POSITION-SPECIFIC ISOTOPE FRACTIONATION OF AMINO ACIDS ON ICE SURFACES: IMPLICATIONS FOR Icy ENVIRONMENTS. A. C. Fox1, E. Martineau2,3, G. S. Remaud1, and K. H. Freeman1, 1Pennsylvania State University, University Park, Pennsylvania, USA, 2SpectroMaitrise, CAPACITES, 26, bd Vincent Gâche - 44200 Nantes, France 3CEISAM UMR 6230, University of Nantes, 2 rue de la Houssinière, Nantes, France.

Introduction: Amino acids (AAs) are prime targets for detection in extraterrestrial environments as their presence on early Earth led to more complex prebiotic compounds essential to life (1). Abiotic formation mechanisms have led to AAs present in many diverse environments, such as meteorites (2), as well as their suspected presence in interstellar space (3) and icy worlds (4).

The presence of AAs in extraterrestrial environments implies they survive high radiative and oxidative conditions. AAs can be protected from oxidation or radiolysis by interactions with mineral surfaces (5). Furthermore, they can concentrate on ice and mineral surfaces, which can promote condensation reactions (6). Therefore understanding the isotopic effects associated with organo-mineral interactions will provide insight on both the preservation and alteration of AAs in extraterrestrial environments.

AAs primarily interact with the surface hydroxyl groups via hydrogen bonding or dipole-dipole interactions (7). While these are relatively weak intermolecular forces, previous work has observed small C isotope effects associated with these forces. Dias and Freeman (1997) found that carboxylic acids sorbed to solid phase microextraction fiber were enriched up to 1.5‰ compared to those in solution (8). More recently, Julien et al. (2017) measured C isotope fractionation during the distillation of alcohols. While global C isotope effects were negligible, there was significant fractionation at C positions closest to the alcohol group (up to 8.6‰). The authors noted correlations between the magnitude of fractionation and physical properties relating to hydrogen bonding (i.e. hydrogen bond acidity), suggesting that the strength of the intermolecular interaction influenced the magnitude of isotopic fractionation (9). Both studies indicate that weak intermolecular interactions can influence the isotopic composition of amino acids that interact with mineral or ice surfaces.

In this work, the interactions between glycine, L-alanine, or L-leucine and ice surfaces were investigated using position-specific isotope analysis (PSIA). PSIA was used to determine the $\Delta^{13}C_{\text{free-ice}}$ (defined here as $\delta^{13}C_{\text{free}} - \delta^{13}C_{\text{ice}}$) of each C position. Using these data, we sought to determine [1] if the preservation of AAs by sorption can impart either a global or position-specific isotope effect, and [2] if position-specific isotope analysis can determine the orientation of sorbed AAs.

Methods: 
Amino Acid – Ice Sorption. Three hundred milligrams of an individual amino acid (Sigma Aldrich) were dissolved in 50 mL of 18.2 MΩ water. The sample was placed in an ice bath kept at 0°C and 50 g ± 5 g of ice were added to the sample. The sample was then agitated for 1 hr using a stir bar. After agitation, the liquid and ice fractions were separated into clean beakers and evaporated to dryness at 95±5°C under an N2 stream.

Isotopic Analysis. Isotopic analyses were performed for global $\delta^{13}C$ and $\delta^{15}N$ and position-specific $\delta^{13}C$. One milligram of sample from the ice and liquid fractions were analyzed by EA-IRMS using a Delta-V Advantage isotope ratio mass spectrometer coupled to an NA2100 elemental analyzer (Thermo Scientific). Intramolecular $\delta^{13}C$ values for each carbon in a given amino acid were calculated using the equation:

$$x_i = x_g \left( \frac{F_i}{F_c} \right)$$

where $x_g$ and $x_i$ are the isotopic abundance of global and of position $i$ of the molecule, respectively, $F_i$ is the molar fraction determined from NMR peak areas, and $F_c$ is the statistical molecular fraction, molar fraction for the carbon position $i$ in case of a homogeneous $^{13}C$ distribution within the molecule ($F_c = 1/6$ for leucine).

Quantitative $^{13}C$ NMR was used to determine the peak areas for each position within a given amino acid. All the NMR acquisitions were performed at 303 K on a Bruker Avance HD 700 spectrometer (Bruker Biospin), at a frequency of 176.09 MHz with a cryogenic $^1H/^13C$ dual probe. For analysis, between 30-50 mg of each amino acid was dissolved in the appropriate ratio (based on solubility) of 18.2 MΩ water and deuterated acetic acid (EURISOTOP). The ratio of the area of peak $i$ to the total area of all peaks were used to determine the molar fraction, $f_i$.

Results: Here we report the $\Delta^{13}C_{\text{free-ice}}$ for all positions within glycine, L-alanine, and L-leucine. In all experiments, recovery was ≥ 95%. Percent sorbed was between 10-20%, consistent will previous sorption studies on a variety of minerals (10, 11).

Global Isotopic Fractionation. In all cases, both C and N isotopic values of free and ice-bound amino acids were within error of each other, indicating that there is no observable fractionation (i.e. $\Delta^{13}C_{\text{free-ice}} = 0$) caused by sorption at the global scale.
Figure 1: Molecular structures and $\Delta^{13}\text{C}_{\text{free-ice}}$ for glycine, L-alanine, and L-leucine, where $\Delta^{13}\text{C}_{\text{free-ice}}$ is defined as $\delta^{13}\text{C}_{\text{free}} - \delta^{13}\text{C}_{\text{ice}}$. Errors were calculated as the standard deviation of 10 runs. Only L-leucine displayed fractionation outside error bars.

Position-Specific Isotopic Fractionation. For glycine and L-alanine, all positions had $\Delta^{13}\text{C}_{\text{free-ice}}$ less than the experimental error of 2 %. In contrast, L-leucine had $\Delta^{13}\text{C}_{\text{free-ice}}$ values between 1 and 8 %, with the highest isotope effect at the carboxyl carbon (Fig 1). The magnitude of $\Delta^{13}\text{C}_{\text{free-ice}}$ in L-leucine is well above the experimental error of 2 %.

Discussion: At the pH of the experiment, (pH = 6) all three AAs will be predominantly present in a neutral, zwitterionic form (Fig 1). As a result, they are expected to interact with the ice surface via similar interactions, likely hydrogen bonding and fleeting dipole-dipole interactions. However, the only observable fractionation occurs at specific positions within L-leucine, primarily the depletion of carboxyl $\delta^{13}\text{C}$ in the ice-bound fraction. These results are consistent with previous work that found both a depletion of sorbed polar compounds (8) and the highest isotope effect on the primary carbon (9).

These data suggest that L-leucine is preferentially sorbed to the ice surface by interaction at the carboxyl C. In contrast, glycine and L-alanine do not have a preferred orientation and are equally likely to interact at carboxyl or amine sites, both of which are capable of forming hydrogen bonds. The preferred orientation of L-leucine could be driven by its aliphatic chain. This nonpolar region could be subject to steric effects that drives the carboxyl carbon towards the surface causing an isotope effect. However, this hypothesis needs to be tested by repeating this experiment with amino acids with different structures and functional groups.

Implications: The focus of this work was two-fold. First, to determine if interactions with ice surfaces caused a global or position-specific isotope effect. For all amino acids tested, there is no global isotope effect. But, a position-specific isotope effect was observed for L-leucine. This suggests that the preservation of neutral amino acids through organic-mineral interactions will not affect interpretations of compound-specific, but can affect intramolecular isotope patterns. This was not an issue for small amino acids, but should be considered when using PSIA for larger amino acids.

Second, to use PSIA to gain information about how amino acids interact with an ice surface. In proposed models of dimerization on clay surfaces on early Earth, amino acids sorb at the carboxyl group. This leaves the amine group free to react with other amino acids via condensation reactions to polymerize and form more complex biomolecules (6). The data presented here suggest that smaller amino acids do not sorb to ice in a preferred orientation, which may inhibit dimerization on icy grains.

Acknowledgments: Thank you to A.-M. Schiporst (CEISAM) for EA-IRMS analysis. This material is based upon research supported by the Chateaubriand Fellowship of the Office for Science & Technology of the Embassy of France in the United States and the National Aeronautics and Space Administration through the NASA Astrobiology Institute under Cooperative Agreement No. 80NSSC18M0094 issued through the Science Mission Directorate.