

A generalizable box model for kinetic clumped isotope effects in the CaCO₃ -DIC-H₂O system

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Abstract

Most Earth surface carbonates precipitate out of isotopic equilibrium with their host solution, complicating the use of stable isotopes in paleoenvironment reconstructions. Disequilibrium can arise from exchange reactions in the DIC-H₂O system as well as during crystal growth reactions in the DIC-CaCO₃ system. Existing models account for kinetic isotope effects in these systems separately but the models have yet to be combined in a general framework. Here, a box model is developed for describing disequilibrium carbon, oxygen, and clumped isotope effects in the CaCO₃-DIC-H₂O system. The model is applied to inorganic calcite precipitation experiments where there is a known CO₂ influx and CaCO₃ outflux. The example provided can be adapted to other situations involving CO₂ absorption (e.g., corals, foraminifera, high-pH travertines) or degassing (e.g., speleothems, low-pH travertines, cryogenic carbonates) and/or mixing with other DIC sources.

A generalizable box model for kinetic clumped isotope effects in the CaCO_3 -DIC- H_2O system

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Key Points:

- We derive a box model for kinetic clumped isotope effects in the CaCO_3 -DIC- H_2O system.
- The model is used to fit and explain extreme $\delta^{18}\text{O}$ and Δ_{47} KIEs in high-pH inorganic calcite precipitation experiments.
- The model can be used to better understand KIEs in biogenic calcifiers if the DIC fluxes are known or constrained.

Keywords: kinetic isotope effects, carbonates, carbon isotopes, oxygen isotopes, clumped isotopes, hydration, hydroxylation

Abstract

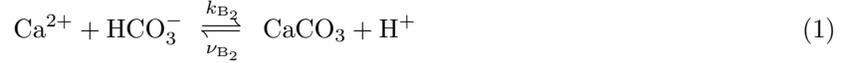
Most Earth surface carbonates precipitate out of isotopic equilibrium with their host solution, complicating the use of stable isotopes in paleoenvironment reconstructions. Disequilibrium can arise from exchange reactions in the DIC- H_2O system as well as during crystal growth reactions in the DIC- CaCO_3 system. Existing models account for kinetic isotope effects in these systems separately but the models have yet to be combined in a general framework. Here, a box model is developed for describing disequilibrium carbon, oxygen, and clumped isotope effects in the CaCO_3 -DIC- H_2O system. The model is applied to inorganic calcite precipitation experiments where there is a known CO_2 influx and CaCO_3 outflux. The example provided can be adapted to other situations involving CO_2 absorption (e.g., corals, foraminifera, high-pH travertines) or degassing (e.g., speleothems, low-pH travertines, cryogenic carbonates) and/or mixing with other DIC sources.

1 Introduction

The oxygen and clumped isotope compositions of carbonate minerals are widely used for paleoenvironment reconstructions. When crystals grow slowly, near equilibrium, oxygen isotope partitioning and ^{13}C - ^{18}O bond ordering (or ‘clumping’) are expected to depend solely on temperature, providing a theoretical foundation for both oxygen isotope and clumped isotope thermometry (Bigeleisen and Mayer, 1947; Urey, 1947; Ghosh et al., 2006; Eiler, 2007). Natural mineral growth, however, typically occurs under non-equilibrium conditions, as does precipitation of calcite in laboratory experiments (Kim and O’Neil, 1997; Dietzel et al., 2009; Gabitov et al., 2012; Watkins et al., 2013; Affek and Zaarur, 2014). The resulting kinetic isotope effects (KIEs) can arise from multiple processes, including but not limited to: (1) diffusive transport of CO_2 through membranes (Thiagarajan et al., 2011; Hansen et al., 2017), (2) crystal growth reactions (DePaolo, 2011; Watkins et al., 2013), and (3) incomplete isotope exchange reactions between dissolved inorganic carbon species ($\text{DIC} = \text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-}$) and water (e.g., Zeebe and Wolf-Gladrow, 2001; Guo, 2008; Uchikawa

37 and Zeebe, 2012; Affek, 2013; Devriendt et al., 2017; Staudigel and Swart, 2018; Bajnai et al., 2018; many
 38 others). Reconstructing environments from disequilibrium isotope compositions first requires knowledge of
 39 the calcification pathways and the KIEs that arise during each step in mineral formation.

Over the past decade, significant progress has been made towards quantifying the sign and magnitude
 of KIEs in the CaCO_3 -DIC- H_2O system. In the simplest scenario of CaCO_3 growth from an isotopically
 equilibrated DIC pool, KIEs can be attributed to the following crystal growth reactions (Watkins et al.,
 2013):



and



40 where the k 's and ν 's are mass-dependent rate constants following the notation of Wolthers et al. (2012).
 41 For calcite, the KIEs attending these reactions can be significant across the full range of growth rate and
 42 pH: $\sim 1\text{-}4\text{‰}$ for $\delta^{18}\text{O}$ and $\sim 0.3\text{‰}$ for Δ_{47} (Watkins et al., 2014; Watkins and Hunt, 2015), which translate
 43 to ΔT of about 4-16 °C and 7-11 °C, respectively (McCrea, 1950; Ghosh et al., 2006; Zaarur et al., 2013).
 44 Although this is a fairly large temperature range, the temperature sensitivities of most empirical calibrations
 45 are probably not compromised by surface reaction-controlled KIEs because they are based on carbonates
 46 precipitated over a narrow range in growth rate and pH (e.g., McCrea, 1950; O'Neil et al., 1969; Kim and
 47 O'Neil, 1997; Ghosh et al., 2006; Dennis and Schrag, 2010; Zaarur et al., 2013; Watkins et al., 2013; Candelier
 48 et al., 2013; Marchitto et al., 2014; Kluge et al., 2015; Kele et al., 2015; Parker et al., 2017; Kelson et al.,
 49 2017, and many others).

50 The picture gets more complicated when CaCO_3 precipitates from a DIC pool that is not isotopically
 51 equilibrated. Here, the key reactions are the relatively slow (de-)hydration and (de-)hydroxylation reactions:



and



52 where the k 's are isotopologue-specific rate constants. If the reactions are unidirectional (either forward or
 53 backward), the KIEs can be an order of magnitude larger than the KIEs attending crystal growth (O'Neil
 54 and Barnes, 1971; Clark and Fontes, 1990; Clark et al., 1992; Guo, 2008; Guo et al., 2009; Mervine et al.,
 55 2014; Falk et al., 2016; Leleu et al., 2016; Devriendt et al., 2017; Guo, 2020; Yumol et al., 2020; Christensen
 56 et al., 2021). More often than not, however, these reactions are bi-directional, and an important challenge
 57 is to be able to estimate the degree of reaction reversibility, which controls the magnitude of the KIEs.

58 Two different approaches have been taken recently to model KIEs in the DIC- H_2O system in the absence
 59 of crystal growth. The IsoDIC model of Guo (2020) tracks all of the reactions ($n = 155$) involving ^{12}C , ^{13}C ,
 60 ^{16}O , ^{17}O , and ^{18}O . This amounts to 32 coupled ordinary differential equations (ODEs) describing changes in
 61 concentration of CO_2 and HCO_3^- isotopologues (Table 1). By contrast, the ExClump38 model of Uchikawa
 62 et al. (2021) only tracks the major isotopologue at each mass, up to mass 63 (Table 1). This has the
 63 advantage of only requiring 8 ODEs for single clumped isotopes (i.e., Δ_{47}) but it includes approximations
 64 that have not been fully explained or validated against the IsoDIC model. Additionally, the ExClump38
 65 model has not been extended to double clumped isotopes (Δ_{48}) or triple clumped isotopes (Δ_{49}).

66 In this contribution, we add important details and explanations to the derivation of the ExClump38
 67 framework first developed by Chen et al. (2018) for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ and subsequently expanded to Δ_{47} by
 68 Uchikawa et al. (2021). The exposition is necessary for: (1) clarifying some ambiguities so that ExClump38
 69 is on a stronger foundation, (2) expanding the model to Δ_{48} and Δ_{49} , and (3) deriving new expressions
 70 that describe the influence of CaCO_3 precipitation. After validating the ExClump38 framework against the
 71 IsoDIC model, we provide an example box model with a CO_2 influx and CaCO_3 outflux for comparison to
 72 recent calcite precipitation experiments. The box model can be adjusted judiciously to describe carbonate
 73 precipitation in other settings.

2 Existing framework for carbon and oxygen isotopes

The ExClump38 framework was first used by Chen et al. (2018) to describe $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ co-variations in corals. The ODEs were then adopted and modified by Christensen et al. (2021) to describe $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ co-variations in alkaline travertines. Differences in the equations used between these two studies were reconciled by Uchikawa et al. (2021). In this section, we provide a step-by-step derivation that builds upon the latest version of ExClump38 (Uchikawa et al., 2021). This leads to slightly different expressions than used by Uchikawa et al. (2021), but importantly, the differences are not significant for single clumped isotopes (^{13}C - ^{18}O). However, the differences are important for double (^{18}O - ^{18}O) and triple (^{13}C - ^{18}O - ^{18}O) clumped isotopes. Additionally, we justify certain approximations that were made and validate the model against analytical expressions available in the literature.

2.1 Isotope versus isotopologue ratios

An essential part of the ExClump38 framework is the built-in conversions between isotope ratios (r) and isotopologue ratios (R). In the case where the isotopes are randomly distributed, we have the following relationships for oxygen isotopes in CO_3^{2-} (ignoring ^{17}O):

$$[\text{C}^{16}\text{O}^{16}\text{O}^{16}\text{O}] = P(16, 16, 16) = (0.998)^3 \approx 0.994 \quad (5)$$

$$[\text{C}^{16}\text{O}^{16}\text{O}^{18}\text{O}] = P(16, 16, 18) = 3(0.998)(0.998)(0.002) \approx 0.005998 \quad (6)$$

$$[\text{C}^{16}\text{O}^{18}\text{O}^{18}\text{O}] = P(16, 18, 18) = 3(0.998)(0.002)(0.002) \approx 0.000012 \quad (7)$$

and

$$[\text{C}^{18}\text{O}^{18}\text{O}^{18}\text{O}] = P(18, 18, 18) = (0.002)^3 \approx 8 \times 10^{-9}, \quad (8)$$

where the P 's refer to probabilities. Without rounding, these sum to exactly 1. The $^{18}\text{O}/^{16}\text{O}$ ratio is related to the isotopologue abundances through

$$r_{\text{CO}_3^{2-}} = \left(\frac{^{18}\text{O}}{^{16}\text{O}}\right)_{\text{CO}_3^{2-}} = \frac{[\text{C}^{16}\text{O}^{16}\text{O}^{18}\text{O}] + 2[\text{C}^{16}\text{O}^{18}\text{O}^{18}\text{O}] + 3[\text{C}^{18}\text{O}^{18}\text{O}^{18}\text{O}]}{3[\text{C}^{16}\text{O}^{16}\text{O}^{16}\text{O}] + 2[\text{C}^{16}\text{O}^{16}\text{O}^{18}\text{O}] + [\text{C}^{16}\text{O}^{18}\text{O}^{18}\text{O}]}. \quad (9)$$

The $^{18}\text{O}/^{16}\text{O}$ ratio can alternatively be expressed using any two different isotopologues without losing any information. For example:

$$r_{\text{CO}_3^{2-}} = \left(\frac{[\text{C}^{18}\text{O}^{18}\text{O}^{18}\text{O}]}{[\text{C}^{16}\text{O}^{16}\text{O}^{16}\text{O}]}\right)^{1/3} = \left(\frac{(0.002)(0.002)(0.002)}{(0.998)(0.998)(0.998)}\right)^{1/3}, \quad (10)$$

or

$$r_{\text{CO}_3^{2-}} = \frac{1}{3} \frac{[\text{C}^{16}\text{O}^{16}\text{O}^{18}\text{O}]}{[\text{C}^{16}\text{O}^{16}\text{O}^{16}\text{O}]} = \frac{3(0.998)(0.998)(0.002)}{3(0.998)(0.998)(0.998)}, \quad (11)$$

or

$$r_{\text{CO}_3^{2-}} = \left(\frac{[\text{C}^{16}\text{O}^{18}\text{O}^{18}\text{O}]}{3[\text{C}^{16}\text{O}^{16}\text{O}^{16}\text{O}]}\right)^{1/2} = \left(\frac{3(0.998)(0.002)(0.002)}{3(0.998)(0.998)(0.998)}\right)^{1/2}. \quad (12)$$

Because the singly-substituted isotopologue is the second most abundant, a sensible choice is to use Eq. 11 as done by Watkins et al. (2014) in their ion-by-ion model for calcite growth from HCO_3^- and CO_3^{2-} isotopologues. To reiterate, Eqs. 10-12 are equivalent to Eq. 9 when the isotopes are randomly distributed.

For CO_2 , we have:

$$[\text{C}^{16}\text{O}^{16}\text{O}] = P(16, 16) = (0.998)^2 \approx 0.996 \quad (13)$$

$$[\text{C}^{16}\text{O}^{18}\text{O}] = P(16, 18) = 2(0.998)(0.002) \approx 0.003992 \quad (14)$$

$$[\text{C}^{18}\text{O}^{18}\text{O}] = P(18, 18) = (0.002)^2 \approx 4 \times 10^{-6} \quad (15)$$

The $^{18}\text{O}/^{16}\text{O}$ ratio can be written as:

$$r_{\text{CO}_2} = \left(\frac{^{18}\text{O}}{^{16}\text{O}} \right)_{\text{CO}_2} = \frac{[\text{C}^{16}\text{O}^{18}\text{O}] + 2[\text{C}^{18}\text{O}^{18}\text{O}]}{2[\text{C}^{16}\text{O}^{16}\text{O}] + [\text{C}^{16}\text{O}^{18}\text{O}]} \quad (16)$$

There are two other ways this can be written:

$$r_{\text{CO}_2} = \left(\frac{[\text{C}^{18}\text{O}^{18}\text{O}]}{[\text{C}^{16}\text{O}^{16}\text{O}]} \right)^{1/2} = \left(\frac{(0.002)(0.002)}{(0.998)(0.998)} \right)^{1/2} \quad (17)$$

or

$$r_{\text{CO}_2} = \frac{1}{2} \frac{[\text{C}^{16}\text{O}^{18}\text{O}]}{[\text{C}^{16}\text{O}^{16}\text{O}]} = \frac{2(0.002)(0.998)}{2(0.998)(0.998)} \quad (18)$$

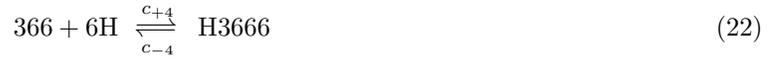
92 Note that these definitions have not taken into account carbon isotopes. If we were to add ^{12}C and ^{13}C , there
 93 would instead be 8 CO_3^{2-} isotopologues and 6 CO_2 isotopologues (again, ignoring ^{17}O). The expressions in Eq.
 94 9 and 16 would thus have more terms but the other expressions involving any two of the isotopologues would
 95 remain the same. Although it is not immediately obvious, these relationships are applicable to problems
 96 involving non-random distributions, or clumping, as will be shown.

97 2.2 Shorthand notation

98 We use the following shorthand notation after Zeebe (2014): $^{12}\text{C} = 2$, $^{13}\text{C} = 3$, $^{16}\text{O} = 6$, and $^{18}\text{O} = 8$.
 99 Additionally, we drop the superscript charges on ionic species. We treat isotopomers as indistinguishable
 100 such that [286] will hereafter refer to the total concentration of singly-substituted CO_2 : [286]+[268]. In what
 101 follows it will be made unambiguous that $d[\text{C}^{18}\text{OO}]/dt$ (notation of Chen et al., 2018) refers to the change
 102 in concentration of the sum of isotopomers ([286]+[268]) as opposed to (a) a single isotopomer or (b) the
 103 total ^{18}O of CO_2 , which includes $\text{C}^{18}\text{O}^{18}\text{O}$.

104 2.3 The Chen et al. (2018) subset of reactions

105 For carbon and oxygen isotope calculations, ExClump38 includes the following exchange reactions:



106 For the reactions involving oxygen isotopes, the 1/3 and 2/3 factors have been added to the rate constants
 107 for isotopic mass balance (Christensen et al., 2021; Uchikawa et al., 2021). To understand these factors
 108 qualitatively, consider, e.g., the two dehydration reactions involving H2866 (reactions 23 and 24). The right-
 109 hand sides of these two reactions are identical, but for every mole of H2866 that undergoes dehydration,
 110 $\sim 2/3$ goes to 286 and $\sim 1/3$ goes to H₂8. The factors are approximate because the isotopes are not exactly
 111 randomly distributed among the isotopologues and also because there is isotopic fractionation attending
 112 these reactions. A more quantitative justification is given in section 2.5.

113 2.4 From reactions to ordinary differential equations (ODEs)

114 The above reactions involve six isotopically-distinct DIC species that need to be tracked. We write HCO₃⁻
 115 and CO₃²⁻ together as EIC (equilibrated inorganic carbon, Chen et al., 2018) and apply the notation to
 116 isotopologues (e.g. H2666 + 2666 = E2666). The ODEs become:

$$\begin{aligned} \frac{d[266]}{dt} = & -k_{+1}[266] + k_{-1}[E2666]\chi[H] \\ & -k_{+4}[266][6H^-] + k_{-4}[E2666]\chi \\ & -a_{+1}[266]r_w + \frac{1}{3}a_{-1}[E2866]^{18}\chi[H] \\ & -a_{+4}[266][8H] + \frac{1}{3}a_{-4}[E2866]^{18}\chi \end{aligned} \quad (27)$$

$$\begin{aligned} \frac{d[E2666]}{dt} = & k_{+1}[266] - k_{-1}[E2666]\chi[H] \\ & k_{+4}[266][6H] - k_{-4}[E2666]\chi \end{aligned} \quad (28)$$

$$\begin{aligned} \frac{d[366]}{dt} = & -c_{+1}[366] + c_{-1}[E3666]^{13}\chi[H] \\ & -c_{+4}[366][6H] + c_{-4}[E3666]^{13}\chi \end{aligned} \quad (29)$$

$$\begin{aligned} \frac{d[E3666]}{dt} = & c_{+1}[366] - c_{-1}[E3666]^{13}\chi[H] \\ & c_{+4}[366][6H] - c_{-4}[E3666]^{13}\chi \end{aligned} \quad (30)$$

$$\begin{aligned} \frac{d[286]}{dt} = & -b_{+1}[286] + \frac{2}{3}b_{-1}[E2866]^{18}\chi[H] \\ & -b_{+4}[286][6H] + \frac{2}{3}b_{-4}[E2866]^{18}\chi \end{aligned} \quad (31)$$

$$\begin{aligned} \frac{d[E2866]}{dt} = & a_{+1}[266]r_w - \frac{1}{3}a_{-1}[E2866]^{18}\chi[H] \\ & + a_{+4}[266][8H] - \frac{1}{3}a_{-4}[E2866]^{18}\chi \\ & + b_{+1}[286] - \frac{2}{3}b_{-1}[E2866]^{18}\chi[H] \\ & + b_{+4}[286][6H] - \frac{2}{3}b_{-4}[E2866]^{18}\chi \end{aligned} \quad (32)$$

117 The last four terms in Eq. 27 were not included in previous iterations of ExClump38, presumably because
 118 they were deemed negligible. While this is generally true, there is an additional more nuanced reason for
 119 not retaining these terms (Supplement S.1).

The χ terms represent the fraction of EIC that is HCO₃⁻:

$$\chi = \frac{[H2666]}{[H2666] + [2666]} = \frac{1}{1 + \frac{K_2}{[H^+]}} \quad (33)$$

$${}^{13}\chi = \frac{[\text{H3666}]}{[\text{H3666}] + [\text{3666}]} = \frac{1}{1 + \frac{K_2 \cdot {}^{13}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}}{[\text{H}^+]}} \quad (34)$$

and

$${}^{18}\chi = \frac{[\text{H2866}]}{[\text{H2866}] + [\text{2866}]} = \frac{1}{1 + \frac{K_2 \cdot {}^{18}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}}{[\text{H}^+]}} \quad (35)$$

where $\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}$ is the equilibrium fractionation factor between CO_3^{2-} and HCO_3^- . In many situations, it is appropriate to treat $[\text{H}_2\text{O}]$, $[\text{6H}]$, $[\text{H}_2\text{S}]$, and $[\text{8H}]$ as constant, implying there is an infinite reservoir of H_2O , and by extension, OH^- .

2.5 Rate constants and EFFs

The forward rate constants are directly related to kinetic fractionation factors. For the hydration reactions, we have

$$\frac{c_{+1}}{k_{+1}} = {}^{13}\alpha_{c_{+1}}^{\text{KFF}} \quad (36)$$

$$\frac{a_{+1}}{k_{+1}} = {}^{18}\alpha_{a_{+1}}^{\text{KFF}} \quad (37)$$

and

$$\frac{b_{+1}}{k_{+1}} = {}^{18}\alpha_{b_{+1}}^{\text{KFF}} \quad (38)$$

For the hydroxylation reactions, we have

$$\frac{c_{+4}}{k_{+4}} = {}^{13}\alpha_{c_{+4}}^{\text{KFF}} \quad (39)$$

$$\frac{a_{+4}}{k_{+4}} = {}^{18}\alpha_{a_{+4}}^{\text{KFF}} \quad (40)$$

and

$$\frac{b_{+4}}{k_{+4}} = {}^{18}\alpha_{b_{+4}}^{\text{KFF}} \quad (41)$$

The backward rate constants must satisfy equilibrium, which leads to:

$$k_{+1}[\text{266}][\text{H}_2\text{6}] = k_{-1}[\text{H2666}][\text{H}], \quad (42)$$

$$c_{+1}[\text{366}][\text{H}_2\text{6}] = c_{-1}[\text{H3666}][\text{H}], \quad (43)$$

$$a_{+1}[\text{266}][\text{H}_2\text{8}] = \frac{1}{3}a_{-1}[\text{H2866}][\text{H}], \quad (44)$$

$$b_{+1}[\text{286}][\text{H}_2\text{6}] = \frac{2}{3}b_{-1}[\text{H2866}][\text{H}], \quad (45)$$

$$k_{+4}[\text{266}][\text{6H}] = k_{-4}[\text{H2666}], \quad (46)$$

$$c_{+4}[\text{366}][\text{6H}] = c_{-4}[\text{H3666}], \quad (47)$$

$$a_{+4}[\text{266}][\text{8H}] = \frac{1}{3}a_{-4}[\text{H2866}], \quad (48)$$

and

$$b_{+4}[\text{286}][\text{6H}] = \frac{2}{3}b_{-4}[\text{H2866}]. \quad (49)$$

Converting isotopologue ratios to isotope ratios (§2.1) and rearranging yields the following relationships between rate constants and equilibrium constants:

$$\frac{k_{+1}}{k_{-1}} = \frac{[\text{H2666}][\text{H}]}{[266][\text{H}_2\text{6}]} = K_1, \quad (50)$$

$$\frac{c_{+1}}{c_{-1}} = \frac{{}^{13}r_{\text{HCO}_3^-}[\text{H2666}][\text{H}]}{{}^{13}r_{\text{CO}_2}[266][\text{H}_2\text{6}]} = K_1 \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}, \quad (51)$$

$$\frac{a_{+1}}{a_{-1}} = \frac{(3){}^{18}r_{\text{HCO}_3^-}[\text{H2666}][\text{H}^+]}{(3)[266][\text{H}_2\text{6}][\text{H}_2\text{8}]} = K_1 \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}, \quad (52)$$

$$\frac{b_{+1}}{b_{-1}} = \frac{(2 \cdot 3){}^{18}r_{\text{HCO}_3^-}[\text{H2666}][\text{H}]}{(3 \cdot 2){}^{18}r_{\text{CO}_2}[266][\text{H}_2\text{6}]} = K_1 \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}, \quad (53)$$

$$\frac{k_{+4}}{k_{-4}} = \frac{[\text{H2666}]}{[266][6\text{H}]} = \frac{K_1}{K_w}, \quad (54)$$

$$\frac{c_{+4}}{c_{-4}} = \frac{{}^{13}r_{\text{HCO}_3^-}[\text{H2666}]}{{}^{13}r_{\text{CO}_2}[266][6\text{H}]} = \frac{K_1}{K_w} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}, \quad (55)$$

$$\frac{a_{+4}}{a_{-4}} = \frac{(3){}^{18}r_{\text{HCO}_3^-}[\text{H2666}]}{(3)r_{\text{OH}^-}[266][6\text{H}]} = \frac{K_1}{K_w} \cdot \frac{{}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}}{{}^{18}\alpha_{\text{OH}^- - \text{H}_2\text{O}}^{\text{eq}}} \quad (56)$$

and

$$\frac{b_{+4}}{b_{-4}} = \frac{(2 \cdot 3){}^{18}r_{\text{HCO}_3^-}[\text{H2666}]}{(3 \cdot 2){}^{18}r_{\text{CO}_2}[266][6\text{H}]} = \frac{K_1}{K_w} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}. \quad (57)$$

129 It is here that the need for 1/3 and 2/3 factors on the rate constants becomes apparent: They cancel with
 130 the factors of 2 and 3 that accompany the conversion from isotopologue ratios to isotope ratios and are
 131 fundamentally related to the statement that isotopomers are indistinguishable; i.e., that [286] and [2866]
 132 refer to the total concentration of singly-substituted CO₂ ([286]+[268]) and HCO₃⁻ ([2866]+[2686]+[2668]).
 133 An up-to-date compilation of rate constants, equilibrium constants, and isotopic fractionation factors is
 134 provided in Table 2.

135 2.6 Model validation

136 The system of ODEs (Eqs. 27-32) has been used to describe the chemical and isotopic evolution of DIC
 137 species in the DIC-H₂O system (Chen et al., 2018; Christensen et al., 2021; Uchikawa et al., 2021) but without
 138 having been validated against experiments and analytical expressions that are available in the literature.

139 Uchikawa and Zeebe (2012) performed time-series experiments where an unequilibrated DIC pool was
 140 precipitated quantitatively as witherite (BaCO₃) and then analyzed for δ¹⁸O. Their data at pH = 8.3 and
 141 8.9 are compared to theoretical and numerical predictions in Fig. 1a. The analytical and numerical curves
 142 are nearly indistinguishable and in excellent agreement with the time series data. The numerical-analytical
 143 comparison is expanded to a broad range of pH for the time required to reach 99% oxygen isotope equilibration
 144 (τ_{99%}) as well as the time to reach 69.3% equilibration for carbon isotopes (τ e-fold) in Figs. 1b and 1c.

145 An interesting result is a large discrepancy between analytical and numerical results when it comes to
 146 chemical equilibration, as shown in Fig. 1d. Numerical experimentation revealed that this discrepancy
 147 is due to the assumption of instantaneous pH adjustment - an assumption that is also employed in the
 148 IsoDIC model (Guo, 2020). Upon adding ODEs that explicitly track d[H⁺]/dt, d[OH⁻]/dt, d[HCO₃⁻]/dt,
 149 and d[CO₃²⁻]/dt (referred to as the ‘Full model’ in the right panels of Fig. 1), the disagreement in chemical
 150 equilibration times is resolved (Fig. 1h). This is an interesting curiosity, but since it has little to no bearing
 151 on isotope-related problems, it will not be discussed further.

152 3 Adding clumped isotopes to the model

153 The above framework was extended to single clumped isotopes by Uchikawa et al. (2021) to describe oxygen
 154 and clumped isotope equilibration in the DIC-H₂O system. Much of what follows has been presented

155 previously (Uchikawa et al., 2021). Here, a re-derivation is provided that explicitly tracks the 1/3 and 2/3
 156 factors for oxygen isotope bookkeeping and does not fold the acid fractionation factors (AFFs) into the
 157 expressions for EIC. Additionally, we clarify a difference in the final expressions for the rate constants that
 158 would otherwise lead to issues when extending the model to double and triple clumped isotopes.

159 3.1 Homogeneous reactions and clumped isotope definitions

Clumped isotope systems involve the equilibrium of ^{13}C - ^{18}O bonding within a single species. For CO_2 , we can write the following isotope exchange reaction:



which has an equilibrium constant

$${}^{47}K_{\text{CO}_2} = \frac{[386][266]}{[366][286]}. \quad (59)$$

160 The abundance of 386 is measured as (Eiler, 2007):

$$\Delta_{47} = \left[\left(\frac{{}^{47}R}{{}^{47}R^*} - 1 \right) - \left(\frac{{}^{46}R}{{}^{46}R^*} - 1 \right) - \left(\frac{{}^{45}R}{{}^{45}R^*} - 1 \right) \right] \times 1000, \quad (60)$$

where ${}^{47}R$, ${}^{46}R$, and ${}^{45}R$ are the abundance ratios of masses 47, 46, and 45 relative to mass 44, and the asterisk denotes the stochastic distribution. In the absence of ^{17}O (as prescribed in the ExClump38 model), this reduces to:

$$\Delta_{47} = \left(\frac{{}^{47}R}{{}^{47}R^*} - 1 \right) \times 1000, \quad (61)$$

where

$${}^{47}R = \frac{[386]}{[266]}. \quad (62)$$

The stochastic ratio, ${}^{47}R^*$, can be calculated from the standard carbon and oxygen isotope ratios following Eq. 30 in Watkins and Hunt (2015). The ${}^{47}K_{\text{CO}_2}$ can be related to Δ_{47} values by first multiplying the top and bottom by [266]:

$${}^{47}K_{\text{CO}_2} = \frac{[386]}{[266]} \frac{[266]}{[366]} \frac{[266]}{[286]} = {}^{47}R \cdot \underbrace{({}^{13}r_{\text{CO}_2})^{-1} \cdot (2 \cdot {}^{18}r_{\text{CO}_2})^{-1}}_{{}^{47}R^{*-1}} = \left(\frac{{}^{47}R}{{}^{47}R^*} \right)_{\text{CO}_2}^{\text{eq}}. \quad (63)$$

Here, the equilibrium constant K is equivalent to R/R^* . As shown in Supplement S.3 and S.4, this is not the case for double and triple clumped isotopes. Combining Eq. 61 with Eq. 63 leads to

$${}^{47}K_{\text{CO}_2} = \left(\frac{{}^{47}R}{{}^{47}R^*} \right)_{\text{CO}_2}^{\text{eq}} = \frac{[386][266]}{[366][286]} = \left(\frac{\Delta_{47, \text{CO}_2}^{\text{eq}}}{1000} + 1 \right). \quad (64)$$

Similar expressions can be written for clumped isotope equilibrium in HCO_3^- and CO_3^{2-} :



and



which have equilibrium constants

$${}^{63}K_{\text{HCO}_3^-} = \frac{[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} = \left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} = \left(\frac{\Delta_{63, \text{HCO}_3^-}^{\text{eq}}}{1000} + 1 \right) \quad (67)$$

and

$${}^{63}K_{\text{CO}_3^{2-}} = \frac{[3866][2666]}{[3666][2866]} = \left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{CO}_3^{2-}}^{\text{eq}} = \left(\frac{\Delta_{63, \text{CO}_3^{2-}}^{\text{eq}}}{1000} + 1 \right). \quad (68)$$

3.2 Heterogeneous reactions involving clumped isotopologues

To add clumped isotopes to the model, we need to include the reactions involving ${}^{13}\text{C}$ - ${}^{18}\text{O}$ ‘clumps’ in CO_2 and HCO_3^- :



Rate constants for the clumped isotope reactions are denoted p for ‘primary’ and s for ‘secondary’ following Guo (2020). In the primary reactions, a clumped isotopologue is created from reactants that are singly-substituted. From these reactions we obtain the following ODEs

$$\begin{aligned} \frac{d[386]}{dt} &= -p_{+1}[386] + \frac{1}{3}p_{-1}[\text{E3866}]{}^{63}\chi[\text{H}] \\ &\quad - s_{+4}[386][6\text{H}] + \frac{2}{3}s_{-4}[\text{E3866}]{}^{63}\chi \end{aligned} \quad (73)$$

$$\begin{aligned} \frac{d[\text{E3866}]}{dt} &= p_{+1}[366]r_w - \frac{1}{3}p_{-1}[\text{E3866}]{}^{63}\chi[\text{H}] \\ &\quad + p_{+4}[266][8\text{H}] - \frac{1}{3}p_{-4}[\text{E3866}]{}^{63}\chi \\ &\quad + s_{+1}[386] - \frac{2}{3}s_{-1}[\text{E3866}]{}^{63}\chi[\text{H}] \\ &\quad + s_{+4}[386][6\text{H}] - \frac{2}{3}s_{-4}[\text{E3866}]{}^{63}\chi \end{aligned} \quad (74)$$

The next task is to determine the rate constants and ${}^{63}\chi$.

3.3 Derivation of ${}^{63}\chi$

The ${}^{63}\chi$ term is analogous to ${}^{13}\chi$ and ${}^{18}\chi$ and will be used to instantaneously redistribute the clumped isotopes between HCO_3^- and CO_3^{2-} so that these two species are in clumped isotopic equilibrium with each other. The expression takes the same form as the other χ terms because the fraction of clumped EIC that is in the form of HCO_3^- has the same pH dependence as for standard isotope ratios (Hill et al., 2014):

$${}^{63}\chi = \frac{1}{1 + \frac{{}^{63}K_2}{[\text{H}^+]}} \quad (75)$$

where ${}^{63}K_2$ is the equilibrium constant for the deprotonation reaction:

$${}^{63}K_2 = \frac{[3866][\text{H}]}{[\text{H3866}]} \quad (76)$$

This can be expressed in terms of the equilibrium Δ_{63} values of HCO_3^- and CO_3^{2-} . First, multiply the top and bottom by a common factor:

$${}^{63}K_2 = \frac{[3866][\text{H}]}{[\text{H}3866]} \cdot \frac{\frac{[\text{H}2666]}{[\text{H}3666][\text{H}2866]}}{\frac{[\text{H}2666]}{[\text{H}3666][\text{H}2866]}} \cdot \frac{\frac{[2666]}{[3666][2866]}}{\frac{[2666]}{[3666][2866]}} \cdot \frac{[2666]}{[2666]} \cdot \frac{[\text{H}2666]}{[\text{H}2666]}, \quad (77)$$

which upon rearrangement yields

$${}^{63}K_2 = \frac{\frac{[3866][2666]}{[3666][2866]}}{\frac{[\text{H}3866][\text{H}2666]}{[\text{H}3666][\text{H}2866]}} \cdot \frac{\frac{[\text{H}2666]}{[\text{H}3666]} \cdot \frac{[\text{H}2666]}{[\text{H}2866]}}{\frac{[2666]}{[3666]} \cdot \frac{[2666]}{[2866]}} \cdot \frac{[2666][\text{H}]}{[\text{H}2666]}. \quad (78)$$

Converting isotopologue ratios to isotope ratios leads to

$${}^{63}K_2 = {}^{63}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot {}^{13}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot {}^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot K_2, \quad (79)$$

where

$${}^{63}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} = \frac{\left(1 + \frac{\Delta_{63,\text{CO}_3^{2-}}^{\text{eq}}}{1000}\right)}{\left(1 + \frac{\Delta_{63,\text{HCO}_3^-}^{\text{eq}}}{1000}\right)}. \quad (80)$$

3.4 Rate constants

Watkins and Hunt (2015) suggested that the kinetic fractionation factor for clumped isotopes could be expressed as the deviation from the product of the carbon and oxygen KFFs:

$${}^{13-18}\alpha_{p+1}^{\text{KFF}} = {}^{18}\alpha_{a+1}^{\text{KFF}} \cdot {}^{13}\alpha_{c+1}^{\text{KFF}} + \epsilon_{a+1}, \quad (81)$$

where ϵ_{a+1} is expected to be small, on the order of 10^{-5} . Guo (2020) also expressed the KFF for clumped isotopes relative to the product of the carbon and oxygen KFFs, but in a different way; i.e.,

$${}^{13-18}\text{KIE}_{p+1} = \frac{{}^{13-18}\alpha_{p+1}^{\text{KFF}}}{{}^{18}\alpha_{a+1}^{\text{KFF}} \cdot {}^{13}\alpha_{c+1}^{\text{KFF}}} = \frac{\frac{p+1}{k_{+1}}}{\frac{c+1}{k_{+1}} \frac{a+1}{k_{+1}}}. \quad (82)$$

A ${}^{13-18}\text{KIE}_{p+1} = 1$ in the Guo (2020) formulation is equivalent to $\epsilon_{a+1} = 0$ in the Watkins and Hunt (2015) formulation of clumped isotope KFFs. Here, we adopt the Guo (2020) formulation and use his theoretical values for the ${}^{13-18}\text{KIE}$ s (Table 3). Rearranging Eq. 82 leads to an expression for p_{+1} :

$$p_{+1} = \frac{{}^{13-18}\text{KIE}_{p+1} c_{+1} a_{+1}}{k_{+1}}, \quad (83)$$

where ${}^{13-18}\text{KIE}_{p+1}$ is treated as a known quantity Guo (2020). To obtain p_{-1} , we use the equilibrium constraint from the corresponding reaction:

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3}[\text{H}3866][\text{H}]}{[366][\text{H}_28]}. \quad (84)$$

By multiplying the top and bottom by a common factor, we can write:

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3}[\text{H}3866][\text{H}]}{[366][\text{H}_28]} \cdot \frac{\frac{[\text{H}2666]}{[\text{H}3666][\text{H}2866]}}{\frac{[\text{H}2666]}{[\text{H}3666][\text{H}2866]}} \cdot \frac{\frac{[\text{H}2666]}{[266]}}{\frac{[\text{H}2666]}{[266]}} \cdot \frac{\frac{1}{\text{H}_26}}{\frac{1}{\text{H}_26}}, \quad (85)$$

which upon rearrangement leads to

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3} \frac{[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{H3666}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{366}]}{[\text{266}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}. \quad (86)$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p_{+1}}{p_{-1}} = \frac{1}{3} \cdot \left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot \frac{{}^{13}r_{\text{HCO}_3^-}}{{}^{13}r_{\text{CO}_2}} \cdot \frac{3 \cdot {}^{18}r_{\text{HCO}_3^-}}{r_w}. \quad (87)$$

The factors of 3 and 1/3 cancel and we get:

$$\frac{p_{+1}}{p_{-1}} = \left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}} \quad (88)$$

169 Similar expressions can be derived for the remaining clumped isotope rate constants (Supplement S.2),
170 ultimately leading to:

$$\frac{s_{+1}}{s_{-1}} = \frac{\left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}}{\left(\frac{{}^{47}R}{{}^{47}R^*} \right)_{\text{CO}_2}^{\text{eq}}}, \quad (89)$$

$$\frac{p_{+4}}{p_{-4}} = \left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{OH}^-}^{\text{eq}}, \quad (90)$$

and

$$\frac{s_{+4}}{s_{-4}} = \frac{\left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}}{\left(\frac{{}^{47}R}{{}^{47}R^*} \right)_{\text{CO}_2}^{\text{eq}}} \quad (91)$$

171 These expressions for the rate constants are nearly identical to those of Uchikawa et al. (2021). The only
172 significant difference is that they use ${}^{47}K_{\text{CO}_2}^{\text{eq}}$ and ${}^{63}K_{\text{HCO}_3^-}^{\text{eq}}$ instead of $({}^{47}R/{}^{47}R^*)_{\text{CO}_2}^{\text{eq}}$ and $({}^{63}R/{}^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}}$,
173 respectively. This works for single clumped isotopes because the quantities are equivalent, but breaks down
174 for double and triple clumped isotopes. A compilation of the parameters needed to solve Eqs. 73-74 is
175 provided in Table 3.

176 4 Adding double and triple clumped isotopes to the model

177 The theoretical study of kinetic clumped isotope fractionation by Guo (2020) showed how paired Δ_{47} - Δ_{48}
178 measurements might provide additional constraints on the sources of KIEs and mechanisms of carbonate
179 formation. Despite the low abundance of the mass 64 isotopologue (Table 1), it is now possible to measure
180 Δ_{48} with sufficient precision to resolve departures from equilibrium in Δ_{47} - Δ_{48} space (Fiebig et al., 2019;
181 Bajnai et al., 2020; Fiebig et al., 2021). Such measurements are being used to correct for kinetic effects and
182 infer temperatures of carbonate formation from samples that were previously thought to be compromised
183 (Bajnai et al., 2020; Fiebig et al., 2021). Anticipating that the ExClump38 model will ultimately be useful for
184 refining these corrections, the derivations of rate constants for double and triple clumped isotope reactions
185 are provided in Supplement S.3 and S.4, with the results summarized in Tables 4 and 5.

186 4.1 Model validation

187 The ExClump38 framework ignores ${}^{17}\text{O}$ and only tracks the major isotopologue at each mass (i.e., masses
188 47, 48 and 49 for CO_2 , and masses 63, 64, and 65 for HCO_3^- and CO_3^{2-}). To instill confidence that these
189 simplifications do not introduce significant errors, we performed numerical experiments for comparison to the
190 IsoDIC simulations of the time evolution of HCO_3^- during CO_2 degassing and CO_2 absorption (Fig. 2). In

191 these simulations, the DIC is initially equilibrated and then the CO₂ concentration and isotopic composition
192 are perturbed from the equilibrium value and held constant. The reassuring outcome is that the curves are
193 indistinguishable from those of Fig. 3 in Guo (2020).

194 4.2 Model uses and next steps

195 Guo (2008) provided the first estimates of the effects of hydration and hydroxylation on the Δ_{47} of DIC
196 using *ab initio* transition state theory calculations. For more than a decade, these first-order estimates
197 served as a valuable guide for interpreting kinetic clumped isotope effects, particularly when paired with
198 $\delta^{18}\text{O}$ measurements (Fig. 3a). The vectors for (de-)hydration and (de-)hydroxylation, however, do not
199 consider many details such as (non-linear) mixing or isotope exchange in the solution.

200 The work by Guo (2020) constitutes a major step forward in characterizing the patterns and controls
201 of clumped isotope KIEs more generally, as summarized in his ‘loop diagrams’ (e.g., Fig. 3b). The loops
202 show how Δ_{47} and $\delta^{18}\text{O}$ co-vary following a perturbation of an initial chemical and isotopic equilibrium by
203 a forced input/output of CO₂ to/from the solution. Each of the loops begins and ends on the equilibrium
204 composition denoted by the black circle and each point on a loop corresponds to a specific time in Fig. 2.
205 In these simulations, CO₂ absorption (hydroxylation) can produce Δ_{63} - $\delta^{18}\text{O}$ co-variations with a negative
206 slope (initial perturbation), just like in the vectors in Fig. 3a, or a slope pointing in any of the other
207 three quadrants depending on the extent of equilibration (cf. Fiebig et al., 2021). The same can be said
208 for a scenario involving CO₂ degassing. The Δ_{63} - $\delta^{18}\text{O}$ patterns that ultimately get recorded in carbonate
209 minerals depend on the degree of reaction reversibility, which in turn depend on the nature of DIC influxes
210 and outfluxes. In other words, determining where on each of these loops a system will land requires a more
211 complicated model in which the CO₂ flux is specified and there is also a CaCO₃ sink.

212 5 Box model and application to inorganic calcite precipitation 213 experiments

214 In this section we build a box model to explain large ($>0.3\%$) kinetic clumped isotope effects from inorganic
215 calcite precipitation experiments at high-pH (Tang et al., 2014). This data set is particularly amenable to
216 a box model because the experimental solutions were well-stirred (homogeneous) and the authors provided
217 information regarding the magnitude and isotopic composition of DIC fluxes. The goals of this exercise are:
218 (1) to provide the first example of a clumped isotope box model with a complete set of reaction kinetics
219 that can be easily adapted to other situations, and (2) to quantitatively evaluate the hypothesis by Tang
220 et al. (2014) that extreme light isotope enrichments in their experiments are due to some combination of
221 CO₂ diffusion through a membrane and the hydroxylation reaction.

222 5.1 Summary of the Tang et al. (2014) experiments

223 The experimental setup is shown in Fig. 4a. A polyethylene (PE) container houses an inner solution with
224 no Ca²⁺ but high CO_{2(aq)}. The PE container is placed in an outer solution with 10 mM CaCl₂ and no DIC
225 initially. The DIC is delivered to the outer solution by CO₂ diffusion through the PE membrane. The CO₂
226 flux varies between experiments because of differences in membrane thickness and in the pH of inner and
227 outer solutions. The pH of the outer solution is held constant through use of an autotitrator with NaOH as
228 the titrant.

229 Tang et al. (2014) describe their experiments as consisting of two stages. During Stage I, there is a CO₂
230 influx and the concentration of DIC in the outer solution increases monotonically until a critical saturation
231 is reached for spontaneous calcite precipitation. During Stage II, there is a CO₂ influx and CaCO₃ outflux.
232 Stage II is characterized by a short period of rapid CaCO₃ nucleation and growth followed by a prolonged
233 period of slower growth under steady state conditions.

234 A modified version of the experimental parameters and results of Tang et al. (2014) is given in Table
235 6. The flux of CO₂ into solution during Stage I was calculated from [DIC] at the end of Stage I and the
236 duration of Stage I (t_1). The flux of CaCO₃ was calculated from the moles of CaCO₃ precipitated (M) and
237 duration of Stage II (t_2). The surface area normalized growth rate of CaCO₃ (moles m⁻² s⁻¹) was calculated

238 by Tang et al. (2014) using a specific surface area for calcite based on particle size distributions. From the
 239 reported growth rates, we report the total reactive surface area (SA) of crystals, which falls in the range of
 240 $0.1 \pm 0.05 \text{ m}^2$ between experiments.

241 5.2 Box model setup

242 The experiments can be described using a box model with two fluxes: F_{CO_2} and F_{CaCO_3} (both in moles L^{-1}
 243 s^{-1}) (Fig. 4b). For simplicity, we treat the problem as a seeded experiment such that the reactive surface
 244 area is constant and equal to a representative value of 0.1 m^2 . This is justified on the basis that at the
 245 onset of Stage II, there is a short period of rapid nucleation and growth that generates “seed” crystals whose
 246 reactive surface area could plausibly be approximated as constant thereafter. Additional details regarding
 247 the treatments of F_{CO_2} and F_{CaCO_3} are described individually below.

248 5.2.1 CO_2 flux

249 A key source of uncertainty is the isotopic composition of CO_2 as it enters solution. In the default case, we
 250 assume that the $\text{CO}_{2(\text{aq})}$ in the inner solution is isotopically equilibrated with water. Under the experimental
 251 conditions ($5 \text{ }^\circ\text{C}$ and pH of inner solution between 7.3-8.1), the equilibration time for oxygen and clumped
 252 isotopes ($t_{99\%}$) ranges from 6 to 35 hours (Uchikawa and Zeebe, 2012; Staudigel and Swart, 2018). Tang
 253 et al. (2014) did not report the pre-experiment dwell time and whether or not it was sufficient to ensure
 254 isotopic equilibration. Even if the $\text{CO}_{2(\text{aq})}$ in the inner solution were equilibrated, it is possible for it to get
 255 isotopically fractionated by diffusion through the PE membrane. To the best of our knowledge, there is no
 256 available data on isotope fractionation by diffusion of $\text{CO}_{2(\text{g})}$ through a PE membrane. However, an upper
 257 bound on these effects is given by the mass dependence on diffusion through a porous medium where the
 258 pore size is smaller than the mean free path of CO_2 molecules (Knudsen diffusion):

$$R_{\text{diffused}} = R_{\text{residue}} \left(\frac{M_i}{M_j} \right)^{0.5}, \quad (92)$$

259 where R is the ratio of the concentration of isotopologue j to that of i , and M_i and M_j are the masses of
 260 isotopologues i and j , respectively. Previous work suggests that this fractionation law with exponent of 0.5
 261 yields a diffused population of CO_2 that is 22.2‰ lower in $\delta^{18}\text{O}$ and 0.5‰ higher in Δ_{47} (Eiler and Schauble,
 262 2004). If the pore size were larger than the mean free path and/or the power law exponent were less than 0.5,
 263 the isotopic effects would be reduced. In any case, there is potential for the CO_2 flux to have significantly
 264 lower $\delta^{18}\text{O}$ and higher Δ_{47} than presumed in the default scenario.

265 5.2.2 CaCO_3 flux

The precipitation of CaCO_3 constitutes a sink of EIC isotopologues that can affect the isotopic composition
 of residual EIC. Chen et al. (2018) provided the CaCO_3 flux terms for carbon and oxygen isotopes. Here,
 we build upon this framework by deriving the CaCO_3 sink term for the E3866 isotopologue. To begin, from
 Eq. 29 in Watkins and Hunt (2015) we have:

$$\left(\frac{{}^{63}R}{{}^{63}R^*} \right) = \frac{\left(\frac{[3866]}{[2666]} \right)}{\left(\frac{[3666]}{[2666]} \right) \left(\frac{[2866]}{[2666]} \right)}. \quad (93)$$

Next, we can define a kinetic clumped isotope fractionation factor:

$${}^{63}\alpha_{\text{CaCO}_3\text{-EIC}} = \frac{\left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{CaCO}_3}}{\left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{EIC}}} = \frac{\left[\frac{\left(\frac{[3866]}{[2666]} \right)_{\text{CaCO}_3}}{\left(\frac{[3666]}{[2666]} \right)_{\text{CaCO}_3} \left(\frac{[2866]}{[2666]} \right)_{\text{CaCO}_3}} \right]}{\left[\frac{\left(\frac{[3866]}{[2666]} \right)_{\text{EIC}}}{\left(\frac{[3666]}{[2666]} \right)_{\text{EIC}} \left(\frac{[2866]}{[2666]} \right)_{\text{EIC}}} \right]}, \quad (94)$$

266 which upon rearrangement leads to

$${}^{63}\alpha_{\text{CaCO}_3\text{-EIC}} = \frac{\left(\frac{[3866]}{[2666]}\right)_{\text{CaCO}_3}}{\left(\frac{[3866]}{[2666]}\right)_{\text{EIC}}} \cdot \frac{\left(\frac{[3666]}{[2666]}\right)_{\text{EIC}}}{\left(\frac{[3666]}{[2666]}\right)_{\text{CaCO}_3}} \cdot \frac{\left(\frac{[2866]}{[2666]}\right)_{\text{EIC}}}{\left(\frac{[2866]}{[2666]}\right)_{\text{CaCO}_3}} = \frac{\left(\frac{[3866]}{[2666]}\right)_{\text{CaCO}_3}}{\left(\frac{[3866]}{[2666]}\right)_{\text{EIC}}} \cdot {}^{13}\alpha_{\text{EIC-CaCO}_3} \cdot {}^{18}\alpha_{\text{EIC-CaCO}_3}. \quad (95)$$

267 Solving for $([3866])_{\text{CaCO}_3}$ leads to

$$([3866])_{\text{CaCO}_3} = ([2666])_{\text{CaCO}_3} \cdot \frac{[\text{E}3866]}{[\text{E}2666]} \cdot {}^{63}\alpha_{\text{CaCO}_3\text{-EIC}} \cdot {}^{13}\alpha_{\text{CaCO}_3\text{-EIC}} \cdot {}^{18}\alpha_{\text{CaCO}_3\text{-EIC}}. \quad (96)$$

268 Considering this expression in terms of the flux of CaCO_3 , we can write:

$$\frac{d([3866])_{\text{CaCO}_3}}{dt} = F_{\text{CaCO}_3} \cdot \frac{[\text{E}3866]}{[\text{E}2666]} \cdot {}^{63}\alpha_{\text{CaCO}_3\text{-EIC}} \cdot {}^{13}\alpha_{\text{CaCO}_3\text{-EIC}} \cdot {}^{18}\alpha_{\text{CaCO}_3\text{-EIC}}. \quad (97)$$

Finally, the effect of calcite precipitation on the clumped isotope composition of residual EIC is given by

$$\frac{d([3866])_{\text{CaCO}_3}}{dt} = -\frac{d([3866])_{\text{EIC}}}{dt}. \quad (98)$$

269 The clumped isotope composition of calcite relative to EIC can be calculated from the ion-by-ion model of
 270 Watkins and Hunt (2015), which describes the attachment and detachment of HCO_3^- and CO_3^{2-} isotopologues
 271 to and from the calcite crystal (Eqs. 1 and 2). Specifically, Eq. 38 of Watkins and Hunt (2015) gives the
 272 ${}^{63}R/{}^{63}R^*$ of the crystal for a given pH and growth rate, which can then be normalized by the ${}^{63}R/{}^{63}R^*$ of
 273 EIC to get ${}^{63}\alpha_{\text{CaCO}_3\text{-EIC}}$ (Eq. 94).

274 5.3 Governing equations

275 The Tang et al. (2014) experiments are modeled by solving the following system of equations:

$$\frac{d[266]}{dt} = \{\text{rxn terms, Eq. 27}\} + \frac{F_{\text{CO}_2}}{V} \quad (99)$$

$$\frac{d[\text{E}2666]}{dt} = \{\text{rxn terms, Eq. 28}\} - \frac{F_{\text{CaCO}_3}}{V} \quad (100)$$

$$\frac{d[366]}{dt} = \{\text{rxn terms, Eq. 29}\} + \frac{F_{\text{CO}_2} \cdot {}^{13}R_{\text{CO}_2}}{V} \quad (101)$$

$$\frac{d[\text{E}3666]}{dt} = \{\text{rxn terms, Eq. 30}\} - \frac{F_{\text{CaCO}_3}}{V} \cdot \frac{[\text{E}3666]}{[\text{E}2666]} \cdot {}^{13}\alpha_{\text{CaCO}_3\text{-EIC}} \quad (102)$$

$$\frac{d[286]}{dt} = \{\text{rxn terms, Eq. 31}\} + \frac{F_{\text{CO}_2} \cdot {}^{18}R_{\text{CO}_2}}{V} \quad (103)$$

$$\frac{d[\text{E}2866]}{dt} = \{\text{rxn terms, Eq. 32}\} - \frac{F_{\text{CaCO}_3}}{V} \cdot \frac{[\text{E}2866]}{[\text{E}2666]} \cdot {}^{18}\alpha_{\text{CaCO}_3\text{-EIC}} \quad (104)$$

$$\frac{d[386]}{dt} = \{\text{rxn terms, Eq. 73}\} + \frac{F_{\text{CO}_2} \cdot {}^{47}R_{\text{CO}_2}}{V} \quad (105)$$

$$\frac{d[\text{E}3866]}{dt} = \{\text{rxn terms, Eq. 74}\} - \frac{F_{\text{CaCO}_3}}{V} \cdot \frac{[\text{E}3866]}{[\text{E}2666]} \cdot {}^{63}\alpha_{\text{CaCO}_3\text{-EIC}} \cdot {}^{13}\alpha_{\text{CaCO}_3\text{-EIC}} \cdot {}^{18}\alpha_{\text{CaCO}_3\text{-EIC}} \quad (106)$$

$$\frac{d[\text{Ca}^{2+}]}{dt} = -\frac{F_{\text{CaCO}_3}}{V} \quad (107)$$

276 Since the ion-by-ion model describes calcite growth and not dissolution, we initialize the model with enough
 277 DIC so that $\Omega = 1$. Hence, the model effectively begins just prior to Stage II of the experiments. The
 278 CO_2 that is fluxed into solution gets converted to HCO_3^- and CO_3^{2-} according to the (de-)hydration and

279 (de-)hydroxylation reactions and calcite grows at a rate that depends on $[Ca^{2+}]$ and $[CO_3^{2-}]$. At each
280 timestep, the H2666 and 2666 species concentrations are calculated from E2666 and are used as inputs into
281 the ion-by-ion model, which returns the surface area normalized growth rate as well as the pH- and growth
282 rate-dependent $^{13}\alpha_{CaCO_3-EIC}$, $^{18}\alpha_{CaCO_3-EIC}$, and $^{63}\alpha_{CaCO_3-EIC}$ values. The growth rate is multiplied by
283 the reactive surface area (0.1 m^2) to get F_{CaCO_3} and the calculation is repeated.

284 5.4 Model behavior

285 An example showing the behavior of the model run to steady state is shown in Fig. 5. The CO_2 flux
286 is held constant at 0.01 mmol/h (Fig. 5a), which is in the middle of the range of the Tang et al. (2014)
287 experiments. The flux of $CaCO_3$ is initially 0 but it increases monotonically until it exactly balances with
288 the specified flux of CO_2 (Fig. 5b). The specified F_{CO_2} determines the time required to reach steady state
289 as well as the steady state growth rate. The oxygen isotope composition of DIC is initially equilibrated
290 but $1000\ln\alpha$ decreases due to the kinetic fractionations attending the hydration and hydroxylation reactions
291 (Fig. 5c). After about 70 hours, the system reaches steady state composition that is far from equilibrium.
292 The emergent behavior of clumped isotopes is that the DIC species become more ordered due to hydration
293 and hydroxylation, and $CaCO_3$ growth is fast enough to inherit the weighted sum of HCO_3^- and CO_3^{2-} , in
294 accordance with the ion-by-ion model (Watkins and Hunt, 2015).

295 5.5 Model application

296 Outputs from the default version of the model are compared to the Tang et al. (2014) data in Fig. 6a-b.
297 Each point on a model curve represents the *steady state* isotopic composition for the specified pH and F_{CO_2} .
298 For oxygen isotopes (Fig. 6a), the model curves bracket the data below $pH = 9.5$, but lie well above the
299 data at higher pH. For clumped isotopes (Fig. 6a), the model correctly predicts an increase in Δ_{47} with
300 increasing pH and the curves bracket most of the data. The model predicts a kinetic limit of $\Delta_{47} \sim 1.00$,
301 which is somewhat lower than the experimental $\Delta_{47} = 1.06$ at $pH = 10.5$.

302 There are two adjustments to the default model that can better fit the data at high pH. The first is to
303 increase the kinetic fractionation factors for the hydroxylation reaction, as shown in Figs. 6c-d. However,
304 fitting the oxygen isotope data requires changing the KFF assigned to OH^- by almost 30% ($a_{+4}/k_{+4} =$
305 $0.9988 \rightarrow 0.9700$) to generate a KIE that is 10‰ greater because OH^- contributes only 1/3 of the oxygen
306 atoms to the hydroxylation reaction. Note that changing the KFF assigned to CO_2 (b_{+4}/k_{+4}) has little
307 effect on the results because the CO_2 is nearly quantitatively converted to HCO_3^- , and consequently, the
308 corresponding KIE isn't expressed. Fitting the clumped isotope data requires changing the direction of the
309 primary clumped isotope KIE ($^{13-18}KIE_{p+4} = 1-0.016/1000 \rightarrow 1+0.180/1000$), which is also unsatisfying.
310 Changing the secondary KIE ($^{13-18}KIE_{s+4}$) has little effect on the results.

311 The alternative option is to relax the assumption that the CO_2 flux is in oxygen and clumped isotope
312 equilibrium with water at $5\text{ }^\circ\text{C}$ (Fig. 6e-f). If the KFFs in the default model are correct, then fitting the
313 oxygen isotope data requires the $\delta^{18}O$ of CO_2 to be 13.7‰ lower whereas the clumped isotope data requires
314 the Δ_{47} of CO_2 to be 0.11‰ higher. As discussed in §5.2.1, both the sign and magnitude of these changes
315 could plausibly be produced by $CO_{2(g)}$ diffusion through a PE membrane. Such diffusive isotope effects
316 should also fractionate carbon isotopes but this cannot be evaluated because Tang et al. (2014) did not
317 report the $\delta^{13}C$ of DIC in the inner solution.

318 In summary, this modeling exercise supports Tang et al. (2014)'s interpretation that low $\delta^{18}O$ and
319 high Δ_{47} values at high pH are due to a combination of diffusion and reaction KIEs. Reaction KIEs alone
320 cannot explain the data unless previously-published KFFs for hydroxylation are significantly underestimated.
321 Modeling results indicate that diffusion of $CO_{2(g)}$ through a PE membrane can efficiently separate the
322 isotopes of oxygen, and presumably also carbon, which may have implications for KIEs arising from CO_2
323 diffusion through other types of membranes in biomineralization models (Zeebe et al., 1999a; Adkins et al.,
324 2003; Erez, 2003). In principle, the model can be used to inform the design and run conditions of future
325 experiments, particularly if the goal is to avoid KIEs attending a particular step during mineral formation.

6 Summary

Kinetic isotope effects are ubiquitous in inorganic and biogenic carbonates. Open-system, reactive-transport models and/or box models have been useful for understanding the cause(s) of kinetic $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ effects in corals (Adkins et al., 2003; Chen et al., 2018), foraminifers (Zeebe et al., 1999a), speleothems (Hansen et al., 2017; Guo and Zhou, 2019a) and alkaline travertines (Christensen et al., 2021). Kinetic clumped isotope effects hold additional information about the conditions of carbonate formation, but only recently has there been sufficient information regarding clumped isotope KFFs to extend the models to Δ_{47} , Δ_{48} and Δ_{49} .

Two approaches have been taken for describing clumped isotope KIEs in the DIC- H_2O system. The IsoDIC model (Guo, 2020) is the most complete and accurate model because it tracks most of the isotopologues (^2H and ^3H are excluded) in the DIC- H_2O system. The ExClump38 model (Chen et al., 2018; Uchikawa et al., 2021) tracks the most abundant isotopologue at each mass, giving it the advantage of fewer equations that need to be adapted for biomineralization and inorganic calcification models.

We expanded the ExClump38 model to double and triple clumped isotopes and validated it against the IsoDIC model. We then showed how to couple the model to a separate model that describes KIEs in the CaCO_3 -DIC system (Watkins and Hunt, 2015). An example box model describing KIEs in the full CaCO_3 -DIC- H_2O system was applied to the inorganic calcite precipitation experiments of Tang et al. (2014). The overall approach should be useful for adding a quantitative element to interpretations of $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ - Δ_{47} - Δ_{48} - Δ_{49} kinetic effects in many inorganic and biogenic carbonate systems.

Remark For this work, we developed codes in MATLAB for the ExClump38-IsoDIC comparison (Figure 2) as well as the box model (Fig. 6) that we intend to make freely accessible on GitHub and/or EarthChem Library.

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References

- Adkins, J., Boyle, E., Curry, W., Lutringer, A., 2003. Stable isotopes in deep-sea corals and a new mechanism for “vital effects”. *Geochimica et Cosmochimica Acta* 67 (6), 1129–1143.
- Affek, H. P., 2013. Clumped isotopic equilibrium and the rate of isotope exchange between CO₂ and water. *American Journal of Science* 313 (4), 309–325.
- Affek, H. P., Zaarur, S., 2014. Kinetic isotope effect in CO₂ degassing: Insight from clumped and oxygen isotopes in laboratory precipitation experiments. *Geochimica et Cosmochimica Acta* 143, 319–330.
- Bajnai, D., Fiebig, J., Tomašových, A., Garcia, S. M., Rollion-Bard, C., Raddatz, J., Löffler, N., Primo-Ramos, C., Brand, U., 2018. Assessing kinetic fractionation in brachiopod calcite using clumped isotopes. *Scientific Reports* 8 (1), 1–12.
- Bajnai, D., Guo, W., Spötl, C., Coplen, T. B., Methner, K., Löffler, N., Krsnik, E., Gischler, E., Hansen, M., Henkel, D., et al., 2020. Dual clumped isotope thermometry resolves kinetic biases in carbonate formation temperatures. *Nature communications* 11 (1), 1–9.
- Beck, W. C., Grossman, E. L., Morse, J. W., 2005. Experimental studies of oxygen isotope fractionation in the carbonic acid system at 15°, 25°, and 40°C. *Geochimica et Cosmochimica Acta* 69 (14), 3493–3503.
- Bigeleisen, J., Mayer, M. G., 1947. Calculation of equilibrium constants for isotopic exchange reactions. *The Journal of Chemical Physics* 15 (5), 261–267.
- Candelier, Y., Minoletti, F., Probert, I., Hermoso, M., 2013. Temperature dependence of oxygen isotope fractionation in coccolith calcite: A culture and core top calibration of the genus *Calcidiscus*. *Geochimica et cosmochimica acta* 100, 264–281.
- Chen, S., Gagnon, A. C., Adkins, J. F., 2018. Carbonic anhydrase, coral calcification and a new model of stable isotope vital effects. *Geochimica et Cosmochimica Acta* 236, 179–197.
- Christensen, J. N., Watkins, J. M., Devriendt, L. S., DePaolo, D. J., Conrad, M. E., Voltolini, M., Yang, W., Dong, W., 2021. Isotopic fractionation accompanying CO₂ hydroxylation and carbonate precipitation from high pH waters at The Cedars, California, USA. *Geochimica et Cosmochimica Acta* 301, 91–115.
- Clark, I. D., Fontes, J.-C., 1990. Paleoclimatic reconstruction in northern Oman based on carbonates from hyperalkaline groundwaters. *Quaternary Research* 33 (3), 320–336.
- Clark, I. D., Fontes, J.-C., Fritz, P., 1992. Stable isotope disequilibria in travertine from high pH waters: laboratory investigations and field observations from Oman. *Geochimica et Cosmochimica Acta* 56 (5), 2041–2050.
- Dennis, K. J., Schrag, D. P., 2010. Clumped isotope thermometry of carbonatites as an indicator of diagenetic alteration. *Geochimica et Cosmochimica Acta* 74 (14), 4110–4122.
- DePaolo, D. J., 2011. Surface kinetic model for isotopic and trace element fractionation during precipitation of calcite from aqueous solutions. *Geochimica et Cosmochimica Acta* 75 (4), 1039–1056.
- Devriendt, L. S., Watkins, J. M., McGregor, H. V., 2017. Oxygen isotope fractionation in the CaCO₃-DIC-H₂O system. *Geochimica et Cosmochimica Acta* 214, 115–142.
- Dietzel, M., Tang, J., Leis, A., Köhler, S. J., 2009. Oxygen isotopic fractionation during inorganic calcite precipitation – Effects of temperature, precipitation rate and pH. *Chemical Geology* 268 (1), 107–115.
- Eiler, J. M., 2007. ‘Clumped-isotope’ geochemistry – The study of naturally-occurring, multiply-substituted isotopologues. *Earth and Planetary Science Letters* 262 (3), 309–327.
- Eiler, J. M., Schauble, E., 2004. ¹⁸O¹³C¹⁶O in Earth’s atmosphere. *Geochimica et Cosmochimica Acta* 68 (23), 4767–4777.
- Erez, J., 2003. The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. *Reviews in mineralogy and geochemistry* 54 (1), 115–149.
- Falk, E., Guo, W., Paukert, A., Matter, J., Mervine, E., Kelemen, P., 2016. Controls on the stable isotope compositions of travertine from hyperalkaline springs in Oman: Insights from clumped isotope measurements. *Geochimica et Cosmochimica Acta* 192, 1–28.
- Fiebig, J., Bajnai, D., Löffler, N., Methner, K., Krsnik, E., Mulch, A., Hofmann, S., 2019. Combined high-precision δ₄₈ and δ₄₇ analysis of carbonates. *Chemical Geology* 522, 186–191.
- Fiebig, J., Daëron, M., Bernecker, M., Guo, W., Schneider, G., Boch, R., Bernasconi, S. M., Jautzy, J.,

- 408 Dietzel, M., 2021. Calibration of the dual clumped isotope thermometer for carbonates. *Geochimica et*
409 *Cosmochimica Acta*, <https://doi.org/10.1016/j.gca.2021.07.012>.
- 410 Gabitov, R. I., Watson, E. B., Sadekov, A., 2012. Oxygen isotope fractionation between calcite and fluid as
411 a function of growth rate and temperature: An *in situ* study. *Chemical Geology* 306, 92–102.
- 412 Ghosh, P., Adkins, J., Affek, H., Balta, B., Guo, W., Schauble, E. A., Schrag, D., Eiler, J. M., 2006. ^{13}C – ^{18}O
413 bonds in carbonate minerals: A new kind of paleothermometer. *Geochimica et Cosmochimica Acta* 70 (6),
414 1439–1456.
- 415 Guo, W., 2008. Carbonate clumped isotope thermometry: application to carbonaceous chondrites & effects
416 of kinetic isotope fractionation. California Institute of Technology, doi:10.7907/BWC2-RH54.
- 417 Guo, W., 2020. Kinetic clumped isotope fractionation in the DIC-H₂O-CO₂ system: patterns, controls, and
418 implications. *Geochimica et Cosmochimica Acta* 268, 230–257.
- 419 Guo, W., Mosenfelder, J. L., Goddard III, W. A., Eiler, J. M., 2009. Isotopic fractionations associated with
420 phosphoric acid digestion of carbonate minerals: Insights from first-principles theoretical modeling and
421 clumped isotope measurements. *Geochimica et Cosmochimica Acta* 73 (24), 7203–7225.
- 422 Guo, W., Zhou, C., 2019a. Patterns and controls of disequilibrium isotope effects in speleothems: insights
423 from an isotope-enabled diffusion-reaction model and implications for quantitative thermometry. *Geochim-*
424 *ica et Cosmochimica Acta* 267, 196–226.
- 425 Guo, W., Zhou, C., 2019b. Triple oxygen isotope fractionation in the DIC-H₂O-CO₂ system: A numerical
426 framework and its implications. *Geochimica et Cosmochimica Acta* 246, 541–564.
- 427 Hansen, M., Scholz, D., Froeschmann, M.-L., Schöne, B. R., Spötl, C., 2017. Carbon isotope exchange
428 between gaseous CO₂ and thin solution films: Artificial cave experiments and a complete diffusion-reaction
429 model. *Geochimica et Cosmochimica Acta* 211, 28–47.
- 430 Hill, P. S., Schauble, E. A., Tripathi, A., 2020. Theoretical constraints on the effects of added cations on
431 clumped, oxygen, and carbon isotope signatures of dissolved inorganic carbon species and minerals.
432 *Geochimica et Cosmochimica Acta* 269, 496–539.
- 433 Hill, P. S., Tripathi, A. K., Schauble, E. A., 2014. Theoretical constraints on the effects of ph, salinity,
434 and temperature on clumped isotope signatures of dissolved inorganic carbon species and precipitating
435 carbonate minerals. *Geochimica et cosmochimica acta* 125, 610–652.
- 436 Kele, S., Breitenbach, S. F., Capezzuoli, E., Meckler, A. N., Ziegler, M., Millan, I. M., Kluge, T., Deák,
437 J., Hanselmann, K., John, C. M., et al., 2015. Temperature dependence of oxygen-and clumped isotope
438 fractionation in carbonates: a study of travertines and tufas in the 6–95 °C temperature range. *Geochimica*
439 *et Cosmochimica Acta* 168, 172–192.
- 440 Kelson, J. R., Huntington, K. W., Schauer, A. J., Saenger, C., Lechler, A. R., 2017. Toward a univer-
441 sal carbonate clumped isotope calibration: Diverse synthesis and preparatory methods suggest a single
442 temperature relationship. *Geochimica et Cosmochimica Acta* 197, 104–131.
- 443 Kim, S.-T., O’Neil, J. R., 1997. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbon-
444 ates. *Geochimica et Cosmochimica Acta* 61 (16), 3461–3475.
- 445 Kluge, T., John, C. M., Jourdan, A.-L., Davis, S., Crawshaw, J., 2015. Laboratory calibration of the calcium
446 carbonate clumped isotope thermometer in the 25–250 °C temperature range. *Geochimica et Cosmochimica*
447 *Acta* 157, 213–227.
- 448 Leleu, T., Chavagnac, V., Delacour, A., Noiriél, C., Ceuleneer, G., Aretz, M., Rommevaux, C., Ventalon,
449 S., 2016. Travertines associated with hyperalkaline springs: evaluation as a proxy for paleoenvironmental
450 conditions and sequestration of atmospheric CO₂. *Journal of Sedimentary Research* 86 (11), 1328–1343.
- 451 Marchitto, T., Curry, W., Lynch-Stieglitz, J., Bryan, S., Cobb, K., Lund, D., 2014. Improved oxygen isotope
452 temperature calibrations for cosmopolitan benthic foraminifera. *Geochimica et Cosmochimica Acta* 130,
453 1–11.
- 454 McCrea, J. M., 1950. On the isotopic chemistry of carbonates and a paleotemperature scale. *The Journal of*
455 *Chemical Physics* 18 (6), 849–857.
- 456 Mervine, E. M., Humphris, S. E., Sims, K. W., Kelemen, P. B., Jenkins, W. J., 2014. Carbonation rates of
457 peridotite in the Samail Ophiolite, Sultanate of Oman, constrained through ^{14}C dating and stable isotopes.
458 *Geochimica et Cosmochimica Acta* 126, 371–397.

- 459 O'Neil, J. R., Barnes, I., 1971. C¹³ and O¹⁸ compositions in some fresh-water carbonates associated with
460 ultramafic rocks and serpentinites: western United States. *Geochimica et Cosmochimica Acta* 35 (7),
461 687–697.
- 462 O'Neil, J. R., Clayton, R. N., Mayeda, T. K., 1969. Oxygen isotope fractionation in divalent metal carbonates.
463 *The Journal of Chemical Physics* 51 (12), 5547–5558.
- 464 Parker, W. G., Yanes, Y., Surge, D., Mesa-Hernández, E., 2017. Calibration of the oxygen isotope ratios
465 of the gastropods *Patella candei crenata* and *Phorcus atratus* as high-resolution paleothermometers from
466 the subtropical eastern Atlantic Ocean. *Palaeogeography, Palaeoclimatology, Palaeoecology* 487, 251–259.
- 467 Sade, Z., Halevy, I., 2018. Corrigendum to “New constraints on kinetic isotope effects during CO₂(aq)
468 hydration and hydroxylation: Revisiting theoretical and experimental data” [*Geochim. Cosmochim. Acta*
469 214 (2017) 246–265]. *Geochimica et Cosmochimica Acta* 225, 237–240.
- 470 Staudigel, P. T., Swart, P. K., 2018. A kinetic difference between ¹²C- and ¹³C-bound oxygen exchange
471 rates results in decoupled δ¹⁸O and Δ₄₇ values of equilibrating DIC solutions. *Geochemistry, Geophysics,*
472 *Geosystems* 19 (8), 2371–2383.
- 473 Tang, J., Dietzel, M., Fernandez, A., Tripathi, A. K., Rosenheim, B. E., 2014. Evaluation of kinetic effects on
474 clumped isotope fractionation (Δ₄₇) during inorganic calcite precipitation. *Geochimica et Cosmochimica*
475 *Acta* 134, 120–136.
- 476 Thiagarajan, N., Adkins, J., Eiler, J., 2011. Carbonate clumped isotope thermometry of deep-sea corals and
477 implications for vital effects. *Geochimica et Cosmochimica Acta* 75 (16), 4416–4425.
- 478 Tripathi, A. K., Hill, P. S., Eagle, R. A., Mosenfelder, J. L., Tang, J., Schauble, E. A., Eiler, J. M., Zeebe,
479 R. E., Uchikawa, J., Coplen, T. B., et al., 2015. Beyond temperature: Clumped isotope signatures in dis-
480 solved inorganic carbon species and the influence of solution chemistry on carbonate mineral composition.
481 *Geochimica et Cosmochimica Acta* 166, 344–371.
- 482 Uchikawa, J., Chen, S., Eiler, J. M., Adkins, J. F., Zeebe, R. E., 2021. Trajectory and timescale of oxygen and
483 clumped isotope equilibration in the dissolved carbonate system under normal and enzymatically-catalyzed
484 conditions at 25 °C. *Geochimica et Cosmochimica Acta*, <https://doi.org/10.1016/j.gca.2021.08.014>.
- 485 Uchikawa, J., Zeebe, R. E., 2012. The effect of carbonic anhydrase on the kinetics and equilibrium of the
486 oxygen isotope exchange in the CO₂–H₂O system: Implications for δ¹⁸O vital effects in biogenic carbonates.
487 *Geochimica et Cosmochimica Acta* 95, 15–34.
- 488 Urey, H. C., 1947. The thermodynamic properties of isotopic substances. *J. Chem. Soc.*, 562–581.
- 489 Wang, Z., Schauble, E. A., Eiler, J. M., 2004. Equilibrium thermodynamics of multiply substituted isotop-
490 ologues of molecular gases. *Geochimica et Cosmochimica Acta* 68 (23), 4779–4797.
- 491 Watkins, J., Hunt, J., 2015. A process-based model for non-equilibrium clumped isotope effects in carbonates.
492 *Earth and Planetary Science Letters* 432, 152–165.
- 493 Watkins, J. M., Hunt, J. D., Ryerson, F. J., DePaolo, D. J., 2014. The influence of temperature, pH, and
494 growth rate on the δ¹⁸O composition of inorganically precipitated calcite. *Earth and Planetary Science*
495 *Letters* 404, 332–343.
- 496 Watkins, J. M., Nielsen, L. C., Ryerson, F. J., DePaolo, D. J., 2013. The influence of kinetics on the oxygen
497 isotope composition of calcium carbonate. *Earth and Planetary Science Letters* 375, 349–360.
- 498 Wolthers, M., Nehrke, G., Gustafsson, J. P., Van Cappellen, P., 2012. Calcite growth kinetics: Modeling the
499 effect of solution stoichiometry. *Geochimica et Cosmochimica Acta* 77, 121–134.
- 500 Yumol, L. M., Uchikawa, J., Zeebe, R. E., 2020. Kinetic isotope effects during CO₂ hydration: Experimental
501 results for carbon and oxygen fractionation. *Geochimica et Cosmochimica Acta* 279, 189–203.
- 502 Zaarur, S., Affek, H. P., Brandon, M. T., 2013. A revised calibration of the clumped isotope thermometer.
503 *Earth and Planetary Science Letters* 382, 47–57.
- 504 Zeebe, R. E., 2014. Kinetic fractionation of carbon and oxygen isotopes during hydration of carbon dioxide.
505 *Geochimica et Cosmochimica Acta* 139, 540–552.
- 506 Zeebe, R. E., 2020. Oxygen isotope fractionation between water and the aqueous hydroxide ion. *Geochimica*
507 *et Cosmochimica Acta* 289, 182–195.
- 508 Zeebe, R. E., Bijma, J., Wolf-Gladrow, D. A., 1999a. A diffusion-reaction model of carbon isotope fraction-
509 ation in foraminifera. *Marine Chemistry* 64 (3), 199–227.

- 510 Zeebe, R. E., Wolf-Gladrow, D., Jansen, H., 1999b. On the time required to establish chemical and isotopic
511 equilibrium in the carbon dioxide system in seawater. *Marine Chemistry* 65 (3), 135–153.
- 512 Zeebe, R. E., Wolf-Gladrow, D. A., 2001. *CO₂ in seawater: equilibrium, kinetics, isotopes*. Vol. 65. Gulf
513 Professional Publishing.
- 514 Zhang, J., Quay, P., Wilbur, D., 1995. Carbon isotope fractionation during gas-water exchange and dissolu-
515 tion of CO₂. *Geochimica et Cosmochimica Acta* 59 (1), 107–114.

Number ID	Isotopologue	Mass (ignoring H)	Permutations	Abundance
<i>Part I: CO₂</i>				
1	¹² C ¹⁶ O ¹⁶ O	44	1	98.40%
2	¹³ C ¹⁶ O ¹⁶ O	45	1	1.11%
3	¹² C ¹⁷ O ¹⁶ O	45	2	748 ppm
4	¹² C ¹⁸ O ¹⁶ O	46	2	0.40%
5	¹³ C ¹⁷ O ¹⁶ O	46	2	8.4 ppm
6	¹² C ¹⁷ O ¹⁷ O	46	1	0.142 ppm
7	¹³ C ¹⁸ O ¹⁶ O	47	2	44.4 ppm
8	¹² C ¹⁷ O ¹⁸ O	47	2	1.50 ppm
9	¹³ C ¹⁷ O ¹⁷ O	47	1	1.60 ppb
10	¹² C ¹⁸ O ¹⁸ O	48	1	3.96 ppm
11	¹³ C ¹⁷ O ¹⁸ O	48	2	16.8 ppb
12	¹³ C ¹⁸ O ¹⁸ O	49	1	44.5 ppb
				100.0%
<i>Part II: HCO₃⁻</i>				
1	H¹²C¹⁶O¹⁶O¹⁶O	60	1	98.20%
2	H ¹³ C ¹⁶ O ¹⁶ O ¹⁶ O	61	1	1.10%
3	H ¹² C ¹⁷ O ¹⁶ O ¹⁶ O	61	3	0.11%
4	H¹²C¹⁸O¹⁶O¹⁶O	62	3	0.60%
5	H ¹³ C ¹⁷ O ¹⁶ O ¹⁶ O	62	3	12 ppm
6	H ¹² C ¹⁷ O ¹⁷ O ¹⁶ O	62	3	405 ppb
7	H¹³C¹⁸O¹⁶O¹⁶O	63	3	67 ppm
8	H ¹² C ¹⁷ O ¹⁸ O ¹⁶ O	63	6	4.4 ppm
9	H ¹³ C ¹⁷ O ¹⁷ O ¹⁶ O	63	3	4.54 ppb
10	H ¹² C ¹⁷ O ¹⁷ O ¹⁷ O	63	1	50 ppt
11	H ¹² C ¹⁸ O ¹⁸ O ¹⁶ O	64	3	12 ppm
12	H ¹³ C ¹⁷ O ¹⁸ O ¹⁶ O	64	6	50 ppb
13	H ¹² C ¹⁷ O ¹⁷ O ¹⁸ O	64	3	828 ppt
14	H ¹³ C ¹⁷ O ¹⁷ O ¹⁷ O	64	1	0.5 ppt
15	H ¹³ C ¹⁸ O ¹⁸ O ¹⁶ O	65	3	138 ppb
16	H ¹² C ¹⁷ O ¹⁸ O ¹⁸ O	65	3	4.5 ppb
17	H ¹³ C ¹⁷ O ¹⁷ O ¹⁸ O	65	3	9 ppt
18	H ¹² C ¹⁸ O ¹⁸ O ¹⁸ O	66	1	8 ppb
19	H ¹³ C ¹⁷ O ¹⁸ O ¹⁸ O	66	3	51 ppt
20	H ¹³ C ¹⁸ O ¹⁸ O ¹⁸ O	67	1	94 ppt
				100.0%

Table 1: List of CO₂ and CO₃²⁻ isotopologues tracked in the IsoDIC model (Guo, 2020). The subset of isotopologues in bold are tracked in the ExClump38 model (Uchikawa et al., 2021). Abundances are from Ghosh et al. (2006).

Table 2: Parameters for conventional isotope ratios.

Symbol	Expression (or value at 25 °C)	Reference/Note
<i>Part I: Chemical parameters</i>		
K_1	$\frac{[H^{2666}][H]}{[266][H_26]}, [H_26] = 1$	$f(T, S)$
K_2	$\frac{[2666][H]}{[H^{2666}]}$	$f(T, S)$
K_w	$\frac{[6H][H]}{[H^+]}$	$f(T, S)$
k_{+1}	$\ln k_{+1} = 1246.98 - \frac{61900}{T_K} - 183.0 \ln T_K$	Uchikawa and Zeebe (2012)
k_{-1}	$k_{-1} = k_{+1} / K_1$	-
k_{+4}	$\ln k_{+4} = 17.67 - \frac{2790.47}{T_K}$	Uchikawa and Zeebe (2012)
k_{-4}	$k_{-4} = k_{+4} \frac{K_w}{K_1}$	-
χ	$\chi = \frac{1}{1 + \frac{K_2}{[H^+]}}$	-
<i>Part II: Carbon isotope parameters</i>		
$^{13}\alpha_{CO_2-HCO_3^-}^{eq}$	$-9.866 T^{-1} + 1.02412$	Zhang et al. (1995)
$^{13}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}$	$-0.867 T^{-1} + 1.00252$	Zhang et al. (1995)
$^{13}\alpha_{c+1}^{KFF}$	0.9872	Yumol et al. (2020)
$^{13}\alpha_{c+4}^{KFF}$	0.9814	Christensen et al. (2021)
c_{+1}	$c_{+1} = ^{13}\alpha_{c+1}^{KFF} \cdot k_{+1}$	-
c_{-1}	$c_{-1} = c_{+1} / (K_1 \cdot ^{13}\alpha_{HCO_3^-CO_2}^{eq})$	-
c_{+4}	$c_{+4} = ^{13}\alpha_{c+4}^{KFF} \cdot k_{+4}$	-
c_{-4}	$c_{-4} = c_{+4} / \left(\frac{K_1}{K_w} \cdot ^{13}\alpha_{HCO_3^-CO_2}^{eq} \right)$	-
$^{13}\chi$	$^{13}\chi = \frac{1}{1 + \frac{K_2 \cdot ^{13}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}}{[H^+]}}$	-
<i>Part III: Oxygen isotope parameters</i>		
$^{18}\alpha_{CO_2-H_2O}^{eq}$	$\exp(2520 T_K^{-2} + 0.01212)$	Beck et al. (2005)
$^{18}\alpha_{HCO_3^-H_2O}^{eq}$	$\exp(2590 T_K^{-2} + 0.00189)$	Beck et al. (2005)
$^{18}\alpha_{CO_3^{2-}-H_2O}^{eq}$	$\exp(2390 T_K^{-2} - 0.00270)$	Beck et al. (2005)
$^{18}\alpha_{OH^-H_2O}^{eq}$	$5.6676 \times 10^{-5} T_K + 0.9622$	based on Zeebe (2020)
$^{18}\alpha_{a+1}^{KFF}$	1.0000	Yumol et al. (2020)
$^{18}\alpha_{b+1}^{KFF}$	0.9812	Yumol et al. (2020)
$^{18}\alpha_{a+4}^{KFF}$	0.9988	Christensen et al. (2021)
$^{18}\alpha_{b+4}^{KFF}$	1.0000	Christensen et al. (2021)
a_{+1}, b_{+1}	$a_{+1} = ^{18}\alpha_{a+1}^{KFF} \cdot k_{+1}$ $b_{+1} = ^{18}\alpha_{b+1}^{KFF} \cdot k_{+1}$	-
a_{-1}, b_{-1}	$a_{-1} = a_{+1} / (K_1 \cdot ^{18}\alpha_{HCO_3^-H_2O}^{eq})$ $b_{-1} = b_{+1} / (K_1 \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq})$	-
a_{+4}, b_{+4}	$a_{+4} = ^{18}\alpha_{a+4}^{KFF} \cdot k_{+4}$ $b_{+4} = ^{18}\alpha_{b+4}^{KFF} \cdot k_{+4}$	-
a_{-4}, b_{-4}	$a_{-4} = a_{+4} / \left(\frac{K_1}{K_w} \frac{^{18}\alpha_{HCO_3^-H_2O}^{eq}}{^{18}\alpha_{OH^-H_2O}^{eq}} \right)$ $b_{-4} = b_{+4} / \left(\frac{K_1}{K_w} ^{18}\alpha_{HCO_3^-CO_2}^{eq} \right)$	-
$^{18}\chi$	$^{18}\chi = \frac{1}{1 + \frac{K_2 \cdot ^{18}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}}{[H^+]}}$	-

Table 3: Parameters for single-clumped isotopes

Symbol	Value	Reference/Note
<i>Part IV: Single-clumped isotope parameters</i>		
Δ_{47,CO_2}^{eq}	$26447/T_K^2 + 285.51/T_K - 0.3004$	Wang et al. (2004)
$\Delta_{63,HCO_3^-}^{eq}$	$43655/T_K^2 - 23.643/T_K - 0.0088$	Hill et al. (2020)
$\Delta_{63,CO_3^{2-}}^{eq}$	$43187/T_K^2 + 34.833/T_K + 0.0007$	Hill et al. (2020)
$(^{47}R/^{47}R^*)_{CO_2}^{eq}$	$\Delta_{47,CO_2}^{eq}/1000 + 1$	
$(^{63}R/^{63}R^*)_{HCO_3^-}^{eq}$	$\Delta_{63,HCO_3^-}^{eq}/1000 + 1$	
$(^{63}R/^{63}R^*)_{CO_3^{2-}}^{eq}$	$\Delta_{63,CO_3^{2-}}^{eq}/1000 + 1$	
$^{63}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}$	$(^{63}R/^{63}R^*)_{CO_3^{2-}}^{eq} / (^{63}R/^{63}R^*)_{HCO_3^-}^{eq}$	-
$^{63}K_2$	$^{63}\alpha_{CO_3^{2-}-HCO_3^-}^{eq} \cdot ^{13}\alpha_{CO_3^{2-}-HCO_3^-}^{eq} \cdot ^{18}\alpha_{CO_3^{2-}-HCO_3^-}^{eq} \cdot K_2$	-
$^{63}\chi$	$\frac{1}{1 + \frac{^{63}K_2 \cdot ^{63}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}}{[H^+]}}$	-
$^{13-18}KIE_{p+1}$	1-0.146/1000	Guo (2020)
$^{13-18}KIE_{s+1}$	1-0.219/1000	Guo (2020)
$^{13-18}KIE_{p+4}$	1-0.016/1000	Guo (2020)
$^{13-18}KIE_{s+4}$	1-0.175/1000	Guo (2020)
p_{+1}, s_{+1}	$p_{+1} = (^{13-18}KIE_{p+1} \cdot c_{+1} \cdot a_{+1})/k_{+1}$ $s_{+1} = (^{13-18}KIE_{s+1} \cdot c_{+1} \cdot b_{+1})/k_{+1}$	Uchikawa et al. (2021) Uchikawa et al. (2021)
p_{-1}, s_{-1}	$p_{-1} = p_{+1} / \left[\left(^{63}R/^{63}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 \cdot ^{13}\alpha_{HCO_3^- - H_2O}^{eq} \cdot ^{18}\alpha_{HCO_3^- - H_2O}^{eq} \right]$ $s_{-1} = s_{+1} \cdot \left[\left(^{47}R/^{47}R^* \right)_{CO_2}^{eq} \right] / \left[\left(^{63}R/^{63}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 \cdot ^{13}\alpha_{HCO_3^- - CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^- - H_2O}^{eq} \right]$	-
p_{+4}, s_{+4}	$p_{+4} = (^{13-18}KIE_{p+4} \cdot c_{+4} \cdot a_{+4})/k_{+4}$ $s_{+4} = (^{13-18}KIE_{s+4} \cdot c_{+4} \cdot b_{+4})/k_{+4}$	Uchikawa et al. (2021) Uchikawa et al. (2021)
p_{-4}, s_{-4}	$p_{-4} = p_{+4} / \left[\left(^{63}R/^{63}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 / K_w \cdot ^{13}\alpha_{HCO_3^- - CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^- - OH}^{eq} \right]$ $s_{-4} = s_{+4} \cdot \left[\left(^{47}R/^{47}R^* \right)_{CO_2}^{eq} \right] / \left[\left(^{63}R/^{63}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 / K_w \cdot ^{13}\alpha_{HCO_3^- - CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^- - CO_2}^{eq} \right]$	-

Table 4: Parameters for double-clumped isotopes

Symbol	Value	Reference/Note
<i>Part V: Double-clumped isotope parameters</i>		
Δ_{48,CO_2}^{eq}	$29306/T_K^2 + 93.885/T_K - 0.2914$	Wang et al. (2004)
$\Delta_{64,HCO_3^-}^{eq}$	$21842/T_K^2 - 50.457/T_K + 0.0291$	Hill et al. (2020)
$\Delta_{64,CO_3^{2-}}^{eq}$	$23492/T_K^2 - 52.842/T_K + 0.0304$	Hill et al. (2020)
$(^{48}R/^{48}R^*)_{CO_2}^{eq}$	$\Delta_{48,CO_2}^{eq}/1000 + 1$	-
$(^{64}R/^{64}R^*)_{HCO_3^-}^{eq}$	$\Delta_{64,HCO_3^-}^{eq}/1000 + 1$	-
$(^{64}R/^{64}R^*)_{CO_3^{2-}}^{eq}$	$\Delta_{64,CO_3^{2-}}^{eq}/1000 + 1$	-
$^{64}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}$	$(^{64}R/^{64}R^*)_{CO_3^{2-}}^{eq} / ((^{64}R/^{64}R^*)_{HCO_3^-}^{eq})$	-
$^{64}K_2$	$^{64}\alpha_{CO_3^{2-}-HCO_3^-}^{eq} \cdot ^{18}\alpha_{CO_3^{2-}-HCO_3^-}^{eq} \cdot ^{18}\alpha_{CO_3^{2-}-HCO_3^-}^{eq} \cdot K_2$	-
$^{64}\chi$	$\frac{1}{1 + \frac{^{64}K_2 \cdot ^{64}\alpha_{CO_3^{2-}-HCO_3^-}^{eq}}{[H^+]}}$	-
$^{18-18}KIE_{p'_{+1}}$	$1 + 0.049/1000$	Guo (2020)
$^{18-18}KIE_{s'_{+1}}$	$1 - 0.146/1000$	Guo (2020)
$^{18-18}KIE_{p'_{+4}}$	$1 - 0.144/1000$	Guo (2020)
$^{18-18}KIE_{s'_{+4}}$	$1 - 0.086/1000$	Guo (2020)
p'_{+1}, s'_{+1}	$p'_{+1} = (^{18-18}KIE_{p'_{+1}} \cdot b_{+1} \cdot a_{+1}) / k_{+1}$ $s'_{+1} = (^{18-18}KIE_{s'_{+1}} \cdot b_{+1} \cdot b_{+1}) / k_{+1}$	-
p'_{-1}, s'_{-1}	$p'_{-1} = p'_{+1} / \left[\left(^{64}R/^{64}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^-H_2O}^{eq} \right]$ $s'_{-1} = s'_{+1} \cdot \left[\left(^{48}R/^{48}R^* \right)_{CO_2}^{eq} \right] / \left[\left(^{64}R/^{64}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq} \right]$	-
p'_{+4}, s'_{+4}	$p'_{+4} = (^{18-18}KIE_{p'_{+4}} \cdot b_{+4} \cdot a_{+4}) / k_{+4}$ $s'_{+4} = (^{18-18}KIE_{s'_{+4}} \cdot b_{+4} \cdot b_{+4}) / k_{+4}$	-
p'_{-4}, s'_{-4}	$p'_{-4} = p'_{+4} / \left[\left(^{64}R/^{64}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 / K_w \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^-OH}^{eq} \right]$ $s'_{-4} = s'_{+4} \cdot \left[\left(^{48}R/^{48}R^* \right)_{CO_2}^{eq} \right] / \left[\left(^{64}R/^{64}R^* \right)_{HCO_3^-}^{eq} \cdot K_1 / K_w \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq} \cdot ^{18}\alpha_{HCO_3^-CO_2}^{eq} \right]$	-

Table 5: Parameters for triple-clumped isotopes

Symbol	Value	Reference/Note
<i>Part VI: Triple-clumped isotope parameters</i>		
$\Delta_{49, \text{CO}_2}^{\text{eq}}$	$108776/T_K^2 + 477.14/T_K - 0.5954$	Wang et al. (2004)
$\Delta_{65, \text{HCO}_3^-}^{\text{eq}}$	$112026/T_K^2 - 97.208/T_K + 0.009$	Hill et al. (2020)
$\Delta_{65, \text{CO}_3^{2-}}^{\text{eq}}$	$112667/T_K^2 - 123.11/T_K + 0.0304$	Hill et al. (2020)
$(^{49}R/^{49}R^*)_{\text{CO}_2}^{\text{eq}}$	$\Delta_{49, \text{CO}_2}^{\text{eq}}/1000 + 1$	-
$(^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}}$	$\Delta_{65, \text{HCO}_3^-}^{\text{eq}}/1000 + 1$	-
$(^{65}R/^{65}R^*)_{\text{CO}_3^{2-}}^{\text{eq}}$	$\Delta_{65, \text{CO}_3^{2-}}^{\text{eq}}/1000 + 1$	-
$^{65}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}$	$(^{65}R/^{65}R^*)_{\text{CO}_3^{2-}}^{\text{eq}} / ((^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}})$	-
$^{65}K_2$	$^{65}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-} \cdot ^{13}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-} \cdot ^{18}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-} \cdot ^{18}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-} \cdot K_2$	-
$^{65}\chi$	$\frac{1}{1 + \frac{^{65}K_2 \cdot ^{65}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}}{[\text{H}^+]}}$	-
$^{13-18-18}\text{KIE}_{p'+1}$	1-0.317/1000	Guo (2020)
$^{13-18-18}\text{KIE}_{s'+1}$	1-0.573/1000	Guo (2020)
$^{13-18-18}\text{KIE}_{p'+4}$	1-0.282/1000	Guo (2020)
$^{13-18-18}\text{KIE}_{s'+4}$	1-0.402/1000	Guo (2020)
p''_{+1}, s''_{+1}	$p''_{+1} = (^{13-18-18}\text{KIE}_{p'+1} \cdot c_{+1} \cdot b_{+1} \cdot a_{+1})/k_{+1}^2$ $s''_{+1} = (^{13-18-18}\text{KIE}_{s'+1} \cdot c_{+1} \cdot b_{+1} \cdot b_{+1})/k_{+1}^2$	-
p''_{-1}, s''_{-1}	$p''_{-1} = p''_{+1} \cdot \left[\frac{(^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}}}{(^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}} \right]$ $s''_{-1} = s''_{+1} \cdot \left[\frac{(^{49}R/^{49}R^*)_{\text{CO}_2}^{\text{eq}}}{(^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2} \right]$	-
p''_{+4}, s''_{+4}	$p''_{+4} = (^{13-18-18}\text{KIE}_{p'+4} \cdot c_{+4} \cdot b_{+4} \cdot a_{+4})/k_{+4}^2$ $s''_{+4} = (^{13-18-18}\text{KIE}_{s'+4} \cdot c_{+4} \cdot b_{+4} \cdot b_{+4})/k_{+4}^2$	-
p''_{-4}, s''_{-4}	$p''_{-4} = p''_{+4} \cdot \left[\frac{(^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}}}{(^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}}} \cdot K_1/K_w \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{OH}^-} \right]$ $s''_{-4} = s''_{+4} \cdot \left[\frac{(^{49}R/^{49}R^*)_{\text{CO}_2}^{\text{eq}}}{(^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}}} \cdot K_1/K_w \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2} \right]$	-

Exp	T (C)	pHout	t1 (hrs)	t2 (hrs)	ttot (hrs)	M (mmol)	[Ca]ini (mM)	[DIC] (mM)	FCO2 (mmol/h)	FCaCO3 (mmol/hr)	SA (m2)	log10R (mol/m2/s)	1000 ln a	D47
1	5	9	194	647	841	5.8	10.1	0.24	0.006	0.009	0.059	-7.38	26.75	0.733
2	5	9	186	402	588	5.2	9.9	0.24	0.006	0.013	0.073	-7.31	28.23	0.735
3	5	9	162	223	385	9.6	10.5	0.33	0.010	0.043	0.188	-7.20	29.49	0.748
4	5	10	119	309	428	6.9	9.9	0.05	0.002	0.022	0.112	-7.26	14.09	0.962
5	5	8.5	438	336	774	3.7	10.2	0.53	0.006	0.011	0.056	-7.27	31.51	0.787
6	5	10.5	48	187	235	12.4	92.5	0.04	0.004	0.066	0.230	-7.10	13.07	1.065
7	5	8.3	72	26	98	6.6	10	2.15	0.149	0.254	0.164	-6.37	31.22	0.765
9	5	8.3	127	65	192	4.8	10.3	1.6	0.063	0.074	0.107	-6.72	31.78	0.753
10	5	8.3	61	51	112	9.3	9.9	1.95	0.160	0.182	0.209	-6.62	30.99	0.752

Table 6: Experimental data from Dietzel et al. (2009) and Tang et al. (2014) used to constrain the input parameters (T , pH, $[Ca^{2+}]$, and SA) and adjustable parameters (F_{CO_2}) used in the model.

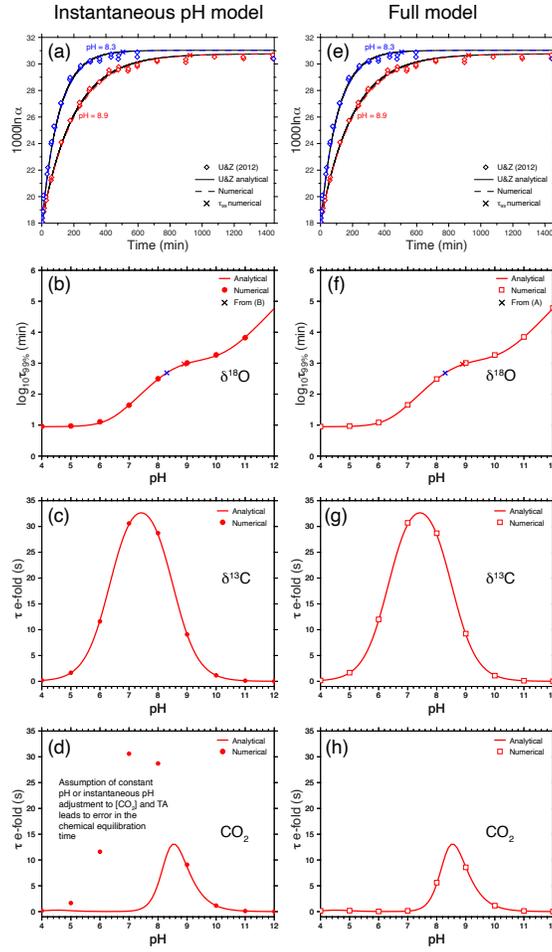


Figure 1: Validation of the ExClump38 model for carbon and oxygen isotopes. In these simulations, the DIC pool is initially equilibrated and then the CO_2 or HCO_3^- is perturbed and the system is allowed to adjust back to equilibrium. The analytical solutions come from Uchikawa and Zeebe (2012) for $\delta^{18}\text{O}$ equilibration, Zeebe and Wolf-Gladrow (2001) for $\delta^{13}\text{C}$ equilibration, and Zeebe et al. (1999b) for chemical equilibration.

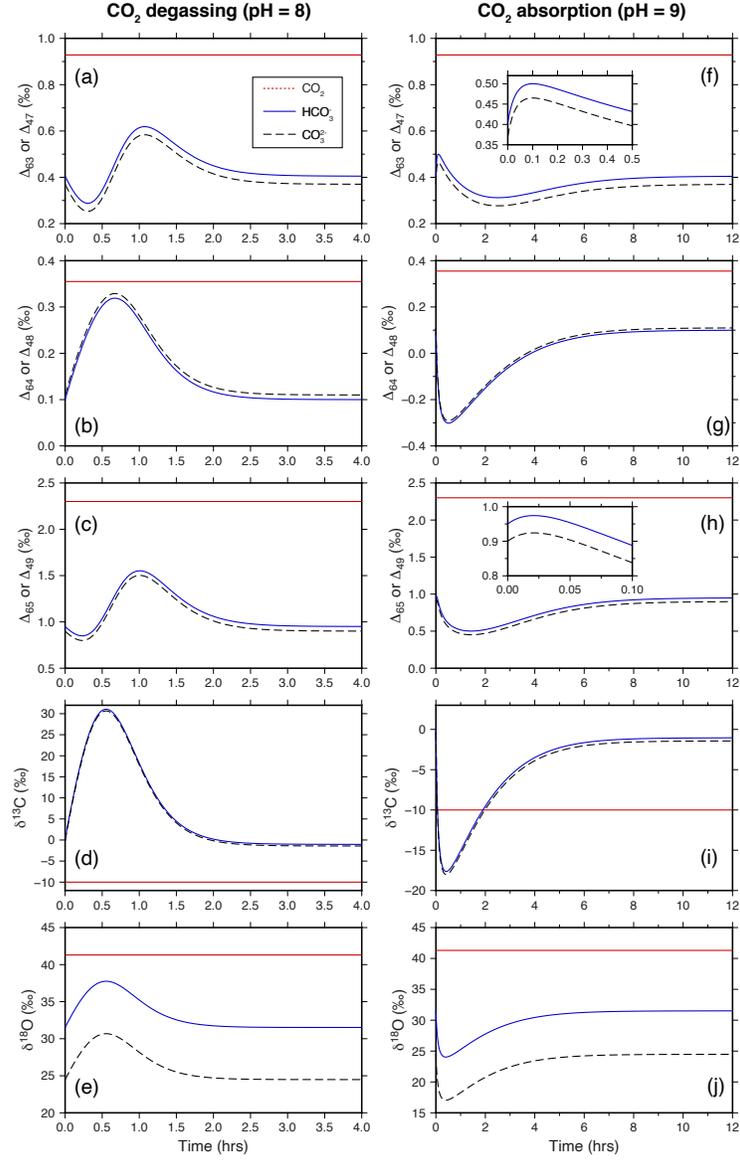


Figure 2: Validation of the ExClump38 model for clumped isotopes. In these simulations, the DIC pool is initially equilibrated and then the CO_2 is perturbed and held constant and the system is allowed to adjust to the new equilibrium. The curves are indistinguishable from those in Fig. 3 of Guo (2020) and were produced using the same rate constants and isotopic fractionation factors for carbon and oxygen isotopes as Guo and Zhou (2019b) and Guo (2020), which differ from those compiled in Table 2.

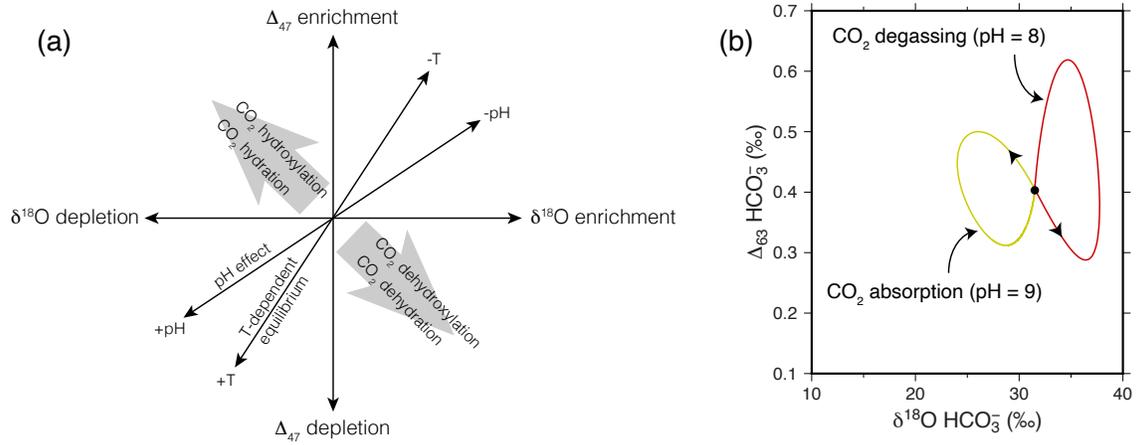
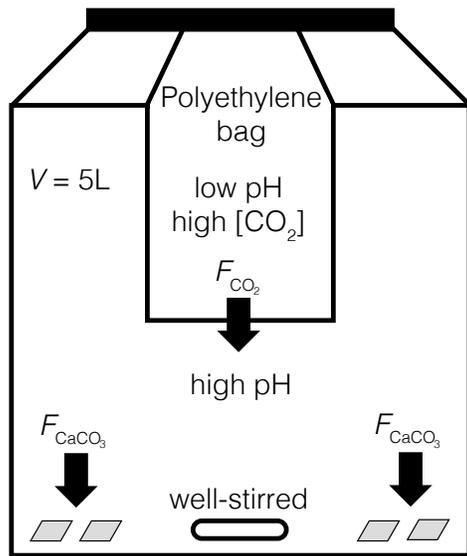


Figure 3: The effects on (de-)hydration and (de-)hydroxylation on Δ_{47} and $\delta^{18}\text{O}$. (a) Vectors based on calculations by Guo (2008) showing the expected direction of departure from equilibrium (modified from Tripathi et al., 2015). (b) Results from the simulations in Fig. 2 showing the direction and magnitude of departure from equilibrium. The initial kinetic effects (i.e., early times in the simulation) are shown by the black arrows. These agree with those depicted qualitatively in (a), but a central question is to what extent the arrows in (a) are generalizable.

(a) Tang et al. (2014) experiments

$T = 5\text{ }^\circ\text{C}$
pH = constant
 $[\text{Ca}^{2+}]_{\text{ini}} = 10\text{ mM}$
 $F_{\text{CO}_2} \sim \text{constant?}$



(b) Isotopic box model

$T = 5\text{ }^\circ\text{C}$
pH = constant
 $[\text{Ca}^{2+}]_{\text{ini}} = 10\text{ mM}$
 $F_{\text{CO}_2} = \text{constant}$

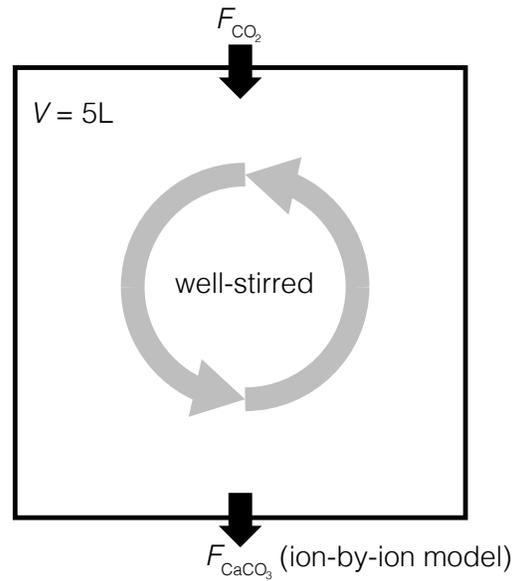


Figure 4: (a) Experimental setup used by Dietzel et al. (2009) and Tang et al. (2014). (b) Setup for the isotopic box model.

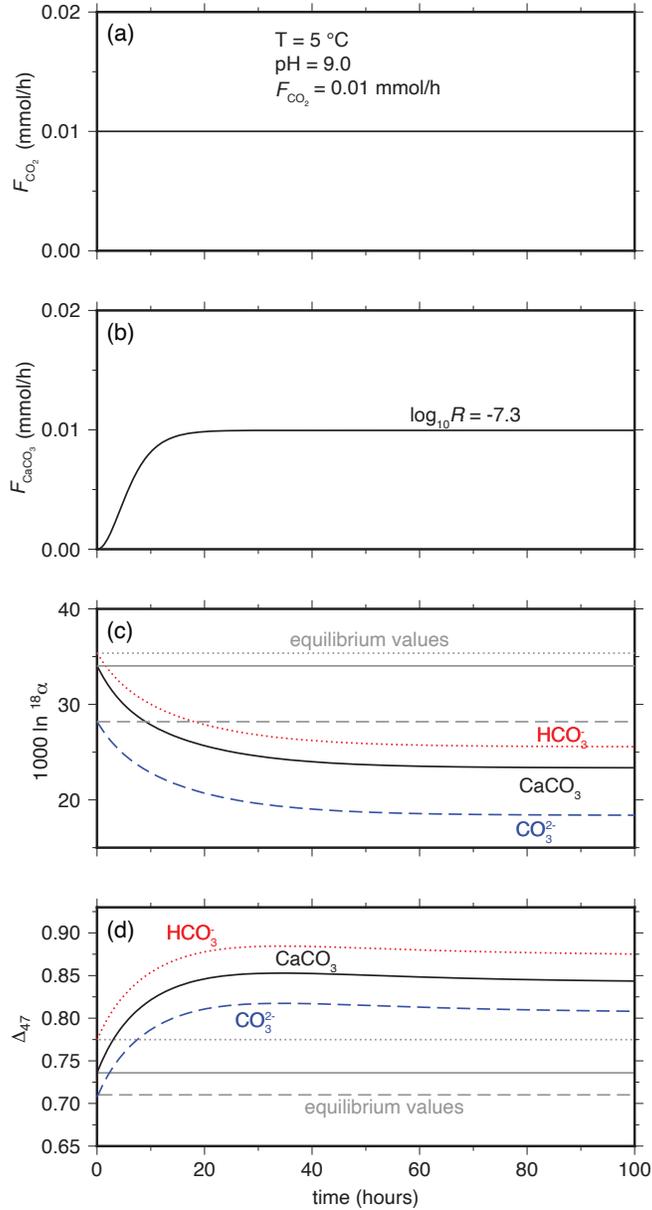


Figure 5: A box model simulation run to steady state. (a) F_{CO_2} is held constant throughout. The $\text{CO}_{2(\text{aq})}$ gets converted to HCO_3^- and CO_3^{2-} , which increases the degree of supersaturation with respect to calcite. (b) F_{CaCO_3} at each timestep is calculated from the ion-by-ion model based on the degree of supersaturation. Initially, $\Omega = 1$ and it increases monotonically until $F_{\text{CaCO}_3} = F_{\text{CO}_2}$. (c) The oxygen isotope composition of HCO_3^- and CO_3^{2-} are initially equilibrated but become isotopically lighter due to hydration and hydroxylation reactions. CaCO_3 inherits the light composition of HCO_3^- and CO_3^{2-} plus an additional fractionation attending the crystal growth reaction in accordance with the ion-by-ion model. (d) The clumped isotope composition of HCO_3^- and CO_3^{2-} increases with time and is also inherited by CaCO_3 plus a small additional effect attending the crystal growth reaction in accordance with the ion-by-ion model.

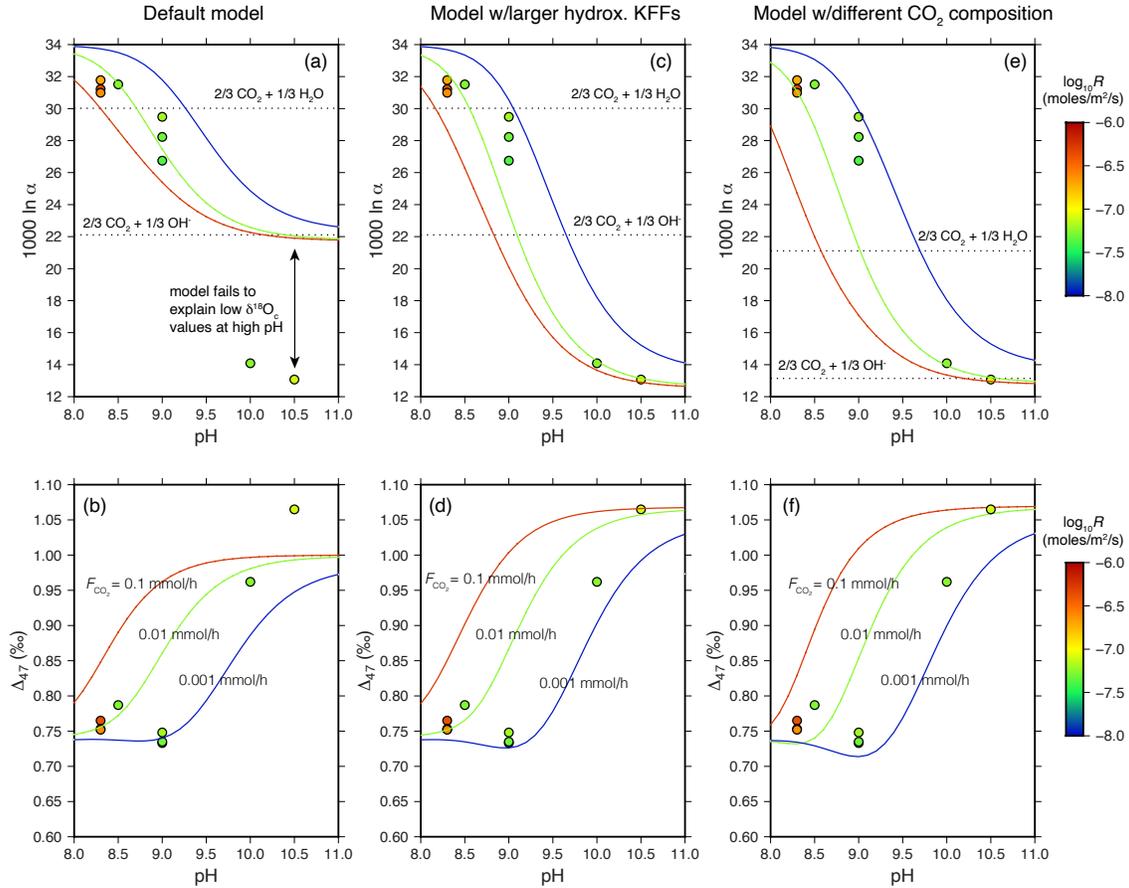


Figure 6: Steady state solutions to the box model for different pH and F_{CO_2} . The data points and curves are color-coded according to the steady state growth rate. In the default model (a-b), the KFFs are treated as “known” (Table 2) and the CO_2 flux is assumed to be in oxygen and clumped isotope equilibrium with water at 5 °C. (c-d) Same as the default model but with larger oxygen isotope KFFs on the hydroxylation reaction. (e-f) Same as the default model but with the CO_2 flux having lower $\delta^{18}\text{O}$ (by 13.7‰) and higher Δ_{47} (by 0.11‰). In the latter scenario, the non-equilibrium CO_2 could be due to insufficient dwell time prior to the onset of an experiment, or more likely by diffusion through the PE membrane.

Supplemental Material

S.1 Justification for omitting certain terms

Chen et al. (2018) compartmentalized the chemical reactions from the isotopic reactions. That is, their equation for $d[266]/dt$ did not include contributions from the isotopic reactions (i.e., the terms in red):

$$\begin{aligned} \frac{d[266]}{dt} = & -k_{+1}[266] + k_{-1}[\text{EIC}]\chi[\text{H}] \\ & -k_{+4}[266][6\text{H}] + k_{-4}[\text{EIC}]\chi \\ & -a_{+1}[266]r_w + \frac{1}{3}a_{-1}[\text{E2866}]^{18}\chi[\text{H}] \\ & -a_{+4}[266][8\text{H}] + \frac{1}{3}a_{-4}[\text{E2866}]^{18}\chi \end{aligned} \quad (\text{S.1})$$

Although these terms are small, they may not be negligible in all cases. Consider the forward CO_2 hydration reaction and the special case where there is no kinetic isotope fractionation (i.e., $a_{+1}/k_{+1} = 1$). In this scenario, the isotopic composition of the flux should exactly match the isotopic composition of reactant CO_2 . That is, $d([286]/[266])/dt$ should equal 0. We can check this by using the pure forward (hydration-only) expressions:

$$\frac{d\left(\frac{[286]}{[266]}\right)}{dt} = \frac{\frac{d[286]}{dt} \cdot [266] - \frac{d[266]}{dt} \cdot [286]}{[266][266]} = \frac{-a_{+1}[286][266] + k_{+1}[266][286]}{[266][266]} = 0, \quad (\text{S.2})$$

which is true. If we were to include the additional contributions from E2866 in $d[266]/dt$ we would have the following:

$$\frac{d\left(\frac{[286]}{[266]}\right)}{dt} = \frac{-a_{+1}[286][266] + k_{+1}[266][286] + \overbrace{a_{+1}[266]r_w[286]}^{\text{extra flux}}}{[266][266]} \neq 0, \quad (\text{S.3})$$

which is problematic. Deleting the terms in red is one way to resolve this problem, but that justification is unsatisfying. As recognized by Sade and Halevy (2018), when one considers the subset of isotopologues 266, 286, H_28 , and H_26 , failure to include the reaction involving both 286 and H_28 can lead to errors that show up in cases of unidirectional reaction. In other words, an argument could be made for including the following reaction involving heavy CO_2 and heavy H_2O :



where p' is one of the rate constants for double clumped isotopes. By including this reaction, the expression would read

$$\frac{d\left(\frac{[286]}{[266]}\right)}{dt} = \frac{-a_{+1}[286][266] - p'_{+1}[286][\text{H}_28][266] + k_{+1}[266][286] + a_{+1}[266][\text{H}_28][286]}{[266][266]} = 0, \quad (\text{S.5})$$

which is true when the forward rate constants are all equal (i.e., no kinetic fractionation).

This presents two options. The first is to include two additional reactions:



$$286 + 8\text{H} \xrightleftharpoons[2/3p'_{-4}]{p'_{+4}} \text{H2886}. \quad (\text{S.7})$$

and explicitly track the double clumped isotopologue and include their contributions to chemical and oxygen isotope equilibration. The second option is to do as was done previously (Chen et al., 2018; Christensen et al., 2021) and treat chemical equilibration independent of isotopic equilibration. Both options recover the correct behavior in the kinetic limit of purely unidirectional reactions. However, the latter option is attractive when it comes to including kinetic effects from CaCO_3 precipitation because the ion-by-ion model or a suitable alternative has yet to be developed for double and triple clumped isotopes.

S.2 Derivation of rate constants for single clumped isotopes

For the primary hydration reaction we have

$$^{13-18}\text{KIE}_{p_{+1}} = \frac{^{13-18}\alpha_{p_{+1}}^{\text{KFF}}}{^{18}\alpha_{a_{+1}}^{\text{KFF}} \cdot ^{13}\alpha_{c_{+1}}^{\text{KFF}}} = \frac{\frac{p_{+1}}{k_{+1}}}{\frac{c_{+1}}{k_{+1}} \frac{a_{+1}}{k_{+1}}}, \quad (\text{S.8})$$

from which we get an expression for p_{+1} :

$$p_{+1} = \frac{^{13-18}\text{KIE}_{p_{+1}} c_{+1} a_{+1}}{k_{+1}} \quad (\text{S.9})$$

To obtain p_{-1} we use the equilibrium constraint from the corresponding hydration reaction:

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3}[\text{H3866}][\text{H}]}{[\text{366}][\text{H}_2\text{8}]}. \quad (\text{S.10})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3}[\text{H3866}][\text{H}]}{[\text{366}][\text{H}_2\text{8}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{1}{\text{H}_2\text{6}}}{\frac{1}{\text{H}_2\text{6}}}, \quad (\text{S.11})$$

which upon rearrangement yields

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{\frac{1}{3}[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]}}{\frac{[\text{366}]}{[\text{266}]} \cdot \frac{[\text{H2666}]}{[\text{H3666}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}, \quad (\text{S.12})$$

Rearranging again yields

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{\frac{1}{3}[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{H3666}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{366}]}{[\text{266}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}, \quad (\text{S.13})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p_{+1}}{p_{-1}} = \frac{1}{3} \cdot \left(\frac{^{63}R}{^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot \frac{^{13}r_{\text{HCO}_3^-}}{^{13}r_{\text{CO}_2}} \cdot \frac{3 \cdot ^{18}r_{\text{HCO}_3^-}}{r_w}. \quad (\text{S.14})$$

The factors of 3 cancel and we end up with

$$\frac{p_{+1}}{p_{-1}} = \left(\frac{^{63}R}{^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}. \quad (\text{S.15})$$

For the secondary clumped isotope hydration reaction, we have

$$^{13-18}\text{KIE}_{s+1} = \frac{^{13-18}\alpha_{s+1}^{\text{KFF}}}{^{18}\alpha_{b+1}^{\text{KFF}} \cdot ^{13}\alpha_{c+1}^{\text{KFF}}} = \frac{\frac{s+1}{k_{+1}}}{\frac{c+1}{k_{+1}} \frac{b+1}{k_{+1}}}, \quad (\text{S.16})$$

from which we get an expression for s_{+1} :

$$s_{+1} = \frac{^{13-18}\text{KIE}_{s+1} c_{+1} b_{+1}}{k_{+1}}. \quad (\text{S.17})$$

To obtain s_{-1} we use the equilibrium constraint:

$$\frac{s_{+1}}{s_{-1}} = \frac{\frac{2}{3}[\text{H3866}][\text{H}]}{[\text{386}][\text{H}_2\text{6}]}. \quad (\text{S.18})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{s_{+1}}{s_{-1}} = \frac{\frac{2}{3}[\text{H3866}][\text{H}]}{[\text{386}][\text{H}_2\text{6}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{266}]}{[\text{366}][\text{286}]}}{\frac{[\text{266}]}{[\text{366}][\text{286}]}}}, \quad (\text{S.19})$$

which upon rearrangement yields

$$\frac{s_{+1}}{s_{-1}} = \frac{\frac{2}{3}[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H}_2\text{6}]} \cdot \frac{[\text{266}]}{[\text{366}][\text{286}]} \cdot \frac{[\text{386}][\text{266}]}{[\text{366}][\text{286}]} \cdot \frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{266}]}. \quad (\text{S.20})$$

Rearranging again yields

$$\frac{s_{+1}}{s_{-1}} = \frac{\frac{2}{3}[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H}_2\text{6}]} \cdot \frac{[\text{H3666}][\text{H2866}]}{[\text{H2666}][\text{H2666}]} \cdot \frac{[\text{386}][\text{266}]}{[\text{366}][\text{286}]} \cdot \frac{[\text{366}][\text{286}]}{[\text{266}][\text{266}]}. \quad (\text{S.21})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{s_{+1}}{s_{-1}} = \frac{\frac{2}{3} \cdot \left(\frac{^{63}\text{R}}{^{63}\text{R}^*}\right)^{\text{eq}}_{\text{HCO}_3^-} \cdot K_1 \cdot ^{13}r_{\text{HCO}_3^-} \cdot 3 \cdot ^{18}r_{\text{HCO}_3^-}}{\left(\frac{^{47}\text{R}}{^{47}\text{R}^*}\right)^{\text{eq}}_{\text{CO}_2} \cdot ^{13}r_{\text{CO}_2} \cdot 2 \cdot ^{18}r_{\text{CO}_2}}. \quad (\text{S.22})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{s_{+1}}{s_{-1}} = \frac{\left(\frac{^{63}\text{R}}{^{63}\text{R}^*}\right)^{\text{eq}}_{\text{HCO}_3^-} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}}{\left(\frac{^{47}\text{R}}{^{47}\text{R}^*}\right)^{\text{eq}}_{\text{CO}_2}}. \quad (\text{S.23})$$

For the primary clumped isotope hydroxylation reaction, we have

$$^{13-18}\text{KIE}_{p+4} = \frac{^{13-18}\alpha_{p+4}^{\text{KFF}}}{^{18}\alpha_{a+4}^{\text{KFF}} \cdot ^{13}\alpha_{c+4}^{\text{KFF}}} = \frac{\frac{p+4}{k_{+4}}}{\frac{c+4}{k_{+4}} \frac{a+4}{k_{+4}}}, \quad (\text{S.24})$$

from which we get an expression for p_{+4} :

$$p_{+4} = \frac{^{13-18}\text{KIE}_{p+4} c_{+4} a_{+4}}{k_{+4}} \quad (\text{S.25})$$

To obtain p_{-4} we use the equilibrium constraint:

$$\frac{p_{+4}}{p_{-4}} = \frac{\frac{1}{3}[\text{H3866}]}{[\text{366}][\text{8H}]} \quad (\text{S.26})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{p_{+4}}{p_{-4}} = \frac{\frac{1}{3}[\text{H3866}]}{[\text{366}][\text{8H}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{H}]}{[\text{6H}]}}{\frac{[\text{H}]}{[\text{6H}]}} \quad (\text{S.27})$$

which upon rearrangement yields

$$\frac{p_{+4}}{p_{-4}} = \frac{\frac{1}{3}[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}]} \cdot \frac{1}{[\text{H}][\text{6H}]} \quad (\text{S.28})$$

Rearranging again yields

$$\frac{p_{+4}}{p_{-4}} = \frac{\frac{1}{3}[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}]} \cdot \frac{1}{[\text{H}][\text{6H}]} \cdot \frac{[\text{H3666}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]} \quad (\text{S.29})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p_{+4}}{p_{-4}} = \frac{\frac{1}{3} \cdot \left(\frac{{}^{63}\text{R}}{{}^{63}\text{R}^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot \frac{1}{K_w} \cdot {}^{13}\text{r}_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}\text{r}_{\text{HCO}_3^-}}{{}^{13}\text{r}_{\text{CO}_2} \cdot r_{\text{OH}^-}} \quad (\text{S.30})$$

The factors of 3 cancel and we end up with

$$\frac{p_{+4}}{p_{-4}} = \left(\frac{{}^{63}\text{R}}{{}^{63}\text{R}^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{OH}^-}^{\text{eq}} \quad (\text{S.31})$$

For the secondary clumped isotope hydroxylation reaction, we have

$${}^{13-18}\text{KIE}_{s_{+4}} = \frac{{}^{13-18}\alpha_{s_{+4}}^{\text{KFF}}}{{}^{18}\alpha_{s_{+4}}^{\text{KFF}} \cdot {}^{13}\alpha_{c_{+4}}^{\text{KFF}}} = \frac{\frac{s_{+4}}{k_{+4}}}{\frac{c_{+4}}{k_{+4}} \cdot \frac{b_{+4}}{k_{+4}}}, \quad (\text{S.32})$$

from which we get an expression for s_{+4} :

$$s_{+4} = \frac{{}^{13-18}\text{KIE}_{s_{+4}} c_{+4} b_{+4}}{k_{+4}} \quad (\text{S.33})$$

To obtain s_{-4} we use the equilibrium constraint:

$$\frac{s_{+4}}{s_{-4}} = \frac{\frac{2}{3}[\text{H3866}]}{[\text{386}][\text{6H}]} \quad (\text{S.34})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{s_{+4}}{s_{-4}} = \frac{\frac{2}{3}[\text{H3866}]}{[\text{386}][\text{6H}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3666}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{266}][\text{266}]}{[\text{366}][\text{286}]}}{\frac{[\text{266}][\text{266}]}{[\text{366}][\text{286}]}} \quad (\text{S.35})$$

which upon rearrangement yields

$$\frac{s_{+4}}{s_{-4}} = \frac{\frac{2}{3} \frac{[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{266}]}{[\text{366}]} \cdot \frac{[\text{266}]}{[\text{286}]} \cdot \frac{[\text{H2666}]}{[\text{266}][\text{6H}]}}{\frac{[\text{386}][\text{266}]}{[\text{366}][\text{286}]} \cdot \frac{[\text{H2666}]}{[\text{H3666}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]}} \quad (\text{S.36})$$

Rearranging again yields

$$\frac{s_{+4}}{s_{-4}} = \frac{\frac{2}{3} \frac{[\text{H3866}][\text{H2666}]}{[\text{H3666}][\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{266}][\text{6H}]} \cdot \frac{[\text{H3666}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{386}][\text{266}]}{[\text{366}][\text{286}]} \cdot \frac{[\text{366}]}{[\text{266}]} \cdot \frac{[\text{286}]}{[\text{266}]}} \quad (\text{S.37})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{s_{+4}}{s_{-4}} = \frac{\frac{2}{3} \cdot \left(\frac{{}^{63}\text{R}}{{}^{63}\text{R}^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{13}\text{r}_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}\text{r}_{\text{HCO}_3^-}}{\left(\frac{{}^{47}\text{R}}{{}^{47}\text{R}^*} \right)_{\text{CO}_2}^{\text{eq}} \cdot {}^{13}\text{r}_{\text{CO}_2} \cdot 2 \cdot {}^{18}\text{r}_{\text{CO}_2}} \quad (\text{S.38})$$

The factors of 2 and 3 cancel and we end up with

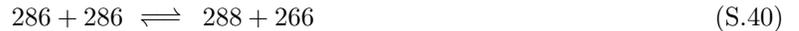
$$\frac{s_{+4}}{s_{-4}} = \frac{\left(\frac{{}^{63}\text{R}}{{}^{63}\text{R}^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}}{\left(\frac{{}^{47}\text{R}}{{}^{47}\text{R}^*} \right)_{\text{CO}_2}^{\text{eq}}} \quad (\text{S.39})$$

535 These expressions for the rate constants are nearly identical to those of Uchikawa et al. (2021). The
 536 only difference is that they wrote ${}^{47}K_{\text{CO}_2}^{\text{eq}}$ and ${}^{63}K_{\text{HCO}_3^-}^{\text{eq}}$ instead of $({}^{47}\text{R}/{}^{47}\text{R}^*)_{\text{CO}_2}^{\text{eq}}$ and $({}^{63}\text{R}/{}^{63}\text{R}^*)_{\text{HCO}_3^-}^{\text{eq}}$,
 537 respectively. This works for single clumped isotopes because the quantities are equivalent, but breaks down
 538 for double and triple clumped isotopes. A compilation of the parameters needed to solve Eqs. 73-74 is
 539 provided in Table 3.

540 S.3 Adding double-clumped isotopes to the model

541 S.3.1 Homogeneous reactions and clumped isotope definitions

Double-clumped isotope systems involve the internal equilibrium of ${}^{18}\text{O}$ - ${}^{18}\text{O}$ bonding within a single species. For CO_2 , we can write the following isotope exchange reaction:



which has an equilibrium constant

$${}^{48}K_{\text{CO}_2} = \frac{[\text{288}][\text{266}]}{[\text{286}][\text{286}]} \quad (\text{S.41})$$

The abundance of 288 is measured as:

$$\Delta_{48} = \left(\frac{{}^{48}\text{R}}{{}^{48}\text{R}^*} - 1 \right) \times 1000, \quad (\text{S.42})$$

where

$${}^{48}\text{R} = \frac{[\text{288}]}{[\text{266}]} \quad (\text{S.43})$$

The asterisk in Eq. S.42 indicates the stochastic distribution, which can be calculated from the standard carbon and oxygen isotope ratios. Unlike the case for single clumped isotopes, the ${}^{48}K_{\text{CO}_2}$ and Δ_{48} are not equivalent:

$${}^{48}K_{\text{CO}_2} = \frac{[\text{288}]}{[\text{266}]} \frac{[\text{266}]}{[\text{286}]} \frac{[\text{266}]}{[\text{286}]} = {}^{48}\text{R} \cdot (2 \cdot {}^{18}\text{r}_{\text{CO}_2})^{-1} \cdot (2 \cdot {}^{18}\text{r}_{\text{CO}_2})^{-1} = \frac{1}{4} \left(\frac{{}^{48}\text{R}}{{}^{48}\text{R}^*} \right)_{\text{CO}_2}^{\text{eq}}, \quad (\text{S.44})$$

where we have used the relationship from §2.1 (Eq. 17) that, for a stochastic distribution,

$$\frac{[288]}{[266]} = {}^{48}R^* = {}^{18}r_{\text{CO}_2}^2. \quad (\text{S.45})$$

542 The factor of 1/4 will ultimately get cancelled in the derivation of the backward rate constants.
Combining Eq. S.42 with Eq. S.44 leads to

$${}^{48}K_{\text{CO}_2} = \frac{[288][266]}{[286][286]} = \frac{1}{4} \left(\frac{{}^{48}R}{{}^{48}R^*} \right)_{\text{CO}_2}^{\text{eq}} = \frac{1}{4} \left(\frac{\Delta_{48,\text{CO}_2}^{\text{eq}}}{1000} + 1 \right) \quad (\text{S.46})$$

Similar expressions can be written for clumped isotope equilibrium in HCO_3^- and CO_3^{2-} :



and



which have equilibrium constants

$${}^{64}K_{\text{HCO}_3^-} = \frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} = \frac{1}{3} \left(\frac{{}^{64}R}{{}^{64}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} = \frac{1}{3} \left(\frac{\Delta_{64,\text{HCO}_3^-}^{\text{eq}}}{1000} + 1 \right) \quad (\text{S.49})$$

and

$${}^{64}K_{\text{CO}_3^{2-}} = \frac{[2886][2666]}{[2866][2866]} = \frac{1}{3} \left(\frac{{}^{64}R}{{}^{64}R^*} \right)_{\text{CO}_3^{2-}}^{\text{eq}} = \frac{1}{3} \left(\frac{\Delta_{64,\text{CO}_3^{2-}}^{\text{eq}}}{1000} + 1 \right). \quad (\text{S.50})$$

543 S.3.2 Heterogeneous reactions involving double-clumped isotopologues

544 To add double-clumped isotopes to the model, we need to include the reactions involving ${}^{18}\text{O}$ - ${}^{18}\text{O}$ ‘clumps’
545 in CO_2 and HCO_3^- :



Note that for double-clumped reactions, the primary reactions have a factor of 2/3 on the back reaction whereas for singly-clumped reactions, the primary reactions had a factor of 1/3. From these reactions we obtain the following ODEs:

$$\begin{aligned} \frac{d[288]}{dt} = & -s'_{+1}[288] + \frac{1}{3}s'_{-1}[\text{E2886}]{}^{64}\chi[\text{H}] \\ & -s'_{+4}[288][6\text{H}] + \frac{1}{3}s'_{-4}[\text{E2886}]{}^{64}\chi \end{aligned} \quad (\text{S.55})$$

$$\begin{aligned}
\frac{d[\text{E2886}]}{dt} &= p'_{+1}[\text{286}]r_w - \frac{2}{3}p'_{-1}[\text{E2886}]^{64}\chi[\text{H}] \\
&\quad + p'_{+4}[\text{286}][\text{8H}] - \frac{2}{3}p'_{-4}[\text{E2886}]^{64}\chi \\
&\quad + s'_{+1}[\text{288}] - \frac{1}{3}s'_{-1}[\text{E2886}]^{64}\chi[\text{H}] \\
&\quad + s'_{+4}[\text{288}][\text{6H}] - \frac{1}{3}s'_{-4}[\text{E2886}]^{64}\chi
\end{aligned} \tag{S.56}$$

546 S.3.3 Derivation of $^{64}\chi$

The $^{64}\chi$ term takes the same form as the other χ terms because the fraction of clumped EIC that is in the form of HCO_3^- has the same pH dependence as for standard isotope ratios (Hill et al., 2014):

$$^{64}\chi = \frac{1}{1 + \frac{^{64}K_2}{[\text{H}^+]}} \tag{S.57}$$

where $^{64}K_2$ is the equilibrium constant for the deprotonation reaction:

$$^{64}K_2 = \frac{[\text{2886}][\text{H}]}{[\text{H2886}]} \tag{S.58}$$

This can be expressed in terms of the equilibrium Δ_{64} values of HCO_3^- and CO_3^{2-} . First, multiply the top and bottom by a common factor:

$$^{64}K_2 = \frac{[\text{2886}][\text{H}]}{[\text{H2886}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}} \cdot \frac{\frac{[\text{2666}][\text{2866}]}{[\text{2866}][\text{2866}]}}{\frac{[\text{2666}][\text{2866}]}{[\text{2866}][\text{2866}]}} \cdot \frac{[\text{2666}]}{[\text{2666}]} \cdot \frac{[\text{H2666}]}{[\text{H2666}]}, \tag{S.59}$$

which upon rearrangement yields

$$^{64}K_2 = \frac{\frac{[\text{2886}][\text{2666}]}{[\text{2866}][\text{2866}]}}{\frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]}}{\frac{[\text{2666}]}{[\text{2866}]} \cdot \frac{[\text{2666}]}{[\text{2866}]}} \cdot \frac{[\text{2666}][\text{H}]}{[\text{H2666}]} \tag{S.60}$$

Converting isotopologue ratios to isotope ratios leads to

$$^{64}K_2 = ^{64}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot K_2, \tag{S.61}$$

where

$$^{64}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} = \frac{\left(1 + \frac{\Delta_{64,\text{CO}_3^{2-}}^{\text{eq}}}{1000}\right)}{\left(1 + \frac{\Delta_{64,\text{HCO}_3^-}^{\text{eq}}}{1000}\right)} \tag{S.62}$$

547 S.3.4 Rate constants

For the primary double-clumped isotope hydration reaction, we have

$$^{18-18}\text{KIE}_{p'_{+1}} = \frac{^{18-18}\alpha_{p'_{+1}}^{\text{KFF}}}{^{18}\alpha_{a+1}^{\text{KFF}} \cdot ^{18}\alpha_{b+1}^{\text{KFF}}} = \frac{\frac{p'_{+1}}{k_{+1}}}{\frac{b_{+1}}{k_{+1}} \cdot \frac{a_{+1}}{k_{+1}}} \tag{S.63}$$

Rearranging leads to

$$p'_{+1} = \frac{^{18-18}\text{KIE}_{p'_{+1}} b_{+1} a_{+1}}{k_{+1}} \tag{S.64}$$

To obtain p'_{-1} we use the equilibrium constraint from the corresponding hydration reaction:

$$\frac{p'_{+1}}{p'_{-1}} = \frac{\frac{2}{3}[\text{H2886}][\text{H}]}{[\text{286}][\text{H}_2\text{8}]}. \quad (\text{S.65})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{p'_{+1}}{p'_{-1}} = \frac{\frac{2}{3}[\text{H2886}][\text{H}]}{[\text{286}][\text{H}_2\text{8}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{1}{\text{H}_2\text{6}}}{\frac{1}{\text{H}_2\text{6}}}, \quad (\text{S.66})$$

which upon rearrangement yields

$$\frac{p'_{+1}}{p'_{-1}} = \frac{\frac{\frac{2}{3}[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]}}{\frac{[\text{286}]}{[\text{266}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}, \quad (\text{S.67})$$

Rearranging again yields

$$\frac{p'_{+1}}{p'_{-1}} = \frac{\frac{\frac{2}{3}[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{286}]}{[\text{266}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}, \quad (\text{S.68})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p'_{+1}}{p'_{-1}} \frac{\frac{2}{3} \cdot \left(\frac{1}{3} \frac{{}^{64}\text{R}}{{}^{64}\text{R}^*}\right)_{\text{HCO}_3^-}^{\text{eq}}}{\frac{2}{3} \cdot \left(\frac{1}{3} \frac{{}^{64}\text{R}}{{}^{64}\text{R}^*}\right)_{\text{HCO}_3^-}^{\text{eq}}} = \frac{K_1 \cdot 3 \cdot {}^{18}\text{r}_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}\text{r}_{\text{HCO}_3^-}}{2 \cdot {}^{18}\text{r}_{\text{CO}_2}}. \quad (\text{S.69})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{p'_{+1}}{p'_{-1}} = \left(\frac{{}^{64}\text{R}}{{}^{64}\text{R}^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}. \quad (\text{S.70})$$

For the secondary double-clumped isotope hydration reaction, we have

$${}^{18-18}\text{KIE}_{s'_{+1}} = \frac{{}^{18-18}\alpha_{s'_{+1}}^{\text{KFF}}}{{}^{18}\alpha_{b_{+1}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+1}}^{\text{KFF}}} = \frac{\frac{s'_{+1}}{k_{+1}}}{\frac{b_{+1}}{k_{+1}} \cdot \frac{b_{+1}}{k_{+1}}}, \quad (\text{S.71})$$

from which we get an expression for s_{+1} :

$$s'_{+1} = \frac{{}^{18-18}\text{KIE}_{s'_{+1}} b_{+1} b_{+1}}{k_{+1}} \quad (\text{S.72})$$

To obtain s'_{-1} we use the equilibrium constraint:

$$\frac{s'_{+1}}{s'_{-1}} = \frac{\frac{1}{3}[\text{H2886}][\text{H}]}{[\text{288}][\text{H}_2\text{6}]}. \quad (\text{S.73})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{s'_{+1}}{s'_{-1}} = \frac{\frac{1}{3}[\text{H2886}][\text{H}]}{[\text{288}][\text{H}_2\text{6}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{266}]}{[\text{286}][\text{286}]}}{\frac{[\text{266}]}{[\text{286}][\text{286}]}}}, \quad (\text{S.74})$$

which upon rearrangement yields

$$\frac{s'_{+1}}{s'_{-1}} = \frac{\frac{1}{3} \frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{266}][\text{266}]}{[\text{286}][\text{286}]}}{\frac{[\text{288}][\text{266}]}{[\text{286}][\text{286}]} \cdot \frac{[\text{H2666}][\text{H2666}]}{[\text{H2866}][\text{H2866}]}}. \quad (\text{S.75})$$

Rearranging again yields

$$\frac{s'_{+1}}{s'_{-1}} = \frac{\frac{1}{3} \frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{H2866}][\text{H2866}]}{[\text{H2666}][\text{H2666}]}}{\frac{[\text{288}][\text{266}]}{[\text{286}][\text{286}]} \cdot \frac{[\text{286}][\text{286}]}{[\text{266}][\text{266}]}}. \quad (\text{S.76})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{s'_{+1}}{s'_{-1}} = \frac{\frac{1}{3} \cdot \left(\frac{1}{3} \frac{{}^{64}\text{R}}{{}^{64}\text{R}^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot 3 \cdot {}^{18}\text{r}_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}\text{r}_{\text{HCO}_3^-}}{\left(\frac{1}{4} \frac{{}^{48}\text{R}}{{}^{48}\text{R}^*} \right)_{\text{CO}_2}^{\text{eq}} \cdot 2 \cdot {}^{18}\text{r}_{\text{CO}_2} \cdot 2 \cdot {}^{18}\text{r}_{\text{CO}_2}}. \quad (\text{S.77})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{s'_{+1}}{s'_{-1}} = \frac{\left(\frac{{}^{64}\text{R}}{{}^{64}\text{R}^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}}{\left(\frac{{}^{48}\text{R}}{{}^{48}\text{R}^*} \right)_{\text{CO}_2}^{\text{eq}}}. \quad (\text{S.78})$$

For the primary double-clumped isotope hydroxylation reaction, we have

$${}^{18-18}\text{KIE}_{p'_{+4}} = \frac{{}^{18-18}\alpha_{p'_{+4}}^{\text{KFF}}}{{}^{18}\alpha_{a_{+4}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+4}}^{\text{KFF}}} = \frac{\frac{p'_{+4}}{k_{+4}}}{\frac{b_{+4}}{k_{+4}} \cdot \frac{a_{+4}}{k_{+4}}}, \quad (\text{S.79})$$

from which we get an expression for p'_{+4} :

$$p'_{+4} = \frac{{}^{18-18}\text{KIE}_{p'_{+4}} b_{+4} a_{+4}}{k_{+4}} \quad (\text{S.80})$$

To obtain p'_{-4} we use the equilibrium constraint:

$$\frac{p'_{+4}}{p'_{-4}} = \frac{\frac{2}{3} [\text{H2886}]}{[\text{286}][\text{8H}]}. \quad (\text{S.81})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{p'_{+4}}{p'_{-4}} = \frac{\frac{2}{3} [\text{H2886}]}{[\text{286}][\text{8H}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{H}]}{[\text{6H}]}}{\frac{[\text{H}]}{[\text{6H}]}}}, \quad (\text{S.82})$$

which upon rearrangement yields

$$\frac{p'_{+4}}{p'_{-4}} = \frac{\frac{2}{3} \frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}]} \cdot \frac{1}{[\text{H}][\text{6H}]}}{\frac{[\text{286}]}{[\text{266}]} \cdot \frac{[\text{8H}]}{[\text{6H}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]}}}. \quad (\text{S.83})$$

Rearranging again yields

$$\frac{p'_{+4}}{p'_{-4}} = \frac{\frac{2}{3} \frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}]} \cdot \frac{1}{[\text{H}][\text{6H}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{286}]}{[\text{266}]} \cdot \frac{[\text{8H}]}{[\text{6H}]}}}. \quad (\text{S.84})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p'_{+4}}{p'_{-4}} = \frac{\frac{2}{3} \cdot \left(\frac{1}{3} \frac{{}^{64}R}{{}^{64}R^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot \frac{1}{K_w} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-}}{2 \cdot {}^{18}r_{\text{CO}_2} \cdot r_{\text{OH}^-}}. \quad (\text{S.85})$$

The factors of 3 cancel and we end up with

$$\frac{p'_{+4}}{p'_{-4}} = \left(\frac{{}^{64}R}{{}^{64}R^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{OH}^-}^{\text{eq}}. \quad (\text{S.86})$$

For the secondary double-clumped isotope hydroxylation reaction, we have

$${}^{18-18}\text{KIE}_{s'_{+4}} = \frac{{}^{18-18}\alpha_{s'_{+4}}^{\text{KFF}}}{{}^{18}\alpha_{b_{+4}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+4}}^{\text{KFF}}} = \frac{\frac{s'_{+4}}{k_{+4}}}{\frac{b_{+4}}{k_{+4}} \cdot \frac{b_{+4}}{k_{+4}}}, \quad (\text{S.87})$$

from which we get an expression for s'_{+4} :

$$s'_{+4} = \frac{{}^{18-18}\text{KIE}_{s'_{+4}} \cdot b_{+4} \cdot b_{+4}}{k_{+4}} \quad (\text{S.88})$$

To obtain s'_{-4} we use the equilibrium constraint:

$$\frac{s'_{+4}}{s'_{-4}} = \frac{\frac{1}{3}[\text{H2886}]}{[\text{288}][\text{6H}]}. \quad (\text{S.89})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{s'_{+4}}{s'_{-4}} = \frac{\frac{1}{3}[\text{H2886}]}{[\text{288}][\text{6H}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H2866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{266}][\text{266}]}{[\text{286}][\text{286}]}}{\frac{[\text{266}][\text{266}]}{[\text{286}][\text{286}]}}}, \quad (\text{S.90})$$

which upon rearrangement yields

$$\frac{s'_{+4}}{s'_{-4}} = \frac{\frac{1}{3}[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{266}]}{[\text{286}]} \cdot \frac{[\text{266}]}{[\text{286}]} \cdot \frac{[\text{H2666}]}{[\text{266}][\text{6H}]}. \quad (\text{S.91})$$

Rearranging again yields

$$\frac{s'_{+4}}{s'_{-4}} = \frac{\frac{1}{3}[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{266}][\text{6H}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}. \quad (\text{S.92})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{s'_{+4}}{s'_{-4}} = \frac{\frac{1}{3} \cdot \left(\frac{1}{3} \frac{{}^{64}R}{{}^{64}R^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-}}{\left(\frac{1}{4} \frac{{}^{48}R}{{}^{48}R^*}\right)_{\text{CO}_2}^{\text{eq}} \cdot 2 \cdot {}^{18}r_{\text{CO}_2} \cdot 2 \cdot {}^{18}r_{\text{CO}_2}}. \quad (\text{S.93})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{s'_{+4}}{s'_{-4}} = \frac{\left(\frac{{}^{64}R}{{}^{64}R^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}}{\left(\frac{{}^{48}R}{{}^{48}R^*}\right)_{\text{CO}_2}^{\text{eq}}}. \quad (\text{S.94})$$

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S.4 Adding triple-clumped isotopes to the model

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This derivation will involve the $[386]/[266]$ and $[3866]/[2666]$ ratios and an important subtlety is that these cannot be treated as stochastic. Recall that for a stochastic distribution we have the following relationships:

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$${}^{47}R^* = \left(\frac{[386]}{[266]} \right)^* = \frac{[366]}{[266]} \cdot \frac{[286]}{[266]} = {}^{13}r_{\text{CO}_2} \cdot 2 \cdot {}^{18}r_{\text{CO}_2} \quad (\text{S.95})$$

and

$${}^{63}R^* = \left(\frac{[3866]}{[2666]} \right)^* = \frac{[3666]}{[2666]} \cdot \frac{[2866]}{[2666]} = {}^{13}r_{\text{CO}_3^{2-}} \cdot 3 \cdot {}^{18}r_{\text{CO}_3^{2-}}. \quad (\text{S.96})$$

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which we can use to express the non-stochastic R 's as follows:

$${}^{47}R = \frac{[386]}{[266]} = \left(\frac{{}^{47}R}{{}^{47}R^*} \right) \cdot {}^{13}r_{\text{CO}_2} \cdot 2 \cdot {}^{18}r_{\text{CO}_2} \quad (\text{S.97})$$

and

$${}^{63}R = \frac{[3866]}{[2666]} = \left(\frac{{}^{63}R}{{}^{63}R^*} \right) \cdot {}^{13}r_{\text{CO}_3^{2-}} \cdot 3 \cdot {}^{18}r_{\text{CO}_3^{2-}}. \quad (\text{S.98})$$

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S.4.1 Homogeneous reactions and clumped isotope definitions

Triple-clumped isotope systems involve the internal equilibrium of ${}^{13}\text{C}$ - ${}^{18}\text{O}$ - ${}^{18}\text{O}$ bonding within a single species. For CO_2 , we can write the following isotope exchange reaction:



which has an equilibrium constant

$${}^{49}K_{\text{CO}_2} = \frac{[388][266]}{[386][286]}. \quad (\text{S.100})$$

The abundance of 388 is measured as:

$$\Delta_{49} = \left(\frac{{}^{49}R}{{}^{49}R^*} - 1 \right) \times 1000, \quad (\text{S.101})$$

where

$${}^{49}R = \frac{[388]}{[266]}. \quad (\text{S.102})$$

By multiplying the top and bottom by $[266]$ we have:

$${}^{49}K_{\text{CO}_2} = \frac{[388]}{[266]} \frac{[266]}{[386]} \frac{[266]}{[286]} = {}^{49}R \cdot \left(\frac{{}^{47}R}{{}^{47}R^*} \right)^{-1} \cdot ({}^{13}r_{\text{CO}_2})^{-1} \cdot (2 \cdot {}^{18}r_{\text{CO}_2})^{-1} \cdot (2 \cdot {}^{18}r_{\text{CO}_2})^{-1} = \frac{\frac{1}{4} \left(\frac{{}^{49}R}{{}^{49}R^*} \right)_{\text{CO}_2}^{\text{eq}}}{\left(\frac{{}^{47}R}{{}^{47}R^*} \right)_{\text{CO}_2}^{\text{eq}}}, \quad (\text{S.103})$$

which is similar to the analogous expression for double-clumped isotopes in that the factor of $1/4$ comes from the oxygen isotope part of the expression. Combining Eq. S.101 with Eq. S.103 leads to

$${}^{49}K_{\text{CO}_2} = \frac{[388][266]}{[386][286]} = \frac{\frac{1}{4} \left(\frac{{}^{49}R}{{}^{49}R^*} \right)_{\text{CO}_2}^{\text{eq}}}{\left(\frac{{}^{47}R}{{}^{47}R^*} \right)_{\text{CO}_2}^{\text{eq}}} = \frac{\frac{1}{4} \left(\frac{\Delta_{49, \text{CO}_2}^{\text{eq}}}{1000} + 1 \right)}{\left(\frac{\Delta_{47, \text{CO}_2}^{\text{eq}}}{1000} + 1 \right)}. \quad (\text{S.104})$$

Similar expressions can be written for clumped isotope equilibrium in HCO_3^- and CO_3^{2-} :



and



which have equilibrium constants

$${}^{65}K_{\text{HCO}_3^-} = \frac{[\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} = \frac{\frac{1}{3} \left(\frac{{}^{65}R}{{}^{65}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}}{\left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}} = \frac{\frac{1}{3} \left(\frac{\Delta^{\text{eq}}_{65, \text{HCO}_3^-}}{1000} + 1 \right)}{\left(\frac{\Delta^{\text{eq}}_{63, \text{HCO}_3^-}}{1000} + 1 \right)} \quad (\text{S.107})$$

and

$${}^{65}K_{\text{CO}_3^{2-}} = \frac{[\text{3886}][\text{2666}]}{[\text{3866}][\text{2866}]} = \frac{\frac{1}{3} \left(\frac{{}^{65}R}{{}^{65}R^*} \right)_{\text{CO}_3^{2-}}^{\text{eq}}}{\left(\frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{CO}_3^{2-}}^{\text{eq}}} = \frac{\frac{1}{3} \left(\frac{\Delta^{\text{eq}}_{65, \text{CO}_3^{2-}}}{1000} + 1 \right)}{\left(\frac{\Delta^{\text{eq}}_{63, \text{CO}_3^{2-}}}{1000} + 1 \right)}. \quad (\text{S.108})$$

553 S.4.2 Heterogeneous reactions involving triple-clumped isotopologues

554 To add triple-clumped isotopes to the model, we need to include the reactions involving ^{13}O - ^{18}O - ^{18}O ‘clumps’
555 in CO_2 and HCO_3^- :



From these reactions we obtain the following ODEs:

$$\begin{aligned} \frac{d[388]}{dt} &= -s''_{+1}[388] + \frac{1}{3}s''_{-1}[\text{E3886}]^{65}\chi[\text{H}] \\ &\quad - s''_{+4}[388][6\text{H}] + \frac{1}{3}s''_{-4}[\text{E3886}]^{65}\chi \end{aligned} \quad (\text{S.113})$$

$$\begin{aligned} \frac{d[\text{E3886}]}{dt} &= p''_{+1}[386]r_w - \frac{2}{3}p''_{-1}[\text{E3886}]^{65}\chi[\text{H}] \\ &\quad + p''_{+4}[386][8\text{H}] - \frac{2}{3}p''_{-4}[\text{E3886}]^{65}\chi \\ &\quad + s''_{+1}[388] - \frac{1}{3}s''_{-1}[\text{E3886}]^{65}\chi[\text{H}] \\ &\quad + s''_{+4}[388][6\text{H}] - \frac{1}{3}s''_{-4}[\text{E3886}]^{65}\chi \end{aligned} \quad (\text{S.114})$$

S.4.3 Derivation of $^{65}\chi$

The $^{65}\chi$ term takes the same form as the other χ terms because the fraction of clumped EIC that is in the form of HCO_3^- has the same pH dependence as for standard isotope ratios (Hill et al., 2014):

$$^{65}\chi = \frac{1}{1 + \frac{^{65}K_2}{[\text{H}^+]}} \quad (\text{S.115})$$

where $^{65}K_2$ is the equilibrium constant for the deprotonation reaction:

$$^{65}K_2 = \frac{[3886][\text{H}]}{[\text{H3886}]} \quad (\text{S.116})$$

This can be expressed in terms of the equilibrium Δ_{65} values of HCO_3^- and CO_3^{2-} . First, multiply the top and bottom by a common factor:

$$^{65}K_2 = \frac{[3886][\text{H}]}{[\text{H3886}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}} \cdot \frac{\frac{[2666]}{[3866][2866]}}{\frac{[2666]}{[3866][2866]}} \cdot \frac{[2666]}{[2666]} \cdot \frac{[\text{H2666}]}{[\text{H2666}]}, \quad (\text{S.117})$$

which upon rearrangement yields

$$^{65}K_2 = \frac{\frac{[3886][2666]}{[3866][2866]}}{\frac{[\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]}}{\frac{[2666]}{[3866]} \cdot \frac{[2666]}{[2866]}} \cdot \frac{[2666][\text{H}]}{[\text{H2666}]} \quad (\text{S.118})$$

Converting isotopologue ratios to isotope ratios leads to

$$^{65}K_2 = ^{65}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{13}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot K_2, \quad (\text{S.119})$$

where

$$^{65}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} = \frac{\left(1 + \frac{\Delta_{65, \text{CO}_3^-}^{\text{eq}}}{1000}\right)}{\left(1 + \frac{\Delta_{65, \text{HCO}_3^-}^{\text{eq}}}{1000}\right)} \quad (\text{S.120})$$

S.4.4 Rate constants

For the primary triple-clumped isotope hydration reaction, we have

$$^{13-18-18}\text{KIE}_{p''_{+1}} = \frac{^{13-18-18}\alpha_{p''_{+1}}^{\text{KFF}}}{^{13}\alpha_{c_{+1}}^{\text{KFF}} \cdot ^{18}\alpha_{a_{+1}}^{\text{KFF}} \cdot ^{18}\alpha_{b_{+1}}^{\text{KIF}}} = \frac{\frac{p''_{+1}}{k_{+1}}}{\frac{c_{+1}}{k_{+1}} \frac{a_{+1}}{k_{+1}} \frac{b_{+1}}{k_{+1}}} \quad (\text{S.121})$$

Rearranging leads to

$$p''_{+1} = \frac{^{13-18-18}\text{KIE}_{p''_{+1}} c_{+1} a_{+1} b_{+1}}{k_{+1} k_{+1}} \quad (\text{S.122})$$

To obtain p''_{-1} we use the equilibrium constraint from the corresponding hydration reaction:

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\frac{2}{3}[\text{H3886}][\text{H}]}{[386][\text{H}_2\text{S}]} \quad (\text{S.123})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\frac{2}{3} [\text{H3886}][\text{H}]}{[\text{386}][\text{H}_2\text{8}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{1}{\text{H}_2\text{6}}}{\frac{1}{\text{H}_2\text{6}}}, \quad (\text{S.124})$$

which upon rearrangement yields

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\frac{\frac{2}{3} [\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]}}{\frac{[\text{386}]}{[\text{266}]} \cdot \frac{[\text{H2666}]}{[\text{H3866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}}. \quad (\text{S.125})$$

Rearranging again yields

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\frac{\frac{2}{3} [\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{H3866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{386}]}{[\text{266}]} \cdot \frac{[\text{H28}]}{[\text{H26}]}}}. \quad (\text{S.126})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\frac{2}{3} \cdot \frac{\frac{1}{3} \left(\frac{65R}{65R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}}{\left(\frac{63R}{63R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}} \cdot K_1 \cdot \left(\frac{63R}{63R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot {}^{13}r_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-}}{\left(\frac{47R}{47R^*} \right)_{\text{CO}_2}^{\text{eq}} \cdot {}^{13}r_{\text{CO}_2} \cdot 2 \cdot {}^{18}r_{\text{CO}_2} \cdot r_w}. \quad (\text{S.127})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\left(\frac{65R}{65R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}}{\left(\frac{47R}{47R^*} \right)_{\text{CO}_2}^{\text{eq}}} \cdot K_1 \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}. \quad (\text{S.128})$$

For the secondary triple-clumped isotope hydration reaction, we have

$${}^{13-18-18}\text{KIE}_{s''_{+1}} = \frac{{}^{13-18-18}\alpha_{s''_{+1}}^{\text{KFF}}}{{}^{13}\alpha_{c_{+1}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+1}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+1}}^{\text{KIF}}} = \frac{\frac{s''_{+1}}{k_{+1}}}{\frac{c_{+1}}{k_{+1}} \cdot \frac{b_{+1}}{k_{+1}} \cdot \frac{b_{+1}}{k_{+1}}}, \quad (\text{S.129})$$

from which we get an expression for s_{+1} :

$$s''_{+1} = \frac{{}^{13-18-18}\text{KIE}_{s''_{+1}} \cdot b_{+1} c_{+1} b_{+1}}{k_{+1} k_{+1}} \quad (\text{S.130})$$

To obtain s''_{-1} we use the equilibrium constraint:

$$\frac{s''_{+1}}{s''_{-1}} = \frac{\frac{1}{3} [\text{H3886}][\text{H}]}{[\text{388}][\text{H}_2\text{6}]}. \quad (\text{S.131})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{s''_{+1}}{s''_{-1}} = \frac{\frac{1}{3} [\text{H3886}][\text{H}]}{[\text{388}][\text{H}_2\text{6}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{266}]}{[\text{386}][\text{286}]}}{\frac{[\text{266}]}{[\text{386}][\text{286}]}}}, \quad (\text{S.132})$$

which upon rearrangement yields

$$\frac{s''_{+1}}{s''_{-1}} = \frac{\frac{1}{3}[\text{H3886}][\text{H2666}] \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{266}][\text{266}]}{[\text{386}][\text{286}]}}{\frac{[\text{388}][\text{266}]}{[\text{386}][\text{286}]} \cdot \frac{[\text{H2666}][\text{H2666}]}{[\text{H3866}][\text{H2866}]}}. \quad (\text{S.133})$$

Rearranging again yields

$$\frac{s''_{+1}}{s''_{-1}} = \frac{\frac{1}{3}[\text{H3886}][\text{H2666}] \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H26}]} \cdot \frac{[\text{H3866}][\text{H2866}]}{[\text{H2666}][\text{H2666}]}}{\frac{[\text{388}][\text{266}]}{[\text{386}][\text{286}]} \cdot \frac{[\text{386}][\text{286}]}{[\text{266}][\text{266}]}}. \quad (\text{S.134})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{s''_{+1}}{s''_{-1}} = \frac{\frac{1}{3} \cdot \frac{\frac{1}{3} \left(\frac{65R}{65R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot \left(\frac{63R}{63R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot {}^{13}r_{\text{HCO}_3^-} \cdot \mathbf{3} \cdot {}^{18}r_{\text{HCO}_3^-} \cdot \mathbf{3} \cdot {}^{18}r_{\text{HCO}_3^-}}{\frac{1}{4} \left(\frac{49R}{49R^*} \right)_{\text{CO}_2}^{\text{eq}} \cdot \left(\frac{47R}{47R^*} \right)_{\text{CO}_2}^{\text{eq}} \cdot {}^{13}r_{\text{CO}_2} \cdot \mathbf{2} \cdot {}^{18}r_{\text{CO}_2} \cdot \mathbf{2} \cdot {}^{18}r_{\text{CO}_2}}. \quad (\text{S.135})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{s''_{+1}}{s''_{-1}} = \frac{\left(\frac{65R}{65R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}}{\left(\frac{49R}{49R^*} \right)_{\text{CO}_2}^{\text{eq}}}. \quad (\text{S.136})$$

For the primary triple-clumped isotope hydroxylation reaction, we have

$${}^{13-18-18}\text{KIE}_{p''_{+4}} = \frac{{}^{13-18-18}\alpha_{p''_{+4}}^{\text{KIF}}}{{}^{13}\alpha_{c_{+4}}^{\text{KFF}} \cdot {}^{18}\alpha_{a_{+4}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+4}}^{\text{KFF}}} = \frac{\frac{p''_{+4}}{k_{+4}}}{\frac{c_{+4}}{k_{+4}} \frac{a_{+4}}{k_{+4}} \frac{b_{+4}}{k_{+4}}}, \quad (\text{S.137})$$

from which we get an expression for p''_{+4} :

$$p''_{+4} = \frac{{}^{13-18-18}\text{KIE}_{p''_{+4}} \cdot c_{+4} a_{+4} b_{+4}}{k_{+4} k_{+4}} \quad (\text{S.138})$$

To obtain p''_{-4} we use the equilibrium constraint:

$$\frac{p''_{+4}}{p''_{-4}} = \frac{\frac{2}{3}[\text{H3886}]}{[\text{386}][\text{8H}]}. \quad (\text{S.139})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{p''_{+4}}{p''_{-4}} = \frac{\frac{2}{3}[\text{H3886}]}{[\text{386}][\text{8H}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[\text{266}]}}{\frac{[\text{H2666}]}{[\text{266}]}} \cdot \frac{\frac{[\text{H}]}{[\text{6H}]}}{\frac{[\text{H}]}{[\text{6H}]}}}, \quad (\text{S.140})$$

which upon rearrangement yields

$$\frac{p''_{+4}}{p''_{-4}} = \frac{\frac{2}{3}[\text{H3886}][\text{H2666}] \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}]} \cdot \frac{1}{[\text{H}][\text{6H}]}}{\frac{[\text{386}] \cdot [\text{8H}]}{[\text{266}]} \cdot \frac{[\text{H2666}]}{[\text{H3866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]}}}. \quad (\text{S.141})$$

Rearranging again yields

$$\frac{p''_{+4}}{p''_{-4}} = \frac{\frac{2}{3} \frac{[\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[266]} \cdot \frac{1}{[\text{H}][6\text{H}]} \cdot \frac{[\text{H3866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[386]}{[266]} \cdot \frac{[8\text{H}]}{[6\text{H}]}}. \quad (\text{S.142})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{p''_{+4}}{p''_{-4}} = \frac{\frac{2}{3} \cdot \frac{\frac{1}{3} \left(\frac{65R}{65R^*}\right)_{\text{HCO}_3^-}^{\text{eq}}}{\left(\frac{63R}{63R^*}\right)_{\text{HCO}_3^-}^{\text{eq}}} \cdot \frac{K_1}{K_w} \cdot \left(\frac{63R}{63R^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot {}^{13}r_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-} \cdot 3 \cdot {}^{18}r_{\text{HCO}_3^-}}{\left(\frac{47R}{47R^*}\right)_{\text{CO}_2}^{\text{eq}} \cdot {}^{13}r_{\text{CO}_2} \cdot 2 \cdot {}^{18}r_{\text{CO}_2} \cdot r_{\text{OH}^-}}. \quad (\text{S.143})$$

The factors of 3 cancel and we end up with

$$\frac{p''_{+4}}{p''_{-4}} = \frac{\left(\frac{65R}{65R^*}\right)_{\text{HCO}_3^-}^{\text{eq}} \cdot \frac{K_1}{K_w} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{OH}^-}^{\text{eq}}}{\left(\frac{47R}{47R^*}\right)_{\text{CO}_2}^{\text{eq}}}. \quad (\text{S.144})$$

For the secondary triple-clumped isotope hydroxylation reaction, we have

$${}^{13-18-18}\text{KIE}_{s''_{+4}} = \frac{{}^{13-18-18}\alpha_{s''_{+4}}^{\text{KFF}}}{{}^{18}\alpha_{c_{+4}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+4}}^{\text{KFF}} \cdot {}^{18}\alpha_{b_{+4}}^{\text{KIF}}} = \frac{\frac{s''_{+4}}{k_{+4}}}{\frac{c_{+4}}{k_{+4}} \frac{b_{+4}}{k_{+4}} \frac{b_{+4}}{k_{+4}}}, \quad (\text{S.145})$$

from which we get an expression for s''_{+4} :

$$s''_{+4} = \frac{{}^{13-18-18}\text{KIE}_{s''_{+4}} c_{+4} b_{+4} b_{+4}}{k_{+4} k_{+4}} \quad (\text{S.146})$$

To obtain s''_{-4} we use the equilibrium constraint:

$$\frac{s''_{+4}}{s''_{-4}} = \frac{\frac{1}{3} [\text{H3886}]}{[388][6\text{H}]}. \quad (\text{S.147})$$

By multiplying the top and bottom by a common factor, we can write

$$\frac{s''_{+4}}{s''_{-4}} = \frac{\frac{1}{3} [\text{H3886}]}{[388][6\text{H}]} \cdot \frac{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}}{\frac{[\text{H2666}]}{[\text{H3866}][\text{H2866}]}} \cdot \frac{\frac{[\text{H2666}]}{[266]}}{\frac{[\text{H2666}]}{[266]}} \cdot \frac{\frac{[266][266]}{[386][286]}}{\frac{[266][266]}{[386][286]}}}, \quad (\text{S.148})$$

which upon rearrangement yields

$$\frac{s''_{+4}}{s''_{-4}} = \frac{\frac{1}{3} \frac{[\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} \cdot \frac{[266]}{[386]} \cdot \frac{[266]}{[286]} \cdot \frac{[\text{H2666}]}{[266][6\text{H}]}}{\frac{[388][266]}{[386][286]} \cdot \frac{[\text{H2666}]}{[\text{H3866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]}}}. \quad (\text{S.149})$$

Rearranging again yields

$$\frac{s''_{+4}}{s''_{-4}} = \frac{\frac{1}{3} \frac{[\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} \cdot \frac{[\text{H2666}]}{[266][6\text{H}]} \cdot \frac{[\text{H3866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[388][266]}{[386][286]} \cdot \frac{[386]}{[266]} \cdot \frac{[286]}{[266]}}}. \quad (\text{S.150})$$

Converting isotopologue ratios to isotope ratios leads to

$$\frac{s''_{+4}}{s''_{-4}} = \frac{\frac{1}{3} \cdot \frac{\left(\frac{65R}{65R^*}\right)^{\text{eq}}_{\text{HCO}_3^-} \cdot \frac{K_1}{K_w} \left(\frac{63R}{63R^*}\right)^{\text{eq}}_{\text{HCO}_3^-} \cdot {}^{13}r_{\text{HCO}_3^-} \cdot \mathbf{3} \cdot {}^{18}r_{\text{HCO}_3^-} \cdot \mathbf{3} \cdot {}^{18}r_{\text{HCO}_3^-}}{\frac{\frac{1}{4} \left(\frac{49R}{49R^*}\right)^{\text{eq}}_{\text{CO}_2}}{\left(\frac{47R}{47R^*}\right)^{\text{eq}}_{\text{CO}_2}} \cdot \left(\frac{47R}{47R^*}\right)^{\text{eq}}_{\text{CO}_2} \cdot {}^{13}r_{\text{CO}_2} \cdot \mathbf{2} \cdot {}^{18}r_{\text{CO}_2} \cdot \mathbf{2} \cdot {}^{18}r_{\text{CO}_2}}. \quad (\text{S.151})$$

The factors of 2 and 3 cancel and we end up with

$$\frac{s''_{+4}}{s''_{-4}} = \frac{\left(\frac{65R}{65R^*}\right)^{\text{eq}}_{\text{HCO}_3^-} \cdot \frac{K_1}{K_w} \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}}}{\left(\frac{49R}{49R^*}\right)^{\text{eq}}_{\text{CO}_2}}. \quad (\text{S.152})$$