Electronic Supplementary Information (ESI)

Heterologous production and functional characterization of *Bradyrhizobium japonicum* copper-containing nitrite reductase and its physiological redox partner cytochrome c_{550}

Julio C. Cristaldi,[a] Felix M. Ferroni,[a] Andrea B. Duré,[a] Cintia S. Ramírez,[a] Sergio D. Dalosto,[b] Alberto C. Rizzi,[a] Pablo J. González,[a] Maria G. Rivas,[a] and Carlos D. Brondino*[a]

[a]Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral and CONICET, S3000ZAA Santa Fe, Argentina

[b]Instituto de Física del Litoral, CONICET-UNL, Güemes 3450, S3000GLN Santa Fe, Argentina

*email: brondino@fbcb.unl.edu.ar, Tel: +54-342-457 5213

URL: https://www.fbcb.unl.edu.ar/dfbioq/

Fig. S1 Size-exclusion chromatography performed on a Superdex 75 column equilibrated with 10 mM Tris-HCl buffer pH 8.0 plus 200 mM NaCl. Calibration curve (Left): Aprotinin (A, 6.5 kDa), Ribonuclease (R, 13.7 kDa), Carbonic Anhidrase (CA, 29 kDa), Ovalbumin (44 kDa) and Conalbumin (75 kDa). M, D and T indicate monomer, dimer, and tetramer, respectively. Right: runs from a diluted (~ 0.25 mg/mL) sample (red) and concentrated (~ 20 mg/mL) sample (black).

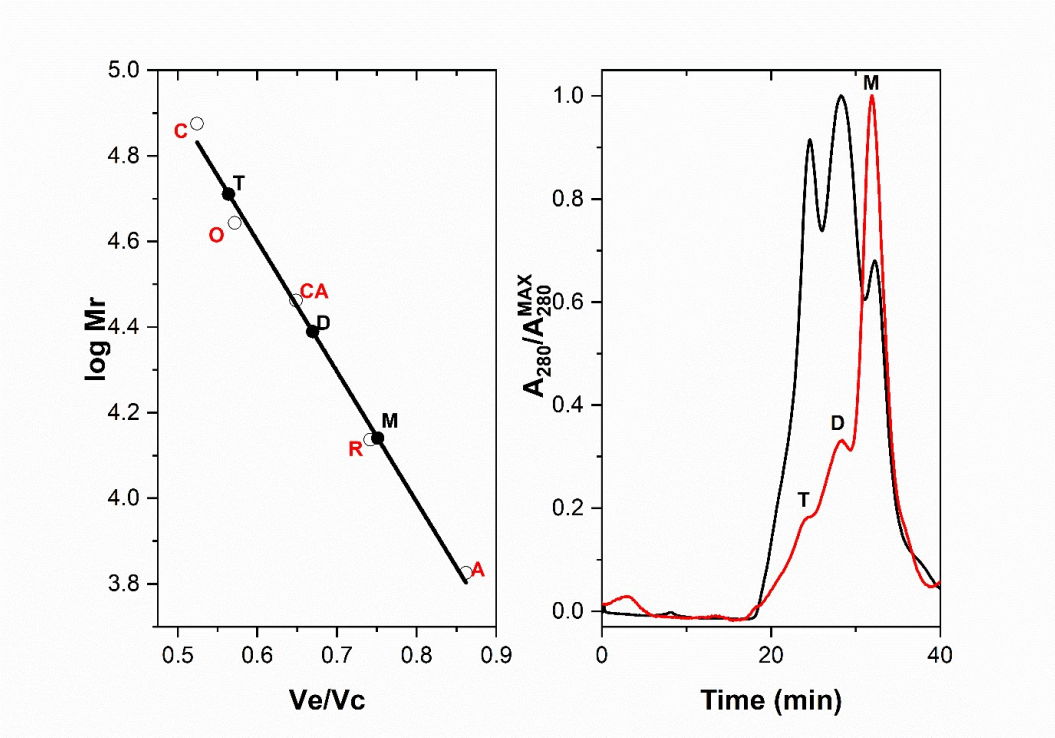
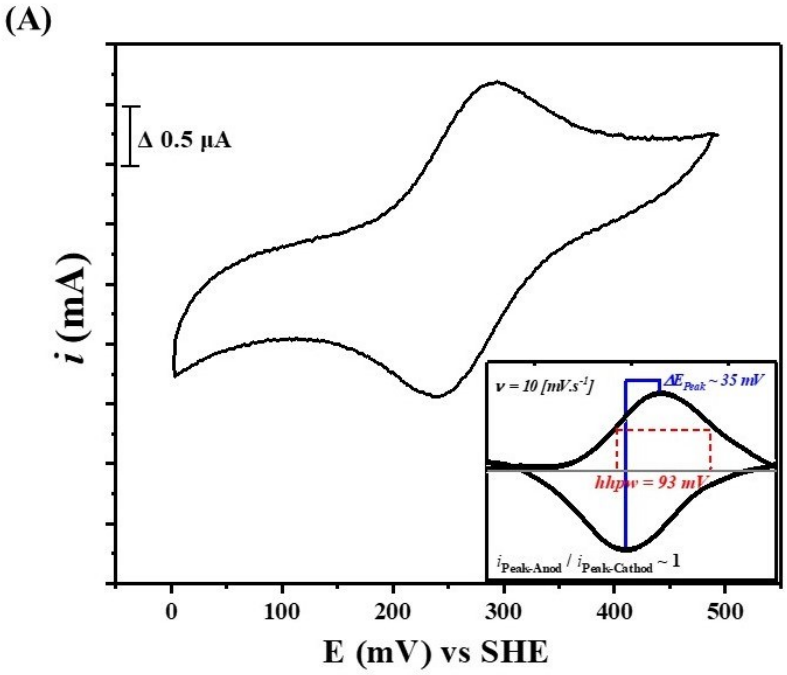
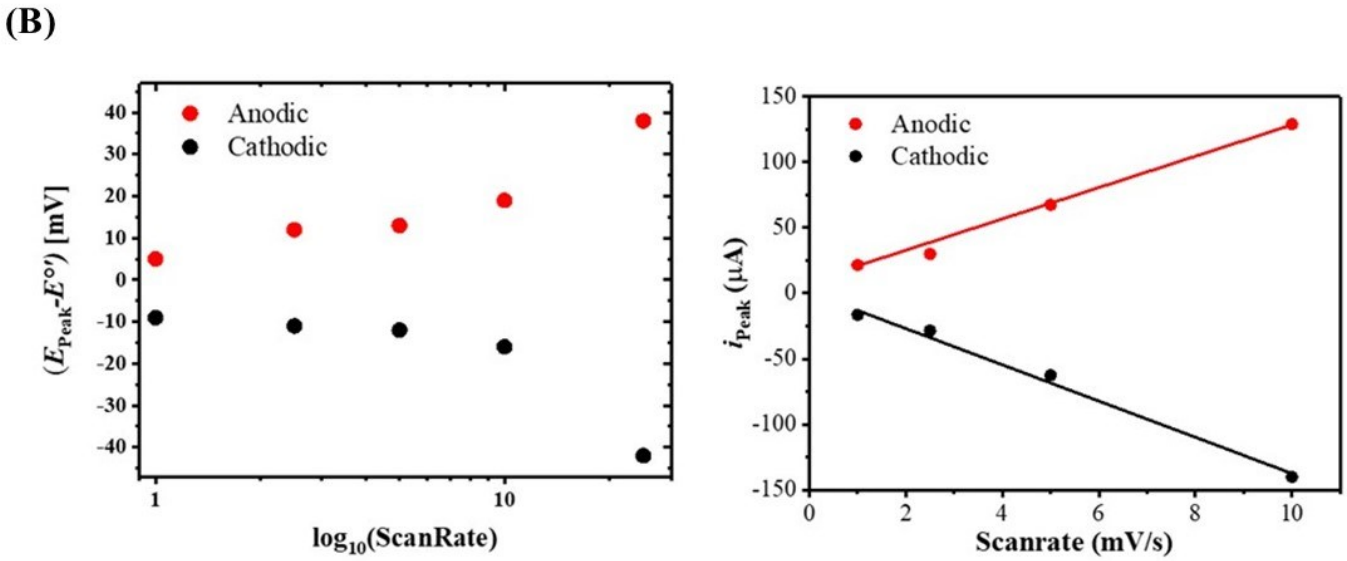


Fig. S2 *Panel A.* Cyclic voltammograms of *Bj*CycA using 1 mM 4,4´-dithiopiridine as promoter of the protein-electrode interaction and 500 mM glycine as protein stabilizer. The inset shows a blank-subtracted voltammogram recorded at v=10 mV·s^{-1}. Typical voltamogram features indicate that the system behaves as thin-layer at this scan rate, e.g. i_{Peak} ratio ~ 1, half-height peak width ~ 90 mV, and ΔE_{Peak} lower than 59/n mV. *Panel B.* Left, Laviron plot showing the variation of the anodic and cathodic peaks potentials (E_{peak}) as a function of the logarithm of the scan rate. Right, Linear fitting of the variation of CV cathodic and anodic peaks current (i_{peak}) with the scan rate (right), adding evidence to the thin-layer

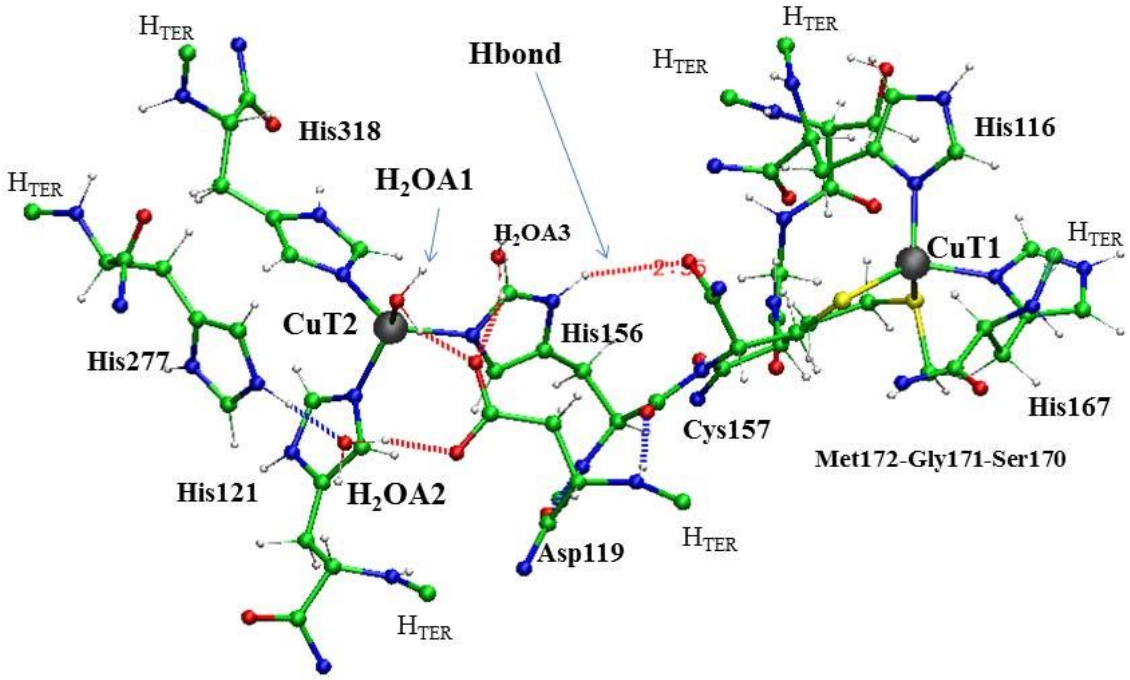




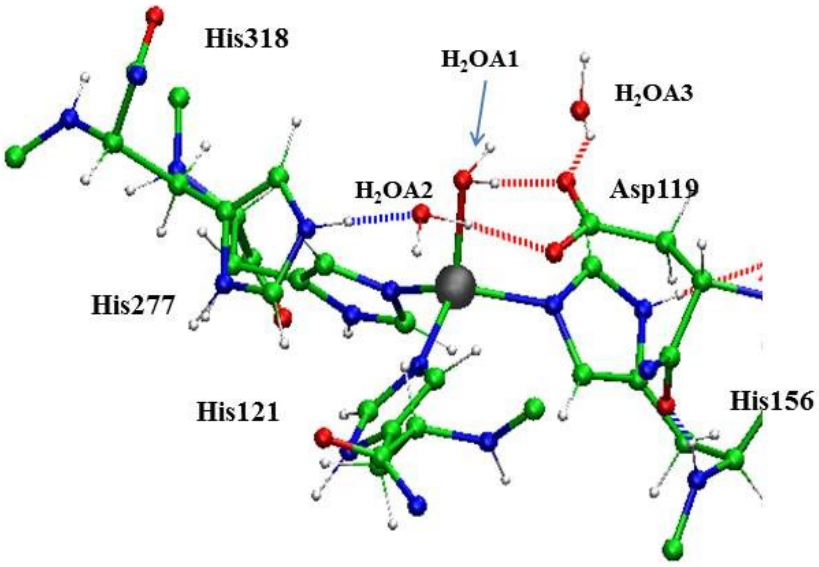
behavior.

Fig. S3 QM part of the QM/MM calculations. Panel A includes residues His328, His121, His156, which are the ligands of the catalytic copper atom T2, His116, His167, Cys157, which are nearest neighbors of copper atom at T1, and the weaker Met 172 ligand, and the second sphere residues Asp119 (Asp_{CAT}), His277 (His_{CAT}), Gly171, and Ser170. The color codes are green for carbon, blue for Nitrogen, red Oxygens, light grey for hydrogen, yellow for sulfur, and grey for Cu. Water hydrogen bond interactions and the hydrogen bond (His156)-$N^{\delta 1}H$…O=C-(Cys157) are indicated as red dashed lines. Three water molecules were included based on structural data of other similar copper-nitrite reductases (PDB 1OE1 and 5F7B), H2OA1 bonded to T2 copper, H2OA2 which is bridging His_{CAT} and Asp_{Cat} and H2OA3 bonded to Asp_{Cat}. His_{CAT} and Asp_{CAT} are protonated and deprotonated, respectively. Panels B and C are overviews in different projection of the of the T1 and T2 sites shown in Panel A, respectively. Cartesian coordinates of the atoms are given at the end of the document.

(A)



(B)


His318
H2OA1
H2OA3
H2OA2
Asp119
His277
His121
His156

(C)

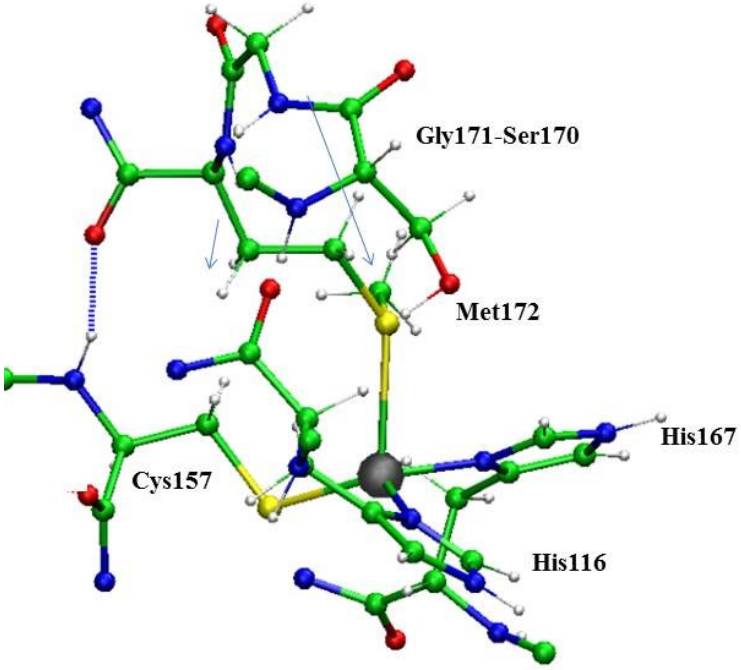

Gly171-Ser170
Met172
His167
Cys157
His116

Fig. S4 Computed T1 UV-vis absorption spectra of *Bj*NirK (pink) and *Sm*NirK (green) determined by TDDFT using the HSEh1PBE functional and the basis set 6-31(d,p). The inset shows the model used in the computation.

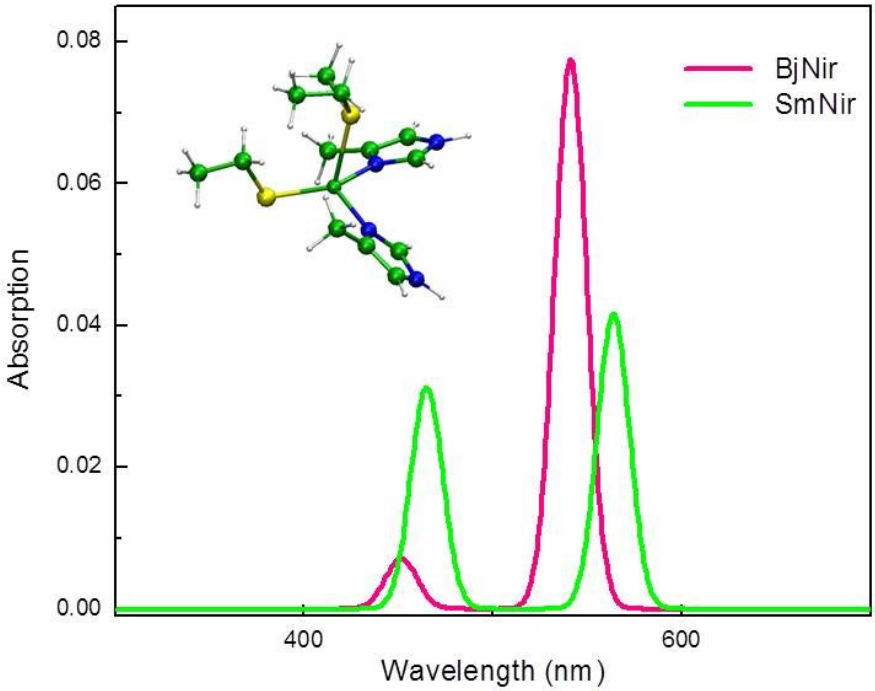


Fig. S5 EPR spectra of *Bj*NirK at pH 10 showing two type 2 copper (II) overlapping spectral components (red and pink). g-values: $g_{1,2,3}$= 2.190, 2.052, 2.023, $A_{1,2,3}$= 7 mT for T1(blue), $g_{1,2,3}$= 2.231, 2.055, 2.040, $A_{1,2=3}$= 20.6 mT for the major component ($T2_I$; red) and $g_{1,2,3}$= 2.345, 2.055, 2.055, $A_{1,2=3}$= 16 mT for the minor component ($T2_{II}$; pink); T1:$T2_I$:$T2_{II}$ ratio of 1:0.8:0.2

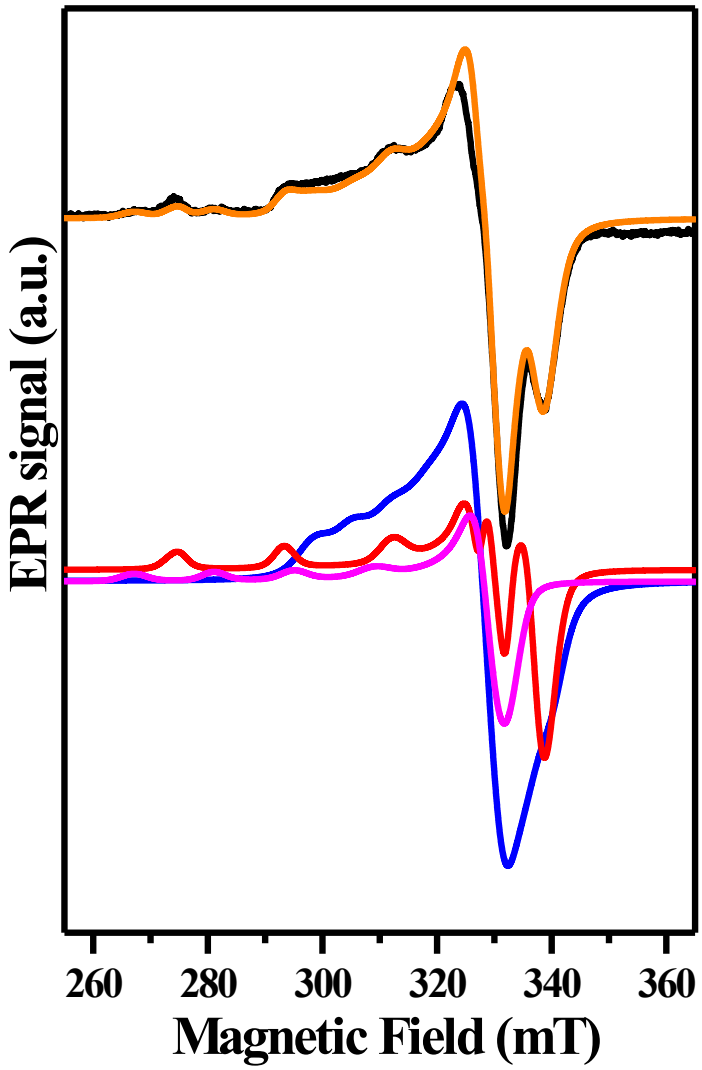
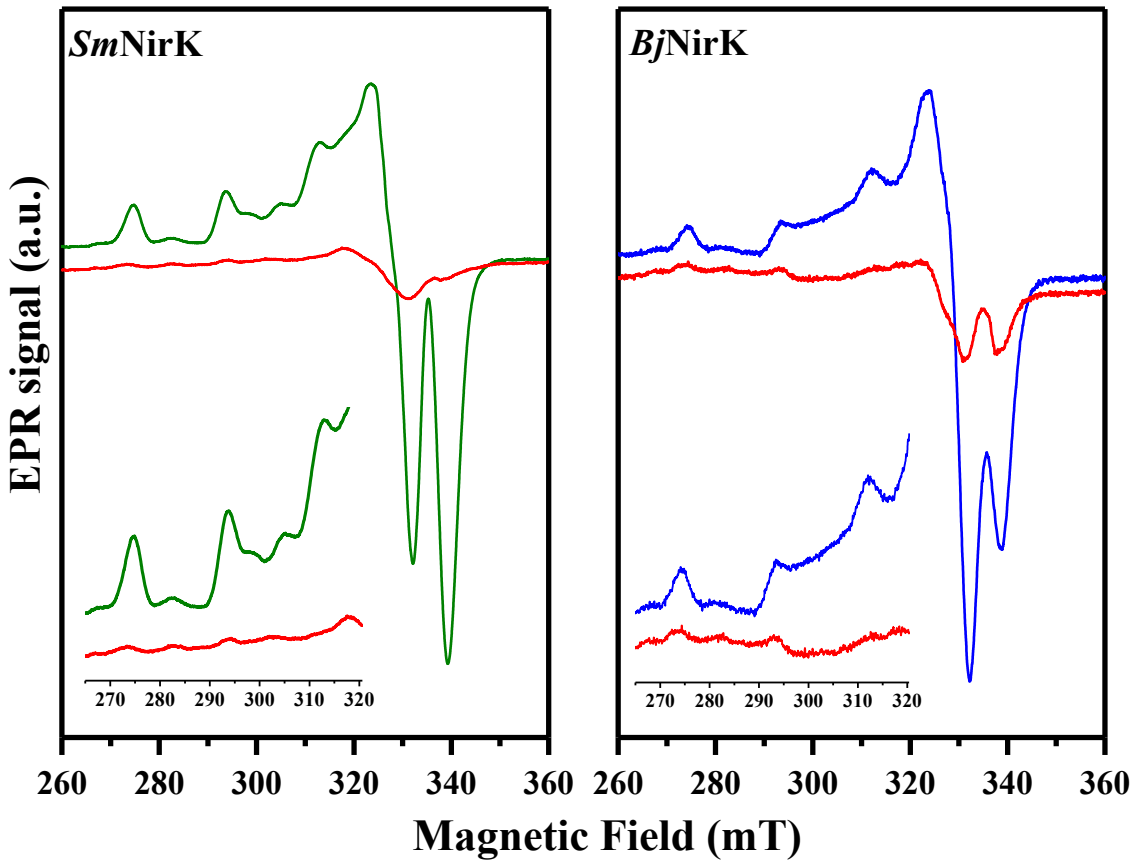


Fig. S6 EPR spectra of as-purified *Bj*NirK (blue) and *Sm*NirK (green) at pH 10 and upon ascorbate reduction (red) under anaerobic conditions.



Cartesian coordinates of the QM part including PDB and residue names and residue numbers.

```
N(PDBName=N,ResName=HSD,ResNum=277_A)    21.062037   31.953008   37.48698
H(PDBName=HN,ResName=HIS,ResNum=277_A)   21.976961   31.537075   37.63645
C(PDBName=CA,ResName=HIS,ResNum=277_A)   20.679312   32.375623   36.18835
H(PDBName=HA,ResName=HIS,ResNum=277_A)   19.615387   32.642539   36.17250
C(PDBName=CB,ResName=HIS,ResNum=277_A)   21.491270   33.617349   35.67343
H(PDBName=HB1,ResName=HIS,ResNum=277_A)  21.372926   34.413826   36.41224
H(PDBName=HB2,ResName=HIS,ResNum=277_A)  22.549487   33.337129   35.68201
N(PDBName=ND1,ResName=HIS,ResNum=277_A)  20.230649   35.164428   34.11443
H(PDBName=HD1,ResName=HIS,ResNum=277_A)  19.831119   35.721784   34.87475
C-CC(PDBName=CG,ResName=HIS,ResNum=277_A)  21.120034   34.128044
34.31374
C(PDBName=CE1,ResName=HIS,ResNum=277_A)   20.109754   35.420939
32.81599
H(PDBName=HE1,ResName=HIS,ResNum=277_A)    19.513757   36.201092
32.37257
N(PDBName=NE2,ResName=HIS,ResNum=277_A)   20.890310   34.577815
32.15229
H(PDBName=HE2,ResName=HIS,ResNum=277_A)     21.012488   34.607102
31.11856
C(PDBName=CD2,ResName=HIS,ResNum=277_A)   21.523249   33.761922
33.06261
H(PDBName=HD2,ResName=HIS,ResNum=277_A)    22.212897   32.995117
32.78116
C(PDBName=C,ResName=HIS,ResNum=277_A)   20.929979   31.227011   35.22300
O(PDBName=O,ResName=HIS,ResNum=277_A)  21.983000   30.586999   35.28899
N(PDBName=N,ResName=HIS,ResNum=328_A)    25.030101   34.558840   39.09624
H(PDBName=HN,ResName=HIS,ResNum=328_A)  24.169288   34.507276   39.62284
C(PDBName=CA,ResName=HIS,ResNum=328_A)  25.324981   35.680342   38.28256
H(PDBName=HA,ResName=HIS,ResNum=328_A)  26.056464   35.395576   37.50604
C(PDBName=CB,ResName=HIS,ResNum=328_A)  24.057863   36.131546   37.52594
H(PDBName=HB1,ResName=HIS,ResNum=328_A)  23.444440   35.235896   37.41466
H(PDBName=HB2,ResName=HIS,ResNum=328_A)  23.489058   36.840457   38.12587
N(PDBName=ND1,ResName=HIS,ResNum=328_A)  25.130335   37.764858   35.94238
H(PDBName=HD1,ResName=HIS,ResNum=328_A)   25.627436   38.250575
36.69698
C(PDBName=CG,ResName=HIS,ResNum=328_A)  24.316506   36.680119   36.17036
C(PDBName=CE1,ResName=HIS,ResNum=328_A)  25.133982   38.037617   34.63122
H(PDBName=HE1,ResName=HIS,ResNum=328_A)  25.674366   38.864341   34.19771
N(PDBName=NE2,ResName=HIS,ResNum=328_A)  24.368902   37.160940   33.98058
C(PDBName=CD2,ResName=HIS,ResNum=328_A)  23.856297   36.308021   34.93803
H(PDBName=HD2,ResName=HIS,ResNum=328_A)  23.204339   35.492732   34.67126
C(PDBName=C,ResName=HIS,ResNum=328_A)  26.096328   36.791902   39.01503
O(PDBName=O,ResName=HIS,ResNum=328_A)  26.090171   37.965751   38.70565
N(PDBName=N,ResName=HIS,ResNum=116_C)  34.630000   39.771006   21.07100
H(PDBName=HN,ResName=HIS,ResNum=116_C)  34.544088   39.875753   20.06799
C(PDBName=CA,ResName=HIS,ResNum=116_C)  33.526313   40.247200   21.88115
H(PDBName=HA,ResName=HIS,ResNum=116_C)  33.641297   39.885150   22.91581
C(PDBName=CB,ResName=HIS,ResNum=116_C)  33.395410   41.752507   21.89088
H(PDBName=HB1,ResName=HIS,ResNum=116_C)  33.407030   41.980759   20.82172
H(PDBName=HB2,ResName=HIS,ResNum=116_C)  32.446621   42.072940   22.34504
N(PDBName=ND1,ResName=HIS,ResNum=116_C)  34.749468   42.436321   23.91646
C(PDBName=CG,ResName=HIS,ResNum=116_C)  34.541315   42.451624   22.54645
C(PDBName=CE1,ResName=HIS,ResNum=116_C)  35.840242   43.170326   24.13344
H(PDBName=HE1,ResName=HIS,ResNum=116_C)  36.263376   43.372051   25.10121
```

N(PDBName=NE2,ResName=HIS,ResNum=116_C) 36.343051   43.630874   22.98246
H(PDBName=HE2,ResName=HIS,ResNum=116_C) 37.201432   44.200827   22.85927
C(PDBName=CD2,ResName=HIS,ResNum=116_C) 35.535968   43.187169   21.96738
H(PDBName=HD2,ResName=HIS,ResNum=116_C) 35.730611   43.440318   20.93928
C(PDBName=C,ResName=HIS,ResNum=116_C) 32.279004   39.564992   21.32800
O(PDBName=O,ResName=HIS,ResNum=116_C) 32.272997   39.122006   20.18399
N(PDBName=N,ResName=ASP,ResNum=119_C) 25.181936   38.576149   24.98501
H(PDBName=HN,ResName=ASP,ResNum=119_C) 25.613286   39.295907   25.54028
C(PDBName=CA,ResName=ASP,ResNum=119_C) 24.229044   37.752994   25.67617
H(PDBName=HA,ResName=ASP,ResNum=119_C) 23.882292   36.949056   25.01888
C(PDBName=CB,ResName=ASP,ResNum=119_C) 24.893261   37.163485   26.94222
H(PDBName=HB1,ResName=ASP,ResNum=119_C) 25.750440   36.562243   26.62664
H(PDBName=HB2,ResName=ASP,ResNum=119_C) 25.306735   38.005258   27.51179
C(PDBName=CG,ResName=ASP,ResNum=119_C) 24.057108   36.341730   27.92050
O(PDBName=OD1,ResName=ASP,ResNum=119_C)   22.809186   36.346580
27.84913
O(PDBName=OD2,ResName=ASP,ResNum=119_C) 24.718328   35.708006   28.81101
C(PDBName=C,ResName=ASP,ResNum=119_C) 23.067981   38.690930   26.03507
O(PDBName=O,ResName=ASP,ResNum=119_C) 23.269029   39.661048   26.76392
N(PDBName=N,ResName=HSD,ResNum=121_C) 19.656986   38.629034   28.10801
H(PDBName=HN,ResName=HSD,ResNum=121_C) 20.057658   39.517469   28.37319
C(PDBName=CA,ResName=HSD,ResNum=121_C) 18.886192   37.905155   29.07469
H(PDBName=HA,ResName=HSD,ResNum=121_C) 19.224070   36.857444   29.16899
C(PDBName=CB,ResName=HSD,ResNum=121_C) 18.987432   38.541173   30.45940
H(PDBName=HB1,ResName=HSD,ResNum=121_C) 18.757439   39.603789   30.40123
H(PDBName=HB2,ResName=HSD,ResNum=121_C) 18.203439   38.090643   31.06915
N(PDBName=ND1,ResName=HSD,ResNum=121_C) 20.391187   38.675428   32.50530
H(PDBName=HD1,ResName=HSD,ResNum=121_C) 19.675951   39.168638   33.03608
C(PDBName=CG,ResName=HSD,ResNum=121_C) 20.294086   38.388349   31.15677
C(PDBName=CE1,ResName=HSD,ResNum=121_C) 21.644149   38.411524   32.91304
H(PDBName=HE1,ResName=HSD,ResNum=121_C) 21.999320   38.540382   33.92082
N(PDBName=NE2,ResName=HSD,ResNum=121_C) 22.361543   37.950811   31.90051
C(PDBName=CD2,ResName=HSD,ResNum=121_C) 21.532328   37.945959   30.78580
H(PDBName=HD2,ResName=HSD,ResNum=121_C) 21.871945   37.642399   29.80353
C(PDBName=C,ResName=HSD,ResNum=121_C) 17.379093   37.845950   28.75006
O(PDBName=O,ResName=HSD,ResNum=121_C) 16.604966   37.075011   29.30196
N(PDBName=N,ResName=HSD,ResNum=156_C) 24.795021   41.653968   28.12612
H(PDBName=HN,ResName=HSD,ResNum=156_C) 24.480343   40.837310   27.62437
C(PDBName=CA,ResName=HSD,ResNum=156_C) 26.196797   41.845758   28.38028
H(PDBName=HA,ResName=HSD,ResNum=156_C) 26.500636   42.886497   28.21983
C(PDBName=CB,ResName=HSD,ResNum=156_C) 26.558581   41.525141   29.85438
H(PDBName=HB1,ResName=HSD,ResNum=156_C) 27.589365   41.803676   30.04661
H(PDBName=HB2,ResName=HSD,ResNum=156_C) 25.948513   42.167878   30.49271
N(PDBName=ND1,ResName=HSD,ResNum=156_C) 27.233612   39.159553   30.43483
H(PDBName=HD1,ResName=HSD,ResNum=156_C) 28.234745   39.309263   30.35947
C(PDBName=CG,ResName=HSD,ResNum=156_C) 26.268214   40.116550   30.22154
C(PDBName=CE1,ResName=HSD,ResNum=156_C) 26.637887   38.017215   30.80795
H(PDBName=HE1,ResName=HSD,ResNum=156_C) 27.167411   37.103441   31.03082
N(PDBName=NE2,ResName=HSD,ResNum=156_C) 25.319055   38.180562   30.82496
C(PDBName=CD2,ResName=HSD,ResNum=156_C) 25.081854   39.479936   30.44428
H(PDBName=HD2,ResName=HSD,ResNum=156_C) 24.079355   39.847274   30.32312
C(PDBName=C,ResName=HSD,ResNum=156_C) 27.104444   40.997978   27.48256
O(PDBName=O,ResName=HSD,ResNum=156_C) 26.704785   39.989999   26.90612
N(PDBName=N,ResName=CYS,ResNum=157_C) 28.408002   41.380450   27.40902
H(PDBName=HN,ResName=CYS,ResNum=157_C) 28.720145   42.187724   27.94102
C(PDBName=CA,ResName=CYS,ResNum=157_C) 29.355029   40.585123   26.64258
H(PDBName=HA,ResName=CYS,ResNum=157_C) 28.845722   40.240309   25.73776

C(PDBName=CB,ResName=CYS,ResNum=157_C) 30.573178   41.428337   26.25193
H(PDBName=HB1,ResName=CYS,ResNum=157_C) 30.259432   42.259999   25.61636
H(PDBName=HB2,ResName=CYS,ResNum=157_C) 31.036981   41.836501   27.15196
S(PDBName=SG,ResName=CYS,ResNum=157_C) 31.817790   40.419575   25.36146
C(PDBName=C,ResName=CYS,ResNum=157_C) 29.840985   39.371018   27.44098
O(PDBName=O,ResName=CYS,ResNum=157_C) 30.105992   39.477990   28.63400
N(PDBName=N,ResName=HIS,ResNum=167_C) 35.067000   41.022003   30.85400
H(PDBName=HN,ResName=HIS,ResNum=167_C) 34.875841   40.033482   30.77571
C(PDBName=CA,ResName=HIS,ResNum=167_C) 34.402774   41.911944   29.93455
H(PDBName=HA,ResName=HIS,ResNum=167_C) 35.090646   42.707505   29.61490
C(PDBName=CB,ResName=HIS,ResNum=167_C) 33.946047   41.160250   28.67232
H(PDBName=HB1,ResName=HIS,ResNum=167_C) 33.369616   40.270809   28.95290
H(PDBName=HB2,ResName=HIS,ResNum=167_C) 33.278779   41.810050   28.09448
N(PDBName=ND1,ResName=HIS,ResNum=167_C) 35.129627   40.961207   26.44040
C(PDBName=CG,ResName=HIS,ResNum=167_C) 35.117547   40.799976   27.81539
C(PDBName=CE1,ResName=HIS,ResNum=167_C) 36.335675   40.605747   26.01973
H(PDBName=HE1,ResName=HIS,ResNum=167_C) 36.675955   40.628640   25.00123
N(PDBName=NE2,ResName=HIS,ResNum=167_C) 37.092278   40.224449   27.05568
H(PDBName=HE2,ResName=HIS,ResNum=167_C) 38.077157   40.005571   27.01508
C(PDBName=CD2,ResName=HIS,ResNum=167_C) 36.346409   40.348230   28.19960
H(PDBName=HD2,ResName=HIS,ResNum=167_C) 36.718377   40.150690   29.18794
C(PDBName=C,ResName=HIS,ResNum=167_C) 33.216467   42.663193   30.54832
O(PDBName=O,ResName=HIS,ResNum=167_C) 33.066991   43.854996   30.35698
N(PDBName=N,ResName=SER,ResNum=170_C) 34.503005   45.246986   33.09200
H(PDBName=HN,ResName=SER,ResNum=170_C) 34.346440   44.337210   32.70041
C(PDBName=CA,ResName=SER,ResNum=170_C) 35.097767   46.262797   32.28148
H(PDBName=HA,ResName=SER,ResNum=170_C) 35.807342   46.857381   32.86236
C(PDBName=CB,ResName=SER,ResNum=170_C) 35.883715   45.679367   31.09955
H(PDBName=HB1,ResName=SER,ResNum=170_C) 35.179544   45.109138   30.47127
H(PDBName=HB2,ResName=SER,ResNum=170_C) 36.265103   46.517751   30.51674
O(PDBName=OG,ResName=SER,ResNum=170_C) 36.996143   44.896552   31.45969
H(PDBName=HG1,ResName=SER,ResNum=170_C) 36.629382   44.123203   31.93972
C(PDBName=C,ResName=SER,ResNum=170_C) 34.095305   47.260140   31.67676
O(PDBName=O,ResName=SER,ResNum=170_C) 34.481005   48.233106   31.02451
N(PDBName=N,ResName=GLY,ResNum=171_C) 32.790575   46.948709   31.85384
H(PDBName=HN,ResName=GLY,ResNum=171_C) 32.605939   46.213558   32.53681
C(PDBName=CA,ResName=GLY,ResNum=171_C) 31.753428   47.846542   31.41385
H(PDBName=HA1,ResName=GLY,ResNum=171_C) 32.245657   48.739721   31.02407
H(PDBName=HA2,ResName=GLY,ResNum=171_C) 31.104778   48.128540   32.24520
C(PDBName=C,ResName=GLY,ResNum=171_C) 30.865854   47.324163   30.30424
O(PDBName=O,ResName=GLY,ResNum=171_C) 29.987963   48.004660   29.78482
N(PDBName=N,ResName=MET,ResNum=172_C) 31.035450   46.018873   29.92594
H(PDBName=HN,ResName=MET,ResNum=172_C) 31.791248   45.472682   30.33283
C(PDBName=CA,ResName=MET,ResNum=172_C) 30.345798   45.562999   28.73737
H(PDBName=HA,ResName=MET,ResNum=172_C) 30.192840   46.451164   28.10517
C(PDBName=CB,ResName=MET,ResNum=172_C) 31.097231   44.497951   27.94659
H(PDBName=HB1,ResName=MET,ResNum=172_C) 31.229448   43.599627   28.55499
H(PDBName=HB2,ResName=MET,ResNum=172_C) 30.462135   44.222622   27.09773
C(PDBName=CG,ResName=MET,ResNum=172_C) 32.440199   45.009101   27.45845
H(PDBName=HG1,ResName=MET,ResNum=172_C) 32.363333   46.050531   27.14346
H(PDBName=HG2,ResName=MET,ResNum=172_C) 33.185642   44.959089   28.25437
S(PDBName=SD,ResName=MET,ResNum=172_C) 33.155825   44.119445   26.04375
C(PDBName=CE,ResName=MET,ResNum=172_C) 32.079558   44.659287   24.69130
H(PDBName=HE1,ResName=MET,ResNum=172_C) 32.509564   44.263267   23.76831
H(PDBName=HE2,ResName=MET,ResNum=172_C) 31.059119   44.288105   24.80346
H(PDBName=HE3,ResName=MET,ResNum=172_C) 32.069733   45.749979   24.63458
C(PDBName=C,ResName=MET,ResNum=172_C) 28.973037   45.047984   29.13499

O(PDBName=O,ResName=MET,ResNum=172_C) 28.708939   43.848006   29.05398
Cu(PDBName=CU,ResName=CU,ResNum=371_C) 33.670940   41.571406   25.32516
Cu(PDBName=CU,ResName=CU,ResNum=372_C) 24.143311   37.176540   32.03380
O(PDBName=OH2,ResName=TIP,ResNum=H2OA1) 24.616881   35.363636   31.36837
H(PDBName=H1,ResName=TIP,ResNum= H2OA1) 25.570500   35.151785   31.32262
H(PDBName=H1,ResName=TIP,ResNum= H2OA1) 24.424233   35.445356   30.38111
O(PDBName=OH2,ResName=TIP,ResNum= H2OA2) 21.163819   34.710027
29.33100
H(PDBName=H2,ResName=TIP,ResNum= H2OA2) 21.870471   35.256894   28.89342
H(PDBName=H1,ResName=TIP,ResNum= H2OA2) 20.366372   35.120051   28.96894
O(PDBName=OH2,ResName=TIP,ResNum= H2OA3) 26.991407   35.186405
30.07555
H(PDBName=H2,ResName=TIP,ResNum= H2OA3) 26.331322   35.459955   29.39188
H(PDBName=H1,ResName=TIP,ResNum= H2OA3) 27.297514   34.319042   29.80244