SUPPORTING INFORMATION

Transiently produced hypochlorite is responsible for the irreversible inhibition of chlorite dismutase

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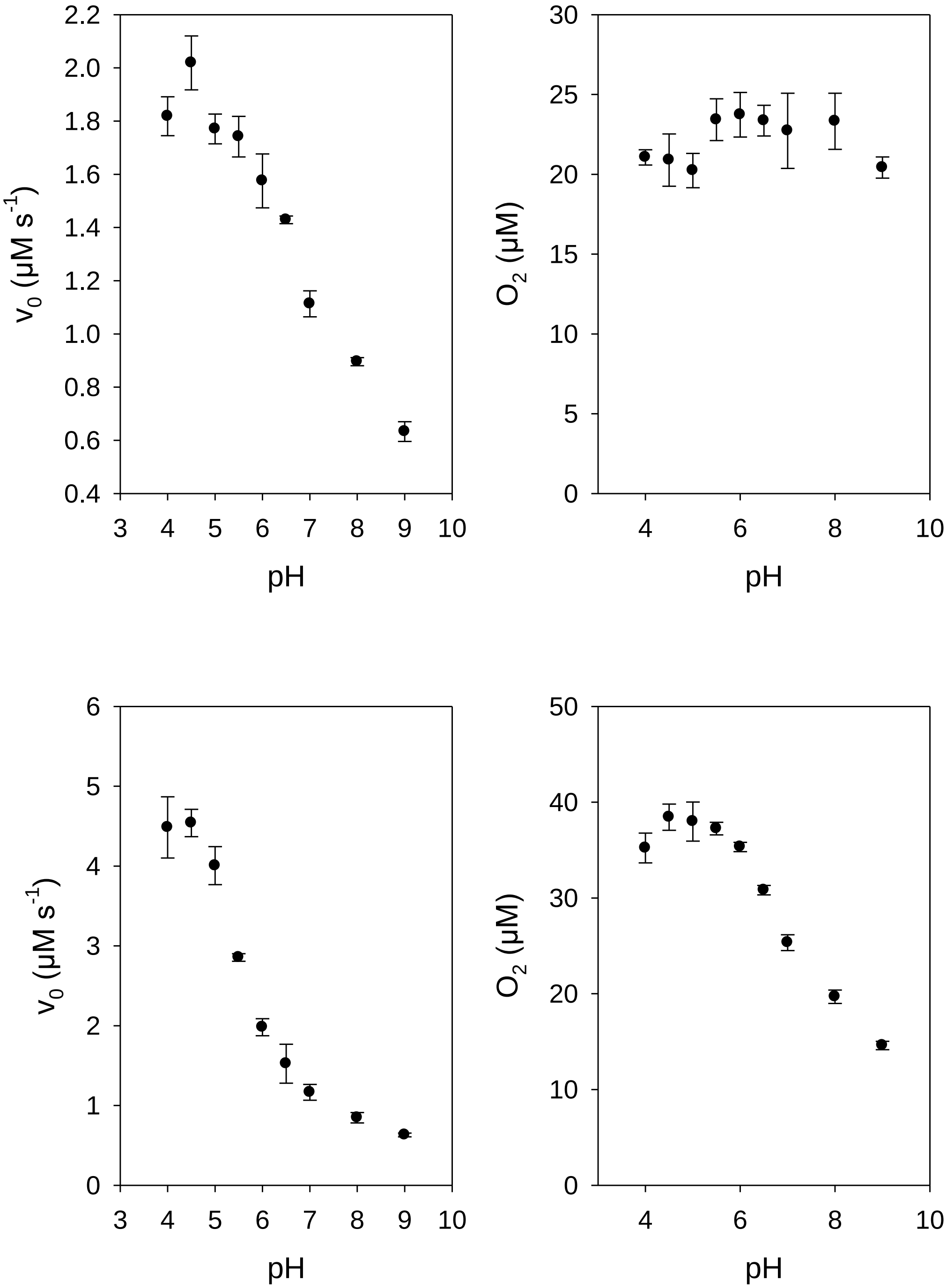
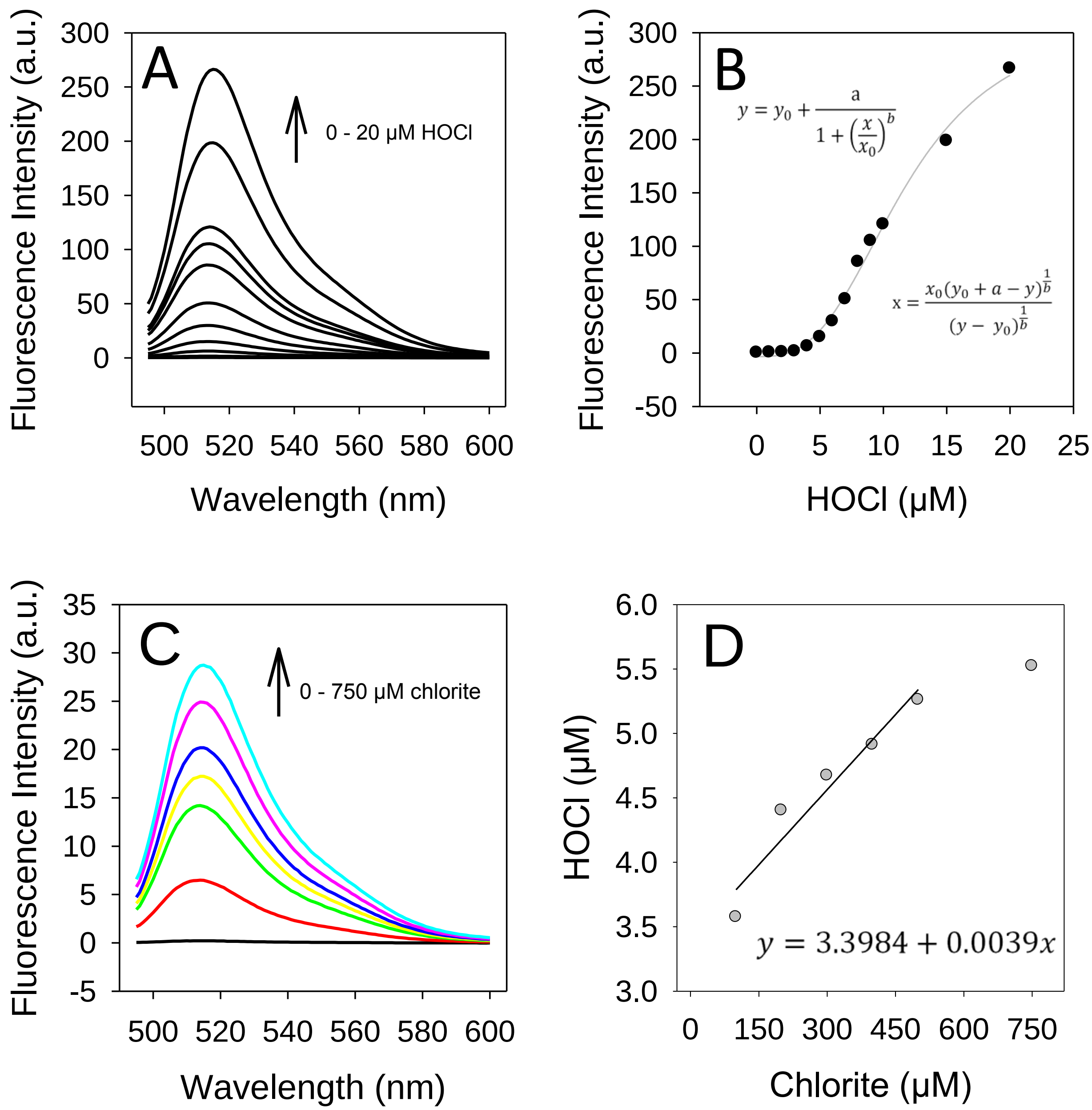
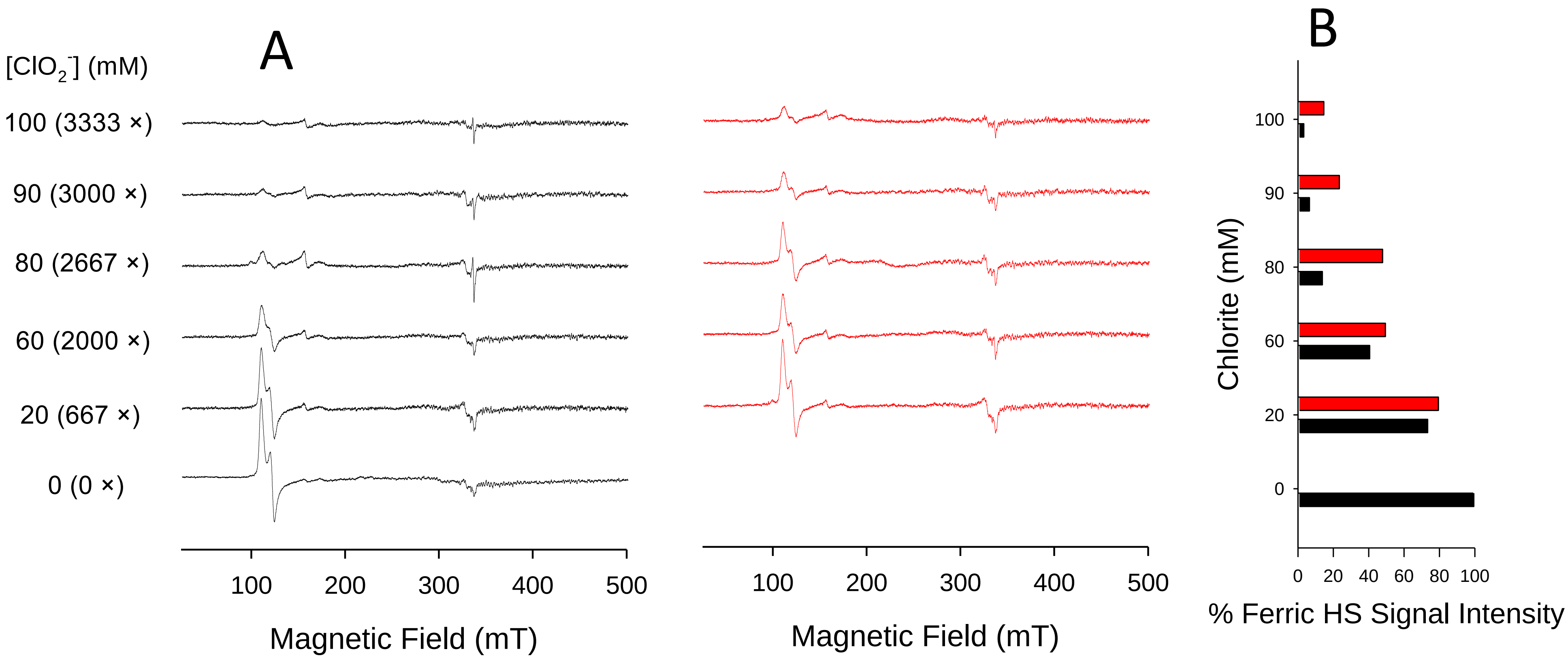
Supplemental Figure 1. pH-dependence of chlorite conversion by NdCld mutants. (A) Initial rates of production of dioxygen from 40 µM chlorite mediated by 200 nM NdCld mutant R173A at different pH-values (50 mM citrate phosphate buffer pH 4 - 7; 50 mM phosphate buffer pH 7 - 8; 50 mM Tris/HCl buffer, pH 8 - 9). (B) Total amount of produced dioxygen in the range of pH 4 - 9. Conditions in (C), where 200 nM NdCld mutant R173K was used resembled conditions from (A); and conditions in (D) were equal to conditions in (B) using 200 nM NdCld mutant R173K.



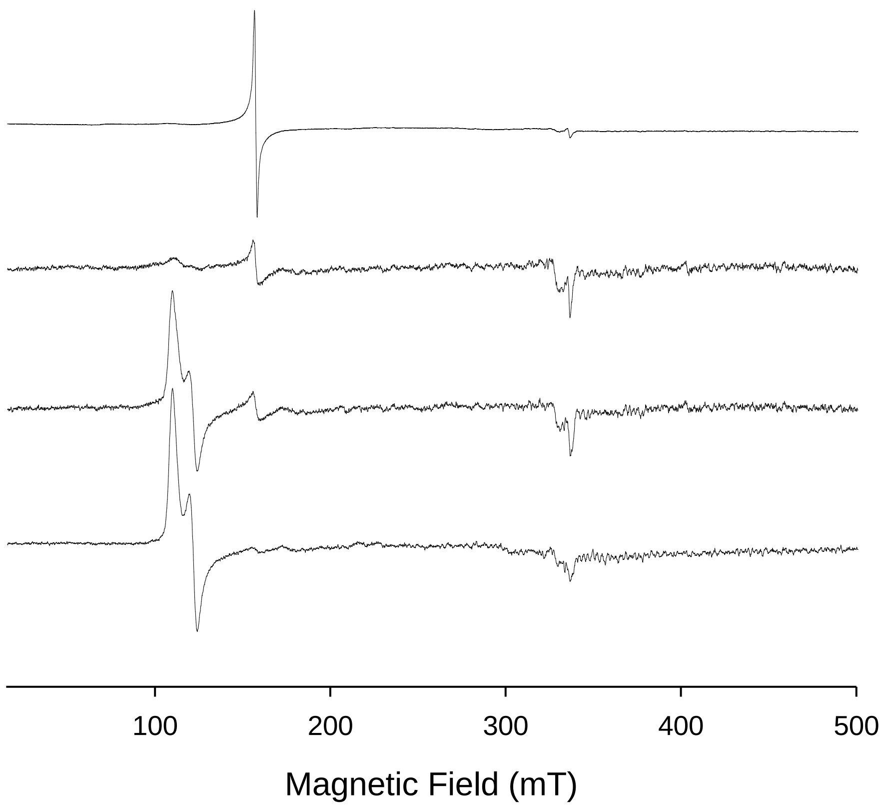
Supplemental Figure 2. Calibration curve of APF fluorescence for hypochlorite. (A) fluorescence spectra of 10 µM APF between 495 and 600 nm ($\lambda_{excitation}$ = 488 nm) after reactions with with 0 µM to 20 µM hypochlorite; 50 mM phosphate buffer pH 7.0. (B) Plot of fluorescence intensity *versus* hypochlorite concentration, showing a sigmoidal fit (logistic, 4 parameter). (C) Fluorescence spectra of 10 µM APF between 495 and 600 nm ($\lambda_{excitation}$ = 488 nm) after reactions with 100 nM NdCld wild-type with 0 µM (black), 100 µM (red), 200 µM (green), 300 µM (yellow), 400 µM (blue), 500 µM (magenta), and 750 µM (cyan) chlorite; 50 mM phosphate buffer pH 7.0. (D) Plot of trapped HOCl concentration versus chlorite concentration, showing a linear fit between 100 and 500 µM chlorite.



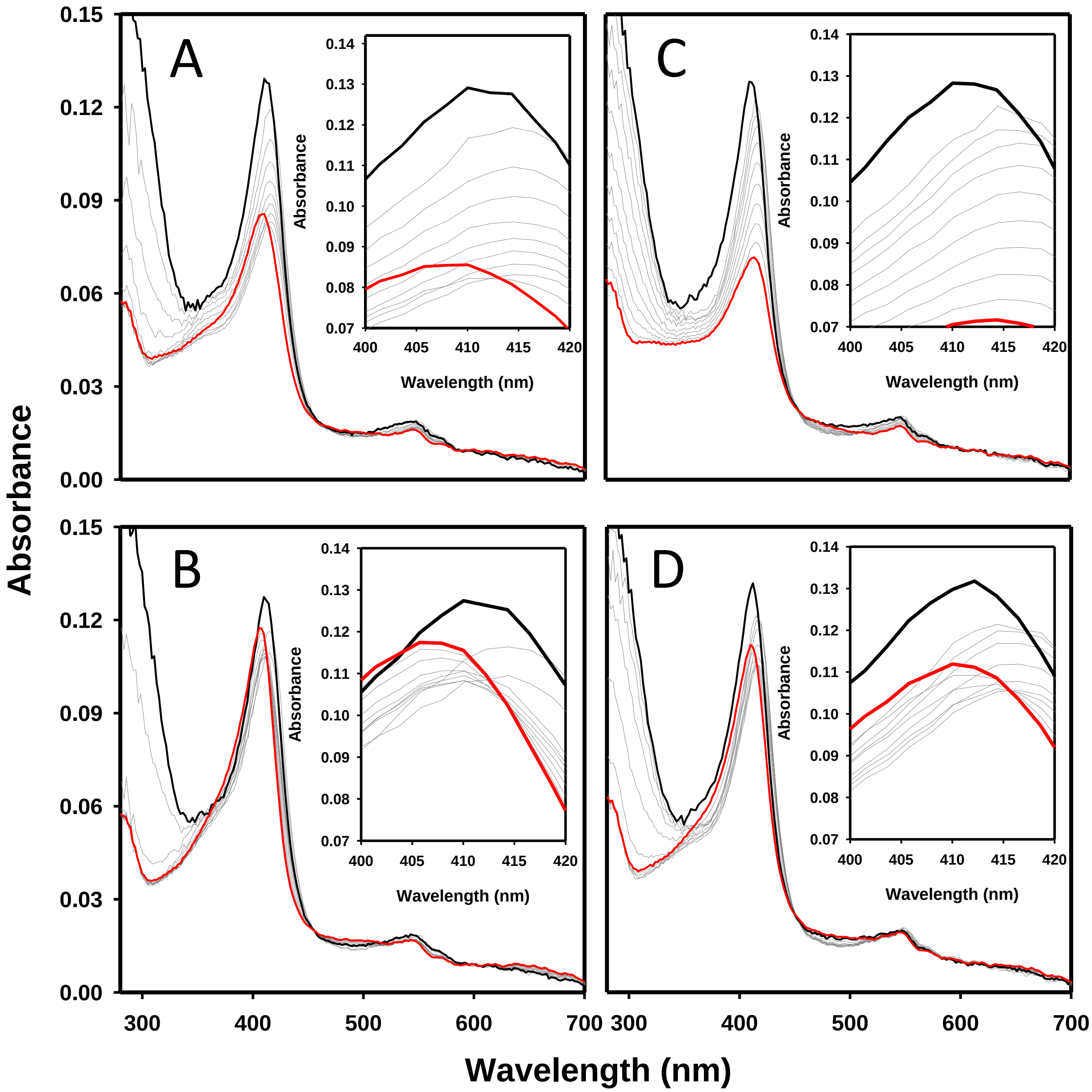
Supplemental Figure 3. (A) Electron Paramagnetic Resonance spectra of 30 µM NdCld in 50 mM MES buffer, pH 5.5., in absence (black lines) and presence (red lines) of 17.5 mM methionine, after reaction with 0 mM (bottom) to 100 mM (top) chlorite. (B) Ferric high spin signal intensity of chlorite treated samples in absence (black bars) and in presence (red bars) of methionine.



Supplemental Figure 4. Influence of hypochlorite to the architecture of the heme-iron of chlorite dismutase from "*Candidatus* Nitrospira defluvii". EPR spectra of 30 µM wild-type NdCld treated with (bottom to top) 0 mM, 1 mM, 16 mM, and 23 mM of hypochlorite



Supplemental Figure 5. The effect of methionine on the interconversion of redox intermediates and heme bleaching. (A) Spectral changes, when 1 µM of wild-type NdCld was mixed with 1 mM of chlorite at pH 5.5 (50 mM citrate-phosphate buffer) in the conventional stopped-flow modus. Spectra were taken at 3 ms (black spectrum), 74 ms, 403 ms, 755 ms, 1352 ms, 2367 ms, 4090 ms, 7018 ms, and 11990 ms, respectively. The final spectrum (20000 ms) is depicted in red. Inset shows enlargement of Soret region. (B) Same reaction as in (A) but in the presence of 5 mM methionine. (C) Same reaction as in (A) but at pH 7.0 (50 mM phosphate buffer), (D) same reaction as (C) but in presence of 5 mM methionine.



Supplemental Table 1. EPR simulation parameters from individual high spin and low spin forms of NdCld at pH 5.5 and pH 7.0, eventually with chlorite and methionine (HS – high spin, LS – low spin, R – rhombicity, I – relative intensity, E/D – rhombic to axial contribution, TI – total intensity with respect to untreated protein).

	HS LS compounds*	g_x^{eff} g_x	g_y^{eff} g_y	g_z^{eff} g_z	E/D	R (%)	I (%)	TI (%)
NdCld pH 5.5	HS1	5.500	6.175	1.990	0.014	4.2	80	100
	HS2	5.950	5.950	1.995			20	
	HS1	5.500	6.175	1.990	0.014	4.2	49	104
NdCld pH 5.5	HS2	5.950	5.950	1.995			26	
+ 17.5 mM methionine	LS1	2.980	2.250	1.875			13	
	LS2	3.150	2.250	1.800			13	
NdCld pH 5.5	HS1	5.625	6.225	1.990	0.013	3.7	43	14
+ 100 mM chlorite	HS2	5.950	5.950	1.995			57	
NdCld pH 5.5	HS1	5.500	6.175	1.990	0.014	4.2	50	36
+ 100 mM chlorite	HS2	5.950	5.950	1.995			28	
+ 17.5 mM methionine	HS3	5.250	6.375	1.975	0.025	7.0	22	
	HS1	5.500	6.175	1.990	0.014	4.2	49	100
NdCld pH 7.0	HS2	5.950	5.950	1.995			19	
	LS1	2.980	2.250	1.875			15	
	LS2	3.150	2.250	1.800			16	
	HS1	5.500	6.175	1.990	0.014	4.2	32	83
NdCld pH 7.0	HS2	5.950	5.950	1.995			28	
+ 17.5 mM methionine	HS3	5.250	6.375	1.975	0.025	7.0	14	
	LS1	2.980	2.250	1.875			13	
	LS2	3.150	2.250	1.800			14	
NdCld pH 7.0	HS1	5.500	6.175	1.990	0.014	4.2	18	11
+ 100 mM chlorite	HS2	5.950	5.950	1.995			64	
	HS3	5.450	6.225	1.990	0.017	4.8	18	
NdCld pH 7.0	HS1	5.500	6.175	1.990	0.014	4.2	41	42
+ 100 mM chlorite	HS2	5.950	5.950	1.995			23	
+ 17.5 mM methionine	HS3	5.250	6.375	1.975	0.025	7.0	36	

*minimum number of high-spin and low-spin compounds used for simulation