

Supporting Material

Computational Method - CP-PAW

The PAW method is an ab-initio k-space all-electron method in the Kohn-Sham scheme [1] of the DFT, where the wave function is expanded into an augmented plane wave basis set. In this case, the system was simulated as a truly isolated molecule using a standard repeated super-cell approach. Wave function overlap and artificial electrostatic interactions between periodic images were prevented using a charge decoupling scheme suggested by Blöchl and keeping a minimum distance of 6 Å between periodic images. Geometry optimizations were performed using 40Ry and 160Ry as “cut-off” energy values in the corresponding expansions of the wave functions and charge densities. The number of projector functions per angular momentum used was one s for hydrogen atom; two s, two p, and one d for carbon, nitrogen and oxygen atoms; and an additional d-projector function was used for copper. This choice of computational parameters has been already shown to be well suited to describe electronic and structural properties in similar compounds using the CP-PAW code.

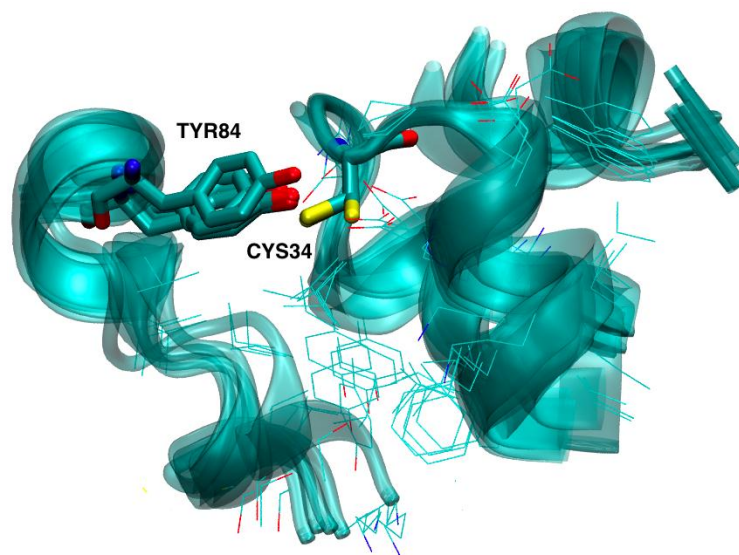


Figure S1 – Representative structures of Cys34 site at HSA, according to pdb files. Used codes: 1BJ5, 1N5U, 1BKE, 1BM0, 1HK5 and 3A73.

Similar experimental data for complex **1**, using bovine serum albumin (BSA)

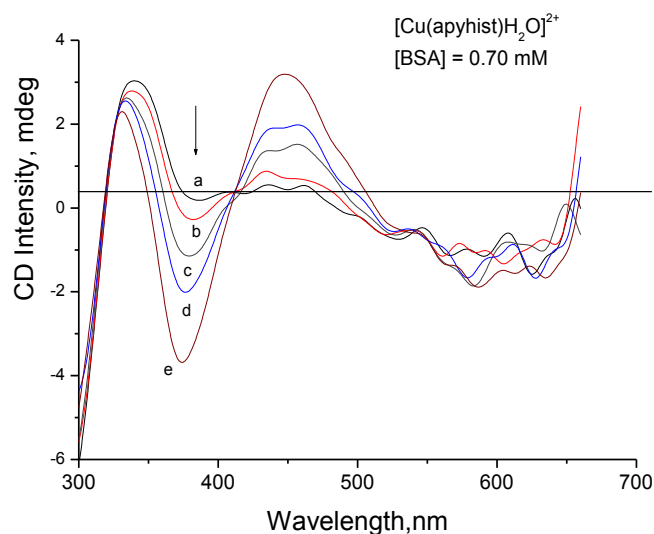
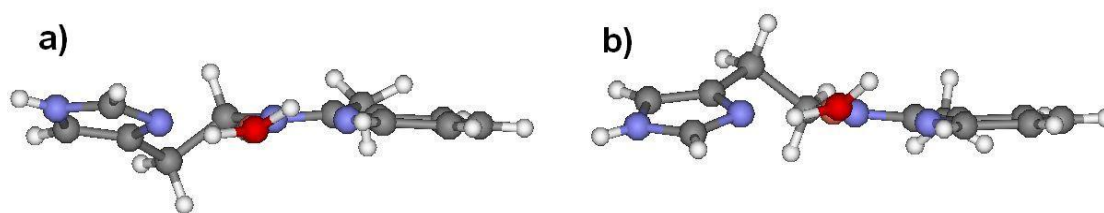


Figure S2 - CD spectra of BSA solution (0.70 mM) after addition of complex $[\text{Cu}(\text{apyhist})\text{H}_2\text{O}]^{2+} **1** (different amounts of a 1.50 mM solution), in phosphate buffer 10 mM (pH = 7.3). $[\text{CuL}]/[\text{BSA}] = 1:10$ (a); 1:5 (b); 1:4 (c); 1:3 (d); 1:2 (e).$

Ligand conformers

The ligand *apyhist* coordinated to copper ion has had its crystal structure resolved in related complexes previously published [2]. Two possible conformational structures were found due to the flexibility of methylene groups $(\text{CH}_2)_2$, connected to the heterocyclic ring showing similar copper coordination, as shown bellow.



Apyhist ligand with two conformational possibilities related to the $-(\text{CH}_2)_2$ -imidazole flexibility structures. a) down conformation b) up conformation.

The geometry optimizations performed here are in very good agreement with experimental results. Therefore, the calculated electronic and energetic properties of these conformers did not show any remarkable difference among the different complexes (data not shown).

Molecular properties analysis

The vertical electron affinity (EA) was calculated as a difference, $EA = {}^0E_{[CuL]^{2+}} - E_{[CuL]^+}$, where the first term is the total energy of the oxidized relaxed geometry of the copper(II) complex and the second is the total energy of the reduced state calculated in the oxidized geometry. Analogously, the vertical potential energy (IP) was calculated as $IP = {}^0E_{[CuL]^+} - E_{[CuL]^{2+}}$, where the first term is the total energy of the relaxed reduced form of the copper complex and the second one is the total energy obtained for the oxidized state in the geometry of the reduced state. All these energies were obtained using the CP-PAW code.

Charge Decomposition Analysis and Energy Decomposition Analysis

In CDA, the Kohn-Sham MOs of complexes are expressed as a linear combination of MOs of ligands (donors) and copper(II) ion (acceptor) in the geometry of the complex. The interaction between occupied orbitals of ligands L with unoccupied orbitals of copper is defined as donation, *d*. According to Frenking and co-workers, [3,4] the term donation denotes not only charge transfer, but also includes an overall reorganization of electronic charges, polarization, exchange repulsion, and charge transfer. In complexes such **1**, containing H₂O as ligand, L denotes the Schiff base and the water molecule as well. The interactions involving occupied orbitals of the metal and vacant orbitals of L are defined as back-donation, *b*. Two other terms derived from CDA are the repulsive polarization, *r*, and the rest term, Δ . The former is related to the overlap between occupied orbitals of the interacting fragments, while the latter is calculated from the overlap of unoccupied orbitals in the fragments. The rest term, Δ , is an indicative of true donor-acceptor character ($\Delta \cong 0$). Deviations from zero indicate that the complex is

more appropriately described by interactions of open-shell fragments, that is, the interactions are more covalent. According to EDA,[5, 6] the following contributions to the electronic interaction energy, ΔE_{int} , between two fragments in a molecule can be defined as electrostatic energy ΔE_{elstat} , exchange repulsion energy ΔE_{exch} , and orbital interaction energy ΔE_{orb} (equation 1)

$$\Delta E_{\text{int}} = \Delta E_{\text{elstat}} + \Delta E_{\text{exch}} + \Delta E_{\text{orb}} \quad (1)$$

The electrostatic energy and exchange repulsion energy can be combined together into a single term named steric ΔE_{steric} , and as a result equation 2 can be defined.

$$\Delta E_{\text{int}} = \Delta E_{\text{steric}} + \Delta E_{\text{orb}} \quad (2)$$

As expected, CDA results have shown that all complexes presented strong electron donation from ligands to the metal (Table 3), while the back-donation is not significant, as indicated by $L \rightarrow Cu$, $L \leftarrow Cu$ and $b/(d+b)$ values. The donation values are quite similar in all complexes, in particular complex **1** exhibits a slightly large donation value 1.373 (e). All r values are negative and close to zero, which is a consequence of the repulsive polarization involving interactions between filled orbitals of ligands and filled or partially filled orbitals of the metal centre. The r values are directly related to back-donation values. It can be noticed that the back-donation increases from **1** to $[Cu(\text{BSA-N}_{\text{terminal}})]^+$, while $[Cu(\text{HSA-N}_{\text{terminal}})]^+$ exhibits an intermediate value. In all cases, the rest term Δ is very close to zero, indicating that the interactions can be described as donor-acceptor type, such as in the Dewar-Chatt-Duncanson model.[7, 8] The EDA results obtained show clearly that the interaction between the copper(II) ion and the ligands is more strong in $[Cu(\text{BSA-N}_{\text{terminal}})]^+$ than in $[Cu(\text{HSA-N}_{\text{terminal}})]^+$ or complex **1**. This happens not only

due to the electrostatic ΔE_{elstat} , but also due to the orbital term ΔE_{orb} , which is dependent on the ligand size. EDA results are also well correlated with the CDA ones, suggesting that in complexes where the back-donation is more effective, the interaction energy is more stabilizing. Despite the intrinsic differences on the electronic structure and charge transfer of the considered complexes, it can be argued that the copper(II) ion in **1** is similarly stabilized, in a close range of magnitude as in $[\text{Cu}(\text{HSA-N}_{\text{terminal}})]^+$ or $[\text{Cu}(\text{BSA-N}_{\text{terminal}})]^+$. Additional CDA and EDA calculations for **3** reveal a similar trend as expected. By comparing CDA and EDA results for this complex and that for **1** (Table S1) a very similar trend is observed, that is, in both complexes the $\text{L} \rightarrow \text{Cu}$ donation is slightly more pronounced than in $[\text{Cu}(\text{BSA-N}_{\text{terminal}})]^+$ or $[\text{Cu}(\text{HSA-N}_{\text{terminal}})]^+$. In both cases the interactions verified can be described as donor-acceptor.

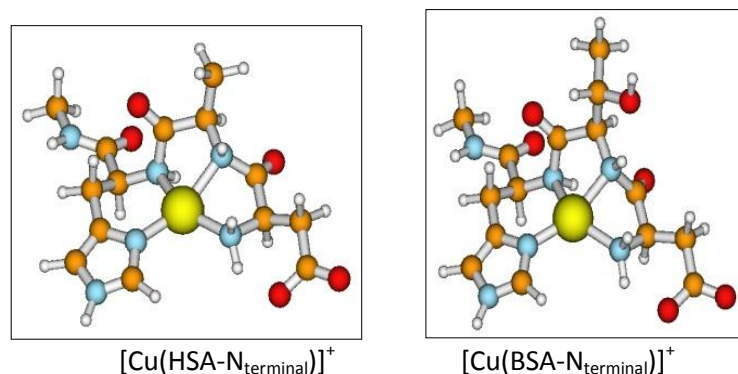


Figure S3 – Optimized structures (BP86/TZVP) for $[\text{Cu}(\text{HSA-N}_{\text{terminal}})]^+$ and $[\text{Cu}(\text{BSA-N}_{\text{terminal}})]^+$, employed to perform CDA and EDA analyses.

Table S1 – Charge decomposition analysis, CDA (e) and energy decomposition analysis, EDA (kcal.mol⁻¹).

Charge Decomposition Analysis CDA*					
Compound	L→Cu (d)	Cu→L (b)	b/(d+b) (%)	<i>r</i>	Δ
[Cu(apyhist)H ₂ O] ²⁺	1.373	0.027	1.93	-0.076	-0.133
[Cu(apyepy)H ₂ O] ²⁺	1.434	0.032	2.18	-0.163	-0.127
[Cu(HSA-N _{terminal})] ⁺	1.170	0.051	4.18	-0.145	-0.095
[Cu(BSA-N _{terminal})] ⁺	1.150	0.064	5.27	-0.160	-0.091

Energy Decomposition Analysis EDA			
Compound	ΔE _{int}	ΔE _{orb}	ΔE _{steric}
[Cu(apyhist)H ₂ O] ²⁺	-451.2	-303.9	-147.3
[Cu(apyepy)H ₂ O] ²⁺	-445.3	-306.7	-138.6
[Cu(HSA-N _{terminal})] ⁺	-571.4	-335.4	-236.0
[Cu(BSA-N _{terminal})] ⁺	-575.1	-336.0	-239.1

***d** denotes donation (expressed in e), **b** represents back-donation (expressed in e), *r* represents repulsive polarization (expressed in e), and Δ denotes the rest term (expressed in e).

Dipole Moment analysis

Dipole calculations were performed using the Gaussian03 package considering the oxidized theoretically optimized structures in the gas phase both with and without the water molecule bound to the fourth copper(II) coordination site. All copper complexes was considered in the same system axes where copper ion is centralized in the basis origin, the X axis in the Cu-O orientation and the Y axis in the Cu-N orientation, i.e. N-site of the pyridine moiety in complexes **1** and **3**, and

proximal N-site of the pyrazine in complexes **2** and **4**. These calculations, excluding the water molecule, were performed in order to avoid the strong influence of the water molecule position on the dipole moment, but also to simulate more closely the possible experimental scenario of interaction of copper species with the sulfur at the Cys34 site.

A further inspection on dipole moment, electronic levels and similarity features analysis was then accomplished in order to provide new insights regarding to the complex **1** interaction with the albumin protein. [Figures S4](#) and [S5](#) shows the dipole orientation, respectively 2.9D, 4.3D, 1.9D and 2.5D for the **1** to **4** complexes, with the water molecule, and 1.3D, 3.9D, 1.8D and 1.2 D for the corresponding complexes not considering the water molecule. Changes in the dipole moment orientation are dependent of the flexibility of methylene groups (CH_2)₂, and also the position of the water molecule. These two factors, besides the ligand characteristic, will influence the final dipole moment orientation. Without the influence of this water molecule, dipole orientation in complexes **1** and **2** points to the pyridyl moiety, and in complex **4** to the pyrazyl moiety. On the contrary, dipole moment in complex **3** points to the opposite direction, i.e. to the flexible pyridyl moiety ([Figure S5](#)). For all these complexes the water inclusion increases the dipole value. Especially complex **1** shows a higher influence of the water, changing the orientation of its dipole.

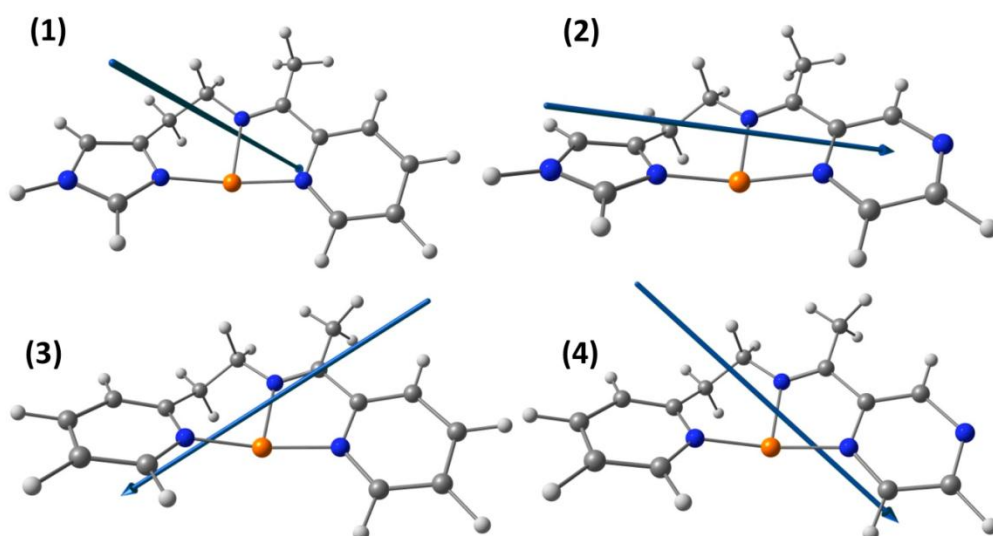


Figure S4 - Representation of the dipole moment for all the complexes, with the labile

H₂O ligand: 1) [Cu(apyhist)H₂O]²⁺; 2) [Cu(apzhist)H₂O]²⁺; 3)

[Cu(apyepy)H₂O]²⁺; 4) [Cu(apzepy)H₂O]²⁺.

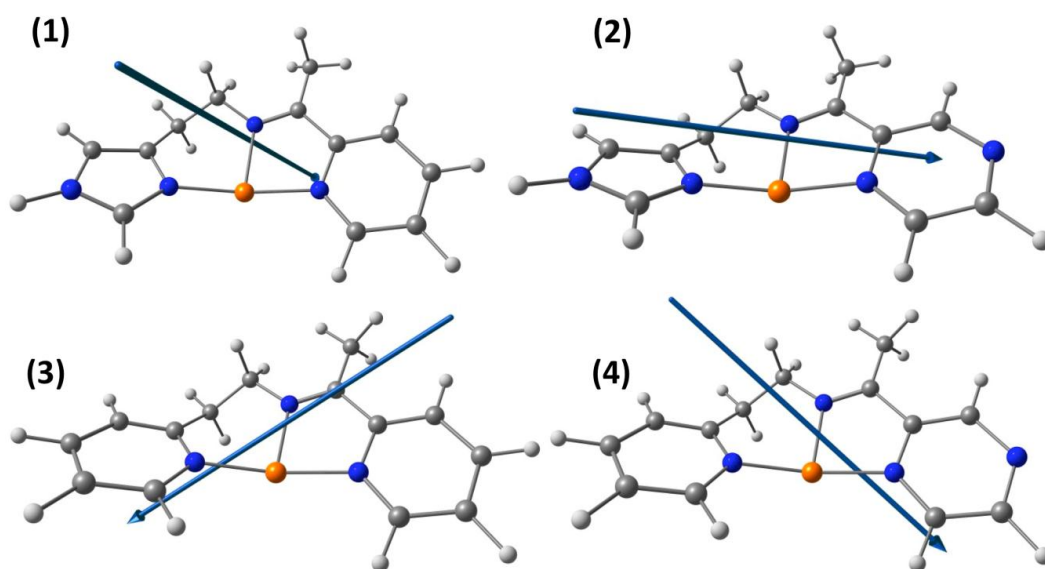


Figure S5 - Representation of the dipole moment for all the complexes, without the labile

H₂O ligand: 1) [Cu(apyhist)]²⁺; 2) [Cu(apzhist)]²⁺; 3) [Cu(apyepy)]²⁺; 4)

[Cu(apzepy)]²⁺.

Orbital and PDOS analyses

The orbital and PDOS inspection of the complexes, with some particular differences, shows mainly the contribution of the *d* orbitals in the copper ion and those orbitals with σ character in the coordinating ligands, as well as π orbitals in the imidazole ring and in N sites at the pyrazine ligands (Figure S6), in the frontier levels. For all complexes, the lowest unoccupied molecular orbital (LUMO) is localized in the metal coordination sphere, showing a $d_{x^2-y^2}$ character in the copper ion, and a σ character in the Nitrogen and Oxygen coordinating bonds. The highest occupied molecular orbital (HOMO) is singly occupied, with copper coordination contributions similar to the LUMO, but also including contributions from the imidazole ring π orbitals in complexes **1** and **2**, and from pyridine ring lone pair orbitals in complexes **2** and **4**. Complex **3** which has two pyridine moieties in the ligand, presents only metal coordination sphere contributions to HOMO. The HOMO-LUMO energy gap in all complexes is around 0.65 eV.

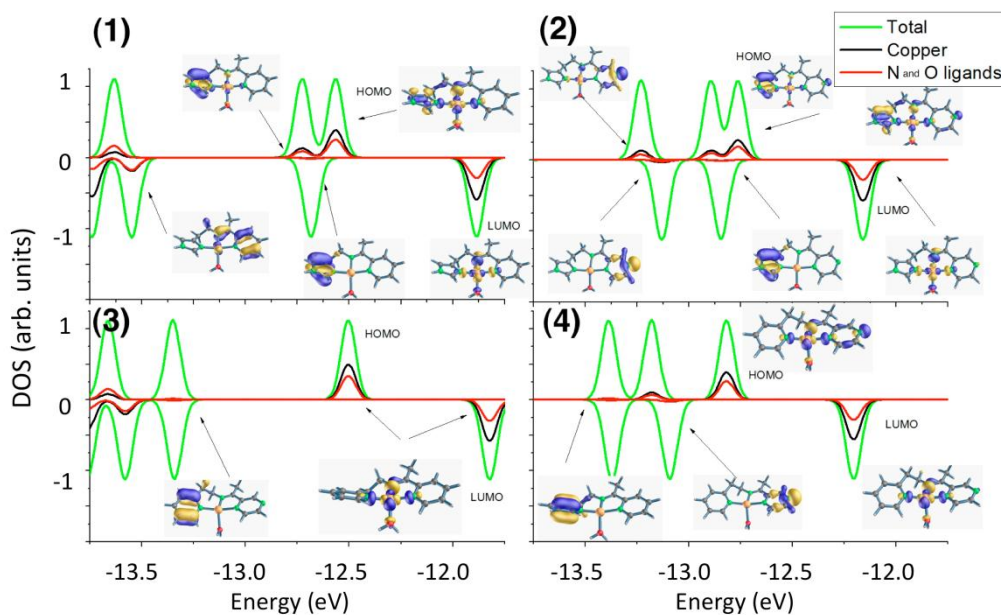


Figure S6 – Wave function representation, total and projected density of states (PDOS) from the first unoccupied electronic level and the most relevant frontier occupied levels from the **1-4** complexes. Green line represents the normalized

total density of states, in black the Copper PDOS and in red the PDOS from Nitrogen sites and Oxygen site from the Copper coordination sphere. The discrete electronic energy levels are plotted using a Gaussian broadening to better visualization.

References

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