Electronic Supplementary Information (ESI) for:

Selective Non-Covalent Triggered Release from Liposomes

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A. Materials and Methods

Materials

All lipids were purchased from Avanti Polar lipids (Alabaster, AL, USA). Dialysis membranes were purchased from SpectrumLabs (Rancho Dominquez, CA, USA). All other reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification.

Instrumentation

Fluorescence measurements were obtained using a Jobin-Yvon FluoroMax 3 fluorometer. Absorbance readings were obtained using a Perkin-Elmer Lambda 25 spectrophotometer. Dynamic light scattering measurements were made using a Beckman Coulter® N4 Plus Submicron Particle Sizer. DSC used a DSC 1 calorimeter (Mettler Toledo, Columbus, OH)

Liposome preparation

Liposomes were prepared by the thin film hydration method, followed by membrane extrusion for size control. The appropriate mixture of phospholipids from $CHCl_3$ based stock solutions were added to a clean and dry test tube and solvent was removed under a stream of nitrogen gas. The resulting lipid films were placed under high vacuum for at least 1 h to remove residual organic solvent. Rehydration at room temperature with the appropriate buffer produced a stock solution of multilamellar vesicles (10 mM total lipid) that were extruded 29 times through a 19 mm polycarbonate Nucleopore filter with 200 nm diameter pores to form unilamellar vesicles with a Basic Liposo-Fast device purchased from Avestin, Inc. (Ottawa, Canada).

Liposome unilamellarity assay [S1]

Fluorescent liposomes (67:28:5:0.5 DPPC:Cholesterol:POPS:NBD-PS) were prepared by the thin film hydration method using an appropriate buffer (5 mM TES, 145 mM NaCl, pH 7.4). To determine vesicle unilamellarity the NBD-PS emission (ex: 470 nm, em: 540 nm) was measured as a function of time after exposure to 1.0 M $Na_2S_2O_4$ (30 µL) in 1.0 M Tris buffer (pH 10) at 60 s and 20 % (v/v) Triton X-100 (20 µL) at 180 s. Only the NBD-PS fluorophore in the outer leaflet of the outer vesicle membrane is chemically quenched by the initial dose of $Na_2S_2O_4$ and all of the NBD-PS is quenched after treatment with Triton. The relative NBD fluorescence at 540 nm was calculated from equation (S1).

Relative NBD fluorescence = $\frac{F_t - F_i}{F_f - F_i} x100$ **Equation S1**

where F_i and F_f are the initial and final fluorescent intensities respectively and F_t is the fluorescent intensity at time t. Figure S1 shows that 70% of the NBD-PS is exposed on the outer surface which is indicative of liposomes that are primarily unilamellar.

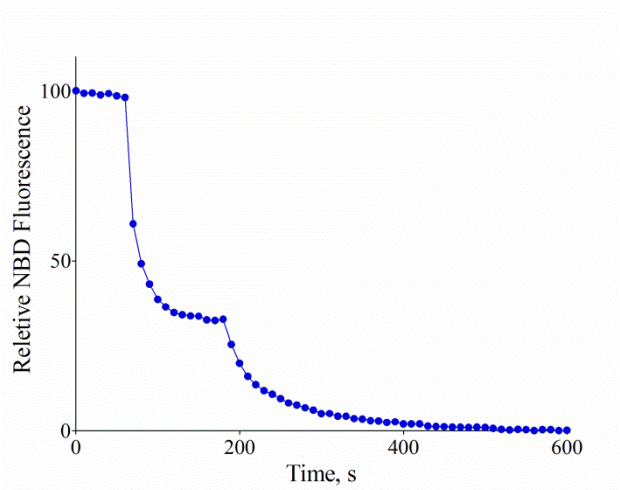


Figure S1: Reduction of NBD-PS labeled liposomes (67:28:5:0.5 DPPC:Cholesterol:POPS:NBD-PS) upon exposure to 1 M $Na_2S_2O_4$ (30 µL) in 1.0 M Tris buffer (pH 10) at 60 s and 20 % (v/v) Triton X-100 (20 µL) at 180 s (ex: 470 nm, em: 540 nm).

Carboxyfluroescein leakage assay [S2]

Liposomes were prepared by the thin film hydration method using an appropriate buffer containing 50 mM carboxyfluorescein (CF). Unencapsulated CF was separated from the liposomes by overnight dialysis (12-14,000 MWCO tubing) against an appropriate buffer. A typical buffer contained 5 mM TES and 145 mM NaCl at pH 7.40. From this stock solution of liposomes, samples (10 µM total lipid) were assayed for leakage (ex: 492 nm, em: 517 nm) upon the addition of chemical trigger **1** (10 µM) at 60 s and liposome lysis using 20 % (v/v) Triton X-100 (20 µL) at 420 s. The % CF Release was calculated from equation (S2):

$$\% \text{ CF Release} = \frac{F_t - F_i}{F_f - F_i} x100 \qquad \textbf{Equation S2}$$

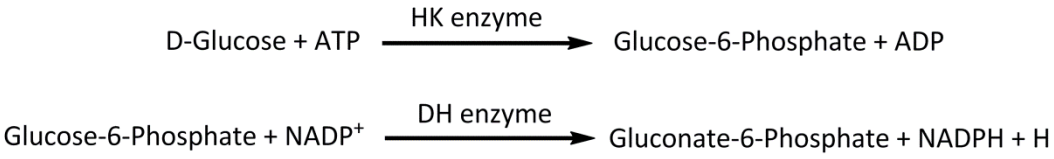
Where F_i and F_f are the initial and final fluorescent intensities and F_t is the fluorescent intensity at 120 s. after addition of chemical trigger. All leakage experiments were reproduced at least three times using at least two separate liposome preparations.

Glucose leakage assay [S3]

A stock solution of liposomes (10 mM total lipid) were prepared by rehydrating lipid films in the presence of 5 mM TES, 145 mM NaCl, pH 7.4 buffer containing 0.3 M D-(+)-glucose. Unencapsulated glucose was removed from the liposomes by overnight dialysis (12-14,000 MWCO tubing). From this stock solution of liposomes, samples (10 μM total lipid) were assayed for leakage of glucose upon addition of chemical trigger **1** (10 μM) at 0 s and 20 % (v/v) Triton X-100 (20 μL) at 500 s. The % Glucose Release was calculated from equation (S3) based on the absorbance of NADPH at 340 nm that was produced by a coupled enzyme assay.

$$\% \text{ Glucose Release} = \frac{A_t}{A_f} x100 \qquad \textbf{Equation S3}$$

where A_t is the absorbance at time t and A_f is the final absorbance of the sample after complete lysis with Triton. The coupled enzyme assay enzymatically converts D-glucose to gluconate-6-phosphate through a two-step process that produces an equimolar amount of NADPH, see Scheme S1.



Scheme S1: Enzymatic conversion of D-Glucose to Gluconate-6-Phosphate

For glucose release to occur, the chemical trigger **1** had to be added before the glucose assay reagents, because high concentrations of phosphorylated species (both ATP and ADP) associate with the ZnDPA **1** and inhibit its ability to trigger leakage. It is important to note that the observed rate of signal appearance in the glucose leakage assay is determined by the rate of enzymatic conversion of glucose to gluconate-6-phosphate which is slower than the rate of glucose leakage.

B. Liposome Size

Vesicle size and percent release of CF was measured at three different points in the leakage experiment using dynamic light scattering (DLS) and fluorescence spectroscopy. The data, shown in Table S1, reflects a negligible change in particle diameter and suggests that liposome fusion is not occurring.

Table S1: Percent release of CF and vesicle size of liposomes (67:28:5 DPPC:Cholesterol:POPS) upon exposure to chemical trigger **1**.

Entry	Sample	Average particle diameter, nm	% Rel.
1	Untreated Liposomes	262 ± 2	0 ± 1
2	After exposure to **1**	288 ± 3	55 ± 3 [a]
3	After treatment with Triton-X-100	77 ± 18	100 ± 1

[a] Percent release at 120 s after addition of chemical trigger **1** at 37 °C in buffer (5 mM TES, 145 mM NaCl, pH 7.4); [total lipid] = [**1**] = 10 μM.

C. CF Leakage Studies

A population of 200 nm liposomes encapsulating 50 mM CF in buffer (5 mM TES, 145 mM NaCl, pH 7.4) were treated with different amounts of chemical trigger **1**, aqueous $Zn(NO_3)_2$, or the apo-ligand of **1** (lacking Zn^{2+}).

Table S2: Percent release of CF from liposomes (67:28:5 DPPC:Chol.:POPS) upon exposure to either chemical trigger **1** or $Zn(NO_3)_2$.[a]

Entry	Chemical Trigger	[Trigger], μM	% Rel.
1	**1**	10	55 ± 3
2	**1**	5	47 ± 3
3	**1**	3	34 ± 9
4	**1**	2	20 ± 8
5	$Zn(NO_3)_2$	20	6 ± 1
6	apo-**1**	10	11 ± 5

[a] Percent release at 120 s after addition of chemical trigger **1** at 37 °C in buffer (5 mM TES, 145 mM NaCl, pH 7.4); [total lipid] = 10 μM.

Leakage triggered by **1** from DPPC:Cholesterol:POPS 67:28:5 liposomes decreases upon warming from 25 °C to 65 °C (Table S3, compare entries 1-3).

Table S3: Percent release of CF from liposomes upon exposure to chemical trigger **1** at various temperatures.[a]

	Membrane composition				
Entry	Chol.	DPPC	POPS	Temp., °C	% Rel.
1	28	67	5	25	58 ± 3
2	28	67	5	37	55 ± 3
3	28	67	5	65	14 ± 1
4	28	67	0	25	3 ± 3
5	28	67	0	37	3 ± 3
6	28	67	0	65	27 ± 2

[a] Percent release at 120 s after addition of chemical trigger **1** in buffer (5 mM TES, 145 mM NaCl, pH 7.4); [total lipid] = [**1**] = 10 μM.

D. Differential Scanning Calorimetry

The effect of chemical trigger **1** on the phase behavior of DPPC:Chol.:POPS 67:28:5 liposomes was measured using a DSC 1 calorimeter (Mettler Toledo, Columbus, OH) with a sample volume of 100 µL. For each phospholipid composition, a suspension of liposomes (5 mM total lipid) was prepared in buffer solution containing 5 mM TES, 145 mM NaCl, at pH 7.4. For samples containing chemical trigger **1**, the mixture was incubated at ambient temperature for approximately 15 minutes before being loaded into the calorimeter cell. Heating scans were collected at a rate of 60 °C per hour using the buffer as a reference. As expected, a sharp gel to liquid phase melting transition was observed at 41 °C for membranes composed entirely of DPPC (Figure S2). This transition disappeared with liposomes composed of DPPC:Chol.:POPS 67:28:5 suggesting that the membrane is in a liquid ordered phase, which is consistent with literature.[S4] The thermogram for a sample containing 5 mM 67:28:5 DPPC:Chol.:POPS liposomes and 1 mM of chemical trigger **1** shows a weak transition at 44.5 °C, suggesting that chemical trigger **1** induces domain formation and perhaps phase separation.

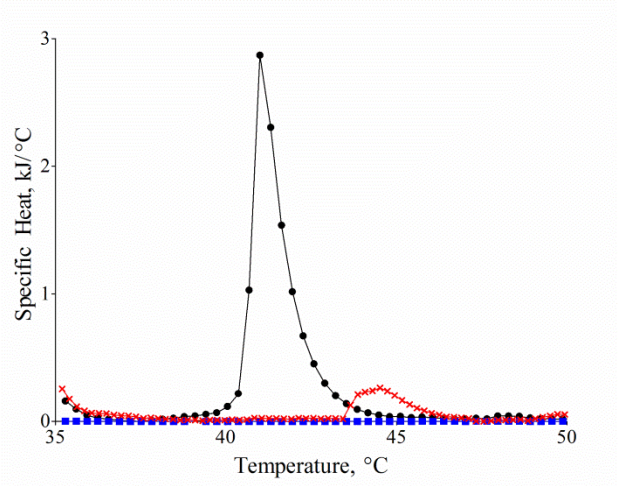


Figure S2: DSC thermograms for liposome mixtures in 5 mM TES, 145 mM NaCl, pH 7.4. (*black circles*) 5 mM DPPC liposomes; (*blue squares*) 5 mM DPPC:Chol.:POPS 67:28:5 liposomes; (*red crosses*) 5 mM DPPC:Chol.:POPS 67:28:5 liposomes in the presence of 1 mM chemical trigger **1**.

E. Cell Toxicity

Quantification of toxicity was measured using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell vitality assay. The number of viable cells is directly correlated to the amount of MTT reduced to formazan. Only active reductase enzymes in living cells can reduce MTT, therefore high levels of cell vitality are indicated by a purple formazan color. The relative toxicity of **1** at different concentrations can be assayed by comparing the absorbance at 570 nm.

MDA-MB-231 human breast cancer cells were purchased from American Type Culture Collection, seeded into 96-microwell plates, and grown to a confluency of 85 % in RPMI media supplemented with 10 % fetal bovine serum, and 1% streptavadin L-glutamate at 37 °C and 5 % CO_2. The Vybrant MTT Cell Proliferation Assay Kit (Invitrogen, Eugene, USA) was performed according to the manufacturer's protocol and validated using 50 μM etoposide as a positive control for high toxicity. The cells were treated with **1** (0-500 μM) and incubated for 18 h at 37 °C. The medium was removed and replaced with 100 μL of RPMI media containing [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, 1.2 mM). An SDS-HCl detergent solution was added and incubated at 37 °C and 5 % CO_2 for an additional 4 hours. The absorbance of each well was read at 570 nm and the normalized data (measured in triplicate) are shown in Figure S3. The results indicate that **1** induces a negligible amount of cell death at concentrations <100 μM. The data indicate that chemical trigger **1** is relatively nontoxic and a potential candidate for *in vivo* studies.

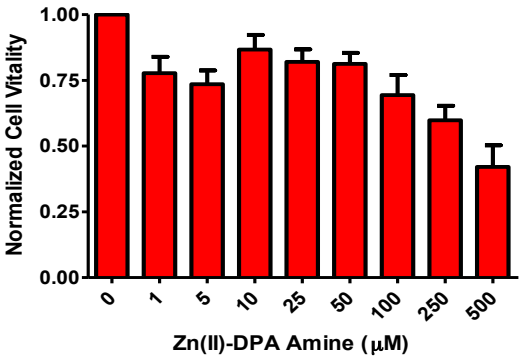


Figure S3: Cell vitality of MDA-MB-231 breast cancer cells treated with Zn(II)-DPA Amine **1**.

D. References

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[S3] P. R. Westmark, B. D. Smith, *J. Am. Chem. Soc.*, 1994, **116**, 9343.
[S4] T. P. W. McMullen, R. N. A. H. Lewis, R. N. McElhaney, *Biochemistry*, 1993, **32**, 516.