ELECTRONIC SUPPORTING INFORMATION

Calculation of the number of integrated peptide amphiphiles and encapsulated material per liposome

The number of both membrane integrated liposome and encapsulated material was calculated as follows.[40] Equation 1 was conducted first to estimate the total number of lipid molecules per vesicle,

$$Ntot = \frac{\left[4\pi\left(\frac{d}{2}\right)^2 + 4\pi\left(\frac{d}{2} - h\right)^2\right]}{a} \tag{eq.1}$$

where *h* is lipid bilayer thickness and taken as 5 nm, *d* is the diameter of a unilamellar vesicle obtained from light scattering measurements, and a is polar head group. Average area per lipid molecule (*a*) was calculated as $a = a_1N_1 + a_2N_2 + ... + a_nN_n$, where; N is the molar fraction of each component. *a* values for each amphipathic molecule used in liposomal formulations were DOPG (69 $Å^2$) and cholesterol (41 $Å^2$).[41-42] For peptide amphiphiles (75 $Å^2$), this value was obtained by using ChemDraw Ultra 8.

(i) Number of peptide amphiphiles per liposome

Total number of lipid molecules per liposome (Ntot) and molar concentration of the membrane integrated peptide amphiphile (Mpep) values were used to calculate theoretical number of membrane integrated peptide amphiphiles and it was formulated in Equation 2 where NA is the Avogadro number,

$$Npep = \frac{Mpep \times NA}{Ntot} \tag{eq.2}$$

(ii) Number of encapsulated material per liposome

The number of liposome per mL (Nlip) for known concentrations of liposomes was calculated by using Equation 3:

$$Nlip = \frac{M(lipid) \times NA}{Ntot \times 1000} \tag{eq.3}$$

where NA is the Avogadro number and it is equal to 6.02E23, M(lipid) is the molar concentration of lipid, and Ntot is the total number of lipids per liposome. Finally, number of encapsulated material (N(Enc)) can be calculated by using Equation 4. M(Enc) is the molar concentration of encapsulated material per mL of the sample.

$$N(Enc) = \frac{M(Enc) \times NA}{Nlip} \tag{eq.4}$$

DOPG:Chol **DOPG:Chol:PA**
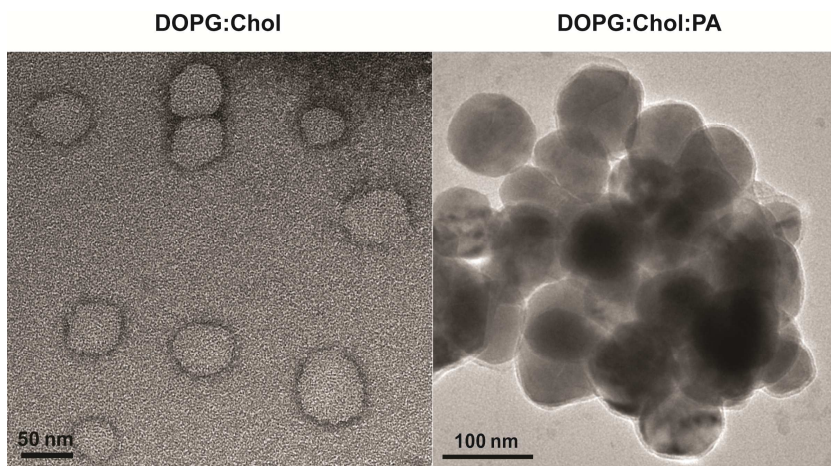

Fig. S1 Transmission electron microscope images of liposomes (scale bars= 50 nm and 100 nm, respectively.
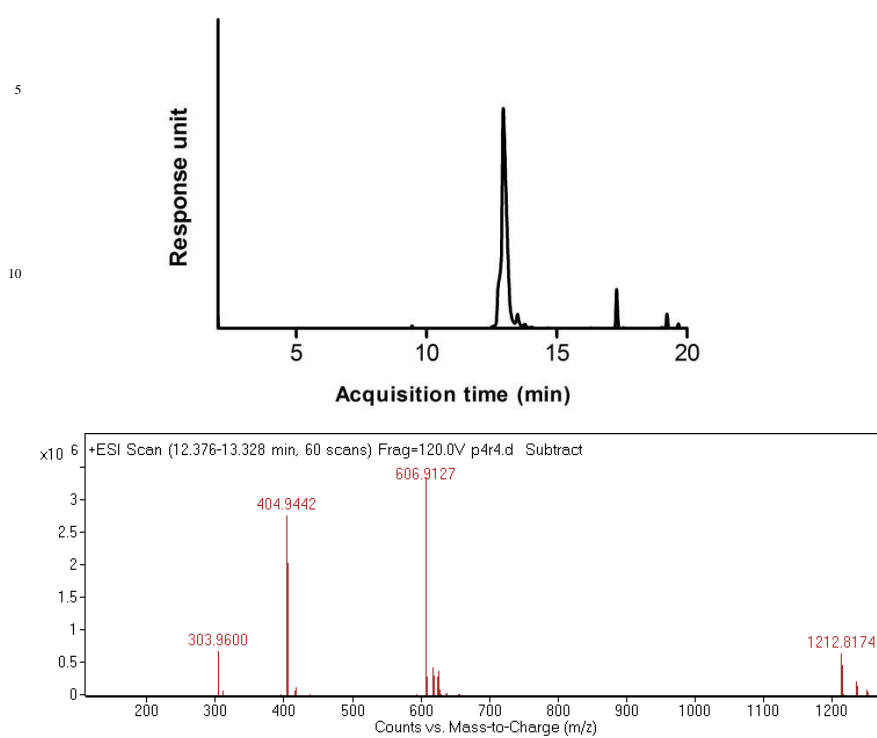
5

10

15 **Fig. S2** (Top) Liquid chromatogram of Lauryl-PPPPRRRR-Am; (Bottom) Mass spectrum of corresponding peptide molecule. Mass data $[M+H]^+$ (calculated) = 1212.54, $[M+H]^+$ (observed) = 1212.82 (observed $[(M+2H)/2]^+$= 606.91, $[(M+3H)/3]^+$= 404.94, $[(M+4H)/4]^+$= 303.96)
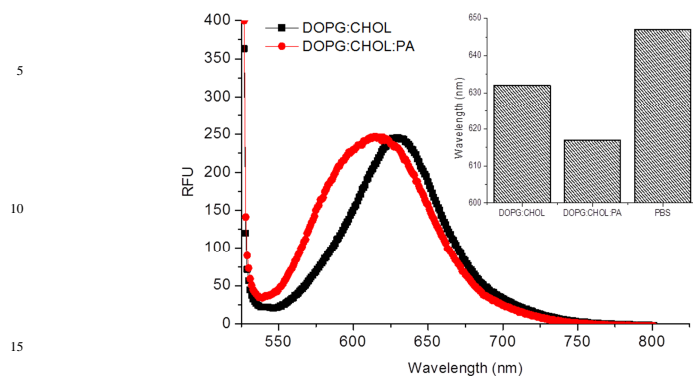
Fig. S3 Polarity change in DOPG:Chol liposomes from polar to nonpolar when peptide amphiphile was integrated to their membrane.
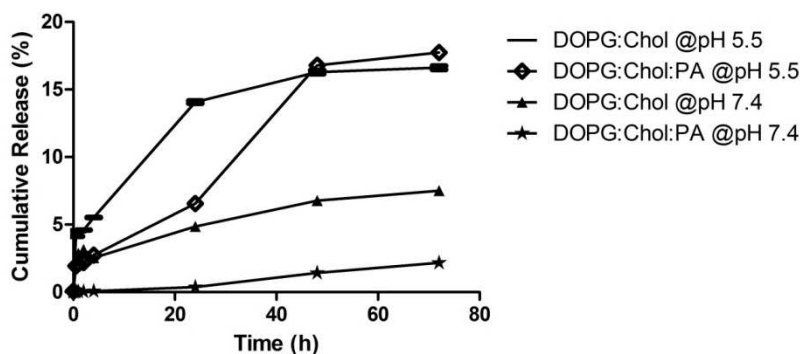

Fig. S4 *In vitro* release profile of DOPG:Chol and DOPG:Chol:PA liposomes at pH 7.4 and pH 5.5. Both liposome formulations are stable at physiological condition while they both show slow release slightly triggered by acidic pH.
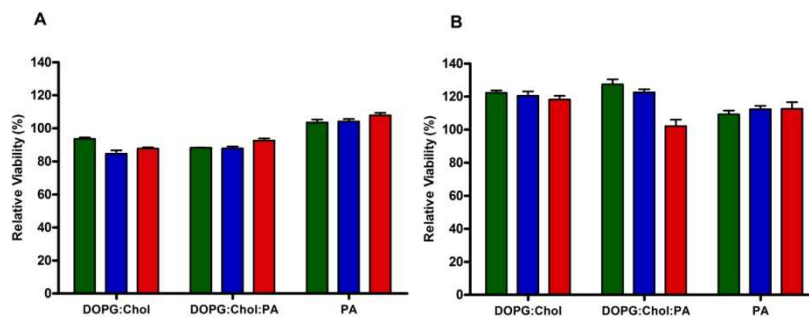

Fig. S5 Viability of MCF7 cells against drug free liposomes after cell exposure to liposomes w/o PA and only PA in free form for **a.** 4 h and **b.** 24 h. Samples were optimized to final peptide concentrations of 250 (green), 25 (blue) and 12.5 (red) μM. Results were normalized to untreated cells in PBS. (n=4)
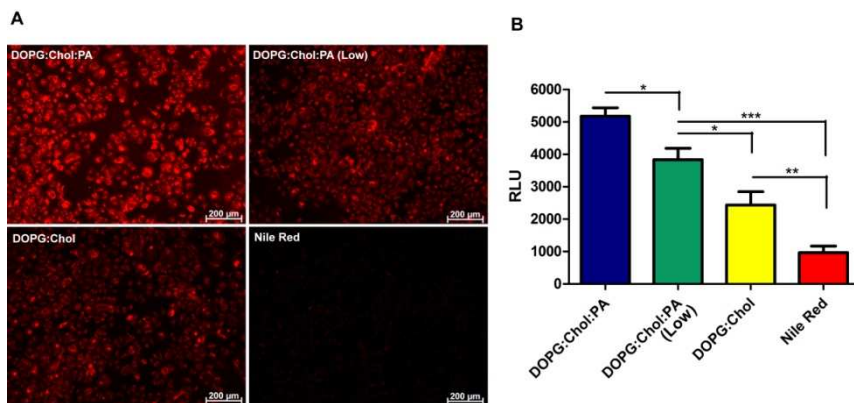
Figure S6. Uptake of 10 µM Nile Red by MCF7 cells. Nile Red was administrated in free or liposome encapsulated form for 3 h. **A.** Fluorescent microscopy images of cells following liposomal DOPG:Chol:PA, DOPG:Chol:PA(Low), DOPG:Chol, and free Nile Red administration. **B.** Quantitative representation of Nile Red uptaken by tumor cells after 3 h of incubation. Following incubation, Nile Red stain from cells collected with ethanol solution and relative flourescence units (RFU) measured to estimate Nile Red uptake. n=4 * stands for p<0.1, ** p<0.5, *** p<0.001
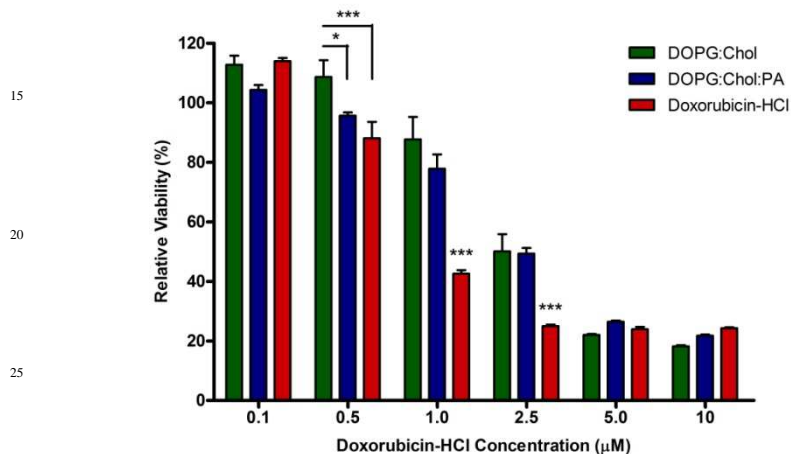

Fig. S7 Dose response of MCF7 cells against free Doxorubicin-HCl and Doxorubicin-HCl loaded DOPG:Chol and DOPG:Chol:PA liposomes. After 24 h of exposure to Doxorubicin-HCl, viability of cells was measured by Alamar Blue. Results were normalized to untreated cells in PBS. (*** stands for $p < 0.001$, ** stands for $p < 0.01$, * stands for $p < 0.05$) (n=4)

Table S1 Physical properties of liposomes

Liposome Formulation	Size(nm)±SDV	PDI	ζ-Pot (mV) ±SDV	Peptide Integration (%)	Integrated PA/Liposome
DOPG:Chol	63.5±8.2	0.43	-41.4±3.9	-	-
DOPG:Chol:PA (7:8:1)	95.26±7.03	0.46	-30.4±2.57	75.40	4568

5

Table S2 Encapsulation of model dyes by liposomes

Liposome Formulation	Encapsulated RHB. (μM)±SDS		RHB. Number/ Liposome		Encapsulated Nile Red (μM)±SDV*	Nile Red Number/ Liposome
	Initial RHB Concentration		Initial RHB Concentration			
	10 μM	100 μM	10 μM	100 μM		
DOPG:Chol	7.90±0.8	36.3±3.9	4.15×10^4	19.1×10^4	150.4±5.2	7.90×10^5
DOPG:Chol:PA	7.00±0.4	30.5±2.2	12.4×10^4	54.0×10^4	176.7±6.7	31.3×10^5

*Initial Nile Red concentration was 0.53 mM.

10

Table S3 Physical properties of DOPG:Chol:PA (7.5:8:0.5)

Liposome Formulation	Size(nm) ± SDV	PDI	ζ-Pot (mV) ± SDV	Peptide Integration (%)	Integrated PA/Liposome
DOPG:Chol:PA (7.5:8:0.5)	98.49 ± 4.9	0.32	-33.2 ± 1.71	77.24	2706

Table S4 Comparison of Doxorubicin-HCl encapsulation capacities of DOPG liposomal
15 formulations

Formulation	Encapsulated Doxorubicin-HCl (mM)±SDV*	Number of Doxorubicin-HCl per liposome	Encapsulation Efficiency (%)
DOPG:Chol	$0.258± 2.6 \times 10^{-4}$	1.36×10^3	75.0
DOPG:Chol:PA	$0.227±1.2 \times 10^{-3}$	4.03×10^3	65.9

*Initial Doxorubicin-HCl concentration of this sample was 0.344 mM.

20

Table S5 Comparison of Paclitaxel encapsulation capacities of liposomal formulations

Formulation	Encapsulated Paclitaxel (mM) *	Number of Paclitaxel per liposome	Encapsulation Efficiency (%)
DOPG:Chol	0.092	4.85×10^5	49.33
DOPG:Chol:PA	0.117	20.7×10^5	62.6

*Initial Paclitaxel concentration of this sample was 0.187 mM

5 **Supporting References:**

40.   A. Güven, M. Ortiz, M. Constanti and C. K. O'Sullivan, *J. Lipos. Res.*, 2009, **19**, 148-154.

41.   I. Brzozowska and Z. A. Figaszewski, *Colloid Surface B*, 2002, **23**, 51-58.

42.   J. Pan, F. A. Heberle, S. Tristram-Nagle, M. Szymanski, M. Koepfinger, J. Katsaras and
10   N. Kučerka, *BBA-Biomembranes*, 2012, **1818**, 2135-2148.