Modulation of Phase Separation at micron scale and nanoscale in Hybrid Polymer/Lipid Giant Unilamellar Vesicles (GHUVs)

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SUPPORTING INFORMATION

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S1. ^{1}H NMR Characterization of Triblock copolymers

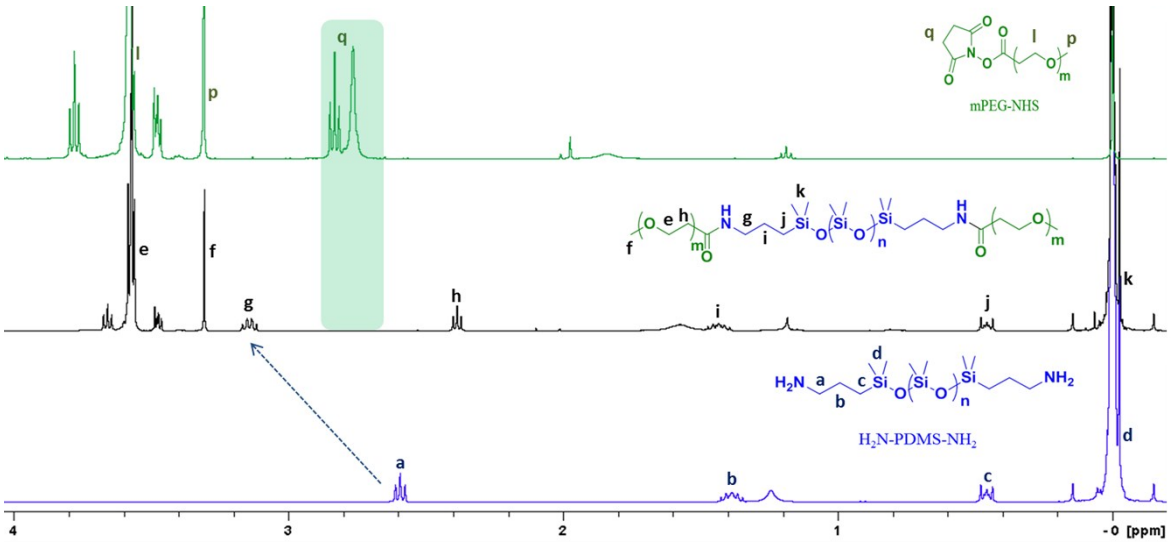

Figure S1. The coupling between the blocks of the copolymers was monitored through disappearance of proton signal from NHS group and the position shift of proton near amine group. From the top to the bottom: ^{1}H NMR spectrum in $CDCl_3$ of PEO-NHS; PEO-*b*-PDMS-*b*-PEO and NH_2-PDMS-NH_2.

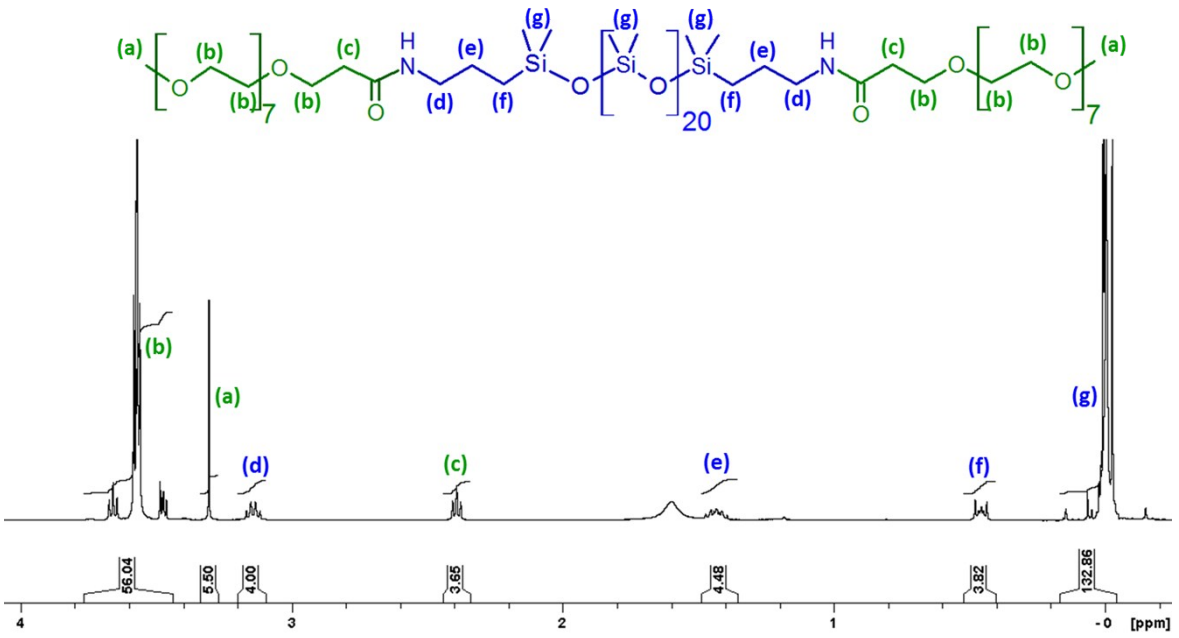

Figure S2. ^{1}H NMR spectrum of triblock copolymer PEO_8-*b*-$PDMS_{22}$-*b*-PEO_8 in $CDCl_3$

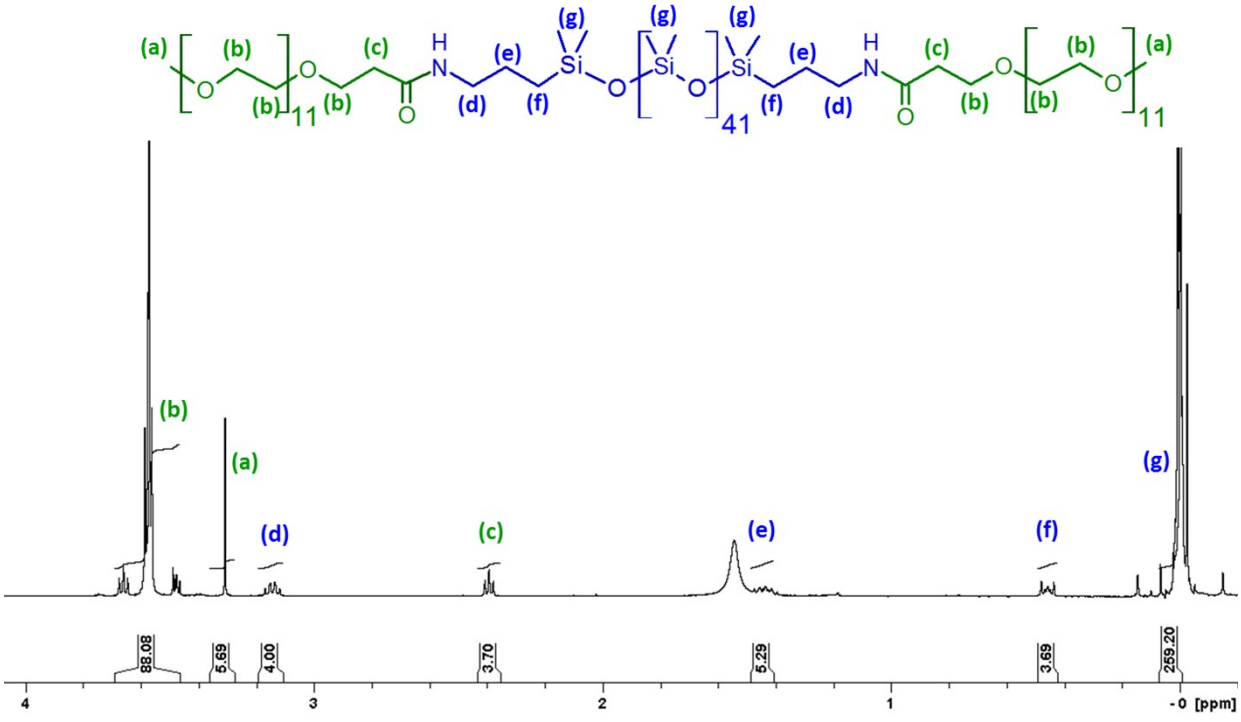

Figure S3. ^{1}H NMR spectrum of triblock copolymer PEO_{12}-*b*-$PDMS_{43}$-*b*-PEO_{12} in $CDCl_3$

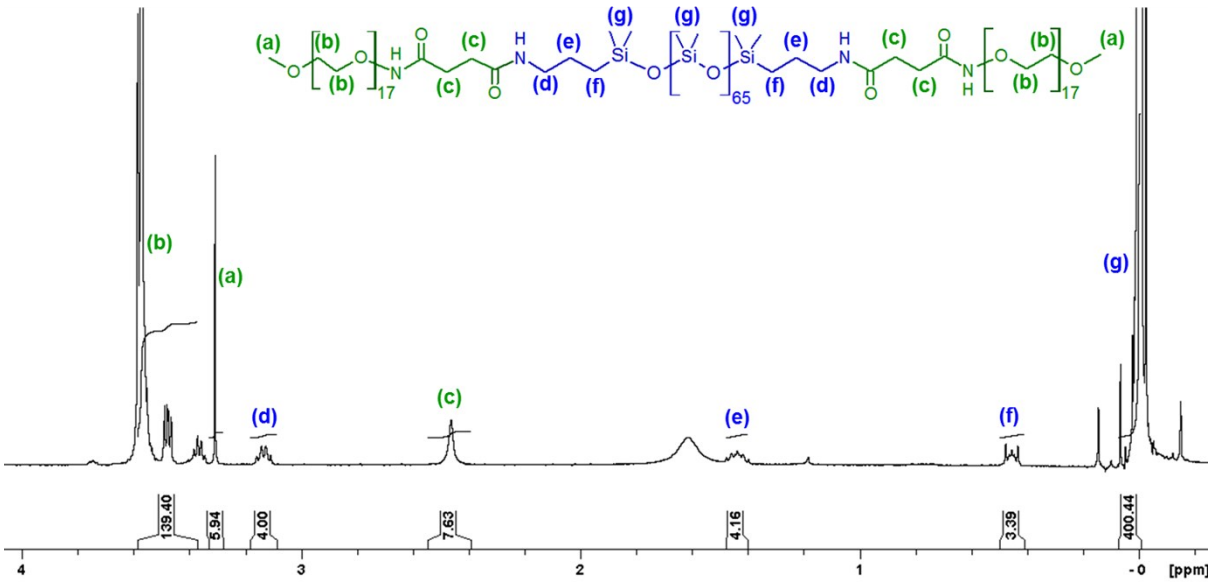

Figure S4. ^{1}H NMR spectrum of triblock copolymer PEO_{17}-*b*-$PDMS_{67}$-*b*-PEO_{17} in $CDCl_3$.

S2. SEC characterization of the triblock copolymers

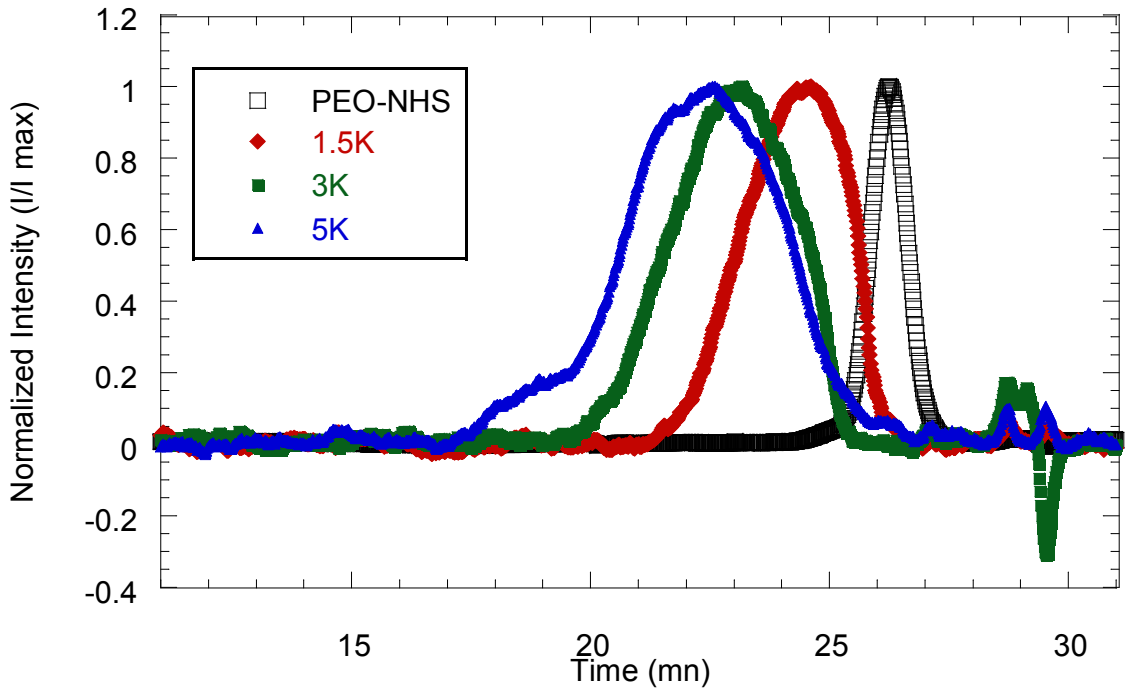

Figure S5. Normalized SEC curves of all triblock copolymers and PEO-NHS in THF. H_2N-PDMS-NH_2 cannot be characterized because its refractive index is very close to the refractive index of THF, thus its RI increment in THF is nearly zero.

SEC analysis demonstrates that all remaining PEO-NHS was totally removed after the dialysis procedure.

S3. Cryo-TEM

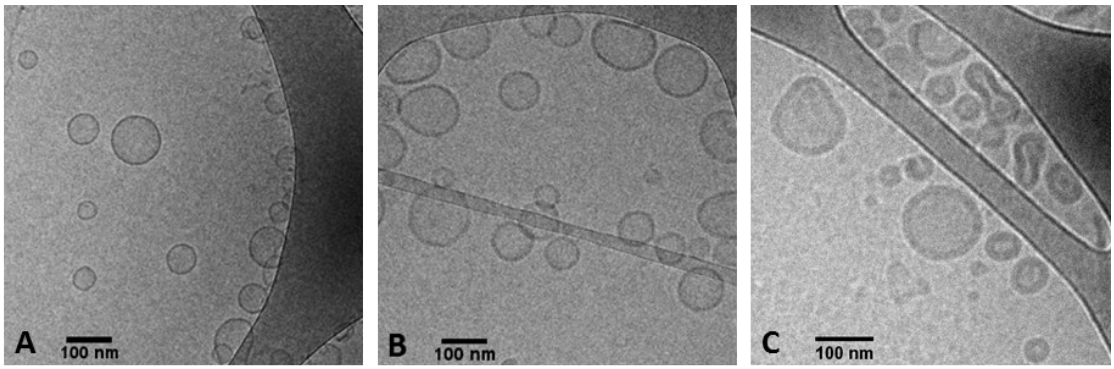

Figure S6. Cryo-TEM pictures of self-assembly from: PEO_8-*b*-$PDMS_{22}$-*b*-PEO_8 (A) ; PEO_{12}-*b*-$PDMS_{43}$-*b*-PEO_{12} (B) and PEO_{17}-*b*-$PDMS_{67}$-*b*-PEO_{17} (C). Their membrane thicknesses vary from comparable to significantly higher than liposome membrane: 5.4±0.4nm; 8.8±0.5 nm and 11.2±1.2 nm respectively.

S4. Confocal imaging

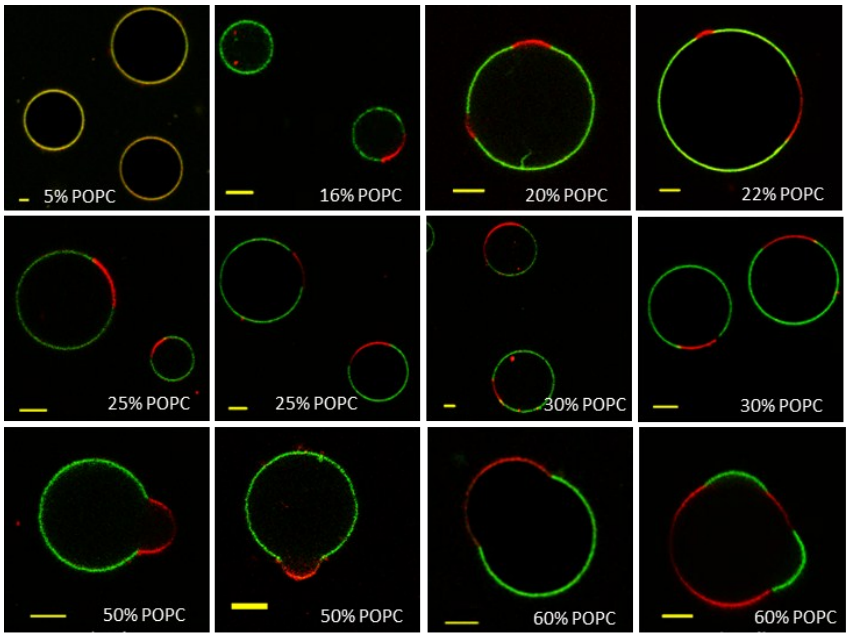

Figure S7. The equatorial z-section images of 1.5K/POPC GHUVs labelled with $PDMS_{26}$-*g*-$(PEO_{12})_2$-FITC (green channel) and DOPE-Rhod (red channel) observed by confocal fluorescence microscopy at room temperature. Scale bars: 5 µm.

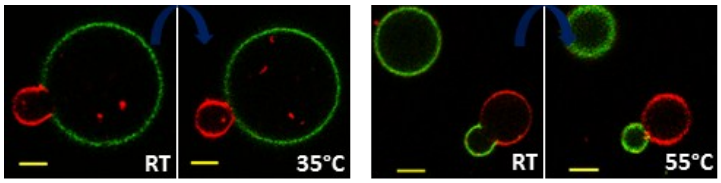

Figure S8. Fission phenomenon occurring in 1.5K/POPC GHUVs at high temperature for both cases of budded lipid domain (left 90 weight % POPC) and budded polymer domain (right: 80 weight % POPC). Scale bars: 5 µm.

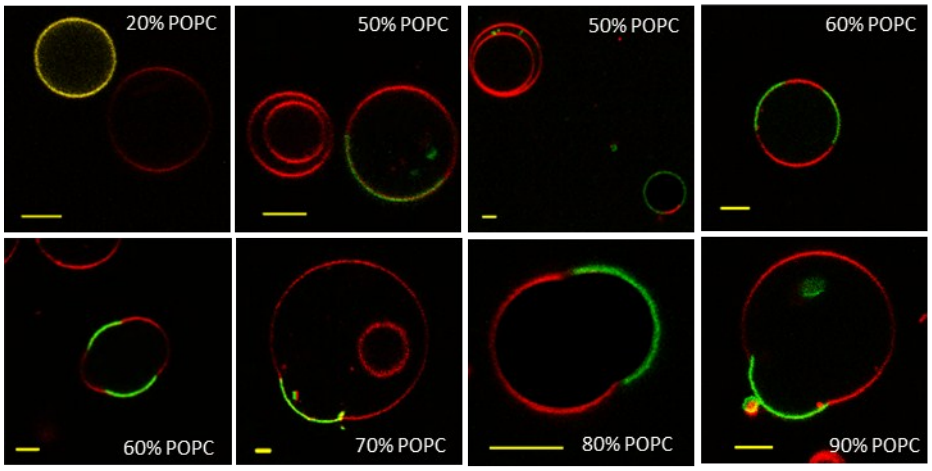

Figure S9. The equatorial z-section images of 3K/POPC GHUVs labelled with $PDMS_{26}$-*g*-$(PEO_{12})_2$-FITC (green channel) and DOPE-Rhod (red channel) observed by confocal fluorescence microscopy at room temperature. Scale bars: 5 µm.

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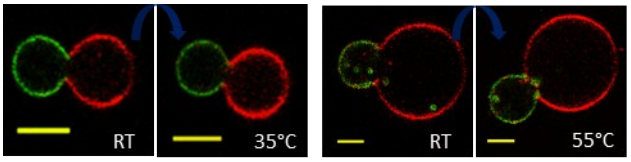

Figure S10. Fission of budded domains in 3K/POPC (90 weight % POPC) GHUVs promoted by temperature increases. Scale bars: 5 µm.

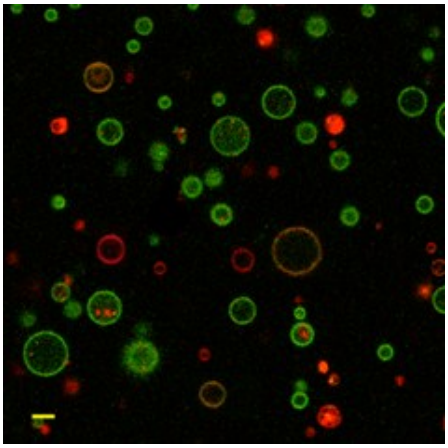

Figure S11. Confocal slice of PEO_{17}-*b*-$PDMS_{67}$-*b*-PEO_{17}/POPC GHUVs obtained from an initial mixture containing 75% POPC (% wt). Polymer is labelled with $PDMS_{26}$-*g*-$(PEO_{12})_2$-FITC and lipid with DOPE-Rhod. scale bars: 5 µm

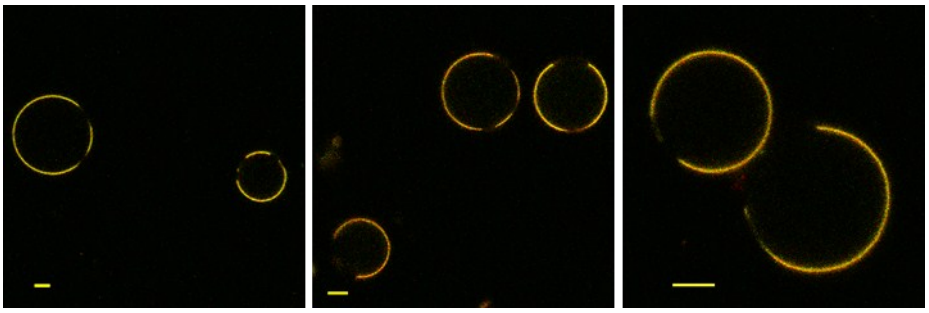

Figure S12. Phase separation in mixture of DPPC and triblock copolymers observed at 5% w/w of DPPC incorporated: from left to right: (1.5K/DPPC,3K/DPPC 5K/DPPC).. Scale bars: 5 µm.

S5. Detection of the nanodomains by FLIM-FRET

Table S1. FITC fluorescence lifetime (unit: ns) in each individual vesicle of 1.5K/POPC obtained from fluorescence single decay measurements (T_D : fluorescence lifetime in GHUV labeled with only donor- $PDMS_{26}$-g-$(PEO_{12})_2$-FITC; T_{DA} : fluorescence lifetime in GHUV labeled with both donor and acceptor-DOPE-Rhod).

1.5K/POPC							
0% POPC		5% POPC		10% POPC		15% POPC	
T_D	T_{DA}	T_D	T_{DA}	T_D	T_{DA}	T_D	T_{DA}
3.228	2.608	3.131	2.572	2.992	2.55	2.827	2.644
3.164	2.618	3.020	2.636	2.996	2.58	2.886	2.631
2.956	2.522	2.914	2.493	2.925	2.573	2.936	2.628
3.054	2.641	3.003	2.594	2.841	2.623	2.947	2.707
3.126	2.625	3.005	2.664	2.888	2.533	2.837	2.637
2.975	2.697	3.057	2.635	2.917	2.543	2.885	2.623
3.277	2.632	3.213	2.668	2.960	2.538	2.931	2.615
3.044	2.664	3.082	2.663	2.889	2.638	2.929	2.58
3.130	2.634	3.159	2.626	2.979	2.503	2.850	2.681
3.067	2.677		2.592	2.925	2.59	2.876	2.611
			2.572				
			2.614				
			2.61				
			2.665				
			2.606				
			2.589				

Table S2. FITC fluorescence lifetime (unit: ns) in each individual vesicles of 3K/POPC obtained from fluorescence single decays measurements (TD : fluorescence lifetime in GHUV labeled with only donor- $PDMS_{26}$-g-$(PEO_{12})_2$-FITC; TDA : fluorescence lifetime in GHUV labeled with both donor and acceptor-DOPE-Rhod).

3K/POPC					
0% POPC		10% POPC		40% POPC	
T_D	T_{DA}	T_D	T_{DA}	T_D	T_{DA}
2.981	2.604	2.868	2.727	2.873	2.808
3.017	2.629	2.831	2.759	2.862	2.771
3.156	2.813	2.856	2.723	2.913	2.866
3.021	2.702	2.826	2.624	2.963	2.749
3.080	2.673	2.879	2.705	2.738	2.863

3.035	2.597	2.823	2.661	2.839	2.811
3.058	2.598	2.873	2.717	2.759	2.804
3.049	2.686	2.747	2.734	2.776	2.733
3.031	2.621	2.844	2.673	2.766	2.708
3.121		2.689	2.552	2.769	2.821
2.979			2.675	2.724	
2.990			2.657	2.725	
3.007				2.731	
3.041				2.696	
2.993				2.786	
2.993					

Table S3. FITC fluorescence lifetime (unit: ns) in each individual vesicle of DOW/POPC obtained from fluorescence single decay measurements (τ_D : fluorescence lifetime in GHUV labeled with only donor- $PDMS_{26}$-g-$(PEO_{12})_2$-FITC; τ_{DA} : fluorescence lifetime in GHUV labeled with both donor and acceptor-DOPE-Rhod).

DOW/POPC					
0% POPC		10% POPC		15% POPC	
τ_D	τ_{DA}	τ_D	τ_{DA}	τ_D	τ_{DA}
3.024	2.676	2.959	2.642	2.695	2.539
3.052	2.642	2.972	2.597	2.723	2.536
2.984	2.629	2.873	2.658	2.715	2.535
2.993	2.743	2.906	2.592	2.738	2.639
3.026	2.652	2.861	2.620	2.774	2.562
3.123	2.739	2.941	2.608	2.793	2.522
3.118	2.650	2.869	2.607	2.752	2.543
2.973	2.711	2.885	2.556	2.710	2.540
3.027	2.723	2.864	2.600	2.711	2.527
2.931	2.645			2.761	

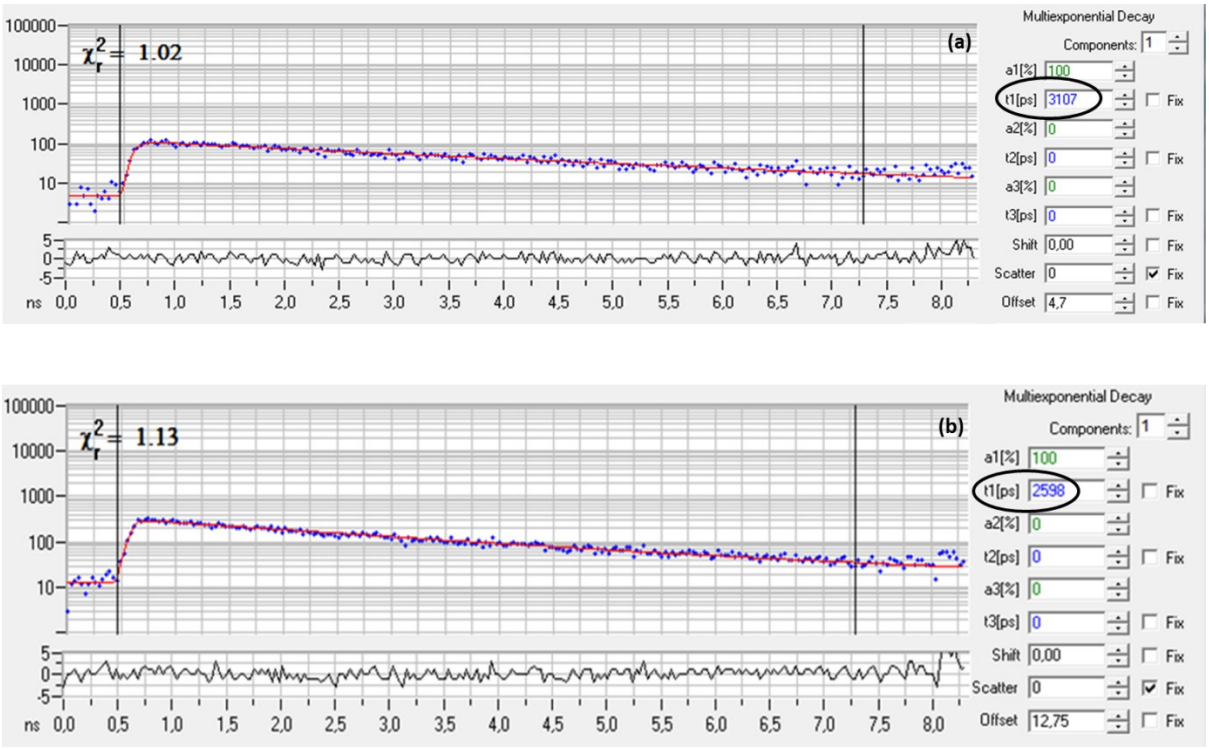

Figure S13. An example of one TCSPC histograms of $PDMS_{26}$-g-$(PEO_{12})_2$-FITC fluorescence: experimental data (blue) and fitted curve (red). Experimental data was well fitted with mono-exponential model (correlation factor χ^2 close to 1 and residuals showing minimal systematic variations). The abscissa is in ns.
(a) 1.5K/POPC 95%/5% GHUV labeled with only donor ($PDMS_{26}$-g-$(PEO_{12})_2$-FITC), with a fluorescence lifetime of ~ 3.1 ns.
(b) 1.5K/POPC 95%/5% GHUV labeled with both donor ($PDMS_{26}$-g-$(PEO_{12})_2$-FITC) and acceptor (DOPE-Rhod), with a fluorescence lifetime of ~ 2.6 ns.

FRET efficiency is determined *via* the changes in fluorescence lifetime of the donor in the absence (τ_D) and presence (τ_{DA}) of the acceptor, obtained from FLIM measurements:

$$E = 1 - \frac{\tau_{DA}}{\tau_D}$$

S6. Natural cooling process of hybrid vesicle suspensions

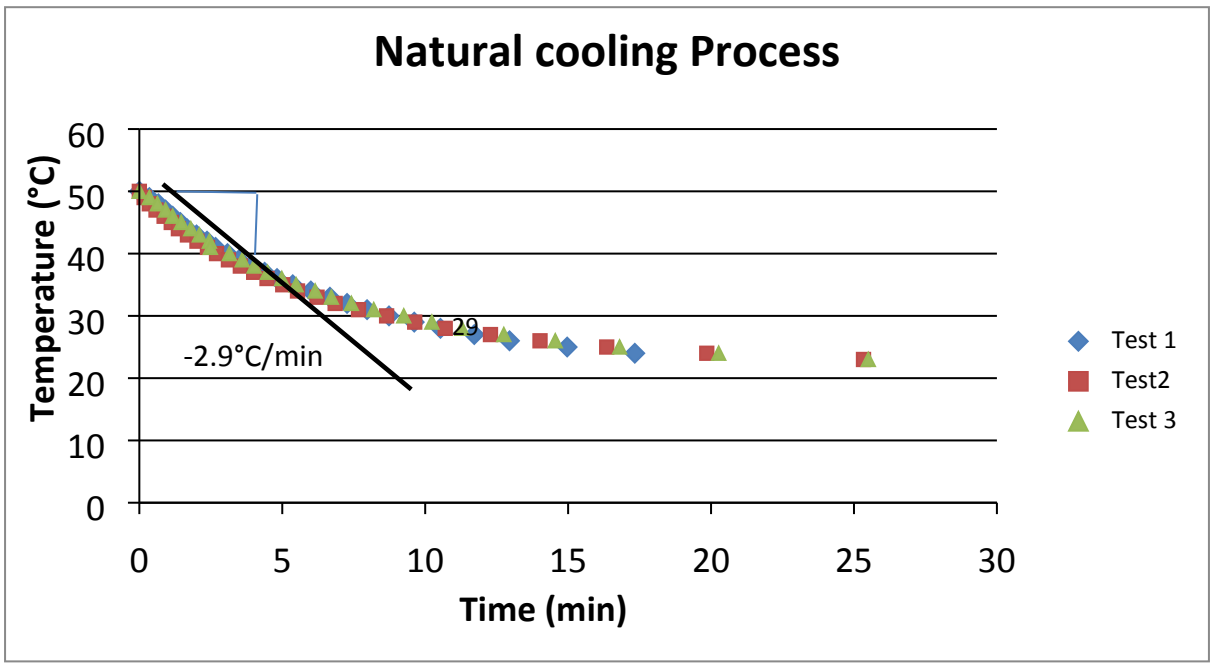

Figure S14. Evolution of temperature in the sample during the "natural" cooling process in room at 22°C.