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## Binding to the model protein lysozyme of the dioxidovanadium(V) complex of aroylhydrazone

Chaired by **Dr. Alfredo Berzal-Herranz**  
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*pharmaceuticals*



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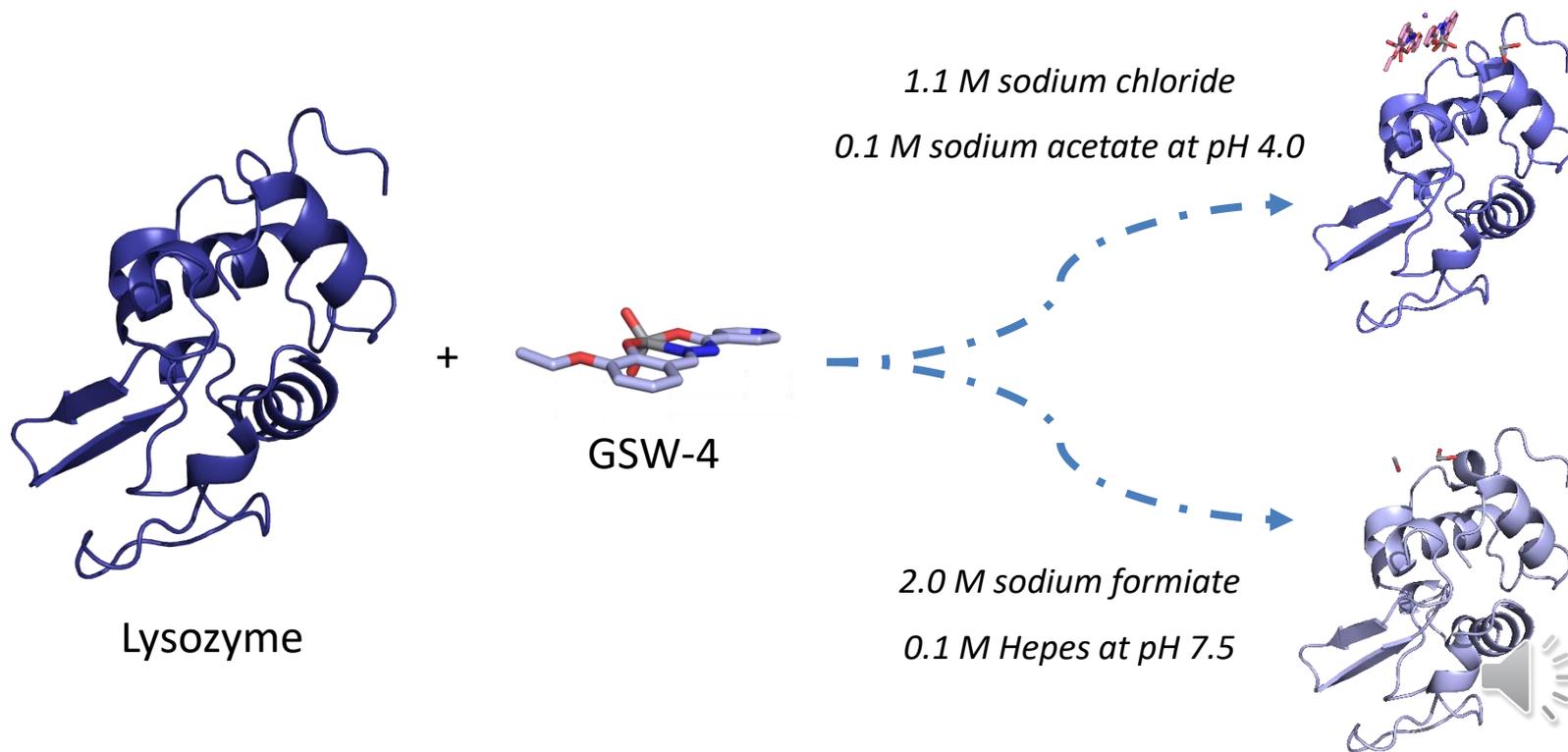
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# Binding to the model protein lysozyme of the dioxidovanadium(V) complex of aroylhydrazone





**Abstract:** VCs show a wide range of pharmacological properties; the most important application in medicine is for the treatment of cancer and diabetes. The VCs therapeutic action may be associated with their binding to proteins. For this reason, the characterization of VCs/protein interactions is important. Among the most promising VCs, dioxidovanadium(V) complex with the aroylhydrazone furan-2-carboxylic acid 3-ethoxy-2-hydroxybenzylidene)hydrazide (GSW-4) deserves to be mentioned. GSW-4 is cytotoxic for several cancer cell lines, including HeLa. The interaction of GSW-4 with the model protein hen egg white lysozyme (HEWL) was studied by X-ray crystallography. X-ray diffraction data, collected under two different experimental conditions, reveal that GSW-4 and V-containing fragments derived from this molecule bind the protein through non-covalent interactions with the side chains of Arg5, Cys6, Glu7 and Lys33. On the protein surface, a GSW-4 molecule forms a supramolecular association with another GSW-4 unit through a Na<sup>+</sup> ion. The supramolecular assembly is stabilized by stacking interactions. The reactivity of GSW-4 with HEWL could help in understanding of transport and mechanisms of action of this molecule, promoting the development of new compounds as therapeutic agents.

**Keywords:** metallodrugs; protein metalation; protein metal compounds interactions; V compounds.

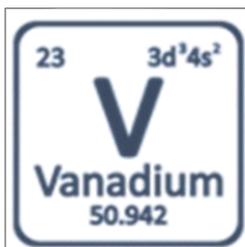




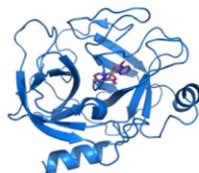
## Introduction



Transition metal-based drugs are extensively used for the treatment of different diseases



Among those, vanadium compounds (VCs) have attracted great interest



Proteins play a significant role in the biospeciation and biotransformation of a VC in the organism





## Vanadium compounds in medicine

Vanadium compounds (VCs) show a variety of pharmacological actions

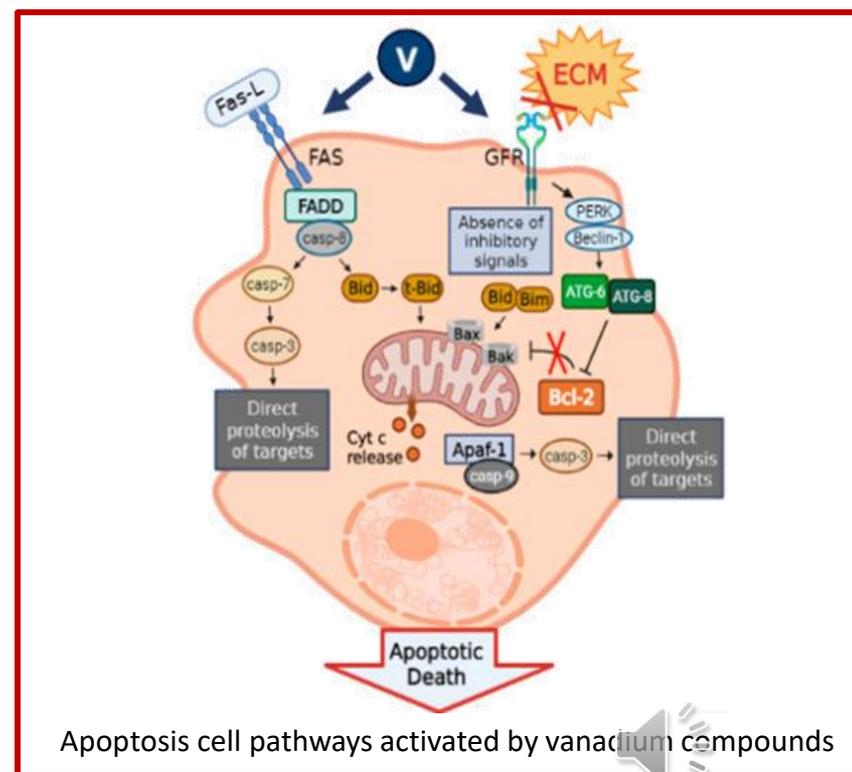
Antiviral activities

Antiparasitic activities

Antituberculosis activities

*Anticancer activities*

Antidiabetic activities





## Vanadium compounds in medicine

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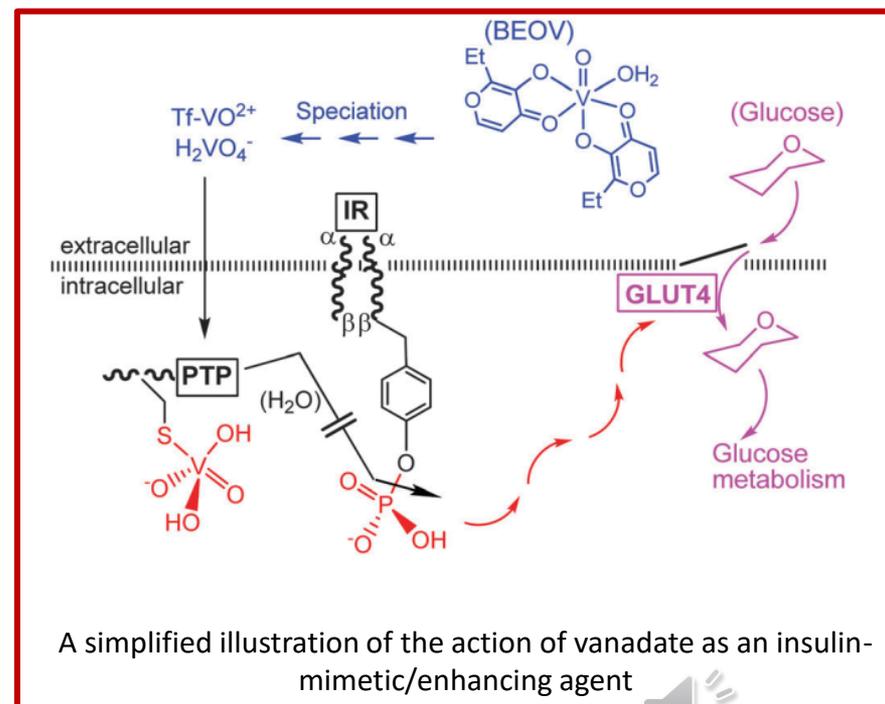
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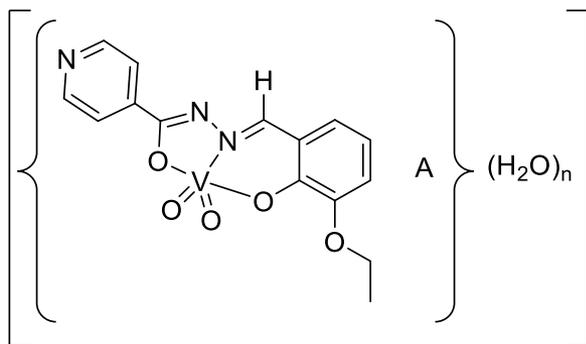
**Antidiabetic activities**



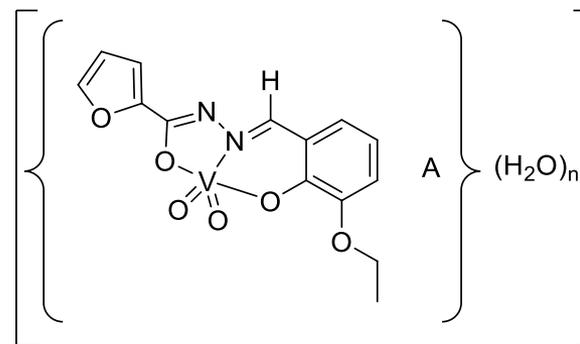


## Dioxidovanadium(V) complexes of aroylhydrazone

Aroylhydrazones tethered with a heterocyclic moiety play a significant role in the development of different medicinal agents



A=Na, n=3, **GSW-1**  
A=K, n=2, **GSW-3**  
A=Cs, n=2, **GSW-5**



A=K, n=2, **GSW-2**  
A=Cs, n=0, **GSW-4**

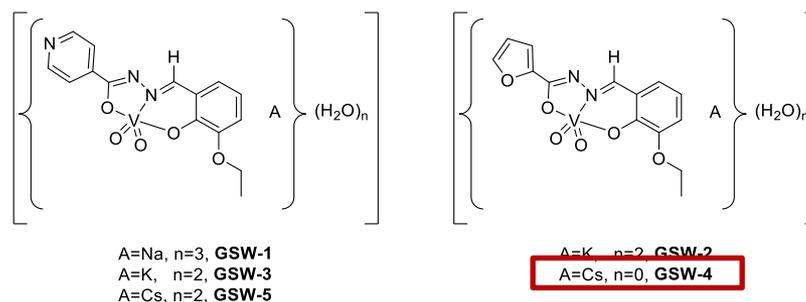
These complexes have exhibited cytotoxicity against various cancer cell lines, including HeLa



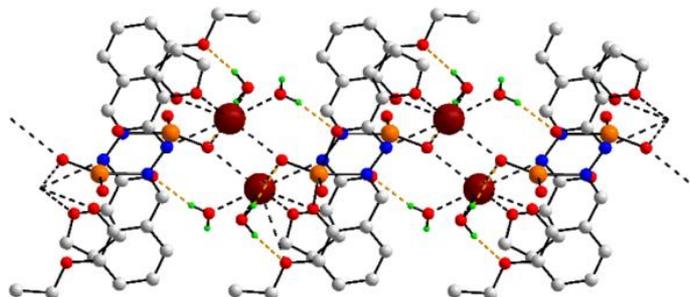


## Dioxidovanadium(V) complexes of aroylhydrazone

Aroylhydrazones tethered with a heterocyclic moiety play a significant role in the development of different medicinal agents



Among the most promising VCs, dioxidovanadium(V) complex with aroylhydrazone furan-2-carboxylic acid (3-ethoxy-2-hydroxybenzylidene)hydrazide (**GSW-4**) deserves to be mentioned

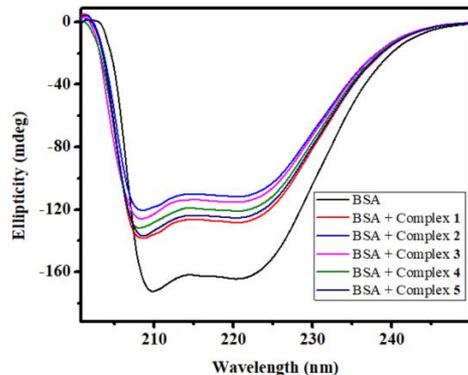




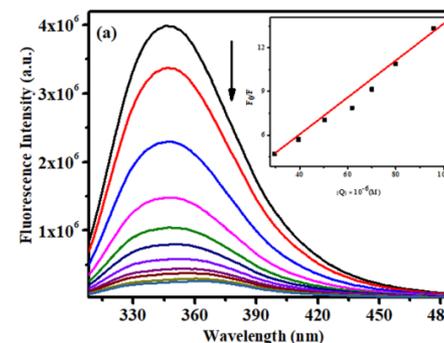
# Interaction of dioxidovanadium(V) complexes of aroylhydrazone with proteins



Proteins play a significant role in the biospeciation and biotransformation of a VC in the organism



CD spectra for free BSA and BSA upon the addition of 6.25  $\mu\text{M}$  of **GSW-1–GSW-5** at pH 8.0

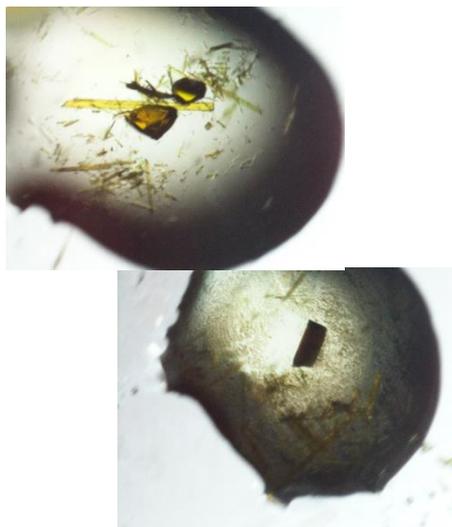


Fluorescence quenching of BSA (10  $\mu\text{M}$ ) by  $[\text{V}^{\text{V}}\text{O}_2(\text{L}_2)\text{K}(\text{H}_2\text{O})_2]$

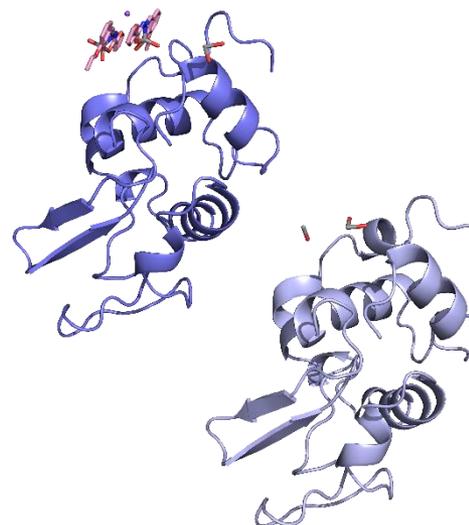


## Aim of the work

Crystallization of the adducts obtained upon the reaction of GSW-4 with the model protein hen egg white lysozyme (HEWL)



Crystallographic refinement of lysozyme structures treated with GSW-4

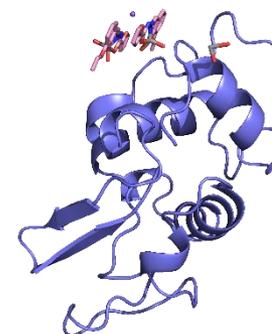




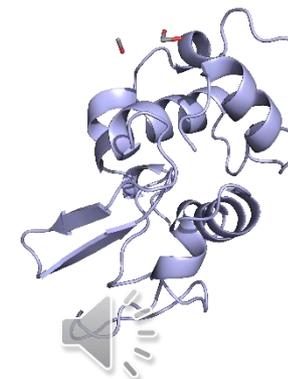
## Results and discussion

### Overall structures of HEWL in the presence of GSW-4

	Structure A	Structure B
	<i>1.1 M sodium chloride</i> <i>0.1 M sodium acetate at pH 4.0</i>	<i>2.0 M sodium formate</i> <i>0.1 M HEPES at pH 7.5</i>
Space group	P4 <sub>3</sub> 2 <sub>1</sub> 2	P4 <sub>3</sub> 2 <sub>1</sub> 2
a (Å)	76.85	76.12
b (Å)	76.85	76.12
c (Å)	36.84	36.83
α/β/γ (°)	90.0/90.0/90.0	90.0/90.0/90.0
Resolution range (Å)	54.34-1.24 (1.26-1.24)	53.82-1.01 (1.03-1.01)
Observations	832405 (40283)	1396592 (36560)
Unique reflections	32110 (1555)	56598 (2777)
Completeness (%)	100.0 (100.0)	99.9 (98.8)
Redundancy	25.9 (25.9)	24.7 (13.2)
Rmerge (%)	0.085 (6.381)	0.089 (2.989)
Average I/σ(I)	15.0 (0.4)	15.6 (0.5)



Structure A



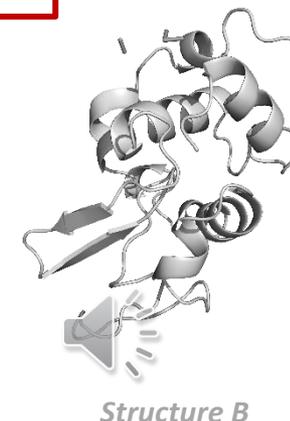
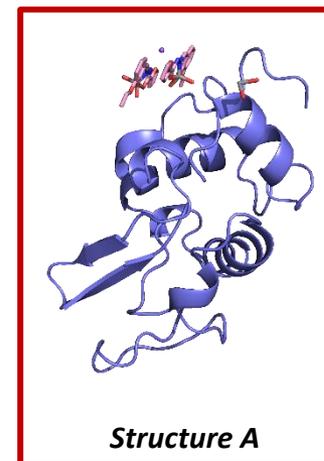
Structure B



## Results and discussion

Overall structures of HEWL in the presence of GSW-4

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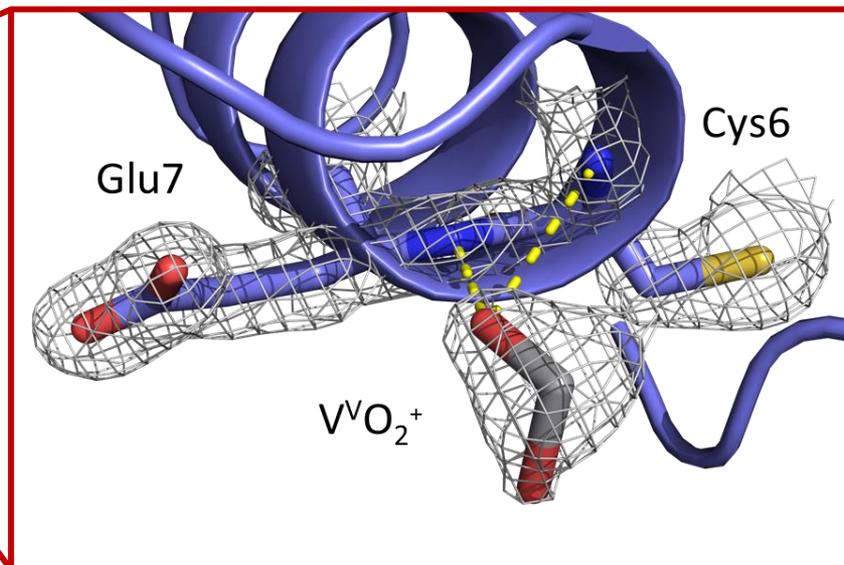
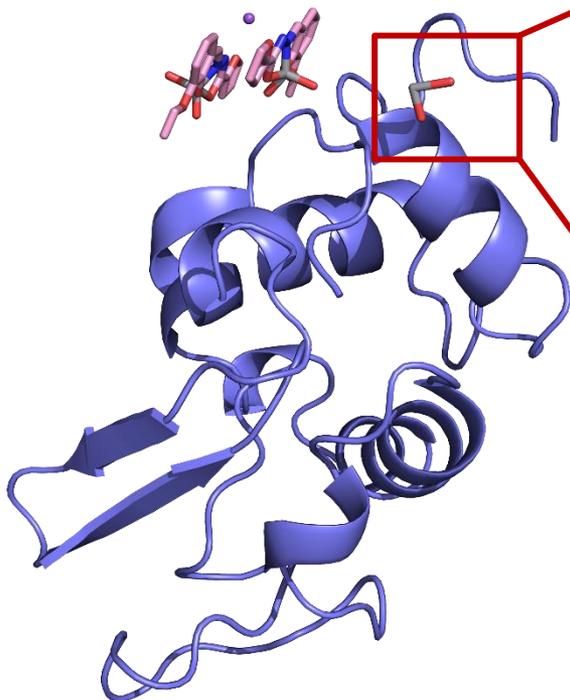


Rfactor/Rfree

0.204/0.251

## Structure A (1.24 Å)

Soaking time 7 days



Non-covalent binding of  $VVO_2^+$   
with the side chains of Cys6 and Glu7

Estimated occupancy of ( $VVO_2^+$ )	0.70
Estimated occupancy of GSW-4	0.70
Estimated occupancy of GSW-4	0.70
B-factor of ( $VVO_2^+$ ) (Å <sup>2</sup> )	38.73
B-factor of GSW-4 (Å <sup>2</sup> )	33.40
B-factor of GSW-4 (Å <sup>2</sup> )	40.92

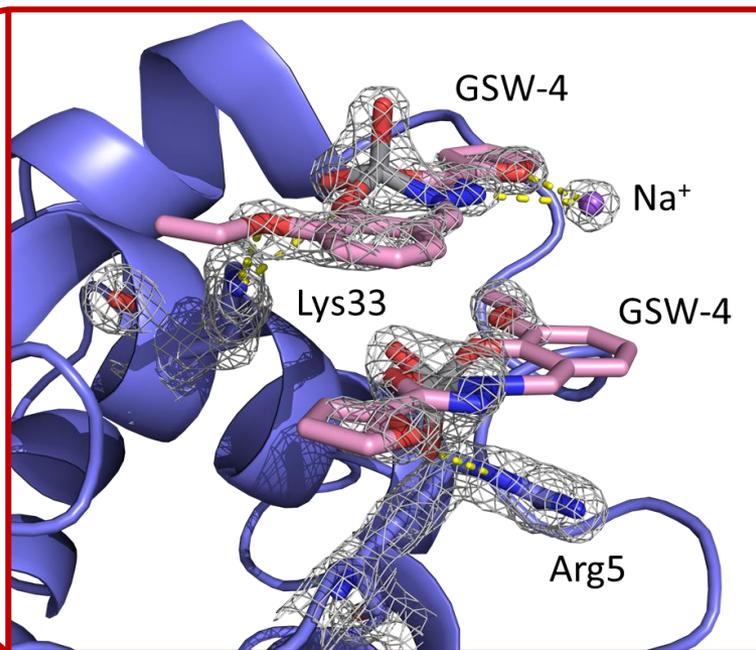
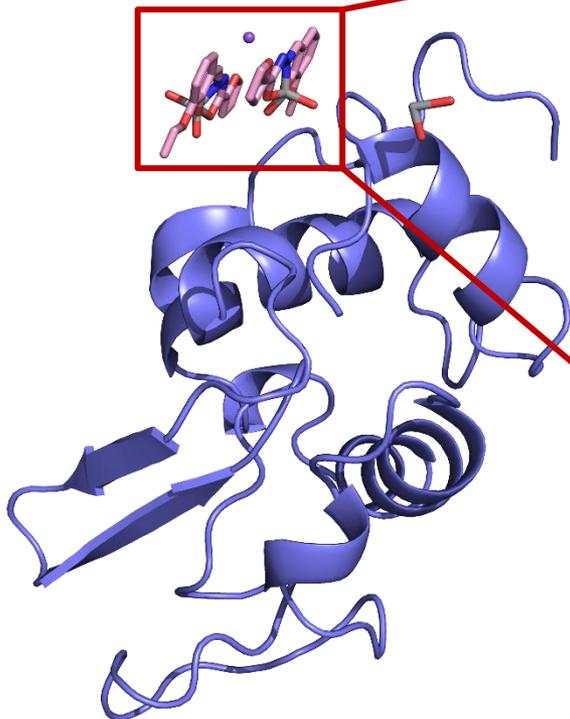


Rfactor/Rfree

0.204/0.251

## Structure A (1.24 Å)

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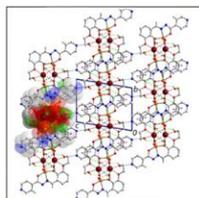


A GSW-4 molecule that forms  
a supramolecular association with another GSW-4  
unit through a Na<sup>+</sup> ion

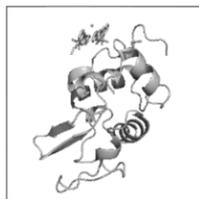
Estimated occupancy of (V <sup>VO</sup> <sub>2</sub> <sup>+</sup> )	0.70
Estimated occupancy of <b>GSW-4</b>	0.70
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B-factor of (V <sup>VO</sup> <sub>2</sub> <sup>+</sup> ) (Å <sup>2</sup> )	38.73
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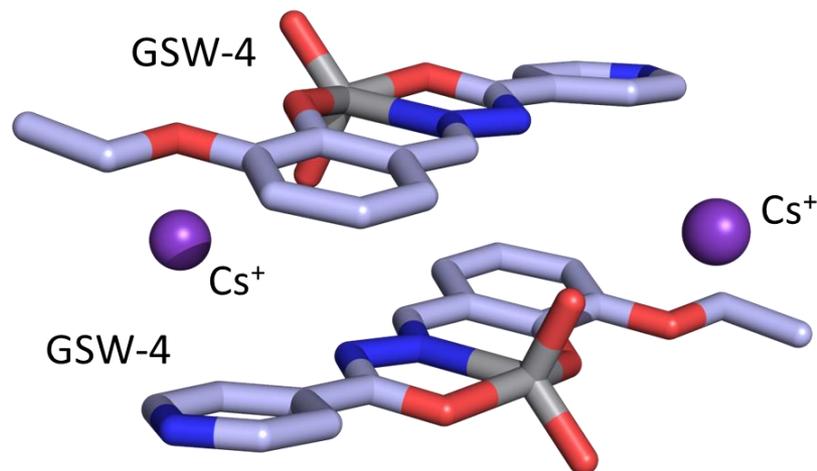
## Supramolecular association of two GSW-4



Supramolecular association of  
GSW-4 with another GSW-4  
unit in the crystal structure of  
the VC



Supramolecular association of  
GSW-4 with another GSW-4  
unit in structure A

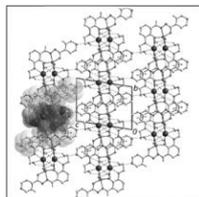


The  $\text{Cs}^+$  ion forms six close interactions with oxygen and one with nitrogen. Each  $\text{Cs}^+$  center forms direct bridges between three anions

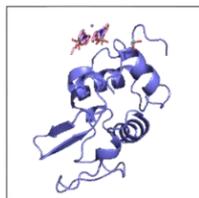




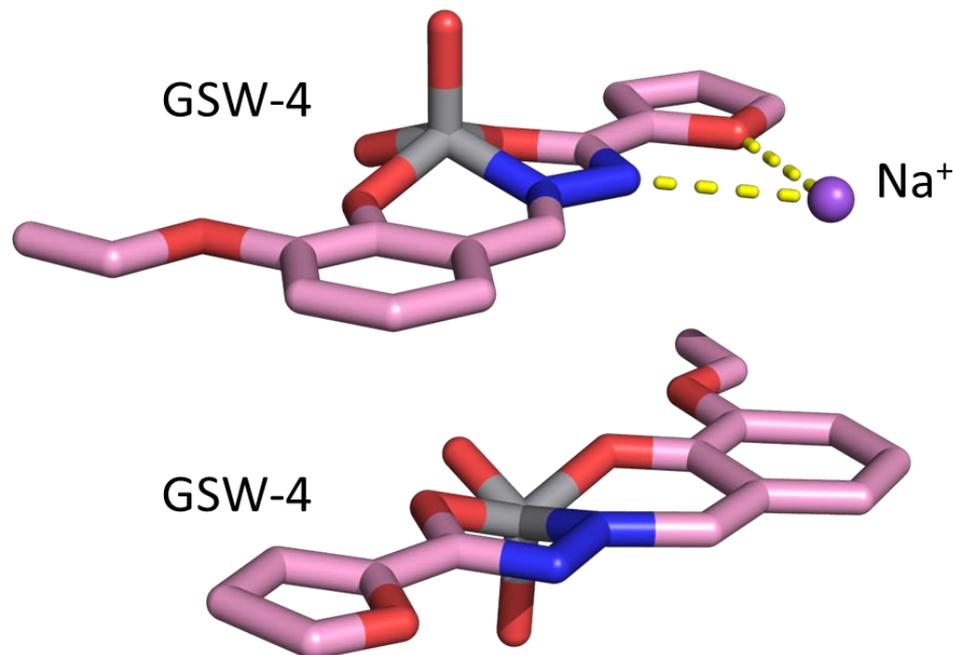
## Supramolecular association of two GSW-4



Supramolecular association of  
GSW-4 with another GSW-4  
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the VC



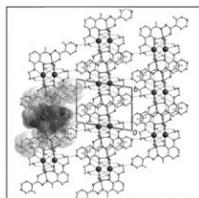
Supramolecular association of  
GSW-4 with another GSW-4  
unit in structure A



The supramolecular assembly is stabilized by stacking interactions



## Supramolecular association of two GSW-4

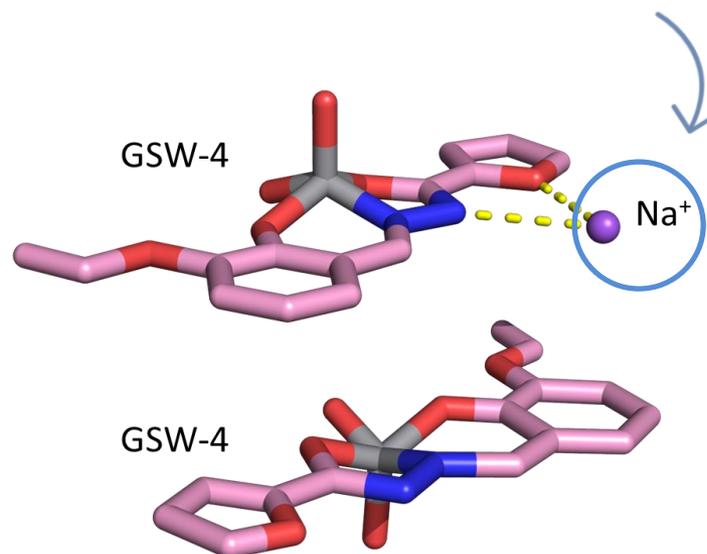


Supramolecular association of  
GSW-4 with another GSW-4  
unit in the crystal structure of  
the VC



Supramolecular association of  
GSW-4 with another GSW-4  
unit in structure A

The cation interacts with the oxygen  
and the nitrogen atoms of one of the two  
dioxidovanadium(V) units



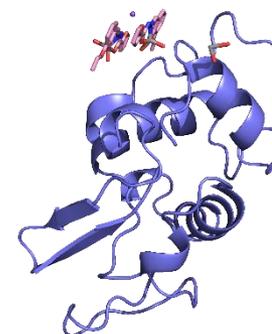
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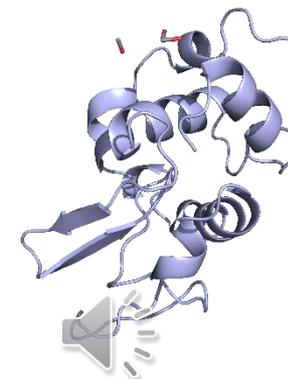
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Structure A



Structure B



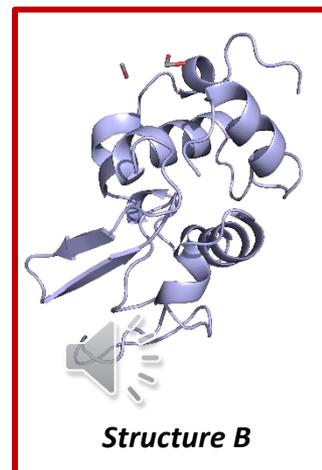
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Structure A



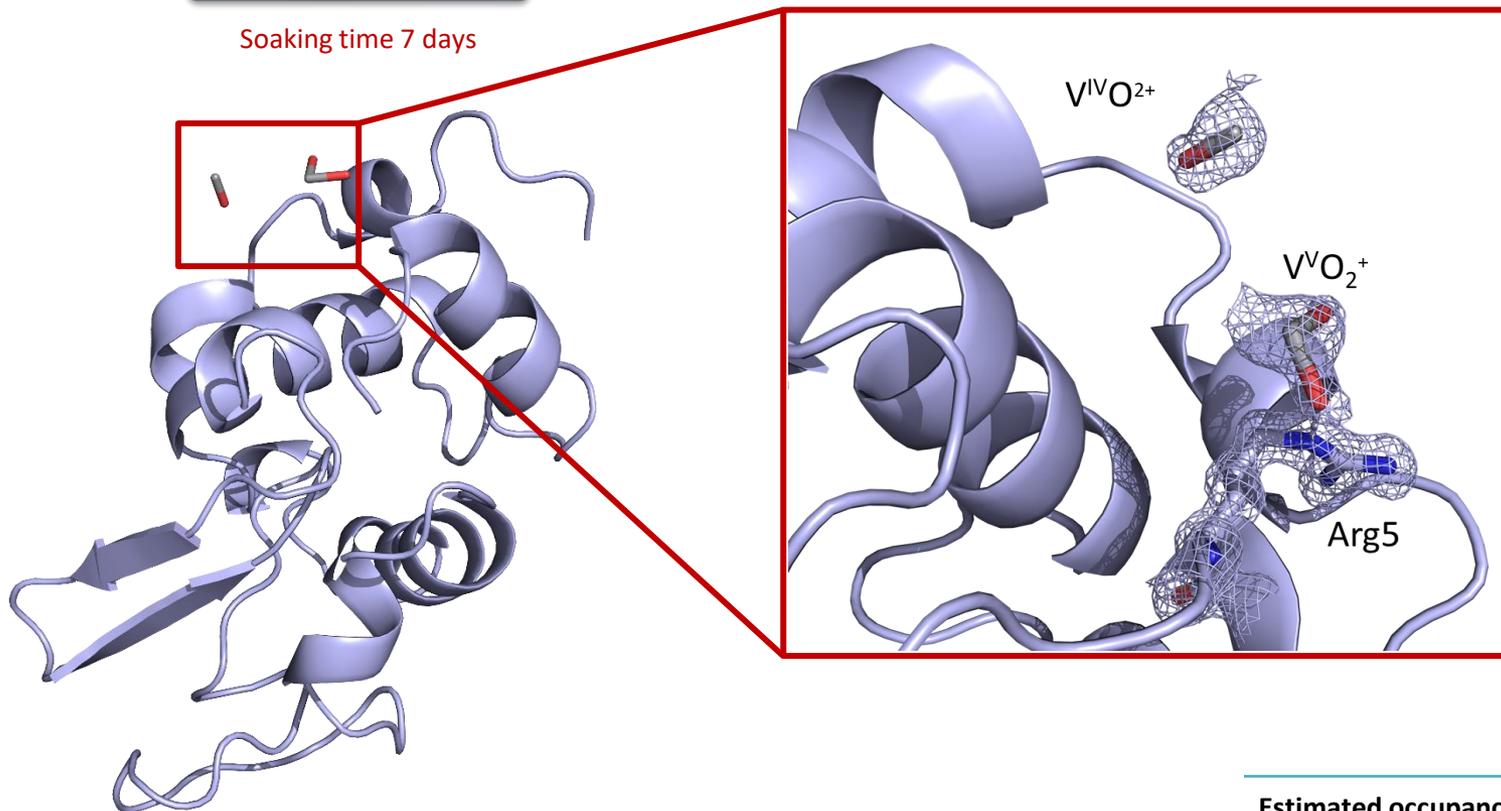
Structure B



Rfactor/Rfree  
0.144/0.164

## Structure B (1.01 Å)

Soaking time 7 days



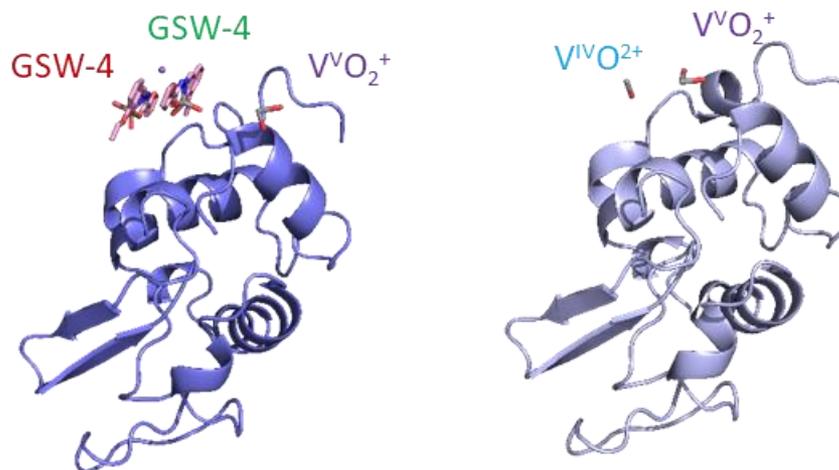
Non-covalent binding of V-containing fragments derived  
from GSW-4 with the side chain of Arg5

Estimated occupancy of ( $V^{IV}O_2^+$ )	0.80
Estimated occupancy of ( $V^{VO}_2^+$ )	0.70
B-factor of ( $V^{VO}_2^+$ ) ( $\text{\AA}^2$ )	40.04
B-factor of ( $V^{VO}_2^+$ ) ( $\text{\AA}^2$ )	49.94



## Conclusions

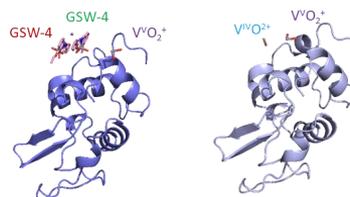
- X-ray diffraction data have shown non-covalent binding of GSW-4, together with V-containing fragments, to the side chains of Arg5, Cys6, Glu7 and Lys33



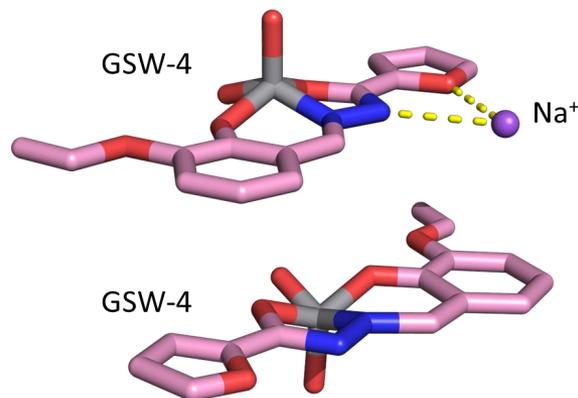


## Conclusions

- X-ray diffraction data have shown non-covalent binding of GSW-4, together with V-containing fragments, to the side chains of Arg5, Cys6, Glu7 and Lys33



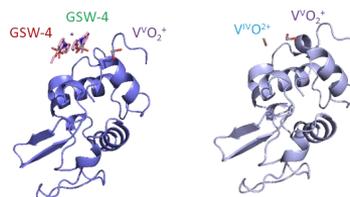
- A molecule of GSW-4 forms a supramolecular association with another GSW-4 unit through a  $\text{Na}^+$  ion



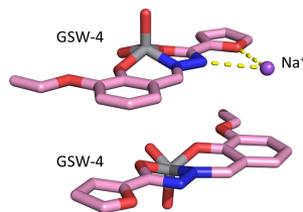


## Conclusions

- X-ray diffraction data have shown non-covalent binding of GSW-4, together with V-containing fragments, to the side chains of Arg5, Cys6, Glu7 and Lys33



- A molecule of GSW-4 forms a supramolecular association with another GSW-4 unit through a Na<sup>+</sup> ion



- The reactivity of GSW-4 with HEWL could help in understanding of transport and mechanisms of action of V, promoting the development of new compounds as therapeutic agents



## Acknowledgments

We gratefully acknowledge the European Synchrotron Radiation Facility (ESRF) staff for their assistance during data collection

