

Interactive comment on “Ideas and perspectives: Same Carbon Different Elements – An Insight into Position-Specific Isotope Patterns Within a Single Compound” by Yuyang He et al.

Anonymous Referee #3

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1 General Comments

In this ideas and perspectives piece, the authors discuss the limitations of interpreting position-specific isotope compositions (or intramolecular isotope distributions, “Intra-IDs”) by comparison with theoretical Intra-IDs calculated for the same compound, assuming an equilibrium distribution of isotopes. In other words, if a compound is measured and displays a close-to-equilibrium distribution of isotopes, is this evidence that reversible processes dominate the synthesis of the compound? Or could the similarity to the equilibrium reference state be coincidental?

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This work is an extension of a longstanding debate in the literature over fundamental controls on isotope distributions within molecules, especially in biological systems. Over several decades, Galimov has argued that apparent correlations between the reduced partition function ($\beta^{13}\text{C}$ -factor) and isotopic compositions ($\delta^{13}\text{C}$ values) of the corresponding compounds in biological systems suggest that thermodynamics, rather than kinetics or biosynthetic pathways, predominantly control the distribution of isotopes within and between biomolecules (Galimov, 1985). However, many other authors including Schmidt, Hayes, Buchachenko and colleagues cited throughout this work have argued for the importance of other, non-thermodynamic factors, which are revisited here, and that any similarities to equilibrium reference states cannot be used as evidence for biochemical reactions favoring an equilibrium state.

The central thesis of this manuscript is that Intra-IDs must be interpreted in context: sources, reaction pathways, and isotope effects (KIEs and EIEs) must be constrained in order to make sense of these Intra-ID signatures. Otherwise, any apparent similarities between position-specific isotope analyses and a predicted equilibrium state may be coincidental, actually arising from the expression of kinetic isotope effects or other features of metabolic pathways. In my opinion, this thesis is not particularly controversial and has been echoed throughout the literature, both in response to Galimov’s hypothesis and elsewhere – for example, Hayes (2004) succinctly stated, “*An isotopic variation does not constitute an interpretable signal unless the mechanism controlling it is known.*” However, since a number of papers have recently highlighted the importance of predicting equilibrium Intra-IDs to provide a baseline for interpreting position-specific data (e.g., Rustad, 2009; He et al., 2018, 2020), and PSIA measurements are becoming more common (e.g., by SNIF-NMR or Fourier Transform mass spectrometry), revisiting these topics seems timely and relevant to the audience of *Biogeosciences*. The authors do a nice job of distinguishing between two concepts of equilibrium that are not sufficiently defined in some of the classic literature, the examples (oxygen-bearing minerals, N_2O , and acetic acid) feel appropriate for illustrating the authors’ points, and the authors center a call to action to characterize more EIEs

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and KIEs, sources, and pathways, for aiding interpretations moving forward.

While the authors provide sufficient literature context for their discussion, my biggest concern with the paper in its current form is that it does not clearly delineate novel insights or findings by the authors from pre-existing literature. In other words, I am not certain where the review of the historical literature ends, and the authors' analysis begins, which makes it difficult to evaluate exactly what the authors' primary contributions are here. For example, to what extent has the N₂O site-preference example already been articulated in the literature? Site-preference measurements have been made for many years, and the authors cite other studies noting that precursor symmetry matters, so for readers less familiar with this application: is this simply an example compiled from existing studies to argue that more context is needed to interpret an Intra-ID, or is some component of the discussion new, like the subsequent discussion of reversibility? Some careful re-wording could clarify this and similar types of questions throughout. Similarly, it would be helpful if the authors could add at least one more sentence at the end of the oxygen-bearing minerals example synthesizing the broadly generalizable point that the authors are trying to convey.

2 Specific Comments

- I do not really understand the meaning behind the “same carbon different elements” portion of the title. Unless further explanation is added to the text, I think it could be easily removed. “Same carbon different positions” or something similar seems better aligned with the focus of the paper, but does not necessarily improve the existing title.
- It is not always clear throughout the text whether the term “Intra-ID” is being used to mean position-specific isotope analysis (i.e., a measurement) or a calculated distribution of isotopes (i.e., a prediction of an equilibrium state). Adding clarifying

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language throughout would be helpful.

- Line 34: the definition of position-specific isotope composition is circular since it uses the words ‘specific’ and ‘position’ again. Perhaps something along the lines of “at particular atomic sites within an individual compound” or “at structurally-distinct atomic sites. . .” would be more clear.
- Line 38: “Intra-ID” is an abbreviation that has been introduced in prior papers, for example, He et al., 2020 GCA. It might be helpful to cite this or the earliest use of this phrase to show precedent.
- Line 39: can the authors clarify what they mean here? “Most common” in what sense? (i.e., in terms of calculations, measurements, publications, or something else)
- Line 47: “to compare to” does not add much, but could be replaced by “for interpreting position-specific isotope measurements” and I believe makes the authors' point clearer.
- Line 54: I do not think “correlate loosely” is sufficiently clear here. Perhaps, “do not correlate well with” or “are poorly correlated with”
- Line 60: the point citing He et al., 2018 seems interesting and highly relevant to the discussion in this paper. Can the authors add one more sentence summarizing the finding of that study and why the correlation approach is invalid?
- Line 97: It would be helpful to add one summary sentence at the end here to clarify what general point the authors hope the readers will take away from this section.
- Line 137: “mechanisms” might be more meaningful than “processes” here?

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- In line 196, I do not understand why any references are needed. It is clear from the chemical formulae, for example, that H₂O consists of H and O atoms and NO₃⁻ consists of N and O. Why are 7 references needed in this sentence?
- I would expect to see Hayes, 2001 cited somewhere in the manuscript as another classic discussion of kinetic and metabolic controls on isotope signatures of biomolecules.

3 Suggested minor technical corrections

- Line 12 - "biosystem" should be plural
- Line 13 - "debates remain" should become "debate remains"
- Line 21 - "roots" → "is rooted"
- Line 24 - "to be isolated and to be controlled" → "to isolate and control"; "effect" should be plural
- Line 39 - "facing" → "faced"
- Line 42 - "termed in" → "described as being in"
- Line 53 - "contrary" → "contrast"
- Line 55 - "are also observed" is not needed
- Line 63 - "between" → "among"
- Line 71 - eliminate "in contrast to existing optimism"
- Line 95 - "can come from different sources"

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- Line 179 - I believe "involving" should be "evolving" here
- Line 206 - "offer" → "offers"
- Line 218 - "at molecular level" → "at the molecular level"

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