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Ru and Rh binding sites in the structure of human serum transferrin with Fe³⁺ bound at the C-lobe

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Ru and Rh binding sites in the structure of human serum transferrin with Fe³⁺ bound at the C-lobe were identified by X-ray crystallography. Several His side chains and one Met are involved in the recognition of the metal ions by the protein.

Introduction

Serum transferrin (hTF) is an abundant human plasma glycoprotein of about 80 kDa that binds Fe³⁺ and transports it throughout the human body, playing a critical role in regulating its biodistribution and homeostasis.¹ The protein is recognized by the cells through the TF receptor (TFR).²

hTF carries two lobes (N- and C-) of about 330 residues, that are connected by a short flexible peptide constituted by residues 331–339 and contain equivalent clefts with the Fe binding site.¹ Each lobe can be further divided into two subdomains: N1 (residues 1–92, 247–330), N2 (residues 93–246), C1 (residues 340–425, 573–679) and C2 (426–572). Upon Fe³⁺ binding, the subdomains undergo a conformational variation that reduces solvent accessibility of the Fe binding sites.³ Thus, the apo (iron-free) conformation is described as “open”, while the holo (Fe₂-hTF) form adopts a “closed” conformation. Beyond the apo- and holo-forms, hTF exists in the monoferric form with Fe³⁺ bound at the C-lobe only (Fe_C-hTF) or at the N-lobe only (Fe_N-hTF).⁴

Apart from iron, hTF can bind and transport other metal ions, including Cu(II), Al(III), Co(III), Cr(III), Ga(III), In(III), Ti(IV), Zn(II), Bi(III), Sm(III), Ru(II), Ru(III), Pu(IV), Cm(III), V(III), V(IV), V(V) and Th(IV).^{5–10}

Given this capability the protein could play a role in detoxification process.^{5,11} Furthermore, since TFR amount is significantly increased in cancer cells,¹² hTF has been proposed as a

potential metallodrug delivery system,^{13,14} although this possible role is still controversial.¹⁵ In this frame, it has been demonstrated that the apo- and Fe_C-hTF forms bind the anticancer agent cisplatin,^{16,17} that the apo-form binds the antiarthritic gold-based drug aurothiomalate¹⁸ and the anticancer agent KP1019,¹⁵ and that Fe_C-hTF binds a divanadate formed upon reaction of the antidiabetic drug [VO(acac)₂] (acac = acetylacetonate) with the protein.¹⁹ These metal-bound forms of hTF remain able to bind TFR, at least *in vitro*. Cisplatin bound form of apo-hTF selectively delivers the anticancer agent to cancer cells *in vitro* and *in vivo*.^{20–24} Similarly, it has been shown that hTF enhances the cytotoxicity of titanocene dichloride.²⁵ Gallium and indium bound forms of hTF inhibit the growth of malignant carcinoma cell lines at least 10 times more than their free salts.²⁶ hTF has been also used to deliver Gd-contrasting agents to the brain.^{27,28} On the other hand, it has been shown that if Ru(III) compounds bind hTF in the iron binding site, Ru uptake by cells is not mediated by hTF, while if it binds close to protein surface residues hTF can promote the transport of the metallodrug to cells, but this process is unlikely in the presence of serum albumin.¹⁵

Crystallographic studies carried out on Fe_C-hTF adducts formed with Os and Ru compounds revealed that the protein binds Ru³⁺ close to side chain of His14, the side chains of His14 and His289, and that of His578,²⁹ and Os³⁺ close to His14/His289, His273, His349/His350, Lys489-Lys490/Glu507, His578/Arg581.²⁹ Structural analyses of the apo-form and Fe_C-hTF adducts with cisplatin have identified platinum binding sites near Met499 and Met256.^{16,17} Gold ions, in contrast, interact with the side chains of multiple residues in apo-hTF, including His25, Asp63, His207, Tyr238, His249, His273, His289, His300, His473, His606, His642, and His858.¹⁸

For Cr(III), Ti(IV), Bi(III), and Yb(III), binding occurs primarily at the iron-binding site of the protein, near residues such as Tyr426, Tyr517, His585, and Asp392, or alternatively Tyr188, Tyr95, Asp63, and His249.³⁰ Notably, residues involved in iron recognition also coordinate V(IV) and V(V)-containing species.^{19,31}

In 2022, by using FT-IR and UV-vis absorption spectroscopy it has been shown that hTF strongly binds Rh³⁺ ions with a

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change in the microenvironment of aromatic residues.³² However, the exact Rh binding sites are unknown. Therefore, the aim of this work is to identify the specific Rh binding sites on human serum transferrin. To achieve this goal, we have solved X-ray structure of the Rh/Fe_C-hTF adduct formed upon reaction of the paddlewheel Rh(II) compound [Rh₂(CH₃COO)₄] with the protein. These results were compared with those obtained when Fe_C-hTF reacts with the analogous Ru(II/III) paddlewheel complex [Ru₂(CH₃COO)₄Cl] to the form the Ru/Fe_C-hTF adduct. Both these compounds and some of their analogues have been already studied in the interaction with aminoacids,³³ peptides,^{34,35} proteins^{36–44} and nucleic acids,^{45–47} due to their biological activity.^{6,48–50}

Results and discussion

Crystals of the Rh/Fe_C-hTF and Ru/Fe_C-hTF adducts were obtained by soaking Fe_C-hTF crystals, grown by hanging drop vapour diffusion method using 15% (w/v) PEG 3350, 16% (v/v) glycerol, 8 mM disodium malonate, and 150 mM Na-PIPES pH 6.5 as a reservoir, in a solution of the reservoir containing the dimetallic compound in <10% DMSO. The presence of DMSO increases the solubility of the compounds and reduces their ability to form oligomeric species.^{40,51} It has been verified using UV-visible absorption spectroscopy that under the investigated experimental conditions both compounds remain stable for days (data not shown). X-ray diffraction data on several crystals of each adduct were collected at 100 K on different European Synchrotrons (SOLEIL and ESRF, see SI for details and Table S1 for data collection statistics). As expected, the crystals of the adducts of Fe_C-hTF with Ru and Rh are isomorphous to those of Fe_C-hTF. Soaking with dirhodium tetraacetate significantly deteriorates the crystals, reducing their diffraction power. The best crystal of Rh/Fe_C-hTF diffracts X-ray at 2.37 Å resolution and was obtained by exposing Rh-free Fe_C-hTF crystals to a solution containing 1.9 mM dirhodium tetraacetate for 2 hours. On the contrary, crystals of Ru/Fe_C-hTF diffract X-ray at high resolution, up to 2.26 Å, even when treated with a reservoir solution containing 5 mM diruthenium tetraacetate for 72 h. The structures were solved by molecular replacement using the program Phaser MR⁵² and the coordinates of Fe_C-hTF structure recently obtained in our group¹⁹ as the search model. Final models for the structures of Rh/Fe_C-hTF and Ru/Fe_C-hTF refine *R*-factors of 0.182/0.186 (*R*_{free} = 0.244/0.234) using REFMAC5⁵³ (Table S2). Notably, the binding of metal-containing fragments to the protein does not significantly alter its overall structure (Fig. 1). Indeed, the C α root mean square deviation of the Rh/Fe_C-hTF adduct from the starting model and from the structure of Ru/Fe_C-hTF are 0.305 Å and 0.299 Å, respectively. Pairwise comparisons performed by calculating the rotation angle (χ) needed to superimpose N2 or C2 subdomains after the best fitting of N1 or C1 subdomains of Rh/Fe_C-hTF or Ru/Fe_C-hTF when compared to apo-hTF further confirm this result. Indeed, data reveal that N-lobes of both Rh/Fe_C-hTF and Ru/Fe_C-hTF structures are

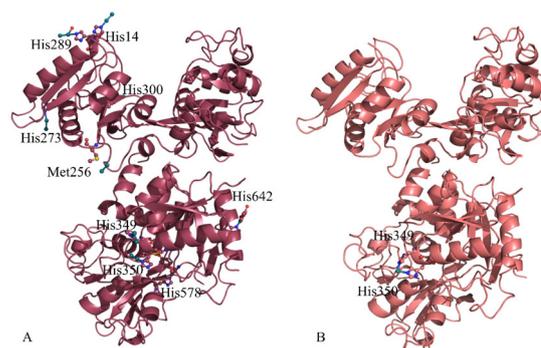


Fig. 1 Overall structures of Rh/Fe_C-hTF (panel A) and of Ru/Fe_C-hTF (panel B) adducts. Rh and Ru binding sites are shown in balls and sticks. Coordinates and structure factors of Rh/Fe_C-hTF and Ru/Fe_C-hTF adducts were deposited in the PDB under the accession codes 28MS and 28MR.

similar to that of apo-hTF (χ values to superimpose N2 subdomains after the best fitting of N1 subdomains between 2.22 and 2.95°) and thus adopt the “open” conformation, while the C-lobes appear “closed” (χ values to superimpose C2 subdomains after the best fitting of C1 subdomains are between 41.9 and 42.5°).

DiRh centers are found close to His14, Met256, His273, His289, His300, His349, His350, His578, and His642 (Fig. 2), while a single Ru atom was found close to the side chains of His349 and His350 (Fig. 3). Identification of Rh binding sites is based on the inspection of Fourier difference ($2F_o - F_c$ and $F_o - F_c$) and anomalous difference electron density (e.d.) maps (Fig. 2). The Ru binding site has been identified analyzing $2F_o - F_c$ and $F_o - F_c$ e.d. maps and inspecting isomorphous difference ($F_o - F_o$) e.d. maps (Fig. 3) calculated using the data of the reported structures and those of the Fe_C-hTF structure that we have previously refined (negative control with DMSO).¹⁹ The assignments are also further supported by refinements of duplicated structures at slightly lower resolution (data not shown).

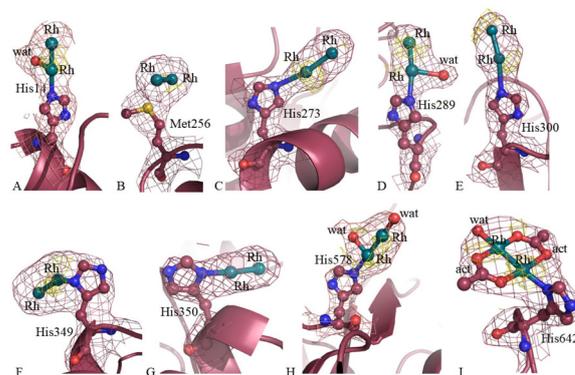


Fig. 2 Rh binding sites in the structure of the adduct that Fe_C-hTF forms with dirhodium tetraacetate. $2F_o - F_c$ electron density map is reported at 1.0σ (ruby). Anomalous difference map is reported in yellow at 2.0σ (yellow).



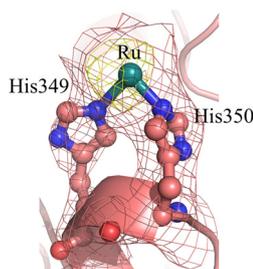


Fig. 3 Ru binding site in the structure of the adduct that Fe_C -hTF forms with diruthenium tetraacetate. $2F_o - F_c$ electron density map is reported at 1.0σ (ruby). Isomorphous difference ($F_o - F_c$) electron density map is reported at 4.0σ (yellow).

DiRh centers are bound to His side chains at the axial binding site, with the only exception of the dimetallic centre anchored to His349 (Fig. 2F). Binding to Met256 occurs at the equatorial site (Fig. 2B). Close to His642, two acetate ligands were modelled (Fig. 2I). At the other metal binding sites, metal ligands were not assigned, if one excludes equatorial water molecules bound to the Rh atoms coordinated to His14 (Fig. 2A), His289 (Fig. 2D) and His578 (Fig. 2H). This is common in the low-resolution structures of adducts of metalodrugs with proteins.^{54,55} His has been frequently found as a potential diRh binding site.³⁹ Met256 has been identified as Pt binding site in the Fe_C -hTF adduct with cisplatin.¹⁷

Binding of a monometallic Ru-containing fragment close to the side chains of His349 and His350 should be due to degradation of the diruthenium complex.

The binding of Ru^{3+} to His350 was hypothesized in the structures of the Ru/ Fe_C -hTF adducts that Fe_C -hTF forms upon reaction with the Ru compounds $\text{Ru}(\text{NTA})_2$ (NTA = nitrilotriacetate) and $\text{Ru}(\text{bpy})\text{OHCl}_2(\text{DMSO})_2$ (bpy = 2,2'-bipyridine) but not modelled in the final structures.²⁹

Occupancy values for Rh atoms are within the range 0.30–0.50, while the Ru atom in the structure of Ru/ Fe_C -hTF has occupancy = 0.70. On the contrary, Fe^{3+} ion has occupancy equal to 1.00. B-factors for the metal centers are high (69.92–88.35 \AA^2 in the case of Rh atoms and 122.47 \AA^2 in the case of Ru), but this is not surprising considering the resolution, partial occupancy and conformational disorder associated with the paddlewheel dimetallic compound fragment binding to protein atoms. Average value for the $\text{N}\cdots\text{Rh}$ distances is 2.44 ± 0.16 \AA , while the observed $\text{N}\cdots\text{Ru}$ bond is 2.28 \AA in the case of His349 and 2.80 \AA in the case of His350. These values are not far from those expected (Rh–N distance should be = 2.23 \AA , while Ru–N distance should be within the range 2.18–2.25 \AA). $\text{Rh}\cdots\text{S}(\text{Met256})$ distance is 3.10 \AA , larger than expected (Rh–S distance should be = 2.57 \AA).

Summary and conclusion

In conclusion, inspired by previous studies on the interaction of metalodrugs with hTF,¹⁷ we have investigated the reactivity

of the isostructural dirhodium and diruthenium tetraacetate complexes with Fe_C -hTF and have identified diRh and Ru binding sites in the structure of the adducts formed by the protein with these two complexes. Our results reveal that:

(i) DiRh binding sites are found close to His14, Met256, His273, His289, His300, His349, His350, His578, and His642, with dirhodium center that prefers axial rather than equatorial binding mode. This is the first structural observation of binding of Rh atoms to hTF. This is in line with previous reactivity of this compound with proteins.^{44,56}

(ii) A Ru binding site has been identified close to the side chains of His349 and His350. This result supports previous finding indicating that Ru ligands play a role in defining the final Ru binding site.^{57,58} Indeed, the structures of Ru/ Fe_C -hTF adducts formed when the protein reacts with the Ru compounds $\text{Ru}(\text{NTA})_2$ and $\text{Ru}(\text{bpy})\text{OHCl}_2(\text{DMSO})_2$ are different from each other²⁹ and from the one obtained here with diruthenium tetraacetate.

Results (i) and (ii) suggest that the Ru and Rh ions bind protein surface residues, although the iron binding site of the N-lobe is unoccupied.

(iii) The binding of Ru and Rh ions to Fe_C -hTF does not significantly affect the overall conformation of the protein that adopts a closed conformation of the C-lobe and an open conformation of the N-lobe. This agrees with what has been previously observed for cisplatin binding to both apo- and Fe_C -hTF forms^{16,17} and with what has been found in the case of adducts with Os²⁹ and V.¹⁹ However, this result differs from previous observations obtained comparing the apo-hTF structure with that of the adducts formed with gold ions from aurothiomalate¹⁸ and with Bi^{3+} .⁵⁹

(iv) Ru and Rh-containing fragments from dimetallic paddlewheel complexes compete for the same binding sites on the hTF structure. Under the same experimental conditions, the dirhodium complex is much more reactive with hTF than its diruthenium analogue.

Previous investigations by P. Lay and coworkers have demonstrated that Ru binding to the surface His side chains does not affect the hTF cycle.³¹ While the Ru/ Fe_C -hTF and Rh/ Fe_C -hTF adducts could theoretically mediate cellular uptake of Ru or Rh, this mechanism might be disrupted by competing proteins like serum albumin. However, further studies are necessary to verify these hypotheses and test the possible use of these hTF adducts as a Trojan Horse to transport and deliver metalodrugs to cancer cells.

Author contributions

All authors have given approval to the final version of the manuscript. A.-S. B. and G. F. equally contribute to this work.

Conflicts of interest

There are no conflicts to declare.



Data availability

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

Supplementary information (SI): Tables S1 and S2. See DOI: <https://doi.org/10.1039/d6dt00205f>.

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References

- H. Sun, H. Li and P. J. Sadler, Transferrin as a Metal Ion Mediator, *Chem. Rev.*, 1999, **99**(9), 2817–2842, DOI: [10.1021/cr980430w](https://doi.org/10.1021/cr980430w).
- L. A. Lambert, Molecular Evolution of the Transferrin Family and Associated Receptors, *Biochim. Biophys. Acta, Gen. Subj.*, 2012, **1820**(3), 244–255, DOI: [10.1016/j.bbagen.2011.06.002](https://doi.org/10.1016/j.bbagen.2011.06.002).
- R. T. A. MacGillivray, S. A. Moore, J. Chen, B. F. Anderson, H. Baker, Y. Luo, M. Bewley, C. A. Smith, M. E. P. Murphy, Y. Wang, A. B. Mason, R. C. Woodworth, G. D. Brayer and E. N. Baker, Two High-Resolution Crystal Structures of the Recombinant N-Lobe of Human Transferrin Reveal a Structural Change Implicated in Iron Release, *Biochemistry*, 1998, **37**(22), 7919–7928, DOI: [10.1021/bi980355j](https://doi.org/10.1021/bi980355j).
- J. Williams and K. Moreton, The Distribution of Iron between the Metal-Binding Sites of Transferrin Human Serum, *Biochem. J.*, 1980, **185**(2), 483–488, DOI: [10.1042/bj1850483](https://doi.org/10.1042/bj1850483).
- J. A. Benjamín-Rivera, A. E. Cardona-Rivera, Á. L. Vázquez-Maldonado, C. Y. Dones-Lassalle, H. L. Pabón-Colon, H. M. Rodríguez-Rivera, I. Rodríguez, J. C. González-Espiet, J. Pazol, J. D. Pérez-Ríos, J. F. Catala-Torres, M. Carrasquillo Rivera, M. G. De Jesus-Soto, N. A. Cordero-Virella, P. M. Cruz-Maldonado, P. González-Pagan, R. Hernández-Ríos, K. Gaur, S. A. Loza-Rosas and A. D. Tinoco, Exploring Serum Transferrin Regulation of Nonferric Metal Therapeutic Function and Toxicity, *Inorganics*, 2020, **8**(9), 48, DOI: [10.3390/inorganics8090048](https://doi.org/10.3390/inorganics8090048).
- W. R. Harris and L. Messori, A Comparative Study of Aluminum(III), Gallium(III), Indium(III), and Thallium(III) Binding to Human Serum Transferrin, *Coord. Chem. Rev.*, 2002, **228**(2), 237–262, DOI: [10.1016/S0010-8545\(02\)00037-1](https://doi.org/10.1016/S0010-8545(02)00037-1).
- J. B. Vincent and S. Love, The Binding and Transport of Alternative Metals by Transferrin, *Biochim. Biophys. Acta, Gen. Subj.*, 2012, **1820**(3), 362–378, DOI: [10.1016/j.bbagen.2011.07.003](https://doi.org/10.1016/j.bbagen.2011.07.003).
- H. A. Alhazmi, Measurement of Interaction Behavior of Six Biologically Important Noble Metal Ions with the Iron(III) Binding Protein, Apo-Transferrin, Using Mobility-Shift Affinity Electrophoresis, *Pharmazie*, 2018, **3**, 143–149, DOI: [10.1691/ph.2018.7179](https://doi.org/10.1691/ph.2018.7179).
- M. Zhang, D. R. Gumerov, I. A. Kaltashov and A. B. Mason, Indirect Detection of Protein-Metal Binding: Interaction of Serum Transferrin with In³⁺ and Bi³⁺, *J. Am. Soc. Mass Spectrom.*, 2004, **15**(11), 1658–1664, DOI: [10.1016/j.jasms.2004.08.009](https://doi.org/10.1016/j.jasms.2004.08.009).
- E. Keshavarz, A.-K. Bordbar and R. Amiri, Interaction of Gallium Maltolate with Apotransferrin: A Spectroscopic and Isothermal Titration Calorimetric Study, *J. Therm. Anal. Calorim.*, 2015, **120**(1), 325–333, DOI: [10.1007/s10973-014-4022-x](https://doi.org/10.1007/s10973-014-4022-x).
- D. J. Reilley, J. T. Fuller, M. R. Nechay, M. Victor, W. Li, J. D. Ruberry, J. I. Mujika, X. Lopez and A. N. Alexandrova, Toxic and Physiological Metal Uptake and Release by Human Serum Transferrin, *Biophys. J.*, 2020, **118**(12), 2979–2988, DOI: [10.1016/j.bpj.2020.05.006](https://doi.org/10.1016/j.bpj.2020.05.006).
- Y. Niitsu, Y. Kohgo, T. Nishisato, H. Kondo, J. Kato, Y. Urushizaki and I. Urushizaki, Transferrin Receptors in Human Cancerous Tissues, *Tohoku J. Exp. Med.*, 1987, **153**(3), 239–243, DOI: [10.1620/tjem.153.239](https://doi.org/10.1620/tjem.153.239).
- S. Tortorella and T. C. Karagiannis, Transferrin Receptor-Mediated Endocytosis: A Useful Target for Cancer Therapy, *J. Membr. Biol.*, 2014, **247**(4), 291–307, DOI: [10.1007/s00232-014-9637-0](https://doi.org/10.1007/s00232-014-9637-0).
- H. S. Oberoi, N. V. Nukolova, A. V. Kabanov and T. K. Bronich, Nanocarriers for Delivery of Platinum Anticancer Drugs, *Adv. Drug Delivery Rev.*, 2013, **65**(13–14), 1667–1685, DOI: [10.1016/j.addr.2013.09.014](https://doi.org/10.1016/j.addr.2013.09.014).
- A. Levina, A. R. M. Chetcuti and P. A. Lay, Controversial Role of Transferrin in the Transport of Ruthenium Anticancer Drugs, *Biomolecules*, 2022, **12**(9), 1319, DOI: [10.3390/biom12091319](https://doi.org/10.3390/biom12091319).
- R. Troisi, F. Galardo, G. Ferraro, F. Sica and A. Merlino, Cisplatin Binding to Human Serum Transferrin: A Crystallographic Study, *Inorg. Chem.*, 2023, **62**(2), 675–678, DOI: [10.1021/acs.inorgchem.2c04206](https://doi.org/10.1021/acs.inorgchem.2c04206).
- R. Troisi, F. Galardo, G. Ferraro, R. Lucignano, D. Picone, A. Marano, M. Trifuoggi, F. Sica and A. Merlino, Cisplatin/Apo-Transferrin Adduct: X-Ray Structure and Binding to the Transferrin Receptor 1, *Inorg. Chem.*, 2025, **64**(1), 761–765, DOI: [10.1021/acs.inorgchem.4c04435](https://doi.org/10.1021/acs.inorgchem.4c04435).
- R. Troisi, F. Galardo, L. Messori, F. Sica and A. Merlino, The X-Ray Structure of the Adduct Formed upon Reaction of Aurothiomalate with Apo-Transferrin: Gold Binding Sites and a Unique Transferrin Structure along the Apo/Holo Transition Pathway, *Inorg. Chem. Front.*, 2025, **12**(7), 2627–2637, DOI: [10.1039/D4QI03184A](https://doi.org/10.1039/D4QI03184A).
- A. S. Banneville, R. Lucignano, M. Paolillo, V. Cuomo, M. Chino, G. Ferraro, D. Picone, E. Garribba, I. Cornaci-Hoffman, A. Pica and A. Merlino, First Crystal Structure of



- an Adduct Formed upon Reaction of a Vanadium Compound with Human Serum Transferrin, *Commun. Chem.*, 2026, **9**, 89, DOI: [10.1038/s42004-026-01891-1](https://doi.org/10.1038/s42004-026-01891-1).
- 20 T. Hoshino, M. Misaki, M. Yamamoto, H. Shimizu, Y. Ogawa and H. Toguchi, In Vitro Cytotoxicities and In Vivo Distribution of Transferrin–Platinum(II) Complex, *J. Pharm. Sci.*, 1995, **84**(2), 216–221, DOI: [10.1002/jps.2600840219](https://doi.org/10.1002/jps.2600840219).
- 21 L.-Z. Luo, H.-W. Jin and H.-Q. Huang, Transferrin–Cisplatin Specifically Deliver Cisplatin to HepG2 Cells in Vitro and Enhance Cisplatin Cytotoxicity, *J. Proteomics*, 2012, **77**, 237–250, DOI: [10.1016/j.jprot.2012.08.023](https://doi.org/10.1016/j.jprot.2012.08.023).
- 22 H. Peng, H. Jin, H. Zhuo and H. Huang, Enhanced Antitumor Efficacy of Cisplatin for Treating Ovarian Cancer *in Vitro* and *in Vivo* via Transferrin Binding, *Oncotarget*, 2017, **8**(28), 45597–45611, DOI: [10.18632/oncotarget.17316](https://doi.org/10.18632/oncotarget.17316).
- 23 J. Will, D. A. Wolters and W. S. Sheldrick, Characterisation of Cisplatin Binding Sites in Human Serum Proteins Using Hyphenated Multidimensional Liquid Chromatography and ESI Tandem Mass Spectrometry, *ChemMedChem*, 2008, **3**(11), 1696–1707, DOI: [10.1002/emdc.200800151](https://doi.org/10.1002/emdc.200800151).
- 24 I. Khalaila, C. S. Allardyce, C. S. Verma and P. J. Dyson, A Mass Spectrometric and Molecular Modelling Study of Cisplatin Binding to Transferrin, *ChemBioChem*, 2005, **6**(10), 1788–1795, DOI: [10.1002/cbic.200500067](https://doi.org/10.1002/cbic.200500067).
- 25 M. Guo, H. Sun, H. J. McArdle, L. Gambling and P. J. Sadler, TiIV Uptake and Release by Human Serum Transferrin and Recognition of TiIV -Transferrin by Cancer Cells: Understanding the Mechanism of Action of the Anticancer Drug Titanocene Dichloride, *Biochemistry*, 2000, **39**(33), 10023–10033, DOI: [10.1021/bi000798z](https://doi.org/10.1021/bi000798z).
- 26 J. F. Head, F. Wang and R. L. Elliott, Antineoplastic Drugs That Interfere with Iron Metabolism in Cancer Cells, *Adv. Enzyme Regul.*, 1997, **37**, 147–169, DOI: [10.1016/S0065-2571\(96\)00010-6](https://doi.org/10.1016/S0065-2571(96)00010-6).
- 27 H. Korkusuz, K. Ulbrich, K. Welzel, V. Koeberle, W. Watcharin, U. Bahr, V. Chernikov, T. Knobloch, S. Petersen, F. Huebner, H. Ackermann, S. Gelperina, W. Kromen, R. Hammerstingl, J. Hauptenthal, F. Gruenwald, J. Fiehler, S. Zeuzem, J. Kreuter, T. J. Vogl and A. Piiper, Transferrin-Coated Gadolinium Nanoparticles as MRI Contrast Agent, *Mol. Imaging Biol.*, 2013, **15**(2), 148–154, DOI: [10.1007/s11307-012-0579-6](https://doi.org/10.1007/s11307-012-0579-6).
- 28 A. Korotcov, L. Shan, H. Meng, T. Wang, R. Sridhar, Y. Zhao, X.-J. Liang and P. C. Wang, A Nanocomplex System as Targeted Contrast Agent Delivery Vehicle for Magnetic Resonance Imaging Dynamic Contrast Enhancement Study, *J. Nanosci. Nanotechnol.*, 2010, **10**(11), 7545–7549, DOI: [10.1166/jnn.2010.2821](https://doi.org/10.1166/jnn.2010.2821).
- 29 M. Wang, H. Wang, X. Xu, T.-P. Lai, Y. Zhou, Q. Hao, H. Li and H. Sun, Binding of Ruthenium and Osmium at Non-iron Sites of Transferrin Accounts for Their Iron-Independent Cellular Uptake, *J. Inorg. Biochem.*, 2022, **234**, 111885, DOI: [10.1016/j.jinorgbio.2022.111885](https://doi.org/10.1016/j.jinorgbio.2022.111885).
- 30 J. P. Curtin, M. Wang, T. Cheng, L. Jin and H. Sun, The Role of Citrate, Lactate and Transferrin in Determining Titanium Release from Surgical Devices into Human Serum, *J. Biol. Inorg. Chem.*, 2018, **23**(3), 471–480, DOI: [10.1007/s00775-018-1557-5](https://doi.org/10.1007/s00775-018-1557-5).
- 31 A. Levina and P. A. Lay, Vanadium(V/IV)–Transferrin Binding Disrupts the Transferrin Cycle and Reduces Vanadium Uptake and Antiproliferative Activity in Human Lung Cancer Cells, *Inorg. Chem.*, 2020, **59**(22), 16143–16153, DOI: [10.1021/acs.inorgchem.0c00926](https://doi.org/10.1021/acs.inorgchem.0c00926).
- 32 M. A. Bratty, H. A. Alhazmi, A. Najmi, M. S. Alam, N. Shubayr, H. Khudishi and S. A. Javed, Spectroscopic Studies for Rhodium(III) Binding to Apo-Transferrin, *J. Chem.*, 2022, **2022**, 1–10, DOI: [10.1155/2022/2879840](https://doi.org/10.1155/2022/2879840).
- 33 R. L. S. R. Santos, R. Van Eldik and D. De Oliveira Silva, Thermodynamics of Axial Substitution and Kinetics of Reactions with Amino Acids for the Paddlewheel Complex Tetrakis(Acetato)Chloridodiruthenium(II,III), *Inorg. Chem.*, 2012, **51**(12), 6615–6625, DOI: [10.1021/ic300168t](https://doi.org/10.1021/ic300168t).
- 34 S. La Manna, C. Di Natale, V. Panzetta, M. Leone, F. A. Mercurio, I. Cipollone, M. Monti, P. A. Netti, G. Ferraro, A. Terán, A. E. Sánchez-Peláez, S. Herrero, A. Merlino and D. Marasco, A Diruthenium Metallodrug as a Potent Inhibitor of Amyloid- β Aggregation: Synergism of Mechanisms of Action, *Inorg. Chem.*, 2024, **63**(1), 564–575, DOI: [10.1021/acs.inorgchem.3c03441](https://doi.org/10.1021/acs.inorgchem.3c03441).
- 35 A. N. Zaykov, B. V. Popp and Z. T. Ball, Helix Induction by Dirhodium: Access to Biocompatible Metallopeptides with Defined Secondary Structure, *Chem. – Eur. J.*, 2010, **16**(22), 6651–6659, DOI: [10.1002/chem.200903092](https://doi.org/10.1002/chem.200903092).
- 36 A. Terán, G. Ferraro, P. Imbimbo, A. E. Sánchez-Peláez, D. M. Monti, S. Herrero and A. Merlino, Steric Hindrance and Charge Influence on the Cytotoxic Activity and Protein Binding Properties of Diruthenium Complexes, *Int. J. Biol. Macromol.*, 2023, **253**, 126666, DOI: [10.1016/j.ijbiomac.2023.126666](https://doi.org/10.1016/j.ijbiomac.2023.126666).
- 37 G. Ferraro, A. Pratesi, L. Messori and A. Merlino, Protein Interactions of Dirhodium Tetraacetate: A Structural Study, *Dalton Trans.*, 2020, **49**(8), 2412–2416, DOI: [10.1039/C9DT04819G](https://doi.org/10.1039/C9DT04819G).
- 38 I. Tolbatov, E. Barresi, S. Taliani, D. La Mendola, T. Marzo and A. Marrone, Diruthenium(II,III) Paddlewheel Complexes: Effects of Bridging and Axial Ligands on Anticancer Properties, *Inorg. Chem. Front.*, 2023, **10**(8), 2226–2238, DOI: [10.1039/D3QI00157A](https://doi.org/10.1039/D3QI00157A).
- 39 D. Loreto, G. Ferraro and A. Merlino, Unusual Structural Features in the Adduct of Dirhodium Tetraacetate with Lysozyme, *Int. J. Mol. Sci.*, 2021, **22**(3), 1496, DOI: [10.3390/ijms22031496](https://doi.org/10.3390/ijms22031496).
- 40 L. Messori, T. Marzo, R. N. F. Sanches, H. U. Rehman, D. de Oliveira Silva and A. Merlino, Unusual Structural Features in the Lysozyme Derivative of the Tetrakis(Acetato) Chloridodiruthenium(II,III) Complex, *Angew. Chem., Int. Ed.*, 2014, **53**(24), 6172–6175, DOI: [10.1002/anie.201403337](https://doi.org/10.1002/anie.201403337).
- 41 G. Ferraro, A. Terán, F. Galardo, R. Lucignano, D. Picone, L. Massai, F. Fasulo, A. B. Muñoz-García, L. Messori, S. Herrero and A. Merlino, Deciphering the Role of Neutral Diruthenium Complexes in Protein Binding, *Int. J. Biol.*



- Macromol.*, 2024, **283**, 137691, DOI: [10.1016/j.ijbiomac.2024.137691](https://doi.org/10.1016/j.ijbiomac.2024.137691).
- 42 I. Tolbatov, P. Umari and A. Marrone, Diruthenium Paddlewheel Complexes Attacking Proteins: Axial versus Equatorial Coordination, *Biomolecules*, 2024, **14**(5), 530, DOI: [10.3390/biom14050530](https://doi.org/10.3390/biom14050530).
- 43 A. Terán, G. Ferraro, A. E. Sánchez-Peláez, S. Herrero and A. Merlino, Effect of Equatorial Ligand Substitution on the Reactivity with Proteins of Paddlewheel Diruthenium Complexes: Structural Studies, *Inorg. Chem.*, 2023, **62**(2), 670–674, DOI: [10.1021/acs.inorgchem.2c04103](https://doi.org/10.1021/acs.inorgchem.2c04103).
- 44 D. Loreto and A. Merlino, The Interaction of Rhodium Compounds with Proteins: A Structural Overview, *Coord. Chem. Rev.*, 2021, **442**, 213999, DOI: [10.1016/j.ccr.2021.213999](https://doi.org/10.1016/j.ccr.2021.213999).
- 45 I. Tolbatov, P. Umari and A. Marrone, The Binding of Diruthenium (II,III) and Dirhodium (II,II) Paddlewheel Complexes at DNA/RNA Nucleobases: Computational Evidences of an Appreciable Selectivity toward the AU Base Pairs, *J. Mol. Graphics Modell.*, 2024, **131**, 108806, DOI: [10.1016/j.jmgm.2024.108806](https://doi.org/10.1016/j.jmgm.2024.108806).
- 46 G. Tito, R. Troisi, G. Ferraro, A. Geri, L. Massai, L. Messori, F. Sica and A. Merlino, Dirhodium Tetraacetate Binding to a B-DNA Double Helical Dodecamer Probed by X-Ray Crystallography and Mass Spectrometry, *Dalton Trans.*, 2023, **52**(21), 6992–6996, DOI: [10.1039/D3DT00320E](https://doi.org/10.1039/D3DT00320E).
- 47 G. Tito, G. Ferraro and A. Merlino, Dirhodium Tetraacetate Binding to Lysozyme at Body Temperature, *Int. J. Mol. Sci.*, 2025, **26**(14), 6582, DOI: [10.3390/ijms26146582](https://doi.org/10.3390/ijms26146582).
- 48 A. M. Angeles-Boza, H. T. Chifotides, J. D. Aguirre, A. Chouai, P. K.-L. Fu, K. R. Dunbar and C. Turro, Dirhodium(II,II) Complexes: Molecular Characteristics That Affect in Vitro Activity, *J. Med. Chem.*, 2006, **49**(23), 6841–6847, DOI: [10.1021/jm060592h](https://doi.org/10.1021/jm060592h).
- 49 R. Hrdina, Dirhodium(II,II) Paddlewheel Complexes, *Eur. J. Inorg. Chem.*, 2021, (6), 501–528, DOI: [10.1002/ejic.202000955](https://doi.org/10.1002/ejic.202000955).
- 50 H. U. Rehman, T. E. Freitas, R. N. Gomes, A. Colquhoun and D. De Oliveira Silva, Axially-Modified Paddlewheel Diruthenium(II,III)-Ibuprofenato Metallodrugs and the Influence of the Structural Modification on U87MG and A172 Human Glioma Cell Proliferation, Apoptosis, Mitosis and Migration, *J. Inorg. Biochem.*, 2016, **165**, 181–191, DOI: [10.1016/j.jinorgbio.2016.10.003](https://doi.org/10.1016/j.jinorgbio.2016.10.003).
- 51 E. Warzecha, T. C. Berto, C. C. Wilkinson and J. F. Berry, Rhodium Rainbow: A Colorful Laboratory Experiment Highlighting Ligand Field Effects of Dirhodium Tetraacetate, *J. Chem. Educ.*, 2019, **96**(3), 571–576, DOI: [10.1021/acs.jchemed.6b00648](https://doi.org/10.1021/acs.jchemed.6b00648).
- 52 A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, Phaser Crystallographic Software, *J. Appl. Crystallogr.*, 2007, **40**(4), 658–674, DOI: [10.1107/S0021889807021206](https://doi.org/10.1107/S0021889807021206).
- 53 G. N. Murshudov, P. Skubák, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls, M. D. Winn, F. Long and A. A. Vagin, REFMAC 5 for the Refinement of Macromolecular Crystal Structures, *Acta Crystallogr., Sect. D: Biol. Crystallogr.*, 2011, **67**(4), 355–367, DOI: [10.1107/S0907444911001314](https://doi.org/10.1107/S0907444911001314).
- 54 G. Ferraro and A. Merlino, Investigation of Metallodrug/Protein Interaction by X-Ray Crystallography and Complementary Biophysical Techniques, *Inorg. Chem. Front.*, 2025, **12**(9), 3345–3366, DOI: [10.1039/D4QI03277B](https://doi.org/10.1039/D4QI03277B).
- 55 I. Russo Krauss, G. Ferraro, A. Pica, J. A. Márquez, J. R. Helliwell and A. Merlino, Principles and Methods Used to Grow and Optimize Crystals of Protein–Metallodrug Adducts, to Determine Metal Binding Sites and to Assign Metal Ligands, *Metallomics*, 2017, **9**(11), 1534–1547, DOI: [10.1039/C7MT00219J](https://doi.org/10.1039/C7MT00219J).
- 56 G. Ferraro, A. Pratesi, L. Messori and A. Merlino, Protein Interactions of Dirhodium Tetraacetate: A Structural Study, *Dalton Trans.*, 2020, **49**(8), 2412–2416, DOI: [10.1039/C9DT04819G](https://doi.org/10.1039/C9DT04819G).
- 57 A. Merlino, Interactions between Proteins and Ru Compounds of Medicinal Interest: A Structural Perspective, *Coord. Chem. Rev.*, 2016, **326**, 111–134, DOI: [10.1016/j.ccr.2016.08.001](https://doi.org/10.1016/j.ccr.2016.08.001).
- 58 L. Messori and A. Merlino, Ruthenium Metalation of Proteins: The X-Ray Structure of the Complex Formed between NAMI-A and Hen Egg White Lysozyme, *Dalton Trans.*, 2014, **43**(16), 6128, DOI: [10.1039/c3dt53582g](https://doi.org/10.1039/c3dt53582g).
- 59 N. Yang, H. Zhang, M. Wang, Q. Hao and H. Sun, Iron and Bismuth Bound Human Serum Transferrin Reveals a Partially-Opened Conformation in the N-Lobe, *Sci. Rep.*, 2012, **2**(1), 999, DOI: [10.1038/srep00999](https://doi.org/10.1038/srep00999).

