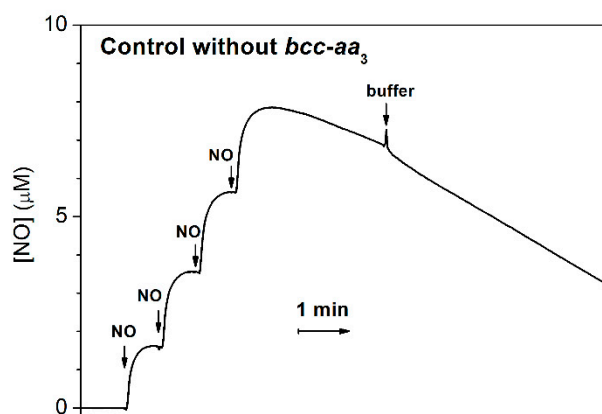


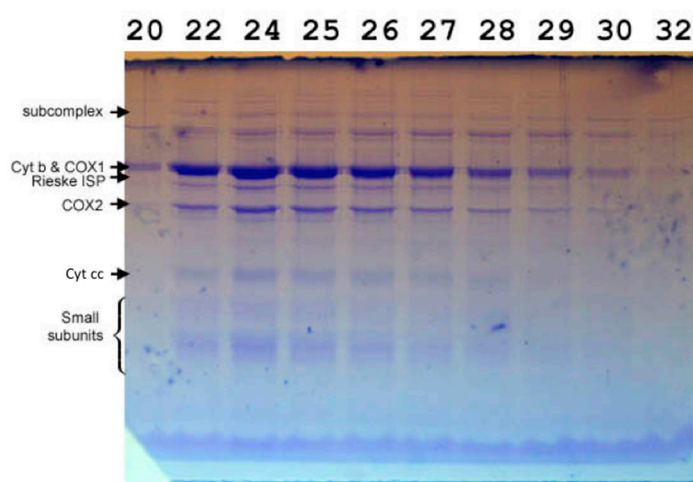


# 1 Supplementary materials for 2 Nitric oxide does not inhibit but is metabolized by the 3 cytochrome *bcc-aa<sub>3</sub>* supercomplex

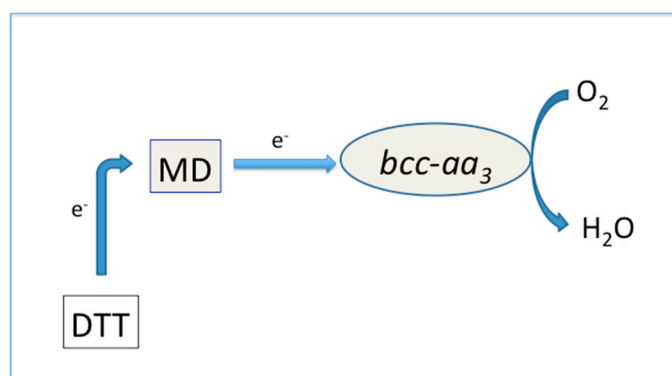
4 Elena Forte, Alessandro Giuffrè, Li-shar Huang, Edward A. Berry, Vitaliy B. Borisov



5  
6 **Supplementary Figure S1.** Control NO trace acquired under anaerobic conditions in the absence of the  
7 supercomplex but in the presence of DTT/MD. Conditions as in Figure 4, except that an equivalent volume (30  
8 μL) of air-equilibrated buffer (20 mM K/MOPS, 100 mM NaCl, 0.5 mM EDTA and 0.01% dodecyl-β-D-maltoside,  
9 pH 7.3) was added instead of the supercomplex.



10  
11 **Supplementary Figure S2.** SDS-PAGE analysis of the purified chimeric *bcc-aa<sub>3</sub>* supercomplex composed of *M.*  
12 *tuberculosis* cytochrome *bcc* and *M. smegmatis* *aa<sub>3</sub>*-type terminal oxidase. Samples are the fractions from final gel-  
13 filtration column. Fractions 22-28 were pooled, concentrated and used for the assays. Under the mild denaturing  
14 conditions used, subunit dissociation is incomplete. Minor bands above cytochrome *b* (cyt *b*) and Cox I  
15 are probably subcomplexes, as is known to be the case for the band so labeled, which stains for heme indicating that  
16 it contains cytochrome *cc* (Cyt *cc*).  
17 ISP: iron-sulfur proteins



18

19 **Supplementary Figure S3.** Scheme illustrating the electron flow from DTT to O<sub>2</sub> via MD and the cytochrome  
20 *bcc-aa<sub>3</sub>* supercomplex. DTT reduces MD, which in the reduced state (menaquinol) binds at the quinol oxidation  
21 site of the supercomplex. In the supercomplex, the electrons donated by reduced MD are intramolecularly  
22 transferred to the heme *a<sub>3</sub>*-Cu<sub>B</sub> binuclear active site, where O<sub>2</sub> is reduced to H<sub>2</sub>O.

### 23 **Supplementary Method**

#### 24 **Gel electrophoresis**

25 Samples were denatured by mixing with an equal volume of the denaturing buffer  
26 described in [1] containing 2% SDS and 2% 2-mercaptoethanol and incubated at room  
27 temperature for several min before loading on a 80 × 60 × 0.75 mm 15% (T+C)  
28 polyacrylamide gel. The gel was run at 150 V in Laemli running buffer until the tracking  
29 dye reached the bottom, then stained first for heme (not shown) and then with Coomassie  
30 R-250 stain.

#### 31 **Reference**

32 1. Laemli, U.K. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4 *Nature*  
33 1970, 227, 680-685

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