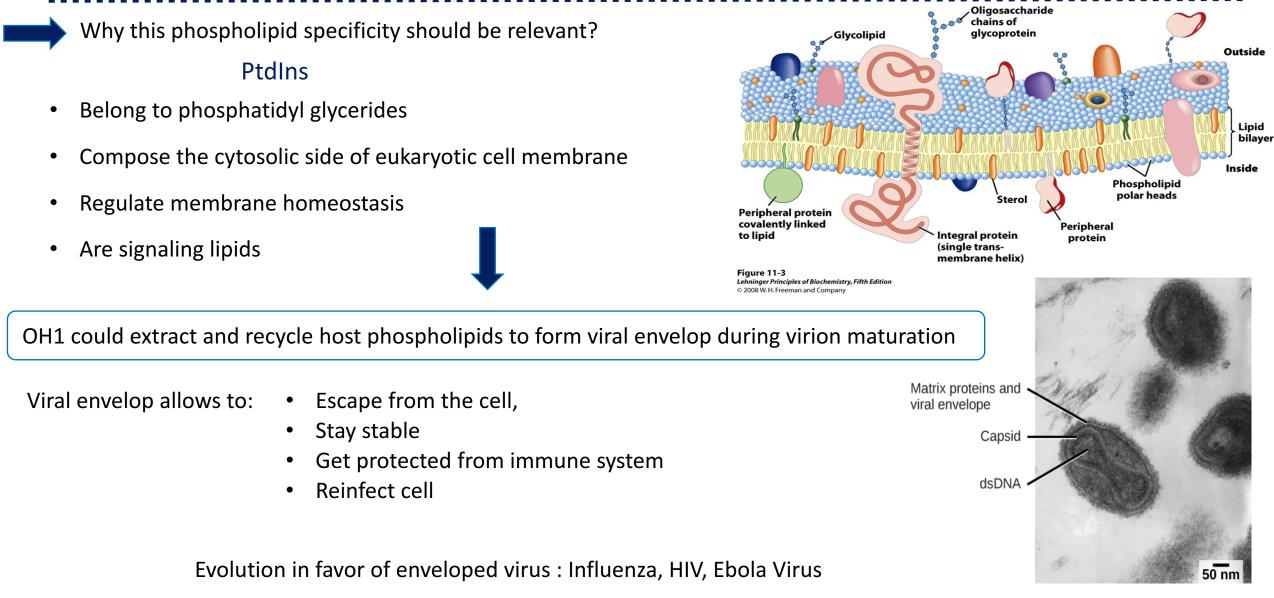
The phospho-group in 5' binds into the active pocket

Relevant physiologically?



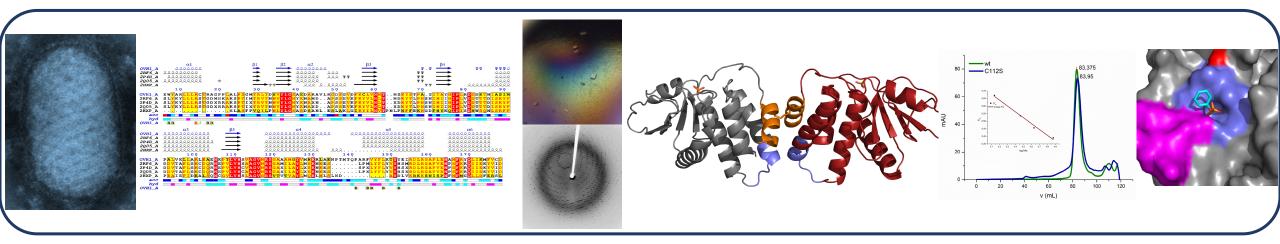


# A new tyrosine phosphatase



**CeCam** 19-20 May





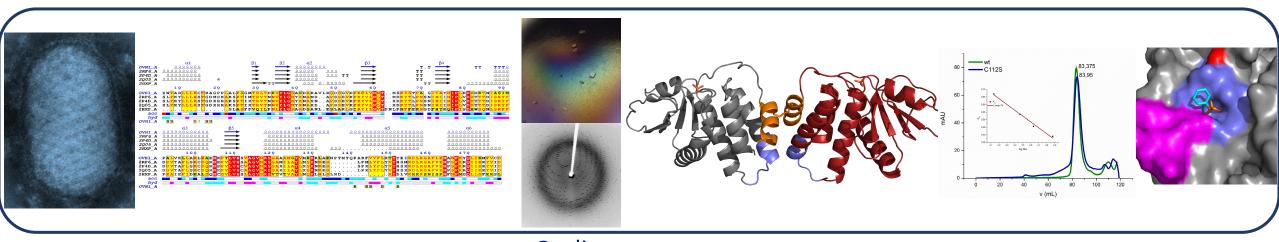
### Highlights

- 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
- 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





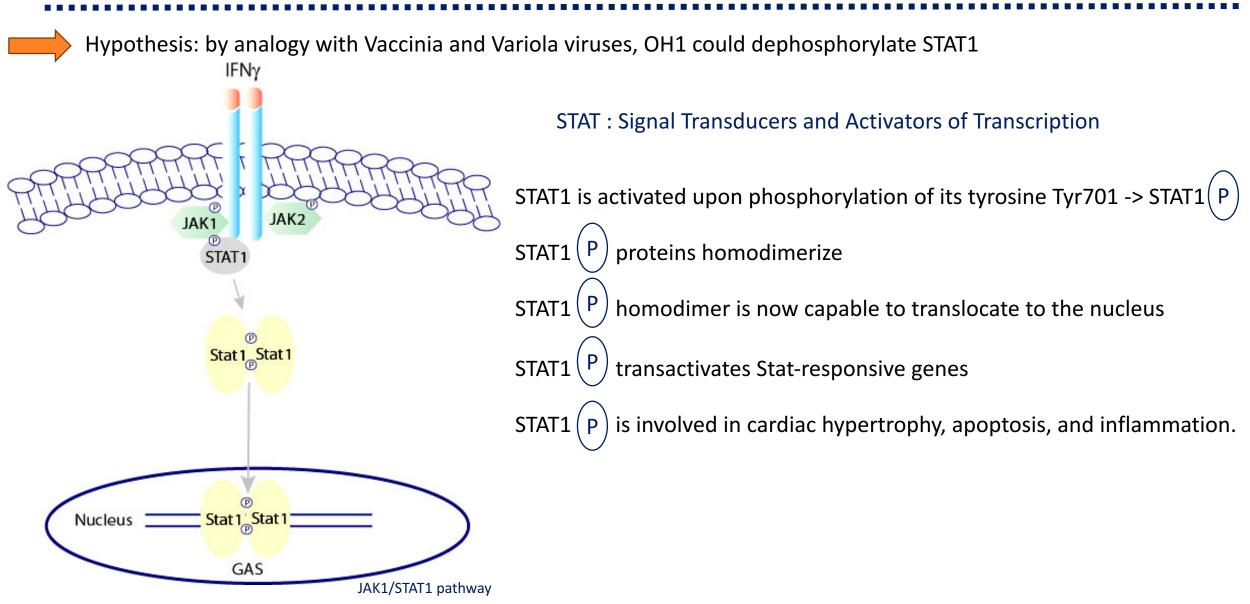
#### Outline

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





### Interaction with STAT1







OrfV dephosphorylates STAT1

JAK1

STAT1

Stat 1\_Stat 1

Stat 1 Stat 1

GAS

IFN<sub>2</sub>

JAK2

### Interaction with STAT1

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<sup>1</sup>, McCaughan C<sup>2</sup>, Wise LM<sup>3</sup>, Mercer AA<sup>4</sup>, Fleming SB<sup>5</sup>.

Author information

Format: Abstract -

#### 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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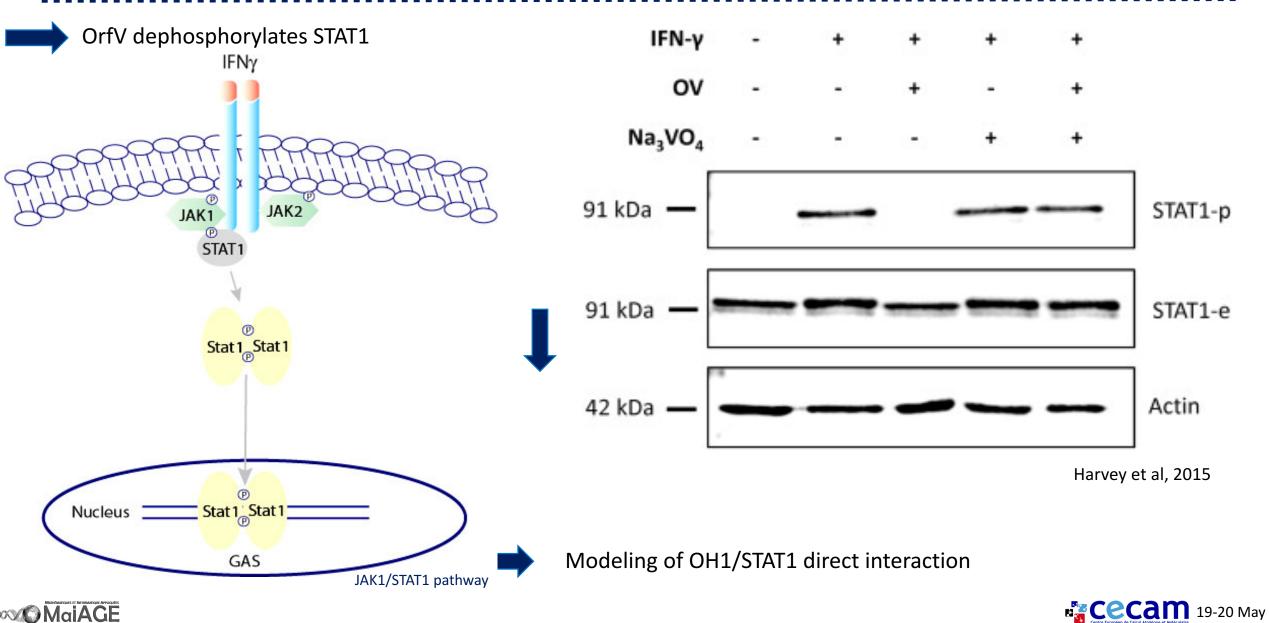
JAK1/STAT1 pathway



Nucleus



### Interaction with STAT1





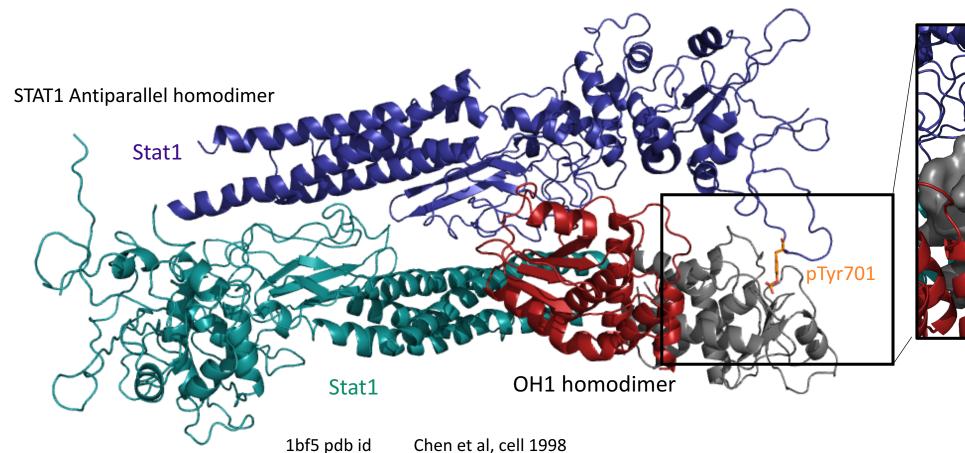
### Interaction with STAT1

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

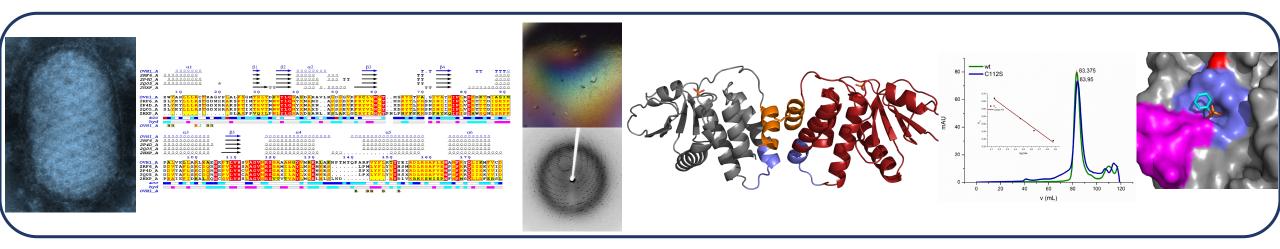


Active site







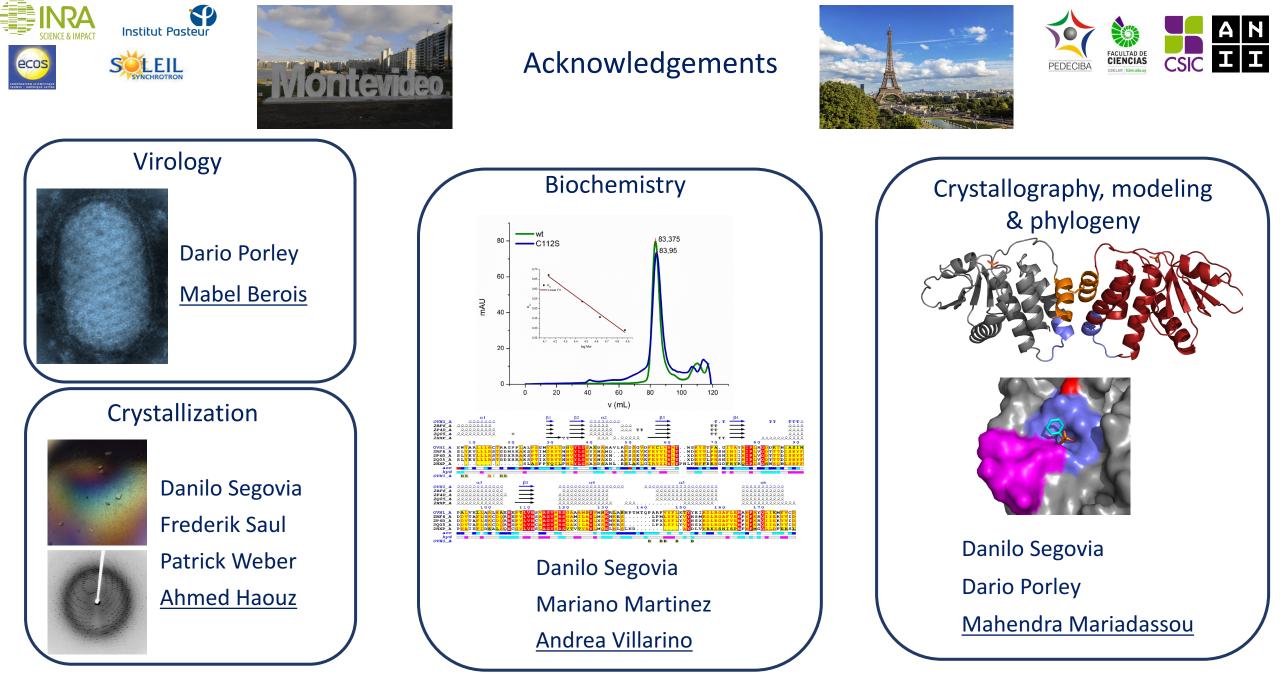


#### Perspectives

- 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

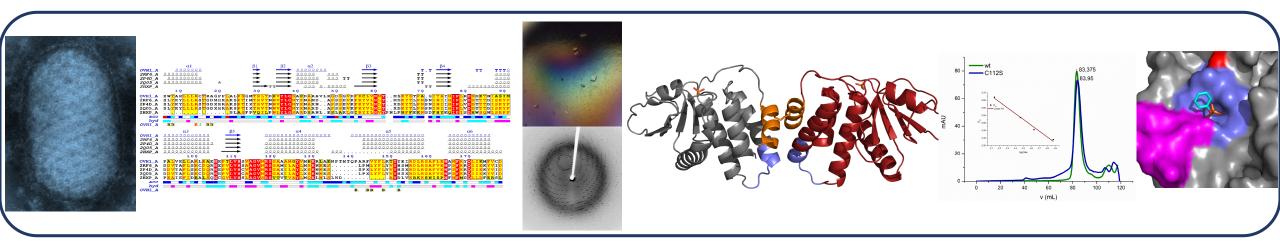








Distinct structural features & triple substrate specificity



Thank you very much for your kind attention



