Supporting Information

Table S1: Protein and metal concentrations in isolated mitochondria. Mitochondria isolated from cells grown on glycerol, galactose, and glucose are designated R# (respiring), RF# (respirofermenting) and F# (fermenting), respectively. Batches F1 – F4 were from cells grown on YPD media while F5 – F16 were from cells grown on minimal media. Sample characterizations are denoted (MB) Mössbauer, (U) UV-vis Spectroscopy, (E) EPR, (P) protein analysis and (M) metal analysis.

Batch	Protein (mg/mL)	Fe (μM)	Mn (μM)	Cu (μM)	Zn (μM)	Characterization
R1	200	750	23	360	420	MB, U, E, P, M
R2	180	600	46	63	320	MB, U, E P, M
R3	110	600	30	59	180	U, P, M
R4	120	670	42	110	82	U, P, M
R5	80	320	6.1	270	200	E, P, M
RF1	200	770	13	80	270	MB, E, P, M
RF2	120	690	8	170	100	U, E, P, M
F1	74	640	30	36	540	U, P, M
F2	64	520	12	95	580	U, P, M
F3	86	650	11	92	220	U, P, M
F4	80	530	27	58	190	U, P, M
F5	120	850	7.2	46	180	MB, P, M
F6	96	280	2.5	14	75	MB, P, M

F7	74	620	6.3	28	220	MB, P, M
F8	120	500	4.8	25	140	MB, P, M
F9	99	560	3.9	22	140	MB, P, M
F10	120	1300	1.6	41	280	E, P, M
F11	89	470	22	4.5	89	E, P, M
F12	N/A	N/A	N/A	N/A	N/A	MB
F13	N/A	N/A	N/A	N/A	N/A	MB
F14	N/A	N/A	N/A	N/A	N/A	MB
F15	N/A	N/A	N/A	N/A	N/A	MB
F16	N/A	N/A	N/A	N/A	N/A	MB

Figure S1. Electronic absorption spectra of heme-containing proteins. A, cytochrome *c* oxidase; B, cytochrome b_5; C, cytochrome *c*. Data were treated as described in *Experimental Procedures*. The heme *b* in cytochrome b_5 is LS, whereas the heme *b* groups in mitochondria are both LS and HS. The spectrum of HS heme *b* differs, but the extinction coefficients in the region used in fitting (~ 560 nm) was insignificantly different. Thus, our estimate of the heme *b* concentration in mitochondria will not be affected by calibrating with a LS center.

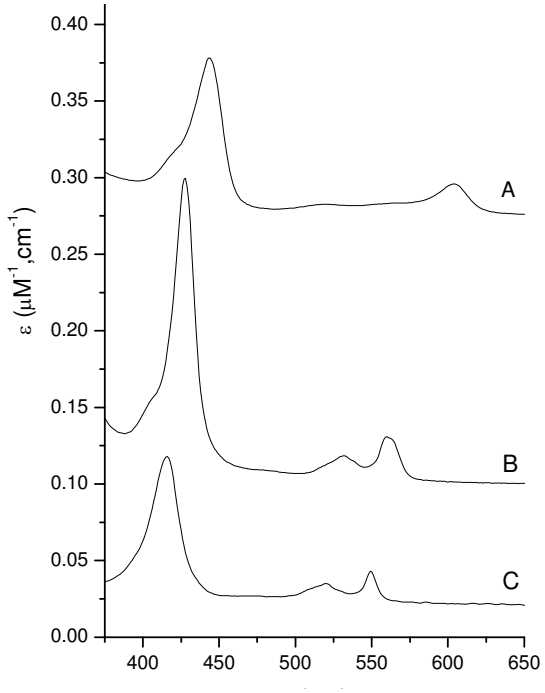

Figure S2. Protection of cytochrome c from protease degradation in isolated mitochondria. A sample of isolated mitochondria (50 μg of protein) was subjected to SDS-PAGE using a 12% polyacrylamide gel. Lane 1, control with no proteinase K (Fisher Scientific) added. Lanes 2 and 3, samples treated with 100 μg/mL and 500 μg/mL of proteinase K, respectively for 30 min. Samples were treated with PMSF (1 mM final concentration) for 30 min to inhibit protease activity and then with 0.5% deoxycholate prior to SDS-PAGE. Lanes 4 and 5, samples treated with 100 μg/mL and 500 μg/mL of proteinase K, respectively, in the presence of 0.5% deoxycholate.

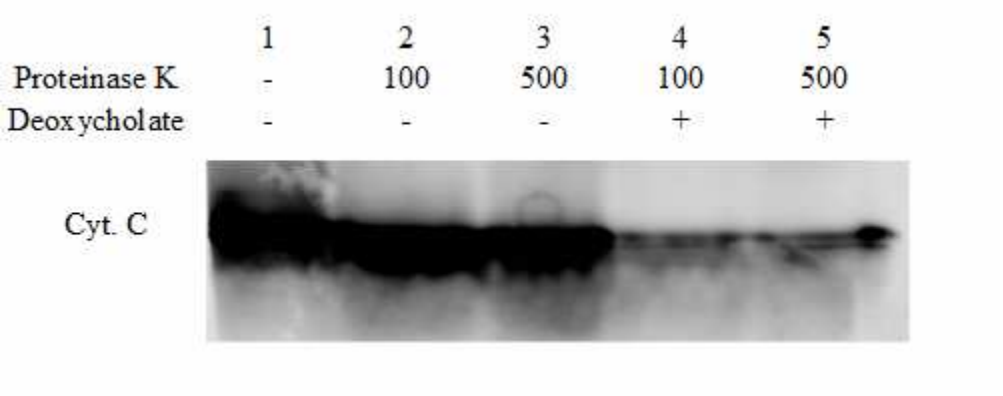

Figure S3. Mössbauer spectra of a respiring mitochondrial batch (Batch R2) not shown in Figure 1 but used in constructing Table 1. A, 4.5 K, 0.04 T parallel field; B, 100 K 0.04 T parallel field; and C, 4.2 K, 8 T applied field.

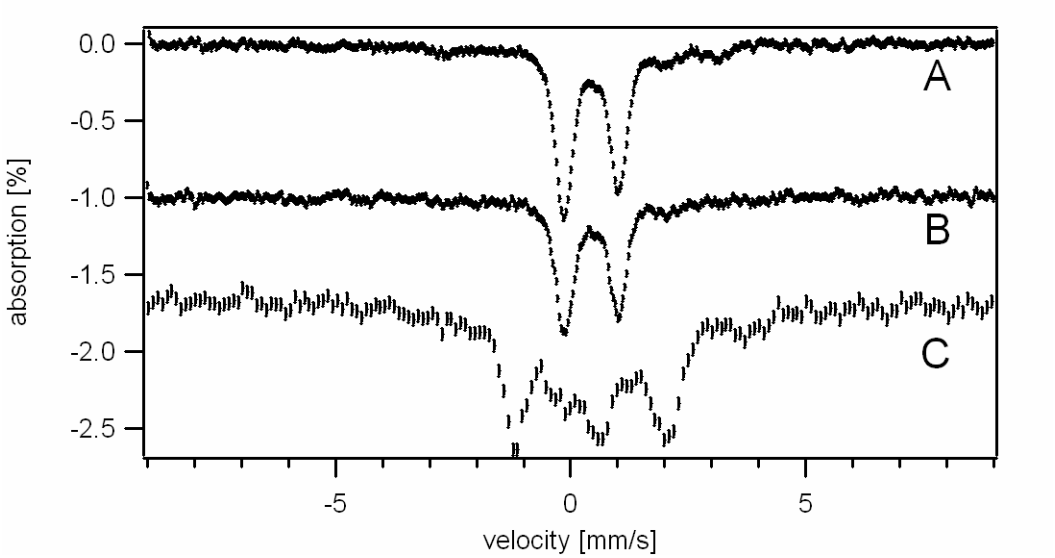

Figure S4. Electronic absorption spectra of respiring mitochondria suspensions. A, Batch R3 (protein concentration, 45 mg/mL); B, Batch R4 (44 mg/mL); and C, Batch R2 (74 mg/mL). Dotted lines are composite spectra using the [Heme *a*], [Heme *b*], and [Heme *c*] concentrations given in Table S2.

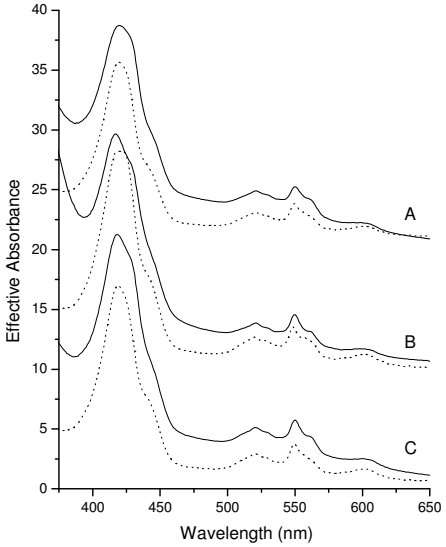

Table S2. Concentrations of each heme component determined for individual mitochondrial samples (in μM). Values indicated are for neat mitochondria. Estimated uncertainties are ± 20% for each entry.

Batch	[Heme *a*]	[Heme *b*]	[Heme *c*]
R1	45	43	110
R2	55	44	130
R3	60	60	130
R4	44	44	120
F1	15	30	85
F2	15	33	85
F3	13	22	61
F4	12	23	61

Figure S5. 10 K EPR spectra of mitochondria batches not shown in Figure 4 but used in the construction of Table 1. Spectra A, B, C and D are from Batches R5, R1, RF2 and F11, respectively. Spectra A, B and D were collected at 0.05 mW microwave power while spectrum C was collected at 0.2 mW. Microwave frequency, 9.468; modulation amplitude, 10 for A, C, D and 5 for B. Spectra A and C were multiplied by 1.5 while D was multiplied by 15.

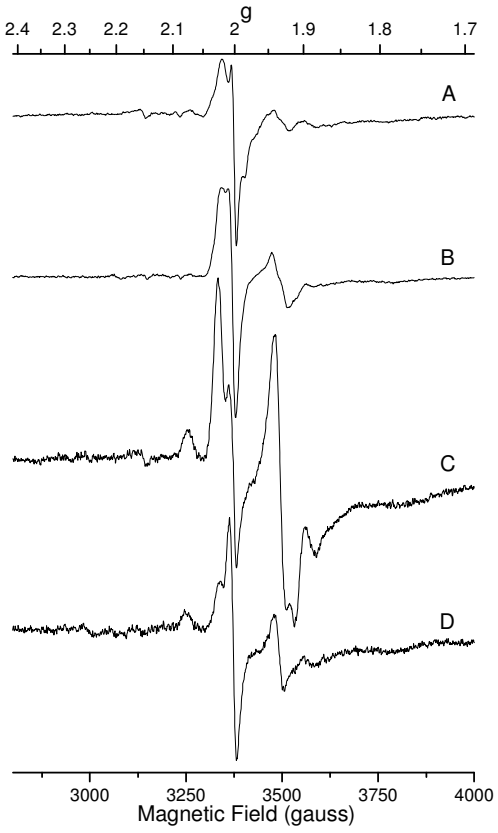

Figure S6. UV-Vis spectra of different batches of fermenting mitochondria. A, Batch F4 (33 mg/mL); B, Batch F1 (30 mg/mL) and C, Batch F2 (27 mg/mL). Dotted lines are composite spectra using the concentrations given in Table S2.