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Energetic control of methane isotopologue systematics revealed by integrated pathway thermodynamics, proteomics, and isotope flow network modeling

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Methane from deep subsurface environments commonly exhibits near-equilibrium carbon ($^{13}C/^{12}C$), hydrogen (D/H), and clumped-isotopologue ($^{13}CH_3D$, $^{12}CH_2D_2$) signatures, despite laboratory hydrogenotrophic methanogenesis typically producing strong kinetic fractionations. Recent high-pressure co-culture experiments demonstrated that near-equilibrium methane isotopologue distributions can be reproduced under low H_2 concentrations, where methanogenesis operates near the thermodynamic limit ($\Delta G \approx -15$ kJ mol^{-1} CH_4) (Mayumi et al., 2024). Independently, pathway thermodynamic and isotopologue flow-network models predict that isotope and isotopologue fractionations are governed by the reversibility of individual enzymatic steps, which is controlled by the availability of free energy and enzyme kinetic parameters (Ono et al., 2022).

Here we present quantitative data independent acquisition (DIA) proteomics of *Methanothermobacter thermautotrophicus* grown under high-energy H_2–CO_2 conditions ($\Delta G \approx -145$ kJ mol^{-1} CH_4) and under energy-limited syntrophic co-culture with an acetate oxidizer at ambient and 10 MPa pressure. H_2-grown cultures exhibit high ribosomal allocation (~23% of the proteome), consistent with rapid growth under strong thermodynamic driving force. In contrast, energy-limited co-cultures show markedly reduced ribosomal investment and redistribution of enzyme allocation within the methanogenesis pathway. When normalized to total methanogenesis proteins, terminal and near-reversible steps, methyl-coenzyme M reductase (MCR) and methylene-H_4MPT reductase (MER), are significantly upregulated under low-energy conditions. This shift is qualitatively consistent with pathway thermodynamic optimization, in which reactions approaching reversibility require increased enzyme allocation to sustain flux.

By integrating isotopologue systematics, pathway thermodynamics, and proteome allocation, we provide empirical evidence that enzyme-level resource optimization links free energy availability to methane isotope signatures. We further introduce a Bayesian framework that jointly constrains enzyme kinetic parameters, reaction reversibilities, and isotope fractionation factors using combined proteomic and isotopologue datasets. This integrative approach provides a quantitative framework for interpreting methane isotope signatures in deep sedimentary environments and natural gas systems operating near energetic limits.

Reference

Mayumi, D. *et al.* (2024) *Science* 386, 1372–1376

Ono et al. (2022) *Geochimica et Cosmochimica Acta* 325, 296–315