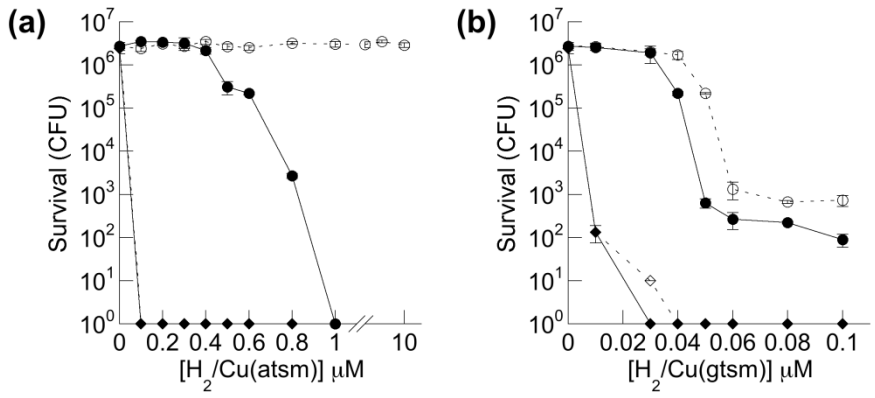
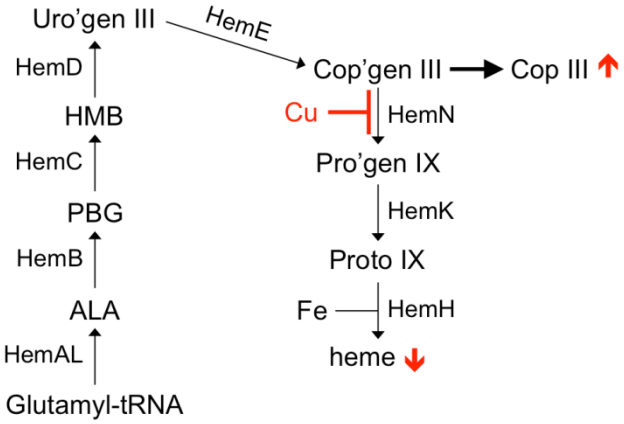
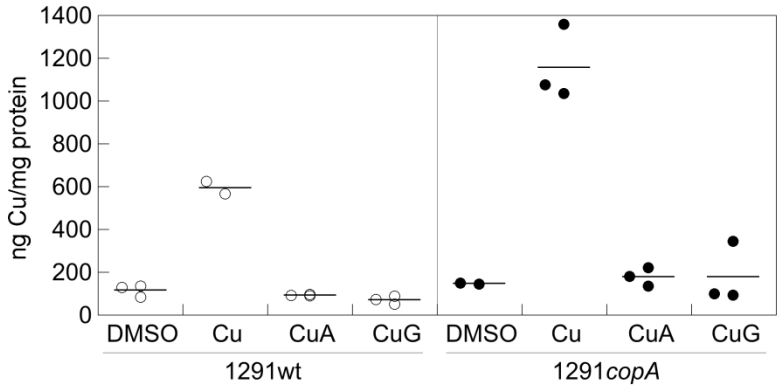
SUPPORTING INFORMATION



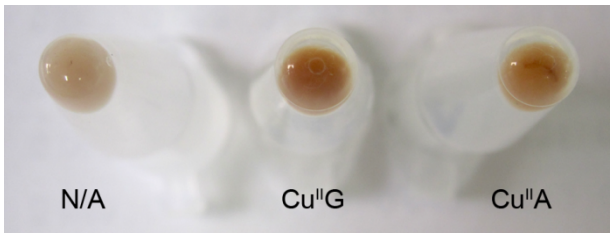
Supporting Figure 1. Copper-free H_2btsc was less toxic than Cu(btsc). Survival of 1291wt (circles ○ and ●) and 1291*copA* (diamonds ◇ and ◆) after exposure to increasing concentrations of: **a)** Cu(atsm) (closed symbols ● and ◆) and H_2atsm (open symbols ○ and ◇), or **b)** Cu(gtsm) (closed symbols ● and ◆) and H_2gtsm (open symbols ○ and ◇) for 20–24 h. Each data point was generated from three independent experiments. Error bars represent ± standard deviation from the mean. It is important to note that H_2atsm and H_2gtsm ligand are metallated by basal amounts of Cu from the growth media to generate the toxic species Cu(atsm) and Cu(gtsm). We have routinely detected between 1 and 10 nM of basal Cu in our media preparations. These concentrations are within the toxic range for Cu(atsm) and Cu(gtsm). Thus, the requirement for Cu is most apparent in panel a). The 1291wt strain was killed only by ~ 1 μM Cu(atsm). In this case, the H_2atsm ligand had no observable effect up to 10 μM.



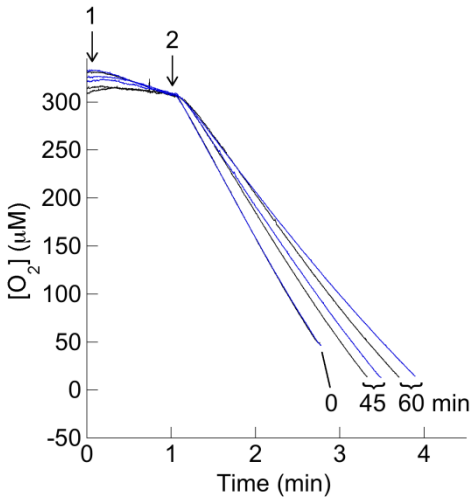
Supporting Figure 2. Excess Cu arrests the heme biosynthesis pathway in *N. gonorrhoeae*. Excess Cu blocks the step catalysed by HemN as indicated in red. As a result, there is a decrease in heme levels (red downwards arrow 🡻) and a concomitant increase of Cop III levels (red upwards arrow 🡹). ALA, aminolevulinic acid; PBG, porphobilinogen; HMB, hydroxymethylbilane; Uro'gen III, uroporphyrinogen III; Cop'gen, coproporphyrinogen III; Cop III, coproporphyrin III; Pro'gen IX, protoporphyrinogen IX; Proto IX, protoporphyrin IX.



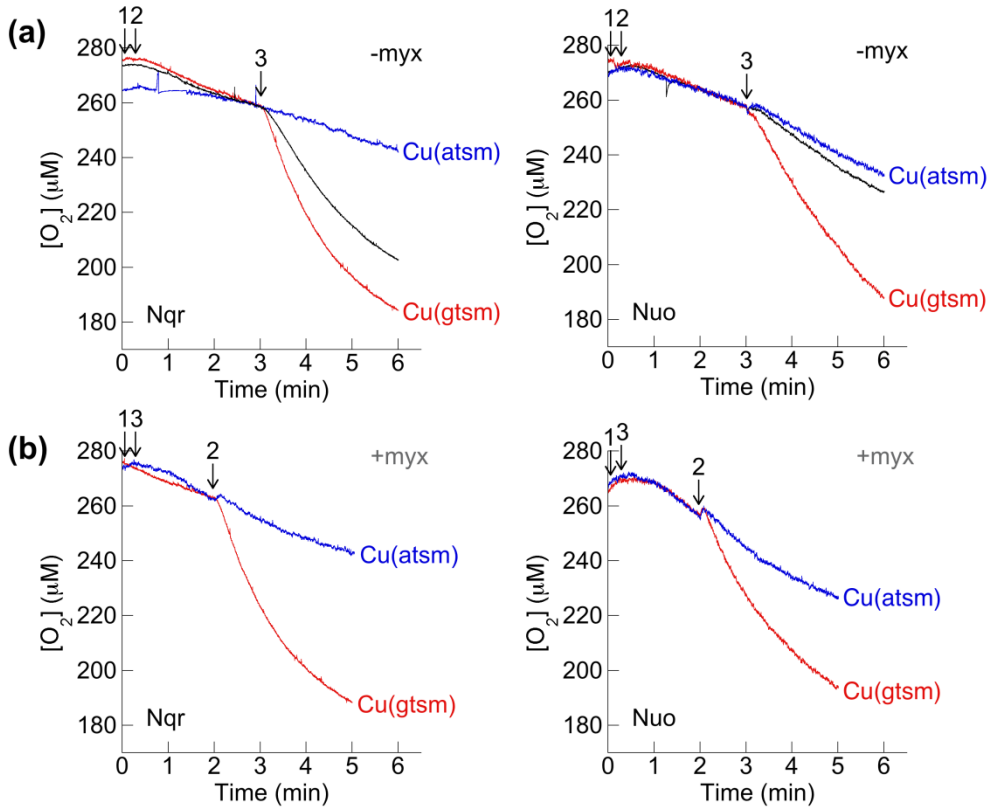
Supporting Figure 3. Treatment with Cu(btsc) did not lead to a detectable increase in intracellular Cu levels. Total amounts of intracellular copper as detected by ICP MS. *N. gonorrhoeae* strains 1291wt and 1291*copA* were treated with DMSO, $Cu(NO_3)_2$ (Cu), Cu(atsm) (CuA), or Cu(gtsm) (CuG) following the conditions shown on Figure 1c. At least two independent replicates were shown for each measurement.



Supporting Figure 4. Association of Cu(btsc) with *N. gonorrhoeae* cell membranes. Cu(gtsm) ($Cu^{II}G$) and Cu(atsm) ($Cu^{II}A$) (50 μM each) was added to a suspension of 1291wt in PBS to reflect the conditions used for the measurement of respiration in intact cells (see Materials and Methods). Cells were sedimented after 1 min and the supernatant was removed. Photos of centrifuged bacterial pellets were taken under ambient light conditions. The untreated control (N/A) was shown for comparison.



Supporting Figure 5. Prolonged incubation with Cu(atsm) did not lead to an enhanced inhibition of aerobic respiration. Pyruvate-driven consumption of O_2 by intact cells after pre-incubation without (black traces) or with 50 μM Cu(atsm) (blue traces) for 0, 45, and 60 min as indicated. Addition of cells (1) and pyruvate (2) are indicated by downward arrows. There was an overall decrease in the rates of pyruvate oxidation regardless of Cu(atsm) treatment, presumably due autolysis of *N. gonorrhoeae*.



Supporting Figure 6. Inhibition of Nuo and Nqr by Cu(atsm) and NADH-dependent redox cycling of Cu(gtsm). NADH-dependent consumption of O_2 by cell-free membrane vesicles of mutant strains containing only one active NADH dehydrogenase. Nqr activity was obtained from 1291*nuoF* mutant, while Nuo activity was measured using 1291*nqrF* mutant. **a)** Effects of Cu(btsc) on the rates of NADH respiration. Rates of O_2 consumption after pre-incubation with 0 (black traces) or 100 μM each of Cu(atsm) (blue traces) or Cu(gtsm) (red traces). Measurements were performed in the absence of myxothiazol (-myx). **b)** NADH-dependent redox cycling of Cu(btsc). Rates of O_2 consumption after pre-incubation with 100 μM each of Cu(atsm) (blue traces) or Cu(gtsm) (red traces). Measurements were performed in presence of 10 μM myxothiazol (+myx). **a-b)** Addition of membrane vesicles (1), DMSO or Cu compounds (2), and NADH (3) are indicated by downward arrows.

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