Supplementary Data

Figure S1. Flow-cytometric analysis of PB_{46}-PEO_{30}/POPC vesicle samples. (**a**) PB-PEO/POPC (75:25); (**b**) PB-PEO/POPC (50:50); (**c**) PB-PEO/POPC (25:75). The preparations have been labeled with TMRho-PB-PEO and Pyrene-PE to confirm the presence of hybrid vesicles in the population; (**d**) Control PB-PEO sample (TMRho-labeled); (**e**) Control PB-PEO sample (pyrene-labeled); and (**f**) Control PB-PEO sample (unlabeled). The *x* and *y* axes of each dot plots represent the fluorescence intensity (units) for Pyrene and TMRho, respectively.

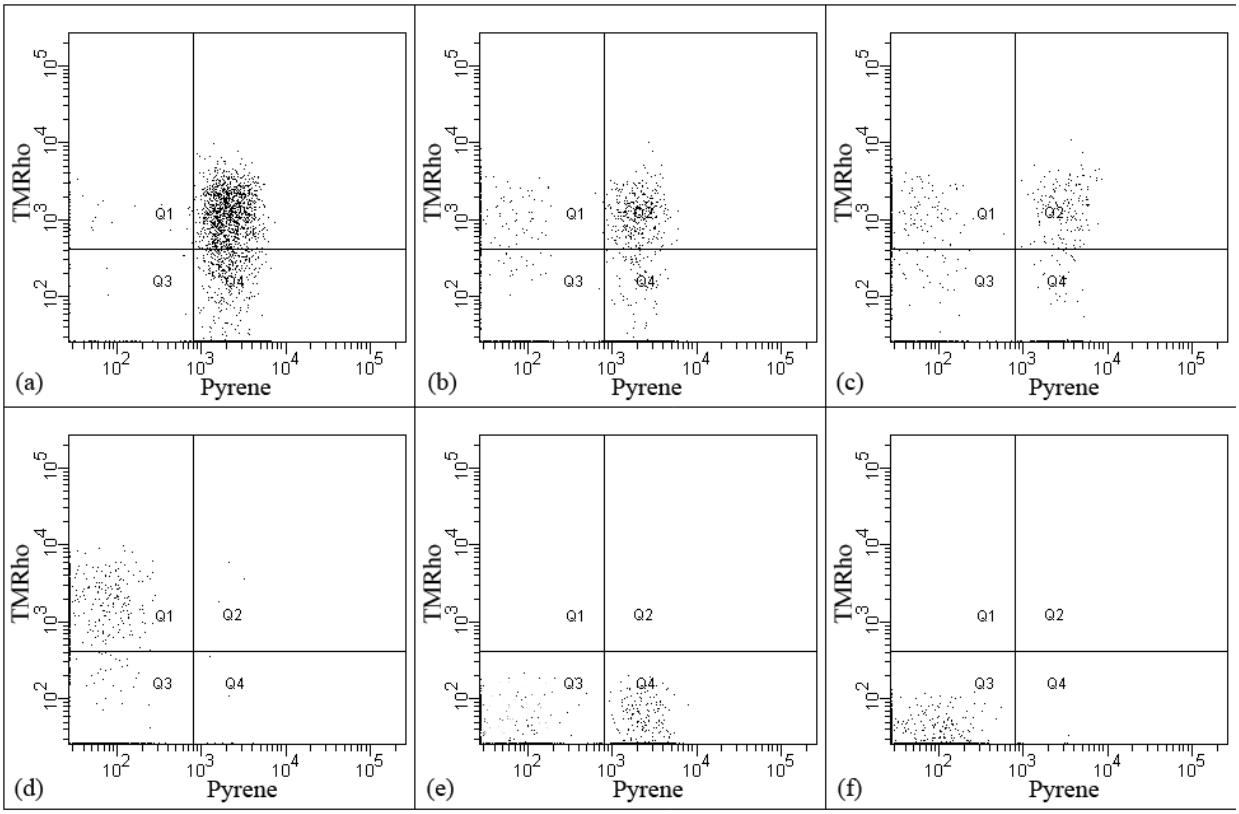
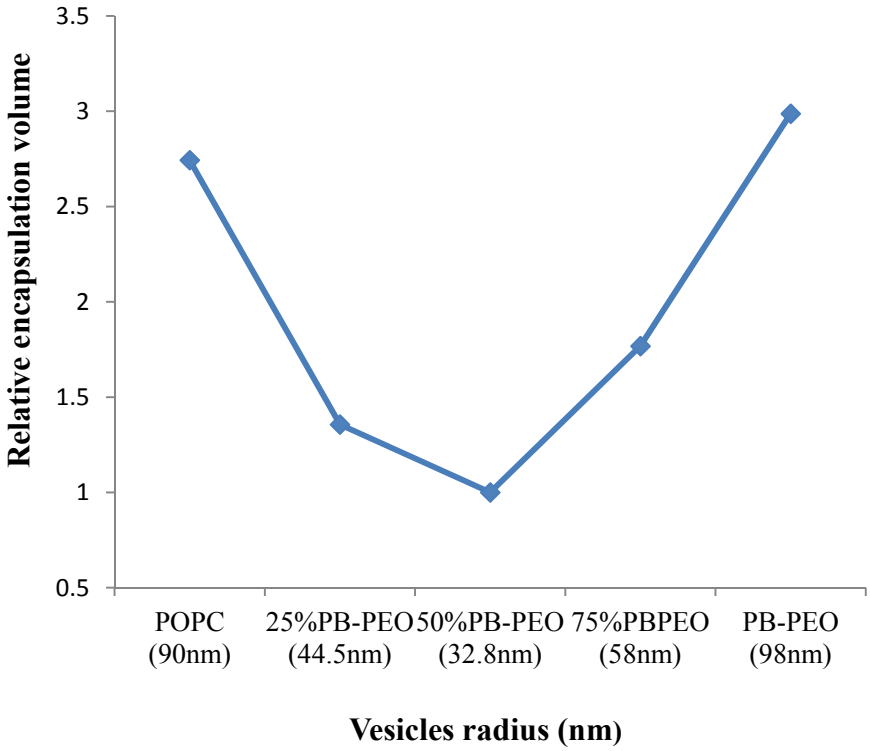


Table S1. Summary of flow-cytometric analysis of PB_{46}-PEO_{30}/POPC vesicle samples. PB-PEO/POPC (75:25); PB-PEO/POPC (50:50); and PB-PEO/POPC (25:75). Only few vesicles (<36% of vesicle population) contain both pyrene and TMRho, suggesting poor hybrid vesicle formation efficiency.

Vesicles	% of Pyrene and TMRho-labeled (hybrids)	% of Pyrene-labeled	% of TMRho-labeled
PB-PEO/POPC (25:75)	17.1	15.6	23.9
PB-PEO/POPC (50:50)	23.1	13.1	37.4
PB-PEO/POPC (75:25)	36.0	60.6	2.1

Figure S2. Calculation of encapsulation volume *vs*. vesicles of different size.



Note on calculation:

Surface area per vesicles (A) = $2 \times 4\pi R^2$, where R is liposome radius;

Encapsulation volume per vesicles (V) = $(4/3)(\pi R^3)$

For equal number X of starting amphiphiles (molecular area = A_0), total number of vesicles formed (N) = $A_0X/A = A_0X/(2 \times 4\pi R^2)$

Total encapsulation volume = $N \times V = (A_0X/8\pi R^2) \times (4/3)(\pi R^3) = 1/6 \times (A_0X \times R)$

∞ $R/6$ for equal number of amphiphiles.

Therefore, the theoretical encapsulation volume of vesicles is propotional to $R/6$. The calculated relative encapsulation volume of our vesicles was shown in Figure S2, assuming equal amphiphile number and molecular area and CF stock concentration.