Ideas and perspectives: Same Carbon Behave Like Different Elements- An Insight into Position-Specific Isotope Distributions

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Abstract. It is expected that information on the source, reaction pathway, and reaction kinetics of an organic compound can
10 be obtained from its position-specific isotope compositions or intramolecular isotope distribution (Intra-ID). To retrieve the information, we could use its predicted equilibrium Intra-ID as a reference for understanding the observed Intra-IDs. Historically, observed, apparently close-to-equilibrium carbon Intra-ID had prompted an open debate on the nature of biosystems and specifically the pervasiveness of reversible biochemical reactions. Much of the debate remains unresolved, and the discussion has not clearly distinguished two states of equilibrium: 1) the equilibrium among the corresponding bond-
15 breaking/forming positions in reactant and product, and 2) the equilibrium among all carbon positions within a compound. For an organic molecule with multiple carbon positions, equilibrium carbon Intra-ID can be attained only when a specific reaction is in equilibrium and the sources of each position are also in equilibrium with each other. An observed Intra-ID provides limited information if the sources and pathways are both unconstrained. Here, we elaborate on this insight using examples of the observed Intra-IDs of hydroxyl-bearing minerals, N_2O, and acetic acid. Research effort aiming at calibrating position-specific
20 equilibrium and kinetic isotope fractionation factors for defined processes will help to interpret observed Intra-IDs of a compound accurately and fully.

1 Introduction

Biosystems are dominated by a series of non-equilibrium kinetic processes. The understanding of biosystems is rooted in the study of the biochemical reaction mechanism. However, a majority of the biochemical reaction mechanisms remains elusive
25 since they are difficult to isolate and control in laboratory experiments. Stable isotope effects can be used to examine the transition-state structure and reversibility of an elementary reaction. Therefore, it can provide information on reaction mechanisms (Bigeleisen, 1949; Galimov, 2006; Bennet, 2012). However, a big organic molecule produced by an organism is the result of complex biochemical reactions that involve multiple kinetic and equilibrium isotope effects (KIE and EIE). KIE and EIE refer to the two intrinsic parameters for interpreting the observed isotope fractionations (Bao et al., 2015). According

30   to the transition-state theory (Eyring, 1935a, b), the KIE of an elementary step can be defined as the equilibrium fractionation factor between transition-state and reactant (Jones and Urbauer, 1991; Bao et al., 2015):

$$KIE = \beta_{TS}/\beta_R$$

where the β factor denotes the reduced partition function ratio of transition-state (TS) or reactant (R). A β factor is the equilibrium isotope fractionation factor between an atom in a specific bond environment and its atomic form that can be
35   predicted theoretically (Urey, 1947, Bigeleisen and Goeppert-Mayer, 1947). For a unidirectional reaction, the KIE of a reaction can also be defined as:

$$KIE = {}^h k / {}^l k$$

where k denotes the reaction rate constant of heavy (h) or light (l) isotopes. To adapt to the convention of geochemists, we define KIE this way so that the normal KIE is less than 1.000, which is the opposite of what Bigeleisen (Bigeleisen, 1949)
40   initially defined.

EIE is the isotope fractionation among reactant and product, which is determined by the bonding environment of the target position or compound:

$$EIE = \beta_P/\beta_R$$

where P denotes the product of a target reaction. It can also be defined as:

45 $$EIE = {}^h K / {}^l K$$

where K denotes the equilibrium constant of a target reaction. At equilibrium, the EIE of a reaction equals to the ratio of forward reaction KIE_f, and backward reaction KIE_b (EIE = KIE_f/KIE_b, Bao et al., 2016).

An organic compound usually contains multiple positions of the same element, such as carbon, hydrogen, oxygen, or nitrogen. Compound-specific isotope composition refers to the bulk isotope composition of an element in an individual compound.
50   Position-specific isotope composition refers to the isotope composition of an element at structurally-distinct atomic positions within an individual compound. Information on sources, reaction pathways, and reaction kinetics of an organic molecule are pertinent to each position. The compound-specific isotope composition averages isotope compositions of all different positions of the same element in a compound, where information contained in position-specific isotope compositions could be lost (Elsner, 2010; Piasecki et al., 2018).
55   We named position-specific isotope compositions in a compound *intramolecular isotope distribution* or *Intra-ID* (He et al., 2018, 2020). Carbon Intra-ID in organic compounds has invoked a long-standing debate about its fundamental controls. When

faced with the observed diverse Intra-IDs, earlier researchers inferred that the patterns "*must be the expression of some logical order*" (Schmidt, 2003), which is controlled by EIE and KIE of biochemical reactions (e.g. Hayes, 2001, 2004; Galimov, 2009; Schmidt et al., 2015; Eiler et al., 2018; Gilbert et al., 2019). The Intra-ID was described as being in *thermodynamic order* or

60 *statistical isotope pattern* when each position in a molecule reaches equilibrium with each other (Galimov, 1985; Schmidt et al., 2015). Here, we name it *equilibrium Intra-ID*. The non-equilibrium state is expected to be a norm for a biochemical system since life is a dissipative system. At equilibrium, the difference in isotope composition between two positions depends on temperature only and therefore the deviation of an observed Intra-ID from its predicted equilibrium state has been considered as an ideal reference for interpreting position-specific isotope compositions (Galimov, 1985; Hayes, 2001, 2004; He et al.,

65 2018, 2020; Rustad, 2009; Piasecki et al., 2016).

It has been reported that different carbon fragments of chlorophyll, different carbon positions in acetoin, malonic acid, citric acid, and purine alkaloid have $^{13}\beta$-$\delta^{13}C$ correlation with regression coefficients in the range of 0.33-0.51 (Galimov, 1985, 2003, 2004, 2006 and references therein). Such a $^{13}\beta$-$\delta^{13}C$ correlation is written as $\delta^{13}C - \delta^{13}C_{ave} = \chi(\beta - \beta_{ave}) \times 10^3$, where χ is the regression coefficient. Galimov interpreted such observed intramolecular $^{13}\beta$-$\delta^{13}C$ correlations as *equilibrium-like* Intra-

70 IDs produced from sets of reversible biochemical reactions at steady-states which are not far from equilibrium. The $^{13}\beta$-$\delta^{13}C$ correlations were used as supporting evidence that the theorem of minimum entropy production can be applied in biochemical systems. However, other groups interpreted the fair-to-good correlation as fortuitous regardless of the presence or absence of complete reversibility of enzymatic reactions (Buchachenko, 2003, 2007; Schmidt, 2003; Schmidt et al., 2015). In contrast to these reported observed equilibrium-like Intra-IDs, measured position-specific $\delta^{13}C$ values are poorly correlated with their

75 predicted $^{13}\beta$ values in organic molecules like glucose, nicotine, and tropine (Rossmann et al., 1991; Gleixner and Schmidt, 1997; Robins et al., 2016; Romek et al., 2016). Such observed non-equilibrium Intra-ID has been termed *non-statistical isotope pattern* (Rossmann et al., 1991; Gleixner and Schmidt, 1997; Schmidt, 2003; Robins et al., 2016; Romek et al., 2016). Buchachenko (2003, 2007) and Schmidt et al. (2004, 2015) argued that the observed $^{13}\beta$-$\delta^{13}C$ correlations are random Intra-ID that only "*simulates*" the thermodynamic state, which cannot be used as evidence for biochemical reactions favoring

80 equilibrium state. The $^{13}\beta$-$\delta^{13}C$ correlation used an unweighted arithmetic mean isotope composition of all the components as the reference of a system. Strictly, only the mass-weighted isotope composition of all components should represent that of a system (Hayes, 2001). In addition, arbitrarily assigning a reference is not mathematically rigorous (He et al., 2018). Therefore, a $^{13}\beta$-$\delta^{13}C$ correlation cannot be used as supporting evidence for Galimov's hypothesis that the theorem of minimum entropy production applies in biochemical systems. Nevertheless, the invalidity of $^{13}\beta$-$\delta^{13}C$ correlations cannot fully quell the

85 controversy on the nature of biosystem.

It should be noted that the debate on isotope equilibrium in biosystems among Galimov, Buchachenko, and Schmidt (and their colleagues) did not clearly distinguish two states of equilibrium: 1) intermolecular isotope equilibrium among the corresponding bond-breaking/forming positions in reactant and product in a defined **process**, and 2) intramolecular isotope equilibrium among all carbon positions in a defined **molecule**. Such a difference might also be overlooked when discussing

90 the Intra-ID or the site preference (SP) value, i.e. the isotope composition difference among two positions. A fully reversible

reaction is necessary for isotope equilibrium between corresponding active positions or functional groups. Similarly, a fully reversible intramolecular exchange mechanism must exist if different positions within a compound are to attain equilibrium. However, the overwhelming majority of biochemical reactions, especially in cases involving large organic molecules, have very few intramolecular exchange pathways. Here, we propose that the utility of parameters like SP value in organic molecules could be limited before we obtain sufficient details on the source, pathway, as well as KIE and EIE of biochemical reactions. To elaborate this point, we present simple cases starting from hydroxyl-bearing minerals in which oxygen occupies more than one position, to the case of N_2O in which uni-directional and fully reversible reactions can produce similar nitrogen Intra-IDs if there exists a symmetric precursor. After presenting the two inorganic cases, we move to examine measured carbon Intra-IDs from the literature of a simple organic molecule, acetic acid, in which Intra-IDs are pathway-dependent.

2 Intramolecular isotope distribution

2.1 Intracrystalline oxygen isotope difference – a rarely effective single mineral geothermometer

The same element, e.g. carbon, occupies different positions in a compound is not a unique feature of organic compounds. Some oxygen-bearing minerals have two or more position-specific oxygens, where the oxygen atoms occupy different positions in a mineral structure and have different chemical properties. Their isotope composition difference was proposed as a potential single mineral geothermometer. For example, it had been proposed that water temperature could be reconstructed from intracrystalline oxygen isotope difference of single mineral copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) (Götz et al., 1975), kaolinite ($Al_2Si_2O_5(OH)_4$), illite ($K_{0.65}Al_{2.0}(Al_{0.65}Si_{3.35}O_{10})(OH)_2$) (Bechtel and Hoernes, 1990), or alunite ($KAl_3(SO_4)_2(OH)_6$) (Arehart et al., 1992). By analyzing the isotope composition difference of different oxygens in the same minerals, the early researchers attempted to reconstruct the precipitation temperatures.

To be a single-mineral geothermometer, different oxygen sites must have attained equilibrium within the single mineral, which can be achieved when different positions in a compound have the same source or initially different sources are in equilibrium with each other. Take alunite precipitation from a solution as an example. Alunite has sulfate and hydroxyl oxygen positions in its structure that precipitate from sulfate and hydroxyl ions in the solution (Fig. 1). Alunite with equilibrium Intra-ID can be produced from an equilibrium precipitation process, only if both the oxygen isotope compositions of sulfate and hydroxyl ions in the solution equilibrated with the same ambient water oxygen at the same temperature. However, sulfate oxygen does not readily exchange with that of water; the isotope equilibration time for SO_4^{2-} and ambient water at Earth's surface condition is greater than 10^6 to 10^7 years (Lloyd, 1968; Turchyn and Schrag, 2004; Turchyn et al., 2010) while the oxygen in OH^- can equilibrate with ambient water instantly and can readily exchange during alunite's later burial and diagenetic processes. Thus, the two oxygen positions in alunite can come from different sources at different temperature, rendering alunite a flawed single-mineral geothermometer. The same is true for gypsum ($CaSO_4 \cdot 2H_2O$) in which sulfate oxygen is not in equilibrium with formation water, and the crystallization water ($\cdot 2H_2O$) oxygen may be in equilibrium with a different type of water.

The use of a single-mineral geothermometer requires that oxygens at two different sites have attained equilibrium by exchanging with each other or with the same source oxygen (e.g. water). Unfortunately, this requirement is difficult to be met for most minerals. It is, therefore, not surprising that few successful single-mineral geothermometers exist if any at all.

2.2 "Equilibrium-like" Intra-ID produced by a kinetic process

For a compound with two different positions of the same element, a simple way to describe its observed Intra-ID is to report the difference between the two isotope compositions, i.e. the site-preference (SP) value. The concept of SP originated from the study of nitrous oxide ($^{\beta}N^{\alpha}NO$), which is defined as the nitrogen isotope composition difference between the central nitrogen ($\delta^{15}N^{\alpha}$) and the terminal nitrogen ($\delta^{15}N^{\beta}$) (Yoshida and Toyoda, 2000). The predicted equilibrium SP value at room temperature in N_2O is 45‰ (Yung and Miller, 1997; Wang et al., 2004; Cao and Liu, 2012). Although most observations fit the equilibrium prediction that ^{15}N preferentially enriches in the $^{\alpha}N$ position by 30-40‰ (Yoshida and Toyoda, 2000; Toyoda et al., 2002; Sutka et al., 2006), negative SP values were observed nevertheless (Yamulki et al., 2001; Sutka et al., 2003).

In previous literature, the difference in SP values was explained by the difference in synthetic pathways associated with symmetrical or asymmetrical precursors (Schmidt et al., 2004; Toyoda et al., 2005; Sutka et al., 2006). If the precursor of N_2O is symmetrical (e.g. -ONNO-, Fig. 2 left), the two nitrogens in the precursor are positionally equivalent; any prior isotope composition and fractionation difference would be erased by the symmetrical structures of the precursor. When producing N_2O from a symmetrical precursor, the $^{\beta}N$ undergoes N-O bond cleavage and therefore has a primary isotope effect which is large, whereas the $^{\alpha}N$ has only a secondary isotope effect which is negligible (close to 1.000, Bigeleisen and Wolfsberg, 1958). Therefore, ^{15}N depletion is expected only on the $^{\beta}N$ of N_2O produced from a symmetrical precursor is expected to have a positive SP value. If the precursor is asymmetrical (e.g. -NH(OH)NO, Fig. 2 right), the two nitrogens in the precursor are not positionally equivalent. It is expected that the two nitrogens in the precursor were produced from different EIEs or KIEs because they went through different reaction pathways and may even have different nitrogen sources. Therefore, during the formation of N_2O from an asymmetrical precursor, the difference in the position-specific $\delta^{15}N$ values of the precursors and the difference in isotope fractionation during the formation processes will be recorded in the SP value of N_2O. Such N_2O can have either SP>0 or SP<0.

Nevertheless, the two previously proposed mechanisms cannot distinguish N_2O with SP>0 produced from the two mechanisms. In addition, for N_2O produced from a symmetric precursor, the SP value cannot provide information on the reaction kinetics, since both fully reversible and uni-directional reactions can produce similar observed SP values. When we state that a compound displays an equilibrium Intra-ID, the underlying assumption is that there exists a mechanism for different positions to exchange isotopes intramolecularly. However, not all observed apparent equilibrium or equilibrium-like Intra-IDs are produced by an intramolecular equilibrium process. For reactions like -ONNO- $\leftrightarrow N_2O$, two types of processes could produce SP>0. First, the N_2O formation reaction is fully reversible and attains an equilibrium. When fully reversible, the two nitrogens in N_2O are scrambled when it forms the symmetrical precursor through the reverse reaction. At equilibrium, the terminal nitrogen in a weaker bond environment is expected to be depleted in heavier isotope than the central nitrogen by 45‰ at

155 surface temperature. Second, the N_2O formation reaction is uni-directional. When uni-directional, only the N-O bond-breaking position ($^\beta$N) undergoes a KIE. Thus, the SP value is approximately equal to the KIE value. In this scenario, if there is a normal KIE, the terminal nitrogen is expected to be depleted in heavier isotope than the central nitrogen by the extent of the KIE value. Such an observed Intra-ID would be similar to the predicted equilibrium Intra-ID, but it is produced by isotope depletion on the uni-directional bond-breaking process. No intramolecular exchange involves. Therefore, even if the N_2O produced by the

160 uni-directional process has SP $\approx$ 45‰, it is not due to a close-to-equilibrium intramolecular isotope exchange. Therefore, it is necessary to distinguish the mechanisms and reaction kinetics that can produce an observed Intra-ID.

Here we see that both fully reversible and uni-directional processes can result in a similar SP value, but the underlying mechanisms are entirely different. Furthermore, a positive SP value can also be achieved through a combination of nitrogen sources and isotope fractionations from an asymmetrical precursor. Thus, without knowing the underlying process, we cannot

165 interpret an observed Intra-ID or SP value uniquely.

2.3 Position-specific isotope fractionations between reactant and product

As illustrated above, the observed Intra-ID of a compound can be used to gauge the degree of internal thermodynamic equilibrium only if we can determine the mechanisms involved in isotope fractionation. It does not mean, however, that position-specific isotope composition is useless. Based on the predicted equilibrium Intra-ID, a predicted isotope fractionation

170 factor (α) of corresponding positions between reactant and product in a process can help to evaluate the thermodynamic state of a system and to decipher reaction pathways. In this section, we use a simple organic molecule, acetic acid (CH_3COOH), and its measured Intra-IDs from literature as examples to illustrate how position-specific isotope fractionation occurs between reactant and product.

The relative isotope enrichment between the carboxyl and methyl carbon in acetic acid is defined as $\ln^{13}\alpha_{carb-met}=$

175 $\ln(^{13}R_{carb}/^{13}R_{met})$. ^{13}R ($=^{13}C/^{12}C$) denotes the carbon isotope molar abundance ratio in a position. Our calculated equilibrium Intra-ID of acetic acid has the carboxyl carbon being 47.3 ‰ heavier than the methyl carbon at 25°C ($\ln^{13}\alpha_{carb-met\,(eq)}=$ 47.3‰, He et al., 2020). The measured $\delta^{13}C_{met}$ values from literature can be lower, higher, or approximately equal to the $\delta^{13}C_{carb}$ values for acetic acids from biological, artificial, or hydrous pyrolysis samples (Table 1). The position-specific $\delta^{13}C$ values of biological, artificial, or hydrous pyrolysis produced acetic acid are largely overlapping on $\delta^{13}C_{met}$-$\delta^{13}C_{carb}$ space. For the

180 majority of biological acetic acids, the $\delta^{13}C_{carb}$ values are several per mil higher than the $\delta^{13}C_{met}$ values (Fig. 3 top, $\ln^{13}\alpha_{carb-met}=$5.1±4.8‰, n=29), with two cases of ~18‰ higher and one case of -2.2‰ lower in $\delta^{13}C_{carb}$ values. It is expected that the metabolic and catabolic pathways and carbon sources are limited for most natural acetic acid. Therefore, the $\ln^{13}\alpha_{carb-met}$ value of 5.1±4.8‰ could be characteristic but not necessarily exclusive for biologically produced acetic acid. Artificial acetic acids have a very large range of $\ln^{13}\alpha_{carb-met}$ values from -30.2‰ to 24.2‰ (Fig. 3 middle, 7.3±14.3‰, n=24). Biological and hydrous

185 pyrolysis produced acetic acids do not have such negative $\ln^{13}\alpha_{carb-met}$ values.

Except for the above-mentioned features, the production of artificial and biological acetic acid has too many unconstrained parameters. Thus, our discussion will focus on the acetic acid derived from hydrous pyrolysis of oil-prone source rocks. The acetic acids produced from the hydrous pyrolysis of oil-prone source rocks have a $\ln^{13}\alpha_{carb\text{-}met}$ value of $18.3\pm7.7‰$ (n=22, Fig. 3 bottom). $\ln^{13}\alpha_{carb\text{-}met}$ values of ~30‰ were produced at 310~350 °C from Mahogany Shale or Black Shale with a proposed mechanism of uni-directional pyrolysis of precursor acid forms (R-CH$_2$COOH ↔ R + CH$_3$COOH, Fig. 4, Dias et al., 2002b). If we consider only the primary KIE between the methylene carbon in R-*CH$_2$COOH and the methyl carbon in acetic acid (*CH$_3$COOH), it is expected that a uni-directional process would lead to a ^{13}C depletion only on the methyl carbon position in acetic acid. The observed Intra-ID of the produced acetic acid should equal to the δ^{13}C value difference between the precursors minus the primary KIE value. The primary KIE value is expected to be more negative than the predicted equilibrium isotope fractionation factor, which is -14‰ (He et al., 2020). Thus, as long as the position-specific δ^{13}C value difference between the methylene and carboxyl carbon in R-CH$_2$COOH is greater than -14‰, the acetic acid produced from uni-directional pyrolysis of such precursor acid should have a carboxyl carbon with a higher δ^{13}C value than that of the methyl carbon. If the carboxyl carbon in the precursor acid has a higher δ^{13}C than that of the methylene carbon, the pyrolysis process can easily produce acetic acid with an apparent $\ln^{13}\alpha_{carb\text{-}met}$ value close to the predicted equilibrium Intra-ID. Such apparently "equilibrium-like" Intra-ID does not involve intramolecular exchange, but it is the product of uni-directional precursor acid pyrolysis.

3 Implications

Life sustains itself by feeding on negative entropy. Persistent efforts are devoted into describing living systems by rigorous mathematics. Boltzmann first considered living organisms from a thermodynamic perspective, and Schrodinger later applied equilibrium thermodynamics to living systems (Popovic, 2018). Those attempts were not pursued further, since, as we all know today, a living system is an open system that is away from thermodynamic equilibrium, i.e. a dissipative system. The establishment of nonequilibrium thermodynamics by Prigogine and his coworkers has guided researchers to the theorem of minimum entropy production in biological systems (Prigogine and Wiame, 1946). The theorem of minimum entropy production in biological systems describes that although a biosystem has increasing entropy, but it is usually stable in a steady state. If the system displaced from the steady state, it tends to return to its original state since the entropy is minimal at the state. Since then, efforts in applying nonequilibrium thermodynamics to living systems have been continued with mixed success (Stoward, 1962; Schneider and Kay, 1994; Hayflick, 2007; Demirel, 2010; Barbacci et al., 2015; Gerber et al., 2016). The theorem of minimum entropy production applies only to linear thermodynamic systems. Therefore, it is necessary to demonstrate that the magnitude of reaction rate on the scale of interest in a living system is linearly dependent on the driving force responsible for the reaction system. It is reasonable to view that a complex interacting and constantly evolving non-linear system is constructed by a series of synergistic reactions, and there should exist local linearity, local steady-state, even local equilibrium (Galimov, 2006).

Local nonequilibrium of biochemical system is potentially significant for the increasing complexity and ordering in the structure of life (Prigogine and Wiame, 1946; Galimov, 2006). Such a system should consist of a set of reversible but not necessarily equilibrium reactions conjugated with energy supplies that maintain in a steady-state not far from equilibrium.

220 Galimov (1985, 2004, 2005) argued that such a close-to-equilibrium steady-state should be expressed as a tendency toward equilibrium inter- and intra-molecular stable isotope distributions, i.e. a linear inter- and intra-molecular $^{13}\beta$-$\delta^{13}C$ correlation with a regression coefficient smaller than but close to 1 . In addition, such "*equilibrium-like*" Intra-ID in organic molecules was proposed as a "*special feature of biological systems*", which could be used as a criterion to identify biologically produced extraterrestrial organic molecules (Galimov, 2003). As we have illustrated above, observed Intra-ID in organic molecules is

225 the product of a set of equilibrium or nonequilibrium processes as well as their source isotope compositions. An observed Intra-ID itself cannot be used as conclusive evidence for the thermodynamic state of a system. Therefore, even if a compound does have a linear intramolecular $^{13}\beta$-$\delta^{13}C$ correlation with a slope of 1, it does not constitute supporting evidence for the existence of an equilibrium state among biochemical reactions in organisms. To apply nonequilibrium thermodynamics on living systems, further solid evidence is needed.

230 A simple comparison of position-specific isotope compositions in one sample, e.g. $\ln^{13}\alpha_{carb-met}$ values of one acetic acid sample, offers little information on the reaction mechanisms and reaction kinetics of the reaction it involves. Although the position-specific atoms are the same element, without an exchange mechanism, they behave independently as different elements. It would be helpful if we could consider the position-specific atoms independently. The isotope fractionation relationship of different elements in the same compound, i.e. $(\alpha_A-1)/(\alpha_B-1)$, $\ln\alpha_A/\ln\alpha_B$, or $\Delta\delta_A/\Delta\delta_B$, (named bonded isotope effect, He and Bao,

235 2019), is often used to characterize a reaction pathway. For instance, δD and $\delta^{18}O$ in H_2O (Dansgaard, 1964;Craig, 1961), $\delta^{15}N$ and $\delta^{18}O$ in NO_3^- (Casciotti and McIlvin, 2007; Wankel et al., 2009), $\delta^{34}S$ and $\delta^{18}O$ in SO_4^{2-} (Antler et al., 2013), or $\delta^{13}C$ and δD in organic compounds (Elsner, 2010; Palau et al., 2017). The isotope composition difference of different elements is only useful if the isotope fractionation relationships are considered and their isotope compositions are normalized, e.g. $\Delta(15,18)=(\delta^{15}N-\delta^{15}N_m)-(^{15}\alpha-1/^{18}\alpha-1)\times(\delta^{18}O-\delta^{18}O_m)$, $\delta^{15}N_m$ and $\delta^{18}O_m$ are the average isotope composition in a given ocean

240 water column (Sigman et al., 2005). The normalization procedure is necessary because the source isotope compositions can affect the values of the product. Similarly, if the same element at different positions have different sources, their source isotope composition difference must also be considered. Position-specific isotope researches can build upon our understanding of bonded isotope effect.

4 Conclusions

245 An organic compound usually has an element, e.g. carbon, at different positions and therefore has an Intra-ID. The deviation of an observed Intra-ID from its equilibrium state has been used to evaluate the thermodynamic state of a system. Our analysis of oxygen-bearing minerals, N_2O, and acetic acids show that both isotope sources and all reaction processes need to be in equilibrium to reach an intramolecular equilibrium state. However, such a condition is rarely satisfied. When different positions

of the same element cannot exchange with each other, these different positions behave independently like different elements.
250 Observed Intra-ID that is apparently similar to the equilibrium one can also be produced from a combination of different sources and uni-directional processes. Thus, an observed Intra-ID itself is not conclusive without adequate information on sources and reaction kinetics. Compared to position-specific isotope compositions, position-specific isotope fractionation of a defined process is more informative to identifying bond-breaking/forming positions of a large molecule, to predicting its transition-state structure, to evaluating the reversibility of a biochemical process, and to determining and qualifying a process
255 in a complex system. All in all, an understanding of a reaction process at the molecular level will always be the first step required for later sound and wide application of stable isotope composition.

Author contribution

All authors contributed ideas. Y. He and H. Bao wrote the manuscript.

Code/Data availability

260 All data, models, and code generated or used during the study appear in the submitted article.

Competing interests

The authors declare that they have no conflict of interest.

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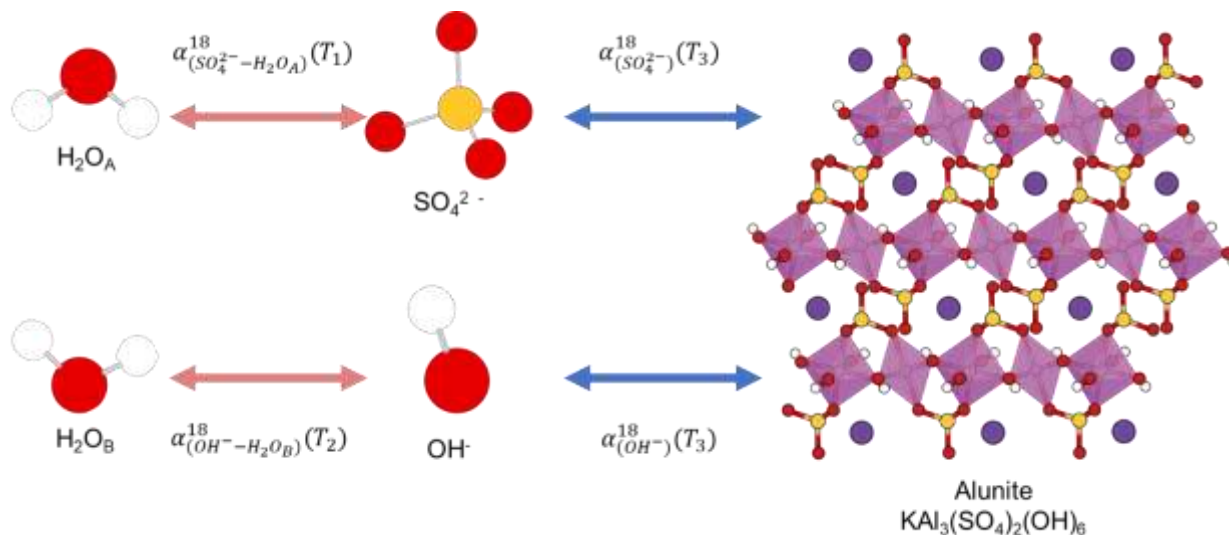
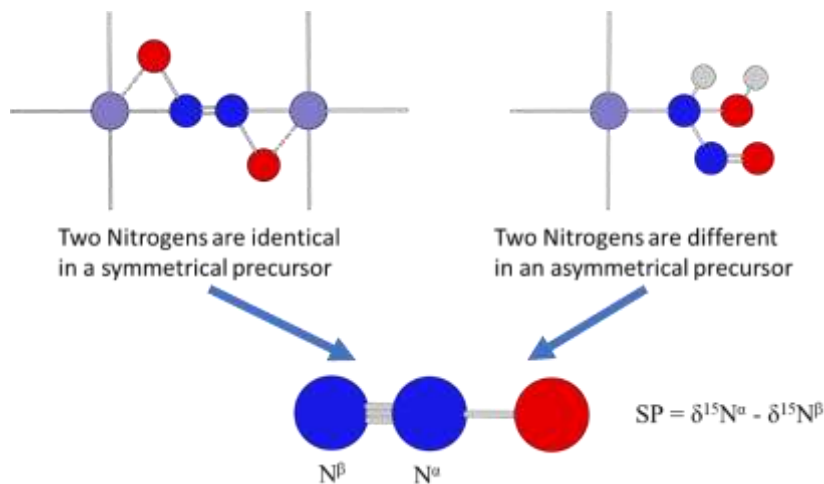

435

Figure 1. Sketch of alunite precipitation from water. The alunite could be a single-mineral geothermometer if three conditions are fully all satisfied: (1) $H_2O_A = H_2O_B$, (2) $T_1=T_2=T_3$, and (3) all the four reactions are fully reversible and attain equilibrium. White, red, yellow, pink, and purple spheres represent hydrogen, oxygen, sulfur, aluminum, and potassium atoms, respectively.

440   **Figure 2. Proposed mechanisms for N_2O formation from symmetrical and asymmetrical precursors (Modified from Schmidt et al., 2004). Light gray, red, blue, and purple spheres represent hydrogen, oxygen, nitrogen, and iron atoms, respectively.**

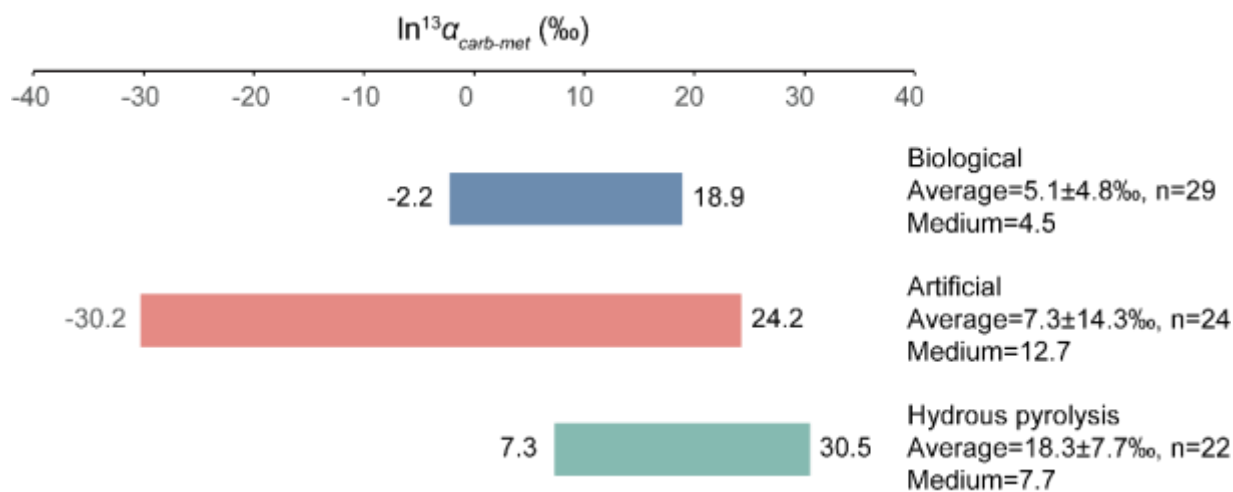



Figure 3. $\ln^{13}\alpha_{carb-met}$ values of biological, artificial, and hydrous pyrolysis produced acetic acid.

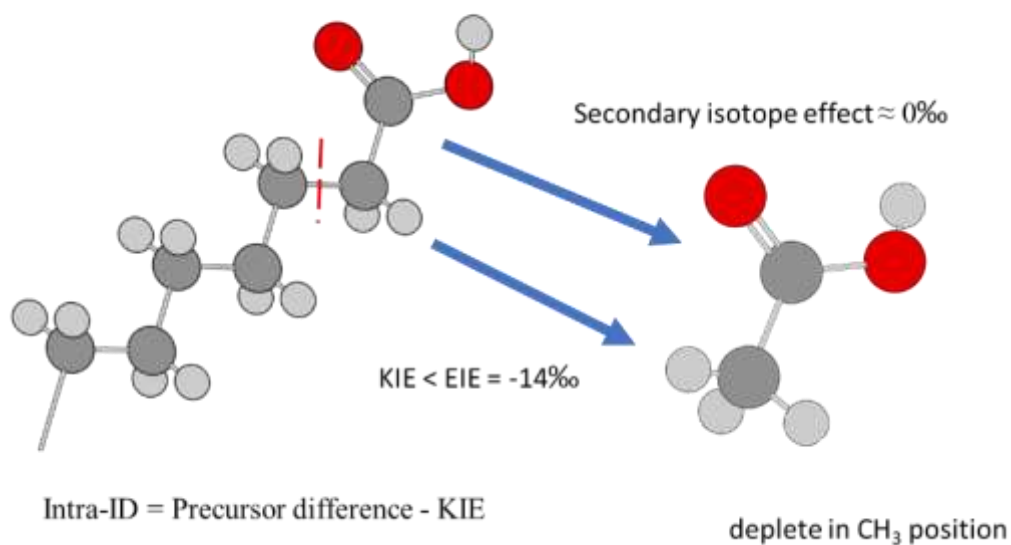



Figure 4. Acetic acid produced from pyrolysis of precursor acid forms has an Intra-ID that is depleted in ^{13}C in the methyl position. Dark gray, light grey, and red spheres represent carbon, hydrogen, and oxygen atoms, respectively.