FAST DETERMINATION OF ABSOLUTE METABOLITE CONCENTRATIONS BY SPATIALLY-ENCODED 2D NMR: APPLICATION TO BREAST CANCER CELL EXTRACTS

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Purpose:

The use of 2D NMR for quantitative purposes is far from being trivial, because of its experiment time and of its subsequent high sensitivity to hardware instabilities affecting its precision. We propose an alternative approach, based on a "multi-scan single shot" (M3S) strategy [1] (derived from the ultrafast 2D NMR [2]), to measure absolute metabolite concentrations in complex mixtures with a high precision in a reasonable time.

Results:

The two acquisition strategies (Figure 1a and 1b) were first compared on a series of model metabolic mixtures whose concentrations ranged from 2 to 10 mM. The spectra acquired in 10 min are represented in Figure 1c and 1d for the 6 mM sample.

The CV, obtained from 15 spectra, were between 1% and 4% for the M3S approach, whereas they vary between 5.5% and 18.3% for the conventional one. The linearity of the two approaches was also assessed and very high coefficients of determination (r^2) were obtained.

The quantitative M3S protocol was then applied to compare the metabolite contents from three different human breast cancer cell lines. The results (obtained from a standard addition procedure [3]) are plotted in Figure 2, showing a large metabolite concentration range, from 10-20 mM for lactate to 0.3 mM for less abundant metabolites.

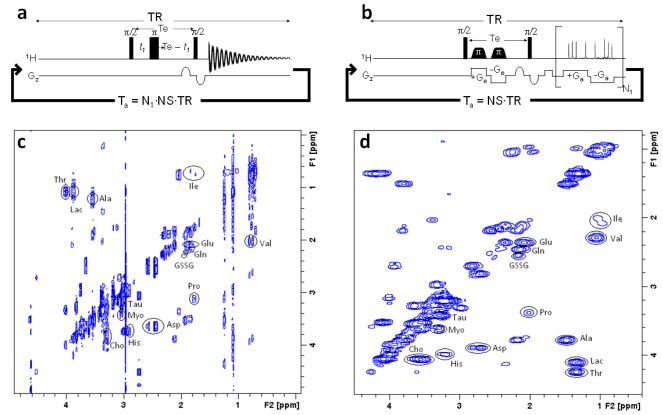

Figure 1: Strategies for recording quantitative 2D NMR spectra: NS scans for each N_1 increment of a conventional 2D sequence (a) ; NS ultrafast experiments (b). The corresponding spectra are shown in (c) and (d) from a 6 mM metabolic mixture (10 minutes on a 500 MHz spectrometer with a cryoprobe).

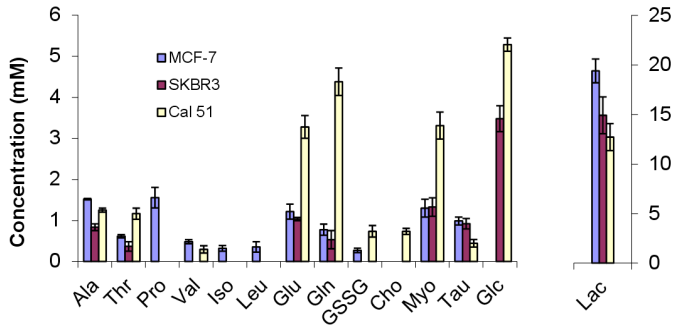

Figure 2: Metabolite concentrations of intracellular extracts from three cell lines: SKBR3, MCF-7 and Cal 51 measured by the M3S COSY protocol.

Discussion:

We observe significant differences between cell lines, which are often higher than the experimental error. The superiority of the M3S approach in terms of analytical performance can be explained by its higher immunity to hardware temporal instabilities [1].

Conclusion:

The results described in this study show the high analytical potential of the M3S approach compared to its conventional counterpart, particularly in terms of precision. The protocol described in this study offers a powerful tool to determine absolute metabolite concentrations in cell extracts, and more generally in complex biological mixtures.

References:

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