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Binding to proteins of the pharmacologically active bis(maltolato)oxidovanadium(IV)

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pharmaceuticals MDPI

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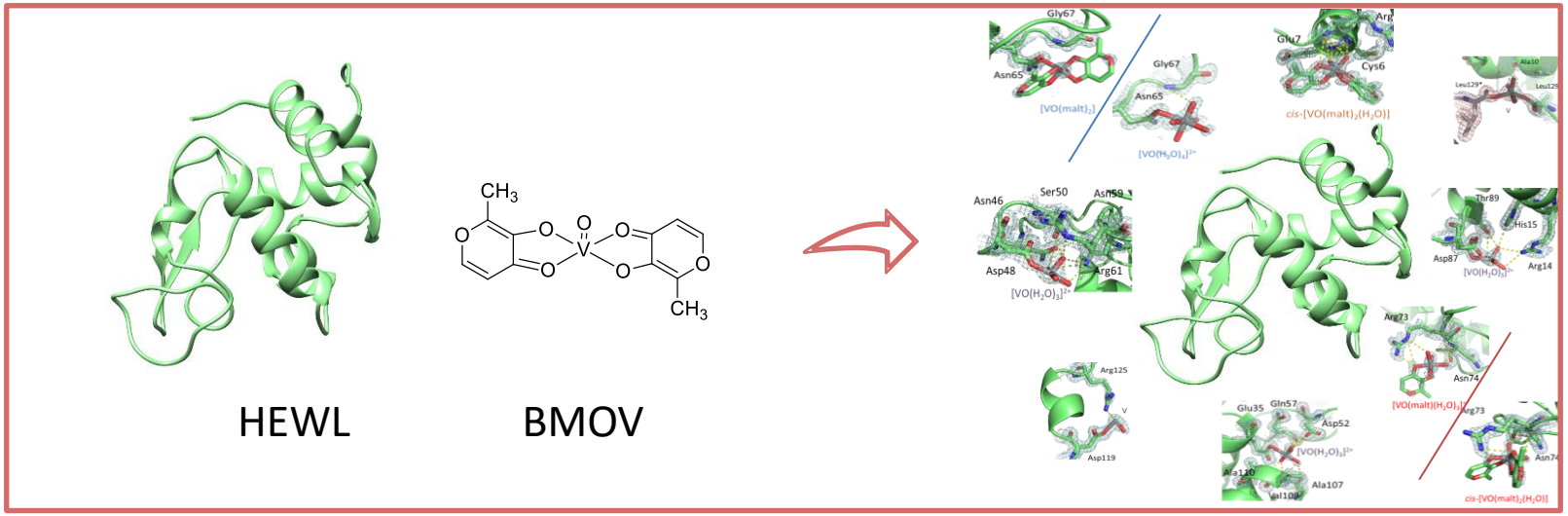
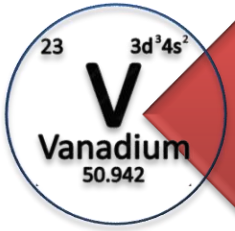
Binding to proteins of the pharmacologically active bis(maltolato)oxidovanadium(IV)



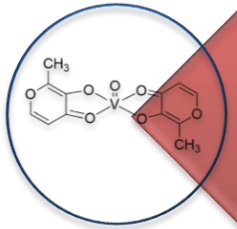
Abstract: Bis(maltolato)oxidovanadium(IV) ([$V^{IV}O(malt)_2$] or BMOV, where malt is maltolato) and bis(ethylmaltolato)oxidovanadium(IV) (BEOV) are among the most potent orally active insulin-mimetic agents. They have undergone extensive pre-clinical testing. Even though these experimentations were temporarily stopped due to renal problems of several patients and financial problems of Akesis Pharmaceuticals, BMOV is usually considered the reference for the new molecules with insulin-mimetic action. Surprisingly, recently the tests on BMOV are continued by CFM Pharma (CFM10, Vanadis) and now it is arrived to the phase II for the treatment of patients with injuries on secondary tissues caused by accidents or fire and with myocardial infarction. Proteins play a central role in the biospeciation of V compounds in the organism, because of both their high affinity toward V and their high concentration in biological fluids. Here, the interaction of BMOV with two model proteins has been analyzed by X-ray crystallography. Data indicate both non-covalent binding of *cis*-[$VO(malt)_2(H_2O)$] and [$VO(malt)(H_2O)_3]^+$ and covalent binding of [$VO(H_2O)_{3-4}]^{2+}$ and *cis*-[$VO(malt)_2$] and other V-containing fragments to the side chains of Glu, Asp and to the C-terminal carboxylate. Thus, our results suggest a multiple and variable interaction of BMOV with proteins. Our data can help to better understand the BMOV solution chemistry and contribute to define the molecular basis of the mechanism of action of this intriguing metallodrug.

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

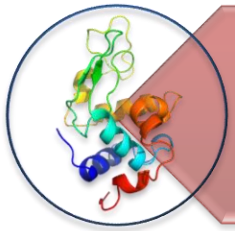
Introduction



Vanadium compounds (VCs) show a wide variety of pharmacological actions

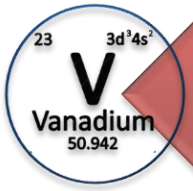


Among the VCs with anti-diabetic properties bis(maltolato)oxidovanadium(IV) (BMOV) is particularly interesting



Interaction with proteins is relevant

Vanadium compounds



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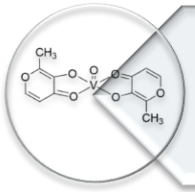

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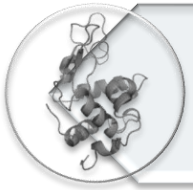
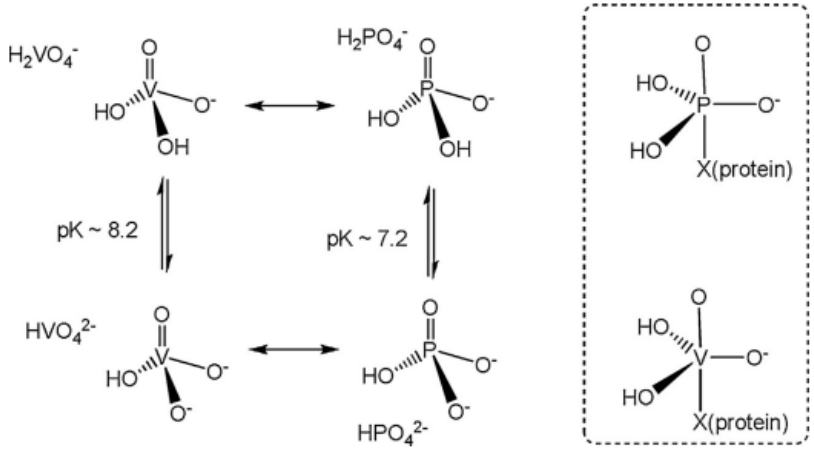


Interaction with proteins is relevant

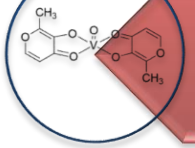


Analogy between phosphate and vanadate: monovanadate and phosphate are structurally very similar sharing similar acid-base equilibria and other types of reactions

Vanadate similarity to phosphate helps to explain its effect on phosphorylation events in the body

Oxidovanadium(IV) compounds

Vanadium compounds (VCs) show a wide variety of pharmacological actions



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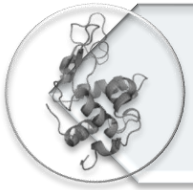
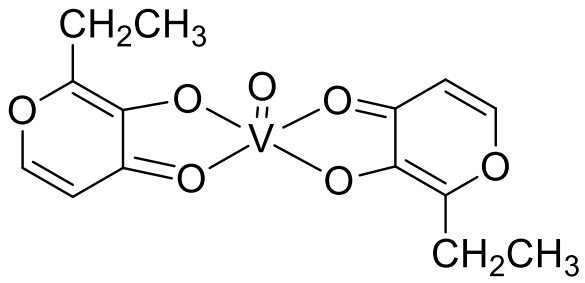
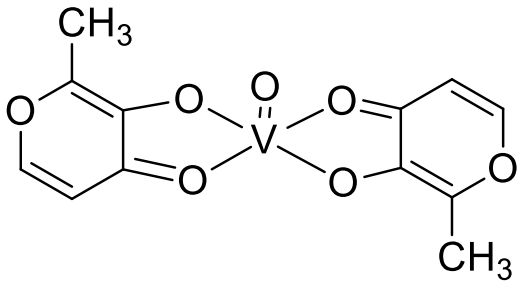


Interaction with proteins is relevant



Structure of bis(ethylmaltolato)oxovanadium(IV) (BEOV)

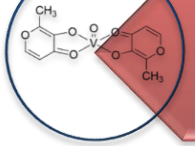


Structure of bis(maltolato)oxovanadium(IV) (BMOV)

BMOV is arrived to the **phase II** for the treatment of patients with injuries on secondary tissues caused by accidents or fire and with myocardial infarction

Bis(maltolato)oxidovanadium(IV) (BMOV)

Vanadium compounds (VCs) show a wide variety of pharmacological actions



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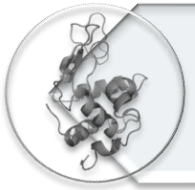
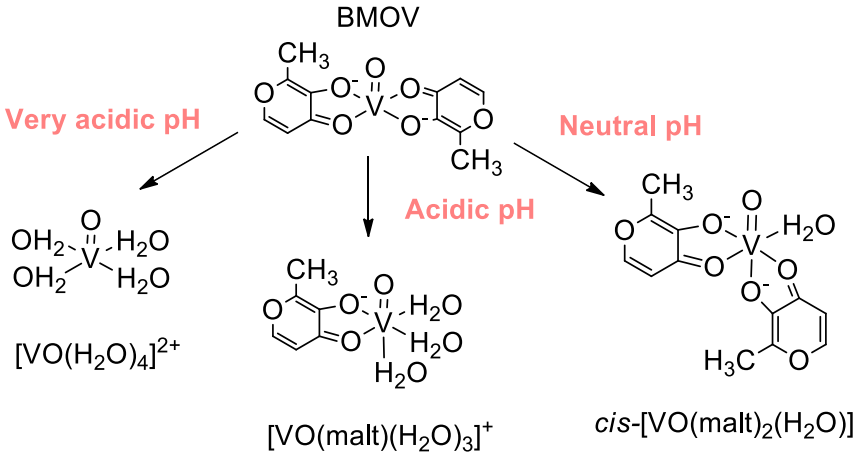


Interaction with proteins is relevant

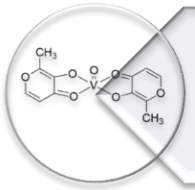


Structure of BMOV and its possible transformations in aqueous solution

In aqueous solutions different species can be formed. The ability to characterize BMOV in these solutions is critical for its possible use in diabetic treatment

Interaction of BMOV with metabolites and proteins

Vanadium compounds (VCs) show a wide variety of pharmacological actions



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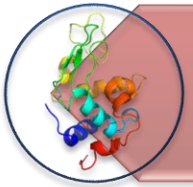
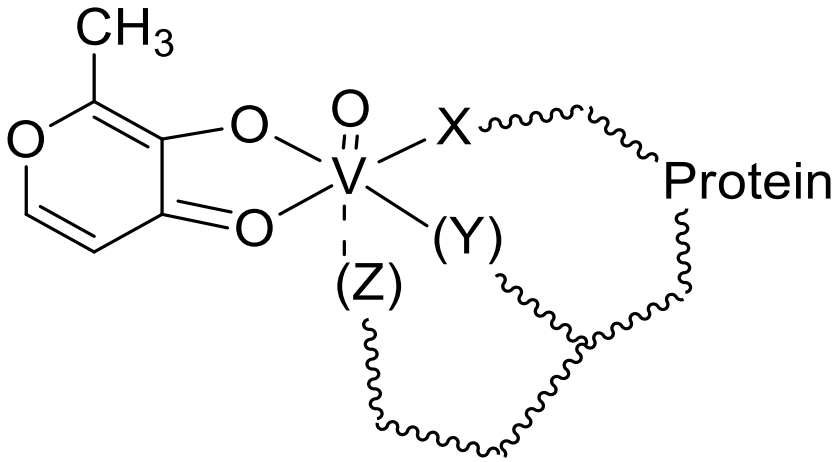


Interaction with proteins is relevant

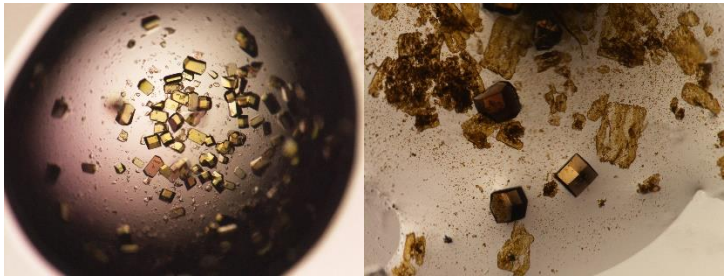


Adduct Formed at the physiological V concentration by BMOV

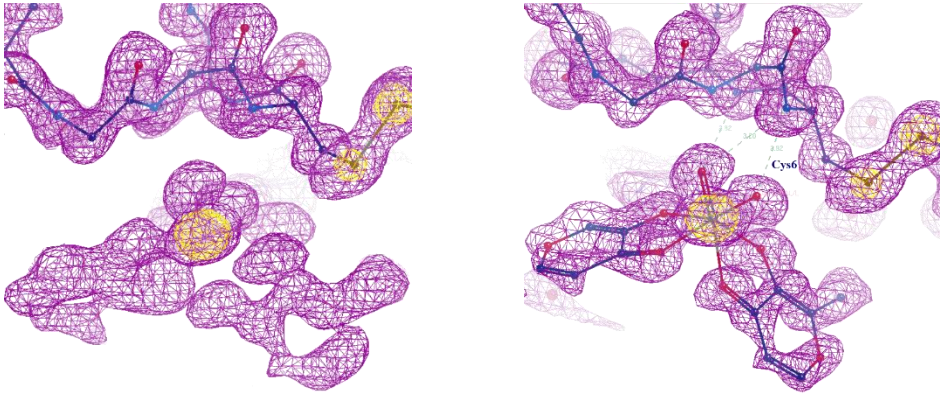
Proteins play a significant role in biospeciation and biotransformation of a vanadium compound in the organism

Aim of the work

Crystallization of the adducts obtained upon reaction of BMOV with the model proteins hen egg white lysozyme (HEWL) bovine pancreatic ribonuclease (RNase A)

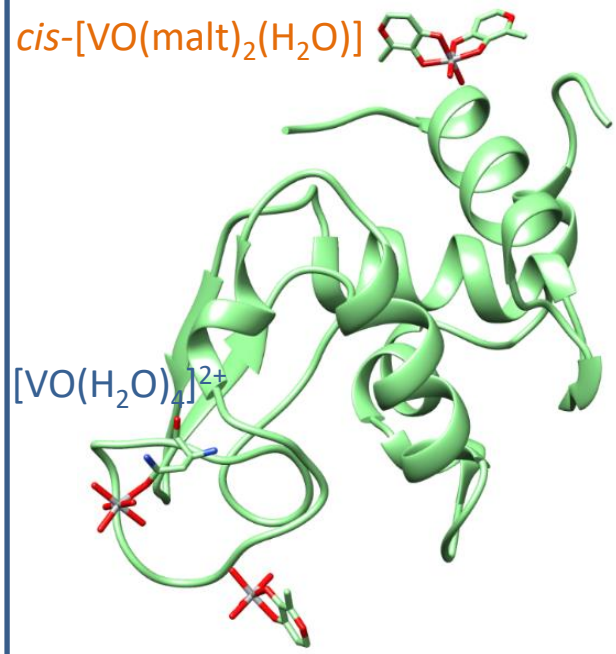


Refinement of crystal structures of HEWL and RNase A treated with BMOV, identification and interpretation of BMOV binding sites



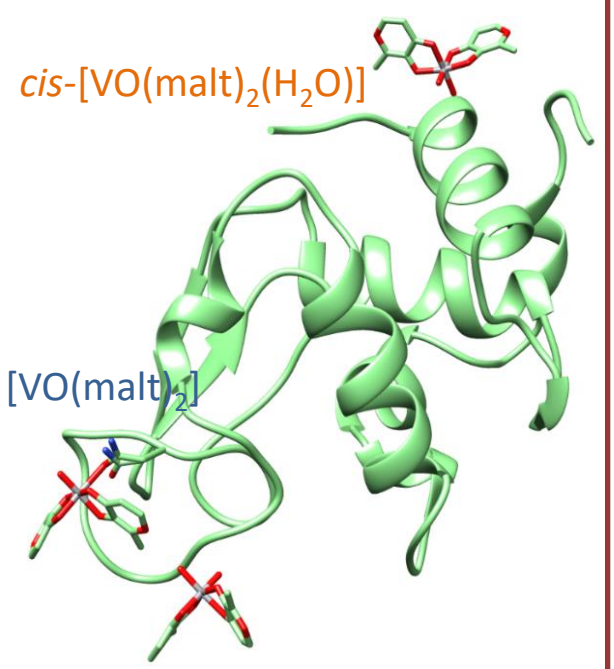
Results and discussion

Overall structures of HEWL in the presence of BMOV



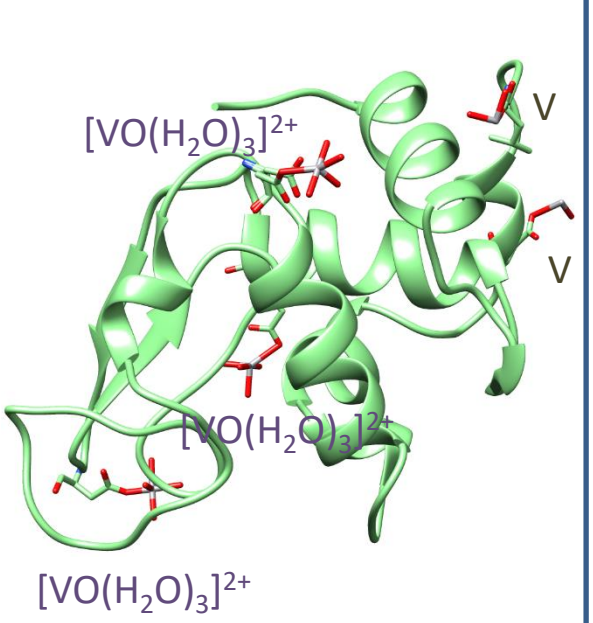
[VO(malt)(H$_2$O)$_3$]$^+$

Structure **A**
2.0 M sodium formate and
0.1 M Hepes buffer at pH 7.5
3 weeks (soaking)



cis-[VO(malt)$_2$(H$_2$O)]

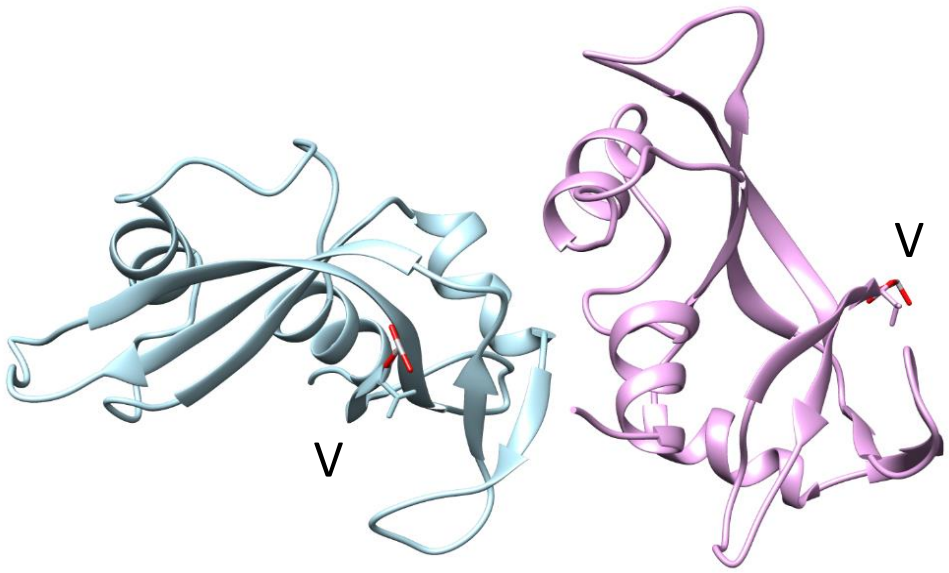
Structure **A'**
2.0 M sodium formate and
0.1 M Hepes buffer at pH 7.5
3 weeks (soaking)



Structure **B**
20% ethylene glycol, 0.1 M
sodium acetate at pH 4.0
and 0.6 M sodium nitrate
3 days (soaking)

Results and discussion

Overall structure of RNase A in the presence of BMOV

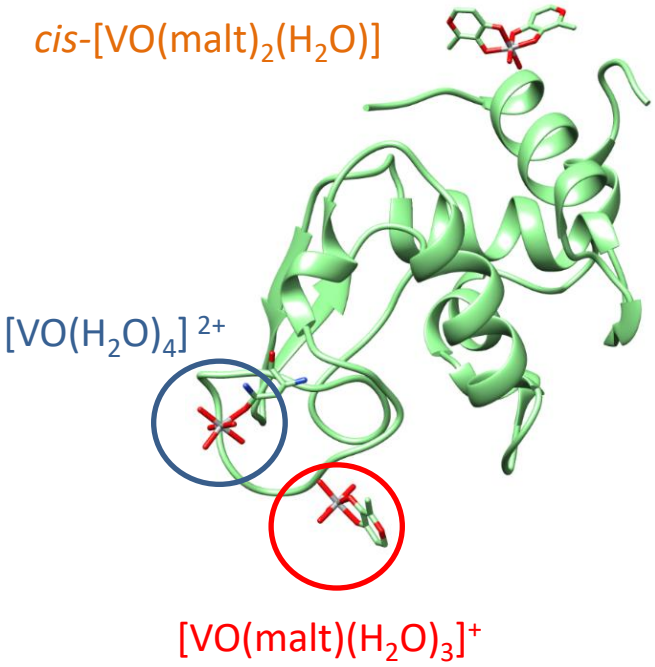


Structure of **RNase A**
22% PEG4K, 0.01 M sodium
citrate pH 5.1
3 days (soaking)

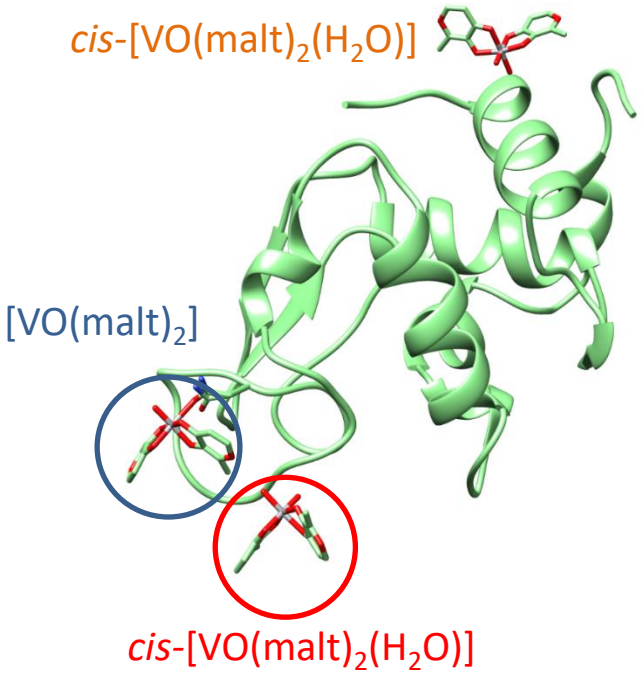
Structures A (1.13 Å) and A’ (1.22 Å)

Analysis of the diffraction data reveal that the structures **A** and **A’** show some local variations

Structure **A**



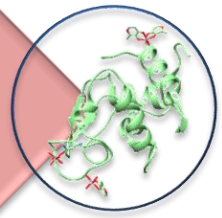
Structure **A’**



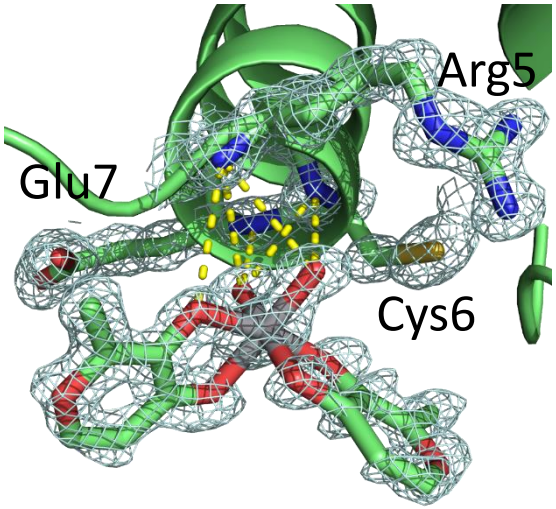
Structure A (1.13 Å)

Structure **A**

R_{factor}/R_{free}
0.199/0.249

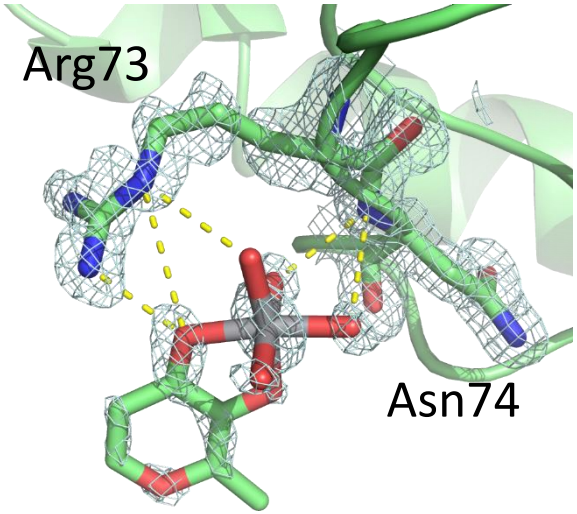


Noncovalent bindings of *cis*-$[VO(malt)_2(H_2O)]$ and $[VO(malt)(H_2O)_3]^+$ together with the covalent binding of $[VO(H_2O)_4]^{2+}$ to the side chain of Asn65 are found



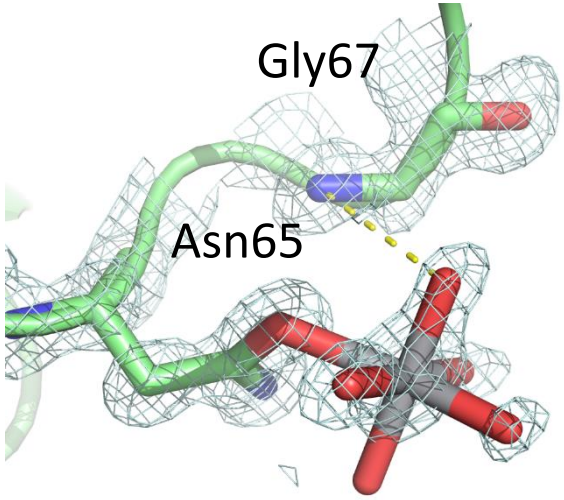
cis-$[VO(malt)_2(H_2O)]$

A



$[VO(malt)(H_2O)_3]^+$

B



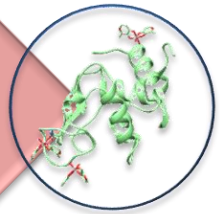
$[VO(H_2O)_4]^{2+}$

C

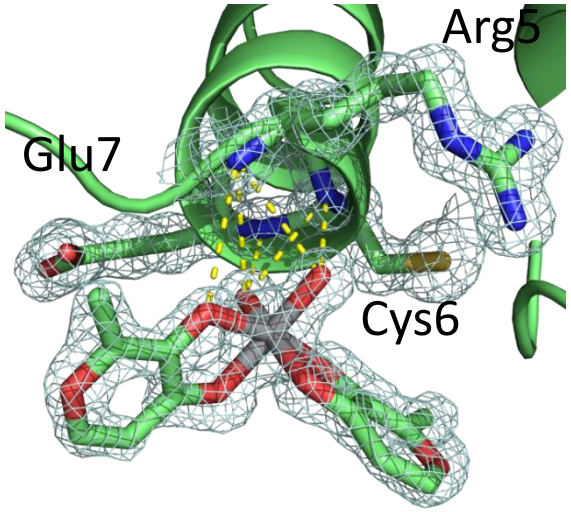
Structure A’ (1.22 Å)

Structure **A’**

R_{factor}/R_{free}
0.178/0.203

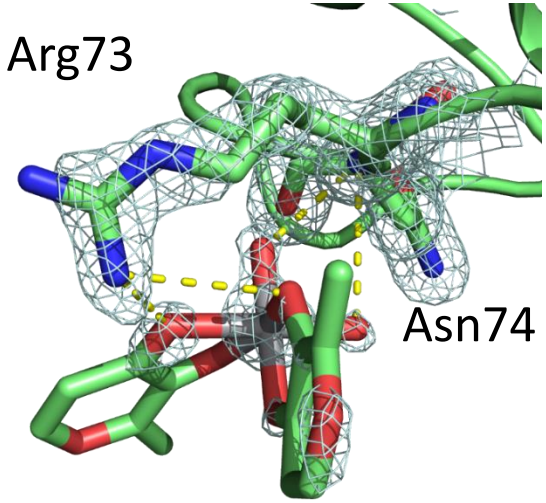


Noncovalent bindings of the two *cis*-[VO(malt)$_2$(H$_2$O)] together with the covalent binding of the [VO(malt)$_2$] to the side chain of Asn65 are found



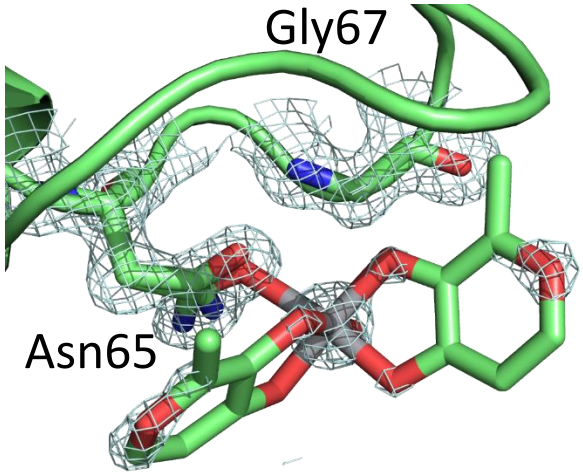
cis-[VO(malt)$_2$(H$_2$O)]

A



cis-[VO(malt)$_2$(H$_2$O)]

B

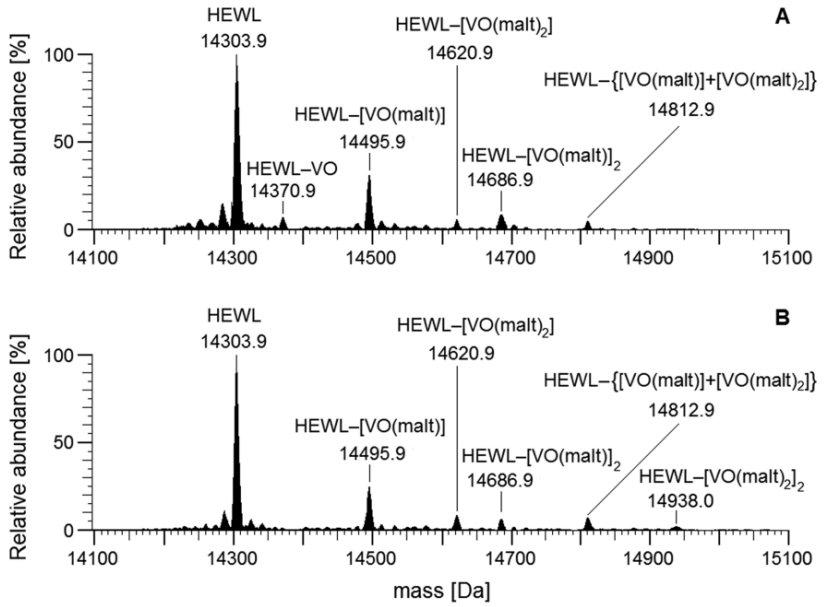


[VO(malt)$_2$]

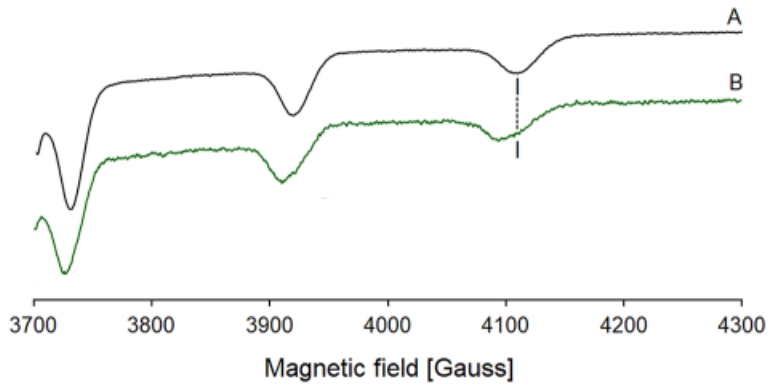
C

Structures A and A’

These data are in perfect agreement with the **ESI-MS experiments** and with the **EPR data**

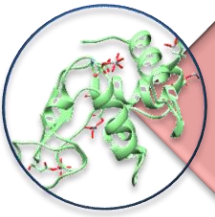


Deconvoluted ESI-MS(+) spectra recorded on the system containing BMOV (C_V=100 µM)/HEWL 2/1: **(A)** pH 5.0 and **(B)** pH 6.5



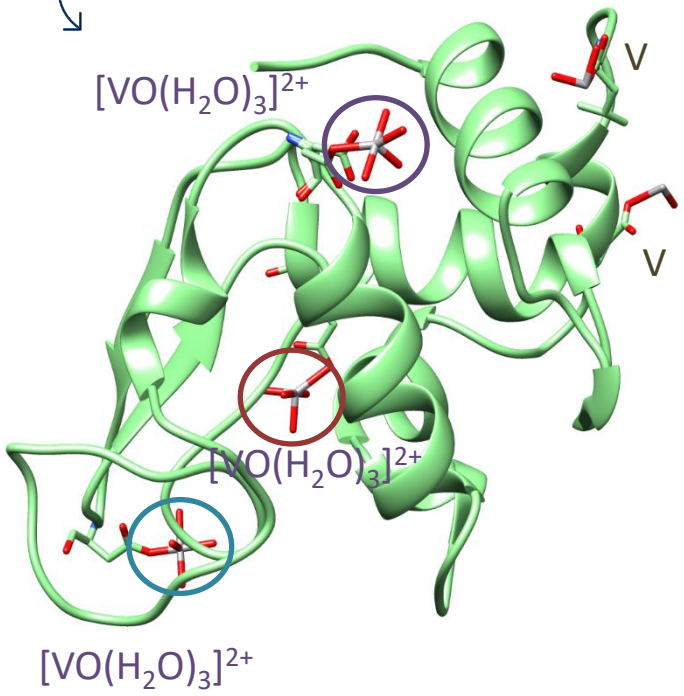
High-field region of anisotropic X-band EPR spectra recorded at pH 7.4 and 120 K in the systems of an aqueous solution containing **(A)** [$V^{IV}O(malt)_2(H_2O)$], **(B)** BMOV/HEWL 2/1. (C_V= 1.0 Mm).
The M_I = 7/2 resonance of [$V^{IV}O(malt)_2(H_2O)$] is indicated with **I**

Structure B (1.31 Å)

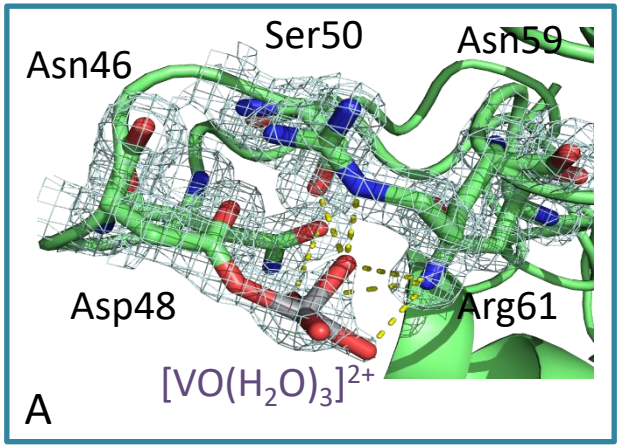


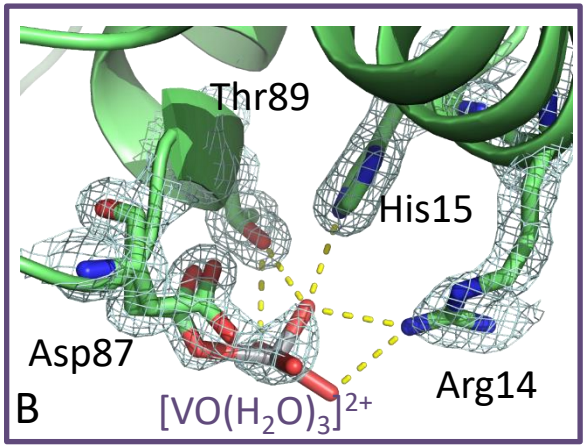
Structure **B**

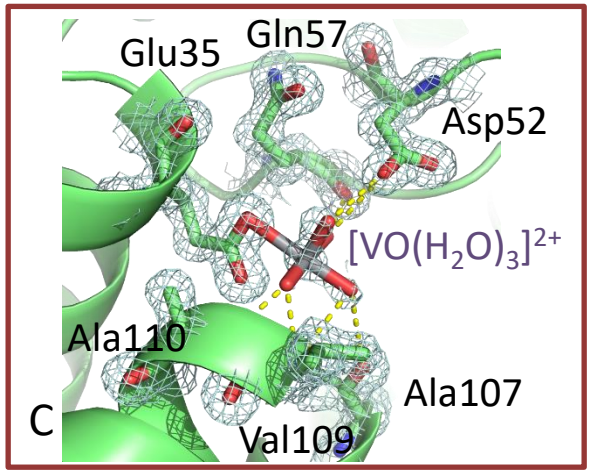
R_{factor}/R_{free}
0.168/0.198



Covalent binding of three $[VO(H_2O)_3]^{2+}$ ions are found







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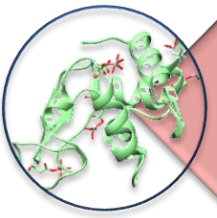

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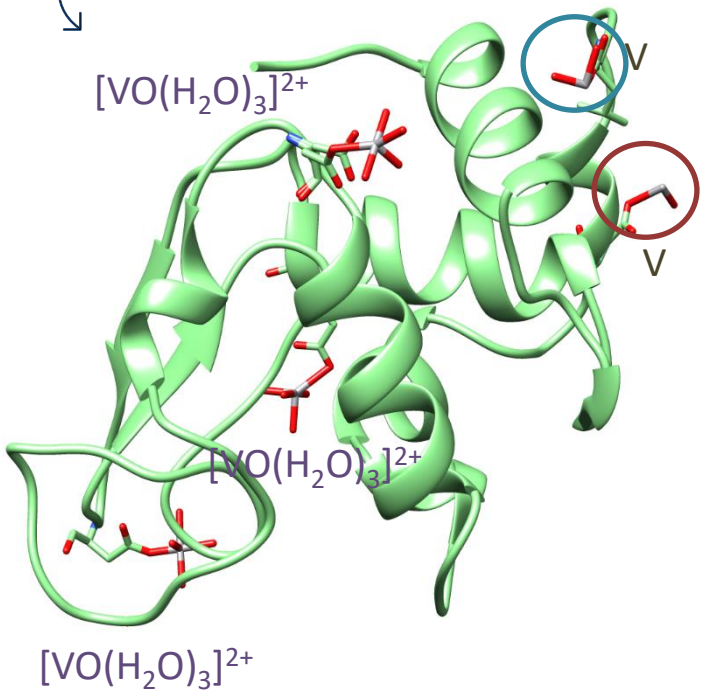
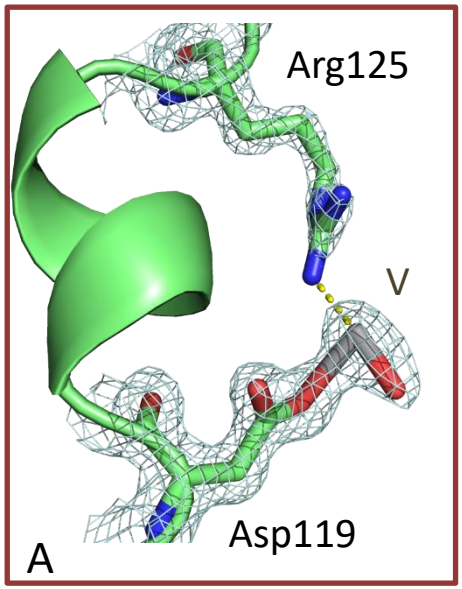
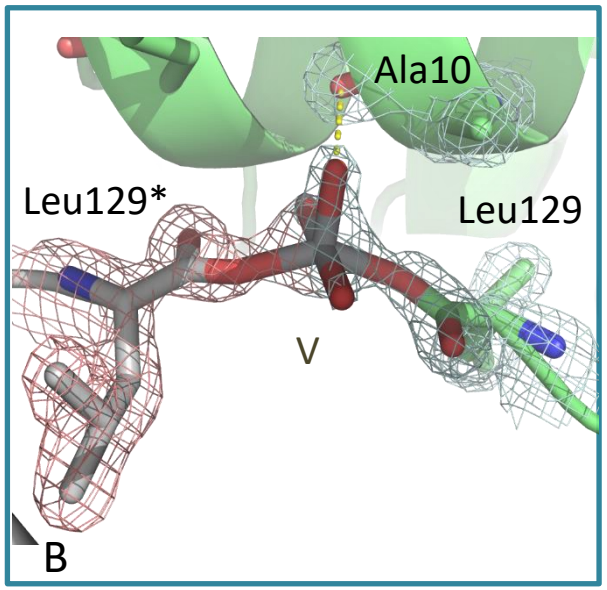


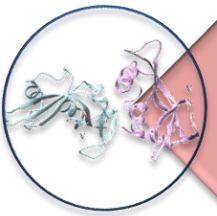
Covalent binding of two additional V atoms, whose geometry is not well defined, is found





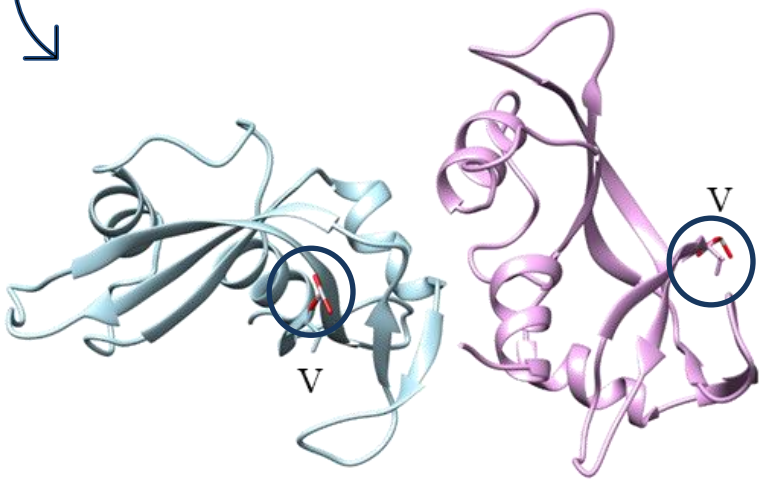
Atoms from symmetry related molecules are highlighted with * and colored in grey; the e.d. map is in red

Structure of RNase A (1.95 Å)

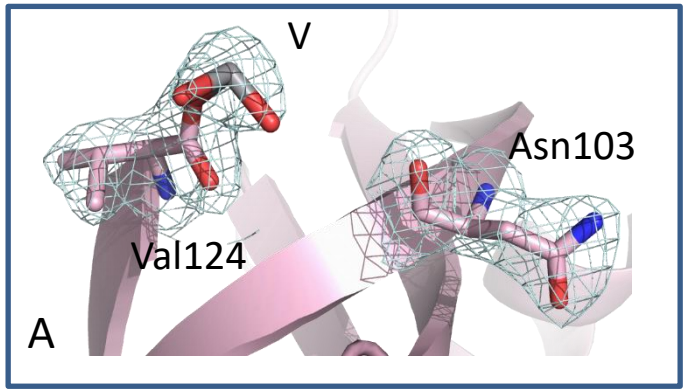


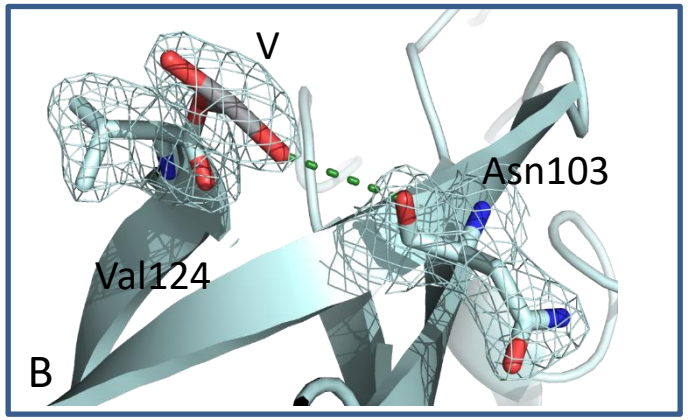
Structure of **RNase A**

R_{factor}/R_{free}
0.191/0.256



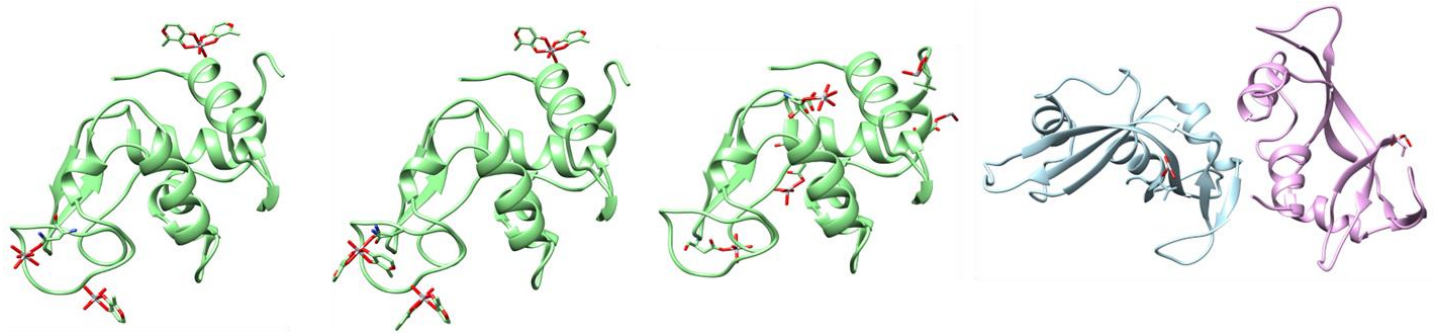
A V-containing fragment is found close to the C-terminal tail in both molecules present in the asymmetric unit





Conclusions

1. BMOV can bind proteins non-covalently, but it can also coordinate residue side chains upon losing one or more of its ligands



2. The protein structures are almost unaffected by the binding of the V compound

3. The reactivity of BMOV with HEWL and RNase A elucidated in this work could help in the understanding of the mechanisms at the basis of the formation of $V^{IV}O$ carrier–protein adducts

Acknowledgments

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