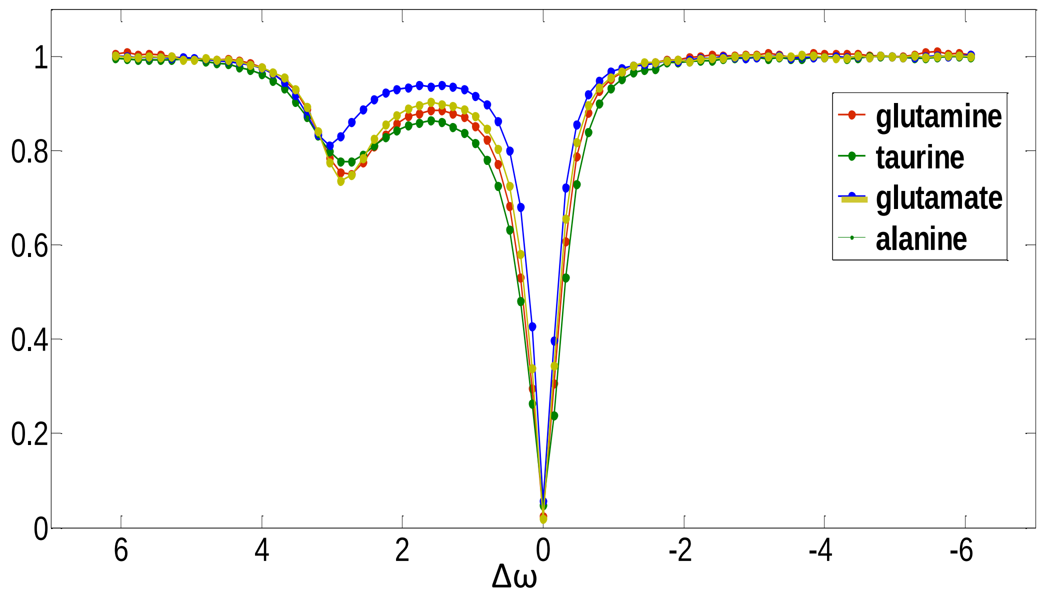
Supplementary data

I.



Supporting Figure S1 Z-spectra obtained at 0.78 μT from 100 mM of glutamine, taurine, glutamate and alanine.

II. Numerical solutions to the six Bloch equations including direct saturation were obtained using the ordinary differential equation solver (ode45) in Matlab. These were solved using the initial conditions of [0, 0, 0, 0, 0, 0] for [Mxs, Mys, Mzs, Mxw, Myw, Mzw], respectively, and with an error tolerance for each integration step of 10^{-12}.

$$\frac{dM_{xs}}{dt} = -\Delta\omega_s M_{ys} - R_{2s}M_{xs} - k_{sw}M_{xs} + k_{ws}M_{xw} \quad \text{(S1)}$$

$$\frac{dM_{ys}}{dt} = \Delta\omega_s M_{xs} + \omega_1 M_{zs} - R_{2s}M_{ys} - k_{sw}M_{ys} + k_{ws}M_{yw} \quad \text{(S2)}$$

$$\frac{dM_{zs}}{dt} = -\omega_1 M_{ys} - R_{1s}(M_{zs} - M_{0s}) - k_{sw}M_{zs} + k_{ws}M_{zw} \quad \text{(S3)}$$

$$\frac{dM_{xw}}{dt} = \Delta\omega_w M_{yw} - R_{2w}M_{xw} + k_{sw}M_{xs} - k_{ws}M_{xw} \quad \text{(S4)}$$

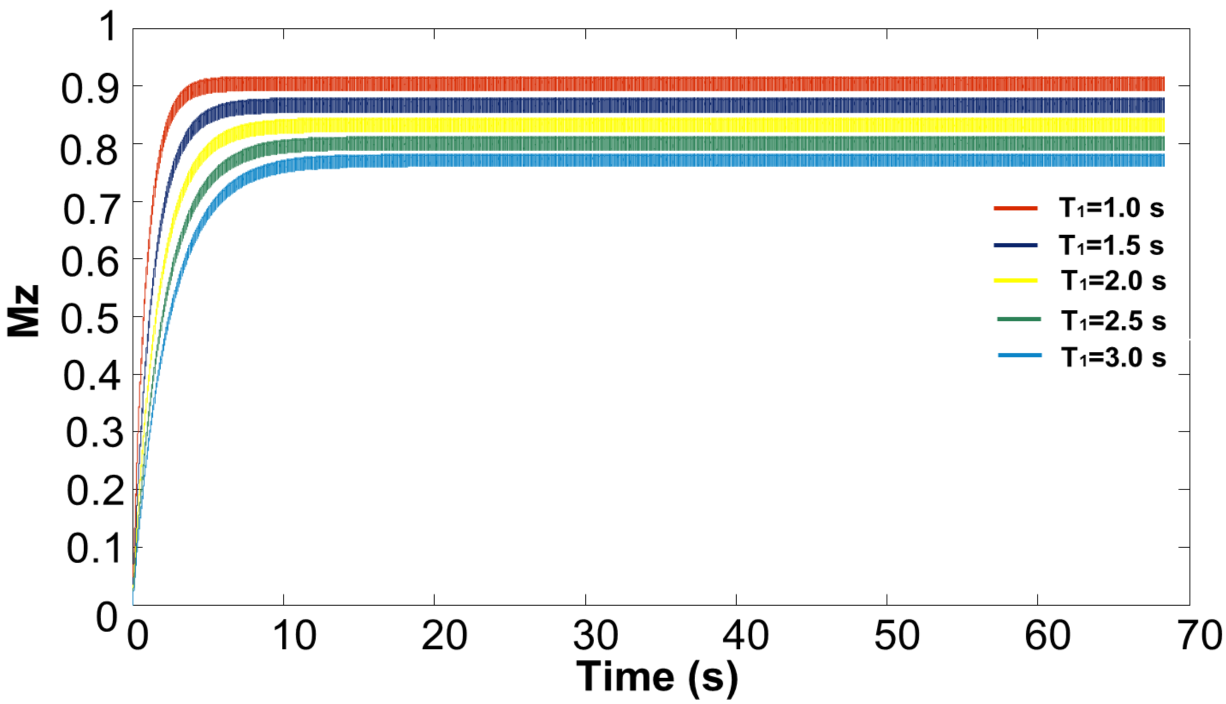
$$\frac{dM_{yw}}{dt} = \Delta\omega_w M_{xw} + \omega_1 M_{zw} - R_{2w}M_{yw} + k_{sw}M_{ys} - k_{ws}M_{yw} \quad \text{(S5)}$$

$$\frac{dM_{zw}}{dt} = -\omega_1 M_{yw} - R_{1w}(M_{zw} - M_{0w}) + k_{sw}M_{zs} - k_{ws}M_{zw} \quad \text{(S6)}$$

where $\omega_0 = \gamma B_0$ and $\omega_1 = \gamma B_1$ (γ is the gyromagnetic ratio, B_0 is the main magnetic field strength and B_1 is the applied RF field on the *x*-axis), $\Delta\omega_s = \omega_s - \omega_0$ and $\Delta\omega_w = \omega_w - \omega_0$ with $\Delta\omega_s$ and $\Delta\omega_w$ the chemical shift differences between the offset frequency of the RF pulse and the solute or water resonance frequency, respectively. Exchange occurs between the two pools at a forward (k_{sw}) and backward rate (k_{ws}). Finally, the system at equilibrium follows the equation: $k_{sw}M_{0s} = k_{ws}M_{0w}$

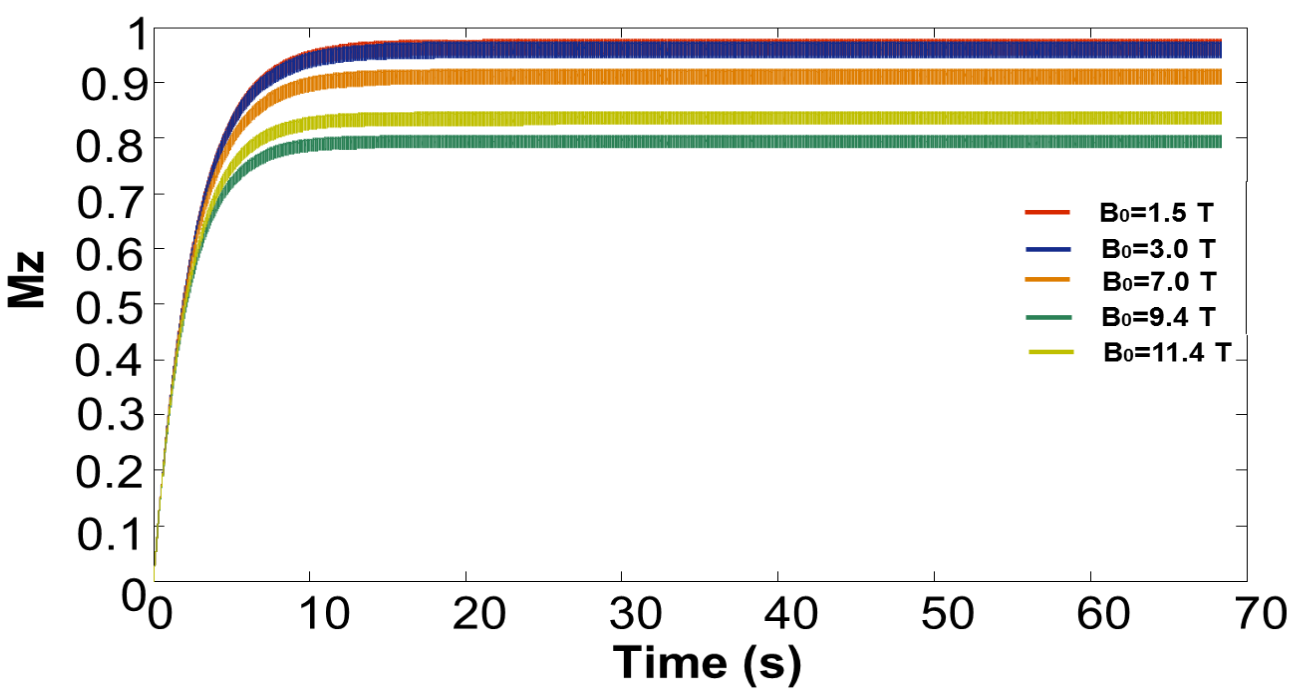
In order to simulate the PRO-QUEST sequence each pulse shape was sampled into 256 segments and it was incorporated into the BM equations by arraying the ω_1 parameter. After the saturation pulse which was applied at 3.0ppm the small flip angle Gaussian pulse was inserted followed by a period with $\omega_1 = 0$. During the read out ($\omega_1 = 0$) the frequency offset was set to zero.The module {asymmetric pulse | Gaussian pulse | Recovery period} was repeated for 300 times using a loop in matlab.

The two-pool Bloch McConnell simulation results using five different longitudinal relaxation times T_1 are shown in Supporting Figure S2. The parameters used for simulation are kb=1000 s^{-1}, offset of the exchangeable protons =3.0ppm, B_0=9.4T, concentration=100mM, ϑ=8 °, B_1= 0.86 μT, pulse shape asymmetric Sinc-Gauss, pulse duration=16.77msec.



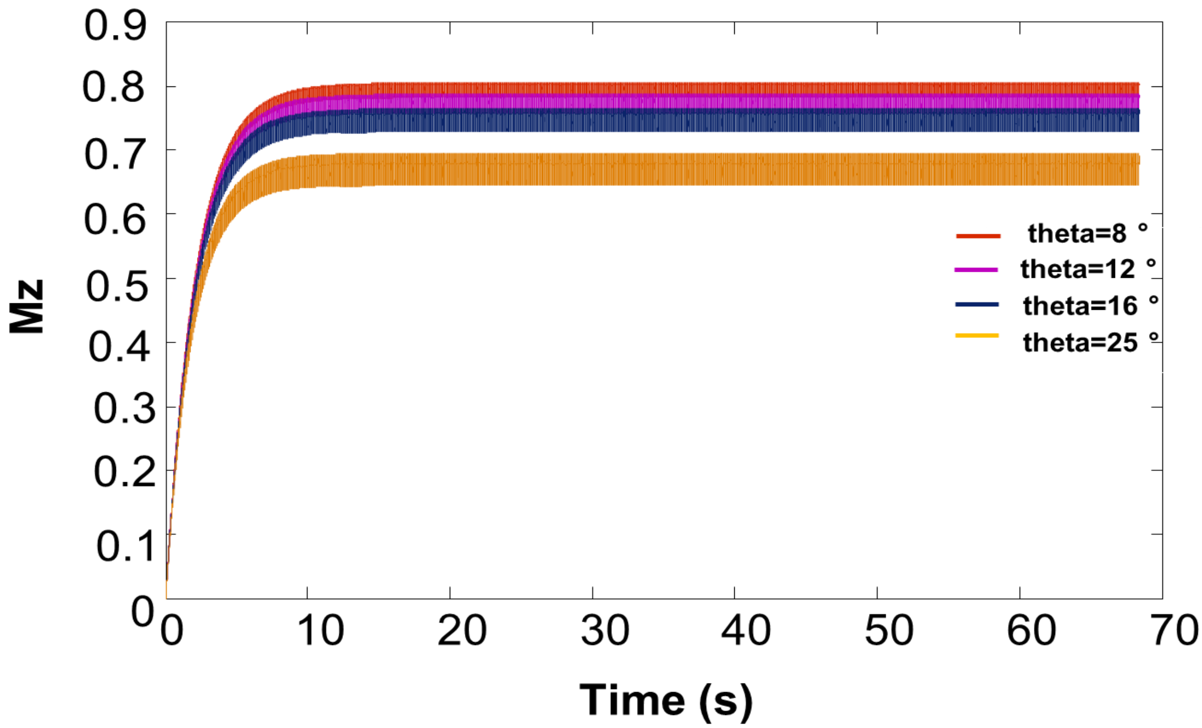
Supporting Figure S2: Simulated 2-pool PRO-QUEST curves at different T_1. The simulation indicates the sensitivity of the PRO-QUEST measurements to changes in T_1. For all the simulations B_0=9.4 T, theta= 8°, kb=1000 s^{-1}, B_1= 0.87 μT.

Supporting Figure S3 shows PRO-QUEST curves obtained at different magnetic field strengths. As opposed to Supporting Figure S1, the curves here are different for different main field strengths B_0.



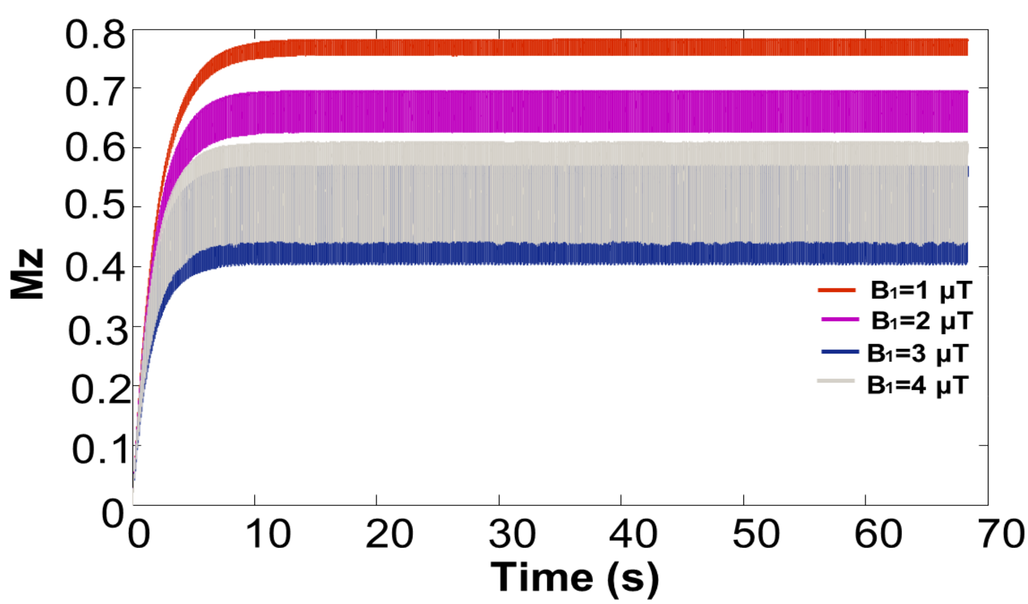
Supporting Figure S3 displays PRO-QUEST curves obtained by simulating a two-pool system at different B_0. For all the simulations T_1 =2.9 sec, theta= 8°, kb=1000 s^{-1}, B_1= 0.87 μT.

Supporting Figure S4 shows the simulated curves when the small tip angle ϑ that was used to tip the Z-magnetization for read out is varied from 8 to 25 degrees. The results indicate that changes in ϑ result in changes to the simulated recovery curves and therefore the value of ϑ should be mapped experimentally with high accuracy.



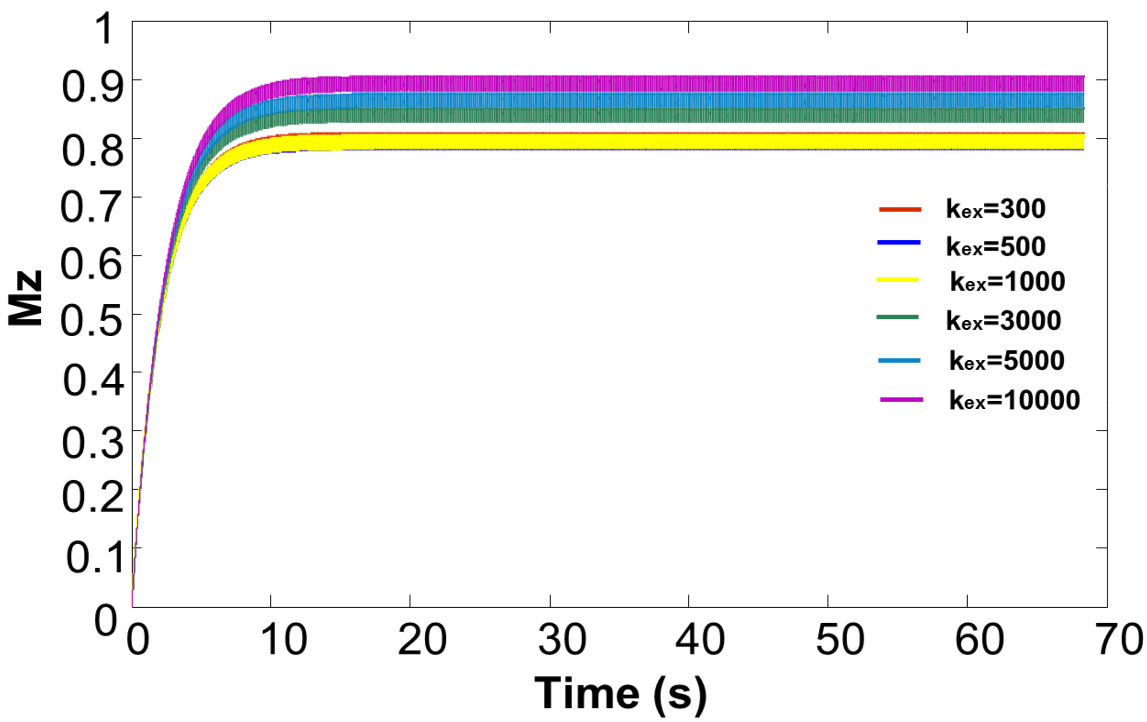
Supporting Figure S4 displays simulated data obtained at various tip angles ϑ. For all the simulations T_1 =2.9 sec, B_0=9.4 T, B_1= 0.87 μT, kb=1000 s^{-1}.

Supporting Figure S5 shows the sensitivity of the PRO-QUEST at various irradiation powers of the RF field ranging from 1-4 μT.



Supporting Figure S5 shows simulated data obtained at different irradiation powers B_1. For all the simulations T_1 =2.9 sec, B_0=9.4 T, theta= 8°, kb=1000 s^{-1}.

Supporting Figure S6 shows the sensitivity of the developed PRO-QUEST method to map changes in exchange rates between 300 and 10000 s^{-1} without changes in T_2 values.



Supporting Figure S6: Simulated two pool saturation recovery curves obtained at various exchange rates. For all the simulations T_1 =2.9 sec, B_0=9.4 T, theta= 8°, B_1= 0.87 μT.

II. QUESP results

Supporting Table S1: Exchange rates in alanine, glutamine, glutamate and taurine at various pH values

Substance			**pH**		
Alanine	**6.02**	**6.33**	**6.74**	**7.06**	**7.42**
k_{ex} $(10^3 s^{-1})$ QUESP	0.54±0.02	1.03±0.02	2.45±0.03	7.12±0.22	11.1±0.71
			pH		
Glutamate	**6.1**	**6.4**	**6.6**	**7.1**	**7.4**
k_{ex} $(10^3 s^{-1})$ QUESP	0.61±0.06	0.68±0.03	0.97±0.03	1.56±0.03	2.87±0.06
			pH		
Glutamine	**6.1**	**6.4**	**6.6**	**7.1**	**7.4**
k_{ex} $(10^3 s^{-1})$ QUESP	0.98±0.02	1.86±0.03	2.55±0.03	9.09±1.01	13.43±2.47
			pH		
Taurine	**5.85**	**6.08**	**6.31**	**6.86**	**7.2**
k_{ex} $(10^3 s^{-1})$ QUESP	2.59±0.07	5.15±0.10	6.56±0.16	17.4±2.63	19.84±40.33

Supporting Table S2: Exchange rates in alanine, glutamine, glutamate and taurine at various concentrations

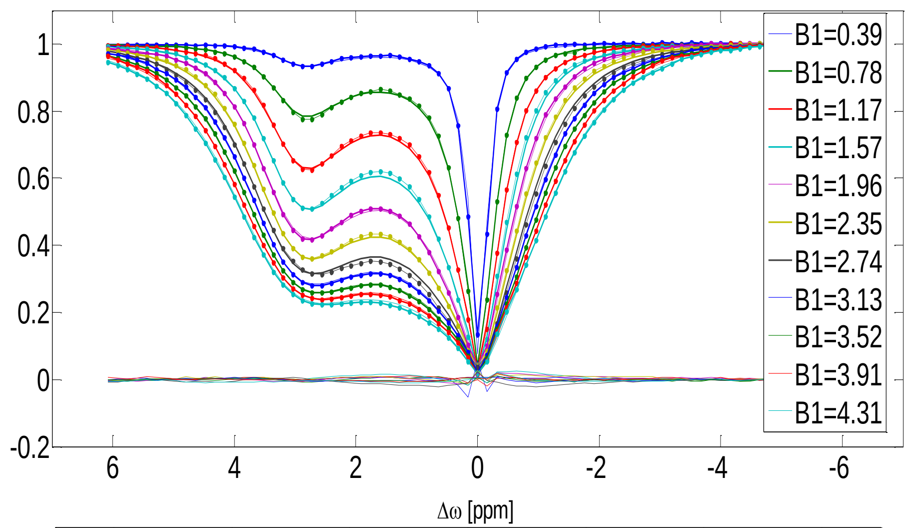
Substance		**Concentration**		
Alanine	**12.5mM**	**25mM**	**50mM**	**100mM**
k_{ex} $(10^3 s^{-1})$ PR0-QUESP	0.38±0.02	0.67±0.07	0.74±0.03	1.24±0.02
		Concentration		
Glutamate	**12.5mM**	**25mM**	**50mM**	**100mM**
k_{ex} $(10^3 s^{-1})$ PR0-QUESP	0.71±0.16	0.69±0.08	0.82±0.03	0.87±0.02
		Concentration		
Glutamine	**12.5mM**	**25mM**	**50mM**	**100mM**
k_{ex} $(10^3 s^{-1})$ PR0-QUESP	0.64±0.02	0.91±0.02	1.10±0.03	1.82±0.02
		Concentration		
Taurine	**12.5mM**	**25mM**	**50mM**	**100mM**
k_{ex} $(10^3 s^{-1})$ PR0-QUESP	1.06±0.13	1.88±0.05	3.29±0.05	6.25±0.02

III.TI values used in samples and *in vivo*

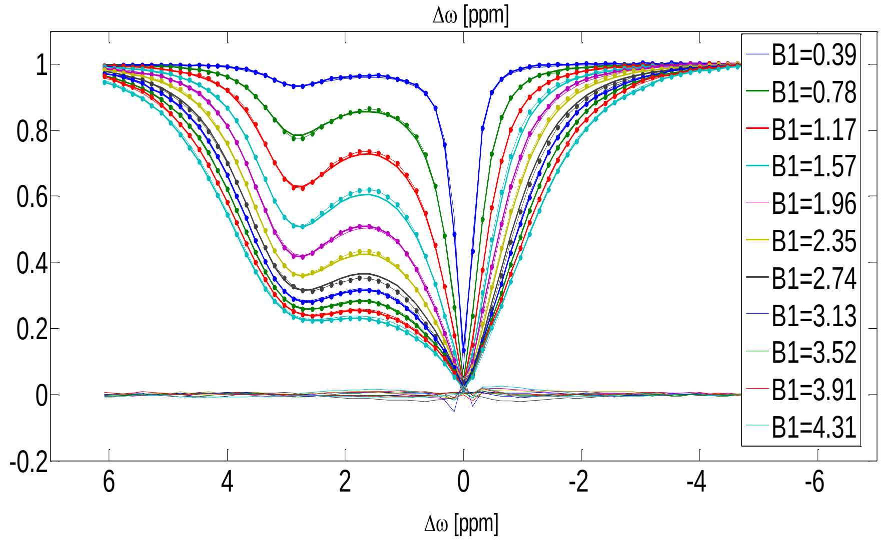
Supporting Table S3

TI values used in phantoms (s)	**TI values used** ***in vivo*** **(s)**
0.008	0.008
0.797	0.017
1.586	0.037
2.375	0.079
3.164	0.169
3.953	0.360
4.742	0.769
5.531	1.640
6.320	3.500
7.109	7.500
7.898	
8.687	
9.476	
10.265	
11.054	
11.843	
12.632	
13.422	
14.211	
15.000	

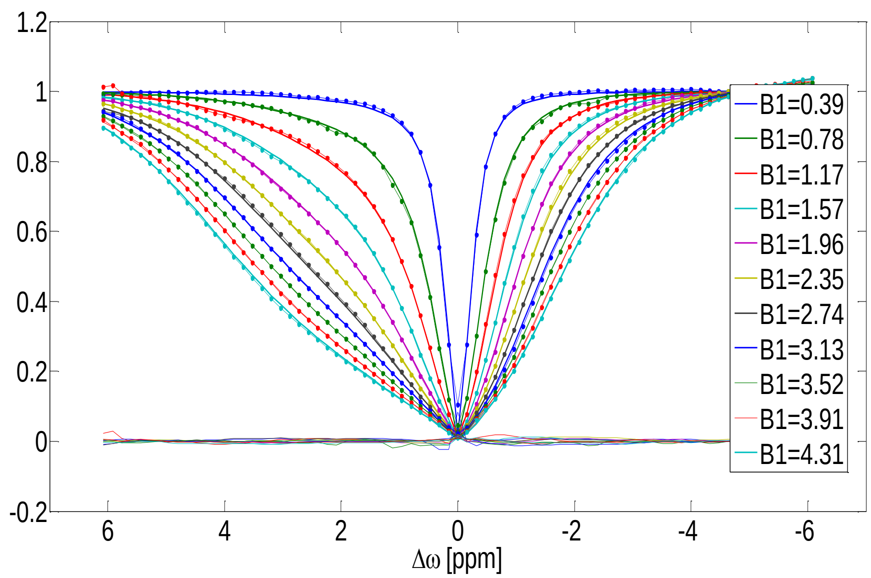
a)



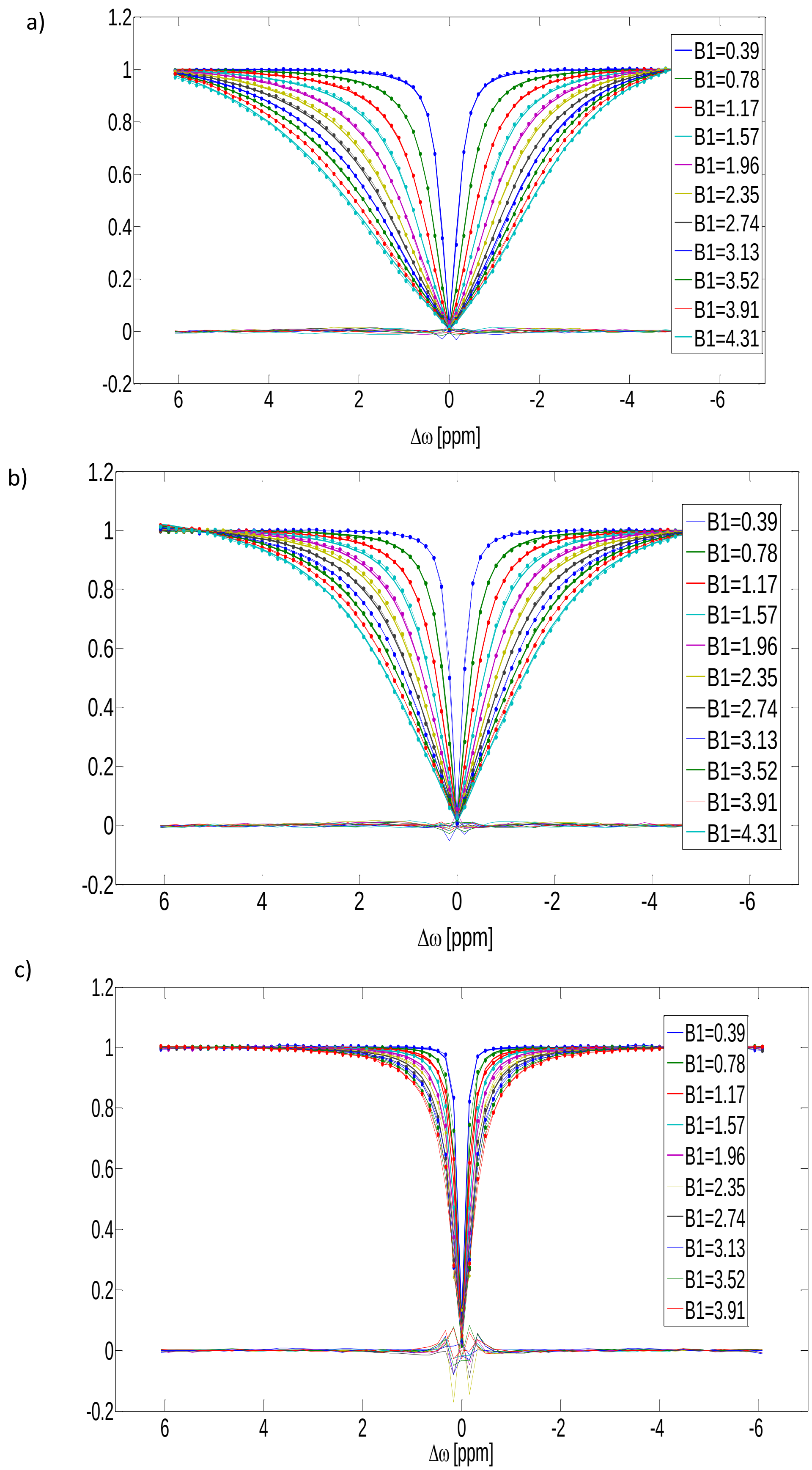
b)



c)



Supporting Figure S7 Z-spectra obtained from taurine at (a) pH=5.85, (b) pH=6.08 and (c) pH= 6.31



Supporting Figure S8 Z-spectra obtained from taurine at (a) pH=6.86, (b) pH=7.2 and (c) PBS