Supplementary Information (Chi Fru et al.)

1.0 Supplementary Methods

1.1. Mb-Cu-Mineral Dissolution Experiments. The minerals assessed included analytical-grade $CuCO_3.Cu(OH)_2$ (Malachite), CuO (Tenorite), Cu_2O (Cuprite), and Cu_2S (Chalcocite) (all from Sigma-Aldrich, UK) as well as Cu-doped SiO_2 synthesized to 1000 ppm Cu (Knapp et al., 2007; Kulczycki et al., 2007). These minerals were chosen to span different solubilities (see Table 1). The solubility of the synthesised Cu-doped silicate was not modelled because appropriate equilibrium constants and enthalpy of reactions were not available.

The abiotic Cu dissolution experiments used acid-washed 50 ml glass vials (5% HNO_3) that contained one mineral, mb (as needed), and 20 ml of 5 mM carbonate-buffered deionised water of pH 8.0. Experiments were performed in duplicate and mineral supplies were always 16.55 µmoles-total Cu per vial. Minerals were pre-crushed and sieved to < 90 µm grain size to ensure consistent surface-to-volume ratios among minerals. Cu-free crude mb (~20% purity) was added at either 0.33 or 1.65 mg per vial, which were designated as the 1x and 5x treatments, respectively. Mb and Cu mineral supply levels were chosen such that Cu was always in excess to compare the impact of different mb levels on Cu release rates. The Cu(I) mineral treatments were prepared using solutions bubbled with oxygen-free N_2 for one hour.

Assays were performed by placing vials on a shaker table (30 rpm) at room temperature and collecting 2-ml samples for soluble Cu analysis after 0, 3 and 6 hours of contact. Mineral only and mb-only (at 5x) controls were maintained to compare Cu dissolution rates with and without mb added. Samples were filtered, processed, and analysed for soluble Cu using the methods described in the main text.

1.2 Effect of dialysis bags on sMMO activity in Cu-free cultures of Methylosinus trichosporium OB3b Growth behaviour of *M. trichosporium* OB3b was compared with or

without the presence of < 1000 Da dialysis bags (Medicell International Ltd, UK) in 1-L replicate flasks (n > 3) containing 200 ml NMS growth media. Equal volumes of mid-exponential phase *M. trichosporium* OB3b with elevated sMMO activity were inoculated into each flask to achieve common OD600 among treatments. The flasks were then sealed with septum-lids, amended with reagent grade methane (4% in the headspace) and placed on a shaker table (150 rpm) maintained at 28°C in the dark. OD600 and headspace CH4 levels were monitored over time until stationary growth was observed. sMMO activity was assessed using the liquid naphthalene-naphthol assay (DiSpirito et al., 1998).

2.0 Supplementary Results and Discussion

2.1 Abiotic Cu mineral-Mb Dissolution Experiments. The relative levels of Cu released from the five minerals with and without mb added (at two different mb levels) after three and six hours exposure were assessed to observe broad differences among mb-mineral interactions (Figure S1). Reported values are the differences in soluble Cu with and without mb present; positive values indicate mb enhanced Cu release, whereas negative values indicate mb quenched Cu release. Background soluble Cu levels for minerals without mb added were always <0.2 mg-Cu L^{-1}, whereas mb-only controls always had < 0.08 mg-Cu L^{-1}.

Both Cu-doped SiO_2 and Cu_2S released less Cu with mb present than when it was absent, which is consistent with Kulczycki et al. 2007, who showed mb tends to coat such surfaces and quench Cu release, similar to other complexing agents with hydrated Cu silicate minerals (Fuerstenau et al., 2000). CuO and Cu_2O had lower Cu release levels with mb present after three hours, but higher levels of Cu release (compared with no mb controls) after six hours with 5x mb. In contrast, $CuCO_3.Cu(OH)_2$ always released higher levels of Cu when 5x mb was provided and was the only mineral with elevated Cu levels at 1x mb. These Cu release patterns are consistent with MMO-related gene expression patterns observed in *M.*

trichosporium OB3b with Cu-Fe oxides and Cu-doped SiO_2 (Knapp et al., 2007), which indicated mb only increases Cu uptake from some minerals. Furthermore, Cu release patterns (Figure S1) broadly agree with predicted aqueous phase Cu equilibrium patterns (Table 1), although these experiments were not performed appropriately to compare directly with the Table 1 predictions.

2.2. Effect of dialysis bags on sMMO activity in Cu-free cultures of Methylosinus trichosporium OB3b. The presence or absence of dialysis bags in Cu–free cultures of *Methylosinus trichosporium* OB3b had no detectable influence on sMMO activity during growth (Figure S2). These results show that the dialysis bags were not potential sources of Cu contamination in subsequent experiments (see Figure 2-4 main text) to assess the influence of cell-mineral contact and mb on cell growth and sMMO Activity.

3.0 References

DiSpirito, A. A., Zahn, J. A., Graham, D. W., Kim, H. J., Larive, C. K., Derrick, T. S., Cox, C. D., and Taylor, A.: Copper-binding compounds from *Methylosinus trichosporium* OB3b, J. Bacteriol., 180, 3606-3613, 1998.

Fuerstenau, D.W., Herrera-Urbina, R., and McGlashan, D. W.: Studies on the applicability of chelating agents as universal collectors for copper minerals, Int. J. Min. Proc., 58, 15-33, 2000.

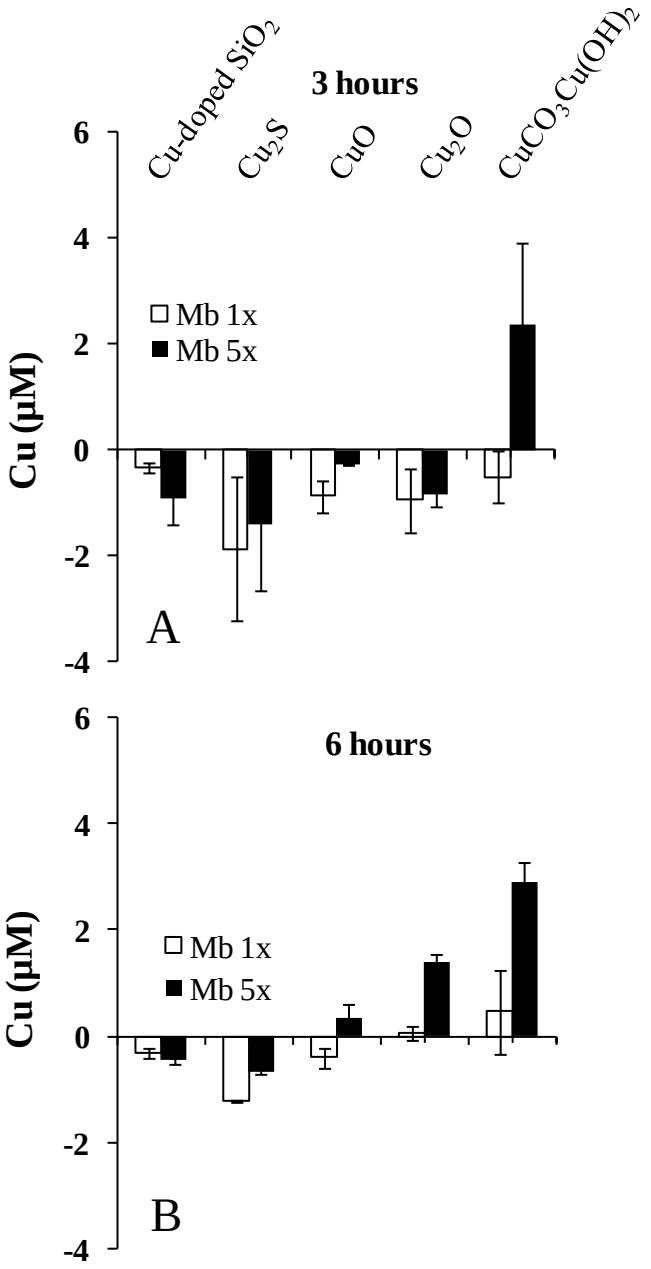
Knapp, C. W., Fowle, D. A., Kulczycki, E., Roberts, J. A., and Graham, D. W.: Methane monooxygenase gene expression mediated by methanobactin in the presence of mineral copper sources, Proc. Nat. Acad. Sci. U.S.A**,** 104**,** 12040-12045, 2007.

Kulczycki, E., Fowle, D. A., Knapp, C., Graham, D. W., and Roberts, J. A.: Methanobactin-promoted dissolution of Cu-substituted borosilicate glass, Geobiol. 5, 251-263, 2007.

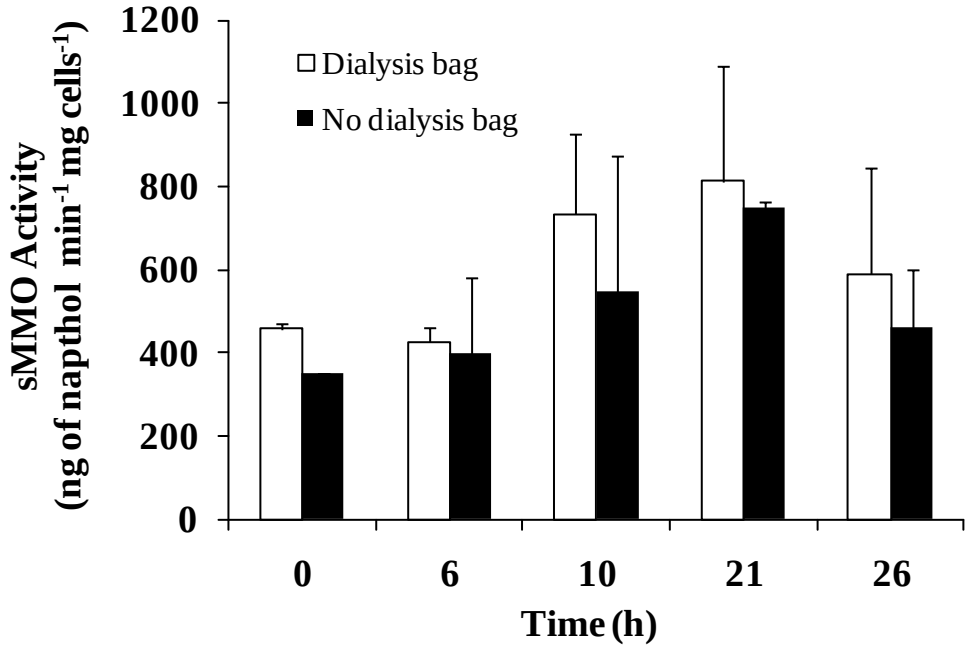
4.0 Supplementary Figure Legends

Supplementary Figure S1: Effect of mb level on Cu release from different Cu solid-phase mineral after (A) 3 and (B) 6 hours contact. Black bars denote experiments with 0.33 mg mb/20 ml (1x) and white bars denote 1.65 mg mb/20 ml (5x). Values are the "relative" dissolved Cu levels obtained by subtracting Cu released from each mineral without mb from the Cu released when mb is provided. Error bars present the range of values from two independent experiments.

Supplementary Figure S2: Effect of the presence or absence of dialysis bags on the sMMO activity of Cu-free cultures of *Methylosinus trichosporium* OB3b. Error bars represent standard deviations (n=3).



Supplementary Figure S1



Supplementary Figure S2