Supplementary Materials

Porphyrin-Phospholipid Liposomes with Tunable Leakiness

Authors: Dandan Luo, Kevin A Carter, Aida Razi, Jumin Geng, Shuai Shao, Cuiyan Lin, Joaquin Ortega, Jonathan F Lovell

Contact: jflovell@buffalo.edu

Supplementary Methods

1. Serum proteins induced destabilization of Dox loaded leaky HPPH liposomes

Dose dependency of protein induced destabilization of leaky HPPH liposomes were performed by incubating leaky HPPH liposomes with 100, 50, 25 and 10mg/ml BSA (dissolved in PBS) or 40, 25,10,5 and 2.5 mg/ml BGG (dissolved in PBS) at 37 °C for 24h. The Bradford method was used to quantify the amount of BSA absorbed to HPPH liposomes and standard liposomes. Briefly, liposomes (~20 mg/mL lipids) were diluted 10 times in 50 mg/mL BSA solution prepared above and incubated for 20 hours. 500 µl incubated samples were separated from free BSA by gel filtration via CL-2B column. Liposome fractions were collected (fractions 4-6) and BSA absorbed to liposomes were determined by BSA standard curve, ensuring no interference from the HPPH absorption. To study if complement destabilized liposomes, HPPH liposomes or std. liposomes were incubated with 50% mature bovine serum (Pel-Freez #37218-5) or 50% heat inactivated bovine serum (56°C for 30min). Dox leakage was determined as described previously.

2. Bilayer thickness measurements

The thickness of the lipid bilayer of Dox loaded stable std. liposomes, leaky HPPH liposomes and empty HPPH liposomes were measured in two different ways. The first approach was by looking through each micrograph and measuring the thickness of the membrane of 50 liposomes form each sample. The average values for the thickness of the lipid bilayer in the standard and leaky HPPH liposomes were ~7.4 nm and 3.6 nm, respectively. In the second method, ~700 individual membranes were boxed in standard and HPPH liposome. To obtain higher signal to noise ratio averages of the membranes delimiting each type of liposomes, a two-dimensional alignment and averaging of those particles were performed. The thickness of each bilayer were measured using the higher signal to noise ratio averages. The result was very much consistent with the results in the first approach. The thickness membrane bilayers from both stable std.

liposomes and leaky HPPH liposomes were ~ 6 nm and bilayer from the empty HPPH liposomes was ~ 4 nm.

3. Light triggered release of Dox from leaky HPPH liposomes

Light release of HPPH liposomes with indicated loading ratios, stable std.(D:L 1:4)+empty HPPH liposomes were performed in real time with a fluorometer (PTI). Irradiation was performed in 50% matured bovine serum with a 665 nm laser (RPMC laser, LDX-3115-665) at 200 mW/cm^2 for 30 min. 5 µL of leaky HPPH liposomes(3.5mg/ml Dox) were diluted into 3 mL 50% bovine serum in a cuvette under stirring at 37 °C. For stable std. lipos+empty HPPH lipos, 5 µl stable std. lipos (3.5mg/ml Dox) and 5 µl of unloaded HPPH liposomes (~20mg/ml lipids) were used. For the control of leaky HPPH liposomes without laser irradiation, 0.25% Triton X-100 was used after 30 min to read the final fluorescence. Release of doxorubicin are calculated as $(F_{Final}-F_{initial})/(F_{x\text{-}100}-F_{initial})\times 100\%$.

4. Serum stability of leaky HPPH liposomes after laser irradiation

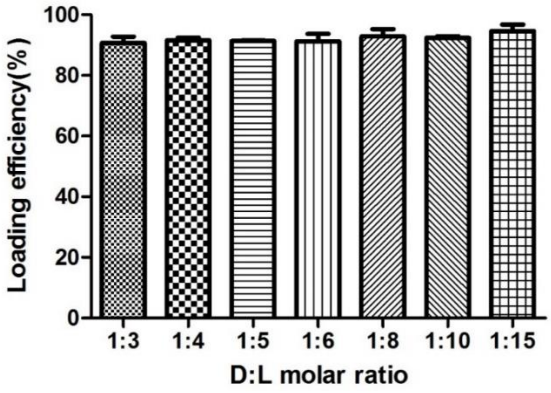
5 µl of leaky HPPH liposomes (3.5mg/ml, 1:4 D:L molar ratio) were diluted into 3ml 50% matured bovine serum in a cuvette under stirred at 37 °C. Initial fluorescence readings were taken and samples were irradiated for 250, 125 and 62.5 seconds with 665 nm laser (200 mW/cm^2). Samples were incubated at 37 °C after taking the fluorescence reading immediately after irradiation. Dox fluorescence were taken every half hour. 0.25% Triton X-100 was added to lyse the liposomes and final fluorescence value were read. Dox release was calculated according to the formula above.

5. Tumoral Dox accumulation devoid of light triggered release

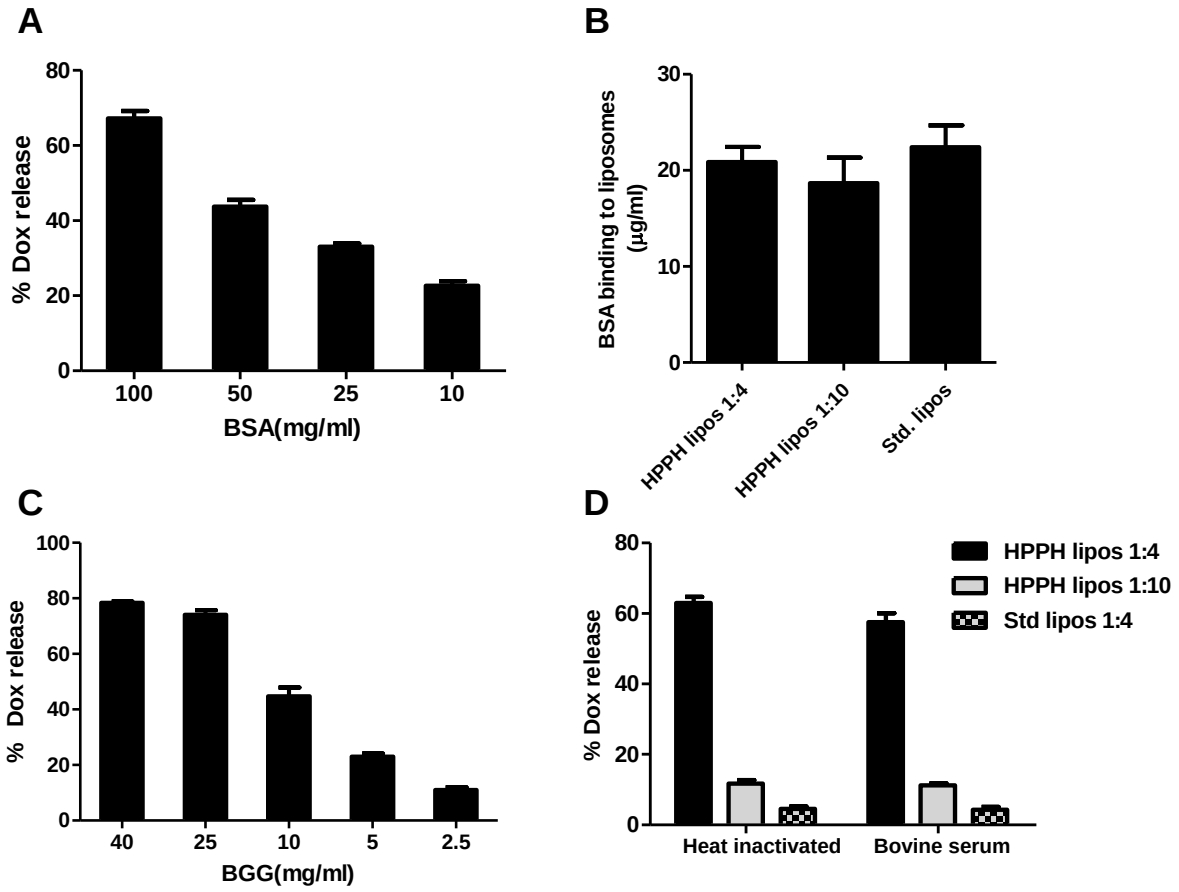
To further test the hypothesis that enhanced Dox accumulation for this particular formulation is attributed to photo-induced tumor vessel permeability, rather than direct light triggered release, a study was designed to separate the effect of PDT-induced accumulation and light triggered release by a sequential two injections method. One group of mice bearing KB tumors (n=5 per group) were first intravenously injected equivalent amount of empty HPPH liposomes (15.5mg/kg HPPH-lipid) alone, laser treatment was performed (200mW/cm^2 for 12.5min) 15min post injection. After laser treatment, mice were again injected with 10mg/kg Dox loaded leaky HPPH liposomes. Dox accumulation in tumors were determined as

described above. Another group of mice were injected with 10mg/kg Dox loaded leaky HPPH liposomes, 15min later laser treatment was performed at 200mW/cm^2 for 12.5min. Tumoral Dox accumulation were determined as described previously.

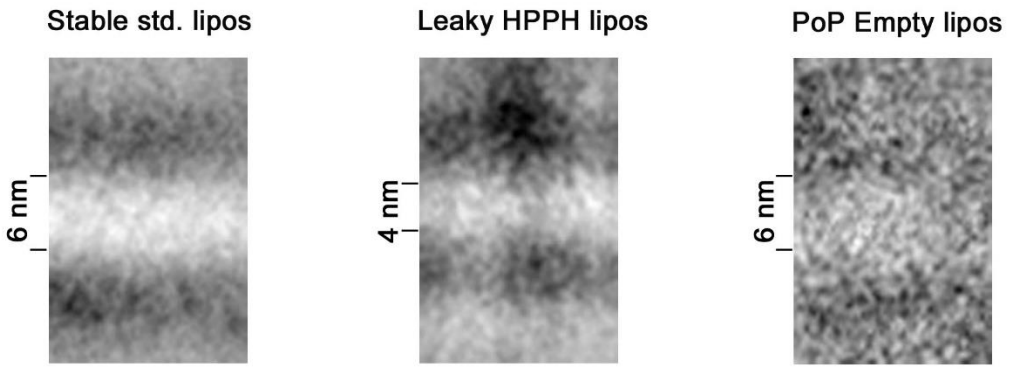
Supplementary Data



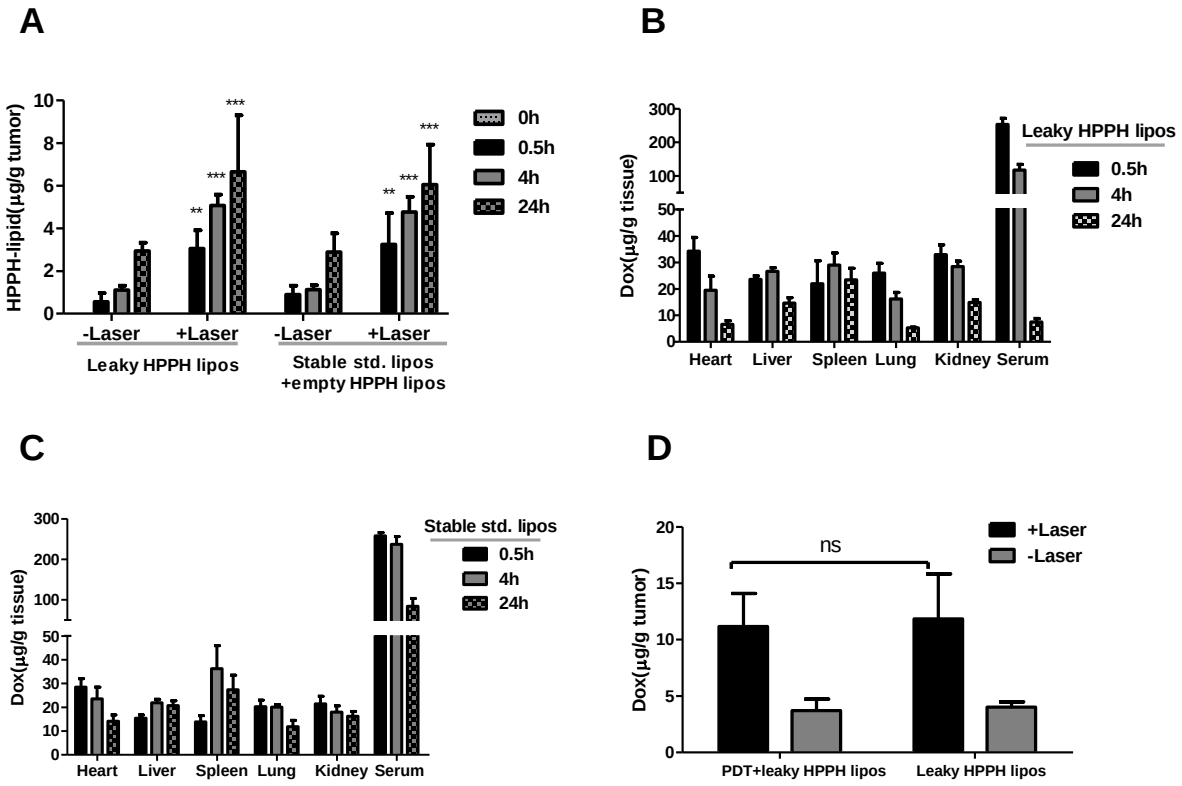
Supporting Figure 1: High loading efficiency of HPPH liposomes at variable loading ratios. Loading efficiency of HPPH liposomes loaded at the D:L ratio of 1:3,1:4, 1:5, 1:6, 1:8, 1: 10 and 1:15. The loading efficiencies were all over 90%. Data represent means ± s. d., n=3.



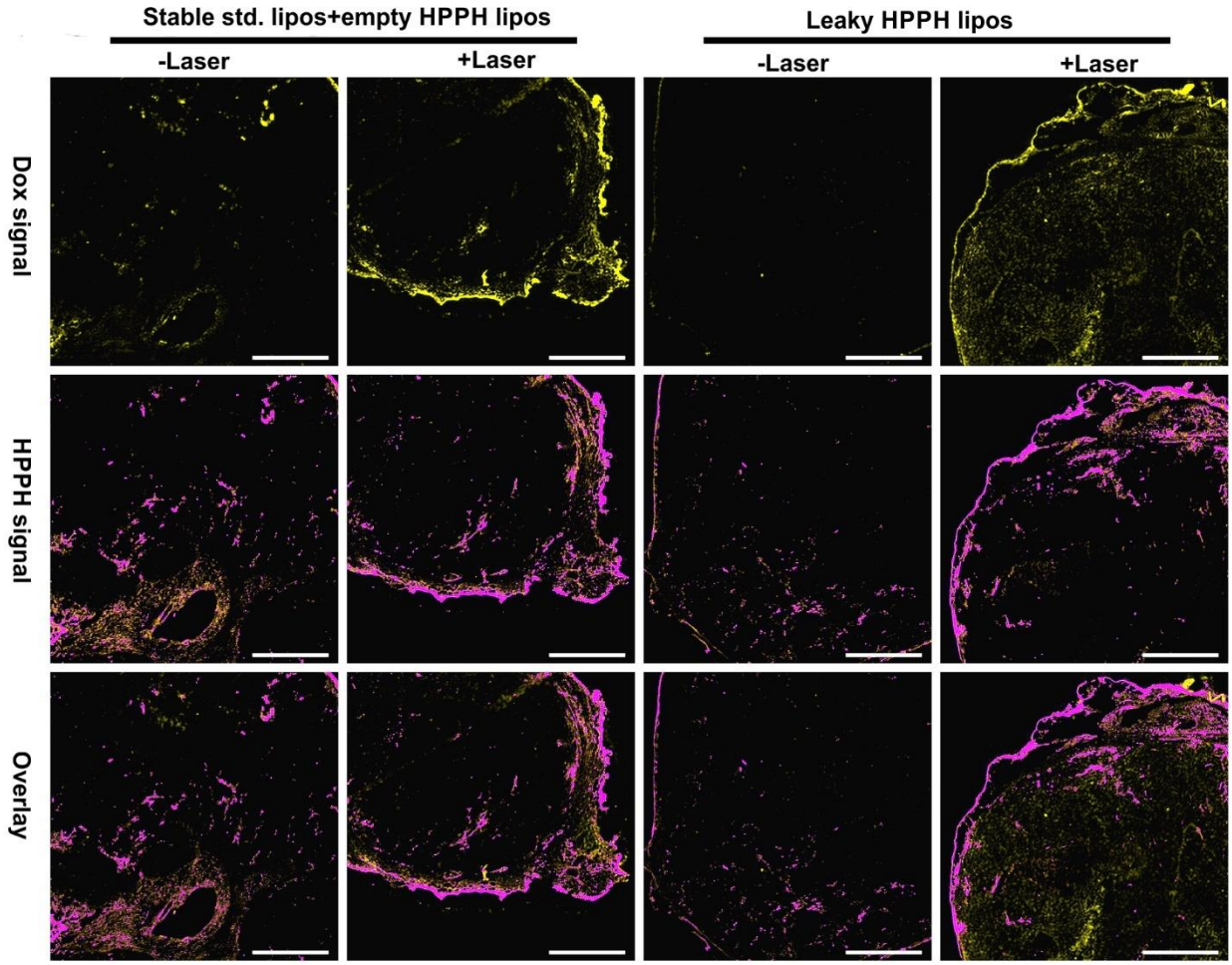
Supporting Figure 2: Serum proteins induced destabilization of HPPH liposomes. (A) Destabilization of HPPH liposomes by BSA is dose dependent. Dox release from leaky HPPH liposomes after 24h incubation with 100, 50, 25 and 10mg/ml BSA. **(B)** Amount of BSA absorbed to 50 µL of indicated liposomes (~20mg/ml lipids) after incubating with 50mg/ml BSA solution for 20h. **(C)** Destabilization of HPPH liposomes by BGG is dose dependent. Dox release from leaky HPPH liposomes after 24h incubation with 40, 25, 10 and 5 and 2.5mg/ml BGG. (**D**) Similar to regular bovine serum, complement inactivated bovine serum can destabilize HPPH liposomes. Complement proteins were inactivated by heating at 65°C for 30min.



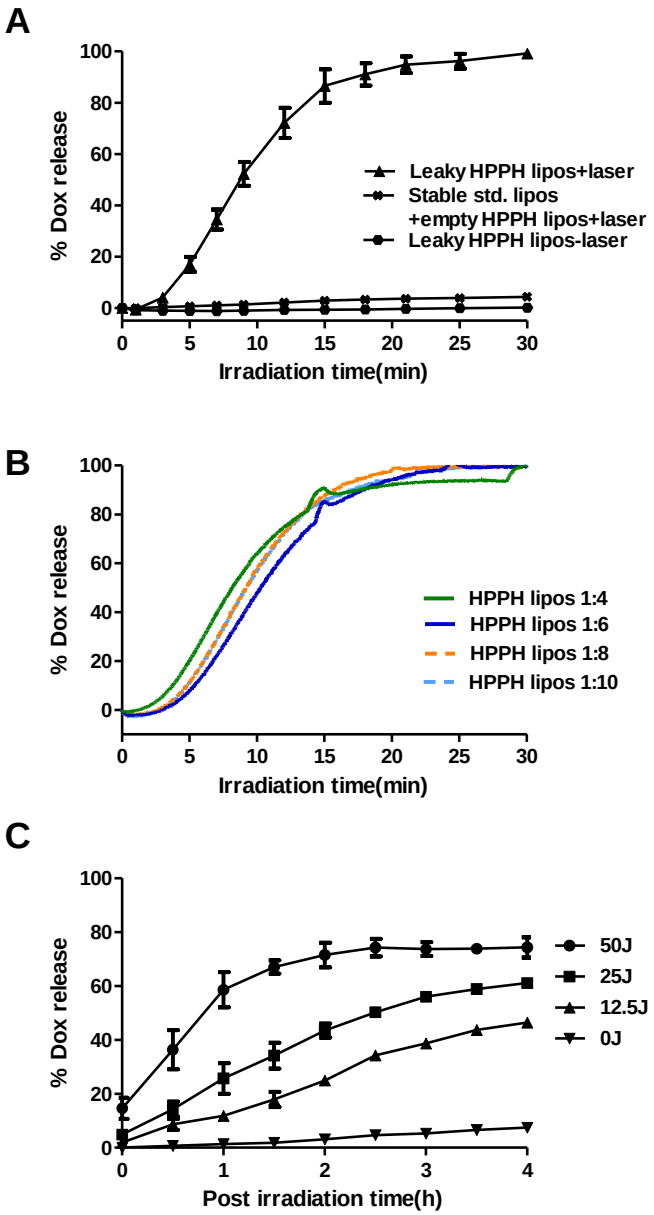
Supporting Figure 3: Representative bilayer thickness of Dox loaded stable std., leaky HPPH liposomes and unloaded HPPH liposomes. These results suggest that Dox loaded leaky HPPH liposomes have a thinner bilayer.



Supporting Figure 4: HPPH-lipid deposition in KB tumors. (A) Both leaky HPPH liposomes and stable std. liposomes +empty HPPH liposomes group were examined at 0, 0.5, 4 and 24 hours with or without laser treatment. There is no significant difference between leaky HPPH liposomes and stable std. liposomes+empty HPPH liposomes in terms of HPPH-lipid deposition. **(B)** Biodistribution of Dox from leaky HPPH liposomes in KB tumors at 0.5h,4h and 24h.**(C)** Biodistribution of Dox from stable std. liposomes in KB tumors at 0.5h,4h and 24h. Results are mean± std. dev. for n=4 mice at each time point. Analysis were performed by Bonferroni post-test, two way ANOVA. (**p < 0.01, ***p < 0.001). **(D)** Following injection with empty HPPH liposomes, mice were treated with laser for 12.5min. Immediately after laser treatment, mice were injected with 10mg/kg Dox loaded leaky HPPH liposomes. One injection of 10mg/kg Dox loaded leaky HPPH liposomes followed by light treatment was used as a regular treatment control. This demonstrated that the enhanced Dox accumulation is attributed to PDT-induced vascular permeability, rather than light triggered release. mean± std. dev. for n=5.



Supporting Figure 5: Dox and HPPH spatial distribution 24 hours after treatment with or without laser irradiation. Following laser irradiation of mice injected with the indicated liposomes at the dose of 10 mg/kg Dox, mice were sacrificed and tumors were collected 24 hours after irradiation. Tumor micrographs from mice injected with the indicated liposomes with or without laser irradiation. The images are the same as Figure 5 except smaller tumor regions are shown. 1 mm scale bars are shown.



Supporting Figure 6: Drug release and destabilization induced by laser irradiation *in vitro*. (A) Release of doxorubicin while laser irradiation (200 mW/cm^2) in 50% mature bovine serum for leaky HPPH liposomes or stable std. liposomes. Leaky HPPH liposomes-laser is a without laser irradiation control. **(B)** Light triggered release of doxorubicin while laser irradiation (200mW/cm^2) in 50% mature bovine serum of HPPH liposomes with various loading ratios. There is no significant difference between different loading ratios for light release rate. **(C)** Continued release of Dox from leaky HPPH liposomes in 50% mature bovine serum following laser irradiation for 250, 125 and 62.5 seconds, corresponding to light dose of 50 J/cm^2, 25 J/cm^2 and 12.5 J/cm^2. Mean ± s.d. for n=3.