GLINT
GLUCOCEST IMAGING IN NEOPLASTIC TUMOURS

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GLINT

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D4.1 Assessing the intra-/extracellular localization of 3OMG by measuring pH

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CO	Confidential, only for members of the consortium (including the Commission Services)	
CI	Classified, as referred to in Commission Decision 2001/844/EC	

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1 Version log

Version	Date	Released by	Nature of Change
V1.0	01/12/2016	G. Navon	First version
V1.1	02/12/2016	K. Krischak	Format changes, spell check
V2.1	13/02/2017	G. Navon	Second version
V2.2	03/03/2017	M. Kim	Formatting the original report into the EC template, Adding acronyms, Revision.
V2.3a	08/03/2017	M. Rivlin	Minor revision
V2.3b	08/03/2017	M. Kim	Final Version in the EC template

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2 Definition and acronyms

Acronyms	Definitions
3OMG	3-O-methyl-D-glucose
MTR_{asym}	asymmetric magnetization transfer ratio
D_2O	Deuterium oxide
mM	millimolar
PB	Phosphate buffer
BM	Bloch-McConnell

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3 Introduction

This report provides an overview of work undertaken to assess the intra/extracellular localization of 3OMG by measuring pH. Although initial challenges were identified in the process, some of new results indicate alternative way of acheiving a goal of the task. The planned experiments are also described.

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4 Activities carried out and results

The pH dependence of the MTR_{asym} for 3OMG was published before at 25°C (Fig. 1) and now we have results at 37°C (Fig. 2). The pH dependence of the MTR_{asym} is not sensitive enough to serve as a marker for the location of the 3OMG in the intracellular and extracellular spaces. However, the protons exchange rate that can be assessed from the dependence of the Z spectra with B_1 rf field is expected to be much more sensitive to the pH. For this goal we have now preliminary results of the exchange rates for both D-glucose and for 3OMG solutions at T=37°C. Furthermore, we have measured the dependence of the exchange rate of the phosphate buffer (PB) concentration. The analysis was done by the algorithm that was based on the fit to Bloch McConnell (BM) equations that was supplied to us by Dr. Moritz Zaiss (MPG). In the fitting we arbitrarily fixed the fractions f_B and f_D to two and one hydroxyl groups at 1.2 and 3ppm from the water signal corresponding to 3.6E-04 and 1.8E-04 respectively for 20mM D-glu solution and 1.8E-04 and 0.9E-04 for 10mM 3OMG solution. The results reported here are preliminary and the fit was not very good. However, this preliminary results show an indication of the monotonous trend of the exchange rate at 1.2 ppm (k_{ba}), which increases by a factor of 1.44 with the increase of pH from 6.48 to 7.37. We are currently continuing to work on the subject.

All CEST spectra presented here were acquired with a 500 MHz Bruker NMR spectrometer with a range of saturation powers.

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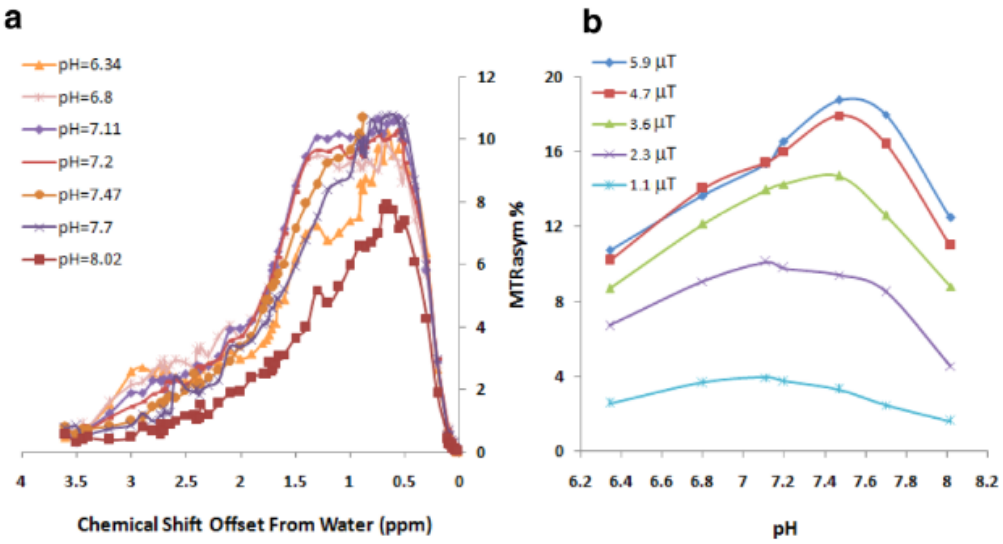

Figure 1: MTR_{asym} plot of a 10 mM 3OMG solution (containing 10 mM phosphate buffer and 10% D_2O) with pH values from 6.3–8 measured at different frequencies offset from water (B_1 = 2.5 µT) (**a**) and at frequency offset of 1.2 ppm as a function of the rf saturation field (B_1) (**b**). (T= 25°C).

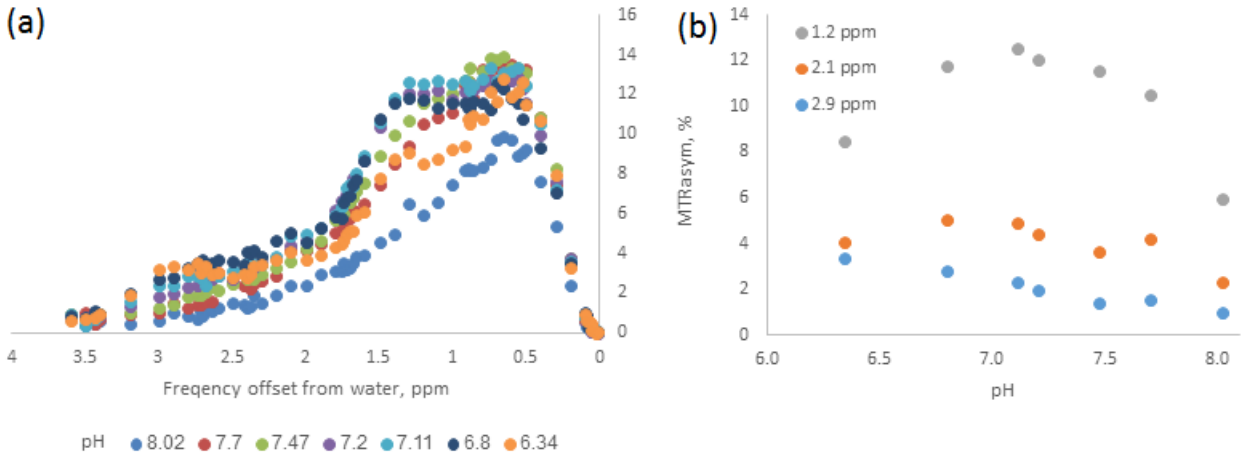

Figure 2: MTR_{asym} plot of a 10 mM 3OMG solution (containing 10 mM phosphate buffer and 10% D_2O) with pH values from 6.34–8.02 measured at different frequencies offset from water (B_1 = 2.5 µT) (**a**) and at frequency offset of 1.2 ppm as a function of the rf saturation field (B_1) (**b**). (T= 37°C)

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I. CEST quantification for a solution of 10mM 3OMG, pH=6.49-7.37, 10mM PB, 10% D_2O, T=37°C

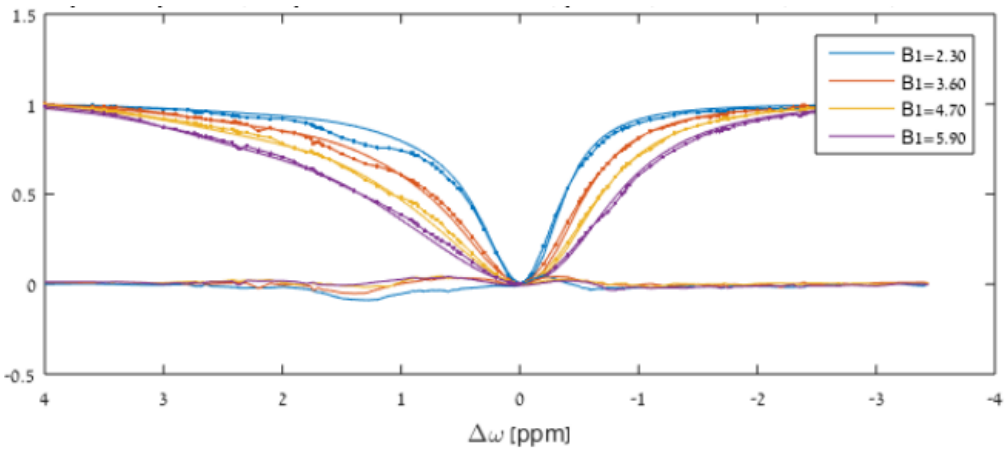

Figure 3: Z spectra with BM fit to 10mM 3OMG solution, 10mM PB, 10%D_2O, pH=6.49, T=37°C

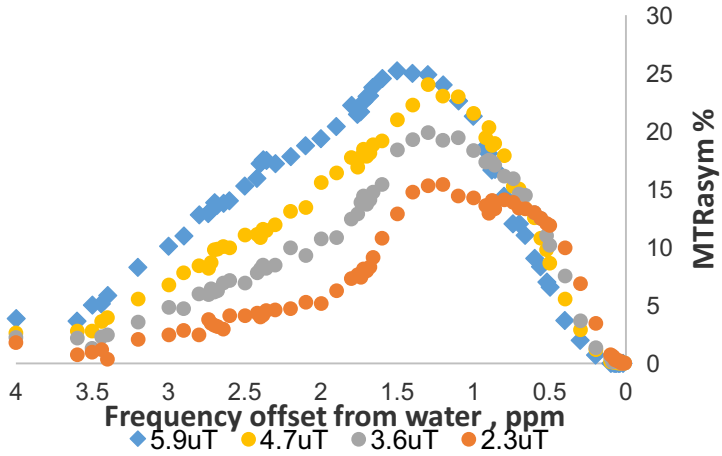

Figure 4: MTRasym plot of 10mM 3OMG solution, 10mM PB, 10%D_2O, pH=6.49, T=37°C

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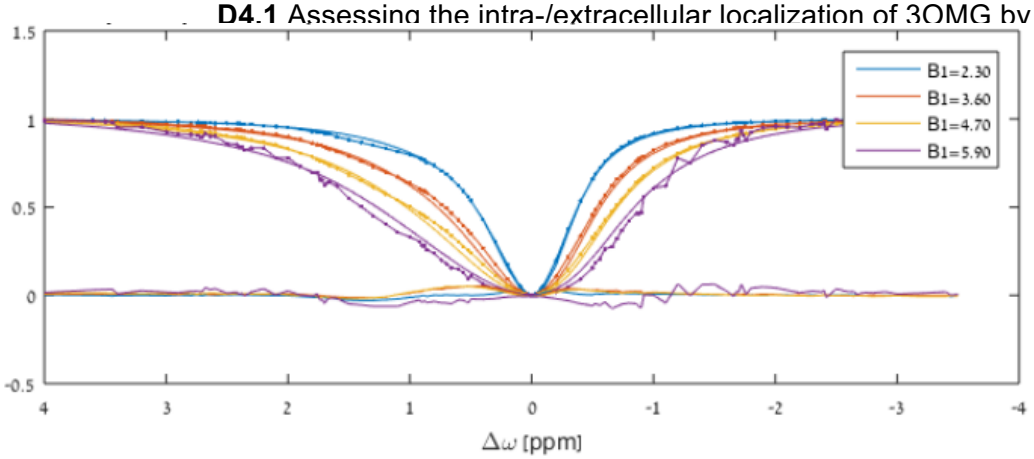

Figure 5: Z spectra with BM fit to 10mM 3OMG solution, 10mM PB, 10%D_2O, pH=6.8, T=37°C

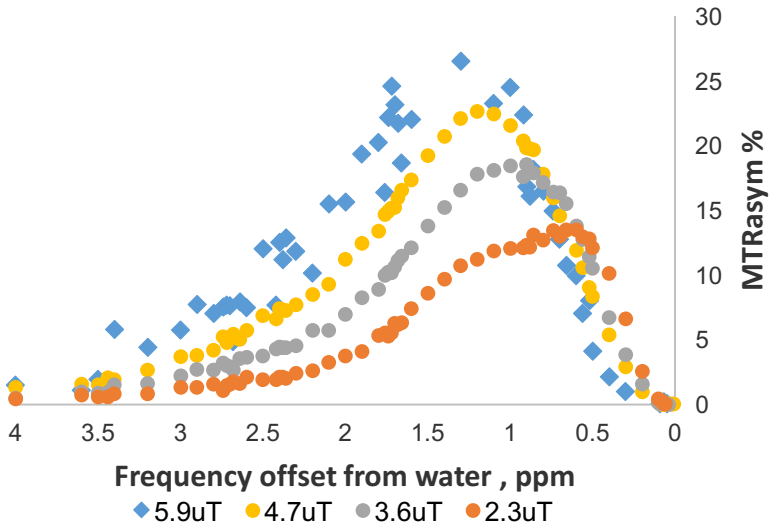

Figure 6: MTRasym plot of 10mM 3OMG solution, 10mM PB, 10%D_2O, pH=6.8, T=37°C

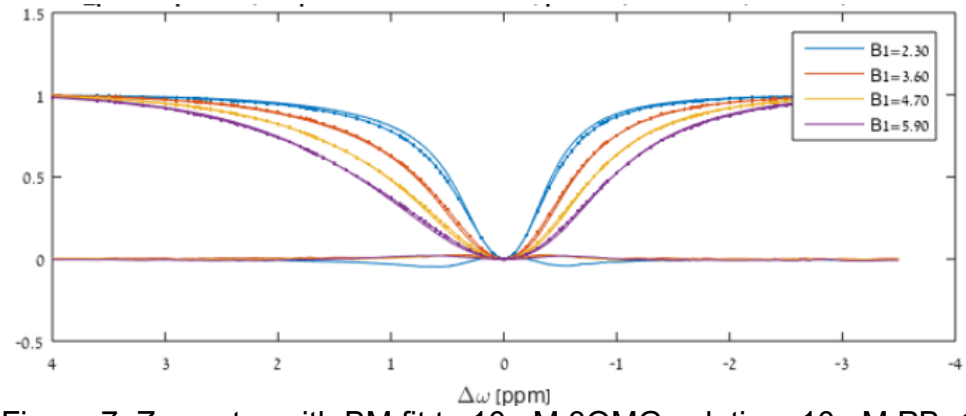

Figure 7: Z spectra with BM fit to 10mM 3OMG solution, 10mM PB, 10%D_2O, pH=7.37, T=37°C

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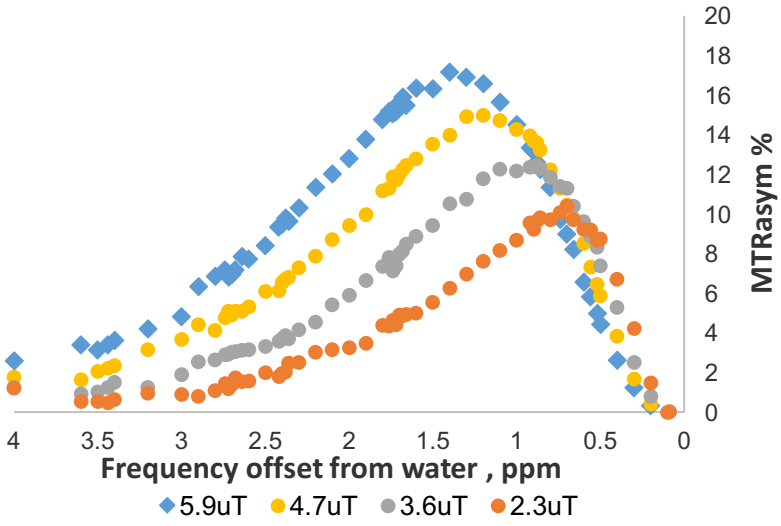

Figure 8: MTRasym plot of 10mM 3OMG solution, 10mM PB, 10%D_2O, pH=7.37, T=37°C

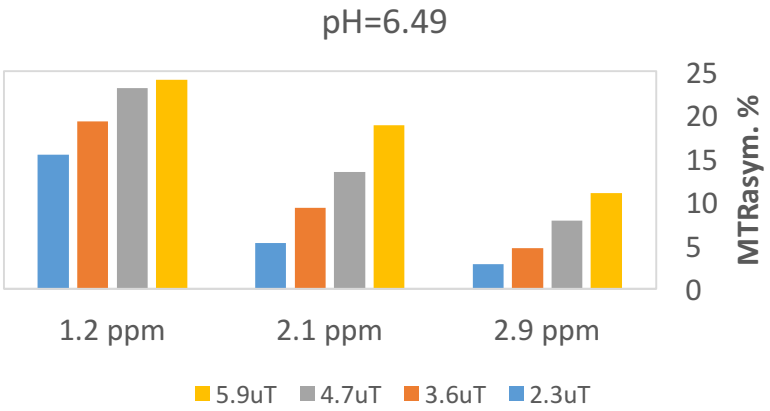

Figure 9: Bar graph showing the % of MTR_{asym} of 10mM 3OMG solution (10% D_2O) at pH=6.49 at frequencies offset of 1.2, 2.1 and 2.9 ppm form the water signal, at T=37°C

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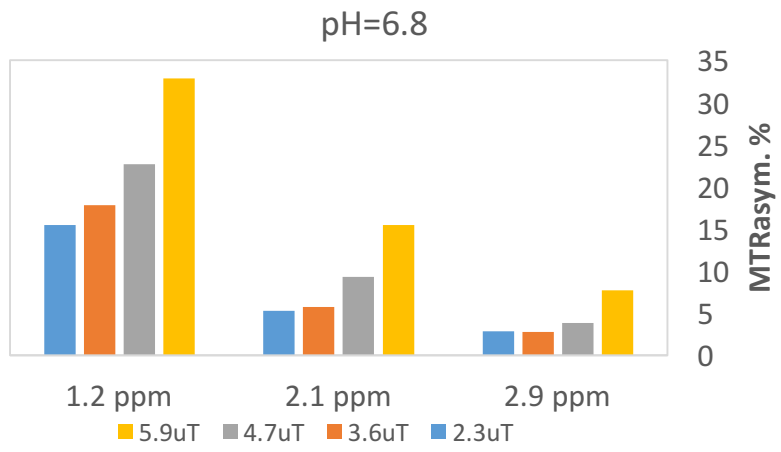

Figure 10: Bar graph showing the % of MTRasym of 10mM 3OMG solution (10% D_2O) at pH=6.8 at frequencies offset of 1.2, 2.1 and 2.9 ppm form the water signal, at T=37°C

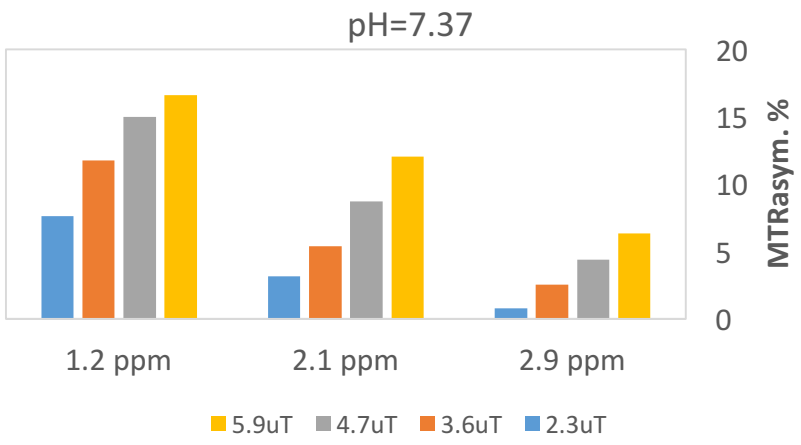

Figure 11: Bar graph showing the % of MTRasym of 10mM 3OMG solution (10% D_2O) at pH=7.37 at frequencies offset of 1.2, 2.1 and 2.9 ppm form the water signal, at T=37°

pH	6.48	6.8	6.99	7.37
Kba (~1.2 ppm)	2320	2641	2869	3334
Kda (~3 ppm)	7608	9590	-	10063

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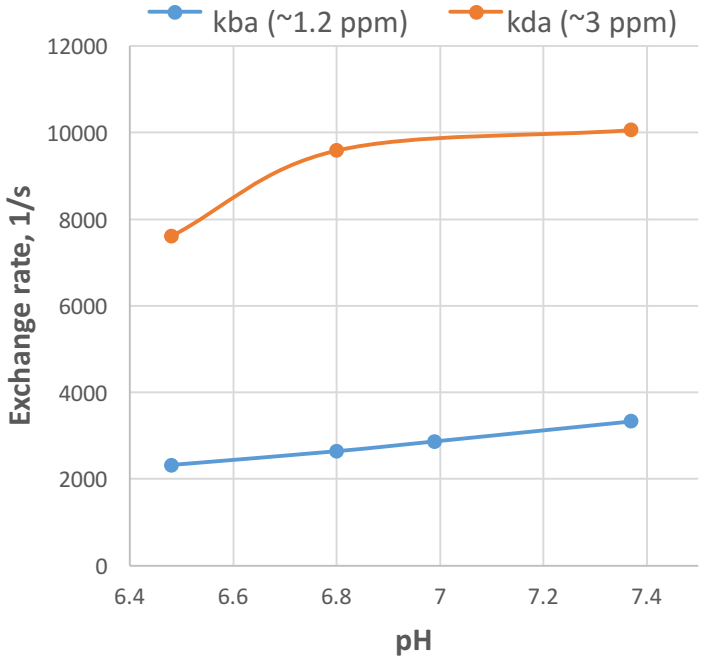

Figure 12: The exchange rates of two hydroxyl metabolites (~1.2 and ~3ppm from the water signal) of 10mM 3OMG solution at different pH values.

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In order to see whether the proton exchange rate of D-Glu depends on the environment conditions, we tested its dependence on the phosphate buffer concentration.

II. CEST quantification for a solution of 20mM D-glucose, pH=7.4, 0-50mM PB, 10% D_2O, T=37°C

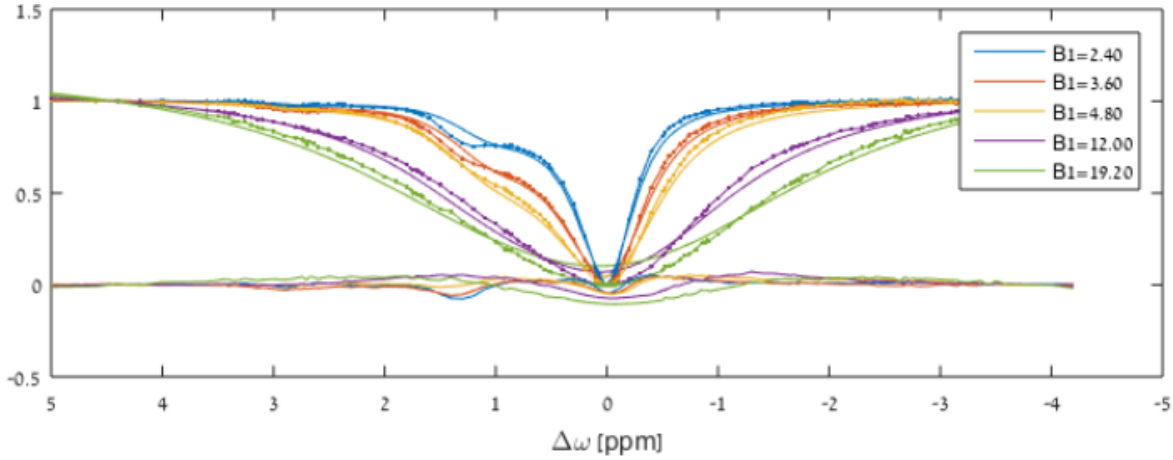

Figure 13: Z spectra with BM fit to 20mM D-Glu solution, 0mM PB, 10%D_2O, pH=7.4, T=37°C

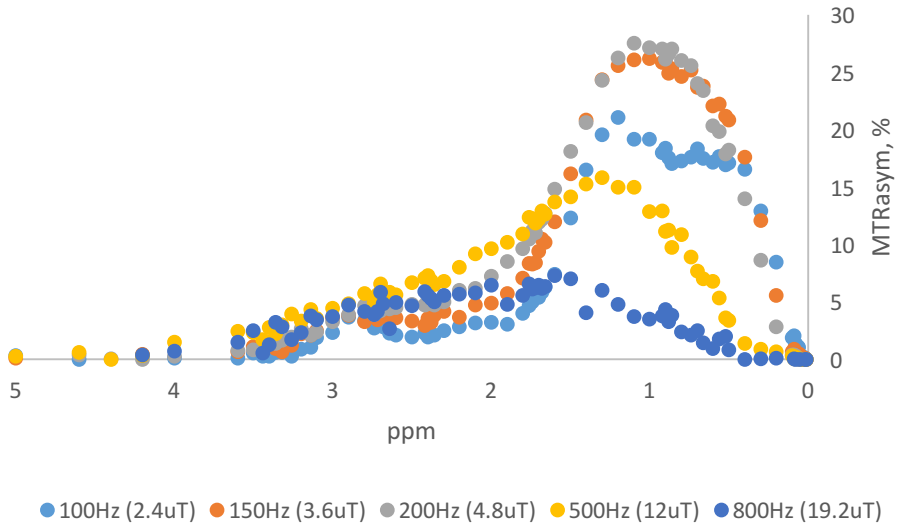

Figure 14: MTR_{asym} plot of 20mM D-Glu solution, 0mM PB, 10%D_2O, pH=7.4, T=37°C

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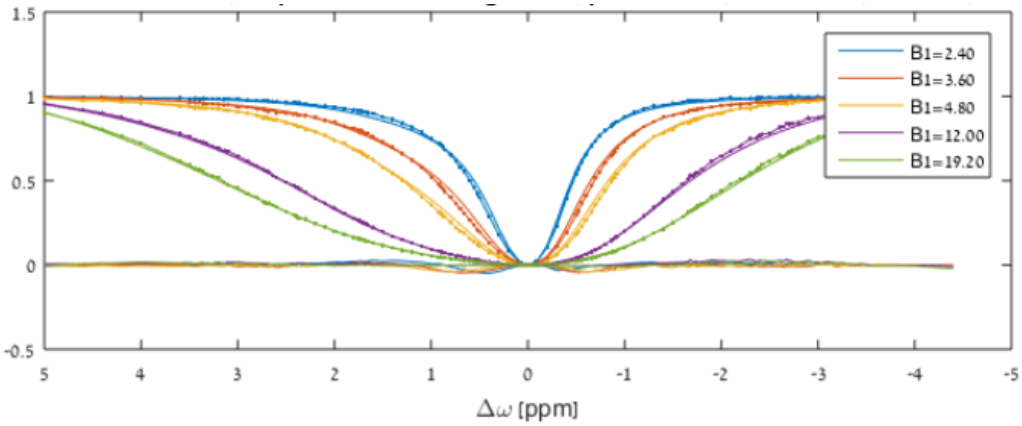

Figure 15: Z spectra with BM fit to 20mM D-Glu solution, 5mM PB, 10%D_2O, pH=7.4, T=37°C

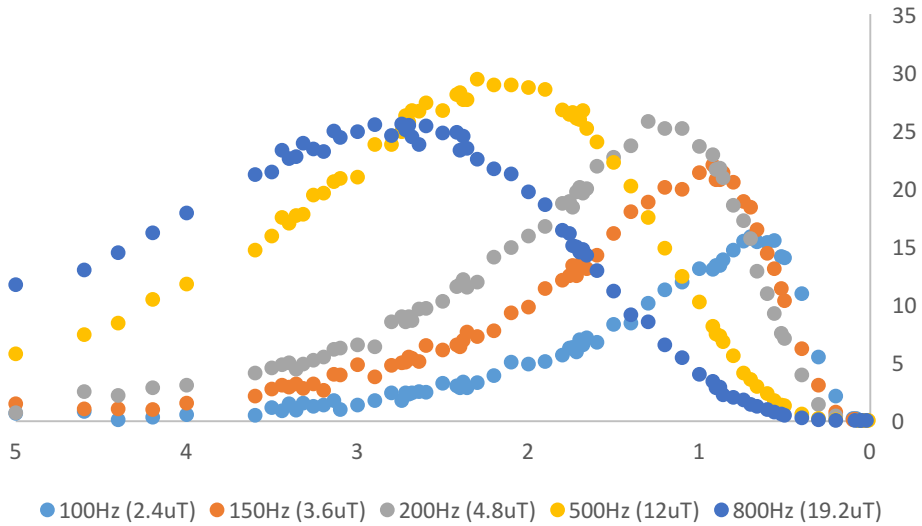

Figure 16: MTRasym plot of 20mM D-Glu solution, 5mM PB, 10%D_2O, pH=7.4, T=37°C

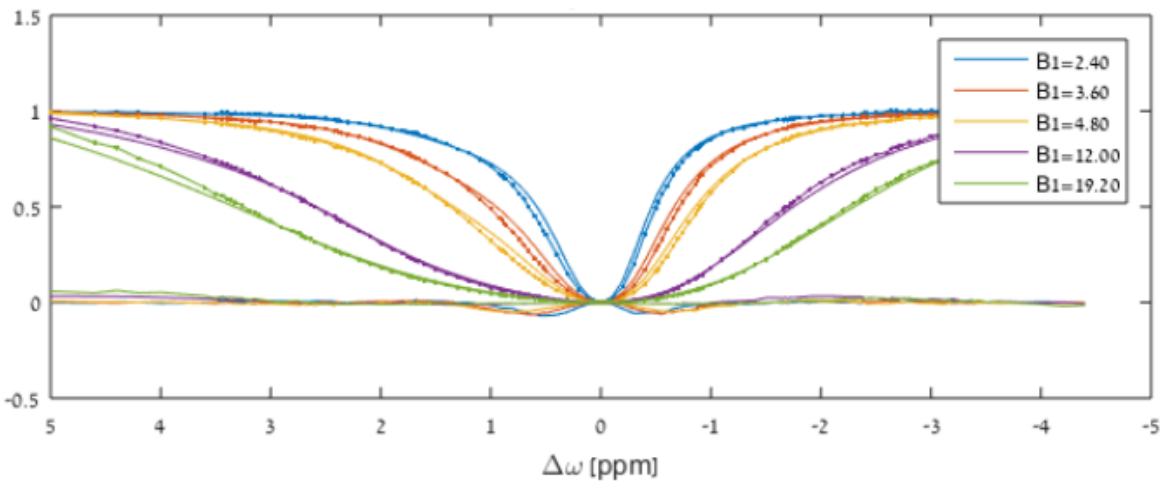

Figure 17: Z spectra with BM fit to 20mM D-Glu solution, 10mM PB, 10%D_2O, pH=7.4, T=37°C

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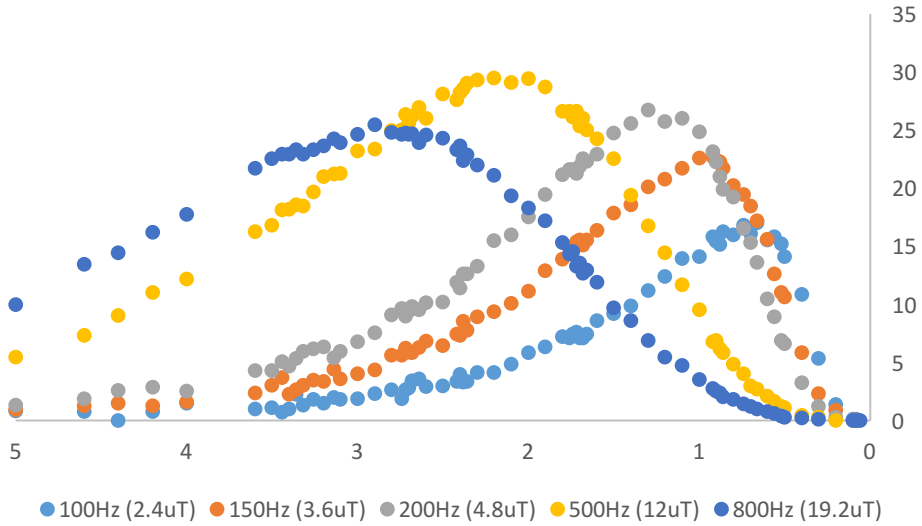

Figure 18: MTRasym plot of 20mM D-Glu solution, 10mM PB, 10%D_2O, pH=7.4, T=37°C

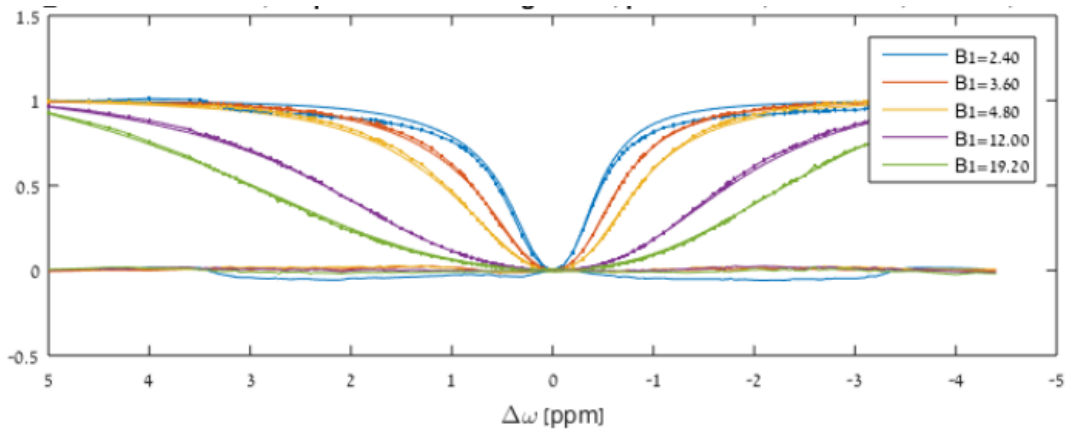

Figure 19: Z spectra with BM fit to 20mM D-Glu solution, 20mM PB, 10%D_2O, pH=7.4, T=37°C

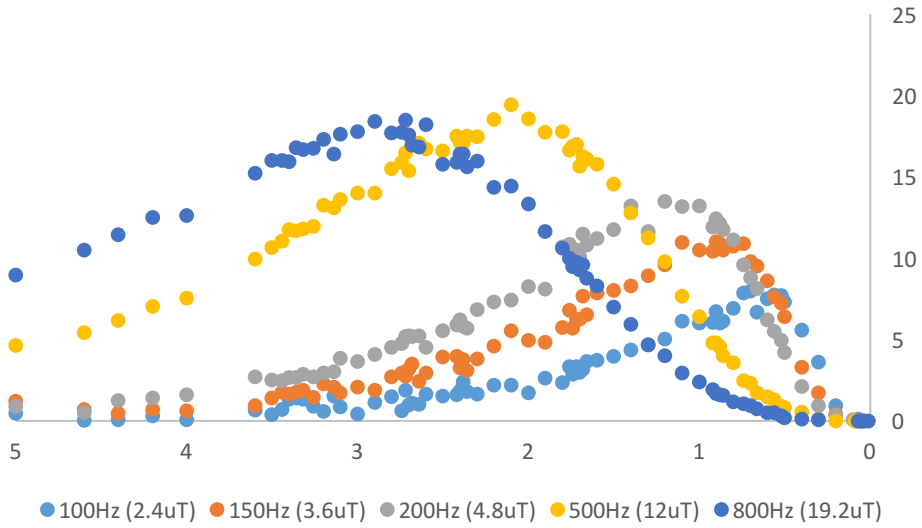

Figure 20: MTRasym plot of 20mM D-Glu solution, 20mM PB, 10%D_2O, pH=7.4, T=37°C

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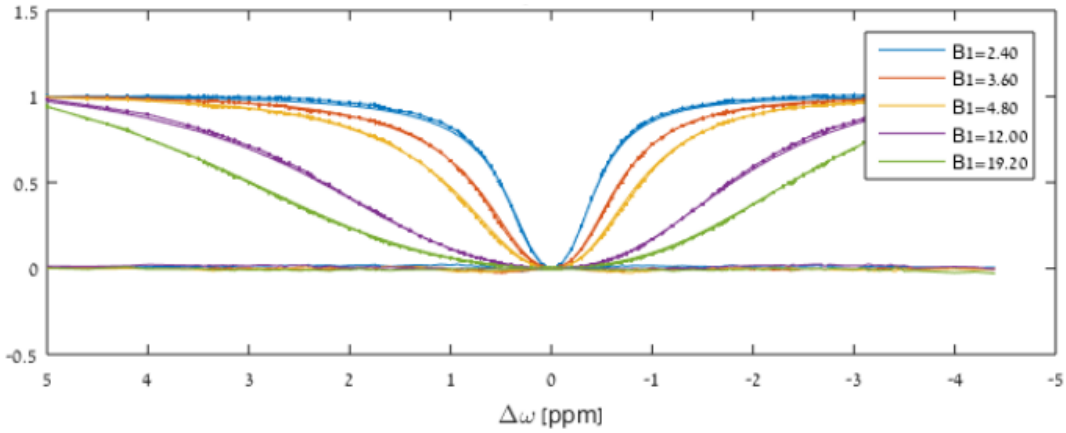

Figure 21: Z spectra with BM fit to 20mM D-Glu solution, 30mM PB, 10%D_2O, pH=7.4, T=37°C

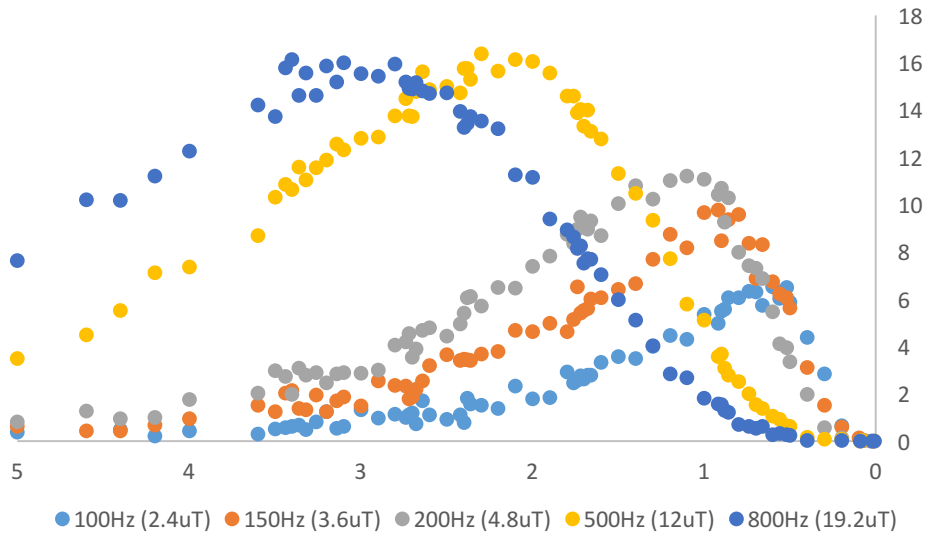

Figure 22: MTRasym plot of 20mM D-Glu solution, 30mM PB, 10%D_2O, pH=7.4, T=37°C

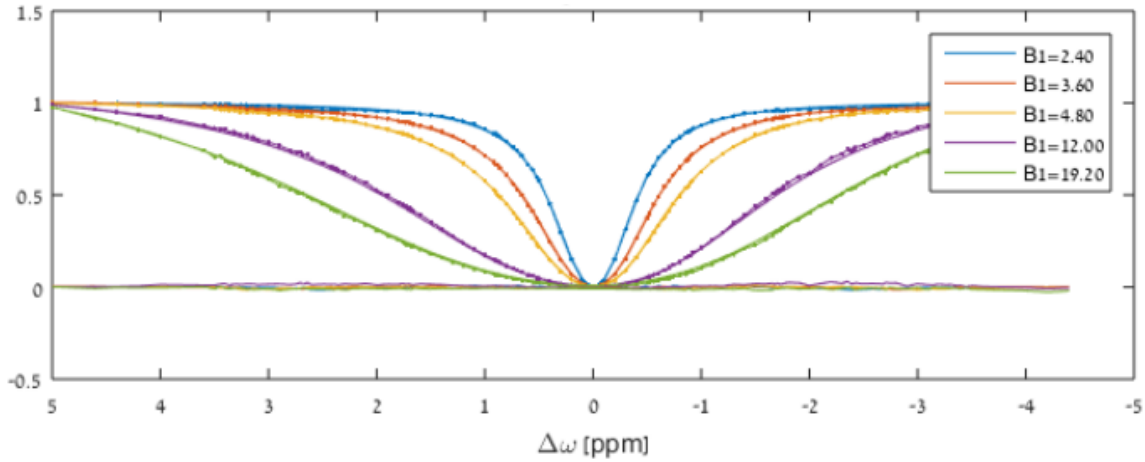

Figure 23: Z spectra with BM fit to 20mM D-Glu solution, 50mM PB, 10%D_2O, pH=7.4, T=37°C

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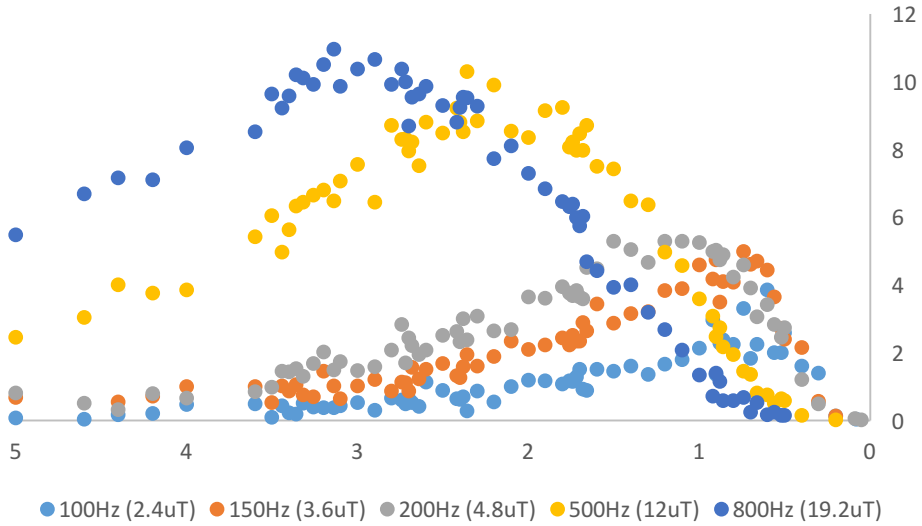

Figure 24: MTRasym plot of 20mM D-Glu solution, 50mM PB, 10%D_2O, pH=7.4, T=37°C

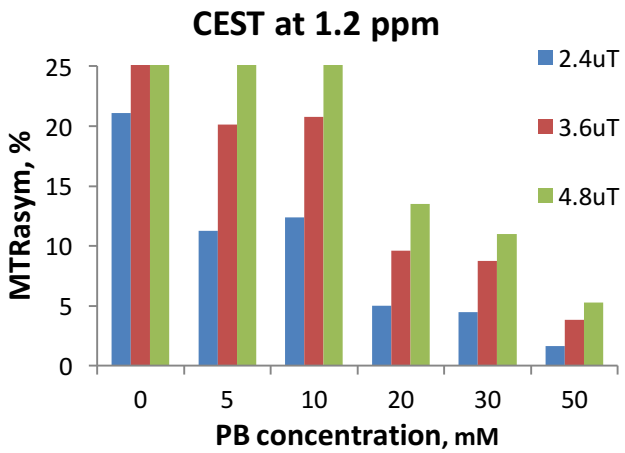

Figure 25: Bar graph showing the % of MTR_{asym} of 20mM D-Glu solution (10% D_2O) at different PB concentrations at frequency offset of 1.2 ppm form the water signal, at T=37°C

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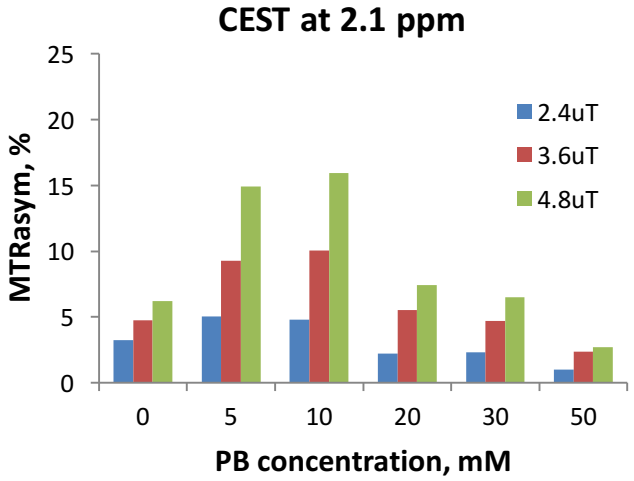

Figure 26: Bar graph showing the % of MTR_{asym} of 20mM D-Glu solution (10% D_2O) at different PB concentrations at frequency offset of 2.1 ppm form the water signal, at T=37°C

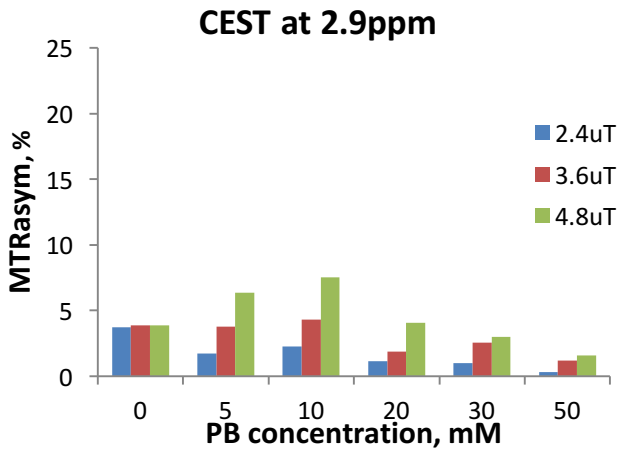

Figure 27: Bar graph showing the % of MTR_{asym} of 20mM D-Glu solution (10% D_2O) at different PB concentrations at frequency offset of 2.9 ppm form the water signal, at T=37°C

PB (mM)	5	10	20	30	50
kba (~1.2 ppm)	3328	2958	3818	5643	7139
kda (~3 ppm)	10015	12872	17169	20479	25045

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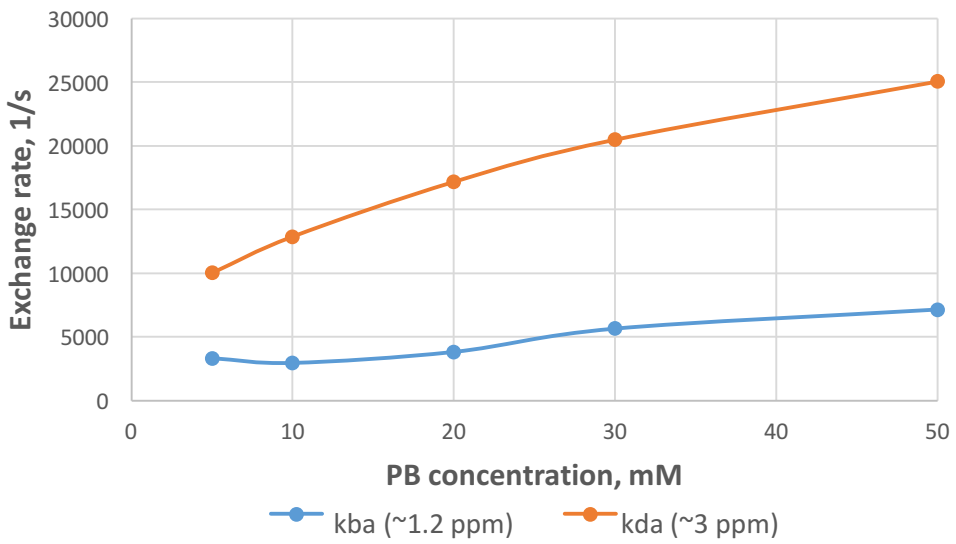

Figure 28: The exchange rates of two hydroxyl metabolites (~1.2 and ~3ppm from the water signal) of 20mM D-Glu solution at different PB concentrations

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5 Conclusions

The changes of the protons exchange rates of 3OMG as a function of pH give us the hope that by the measurements of these rates we will be able to locate the 3OMG to the intra or extra cellular compartments. However, the dependence of the exchange rate on the PB concentrations will make this assignment less straightforward. In parallel, we started to investigate this problem by different route, i.e. by the modification of the MTR_{asym} intensities by paramagnetic gadolinium complexes which reside exclusively at the extra cellular compartments.

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