Supporting Information

THE CATALYTIC MECHANISM OF HUMAN ALDEHYDE OXIDASE

P. Ferreira[a], N.M.F.S.A. Cerqueira[a,b], P.A. Fernandes[a], M.J. Romão[c] and M. J. Ramos[a]*.

[a]LAQV@REQUIMTE, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal; [b] present address: UCIBIO@REQUIMTE, BioSIM - Departamento de Biomedicina, Faculdade de Medicina, Universidade do Porto, Alameda Professor Hernani Monteiro, 4200-319 Porto, Portugal; [c]UCIBIO@REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

*corresponding author: mjramos@fc.up.pt

Details regarding the MD simulation conducted previously for the study of the catalytic mechanism of *h*AOX:

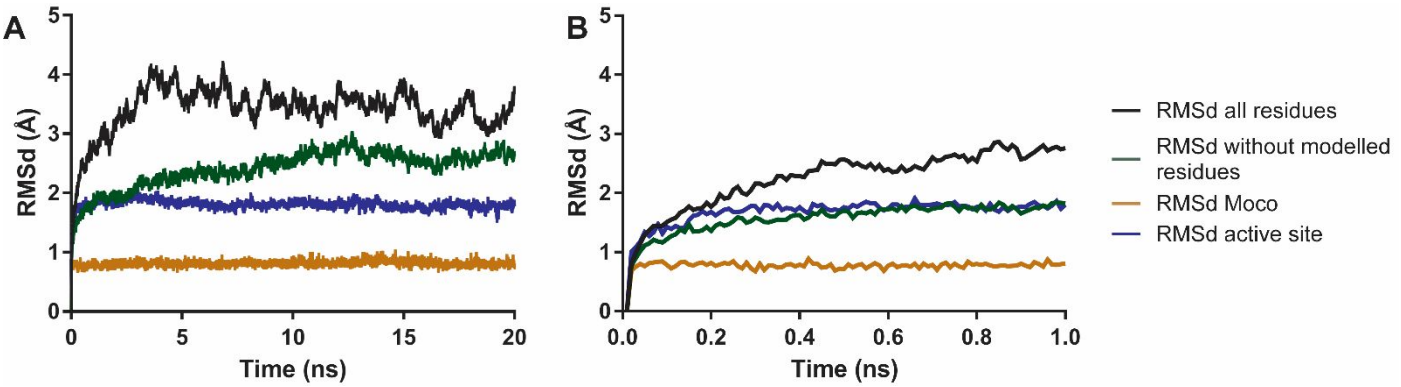

Figure S1. A. RMSd values during the 20 ns long MD simulation using as reference the minimized structure. B. RMSd values during the first nanosecond of the MD simulation from which the frame used for the study of the mechanism was taken. The values presented in the chart are those taking into account the whole system (black line), the RMSd excluding the values of the residues that were modelled (green line), the RMSd of the residues that make up the active site (purple line), and the RMSd values of the Moco (orange line).

In figure S1, we exhibit four different RMSd plots. One taking into account all residues of the enzyme (black line), another taking into account only the contribution of the crystallographic residues and not of those that were modelled (green line), another considering the residues that make up the active site (purple line) and a fourth one taking into account only the RMSd value of the Moco (brown line) throughout the MD simulation. As expected, the contribution of the modelled residues increase the overall RMSd value. This is justified by the fact that the modelled residues had to relax to a conformation of optimal energy as well as belonging to

external and mobile parts of the enzyme, particularly the 167-199 loop. The plot of the RMSd of the Moco revealed that it remained constant and always below 1 Å. Moreover, the active site also remained stable since the beginning of the simulation with a RMSd value below 2 Å. The RMSd plots (Figure S1) revealed that the system equilibrated around the 5 ns and that the residues of the active site and the Moco stabilized within the first nanosecond of the MD simulation.

During the initial picoseconds of the MD simulation, the PHT approached the Moco and positioned itself favourably to begin the reaction (Figure S2). The Lys893 approached the PHT and established with its N2 atom a strong hydrogen bond. Another strong hydrogen bond was established between Lys893 and Glu1270. Therefore, the model evolved from a structure that resembled a non-productive conformation to a conformation ready to begin the catalysis.

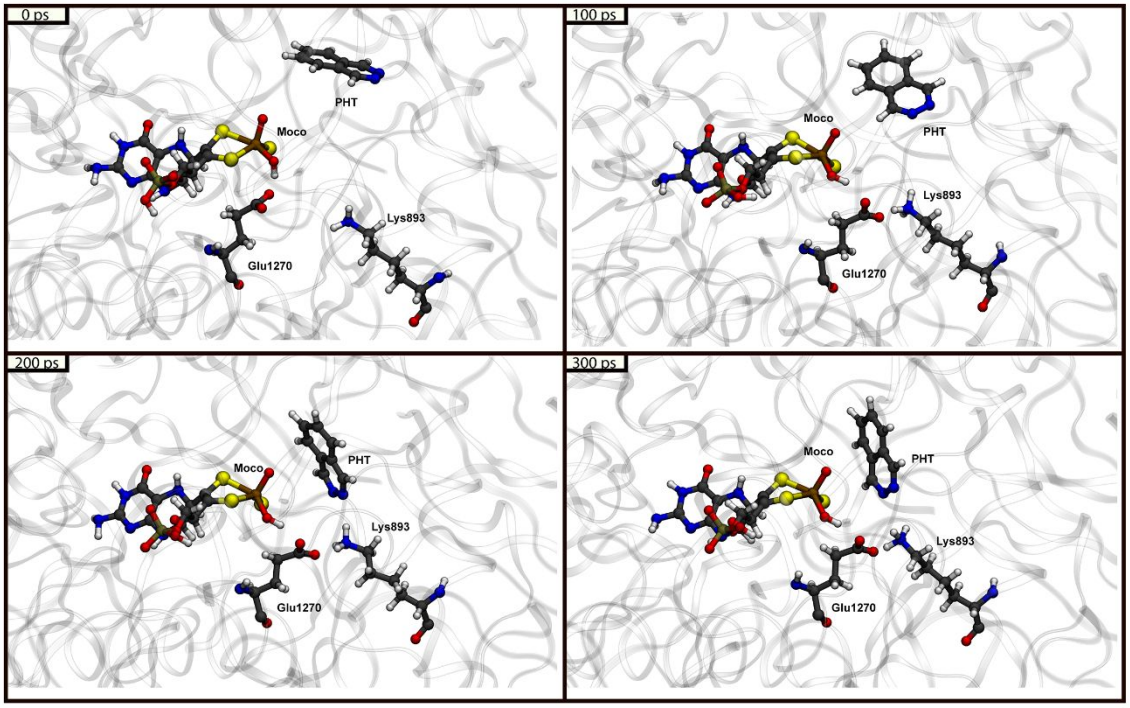

Figure S2. 3D representation of the conformation of the *h*AOX active site in the initial 300 ps of the 20 ns long MD simulation. The snapshots were taken at each 100 ps. During this time frame, the substrate (PHT) approached the Moco, evolving from a non-productive conformation (at 0 ps) to a conformation ready to initiate the catalysis (at 300 ps).

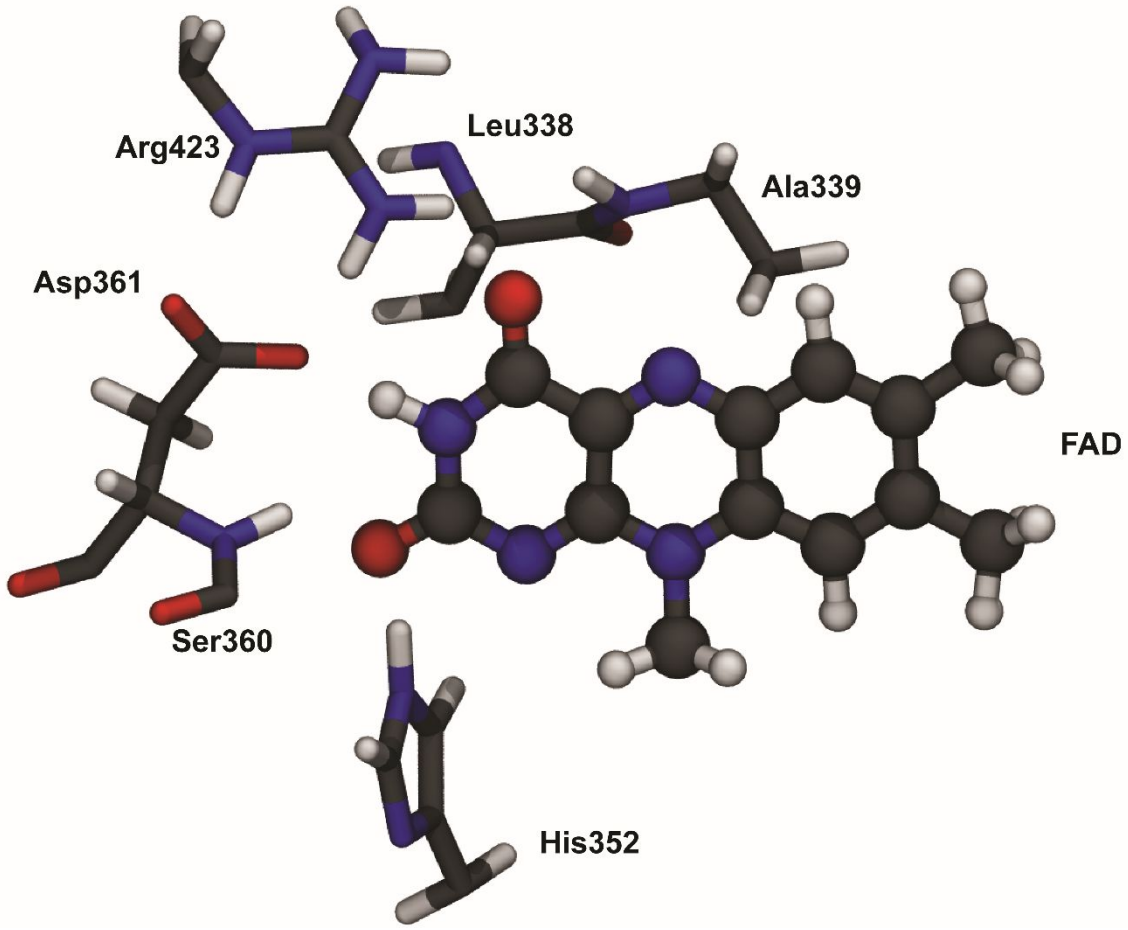

Figure S3. Representation of the QM layer used for the calculations of the Gibbs free energies of all redox half-reactions involving FAD. The QM layer was composed by 84 atoms. The methodology and level of theory used to calculate the energies were the same as those used to study the reaction mechanism, explained in the methodology section of the manuscript.

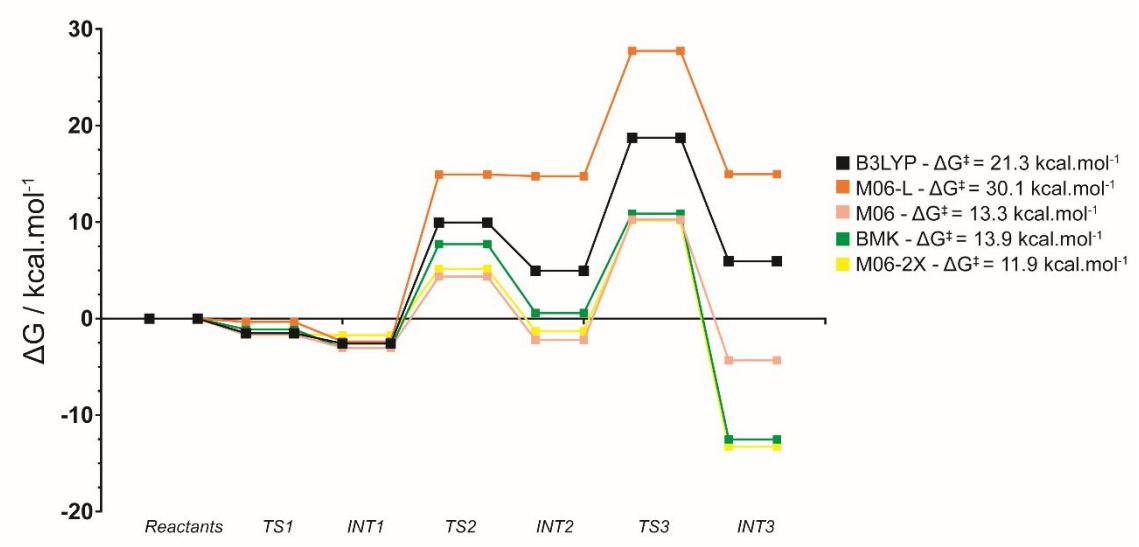

Figure S4. Comparison of potential energy surface of the oxidation of phthalazine by *h*AOX obtained with single point calculations with 5 different DFT functionals. The geometry of stationary points, in which the single point calculations with the different functionals were conducted, were obtained at the B3LYP/6–31G(d)/SDD:FF99SB level of theory. At the same level of theory, the zero-point energy and thermal corrections were determined to obtain the Gibbs free energies. The B3LYP was the one that returned the free activation energy closer to the experimental one, which is 20.96 kcal.mol^{-1}. All the others returned activation energies farther from the experimental values than B3LYP. However, despite the energy differences between the functionals used (ranging from 7 to 10 kcal.mol^{-1}), the overall shape of the PES and the rate-limiting step were the same.

Table S1. Thermochemical profile for the oxidation of phthalazine by hAOX1. The enthalpy values include the zero-point energy corrections and corrections to the internal thermal energy.

Stationary State	**ΔH / kcal.mol^{-1}**	**-TΔS kcal.mol^{-1}**	**ΔG / kcal.mol^{-1}**
Reactants	0	0	0
TS1	-1.5	0.0	-1.5
INT1	-2.3	-0.3	-2.6
TS2	8.0	2.0	10.0
INT2	0.5	4.5	5.0
TS3	15.1	3.7	18.8
INT3	4.2	1.8	6.0
Products	-13.9	2.2	-11.7

Force field parameters and files

FAD.lib

```
!!index array str
 "FAD"
!entry.FAD.unit.atoms table  str name  str type  int typex  int resx  int flags  int seq  int elmnt  dbl chg
 "N1" "nc" 0 1 131072 1 -1 -0.757470
 "C10" "cd" 0 1 131072 2 -1 0.399640
 "C2" "c" 0 1 131072 3 -1 0.993610
 "O2" "o" 0 1 131072 4 -1 -0.670980
 "N3" "n" 0 1 131072 5 -1 -0.770530
 "H136" "hn" 0 1 131072 6 -1 0.376140
 "C4" "c" 0 1 131072 7 -1 0.712580
 "O4" "o" 0 1 131072 8 -1 -0.606550
 "C4X" "cd" 0 1 131072 9 -1 0.219190
 "N5" "ne" 0 1 131072 10 -1 -0.561450
 "C5X" "ca" 0 1 131072 11 -1 0.500380
 "C6" "ca" 0 1 131072 12 -1 -0.452920
 "H138" "ha" 0 1 131072 13 -1 0.208200
 "C7" "ca" 0 1 131072 14 -1 0.189550
 "C7M" "c3" 0 1 131072 15 -1 -0.357400
 "H140" "hc" 0 1 131072 16 -1 0.096350
 "H142" "hc" 0 1 131072 17 -1 0.096350
 "H144" "hc" 0 1 131072 18 -1 0.096350
 "C8" "ca" 0 1 131072 19 -1 0.090190
 "C8M" "c3" 0 1 131072 20 -1 -0.206390
 "H146" "hc" 0 1 131072 21 -1 0.105850
 "H148" "hc" 0 1 131072 22 -1 0.105850
 "H150" "hc" 0 1 131072 23 -1 0.105850
 "C9" "ca" 0 1 131072 24 -1 -0.296590
 "H152" "ha" 0 1 131072 25 -1 0.213570
 "C9A" "ca" 0 1 131072 26 -1 -0.098630
 "N10" "nh" 0 1 131072 27 -1 -0.043610
 "C1'" "c3" 0 1 131072 28 -1 0.113590
 "H154" "h1" 0 1 131072 29 -1 0.033180
 "H156" "h1" 0 1 131072 30 -1 0.033180
 "C2'" "c3" 0 1 131072 31 -1 0.370170
 "O2'" "oh" 0 1 131072 32 -1 -0.727170
 "H160" "ho" 0 1 131072 33 -1 0.410650
 "H158" "h1" 0 1 131072 34 -1 3.000000E-05
 "C3'" "c3" 0 1 131072 35 -1 0.051170
 "O3'" "oh" 0 1 131072 36 -1 -0.687680
 "H164" "ho" 0 1 131072 37 -1 0.492840
 "H162" "h1" 0 1 131072 38 -1 0.049990
 "C4'" "c3" 0 1 131072 39 -1 0.363640
 "O4'" "oh" 0 1 131072 40 -1 -0.741340
 "H168" "ho" 0 1 131072 41 -1 0.391490
 "H166" "h1" 0 1 131072 42 -1 0.075060
 "C5'" "c3" 0 1 131072 43 -1 0.181830
 "H170" "h1" 0 1 131072 44 -1 0.009420
 "H172" "h1" 0 1 131072 45 -1 0.009420
 "O5'" "os" 0 1 131072 46 -1 -0.556330
 "P" "p5" 0 1 131072 47 -1 1.264660
 "O2P" "o" 0 1 131072 48 -1 -0.803550
 "O3P" "os" 0 1 131072 49 -1 -0.453190
 "PA" "p5" 0 1 131072 50 -1 1.183400
 "O1A" "o" 0 1 131072 51 -1 -0.780140
```

"O2A" "o" 0 1 131072 52 -1 -0.780140
"O5B" "os" 0 1 131072 53 -1 -0.536970
"C5B" "c3" 0 1 131072 54 -1 -0.014160
"C4B" "c3" 0 1 131072 55 -1 0.217620
"O4B" "os" 0 1 131072 56 -1 -0.539570
"C1B" "c3" 0 1 131072 57 -1 0.448600
"C2B" "c3" 0 1 131072 58 -1 0.274100
"C3B" "c3" 0 1 131072 59 -1 0.087940
"O3B" "oh" 0 1 131072 60 -1 -0.733380
"H120" "ho" 0 1 131072 61 -1 0.442610
"H118" "h1" 0 1 131072 62 -1 0.093580
"O2B" "oh" 0 1 131072 63 -1 -0.715350
"H124" "ho" 0 1 131072 64 -1 0.443370
"H122" "h1" 0 1 131072 65 -1 0.113290
"N9A" "na" 0 1 131072 66 -1 -0.281720
"C8A" "cc" 0 1 131072 67 -1 0.339830
"N7A" "nd" 0 1 131072 68 -1 -0.618880
"C5A" "ca" 0 1 131072 69 -1 -0.057760
"C6A" "ca" 0 1 131072 70 -1 0.766360
"N6A" "nh" 0 1 131072 71 -1 -0.930850
"H130" "hn" 0 1 131072 72 -1 0.392820
"H132" "hn" 0 1 131072 73 -1 0.392820
"N1A" "nb" 0 1 131072 74 -1 -0.846340
"C2A" "ca" 0 1 131072 75 -1 0.630870
"N3A" "nb" 0 1 131072 76 -1 -0.815180
"C4A" "ca" 0 1 131072 77 -1 0.554700
"H134" "h5" 0 1 131072 78 -1 0.028140
"H128" "h5" 0 1 131072 79 -1 0.162960
"H126" "h2" 0 1 131072 80 -1 0.045030
"H116" "h1" 0 1 131072 81 -1 0.028160
"H112" "h1" 0 1 131072 82 -1 0.119840
"H114" "h1" 0 1 131072 83 -1 0.119840
"O1P" "o" 0 1 131072 84 -1 -0.803550
!entry.FAD.unit.atomspertinfo table str pname str ptype int ptypex int pelmnt dbl pchg
"N1" "nc" 0 -1 0.0
"C10" "cd" 0 -1 0.0
"C2" "c" 0 -1 0.0
"O2" "o" 0 -1 0.0
"N3" "n" 0 -1 0.0
"H136" "hn" 0 -1 0.0
"C4" "c" 0 -1 0.0
"O4" "o" 0 -1 0.0
"C4X" "cd" 0 -1 0.0
"N5" "ne" 0 -1 0.0
"C5X" "ca" 0 -1 0.0
"C6" "ca" 0 -1 0.0
"H138" "ha" 0 -1 0.0
"C7" "ca" 0 -1 0.0
"C7M" "c3" 0 -1 0.0
"H140" "hc" 0 -1 0.0
"H142" "hc" 0 -1 0.0
"H144" "hc" 0 -1 0.0
"C8" "ca" 0 -1 0.0
"C8M" "c3" 0 -1 0.0
"H146" "hc" 0 -1 0.0
"H148" "hc" 0 -1 0.0
"H150" "hc" 0 -1 0.0
"C9" "ca" 0 -1 0.0
"H152" "ha" 0 -1 0.0
"C9A" "ca" 0 -1 0.0

"N10" "nh" 0 -1 0.0
"C1'" "c3" 0 -1 0.0
"H154" "h1" 0 -1 0.0
"H156" "h1" 0 -1 0.0
"C2'" "c3" 0 -1 0.0
"O2'" "oh" 0 -1 0.0
"H160" "ho" 0 -1 0.0
"H158" "h1" 0 -1 0.0
"C3'" "c3" 0 -1 0.0
"O3'" "oh" 0 -1 0.0
"H164" "ho" 0 -1 0.0
"H162" "h1" 0 -1 0.0
"C4'" "c3" 0 -1 0.0
"O4'" "oh" 0 -1 0.0
"H168" "ho" 0 -1 0.0
"H166" "h1" 0 -1 0.0
"C5'" "c3" 0 -1 0.0
"H170" "h1" 0 -1 0.0
"H172" "h1" 0 -1 0.0
"O5'" "os" 0 -1 0.0
"P" "p5" 0 -1 0.0
"O2P" "o" 0 -1 0.0
"O3P" "os" 0 -1 0.0
"PA" "p5" 0 -1 0.0
"O1A" "o" 0 -1 0.0
"O2A" "o" 0 -1 0.0
"O5B" "os" 0 -1 0.0
"C5B" "c3" 0 -1 0.0
"C4B" "c3" 0 -1 0.0
"O4B" "os" 0 -1 0.0
"C1B" "c3" 0 -1 0.0
"C2B" "c3" 0 -1 0.0
"C3B" "c3" 0 -1 0.0
"O3B" "oh" 0 -1 0.0
"H120" "ho" 0 -1 0.0
"H118" "h1" 0 -1 0.0
"O2B" "oh" 0 -1 0.0
"H124" "ho" 0 -1 0.0
"H122" "h1" 0 -1 0.0
"N9A" "na" 0 -1 0.0
"C8A" "cc" 0 -1 0.0
"N7A" "nd" 0 -1 0.0
"C5A" "ca" 0 -1 0.0
"C6A" "ca" 0 -1 0.0
"N6A" "nh" 0 -1 0.0
"H130" "hn" 0 -1 0.0
"H132" "hn" 0 -1 0.0
"N1A" "nb" 0 -1 0.0
"C2A" "ca" 0 -1 0.0
"N3A" "nb" 0 -1 0.0
"C4A" "ca" 0 -1 0.0
"H134" "h5" 0 -1 0.0
"H128" "h5" 0 -1 0.0
"H126" "h2" 0 -1 0.0
"H116" "h1" 0 -1 0.0
"H112" "h1" 0 -1 0.0
"H114" "h1" 0 -1 0.0
"O1P" "o" 0 -1 0.0
!entry.FAD.unit.boundbox array dbl
-1.000000

0.0
0.0
0.0
0.0
!entry.FAD.unit.childsequence single int
2
!entry.FAD.unit.connect array int
1
84
!entry.FAD.unit.connectivity table int atom1x int atom2x int flags
1 2 1
1 3 1
2 9 1
2 27 1
3 4 1
3 5 1
5 6 1
5 7 1
7 8 1
7 9 1
9 10 1
10 11 1
11 12 1
11 26 1
12 13 1
12 14 1
14 15 1
14 19 1
15 16 1
15 17 1
15 18 1
19 20 1
19 24 1
20 21 1
20 22 1
20 23 1
24 25 1
24 26 1
26 27 1
27 28 1
28 29 1
28 30 1
28 31 1
31 32 1
31 34 1
31 35 1
32 33 1
35 36 1
35 38 1
35 39 1
36 37 1
39 40 1
39 42 1
39 43 1
40 41 1
43 44 1
43 45 1
43 46 1
46 47 1
47 48 1

47 49 1
47 84 1
49 50 1
50 51 1
50 52 1
50 53 1
53 54 1
54 55 1
54 82 1
54 83 1
55 56 1
55 81 1
55 59 1
56 57 1
57 58 1
57 66 1
57 80 1
58 59 1
58 63 1
58 65 1
59 60 1
59 62 1
60 61 1
63 64 1
66 67 1
66 77 1
67 68 1
67 79 1
68 69 1
69 70 1
69 77 1
70 71 1
70 74 1
71 72 1
71 73 1
74 75 1
75 76 1
75 78 1
76 77 1
!entry.FAD.unit.hierarchy table str abovetype int abovex str belowtype int belowx
 "U" 0 "R" 1
 "R" 1 "A" 1
 "R" 1 "A" 2
 "R" 1 "A" 3
 "R" 1 "A" 4
 "R" 1 "A" 5
 "R" 1 "A" 6
 "R" 1 "A" 7
 "R" 1 "A" 8
 "R" 1 "A" 9
 "R" 1 "A" 10
 "R" 1 "A" 11
 "R" 1 "A" 12
 "R" 1 "A" 13
 "R" 1 "A" 14
 "R" 1 "A" 15
 "R" 1 "A" 16
 "R" 1 "A" 17
 "R" 1 "A" 18
 "R" 1 "A" 19

"R" 1 "A" 20
"R" 1 "A" 21
"R" 1 "A" 22
"R" 1 "A" 23
"R" 1 "A" 24
"R" 1 "A" 25
"R" 1 "A" 26
"R" 1 "A" 27
"R" 1 "A" 28
"R" 1 "A" 29
"R" 1 "A" 30
"R" 1 "A" 31
"R" 1 "A" 32
"R" 1 "A" 33
"R" 1 "A" 34
"R" 1 "A" 35
"R" 1 "A" 36
"R" 1 "A" 37
"R" 1 "A" 38
"R" 1 "A" 39
"R" 1 "A" 40
"R" 1 "A" 41
"R" 1 "A" 42
"R" 1 "A" 43
"R" 1 "A" 44
"R" 1 "A" 45
"R" 1 "A" 46
"R" 1 "A" 47
"R" 1 "A" 48
"R" 1 "A" 49
"R" 1 "A" 50
"R" 1 "A" 51
"R" 1 "A" 52
"R" 1 "A" 53
"R" 1 "A" 54
"R" 1 "A" 55
"R" 1 "A" 56
"R" 1 "A" 57
"R" 1 "A" 58
"R" 1 "A" 59
"R" 1 "A" 60
"R" 1 "A" 61
"R" 1 "A" 62
"R" 1 "A" 63
"R" 1 "A" 64
"R" 1 "A" 65
"R" 1 "A" 66
"R" 1 "A" 67
"R" 1 "A" 68
"R" 1 "A" 69
"R" 1 "A" 70
"R" 1 "A" 71
"R" 1 "A" 72
"R" 1 "A" 73
"R" 1 "A" 74
"R" 1 "A" 75
"R" 1 "A" 76
"R" 1 "A" 77
"R" 1 "A" 78
"R" 1 "A" 79

"R" 1 "A" 80
"R" 1 "A" 81
"R" 1 "A" 82
"R" 1 "A" 83
"R" 1 "A" 84
!entry.FAD.unit.name single str
"FAD"
!entry.FAD.unit.positions table dbl x dbl y dbl z
-19.021000 2.395000 2.304000
-17.892000 2.343000 1.578000
-19.001000 2.723000 3.616000
-20.079000 2.754000 4.238000
-17.874000 3.016000 4.278000
-17.933000 3.267000 5.316000
-16.682000 3.004000 3.682000
-15.648000 3.278000 4.315000
-16.618000 2.657000 2.249000
-15.452000 2.623000 1.583000
-15.421000 2.302000 0.276000
-14.199000 2.281000 -0.373000
-13.287000 2.521000 0.173000
-14.136000 1.955000 -1.715000
-12.807000 1.932000 -2.414000
-12.950000 1.656000 -3.459000
-12.155000 1.203000 -1.933000
-12.350000 2.920000 -2.359000
-15.377000 1.628000 -2.456000
-15.292000 1.275000 -3.914000
-16.291000 1.065000 -4.296000
-14.663000 0.394000 -4.039000
-14.860000 2.110000 -4.466000
-16.606000 1.648000 -1.809000
-17.517000 1.407000 -2.357000
-16.671000 1.976000 -0.464000
-17.903000 2.015000 0.215000
-19.170000 1.677000 -0.438000
-19.013000 0.771000 -1.024000
-19.907000 1.483000 0.342000
-19.739000 2.735000 -1.362000
-20.377000 3.746000 -0.584000
-21.153000 3.317000 -0.021000
-18.942000 3.186000 -1.954000
-20.762000 2.078000 -2.280000
-20.087000 1.441000 -3.362000
-19.539000 2.155000 -3.902000
-21.318000 1.361000 -1.675000
-21.746000 3.079000 -2.867000
-22.441000 3.741000 -1.813000
-22.964000 3.034000 -1.239000
-21.197000 3.814000 -3.455000
-22.739000 2.369000 -3.774000
-22.256000 1.504000 -4.229000
-23.594000 2.040000 -3.184000
-23.184000 3.249000 -4.794000
-23.217000 2.814000 -6.336000
-21.799000 2.667000 -6.814000
-23.898000 1.361000 -6.353000
-24.078000 0.561000 -7.730000
-22.902000 -0.353000 -7.880000
-24.407000 1.526000 -8.826000

```
 -25.375000 -0.349000 -7.467000
 -25.954000 -0.488000 -6.171000
 -27.140000 -1.447000 -6.209000
 -28.333000 -0.743000 -5.875000
 -29.236000 -0.693000 -6.978000
 -28.501000 -1.257000 -8.174000
 -27.352000 -2.033000 -7.590000
 -27.739000 -3.406000 -7.488000
 -26.985000 -3.947000 -6.997000
 -26.446000 -1.975000 -8.193000
 -29.340000 -2.130000 -8.922000
 -28.786000 -2.568000 -9.699000
 -28.177000 -0.469000 -8.854000
 -29.611000 0.714000 -7.231000
 -28.760000 1.697000 -7.553000
 -29.413000 2.871000 -7.713000
 -30.715000 2.645000 -7.493000
 -31.953000 3.455000 -7.499000
 -31.923000 4.777000 -7.784000
 -30.993000 5.255000 -8.010000
 -32.830000 5.345000 -7.783000
 -33.111000 2.829000 -7.213000
 -33.150000 1.518000 -6.932000
 -32.069000 0.726000 -6.908000
 -30.841000 1.220000 -7.174000
 -34.119000 1.070000 -6.710000
 -27.684000 1.568000 -7.670000
 -30.143000 -1.264000 -6.780000
 -26.923000 -2.246000 -5.500000
 -25.202000 -0.876000 -5.483000
 -26.294000 0.488000 -5.825000
 -24.136000 3.754000 -7.058000
!entry.FAD.unit.residueconnect table  int c1x  int c2x  int c3x  int c4x  int c5x  int c6x
 1 84 0 0 0 0
!entry.FAD.unit.residues table  str name  int seq  int childseq  int startatomx  str restype  int imagingx
 "FAD" 1 85 1 "?" 0
!entry.FAD.unit.residuesPdbSequenceNumber array int
 0
!entry.FAD.unit.solventcap array dbl
 -1.000000
 0.0
 0.0
 0.0
 0.0
!entry.FAD.unit.velocities table  dbl x  dbl y  dbl z
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
```

0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0

0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0
0.0 0.0 0.0

FAD.frcmod

BOND
cd-ne 381.80   1.414

ANGLE
cd-cd-ne   69.300   121.150
cd-nh-ca   65.500   123.660
c -cd-ne   65.257   121.360
c -cd-ne   68.400   120.890
cd-ne-ca   66.700   118.670

DIHE
cd-cd-ne-ca   1   0.800   180.000   2.000
c -cd-ne-ca   1   0.800   180.000   2.000

IMPROPER
cd-nc-cd-nh   1.1   180.0   2.0
n -nc-c -o   10.5   180.0   2.0
c -c -n -hn   1.1   180.0   2.0
cd-n -c -o   10.5   180.0   2.0
c -cd-cd-ne   1.1   180.0   2.0
ca-ca-ca-ne   1.1   180.0   2.0
ca-ca-ca-ha   1.1   180.0   2.0
ca-ca-ca-nh   1.1   180.0   2.0
c3-ca-nh-cd   1.1   180.0   2.0
c3-ca-na-cc   1.1   180.0   2.0
h5-na-cc-nd   1.1   180.0   2.0
ca-ca-ca-nd   1.1   180.0   2.0
ca-nb-ca-nh   1.1   180.0   2.0
ca-hn-nh-hn   1.1   180.0   2.0
h5-nb-ca-nb   1.1   180.0   2.0
ca-na-ca-nb   1.1   180.0   2.0

PHT.lib

!!index array str
 "PHT"
!entry.PHT.unit.atoms table str name str type int typex int resx int flags int seq int elmnt dbl chg
 "C1" "ca" 0 1 131075 1 6 0.233278
 "N1" "nb" 0 1 131075 2 7 -0.322793
 "N2" "nb" 0 1 131075 3 7 -0.322793
 "C8" "ca" 0 1 131075 4 6 0.233278
 "H6" "h4" 0 1 131075 5 1 0.032126
 "H1" "h4" 0 1 131075 6 1 0.032126
 "C2" "ca" 0 1 131075 7 6 0.138014

"C3" "ca" 0 1 131075 8 6 0.138014
"C4" "ca" 0 1 131075 9 6 -0.333391
"H2" "ha" 0 1 131075 10 1 0.176854
"C5" "ca" 0 1 131075 11 6 -0.038360
"H3" "ha" 0 1 131075 12 1 0.114270
"C6" "ca" 0 1 131075 13 6 -0.038360
"H4" "ha" 0 1 131075 14 1 0.114270
"C7" "ca" 0 1 131075 15 6 -0.333391
"H5" "ha" 0 1 131075 16 1 0.176854
!entry.PHT.unit.atomspertinfo table str pname str ptype int ptypex int pelmnt dbl pchg
"C1" "ca" 0 -1 0.0
"N1" "nb" 0 -1 0.0
"N2" "nb" 0 -1 0.0
"C8" "ca" 0 -1 0.0
"H6" "h4" 0 -1 0.0
"H1" "h4" 0 -1 0.0
"C2" "ca" 0 -1 0.0
"C3" "ca" 0 -1 0.0
"C4" "ca" 0 -1 0.0
"H2" "ha" 0 -1 0.0
"C5" "ca" 0 -1 0.0
"H3" "ha" 0 -1 0.0
"C6" "ca" 0 -1 0.0
"H4" "ha" 0 -1 0.0
"C7" "ca" 0 -1 0.0
"H5" "ha" 0 -1 0.0
!entry.PHT.unit.boundbox array dbl
-1.000000
0.0
0.0
0.0
0.0
!entry.PHT.unit.childsequence single int
2
!entry.PHT.unit.connect array int
1
15
!entry.PHT.unit.connectivity table int atom1x int atom2x int flags
1 2 1
1 6 1
1 7 1
2 3 1
3 4 1
4 5 1
4 8 1
7 8 1
7 15 1
8 9 1
9 10 1
9 11 1
11 12 1
11 13 1
13 14 1
13 15 1
15 16 1
!entry.PHT.unit.hierarchy table str abovetype int abovex str belowtype int belowx
"U" 0 "R" 1
"R" 1 "A" 1
"R" 1 "A" 2
"R" 1 "A" 3

```
 "R" 1 "A" 4
 "R" 1 "A" 5
 "R" 1 "A" 6
 "R" 1 "A" 7
 "R" 1 "A" 8
 "R" 1 "A" 9
 "R" 1 "A" 10
 "R" 1 "A" 11
 "R" 1 "A" 12
 "R" 1 "A" 13
 "R" 1 "A" 14
 "R" 1 "A" 15
 "R" 1 "A" 16
!entry.PHT.unit.name single str
 "PHT"
!entry.PHT.unit.positions table  dbl x  dbl y  dbl z
 -1.294000 1.329000 0.0
 -2.439000 0.684000 0.0
 -2.439000 -0.684000 0.0
 -1.294000 -1.329000 0.0
 -1.369000 -2.416000 0.0
 -1.369000 2.416000 0.0
 -0.012000 0.709000 0.0
 -0.012000 -0.709000 0.0
 1.216000 -1.411000 0.0
 1.211000 -2.498000 0.0
 2.403000 -0.708000 0.0
 3.349000 -1.240000 0.0
 2.403000 0.708000 0.0
 3.350000 1.240000 0.0
 1.216000 1.411000 0.0
 1.211000 2.498000 0.0
!entry.PHT.unit.residueconnect table  int c1x  int c2x  int c3x  int c4x  int c5x  int c6x
 1 15 0 0 0 0
!entry.PHT.unit.residues table  str name  int seq  int childseq  int startatomx  str restype  int imagingx
 "PHT" 1 17 1 "?" 0
!entry.PHT.unit.residuesPdbSequenceNumber array int
 0
!entry.PHT.unit.solventcap array dbl
 -1.000000
 0.0
 0.0
 0.0
 0.0
!entry.PHT.unit.velocities table  dbl x  dbl y  dbl z
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
```

0.0 0.0 0.0

PHT.frcmod

MASS

BOND

ANGLE

DIHE
ca-nb-nb-ca 1 3.000 180.000 -2.000
ca-nb-nb-ca 1 2.800 0.000 1.000

IMPROPER
ca-h4-ca-nb 1.1 180.0 2.0
ca-ca-ca-ca 1.1 180.0 2.0
ca-ca-ca-ha 1.1 180.0 2.0

FES.lib[1]

```
!!index array str
 "FES"
!entry.FES.unit.atoms table  str name  str type  int typex  int resx  int flags  int seq  int elmnt  dbl chg
 "FE1" "FE" 0 1 131075 1 26 0.0
 "FE2" "FE" 0 1 131075 2 26 0.0
 "S1" "S" 0 1 131075 3 16 0.0
 "S2" "S" 0 1 131075 4 16 0.0
!entry.FES.unit.atomspertinfo table  str pname  str ptype  int ptypex  int pelmnt  dbl pchg
 "FE1" "FE" 0 -1 0.600400
 "FE2" "FE" 0 -1 -0.516400
 "S1" "S" 0 -1 -0.516400
 "S2" "S" 0 -1 0.600400
!entry.FES.unit.boundbox array dbl
 -1.000000
 0.0
 0.0
 0.0
 0.0
!entry.FES.unit.childsequence single int
 2
!entry.FES.unit.connect array int
 0
 0
!entry.FES.unit.connectivity table  int atom1x  int atom2x  int flags
 1 4 1
 1 3 1
 2 4 1
 2 3 1
!entry.FES.unit.hierarchy table  str abovetype  int abovex  str belowtype  int belowx
 "U" 0 "R" 1
 "R" 1 "A" 1
 "R" 1 "A" 2
 "R" 1 "A" 3
 "R" 1 "A" 4
!entry.FES.unit.name single str
 "FES"
```

```
!entry.FES.unit.positions table  dbl x  dbl y  dbl z
 -46.072000 -17.691000 -47.369000
 -43.131000 -18.246000 -47.529000
 -44.468000 -16.853000 -48.658000
 -44.800000 -19.268000 -46.487000
!entry.FES.unit.residueconnect table  int c1x  int c2x  int c3x  int c4x  int c5x  int c6x
 0 0 0 0 0 0
!entry.FES.unit.residues table  str name  int seq  int childseq  int startatomx  str restype  int imagingx
 "FES" 1 6 1 "?" 0
!entry.FES.unit.residuesPdbSequenceNumber array int
 0
!entry.FES.unit.solventcap array dbl
 -1.000000
 0.0
 0.0
 0.0
 0.0
!entry.FES.unit.velocities table  dbl x  dbl y  dbl z
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
 0.0 0.0 0.0
```

FES.frcmod[1]

```
# param FE

MASS
FE 55.0
S 32.06
SH 32.06

BOND
FE-S  93.4 2.17
FE-SH 82.6 2.23

ANGLE
FE-SH-CT 65.5 108.44
S-FE-S   17.3 102.84
HS-SH-FE 65.5 108.44
SH-FE-SH 65.5 108.44
SH-FE-S  17.3 102.84
FE-S-FE  17.3 77.200

DIHE
FE-S-FE-S  1 0.0 0.0  0.0
S-FE-SH-CT 1 0.0 0.0  0.0
HS-SH-FE-SH 1 0.0 0.0  0.0
HS-SH-FE-S 1 0.0 0.0  0.0
SH-FE-SH-CT 1 0.0 0.0  0.0
SH-FE-SH-HS 1 0.0 0.0  0.0
SH-FE-S-FE 1 0.0 0.0  0.0
CT-SH-FE-SH 1 0.0 0.0  0.0
HS-SH-FE-S 1 0.0 0.0  0.0

NONBOND
FE  2.65 0.045
S   2.0000  0.2500                    #W. Cornell CH3SH and CH3SCH3 FEP's
```

SH 2.0000 0.2500 #W. Cornell CH3SH and CH3SCH3 FEP's

Table S2. Equilibrium values and force constants obtained for each bond and angle of the AOX model. Values were taken from our previous work[2]

Bond	**l_0 / Å**	**K_l / kcal mol^{-1} Å^{-2}**
MO-OT	1.711	545.30
MO-SO	2.200	232.30
MO-OR	1.965	206.90
MO-S1	2.466	93.02
MO-S2	2.509	80.08
Angle	**θ_0 / deg**	**K_θ / kcal.mol^{-1}.rad^{-2}**
S1-MO-S2	78.70	289.40
S1-MO-SO	89.08	53.63
S1-MO-OT	100.12	35.23
S1-MO-OR	149.28	34.82
S2-MO-SO	141.45	16.12
S2-MO-OT	108.85	20.20
S2-MO-OR	76.32	125.50
OR-MO-SO	99.47	63.83
SO-MO-OT	109.24	67.92
OT-MO-OR	104.65	53.11
HR-OR-MO	117.06	21.07
C2-S2-MO	109.03	122.00
C2-S1-MO	108.50	119.80

REFERENCES

1. Carvalho, A. T. P.; Teixeira, A. F. S.; Ramos, M. J., Parameters for Molecular Dynamics Simulations of Iron-sulfur Proteins. *J. Chem. Theory Comput.* **2013,** *34*, 1540-1548.
2. Ferreira, P.; Cerqueira, N. M. F. S. A.; Brás, N. F.; Fernandes, P. A.; Ramos, M. J., Parametrization of Molybdenum Cofactors for the AMBER Force Field. *J. Chem. Theory Comput.* **2018**, 14, 2538-2548.