Extra-cranial measurements of amide proton transfer using exchange-modulated point-resolved spectroscopy (EXPRESS)

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Introduction Amide proton transfer (APT) measurements utilise the reduction in water signal observed by selectively saturating exchangeable protons in amide groups. This mechanism has found utility in the measurement of protein accumulation and pH (1,2,3), although confounding factors include water concentration, T_1 and T_2, 'classical' magnetisation transfer, blood flow and oxygenation, each of which can be compensated for by the acquisition of a full z-spectrum (1). This requires multiple acquisition iterations of a chemical exchange saturation transfer (CEST) imaging sequence, which can extend the total acquisition time considerably. As such, fast imaging sequences are generally utilised in CEST experiments, although measurement of a complete z-spectrum would still generally be considered unfeasible in a clinical setting. In this study, a novel acquisition method is presented named exchange-modulated point-resolved spectroscopy (EXPRESS), which 1) allows the fast, high signal-to-noise measurement of full z-spectra *in vivo* and 2) by acquiring two-dimensional chemical shift and saturation offset spectra, can correct extra-cranial APT measurements that contain a confounding fat signal.

Materials and Method The EXPRESS sequence consisted of a saturation preparation (100, 56ms Gaussian saturation pulses with a 4ms inter-pulse delay, effective bandwidth=50Hz) followed by a conventional PRESS acquisition (4), with TE=22.2ms. Saturation pulses centred at ±3500Hz from the nominal water peak were applied at 50Hz intervals, giving a total of 145 saturation frequency offsets. Five averages of a reference saturation frequency offset of 80,000Hz were also acquired and used to normalise z-spectra. Following sampling of the spin-echo signal (200 points, receiver bandwidth=4000 Hz), crusher gradients were applied in the each direction and the next iteration of the saturation loop initiated following an optional TR delay.

In vitro experiments: A 9.4T Varian horizontal bore system (Varian Inc. Palo Alto, CA) was used to scan two samples containing 1ml of 1mM poly-L-lysine (PLL) in PBS. 1ml of cooking oil was added to phantom 2 to simulate the presence of fat *in vivo*. Each phantom was placed in the centre of the scanner and warmed to a temperature of 37°C. 1.5cm^3 voxels were defined over a uniform region of the phantom and shimming was performed using an automated 3D gradient echo shimming sequence (ge3dshim). All measurements were performed using a 39mm birdcage coil (Rapid MR International, Columbus, Ohio).

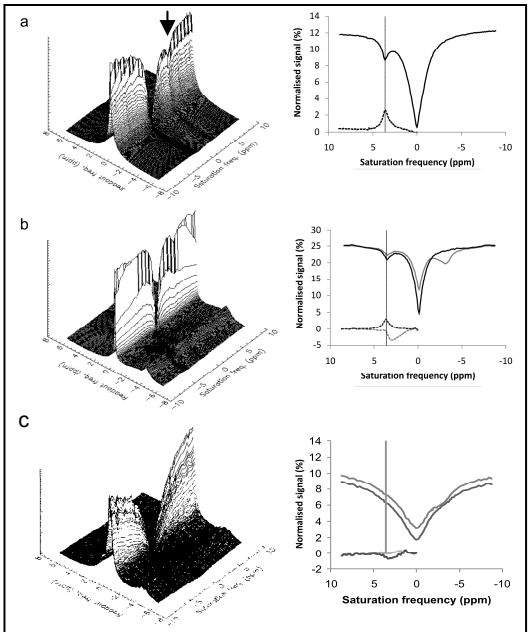

Figure 1: Two-dimensional EXPRESS spectra for phantom 1 (PBS with 1 mM poly-L-lysine), phantom 2 (poly-L-lysine with PBS and oil) and an example tumour xenograft (a-c, respectively). Graphs to the right show z-spectra (solid line) and MTR_{asym} spectra (dashed line) calculated from the data shown in the EXPRESS spectra to the left. In b and c, z- and MTR_{asym} spectra are shown both without fat correction (grey) and with fat correction (black). Use of the spectral information provided by EXPRESS allows the confounding effect of fat on the z-spectrum to be effectively corrected.

In vivo experiments: 5×10^6 SW1222 human colorectal carcinoma cells were injected subcutaneously into the flanks of four MF1 nude (nu/nu) mice. These were allowed to grow for 14 days, at which point the average tumour volume was 1.7±0.3cm^3. During scanning, core temperature was maintained at 37°C using a warm air blower. Shimming was performed as for *in vitro* experiments, using voxels that encompassed the entire tumour extent (1.5 to 2.0cm^3 voxel volume). EXPRESS data were then acquired from the same voxel as was used for shimming. Total scan duration for the EXPRESS sequence was 14 minutes.

Post-processing and fat correction: Two-dimensional EXPRESS spectra were produced that consisted of both chemical shift and saturation offset frequency dimensions. Z-spectra were calculated by integrating EXPRESS spectra in the chemical shift direction to evaluate the total measured proton signal (i.e. between -6 and +6ppm). These were expressed as a percentage of the averaged reference measurement at 200ppm (80,000Hz). To correct for the presence of fat in the z-spectrum, EXPRESS spectra were instead integrated between -1.5 and 6ppm, thus removing the fat signal from the analysis.

Results *In vitro experiments:* Two-dimensional EXPRESS spectra and z-spectra for phantoms 1 (1 mM PLL) and 2 (1 mM PLL with oil) are shown in Fig. 1. In the EXPRESS spectrum for phantom 1, as expected, the proton exchange between amide groups and water introduced greater water saturation at a saturation offset frequency of 3.5ppm (arrow), compared with -3.5ppm. For phantom 2, (Fig.1b), the presence of oil introduced a second peak in the EXPRESS spectrum, centred at a chemical shift frequency of -3.8ppm. A dip in the intensity of this peak can be seen at a saturation offset of -3.8ppm, corresponding to direct saturation of the oil. When converted to a z-spectrum by integrating the chemical shift dimension, the presence of the oil has a number of confounding effects. Firstly, the apparent intensity of the amide transfer dip at 3.5ppm is reduced; secondly, the apparent intensity of the direct water saturation at 0ppm is reduced due to the presence of unsaturated oil; and thirdly, a third dip is introduced, centred at -3.8ppm, corresponding to direct saturation of the oil. The MTR_{asym} spectrum calculated without fat correction is dominated at 3.5ppm by the direct oil saturation, resulting in an apparent APT enhancement of -0.2%. With fat correction, a CEST enhancement of 2.6% was measured, which is comparable with that measured in phantom 1. The shapes of the MTR_{asym} spectrum from phantom 1 and fat-corrected spectrum phantom 2 are also comparable.

In vivo experiments: A typical EXPRESS spectrum from a subcutaneous colorectal tumour xenograft is shown in Fig. 1c. Water and fat peaks are clearly evident in this spectrum, the latter of which is likely to be principally due to subcutaneous fat included in the voxel. Direct saturation of both peaks can also be observed. Across all tumours, a CEST enhancement of (0.04±0.01)% was measured at 3.5ppm, when using fat correction. Without excluding fat from the analysis, this value was (-0.5±0.1)%, which was significantly lower than the corrected value ($p<0.01$, Wilcoxon rank sum).

Discussion & Conclusion The EXPRESS sequence presented here allows the fast measurement of CEST effects *in vivo*, with complete sampling of the z-spectrum in less than 15 minutes and with high frequency sampling rate and signal-to-noise characteristics (5). The acquisition of complete z-spectra is essential for meaningful quantification of CEST effects and for subsequent comparison between studies. A further advantage of the EXPRESS sequence is its ability to isolate the confounding influence of multiple fat peaks that, as demonstrated here, can entirely obscure amide proton transfer (APT) effects. As the majority of organs outside the brain contain a proportion of fat, EXPRESS enables precise extra-cranial CEST studies to be performed without elaborated methodological steps.

Acknowledgements: King's College London and UCL Comprehensive Cancer Imaging Centre CR-UK & EPSRC, in association with the MRC and DoH (England), and the British Heart Foundation. **References:** [1] Sun PZ *et al.*, Magn Reson Med, 2008;59:1175-82 [2] Gilad AA, *et* al.Nature Biotechnology, 2007;25:217-9 [3] Zhou J *et al.* Magn Reson Med, 2003;50:1120-6 [4] Haacke EM *et al.*, Magnetic Resonance Imaging: Physical Principles and Sequence Design: Wiley-Blackwell; 1999 [5] Moonen CT *et al.* NMR Biomed 1989;2:201-8