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SUPPLEMENTARY INFORMATION

Inhibition of respiratory Complex I by copper(II)-*bis*(thiosemicarbazonato) complexes

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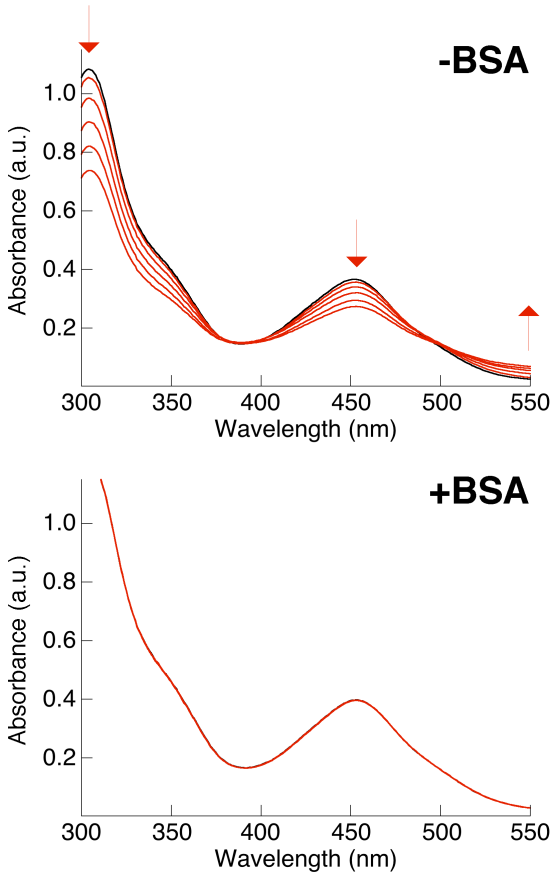
SUPPLEMENTARY TABLES

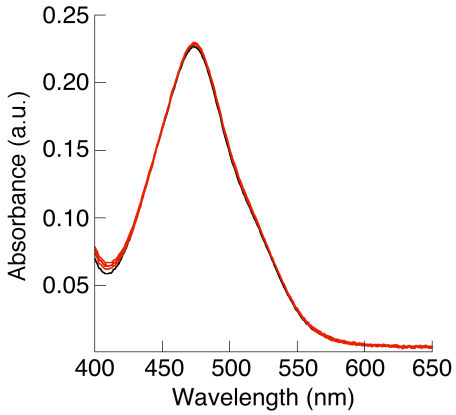
Supplementary Table 1. Fit parameters for all dose-response curves. Each curve was generated from at least five data points. Data were fitted to Eq. 1 in Methods. Standard deviations are shown in brackets.

Inhibitor	*m*		*n*	
	Values	**Average**	**Values**	**Average**
Cu(atsm)	> 200		0.8 (0.3)	
Cu(gtsm)	14.0 (1.0) 13.2 (1.0) 15.4 (0.8) 16.1 (0.6) 19.2 (1.3)	15.6	1.1 (0.1) 1.3 (0.1) 1.2 (0.1) 1.0 (0.1) 0.8 (0.1)	1.1
Cu^{2+}_{aq}	18.7 (1.0) 15.4 (1.2) 17.7 (1.5) 12.9 (0.5)	16.2	2.5 (0.3) 2.8 (0.5) 2.8 (0.5) 2.6 (0.2)	2.7
Zn(gtsm)	41.7 (2.6) 30.8 (2.7) 35.4 (3.7)	36.4	1.0 (0.1) 1.1 (0.1) 1.2 (0.2)	1.1

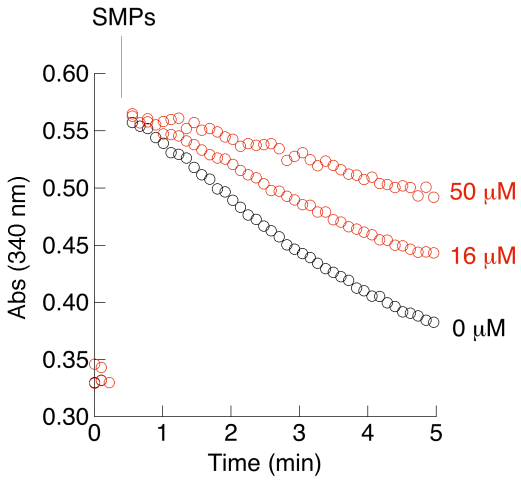
SUPPLEMENTARY FIGURES



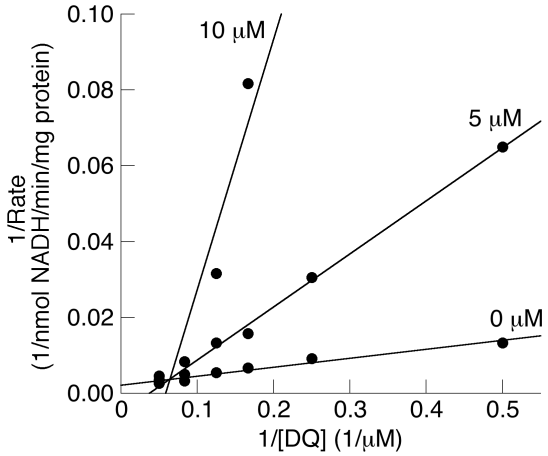
Supplementary Figure 1. Aggregation of Cu(atsm). Solution spectrum of Cu(atsm) (60 μM, λ_{max}, 457 nm; ε_{457}, 7200 M^{-1} cm^{-1}) in NADH:O_2 OR activity assay buffer in the absence (-BSA) and presence (+BSA) of BSA (1 mg mL^{-1}). Spectral data were collected at 0 min (black trace) and 1 – 10 min (red traces). Downward and upward arrows indicate time-dependent decrease and increase in absorbance intensity, respectively.



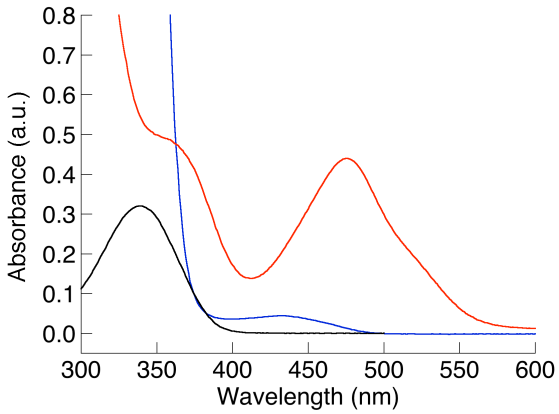
Supplementary Figure 2. Undetectable reduction of Cu^{II}(gtsm) or dissociation of bio-available Cu^{I} ions. Solution spectrum of Cu(gtsm) (25 μM, λ_{max} 478 nm, ε_{478} 8700 M^{-1} cm^{-1}) in NADH:O_2 OR activity assay buffer containing bicinchoninic acid (100 μM, λ_{max} $[Cu^{I}(Bca)_2]^{3-}$ 562 nm, ε_{562} 8000 M^{-1} cm^{-1}). Spectral data were recorded at 0 min (black trace) and 1 – 10 min (red traces) after addition of SMPs (10 μg mL^{-1}).



Supplementary Figure 3. Progress traces for the oxidation of NADH by SMPs. Each reaction contained NADH (50 μM) and Cu(gtsm) (0, 16, or 50 μM as indicated). SMPs (10 μg mL^{-1}) were added to the reaction mixture as indicated. The decrease in solution absorbance at 340 nm was monitored for up to 5 min. The absorbance of Cu(gtsm) in the assay buffer without any NADH or SMP was used to zero the instrument prior to each assay (also see Supplementary Figure 5).



Supplementary Figure 4. Kinetic analyses of Complex I inhibition by Cu(gtsm). Double reciprocal plots of the initial rates of NADH oxidation in the presence of increasing amounts of decylubiquinone as the electron acceptor. Cu(gtsm) was added at a final concentration of 0, 5, and 10 μM as indicated. NADH was used at a final concentration of 50 μM. These plots resemble competitive inhibition plots but the intersect occurs in the first quadrant instead of on the *y*-axis.



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3 **Supplementary Figure 5.** Spectral overlap between NADH (black trace), Cu(gtsm) (red

4 trace) and Zn(gtsm) (blue trace) (ca. 50 μM each).