Metabolic Modelling

Relative fluxes can be obtained using the ^{13}C enrichment values from ^{1}H spectra (**Fig. 1**) and peak ratios from ^{13}C spectra (**Fig. 2**) and incorporating these measures in appropriate metabolic models. The authors use tcaCALC ***(1)*** for fitting the peak ratios to appropriate models. The program tcaCALC is freely available through the UT Southwestern P41 research resource funded web site. As with all modeling paradigms, superior signal-to-noise ratio (SNR) data will produce more reproducible and accurate estimates of the metabolic variables. If the researcher wants to use labeling schemes other than those suggested above, they should also download the tcaSIM package, which allows simulations of the expected spectra assuming a given substrate mix. This effort is worthwhile, as repeated simulations can help generate a "feel" for the expected results based on the driving hypothesis about substrate selection.

In several cases, providing ^{13}C enrichment values to the model yields better results. This value can be obtained from the ^{1}H spectrum of the same extract. The ratio of ^{13}C satellites (indicated as A_s in **Fig. 1**) to central peak (indicated as A in **Fig. 1**) provides the absolute ^{13}C enrichment.

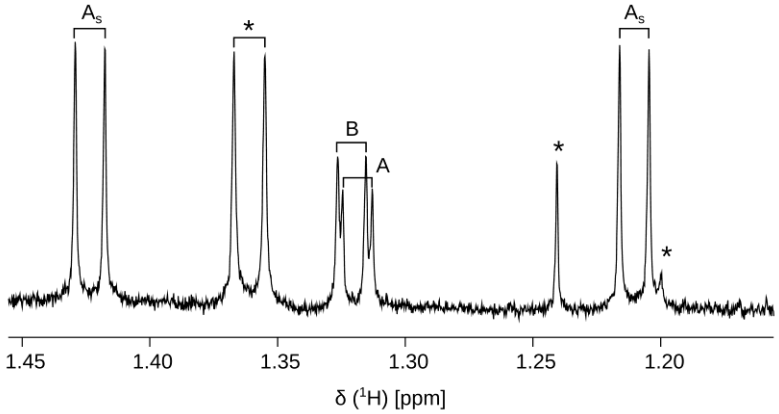

Figure 1: *^{1}H spectrum showing ^{13}C enrichment in C-3 position of lactate indicated as* A. *^{13}C satellites of lactate are indicated as* A_s. *Also present in the region of the spectrum are threonine (*B*) and other unknown resonances (*). The importance of good lineshapes in ^{1}H spectra to estimate ^{13}C enrichment from ^{1}H spectra accurately can be seen from the small difference in chemical shift between lactate and threonine.*

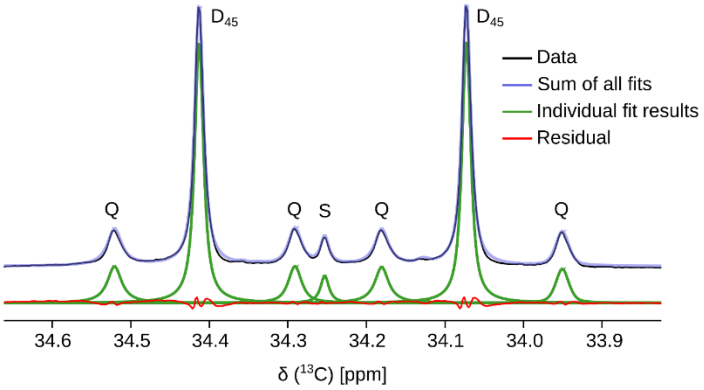

Figure 2: *^{13}C spectrum showing C-4 glutamate fitted to a mixed Gaussian-Lorentzian line shape. NMR spectra (black) and sum of fits (purple) are shifted vertically for clarity. Peaks are fit simultaneously constrained only for the chemical shifts. Peak intensity and linewidth of the peaks are allowed to vary during fitting and residuals are minimized.*

Refs

1. Sherry AD, Jeffrey FMH, and Malloy CR (2004) Analytical solutions for ^{13}C isotopomer analysis of complex metabolic conditions: substrate oxidation, multiple pyruvate cycles, and gluconeogenesis. Metab Eng 6:12–24