INORGANIC CHEMISTRY FRONTIERS

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Inorg. Chem. Front.*, 2025, **12**, 6870

Spherical mixed-valence pentadecavanadate binding to human H-chain ferritin

Rosanna Lucignano,†[a] Gabriella Tito,†[a] Giarita Ferraro,[a] Delia Picone,[a] Federico Pisanu,[b] Eugenio Garribba *[b] and Antonello Merlino *[a]

Received 11th August 2025,
Accepted 20th August 2025

DOI: 10.1039/d5qi01694k

rsc.li/frontiers-inorganic

The interaction of $[V^{IV}O(acac)_2]$, acac = acetylacetonato, with H-chain ferritin has been studied. After dissociation and partial oxidation, $[V^{IV}O(acac)_2]$ forms a mixed-valence $[V_{15}O_{36}(Cl)]^{6-}$ ion, which non-covalently binds with the protein nanocage. This polyoxidovanadate is formed at alkaline pH, showing that proteins can favor the formation and stabilization of species hardly isolable in solution.

Polyoxidometalates (POMs), polyanion clusters formed by early transition metals linked together by shared oxygen atoms, are fascinating systems due to their variety in size and structure which reflects an equal variety of applications spacing from catalysis,[1,2] photo- and electrochemistry,[3,4] nanomaterial science,[5,6] to protein crystallography,[7,8] and medicinal chemistry.[9,10] Among POMs, polyoxidovanadates (POVs) have been extensively studied in the past decades owing to their ability to act as insulin mimetics for diabetes treatment, ROS scavengers for oxidative stress balancing,[6] antimicrobial agents for neutralization of antibiotic-resistant bacterial strains,[11–14] potential therapeutics for neurodegenerative[15,16] and metabolic diseases,[17–20] and for cancer treatment.[17]

The mechanisms of action at the basis of their multiple biological roles are not well known and remain to be clarified. However, it has been suggested that the main factor of POV biological activities arises from their capability to establish non-covalent interactions with biomolecules.[21,22] Indeed, because of their negative charge, POVs have a strong tendency to bind to neutral or hydrophilic surfaces, becoming, in this way, potential partners for DNA, peptides and proteins. Their affinity for proteins can be exploited for different reasons. For example, proteins could take part in POV transport through the bloodstream, as well as their targeting, uptake, and biospeciation.[23–25] Furthermore, functionalization of POVs with proteins could be a useful tool to optimize and increase their stability and biocompatibility,[26–28] enhancing their potential as metallodrugs. In this scenario, as pointed out previously,[29] a deep comprehension of the interaction occurring between POVs and proteins represents the first step for future physiological and pathological applications and for a more rational design of new potential drugs belonging to this family of compounds.

Recently, in our extensive effort to study the interaction of V compounds and proteins,[30–37] we found that upon reaction of the potential drug $[V^{IV}O(acac)_2]$, where acac is the monoanionic acetylacetonato ligand (Fig. 1), with the model protein lysozyme, under different experimental conditions, different POVs can be formed, including mixed-valence cage-like structures with fifteen ($[V_{15}O_{36}(H_2O)]^{5-}$ and $[V_{15}O_{33}(H_2O)]^{+}$)[35,36] and twenty ($[V_{20}O_{51}(NO_3)]^{n-}$ and $[V_{20}O_{54}(H_2O)]^{n-}$)[35,36] vanadium atoms.

Ferritin is a highly conserved protein that plays a major role in iron storage and metabolism.[38,39] Mammalian ferritins are composed of 24 subunits (H- or L-chains) that self-assemble to form a spherical hollow nanocage.[40] The inner cavity connects to the external surface *via* two types of channels, the four-fold hydrophobic channels (C4) and the three-fold hydrophilic channels (C3).[41,42] Fe^{2+} ions cross these channels to be mineralized and stored within ferritin bulk in the form of hydrous ferric oxide.[43–45] The spherical architecture and the internal hollow cavity, coupled to lack of immunogenicity and high biocompatibility, make ferritin the perfect candidate for the delivery of different types of molecules.[46] This possibility is strongly empowered by the finding that cells can recognize and internalize ferritin by specific receptors, such as TfR1 (transferrin

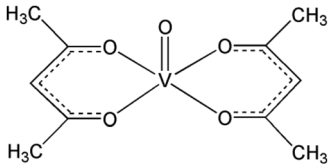

Fig. 1 Structure of $[V^{IV}O(acac)_2]$.

[a]*Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, via Cinthia 26, 80126 Naples, Italy. E-mail: antonello.merlino@unina.it*
[b]*Dipartimento di Medicina, Chirurgia e Farmacia, Università di Sassari, Viale San Pietro, I-07100 Sassari, Italy. E-mail: garribba@uniss.it*
† These authors contributed equally to this work.

receptor 1) which specifically binds H-chains.[47] Given its central role in cellular metabolism and its potential action as a nanocarrier, the human H-chain ferritin (HuHf) represents an ideal model for the study of the interaction of POVs with proteins of physiological interest.

Thus, as $[V^{IV}O(acac)_2]$ reacts with proteins undergoing progressive and relatively rapid transformations (ligand loss and oxidation from V^{IV} to V^{V}) that lead to the formation of various POVs,[35,36,48] we decided to study its reaction with recombinant HuHf.

The aim of this work is to extend the results of the previous studies with the model protein lysozyme and unveil the binding of $[V^{IV}O(acac)_2]$ to a protein of physiological relevance. Notably, the study provides a rather exhaustive description, at the molecular level, of the interaction between HuHf and a POV formed upon the transformation in an aqueous solution of $[V^{IV}O(acac)_2]$ under the used experimental conditions.

HuHf crystals were grown in 2.0 M magnesium chloride and 0.1 M bicine (pH 9.0) and incubated overnight (structure **A**), for 24 h (structure **B**) and for 6 days (structure **C**) with a reservoir solution saturated with $[V^{IV}O(acac)_2]$ (concentration about 300 μM[48]). X-ray diffraction data were collected on these crystals at high resolution, *i.e.* at 1.38, 1.50 and 1.54 Å resolution, respectively (Table S1 of the SI). The three structures were refined with *R*-factor values within the range of 0.162–0.177 (R_{free} 0.177–0.198) and their coordinates, together with their structure factors, are deposited in the Protein Data Bank (PDB) under the accession codes 8RGH, 9RGJ and 8RGI (Fig. 2 and Fig. S1). The three structures are identical: the models contain residues 4–176 of HuHf, 6 Mg^{2+} ions, and a $[V_{15}O_{36}(Cl)]^{6-}$ ion (Fig. 2A–C). HuHf chains adopt the four-helix bundle structure typical of the ferritin fold and can be superimposed to those of metal-free HuHf with the Cα root mean square deviation (rmsd) within the range of 0.41–0.53 Å. 2Fo–Fc and anomalous difference electron density (e.d.) maps of the $[V_{15}O_{36}(Cl)]^{6-}$ ion are shown in Fig. 2C for the structure solved at the highest resolution and in Fig. S2 for the other two structures. The POV interacts with residues located on the protein surface establishing H-bonds and electrostatic interactions with residues Thr5, Arg9, Gln10, Asn11, Tyr12, His13 and Gln14 and with five water molecules. These residues are not involved in iron recognition by the protein.[49] Occupancy of the POV in the three structures is within the range of 0.50–0.60; this suggests that HuHf binds *ca.* 12–14 $[V_{15}O_{36}(Cl)]^{6-}$ anions per cage.

$[V_{15}O_{36}]^{5-}$ was characterized for the first time by Muller *et al.*, who described it as "formed by linkage of fifteen tetragonal VO_5 pyramids" and "arranged on the surface of a sphere at a distance of 3.43 ± 10 pm".[50] The bottom of the pyramids points to the center of the cluster, which is occupied by the so-called guest anion,[51] a chloride ion in our case (Fig. 2C). According to Muller *et al.*,[50] the $\{V_{15}O_{36}\}$ cage is a mixed-valence cluster where V^{V} and V^{IV} coexist in a ratio depending on the average value of the bond valence sum (BVS) and of the cage radius. If the BVS calculations are applied to our systems, the anion bound to HuHf can be assigned to a $V^{V}_{7}V^{IV}_{8}$ system with a charge of −5 (Table S2). Indeed, the fifteen V atoms in our structures **A–C** are arranged at the cage surface with radii of 3.39 ± 0.11 Å, 3.37 ± 0.17 Å and 3.36 ± 0.17 Å from the central chloride ion, and average BVS values of 4.44 ± 0.25 Å, 4.47 ± 0.23 Å and 4.49 ± 0.23 Å, respectively (Table S2), compared with the average experimentally observed value of 3.45 ± 0.01 Å and the theoretical value of 4.47 Å, respectively.[50,52] Notably, although decavanadate has been observed at pH 10 and is kinetically stable for months at 1 mM concentration,[53] the formation of the $\{V_{15}O_{36}\}$ cage at alkaline pH is unexpected,[22,24,25,51] since under this condition the main species should be the orthovanadates $[H_2V^{V}O_4]^-$ and $[HV^{V}O_4]^{2-}$.[54]

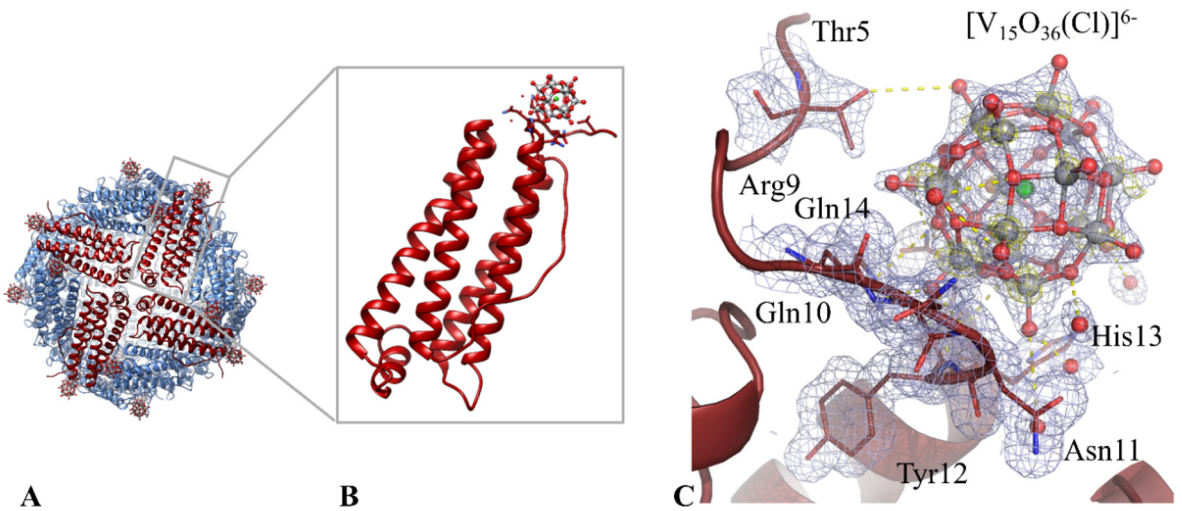

Fig. 2 Cartoon representation of the HuHf nanocage (A) and of the HuHf chain (B) with $[V_{15}O_{36}(Cl)]^{6-}$ binding site in structure **A**. The interactions of POV with the protein residues are reported in (C). 2Fo–Fc e.d. map is contoured at 1.0σ level in light blue. Anomalous difference e.d. map is contoured at 3.0σ level in yellow.

To obtain further information on the interaction of $[V^{IV}O(acac)_2]$-derived species with HuHf, in-solution studies were also carried out. Circular dichroism spectra of HuHf in the presence of $[V^{IV}O(acac)_2]$ (saturated conditions) were collected at 25 °C at four different incubation times (T_0, overnight, after 24 h and 6 days), under the experimental conditions used to obtain the crystals of HuHf treated with the vanadium compound. As a negative control, a sample of V-free HuHf was incubated under the same experimental conditions. The spectral profile of V-free HuHf displays the features of a protein with high alpha-helix content, with two broad minima at 208 and 222 nm and a maximum at about 200 nm (Fig. 3A). CD spectra of the samples incubated with $[V^{IV}O(acac)_2]$ show a progressive reduction of the CD signal, which is indicative of sample aggregation and/or precipitation. These phenomena are compatible with the binding of V-containing fragments on the external surface of the HuHf cage: these fragments can act as cross-linkers and/or interact with two or more different cages, driving aggregation and precipitation phenomena. This agrees well with the crystallographic observation of the interaction between the external HuHf cage surface and POV.

Moreover, the behavior of the same HuHf samples was compared to that of the protein in 20 mM Tris-HCl (pH 7.4) through polyacrylamide non-denaturing gel electrophoresis (Fig. 3B). These data are also compatible with crystallographic results. Indeed, inspection of Fig. 3B indicates that, when incubated in 2.0 M magnesium chloride and 0.1 M bicine (pH 9.0) in the absence (lane 2) and in the presence of vanadium at time zero (lane 3), HuHf shows a migration that is slightly lower than that observed for the HuHf samples incubated for longer times in the presence of the vanadium complex (lanes 4, 5 and 6) and lower than that of HuHf in 20 mM Tris-HCl (pH 7.4) (lane 1). The reduced migration of the V-free sample at pH 9.0 when compared to the same sample at pH 7.4 could be explained by considering the binding of Mg^{2+} ions on the protein cage surface, which shields its negative charges when compared to the Mg^{2+}-free protein. Conversely, the different migration of the samples in the absence (lane 2) and in the presence of $[V^{IV}O(acac)_2]$ at time zero (lane 3) compared to the samples incubated for longer times (lanes 4, 5 and 6) could be attributed to the binding of POV species to the protein surface, which in some way balances the positive charge of Mg^{2+} ions, increasing the protein sample migration towards the positive pole. Finally, after 6 d of incubation, a reduction of the intensity of the band (lane 6) was observed, in accordance with CD data, confirming, once again, the occurrence of aggregation and/or precipitation phenomena.

Molecular docking confirms the observed binding of $[V_{15}O_{36}(Cl)]^{6-}$ to HuHf and allows generalization of the results. Docking calculations were carried out using different models for $[V_{15}O_{36}(Cl)]^{6-}$ and one single H-chain, both taken from different crystal structures (see the SI for further details). In all cases, the most populated pose is in excellent agreement with that experimentally observed and shows the same network of H-bonds (Fig. 4A and Table S3). This means that the revealed binding site is the result of the interaction of $[V_{15}O_{36}(Cl)]^{6-}$ with only one single H-chain independently of the other chains of HuHf. To validate this result, additional docking simulations were carried out considering six HuHf chains, whose assembly well represents the full protein structure due to its inherent symmetry. The results of this analysis indicate that two possible $[V_{15}O_{36}(Cl)]^{6-}$ binding sites (indicated with I, the experimental one, and II in Table S4) can be identified on the HuHf structure with the most populated site that corresponds to that experimentally observed (Fig. S3). These data confirm that the stabilization of $[V_{15}O_{36}(Cl)]^{6-}$ is determined by the interaction with one HuHf chain and that the protein–protein interactions – used to explain the formation of other V-protein adducts[32] – are not relevant for the POV recognition by ferritin.

In conclusion, in this study we have investigated the interaction of the potential drug $[V^{IV}O(acac)_2]$ with human H-chain ferritin. This is the first structure of a V compound with human ferritin. Data indicate that $[V^{IV}O(acac)_2]$ transforms in

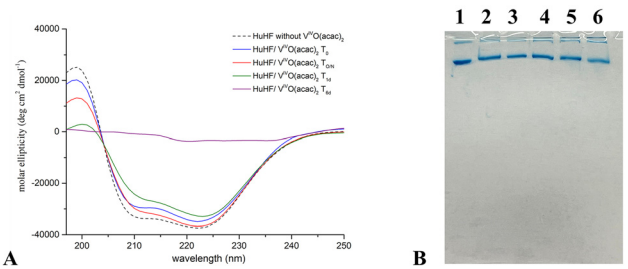

Fig. 3 (A) Far UV-CD spectra of 0.05 mg mL^{-1} HuHf in 2.0 M magnesium chloride and 0.1 M bicine buffer (pH 9.0) in the absence (black line) and in the presence of $[V^{IV}O(acac)_2]$ (saturated condition) incubated at time zero (T_0, blue line), overnight (red line), 1 d (green line) and 6 d (purple line). (B) Non-denaturing gel electrophoresis of HuHf in 20 mM Tris-HCl (pH 7.4) (lane 1) and HuHf incubated in 2.0 M magnesium chloride and 0.1 M bicine buffer (pH 9.0) in the absence (lane 2) and in the presence of $[V^{IV}O(acac)_2]$ at T_0 (lane 3), overnight (lane 4), 1 d (lane 5) and 6 d (lane 6). Other experimental details are reported in the SI.

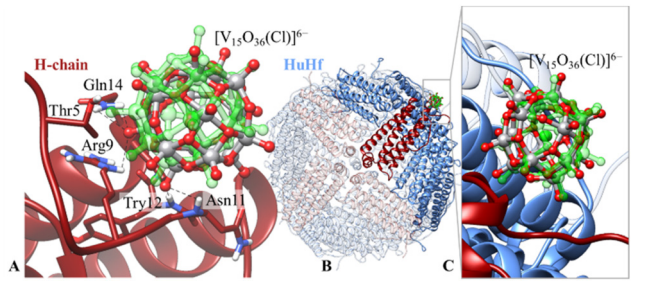

Fig. 4 Cartoon representation of site I (the experimental one) obtained by docking simulations of $[V_{15}O_{36}(Cl)]^{6-}$ to HuHf (both from the crystal structure here reported, PDB code 9RGHS1). (A) Docking to single H-chain and (B and C) docking to the HuHf six-chain assembly. For comparison, the experimental position of $[V_{15}O_{36}(Cl)]^{6-}$, taken from its crystallographic coordinates, is reported in green and as transparent. Dashed lines indicate H-bonds.

the presence of HuHf, similarly to when this V compound reacts with lysozyme under different experimental conditions.[35,36] The V center loses the acac ligands and in part oxidizes to V^V. While with lysozyme $\{V_{15}O_{33}\}$, $\{V_{20}O_{51}\}$ and $\{V_{20}O_{54}\}$ cages are also obtained,[35,36,48] a spherical $[V^V_7V^{IV}_8O_{36}(Cl)]^{6-}$ ion is formed, which has several biological actions, for example, cytotoxic activity due to oxidative stress and lipid peroxidation,[55] and chemoprotective effect against DNA plasmid pUC19 alkylating agents diethyl sulphate and dimethyl sulphate.[11,56] $[V_{15}O_{36}(Cl)]^{6-}$ interacts with the HuHf outer cage surface *via* hydrogen bonds and electrostatic interactions. These findings confirm previous data indicating that mixed-valence POVs can be formed starting from V^{IV} compounds in the presence of proteins and that the spherical $[V_{15}O_{36}(Cl)]^{6-}$ ion can interact with proteins *via* non-covalent interactions.[37] More importantly, these new data show that (i) mixed valence POVs can be formed and remain stable even at basic pH; (ii) $[V_{15}O_{36}(Cl)]^{6-}$ ion can be formed in the presence of a physiologically relevant protein; and (iii) $[V_{15}O_{36}(Cl)]^{6-}$ can be synthesized at room temperature. In this respect, it is useful to recall that to synthesize $[V_{15}O_{36}(Cl)]^{6-}$, high temperatures, *ca.* 70 °C, and reflux are necessary.[11,55]

Overall, these findings suggest that POVs could be in principle transported in body fluids by ferritin. Furthermore, considering the role of ferritin in iron metabolism, this protein could be exploited as a carrier for POVs with potential biological properties. To this, it should be added that POVs can exist in solution when they are administered in such a form or when they are formed after the transformation of mononuclear V^{IV} or V^V compounds and stabilization by interaction with proteins. Therefore, the possibility that the formation of POV–protein adducts may be more important than believed to date and play significant biological roles should not be underestimated.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data are all available in the literature or deposited in the PDB.

Supplementary information is available. Experimental procedures, supplementary figures and tables. See DOI: **https://doi.org/10.1039/d5qi01694k**.

Acknowledgements

The authors thank Elettra staff for their assistance during data collection. A.M. thanks MIUR PRIN 2022 – Cod. 2022JMFC3X "Protein Metalation by Anticancer Metal-based Drugs", and E. G. thanks MIUR PRIN 2022 – Cod. 2022APCTNA "TRILLI – TRansforming metal Ions and Low-cost LIgands into next generation metallodrugs. A thermodynamic, spectroscopic and biological approach for their rational design".

References

1 S.-S. Wang and G.-Y. Yang, Recent advances in polyoxometalate-catalyzed reactions, *Chem. Rev.*, 2015, **115**, 4893–4962.
2 J. Zhong, J. Pérez-Ramírez and N. Yan, Biomass valorisation over polyoxometalate-based catalysts, *Green Chem.*, 2021, **23**, 18–36.
3 N. Li, J. Liu, B. Dong and Y. Lan, Polyoxometalate–based compounds for photo–and electrocatalytic applications, *Angew. Chem., Int. Ed.*, 2020, **59**, 20779–20793.
4 Y. Zhang, J. Liu, S.-L. Li, Z.-M. Su and Y.-Q. Lan, Polyoxometalate-based materials for sustainable and clean energy conversion and storage, *EnergyChem*, 2019, **1**, 100021.
5 Y.-F. Song and R. Tsunashima, Recent advances on polyoxometalate-based molecular and composite materials, *Chem. Soc. Rev.*, 2012, **41**, 7384.
6 J. Liu, M. Huang, X. Zhang, Z. Hua, Z. Feng, Y. Dong, T. Sun, X. Sun and C. Chen, Polyoxometalate nanomaterials for enhanced reactive oxygen species theranostics, *Coord. Chem. Rev.*, 2022, **472**, 214785.
7 A. Bijelic and A. Rompel, The use of polyoxometalates in protein crystallography–An attempt to widen a well-known bottleneck, *Coord. Chem. Rev.*, 2015, **299**, 22–38.
8 S. Lentink, D. E. Salazar Marcano, M. A. Moussawi and T. N. Parac-Vogt, Exploiting interactions between polyoxometalates and proteins for applications in (bio)chemistry and medicine, *Angew. Chem., Int. Ed.*, 2023, **62**, e202303817.
9 A. Bijelic, M. Aureliano and A. Rompel, The antibacterial activity of polyoxometalates: structures, antibiotic effects and future perspectives, *Chem. Commun.*, 2018, **54**, 1153–1169.
10 M. B. Čolović, M. Lacković, J. Lalatović, A. S. Mougharbel, U. Kortz and D. Z. Krstić, Polyoxometalates in biomedicine: Update and overview, *Curr. Med. Chem.*, 2020, **27**, 362–379.
11 G. G. Nunes, A. C. Bonatto, C. G. De Albuquerque, A. Barison, R. R. Ribeiro, D. F. Back, A. V. C. Andrade, E. L. De Sá, F. D. O. Pedrosa, J. F. Soares and E. M. De Souza, Synthesis, characterization and chemoprotective activity of polyoxovanadates against DNA alkylation, *J. Inorg. Biochem.*, 2012, **108**, 36–46.
12 M. Aureliano, N. I. Gumerova, G. Sciortino, E. Garribba, A. Rompel and D. C. Crans, Polyoxovanadates with emerging biomedical activities, *Coord. Chem. Rev.*, 2021, **447**, 214143.
13 M. T. Pope and A. Müller, Polyoxometalate chemistry: an old field with new dimensions in several disciplines, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 34–48.
14 N. I. Gumerova and A. Rompel, Speciation atlas of polyoxometalates in aqueous solutions, *Sci. Adv.*, 2023, **9**, eadi0814.
15 A. Díaz, R. Vázquez-Roque, K. Carreto-Meneses, D. Moroni-González, J. A. Moreno-Rodríguez and S. Treviño, Polyoxidovanadates as a pharmacological option against brain aging, *J. Chem. Neuroanat.*, 2023, **129**, 102256.

16 M. Aureliano, The future is bright for polyoxometalates, *BioChem*, 2022, **2**, 8–26.
17 E. Kioseoglou, S. Petanidis, C. Gabriel and A. Salifoglou, The chemistry and biology of vanadium compounds in cancer therapeutics, *Coord. Chem. Rev.*, 2015, **301–302**, 87–105.
18 W. Qi, B. Zhang, Y. Qi, S. Guo, R. Tian, J. Sun and M. Zhao, The Anti-Proliferation Activity and Mechanism of Action of $K_{12}[V_{18}O_{42}(H_2O)]\cdot 6H_2O$ on Breast Cancer Cell Lines, *Molecules*, 2017, **22**, 1535.
19 L. Roubatis, N. C. Anastasiadis, C. Paratriantafyllopoulou, E. Moushi, A. J. Tasiopoulos, S. Ch. Karkabounas, P. G. Veltsistas, S. P. Perlepes and A. M. Evangelou, A missing oxidation-state level in the family of polyoxo(azide) octadecavanadate(IV/V) clusters: Synthesis, structure and antitumoural properties of $[V^{IV}_{11}V^{V}_{7}O_{44}(N_3)]^{10-}$ in a sodium containing-3D architecture, *Inorg. Chem. Commun.*, 2016, **69**, 85–88.
20 F. Carvalho and M. Aureliano, Polyoxometalates impact as anticancer agents, *Int. J. Mol. Sci.*, 2023, **24**, 5043.
21 M. Arefian, M. Mirzaei, H. Eshtiagh-Hosseini and A. Frontera, A survey of the different roles of polyoxometalates in their interaction with amino acids, peptides and proteins, *Dalton Trans.*, 2017, **46**, 6812–6829.
22 M. Aureliano, N. I. Gumerova, G. Sciortino, E. Garribba, C. C. McLauchlan, A. Rompel and D. C. Crans, Polyoxidovanadates' interactions with proteins: An overview, *Coord. Chem. Rev.*, 2022, **454**, 214344.
23 J. Costa Pessoa, E. Garribba, M. F. A. Santos and T. Santos-Silva, Vanadium and proteins: Uptake, transport, structure, activity and function, *Coord. Chem. Rev.*, 2015, **301–302**, 49–86.
24 J. Costa Pessoa, M. F. A. Santos, I. Correia, D. Sanna, G. Sciortino and E. Garribba, Binding of vanadium ions and complexes to proteins and enzymes in aqueous solution, *Coord. Chem. Rev.*, 2021, **449**, 214192.
25 R. Dinda, E. Garribba, D. Sanna, D. C. Crans and J. Costa Pessoa, Hydrolysis, Ligand Exchange, and Redox Properties of Vanadium Compounds: Implications of Solution Transformation on Biological, Therapeutic, and Environmental Applications, *Chem. Rev.*, 2025, **125**, 1468–1603.
26 S. Ramos, M. Manuel, T. Tiago, R. Duarte, J. Martins, C. Gutiérrez-Merino, J. J. G. Moura and M. Aureliano, Decavanadate interactions with actin: inhibition of G-actin polymerization and stabilization of decameric vanadate, *J. Inorg. Biochem.*, 2006, **100**, 1734–1743.
27 S. Ramos, J. J. G. Moura and M. Aureliano, Actin as a potential target for decavanadate, *J. Inorg. Biochem.*, 2010, **104**, 1234–1239.
28 S. Ramos, R. M. Almeida, J. J. G. Moura and M. Aureliano, Implications of oxidovanadium(IV) binding to actin, *J. Inorg. Biochem.*, 2011, **105**, 777–783.
29 G. Sciortino, M. Aureliano and E. Garribba, Rationalizing the Decavanadate(V) and Oxidovanadium(IV) Binding to G-Actin and the Competition with Decaniobate(V) and ATP, *Inorg. Chem.*, 2021, **60**, 334–344.
30 M. Paolillo, G. Ferraro, G. Sahu, P. D. Pattanayak, E. Garribba, S. Halder, R. Ghosh, B. Mondal, P. B. Chatterjee, R. Dinda and A. Merlino, Interaction of $V^{V}O_2$–hydrazonates with lysozyme, *J. Inorg. Biochem.*, 2025, **264**, 112787.
31 M. Paolillo, G. Ferraro, I. Cipollone, E. Garribba, M. Monti and A. Merlino, Unexpected in crystallo reactivity of the potential drug bis(maltolato)oxidovanadium(IV) with lysozyme, *Inorg. Chem. Front.*, 2024, **11**, 6307–6315.
32 M. Paolillo, G. Ferraro, F. Pisanu, J. Maréchal, G. Sciortino, E. Garribba and A. Merlino, Protein–Protein Stabilization in $V^{IV}O$/8-Hydroxyquinoline–Lysozyme Adducts, *Chem. – Eur. J.*, 2024, **30**, e202401712.
33 G. Ferraro, M. Paolillo, G. Sciortino, E. Garribba and A. Merlino, Multiple and variable binding of pharmacologically active bis (maltolato) oxidovanadium(IV) to lysozyme, *Inorg. Chem.*, 2022, **61**, 16458–16467.
34 G. Ferraro, M. Paolillo, G. Sciortino, F. Pisanu, E. Garribba and A. Merlino, Implications of Protein Interaction in the Speciation of Potential $V^{IV}O$–Pyridinone Drugs, *Inorg. Chem.*, 2023, **62**, 8407–8417.
35 G. Ferraro, G. Tito, G. Sciortino, E. Garribba and A. Merlino, Stabilization and Binding of $[V_4O_{12}]^{4-}$ and Unprecedented $[V_{20}O_{54}(NO_3)]^{n-}$ to Lysozyme upon Loss of Ligands and Oxidation of the Potential Drug $V^{IV}O$(acetylacetonato)$_2$, *Angew. Chem., Int. Ed.*, 2023, **62**(50), e202310655.
36 G. Tito, G. Ferraro, F. Pisanu, E. Garribba and A. Merlino, Non–Covalent and Covalent Binding of New Mixed–Valence Cage–like Polyoxidovanadate Clusters to Lysozyme, *Angew. Chem., Int. Ed.*, 2024, **63**, e202406669.
37 G. Ferraro, E. Garribba and A. Merlino, Exploring polyoxidovanadate–protein interaction, *Trends Chem.*, 2024, **7**, 3–6.
38 J. Wang and K. Pantopoulos, Regulation of cellular iron metabolism, *Biochem. J.*, 2011, **434**, 365–381.
39 E. L. Mackenzie, K. Iwasaki and Y. Tsuji, Intracellular iron transport and storage: from molecular mechanisms to health implications, *Antioxid. Redox Signal.*, 2008, **10**, 997–1030.
40 M. Plays, S. Müller and R. Rodriguez, Chemistry and biology of ferritin, *Metallomics*, 2021, **13**, mfab021.
41 S. C. Andrews, P. Arosio, W. Bottke, J. F. Briat, M. von Darl, P. M. Harrison, J. P. Laulhère, S. Levi, S. Lobreaux and S. J. Yewdall, Structure, function, and evolution of ferritins, *J. Inorg. Biochem.*, 1992, **47**, 161–174.
42 K. J. Hintze and E. C. Theil, Cellular regulation and molecular interactions of the ferritins, *Cell. Mol. Life Sci.*, 2006, **63**, 591–600.
43 D. He, S. Hughes, S. Vanden-Hehir, A. Georgiev, K. Altenbach, E. Tarrant, C. L. Mackay, K. J. Waldron, D. J. Clarke and J. Marles-Wright, Structural characterization of encapsulated ferritin provides insight into iron storage in bacterial nanocompartments, *eLife*, 2016, **5**, e18972.
44 I. Yanatori, F. Kishi and S. Toyokuni, New iron export pathways acting via holo-ferritin secretion, *Arch. Biochem. Biophys.*, 2023, **746**, 109737.

45 I. Yanatori, S. Nishina, F. Kishi and K. Hino, Newly uncovered biochemical and functional aspects of ferritin, *FASEB J.*, 2023, **37**, e23095.

46 M. Uchida, S. Kang, C. Reichhardt, K. Harlen and T. Douglas, The ferritin superfamily: Supramolecular templates for materials synthesis, *Biochim. Biophys. Acta, Gen. Subj.*, 2010, **1800**, 834–845.

47 L. Li, C. J. Fang, J. C. Ryan, E. C. Niemi, J. A. Lebrón, P. J. Björkman, H. Arase, F. M. Torti, S. V. Torti, M. C. Nakamura and W. E. Seaman, Binding and uptake of H-ferritin are mediated by human transferrin receptor-1, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 3505–3510.

48 G. Tito, G. Ferraro, E. Garribba and A. Merlino, Formation of Mixed-Valence Cage-Like Polyoxidovanadates at 37 °C Upon Reaction of $V^{IV}O$(acetylacetonato)$_2$ With Lysozyme, *Chem. – Eur. J.*, 2025, **31**, e202500488.

49 C. Pozzi, F. Di Pisa, C. Bernacchioni, S. Ciambellotti, P. Turano and S. Mangani, Iron binding to human heavy-chain ferritin, *Acta Crystallogr., Sect. D:Biol. Crystallogr.*, 2015, **71**, 1909–1920.

50 A. Müller, E. Krickemeyer, M. Penk, H. Walberg and H. Bögge, Spherical Mixed–Valence $[V_{15}O_{36}]^{5-}$, an Example from an Unusual Cluster Family, *Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 1045–1046.

51 Y. Hayashi, Hetero and lacunary polyoxovanadate chemistry: Synthesis, reactivity and structural aspects, *Coord. Chem. Rev.*, 2011, **255**, 2270–2280.

52 C.-D. Zhang, S.-X. Liu, B. Gao, C.-Y. Sun, L.-H. Xie, M. Yu and J. Peng, Hybrid materials based on metal–organic coordination complexes and cage-like polyoxovanadate clusters: Synthesis, characterization and magnetic properties, *Polyhedron*, 2007, **26**, 1514–1522.

53 M. Aureliano, C. A. Ohlin, M. O. Vieira, M. P. M. Marques, W. H. Casey and L. A. E. Batista De Carvalho, Characterization of decavanadate and decaniobate solutions by Raman spectroscopy, *Dalton Trans.*, 2016, **45**, 7391–7399.

54 H. Schmidt, I. Andersson, D. Rehder and L. Pettersson, A Potentiometric and ^{51}V NMR Study of the Aqueous H^+/$H_2VO_4^-$/H_2O_2/L–α–Alanyl–L–histidine System, *Chem. – Eur. J.*, 2001, **7**, 251–257.

55 M. D. M. Barbosa, L. M. A. D. Lima, W. A. D. S. Alves, E. K. B. D. Lima, L. A. D. Silva, T. D. D. Silva, K. Postal, M. Ramadan, K. Kostenkova, D. A. Gomes, G. G. Nunes, M. C. Pereira, W. E. D. Silva, M. F. Belian, D. C. Crans and E. C. Lira, In vitro, oral acute, and repeated 28-day oral dose toxicity of a mixed-valence polyoxovanadate cluster, *Pharmaceuticals*, 2023, **16**, 1232.

56 K. Postal, F. S. Santana, D. L. Hughes, A. L. Rüdiger, R. R. Ribeiro, E. L. Sá, E. M. De Souza, J. F. Soares and G. G. Nunes, Stability in solution and chemoprotection by octadecavanadates(IV/V) in *E. coli* cultures, *J. Inorg. Biochem.*, 2021, **219**, 111438.