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

Norma Cecilia Martinez-Gomez[1*], Huong N. Vu[2], and Elizabeth Skovran[2]

[1] Department of Microbiology and Molecular Genetics, Michigan State University, 567 Wilson Road, East Lansing, MI 48824

[2] Department of Biological Sciences, San José State University, 1 Washington Square, San José, CA 95192

[*] To whom correspondence should be addressed: Dr. N. Cecilia Martinez-Gomez, Department of Microbiology and Molecular Genetics, Michigan State University School of Natural Sciences, 6198 Biomedical Physical Sciences Building, 567 Wilson Road, East Lansing, MI 48824, Telephone: (517) 884-5406, E-mail: mart1754@msu.edu

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 29ºC in modified *Methylobacterium* PIPES (MP) medium containing 20 μM $CaCl_2$ as previously described[1,2] without or with addition of 2 μM $NdCl_3$, 2 μM $FeCl_3$, 2 μM $NiCl_2$, 2 μM $NaBH_4$, 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 lanthanides[2].

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 60º-angled tube racks as previously described[2] or in 250 mL glass Erlenmeyer flasks (100 mL cultures). Cultures were shaken at 180 rpm in an Excella E25 shaking incubator at 29ºC (New Brunswick Scientific, Edison, NJ). Wild-type strains carrying the *xox1-venus* fluorescent transcriptional reporter fusion plasmid pES503[3] or the promoterless control vector pAP5[3] 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*[4] 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

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