

The Essential Role of the Cu(II) State of Sco in the Maturation of the Cu_A Center of Cytochrome Oxidase. Evidence from H135Met and H135SeM Variants of the *Bacillus subtilis* Sco

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Supplementary Material

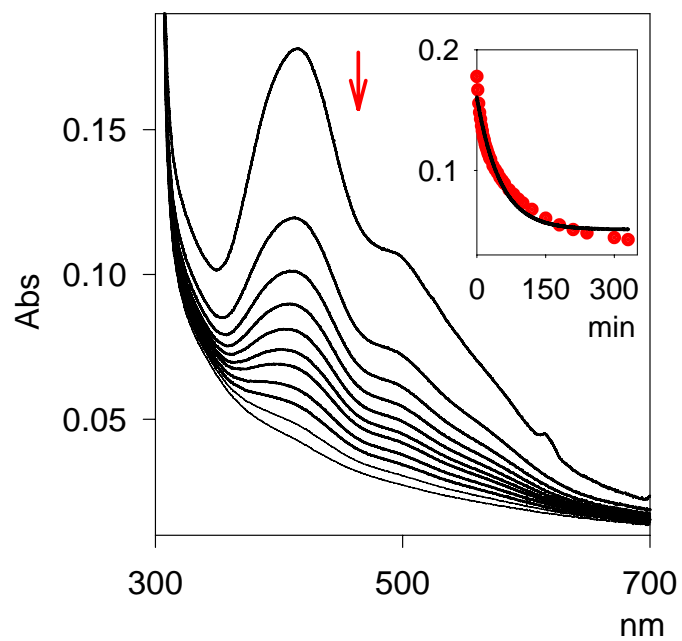


Figure S1. Selected kinetic traces showing the auto-reduction of Cu(II)-H135SeM ($80 \mu\text{M}$) to Cu(I)-H135SeM with time. *Inset* shows the exponential nature of auto-reduction.

X-band EPR of M52/M56/H135SeM-Cu²⁺

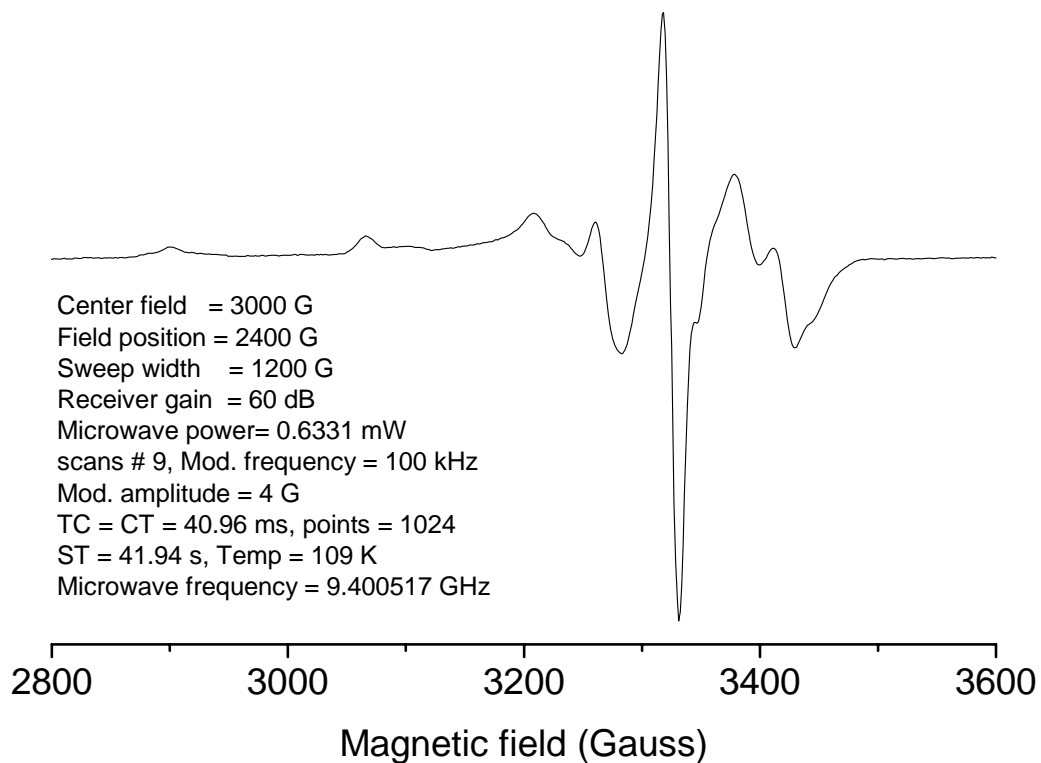


Figure S2. Continuous wave first-derivative X-band EPR spectra of Cu(II)-H135SeM. The experimental parameters are center field 3000 G, field position 2400 G, sweep width 1200 G, modulation amplitude 4G, modulation frequency 100 kHz, number of points 1024, receiver gain 60 dB, time constant = conversion time 40.96 ms, sweep time 41.94 s, scans 3, temperature 109 K, microwave power 0.63 mW, microwave frequency 9.4 GHz.

Table S1. Mass spectrometric data for WT, H135M and H135SeM derivatives of BSco

Sample	Observed Mass	Calculated Mass + N-terminal Met	Calculated Mass - N-terminal Met
Wild Type	19757	19766	19635
H135M	19773	19762	19629
H135SeM	19948	19950	19770

¹ Masses were calculated assuming a single disulfide between C45 and C49

Table S2. Association constants for WT Sco and its H135 variants. Cu(I) binding constants of BSco proteins were measured at 23°C by competitive titration against bicinchoninic acid (BCA) as described in the text. The fitting error is the statistical error derived from the simulation of each data set, and does not take into account the experimental errors associated with data collection which are generally much larger. A more realistic estimate of the error is the standard deviation of the average of K_{Assoc} which is given in Table 1 of the main text.

Sample	[Sco_Variant]	[Cu]	[BCA]	K_{Assoc}	Fitting Error (%)
WT	Titrated	10	100	2.1×10^{12}	30
	Titrated	10	200	5.4×10^{12}	11
	Titrated	10	350	7.4×10^{12}	13
	Titrated	10	500	1.7×10^{13}	10
	35	20	Titrated	2.5×10^{13}	15
H135A	Titrated	10	100	7.5×10^{10}	12
	Titrated	20	100	5.6×10^{10}	36
	25	10	Titrated	1.1×10^{11}	23
H135M	Titrated	10	50	4.4×10^{11}	7
	Titrated	10	100	6.9×10^{12}	14
	Titrated	10	300	9.0×10^{12}	11
	Titrated	10	500	1.0×10^{13}	4
	25	10	Titrated	3.7×10^{12}	10
H135SeM	Titrated	10	100	2.1×10^{12}	13
	Titrated	10	300	2.1×10^{12}	10
M52IM56IH135M	Titrated	10	100	4.3×10^{12}	20
	Titrated	10	300	1.8×10^{12}	4
M52IM56IH135SeM	Titrated	10	50	3.1×10^{12}	42
		10	100	2.5×10^{12}	30