Quantification of High-Resolution 1H[13C] NMR Spectra from Rat Brain Extracts

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Introduction – $^{1}H[^{13}C]$ NMR, also known as Proton-Observed, Carbon-Edited (POCE) NMR, is a powerful technique to detect ^{13}C label incorporation from ^{13}C-labeled substrates into metabolic products with the sensitivity of proton detection [1]. $^{1}H[^{13}C]$ NMR can be used directly *in vivo*, but is also frequently applied to study ^{13}C labeling patterns in brain extracts *in vitro*. Recently, a novel spectral fitting algorithm was described for the spectral quantification of high-resolution ^{1}H NMR spectra from rat brain extracts *in vitro* [2]. Various aspects of $^{1}H[^{13}C]$ NMR spectra are different from regular ^{1}H NMR spectra and pertain to ^{13}C isotope shifts, decoupling sidebands and different amplitudes for various multiplets within the same molecule. Here an extension of spectral fitting to the quantification of high-resolution $^{1}H[^{13}C]$ NMR spectra is described.

Methods – All experiments were performed on a Bruker 11.74 T magnet. Rats were infused with [1,6-$^{13}C_2$]-glucose for 15, 30 or 60 min after which metabolism was stopped by focused-beam microwave irradiation. Ethanol-based extracts were made from the medial prefrontal cortex. Following lyophilization, extracts were dissolved in 500 uL of a 50 mM phosphate buffer (85 % D_2O) containing 0.5 mM DSS. $^{1}H[^{13}C]$ NMR spectra were acquired with an adiabatic spin-echo sequence (TR/TE = 15,000/8 ms) in the presence of adiabatic broadband decoupling during acquisition. $^{1}H[^{13}C]$ difference spectra were calculated as the difference between scans with and without a ^{13}C inversion pulse.

Results – All metabolites displayed a measurable ^{13}C isotope shift of –2.02 ± 0.26 ppb relative to the ^{12}C multiplet. Given the narrow line widths *in vitro* (1.18 ± 0.23 Hz), the ^{13}C isotope shifts essentially lead to shifted multiplets that cannot easily be approximated with a single basisset. In the $^{1}H[^{13}C]$ NMR difference spectrum, the ^{13}C isotope shift is not relevant and the spectrum can be fitted as a regular ^{1}H NMR spectrum (Fig. 1A-C), with the exception that the multiplet amplitudes must be independent (e.g. the amplitude of [4-^{13}C]-Glu does not necessarily have to be equal to that of [3-^{13}C]-Glu). The fitted $^{1}H[^{13}C]$ NMR difference spectrum (Figs. 1B/E) can then be subtracted from the ^{1}H NMR spectrum acquired without ^{13}C inversion (i.e. $^{1}H[^{12}C+^{13}C]$ NMR spectrum, Fig. 1D) to give a $^{1}H[^{12}C]$ NMR spectrum (Fig. 1F). In this spectrum the ^{13}C isotope shift is not present, such that it can be fitted as a regular ^{1}H NMR spectrum (Fig. 1G), but with the exception that the total metabolite amplitudes (e.g. Glu) should be corrected for the amount of ^{13}C-label fitted in Fig. 1B (i.e. [4-^{12}C]-Glu = Glu – [4-^{13}C]-Glu and [3-^{12}C]-Glu = Glu – [3-^{13}C]-Glu).

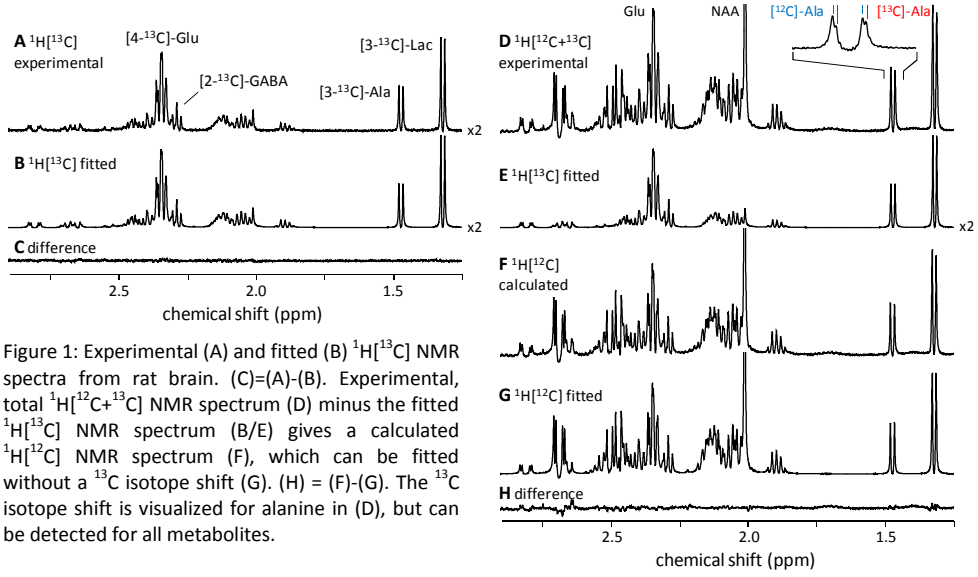

Figure 1: Experimental (A) and fitted (B) $^{1}H[^{13}C]$ NMR spectra from rat brain. (C)=(A)-(B). Experimental, total $^{1}H[^{12}C+^{13}C]$ NMR spectrum (D) minus the fitted $^{1}H[^{13}C]$ NMR spectrum (B/E) gives a calculated $^{1}H[^{12}C]$ NMR spectrum (F), which can be fitted without a ^{13}C isotope shift (G). (H) = (F)-(G). The ^{13}C isotope shift is visualized for alanine in (D), but can be detected for all metabolites.

Discussion – A spectral fitting algorithm has been described to account for the specific features of $^{1}H[^{13}C]$ NMR spectra. The most noticeable feature is a significant ^{13}C isotope shift, which is readily dealt with by sequentially fitting $^{1}H[^{13}C]$ and $^{1}H[^{12}C]$ NMR sub-spectra in which the ^{13}C isotope shift is not relevant. Additional features are the presence of decoupling sidebands and ^{13}C natural abundance signals and the need for independent multiplet amplitudes due to varying ^{13}C labeling. All of these features can be quantitatively dealt with by the spectral fitting algorithm. While the focus was on high-resolution $^{1}H[^{13}C]$ NMR, the algorithm is equally suited for *in vivo* $^{1}H[^{13}C]$ NMR where the ^{13}C isotope shifts represent a much smaller fraction of the spectral line widths.

[1] R. A. de Graaf et al, NMR Biomed. 24, 958 (2011) [2] R. A. de Graaf et al, Anal. Chem. 83, 216 (2011)

This research is partially sponsored by NIH grant R01 MH095104.