

# A generalizable box model for kinetic clumped isotope effects in the $\text{CaCO}_3$ -DIC- $\text{H}_2\text{O}$ system

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## Key Points:

- We derive a box model for kinetic clumped isotope effects in the  $\text{CaCO}_3$ -DIC- $\text{H}_2\text{O}$  system.
- The model is used to fit and explain extreme  $\delta^{18}\text{O}$  and  $\Delta_{47}$  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

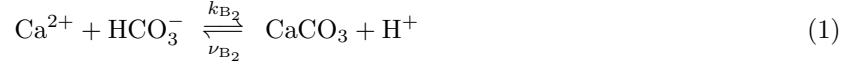
Most Earth surface carbonates precipitate out of isotopic equilibrium with their host solution, complicating the use of stable isotopes in paleoenvironment reconstructions. Disequilibrium can arise from exchange reactions in the DIC- $\text{H}_2\text{O}$  system as well as during crystal growth reactions in the DIC- $\text{CaCO}_3$  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  $\text{CaCO}_3$ -DIC- $\text{H}_2\text{O}$  system. The model is applied to inorganic calcite precipitation experiments where there is a known  $\text{CO}_2$  influx and  $\text{CaCO}_3$  outflux. The example provided can be adapted to other situations involving  $\text{CO}_2$  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  $^{13}\text{C}$ - $^{18}\text{O}$  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  $\text{CO}_2$  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

and Zeebe, 2012; Affek, 2013; Devriendt et al., 2017; Staudigel and Swart, 2018; Bajnai et al., 2018; many others). Reconstructing environments from disequilibrium isotope compositions first requires knowledge of 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  $\text{CaCO}_3$ -DIC- $\text{H}_2\text{O}$  system. In the simplest scenario of  $\text{CaCO}_3$  growth from an isotopically equilibrated DIC pool, KIEs can be attributed to the following crystal growth reactions (Watkins et al., 2013):



and



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

The picture gets more complicated when  $\text{CaCO}_3$  precipitates from a DIC pool that is not isotopically equilibrated. Here, the key reactions are the relatively slow (de-)hydration and (de-)hydroxylation reactions:



and



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

Two different approaches have been taken recently to model KIEs in the DIC- $\text{H}_2\text{O}$  system in the absence of crystal growth. The IsoDIC model of Guo (2020) tracks all of the reactions ( $n = 155$ ) involving  $^{12}\text{C}$ ,  $^{13}\text{C}$ ,  $^{16}\text{O}$ ,  $^{17}\text{O}$ , and  $^{18}\text{O}$ . This amounts to 32 coupled ordinary differential equations (ODEs) describing changes in concentration of  $\text{CO}_2$  and  $\text{HCO}_3^-$  isotopologues (Table 1). By contrast, the ExClump38 model of Uchikawa et al. (2021) only tracks the major isotopologue at each mass, up to mass 63 (Table 1). This has the advantage of only requiring 8 ODEs for single clumped isotopes (i.e.,  $\Delta_{47}$ ) but it includes approximations that have not been fully explained or validated against the IsoDIC model. Additionally, the ExClump38 model has not been extended to double clumped isotopes ( $\Delta_{48}$ ) or triple clumped isotopes ( $\Delta_{49}$ ).

In this contribution, we add important details and explanations to the derivation of the ExClump38 framework first developed by Chen et al. (2018) for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  and subsequently expanded to  $\Delta_{47}$  by Uchikawa et al. (2021). The exposition is necessary for: (1) clarifying some ambiguities so that ExClump38 is on a stronger foundation, (2) expanding the model to  $\Delta_{48}$  and  $\Delta_{49}$ , and (3) deriving new expressions that describe the influence of  $\text{CaCO}_3$  precipitation. After validating the ExClump38 framework against the IsoDIC model, we provide an example box model with a  $\text{CO}_2$  influx and  $\text{CaCO}_3$  outflux for comparison to recent calcite precipitation experiments. The box model can be adjusted judiciously to describe carbonate 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 ( $^{13}\text{C}$ - $^{18}\text{O}$ ). However, the differences are important for double ( $^{18}\text{O}$ - $^{18}\text{O}$ ) and triple ( $^{13}\text{C}$ - $^{18}\text{O}$ - $^{18}\text{O}$ ) 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  $\text{CO}_3^{2-}$  (ignoring  $^{17}\text{O}$ ):

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

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

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

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  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  isotopologues. To reiterate, Eqs. 10-12 are equivalent to Eq. 9 when the isotopes are randomly distributed.

For  $\text{CO}_2$ , 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

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

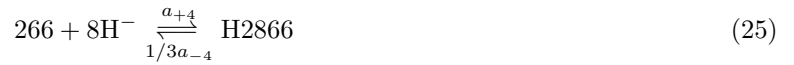
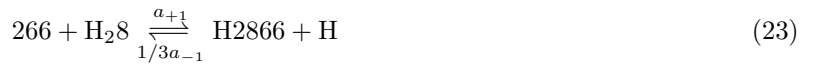
Note that these definitions have not taken into account carbon isotopes. If we were to add  $^{12}\text{C}$  and  $^{13}\text{C}$ , there would instead be 8  $\text{CO}_3^{2-}$  isotopologues and 6  $\text{CO}_2$  isotopologues (again, ignoring  $^{17}\text{O}$ ). The expressions in Eq. 9 and 16 would thus have more terms but the other expressions involving any two of the isotopologues would remain the same. Although it is not immediately obvious, these relationships are applicable to problems involving non-random distributions, or clumping, as will be shown.

## 2.2 Shorthand notation

We use the following shorthand notation after Zeebe (2014):  $^{12}\text{C} = 2$ ,  $^{13}\text{C} = 3$ ,  $^{16}\text{O} = 6$ , and  $^{18}\text{O} = 8$ . Additionally, we drop the superscript charges on ionic species. We treat isotopomers as indistinguishable such that [286] will hereafter refer to the total concentration of singly-substituted  $\text{CO}_2$ : [286]+[268]. In what follows it will be made unambiguous that  $d[\text{C}^{18}\text{OO}]/dt$  (notation of Chen et al., 2018) refers to the change in concentration of the sum of isotopomers ([286]+[268]) as opposed to (a) a single isotopomer or (b) the total  $^{18}\text{O}$  of  $\text{CO}_2$ , which includes  $\text{C}^{18}\text{O}^{18}\text{O}$ .

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

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



For the reactions involving oxygen isotopes, the 1/3 and 2/3 factors have been added to the rate constants for isotopic mass balance (Christensen et al., 2021; Uchikawa et al., 2021). To understand these factors qualitatively, consider, e.g., the two dehydration reactions involving H2866 (reactions 23 and 24). The right-hand sides of these two reactions are identical, but for every mole of H2866 that undergoes dehydration,  $\sim 2/3$  goes to 286 and  $\sim 1/3$  goes to H<sub>2</sub>8. The factors are approximate because the isotopes are not exactly randomly distributed among the isotopologues and also because there is isotopic fractionation attending these reactions. A more quantitative justification is given in section 2.5.

## 2.4 From reactions to ordinary differential equations (ODEs)

The above reactions involve six isotopically-distinct DIC species that need to be tracked. We write  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  together as EIC (equilibrated inorganic carbon, Chen et al., 2018) and apply the notation to isotopologues (e.g.  $\text{H2666} + \text{2666} = \text{E2666}$ ). The ODEs become:

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

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

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

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

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

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

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

The  $\chi$  terms represent the fraction of EIC that is  $\text{HCO}_3^-$ :

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

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

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  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$ . In many situations, it is appropriate to treat  $[\text{H}_2\text{6}]$ ,  $[\text{6H}]$ ,  $[\text{H}_2\text{8}]$ , and  $[\text{8H}]$  as constant, implying there is an infinite reservoir of  $\text{H}_2\text{O}$ , and by extension,  $\text{OH}^-$ .

## 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

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

It is here that the need for 1/3 and 2/3 factors on the rate constants becomes apparent: They cancel with the factors of 2 and 3 that accompany the conversion from isotopologue ratios to isotope ratios and are fundamentally related to the statement that isotopomers are indistinguishable; i.e., that [286] and [2866] refer to the total concentration of singly-substituted  $\text{CO}_2$  ([286]+[268]) and  $\text{HCO}_3^-$  ([2866]+[2686]+[2668]). An up-to-date compilation of rate constants, equilibrium constants, and isotopic fractionation factors is provided in Table 2.

## 2.6 Model validation

The system of ODEs (Eqs. 27-32) has been used to describe the chemical and isotopic evolution of DIC species in the DIC- $\text{H}_2\text{O}$  system (Chen et al., 2018; Christensen et al., 2021; Uchikawa et al., 2021) but without having been validated against experiments and analytical expressions that are available in the literature.

Uchikawa and Zeebe (2012) performed time-series experiments where an unequilibrated DIC pool was precipitated quantitatively as witherite ( $\text{BaCO}_3$ ) and then analyzed for  $\delta^{18}\text{O}$ . Their data at pH = 8.3 and 8.9 are compared to theoretical and numerical predictions in Fig. 1a. The analytical and numerical curves are nearly indistinguishable and in excellent agreement with the time series data. The numerical-analytical comparison is expanded to a broad range of pH for the time required to reach 99% oxygen isotope equilibration ( $\tau_{99\%}$ ) as well as the time to reach 69.3% equilibration for carbon isotopes ( $\tau$  e-fold) in Figs. 1b and 1c.

An interesting result is a large discrepancy between analytical and numerical results when it comes to chemical equilibration, as shown in Fig. 1d. Numerical experimentation revealed that this discrepancy is due to the assumption of instantaneous pH adjustment - an assumption that is also employed in the IsoDIC model (Guo, 2020). Upon adding ODEs that explicitly track  $d[\text{H}^+]/dt$ ,  $d[\text{OH}^-]/dt$ ,  $d[\text{HCO}_3^-]/dt$ , and  $d[\text{CO}_3^{2-}]/dt$  (referred to as the ‘Full model’ in the right panels of Fig. 1), the disagreement in chemical equilibration times is resolved (Fig. 1h). This is an interesting curiosity, but since it has little to no bearing on isotope-related problems, it will not be discussed further.

## 3 Adding clumped isotopes to the model

The above framework was extended to single clumped isotopes by Uchikawa et al. (2021) to describe oxygen and clumped isotope equilibration in the DIC- $\text{H}_2\text{O}$  system. Much of what follows has been presented

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

### 3.1 Homogeneous reactions and clumped isotope definitions

Clumped isotope systems involve the equilibrium of  $^{13}\text{C}$ - $^{18}\text{O}$  bonding within a single species. For  $\text{CO}_2$ , we can write the following isotope exchange reaction:



which has an equilibrium constant

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

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  $^{17}\text{O}$  (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  $\Delta_{47}$  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  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ :



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  $\text{CO}_2$  and  $\text{HCO}_3^-$ :



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

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

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

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  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  so that these two species are in clumped isotopic equilibrium with each other. The expression takes the same form as the other  $\chi$  terms because the fraction of clumped EIC that is in the form of  $\text{HCO}_3^-$  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  $\Delta_{63}$  values of  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . 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  $\epsilon_{a+1}$  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  ${}^{13-18}\text{KIEs}$  (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

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

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:

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

Similar expressions can be derived for the remaining clumped isotope rate constants (Supplement S.2), 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

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

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

## 4 Adding double and triple clumped isotopes to the model

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

### 4.1 Model validation

The ExClump38 framework ignores  ${}^{17}\text{O}$  and only tracks the major isotopologue at each mass (i.e., masses 47, 48 and 49 for  $\text{CO}_2$ , and masses 63, 64, and 65 for  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ). To instill confidence that these simplifications do not introduce significant errors, we performed numerical experiments for comparison to the IsoDIC simulations of the time evolution of  $\text{HCO}_3^-$  during  $\text{CO}_2$  degassing and  $\text{CO}_2$  absorption (Fig. 2). In

these simulations, the DIC is initially equilibrated and then the  $\text{CO}_2$  concentration and isotopic composition are perturbed from the equilibrium value and held constant. The reassuring outcome is that the curves are indistinguishable from those of Fig. 3 in Guo (2020).

## 4.2 Model uses and next steps

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

The work by Guo (2020) constitutes a major step forward in characterizing the patterns and controls of clumped isotope KIEs more generally, as summarized in his ‘loop diagrams’ (e.g., Fig. 3b). The loops show how  $\Delta_{47}$  and  $\delta^{18}\text{O}$  co-vary following a perturbation of an initial chemical and isotopic equilibrium by a forced input/output of  $\text{CO}_2$  to/from the solution. Each of the loops begins and ends on the equilibrium composition denoted by the black circle and each point on a loop corresponds to a specific time in Fig. 2. In these simulations,  $\text{CO}_2$  absorption (hydroxylation) can produce  $\Delta_{63}$ - $\delta^{18}\text{O}$  co-variations with a negative slope (initial perturbation), just like in the vectors in Fig. 3a, or a slope pointing in any of the other three quadrants depending on the extent of equilibration (cf. Fiebig et al., 2021). The same can be said for a scenario involving  $\text{CO}_2$  degassing. The  $\Delta_{63}$ - $\delta^{18}\text{O}$  patterns that ultimately get recorded in carbonate minerals depend on the degree of reaction reversibility, which in turn depend on the nature of DIC influxes and outfluxes. In other words, determining where on each of these loops a system will land requires a more complicated model in which the  $\text{CO}_2$  flux is specified and there is also a  $\text{CaCO}_3$  sink.

## 5 Box model and application to inorganic calcite precipitation experiments

In this section we build a box model to explain large ( $>0.3\text{‰}$ ) kinetic clumped isotope effects from inorganic calcite precipitation experiments at high-pH (Tang et al., 2014). This data set is particularly amenable to a box model because the experimental solutions were well-stirred (homogeneous) and the authors provided information regarding the magnitude and isotopic composition of DIC fluxes. The goals of this exercise are: (1) to provide the first example of a clumped isotope box model with a complete set of reaction kinetics that can be easily adapted to other situations, and (2) to quantitatively evaluate the hypothesis by Tang et al. (2014) that extreme light isotope enrichments in their experiments are due to some combination of  $\text{CO}_2$  diffusion through a membrane and the hydroxylation reaction.

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

The experimental setup is shown in Fig. 4a. A polyethylene (PE) container houses an inner solution with no  $\text{Ca}^{2+}$  but high  $\text{CO}_{2(\text{aq})}$ . The PE container is placed in an outer solution with 10 mM  $\text{CaCl}_2$  and no DIC initially. The DIC is delivered to the outer solution by  $\text{CO}_2$  diffusion through the PE membrane. The  $\text{CO}_2$  flux varies between experiments because of differences in membrane thickness and in the pH of inner and outer solutions. The pH of the outer solution is held constant through use of an autotitrator with NaOH as the titrant.

Tang et al. (2014) describe their experiments as consisting of two stages. During Stage I, there is a  $\text{CO}_2$  influx and the concentration of DIC in the outer solution increases monotonically until a critical saturation is reached for spontaneous calcite precipitation. During Stage II, there is a  $\text{CO}_2$  influx and  $\text{CaCO}_3$  outflux. Stage II is characterized by a short period of rapid  $\text{CaCO}_3$  nucleation and growth followed by a prolonged period of slower growth under steady state conditions.

A modified version of the experimental parameters and results of Tang et al. (2014) is given in Table 6. The flux of  $\text{CO}_2$  into solution during Stage I was calculated from  $[\text{DIC}]$  at the end of Stage I and the duration of Stage I ( $t_1$ ). The flux of  $\text{CaCO}_3$  was calculated from the moles of  $\text{CaCO}_3$  precipitated ( $M$ ) and duration of Stage II ( $t_2$ ). The surface area normalized growth rate of  $\text{CaCO}_3$  ( $\text{moles m}^{-2} \text{s}^{-1}$ ) was calculated

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

## 5.2 Box model setup

The experiments can be described using a box model with two fluxes:  $F_{\text{CO}_2}$  and  $F_{\text{CaCO}_3}$  (both in moles  $\text{L}^{-1} \text{ s}^{-1}$ ) (Fig. 4b). For simplicity, we treat the problem as a seeded experiment such that the reactive surface area is constant and equal to a representative value of  $0.1 \text{ m}^2$ . This is justified on the basis that at the onset of Stage II, there is a short period of rapid nucleation and growth that generates “seed” crystals whose reactive surface area could plausibly be approximated as constant thereafter. Additional details regarding the treatments of  $F_{\text{CO}_2}$  and  $F_{\text{CaCO}_3}$  are described individually below.

### 5.2.1 $\text{CO}_2$ flux

A key source of uncertainty is the isotopic composition of  $\text{CO}_2$  as it enters solution. In the default case, we assume that the  $\text{CO}_{2(\text{aq})}$  in the inner solution is isotopically equilibrated with water. Under the experimental conditions ( $5^\circ \text{C}$  and pH of inner solution between 7.3-8.1), the equilibration time for oxygen and clumped isotopes ( $t_{99\%}$ ) ranges from 6 to 35 hours (Uchikawa and Zeebe, 2012; Staudigel and Swart, 2018). Tang et al. (2014) did not report the pre-experiment dwell time and whether or not it was sufficient to ensure isotopic equilibration. Even if the  $\text{CO}_{2(\text{aq})}$  in the inner solution were equilibrated, it is possible for it to get isotopically fractionated by diffusion through the PE membrane. To the best of our knowledge, there is no available data on isotope fractionation by diffusion of  $\text{CO}_{2(\text{g})}$  through a PE membrane. However, an upper bound on these effects is given by the mass dependence on diffusion through a porous medium where the pore size is smaller than the mean free path of  $\text{CO}_2$  molecules (Knudsen diffusion):

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

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 isotopologues  $i$  and  $j$ , respectively. Previous work suggests that this fractionation law with exponent of 0.5 yields a diffused population of  $\text{CO}_2$  that is 22.2‰ lower in  $\delta^{18}\text{O}$  and 0.5‰ higher in  $\Delta_{47}$  (Eiler and Schauble, 2004). If the pore size were larger than the mean free path and/or the power law exponent were less than 0.5, the isotopic effects would be reduced. In any case, there is potential for the  $\text{CO}_2$  flux to have significantly lower  $\delta^{18}\text{O}$  and higher  $\Delta_{47}$  than presumed in the default scenario.

### 5.2.2 $\text{CaCO}_3$ flux

The precipitation of  $\text{CaCO}_3$  constitutes a sink of EIC isotopologues that can affect the isotopic composition of residual EIC. Chen et al. (2018) provided the  $\text{CaCO}_3$  flux terms for carbon and oxygen isotopes. Here, we build upon this framework by deriving the  $\text{CaCO}_3$  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:

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

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  $\text{CaCO}_3$ , 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  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  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  $\text{CO}_2$  that is fluxed into solution gets converted to  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  according to the (de-)hydration and

(de-)hydroxylation reactions and calcite grows at a rate that depends on  $[\text{Ca}^{2+}]$  and  $[\text{CO}_3^{2-}]$ . At each timestep, the H2666 and 2666 species concentrations are calculated from E2666 and are used as inputs into the ion-by-ion model, which returns the surface area normalized growth rate as well as the pH- and growth rate-dependent  $^{13}\alpha_{\text{CaCO}_3\text{-EIC}}$ ,  $^{18}\alpha_{\text{CaCO}_3\text{-EIC}}$ , and  $^{63}\alpha_{\text{CaCO}_3\text{-EIC}}$  values. The growth rate is multiplied by the reactive surface area ( $0.1 \text{ m}^2$ ) to get  $F_{\text{CaCO}_3}$  and the calculation is repeated.

## 5.4 Model behavior

An example showing the behavior of the model run to steady state is shown in Fig. 5. The  $\text{CO}_2$  flux is held constant at  $0.01 \text{ mmol/h}$  (Fig. 5a), which is in the middle of the range of the Tang et al. (2014) experiments. The flux of  $\text{CaCO}_3$  is initially 0 but it increases monotonically until it exactly balances with the specified flux of  $\text{CO}_2$  (Fig. 5b). The specified  $F_{\text{CO}_2}$  determines the time required to reach steady state as well as the steady state growth rate. The oxygen isotope composition of DIC is initially equilibrated but  $1000\ln\alpha$  decreases due to the kinetic fractionations attending the hydration and hydroxylation reactions (Fig. 5c). After about 70 hours, the system reaches steady state composition that is far from equilibrium. The emergent behavior of clumped isotopes is that the DIC species become more ordered due to hydration and hydroxylation, and  $\text{CaCO}_3$  growth is fast enough to inherit the weighted sum of  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ , in accordance with the ion-by-ion model (Watkins and Hunt, 2015).

## 5.5 Model application

Outputs from the default version of the model are compared to the Tang et al. (2014) data in Fig. 6a-b. Each point on a model curve represents the *steady state* isotopic composition for the specified pH and  $F_{\text{CO}_2}$ . For oxygen isotopes (Fig. 6a), the model curves bracket the data below  $\text{pH} = 9.5$ , but lie well above the data at higher pH. For clumped isotopes (Fig. 6a), the model correctly predicts an increase in  $\Delta_{47}$  with increasing pH and the curves bracket most of the data. The model predicts a kinetic limit of  $\Delta_{47} \sim 1.00$ , which is somewhat lower than the experimental  $\Delta_{47} = 1.06$  at  $\text{pH} = 10.5$ .

There are two adjustments to the default model that can better fit the data at high pH. The first is to increase the kinetic fractionation factors for the hydroxylation reaction, as shown in Figs. 6c-d. However, fitting the oxygen isotope data requires changing the KFF assigned to  $\text{OH}^-$  by almost 30% ( $a_{+4}/k_{+4} = 0.9988 \rightarrow 0.9700$ ) to generate a KIE that is 10‰ greater because  $\text{OH}^-$  contributes only 1/3 of the oxygen atoms to the hydroxylation reaction. Note that changing the KFF assigned to  $\text{CO}_2$  ( $b_{+4}/k_{+4}$ ) has little effect on the results because the  $\text{CO}_2$  is nearly quantitatively converted to  $\text{HCO}_3^-$ , and consequently, the corresponding KIE isn't expressed. Fitting the clumped isotope data requires changing the direction of the primary clumped isotope KIE ( $^{13-18}\text{KIE}_{p+4} = 1-0.016/1000 \rightarrow 1+0.180/1000$ ), which is also unsatisfying. Changing the secondary KIE ( $^{13-18}\text{KIE}_{s+4}$ ) has little effect on the results.

The alternative option is to relax the assumption that the  $\text{CO}_2$  flux is in oxygen and clumped isotope equilibrium with water at  $5^\circ\text{C}$  (Fig. 6e-f). If the KFFs in the default model are correct, then fitting the oxygen isotope data requires the  $\delta^{18}\text{O}$  of  $\text{CO}_2$  to be 13.7‰ lower whereas the clumped isotope data requires the  $\Delta_{47}$  of  $\text{CO}_2$  to be 0.11‰ higher. As discussed in §5.2.1, both the sign and magnitude of these changes could plausibly be produced by  $\text{CO}_{2(g)}$  diffusion through a PE membrane. Such diffusive isotope effects should also fractionate carbon isotopes but this cannot be evaluated because Tang et al. (2014) did not report the  $\delta^{13}\text{C}$  of DIC in the inner solution.

In summary, this modeling exercise supports Tang et al. (2014)'s interpretation that low  $\delta^{18}\text{O}$  and high  $\Delta_{47}$  values at high pH are due to a combination of diffusion and reaction KIEs. Reaction KIEs alone cannot explain the data unless previously-published KFFs for hydroxylation are significantly underestimated. Modeling results indicate that diffusion of  $\text{CO}_{2(g)}$  through a PE membrane can efficiently separate the isotopes of oxygen, and presumably also carbon, which may have implications for KIEs arising from  $\text{CO}_2$  diffusion through other types of membranes in biomineralization models (Zeebe et al., 1999a; Adkins et al., 2003; Erez, 2003). In principle, the model can be used to inform the design and run conditions of future 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  $\Delta_{47}$ ,  $\Delta_{48}$  and  $\Delta_{49}$ .

Two approaches have been taken for describing clumped isotope KIEs in the DIC- $\text{H}_2\text{O}$  system. The IsoDIC model (Guo, 2020) is the most complete and accurate model because it tracks most of the isotopologues ( $^2\text{H}$  and  $^3\text{H}$  are excluded) in the DIC- $\text{H}_2\text{O}$  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  $\text{CaCO}_3$ -DIC system (Watkins and Hunt, 2015). An example box model describing KIEs in the full  $\text{CaCO}_3$ -DIC- $\text{H}_2\text{O}$  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  $\delta^{13}\text{C}$ - $\delta^{18}\text{O}$ - $\Delta_{47}$ - $\Delta_{48}$ - $\Delta_{49}$  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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515 tion of CO<sub>2</sub>. *Geochimica et Cosmochimica Acta* 59 (1), 107–114.

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

Table 1: List of CO<sub>2</sub> and CO<sub>3</sub><sup>2-</sup> 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>		
$K_1$	$\frac{[\text{H}^{2666}][\text{H}]}{[\text{H}_2\text{O}][\text{H}_2\text{O}]} \cdot [\text{H}_2\text{O}] = 1$	$f(T, S)$
$K_2$	$\frac{[\text{H}^{2666}][\text{H}]}{[\text{H}_2\text{O}][\text{H}_2\text{O}]}$	$f(T, S)$
$K_w$	$\frac{[\text{H}][\text{H}]}{[\text{H}_2\text{O}]}$	$f(T, S)$
$k_{+1}$	$\ln k_{+1} = 1246.98 - \frac{61900}{T_K} - 183.0 \ln T_K$	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)
$k_{-4}$	$k_{-4} = k_{+4} \frac{K_w}{K_1}$	-
$\chi$	$\chi = \frac{1}{1 + \frac{K_2}{[\text{H}^+]}}$	-
<i>Part II: Carbon isotope parameters</i>		
$^{13}\alpha_{\text{CO}_2-\text{HCO}_3^-}^{\text{eq}}$	$-9.866 T^{-1} + 1.02412$	Zhang et al. (1995)
$^{13}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}}$	$-0.867 T^{-1} + 1.00252$	Zhang et al. (1995)
$^{13}\alpha_{\text{KFF}}^{c+1}$	0.9872	Yumol et al. (2020)
$^{13}\alpha_{\text{KFF}}^{c+4}$	0.9814	Christensen et al. (2021)
$c_{+1}$	$c_{+1} = ^{13}\alpha_{\text{KFF}}^{c+1} \cdot k_{+1}$	-
$c_{-1}$	$c_{-1} = c_{+1} / (K_1 \cdot ^{13}\alpha_{\text{HCO}_3-\text{CO}_2}^{\text{eq}})$	-
$c_{+4}$	$c_{+4} = ^{13}\alpha_{\text{KFF}}^{c+4} \cdot k_{+4}$	-
$c_{-4}$	$c_{-4} = c_{+4} / \left( \frac{K_1}{K_w} \cdot ^{13}\alpha_{\text{HCO}_3-\text{CO}_2}^{\text{eq}} \right)$	-
$^{13}\chi$	$^{13}\chi = \frac{1}{1 + \frac{K_2 \cdot ^{13}\alpha_{\text{CO}_3-\text{HCO}_3}^{\text{eq}}}{[\text{H}^+]}}$	-
<i>Part III: Oxygen isotope parameters</i>		
$^{18}\alpha_{\text{CO}_2-\text{H}_2\text{O}}^{\text{eq}}$	$\exp(2520 T_K^{-2} + 0.01212)$	Beck et al. (2005)
$^{18}\alpha_{\text{HCO}_3-\text{H}_2\text{O}}^{\text{eq}}$	$\exp(2590 T_K^{-2} + 0.00189)$	Beck et al. (2005)
$^{18}\alpha_{\text{CO}_3^{2-}-\text{H}_2\text{O}}^{\text{eq}}$	$\exp(2390 T_K^{-2} - 0.00270)$	Beck et al. (2005)
$^{18}\alpha_{\text{OH}-\text{H}_2\text{O}}^{\text{eq}}$	$5.6676 \times 10^{-5} T_K + 0.9622$	based on Zeebe (2020)
$^{18}\alpha_{\text{KFF}}^{a+1}$	1.0000	Yumol et al. (2020)
$^{18}\alpha_{\text{KFF}}^{b+1}$	0.9812	Yumol et al. (2020)
$^{18}\alpha_{\text{KFF}}^{a+4}$	0.9988	Christensen et al. (2021)
$^{18}\alpha_{\text{KFF}}^{b+4}$	1.0000	Christensen et al. (2021)
$a_{+1}, b_{+1}$	$a_{+1} = ^{18}\alpha_{\text{KFF}}^{a+1} \cdot k_{+1}$ $b_{+1} = ^{18}\alpha_{\text{KFF}}^{b+1} \cdot k_{+1}$	-
$a_{-1}, b_{-1}$	$a_{-1} = a_{+1} / (K_1 \cdot ^{18}\alpha_{\text{HCO}_3-\text{H}_2\text{O}}^{\text{eq}})$ $b_{-1} = b_{+1} / (K_1 \cdot ^{18}\alpha_{\text{HCO}_3-\text{CO}_2}^{\text{eq}})$	-
$a_{+4}, b_{+4}$	$a_{+4} = ^{18}\alpha_{\text{KFF}}^{a+4} \cdot k_{+4}$ $b_{+4} = ^{18}\alpha_{\text{KFF}}^{b+4} \cdot k_{+4}$	-
$a_{-4}, b_{-4}$	$a_{-4} = a_{+4} / \left( \frac{K_1}{K_w} \cdot ^{18}\alpha_{\text{HCO}_3-\text{H}_2\text{O}}^{\text{eq}} \right)$ $b_{-4} = b_{+4} / \left( \frac{K_1}{K_w} \cdot ^{18}\alpha_{\text{HCO}_3-\text{CO}_2}^{\text{eq}} \right)$	-
$^{18}\chi$	$^{18}\chi = \frac{1}{1 + \frac{K_2 \cdot ^{18}\alpha_{\text{CO}_3-\text{HCO}_3}^{\text{eq}}}{[\text{H}^+]}}$	-

Table 3: Parameters for single-clumped isotopes

Symbol	Value	Reference/Note
<i>Part IV: Single-clumped isotope parameters</i>		
$\Delta_{47, \text{CO}_2}^{\text{eq}}$	$26447/T_K^2 + 285.51/T_K - 0.3004$	Wang et al. (2004)
$\Delta_{63, \text{HCO}_3^-}^{\text{eq}}$	$43655/T_K^2 - 23.643/T_K - 0.0088$	Hill et al. (2020)
$\Delta_{63, \text{CO}_3^{2-}}^{\text{eq}}$	$43187/T_K^2 + 34.833/T_K + 0.0007$	Hill et al. (2020)
$(^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}}$	$\Delta_{47, \text{CO}_2}^{\text{eq}}/1000 + 1$	
$(^{63}R/^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}}$	$\Delta_{63, \text{HCO}_3^-}^{\text{eq}}/1000 + 1$	
$(^{63}R/^{63}R^*)_{\text{CO}_3^{2-}}^{\text{eq}}$	$\Delta_{63, \text{CO}_3^{2-}}^{\text{eq}}/1000 + 1$	
$^{63}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}$	$(^{63}R/^{63}R^*)_{\text{CO}_3^{2-}}^{\text{eq}} / (^{63}R/^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}}$	-
$^{63}K_2$	$^{63}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot ^{13}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot K_2$	-
$^{63}\chi$	$\frac{1}{1 + \frac{^{63}K_2 \cdot ^{63}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}}{[\text{H}^+]}}$	-
$^{13-18}\text{KIE}_{p+1}$	1-0.146/1000	Guo (2020)
$^{13-18}\text{KIE}_{s+1}$	1-0.219/1000	Guo (2020)
$^{13-18}\text{KIE}_{p+4}$	1-0.016/1000	Guo (2020)
$^{13-18}\text{KIE}_{s+4}$	1-0.175/1000	Guo (2020)
$p_{+1}, s_{+1}$	$p_{+1} = (^{13-18}\text{KIE}_{p+1} \cdot c_{+1} \cdot a_{+1})/k_{+1}$ $s_{+1} = (^{13-18}\text{KIE}_{s+1} \cdot c_{+1} \cdot b_{+1})/k_{+1}$	Uchikawa et al. (2021) Uchikawa et al. (2021)
$p_{-1}, s_{-1}$	$p_{-1} = p_{+1} / \left[ (^{63}R/^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}} \right]$ $s_{-1} = s_{+1} \cdot \left[ (^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}} \right] / \left[ (^{63}R/^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}} \right]$	- -
$p_{+4}, s_{+4}$	$p_{+4} = (^{13-18}\text{KIE}_{p+4} \cdot c_{+4} \cdot a_{+4})/k_{+4}$ $s_{+4} = (^{13-18}\text{KIE}_{s+4} \cdot c_{+4} \cdot b_{+4})/k_{+4}$	Uchikawa et al. (2021) Uchikawa et al. (2021)
$p_{-4}, s_{-4}$	$p_{-4} = p_{+4} / \left[ (^{63}R/^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1/K_w \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{OH}^-}^{\text{eq}} \right]$ $s_{-4} = s_{+4} \cdot \left[ (^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}} \right] / \left[ (^{63}R/^{63}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1/K_w \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \right]$	- -

Table 4: Parameters for double-clumped isotopes

Symbol	Value	Reference/Note
<i>Part V: Double-clumped isotope parameters</i>		
$\Delta_{48,\text{CO}_2}^{\text{eq}}$	$29306/T_K^2 + 93.885/T_K - 0.2914$	Wang et al. (2004)
$\Delta_{64,\text{HCO}_3^-}^{\text{eq}}$	$21842/T_K^2 - 50.457/T_K + 0.0291$	Hill et al. (2020)
$\Delta_{64,\text{CO}_3^{2-}}^{\text{eq}}$	$23492/T_K^2 - 52.842/T_K + 0.0304$	Hill et al. (2020)
$(^{48}R/^{48}R^*)_{\text{CO}_2}^{\text{eq}}$	$\Delta_{48,\text{CO}_2}^{\text{eq}}/1000 + 1$	-
$(^{64}R/^{64}R^*)_{\text{HCO}_3^-}^{\text{eq}}$	$\Delta_{64,\text{HCO}_3^-}^{\text{eq}}/1000 + 1$	-
$(^{64}R/^{64}R^*)_{\text{CO}_3^{2-}}^{\text{eq}}$	$\Delta_{64,\text{CO}_3^{2-}}^{\text{eq}}/1000 + 1$	-
$^{64}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}}$	$(^{64}R/^{64}R^*)_{\text{CO}_3^{2-}}^{\text{eq}} / ((^{64}R/^{64}R^*)_{\text{HCO}_3^-}^{\text{eq}})$	-
$^{64}K_2$	$^{64}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}} \cdot K_2$	-
$^{64}\chi$	$\frac{1}{1 + \frac{^{64}K_2 \cdot ^{64}\alpha_{\text{CO}_3^{2-}-\text{HCO}_3^-}^{\text{eq}}}{[\text{H}^+]}}$	-
$^{18-18}\text{KIE}_{p'_{+1}}$	$1 + 0.049/1000$	Guo (2020)
$^{18-18}\text{KIE}_{s'_{+1}}$	$1 - 0.146/1000$	Guo (2020)
$^{18-18}\text{KIE}_{p'_{+4}}$	$1 - 0.144/1000$	Guo (2020)
$^{18-18}\text{KIE}_{s'_{+4}}$	$1 - 0.086/1000$	Guo (2020)
$p'_{+1}, s'_{+1}$	$p'_{+1} = (^{18-18}\text{KIE}_{p'_{+1}} \cdot b_{+1} \cdot a_{+1})/k_{+1}$ $s'_{+1} = (^{18-18}\text{KIE}_{s'_{+1}} \cdot b_{+1} \cdot b_{+1})/k_{+1}$	-
$p'_{-1}, s'_{-1}$	$p'_{-1} = p'_{+1} / \left[ (^{64}R/^{64}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{18}\alpha_{\text{HCO}_3^--\text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^--\text{H}_2\text{O}}^{\text{eq}} \right]$ $s'_{-1} = s'_{+1} \cdot \left[ (^{48}R/^{48}R^*)_{\text{CO}_2}^{\text{eq}} \right] / \left[ (^{64}R/^{64}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{18}\alpha_{\text{HCO}_3^--\text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^--\text{CO}_2}^{\text{eq}} \right]$	-
$p'_{+4}, s'_{+4}$	$p'_{+4} = (^{18-18}\text{KIE}_{p'_{+4}} \cdot b_{+4} \cdot a_{+4})/k_{+4}$ $s'_{+4} = (^{18-18}\text{KIE}_{s'_{+4}} \cdot b_{+4} \cdot b_{+4})/k_{+4}$	-
$p'_{-4}, s'_{-4}$	$p'_{-4} = p'_{+4} / \left[ (^{64}R/^{64}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1/K_w \cdot ^{18}\alpha_{\text{HCO}_3^--\text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^--\text{OH}-}^{\text{eq}} \right]$ $s'_{-4} = s'_{+4} \cdot \left[ (^{48}R/^{48}R^*)_{\text{CO}_2}^{\text{eq}} \right] / \left[ (^{64}R/^{64}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1/K_w \cdot ^{18}\alpha_{\text{HCO}_3^--\text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^--\text{CO}_2}^{\text{eq}} \right]$	-



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)
$(^{49}R/^{49}R^*)_{\text{CO}_2}^{\text{eq}}$	$\Delta_{49, \text{CO}_2}^{\text{eq}}/1000 + 1$	-
$(^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}}$	$\Delta_{65, \text{HCO}_3^-}^{\text{eq}}/1000 + 1$	-
$(^{65}R/^{65}R^*)_{\text{CO}_3^{2-}}^{\text{eq}}$	$\Delta_{65, \text{CO}_3^{2-}}^{\text{eq}}/1000 + 1$	-
$^{65}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}$	$(^{65}R/^{65}R^*)_{\text{CO}_3^{2-}}^{\text{eq}} / ((^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}})$	-
$^{65}K_2$	$^{65}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot ^{13}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot ^{18}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}} \cdot K_2$	-
$^{65}\chi$	$\frac{1}{1 + \frac{^{65}K_2 \cdot ^{65}\alpha_{\text{CO}_3^{2-} - \text{HCO}_3^-}^{\text{eq}}}{[\text{H}^+]}}$	-
$^{13-18-18}\text{KIE}_{p'_{+1}}$	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)
$p''_{+1}, s''_{+1}$	$p''_{+1} = (^{13-18-18}\text{KIE}_{p'_{+1}} \cdot c_{+1} \cdot b_{+1} \cdot a_{+1})/k_{+1}^2$ $s''_{+1} = (^{13-18-18}\text{KIE}_{s'_{+1}} \cdot c_{+1} \cdot b_{+1} \cdot b_{+1})/k_{+1}^2$	-
$p''_{-1}, s''_{-1}$	$p''_{-1} = p''_{+1} \cdot \left[ (^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}} / \left[ (^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}} \right] \right]$ $s''_{-1} = s''_{+1} \cdot \left[ (^{49}R/^{49}R^*)_{\text{CO}_2}^{\text{eq}} / \left[ (^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1 \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \right] \right]$	-
$p''_{+4}, s''_{+4}$	$p''_{+4} = (^{13-18-18}\text{KIE}_{p'_{+4}} \cdot c_{+4} \cdot b_{+4} \cdot a_{+4})/k_{+4}^2$ $s''_{+4} = (^{13-18-18}\text{KIE}_{s'_{+4}} \cdot c_{+4} \cdot b_{+4} \cdot b_{+4})/k_{+4}^2$	-
$p''_{-4}, s''_{-4}$	$p''_{-4} = p''_{+4} \cdot \left[ (^{47}R/^{47}R^*)_{\text{CO}_2}^{\text{eq}} / \left[ (^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1/K_w \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{OH}}^{\text{eq}} \right] \right]$ $s''_{-4} = s''_{+4} \cdot \left[ (^{49}R/^{49}R^*)_{\text{CO}_2}^{\text{eq}} / \left[ (^{65}R/^{65}R^*)_{\text{HCO}_3^-}^{\text{eq}} \cdot K_1/K_w \cdot ^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot ^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \right] \right]$	-

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,  $[\text{Ca}^{2+}]$ , and SA) and adjustable parameters ( $F_{\text{CO}_2}$ ) used in the model.

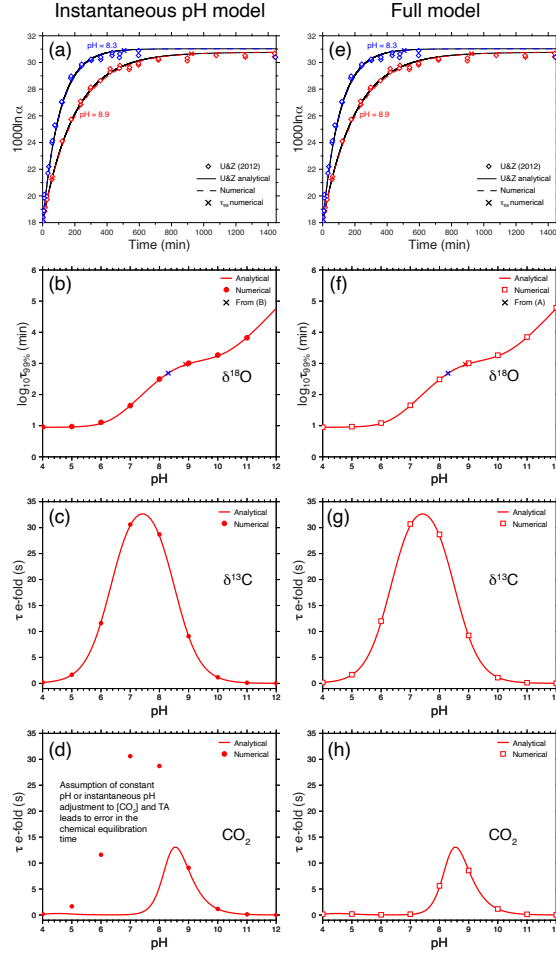


Figure 1: Validation of the ExClump38 model for carbon and oxygen isotopes. In these simulations, the DIC pool is initially equilibrated and then the  $\text{CO}_2$  or  $\text{HCO}_3^-$  is perturbed and the system is allowed to adjust back to equilibrium. The analytical solutions come from Uchikawa 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.

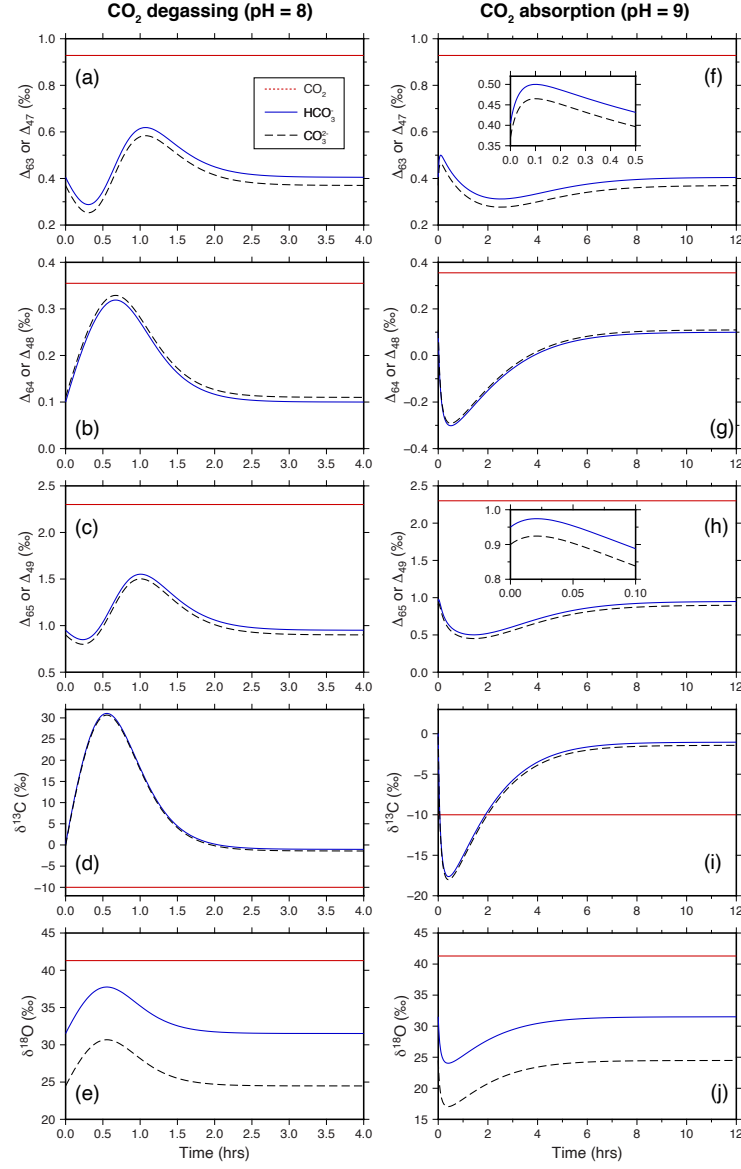


Figure 2: Validation of the ExClump38 model for clumped isotopes. In these simulations, the DIC pool is initially equilibrated and then the  $\text{CO}_2$  is perturbed and held constant and the system is allowed to adjust to the new equilibrium. The curves are indistinguishable from those in Fig. 3 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.

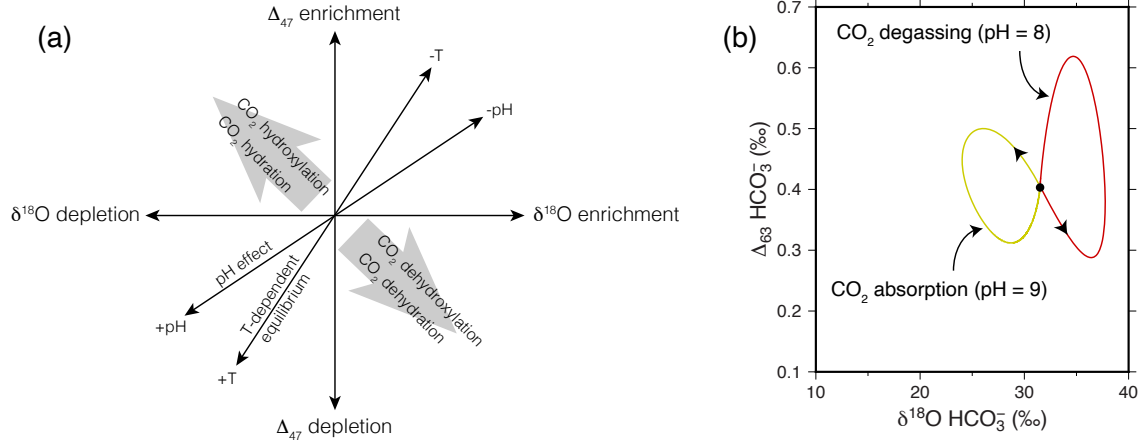
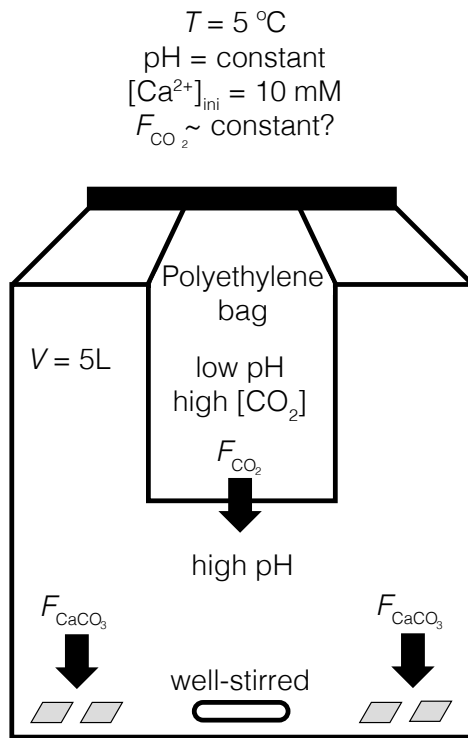


Figure 3: The effects on (de-)hydration and (de-)hydroxylation on  $\Delta_{47}$  and  $\delta^{18}\text{O}$ . (a) Vectors based on calculations by Guo (2008) showing the expected direction of departure from equilibrium (modified from Tripathi et al., 2015). (b) Results from the simulations in Fig. 2 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**



**(b) Isotopic box model**

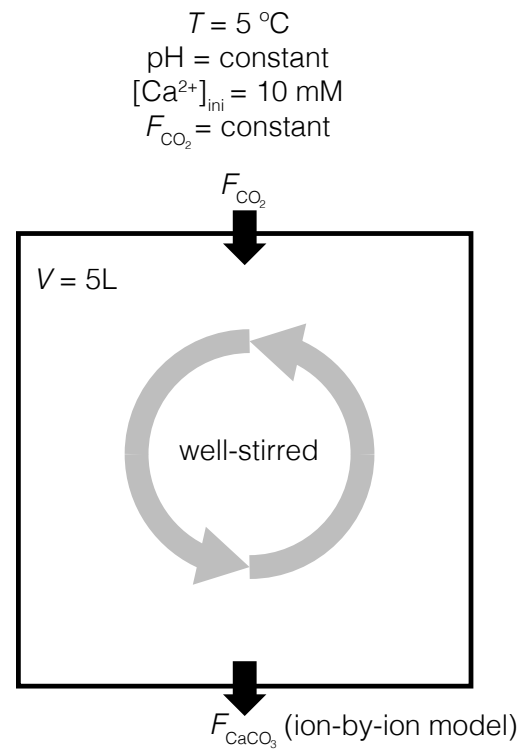


Figure 4: (a) Experimental setup used by Dietzel et al. (2009) and Tang et al. (2014). (b) Setup for the isotopic box model.

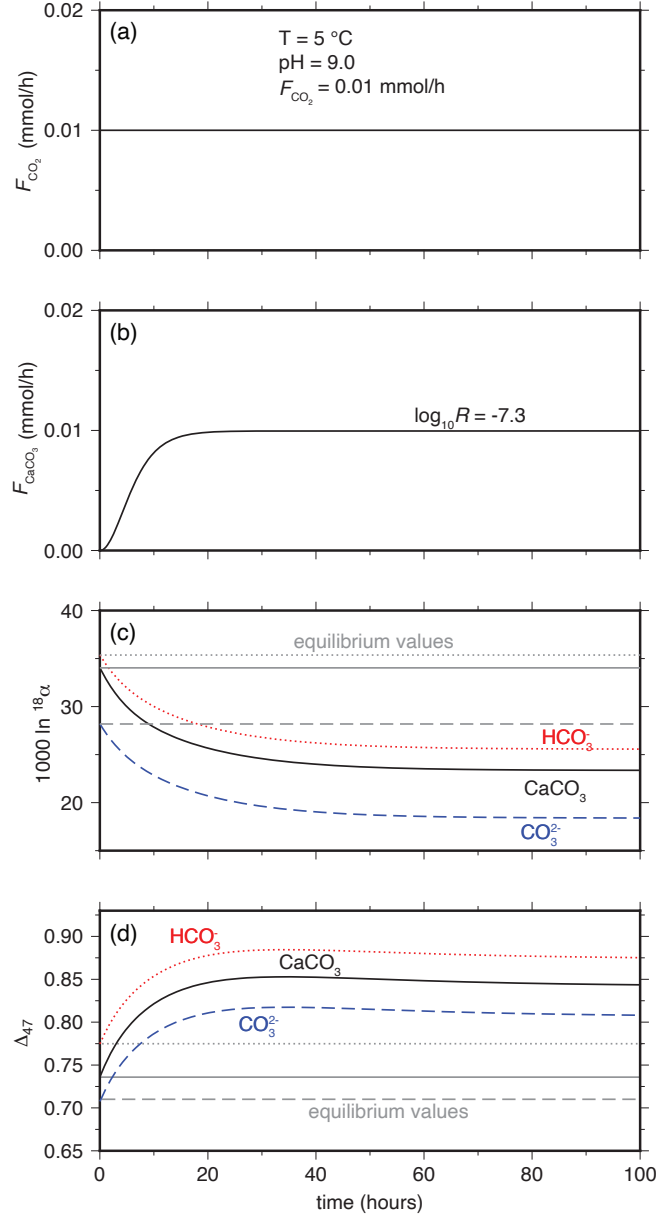


Figure 5: A box model simulation run to steady state. (a)  $F_{\text{CO}_2}$  is held constant throughout. The  $\text{CO}_{2(\text{aq})}$  gets converted to  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ , which increases the degree of supersaturation with respect to calcite. (b)  $F_{\text{CaCO}_3}$  at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

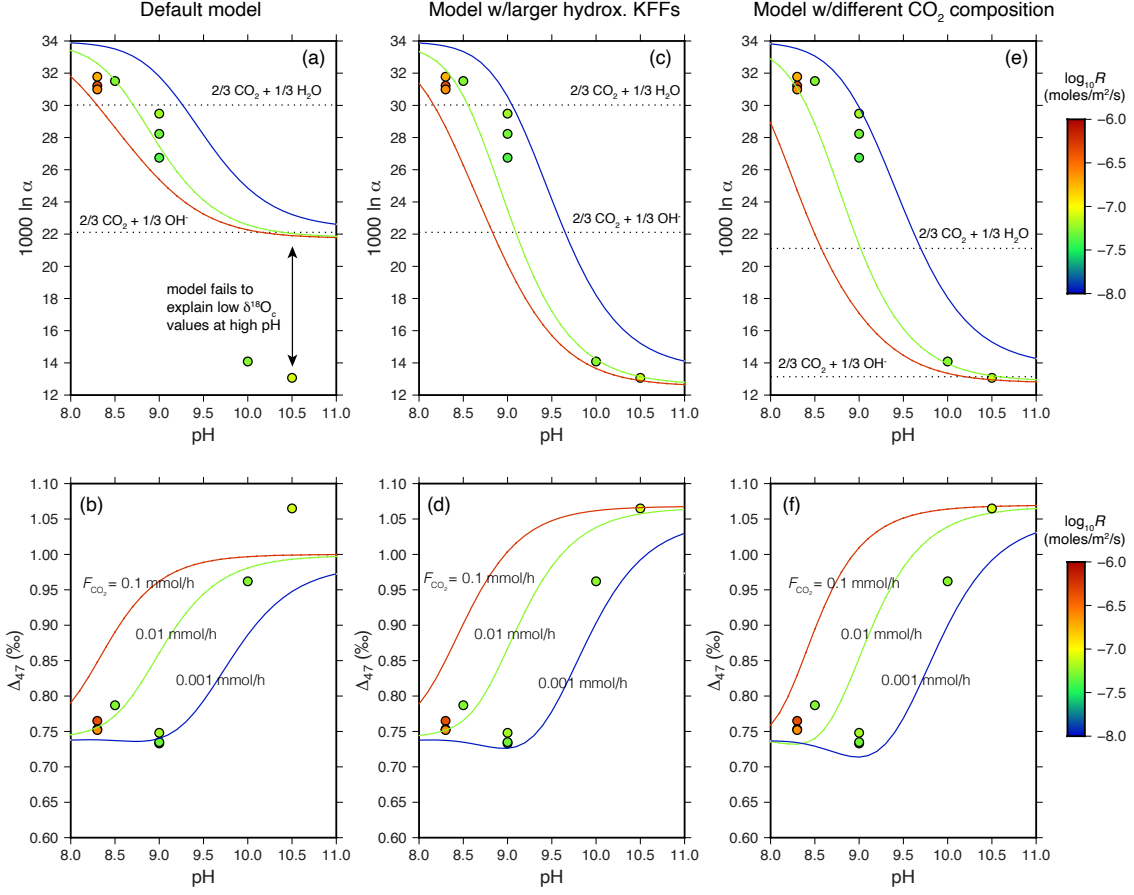


Figure 6: Steady state solutions to the box model for different pH and  $F_{\text{CO}_2}$ . The data points and curves are color-coded according to the steady state growth rate. In the default model (a-b), the KFFs are treated as “known” (Table 2) and the  $\text{CO}_2$  flux is assumed to be in oxygen 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  $\text{CO}_2$  flux having lower  $\delta^{18}\text{O}$  (by 13.7‰) and higher  $\Delta_{47}$  (by 0.11‰). In the latter scenario, the non-equilibrium  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{CO}_2$ . 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,  $\text{H}_28$ , and  $\text{H}_26$ , failure to include the reaction involving both 286 and  $\text{H}_28$  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  $\text{CO}_2$  and heavy  $\text{H}_2\text{O}$ :



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  $\text{CaCO}_3$  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

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3} [\text{H3866}] [\text{H2666}]}{[\text{H3666}] [\text{H2866}]} \cdot \frac{[\text{H2666}] [\text{H}]}{[\text{266}] [\text{H}_2\text{6}]} \cdot \frac{[\text{H2666}]}{[\text{366}]} \cdot \frac{[\text{H2666}]}{[\text{H3666}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H}_2\text{8}]}{[\text{H}_2\text{6}]}. \quad (\text{S.12})$$

Rearranging again yields

$$\frac{p_{+1}}{p_{-1}} = \frac{\frac{1}{3} [\text{H3866}] [\text{H2666}]}{[\text{H3666}] [\text{H2866}]} \cdot \frac{[\text{H2666}] [\text{H}]}{[\text{266}] [\text{H}_2\text{6}]} \cdot \frac{[\text{H3666}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]} \cdot \frac{[\text{366}]}{[\text{266}]} \cdot \frac{[\text{H}_2\text{8}]}{[\text{H}_2\text{6}]}. \quad (\text{S.13})$$

Converting isotopologue ratios to isotope ratios leads to

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

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

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

which upon rearrangement yields

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

Rearranging again yields

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

Converting isotopologue ratios to isotope ratios leads to

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

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

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

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

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

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

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

Rearranging again yields

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

Converting isotopologue ratios to isotope ratios leads to

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

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

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

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

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

### 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  $\text{CO}_2$ , we can write the following isotope exchange reaction:



which has an equilibrium constant

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

The abundance of 288 is measured as:

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

where

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

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  $\Delta_{48}$  are not equivalent:

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

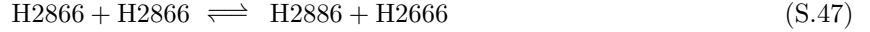
where we have used the relationship from §2.1 (Eq. 17) that, for a stochastic distribution,

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

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  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ :



and



which have equilibrium constants

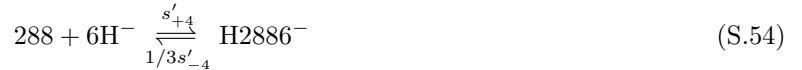
$${}^{64}K_{\text{HCO}_3^-} = \frac{[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} = \frac{1}{3} \left( \frac{{}^{64}R}{{}^{64}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}} = \frac{1}{3} \left( \frac{\Delta_{64,\text{HCO}_3^-}^{\text{eq}}}{1000} + 1 \right) \quad (\text{S.49})$$

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  $\text{CO}_2$  and  $\text{HCO}_3^-$ :



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  $\chi$  terms because the fraction of clumped EIC that is in the form of  $\text{HCO}_3^-$  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  $\Delta_{64}$  values of  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . First, multiply the top and bottom by a common factor:

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

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

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

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

$$\frac{p'_{+1}}{p'_{-1}} = \frac{\frac{\frac{2}{3}[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H}_2\text{6}]}}{\frac{[\text{286}]}{[\text{266}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H2666}]}{[\text{H2866}]} \cdot \frac{[\text{H}_2\text{8}]}{[\text{H}_2\text{6}]}}. \quad (\text{S.67})$$

Rearranging again yields

$$\frac{p'_{+1}}{p'_{-1}} = \frac{\frac{\frac{2}{3}[\text{H2886}][\text{H2666}]}{[\text{H2866}][\text{H2866}]} \cdot \frac{[\text{H2666}][\text{H}]}{[\text{266}][\text{H}_2\text{6}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]} \cdot \frac{[\text{H2866}]}{[\text{H2666}]}}{\frac{[\text{286}]}{[\text{266}]} \cdot \frac{[\text{H}_2\text{8}]}{[\text{H}_2\text{6}]}}. \quad (\text{S.68})$$

Converting isotopologue ratios to isotope ratios leads to

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

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

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



which upon rearrangement yields

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

Rearranging again yields

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

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  $p'_{+4}$ :

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

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

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

which upon rearrangement yields

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

Rearranging again yields

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

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  $s'_{+4}$ :

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

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

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

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

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

which upon rearrangement yields

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

Rearranging again yields

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

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

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

## S.4 Adding triple-clumped isotopes to the model

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:

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

and

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

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

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

### 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  $\text{CO}_2$ , 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

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

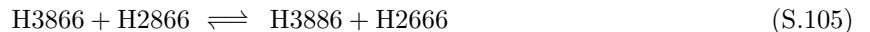
By multiplying the top and bottom by  $[266]$  we have:

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

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  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ :



and



which have equilibrium constants

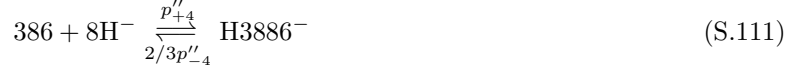
$${}^{65}K_{\text{HCO}_3^-} = \frac{[\text{H3886}][\text{H2666}]}{[\text{H3866}][\text{H2866}]} = \frac{\frac{1}{3} \left( \frac{{}^{65}R}{{}^{65}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}}{\left( \frac{{}^{63}R}{{}^{63}R^*} \right)_{\text{HCO}_3^-}^{\text{eq}}} = \frac{\frac{1}{3} \left( \frac{\Delta_{65,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

and

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

### 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  ${}^{13}\text{O}$ - ${}^{18}\text{O}$ - ${}^{18}\text{O}$  ‘clumps’  
 555 in  $\text{CO}_2$  and  $\text{HCO}_3^-$ :



From these reactions we obtain the following ODEs:

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

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

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

The  $^{65}\chi$  term takes the same form as the other  $\chi$  terms because the fraction of clumped EIC that is in the form of  $\text{HCO}_3^-$  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:

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

This can be expressed in terms of the equilibrium  $\Delta_{65}$  values of  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ . First, multiply the top and bottom by a common factor:

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

which upon rearrangement yields

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

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

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

Rearranging leads to

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

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

$$\frac{p_{+1}''}{p_{-1}''} = \frac{\frac{2}{3}[\text{H}3886][\text{H}]}{[386][\text{H}_28]}. \quad (\text{S.123})$$

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

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

which upon rearrangement yields

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

Rearranging again yields

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

Converting isotopologue ratios to isotope ratios leads to

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

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

$$\frac{p''_{+1}}{p''_{-1}} = \frac{\left( \frac{65}{63} R \right)_{\text{HCO}_3^-}^{\text{eq}}}{\left( \frac{47}{47} R \right)_{\text{CO}_2}^{\text{eq}}} \cdot K_1 \cdot {}^{13}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{CO}_2}^{\text{eq}} \cdot {}^{18}\alpha_{\text{HCO}_3^- - \text{H}_2\text{O}}^{\text{eq}}. \quad (\text{S.128})$$

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:

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

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

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

which upon rearrangement yields

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

Rearranging again yields

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

Converting isotopologue ratios to isotope ratios leads to

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

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  $p''_{+4}$ :

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

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

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

which upon rearrangement yields

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

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

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

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

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

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

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

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

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

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

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