Supporting Information

Computing Proton-Coupled Redox Potentials of Fluorotyrosines in a Protein Environment

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Computational Details

Protein preparation and equilibration

The equilibration of the system for molecular dynamics (MD) consisted of a stepwise process. First, the positions of the solvent and ions were energy minimized with 5000 steps steepest descent (SD) while the protein was effectively fixed by restraints with force constants of 500 kcal mol^{-1} $Å^{-2}$. Next, the solvent was equilibrated at 300 K for 500 ps in the NVT ensemble followed by 500 ps in the NPT ensemble with the Berendsen barostat,[1] while the protein remained fixed with the same restraints. After this equilibration of the solvent and ions, the protein was energy minimized while the solvent and ions remained fixed. First, the protein hydrogen atoms were minimized with 2000 steps SD, followed by 3000 steps conjugate gradient (CG), while the remaining atoms of the protein were restrained with 100 kcal mol^{-1} $Å^{-2}$ force constants. This step is not necessary for the standard NMR structure, as it has hydrogen atoms present, but was performed to be consistent with standard protocol. The restraints on the sidechains were then released, and the protein was minimized with another cycle of 2000 steps SD and 3000 steps CG minimization. Next, the sidechains were minimized while the backbone was restrained with the same force constants. This procedure was repeated with the backbone restraints decreased to force constants of 50 kcal mol^{-1} $Å^{-2}$ and then 10 kcal mol^{-1} $Å^{-2}$. Finally, all protein restraints were released for a final minimization with 2500 steps SD followed by 2500 steps CG.

The minimized structure was heated from 0 K to 300 K over 360 ps, where heating by 50 K increments occurred over 10 ps, followed by a 50 ps equilibrating stage. The system was then equilibrated for 20 ns in the NPT ensemble, followed by another 100 ns after switching to the NVT ensemble. After this equilibration, the production simulations were conducted for 1 μs for the Y-OH systems and 100 ns for the Y-O$^{\bullet}$ systems. In all simulations, a time step of 1 fs was used, with a collision frequency of 2.0 ps^{-1} for the Langevin thermostat. Electrostatic interactions were treated with the Particle Mesh Ewald[2] method with the cut-off set to 10 Å. The minimizations were conducted with pmemd.MPI, and the MD simulations were performed with the pmemd.cuda[3] program in Amber 18.[4] In the protein, bond lengths involving hydrogen atoms were constrained with the SHAKE algorithm,[5] and the triangulated TIP3P waters were constrained with the SETTLE algorithm.[6] CPPTRAJ was used to analyze the trajectories.[7] Fluorinated tyrosine simulations followed exactly the same procedure, and the number of water molecules and ions were the same as the wild-type system to ensure uniform system size.

As an additional test of the force field parameters, we also simulated an individual tyrosyl radical in aqueous solution. This simulation was conducted by building a neutral tyrosyl radical residue with N- and C- termini capping groups (ACE-YRA-NME), solvating the system with 3827 explicit TIP3P water molecules and no counterions in a periodic rectilinear box, and following the equilibration procedure described above. Then we analyzed the hydrogen-bonding interactions between the tyrosyl radical and water for a 100 ns trajectory in the NVT ensemble. Using a 3.0 Å donor-acceptor distance cut-off resulted in a hydrogen bond for 65.1% of the trajectory, and increasing the cut-off to 3.2 Å resulted in a hydrogen bond for 92.5% of the trajectory. This simulation demonstrates that the tyrosyl radical is able to form hydrogen bonds with water.

Calculation of Proton-Coupled Redox Potential in the Gas Phase

Redox potentials of the fluorinated tyrosine side chains in the gas phase were calculated relative to phenol to mimic the tyrosine side chain. The asymmetric fluorination pattern of the phenol ring exerts a preference to the orientation of the hydroxyl proton, and it is important to probe both cases for bias towards starting structures during optimization. Typically, the more favorable conformation corresponds to the hydroxyl proton oriented on the side of the ring that is fluorinated. In the gas phase, this effect can lead to shifts in the redox potential greater than the difference between the species themselves if the proper conformation is not chosen. The geometry optimizations were performed with QCHEM 5.1[8] with no symmetry assumed and 10^{-8} tolerance for the SCF convergence. The threshold for neglecting two-electron integrals was set to 10^{-11}. Sample input files for these calculations are provided below.

```
# QCHEM Input file
$comment
YH Optimization
$end

$molecule
0 1
 H          0.20998700   0.00000000   0.00000000
 C          1.29998700   0.00000000   0.00000000
 C          2.01967000   1.20498000   0.00000000
 H          1.47912900   2.14809100   0.00887300
 C          3.41134900   1.23000400  -0.01143500
 H          3.95940700   2.16651100  -0.01292400
 C          4.11159600   0.02185900  -0.02211800
 O          5.49287800   0.08822400  -0.02944400
 H          5.87230300  -0.79787000  -0.08413900
 C          3.42642900  -1.19128700  -0.02287000
 H          3.97221000  -2.13202900  -0.02973900
 C          2.02837400  -1.19236700  -0.01200500
 H          1.50208000  -2.14281400  -0.01110400
$end

$rem
  BASIS = 6-31+G**
  THRESH = 11
  DFT_D = D3_BJ
  GEOM_OPT_SYMMETRY = 0
  GUI = 2
  JOB_TYPE = Optimization
  GEOM_OPT_COORDS = 0
  METHOD = B3LYP
  SCF_CONVERGENCE = 8
  SCF_MAX_CYCLES = 100
  SYMMETRY_IGNORE = 1
$end
```

```
@@@

$comment
YH Frequencies
$end

$molecule
read
$end

$rem
  BASIS = 6-31+G**
  THRESH = 11
  DFT_D = D3_BJ
  GEOM_OPT_SYMMETRY = 0
  GUI = 2
  JOB_TYPE = Frequency
  METHOD = B3LYP
  SCF_CONVERGENCE = 8
  SCF_MAX_CYCLES = 100
  SYMMETRY_IGNORE = 1
$end
```

Calculation of Proton-Coupled Redox Potential in the Protein Environment

Redox potentials in the protein environment were calculated from 10,000 conformations. For the oxidized radical Y-O• state, the trajectories were 100 ns with conformations obtained every 10 ps, and for the reduced Y-OH state, the trajectories were 1 μs with conformations obtained every 100 ps. The trajectories were first imaged and recentered to properly place the protein in the center of the box so the solvent molecules and ions were even on each side, ensuring a balanced electric field. After the trajectories were centered, an automated procedure was used to abstract individual frames, add the hydrogen link atom with AMBER, and write a Q-Chem input file specifying optimization and no symmetry. The SCF convergence threshold was set to 10^{-8}, and the threshold for neglecting two-electron integrals was set to 10^{-11}. To generate the radical species from the reduced trajectory, the same procedure was followed except the tyrosine hydroxyl proton was deleted, and the spin multiplicity was updated. To generate the reduced species from the oxidized trajectory, the conformation was saved as a PDB file, and the tyrosine was renamed to the reduced form. This PDB file was then input into the tLEaP program in AmberTools,[9] where the proton was built from the internal coordinates present in the library file. No other manipulations to the coordinates were performed to ensure that the environment was exactly the same.

After the geometry optimizations were completed, the optimized structures of the tyrosine with the protein environment were subjected to a frequency calculation to verify that the optimization had identified a minimum by checking for imaginary frequencies. These files were then processed, adding zero-point energy and entropic contributions to generate the free energies

used to compute the proton-coupled redox potentials. This step included a check for incomplete optimizations and imaginary frequencies and for jobs that terminated abnormally. These problematic structures typically amounted to <1 % of the calculations per data set, and their associated redox potentials were excluded from the final value.
Sample optimization input file for the protein containing system with coordinates and charges removed due to size limitations.

```
$molecule
$end

$external_charges
$end

$rem
SYMMETRY false
SYM_IGNORE true
JOBTYPE opt
EXCHANGE B3LYP
BASIS 6-31+G**
SCF_CONVERGENCE 8
MAX_SCF_CYCLES 250
THRESH 11
DFT_D D3_BJ
$end
```

Table S1. Partial Charges and Atom Types of Residue 2,3,6-F_3Y

2,3,6-F_3Y

		OH	O•
Atom name	Atom type	Charge	Charge
N	N	-0.415700	-0.415700
H	H	0.271900	0.271900
CA	CX	0.087060	0.001687
HA	H1	0.079580	0.108027
CB	CT	-0.064203	0.079594
HB2	HC	0.051499	0.030583
HB3	HC	0.051499	0.030583
CG	CA	-0.219137	-0.230231
CD1	CA	0.290052	0.216714
F	F	-0.136323	-0.132714
CE1	CA	-0.005128	0.061959
F1	F	-0.161669	-0.153578
CZ	C	0.355753	0.551009
OH	OH	-0.577018	-0.378542
HH	HO	0.452109	N/A
CE2	CA	-0.439941	-0.510283
HE2	HA	0.260172	0.246765
CD2	CA	0.251899	0.390043
F2	F	-0.161804	-0.197216
C	C	0.597300	0.597300
O	O	-0.567900	-0.567900

Table S2. Partial Charges and Atom Types of Residue 2,3,5-F_3Y

2,3,5-F_3Y

		OH	O•
Atom name	Atom type	Charge	Charge
N	N	-0.415700	-0.415700
H	H	0.271900	0.271900
CA	CX	-0.078983	0.041042
HA	H1	0.128087	0.082290
CB	CT	0.070507	0.005282
HB2	HC	0.046383	0.038939
HB3	HC	0.046383	0.038939
CG	CA	-0.085532	-0.040458
CD1	CA	0.175082	0.156150
F	F	-0.154469	-0.133342
CE1	CA	0.087331	0.103673
F1	F	-0.161799	-0.150411
CZ	C	0.182086	0.385325
OH	OH	-0.554346	-0.346546
HH	HO	0.449891	N/A
CE2	CA	0.254969	0.215005
F2	F	-0.162430	-0.175207
CD2	CA	-0.362373	-0.311667
HD2	HA	0.233613	0.205386
C	C	0.597300	0.597300
O	O	-0.567900	-0.567900

Table S3. Partial Charges and Atom Types of Residue 2,3-F_2Y

2,3-F_2Y

		OH	O•
Atom name	Atom type	Charge	Charge
N	N	-0.4157	-0.4157
H	H	0.2719	0.2719
CA	CX	-0.03267	0.03646
HA	H1	0.140248	0.066417
CB	CT	0.040834	0.069246
HB2	HC	0.009922	0.01227
HB3	HC	0.009922	0.01227
CG	CA	-0.060725	-0.020208
CD1	CA	0.226685	0.151363
F	F	-0.165122	-0.160725
CE1	CA	0.034122	0.122281
F1	F	-0.169496	-0.166411
CZ	C	0.329479	0.499282
OH	OH	-0.598274	-0.386857
HH	HO	0.453941	N/A
CE2	CA	-0.311009	-0.330946
HE2	HA	0.224905	0.197273
CD2	CA	-0.220128	-0.17095
HD2	HA	0.201766	0.183636
C	C	0.5973	0.5973
O	O	-0.5679	-0.5679

Table S4. Partial Charges and Atom Types of Residue 3,5-F_2Y

3,5-F_2Y

		OH	O•
Atom name	Atom type	Charge	Charge
N	N	-0.4157	-0.4157
H	H	0.2719	0.2719
CA	CX	0.001054	0.030889
HA	H1	0.09768	0.09278
CB	CT	0.024176	-0.062887
HB2	HC	0.018228	0.044232
HB3	HC	0.018228	0.044232
CG	CA	0.132814	0.110353
CD1	CA	-0.428609	-0.33709
HE1	HA	0.219861	0.211895
CE1	CA	0.307876	0.222601
F	F	-0.182804	-0.182434
CZ	C	0.090567	0.38952
OH	OH	-0.492907	-0.363104
HH	HO	0.391912	N/A
CE2	CA	0.307876	0.222601
F1	F	-0.182804	-0.182434
CD2	CA	-0.428609	-0.33709
HD2	HA	0.219861	0.211895
C	C	0.5973	0.5973
O	O	-0.5679	-0.5679

Table S5. Associated Force Field Parameters Adapted from GAFF for Fluorinated Tyrosines

```
ANGL
C -CA -F    70.000  121.000 ! Taken by analogy to CA-CA-F
F -CA -C    70.000  121.000 ! Taken by analogy to CA-CA-F
```

Table S6. Root-Mean-Square Deviations (RMSDs) for Molecular Dynamics Trajectories

System[a]	RMSD (C_α)
α_3(2,3,6)F_3Y-OH	1.8 ± 0.4 Å
α_3(2,3,6)F_3Y-O$^\bullet$	1.3 ± 0.3 Å
α_3(2,3)F_2Y-OH	1.4 ± 0.3 Å
α_3(2,3)F_2Y-O$^\bullet$	1.1 ± 0.3 Å
α_3(2,3,5)F_3Y-OH	1.4 ± 0.3 Å
α_3(2,3,5)F_3Y-O$^\bullet$	1.1 ± 0.2 Å
α_3Y	1.3 ± 0.4 Å
α_3Y-O$^\bullet$	1.3 ± 0.2 Å
α_3(3,5)F_2Y	1.6 ± 0.2 Å
α_3(3,5)F_2Y-O$^\bullet$	1.2 ± 0.2 Å

[a]RMSD values were calculated with respect to the starting NMR structure. Each value for the Y-OH systems is the average and standard deviation from 1 μs trajectories, and each value for the Y-O$^\bullet$ systems is the average and standard deviation from 100 ns trajectories.

Table S7. Proton-Coupled Redox Potentials for Y and F_nY Computed in the Gas Phase with Different Functionals and Basis Sets[a]

System	$\Delta E°_{expt}$	$\Delta E°$ B3LYP-D3(BJ) 6-31G(d,p)	$\Delta E°$ B3LYP-D3(BJ) 6-31+G(d,p)	$\Delta E°$ B3LYP-D3(BJ) 6-31++G(d,p)	$\Delta E°$ B3LYP 6-31++G(d,p)	$\Delta E°$ ωB97X-D 6-31+G(d,p)
2,3,6-F_3Y	135	123	142	141	139	140
2,3-F_2Y	70	86	102	89	99	129
2,3,5-F_3Y	39	8	46	45	43	33
Y	0	0	0	0	0	0
3,5-F_2Y	–25	–31	10	9	7	–26
MUE		16	20	16	17	18

[a]All values are reported in mV relative to phenol at the given level of theory. The mean unsigned error (MUE) relative to experiment is given for each level of theory. The experimental values are cited in Table 1 in the main paper.

Table S8. Computed Proton-Coupled Redox Potentials in the Protein Environment Relative to the Gas-phase Value for Phenol[a]

System	$\Delta E^{o}_{\text{prot-red}}$ [b]	$\Delta E^{o}_{\text{prot-red,all}}$ [c]	$\Delta E^{o}_{\text{prot-ox}}$ [d]	$\Delta E^{o}_{\text{prot-ave}}$ [e]
$\alpha_3(2,3,6)F_3Y$	664	547	–700	–18
$\alpha_3(2,3)F_2Y$	629	403	–888	–129
$\alpha_3(2,3,5)F_3Y$	555	299	–956	–200
α_3Y	446	425	–831	–192
$\alpha_3(3,5)F_2Y$	479	138	–908	–214

[a]All values are reported in mV relative to the gas phase phenol value, where phenol represents Y. Note that the values obtained from reduced (Y-OH) trajectories are greater than the value for phenol, whereas the values obtained from oxidized (Y-O•) trajectories are less than the value for phenol because sampling in a particular state stabilizes that state relative to the other state. Thus, the proton-coupled redox potentials are only meaningful relative to the α_3Y value obtained by sampling in the same state (i.e., reduced or oxidized).
[b]These values were obtained by averaging over all conformations with Y-OH hydrogen bonded to at least one water molecule among 10,000 conformations equally distributed along the 1 μs trajectory with Y-OH for each system.
[c]These values were obtained by averaging over all conformations equally distributed along the 1 μs trajectory with Y-OH for each system.
[d]These values were obtained by averaging over 10,000 conformations equally distributed along the 100 ns trajectory with Y-O• for each system.
[e]These values were obtained by averaging the previous two columns (data columns 1 and column 3) corresponding to the hydrogen bonded conformations from Y-OH and Y-O• for each system.

Table S9. Experimental and Computed Relative Proton-Coupled Redox Potentials in the Protein Environment with Standard Deviations Using B3LYP-D3(BJ) Functional[a]

System	ΔE^{o}_{expt} [b]	$\Delta E^{o}_{prot\text{-}red}$ [c]	$\Delta E^{o}_{prot\text{-}red,all}$ [d]	$\Delta E^{o}_{prot\text{-}ox}$ [e]	$\Delta E^{o}_{prot\text{-}ave}$ [f]
$\alpha_3(2,3,6)F_3Y$	135	218 ± 207	122 ± 238	130 ± 218	174 ± 212
$\alpha_3(2,3)F_2Y$	70	183 ± 208	–22 ± 206	–57 ± 203	63 ± 205
$\alpha_3(2,3,5)F_3Y$	39	110 ± 206	–125 ± 206	–124 ± 205	–7 ± 205
α_3Y	0	0 ± 195	0 ± 203	0 ± 224	0 ± 210
$\alpha_3(3,5)F_2Y$	–25	33 ± 198	–286 ± 204	–77 ± 205	–22 ± 202
MUE		81	133	87	24

[a]All values are reported in mV relative to α_3Y in the associated column. The mean unsigned error (MUE) relative to experiment is given for each method.
[b]The experimental values were obtained from Ref. 10.
[c]These values were obtained by averaging over all conformations with Y-OH hydrogen bonded to at least one water molecule among 10,000 conformations equally distributed along the 1 μs trajectory for each system.
[d]These values were obtained by averaging over all conformations equally distributed along the 1 μs trajectory with Y-OH for each system.
[e]These values were obtained by averaging over 10,000 conformations equally distributed along the 100 ns trajectory with Y-O$^{\bullet}$ for each system. Values computed for 10,000 conformations equally distributed along the subsequent 100 ns of an extended 200 ns trajectory for the $\alpha_3(2,3,5)F_3Y$-O$^{\bullet}$ and $\alpha_3(2,3,6)F_3Y$-O$^{\bullet}$ systems were –125 ± 213 mV and 125 ± 226 mV, respectively, exhibiting consistency to within 5 mV.
[f]These values were obtained by averaging the previous two columns (data columns 2 and column 4) corresponding to Y-OH and Y-O$^{\bullet}$ for each system.

Table S10. Experimental and Computed Relative Proton-Coupled Redox Potentials in the Protein Environment with Standard Deviations Using ωB97X-D Functional[a]

System	ΔE^{o}_{expt} [b]	$\Delta E^{o}_{prot\text{-}red}$ [c]	$\Delta E^{o}_{prot\text{-}red,all}$ [d]	$\Delta E^{o}_{prot\text{-}ox}$ [e]	$\Delta E^{o}_{prot\text{-}ave}$ [f]
$\alpha_3(2,3,6)F_3Y$	135	222 ± 214	136 ± 200	125 ± 223	173 ± 218
$\alpha_3(2,3)F_2Y$	70	167 ± 209	34 ± 217	–55 ± 213	56 ± 211
$\alpha_3(2,3,5)F_3Y$	39	71 ± 212	–125 ± 214	–118 ± 209	–23 ± 210
α_3Y	0	0 ± 219	0 ± 206	0 ± 221	0 ± 220
$\alpha_3(3,5)F_2Y$	–25	–9 ± 224	–301 ± 243	–82 ± 207	–45 ± 211
MUE		58	120	87	34

[a]All values are reported in mV relative to α_3Y in the associated column. The mean unsigned error (MUE) relative to experiment is given for each method.
[b]The experimental values were obtained from Ref. 10.
[c]These values were obtained by averaging over all conformations with Y-OH hydrogen bonded to at least one water molecule among 10,000 conformations equally distributed along the 1 μs trajectory for each system.
[d]These values were obtained by averaging over all conformations equally distributed along the 1 μs trajectory with Y-OH for each system.
[e]These values were obtained by averaging over 10,000 conformations equally distributed along the 100 ns trajectory with Y-O$^{\bullet}$ for each system.
[f]These values were obtained by averaging the previous two columns (data columns 2 and column 4) corresponding to Y-OH and Y-O$^{\bullet}$ for each system.

Table S11. Hydrogen-Bonding Interactions Between Y-OH and Water for MD Trajectories of α_3Y and α_3F_nY Systems[a]

System	Y-OH(total)	Y-OH(donor)	Y-OH(acceptor)
$\alpha_3(2,3,6)F_3Y$	58.2%	52.2%	6%
$\alpha_3(2,3)F_2Y$	27.5%	22.5%	5%
$\alpha_3(2,3,5)F_3Y$	17.4%	16.1%	1.3%
α_3Y	38.2%	5%	33.2%
α_3Y-O•	6.1 %	NA	6.1 %
$\alpha_3(3,5)F_2Y$	5.1%	1.7%	3.4%

[a]Each value is the percentage of conformations with Y-OH hydrogen bonded to a water molecule, Y-OH(total), also divided into the Y-OH serving as the hydrogen bond donor, Y-OH(donor), or acceptor, Y-OH(acceptor), over a 1 μs trajectory. In 90.4% of occurrences that Y was serving as a hydrogen bond acceptor, it was found to be also donating a hydrogen bond to the backbone carbonyl of V9 or to E13. The data for the α_3Y-O• trajectory is also given, with NA representing "Not Applicable" as the tyrosine radical cannot donate a hydrogen bond to water.

References

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