Lanthanide chemistry: From coordination in chemical complexes shaping our technology to coordination in enzymes shaping bacterial metabolism

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SUPPLEMENTARY MATERIALS AND METHODS

Chemicals. All chemicals were purchased through Fisher Scientific (Pittsburgh, PA). Nd-containing magnets removed from discarded computer hard-drives at San José State University were crushed into powder using a hammer and a Magic Bullet blender (Homeland Housewares, LLC, Los Angeles, CA).

Growth conditions. Methylobacterium extorquens AM1 strains were grown at 29°C in modified Methylobacterium PIPES (MP) medium containing 20 µM CaCl₂ as previously described¹,² without or with addition of 2 µM NdCl₃, 2 µM FeCl₃, 2 µM NiCl₂, 2 µM NaBH₄, or different amounts of crushed computer hard-drive magnets as indicated in the text. Succinate (0.4%) or methanol (0.5%) was added to growth medium as a carbon source. Tetracycline (Tc) was added to MP medium at a final concentration of 5 µg/ml when appropriate.

Preparation of glassware. Glassware was treated as previously described to minimize contamination of trace lanthanides².

Transcriptional reporter fusion assays. Cells were grown in MP medium containing Tc in borosilicate glass tubes (2 mL cultures in 16 x 125 mm tubes or 10 mL cultures in 25 x 150 mm tubes) placed in 60°-angled tube racks as previously described² or in 250 mL glass Erlenmeyer flasks (100 mL cultures). Cultures were shaken at 180 rpm in an Excella E25 shaking incubator at 29°C (New Brunswick Scientific, Edison, NJ). Wild-type strains carrying the xox1-venus fluorescent transcriptional reporter fusion plasmid pES503³ or the promoterless control vector pAP5³ were grown in 2 mL MP succinate medium to late-exponential phase, then subcultured into 100 mL MP methanol medium. When methanol cultures reached mid-exponential phase, 10 mL aliquots of cultures or MP methanol medium lacking cells were added to tubes without
lanthanides or with NdCl$_3$, FeCl$_3$, NiCl$_2$, NaBH$_4$, or crushed computer hard-drive magnets. Every two hours, 200 µL aliquots were removed and the relative fluorescent units (RFU) were measured in clear-bottom black 96-well plates using a Spectramax M2 plate reader (Molecular Devices, Sunnyvale, CA). Excitation and emission spectra were at 490 nm and 540 nm, respectively.

**Growth curve analysis.** Cultures were grown in tubes as described above, except Tc was omitted from MP medium. An *M. extorquens* AM1 strain lacking the Ca-dependent methanol dehydrogenase gene *mxaF* was grown to late-exponential phase in 2 mL MP succinate medium, then subcultured (300 µL) into 10 mL MP methanol medium lacking lanthanides or containing NdCl$_3$, FeCl$_3$, NiCl$_2$, NaBH$_4$, or crushed computer hard-drive magnets. Every three hours, optical densities (OD$_{600}$) of cultures and media lacking cells were recorded at 600 nm in standard clear 96-well plates using a Spectramax M2 plate reader (Molecular Devices, Sunnyvale, CA). To account for the increase in OD$_{600}$ due to dissolution of magnets, the OD$_{600}$ of the no cell controls was subtracted from the OD$_{600}$ of the cultures.
References


