Quantification of 1H NMR Spectra from Human Blood Plasma

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Introduction – Blood plasma is a biofluid that is high in information content, making it an excellent candidate for metabolomic studies [1]. Besides MS-based methods, ^{1}H NMR has been a popular technique to detect several dozen metabolites in blood plasma. However, in order to become a quantitative and high-throughput method ^{1}H NMR has several challenges to overcome. Firstly, the bulk of signal intensity in a typical ^{1}H NMR spectrum arises from lipoproteins, thereby complicating the detection and quantification of metabolites. Ultrafiltration is a routine method to remove lipoproteins, but is also a time and labor-intensive procedure. Secondly, spectral overlap between different metabolites often leads to ambiguities in quantification when spectral integration or binning is used. Here we present an extension of a previously described method to separate metabolites and lipoproteins based on differences in diffusion [2] and combine it with a recently described spectral fitting algorithm [3]. It is anticipated that the combination of minimal sample preparation together with minimal user interaction during processing and quantification will provide a metabolomic technique for automated, quantitative ^{1}H NMR of blood plasma.

Methods – All experiments were performed on a Bruker 500 MHz magnet equipped with triple-axis gradients. Human plasma (300 uL) was diluted with 300 uL solution containing 250 mM phosphate buffer (pH 7.4), 5 mM formate and 10% D_2O. The MR pulse sequence consisted of an adiabatic double-spin echo with 4 ms magnetic field gradients on all three axes, giving a maximum b-value of 15 ms/um^2 at an echo-time TE of 17.28 ms. All samples were measured at 298 K with 96 averages and a repetition time TR of 6,000/3,000 ms for low/high b-value acquisitions.

Results – Fig 1A shows a typical ^{1}H NMR spectrum from human blood plasma with low diffusion sensitization (b = 0.01 ms/um^2) such that both metabolites and lipoproteins are visible. At high diffusion sensitization (b = 15 ms/um^2), only the signals from lipoproteins remain (Fig 1B). Variation in lipoprotein diffusion coefficients [2] will lead to small (+/- 20%) amplitude variations, such that the lipoprotein spectrum does not exactly match the lipoprotein background signals in Fig 1A. Following parameterization of the lipoprotein spectrum (Fig 1C), the individual lipoprotein signal amplitudes are allowed to vary slightly (+/- 20%) during the spectral fitting algorithm as to provide the best fit (Fig 1E) to the experimental ^{1}H NMR spectrum (Fig 1D). The parameterized lipoprotein line widths, phases and frequency offsets are held constant. Fig 1F shows the residual between the experimental (Fig 1D) and fitted (Fig 1E) ^{1}H NMR spectra.

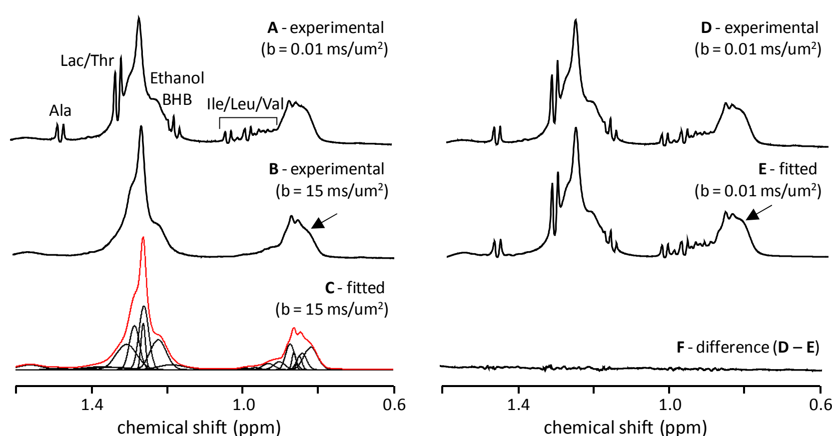

Figure 1: (A) Experimental ^{1}H NMR spectrum of human blood plasma acquired with low diffusion sensitivity (b = 0.01 ms/um^2). The broad lipoprotein resonances dominate the spectrum and overlap with all metabolite resonances. (B) Experimental ^{1}H NMR spectrum acquired with high diffusion sensitivity (b = 15 ms/um^2). Signals from low molecular weight metabolites are eliminated, leaving only lipoprotein signals. (C) Spectral decomposition of (B) into a limited number of Gaussian lines. The red line indicates the total spectral fit, i.e. the sum of all fitted Gaussian lines. (D) = (A). (E) Fitted ^{1}H NMR spectrum using a basis set composed of metabolites (Ala, BHB, ethanol, Ile, Lac, Leu, Thr and Val) and the Gaussian lines obtain from (C). Note that small amplitude variations allowed on the Gaussian lines are used to accommodate different diffusion coefficients among lipoprotein resonances (e.g. compare the shoulders indicated by the arrows in (B) and (E)). (F) = (D) – (E).

Discussion – Here a novel workflow for the acquisition and processing of metabolomic ^{1}H NMR data from human blood plasma is presented. Sample preparation is minimal, whereas the acquisition of additional (high b-value) data is limited to 5 min per sample. The processing of the low/high b-value data combination can be fully automated, such that ^{1}H NMR of blood plasma is ready to become a high throughput technique for metabolomic studies.

[1] N. Psychogios et al, PloS One 6, e16957 (2011) [2] R. A. de Graaf and K. L. Behar, Anal. Chem. 75, 2100 (2003) [3] R. A. de Graaf et al, Anal. Chem. 83, 216 (2011)

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