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A novel methodology to investigate isotopic biosignatures

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An enduring goal of trace metal isotopic studies of Earth History is to find isotopic 'fingerprints' of life or of life's individual physiochemical processes. Generally, such signatures are sought by relating an isotopic effect observed in controlled laboratory conditions or a well-characterized environment to a more complex system or the geological record. However, such an approach is ultimately limited because life exerts numerous isotopic fractionations on any one element so it is hard to dissect the resultant net fractionation into its individual components. Further, different organisms, often with the same apparent cellular function, can express different isotopic fractionation factors.

We have used a novel method to investigate the isotopic fractionation associated with a single physiological process—enzyme specific isotopic fractionation. We selected Cd isotopes since only one biological use of Cd is known, CdCA (a Cd/Zn carbonic anhydrase from the coastal diatom T. Weissflogii). Thus, our investigation can also inform the long standing mystery as to why this generally toxic element appears to have a nutrient-like dissolved isotopic and concentration profile in the oceans.

We used the pET-15b plasmid to insert the CdCA gene into the *E. coli* genome. There is no known biochemical function for Cd in *E. coli*, making it an ideal vector for studying distinct physiological processes within a single organism. The uptake of Cd and associated isotopic fractionation was determined for both normal cells and those expressing CdCA. It was found that whole cells always exhibited a preference for the light isotopes of Cd, regardless of the expression of CdCA; adsorption of Cd to cell surfaces was not seen to cause isotopic fractionation. However, the cleaning procedure employed exerted a strong control on the observed isotopic composition of cells.

Using existing protein purification techniques, we measured the Cd isotopic composition of different subcellular fractions of *E. coli* (e.g. membranes, cytosol, etc.), including the catalytic metal atoms within CdCA. These experiments allow isotopic exchange reactions to be observed in biological systems at an unparalleled resolution, demonstrating that isotopic fractionation can occur, in vivo, on length scales as small as a few Å. We will explore future applications of this technique using the marine geochemistry of Cd as a case study. This experimental approach has great promise for studying the individual isotopic biosignatures of other biochemical reactions, in particular those which may have been active during early Earth History.