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Binding of pharmacologically active diacetylacetonateoxidovanadium(IV) to the model protein lysozyme: structural studies

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pharmaceuticals MDPI

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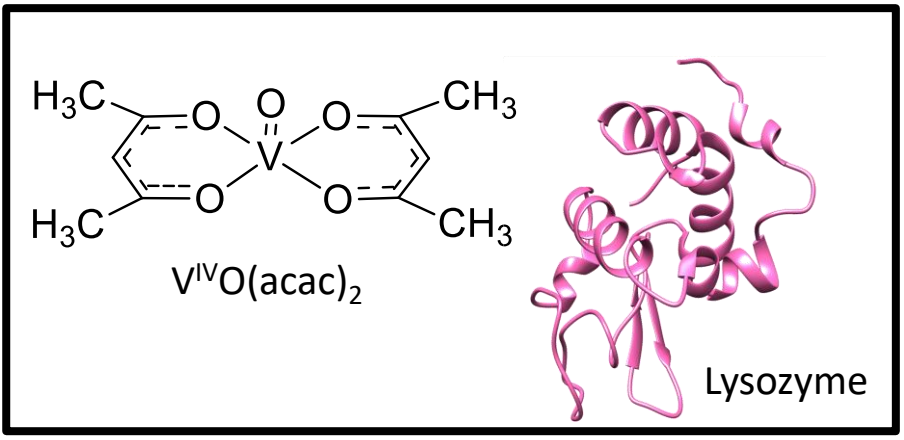
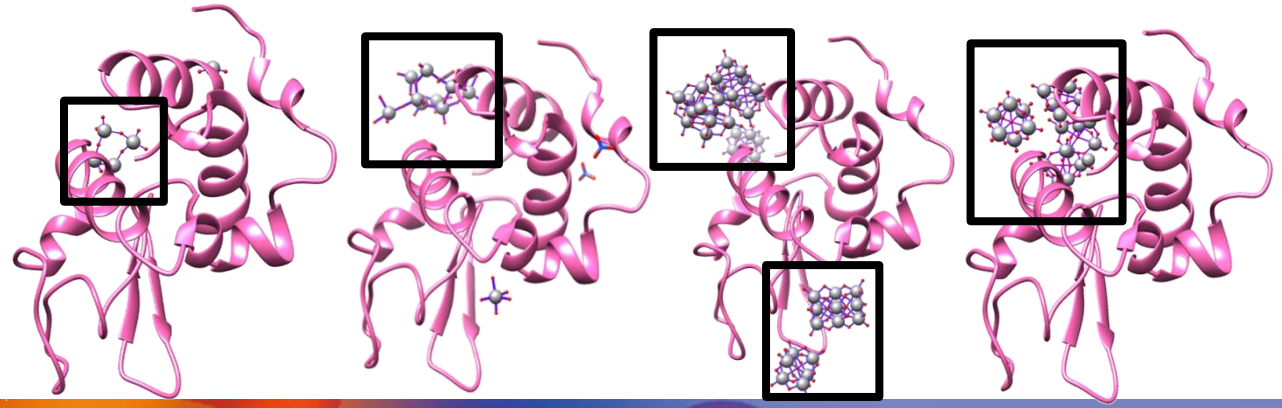
Binding of pharmacologically active diacetylacetonateoxidovanadium(IV) to the model protein lysozyme: structural studies





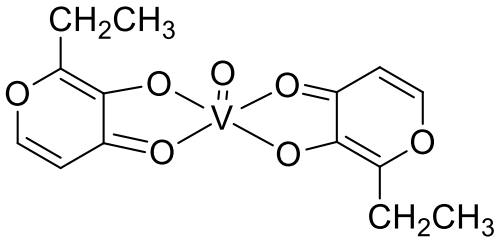
Abstract: Some V complexes are considered as promising antidiabetics. These compounds are able to mimic most of the biological effects of insulin in different organisms. Diacetylacetonateoxidovanadium(IV) [$V^{IV}O(acac)_2$] decreases glucose concentration in blood and enhances the kinase activity of the insulin receptor in cells more than other V compounds. Proteins play a major role in the definition of the mechanism of action of metallodrugs because of their high concentration in biological fluids and their high affinity for metal ions. To better understand the mechanism of action of [$V^{IV}O(acac)_2$] it is important to define its reaction with proteins. Here, we report the structures obtained upon reaction of [$V^{IV}O(acac)_2$] with the model protein lysozyme. The crystallographic study reveals the loss of the ligands, the oxidation of V^{IV} to V^{V}, and the subsequent formation of fascinating adducts of the protein with different polyoxidovanadates (POVs). POVs constitute a sub-class of the vast polyoxidometalates (POMs) family that exerts various biological activities, such as anti-Alzheimer's disease, antibacterial, anti-cancer, anti-diabetes, anti-virus, and so on. The obtained structural data expand the repertoire of structures of known protein-POM complexes and provide useful information on the recognition of POVs by proteins.

Keywords: vanadium; polyoxidovanadate; lysozyme; protein interaction; polyhedra

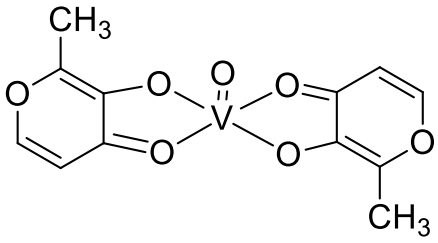
Vanadium compounds in medicine

Vanadium compounds have attracted great interest for their pharmacological properties.

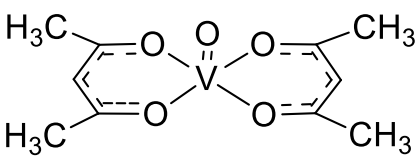
Some VCs show antidiabetic effects.



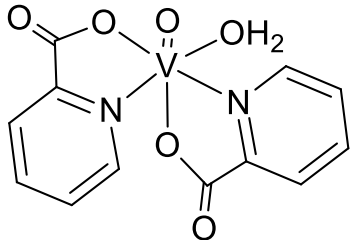
Bis(ethyl maltolato)oxidovanadium (BEOV)



Bis(maltolato)oxidovanadium (BMOV)



Diacetylacetonateoxidovanadium

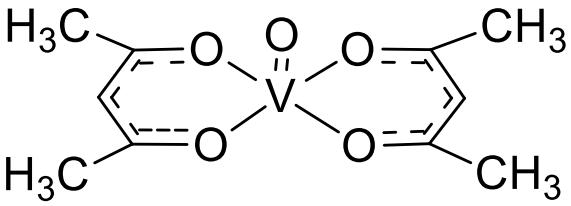


Bis(picolinato)oxidovanadium

- ✓ Increase glucose transport and oxidation
- ✓ Inhibit gluconeogenesis
- ✓ Regulate multiple enzymes.

Diacetylacetonateoxidovanadium(IV) [$V^{IV}O(acac)_2$]

Among vanadium compounds with potential medicinal applications, diacetylacetonateoxidovanadium(IV) [$V^{IV}O(acac)_2$] is one of the most promising for its antidiabetic and anticancer activities.



Structure of [$V^{IV}O(acac)_2$]

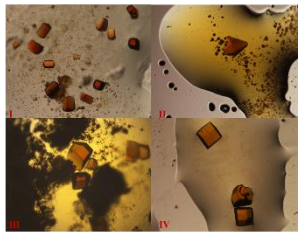
- It favors the decrease of glucose concentration in blood circulation.
- It enhances the insulin receptor kinase activity more than other $V^{IV}O$ chelate.

In bio-speciation of potential drugs proteins play an important role because of their high concentration in biological fluid and their high affinity for metal ions.

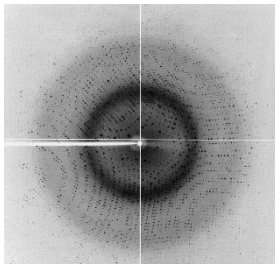
Aim of the project

- Preparation of lysozyme crystals treated with $[V^{IV}O(acac)_2]$ in four different experimental conditions.
- Refinement, validation and analysis of crystallographic structures obtained upon reaction of $[V^{IV}O(acac)_2]$ with lysozyme.

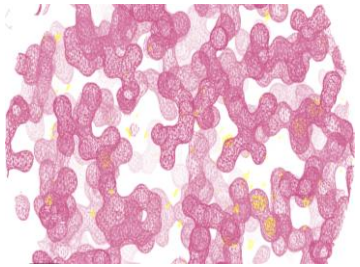
Crystal



Diffraction pattern



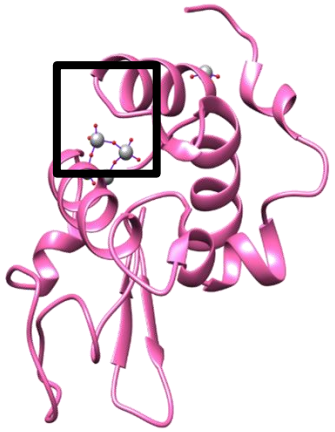
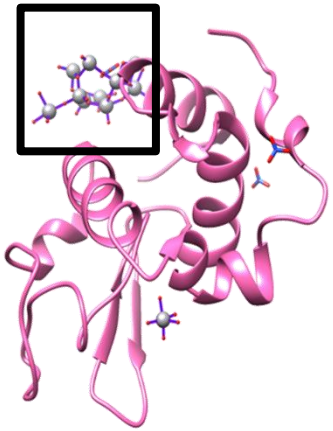
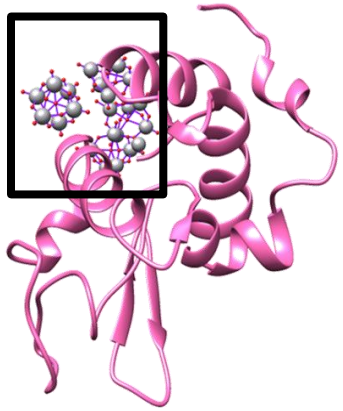
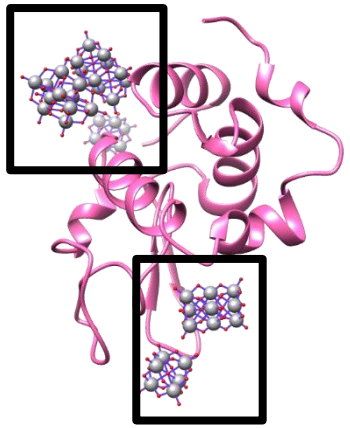
Electron density map



Atomic structure model

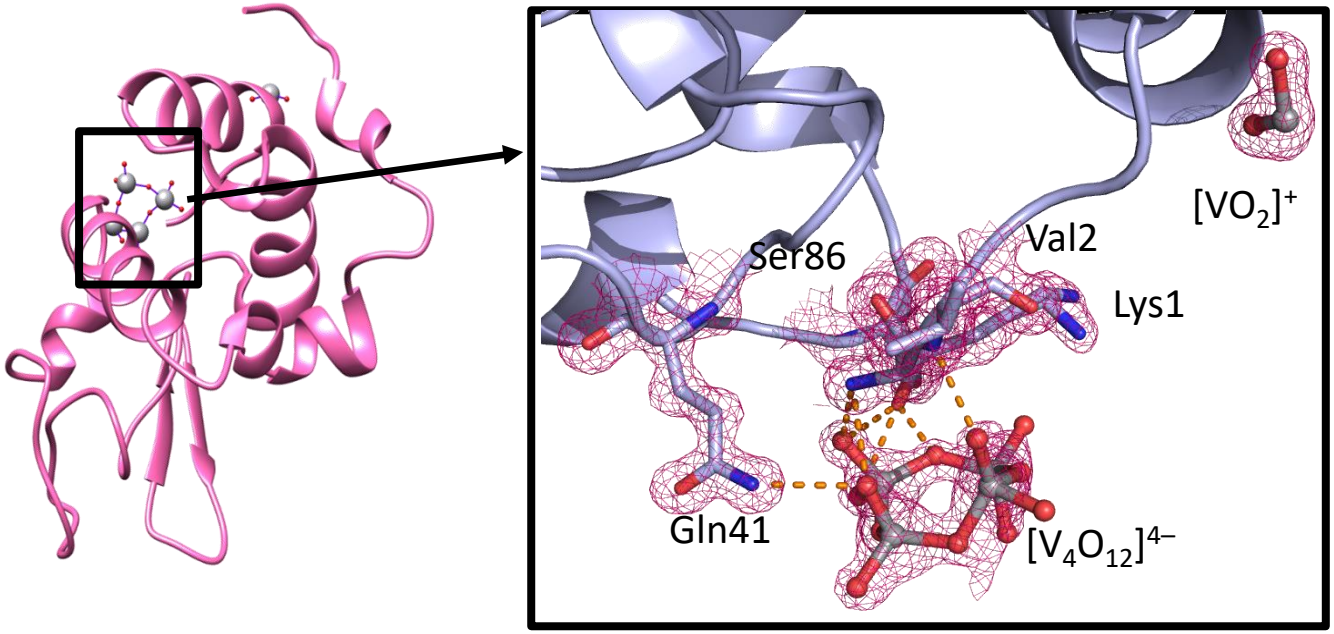
Results and discussion

Inspection of Fourier difference and anomalous electron density maps revealed the presence of different **polyoxovanadates (POVs)** species in all the analysed conditions, close to the protein surface.

Structure A	Structure B	Structure C	Structure D
			
2.0 M sodium formate, 0.1 M Hepes pH 7.5	*20% ethylene glycol, 0.1 M sodium acetate pH 4.0, 0.6 M sodium nitrate*	*0.8 M succinic acid pH 7.0*	*1.1 M sodium chloride, 0.1 M sodium acetate pH 4.0*
22 days (soaking)	*3 days (soaking)*	*22 days (soaking)*	*4 days (soaking)*

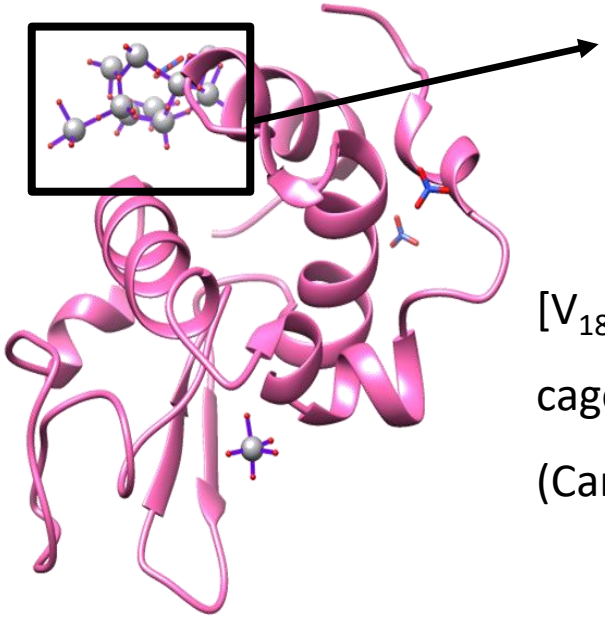
Structure A (pH 7.5)

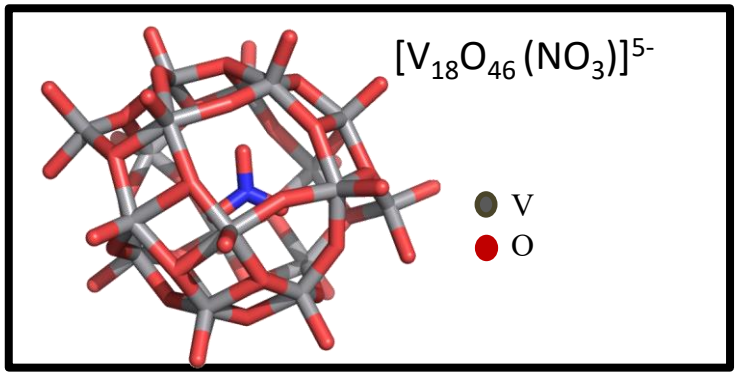
Non-covalent binding of $[V_4O_{12}]^{4-}$ anion on the protein surface has been found. Under the investigated experimental conditions, the metal oxidation state has shifted from +IV to +V.



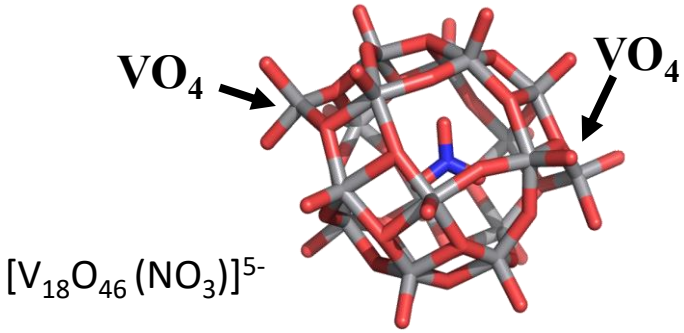
- Cyclic structure with V^V in a tetrahedral geometry.

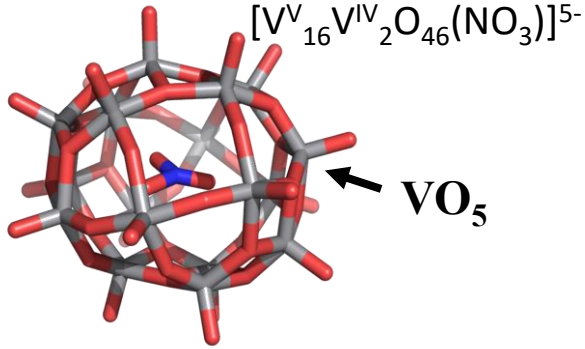
Structure B (pH 4.0)





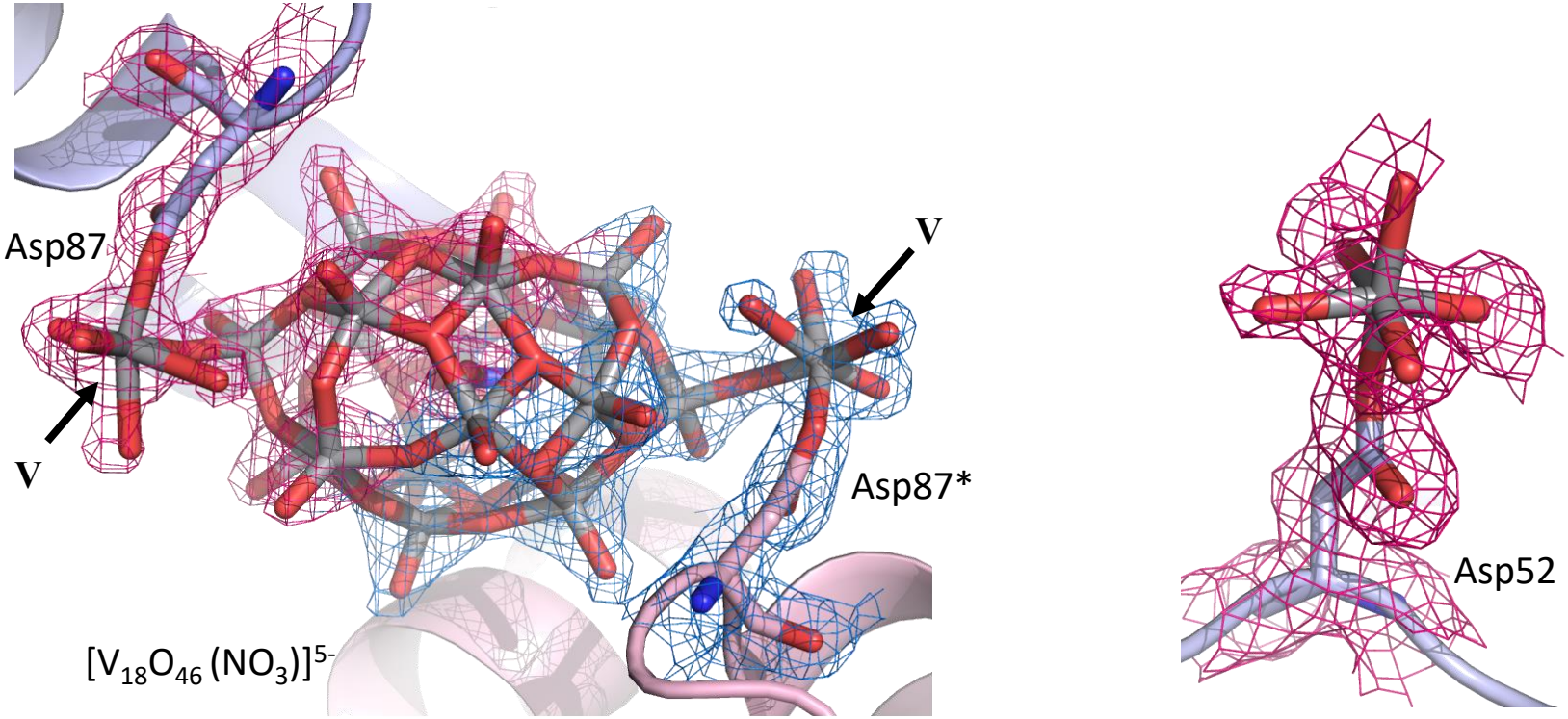
$[V_{18}O_{46}(NO_3)]^{5-}$ anion observed in this structure resembles the V18 cage with structure $[V^{V}_{16}V^{IV}_{2}O_{46}(NO_3)]^{5-}$ reported in the literature (Cambridge Structural Database code UNIQAC).





Structure B (pH 4.0)

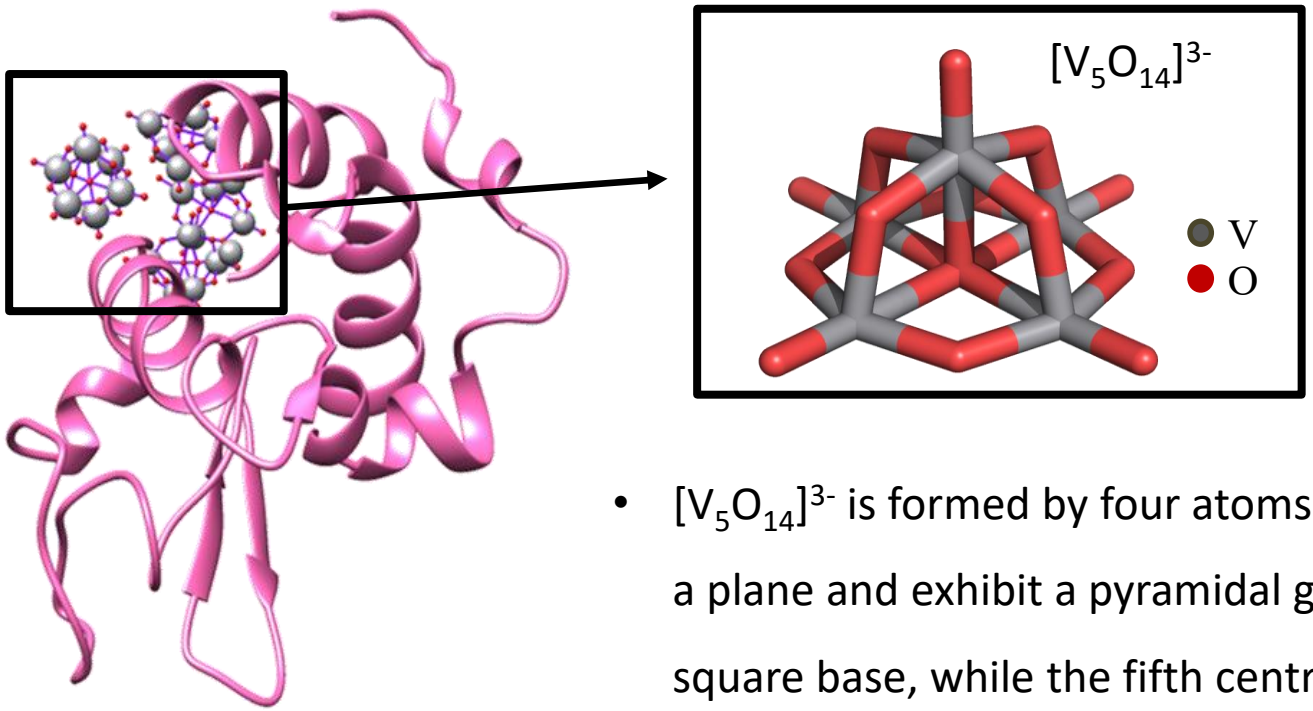
The $[V_{18}O_{46}(NO_3)]^{5-}$ anion, which has been refined with an occupancy of 0.60, is held in its position by a **covalent bond** formed with a VO_5 group that in turn is bound to the side chain of Asp87 and of Asp87*.



Atoms from symmetry-related molecules are labeled with *.

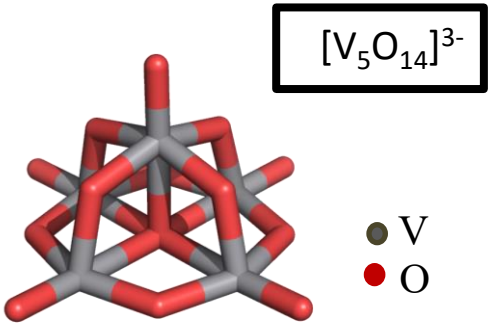
Structure C (pH 7.0)

Non-covalent bindings of $[V_5O_{14}]^{3-}$ anions on the protein surface have been found. Thus, also in this structure, the oxidation of the V centre from +IV to +V occurs.

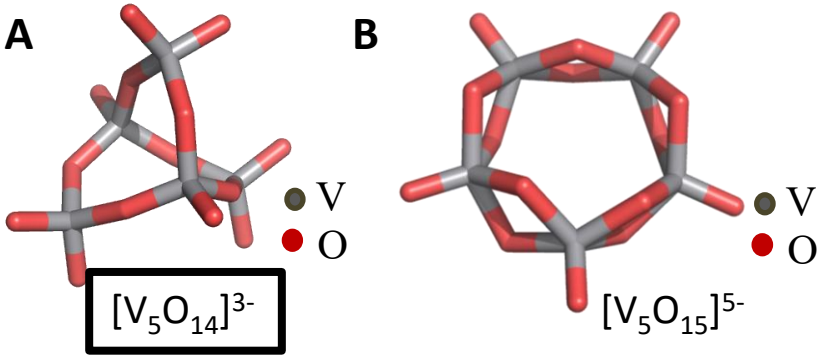


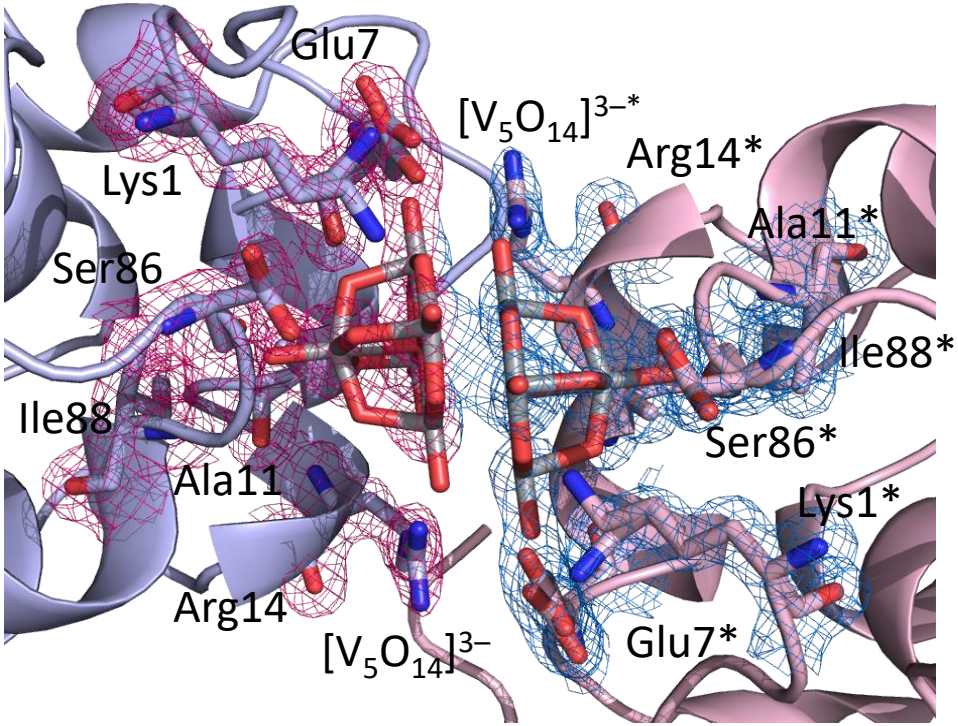
- $[V_5O_{14}]^{3-}$ is formed by four atoms of vanadium lying on a plane and exhibit a pyramidal geometry with a square base, while the fifth centre of V occupies the apex of a square pyramid.

Structure C



In the literature, the pentavanadate is reported in two cyclic forms:

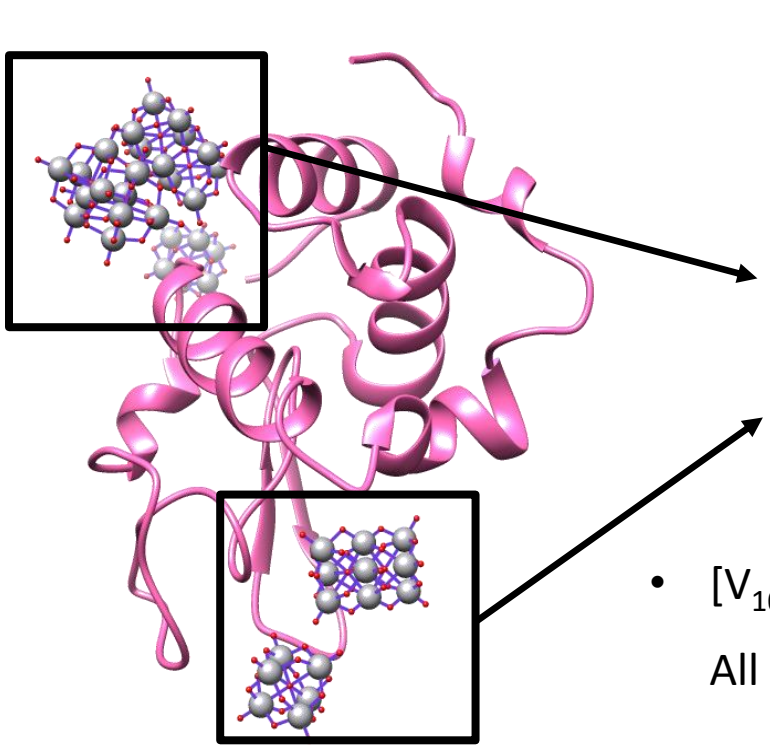


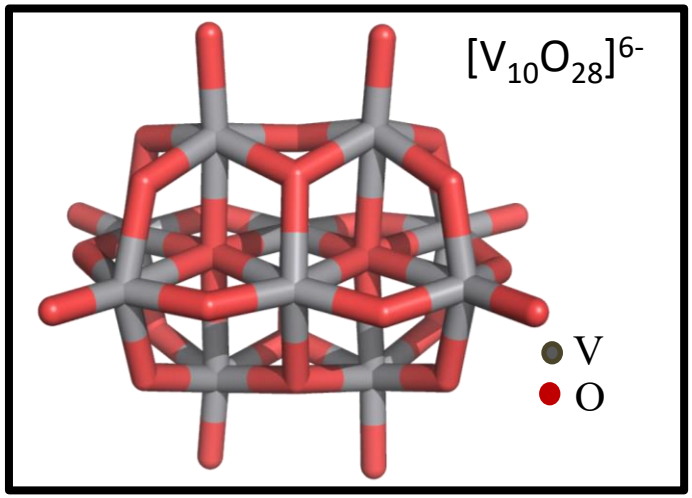


The cyclic pentavanadate $[V_5O_{14}]^{3-}$ observed in this structure has a molecular formula equal to that of the structure reported in Figure A **but has a totally different structure**.

Structure D (pH 4.0)

Non-covalent bindings of $[V_{10}O_{28}]^{6-}$ anions on the protein surface have been found. Thus, also in this structure, the oxidation of the V centre from +IV to +V occurs.



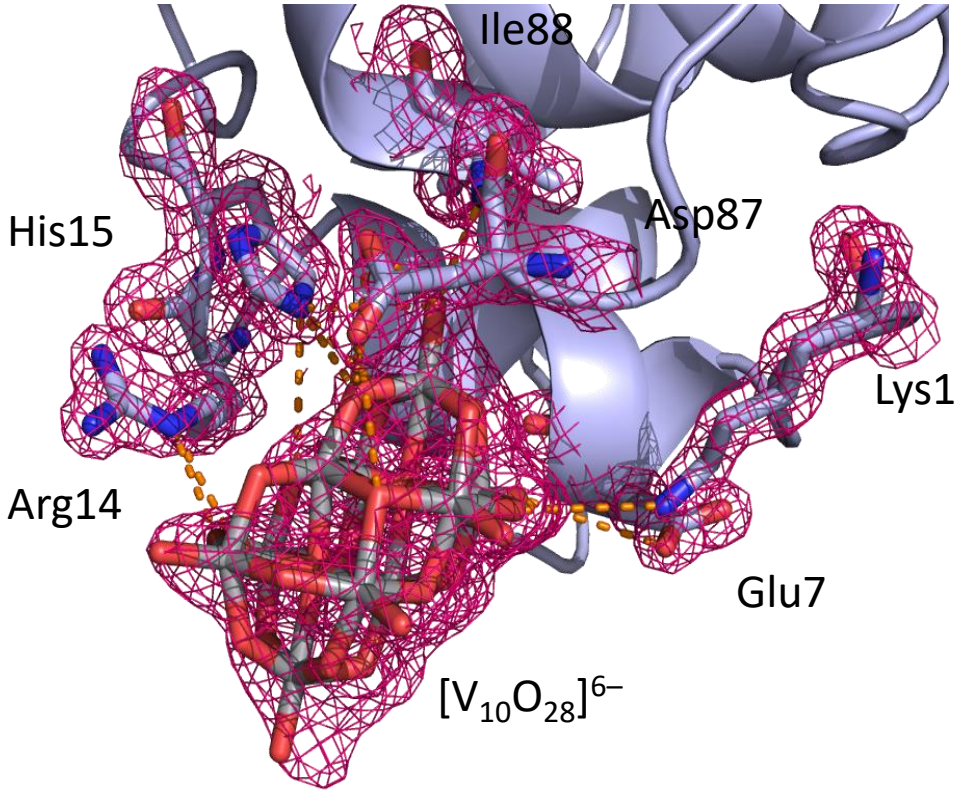


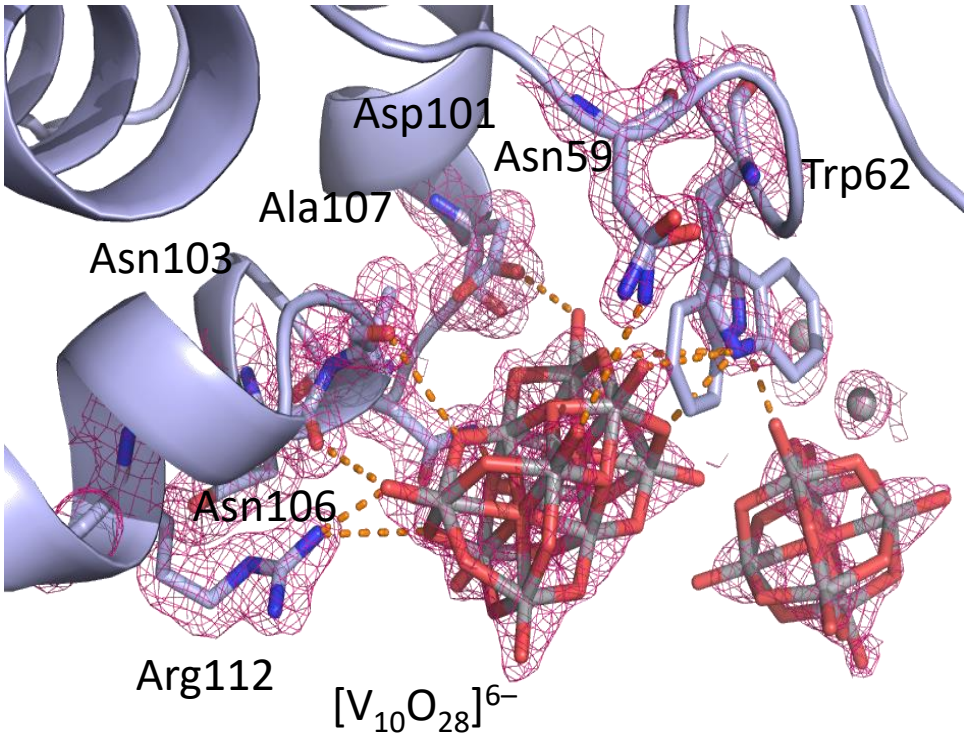
- $[V_{10}O_{28}]^{6-}$ is formed by 10 condensed $V^{V}O_6$ polyhedra. All vanadium atoms show distorted octahedral coordination.

Structure D

The former **$[V_{10}O_{28}]^{6-}$** ion is found almost in the same position adopted by the first $[V_5O_{14}]^{3-}$ ion in the structure **C**.

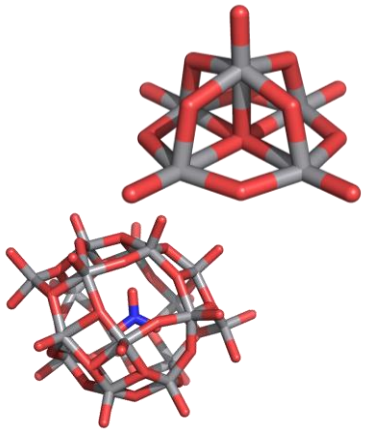
The latter **$[V_{10}O_{28}]^{6-}$** ion forms H-bonds with the residues of the protein.





Conclusions

- ✓ The results of this crystallographic study indicate that under the investigated experimental conditions [$V^{IV}O(acac)_2$] undergoes dissociation, V oxidation and reassembly, forming POVs with four, five, ten and eighteen V atoms.
- ✓ The obtained structures provide nice examples of stabilization of unrevealed POV structures. $[V_5O_{14}]^{3-}$ and $[V_{18}O_{46}(NO_3)]^{5-}$ have never been isolated and these structures are different from those previously reported.



- ✓ Finally, the obtained results provide a new structural basis for interpreting of experiments carried out with the potential drug [$V^{IV}O(acac)_2$] and for understanding their significant biological properties.

Acknowledgments

We gratefully acknowledge the Elettra Synchrotron staff for their assistance during data collection.