

Localized Proton Magnetic Resonance Spectroscopy at 7 Tesla in the Rat Brain: Metabolite Concentration Estimates Using QUEST and Two Approaches to Accommodate the Background

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Abstract — Localized proton Magnetic Resonance Spectroscopy (MRS) brain signals acquired at short echo-time contain contributions from metabolites, water and a ‘background’ which mainly originates from macromolecules and lipids. The purpose of the present study was to compare the influence of the background-accommodation strategy on the metabolite concentration estimates. The metabolite concentrations were estimated with the semi-parametric method QUEST combined with an *in vitro* metabolite basis-set. Two strategies were investigated to accommodate the background, 1) the measured background signal was incorporated in the metabolite basis set; and 2) the background signal was estimated and subtracted from the *in vivo* signal using ‘Subtract’-QUEST. For the considered signals of this study, the influence of the background on the metabolite estimates was reduced by including the background signal in the metabolite basis-set. The background estimates obtained with Subtract-QUEST was always underestimated leading to overestimated metabolite concentration estimates. However, the main advantage of the ‘Subtract’ approach is that it obviates the extra acquisition time needed to acquire the *in vivo* background signal.

Key words — Magnetic Resonance Spectroscopy (MRS), Signal Processing, Rat Brain metabolites, Background, Quantification.

I. INTRODUCTION

Localized proton Magnetic Resonance Spectroscopy (MRS) brain signals acquired at short echo-time contain contributions from metabolites, water and a ‘background’ which mainly originates from macromolecules and lipids. As the ‘background’ is *a priori* of unknown shape and intensity, it

poses a problem if one wants to quantify the overlying signals from the metabolites. On the other hand, the ‘background’ contributions may provide relevant clinical information.

Several methods have been proposed to accommodate the ‘background’:

- When the signal of the ‘background’ is known, *i.e.* separately acquired using the inversion recovery method, the ‘background’ signal can be added to the basis set [1-5] or subtracted from the total signal [6]. This second approach is treated by us in a separate ProRISC 2006 paper.
- When the signal of the ‘background’ is unknown, it can be mathematically approximated. Two kinds of approaches can be distinguished:
 1. ‘Background’ in the model function: the ‘background’ signal is approximated by a mathematical function (a sum of splines, wavelets, sinusoids or polynomials with adjustable parameters) and then included in the parametric nonlinear least-squares fit [7-12].
 2. ‘Background’ separately accommodated in a pre-processing step:
 - Modelling of the ‘background’ in the frequency domain with wavelets [9, 13] or in the time-domain using singular value decomposition [14, 15] and subsequent subtraction.
 - Truncation of initial data points [16, 17].
 - ‘Subtract’ approach [18, 19].

The purpose of the present study was to compare the influence of the background-accommodation strategy on the metabolite concentration estimates. Two strategies were investigated: 1) the measured background signal was

incorporated in the metabolite basis-set; and 2) the background signal was estimated and subtracted from the investigated signal using ‘Subtract’-QUEST [18, 19].

II. METHOD

A. *In vivo* background measurements in rat brain

The experiments were performed on a 7 Tesla Biospec system (BRUKER BioSpin MRI, Germany), equipped with a 12 cm diameter gradient coil (400 mT/m maximum amplitude). A transmit cylindrical bird cage coil (72 mm inner diameter) and a receiver surface coil (15 mm diameter) were used.

Healthy adult rats (Sprague-Dawley, 200g in weight, four animals) were anesthetized by inhalation of isoflurane (Abbott Laboratories, Rungis, France) with 2.5% concentration in a mixture of 50% oxygen and 50% nitrous oxide. The body temperature was maintained at 37°C by warm water circulation. The respiratory cycle was monitored using a pressure probe. The experiments were conducted according to the procedures approved by the Institutional Animal Care and Ethical Committee of our University.

Acquisitions were performed using a short-echo time PRESS sequence (Point Resolved Spectroscopy) combined with outer volume suppression (echo-time (TE)=20ms, repetition time (TR)=3.5s, bandwidth of 4kHz, 4096 data-points). The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOR) [20]. All first- and second- order shim terms were adjusted using FASTMAP (Fast, Automatic Shimming technique by Mapping Along Projections) [21] for each volume of interest (VOI) centred in the hippocampus (left size of the brain, 3.2x2x3.2mm³) of the healthy rats. Shimming resulted in unsuppressed water spectral linewidths of 7-11 Hz.

An Inversion-Recovery module was included prior to the PRESS sequence in order to measure the background signal (the metabolite-nulled signal) (see Figure 1). The inversion time (IT) after the inversion pulse was set to 675ms.

B. *In vitro* metabolite basis-set

To set up an *in vitro* metabolite basis-set [22-24], eleven metabolite solutions were prepared: aspartate (Asp), creatine (Cr), choline (Cho), γ -aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), N-acetylaspartate (NAA), taurine (Tau), lactate (Lac) and myo-inositol (Ins). The metabolites were dissolved separately in aqueous solutions buffered with phosphate at pH=7.0±0.1 (100mM, 10ml). The metabolites were purchased from Sigma-Aldrich. The *in vitro* basis-set signals were acquired at 7 Tesla using a short-echo time PRESS sequence (TE=20ms, TR=10s, bandwidth of 4 kHz, 4096 data-points, 128 averages). It is essential that the *in vitro* metabolite signals be measured using acquisition parameters identical to the *in vivo* ones and with the same pH and temperature conditions.

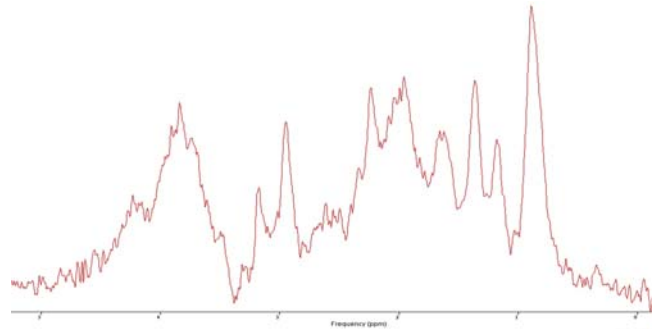


Figure 1: *In vivo* background (metabolite-nulled) ¹H NMR spectrum in the rat brain, obtained at TE=20ms, TR=3.5s, IT=675ms.

C. Metabolite quantifications

The signals were processed using the jMRUI software [25, 26]. Removal of residual water components was performed in a preprocessing step using the Hankel-Lanczos Singular Value Decomposition algorithm - HLSVD (25 spectral components were used for modelling).

The eleven metabolite concentrations were estimated with the semi-parametric method QUEST combined with an *in vitro* metabolite basis set.

When the Subtract-QUEST method was invoked, the background signal was estimated from the first 24 data-points of the signal and modelled using a maximum of $K=12$ spectral components. The number K depends of the number of truncated data-points and can not be modified by the user. The number of data-points used for the background estimation was chosen using an empirical way so that the phased real-part of the background spectrum estimates is positive.

The zero-order phase and the dead time were fixed to zero in the quantification procedure for the two approaches.

The reliability of the quantification was assessed using the Cramér-Rao lower bounds (CRBs) [27]. An estimate was considered as relevant when the corresponding CRB was found below 15% of the estimate.

D. Monte Carlo studies

The influence of the background-accommodation strategy on the metabolite concentration estimates was addressed with the aid of Monte-Carlo studies. A signal mimicking an *in vivo* rat brain signal acquired at 7 Tesla was created. It consists of a weighted sum of the described eleven *in vitro* measured metabolite signals (after residual water removing with HLSVD).

Each summed signal was weighted according to the *in vivo* intensity ratios [5, 28]: NAA : Cr : Cho : Ins : Glu : Gln : GABA : Glc : Asp : Lac : Tau = 8.5 : 7.5 : 1.9 : 3.5 : 6.5 : 3.5 : 2 : 1.6 : 1.9 : 0.6 : 5.8.

The measured *in vivo* background signal presented in Figure 1 was added too. The latter was intensity-weighted to correspond, on a visual inspection, to the background signal observed in our *in vivo* rat brain signals.

The resulting low-noise signal was Lorentzian line broadened to mimic an *in vivo* signal acquired at 7 Tesla in the rat brain (damping factors of 30 Hz), see Figure 2. Two different linewidths were chosen. Each extra damping value (5 and 10 Hz) led to total damping values of 35Hz and 40Hz,

respectively. Note that this choice of surrogate signal was made in order to be close to the reality, in terms of lineshapes and background signal. A total of 270 realizations of a white Gaussian distributed noise were added to the low-noise signal. Two noise levels were chosen corresponding to signal to noise ratio (SNRs) of 60:1 and 24:1 compared to the creatine amplitude.

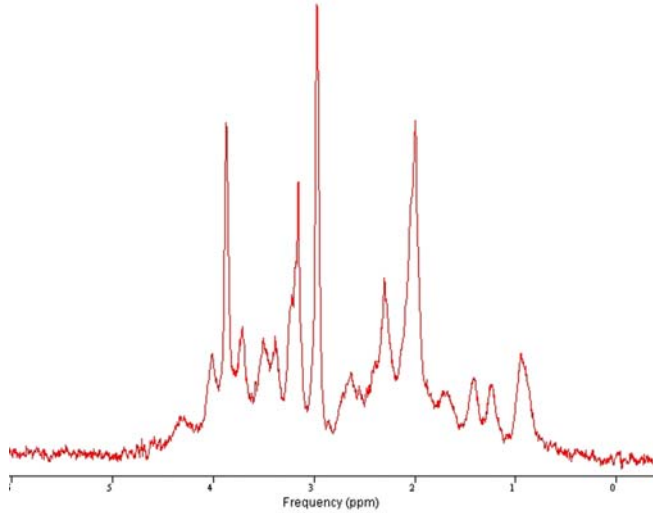


Figure 2: Spectrum of the weighted sum of the eleven *in vitro* metabolite spectra and of the measured *in vivo* background spectrum used for Monte Carlo studies (SNR 60:1, $\Delta\alpha = 10\text{Hz}$).

For each set of 270 signals, the metabolite concentrations were estimated with QUEST combined with the two mentioned approaches to accommodate the background. The mean of the relevant estimates for the Monte Carlo studies and the corresponding error bars ($\pm 2\text{SD}$) were computed, corresponding to a 95.4% confidence interval (see Figure 4).

III. RESULTS AND DISCUSSIONS

Our *in vivo* measured background signal was consistent with the literature [28, 29] (Figure 1). Note that when the background signal (macromolecule + lipids) is added to the metabolite-only signal, a strong correlation between the background and metabolite parameters appears due to spectral overlap.

- Background in the metabolite basis set:
The eleven metabolites were successfully estimated using QUEST combined with a metabolite basis set that includes the measured *in vivo* background signal, even for low SNRs and large damping factors (Figure 4a, b). As expected a slightly lower accuracy was obtained from the lower SNR signals and with the larger linewidths. Small biases and standard deviations were observed.
- Mathematical estimation of the background and subtraction using Subtract-QUEST:

In Figure 3, the measured *in vivo* background spectrum is superimposed on all the 270 estimated background spectra. As

it can be seen, the important resonances of the background signal were well identified. However, all the estimated background spectra were underestimated. The quality of the background estimates depends on the number of truncated data-points and the number of components used for modelling. As expected, when the number of truncated points was chosen too small (below 24 data-points), the background estimates were underestimated. A major background resonance was missing. When the number of truncated points was chosen larger than 24 data-points, a part of information about the metabolites was lost and we observed that the background estimates became negative around 3.2 ppm.

The underestimation of the background signals led to an overestimation of the majority of the metabolite concentrations (Figure 4). The glucose, which is a metabolite weakly represented, was not correctly identified and quantified.

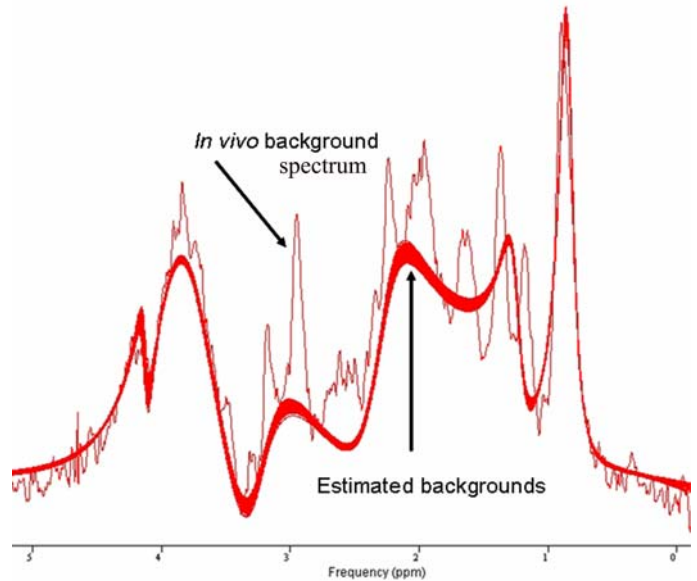


Figure 3: Measured *in vivo* background spectrum superimposed on the estimated background signals for 270 realizations of added noise, obtained using the ‘Subtract’ approach from QUEST.

IV. CONCLUSIONS

For the considered signals of this study, the influence of the background on the metabolite estimates was reduced by including the background signal in the metabolite basis-set.

Second, the background estimation using Subtract-QUEST with the chosen number of truncated data-points was always underestimated leading to overestimated metabolite concentration estimates. An automatic and robust way to select the number of truncated data-points and spectral components used for background modelling is in progress. First results are presented in the contribution of Rabeson H *et al*, ProRISC 2006. Also we point out that the main advantage of the ‘Subtract’ approach is that it obviates repeating tedious experimental work needed for acquiring the *in vivo* background signal.

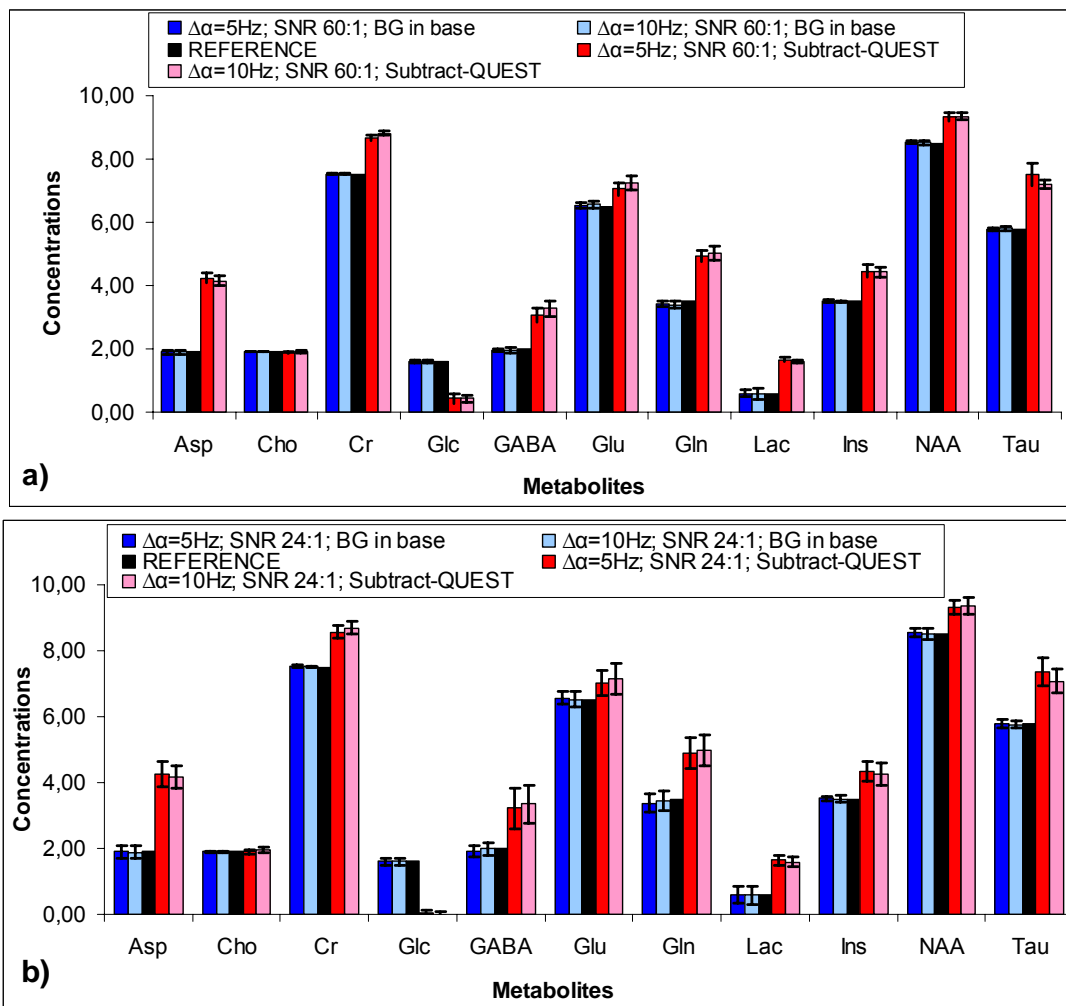


Figure 4: Monte Carlo quantification results. Quantifications were performed with QUEST combined with two approaches to accommodate the background, 1) the measured background signal was incorporated in the metabolite basis set (dark and light blue bars); and 2) the background signal was estimated and subtracted from the *in vivo* signal using 'Subtract'-QUEST (dark and light red bars), for: a) SNR 60:1; b) SNR 24:1.

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