The Role of Conserved Tyrosine Residues in NiSOD Catalysis: A Case of Convergent Evolution

Robert W. Herbst, Abigail Guce, Peter A. Bryngelson, Khadine A. Higgins, Kelly C. Ryan Diane E. Cabelli, Scott Garman and Michael J. Maroney[†]**

SUPPORTING INFORMATION

Figure S1. Amino acid sequence alignment of NiSODs. Strictly conserved residues are colored; green residues are the topic of this paper. The boxed residues constitute the "Ni hook" domain. The N-terminus of the mature polypeptide is indicated by the arrow, and residue numbering in the text begins with this residue.

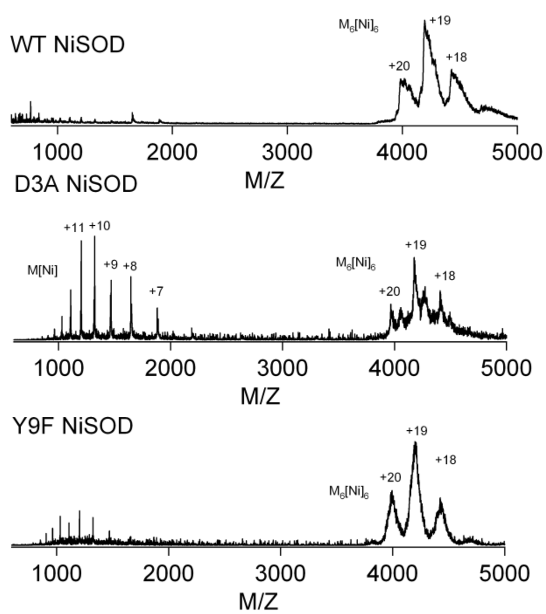

Figure S2. ESI-MS taken under non-denaturing conditions. Monomeric species correspond to peaks centered at M/Z ~ 1500, and hexameric species correspond to M/Z ~ 3800 – 4500.

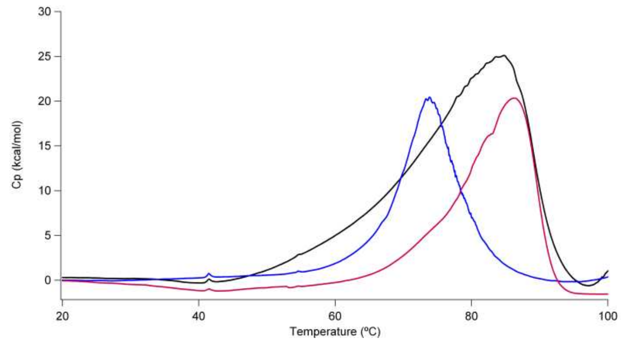

Figure S3. DSC Thermograms. Black – WT-NiSOD; Blue – D3A-NiSOD; Red – Y9F-NiSOD.

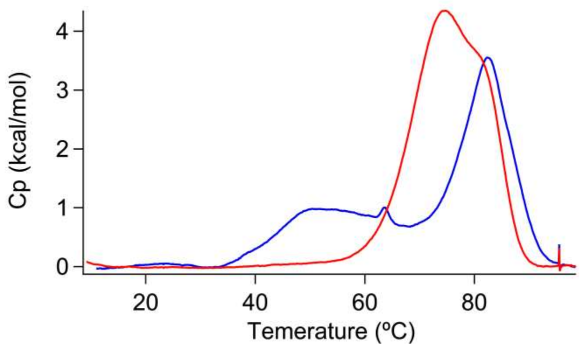

Figure S4. DSC Thermograms. Blue – Y62F-NiSOD; Red – Y9FY62F-NiSOD.

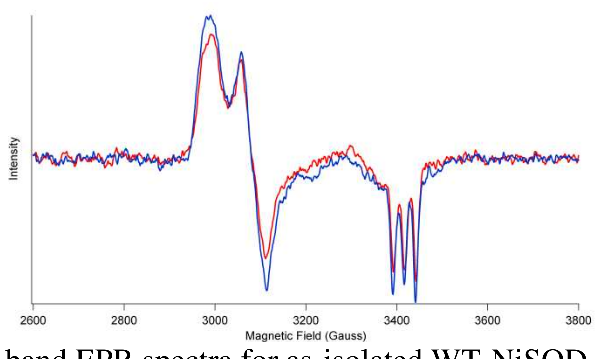

Figure S5. Overlay of X-band EPR spectra for as-isolated WT-NiSOD (Red) and after addition of $KMnO_4$ (Blue).

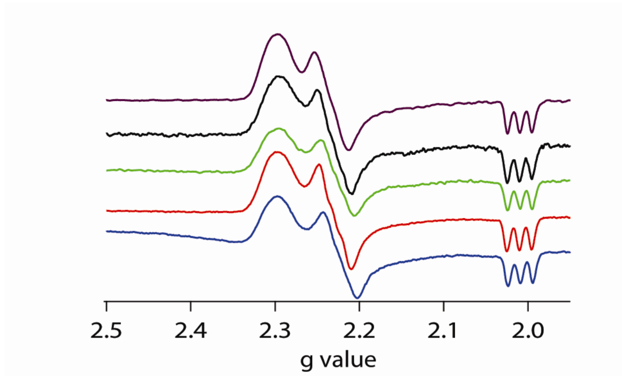

Figure S6. X-band EPR spectra of WT and mutant strains of NiSOD. Spectra were collected at 77K with a microwave frequency of 9.46 GHz. WT-(Blue), Y9F-NiSOD (Red), Y62F-NiSOD (Green), Y9FY62F-NiSOD (Black), and D3A-NiSOD (Purple).

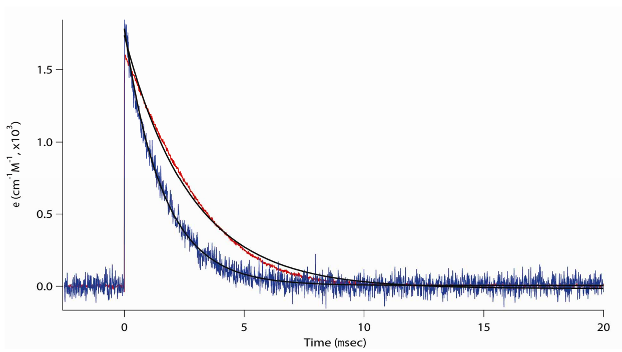

Figure S7. Kinetic data monitoring the disappearance of superoxide at 260 nm. The red trace is the activity of Y9F-NiSOD when 48.3 mM of O_2^- is generated. The blue trace is the activity of Y9F-NiSOD when 6.47 mM of O_2^- is generated. The black traces are both first-order fits to the data. A first-order fit is not sufficient to fit the data when large amounts of O_2^- are generated. This is because of saturation effects. Product inhibition can be ruled out because the data depicted by the blue trace (low substrate concentration) was taken on the same sample after the data in the red trace, and thus more products were present.

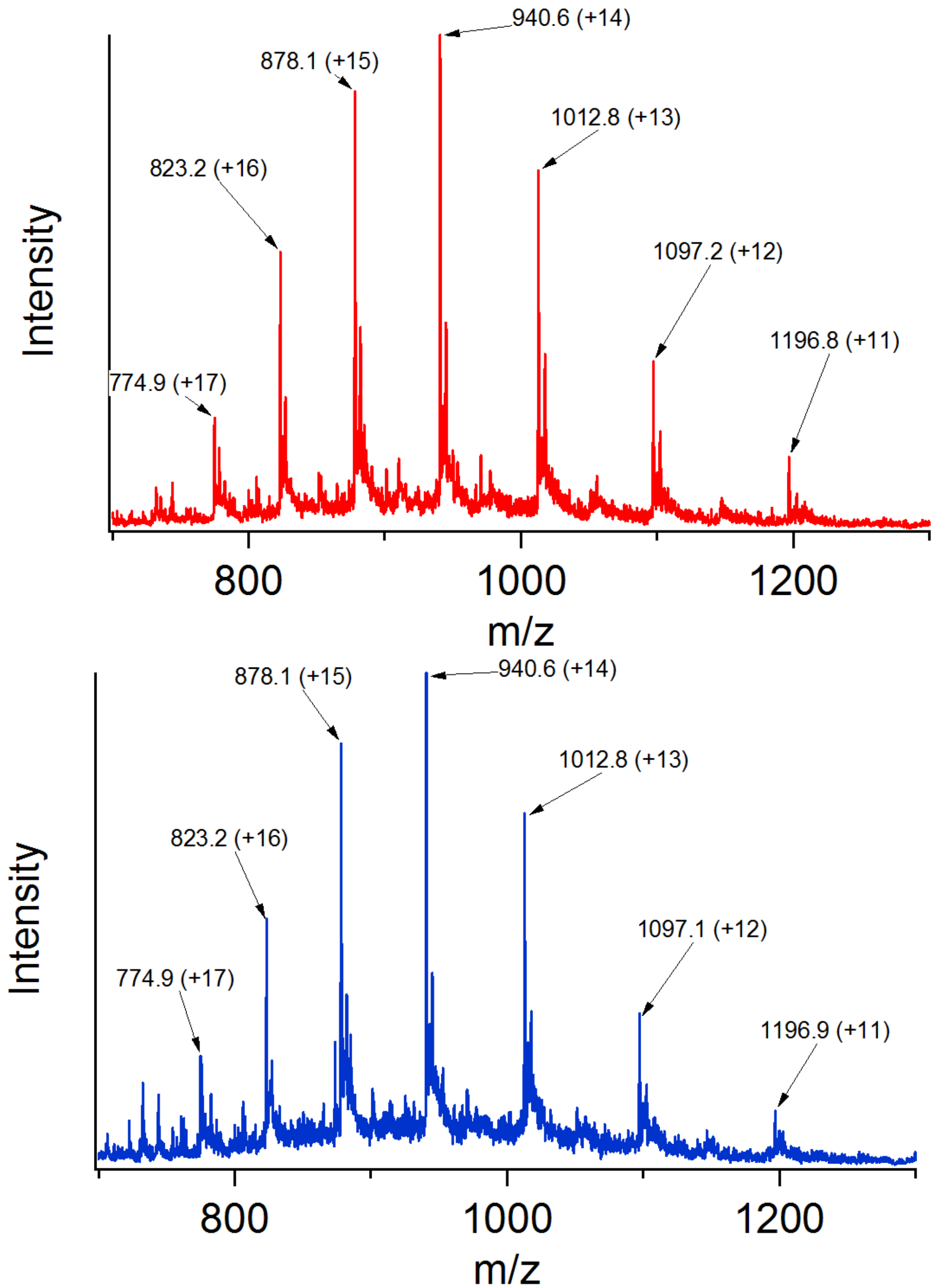

Figure S8. ESI-MS of D3A-NiSOD before (red, top) and after (blue, bottom) reaction with H_2O_2.

Table S1. Y9F First Shell XAS Fits

Ni-N 2.00

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
3N	2.04(2)	6(2)	0(3)	58.2	0.2752
4N	2.03(2)	9(2)	-2(3)	55.1	0.2609
5N	2.02(2)	11(2)	-3(3)	57.54	0.2723
6N	2.01(3)	13(2)	-4(3)	62.8	0.297

Ni-S 2.30

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.16(3)	8(1)	-12(5)	51.2	0.2423
3S	2.16(2)	11(1)	-14(4)	44	0.2082
4S	2.15(2)	14(1)	-16(4)	43.5	0.2057
5S	2.14(2)	16(2)	-17(4)	45.9	0.217
6S	2.14(3)	18(2)	-18(4)	49.5	0.2343

Ni-N 2.00 Ni-S 2.30

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	1.88(6)	9(5)			
2S	2.18(3)	7(2)	-14(6)	63.7	0.1497
3N	2.0(2)	21(12)			
2S	2.17(4)	9(4)	-11(12)	67.5	0.1588
3N	1.96(3)	9(3)			
1S	2.24(3)	4(2)	-7(4)	37.4	0.0661
1S	2.54(3)	7(4)			

Table S2. Y62F First Shell XAS Fits

Ni-N 2.00

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
3N	2.03(2)	4(1)	1(2)	32.8	0.1081
4N	2.03(2)	7(2)	-1(3)	39.21	0.1292
5N	2.03(2)	9(2)	-2(3)	54.3	0.1789
6N	2.02(3)	12(3)	-3(3)	72.7	0.2396

Ni-S 2.30

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.14(2)	6(1)	-14(4)	26.7	0.0884
3S	2.14(2)	10(1)	-15(4)	25.4	0.0837
4S	2.14(2)	12(1)	-16(4)	33.4	0.11

Ni-N 2.0 Ni-S 2.2

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	1.9(2)	21(16)			
2S	1.15(3)	6(2)	-14(7)	27.8	0.0645
3N	2.0(3)	30(18)	-12(9)	27.3	0.0635

2S	2.15(3)	7(2)			
2N					
3S					N/A
4N	2.07(6)	15(7)			
1S	2.18(1)	4(2)	-2(5)	17.9	0.0417
3N	1.8(2)	39(51)			
3S	2.14(3)	9(2)	-17(6)	34	0.0792
4N	2.09(9)	5(4)			
2S	2.13(6)	8(8)	-2(4)	20.2	0.047
5N	2.09(5)	19(6)			
1S	2.18(1)	3(1)	-1(4)	16.1	0.0373

Ni-N 1.8 Ni-N 2.0

	R	**σ^2 (x10^{-3})**	**E0**	**red chi**	**r-factor**
2N	1.93(3)	4(4)			
2N	2.06(2)	7e-7(2)	-2(3)	32.2	0.0759
2N	1.90(6)	9(9)			
3N	2.04(2)	3(3)	-4(3)	46	0.1071
3N	1.94(5)	10(7)			
2N	2.05(2)	1(2)	-4(3)	42.6	0.0991
3N	1.90(8)	20(17)			
3N	2.03(2)	4(2)	-4(4)	52.7	0.1226
4N	1.94(7)	17(10)			
2N	2.04(2)	1(2)	-5(4)	52	0.121

Ni-S 2.2 Ni-S 2.4

	R	**σ^2 (x10^{-3})**	**E0**	**red chi**	**r-factor**
2S	2.16(2)	6(1)			
2S	2.4(1)	32(33)	-11(6)	27.6	0.0641
3S	2.16(2)	9(1)			
2S	2.45(4)	19(7)	-10(3)	18.9	0.0439
3S	2.17(2)	8.5(9)			
3S	2.45(4)	24(7)	-9(3)	15.5	0.0359
4S	2.18(2)	11(1)			
2S	2.47(3)	14(4)	-11(3)	16.2	0.0376

Table S3. Y9FY62F First Shell XAS Fits

Ni-N 2.00

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
3N	2.06(2)	4(2)	1(3)	59.3	0.2331
4N	2.06(3)	7(3)	0(3)	75.4	0.2966
5N	2.05(4)	11(4)	-1(4)	93.7	0.3683
6N	2.05(6)	16(6)	-1(4)	108.7	0.4274

Ni-S 2.30

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.16(3)	6(2)	-14(6)	76.2	0.2994
3S	2.16(4)	10(3)	-15(7)	89.9	0.3532
4S	2.16(5)	14(3)	-16(7)	102.5	0.4029

Ni-N 2.0 Ni-S 2.2

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	2.07(5)	9e-7(3)			
2S	2.10(5)	7(8)	-12(10)	88.1	0.1781
3N	2.08(4)	0.4(26)			
2S	2.09(4)	6(6)	-6(7)	80	0.1617
2N	1.67(9)	18(18)			
3S	2.13(4)	10(2)	-20(8)	123.9	0.2504
4N	2.07(3)	3e-7(4)			
1S	2.05(5)	1(7)	0(3)	48.8	0.0987
3N	2.27(4)	0.1(13)			
3S	2.0(3)	32(25)	-43(16	86.6	0.175
4N	2.10(3)	0.3(24)			
2S	2.10(3)	4(4)	-2(3)	55	0.1111
5N	2.03(3)	0.7(24)			
1S	1.99(4)	3e-6(3)	-2(3)	76.5	0.1546

Ni-N 1.8 Ni-N 2.0

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	1.85(8)	17(17)			
3N	2.05(2)	3(2)	-3(5)	97.4	0.1968
3N	1.85(9)	27(25)			
3N	2.04(3)	4(2)	-4(5)	99.4	0.201
4N	2.04(3)	5(2)			
2N	1.80(8)	19(18)	-3(5)	105.4	0.2131

Ni-S 2.2 Ni-S 2.4

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.19(2)	5(1)			
2S	2.44(4)	11(4)	-8(4)	52.2	0.1055
2S	2.18(2)	5(1)			
3S	2.44(4)	17(5)	-7(4)	48.8	0.0986
3S	2.19(2)	8(1)			
3S	2.46(3)	13(4)	-8(4)	51.7	0.1046
4S	2.20(2)	11(2)			
2S	2.47(3)	7(3)	-9(4)	58.1	0.1175

Table S4. D3A First Shell XAS Fits

Ni-N 2.00

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
3N	2.06(2)	2(1)	1(3)	65	0.1742
4N	2.06(2)	4(2)	0(4)	94.6	0.2534
5N	2.06(3)	7(3)	-1(4)	129.6	0.3473
6N	2.05(4)	10(4)	-3(5)	161.5	0.4326

Ni-S 2.30

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.16(2)	4(1)	-15(5)	51.7	0.1384
3S	2.16(3)	7(2)	-16(5)	77.1	0.2065
4S	2.16(4)	10(2)	-17(6)	104.3	0.2794

Ni-N 2.0 Ni-S 2.2

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	1.80(3)	12(5)			
2S	2.14(1)	4.5(6)	-20(3)	20	0.0329
3N	2.08(3)	2(1)			
2S	2.12(2)	8(4)	-6(2)	16.3	0.0269
2N	2.2(2)	7(40)			
3S	2.17(2)	6(7)	-13(12)	41.9	0.0691
4N	2.0(1)	23(8)			
1S	2.18(2)	1.5(6)	-8(7)	13.3	0.022
3N	2.06(1)	1.8(9)			
3S	2.11(2)	10(3)	-11(3)	16.3	0.0268
4N	2.09(3)	2(1)			
2S	2.11(2)	6(4)	-5(2)	14.2	0.0234
5N	2.0(1)	28(7)	-8(7)	14.5	0.0239

1S	2.17(2)	1.4(6)

Ni-N 1.8 Ni-N 2.0

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	1.87(4)	9(7)			
3N	2.04(2)	2(2)	-7(3)	69.3	0.1142
3N	1.88(4)	9(6)			
3N	2.04(2)	1(2)	-8(4)	112.1	0.1424
4N	2.03(2)	3(1)			
2N	1.84(4)	8(6)	-7(3)	80	0.1319

Ni-S 2.2 Ni-S 2.4

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.17(1)	4.6(6)			
2S	2.41(5)	24(10)	-11(3)	19.8	0.0326
2S	2.17(1)	4.6(6)			
3S	2.42(4)	29(8)	-10(2)	15.5	0.027
3S	2.18(1)	7.2(6)			
3S	2.45(2)	19(4)	-11(2)	17.9	0.0295
4S	2.18(2)	9.7(9)			
2S	2.46(2)	10(3)	-13(3)	28.8	0.0474

Table S5. WT First Shell XAS Fits

Ni-N 2.00

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
3N	2.03(3)	6(2)	0(4)	75.6	0.22
4N	2.02(3)	9(3)	-2(4)	84	0.24
5N	2.01(4)	12(3)	-4(4)	97.3	0.28
6N	2.00(4)	15(4)	-6(5)	111.8	0.32

Ni-S 2.30

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2S	2.16(3)	8(1)	-13(5)	49.6	0.14
3S	2.15(3)	11(2)	-15(5)	51.6	0.15
4S	2.14(3)	14(2)	-17(5)	59.8	0.1717

Ni-N 2.0 Ni-S 2.2

	R	σ^2 (x10^{-3})	E0	red chi	r-factor
2N	1.82(6)	10(6)			
2S	2.15(3)	6(2)	-18(6)	48.9	0.099
3N	2.1(4)	29(140)			
2S	2.17(9)	8(4)	-8(33)	54.3	0.11

2N	2.08(2)	1.5e-6(2)			
3S	2.10(2)	9(2)	-13(5)	26.5	0.054
4N	1.90(8)	18(6)			
1S	2.17(2)	3(2)	-14(6)	37	0.075
3N	2.09(2)	1.5e-6(1)			
3S	2.10(2)	7(2)	-12(4)	22	0.045
4N	2.16(6)	21(22)			
2S	2.19(3)	8(2)	-2(8)	48.5	0.098
5N	2.1(2)	30(15)			
1S	2.18(3)	3(1)	4(11)	37.7	0.076

Ni-N 1.8 Ni-N 2.0

	R	**σ^2 (x10^{-3})**	**E0**	**red chi**	**r-factor**
2N	1.91(3)	4(4)			
2N	2.06(2)	1.5e-6(2)	-4(3)	52.1	0.11
2N	1.87(3)	3(3)			
3N	2.04(2)	1(2)	-6(4)	72.1	0.15
3N	1.91(3)	6(4)			
2N	2.06(2)	3.3e-7(2)	-6(4)	61.9	0.13
3N	1.88(4)	7(5)			
3N	2.04(3)	2(2)	-8(4)	87.9	0.18
4N	1.91(4)	10(4)			
2N	2.05(3)	0.3(21)	-8(4)	77.1	0.16

Ni-S 2.2 Ni-S 2.4

	R	**σ^2 (x10^{-3})**	**E0**	**red chi**	**r-factor**
2S	1.98(3)	8(2)			
2S	2.14(2)	3(1)	-25(4)	31	0.063
3S	2.18(3)	10(2)			
2S	2.48(4)	16(7)	-9(5)	42.5	0.086
3S	2.47(5)	20(7)			
3S	2.18(3)	10(2)	-9(5)	43.7	0.089
4S	2.18(3)	13(2)			
2S	2.49(3)	12(4)	-11(4)	37.5	0.076