Electronic Supplementary Material (ESI) for Analyst.

The use of a personal glucose meter for detecting procalcitonin through glucose encapsulated within liposomes

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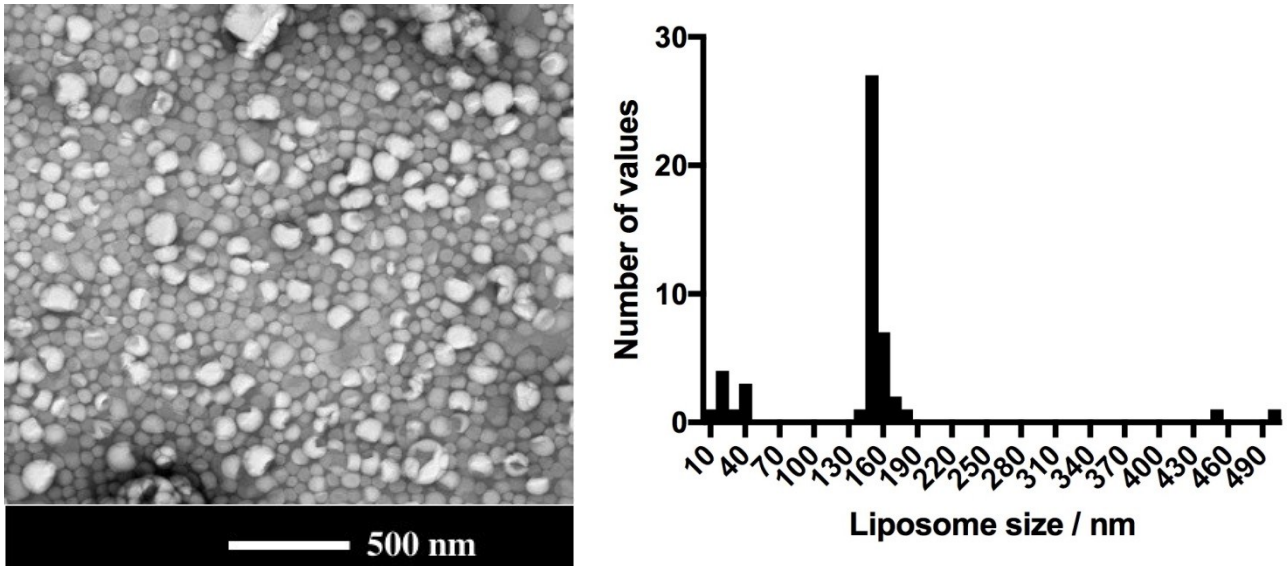
TEM images of the liposomes



A **B**

Figure S1. (**A**) TEM image of the liposomes with encapsulated glucose. (**B**) Histogram of the sizes built from 50 particles on the TEM image. The particle size was measured using the Threshold function on ImageJ. The average size is 144 ± 85 nm.

Lipsomes were incubated on glow discharged (EasiGlow; Ted Pella Inc, USA) carbon coated grids (Ted Pella Inc, USA), washed with double distilled water and negatively stained with 1% uranyl acetate (w/v). Grid were imaged unbinned on a Tecnai G2 20 transmission electron microscope (FEI) fitted with a BM Eagle 2k x2k camera under the control of Tecnai Imaging and Analysis software.

Nanotracking analysis

The liposome concentration was measured using a NanoSight NS300 instrument (Malvern), using the Nanoparticle Tracking Analysis technique. The concentration of a typical sample of 200 nm liposomes after dialysis was found to be about $2.75 \cdot 10^{14}$ particle per mL.

Liposome stability study

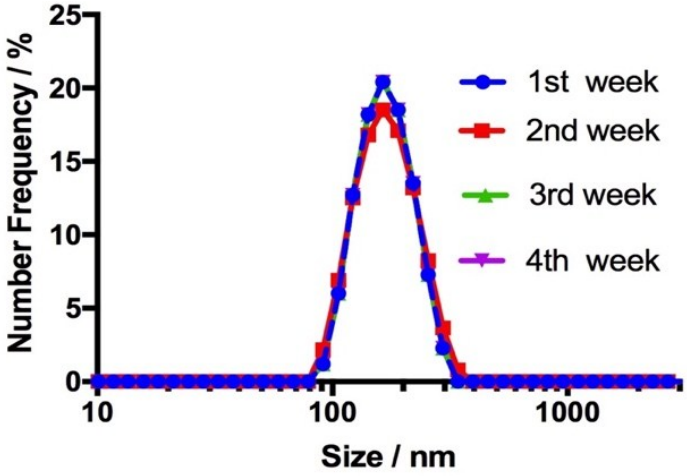


Figure S2. DLS traces of a sample of liposomes stored for one month at 4 °C. The size of the liposomes and the concentration of glucose leaked in solution (measured using a PGM) were monitored once a week for four weeks. Across this period of time the size of the liposomes did not change and glucose was never detected from the solution. This proved that the liposomes prepared in this study are stable and can be stored for at least one month without any glucose leakage. This result is in agreement with the literature, which reports that glucose cannot permeate the liposome membrane at temperatures below the gel-liquid crystal transition temperature (Tm) of the lipid, not even in presence of cholesterol.[1] The main components of the liposomes prepared in this study is HSPC, with a Tm of about 55 °C, thus well above the temperature used during this stability study.

Electrochemical determination of glucose

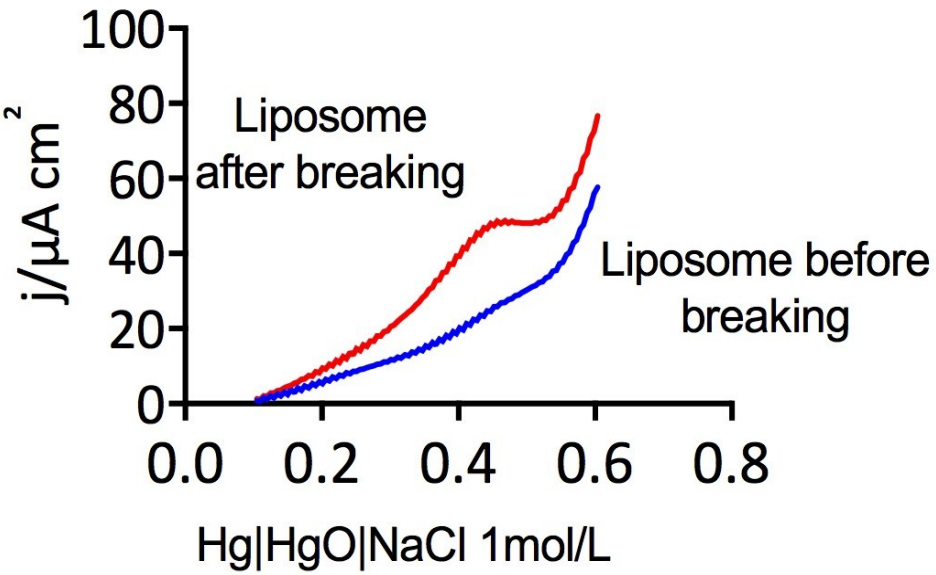


Figure S3. An electrochemical assay was used to further confirm the release of glucose. Plot of current (j/µA cm^2) vs potential (V). The rupture of the liposomes and release of glucose was confirmed by the oxidation of the glucose on Au NPs (5 nm nanoparticles, 5 µL of a solution $5.5 \cdot 10^{13}$ particles/mL) on glassy carbon surface with 0.07 cm^2 as a working electrode, and Hg|HgO|NaCl 1mol/L as reference electrode and Pt mesh as counter-electrode. Two samples of liposomes with encapsulated glucose were compared, after dialysis against PBS. The first sample is the liposomes with encapsulated glucose before breaking and the second is the same liposomes after adding Triton X-100. A current attributed to the oxidation of glucose appears only after breaking the liposomes (red line), and no glucose signal is present before adding the Triton X-100 (blue line), confirming that the dialysis process is good enough to remove all the un-encapsulated glucose and also confirming the glucose releasing process.

ELISA controls

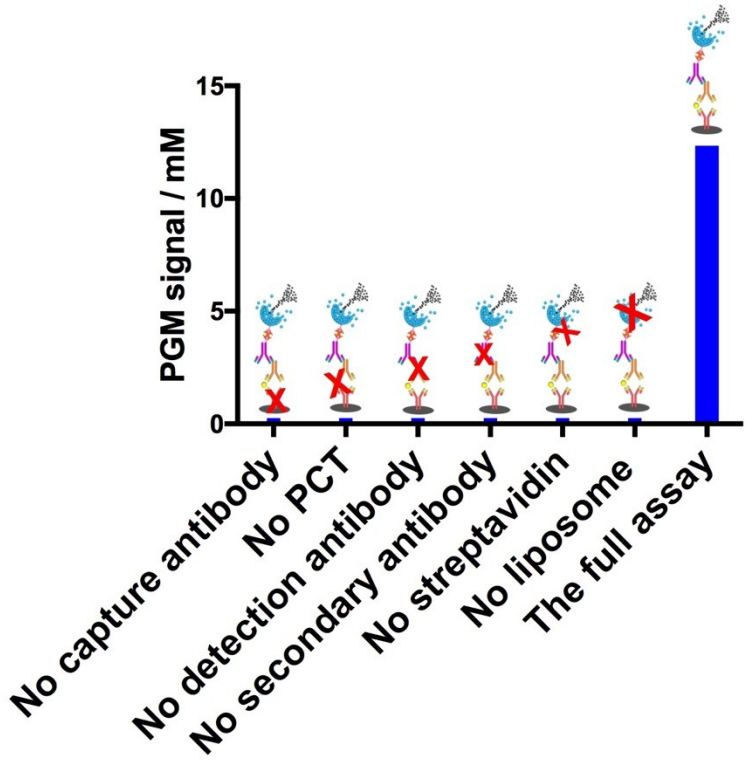


Figure S4. PGM response of the liposomes-encapsulated glucose assay for PCT, in absence of specific components. The assay does not generate any glucose signal if one component is not present. From left to right: no capture antibody, no analyte (PCT), no detection antibody, no secondary antibody, no streptavidin, and no liposomes with encapsulated glucose. The signal was only produced in presence of all components (full assay, last column). These results indicated that the generation of glucose signal results from the specific antigen-antibody interaction.

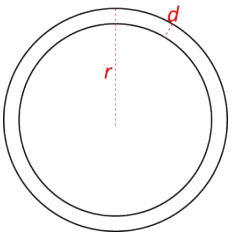
Theoretical liposome concentration and mol of glucose released

The concentration and inner volume of liposomes can be calculated from the amount of lipid used and the physical constants of such lipids. The calculations here reported are based on the article by C. Huang.[2]

The mole percents of the components of the liposomes prepared in this study are: HSPC = 70%, cholesterol = 29%, DSPE-PEG200-biotin = 1%. HSPC is a mixture of lipids, but mostly the 18:0 lipid (https://avantilipids.com/product/840058), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). DSPE is also a 18:0 lipid, but present in much lower amount compared to HSPC. Therefore, it was assumed the thickness of the liposome membrane to be the same of pure DSPC at 25 °C, about 5.03 nm.[3] [4] The presence of cholesterol further increases the bilayer thickness of about 4%, [5] thus giving an estimated thickness of 5.23 nm.

Despite DSPC and DSPE have different head groups, DSPC is more abundant and therefore its 'footprint' (i.e. the area occupied by one lipid molecule on the membrane) of 0.55 nm at 25 °C was used for the following calculations. [3]

Other three assumptions used for these calculations are that 1) there are no losses of lipids during synthesis and purification, 2) all liposomes have the same radius *r*, and 3) the dispersion has unilamellar vesicles only.



The inner volume V_i (in nm^3) of one liposome, where the glucose solution is encapsulated, is:

$$V_i = \frac{4}{3}\pi(r-d)^3 = \frac{4}{3}\pi(r-5.23)^3$$

The inner (S_i) and outer (S_o) surface (in nm^2) of one liposome are:

$$S_i = 4\pi(r-d)^2 = 4\pi(r-5.23)^2$$

$$S_o = 4\pi r^2$$

The number of lipids in one liposome (*n*) can be calculated by dividing the total surface area by the lipid footprint:

$$n = \frac{S_i + S_o}{footprint} = \frac{S_i + S_o}{0.55}$$

The number of liposomes of radius *r* (N_r) that can be prepared from *x* mol of lipids can be calculated by dividing the total number of lipid molecules by the number of lipids in one liposome. All the liposomes in this study were prepared using 0.158 mmol of HSPC and 0.002 mmol of DSPE-PEG2000-biotin, for a total of 0.160 mmol of lipids. Therefore, the total number of liposomes is:

$$N_r = \frac{x \cdot N_A}{\frac{S_i + S_o}{footprint}} = \frac{0.55 \cdot 1.6 \cdot 10^{-4} \cdot 6.022 \cdot 10^{23}}{S_i + S_o}$$

Where $N_A = 6.022 \cdot 10^{23}$ is Avogadro's number. The concentration of liposomes in solution will be the number of liposomes N_r divided by the volume where such liposomes are dispersed, N_r/V_s.

Regarding the encapsulation of glucose, it was reported by Nicholas *et al.* that the presence of PEGylated lipids decreases the encapsulation efficiency of glucose, possibly because the PEG chains restrict the free volume available inside the liposomes for carrying glucose. [6] Nicholas *et al.* showed that liposomes prepared with 1% mol of DSPE-PEG2000 (same composition than in this study) can only encapsulate 65% of glucose compared to liposomes prepared without DSPE-PEG2000. Therefore, the volume of glucose solution released by an aliquot *V'* of liposome of radius *r* is:

$$volume\ of\ glucose\ released = V_i \cdot 0.65 \cdot \frac{N_r}{V_s} \cdot V'$$

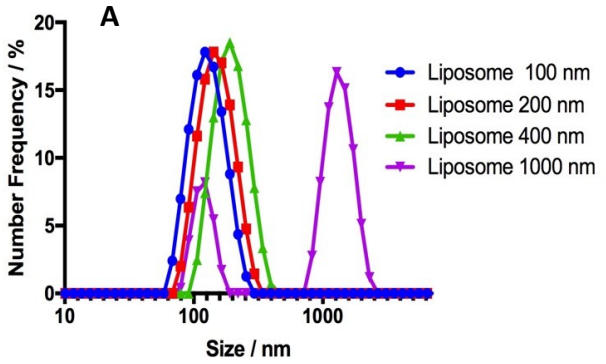
To calculate the mol of glucose released, it was assumed that the concentration of glucose inside the liposomes was the same of the solution used to rehydrate the lipid film, C_g.

$$mol\ of\ glucose\ released = C_g \cdot V_i \cdot \frac{N_r}{V_s} \cdot V'$$

The formula above were used to calculate liposome concentration and mol of glucose released for the "liposome size" and "glucose concentration" studies.

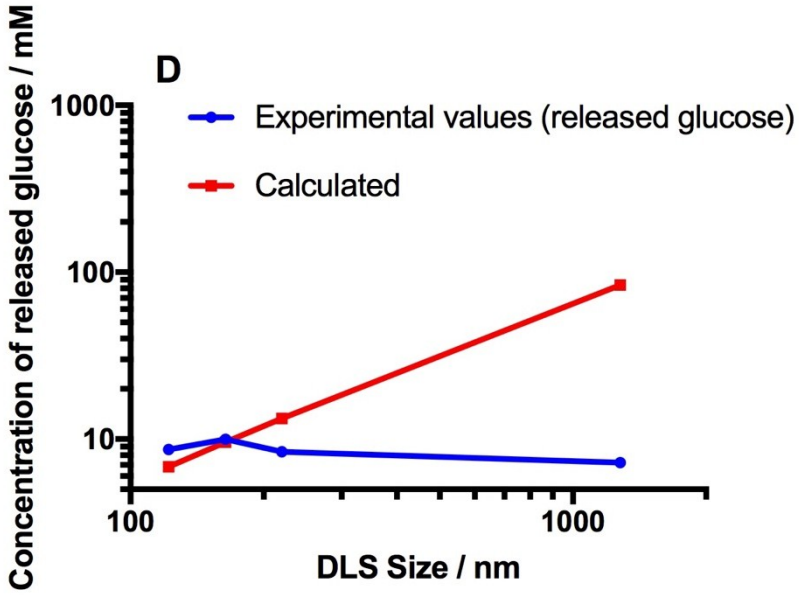
Liposome size study

B



C

DLS size / nm	Calculated liposome concentration / mL^{-1}	Calculated volume of glucose released / L	Calculated mol of glucose released / mM	Calculated concentration of glucose released / mM
122	$6.17 \cdot 10^{14}$	$2.94 \cdot 10^{-5}$	$8.18 \cdot 10^{-7}$	6.82
164	$3.34 \cdot 10^{14}$	$4.15 \cdot 10^{-5}$	$1.15 \cdot 10^{-6}$	9.61
220	$1.83 \cdot 10^{14}$	$5.75 \cdot 10^{-5}$	$1.60 \cdot 10^{-6}$	13.32
1280	$5.19 \cdot 10^{12}$	$3.62 \cdot 10^{-4}$	$1.01 \cdot 10^{-5}$	83.80

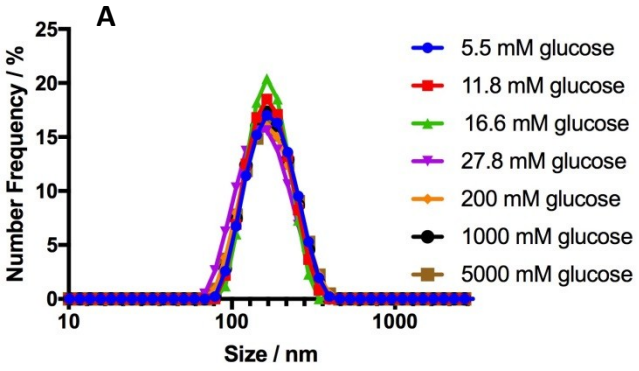


D

Figure S5. Investigate the effect of liposome size on the PGM reading. (**A**) DLS traces for liposomes prepared using a solution of glucose 27.8 mM and extruded with membranes of different nominal size (100, 200, 400, and 1000 nm). The samples were dialysed against PBS and then measured. After the extrusion with the 1000 nm membrane, a relevant fraction of smaller liposomes is also present; the reason is that the extrusion with a membrane of a given porosity cannot alter the size of liposomes smaller than those pores. (**B**) Table summarising the nominal pre size of the membranes used for the extrusion, the liposome sizes measured via DLS and the glucose concentrations measured via PGM. To measure the glucose concentration, 100 µL of liposome solution was added with 20 µL of 10% (w/v) Triton X-100 to break the liposomes and release the glucose in a final volume of 120 µL. (**C**) Calculated liposomes concentrations, glucose released and final glucose concentrations calculated using the equations reported above. It is

worth noting that the calculated liposome concentration obtained for particles extruded with the 200 nm membrane ($3.13 \cdot 10^{14}$ liposomes·mL^{-1}) matches very well with the experimental concentration measured using the Malvern NanoSight ($2.75 \cdot 10^{14}$ liposomes·mL^{-1}). (**D**) Comparison between theoretical and measured concentrations (three replicates) of glucose released in solution after breaking the liposomes, generated from liposomes of different sizes. The experimental and theoretical values match quite well for liposomes extruded using 100 and 200 nm membranes, but start to deviate for bigger liposomes, which release an amount of glucose lower than expected. The explanation is probably because liposomes bigger than 200 nm are likely not unilamellar but multilamellar (as mentioned on the website of the manufacturer of the extruder, https://avantilipids.com/divisions/equipment-products/mini-extruder-extrusion-technique/). It is reported in the literature that the encapsulation efficiency of multilamellar liposomes is lower than for unilamellar ones.[7] This lower volume available to encapsulate glucose might explain the decrease in glucose signal found in this study.

Glucose concentration study



B

Initial glucose concentration / mM	DLS size / nm	Average PGM reading / mM
5.5	156 ± 15.3	2.63 ± 0.41
11.8	161 ± 23.2	4.71 ± 0.20
16.6	168 ± 18.4	6.91 ± 0.058
27.8	152 ± 24.7	11.11 ± 0.057
200	154 ± 22.5	10.713 ± 0.15
1000	153 ± 24.8	10.760 ± 0.18
5000	155 ± 28.5	11.37 ± 0.15

C

Initial glucose concentration / mM	Calculated liposome concentration / mL^{-1}	Calculated volume of glucose released / L	Calculated mol of glucose released / mM	Calculated concentration of glucose released / mM
5.5	$3.71 \cdot 10^{14}$	$3.92 \cdot 10^{-5}$	$2.15 \cdot 10^{-7}$	1.80
11.8	$3.47 \cdot 10^{14}$	$4.06 \cdot 10^{-5}$	$4.79 \cdot 10^{-7}$	3.99
16.6	$3.18 \cdot 10^{14}$	$4.26 \cdot 10^{-5}$	$7.07 \cdot 10^{-7}$	5.89
27.8	$3.91 \cdot 10^{14}$	$3.80 \cdot 10^{-5}$	$1.06 \cdot 10^{-6}$	8.81
200	$3.81 \cdot 10^{14}$	$3.86 \cdot 10^{-5}$	$7.72 \cdot 10^{-6}$	64.33
1000	$3.86 \cdot 10^{14}$	$3.83 \cdot 10^{-5}$	$3.83 \cdot 10^{-5}$	319.27
5000	$3.76 \cdot 10^{14}$	$3.89 \cdot 10^{-5}$	$1.94 \cdot 10^{-4}$	1620.21

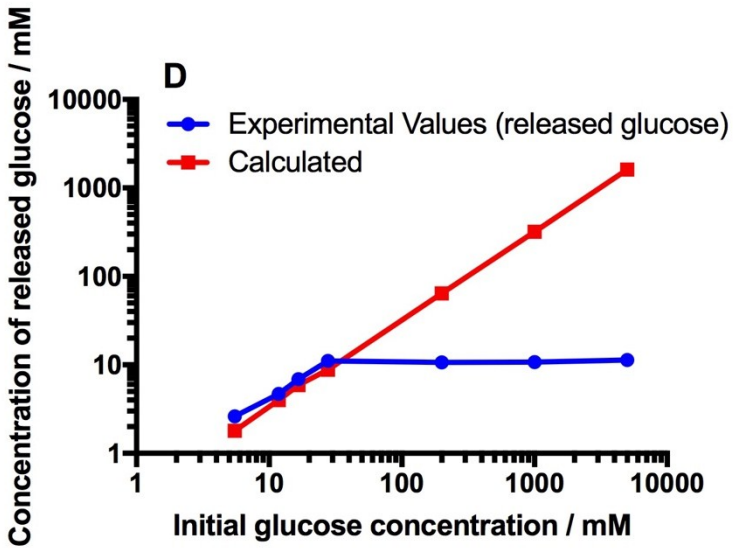


Figure S6. The effect of the initial glucose concentration on the signal recorded with a PGM. This glucose concentration is the one of the solution used to rehydrate the lipid film during the liposome preparation. (**A**) DLS traces of liposomes prepared using glucose solutions of concentration 5.5, 11.8, 16.6, 27.8, 200, 1000 and 5000 mM (solubility of glucose in water at 25 °C is about 5.05 M). The samples were extruded

using a 200 nm membrane, dialysed against PBS and then measured. (**B**) Table summarising initial glucose concentration used to prepare the liposomes, the size measured via DLS and the concentration of glucose generated in solution measured via PGM. To measure the glucose concentration, 100 µL of liposome solution was added with 20 µL of 10% (w/v) Triton X-100 to break the liposomes and release the glucose in a final volume of 120 µL. (**C**) Calculated liposomes concentrations, glucose released and final glucose concentrations calculated using the equations reported above. (**D**) Comparison between theoretical and measured concentrations (three replicates) of glucose released in solution after breaking the liposomes. The experimental and theoretical values match very well for initial glucose concentration up to 27.8 mM (5 mg/mL), and then the PGM signal goes to a plateau. This behaviour suggests that during the dialysis process the content of liposomes prepared with glucose concentrations higher than 27.8 mM leaches out, and such liposomes become equivalent (in terms of size and amount of encapsulated glucose) to those prepared with 27.8 mM glucose. A possible explanation for this observation is the difference in osmolaritiy (and thus osmotic pressure) between inside and outside the membrane.

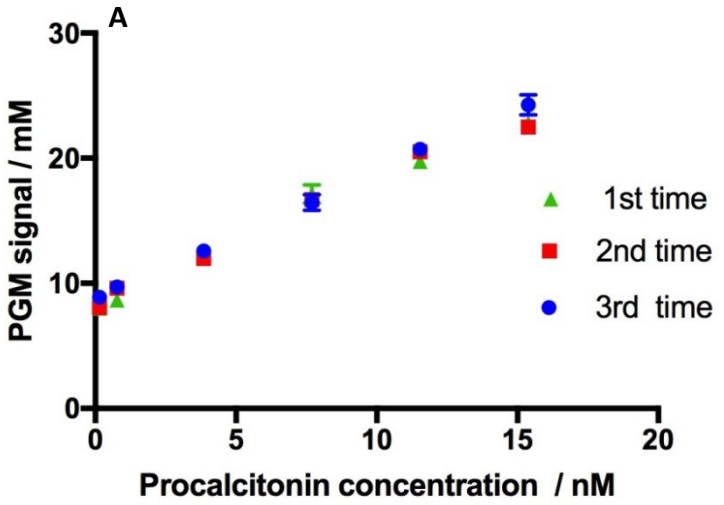
The dialysis of the liposomes with encapsulated glucose was performed in PBS, which has an osmolaritiy of about 300 Osm/L (https://www.thermofisher.com/order/catalog/product/14190250). Glucose and ions from the buffer cannot permeate the HSPC membrane at room temperature, but water can. In this condition the internal glucose solution inside the liposome will be hypo-, iso- or hyperosmotic with respect to the external PBS solution, depending on the glucose concentration.

Glucose concentration / mM	**Osmolarity of glucose solution (calculated) / Osm/L**	**Osmolarity of PBS / Osm/L**	**Osmotic differential / Osm/L**	**PBS respect to the internal glucose solution**
5.5	0.0055	0.300	-0.301	Hyperosmotic
11.8	0.0118	0.300	-0.295	Hyperosmotic
16.6	0.0166	0.300	-0.290	Hyperosmotic
27.8	0.0278	0.300	-0.279	Hyperosmotic
200	0.2	0.300	-0.106	Hyperosmotic
1000	1	0.300	0.694	Hypoosmotic
5000	5	0.300	4.694	Hypoosmotic

It has been reported several times in the literature that exposing liposomes to hypo- or hyperosmotic conditions can affect their size, shape and content of encapsulated molecule. In particular, when liposomes are in a hypoosmotic media (with respect to the internal solution), transient pores are formed that cause the release of the solute until the osmotic gradient is below a certain value (that depends on the liposome formulation being used). [8-9] This process explains the decrease in encapsulated glucose for the samples prepared with 1000 and 5000 mM of glucose, but not for the 200 mM sample, which is almost isoosmotic to PBS.

When liposomes are in a hyperosmotic media (with respect to the internal solution), the liposome shrinks if the osmotic gradient is above a certain value. [10] However, this shrinking process is not observed in this system, as shown by the DLS traces. The reason is probably that the lipids used in this study are in the 'gel' phase (i.e. their phase transition temperature T_m is above room temperature), meaning that the membrane is more rigid. On the other hand, publications that have reported shrinking of liposomes used lipids with phase transition temperatures below room temperature, causing the liposomes to be in their 'liquid', more fluid, phase. [9-11]

Inter- and intra-variability of the assay and spike-and-recover assay



B

PCT concentration / nM	CV% (n=3) of 1st curve	CV% (n=3) of 2nd curve	CV% (n=3) of 3rd curve	CV% for the 3 curves
0.153	1.7	7.0	0	5.9
0.769	1.3	2.3	0	6.3
3.846	1.3	0.9	1.3	2.7
7.692	4.3	3.3	3.9	2.0
11.538	0.8	2.2	1.5	2.6
15.385	2.5	2.3	3.4	4.0

Figure S7. Calibration curves repeated in buffer using different batches of liposomes with encapsulated glucose. The intra-assay variability was calculated for low, middle and high concentrations of PCT by dividing the standard deviation of the PGM reading by their mean. The inter-variability of the assay was calculated, for the three PCT concentrations, by averaging the CV obtained for the three calibration curves.

The spike-and-recovery assay was performed by measuring two blind samples (of 'true' concentration 2.3 and 9.8 nM PCT) with the liposome immunoassay to get a glucose signal, and then interpolate the calibration curve to obtain the corresponding 'experimental' PCT concentration. The calibration curve in buffer of **Figure 5** could be fitted by the linear equation: $[glucose] = 1.0125 \cdot [PCT] + 8.7909$. Using this equation, the recovery was calculated as: $1 - \frac{True\ PCT\ conc.\ -\ Experimental\ PCT\ conc.}{True\ PCT\ conc.}$.

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