

METHODOLOGY

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# A synthetic standard for the analysis of carbon isotopes of carbon in silicates, and the observation of a significant water-associated matrix effect

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## Abstract

**Background:** Due to the biogeochemical fractionation of isotopes, organic material can be heterogeneous at the microscale. Because this heterogeneity preserves in the rock record, the microscale measurement of carbon isotopes is an important frontier of geobiology. Such analyses via secondary ion mass spectrometry (SIMS) have been, however, held back by the lack of an appropriate homogeneous synthetic standard that can be shared between laboratories. Such a standard would need to yield a carbon signal intensity within the same instrument dynamic range as that found for typical rocks, exhibit minimal matrix effects under typical SIMS conditions, and be widely available. In this work, five possible standards were tested with repeated  $\delta^{13}\text{C}$  ion microprobe measurements against the PPRG #215-1 Precambrian chert that has been used as a working standard for these types of analyses by several laboratories.

**Results:** Results showed that silica powder, pressed, and bonded with Ceramacast 905 produced a useful synthetic standard. The material produced has a secondary ion carbon yield of only about 15× that of the PPRG #215-1 organic-rich chert. Finally, the material, once dried sufficiently, did not demonstrate an observable matrix effect when the carbon isotopes were measured. Another similar material (silica nanopowder, pressed, and bonded with Aremco-Bond 526N) appears to have retained its hydration after a substantial effort to dry it. The isotopes measurements of this more hydrated material showed a significant matrix effect that was diminished by intense pre-sputtering. The results indicate water can affect SIMS carbon isotopic measurements, and an intense beam reduces the effect. A hydrated standard might be useful to monitor the effect.

**Conclusions:** A suitable artificial standard for SIMS isotopic measurements of organic material in rocks has been found, and it will allow an acute growth in both the quantity and quality of studies of ancient carbon at the microscale. Also, this work has revealed a novel water-associated matrix effect for carbon isotopes. This newly revealed matrix effect is important because it might have misled previous research. The effect could lead to increased observed heterogeneity of partially hydrated samples and/or produced systematic differences between natural targets and the standards used.

**Keywords:** Carbon isotopes, SIMS, Standardization, Matrix effect

## Background

Efforts to understand the long Precambrian history of life on Earth have generally focused on either the

morphology of microfossils preserved, the geochemical signatures of specific metabolisms, or the preservation of organic material in sedimentary rocks. In particular, the carbon isotopic analyses of bulk organic material extracted from rocks has been particularly important for showing the antiquity of life on Earth and major perturbations of the global carbon cycle. Interpretations of

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bulk carbon isotope measurements are, however, limited because they represent an average of carbon in rocks, which is a mix of carbon from indigenous organisms, reworked organic material, and potentially post-depositional contaminants. Microanalysis of carbon isotopes allows researchers to obtain carbon isotopic information for individual microfossils, as well as reveal diversity of microbial metabolisms in an environment from carbon isotopic microscale heterogeneity. The work published to date demonstrates that there is a great deal of such heterogeneity that is masked in a bulk analysis (e.g., [1–7]). This heterogeneity could, if measured, be a signature of particular microbial processes occurring when the sediments were deposited. For example, different carbon fixation pathways impart demonstrated different carbon isotopic fractionations [8–10], and different sources of carbon could result in sedimentary organic material with distinct carbon isotopic compositions (e.g., [11–13]).

In spite of the high value carbon isotopic microanalyses have for the fields of biogeochemistry and geobiology, the number of isotope studies of ancient sedimentary carbon conducted at the microscale remains quite small. The primary barrier to a substantial increase in work on this topic has been the lack of an appropriate homogeneous synthetic standard that can be shared between laboratories worldwide. In SIMS carbon isotope research, a standard of known isotopic composition is needed that can be analyzed in parallel with the materials being studied. The lack of an ideal standard has both hindered experienced secondary ion mass spectrometry (SIMS) laboratories and new potential researchers to the field.

For past work, several studies have used powdered graphite to study natural graphite or organic material in silicate rocks [14–16]. Under at least some SIMS conditions, however, there appears to be a matrix effect between graphite and organic material [3, 5], and there are reports that H/C can correlate with a matrix effect [17, 18]. Diamond has also been used as a standard for SIMS work [19], and it remains a viable option for these types of measurements. The possible limitations with diamond are that there might be a matrix effect between diamond and sedimentary organic material, and that diamond is hard to share between disparate laboratories. Also, while not well documented in the literature, several groups have tried using either epoxy or plastics as a carbon standard for organic material. This approach could be useful as an isotope standard for analyses of very organic-rich materials, but the approach is problematic for analyses of typical sedimentary rocks that contain only a few percent carbon. The problem is that the carbon-rich standard gives a much stronger signal than the unknowns and therefore must be analyzed

under different conditions and potentially with a different detector set-up. Several groups have used the Precambrian rock chip PPRG #215-1 as a working standard for the analysis of carbon isotopes of organic material in Precambrian cherts [3, 7, 20, 21]. The advantage of this approach has been the lack of any significant matrix effect between the standard and other natural unknown samples. The downsides have been that it is hard to share between disparate laboratories, and that there is some isotopic heterogeneity making it hard to use for the evaluation of ion microprobe spot-to-spot reproducibility. Also, the carbon secondary ion signal obtained from PPRG #215-1 is often less than that obtained from large microfossils with the same SIMS conditions.

The ideal standard for isotope measurements via SIMS would yield a secondary carbon signal intensity within the same instrument dynamic range as that typically found for microfossils in sedimentary rocks, exhibit minimal matrix effects under typical SIMS conditions used, and be available to the whole geochemical community. Here, we have investigated five possible carbon SIMS standards. The candidate materials were either synthesized, purchased, or donated and were tested with repeated  $\delta^{13}\text{C}$  ion microprobe measurements against the PPRG#215-1 Precambrian rock chip. We report, here, that one of these materials tested is quite suitable for SIMS work. During the testing, I discovered a matrix effect associated with the degree of hydration of the material being analyzed, and I found that an intense pre-sputter reduces the observed effect.

## Results and discussion

### Secondary ion yields

Table 1 shows the yield of carbon secondary ions (in this case  $\text{C}_2^-$ ) for each of the five materials studied here along with the yield for PPRG #215-1. This initial test demonstrated that two of the five materials (Ceramacast 905 + silica and Aremco-Bond 526N + silica) produce secondary ion yields in a similar magnitude to typical natural organic-rich sedimentary rocks. From these measurements, it appears that one of these materials could be a useful isotope standard for natural samples. The other three materials produced secondary ion yields that were a few orders of magnitude higher. From these results, it is quite possible that these other materials could be useful standards when analyzing very carbon-rich materials. In particular, because PEEK GF30 is a commercially available high performance glass-filled plastic, its overall elemental composition is similar to natural samples and it is readily available. Thus, PEEK GF30 (with or without added surface silica) would likely be a useful standard for analyses of coal or large particulate organic material

**Table 1** Observed carbon ion yields for different materials

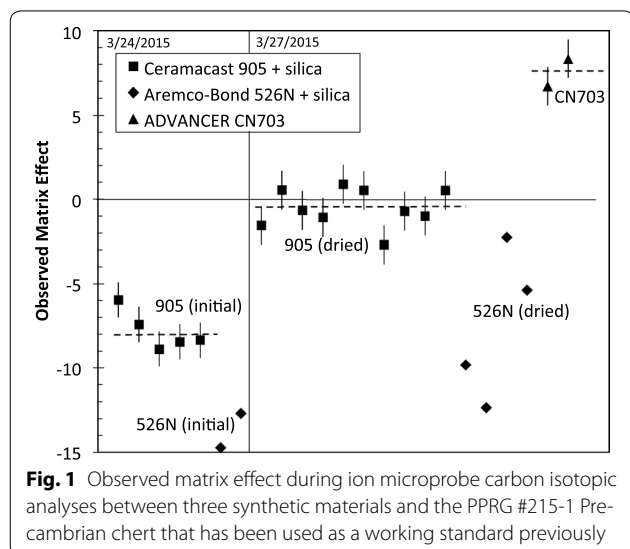
Possible standard material	Approx. C <sub>2</sub> yield (10 <sup>6</sup> cps per nA)
PPRG #215-1	0.1 <sup>a</sup>
PEEK GF30 + silica	75
Ceramacast 905 + silica	1.5
Aremco-Bond 526N + silica	1.5
ADVANCER CN703 (bonded SiC)	35
CARBOFRAX A (bonded SiC)	50

<sup>a</sup> Signal varies between different spots by about an order of magnitude on this natural rock chip

extracted from rocks (including, for example, extracted Acritarchs). Also, with more silica nanopowder adhered to the surface, PEEK might be an excellent standard for the SIMS analyses of a variety of targets. However, additional work is needed on a procedure to produce such a material.

### Carbon isotopic measurements

In order to investigate the utility of the three materials with carbon signals most similar to that of natural sedimentary silicates (i.e., Ceramacast 905 + silica, Aremco-Bond 526N + silica, and ADVANCER CN703), we used an ion microprobe to measure their carbon isotopic composition using the PPRG #215-1 as a standard. By using a natural rock standard, this test was a measure of observable matrix effects during the isotope measurement by SIMS. In general, SIMS is known for potentially having large instrumental mass fractionations that need to be corrected by a parallel analysis of a standard. Figure 1 shows the observed matrix effects of each material on two different days. The first day of analyses (March 24th)



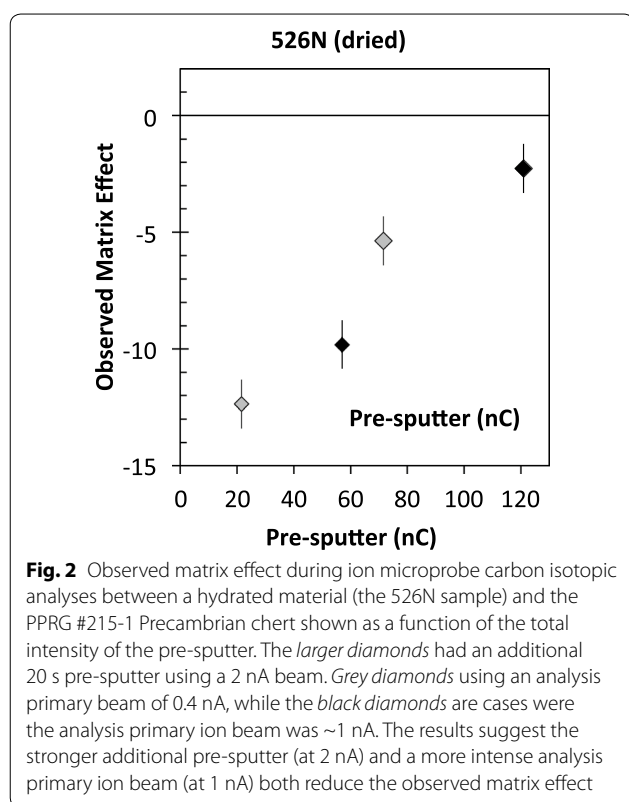
was shortly after synthesizing, mounting, and polishing the materials. The second set of analyses was conducted 3 days later. Between these two ion microprobe sessions, the materials were stored in a heated vacuum oven.

The  $\delta^{13}\text{C}$  results from March 24th clearly show a large matrix effect with the 905 and 526N samples appearing more depleted in  $^{13}\text{C}$  than they are. The matrix effect observed for the 526N sample was larger than that of 905 at up to 14%. Because I had observed swelling of the 526N sample during polishing and rinsing (see experimental methods), I hypothesized that the matrix effect could be associated with hydration.

Based on the hypothesis that at least part of the matrix effect observed was associated with water, the samples were reanalyzed after being in the vacuum oven. The  $\delta^{13}\text{C}$  results from March 27th provide support for the notion that water is associated with a substantial matrix effect. The later results from Ceramacast 905 + silica showed no significant matrix effects and were constant over the course of each run. In contrast, the more hydrated Aremco-Bond 526N + silica again showed a large matrix effect (with the carbon appearing to be up to 12% more depleted in  $^{13}\text{C}$  than it is). Because a more intense primary ion beam appeared to reduce the observed effect (Fig. 1), I added an intense pre-sputtering using a  $\sim 3$  nA beam before the final two  $\delta^{13}\text{C}$  measurements of the 526N sample. The March 27th results from the 526N sample show that both an increased primary ion beam and the addition of an intense pre-sputter greatly reduce the observed matrix effect (Fig. 2). These results suggest that the effect is due to hydration that occurred during sample preparation. The synthetic standards, generally, did not show significant trends toward more  $^{13}\text{C}$ -depleted values over the course of individual analysis runs, as might be expected if the pre-sputter was simply removing the hydrated surface. Overall, the results still indicate that sample drying, adequate intense pre-sputtering, and an intense primary ion beam reduce matrix effects between the different samples.

At this time, it is not clear how water generates such a large matrix effect. We can, however, rule out some possible mechanistic options. First, this is not due to interference from the molecular hydride ion, as that would result in the appearance of  $^{13}\text{C}$ -enrichment rather than  $^{13}\text{C}$ -depletion. Likewise, this is not exclusively a sample chamber vacuum issue, because the effect was observed between two materials (PPRG #215-1 and Ceramacast 905) mounted on the same grain mount.

The effect observed here does operate in the same direction as the matrix effect correlated with H/C observed by [17, 18] where higher hydrogen was correlated with spots appearing to be more  $^{13}\text{C}$ -depleted. These various results might be related if these matrix



effects result from increased ionization of  $^{12}\text{C}$  and  $\text{C}_2$ , which could occur in the presence of both water and hydrogen. It should be noted, though, that the organic materials used in this present study are quite rich in hydrogen and so it does not appear that H/C was associated with a matrix effect for the SIMS conditions used here. Also, based on the observation that a 3 nA pre-sputter was effective at reducing the matrix effect observed here, we might not expect this effect under the conditions used by [17] which had primary ion beams of between 2 and 20 nA.

It is also unclear why an intense pre-sputter lessens or eliminates the matrix effect observed here. If the matrix effect is related to ionization efficiency, the cesium added during the intense pre-sputter might moderate the influence of the water present. This explanation, however, seems erroneous because the intense pre-sputter is resulting in a material that acts like the water-poor PPRG 215-1 even though cesium increases overall ionization. Another possible explanation is that some hydration slightly increases the electrolytic conductivity of the surface. In this explanation, an intense pre-sputter could reduce the observed matrix effect by a combination of removing surface layers that are particularly wet and dehydrating a zone beneath the pit through ion milling. Regardless of the cause of the matrix effect, it might be

possible to incorporate the Aremco-Bond 526N + silica material into a working test of SIMS conditions prior to the analysis of important samples to ensure that the conditions used are suitable for minimizing water-associated matrix effects. For the Ceramacast 905 plus silica material, 60 s of pre-sputter was used on March 27th, which was sufficient for the results reported, and so it is unclear if a more intense pre-sputter of the Ceramacast 905 would have removed the about 0.5‰ difference in instrumental mass fractionation seen in the reported data (Fig. 1).

Last, the ADVANCER CN703 sample was analyzed. Although this silicon carbide material had demonstrated a high carbon secondary ion signal, its carbon signal could be analyzed using the same ion microprobe configuration by greatly reducing the intensity of the primary ion beam. The results shown in Fig. 1 demonstrate a significant matrix effect with the material appearing more enriched in  $^{13}\text{C}$  than its actual composition by almost 6%. At face value, these results indicate that like graphite, SiC is not a proper SIMS standard for organic carbon.

## Experimental

I chose five materials to be tested for their suitability as isotope standards for  $\delta^{13}\text{C}$  analyses of carbon in silicate sedimentary rocks. These materials either were purchased, donated, or synthesized for this research. The materials were then analyzed for their  $\delta^{13}\text{C}$  composition using the UCLA CAMECA ims 1270 ion microprobe.

### PEEK GF30 + silica synthesis

A non-annealed, one inch-diameter rod of PEEK GF30 was donated to the project by Quantum Polymers. PEEK was selected because it is a high performance thermoplastic reducing the possibility that it would soften or ablate during the sputtering process or if mistakenly hit with high-energy electrons. A glass-filled variety was selected to have  $\text{SiO}_2$  be a major component of the material, as is the case for sedimentary silicates. During previous research, we had found that PEEK was isotopically homogeneous, but generates too strong a secondary ion beam for comparison with Proterozoic cherts (Peng, Guo, House, Chen, and Ta, unpublished observations). In this work, I attempted to reduce the secondary ion signal from PEEK to a level similar to that of natural sedimentary silicates by adding a mask of silica to the top of the GF30 disk. To do this, in short, I placed a PEEK disk into silica powder and then heated the disk beyond the melting point of PEEK so that some of the silica would adhere to the plastic surface.

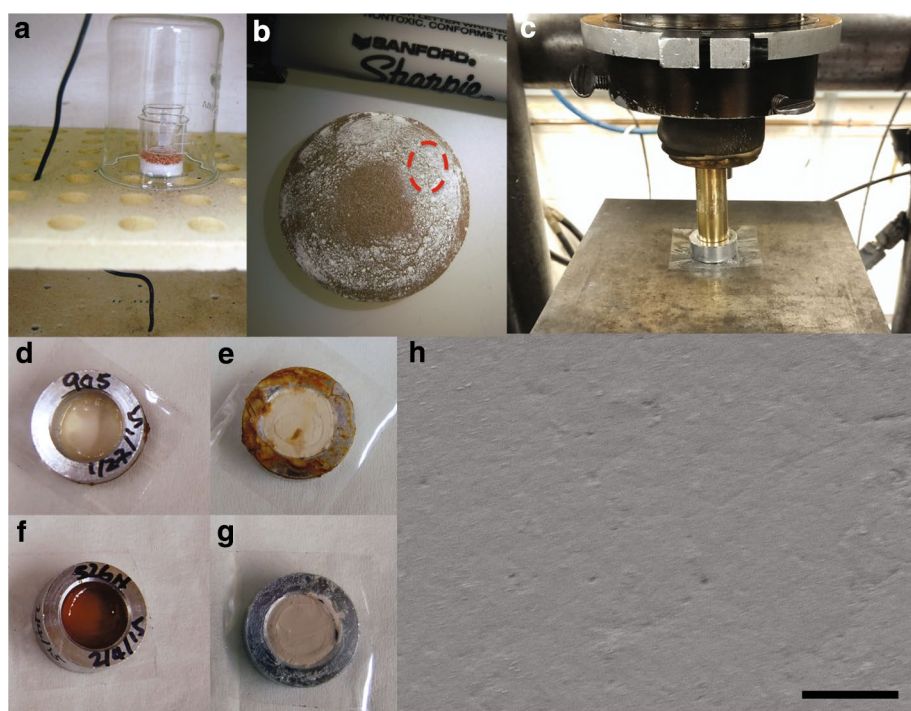
First, a ~0.5 cm long piece of the PEEK GF20 rod was cut forming a disk. A bed of 0.5 g of silica nanopowder (Sigma-Aldrich item #637238) was placed into

a 25-mL Pyrex beaker. The PEEK disk was then placed onto this bed of silica powder, and another 0.5 g of silica nanopowder was placed on top of the disk, covering it completely. A 15-mL beaker was then placed on top of the silica, and approximately one gram of copper filings was added to that beaker to act as an oxygen scrubber. The whole set-up was then placed into an ashing furnace covered by a large beaker (Fig. 3a). A copper line was run into the furnace to continuously flow nitrogen through the oven space. The oven temperature was then raised to 345 °C over the course of a couple of hours. Then, the oven temperature was held at 345 °C for 1 h. The melting point of PEEK is 343 °C and so this procedure was meant to ensure that the PEEK disk exceeded its melting point while surrounded by silica powder that could then adhere to the plastic (Fig. 3b). This procedure was not optimized, and certainly could use improvement if this material is to become a widely used standard. For example, placing the sample between two metal blocks would likely provide more even heating and pressure (Katherine Freeman, personal communication).

#### Ceramacast 905 + silica synthesis

Ceramacast 905 is a one component high temperature casting silicone rubber (purchased from Aremco). This casting material was used to bond the grains of a pressed silica powder producing a solid disk of mostly silica and secondarily rubber.

First, a 1-inch OD aluminum ring (“aluminum unthreaded spacer,” item #92510A818 from McMaster-Carr) was taped on one side with acrylic packing tape. 0.5 g of silica nanopowder (Sigma-Aldrich item #637238) was then pressed into the ring at ~25 MPa by a brass piston using a biaxial loading frame (Fig. 3c). Then, 0.5 g of the Ceramacast 905 liquid reagent was added to the ring (Fig. 3d). The ring was set aside for 24 h to air dry (allowing the volatile component of the reagent to leave). The ring was then heated for 2 h at 230 °C to cure the rubber. This one-step curing approach was used rather than the manufacturer’s directions because it appeared that by going to 230 °C immediately the rubber migrated throughout the silica before hardening. After curing, the tape was carefully removed from the underside of the sample (Fig. 3e), and the exposed surface was



**Fig. 3** Images showing the syntheses of three of the materials used in this study. **a** A PEEK GF30 disk surrounded by silica nanopowder and covered by a layer of copper filings, in an oven to be heated to 345 °C under a nitrogen flow. **b** PEEK GF30 disk after removal from the oven showing silica nanopowder adhered to the surface. The red circle shows the approximate piece cut out and used for the test. **c** Silica nanopowder in an aluminum ring being pressed at 25 MPa. **d** Pressed silica nanopowder with Ceramacast 905 added. **e** Same sample as D turned over to show side that will be polished after the packing tape is removed. **f** Pressed silica nanopowder with Aremco-Bond 526N added. **g** Same sample as F turned over to show side that will be polished after the packing tape is removed. **h** SEM image of the surface of the polished Ceramacast 905 + silica sample used for SIMS. Scale bar is 20  $\mu$ m

then polished by hand using a slurry of 3-micron alumina (Buehler) on a polishing cloth adhered to a glass plate. The polished material was later removed from the aluminum ring and placed in an epoxy grain mount.

Figure 3h shows a scanning electron microscope (SEM) image of the polished surface of the Ceramacast 905 + silica sample. The SEM image shows that the silica grains are small relative to a typical dynamic SIMS primary ion beam. However, the surface, as prepared here, may not be suitable for NanoSIMS without additional refinement because NanoSIMS is often operated with sub-micrometer-sized primary ion beams.

#### Aremco-Bond 526N + silica synthesis

Aremco-Bond 526N is a two component high temperature epoxy (purchased from Aremco). This epoxy was used to bond the grains of a pressed silica powder producing a solid disk of mostly silica and secondarily rubber.

First, another 1-inch OD aluminum ring (item #92510A818 from McMaster-Carr) was taped on one side with acrylic packing tape. 0.5 g of silica nanopowder (Sigma-Aldrich item #637238) was then pressed into the ring at ~25 Mpa by a brass piston using a biaxial loading frame. Then, 0.6 g of the Aremco-Bond 526N epoxy mixture was added to the ring (Fig. 3f). The ring was then heated at 170 °C for 3.5 h to cure the epoxy. After curing, the tape was carefully removed from the underside of the sample (Fig. 3g), and the exposed surface was then polished by hand using a slurry of 3-micron alumina on a polishing cloth adhered to a glass plate. In this case, the aluminum ring was cut in half to decrease the depth of the sample and allow the whole mount to fit into a typical SIMS sample holder.

During the polishing step, the sample swelled (as the silica apparently hydrated), and then popped out of the aluminum ring due to its swelling. In contrast, this clear swelling was not observed for the 905 sample when it was similarly polished.

#### ADVANCER CN703 and CARBOFRAX a procurement

ADVANCER CN703 and CARBOFRAX A ceramic plates were donated by Saint-Gobain Materials. 1-cm size squares were cut from this silicon carbide-based material. The cut-surfaces were used for the ion microprobe analyses after being placed in an epoxy grain mount.

#### Conclusions

This study has shown that silica powder, pressed, and then bonded with Ceramacast 905 produced a useful synthetic standard. The material produced has a secondary ion carbon yield of only about 15× that of the organic-rich chert PPRG #215-1 and similar to large microfossils in polished chert sections. Finally, the material,

once dried sufficiently, did not demonstrate a significant matrix effect when the carbon isotopes were determined. The resultant suitable carbon isotope standard for SIMS measurements of organic material in rocks will allow an acute growth in both the quantity and quality of studies of ancient carbon at the microscale.

The project has revealed a strong matrix effect associated with hydration of two of the materials studied. Especially, silica powder, pressed, and then bonded with Aremco-Bond 526N clearly showed this water-associated matrix effect, and repeated analyses of the 526N sample demonstrated that the effect was greatly reduced by an intense pre-sputter. This newly revealed matrix effect is important because it might have misled previous research. The effect could lead to increased observed heterogeneity of partially hydrated samples and/or produced systematic differences between natural targets and the standards used.

#### Methods

##### Ion microprobe analyses

$\delta^{13}\text{C}$  compositions were determined with the University of California–Los Angeles (UCLA) Cameca 1270 using a multicollector configuration with  $^{12}\text{C}_2^-$  detected by an off-axis electron multiplier (EM) and  $^{12}\text{C}^{13}\text{C}^-$  measured using the central EM. The molecular ion ( $\text{C}_2^-$ ) produces a stronger count rate than the atomic ion ( $\text{C}^-$ ), and its mass permits the on-axis detector to be used. SIMS was performed using a 0.03–1.2 nA, ~8  $\mu\text{m}$ ,  $\text{Cs}^+$  primary beam (with lower intensities required for ADVANCER CN703 and a range of intensities used for the PPRG #215-1). The mass resolving power (M/M) was about 6000, and charge compensation was achieved using a normal incident electron gun and a gold coat. The analyses (Table 2) were calibrated against repeated analyses of PPRG#215-1 Precambrian chert, as previously used for Precambrian microfossils [3, 7, 20, 21]. The instrumental mass fractionation was found to be similar to past experience with these conditions. Under these conditions, quasi-simultaneous arrival effects are expected to be small because the production of  $\text{C}_2^-$  is relatively ineffective with ionization around a few percent. In most cases, a pre-sputter of the analysis primary ion beam was used. These conventional pre-sputter times are listed in Table 2. For the final four analyses of Aremco-Bond 526N + silica, pre-sputter was added with combinations of 60 s of a 0.4 nA primary ion beam and/or 20 s of a ~3 nA primary ion beam.

##### Scanning electron microscopy

An SEM image at 2500× magnification was taken of the polished and gold-coated surface of the Ceramacast 905 + silica sample. The SEM high voltage was 20 kV, the working distance was 10 mm, the spot diameter was 5 nm, and the horizontal field width was 0.11 mm.

**Table 2 Carbon isotope analyses by ion microprobe of possible synthetic standards**

Sample material	Primary Cs <sup>+</sup> intensity	Pre sputter (s)	C <sub>2</sub> <sup>-</sup> intensity (cps)	Observed δ <sup>13</sup> C ± σ	PPRG #215-1 Instrument mass fractionation factor α	Corrected δ <sup>13</sup> C	True δ <sup>13</sup> C	Observed matrix effect
905	6.7E-10	15	1.7E+05	-33.1 ± 1.1	1.0006 (0.6 ‰)	-33.7	-27.9	-6.0
905	6.7E-10	15	3.4E+05	-34.5 ± 0.7	1.0006 (0.6 ‰)	-35.1	-27.9	-7.4
905	2.2E-10	15	3.7E+05	-35.9 ± 0.7	1.0006 (0.6 ‰)	-36.5	-27.9	-8.9
905	2.2E-10	45	2.6E+05	-35.5 ± 0.7	1.0006 (0.6 ‰)	-36.1	-27.9	-8.4
905	2.2E-10	45	2.3E+05	-35.4 ± 1.1	1.0006 (0.6 ‰)	-36.0	-27.9	-8.4
					Weighted mean	-35.7		-8.0
					Weighted standard deviation	1.0		
526N	8.30E-11	45	1.3E+05	-41.9 ± 0.7	1.0006 (0.6 ‰)	-41.9	-27.7	-14.7
526N	8.40E-11	45	7.6E+05	-40.0 ± 1.1	1.0006 (0.6 ‰)	-40.0	-27.7	-12.7
					Weighted mean	-40.4		-13.1
					Weighted standard deviation	1.4		
Sample material	Primary Cs <sup>+</sup> intensity	PrE-sputter (s)	C <sub>2</sub> <sup>-</sup> intensity (cps)	Observed δ <sup>13</sup> C ± σ	PPRG #215-1 Instrument mass fractionation factor α	Corrected δ <sup>13</sup> C	True δ <sup>13</sup> C	Observed matrix effect
905	7.5E-11	60	1.2E+05	-24.9 ± 1.4	1.0046 (4.6 ‰)	-29.4	-27.9	-1.6
905	1.1E-10	60	2.6E+05	-22.9 ± 0.8	1.0046 (4.6 ‰)	-27.4	-27.9	0.5
905	5E-11	60	1.4E+05	-24.0 ± 1.2	1.0046 (4.6 ‰)	-28.5	-27.9	-0.7
905	5.2E-11	60	1.6E+05	-24.4 ± 1.0	1.0046 (4.6 ‰)	-29.0	-27.9	-1.1
905	4.1E-11	60	1.4E+05	-22.5 ± 1.4	1.0046 (4.6 ‰)	-27.0	-27.9	0.9
905	5E-11	60	1.6E+05	-22.9 ± 0.8	1.0046 (4.6 ‰)	-27.4	-27.9	0.5
905	1E-10	60	2.1 E+05	-26.0 ± 0.8	1.0046 (4.6 ‰)	-30.5	-27.9	-2.7
905	1E-10	60	2.0E+05	-24.1 ± 1.1	1.0046 (4.6 ‰)	-28.6	-27.9	-0.7
905	1.6E-10	60	2.4E+05	-24.4 ± 1.1	1.0046 (4.6 ‰)	-28.9	-27.9	-1.0
905	1.6E-10	60	3.0E+05	-22.9 ± 0.8	1.0046 (4.6 ‰)	-27.4	-27.9	0.5
					Weighted mean	-28.4		-0.5
					Weighted standard deviation	1.1		
526N	9.5E-10	60	1.2E+05	-32.8 ± 2.0	1.0046 (4.6 ‰)	-37.2	-27.7	-9.8
526N	3.6E-10	60	3.9E+04	-35.2 ± 3.5	1.0046 (4.6 ‰)	-39.7	-27.7	-12.4
526N	9.4E-10	60+	2.7E+05	-25.4 ± 1.2	1.0046 (4.6 ‰)	-29.9	-27.7	-2.3
526N	3.6E-10	60+	6.2E+04	-28.4 ± 2.1	1.0046 (4.6 ‰)	-32.9	-27.7	-5.4
					Weighted mean	-32.5		-4.9
					Weighted standard deviation	4.5		
CN703	3.1E-11	60	4.5E+05	-17.5 ± 1.1	1.0046 (4.6 ‰)	-22.1	0.0	6.7
CN703	3.1E-11	60	2.5E+05	-15.9 ± 1.2	1.0046 (4.6 ‰)	-20.5	0.0	8.3
					Weighted mean	-21.3		7.4
					Weighted standard deviation	1.1		

Instrument mass fractionation (IMF) factor  $\alpha = (\delta^{13}\text{C}_{\text{obs}} + 1000)/(\delta^{13}\text{C}_{\text{Exp}} + 1000)$

IMF in ‰ =  $1000 * \ln(\alpha)$ . Corrected  $\delta^{13}\text{C} = ((\delta^{13}\text{C}_{\text{obs}} + 1000/\alpha) - 1000)$

True  $\delta^{13}\text{C}$  is that measured by conventional elemental analyzer-isotope ratio mass spectrometry (EA-IRMS), apparent matrix effect (AS) = Corrected  $\delta^{13}\text{C}$  - True  $\delta^{13}\text{C}$

The last two 526N samples add the following additional pre-sputter respectively: 20 seconds of an ~2 nA beam and 60 s of an ~0.4 nA beam; 20 s of an ~2 nA beam

### Abbreviations

SIMS: secondary ion mass spectrometry is a form of mass spectrometry where a focused primary ion beam is used to sputter the surface of a specimen ejecting secondary ions. The secondary ion beam generated is then analyzed by measuring the mass-to-charge ratio; PEEK: polyether ether ketone is a colorless high-performance thermoplastic polymer; SEM: scanning electron microscope is a microscope that forms a image by scanning a target with a focused beam of electrons.

### Authors' information

CHH has been testing possible synthetic standards for the carbon isotopic analysis of microfossils in Precambrian chert since looking for such a standard during his Ph.D. dissertation research. The work here, therefore, represents the successful end of a long search.

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### Compliance with ethical guidelines

### Competing interests

The author declares that he has no competing interests.

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### References

- Hinrichs KU, Hayes JM, Sylva SP, Brewer PG, DeLong EF (1999) Methane-consuming archaeobacteria in marine sediments. *Nature* 398:802–805
- House CH, Orphan VJ, Turk KA, Thomas B, Pernthaler A, Vrentas JM, Joye SB (2009) Extensive carbon isotopic heterogeneity among methane seep microbiota. *Environ Microbiol* 11:2207–2215
- House CH, Schopf JW, McKeegan KD, Coath CD, Harrison TM, Stetter KO (2000) Carbon isotopic composition of individual Precambrian microfossils. *Geology* 28:707–710
- Kilburn MR, Wacey D (2011) Elemental and isotopic analysis by NanoSIMS: insights for the study of stromatolites and early life on Earth. In: Tewari V, Tewari VC, Seckbach J (eds) *Stromatolites: interaction of microbes with sediments*. Springer, Dordrecht, Netherlands, pp 463–493
- Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF (2001) Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science* 293:484–487
- Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF (2002) Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proc Natl Acad Sci* 99:7663–7668
- Williford KH, Ushikubo T, Schopf JW, Lepot K, Kitajima K, Valley JW (2013) Preservation and detection of microstructural and taxonomic correlations in the carbon isotopic compositions of individual Precambrian microfossils. *Geochim Cosmochim Acta* 104:165–182
- House CH, Schopf JW, Stetter KO (2003) Carbon isotopic fractionation by Archaeans and other thermophilic prokaryotes. *Org Geochem* 34:345–356
- Londry KL, Des Marais DJ (2003) Stable carbon isotope fractionation by sulfate-reducing bacteria. *Appl Environ Microbiol* 69:2942–2949
- Preuß A, Schauder R, Fuchs G, Stichler W (1989) Carbon isotope fractionation by autotrophic bacteria with 3 different CO<sub>2</sub> fixation pathways. *Z Naturforsch C J Biosci* 44:397–402
- Hayes JM (1994) Global methanotrophy at the Archean-Proterozoic transition. In: Bengtson S (ed) *Early life on Earth: Nobel Symposium 84*. Columbia University Press, New York, pp 220–236
- Hinrichs KU (2002) Microbial fixation of methane carbon at 2.7 Ga: was an anaerobic mechanism possible? *Geochem Geophys Geosyst* 3:1–10
- Zerkle AL, House CH, Brantley SL (2005) Biogeochemical signatures through time as inferred from whole microbial genomes. *Am J Sci* 305:467–502
- Farquhar J, Hauri E, Wang J (1999) New insights into carbon fluid chemistry and graphite precipitation: SIMS analysis of granulite facies graphite from Ponmudi, South India. *Earth Planet Sci Lett* 171:607–621
- Mojzsis SJ, Arrhenius G, McKeegan KD, Harrison TM, Nutman AP, Friend CR (1996) Evidence for life on Earth before 3,800 million years ago. *Nature* 384:55–59
- Ueno Y, Isozaki Y, Yurimoto H, Maruyama S (2001) Carbon isotopic signatures of individual Archean microfossils (?) from Western Australia. *Int Geol Rev* 43:196–212
- Sangély L, Chaussidon M, Michels R, Huault V (2005) Microanalysis of carbon isotope composition in organic matter by secondary ion mass spectrometry. *Chem Geol* 223:179–195
- Williford KH, Ushikubo T, Lepot K, Hallmann C, Spicuzza MJ, Eigenbrode JL, Summons RE, Valley JW (2011) In situ carbon isotope analysis of Archean organic matter with SIMS. In: *AGU Fall Meeting Abstracts*
- Kaufman AJ, Xiao S (2003) High CO<sub>2</sub> levels in the Proterozoic atmosphere estimated from analyses of individual microfossils. *Nature* 425:279–282
- House CH, Oehler DZ, Sugitani K, Mimura K (2013) Carbon isotopic analyses of ca. 3.0 Ga microstructures imply planktonic autotrophs inhabited Earth's early oceans. *Geology* 41:651–654
- Lepot K, Williford KH, Ushikubo T, Sugitani K, Mimura K, Spicuzza MJ, Valley JW (2013) Texture-specific isotopic compositions in 3.4 Gyr old organic matter support selective preservation in cell-like structures. *Geochim Cosmochim Acta* 112:66–86

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