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SUPPORTING INFORMATION

QM/MM MD Simulations Reveal an Asynchronous PCET mechanism for the Nitrite Reduction by Copper Nitrite Reductase

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Force field of CuNiR used in this study

REMARK GOES HERE, THIS FILE IS GENERATED BY MCPB.PY

MASS

M1 63.55 Cu ion

Y1 14.01 0.530 sp2 N in 5 memb.ring w/LP (HIS,ADE,GUA)

Y2 32.06 2.900 S in cystine

Y3 14.01 0.530 sp2 N in 5 memb.ring w/LP (HIS,ADE,GUA)

Y4 32.06 2.900 S in disulfide linkage,pol:JPC,102,2399,98

BOND

Y1-M1 71.0 2.0676 Created by Seminario method using MCPB.py

Y2-M1 70.8 2.3055 Created by Seminario method using MCPB.py

Y3-M1 65.1 2.0404 Created by Seminario method using MCPB.py

Y4-M1 17.8 2.4998 Created by Seminario method using MCPB.py

2C-Y2 237.0 1.8100

2C-Y4 227.0 1.8100

CC-Y1 410.0 1.394 JCC,7,(1986),230; HIS

CC-Y3 410.0 1.394 JCC,7,(1986),230; HIS

Y1-CR 488.0 1.335 JCC,7,(1986),230; HIS

Y3-CR 488.0 1.335 JCC,7,(1986),230; HIS

Y4-CT 227.0 1.810 changed from 222.0 based on dimethylS nmodes

ANGL

2C-Y2-M1 49.52 113.60 Created by Seminario method using MCPB.py

2C-Y4-M1 65.94 109.00 Created by Seminario method using MCPB.py

CC-Y1-M1 106.81 123.68 Created by Seminario method using MCPB.py

CC-Y3-M1 81.60 129.98 Created by Seminario method using MCPB.py

M1-Y1-CR 98.93 128.80 Created by Seminario method using MCPB.py

M1-Y3-CR 79.36 122.47 Created by Seminario method using MCPB.py

M1-Y4-CT 21.55 100.39 Created by Seminario method using MCPB.py

Y1-M1-Y2 42.65 142.18 Created by Seminario method using MCPB.py

Y1-M1-Y3 48.49 94.15 Created by Seminario method using MCPB.py

Y1-M1-Y4 50.64 85.33 Created by Seminario method using MCPB.py

Y2-M1-Y3 33.65 96.78 Created by Seminario method using MCPB.py

Y2-M1-Y4 46.62 103.81 Created by Seminario method using MCPB.py

Y3-M1-Y4 21.54 146.86 Created by Seminario method using MCPB.py

2C-2C-Y4 50.0 114.70

2C-Y4-CT 62.0 98.90

CC-Y1-CR 70.0 117.00 AA his

CC-Y3-CR 70.0 117.00 AA his

CT-CC-Y1 70.0 120.00 AA his

CT-CC-Y3 70.0 120.00 AA his

CW-CC-Y1 70.0 120.00 AA his

CW-CC-Y3 70.0 120.00 AA his

CX-2C-Y2 50.0 108.60

H1-2C-Y2 50.0 109.50

H1-2C-Y4 50.0 109.50

Y1-CR-H5 50.0 120.00 AA his

Y1-CR-NA 70.0 120.00 AA his

Y3-CR-H5 50.0 120.00 AA his

Y3-CR-NA 70.0 120.00 AA his

Y4-CT-H1 50.0 109.50 AA cys changed based on NMA nmodes

DIHE

X -2C-Y4-X 3 1.0 0.0 3.0 JCC,7,(1986),230 (X -CT-S -X)

X -CC-Y1-X 2 4.8 180.0 2.0 JCC,7,(1986),230

X -CC-Y3-X 2 4.8 180.0 2.0 JCC,7,(1986),230

X -Y1-CR-X 2 10.0 180.0 2.0 JCC,7,(1986),230

X -Y3-CR-X 2 10.0 180.0 2.0 JCC,7,(1986),230

2C-2C-Y4-CT 1 0.057 0.0 -4.0

2C-2C-Y4-CT 1 0.414 0.0 -3.0

2C-2C-Y4-CT 1 0.442 0.0 -2.0

2C-2C-Y4-CT 1 0.247 180.0 1.0

2C-2C-Y4-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

2C-Y2-M1-Y3 3 0.00 0.00 3.0 Treat as zero by MCPB.py

2C-Y2-M1-Y4 3 0.00 0.00 3.0 Treat as zero by MCPB.py

2C-Y4-CT-H1 3 1.0 0.0 3.0

CC-Y1-M1-Y2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CC-Y1-M1-Y3 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CC-Y1-M1-Y4 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CC-Y3-M1-Y4 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CT-CC-Y1-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CT-CC-Y3-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CW-CC-Y1-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CW-CC-Y3-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CX-2C-2C-Y4 1 0.028 0.0 -4.0

CX-2C-2C-Y4 1 0.016 0.0 -3.0

CX-2C-2C-Y4 1 0.245 0.0 -2.0

CX-2C-2C-Y4 1 0.417 0.0 1.0

CX-2C-Y2-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CX-CT-CC-Y1 1 0.047 180.0 -4.0

CX-CT-CC-Y1 1 0.74 0.0 -3.0

CX-CT-CC-Y1 1 0.204 0.0 -2.0

CX-CT-CC-Y1 1 0.69 0.0 1.0

CX-CT-CC-Y3 1 0.047 180.0 -4.0

CX-CT-CC-Y3 1 0.74 0.0 -3.0

CX-CT-CC-Y3 1 0.204 0.0 -2.0

CX-CT-CC-Y3 1 0.69 0.0 1.0

H1-2C-Y2-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H1-2C-Y4-M1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M1-Y1-CR-H5 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M1-Y1-CR-NA 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M1-Y3-CR-H5 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M1-Y3-CR-NA 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M1-Y4-CT-H1 3 0.00 0.00 3.0 Treat as zero by MCPB.py

```
N -CX-2C-Y2    1      0.033        0.0  -4.0
N -CX-2C-Y2    1      0.251        0.0  -3.0
N -CX-2C-Y2    1      0.486      180.0  -2.0
N -CX-2C-Y2    1      0.154        0.0   1.0
Y1-M1-Y2-2C    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y1-M1-Y3-CC    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y1-M1-Y3-CR    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y1-M1-Y4-2C    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y1-M1-Y4-CT    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y2-2C-CX-C     1      0.075        0.0  -4.0       C
Y2-2C-CX-C     1      0.251        0.0  -3.0
Y2-2C-CX-C     1      0.337      180.0  -2.0
Y2-2C-CX-C     1      0.269      180.0   1.0
Y2-M1-Y1-CR    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y2-M1-Y3-CC    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y2-M1-Y3-CR    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y2-M1-Y4-2C    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y2-M1-Y4-CT    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y3-M1-Y1-CR    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y3-M1-Y4-2C    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y3-M1-Y4-CT    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y4-M1-Y1-CR    3       0.00       0.00   3.0    Treat as zero by MCPB.py
Y4-M1-Y3-CR    3       0.00       0.00   3.0    Treat as zero by MCPB.py

IMPR
CT-CW-CC-Y3        1.1        180.         2.
CT-CW-CC-Y1        1.1        180.         2.

NONB
 M1         1.2140  0.0013919600       IOD set for Cu+ ion from Li et al. JCTC, 2015, 11, 1645
 Y1         1.8240  0.1700             OPLS
 Y2         2.0000  0.2500             W. Cornell CH3SH and CH3SCH3 FEP's
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Y3 1.8240 0.1700 OPLS

Y4 2.0000 0.2500 W. Cornell CH3SH and CH3SCH3 FEP's

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MASS

M2 63.55 Cu ion

Y5 14.01 0.530 sp2 N in 5 memb.ring w/LP (HIS,ADE,GUA)

Y6 14.01 0.530 sp2 N in 5 memb.ring w/LP (HIS,ADE,GUA)

Y7 14.01 0.530 sp2 N in 5 memb.ring w/LP (HIS,ADE,GUA)

Y8 16.00 0.434 Oxygen with one connected atom

Y9 16.00 0.434 Oxygen with one connected atom

BOND

M2-Y8 45.3 2.0754 Created by Seminario method using MCPB.py

M2-Y9 7.3 2.8038 Created by Seminario method using MCPB.py

Y5-M2 60.9 2.0563 Created by Seminario method using MCPB.py

Y6-M2 80.7 2.0139 Created by Seminario method using MCPB.py

Y7-M2 81.2 2.0191 Created by Seminario method using MCPB.py

CR-Y5 488.0 1.335 JCC,7,(1986),230; HIS

CR-Y6 488.0 1.335 JCC,7,(1986),230; HIS

CR-Y7 488.0 1.335 JCC,7,(1986),230; HIS

CV-Y5 410.0 1.394 JCC,7,(1986),230; HIS

CV-Y6 410.0 1.394 JCC,7,(1986),230; HIS

CV-Y7 410.0 1.394 JCC,7,(1986),230; HIS

n2-Y8 766.3 1.2172 SOURCE3_SOURCE5 112 0.0102

n2-Y9 766.3 1.2172 SOURCE3_SOURCE5 112 0.0102

ANGL

CR-Y5-M2 73.50 128.91 Created by Seminario method using MCPB.py

CR-Y6-M2 99.65 126.19 Created by Seminario method using MCPB.py

CR-Y7-M2 90.03 129.50 Created by Seminario method using MCPB.py

CV-Y5-M2 77.55 124.49 Created by Seminario method using MCPB.py

CV-Y6-M2 103.98 125.79 Created by Seminario method using MCPB.py

CV-Y7-M2 93.35 123.45 Created by Seminario method using MCPB.py

M2-Y8-n2 110.10 115.13 Created by Seminario method using MCPB.py

M2-Y9-n2 84.55 79.78 Created by Seminario method using MCPB.py

Y5-M2-Y6 62.00 90.99 Created by Seminario method using MCPB.py

Y5-M2-Y7 59.97 94.65 Created by Seminario method using MCPB.py

Y5-M2-Y8 58.72 170.69 Created by Seminario method using MCPB.py

Y5-M2-Y9 48.38 121.89 Created by Seminario method using MCPB.py

Y6-M2-Y7 55.48 169.31 Created by Seminario method using MCPB.py

Y6-M2-Y8 54.63 88.24 Created by Seminario method using MCPB.py

Y6-M2-Y9 44.53 89.67 Created by Seminario method using MCPB.py

Y7-M2-Y8 56.99 87.64 Created by Seminario method using MCPB.py

Y7-M2-Y9 43.71 95.02 Created by Seminario method using MCPB.py

Y9-M2-Y8 70.93 48.84 Created by Seminario method using MCPB.py

CC-CV-Y5 70.0 120.00 AA his

CC-CV-Y6 70.0 120.00 AA his

CC-CV-Y7 70.0 120.00 AA his

CV-Y5-CR 70.0 117.00 AA his

CV-Y6-CR 70.0 117.00 AA his

CV-Y7-CR 70.0 117.00 AA his

H4-CV-Y5 50.0 120.00 AA his

H4-CV-Y6 50.0 120.00 AA his

H4-CV-Y7 50.0 120.00 AA his

H5-CR-Y5 50.0 120.00 AA his

H5-CR-Y6 50.0 120.00 AA his

H5-CR-Y7 50.0 120.00 AA his

NA-CR-Y5 70.0 120.00 AA his

NA-CR-Y6 70.0 120.00 AA his

NA-CR-Y7 70.0 120.00 AA his

Y9-n2-Y8 80.5 115.37 SOURCE3 1

DIHE

X -CR-Y5-X 2 10.0 180.0 2.0 JCC,7,(1986),230

X -CR-Y6-X 2 10.0 180.0 2.0 JCC,7,(1986),230

X -CR-Y7-X 2 10.0 180.0 2.0 JCC,7,(1986),230

X -CV-Y5-X 2 4.8 180.0 2.0 JCC,7,(1986),230

X -CV-Y6-X 2 4.8 180.0 2.0 JCC,7,(1986),230

X -CV-Y7-X 2 4.8 180.0 2.0 JCC,7,(1986),230

CC-CV-Y5-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CC-CV-Y6-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CC-CV-Y7-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y5-M2-Y6 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y5-M2-Y7 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y5-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y5-M2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y6-M2-Y7 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y6-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y6-M2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y7-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CR-Y7-M2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y5-M2-Y6 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y5-M2-Y7 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y5-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y5-M2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y6-M2-Y7 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y6-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y6-M2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y7-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

CV-Y7-M2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H4-CV-Y5-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H4-CV-Y6-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H4-CV-Y7-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H5-CR-Y5-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H5-CR-Y6-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

H5-CR-Y7-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M2-Y8-n2-Y9 3 0.00 0.00 3.0 Treat as zero by MCPB.py

M2-Y9-n2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

NA-CR-Y5-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

NA-CR-Y6-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

NA-CR-Y7-M2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y5-M2-Y6-CR 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y5-M2-Y6-CV 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y5-M2-Y7-CR 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y5-M2-Y7-CV 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y5-M2-Y8-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y5-M2-Y9-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y6-M2-Y7-CR 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y6-M2-Y7-CV 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y6-M2-Y8-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y6-M2-Y9-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y7-M2-Y8-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y7-M2-Y9-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

Y9-M2-Y8-n2 3 0.00 0.00 3.0 Treat as zero by MCPB.py

n2-Y9-M2-Y8 3 0.00 0.00 3.0 Treat as zero by MCPB.py

IMPR

NONB

M2 1.4090 0.0172100000 IOD set for Cu2+ ion from Li et al. JCTC, 2013, 9, 2733

Y5 1.8240 0.1700 OPLS

Y6 1.8240 0.1700 OPLS

Y7 1.8240 0.1700 OPLS

Y8 1.6612 0.2100 OPLS

Y9 1.6612 0.2100 OPLS

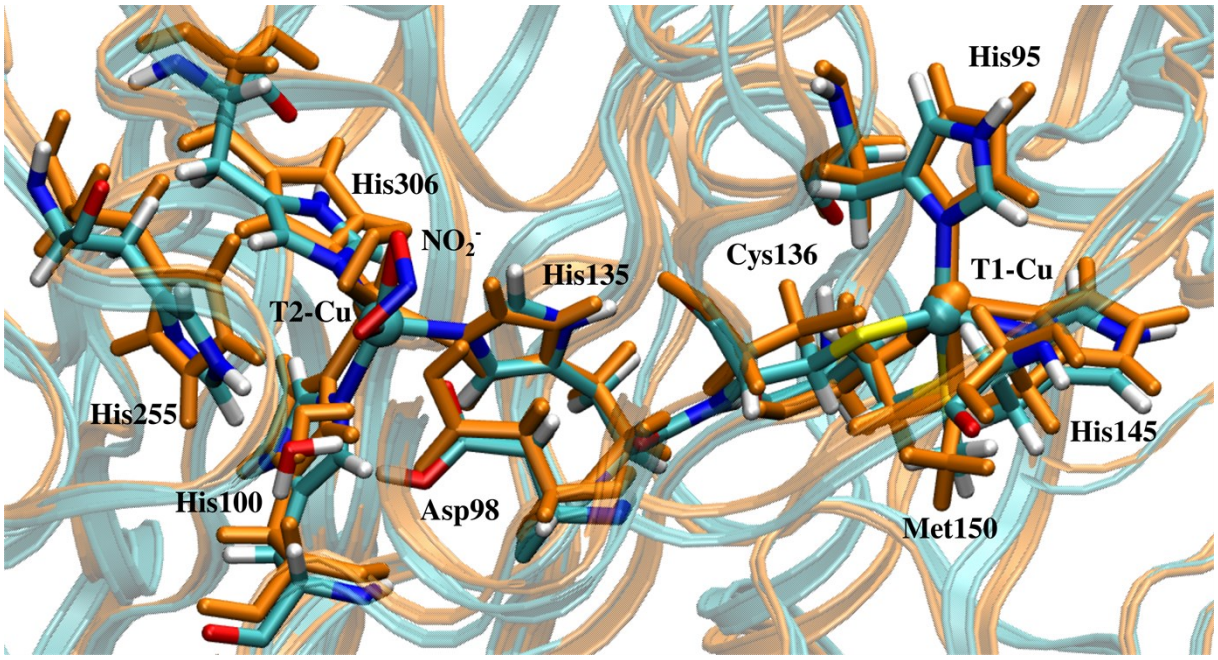

Figure S1. Comparison of the crystal structure of nitrite-bound *A. faecalis* CuNiR (PDB ID: 1SJM; depicted in cyan), and the nitrite-bound *A. faecalis* CuNiR after classical molecular dynamics simulations (depicted in orange).

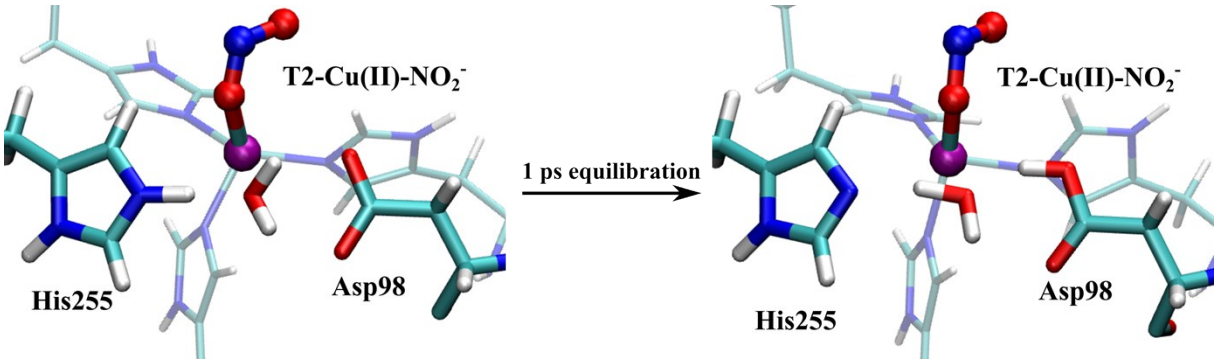

Figure S2. A representation of the nitrite-bound *A. faecalis* CuNiR at the ASP-HIP protonation state where Asp98 is deprotonated and His255 is protonated. After 1 ps of QM/MM equilibration, convergence to the ASH-HID protonation state where Asp98 is protonated and His255 is protonated, is observed.

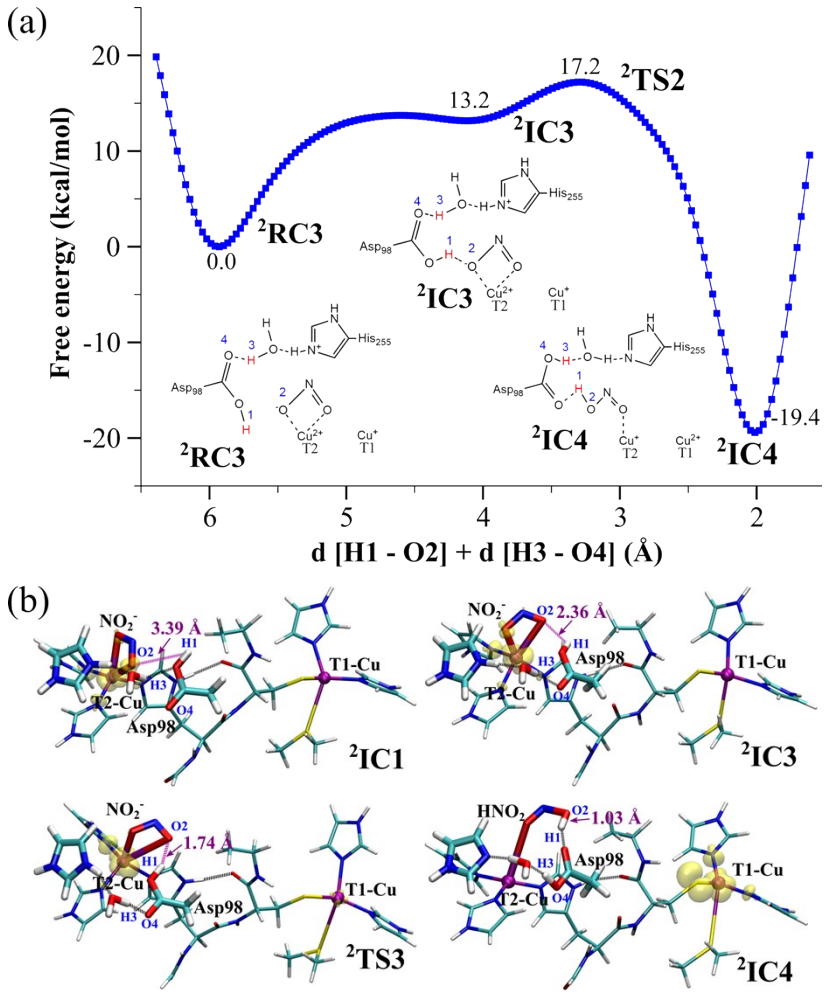

Figure S3. (a) The QM(B3LYP)/MM-MetD calculated free-energy profile for proton transfer from Asp98 in CuNiR using the TZVP basis set. The reaction coordinate is defined as the sum of the distance between the H1 of Asp98 and the O2 of nitrite, and the distance between H3 of bridging water and O4 of Asp98. The width of the Gaussian shaped potential hills was set to 0.3 Å. (b) The representative structures of the QM region along the reaction pathway, with spin-up isodensity surfaces plotted in yellow. Slightly different with the free energy profile obtained at DZVP basis set (Figure 5), here we sampled an intermediate ^{2}IC2 corresponding to the proximal conformer of Asp98.

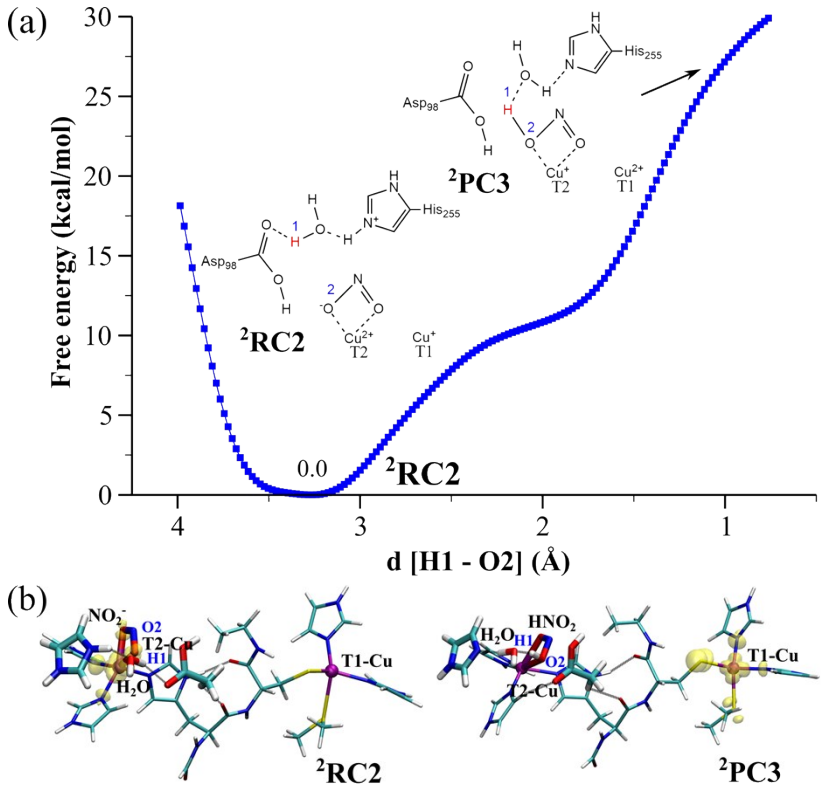

Figure S4. The QM(B3LYP)/MM-MetD calculated free-energy profile for proton transfer from bridging water in CuNiR. The reaction coordinate is defined as the distance di□erence between O2 of nitrite and H1 of bridging water. (b) The representative structures of the QM region along the reaction pathway, with spin-up isodensity surfaces plotted in yellow.

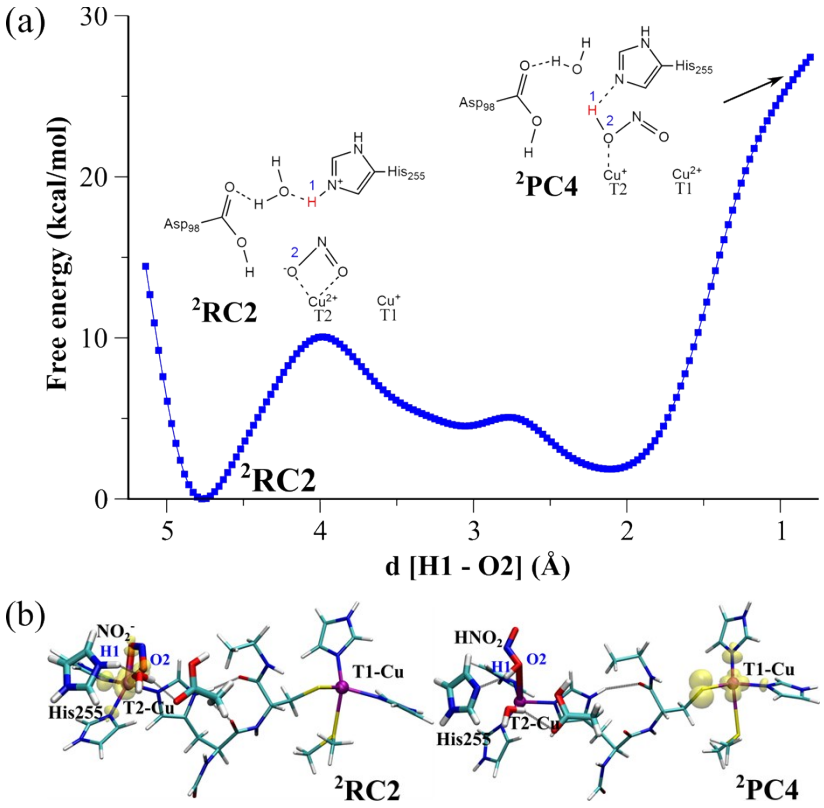

Figure S5. (a) The QM(B3LYP)/MM-MetD calculated free-energy profile for proton transfer from His255 in CuNiR. The reaction coordinate is defined as the distance di□erence between O2 of nitrite and H1 of His255. (b) The representative structures of the QM region along the reaction pathway, with spin-up isodensity surfaces plotted in yellow.

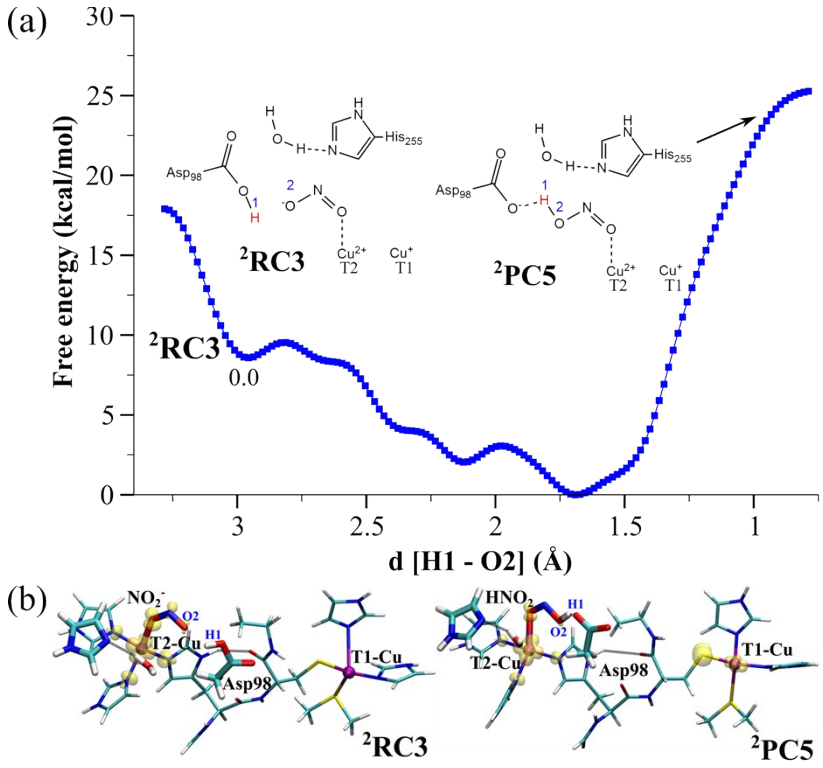

Figure S6. The QM(B3LYP)/MM-MetD calculated free-energy profile for proton transfer from Asp98 in CuNiR at the ASH-HID protonation state, where Asp98 is protonated and His255 is deprotonated. The reaction coordinate is defined as the distance di□erence between O2 of nitrite and H1 of Asp98. (b) The representative structures of the QM region along the reaction pathway, with spin-up isodensity surfaces plotted in yellow.

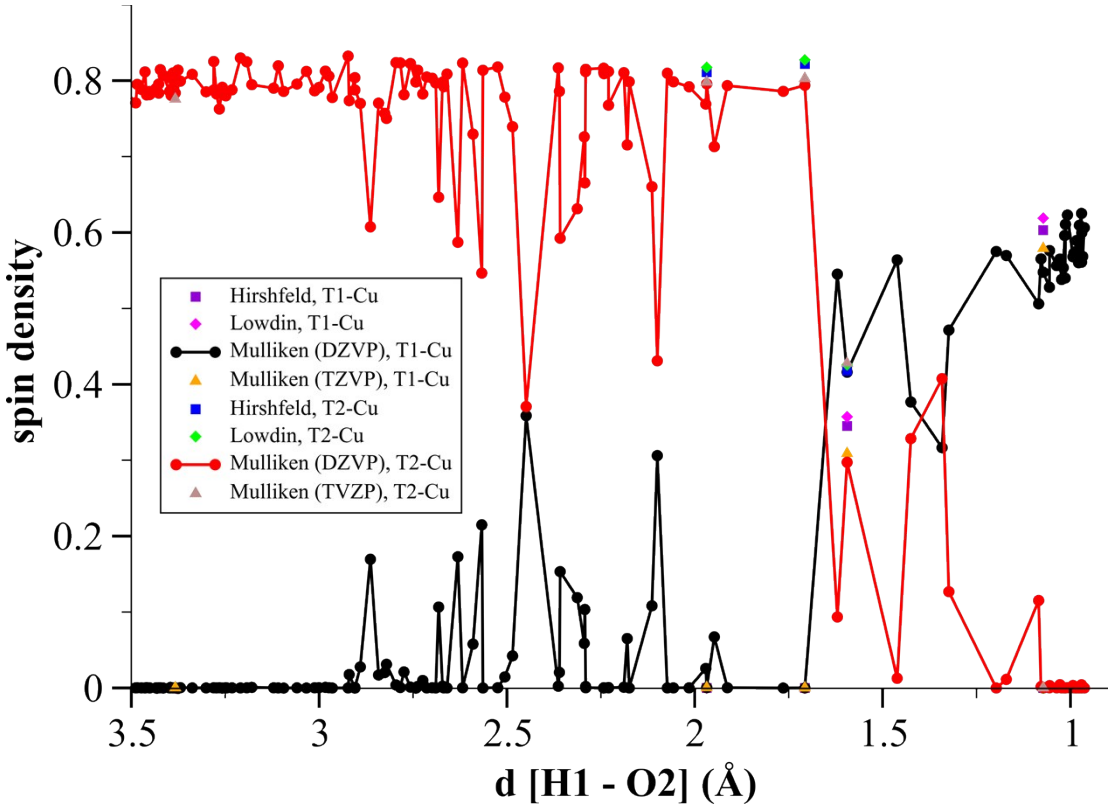

Figure S7. Calculated spin density at snapshots of interest of T1-Cu and T2-Cu with different population analysis methods, overlaid with Mulliken population analysis results using the DZVP basis set. Mulliken population analysis was performed with the DZVP and TZVP basis sets.

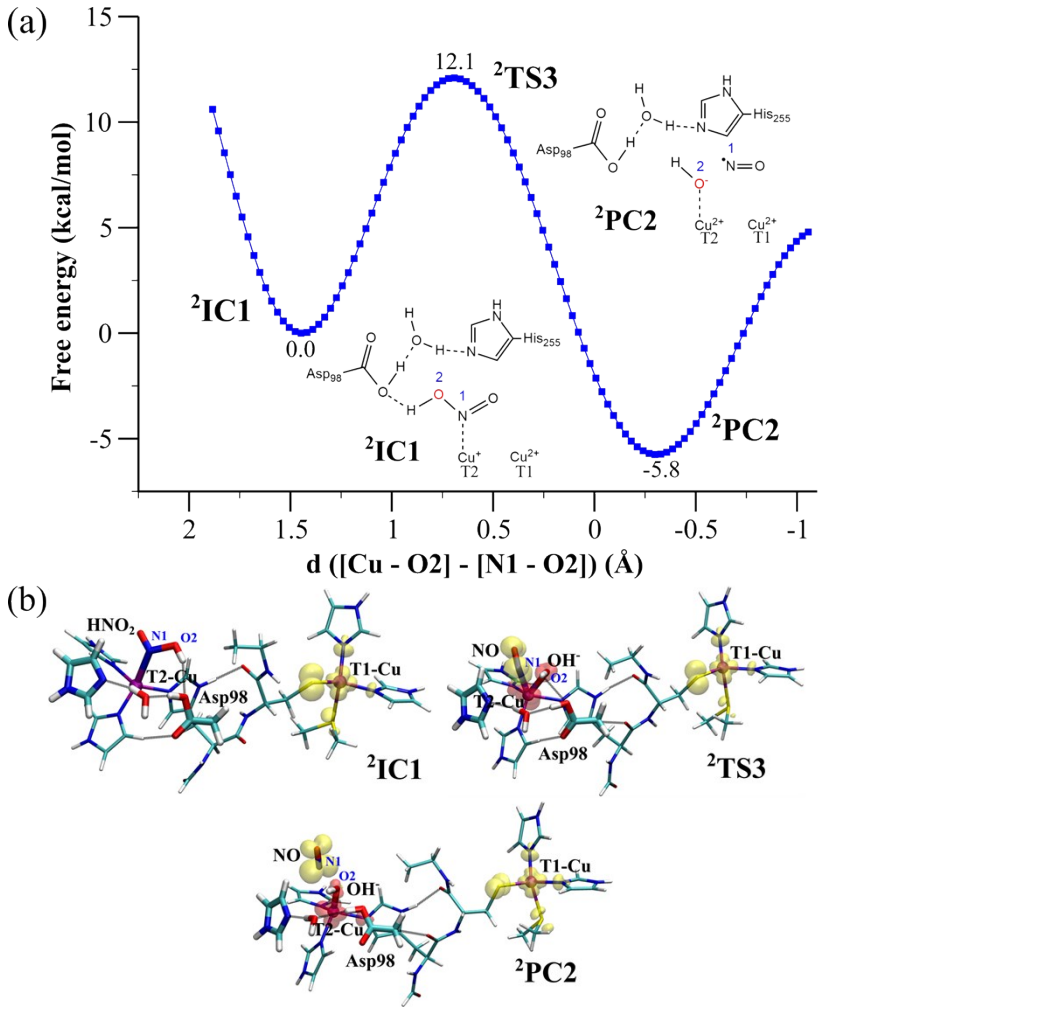

Figure S8. (a) The QM(B3LYP)/MM-MetD calculated free-energy profile for nitric oxide product release in CuNiR. The reaction coordinate is defined as the distance di□erence between T2-Cu and O2 of nitrite (d1) and that between N1 of nitrite to O2 of nitrite (d2). The width of the Gaussian shaped potential hills was set to 0.3 Å. (b) The representative structures of the QM region along the reaction pathway, with spin-up and spin-down isodensity surfaces plotted in yellow and red, respectively.