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):
\[
\frac{dM_{xx}}{dt} = -2\pi\Delta M_{ys} + \left( -\frac{1}{T_{2s}} - R_{cest}M_0^w \right)M_{xs} + R_{cest}M_0^s M_{xw} \quad (S1)
\]
\[
\frac{dM_{ys}}{dt} = 2\pi\Delta M_{xs} + \omega_1 M_{zs} + \left( -\frac{1}{T_{2s}} - R_{cest}M_0^w \right)M_{ys} + R_{cest}M_0^s M_{yw} \quad (S2)
\]
\[
\frac{dM_{zs}}{dt} = -\omega_1 M_{ys} + (-R_{1s} - R_{cest}M_0^w)M_{zs} + R_{1s}M_0^s + R_{cest}M_0^s M_{zw} \quad (S3)
\]
\[
\frac{dM_{zw}}{dt} = -2\pi\Delta M_{yw} + \left( -\frac{1}{T_{2w}} - R_{cest}M_0^s \right)M_{zw} + R_{cest}M_0^w M_{xs} \quad (S4)
\]
\[
\frac{dM_{yw}}{dt} = 2\pi\Delta M_{xw} + \omega_1 M_{zw} + \left( -\frac{1}{T_{2w}} - R_{cest}M_0^s \right)M_{yw} + R_{cest}M_0^w M_{ys} \quad (S5)
\]
\[
\frac{dM_{xw}}{dt} = -\omega_1 M_{yw} + (-R_{1w} - R_{cest}M_0^w)M_{xw} + R_{1w}M_0^w + R_{cest}M_0^w M_{zs} \quad (S6)
\]

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: \([\frac{dM_{xx}}{dt}, \frac{dM_{ys}}{dt}, \frac{dM_{zs}}{dt}, \frac{dM_{zw}}{dt}, \frac{dM_{yw}}{dt}, \frac{dM_{xw}}{dt}] = [0,0,0,0,0,0] \). \( \omega_1 = \gamma B_1 \) (\( \gamma \) is the gyromagnetic ratio and \( B_1 \) is the applied RF field on the x-axis), \( \Delta \) 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 S1-6 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: \( M_{zs} = \frac{R_{cest}M_0^s M_{xw} - \omega_1 M_{ys} + R_{1s}M_0^s}{R_{1s} + R_{cest}M_0^w} \) \( (S9) \)

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: \( M_{zs} = \frac{\omega_1 M_{yw} + (R_{1w} + R_{cest}M_0^w)M_{z} - R_{1w}M_0^w}{R_{cest}M_0^w} \) \( (S12) \)
Combing equations (S7) and (S8) we have:

\[ M_{xs} = \frac{R_{\text{cest}} M_0^S T_{2s} M_{zw} - 2 \pi \Delta (T_{2s})^2 \omega_1 M_{zw}}{1 + (2 \pi \Delta T_{2s})^2 + R_{\text{cest}} M_0^S T_{2s}} \]  

(S13)

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

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

(S14):

Finally using (S12) and (S14):

\[ M_{zs} = \frac{\alpha M_{zw} + b R_{1s} M_0^S}{c} \]  

(S15)

where

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

\[
b = \frac{-\left( \frac{R_{\text{cest}} M_0^w}{\omega_1^2 T_{2w} (2 \pi \Delta)^2} \left( \frac{1}{T_{2s}} + R_{\text{olest}} M_0^w \right) - \frac{1}{R_{\text{cest}} M_0^w \omega_1^2 (2 \pi \Delta T_{2w})^2} \right) \{1 + (2 \pi \Delta T_{2w})^2 + R_{\text{cest}} M_0^S \} \left[ \left( \frac{1}{T_{2s}} + R_{\text{cest}} M_0^w \right)^2 + (2 \pi \Delta)^2 \right] \}

\]

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

Equation (S15) can then be written as follows:

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

\[ M_{zs} = \frac{1}{R_{1s} + R_{\text{cest}} M_0^w} (R_{\text{cest}} M_0^S M_{zw} + R_{1s} M_0^S) \]

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

\[ S3 \]
\[ \frac{M_{22}^{DF}(\Delta \omega)}{M_0} = \frac{R_{1A}}{R_{1A}(\Delta \omega)_{DC} + R_{1A}(1-DC)} \quad (S16) \]

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:

\[
\overline{R}_{1,\rho} = \frac{1}{t_p} \int_0^{t_p} R_{1,\rho}(t) dt = R_{1w} + \frac{1}{t_p} \int_0^{t_p} (R_{2w} - R_{1w}) \frac{\omega_2^2(t)}{\Delta^2 + \omega_1^2(t)} dt + \frac{1}{t_p} \int_0^{t_p} f_B k_{BA} \frac{\omega_2^2(t)}{k_{BA}(k_{BA} + R_{2B})^2 + \omega_1^2(t)} \frac{\omega_s^2}{\Delta^2 + \omega_1^2(t)} dt \quad (S17)
\]

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.

\[
\frac{1}{t_p} \int_0^{t_p} f_c k_{CA} \frac{\omega_1^2(t)}{k_{CA}(k_{CA} + R_{2B})^2 + \omega_1^2(t)} \frac{\omega_2^2}{\Delta^2 + \omega_1^2(t)} dt \quad \text{to Equation (S17). Finally, (S17) is substituted to (S16) for calculating the exchange rates of hydroxyl groups in glucose or 2-DG.}
\]

**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 (Å) | 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 ($\sigma$) values. When the particle size distribution can be fitted to a Gaussian distribution, the relationship between PdI and $\sigma$ and the average hydrodynamic radius ($r$) can be described by the following equation:

$$PdI = \frac{\sigma^2}{r^2}$$

**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 H₂SO₄ (1.0 mL) was added to terminate the reaction. The A₅₄₀ 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.** ¹H 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±55 | 1146±400 | 1179±429 | 1693±645 | 1567±517 | 1836±827 |
| 1.28 ppm   | 1341±26 | 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 | 2-DG 25 °C | pH=6.0 | pH=7.0 |
|---------------|--------|--------|------------|--------|--------|
| 0.66 ppm      | 820±105| 786±107| 0.66 ppm   | 677±152| 477±165|
| 1.28 ppm      | 392±11 | 1257±23| 1.28 ppm   | 438±24 | 1128±70|
| 2.08 ppm      | 242±46 | 2465±86| 2.08 ppm   | 113±27 | 2304±109|
| 2.88 ppm      | 297±26 | 2190±44| 2.88 ppm   | 246±70 | 886±76 |

Table S7.

| Glucose 37 °C | pH=6.0 | pH=7.0 | 2-DG 37 °C | pH=6.0 | pH=7.0 |
|---------------|--------|--------|------------|--------|--------|
| 0.66 ppm      | 926±114| 50±11  | 0.66 ppm   | 610±144| 896±148|
| 1.28 ppm      | 823±18 | 3554±75| 1.28 ppm   | 994±50 | 2910±108|
| 2.08 ppm      | 905±66 | 5551±546| 2.08 ppm   | 371±42 | 4760±206|
| 2.88 ppm      | 831±19 | 7165±372| 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**

| Concentration (ppm) | 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**

| Concentration (ppm) | 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**

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

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### Table S15.

| 2DG-liposomes | 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       | 37 °C   | 34 °C   | 31 °C   | 28 °C   | 25 °C   |
|------------|---------|---------|---------|---------|---------|
| liposomes  |         |         |         |         |         |
| 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 °C and agitated at 350 rpm using a BIOER mixing block.

Release at 2 h:
- Glc from 3% PEG: 1.4% $\rightarrow$ exterior Glc conc of 0.84 mM
- 2-DG from 3% PEG: 10% $\rightarrow$ exterior 2-DG conc of 3.8 mM
- 2-DG from DPPC: 15% $\rightarrow$ exterior 2-DG conc of 4.5 mM
VII. Simulations of the liposomal system

Figure S3. Simulated Z-spectra obtained from a six-site chemical exchange with $R_{\text{cest}} = 0$, $B_1 = 1.5 \, \mu T$
Figure S4. Simulated Z-spectra obtained from a six-site chemical exchange with $R_{\text{cest}} = 10$, $B_1 = 1.5 \, \mu\text{T}$. 
Figure S5. Simulated Z-spectra obtained from a six-site chemical exchange with $R_{\text{cest}} = 10$, $B_1 = 5.06 \mu T$. 

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**Figure S6.** Simulated Z-spectra obtained from a six-site chemical exchange for various \( R_{\text{cest}} \) at \( B_1 = 1.5 \mu T \) and 5.06 \( \mu T \). 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
Free glucose pH=6.5
Free glucose pH = 6.75
Free glucose pH=7.0
Free glucose pH=7.4
Gluco-liposomes 37 °C
Gluco-liposomes 34 °C
Gluco-liposomes 31 °C
Gluco-liposomes 28 °C
Gluco-liposomes 25 °C
Fitted and experimental data for calculating the chemical exchange rate of 0.25 M of glucose at physiological conditions.