SUPPORTING INFORMATION

Distinguishing Nitro vs Nitrito Coordination in Cytochrome *c′* using Vibrational Spectroscopy and Density Functional Theory

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Supplementary Data

Figure S1. Porphyrin marker RR bands of Fe(III) L16G AXCP (pH 7.0, room temperature) in the absence and presence of nitrite.

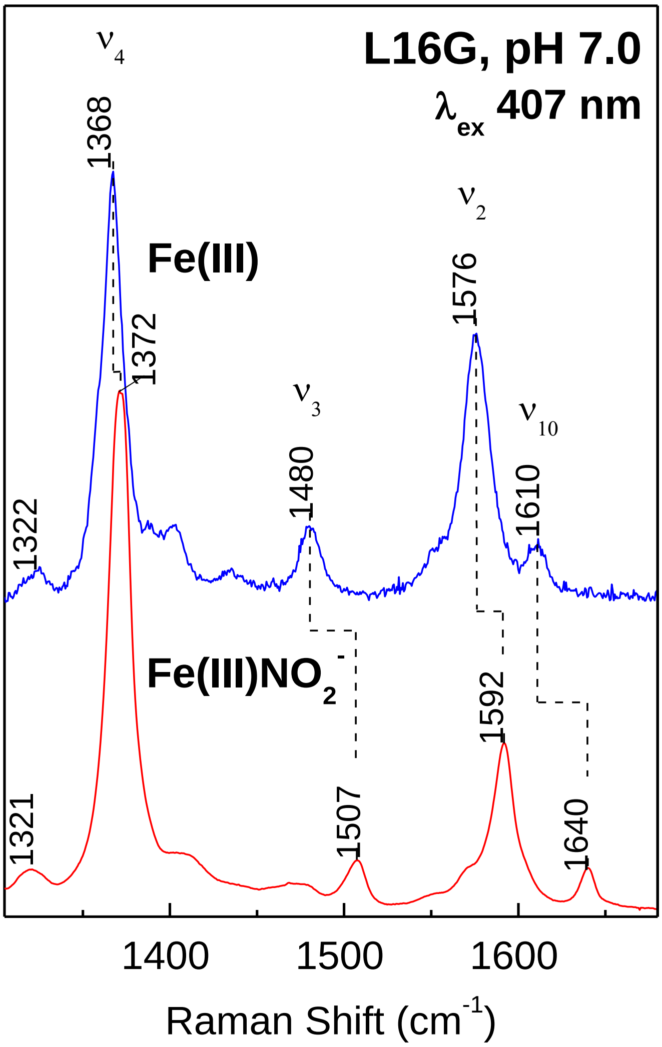


Figure S2. RR spectra of Fe(III)-nitrite L16G AXCP obtained at 110 K. Left panel shows the porphyrin marker band region. Right panel shows the low-frequency region and the identification of the δ(NO_2) vibration via isotopic substitution (inset).

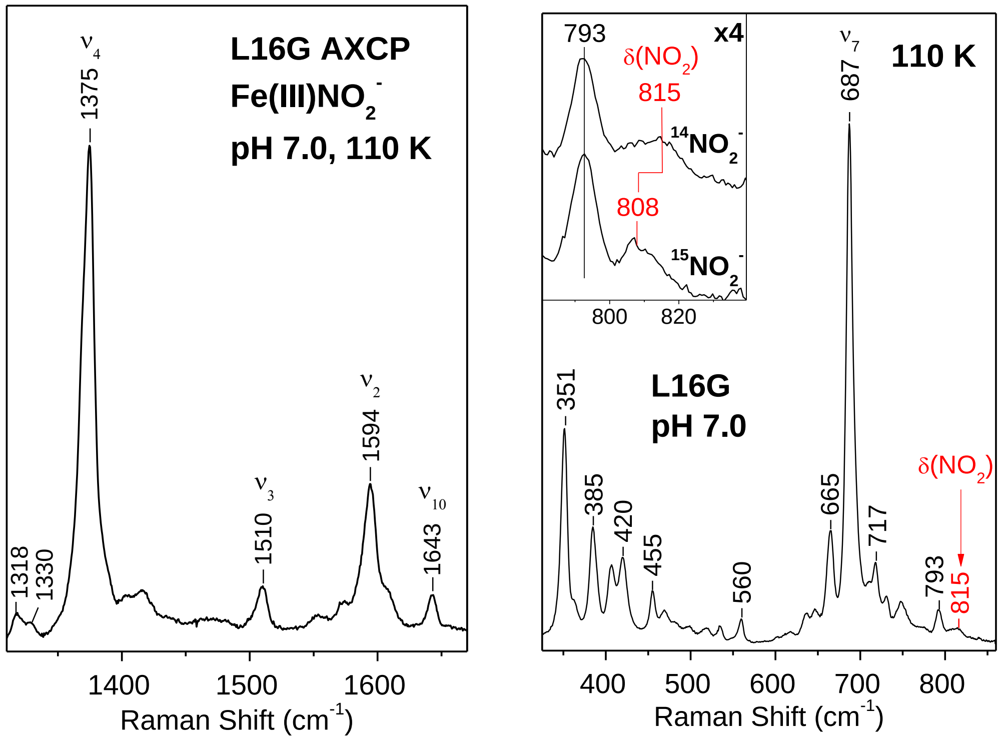


Figure S3. EPR spectrum of the nitrite complex of Fe(III) L16G AXCP (100 μM in heme, 20 mM nitrite) obtained at 10 K. Dominant low-spin rhombic *g* values are observed at 2.84, 2.36 and 1.56. Lower intensity signals at *g* = 3.25 and 2.2 are suggestive of minor conformational heterogeneity in the low-spin nitrite complex, while the signal around *g* = 2.07 is likely to reflect some Cu(II) impurity.

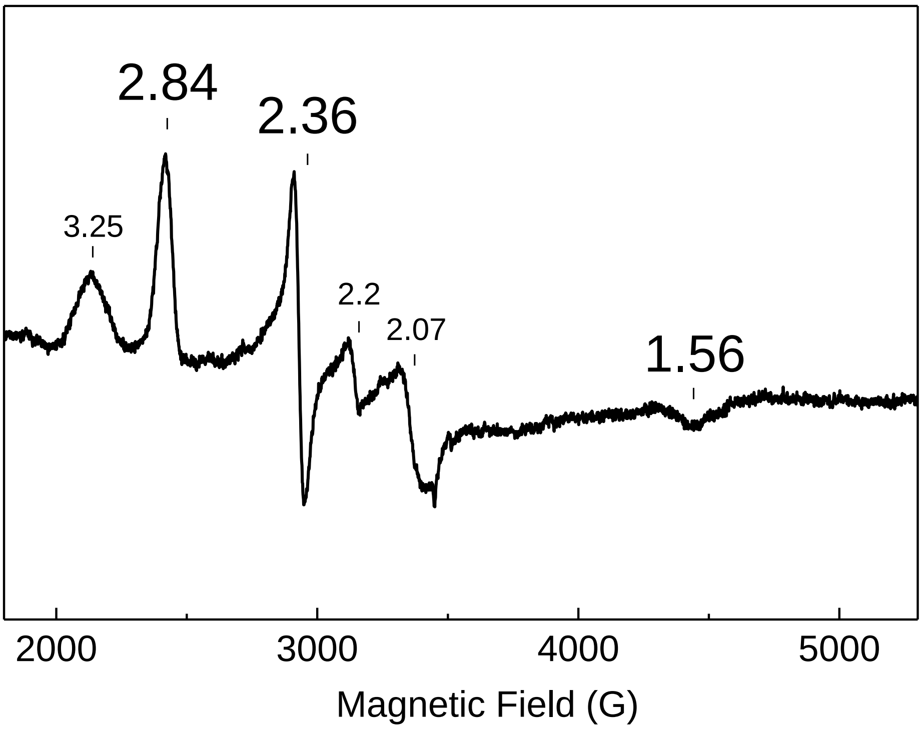


Figure S4. Titration of Fe(III) L16G AXCP (pH 7.0) with nitrite to determine K_d.

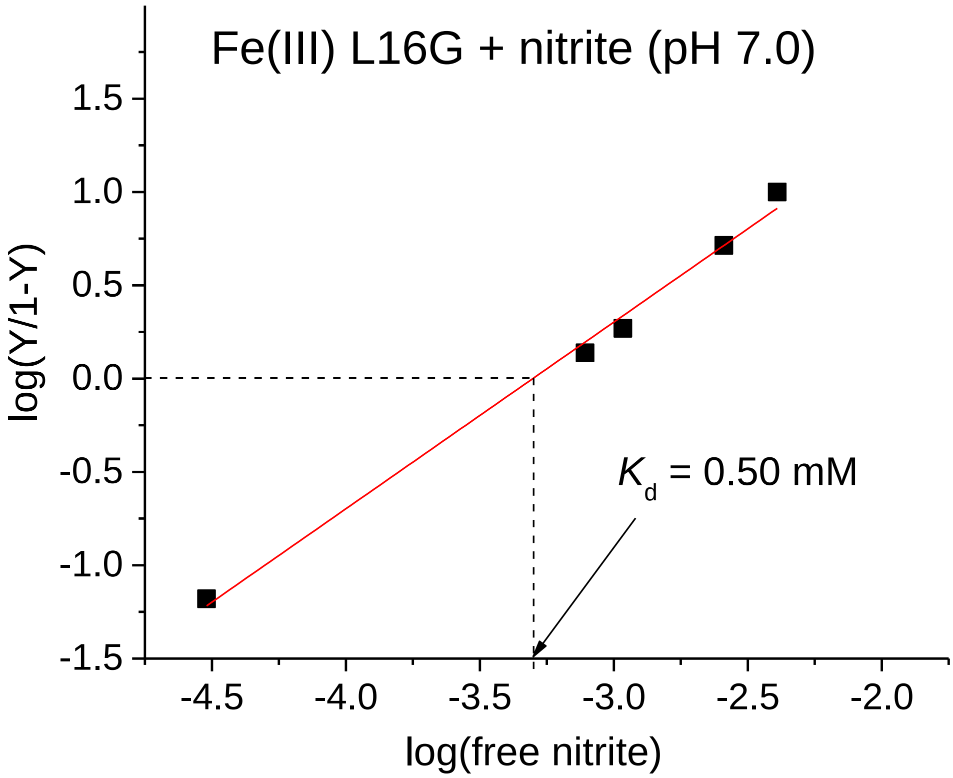


Figure S5. Absorption time courses at 415 nm showing the reaction of Fe(III) L16G AXCP with nitrite. Overlaid (and mostly obscured) are kinetic fits to single exponential functions

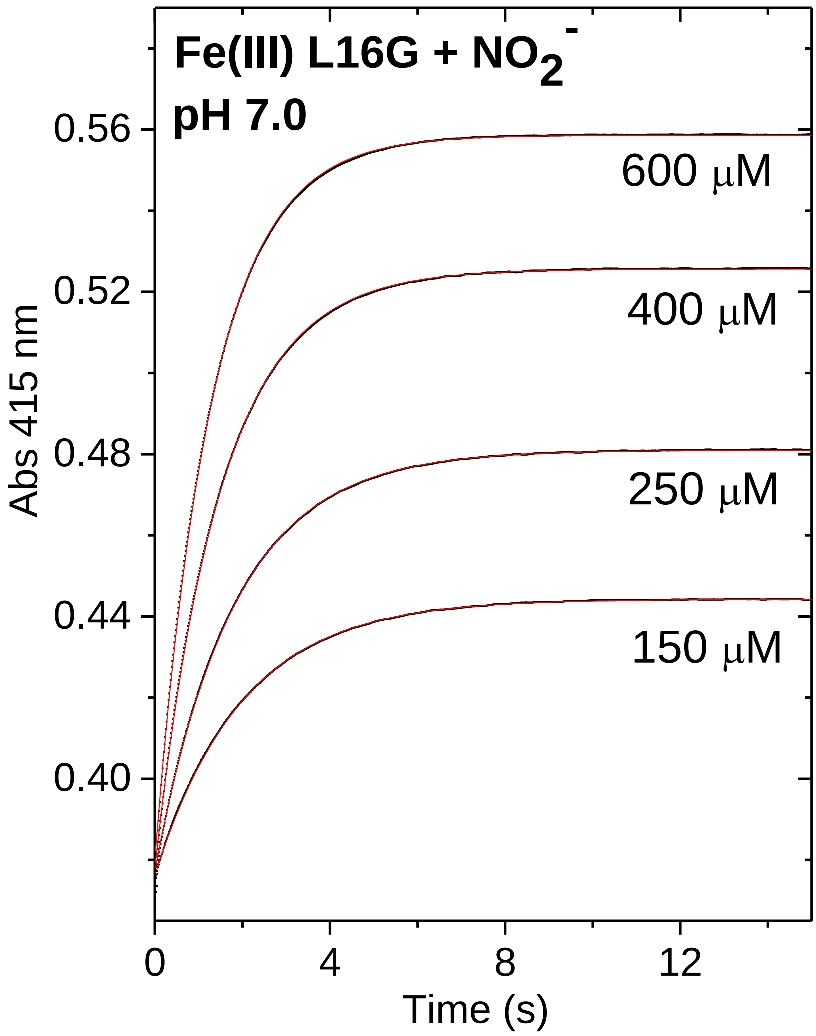


Figure S6. Determination of k_{on} and k_{off} for the Fe(III)-nitrite complex of L16G AXCP from the dependence of k_{obs} on nitrite concentration.

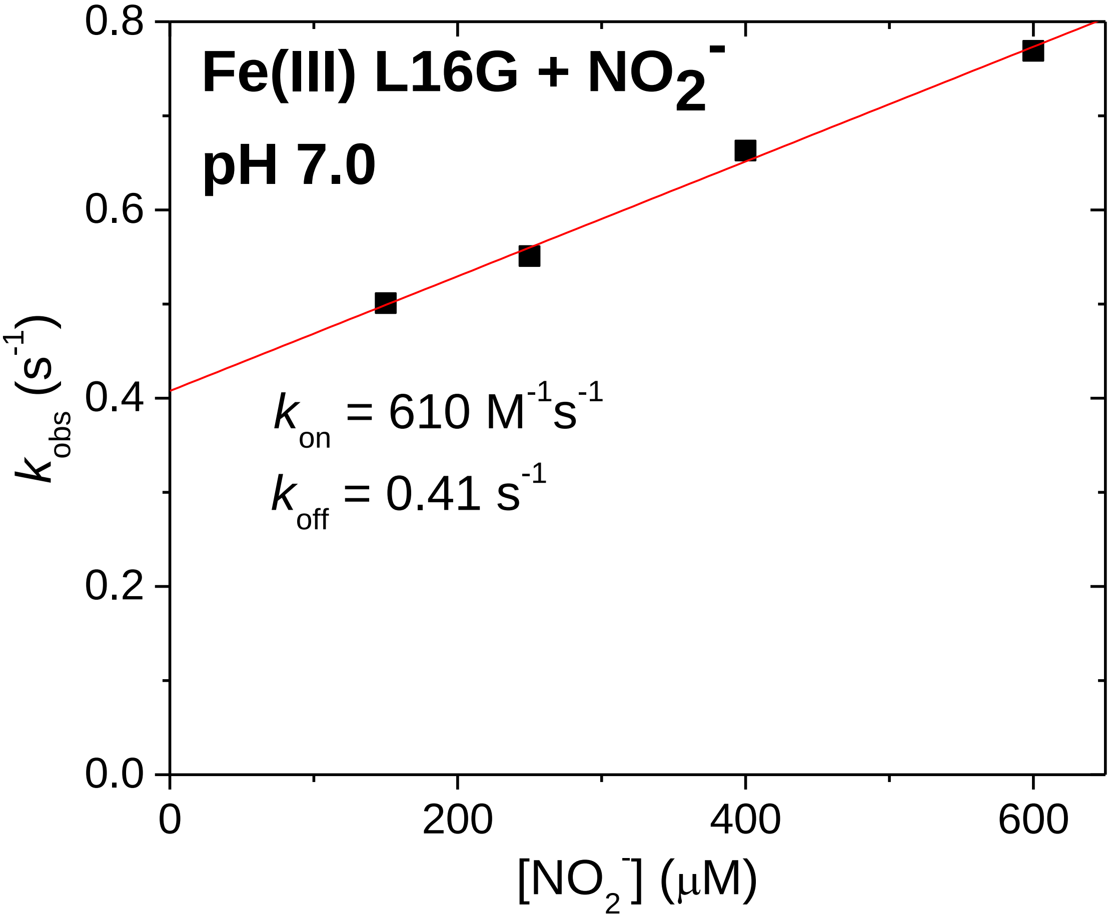
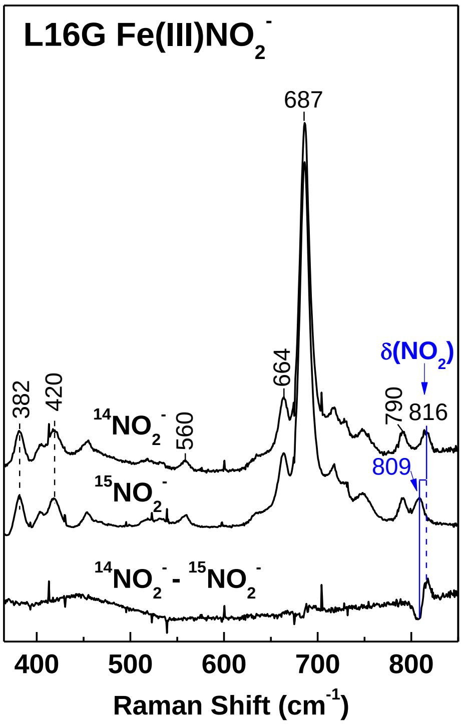
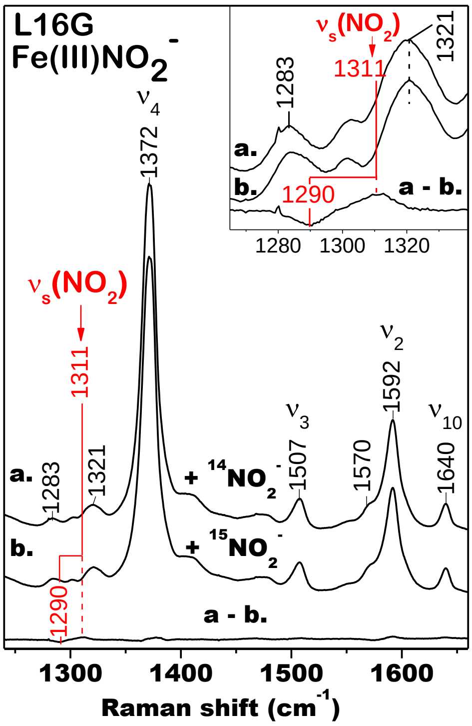


Figure S7. Room-temperature RR spectra of Fe(III)-nitrite L16G AXCP prepared with $^{14}NO_2^-$ and $^{15}NO_2^-$, recorded in different frequency windows: (A) 370 – 850-cm^{-1} , (B) 1240 – 1660 cm^{-1}, and (C) 760 – 1350 cm^{-1}.

(A)



(B)



(C)

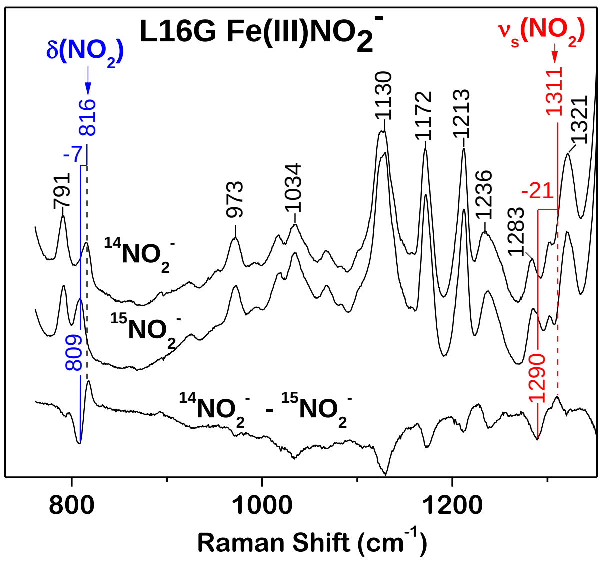


Figure S8. Top panel: UV-vis spectra of L16G AXCP $\{FeNO\}^6$ (magenta, solid line) and $\{FeNO\}^7$ (blue, dashed line) complexes (pH 7.0), formed by the reaction of NO with Fe(III) and Fe(II) L16G AXCP, respectively. Bottom panel: UV-vis spectra showing the photoconversion of the L16G AXCP $Fe(III)NO_2^-$ complex (red line) to the $\{FeNO\}^7$ complex (blue line) upon 200 s illumination with a Xe arc lamp. The spectrum recorded after 50 s illumination (green line) is consistent with a mixture of species that includes the $\{FeNO\}^6$ complex.

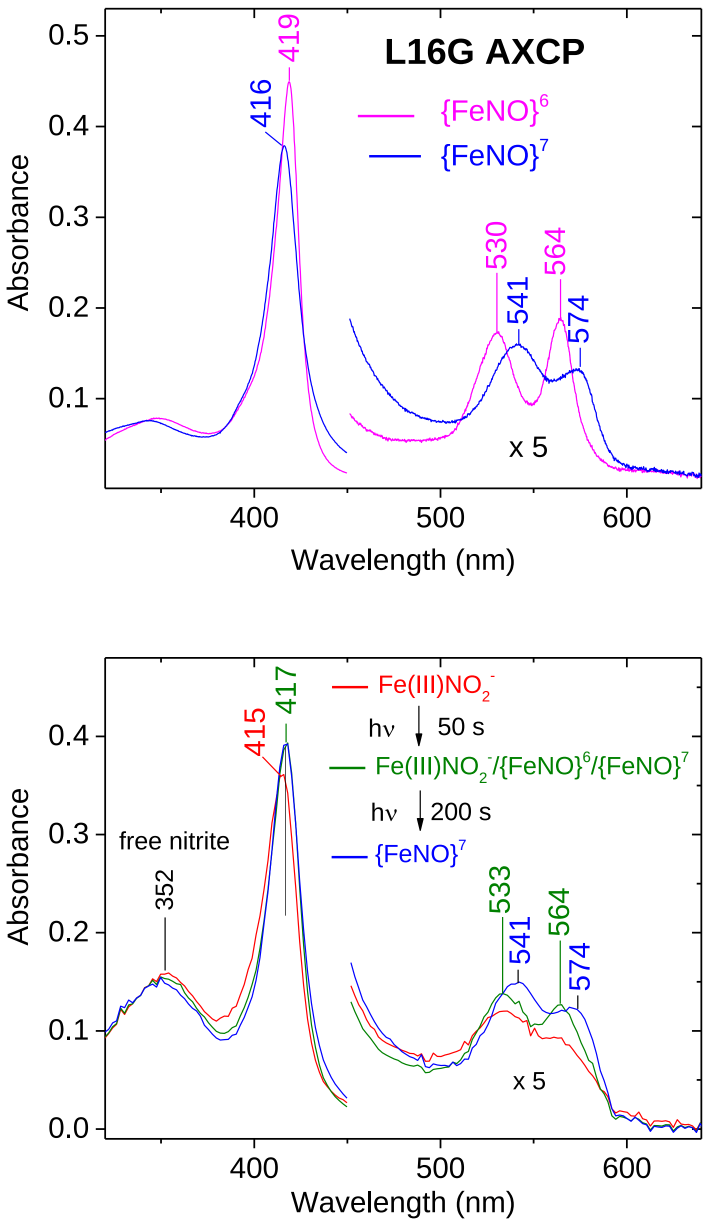


Figure S9. RR spectra of {FeNO}^{7} L16G AXCP in the high frequency (left panel) and low frequency (right panel) regions. Samples were measured at room temperature (rt) with $^{14}N^{16}O$ (A) and $^{15}N^{18}O$ (B) as well as at 100 K with $^{14}N^{16}O$ (C) and $^{15}N^{18}O$ (D). Vibrational assignments are based on isotopic substitution difference spectra at room temperature (A-B) and 100 K (C-D).

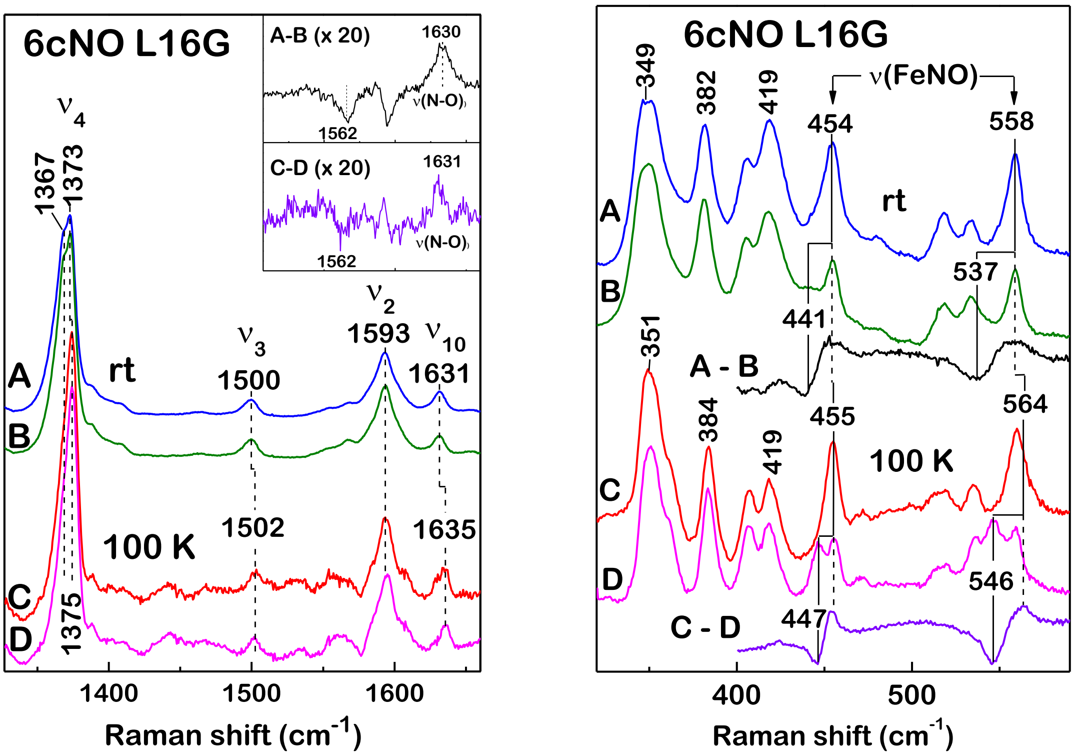
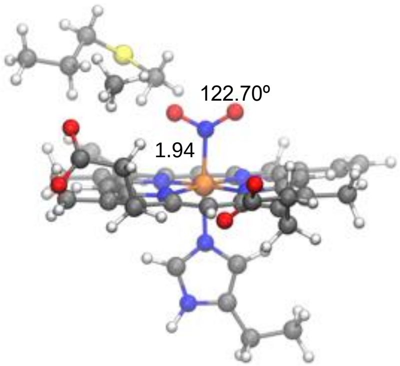
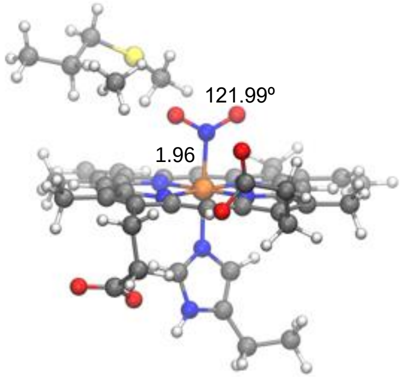


Figure S10. L16G AXCP N-bound and O-bound NO_2^- structures with different propionate configurations, relaxed from the crystal structure and flipped to the proximal side of the heme. Absolute energies are in hartrees (1 hartree = 2625.5 kJ mol^{-1}), bond lengths in Å.

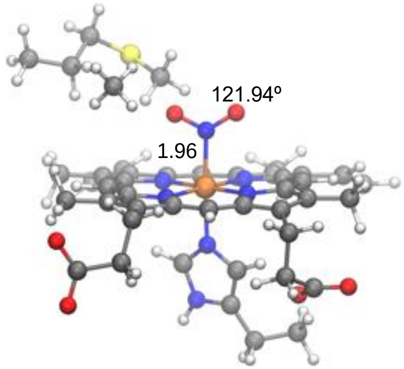
N-bound



Propionates as in crystal structure
Absolute Energy: -4201.285 H

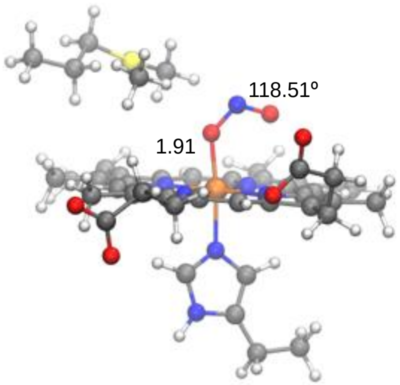


One propionate flipped to the proximal side
Absolute Energy: -4201.308 H

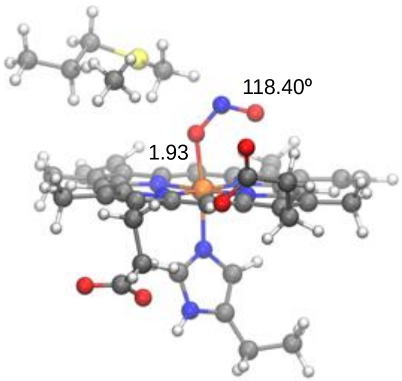


Both propionates flipped to the proximal side
Absolute Energy: -4201.312 H

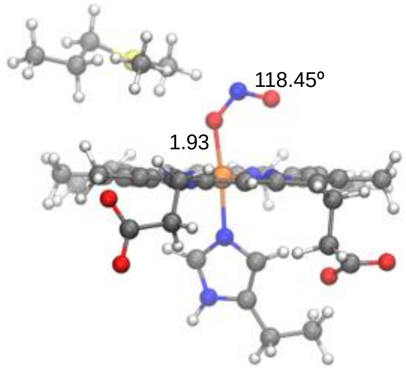
O-bound



Propionates as in crystal structure
Absolute Energy: -4201.281 H



One propionate flipped to the proximal side
Absolute Energy: -4201.307 H



Both propionates flipped to the proximal side
Absolute Energy: -4201.309 H

Figure S11. Calculated normal-mode eigenvectors for vibrational modes of the L16G AXCP Fe(III)-nitrite complex.

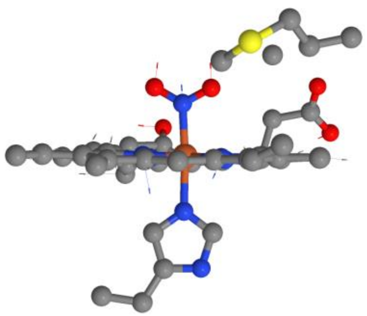
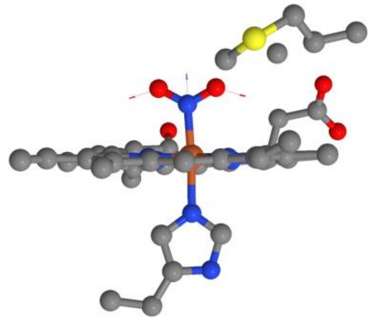
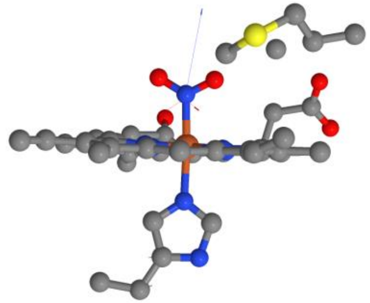
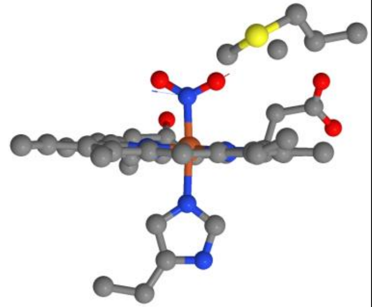
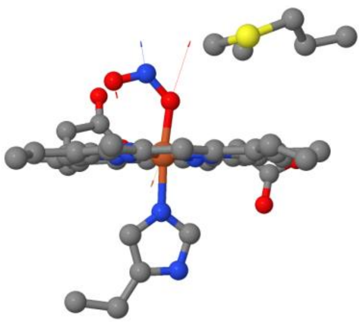
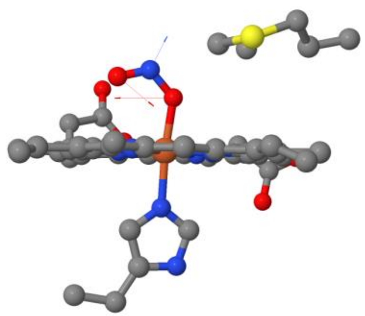
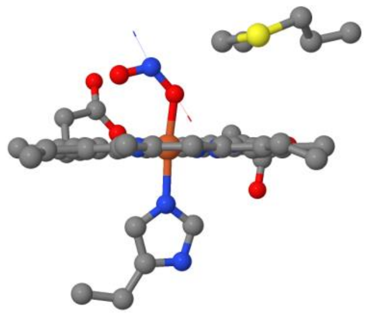
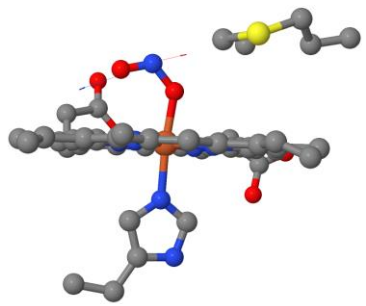
N-bound			
			
$\nu(FeNO_2)$	$\delta(ONO)$	$\nu_s(NO_2)$	$\nu_{as}(NO_2)$
O-bound			
			
$\nu(FeNO_2)$	$\delta(ONO)$	ν(N-O)	ν(N=O)

Table S1. Crystallographic data collection, processing and refinement statistics for Fe(III)-nitrite L16G AXCP. Values in parentheses are for highest resolution shells. Data were processed using CC-1/2 ≧0.5 and I/σ(I) ≧1.0 (outer shell) cut off.

Structure	L16G-NO_2 AXCP
Space Group	$P6_522$
Cell axis (Å)	53.91, 53.91, 183.21
Resolution (Å)	45.24 – 1.06 (1.08 – 1.06)
Unique reflections	70168 (3511)
Redundancy	7.7 (5.1)
R_{pim} (%)	2.3 (49.3)
CC-1/2 outer shell	0.59
I/σ(I)	13.1 (1.2)
Completeness (%)	96.8 (99.0)
Wilson B-factor ($Å^2$)	8.5
Refinement	
R_{work}/R_{free} (%)	17.8 / 20.0
RMSD Bond Length (Å)	0.016
RMDS Bond Angle (°)	2.18
ML Based ESU (Å)	0.024
Average Protein B-factor ($Å^2$)	13.3
Average Water B-factor ($Å^2$)	28.7
Ramachandran (%)	
Favored Regions	96
Allowed Regions	4
PDB Code	5NGX

Table S2. Effect of distal coordination and redox state on the electronic properties of L16G AXCP, pH 7.0.

	spin state	λ_{max} (nm)			ref
Fe(III)(OH_2)	6cHS	405	500	627	this work
Fe(III)(NO_2^-)	6cLS	415	535	571	this work
$\{FeNO\}^6$	6cLS	419	530	564	this work
$\{FeNO\}^7$	6cLS	416	541	574	this work

Table S3. Porphyrin marker vibrational frequencies (cm^{-1}) of aqua and nitrite complexes of Fe(III) heme proteins.

	temp	spin-state	ν_4	ν_3	ν_2	ν_{10}	ref
L16G AXCP, pH 7.0							
$Fe(III)(OH_2)$	rt	6cHS	1368	1480	1576	1610	this work
$Fe(III)(NO_2^-)$	rt	6cLS	1372	1507	1592	1640	this work
	110 K	6cLS	1375	1510	1594	1643	this work
NP4, pH 7.0							
$Fe(III)(OH_2)$	298 K	6cHS	1372	1481	1560	1610	[1]
$Fe(III)(NO_2^-)$	77 K	6cLS	1375	1507	1584	1638	[2]
Hb, pH 6.6							
$Fe(III)(OH_2)$	298 K	6cHS	1373	1481	1561	1610	[3]
$Fe(III)(NO_2^-)$	77 K	6cLS	1375	1507	1585	1640	[2]

Table S4. Kinetic parameters and dissociation constants for heme protein Fe(III)nitrite complexes

protein		k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (mM)[a]	ref
L16G AXCP	pH 7.0, 25 °C	610	0.41	0.67, *0.50*	this work
Mb[b]	pH 7.4, 20 °C	156	2.6	16.7	[4]
Hb[c]	pH 7.4, 25 °C			*3.0*	[5]
Hb[c]	pH 7.4, 25 °C			*1.1*	[6]
Hb[c]	pH 6.9, 20 °C	140[e]			[7]
		20[f]			
NP4[d]	pH 7.0, 30 °C	0.13[e]	0.0084[e]	67[e]	[2]
		0.01[f]	0.00047[f]	47[f]	
NP7[d]	pH 7.0, 30 °C	0.076[e]	0.00040[e]	5.2[e]	[2]
		0.0088[f]	0.00013[f]	15[f]	

[a]Dissociation constants, K_d, calculated from the ratio of k_{off}/k_{on}, except for values in italics determined by titration. [b]Horse Mb. [c]Human Hb. [d]nitrophorin from *Rhodnius prolixus*. [e]Slow component of biphasic reaction. [f]Fast component of biphasic reaction.

References

1. Zareba, A. A. Resonance Raman spectroscopic characterization of nitrophorins from *Rhodnius prolixus* and *Cimex lectularius*. University of Houston, 2006.
2. He, C.; Ogata, H.; Knipp, M., Formation of the complex of nitrite with the ferriheme b beta-barrel proteins nitrophorin 4 and nitrophorin 7. *Biochemistry* **2010,** *49*, 5841-5851.
3. Spiro, T. G.; Stong, J. D.; Stein, P., Porphyrin core expansion and doming in heme proteins. New evidence from resonance Raman spectra of six-coordinate high-spin iron(III) hemes. *J. Am. Chem. Soc.* **1979,** *101*, 2648-2655.
4. Wanat, A.; Gdul-Argasinska, J.; Rutkowska-Zbik, D.; Witko, M.; Stochel, G.; van Eldik, R., Nitrite binding to metmyoglobin and methemoglobin in comparison to nitric oxide binding. *J. Biol. Inorg. Chem.* **2002,** *7*, 165-176.
5. Smith, R. P., The nitrite methemoglobin complex: Its significance in methemoglobin analyses and its possible role in methemoglobinemia. *Biochem. Pharmacol.* **1967,** *16*, 1655-1664.
6. Rodkey, K. L., A mechanism for the conversion of oxyhemoglobin to methemoglobin by nitrite. *Clin. Chem.* **1976,** *22*, 1986-1990.
7. Gibson, Q. H.; Parkhurst, L. J.; Geraci, G., The reaction of methemoglobin with some ligands. *J. Biol. Chem.* **1969,** *244*, 4668-4676.

Cartesian coordinates of the optimized nitro and nitrito complexes

Nitro complex

C	-24.73858	21.14488	12.13954
H	-24.86072	20.86432	11.08457
H	-25.68462	21.50676	12.57904
H	-24.37897	20.27187	12.71147
H	-23.98557	21.94429	12.18731
C	-20.94984	20.41766	14.94521
H	-21.31938	19.39577	15.14034
H	-21.67519	21.11528	15.39254
H	-19.98949	20.53862	15.47738
C	-20.78658	20.68503	13.44767
H	-21.76586	20.65900	12.94500
H	-20.40026	21.70767	13.30992
C	-19.81903	19.70956	12.76384
H	-19.04469	19.36820	13.47261
H	-20.35537	18.80867	12.41724
S	-18.84700	20.41808	11.38320
C	-20.16030	21.12760	10.33856
H	-19.65823	21.63035	9.49995
H	-20.74737	21.88015	10.87637
H	-20.84428	20.36141	9.94527
C	-22.33207	29.80868	5.66733
H	-21.45090	29.19899	5.41290
H	-22.27080	30.75242	5.10405
H	-23.22148	29.25902	5.32250
C	-22.40079	30.07600	7.17012
H	-23.29877	30.68347	7.39271
H	-21.53956	30.70427	7.47034
C	-22.43347	28.83650	7.99210
N	-22.52051	28.84834	9.37693
H	-22.51360	29.66992	9.96748
C	-22.67233	27.56717	9.80912
H	-22.78026	27.26792	10.84943
N	-22.67995	26.75428	8.77497
C	-22.53501	27.51338	7.63804
H	-22.53527	27.06097	6.65138
C	-26.30041	25.31193	8.92823
H	-27.38969	25.47903	8.91641
C	-25.69800	25.21279	7.68266
C	-26.41178	25.35467	6.42090
C	-25.51045	25.01529	5.44085
C	-24.23670	24.76850	6.10343
N	-24.37397	24.91580	7.45891
C	-23.04269	24.52975	5.44144
H	-23.09447	24.42267	4.35936
C	-21.78197	24.47840	6.03433
C	-20.52877	24.42217	5.29310
C	-19.51559	24.49704	6.24301
C	-20.16471	24.56294	7.52834
N	-21.53398	24.56730	7.37473
C	-19.49484	24.57895	8.75047

H	-18.40705	24.52533	8.72527
C	-20.09838	24.60750	9.99992
C	-19.37894	24.53647	11.26501
C	-20.34193	24.59039	12.26011
C	-21.61944	24.69089	11.58744
N	-21.44696	24.68244	10.22646
C	-22.83872	24.83155	12.24782
H	-22.82725	24.82116	13.33688
C	-24.07700	24.98601	11.64415
C	-25.32104	25.14182	12.38969
C	-26.32879	25.25207	11.45721
C	-25.67098	25.19740	10.15770
N	-24.30501	25.02147	10.29224
C	-27.81368	25.23173	11.66410
H	-28.10115	25.90809	12.48572
H	-28.33655	25.54628	10.74874
C	-28.29048	23.82225	12.08834
H	-29.37868	23.76857	11.90896
H	-27.81209	23.04896	11.46802
C	-28.02058	23.54772	13.60032
O	-27.38056	22.50712	13.88573
O	-28.44406	24.43238	14.38843
C	-25.41568	25.09583	13.87950
H	-24.62960	25.70674	14.35867
H	-26.40842	25.41457	14.22735
H	-25.32580	24.05455	14.22994
C	-20.14200	24.60746	13.74142
H	-20.95902	25.13517	14.25478
H	-20.10349	23.58882	14.16508
H	-19.19231	25.10044	14.00322
C	-17.93331	24.43207	11.39249
H	-17.35143	24.84449	10.55826
C	-17.25438	23.87342	12.41133
H	-16.16067	23.85049	12.41064
H	-17.75996	23.37408	13.23911
C	-18.03958	24.53694	6.00769
H	-17.65626	23.58023	5.60896
H	-17.77770	25.31451	5.26966
H	-17.48491	24.76068	6.93024
C	-20.44863	24.34289	3.84637
H	-21.41686	24.33864	3.33460
C	-19.35859	24.27142	3.05424
H	-19.47329	24.21725	1.96778
H	-18.33959	24.25348	3.44175
C	-25.78559	24.95459	3.96996
H	-24.90018	24.66201	3.38588
H	-26.58208	24.22352	3.75271
H	-26.14305	25.92523	3.58099
C	-27.80521	25.87260	6.23066
H	-28.01858	26.61784	7.01319
H	-27.82668	26.39054	5.25513
C	-28.96438	24.85398	6.30295
H	-29.66957	25.01680	5.47038
H	-28.59644	23.81872	6.19474
C	-29.81777	24.92714	7.63220
O	-30.88219	24.29277	7.59739
O	-29.32371	25.61716	8.56495

Fe	-22.93155	24.73504	8.84076
N	-23.11649	22.80123	8.78567
O	-22.95114	22.23535	9.86279
O	-23.33339	22.19523	7.74877

Nitrito Complex

C	-24.52278	20.94459	11.90327
H	-25.33246	20.20042	11.89779
H	-23.65914	20.53692	12.45078
H	-24.22411	21.19805	10.87610
H	-24.87511	21.85881	12.40193
C	-20.69658	20.27169	14.66448
H	-21.02967	19.22809	14.80290
H	-21.44490	20.92226	15.14384
H	-19.74364	20.39988	15.20701
C	-20.54473	20.62624	13.17957
H	-21.52404	20.55428	12.68168
H	-20.23350	21.67757	13.08403
C	-19.53406	19.76418	12.42738
H	-18.58326	19.71067	12.98543
H	-19.90918	18.72985	12.32056
S	-19.03374	20.40427	10.78213
C	-20.64067	20.74767	9.99098
H	-20.41934	21.01274	8.94798
H	-21.16235	21.60047	10.44415
H	-21.29472	19.86331	9.99603
C	-22.57443	30.03035	5.87065
H	-21.63087	29.54084	5.58253
H	-22.63695	30.99966	5.35277
H	-23.39886	29.39713	5.50880
C	-22.65941	30.21801	7.38785
H	-23.59299	30.75459	7.64035
H	-21.83521	30.87193	7.73070
C	-22.62614	28.91845	8.13788
N	-22.71955	28.84342	9.52291
H	-22.80246	29.62701	10.15853
C	-22.77357	27.53603	9.88686
H	-22.87868	27.17986	10.90950
N	-22.70706	26.77327	8.81383
C	-22.61377	27.60758	7.71789
H	-22.56520	27.20850	6.70958
C	-26.30039	25.16908	8.88515
H	-27.39654	25.25539	8.87915
C	-25.68895	25.16559	7.63543
C	-26.38813	25.32979	6.36719
C	-25.43425	25.18530	5.38707
C	-24.16242	24.97586	6.05845
N	-24.34572	24.95082	7.41850
C	-22.93825	24.87788	5.41157
H	-22.94943	24.93367	4.32394

C	-21.70324	24.70620	6.03374
C	-20.43025	24.59146	5.33401
C	-19.46638	24.39791	6.31665
C	-20.16049	24.42922	7.58154
N	-21.50913	24.62000	7.38442
C	-19.52809	24.30565	8.81789
H	-18.44956	24.15184	8.81416
C	-20.14697	24.37387	10.05761
C	-19.45017	24.25063	11.33277
C	-20.41823	24.37169	12.31544
C	-21.67536	24.55806	11.62518
N	-21.48446	24.57971	10.26727
C	-22.90343	24.66099	12.27106
H	-22.90271	24.60303	13.35765
C	-24.13282	24.79280	11.64655
C	-25.40367	24.84944	12.35690
C	-26.38151	24.98731	11.39346
C	-25.66743	25.02762	10.11104
N	-24.31068	24.91631	10.28887
C	-27.85230	25.25601	11.52437
H	-27.96717	26.35811	11.52331
H	-28.38132	24.88463	10.63162
C	-28.54313	24.79056	12.80783
H	-29.63066	24.78317	12.61152
H	-28.28362	23.75092	13.07203
C	-28.32729	25.70895	14.06141
O	-28.53277	25.16419	15.16915
O	-27.95446	26.88680	13.82390
C	-25.52133	24.88227	13.84832
H	-24.59946	24.53911	14.34286
H	-25.76301	25.90675	14.17739
H	-26.35409	24.27804	14.22718
C	-20.26117	24.27303	13.79940
H	-21.14615	23.81741	14.26881
H	-19.38352	23.66306	14.06175
H	-20.12146	25.26253	14.27186
C	-18.02567	23.99473	11.48590
H	-17.56031	23.42013	10.67685
C	-17.25295	24.36706	12.52385
H	-16.19701	24.08395	12.56893
H	-17.64268	24.96526	13.35098
C	-17.99954	24.16651	6.13761
H	-17.81030	23.44007	5.33029
H	-17.45911	25.09174	5.86407
H	-17.53413	23.77343	7.05299
C	-20.28174	24.66140	3.89166
H	-21.22128	24.62412	3.32915
C	-19.14914	24.76688	3.16577
H	-19.19241	24.80062	2.07337
H	-18.15920	24.83257	3.61910
C	-25.67135	25.21339	3.90784
H	-24.77088	24.94247	3.33613
H	-26.46416	24.49995	3.62930
H	-26.00758	26.20719	3.55937
C	-27.84589	25.60028	6.16271
H	-28.22813	26.25310	6.96358
H	-27.95098	26.13641	5.20287

C -28.76029 24.35643 6.17958
H -29.56441 24.47702 5.43146
H -28.21573 23.44097 5.89424
C -29.48618 24.08154 7.55702
O -30.14412 23.03119 7.58730
O -29.33556 24.96359 8.44537
Fe -22.92533 24.73678 8.83161
O -23.11625 22.84199 9.03055
N -23.17815 21.90176 8.12958
O -23.13930 22.21555 6.96756

Vibrational Frequencies (cm^{-1})

Nitro Complex

0: 0.00
1: 0.00
2: 0.00
3: 0.00
4: 0.00
5: 0.00
6: -99.66
7: -36.62
8: -28.61
9: -20.57
10: -13.70
11: 3.64
12: 5.75
13: 13.36
14: 16.97
15: 20.69
16: 22.02
17: 28.78
18: 30.70
19: 31.41
20: 37.35
21: 40.39
22: 42.08
23: 50.20
24: 54.33
25: 55.26
26: 61.67

27: 65.30
28: 70.60
29: 72.50
30: 81.32
31: 83.68
32: 89.84
33: 94.13
34: 97.00
35: 100.20
36: 105.08
37: 106.86
38: 112.92
39: 116.79
40: 122.02
41: 129.42
42: 132.13
43: 135.74
44: 141.79
45: 144.26
46: 155.34
47: 157.63
48: 163.65
49: 173.22
50: 183.21
51: 188.15
52: 192.22
53: 194.94
54: 197.59
55: 205.57
56: 209.93
57: 214.13
58: 215.41
59: 220.18
60: 222.12
61: 226.83
62: 233.50
63: 239.52
64: 239.74
65: 246.99
66: 252.64
67: 259.20
68: 266.47
69: 272.28
70: 281.78
71: 283.64
72: 291.02
73: 296.00
74: 308.88
75: 310.06
76: 310.81
77: 318.09
78: 324.46
79: 330.88
80: 336.79

81: 340.05
82: 343.94
83: 347.77
84: 358.81
85: 376.30
86: 379.23
87: 390.25
88: 399.48
89: 414.09
90: 427.20
91: 430.37
92: 435.92
93: 458.76
94: 469.05
95: 493.45
96: 506.17
97: 514.59
98: 521.63
99: 523.39
100: 544.60
101: 561.09
102: 571.07
103: 587.02
104: 607.20
105: 609.54
106: 613.96
107: 632.23
108: 641.70
109: 641.78
110: 651.67
111: 659.99
112: 674.49
113: 678.94
114: 679.79
115: 685.59
116: 693.32
117: 714.54
118: 718.31
119: 718.79
120: 725.13
121: 730.32
122: 732.64
123: 739.32
124: 741.29
125: 744.39
126: 748.17
127: 765.91
128: 768.06
129: 770.54
130: 778.33
131: 782.85
132: 783.72
133: 795.44
134: 802.98

135: 812.76
136: 815.83
137: 835.64
138: 839.93
139: 844.65
140: 846.74
141: 848.79
142: 850.63
143: 857.92
144: 862.62
145: 872.93
146: 874.95
147: 887.26
148: 905.84
149: 923.47
150: 942.20
151: 944.96
152: 952.91
153: 957.97
154: 966.22
155: 971.28
156: 972.95
157: 977.06
158: 984.31
159: 995.05
160: 1009.07
161: 1010.73
162: 1017.84
163: 1018.77
164: 1021.62
165: 1024.97
166: 1028.76
167: 1035.38
168: 1035.78
169: 1038.05
170: 1039.80
171: 1045.03
172: 1052.45
173: 1057.94
174: 1058.16
175: 1077.76
176: 1084.00
177: 1085.09
178: 1101.60
179: 1105.15
180: 1106.01
181: 1118.58
182: 1128.73
183: 1134.77
184: 1149.58
185: 1153.74
186: 1156.93
187: 1160.54
188: 1164.39

189: 1167.57
190: 1170.86
191: 1184.28
192: 1200.68
193: 1215.98
194: 1218.25
195: 1228.89
196: 1232.28
197: 1233.93
198: 1244.97
199: 1253.60
200: 1266.25
201: 1274.01
202: 1274.83
203: 1291.03
204: 1291.40
205: 1293.91
206: 1299.57
207: 1308.59
208: 1312.89
209: 1315.72
210: 1323.18
211: 1331.00
212: 1331.22
213: 1339.79
214: 1344.03
215: 1352.67
216: 1361.63
217: 1365.13
218: 1369.08
219: 1370.85
220: 1378.46
221: 1379.04
222: 1388.51
223: 1392.70
224: 1398.22
225: 1398.57
226: 1399.40
227: 1401.90
228: 1411.00
229: 1413.43
230: 1417.51
231: 1420.49
232: 1425.78
233: 1427.62
234: 1429.38
235: 1430.17
236: 1434.45
237: 1437.48
238: 1443.52
239: 1446.09
240: 1446.52
241: 1449.99
242: 1451.38

243: 1452.19
244: 1454.88
245: 1455.76
246: 1461.88
247: 1463.70
248: 1464.70
249: 1465.96
250: 1466.03
251: 1466.18
252: 1466.70
253: 1467.27
254: 1472.16
255: 1472.60
256: 1473.47
257: 1481.65
258: 1485.93
259: 1496.22
260: 1517.91
261: 1527.47
262: 1532.33
263: 1537.95
264: 1545.97
265: 1548.72
266: 1564.66
267: 1575.82
268: 1588.47
269: 1599.09
270: 1615.40
271: 1624.75
272: 1628.04
273: 1646.50
274: 1654.25
275: 1657.63
276: 1679.50
277: 1686.67
278: 1713.11
279: 1716.56
280: 1764.06
281: 2981.88
282: 2988.73
283: 2997.93
284: 3001.37
285: 3005.13
286: 3007.84
287: 3009.14
288: 3017.62
289: 3017.83
290: 3019.24
291: 3019.72
292: 3026.80
293: 3029.72
294: 3036.51
295: 3040.99
296: 3054.71

297: 3057.72
298: 3062.08
299: 3065.52
300: 3070.38
301: 3072.12
302: 3075.81
303: 3075.94
304: 3082.54
305: 3084.51
306: 3090.82
307: 3093.96
308: 3106.20
309: 3107.03
310: 3110.09
311: 3113.78
312: 3115.39
313: 3117.17
314: 3117.90
315: 3118.11
316: 3119.26
317: 3122.49
318: 3139.21
319: 3140.22
320: 3145.44
321: 3148.67
322: 3153.31
323: 3157.63
324: 3187.07
325: 3206.91
326: 3213.20
327: 3216.21
328: 3245.50
329: 3247.67
330: 3264.11
331: 3278.41
332: 3654.37

Nitrito Complex

0: 0.00
1: 0.00
2: 0.00
3: 0.00
4: 0.00
5: 0.00
6: -86.85
7: -67.69
8: -43.90
9: -34.25

10: -13.69
11: -5.12
12: 13.90
13: 14.87
14: 18.41
15: 20.53
16: 24.75
17: 27.39
18: 29.61
19: 35.19
20: 37.41
21: 40.65
22: 45.43
23: 47.27
24: 51.67
25: 55.03
26: 57.86
27: 63.98
28: 66.46
29: 68.92
30: 69.68
31: 74.20
32: 76.32
33: 81.69
34: 87.81
35: 92.79
36: 98.78
37: 102.35
38: 107.13
39: 110.49
40: 112.73
41: 117.16
42: 121.87
43: 124.39
44: 128.09
45: 131.24
46: 136.56
47: 148.16
48: 158.11
49: 161.33
50: 171.13
51: 174.79
52: 178.08
53: 187.75
54: 191.56
55: 195.85
56: 197.46

57: 203.53
58: 204.82
59: 206.67
60: 216.52
61: 219.64
62: 226.23
63: 234.09
64: 244.84
65: 246.32
66: 249.05
67: 252.46
68: 261.86
69: 268.80
70: 274.56
71: 279.99
72: 285.87
73: 288.30
74: 294.74
75: 300.41
76: 304.83
77: 315.09
78: 329.14
79: 333.47
80: 336.09
81: 343.78
82: 349.62
83: 352.49
84: 359.29
85: 373.37
86: 375.52
87: 376.92
88: 380.53
89: 407.10
90: 414.99
91: 433.32
92: 436.05
93: 450.99
94: 474.72
95: 480.07
96: 490.02
97: 508.91
98: 515.24
99: 524.69
100: 534.58
101: 541.51
102: 560.87
103: 567.77

104: 575.82
105: 587.94
106: 614.31
107: 635.73
108: 642.79
109: 643.57
110: 644.51
111: 658.83
112: 668.30
113: 674.23
114: 677.09
115: 678.61
116: 686.04
117: 695.47
118: 715.05
119: 718.73
120: 725.39
121: 726.87
122: 729.32
123: 731.59
124: 739.83
125: 743.17
126: 749.36
127: 754.22
128: 758.50
129: 771.53
130: 775.37
131: 782.17
132: 783.58
133: 795.27
134: 802.04
135: 813.26
136: 818.26
137: 843.04
138: 845.33
139: 846.11
140: 847.60
141: 848.06
142: 848.44
143: 854.14
144: 868.32
145: 874.24
146: 875.35
147: 888.71
148: 903.27
149: 924.51
150: 936.81

151: 946.95
152: 950.82
153: 952.13
154: 960.29
155: 961.83
156: 967.70
157: 975.76
158: 983.04
159: 991.16
160: 1007.05
161: 1008.28
162: 1015.17
163: 1018.03
164: 1024.33
165: 1026.63
166: 1027.99
167: 1029.90
168: 1032.54
169: 1035.16
170: 1040.29
171: 1042.25
172: 1048.73
173: 1053.27
174: 1067.18
175: 1075.79
176: 1079.61
177: 1090.28
178: 1093.47
179: 1099.53
180: 1104.12
181: 1112.17
182: 1118.36
183: 1138.29
184: 1142.09
185: 1151.17
186: 1154.36
187: 1155.68
188: 1156.91
189: 1161.76
190: 1162.72
191: 1169.34
192: 1184.17
193: 1200.85
194: 1215.74
195: 1216.71
196: 1226.05
197: 1233.88

198: 1244.59
199: 1247.13
200: 1248.81
201: 1266.36
202: 1271.00
203: 1274.60
204: 1286.50
205: 1295.29
206: 1296.30
207: 1305.04
208: 1305.74
209: 1312.33
210: 1313.61
211: 1320.83
212: 1323.80
213: 1332.87
214: 1333.70
215: 1336.28
216: 1344.66
217: 1350.08
218: 1366.49
219: 1367.98
220: 1369.10
221: 1377.89
222: 1379.66
223: 1384.90
224: 1389.74
225: 1395.34
226: 1398.03
227: 1400.94
228: 1403.29
229: 1410.56
230: 1413.63
231: 1417.38
232: 1417.97
233: 1420.61
234: 1422.91
235: 1427.40
236: 1430.88
237: 1433.87
238: 1437.08
239: 1443.95
240: 1445.91
241: 1448.06
242: 1449.33
243: 1451.72
244: 1453.66

245: 1456.99
246: 1459.95
247: 1460.59
248: 1462.28
249: 1462.92
250: 1463.38
251: 1467.23
252: 1467.92
253: 1469.12
254: 1470.24
255: 1471.28
256: 1473.49
257: 1483.55
258: 1483.70
259: 1491.55
260: 1515.54
261: 1524.57
262: 1529.57
263: 1531.55
264: 1534.49
265: 1540.47
266: 1563.98
267: 1578.60
268: 1592.93
269: 1607.55
270: 1616.06
271: 1620.49
272: 1623.40
273: 1638.57
274: 1646.61
275: 1657.76
276: 1678.68
277: 1683.97
278: 1712.01
279: 1731.69
280: 1760.11
281: 2989.75
282: 2998.84
283: 3001.43
284: 3001.72
285: 3006.20
286: 3007.02
287: 3007.47
288: 3011.56
289: 3014.38
290: 3017.13
291: 3021.48

292: 3025.57
293: 3030.50
294: 3036.72
295: 3049.98
296: 3057.68
297: 3060.28
298: 3063.89
299: 3067.91
300: 3071.19
301: 3071.28
302: 3072.35
303: 3074.04
304: 3077.73
305: 3077.89
306: 3079.20
307: 3097.43
308: 3106.51
309: 3109.44
310: 3116.67
311: 3117.43
312: 3118.88
313: 3124.17
314: 3124.19
315: 3132.63
316: 3135.30
317: 3137.73
318: 3144.81
319: 3147.78
320: 3149.75
321: 3150.46
322: 3153.03
323: 3163.76
324: 3170.35
325: 3207.45
326: 3208.12
327: 3223.36
328: 3229.30
329: 3243.44
330: 3265.18
331: 3276.65
332: 3649.25

The first 6 frequencies correspond to translational and rotational ones and so are 0.00 cm^{-1}

The imaginary frequencies are a result of the fragment optimization methodology and are associated with the atoms whose coordinates are kept constrained.