Supplementary Information for

X-ray Crystallographic Evidence for the Simultaneous Presence of Axial and Rhombic Sites in Cupredoxins: Atomic Resolution X-ray Crystal Structure Analysis of Pseudoazurin and DFT Modelling

T. Yamaguchi,[a] K. Akao,[a] A. Takashina,[a] S. Asamura,[a] M. Unno,[a,b]
R. K. Szilagyi,[c*] and T. Kohzuma[a,b*]

[a]*Institute of Quantum Beam Science, Ibaraki University, Bunkyo 2-1-1, Mito, Ibaraki 310-8512, Japan*

[b] *Frontier Research Center for Applied Atomic Sciences, Ibaraki University, Shirakata 162-1,Tokai, Ibaraki, 319-1106, Japan*

[c]*Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59718, U.S.A. and MTA-ELTE "Momentum" Chemical Structure/Function Laboratory, Budapest, 1117, Hungary*

Selection of final structure model in given crystallographic analysis

The final structure model was selected based on disappearing of F_o-F_c residual density map in Figure S1. The F_o-F_c density map was minimalized when the occupancy of positions1 and positions2 is 40 % and 60 %, respectively.

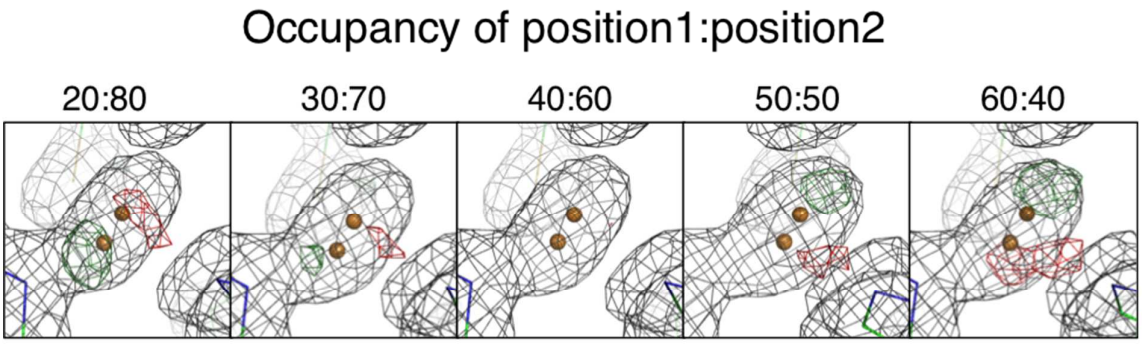

Figure S1: $2F_o$-F_c (gray: 1.5 σ) and F_o-F_c (green: 4.0 σ, red: -4.0 σ) density maps as a function of occupancy of position1:position2 for Cu ion.

Table S1. Cu-Ligand distances between oxidized and reduced form of $[N_2S_2]$ type 1 Cu site

#1. PlastocyaninA pH 6			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His37)	1.94	1.91	-0.03
Cu-S(Cys84)	2.16	2.15	-0.01
Cu-N(His87)	1.99	2.54	0.55
Cu-S(Met92)	2.78	2.62	-0.16
PDB ID - resolution	4DP9 - 1.00 Å	4DPA - 1.05 Å	
#2. PlastocyaninB pH 6			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His37)	2.07	2.06	-0.01
Cu-S(Cys84)	2.14	2.15	0.01
Cu-N(His87)	2.11	2.19	0.08
Cu-S(Met92)	2.74	2.71	-0.03
PDB ID - resolution	4DP2 - 1.80 Å	4DP4 - 1.54 Å	
#3. PlastocyaniA pH 8			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His37)	1.97	1.98	0.01
Cu-S(Cys84)	2.17	2.18	0.01
Cu-N(His87)	2.02	2.1	0.08
Cu-S(Met92)	2.76	2.71	-0.05
PDB ID - resolution	4DPB - 1.00 Å	4DPC - 1.06 Å	
#4. PlastocyaninB pH 8			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His37)	2.04	2.04	0.00
Cu-S(Cys84)	2.38	2.2	-0.18
Cu-N(His87)	2.24	2.21	-0.03
Cu-S(Met92)	2.78	2.74	-0.04
PDB ID - resolution	4DP5 - 1.88 Å	4DP6 - 1.67 Å	
#5. Pseudoazurin pH6.0			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	1.95	2.04	0.09
Cu-S(Cys78)	2.13	2.19	0.06
Cu-N(His81)	1.92	2.11	0.19
Cu-S(Met86)	2.71	2.85	0.14
PDB ID - resolution	1BQK - 1.35 Å	1BQR - 1.60 Å	
#6. Pseudoazurin pH 6.0			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	1.88	2.23	0.35
Cu-S(Cys78)	2.14	2.24	0.10
Cu-N(His81)	1.99	2.36	0.37
Cu-S(Met86)	2.71	2.76	0.05
PDB ID - resolution	1ZIA - 1.54 Å	1ZIB - 2.00 Å	
#7. Pseudoazurin pH7.0			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	2.01	2.1	0.09
Cu-S(Cys78)	2.13	2.17	0.04
Cu-N(His81)	2.01	2.31	0.30
Cu-S(Met86)	2.71	2.82	0.11
PDB ID - resolution	8PAZ - 1.60 Å	3PAZ - 1.73 Å	

Table S1. continued

#8. Pseudoazurin pH7.8			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	2.16	2.16	0.00
Cu-S(Cys78)	2.16	2.17	0.01
Cu-N(His81)	2.12	2.29	0.17
Cu-S(Met86)	2.76	2.91	0.15
PDB ID - resolution	1PAZ - 1.55 Å	1PZA - 1.80 Å	
#9. Pseudoazurin P80I			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	2.01	2.04	0.03
Cu-S(Cys78)	2.13	2.15	0.02
Cu-N(His81)	2.07	2.13	0.06
Cu-S(Met86)	2.9	2.95	0.05
PDB ID - resolution	6PAZ- 1.91 Å	7PAZ- 2.00 Å	
#10. Pseudoazurin P80A			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	1.98	2.06	0.08
Cu-S(Cys78)	2.14	2.19	0.05
Cu-N(His81)	1.96	2.02	0.06
Cu-S(Met86)	2.76	3.00	0.24
PDB ID - resolution	4PAZ- 1.76 Å	5PAZ- 1.76 Å	
#11. Amycyanin P94F pH 5.6			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His53)	1.97	1.98	0.01
Cu-S(Cys92)	2.22	2.2	-0.02
Cu-N(His95)	2.05	2.04	-0.01
Cu-S(Met98)	2.8	2.92	0.12
PDB ID - resolution	1SFD - 0.99 Å	1SFH- 1.05 Å	
#12. Pseudoazurin with engineered Amicyanin pH 7.5			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	2.09	2.05	-0.04
Cu-S(Cys78)	2.22	2.1	-0.12
Cu-N(His81)	2.09	2.5	0.41
Cu-S(Met86)	2.39	2.37	-0.02
PDB ID - resolution	2UX6- 1.30 Å	2UX7- 1.30 Å	
#13 Pseudoazurin with engineered Amicyanin pH 5.5			
Cu-Ligand	oxidized / Å	reduced / Å	elongation due to reduction / Å
Cu-N(His40)	2.1	2.16	0.06
Cu-S(Cys78)	2.27	2.14	-0.13
Cu-N(His81)	2.24	2.64	0.4
Cu-S(Met86)	2.38	2.43	0.05
PDB ID - resolution	2UXF - 2.00 Å	2UXG- 1.99 Å	

Fully optimized oxidized site at BP86/def2TZVP level (Fig. 3A)

Cu	7.252537000	9.614021000	19.965134000
H	10.992664000	13.094688000	18.455666000
C	10.928016000	12.269996000	19.184987000
C	9.601964000	11.658014000	19.143017000
S	9.372542000	10.238205000	20.306258000
H	11.727899000	11.553663000	18.938882000
H	11.165197000	12.695300000	20.173366000
H	8.810842000	12.391050000	19.358848000
H	9.395475000	11.257477000	18.139451000
H	6.142258000	7.962957000	25.106638000
C	6.782999000	8.530997000	24.395004000
O	7.647998000	8.001002000	23.719996000
N	6.529990000	9.838997000	24.398997000
C	7.161018000	10.725020000	23.456007000
C	6.115037000	11.431956000	22.615922000
C	5.128137000	10.520717000	21.948447000
N	5.462014000	9.689979000	20.878227000
C	4.362566000	9.004121000	20.553693000
N	3.342589000	9.357861000	21.369862000
C	3.801852000	10.310804000	22.257465000
C	7.986984000	11.822002000	24.160028000
O	7.801008000	12.125001000	25.327015000
H	5.839202000	10.214385000	25.045828000
H	7.815562000	10.103520000	22.828420000
H	5.551324000	12.133944000	23.252313000
H	6.620233000	12.045785000	21.852285000
H	4.275241000	8.277322000	19.753177000
H	3.162413000	10.749090000	23.015275000
H	2.396344000	8.985952000	21.327865000
N	8.919997000	12.348002000	23.379996000
C	9.633998000	13.533000000	23.847998000
H	10.273518000	13.294221000	24.710461000
H	10.264010000	13.914225000	23.035707000
H	9.090916000	11.970963000	22.446404000

H	8.922852000	14.314566000	24.150278000
H	12.314347000	6.916541000	20.072871000
C	11.839011000	6.636991000	19.102015000
O	12.047000000	5.578995000	18.556995000
N	11.049998000	7.563047000	18.630992000
C	10.281002000	7.367926000	17.415968000
C	8.829989000	7.749063000	17.596066000
C	8.148126000	6.981882000	18.693083000
N	7.496928000	7.619876000	19.746348000
C	7.066096000	6.672857000	20.583627000
N	7.403554000	5.454342000	20.102401000
C	8.084404000	5.622033000	18.911967000
H	10.716154000	7.957818000	16.590659000
H	10.868716000	8.406021000	19.187424000
H	10.385308000	6.310246000	17.139609000
H	8.742240000	8.819175000	17.836643000
H	8.308395000	7.601346000	16.636134000
H	6.565566000	6.837741000	21.531421000
H	8.463046000	4.779746000	18.344585000
H	7.206981000	4.565722000	20.557102000
H	8.850376000	10.668739000	13.732614000
C	8.241982000	11.328018000	14.370980000
C	7.950010000	10.700971000	15.672042000
C	7.132892000	11.645766000	16.566226000
S	6.247276000	10.799392000	17.965926000
C	5.320599000	12.251199000	18.604490000
H	8.800737000	12.272471000	14.479369000
H	7.320498000	11.558927000	13.812240000
H	7.381854000	9.766874000	15.523517000
H	8.882224000	10.416063000	16.186323000
H	7.755687000	12.449995000	16.983770000
H	6.323737000	12.107638000	15.979521000
H	4.715089000	11.908762000	19.450313000
H	4.664434000	12.630456000	17.811304000
H	6.011974000	13.035199000	18.936430000

Fully optimized oxidized site at B3LYP/def2TZVP level (Fig. 3B)

Cu	7.281578000	9.592163000	20.010296000
H	10.994097000	13.084257000	18.456958000
C	10.928015000	12.269994000	19.184996000
C	9.601955000	11.658012000	19.143013000
S	9.395072000	10.257244000	20.328480000
H	11.719662000	11.555310000	18.946838000
H	11.159316000	12.695780000	20.165051000
H	8.819162000	12.387715000	19.351371000
H	9.404452000	11.252666000	18.151903000
H	6.150997000	7.963262000	25.095547000
C	6.783004000	8.530991000	24.395012000
O	7.647998000	8.001003000	23.719986000
N	6.529979000	9.838997000	24.399016000
C	7.160989000	10.725013000	23.456043000
C	6.115108000	11.431954000	22.615809000
C	5.129466000	10.523217000	21.950191000
N	5.467186000	9.672038000	20.906293000
C	4.371673000	9.004033000	20.577664000
N	3.348431000	9.385078000	21.360470000
C	3.805392000	10.343719000	22.235082000
C	7.986973000	11.821999000	24.160045000
O	7.801016000	12.125001000	25.327021000
H	5.842913000	10.207441000	25.039501000
H	7.816228000	10.114999000	22.839554000
H	5.555138000	12.122147000	23.252160000
H	6.612449000	12.048294000	21.863195000
H	4.288027000	8.268589000	19.796098000
H	3.165880000	10.803233000	22.967507000
H	2.405467000	9.029027000	21.307785000
N	8.919991000	12.348003000	23.379995000
C	9.633995000	13.533003000	23.847996000
H	10.252299000	13.298878000	24.715381000
H	10.274159000	13.896952000	23.047180000
H	9.091036000	11.975127000	22.456154000

H	8.929274000	14.316540000	24.126987000
H	12.313119000	6.907802000	20.061532000
C	11.839010000	6.636983000	19.102009000
O	12.047001000	5.578984000	18.556995000
N	11.050000000	7.563068000	18.630996000
C	10.281015000	7.367954000	17.416005000
C	8.829961000	7.749019000	17.596025000
C	8.148509000	6.975690000	18.685371000
N	7.511327000	7.599023000	19.747398000
C	7.082500000	6.649910000	20.565908000
N	7.403374000	5.443953000	20.069738000
C	8.076066000	5.624506000	18.882732000
H	10.711563000	7.957327000	16.600656000
H	10.884717000	8.400621000	19.179330000
H	10.380857000	6.321164000	17.138276000
H	8.747880000	8.808686000	17.830434000
H	8.316098000	7.600705000	16.642721000
H	6.587075000	6.802900000	21.508688000
H	8.445081000	4.795999000	18.305325000
H	7.202992000	4.558165000	20.509476000
H	8.871799000	10.686481000	13.749901000
C	8.242004000	11.328047000	14.370977000
C	7.949952000	10.700941000	15.672082000
C	7.103079000	11.636487000	16.541098000
S	6.215842000	10.795018000	17.930640000
C	5.292542000	12.242195000	18.573108000
H	8.769092000	12.280302000	14.482711000
H	7.328171000	11.528308000	13.805147000
H	7.409209000	9.762182000	15.520837000
H	8.875253000	10.445073000	16.192760000
H	7.704159000	12.446047000	16.954852000
H	6.308226000	12.077957000	15.938024000
H	4.674833000	11.900362000	19.398574000
H	4.655953000	12.635133000	17.782830000
H	5.978727000	13.011471000	18.921649000

Fully optimized oxidized site at B38HFP86/def2TZVP level (Fig. 3C)

Cu	7.353587000	9.617781000	20.015001000
H	10.998264000	13.087064000	18.464310000
C	10.928009000	12.270007000	19.184999000
C	9.601979000	11.658003000	19.142994000
S	9.439667000	10.276548000	20.329430000
H	11.713315000	11.552370000	18.944768000
H	11.156186000	12.683223000	20.168606000
H	8.825390000	12.389745000	19.359823000
H	9.399352000	11.260051000	18.150794000
H	6.154164000	7.963556000	25.092336000
C	6.783001000	8.530996000	24.395000000
O	7.648001000	8.000999000	23.719998000
N	6.529997000	9.839008000	24.399000000
C	7.160996000	10.725000000	23.456013000
C	6.115001000	11.431999000	22.615995000
C	5.177572000	10.509787000	21.917150000
N	5.555652000	9.678874000	20.880194000
C	4.487598000	8.999401000	20.519533000
N	3.446860000	9.350052000	21.271873000
C	3.858204000	10.301436000	22.162506000
C	7.987000000	11.821994000	24.160001000
O	7.801001000	12.124999000	25.327002000
H	5.839926000	10.205575000	25.030589000
H	7.819646000	10.116390000	22.840572000
H	5.527114000	12.089989000	23.257379000
H	6.613133000	12.075513000	21.888474000
H	4.440062000	8.271915000	19.729064000
H	3.192924000	10.741936000	22.881320000
H	2.516806000	8.976787000	21.190650000
N	8.920006000	12.348004000	23.379994000
C	9.633996000	13.533000000	23.848000000
H	10.264105000	13.293369000	24.702476000
H	10.257364000	13.906271000	23.041339000
H	9.099582000	11.960587000	22.466055000

H	8.926011000	14.304596000	24.142087000
H	12.311664000	6.905033000	20.058117000
C	11.839007000	6.636984000	19.102008000
O	12.047003000	5.578994000	18.556995000
N	11.049987000	7.563043000	18.631003000
C	10.281009000	7.367970000	17.415982000
C	8.830001000	7.749012000	17.596026000
C	8.177550000	7.012196000	18.716823000
N	7.550142000	7.656106000	19.761637000
C	7.153753000	6.733429000	20.612060000
N	7.483907000	5.527147000	20.155916000
C	8.130771000	5.675768000	18.960646000
H	10.711686000	7.952254000	16.600884000
H	10.874977000	8.393359000	19.181629000
H	10.378298000	6.319597000	17.147194000
H	8.750778000	8.815439000	17.803582000
H	8.305478000	7.571491000	16.656419000
H	6.674465000	6.913535000	21.557818000
H	8.504125000	4.836222000	18.404956000
H	7.305567000	4.657894000	20.627844000
H	8.836099000	10.669282000	13.737748000
C	8.242008000	11.328014000	14.370985000
C	7.949987000	10.700975000	15.672015000
C	7.155381000	11.640406000	16.567410000
S	6.304887000	10.803435000	17.953915000
C	5.398882000	12.224015000	18.617798000
H	8.802650000	12.257785000	14.484364000
H	7.326499000	11.564413000	13.826651000
H	7.378130000	9.782421000	15.522162000
H	8.875500000	10.409422000	16.171110000
H	7.783887000	12.432999000	16.970292000
H	6.352789000	12.102997000	15.993192000
H	4.814718000	11.879438000	19.465403000
H	4.729664000	12.608553000	17.853095000
H	6.085185000	13.001894000	18.940831000

Fully optimized reduced site at BP86/def2TZVP level (Fig. 3D)

Cu	7.249441000	9.549508000	19.960915000
H	11.058216000	13.015447000	18.379055000
C	10.928025000	12.270015000	19.184989000
C	9.601958000	11.657991000	19.143028000
S	9.333020000	10.381754000	20.478702000
H	11.731408000	11.523492000	19.068608000
H	11.115112000	12.790737000	20.139127000
H	8.807961000	12.414759000	19.242586000
H	9.431560000	11.159129000	18.177340000
H	6.137972000	7.962001000	25.104210000
C	6.782999000	8.530995000	24.395017000
O	7.647995000	8.001000000	23.719980000
N	6.529977000	9.838987000	24.399015000
C	7.161025000	10.725055000	23.456040000
C	6.115069000	11.431942000	22.615837000
C	5.106838000	10.530949000	21.977832000
N	5.446803000	9.662505000	20.945927000
C	4.329461000	9.013452000	20.614675000
N	3.291593000	9.424558000	21.391951000
C	3.764969000	10.386082000	22.264591000
C	7.986957000	11.821992000	24.160052000
O	7.801023000	12.124995000	25.327016000
H	5.829660000	10.211508000	25.036636000
H	7.806779000	10.112851000	22.810740000
H	5.576902000	12.166651000	23.239131000
H	6.636121000	11.999344000	21.827381000
H	4.232987000	8.269250000	19.830900000
H	3.121766000	10.872238000	22.990031000
H	2.333807000	9.087830000	21.333588000
N	8.919995000	12.348008000	23.379983000
C	9.633998000	13.533001000	23.847998000
H	10.287583000	13.297685000	24.702207000
H	10.251742000	13.922114000	23.029231000
H	9.112178000	11.930001000	22.450971000

H	8.923627000	14.310490000	24.163932000
H	12.309375000	6.914393000	20.076277000
C	11.838988000	6.636973000	19.102035000
O	12.047015000	5.578985000	18.556979000
N	11.050008000	7.563144000	18.630968000
C	10.280984000	7.367799000	17.415944000
C	8.830003000	7.749152000	17.596156000
C	8.078355000	6.894461000	18.570594000
N	7.262782000	7.481657000	19.531268000
C	6.746477000	6.485328000	20.249603000
N	7.192655000	5.285677000	19.788873000
C	8.039894000	5.522942000	18.722348000
H	10.716835000	7.969941000	16.598917000
H	10.822020000	8.384856000	19.210749000
H	10.393823000	6.313913000	17.127307000
H	8.749787000	8.786823000	17.962028000
H	8.341433000	7.724094000	16.606999000
H	6.084445000	6.588088000	21.102902000
H	8.530776000	4.714433000	18.192084000
H	6.965216000	4.374817000	20.179363000
H	8.935166000	10.719940000	13.767714000
C	8.242048000	11.328027000	14.370970000
C	7.949904000	10.700985000	15.672050000
C	7.017463000	11.587533000	16.507681000
S	6.183968000	10.683447000	17.899890000
C	5.252384000	12.116617000	18.572249000
H	8.707018000	12.322096000	14.484558000
H	7.328333000	11.467380000	13.769868000
H	7.468686000	9.719305000	15.518913000
H	8.875466000	10.503846000	16.236073000
H	7.565416000	12.442698000	16.931885000
H	6.208204000	11.979359000	15.870972000
H	4.661595000	11.755096000	19.421195000
H	4.580277000	12.521622000	17.803724000
H	5.943741000	12.896834000	18.918025000

Fully optimized reduced site at B3LYP/def2TZVP level (Fig. 3E)

Cu	7.347343000	9.387033000	20.161025000
H	11.049037000	13.025937000	18.399005000
C	10.928038000	12.269971000	19.184978000
C	9.601909000	11.658036000	19.143068000
S	9.402905000	10.373689000	20.479912000
H	11.721957000	11.530736000	19.046385000
H	11.119304000	12.766413000	20.140669000
H	8.814607000	12.404299000	19.265953000
H	9.426651000	11.173121000	18.183674000
H	6.146895000	7.962211000	25.092709000
C	6.783001000	8.530984000	24.395020000
O	7.647992000	8.001010000	23.719983000
N	6.529979000	9.838979000	24.399005000
C	7.160996000	10.725053000	23.456082000
C	6.115144000	11.431910000	22.615680000
C	5.112810000	10.527692000	21.978200000
N	5.468003000	9.602130000	21.014439000
C	4.359772000	8.970366000	20.671915000
N	3.306178000	9.445457000	21.371237000
C	3.767225000	10.435706000	22.209601000
C	7.986952000	11.822002000	24.160083000
O	7.801033000	12.124997000	25.327031000
H	5.832317000	10.204157000	25.029304000
H	7.807163000	10.122004000	22.823903000
H	5.576173000	12.152278000	23.238407000
H	6.627263000	12.005504000	21.839936000
H	4.278174000	8.191362000	19.933276000
H	3.115212000	10.972506000	22.875968000
H	2.351646000	9.130681000	21.289074000
N	8.919983000	12.348011000	23.379991000
C	9.633997000	13.533002000	23.847993000
H	10.265963000	13.301843000	24.707507000
H	10.262174000	13.905220000	23.040903000
H	9.117125000	11.935799000	22.465531000

H	8.929296000	14.312192000	24.140176000
H	12.308365000	6.905589000	20.064803000
C	11.838995000	6.636988000	19.102036000
O	12.047003000	5.578987000	18.556987000
N	11.050025000	7.563158000	18.630937000
C	10.280962000	7.367717000	17.415920000
C	8.829999000	7.749241000	17.596262000
C	8.082454000	6.841948000	18.525990000
N	7.322719000	7.349051000	19.563716000
C	6.812141000	6.312289000	20.201986000
N	7.203355000	5.157560000	19.620464000
C	8.012252000	5.473822000	18.552305000
H	10.714130000	7.969848000	16.610119000
H	10.835819000	8.379751000	19.203042000
H	10.387936000	6.325246000	17.124725000
H	8.751367000	8.762777000	17.989800000
H	8.355142000	7.751612000	16.611249000
H	6.186514000	6.351505000	21.077266000
H	8.457182000	4.714948000	17.933403000
H	6.961101000	4.228211000	19.927588000
H	8.991769000	10.767453000	13.805580000
C	8.242016000	11.328016000	14.370964000
C	7.949918000	10.701010000	15.672100000
C	6.937311000	11.555237000	16.443964000
S	6.058830000	10.621597000	17.763248000
C	5.052248000	11.994228000	18.436054000
H	8.627138000	12.346276000	14.485320000
H	7.347803000	11.391042000	13.744614000
H	7.535160000	9.700462000	15.518213000
H	8.857386000	10.573104000	16.263940000
H	7.431034000	12.414775000	16.900934000
H	6.176247000	11.930105000	15.756243000
H	4.427268000	11.585108000	19.226020000
H	4.416962000	12.414814000	17.657104000
H	5.692354000	12.770692000	18.853526000

Fully optimized reduced site at B38HFP86/def2TZVP level (Fig. 3F)

Cu	7.332558000	9.487853000	20.040487000
H	11.059627000	13.004430000	18.384580000
C	10.928014000	12.270010000	19.184994000
C	9.601977000	11.657994000	19.143000000
S	9.378319000	10.420488000	20.482554000
H	11.717496000	11.524299000	19.076727000
H	11.104239000	12.785131000	20.131081000
H	8.818870000	12.411494000	19.234924000
H	9.436906000	11.158662000	18.189101000
H	6.150018000	7.962596000	25.089459000
C	6.783006000	8.531000000	24.395002000
O	7.647996000	8.000998000	23.719995000
N	6.529995000	9.839005000	24.399002000
C	7.160983000	10.725011000	23.456028000
C	6.115016000	11.431976000	22.615975000
C	5.134634000	10.524881000	21.969994000
N	5.496239000	9.646581000	20.979124000
C	4.405280000	9.008540000	20.630469000
N	3.357063000	9.430626000	21.351124000
C	3.801153000	10.396124000	22.211429000
C	7.986991000	11.821998000	24.160009000
O	7.801012000	12.124992000	25.327001000
H	5.830144000	10.201706000	25.021301000
H	7.806648000	10.122479000	22.822256000
H	5.569262000	12.141418000	23.241698000
H	6.630656000	12.011146000	21.848461000
H	4.334550000	8.255257000	19.865764000
H	3.146944000	10.896864000	22.901055000
H	2.413195000	9.098140000	21.266868000
N	8.920002000	12.348012000	23.379986000
C	9.634000000	13.532999000	23.848002000
H	10.269072000	13.296567000	24.700563000
H	10.254142000	13.907839000	23.039035000
H	9.104081000	11.940386000	22.464290000

H	8.927560000	14.305158000	24.146026000
H	12.307392000	6.902796000	20.061044000
C	11.838970000	6.636947000	19.102036000
O	12.047044000	5.578999000	18.556960000
N	11.049995000	7.563142000	18.631013000
C	10.281001000	7.367830000	17.415968000
C	8.830003000	7.749105000	17.596036000
C	8.101125000	6.886971000	18.564604000
N	7.302515000	7.440549000	19.533276000
C	6.814739000	6.443785000	20.230154000
N	7.256032000	5.271476000	19.753735000
C	8.078548000	5.530858000	18.692448000
H	10.713939000	7.964315000	16.610028000
H	10.833463000	8.372201000	19.203582000
H	10.386472000	6.323424000	17.134697000
H	8.756553000	8.776258000	17.956391000
H	8.346316000	7.719821000	16.617789000
H	6.166479000	6.528185000	21.084199000
H	8.567812000	4.746580000	18.145261000
H	7.038791000	4.365210000	20.127298000
H	8.913810000	10.715684000	13.768332000
C	8.242002000	11.328011000	14.370986000
C	7.949994000	10.700978000	15.672013000
C	7.050960000	11.595565000	16.510266000
S	6.233307000	10.705970000	17.873004000
C	5.356140000	12.110159000	18.599890000
H	8.717233000	12.304114000	14.489838000
H	7.331661000	11.480621000	13.788703000
H	7.453434000	9.739964000	15.516966000
H	8.869135000	10.486581000	16.218346000
H	7.619439000	12.425430000	16.930529000
H	6.260368000	12.011090000	15.884156000
H	4.779152000	11.739486000	19.441841000
H	4.683085000	12.552847000	17.868896000
H	6.061151000	12.859090000	18.954503000

Optimized inner-sphere ligands for oxidized, axial site at B38HFP86/def2TZVP level (Fig. 4A)

Cu	7.349299000	9.620275000	20.014537000
H	10.997606000	13.087789000	18.465061000
C	10.928027000	12.270003000	19.184981000
C	9.601973000	11.658023000	19.143016000
S	9.436988000	10.276347000	20.328699000
H	11.713411000	11.552762000	18.943762000
H	11.156764000	12.682297000	20.168895000
H	8.825705000	12.390209000	19.359272000
H	9.399388000	11.260569000	18.150479000
H	6.154072000	7.963589000	25.092297000
C	6.783015000	8.531001000	24.395007000
O	7.647999000	8.000995000	23.719997000
N	6.529995000	9.839001000	24.398995000
C	7.160955000	10.724987000	23.456041000
C	6.115024000	11.432039000	22.615991000
C	5.176315000	10.510357000	21.918221000
N	5.551920000	9.683102000	20.877474000
C	4.483551000	9.004053000	20.517590000
N	3.444634000	9.351162000	21.274173000
C	3.857781000	10.300158000	22.166628000
C	7.986990000	11.821971000	24.159996000
O	7.801006000	12.125000000	25.326989000
H	5.840303000	10.205576000	25.031050000
H	7.819541000	10.116345000	22.840643000
H	5.528272000	12.091658000	23.256803000
H	6.613542000	12.073914000	21.887338000
H	4.434798000	8.278140000	19.725762000
H	3.193875000	10.738436000	22.888122000
H	2.514850000	8.976720000	21.195065000
N	8.920011000	12.348024000	23.379993000
C	9.633998000	13.532996000	23.847999000
H	10.266254000	13.292913000	24.700780000
H	10.255204000	13.907969000	23.040449000
H	9.100024000	11.960204000	22.466285000

H	8.925862000	14.303442000	24.144657000
H	12.311701000	6.905013000	20.058111000
C	11.839004000	6.636988000	19.102020000
O	12.047008000	5.579000000	18.556989000
N	11.049971000	7.562977000	18.630999000
C	10.281020000	7.368067000	17.415998000
C	8.830003000	7.748942000	17.595965000
C	8.177097000	7.013150000	18.717019000
N	7.544089000	7.657037000	19.758491000
C	7.148315000	6.734529000	20.609212000
N	7.483512000	5.528315000	20.156412000
C	8.133745000	5.676990000	18.962917000
H	10.711613000	7.952689000	16.601071000
H	10.874914000	8.393202000	19.181691000
H	10.378599000	6.319827000	17.146937000
H	8.750645000	8.815520000	17.802492000
H	8.305513000	7.570803000	16.656364000
H	6.664820000	6.914536000	21.552805000
H	8.510997000	4.837576000	18.409632000
H	7.306295000	4.659324000	20.629282000
H	8.836322000	10.669354000	13.737877000
C	8.242017000	11.328004000	14.370986000
C	7.949975000	10.700999000	15.672015000
C	7.155059000	11.640946000	16.566910000
S	6.301546000	10.804984000	17.952514000
C	5.392973000	12.226224000	18.611825000
H	8.802437000	12.257883000	14.484380000
H	7.326525000	11.564131000	13.826499000
H	7.378115000	9.782451000	15.522264000
H	8.875412000	10.409668000	16.171345000
H	7.783760000	12.432899000	16.970630000
H	6.353634000	12.104255000	15.991672000
H	4.796121000	11.883032000	19.451339000
H	4.734411000	12.615377000	17.840152000
H	6.077989000	13.001009000	18.944926000

Optimized inner-sphere ligands for oxidized, rhombic site at B38HFP86/def2TZVP level (Fig. 4B)

Cu	7.353587000	9.617781000	20.015001000
H	10.998264000	13.087064000	18.464310000
C	10.928009000	12.270007000	19.184999000
C	9.601979000	11.658003000	19.142994000
S	9.439667000	10.276548000	20.329430000
H	11.713315000	11.552370000	18.944768000
H	11.156186000	12.683223000	20.168606000
H	8.825390000	12.389745000	19.359823000
H	9.399352000	11.260051000	18.150794000
H	6.154164000	7.963556000	25.092336000
C	6.783001000	8.530996000	24.395000000
O	7.648001000	8.000999000	23.719998000
N	6.529997000	9.839008000	24.399000000
C	7.160996000	10.725000000	23.456013000
C	6.115001000	11.431999000	22.615995000
C	5.177572000	10.509787000	21.917150000
N	5.555652000	9.678874000	20.880194000
C	4.487598000	8.999401000	20.519533000
N	3.446860000	9.350052000	21.271873000
C	3.858204000	10.301436000	22.162506000
C	7.987000000	11.821994000	24.160001000
O	7.801001000	12.124999000	25.327002000
H	5.839926000	10.205575000	25.030589000
H	7.819646000	10.116390000	22.840572000
H	5.527114000	12.089989000	23.257379000
H	6.613133000	12.075513000	21.888474000
H	4.440062000	8.271915000	19.729064000
H	3.192924000	10.741936000	22.881320000
H	2.516806000	8.976787000	21.190650000
N	8.920006000	12.348004000	23.379994000
C	9.633996000	13.533000000	23.848000000
H	10.264105000	13.293369000	24.702476000
H	10.257364000	13.906271000	23.041339000
H	9.099582000	11.960587000	22.466055000

H	8.926011000	14.304596000	24.142087000
H	12.311664000	6.905033000	20.058117000
C	11.839007000	6.636984000	19.102008000
O	12.047003000	5.578994000	18.556995000
N	11.049987000	7.563043000	18.631003000
C	10.281009000	7.367970000	17.415982000
C	8.830001000	7.749012000	17.596026000
C	8.177550000	7.012196000	18.716823000
N	7.550142000	7.656106000	19.761637000
C	7.153753000	6.733429000	20.612060000
N	7.483907000	5.527147000	20.155916000
C	8.130771000	5.675768000	18.960646000
H	10.711686000	7.952254000	16.600884000
H	10.874977000	8.393359000	19.181629000
H	10.378298000	6.319597000	17.147194000
H	8.750778000	8.815439000	17.803582000
H	8.305478000	7.571491000	16.656419000
H	6.674465000	6.913535000	21.557818000
H	8.504125000	4.836222000	18.404956000
H	7.305567000	4.657894000	20.627844000
H	8.836099000	10.669282000	13.737748000
C	8.242008000	11.328014000	14.370985000
C	7.949987000	10.700975000	15.672015000
C	7.155381000	11.640406000	16.567410000
S	6.304887000	10.803435000	17.953915000
C	5.398882000	12.224015000	18.617798000
H	8.802650000	12.257785000	14.484364000
H	7.326499000	11.564413000	13.826651000
H	7.378130000	9.782421000	15.522162000
H	8.875500000	10.409422000	16.171110000
H	7.783887000	12.432999000	16.970292000
H	6.352789000	12.102997000	15.993192000
H	4.814718000	11.879438000	19.465403000
H	4.729664000	12.608553000	17.853095000
H	6.085185000	13.001894000	18.940831000

Optimized inner-sphere ligands for reduced, axial site at B38HFP86/def2TZVP level (Fig. 6A)

Cu	7.646982000	9.136992000	20.347984000
H	11.055324000	13.024763000	18.401668000
C	10.928037000	12.270011000	19.184994000
C	9.601955000	11.657995000	19.143008000
S	9.509115000	10.416088000	20.512346000
H	11.714534000	11.526344000	19.051782000
H	11.111282000	12.761604000	20.141454000
H	8.807330000	12.392438000	19.270010000
H	9.419049000	11.150009000	18.198756000
H	6.150487000	7.962550000	25.089718000
C	6.783012000	8.531001000	24.395000000
O	7.647986000	8.000995000	23.719991000
N	6.530016000	9.838977000	24.398987000
C	7.160911000	10.725043000	23.456181000
C	6.115093000	11.431969000	22.615827000
C	5.221627000	10.481232000	21.903855000
N	5.705728000	9.510892000	21.058874000
C	4.661594000	8.854019000	20.615436000
N	3.528888000	9.348881000	21.133047000
C	3.865273000	10.384512000	21.960753000
C	7.986969000	11.821998000	24.160032000
O	7.801042000	12.124967000	25.326989000
H	5.828130000	10.201780000	25.019066000
H	7.807880000	10.125258000	22.820919000
H	5.509369000	12.083630000	23.248083000
H	6.626178000	12.068719000	21.892041000
H	4.685231000	8.029397000	19.925070000
H	3.130246000	10.949830000	22.503477000
H	2.600576000	9.015214000	20.944794000
N	8.919995000	12.348037000	23.379968000
C	9.634001000	13.532998000	23.848002000
H	10.271823000	13.296987000	24.698724000
H	10.251176000	13.910027000	23.037780000
H	9.119319000	11.931658000	22.471657000

H	8.926842000	14.303399000	24.148622000
H	12.308358000	6.903453000	20.060462000
C	11.838985000	6.636960000	19.102043000
O	12.047023000	5.578997000	18.556973000
N	11.049999000	7.563113000	18.630975000
C	10.280994000	7.367869000	17.415968000
C	8.830004000	7.749092000	17.596060000
C	8.105575000	6.804066000	18.493746000
N	7.567892000	7.210244000	19.689085000
C	7.050020000	6.146042000	20.254104000
N	7.225656000	5.069962000	19.476422000
C	7.895100000	5.464994000	18.352314000
H	10.716941000	7.967158000	16.612973000
H	10.844800000	8.380236000	19.197690000
H	10.382507000	6.324022000	17.133460000
H	8.746839000	8.751879000	18.016605000
H	8.358908000	7.770205000	16.612853000
H	6.562233000	6.114480000	21.212048000
H	8.159150000	4.773797000	17.573482000
H	6.929861000	4.134824000	19.691898000
H	8.993865000	10.771147000	13.810093000
C	8.242017000	11.328021000	14.370954000
C	7.949965000	10.700985000	15.672062000
C	6.930028000	11.523572000	16.441066000
S	6.232281000	10.607953000	17.844389000
C	5.208733000	11.925747000	18.539727000
H	8.619137000	12.346144000	14.489421000
H	7.348792000	11.385408000	13.746540000
H	7.553926000	9.694331000	15.518470000
H	8.857389000	10.588316000	16.265660000
H	7.389162000	12.438285000	16.818966000
H	6.111188000	11.807577000	15.778369000
H	4.730187000	11.537432000	19.434431000
H	4.445129000	12.236610000	17.829675000
H	5.825089000	12.779257000	18.814954000

Optimized inner-sphere ligands for reduced, rhombic site at B38HFP86/def2TZVP level (Fig. 6B)

Cu	7.379000000	9.640022000	20.043031000
H	11.076770000	12.984380000	18.368867000
C	10.928007000	12.270007000	19.184996000
C	9.601983000	11.658005000	19.142995000
S	9.412753000	10.466381000	20.523152000
H	11.716054000	11.519139000	19.107878000
H	11.090929000	12.807801000	20.120604000
H	8.814656000	12.408593000	19.215323000
H	9.444434000	11.128848000	18.204429000
H	6.150036000	7.962583000	25.089473000
C	6.783001000	8.530996000	24.394998000
O	7.648002000	8.000999000	23.720001000
N	6.530000000	9.839012000	24.399000000
C	7.160993000	10.724991000	23.456005000
C	6.114992000	11.432004000	22.616013000
C	5.142318000	10.526223000	21.959949000
N	5.520221000	9.684190000	20.946011000
C	4.444680000	9.027985000	20.584246000
N	3.389989000	9.405364000	21.320616000
C	3.813520000	10.359753000	22.203921000
C	7.987006000	11.821995000	24.159993000
O	7.800998000	12.125001000	25.327003000
H	5.831162000	10.201971000	25.022317000
H	7.806228000	10.122304000	22.821849000
H	5.566500000	12.137061000	23.244575000
H	6.631022000	12.016621000	21.852956000
H	4.390094000	8.292390000	19.801390000
H	3.149919000	10.826331000	22.908313000
H	2.454306000	9.051691000	21.231845000
N	8.920006000	12.348004000	23.379997000
C	9.633997000	13.532999000	23.848000000
H	10.264685000	13.298115000	24.704284000
H	10.258773000	13.903825000	23.040668000
H	9.106619000	11.940499000	22.463279000

H	8.928196000	14.308020000	24.140400000
H	12.307998000	6.903169000	20.060618000
C	11.839005000	6.636980000	19.102007000
O	12.047009000	5.578991000	18.556991000
N	11.049983000	7.563039000	18.631019000
C	10.281020000	7.367998000	17.415991000
C	8.829990000	7.748980000	17.595987000
C	8.121628000	6.934409000	18.619787000
N	7.358533000	7.537630000	19.586666000
C	6.900703000	6.578644000	20.352275000
N	7.324653000	5.381941000	19.920275000
C	8.106218000	5.586163000	18.816783000
H	10.714152000	7.961188000	16.607771000
H	10.839341000	8.373548000	19.202531000
H	10.384738000	6.322371000	17.138399000
H	8.754516000	8.791519000	17.907979000
H	8.334768000	7.671686000	16.626135000
H	6.290852000	6.709110000	21.228555000
H	8.576650000	4.774785000	18.293021000
H	7.124852000	4.496539000	20.349304000
H	8.902593000	10.709175000	13.762632000
C	8.241999000	11.328009000	14.370986000
C	7.950001000	10.700979000	15.672011000
C	7.067102000	11.606743000	16.515090000
S	6.256908000	10.734857000	17.893524000
C	5.363460000	12.144704000	18.589600000
H	8.730010000	12.297735000	14.489904000
H	7.330256000	11.493511000	13.794446000
H	7.441311000	9.746077000	15.519192000
H	8.870391000	10.476156000	16.212117000
H	7.645274000	12.436817000	16.921378000
H	6.271090000	12.021629000	15.895497000
H	4.789094000	11.785914000	19.438447000
H	4.687027000	12.563338000	17.847691000
H	6.059467000	12.908984000	18.928724000

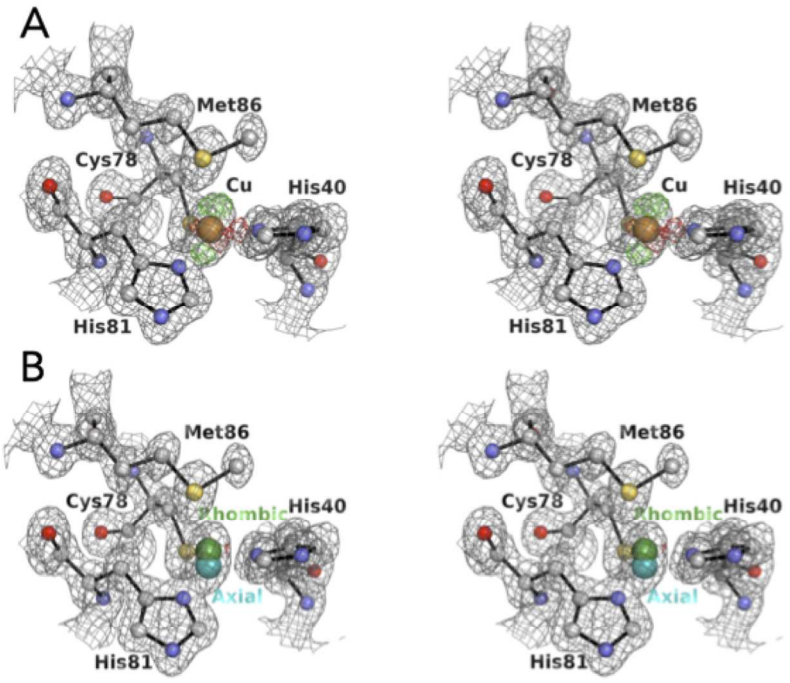

Figure 1. The stereo view of 2Fo-Fc (grey at 1.5σ level) and Fo-Fc (green at 4.0σ and red, -4.0σ levels) maps around the Cu site of AcPAz modelled with a single Cu position (A) and dual Cu positions (B). Positions 1 and 2 are labelled as 'Axial' and 'Rhombic' in Figure 1B, respectively.

254x190mm (300 x 300 DPI)

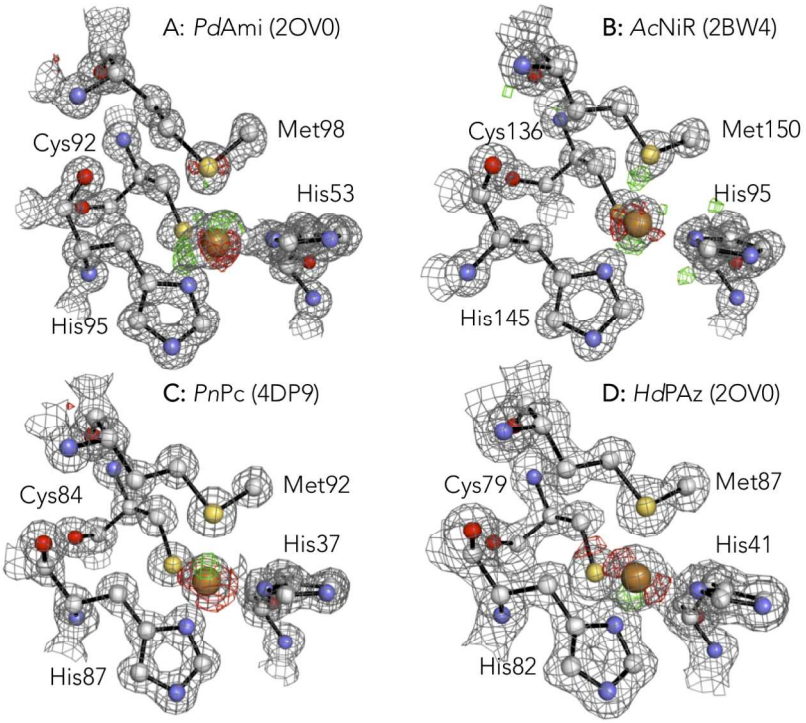

Figure 2. 2Fo-Fc (grey at 1.5σlevel) and Fo-Fc (green at 4.0σ and red -4.0σlevels) maps at the Cu binding site of PdAmi(A, 2OV0), AcNiR(B, 2BW4), PnPc(C, 4DP9), and HdPAz(D, 3EF4). Maps were generated from structures and electron density maps deposited in the Protein Data Bank.

254x190mm (300 x 300 DPI)

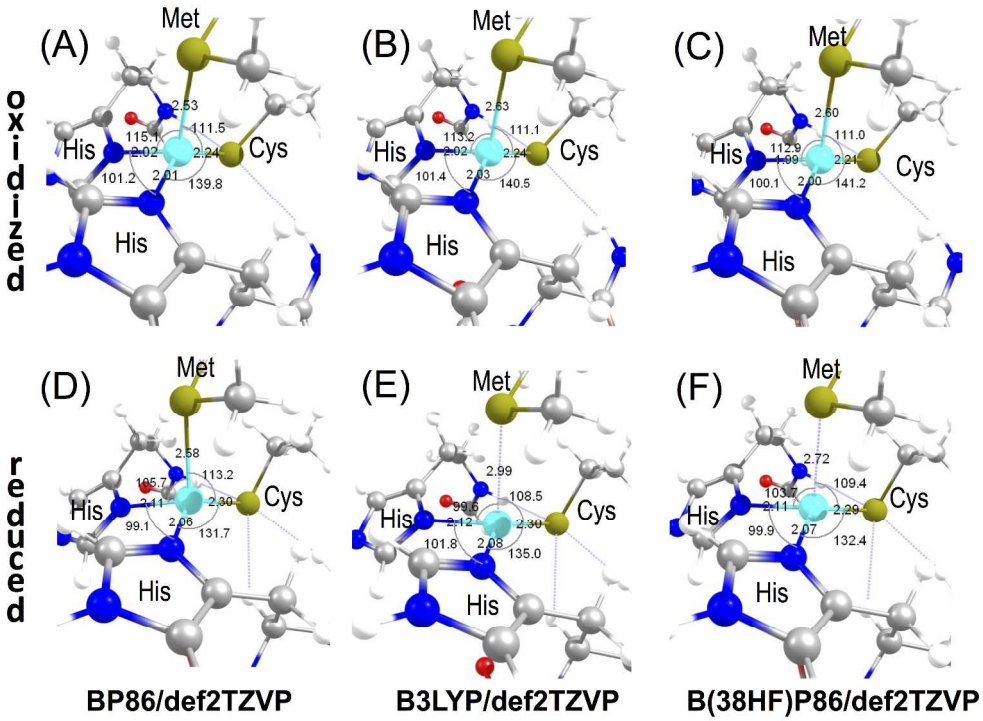

Figure 3. Optimized inner sphere environment for the oxidized (A-C) and reduced (D-F) Cu sites in AcPAz starting from separate axial and rhombic sites, but converging to a single site geometry using BP86 (A,D), B3LYP (B,E), and B38HFP86 (C,F) functionals with def2TZVP basis sets with all α- and β-carbon atoms fixed at their crystallographic positions.

454x339mm (300 x 300 DPI)

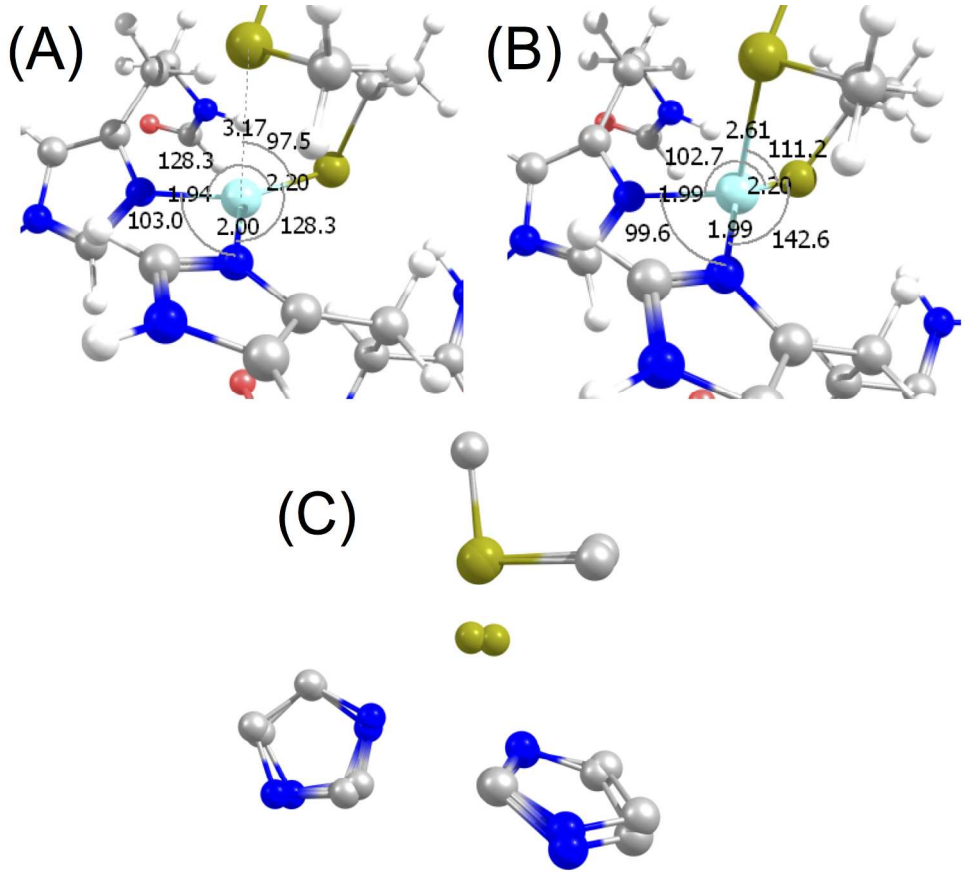

Figure 4. Partially optimized structures for the oxidized (A) axial and (B) rhombic Cu sites of AcPAz at B38HFP86/def2TZVP level with Cartesian coordinates of the Cu2+ ion, all α- and β-carbon atoms kept fixed at their crystallographic values. Panel (C) shows the overlay of the optimized atomic positions for the inner sphere ligands.

218x189mm (300 x 300 DPI)

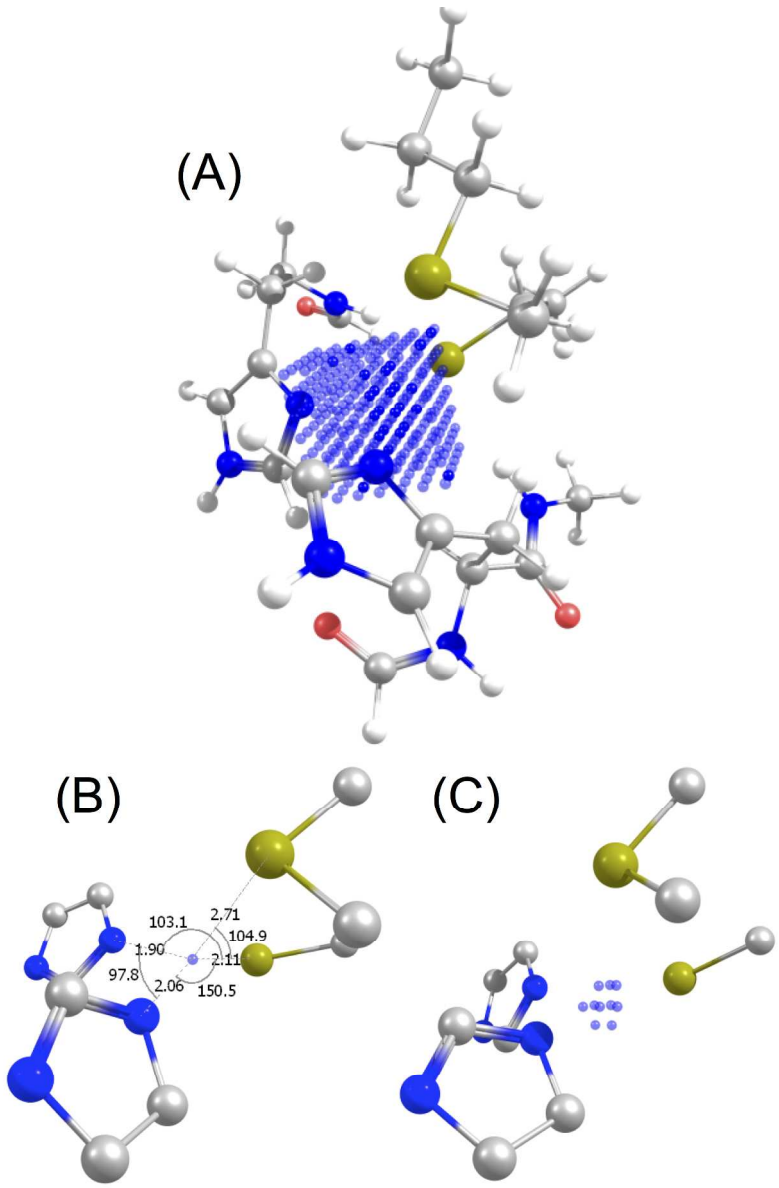

Figure 5. Molecular field analysis of the inner sphere environment at the metal binding site of AcPAz using Cu2+ ion as probe (A: Cu positions evaluated in MFA, B: the lowest energy structure, C: range of low energy structures within 20 kJ mol-1). The blue dots correspond to the position of Cu2+ tested in MFA.

203x304mm (300 x 300 DPI)

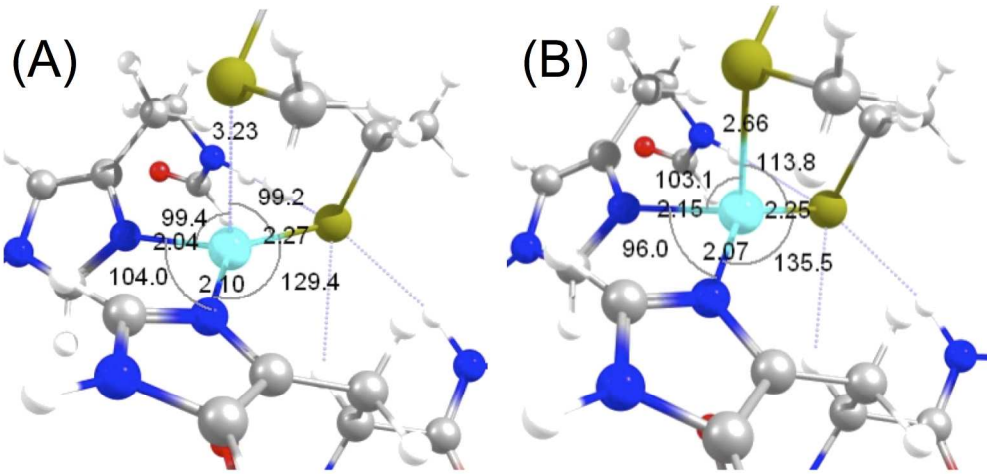

Figure 6. Partially optimized structures for the reduced (A) axial and (B) rhombic Cu sites of AcPAz at B38HFP86/def2TZVP level with Cartesian coordinates of the Cu2+ ion, all α- and β-carbon kept fixed at their crystallographic values.

251x122mm (300 x 300 DPI)