The Effect of Liposomal Encapsulation on the Chemical Exchange Properties of Diamagnetic CEST Agents

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Supporting Information

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I. Derivation of a six-site exchange model

For the derivation of a six-site model we consider the chemical exchange of the intra-liposomal water magnetization with the extra-liposomal water magnetization. The intra-liposomal magnetization is described by a five-site chemical exchange while the chemical exchange of the intra-liposomal water magnetization from a single hydroxyl group of glucose or 2-DG with the extra-liposomal water can be consider as a two-site system because it is described by a single exchange rate known as the intermembrane-exchange rate.

A two-site exchange model is described by a set of differential equations known as Bloch–McConnell equations (S1-S6):

$$\frac{dM_{xs}}{dt} = -2\pi\Delta M_{ys} + (\frac{-1}{T_{2s}} - R_{cest}M_0^w)M_{xs} + R_{cest}M_0^s M_{xw} \quad (S1)$$

$$\frac{dM_{ys}}{dt} = 2\pi\Delta M_{xs} + \omega_1 M_{zs} + \left(\frac{-1}{T_{2s}} - R_{cest}M_0^w\right)M_{ys} + R_{cest}M_0^s M_{yw} \quad (S2)$$

$$\frac{dM_{zs}}{dt} = -\omega_1 M_{ys} + (-R_{1s} - R_{cest}M_0^w)M_{zs} + R_{1s}M_0^s + R_{cest}M_0^s M_{zw} \quad (S3)$$

$$\frac{dM_{xw}}{dt} = -2\pi\Delta M_{yw} + \left(\frac{-1}{T_{2w}} - R_{cest}M_0^s\right)M_{xw} + R_{cest}M_0^w M_{xs} \quad (S4)$$

$$\frac{dM_{yw}}{dt} = 2\pi\Delta M_{xw} + \omega_1 M_{zw} + \left(\frac{-1}{T_{2w}} - R_{cest}M_0^s\right)M_{yw} + R_{cest}M_0^w M_{ys} \quad (S5)$$

$$\frac{dM_{zw}}{dt} = -\omega_1 M_{yw} + (-R_{1w} - R_{cest}M_0^s)M_{zw} + R_{1w}M_0^w + R_{cest}M_0^w M_{zs} \quad (S6)$$

S1-3 represent the extra liposomal water magnetization and S4-6 represent the intra liposomal water magnetization. For steady state conditions the differential terms are all zero: $[\frac{dM_{xs}}{dt}, \frac{dM_{ys}}{dt}, \frac{dM_{zs}}{dt}, \frac{dM_{xw}}{dt}, \frac{dM_{yw}}{dt}, \frac{dM_{zw}}{dt}]$ = [0,0,0,0,0,0]. $\omega_1 = \gamma B_1$, (γ is the gyromagnetic ratio and B_1 is the applied RF field on the *x*-axis), Δ is the frequency offset of the applied off-resonance saturation pulse, $R_{1w,1s}$ and $\frac{1}{T_{2w,2s}}$ are the longitudinal and transverse relaxation rate of intra-liposomal and extra-liposomal water and $R_{cest}M_0^s M_0^w = k_{sw}M_0^s = k_{ws}M_0^w$ where k_{sw} is the exchange rate of the water from the extra liposomal to intra liposomal space and k_{ws} represents the exchange rate of intra liposomal to extra liposomal water.

From our simulations, we found that by setting $R_{cest}M_0^s M_{yw} = R_{cest}M_0^w M_{ys} = 0$ the resulting z-Magnetization will not change from its theoretical value. Taking this into account S1-S6 can be solved as follows:

From S1 we derive: $M_{yS} = \frac{1}{2\pi\Delta}\left(-\frac{1}{T_{2s}} - R_{cest}M_0^w\right)M_{xs} + \frac{R_{cest}M_0^s M_{xw}}{2\pi\Delta}$ (S7)

From S2 we derive: $M_{ys} = (2\pi\Delta M_{xs} + \omega_1 M_{zs})T_{2w}$ (S8)

From S3 we derive: $M_{zs} = \frac{R_{cest}M_0^s M_{zw} - \omega_1 M_{ys} + R_{1s}M_0^s}{R_{1s} + R_{cest}M_0^w}$ (S9)

From S4 we derive: $M_{xw} = \frac{R_{cest}M_0^w M_{xs} - 2\pi\Delta M_{yw}}{\frac{1}{T_{2w}} + R_{cest}M_0^s}$ (S10)

From S5 we derive: $M_{xw} = \frac{1}{2\pi\Delta}\left(\frac{1}{T_{2w}} + R_{cest}M_0^s\right)M_{yw} - \frac{\omega_1 M_{zw}}{2\pi\Delta}$ (S11)

From S6 we derive: $M_{zs} = \frac{\omega_1 M_{yw} + (R_{1w} + R_{cest}M_0^s)M_{zw} - R_{1w}M_0^w}{R_{cest}M_0^w}$ (S12)

Combing equations (S7) and (S8) we have:

$$M_{xs} = \frac{R_{cest}M_0^S T_{2s} M_{xw} - 2\pi\Delta(T_{2s})^2 \omega_1 M_{zs}}{1+(2\pi\Delta T_{2s})^2 + R_{cest}M_0^S T_{2s}} \quad \text{(S13)}$$

Then combing equations (S13) and (S10) we have $M_{zs} = f(M_{xs}, M_{yw})$ and using equation (S10) and (S11) $M_{xs} = f(M_{yw}, M_{zw})$ which will lead to $M_{zs} = f\left(M_{zw}, M_{yw}\right)$ (S14).

(S14): $M_{zs} = \{\frac{R_{cest}M_0^S}{\omega_1 T_{2s}(2\pi\Delta)^2}(\frac{1}{T_{2w}} + R_{cest}M_0^S) - \frac{1}{R_{cest}M_0^W \omega_1 (2\pi\Delta T_{2s})^2}(\frac{1}{T_{2w}} + R_{cest}M_0^S)^2 [1 + (2\pi\Delta T_{2s})^2 + R_{cest}M_0^W T_{2s}] - \frac{1}{R_{cest}M_0^W \omega_1 (T_{2s})^2}[1 + (2\pi\Delta T_{2s})^2 + R_{cest}M_0^W T_{2s}]\} M_{yw} - [\frac{R_{cest}M_0^S}{T_{2s}(2\pi\Delta)^2} - \frac{1}{R_{cest}M_0^W (2\pi\Delta T_{2s})^2}(\frac{1}{T_{2w}} + R_{cest}M_0^S)[1 + (2\pi\Delta T_{2s})^2 + R_{cest}M_0^W T_{2s}]] M_{zw}$

Finally using (S12) and (S14):

$$M_{zs} = \frac{\alpha M_{zw} + bR_{1s}M_0^S}{c} \quad (S15)$$

where

$$\alpha = 1 - \frac{(R_{cest})^2 M_0^S M_0^W}{\omega_1{}^2 T_{2w}(2\pi\Delta)^2}\left(\frac{1}{T_{2s}} + R_{cest}M_0^W\right) + \frac{1}{\omega_1{}^2(2\pi\Delta T_{2w})^2}\{1 + (2\pi\Delta T_{2w})^2 + R_{cest}M_0^S\}.\{\left(\frac{1}{T_{2s}} + R_{cest}M_0^W\right)^2 + (2\pi\Delta)^2\}$$

$$b = -\{\frac{R_{cest}M_0^W}{\omega_1{}^2 T_{2w}(2\pi\Delta)^2}\left(\frac{1}{T_{2s}} + R_{cest}M_0^W\right) - \frac{1}{R_{cest}M_0^S \omega_1{}^2(2\pi\Delta T_{2w})^2}\{1 + (2\pi\Delta T_{2w})^2 + R_{cest}M_0^S\}.\{\left(\frac{1}{T_{2s}} + R_{cest}M_0^W\right)^2 + (2\pi\Delta)^2\}\}$$

where $\alpha = 1 + R_{cest}M_0^S b$ and c= $(R_{1w} + R_{cest}M_0^S)b$

Equation $(S15)$ can then be written as follows:

$M_{zs} = \frac{M_{zw}}{c} + \frac{b}{c}\ (R_{cest}M_0^S M_{zw} + R_{1s}M_0^S)$ which can be simplified to

$$M_{zs} = \frac{1}{R_{1s} + R_{cest}M_0^W}\ (R_{cest}M_0^S M_{zw} + R_{1s}M_0^S)$$

The longitudinal water magnetization for a five-site exchange model was derived from previous work[1] and can be written as follows:

$$\frac{M_{WZ}^{SS}(\Delta\omega)}{M_0} = \frac{R_{1A}}{\bar{R}_{1\rho}(\Delta\omega)DC + R_{1A}(1-DC)} \quad (S16)$$

where DC is the duty cycle defined as DC=$tp/(tp+td)$. For shaped RF pulses $R_{1\rho}$ is described by the average $\overline{R_{1\rho}}$ defined as follows:

$$\overline{R_{1\rho}} = \frac{1}{t_p}\int_0^{t_p} R_{1\rho}(t)dt = R_{1w} + \frac{1}{t_p}\int_0^{t_p}(R_{2w}-R_{1w})\frac{\omega_1^2(t)}{\Delta^2+\omega_1^2(t)}dt + \frac{1}{t_p}\int_0^{t_p} f_{\mathrm{B}}k_{\mathrm{BA}}\frac{\omega_1^2(t)}{k_{\mathrm{BA}}(k_{\mathrm{BA}}+R_{2\mathrm{B}})^2+\omega_1^2(t)}\frac{\omega_s^2}{\Delta^2+\omega_1^2(t)}dt \quad (S17)$$

where f_{B} is the fractional concentration of a single hydroxyl group from glucose or 2-DG and k_{BA} its chemical exchange rate with water. To expand this into a five site-exchange system we simply add another 3 terms (for the rest of the hydroxyl protons in glucose or 2-DG) i.e. $\frac{1}{t_p}\int_0^{t_p} f_{\mathrm{C}}k_{\mathrm{CA}}\frac{\omega_1^2(t)}{k_{\mathrm{CA}}(k_{\mathrm{CA}}+R_{2\mathrm{B}})^2+\omega_1^2(t)}\frac{\omega_s^2}{\Delta^2+\omega_1^2(t)}dt$ to Equation *(S17)*. Finally, *(S17)* is substituted to *(S16)* for calculating the exchange rates of hydroxyl groups in glucose or 2-DG.

II. Calculation of encapsulation efficiency

Table S1. Parameters used to calculate and the calculated internal volume of each liposome sample.

Liposome sample	Z-Ave (d.nm)	Size distribution (σ)	Bilayer thickness (*d*)	Average area per lipid (*A*)	Lipid concentration (mM)	Internal volume
1	180	28.5	4.6	47.3	30	12%
2	178	33.3	4.6	47.3	30	13%
3	168	40.3	4.6	47.3	30	13%
4	155	30.0	4.6	47.3	30	11%
5	151	32.0	4.6	47.3	30	11%
6	184	30.5	4.6	47.3	30	13%
7	147	24.4	5.1	47.3	30	10%
8	146	25.9	5.1	47.3	30	10%
9	152	25.2	5.1	47.3	30	10%
10	164	34.8	5.1	47.3	30	12%

PdI values measured by DLS must be converted into standard deviation (σ) values. When the particle size distribution can be fitted to a Gaussian distribution, the relationship between PdI and σ and the average hydrodynamic radius (r) can be described by the following equation:

$$\mathrm{PdI} = \sigma^2/\mathrm{r}^2$$

III. Determining monosaccharide concentrations of liposomal samples

Overall and exterior glucose and 2-DG concentrations for liposome formulations were obtained using the Glucose GO Assay Kit® supplied by Sigma-Aldrich. The kit is an enzymatic, colorimetric assay intended to measure glucose concentration utilising the enzyme, glucose oxidase. The assay reagent contains glucose oxidase (500 units), peroxidase (horseradish, 100 purpurogallin units), o-dianisidine dihydrochloride (4 mg), buffer salts and 40 mL DI water. When the assay reagent is added to glucose/2-DG solutions the glucose/2-DG is oxidised by glucose oxidase producing hydrogen peroxide as a side product. Hydrogen peroxidase reacts with o-dianisidine in the presence of peroxidase to form a coloured product. To terminate the assay sulfuric acid is added to react with oxidised o-dianisidine and form a more stable coloured product. The intensity of this pink colour measured at 540 nm (A_{540}) is proportional to the glucose/2-DG concentration.

Overall sugar concentrations for liposomal samples were measured after addition of Triton X-100 which was used to disrupt the liposome bilayer and cause uniform dispersion of the encapsulated contents throughout the total sample volume. Overall concentration test solutions consisted of DI water (490 µL), 3% Triton X-100 (5 µL) and liposomal sample (5 µL).

The assay reagent conditions were found to cause monosaccharide leakage from liposomes so in order to measure exterior sugar concentrations liposome samples were centrifuged at 4000 rpm for 5 minutes. When subjected to centrifugation liposomes formed a pellet and did not release encapsulated monosaccharide allowing 5 µL of the supernatant (or exterior liposome solution) to be carefully removed. The 5 µL of supernatant was added to DI water (495 µL) to create the test solution for exterior monosaccharide concentration.

A new calibration curve was constructed alongside every run of the assay to correct for slight differences in lab temperature, assay run length and time passed since assay reagent was prepared (assay reagent is viable for up to 1 month according to manufacturer's instructions).

Assay reagent (1.0 mL) was added to each calibration or test solution and agitated for exactly 30 minutes at room temperature *via* shaking on an IKA KS130 basic platform shaker at 320 rpm. After this time 6 M H_2SO_4 (1.0 mL) was added to terminate the reaction. The A_{540} was measured for test and calibration solutions using an Agilent Cary 100 spectrophotometer and unknown concentration values were derived using the constructed calibration curves, considering dilution factors.

IV. NMR shifts of hydroxyl protons in glucose and 2-DG at varying concentrations

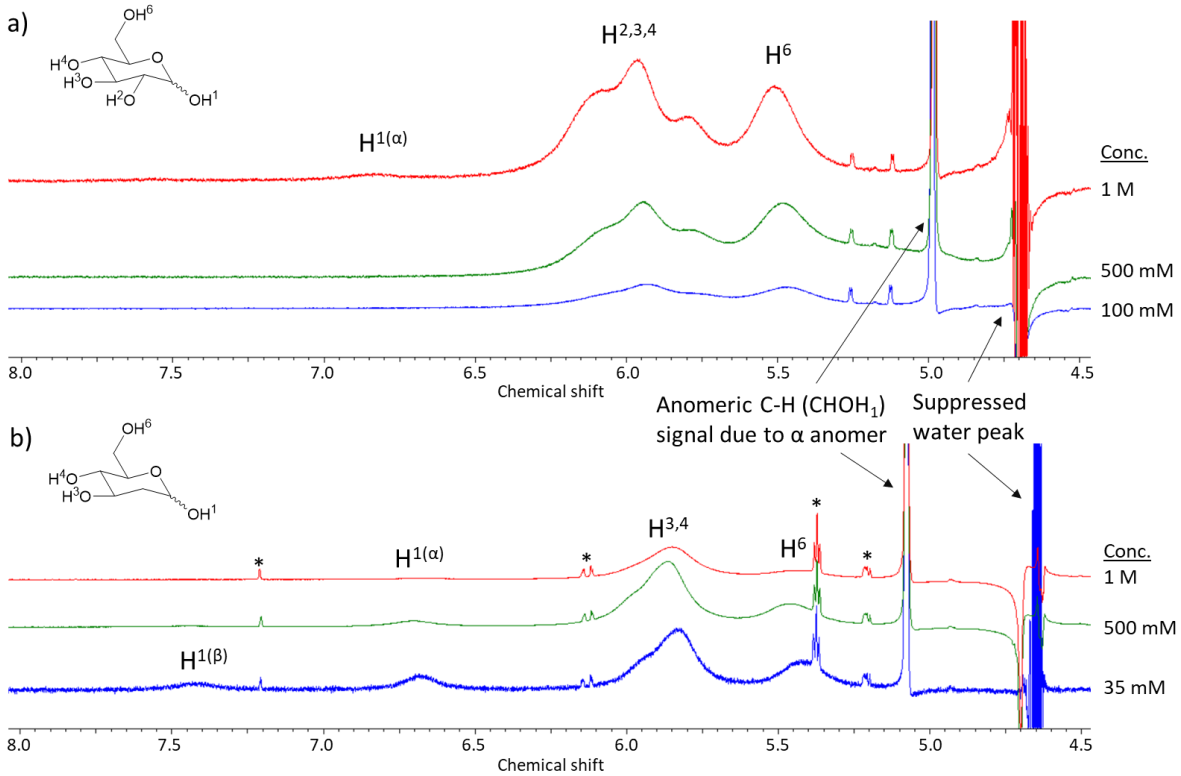


Figure S1. ^{1}H NMR spectra of monosaccharides in DI water with 20% PBS at pH 7, a) glucose at concentrations of 1 M, 500 mM and 100 mM, and b) 2-DG at concentrations of 1 M, 500 mM and 35 mM. The suppressed water and anomeric C-H signals are labelled and asterisks mark impurities present in the commercially available 2-DG

V. Results of exchange rates for free monosaccharides and monosaccharides encapsulated inside liposomes with five and six site exchange model

Table S2.

Glucose 25 °C	*pH=6.0*	*pH=6.25*	*pH=6.5*	*pH=6.75*	*pH=7.0*	*pH=7.4*
0.66 ppm	1266±392	1353±426	1422±369	1311±314	1907±623	1493±362
1.28 ppm	725±55	688±56	746±46	972±48	1424±67	2675±105
2.08 ppm	50±120	204±211	320±170	1733±275	2493±296	3869±429
2.88 ppm	250±151	416±141	608±114	885±88	1308±177	3692±534

Table S3.

Glucose 37 °C	*pH=6.0*	*pH=6.25*	*pH=6.5*	*pH=6.75*	*pH=7.0*	*pH=7.4*
0.66 ppm	2069±607	1104±280	1062±256	1272±431	2241±437	2485±496
1.28 ppm	958±53	907±44	1163±44	1670±79	2596±137	6289±419
2.08 ppm	189±102	1179±281	1796±181	2565±279	3940±648	9110±640
2.88 ppm	501±107	683±54	1106±96	2197±283	3838±643	8000±181

Table S4.

2-DG 25 °C	*pH=6.0*	*pH=6.25*	*pH=6.5*	*pH=6.75*	*pH=7.0*	*pH=7.4*
0.66 ppm	1022±455	1146±400	1179±429	1693±645	1567±517	1836±827
1.28 ppm	1341±126	1021±91	1286±106	1405±117	1619±134	2819±317
2.08 ppm	50±452	88±91	114±94	209±96	1469±263	3652±791
2.88 ppm	88±166	324±221	400±225	626±238	940±241	4275±1497

Table S5.

2-DG 37 °C	*pH=6.0*	*pH=6.25*	*pH=6.5*	*pH=6.75*	*pH=7.0*	*pH=7.4*
0.66 ppm	912±238	920±314	916±306	1068±442	1460±535	2022±508
1.28 ppm	1293±68	1286±84	1419±94	1671±129	2335±170	6994±801
2.08 ppm	105±60	255±56	1465±267	2134±280	2954±433	9133±709
2.88 ppm	358±131	611±178	406±60	2330±335	3488±605	8000±6636

Table S6.

Glucose 25 °C	*pH=6.0*	*pH=7.0*
0.66 ppm	820±105	786±107
1.28 ppm	392±11	1257±23
2.08 ppm	242±46	2465±86
2.88 ppm	297±26	2190±44

2-DG 25 °C	*pH=6.0*	*pH=7.0*
0.66 ppm	677±152	477±165
1.28 ppm	438±24	1128±70
2.08 ppm	113±27	2304±109
2.88 ppm	246±70	886±76

Table S7.

Glucose 37 °C	*pH=6.0*	*pH=7.0*
0.66 ppm	926±114	50±11
1.28 ppm	823±18	3554±75
2.08 ppm	905±66	5551±546
2.88 ppm	831±19	7165±372

2-DG 37 °C	*pH=6.0*	*pH=7.0*
0.66 ppm	610±144	896±148
1.28 ppm	994±50	2910±108
2.08 ppm	371±42	4760±206
2.88 ppm	443±41	3268±250

five-site vs six-site exchange model

five-site model

Table S8.

Gluco-liposomes 25 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1039±313	1093±356	745±344	1067±395
1.28 ppm	301±16	468±29	180±19	210±19
2.08 ppm	356±171	541±298	483±181	423±336
2.88 ppm	570±170	303±85	1161±325	318±160

Table S9.

Gluco-liposomes 37 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1443±300	1453±325	556±274	50±41
1.28 ppm	1249±45	1570±56	1652±92	1762±76
2.08 ppm	413±67	747±199	157±60	253±160
2.88 ppm	2200±118	691±79	1841±244	509±134

six-site model

Table S10.

Gluco-liposomes 25 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1115±463	1352±603	857±409	1118±455
1.28 ppm	442±59	1109±81	135±35	159±49
2.08 ppm	378±253	620±389	278±126	419±332
2.88 ppm	661±182	399±86	1145±311	196±113
$R_{intermembrane}$	52±59	50±64	39±45	44±44

Table S11.

Gluco-liposomes 37 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1072±507	1087±582	898±592	1223±503
1.28 ppm	1216±83	1365±106	1453±119	1387±85
2.08 ppm	1884±363	1575±423	2061±484	802±387
2.88 ppm	1834±264	882±163	2169±419	738±105
$R_{intermembrane}$	61±63	95±134	66±79	59±67

five-site model

Table S12.

2DG-liposomes 25 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1249±390	1054±470	942±362	1052±345
1.28 ppm	52±20	411±30	137±18	161±18
2.08 ppm	358±596	127±127	172±70	158±85
2.88 ppm	84±54	394±394	803±452	428±369

Table S13.

2DG-liposomes 37 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1502±507	1196±408	154±154	1168±503
1.28 ppm	521±83	1674±80	2218±2218	395±85
2.08 ppm	86±363	289±35	94±94	267±387
2.88 ppm	685±264	1386±167	1759±419	756±105

six-site model

Table S14.

2DG-liposomes 25 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1595±687	1461±642	2025±703	2157±730
1.28 ppm	123±27	117±39	30±22	48±24
2.08 ppm	147±235	547±926	112±161	148±390
2.88 ppm	351±834	101±73	432±954	202±821
$R_{intermembrane}$	52±59	50±64	39±45	44±44

Table S15.

2DG-liposomes 37 °C	*DPPC pH=6.0*	*DPPC pH=7.0*	*DSPC pH=7.0*	*DSPC pH=6.0*
0.66 ppm	1352±608	986±474	1067±525	1184±530
1.28 ppm	502±36	1885±108	252±24	267±32
2.08 ppm	272±104	2635±161	346±60	674±259
2.88 ppm	635±321	1504±149	1483±290	639±221
$R_{intermembrane}$	61±63	95±134	66±79	59±67

six-site model

Table S16.

glucose	*37 °C*	*34 °C*	*31 °C*	*28 °C*	*25 °C*
0.66 ppm	1363±480	1300±317	1181±418	1037±813	1090±340
1.28 ppm	3049±207	2555±95	2207±113	1711±124	1401±64
2.08 ppm	4473±824	3447±466	3449±350	2329±893	1883±267
2.88 ppm	4014±1254	3449±360	2339±473	1933±227	1492±189

Table S17.

Gluco-liposomes	*37 °C*	*34 °C*	*31 °C*	*28 °C*	*25 °C*
0.66 ppm	2425±2487	909±753	877±769	975±370	340±768
1.28 ppm	1544±192	972±123	871±116	846±80	64±98
2.08 ppm	2875±300	1354±299	1138±852	1073±274	267±604
2.88 ppm	1430±445	1380±300	1077±229	1028±272	189±304
$R_{intermembrane}$	92±34	79±66	65±13	56±78	45±46

Table S18.

2-DG	*37 °C*	*34 °C*	*31 °C*	*28 °C*	*25 °C*
0.66 ppm	805±534	884±442	809±815	896±399	935±448
1.28 ppm	1876±98	1924±105	1349±158	1455±90	1241±99
2.08 ppm	2918±172	2560±154	1696±327	1510±37	413±116
2.88 ppm	1764±218	967±199	1096±238	587±178	823±210

Table S19.

2-DG liposomes	*37 °C*	*34 °C*	*31 °C*	*28 °C*	*25 °C*
0.66 ppm	1017±902	938±1124	907±442	1017±929	1096±321
1.28 ppm	1566±193	1308±218	1230±105	1191±183	161±43
2.08 ppm	2063±265	1843±406	304±154	194±219	56±51
2.88 ppm	1155±343	792±349	547±199	427±401	256±487
$R_{intermembrane}$	92±34	79±66	65±13	56±78	45±46

VI. Release over time experiments

Table S20. Table of liposome sample bilayer composition, monosaccharide contents, diameter, hydrodynamic size distribution, overall and exterior monosaccharide concentrations and pH. Liposomes were formulated as detailed in the experimental section of the main paper. They were dialysed into 0.25 M NaCl with 20% PBS.

Liposome sample	Lipid composition	[lipid]	encapsulated	Z-Ave (d.nm) (Std dev)	PdI (Std Dev)	Overall [monosaccharide] (mM) (of which exterior (mM))	pH
L1	3% DPPE-PEG2000, 97% DPPC	> 35 mM*	glucose	156 (4.0)	0.18 (0.01)	60 (0.3)	7
L2	3% DPPE-PEG2000, 97% DPPC	30 mM	2-DG	166 (1.6)	0.10 (0.02)	38 (0.7)	7
L3	100% DPPC	30 mM	2-DG	197 (3.9)	0.16 (0.01)	30 (0.5)	7

*this liposome sample had been centrifuged and some exterior solution pipetted off to increase lipid and monosaccharide concentration. This was carried out to aid detection of small quantities of leakage in the early stages of the experiment.

Glucose (**L1**) and 2-DG (**L2** and **L3**) liposomes were incubated at 37 °C using a BIOER mixing block with slow agitation at 350 rpm. Before the start of an experiment the initial exterior monosaccharide concentration was confirmed to be negligible (< 1 mM) using the Glucose GO Assay® and an aliquot of exterior solution was kept aside to obtain a 0 min data point in the assay conducted at the end of the experiment. Overall monosaccharide test solutions were obtained as usual (5 µL liposomes, 5 µL 3% Triton).

Once heating at 37 °C was commenced, aliquots (40 µL) were taken from the incubated liposome sample at regular time points, decanted into a 0.35 mL Eppendorf, dipped in an ice bath to immediately stop leakage and then stored in the fridge until the end of the experiment. Once all time points aliquots had been collected, the aliquots were centrifuged at 10,400 rpm and 4 °C for 1 h, and 5 µL of supernatant was pipetted off to be used in the Glucose GO Assay® to determine exterior monosaccharide concentration. Determining exterior concentrations for all time points in a single assay was found to be more accurate than conducting several assays throughout the experiment. Following completion of the assay, the A_{540} of test solutions were measured in triplicate and readings obtained for the original exterior monosaccharide and overall monosaccharide

concentrations (measured in the same assay) were used to convert each time point A_{540} reading into a percentage leakage value (Figure S2).

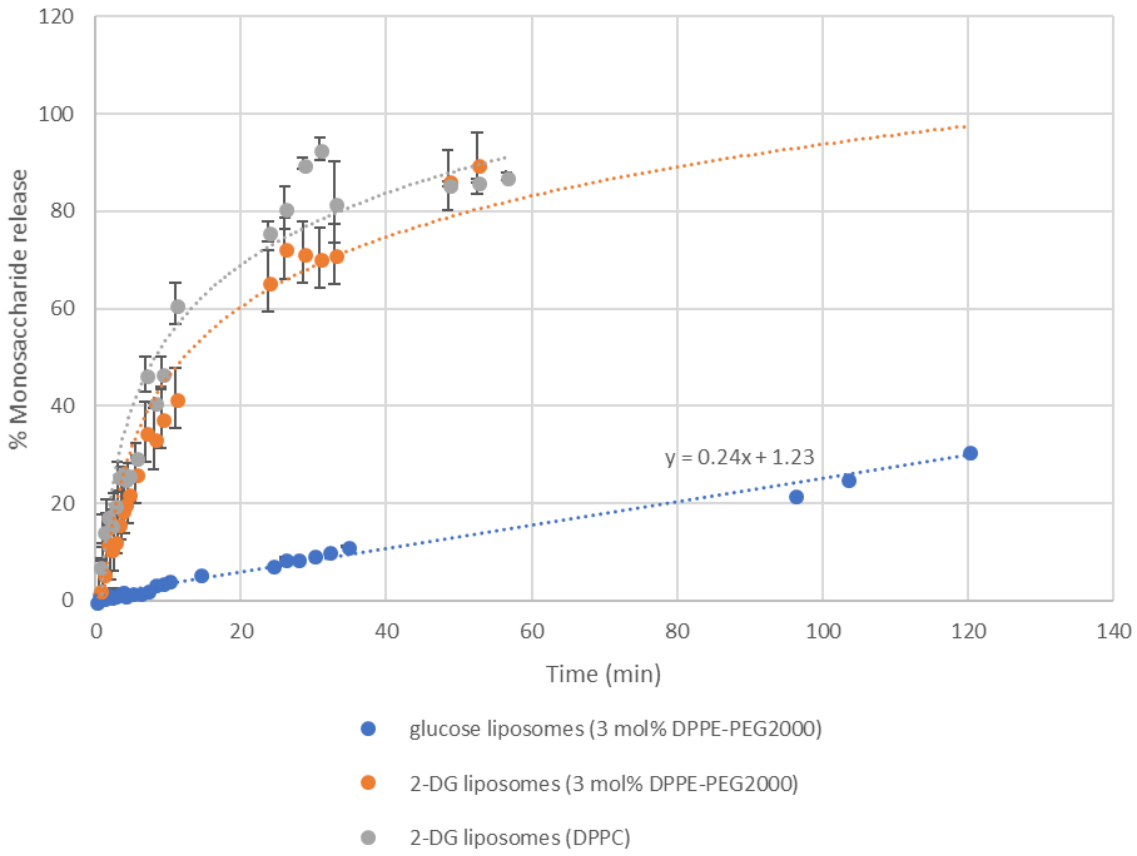


Figure S2. Release of glucose 2-DG from liposome formulations **L1-3** (Table S20) when incubated at 37 ^{o}C and agitated at 350 rpm using a BIOER mixing block.

Release at 2 h:

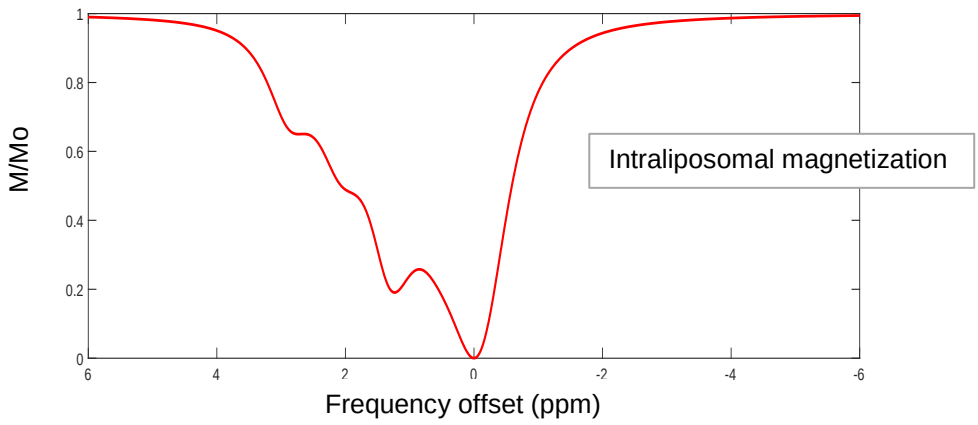
Glc from 3% PEG: 1.4% → exterior Glc conc of 0.84 mM

2-DG from 3% PEG: 10% →exterior 2-DG conc of 3.8 mM

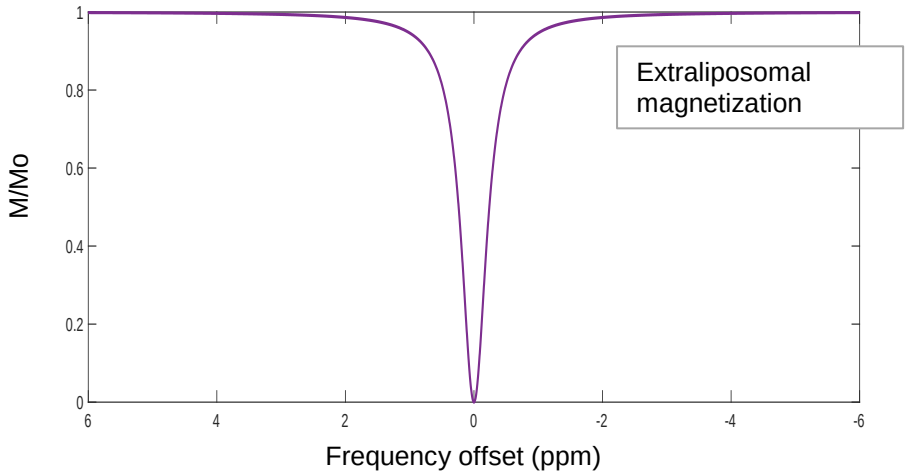
2-DG from DPPC: 15% → exterior 2-DG conc of 4.5 mM

VII. Simulations of the liposomal system

a



b



c

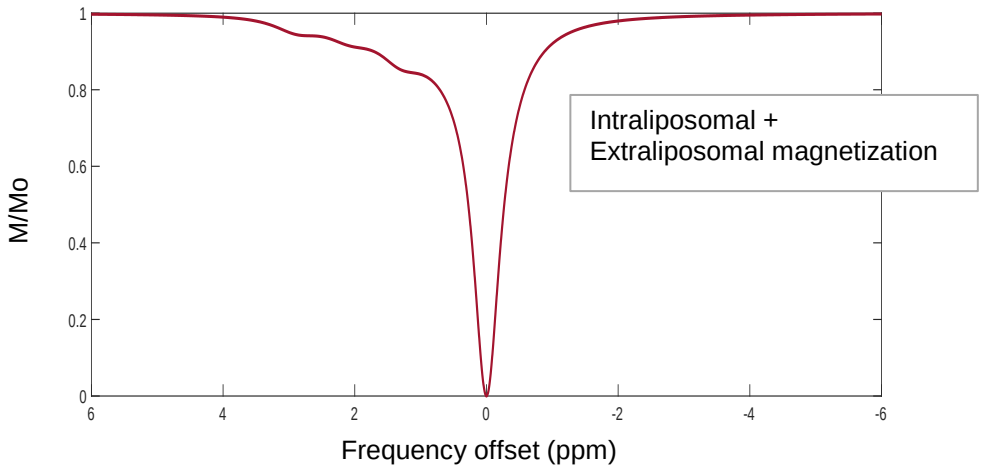
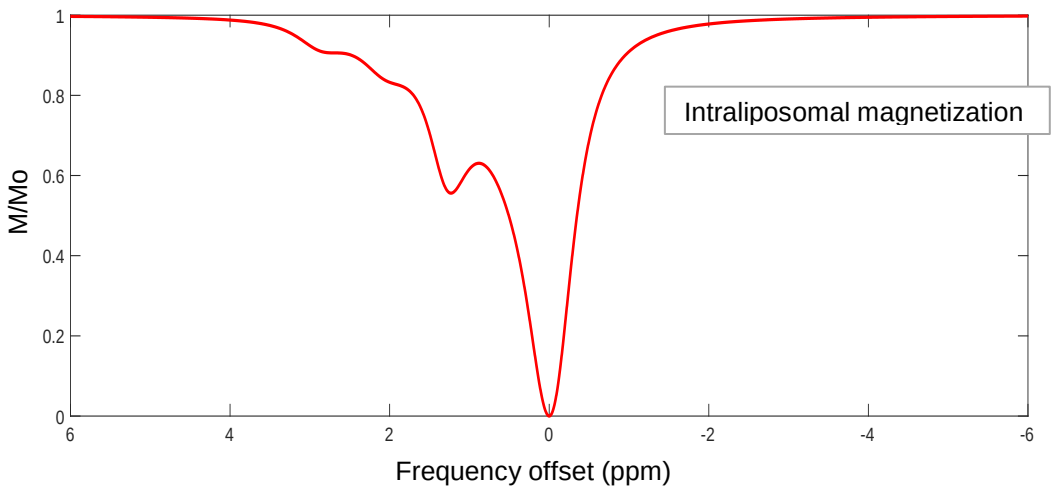
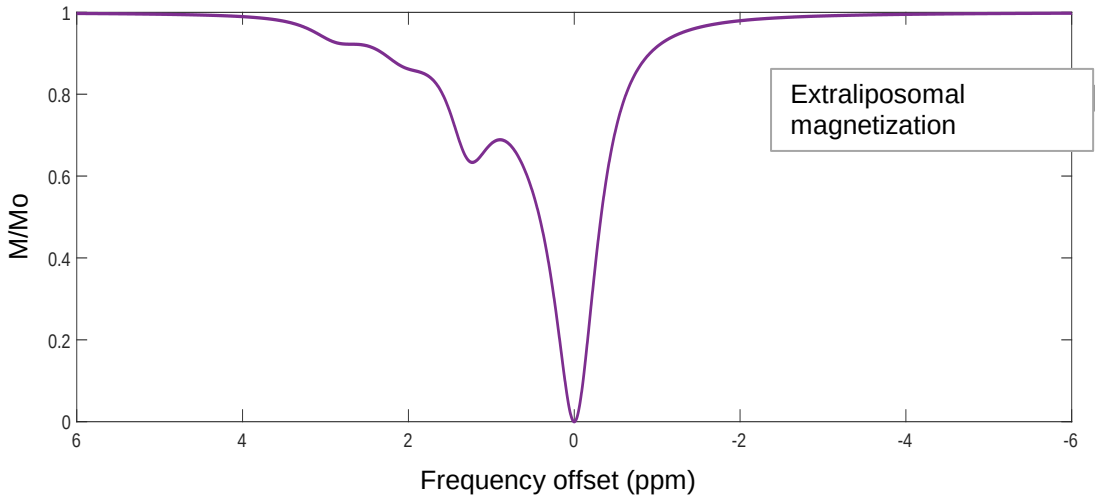


Figure S3. Simulated Z-spectra obtained from a six-site chemical exchange with Rcest = 0, B_1 = 1.5 µT

a



b



c

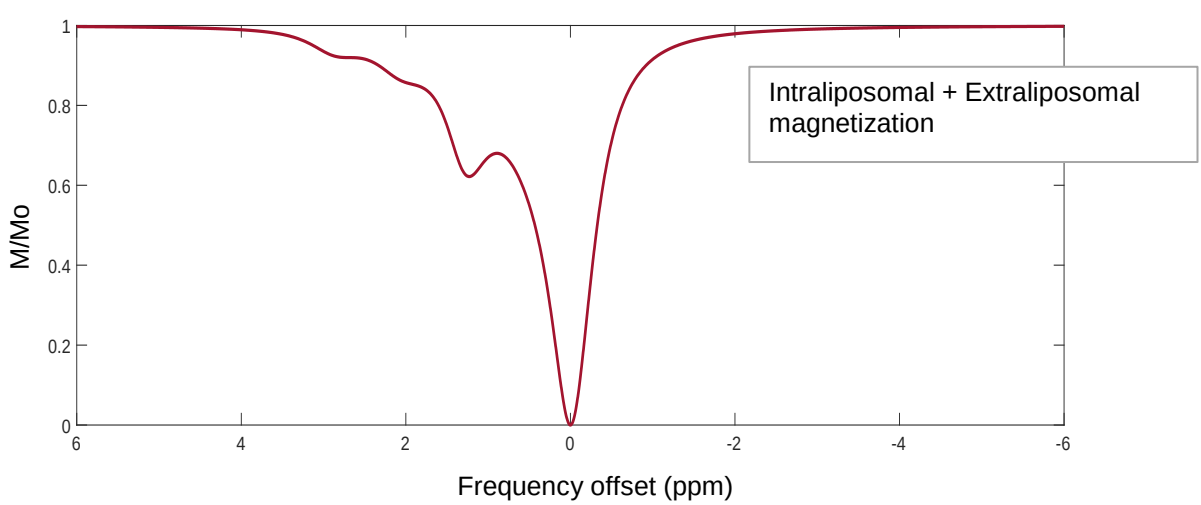
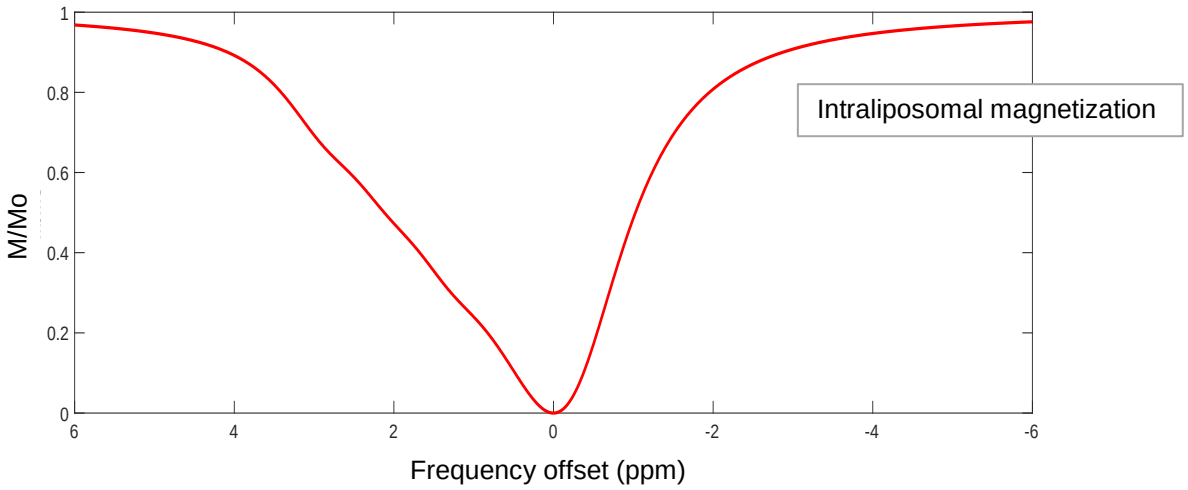
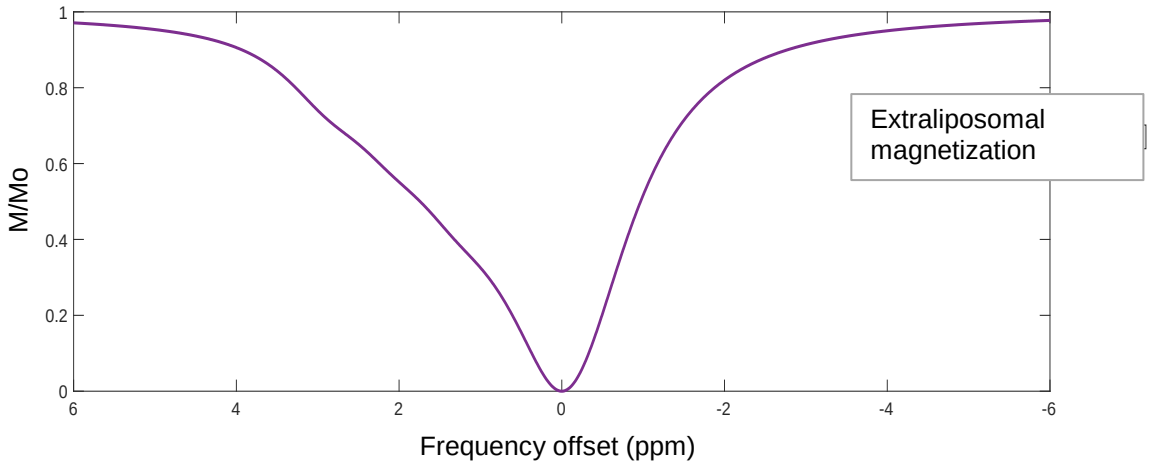


Figure S4. Simulated Z-spectra obtained from a six-site chemical exchange with Rcest = 10**,** B_1 = 1.5 μT.

a



b



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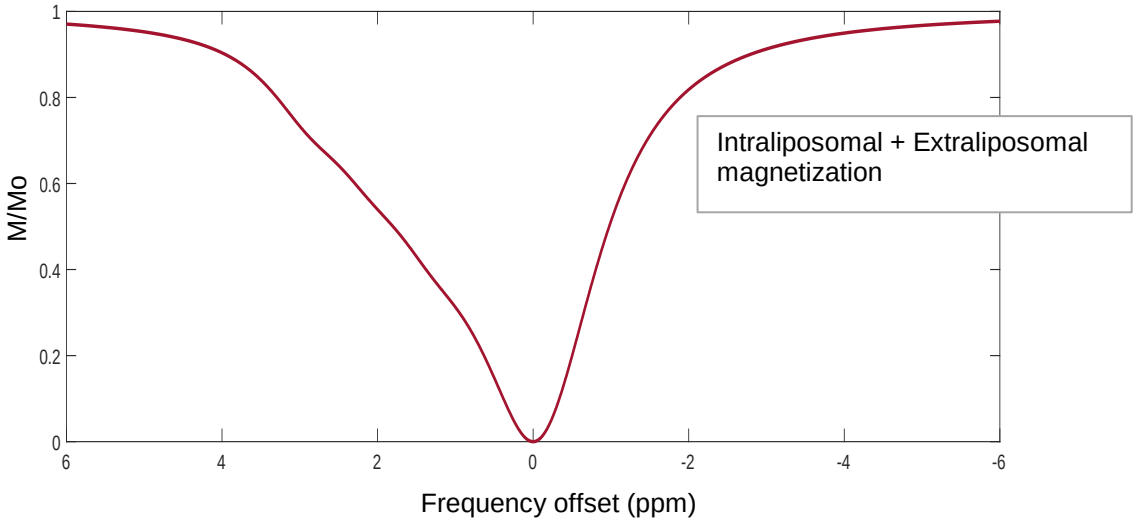
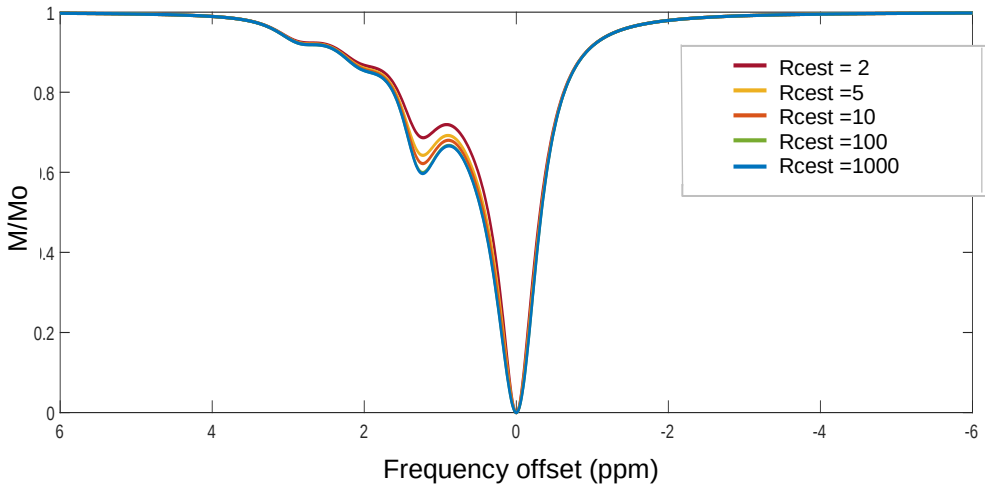
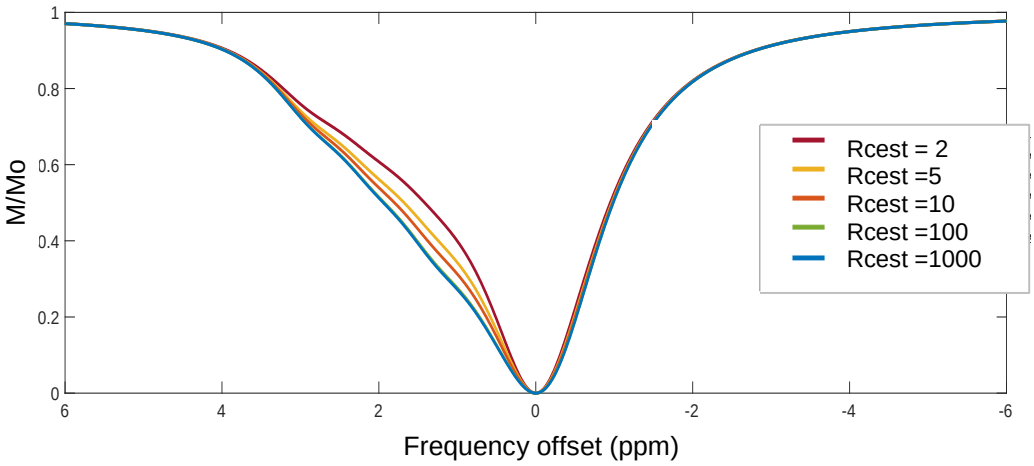


Figure S5. Simulated Z-spectra obtained from a six-site chemical exchange with Rcest = 10, B_1 = 5.06 μT.

a



b



c

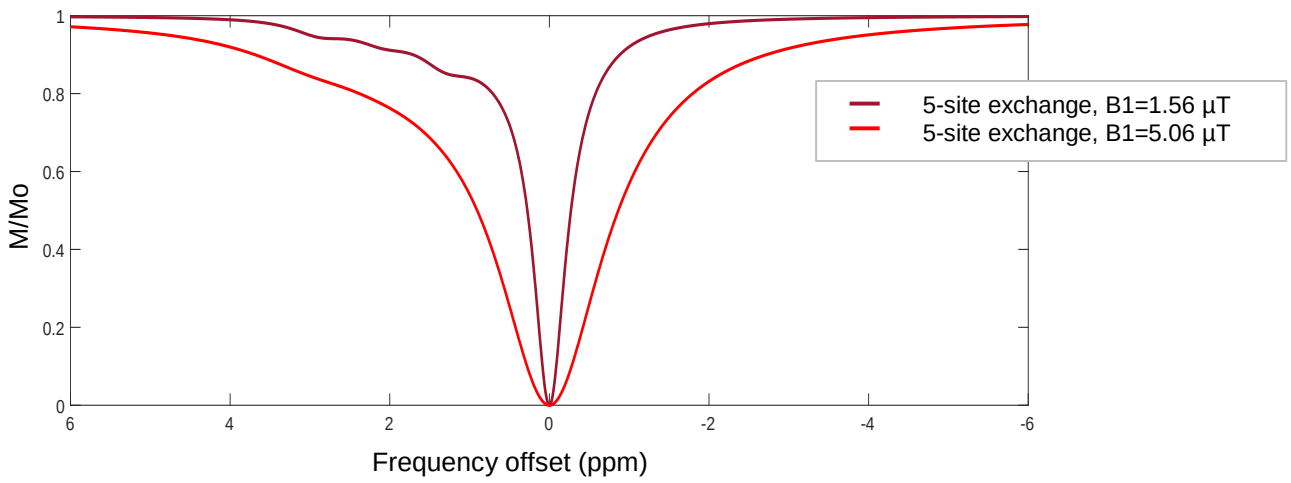
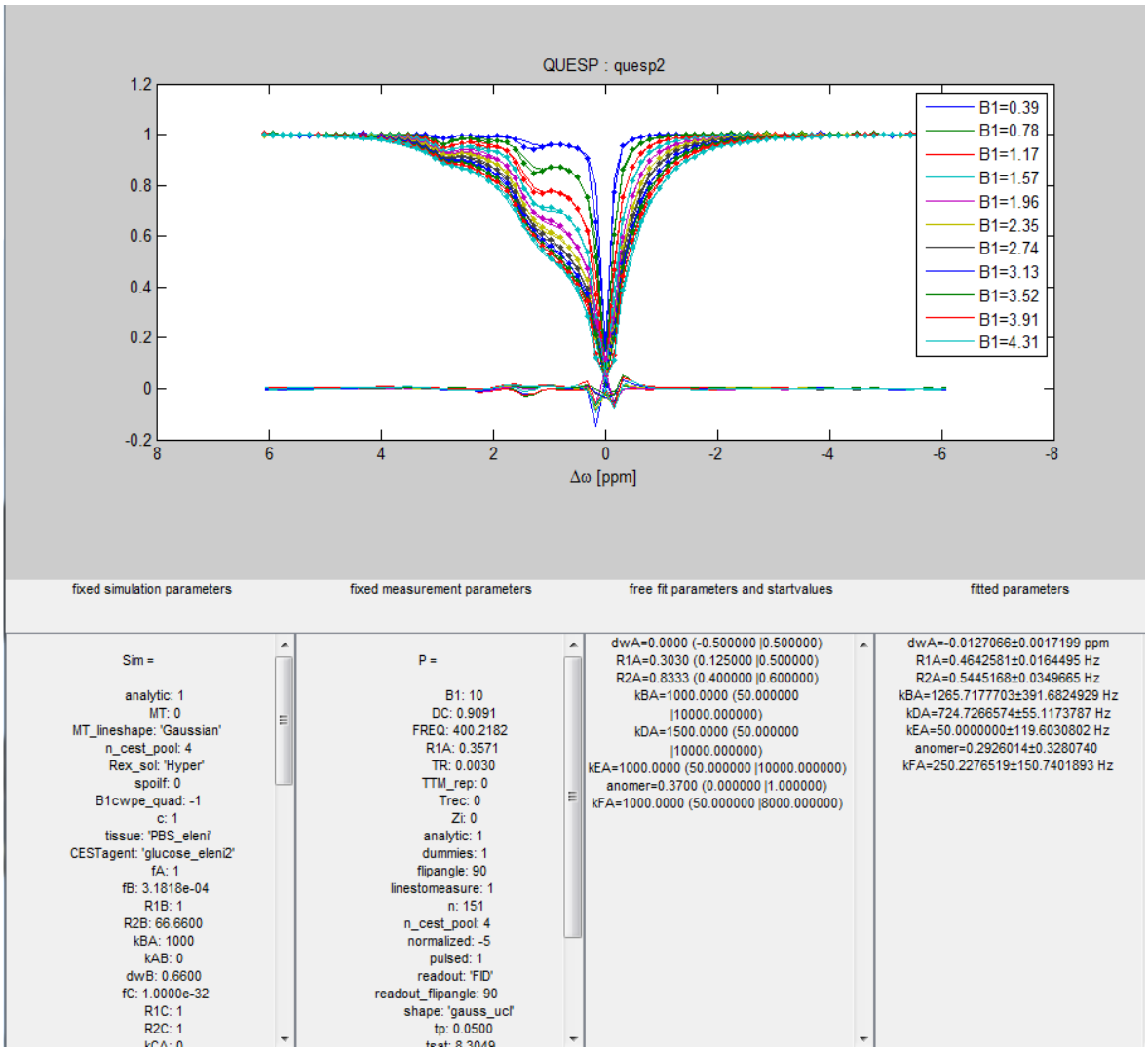


Figure S6. Simulated Z-spectra obtained from a six-site chemical exchange for various Rcest at B_1 = 1.5 µT and 5.06 µT. 5-site Z-spectra are displayed for comparison.

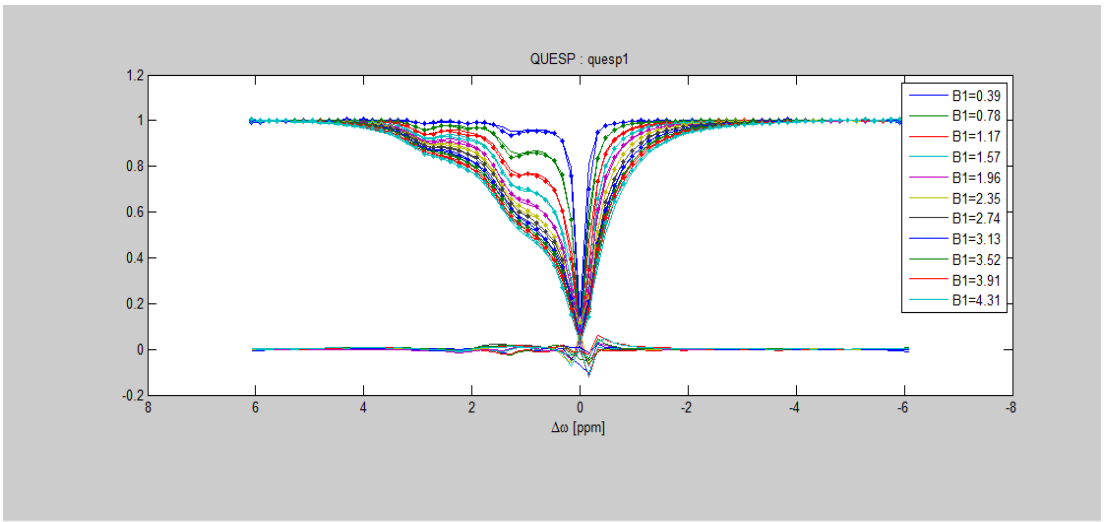
VIII. Examples of fitted Z-spectra for calculating the chemical exchange rates

With dots are the experimental data, with lines the fitting results and the line at 0ppm represents the difference between the fitted results and the experimental data. The measured exchange rates are listed under the fitted parameters column.

Free glucose pH=6.0

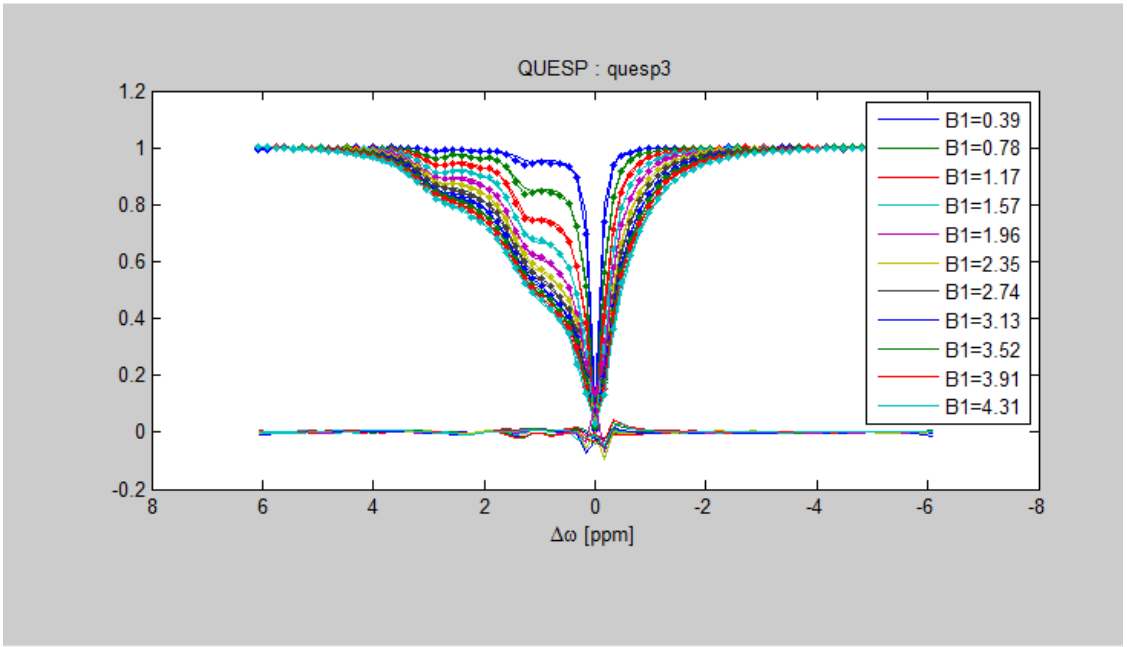


Free glucose pH=6.25



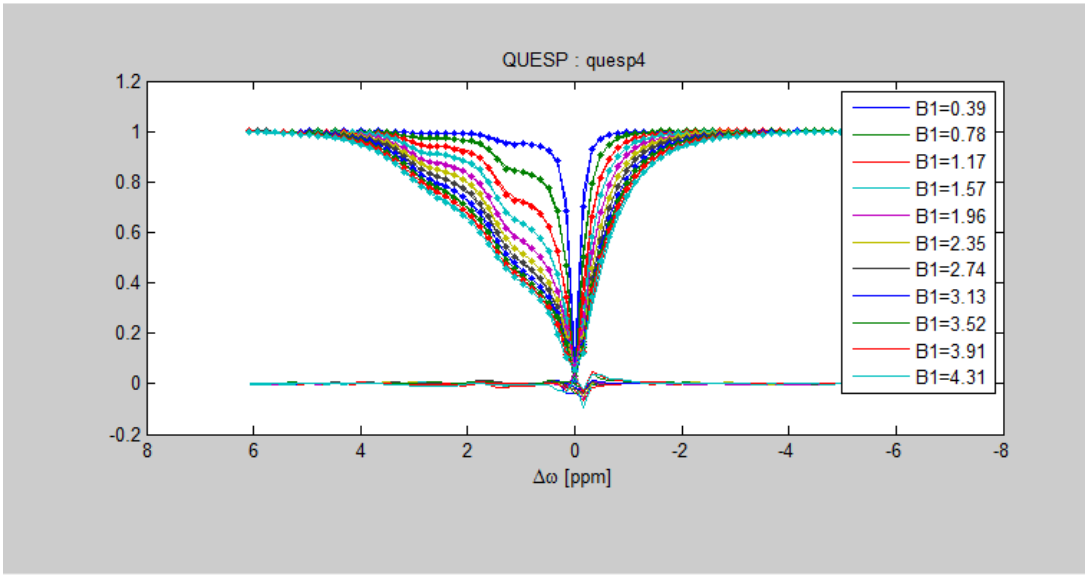
fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim =	P =	dwA=0.0000 (-0.500000 |0.500000)	dwA=-0.0134056±0.0018063 ppm
analytic: 1	B1: 10	R1A=0.3030 (0.125000 |0.500000)	R1A=0.4496517±0.0176913 Hz
MT: 0	DC: 0.9091	R2A=0.8333 (0.400000 |0.600000)	R2A=0.5606202±0.0378718 Hz
MT_lineshape: 'Gaussian'	FREQ: 400.2182	kBA=1000.0000 (50.000000 |10000.000000)	kBA=1353.2016312±426.4250409 Hz
n_cest_pool: 4	R1A: 0.3571	kDA=1500.0000 (50.000000 |10000.000000)	kDA=688.6827441±56.1959715 Hz
Rex_sol: 'Hyper'	TR: 0.0030	kEA=1000.0000 (50.000000 |10000.000000)	kEA=203.7681136±210.9283866 Hz
spoilf: 0	TTM_rep: 0	anomer=0.3700 (0.000000 |1.000000)	anomer=0.3043755±0.1598027
B1cwpe_quad: -1	Trec: 0	kFA=1000.0000 (50.000000 |6000.000000)	kFA=416.0549151±140.8548371 Hz
c: 1	Zi: 0		
tissue: 'PBS_eleni'	dummies: 1		
CESTagent: 'glucose_eleni2'	flipangle: 90		
fA: 1	linestomeasure: 1		
fB: 3.1818e-04	n: 151		
R1B: 1	normalized: -5		
R2B: 66.6600	pulsed: 1		
kBA: 1000	readout: 'FID'		
kAB: 0	readout_flipangle: 90		
dwB: 0.6600	shape: 'gauss_ucl'		
fC: 1.0000e-32	tp: 0.0500		
R1C: 1	tsat: 8.3049		
R2C: 1	vary: 'B1'		
kCA: 0	varyval: [0.3915 0.7829 1.1744 1.5658		

Free glucose pH=6.5



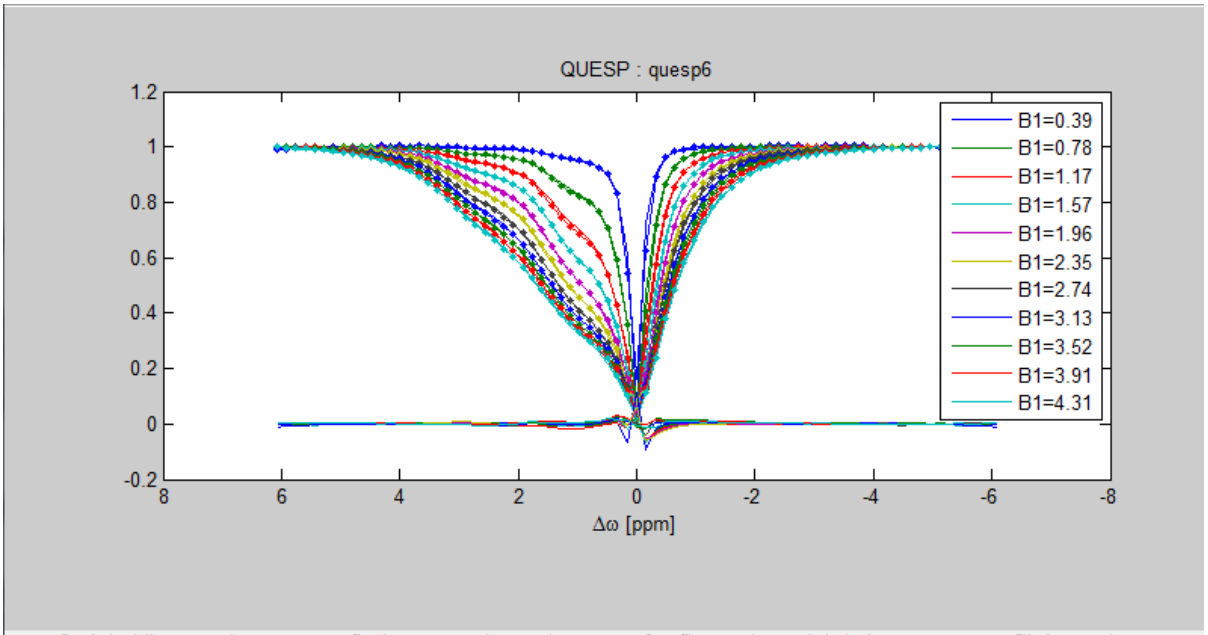
fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim = analytic: 1 MT: 0 MT_lineshape: 'Gaussian' n_cest_pool: 4 Rex_sol: 'Hyper' spoilf: 0 B1cwpe_quad: -1 c: 1 tissue: 'PBS_eleni' CESTagent: 'glucose_eleni2' fA: 1 fB: 3.1818e-04 R1B: 1 R2B: 66.6600 kBA: 1000 kAB: 0 dwB: 0.6600 fC: 1.0000e-32	P = B1: 10 DC: 0.9091 FREQ: 400.2182 R1A: 0.3571 TR: 0.0030 TTM_rep: 0 Trec: 0 Zi: 0 analytic: 1 dummies: 1 flipangle: 90 linestomeasure: 1 n: 151 n_cest_pool: 4 normalized: -5 pulsed: 1 readout: 'FID' readout_flipangle: 90	dwA=0.0000 (-0.500000 |0.500000) R1A=0.3030 (0.125000 |0.500000) R2A=0.8333 (0.400000 |0.600000) kBA=1000.0000 (50.000000 |10000.000000) kDA=1500.0000 (50.000000 |10000.000000) kEA=1000.0000 (50.000000 |10000.000000) anomer=0.3700 (0.000000 |1.000000) kFA=1000.0000 (50.000000 |8000.000000)	dwA=-0.0098718±0.0015887 ppm R1A=0.4125549±0.0121207 Hz R2A=0.4712507±0.0302154 Hz kBA=1421.8221432±369.2652309 Hz kDA=746.3759631±45.5353183 Hz kEA=318.8835059±169.9071136 Hz anomer=0.3415427±0.0699051 kFA=608.1884283±113.8781366 Hz

Free glucose pH=6.75



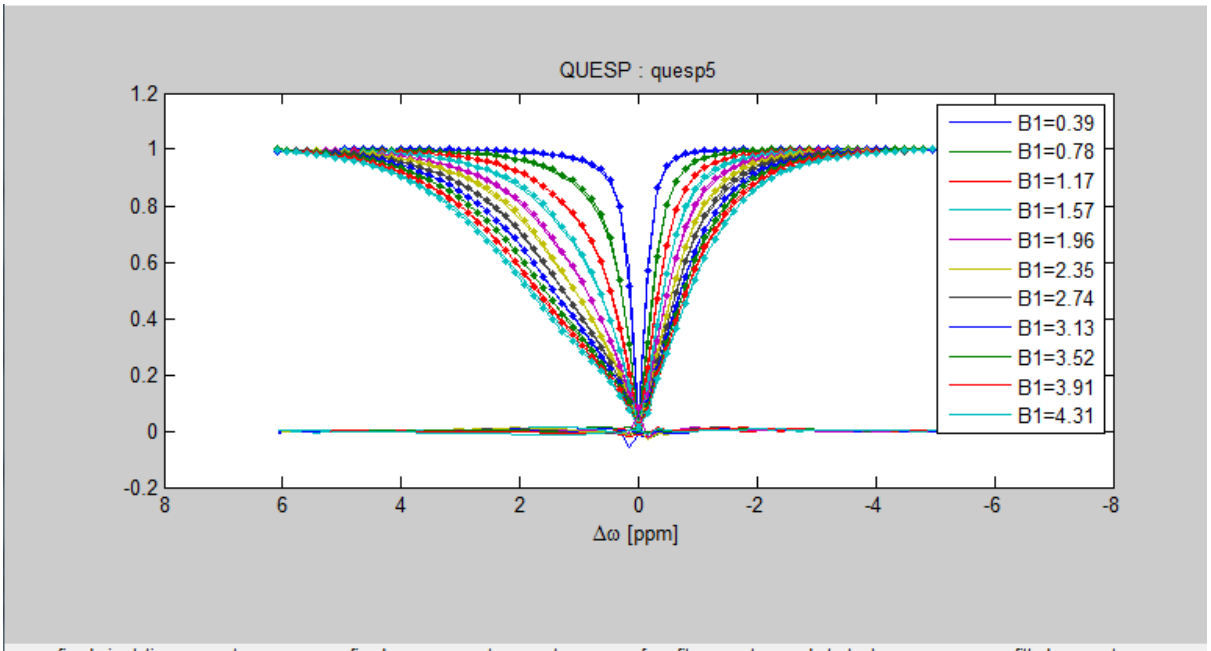
fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim =	P =	dwA=0.0000 (-0.500000 |0.500000)	dwA=-0.0104624±0.0014793 ppm
analytic: 1	B1: 10	R1A=0.3030 (0.125000 |0.500000)	R1A=0.4018908±0.0071596 Hz
MT: 0	DC: 0.9091	R2A=0.8333 (0.400000 |0.600000)	R2A=0.4000000±0.0254995 Hz
MT_lineshape: 'Gaussian'	FREQ: 400.2182	kBA=1000.0000 (50.000000 |10000.000000)	kBA=1310.5074673±313.5049552 Hz
n_cest_pool: 4	R1A: 0.3571	kDA=1500.0000 (50.000000 |10000.000000)	kDA=971.8550889±48.0016639 Hz
Rex_sol: 'Hyper'	TR: 0.0030	kEA=1000.0000 (50.000000 |10000.000000)	kEA=1732.9371863±274.6080823 Hz
spoilf: 0	TTM_rep: 0	anomer=0.3700 (0.000000 |1.000000)	anomer=0.4149372±0.0298988
B1cwpe_quad: -1	Trec: 0	kFA=1000.0000 (50.000000 |8000.000000)	kFA=885.3982200±87.8827168 Hz
c: 1	Zi: 0		
tissue: 'PBS_eleni'	analytic: 1		
CESTagent: 'glucose_eleni2'	dummies: 1		
fA: 1	flipangle: 90		
fB: 3.1818e-04	linestomeasure: 1		
R1B: 1	n: 151		
R2B: 66.6600	n_cest_pool: 4		
kBA: 1000	normalized: -5		
kAB: 0	pulsed: 1		
dwB: 0.6600	readout: 'FID'		

Free glucose pH=7.0



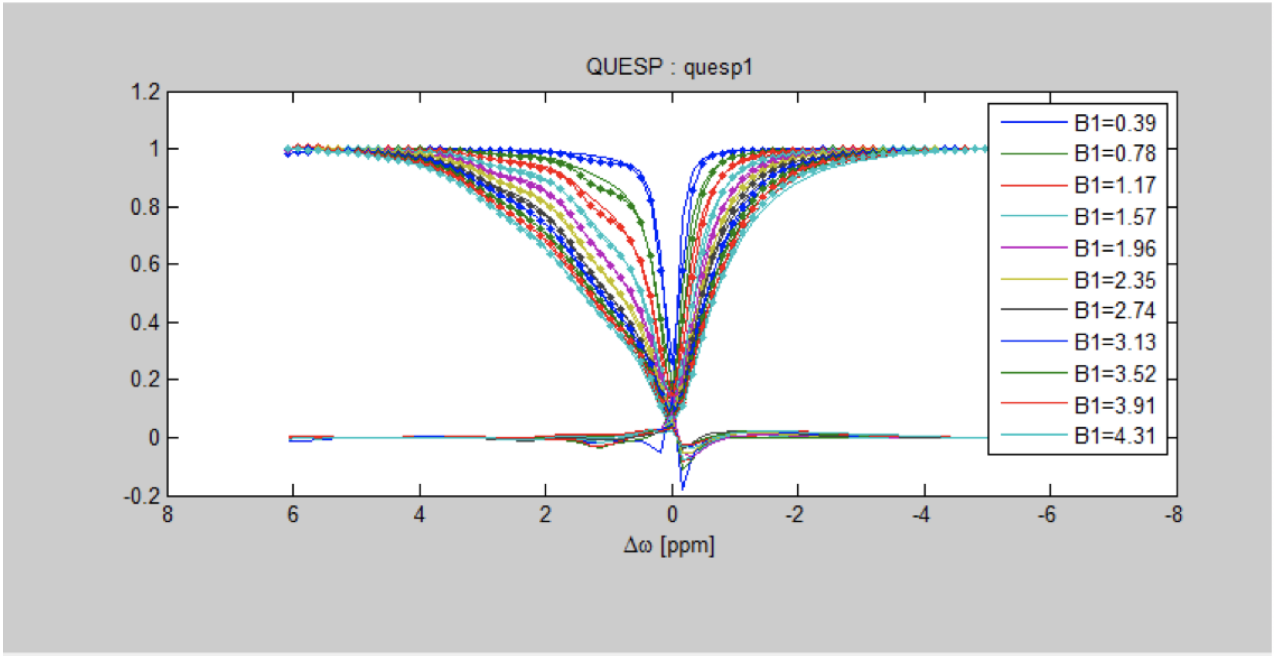
fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim =	P =	dwA=0.0000 (-0.500000 |0.500000)	dwA=0.0084178±0.0021423 ppm
analytic: 1	B1: 10	R1A=0.3030 (0.125000 |0.500000)	R1A=0.3556163±0.0068834 Hz
MT: 0	DC: 0.9091	R2A=0.8333 (0.400000 |0.600000)	R2A=0.4000000±0.0477654 Hz
MT_lineshape: 'Gaussian'	FREQ: 400.2182	kBA=1000.0000 (50.000000 |10000.000000)	kBA=1907.3595794±623.3579289 Hz
n_cest_pool: 4	R1A: 0.3571	kDA=1500.0000 (50.000000 |10000.000000)	kDA=1424.3247487±67.2285601 Hz
Rex_sol: 'Hyper'	TR: 0.0030	kEA=1000.0000 (50.000000 |10000.000000)	kEA=2492.6821986±295.8187134 Hz
spoilf: 0	TTM_rep: 0	anomer=0.3700 (0.000000 |1.000000)	anomer=0.5363394±0.0255307
B1cwpe_quad: -1	Trec: 0	kFA=1000.0000 (50.000000 |8000.000000)	kFA=1307.6957087±177.3181333 Hz
c: 1	Zi: 0		
tissue: 'PBS_eleni'	analytic: 1		
CESTagent: 'glucose_eleni2'	dummies: 1		
fA: 1	flipangle: 90		
fB: 3.1818e-04	linestomeasure: 1		
R1B: 1	n: 151		
R2B: 66.6600	n_cest_pool: 4		
kBA: 1000	normalized: -5		
kAB: 0	pulsed: 1		
dwB: 0.6600	readout: 'FID'		
fC: 1.0000e-32	readout_flipangle: 90		

Free glucose pH=7.4



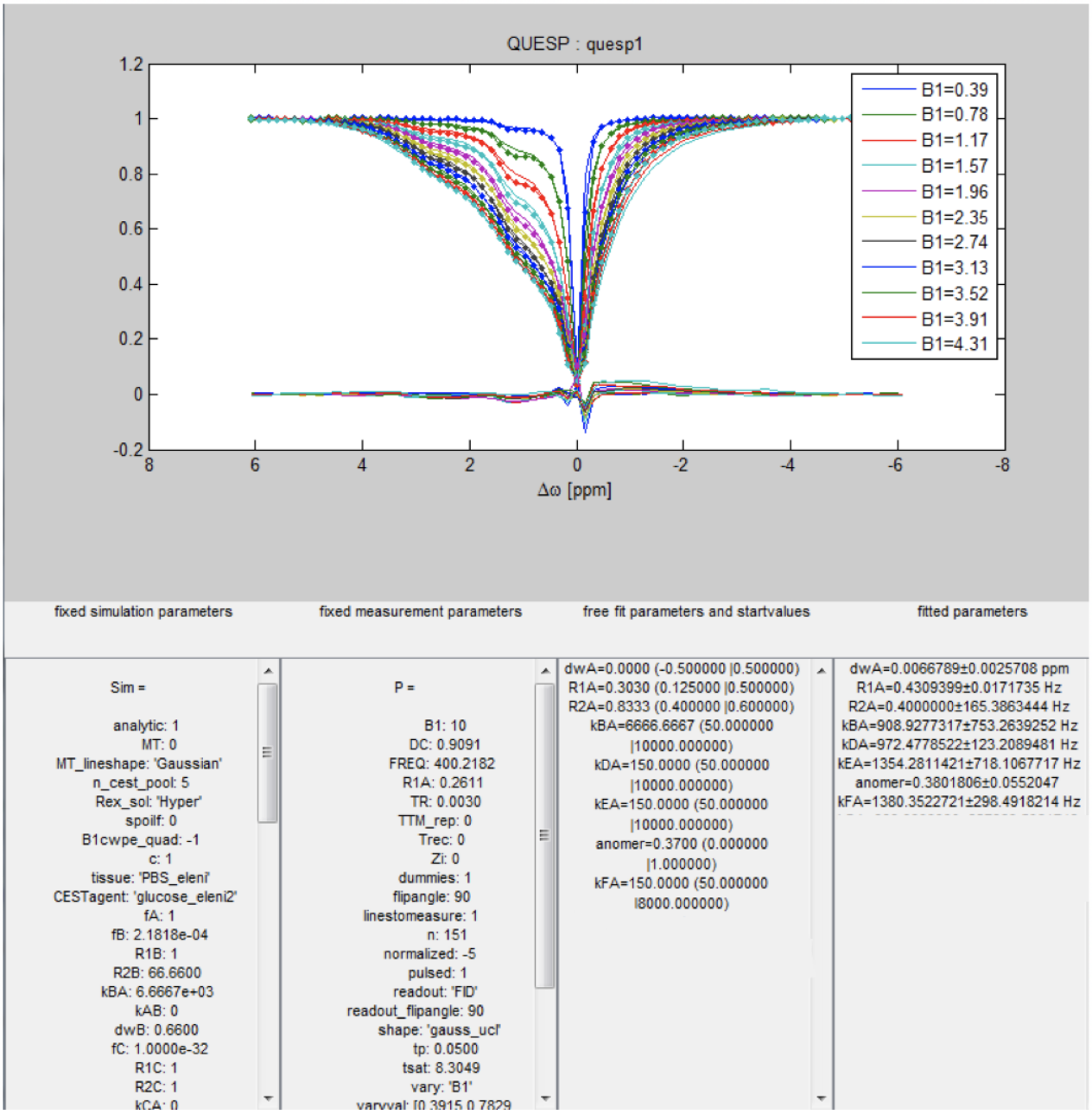
fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim = analytic: 1 MT: 0 MT_lineshape: 'Gaussian' n_cest_pool: 4 Rex_sol: 'Hyper' spoilf: 0 B1cwpe_quad: -1 c: 1 tissue: 'PBS_eleni' CESTagent: 'glucose_eleni2' fA: 1 fB: 3.1818e-04 R1B: 1 R2B: 66.6600 kBA: 1000 kAB: 0 dwB: 0.6600 fC: 1.0000e-32	P = B1: 10 DC: 0.9091 FREQ: 400.2182 R1A: 0.3571 TR: 0.0030 TTM_rep: 0 Trec: 0 Zi: 0 analytic: 1 dummies: 1 flipangle: 90 linestomeasure: 1 n: 151 n_cest_pool: 4 normalized: -5 pulsed: 1 readout: 'FID' readout_flipangle: 90	dwA=0.0000 (-0.500000 |0.500000) R1A=0.3030 (0.125000 |0.500000) R2A=0.8333 (0.400000 |0.600000) kBA=1000.0000 (50.000000 |10000.000000) kDA=1500.0000 (50.000000 |10000.000000) kEA=1000.0000 (50.000000 |10000.000000) anomer=0.3700 (0.000000 |1.000000) kFA=1000.0000 (50.000000 |8000.000000)	dwA=-0.0089444±0.0015990 ppm R1A=0.3345828±0.0065056 Hz R2A=0.4000000±0.0433420 Hz kBA=1492.9182184±361.5354426 Hz kDA=2674.5899133±105.0335076 Hz kEA=3868.5163261±428.7809119 Hz anomer=0.6390289±0.0548189 kFA=3692.0962686±534.4546512 Hz

Gluco-liposomes 37 ºC

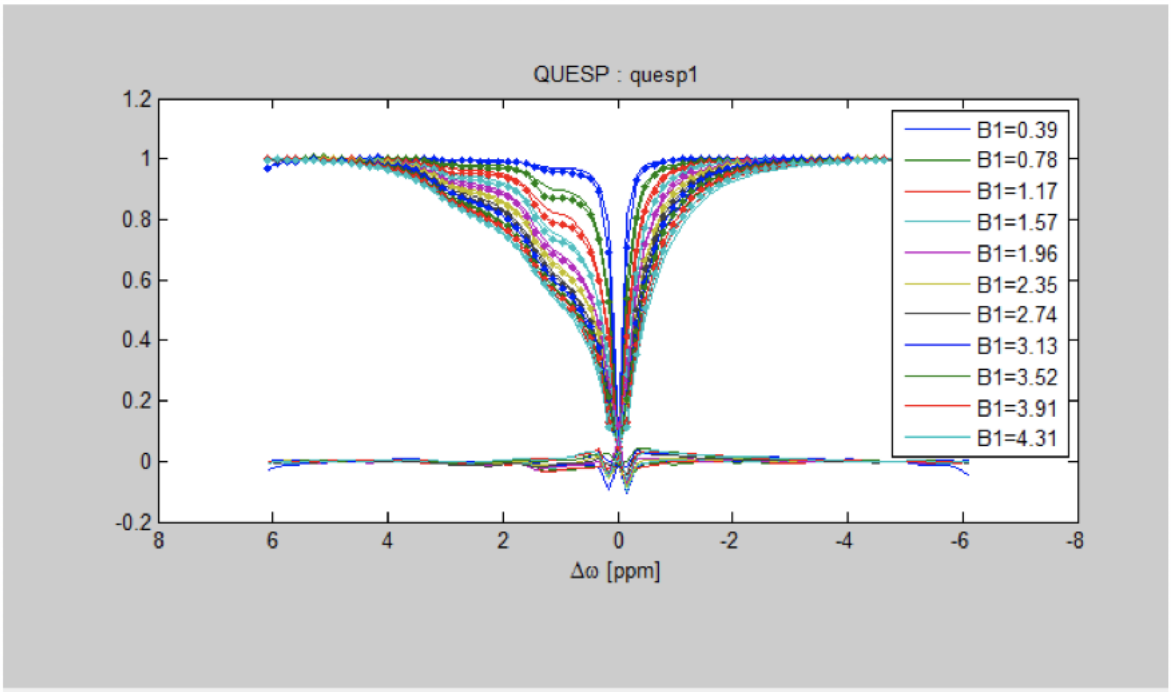


fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim = analytic: 1 MT: 0 MT_lineshape: 'Gaussian' n_cest_pool: 5 Rex_sol: 'Hyper' spoilf: 0 B1cwpe_quad: -1 c: 1 tissue: 'PBS_eleni' CESTagent: 'glucose_eleni2' fA: 1 fB: 2.1818e-04 R1B: 1 R2B: 66.6600 kBA: 6.6667e+03 kAB: 0 dwB: 0.6600	P = B1: 10 DC: 0.9091 FREQ: 400.2182 R1A: 0.2415 TR: 0.0030 TTM_rep: 0 Trec: 0 Zi: 0 dummies: 1 flipangle: 90 linestomeasure: 1 n: 151 normalized: -5 pulsed: 1 readout: 'FID' readout_flipangle: 90 shape: 'gauss_ucl'	dwA=0.0000 (-0.500000 |0.500000) R1A=0.3030 (0.125000 |0.500000) R2A=0.8333 (0.400000 |0.600000) kBA=6666.6667 (50.000000 |10000.000000) kDA=150.0000 (50.000000 |10000.000000) kEA=150.0000 (50.000000 |10000.000000) anomer=0.3700 (0.000000 |1.000000) kFA=150.0000 (50.000000 |8000.000000)	dwA=0.0184340±0.0033863 ppm R1A=0.3912638±0.0274214 Hz R2A=0.4000000±227.5773459 Hz kBA=2424.9412625±2294.7315343 Hz kDA=1543.9948632±209.4382851 Hz kEA=2875.0922253±1090.4834761 Hz anomer=0.5280080±0.0811726 kFA=1429.9537344±501.5914102 Hz

Gluco-liposomes 34 ºC

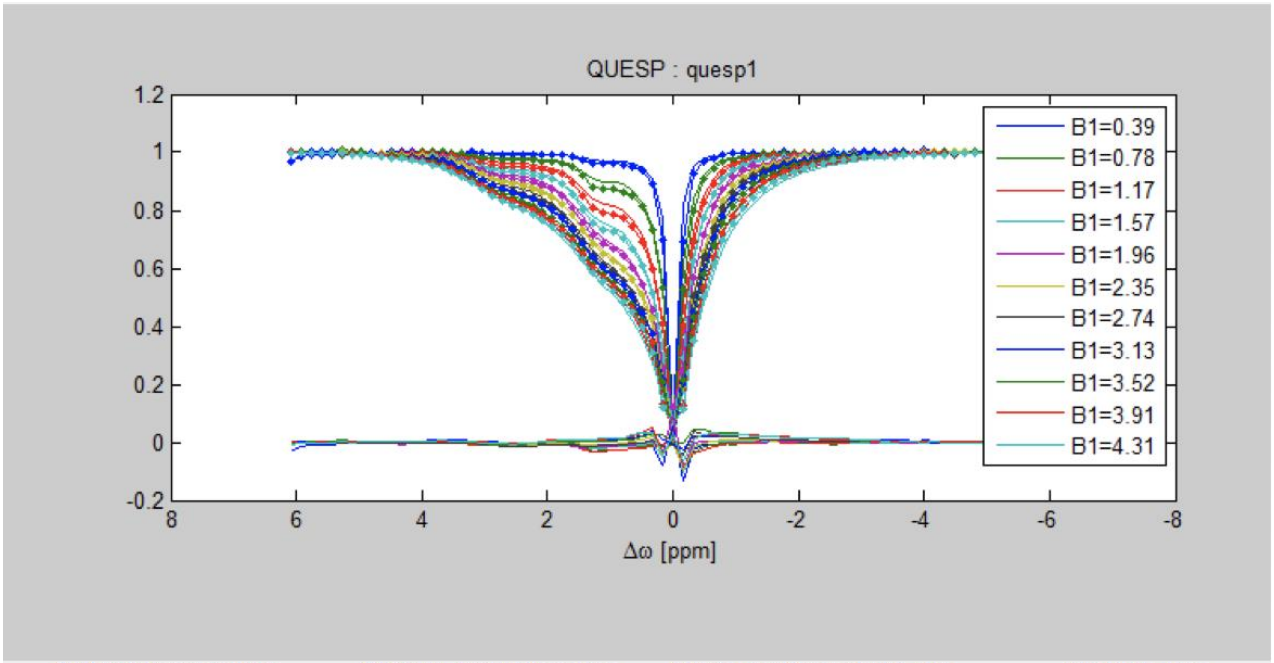


Gluco-liposomes 31 ºC



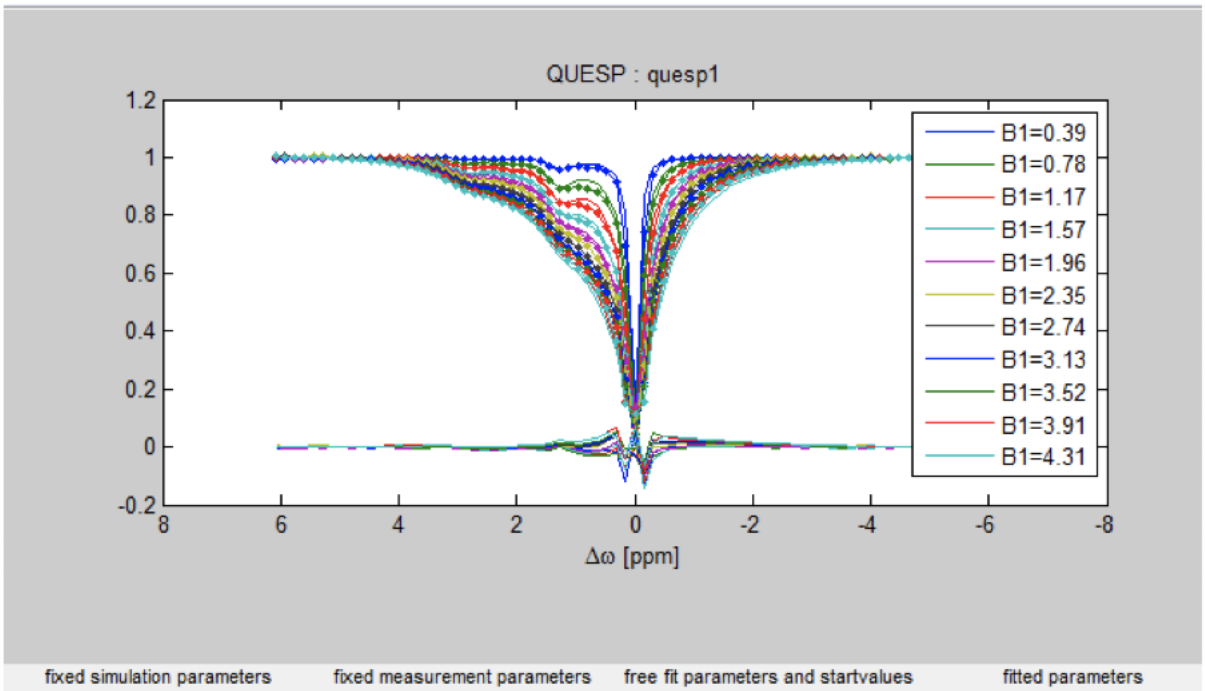
fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim = analytic: 1 MT: 0 MT_lineshape: 'Gaussian' n_cest_pool: 5 Rex_sol: 'Hyper' spoilf: 0 B1cwpe_quad: -1 c: 1 tissue: 'PBS_eleni' CESTagent: 'glucose_eleni2' fA: 1 fB: 2.1818e-04 R1B: 1 R2B: 66.6600 kBA: 6.6667e+03 kAB: 0 dwB: 0.6600 fC: 1.0000e-32	P = B1: 10 DC: 0.9091 FREQ: 400.2182 R1A: 0.2778 TR: 0.0030 TTM_rep: 0 Trec: 0 Zi: 0 dummies: 1 flipangle: 90 linestomeasure: 1 n: 151 normalized: -5 pulsed: 1 readout: 'FID' readout_flipangle: 90 shape: 'gauss_ucl' tp: 0.0500	dwA=0.0000 (-0.500000 |0.500000) R1A=0.3030 (0.125000 |0.500000) R2A=0.8333 (0.400000 |0.600000) kBA=6666.6667 (50.000000 |10000.000000) kDA=150.0000 (50.000000 |10000.000000) kEA=150.0000 (50.000000 |10000.000000) anomer=0.3700 (0.000000 |1.000000) kFA=150.0000 (50.000000 |8000.000000)	dwA=-0.0058950±0.0023923 ppm R1A=0.4998987±0.0260365 Hz R2A=0.4000000±172.9342383 Hz kBA=876.7992587±768.7940922 Hz kDA=871.0871557±115.9339230 Hz kEA=1137.6766614±852.847229 Hz anomer=0.3596106±0.0646491 kFA=1076.5179891±229.407267 Hz

Gluco-liposomes 28 ºC



fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim = analytic: 1 MT: 0 MT_lineshape: 'Gaussian' n_cest_pool: 5 Rex_sol: 'Hyper' spoilf: 0 B1cwpe_quad: -1 c: 1 tissue: 'PBS_eleni' CESTagent: 'glucose_eleni2' fA: 1 fB: 2.1818e-04 R1B: 1 R2B: 66.6600 kBA: 6.6667e+03 kAB: 0 dwB: 0.6600	P = B1: 10 DC: 0.9091 FREQ: 400.2182 R1A: 0.2778 TR: 0.0030 TTM_rep: 0 Trec: 0 Zi: 0 dummies: 1 flipangle: 90 linestomeasure: 1 n: 151 normalized: -5 pulsed: 1 readout: 'FID' readout_flipangle: 90 shape: 'gauss_ucl'	dwA=0.0000 (-0.500000 |0.500000) R1A=0.3030 (0.125000 |0.500000) R2A=0.8333 (0.400000 |0.600000) kBA=6666.6667 (50.000000 |10000.000000) kDA=150.0000 (50.000000 |10000.000000) kEA=150.0000 (50.000000 |10000.000000) anomer=0.3700 (0.000000 |1.000000) kFA=150.0000 (50.000000 |8000.000000)	dwA=0.0002710±0.0026802 ppm R1A=0.4936990±0.0279388 Hz R2A=0.4000000±162.5841320 Hz kBA=974.5774934±813.1046239 Hz kDA=845.7668857±123.8968860 Hz kEA=1073.1477289±893.3077527 Hz anomer=0.3561892±0.0679981 kFA=1027.4898984±226.7044148 Hz

Gluco-liposomes 25 ºC



fixed simulation parameters	fixed measurement parameters	free fit parameters and startvalues	fitted parameters
Sim = analytic: 1 MT: 0 MT_lineshape: 'Gaussian' n_cest_pool: 5 Rex_sol: 'Hyper' spoilf: 0 B1cwpe_quad: -1 c: 1 tissue: 'PBS_eleni' CESTagent: 'glucose_eleni2' fA: 1 fB: 2.1818e-04 R1B: 1 R2B: 66.6600 kBA: 6.6667e+03	P = B1: 10 DC: 0.9091 FREQ: 400.2182 R1A: 0.3155 TR: 0.0030 TTM_rep: 0 Trec: 0 Zi: 0 dummies: 1 flipangle: 90 linestomeasure: 1 n: 151 normalized: -5 pulsed: 1 readout: 'FID' readout_flipangle: 90	dwA=0.0000 (-0.500000 |0.500000) R1A=0.3030 (0.125000 |0.500000) R2A=0.8333 (0.400000 |0.600000) kBA=6666.6667 (50.000000 |10000.000000) kDA=150.0000 (50.000000 |10000.000000) kEA=150.0000 (50.000000 |10000.000000) anomer=0.3700 (0.000000 |1.000000) kFA=150.0000 (50.000000 |8000.000000)	dwA=0.0083904±0.0022953 ppm R1A=0.5000000±0.0779702 Hz R2A=0.4000000±176.0221469 Hz kBA=1244.8088920±768.37783 Hz kDA=378.8407068±98.4881413 Hz kEA=461.4577216±604.3881866 Hz anomer=0.3608864±0.1774159 kFA=585.1397016±304.0041443 Hz

Fitted and experimental data for calculating the chemical exchange rate of 0.25 M of glucose at physiological conditions.