NMR Methods for Quantitative Isotopomer Rates in Real-Time Metabolism of Cells

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NMR Methods for Quantitative Isotopomer Rates in Real-Time Metabolism of Cells

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Abstract: Tracer-based metabolism is becoming increasingly important to study metabolic mechanisms in cells. NMR offers several approaches to measure label incorporation in metabolites, including ^{13}C and ^1H -detected spectra. The latter are generally more sensitive but quantification depends on the proton carbon $^1J_{\text{CH}}$ coupling constant which varies significantly between different metabolites. It is therefore not possible to have one experiment optimised for all metabolites and quantification of ^1H -edited spectra such as HSQCs requires precise knowledge of coupling constants. Increasing interest in tracer-based and metabolic flux analysis requires robust analyses with reasonably small acquisition times. Here we compare ^{13}C -filtered and ^{13}C -edited methods for quantification and show the applicability of the method for real-time NMR of cancer cell metabolism where label incorporations are subject to constant flux. We find an approach using a double-filter most suitable and sufficiently robust to reliably obtain ^{13}C -incorporations from difference spectra. This is demonstrated for JJN3 multiple myeloma cells processing glucose over 24h. The proposed method is equally well suited for calculating label incorporation levels in labelled cell extracts in the context of metabolic flux analysis.

Cellular metabolism changes extensively during normal proliferation and differentiation, and in response to disease and drug treatment. As the biological understanding of metabolism is vastly growing, there is also increasing interest in tracer-based experiments to study metabolic mechanisms and to carry out metabolic flux analysis. The two most common analytical technologies in this context are mass spectrometry (MS)^[1] and Nuclear Magnetic Resonance (NMR) spectroscopy. NMR is becoming an increasingly important method in this context^[2] as it has the ability to quantify site-specific label incorporation and is suitable for detecting metabolism in primary patient cells placed in an NMR tube to measure metabolism in real-time^[2–4] and to analyze labelled cell extracts in reasonable high-throughput^[5–10].

NMR offers several options to measure ^{13}C or ^{15}N isotope incorporation. For low-concentration metabolism samples, ^{13}C -observed spectra require long acquisition times because of the inherently lower sensitivity of ^{13}C vs ^1H . Moreover, long ^{13}C relaxation times contribute to increased acquisition times, a factor that can be reduced using relaxation agents, although these substances are not desirable for use in cell cultures. For this reason, most studies of cell samples have used proton observed spectra. From ^{13}C -observed spectra it is generally difficult to

quantify label incorporation. Due to the NOE arising from proton decoupling, intensities are unsuitable for quantification. But most importantly, ^1H -observe spectra can detect ^{13}C and ^{12}C -linked protons which enables a proper calculation of label incorporation. This is not possible with ^{13}C -observed spectra. This is why proton-observed methods have become the method of choice to quantify label incorporation.

Fan and co-workers have successfully used TOCSY spectra to study label incorporation in metabolites^[6,11], but these are time-consuming and not suitable for high-throughput or real-time analyses, although recently developed fast acquisition schemes may soon overcome this limitation^[11]. The most common approach for NMR based flux analysis is the use of ^1H - ^{13}C -HSQC spectra, as originally suggested by Szyperski et al^[12–14]. Several recent publications, including our own, show the true potential of HSQC spectra for a comprehensive tracer-based metabolic flux analysis^[7,15,16]. However, intensities in HSQC spectra are unhelpfully modulated by the large variation of coupling constants in metabolites, which can range between 120 and 210 Hz. Fast acquisition schemes such as ALSOFast considerably reduce acquisition times, however quantification is compromised due to the use of an Ernst angle approach^[17]. There is considerable information content in CC coupling constants but this again requires long acquisition times^[7,15].

The use of simple proton observed 1D spectra has recently been proposed for high-throughput screening of label incorporation into metabolites^[18]. In such 1D- ^1H spectra, the unlabelled ^{12}C -species appears as a central peak, flanked by the ^{13}C -coupled signals of the same proton. Vinaixa *et al.* suggested simply using the reduced intensity of the central signal to indicate label incorporation, but this approach is prone to large errors, most probably arising from signal overlap^[18]. Gialleonardo proposed comparing decoupled and undecoupled spectra for high-throughput quantification of enriched metabolites^[8]. However it is not clear what level of accuracy can be achieved owing to overlap with ^{13}C -coupled ^1H -signals in the undecoupled spectrum. Alternatively, Wan *et al.*^[19] suggested to calculate accurate concentrations from 1D- ^1H - ^{13}C -HSQC spectra using observed intensities and the pulse sequence specific transfer function. This is only possible if the $^1J_{\text{CH}}$ coupling constant and relaxation rates are known which is unfortunately not the case for most metabolites. There have been earlier efforts to obtain quantitative HSQCs that cover a wide range of coupling constants. Q-HSQC uses a constant-time approach, which has the inherent disadvantage of lower sensitivity^[20].

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Using a one-dimensional version of ^{13}C -edited spectra can be very useful for samples with only a sparse set of metabolites. For such 1D-spectra, it is challenging to achieve artefact-free ^{13}C -decoupling, and this is increasingly difficult at higher field strengths. In order to derive $^{13}\text{C}/^{12}\text{C}$ ratios it is also essential to acquire reference spectra that show all protons using a pulse sequence with equal transverse magnetization times in order to minimize relaxation-induced errors^[7]. This allows intensities in spectra to be used directly without further scaling to calculate the proportions of $^1\text{H}\{^{12}\text{C}\}$ or $^1\text{H}\{^{13}\text{C}\}$.

For one-dimensional approaches, there is a choice of using ^{13}C -edited spectra, measuring all ^{13}C -bound protons ($^1\text{H}\{^{13}\text{C}\}$) at a particular locus of a metabolite, or ^{13}C -filtered spectra yielding the counterpart of protons attached to ^{12}C only ($^1\text{H}\{^{12}\text{C}\}$). Such filtering is typically achieved using a BIRD sequence^[21]. Our goal here is to quantify label incorporation in metabolic flux experiments using NMR methods in a manner that is suitable for high-throughput analyses or real-time NMR of living cells^[2]. For this we have explored edited and filtered one-dimensional NMR methods. We have specifically looked at GBIRD^[22] as an option to quantify label incorporation in a range of metabolites with varying and unknown coupling constants.

Results

We decided to compare what we saw as the most promising editing and filtering sequences and to look for the most robust option that works for a wide range of J_{CH} couplings, providing high-resolution data. Theoretically the difference between an all-proton experiment ($^1\text{H}\{^{12}\text{C}\} + ^1\text{H}\{^{13}\text{C}\}$) and a ^{13}C -filtered experiment should yield the ^{13}C -edited spectrum and vice versa, the ^{13}C -edited subtracted from the all-proton experiment should yield the ^{12}C -counterpart. This also requires that all spectra except the filtered are ^{13}C -decoupled, although in practice it is advisable to also decouple the filtered spectrum in order to have exactly the same heat load arising from the decoupling sequence during acquisition thus avoiding difference artefacts.

Figure 1. Editing and filtering efficiencies. Extracts from 1D spectra showing the anomeric ^1H from α -D-glucose ($J_{\text{CH}}=168\text{Hz}$) for a 48% U- ^{13}C -glucose and 52% natural abundance glucose in RPMI cell culture medium. (A) 1D ^1H -NOESY

spectrum with the $^1\text{H}\{^{12}\text{C}\}$ and $^1\text{H}\{^{13}\text{C}\}$ resonances highlighted in blue and red respectively. (B) Overlay of 1D 'all- ^1H ' (solid lines) and 1D $^1\text{H}\{^{13}\text{C}\}$ -edited (dotted lines) spectra showing reduced accuracy as the J_{F} value determining the evolution periods ($2\delta=2\epsilon=1/J_{\text{F}}$) deviates increasingly from the optimal value of 168Hz. (C) and (D): Overlays of 1D 'all- ^1H ' (solid lines) and 1D $^1\text{H}\{^{12}\text{C}\}$ ^{13}C -filtered (dotted lines) spectra showing the efficiency of the double-BIRD filter with two identical filter periods (C) or with two different filter periods (D).

For the editing experiments, we chose the recently published pulse sequence of Smith *et al.*^[7] (Figure S1A) as this sequence is already optimized for 1D-application and has a higher sensitivity than a 1D-HSQC. The chosen pulse sequence also allows a switch from editing $^1\text{H}\{^{13}\text{C}\}$ to all- ^1H by omitting INEPT-type ^{13}C -pulses together with changes in the phase cycle. For filtering experiments, we implemented a double gradient-BIRD approach using two BIRD operators with delays $2\delta=1/J_{\text{F1}}$ and $2\epsilon=1/J_{\text{F2}}$ set for two different coupling constants^[21] (Figure S1B)^[22] to which we have added a bilinear adiabatic decoupling scheme. (See Supplementary Material and Figure S1 for technical details of this implementation). With this sequence, the corresponding all- ^1H experiment could be obtained by simply omitting selective ^{13}C -pulses.

As an initial test sample we used a mixture of 48% [U- ^{13}C]glucose and 52% natural abundance glucose. The amount of label incorporation was confirmed using an uncoupled 1D spectrum by integrating the central $^1\text{H}\{^{12}\text{C}\}$ and outer $^1\text{H}\{^{13}\text{C}\}$ signals of the anomeric proton of glucose (Figure 1a). With decoupled spectra (Figure 1b-d and S2), the overall line shape with a resolution of 32k points for the all-proton spectra is complex as the signals of $^1\text{H}\{^{12}\text{C}\}$ and $^1\text{H}\{^{13}\text{C}\}$ are separated by a small 1.5-2Hz isotope shift. Filtered spectra yield narrower lines because the $^1\text{H}\{^{12}\text{C}\}$ resonance is not subject to ^{13}C -mediated relaxation.

To address the question of how effective the filtering approach is for sub-optimal evolution periods, we arrayed the filtering periods without ^{13}C -decoupling during acquisition to observe residual $^1\text{H}\{^{13}\text{C}\}$ resonances. This eliminates potential errors in intensities arising from suboptimal ^{13}C -decoupling. For this we focussed on the well-resolved resonances of the anomeric ^1H of α -D-glucose with a J_{CH} coupling constant of 170Hz using the double filter GBIRD with either both filter periods δ and ϵ set to the same value (Figure 2, blue line and Figure S3a), or with the two filter periods set to J values separated by 50Hz (Figure 2, black line and Figure S3b).

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Figure 2. Filter failure, the proportion of $^1\text{H}\{^{13}\text{C}\}$ resonance intensity of the anomeric ^1H from α -D-glucose that survives the filter in undecoupled ^{13}C -filtered spectra. Double GBIRD filter with two equal filter periods $2\delta=2\varepsilon=1/J_{\text{HH}}$ (blue line) and double GBIRD filter with two unequal filter periods, $2\delta=1/J_{F1}$ and $2\varepsilon=1/J_{F2}$, separated by 50 Hz (black line). Filter failures were calculated from the areas under the curve for the $^1\text{H}\{^{13}\text{C}\}$ resonances as $100 \cdot (\text{AUC}/\text{AUC}_{J_{85\text{Hz}}})$.

For the experiments with both delays set to the same value, the filter efficiency suffers for large offsets of these delays from the actual coupling constant. For two unequal delays δ and ε set 50 Hz apart, the filter becomes considerably more tolerant towards suboptimal settings, in fact, the $^1\text{H}\{^{13}\text{C}\}$ resonance always appears to be well suppressed. The filter is somewhat more robust towards delays set for larger J values than smaller. Generally, the double filter GBIRD with two different filter periods separated by 50 Hz is a very robust filter, well suited to isotopomer quantification in metabolites.

With decoupling during acquisition, the situation is somewhat more complicated, mainly owing to the isotope shift that leads to two sets of overlapping doublets. Figure 1a shows the overall ^1H -NOESY signal without decoupling, whereas panels b-d show experiments with decoupling. The results again illustrate the greater robustness of the double filtering approach. As expected, editing shows a variation of signal intensity for varying settings of the evolution delay owing to the transfer function. The filtering experiments proved much more robust, although with decoupling there is a less obvious difference in the quality of filtering for equal and different BIRD periods with non-ideal evolution constants (Figure 1c and Figure 1d).

A remaining overestimation of the proportion of $^1\text{H}\{^{12}\text{C}\}$ in decoupled experiments is observed for longer filtering delays (smaller J_F). This was puzzling as the undecoupled experiment (Figure 2 and S3) indicated that the filter efficiency was very high. The likely explanation relates to the faster relaxation of $^1\text{H}\{^{13}\text{C}\}$ compared with $^1\text{H}\{^{12}\text{C}\}$. For smaller J_F , i.e. longer δ and ε , the transverse magnetization period is increased. The all- ^1H resonance intensity is more affected by transverse relaxation than the $^1\text{H}\{^{12}\text{C}\}$ and so for lower values of J_F , there is a small

overestimate of the proportion of $^1\text{H}\{^{12}\text{C}\}$ present. The faster relaxation of $^1\text{H}\{^{13}\text{C}\}$ vs $^1\text{H}\{^{12}\text{C}\}$ is clearly reflected in the greater line widths (Figure 1B-D). Nonetheless, the filtering approach is much more robust to non-ideal J than the editing approach.

We tested the filtering approach in real-time NMR spectra using JJN3 multiple myeloma cells fed with $[\text{U-}^{13}\text{C}]\text{glucose}$ as a metabolic precursor. For the acquisition of these spectra, JJN3 cells were embedded into a matrix of agarose as previously described^[2]. Many previous NMR studies of cells have targeted tissue densities to enhance NMR signal^[23–25], but by using our pulse sequence we can detect real-time metabolism from cell densities similar to those used in standard cell culture conditions. In this model-system the agarose acts mainly to keep small numbers of cells afloat while nutrients are sufficient for longer experiments. In such experiments cellular metabolic turnover can be observed, but not intra-cellular metabolism. As real-time spectra change over time, the pulse sequence had to be modified to acquire the filtered and all-proton reference spectrum in a scan interleaved mode. Decoupling was applied for both spectra in order to have the same heating effect for both, even though the ^{13}C -filtered spectrum does not benefit from decoupling. The acquisition time for each pair of spectra was 15 m for a sample containing approximately half a million cells. The time course as shown in Figure 3 shows the difference spectra of all- ^1H and $^1\text{H}\{^{12}\text{C}\}$ spectra, demonstrating the suitability and high reproducibility of this approach for real time acquisition of metabolic changes using a tracer-based approach.

Figure 3. Real-time experiment with JJN3 cells. Spectra were acquired using the double GBIRD filter with $\delta=1/128\text{Hz}$ and $\varepsilon=1/168\text{Hz}$. All ^1H and $^1\text{H}\{^{12}\text{C}\}$ spectra were acquired in scan-interleaved mode. The spectra shown,

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representing $^1\text{H}\{^{13}\text{C}\}$, were calculated by taking the difference between all- ^1H and $^1\text{H}\{^{12}\text{C}\}$ spectra. As the decoupling bandwidth did not cover the region around 200ppm we did not decouple lactate carboxylic acid carbons, hence the lactate CH_3 signal (B) shows the extra $^3J_{\text{CH}}$ coupling from COO to the methyl protons (further experimental details in Supplementary Material).

It should be noted that decoupling restricts the number of points that can be acquired. At 600MHz and a spectral width of 12ppm, 32K points could be acquired on both room temperature and 5mm cryoprobes. The room temperature probe is less able to compensate for heating effects of adiabatic decoupling and, therefore compromises were made as detailed in the methods section. We have also used this approach using a 1.7mm cryoprobe at 600MHz which has a superconducting ^1H receiver coil that is more sensitive to heating. Nevertheless, good quality spectra could be obtained for 16k data points.

Moreover, this technique is equally important for metabolic flux analysis of cell extracts in combination with other methods such as 2D-HSQC spectra^[10]. HSQC spectra suffer a similar drawback as ^{13}C -observed spectra as they only observe ^{13}C -bound protons. Moreover, intensities in HSQC spectra are proportional to label incorporation and concentrations. We have previously suggested that HSQCS could be scaled by 1D spectra. Filtered 1D-spectra represent a perfect complement for HSQC data to accurately calculate label incorporations. 2D-HSQC spectra benefit from larger spectral dispersion and this usually allows quantification of label incorporations for all proton-bound ^{13}C -atoms. Filtered spectra can however help to augment such data, particularly for metabolites where resonances are absent in the natural abundance HSQC reference spectrum.

The ^{13}C -filtered approach is particularly useful to measure label incorporation in metabolites with unknown and varying J_{CH} coupling constants. The maximum filter leakage is 2% when the filter periods are mis-set by $\pm 50\text{Hz}$ from the ideal values for a particular $^1\text{H}\{^{13}\text{C}\}$ resonance.

When coupling constants are known, editing becomes a more realistic option for the quantification of label incorporations as the overall transfer function can be calculated based on the $^1J_{\text{CH}}$ and T_2 transverse relaxation times, as recently shown by Wan et al. for 1D-HMQC^[19]. However, this requires precise knowledge of these parameters. Moreover, for real-time approaches, where the same metabolites are compared over a longer time period, the filter approach yields highly reproducible spectra with a good baseline and water suppression, without losses incurred by echo-anti-echo selection.

In summary, we have shown that label incorporation in sparsely labelled metabolism samples can be obtained using ^{13}C -filtered one-dimensional spectra using a sequence with two filter constants that is highly tolerant towards deviations of the set delays from the actual coupling constant. With this approach, label incorporations can be obtained with a relatively high accuracy. This method requires the availability of isolated signals in a 1D spectrum, although such filters could be combined with two-dimensional spectra to reduce signal overlap.

- [1] M. Cascante, S. Marin, *Essays In Biochemistry* **2008**, *45*, 67–82.
- [2] K. M. Koczula, C. Ludwig, R. Hayden, L. Cronin, G. Pratt, H. Parry, D. Tennant, M. Drayson, C. M. Bunce, F. L. Khanim, et al., *Leukemia* **2016**, *30*, 65–73.
- [3] A. P. Tikunov, Y.-S. Shim, N. Bhattarai, S. Q. Siler, V. Soldatow, E. L. LeCluyse, J. E. McDunn, P. B. Watkins, J. M. Macdonald, *Toxicology in Vitro* **2017**, *45*, 119–127.
- [4] M. T. Judge, Y. Wu, F. Tayyari, A. Hattori, J. Glushka, T. Ito, J. Arnold, A. S. Edison, *bioRxiv* **2018**, DOI 10.1101/501577.
- [5] Q. Wan, Y. Wang, H. Tang, *Anal. Chem.* **2017**, *89*, 3293–3299.
- [6] T. W.-M. Fan, A. N. Lane, *Progress in Nuclear Magnetic Resonance Spectroscopy* **2008**, *52*, 69–117.
- [7] T. B. Smith, K. Patel, H. Munford, A. Peet, D. A. Tennant, M. Jeeves, C. Ludwig, *Wellcome Open Research* **2018**, *3*, 5.
- [8] V. Di Gialleonardo, S. S. Tee, H. N. Aldeborgh, V. Z. Miloushev, L. S. Cunha, G. D. Sukenick, K. R. Keshari, *Analytical Chemistry* **2016**, *88*, 11147–11153.
- [9] M. Vinaixa, M. A. Rodríguez, S. Aivio, J. Capellades, J. Gómez, N. Canyellas, T. H. Stracker, O. Yanes, *Angewandte Chemie International Edition* **n.d.**, *56*, 3531–3535.
- [10] R. Saborano, Z. Eraslan, J. Roberts, F. L. Khanim, P. F. Lalor, M. A. C. Reed, U. L. Günther, *Scientific Reports* **2019**, *9*, DOI 10.1038/s41598-018-37525-3.
- [11] K. Bingol, L. Bruschweiler-Li, D.-W. Li, R. Bruschweiler, *Anal Chem* **2014**, *86*, 5494–5501.
- [12] T. Szyperski, *Eur. J. Biochem.* **1995**, *232*, 433–448.
- [13] T. Szyperski, J. E. Bailey, K. Wüthrich, *Trends in Biotechnology* **1996**, *14*, 453–459.
- [14] T. Szyperski, R. W. Glaser, M. Hochuli, J. Fiaux, U. Sauer, J. E. Bailey, K. Wüthrich, *Metabolic Engineering* **1999**, *1*, 189–197.
- [15] M. Chong, A. Jayaraman, S. Marin, V. Selivanov, P. R. de Atauri Carulla, D. A. Tennant, M. Cascante, U. L. Günther, C. Ludwig, *Angewandte Chemie* **2017**, *129*, 4204–4208.
- [16] R. Saborano, Z. Eraslan, J. Roberts, F. Khanim, P. Lalor, M. Reed, *submitted* **2018**.
- [17] M. P. Schätzlein, J. Becker, D. Schulze-Sünninghausen, A. Pineda-Lucena, J. R. Herance, B. Luy, *Anal Bioanal Chem* **2018**, *410*, 2793–2804.
- [18] M. Vinaixa, M. A. Rodríguez, S. Aivio, J. Capellades, J. Gómez, N. Canyellas, T. H. Stracker, O. Yanes, *Angewandte Chemie International Edition* **2017**, *56*, 3531–3535.
- [19] Q. Wan, Y. Wang, H. Tang, *Analytical Chemistry* **2017**, *89*, 3293–3299.
- [20] S. Heikkinen, M. M. Toikka, P. T. Karhunen, I. A. Kilpeläinen, *Journal of the American Chemical Society* **2003**, *125*, 4362–4367.
- [21] J. R. Garbow, D. P. Weitekamp, A. Pines, *Chemical Physics Letters* **1982**, *93*, 504–509.
- [22] S. Heikkinen, I. Kilpeläinen, *Journal of Magnetic Resonance* **1999**, *137*, 93–99.
- [23] S. M. Cohen, S. Ogawa, R. G. Shulman, *Proceedings of the National Academy of Sciences* **1979**, *76*, 1603–1607.
- [24] R. J. Gillies, J.-P. Galons, K. A. McGovern, P. G. Scherer, Y.-H. Lien, C. Job, R. Ratcliff, F. Chapa, S. Cerdan, B. E. Dale, *NMR in Biomedicine* **1993**, *6*, 95–104.
- [25] J. Ruiz-Cabello, J. S. Cohen, in *International Review of Cytology*, Elsevier, **1993**, pp. 1–63.

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Michelle AC Reed, Jennie Roberts, Peter Gierth, Ěriks Kupĉe, Ulrich L Gnther*

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