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Issue Article

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BINDING OF Co(II) AND Cu(II) COMPLEXES BASED ON 3-AMINO-1,2,4-TRIAZOLE AND PYROMELLITIC ACID TO THE β-DOMAIN OF METALLOTHIONEIN-2: A MOLECULAR DOCKING STUDY

Abstract: *Metallothioneins are small cysteine-rich proteins that act as the intracellular reservoir of zinc and copper and as a detoxification system for heavy metals. In this work the binding of Co(II) and Cu(II) coordination compounds based on 3-amino-1,2,4-triazole and pyromellitic acid to the β-domain of human metallothionein-2 (PDB 2MHU) was studied by molecular docking. Both free ligands were docked separately for comparison. Calculations were carried out in CB-Dock2 independently for each of the five cavities detected on the protein surface. The free ligands selected cavity C2 and gave low scores: −4.8 kcal mol⁻¹ for pyromellitic acid and −3.4 kcal mol⁻¹ for the triazole. Coordination to a metal centre markedly enhanced binding: −6.4 kcal mol⁻¹ for the Cu(II) complex in C2 and −6.6 kcal mol⁻¹ for the Co(II) complex in C1. This advantage was preserved in all five cavities without exception. The 0.2 kcal mol⁻¹ difference between the complexes lies below the resolution of the method, so the two were regarded as equipotent; they differ in binding site and interaction type. The Cu(II) complex occupies the groove above the thiolate cluster and forms π-type contacts with Cys7, Cys13 and Cys26, whereas the Co(II) complex shifts to the C1 surface and binds through hydrogen bonds alone. The difference was interpreted within Pearson's theory. The results indicate that coordination compounds may act on metallothionein through a mechanism distinct from that of conventional chelators — as preorganised recognition elements.*

Key words: *metallothionein-2, molecular docking, 3-amino-1,2,4-triazole, pyromellitic acid, coordination compound, π-sulfur interaction, Pearson's theory.*

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Introduction

UDC: 541.49. 547.792. 544.18. 577.112

Contamination with heavy metals remains a serious ecological and medical problem for industrialised regions. The fate of copper, cadmium, zinc and mercury ions entering the body is largely determined by the activity of a single protein family — the metallothioneins [1]. Cysteine accounts for almost one third of the composition of these proteins, and it is precisely these cysteine sulfurs that bind metal ions through thiolate bridges, forming a cluster.

Mammalian metallothionein-1 and -2 consist of two independent domains. The C-terminal α-domain binds four divalent ions through eleven cysteines, while the N-terminal β-domain binds three ions through nine cysteines [2]. The two domains differ not only in size but also in behaviour: the β-domain exchanges metal considerably faster, and it is through this domain that the protein transfers zinc to apo-enzymes and transcription factors [2,3]. Ion-mobility mass spectrometry and molecular dynamics studies carried out in recent years have shown that the domains are not independent of one another, and that their connection differentiates the affinity for Zn(II) [3,4]. The β-domain is therefore the most accessible target for external intervention in metallothionein function.

Compounds studied as agents acting on metallothionein have usually been free chelators — polyaminocarboxylic acids, dithiocarbamates and thiol reagents. Their mechanism of action is uniform: withdrawal of a metal ion from the cluster [5]. Selectivity in this approach is low, however, since such reagents act equally on other metal-containing structures within the cell.

An alternative route is the use of a preformed coordination compound. In such a compound the metal is already saturated by its own ligands, which means that it cannot withdraw metal from the protein cluster. If such a compound nevertheless binds the protein, it must be recognised as a shape. Shape, in turn, is a property that can be controlled far more precisely than the composition of a chelator, and this opens a route to selectivity. Despite this, the direction has remained almost unexplored in the literature.

3-Amino-1,2,4-triazole and pyromellitic acid were selected as ligands for this study. The triazole coordinates through ring nitrogen and pyromellitic acid through carboxylate oxygen; together they provide the mixed N,O environment characteristic of many metalloenzymes. Metal complexes of triazole derivatives have repeatedly been reported to show higher biological activity than the free heterocycle [6,7], while pyromellitic acid is known to give stable coordination structures with Co(II) and Cu(II) [8,9]. Importantly, neither compound on its own has any pronounced affinity for a protein surface: the triazole is very small, and pyromellitic acid is rigid and highly charged. If their complex nevertheless binds the domain better than either component, the gain must arise from the assembly itself.

The aim of the work — to study, by molecular docking, the binding of Co(II) and Cu(II) complexes based on 3-amino-1,2,4-triazole and pyromellitic acid to the β-domain of metallothionein-2, and to compare this binding with that of the free ligands. The following tasks were set: (a) to identify the possible binding sites on the protein surface; (b) to obtain and compare docking scores for the four species; (c) to analyse the set of interactions for each best pose; (d) to interpret the difference between the Co(II) and Cu(II) complexes on the basis of coordination-chemistry principles.

Research objects and methods

Receptor

The receptor was taken from the Protein Data Bank, entry 2MHU — the solution structure of [$^{113}Cd_7$]-metallothionein-2 determined by heteronuclear NMR spectroscopy [10]. The calculation used

chain A, that is the β-domain (Met1–Lys30, sequence MDPNCSCAAGDSCTCAGSCKCKECKCTSCK). The three cadmium ions of the Cd_3S_9 cluster were retained: their removal would disrupt the protein fold and eliminate the binding sites on the surface. The first model of the NMR ensemble was selected. Polar hydrogens were added and Gasteiger charges assigned automatically by the server.

Ligands

Four objects were prepared. The free ligands — 3-amino-1,2,4-triazole (hereafter L1) and pyromellitic acid (L2) — were built from standard structures and their geometries optimised. The Co(II) and Cu(II) complexes were extracted from the corresponding single-crystal X-ray structures with the full coordination environment retained: in both cases the metal centre is bound by the nitrogen atoms of two triazole rings and by the carboxylate oxygen atoms of two pyromellitate residues. Water of crystallisation was deleted while coordinated species were kept. Hydrogens were added in idealised positions and the structures saved in MOL2 format, which carries bond orders and metal–donor connectivity through to the next stage.

Docking

Calculations were performed on the CB-Dock2 web server [11]. This tool detects cavities on the protein surface by curvature (the CurPocket

algorithm) and docks into each cavity using the AutoDock Vina scoring function [12,13]. Cavity detection was carried out once and returned five sites ranked by volume. Each ligand was then docked separately into each of the five cavities — twenty independent calculations in total. Box size was assigned by the server according to ligand size: 14 Å for L1, 19 Å for L2 and 27 Å for the complexes. Cavity centres remained unchanged across all calculations, which allowed the scores to be compared directly. The protein was treated as rigid and the torsion angles of the ligand as free.

Interaction analysis

The best-scoring poses were analysed in BIOVIA Discovery Studio Visualizer [14]. Receptor and ligand were defined separately, and non-bonded contacts were identified using the default criteria of the program: hydrogen bonds, hydrophobic contacts, π-sulfur, π-alkyl, π-sigma, amide-π stacking and van der Waals interactions. Two- and three-dimensional representations were generated from the same coordinates.

Received results and their analysis

Cavities on the protein surface

The CurPocket algorithm detected five cavities on the surface of the β-domain (Table 1). Two of them dominate in volume: C1 (82 Å³) and C2 (72 Å³); the remaining three are shallow depressions of 4–6 Å³ and are of no practical significance.

The walls of both major cavities are formed mainly by cysteine residues: seven of nine in C1 and six in C2. The remainder is occupied by small residues (Gly, Ala, Ser, Thr) — precisely those that give the β-domain its loose, solvent-exposed fold. It should be emphasised that neither cavity is a buried pocket comparable to an enzyme active site; both are surface features associated with the metal–thiolate cluster.

C1 and C2 differ from one another in shape. The contact list of C1 runs from Met1 to Cys29, that is, it covers almost the entire polypeptide — a signature of a broad, flat surface. The contact set of C2 is more compactly gathered between Cys5 and Cys24 and lies directly above the Cd_3S_9 cluster; here the chain wraps around the metals and generates genuine concavity.

Table 1. Description of the cavities detected on the surface of the β-domain

Cavity	Volume, Å³	Centre (x, y, z), Å	Cys in wall	Shape
C1	82	−2.51; −4.88; −2.23	7 of 9	Broad flat surface
C2	72	4.54; −1.75; −6.21	6 of 9	Groove above the cluster
C3	6	−4.25; −5.41; −12.30	7 of 9	Shallow depression
C4	6	−3.11; 2.04; −7.79	6 of 9	Shallow depression
C5	4	−0.06; −10.67; −8.03	6 of 9	Shallow depression

Docking scores

The results of the twenty calculations are collected in Table 2. They reveal two patterns.

The first is the chelation effect. Both complexes scored 1.6–1.8 kcal mol⁻¹ below the better free ligand and 3.0–3.2 kcal mol⁻¹ below the weaker one. This ordering is preserved in all five cavities — it is not broken once in twenty calculations. Such consistency cannot be attributed to chance or to computational error.

The second is the difference in site selection. Both free ligands and the Cu(II) complex gave their best result in cavity C2 (−3.4, −4.8 and −6.4 kcal mol⁻¹ respectively). The Co(II) complex departs from this: its best score is in C1 (−6.6 kcal mol⁻¹), while C2 ranks fourth of the five sites. The reason for this difference is analysed below.

Table 2. AutoDock Vina scores for the four species in each cavity, kcal mol⁻¹ (best values in bold)

Cavity	L1 (triazole)	L2 (H_4pma)	Cu(II) complex	Co(II) complex
C1	−2.4	−4.2	−6.1	**−6.6**
C2	**−3.4**	**−4.8**	**−6.4**	−6.0
C3	−2.3	−3.8	−6.1	−5.9
C4	−2.5	−4.2	−5.7	−5.5
C5	−2.6	−4.1	−5.6	−6.0

Cavity	L1 (triazole)	L2 (H_4pma)	Cu(II) complex	Co(II) complex
Search box edge, Å	14	19	27	27

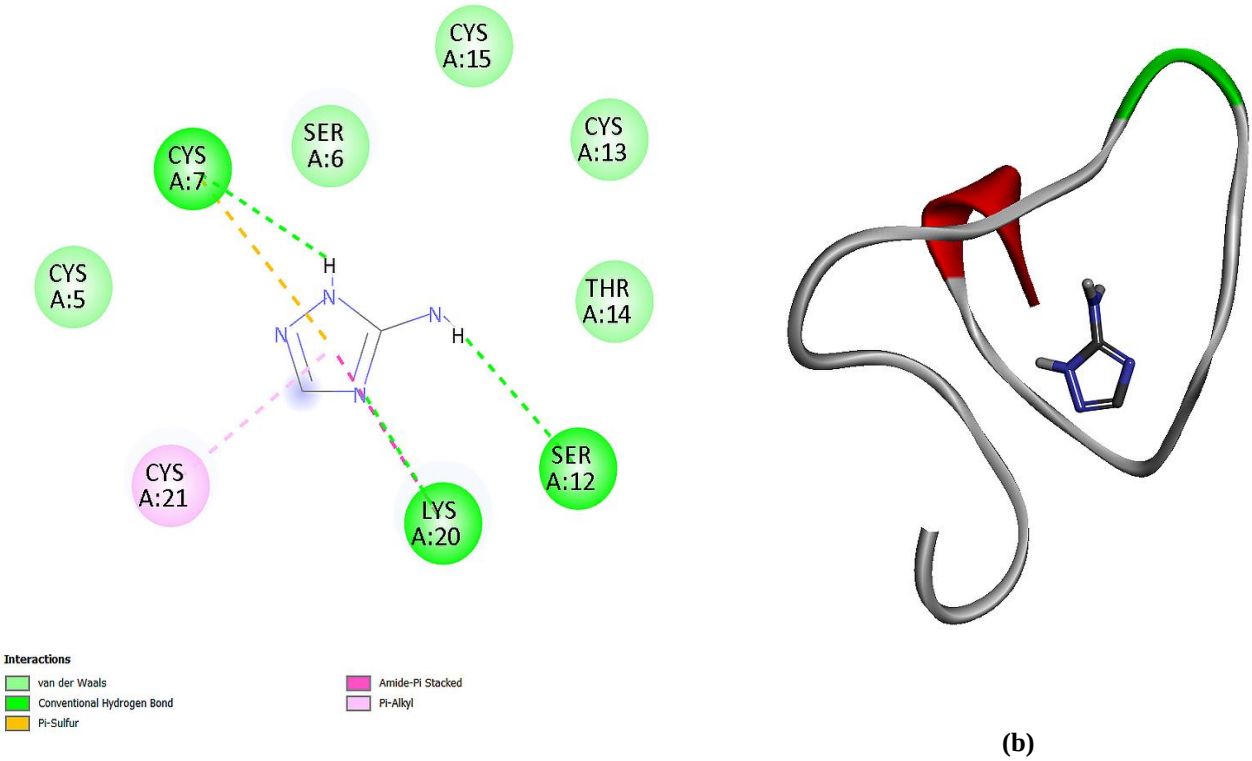
The difference between the best scores of the two complexes amounts to only 0.2 kcal mol⁻¹. The average error of the AutoDock Vina scoring function is an order of magnitude larger [12,13], so this difference cannot be used to claim that one binds more tightly than the other. By score, the complexes are regarded as equipotent. The real difference between them lies elsewhere — in the site and type of binding — and this difference, as shown below, is considerably more robust.

A further feature of Table 2 deserves comment: the spread of scores across the five cavities differs between species. For 3-amino-1,2,4-triazole the range is 1.1 kcal mol⁻¹ and for pyromellitic acid 1.0, whereas for the complexes it is 0.8 for Cu(II) and 1.1 for Co(II). In absolute terms these values are comparable, but relative to the binding score itself the spread is far smaller for the complexes. The free ligands therefore discriminate between sites more sharply than the complexes do.

This is a direct consequence of box size. The search box assigned to the complexes (27 Å) exceeds the cross-section of the domain, so the five runs sample overlapping regions of the surface rather than five distinct sites. For the smaller free ligands, with boxes of 14 and 19 Å, the cavities remain genuinely separate. The point is taken up again among the limitations of the method, and it is the reason why the assignment of a complex to one particular cavity is reported here as indicative rather than definitive.

Binding of the free ligands

3-Amino-1,2,4-triazole proved to be the weakest binder of the whole series (−3.4 kcal mol⁻¹). The forces holding it in place are: three hydrogen bonds — from the ring N–H group to Cys7, from the exocyclic amino group to Ser12 and from a ring nitrogen to Lys20; a π-sulfur contact with Cys7; and a π-alkyl contact with Cys21 (Fig. 1). The van der Waals environment comprises Cys5, Ser6, Cys13, Cys15 and Thr14. The molecule falls within the groove but occupies only a small part of it — which is the reason for the low score.



(a) **(b)**

Figure 1. The best pose of 3-amino-1,2,4-triazole in the C2 cavity (−3.4 kcal mol⁻¹): (a) two-dimensional interaction diagram; (b) three-dimensional view (protein — ribbon, ligand — sticks)

Pyromellitic acid binds appreciably more strongly (−4.8 kcal mol⁻¹). Its four carboxyl groups radiate in four directions and form six hydrogen bonds — with Met1, Asp2, Pro3, Ser6, Cys21 and Lys22. To these are added a π-sulfur contact with Met1 and amide-π stacking with Asn4 (Fig. 2). The nature of the binding is chiefly electrostatic: the aromatic core lies flat against the surface while the carboxylates reach towards backbone amides and the ammonium group of Lys22. It should be noted that in this pose the molecule is placed at the N-terminal end of the domain and engages only one cysteine (Cys21) through hydrogen bonding.

The 1.4 kcal mol⁻¹ gap between the two free ligands has a straightforward structural basis. Pyromellitic acid presents four carboxyl groups on a rigid aromatic core, so it offers eight potential oxygen donors distributed over a wide arc, and its deprotonated form carries a negative charge complementary to the lysine side chains at the rim of the site. 3-Amino-1,2,4-triazole offers three nitrogen donors and one amino group on a five-membered ring, and is neutral at physiological pH. The larger contact area and the electrostatic complementarity together account for the difference.

Taken together, the two free-ligand poses establish the baseline against which the complexes must be judged. Both ligands bind in the same cavity, both bind weakly, and both rely predominantly on hydrogen bonding. Neither reaches more than one cysteine sulfur through a directional contact. Whatever the complexes achieve beyond this must therefore be attributed to the assembly rather than to the donors themselves.

One detail of the triazole pose deserves note in advance of the comparison with the complexes. The single π-sulfur contact it forms, with Cys7, involves the same residue that the Cu(II) complex later engages twice over. The free heterocycle therefore already "finds" the correct sulfur; what it cannot do is hold a second ring in position to reach a second one. This observation anticipates the interpretation developed below, in which the role of coordination is not to create a new type of contact but to make an existing one occur twice at once.

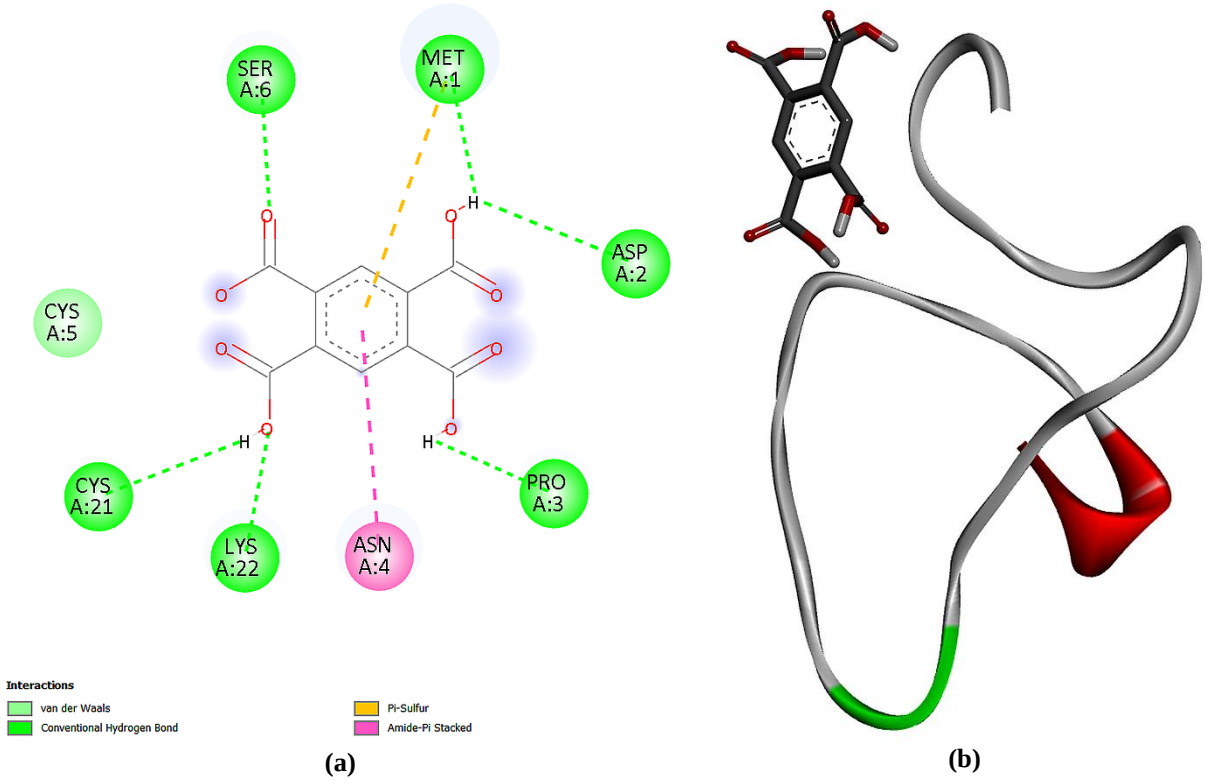


Figure 2. The best pose of pyromellitic acid in the C2 cavity (−4.8 kcal mol⁻¹): (a) two-dimensional interaction diagram; (b) three-dimensional view

Binding of the metal complexes

The Cu(II) complex gives its best result in C2 (−6.4 kcal mol⁻¹) — the same groove selected by both free ligands. In this pose (Fig. 3) the two pyromellitate arms lie along the groove while the two triazole rings face the thiolate cluster. Four hydrogen bonds are formed, involving Lys25, Ser28, Cys26 and Cys7. The latter two are of particular interest, since these cysteines serve as cluster ligands in the native protein.

Beyond hydrogen bonding, the Cu(II) complex engages a set of π-type contacts that no free ligand achieves: π-sulfur interactions with two cysteines simultaneously (Cys7 and Cys26), a π-alkyl contact with Cys13, and π-sigma contacts with Ala8 and Thr27. Free triazole also forms a π-sulfur contact, but with a single sulfur only — the simultaneous engagement of two is what coordination makes possible. The whole assembly is enclosed by a broad van der Waals shell (Gly10, Ser12, Thr14, Ala9, Asp11, Cys29).

The spatial arrangement of this pose is worth describing in more detail, since it is what distinguishes the copper species from every other structure examined here. The metal centre sits at the mouth of the groove rather than inside it. From this position the coordination sphere projects in two directions: the pyromellitate units extend outwards along the walls of the groove, where their free carboxyl groups are exposed to solvent and to the backbone, while the

triazole rings point inwards and downwards, towards the floor of the groove formed by the thiolate cluster. The assembly therefore straddles the site rather than occupying it, and the two halves of the ligand shell perform different roles.

This division of labour is reflected in the contacts themselves. All four hydrogen bonds are formed by carboxylate oxygens of the pyromellitate arms; the triazole rings form none. Conversely, all five π-type contacts involve the triazole rings; the pyromellitate units contribute none. The two ligand types are thus not interchangeable partners in the binding event but complementary components, one anchoring the complex to the backbone and the other engaging the sulfur-rich floor of the site.

Two residues, Cys7 and Cys26, are engaged twice over — once by a hydrogen bond from a carboxylate oxygen and once by a π-sulfur interaction with a triazole ring. Double engagement of this kind is not observed for any other residue, nor for any other species in the series. Since Cys7 and Cys26 are both cluster ligands in the native protein, this is the point at which the docked complex comes closest to the metal-binding apparatus itself.

Whether such proximity would translate into an effect on cluster stability cannot be decided from a docking calculation. What can be said is that the geometry required for such an effect is present in this pose and absent from the other three.

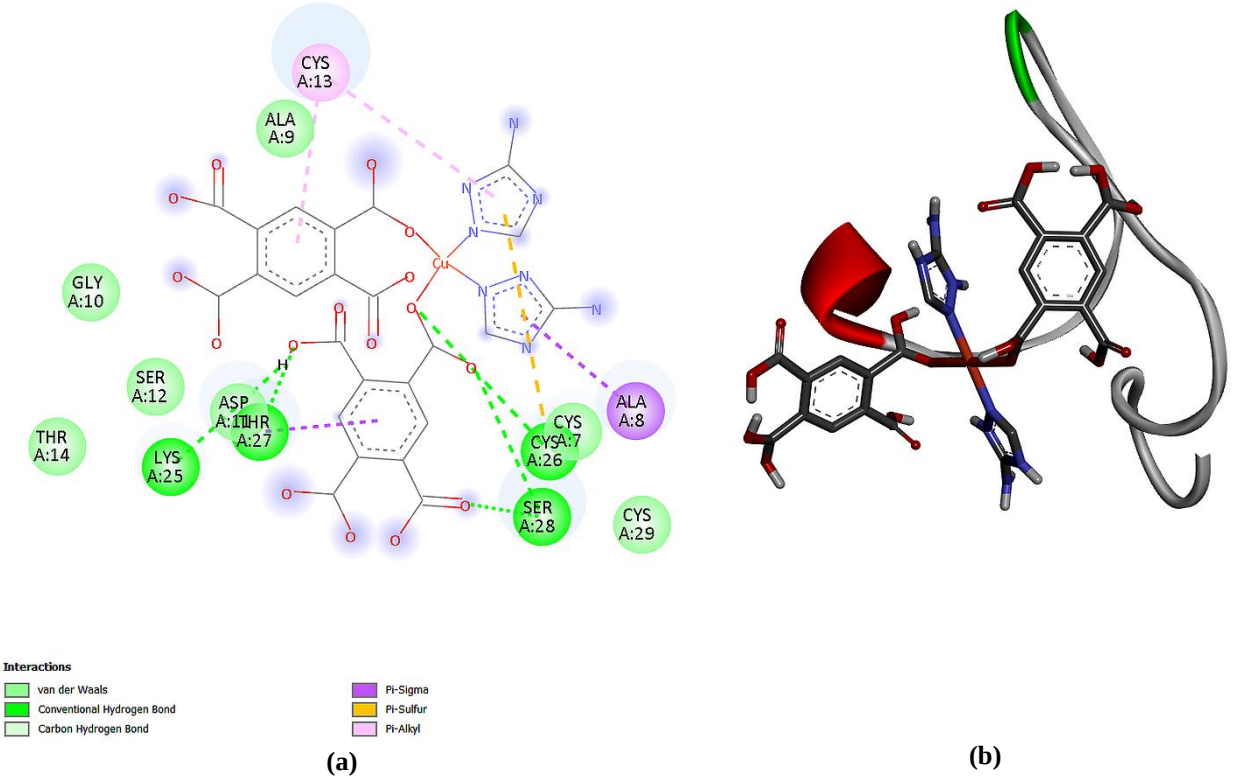


Figure 3. The best pose of the Cu(II) complex in the C2 cavity (−6.4 kcal mol⁻¹): (a) interaction diagram, with π-sulfur contacts to Cys7 and Cys26 shown as orange lines; (b) three-dimensional view, with the Cu–N and Cu–O coordination bonds of the complex visible at the centre

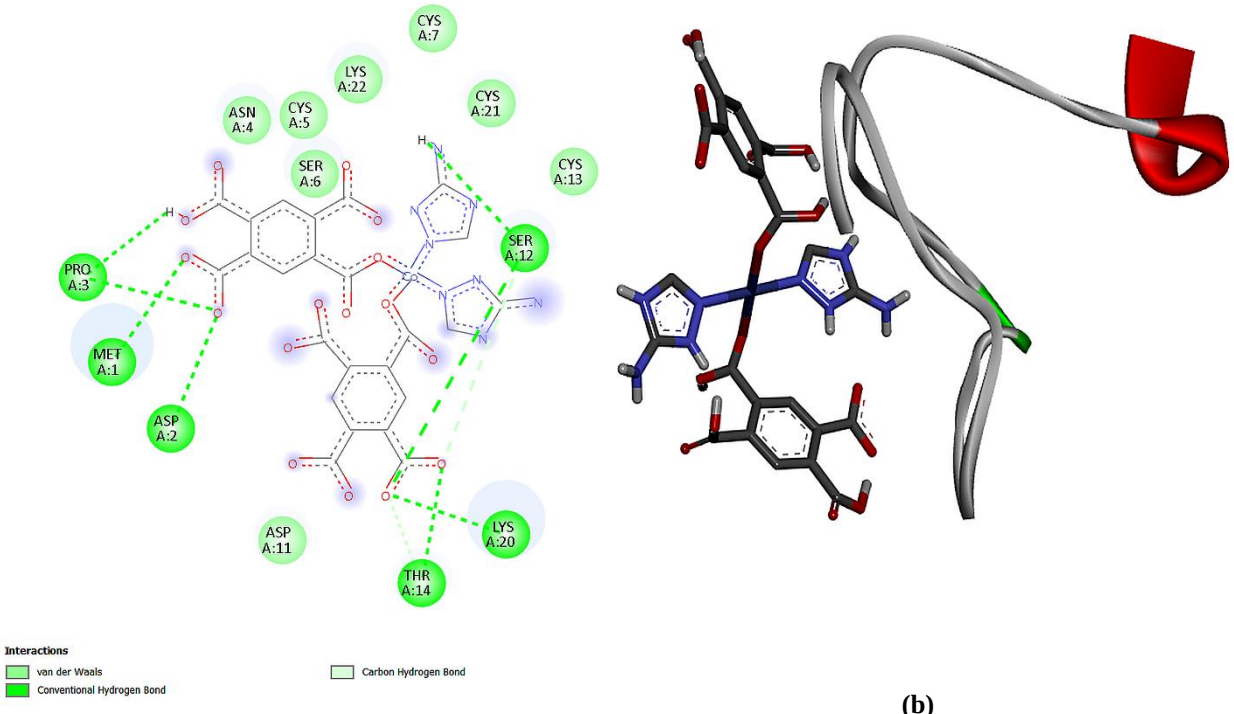
The Co(II) complex behaves differently. Its best pose is found in C1 (−6.6 kcal mol⁻¹) and its interaction profile is qualitatively distinct (Fig. 4). Six conventional hydrogen bonds are formed — with Met1, Asp2, Pro3, Ser12, Thr14 and Lys20 — together with one carbon–hydrogen bond to Thr14 and a van der Waals shell comprising eight residues. Critically, not a single contact of the π-sulfur, π-alkyl or π-sigma type is present. It is the carboxylate arms, not the triazole rings, that face the protein; the triazoles point towards the solvent. The binding is therefore of the same purely electrostatic character as that of free pyromellitic acid, merely amplified by the larger molecular surface.

The contrast between the two poses is seen most clearly in the orientation of the triazole rings. In the Cu(II) complex they are turned inward, towards the sulfur-rich wall of the groove, and account for all five π-type contacts of that pose. In the Co(II) complex the same rings are turned outward, into the solvent, and contribute nothing to the binding. The pyromellitate arms, by contrast, behave similarly in both cases, supplying carboxylate oxygens for hydrogen bonding to backbone amides. What differs is therefore not which ligand binds, but which face of the assembly is presented to the protein.

It is also worth noting how each complex relates to its own components. The Cu(II) complex occupies the same cavity as both free ligands and reproduces two of their contacts — the hydrogen bond to Cys7 formed by free triazole and the π-sulfur interaction of the same ring — while adding contacts that neither ligand achieves alone. The Co(II) complex, by contrast, reproduces the pose of free pyromellitic acid almost exactly: the same region of the surface, the same hydrogen bonds to Met1, Asp2 and Pro3, and the same absence of π-contacts. In this sense the cobalt species behaves as an enlarged pyromellitate, whereas the copper species behaves as a genuinely new binding entity.

The geometric reason for this behaviour can be read directly from the pose. In the Co(II) structure the metal centre lies further from the cluster than in the copper case, and the plane defined by the two triazole rings is rotated away from the protein surface rather than towards it. Whatever contact the rings might have made is thereby lost, and binding falls entirely to the carboxylate arms. Since those arms are chemically identical in both complexes, the Co(II) species can do no better than the free acid does on its own, apart from the additional surface its bulk provides.

The same reasoning explains why the cobalt species prefers a different cavity. Binding that depends only on carboxylate hydrogen bonds is indifferent to the presence of sulfur and responds instead to the extent of accessible backbone, which is greater in C1 than in C2.



(a) **(b)**

Figure 4. The best pose of the Co(II) complex in the C1 cavity (−6.6 kcal mol⁻¹): (a) interaction diagram, showing only hydrogen bonds and van der Waals contacts; (b) three-dimensional view, with the triazole rings directed away from the protein

Table 3. Non-bonded contacts in the best poses (residues numbered according to chain A of 2MHU)

Contact type	L1, C2	L2, C2	Cu (II), C2	Co(II), C1
Hydrogen bond	Cys7, Ser12, Lys20	Met1, Asp2, Pro3, Ser6, Cys21, Lys22	Cys7, Cys26, Lys25, Ser28	Met1, Asp2, Pro3, Ser12, Thr14, Lys20
C–H hydrogen bond	–	–	–	Thr14
π-Sulfur	Cys7	Met1	Cys7, Cys26	–
π-Alkyl	Cys21	–	Cys13	–
π-Sigma	–	–	Ala8, Thr27	–
Amide-π stacking	Lys20 region	Asn4	–	–
van der Waals	Cys5, Ser6, Cys13, Cys15, Thr14	Cys5	Gly10, Ser12, Thr14, Ala9, Asp11, Cys29	Asn4, Cys5, Ser6, Cys7, Cys13, Cys21, Lys22, Asp11
Cys engaged by π-contact	2	0	3	0

Quantitative comparison of contact profiles

The contacts listed in Table 3 can be grouped by their directional character. Hydrogen bonds and π-type interactions impose geometric requirements on the ligand, forming only at defined angles and distances. Van der Waals contacts carry no such requirement and increase in proportion to molecular size. Contacts of the first group therefore reflect the selectivity of binding, those of the second only its extent.

Counted this way, the four species differ sharply. 3-Amino-1,2,4-triazole forms six directional contacts, pyromellitic acid eight, the Cu(II) complex nine and the Co(II) complex seven (Table 4). The decisive difference, however, lies in composition rather than number: of the nine directional contacts of the Cu(II) complex, five are of π-type, while the Co(II) complex forms none at all. The Cu(II) complex therefore achieves its higher directional count through an interaction class that is entirely unavailable to its cobalt analogue.

Table 4. Comparison of directional and non-directional contact numbers

Parameter	L1	L2	Cu(II)	Co(II)
Hydrogen bonds	3	6	4	**7**
π-type contacts	**3**	**2**	**5**	0
Total directional	6	8	9	7
Share of π-contacts, %	50	25	56	0
van der Waals contacts	5	1	6	8

Engagement of the cluster-forming cysteines

All nine cysteines of the β-domain — Cys5, Cys7, Cys13, Cys15, Cys19, Cys21, Cys24, Cys26 and Cys29 — participate in the Cd_3S_9 cluster; none is a spectator residue. It is therefore informative to ask which of them each species reaches, and by what kind of force.

The Cu(II) complex engages three cysteines — Cys7, Cys13 and Cys26 — through π-type interactions, and reinforces two of these (Cys7 and Cys26) with hydrogen bonds. One third of the cluster-forming cysteines are thus held by directional forces. The Co(II) complex reaches four cysteines (Cys5, Cys7, Cys13 and Cys21), but exclusively through van der Waals contact, which carries no geometric preference.

The significance of this difference is that perturbing the coordination environment of a cluster metal is, in principle, possible only through directional contact with a thiolate sulfur. A van der Waals shell holds the molecule at the surface but does not affect the electronic state of the sulfur. Thus, although both complexes bind the protein with equal strength, only the Cu(II) complex possesses contacts of a type capable of interfering with cluster function.

The nature of the chelation effect

At first sight the advantage of the complexes might be explained by size: they are the largest species examined, and AutoDock Vina rewards buried surface. Analysis of the contacts, however, shows this explanation to be insufficient. Free pyromellitic acid forms six hydrogen bonds and free triazole three. Had the complex presented the same donors in the same manner, one would expect a hydrogen-bond-rich profile approximating the union of the two residue sets. In practice the Cu(II) complex forms fewer hydrogen bonds than pyromellitic acid — four against six — yet still binds more strongly.

The reason is the emergence of a new contact class. Coordination fixes the two triazole rings on opposite sides of the metal centre at a defined separation. As a result the rings can engage two cysteine sulfurs at the same time, because their mutual arrangement is not sampled at random but enforced by the coordination environment. This is the preorganisation effect familiar from macrocyclic chemistry, transposed onto a protein surface. Free triazole, lacking such a constraint, achieves only one π-sulfur contact.

The difference between the Co(II) and Cu(II) complexes

That two compounds identical in composition and differing only in the central ion are recognised by the same protein in two different ways is the most interesting result of this work. The Cu(II) complex directs its triazole rings towards the thiolate cluster and enters into π-type contacts with three (Cys7, Cys13, Cys26) of the nine cysteines that constitute the metal-binding apparatus of the domain. The Co(II) complex turns the opposite face to the protein, selects a different cavity and forms no π-contacts at all.

This difference cannot be called accidental, since it accords with two established principles of coordination chemistry. First, according to Pearson's theory of hard and soft acids and bases, thiolate sulfur is a typical soft base. Cu(II) is a borderline acid inclined towards softness, whereas Co(II) is harder. The contact of the ligand shell of the Cu centre with a sulfur-rich wall, and the turn of the Co centre towards carboxylate and backbone oxygen, are therefore the expected outcome.

Second, in accordance with the Irving–Williams series, Cu(II) binds metallothionein thiolates with an affinity several orders of magnitude higher than Co(II) — an experimentally established fact. The docking result agrees with this independently: it is the Cu complex that selects the cluster region.

A third factor is coordination geometry. Cu(II), with its d^9 configuration, is subject to Jahn–Teller distortion and adopts an axially elongated, anisotropic environment. In such an environment the two triazole rings are presented as a distinct protruding face able to contact a sulfur-rich wall. Co(II), with d^7, prefers a more regular geometry in which the ligands are evenly distributed and no such face arises. It should be emphasised that this third explanation, although consistent with the available crystallographic data, was not verified computationally in the present work and is regarded as a hypothesis.

The difference in site selection obeys the same logic. A species binding through hydrogen bonds formed by peripheral carboxylate arms does not require a concave site; it requires a broad, accessible area of backbone — exactly what C1 provides. A species binding through π-contacts requires proximity to the thiolate cluster and therefore remains in C2. Binding type and site selection are thus two consequences of a single cause.

Limitations of the method

The following must be taken into account when interpreting the results obtained.

– The AutoDock Vina scoring function contains no explicit term for metal–ligand coordination: the metal is treated as an ordinary atom. This is a recognised shortcoming of general-purpose docking programs applied to inorganic systems, and it has prompted the development of dedicated tools for metal complexes [15,16]. The scores presented here are therefore an empirical ranking measure rather than binding free energies.

– The search box for the complexes measured 27 Å, while the cross-section of the β-domain is about 25 Å. That is, the five calculations for the complexes effectively covered the whole protein and the cavities are not fully independent. This is confirmed by the narrow spread of scores (1.1 for Co and 0.8 kcal mol^{-1} for Cu). Assignment of a complex to a particular cavity should therefore be taken as indicative rather than as a firm conclusion.

– The protein was treated as rigid. Metallothioneins, however, are conformationally mobile structures, and the β-domain samples a broad set of states in solution [4,5]. Induced-fit effects were not taken into account.

– The receptor retains its native Cd_3S_9 cluster, so binding was computed with respect to a metal-loaded rather than an apo protein. This corresponds to the physiological state, but it means that processes involving metal exchange lie outside the scope of the calculation.

– Only the β-domain (residues 1–30) was studied. The α-domain, which binds four metals through eleven cysteines, may behave differently.

– The computational results have not been confirmed by experimental measurement. Isothermal titration calorimetry, a competitive displacement assay or ^{105}Cd NMR titration would be required for this purpose.

Nevertheless, the internal comparative logic of the work is preserved: the four species were docked to the same protein, into the same cavities and with the

same scoring function. The relative ordering — and, more importantly, the qualitative difference in the set of interactions — is therefore independent of the systematic errors affecting the absolute values.

Practical implications

If the pattern described here is confirmed experimentally, it points to a design principle rather than to a single compound. The active element is neither the metal alone nor the ligand alone, but the geometry that coordination imposes on the ligand set. Two rings held at a fixed separation reach two thiolate sulfurs; the same rings, free in solution, reach one.

It follows that the spacing and mutual orientation of aromatic donors — quantities governed by the coordination number and geometry of the central ion — are the parameters worth varying in a systematic search for metallothionein-selective compounds. The logical next step would be to test complexes of higher coordination number, or structures with a rigid chelating backbone that fixes the ring separation. Such an approach has an advantage over conventional chelators in that it controls selectivity through the shape of the compound rather than its composition.

Conclusions

1. Co(II) and Cu(II) complexes based on 3-amino-1,2,4-triazole and pyromellitic acid bind the β-domain of metallothionein-2 appreciably more strongly than the free ligands: the difference amounts to 1.6–1.8 kcal mol⁻¹ relative to the better free ligand and is preserved in all five cavities without exception.

2. The reason for this advantage is not a simple accumulation of interactions but a change in the type of binding. The Cu(II) complex forms fewer hydrogen bonds than free pyromellitic acid yet binds more strongly, because coordination fixes its triazole rings in a geometry that permits simultaneous π-sulfur contact with Cys7 and Cys26.

3. The two complexes are equipotent by docking score (a difference of 0.2 kcal mol⁻¹, below the resolution of the method), but differ clearly in binding site and type: the Cu(II) complex selects the C2 groove above the thiolate cluster and forms π-contacts with three cysteines, whereas the Co(II) complex selects the flatter C1 surface and is confined to hydrogen bonds.

4. This difference accords with Pearson's theory of hard and soft acids and bases and with the Irving–Williams series: soft thiolate sulfur contacts the ligand shell of the softer Cu(II) centre. The nature of the central ion therefore determines how the compound is recognised rather than how tightly it binds.

5. Comparison with the free-ligand poses shows that the two complexes stand in different relations to their own components. The Cu(II) complex reproduces two contacts of free triazole and adds contacts that neither ligand achieves alone, whereas the Co(II) complex reproduces the pose of free pyromellitic acid almost exactly. The cobalt species therefore behaves as an enlarged pyromellitate, while the copper species behaves as a new binding entity.

6. Of the nine cluster-forming cysteines, the Cu(II) complex holds three (Cys7, Cys13 and Cys26) by directional π-contacts, while the Co(II) complex reaches four but only through van der Waals contact. Since the coordination environment of a cluster metal can be perturbed only by directional contact with a thiolate sulfur, this distinction is of practical significance.

7. The results obtained show that coordination compounds may act on metallothionein through a mechanism distinct from that of conventional chelators — as preorganised recognition elements. Developing this direction requires experimental confirmation, study of the α-domain and the use of a scoring scheme parameterised for metal centres.

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