Multilayered Modelling of the Metallation of Biological Targets

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Abstract. The unique property of metals – the remarkable ability to modulate the electronic structure of both metal center and bound ligands – is the reason for their omnipresence in enzymes and in metal-coordinating biological factors. Modern metallodrug chemistry began with the serendipitous unveiling of the antitumour properties of cisplatin, followed by an avalanche of synthesized novel metallodrugs. The metallation of biological targets has then become a new paradigm in the field of bioinorganic chemistry, and a plethora of computational approaches have been developed and utilized to ease the detailed comprehension of its mechnisms with a focus on medical applications. Studies of the electronic structure of metallodrugs and of the coordination of metal elements with biomolecular ligands, as well as an accurate description of both thermodynamics and kinetics of reactions with biomolecules, are crucial for development of novel metallodrugs with improved therapeutic profiles. Here, we provide an account of the application of multilayered computational schemes developed in our group for the study of processes leading and/or culminating with the metallation of biomolecules, the key step in the mechanism of action of metallodrugs.

1 Introduction

The essential role of metals in biological systems demonstrates the exceptional adaptability of coordinative bond, which causes reciprocal adjustment in the electronic structure of both the metal center and its ligands, thus allowing enzymes and/or metal-coordinating biological factors to be players in a very fine chemistry. The same reasons motivate the interest for metal-based drugs or metallodrugs in which the peculiar interaction between the metal and the biological targets may lead and modulate the therapeutical benefit.

Since the serendipitous finding [1] of cisplatin with its still wide employment in the anticancer therapy [2], a growing interest has been focused on the identification of novel metallodrugs, leading to a vast body of literature data. One of the most appealing aspects of cisplatin pharmacodynamics is represented by its capability of binding at DNA nucleobases: the Pt(II) center of cisplatin is mostly coordinated at two consecutive

© Springer Nature Switzerland AG 2021
O. Gervasi et al. (Eds.): ICCSA 2021, LNCS 12958, pp. 398–412, 2021.
https://doi.org/10.1007/978-3-030-87016-4_30

intrastrand GG or AG nucleobases [3] in the major groove of the DNA duplex, and causing a specific structural aberration capable of inducing apoptosis [4].

The metallation of biological targets has thus become a new paradigm in the domain of bioinorganic chemistry, and many endeavours have been devoted to achieve a comprehensive understanding of how this process could be therapeutically exploited [5].

The match between the multifacet chemistry disclosed by metal compounds with the extreme complexity of biological systems makes the comprehension of the metallation process a particularly challenging task even in the in vitro context. Again, the cisplatin example has taught to us that, despite metallation eventuates in the coordination of a metal center to a specific biological target, this process is the result of an intricate sequence of chemical events that are triggered when the metallodrug starts to interact with the components of the biological medium: the cisplatin aquation equilibrium taking place in the cytoplasm has been recognized as responsible of its activation [6].

The studies of electronic structure and metal coordination for metallodrugs and biomolecules, as well as an in-depth characterization of both thermodynamics and kinetics of the involved reactions, are of utmost importance at the initial stages of investigations and are followed by the quantum mechanical (QM) methods which target the particular bioinorganic systems.

Based on a consolidated expertise in the bioinorganic computational chemistry [7, 8], several computational strategies were designed and adopted by our research group to include a QM description of the structure and/or reactivity of metallodrugs, most of them implying the combination and/or hybridization of different level of theories.

In this report, we provide for an account of applications of multilayered computational approaches for the investigation of the processes leading and/or culminating with the metallation of biomolecules implicated in the mechanism of action of metallodrugs (Fig. 1). The word "multilayered" will be used herein with a twofold meaning, but with a unique address (Fig. 2). On one hand, a multilayered representation of the whole system, generally made of the metallodrug, the bulk, and the biological target, may support the extraction of reduced models: for example, the activation processes taking place before the contact between the metallodrug Pt-bispidine (vide infra) with its target – in the bulk layer – as well as the reaction of the aquo forms of Pt-bispidine with guanine sites – in the target layer – may be both investigated by means of QM methods (Fig. 2a). On the other hand, the term "multilayered" can also be used to indicate the treatment of different portion of the system with different level of theories, thus, referring to a "layering" of the introspection accuracy: for example, the use of QM/MM approaches allowing to investigate the reaction within a metallodrug with its binding site at QM level of theory, while describing the remaining portions of the system within an empirical force field (Fig. 2b).

In either cases the multilayered approach is used to treat the most chemically relevant portion of the system at high level of theory, thus allowing to study the thermodynamics and kinetics of the processes involved in the metallation of biological targets.

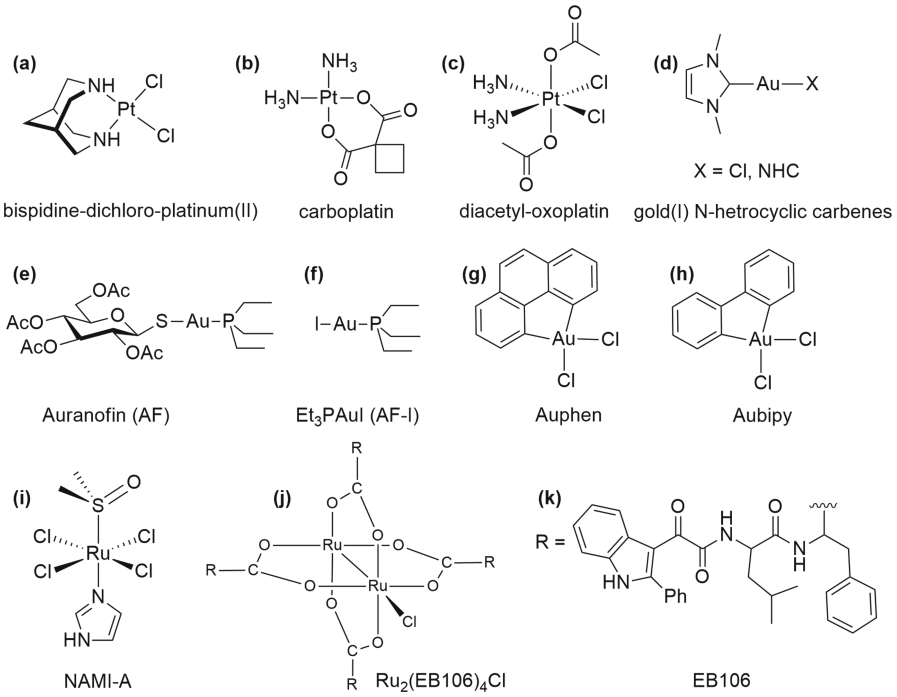

Fig. 1. The Pt, Au, and Ru-based metallodrugs described in the present report.

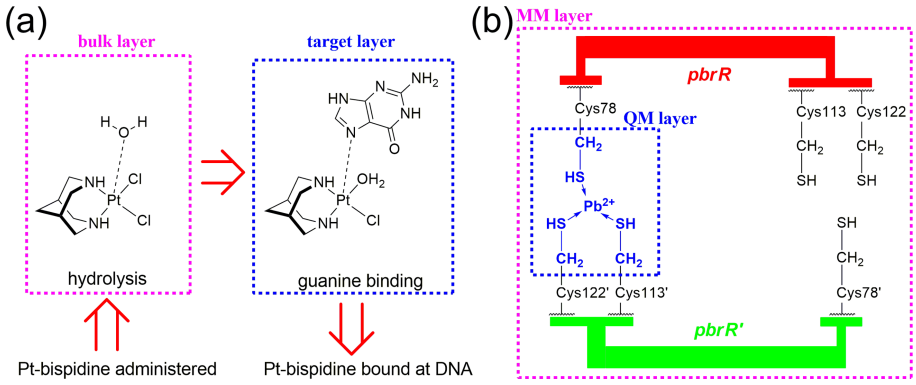

Fig. 2. Rendition of the twofold meaning of the word "multilayer". (a) The administered metallodrug Pt-bispidine undergoes to hydrolytic activation, a process occurring in the bulk layer. Afterwards, the aquo forms of Pt-bispidine react with guanine sites at DNA, a process occurring in the target layer. (b) The description of the coordinative bonds between a lead ion and the cysteine triad formed by a pbrR dimer requires a high-level of theory, hence the Pb^{2+} ion and the side chains of Cys78, Cys113', and Cys122' are included in the QM layer. On the other hand, the lasting protein environment, exerting an electrostatic and steric influence on the lead binding, can be described by an empirical force field, hence included in the MM layer.

2 Pt(II) and Pt(IV) Metallodrugs

A body of evidence has definitely ascertained the crucial role played by aquation in the mode of action of cisplatin [9, 10], and helped the elucidation of the structure-activity relationships affecting the cisplatin-like Pt(II) anticancer complexes and the possibility of its targeted release through different supports [11, 12]. In recent studies, it was found that cisplatin and its iodide analogue cis-[$PtI_2(NH_3)_2$] display remarkable dissimilarities in their reactivity with the model proteins cytochrome c and lysozyme [13–15]. In order to unveil the molecular rationale for such comportment, a computational DFT approach was utilized for the investigation of the reactions of cis-[$PtCl_2(NH_3)_2$] and cis-[$PtI_2(NH_3)_2$] with molecular models (Ls) of the aminoacid sidechains – cysteine, methionine, histidine [14], a procedure which was found to give relevant insights on the reaction mechanism of cisplatin even in a solvent-free environment [16, 17]. It was concluded that the reaction of such Pt(II) complexes with sulfur-containing amino acids leads to a prompt loss of their halide ligands, which is followed by the loss of ammonia by the resultant cis-[$Pt(L)_2(NH_3)_2$] complexes in reaction with a ligand excess. On the other hand, the reaction of cis-[$PtCl_2(NH_3)_2$] and cis-[$PtI_2(NH_3)_2$] with imidazole results into the loss of their halide ligands. Nevertheless, the formed cis-[$PtL_2(NH_3)_2$] complex does not release ammonia in reaction with imidazole in excess. The differences in reactivity of these two platinum complexes with small models of amino acid side chains correspond well to their behavior observed in reactions with model proteins. This allowed to conclude that the role played by protein itself is crucial in activating the anomalous reactivity of the cis-[$PtI_2(NH_3)_2$] and in determining the formed protein adducts.

Bispidine is an anticancer drug with propitious qualities, such as minor side effects, ability to overcome inherent or acquired resistance, improved DNA targeting, and a simplified drug administration [18] (Fig. 1a). In order to shed light upon its reactivity and mode of action, we carried out a DFT study [19] focusing on the analysis of the first and second aquation steps as well as of the ensuing reactions with guanine, the primary objective of the platinum-based antitumor compounds. Consistently with the experimental data, calculations indicate that the bispidine is substantially less reactive with water molecules compared to cisplatin, its kinetic constant being fivefold smaller than the corresponding value for cisplatin [20]. Nevertheless, it was shown that the aquated complexes demonstrate an exceptional reactivity with guanine with the activation free energies proximate to those for the analogous aquated complexes of cisplatin, 20.8 vs 20.4 kcal mol^{-1} and 21.3 vs 20.9 kcal mol^{-1}, for the binding of the mono- and diaqua complex of the bispidine, respectively [19] (Fig. 2a).

Nowadays, carboplatin is the anticancer drug that is used ubiquitously, since it causes less side effects than cisplatin [21] (Fig. 1b). The scaffold of carbolatin resembles much cisplatin with the two attached stable cis ammino ligands, however, carbolatin possesses a dicarboxylate chelate ring instead of two labile chloro ligands of cisplatin. Both metallodrugs operate in the analogous way, indeed, carboplatin was found to form the $Pt(NH_3)_2{}^{2+}$-DNA adducts in vivo [22]. However, the bidentate malonate ring is less labile than chloride ions, causing the lesser reactivity of carboplatin than cisplatin. For the purpose of studying the mode of action of carboplatin, we carried out an ab initio study on the mechanism of carboplatin activation by chloride ions in different pH conditions [23], demonstrating that in neutral conditions, or in weak acidic conditions

characteristic for the hypoxic tumor milieu, the process is unlikely to take place at physiological temperature, an indication that is not involved in the mechanism of carboplatin activation. Stronger nucleophiles than chloride are needed to activate carboplatin.

Carbonate, an omnipresent species in biological systems, existing in considerable concentration (24 mM) in blood plasma, was thus considered to play a possible role in carboplatin activation mechanism. A computational study was carried out with a focus on the initial steps of the process, consisting of the detachment of one arm of the malonato chelating ring of carboplatin accompanied by the decarboxylation, yielding cis-$[Pt(CBDCA\text{-}O)(OH)(NH_3)_2]^-$ [24]. We considered both carbonate and bicarbonate ions as attacking species, since they are both present in the carbonate buffer at physiological pH. The ring opening by the carbonate ion has the reaction free energy and activation enthalpy values lower (by ca. 2.4–4.8 kcal mol^{-1} at various levels of theory) than the corresponding reaction with bicarbonate, thus proving that the carboplatin activation should be ascribed to the carbonate ion, consistently with existing experimental data [25]. As in the case of chloride ions, the effect of the environment acidity was taken into consideration and indicated that carboplatin activation occurs via the protonation of the carbonate to a bicarbonate complex, which is subsequently decarboxylated to the ultimate hydroxo complex. The rate-determining step of the entire process is the opening of the ring, immediately followed by the protonation to the corresponding bicarbonate complex. After a facile decarboxylation by means of a mechanism of bicarbonate rotation, a hydroxo-coordinated bicarbonate intermediate is produced, which promptly undergoes the final CO_2 dissociation (activation enthalpy and free energy below 4.8 kcal mol^{-1}). The whole process, is exoergonic with a rather low free energy of activation (less than 28.7 kcal mol^{-1}) suggesting that the reaction with carbonate might be a feasible and plausible pathway for the activation of carboplatin in the biological milieu [24].

An auspicious approach to circumvent one of the main disadvantages of cisplatin, i.e. its severe side effects [26], is the use of six-coordinate Pt(IV) complexes which can act as prodrugs after their permeation into the target cell, where they are reduced by glutathione or ascorbate, to the active four-coordinate square planar Pt(II) analogues which, in turn, form the adduct with the final DNA target [27]. The main side effects of Pt(II) complexes, early hydrolysis and subsequent binding to proteins of plasma, are impeded due to substantial inertness of low spin d^6 Pt(IV) complexes, a feature which might even enable an oral administration of the drug. The Pt(IV) to Pt(II) reduction occurs simultaneously to the detachment of two axial ligands and, being the key step for the drug activation, was thoroughly studied by electrochemical and chemical approaches [28]. Cyclic voltammetry was comprehensively utilized to investigate the reduction–oxidation process in Pt(IV) antitumor complexes, and only a single irreversible reduction event was demonstrated for this irreversible reduction instead of the standard redox potential for the Pt(IV)/Pt(II) couple. An insight on the two-electron reduction mechanism of the $[Pt^{IV}(NH_3)_2(CH_3COO)_2Cl_2]$ complex was recently obtained from a theoretical DFT study [29] (Fig. 1c). It disentangled the stepwise mechanism consisting of the initial one-electron reduction of the $[Pt^{IV}(NH_3)_2(CH_3COO)_2Cl_2]$ complex, producing the hexacoordinated platinum(III) $[Pt^{III}(NH_3)_2(CH_3COO)_2Cl_2]^-$ intermediate and the detached acetate, with an activation free energy of 6.46 kcal mol^{-1}. On the contrary, the formed pentacoordinated $[Pt^{III}(NH_3)_2(CH_3COO)Cl_2]$ species is reduced by the second electron

by means of a barrierless concerted process to the eventual $[Pt^{II}(NH_3)_2(CH_3COO)_2]$ derivative.

3 Au(I) and Au(III) Metallodrugs

Gold(I) N–heterocyclic carbenes (NHC) [Au(I)(NHC)R], in which R can be either an anionic or a neutral ligand, e.g. Cl^-, PH_3, or NHC, are ubiquitously used in medicinal chemistry [30] as anti-arthritic, antibacterial, and anticancer agents [31, 32] (Fig. 1d). Nevertheless, comprehensive understanding of the reaction mechanism of these gold carbene compounds with amino acids and fragments of proteins linger predominantly unrevealed. In a recent investigation [33], the reactivity of [Au(I)(NHC)Cl] with various nucleophiles was evaluated, including water, N-terminal of any amino acid, as well as the side chains of cysteine, selenocysteine, methionine, histidine, arginine, lysine. The computed results demonstrate that the substitution of chloride by the examined residues is slightly exergonic. It was found that the hydration of the [Au(I)(NHC)Cl] complex happens relatively fast with the free energy of activation of 23.0 kcal mol^{-1}. Nevertheless such barrier is considerably higher than the barriers for the amino acid side chains, implying that [Au(I)(NHC)Cl] complexes interact with their biomolecular targets before being hydrolyzed. The smallest barriers were computed for cysteine and selenocysteine, thus substantiating the predominant binding of $[Au(I)(NHC)]^+$ to the cysteine and selenocysteine residues of thioredoxine in agreement with experimental data [34]. Low barriers were also computed for the nitrogen-based nucleophile sites: arginine, lysine, and N-terminal amine (16–19 kcal mol^{-1}). This indicates that there is only a weak differentiation in reactivity between S/Se and N-based nucleophile sites. As a consequence, when no cystein and selenocysteine residues are available or when they are buried in the protein, the $[Au(I)(NHC)]^+$ moiety could also bind to N-based sites, suggesting that accessibility on the protein surface might play a relevant role on the binding preference of such compounds.

Recently, it was experimentally shown that the biscarbene Au(I)-NHC complexes $[(Me_2Im)_2Au]^+$ activate the cell apoptosis mechanism by thioredoxin reductase (TrxR) inhibition. During this process they consecutively release two NHC ligands in a two-step reaction, forming a $[Au(Cys)_2]$ complex [30]. A DFT study focused on characterization of the first steps of the mechanism of action of these anticancer complexes was carried out, addressing the substitution of carbene ligand by capped cysteine and selenocysteine which modeled the side chains of respective protein residues [35]. Numerous reaction mechanisms were investigated, including those involving the protonation of the detached carbene, as well as both neutral and deprotonated forms of cysteine and selenocysteine, i.e. the examined nucleophiles in the reaction of ligand exchange on the metal center. Results indicate that the reaction can only happen with anionic thiolate or selenothiolate, together with a concomitant transfer of proton to the detached carbene from the acidic constituent of the buffer. The availability of a buffer is indeed necessary in experiments in physiological conditions, so that experimental and theoretical results converge and indicate the importance of the biological environment for such reactions to take place. The substitution of carbene is a marginally exergonic process with fairly low barriers of 21.3 kcal mol^{-1} and 19.6 kcal mol^{-1}, respectively, for capped cysteine and selenocysteine, consistently with the available kinetic experimental results for free amino acids [36].

Auranofin (AF) is the gold-based metallodrug for the therapy of rheumatoid arthritis [37]; and it is being currently reevaluated as antibacterial, antiviral, antifungal, and antiparasitic agent [38] (Fig. 1e). Its iodide analogue $Au(PEt_3)I$, in which iodide substitutes the thiosugar, is efficacious in vitro against ovarian and colorectal cancer due to its increased cytotoxicity [39] (Fig. 1f). Reactivity of AF and AF-I complexes with selected aminoacids and protein fragments were recently investigated in a joint experimental and theoretical study [40]. It was demonstrated that the aquation of AF and AF-I is thermodynamically reprobated, hence both complexes interact with the biomolecules, loosing either thiosugar or iodide, respectively. DFT calculations for the ligand exchange reactions of AF and AF-I with the models of side chains of Cys, Sec, Met, and His displayed the thermodynamical and kinetic unfavorability of substitution of either thiosugar or iodide. It was demonstrated that only anionic cysteine and selenocysteine interact with these Au-based metallodrugs, exhibiting the barriers for substitution of iodide as being substantially lower than the corresponding values for thiosugar [40].

The bipyridyl gold(III) complexes $[Au(phen)Cl_2]Cl$ phen = phenantroline and $[Au(bpy)Cl_2]PF_6$ bpy = bipyridine, named auphen and aubipy, respectively, have been extensively investigated due to their capability of selectively blocking the aquaglyceroporin AQP-3 [41], the latter channel protein being recognized to be involved in the tumorigenesis [42] (Fig. 1g). Aiming to attain a detailed understanding of the Aubipy binding and its effects on the performance of AQP3, the classical MD simulations were employed for the studies of the structures of non-covalent and covalent Aubipy–AQP3 adducts [43] (Fig. 1h). Substantial structural and functional changes in AQP3, caused by the attachment of the gold-containing fragment, were discovered through the simulation of unbound, non-covalently and covalently bound adducts. Peculiarly, the extracellular pores of both the non-covalently and covalently bound complexes experience changes in size and shape decreasing the flux of water molecules. This finding is confirmed by examination of the channel radius and water density along the transmembrane, and by evaluating the flux of water in the monomer of AQP3 bound to gold. Indeed, the water permeability is prevented by the binding of the gold complex causing a contraction of the pore at the extracellular boundary due to conformational rearrangement produced by the metal complex. These results are of an utmost value for the design of novel Au(III) complexes for the selective inhibition of AQP3, for instance, permitting the determination of aromatic ligands with asymmetric substitution arrangement in the two pyridil rings that might additionally stabilize the coordinative adduct. Moreover, the increase of binding of the Au(III) complex binding can be obtained by optimizing the hydrophobic and π-stacking interactions. Meticulous refinement of the interactions between the metallacycle and pore may augment the selectivity of the compound for inhibition of AQP3 juxtaposed to other isoforms of aquaglyceroporin [43].

The mechanism of inhibition of AQP3 by Au(III) complexes with phenanthroline (phen) ligands was clarified at the molecular level in a recent study via a combination of biophysical techniques and advanced computational methods [44]. A set of novel Au(III)-based complexes with phen ligands was synthesized, and their stability in water solution was studied together with their antitumoral properties in vitro. Their capability to inhibit the permeation of glycerol via AQP3 was studied by stopped-flow spectroscopy,

by which it was found that several complexes possess AQP3 inhibition properties surpassing the cytotoxicity of benchmark compounds Aubipy and Auphen [44]. It was shown that the dichloro form of the studied Auphen-like complexes is stable under physiological conditions whereas Aubipy is found mostly in the chloro hydroxo form [43]. This contrasting speciation principally influences the noncoordinative interaction with AQP3. Indeed, both Auphen and its 5-chloro derivative bind at the side of extracellular pore analogously to Aubipy, yet they reach dissimilar residues and obtain a different orientation. Auphen-like complexes protrude the metal atom toward the side chain of the gold binding site Cys40, at a reduced distance in comparison to Aubipy, thus indicating that these complexes are able to access their final coordinative site more efficiently. These conclusions agree with the better inhibition of glycerol permeation by Auphen than by Aubipy. Moreover, it was indicated that the coordinative adducts formed between Auphen and AQP3 have a structural correspondance with those formed by Aubipy, they produce conformational rearrangements of the protein, which ultimately result in pore closure and the inhibition of AQP3. These results form a crucial foundation for the future conception of gold-based inhibitors which attack selectively different AQP isoforms [44].

4 Ru(II) and Ru(III) Anticancer Drugs

Several Ru(II) and Ru(III) complexes with nitrogen or aromatic ligands were studied for their remarkable in vitro antitumor activity and their preferential localization in tumor tissues [45, 46]. Despite the body of data testifying the anticancer potential of ruthenium complexes, a full atomistic understanding of their mode of action remains elusive, thus, feeding the interest in the modelling of their binding at biological targets.

The thermodynamics of the binding of $[Ru(NH_3)_5]^{2+}$ and $[Ru(NH_3)_5]^{3+}$ at DNA nucleobases and peptides has been computationally studied to assess the affinity of Ru(II) and Ru(III) monofunctional scaffolds toward possible biological targets [47]. In particular, the computed enthalpies and free energies for the bond dissociation show that the Ru(II) complexes, in analogy with cisplatin, disclose a high affinity for the N7 of guanine, although either histidine or sulfur-containing residues, cysteine and methionine, are also favorite binding sites, though to a lesser extent. A different profile of binding affinity was computed for Ru(III) complexes characterized by their stronger coordination to the dimethyl-phosphate anion $[(MeO)_2PO_2]^-$, a model of the phosphodiester backbone of the DNA double helix. This outcome markedly differentiates Ru(III) from Ru(II), whose binding at the phosphate moiety has been estimated to be labile in solution, and highlights the preferential targeting of Ru(III) complexes for the DNA backbone or anionic protein groups. The weak antitumor activity of Ru(III) species, observed experimentally, seems to be corroborated by the computational data, and, in accordance, postulates the Ru(III) → Ru(II) reduction as the crucial activating step.

Bifunctional complexes such as $[Ru(NH_3)_4Cl_2]$, $[Ru(NH_3)_4(H_2O)_2]^{2+}$, [cis-[cis-$RuCl_2(azpy)_2$], and $RuCl_2(bpy)_2$] were evaluated in terms of their affinity toward DNA or protein sites. The presence of a chloro or aquo ligand was found to only marginally affect the enthalpy of dissociation and free energy in solution of the bond between Ru(II) and guanine, whereas the arylazopyridine or polypyridyl ligands induced only a slight diminishing of these affinity parameters.

Among the most promising ruthenium anticancer drugs [48], the New Anti-tumor Metastasis Inhibitor (NAMI-A) has been investigated in details to provide a complete mechanistic insight of its activation via aquation by using DFT methods [49] (Fig. 1i). In the study three water molecules were included explicitly to improve the Poisson-Boltzmann (PB) implicit solvation energies and better represent the water assistance to the chloro-aquo ligand exchange. The calculations show that the aquation of the considered Ru(III) octahedral complex passes via a 7-coordinated transition state. The values for the activation free energies of the first and second aquation steps of 24.8 and 30.5 kcal·mol^{-1}, correspond well with the experimental evidence indicating that the first aquation of this complex is kinetically controlled while the second aquation is much slower.

Recently, a DFT study was performed with the aim to disentangle the anomalous stability of a ruthenium complex [Ru_2(EB106)$_4$Cl] (Fig. 1j), where EB106 is the antitumor drug (2-phenylindol-3-yl)glyoxyl-L-leucine-L-phenylalanine), which was synthesized in order to conjoin the antitumoral effects of EB106 and the therapeutic capabilities of the ruthenium mixed valence (II, III) core, thus being designed for the transport of diverse cytotoxic agents [50] (Fig. 1k). Surprisingly, [Ru_2(EB106)$_4$Cl] displayed a complete absence of reactivity toward protein targets, being utterly non-cytotoxic. The DFT study and the analysis of the solvent-accessible and van der Waals surfaces allowed to conclude that for the paddle-wheel shaped complexes of ruthenium, the selection of ligand is pivotal and its structural features are to be taken into account over the plain availability of the carboxylic group crucial for the attachment to the Ru_2(II,III) core. Certainly, the multidrug delivery systems improve their therapeutic properties. However, the ligand structure may exert a pronounced influence on the entire chemical profile, damaging the activation of drug, i.e. the release of ligand and eventually the medicinal effects. This investigation indicates that the chemical properties of the ligand designed for the coordination to the di-rithenium core should be carefully evaluated when a prodrug is conceived. Ideally, the reactivity should not be disrupted by the usage of ligands with a proper steric hindrance. Moreover, it can be concluded that the increase of the ligands occupancy may cause a diminished or even absent reactivity. That is why computational methods are quintessential for the decision on the appropriateness of the selected ligand as they help in the prediction of the bimetallic center reactivity and provide the mechanistic comprehension of its mode of action, which is of utmost importance for considering its therapeutic application. Remarkably, the careful evaluation of this balance has significant benefits in optimization of drug transport to its biomolecular target and of the intended pharmaceutical effects. Additionally, it is possible to accurately modulate the stability of the designed complexes by coordinating them to the Ru_2 core, thus circumventing undesirable side-reactivity and ameliorating the medication [50].

5 Metal Cations Targeting Proteins: *golB*, *pbrR*

It is long known that Au(I) cations display antibacterial properties [51], by displacing essential metals, thus obtruding with the correct metabolism, obstructing the redox equilibrium and damaging the cell membrane, causing the death of cell [52]. This leads several bacterial species to evolve their protein systems controlling the concentration of

intracellular gold ion [53]. For example, Au-specific transcriptional regulators of *merR*-type developed by bacteria such as *Cupriavidus metallidurans* and *Salmonella enterica* and spot gold ions and regulate the production of specific resistance factors [54]. One of these proteins, *golB*, possesing an exceptional selectivity for Au(I) ions was studied recently, and the X-ray structures of its apo and Au(I)-bound forms were reported [55], whereas its exceptional affinity toward Au(I) cations was studied computationally [56]. Molecular dynamic simulations allowed the sampling of the conformations of apo protein, which are favorable for the sequestration of Au(I) into the Cys-XX-Cys motif. Subsequently, the assessment of the structural features determining the Au(I) chelation was obtained by the usage of QM/MM calculations on metal-bound structures of golB, and the most probable protonation state for gold binding motif was determined. Consistently with experimental data, it was concluded that the Au(I) affinity of *golB* is regulated by changes of protein conformations, which influence the Cys10–Cys13 distance, thus serving as a molecular switch between the states responsible for the Au(I) sequestration or release in reaction to exterior stimuli. Interestingly it was noted that the structure of protein around the metal binding motif accomodates for the protonation state of Au(I)-*golB* with one cysteine being neutral and the other one deprotonated, consequently augmenting the selectivity for binding of Au(I) over other metal cations [56].

Industrialization increases the contamination of the ecosphere by lead (Pb) which, accretes in the ecosystem and causes the poisoning of food chain [57]. The studies of various mechanisms allowing bacteria to preserving homeostasis in conditions of the lead excess [58] is of utmost importance for the conception of new approaches for the isolation of Pb and its ensuing removal from polluted milieu. A unique protein which grants the resistance toward lead was found in *Cupriavidus metallidurans*, and it is the sole known metalloprotein in nature with a specific selectivity toward Pb(II), which permits the binding of Pb(II) with a thousandfold affinity as compared to other heavy metals [59]. Recently, the X-ray structures of apo and Pb(II)-bound *pbrR*, a Pb-responsive protein responsible of the modulation of the *pbr* operon in bacteria [60] were reported [61], and the binding of the cation Pb(II) at pbrR was studied computationally [62] (Fig. 2b). It was shown that the tris-cysteine motif formed by two helices of two packed *pbrR* monomers yields the conformations liable for the Pb(II) sequestration. Moreover, the most probable protonation state for the lead binding motif was defined by QM/MM computations, and its structural properties responsible for Pb(II) complexation were determined. Additionally, it was demonstrated that the binding affinity for Pb(II) is augmented by the formation of the thiol–thiolate–thiolate protonation state in Pb(II)-*pbrR*, which is prompted by the protein structure around the metal binding motif. Consistently with experimental results, *pbrR* switches on the Pb(II) sequestration/release-prone states, by changing the protonation states of Cys78′ and Cys122 aa well as the distance between those residues in response to variation of pH of its environment [62]. Moreover, the coordination of Pb(II) in the metal-binding motif of *pbrR* was investigated by the usage of topological schemes based on cationic dummy atoms and the classical molecular dynamics simulations [63], underlining again the crucial role of a tri-cysteine motif for the emergence of the selectivity toward the Pb(II) cation.

Summary

The extraordinary capability of metals to coordinate with various biomolecular targets presents them as an inestimable supply of active molecules in the field of medicinal chemistry. Due to the availability of the metal center, their properties cannot be facilely imitated by organic molecules, thus resulting in metal-based complexes being a separate class of medicinally applicable compounds with respect to organic drugs. Therefore, the optimization of the strategies of the computational modelling of their interaction with biomolecules is of utmost importance, in order to have the possibility to fine-tune their therapeutic and medicinal properties.

In this short review, we discussed the strategies developed in our group for multilayered modelling of the metallation of biological targets, which, being usually the parts of proteins and DNA, are too computationally expensive to be studied with a single approach due to their sheer size of up to several thousands atoms. That is why the usage of multilayered approaches is essential. The most chemically relevant portion of the system is done at the highest level of theory, thus allowing to study the thermodynamics and kinetics of the processes involved in the metallation of biological targets. On the other hand, the effects of the surrounding areas are taken into account by the use of an empirical force field.

Even with the permanent development of computational resources, the separation of studied systems into the zones treated with an increased accuracy and the greater regions, the effect of which is taken into account with the employment of a less precise yet better scalable methodology, will continue to be the modus opernadi of choice in this field.

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