

# Comparison of Experimental vs Theoretical Abundances of $^{13}\text{CH}_3\text{D}$ and $^{12}\text{CH}_2\text{D}_2$ for Isotopically Equilibrated Systems from 1 to 500 °C

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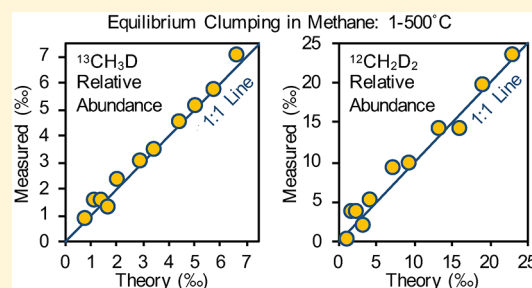
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## S Supporting Information

**ABSTRACT:** Methane is produced and consumed via numerous microbial and chemical reactions in atmospheric, hydrothermal, and magmatic reactions. The stable isotopic composition of methane has been used extensively for decades to constrain the source of methane in the environment. A recently introduced isotopic parameter used to study the formation temperature and formational conditions of methane is the measurement of molecules of methane with multiple rare, heavy isotopes ("clumped") such as  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$ . In order to place methane-clumped isotope measurements into a thermodynamic reference frame that allows calculations of clumped isotope-based temperatures (geothermometry) and comparison between laboratories, all past studies have



calibrated their measurements using a combination of experiment and theory based on the temperature dependence of clumped isotopologue distributions for isotopically equilibrated systems. These have previously been performed at relatively high temperatures (>150 °C). Given that many natural occurrences of methane form below these temperatures, previous calibrations require extrapolation when calculating clumped isotope-based temperatures outside of this calibration range. We provide a new experimental calibration of the relative equilibrium abundances of  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  from 1 to 500 °C using a combination of  $\gamma\text{-Al}_2\text{O}_3$ - and Ni-based catalysts and compare them to new theoretical computations using Path Integral Monte Carlo (PIMC) methods and find 1:1 agreement (within  $\pm 1$  standard error) for the observed temperature dependence of clumping between experiment and theory over this range. This demonstrates that measurements, experiments, and theory agree from 1 to 500 °C, providing confidence in the overall approaches. Polynomial fits to PIMC computations, which are considered the most rigorous theoretical approach available, are given as follows (valid  $T \geq 270$  K):  $\Delta^{13}\text{CH}_3\text{D} \cong 1000 \times \ln(K^{13}\text{CH}_3\text{D}) = (1.47348 \times 10^{19})/T^7 - (2.08648 \times 10^{17})/T^6 + (1.19810 \times 10^{15})/T^5 - (3.54757 \times 10^{12})/T^4 + (5.54476 \times 10^9)/T^3 - (3.49294 \times 10^6)/T^2 + (8.89370 \times 10^2)/T$  and  $\Delta^{12}\text{CH}_2\text{D}_2 \cong 1000 \times \ln(8/3K^{12}\text{CH}_2\text{D}_2) = -(9.67634 \times 10^{15})/T^6 + (1.71917 \times 10^{14})/T^5 - (1.24819 \times 10^{12})/T^4 + (4.30283 \times 10^9)/T^3 - (4.48660 \times 10^6)/T^2 + (1.86258 \times 10^3)/T$ . We additionally compare PIMC computations to those performed utilizing traditional approaches that are the basis of most previous calibrations (Bigeleisen, Mayer, and Urey model, BMU) and discuss the potential sources of error in the BMU model relative to PIMC computations.

**KEYWORDS:** Methane Clumped Isotopes, Methane Isotope Equilibration, Methane Geochemistry, Path Integral Monte Carlo Calculations, 253 Ultra

## 1. INTRODUCTION

Methane is a product and reactant in atmospheric, hydrothermal, and magmatic chemical reactions and in microbial metabolisms. It is also a major component of commercial hydrocarbon deposits. A common first step in the study of methane in the environment, regardless of the application, is to constrain its source. A long-standing approach for this is to use the stable isotopic composition of a methane sample either through comparison of methane  $^{13}\text{C}/^{12}\text{C}$  vs D/H ratios to each other [given as  $\delta^{13}\text{C}$  and  $\delta\text{D}$  values ( $\delta\text{D}_{\text{VSMOW}} = (R_{\text{wg}}/R_{\text{VSMOW}} - 1) \times 1000$  and  $\delta^{13}\text{C}_{\text{VPDB}} = (R_{\text{wg}}/R_{\text{VPDB}} - 1) \times$

$1000$ ;  $R = [\text{D}]/[\text{H}]$  and  $R = [^{13}\text{C}]/[^{12}\text{C}]$ ] (e.g., refs 1 and 2), to the concentration of alkane gases (e.g., methane, ethane, propane, and butane, e.g., ref 3), or to the stable isotopic composition of larger alkane gases (e.g., ref 4).

The measurement of molecules of methane with multiple rare, heavy ("clumped") isotopes such as  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  has

Received: September 16, 2019

Revised: October 26, 2019

Accepted: October 28, 2019

Published: October 28, 2019

provided a new way to study the formational conditions of methane.<sup>5–7</sup> For an isotopically equilibrated system, the abundance of these clumped isotopologues relative to that expected for a random distribution of isotopes among all methane molecules is a monotonic function of temperature.<sup>5–11</sup> Thus, measurement of methane-clumped isotope compositions (relative to a random isotopic distribution) can in principle be used as a geothermometer and to study departures of samples from isotopic equilibrium. Applications of methane-clumped isotope studies include the determination of apparent formation (or re-equilibration) temperatures of methane in subsurface reservoirs and to fingerprint abiogenic, biogenic, and thermogenic methane.<sup>7,12,21,22,13–20</sup>

These capabilities arise from the ability to precisely measure (order per mil) the relative abundances of unsubstituted ( $^{12}\text{CH}_4$ ), singly substituted ( $^{12}\text{CH}_3\text{D}$ ,  $^{13}\text{CH}_4$ ), and multiply substituted isotopologues of methane ( $^{13}\text{CH}_3\text{D}$ ,  $^{12}\text{CH}_2\text{D}_2$ ) using either high-resolution gas-source isotope-ratio mass spectrometers (e.g., refs 5, 7, 23, and 24) or laser absorption spectrometers.<sup>6</sup> Regardless of the technique, measurements are performed relative to commercial high-purity methane “working gases”, which have a priori unknown clumped isotope compositions. As a result, measured methane-clumped isotope compositions are not inherently anchored to an external reference frame such as that set by international standards (which are not available) or set by theoretical expectations of the equilibrium temperature dependence of methane clumping. Past studies have combined experiment and theory to place measured clumped isotope compositions into a reference frame anchored by theoretical expectations of the equilibrium temperature dependence of  $^{13}\text{CH}_3\text{D}$  or  $^{12}\text{CH}_2\text{D}_2$  concentrations vs their expected concentrations for a system in isotopic equilibrium with a random distribution of isotopes.<sup>5–7,14</sup> To accomplish this, previous studies isotopically equilibrated methane isotopologues at temperatures greater than 150 °C in the presence of catalysts that promote C–H bond activation and hydrogen isotope exchange. The measured differences between samples equilibrated at different temperatures were then compared to statistical mechanical-based theoretical calculations of these expected differences (e.g., refs 5–7). All measurements of clumped methane compositions are based on this approach and are performed on a lab-by-lab basis. The accuracy of such “heated gas” calibrations and thus measured methane-clumped isotope compositions and apparent temperatures depends on the accuracy of the theoretical calculations, the experiments, and the isotopic measurements.

### 1.1. Isotope-Exchange Reactions and Nomenclature.

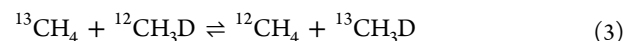
Two clumped methane isotopologues ( $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$ ) have been measured at precisions necessary to calculate clumped isotope-based temperatures at useful precisions ( $\pm 25$  °C) at temperatures < 200 °C for samples with natural abundances of stable isotopes. The abundances of these species for a given measurement are reported using  $\Delta$  notation<sup>25</sup> such that

$$\Delta^{13}\text{CH}_3\text{D} = 1000 \times \left( \frac{[\text{CH}_3\text{D}]/[\text{CH}_4]}{[\text{CH}_3\text{D}]^*/[\text{CH}_4]^*} - 1 \right) \quad (1)$$

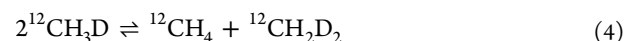
and

$$\Delta^{12}\text{CH}_2\text{D}_2 = 1000 \times \left( \frac{[\text{CH}_2\text{D}_2]/[\text{CH}_4]}{[\text{CH}_2\text{D}_2]^*/[\text{CH}_4]^*} - 1 \right) \quad (2)$$

In eqs 1 and 2, the brackets denote concentrations relative to all other methane isotopologues and the asterisk denotes the calculated concentration of an isotopologue assuming all isotopes of carbon and hydrogen are randomly distributed among all isotopologues (see ref 25 for a more detailed review). These  $\Delta$  values can be related to the following isotope-exchange reactions



and



$K^{13}\text{CH}_3\text{D}$  and  $K^{12}\text{CH}_2\text{D}_2$  describe the equilibrium constants for eqs 3 and 4, respectively.

For isotopically equilibrated systems,  $\Delta$  and  $K$  values are related through the following equations (see derivation in ref 25)

$$\Delta^{13}\text{CH}_3\text{D} \cong 1000 \times \ln(K^{13}\text{CH}_3\text{D}) \quad (5)$$

and

$$\Delta^{12}\text{CH}_2\text{D}_2 \cong 1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right) \quad (6)$$

The 8/3 value is present in eq 6 due to the differing symmetry numbers of the various methane isotopologues in eq 4. The approximate signs are present because we assume that the concentrations of the  $^{13}\text{CH}_4$  and  $^{12}\text{CH}_3\text{D}$  isotopologues are equal to values expected for a random isotopic distribution. This is only approximately true, but as discussed in ref 5, this approximation is valid for our purposes given both the measurement precisions ( $\pm 1$  s.e.) that will be reported below for  $\Delta^{13}\text{CH}_3\text{D}$  ( $\pm 0.25$ – $0.3\%$ ) and  $\Delta^{12}\text{CH}_2\text{D}_2$  ( $\pm 1$ – $1.5\%$ ) as well as the typical  $\delta^{13}\text{C}$  and  $\delta\text{D}$  ranges of environmental samples ( $\sim 70\%$  and  $\sim 500\%$ , respectively).

We note that an additional parameter that was used for the first methane-clumped isotope measurements is  $\Delta_{18}$ .<sup>5</sup> This represents the combined measurements of  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  vs  $^{12}\text{CH}_4$  compared to a random isotopic distribution (see ref 5).  $\Delta_{18}$  values are largely equivalent to  $\Delta^{13}\text{CH}_3\text{D}$  values because 98% of the cardinal mass-18 methane isotopologues are  $^{13}\text{CH}_3\text{D}$  and 2% are  $^{12}\text{CH}_2\text{D}_2$ .

The key point for our purposes here is that the measured  $\Delta$  quantities are directly related to temperature-dependent equilibrium isotope-exchange reactions for isotopically equilibrated systems (i.e., in homogeneous phase equilibrium). Thus, if samples can be isotopically equilibrated at known temperatures and the theoretically expected differences calculated then the  $\Delta$  value of samples can be converted into apparent temperatures based on well-understood quantum-statistical-mechanical theories regardless of the clumped isotopic composition of the reference gas used during measurements.

**1.2. Previous Experimental and Theoretical Determinations of the Temperature Dependence of  $\Delta$  Values for Isotopically Equilibrated Systems.** Experimental calibrations and temperature dependencies of  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  for isotopically equilibrated systems have been conducted at temperatures above 150<sup>14</sup> and 300 °C,<sup>7</sup> respectively, and above 200 °C for  $\Delta_{18}$  values.<sup>5</sup> In contrast, formation temperatures of biogenic gases on earth are typically thought to be below 80 °C (e.g., refs 26 and 27), while thermogenic gases are thought to begin forming as low as 60 °C.<sup>28</sup> Thus, the

potential range of expected gas-formation temperatures in nature is commonly outside of these calibrated ranges. This requires extrapolation of calibrations to lower temperatures and higher  $\Delta$  values to calculate clumped isotope-based temperatures. Stolper and co-workers<sup>5</sup> calibrated equilibrium  $\Delta_{18}$  values at four temperatures (200, 300, 400, and 500 °C) using a nickel-based catalyst that represented a total measured range in  $\Delta_{18}$  of 1.8‰ (quoted internal precision of  $\pm 0.25$ – $0.3$ ‰,  $\pm 1$  s.e., and external precision of  $\pm 0.25$ – $0.3$ ‰,  $\pm 1\sigma$ ). Following this Ono and co-workers<sup>6</sup> calibrated equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  values at three temperatures (200, 300, and 400 °C) using a platinum-based catalyst that represented a total measured range in  $\Delta^{13}\text{CH}_3\text{D}$  of about 1.4‰ (quoted  $\pm 1\sigma$  internal precision of  $\pm 0.1$ ‰ and external  $\pm 1\sigma$  precision of  $\pm 0.35$ ‰; note the external precision incorporates both reproducibility and accuracy;  $\sigma$  = standard deviation). In the same laboratory, Wang and co-workers<sup>14</sup> performed a similar calibration using a platinum catalyst at three temperatures (150, 170, and 250 °C) and a total measured range in  $\Delta^{13}\text{CH}_3\text{D}$  of about 1.2‰. They<sup>14</sup> additionally measured a sample at 400 °C, but this data point was not included in their calibration because it did not fit with the expected theoretical temperature dependence. It was proposed that the sample may have been compromised by potential quench effects. Finally, Young and co-workers<sup>7</sup> calibrated equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values at three temperatures (300, 400, and 500 °C) using a platinum-based catalyst representing a total measured range in  $\Delta^{13}\text{CH}_3\text{D}$  of 1.0‰ and  $\Delta^{12}\text{CH}_2\text{D}_2$  of about 2.2‰ (quoted internal  $\pm 1$  s.e. precision  $\pm 0.15$ ‰ and  $\pm 0.35$ ‰, respectively). The external precision ( $\pm 1\sigma$ ; note this incorporates both reproducibility and accuracy) of these measurements from the same lab was stated to be  $\pm 0.3$ ‰ and  $\pm 1.0$ ‰ for  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ , respectively, in ref 12.

The lack of samples equilibrated at temperatures < 150 °C, despite expectations that biogenic and thermogenic gases could form at such temperatures, is due to the usage of catalysts (nickel and platinum based) that do not facilitate reaction at temperatures < 150 °C over laboratory time scales. For example, the calibration of the equilibrium value for  $\Delta^{13}\text{CH}_3\text{D}$  at 150 °C (representing the lowest clumped methane calibration temperature reported in the above studies) is based on a single experiment that was allowed to react/equilibrate for 110 days.<sup>14</sup> The ability to extend calibrations to lower temperatures using methane equilibrated in the laboratory would allow for more detailed comparisons between theory and experiment and allow apparent clumped isotope-based temperatures to be calculated based on interpolation of calibrations as opposed to extrapolations.

Previous theoretical calculations of equilibrium

$$\Delta^{13}\text{CH}_3\text{D} \cong 1000 \times \ln(K^{13}\text{CH}_3\text{D})$$

and

$$\Delta^{12}\text{CH}_2\text{D}_2 \cong 1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right)$$

values are based on one of two theoretical approaches: (i) the Bigeleisen and Mayer/Urey model<sup>29,30</sup> (BMU, e.g., refs 7–10, 14, and 31), which in practice involves calculations of so-called reduced partition function ratios (RPFs) using a harmonic approximation for the treatment of the vibrational partition function and classical expressions for rotational and translational

partition functions, and (ii) the Path Integral Monte Carlo (PIMC) simulations that avoid the major approximations in the BMU model yielding a fully anharmonic and quantum mechanical description of the partition function ratios.<sup>11</sup>

Both approaches require independent computations of the electronic potential energy surface for methane, which are typically taken from electronic structure calculations based on density functional theory (DFT) or more accurate ab initio wave function theories, such as coupled-cluster theory. Differences in previous theoretical calculations of equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  values given as  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  as a function of temperature based on the BMU model using harmonic frequencies are comparable to the typical internal precision of  $\Delta^{13}\text{CH}_3\text{D}$  measurements ( $\leq 0.2$ ‰ for  $T \geq 0$  °C, e.g., refs 8–10). Cao and Liu<sup>31</sup> initially assessed the effect of ex post facto anharmonic corrections to  $\Delta^{13}\text{CH}_3\text{D}$  and found deviations (up to  $\sim 0.2$ ‰) that are comparable to typical internal precision of  $\Delta^{13}\text{CH}_3\text{D}$  measurements. A later study<sup>9</sup> from the same group applied a series of ex post facto corrections to harmonic RPFs to account for the effects of anharmonicity and many of the other major approximations inherent to the BMU model using computed isotopologue-specific molecular constants following approaches summarized in refs 32 and 33. In this study,<sup>9</sup> smaller differences were found in computed  $\Delta^{13}\text{CH}_3\text{D}$  values given as  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  relative to uncorrected values based on harmonic RPFs, but the differences may be systematic in nature (i.e., from +0.04‰ at 0 °C to +0.07‰ at 500 °C).

Webb and Miller<sup>11</sup> performed theoretical calculations of  $\Delta^{13}\text{CH}_3\text{D}$  values given as  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  using both PIMC and BMU approaches (with and without anharmonic corrections for the BMU approach) based on the same computed electronic potential energy surface<sup>34</sup> for all calculations from 27 to 327 °C. Calculations of  $\Delta^{13}\text{CH}_3\text{D}$  values using both PIMC and BMU-harmonic approaches yielded similar results over the temperature ranges studied (i.e., all are within  $\leq 0.06$ ‰ over 27–327 °C). However, they<sup>11</sup> illustrated that the apparent agreement between the BMU-harmonic and PIMC calculations arises due to a precise cancelation of errors in the harmonically computed RPFs during computation of the equilibrium constant. They<sup>11</sup> further demonstrated that an ex post facto anharmonic correction to the vibrational zero-point energy resulted in comparatively worse agreement (e.g., 0.2–0.4‰ differences in  $\Delta^{13}\text{CH}_3\text{D}$  relative to PIMC<sup>11</sup>). It is important to note that precise error cancelation between PFRs was not universally observed by Webb and Miller.<sup>11</sup> For example, in the isotope-exchange reaction describing position-specific isotope abundances for an isotopically equilibrated system between  $^{14}\text{N}^{15}\text{N}^{16}\text{O}$  and  $^{15}\text{N}^{14}\text{N}^{16}\text{O}$ , an anharmonic correction did yield overall better agreement with PIMC results. This indicates that the partial corrections to the BMU model may or may not improve accuracy of results.

Theoretical calculations of equilibrium  $\Delta^{12}\text{CH}_2\text{D}_2$  values as given by  $1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right)$  as a function of temperature have been performed in two studies based solely on the harmonic BMU model.<sup>7,10</sup> The calculated  $\Delta^{12}\text{CH}_2\text{D}_2$  values from these two studies as a function of temperature are similar (differ  $\leq 0.44$ ‰ for temperatures  $\geq 0$  °C).

**1.3. This Study.** Here, we provide an experimental calibration of equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values from 1 to 500 °C and compare this calibration to new theoretical computations of equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values as a function of temperature using PIMC methods<sup>11,35</sup> and BMU calculations based on the same electronic potential energy surface to facilitate direct comparison. To achieve isotopic equilibrium on laboratory time scales, we use a  $\gamma\text{-Al}_2\text{O}_3$  catalyst to equilibrate methane from 1 to 165 °C and a nickel-based catalyst for higher temperatures (250–500 °C). We then compare these results to the expected differences using different theoretical approaches for computing clumped methane compositions (i.e., PIMC and BMU). We show that the theoretical and experimental measurements are in 1:1 agreement from 1 to 500 °C and thus provide a calibration for the relative equilibrium abundances of both  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  over this temperature range validated by both experiment and theory.

This work including experimental techniques was originally described and presented in two abstracts.<sup>36,37</sup> Following presentation of this work and during the drafting of this manuscript we became aware that Wang et al. (accepted)<sup>38</sup> also recently used  $\gamma\text{-Al}_2\text{O}_3$  catalysts to equilibrate  $\Delta^{13}\text{CH}_3\text{D}$  values of methane at 25 and 100 °C though using a different approach. Their total measured  $\Delta^{13}\text{CH}_3\text{D}$  range is 1.90‰ with analytical precisions of generally  $\pm 1\%$  (95% confidence interval).<sup>38</sup> Both their and our success in equilibrating  $\Delta^{13}\text{CH}_3\text{D}$  using this catalyst demonstrates its general ability to equilibrate methane-clumped isotopic compositions at low (<150 °C) temperatures.

## 2. METHODS

**2.1. House Methane Working Gas, In-House Standards, and Calibration to VSMOW and VPDB.** All methane used in this study was prepared from a single tank of 99.999% pure compressed methane (5.0 Research grade; Praxair). This gas is referred to as either the house methane in the context of experimental preparation or the working gas (wg) in the context of mass spectrometric measurements. Internal reference standards having higher  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ , or  $\Delta^{12}\text{CH}_2\text{D}_2$  values than the house methane were prepared by adding labeled methane to 500 mL glass bottles filled with  $\sim 1$  atm of the house methane. Specifically,  $^{12}\text{CH}_3\text{D}$  (98 atom % D; Sigma-Aldrich),  $^{13}\text{CH}_4$  (99 atom %  $^{13}\text{C}$ ; Sigma-Aldrich), or  $^{12}\text{CH}_2\text{D}_2$  (98 atom % D; Sigma-Aldrich) were added to the house methane to make internal standards with the desired isotopic compositions.

The  $\delta\text{D}$  and  $\delta^{13}\text{C}$  values of the house methane and internal in-house standards were independently determined at the Stable Isotope Facility in the Department of Plant Sciences at UC Davis (SIF-UCD) using standard combustion and pyrolysis techniques previously described.<sup>39</sup>

**2.2. Equilibrated Gas Experiments.** **2.2.1.  $\gamma\text{-Al}_2\text{O}_3$ -Catalyzed Experiments from 1 to 165 °C.** Methane samples were equilibrated from 1 to 165 °C using  $\gamma\text{-Al}_2\text{O}_3$  as a catalyst to activate methane C–H bonds.<sup>40,41</sup> Pellets of  $\gamma\text{-Al}_2\text{O}_3$  (Alfa Aesar) were purchased and activated following a procedure modified from Robertson and co-workers<sup>41</sup> and based on that described by Turner and co-workers.<sup>37</sup>  $\gamma\text{-Al}_2\text{O}_3$  pellets were added to quartz tubes (10 pellets or 0.24–0.28 g per tube) and torched under vacuum to baseline ( $<10^{-3}$  Torr) to drive off adsorbed air and water (10–20 min). Tubes with pellets were then heated at  $\sim 550$  °C in the presence of  $\text{O}_2$  ( $\sim 100$  Torr) for 5

h and then under vacuum (while still held at 550 °C) for 12–14 additional hours prior to being flame sealed by torch under vacuum in the tubes. The activated  $\gamma\text{-Al}_2\text{O}_3$  pellets were stored in the sealed quartz tubes under vacuum at room temperature until used.

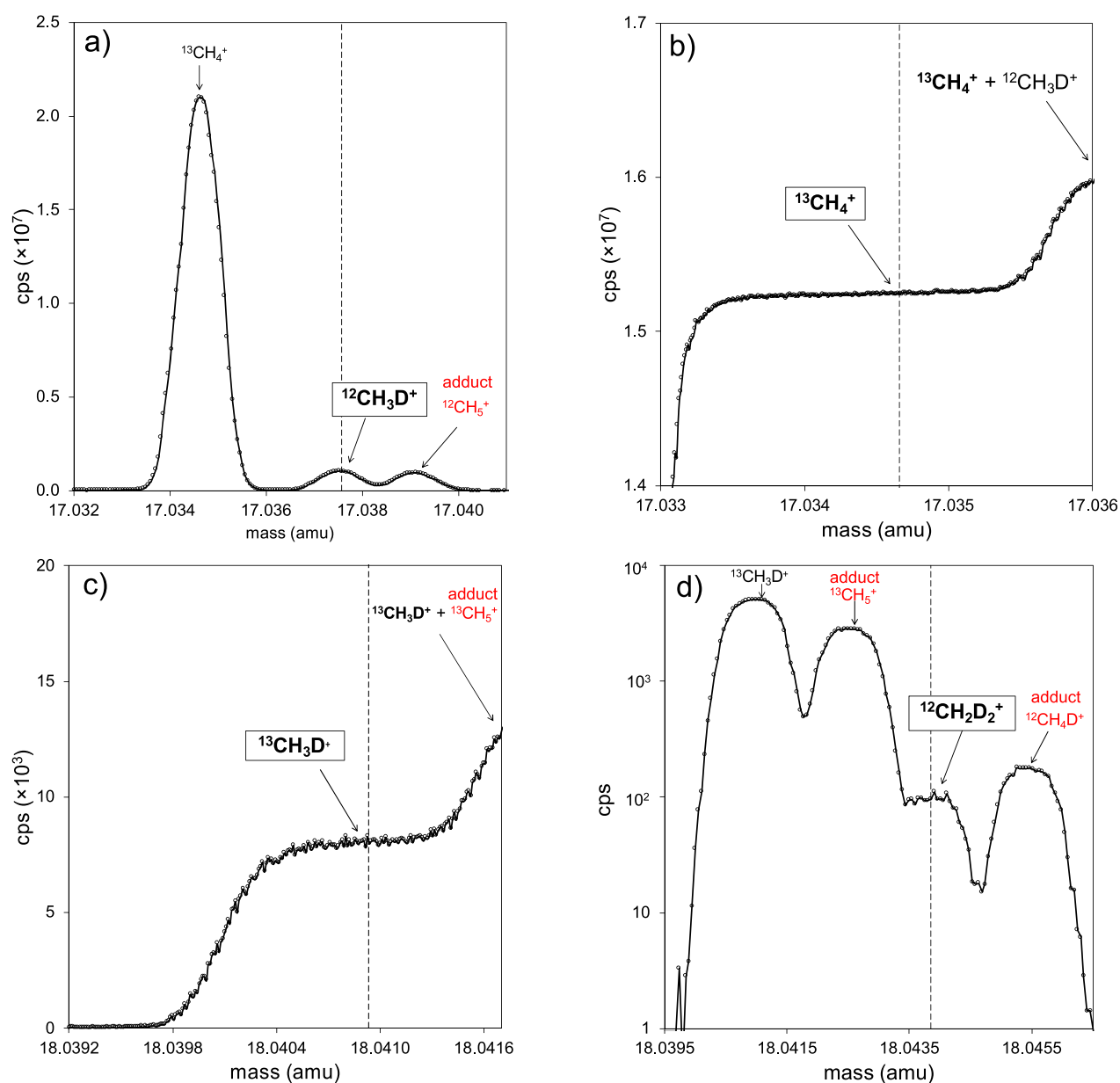
For a given methane equilibration experiment,  $\sim 0.5$  g of activated  $\gamma\text{-Al}_2\text{O}_3$  pellets (20 pellets) was added to a 20 mL borosilicate crimp top headspace vial (SUN SRi), sealed with a crimped septum stopper (blue chlorobutyl; Bellco Glass), and immediately evacuated to baseline ( $<10^{-3}$  Torr) through a needle attached to the vacuum line via an Ultra-Torr fitting (Swagelok). After removal from the vacuum line, the vial was immediately injected with  $\sim 30$  mL of house methane (STP) taken from prefilled Tedlar gas bag (SKC Inc.). The vial was then placed in a temperature-controlled apparatus. For the 50–165 °C experiments, samples were heated using dry-block heaters with digital temperature set points (VWR); for the 25 °C experiment a 5 L water bath was used with a digital temperature set point; for the 1 °C experiment a convertible refrigerator/freezer was used (572 L capacity; Kenmore).

For the 25–165 °C experiments, temperature was monitored using a Type K Chromel/Alumel thermocouple. For the 1 °C experiment, a USB temperature datalogger (Extech) was used. The time allowed for equilibration prior to subsampling was chosen to be in excess of the apparent equilibration times required to attain hydrogen isotope equilibrium between  $\text{CH}_4$  and  $\text{H}_2$  catalyzed by activated  $\gamma\text{-Al}_2\text{O}_3$  determined by Turner and co-workers<sup>37</sup> using a bracketing approach.<sup>42</sup> We assumed here that internal isotopic equilibrium of methane is reached over comparable time scales; we consider this a valid assumption because the equilibration of hydrogen isotopes between methane and hydrogen gas has been demonstrated to proceed at similar rates as between methane isotopologues (cf. ref 43).

Methane samples (3–5 mL) were extracted from vials using gastight syringes (VICI) and immediately injected into a vacuum line for cryogenic purification (see below). Sampling was conducted without removing the vial from the temperature control apparatus for the 25–165 °C experiments in order to avoid temperature perturbations during sampling (i.e., the catalyst remained at the reported temperature during sampling). We minimized the effect of sampling on temperature during the sampling of the refrigeration experiment (1 °C) by completing sampling within  $\sim 1$ –2 min of opening the refrigerator door.

**2.2.2. Catalyzed Experiments from 250 to 500 °C Using Nickel.** Equilibration experiments of methane at higher temperatures (250–500 °C) were performed using a nickel catalyst ( $\sim 66\%$  nickel on silica–alumina; Alfa Aesar) in Pyrex tube experiments following procedures described previously.<sup>5</sup> Briefly, nickel catalyst powder was added to Pyrex tubes and packed with glass wool (Sigma-Aldrich); 5 Å molecular sieve (10 pellets, 1–2 mm diameter by  $\sim 5$  mm in length; Alfa Aesar) was loaded on top of the glass wool. Both nickel and sieve were torched under vacuum to drive off sorbed gases (20 min) prior to quantitatively condensing methane on the sieve using liquid nitrogen and flame sealing the Pyrex tubes. Experiments were placed in the center position of a box furnace (Lindberg/Blue M; ThermoFisher Scientific) and maintained at the designated temperature (250–500 °C) for the specified duration. At the end of the experiment, samples were quenched to room temperature in  $<30$  s using compressed air.

**2.3. Methane Purification and Introduction to the Mass Spectrometer.** Methane from either type of equilibration experiment ( $\gamma\text{-Al}_2\text{O}_3$  or Ni) was purified on a glass vacuum



**Figure 1.** (a–d) Mass scans performed under typical measurement conditions (cps = counts per second). Vertical dashed lines indicate where measurements are made. Boxed species are those that are measured and adducts are highlighted in red text (unboxed species in black are other species). (a) mass-17 peaks; (b) flat  $^{13}\text{CH}_4^+$  shoulder; (c) flat  $^{13}\text{CH}_3\text{D}^+$  shoulder; (d) mass-18 scan showing resolved  $^{12}\text{CH}_2\text{D}_2^+$  (note the logarithmic scale of the y axis for (d)).

line using a cryostat (Cryodyne Refrigerator System; CTI-Cryogenics, Brooks Automation, Inc.) prior to isotopic analysis following a protocol based on previous studies.<sup>5,15</sup> Briefly, methane is first frozen at 20 K in the cryostat (10–15 min equilibration time), and any noncondensable gases present in the headspace of the line are evacuated ( $\leq 2$  min; i.e.,  $\text{H}_2$  produced during the nickel experiments). Remaining noncondensable gases trapped in the condensed methane (i.e.,  $\text{H}_2$  in the nickel experiments or trace air in the  $\gamma\text{-Al}_2\text{O}_3$  experiments introduced during syringe sampling) are then removed by first bringing the cryostat to 45 K, evacuating the noncondensable gases released at this temperature (0.5–2 min), and then implementing a thaw–freeze–evacuation procedure 3–5 times until release of noncondensable gas from condensed methane is no longer detected ( $<10^{-3}$  Torr). Finally, the methane is quantitatively distilled from the cryostat at 70 K to 5 Å molecular

sieve (10 pellets; pretreated by torch under vacuum for 20 min) contained in Pyrex submerged in liquid nitrogen and flame sealed and stored until analysis. Methane frozen to sieve is introduced into the inlet of the 253 Ultra for IRMS analysis using a break seal after heating the sieve at  $\sim 150^\circ\text{C}$  for 3–4 h.<sup>5</sup>

**2.4. Mass Spectrometry: 253 Ultra.** In this section we describe how the isotopic measurements are made. This is done in detail because resolved  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  methane-clumped isotope measurements on the mass spectrometer used (ThermoFisher 253 Ultra) have not yet been described outside of conference proceedings.<sup>36,44</sup> The mass spectrometry schemes described here were originally created to measure fragment methyl groups derived from larger molecules as described by Lloyd and co-workers<sup>45</sup> and adopted for methane measurements. Measurements are organized into “blocks”. At the start of a block, the sample and standard are pressure balanced.

Following this, the working gas standard and sample are measured in a series of “cycles” comprised of integrations and subintegrations following typical sample/standard bracketing techniques for dual inlet isotope-ratio measurements. The total measurement time for  $\delta D$ ,  $\delta^{13}C$ ,  $\Delta^{13}CH_3D$ , and  $\Delta^{12}CH_2D_2$  for a single methane sample is approximately 20–21 h (i.e., 1 sample per day).

**2.4.1. Measurement of  $[^{12}CH_3D^+]/[^{12}CH_4^+ + ^{12}CH_2D^+]$ .** The ratio of  $[^{12}CH_3D^+]/[^{12}CH_4^+ + ^{12}CH_2D^+]$  is determined to derive sample  $\delta D$  values. This measurement is performed in medium-resolution mode ( $\sim 16 \mu m$  entrance slit) with the aperture set to HR+. The mass-16 ion beam consists of  $^{12}CH_4^+ + ^{12}CH_2D^+$  and is measured on the L1 Faraday cup using a  $10^{10} \Omega$  amplifier with a typical intensity of  $2.5\text{--}2.8 \times 10^9$  counts per second (cps). The mass-17 ion beam consists of  $^{12}CH_3D^+$  and is measured on the H4 Faraday cup using a  $10^{12} \Omega$  amplifier with a typical intensity of  $1 \times 10^6$  cps. The mass resolving power (5–95%) is tuned to 20–25 000. At these resolutions, the H4 cup’s narrow exit slit (0.04 mm) allows  $^{12}CH_3D^+$  to be resolved from other proximal mass-17 ions such as  $^{13}CH_4^+$  and the  $^{12}CH_5^+$  adduct (Figure 1a). Mass-16 is measured on the flat shoulder of  $^{12}CH_4^+$  where there is a contribution of the  $^{12}CH_2D^+$  fragment. This is accounted for during data processing as described in section SI.1.

Four measurement blocks consisting of 21 integrations are performed (corresponding to 10 cycles of sample/standard brackets) and begin with an automatic pressure balance. Each integration is comprised of a peak center on  $^{12}CH_3D^+$  that is followed by 75 subintegrations of 0.524 s. We peak center on every integration to ensure the measurement remains on peak due to the narrow region of peak flatness on the H4 cup (Figure 1a). Following the four measurement blocks, the background is measured at higher mass (+0.1 Da) while gas is flowing in for the working gas and the sample. The mean background of both is removed from the measured ion beam intensities of each. The total analysis time for this measurement is approximately 2.5 h. A diagram summarizing this measurement is provided in Figure SI.2.

**2.4.2. Measurement of  $[^{13}CH_4^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$  and  $[^{13}CH_3D^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$ .** The ratios of  $[^{13}CH_4^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$  and  $[^{13}CH_3D^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$  are measured together and used to determine the  $\delta^{13}C$  and  $\Delta^{13}CH_3D$  values of sample. This measurement is performed in high-resolution mode ( $5 \mu m$  entrance slit) with the aperture set to standard. The mass-16 ion beam is made up of  $^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+$  and is registered on the L4 Faraday cup using a  $10^{10} \Omega$  amplifier with a typical signal intensity of  $1.4\text{--}1.6 \times 10^9$  cps. The mass-17 ion beam consists solely of  $^{13}CH_4^+$  and is registered on the L2 Faraday cup through a  $10^{12} \Omega$  amplifier with a typical signal intensity of  $2 \times 10^7$  cps. Finally, the mass-18 ion beam consists solely of  $^{13}CH_3D^+$  and is registered on the H3 compact discrete dynode (CDD) secondary electron multiplier with a typical signal intensity of 8000 cps. The mass-resolving power is tuned to 28–30 000 (5–95%) such that  $^{13}CH_4^+$  and  $^{13}CH_3D^+$  are measurable on flat shoulders (Figure 1b and 1c). The measurement is performed in 8 blocks where each block is comprised of 21 standard/sample integrations (corresponding to 10 cycles per block that represent sample/standard brackets). Each integration consists of 60 discretized 1.05 s subintegrations. Measurements are centered on the flat shoulder of  $^{13}CH_3D^+$ . This center position is determined every two blocks

(4× total) beginning with the first block. Background measurements for all beams (masses 16, 17, and 18) for the sample and then the working gas are performed at the end of every two blocks (4× total) by moving down-mass ( $-0.0089$  Da) while gas is flowing into the source. In the case of each ion beam, the mean background of the sample and working gas is taken and then subtracted from the raw intensities of both sample and working gas for each block pair. The presence of the  $^{13}CH_3^+ + ^{12}CH_2D^+$  fragments on the mass-16 intensity are corrected for as described in section SI.1. The total analysis time for this measurement is approximately 5.5 h. A diagram summarizing this measurement is provided in Figure SI.3.

**2.4.3. Measurement of  $[^{12}CH_2D_2^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$ .** The  $[^{12}CH_2D_2^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$  ratio is measured to determine the  $\Delta^{12}CH_2D_2$  value of a sample. This measurement is performed in high-resolution mode ( $5 \mu m$  entrance slit) with the aperture set to standard. The mass-16 ion beam consists of  $^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+$  and is registered on the L3 Faraday cup with a  $10^{10} \Omega$  amplifier with a typical signal size of  $1.4\text{--}1.8 \times 10^9$  cps. The mass-18 ion beam consists of  $^{12}CH_2D_2^+$  and is registered on the H4 CDD with a typical signal size of 60–100 cps. The mass-resolving power is tuned to 28–32 000 (5–95%) such that  $^{12}CH_2D_2^+$  is separated from proximal mass-18 adducts ( $^{13}CH_5^+$  and  $^{12}CH_4D^+$ ; Figure 1d).

The measurement is performed in 18 blocks where each block is comprised of 21 standard/sample integrations (i.e., 10 cycles of sample/standard brackets). Each integration begins with a peak center on  $^{13}CH_3D^+$  that is followed by a magnet peak hop of 0.00292 Da to the center of  $^{12}CH_2D_2^+$ . We then perform 60 discretized 1.05 s subintegrations. A background measurement is taken at the end of the last block by moving up in mass (+0.1 Da) while gas is flowing. The mean background for sample and working gas is determined and then subtracted from both the sample and the working gas ion-beam intensities. The total analysis time for this measurement is approximately 12–13 h. Finally, we correct for the contributions of peak tailing of the adjacent  $^{13}CH_3D^+$  and  $^{13}CH_5^+$  peaks to the total background at  $^{12}CH_2D_2^+$  based on the methodology originally devised and presented by Xie and co-workers.<sup>44</sup> These corrections are typically  $\sim 0.35$  cps. A description of this correction is provided in detail in section SI.2. A diagram summarizing this measurement is provided in Figure SI.4.

#### 2.4.4. Calculation of $\delta D$ , $\delta^{13}C$ , $\Delta^{13}CH_3D$ , and $\Delta^{12}CH_2D_2$ Values.

The calculation of  $\delta D$ ,  $\delta^{13}C$ ,  $\Delta^{13}CH_3D$ , and  $\Delta^{12}CH_2D_2$  values from the above ratios requires a correction be made for the  $^{12}CH_2D^+$  and  $^{13}CH_3^+$  fragments present in the measurements. This is done by determining a fragmentation ratio that describes the relative abundance of a fragment methyl ion vs an unfragmented methane ion. The fragmentation ratio for methyl groups from methane is determined operationally during the  $[^{13}CH_4^+]/[^{12}CH_4^+ + ^{13}CH_3^+ + ^{12}CH_2D^+]$  measurement (see section 2.4.2) by measuring the  $[^{13}CH_3^+]/[^{13}CH_4^+]$  ratio after every two blocks before the background measurements are taken (4× total). The fragmentation ratio for the 253 Ultra at UC Berkeley is  $\sim 0.8$ , which is the same as that measured by Stolper and co-workers<sup>5</sup> on the 253 Ultra prototype instrument. From the measured ion ratios above and the determined fragmentation ratio, the  $\delta D$ ,  $\delta^{13}C$ ,  $\Delta^{13}CH_3D$ , and  $\Delta^{12}CH_2D_2$  values can be determined. The equations used to do this are given in section SI.1.

Calculation of  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{13}\text{CH}_2\text{D}_2$  for a measured sample requires knowledge of the  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  composition of the working gas (house methane), which a priori is unknown. Therefore, measurements are initially placed into a working gas reference frame by assuming that the compositions of the working gas correspond to  $\Delta^{13}\text{CH}_3\text{D} = 0\text{‰}$  and  $\Delta^{12}\text{CH}_2\text{D}_2 = 0\text{‰}$ . Measurements reported in the working gas reference frame in this study will be denoted with wg for clarity (i.e.,  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$  and  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})$ ). We emphasize that measurements reported relative to a working gas are in an arbitrary reference frame that is not rooted in thermodynamics or internationally recognized standards (which do not exist for methane-clumped isotope measurements). Such measurements can and will be converted to the thermodynamic (or absolute) reference frame as covered in section 4.1 based on our experiments and theoretical calculations.

**2.5. Theoretical Calculations of Equilibrium Methane Clumping.** This section briefly describes how partition function ratios (PFRs) are computed using the PIMC (e.g., refs 11 and 35) and BMU<sup>29,30</sup> theoretical frameworks. In this study, the potential energy surface for methane, which is required for both the PIMC and the BMU calculations, is taken from Lee and co-workers;<sup>34</sup> this potential energy surface is constructed from calculations at the CCSD(T) level of theory using cc-pVTZ and cc-pVQZ basis sets (see Lee and co-workers<sup>34</sup> for additional details regarding the construction of the potential energy surface). We base our PIMC and BMU theoretical calculations on the same potential energy surface for methane<sup>34</sup> in order to obtain a direct comparison between the two theoretical approaches for computing PFRs.<sup>11</sup>

**2.5.1. Path Integral Monte Carlo (PIMC) Calculations.** The Path Integral Monte Carlo (PIMC) technique employs the imaginary-time path integral formalism<sup>46</sup> to map the quantum mechanical partition function (PF) onto a classical PF<sup>47</sup>

$$Q(N, \beta) = \lim_{P \rightarrow \infty} \frac{1}{\sigma} \prod_{i=1}^N \left( \frac{m_i P}{2\pi\beta\hbar^2} \right)^{3P/2} \int \prod_{j=1}^N \prod_{k=1}^P d\mathbf{r}_j^{(k)} e^{-\beta_p U_p(\{\mathbf{r}_j^{(k)}\})} \quad (7)$$

such that the quantum Boltzmann statistics of the  $N$ -particle system is obtained from the classical statistics of a ring polymer with  $P$  beads at inverse temperature  $\beta_p = \beta/P$  that interact via an effective potential

$$U_p(\{\mathbf{r}_j^{(k)}\}) = \sum_{j=1}^N \sum_{k=1}^P \frac{m_j \omega_p^2}{2} (\mathbf{r}_j^{(k)} - \mathbf{r}_j^{(k-1)})^2 + \sum_{k=1}^P U(\mathbf{r}_1^{(k)}, \dots, \mathbf{r}_N^{(k)}) \quad (8)$$

Here,  $\mathbf{r}_j^{(k)}$  indicates the position of the  $j$ th atom in the  $k$ th ring polymer bead,  $\omega_p = 1/(\beta_p \hbar)$  is the intrabead vibrational frequency,  $\mathbf{r}_j^{(0)} = \mathbf{r}_j^{(P)}$ , and  $U(\mathbf{r}_1, \dots, \mathbf{r}_N)$  is the Born–Oppenheimer potential energy surface for the molecular system. Note that the indistinguishability of identical nuclei in the PI calculations in eq 7 is treated using the classical rotational symmetry number,  $\sigma$ , since effects related to nuclear exchange statistics are expected to be negligible for the temperatures and isotope-exchange reactions considered in this study.

The methodology for computing PFRs and equilibrium constants in this study follows that employed by Webb and co-workers<sup>35</sup> and is only briefly reviewed here. In short, a direct scaled-coordinate estimator<sup>48</sup> is used to calculate the PFRs. Heavy isotopologue configurations are sampled with PIMC in

Cartesian coordinates with an explicit staging transformation.<sup>49</sup> The staging length,  $j$ , is set such that 38–42% of all proposed staging moves are accepted. Prior to any data collection, each sampling trajectory is equilibrated for  $10^5$  MC steps, with  $P/j$  staging moves (rounded up to the nearest integer) attempted per MC step. Thereafter, ring-polymer configurations are sampled every 10 MC steps. The total number of MC moves for each PFR calculation is  $2 \times 10^8$  ( $10^9$  for select  $T$  values: 1.2, 50.5, 75.7, 127.8, and 165.4 °C). There are two primary sources of error in the PIMC calculations, aside from any errors due to the potential energy surface. The first is systematic error related to convergence of the PFs (or derived quantities) with the number of beads; this error vanishes in the limit of infinite beads as expressed in eq 7. The second is statistical error related to sampling of the direct scaled-coordinate estimator for the PFRs; this error vanishes in the limit of infinite sampling. In this study, the number of beads employed in the PIMC calculations is determined based on explicit convergence tests for the individual PFRs (see Figure SI.5) and summarized in Table SI.1. All errors reported for the PIMC calculations reflect standard errors related to statistical uncertainty with the Monte Carlo sampling method.

**2.5.2. Bigeleisen and Mayer/Urey Model Calculations.** The partition function ratio of an isotopologue pair based on the Bigeleisen and Mayer/Urey (BMU) model<sup>29,30</sup> is given by<sup>11,35</sup>

$$\text{PFR}_{\text{BMU}} = \frac{Q^*}{Q} = \frac{\sigma}{\sigma^*} e^{-(E_0^* - E_0)/k_B T} \prod_{i=1}^N \left( \frac{m_i^*}{m_i} \right)^{3/2} \prod_{j=1}^a \frac{\omega_j^*}{\omega_j} \frac{1 - e^{-\hbar\omega_j/k_B T}}{1 - e^{-\hbar\omega_j^*/k_B T}} \quad (9)$$

where  $\sigma$  are rotational symmetry numbers,  $E_0$  is the zero-point energy,  $m_i$  is the mass of the  $i$ th atom in a molecule of  $N$  atoms,  $\omega_j$  is the harmonic frequency (given as wavenumber) of the  $j$ th normal mode,  $a$  is the total number of vibrational modes ( $a = 3N - 5$  for linear molecules,  $a = 3N - 6$  for nonlinear molecules), and the asterisk indicates the isotopically substituted molecule. The BMU model is arranged to compute PFRs in terms of substituted isotopologues (i.e.,  $^{12}\text{CH}_3\text{D}$ ,  $^{13}\text{CH}_4$ ,  $^{13}\text{CH}_3\text{D}$ ,  $^{12}\text{CH}_2\text{D}_2$ ) relative to the unsubstituted isotopologue ( $^{12}\text{CH}_4$ ). In this study, we will diverge from this convention in reporting PFRs (after ref 11) and will instead specify the isotopologues comprising a PFR.

The so-called reduced partition function ratio (RPFR) that is commonly reported in the stable isotope geochemistry literature (e.g., refs 9, 10, 31–33, and 50) was first recommended by the original authors of the BMU model for simplicity<sup>29,30</sup> and is defined by convention as the above (eq 9) but is normalized for the mass terms<sup>50</sup> and the rotational symmetry numbers. The reasons for these normalizations come from the traditional application of the BMU model to problems of single isotope-exchange reactions among molecules and the observation that the mass terms always cancel in the computation of fractionation factors and/or isotope exchange equilibrium constants and that the rotational symmetry numbers are not responsible for relative isotopic differences between different phases or species.<sup>29</sup> We compute and discuss PFRs rather than RPFRs in this work. We provide computations of RPFRs for both PIMC and BMU calculations in the Supporting Information (see Table SI.5). We additionally provide harmonic vibrational frequencies for methane isotopologues derived from the potential energy surface of methane of Lee and co-workers<sup>34</sup> in Table SI.6 that were utilized for our BMU calculations.

### 3. RESULTS

**3.1. Accuracy and Precision.** In order to determine both our external precision as well as our accuracy, three gas standards with different bulk and clumped isotopic compositions were prepared and measured in multiple analytical sessions. The standards “PlusD” and “PlusD-200” were prepared to have higher  $\delta D$  values relative to the working gas (+88‰ and +208‰, respectively), and the “Plus<sup>13</sup>C” standard was prepared to have a higher  $\delta^{13}C$  value relative to the working gas (+34‰). They were replicated 9, 8, and 4 times, respectively, over different analytical sessions. Eight distinct analytical sessions are represented in our data set. All individual replicate measurements are provided in Table SI.2. A summary table of the average measured values along with external precisions is given in Table 1.

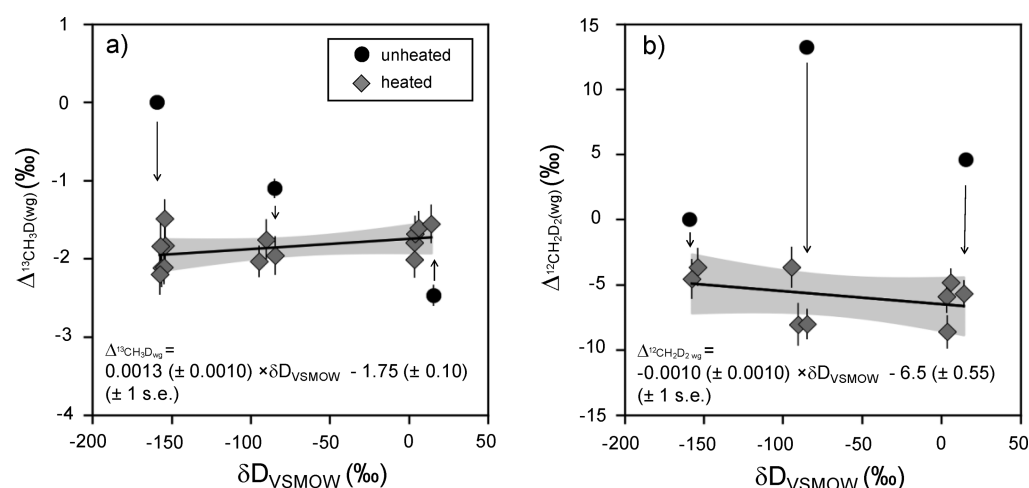
On the basis of the reproducibility of these in-house standards, we estimate that our  $\pm 1\sigma$  external precision for each measured isotopic parameter is  $\delta D = \pm 0.15\text{‰}$ ,  $\delta^{13}C = \pm 0.02\text{‰}$ ,  $\Delta^{13}CH_3D = \pm 0.33\text{‰}$ , and  $\Delta^{12}CH_2D_2 = \pm 1.35\text{‰}$ . These are similar to the measured internal precisions:  $\delta D = \pm 0.12\text{‰}$ ,  $\delta^{13}C = \pm 0.01\text{‰}$ ,  $\Delta^{13}CH_3D = \pm 0.25\text{‰}$ ,  $\Delta^{12}CH_2D_2 = \pm 1.35\text{‰}$  ( $\pm 1$  s.e.).

In order to place our  $\delta D$  and  $\delta^{13}C$  measurements on the VSMOW and VPDB reference scales and establish the accuracy of our measurements, the  $\delta D$  and  $\delta^{13}C$  values of the house methane (working gas) and in-house reference standards were measured at the Stable Isotope Facility of UC Davis following methods described in ref 39. At this lab methane stable isotope measurements are anchored to international methane standards with known  $\delta D$  and  $\delta^{13}C$  values. At UC Davis our reference gas was triplicated for both  $\delta D$  and  $\delta^{13}C$ , while the other standards were duplicated for these parameters. The  $\delta D$  and  $\delta^{13}C$  values of these in-house standards as determined at UC Davis relative to the VSMOW and VPDB scales are presented in Table 1. The working gas was determined to have a  $\delta D_{VSMOW} = -159.3\text{‰}$  ( $\pm 2.4\text{‰}$ ,  $\pm 1\sigma$ ;  $\pm 1.4\text{‰}$ ,  $\pm 1$  s.e.) and  $\delta^{13}C_{VPDB} = -38.37\text{‰}$  ( $\pm 0.04\text{‰}$ ,  $\pm 1\sigma$ ;  $\pm 0.02$ ,  $\pm 1$  s.e.). We assign these values to the UC Berkeley working gas. With this assignment we can directly compare determinations of  $\delta D$  and  $\delta^{13}C$  between samples (PlusD, PlusD-200, and Plus<sup>13</sup>C) measured on the 253 Ultra at UC Berkeley and measured using conventional pyrolysis and combustion techniques at UC Davis (Table 1). The values of  $\delta D_{VSMOW}$  as measured on the 253 Ultra are within  $\leq 4.4\text{‰}$  of the conventional analyses (note: typical  $\pm 1\sigma$  external precision for  $\delta D-CH_4$  at UC Davis is  $\pm 4\text{‰}$ <sup>39</sup>). A least-squares linear regression of  $\delta D_{VSMOW}$  (conventional-UC Davis) vs  $\delta D_{VSMOW}$  (253 Ultra-UC Berkeley) yields a slope of  $1.01 \pm 0.02$  (1 s.e.). The 253 Ultra measurements of  $\delta^{13}C_{VPDB}$  are within  $\leq 0.56\text{‰}$  of the conventional measurements (note: typical  $\pm 1\sigma$  external precision for  $\delta^{13}C-CH_4$  at UC Davis is  $\pm 0.2\text{‰}$ <sup>39</sup>). The distribution in values of  $\delta^{13}C_{VPDB}$  for the in-house standards is insufficient to perform a meaningful regression for additional comparison (i.e., only one of the three standards differs greatly in  $\delta^{13}C_{VPDB}$ ). On the basis of these comparisons we consider our measurements of  $\delta D$  and  $\delta^{13}C$  values using the 253 Ultra to be accurate.

We test an aspect of our  $\Delta^{13}CH_3D$  or  $\Delta^{12}CH_2D_2$  accuracy as follows. Correctly measured  $\Delta^{13}CH_3D$  or  $\Delta^{12}CH_2D_2$  for samples isotopically equilibrated at the same temperature should be constant and should not depend on  $\delta D$  or  $\delta^{13}C$  values. To demonstrate this we follow the approach outlined previously<sup>5</sup>

Table 1. Measurements of In-House Reference Standards Including the Comparison between Conventional (UC Davis) and 253 Ultra Measurements<sup>a</sup>

| conventional (UC Davis)  |     |                    |             |                 |                       |             |                 |     |                    | 253 Ultra (UC Berkeley) |                 |                       |           |                 |                        |                        |                      |           |                 | $n^f$ |  |
|--|-----|--------------------|-------------|-----------------|-----------------------|-------------|-----------------|-----|--------------------|-------------------------|-----------------|-----------------------|-----------|-----------------|------------------------|------------------------|----------------------|-----------|-----------------|-------|--|
| sample   | $n$ | $\delta D_{VSMOW}$ | $1\sigma^b$ | $1\text{ s.e.}$ | $\delta^{13}C_{VPDB}$ | $1\sigma^c$ | $1\text{ s.e.}$ | $n$ | $\delta D_{VSMOW}$ | $1\sigma$               | $1\text{ s.e.}$ | $\delta^{13}C_{VPDB}$ | $1\sigma$ | $1\text{ s.e.}$ | $\Delta^{13}CH_3D(wg)$ | $\Delta^{13}CH_3D(wg)$ | $\Delta^{12}CH_2D_2$ | $1\sigma$ | $1\text{ s.e.}$ | $n^f$ |  |
| house  | 3   | -159.3             | 2.4         | 1.4             | -38.37                | 0.04        | 0.02            |     | -159.3             | 0.13                    | 0.04            | (-38.37)              | 0.03      | 0.01            | (0) <sup>d</sup>       | (0) <sup>d</sup>       | (5.86) <sup>e</sup>  | 1.63      | 0.58            | 8     |  |
| PlusD  | 2   | -83.2              | 1.1         | 0.8             | -38.34                | 0.14        | 0.10            | 9   | -84.93             | 0.20                    | 0.07            | -38.37                | 0.01      | 0.00            | -1.10                  | 13.22                  | 19.10                | 1.41      | 0.50            | 8     |  |
| PlusD-200  | 2   | 20.0               | 0.1         | 0.1             | -38.02                | 0.33        | 0.23            | 8   | 15.49              | 0.08                    | 0.04            | -38.33                | 0.01      | 0.00            | -2.47                  | 4.58                   | 10.39                | 1.41      | 0.50            | 8     |  |
| Plus <sup>13</sup> C   | 2   | -155.5             | 1.5         | 1.1             | -5.26                 | 0.22        | 0.16            | 4   | -158.82            | 0.08                    | 0.04            | -5.82                 | 0.02      | 0.01            | 4.56                   | 1.73                   | 7.60                 | 0.26      | 0.15            | 3     |  |
| <sup>a</sup> All compositions reported in units of permil (‰). Assigned values for the house methane are in parentheses. $\sigma$ = standard deviation. <sup>b</sup> Typical external precision for $\delta D-CH_4$ measurements at UC Davis is $\pm 4\text{‰}$ ( $\pm 1\sigma$ ). <sup>c</sup> Typical external precision for $\delta^{13}C-CH_4$ measurements at UC Davis is $\pm 0.2\text{‰}$ ( $\pm 1\sigma$ ). <sup>d</sup> Compositions reported in the working gas reference frame where the composition of the working gas (house methane) is assumed to be $\Delta^{13}CH_3D = \Delta^{12}CH_2D_2 = 0\text{‰}$ (see section 2.4.4). <sup>e</sup> Compositions reported in the thermodynamic reference frame where working gas (house methane) is assigned $\Delta^{13}CH_3D = 2.59\text{‰}$ and $\Delta^{12}CH_2D_2 = 5.86\text{‰}$ based on the calibration provided in this study (see section 4.1). <sup>f</sup> A separate $n$ is reported for the $\Delta^{13}CH_3D_3$ measurements because one run each of the PlusD and Plus <sup>13</sup> C standards did not include this measurement. |     |                    |             |                 |                       |             |                 |     |                    |                         |                 |                       |           |                 |                        |                        |                      |           |                 |       |  |



**Figure 2.** (a and b) Measurements of  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$  and  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})$  as a function of  $\delta\text{D}_{\text{VSMOW}}$  values for 500 °C heated gas experiments. Black circles indicate the compositions of the starting gases (which correspond to the in-house reference standards). Gray diamonds represent the compositions measured after heating at 500 °C in the presence of Ni-based catalysts. All error bars are  $\pm 1$  s.e. and reflect the internal precision of the individual measurements. Least-squares linear regression (black line, with gray 95% confidence intervals) yields no significant dependence of either  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$  or  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})$  on the  $\delta\text{D}_{\text{VSMOW}}$  of the gas.

**Table 2. Summary of Equilibrated Methane Experiments<sup>a</sup>**

| <i>T</i> (°C)    | catalyst                       | duration (h) | $\Delta^{13}\text{CH}_3\text{D}(\text{wg})^c$ | $\Delta^{13}\text{CH}_3\text{D}^d$ | 1 $\sigma$ | 1 s.e. | <i>n</i> | $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})^c$ | $\Delta^{12}\text{CH}_2\text{D}_2^d$ | 1 $\sigma$ | 1 s.e.            | <i>n</i> |
|------------------|--------------------------------|--------------|---|------------------------------------|------------|--------|----------|---|--------------------------------------|------------|-------------------|----------|
| 500 <sup>b</sup> | Ni                             | 1–4          | −1.82   | 0.76                               | 0.23       | 0.06   | 16       | −5.88   | −0.06                                | 1.92       | 0.64              | 9        |
| 400              | Ni                             | 4–6          | −1.14   | 1.45                               | 0.51       | 0.36   | 2        | −2.39   | 3.45                                 | 0.40       | 0.28              | 2        |
| 350              | Ni                             | 9–12         | −1.12   | 1.46                               | 0.17       | 0.10   | 3        | −2.41   | 3.44                                 |            | 1.21 <sup>e</sup> | 1        |
| 300              | Ni                             | 24–65        | −1.33   | 1.26                               | 0.16       | 0.11   | 2        | −3.98   | 1.86                                 | 1.71       | 1.21              | 2        |
| 250 <sup>b</sup> | Ni                             | 72–184       | −0.31   | 2.27                               | 0.34       | 0.19   | 3        | −0.85   | 5.00                                 | 2.48       | 1.43              | 3        |
| 165.4            | $\gamma\text{-Al}_2\text{O}_3$ | 6.5–8.5      | 0.35  | 2.94                               | 0.30       | 0.21   | 2        | 3.00  | 8.88                                 | 1.43       | 1.01              | 2        |
| 127.8            | $\gamma\text{-Al}_2\text{O}_3$ | 23–25.5      | 0.77  | 3.36                               | 0.11       | 0.08   | 2        | 3.76  | 9.64                                 | 0.58       | 0.41              | 2        |
| 75.7             | $\gamma\text{-Al}_2\text{O}_3$ | 233–288      | 1.89  | 4.48                               | 0.21       | 0.15   | 2        | 8.14  | 14.05                                | 1.96       | 1.39              | 2        |
| 50.5             | $\gamma\text{-Al}_2\text{O}_3$ | 292–478.5    | 2.49  | 5.09                               | 0.24       | 0.17   | 2        | 8.18  | 14.09                                | 0.46       | 0.33              | 2        |
| 25               | $\gamma\text{-Al}_2\text{O}_3$ | 551–622      | 3.04  | 5.64                               | 0.07       | 0.05   | 2        | 13.70   | 19.64                                | 0.95       | 0.67              | 2        |
| 1.2              | $\gamma\text{-Al}_2\text{O}_3$ | 503.5–816    | 4.42  | 7.02                               | 0.09       | 0.06   | 2        | 17.54   | 23.50                                | 1.54       | 1.09              | 2        |

<sup>a</sup>Compositions reported in units of permil (‰).  $\sigma$  = standard deviation. <sup>b</sup>Includes experiments performed with methane other than the house methane (see Table SI.2 for a detailed listing). <sup>c</sup>Reported in the working gas reference frame where the composition of the working gas (house methane) is assumed to be  $\Delta^{13}\text{CH}_3\text{D} = \Delta^{12}\text{CH}_2\text{D}_2 = 0$  ‰ (see section 2.4.4). <sup>d</sup>Reported in the thermodynamic reference frame where working gas is assigned  $\Delta^{13}\text{CH}_3\text{D} = 2.59$  ‰ and  $\Delta^{12}\text{CH}_2\text{D}_2 = 5.86$  ‰ based on the calibration of this study (see section 4.1). <sup>e</sup>In this instance, error is internal precision ( $\pm 1$  s.e.) because the value represents a single measurement.

(and based on approaches developed for CO<sub>2</sub> clumped isotope analyses, e.g., ref<sup>51</sup>) in which samples with varying bulk isotopic compositions are equilibrated at the same temperature. Specifically, samples of the house methane, PlusD, PlusD-200, and Plus<sup>13</sup>C in-house standards were equilibrated at 500 °C. We use  $\delta\text{D}$  values to test for this as they vary the most (208‰) for the standards as compared to the  $\delta^{13}\text{C}$  variation (38.4‰). The results of these experiments are included in Table SI.3 and are plotted in Figure 2.

Over a  $\delta\text{D}$  range of  $\sim 204$ ‰, the slope of  $\Delta^{13}\text{CH}_3\text{D}$  vs  $\delta\text{D}$  is  $0.0013 \pm 0.0010$  (1 s.e.) and  $\Delta^{12}\text{CH}_2\text{D}_2$  vs  $\delta\text{D}$  is  $-0.010 \pm 0.010$  (1 s.e.). As these slopes are statistically indistinguishable from 0 at the 2 s.e. level, we conclude our measurements are relatively precise over  $\sim 200$ ‰  $\delta\text{D}$  ranges with no compositional dependence.

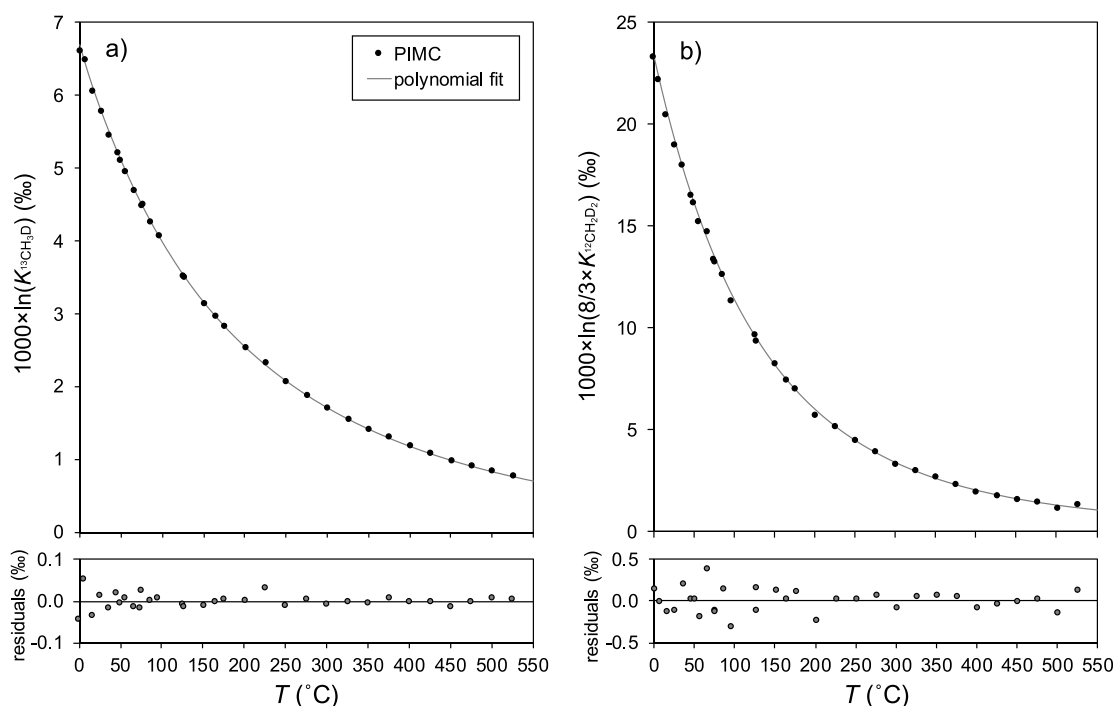
**3.2. Equilibrated Gas Experiments.** The average  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values of the equilibrated gas experiments from 1 to 500 °C in the working gas reference frame (i.e., where 0‰ is the

$\Delta$  value of the working gas and denoted with wg) are presented in Table 2. Individual measurements of sample  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$ , and  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})$  values are given in Table SI.3. All experiments except those at 500 °C discussed above and at 250 °C were performed using the house methane (working gas). For the 250 °C experiments, measured  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  using the house methane (working gas) as the starting gas yielded values within  $\pm 1$  s.e. (note: this is internal precision) of unheated working gas ( $\Delta^{13}\text{CH}_3\text{D}(\text{wg}) = -0.22 \pm 0.25$ ‰,  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg}) = 1.4 \pm 1.4$ ‰,  $\pm 1$  s.e.; Table SI.3). To verify that isotopic equilibrium was indeed reached at 250 °C, we performed two additional experiments using a starting methane with elevated  $\Delta^{12}\text{CH}_2\text{D}_2$  value relative to the working gas ( $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg}) = 872.7 \pm 1.9$ ‰,  $\pm 1$  s.e., internal precision) but otherwise similar  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ , and  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$  compositions vs the working gas (Plus<sup>12</sup>CH<sub>2</sub>D<sub>2</sub> in Table SI.2). These experiments yielded final  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$  and  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})$  values within  $\pm 2$  s.e. of

Table 3. Results of PIMC Calculations of Equilibrium Partition Function Ratios (PFRs)<sup>a</sup> and Equilibrium Constants (K)

| T (°C) | no. of beads | PFR <sub>PIMC</sub>  |   |  |  |  | PIMC   |  |  |  |  |
|--------|--------------|--|---|--|--|--|--|--|--|--|--|
|        |              | <sup>12</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>4</sub> | <sup>13</sup> CH <sub>4</sub> / <sup>12</sup> CH <sub>4</sub> | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>12</sup> CH <sub>2</sub> D <sub>2</sub> / <sup>12</sup> CH <sub>3</sub> D | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D |
| −3.1   | 420          | 165.119  | 1.27411   | 1.28285  | 63.4149  | 1.006859   | 0.384056   | 6.836  | 0.028  | 23.86  | 0.33   |
| 1.2    | 414          | 156.615  | 1.27111   | 1.27953  | 60.1139  | 1.006620   | 0.383834   | 6.598  | 0.031  | 23.28  | 0.13   |
| 6.9    | 408          | 146.643  | 1.26726   | 1.27550  | 56.2243  | 1.006498   | 0.383409   | 6.477  | 0.021  | 22.18  | 0.22   |
| 16.9   | 396          | 131.403  | 1.26100   | 1.26863  | 50.2938  | 1.006054   | 0.382743   | 6.036  | 0.030  | 20.44  | 0.33   |
| 26.9   | 381          | 118.598  | 1.25512   | 1.26238  | 45.3268  | 1.005781   | 0.382190   | 5.764  | 0.029  | 18.99  | 0.26   |
| 36.9   | 372          | 107.730  | 1.24967   | 1.25649  | 41.1306  | 1.005456   | 0.381795   | 5.441  | 0.026  | 17.96  | 0.24   |
| 46.9   | 360          | 98.4964  | 1.24457   | 1.25106  | 37.5515  | 1.005215   | 0.381247   | 5.201  | 0.023  | 16.52  | 0.24   |
| 50.5   | 357          | 95.4660  | 1.24281   | 1.24914  | 36.3808  | 1.005094   | 0.381087   | 5.081  | 0.029  | 16.10  | 0.10   |
| 56.9   | 351          | 90.5717  | 1.23984   | 1.24598  | 34.4838  | 1.004946   | 0.380735   | 4.934  | 0.023  | 15.18  | 0.21   |
| 66.9   | 342          | 83.6501  | 1.23540   | 1.24118  | 31.8332  | 1.004682   | 0.380552   | 4.671  | 0.020  | 14.70  | 0.22   |
| 75.7   | 333          | 78.3302  | 1.23171   | 1.23723  | 29.7684  | 1.004477   | 0.380037   | 4.467  | 0.018  | 13.34  | 0.09   |
| 76.9   | 333          | 77.6800  | 1.23126   | 1.23680  | 29.5173  | 1.004494   | 0.379986   | 4.484  | 0.024  | 13.21  | 0.17   |
| 86.9   | 324          | 72.3917  | 1.22736   | 1.23258  | 27.4908  | 1.004256   | 0.379750   | 4.246  | 0.019  | 12.59  | 0.20   |
| 96.9   | 315          | 67.7750  | 1.22367   | 1.22864  | 25.7050  | 1.004062   | 0.379270   | 4.054  | 0.018  | 11.32  | 0.21   |
| 126.9  | 294          | 56.7170  | 1.21384   | 1.21810  | 21.4757  | 1.003510   | 0.378647   | 3.504  | 0.017  | 9.68   | 0.16   |
| 127.8  | 294          | 56.4316  | 1.21357   | 1.21781  | 21.3605  | 1.003489   | 0.378520   | 3.483  | 0.016  | 9.34   | 0.13   |
| 151.9  | 279          | 49.8849  | 1.20681   | 1.21059  | 18.8612  | 1.003128   | 0.378095   | 3.123  | 0.013  | 8.22   | 0.13   |
| 165.4  | 273          | 46.8367  | 1.20341   | 1.20696  | 17.6950  | 1.002952   | 0.377803   | 2.947  | 0.017  | 7.45   | 0.07   |
| 176.9  | 267          | 44.5391  | 1.20064   | 1.20402  | 16.8198  | 1.002813   | 0.377642   | 2.809  | 0.016  | 7.02   | 0.10   |
| 201.9  | 255          | 40.2836  | 1.19520   | 1.19822  | 15.1926  | 1.002525   | 0.377141   | 2.522  | 0.014  | 5.69   | 0.11   |
| 226.9  | 243          | 36.8111  | 1.19035   | 1.19309  | 13.8752  | 1.002306   | 0.376931   | 2.303  | 0.016  | 5.14   | 0.12   |
| 251.9  | 234          | 33.9564  | 1.18605   | 1.18848  | 12.7903  | 1.002045   | 0.376669   | 2.042  | 0.011  | 4.44   | 0.11   |
| 276.9  | 225          | 31.5734  | 1.18216   | 1.18436  | 11.8863  | 1.001869   | 0.376466   | 1.867  | 0.009  | 3.90   | 0.13   |
| 301.9  | 216          | 29.5629  | 1.17866   | 1.18065  | 11.1224  | 1.001685   | 0.376228   | 1.684  | 0.009  | 3.27   | 0.13   |
| 326.9  | 207          | 27.8446  | 1.17549   | 1.17729  | 10.4729  | 1.001537   | 0.376121   | 1.536  | 0.012  | 2.98   | 0.11   |
| 351.9  | 201          | 26.3684  | 1.17263   | 1.17427  | 9.91438  | 1.001400   | 0.375995   | 1.399  | 0.007  | 2.65   | 0.12   |
| 376.9  | 195          | 25.0874  | 1.16998   | 1.17148  | 9.42967  | 1.001289   | 0.375873   | 1.288  | 0.010  | 2.33   | 0.10   |
| 401.9  | 189          | 23.9686  | 1.16758   | 1.16894  | 9.00554  | 1.001169   | 0.375723   | 1.169  | 0.008  | 1.93   | 0.10   |
| 426.9  | 183          | 22.9861  | 1.16538   | 1.16663  | 8.63492  | 1.001067   | 0.375659   | 1.066  | 0.007  | 1.75   | 0.10   |
| 451.9  | 177          | 22.1140  | 1.16335   | 1.16448  | 8.30600  | 1.000966   | 0.375599   | 0.966  | 0.006  | 1.60   | 0.08   |
| 476.9  | 174          | 21.3370  | 1.16148   | 1.16252  | 8.01303  | 1.000893   | 0.375547   | 0.892  | 0.007  | 1.46   | 0.09   |
| 501.9  | 168          | 20.6483  | 1.15975   | 1.16071  | 7.75197  | 1.000828   | 0.375430   | 0.828  | 0.006  | 1.15   | 0.06   |
| 526.9  | 165          | 20.0243  | 1.15816   | 1.15903  | 7.51883  | 1.000752   | 0.375485   | 0.752  | 0.007  | 1.29   | 0.09   |

<sup>a</sup>See also Table S1.5 (Supporting Information) where PFRs are converted into RPRs in the conventional format. <sup>b</sup> $K_{\text{CH}_3\text{D}_2}$  converges to 3/8 = 0.375 in the high-temperature limit, whereas  $K_{\text{CH}_3\text{D}}$  converges to 1.



**Figure 3.** (a and b) Results of PIMC calculations relative to the polynomial fits (eqs 10 and 11) plotted as a function of temperature (see main text for further details). Error bars on PIMC calculations ( $\pm 1$  s.e.) are smaller than the data points.

the value obtained for the house methane experiment and thus show the nickel catalyst is sufficiently active at 250 °C to equilibrate clumped methane compositions over the time scales used ( $\leq 184$  h; Table SI.3).

All other experiments were performed with the house methane at a given temperature and were replicated at least twice and for different time durations (Tables 2 and SI.3). Experiments performed at the same temperature but for different durations do not exhibit time dependence in values of  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  within  $\pm 2$  s.e. of the measurements (internal precision), which is evidence that internal isotopic equilibrium was reached. House methane allowed to react in the presence of the nickel catalyst (250–500 °C) exhibits bulk  $\delta\text{D}$  and  $\delta^{13}\text{C}$  compositions that are comparable to the starting gas (i.e., within  $\leq 8\text{‰}$  for  $\delta\text{D}$  and  $\leq 1.3\text{‰}$  for  $\delta^{13}\text{C}$ ). This is also true for the  $\gamma\text{-Al}_2\text{O}_3$  experiments (1–165.4 °C) in which changes in  $\delta\text{D}$  are within  $\leq 5\text{‰}$  and changes in  $\delta^{13}\text{C}$  are within  $\leq 0.5\text{‰}$  (Table SI.3). The external precision of  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  as estimated solely from the experimental replicates at a given temperature is  $\pm 0.20\text{‰}$  for  $\Delta^{13}\text{CH}_3\text{D}$  and  $\pm 1.78\text{‰}$  for  $\Delta^{12}\text{CH}_2\text{D}_2$  (1 $\sigma$ ; Table SI.3), which are similar to internal precisions and the external precisions as estimated from the long-term reproducibility of in-house reference standards ( $\pm 0.33\text{‰}$  and  $\pm 1.35\text{‰}$ , respectively,  $\pm 1\sigma$ ; Table 1). The total range in mean clumped compositions measured from experiments conducted over 1–500 °C is 6.3‰ for  $\Delta^{13}\text{CH}_3\text{D}$  and 23.6‰ for  $\Delta^{12}\text{CH}_2\text{D}_2$  (Table 2). For reference, previous calibrations of these values ranged from 1.0‰ to 2.2‰ for  $\Delta^{13}\text{CH}_3\text{D}$ <sup>5–7,14,38</sup> and 2.2‰ for  $\Delta^{12}\text{CH}_2\text{D}_2$ <sup>7</sup> (see sections 1.2 and 1.3).

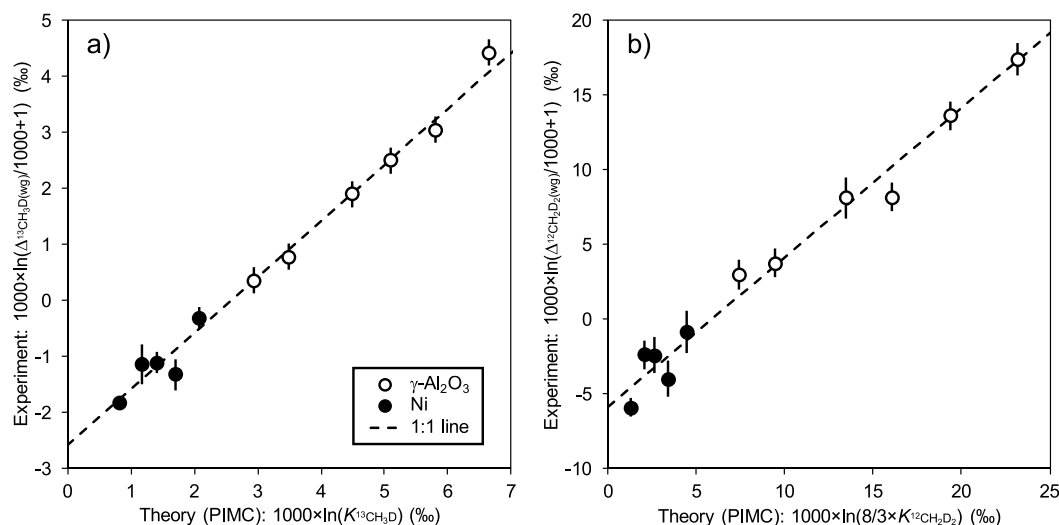
Finally, the 500 °C experiments performed with the methane of varying  $\delta\text{D}$  (house gas, PlusD, and PlusD-200) comprise a bracketing experiment with respect to  $\Delta^{13}\text{CH}_3\text{D}$  (Figure 2a) where final values determined after heating were approached from both

higher and lower starting  $\Delta^{13}\text{CH}_3\text{D}$  values. This is further supporting evidence for achieving equilibrium at 500 °C.

**3.3. PIMC and BMU Calculations.** Results of the PIMC calculations are presented in Table 3, and comparable calculations using the BMU model<sup>29,30</sup> are presented in Table 4. As discussed, both sets of calculations utilize the same electronic potential energy surface for methane,<sup>34</sup> which is constructed at the CCSD(T) level of theory (see Methods for more details). Values of  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  and  $1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right)$  using the PIMC methods have been computed over a temperature range from  $-3$  to 527 °C (270–800 K). The errors ( $\pm 1$  s.e.) on individual PIMC computations are  $\leq 0.03\text{‰}$  for  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  and  $\leq 0.33\text{‰}$  for  $1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right)$ .

Polynomial fits to  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  and  $1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right)$  values as a function of  $T^{-1}$  (sixth or seventh order, respectively, with both fits forced through an intercept of 0‰ at infinite temperature) have been applied to the PIMC results to allow interpolation between computed temperatures (from  $-3$  to 527 °C) and extrapolation above the highest computed temperature

$$\begin{aligned} \Delta^{13}\text{CH}_3\text{D} &\cong 1000 \times \ln(K^{13}\text{CH}_3\text{D}) \\ &= \frac{1.47348 \times 10^{19}}{T^7} - \frac{2.08648 \times 10^{17}}{T^6} \\ &\quad + \frac{1.19810 \times 10^{15}}{T^5} - \frac{3.54757 \times 10^{12}}{T^4} \\ &\quad + \frac{5.54476 \times 10^9}{T^3} - \frac{3.49294 \times 10^6}{T^2} \\ &\quad + \frac{8.89370 \times 10^2}{T} \end{aligned} \quad (10)$$



**Figure 4.** (a and b) Comparison between theoretical calculations and experimental measurements of methane clumping as a function of temperature:  $1000 \times \ln(\Delta_{\text{wg}}/1000 + 1)$  values from experiments (y axis) vs  $1000 \times \ln(K_{13}\text{CH}_3\text{D})$  (a) and  $1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right)$  values (b) theoretically computed from the PIMC calculations at the experimental temperatures (computed from eqs 10 and 11) from 1 to 500 °C. Error bars for replicated experimental data points from this study represent either the  $\pm 1$  s.e. of replicates (i.e., experimental reproducibility) or the expected  $\pm 1$  s.e. based on the observed external precision of standards ( $\sigma_{\text{external}}$ ) and the number of experimental replicates (i.e.,  $\sigma_{\text{external}}/\sqrt{n}$ , where  $n$  = number of experimental replicates), whichever is larger. Error bar on one experimental data point from this study that has not been replicated (the  $\Delta^{12}\text{CH}_2\text{D}_2$  value at 350 °C) represents  $\pm 1$  s.e. internal precision (see Table 2). Error bars in the PIMC calculations (x axis error bars,  $\pm 1$  s.e.) are smaller than the symbols. Since experimentally measured values are shown in the working gas reference frame (where the working gas is assumed to have  $\Delta = 0$ ), the key aspect of this comparison is the relative difference between theory and experiment as a function of temperature. Experiments vs theory are consistent with a 1:1 line (dashed line) with respect to the temperature differences. Composition of the working gas can be constrained by interpolation (e.g., where the 1:1 dashed line intersects the x axis) for the use of reporting measurements in the thermodynamic reference frame (absolute) represented by the PIMC calculations.

$$\begin{aligned} \Delta^{12}\text{CH}_2\text{D}_2 &\cong 1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right) \\ &= -\frac{9.67634 \times 10^{15}}{T^6} + \frac{1.71917 \times 10^{14}}{T^5} \\ &\quad - \frac{1.24819 \times 10^{12}}{T^4} + \frac{4.30283 \times 10^9}{T^3} \\ &\quad - \frac{4.48660 \times 10^6}{T^2} + \frac{1.86258 \times 10^3}{T} \end{aligned} \quad (11)$$

Values computed from these equations are strictly valid over 270–800 K but are also likely valid >800 K due to the requirement that these values (as defined) must approach 0‰ at the high-temperature limit. Note that the range in values of  $1000 \times \ln(K_{13}\text{CH}_3\text{D})$  and  $1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right)$  extrapolated above 800 K to the high-temperature limit are  $\leq 0.75\%$  and  $\leq 1.3\%$ , respectively.

Computed values from the polynomial fits are shown in Figure 3 along with the residuals. The computed  $\pm 1\sigma$  of the residuals are  $\pm 0.02\%$  and  $\pm 0.14\%$  for the  $1000 \times \ln(K_{13}\text{CH}_3\text{D})$  and  $1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right)$  polynomial fits, respectively, which are comparable to the stated precision of the calculations at any given temperature (see Table 3 and Figure 3).

Computations of equilibrium constants ( $K_{13}\text{CH}_3\text{D}$  and  $K_{12}\text{CH}_2\text{D}_2$ ) and derived equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  given as  $1000 \times \ln(K_{13}\text{CH}_3\text{D})$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  given as  $1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right)$  using eqs 5 and 6 are comparable between the PIMC and the BMU approaches (Tables 3 and 4). For example, values of  $1000 \times \ln(K_{13}\text{CH}_3\text{D})$  computed using the BMU model are within

$\leq 0.10\%$  of the PIMC values over all computed temperatures (from  $-3$  to 527 °C). Similarly, values of  $1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right)$  computed using the BMU model are within  $\leq 0.37\%$  of the PIMC values.

Despite this agreement, the BMU-based PFRs, which are used to calculate the BMU-based  $1000 \times \ln(K_{13}\text{CH}_3\text{D})$  and  $1000 \times \ln\left(\frac{8}{3}K_{12}\text{CH}_2\text{D}_2\right)$  values, are systematically higher than the PIMC PFRs. Table SI.4 contains computations of the differences between the two approaches in  $\delta$ -notation:  $\delta\text{PFR}_{(\text{BMU-PIMC})} = 1000 \times (\text{PFR}_{\text{BMU}}/\text{PFR}_{\text{PIMC}} - 1)$  and  $\delta K_{(\text{BMU-PIMC})} = 1000 \times (K_{\text{BMU}}/K_{\text{PIMC}} - 1)$  (reported in units of ‰). The  $\delta\text{PFR}_{(\text{BMU-PIMC})}$  are as high as 5–6‰ for the  $^{13}\text{CH}_4/^{12}\text{CH}_4$  and  $^{13}\text{CH}_3\text{D}/^{12}\text{CH}_3\text{D}$  PFRs and 29–95‰ for the  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  and  $^{12}\text{CH}_2\text{D}_2/^{12}\text{CH}_3\text{D}$  PFRs over the computed temperature range. We note that comparable computations of RPFRs for  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  and  $^{13}\text{CH}_4/^{12}\text{CH}_4$  (Table SI.5) yield the same relative differences between PIMC and BMU approaches (in terms of analogous  $\delta\text{RPFR}_{(\text{BMU-PIMC})}$  comparisons) as expected given the cancellation of rotational symmetry numbers and the mass terms in values of  $\delta\text{PFR}_{(\text{BMU-PIMC})}$  that are normalized out in the RPFR calculations by convention.

## 4. DISCUSSION

**4.1. Experiment vs Theory (PIMC): Working Gas Calibration.** Figure 4 compares measured vs PIMC theoretical clumped isotope compositions computed at the experimental temperatures. For this comparison, we compare experimentally measured values as  $1000 \times \ln(\Delta_{\text{wg}}/1000 + 1)$  values (where the  $\Delta_{\text{wg}}$  value represents  $\Delta^{13}\text{CH}_3\text{D}(\text{wg})$  or  $\Delta^{12}\text{CH}_2\text{D}_2(\text{wg})$  values as

Table 4. Results of BMU Calculations of Equilibrium Partition Function Ratios (PFRs)<sup>a</sup> and Equilibrium Constants (K)

| T (°C) | PFR <sub>BMU</sub>   |   |  |  | BMU                              |                                    |  |   |
|--------|--|---|--|--|----------------------------------|------------------------------------|--|---|
|        | <sup>12</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>4</sub> | <sup>13</sup> CH <sub>4</sub> / <sup>12</sup> CH <sub>4</sub> | <sup>13</sup> CH <sub>3</sub> D/ <sup>12</sup> CH <sub>3</sub> D | <sup>12</sup> CH <sub>2</sub> D <sub>2</sub> / <sup>12</sup> CH <sub>3</sub> D | $K_{^{13}\text{CH}_3\text{D}}^b$ | $K_{^{12}\text{CH}_2\text{D}_2}^b$ | $1000 \times \ln(K_{^{13}\text{CH}_3\text{D}})$<br>(‰) | $1000 \times \ln(8/3 \times K_{^{12}\text{CH}_2\text{D}_2})$<br>(‰) |
| −3.1   | 180.660  | 1.28108   | 1.28976  | 69.4098  | 1.006779                         | 0.384201                           | 6.757  | 24.24   |
| 1.2    | 171.175  | 1.27792   | 1.28637  | 65.7119  | 1.006611                         | 0.383887                           | 6.589  | 23.42   |
| 6.9    | 160.000  | 1.27397   | 1.28212  | 61.3595  | 1.006400                         | 0.383498                           | 6.380  | 22.41   |
| 16.9   | 142.909  | 1.26739   | 1.27506  | 54.7135  | 1.006050                         | 0.382856                           | 6.032  | 20.73   |
| 26.9   | 128.622  | 1.26129   | 1.26851  | 49.1685  | 1.005725                         | 0.382271                           | 5.709  | 19.20   |
| 36.9   | 116.567  | 1.25562   | 1.26243  | 44.4976  | 1.005424                         | 0.381735                           | 5.409  | 17.80   |
| 46.9   | 106.305  | 1.25033   | 1.25676  | 40.5283  | 1.005143                         | 0.381245                           | 5.130  | 16.52   |
| 50.5   | 102.938  | 1.24849   | 1.25479  | 39.2273  | 1.005046                         | 0.381076                           | 5.033  | 16.07   |
| 56.9   | 97.5017  | 1.24540   | 1.25148  | 37.1282  | 1.004882                         | 0.380796                           | 4.870  | 15.34   |
| 66.9   | 89.8940  | 1.24077   | 1.24653  | 34.1942  | 1.004638                         | 0.380384                           | 4.628  | 14.25   |
| 75.7   | 83.9921  | 1.23692   | 1.24241  | 31.9210  | 1.004436                         | 0.380047                           | 4.426  | 13.37   |
| 76.9   | 83.2758  | 1.23644   | 1.24189  | 31.6453  | 1.004411                         | 0.380006                           | 4.401  | 13.26   |
| 86.9   | 77.4829  | 1.23237   | 1.23754  | 29.4170  | 1.004197                         | 0.379658                           | 4.189  | 12.34   |
| 96.9   | 72.3835  | 1.22854   | 1.23345  | 27.4578  | 1.003997                         | 0.379338                           | 3.989  | 11.50   |
| 126.9  | 60.2730  | 1.21830   | 1.22252  | 22.8147  | 1.003468                         | 0.378523                           | 3.462  | 9.35  |
| 127.8  | 59.9524  | 1.21800   | 1.22221  | 22.6920  | 1.003452                         | 0.378500                           | 3.446  | 9.29  |
| 151.9  | 52.8184  | 1.21098   | 1.21472  | 19.9642  | 1.003093                         | 0.377978                           | 3.088  | 7.91  |
| 165.4  | 49.4964  | 1.20739   | 1.21091  | 18.6960  | 1.002912                         | 0.377724                           | 2.907  | 7.24  |
| 176.9  | 47.0036  | 1.20455   | 1.20788  | 17.7453  | 1.002769                         | 0.377530                           | 2.765  | 6.72  |
| 201.9  | 42.3752  | 1.19887   | 1.20185  | 15.9822  | 1.002485                         | 0.377159                           | 2.482  | 5.74  |
| 226.9  | 38.6264  | 1.19382   | 1.19649  | 14.5564  | 1.002237                         | 0.376851                           | 2.235  | 4.93  |
| 251.9  | 35.5440  | 1.18931   | 1.19171  | 13.3857  | 1.002019                         | 0.376595                           | 2.016  | 4.24  |
| 276.9  | 32.9760  | 1.18525   | 1.18742  | 12.4115  | 1.001825                         | 0.376379                           | 1.824  | 3.67  |
| 301.9  | 30.8116  | 1.18160   | 1.18355  | 11.5912  | 1.001654                         | 0.376198                           | 1.652  | 3.19  |
| 326.9  | 28.9685  | 1.17828   | 1.18005  | 10.8934  | 1.001501                         | 0.376044                           | 1.500  | 2.78  |
| 351.9  | 27.3848  | 1.17527   | 1.17688  | 10.2943  | 1.001365                         | 0.375914                           | 1.364  | 2.43  |
| 376.9  | 26.0127  | 1.17253   | 1.17398  | 9.77564  | 1.001243                         | 0.375802                           | 1.242  | 2.14  |
| 401.9  | 24.8153  | 1.17001   | 1.17134  | 9.32329  | 1.001134                         | 0.375707                           | 1.133  | 1.88  |
| 426.9  | 23.7634  | 1.16771   | 1.16892  | 8.92614  | 1.001036                         | 0.375625                           | 1.035  | 1.67  |
| 451.9  | 22.8338  | 1.16559   | 1.16669  | 8.57534  | 1.000948                         | 0.375554                           | 0.947  | 1.48  |
| 476.9  | 22.0077  | 1.16363   | 1.16464  | 8.26376  | 1.000868                         | 0.375493                           | 0.868  | 1.31  |
| 501.9  | 21.2700  | 1.16182   | 1.16275  | 7.98563  | 1.000796                         | 0.375440                           | 0.796  | 1.17  |
| 526.9  | 20.6082  | 1.16015   | 1.16099  | 7.73620  | 1.000731                         | 0.375394                           | 0.731  | 1.05  |

<sup>a</sup>See also Table SI.5 (Supporting Information) where PFRs are converted into RPFRs in the conventional format. <sup>b</sup> $K_{^{12}\text{CH}_2\text{D}_2}$  converges to  $3/8 = 0.375$  in the high-temperature limit, whereas  $K_{^{13}\text{CH}_3\text{D}}$  converges to 1.

measured and reported in Tables 2 and SI.3) vs computed values of  $1000 \times \ln(K_{^{13}\text{CH}_3\text{D}})$  and  $1000 \times \ln\left(\frac{8}{3}K_{^{12}\text{CH}_2\text{D}_2}\right)$ . This comparison limits approximations associated with measured vs theoretical clumped isotope compositions (see ref 25 and our eqs 5 and 6) [Note: The  $1000 \times (R/R^* - 1) \cong 1000 \times \ln(R/R^*)$  approximation holds for the measured compositional range of  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  (i.e., differences between the two notations are ca.  $\geq 10\times$  smaller than the precision of the measurements), and our choice makes no significant difference on the results or interpretations of our calibration. Nevertheless, the  $1000 \times \ln(R/R^*)$  vs  $1000 \times \ln(K_{^{13}\text{CH}_3\text{D}})$  or  $1000 \times \ln\left(\frac{8}{3}K_{^{12}\text{CH}_2\text{D}_2}\right)$  comparisons of measurements vs theory for isotopically equilibrated systems is more accurate.]

A least-squares linear regression through each measured vs theory (PIMC) data set yields a slope of  $1.02 \pm 0.04$  for the  $\Delta^{13}\text{CH}_3\text{D}$ -based comparison and  $0.98 \pm 0.05$  for the  $\Delta^{12}\text{CH}_2\text{D}_2$ -based comparison ( $\pm 1$  s.e.). Thus, both slopes are within  $\pm 1$  s.e. of 1 over a temperature range of 1–500 °C. Given this 1:1 agreement between experiment and theory, lines with

slopes  $\equiv 1$  are used to infer the intercept in each plot to obtain estimates of the working gas composition. These yield  $\Delta^{13}\text{CH}_3\text{D} = 2.59 \pm 0.14\text{‰}$  and  $\Delta^{12}\text{CH}_2\text{D}_2 = 5.86 \pm 0.60\text{‰}$  ( $\pm 1$  s.e.) [Note: These values are in the  $\Delta = 1000 \times (R/R^* - 1)$  notation.]. We emphasize that these values for the working gas are in the thermodynamic reference frame where  $\Delta = 0\text{‰}$  occurs at infinite temperature.

Table 2 contains the average  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values of our experimental gases equilibrated at different temperatures (individual experimental data in Table SI.3) converted to the thermodynamic reference frame using the  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values determined for the working gas. These values are further converted to  $1000 \times \ln(\Delta/1000 + 1)$  values and compared to PIMC calculations of  $1000 \times \ln(K_{^{13}\text{CH}_3\text{D}})$  and  $1000 \times \ln\left(\frac{8}{3}K_{^{12}\text{CH}_2\text{D}_2}\right)$  in Table 5 and Figure 5. As expected from the 1:1 agreement in the temperature dependence indicated in Figure 4, the experimental data match the predicted temperature dependence from the PIMC calculations over 1–500 °C. The computed  $\pm 1\sigma$  of the residuals are  $\pm 0.22\text{‰}$  and  $\pm 1.17\text{‰}$  for

Table 5. Comparison between Theoretical (PIMC) and Experimental Equilibrium Clumped Methane Compositions as a Function of Temperature<sup>a</sup>

| T (°C) | PIMC   |  |  | experiment                                   |  |  | PIMC   |  |  | experiment                                   |  |  | residuals |
|--------|--|--|--|--|--|--|--|--|--|--|--|--|-----------|
|        | $1000 \times \ln(K^{\text{CH}_3\text{D}})^b$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(K^{\text{CH}_3\text{D}})^b$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(K^{\text{CH}_3\text{D}})^b$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(K^{\text{CH}_3\text{D}})^b$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ | $1000 \times \ln(\Delta^{13}\text{CH}_3\text{D}/1000 + 1)$ |           |
| 500    | 0.82   | 0.76   | 0.23   | 0.06   | -0.06  | 1.30   | 1.30   | -0.06  | 1.93   | 0.64   | -1.36  | 1.93   | -1.36     |
| 400    | 1.18   | 1.44   | 0.51   | 0.36   | 0.27   | 2.03   | 2.03   | 3.45   | 0.40   | 0.28   | 1.42   | 0.40   | 1.42      |
| 350    | 1.41   | 1.46   | 0.17   | 0.10   | 0.05   | 2.60   | 2.60   | 3.43   |  | 1.21 <sup>c</sup>                            | 0.83   |  | 0.83      |
| 300    | 1.70   | 1.26   | 0.16   | 0.11   | -0.45  | 3.39   | 3.39   | 1.86   | 1.72   | 1.22   | -1.52  | 1.72   | -1.52     |
| 250    | 2.07   | 2.27   | 0.34   | 0.20   | 0.20   | 4.47   | 4.47   | 4.99   | 2.49   | 1.44   | 0.52   | 2.49   | 0.52      |
| 165.4  | 2.95   | 2.93   | 0.30   | 0.21   | -0.02  | 7.43   | 7.43   | 8.84   | 1.44   | 1.02   | 1.42   | 1.44   | 1.42      |
| 127.8  | 3.50   | 3.35   | 0.11   | 0.08   | -0.14  | 9.46   | 9.46   | 9.59   | 0.59   | 0.42   | 0.14   | 0.59   | 0.14      |
| 75.7   | 4.48   | 4.47   | 0.21   | 0.15   | -0.01  | 13.45  | 13.45  | 13.95  | 1.98   | 1.40   | 0.50   | 1.98   | 0.50      |
| 50.5   | 5.09   | 5.07   | 0.24   | 0.17   | -0.01  | 16.08  | 16.08  | 13.99  | 0.47   | 0.33   | -2.09  | 0.47   | -2.09     |
| 25     | 5.81   | 5.62   | 0.07   | 0.05   | -0.19  | 19.36  | 19.36  | 19.45  | 0.95   | 0.67   | 0.08   | 0.95   | 0.08      |
| 1.2    | 6.64   | 7.00   | 0.09   | 0.06   | 0.36   | 23.15  | 23.15  | 23.23  | 1.55   | 1.10   | 0.08   | 1.55   | 0.08      |
| 1σ     |  |  |  |  |  | 0.22   |  |  |  |  | 1.17   |  | 1.17      |

<sup>a</sup>Listing follows Table 2. <sup>b</sup>Computed from eq 10 or 11 in the main text (polynomial fits to PIMC calculations). <sup>c</sup>In this instance, error is given as the internal precision ( $\pm 1$  s.e.) because the value represents a single measurement.

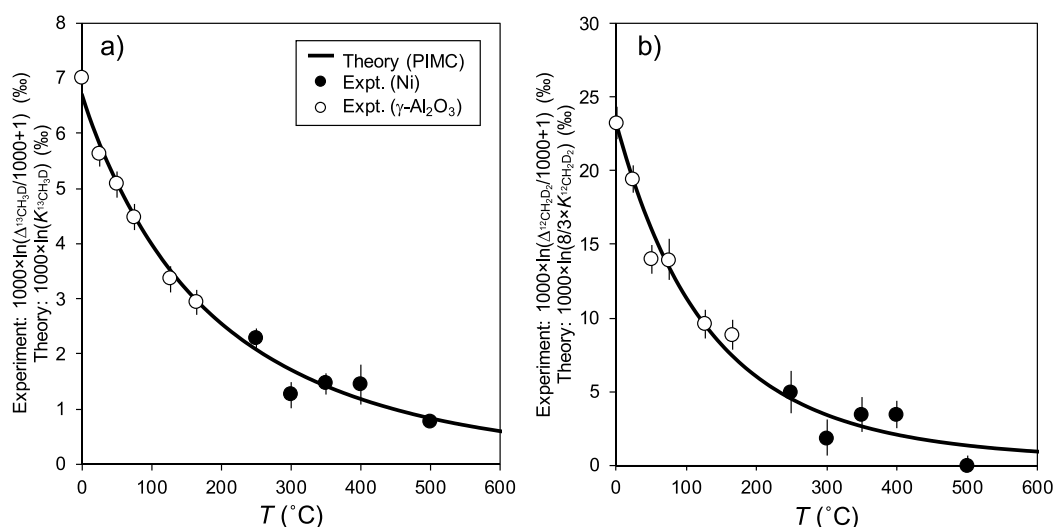
the  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  comparisons, respectively (Table 5). These are similar to the external precision estimated solely from the experimental replicates at a given temperature ( $\pm 0.20\%$  for  $\Delta^{13}\text{CH}_3\text{D}$  and  $\pm 1.78\%$  for  $\Delta^{12}\text{CH}_2\text{D}_2$ ; 1σ; section 3.2). Finally, we provide a comparison of previously measured  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values for samples experimentally equilibrated at known temperatures (refs 6, 7, 14, and 38) vs our data and PIMC theoretical curves in Figure SL6. The comparison shown in Figure SL6 of experimentally determined equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values as a function of temperature also shows agreement with the theoretical equilibrium temperature dependence of methane clumping presented here (i.e., determinations from other laboratories<sup>6,7,14,38</sup> are within  $\sim 0.2\%$  for both  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ , respectively, over the determined temperature ranges).

This work yields the important and satisfying result that theoretically calculated  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values using the most rigorous theoretical approach available (PIMC) are in 1:1 agreement (at the  $\pm 1$  s.e. level) with experimental determinations of equilibrium  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ . This provides confidence in the theory, experiments, and measurement techniques over essentially the full range of formation temperatures of microbial and thermogenic gases on earth. Furthermore, agreement in  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  values of experimentally equilibrated samples between various laboratories (Figure SL6) despite different measurement techniques, working gases, and theoretical calculations utilized for calibrations is encouraging and indicates that measurements between laboratories can likely be directly compared.

Finally, the working gas clumped compositions converted appropriately to  $1000 \times \ln(\Delta/1000 + 1)$  values yield apparent methane-clumped isotope temperatures of  $196 \pm 13$  °C for  $\Delta^{13}\text{CH}_3\text{D}$  and  $204 \pm 17$  °C for  $\Delta^{12}\text{CH}_2\text{D}_2$  ( $\pm 1$  s.e.) using the polynomial fits to the PIMC calculations (eqs 10 and 11). On the basis of the  $\delta\text{D}_{\text{VSMOW}}$  ( $-159.3 \pm 2.4\%$ ,  $\pm 1\sigma$ ) and  $\delta^{13}\text{C}_{\text{VPDB}}$  ( $-38.37 \pm 0.04\%$ ,  $\pm 1\sigma$ ) values of the working gas, the cylinder gas is likely thermogenic in origin.<sup>1,2</sup> Such temperatures are reasonable potential gas formation temperatures<sup>28</sup> and are consistent with the common observation that apparent methane-clumped isotope temperatures of thermogenic methane are compatible with expectations of thermogenic gas formation temperatures.<sup>7,12,14,15,20</sup>

The  $\Delta^{13}\text{CH}_3\text{D}$ - and  $\Delta^{12}\text{CH}_2\text{D}_2$ -based temperatures are within  $\pm 1$  s.e. of each other. Such agreement has been previously seen in assumed thermogenic gases from commercially purchased cylinders<sup>24</sup> as well as thermogenic gases from natural gas deposits.<sup>7,12</sup> Such agreement has been taken as additional evidence that thermogenic gases may form in clumped isotope equilibrium and that  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  may represent formation temperatures of thermogenic gases (or at least re-equilibration temperatures).

Given the agreement in clumped-based temperatures of our working gas inferred for both  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$ , we could choose to force our working gas to have a  $\Delta^{12}\text{CH}_2\text{D}_2$  composition that corresponds to the temperature derived from the  $\Delta^{13}\text{CH}_3\text{D}$  calibration ( $\sim 196$  °C) given that the  $\Delta^{13}\text{CH}_3\text{D}$  measurements are more precise. This exercise would yield a  $\Delta^{12}\text{CH}_2\text{D}_2$  value of  $\sim 6.17\%$  for our working gas derived from eq 11, which is about



**Figure 5.** (a and b) Plots of experimental vs theoretical values of equilibrium clumping in methane as a function of temperature. Note that the theoretical calculations (black curves) are computed following eq 10 as  $1000 \times \ln(K^{13}\text{CH}_3\text{D})$  values (a) and following eq 11 as  $1000 \times \ln\left(\frac{8}{3}K^{12}\text{CH}_2\text{D}_2\right)$  values (b). Accordingly, the experimental data have been converted into the  $1000 \times \ln(\Delta/1000 + 1)$  format to facilitate direct comparison to theory (see also Table 5). Additionally, the experimental values are based on converting  $\Delta_{(\text{wg})}$  (reported in the working gas reference frame) into  $\Delta$  values in the thermodynamic reference frame by taking into consideration the constrained  $\Delta$  compositions of the working gas ( $\Delta^{13}\text{CH}_3\text{D} = 2.59 \pm 0.14\text{‰}$  and  $\Delta^{12}\text{CH}_2\text{D}_2 = 5.86 \pm 0.60\text{‰}$ ,  $\pm 1$  s.e.; see section 4.1). Error bars on experimental points are as described in Figure 4.

0.31‰ higher than but within 1 s.e. of what we directly infer from our calibration ( $5.86 \pm 0.60\text{‰}$ , 1 s.e.). This may mean that our future measurements of  $\Delta^{12}\text{CH}_2\text{D}_2$  could be biased to ca. 0.3‰ lower values based on our calibration if our working gas is actually in internal isotopic equilibrium (which is not known). Given our current typical external precision ( $1.35\text{‰}$ ,  $\pm 1\sigma$ ) we do not expect that any such bias would change any interpretations of environmental or experimental samples.

**4.2. PIMC Methods vs Traditional BMU Models.** PIMC calculations provide a way to compute stable isotope fractionation factors independent of the traditionally implemented BMU model. As the PIMC calculations inherently include a fully anharmonic and quantum mechanical description of the partition function ratios, they are considered to be more rigorous than BMU calculations such that comparison of BMU and PIMC calculations can be used to identify errors in BMU calculations.<sup>11,35</sup> In the current study, all BMU-PFRs exhibit significant departures from the PIMC-PFRs: up to 5–6‰ for  $^{13}\text{CH}_4/^{12}\text{CH}_4$  and  $^{13}\text{CH}_3\text{D}/^{12}\text{CH}_3\text{D}$  PFRs and 95–29‰ for the  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  and  $^{12}\text{CH}_2\text{D}_2/^{12}\text{CH}_3\text{D}$  PFRs over the computed temperature range (from  $-3$  to  $527^\circ\text{C}$ ). As noted earlier, the same relative differences are preserved when  $^{13}\text{CH}_4/^{12}\text{CH}_4$  and  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  PFRs are transformed into comparable RPFs that are more commonly reported in the stable isotope literature (e.g., refs 9, 10, and 31–33, Tables SI.4 and SI.5). Given that both BMU and PIMC computations were performed using the same electronic potential energy surface for methane computed using high-level coupled cluster theory,<sup>34</sup> these are true differences between the BMU and the PIMC theoretical treatments as opposed to differences due to different potential energy surfaces. The  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  PFRs calculated using the BMU vs PIMC approach exhibit 5–20× larger relative differences than  $^{13}\text{CH}_4/^{12}\text{CH}_4$  PFRs. We propose that the larger discrepancy present for D/H exchange is likely due to the long-recognized inadequacies in the simplified treatments of partition functions in the BMU model to account properly for

D/H exchange (e.g., harmonic vibrational PF and classical rotational PF, e.g., refs 29, 30, 32, and 33). The PIMC calculations inherently account for vibrational anharmonicity and quantize the rotational motions, and therefore avoid these well-understood approximations inherent to the BMU approach.<sup>29,30,32,33</sup>

Additional insight into the problem may be given by comparisons between the calculations of the present study (BMU vs PIMC) and previous BMU calculations of methane performed without and with ex post facto corrections reported by Liu and Liu<sup>9</sup> following earlier correction schemes.<sup>32,33</sup> We first note that such a comparison is ultimately inexact because their<sup>9</sup> calculations are based on an electronic potential energy surface for methane computed at the MP2 level of theory (aug-cc-pVTZ basis set) rather than the more accurate couple cluster theories of the present study (CCSD(T), cc-pVTZ, and cc-pVQZ basis sets; see ref 34 for more detail). The corrections implemented by Liu and Liu<sup>9</sup> include those for vibrational anharmonicity (e.g., anharmonic contributions to the zero-point energy and anharmonic contributions to vibrational excited states) and quantum corrections to rotational motions among others,<sup>9,33</sup> which are applied ex post facto to harmonically computed RPFs.<sup>9,33</sup> The relative difference between uncorrected and corrected  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  RPFs (from 104‰ to 34‰ over from 0 to  $500^\circ\text{C}$ , respectively) and  $^{13}\text{CH}_4/^{12}\text{CH}_4$  RPFs (from 5.4‰ to 1.9‰ over from 0 to  $500^\circ\text{C}$ , respectively) using the BMU model by Liu and Liu<sup>9</sup> is of similar magnitude and sign in both cases to what we observe between BMU and PIMC calculations in this study (93 to 30‰ and 5.4–1.8‰ for  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  and  $^{13}\text{CH}_4/^{12}\text{CH}_4$ , respectively, over the comparable  $T$  range from 1.2 to  $501.9^\circ\text{C}$ ; Tables SI.4 and SI.5). In the case of both the  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  and the  $^{13}\text{CH}_4/^{12}\text{CH}_4$  RPFs, the total correction given by Liu and Liu<sup>9</sup> (multiplicative factors of  $\sim 0.906$  to  $\sim 0.968$  for  $^{12}\text{CH}_3\text{D}/^{12}\text{CH}_4$  from  $0^\circ\text{C}$  to  $500^\circ\text{C}$  and similarly 0.995 to 0.998 for  $^{13}\text{CH}_4/^{12}\text{CH}_4$ ) is almost entirely driven by the correction for the anharmonic contributions to the zero-point energy (see

their<sup>9</sup> Table 3). This may suggest that the harmonic treatment of the vibrational partition function may be the source of much of the error in BMU-based computations for methane.

Regardless of the precise source of the errors in the BMU model, the contrastingly small ( $\leq 0.1$ – $0.4\%$ ) relative differences in the computed equilibrium constants and related  $\Delta$  values describing equilibrium clumping in methane from BMU-based PFRs arises from a cancelation of errors in component PFRs as observed by Webb and Miller.<sup>11</sup> One likely reason for this precise cancelation of errors may be due to inherent symmetry preserved in these isotopic clumping reactions. In particular, any errors present in the  $^{13}\text{CH}_4/^{12}\text{CH}_4$  PFR are expected to be similar in nature and magnitude to those present in the  $^{13}\text{CH}_3\text{D}/^{12}\text{CH}_3\text{D}$  PFR, since the PFRs reflect the same type of isotopic substitution. The same cannot be said for some exchange reactions involving isotopomers (e.g.,  $^{14}\text{N}^{15}\text{N}^{16}\text{O} \rightleftharpoons ^{15}\text{N}^{14}\text{N}^{16}\text{O}$ ) for which BMU calculations have been shown to only benefit from a partial cancelation of errors.<sup>11</sup> Although the PFR errors appear significant when compared on a per mil scale, such errors only amount to relative free energy differences of approximately  $10^{-3}$  and  $2 \times 10^{-2}$  kcal/mol for the  $^{13}\text{C}/^{12}\text{C}$ -related and D/H-related PFRs, respectively.

As a final note, previous theoretical calculations of clumped methane equilibrium constants based on the BMU model that have served as the basis of previous heated gas calibrations (e.g., refs 6, 7, and 14) or that have otherwise been reported<sup>8–10,31</sup> compare well to the values of the present study that are based on PIMC approaches due to this cancelation of errors in individual BMU partition function ratios. For example, theoretical calculations of  $\Delta^{13}\text{CH}_3\text{D}$  values as given by  $1000 \times \ln(K_{^{13}\text{CH}_3\text{D}})$  from refs 6–10 are all within  $\leq 0.2\%$  of the PIMC calculations presented here (eq 10;  $T \geq 0^\circ\text{C}$ ). Similarly, the  $\Delta^{12}\text{CH}_2\text{D}_2$  values as given by  $1000 \times \ln\left(\frac{8}{3}K_{^{12}\text{CH}_2\text{D}_2}\right)$  based on the calculations of Young and co-workers<sup>7</sup> and Piasecki and co-workers<sup>10</sup> are within  $\leq 0.35\%$  and  $\leq 0.1\%$ , respectively, of the PIMC calculations presented here (eq 11;  $T \geq 0^\circ\text{C}$ ). It is encouraging that different theoretical treatments arrive at similar values for clumped methane equilibrium constants upon which all previous clumped methane isotope measurements are anchored.<sup>5–7,14</sup> However, the reliance upon error cancelation to achieve relative accuracy will have to be carefully considered when applying BMU treatments of partition function ratios to problems of calculating fractionation factors between methane and other compounds (e.g., equilibrium D/H partitioning in  $\text{CH}_4$  vs  $\text{H}_2$ )<sup>37</sup> given that error cancellation may not be as precise when comparing different molecular substances.

## 5. CONCLUSIONS

We summarize the conclusions of this study as follows.

- (1) We presented a new mass-spectrometric technique to measure methane  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\Delta^{13}\text{CH}_3\text{D}$ , and  $\Delta^{12}\text{CH}_2\text{D}_2$  with external precisions ( $\pm 1\sigma$ ) of  $0.15\%$ ,  $0.02\%$ ,  $0.33\%$ , and  $1.35\%$ , respectively. Accuracy for  $\delta\text{D}$  and  $\delta^{13}\text{C}$  was determined through measurement of in-house standards with independently measured values. Accuracy for  $\Delta^{13}\text{CH}_3\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  was determined by demonstrating that gases with different  $\delta\text{D}$  values (204‰ range) equilibrated at  $500^\circ\text{C}$  show no statistically resolvable ( $\pm 2$  s.e.) dependence of  $\Delta$  values on bulk  $\delta\text{D}$  values.

- (2) We presented a new experimental and theoretical working gas calibration method utilizing both  $\gamma\text{-Al}_2\text{O}_3$  and Ni catalysts to allow for both  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  equilibrations from 1 to  $500^\circ\text{C}$ , covering the range of expected thermogenic and microbial gas formation temperatures on earth.
- (3) We presented new path integral calculations (PIMC) of equilibrium clumping in methane over the same temperature range for both  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$ .
- (4) We observed 1:1 agreement (within  $\pm 1$  s.e.) between measured differences in  $^{13}\text{CH}_3\text{D}$  and  $^{12}\text{CH}_2\text{D}_2$  abundances for samples equilibrated from 1 to  $500^\circ\text{C}$  vs those theoretically computed (PIMC) over the same temperature range.
- (5) The PIMC calculations can be used to gain insight into the potential sources of error in traditional approaches (BMU model), which appear to arise predominantly from the simplified treatment of partition functions in the BMU model (i.e., harmonic vibrational PF and classical rotational PF). Such insights are important for considering theoretical calculations of hydrogen isotope fractionation factors for different molecules (e.g.,  $\text{CH}_4$  vs  $\text{H}_2$ ) based on the BMU model.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsearthspacechem.9b00244.

Description of the fragment-correction scheme to determine  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\Delta^{13}\text{CH}_3\text{D}$ , and  $\Delta^{12}\text{CH}_2\text{D}_2$  from measured isotopologue ratios that include fragments; description of peak tailing corrections on  $^{12}\text{CH}_2\text{D}_2^+$  intensity using water peak scans (including Figure SI.1 illustrating the water peak exercise); description of the data filtering rationale used for  $\delta\text{D}$  and  $\Delta^{12}\text{CH}_2\text{D}_2$  measurements on the 253 Ultra;  $\delta\text{D}$  measurement and data processing in a flow-chart; combined  $\delta^{13}\text{C}$  and  $\Delta^{13}\text{CH}_3\text{D}$  measurement and data processing in a flow-chart;  $\Delta^{12}\text{CH}_2\text{D}_2$  measurement and data processing in a flow chart; convergence of PIMC PFRs as a function of the number of beads; comparison of the experiment vs theory calibration of the present study to those published from other laboratories (PDF)

Convergence of PIMC PFRs as a function of the number of beads (data for Figure SI.5); individual analyses of methane isotope compositions of in-house reference standards; individual isotope analyses of methane from equilibration experiments; comparison between traditional BMU calculations and PIMC methods in  $\delta$  notation; theoretically calculated reduced partition function ratios (RPFRs) from both PIMC and BMU calculations derived from the complete partition function ratios (PFRs) presented in the main text; harmonic vibrational frequencies of methane isotopologues derived from the potential energy surface of Lee and co-workers<sup>34</sup> used in the BMU calculations of the present study (XLSX)

Script (written in Python) used for processing Qtegra LabBook output .csv files on the 253 Ultra at UC Berkeley (PDF)

Script (written in Python) used at UC Berkeley to calculate  $\delta$  and  $\Delta$  values from measured isotopologue ratios. (PDF)

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### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

D.L.E. and D.A.S. acknowledge research support from the Laboratory Directed Research and Development Program of Lawrence Berkeley National Laboratory under U.S. Department of Energy Contract No. DE-AC02-05CH11231. D.A.S. acknowledges support for ACT from the National Science Foundation under Grant No. EAR-1911296 and the Donors of the American Chemical Society Petroleum Research Fund. M.K.L. acknowledges support from the Agouron Institute. T.F.M. acknowledges support from the National Science Foundation under Grant No. CHE-1611581. The 253 Ultra Mass Spectrometer was funded by the Heising-Simons Foundation and the University of California, Berkeley. Mark Conrad and Markus Bill (LBNL) are thanked for helpful conversations regarding standardization. Xuecheng Tao (Caltech) is thanked for helpful conversations regarding PIMC calculations.

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