**Electronic Supplementary Information:**


**A combined molecular dynamics simulation and quantum chemical study on the mechanism for activation of the OxyR transcription factor by hydrogen peroxide**

Juraj Kóňa [a,b] and Tore Brinck *[a]


[a] Physical Chemistry, Royal Institute of Technology, SE-10044 Stockholm, Sweden

[b] Institute of Chemistry, Slovak Academy of Sciences, Dúbravska cesta 9, SK-845 38 Bratislava, Slovak Republic


* Correspondence to: Tore Brinck, Physical Chemistry, Royal Institute of Technology, SE-10044 Stockholm, Sweden

fax: +46-8-7908207
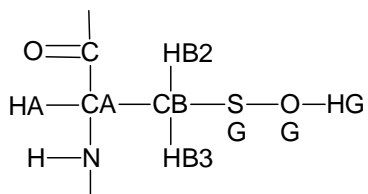
e-mail: tore@physchem.kth.se

```
O═C       HB2
          |
HA—CA—CB—S—O—HG
   |      |  G  G
H—N      HB3
   |
```

**Table S1** Atom types and charges derived for the sulfenic acid (CYA) residue.

| Atom name | Atom type | Charge |
| --- | --- | --- |
| N | N | -0.4157 |
| H | H | 0.2719 |
| CA | CT | 0.0429 |
| HA | H1 | 0.0766 |
| CB | CT | -0.0790 |
| HB2 | H1 | 0.0910 |
| HB3 | H1 | 0.0910 |
| SG | S | -0.1081 |
| OG | OH | -0.4500 |
| HG | HO | 0.4500 |
| C | C | 0.5973 |
| O | O | -0.5679 |

**Table S2** Bond (BOND), angle (ANGLE), dihedral (DIHE) and van der Waals (NONB) parameters for the CYA residue.

| BOND | | | | |
|---|---|---|---|---|
| S-OH | 230.0 | 1.678 | | |
| OH-HO | 553.0 | 0.960 | | |
| **ANGLE** | | | | |
| CT-S-OH | 68.0 | 103.0 | | |
| S-OH-HO | 55.0 | 108.5 | | |
| **DIHE** | | | | |
| CT-S-OH-HO | 1.0 | 3.5 | 0.0 | -2.0 |
| CT-S-OH-HO | 1.0 | 0.6 | 0.0 | 3.0 |
| **NONB** | | | | |
| S | 2.000 | 0.250 | | |
| OH | 1.721 | 0.2104 | | |
| HO | 0.0 | 0.0 | | |

**Table S3** The RMSD of the Cα carbons of OxyR during 1 ns (**MD_1**, **MD_2**, **MD_4** and **MD_5**) and during 4 ns MD simulations (**MD_3**).

| | MD_1 | MD_2 | MD_3 | MD_4 | MD_5 |
|---|---|---|---|---|---|
| Cα carbons of all residues of OxyR (87-298) | | | | | |
| RMSD average | 1.4 ± 0.2 | 1.6 ± 0.3 | 1.4 ± 0.2 | 1.2 ± 0.2 | 1.6 ± 0.3 |
| min | 0.6 | 0.7 | 0.6 | 0.6 | 0.6 |
| max | 2.3 | 2.2 | 2.0 | 1.6 | 2.1 |
| Cα carbons of residues of the redox-active loop of OxyR (189-225) | | | | | |
| average | 1.1 ± 0.2 | 1.1 ± 0.2 | 1.3 ± 0.4 | 1.2 ± 0.2 | 1.5 ± 0.5 |
| min | 0.5 | 0.5 | 0.5 | 0.4 | 0.4 |
| max | 1.8 | 1.8 | 2.6 | 1.8 | 2.5 |
| Cα carbons of all residues but the redox-active loop (87-188, 226-298) | | | | | |
| average | 1.3 ± 0.3 | 1.5 ± 0.4 | 1.2 ± 0.2 | 1.1 ± 0.1 | 1.5 ± 0.2 |
| min | 0.5 | 0.7 | 0.5 | 0.6 | 0.5 |
| max | 2.2 | 2.3 | 1.8 | 1.5 | 1.9 |
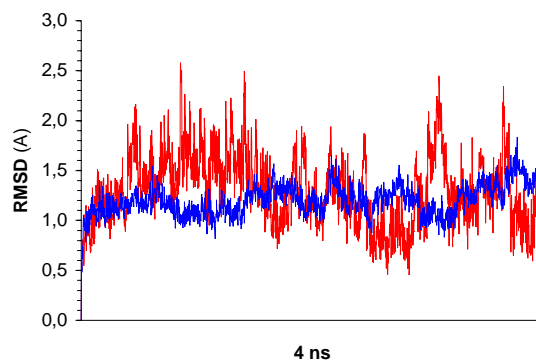
**Fig. S1** RMSD (in Å) of Cα carbons of reduced form of OxyR with ionized Cys199 (**MD_3**, HIP198-CYM199-CYS208) during the 4 ns MD simulation. (red line - Cα carbons of redox-active loop, blue line - other Cα carbons).
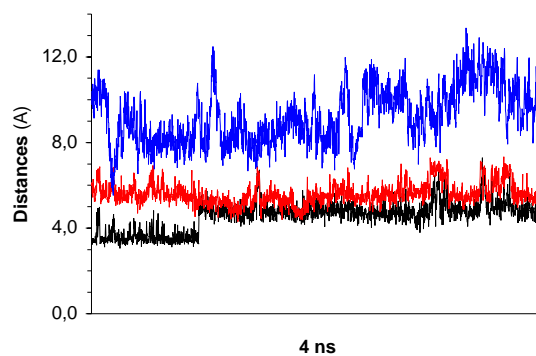
**Fig. S2** Distances d(Cys199-Sγ...Sγ-Cys208) (black), d(Cys199-Sγ…Nδ1-His198) (red) and d(Cys199-Sγ…Cζ-Arg220) (blue) in Å in the reduced form of OxyR (with configuration HIP198-CYM199-CYS208) during the 4 ns simulations (**MD_3**).

**Table S4** Total energies (*E*), ZPE corrections (*ZPE*), thermal corrections to enthalpy (*H*) and Gibbs free energy (*G*) for the first redox step (reaction of Cys199 of the OxyR with hydrogen peroxide) in the gas phase in a.u. [B3LYP/6-31(+S)G(d)]

|     | *E* | *ZPE* | *H* | *G* |
|-----|-----|-------|-----|-----|
| **1** | -1022.2760 | 0.2397 | 0.2597 | 0.1885 |
| **2TS** | -1022.2507 | 0.2389 | 0.2584 | 0.1886 |
| **3** | -1022.3662 | 0.2399 | 0.2601 | 0.1867 |

**Table S5** Total energies ($E$) for the first redox step (reaction of Cys199 of the OxyR with hydrogen peroxide) in the gas phase and aqueous solution in a.u.

|  | $E^a$ | $E^b$ | $E^c$ |
|---|---|---|---|
| **1** | -1022.3486 | -1019.9749 | -1022.4289 |
| **2TS** | -1022.3240 | -1019.9508 | -1022.4066 |
| **3** | -1022.4461 | -1020.0872 | -1022.5201 |

[a] B3LYP/6-31+G(d,p)//B3LYP/6-31(+S)G(d). [b] MP2/6-31+G(d,p)//B3LYP/6-31(+S)G(d). [c] PCM-B3LYP/6-31+G(d,p)//B3LYP/6-31(+S)G(d).

**Table S6** Total energies (*E*), ZPE corrections (*ZPE*), thermal corrections to enthalpy (*H*) and Gibbs free energy (*G*) for the proton-transfer reaction (deprotonation of Cys208 by His198) and the second redox step (reaction of Cys199-S-OH with Cys208 of the OxyR) in the gas phase in a.u. [B3LYP/6-31(+S)G(d)]

|       | *E*        | *ZPE*   | *H*     | *G*     |
|-------|------------|---------|---------|---------|
| **4**   | -1461.0708 | 0.2879  | 0.3139  | 0.2230  |
| **5TS** | -1461.0659 | 0.2853  | 0.3107  | 0.2218  |
| **6**   | -1461.0667 | 0.2887  | 0.3142  | 0.2242  |
| **7**   | -1461.0665 | 0.2903  | 0.3156  | 0.2281  |
| **8TS** | -1461.0488 | 0.2906  | 0.3150  | 0.2326  |
| **9**   | -1461.0877 | 0.2895  | 0.3160  | 0.2249  |

**Table S7** Total energies ($E$) for the proton-transfer reaction (deprotonation of Cys208 by His198) and the second redox step (reaction of Cys199-S-OH with Cys208 of the OxyR) in the gas phase and aqueous solution in a.u.

| | $E^a$ | $E^b$ | $E^c$ |
|---|---|---|---|
| **4** | -1461.1569 | -1458.0841 | -1461.2229 |
| **5TS** | -1461.1528 | -1458.0780 | -1461.2229 |
| **6** | -1461.15311 | -1458.0775 | -1461.2242 |
| **7** | -1461.1534 | -1458.0770 | -1461.2250 |
| **8TS** | -1461.1363 | -1458.0610 | -1461.2094 |
| **9** | -1461.1811 | -1458.1078 | -1461.2556 |

[a] B3LYP/6-31+G(d,p)//B3LYP/6-31(+S)G(d). [b] MP2/6-31+G(d,p)//B3LYP/6-31(+S)G(d). [c] PCM-B3LYP/6-31+G(d,p)//B3LYP/6-31(+S)G(d).

#B3LYP/6-31G(d) extrabasis opt freq

Complex 1

1,1
```
 7     -0.633167    -0.582110    -4.210011
 6     -0.531076    -0.587728    -2.870246
 7      0.760515    -0.589951    -2.561545
 6      1.509169    -0.585013    -3.718598
 6      0.634425    -0.581283    -4.766662
 8     -2.439707    -0.600376    -0.160002
 8     -1.702202    -0.845933     1.073359
 7     -1.529790     1.501694     2.670565
 6     -0.428581     1.920627     3.295843
 7     -0.531205     2.906747     4.214713
 7      0.759907     1.394771     3.016221
16      1.250471    -0.523416     0.453030
 6      2.051517    -2.095945     0.998263
 1      0.292179     3.302921     4.642966
 1     -1.417952     3.117450     4.648561
 1      1.561522     1.616003     3.589605
 1      0.924783     0.778507     2.191108
 1     -1.550548     0.665368     2.063226
 1     -2.385272     2.030941     2.755886
 1     -0.734290    -0.880312     0.746890
 1      3.137771    -2.001907     0.928264
 1      1.785094    -2.316024     2.035387
 1      1.727221    -2.933921     0.375216
 1     -3.045639    -1.362336    -0.135009
 1     -1.339888    -0.587914    -2.148116
 1      1.097686    -0.584101    -1.530020
 1      2.587704    -0.584529    -3.701536
 1      0.794867    -0.578975    -5.833231
 1     -1.504042    -0.578039    -4.725739
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt=(ts,calcfc,noeigentest) freq

Transition state 2TS

1,1
```
 6   -3.392808   0.327474   1.942201
 6   -3.403714   0.353246   3.305799
 7   -2.077886   0.302493   3.701255
 6   -1.282150   0.243730   2.621366
 7   -2.072353   0.258070   1.548600
 8    1.673254   0.089802   1.775139
 8    0.636972  -0.054933   0.402036
16   -1.020793   0.108738  -1.460029
 6   -1.604400  -1.636119  -1.584615
 7    3.048510   0.043516  -1.056925
 6    3.025495   0.080945  -2.390591
 7    1.870773  -0.034920  -3.042287
 7    4.179435   0.232975  -3.072005
 1    4.185311   0.334152  -4.075947
 1    5.071096   0.101707  -2.617463
 1    1.853982  -0.158491  -4.044536
 1    0.957032   0.004447  -2.548358
 1    2.175214  -0.077145  -0.521774
 1    3.854732   0.380841  -0.550866
 1    0.537780   0.910884   0.320064
 1   -2.142016  -1.752461  -2.530124
 1   -0.772860  -2.345559  -1.564672
 1   -2.293330  -1.882227  -0.770709
 1    1.805480  -0.870055   1.876298
 1   -0.192242   0.196719   2.605011
 1   -1.720184   0.242861   0.551644
 1   -4.200810   0.351096   1.228065
 1   -4.216018   0.400866   4.013411
 1   -1.746189   0.313008   4.657605
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt freq

Intermediate 2

1,1
```
 6    -2.732820   -0.004105    3.150861
 6    -2.851125    0.160704    4.504558
 7    -1.574184    0.429015    4.953581
 6    -0.742396    0.418474    3.882498
 7    -1.414474    0.158017    2.774300
 8    -0.084017   -2.237234   -1.254897
 8    -0.557695   -0.049135    0.265823
16     0.646434    1.090886    0.003884
 6    -0.313607    2.501904   -0.627381
 7     1.007709   -1.687762   -3.672380
 6     1.995586   -0.819376   -3.841799
 7     2.331405    0.023717   -2.857493
 7     2.681817   -0.774649   -5.001186
 1     3.243706    0.027467   -5.245770
 1     2.563160   -1.489040   -5.704393
 1     3.220158    0.502786   -2.874253
 1     1.742858    0.157667   -2.030345
 1     0.599657   -1.884932   -2.728539
 1     0.622617   -2.175934   -4.468922
 1    -0.373246   -1.497098   -0.665442
 1     0.414015    3.284329   -0.873174
 1    -0.873609    2.227468   -1.524926
 1    -0.993189    2.878357    0.140472
 1     0.319456   -2.896934   -0.672238
 1     0.319709    0.603136    3.952691
 1    -0.867790    0.024859    1.244115
 1    -3.503634   -0.227979    2.428071
 1    -3.698013    0.113627    5.171197
 1    -1.304843    0.605023    5.911721
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

13

#B3LYP/6-31G(d) extrabasis opt freq

Intermediate 4

1,1
```
 6     -5.572017   -0.114635   -0.859440
 7     -4.635535    0.499750   -0.053102
 6     -5.100717    1.712837    0.181635
 7     -6.294353    1.904688   -0.439444
 6     -6.611034    0.741819   -1.111209
16     -1.926901   -0.875608    1.119922
 6     -2.695007   -1.704562    2.567395
 7      0.338538   -2.323620   -0.925206
 6      1.446070   -1.635560   -0.618740
 7      1.395990   -0.625286    0.251394
 7      2.625935   -1.953051   -1.171971
 8      4.162198    0.204359   -0.033462
16      4.707898    1.378936   -1.150815
 6      4.195558    2.915187   -0.329191
 8      6.326918   -0.317292    1.483728
 1     -1.908785   -2.258161    3.085287
 1     -3.471448   -2.400272    2.244580
 1     -3.113844   -0.961151    3.247932
 1     -3.054365   -0.260952    0.594608
 1     -5.439818   -1.129730   -1.205431
 1     -7.518364    0.638138   -1.685912
 1     -6.851969    2.746962   -0.411123
 1     -4.620618    2.472412    0.782325
 1     -0.540351   -2.114556   -0.446966
 1      0.397446   -3.180706   -1.454950
 1      2.671625   -2.565458   -1.972605
 1      3.424811   -1.352053   -0.963751
 1      0.532644   -0.399067    0.735772
 1      2.240194   -0.077291    0.407965
 1      4.922049    0.013275    0.583497
 1      4.488831    3.720588   -1.012980
 1      3.111792    2.950129   -0.191486
 1      4.710960    3.059155    0.623882
 1      7.148272   -0.187123    0.984107
 1      6.470911    0.128195    2.333020
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt=(ts,noeigentest,calcfc) freq

Transition state 5TS

1,1
```
6      3.017156    1.562641   -4.542701
7      3.014000    1.553187   -3.164918
6      4.272808    1.530978   -2.757966
7      5.090577    1.525274   -3.832410
6      4.314062    1.545180   -4.975511
16     0.668970    1.611598   -1.386248
6      0.442769    3.435963   -1.240157
7     -2.206439   -0.015986   -1.298127
6     -2.233235   -0.387225   -0.011219
7     -1.182818   -0.152071    0.773926
7     -3.316508   -0.992818    0.506940
8     -2.219937   -1.587256    3.119140
16    -1.955597   -3.247188    3.424006
6     -0.237362   -3.229644    4.011767
8     -3.399455   -0.682815    5.373743
1     -0.433600    3.640050   -0.619433
1      0.283106    3.888574   -2.222132
1      1.313204    3.895752   -0.765274
1      1.977357    1.564350   -2.386879
1      2.098813    1.580504   -5.109651
1      4.746180    1.545554   -5.963946
1      6.101682    1.510506   -3.802100
1      4.605024    1.518429   -1.730572
1     -1.402218    0.529941   -1.635785
1     -3.062175    0.012070   -1.832954
1     -4.005156   -1.405205   -0.104796
1     -3.247413   -1.340159    1.464023
1     -0.393493    0.390499    0.415512
1     -1.190796   -0.517683    1.722599
1     -2.618766   -1.199190    3.945492
1      0.021027   -4.280771    4.186601
1      0.436070   -2.824496    3.252098
1     -0.133279   -2.681102    4.951505
1     -3.930029   -1.375988    5.796912
1     -2.873239   -0.292099    6.088432
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt freq

Intermediate 6

1,1
```
6      3.055016    1.413510   -4.707765
7      3.048993    1.382184   -3.330875
6      4.301380    1.355619   -2.898612
7      5.120979    1.368439   -3.965825
6      4.357677    1.404914   -5.118853
16     0.675073    1.402867   -1.498074
6      0.384299    3.215451   -1.279771
7     -2.237977   -0.085199   -1.283443
6     -2.251734   -0.374756    0.024707
7     -1.168087   -0.149178    0.764358
7     -3.353684   -0.886521    0.605676
8     -2.222263   -1.389360    3.230527
16    -2.048709   -3.035139    3.649596
6     -0.299163   -3.092721    4.133651
8     -3.240995   -0.260521    5.465373
1     -0.488819    3.376422   -0.640679
1      0.196075    3.701511   -2.241147
1      1.244501    3.692202   -0.801550
1      2.137104    1.385715   -2.653729
1      2.138622    1.438630   -5.276627
1      4.801527    1.421300   -6.101758
1      6.132259    1.354760   -3.925096
1      4.611164    1.327781   -1.864977
1     -1.412024    0.410198   -1.657597
1     -3.108489   -0.019601   -1.790700
1     -4.068721   -1.311905    0.034370
1     -3.274717   -1.182594    1.578698
1     -0.366024    0.333731    0.341043
1     -1.163821   -0.455964    1.732814
1     -2.558066   -0.917936    4.040838
1     -0.099835   -4.141646    4.383049
1      0.349432   -2.799633    3.304088
1     -0.099068   -2.477574    5.014850
1     -3.785877   -0.897644    5.953472
1     -2.657427    0.137469    6.129701
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt freq

Intermediate 7

1,1
```
16     0.104886    0.120415   -0.781202
16     0.116507    0.151912    3.422329
8      1.205296    0.129531    2.107259
7      2.504414   -2.095826    1.399480
6      2.207109   -2.502690    0.168075
7      2.942560   -3.597040   -0.082786
6      3.727969   -3.892627    1.019218
6      3.446059   -2.938766    1.952886
6      1.368174    1.198117   -1.601323
7     -2.537093    1.830709   -1.774258
6     -2.818255    1.142272   -2.877069
7     -2.041166    0.107226   -3.223083
7     -3.879316    1.487498   -3.633020
1      1.461636    2.153727   -1.078496
1      1.083989    1.398302   -2.639333
1      2.350161    0.714875   -1.609811
1     -0.093722    1.862293    5.038345
1      0.257667    2.584505    3.445159
1      1.572435    1.829764    4.407546
1      0.704666    0.287524    1.240207
1      2.051338   -1.237253    1.821976
1      1.495501   -1.991985   -0.483201
1      2.919251   -4.118388   -0.949581
1      4.400975   -4.734938    1.039530
1      3.831888   -2.793952    2.949499
1     -2.367989   -0.565729   -3.900765
1     -1.292972   -0.166028   -2.561465
1     -1.762600    1.501699   -1.177046
1     -3.074175    2.663739   -1.556483
1     -4.388701    2.333868   -3.386494
1     -3.956535    1.139909   -4.577247
6      0.516454    1.774906    4.131683
8     -4.726664    3.805476   -2.102976
1     -4.655953    4.745497   -2.333451
1     -5.519057    3.740051   -1.546350
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt=(ts,calcfc,noeigentest) freq

Transition state 8TS

1,1
```
 6      1.978801   -0.054541    2.739537
 7      1.923676   -0.140493    4.076913
 6      3.202828   -0.221878    4.599650
 6      4.049938   -0.183825    3.532177
 7      3.269362   -0.080817    2.397341
 8      3.765999    0.208189   -0.318908
16      1.977853    0.165325   -0.504141
16     -0.818728   -0.193035    0.221455
 6     -0.599391   -2.025097    0.204105
 6      1.811848    1.841815   -1.202000
 7     -2.798308    0.011647   -2.371960
 6     -3.965948    0.164806   -1.753125
 7     -5.086424    0.376657   -2.473429
 7     -4.012066    0.109747   -0.416099
 1     -5.989422    0.239797   -2.043880
 1     -5.021898    0.306737   -3.486870
 1     -4.818729    0.460341    0.079309
 1     -3.108557    0.026294    0.084229
 1     -2.773768   -0.034502   -3.385273
 1     -1.943714   -0.038838   -1.790900
 1     -1.101788   -2.482083   -0.654523
 1     -1.000671   -2.474365    1.117387
 1      0.467351   -2.273278    0.135978
 1      0.741568    2.039092   -1.286482
 1      2.274798    1.890810   -2.190523
 1      2.270768    2.577612   -0.537053
 1      3.610940   -0.027638    1.411881
 1      5.126877   -0.218581    3.486694
 1      3.392254   -0.296869    5.658492
 1      1.061862   -0.142558    4.607978
 1      1.129187    0.019564    2.059315
 1      4.145437   -0.378025   -0.995524
 8     -3.910560    0.056287   -5.122842
 1     -4.000918   -0.728409   -5.686620
 1     -3.793229    0.797597   -5.738054
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****

#B3LYP/6-31G(d) extrabasis opt freq

Product 9

1,1
```
6     5.028333   -1.148546   -1.537729
7     4.842076   -0.177805   -0.575576
6     5.973164    0.499225   -0.523918
7     6.879330    0.005995   -1.410030
6     6.287887   -1.052674   -2.068614
16    0.220073    0.433230   -0.113081
6     0.444296    2.254199   -0.043251
16   -1.676327    0.279902   -1.014709
6    -1.380472    0.290712   -2.826194
7    -4.384572    0.151732    1.079225
6    -4.265761   -1.115401    1.482212
7    -5.062403   -1.564172    2.462703
7    -3.370521   -1.926512    0.904375
8    -6.567562    0.875131    2.866851
1     4.247314   -1.852968   -1.785729
1     6.804768   -1.618741   -2.828050
1     7.817365    0.350830   -1.557423
1     6.180505    1.337472    0.126274
1     1.383285    2.382623    0.497947
1    -0.376201    2.722869    0.504076
1     0.527914    2.677409   -1.046452
1    -2.350770    0.125869   -3.304898
1    -0.692360   -0.511224   -3.099617
1    -0.982350    1.258207   -3.138211
1    -5.760775   -0.934007    2.853985
1    -5.108134   -2.546875    2.686758
1    -3.153322   -2.825198    1.309866
1    -2.727148   -1.552753    0.204295
1    -3.729264    0.543304    0.409331
1    -5.096082    0.740624    1.505968
1    -7.483958    1.017585    2.579222
1    -6.452347    1.450267    3.640684
8     2.726041    0.602665    1.138778
1     2.716113   -0.080959    1.824510
1     3.415626    0.293431    0.497164
```

S 0
SP 1 1.00
0.0405 1.0 1.0
****