A method for T_2-selective saturation to cancel out macromolecular magnetization transfer in Z-spectroscopy

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Z-spectroscopy provides a tool to investigate pH-dependent chemical exchange *in vivo* [1]. The exchanging species, mainly amides, resonate around 3.5 ppm from free water and result in a negative peak in the Z-spectrum. The analysis of Z-spectra relies on the calculation of asymmetry between the halves of the Z-spectrum, to cancel out the effects of direct saturation and macromolecular magnetization transfer (MT) at the offset frequency of interest. The method is very sensitive to the estimation of the water frequency, and requires assumptions about the symmetry of the MT about this frequency. Furthermore, if the shielded (low chemical shift) side of the Z-spectrum contains other features, this will bias the analysis of asymmetry. We present a method for measuring directly the MT contribution in order to improve the quantification of exchange information.

Theory The phase of the low amplitude saturating irradiation is manipulated to discriminate between short and long T_2 species. If the irradiation uses constant phase (Cφ) a normal Z-spectrum is obtained. If the phase of the irradiation is alternated (Aφ) φ, -φ, φ, -φ... at fixed intervals τ, the degree of saturation of signals with $T_2 << \tau$ is unaffected, whereas long T_2 signals on resonance are unaffected and saturation of such signals is instead seen at the sideband frequencies $f = \pm(n+½)/\tau$, where n = 0,1,2... , as in a DANTE experiment [2]. The result is that the normal Z-spectral features for long T_2 species are shifted to either side of the irradiation frequency while MT is unaffected. A similar strategy for T_2 discrimination based on binomial pulse sequences has been presented previously [3,4]

Methods Z-spectra were acquired *in vitro* from boiled egg white and *in vivo* from rat brain. *In vitro* data were acquired on a 9.4 T Varian INOVA 400 spectrometer using saturation RF amplitudes ($\gamma B_1/2\pi$) of 25, 50 and 100 Hz, with a saturation time of 10 s, a phase shift interval τ of 100 μs, a 10° flip-angle detection pulse, and a relaxation delay of 13 s. The saturation frequency was varied from -100 kHz to 100 kHz in 273 steps. *In vivo* data was measured using a Magnex 4.7 T magnet interfaced to a Varian Inova console with a quadrature volume coil transmit, surface coil receive. Saturation time was 5 s, relaxation delay 7 s and saturation ($\gamma B_1/2\pi$ = 18 Hz and 75 Hz) applied at 143 offsets from -50 kHz to 50 kHz. The data were read out along a 3 mm square column and 7 voxels (= 2 mm) from the cortical region were averaged.

Results Boiled egg white data are shown in Fig. 1. The Cφ experiments (solid lines) with three different B_1s display the features of conventional Z-spectra: the MT effect and direct saturation width increase as B_1 is increased. The MT bandshape is narrow compared to that in tissue because of the relatively rapid segmental motion of the denatured protein. The Aφ Z-spectra (dotted lines) show the same MT background as the normal Z-spectrum, but the sharp features from amide and H_2O are displaced to the sideband frequencies ± 12.5 ppm, ± 37.5 ppm etc. as expected. Thus, for example, the peak at -9 ppm in Fig. 1B arises from the amide response shifted by -12.5 ppm. The *in vivo* data (Fig. 2) confirm that the experiment can be applied to a heterogeneous sample at lower field. All data are normalized to the Cφ results at -250 ppm. Interestingly, both the conventional Cφ and the Aφ data for low B_1 show exchange of magnetization between water and aliphatic protons, an effect currently being investigated.

Discussion Subtracting the results of an Aφ experiment from those of a Cφ provides a way to cancel out the MT background of a Z-spectrum in a few ppm range around H_2O resonance. It may also offer a faster way to estimate the rate of amide exchange, avoiding the need to measure the Z-spectral asymmetry, if the direct saturation is either minimised (low B_1, good shim) or can be calculated (e.g. by fitting the water region or by calculating the saturation shape from relaxation times, shim and B_1). One possible complication is the effect of direct saturation from the sidebands, which can be minimized by the choice of B_1 and τ. B_1 requires a trade-off between sensitivity to exchange and direct saturation width [1]; the RF pulse interval τ must be kept short relative to water and amide T_2 (tens of ms) and long relative to macromolecular T_2 (tens of μs). In addition to exchange studies, the method may be useful in creating and quantifying conventional macromolecular MT contrast. Contrast based on the Aφ experiment at zero offset might provide cleaner MT data.

1. Sun, P.Z. et al. J. Mag. Res. 175, 2005, 2. Morris, G.A. and Freeman, R., J. Mag. Res. 29, 1978
3. Pachot-Clouard, M. and Darrasse, L., MRM 34,1995, 4. Davies, N.P. et al., JMRI 11, 2000

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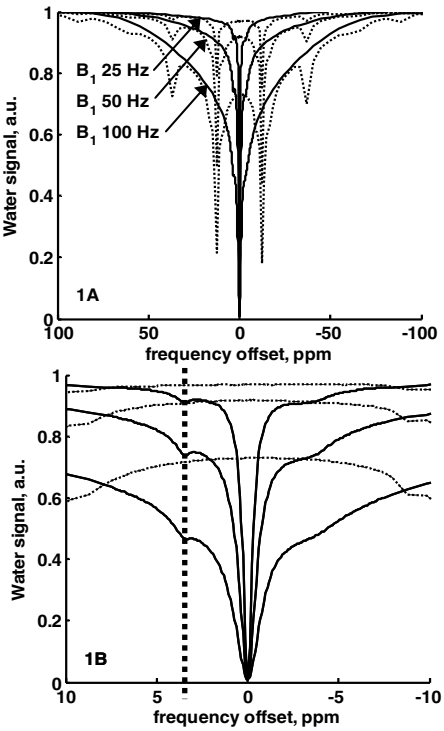

Fig. 1 Egg white Z-spectrum (1A), Cφ experiments are plotted in solid lines, Aφ Z-spectra in dotted. Magnification of data near water resonance is shown in 1B. Amide signal position is marked.

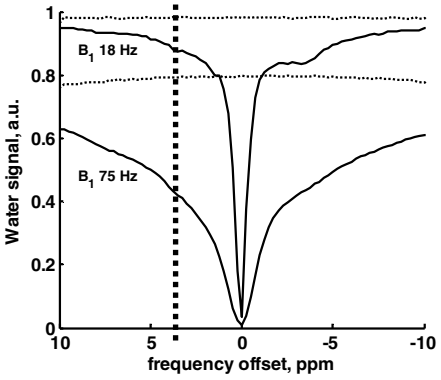

Fig. 2 Rat brain *in vivo* Z-spectrum. Cφ experiments are plotted in solid lines, Aφ Z-spectra in dotted. Amide signal position is marked.