

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 #2

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The authors propose a method to receive added value out of the knowledge of site-specific or position-specific isotopic compositions in more or less complex (organic) molecules (or inorganic minerals). They suggest to compare measured “intramolecular isotope distributions” (abbreviated as “Intra-ID”) in (organic) molecules with theoretically calculated isotope distributions assuming a synthesis reaction under thermodynamic control thereby accomplishing (chemical) equilibrium. The manuscript has a sort of review character as obviously all measured intra-IDs have been taken from literature. The authors would like to test the theory that “information on the source, reaction pathway, and kinetics of an organic compound can be obtained from its position-specific isotope compositions” and end up with the familiar and already well-known conclusion

C1

that inter- and intramolecular isotopic compositions alone are an inadequate means to reach this goal. A basic idea on the involved synthesis/breakdown reactions, flux rates and regulation points of the involved reaction pathway(s) in addition to knowledge on kinetic or equilibrium isotope effects introducing isotope fractionations and thereby sharpening the observed intra-IDs is needed. The manuscript is interesting and innovative, but needs major amendment. There might be even the need for more than this “review round”. Especially, there is a need to work out a sort of a “Take home message” for the reader (in the conclusion part?). The subchapter “3 Implications” needs a complete revision. Instead of discussing equilibrium and non-equilibrium issues in terrestrial or extraterrestrial material there is need to present here which additional info is needed to interpret the Intra-IDs. Best would be here to connect e.g. N₂O (site-preference) data with the schematics of a metabolism pathway producing N₂O from a defined origin (and/or the acetic acid part can be elaborated in an analogues manner).

Specific comments: 1) Title: I do not understand the title. What is the meaning of “same carbon different elements” ??? → interesting terms here might be “functional groups”, “carbon molecule positions”, “different bond types” ??? 2) Title: “isotope pattern” not mentioned in the text of the manuscript. What is the difference between “isotope pattern” and “isotope distribution”? Pls use only one description. 3) Line 9: Only “kinetics”, no “thermodynamics” ??? kinetically controlled reactions and equilibrium, isn't that a contradiction? 4) Line 25ff: KIE give info on transition state / mechanism (rate-determining step) of a reaction, whereas EIE give info on the stiffness of the bond of the corresponding isotope in educt and product (change in bonding of the isotope in question). Your “transition-state and reversibility” is too much abridged here. Best would be to mention here in this context also the connection of KIE and EIE. EIE is equal to the ratio of the KIEs on forward and backward reaction in case the chemical (and isotopic) equilibrium has been accomplished. In kinetically controlled reactions the step between educt and the intermediary transition state (TS) is reversible and the reaction from TS towards product is irreversible. The term “equilibrium” is not helpful when talking about KIE. Please change wording correspondingly. Addition-

C2

ally also info on EIE would be needed here. 5) Line 30: Please use terminology of Coplen (2011, <https://doi.org/10.1002/rcm.5129>). As there are different definitions of KIE numbers used in geochemistry and other disciplines, it would be a good idea to also write the corresponding equations for KIE and EIE and clearly state which number corresponds to normal and inverse IE. Perhaps in a footnote or Appendix (as the editor recommends). A KIE of e.g. 1.01 means that the product is depleted or enriched in the heavy isotope relative to the educt? Please check with Coplen (2011) or define via own equation(s). But a definition is needed. 6) Line 36: "Pls replace "... of all different positions in a compound" by "... of all different positions of the same element in a compound". That is what you mean? 7) Line 39: There are many more paper on hydrogen isotope distribution in organic molecules. See e.g. Martin et al. (<https://doi.org/10.1111/j.1365-3040.1992.tb01654.x>). ¹³C intra ID by NMR is a relative recent approach. 8) Line 42: According to my opinion, the term "statistical" was chosen by Schmidt to explain that the distribution of the heavier isotopes in an isotopomer compounds is not a stochastic distribution but follows certain rules. In the articles by Schmidt the term "non-statistical" states that the distribution is not guided by chance, but follows a logical order. It is not stated, whether this order is under thermodynamic or kinetic control. Please adapt. In case, the text passage in italics is a direct citation, most probably Galimov or Schmidt (not both) have stated that. See also line 56. 9) Line 61: "averages": Do you mean average d-value of the whole molecule? The Intermolecular isotopic composition? 10) Line 64ff: I do not understand your differentiation between your point 1) and your point 2). Let's assume the reaction sequence ... A → B → C ⇌ D → E → F... (and a branching point at C and/ or D according to Hayes and Schmidt). The system should also be "regulated" on the reaction from A/B and E/F ("bottleneck" as an analogy), so that the reaction between C and D approaches or even accomplishes chemical equilibrium. The reaction between C and D should "own" an EIE (e.g. ¹³C EIE). Then only the carbon atoms in molecule C and molecule D can be "isotopically" equilibrated that are influenced by the primary and secondary (tertiary ??) thermodynamic isotope effects on the equilibration reaction

C3

(Secondary isotope effects: <https://goldbook.iupac.org/terms/view/S05523>). It is useless (without a value, not applicable) to make a statement on the carbon atoms in C and D, that are not touched by any equilibrium isotope effects. Even secondary IE (for the heavy elements beside 2H) are normally very small. 11) Line 71: "few intramolecular exchange pathways". This statement needs either a literature citation or there is need to present own data as a proof. 12) Line 86/87: You should state here that oxygen can be bonded in different functional groups that have different chemical properties. A way out would be a position-specific analysis of the oxygen isotopic composition. 13) Line 129: Would it be possible to present a typical example for N₂O produced from equilibrium or from a non-reversible reaction here? 14) Line 143: Pls define alpha with an equation (is it isotope fractionation factor ? Pls see also Coplen 2011). The factor 1000 in the alpha formula is related to the d¹³C formula? Meanwhile the factor 1000 is deprecated in the e.g. d¹³C formula. Needs to be communicated also in the text and foot note / appendix. 15) Line 151: What is the meaning of "man-made"? Produced by chemical synthesis ? 16) Line 161: "Intra-ID" should be equal to the d¹³C value difference between the precursor minus the primary KIE". Do you have information on the original Intra-ID of the oil from the "oil-prone source rocks"? 17) Line 162: The fact, that numbers for KIEs are higher as corresponding EIE values is commonly known. But what is a negative KIE? Please define also the equilibrium isotope fractionation factor. 18) Lines 170 to roughly 190 should be shortened. Non-essential rather distracting information is given here. The focus of the manuscript by He et al. is not to present a proof of the Galimov theory, or? 19) BTW, I do not understand the text part starting in line 196. H₂O consists out of H and O, yes. Given info also true for nitrate and sulfate. There are no isotopomer water molecules. Are there isotopomer molecules of sulfate and nitrate with an Intra-ID? What idea is behind this Paragraph? It would be interesting to compare Intra-IDs of e.g. carbon and oxygen or carbon and hydrogen in organic molecules like glucose. 2H isotopomer distribution and ¹³C isotopomer distribution of glucose have been published already. 20) Line 44/45: It should read "Bigeleisen and Goepfert-Mayer" (with or without hyphen). Jacob Bigeleisen and Maria Goepfert

C4

Mayer <https://aip.scitation.org/doi/10.1063/1.1746492>

21) Comment on the Galimov theory The above mentioned calculations for the "Equilibrium Intra IDs" are based on the framework elaborated by Galimov, who assumed that inter- and intramolecular isotope distributions in molecules of metabolic reaction networks in Nature are under thermodynamic control. The theoretically calculated b-factors (e.g. b^{13C} for carbon) according to Galimov are compared with measured and reported d-values (e.g. d^{13C}). The theory of Galimov on thermodynamic factors controlling the intra-IDs has been contradicted by many researchers. Additionally, to the already cited manuscripts by Buchachenko, Schmidt (and coworkers), Hayes, also Monson and Hayes (1982 *Geochim Cosmochim Acta* 46, 139ff), O'Leary and Yapp (1978 *Biochem Biophys Res Commun* 80, 155ff) and Varshavskii (1988, *Biophysics* 33(2), 377ff. Elsevier Pergamon Article in english) could be listed there. Dynamic reaction networks in living organism are kinetically controlled. Chemical (and isotopic) compositions of molecules at diverse levels are controlled in a steady state that allows continuous flow of mass and energy followed by a constant but adjustable flux through biochemical pathways including continuous synthesis and degradation reactions of compound molecules involved. In contrast, a system at chemical (and isotopic) equilibrium would approach a stable state and be a closed system not exchanging matter with the environment. The Gibbs free energy will then come to a minimum approaching zero. The Galimov theory is not compatible to how the biochemical pathways are explained in (plant) biochemistry text books.

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