Electronic supplementary information for

"Polymeric vesicle formation *via* temperature-assisted nanoprecipitation"

Junli Zhou[a*], Rong Ni[b, c*] and Ying Chau[a,b]

[a] Department of Chemical and Biomolecular Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

[b.] Division of Biomedical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

[c.] Institute for Advanced Study, the Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

* Equal contribution

E-mail: ying.chau@ust.hk

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1. EXPERIMENTAL METHONDS

1) Materials

Methoxypolyethylene glycol (M_n = 2000 and 5000), ε-caprolactone (97%), stannous octoate (95%), chloroform-d and polystyrene analytical standards (for GPC) were purchased from Sigma-Aldrich (St. Louis, USA). Diethyl ether was purchased from Scharlau (Sentmenat, Spain). Tetrahydrofuran (THF) and chloroform were purchased from Fisher Scientific (Waltham, USA).

2) Polymer synthesis

Methoxyl-PEG-b-PCL block copolymers with various molecular weights were synthesized by ring-opening polymerization.[1,2] Briefly, methoxypolyethylene glycol was first added into a clean vial and dehydrated under vacuum above 100 °C for three hours before further use. After that, a given amount of ε-caprolactone and catalyst stannous octoate was injected under nitrogen by a syringe. The reaction vial was then immersed into oil bath at 130 °C with stirring for at least 36 h. Afterward, the product was precipitated into cold diethyl ether to yield the final product.

3) Polymer characterization

Molecular weight and chemical structure of block copolymers were analyzed by nuclear magnetic resonance (NMR). 5-10 mg of each polymer was dissolved in 600 μL chloroform-d. All samples were centrifuged before the test. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer.

Polydispersity and molecular weight of polymers were characterized by gel permeation chromatography (GPC). The experiment was performed on a Waters (USA) GPC system with a 2695 separation module and a Waters 2414 refractive index detector, using tetrahydrofuran (THF) as the eluent at 25 °C with an

elution rate of 0.3 mL/min. Two Styragel columns (HR 3 THF, Waters, USA) in series were calibrated by polystyrene ($M_n \approx 3100, 8950, 22500$) of low polydispersity as standards.

Differential scanning calorimetry (DSC) was performed on a TA Instruments Q2000 Differential Scanning Calorimeter (New Castle, DE) using aluminum sample pans. Polymer samples were first heated at 20 °C min^{-1} to 100 °C, held at that temperature for 5 min, then cooled at 5 °C min^{-1} to -80 °C, and finally after a 5 min isotherm, heated to 100 °C at 5 °C min^{-1}.[3]

4) Particle preparation methods

For the normal nanoprecipitation, designed amount of polymer was first dissolved in organic solvent (e.g. THF) to reach the stock polymer concentration of 10 mg/m. 100µl polymer stock solution was then quickly added into 1ml Millipore water while stirring (maximal stirring speed on a stirring plate). The mixing was allowed to proceed for 30 min. After the mixing, THF was removed under vacuum in the desiccator for 1hr. The whole process was conducted under room temperature (20°C in this study).

For temperature-assisted nanoprecipitation (TAN) method, besides the water bath was heated up to 80°C before the addition of polymer stock solution (10mg/ml), the rest steps remain the same as the normal nanoprecipitation described above. The stirring lasted for 30min under 80°C with the viral open.

For the film rehydration, polymers were dissolved in chloroform to reach stock polymer concentration of 10mg/ml. 100µl polymer stock solution was added into a 10 mL centrifuge tube and chloroform was gently removed by purging nitrogen into the tube until a thin polymer film was formed. Then the film was further dried in vacuum overnight to completely remove trace of chloroform. 1ml hot millipore water (80°C) was added into the tube to rehydrate the film, and the stirring was allowed to proceed for one hour at 80°C.

5) Dynamic Light Scattering (DLS)

The particle size and polydispersity was measured by dynamic light scattering (DLS) with a particle size analyzer (90Plus, Brookhaven Instruments Corporation, NY, USA). Particles prepared by film rehydration were centrifuged at 5000 rpm for three minutes to exclude the micron size particles before the DLS test**.** 5 scans were averaged .

6) Cryo-transmission electron microscopy (cryo-TEM)

C-flat™ Holey Carbon Grids (Electron Microscopy Sciences) were glow discharged for 2min before use. Vitrobot (FEI) was used for sample preparation. The grid was first fixed on the plunge tweezers and then sent into humid chamber. 3 µL sample was deposited on the grid, then the sample was blotted for 1.5 seconds with filter papers before it was promptly vitrified by liquid ethane. Afterwards, sample was transferred to a grid box and stored in liquid nitrogen refrigerator before observation. All samples were observed using JEM 1000F (JEOL, Japan), 200kV.[3]

7). Confocal microscopy

Nile red was used as the fluorescent probe for confocal imaging. 2% mass ratio of Nile red was added into the polymer stock solution first, followed by the same particle or vesicle preparation methods mentioned in step 4). Then, 20µl particle sample was added on a clean glass slide, which was covered with a cover slide and sealed with transparent nail polish. The slides were viewed on confocal microscope with 100X oil lens.

2. NMR ANALYSIS

Molecular weight and chemical structure of block copolymers were analyzed by nuclear magnetic resonance (NMR). 5-10 mg samples were dissolved in 660 µL Chloroform-d and centrifuged before the

test. 1H NMR spectra were recorded on a Varian 300 MHz spectrometer. The monomer ratio in copolymers and copolymer molecule weights were calculated using the results.

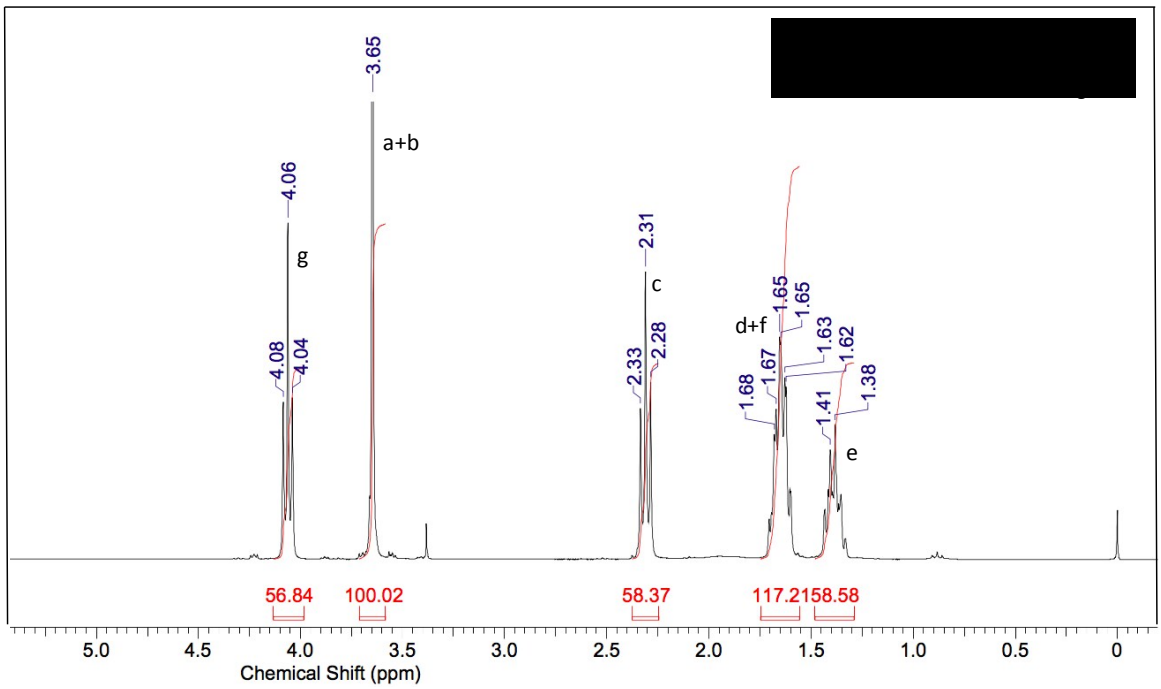


Fig. S1 [1]H NMR Spectra of mPEG(2k)-b-PCL(6k) (CL26).

CL26 was selected as an example to show the NMR spectra (Fig. 1) as well as the molecular weight calculation method. Molecular weights of two poly(ethylene glycol) methyl ether polymers are M_n=2,000 and M_n=5,000 respectively. Integral for EO proton (a+b) and CL proton (c) were calculated using ACD/NMR Processor Academic Edition software. Afterwards, the relative moles of EO, percentage of molecular weight of EO can be calculated. Finally, the molecular weight of copolymer can be calculated. All polymer molecular weights were calculated using this method and the results were shown in Table 1.

Table S1 Molecular weight calculation results of experimental polymers from [1]H NMR spectra

Polymer label	PEG M_n	Integral for EO protons	Integral for CL protons	EO%	Copolymer M_n
CL26	2000	100	56.84	25.32	0.80×10^4
CL212	2000	100	143.40	11.85	1.69×10^4
CL218	2000	100	183.40	9.51	2.10×10^4

3. GPC ANALYSIS

Polydispersity and molecular weight was characterized by gel permeation chromatography (GPC). The experiment was performed on a Waters (USA) GPC system with a 2695 separation module and a Waters 2414 refractive index detector, using tetrahydrofuran (THF) as the eluent at 25 °C with an elution rate of 1.0 mL/min. Two Styragel columns (HR 3 THF, Waters, USA) in series were calibrated by polystyrene (M_W= 3000, 10000, 20000) of low polydispersity as standard.

All data was processed using Empower 2 software according to the instructions. The processed results are listed in Table 2.

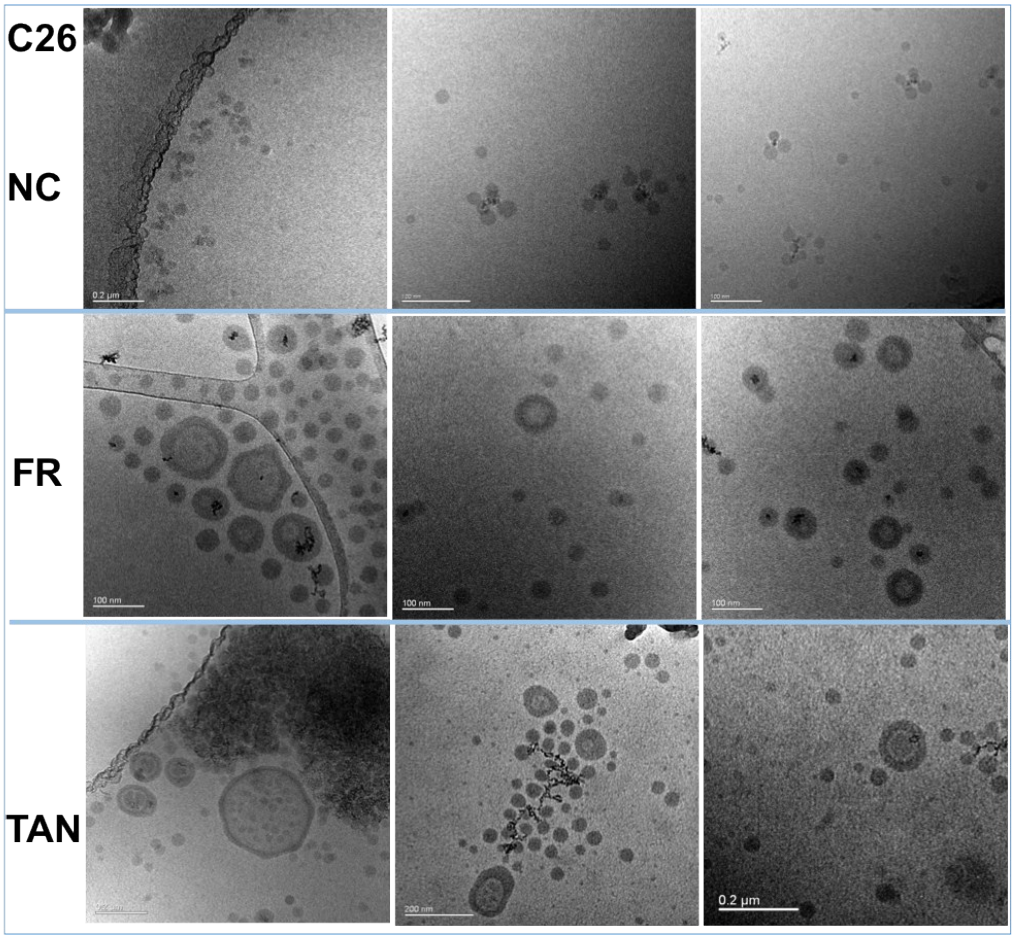
Table S2 Molecular weight and polydispersity results of experimental polymers from GPC

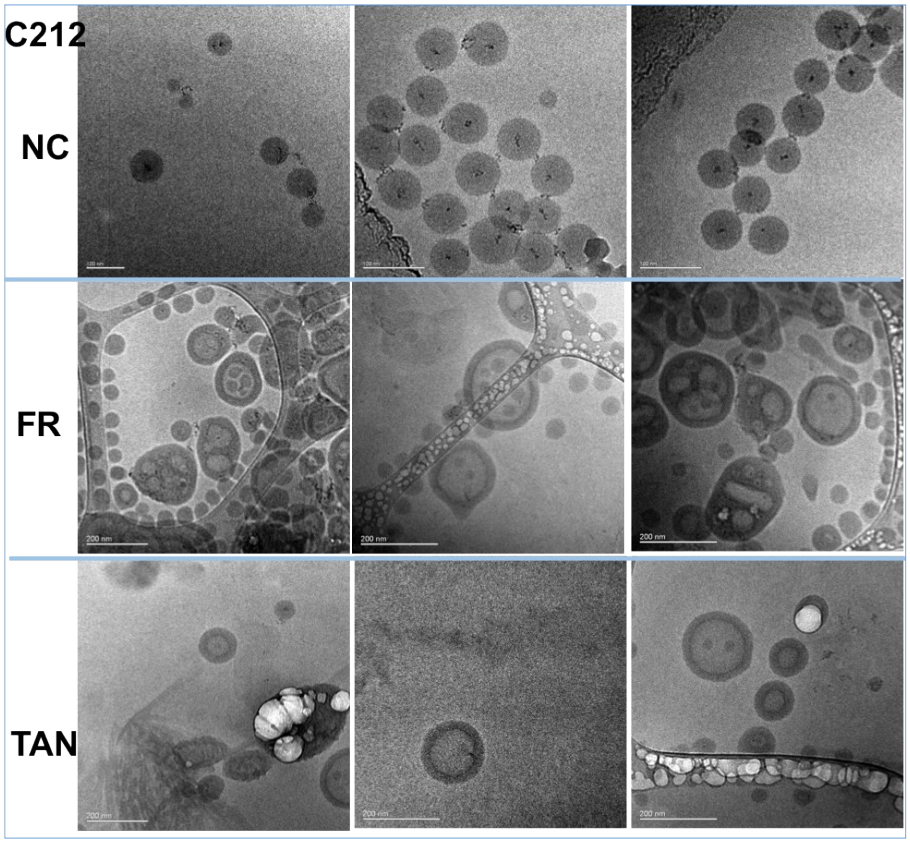
Polymer name	M_n	M_w	M_p	M_z	Polydispersity
CL26	8415	12861	12577	17823	1.53
CL212	10925	14451	13959	18218	1.32
CL218	18683	25060	24296	32227	1.34

4. CRYO-TEM IMAGES

Table S3. Calculated vesicle yield for both film rehydration and TAN methods based on Cryo-TEM images in Fig. S2 and the observation during imaging process.

Polymer	Method	Vesicle Ratio % (vesicle/particles)
CL26	FR	14% (10/71)
	TAN	13% (8/64)
CL212	FR	46% (50/109)
	TAN	54% (29/54)
CL218	FR	20% (7/35)
	TAN	18% (8/44)


C26
NC
FR
TAN
0.2 µm
100 nm
200 nm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FR
TAN
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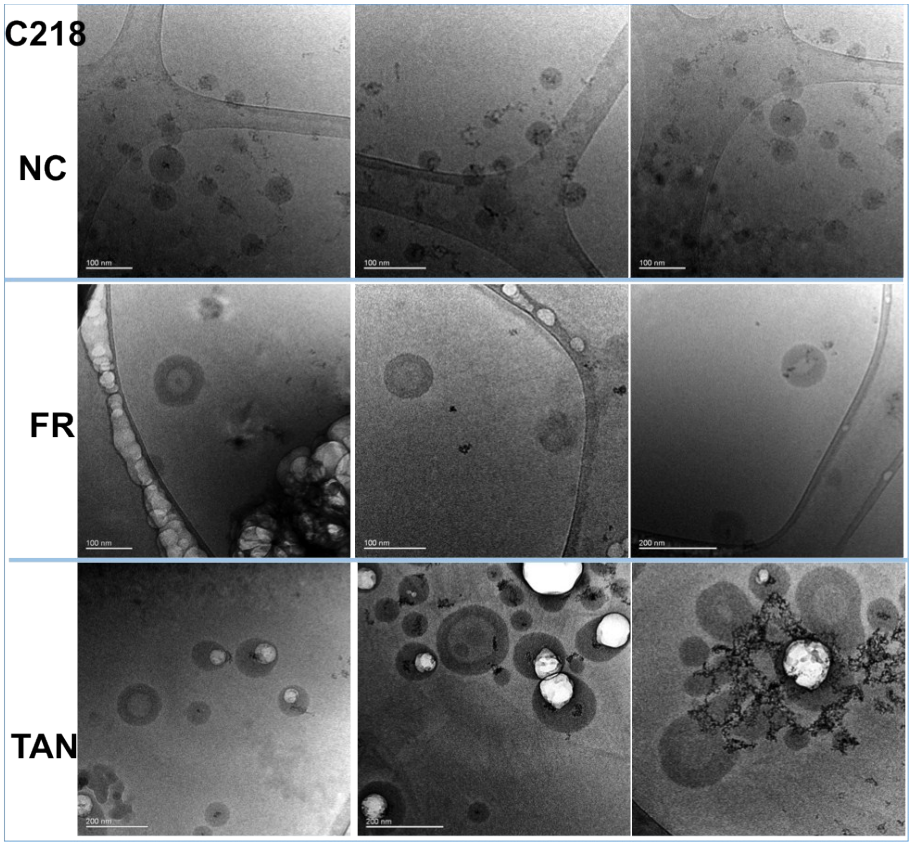


Fig. S2 More representative cryo-TEM images of particles prepared using nanoprecipitation (NP: T= 20 ºC), Film rehydration (FR) and temperature-assisted nanoprecipitation (TAN, T=80ºC).

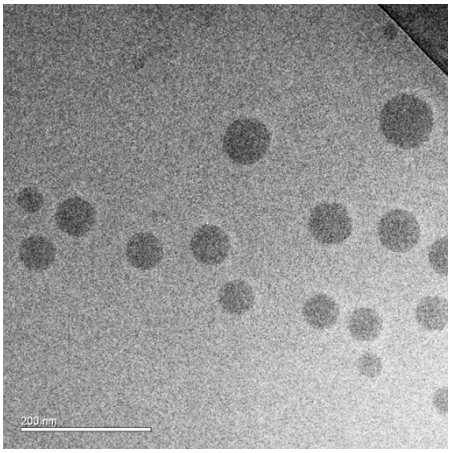
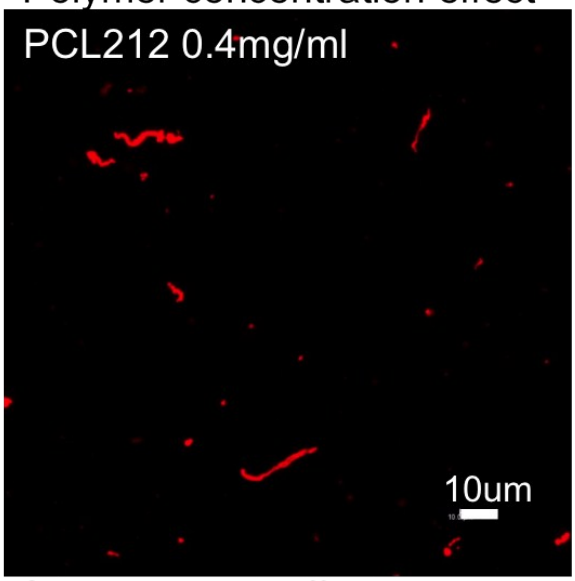
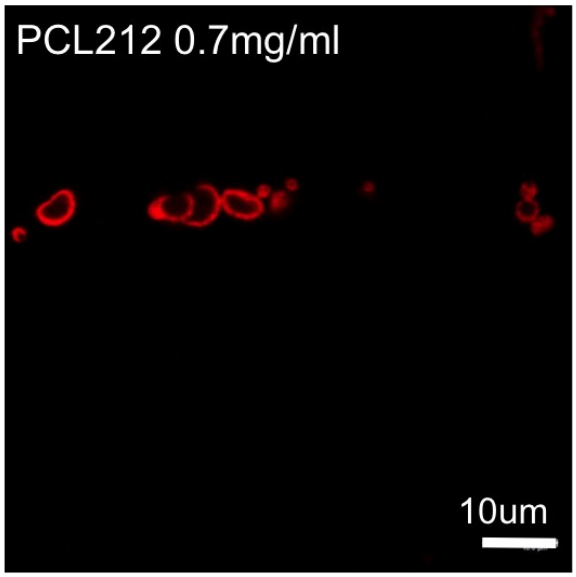


Fig. S3 Cryo-TEM images of C212 particles prepared using nanoprecipitation (T = 50 ºC). Scale bar = 200 nm.

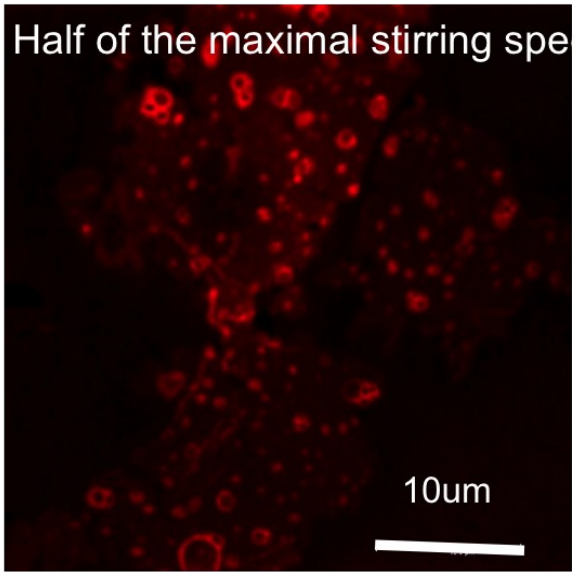
5. CONFOCAL MICROSCOPE IMAGES

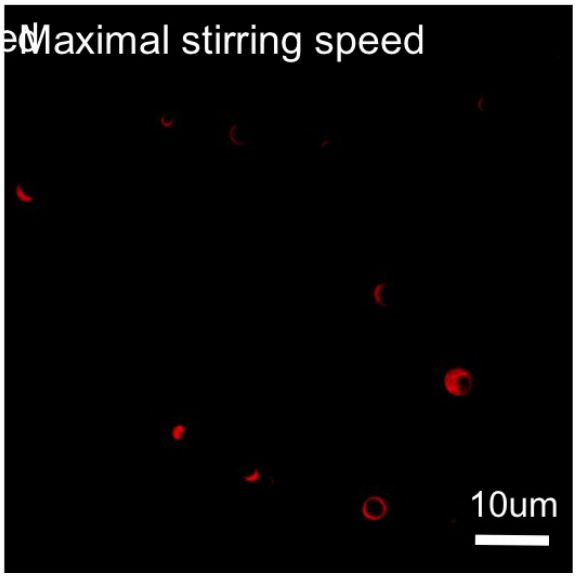
Polymer concentration effect





Stirring speed effect





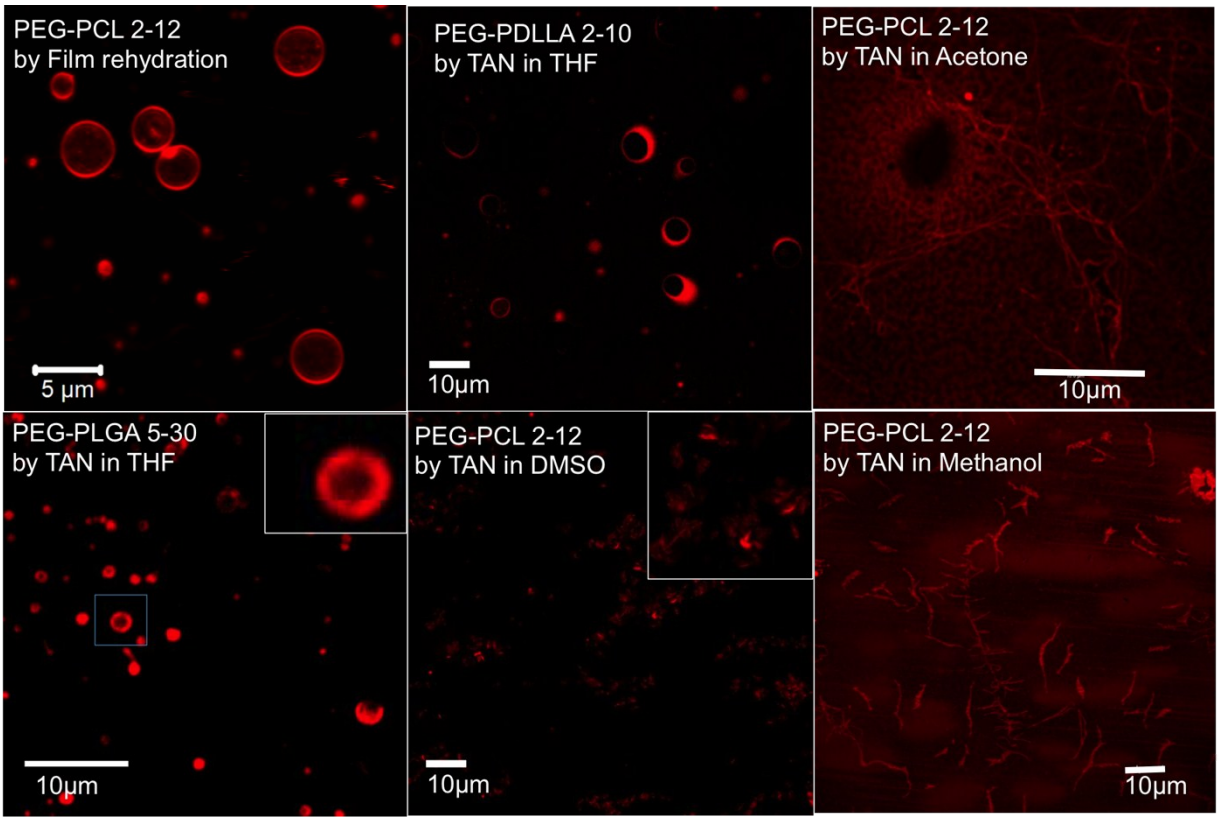


Fig.S4 Confocal microscope images of micron-size vesicles fabricated by film rehydration and TAN.

Reference

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