

# Giant vesicle formation through self-assembly of Chitooligosaccharide-based graft copolymers

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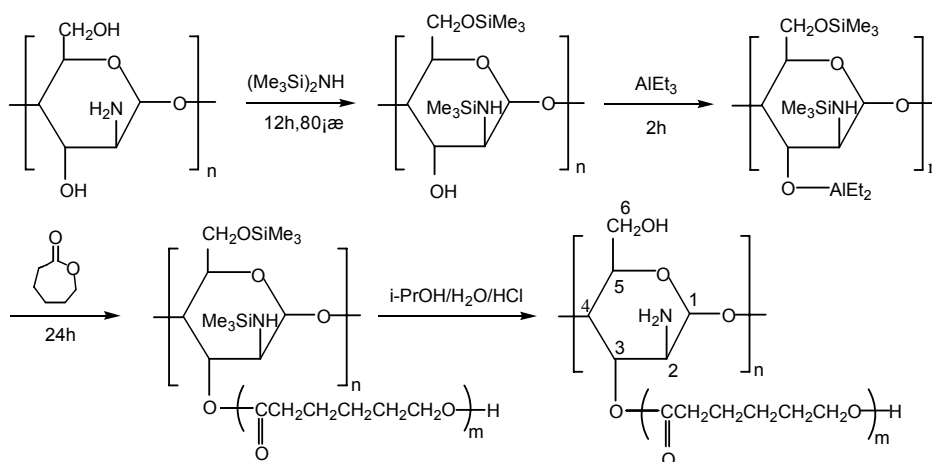
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## 1) Chemicals

Chitooligosaccharide ( $M_n=1800$ , the degree of deacetylation is 96%), purchased from Shenzhen Bright Way Novel Biomaterials Tech. Co. Ltd. (P. R. China), was dried at 60°C under vacuum.  $\epsilon$ -Caprolactone (Acros Organics, 99%) was purified by vacuum distillation over CaH<sub>2</sub> and the fraction collected at 96-98°C was used in polymerization. Triethylaluminum was obtained from Fluka Company and used as a 1.0M solution in toluene. Chloroform and pyridine were purified by usual distillation method. Hexamethyldisilane (HMDS) and other reagents were used as received without further purification.

## 2) The preparation of COS-g-PCL

In the work, the following three-step procedure was used to prepare the targeted copolymer, as shown in scheme S1.



Scheme S1. Synthetic route to COS-g-PCL

## Trimethylsilylation of Chitooligosaccharide

Chitooligosaccharide (5.0g, 30.9mmol pyranose) was placed in a dried glass reactor that was previously flushed with high purity N<sub>2</sub> for 30 min. and added 50ml of DMSO. Under vigorous stirring, the dispersion was heated at 70°C for 6h to form a clear solution and then allowed to cool to room temperature. Predetermined amount of hexamethyldisilane (the molar ratio of hexamethyldisilane to pyranose unit was 2.0) were added, and after being heated at 80°C for 12h with stirring, the mixture was poured into 150ml of acetone. The precipitate was filtered, washed with water, and dried in *vacuo* to give pale tan powder. The degree of substitution (DS) of COS was calculated from the C/N value of elemental analysis to be 1.1.

## Graft Copolymerization and Deprotection

The typical of procedure was described as follows: 1.0 g trimethylsilylChitooligosaccharide

TMSCOS with DS=1.1 was dissolved in Chloroform of 50ml and then a certain amount of triethylaluminum in toluene (the molar ration of Et<sub>3</sub>Al to OH contained in TMSCOS was 1.2:1) was added dropwise via a syringe through a rubber septum. The reaction was kept for two hours and then a desired amount of the  $\epsilon$ -caprolactone monomer was transferred into the reactor. Under N<sub>2</sub>, the graft copolymerization was allowed to proceed for 24h at ambient temperature while stirred. When the setting reaction time of 24h elapsed, 5ml mixture of isopropyl alcohol/H<sub>2</sub>O/HCl was added and the reactor was stirred for further 4h to remove the TMS group. The resulting polymer was purified by repeated precipitation (3 times) using 10ml of chloroform and 300ml alcohol as a solvent and precipitant pair. It was dried for 72h at 50°C in *vacuo* to give the graft copolymer g<sub>1</sub> and g<sub>2</sub>.

### 3) $^1\text{H}$ NMR determination

$^1\text{H}$ -NMR spectra for  $\text{g}_1$  were taken in  $\text{CDCl}_3$  by a Bruker AV 400 spectrometer operating at 400(Figure S1). In the spectra of  $\text{g}_1$ , the signal assignment was concluded as 1.24, 1.46, 2.26, and 3.49ppm, respectively referring to the  $\gamma$ -,  $\delta + \beta$ -,  $\alpha$ -, and  $\epsilon$ -methylene protons next to the carbonyl group of the PCL branch.

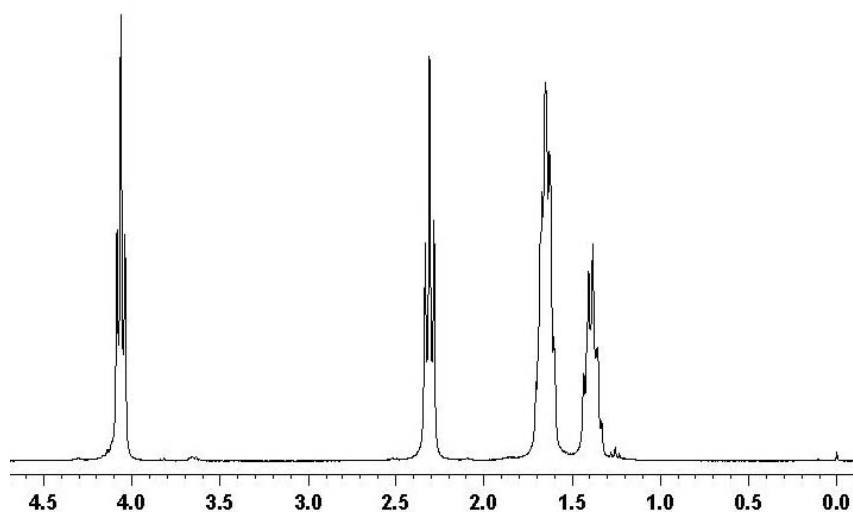


Figure S1.  $^1\text{H}$ -NMR spectra of the graft copolymer  $\text{g}_1$

### 4) GPC determination

The molecular weight ( $M_n$ ) and molecular weight distribution ( $M_w/M_n$ ) were measured with a Waters 515 GPC instrument equipped with Styragel columns (101-, 102-, and 103-nm pore sizes) with tetrahydrofuran(THF) as the mobile phase. Polystyrene and Waters Millennium 32 were used as calibration standards and data-processing software, respectively.

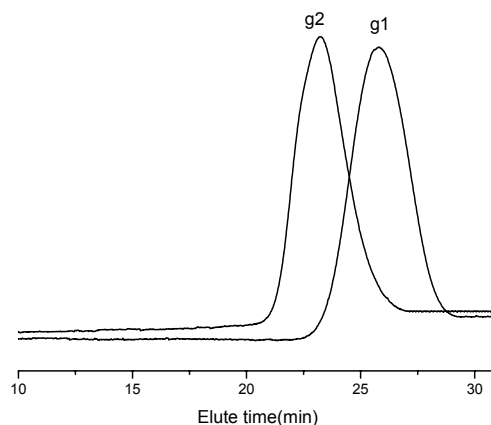


Figure S2 GPC traces of the resultant  $\text{g}_1$  and  $\text{g}_2$

Table S1. Structure details of vesicle-forming graft copolymer<sup>[a]</sup>

Sample	$\overline{M}_n$ [ $\text{g} \cdot \text{mol}^{-1}$ ]	PDI <sup>[b]</sup>	DP <sup>[c]</sup>
<b>g1</b>	13800	1.7	61
<b>g2</b>	29600	1.7	100

[a] The degree of polymerization of COS used for grafting is 11. [b] PDI: the polydispersity index of COS-g-PCL. [c] DP: the degree of polymerization of PCL branch.  $\overline{M}_n$  and PDI were determined from GPC with tetrahydrofuran (THF) as an elution solvent and monodisperse poly(styrene) as standards, and DP for PCL was calculated from the signals at 4.06 ( $-\text{CH}_2\text{O}-$ , repeating unit) and 3.65 ( $-\text{CH}_2\text{OH}$ , end group).

### 5) The stability of the prepared GVs

The GVs suspension was transferred into an observation chamber and observed with a phase-contrast microscope (Olympus BX-51), equipped with a JVC color video camera and connected to an image-recording system. To determine the effect of temperature on the stability of GVs. Slow uniform heating was achieved with a resistive heating stage constructed in our lab, consisting of a backside metal coated microscope coverslip and a current limiting standard power supply. Temperature in the droplet close to the vesicle was measured using a calibrated micron-sized Cu/Ni thermoelement. The temperature measurement was time synchronized with the time readings on the videotape. The results are displayed in Figure S3. It indicates that the GVs in suspension are stable over the temperature spanning from 25 °C to 50 °C. At temperature above 60 °C, the GVs gradually dissolve in 1, 4-dioxane/water mixtures.

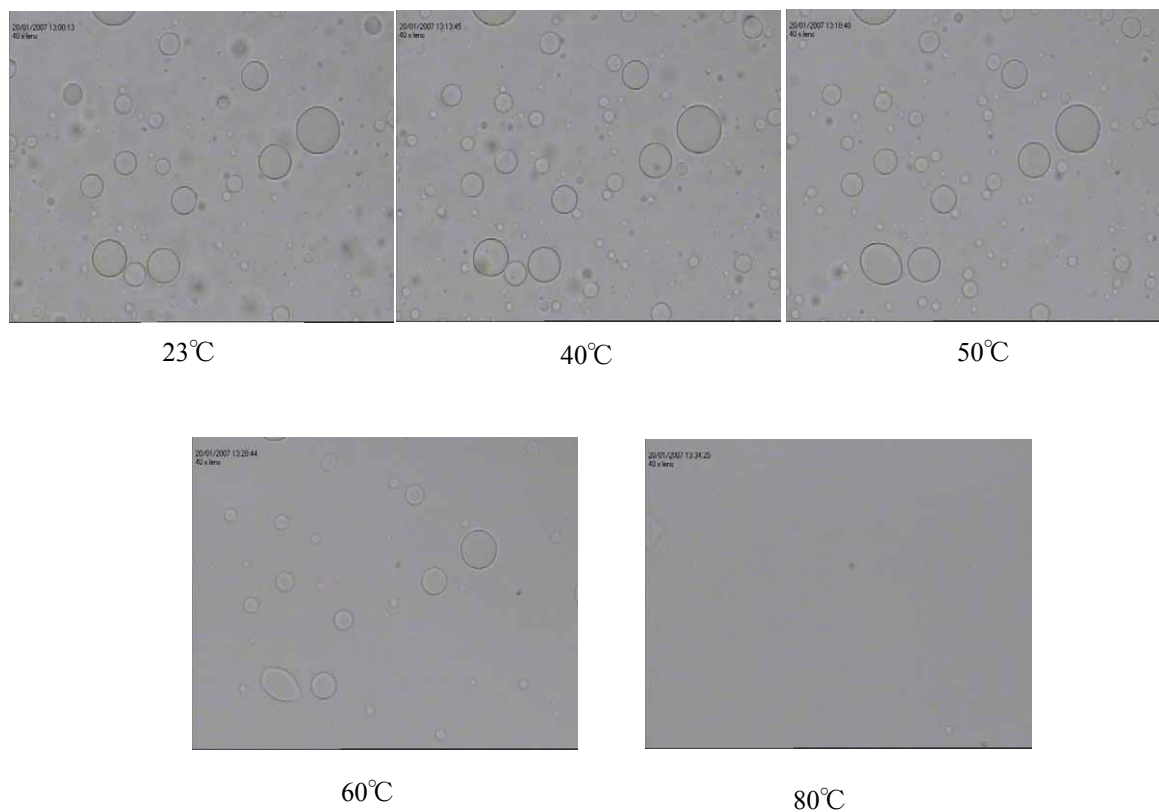


Figure S3 The shape change of GVs at different temperature.

### 6) DSC determination of GVs suspension

The thermal phase behavior of GVs self-assembled from  $g_1$  and  $g_2$  were recorded using DSC heating scans (PerkinElmer Thermal Analysis) at a rate of 30°C/h with final GVs concentration of 0.5wt% in dioxane/water mixture in the DSC cell (Figure S4). For GVs obtained from  $g_1$ , the enthalpy is 0.14J/g with a maximum heat capacity at  $T=53.9$ . For GVs obtained from  $g_2$ , the enthalpy is 0.21 with a maximum heat capacity at  $T=56.9$ . The results show that the both the melting points and the degree of crystalline of GVs increase with PCL branch length.

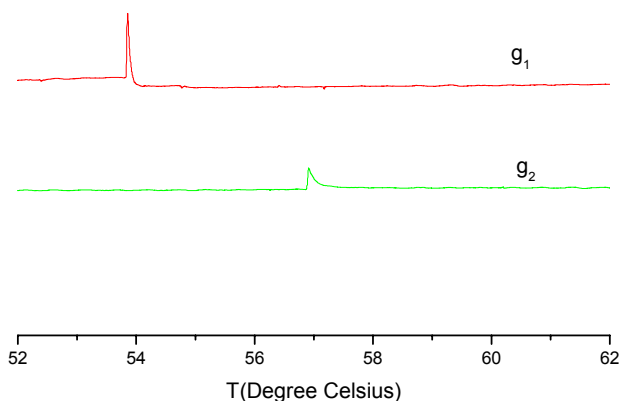


Figure S4. The DSC traces of GVs self-assembled from  $g_1$  and  $g_2$  in dioxane/water mixture

### 7) Confocal fluorescence microscopy of GVs

The Nile Red solution in acetone (1mM, prepared prior to use) was added to the suspension of GVs in dioxane/water mixture to make a Nile Red concentration of 1 $\mu$ M. Fluorescence images were recorded using the microscopy (Olympus BX 61, Olympus Optical Co., Tokyo, Japan) with a confocal optical scanner (Yokogawa Inc., Tokyo, Japan) and an argon ion laser with 550nm excitation wavelength. Images were recorded with a digital CCD camera interfaced to a computer, and operated by the software provided by the camera manufacturer.

### 8) TEM of the formed aggregates

A drop of **g**<sub>1</sub> and **g**<sub>2</sub> GVs suspension was deposited, respectively, on a copper grid coated with a carbon film and stained with a drop of phosphorous tungstic acid in water (2 wt%). The copper grid was then dried at room temperature prior to measurement. TEM was performed on a JEOL JEM-1200 electron microscope at an acceleration voltage of 120KV. The TEM image clearly reveals the vesicular structure, with lamella thickness of ca.12.1nm for **g**<sub>1</sub> vesicle and ca.23.0nm for **g**<sub>2</sub> vesicle (Figure S5).

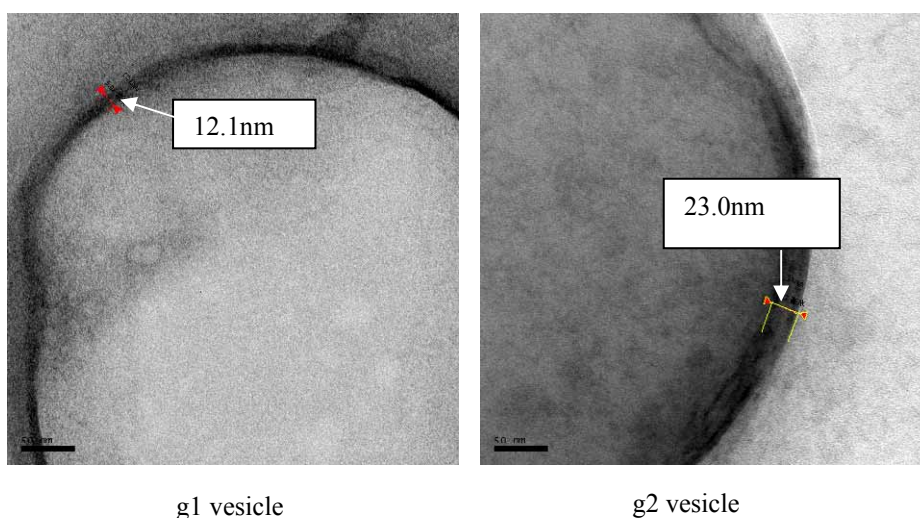


Figure S5 TEM images of **g**<sub>1</sub> and **g**<sub>2</sub> vesicle

### 9) Self-assembly mechanism section

The COS-g-PCL molecule has a hydrophilic COS backbone and hydrophobic PCL branches, so the aggregation of COS-g-PCL molecules driven by the hydrophobic interaction in water/dioxane mixture is well expected. On the other hand, there are a lot of hydroxyl and amino groups along the backbone of COS, the formation of hydrogen bonds is another potential interaction for the self-assembly. To verify this, trimethylsilyl chitoooligosaccharide-graft-poly(caprolactone) (TMSCOS-g-PCL) with trimethylsilylation of amino and hydroxyl groups were utilized to self-assemble. The results are shown in Figure S6. As shown in Figure S6, no GVs were obtained.

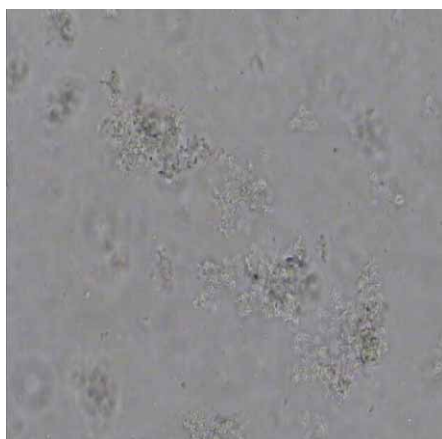
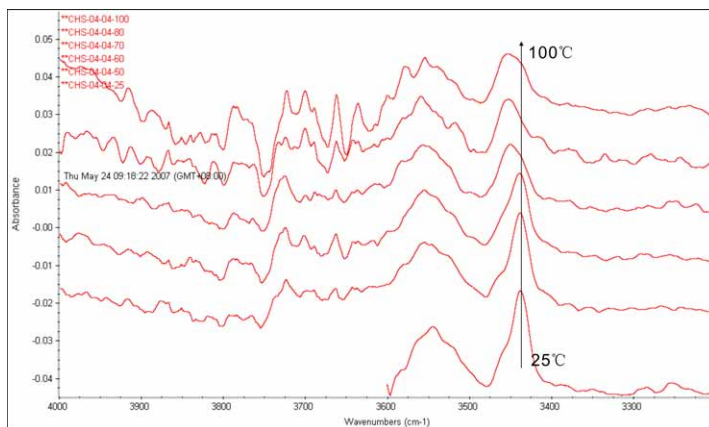


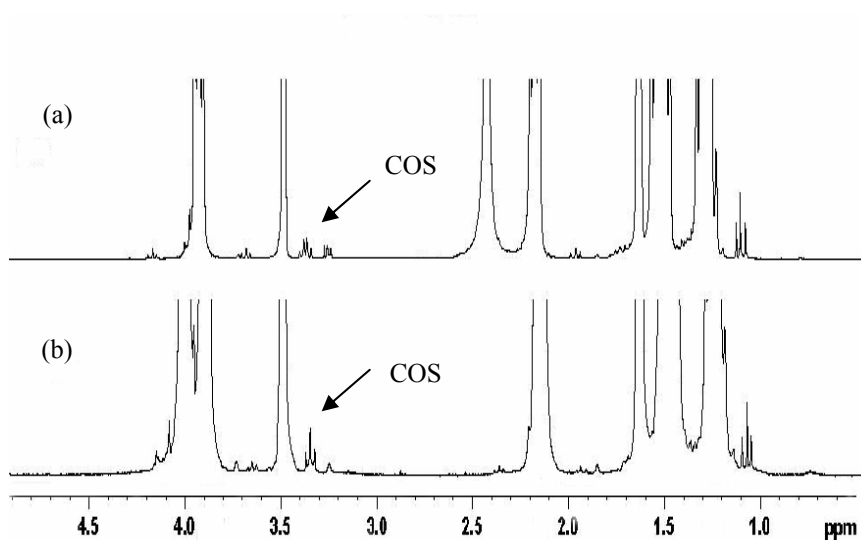
Figure S6. The aggregates formed from the solution of TMS-COS-g-PCL

Variable temperature FTIR was used to detect the hydrogen bonds in the resulting dried vesicles. Variable temperature FTIR was conducted on Nicolet Avatar 360 under N<sub>2</sub> from 25 °C to 80 °C, and the vesicle solution was coated on the KBr crystal wafer for the measurement. The samples were carefully dried before the measurement. The results are provided in Figure S7. It was found that with increasing temperature from 25 °C to 80 °C, the peak of the hydroxyl groups gradually becomes weaker and shifts to the high wavelength side, which clearly indicates that a certain amount of hydroxyl groups have formed hydrogen bonds in the dried vesicles originated from the self-assembly of COS-g-PCL molecules.



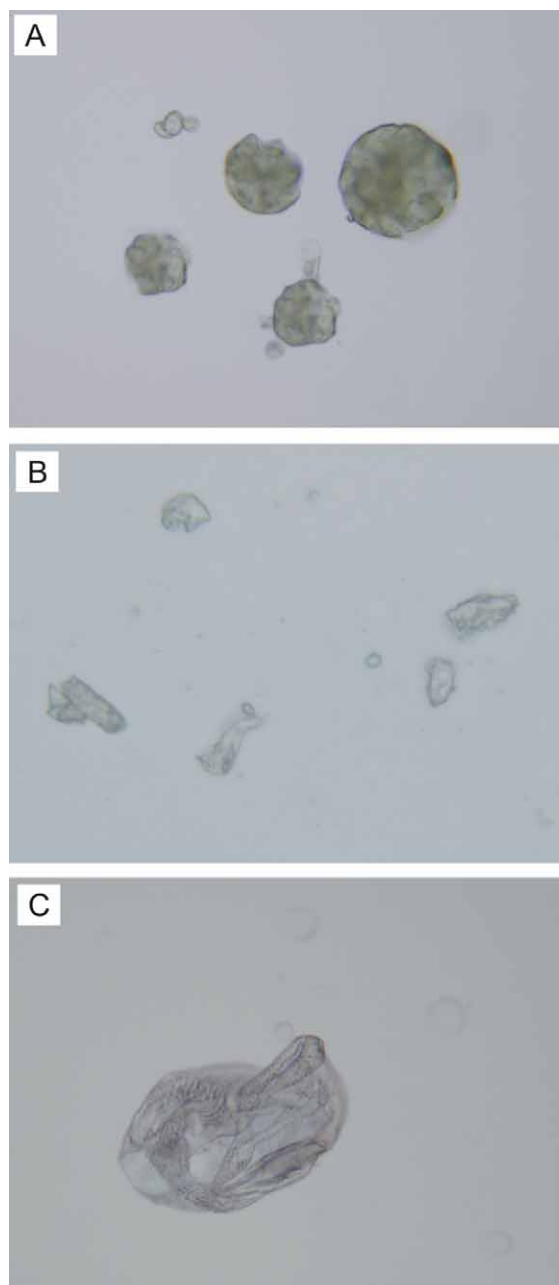
**Figure S7.** Variable temperature FTIR spectra of dried giant vesicles from COS-g-PCL molecules. The temperature of the related curve from the bottom to the top is 25 °C, 50 °C, 60 °C, 70 °C, 80 °C and 100 °C, respectively.

The formation of the vesicles in 1,4-dioxane/water mixtures is evidenced by their <sup>1</sup>H NMR spectra (Figure S8). Different from that measured in 1,4-dioxane (Figure S8a), the peaks related to COS become stronger (Figure S8b). It implies that the PCL segments form the middle layer of the vesicle wall, and the COS segments form the outer layers, which remain in a solvated state.



**Figure S8.** <sup>1</sup>H NMR spectra of copolymer g1 in different solvent. (a) Spectra of g1 copolymer solution in dioxane; (b) Spectra of g1 vesicles in 1,4-dioxane/water mixture with 25wt% water content.

### 10) The effect of crosslinking on the stability of GVs in different solvents

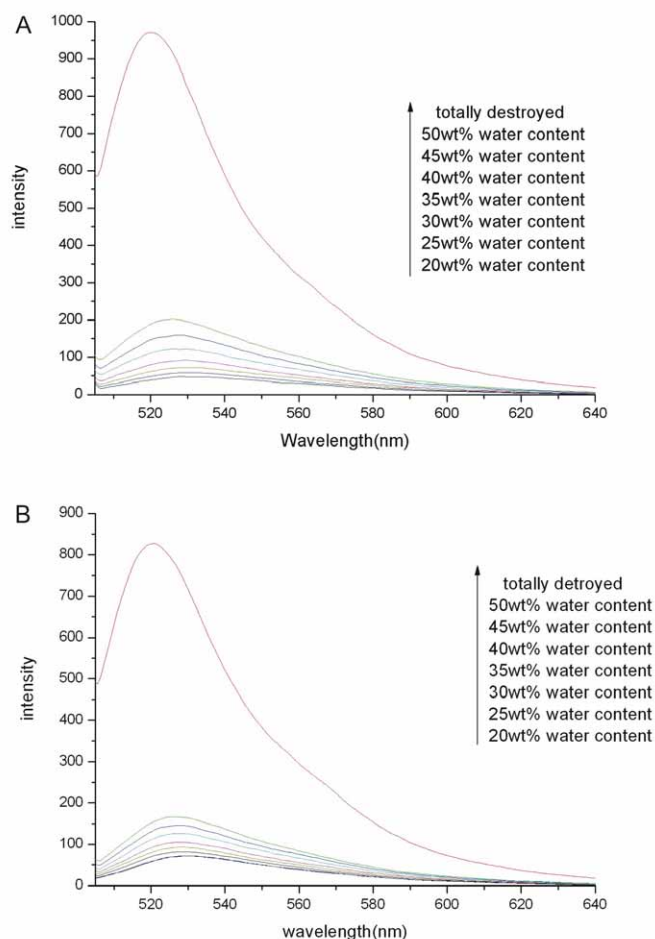


**Figure S9.** Photographs of the crosslinked vesicles in water (A), dioxane (B) and ethanol (C)

### 11) Quantitative information about release of the prepared vesicles in the course of adding water into vesicle suspension

The quantitative information about release of the prepared vesicles in the course of adding water into vesicle suspension was evaluated according to the commonly used method (A. D. Tirrell: *Langmuir* **2000**, 16, 122). To achieve this, calcein loaded vesicles were prepared by adding water into the mixture of 3ml graft copolymer solution (0.5wt% in dioxane) and 30 $\mu$ l calcein solution (25mM in water) to reach 20wt% water content. Then, appropriate amount of CoCl<sub>2</sub> (50mM in water) was introduced to quench the fluorescence of unencapsulated calcein molecules. Efflux of calcein was monitored by measuring the increase of fluorescence emission at 525 nm (excited at 495 nm). The maximum fluorescence intensity was given by the complete release of the encapsulated calcein caused by addition of 3-fold excess water into the suspension.

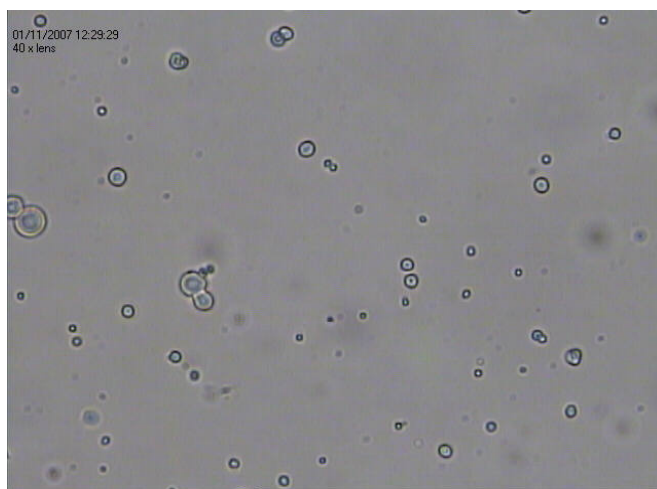
We found that a relatively low release of calcein occurred in the course of adding water into the vesicle suspension, suggesting a high stability of the prepared vesicles.



**Figure S10** The release of calcein from the prepared vesicles in the course of adding water into vesicle suspension: (A) **g1** vesicles; (B) **g2** vesicles

## 12) Formation of the giant vesicles in total water medium

The formation of the giant vesicles in total water medium was also achieved. The preparation procedure is as follows: the synthesized copolymer was first dissolved in dioxane, and 3ml dioxane solution was evaporated using a rotary evaporator to get thin polymer film in a smaller vessel. Then, the thin film was covered with pure water (10 mL of Milli-Q water) and ultrasonicated with a tip for 30 min at room temperature for the formation of the vesicles.



**Figure S11** Photograph of the prepared giant vesicles in total water medium