Copper(I)-alkyl sulfide and -cysteine tri-nuclear clusters as models for metallo proteins: a structural density functional analysis

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Communicated by Ramaswamy H. Sarma

(Received 23 January 2012; final version received 8 April 2012)

After having set up the computational methodology for Cu(I)-sulfur systems as models for copper proteins, namely using the simple ligands H2S, HS−, CH3SH, and CH3S−, the Cu(I)-Cysteine systems have been investigated: [CuI(H2Cys)n]+ (H2Cys, cysteine, NH2,SH,COOH) [CuI(HCys)n]1−a (NH2,SH,COOH). Finally, the structures for bi-nuclear [CuI(S−S)2]2+ (Et, CH2CH2), [CuI(S−SEt)2]2+ and tri-nuclear [CuI(S−SH)3]3−, [CuI(S−SEt)3]3−, [CuI(S−H2Cys)3]3− (NH2,SH,COOH), [CuI(S−HCys)3]3− (NH2,S−, COOH, and NH2,SH,COO−), as well as [CuI(S−Cys)6]9− (NH2,S−,COO−), were also optimized to mimic the active center for a metallo-chaperone copper transport protein (CopZ). The X-ray structures for the biomolecules were matched fairly well as regards the Cu−S bond distances and Cu…Cu contact distances in the case the model cysteine S atom is deprotonated. Upon protonation of ligand S atoms, the conformation of clusters is altered and might bring about the di- and tri-nuclear core breakage. These findings suggest that subtle protonation/deprotonation steps, i.e. small and/or local pH changes play a significant role for copper transport processes.

Keywords: density functional; copper transport; copper proteins; molecular modeling

Introduction

Copper ions occur as inorganic cofactors in a variety of enzymes and metallo-proteins implicated in vital cellular functions ranging from small molecule activation and subsequent redox chemistry (Gorelsky, Ghosh, & Solomon, 2006; Iwata, 1998; Iwata, Ostermeier, Ludwig, & Michel, 1995; Tolman, 2010) to electron transfer (Solomon, Szilagyi, DeBeer, & Basumallick, 2004; Solomon, Xie, & Dey, 2008). Many proteins are also dedicated to copper transport in and out of cells and between cellular compartments, to activate copper-dependent metallo-proteins, or for copper storage (Boal & Rosenzweig, 2009; Calderone et al., 2005; Cobine, Pierrel, & Winge, 2006; Culotta et al., 1999; Huffman & O’Halloran, 2001; Lutsenko, Barnes, Bartee, & Dmitriev, 2007). It is only a recent achievement that all such different copper-dependent functions are deeply interrelated as cellular copper homeostasis involves a balance between intake and efflux systems and depends on a complex machinery whose tools are proteins able to shuttle copper(I) ions from uptake and storage sites to target enzymes and proteins (Banci, Bertini, Cantini, & Cioffi-Baffoni, 2010; Banci et al., 2010; Banci & Rosato, 2003; Bertini & Cavallaro, 2008; Finney & O’Halloran, 2003). The trafficking of copper ions within the cell is regulated by specific proteins called copper-chaperones able to bind copper and to deliver it to partner proteins and enzymes (Banci et al., 2010; Elam et al., 2002; Harris, 2000; Harrison, Jones, Solioz, & Dameron, 2000; Horn & Barrientos, 2008; Kim, Nevitt, & Thiele, 2008; Lu & Solioz, 2002; O’Halloran & Culotta, 2000; Prohaska & Gyzina, 2004; Puig & Thiele, 2002; Robinson & Winge, 2010; Rosenzweig, 2001; Rosenzweig & O’Halloran, 2000; Tottey, Harvie, & Robinson, 2005).

Besides the recent discovery of the role of Cu(I) thioether chemistry in copper transport (Arnesano et al., 2003; Davis & O’Halloran, 2008; Peariso, Huffman, Penner-Hahn, & O’Halloran, 2003), the most common motif able to recognize and bind copper in copper-chaperones requires cysteine residues in CXXC arrangement both in prokaryotic and eukaryotic organisms (Arnesano et al., 2002; Banci, Bertini, & Cioffi-Baffoni, 2009). As a
consequence, the chemistry of copper transfer in copper-chaperones ultimately depends on the properties of Cu(I)-thiolate bonds that allow the thermodynamic and kinetic control of copper-dependent cellular processes. In addition to the interest related to the role of the metal sulfur (thiolate) bonds in the activity of many metallo-enzymes, much effort has been dedicated to explore metallo-chaperone copper transport proteins in solution and at the solid state (Banci, Bertini, Cantini, & Ciofi-Baffoni, 2010; Calderone et al., 2005; Davis & O’Halloran, 2008; Horn & Barrientos, 2008; Huffman & O’Halloran, 2001; Puig & Thiele, 2002; Robinson & Winge, 2010; Rosenzweig, 2001; Rosenzweig & O’Halloran, 2000; Tottey, Harvie, & Robinson, 2005). These studies resulted in mechanistic proposal(s) for copper transfer that appears to happen through affinity gradients and low activation barriers achieved by a network of metal-donor covalent, electrostatic, hydrogen bond interactions in the vicinity of the metal binding site (Banci, Bertini, Cantini, & Ciofi-Baffoni, 2010; Boal & Rosenzweig, 2009; Calderone et al., 2005; Cobine, Pierrel, & Winge, 2006; Ceotto et al., 1999; Lutsenko, Barnes, Bartee, & Dmitriev, 2007; Huffman & O’Halloran, 2001). Despite the large amount of experimental data available on Cu(I)-metallo-proteins, very few or no theoretical studies are available at present in the literature about the description of Cu(I)-thiolate bonds that can be used to deeply understand the copper trafficking pathways in proteins at our knowledge. Density functional methods, especially the hybrid B3LYP exchange-correlation functional, were proven to be accurate and fast for investigating adequate models for the active sites of metallo-enzymes (Siegbahn, 2006; Siegbahn & Himo, 2009).

We wish to report here on selected results from a density functional structural study relevant to Cu(I) complexes of di- and mono-hydrogen-sulfide, methyl sulfide, and cysteine ligands in order to shed light on the mechanism of copper transfer processes in proteins. We do not intend to analyze the mechanism of copper trafficking itself, but rather to get some understanding on the processes via structural optimizations.

### Molecular modeling details

All the density functional theory (DFT) computations were performed by using the Gaussian09 (Frisch et al., 2010) packages implemented on IBM SP5 and SP6 machines at CINECA (Inter-University Computing Center, Casalecchio di Reno, Bologna, Italy). All the structures were fully optimized at the gas phase by using the B3LYP method (Frisch & Frisch, 1998) and the 6–31G** (Frisch & Frisch, 1998) basis set for all the atoms (BS1). The ligands and most of the complex molecules were also calculated at higher level of theory, B3LYP/6–311+G** (BS2) (Frisch & Frisch, 1998) for comparative purposes. The solvent (water) was taken into account by using Polarizable Continuum Model (PCM) method (Tomasi, Mennucci, & Cammi, 2005) and the structures were fully optimized at BS1 W (BS1 + PCMwater) and often even at BS2 W (BS2 + PCMwater) levels of theory.

All the Cu(I) complex structures were computed at spin multiplicity 1, whereas the Cu(II) complex structures were computed at spin multiplicity 2 (an unpaired electron).

The optimization was carried out up to reaching the convergence criteria implemented on Gaussian03:09 (maximum force, 0.000450 Hartrees/Bohr; RMS Force 0.000300 Hartrees/Bohr; maximum displacement 0.001800 Å; and RMS displacement 0.001200 Å). The hessian analysis was also performed and the structure was accepted as ‘fully optimized’ at no imaginary frequencies. Molecular drawings were obtained by using GaussView03 software (Dennington et al., 2003) implemented on Pentium IV machines.

The analyzed simple models, used for setting up the computational strategy were: H2S, HS−, [Cu(I)(S-SH)2]+, [Cu(I)(S-SH)n]1−n (n = 1–4), [Cu(II)(S-SH)2]+, [Cu(II)(S-SH)n]2−, CH3SH, CH3S−, [Cu(II)(S(H)CH3)n], [Cu(II)(SCH3)n]1−n, and (n = 1–4). The summary for this part of the work is just here after listed.

**H2S and HS−**

The free ligands H2S and HS− were optimized at BS1 and BS2 levels of theory (see Supporting material Table S1 and Figure S1) and showed a very good agreement between the two basis sets. The two free ligands were also optimized taking into account the solvent (water) effect through PCM (Tomasi, Mennucci, & Cammi, 2005) The calculated bond distances and angles at BS1 W level agree very well for protonated and not protonated ligands. In conclusion, the changes of basis set do not alter significantly bond distances and angles.

$$[\text{Cu}(\text{S-SH})_n]^+$$ and $$[\text{Cu}(\text{S-SH})_n]^{1−n} (n = 1–3)$$

The mono-, bi-, and tri-coordinate models (Figure S2(a–c) and Table S2) showed elongations by 0.090 Å (or smaller, BS2) on adding an extra ligand. The bi-coordinate species, fully optimized to an almost C2 symmetry (S–Cu–S, 175.7°, BS2), whereas the structure optimization of a tri-coordinate molecule has a quasi-C3h symmetry (S–Cu–S, 119.9° (av), BS2). The data reported above for the di-hydrogen-sulfide derivatives are in agreement with those previously published by others (Pavelka, Šimánek, Šponer, & Burda, 2006). The computed Cu–S bond distances for $$[\text{Cu}(\text{S-SH})_n]^{1−n} (n = 1–3)$$ models are reported in Figure S2(d–f) and Table S2 (Supporting). By starting from different input structures, the same final quasi-C2 symmetry was obtained for the bi-(mono-hydrogen-sulfide). The tri-
coordinate derivative, by starting from a C₄ structure, converged to a C₃ₙh symmetry. The computations at BS2 give Cu–S vectors significantly longer than the corresponding values at BS1. These structures were also confirmed when the water solvent effect was considered (both BS1 W and BS2 W).

\[ \text{[Cu}^{II}(S-SH)_{4}]^{2+} \text{ and [Cu}^{II}(S-SH)_{4}]^{3--2--} \]

The complex models for the tetra-coordinate species were computed both as Cu(I) and Cu(II) metal center. Considering a Cu(I) complex, on starting from a planar arrangement, the computations converge to a H₂S… [Cu'(S-SH₂)₂]…H₂S system (BS2), whereas starting from a pseudo-tetrahedral arrangement, it fully converges to a pseudo-tetrahedron with Cu-S by 2.327 Å (av, Figure S3; 2.428 Å, av, BS2 W). For comparison purposes, the Copper(II) species was also computed and a pseudo-tetrahedral arrangement was optimized (Figure 1(a)). At BS2 W, the convergence is rather oscillating having Cu–S distance by 2.417 Å (av). Several attempts aimed at refining [Cu'(S-SH)₄]³⁻ (by starting from both pseudo-tetrahedral and planar arrangements) do not converge and the structures collapse, always dissociating two HS groups and originating a [Cu'(S-SH)₃]²⁻ (C₃ₙb) system. On the contrary, when starting from a pseudo-tetrahedral [Cu'(S-SH)₄]²⁻ (C₁) structure, fully convergence is obtained, with the optimized structures in a compressed tetrahedral arrangement of the donors (pseudo-D₂, Figure 1(b)).

The results show that the usage of BS2 and BS2 W basis sets is recommended for low coordination number complexes and small overall number of atoms. See Table S3 (Supporting) for more geometrical parameters.

CH₃SH and CH₃S⁻

The computed S–C, S–H, and C–S–H bond parameters for CH₃SH free molecule are well reproduced and the change of basis set and inclusion of solvent effects do not alter significantly the results. Instead significant effects are recorded for CH₃S⁻ free anion (Figure S4 and Table S4).

\[ \text{[Cu}^{II}(S-SH)_{4}]^{+} \text{ and [Cu}^{II}(S-SCH)_{3}]^{1--n} \text{ and (n = 1–4)} \]

Computed Cu–S distances for protonated and not protonated derivatives (n = 1–3) are reported in Tables 1 and S5 and 2 and 2 and show an elongation of the coordination bond upon increasing the coordination number and upon protonation. Figures 2(a) and (b) show the drawings for compounds [Cu'(S-S(H)CH₃)]⁺ and [Cu'(S-SC H₃)]³⁻. It has to be noticed that computations for [Cu'(S-SCH₂CH₃)₂]⁻, [Cu'(S-S(H)CH₃)₂(S-SCH₂ CH₃)], and [Cu'(S-S(H)CH₂CH₃)]⁻ used as models for the copper efflux regulator (CueR) in bacteria (Rao, Cui, & Xu, 2010), performed via density functional methods with effective core potentials (ECP) for metal and 6–31+G* basis set for main group elements (sometimes improved up to 6–311++G**), produced structural arrangements similar to those from the present work and even bond distances agree well. A certain degree of elongation by 0.03–0.06 Å is recorded at ECP level.

The calculated Cu–S–C bond angle for [Cu'(S-S(H)CH₃)]⁺ is in agreement with the finding from classical VSEPR model (Gillespie, 1963) (repulsions decrease in the order: Lone pair (LP)-LP > LP-Bond Pair (BP) > BP-BP) (BS2: Cu–S–C, 108.0° for S-protonated and 104.7° for S-deprotonated). A tri-coordinate CH₃S⁻ derivative, C₁, pseudo-C₃ₙb, has S–Cu–S and Cu–S–C optimized bond angles of 120.0 and 103.6° (av, BS2), respectively. The protonated bi- and tri-coordinate derivatives also converge nicely. Instead the complex with four SCH₃ anions does not converge successfully (gas phase and water continuum) going toward dissociation of a ligand, when starting from a tetrahedral input structure. When the solvent treatment (BS2 W) is applied, the [Cu'(S-S (H)CH₃)]⁺ species successfully converges to a pseudo tetrahedral arrangement (Figure 2(c)). Search on the Cambridge Structural DataBase (Allen, 2002) (queries: Cu–S–CH and Cu-SH–CH residues) reveals that most of the experimental structures have a chelate arrangement (S,X-Cu, and X=N,O) from different ligands. However, the Cu–S and S–C bond distances for Cu(I) complexes range 2.26–2.34 and 1.77–1.84 Å, respectively, showing an acceptable agreement with computed parameters. Catena-[bis(μ²-propane-1,3-dithiolato)copper(I) iodide]

![Figure 1](image.png)
Table 1. Selected average bond distances (Å) and angles (°) for fully optimized \([\text{Cu}(\text{S}-\text{S(H)CH}_3)_n]^+\) \((n=1–4)\) models as calculated via DFT methods at BS1, (BS2), [BS1 W], and {BS2 W} levels of theory.

| Vector(s) | \(n=1\) | \(n=2\) | \(n=3\) | \(n=4\) |
|-----------|---------|---------|---------|---------|
| \(\text{Cu–S}\) | 2.069   | 2.137   | 2.207   | 2.319   |
|           | (2.235) | (2.243) | (2.329) | (2.419) |
|           | [2.060] | [2.137] | [2.211] | [2.318] |
|           | {2.231} | {2.241} | {2.324} | {2.344} |
| \(\text{S–C}\) | 1.842   | 1.847   | 1.838   | 1.838   |
|           | (1.851) | (1.847) | (1.842) | (1.841) |
|           | [1.836] | [1.837] | [1.835] | [1.835] |
|           | {1.843} | {1.842} | {1.840} | {1.838} |
| \(\text{Cu–S–C}\) | 112.4   | 113.8   | 115.6   | 114.0   |
|           | (108.0) | (108.8) | (111.6) | (111.0) |
|           | [111.2] | [110.8] | [112.8] | [112.1] |
|           | {106.3} | {106.7} | {108.3} | {108.7} |
| \(\text{S–Cu–S}\) | 167.0   | 118.5   | 109.5   | 109.5   |
|           | (173.7) | (119.6) | (109.4) | (109.4) |
|           | [169.6] | [119.0] | [109.5] | [109.5] |
|           | {175.8} | {119.9} | {109.4} | {109.4} |

Note: See text for details.
Table 2. Selected average bond distances (Å) and angles (°) for fully optimized \([\text{Cu}(\text{S-SCH}_3)_n]^{1-n}\) \((n=1-4)\) models as calculated via DFT methods at BS1, (BS2), [BS1 W], and \{BS2 W\} levels of theory.

| Vector(s) | \(n = 1\) | \(n = 2\) | \(n = 3\) | \(n = 4\) |
|-----------|-----------|-----------|-----------|-----------|
| \(\text{Cu–S}\) | 2.024 | 2.121 | 2.274 | dnc |
| \(\text{(2.147)}\) | (2.205) | (2.369) | (dnc) | (dnc) |
| \(\text{[2.042]}\) | [2.114] | [2.233] | \([2.433]\) |
| \(\text{[2.166]}\) | \(\{2.196\}\) | \{2.320\} | \{2.504\} |
| \(\text{S–Cu–S}\) | 173.2 | 120.0 | dnc |
| \(\text{(178.3)}\) | (120.0) | (dnc) | (dnc) |
| \(\text{[171.4]}\) | \[120.0]\) | \[105.8/117.0]\) |
| \(\text{[177.3]}\) | \{120.0\} | \{107.8/112.8\} |

Note: See text for details; dnc, did not converge.
(Heller & Sheldrick, 2004) shows Cu–S distances by 2.336 Å in good agreement with computed parameters.

On the basis of the setups for computational details just described, more complex systems were studied: Cysteine (H₂Cys, some conformers), HCys⁻ (NH₂S⁻, COOH), HCys⁻ (NH₂SH,COO⁻), [CuI(S,O-HCys)] (NH₂S⁻,COOH), [CuI(S-O-HCys)]⁺ (NH₂SH,COOH), [CuI(S-H₂Cys)]⁺ (NH₂SH,COOH), [CuI(S-H₂Cys)]⁻ (NH₂S⁻,COOH), [CuI(S-N-HCys)₃⁻ (NH₂S⁻,COOH), [CuI(S-HCys)]⁻ (NH₂S⁻,COOH), and (n = 3,4) (see Scheme 1). In order to model a metallo-chaperone with a tri-nuclear Cu(I)-cluster and with a core bi-nuclear cluster (as part of a tetra-nuclear one) hereafter copper-chaperone, CopZ3, and CopZ4 (Hearnshaw et al., 2009; Singleton et al., 2009; see also Scheme 2), several molecules were also structurally computed: [CuI(S – S(H)Et₂)]²⁺ (Et, CH₂CH₃), [CuI(S – S(H)Et₂)]³⁺ (NH₂SH), [CuI(S – SH)]⁻, [CuI(S – S(H)Et₂)]⁺, [CuI(S – SH)]⁻, [CuI(S – S(H)Et₂)]²⁺, [CuI(S – SH)]⁻, [CuI(S – S(H)Et₂)]³⁺ (NH₂SH,COOH), [CuI(S – H₂Cys)]²⁻ (NH₂SH,COOH), [CuI(S – CyS)]³⁻ (NH₂SH,COOH), [CuI(S – CyS)]²⁻ (NH₂SH,COOH).

**Results and discussion**

After the computational methodology was constructed on the basis of what is reported in molecular modeling details section, the aminoacid cysteine was selected for further calculations in order to study the effect of ligand size expansion and with the aim to include biologically significant ligands (see just below). Selected free ligand and copper(I) and copper(II) (for a few cases) complex...
optimized structures are listed in Table S13 (Supporting).

Selected bond parameters are reported in Figures 3–10 and Tables 3–8 called under the respective sections.

**Structures**

\[ L-H_2Cys, \quad -HCys^- (S^- or COO^-), \quad \text{and} \quad -Cys_2^- (S^-, COO^-) \]

Cysteine is a very flexible molecule and therefore, several possible orientations can be assumed by the backbone. Some computational studies on the selected possible conformations have been reported by others (Dobrowolski, Rode, & Sadlej, 2007; Fernández-Ramos et al., 2000). Here, we wish to present and discuss geometrical parameters for a particular conformation of \( H_2Cys (NH_2,SH,COOH) \) computed starting from a zwitterionic structure (\( NH_2^+,SH,COO^- \)) (Figure 3(a)) and fully optimized at gas phase, with a S-H…O(=C) intra-molecular interaction (Figure 3(b); O…S, 3.278 Å and S-H…O, 12.63° (BS2), Tables 3 and S7) and those for the corresponding conformation for \( HCys^- (NH_2,S^-,COOH) \) (Figure 3(c), BS2), where a S…H-O interaction takes place (O…S, 2.936 Å and O-H…S, 159.7°, BS2). Both models presented also an intra-molecular N…O hydrogen bond interaction. The N atom acts as H-acceptor and as H-donor in cysteine and cysteinate, respectively (N…O, 2.610 and 2.691 Å and H, 123.5 and 110.4°, BS2). A Cys\(^2^-\) (NH_2,S^-, COO^-) model similar to that found via X-ray diffraction for the zwitterionic form (input, Figure 3(d)) after removal of SH proton and a proton from the amine function was optimized (see Figure 3(e) for BS2). The geometrical parameters for the ligand are in good agreement with the corresponding ones found for the protonated species. Optimized S–C and (S)C–C bond distances were 1.877 and 1.531 Å (BS2) and 1.861 and 1.539 Å (BS2 W). Calculated geometrical parameters for \( H_2Cys(NH_2,SH,COOH) \) model are also in good agreement with the X-ray structure of the zwitterionic form (Tables 3 and S7) (Moggach, Clark, & Parsons, 2005). It is interesting to note that the minimum energy optimized structure (gas phase) for \( H_2Cys \) is obtained by starting from the solid state zwitterionic form (Moggach, Clark, & Parsons, 2005; Figure 3(f)) and that the output is not zwitterionic. On the contrary, upon optimizing the zwitterionic structure from Figure 3(a) and on adding the treatment of solvent effect, the fully optimized structures maintain zwitterionic form (Figure 3(g)). Computed bond parameters are in excellent agreement with the experimental values. Other findings indicate that the final structures were significantly dependent on the starting structures as regards the orientation of thiol, amine, and carboxyl groups and were much dependent on the presence of solvent and on protonation modes.

The Cu(I) complex species went to full convergence (at BS1/W, BS2/W levels), for mono-, bi-, and tri-coordinate models with both \( H_2Cys(NH_2,SH,COOH) \) and \( HCys^- (NH_2,S^-,COOH) \) ligands. Optimized structure of \([Cu(L-S-H_2Cys)]^+ \) at BS2 level has Cu–S and S–C bond distances by 2.231 and 1.856 Å (BS2 W; see Tables 4 and S8). Comments on other Cu(I)-H2Cys complexes are reported below in this section (Figure 4). The results for \( H_2Cys (NH_2,S^-,COOH) \) derivatives are in agreement with the geometrical parameters reported in section ‘Molecular modeling details’ for small ligands, in the case of monodentate model, and show a linear and a trigonal arrangement for \([Cu(L-S-HCys)]_n^- \) and \([Cu(L-S-HCys)]_n^2^- \) (Figure 5(a) and (b)), where S–Cu–S bond angles are 179.9 and 120.0° (av, BS2), respectively (see Tables 5 and S9). The calculated Cu–S bond distances are 2.160, 2.202, and 2.331 Å (av, BS2) for the mono-, bi-, and tri-coordinate complexes, respectively. Even for the metal species, O–H…S hydrogen bonds play significant roles in stabilizing the structures. It is important to note that the mono-coordinate species has been fully optimized both as mono-dentate \([Cu(L-S-HCys)]_n^- \) and chelate \([Cu(L-S-HCys)]_n^2^- \) (Figure 5(c)), the latter model being more stable by 12.61 (BS2) and 7.82 kcal/mol (BS2 W). Chelate molecule shows Cu–S and Cu–O bond distances by 2.174 and 2.036 Å (BS2) and 2.187 and 2.042 Å (BS2 W). The
model is stabilized by a strong N–O intermolecular interaction (N–O, 2.528, and 2.498 Å at BS2 and BS2 W, respectively). Going back to the Cu I–H2Cys derivatives, on starting from the structure shown in Figure 4(a) the fully optimized model at BS2 level converges to the molecule depicted in Figure 4(b) (Cu–S, 2.241 Å; S–Cu–S, 176.4°, av). The structure is stabilized by O–S and O–N intermolecular interactions (O–S, 3.171 Å, and N–O, 2.665 Å). The solvent effects do not have significant importance at least in the coordination sphere region. Noteworthy, the optimized structure (BS2) for tri-cysteine derivative shows a trigonal arrangement (Figure 4(c)) similar to the other tri-coordinate computed species with smaller ligands (see above). Computed (BS2) Cu–S, S–C, and (S)C–C bond distances are 2.344, 1.860, and 1.539 Å.

On starting from a certain mono-dentate structure (Figure 4(d)) the computations converge to a chelate system [CuI(S,O-H2Cys)]+ (Figure 4(e)) forming an intramolecular hydrogen bond interaction between O–H (donor) and NH3+ (acceptor) residues (O...N, 2.556 Å, BS2). A similar behavior is shown by the bis-H2Cys derivative, that, starting from a mono-coordinate (S donor) arrangement (Figure 4(f)), converges to a pseudo-tetrahedral S, O-chelate (Figure 4(g)) (Cu–S and Cu–O, 2.290 and 2.244 Å, BS2). A search in CSD (Allen, 2002) for the Cu5,S,S',N,N'-Cysteine backbone (Cu–S–C–C(N)–C(O)–O), found only five structures. Among these, only one is related to a mono-nuclear Cu(II) complex: [CuIII(S,N-L)]2- (L, dimethyl-N,N'-ethylene-bis(L-cysteinato)) (Bhardwaj, Potenza, & Schugar, 1986). However, for comparison reasons, the Cu(I) analog [CuI(S,N-HCys)2]– model was also computed. The structure was fully optimized at BS2 W level and converges to a pseudo-tetrahedral S,N-chelate (Cu–S, Cu–N and S–C by 2.296, 2.402, and 1.847 Å (av), respectively).

The tetra-coordinate H2Cys derivative was calculated up to BS2/BS2 W levels and the structures converged to pseudo-tetrahedral arrangements (Figure 6(a)). The Cu–S bond length is 2.426 Å (av, BS2 W). The structure is stabilized by several hydrogen bonds interactions between different cysteine residues.

The computed [CuI(S-HCys)4]3– model level shows a certain elongation of a HCys– residue: Cu–S bond dis-
Table 3. Selected bond distances (Å) and angles (°) for H$_2$Cys(NH$_3$$^+$,SH,COOH), HCys$^-$ (NH$_2$S$^-$,COOH), and Cys$^{2-}$ (NH$_2$S$^-$,COO$^-$) free ligands (selected conformations) as calculated via DFT methods at BS1, (BS2), [BS1 W], and {BS2 W} levels of theory.

| Vector(s) | H$_2$Cys X-ray | H$_2$Cys$^a$ | H$_2$Cys$^b$ | H$_2$Cys$^c$ | HCys$^-$ (NH$_2$S$^-$,COOH)$^b$ | HCys$^-$ (NH$_2$S$^-$,COO)$^b$ | Cys$^{2-}$ (NH$_2$S$^-$,COO$^-$)$^c$ |
|-----------|----------------|-------------|-------------|-------------|-------------------|-------------------|-------------------|
| S–C       | 1.824(1)       | 1.839       | 1.843       | 1.851       | 1.849             | 1.848             | 1.849             |
|           | (1.837)        | (1.941)     | (1.849)     | (1.850)     | (1.850)           | (1.846)           | (1.877)           |
|           | [1.841]        | [1.844]     | [1.854]     | [1.853]     | [1.855]           | [1.864]           | [1.864]           |
|           | {1.836}        | {1.842}     | {1.853}     | {1.853}     | {1.853}           | {1.861}           | {1.861}           |
| C–O       | 1.244(1)       | 1.211       | 1.210       | 1.208       | 1.237             | 1.227             | 1.261             |
|           | (1.204)        | (1.204)     | (1.201)     | (1.238)     | (1.221)           | (1.257)           | (1.261)           |
|           | [1.247]        | [1.243]     | [1.216]     | [1.246]     | [1.223]           | [1.261]           | [1.261]           |
|           | {1.245}        | {1.242}     | {1.210}     | {1.218}     | {1.261}           | {1.261}           | {1.261}           |
| C–O(H) or C–O(...H) | 1.262(1)       | 1.338       | 1.337       | 1.342       | 1.2779            | 1.320             | 1.268             |
|           | (1.339)        | (1.336)     | (1.343)     | (1.268)     | (1.320)           | (1.267)           | (1.267)           |
|           | [1.266]        | [1.267]     | [1.335]     | [1.269]     | [1.330]           | [1.266]           | [1.266]           |
|           | {1.259}        | {1.259}     | {1.335}     | {1.329}     | {1.261}           | {1.261}           | {1.261}           |

Notes: See text for details. Data compared to X-ray structure for H$_2$Cys (NH$_3$$^+$,SH,COO$^-$) (Moggach, Clark, & Parsons, 2005).

$^a$Fully optimized structure from input structure reported in Figure 3(a), gas phase treatment optimized a not zwitterionic form, solvent treatments optimized a zwitterionic form.

$^b$Fully optimized structure from input structure as reported in Moggach, Clark, & Parsons, 2005, X-ray experimental structures for H$_2$Cys (NH$_3$$^+$,SH,COO$^-$).

$^c$Fully optimized structure having conformation similar to the complexes.
Table 4. Selected average bond distances (Å) and angles (°) for the models \([\text{Cu(S-H2Cys)}_n]^+\) \((n = 1, 4)\) and \([\text{Cu(S,O-H2Cys)}_n]^+\) \((n = 1, 2)\) as calculated via DFT methods at BS1, (BS2), [BS1 W], and {BS2 W} levels of theory.

| Vector(s) | \([\text{Cu(S-H2Cys)}_n]^+\) | \([\text{Cu(S,O-H2Cys)}_n]^+\) |
|-----------|-----------------|-----------------|
|           | \(n = 1\)       | \(n = 2\)       | \(n = 3\)       | \(n = 4\)       | \(n = 1\)       | \(n = 2\)       |
| Cu–S      | 2.040           | 2.133           | 2.228           | 2.340           | 2.103           | 2.256           |
|           | (2.219)         | (2.241)         | (2.344)         | (2.453)         | (2.262)         | (2.290)         |
|           | [2.055]         | [2.135]         | [2.218]         | [2.325]         | [2.259]         | [2.259]         |
|           | \{2.231\}       | \{2.244\}       | \{2.330\}       | \{2.426\}       |                 |                 |
| Cu–O      | 1.839           |                 |                 |                 | 2.001           |                 |
|           | (1.980)         |                 |                 |                 | (2.244)         |                 |
|           |                 |                 |                 |                 | (2.001)         |                 |
| S–C       | 1.855           | 1.856           | 1.854           | 1.851           | 1.876           | 1.851           |
|           | (1.872)         | (1.865)         | (1.860)         | (1.853)         | (1.873)         | (1.860)         |
|           | [1.849]         | [1.850]         | [1.853]         | [1.847]         | [1.849]         | [1.849]         |
|           | \{1.856\}       | \{1.858\}       | \{1.860\}       | \{1.849\}       |                 |                 |
| C–O       | 1.270           | 1.221           | 1.216           | 1.218           | 1.245           | 1.228           |
|           | (1.220)         | (1.208)         | (1.210)         | (1.210)         | (1.231)         | (1.218)         |
|           | \{1.212\}       | \{1.210\}       | \{1.212\}       | \{1.208\}       |                 |                 |
| C–O(H)    | 1.274           | 1.318           | 1.324           | 1.333           | 1.291           | 1.311           |
|           | (1.310)         | (1.325)         | (1.325)         | (1.333)         | (1.298)         | (1.316)         |
|           | [1.326]         | [1.332]         | [1.329]         | [1.344]         | [1.313]         |                 |
|           | \{1.329\}       | \{1.331\}       | \{1.330\}       | \{1.344\}       |                 |                 |
| S–Cu–S    | 169.8           | 117.5           | 99.2/99.4/102.1/106.2/116.7/134.2 | 124.1 |
|           | (176.4)         | (118.5)         | (92.5/102.7/106.7/110.7/114.4/129.0) | (156.4) |
|           | [171.0]         | [119.0]         | [104.4/108.2/109.3/110.8/111.1/112.9] | [114.3] |
|           | \{177.1\}       | \{119.9\}       | \{105.9/107.0/108.3/108.1/112.0/114.6\} |       |

Note: See text for details.
Table 5. Selected average bond distances (Å) and angles (°) for the models \([Cu(S-HCys)]^{1-n}\) \((n=1,4)\), \([Cu(S,O-HCys)]^{1-n}\) \((n=1,2)\), \([Cu(S-Cys)]^-\) and \([Cu(S,O-Cys)]^-\) as calculated via DFT methods at BS1, (BS2), [BS1 W], and {BS2 W} levels of theory.

| Vector(s)       | \([Cu(S-HCys)]_n\)^{n=1} | \([Cu(S,O-HCys)]_n\)^{n=1} | \([Cu(S,N-HCys)]_2\) | \([Cu(S-Cys)]^-\) | \([Cu(S,O-Cys)]^-\) |
|-----------------|---------------------------|-----------------------------|-----------------------|-------------------|-------------------|
|                 | \(n=1\) | \(n=2\) | \(n=3\) | \(n=4\) | \(n=1\) | \(n=2\) | \(n=1\) | \(n=1\) | \(n=1\) |
| Cu–S            | 2.027 | 2.115 | 2.224 | dnc | 2.085 | 2.185 | 2.315 | 2.030 | 2.101 |
|                 | (2.160) | (2.202) | (2.331) | (2.503) | (2.174) | (dnc) | (dnc) | (2.158) | (2.177) |
|                 | \{2.042\} | \{2.113\} | \{2.211\} | \{2.384\} | \{2.187\} | \{2.213\} | \{2.296\} | \{2.165\} | \{2.190\} |
| Cu–O/Cu–N       | 1.865 | 2.359 | 2.096 | 1.833 | 1.865 | 2.146 | 2.055 | 2.042 | 1.937 |
|                 | (2.036) | (dnc) | (dnc) | (1.937) | (2.042) | \{3.006\} | \{2.402\} | (1.963) | (1.963) |
| S–C             | 1.857 | 1.854 | 1.852 | dnc | 1.872 | 1.837 | 1.839 | 1.855 | 1.888 |
|                 | (1.861) | (1.856) | (1.849) | (1.844) | (1.857) | (dnc) | (dnc) | (1.860) | (1.880) |
|                 | \{1.857\} | \{1.859\} | \{1.856\} | \{1.852\} | \{1.878\} | \{1.840\} | \{1.843\} | \{1.862\} | \{1.878\} |
|                 | \{1.863\} | \{1.862\} | \{1.852\} | \{1.862\} | \{1.862\} | \{1.847\} | \{1.862\} | \{1.875\} | \{1.875\} |
| C–O             | 1.210 | 1.218 | 1.225 | dnc | 1.242 | 1.212 | 1.218 | 1.255 | 1.307 |
|                 | (1.204) | (1.212) | (1.219) | (1.225) | (1.227) | (dnc) | (dnc) | (1.253) | (1.296) |
|                 | \{1.216\} | \{1.217\} | \{1.220\} | \{1.222\} | \{1.248\} | \{1.227\} | \{1.217\} | \{1.266\} | \{1.322\} |
|                 | \{1.212\} | \{1.212\} | \{1.217\} | \{1.231\} | \{1.210\} | \{1.212\} | \{1.259\} | \{1.294\} | \{1.294\} |
| C–O(H)          | 1.345 | 1.335 | 1.325 | dnc | 1.307 | 1.349 | 1.367 | 1.261 | 1.234 |
|                 | (1.349) | (1.334) | (1.325) | (1.312) | (1.315) | (dnc) | (dnc) | (1.254) | (1.234) |
|                 | \{1.341\} | \{1.340\} | \{1.336\} | \{1.332\} | \{1.300\} | \{1.331\} | \{1.350\} | \{1.259\} | \{1.240\} |
|                 | \{1.340\} | \{1.339\} | \{1.330\} | \{1.312\} | \{1.339\} | \{1.348\} | \{1.260\} | \{1.239\} | \{1.239\} |
| S–Cu–S          | 179.0 | 119.5 | dnc | 159.5 | 144.9 |
|                 | (179.9) | (120.0) | (109.3) | (dnc) | (dnc) |
|                 | \{175.5\} | \{119.7\} | \{109.5\} | \{135.1\} | \{125.6\} |
|                 | \{178.3\} | \{111.2\} | \{168.1\} | \{146.2\} | \{146.2\} |

Note: See text for details; dnc, did not converge.
distance are 2.336, 2.368, 2.341, and 2.488 Å (BS1 W). The structure is stabilized by O–H...S intra-cysteine hydrogen bonds. Similar structures are obtained through computations at BS2 and BS2 W levels (Cu–S, 2.503 Å av, BS2, Figure 6(b) and 2.464 Å av, BS2 W). Conclusion: Cu(I)-cysteine complexes were investigated in order to simulate the behavior of the aminoacid residue as ligand in enzymes and proteins as regards the coordination properties of SH and S\(^{-}\) functions. Of course, the models fail to reproduce exactly the conformations around the amides functions through NH\(_2\) and COOH groups. As regards the bi-, tri-, and tetra-H\(_2\)Cys and HCys\(^{-}\) derivatives, the easiest models to manage were those for the tri-derivatives. In all cases, full convergence is quickly reached and the CuS\(_3\) core has almost C\(_{3h}\) symmetry. The equivalence of the coordination bonds does not occur for the tetra-coordinate complexes. This finding suggests that the tri-cysteine models are the most...
reliable ones and that Cu(I) centers encountered in biological molecules tend to reach that type of arrangement.

CopZ Dimer and Trimer Models, $[\text{CuI}(S - H)\text{Et}]_2^{2+}$, $[\text{CuI}(S - \text{SEt})]_2^{2-}$, $[\text{CuI}(S\text{-SH})]_3^+$, $[\text{CuI}(S - S\text{Et})]_2^-$, $[\text{CuI}(S - H\text{Cys})]_2^{3+}$, $[\text{CuI}(S - H\text{Cys})]_2^{3-}$.

Recently, different crystal structures of the copper-chaperone CopZ (Protein Data Bank codes, 2QIF and 3I9Z) were reported (Hearnshaw et al., 2009; Singleton et al., 2009). In the solid state, the protein shows a trinuclear Cu(I)$_3$(S-Cys)$_6$ cluster arising from the interaction of three CopZ molecules where the Cys residues come from the CXXC motif (Scheme 2(b); Singleton et al., 2009; 3I9Z). The three Cu(I) ions are bound to three bridging (b) S-Cys and three S-Cys terminal (peripheral, p) sulfur atoms in a trigonal geometry. In a different crystal form of the same protein, a tetranuclear Cu(I)$_4$(S-Cys)$_6$ cluster is observed (Protein Data Bank code, 2QIF (Hearnshaw et al., 2009)). In this latter case, the tetranuclear entity consists of an inner bi-nuclear Cu(I) core bound to two S-Cys (b) from each subunit (CXXC motif) and of two additional Cu(I) atoms in a 2 S-Cys, N-His O-water four-coordination. Here, two Cu(I) ions (b) display distorted diagonal coordination and two Cu(I) ions (p) display tetrahedral coordination. Earlier structure determinations on Cu(I)-CopZ were performed by X-ray absorption spectroscopy (XAS) in solution (Banci et al., 2003). In diluted solution Cu(I)-CopZ dimeric forms of the protein were observed only in high ionic strength where the dinuclear Cu(I)$_2$(S-Cys)$_4$ clusters with trigonal coordination are formed. In the trimeric form the overall number of cysteine ligands is six, while it is four in the CopZ dimer (see Scheme 2). Several models for the dimeric and trimeric CopZ were optimized (Figures 7(a) and (b), 8(a)–(d), 9(a) and (b), 10(a) and (b)) in order to shed light on the structure of the cores. The experimental Cu...Cu contact distances are 3.12 Å (estimated standard deviation, esd, c. 0.14 Å) for the trimer and 2.74 Å (esd c.0.1) for the dimer. The optimized structure (BS2 W, 22nd cycle of refinement) for the di-nuclear model $[\text{CuI}(S - S\text{Et})]_2^{2+}$, (Et, CH$_2$CH$_3$) has Cu...Cu contact distance by 3.591 Å, and Cu-S(b) and Cu-S(p) bond distances by 2.377–2.405 and 2.254 Å (av). The computed intermetallic distance is in excellent agreement with the ones found by XAS (Banci et al., 2003) and even the computed Cu–S distances agree well with the found ones. Even when computations are performed in the gas phase (BS2) the cluster arrangement goes to full convergence. The calculated Cu...Cu, Cu–S(b), and Cu–
S(p) bond distances being 2.912, 2.415, and 2.281 Å (av), respectively. Conclusion: the real cluster is well simulated by the thiolate model (BS2 W). The protonation of the ligand at sulfur reasonably brings about instability and easy dissociation of the cluster.

The structure for the basic tri-nuclear model \([\text{CuI} (\text{S}-\text{SH})]_3\) was fully optimized at BS2 and BS2 W (Figure 8(a) and Tables 7 and S11) and Cu…Cu and Cu–S distances are 2.700 and 2.248 Å and 2.693 and 2.252 Å, respectively, in fairly good agreement with experiment (Singleton et al., 2009). The basic six-membered cycle with three peripheral ligands model \([\text{CuI} (\text{S}\text{–SH})_2]^2_+\) was fully optimized via BS2 level, to an arrangement having long Cu…Cu distances (4.073 Å) (Figure 8(b) and Tables 7 and S11). The S–Cu–S bond angles (BS2) were 109.8° (endo-cycle) and 123.1 and 126.8° (exo-cycle) in good agreement with the experimental ones (108.3°, 124.6°, and 127.0°, respectively) (Singleton et al., 2009). The computed cyclic structure is much less puckered than the experimental one. In case the water solvent treatment was taken into account (BS2 W), the convergence of the model is full and the computed Cu…Cu bond distance is 3.493 Å, in acceptable agreement with experiment. Also Cu–S–Cu–S torsion angles (72° (av)) are close to the experimental ones.

The \([\text{CuI} (\text{S–SH})_2]^3_+\) model was fully optimized at BS1 level (Figure 8(c)), but when computed at BS2 level undergoes complete dissociation of the cluster after 34 cycles. A \([\text{CuI} (\text{S–SH})_6 (\text{S–SH})_3]_3^-\) model was also calculated at BS2 level giving a partially refined structure (after c. 90 cycles), where the Cu…Cu, Cu–S(b), and Cu–S(p) distances are 2.756, 2.265, and 3.063 Å (av), respectively (Figure 8(d)). Interestingly, this analysis shows that on increasing the protonation at sulfur starting from \([\text{CuI} (\text{S–SH})_2]^3^-\), the core arrangement relaxes.

Then the models were upgraded to larger ones, but in some cases the computations were performed at the BS1 W level instead of BS2 W, in order to reduce time of convergence (computational costs).

The model \([\text{CuI} (\text{S–S(H)})_2]^3_+\) (BS1 W, Figure 9(a)) converges to Cu…Cu contact distance by 3.632 Å (against 3.12 Å, experimental; Tables 8 and S12). The Cu–S bond distances are shorter than the experimental ones, averaging 2.220(b) and 2.225(p)Å. When the \([\text{CuI} (\text{S–SEt})_2]^3^-\) model is computed, Cu…Cu distances
converge to much lower value 2.407 Å (av, BS1 W) (Figure 9(b)). The Cu–S bond distances are 2.299(b) and 2.295(p) Å.

The computed structure for \(\frac{1}{2}\)CuI\((\text{S}/\text{C0-SH}2\text{Cys})_2\)\(\text{2}^+\), (NH2,SH,COOH) (BS1 W) converges to a relaxed trimer (Figure 10(a)) in which the Cu–Cu, and Cu–S(b) and Cu–S(p) distances are 3.573, and 2.221 and 2.234 Å (av, see Tables 8 and S12), respectively, in good agreement with experimental data for CopZ protein (it has to be noted that optimization was halted after 490 cycles, when the energy trend was flat for the last 160 cycles and structural parameters did not change appreciably). The simulation for \(\frac{1}{2}\)CuI\((\text{S}/\text{HCys})_2\)\(\text{2}^+\), (NH2,S–C03,C,H–COOH) (BS1 W) successfully converges (Figure 10(b)) and the trimer has Cu–Cu and Cu–S(b) distances of 2.429 and 2.289 Å, respectively.

In order to reach a better simulation for the trimuclear core in the protein, the cysteine molecules were replaced by di-amidate forms (HS–CH2–C(H)–C(=O)–N (H)–CH3)–N(H)–C(=O)–CH3) and structurally optimized at BS1 level (preliminary computations). Interestingly, the main characteristics of the structure agree very well with the not amidate H2Cys derivative. For example, selected distances range: Cu–Cu, 3.806–4.030 Å; Cu–S(b), 2.233–2.338 Å; and Cu–S(p), 2.284–2.317 Å. The results suggest that the not amidate models represent fairly well the structural properties of the metal cluster of the protein active site. Conclusion: this section shows that deprotonation at all sulfur atoms causes a significant shrinking of the core of trimer. This fact should correspond to a significant stabilization of the core; on the contrary, stepwise protonation at sulfur brings about a swelling at nucleus and a trend toward dissociation.

**Gross energy aspects.** The values of total electronic energies for selected computed models are reported in Table S13 (Supporting) and selected electronic formation and protonation energies are reported below under the respective sections.

\([\text{CuI}(\text{S}/\text{H2S})_n]^{2+}\) (n = 1–4)

The computed electronic energies of formation are listed in Table 9. On adding a second H2S ligand to Cu(I) the bond formation energy is still high (~26.38 kcal/mol, BS2 W). The effect of the BSSE (basis set superposition error) correction is small at BS2 level (~2%). The values for the mono-coordinate derivative compare well with corresponding value computed previously from this laboratory for Zn(II) derivatives (Cini, 1999). The larger bond energy for the Zn(II) derivative is reasonably due to the larger charge at metal. Going back to Cu(I), the formation energies for the fourth Cu–S bond approach zero (4.08 kcal/mol, BS2 W).
The electronic energies of formation are listed in Table 10. Once the BSSE correction is performed, the overall formation energy for the tri-coordinate species \([Cu^{1}(S-SH)]_{2}^{3-}\) changes by a mere 0.1%. The addition of the third hydrogen sulfide anion to the bi-coordinate derivative is slightly *exo*-energetic (7.42 kcal/mol) at BS1 W. It should be recalled that the structure for the tetra-coordinate species (overall charge 3−) did not converge (BS1, BS2). The addition of the fourth HS− ligand is *endo*-energetic by +8.66 kcal/mol. Therefore, the formation of tetra-sulfide is not favored from the overall electronic energy standpoint both in the gas phase and in aqueous sys-
Table 7. Selected average bond distances (Å) and angles (°) for fully optimized CopZ trimer models: \([\text{Cu}^1(\text{S-SH})_3], [\text{Cu}^1(\text{S-SH})_2]^-, [\text{Cu}^1(\text{S-SH})_2]^{3+}\) as calculated via DFT methods at BS1, (BS2), [BS1 W], and {BS2 W} levels of theory.

| Vector(s) | \([\text{Cu}^1(\text{S-SH})_3]_3\) | \([\text{Cu}^1(\text{S-SH})_2]^+_3\) | \([\text{Cu}^1(\text{S-SH})_2]^{3+}\) |
|-----------|---------------------------------|---------------------------------|---------------------------------|
| Cu–Cu     | 2.300 (2.700) [2.296] [2.693]    | 3.869 (4.073) [2.366] [3.493]    | 3.925 (4.279)                   |
| Cu–S      | 2.185 (2.248) [2.192] [2.252]    | 2.228 (2.351) [2.296] [2.347]    | 2.274/2.238 (2.348/2.305)      |
| Cu…Cu…Cu | 60.0 (60.0) [60.0] [60.0]        | 60.0 (60.0) [60.0] [60.0]        | 60.0 (60.0)                    |
| S–Cu–S   | 170.2 (165.9) [170.1] [166.2]    | 108.6, 123.6, 127.3 (109.8, 123.1, 126.8) | 107.6/125.0 (109.5/125.1)    |
| Cu–S–Cu  | 63.5 (73.8) [63.2] [73.4]        | 119.0 (117.8) [62.0] [96.1]        | 119.3 (114.2)                  |

Notes: See text for details; dnc, did not converge.

*aStructure partially optimized.
tems. In contrast to Cu(I)-sulfide derivatives, Cu(II)-sulfide tetra-coordinated species have high overall formation energies: $-232.53$ kcal/mol (BS1 W), $-666.82$ kcal/mol (BS2), and $-230.47$ kcal/mmol (BS2 W). Notwithstanding, these data do not mean that the step formation for tetra-coordinate Cu(II) species is exo-energetic. In fact, the

### Table 8. Selected average bond distances (Å) and angles (°) for fully optimized CopZ trimer models:

| Vector(s)   | $[\text{Cu}(S - S(H)Et)_2]^{3+}$ | $[\text{Cu}(S - SEt)_2]^{3-}$ |
|-------------|---------------------------------|--------------------------------|
| Cu…Cu       | 3.736                           | 2.412                          |
|             | [3.632]$^a$                     | [2.407]$^a$                    |
| Cu–S        | 2.245/2.215                     | 2.308/2.384                    |
|             | [2.220/2.225]$^a$               | [2.299/2.295]$^a$              |
| Cu…Cu…Cu   | 60.0                            | 60.0                           |
|             | [60.0]$^a$                      | [60.0]$^a$                     |
| S–Cu–S     | 107.8/121.1/130.0               | 110.7/119.4/123.5              |
|             | [109.0/123.1/127.7]$^a$         | [117.5/111.1/126.1]$^a$        |
| Cu–S–Cu    | 112.5                           | 63.0                           |
|             | [109.8]$^a$                     | [63.1]$^a$                     |
| Cu–S–C     | 117.1                           | 108.3                          |
|             | [115.1]$^a$                     | [112.0]$^a$                    |

| Vector(s)   | $[\text{Cu}(S – H_2Cys)_2]^{3+}$ | $[\text{Cu}(S – HCys)_2]^{3-}$ | $[\text{Cu}(S – Cys)_2]^{6-}$ |
|-------------|---------------------------------|--------------------------------|--------------------------------|
| Cu…Cu       | 4.035/4.747/4.831               | 2.458                          | dnc                            |
|             | [3.573]$^a$                     | [2.429]$^a$                    | [2.417]$^a$                     |
| Cu–S        | 2.221–2.909/2.209–2.373         | 2.282                          | dnc                            |
|             | [2.221/2.234]$^a$               | [2.298]$^a$                    | [2.306]$^a$                     |
| Cu…Cu…Cu   | 49.8/64.0/66.2                  | 60.0                           | dnc                            |
|             | [60.0]$^a$                      | [60.0]$^a$                     | [60.0]$^a$                     |
| S–Cu–S     | 227.1/106.5/77.8                | 135.6/107.0/113.2              | dnc                            |
|             | [109.0/125.2]$^a$               | [110.6/116.0/116.9]$^a$        | [111.8/126.4/116.5]$^a$        |
| Cu–S–Cu    | 125.9/141.7                     | 64.4                           | dnc                            |
|             | [107.2]$^a$                     | [64.1]$^a$                     | [63.2]$^a$                     |
| Cu–S–C     | 87.8/119.2/133.2                | 112.2                          | dnc                            |
|             | [128.2]$^a$                     | [111.5]$^a$                    | [112.0]$^a$                    |

Notes: See text for details; dnc, did not converge.

*Structure partially optimized.

Figure 10. Computed structures for CopZ trimer models: (a) $[\text{Cu}(S – H_2Cys)_2]^{3+}$ (NH$_2$SH,COOH) and (b) $[\text{Cu}(S – HCys)_2]^{3-}$ (NH$_2$S$^-$,COOH) (BS1 W).
that formation of four-coordinate M(S–H(C2H5))4 is uncertain because the electronic energy contribution approaches to zero or is even positive.

Table 9. Electronic formation energies (\(\Delta E\), kcal/mol) for \([\text{Cu}(\text{S–H}_2)_n]^+\) (\(n = 1–4\)) as computed at different levels of theory.

| \(\Delta E\)  | \(\Delta E^{\text{corr}/\text{BSSE}}\) | \(\Delta E\)  | \(\Delta E^{\text{corr}/\text{BSSE}}\) |
|---------------|------------------|---------------|------------------|
| BS1           | −69.22           | −42.71        | −117.45          | −78.82           |
| BS1 W         | −53.10           | −45.71        | −86.62           | −85.18           |
| BS2           | −46.37           | −52.45        | −86.62           | −85.18           |
| BS2 W         | −26.07           | −52.45        | −86.62           | −85.18           |

Note: The values corrected for BSSE effect are also reported (\(\Delta E^{\text{corr}/\text{BSSE}}\), kcal/mol); dnc, did not converge.

Table 10. Electronic formation energies (\(\Delta E\), kcal/mol) for \([\text{Cu}(\text{S–H}_2)_n]^{1–n}\) (\(n = 1–4\)) as computed at different levels of theory.

| \(\Delta E\)  | \(\Delta E^{\text{corr}/\text{BSSE}}\) | \(\Delta E\)  | \(\Delta E^{\text{corr}/\text{BSSE}}\) |
|---------------|------------------|---------------|------------------|
| BS1           | −226.17          | −186.34       | −309.23          | −261.94          |
| BS1 W         | −80.45           | −131.51       | −261.79          | −260.69          |
| BS2           | −193.81          | −92.15        | −126.34 *        | dnc              |
| BS2 W         | −51.05           | −126.34 *     | −85.45 *         | dnc              |

Note: The values corrected for BSSE effect are also reported (\(\Delta E^{\text{corr}/\text{BSSE}}\), kcal/mol); dnc, did not converge.

Structure partially optimized.

Overall formation energy for the trigonal \([\text{Cu}^{II}\text{(S–H}_2)_3]\) is −222.75 kcal/mol (BS2 W). Hence, the formation of the tetra-coordinate species from tri-coordinate is \(\text{exo-energetic}\) by just −7.72 kcal/mol (BS2 W). Conclusion: from this section, it may be said that formation of four-coordinate \(\text{M(S–L}_4\) species is not favorite with small sized mono-valent (and even di-valent) metal ions. Tri-coordination seems to be a sort of upper-limit.

\([\text{Cu}(\text{S–H(C2H5)}_n]^+\) and \([\text{Cu}(\text{S–CH}_n)\text{H})_n]^{1–n}\) (\(n = 1–4\))

The overall electronic formation energies (Table 11) are in the range −28.95/−70.71 kcal/mol (BS2 W). The bond formation energies for the partial processes that bring about the second, third, and fourth Cu–S coordinate linkages are −28.72, −8.75, and −4.29 kcal/mol (BS2 W); whereas the step bond formation energies for \([\text{Cu}(\text{S–S}CH_3)_n]^{1–n}\) (\(n = 2–4\), BS2 W) are −43.20, −2.60, +9.92 kcal/mol. Conclusion: the results presented so far show that the formation of up to tri-coordinate species both for the neutral H2S and CH3SH ligands, as well as for the anionic forms is predictable because significantly stabilized in terms of electronic energy (once the solvent effect is considered). The formation of the tetra-coordinate species is uncertain because the electronic energy contribution approaches to zero or is even positive.

\([\text{Cu}(\text{H}_2\text{Cys}_n)]^+\) and \([\text{Cu}(\text{H-Cys}_n)]^{1–n}\) (\(n = 1–4\))

The overall electronic formation energies are listed in Table 12. The value for \([\text{Cu}(\text{H}_2\text{Cys})]^+\) is −65.94 at BS1/BSSE and −61.28 kcal/mol at BS2 W (no BSSE). The value for \([\text{Cu}(\text{S–H}_2\text{Cys})]^{2–}\) is −104.32 kcal/mol (BS1/BSSE), whereas the step formation energy at BS2 W is −29.14 kcal/mol (no BSSE). A similar pattern is found also when the mono- and bi-coordinated models are investigated for the H2Cys− (NH2, S, COOH) ligand. The computed electronic formation energies increase (as absolute value) on passing from a H2Cys derivative to a H2Cys− one and from H2Cys− to Cys2− (NH2, S, COO−). The protonation energy at sulfur for \([\text{Cu}(\text{S–HCys})]^+\) to
Table 11. Electronic formation energies (ΔEc, kcal/mol) for \([\text{Cu}^I(S-S(H)CH_3)_n]^+\) and \([\text{Cu}^I(S-SCH_3)_n]^{1–n}\) (n = 1–4) as computed at different levels of theory.

|         | ΔEc          | ΔEccorr/BSSE | ΔEc          | ΔEccorr/BSSE |
|---------|--------------|--------------|--------------|--------------|
| BS1     | −80.02       | −51.51       | −134.16      | −93.03       |
| BS1 W   | −58.91       | −95.61       | −151.22      | −96.92       |
| BS2     | −53.93       | −53.35       | −98.22       | −57.67       |
| BS2 W   | −28.95       | −70.71       | −120.19      | -             |
| BS1     | −159.73      | −103.39      | −169.74      | −109.50      |
| BS1 W   | −113.73      | −117.32      | −123.09      | −70.71       |
| BS2     | −113.27      | −111.28      | −123.09      | −120.19      |
| BS2 W   | −66.42       | −70.71       | −120.19      | -             |
| BS1     | −231.08      | −198.76      | −113.48      | −264.00      |
| BS1 W   | −87.04       | −141.36      | −265.09      | -             |
| BS2     | −197.40      | −196.94      | −265.09      | -             |
| BS2 W   | −54.89       | −89.09       | -            | -             |
| BS1     | −260.75      | −199.44      | dnc          | dnc           |
| BS1 W   | −151.22      | −139.23      | dnc          | dnc           |
| BS2     | −207.90      | −206.22      | dnc          | dnc           |
| BS2 W   | −100.70      | −90.78       | dnc          | dnc           |

Note: The values corrected for BSSE effect are also reported (ΔEccorr/BSSE, kcal/mol); dnc, did not converge

*Structure partially optimized.

\([\text{Cu}^I(S-HCys)]^+\) is +20.38 kcal/mol (BS1 W) and the value is comparable to that for the protonation of \([\text{Cu}^I(S-SCH}_3])\) to \([\text{Cu}^I(S-S(H)CH}_3])^+\) (+28.13 kcal/mol) and to that for the protonation energy for \([\text{Cu}^I(S-SH)_2]) to \([\text{Cu}^I(S-SH)_2])^+\) (+72.35 kcal/mol). This result may imply a role of the protonation and deprotonation mechanisms, on the copper transfer in the biomolecules (see below, dimer and trimer models).

CopZ. Dimer and Trimer Models, \([\text{Cu}^I(S-S(H)Et}_2]^{2+}\), \([\text{Cu}^I(S-S(Et))_2]^{2+}\), \([\text{Cu}^I(S-SH)]_3\), \([\text{Cu}^I(S-SH)_2]^{3+}\), \([\text{Cu}^I(S-SH)_3]^{1+}\), \([\text{Cu}^I(S-SH)_2]^{3+}\), \([\text{Cu}^I(S-SH)_2]^{3+}\), \([\text{Cu}^I(S-H_2Cys)_2]^{3+}\), \([\text{Cu}^I(S-H_2Cys)_2]^{3+}\).

The overall formation electronic energies for the bi-nuclear species (Table 13) as computed at BS2 W level, for the EtSH and EtS\(^-\) derivatives, are −112.52 and −193.83 kcal/mol. It has to be noted that the contribution to the overall formation energy for one Cu(I) center e. −56 and −97 kcal/mol (BS2 W) for the EtSH and EtS\(^-\) species (see below, corresponding analysis for the trimers).

The overall formation energy for \([\text{Cu}^I(S-SH)]_3\), (see Table 13) computed at BS2 and BS2 W are −701.35 and −246.68 kcal/mol, whereas for computations at BS2/BSSE the value is −697.44 kcal/mol. When a HS\(^-\) ligand (peripheral) is added to each Cu(I) center the overall formation energy for \([\text{Cu}^I(S-SH)_2]^{3+}\) is −627.92 and −622.30 kcal/mol at BS2 and BS2/BSSE, respectively. One can state that: (i) the solvent effect is very effective, (ii) computations at BS2 and BS2/BSSE levels reveal that the addition of the three \(\text{exo-}\) cyclic HS\(^-\) functions is \(\text{endo-}\) energetic by c. +75 kcal/mol, and (iii) the basis set superposition effect is not large for such cluster molecules.

The models have been then expanded to EtS\(^-\) and EtS\(^-\) ligands, and even to cysteine/cysteinate ligands. For these latter systems, the computations were carried out to BS1 W only for reducing computational times.

Taking into account EtS\(^-\) ligand the overall formation energy for \([\text{Cu}^I(S-S(Et))_2]^{3+}\) is −594.88 kcal/mol (BS1/BSSE), whereas the corresponding value at BS1 W is −482.50 kcal/mol. The effect of solvent reduces the overall formation energy by 70%, in agreement with the corresponding solvent effect for the HS\(^-\) derivative (76%). As regards EtSH, the overall formation energy for \([\text{Cu}^I(S-S(H)Et}_2]^{3+}\) is −133.38 kcal/mol (BS1/BSSE) and −338.19 kcal/mol (BS1 W, partially optimized). The overall formation energy per Cu(I) center for the EtSH tri-nuclear derivative is therefore −112.73 kcal/mol (BS1 W), whereas the corresponding value for the EtS\(^-\) species is −160.83 kcal/mol (BS1 W), these latter values being some 6–9 kcal/mol more \(\text{exo-}\) energetic than the corresponding value for the di-nuclear species. These data are in agreement with lower strain tensions for six-membered cycles with respect to four-membered cycles.

The overall formation energy for \([\text{Cu}^I(S-SH)_2]^{3+}\) is −163.09 (BS1/BSSE) and −338.76 kcal/
Table 12. Electronic formation energies ($\Delta E$, kcal/mol) for \([\text{Cu}^I(S\text{-H}_2\text{Cys})]^{n+}\), \([\text{Cu}^I(S\text{-HCys})]^{2+}\), and \([\text{Cu}^I(S\text{-O-HCys})]^{3+}\) as computed at different levels of theory.

|        | $[\text{Cu}^I(S\text{-H}_2\text{Cys})]^{n+}$ | $[\text{Cu}^I(S\text{-HCys})]^{2+}$ | $[\text{Cu}^I(S\text{-O-HCys})]^{3+}$ |
|--------|----------------------------------|---------------------|---------------------|
| BS1    | -96.57                           | 147.42              | 185.22              |
| BS1 W  | -61.33                           | 97.83               | 118.29              |
| BS2    | -61.28                           | 106.10              | 133.28              |
| BS2 W  | -29.56                           | 58.70               | 65.66               |

Note: Selected values corrected for BSSE are reported in the text. The $\text{H}_2\text{Cys}$ ($\text{NH}_2\text{SH}_2\text{COOH}$) conformation was selected on the basis of the ligand conformation onto the copper(I)-complexes (this is not minimum of energy); dnc, did not converge.

AStructure partially optimized.

**General conclusion**

The work shows that the existence of bi-nuclear \([\text{Cu}^I(S\text{-SX})]^{2+}\) and tri-nuclear \([\text{Cu}^I(S\text{-SX})]^{3+}\) species for important biological species related to copper trafficking can be predicted and rationalized through structure optimization DFT methods. Furthermore, the work shows the importance of protonation equilibria on $\text{XS}^-$ to modulate the stability of Cu(I)-clusters in such a way to guarantee the accumulation-storage-transport-release mechanisms for copper. At this regard, it has to be emphasized that a recent experimental (thermodynamic) work (Badarau & Dennison, 2011a) showed that the Cu(I) transfer in protein systems ‘are dependent from pH and become less favorable as the pH is decreased below,’ confirming the findings from the present theoretical study. The concept that relates the release of Copper(I) through cluster breakage via protonation at sulfur atoms is pictured in Scheme 3. The protonation status at sulfur donors for the dimers and trimers found in solid state for CopZ were probably mixed ones, being Cu...Cu distances c. 3.1 Å from experimental data, c. 2.4 Å from computed not protonated models, and c. 3.6 Å totally protonated computed models (BS1 W) for cysteine derivatives. The increase of protonation at anionic $S$-ligands can be obtained by...
effect are also reported (BS2 W/C0 BS2 dnc dnc BS2 W/C0 BS1 W/C0 BS1 W/C0 BS2 W/C0 BS2 W/C0) 

breakage of Cu2S4 or Cu3S6 clusters followed by copper protein conformational changes and can cause the partial release. Hence, the preferred forms of 

Table 13. Electronic formation energies (ΔEe, kcal/mol) for CopZ dimer and trimer models, [CuI(S - S(H)Et)2]2+, [CuI(S - SEt)2]2+, [CuI(S - SH)2]3+, [CuI(S - SH)3]3+, [CuI(S - S(H)Et)3]3+, [CuI(S - SEt)3]3+, [CuI(S - H2Cys)3]3+, (NH2,SH,COOH), [CuI(S - HCys)3]3+ (NH2,S,COOH) as computed at different levels of theory. The values corrected for BSSE effect are also reported (ΔEecorr/BSSE, kcal/mol); dnc, did not converge.

|       | ΔEe       | ΔEecorr/BSSE | ΔEe       | ΔEecorr/BSSE |
|-------|-----------|--------------|-----------|--------------|
| BS1 W | dnc       | dnc          | -586.13   |              |
| BS1 W | -207.64   | dnc          | -309.85   |              |
| BS2 W | dnc       |              | -466.40   |              |
| BS2 W | -112.52   |              | -193.83   |              |
| [CuI(S - SH)3] |              |              | -701.35   |              |
| BS1 W | -872.42   | -681.91      | -208.93   |              |
| BS1 W | -407.54   |              | -292.33   |              |
| BS2 W | -701.35   | -697.44      | -292.33   |              |
| BS2 W | -246.68   |              | -292.33   | -292.33     |
| [CuI(S - SH)2]3+ |              |              | -790.90   | -212.03     |
| BS1 W | -449.49   | -595.33      | -212.03   |              |
| BS1 W | -701.35   | -622.30      | -595.33   |              |
| BS2 W | -277.40   |              | -595.33   |              |
| BS2 W | -277.40   |              | -595.33   |              |
| [CuI(S - S(H)Et)3]3+ |              |              | -311.18   | -821.99     |
| BS1 W | -338.19   | -133.38      | -821.99   |              |
| BS1 W | -338.19   |              | -821.99   |              |
| BS2 W | -338.19   |              | -821.99   |              |
| BS2 W | -338.19   |              | -821.99   |              |
| [CuI(S - H2Cys)3]3+ |              |              | -360.52   | -594.88     |
| BS1 W | -338.76   | -163.09      | -594.88   |              |
| BS1 W | -338.76   |              | -594.88   |              |
| [CuI(S - HCys)3]3+ |              |              | -338.19   | -482.50     |
| BS1 W | -338.19   |              | -482.50   |              |
| BS1 W | -338.19   |              | -482.50   |              |

*Structure partially optimized.

Scheme 3. The drawing shows the swelling of the Cu(I)-cluster upon protonation at cysteine sulfur atoms.

protein conformational changes and can cause the partial breakage of Cu2S4 or Cu3S6 clusters followed by copper release. Hence, the preferred forms of S-ligands are predictably not protonated at Cu(I)-transport steps and are protonated at Cu(I)-release steps. Extension on further studies on nature of chemical bond at clusters and on effect of local functionals like BLYP, as well as of salvation effect at attenuated ε values (with respect to 78.5, water), are in progress in this laboratory.

As one of the reviewer suggested that relativistic effect could play a role in transition metal compounds, it can be noted that Cu2 dimers in the singlet ground state have small relativistic effects when compared to excited triplet state and even much smaller than those for Au2, as reported in literature (Danovich & Filatov, 2008). Furthermore, the models for copper–proteins we treated are not simple metal clusters but they have RS– and RSH groupings (ethylthiol and cysteine derivatives) that
bridge the metals, thus relativistic effects should be reduced with respect to compact solid materials. The goal of the present work is not that of reaching the highest degree of accuracy for bond distances and formation energies; rather, we look for trends upon protonation at cysteine sulfur atom. On the other side, it is worth mentioning that the computed structures from the present work compare very well with copper trafficking sites in metal–protein systems reported by Badarau and Dennison (2011b) and Badarau et al. (2010). For example, in a metallo-chaperone Atx1 (Badarau et al., 2010) the H₂Cys{Cu$(\mu$S-H₂Cys)}₂H₂Cys unit has bridging Cu–S bond distances by 2.25–2.39 Å: this means a good reliability of the present computational strategy for simulating copper chaperons and copper trafficking sites.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2012.689703.

Acknowledgments

CINECA (Inter-University Consortium for Scientific Computations, Casalecchio di Reno, Bologna, Italy) is gratefully acknowledged for allowing the computations through IBM SP6 machines via the ISCRA Grant 2010 HP10CV16XC.

References

Allen, F.H. (2002). The Cambridge Structural Database: A quarter of a million crystal structures and rising. Acta Crystallographica Section B: Structural Science, 58, 380–388. The Cambridge Crystallographic Data Centre (CCDC), The Cambridge Structural Database CSD, 12 Union Road, Cambridge, CB2 1EZ, UK, Release July 2010, http://www.ccdc.cam.ac.uk/products/csd/

Arnesano, F., Banci, L., Bertini, I., Ciofi-Baffoni, S., Molteni, E., Huffman, D.L., & O’Halloran, T.V. (2002). Metallocchaperones and metal-transporting ATPases: A comparative analysis of sequences and structures. Genome Research, 12, 255–271.

Arnesano, F., Banci, L., Bertini, I., Mangani, S., & Thompsett, A.R. (2003). A redox switch in CopC: An intriguing copper trafficking protein that binds copper(I) and copper(II) at different sites. Proceedings of the National Academy of Sciences USA, 10, 3814–3819.

Badarau, A., & Dennison, C. (2011a). Copper trafficking mechanism of CXXC-containing domains: Insight from the pH-dependence of their Cu(I) affinities. Journal of the American Chemical Society, 133, 2983–2988.

Badarau, A., & Dennison, C. (2011b). Thermodynamics of copper and zinc distribution in the cyanobacterium Synechocystis PCC6803. Proceedings of the National Academy of Sciences USA, 108, 13007–13012.

Badarau, A., Firbank, S.J., McCarthy, A.A., Banfield, M.J., & Dennison, C. (2010). Visualizing the metal-binding versatility of copper trafficking sites. Biochemistry, 49, 7798–7810.

Banci, L., Bertini, I., Cantini, F., & Ciofi-Baffoni, S. (2010). Cellular copper distribution: A mechanistic systems biology approach. Cellular and Molecular Life Sciences, 67, 2563–2589.

Banci, L., Bertini, I., & Ciofi-Baffoni, S. (2009). Copper trafficking in biology: An NMR approach. Human Frontier Science Program Journal, 3, 165–175.

Banci, L., Bertini, I., Ciofi-Baffoni, S., Kozyreva, T., Zovo, K., & Palumaa, P. (2010). Affinity gradients drive copper to cellular destinations. Nature, 465, 645–648.

Banci, L., Bertini, I., Del Conte, R., Mangani, S., & Meyer-Klaucke, W. (2003). X-ray absorption and NMR spectroscopic studies of CopZ, a copper chaperone in Bacillus subtilis: The coordination properties of the copper ion. Biochemistry, 42, 2467–2474.

Banci, L., & Rosato, A. (2003). Structural genomics of proteins involved in copper homeostasis. Accounts of Chemical Research, 36, 215–221.

Bertini, I., & Cavallaro, G. (2008). Metals in the ‘omics’ world: Copper homeostasis and cysteine e oxidase assembly in a new light. Journal of Biological Inorganic Chemistry, 13, 3–14.

Bharadwaj, P.K., Potenza, J.A., & Schugar, H.J. (1986). Characterization of [dimethyl N,N’-ethylenebis(L-cysteinate)2–S,S’]copper(II), a stable copper(II) aliphatic dithiolate. Journal of the American Chemical Society, 108, 3151–3156.

Boal, A.K., & Rosenzweig, A.C. (2009). Structural biology of copper trafficking. Chemical Reviews, 109, 4760–4779.

Calderon, V., Dolderer, B., Hartmann, H.J., Echner, H., Luchinat, C., Del Bianco, C., Mangani, S., & Weser, U. (2005). The crystal structure of yeast copper thiocin: The solution of a long-lasting enigma. Proceedings of the National Academy of Sciences USA, 102, 51–56.

Cini, R. (1999). Molecular orbital study of complexes of zinc(II) with sulphide, thiomethanol, thiomethanol, dimethylthioether, thionophenate, formiate, acetate, carbonate, hydrogen carbonate, iminomethane and imidazole. Relationships with structural and catalytic zinc in some metallo-enzymes. Journal of Biomolecular Structure and Dynamics, 16, 1225–1237.

Cobine, P.A., Pierrel, F., & Winge, D.R. (2006). Copper trafficking to the mitochondrion and assembly of copper metalloenzymes. Biochimica et Biophysica Acta, 1763, 759–772.

Culotta, V.C., Lin, S.J., Schmidt, P., Klomp, L.W., Casarenino, R.L., & Gillin, J. (1999). Intracellular pathways of copper trafficking in yeast and humans. Advances in Experimental Medicine and Biology, 448, 247–254.

Danovich, D., & Filatov, M. (2008). No-pair bonding in coinage metal dimers. Journal of Physical Chemistry A, 112, 12995–13001.

Davis, A.V., & O’Halloran, T.V. (2008). A place for thioether chemistry in cellular copper ion recognition and trafficking. Nature Chemical Biology, 4, 148–151.

Dennington, R. R., Keith, T., Millam, J., Eppinnett, K., Hovell, W.L., & Gilliland, R. (2003). GaussView, Version 3.03. Shawnee Mission, KS: Semichem.

Dobrowolski, J. Cz., Rode, J.E., & Sadlej, J. (2007). Cysteine conformations revisited. Journal of Molecular Structure: Theoreon, 810, 129–134.

Elam, J.S., Thomas, S.T., Holloway, S.P., Taylor, A.B., & Hart, P.J. (2002). Copper chaperones. Advances in Protein Chemistry, 60, 151–219.

Fernández-Ramos, A., Cabaleiro-Lago, E., Hermida-Ramón, J. M., Martinez-Núñez, E., & Peña-Gallego, A. (2000). DFT conformational study of cysteine in gas phase and aqueous solution. Journal of Molecular Structure: Theoreon, 498, 191–200.
Finney, L.A., & O’Halloran, T.V. (2003). Transition metal speciation in the cell: Insights from the chemistry of metal ion receptors. *Science, 300*, 931–936.

Frisch, A., & Frisch, M.J. (1998). *Gaussian98, User’s Reference (2nd ed.).* Pittsburgh, PA, 15106: Gaussian, Carnegie Office Park, Building 6.

Frisch, M.J., Trucks, G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., … & Pope, J.A. (2010). *Gaussian09, Revision B.01*. Wallingford, CT: Gaussian.

Gillespie, R.J. (1963). The valence-shell electron-pair repulsion (VSEPR) theory of directed valence. *Journal of Chemical Education, 40*, 295–301.

Gorelsky, S.I., Ghosh, S., & Solomon, E.I. (2006). Mechanism of N₂O reduction by the mu4-S tetranuclear CuZ cluster of nitrous oxide reductase. *Journal of the American Chemical Society, 128*, 278–290.

Harriss, E.D. (2000). Cellular copper transport and metabolism. *Annual Review of Nutrition, 20*, 291–310.

Harrison, M.D., Jones, C.E., Solioz, M., & Dameron, C.T. (2000). Intracellular copper routing: The role of copper chaperones. *Trends in Biochemical Sciences, 29*, 29–32.

Hearmshaw, S., West, C., Zhou, L., Kihlken, M.A., Strange, R.W., Le Brun, N.E., & Hemmings, A.M. (2009). A tetranuclear Cu(I) cluster in the metallochaperone protein CopZ. *Biochemistry, 48*, 9324–9326.

Heller, M., & Sheldrick, W.S. (2004). Copper(I) Coordination polymers with alkanedithiol and -dinitrile bridging ligands. *Zeitschrift für Anorganische und Allgemeine Chemie, 630*, 1869–1874.

Horn, D., & Barrientos, A. (2008). Mitochondrial copper metabolism and delivery to cytochrome c oxidase. *International Union of Biochemistry and Molecular Biology Life, 60*, 421–429.

Huffman, D.L., & O’Halloran, T.V. (2001). Function, structure, and mechanism of intracellular copper trafficking proteins. *Annual Review of Biochemistry, 70*, 677–701.

Iwata, S. (1998). Structure and function of bacterial cytochrome c oxidase. *Journal of Biochemistry, 123*, 369–375.

Iwata, S., Ostermeier, C., Ludwig, B., & Michel, H. (1995). Structure at 2.8 Å resolution of cytochrome c oxidase from Paracoccus denitrificans. *Nature, 376*, 660–669.

Kim, B.E., Nevitt, T., & Thiele, D.J. (2008). Mechanisms for copper acquisition, distribution and regulation. *Nature Chemical Biology, 4*, 176–185.

Lu, Z.H., & Solioz, M. (2002). Bacterial copper transport. *Advances in Protein Chemistry, 60*, 93–121.

Lutsenko, S., Barnes, N.L., Bartee, M.Y., & Dmitriev, O.Y. (2007). Function and regulation of human copper-transporting ATPases. *Physiological Reviews, 87*, 1011–1046.

Moggach, S.A., Clark, S.J., & Parsons, S. (2005). L-Cysteine-I at 30 K. *Acta Crystallographica Section E: Structure Reports Online, 61*, 2739–2742.

O’Halloran, T.V., & Culotta, V.C. (2000). Metallochaperones, an intracellular shuttle service for metal ions. *Journal of Biological Chemistry, 275*, 25057–25060.

Pavelka, M., Šimánek, M., Šponer, J., & Burda, J.V. (2006). Copper cation interactions with biologically essential types of ligands: A computational DFT study. *Journal of Physical Chemistry A, 110*, 4795–4809.

Peariso, K., Huffman, D.L., Penner-Hahn, J.E., & O’Halloran, T.V. (2003). The PcoC copper resistance protein coordinates Cu(I) via novel S-methionine interactions. *Journal of the American Chemical Society, 125*, 342–343.

Prohaska, J.R., & Gybina, A.A. (2004). Intracellular copper transport in mammals. *Journal of Nutrition, 134*, 1003–1006.

Puig, S., & Thiele, D.J. (2002). Molecular mechanisms of copper uptake and distribution. *Current Opinion in Chemical Biology, 6*, 171–180.

Rao, L., Cui, Q., & Xu, X. (2010). Electronic properties and desolvation penalties of metal ions plus protein electrostatics dictate the metal binding affinity and selectivity in the copper efflux regulator. *Journal of the American Chemical Society, 132*, 18092–18102.

Robinson, N.J., & Winge, D.R. (2010). Copper metallochaperones. *Annual Review of Biochemistry, 79*, 537–562.

Rosenzweig, A.C. (2001). Copper delivery by metallochaperone proteins. *Accounts of Chemical Research, 34*, 119–128.

Rosenzweig, A.C., & O’Halloran, T.V. (2000). Structure and chemistry of the copper chaperone proteins. *Current Opinion in Chemical Biology, 4*, 140–147.

Siegbahn, P.E. (2006). The performance of hybrid DFT for mechanisms involving transition metal complexes in enzymes. *Journal of Biological Inorganic Chemistry, 11*, 695–701.

Siegbahn, P.E., & Himo, F. (2009). Recent developments of the quantum chemical cluster approach for modeling enzyme reactions. *Journal of Biological Inorganic Chemistry, 14*, 643–651.

Singleton, C., Hearmshaw, S., Zhou, L., Le Brun, N.E., & Hemmings, A.M. (2009). Mechanistic insights into Cu(I) cluster transfer between the chaperone CopZ and its cognate Cu(I)-transporting P-type ATPase, CopA. *Biochemical Journal, 424*, 347–356.

Solomon, E.I., Szilagyi, R.K., DeBeer, G.S., & Basumallick, L. (2004). Electronic structures of metal sites in proteins and models: Contributions to function in blue copper protein. *Chemical Reviews, 104*, 419–458.

Solomon, E.I., Xie, X., & Dey, A. (2008). Mixed valent sites and the metal coordination environment and selectivity in the copper efﬁxus regulator. *Journal of the American Chemical Society, 130*, 18092–18102.

Tome, R.W. (2010). Binding and activation of N₂O at transition-metal centers: Recent mechanistic insights. *Angewandte Chemie International Edition, 49*, 1018–1024.

Tome, J., Mennucci, B., & Cammi, R. (2005). Quantum mechanical continuum solvation models. *Chemical Reviews, 105*, 2999–3093.

Tetley, S., Harvie, D.R., & Robinson, N.J. (2005). Understanding how cells allocate metals using metal sensors and metallochaperones. *Accounts of Chemical Research, 38*, 775–783.