Metabolite Profiles obtained with QUEST from HRMAS-NMR signals of Rat Brains

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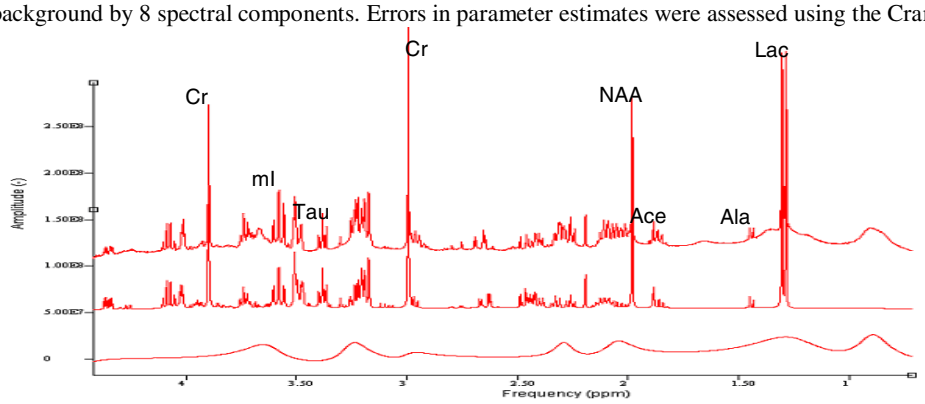
Introduction

High-resolution magic angle spinning (HRMAS) ^{1}H spectroscopy is playing an increasingly important role for diagnosis. This technique enables setting up metabolite profiles of *ex vivo* pathological and healthy tissue, see *e.g.* [1]. Automatic quantitation of HRMAS signals will provide reliable reference profiles to monitor diseases and pharmaceutical follow-up. We show that quantitation of HRMAS signals of rat brains acquired *without* suppression of lipids and macromolecules to avoid metabolite signal loss, is possible with the time-domain *semi*-parametric algorithm QUEST.

Method

Biopsies of 15 to 20mg of tissue were split from rat-brain left hippocampus and rapidly placed in zirconium oxide 4mm rotors with spherical insert, and 50µl of a 3mM TSP solution in pure D_2O was added. The rotors were immediately transferred into the HRMAS probe and acquisition started after 5min of rotation/temperature equilibrium. The HRMAS ^{1}H-NMR experiments were performed at 4°C on a Bruker DRX avance 400 (proton frequency 400.13MHz). Samples were spun at 4000Hz. Signals were acquired without, and with a Carr-Purcell-Meibom-Gill (CPMG) pulse sequence [90-(τ-180-τ)$_n$-acquisition] of 500 ms, enabling lipid/macromolecule suppression ('T_2-Filter`) but at the expense of metabolite amplitudes.

The HRMAS signals were quantitated in the *time-domain* with QUEST combined with 'Subtract' for background modelling [2]. The metabolite basis-set signals were simulated with NMR-SCOPE [3] using spin parameters given in [4]. Twenty-three metabolites (acetate (Ace), alanine (Ala), aspartate (Asp), creatine (Cr), choline (Cho), cysteine (Cys), ethanolamine (Eth), γ-amino-butyrique acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gl), glycine (Gly), glycero-phosphoryl-choline (GPC), lactate (Lac), myo-Inositol (mI), N-acetylaspartate (NAA), phosphoryl-choline (PC), phosphocreatine (PCr), phenylalanine (Phe), scyllo-inositol (sI), serine (Ser), succinate (Suc), taurine (Tau)) have been included in the basis. In a preprocessing step, water signal was first removed using HLSVD-Filter, then the spectral region of interest (0.5, 4.5 ppm) was selected using ER-Filter leading to filtered signals of about 2200 data-points. *Filtered* basis models were then fitted to filtered signals. Disentangling the metabolite from the background signals was achieved by `Subtract-QUEST', using the first 16 data-points (corresponding to a duration of 10 ms) for modelling the background by 8 spectral components. Errors in parameter estimates were assessed using the Cramér-Rao lower bounds (CRBs).



Results

HRMAS signals from hippocampus of rat brains were quantitated (see Fig.1). The background signal is well modelled; both lipid resonances (0.9 and 1.3ppm) and the four principal resonances of macromolecules (around 2.1ppm, 2.3ppm, 3.2ppm and 3.6ppm) are well identified. Quantitation results, expressed in % of total Cr, are compared in Fig.2 for signals acquired with and without the CPMG pulse sequence. A rigorous comparison requires knowledge of T_2 values of all metabolites. Results are in good agreement. The CRBs on amplitudes were found in the range of 1% to 10% for most metabolites.

Fig.1: HRMAS spectra of a rat brain (without CPMG), quantitated with Subtract-QUEST. Raw (top), estimated (middle) spectra and estimated background (bottom).

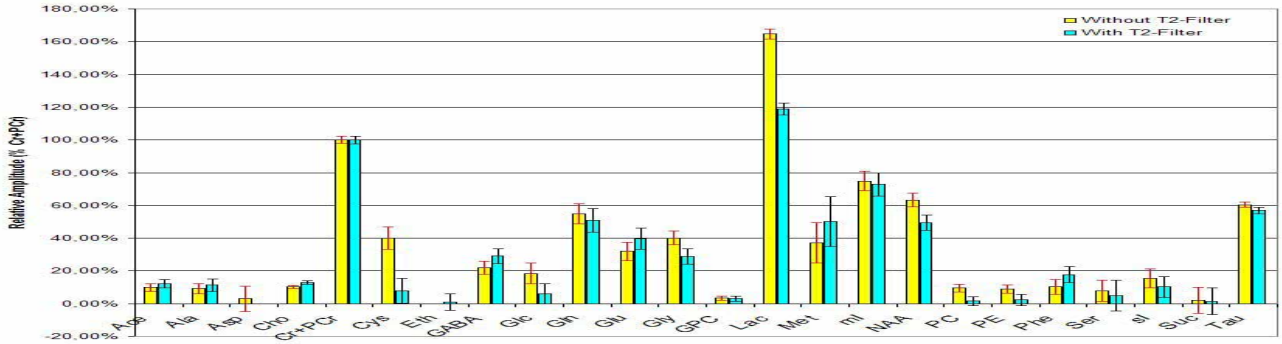


Fig.2: *Estimated amplitudes of 23 metabolites in a rat brain and corresponding 2 CRBs, obtained with QUEST from HRMAS signals acquired with and without CPMG.*

Conclusions

The algorithm QUEST is well suited for automatic quantitation of HRMAS signals, even in the presence of macromolecules and lipids. About twenty metabolites have been reliably quantitated with a simulated basis set, in rat brains and their concentrations can be used to establish reference metabolite profiles of *ex vivo* tissues.

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