***Support Information***

**Refining Details of the Structural and Electronic Properties of the CuB Site in pMMO Enzyme through Sequential Molecular Dynamics/CPKS-EPR Calculations.**

William Daniel B. Da Silva,a Roberta P. Dias,b and Júlio C.S. Da Silvaa\*

*a LQCBio: Laboratório de Química Computacional e Modelagem de Biomoléculas*

Instituto de Química e Biotecnologia, IQB, Universidade Federal de Alagoas

Campus A. C. Simões, 57072-900, Maceió, AL, Brazil.

*bGIMMM*: *Grupo Interdisciplinar de Modelagem Molecular e Simulação de Materiais*

Núcleo Interdisciplinar de Ciências Exatas e da Natureza - NICEN, Campus do Agreste, Universidade Federal de Pernambuco

55002-970, Caruaru, PE, Brazil.

**\***Corresponding authors.

*E-mail adresses:* julio.silva@iqb.ufal.br(J. C. S. Da Silva).

**Table S1**: Calculated EPR parameters g and ACu(MHz) tensors, Aiso and contributions from the Fermi contact (AFC) at the DFT/def2-TZVP level considering different integration grid (Grid5 and Grid7). Calculation carried out on the structure named as frame1 (see figure S1). CGTOs is the number of contracted Gaussian Type Orbitals functions in the basis set.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **B1LYP** | | | | | | | | | |
| **Grid** | **CGTOs** | **g1** | **g2** | **g3** | **AFC** | **Aiso** | **A1** | **A2** | **A3** |
| Labela | 2153 | gy | gx | gz |  |  | Ax | Ay | Az |
| Grid5 | 2.050 | 2.088 | 2.207 | 110.5 | 269.12 | -175.3 | 408.1 | 574.5 |
| Grid7 | 2.050 | 2.088 | 2.207 | 110.9 | 269.52 | -174.9 | 408.5 | 574.9 |
| **BHandHLYP** | | | | | | | | | |
| **Grid** | **CGTOs** | **g1** | **g2** | **g3** | **AFC** | **Aiso** | **A1** | **A2** | **A3** |
| Labela | 2153 | gy | gx | gz |  |  | Ax | Ay | Az |
| Grid5 | 2.068 | 2.122 | 2.301 | 103.8 | 323.5 | -126.4 | 464.5 | 632.2 |
| Grid7 | 2.069 | 2.122 | 2.301 | 104.4 | 324.0 | -125.9 | 465.0 | 632.8 |

a The labels were assigned according to the eigenvectors orientation concerning to the molecular reference model (frame1).

Uma imagem contendo voando, ar, pipa, diferente

Descrição gerada automaticamente

**Figure S1:** Structure of the frame1 cluster and the representation of the calculated relative orientation of the g-factor matrix (red) and ACu (green) tensor. The representation in the sticks corresponds to the water molecules within 5 Å from the copper ion generated from the MD simulation. The two water molecules directly bound to the copper are represented in ball stick format.

**Table S2:** Calculated EPR parameter g tensor with the hybrid GGA B1LYP and B3LYP functionals.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Frame** |  |  |  |  |  |  |
| 1 | 2.088248 | 2.05055 | 2.2070144 | 2.0822383 | 2.04741 | 2.1925613 |
| 2 | 2.077995 | 2.05693 | 2.2110672 | 2.0732250 | 2.05259 | 2.1962594 |
| 3 | 2.062173 | 2.05906 | 2.1898775 | 2.0577102 | 2.05512 | 2.1760463 |
| 4 | 2.111881 | 2.03975 | 2.2118957 | 2.1040964 | 2.03772 | 2.1972458 |
| 5 | 2.076979 | 2.06017 | 2.2042178 | 2.0713641 | 2.05670 | 2.1897702 |
| 6 | 2.085379 | 2.05504 | 2.2073561 | 2.0801633 | 2.05103 | 2.1927619 |
| 7 | 2.081666 | 2.05113 | 2.2067931 | 2.0763037 | 2.04761 | 2.1923473 |
| 8 | 2.074463 | 2.06031 | 2.2074748 | 2.0690118 | 2.05638 | 2.1924660 |
| 9 | 2.075374 | 2.05939 | 2.2085719 | 2.0708378 | 2.05508 | 2.1940430 |
| 10 | 2.062735 | 2.05293 | 2.1868366 | 2.0584647 | 2.04940 | 2.1736628 |
| 11 | 2.068901 | 2.05111 | 2.1865245 | 2.0643635 | 2.04768 | 2.1732970 |
| 12 | 2.073721 | 2.05508 | 2.1957326 | 2.068104 | 2.05171 | 2.1812602 |
| 13 | 2.090175 | 2.05768 | 2.2166443 | 2.0831183 | 2.05513 | 2.2018538 |
| 14 | 2.065036 | 2.05262 | 2.1849085 | 2.0606142 | 2.04915 | 2.1717067 |
| 15 | 2.057345 | 2.04836 | 2.1746089 | 2.0532248 | 2.04511 | 2.1616325 |
| 16 | 2.066574 | 2.06260 | 2.2025280 | 2.0624065 | 2.05810 | 2.1882826 |
| 17 | 2.077967 | 2.04935 | 2.1962450 | 2.0732139 | 2.04564 | 2.1823477 |
| 18 | 2.079823 | 2.05275 | 2.2068718 | 2.0737619 | 2.04942 | 2.1919438 |
| 19 | 2.070578 | 2.05302 | 2.1914905 | 2.0655346 | 2.04963 | 2.1776578 |
| 20 | 2.068157 | 2.05480 | 2.1921036 | 2.0639389 | 2.05056 | 2.1779326 |
| ***Average*** | 2.070 ± 0.012 | 2.054 ± 0.005 | 2.199 ± 0.011 | 2.071 ± 0.011 | 2.051 ± 0.005 | 2.190 ± 0.012 |

**Table S3:** Calculated copper hyperfine constant with the hybrid GGA B1LYP and B3LYP functionals.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Frame** |  |  |  |  |  |  |
| 1 | -175.2656 | 408.0851 | 574.5462 | -172,7126 | 395,9341 | 557,8312 |
| 2 | -197.6296 | 424.6508 | 499.0071 | -194,3778 | 409,0302 | 484,4900 |
| 3 | -229.4667 | 424.2303 | 453.2722 | -225,0747 | 410,2617 | 438,1672 |
| 4 | -139.7708 | 387.1834 | 620.8042 | -139,1739 | 377,2095 | 600,4918 |
| 5 | -209.1124 | 436.4979 | 494.1252 | -206,8777 | 423,4280 | 475,6916 |
| 6 | -184.8143 | 419.6112 | 548.5471 | -181,2406 | 404,6258 | 533,8053 |
| 7 | -179.6954 | 419.5988 | 552.0397 | -175,6672 | 407,4609 | 537,4652 |
| 8 | -209.1740 | 417.0670 | 492.5562 | -206,5260 | 403,1815 | 475,1085 |
| 9 | -208.5272 | 432.4792 | 494.4348 | -205,5103 | 417,4023 | 480,8352 |
| 10 | -240.7646 | 399.8171 | 462.4378 | -235,9108 | 387,6987 | 448,3667 |
| 11 | -213.6461 | 419.8489 | 509.9715 | -207,6050 | 408,6765 | 497,3392 |
| 12 | -214.4641 | 406.9640 | 500.2667 | -211,4148 | 394,0633 | 482,1878 |
| 13 | -178.3322 | 432.9494 | 548.6103 | -177,5686 | 423,2432 | 527,2382 |
| 14 | -238.2500 | 407.1167 | 466.6708 | -233,4614 | 394,7261 | 451,8385 |
| 15 | -242.5334 | 390.3982 | 455.4658 | -235,4694 | 380,1962 | 442,5480 |
| 16 | -227.1144 | 434.2001 | 460.6529 | -224,1504 | 418,7112 | 447,3540 |
| 17 | -206.5015 | 404.6149 | 522.4089 | -201,4017 | 391,4164 | 509,6239 |
| 18 | -192.8512 | 406.0293 | 516.8195 | -189,8430 | 393,5420 | 497,6444 |
| 19 | -223.4126 | 411.3579 | 488.9988 | -219,3305 | 398,9972 | 472,8809 |
| 20 | -232.7342 | 410.4827 | 467.0595 | -228,3800 | 394,9477 | 453,8482 |
| ***Average*** |  |  | 506.0 ± 44.2 |  |  | 491.0 ± 43.0 |

Diagrama

Descrição gerada automaticamente

**Figure S2:** Oniom QM/MM optimized structure with highlights to the QM region.