

Supporting Information

Probing kojic acid binding to Tyrosinase enzyme: insights from model complex and QM/MM calculations

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Experimental

All starting materials were commercially available and used as purchased, unless stated otherwise. Solvents were purified by standard methods before use. Copper(II) complex **1** was synthesized as previously described¹. **Caution:** Although no problems were encountered during the preparation of perchlorate salts, suitable care should be taken when handling such potentially hazardous compounds.

Elemental analyses were performed by the Service Central d'Analyse du CNRS in Lyon, Solaize, France. Electrospray mass spectra were recorded on an Esquire 3000 plus Bruker Daltonics with nanospray inlet. UV-visible spectra were recorded using quartz cuvette (1.0 cm) and a Varian Cary 50 UV-Visible spectrophotometer equipped with a Peltier thermostating accessory.

*1. Synthesis of the copper(II) complex [Cu₂(BPMP)(KA)] (ClO₄)₂ (**1-KA**).*

To a solution of complex **1** (19 mg, 0.02 mmol, 1 eq) in methanol (3 ml) and a small amount of acetone was added a solution of kojic acid (64 mg, 0.45 mmol, 22 eq.) in methanol (3 ml) and a small amount of water. The solution was stirred 30 min and filtered. The green filtrate is slow evaporated and after 3 weeks, pale green crystals of **1-KA** suitable for X-Ray diffraction

¹S. Torelli, C. Belle, I. Gautier-Luneau, J.-L. Pierre, E. Saint Aman, J.-M. Latour, L. Le Pape, D. Luneau, *Inorg. Chem.* **2000**, 39, 3526.

analysis were obtained in presence of white crystals of kojic acid and dark green crystals from complex **1**. Pale green crystals are collected one from the batch. Anal. Calcd. for $C_{39}H_{38}Cl_2Cu_2N_6O_{16} \cdot CH_3OH \cdot 2H_2O$: C, 45.12; H, 4.35; N, 7.89. Found C, 45.02; H, 4.27; N, 7.43. ESI-MS (CH_3CN), m/z : $z = 1$, 897 = (M - ClO_4^-); $z = 2$, 399 = (M - $2ClO_4^-$). UV-Vis (acetone) /nm ($\epsilon/M^{-1} cm^{-1}$): 420 (1584), 640 (400).

2. X-ray structure determination of copper(II) complex (**1-KA**)

Measurement were made on a Bruker-Nonius KappaCCD diffractometer with graphite monochromatized Mo($K\alpha$) radiation ($\lambda = 0.71073 \text{ \AA}$).

Crystal data for (1-KA): $C_{39}H_{38}Cu_2N_6O_5 \cdot 2(ClO_4) \cdot 2(CH_3OH) \cdot H_2O$, Mw = 1078.83, crystal dimensions (mm) : $0.40 \times 0.30 \times 0.10$, crystal system : triclinic, space group : P-1, unit-cell dimensions and volume : $a = 10.807(2) \text{ \AA}$, $b = 11.252(2) \text{ \AA}$, $c = 20.073(4) \text{ \AA}$, $\alpha = 76.82^\circ$, $\beta = 75.42^\circ$, $\gamma = 85.76^\circ$, $V = 2299.8(8) \text{ \AA}^3$, no. of formula units in the unit cell $Z = 2$, calculated density $\rho_{calcd} = 1.558 \text{ g cm}^{-3}$, linear absorption coefficient μ : 1.118 mm^{-1} , temperature of measurement: $T = 200 \text{ K}$, $2 \theta_{max} 55^\circ$, no. of measured and independent reflections : 34170 and 9811, no. of reflections with $I > 2\sigma(I)$: 7899, no. of parameters and restraints : 633 and 41, Rint : 0.027, R = 0.0701, wR = 0.1806, residual electron density: 1.984.

Data were collected using phi and omega scans. Data reduction, cell determination and refinement were performed using EvalCCD². Data were corrected for absorption using SADABS-2004/1 (Bruker, 2004). The structure was solved using SIR-92³, and refined using SHELXL⁴, run under Olex2i. C, N, O, Cl and Cu atoms were refined anisotropically by the full matrix least-squares method. H atoms were set geometrically and constrained to their

²A.J.M. Duisenberg, L.M.J. Kroon-Batenburg, A.M.M. Schreurs, *J. Appl. Cryst.*, **2003**, 36, 220.

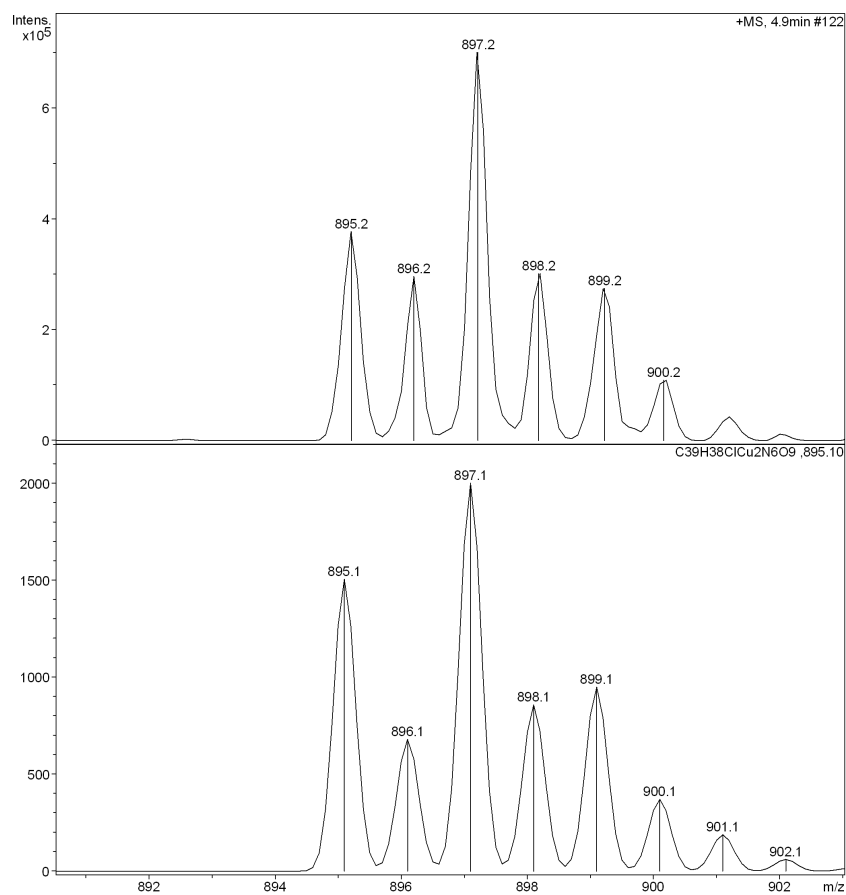
³A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, *J Appl Cryst.*, **1993**, 26, 343.

⁴G.M. Sheldrick, *Acta Cryst.*, **2008**, A64, 112.

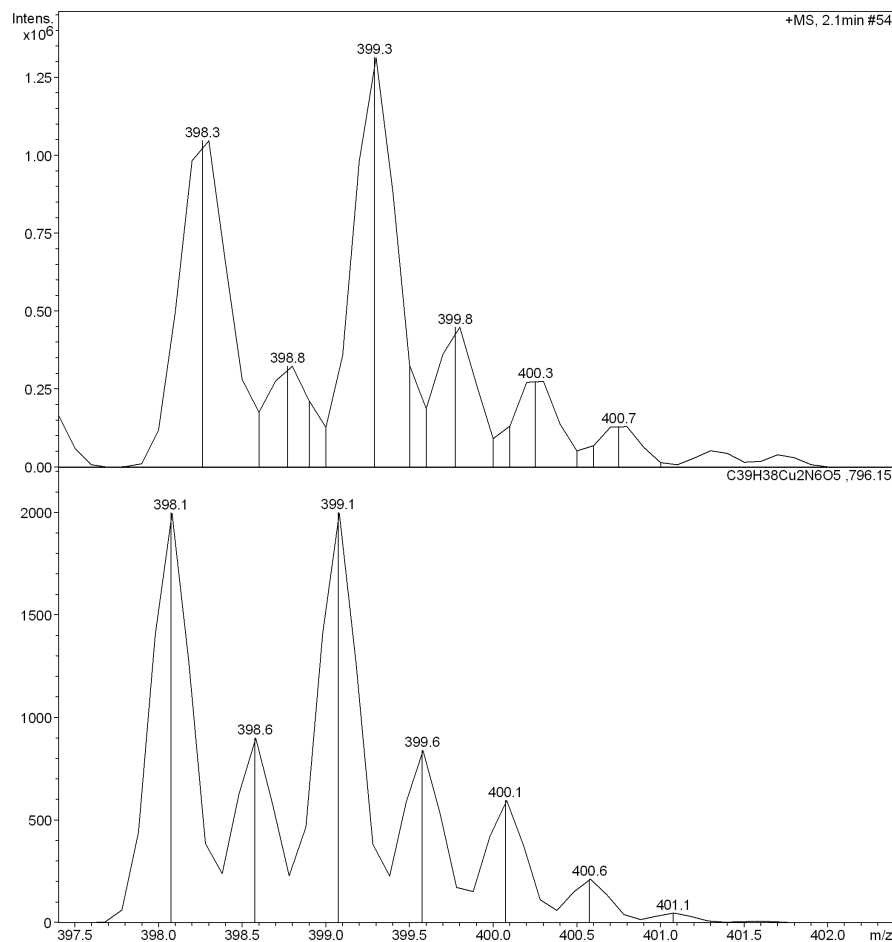
bearing atoms. The CIF file with data for (**1-KA**) has been deposited at the Cambridge Crystallographic Data Centre, CCDC871870. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK. Fax (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk

Figure S1: Experimental (top) and theoretical (bottom) isotopic patterns of (a) $[\text{Cu}_2(\text{BPMP})(\text{KA})](\text{ClO}_4)]^+$ ion(**1-KA**)- ClO_4^+ and (b) $[\text{Cu}_2(\text{BPMP})(\text{KA})]^{2+}$ ion(**1-KA-2ClO}_4)^{2+} in acetone.**

(a)



(b)



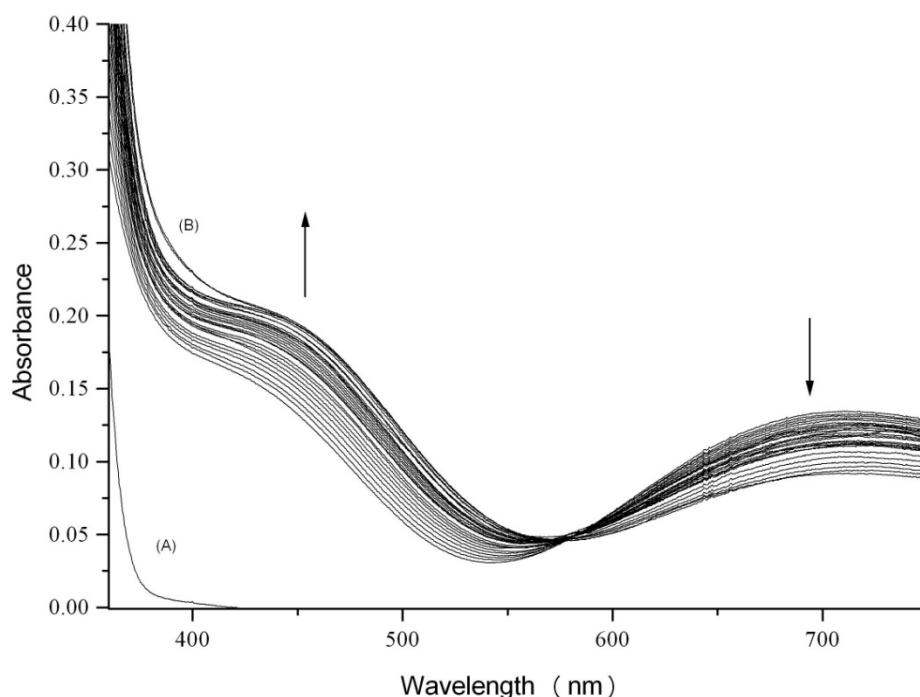
3. Spectrophotometric titration of complex **1** by kojic acid

The titration was carried out by adding small aliquots of a concentrate solution of kojic acid (100 mM) to solution of complex **1** (0.57 mM) in water/DMSO 95/5 (v/v) buffered at pH 7.0 with 50 mM HEPES. The spectral data were processed with the SPECFIT/32 Global Analysis System (Spectrum Software Associates),⁵ adjusting the equilibrium constant and the corresponding molar extinction coefficient of the species at equilibrium. The spectra exhibit an isosbestic point at $\lambda = 577\text{nm}$ indicating that an equilibrium is established between only two different species. Analysis of the spectral data by the Specfit program gave the best fit for

⁵(a) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1985, **32**, 257; (b) H. Gampp, M. Maeder, C. J. Meyer and A. D. Zuberbühler, *Talanta*, 1986, **33**, 943.

a 1:1 stoichiometry of the adduct formation between the complex and kojic acid. The range of values for the residual-squares sum ($\Sigma(A_{\text{exp}} - A_{\text{calc}})^2$) of the fit was $2 \cdot 10^{-2}$.

Figure S2: (A) UV/Vis spectrum of kojic acid (11.4 mM at 25 °C in aqueous mixture (DMSO 5%) buffered at pH 7.0, Hepes 50 mM); (B) Changes in UV/Vis spectrum upon addition of 50 equivalent of kojic acid (100 mM, 1 equiv. by 1 equiv. until 20 equiv, 2 equiv until 22 equiv. and 5 equiv. by 5 equiv. from 25 equiv. to 50 equiv.) to a solution of **1** (0.57 mM at 25°C in aqueous mixture (DMSO 5%) buffered at pH, Hepes 50mM).



4. Computational details:

The initial structure for the enzyme is based on the crystal structure of the bacterial tyrosinase complexed with the ORF38 caddie protein (PDB code: 2ahk). The caddie protein and the water molecules, with the exception of three water molecules close to the active site, were removed. The addition of H atoms leads to the protonation of all histidines on the δ nitrogen atom, whereas the aspartate and glutamate residues remain deprotonated, arginine and lysine residues are positively charged. The X-ray structure of **1**-KA was then aligned with the active

site of the enzyme with the ‘fit atom’ tool of Sybyl version 8.0.⁶ Alignment was done between the His N ϵ and Cu atoms from the enzyme and the N and Cu atoms of **1**-KA. Structure was solvated with a TIP3P water rectangular box (10 Å radius). Equilibration was performed with AMBER10⁷ with the same procedure and parameters described in our previous publication.⁸ Then QM/MM calculations were performed by using the Gaussian03 package⁹ for the QM part and Tinker4.2¹⁰ for the MM part. Both mechanical and electrostatic embedding (direct polarization) schemes were used to describe the interactions between the QM and the MM part, thus ensuring a good description of the effects of the environment of the active site. The QM part contains the two copper (in triplet state), the hydroxyl ion, KA, the side chain of the six histidines linked to the copper. The rest of the protein and the solvent were computed at MM level. The partition between QM and MM parts has led to the cut of six C α -C β bonds of the histidines. The Link atom scheme was used to treat the QM/MM boundary. The QM part was computed with the B3LYP/6-31G* level of theory and the MM part with amber99SB force field parameters. Geometry optimizations were firstly performed with only the residues at 4 Å of the QM part allowed to move. The other residues were frozen. Afterwards, QM/MM dynamics were carried out. The QM/MM Born-Oppenheimer molecular dynamics was run in the NVT ensemble at 298K. The time step was increased by using mass-scaling molecular dynamics. The hydrogen mass was set at 10 amu in order to “freeze” the X-H vibrations and allow us to take a time step of 3 fs for the dynamics.¹¹ For the Tyrosinase coordinated by KA

⁶SYBYL 8.0, Tripos Associates, Inc., Saint Louis, MO, 2007.

⁷Case, D.A.; Darden, T.A.; Cheatham, T.E. III; Simmerling, C.L.; Wang, J.; Duke, R.E.; Luo, R.; Crowley, M.; Walker, R.C.; Zhang, W.; Merz, K.M.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossváry, I.; Wong, K.F.; Paesani, F.; Vanicek, J.; Wu, X.; Brozell, S.R.; Steinbrecher, T.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Mathews, D.H.; Seetin, M.G.; Sagui, C.; Babin, V.; Kollman, P.A.; AMBER 10, University of California, San Francisco, **2008**.

⁸C.Bochet,E. Favre,C. Dubois,B. Baptiste,L. Bubacco,P.A. Carrupt,G. Gellon,R Hardré, D Luneau, Y Moreau, A Nurisso,M Réglier,G Serratrice,C Belle, H. Jamet,*Chem. Eur. J.*, **2013**, 19, 11, 3655.

⁹M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, J. R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. Petersson, A. P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, J. A. Pople, revision A.1. ed., Gaussian 03 Revision A1, Gaussian Inc., Pittsburg PA., **2003**.

⁹ J.W.Ponder, Tinker 4.2 ed., Washington University, Saint Louis, **2004**.

¹⁰ H.Zheng, S.Wang, Y. Zhang, *J. Comput. Chem.***2009**, 16, 2706.

(Ty-KA) with CuA bidentate, a 2.5 ps dynamic was performed and the position is found to be stable (Fig. S3a,b). For system with CuB bidentate, oxygen atom corresponding to O₃ in KA shifts from the first 0.05 ps of the simulation (Fig.S3c).

The exchange constant coupling J was computed using the broken symmetry approach with the Yamaguchi formula.¹² For that purpose, additional single-point high-spin (HS) and broken-symmetry (BS) calculations were done on the QM part of the last structure obtained after the simulation of Ty-KA with CuA bidentate and on the 1-KA structure. QM single point calculations (B3LYP in combination with the TZVP basis set¹³) were performed with the software ORCA,¹⁴ which provides directly the analysis of the unrestricted corresponding orbitals (UCOs). This analysis involves a unitary transformation of the α and β orbitals to create magnetic orbital. Figure S4 shows the magnetic orbitals along with their spatial overlaps S for 1-KA and for the QM part of Ty-KA. Finally, for the QM/MM calculation of J , we still used Gaussian03 package for the QM part and Tinker4.2 for the MM part. Calculations were performed at level B3LYP/6-311g*, using the high-spin wave function and the guess=(read, mix) keyword to generate the unrestricted broken symmetry singlet wave function.

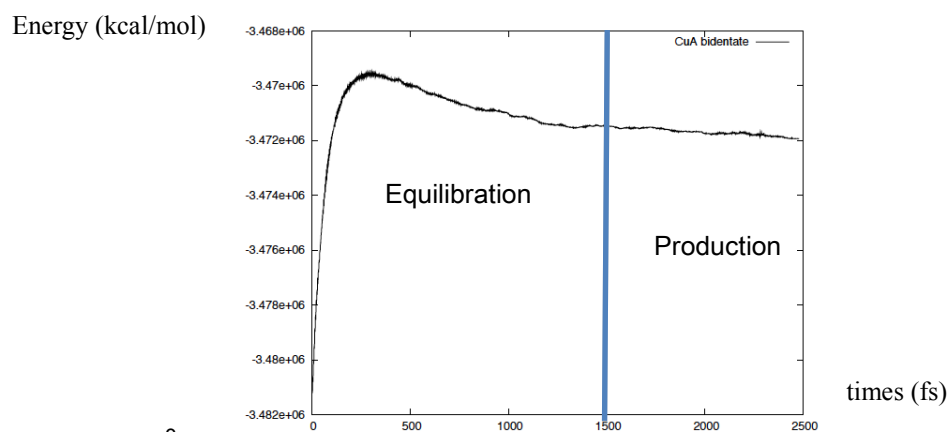
¹²T. Soda, Y. Kitagawa, T. Onishi, Y. Takano, Y. Shigeta, H. Nagao, Y. Yoshioka, K. Yamaguchi *Chem Phys Lett.* **2000**, 319, 223.

¹³A. Schaefer, H. Horn, R. Ahlrichs, *J. Chem. Phys.* **1992**, 97, 2571.

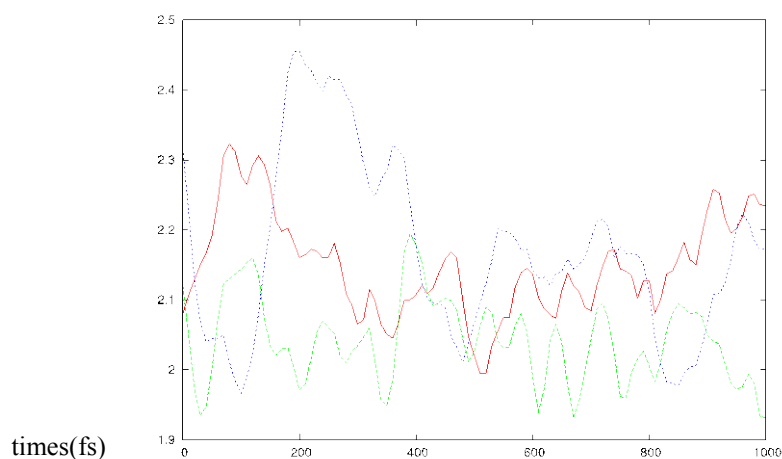
¹⁴ORCA Version 2.6, revision 35. Developed by Neese, F. with contributions from Becker, U.; Ganiouchine, D.; Kößmann, S.; Petrenko, T.; Riplinger, C.; Wennmohs, F., see: <http://www.thch.uni-bonn.de/tc/orca/>.

Figure S3: QM(B3lyp/6-31g*)/MM(amber99Sb) dynamics (a) plot of total energy as a function of time for Ty-KA with CuA bidentate (b) Evolution of Cu-O (CuB..O2 in red, CuA...O2 in green, CuA...O3 in blue) in distances during the last 1ps of the simulation a (c) Evolution of CuB-O3/CuA-O3 distances during the first 0.05 ps of the simulation for Ty-KA, with CuB bidentate.

(a)



(b) Distances(Å)



(c)

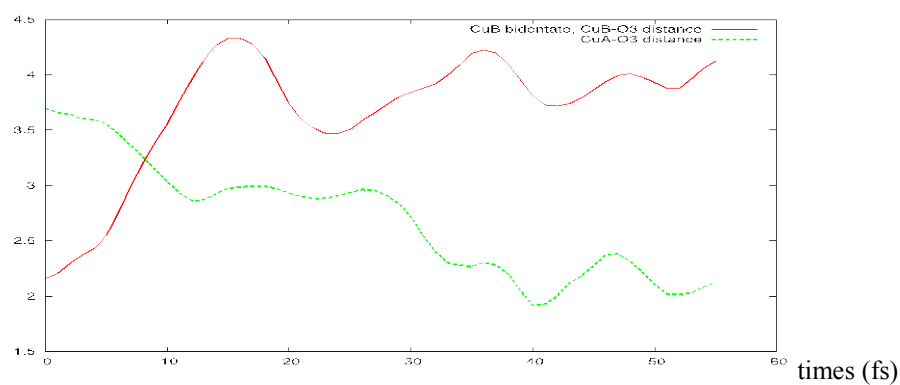
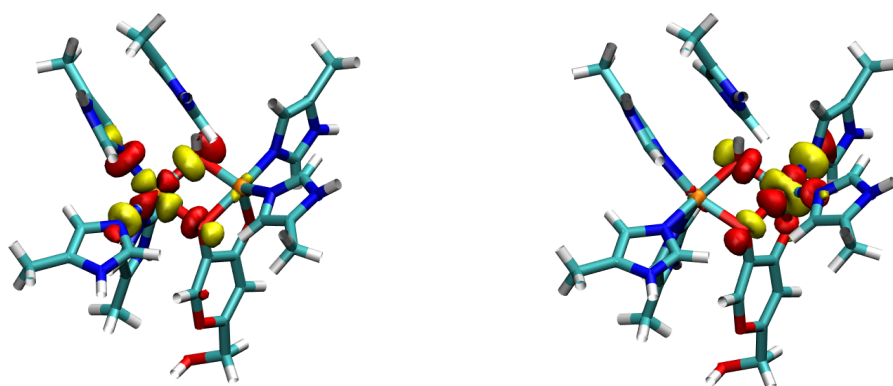


Figure S4:(a) Magnetic orbitals for the broken symmetry states of the QM part of Ty-KA. Spatial overlap between the two orbitals equal to 0.156 (b) Magnetic orbitals for the broken symmetry states of **1**-KA. Spatial overlap between the two orbitals equal to 0.079. Color scheme: Cu : orange; O : red; C : cyan; N : blue, H : white.

(a)



(b)

