Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information to:

Effect of H bond removal and changes in the position of the iron-sulphur head domain on the spin-lattice relaxation properties of [2Fe-2S]$^{2+}$ Rieske cluster in cytochrome bc_1

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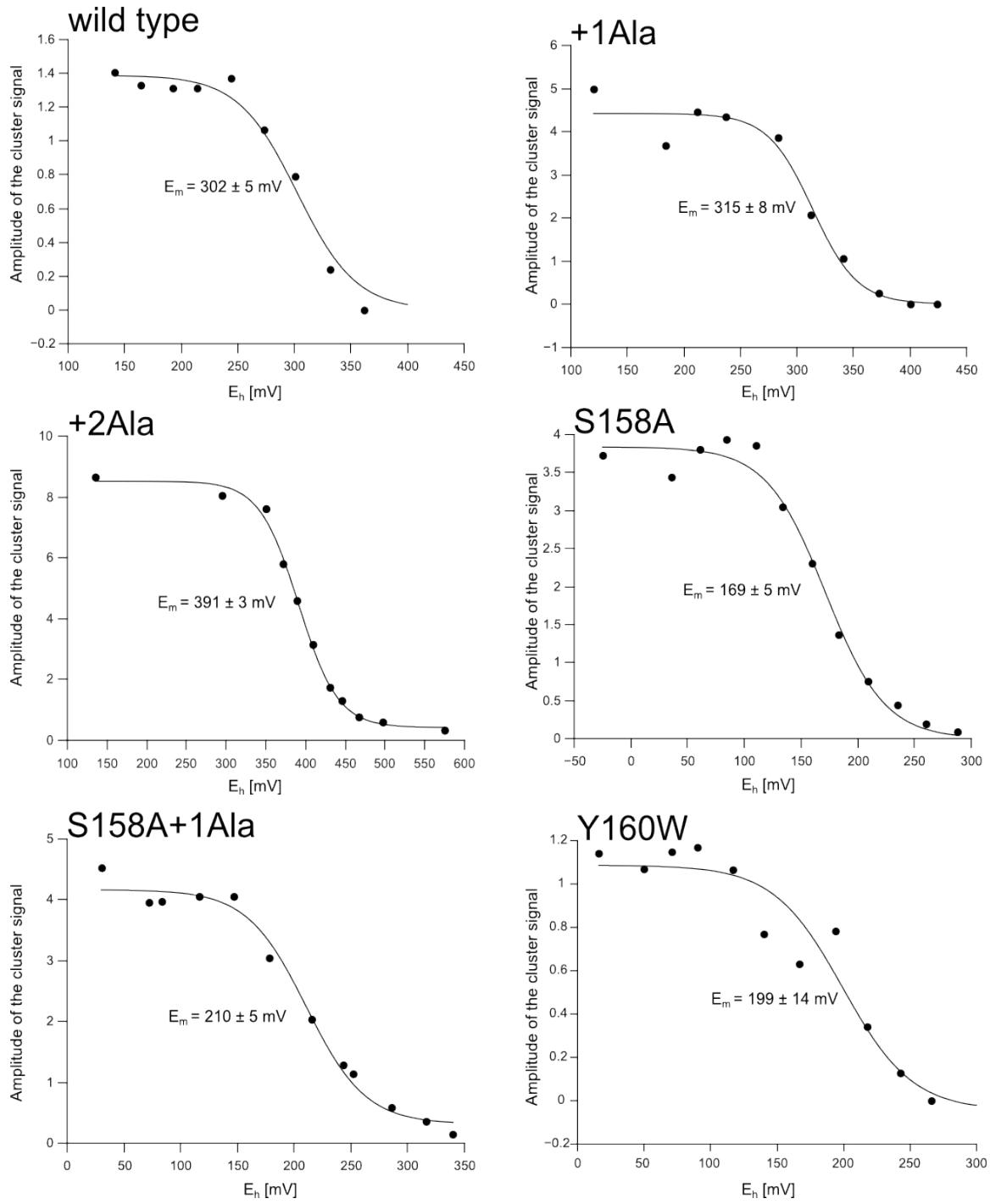

Fig. S1. Results of equilibrium redox titration of the Fe-S cluster for wild type, +1Ala, +2Ala, S158A, S158A+1Ala and Y160W mutants of cytochrome bc_1 at pH 8.0. The amplitude of the CW EPR signal of the Fe-S cluster was measured as a function of the ambient potential (E_h) and fitted using the well-known Nernst equation, yielding the value of the redox midpoint potential $E_{m,pH8}$. The concentration of cytochrome bc_1 was individually set in each experiment, what caused the differences in absolute values of measured signal amplitudes.

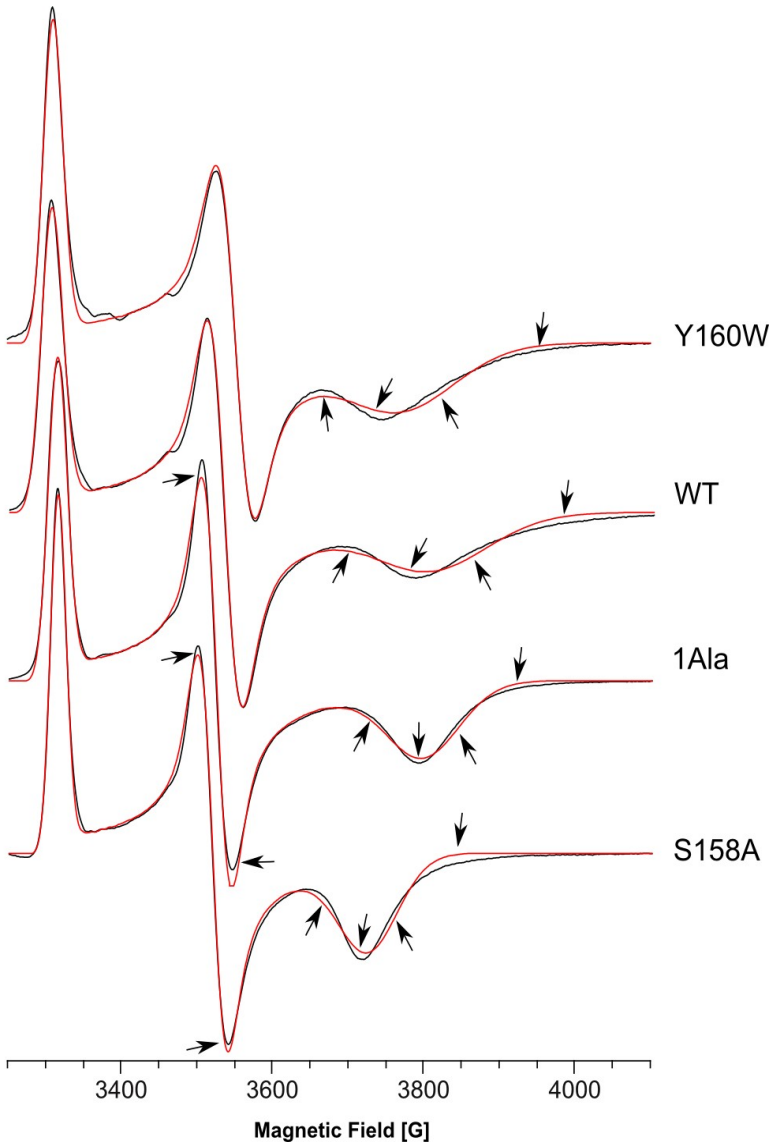

Fig. S2. Selected results of simulations of X-band CW EPR spectra of the Rieske cluster recorded at 25 K. The black lines represent the spectra measured for wild type (WT), Y160W, +1Ala, and S158A mutants of cytochrome bc_1. The red lines depict the simulated traces using a model of only one spectral component: defined by one **g**-tensor and one anisotropic ***g*-strain** tensor. Arrows indicate the regions where the largest discrepancies between experimental and simulated spectra are observed. The quality of the fits is improved significantly by assuming the presence of two components, defined by different **g** and **g-strain** tensors, as enumerated in the following Table S1.

Sample	g value		g-strain	
	component 1	component 2	component 1	component 2
WT	45% 2.0265 1.8962 1.7670	55% 2.0294 1.8975 1.7253	 0.0126 0.0117 0.0259	 0.0077 0.0099 0.0481
WT + tds	100% 2.0231 1.8921 1.7624	-	0.0055 0.0081 0.0209	-
WT + ant	43% 2.0256 1.9008 1.7690	57% 2.0245 1.8952 1.7377	 0.0096 0.0083 0.0195	 0.0099 0.0114 0.0443
WT + myx	41% 2.0279 1.8969 1.7128	59% 2.0258 1.8952 1.7676	 0.0079 0.0095 0.0368	 0.0115 0.0112 0.0268
WT + ato	18% 2.0208 1.8953 1.7685	82% 2.0306 1.8950 1.7518	 0.0099 0.0112 0.0181	 0.0086 0.0111 0.0400
WT + fam	45% 2.0300 1.8984 1.7775	55% 2.0279 1.8971 1.7495	 0.0056 0.0090 0.0197	 0.0118 0.0112 0.0472
WT + ant+myx	46% 2.0275 1.8952 1.7712	54% 2.0252 1.8969 1.7254	 0.0080 0.0110 0.0226	 0.0112 0.0096 0.0484
+1ALA	37% 2.0237 1.9054 1.7685	63% 2.0236 1.9002 1.7571	 0.0077 0.0066 0.0170	 0.0085 0.0103 0.0307
+2ALA	100% 2.0250 1.8989 1.7727	-	0.0077 0.0102 0.0252	-
S158A	46% 2.0216 1.9066 1.7821	54% 2.0238 1.9034 1.8029	 0.0080 0.0077 0.0299	 0.0052 0.0104 0.0157
S158A + tds	100% 2.0208 1.8978 1.7997	-	0.0045 0.0074 0.0159	-
S158A + myx	48% 2.0234 1.9030 1.7824	52% 2.0242 1.9051 1.8079	 0.0087 0.0107 0.0311	 0.0050 0.0073 0.0135
S158A+1ALA	47% 2.0224 1.9051 1.7911	53% 2.0196 1.9096 1.8010	 0.0047 0.0099 0.0237	 0.0077 0.0069 0.0143
S158A+2ALA	100% 2.0223 1.9053 1.8045	-	0.0055 0.0087 0.0181	-
Y160W	46% 2.0268 1.8874 1.7861	54% 2.0239 1.8871 1.7472	 0.0060 0.0109 0.0239	 0.0109 0.0132 0.0413
Y160W + tds	100% 2.0221 1.8862 1.7928	-	0.0050 0.0084 0.0181	-
Y160W + myx	46% 2.0282 1.8900 1.7901	54% 2.0261 1.8896 1.7473	 0.0067 0.0096 0.0237	 0.0129 0.0165 0.0459

Table S1. The values of **g** and **g-tensor** components determined from fitting the two-component model to X-band CW EPR spectra (25 K) for different forms of cytochrome bc_1.

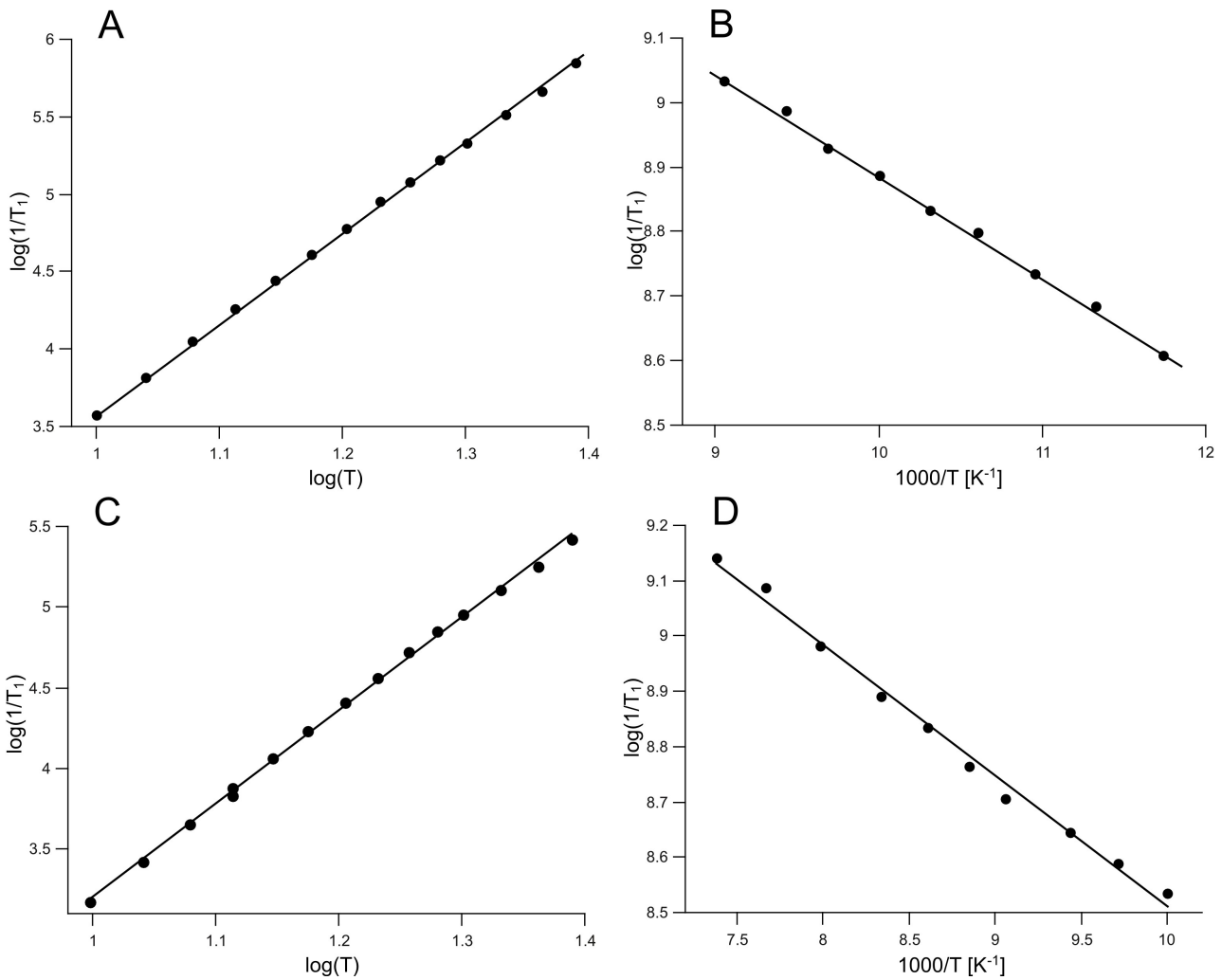

Fig. S3: Temperature dependence of the spin-lattice relaxation rate ($1/T_1$) of the Rieske cluster for tds-inhibited WT (A, B) and S158A mutant (C, D) of cytochrome bc_1. The data displayed in the left panel (A and C), within $\log(1/T_1)$ – log(T) plot, show the region (10 – 24 K), where the Raman process is the dominating mechanism of the relaxation. The data displayed in the right panel (B and D), within $\log(1/T_1)$ – (1000/T) plot, show the region (85 – 110 K for B and 100 – 135 for D), where the Orbach is the dominant process. The coefficients of superimposed linear fits clearly demonstrate differences/similarities between the analyzed samples.