Formation and structure of the ferryl [Fe=O] intermediate in the non-haem iron halogenase SyrB2: classical and QM/MM modelling agree

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1 Supplementary Computational Details

1.1 Protein structure preparation

The complete protein structure was prepared by A. Jarnuczak[1] using the program Modeller[2,3], which creates potential structures for missing loops by constructing an initial loop model, randomly displacing it to generate a number of structures, then carrying out an optimisation of each of these structures at the MM level. 100 such optimised structures were generated, and the most favourable selected according to its DOPE-HR[4] score and its RMSD from 2FCT. Next, hydrogen atoms were added and Asn/Gln/His residues checked for flips using Reduce[5,6] via the MolProbity server[7]. The residues Gln11, Asn95, Gln129, and Gln245 were flipped from their conformation in the crystal structure. Finally, the protonation states of all titratable residues were calculated using PropKa[8,9]. Protonation states of histidine residues are shown in Table S1; all other residues were left in their standard protonation states.

Table S1: Protonation states of histidine residues

Residue	Protonation State
His69	N^{ϵ}
His78	N^{ϵ}
His116	N^{δ}
His235	N^{δ}
His240	N^{ϵ}
His261	N^{δ}
His268	N^{ϵ}
His300	N^{ϵ}

1.2 QM calculations on model compounds, atomic charges for non-standard residues

Partial charges for non-standard residues were obtained at the DFT level by fitting to the molecular electrostatic potential according to the Merz–Singh–Kollmann scheme[10]; structures were fully optimised at the same level of theory unless indicated otherwise. For compatibility with the Amber ff03 force field[11], charges were determined in a polarisable continuum solvent. For the singlet Fe(II) complexes, charges were taken from TPSS-D/def2-TZVP/COSMO($\epsilon = 80$) calculations run with TURBOMOLE[12–14]; charges for the Fe(IV) complexes in the quintet state from B3LYP/def2-TZVP+/PCM($\epsilon = 3.9$) calculations in Gaussian[15]; and charges for the substrates from B97-D/def2-TZVP/PCM(water) calculations with Gaussian. For **ABA** and **NVA**, a distance constraint was used to ensure that the pantetheine was in the same extended conformation as for **THR**. It was verified that the slightly different choices of exchange–correlation functional and value of ϵ had negligible effect on the fitted charges.

For residues cut by a QM–MM boundary, the charges were corrected such as to ensure that the total charge of the atoms that would become QM atoms summed up to an integer value; by construction, the total charge of the remaining MM atoms is then also an integer value. The corrections required to ensure charge integrity were small; the imbalance was distributed over all atoms of the residue, leading to very small ($< 0.01e$) changes of the original atomic charges.

1.3 MD simulations

1.3.1 Simulation parameters

MD simulations were run under periodic boundary conditions in a rhombic-dodecahedral box with an image distance of $d \approx 7.88$ nm, chosen to give a minimal simulation volume while maintaining a minimum distance of 1 nm to the solute. The simulation systems were neutralised by the addition of counterions (14 Na^+ for systems containing substrate). Electrostatic interactions were treated with the particle–mesh Ewald (PME) method ($r_C = 1.0$ nm, fourth-order interpolation, tolerance 10^{-5}, grid spacing 0.12 nm). Van der Waals interactions were cut off at $r_{vdW} = 1.0$ nm. Energies and pressures were corrected for long-range dispersion effects.

The simulation stages and parameters are summarised in Table S2. A time step of 2 fs was used throughout. Overall equilibration was judged by convergence of the backbone RMSD according to a series of statistical tests [16,17]. The production runs were continued to about 20 ns or until conformational equilibration for the active site residues was established. Each trajectory spanned up to 40 ns in total.

Table S2: Equilibration procedure for MD simulations with Fe(II) active-site complex

Parameter	**Energy Minimisation**	**Temperature Equilibration**	**Equilibration Stage 1**	**Equilibration Stage 2**	**Equilibration Stage 3**	**Final Equilibration/Production**
Ensemble	–	NVT	NPT	NPT	NPT	NVT
Integrator	Steepest descent			Leap-frog		
Duration (ps)	–	100	400	100	400	until equilibration
Constraints	all bonds to hydrogen			all bonds		
Position restraints on non-H (kJ mol^{-1} nm^{-1})	1000	1000	1000	100	0	0
τ_T (ps)	–	0.1	0.1	0.1	0.1	2
τ_p (ps)	–	–	1	1	1	–

1.3.2 Force-field parameters

For the residues containing atoms with non-standard charges, we list here the [atoms] blocks in GROMACS topology format. All atom types (and therefore van der Waals parameters) were standard atom types from either Amber ff03 or GAFF.

A-H_2O

```
; id    at type         res nr  residu name     at name  cg nr  charge
1       FE              1       IRO              IRO       1     0.784449

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.706289

; AKG = 2OG
;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
      1    c  1209   AKG     C    1     0.771763     12.01000 ; qtot -0.083
      2    o  1209   AKG     O    2    -0.683526     16.00000 ; qtot -0.184
      3    o  1209   AKG    O1    3     -0.70403     16.00000 ; qtot -0.252
      4    c  1209   AKG    C1    4     0.328831     12.01000 ; qtot -0.319
      5    o  1209   AKG    O2    5    -0.446477     16.00000 ; qtot -0.375
      6   c3 1209   AKG    C2    6    -0.050528     12.01000 ; qtot -0.469
      7   c3 1209   AKG    C3    7    -0.055613     12.01000 ; qtot -0.551
      8    c  1209   AKG    C4    8     0.856546     12.01000 ; qtot -0.655
      9    o  1209   AKG    O3    9    -0.869579     16.00000 ; qtot -0.752
      10   o  1209   AKG    O4   10    -0.829547     16.00000 ; qtot -0.649
      11  hc 1209   AKG     H   11    -0.007143     1.00800 ; qtot -0.760
      12  hc 1209   AKG    H1   12    -0.002513     1.00800 ; qtot -0.862
      13  hc 1209   AKG    H2   13     0.030762     1.00800 ; qtot -0.931
      14  hc 1209   AKG    H3   14     0.069932     1.00800 ; qtot -1.000

; VOD = coordinated water
; id  at type     res nr  res name  at name  cg nr  charge    mass
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```
  1   OW          1       VOD       OW       1     -0.799831    16.00000
  2   HW          1       VOD       HW1      1      0.378185    1.00800
  3   HW          1       VOD       HW2      1      0.42859    1.00800

; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.340433      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.340626      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.349008      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826  -0.085004      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.175775      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.117347      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.354558      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.156461      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.314181      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.307132      14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.350125      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665  -0.201184      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.199216      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667   0.134339      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.428564      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669   0.186526      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
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A

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; id    at type         res nr  residu name     at name  cg nr  charge
1       FE              1       IRO              IRO       1     0.69874

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.64616

; AKG = 2OG
;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
      1    c  1209   AKG     C    1     0.700254     12.01000 ; qtot -0.111771
      2    o  1209   AKG     O    2    -0.663076     16.00000 ; qtot -0.133799
      3    o  1209   AKG    O1    3    -0.634775     16.00000 ; qtot -0.100448
      4    c  1209   AKG    C1    4     0.376036     12.01000 ; qtot -0.094001
      5    o  1209   AKG    O2    5    -0.451993     16.00000 ; qtot -0.071065
      6   c3  1209   AKG    C2    6    -0.084148     12.01000 ; qtot -0.105823
      7   c3  1209   AKG    C3    7    -0.053219     12.01000 ; qtot -0.080459
      8    c  1209   AKG    C4    8     0.860231     12.01000 ; qtot -0.099629
      9    o  1209   AKG    O3    9    -0.870025     16.00000 ; qtot -0.130951
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      10   o  1209   AKG    O4   10    -0.828007     16.00000 ; qtot -0.081349
      11   hc 1209   AKG     H   11    -0.006638      1.00800 ; qtot -0.06559
      12   hc 1209   AKG    H1   12    -0.004849      1.00800 ; qtot -0.060775
      13   hc 1209   AKG    H2   13      0.04114      1.00800 ; qtot -0.129752
      14   hc 1209   AKG    H3   14     0.076491      1.00800 ; qtot -0.115577

; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.357995      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.349642      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.353283      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826   -0.11849      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.191526      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.042566      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.392046      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.181364      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.303336      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.329661      14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.347334      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665  -0.031523      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.143737      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667   -0.11523      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.294443      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669    0.15065      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
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Substrate-D1

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; id    at type         res nr  residu name     at name  cg nr  charge
1      FE              1       FER             IRO       1     0.684248
2       O              1       FER             FEO       1    -0.445369

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.531291

;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
     1    o     1   SUC     O    1    -0.636592     16.00000 ; qtot -0.246
     2    c     1   SUC     C    2     0.598324     12.01000 ; qtot 0.110
     3    o     1   SUC    O1    3    -0.345319     16.00000 ; qtot -0.136
     4   c3     1   SUC    C1    4    -0.127374     12.01000 ; qtot -0.077
     5   c3     1   SUC    C2    5    -0.056894     12.01000 ; qtot -0.017
     6    c     1   SUC    C3    6     0.862099     12.01000 ; qtot 0.339
     7    o     1   SUC    O2    7    -0.882571     16.00000 ; qtot 0.093
     8    o     1   SUC    O3    8    -0.812840     16.00000 ; qtot -0.154
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     9   hc     1   SUC     H    9    -0.007683      1.00800 ; qtot -0.115
    10   hc     1   SUC    H1   10     0.017870      1.00800 ; qtot -0.077
    11   hc     1   SUC    H2   11     0.030399      1.00800 ; qtot -0.038
    12   hc     1   SUC    H3   12     0.029686      1.00800 ; qtot -0.000

; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.358772      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.372509      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.361030      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826   0.020964      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.157317      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.115046      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.332913      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.178074      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.362487      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.401630     14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.354126      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665   0.006603      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.146663      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667  -0.098290      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.366903      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669   0.230197      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
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Substrate-D2

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2       O              1       FER             FEO       1    -0.445369

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.531291

;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
     1    o     1   SUC     O    1    -0.636592     16.00000 ; qtot -0.246
     2    c     1   SUC     C    2     0.598324     12.01000 ; qtot 0.110
     3    o     1   SUC    O1    3    -0.345319     16.00000 ; qtot -0.136
     4   c3     1   SUC    C1    4    -0.127374     12.01000 ; qtot -0.077
     5   c3     1   SUC    C2    5    -0.056894     12.01000 ; qtot -0.017
     6    c     1   SUC    C3    6     0.862099     12.01000 ; qtot 0.339
     7    o     1   SUC    O2    7    -0.882571     16.00000 ; qtot 0.093
     8    o     1   SUC    O3    8    -0.812840     16.00000 ; qtot -0.154
     9   hc     1   SUC     H    9    -0.007683      1.00800 ; qtot -0.115
```

```
    10   hc     1   SUC    H1   10     0.017870      1.00800 ; qtot -0.077
    11   hc     1   SUC    H2   11     0.030399      1.00800 ; qtot -0.038
    12   hc     1   SUC    H3   12     0.029686      1.00800 ; qtot -0.000

; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.358772      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.372509      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.361030      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826   0.020964      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.157317      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.115046      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.332913      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.178074      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.362487      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.401630     14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.354126      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665   0.006603      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.146663      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667  -0.098290      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.366903      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669   0.230197      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
```

Substrate-D3

```
; id    at type         res nr  residu name     at name  cg nr  charge
1      FE              1       FER              IRO       1     1.000264
2       O              1       FER              FEO       1     -0.50310004

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.575058

;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
     1    o     1   SUC     O    1    -0.647829     16.00000 ; qtot -0.246
     2    c     1   SUC     C    2     0.744445     12.01000 ; qtot 0.110
     3    o     1   SUC    O1    3    -0.652057     16.00000 ; qtot -0.136
     4   c3     1   SUC    C1    4    -0.113137     12.01000 ; qtot -0.077
     5   c3     1   SUC    C2    5    -0.037569     12.01000 ; qtot -0.017
     6    c     1   SUC    C3    6     0.855608     12.01000 ; qtot 0.339
     7    o     1   SUC    O2    7    -0.837781     16.00000 ; qtot 0.093
     8    o     1   SUC    O3    8    -0.878422     16.00000 ; qtot -0.154
     9   hc     1   SUC     H    9     0.000406     1.00800 ; qtot -0.115
    10   hc     1   SUC    H1   10    -0.008403     1.00800 ; qtot -0.077
```

```
    11   hc     1   SUC    H2   11     0.032984     1.00800 ; qtot -0.038
    12   hc     1   SUC    H3   12     0.053375     1.00800 ; qtot -0.000

; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.442784      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.444119      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.368646      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826   0.053689      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.155198      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.112765      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.449950      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.194227      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.308498      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.302183     14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.343071      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665  -0.102242      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.171566      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667  -0.016128      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.351546      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669   0.173122      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
```

Substrate-D4

```
; id    at type         res nr  residu name     at name  cg nr  charge
1      FE              1       FER              IRO       1     1.034717
2       O              1       FER              FEO       1    -0.574945

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.550890

;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
     1    o     1   SUC     O    1    -0.669822     16.00000 ; qtot -0.246
     2    c     1   SUC     C    2     0.764680     12.01000 ; qtot 0.110
     3    o     1   SUC    O1    3    -0.659302     16.00000 ; qtot -0.136
     4   c3     1   SUC    C1    4    -0.125507     12.01000 ; qtot -0.077
     5   c3     1   SUC    C2    5    -0.002997     12.01000 ; qtot -0.017
     6    c     1   SUC    C3    6     0.854765     12.01000 ; qtot 0.339
     7    o     1   SUC    O2    7    -0.874124     16.00000 ; qtot 0.093
     8    o     1   SUC    O3    8    -0.846563     16.00000 ; qtot -0.154
     9   hc     1   SUC     H    9    -0.019375     1.00800 ; qtot -0.115
    10   hc     1   SUC    H1   10    -0.013920     1.00800 ; qtot -0.077
    11   hc     1   SUC    H2   11     0.059759     1.00800 ; qtot -0.038
```

```
    12   hc     1   SUC    H3   12     0.030117     1.00800 ; qtot -0.000

; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.392287      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.365249      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.353521      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826  -0.036499      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.183276      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.078236      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.440544      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.196387      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.369485      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.348861     14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.351716      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665  -0.066350      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.172700      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667   0.008000      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.446081      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669   0.213484      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
```

Substrate-D4

```
; id    at type         res nr  residu name     at name  cg nr  charge
1      FE              1       FER              IRO       1     0.793927
2       O              1       FER              FEO       1    -0.578432

; id    at type         res nr  residu name     at name  cg nr  charge
1       Cl              1       CLO              CLO       1     -0.500775

;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot
     1    o     1   LIG     O    1    -0.709408     16.00000 ; qtot -0.246
     2    c     1   LIG     C    2     0.787417     12.01000 ; qtot 0.110
     3    o     1   LIG    O1    3    -0.515985     16.00000 ; qtot -0.136
     4   c3     1   LIG    C1    4    -0.309409     12.01000 ; qtot -0.077
     5   c3     1   LIG    C2    5     0.156446     12.01000 ; qtot -0.017
     6    c     1   LIG    C3    6     0.748318     12.01000 ; qtot 0.339
     7    o     1   LIG    O2    7    -0.852347     16.00000 ; qtot 0.093
     8    o     1   LIG    O3    8    -0.807292     16.00000 ; qtot -0.154
     9   hc     1   LIG     H    9    -0.031508       1.00800 ; qtot -0.115
    10   hc     1   LIG    H1   10    -0.067819       1.00800 ; qtot -0.077
    11   hc     1   LIG    H2   11     0.063750       1.00800 ; qtot -0.038
    12   hc     1   LIG    H3   12     0.059706       1.00800 ; qtot -0.000
```

```
; residue 116 HIQ rtp HIQ  q  0.0
  1816          N    116    HIQ      N   1816  -0.506799      14.01   ; qtot -4.507
  1817          H    116    HIQ      H   1817   0.351021      1.008   ; qtot -4.156
  1818         CT    116    HIQ     CA   1818   0.119066      12.01   ; qtot -4.037
  1819         H1    116    HIQ     HA   1819   0.137761      1.008   ; qtot -3.899
  1820         CT    116    HIQ     CB   1820  -0.122638      12.01   ; qtot -4.022
  1821         HC    116    HIQ    HB1   1821   0.086329      1.008   ; qtot -3.935
  1822         HC    116    HIQ    HB2   1822   0.086329      1.008   ; qtot -3.849
  1823         CC    116    HIQ     CG   1823   0.339699      12.01   ; qtot -3.85
  1824         NA    116    HIQ    ND1   1824  -0.312577      14.01   ; qtot -4.056
  1825          H    116    HIQ    HD1   1825   0.338124      1.008   ; qtot -3.738
  1826         CR    116    HIQ    CE1   1826  -0.090369      12.01   ; qtot -3.591
  1827         H5    116    HIQ    HE1   1827   0.179567      1.008   ; qtot -3.469
  1828         NB    116    HIQ    NE2   1828  -0.116037      14.01   ; qtot -4.07
  1829         CV    116    HIQ    CD2   1829  -0.266276      12.01   ; qtot -4.026
  1830         H4    116    HIQ    HD2   1830   0.193220      1.008   ; qtot -3.916
  1831          C    116    HIQ      C   1831   0.515947      12.01   ; qtot -3.4
  1832          O    116    HIQ      O   1832  -0.599831         16   ; qtot -4

; residue 235 HIX rtp HIX  q  0.0
  3655          N    235    HIX      N   3655  -0.506799      14.01   ; qtot -10.51
  3656          H    235    HIX      H   3656   0.351021      1.008   ; qtot -10.16
  3657         CT    235    HIX     CA   3657   0.119066      12.01   ; qtot -10.04
  3658         H1    235    HIX     HA   3658   0.137761      1.008   ; qtot -9.899
  3659         CT    235    HIX     CB   3659  -0.122638      12.01   ; qtot -10.02
  3660         HC    235    HIX    HB1   3660   0.086329      1.008   ; qtot -9.935
  3661         HC    235    HIX    HB2   3661   0.086329      1.008   ; qtot -9.849
  3662         CC    235    HIX     CG   3662   0.358329      12.01   ; qtot -9.85
  3663         NA    235    HIX    ND1   3663  -0.324563      14.01   ; qtot -10.06
  3664          H    235    HIX    HD1   3664   0.343920      1.008   ; qtot -9.738
  3665         CR    235    HIX    CE1   3665  -0.145887      12.01   ; qtot -9.591
  3666         H5    235    HIX    HE1   3666   0.192682      1.008   ; qtot -9.469
  3667         NB    235    HIX    NE2   3667   0.139527      14.01   ; qtot -10.07
  3668         CV    235    HIX    CD2   3668  -0.474968      12.01   ; qtot -10.03
  3669         H4    235    HIX    HD2   3669   0.274650      1.008   ; qtot -9.916
  3670          C    235    HIX      C   3670   0.515947      12.01   ; qtot -9.4
  3671          O    235    HIX      O   3671  -0.599831         16   ; qtot -10
```

THR

```
;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot   bond_type
     1   c3     1   LIG     C    1     0.028003     12.01000 ; qtot 0.028
     2   os     1   LIG     O    2    -0.185718     16.00000 ; qtot -0.158
     3   c3     1   LIG     C    3    -0.317708     12.01000 ; qtot -0.475
     4   c3     1   LIG     C    4     0.411177     12.01000 ; qtot -0.064
     5   c3     1   LIG     C    5    -0.404402     12.01000 ; qtot -0.469
     6   c3     1   LIG     C    6    -0.289491     12.01000 ; qtot -0.758
     7   c3     1   LIG     C    7    -0.195934     12.01000 ; qtot -0.954
     8   oh     1   LIG     O    8    -0.580049     16.00000 ; qtot -1.534
     9   ho     1   LIG     H    9     0.396549      1.00800 ; qtot -1.138
    10    c     1   LIG     C   10     0.704642     12.01000 ; qtot -0.433
    11    o     1   LIG     O   11    -0.621570     16.00000 ; qtot -1.055
    12    n     1   LIG     N   12    -0.498288     14.01000 ; qtot -1.553
    13   hn     1   LIG     H   13     0.323545      1.00800 ; qtot -1.229
    14   c3     1   LIG     C   14     0.332134     12.01000 ; qtot -0.897
    15   c3     1   LIG     C   15    -0.540514     12.01000 ; qtot -1.438
    16    c     1   LIG     C   16     0.726795     12.01000 ; qtot -0.711
    17    n     1   LIG     N   17    -0.718367     14.01000 ; qtot -1.429
    18   hn     1   LIG     H   18     0.459514      1.00800 ; qtot -0.970
    19    o     1   LIG     O   19    -0.546523     16.00000 ; qtot -1.516
    20   c3     1   LIG     C   20     0.064949     12.01000 ; qtot -1.451
```

```
    21   c3     1   LIG     C   21     0.116089     12.01000 ; qtot -1.335
    22   ss     1   LIG     S   22    -0.220764     32.06000 ; qtot -1.556
    23    c     1   LIG     C   23     0.283013     12.01000 ; qtot -1.273
    24    o     1   LIG     O   24    -0.286551     16.00000 ; qtot -1.559
    25   c3     1   LIG     C   25    -0.199664     12.01000 ; qtot -1.759
    26   n4     1   LIG     N   26    -0.379375     14.01000 ; qtot -2.139
    27   hn     1   LIG     H   27     0.326271      1.00800 ; qtot -1.812
    28   hn     1   LIG     H   28     0.348370      1.00800 ; qtot -1.464
    29   hn     1   LIG     H   29     0.353612      1.00800 ; qtot -1.110
    30   c3     1   LIG     C   30     0.256880     12.01000 ; qtot -0.853
    31   c3     1   LIG     C   31    -0.545099     12.01000 ; qtot -1.398
    32   oh     1   LIG     O   32    -0.623469     16.00000 ; qtot -2.022
    33   ho     1   LIG     H   33     0.458110      1.00800 ; qtot -1.564
    34   h1     1   LIG     H   34     0.023394      1.00800 ; qtot -1.540
    35   h1     1   LIG     H   35     0.035041      1.00800 ; qtot -1.505
    36   h1     1   LIG     H   36     0.160190      1.00800 ; qtot -1.345
    37   h1     1   LIG     H   37     0.121151      1.00800 ; qtot -1.224
    38   h1     1   LIG     H   38     0.117080      1.00800 ; qtot -1.107
    39   hc     1   LIG     H   39     0.123884      1.00800 ; qtot -0.983
    40   hc     1   LIG     H   40     0.092629      1.00800 ; qtot -0.890
    41   hc     1   LIG     H   41     0.089118      1.00800 ; qtot -0.801
    42   hc     1   LIG     H   42     0.057960      1.00800 ; qtot -0.743
    43   hc     1   LIG     H   43     0.041257      1.00800 ; qtot -0.702
    44   hc     1   LIG     H   44     0.077798      1.00800 ; qtot -0.624
    45   h1     1   LIG     H   45     0.100299      1.00800 ; qtot -0.524
    46   h1     1   LIG     H   46    -0.020691      1.00800 ; qtot -0.545
    47   h1     1   LIG     H   47     0.026070      1.00800 ; qtot -0.519
    48   hc     1   LIG     H   48     0.151272      1.00800 ; qtot -0.367
    49   hc     1   LIG     H   49     0.164587      1.00800 ; qtot -0.203
    50   h1     1   LIG     H   50     0.093264      1.00800 ; qtot -0.110
    51   h1     1   LIG     H   51     0.079883      1.00800 ; qtot -0.030
    52   h1     1   LIG     H   52     0.100083      1.00800 ; qtot 0.070
    53   h1     1   LIG     H   53     0.078269      1.00800 ; qtot 0.149
    54   hx     1   LIG     H   54     0.220092      1.00800 ; qtot 0.369
    55   h1     1   LIG     H   55     0.121156      1.00800 ; qtot 0.490
    56   hc     1   LIG     H   56     0.168836      1.00800 ; qtot 0.659
    57   hc     1   LIG     H   57     0.191533      1.00800 ; qtot 0.850
    58   hc     1   LIG     H   58     0.149672      1.00800 ; qtot 1.000
```

ABA

```
;    nr  type  resi  res  atom  cgnr     charge      mass       ; qtot   bond_type
     1    c     1   LIG     C    1     0.563045     12.01000 ; qtot 0.563
     2    n     1   LIG     N    2    -0.541920     14.01000 ; qtot 0.021
     3   hn     1   LIG     H    3     0.367593      1.00800 ; qtot 0.389
     4    o     1   LIG     O    4    -0.527199     16.00000 ; qtot -0.138
     5   c3     1   LIG    C1    5    -0.219109     12.01000 ; qtot -0.358
     6   c3     1   LIG    C2    6     0.100436     12.01000 ; qtot -0.257
     7    n     1   LIG    N1    7    -0.315634     14.01000 ; qtot -0.573
     8   hn     1   LIG    H1    8     0.225383      1.00800 ; qtot -0.347
     9    c     1   LIG    C3    9     0.681581     12.01000 ; qtot 0.334
    10    o     1   LIG    O1   10    -0.667566     16.00000 ; qtot -0.333
    11   c3     1   LIG    C4   11    -0.161290     12.01000 ; qtot -0.495
    12   c3     1   LIG    C5   12     0.424332     12.01000 ; qtot -0.070
    13   c3     1   LIG    C6   13    -0.309853     12.01000 ; qtot -0.380
    14   c3     1   LIG    C7   14    -0.320006     12.01000 ; qtot -0.700
    15   c3     1   LIG    C8   15     0.031176     12.01000 ; qtot -0.669
    16   os     1   LIG    O2   16    -0.271069     16.00000 ; qtot -0.940
    17   c3     1   LIG    C9   17    -0.194687     12.01000 ; qtot -1.135
    18   oh     1   LIG    O3   18    -0.610788     16.00000 ; qtot -1.746
    19   ho     1   LIG    H2   19     0.412076      1.00800 ; qtot -1.333
    20   c3     1   LIG   C10   20     0.017000     12.01000 ; qtot -1.316
```

```
    21   c3     1   LIG   C11   21     0.013460     12.01000 ; qtot -1.303
    22   ss     1   LIG     S   22    -0.141459     32.06000 ; qtot -1.444
    23    c     1   LIG   C12   23     0.236091     12.01000 ; qtot -1.208
    24    o     1   LIG    O4   24    -0.363981     16.00000 ; qtot -1.572
    25   c3     1   LIG   C13   25     0.122405     12.01000 ; qtot -1.450
    26   n4     1   LIG    N2   26    -0.439849     14.01000 ; qtot -1.890
    27   hn     1   LIG    H3   27     0.356848      1.00800 ; qtot -1.533
    28   hn     1   LIG    H4   28     0.381851      1.00800 ; qtot -1.151
    29   hn     1   LIG    H5   29     0.334026      1.00800 ; qtot -0.817
    30   c3     1   LIG   C14   30     0.179588     12.01000 ; qtot -0.638
    31   c3     1   LIG   C15   31    -0.303564     12.01000 ; qtot -0.941
    32   hc     1   LIG    H6   32     0.089303      1.00800 ; qtot -0.852
    33   hc     1   LIG    H7   33     0.084175      1.00800 ; qtot -0.768
    34   h1     1   LIG    H8   34     0.063493      1.00800 ; qtot -0.704
    35   h1     1   LIG    H9   35     0.031689      1.00800 ; qtot -0.672
    36   h1     1   LIG   H10   36     0.086124      1.00800 ; qtot -0.586
    37   hc     1   LIG   H11   37     0.055773      1.00800 ; qtot -0.531
    38   hc     1   LIG   H12   38     0.075055      1.00800 ; qtot -0.455
    39   hc     1   LIG   H13   39     0.058690      1.00800 ; qtot -0.397
    40   hc     1   LIG   H14   40     0.061600      1.00800 ; qtot -0.335
    41   hc     1   LIG   H15   41     0.073139      1.00800 ; qtot -0.262
    42   hc     1   LIG   H16   42     0.064882      1.00800 ; qtot -0.197
    43   h1     1   LIG   H17   43     0.046192      1.00800 ; qtot -0.151
    44   h1     1   LIG   H18   44     0.045894      1.00800 ; qtot -0.105
    45   h1     1   LIG   H19   45     0.123584      1.00800 ; qtot 0.019
    46   h1     1   LIG   H20   46     0.093463      1.00800 ; qtot 0.112
    47   h1     1   LIG   H21   47     0.091840      1.00800 ; qtot 0.204
    48   h1     1   LIG   H22   48     0.092664      1.00800 ; qtot 0.296
    49   h1     1   LIG   H23   49     0.092444      1.00800 ; qtot 0.389
    50   h1     1   LIG   H24   50     0.130940      1.00800 ; qtot 0.520
    51   h1     1   LIG   H25   51     0.077285      1.00800 ; qtot 0.597
    52   hx     1   LIG   H26   52     0.112270      1.00800 ; qtot 0.709
    53   hc     1   LIG   H27   53     0.022432      1.00800 ; qtot 0.732
    54   hc     1   LIG   H28   54    -0.004045      1.00800 ; qtot 0.728
    55   hc     1   LIG   H29   55     0.079242      1.00800 ; qtot 0.807
    56   hc     1   LIG   H30   56     0.084802      1.00800 ; qtot 0.892
    57   hc     1   LIG   H31   57     0.108150      1.00800 ; qtot 1.000
```

NVA

```
;   nr  type  resi  res  atom  cgnr     charge      mass       ; qtot   bond_type
     1    c     1   LIG     C    1     0.594353     12.01000 ; qtot 0.594
     2    n     1   LIG     N    2    -0.642777     14.01000 ; qtot -0.048
     3   hn     1   LIG     H    3     0.377152      1.00800 ; qtot 0.329
     4    o     1   LIG     O    4    -0.533358     16.00000 ; qtot -0.205
     5   c3     1   LIG    C1    5    -0.221485     12.01000 ; qtot -0.426
     6   c3     1   LIG    C2    6     0.076077     12.01000 ; qtot -0.350
     7    n     1   LIG    N1    7    -0.353068     14.01000 ; qtot -0.703
     8   hn     1   LIG    H1    8     0.245176      1.00800 ; qtot -0.458
     9    c     1   LIG    C3    9     0.689601     12.01000 ; qtot 0.232
    10    o     1   LIG    O1   10    -0.664014     16.00000 ; qtot -0.432
    11   c3     1   LIG    C4   11    -0.160891     12.01000 ; qtot -0.593
    12   c3     1   LIG    C5   12     0.363843     12.01000 ; qtot -0.229
    13   c3     1   LIG    C6   13    -0.351007     12.01000 ; qtot -0.580
    14   c3     1   LIG    C7   14    -0.345587     12.01000 ; qtot -0.926
    15   c3     1   LIG    C8   15     0.143370     12.01000 ; qtot -0.783
    16   os     1   LIG    O2   16    -0.289542     16.00000 ; qtot -1.072
    17   c3     1   LIG    C9   17    -0.208274     12.01000 ; qtot -1.280
    18   oh     1   LIG    O3   18    -0.603889     16.00000 ; qtot -1.884
    19   ho     1   LIG    H2   19     0.406190      1.00800 ; qtot -1.478
    20   c3     1   LIG   C10   20     0.229184     12.01000 ; qtot -1.249
    21   c3     1   LIG   C11   21    -0.019238     12.01000 ; qtot -1.268
```

```
22   ss     1   LIG     S   22    -0.149641     32.06000 ; qtot -1.418
23    c     1   LIG   C12   23     0.314334     12.01000 ; qtot -1.103
24    o     1   LIG   O4    24    -0.387219     16.00000 ; qtot -1.491
25   c3     1   LIG   C13   25     0.021667     12.01000 ; qtot -1.469
26   n4     1   LIG    N2   26    -0.534559     14.01000 ; qtot -2.004
27   hn     1   LIG    H3   27     0.375569      1.00800 ; qtot -1.628
28   hn     1   LIG    H4   28     0.412932      1.00800 ; qtot -1.215
29   hn     1   LIG    H5   29     0.373633      1.00800 ; qtot -0.841
30   c3     1   LIG   C14   30    -0.032791     12.01000 ; qtot -0.874
31   c3     1   LIG   C15   31     0.303287     12.01000 ; qtot -0.571
32   c3     1   LIG   C16   32    -0.372728     12.01000 ; qtot -0.944
33   hc     1   LIG    H6   33     0.095783      1.00800 ; qtot -0.848
34   hc     1   LIG    H7   34     0.088785      1.00800 ; qtot -0.759
35   h1     1   LIG    H8   35     0.073853      1.00800 ; qtot -0.685
36   h1     1   LIG    H9   36     0.040517      1.00800 ; qtot -0.645
37   h1     1   LIG   H10   37     0.095754      1.00800 ; qtot -0.549
38   hc     1   LIG   H11   38     0.069619      1.00800 ; qtot -0.479
39   hc     1   LIG   H12   39     0.085855      1.00800 ; qtot -0.394
40   hc     1   LIG   H13   40     0.079225      1.00800 ; qtot -0.314
41   hc     1   LIG   H14   41     0.067648      1.00800 ; qtot -0.247
42   hc     1   LIG   H15   42     0.090665      1.00800 ; qtot -0.156
43   hc     1   LIG   H16   43     0.071043      1.00800 ; qtot -0.085
44   h1     1   LIG   H17   44     0.026701      1.00800 ; qtot -0.058
45   h1     1   LIG   H18   45     0.020637      1.00800 ; qtot -0.038
46   h1     1   LIG   H19   46     0.129349      1.00800 ; qtot 0.092
47   h1     1   LIG   H20   47     0.095040      1.00800 ; qtot 0.187
48   h1     1   LIG   H21   48     0.092330      1.00800 ; qtot 0.279
49   h1     1   LIG   H22   49     0.037340      1.00800 ; qtot 0.316
50   h1     1   LIG   H23   50     0.050219      1.00800 ; qtot 0.367
51   h1     1   LIG   H24   51     0.128813      1.00800 ; qtot 0.495
52   h1     1   LIG   H25   52     0.072863      1.00800 ; qtot 0.568
53   hx     1   LIG   H26   53     0.145235      1.00800 ; qtot 0.714
54   hc     1   LIG   H27   54     0.017256      1.00800 ; qtot 0.731
55   hc     1   LIG   H28   55     0.065916      1.00800 ; qtot 0.797
56   hc     1   LIG   H29   56    -0.036007      1.00800 ; qtot 0.761
57   hc     1   LIG   H30   57    -0.036148      1.00800 ; qtot 0.725
58   hc     1   LIG   H31   58     0.091636      1.00800 ; qtot 0.816
59   hc     1   LIG   H32   59     0.098584      1.00800 ; qtot 0.915
60   hc     1   LIG   H33   60     0.085185      1.00800 ; qtot 1.000
```

1.4 QM/MM setup

For the purposes of the microiterative QM/MM optimisations[18], the system is divided into an inner region, which is updated less frequently, and an outer region, which is optimised to convergence for every step taken in the inner region. The inner region comprised all residues with at least one QM atom (106 atoms for **THR**). Only atoms in the active region are allowed to move in optimisations; the active region included all residues within 8 Å of any atom in the inner region (1550 atoms for **THR**). The HDLC residues used for optimisations coincided with the standard amino-acid resides of the protein; non-standard components (substrate, active-site iron complex) were grouped into chemically meaningful HDLC residues (substrate, [FeCl(2OG)(O_2)]).

The reaction coordinate was implemented by a harmonic restraint with a force constant of 3.0 $E_\mathrm{h} a_0^{-2}$.

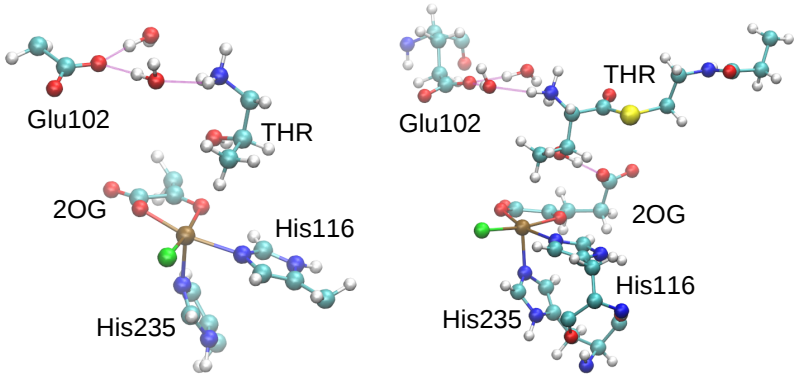

Figure S1: Partitioning of the system for QM/MM calculations and microiterative optimisations (illustrated for **THR**). Left: QM region (without link atoms). Right: Inner region for microiterative optimisations.

2 Supplementary Results

2.1 Docking

Table S3: The ten docked poses of NVA with highest affinity

Pose	**Affinity (kJ/mol)**	**RMSD (Å)**
1	–31.8	0.00
2	–31.4	1.69
3	–31.0	1.86
4	–30.1	2.26
5	–28.9	2.06
6	–28.5	9.75
7	–28.0	9.60
8	–28.0	5.46
9	–28.0	9.43
10	–28.0	7.86

Table S4: The ten docked poses of ABA with highest affinity

Pose	Affinity (kJ/mol)	RMSD (Å)
1	–32.6	0.00
2	–32.2	1.88
3	–31.4	2.08
4	–30.1	2.44
5	–29.7	9.33
6	–29.3	5.19
7	–29.3	9.51
8	–29.3	9.59
9	–29.3	9.67
10	–29.3	9.704

2.2 MD simulations

2.2.1 Simulations of the apoprotein, holoprotein, and substrate–A complexes

In addition to the three substrate–**A** complexes, the holoprotein (**A-H_2O**, containing the active-site iron complex with 2OG, Cl, and H_2O ligands, but no substrate) and the apoprotein (i.e., the "bare" protein) were also simulated using the same protocol. In all cases, the equilibrated structures remained very close to the crystal structure of the holoprotein (PDB code 2FCT): the RMSDs (calculated for backbone atoms, averaged over the equilibrated section of the trajectory) were 1.24 Å (apoprotein), 1.15 Å (holoprotein), 1.95 Å (**THR-A**), 1.33 Å (**ABA-A**), 1.24 Å (**NVA-A**).

The conformation of the benzyl side-chain of the "gate-keeper" residue Phe196 is determined by the C–C^{α}–C^{β}–C^{γ} and C^{α}–C^{β}–C^{γ}–C^{δ} torsions (see Table S5). The side-chain conformations for the three docked structures are nearly identical, with the phenyl ring being positioned such as to allow the substrate access to the active-site channel. In the MD simulations, we find different rotamers for the different systems, including cases where two rotamers are significantly populated (Figure S4). As the protein is fully flexible in the MD simulations, the channel and "gate" on the one hand and the bound substrate on the other are able to adjust their conformations to allow a best (induced) fit; for instance, the Phe196 side-chain adopts the "closed" conformation of the holoprotein in **THR-A**. The "gate" is thus more aptly characterised as a "curtain", which can flexibly wrap around, and yield to, an obstacle placed in the doorway.

Table S5: Side-chain torsions of the "gate-keeper" residue Phe196; values from docking refer to the top pose, MD values are converged averages.

	Method	C–C^{α}–C^{β}–C^{γ} (°)	C^{α}–C^{β}–C^{γ}–C^{δ} (°)
A-H_2O (holoprotein)	X-ray	–167	–77
	MD	–170	–68
THR-A	Docking	135	–31
	MD	175	–70
ABA-A	Docking	134	–35
	MD	68, 156	–50
NVA-A	Docking	133	–33
	MD	75	90

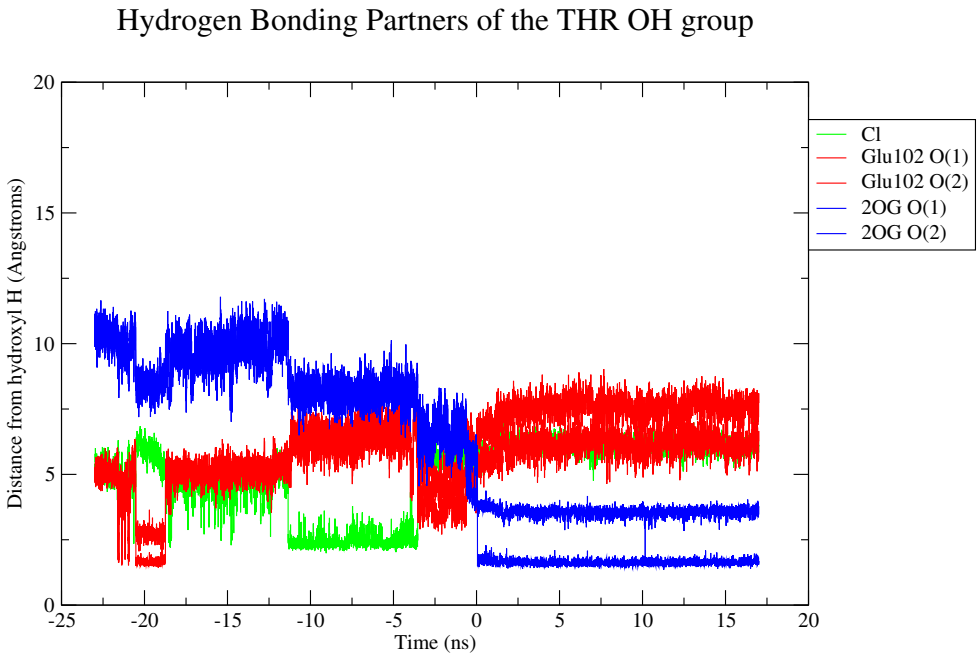

Figure S2: Hydrogen-bonding partners of the **THR-A** OH group over the course of the MD trajectory. Time zero refers to the start of the equilibrated section of the trajectory. 2OG O(1) and O(2) refer to the oxygen atoms of the 2OG tail carboxylate; Glu102 O(1) and O(2) to the carboxylate oxygens of Glu102.

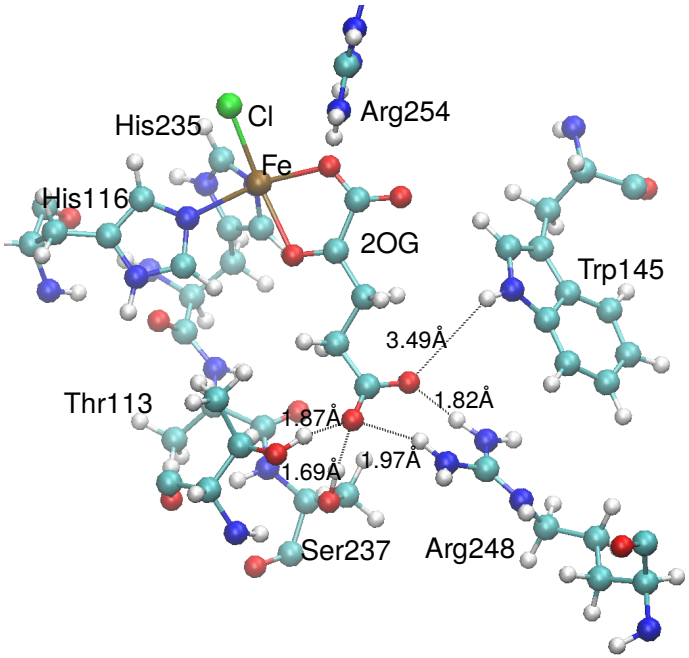

Figure S3: Representative snapshot illustrating the hydrogen-bonding network around the 2OG carboxylate tail as found in the equilibrated structures of **ABA-A** and **NVA-A**.

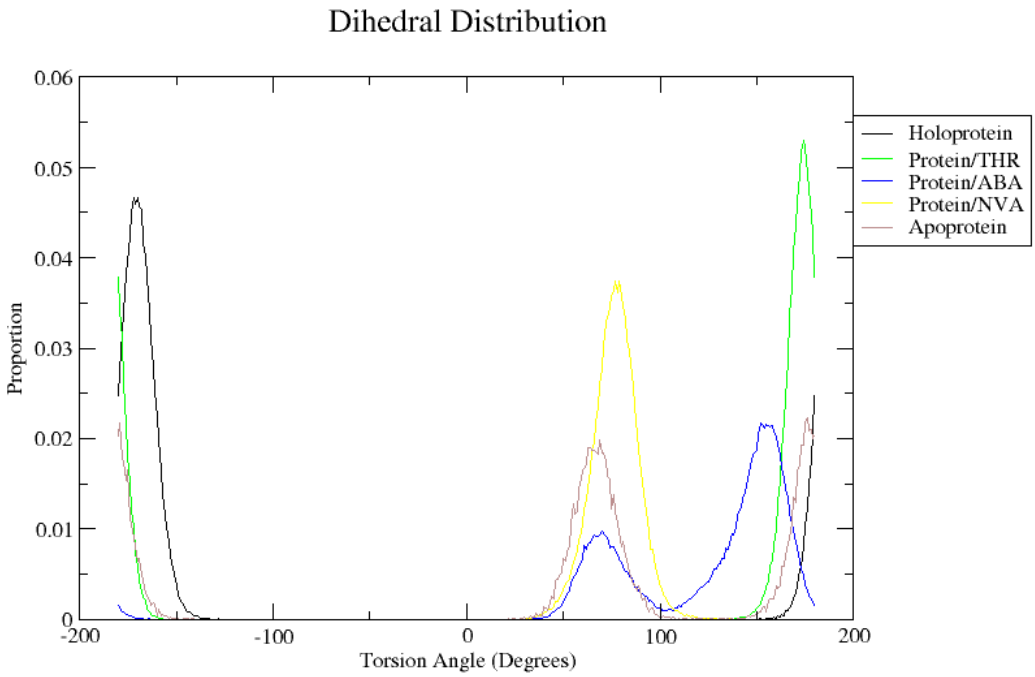

Figure S4: Distribution of the C–C^{α}–C^{β}–C^{γ} dihedral angle of Phe196. In the crystal structure of the SyrB2 holoprotein (PDB 2FCT), the value of this torsion is –167°.

2.2.2 Accessibility of the active site for small molecules

Over the course of the catalytic cycle, O_2 is consumed and CO_2 is generated. The crystal structure of the SyrB2 holoprotein features two channels connecting the active-site cavity to the surface: the main substrate channel (T_1) and an allosteric channel (T_2); see Figure S5. Two questions arise as to the accessibility of the active site for small molecules: (i) Do the channels identified in the crystal structure remain intact and open during the course of MD? (ii) Does the bound substrate plug the main channel, leaving only (T_2) as a route for small molecules to access the active-site cavity?

The MD trajectories for the holoprotein and the enzyme–substrate complexes were analysed using the program Caver.[19] Caver searches the trajectory frame-by-frame for tunnels, then groups related tunnels from different frames into channels; a tunnel is represented as an "elastic hose", whose shape, length, and diameter vary over the course of the trajectory, reflecting the conformational dynamics of the protein. Each channel is assigned an importance (priority) value according to its length, width, and the duration for which it persists. This value is calculated from a cost function (Eq. 1), which depends on overall length L and the varying radius along the tunnel, $r(s)$; long, narrow tunnels have a high cost. The cost may be understood as a measure of the energy required to traverse the tunnel. The priority (Eq. 2) is then calculated from the cost by Boltzmann-weighting and averaging over time (N is the number of frames). The priority value effectively measures the transport capacity of a channel.

$$C = \int_0^L r(s)^{-2} \mathrm{d}s \tag{1}$$

$$\text{Priority} = \frac{1}{N} \sum_{i=1}^{N} \mathrm{e}^{-C_i} \tag{2}$$

The results of this analysis are shown in Table S6. No new channels opened during the MD simulations, but both T_1 and T_2 remained open in all simulations, even when the substrate was bound.

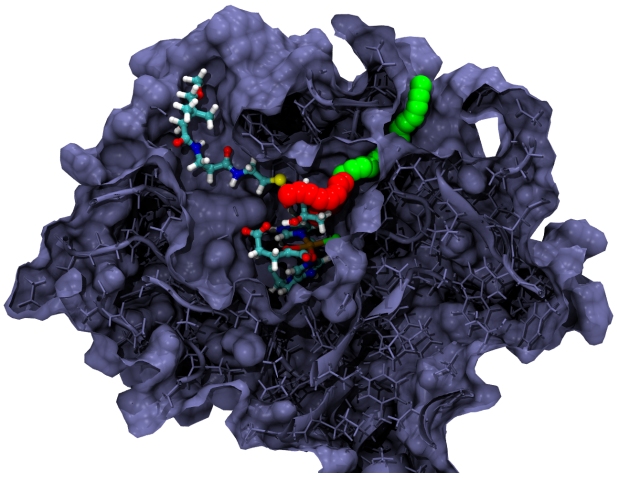

Figure S5: Channels in SyrB2 **THR-A** (T_1 shown in red, T_2 shown in green).

Table S6: Channels leading to active site. $r_{\min}$ is the bottleneck radius, L is the average length. **ABA** yielded two routes through the main channel, above and below the substrate.

Simulation	T_1			T_2		
	Priority	$r_{\min}$ (Å)	L (Å)	Priority	$r_{\min}$ (Å)	L (Å)
Holoprotein	0.192	0.96	21.7	0.081	0.96	23.6
THR	0.280	1.00	16.1	0.203	1.00	22.3
ABA	0.031	0.96	14.8	0.008	0.96	25.1
	0.009	0.93	29.1			
NVA	0.008	0.96	15.7	0.028	0.94	22.7

2.2.3 Simulations of substrate–D complexes

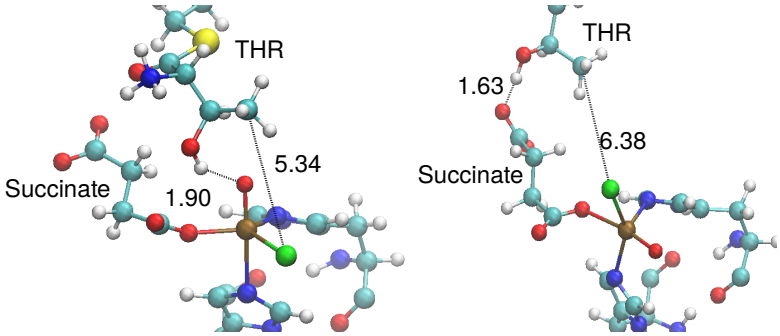

Figure S6: Representative snapshots of **THR-D2** (left) and **THR-D5** (right).

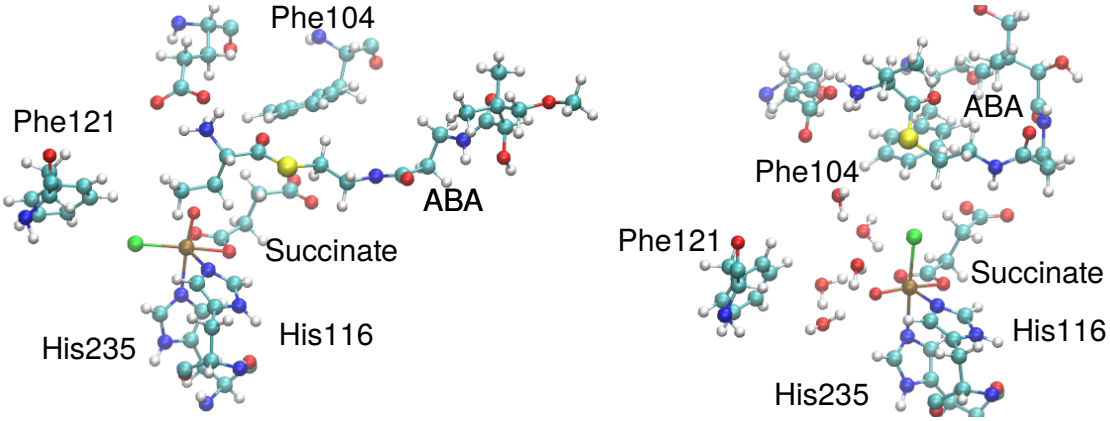

Figure S7: Representative snapshots of **ABA-D3** (left), which adopts a stable conformation, and **ABA-D4** (right), which did not settle into a stable conformation during the simulations.

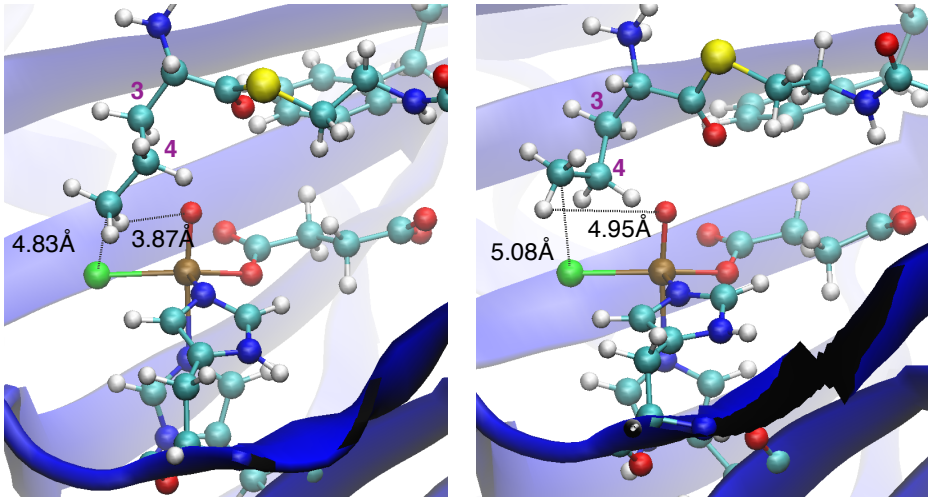

Figure S8: Representative snapshots of **NVA-D2** in the major (left) and minor (right) C^3–C^4 rotamer.

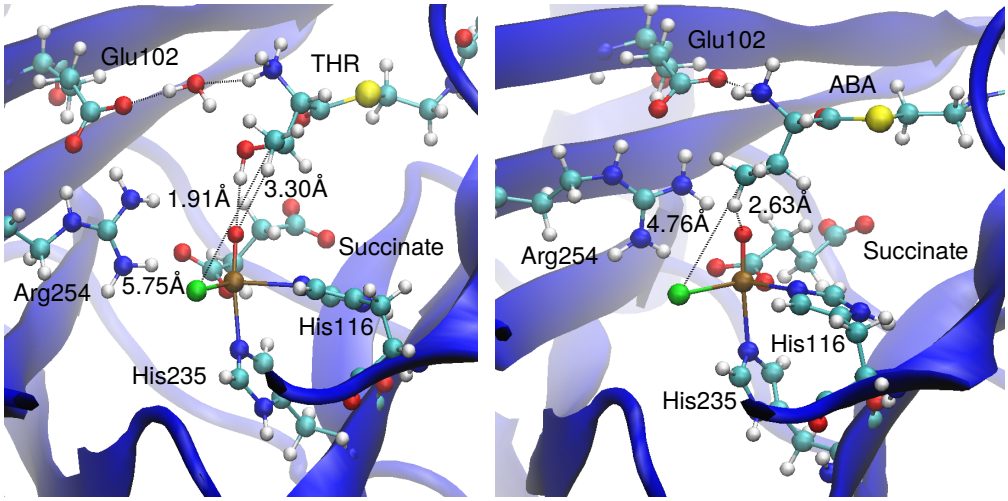

Figure S9: Representative snapshots of **THR-D2** (left) and **ABA-D2** (right). As seen already in the simulations with **B**, **ABA-D** maintains the direct salt bridge between the ammonium group and Glu102 whilst for **THR-D** this salt bridge is indirect, i.e., mediated by a water molecule.

2.2.4 Hydrogen-bond coordination number of the oxido oxygen

In the Mössbauer spectrum of SyrB2 with its native **THR** substrate, two distinct [Fe=O] species are observed[20]. This was explained by Wong *et al.*[21] by different numbers of hydrogen bonds to the oxido ligand, rather than by geometrical isomerism[22]. We therefore analysed the trajectories with the isomers **D1** and **D2** with respect to the hydrogen-bond coordination numbers of the oxido ligand (see Table S7). If we presume that the two species differ by one hydrogen bond, none of the ratios for **THR** (1 vs. 0 and 2 vs. 1 H-bonds, respectively) matches particularly well with the experimental ratio between the two [Fe=O] species of 4:1. For **ABA**, the experimental ratio is 7:1, which agrees nicely with the ratio of 8:1 for 0 vs. 1 H-bond in **ABA-D2**. However, based on these limited data, we can neither support nor reject Wong *et al.*'s proposal.

Table S7: Prevalence of hydrogen-bond coordination numbers to oxido.

Substrate	Isomer	% of frames with n H-bonds to oxido		
		$n = 0$	$n = 1$	$n = 2$
THR	**D1**	38	55	7
	D2	26	68	6
ABA	**D1**	33	52	14
	D2	88	11	0
NVA	**D1**	26	68	6
	D2	33	52	14

2.3 QM/MM calculations

2.3.1 O_2 complexes

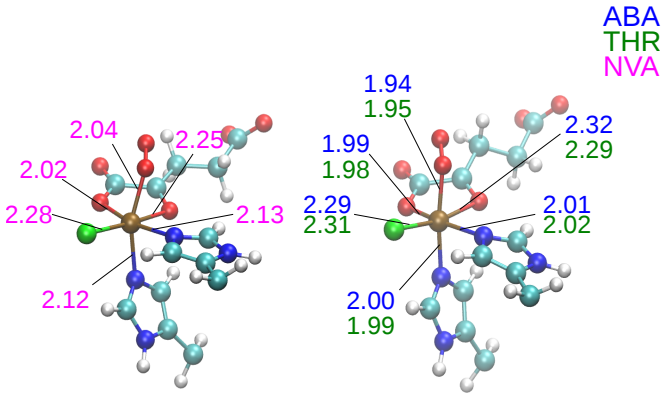

Figure S10: QM/MM-optimised structures of the O_2 complexes in the quintet state, ^{5}**B**. Selected bond lengths are given in Å.

Table S8: Selected structural parameters of the O_2 complexes **B**. (*The structure of **THR-B** ($S = 1$) was optimised at def2-SVP level.)

Parameter	THR			ABA			NVA
	S=1*	S=2	S=3	S=1	S=2	S=3	S=2
Fe–N_{His235} (Å)	2.02	1.99	2.05	1.98	2.00	2.09	2.12
Fe–N_{His116} (Å)	2.11	2.02	2.12	2.00	2.01	2.11	2.13
Fe–Cl (Å)	2.32	2.31	2.27	2.24	2.29	2.24	2.28
Fe–O_P (Å)	2.01	1.95	2.22	1.95	1.94	2.13	2.04
Fe–O_{A1} (Å)	2.06	1.98	2.06	1.95	1.99	2.05	2.02
Fe–O_K (Å)	2.30	2.29	2.08	1.99	2.32	2.28	2.25
O–O (Å)	1.24	1.28	1.28	1.29	1.29	1.28	1.28
N_{His235}–Fe–O_P (°)	169	164	157	178	171	164	158

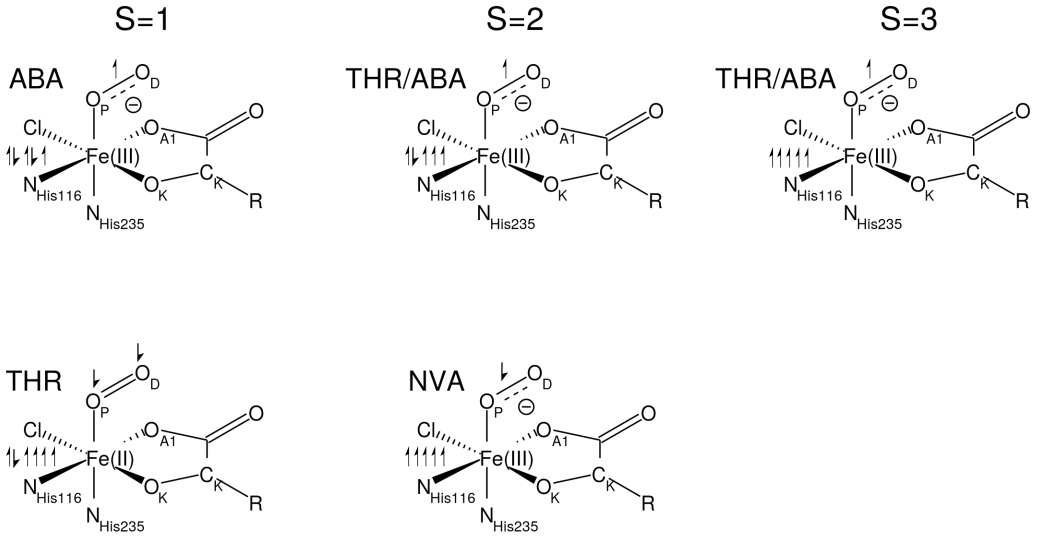

Figure S11: Schematic representation of the electronic structures of the O_2 complexes with $S = 1, 2, 3$. For **THR** and **ABA** ($S = 3$) and **NVA** ($S = 2$), the other direct donor atoms together carry another ∼0.5 majority spins, which is included in the Fe spin count in the schematic.

2.3.2 O_2 complexes: QM/MM vs. HYSCORE

The pulsed-EPR (HYSCORE) experiments of Martinie *et al.*[23] provide distance and angle measurements between O_p, Fe, and specific hydrogens of the substrate; see Figure S12. In the experiment, NO is used as a non-reactive, EPR-active structural mimick of O_2, and substrates need to be deuterated at the position to be measured. Comparing to QM/MM-optimised structures, distances agree well, but angles do not (Table S12).

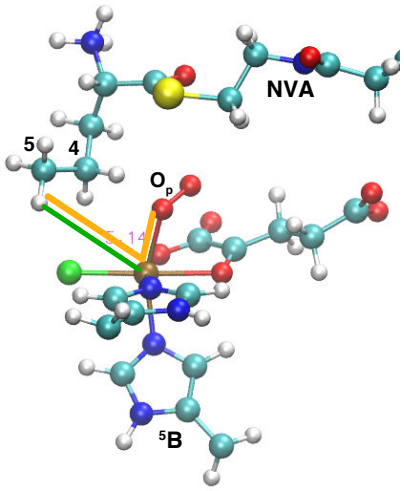

Figure S12: Structure of **NVA-^{5}B**, with the Fe–(C^5)H distance and the O_p–Fe–(C^5)H angle highlighted in green and orange, respectively.

Table S9: Fe–H distances and O–Fe–H angles in O_2 complexes **B** from QM/MM optimisations and HYSCORE experiments[23].

		THR		**ABA**		**NVA**	
		(C^3)H	(C^4)H	(C^3)H	(C^4)H	(C^4)H	(C^5)H
Fe–H (Å)	QM/MM	4.42	4.11	4.3	3.9	3.2	5.14
	Expt	4.7 ± 0.4	4.2 ± 0.3	4.7 ± 0.3	3.7 ± 0.2	3.7 ± 0.3	3.4 ± 0.3
O_p–Fe–(C^3)H (°)	QM/MM	10	24	39	25	50	65
	Expt	81 ± 10	85 ± 10	85 ± 10	85 ± 10	64 ± 7	81 ± 15

2.3.3 O_2 activation/decarboxylation intermediates

Table S10: Selected structural parameters of intermediates along the O_2 activation/decarboxylation pathway, optimised at B3LYP-D3/def-TZVP/MM level. C^2 designates the keto-carbon of 2OG, labelled as C_K above. (*The structure of **THR-^{3}B** was optimised at def2-SVP level.)

Substrate	Parameter	^{3}**B**	^{3}**C1**	^{3}**C2a**	^{3}**D**	^{5}**B**	^{5}**C1**	^{5}**C2a**	^{5}**C2c**	^{5}**D**	^{7}**B**	^{7}**C2b**	^{7}**D**
THR	Fe–N_{235} (Å)	2.05*	1.98	2.27	1.94	1.99	2.09	2.15	—	2.10	2.05	2.09	2.07
	Fe–N_{116} (Å)	2.12*	1.98	2.02	2.10	2.02	2.01	2.13	—	2.10	2.12	2.11	2.16
	Fe–Cl (Å)	2.27*	2.27	2.28	2.29	2.31	2.25	2.32	—	2.25	2.27	2.25	2.32
	Fe–O_p (Å)	2.22*	1.82	1.91	1.60	1.95	2.40	2.05	—	1.63	2.22	1.94	1.90
	Fe–O_k (Å)	2.08*	1.84	2.01	1.96	2.29	1.91	2.26	—	1.93	2.08	2.29	1.92
	O_p–O_d (Å)	1.28*	1.41	1.48	—	1.28	1.30	1.44	—	—	1.28	—	—
	O_d–C^2 (Å)	2.57*	1.45	1.91	1.22	2.25	1.60	1.32	—	1.22	1.93	—	1.22
	N_{235}–Fe–O_p (°)	157*	171	165	111	164	157	114	—	95	157	155	106
ABA	Fe–N_{235} (Å)	1.98	—	—	2.08	2.00	2.11	2.12	2.10	2.07	2.09	2.11	2.07
	Fe–N_{116} (Å)	2.00	—	—	1.96	2.01	1.99	2.11	2.10	2.10	2.11	2.17	2.12
	Fe–Cl (Å)	2.24	—	—	2.33	2.29	2.26	2.35	2.33	2.26	2.24	2.24	2.36
	Fe–O_p (Å)	1.95	—	—	1.59	1.94	2.27	2.02	1.77	1.64	2.13	1.98	1.87
	Fe–O_k (Å)	1.99	—	—	1.99	2.32	2.01	2.33	2.17	1.94	2.28	2.21	1.95
	O_p–O_d (Å)	1.29	—	—	—	1.29	1.28	1.44	—	—	1.28	—	—
	O_d–C^2 (Å)	2.23	—	—	1.23	2.47	2.07	1.32	—	1.22	2.43	—	1.23
	N_{235}–Fe–O_p (°)	178	—	—	156	171	169	139	127	93	164	166	101
NVA	Fe–N_{235} (Å)	—	—	—	—	2.12	—	2.10	—	2.05	—	—	—
	Fe–N_{116} (Å)	—	—	—	—	2.13	—	2.11	—	2.12	—	—	—
	Fe–Cl (Å)	—	—	—	—	2.28	—	2.37	—	2.29	—	—	—
	Fe–O_p (Å)	—	—	—	—	2.04	—	2.00	—	1.63	—	—	—
	Fe–O_k (Å)	—	—	—	—	2.25	—	2.33	—	1.95	—	—	—
	O_p–O_d (Å)	—	—	—	—	1.28	—	1.45	—	—	—	—	—
	O_d–C^2 (Å)	—	—	—	—	2.47	—	1.32	—	1.22	—	—	—
	N_{235}–Fe–O_p (°)	—	—	—	—	158	—	132	—	96	—	—	—

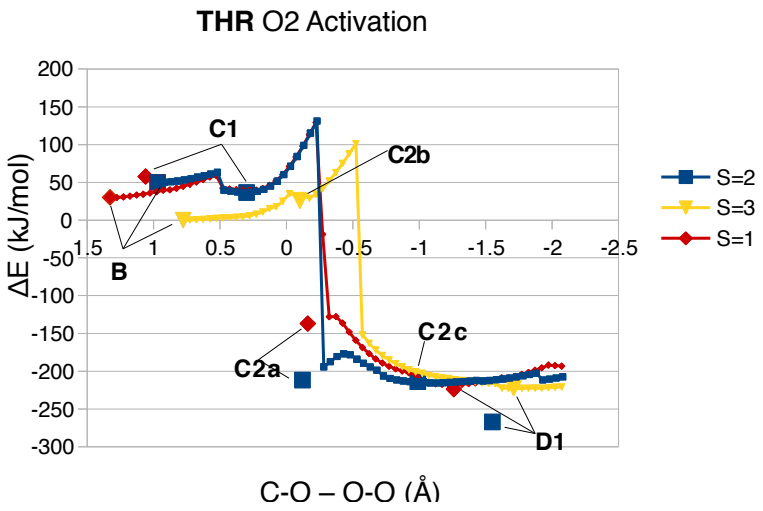

Figure S13: Energetic profiles of the reaction coordinate scans for **THR** on the triplet, quintet, and septet surfaces. Large, labelled symbols denote optimised intermediates. Scans and optimisations done at B3LYP-D3/def2-SVP/MM level.

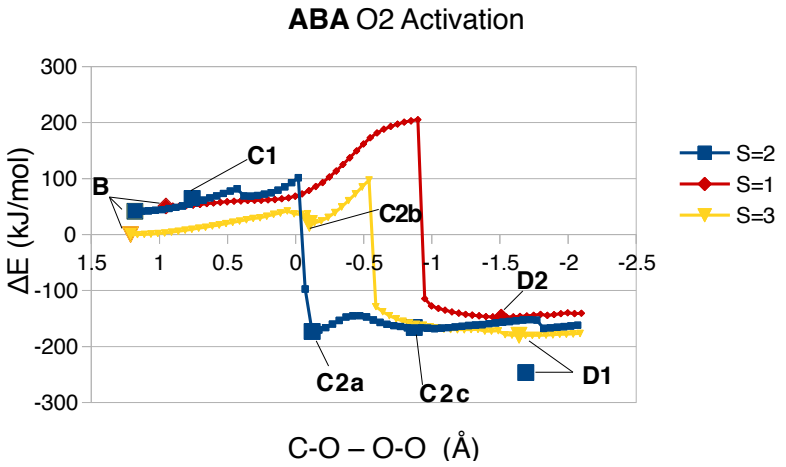

Figure S14: Energetic profiles of the reaction coordinate scans for **ABA** on the triplet, quintet, and septet surfaces. Large, labelled symbols denote optimised intermediates. Scans and optimisations done at B3LYP-D3/def2-SVP/MM level.

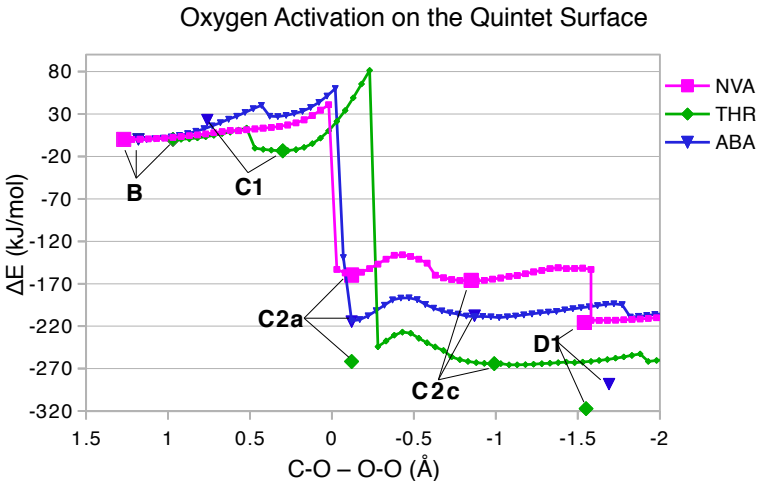

Figure S15: Energetic profiles of the reaction coordinate scans for **THR**, **ABA**, and **NVA** on the quintet surface. Large, labelled symbols denote optimised intermediates. Scans and optimisations done at B3LYP-D3/def2-SVP/MM level.

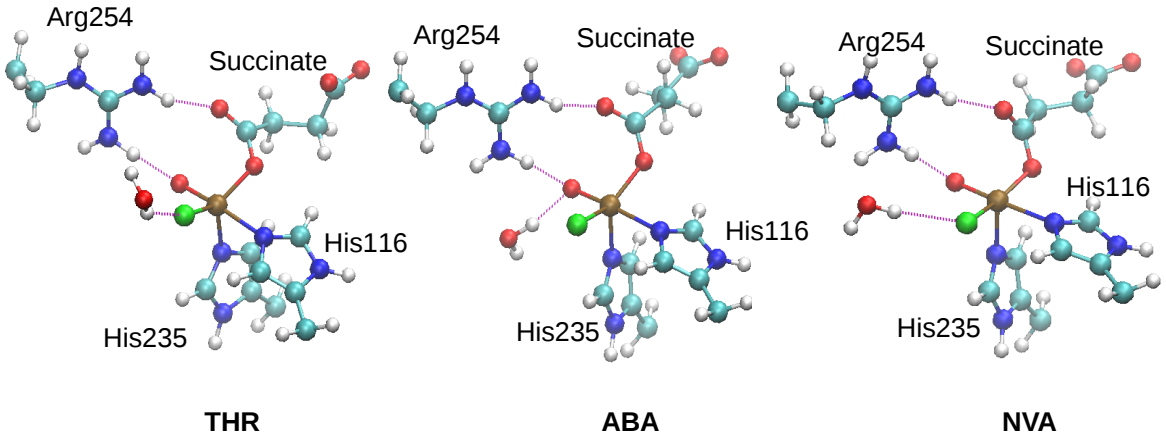

Figure S16: QM/MM-optimised structures of the preferred [Fe=O] species, ^{5}**D1**, for the three substrates. In all three structures, Arg254 is hydrogen-bonding with one "arm" each to the oxido oxygen and the free carbonyl oxygen of the succinate ligand.

References

[1] A. Jarnuczak, *MSci Thesis*, School of Chemistry, University of Glasgow, 2011.

[2] A. Šali and T. L. Blundell, *J. Mol. Biol.*, 1993, **234**, 779–815.

[3] *Modeller 9v8*, 2010.

[4] A. Fiser, R. K. G. Do and A. Šali, *Protein Sci.*, 2000, **9**, 1753–1773.

[5] J. Word, S. C. Lovell, J. S. Richardson and D. C. Richardson, *J. Mol. Biol.*, 1999, **285**, 1735–1747.

[6] *Reduce 3.14*, 2010.

[7] V. B. Chen, W. B. Arendall, J. J. Headd, D. A. Keedy, R. M. Immormino, G. J. Kapral, L. W. Murray, J. S. Richardson and D. C. Richardson, *Acta Crystallogr. Sect. D-Biol. Crystallogr.*, 2009, **66**, 12–21.

[8] H. Li, A. D. Robertson and J. H. Jensen, *Proteins: Struct., Funct., Bioinf.*, 2005, **61**, 704–721.

[9] *PropKa 2.0*, 2008.

[10] U. C. Singh and P. A. Kollman, *J. Comput. Chem.*, 1984, **5**, 129–145.

[11] Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo, T. Lee, J. Caldwell, J. Wang and P. Kollman, *J. Comput. Chem.*, 2003, **24**, 1999–2012.

[12] O. Treutler and R. Ahlrichs, *J. Chem. Phys.*, 1995, **102**, 346–354.

[13] M. Von Arnim and R. Ahlrichs, *J. Comput. Chem.*, 1998, **19**, 1746–1757.

[14] TURBOMOLE V6.4 2012, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989-2007, TURBOMOLE GmbH, since 2007; available from `http://www.turbomole.com`.

[15] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09 Rev. A.02*, Gaussian Inc., Wallingford, CT, 2009.

[16] H. M. Senn, S. Thiel and W. Thiel, *J. Chem. Theory Comput.*, 2005, **1**, 494–505.

[17] S. K. Schiferl and D. C. Wallace, *J. Chem. Phys.*, 1985, **83**, 5203–5209.

[18] J. Kästner, S. Thiel, H. M. Senn, P. Sherwood and W. Thiel, *J. Chem. Theory Comput.*, 2007, **3**, 1064–1072.

[19] E. Chovancova, A. Pavelka, P. Benes, O. Strnad, J. Brezovsky, B. Kozlikova, A. Gora, V. Sustr, M. Klvana, P. Medek, L. Biedermannova, J. Sochor and J. Damborsky, *PLoS Comput. Biol.*, 2012, **8**, e1002708.

[20] M. Matthews, C. Krest, E. Barr, F. Vaillancourt, C. Walsh, M. Green, C. Krebs and J. Bollinger Jr, *Biochemistry*, 2009, **48**, 4331–4343.

[21] S. D. Wong, M. Srnec, M. L. Matthews, L. V. Liu, Y. Kwak, K. Park, C. B. Bell III, E. E. Alp, J. Zhao, Y. Yoda, S. Kitao, M. Seto, C. Krebs, J. M. Bollinger and E. I. Solomon, *Nature*, 2013, **499**, 320–323.

[22] T. Borowski, H. Noack, M. Radoṅ, K. Zych and P. E. M. Siegbahn, *J. Am. Chem. Soc.*, 2010, **132**, 12887–12898.

[23] R. J. Martinie, J. Livada, W. C. Chang, M. T. Green, C. Krebs, J. Bollinger, J. M. and A. Silakov, *J. Am. Chem. Soc.*, 2015, **137**, 6912–6919.