



Interaction of Vanadium Complexes with Proteins: Revisiting the Reported Structures in the Protein Data Bank (PDB) since 2015

Marino F. A. Santos ^{1,2,3,*} and João Costa Pessoa ^{3,*}

- ¹ Associate Laboratory i4HB—Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
- ² UCIBIO—Applied Molecular Biosciences Unit, Chemistry Department, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
- ³ Centro de Química Estrutural, Departamento de Engenharia Química, Institute of Molecular Sciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
- Correspondence: mf.santos@campus.fct.unl.pt (M.F.A.S.); joao.pessoa@ist.utl.pt (J.C.P.)

Abstract: The structural determination and characterization of molecules, namely proteins and enzymes, is crucial to gaining a better understanding of their role in different chemical and biological processes. The continuous technical developments in the experimental and computational resources of X-ray diffraction (XRD) and, more recently, cryogenic Electron Microscopy (cryo-EM) led to an enormous growth in the number of structures deposited in the Protein Data Bank (PDB). Bioinorganic chemistry arose as a relevant discipline in biology and therapeutics, with a massive number of studies reporting the effects of metal complexes on biological systems, with vanadium complexes being one of the relevant systems addressed. In this review, we focus on the interactions of vanadium compounds (VCs) with proteins. Several types of binding are established between VCs and proteins/enzymes. Considering that the V-species that bind may differ from those initially added, the mentioned structural techniques are pivotal to clarifying the nature and variety of interactions of VCs with proteins and to proposing the mechanisms involved either in enzymatic inhibition or catalysis. As such, we provide an account of the available structural information of VCs bound to proteins obtained by both XRD and/or cryo-EM, mainly exploring the more recent structures, particularly those containing organic-based vanadium complexes.

Keywords: X-ray crystallography; cryogenic Electron Microscopy (cryo-EM); protein data bank (PDB); vanadium and vanadium complexes; vanadium-containing proteins

1. Introduction

Many studies have addressed vanadium bioinorganic chemistry and the effects of vanadium compounds (VCs) on living beings, one of the driving forces of these research activities being their possible applications in therapeutics [1–6]. This has led to an interest in understanding interactions of VCs with proteins [1,7–16]. Namely, efforts to understand the transport of VCs in blood led to many studies of the interaction of vanadium compounds with human serum transferrin (HTF), human serum albumin (HSA), hemoglobin (Hb) and immunoglobulins [17–23].

For some time, studies of vanadium haloperoxidases and nitrogenases, as well as of hyper-accumulators of vanadium (e.g., some ascidians), were among the main foci of researchers, leading to the full characterization of several vanadium-containing proteins through X-ray diffraction methods (SC-XRD) [10,24–31]. The similarity between mono-vanadate and phosphate and studies associated with the clarification of the inhibition of ATP-dependent enzymes (ATP, adenosine triphosphate) and phosphatases by vanadate [10] also gave rise to the publication of many SC-XRD structures, including vanadate species bound to several phosphatases [10,27,28,32].



Citation: Santos, M.F.A.; Pessoa, J.C. Interaction of Vanadium Complexes with Proteins: Revisiting the Reported Structures in the Protein Data Bank (PDB) since 2015. *Molecules* 2023, 28, 6538. https://doi.org/10.3390/ molecules28186538

Academic Editor: Massimiliano F. Peana

Received: 27 July 2023 Revised: 4 September 2023 Accepted: 5 September 2023 Published: 9 September 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). The belief that the therapeutic action of VCs may be associated with the binding of vanadium compounds to proteins has led to an increase in interest in the characterization of such interactions, namely by techniques such as single crystal X-ray diffraction analysis

of such interactions, namely by techniques such as single crystal X-ray diffraction analysis (SC-XRD) and cryogenic Electron Microscopy (cryo-EM). In fact, the accurate characterization of V-protein interactions is crucial to clarifying the putative role of vanadium and vanadium compounds, namely, but not exclusively, as therapeutic agents. Despite the substantial amount of information obtained using a wide range of biochemical and biophysical methods—e.g., Electron Paramagnetic Resonance (EPR), Circular Dichroism (CD), ⁵¹V Nuclear Magnetic Resonance (⁵¹V NMR), denaturing urea polyacrylamide gel electrophoresis (urea-PAGE), Small-angle X-ray Scattering (SAXS) and electrochemistry [17,33–35]—as recently reviewed by us [15], structural data, freely available in the Protein Data Bank (PDB), is often required to complement it [36–38].

PDB is nowadays a vital tool for anyone involved in protein studies from biological, biochemical or therapeutic points of view, in addition to educational purposes, as recently highlighted by the COVID-19 pandemic [39]. Following the first X-ray protein structures discovered in the 1950s and 1960s (namely myoglobin and hemoglobin by Kendrew and Perutz, respectively, who shared the 1962 Nobel Prize in Chemistry), the need for an open repository for crystallographic data rapidly arose. In 1971, the PDB was launched shortly after the Cold Spring Harbor Symposium, entitled "Structure and Function of Proteins at the Three-Dimensional Level" [40]. Since then, the number of deposited structures has exponentially increased, reaching 100,000 entries in 2014, 150,000 in 2019 and 200,000 in January 2023 (currently, in September 2023, there are more than 209,000 entries). For more detailed insights on the subject, we recommend several papers by Berman, co-founder and director emerita of the PDB, namely those published within the scope of the 50th anniversary of the PDB celebrated in 2021 [41–43].

The massive increase in number of available structures in the PDB is associated with the astounding technical development of structural experimental methods. In 2015 and 2021, we had the opportunity to delve into the then deposited structures of vanadiumrelated proteins in the PDB [10,15]. Since then, reflecting the substantial evolution of the structural biology field itself, a considerable number of new V-containing protein structures have been released. Most researchers are now more aware of the several modifications that complexes of labile metal ions may undergo when added to biological media [44–49]. In fact, the action of proteins/enzymes may be inhibited and/or modified, and the structure of the original complex may change upon binding to proteins. Thus, sorting out the changes occurring and the several types of binding that may be established between VCs and proteins/enzymes is very important, and the objective of the present review is mainly to provide an account of the presently available structural information on vanadium complexes bound to proteins. As such, in this review, we mainly propose to explore the more recent structures—obtained by both X-ray crystallography and cryo-EM—particularly those containing organic-based vanadium complexes, highlighting their contribution to a more effective knowledge on the roles and potential use of this versatile metal.

2. Vanadium-Containing Proteins and X-ray Crystallography

Despite the recent advent of cryo-EM, X-ray crystallography is still the top structural methodology, taking advantage of its continuous development since 1895, when Röntgen identified X-rays, and the subsequent first years of the 20th century, when its bases were postulated [50]. Such developments are recognized at different levels, namely, but not exclusively, at the level of (1) protein production and purification protocols (e.g., the use of bacterial, insect and mammal expression systems), (2) crystallization processes (e.g., automated crystallization and new crystallization additives) and (3) instrument and data-processing software improvements (e.g., brilliant synchrotron sources and fast detectors) [51–54].

The correct determination of different X-ray structures has been pivotal in many relevant chemical, biological and biochemical achievements, as attested by the numerous

Nobel Prize winners in Physics, Chemistry and Medicine associated with the technique. Similarly, it is not surprising to confirm that over 85% of the total number of PDB entries have been determined using single crystal X-ray crystallography. V-containing proteins are not an exception, and most of the currently available structures in the PDB with both inorganic- and organic-based V complexes were successfully crystallized and later properly solved by X-ray crystallography. In this work, we will mainly address proteins containing bound vanadium complexes with organic molecules as ligands published since 2015.

2.1. Inorganic-Based Vanadium Complexes

2.1.1. Monovanadates

Table 1 summarizes the released V-related crystallographic protein structures since 2015, and, with no surprise, the predominance of ortho- and metavanadate species is notorious. In fact, the similarity between monovanadate and phosphate and studies associated with the clarification of the inhibition of ATP-dependent enzymes and phosphatases by vanadate [10] also gave rise to the publication of many SC-XRD structures, including vanadate species bound to several phosphatases, namely protein tyrosine phosphatases (PTPs) [10,27,28].

Table 1. SC-XRD structures of monovanadate-containing proteins available in the PDB since 2015. The identifier and the name/chemical structure of each V compound, as well as the respective PDB codes, are provided. ^a The publication associated with the PDB entry is not available.

V Species Identifier	V Species Name/Chemical Structure	PDB Codes
V	Vanadium ion	6DYH [55], 6DYL [55], 7Q0T [16]
VO4	Orthovanadate $V^{V}O_{4}^{3-}$ $-O - V - O^{-}$ $O - O^{-}$	4RI4 [56], 4ZG4 [57], 4ZI4 [58], 4ZN5 [58], 5AA6 ^a , 5BZX [59], 5HMP [60], 5I0I [60], 5JNW [61], 5N69 [62], 5OFR [63], 6DR7 [64], 6DT6 [64], 6I7E [65], 6LVQ [66], 6NPZ [67], 6PG0 [68], 6PGT [68], 6PHA [68], 6PHS [68], 6PM8 [68], 6S3N [69], 6XEA [70], 6XEF [70], 6YCX [71], 6YSY [72], 7EZN ^a , 7L0H [73], 7L0M [73], 7MPC [74], 7QWI ^a , 8AJ5 [75]
6BR	Threenine-vanadate $V^VC_4H_8NO_7$ $V^VC_4H_8NO_7$ $V^VC_4H_8NO_7$ $V^VC_4H_8NO_7$ $V^VC_4H_8NO_7$ $V^VC_4H_8NO_7$	5IJS [76]
VN4	Metavanadate V ^V O ₃ -	4RI5 [56], 5A3Q [77], 5A3S [77], 5DMP [78], 6YSO [79]



PTPs reverse the effect of protein tyrosine kinases by removing the phosphate group from phosphorylated tyrosine residues, controlling several cell signaling mechanisms [91–93]. This is the case for the low-molecular-weight protein tyrosine phosphatase (LMPTP) of the insulin receptor, putatively implicated as an anti-diabetic (type 2) biological target [94]. Stanford and collaborators solved a 1.86 Å resolution structure of the double mutated (W49Y and N50E) bovine LMPTP in the complex with vanadate and an uncompetitive

Table 1. Cont.

inhibitor (PDB: 5JNW). Vanadate, covalently bound to the sulfur moiety of the active site, Cys12, in a tetragonal VO₃ adduct, mimics the phosphocysteine intermediate, contributing, combined with other experimental approaches, to revealing the uncompetitive nature of the inhibitor that is nearly bound (Figure 1) [61]. As previously highlighted by Crans and coworkers [95], whether the reduction in V^V takes place or not depends on a combination of parameters, namely pH and oxidovanadium(V) concentration, as well as the presence and concentrations of other complexing ligands. The structure depicted in Figure 1 illustrates a V^V-thiol binding that certainly affects the possibility of its participation in redox reactions.



Figure 1. Structural representation of the VO₃ adduct covalently bound to the Cys12 residues of bovine LMPTP (PDB: 5JNW). The axial oxygen of orthovanadate is not present, leading to a tetrahedral geometry. The adduct is also stabilized by multiple H-bonds with the residues of the so-called P-loop, a conserved active-site sequence motif, $C(X)_5R$, among all PTPs (distances are represented as dashed lines and given in Å).

Similarly, human PTP1B (PDB: 7L0H) and *Yersinia pestis* YopH (PDB: 7L0M) phosphatase variants have been co-crystallized with vanadate to investigate the role of the characteristic WPD loop in the catalytic performance [73]. Shen and collaborators proved, through kinetic studies, that both variants preserve their catalytic activity, although with a different pH activity range. Both co-crystallized 2.1 Å and 2.0 Å resolution structures support the presence of a WPD-loop closed conformation, which was later confirmed by MD (Molecular dynamic) simulations [73].

There is also an entry of a 2.15 Å resolution structure of vanadate-bound bromoperoxidase from *Corallina pilulifera* (PDB: 7QWI), which was recently released. However, the entry still lacks an associated publication.

From a different perspective, vanadate has been used as a crystallographic phasing agent. One of the main experimental challenges of X-ray crystallography is solving the so-called "phase problem" to calculate an electron density map and obtain the respective structure. Currently, due to the massive number of PDB entries, most of the new structures are solved through molecular replacement [96,97]. If no similar proteins are found, alternative methods can be used, including Single/Multiple Anomalous Dispersion (SAD/MAD) and the use of different heavy atoms (e.g., mercury, gold and platinum) for single/multiple isomorphous replacement (SIR/MIR). For a more detailed overview on those methods, including their advantages and disadvantages, we recommend specialized

publications [98–102]. In 2020, Omari and collaborators proposed the use of vanadate to obtain experimental phases while circumventing some of the drawbacks of the traditional phasing methods (e.g., eliminating the use of selenomethionine variants). The integral membrane rabbit sarcoplasmic reticulum Ca²⁺-ATPase was incubated with orthovanadate, and a 3.13 Å resolution structure (PDB: 6YSO) was obtained from twelve different angle 360° datasets collected at a wavelength of 2.2604 Å. Despite this low resolution, the authors were able to identify two vanadium atoms in the structure (a ratio of one vanadium per 994 protein residues) combined with some other identified sulfur atoms. This strategy allowed the protein initial phases to be calculated and further improved by the subsequent steps of model building and refinement [79].

Although they are less common, some protein structures (namely oxidoreductases) with oxidovanadium(IV) are also available, including two different iron(II)- and 2-(oxo)glutarate-dependent (Fe/2OG) oxygenases known to O₂-functionalize C-H bonds: Larginine 3-hydroxylase (VioC) from Streptomyces vinaceus, which hydroxylates the C3 of the L-arginine of the antibiotic viomycin (PDB: 6ALR) [83], and the taurine 2OG dioxygenase (TauD) from Escherichia coli (PDB: 6EDH) [85]. Briefly, Fe/2OG oxygenases contain a Fe²⁺ cofactor that is oxidized to a ferryl intermediate (Fe^{IV}-oxido), while 2-oxoglutarate is reduced to succinate. In the first case, several crystallographic VioC structures with different bound substrates and products were compared, including a 1.55 A resolution VioC-vanadyl structure (also containing L-arginine and succinate) that mimics the unstable ferryl intermediate. The well-defined electron density of the VIVO2+ moiety exhibits a significantly distorted octahedral geometry interacting with two His and a Glu residues and with one of the O-atoms of the succinate molecule, while the sixth position is not completed (Figure 2). Further computational analysis was carried out revealing the role of Arg334 in the H-bond stabilization of the intermediate [83]. Interestingly, the V=O bond distance was determined to be 1.87 Å, which is notably longer than the theoretically established values [103], which, as reported before [104], is an artefact due to the photoreduction of vanadium during the X-ray data collection.



Figure 2. VioC-vanadyl adduct mimicking the ferryl intermediate (PDB: 6ALR). The V moiety is interacting with the side chains of three residues (His168, Glu170 and His316) as well as with one O-atom of the succinate molecule. No electron density (not represented for better clarity of the adduct) was found for the sixth coordination position of V.

Finally, it should be highlighted that vanadium is also found in V-nitrogenases (V-Nases) as part of the catalytic FeVco cofactor. Nitrogenases can catalyze a reduction in atmospheric N₂ to NH₄⁺, thereby playing a relevant role in the nitrogen cycle. Moreover, nitrogenases can also catalyze Haber–Bosch and Fischer–Tropsch reactions under mild ambient conditions, opening up prospective industrial applications [7,105,106]. In 2021, we reviewed the most recent structural insights of V-Nases, and further information on the topic can be found in this work and the references therein [15]. Since then, a novel 1.05 Å resolution structure of the high-CO state V-Nase from *Azotobacter vinelandii* (PDB: 7AIZ) has become available. This structure presents, for the first time, a terminal CO bound to the FeVco cofactor (iron Fe6), in addition to the already characterized μ 2-bridging CO (iron Fe2 and Fe6), allowing the catalytic process at the level of CO reduction and hydrocarbon formation to be seen [89].

2.1.2. Polyoxidovanadates

Polyoxidovanadates (POVs), as well as polyoxidometalates in general, have been attracting increasing interest from the research community, and several prospective biological and therapeutic functions have been proposed, namely as insulin-enhancing agents [107–109]. Herein, we do not intend to further explore this subject, but we recommend the informative publication by Aureliano (and references therein) on the subject [110].

As expected, protein–POV interactions are crucial to their biological effects. Recent reviews explore this, including a complete structural analysis of the entries in the PDB prior 2015 (e.g., PhoE and YopH phosphatases, ecto-nucleoside triphosphate diphosphohydrolase (NTPDase) and tyrosine kinase) [15,110,111]. Protein–decavanadate ($[H_nV_{10}O_{28}]^{-(6-n)}$, V_{10}) interactions are reported particularly often, but smaller POVs, including dimeric (V_2), trimeric (V_3) and tetrameric (V_4) vanadates, are also reported on.

To the best of our knowledge, the currently available SC-XRD protein- V_{10} structures have all been released prior 2015. However, since then, as shown in Table 2, four additional SC-XRD structures with smaller POVs have been deposited and are briefly covered next.

Moise and coworkers were able to obtain two high-resolution (1.12 A) variant structures (W354H and W354Y) of the PTP YopH from *Yersinia enterocolitica* with a bound V₂ adduct (PDBs: 4ZI4 and 4ZN5). In both examples, YopH was co-crystallized with metavanadate (VO_4^{3-}) and a divanadate ester with glycerol, the latter added as a crystal cryo-protectant. The latter is observed at the active center, with one of the V moieties interacting with Cys403. The structural results, supplemented with kinetic assays using p-nitrophenyl phosphate (p-NPP) as the substrate, showed that the WPD loop is in a quasi-open position that is not compatible with a catalytically active form of YopH. As such, taking advantage of the inactive state of the protein, the adduct was not decomposed and was further characterized using computational approaches (e.g., natural bond orbital (NBO)), revealing that POVs can interact with phosphatases [58].

A 1.18 Å resolution structure of lysozyme with a cyclo-tetrametavanadate adduct (PDB: 7ZU6) was recently reported by Tito and collaborators but, so far, the associated publication is not available. A similar compound is found in other two previous structures: the ABC transporter BtuCD from *Escherichia coli* (PDB: 1L7V) and the transferase C3 exoenzyme for Clostridium botulinum (PDB 1UZI) [112,113].

Finally, Feder et al. published a 1.95 Å resolution structure of red kidney bean purple acid phosphatase bound to adenosine divanadate (see Section 2.2.1 for further details), which also contains two POV (hexa- and heptavanadate) species at the protein surface (PDB: 6HWR). Heptavanadate is known to be a partial hydrolytic product of V_{10} , as reviewed by Aureliano and co-authors [111]. However, these moieties were not discussed by Feder et al., and their potential biological role was not assigned [81].

Table 2. Single crystal X-ray structures of polyoxidovanadates-containing proteins available in the PDB since 2015. The identifier and the name/chemical structure of each V compound, as well as the respective PDB codes, are provided. ^a The publication associated with the PDB entry is not available.



2.2. Organic-Based Vanadium Complexes

2.2.1. Nucleotides

As previously indicated, vanadium is often used as a substrate analogue or inhibitor of a great range of enzymes. In this sense, there are quite a few examples of SC-XRD protein structures containing V moieties attached to nucleosides (replacing the phosphate group) or to nucleotides, namely adenosine diphosphate (ADP), as summarized in Table 3. All these structures contain oxidovanadates (V)-replacing phosphate, so they correspond to vanadium-substituted nucleotides. **Table 3.** Single crystal X-ray structures of V-nucleoside and V-nucleotide protein structures available in the PDB since 2015. The identifier and the name/chemical structure of each V compound, as well as the respective PDB codes, are provided. ^a The publication associated with the PDB entry is not available.





As indicated in Table 3, there are two structures in which vanadate forms an adduct with adenosine: adenosine-2',3'-vanadate and adenosine divanadate. In the first case, Pinto and colleagues were able to obtain a 2.10 Å resolution structure (PDB: 6RVZ) of the human deadenylase angel homolog 2 (ANGEL2) soaked with adenosine-2',3'-vanadate, and compared it with the respective 1.45 Å resolution apo-protein structure (PDB: 6RW0), revealing, combined with a biochemical approach, its function as a 2',3'-cyclic phosphatase [114]. During its use by the cell, RNA is known to exhibit different chemical groups at the 3' end, namely a 2',3'-cyclic phosphate group that is then removed by ANGEL2. Adenosine-2',3'-vanadate, previously characterized in solution [121] and by X-ray analysis [122], mimics the transition state assumed upon 2',3'-cyclic phosphate hydrolysis and sits at the active site next to one Mg²⁺ ion in a positively charged groove putatively involved in the binding of the RNA ribose–phosphate backbone. The adenine base establishes a π -stacking interaction with Tyr313, and the rest of the adenosine-2',3'-vanadate moiety is stabilized by a direct

coordination to Mg²⁺ and a H-network involving different (Asn353, His310 and His533) protein residues (Figure 3) [114].



Figure 3. Structural representation of the adenosine-2',3'-vanadate adduct bound to human deadenylase, ANGEL2 (PDB: 6RVZ). Different O-atoms of vanadate and ribose moieties are interacting with a Mg²⁺ ion and H-bonded to N-atoms of three protein residues: His310, Asn353 and His533 (distances are represented as dashed lines and given in Å). Tyr313 (also represented) stabilizes the adenine moiety via a π -stacking interaction.

In addition, as previously mentioned in Section 2.1.2, a 1.95 Å resolution structure of red kidney bean purple acid phosphatase (rkbPAP) bound to adenosine divanadate (PDB: 6HWR) is also available [81]. Purple acid phosphatases can catalyze different substrates, including ATP and ADP containing a heterovalent Fe³⁺-M²⁺ center (in which M can correspond to Fe, Zn or Mn). The apo-rkbPAP crystals were soaked with vanadate and adenosine, which formed in situ an adenosine divanadate moiety, which, in turn, is an analogue of the ADP hydrolysis to adenosine monophosphate (AMP), mimicking the expected transition state. Although the adenine base is not included in the model due to its lack of electron density considering that it is not stabilized by protein residues, docking studies supported its presence. Both divanadate and ribose moieties are visible at the active site and are stabilized by both Fe³⁺ and Zn²⁺ ions (proximal vanadate) and H-bonded to different protein residues in a five-coordinate trigonal bipyramidal geometry (proximal vanadate) and a four-coordinate tetrahedral (distal vanadate) geometry. Based on such observations, the authors proposed a rkbPAP catalytic model by which the nucleophilic O, the one that interacts with Fe^{3+} and Zn^{2+} , binds to the P moiety of the substrate, allowing the opposite bond to be broken and for it to be released [81].

Uridine-2',3'-vanadate, previously characterized in solution [121], was found in two different structures in the PDB. In the first case, uridine-2',3'-vanadate was used as a SAD phasing agent to solve the structure of bovine pancreatic ribonuclease A (RNase), yielding a 1.90 Å resolution structure (PDB: 6YO1) (for further insights on the use of V-based compounds as crystallographic phasing agents, please consult Section 2.1.1). The data were collected at a specific V wavelength (2.2604 Å), and the protein structure was easily solved. A second data collection was carried out at a standard synchrotron wavelength (1.7711 Å), and it was still possible to determine the structure, opening up the possibility of the regular use of V-SAD phasing agents [79].

The second case is a co-crystallization 2.25 Å resolution structure of the uridinespecific Nsp15 endoribonuclease from Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2, PDB: 7K1L). Kim and collaborators investigated its potential role as a therapeutic target during the recent COVID-19 pandemic, using a combined biochemical (nuclease activity assays), biophysical (differential scanning fluorimetry, DSF) and structural rationale [115]. The authors solved five SC-XRD Nsp15 structures with three different nucleotides (5'UMP, 3'UMP and 5'GpU), tipiracil (an uracil derivative synthetic substrate analogue) and uridine-2',3'-vanadate as a transition state analogue. The last structure revealed uridine-2',3'-vanadate moiety at the active site. The uracil base interacts with residues Ser294 and Ser346; the vanadate, covalently bound to the ribose moiety, sits where the 3'-phosphoryl group was observed (5'GpU and tipiracil structures) and is H-bonded to protein residues (His250, His235, Lys290, Thr341 and Gly247) and water molecules. Based on structural data, a two-step reaction mechanism was proposed (3'-uridine monophosphate as final product), contributing to a better understanding of the putative inhibition of the enzyme as a viable therapeutic option [115].

Both ortho- and metavanadate moieties bound to ADP molecules have been obtained, leading to a well-known model for ATP. One example is the case of a 2.25 Å resolution structure of the pre-powerstroke state of the human nonmuscle myosin-2C motor domain (PDB: 5I4E), which, using complementary mutagenesis, kinetics and molecular dynamics techniques, provided further insights into the structural changes upon F-actin binding (e.g., the existence of an allosteric communication between the distal end of the domain and the active site) [116].

More recently, the myosin-2 motor domain from the model organism *Dictyostelium discoideum* was studied by Franz and coworkers [118], who obtained two variants by replacing the two hotspot threonine residues in the connecting loop (W-loop) with two alanine residues (M765^{AA}) or three glycine residues (M765^{GGG}). Both M765^{AA} and M765^{GGG} variants were co-crystallized with 2 mM ADP, 2 mM metavanadate and 2 mM MgCl₂, and two pre-powerstroke state structures were solved and deposited at a 2.60 Å resolution (PDB: 7B1A) and 2.55 Å resolution (PDB: 7B19), respectively. A structural analysis revealed a helix-mediated (W-helix) communication pathway during ATP hydrolysis, controlling the product release, while kinetics and in vitro motility assays showed that the M765^{GGG} variant significantly increased its ATPase activity, decreasing its motor ability [118].

Interestingly, the authors of the described red kidney bean purple acid phosphatase (rkbPAP) structure with adenosine divanadate (PDB: 6HWR), were also able to crystallize a 2.20 Å resolution structure of rkbPAP soaked with vanadate and ADP, leading to the in crystallo formation of ADP metavanadate (PDB: 6PY9). This substrate analogue was found at the active site and the vanadate moiety, H-interacting with both ions of the metal center (Zn^{2+} and Fe^{3+}) and Asn201 through oxygen atoms and adopting a four-coordinate tetrahedral geometry that mimics the substrate-bound state. The rest of the molecule are stabilized by a H-bond network with protein residues and water molecules, even though the ribose moiety lacks them. The structure was later used in complementary docking studies with ADP and ATP, revealing additional insights into the substrate binding, which will likely be useful for future biotechnological applications [117].

Related complexes with other nucleotides—cytidine-5'-monophosphate-2',3'-vanadate and guanosine-5'-monophosphate-2',3'-vanadate—are also found in the PDB, interacting with ribozymes, which are well-known RNA molecules that can catalyze some biochemical reactions. We are focused on proteins, so a detailed characterization of those cases will not be provided here. Nevertheless, we note that both molecules were used as transition state analogues, enlightening the catalytic mechanisms of the respective ribozymes. Cytidine-5'monophosphate-2',3'-vanadate is present in two low-resolution crystallographic structures of the hammerhead ribozyme, at 2.99 Å (PDB: 5EAO) and 3.20 Å (PDB: 5EAQ) resolutions [119]. In turn, guanosine-5'-monophosphate-2',3'-vanadate is present in two equally low-resolution structures of the pistol ribozyme, at 2.80 Å (PDB: 6UEY) and 3.10 Å (PDB: 6UF1) resolutions [120].

2.2.2. Putative Therapeutic V-Complexes and Model Proteins

The use of vanadium compounds (VCs) with small organic ligands, as a means to overcome the low oral absorption rate of inorganic salts, has been common in the last

few decades [123–126]. Several carrier ligands have been proposed, and an extensive number of publications cover their promising therapeutic results against different pathologies, including diabetes and several types of cancer (e.g., breast, ovarian, prostate and testicular) [1,127–129].

Despite the considerable amount of research on the interactions of VCs with proteins namely blood transporters such as HTF and HSA—using a great range of experimental techniques in solution [15,17,21,130,131], the respective SC-XRD characterization has been significantly hampered. However, the use of crystallographic protein models allowed for relevant structural insights, overcoming the crystallization bottlenecks found with those blood transporters and other proteins. In the last years, some SC-XRD studies of oxidovanadium(IV and V) complexes bound to proteins have been reported, namely involving the V^{IV}O²⁺ center. The first SC-XRD structure involving the oxidovanadium(IV) ion was reported in 2014 [104] and a few others have been reported since 2021 [16,75,132–134]. Table 4 summarizes the six VCs' organic ligands bound to three different model proteins (lysozyme, RNase and trypsin) in the PDB, published since 2015, which will be discussed in the next sections.

Hen Egg White Lysozyme (HEWL)

Hen Egg White Lysozyme (HEWL) is a small anti-microbial glycoside hydrolase able to hydrolyze peptidoglycan found in bacteria's cell walls [135]. HEWL is perhaps the most common crystallographic protein model known to produce robust and well-diffracting crystals in diverse experimental conditions (e.g., different precipitant agents and pH values). As such, there are numerous examples of soaked HEWL structures in the PDB containing different compounds, namely metal-based complexes such as those of Pt, Au, Ru, Mn and Re [136–141].

From a chronological point of view, 2014 marks the release of the first HEWL–vanadium structure: a 1.28 Å resolution structure of HEWL-V^{IV}O(picolinato)₂ (PDB: 4C3W). Briefly, we were able to model an adduct at the active site bound to the side chain of Asp52 in a distorted octahedral geometry; further EPR and DFT studies confirmed the oxidation state of the metal despite the detected long V=O_{oxido} distance due to its reduction in V^{IV} to V^{III} during the data collection [104].

Following this pioneering crystallographic structure, the first to confirm a protein– V^{IV} binding by SC-XRD, an increasing attention has been devoted to HEWL. In 2022, a thorough experimental and computational study on the binding of $V^{IV}O^{2+}$, $V^{IV}OL$, $V^{IV}OL_2$ and V^VO_2L moieties to proteins was published, which includes the characterization of two high-resolution SC-XRDs of HEWL soaked with $V^{IV}OSO_4$, 2,2'-bipyridine (bipy) and 1,10-phenanthroline (phen) [16]. Complexes with both ligands were previously characterized, showing interesting anticancer and antiparasitic properties [1,46,127,142–144]. To further clarify their potential behavior upon protein binding, both $V^{IV}O$ -complexes with bipy and phen were used in soaking experiments with HEWL, and two $V^{IV}OL$ structures were obtained at 1.19 Å (PDB: 7Q0U) and 1.12 Å (PDB: 7Q0V) resolutions, respectively.

The first structure presents a single V^{IV}O(H₂O)(bipy) adduct at the active site (Figure 4). V adopts a nearly octahedral geometry bound to the O-donors of the side chains of Asp52 and Asn46, the two N-donors of bipy, the O_{oxido} atom and a water molecule. The adduct is also stabilized by H-bonds (Asp52 and Asn46) and by hydrophobic interactions (Glu35, Gln57 and Val109). The second structure exhibits more than one putative binding site. Similarly to the previous structure, a V^{IV}O(H₂O)(phen) adduct also sits at the active site bound to the side chains of residues Asn46 and Asp52, although with a lower occupancy and higher B factors, suggesting a more disordered moiety. It should be highlighted that additional V atoms were located next to different aspartate residues (Asp101 and Asp119), but the presence of phen moieties was not clearly revealed by the electron density [16]. Globally, this also confirms the previously indicated potential of Asp side groups to bind V^{IV}O²⁺ centers [145].



Table 4. Structures of organic-based vanadium-complexes-containing proteins, obtained by single crystal X-ray diffraction analysis, available in the PDB since 2015.



Figure 4. Structural representation of the V^{IV}O(H₂O)(bipy) adduct covalently bound to the Asn46 and Asp52 residues of HEWL (PDB: 7Q0U), determined by X-ray crystallography.

The structural results described were complemented and validated by several different theoretical and experimental techniques. In brief, (1) docking simulations proved the role of microsolvation for the chiral discrimination of the binding region; (2) QM/MM experiments favored the proposed V^{IV}O(H₂O)(phen) adduct ($\Delta\Delta G_{aq}$ value of 5.8 kcal mol⁻¹), despite the partial lack of electron density in one of the rings of phen; (3) EPR data of HEWL crystals incubated with V^{IV}OSO₄ and phen/bipy, similar to the one recorded in solution, corroborate the existence of the modeled crystallographic adducts [16].

Shortly after, another work was published focusing on the ligand maltol (3-hydroxy-2-methyl-4H-pyran-4-onato) [75]. V^{IV}O(maltolato)₂ (BMOV) and V^{IV}O(ethylmaltolato)₂ (BEOV) have been among the most studied V-containing systems, including in pre-clinical tests [146,147]. Several HEWL crystals soaked with [V^{IV}O(maltol)₂] were analyzed. Two isomorphous crystals obtained from the same crystallization condition at a pH of 7.5 were tested to obtain two high-resolution structures—named A (1.13 Å resolution, PDB: 8AJ3) and A' (1.22 Å resolution, PDB: 8AJ4)—that revealed three different binding sites (Figure 5). Three binding sites were assigned, but the nature and the occupancy of each adduct depend on the structure. Interestingly, three different moieties were found: V^{IV}O²⁺, V^{IV}O(maltol)⁺ and V^{IV}O(maltol)₂, arising from the original soaked compound. Structure A contains the adducts $[V^{IV}O(maltol)_2(H_2O)]$ (site 1), $[V^{IV}O(H_2O)_4]^{2+}$ (site 2) and $[V^{IV}O(maltol)(H_2O)_3]^+$ (site 3), while structure A' presents the adducts [V^{IV}O(maltol)₂(H₂O)] (sites 1 and 3) and $[V^{IV}O(maltol)_2]$ (site 2) [75]. The authors discussed the binding features of each adduct, highlighting the importance of a H-network to stabilize them, which is reinforced by the covalent binding of the V moiety to Asn65 in the case of binding site 2. The EPR and ESI-MS results supported the crystallographic findings, including the +IV oxidation state. A second crystallization condition at a pH of 4 was also used for a soaking experiment

with $V^{IV}O(maltol)_2$. However, the resulting 1.31 Å resolution structure (PDB: 8AJ5) did not present maltol moieties but three $[V^{IV}O(H_2O)_3]^{2+}$ ions bound to the side chains of Asp48, Asp87 and Glu35, reinforcing the labile nature of VCs upon protein binding [75].



Figure 5. Structural representation of the adducts found in HEWL soaked with $[V^{IV}O(maltol)_2]$ (structure A, PDB: 8AJ3). The three depicted binding sites present different V-based species, as referred to in the text, from which only the site 2 is covalently bound. H-bonds (distances are represented as dashed lines and given in Å) are important in stabilizing the adducts.

More recently, HEWL was used to investigate the ligand 1-methyl-2-ethyl-3-hydroxy-4(1H)-pyridinone (empp) using a combined spectrometric, spectroscopic and structural approach [133]. Empp is a pyridinone derivative known for its insulin-mimetic activity by inhibiting the free fatty acid (FFA) release from isolated rat adipocytes [148]. Complex [V^{IV}O(empp)₂] was soaked into HEWL crystals obtained from distinct crystallization

conditions, and three structures were determined at different resolutions: 1.08 Å (PDB: 80M8), 1.10 Å (PDB: 80MS) and 1.10 Å (PDB: 80MT), designated by structures A, B and C, respectively.

Structure A, obtained at a pH of 4.0, exhibits a trinuclear oxidovanadium(V) adduct $[V_{3}^{V}O_{6}(empp)_{3}(H_{2}O)]$ as well as a $[V^{IV}O(empp)(H_{2}O)]^{+}$ adduct. The first one, proving that V^{IV} can be oxidized to V^V under the used experimental conditions, is not covalently bound to the protein, and it is stabilized through both stacking (Trp123) and H-bond interactions (Arg73, Lys33, #Arg73 and #Asp101, where # represents a symmetry-related molecule). One of the V^{V} -atoms is penta-coordinated (distorted square pyramidal geometry), while the remaining V^{V} -atoms are hexa-coordinated (distorted octahedral geometry). The second one is covalently bound to the side chain of Asp48 and H-bonded to Ser50, Asn59, Arg61, #Gln121 and #Asp125. Structure B, obtained at a pH of 7.0, exhibits a single $[V^{IV}O(empp)_2(H_2O)]$ adduct, which is H-bonded to the main chain of Arg5, Cys6, Glu7 and #Arg14 residues. Structure C, obtained at a pH of 7.5, contains a similar [V^{IV}O(empp)₂(H₂O)] adduct, but a second $[V^{IV}O(empp)_2(H_2O)_2]^+$ adduct is also found to be H-bonded to the side chain of Arg125. Altogether, supported by ESI-MS and EPR data, these results emphasize the speciation of [V^{IV}OL₂] compounds upon protein interactions and the possible binding of different V-fragments, which is important to better understanding their transport in the bloodstream and possible binding to proteins inside cells [133].

Bovine Pancreatic Ribonuclease A (RNase A)

Bovine pancreatic ribonuclease A (RNase A) is one of the most representative ribonucleases and has been extensively discussed in the literature over the years [149–151]. Its relevance is reflected by the attribution of the Chemistry Nobel Prize in 1972 (Anfinsen, Moore and Stein) and 1984 (Merrifield) to research involving this protein.

RNase A is widely used as a model protein, including in metalation- and crystallizationrelated investigations [152–154]. To the best of our knowledge, there are currently two SC-XRD structures of RNase A soaked with an organic-based vanadium compound. The first one is a 1.27 Å resolution structure (PDB: 7P8R) [132]. This structure exhibits two molecules (A and B) in the asymmetric unit, but only one adduct is bound to the side chain of the Glu111 residue of molecule A. V^{IV} presents a slightly distorted octahedral geometry with a bidentate coordination of the two picolinato ligands, an Ooxido atom and an O atom from the referred Glu111 and is further stabilized by H-bonds. The bond lengths within the adduct present values in the expected range, even if the V^{IV}=O_{oxido} (1.68 Å) is marginally larger than the theoretical values, as found in the concomitant HEWL-V^{IV}O(picolinato)₂ structure [132]. Globally, this also confirms the previously indicated potential of Glu side groups to bind $V^{IV}O^{2+}$ centers [155]. The authors used different experimental methodsnamely ESI-MS, CD and EPR-to consubstantiate these structural results. Briefly, ESI-MS proved that the adduct RNase A-[V^{IV}O(pic)₂]–[phosphate/sulfate] is formed at 14,088.5 Da; CD showed that the secondary structure of the protein is not altered upon ligand binding and EPR suggested that the equatorial water molecule of the original compound [V^{IV}O(pic)₂(H₂O)] is replaced by the protein residues Asp/Glu or His, depending on the pH (acidic or neutral, respectively). Further computational approaches confirmed its binding to Glu111 at acidic pH values while also predicting its binding to His105 and/or His119 at physiological pH values. Interestingly, the authors also explored the potential protein inhibition caused by [V^{IV}O(pic)₂(H₂O)], verifying that the catalytic activity of RNase A is significantly reduced in its presence. This finding agrees with the structural data, as the adduct sits near the active site of the protein [132].

The second is a 1.57 Å resolution structure (PDB: 7QWH), obtained in a recently published relevant work from Ferraro et al., involving UV-vis, circular dichroism, EPR, computational and X-ray crystallographic studies on the system bovine RNase A with $[V^{IV}O(8HQ)_2]$ (8HQ = 8-hydroxyquinolinato) [134]. From the XRD analysis, it was found that the geometry around the vanadium center corresponds to a slightly distorted square pyramid; one of the 8HQ ligands is replaced by a water ligand of the OE1 of Glu111,

yielding a $[V^{IV}O(8HQ)(H_2O)]^+$ -RNase A adduct. The 8HQ is maintained in its position through stacking interactions with the His119 side chain. The O-atoms within the vanadium coordination sphere are H-bonded to the N-atoms of the side chains of Asn71 and Gln69 and to H₂O molecules. The $V^{IV} = O_{\text{oxido}}$ distance is 1.66 Å, thus within the expected range for these bonds. DFT and docking calculations allowed for a deeper understanding of the system; namely, from the crystallographic data at a pH of 5.1, it was concluded that the formation of the $[V^{I}O(8HQ)(H_2O)] + -RNase A$ adduct explains/results in the inhibition of the RNase A activity [134].

Bovine Trypsin

Serine proteases, which are able to hydrolyze peptide bonds within proteins, are an important family of enzymes that are involved in multiple biological processes and signal transduction pathways, including digestion, immune response, blood coagulation and apoptosis [156–159]. Bovine pancreatic trypsin is one of the most typical serine proteases and is commonly used as a model in related studies. From a crystallographic point of view, trypsin is also an interesting model as it produces well-diffracting crystals under a significant range of experimental conditions.

There are currently two X-ray bovine trypsin structures deposited in the PDB containing vanadium compounds (picolinato and 1,10-phenanthroline), released in 2022 [16]. Before 2022, only a 1.5 Å resolution structure of the related bovine chymotrypsin with vanadate and benzohydroxamic acid (PDB: 2P8O) was available; this showed an adduct at the active site with a distorted octahedral geometry and the V atom covalently bound to the O atom of Ser195 [160]. We highlight that the binding to the side group of serine confirms the findings of earlier studies indicating its potential to bind V^{IV}-centers [161].

Focusing on the available V-trypsin structures, a co-crystallization strategy with $V^{IV}OSO_4$ + picolinato was followed, and a 1.09 Å resolution structure was obtained (PDB: 7Q0X). A single $V^{IV}O$ (picolinato)₂ adduct, very similar to the one described with HEWL, sits at the active site, with V^{IV} presenting a distorted octahedral geometry bound to Ser195, an O_{oxido} and two picolinato anions through the N and O atoms. The adduct is further stabilized by hydrogen bonds (Ser195 and Gln193) and hydrophobic interactions (Phe41, Cys42, Cys191, Gln192 and Phe215). Despite its resemblance with the HEWL adduct, the $V^{IV} = O_{\text{oxido}}$ bond length is significantly shorter (1.70 Å versus 1.82 Å); being slightly above the usual values (1.57 to 1.65 Å) [71], the result was interpreted as being due to a partial reduction of the metal during the data collection. Additional docking studies corroborate the structural results when both buried and surface water molecules were included, proving, as before for HEWL, the essential role of microsolvation for the chiral discrimination of the binding region [16].

The second V-trypsin structure was motivated by the fact that the previously referredto HEWL-V^{IV}O(H₂O)(phen) adduct is partially lacking in electron density for the phen moiety. A successful co-crystallization experiment with bovine trypsin resulted in a 1.20 Å resolution structure (PDB: 7Q0W). The structure revealed multiple imidazole (Im) molecules arising from the crystallization condition and a single V adduct at the active site with a distorted octahedral geometry (Figure 6). V is bound to the O-atom of the side chain of the Ser195 residue, to the two N-atoms of the phen moiety, to the N-atom of an imidazole molecule and to two O-atoms. Similarly to picolinato, hydrogen and hydrophobic interactions have a relevant role in stabilizing the adduct [16].

The distances between the V moiety and those O-atoms (1.70 and 1.71 Å) did not reveal the adduct as V^VO_2 or $V^{IV}O(OH)$. Computational methods—DFT and docking simulations—were used to address this point, showing that the presence of two O_{oxido} or a (O_{oxido} , OH) couple is equally possible (similar energies, although the V^V moiety is slightly preferred) [16]. The finding that the conversion energy between the two common vanadium oxidation states (IV and V) is low suggests that the interchange of the two forms of the VO(phen)-trypsin complex is easy, anticipating consequences for the ease of ROS



production. It is not yet known whether this will happen for other complexes and proteins, but this is a subject to be properly explored by researchers in the near future.

Figure 6. Structural representation of the V^VO₂(phen)(imidazole) adduct covalently bound to the Ser195 residue of bovine trypsin (PDB: 7Q0W), determined by X-ray crystallography.

3. Vanadium-Containing Proteins and Cryo-EM

Despite the still vast prevalence of structures determined by X-ray diffraction techniques when compared with those determined by other methods, structural biology is witnessing a paradigm shift: seven years ago, 89% of the total number of PDB entries were solved by X-ray crystallography, while, in 2023, this percentage decreased to 85%.

One of the main reasons for this scenario is the rise of cryo-EM techniques—in particular, single-particle analysis and cryo-electron tomography—which were further recognized by the attribution of the Chemistry Nobel Prize in 2017 to Dubochet, Frank and Henderson. In the recent years, cryo-EM has made remarkable technical progresses and much higher resolutions are now possible. Simultaneously, access to cutting-edge equipment is now facilitated and diffused throughout the worldwide community dedicated to structural resolution. Altogether, considering its potential to better mimic physiological conditions as it may be applied to samples in solution, cryo-EM has become one of the most popular structural methodologies. In fact, as expressed in Figure 7, there is an exponential increase in the number of PDB entries solved by cryo-EM methods, and approximately 74% of them have been deposited since 2020. For a more detailed description on the advances and potential uses of cryo-EM (including in drug design projects), we recommend several publications [162–166].

Table 5 summarizes the vanadium-containing protein structures that have been deposited in the PDB since 2015. These show a clear incidence of ADP-vanadate species that are used to mimic the intermediate state of the ATP hydrolysis of several ATP-binding cassette (ABC) transporters.



Table 5. Cryo-EM structures of V-containing proteins available in the PDB since 2015. The identifier and name/chemical structure of each vanadium compound, as well as the respective PDB codes, are provided.

It should be noted that many of the structures identified as containing inorganic orthovanadate (identifier VO4) also contain an associated ADP moiety, as the authors did not deposit the ADP-vanadate as a single molecule (identifiers AOV or AD9). In fact, from the eleven VO4 structures indicated in Table 5, only one contains exclusively orthovanadate; it is a 3.30 Å resolution structure of an *E. coli* potassium uptake transporter KdpFABC (PDB: 7ZRD). KdpFABC contains different subunits, namely the P-type ATPase KdpB, in which a serine residue (Ser162) is phosphorylated when no more potassium is needed, leading to the inhibition of the transporter. To further elucidate this inhibition from a structural point of view, Silberberg and collaborators characterized a vast range of cryo-EM wild-type and variant KdpFABC structures resulting from inhibiting and non-inhibiting conditions, and a new inhibited KdpFABC state (named E1P tight) was found [174].

Taking advantage on its well-known structural similarity with phosphate, orthovanadate (able to stabilize P-type ATPases in an E2P state) was incubated with a wild-type KdpFABC sample (PDB: 7ZRD). The orthovanadate was found next to the residue Asp307



of KdpB, known to be bound to the γ -phosphate of ATP. Interestingly, the cryo-EM structure was determined in an E1P-tight state similar to the one under turnover conditions (PDB: 7ZRE), which was interpreted as the conformation adopted after ADP release [174].

Figure 7. Evolution of the cryo-EM structures deposited in the PDB up to July 2023 over 4-year periods.

Regarding ADP-vanadate intermediate conformation form structures, we recently listed some of them [15]. Our review encompassed the single particle cryo-EM structures of the following ABC transporters: (1) LptB2FG and the complex LptB2FG-LptC from *Escherichia coli* (PDBs: 6MHZ and 6MI8) [176]; (2) TmrAB from *Thermus thermophilus* in two different conformations (PDBs: 6RAK and 6RAJ) [177]; (3) NaAtm1 from *Novosphingobium aromaticivorans* (PDB: 6VQT) [167]; and (4) MIaFEDB from *Acinetobacter baumannii* (PDB: 7D0A) [169].

Since then, a considerable amount of other bacterial ABC transporters cryo-EM structures containing an ADP-orthovanadate adduct have been made available. This is the case for a 3.50 Å resolution structure of the *E. coli* ABC transporter complex LolCDE (PDB: 7MDY), which is able to transport lipoproteins from the inner membrane to the outer membrane. The authors were able to obtain an intermediate conformation of the ATPase with one ADP-vanadate moiety in each of the two ATP sites at the interface of a dimer of the LolD domain. This binding led to the rearrangement of the transmembrane helices (TM2) in the domains LolC and LolE which, in turn, released the bound lipoprotein to the periplasm [178].

There is also an example of a cryo-EM entry containing an ADP-metavanadate adduct, the 3.90 Å resolution structure of the ABC transporter MlaFEBD from *Pseudomonas areuginosa* (PDB: 7CH8), which participates in the transport of phospholipids to the inner leaflet of the outer membrane in Gram-negative bacteria. To gain further insights into the transport mechanism, the authors determined and compared three distinct cryo-EM MlaFEBD structures: the apo-form (nucleotide-free), the previously referred-to ADP-vanadate (mimicking the ADP-bound post-hydrolysis conformation) and the ATP analogue AMPPNP (mimicking the ATP-bound state). Unexpectedly, significant conformational modifications upon nucleotide binding were not detected. An ADP-vanadate moiety was found at the ATP binding site of each copy of the MlaF dimer. The adenine and the ribose moieties are coordinated to Arg18 and Arg21, respectively, while the phosphate moieties are coordinated to Arg18 and Arg21, respectively, while the phosphate moieties are coordinated to Arg18 and Arg21, respectively.

dinated to Lys47 and a magnesium ion, and the vanadate is close to the catalytic Glu170 residue. Several phospholipids were found to bind to the substrate-binding pocket of the MlaE, suggesting that this step can be a passive diffusion mechanism rather than an ATP-dependent one [181].

Much less commonly, an eucaryotic ABC transporter with ADP-orthovanadate is also found in the PDB: a single particle 3.77 Å resolution structure of Pdr5 from *Saccharomyces cerevisiae* (PDB: 7P06). Pdr5 is an efflux pump belonging to the PDR (pleiotropic drug resistance) subfamily, which is deeply involved in multi-drug resistance as it can transport a great range of xenobiotics. Known as a model for pathogen fungi (e.g., *Candida albicans*) homologues, different apo- and nucleotide-bound Pdr5 were characterized. An ADP-orthovanadate structure was found in an outward-facing conformation, corresponding to intermediate step of the ATP hydrolysis. The authors further explored the structural rearrangements in both transmembrane and extracellular domains, revealing an asymmetric ATP hydrolysis as conformational changes are much more pronounced in one half of Pdr5, supporting a peristaltic movement of the xenobiotic [179].

Finally, cryo-EM was also used to characterize a system containing a polyoxidovanadate. Their putative biological and therapeutic relevance were mentioned in Section 2.1.2 [107–111]. Among polyoxidovanadates, decavanadate (V₁₀) has been particularly studied [182]. Winkler and collaborators were able to solve a 3.80 Å resolution single-particle structure of the human ion channel TRPM4 complexed with V₁₀ (PDB: 5WP6) [175]. TRPM4, belonging to the TRPM (Transient Receptor Potential Melastatin) subfamily, is a Ca²⁺-activated nonselective channel that transports sodium and/or potassium to depolarize the cell when the intracellular calcium level increases, participating in countless bioprocesses such as the cardiac rhythm. The study aimed to characterize the V₁₀ binding to the protein as V₁₀, being highly negatively charged, interferes with the membrane potential, modulating the voltage dependence of TRPM4. Two V₁₀ binding sites were found (exhibiting several positive residues), at the turn of the C-terminal domain and at the interface of MHR1/2 and the MHR3 of the MHR (N-terminal TRPM homology region) domain. The different structural aspects were discussed (e.g., the role of Gln977 in the Ca²⁺ permeability of the protein), contributing to elucidating the function of the TRPM ion channels [175].

4. Conclusions and Future Directions

As widely described, X-ray crystallography and, more recently, cryo-EM have played pivotal roles in shedding light on the current state of the art of several biological processes. Despite the massive advances in the last decades, much more is expected and will certainly be achieved in the next few years, namely by the introduction of artificial intelligence (AI) tools, currently mainly represented by AlphaFold software (https://alphafold.ebi.ac.uk/, accessed on 7 September 2023) [183].

In fact, the technical development of both experimental and computational structural methods and the understanding that the biological effects of metal complexes on biological systems, namely of vanadium complexes, may be associated with interactions of the metal compounds with proteins, led to an enormous growth in the number of reported structures in the PDB. Concomitantly, this led to a much better understanding of the nature and variety of interactions of metal complexes with proteins, as well as the mechanisms of many reactions involved either in inhibition or catalysis of enzymes by metal ions or in many other types of involvement of metal complexes (e.g., structural or toxic effects) that impact on proteins.

Regarding vanadium compounds, besides halogenases, nitrogenases and vanabins, for which structural information has been available for some time [1,7,10,24–31], and the interference of vanadate in several biological processes [6,7,9,184], it is presently recognized that the biological effects of VCs may be due to their interaction with proteins, and several studies have addressed this topic. The number of structural studies addressing the interaction of vanadium compounds with proteins will undoubtedly increase in the near

future and will provide new information and clues to help understand the biological and therapeutic effects of VCs.

Notwithstanding, despite the relevance of information provided by the several methodologies used, it is known that in biological media, VCs undergo hydrolysis and will certainly be involved in several ligand exchange and redox processes, as well as modifications in geometry, coordination number and nuclearity [15,111]. Additionally, at the low concentrations prevailing, the species formed may totally differ from the compound initially introduced in the media; thus, if a biological effect is reported, the mechanism of action proposed must take into account the species that are effectively formed. Most researchers are now more aware of the complex speciation that is established in biological media [44–49]. Thus, the understanding of the changes occurring and of the several types of binding that may be established between VCs and proteins/enzymes is very important, not only because their action may be inhibited and/or modified but also because the structure of the original complex may have changed upon binding to the protein. The present review provides an updated account of the presently available structural information of vanadium complexes bound to proteins, and it was indeed confirmed that in many cases, the V-containing species that is found to be bound to the protein differs from the one initially added to the crystallization media.

Author Contributions: M.F.A.S. and J.C.P. equally contributed to its conceptualization, methodology, investigation, writing (original draft preparation and review and editing) and supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Applied Molecular Biosciences Unit (UCIBIO) (projects UIDB/04378/2020 and UIDP/04378/2020), Centro de Química Estrutural (CQE) (projects UIDB/00100/2020 and UIDP/00100/2020), the Associate Laboratory Institute for Health and Bioeconomy (i4HB) (project LA/P/0140/2020) and the Associate Laboratory Institute of Molecular Sciences (IMS) (project LA/P/0056/2020), which are financed by National Funds from Fundação para a Ciência e Tecnologia (FCT).

Acknowledgments: The authors would like to thank Rui M. C. Portela for his initial assistance in the PDB searching.

Conflicts of Interest: Both authors declare that there are no conflict of interest.

Sample Availability: Not applicable.

References

- Costa Pessoa, J.; Etcheverry, S.; Gambino, D. Vanadium compounds in medicine. *Coord. Chem. Rev.* 2015, 301–302, 24–48. [CrossRef] [PubMed]
- 2. Rehder, D. Vanadium in biological systems and medicinal applications. *Inorg. Chim. Acta* 2023, 549, 121387. [CrossRef]
- Jakusch, T.; Kiss, T. In vitro study of the antidiabetic behavior of vanadium compounds. *Coord. Chem. Rev.* 2017, 351, 118–126. [CrossRef]
- Korbecki, J.; Baranowska-Bosiacka, I.; Gutowska, I.; Chlubek, D. Vanadium Compounds as Pro-Inflammatory Agents: Effects on Cyclooxygenases. Int. J. Mol. Sci. 2015, 16, 12648–12668. [CrossRef] [PubMed]
- 5. Kioseoglou, E.; Petanidis, S.; Gabriel, C.; Salifoglou, A. The chemistry and biology of vanadium compounds in cancer therapeutics. *Coord. Chem. Rev.* 2015, 301–302, 87–105. [CrossRef]
- Scior, T.; Guevara-Garcia, J.A.; Do, Q.-T.; Bernard, P.; Laufer, S. Why Antidiabetic Vanadium Complexes are Not in the Pipeline of "Big Pharma" Drug Research? A Critical Review. *Curr. Med. Chem.* 2016, 23, 2874–2891. [CrossRef]
- Crans, D.C.; Smee, J.J.; Gaidamauskas, E.; Yang, L. The chemistry and biochemistry of vanadium and the biological activities exerted by vanadium compounds. *Chem. Rev.* 2004, 104, 849–902. [CrossRef]
- Chasteen, N.D. Vanadium-protein interactions. In *Metal Ions in Biological Systems*; Sigel, H., Sigel, A., Eds.; Marcel Dekker Inc: New York, NY, USA, 1995; Volume 31, pp. 231–247.
- 9. Korbecki, J.; Baranowska-Bosiacka, I.; Gutowska, I.; Chlubek, D. Biochemical and medical importance of vanadium compounds. *Acta Chim. Pol.* **2012**, *59*, 195–200. [CrossRef]
- 10. Costa Pessoa, L.; Garribba, E.; Santos, M.F.A.; Santos-Silva, T. Vanadium and proteins: Uptake, transport, structure, activity and function. *Coord. Chem. Rev.* 2015, 301–302, 49–86. [CrossRef]
- 11. Sciortino, G.; Garribba, E. The binding modes of V^{IV}O²⁺ ions in blood proteins and enzymes. *Chem. Commun.* **2020**, *56*, 12218–12221. [CrossRef]

- 12. Ugone, V.; Sanna, D.; Sciortino, G.; Maréchal, J.-D.; Garribba, E. Interaction of Vanadium(IV) Species with Ubiquitin: A Combined Instrumental and Computational Approach. *Inorg. Chem.* **2019**, *58*, 8064–8078. [CrossRef]
- Ugone, V.; Sanna, D.; Sciortino, G.; Crans, D.C.; Garribba, E. ESI-MS Study of the Interaction of Potential Oxidovanadium(IV) Drugs and Amavadin with Model Proteins. *Inorg. Chem.* 2020, 59, 9739–9755. [CrossRef]
- Ugone, V.; Sanna, D.; Ruggiu, S.; Sciortino, G.; Garribba, E. Covalent and non-covalent binding in vanadium–protein adducts. *Inorg. Chem. Front.* 2021, *8*, 1189–1196. [CrossRef]
- 15. Costa Pessoa, J.; Santos, M.F.A.; Correia, I.; Sanna, D.; Sciortino, G.; Garribba, E. Binding of vanadium ions and complexes to proteins and enzymes in aqueous solution. *Coord. Chem. Rev.* **2021**, *449*, 214192. [CrossRef]
- Santos, M.F.A.; Sciortino, G.; Correia, I.; Fernandes, A.C.P.; Santos-Silva, T.; Pisanu, F.; Garribba, E.; Costa Pessoa, J. Binding of V^{IV}O²⁺, V^{IV}OL⁺, V^{IV}OL₂ and V^VO₂L moieties to proteins: X-ray/theoretical characterization and biological implications. *Chem. Eur. J.* 2022, *28*, e202200105. [CrossRef] [PubMed]
- 17. Mehtab, S.; Gonçalves, G.; Roy, S.; Tomaz, A.I.; Santos-Silva, T.; Santos, M.F.A.; Romão, M.J.; Jakusch, T.; Kiss, T.; Costa Pessoa, J. Interaction of vanadium(IV) with human serum apo-transferrin. *J. Inorg. Biochem.* **2013**, *121*, 187–195. [CrossRef] [PubMed]
- Sanna, D.; Micera, G.; Garribba, E. Interaction of VO²⁺ Ion and Some Insulin-Enhancing Compounds with Immunoglobulin G. *Inorg. Chem.* 2011, 50, 3717–3728. [CrossRef]
- Correia, I.; Jakusch, T.; Cobbinna, E.; Mehtab, S.; Tomaz, I.; Nagy, N.V.; Rockenbauer, A.; Costa Pessoa, J.; Kiss, T. Evaluation of the binding of oxovanadium(IV) to human serum albumin. *Dalton Trans.* 2012, 41, 6477–6487. [CrossRef]
- Sciortino, G.; Sanna, D.; Ugone, V.; Maréchal, J.-D.; Alemany-Chavarria, M.; Garribba, E. Effect of secondary interactions, steric hindrance and electric charge on the interaction of V^{IV}O species with proteins. *New J. Chem.* 2019, 43, 17647–17660. [CrossRef]
- 21. Sanna, D.; Serra, M.; Micera, G.; Garribba, E. Interaction of Antidiabetic Vanadium Compounds with Hemoglobin and Red Blood Cells and Their Distribution between Plasma and Erythrocytes. *Inorg. Chem.* **2014**, *53*, 1449–1464. [CrossRef]
- Jakusch, T.; Hollender, D.; Enyedy, É.A.; González, C.S.; Montes-Bayón, M.; Sanz-Medel, A.; Costa Pessoa, J.; Tomaz, I.; Kiss, T. Biospeciation of different antidiabetic V^{IV}O compounds in serum. *Dalton Trans.* 2009, 13, 2428–2437. [CrossRef] [PubMed]
- Kiss, T.; Jakusch, T.; Hollender, D.; Dörnyei, A.; Enyedy, E.A.; Costa Pessoa, J.; Sakurai, H.; Sanz-Medel, A. Biospeciation of antidiabetic VO(IV) complexes. *Coord. Chem. Rev.* 2008, 252, 1153–1162. [CrossRef]
- 24. Hu, Y.; Ribbe, M.W. Nitrogenases-A Tale of Carbon Atom(s). Angew. Chem. Int. Ed. 2016, 55, 8216–8226. [CrossRef]
- Sippel, D.; Schlesier, J.; Rohde, M.; Trncik, C.; Decamps, L.; Djurdjevic, I.; Spatzal, T.; Andrade, S.L.A.; Einsle, O. Production and isolation of vanadium nitrogenase from Azotobacter vinelandii by molybdenum depletion. *J. Biol. Inorg. Chem.* 2017, 22, 161–168. [CrossRef]
- 26. Wever, R.; Barnett, P. Vanadium Chloroperoxidases: The Missing Link in the Formation of Chlorinated Compounds and Chloroform in the Terrestrial Environment? *Chem. Asian J.* **2017**, *12*, 1997–2007. [CrossRef]
- McLauchlan, C.C.; Murakami, H.A.; Wallace, C.A.; Crans, D.C. Coordination environment changes of the vanadium in vanadiumdependent haloperoxidase enzymes. J. Inorg. Biochem. 2018, 186, 267–279. [CrossRef]
- McLauchlan, C.C.; Peters, B.J.; Willsky, G.R.; Crans, D.C. Vanadium–phosphatase complexes: Phosphatase inhibitors favor the trigonal bipyramidal transition state geometries. *Coord. Chem. Rev.* 2015, 301–302, 163–199. [CrossRef]
- Leblanc, C.; Vilter, H.; Fournier, J.-B.; Delage, L.; Potin, P.; Rebuffet, E.; Michel, G.; Solari, P.L.; Feiters, M.C.; Czjzek, M. Vanadium haloperoxidases: From the discovery 30 years ago to X-ray crystallographic and V K-edge absorption spectroscopic studies. *Coord. Chem. Rev.* 2015, 301–302, 134–146. [CrossRef]
- Ueki, T.; Michibata, H. Molecular mechanism of the transport and reduction pathway of vanadium in ascidians. *Coord. Chem. Rev.* 2011, 255, 2249–2257. [CrossRef]
- Ueki, T.; Yamaguchi, N.; Romaidi; Isago, Y.; Tanahashi, H. Vanadium accumulation in ascidians: A system overview. *Coord. Chem. Rev.* 2015, 301–302, 300–308. [CrossRef]
- 32. Akabayov, S.R.; Akabayov, B. Vanadate in structural biology. Inorg. Chim. Acta 2014, 420, 16–23. [CrossRef]
- 33. Costa Pessoa, J.; Gonçalves, G.; Roy, S.; Correia, I.; Mehtab, S.; Santos, M.F.A.; Santos-Silva, T. New insights on vanadium binding to human serum transferrin. *Inorg. Chim. Acta* 2014, 420, 60–68. [CrossRef]
- Correia, I.; Chorna, I.; Cavaco, I.; Roy, S.; Kuznetsov, M.L.; Ribeiro, N.; Justino, G.; Marques, F.; Santos-Silva, T.; Santos, M.F.A.; et al. Interaction of [V^{IV}O(acac)₂] with Human Serum Transferrin and Albumin. *Chem. Asian J.* 2017, 12, 2062–2084. [CrossRef]
- Azevedo, C.G.; Correia, I.; Santos, M.M.C.; Santos, M.F.A.; Santos-Silva, J.; Doutch, J.; Fernandes, L.; Santos, H.M.; Capelo, J.L.; Costa Pessoa, J. Binding of vanadium to human serum transferrin—Voltammetric and spectrometric studies. *J. Inorg. Biochem.* 2018, 180, 211–221. [CrossRef] [PubMed]
- Burley, S.K.; Berman, H.M.; Kleywegt, G.J.; Markley, J.L.; Nakamura, H.; Velankar, S. Protein Data Bank (PDB): The Single Global Macromolecular Structure Archive. In *Protein Crystallography: Methods in Molecular Biology*; Wlodawer, A., Dauter, Z., Jaskolski, M., Eds.; Humana Press: New York, NY, USA, 2017; Volume 1607, pp. 627–641. [CrossRef]
- Velankar, S.; Burley, S.K.; Kurisu, G.; Hoch, J.C.; Markley, J.L. The Protein Data Bank Archive. In *Structural Proteomics: Methods in Molecular Biology*; Owens, R.J., Ed.; Humana Press: New York, NY, USA, 2021; Volume 2305, pp. 3–21. [CrossRef]
- Burley, S.K.; Bhikadiya, C.; Bi, C.; Bittrich, S.; Chao, H.; Chen, L.; Craig, P.A.; Crichlow, G.V.; Dalenberg, K.; Duarte, J.M.; et al. RCSB Protein Data bank: Tools for visualizing and understanding biological macromolecules in 3D. *Protein Sci.* 2022, 31, e4482. [CrossRef]
- 39. Kearns, S. Structure of the Pandemic. Structure 2020, 28, 874–878. [CrossRef] [PubMed]

- 40. Bank, P.D. Crystallography: Protein Data Bank. Nat. New Biol. 1971, 233, 223. [CrossRef]
- 41. Berman, H.M. The Protein Data Bank: A historical perspective. Acta Crystallogr. 2008, A64, 88–95. [CrossRef]
- 42. Berman, H.M.; Gierasch, L.M. How the Protein Data Bank changed biology: An introduction to the JBC Reviews thematic series, part 1. J. Biol. Chem. 2021, 296, 100608. [CrossRef]
- Gierasch, L.M.; Berman, H.M. How the Protein Data Bank changed biology: An introduction to the JBC Reviews thematic series, part 2. J. Biol. Chem. 2021, 296, 100748. [CrossRef]
- 44. Levina, A.; Crans, D.C.; Lay, P.A. Speciation of metal drugs, supplements and toxins in media and bodily fluids controls in vitro activities. *Coord. Chem. Rev.* 2017, 352, 473–498. [CrossRef]
- Levina, A.; Lay, P.A. Stabilities and Biological Activities of Vanadium Drugs: What is the Nature of the Active Species? *Chem. Asian J.* 2017, 12, 1692–1699. [CrossRef] [PubMed]
- Nunes, P.; Correia, I.; Cavaco, I.; Marques, F.; Pinheiro, T.; Avecilla, F.; Costa Pessoa, J. Therapeutic potential of vanadium complexes with 1,10-phenanthroline ligands, quo vadis? Fate of complexes in cell media and cancer cells. *Inorg. Biochem.* 2021, 217, 111350. [CrossRef] [PubMed]
- 47. Sanna, D.; Ugone, V.; Micera, G.; Buglyó, P.; Bíró, L.; Garribba, E. Speciation in human blood of Metvan, a vanadium based potential anti-tumor drug. *Dalton Trans.* **2017**, *46*, 8950–8967. [CrossRef]
- Sanna, D.; Ugone, V.; Serra, M.; Garribba, E. Speciation of potential anti-diabetic vanadium complexes in real serum samples. J. Inorg. Biochem. 2017, 173, 52–65. [CrossRef]
- Nunes, P.; Correia, I.; Marques, F.; Matos, A.P.; Santos, M.M.C.; Azevedo, C.G.; Capelo, J.-L.; Santos, H.M.; Gama, S.; Pinheiro, T.; et al. Copper complexes with 1,10-phenanthroline derivatives: Underlying factors affecting their cytotoxicity. *Inorg. Chem.* 2020, 59, 9116–9134. [CrossRef]
- 50. Thomas, J.M. The birth of X-ray crystallography. Nature 2012, 491, 186–187. [CrossRef]
- 51. Helliwell, J.R. New developments in crystallography: Exploring its technology, methods and scope in the molecular biosciences. *Biosci. Rep.* 2017, 37, BSR20170204. [CrossRef]
- 52. Rathore, I.; Mishra, V.; Bhaumik, P. Advancements in macromolecular crystallography: From past to present. *Emerg. Top. Life Sci.* **2021**, *5*, 127–149. [CrossRef]
- Kermani, A.A.; Aggarwal, S.; Ghanbarpour, A. Advances in X-ray crystallography methods to study structural dynamics of macromolecules. In *Advanced Spectroscopic Methods to Study Biomolecular Structure and Dynamics*; Saudagar, P., Tripathi, T., Eds.; Academic Press: London, UK, 2023; Volume 2305, pp. 309–355. [CrossRef]
- 54. Cohen, A.E. A new era of synchrotron-enabled macromolecular crystallography. Nat. Methods 2021, 18, 433–434. [CrossRef]
- 55. Rittle, J.; Field, M.J.; Green, M.T.; Tezcan, F.A. An efficient, step-economical strategy for the design of functional metalloproteins. *Nat. Chem.* **2019**, *11*, 434–441. [CrossRef] [PubMed]
- Chen, K.-E.; Li, M.-Y.; Chou, C.-C.; Ho, M.-R.; Chen, G.-C.; Meng, T.-C.; Wang, A. H-J. Substrate Specificity and Plasticity of FERM-Containing Protein Tyrosine Phosphatases. *Structure* 2015, 23, 653–664. [CrossRef]
- 57. Wulf, S.F.; Ropars, V.; Fujita-Becker, S.; Oster, M.; Hofhaus, G.; Trabuco, L.G.; Pylypenko, O.; Sweeney, H.L.; Houdusse, A.M.; Schröder, R.R. Force-producing ADP state of myosin bound to actin. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E1844–E1852. [CrossRef]
- Moise, G.; Gallup, N.M.; Alexandrova, A.N.; Hengge, A.C.; Johnson, S.J. Conservative Tryptophan Mutants of the Protein Tyrosine Phosphatase YopH Exhibit Impaired WPD-Loop Function and Crystallize with Divanadate Esters in Their Active Sites. *Biochemistry* 2015, 54, 6490–6500. [CrossRef]
- Lee, C.-U.; Hahne, G.; Hanske, J.; Bange, T.; Bier, D.; Rademacher, C.; Hennig, S.; Grossmann, T.N. Redox Modulation of PTEN Phosphatase Activity by Hydrogen Peroxide and Bisperoxidovanadium Complexes. *Angew. Chem. Int. Ed.* 2015, 54, 13796–13800. [CrossRef]
- 60. Ropars, V.; Yang, Z.; Isabet, T.; Blanc, F.; Zhou, K.; Lin, T.; Liu, X.; Hissier, P.; Samazan, F.; Amigues, B.; et al. The myosin X motor is optimized for movement on actin bundles. *Nat. Commun.* **2016**, *7*, 12456. [CrossRef] [PubMed]
- Stanford, S.M.; Aleshin, A.E.; Zhang, V.; Ardecky, R.J.; Hedrick, M.P.; Zou, J.; Ganji, S.R.; Bliss, M.R.; Yamamoto, F.; Bobkov, A.A.; et al. Diabetes reversal by inhibition of the low-molecular-weight tyrosine phosphatase. *Nat. Chem. Biol.* 2017, 13, 624–632. [CrossRef]
- 62. Planelles-Herrero, V.J.; Hartman, J.J.; Robert-Paganin, J.; Malik, F.I.; Houdusse, A. Mechanistic and structural basis for activation of cardiac myosin force production by omecamtiv mecarbil. *Nat. Commun.* **2017**, *8*, 190. [CrossRef] [PubMed]
- Bountra, K.; Hagelueken, G.; Choudhury, H.G.; Corradi, V.; El Omari, K.; Wagner, A.; Mathavan, I.; Zirah, S.; Wahlgren, W.Y.; Tieleman, D.P.; et al. Structural basis for antibacterial peptide self-immunity by the bacterial ABC transporter McjD. *EMBO J.* 2017, *36*, 3062–3079. [CrossRef]
- Moise, G.; Morales, Y.; Beaumont, V.; Caradonna, T.; Loria, J.P.; Johnson, S.J.; Hengge, A.C. YopH PTP1B Chimera Shows the Importance of the WPD-Loop Sequence to the Activity, Structure, and Dynamics of Protein Tyrosine Phosphatases. *Biochemistry* 2018, 57, 5315–5326. [CrossRef]
- 65. Robert-Paganin, J.; Robblee, J.P.; Auguin, D.; Blake, T.C.A.; Bookwalter, C.S.; Krementsova, E.B.; Moussaoui, D.; Previs, M.J.; Jousset, G.; Baum, J.; et al. Plasmodium myosin A drives parasite invasion by an atypical force generating mechanism. *Nat. Commun.* **2019**, *10*, 3286. [CrossRef] [PubMed]
- Lai, C.-H.; Chang, C.-C.; Chuang, H.-C.; Tan, T.-H.; Lyu, P.-C. Structural Insights into the Active Site Formation of DUSP22 in N-loop-containing Protein Tyrosine Phosphatases. *Int. J. Mol. Sci.* 2020, 21, 7515.e14. [CrossRef] [PubMed]

- 67. Chu, N.; Salguero, A.L.; Liu, A.Z.; Chen, Z.; Dempsey, D.R.; Ficarro, S.B.; Alexander, W.M.; Marto, J.A.; Li, Y.; Amzel, L.M.; et al. Akt Kinase Activation Mechanisms Revealed Using Protein Semisynthesis. *Cell* **2018**, *174*, 897–907.e14. [CrossRef]
- Cui, D.S.; Lipchock, J.M.; Brookner, D.; Loria, J.P. Uncovering the Molecular Interactions in the Catalytic Loop That Modulate the Conformational Dynamics in Protein Tyrosine Phosphatase 1B. J. Am. Chem. Soc. 2019, 141, 12634–12647. [CrossRef] [PubMed]
 Dei M.Y. Chem. Soc. 2019, 141, 12634–12647. [CrossRef] [PubMed]
- Dai, Y.X.; Chen, W.F.; Liu, N.N.; Teng, F.Y.; Guo, H.L.; Hou, X.M.; Dou, S.X.; Rety, S.; Xi, X.G. Structural and functional studies of SF1B Pif1 from Thermus oshimai reveal dimerization-induced helicase inhibition. *Nucleic Acids Res.* 2021, 49, 4129–4143. [CrossRef]
- Shen, R.; Crean, R.M.; Olsen, K.J.; Corbella, M.; Calixto, A.R.; Richan, T.; Brandão, T.A.S.; Berry, R.D.; Tolman, A.; Loria, J.P.; et al. Insights into the importance of WPD-loop sequence for activity and structure in protein tyrosine phosphatases. *Chem. Sci.* 2022, 13, 13524–13540. [CrossRef]
- Moussaoui, D.; Robblee, J.P.; Auguin, D.; Krementsova, E.B.; Haase, S.; Blake, T.C.A.; Baum, J.; Robert-Paganin, J.; Trybus, K.M.; Houdusse, A. Full-length Plasmodium falciparum myosin A and essential light chain PfELC structures provide new anti-malarial targets. *eLife* 2020, 9, e60581. [CrossRef]
- 72. Gyimesi, M.; Horvath, A.I.; Turos, D.; Suthar, S.K.; Penzes, M.; Kurdi, C.; Canon, L.; Kikuti, C.; Ruppel, K.M.; Trivedi, D.V.; et al. Single Residue Variation in Skeletal Muscle Myosin Enables Direct and Selective Drug Targeting for Spasticity and Muscle Stiffness. *Cell* **2020**, *183*, 335–346.e13. [CrossRef]
- 73. Shen, R.; Crean, R.M.; Johnson, S.J.; Kamerlin, S.C.L.; Hengge, A.C. Single Residue on the WPD-Loop Affects the pH Dependency of Catalysis in Protein Tyrosine Phosphatases. *JACS Au* 2021, *1*, 646–659. [CrossRef]
- Pinkston, J.; Jo, J.; Olsen, K.J.; Comer, D.; Glaittli, C.A.; Loria, J.P.; Johnson, S.J.; Hengge, A.C. Significant Loop Motions in the SsoPTP Protein Tyrosine Phosphatase Allow for Dual General Acid Functionality. *Biochemistry* 2021, 60, 2888–2901. [CrossRef]
- 75. Ferraro, G.; Paolillo, M.; Sciortino, G.; Garribba, E.; Merlino, A. Multiple and Variable Binding of Pharmacologically Active Bis(maltolato)oxidovanadium(IV) to Lysozyme. *Inorg. Chem.* **2022**, *61*, 16458–16467. [CrossRef]
- Hausmann, J.; Keune, W.J.; Hipgrave Ederveen, A.L.; van Zeijl, L.; Joosten, R.P.; Perrakis, A. Structural snapshots of the catalytic cycle of the phosphodiesterase Autotaxin. *J. Struct. Biol.* 2016, 195, 199–206. [CrossRef]
- 77. Clausen, J.D.; Bublitz, M.; Arnou, B.; Olesen, C.; Andersen, J.P.; Moller, J.V.; Nissen, P. Crystal Structure of the Vanadate-Inhibited Ca(2+)-ATPase. *Structure* **2016**, *24*, 617–623. [CrossRef]
- Bartlett, E.J.; Brissett, N.C.; Plocinski, P.; Carlberg, T.; Doherty, A.J. Molecular basis for DNA strand displacement by NHEJ repair polymerases. *Nucleic Acids Res.* 2016, 44, 2173–2186. [CrossRef] [PubMed]
- El Omari, K.; Mohamad, N.; Bountra, K.; Duman, R.; Romano, M.; Schlegel, K.; Kwong, H.-S.; Mykhaylyk, V.; Olesen, C.; Moller, J.V.; et al. Experimental phasing with vanadium and application to nucleotide-binding membrane proteins. *IUCrJ* 2020, 7, 1092–1101. [CrossRef] [PubMed]
- 80. Yun, H.Y.; Lee, J.; Kim, H.; Ryu, H.; Shin, H.C.; Oh, B.H.; Ku, B.; Kim, S.J. Structural study reveals the temperature-dependent conformational flexibility of Tk-PTP, a protein tyrosine phosphatase from *Thermococcus kodakaraensis* KOD1. *PLoS ONE* **2018**, *13*, e0197635. [CrossRef]
- 81. Feder, D.; Gahan, L.R.; McGeary, R.P.; Guddat, L.W.; Schenk, G. The binding mode of an ADP analog to a metallohydrolase mimics the likely transition state. *Chembiochem* **2019**, *20*, 1536–1540. [CrossRef] [PubMed]
- Chekan, J.R.; Ongpipattanakul, C.; Wright, T.R.; Zhang, B.; Bollinger, J.M., Jr.; Rajakovich, L.J.; Krebs, C.; Cicchillo, R.M.; Nair, S.K. Molecular basis for enantioselective herbicide degradation imparted by aryloxyalkanoate dioxygenases in transgenic plants. *Proc. Natl. Acad. Sci. USA* 2019, 116, 13299–13304. [CrossRef]
- Mitchell, A.J.; Dunham, N.P.; Martinie, R.J.; Bergman, J.A.; Pollock, C.J.; Hu, K.; Allen, B.D.; Chang, W.C.; Silakov, A.; Bollinger, J.M.; et al. Visualizing the Reaction Cycle in an Iron(II)- and 2-(Oxo)-glutarate-Dependent Hydroxylase. *J. Am. Chem. Soc.* 2017, 139, 13830–13836. [CrossRef] [PubMed]
- Dunham, N.P.; Chang, W.C.; Mitchell, A.J.; Martinie, R.J.; Zhang, B.; Bergman, J.A.; Rajakovich, L.J.; Wang, B.; Silakov, A.; Krebs, C.; et al. Two Distinct Mechanisms for C-C Desaturation by Iron(II)- and 2-(Oxo)glutarate-Dependent Oxygenases: Importance of alpha-Heteroatom Assistance. J. Am. Chem. Soc. 2018, 140, 7116–7126. [CrossRef]
- 85. Davis, K.M.; Altmyer, M.; Martinie, R.J.; Schaperdoth, I.; Krebs, C.; Bollinger, J.M., Jr.; Boal, A.K. Structure of a Ferryl Mimic in the Archetypal Iron(II)- and 2-(Oxo)-glutarate-Dependent Dioxygenase, TauD. *Biochemistry* **2019**, *58*, 4218–4223. [CrossRef]
- Jonnalagadda, R.; Del Rio Flores, A.; Cai, W.; Mehmood, R.; Narayanamoorthy, M.; Ren, C.; Zaragoza, J.P.T.; Kulik, H.J.; Zhang, W.; Drennan, C.L. Biochemical and crystallographic investigations into isonitrile formation by a nonheme iron-dependent oxidase/decarboxylase. J. Biol. Chem. 2021, 296, 100231. [CrossRef]
- 87. Sippel, D.; Rohde, M.; Netzer, J.; Trncik, C.; Gies, J.; Grunau, K.; Djurdjevic, I.; Decamps, L.; Andrade, S.L.A.; Einsle, O. A bound reaction intermediate sheds light on the mechanism of nitrogenase. *Science* **2018**, *359*, 1484–1489. [CrossRef]
- Rohde, M.; Grunau, K.; Einsle, O. CO Binding to the FeV Cofactor of CO-Reducing Vanadium Nitrogenase at Atomic Resolution. Angew. Chem. Int. Ed. 2020, 59, 23626–23630. [CrossRef]
- 89. Rohde, M.; Laun, K.; Zebger, I.; Stripp, S.T.; Einsle, O. Two ligand-binding sites in CO-reducing V nitrogenase reveal a general mechanistic principle. *Sci. Adv.* **2021**, *7*, eabg4474. [CrossRef] [PubMed]
- 90. Sippel, D.; Einsle, O. The structure of vanadium nitrogenase reveals an unusual bridging ligand. *Nat. Chem. Biol.* **2017**, *13*, 956–960. [CrossRef] [PubMed]

- 91. Maccari, R.; Ottanà, R. Low molecular weight phosphotyrosine protein phosphatases as emerging targets for the design of novel therapeutic agents. *J. Med. Chem.* 2012, 55, 2–22. [CrossRef] [PubMed]
- 92. Ardito, F.; Giuliani, M.; Perrone, D.; Troiano, G.; Muzio, L. The crucial of protein phosphorylation in cell signaling and its use as targeted therapy. *Int. J. Mol. Med.* 2017, 40, 271–280. [CrossRef]
- Feng, B.; Dong, Y.; Shang, B.; Zhang, B.; Crans, D.C.; Yang, X. Convergent Protein Phosphatase Inhibitor Design for PTP1B and TCPTP: Exchangeable Vanadium Coordination Complexes on Graphene Quantum Dots. *Adv. Funct. Mater.* 2021, 32, 2108645. [CrossRef]
- 94. Chiarugi, P.; Cirri, P.; Marra, F.; Raugei, G.; Camici, G.; Manao, G.; Ramponi, G. LMW-PTP is a Negative Regulator of Insulinmediated Mitotic and Metabolic Signaling. *Biochem. Biophys. Res. Commun.* **1997**, 238, 676–682. [CrossRef]
- Crans, D.C.; Zhang, B.; Gaidamauskas, E.; Keramidas, A.D.; Willsky, G.R.; Roberts, C.R. Is vanadate reduced by thiols under biological conditions? Changing the redox potential of V(V)/V(IV) by complexation in aqueous solution. *Inorg. Chem.* 2010, 49, 4245–4256. [CrossRef]
- 96. Evans, P.; McCoy, A. An introduction to molecular replacement. Acta Crystallogr. 2008, D64, 1–10. [CrossRef] [PubMed]
- 97. Dodson, E. Introduction to molecular replacement: A time perspective. Acta Crystallogr. 2021, 77, 867–879. [CrossRef]
- Hendrickson, W.A. Anomalous diffraction in crystallographic phase evaluation. *Q. Rev. Biophys.* 2014, 47, 49–93. [CrossRef] [PubMed]
- 99. Rose, J.P.; Wang, B.-C. SAD phasing: History, current impact and future opportunities. *Arch. Biochem. Biophys.* **2016**, 602, 80–94. [CrossRef] [PubMed]
- 100. Taylor, G.L. Introduction to phasing. Acta Crystallogr. 2010, 66, 325–338. [CrossRef] [PubMed]
- Wang, J.; Li, Y.; Modis, Y. Exploiting subtle structural differences in heavy-atom derivatives for experimental phasing. *Acta Crystallogr.* 2014, 70, 1873–1883. [CrossRef] [PubMed]
- Dauter, M.; Dauter, Z. Many Ways to Derivatize Macromolecules and Their Crystals for Phasing. In *Protein Crystallography. Methods in Molecular Biology*; Wlodawer, A., Dauter, Z., Jaskolski, M., Eds.; Humana Press: New York, NY, USA, 2017; Volume 1607, pp. 349–356. [CrossRef]
- Vilas Boas, L.F.; Costa Pessoa, J. Vanadium. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R.D., McCleverty, J.A., Eds.; Pergamon: Oxford, UK, 1987; Volume 3, pp. 453–583.
- 104. Santos, M.F.A.; Correia, I.; Oliveira, A.R.; Garribba, E.; Costa Pessoa, J.; Santos-Silva, T. Vanadium Complexes as Prospective Therapeutics: Structural Characterization of a V^{IV} Lysozyme Adduct. *Eur. J. Inorg. Chem.* 2014, 2014, 3293–3297. [CrossRef]
- Lee, C.C.; Hu, Y.; Ribbe, M.W. Tracing the Hydrogen Source of Hydrocarbons Formed by Vanadium Nitrogenase. Angew. Chem. Int. Ed. 2011, 50, 5545–5547. [CrossRef]
- 106. Eady, R.R. Current status of structure function relationships of vanadium nitrogenase. *Coord. Chem. Rev.* 2003, 237, 23–30. [CrossRef]
- 107. Aureliano, M. The Future Is Bright for Polyoxometalates. BioChem 2022, 2, 8–26. [CrossRef]
- Pereira, M.J.; Carvalho, E.; Eriksson, J.W.; Crans, D.C.; Aureliano, M. Effects of decavanadate and insulin enhancing vanadium compounds on glucose uptake in isolated rat adipocytes. *J. Inorg. Biochem.* 2009, 103, 1687–1692. [CrossRef] [PubMed]
- 109. Treviño, S.; Sánchez-Lara, E.; Sarmiento-Ortega, V.E.; Sánchez-Lombardo, I.; Flores-Hernández, J.Á.; Pérez-Benítez, A.; Brambila-Colombres, E.; González-Vergara, E. Hypoglycemic, lipid-lowering and metabolic regulation activities of metforminium deca-vanadate (H₂Metf)₃[V₁₀O₂₈]·8H₂O using hypercaloric-induced carbohydrate and lipid deregulation in Wistar rats as biological model. *J. Inorg. Biochem.* 2015, 147, 85–92. [CrossRef]
- 110. Aureliano, M.; Gumerova, N.I.; Sciortino, G.; Garribba, E.; Rompel, A.; Crans, D.C. Polyoxovanadates with emerging biomedical activities. *Coord. Chem. Rev.* 2021, 447, 214143. [CrossRef]
- 111. Aureliano, M.; Gumerova, N.I.; Sciortino, G.; Garribba, E.; McLauchlan, C.C.; Rompel, A.; Crans, D.C. Polyoxidovanadates' interactions with proteins: An overview. *Coord. Chem. Rev.* 2022, 454, 214344. [CrossRef]
- 112. Locher, K.P.; Lee, A.T.; Rees, D.C. The *E. coli* BtuCD structure: A framework for ABC transporter architecture and mechanism. *Science* 2002, 296, 1091–1098. [CrossRef]
- Evans, H.R.; Holloway, D.E.; Sutton, J.M.; Ayriss, J.; Shone, C.C.; Acharya, K.R. C3 Exoenzyme from *Clostridium botulinum*: Structure of a Tetragonal Crystal Form and a Reassessment of Nad-Induced Flexure. *Acta Crystallogr.* 2004, D60, 1502–1505. [CrossRef]
- 114. Pinto, P.H.; Kroupova, A.; Schleiffer, A.; Mechtler, K.; Jinek, M.; Weitzer, S.; Martinez, J. ANGEL2 is a member of the CCR4 family of deadenylases with 2',3'-cyclic phosphatase activity. *Science* 2020, *369*, 524–530. [CrossRef]
- 115. Kim, Y.; Wower, J.; Maltseva, N.; Chang, C.; Jedrzejczak, R.; Wilamowski, M.; Kang, S.; Nicolaescu, V.; Randall, G.; Michalska, K.; et al. Tipiracil binds to uridine site and inhibits Nsp15 endoribonuclease NendoU from SARS-CoV-2. *Commun. Biol.* 2021, 4, 193. [CrossRef] [PubMed]
- Chinthalapudi, K.; Heissler, S.M.; Preller, M.; Sellers, J.R.; Manstein, D.J. Mechanistic insights into the active site and allosteric communication pathways in human nonmuscle myosin-2C. *eLife* 2017, 6, e32742. [CrossRef] [PubMed]
- 117. Feder, D.; McGeary, R.P.; Mitić, N.; Lonhienne, T.; Furtado, A.; Schulz, B.L.; Henry, R.J.; Schmidt, S.; Guddat, L.W.; Schenk, G. Structural elements that modulate the substrate specificity of plant purple acid phosphatases: Avenues for improved phosphorus acquisition in crops. *Plant Sci.* 2020, 294, 110445. [CrossRef] [PubMed]

- 118. Franz, P.; Ewert, W.; Preller, M.; Tsiavaliaris, G. Unraveling a Force-Generating Allosteric Pathway of Actomyosin Communication Associated with ADP and Pi Release. *Int. J. Mol. Sci.* **2021**, *22*, 104. [CrossRef] [PubMed]
- Mir, A.; Golden, B.L. Two Active Site Divalent Ions in the Crystal Structure of the Hammerhead Ribozyme Bound to a Transition State Analogue. *Biochemistry* 2016, 55, 633–636. [CrossRef] [PubMed]
- 120. Teplova, M.; Falschlunger, C.; Krasheninina, O.; Egger, M.; Ren, A.; Patel, D.J.; Micura, R. Crucial Roles of Two Hydrated Mg²⁺ Ions in Reaction Catalysis of the Pistol Ribozyme. *Angew. Chem. Int. Ed.* **2020**, *59*, 2837–2843. [CrossRef]
- Elvingson, K.; Keramidas, A.D.; Crans, D.C.; Pettersson, L. Speciation in Vanadium Bioinorganic Systems. 5. Interactions between Vanadate, Uridine and Imidazole—An Aqueous Potentiometric, ⁵¹V, ¹⁷O and ¹³C NMR Study. *Inorg. Chem.* **1998**, *37*, 6153–6160. [CrossRef]
- 122. Angus-Dunne, S.J.; Batchelor, R.J.; Tracey, A.S.; Einstein, F.W.B. The Crystal and Solution Structures of the Major Products of the Reaction of Vanadate with Adenosine. *J. Am. Chem. Soc.* **1995**, *117*, 5292–5296. [CrossRef]
- 123. McNeill, J.H.; Yuen, V.G.; Dai, S.; Orvig, C. Increased potency of vanadium using organic ligands. *Mol. Cell. Biochem.* **1995**, 153, 175–180. [CrossRef]
- 124. Thompson, K.H.; Liboiron, B.D.; Sun, Y.; Bellman, K.D.D.; Setyawati, I.A.; Patrick, B.O.; Karunaratne, V.; Rawji, G.; Wheeler, J.; Sutton, K.; et al. Preparation and characterization of vanadyl complexes with bidentate maltol type ligands; in vivo comparisons of anti-diabetic therapeutic potential. *J. Biol. Inorg. Chem.* 2003, *8*, 66–74. [CrossRef]
- 125. Sakurai, H.; Yoshikawa, Y.; Yasui, H. Current state for the development of metallopharmaceutics and anti-diabetic metal complexes. *Chem. Soc. Rev.* 2008, *37*, 2383–2392. [CrossRef]
- 126. Sakurai, H.; Fugono, J.; Yasui, H. Pharmacokinetic Study and Trial for Preparation of Enteric-Coated Capsule Containing Insulinomimetic Vanadyl Compounds: Implications for Clinical Use. *Mini-Rev. Med. Chem.* **2004**, *4*, 41–48. [CrossRef]
- 127. Rehder, D. Perspectives for vanadium in health issues. Future Med. Chem. 2016, 8, 325–338. [CrossRef]
- Selvaraj, S.; Krishnan, U.M. Vanadium-Flavonoid Complexes: A Promising Class of Molecules for Therapeutic Applications. J. Med. Chem. 2021, 64, 12435–12452. [CrossRef]
- 129. Sharfalddin, A.A.; Al-Younis, I.M.; Mohammed, H.A.; Dhahri, M.; Mouffouk, F.; Ali, H.A.; Anwar, M.J.; Qureshi, K.A.; Hussien, M.A.; Alghrably, M.; et al. Therapeutic Properties of Vanadium Complexes. *Inorganics* 2022, 10, 244. [CrossRef]
- Jakusch, T.; Costa Pessoa, J.; Kiss, T. The Speciation of Vanadium in Human Serum. Coord. Chem. Rev. 2011, 255, 2218–2226.
 [CrossRef]
- 131. Costa Pessoa, J.; Tomaz, I. Transport of Therapeutic Vanadium and Ruthenium Complexes by Blood Plasma Components. *Curr. Med. Chem.* **2010**, *17*, 3701–3738. [CrossRef] [PubMed]
- Ferraro, G.; Demitri, N.; Vitale, L.; Sciortino, G.; Sanna, D.; Ugone, V.; Garribba, E.; Merlino, A. Spectroscopic/Computational Characterization and the X-ray Structure of the Adduct of the V^{IV}O–Picolinato Complex with Rnase A. *Inorg. Chem.* 2021, 60, 19098–19109. [CrossRef]
- Ferraro, G.; Paolillo, M.; Sciortino, G.; Pisanu, F.; Garribba, E.; Merlino, A. Implications of Protein Interaction in the Speciation of Potential V^{IV}O–Pyridinone Drugs. *Inorg. Chem.* 2023, 62, 8407–8417. [CrossRef]
- 134. Ferraro, G.; Vitale, L.; Sciortino, G.; Pisanu, F.; Garribba, E.; Merlino, A. Interaction of V^{IV}O-8-hydroxyquinoline species with RNase A: The effect of metal ligands in the protein adduct stabilization. *Inorg. Chem. Front.* **2023**, *10*, 5186–5198. [CrossRef]
- 135. Ragland, S.A.; Criss, A.K. From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS Pathog.* **2017**, *13*, e1006512. [CrossRef]
- Razavet, M.; Artero, V.; Cavazza, C.; Oudart, Y.; Lebrun, C.; Fontecilla-Camps, J.C.; Fontecave, M. Tricarbonylmanganese(I)– lysozyme complex: A structurally characterized organometallic protein. *Chem. Commun.* 2007, 27, 2805–2807. [CrossRef]
- 137. Casini, A.; Mastrobuoni, G.; Temperini, C.; Gabbiani, C.; Francese, S.; Moneti, G.; Supuran, C.T.; Scozzafava, A.; Messori, L. ESI mass spectrometry and X-ray diffraction studies of adducts between anticancer platinum drugs and hen egg white lysozyme. *Chem. Commun.* 2007, *2*, 156–158. [CrossRef]
- 138. Binkley, S.L.; Ziegler, C.J.; Herrick, R.S.; Rowlett, R.S. Specific derivatization of lysozyme in aqueous solution with Re(CO)₃(H₂O)³⁺. *Chem. Commun.* **2010**, *46*, 1203–1205. [CrossRef]
- 139. Santos, M.F.A.; Seixas, J.D.; Coelho, A.C.; Mukhopadhyay, A.; Reis, P.M.; Romão, M.J.; Romão, C.C.; Santos-Silva, T. New insights into the chemistry of fac-[Ru(CO)₃]²⁺ fragments in biologically relevant conditions: The CO releasing activity of [Ru(CO)₃Cl₂(1,3-thiazole)], and the X-ray crystal structure of its adduct with lysozyme. *J. Inorg. Biochem.* 2012, 117, 285–291. [CrossRef] [PubMed]
- 140. Seixas, J.D.; Santos, M.F.A.; Mukhopadhyay, A.; Coelho, A.C.; Reis, P.M.; Veiros, L.F.; Marques, A.R.; Penacho, N.; Gonçalves, A.M.L.; Romão, M.J.; et al. A contribution to the rational design of Ru(CO)₃Cl₂L complexes for in vivo delivery of CO. *Dalton Trans.* 2015, 44, 5058–5075. [CrossRef] [PubMed]
- Ferraro, G.; Giorgio, A.; Mansour, A.M.; Merlino, A. Protein-mediated disproportionation of Au(i): Insights from the structures of adducts of Au(iii) compounds bearing N,N-pyridylbenzimidazole derivatives with lysozyme. *Dalton Trans.* 2019, 48, 14027–14035. [CrossRef]
- 142. Gambino, D. Potentiality of vanadium compounds as anti-parasitic agents. Coord. Chem. Rev. 2011, 255, 2193–2203. [CrossRef]
- Benitez, J.; Becco, L.; Correia, I.; Leal, S.M.; Guiset, H.; Costa Pessoa, J.; Lorenzo, J.; Tanco, S.; Escobar, P.; Moreno, V.; et al. Vanadium polypyridyl compounds as potential antiparasitic and antitumoral agents: New achievements. *J. Inorg. Biochem.* 2011, 105, 303–312. [CrossRef]

- 144. Thompson, K.H.; Orvig, C. Vanadium in diabetes: 100 years from Phase 0 to Phase I. J. Inorg. Biochem. 2006, 100, 1925–1935. [CrossRef]
- 145. Costa Pessoa, J.; Marques, R.L.; Vilas Boas, L.F.; Gillard, R.D. Oxovanadium(IV) and Aminoacids—III. The system L-Aspartic Acid + VO²⁺; A Potentiometric and Spectroscopic Study. *Polyhedron* 1990, *9*, 81–99. [CrossRef]
- 146. Thompson, K.H.; Lichter, J.; LeBel, C.; Scaife, M.C.; McNeill, J.H.; Orvig, C. Vanadium treatment of type 2 diabetes: A view to the future. *J. Inorg. Biochem.* 2009, 103, 554–558. [CrossRef]
- 147. Liboiron, B.D.; Thompson, K.H.; Hanson, G.R.; Lam, E.; Aebischer, N.; Orvig, C. New insights into the Interactions of Serum Proteins with Bis(maltolato)oxovanadium(IV): Transport and Biotransformation of Insulin-Enhancing Vanadium Pharmaceuticals. J. Am. Chem. Soc. 2005, 127, 5104–5115. [CrossRef] [PubMed]
- 148. Rangel, M.; Tamura, A.; Fukushima, C.; Sakurai, H. In vitro study of the insulin-like action of vanadyl-pyrone and -pyridinone complexes with a VO(O₄) coordination mode. *J. Biol. Inorg. Chem.* **2001**, *6*, 128–132. [CrossRef]
- 149. Raines, R.T. Ribonuclease A. Chem. Rev. 1998, 98, 1045–1065. [CrossRef]
- 150. Marshall, G.R.; Feng, J.A.; Kuster, D.J. Back to the Future: Ribonuclease A. Pept. Sci. 2008, 90, 259–277. [CrossRef]
- 151. Cuchillo, C.M.; Nogués, M.V.; Raines, R.T. Bovine Pancreatic Ribonuclease: 50 Years of the First Enzymatic Reaction Mechanism. *Biochemistry* 2011, 50, 7835–7841. [CrossRef]
- 152. Merlino, A. Recent advances in protein metalation: Structural studies. Chem. Commun. 2021, 57, 1295–1307. [CrossRef] [PubMed]
- Messori, L.; Merlino, A. Protein metalation by metal-based drugs: X-ray crystallography and mass spectrometry studies. *Chem. Commun.* 2017, 53, 11622–11633. [CrossRef]
- Kowacz, M.; Mukhopadhyay, A.; Carvalho, A.L.; Esperança, J.M.S.S.; Romão, M.J.; Rebelo, L.P.N. Hofmeister effects of ionic liquids in protein crystallization: Direct and water-mediated interactions. *CrystEngComm* 2012, 14, 4912–4921. [CrossRef]
- 155. Costa Pessoa, J.; Antunes, J.L.; Vilas Boas, L.F.; Gillard, R.D. Oxovanadium(IV) and aminoacids—V. The System L-Glutamic Acid + VO²⁺; A Potentiometric and Spectroscopic Study. *Polyhedron* **1992**, *11*, 1449–1461. [CrossRef]
- 156. Page, M.J.; Di Cera, E. Serine peptidases: Classification, structure and function. Cell. Mol. Life Sci. 2008, 65, 1220–1236. [CrossRef]
- 157. Di Cera, E. Serine Proteases. IUBMB Life 2009, 61, 510–515. [CrossRef]
- 158. Turk, B.; Turk, D.; Turk, V. Protease signaling: The cutting edge. EMBO J. 2012, 31, 1630–1643. [CrossRef]
- 159. Bond, J.S. Proteases: History, discovery, and roles in health and disease. J. Biol. Chem. 2019, 294, 1643–1651. [CrossRef]
- 160. Moulin, A.; Bell, J.H.; Pratt, R.F.; Ringe, D. Inhibition of Chymotrypsin by a Complex of Ortho-Vanadate and Benzohydroxamic Acid: Structure of the Inert Complex and Its Mechanistic Interpretation. *Biochemistry* **2007**, *46*, 5982–5990. [CrossRef] [PubMed]
- Costa Pessoa, J.; Vilas Boas, L.F.; Gillard, R.D. Oxovanadium(IV) and Aminoacids—II. The systems L-Serine and L-Threonine+VO²⁺. A Potentiometric and Spectroscopic Study. *Polyhedron* 1989, *8*, 1173–1199. [CrossRef]
- 162. Chiu, W.; Schmid, M.F.; Pintilie, G.D.; Lawson, C.L. Evolution of standardization and dissemination of cryo-EM structures and data jointly by the community, PDB, and EMDB. *J. Biol. Chem.* **2021**, *296*, 100560. [CrossRef] [PubMed]
- Guaita, M.; Watters, S.C.; Loerch, S. Recent advances and current trends in cryo-electron Microscopy. *Curr. Opin. Struct. Biol.* 2022, 77, 102484. [CrossRef]
- 164. Chua, E.Y.D.; Mendez, J.H.; Rapp, M.; Ilca, S.L.; Tan, Y.Z.; Maruthi, K.; Kuang, H.; Zimanyi, C.M.; Chen, A.; Eng, E.T.; et al. Better, faster, cheaper: Recent advances in cryo–electron microscopy. *Annu. Rev. Biochem.* **2022**, *91*, 1–32. [CrossRef]
- 165. Lees, J.A.; Dias, J.M.; Han, S. Applications of Cryo-EM in small molecule and biologics drug design. *Biochem. Soc. Trans.* 2021, 49, 2627–2638. [CrossRef] [PubMed]
- 166. Zhu, K.-F.; Yuan, C.; Du, Y.-M.; Sun, K.-L.; Zhang, X.-K.; Vogel, H.; Jia, X.-D.; Gao, Y.-Z.; Zhang, Q.-F.; Wang, D.-P.; et al. Applications and prospects of cryo-EM in drug discovery. *Mil. Med. Res.* **2023**, *10*, 10. [CrossRef]
- 167. Fan, C.; Kaiser, J.T.; Rees, D.C. A structural framework for unidirectional transport by a bacterial ABC exporter. *Proc. Natl. Acad. Sci. USA* 2020, 117, 19228–19236. [CrossRef] [PubMed]
- Kehlenbeck, D.M.; Traore, D.A.K.; Josts, I.; Sander, S.; Moulin, M.; Haertlein, M.; Prevost, S.; Forsyth, V.T.; Tidow, H. Cryo-EM structure of MsbA in saposin-lipid nanoparticles (Salipro) provides insights into nucleotide coordination. *FEBS J.* 2022, 289, 2959–2970. [CrossRef]
- Zhang, Y.; Fan, Q.; Chi, X.; Zhou, Q.; Li, Y. Cryo-EM structures of Acinetobacter baumannii glycerophospholipid transporter. *Cell Discov.* 2020, *6*, 86. [CrossRef]
- 170. Cui, Z.; Li, X.; Shin, J.; Gamper, H.; Hou, Y.M.; Sacchettini, J.C.; Zhang, J. Interplay between an ATP-binding cassette F protein and the ribosome from *Mycobacterium tuberculosis*. *Nat. Commun.* **2022**, *13*, 432. [CrossRef]
- 171. Fan, C.; Rees, D.C. Glutathione binding to the plant AtAtm3 transporter and implications for the conformational coupling of ABC transporters. *eLife* **2022**, *11*, e76140. [CrossRef]
- 172. Borsellini, A.; Kunetsky, V.; Friedhoff, P.; Lamers, M.H. Cryogenic electron microscopy structures reveal how ATP and DNA binding in MutS coordinates sequential steps of DNA mismatch repair. *Nat. Struct. Mol. Biol.* 2022, 29, 59–66. [CrossRef] [PubMed]
- Sedzicki, J.; Ni, D.; Lehmann, F.; Wu, N.; Zenobi, R.; Jung, S.; Stahlberg, H.; Dehio, C. Mechanism of cyclic beta-glucan export by ABC transporter Cgt of Brucella. *Nat. Struct. Mol. Biol.* 2022, 29, 1170–1177. [CrossRef] [PubMed]
- 174. Silberberg, J.M.; Stock, C.; Hielkema, L.; Corey, R.A.; Rheinberger, J.; Wunnicke, D.; Dubach, V.R.A.; Stansfeld, P.J.; Hänelt, I.; Paulino, C. Inhibited KdpFABC transitions into an E1 off-cycle state. *eLife* **2022**, *11*, e80988. [CrossRef]

- 175. Winkler, P.A.; Huang, Y.; Sun, W.; Du, J.; Lü, W. Electron cryo-microscopy structure of a human TRPM4 channel. *Nature* **2017**, *552*, 200–204. [CrossRef] [PubMed]
- 176. Li, Y.; Orlando, B.J.; Liao, M. Structural basis of lipopolysaccharide extraction by the LptB₂FGC complex. *Nature* **2019**, *567*, 486–490. [CrossRef] [PubMed]
- 177. Hofmann, S.; Januliene, D.; Mehdipour, A.R.; Thomas, C.; Stefan, E.; Brüchert, S.; Kuhn, B.T.; Geertsma, E.R.; Hummer, G.; Tampé, R.; et al. Conformation space of a heterodimeric ABC exporter under turnover conditions. *Nature* 2019, 580, 580–583. [CrossRef] [PubMed]
- 178. Sharma, S.; Zhou, R.; Wan, L.; Feng, S.; Song, K.; Xu, C.; Li, Y.; Liao, M. Mechanism of LolCDE as a molecular extruder of bacterial triacylated lipoproteins. *Nat. Commun.* **2021**, *12*, 4687. [CrossRef]
- 179. Harris, A.; Wagner, M.; Du, D.; Raschka, S.; Nentwig, L.-M.; Gohlke, H.; Smits, S.H.J.; Luisi, B.F.; Schmitt, L. Structure and efflux mechanism of the yeast pleiotropic drug resistance transporter Pdr5. *Nat. Commun.* **2021**, *12*, 5254. [CrossRef]
- 180. Lyu, J.; Liu, C.; Zhang, T.; Schrecke, S.; Elam, N.P.; Packianathan, C.; Hochberg, G.K.A.; Russell, D.; Zhao, M.; Laganowsky, A. Structural basis for lipid and copper regulation of the ABC transporter MsbA. *Nat. Commun.* **2022**, *13*, 7291. [CrossRef]
- 181. Zhou, C.; Shi, H.; Zhang, M.; Zhou, L.; Xiao, L.; Feng, S.; Im, W.; Zhou, M.; Zhang, X.; Huang, Y. Structural Insight into Phospholipid Transport by the MlaFEBD Complex from P. aeruginosa. *J. Mol. Biol.* **2021**, *433*, 166986. [CrossRef]
- 182. Aureliano, M.; André Ohlin, C. Decavanadate in vitro and in vivo effects: Facts and opinions. J. Inorg. Biochem. 2014, 137, 123–130. [CrossRef] [PubMed]
- 183. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly accurate protein structure prediction with AlphaFold. *Nature* **2021**, *596*, 583–589. [CrossRef] [PubMed]
- 184. Samart, N.; Althumairy, D.; Zhang, D.; Roess, D.A.; Crans, D.C. Initiation of a novel mode of membrane signaling: Vanadium facilitated signal transduction. *Coord. Chem. Rev.* 2020, *416*, 213286. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.