Electronic supplementary information of:

Dioxygen activation in Cu-amyloid β complex

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Computational details

In this section we report details about the following procedures:

- The construction of the 14 initial configurations used for the calculations performed at the DFT level reported in the main document.
- The DFT calculations of simplified (truncated) models.
- The Bond Valence Sum (BVS) calculations.

Construction of initial configurations

Models **Ia**, **IIa** and **IIc** were obtained by adding dioxygen to the Cu⁺-A β (1-16) structures previously obtained in Ref. 1 after reduction of Cu²⁺-A β (1-16). These latter models were built combining homology modeling (HM) with quantum mechanics based (QM) methods and enclose the different metal coordination spheres proposed experimentally at different pH by EPR spectroscopy (see Introduction in the main document). In order to isolate the effects of the first coordination sphere, the models were truncated keeping only the most relevant residues; that is, Asp1, His6, His13 and His14 for **Ia** and **IIa** and Asp1, Ala2 and His 6 for **IIc**. The obtained configurations were partially optimized at the DFT level (see below).

For models **1-15**, we considered the Cu-bound states of hydroperoxide in Cu(I)-A β (1-16) complexes, from Ref 2, and substituted hydroperoxide by dioxygen keeping the bath of 311 water molecules. The A β peptide is represented, as in the 16 models of Ref. 2, by the two peptides with sequence **DGGGGHD**–NHCH₃ and CH₃CO–**HH**–NHCH₃ model peptides, respectively. Among the 16 configurations, only those with O₂ in the first Cu-coordination sphere were considered whereas configurations with O₂ in the second sphere (**3**, **4**, **6**, **12** and **16**) have not been used as models for the dioxygen binding to Cu(I). The configuration numbering of Ref. 2 is here kept for an easier connection with that work.

Short Car-Parrinello damped molecular dynamics (CP-MD) simulations^{3,4} of 100 fs were performed on the 11 systems to adapt the configuration to the change of oxidation state. The parallel version of the Quantum-Espresso package⁵, which incorporates Vanderbilt ultrasoft pseudo-potentials⁶ and the PBE exchange-correlation functional⁷, was used in all CP-MD simulations. Electronic wave functions were expanded in plane waves up to an energy cutoff of 25 Ry, while a 250 Ry cutoff was used for the expansion of the augmented charge density

in the proximity of the atoms, as required in the ultra-soft pseudo-potential scheme. To minimize finite volume effects, periodic boundary conditions are imposed to the system. Each initial model for the Cu-peptide complex is inserted in a super-cell with sufficiently large linear dimensions to maximize the separation between nearest-neighbor replicas of the system so as to have minimal spurious self-interactions. The super-cell was orthorhombic with sides of 2.24, 2.05 and 2.15 nm in the three directions of space. All the DFT calculations were performed with separated spin effective mono-electronic states, within the local spin density approximation. Simulations have been carried out according to the following general protocol consisting of the two sequential steps: 1) minimization of electronic energy with fixed atomic positions; 2) minimization of total energy as a function of both atomic and electronic degrees of freedom. The energy minimization of steps 1-2 are performed via damped CP-MD, with a damping frequency for all the degrees of freedom of $1/(10\delta t)$ and with δt the time-step of 0.12 fs used for all the CP-MD simulations. The maximal force in the final configuration was always within 0.001 Ry/bohr and the number of minimization timesteps was in the range of 100-200, depending on the system size. It must be noticed that this minimization does not yield a minimum in the total energy, as expected in geometry optimizations, because of the large number of degrees of freedom involved.

The final configurations obtained with this basic minimization were used for constrained geometry optimizations based on truncated models that include only the relevant residues for coordination (Asp1, His6, His13 and His14) and water molecules within a range of 2 Å from the dioxygen molecule using a hybrid DFT functional. Cu ligands were simplified by substituting His sidechains by 4-methyl-imidazole, and Ala 2 by NHCH₃. The C β atoms of His sidechains and Asp 1 were kept fixed during geometry optimizations to partially keep the geometrical constraints due to the original peptides.

To estimate differences in formation energy for the 14 configurations, the molecule formed by Cu(I) bound to N δ / ϵ (N3) in 4-methyl-imidazole is assumed as a reference, mimicking the initially formed Cu(I)-His complex. The formation energy of each A β –Cu(I)–O₂ complex (E_f) is defined as the energy difference for the reaction:

$$(\text{metIm}_{\delta/\epsilon}) - \text{Cu}(I) - (H_2O)_3 + L + O_2 \rightarrow (\text{metIm}_{\delta/\epsilon}) L \text{Cu}(I) - O_2 + 3H_2O$$
(1)

where the L ligands are those additional to 4-methyl-imidazole (metIm_{δ/ϵ}) in each of the Cu-A β truncated model analyzed, (metIm_{δ/ϵ})L₂Cu(I) mimicking Cu(I)-A β .

Electronic structure methods

The 14 truncated configurations obtained as described above, were used for the investigation of O₂ activation by means of density functional theory methods. The functional chosen for this work needs to properly describe the coordination properties at the metal site, particularly the formation of the charged transfer $Cu^{2+}-A\beta \cdot \cdot O_2^{-}$ superoxide complex. For that, both the second ionization energy (IE) of Cu and the O₂ electron affinity (EA) needs to be properly described by the chosen functional. Table S1 shows the second IE of Cu and the O2 EA computed with different functionals and with the basis set (SB) used for the Cu-AB complexes; i.e., the LANL2DZ pseudopotential and its associated basis set for copper (5s5p5d)/ [3s3p2d]⁸ and the standard 6-31+G(d) basis set for the rest of atoms. Moreover, in order to analyze the effect of further enlarging the basis set on these two properties we used the triple-zeta quality basis set LANL2TZ (5s5p5d)/ [5s5p3d] basis set for Cu⁹ supplemented with an f function¹⁰ and the 6-311++G(d,p) for other atoms (LB). Results show that hybrid functionals (M06, M06-2X and B3LYP) provide similar IE, around 0.3(0.2) eV too large with the SB(LB) basis sets compared to the experimental value, while M06-L provides an IE that is 0.1 eV lower. However, concerning the electron affinity, M06-2X seems to be the one that better compares to experiment. Moreover, M06-2X functional has been shown to provide a good description of charge transfer complexes¹¹ and is able to account for mid-range dispersion forces¹². Additionally, previous studies on Cu²⁺ complexes^{13,14} have shown that GGA functionals or hybrid functionals with a low percentage of exact exchange tend to exhibit too large spin delocalization, situations that are overstabilized due to a bad cancellation of the self-interaction part by the exchange functional. Thus, M06-2X functional was chosen to analyze O_2 activation by Cu^+ -A β .

Geometries optimizations were done considering solvent (aqueous) effects using the SMD¹⁵ implicit solvation model and thus, residues were considered in the protonation states expected in solution at physiological pH. Noteworthy, calculations for a model system show that optimized geometrical parameters exhibit minor differences regardless we use one functional or another or whether we use the SB or LB. (see Table S2).

Open-shell calculations were based on an unrestricted formalism. All electronic structure calculations have been performed with the Gaussian 09 set of programs.¹⁶

Bond Valence Sum calculations

The bond valence parameter¹⁷ is given by the following expression:

Bond Valence =
$$e^{\left(\frac{R_0 - R}{B}\right)}$$

where R is the length of a bond between the two given atoms and R_0 and B are parameters (in Å) reported in the literature.¹⁸ The bond valence has the property that its sum (BVS) around each atom in a compound is equal to the valence (oxidation state) of that atom.

For all the 14 models described in Table 1 of the main document and shown in Figures S2 and S3 the bond valence sum (BVS) parameter is calculated using the following input parameters (in Å):

$$R_0(Cu(1)-N(-3)) = 1.52 \text{ (coordination = 3)}$$

$$R_0(Cu(1)-N(-3)) = 1.48 \text{ (coordination = 2)}$$

$$R_0(Cu(2)-N(-3)) = 1.763 \text{ (coordination = 4)}$$

$$R_0(Cu(2)-O(-2)) = 1.679 \text{ (coordination = 4)}$$

$$B = 0.37$$

cut-off for Cu-N/O ligand atoms = 2.5

The obtained values are in agreement with the expected formal oxidation state of the complexes. Moreover, the comparison with the crystal structures of Cu proteins¹⁹ shows that the computed BVS values for all of the structures here reported are acceptable.

]	E	EA		
	SB	LB	SB	LB	
Exp.	20	.29ª	-0.448 ^b		
M06-L	20.17	20.12	0.22	0.21	
M06	20.65	20.56	0.55	0.51	
M06-2X	20.60	20.52	0.35	0.37	
B3LYP	20.78	20.57	0.59	0.58	

Table S1. Ionization Energy (IE) of Cu and Electronic Affinity (EA) of O_2 in gas phase in eV at different levels of calculation with the small basis (SB) and the large basis (LB) sets. The references for the experimental data are a) Ref. 20 b) Ref. 21.



	Distances					Angles					Cu spin	
	dı	d ₂	d ₃	\mathbf{d}_4	\mathbf{d}_{5}	\mathbf{a}_1	\mathbf{a}_2	a ₃	\mathbf{a}_4	\mathbf{a}_5	\mathbf{a}_6	density
M06-L	2.13	2.02	2.06	2.03	1.28	91.9	88.8	89.6	95.4	157.5	164.3	0.44
M06	2.11	2.01	2.04	2.00	1.28	93.3	86.2	87.0	94.1	172.8	171.6	0.44
M06-2X	2.09	2.05	2.02	2.05	1.31	90.4	87.6	90.2	93.1	167.0	173.2	0.71
	(2.09)	(2.06)	(2.01)	(2.06)	(1.31)	(91.1)	(87.7)	(88.3)	(93.3)	(173.6)	(175.0)	0.71
B3LYP	2.12	2.04	2.05	2.04	1.31	92.5	87.6	88.1	93.8	169.5	167.9	0.53

Figure S1. Model considered for coordination Ia calculated at different levels of theory with the LB(SB) basis set. Cu atom is in orange, C atoms are in yellow, N atoms are in blue, O atoms are in red and H atoms are in white. Distances are in Angstroms and angles in degrees.



Figure S2. $Cu^+A\beta\cdots O_2 - Cu^{2+}A\beta O_2^-$ structures for models **Ia**, **IIa** and **IIc**. Cu atom is in orange, C atoms are in yellow, N atoms are in blue, O atoms are in red and H atoms are in white. Distances are in angstroms and angles in degrees.





Figure S3. $Cu^+A\beta\cdots O_2 - Cu^{2+}A\beta O_2^-$ structures for models **1-15**. One model per page is displayed. Cu atom is in orange, C atoms are in yellow, N atoms are in blue, O atoms are in red and H atoms are in white. Distances are in angstroms and angles in degrees.

Model 2

 $\mathbf{Cu}^{+}\mathbf{A\beta}\cdots\mathbf{O}_{2}$



Figure S3. Continuation.















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Figure S3. End.

References

¹ J. Alí-Torres, A. Mirats, J.-D. Maréchal, L. Rodríguez-Santiago and M. Sodupe, *J. Phys. Chem. B*, 2014, **118**, 4840–4850.

² G. La Penna, C. Hureau, O. Andreussi and F. Peter, *J. Phys. Chem. B*, 2013, **117**, 16455–16467.

³ R. Car and M. Parrinello, *Phys. Rev. Lett.*, 1985, **55**, 2471–2474.

⁴ D. Marx and J. Hutter, *Ab Initio Molecular Dynamics: Basic Theory and Advanced Methods*, Cambridge University Press, Cambridge, 2009.

⁵ P. Giannozzi, S. Baroni, N. Bonini, M. Calandra, R. Car, C. Cavazzoni, D. Ceresoli, G. L.

Chiarotti, M. Cococcioni, I. Dabo, A. Dal Corso, S. de Gironcoli, S. Fabris, G. Fratesi,

R. Gebauer, U. Gerstmann, C. Gougoussis, A. Kokalj, M. Lazzeri, L. Martin-Samos, N. Marzari, F. Mauri, R. Mazzarello, S. Paolini, A. Pasquarello, L. Paulatto, C. Sbraccia,

S. Scandolo, G. Sclauzero, A. P. Seitsonen, A. Smogunov, U. Paolo and R. M. Wentzcovitch, *J. Phys.: Condens. Matter*, 2009, **21**, 395502.

⁶ D. Vanderbilt, *Phys. Rev. B*, 1990, **41**, 7892–7895.

⁷ J. P. Perdew, K. Burke and M. Ernzerhof, *Phys. Rev. Lett.*, 1996, **77**, 3865–3868.

⁸ P. J. Hay, W. R. Wadt, J. Chem. Phys. 1985, **82**, 299-310.

⁹ L. E. Roy, P. J. Hay and R. L. Martin J. Chem. Theory Comput. 2008, 4, 1029–1031.

¹⁰ A. W. Ehlers, M. Böhme, S. Dapprich, A. Gobbi, A. Höllwarth, V. Jonas, K. F. Köhler, R. Stegmann, A. Veldkamp and G. Frenking *Chem. Phys. Lett.* 1993, **208**, 111–114.

¹¹ S. N. Steinmann, C. Piemontesi, A. Delachat and C. Corminboeuf *J. Chem. Theory Comput.* 2012, **8**, 1629–1640.

¹² Y. Zhao and D. G. Thrular *Theor. Chem. Acc.* 2008, **120**, 215–241.

¹³ R. Rios-Font, M. Sodupe, L. Rodriguez-Santiago and P. R. Taylor *J. Phys. Chem. A* 2010, **114**, 10857–10863.

¹⁴ I. Georgieva, N. Trendafilova, L. Rodriguez-Santiago and M., Sodupe *J. Phys. Chem. A* 2005, **109**, 5668–5676.

¹⁵ A. V. Marenich; C. J. Cramer; D. G. Truhlar J. Phys. Chem. B 2009, **113**, 6378–6396.

¹⁶ M. J. Frisch; G. W. Trucks; H. B. Schlegel; G. E. Scuseria; M. A. Robb; J. R. Cheeseman;

G. Scalmani; V. Barone; B. Mennucci; G. A. Petersson; H. Nakatsuji; M. Caricato; X. Li; H.

P. Hratchian; A. F. Izmaylov; J. Bloino; G. Zheng; J. L. Sonnenberg; M. Hada; M. Ehara; K.

Toyota; R. Fukuda; J. Hasegawa; M. Ishida; T. Nakajima; Y. Honda; O. Kitao; H. Nakai; T. Vreven; J. J. A. Montgomery; J. E. Peralta; F. Ogliaro; M. Bearpark; J. J. Heyd; E. Brothers; K. N. Kudin; V. N. Staroverov; R. Kobayashi; J. Normand; K. Raghavachari; A. Rendell; J. C. Burant; S. S. Iyengar; J. Tomasi; M. Cossi; N. Rega; J. M. Millam; M. Klene; J. E Knox.; J. B. Cross; V. Bakken; C. Adamo; J. Jaramillo; R. Gomperts; R. E. Stratmann; O. Yazyev; A. J. Austin; R. Cammi; C. Pomelli; J. W. Ochterski; R. L. Martin; K. Morokuma; V. G. Zakrzewski; G. A. Voth; P. Salvador; J. J. Dannenberg; S. Dapprich; A. D. Daniels; O. Farkas; J. B. Foresman; J. V. Ortiz; J. Cioslowski; D. J. Fox, Gaussian 09, Revision A.2. *Gaussian Inc Wallingford CT*, 2009, **34**, Wallingford CT.

¹⁸ I. D. Brown, IUCr data-set updated in 2013:

http://www.iucr.org/resources/data/data-sets/bond-valence-parameters

¹⁹ H. Zheng; M.D. Chordia; D.R. Cooper; M. Chruszcz; P. Müller; G.M. Sheldrick; W. Minor *Nat. Protocols* 2014, **9**, 156–170, http://csgid.org/csgid/metal_sites/

²⁰ National Institute of Standards and Technology (accessed 04/10/2014).

http://physics.nist.gov/PhysRefData/Handbook/Tables/coppertable1.htm

²¹ K. M., Ervin; W., Anusiewicz; P., Skurski; J., Simons; W. C., Lineberger *J. Phys. Chem. A* 2003, **107**, 8521–8529.