Alanine M+1 results were processed according to the algorithm presented in “High-Dimensional Isotomics Part 1: A Mathematical Framework for Isotomics” [CITE]. Briefly, we define alanine as having six sites, as shown in Table X, where a site is defined as a subset of atoms of the same element within a molecule. All nonhydrogen sites correspond to specific positions of alanine. The hydrogen sites include the seven hydrogens in the alanine molecule and a hydrogen picked up upon ionization. We do not associate these with specific hydrogen positions, as the way that fragmentation samples these positions is unknown; we refer to them instead as being “retained” or “lost” upon fragmentation. We observed two subsets of these sites; first all at once in the full molecule, and second, the subset retained upon decarboxylation, which we label the “44” fragment, as the unsubstituted version of this fragment has a cardinal mass of 44.

**Table X: Alanine Sites and Fragmentation**

|  |  |  |  |
| --- | --- | --- | --- |
| **Site Name** | # of atoms | Observed in Full | Observed in 44 |
| Calphabeta | 2 | Yes | Yes |
| Ccarboxyl | 1 | Yes | No |
| Ocarboxyl | 2 | Yes | No |
| Namine | 1 | Yes | Yes |
| Hretrained | 6 | Yes | Yes |
| Hlost | 2 | Yes | No |

In each case, we proceeded via a “M+1” experiment, where only the isotopologues with a cardinal mass of 1 above the unsubstituted isotopologue were observed. We quantified our observations as M+1 relative abundances, defined as the relative abundance of the substitution of interest to the entire population of M+1 substituted isotopologues. For example, for the 44 fragment, our observations take the form

Where gives the M+1 relative abundance of 13C in the 44 fragment and gives the observed abundance of 13C in the 44 fragment.

These observations map onto the M+1 relative abundances of the parent population of isotopologues. With the sites we have defined, there are six M+1 isotopologues, corresponding to the relevant M+1 substitution (13C, 15N, 17O, or 2H) at the site of interest; their M+1 relative abundances have the form

Where gives the isotopologue concentration of the isotopologue with a 13C substitution at the carboxyl site and the abundant isotope (12C, 14N, 16O, 1H) at all other positions. For sites with multiple atoms, such as Ocarboxyl, the isotopologue of interest has a single 17O substitution.

With our isotopologues of interest and observations quantified in M+1 relative abundance space, we computed abundances of individual sites via matrix inversion. In particular, we solved the matrix inversion problem

Where the center column gives the isotopologues of interest, the right column gives our observations, and the left matrix determines how our observations sample the isotopologues. We can solve this problem analytically or numerically. For example, an analytical solution gives:

With and not explicitly used (although we note that all observations of M+1 relative abundances for the same fragment are related). Numerically, we may find a least-squares solution to the matrix equation. Our algorithm opts for the numerical approach, implemented via the *numpy* python library and the np.linalg.lstsq function.

After finding the M+1 relative abundances, we combined this data with observations of U13C, defined as

where [Unsub] gives the concentration of the unsubstituted isotopologue. (U values are a new type of isotope ratio introduced in [CITE] where the numerator gives the sums of concentrations of some set of isotopologues and the denominator gives the concentration of the unsubstituted isotopologue). U13C values were either determined via direct Orbitrap measurement or via EA-IRMS observations of R13C (which we treat as equivalent to U13C). By dividing U13C by the M+1 relative abundance of all carbons, we may calculate the UM+1 value:

Whose numerator is the sum of the concentrations of all M+1 substituted isotopologues and denominator is the unsubstituted isotopologue. Then, for each M+1 relative abundance we constrain, we may calculate the associated site-specific U value via

Where i is the isotope of interest and site is the site label. Following [CITE], we treat these site-specific U values as equivalent to the corresponding site-specific isotope ratios. Our treatment of experimental issues, fractionation, standardization, and error propagation follows that discussed in [CITE]; we use an abundance correction factor of σ = 0.0003, determined via forward modelling of this system.