Supporting Information

SPECTROSCOPIC AND COMPUTATIONAL INVESTIGATION OF THE H155A VARIANT OF CYSTEINE DIOXYGENASE: GEOMETRIC AND ELECTRONIC CONSEQUENCES OF A THIRD-SPHERE AMINO ACID SUBSTITUTION

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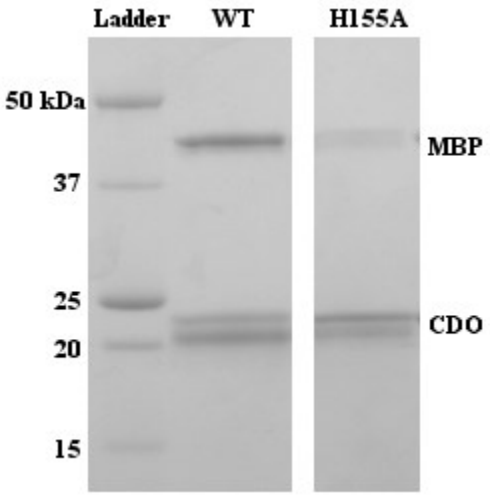

Figure S1: Fragment of an SDS-PAGE gel illustrating protein purity and degree of crosslink formation for typical isolations of WT and H155A CDO. Note that residual maltose binding protein (MBP) present does not interfere with our spectroscopic methods.

Table S1: Experimentally derived and computationally predicted EPR parameters for the (OH^-/Cys) adducts of H155A and WT Fe(III)CDO. Values for WT CDO are reproduced from Ref. 17.

	g_1	g_2	g_3	D (cm^{-1})	E/D
WT, exp.	2.030	2.030	2.030	| 0.726 |	0.317
WT, comp.	2.011	2.016	2.028	0.868	0.207
H155A, exp.	2.030	2.030	2.030	| 0.726 |	0.317
H155A, comp.	2.010	2.015	2.027	0.954	0.272

Table S2: Experimentally derived and computationally predicted EPR parameters for the (NO/Cys) adducts of H155A and WT Fe(II)CDO. Hyperfine coupling constants for the ^{14}N nucleus of the NO ligand are given in MHz. All values for WT CDO are reproduced from Ref. 20.

Species	g_1	g_2	g_3	A_1	A_2	A_3
WT, exp.	1.979	2.028	2.071	<30	100	<30
WT, comp.	1.962	1.990	2.016	15	90	11
H155A, exp.	1.974	2.015	2.075	<30	57	<30
H155A, comp.	1.952	1.997	2.036	12	81	10

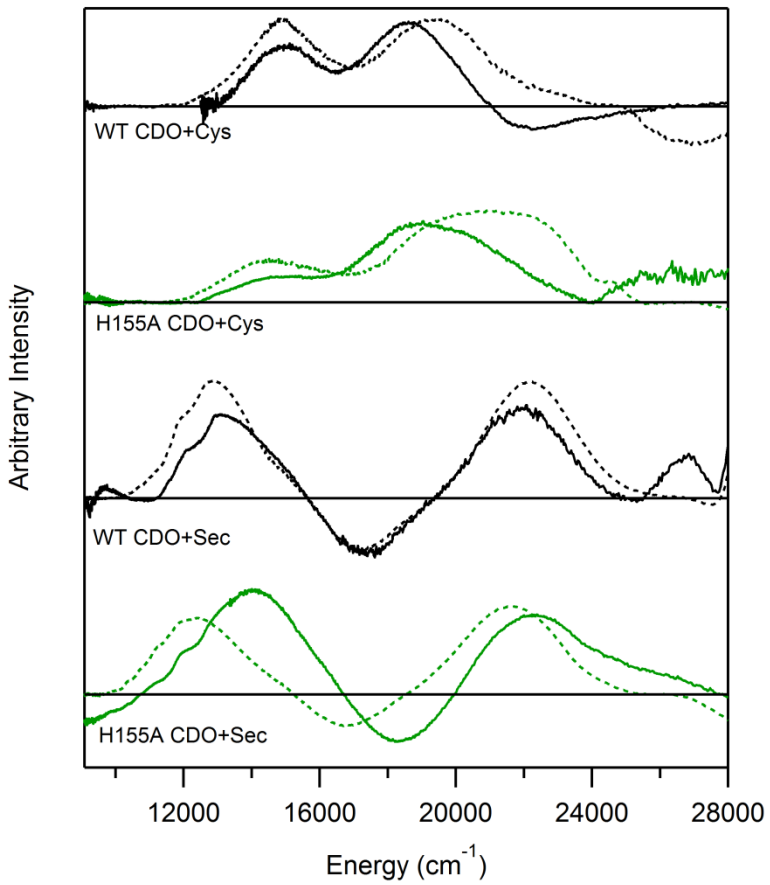

Figure S2: MCD spectra (4.5 K, 7 T) of as-isolated Cys- or Sec-bound WT and H155A CDO in the presence (dotted lines) and absence (solid lines) of sodium azide.

Table S3. Bond distances (in Angstroms) for various Sec-bound CDO species as obtained by whole-protein QM/MM optimizations, as well as for the crystallographically characterized Cys-adduct of WT Fe(II)CDO, where X refers to the ligating atom of the sixth ligand present at the Fe center (where applicable).

Species	Fe-N_{H86}	Fe-N_{H88}	Fe-N_{H140}	Fe-Se_{Sec}	Fe-N_{Sec}	Fe-X	Sec-R60, 1	Sec-R60, 2	Sec-Y157
4JTO, WT (Cys)-Fe(II)CDO[a]	1.893	2.199	2.110	2.291	2.262	N/A	N/A	N/A	N/A
WT (Sec)-Fe(II)CDO	2.166	2.171	2.149	2.529	2.250	N/A	1.769	1.940	1.525
H155A (Sec)-Fe(II)CDO	2.158	2.203	2.174	2.503	2.256	N/A	1.712	2.101	1.552
H155A (H_2O/Sec)-Fe(II)CDO	2.191	2.294	2.166	2.654	2.24	2.283	1.805	1.680	1.509
WT (OH^-/Sec)-Fe(III)CDO	2.330	2.295	2.108	2.599	2.226	1.877	1.981	1.785	1.535
H155A (OH^-/Sec)-Fe(III)CDO	2.334	2.259	2.116	2.601	2.232	1.86	1.91	1.824	1.571
WT (NO/Sec)-Fe(II)CDO	2.116	2.102	2.015	2.506	2.131	1.775	1.768	1.996	1.529
H155A (NO/Sec)-Fe(II)CDO	2.141	2.159	2.032	2.490	2.073	1.778	1.658	1.918	1.545

a Crystal structure at 2.00 Å resolution, from Ref. 14.

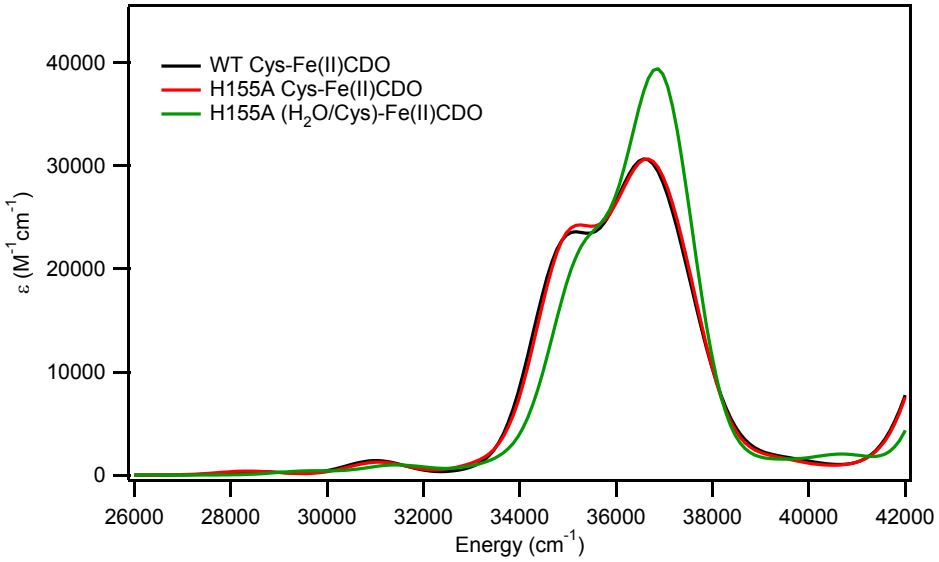

Figure S3: INDO/S-CI predicted Abs spectra for the Cys adducts of WT and H155A Fe(II)CDO.

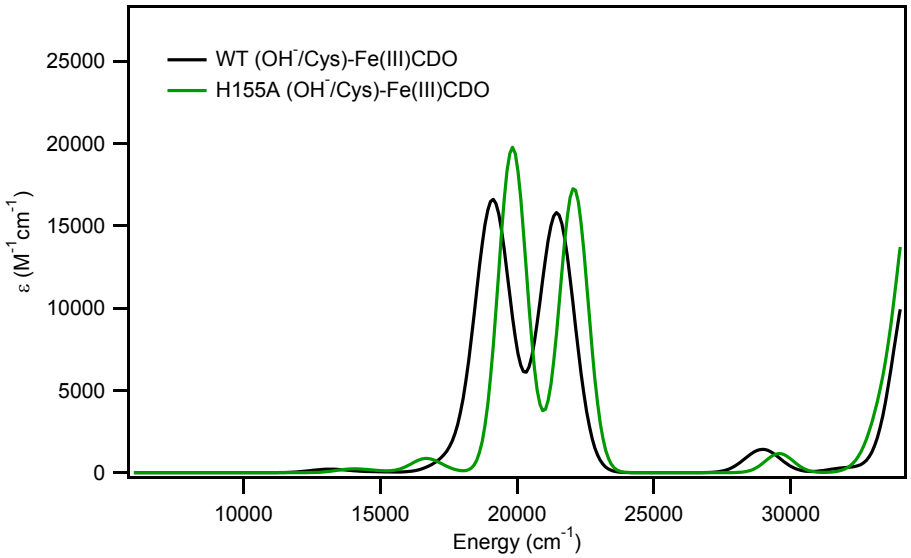

Figure S4: INDO/S-CI predicted Abs spectra for the (OH^-/Cys) adducts of WT and H155A Fe(III)CDO.

Table S4: Cartesian coordinates (in Å) for the QM/MM-optimized active site of WT Cys-Fe(II)CDO (SCF Energy = -4612.77465483 Eh)

Atom	x	y	z	Atom	x	y	z
C	-2.594452	-0.263107	4.972992	C	0.394608	-4.355850	0.557083
C	-1.462051	-0.158005	4.015366	C	-0.170700	-3.139679	0.870178
C	-1.445435	0.325180	2.731537	N	1.208755	-4.083466	-0.542450
N	-0.189835	-0.700027	4.204865	C	1.116089	-2.758606	-0.845444
C	0.522522	-0.550064	3.045532	N	0.294693	-2.156937	0.001389
N	-0.217407	0.073624	2.140366	H	-0.221725	-5.830261	2.153702
H	-3.079620	-1.312134	5.005951	H	1.281403	-6.176620	1.194229
H	-3.370377	0.449097	4.673660	H	-0.271378	-6.376450	0.416672
H	-2.301346	0.010376	5.992416	H	-0.865829	-2.898819	1.658356
H	-2.204559	0.876160	2.208725	H	1.732407	-4.753082	-1.106659
H	0.135086	-1.185974	5.030960	H	1.665146	-2.284042	-1.641342
H	1.533478	-0.889526	2.894700	Fe	0.000000	0.000000	0.000000
C	-5.334076	0.429443	-2.196075	N	0.000015	2.249649	-0.000015
C	-3.923981	-0.048172	-1.986786	C	1.245178	2.742264	0.671204
C	-2.746262	0.625763	-1.707870	C	1.394104	4.267990	0.561356
N	-3.632095	-1.376373	-1.721024	O	1.205185	4.793274	-0.624588
C	-2.358017	-1.463058	-1.266937	C	2.494705	2.074524	0.054779
N	-1.776489	-0.264557	-1.242218	S	2.354416	0.238129	-0.072891
H	-5.801163	0.738892	-1.185104	O	1.752502	4.949829	1.574234
H	-5.959793	-0.347687	-2.647354	H	-0.808365	2.699921	0.433075
H	-5.360291	1.297546	-2.861053	H	0.027527	2.529068	-0.982956
H	-2.501633	1.653931	-1.916351	H	1.163651	2.476288	1.727081
H	-4.212112	-2.215469	-1.827881	H	3.363251	2.326767	0.666214
H	-1.912216	-2.393066	-0.968338	H	2.665665	2.461792	-0.950455
C	0.280731	-5.735596	1.114685				

Table S5: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A Cys-Fe(II)CDO (SCF Energy = -4387.83931423 Eh)

Atom	x	y	z	Atom	x	y	z
C	-2.816757	-0.244461	4.883118	C	0.479538	-4.321960	0.655396
C	-1.654755	-0.137177	3.958527	C	-0.102341	-3.107208	0.939056
C	-1.604584	0.302582	2.659363	N	1.269241	-4.072556	-0.466782
N	-0.366150	-0.605057	4.219696	C	1.150482	-2.759155	-0.808563
C	0.389420	-0.455124	3.087952	N	0.331528	-2.146057	0.032349
N	-0.339783	0.096863	2.128891	H	-0.138092	-5.748093	2.286102
H	-3.279541	-1.303253	4.945068	H	1.425705	-6.061554	1.422700
H	-3.601196	0.436371	4.536392	H	-0.074661	-6.379196	0.582184
H	-2.561142	0.074173	5.899643	H	-0.791550	-2.853012	1.728180
H	-2.363480	0.803619	2.089066	H	1.798600	-4.752655	-1.012939
H	-0.046478	-1.039886	5.075684	H	1.681213	-2.299667	-1.625137
H	1.422531	-0.745789	2.995834	Fe	0.000000	0.000000	0.000000
C	-5.334747	0.191833	-2.328705	N	0.000015	2.255692	0.000015
C	-3.915207	-0.219574	-2.040741	C	1.273865	2.721390	0.640839
C	-2.754227	0.495789	-1.787079	C	1.414780	4.251816	0.614532
N	-3.599838	-1.522217	-1.686005	O	1.161133	4.841888	-0.529984
C	-2.331665	-1.553421	-1.211441	C	2.473663	2.068176	-0.077179
N	-1.772690	-0.343567	-1.253723	S	2.359924	0.226013	-0.059250
H	-5.870804	0.518967	-1.356369	O	1.790573	4.874710	1.657883
H	-5.901382	-0.632095	-2.775803	H	-0.794540	2.697098	0.466141
H	-5.368744	1.026062	-3.033844	H	0.002609	2.558075	-0.977249
H	-2.520554	1.514786	-2.046326	H	1.246979	2.396011	1.682693
H	-4.167267	-2.377853	-1.765594	H	3.396713	2.381241	0.414444
H	-1.869644	-2.453705	-0.852585	H	2.504562	2.392731	-1.119385
C	0.410141	-5.677170	1.270996				

Table S6: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A (H_2O/Cys)-Fe(II)CDO (SCF Energy = -4387.90216576 Eh)

Atom	x	y	z	Atom	x	y	z
C	-2.201553	-5.305695	0.231491	C	-0.148453	-1.150482	-2.990600
C	-1.196060	-4.204956	0.195007	N	1.000488	0.317841	-4.137604
C	-1.324783	-2.871140	0.495453	C	0.920441	0.742172	-2.846313
N	0.109253	-4.326248	-0.284744	N	0.244003	-0.138306	-2.123642
C	0.696838	-3.090469	-0.271255	H	-0.181381	-2.619461	-5.514709
N	-0.153610	-2.190796	0.202484	H	1.127533	-1.578644	-6.094849
H	-2.666900	-5.524506	-0.740952	H	-0.532333	-1.028229	-6.207794
H	-3.001266	-5.027756	0.926331	H	-0.746384	-1.980087	-2.650497
H	-1.765915	-6.234604	0.617233	H	1.403198	0.877900	-4.888245
H	-2.143341	-2.361847	0.963028	H	1.341125	1.664017	-2.487961
H	0.540359	-5.166962	-0.648819	O	0.699905	2.178146	-0.326355
H	1.707169	-2.884293	-0.582077	H	1.585571	1.861923	-0.041641
C	-5.716782	1.391861	0.509674	H	0.338898	2.817810	0.314713
C	-4.313385	1.286209	-0.009918	Fe	0.000000	0.000000	0.000000
C	-3.063721	1.337845	0.579514	N	-0.000015	-0.000015	2.278076
N	-4.060150	0.800491	-1.288284	C	1.267044	-0.593796	2.809723
C	-2.725525	0.540939	-1.417450	C	1.416672	-0.471298	4.333679
N	-2.085587	0.854813	-0.295700	O	1.040741	0.659515	4.885696
H	-6.076157	0.427292	0.897537	C	2.480667	0.091568	2.146683
H	-6.400772	1.727127	-0.278915	S	2.497803	-0.128296	0.313583
H	-5.784515	2.128204	1.312332	O	1.959549	-1.417709	4.985779
H	-2.789047	1.759735	1.532761	H	-0.798523	-0.494888	2.680817
H	-4.772110	0.578644	-1.985336	H	-0.060272	0.973740	2.585938
H	-2.321396	0.124405	-2.322037	H	1.260803	-1.653992	2.550369
C	0.167145	-1.577347	-5.564789	H	3.395111	-0.324738	2.570953
C	0.315796	-0.889526	-4.257600	H	2.453339	1.161377	2.380081

Table S7: Cartesian coordinates (in Å) for the QM/MM-optimized active site of WT (OH^-/Cys)-Fe(III)CDO (SCF Energy = -4688.60450292 Eh)

Atom	x	y	z	Atom	x	y	z
C	-0.980774	0.472046	5.835953	C	-0.143906	-2.892456	1.454819
C	-0.308365	0.322632	4.511322	N	0.427994	-4.162094	-0.232864
C	-0.737732	0.620193	3.237900	C	0.447083	-2.871475	-0.646957
N	0.952087	-0.250702	4.328491	N	0.123871	-2.075806	0.365463
C	1.22435	-0.281158	2.988846	H	-0.089081	-5.389771	2.991745
N	0.222046	0.251053	2.302200	H	0.734879	-6.152161	1.569138
H	-1.485229	-0.489059	6.236908	H	-1.015900	-6.019043	1.558960
H	-1.760239	1.235992	5.751831	H	-0.482758	-2.485611	2.392395
H	-0.281342	0.835068	6.596207	H	0.605743	-4.931168	-0.875977
H	-1.634415	1.128311	2.934296	H	0.669266	-2.554504	-1.649292
H	1.515427	-0.696060	5.041122	O	-0.167694	-0.365800	-1.836044
H	2.133759	-0.667221	2.561707	Fe	0.000000	0.000000	0.000000
C	-5.914215	0.933900	0.250595	H	0.598404	-0.095795	-2.352814
C	-4.543442	0.340485	0.128326	N	-0.000015	2.222534	0.000000
C	-3.301758	0.862213	-0.178391	C	1.332733	2.821548	0.322617
N	-4.264694	-0.944138	0.587387	C	1.423538	4.328537	0.039000
C	-2.913040	-1.137482	0.587479	O	0.779968	4.793839	-1.000900
N	-2.294861	-0.054993	0.130676	C	2.419693	2.096710	-0.488312
H	-6.120178	1.357803	1.312042	S	2.423676	0.288666	-0.140335
H	-6.682205	0.185028	0.030014	O	2.190704	5.040039	0.764206
H	-6.048874	1.740753	-0.473770	H	-0.706345	2.622391	0.621643
H	-3.076920	1.787476	-0.683182	H	-0.267731	2.458145	-0.961441
H	-4.947250	-1.600067	0.973862	H	1.506638	2.659042	1.387283
H	-2.478683	-2.063004	0.925613	H	3.397705	2.509689	-0.235641
C	-0.095398	-5.490402	1.844604	H	2.248383	2.251556	-1.558273
C	0.040543	-4.208664	1.104935				

Table S8: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A (OH^-/Cys)-Fe(III)CDO (SCF Energy = -4463.69981138 Eh)

Atom	x	y	z	Atom	x	y	z
C	-1.162369	0.731064	5.785446	C	0.048004	-2.811951	1.609375
C	-0.450714	0.561050	4.482178	N	0.547073	-4.163467	-0.039612
C	-0.915985	0.403427	3.196289	C	0.468903	-2.902023	-0.534027
N	0.936066	0.514252	4.356064	N	0.183121	-2.059830	0.449890
C	1.250076	0.345000	3.035126	H	0.091278	-5.258362	3.213623
N	0.142517	0.287720	2.305832	H	1.184235	-5.933670	1.994110
H	-1.576981	-0.197281	6.205383	H	-0.545563	-6.053436	1.764236
H	-1.990479	1.438461	5.664597	H	-0.204086	-2.344604	2.545517
H	-0.496460	1.173462	6.534332	H	0.711151	-4.984300	-0.622070
H	-1.928665	0.327621	2.847443	H	0.597946	-2.627228	-1.565842
H	1.597397	0.485565	5.122269	O	-0.221207	-0.451736	-1.803070
H	2.251205	0.279175	2.642532	H	0.352249	-0.027451	-2.449829
C	-5.953659	0.684433	0.095688	Fe	0.000000	0.000000	0.000000
C	-4.547043	0.155487	0.023972	N	0.000000	2.236511	-0.000031
C	-3.324707	0.761047	-0.227051	C	1.356888	2.819992	0.235977
N	-4.216690	-1.097519	0.517410	C	1.466476	4.326492	-0.057388
C	-2.864120	-1.194778	0.587173	O	0.822037	4.783844	-1.099121
N	-2.280594	-0.081726	0.153214	C	2.364212	2.046677	-0.633728
H	-6.152023	1.165802	1.064682	S	2.409012	0.273331	-0.134979
H	-6.682846	-0.121200	-0.045029	O	2.219284	5.044006	0.674942
H	-6.137131	1.419861	-0.692505	H	-0.668488	2.610947	0.679657
H	-3.130630	1.683746	-0.750137	H	-0.325882	2.482086	-0.941376
H	-4.825226	-1.890793	0.759018	H	1.592850	2.664825	1.289612
H	-2.368378	-2.077988	0.948471	H	3.361374	2.473724	-0.522385
C	0.243652	-5.387192	2.131882	H	2.069870	2.121841	-1.686295
C	0.272690	-4.138031	1.327759				

Table S9: Cartesian coordinates (in Å) for the QM/MM-optimized active site of WT (NO/Cys)-Fe(II)CDO (SCF Energy = -4742.70683861 Eh)

Atom	x	y	z	Atom	x	y	z
C	-1.326538	0.508896	-5.540115	C	-0.388947	2.987747	-0.654358
C	-0.565170	0.462311	-4.258728	N	0.422546	3.976288	1.117371
C	-0.891129	-0.059341	-3.027863	C	0.533936	2.633606	1.289063
N	0.658325	1.101181	-4.054596	N	0.056213	1.999786	0.225784
C	1.011108	0.956924	-2.742783	N	0.197021	-0.294128	1.732925
N	0.094803	0.247025	-2.096573	O	0.536331	0.146698	2.762512
H	-1.920715	1.488678	-5.698685	Fe	0.000000	0.000000	0.000000
H	-2.046707	-0.315079	-5.559464	N	0.164352	-2.073578	-0.384247
H	-0.669037	0.359589	-6.402344	C	1.535553	-2.504578	-0.824707
H	-1.732758	-0.670303	-2.760193	C	1.739563	-4.027649	-0.796326
H	1.137955	1.700363	-4.713196	O	1.184341	-4.694946	0.183762
H	1.914490	1.346191	-2.309052	C	2.571457	-1.825836	0.083496
C	-5.654907	-1.302795	0.213181	S	2.369034	0.000046	-0.000015
C	-4.309600	-0.633560	0.280289	O	2.502213	-4.563187	-1.663513
C	-3.001938	-1.081955	0.340286	H	-0.531967	5.789139	-1.703323
N	-4.182312	0.732727	0.048386	H	0.357100	6.280090	-0.205490
C	-2.861053	1.052567	-0.068649	H	-1.374985	6.011490	-0.107437
N	-2.107544	-0.029602	0.098526	H	-0.847977	2.742874	-1.596619
H	-5.986557	-1.544510	-0.875381	H	0.662262	4.628403	1.861115
H	-6.419891	-0.659302	0.662094	H	0.970642	2.188858	2.163803
H	-5.655396	-2.232056	0.786850	H	-0.517593	-2.403534	-1.071457
H	-2.648895	-2.055984	0.636398	H	-0.055618	-2.537796	0.500305
H	-4.951172	1.381149	-0.135193	H	1.667328	-2.155000	-1.849472
H	-2.545807	2.062531	-0.261780	H	3.580048	-2.093018	-0.236800
C	-0.450729	5.629776	-0.563568	H	2.436661	-2.161713	1.116837
C	-0.172440	4.234619	-0.112991				

Table S10: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A (NO/Cys)-Fe(II)CDO (SCF Energy = -4517.79844890 Eh)

Atom	x	y	z	Atom	x	y	z
C	-1.175125	-0.149490	-5.631866	C	-0.093338	2.900482	-1.097031
C	-0.455490	-0.097534	-4.325897	N	0.434189	4.084122	0.665649
C	-0.910233	-0.117737	-3.027740	C	0.410950	2.771362	1.020416
N	0.922852	0.056534	-4.208100	N	0.103363	2.023468	-0.029816
C	1.246231	0.115448	-2.881714	H	-0.121735	5.569275	-2.472183
N	0.150726	-0.002563	-2.139877	H	0.948456	6.100021	-1.110809
H	-1.642960	0.857346	-5.947037	H	-0.784088	6.122772	-0.877000
H	-1.980469	-0.890778	-5.585556	H	-0.362381	2.534607	-2.072479
H	-0.505630	-0.480164	-6.433273	H	0.594864	4.842163	1.328690
H	-1.917389	-0.166489	-2.662476	H	0.635178	2.428558	2.013916
H	1.569200	0.220840	-4.969849	N	0.000000	-0.000015	1.775909
H	2.242065	0.225174	-2.486282	O	0.676666	-0.205475	2.701416
C	-5.810608	-0.890182	0.070526	Fe	0.000000	0.000000	0.000000
C	-4.414032	-0.320923	0.137421	N	0.098938	-2.075043	-0.158173
C	-3.153458	-0.884842	0.288483	C	1.481232	-2.588562	-0.452408
N	-4.158905	0.994949	-0.216583	C	1.650558	-4.109543	-0.284531
C	-2.815613	1.172928	-0.303513	O	1.005768	-4.688522	0.696106
N	-2.159027	0.052368	-0.013367	C	2.455307	-1.835007	0.465225
H	-6.072357	-1.289291	-0.989578	S	2.336258	-0.032761	0.107956
H	-6.548645	-0.125427	0.338165	O	2.452164	-4.725342	-1.055725
H	-5.935500	-1.710663	0.782700	H	-0.546249	-2.406815	-0.881714
H	-2.900833	-1.848312	0.701035	H	-0.217102	-2.464996	0.734436
H	-4.815613	1.777557	-0.342697	H	1.697250	-2.342438	-1.492615
H	-2.379776	2.116638	-0.575790	H	3.475281	-2.180786	0.295395
C	0.029434	5.541962	-1.327988	H	2.193176	-2.018936	1.513107
C	0.110703	4.198364	-0.683350				

Table S11: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A (O_2/Cys)-Fe(II) CDO, singlet ground state (SCF Energy = -4538.20273185 Eh)

Atom	x	y	z	Atom	x	y	z
C	-1.480225	0.075302	5.503098	C	-0.027588	-2.914597	1.070969
C	-0.688995	0.073853	4.236969	N	0.631302	-4.036255	-0.688766
C	-1.072144	0.119110	2.916641	C	0.560440	-2.719589	-1.022247
N	0.696625	-0.054794	4.191406	N	0.172714	-2.010864	0.029541
C	1.093521	-0.080307	2.885193	O	0.016327	-0.160110	-1.783890
N	0.038177	0.041916	2.085678	O	0.918503	0.437958	-2.461044
H	-1.887527	-0.908463	5.779343	Fe	0.000000	0.000000	0.000000
H	-2.322296	0.770157	5.413498	N	0.000000	2.060287	0.000015
H	-0.875427	0.440704	6.340179	C	1.294891	2.651474	0.466629
H	-2.055374	0.178314	2.495728	C	1.460953	4.182083	0.325668
H	1.306931	-0.224976	4.981430	O	0.812759	4.822922	-0.614929
H	2.111389	-0.184784	2.551392	C	2.424637	1.946411	-0.294815
C	-5.765015	0.681534	-0.447937	S	2.291336	0.130768	-0.050278
C	-4.351791	0.157166	-0.423523	O	2.308487	4.719467	1.104400
C	-3.105881	0.767914	-0.447510	H	0.021149	-5.585907	2.350327
N	-4.076996	-1.167969	-0.120041	H	1.177979	-6.049622	1.096329
C	-2.739212	-1.309296	0.058472	H	-0.535629	-6.167618	0.772156
N	-2.105042	-0.153137	-0.120972	H	-0.363098	-2.580643	2.037613
H	-6.115097	1.001816	0.544464	H	0.860855	-4.773132	-1.355637
H	-6.453796	-0.085846	-0.819885	H	0.793442	-2.334824	-1.998444
H	-5.849350	1.538620	-1.122894	H	-0.756454	2.436172	0.576874
H	-2.858612	1.755295	-0.800247	H	-0.175385	2.344147	-0.973022
H	-4.703705	-1.981522	-0.107407	H	1.376617	2.429153	1.532516
H	-2.291534	-2.252548	0.310364	H	3.389420	2.307938	0.058029
C	0.218964	-5.546005	1.268997	H	2.320145	2.138763	-1.367661
C	0.256165	-4.192520	0.643250				

Table S12: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A (O_2/Cys)-Fe(II) CDO, triplet ground state (SCF Energy = -4538.22156598 Eh)

Atom	x	y	z	Atom	x	y	z
C	-1.626221	0.072342	5.430954	C	-0.123123	-2.910583	1.008270
C	-0.782028	0.064285	4.200821	N	0.550079	-4.030090	-0.746338
C	-1.114975	0.146912	2.869629	C	0.543594	-2.707214	-1.062073
N	0.597610	-0.121628	4.203644	N	0.149323	-1.999252	-0.011475
C	1.041794	-0.140594	2.912048	O	-0.020798	-0.021530	-1.893539
N	0.021057	0.033737	2.079437	O	0.956619	-0.175018	-2.705261
H	-2.073593	-0.903656	5.670181	Fe	0.000000	0.000000	0.000000
H	-2.442841	0.793152	5.315308	N	0.000000	2.080475	-0.000015
H	-1.049103	0.405640	6.300293	C	1.326126	2.637604	0.417145
H	-2.078735	0.262924	2.416931	C	1.520508	4.164841	0.297256
H	1.171783	-0.323532	5.012939	O	0.915527	4.816681	-0.662781
H	2.067886	-0.266403	2.613907	C	2.392044	1.897552	-0.402969
C	-5.740875	0.819519	-0.647202	S	2.248306	0.099792	-0.038116
C	-4.342300	0.258774	-0.578979	O	2.344055	4.689606	1.109985
C	-3.082977	0.839874	-0.560318	H	-0.200043	-5.602448	2.249527
N	-4.109253	-1.072510	-0.261078	H	0.975128	-6.083710	1.020340
C	-2.783829	-1.243759	-0.031281	H	-0.731659	-6.143021	0.648529
N	-2.117645	-0.102188	-0.190369	H	-0.477325	-2.578200	1.968872
H	-6.112274	1.149231	0.334305	H	0.772461	-4.765564	-1.417267
H	-6.437851	0.070313	-1.040329	H	0.843781	-2.318237	-2.018585
H	-5.781647	1.678100	-1.324265	H	-0.729965	2.460129	0.608246
H	-2.799042	1.818481	-0.909485	H	-0.203110	2.371292	-0.964203
H	-4.755341	-1.871872	-0.273376	H	1.450700	2.394684	1.473846
H	-2.365433	-2.195267	0.241226	H	3.387390	2.255951	-0.141876
C	0.027924	-5.552353	1.174576	H	2.217651	2.050079	-1.473312
C	0.126343	-4.191971	0.570114				

Table S13: Cartesian coordinates (in Å) for the QM/MM-optimized active site of H155A (O_2/Cys)-Fe(II) CDO, quintet ground state (SCF Energy = -4538.19460937 Eh)

Atom	x	y	z	Atom	x	y	z
C	-1.734253	0.343400	5.661728	C	-0.121674	-2.852493	1.272980
C	-0.867371	0.235168	4.454437	N	1.023056	-4.032500	-0.173080
C	-1.181686	0.256516	3.114304	C	0.998367	-2.741577	-0.598358
N	0.510132	0.032074	4.486404	N	0.304855	-2.003937	0.258423
C	0.962662	-0.049164	3.192856	H	0.150009	-5.280716	2.878021
N	-0.041245	0.101181	2.344757	H	1.231308	-5.930008	1.641922
H	-2.208694	-0.600830	5.967285	H	-0.504913	-6.072845	1.427139
H	-2.540649	1.057022	5.469391	H	-0.698090	-2.483963	2.104340
H	-1.175507	0.740997	6.514618	H	1.498016	-4.766861	-0.691116
H	-2.146164	0.310852	2.645828	H	1.477676	-2.395554	-1.496902
H	1.057281	-0.161163	5.312714	O	-0.137848	-0.652740	-2.133011
H	1.990356	-0.207886	2.913559	O	-0.900116	-1.594574	-2.543640
C	-5.727295	0.555298	-0.618378	Fe	0.000000	0.000000	0.000000
C	-4.296570	0.105453	-0.522324	N	0.000000	2.089844	0.000000
C	-3.101669	0.755844	-0.287857	C	1.279739	2.672485	0.550888
N	-3.968475	-1.243300	-0.482666	C	1.353317	4.215363	0.533813
C	-2.642380	-1.375061	-0.236435	O	1.014984	4.812271	-0.585220
N	-2.072418	-0.177582	-0.110275	C	2.411545	2.023895	-0.250259
H	-6.106949	0.943375	0.338333	S	2.242798	0.195053	-0.066818
H	-6.364624	-0.281067	-0.926010	O	1.766876	4.830032	1.562485
H	-5.853241	1.334595	-1.373032	H	-0.803391	2.463791	0.517883
H	-2.911850	1.811707	-0.239120	H	-0.106979	2.375000	-0.984558
H	-4.585236	-2.039307	-0.573578	H	1.344284	2.362061	1.594849
H	-2.172806	-2.332962	-0.161041	H	3.391434	2.325821	0.123383
C	0.284637	-5.401123	1.792984	H	2.334473	2.284302	-1.309906
C	0.330994	-4.128235	1.028549				