Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2012

Supporting Information

Novel Drug Carriers: From Grafted Copolymers to Cross-linked Vesicles

Jiangtao Xu,[a] *Qiang Fu,*[a] *Jing M. Ren,*[a] *Gary Bryant,*[b] *Greg G. Qiao*[a,]*

[a] Department of Chemical and Biomolecular Engineering, The University of Melbourne, Parkville, Victoria 3010, Australia

[b] School of Applied Sciences, RMIT University, Melbourne, Victoria 3000, Australia

*Corresponding author. E-mail: gregghq@unimelb.edu.au

Experimental Section

Materials: poly(ethylene glycol) monoether (Mn ~ 2000 Da), *N*-propargylamine (98%), copper(I) bromide (CuBr, 98%), *N*,*N*,*N'*,*N''*,*N''*-pentamethyldiethylenetriamine (PMDETA, 99%), Grubbs catalyst (2nd Generation) $(H_2IMes)(PCy_3)(Cl)_2RuCHPh$, *cis*-dichlorodiaminoplatinum (II) (CDDP, 99.9%), pentaerythritol tetrakis(3-mercaptopropionate) (PETMP, >95%), 2,2-dimethoxy-2-phenyl acetophenone (DMPA, 99%) and anhydrous pyridine (99.8%) were all purchased from Aldrich and used as received. *N*,*N*-dimethylformamide (99.8%, Merck), *n*-hexane (AR, Chem-Supply), diethyl ether (AR, Chem-Supply), sodium azide (NaN_3, 99%, Chem-Supply), 1,8,9-anthrancenetriol (DIT, puriss, Fluka) and trans-2-[3-(4-tert.-butylphenyl)-2-methyl-2-propenylidene]-malononitrile (DCTB, puriss, Fluka) were also used as received. Exo-7-oxabicyclo[2.2.1] hept-5-ene-2,3-dicarboxylic anhydride (oxa-norbornenyl anhydride, ONBAn)[1], azido-terminated poly(ethylene glycol) monomethyl ether with

molecular weight 2000 Da (PEG2K-N_3),[2,3] and pyridine modified Grubbs Catalyst (2nd Generation) $(H_2IMes)(pyr)_2(Cl)_2RuCHPh$ [4] were synthesized according to literature procedures.

Instrumentation. Gel permeation chromatography (GPC) (THF as eluent) was performed on a Shimadzu liquid chromatography system fitted with a Wyatt DAWN EOS multi-angle laser light scattering (MALLS) detector (690 nm, 30 mW) and a Wyatt OPTILAB DSP interferometric refractometer (690 nm), using three Phenomenex Phenogel columns (500, 104, and 106 Å porosity; 5 μm bead size) operated at 1 mL/min with column temperature set at 40 ^oC. Astra software (Wyatt Technology Corp.) was used to process the data to determine the molecular weights based on the assumption of 100% mass recovery of the polymer where the dn/dc value was unknown.

^1H NMR spectroscopic analysis was performed on a Varian Unity Plus 400 MHz spectrometer using the deuterated solvent as reference.

Hydrodynamic diameters (D_h) and size distributions of the nanoparticles in aqueous solutions were determined by dynamic light scattering (DLS). DLS measurements were performed using a Malvern high performance particle sizer (HPPS) with a 3.0 mW He-Ne laser operated at 633 nm at an angle of 173^o (back scattering) and a constant temperature of 25 ± 0.1 ^oC.

Static light scattering (SLS) measurements were performed using a commercially available laser light scattering spectrometer (ALV-DLS/SLS-5022F) equipped with an ALV 6010 multiple Tau correlator and Avalanche Photodiode Detectors (APD). Samples were prepared in millipore water, filtered with 0.2 μm filters, at concentrations from 0.5 ~ 2 mg/mL. Samples were transferred to cylindrical glass scattering cells, and placed in an index matching fluid (ethanol) in a temperature control at 25 ^oC. A He-Ne laser of wavelength 632.8 nm illuminates the sample, and an avalanche photodiode detector (APD), located on a goniometer, measures the scattered intensity at a specific scattering angle θ. The accessible angular range is 15^o ~ 150^o, corresponding to a q range of 3.8 ~ 28μm^{-1}, encompassing the peak in the

structure factor at $q \sim 19\ \mu m^{-1}$. The data acquisition was carried out using the ALV-Correlator Control Software, and the accumulation times at each angle were 300 s to 600 s, depending on concentration.

Matrix-assisted laser desorption/ionization Time of Flight (MALDI-ToF) mass spectroscopy was performed on a Bruker Autoflex III Mass Spectrometer operating in positive linear mode; the analyte, matrix (DCTB) and cationisation agent (NaI) were dissolved in THF at concentrations of 10, 10, and 1 mg/mL, respectively, and then mixed in a ratio of 10:1:1. Then 0.3 μL of this solution was spotted onto a ground steel target plate and the solvent was allowed to evaporate prior to analysis. FlexAnalysis (Bruker) was used to analyze the data.

UV-vis spectrometry was performed on a Shimadzu UV-2101PC spectrometer using quartz cuvettes with a 1 cm path length.

Transmission electron microscopy (TEM) samples were prepared by directly depositing a droplet of sample solution on copper grid coated by Formvar/Carbon film and draining excess solution by using filter paper after 60 s. To negatively stain the sample, a droplet of 2% (w/v) uranyl acetate solution was placed on the copper grid for 30 s before being drained with filter paper. The grid was then dried in the air for another 20 min. The images were taken using a FEI Tecnai TF20 transmission electron microscope operated at 100 kV. Images were acquired digitally with a Gatan US1000 2k × 2k CCD Camera.

Cryo-transmission electron microscopy (Cryo-TEM) samples were prepared and observed as described by Eisenberg.[5] Frozen hydrated grids of polymer samples were observed on a FEI Tecnai F30 microscope operating at 200kV. Samples were applied to glow-discharged Quantifoil grids (Quantifoil Micro Tools GmbH), blotted and frozen in liquid ethane slush and stored in liquid nitrogen until loaded onto a cryogenic sample holder (Gatan 626). Images recorded on a Gatan Ultrascan 4k × 4k Digital (CCD) Camera System under low dose conditions (~10 $e^- Å^{-2}$) at a nominal magnification of 50,000×.

Atomic Force Microscopy (AFM) sample was prepared by depositing a droplet of sample solution on silicon wafer which was treated by Piranha solution. After drying in the air, it was subjected directly for measurement. The images were recorded in air with a JPK microscope operating in dry Tapping mode. The probes were commercially available silicon tips (MicroMasch) with a spring constant of 42 N/m, a resonance frequency of 285 kHz and a typical radius of curvature in the 10~12 nm range. Silicon wafer freshly treated by Piranha solution was used as sample substrate materials.

Thermogravimetric analysis (TGA) was performed on a PerkinElmer Pyris-1 thermogravimetric analyzer, and the samples were heated from 70 to 700 ^{o}C at a heating rate of 10 ^{o}C/min under a atmosphere flow (20 mL/min).

Synthesis of *ω*-oxanorbornenyl alkyne (ONB-alkyne), 1. Precursor ONB-alkyne was prepared from ONBAn as reported in the literature.[6] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.55 (s, 2H, CH*C***H**=*C***H**CH), 5.31 (s, 2H, O*C***H**CH=CH*C***H**O), 4.24 (d, 2H, N*C***H**$_2$CCH), 2.90 (s, 2H, *C***H**CON), 2.19 (s, 1H, NCH$_2$C*C***H**). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 174.3, 137.1, 81.1, 75.2, 70.6, 46.9, 27.1.

Synthesis of *ω*-oxanorbornenyl poly(ethylene oxide) (M_n = 2000) macromonomer (ONB-PEG2K, 2) via "click" coupling reaction between ONB-alkyne, 1 and PEG2K-N_3. The general procedure was followed as reported in the literature.[3,6] The ^{1}H NMR and MALDI-TOF mass characterization of the final product was utilized to confirm the integrity of polymerizable oxanorbornene functionality.

Linear-brush type diblock copolymers of poly(ONBAn)-*b*-poly(ONB-PEG2K), 3, by ring-opening metathesis polymerization (ROMP). In a typical experiment, 8 mL of distilled THF was degassed by three freeze-pump-thaw cycles. First monomer ONBAn (0.12 g), second monomer ONB-PEG2K (0.162 g) and pyridine modified 2nd generation Grubbs' catalyst (21 mg) were placed in three reaction vessels and evacuated by argon for 5 min. 2 mL of THF was added into each monomer flask

and 4 mL into catalyst. After a few minutes, the catalyst solution was added into monomer solution under vigorously stirring. After 5 min, second monomer solution was injected rapidly into the reaction mixture. The reaction mixture was stirred for further 3 hours. The polymerization was terminated by the addition of a few droplets of ethyl vinyl ether. The polymer was then precipitated from diethyl ether twice to yield a grey solid powder. Yield = 100%.

The first monomer ONBAn was easily polymerized to a nearly quantitative yield in 5 min, which was confirmed by the total disappearance of vinyl proton signal at δ 6.51 ppm attributed to the oxanorbornene monomer in ^{1}H NMR spectrum (data not shown). The second monomer was added quickly into the reaction solution right after 5 min and then polymerized for 3 hrs. The conversion was confirmed by ^{1}H NMR as well to make sure it was nearly 100%. After termination and purification, it gave light grey powder, **3**: M_n (GPC) = 14,500, M_w/M_n = 1.14 for P(ONBAn)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (the number in the subscript is the repeating units of monomers) and M_n (GPC) = 19,400K, M_w/M_n = 1.16 for P(ONBAn)$_{50}$-*b*-P(ONB-PEG2K)$_5$.

Hydrolysis of diblock copolymers, 3 to yield poly(ONBA)-*b*-poly(ONB-PEG2K), 4. The block copolymer (0.31 g) was added into 15 mL 0.1 M NaOH solution. This solution was stirred for 1 hour until it turned to be clear. The solution was then dialyzed against distilled water for 2 days to remove NaOH using Spectra/Por (Spectrum Laboratories Inc.) regenerated cellulose dialysis membrane with a molecular weight cutoff of 2000 Da. After freeze-drying, grey viscous product, **4**, was obtained for further self-assembly without treatment: P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (denoted as DP25) and P(ONB-diacid)$_{50}$-*b*-P(ONB-PEG2K)$_5$ (denoted as DP50), respectively.

Preparation and cross-linking of polymer vesicles from poly(ONB-diacid)$_m$-*b*-poly(ONB-PEG2K)$_n$, 4. The diblock copolymer, **4** (M_n = 14,500, 30 mg, 2.07 × 10^{-6} mol) was dissolved in 10 mL milli-Q water at a concentration of 3 mg/mL. pH value of the solution was adjusted to be ~12 (measured by pH meter) by adding 2M NaOH solution (~50 μL). Sample was then filtered through a PTFE filter

(pore size 0.45 μm). Subsequently, 2 M HCl solution was added dropwise into the solution until the pH value to 4. The particle size was confirmed by dynamic light scattering (DLS).

A solution of cross-linker PETMP (7 mg, 1.43×10^{-5} mol) and photo initiator DMPA (1.6 mg, 6.24×10^{-6} mol) was prepared in 2 mL distilled THF. An aliquot of 200 μL solution was withdrawn and added slowly into above polymer micellar solution with stirring. The mixture was kept stirring for extra 4 hours under dark and then radiated under 365 nm UV lamp for overnight. The final solution was dialyzed again milli-Q water for 2 days to remove all impurities including excess THF and some salts using regenerated cellulose dialysis membrane with a molecular weight cutoff of 8000 Da. At the meantime, pH value was increased to 7. Morphological observation of the cross-linked polymer micellar suspension was performed by DLS, SLS, TEM and AFM.

Drug conjugation of *cis*-platinum to cross-linked polymer vesicles. The conjugation of *cis*-platinum to the polymer vesicles was followed by modified procedure as reported in the literatures.[7,8] The complex can be obtained either by adding *cis*-dichlorodiamine-platinum(II) (CDDP), one of popular forms of *cis*-platinum drugs, directly to the aqueous solution of cross-linked polymer vesicles, or via the reaction with *cis*-diaminediaqua platinum(II) complex obtained by hydrolysis of CDDP by adding $AgNO_3$. The later has much higher rate of complexation than the former.

CDDP (10 mg) was suspended in 10 mL distilled water and mixed with silver nitrate ($[AgNO_3]/[CDDP] = 1.955$) to form the aqueous complex. The solution was stirred in the dark at room temperature for 4 h. White precipitate of silver chloride was observed indicative of the proceeding reaction. The mixture was then centrifuged at 4400 rpm for 1 hour to remove the AgCl precipitates and the supernatant was purified by passing through a 0.22 μm filter. Polymers vesicles (38 mg, dissolved in 3 mL of NaOH solution (1 mg/mL) and stirred for half an hour) were added to the above CDDP aqueous solution and left to react in a water bath at 37 ^{o}C for 12 h with gentle shaking resulting in polymer-platinum conjugates. The resultant conjugates were purified by dialysis against distilled water

using regenerated cellulose dialysis membrane with a molecular weight cut-off of 8000 Da, followed by freeze-drying, yielding light brown powder.

The platinum conjugation efficiency to polymer nanoparticles was defined as followed and determined by TGA:

$$f = \frac{m_{Pt,exp}}{m_{Pt,theo}} \times 100\% = \frac{W_{Pt}/M_{Pt}}{W_{di-acid}/M_{di-acid}} \times 100\%$$

where $m_{Pt,\ exp}$: the molar amount of Pt determined by experimental data; $m_{Pt,\ theo}$: the theoretical molar amount of Pt in 100% conversion (assume that one Pt molecule forms complex with one di-acid repeating unit); W_{Pt}: weight percent of Pt measured by TGA; M_{Pt}: molecular weight of Pt; $W_{di\text{-}acid}$: weight percent of di-acid repeat unit calculated by TGA data; $M_{di\text{-}acid}$: molecular weight of di-acid repeating unit.

The platinum loading content to polymer nanoparticles was defined as:

$$Loading\ content = \frac{amount\ of\ loaded\ CDDP}{amount\ of\ polymer\ carrier + amount\ of\ loaded\ CDDP} \times 100\%$$

Release of platinum drug from polymer-platinum conjugates. The release of the platinum drug from the polymer vesicles can be triggered by the presence of chloride ions, which will lead to ligand exchange of the platinum complex from carboxylato ligand to chloride ligand. The drug is then cleaved from the polymer vesicles as CDDP. This process is favoured in the presence of high amounts of chlorides, but not in chloride free buffers. Buffers such as the chloride containing phosphate buffered saline (PBS) or simple saline (0.9%) can force the release of CDDP. The *in vitro* release of the CDDP from polymer vesicle solution was performed by a dialysis method. The polymer-platinum conjugate (*cis*-platinum loaded CPV25) at a concentration of 5 mg/mL was added to dialysis tubing and dialyzed at 37 ^{o}C against 0.9% saline solution of pH 7.4, which mimics the environment in plasma. At certain

time intervals, the release medium was sampled and analysed by means of *o*-phenylenediamine colorimetric assay (*o*-PDA).

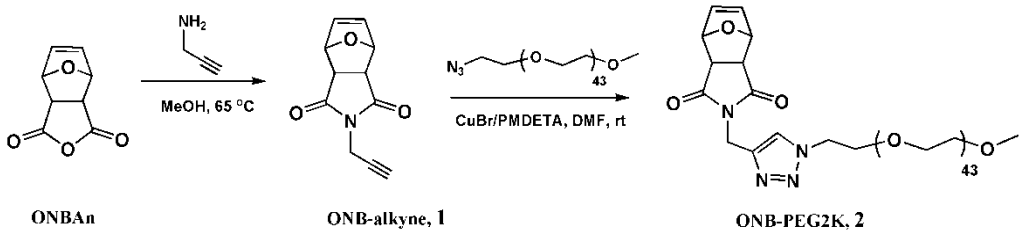
Polymeric platinum conjugates (10 mg) were dissolved in 2 mL saline solution 0.9% NaCl (pH 7.4) and dialyzed (cellulose tubing with molecular weight cut-off of approximately 8000 Da) against saline 0.9% solution (1 L) at 37 ^{o}C. Aliquots of 1 mL were taken at regular time intervals from the dialysate over 143.5 h. The amount of released Pt was determined using the *o*-phenylenediamine colorimetric assay (*o*-PDA) carried out according to a previously published method.[8-10]

Samples with an unknown Pt content were added to 1 mL of *o*-PDA solution in *N,N'*-dimethylformamide (DMF) 2 mg/mL) and heated for 10 min at 100 ^{o}C. The amount of Pt present in the sample was determined by measuring the absorbance at 703 nm using CDDP as a standard curve. The concentration of Pt released from the conjugate was expressed as a ratio of the amount platinum in the releasing solution and that in the initial sample. The percentage of Pt released was calculated using the equation below.

$$\%\ CDDP\ released = \frac{V_{total}(t) \times C + Y}{Z}$$

where $V_{total}(t)$: remaining volume in the releasing container at time *t*, mL; *C*: concentration of platinum determined from UV−vis measurements, μg/mL; Y: the amount of platinum that has already been collected, μg; Z: total amount of platinum at $t = 0$ present in the dialysis bag, μg.

Synthesis and Characterization of Diblock Copolymer



Scheme S1 Synthesis of ω-oxanorbornenyl poly(ethylene oxide) (M_n = 2000) macromonomer (ONB-PEG2K), **2**.

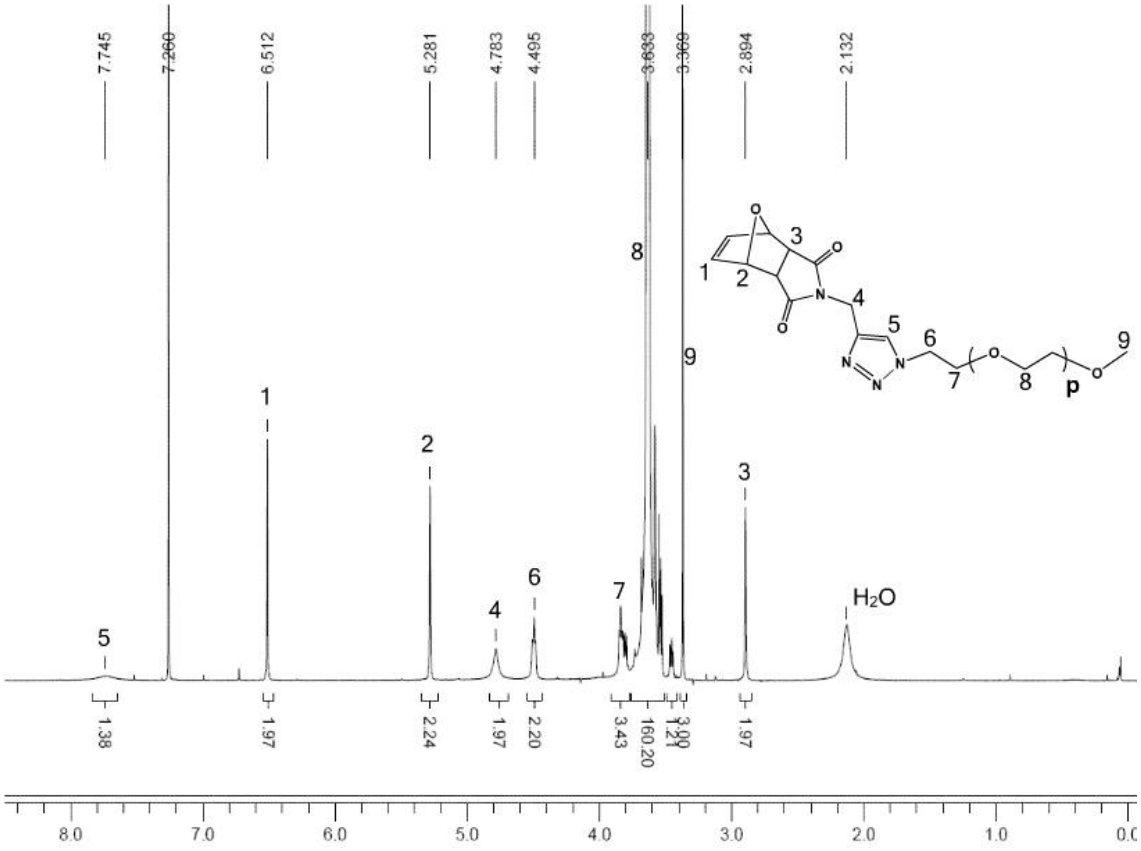


Fig. S1 ^{1}H NMR spectrum of macromonomer ONB-PEG2K, **2**.

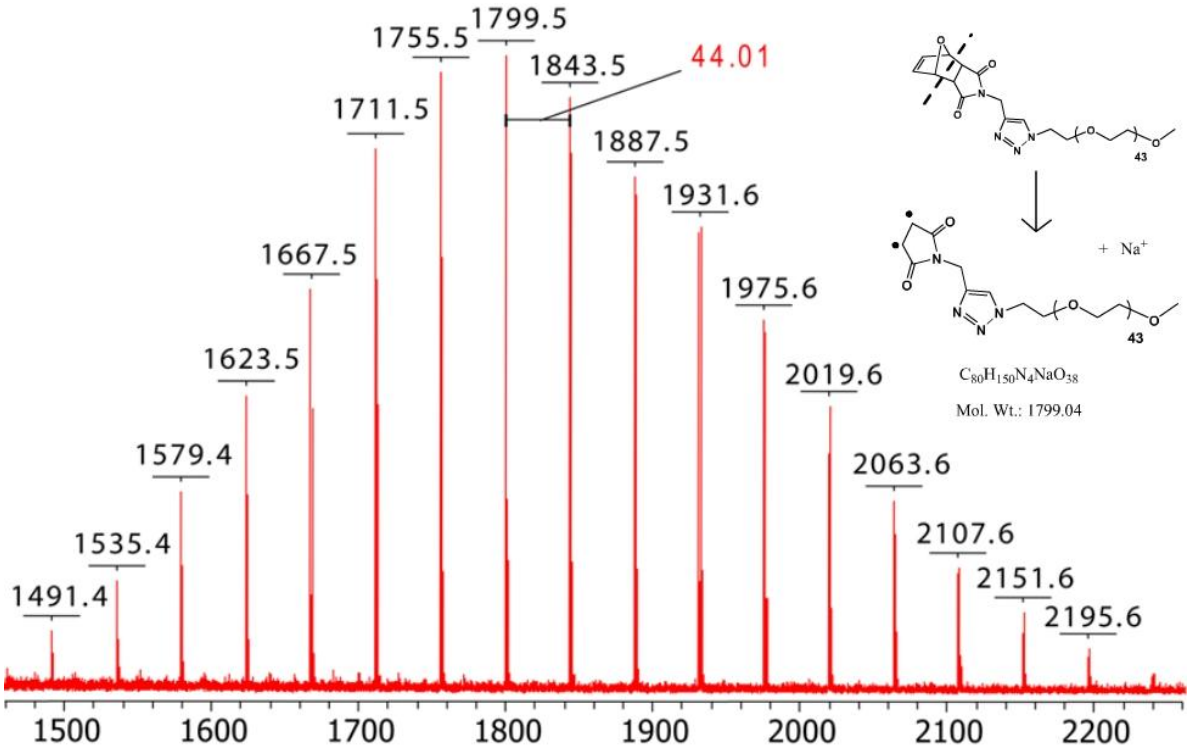
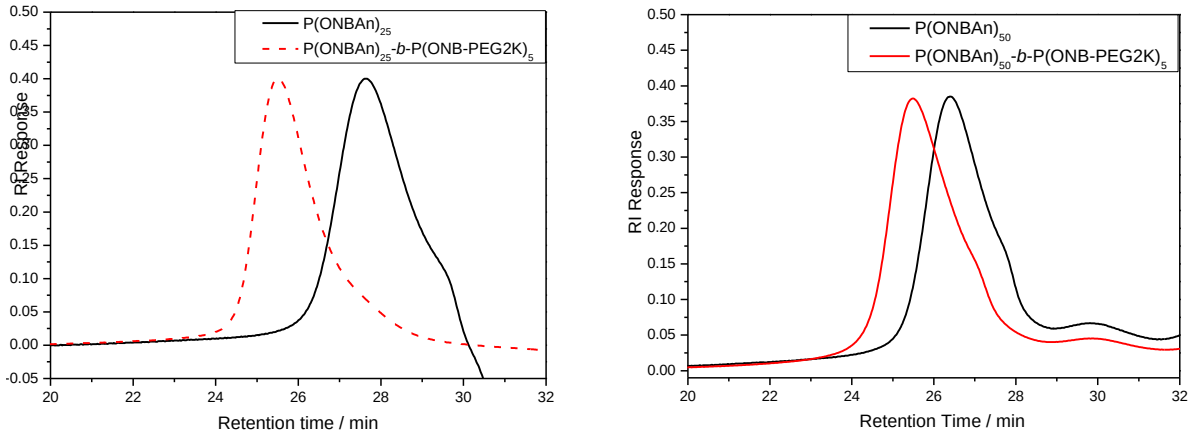


Fig. S2 MALDI-TOF mass spectrum of macromonomer ONB-PEG2K, **2**.



$P(ONBAn)_{25}$-*b*-$P(ONB\text{-}PEG2K)_5$ $P(ONBAn)_{50}$-*b*-$P(ONB\text{-}PEG2K)_5$

Fig. S3 GPC traces of diblock copolymers $P(ONBAn)_m$-*b*-$P(ONB\text{-}PEG2K)_n$, **3**.

This journal is © The Royal Society of Chemistry 2012

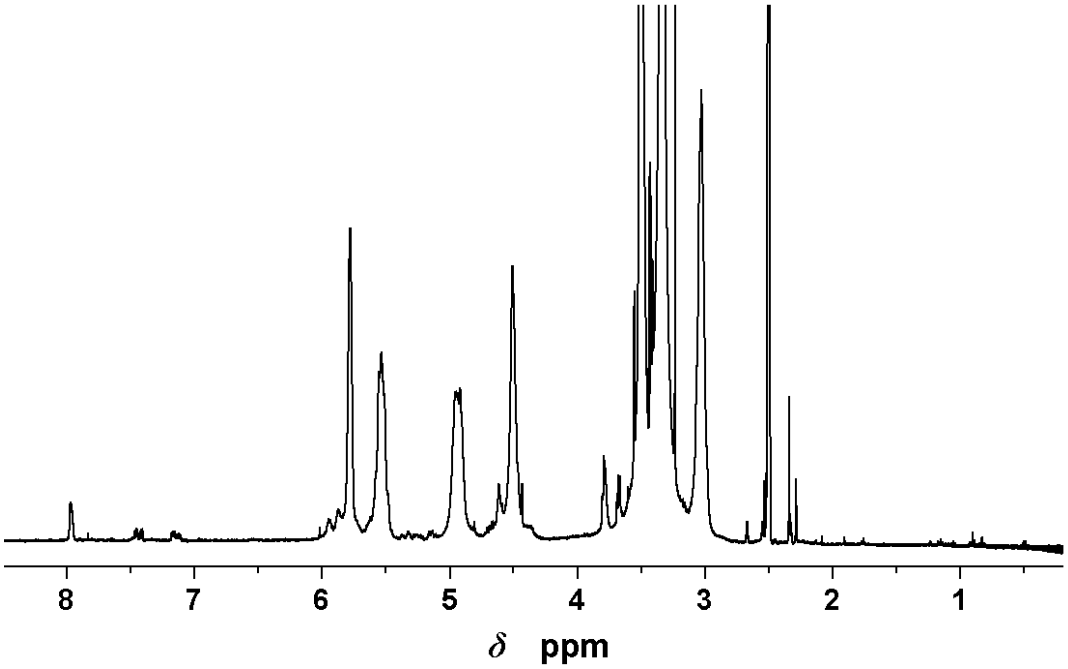


Fig. S4 Typical ^{1}H NMR spectrum of diblock copolymers P(ONBAn)$_m$-*b*-P(ONB-PEG2K)$_n$, **3**.

Dynamic and Static Light Scattering (DLS & SLS) Measurements

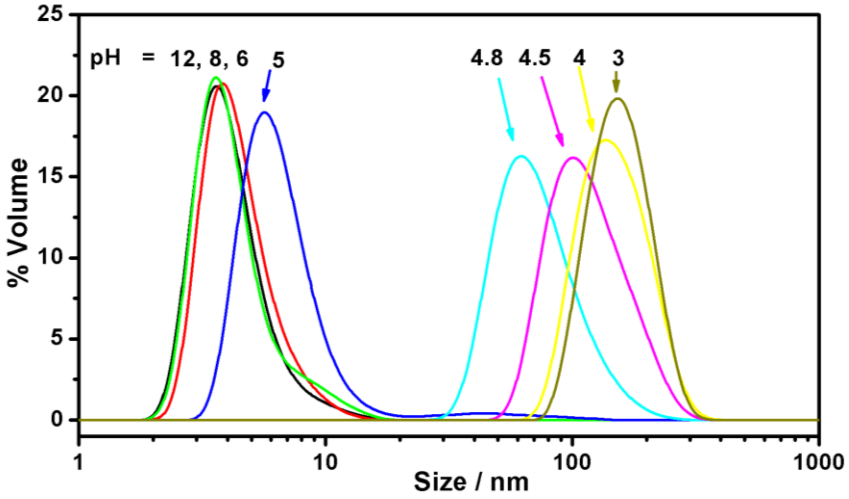
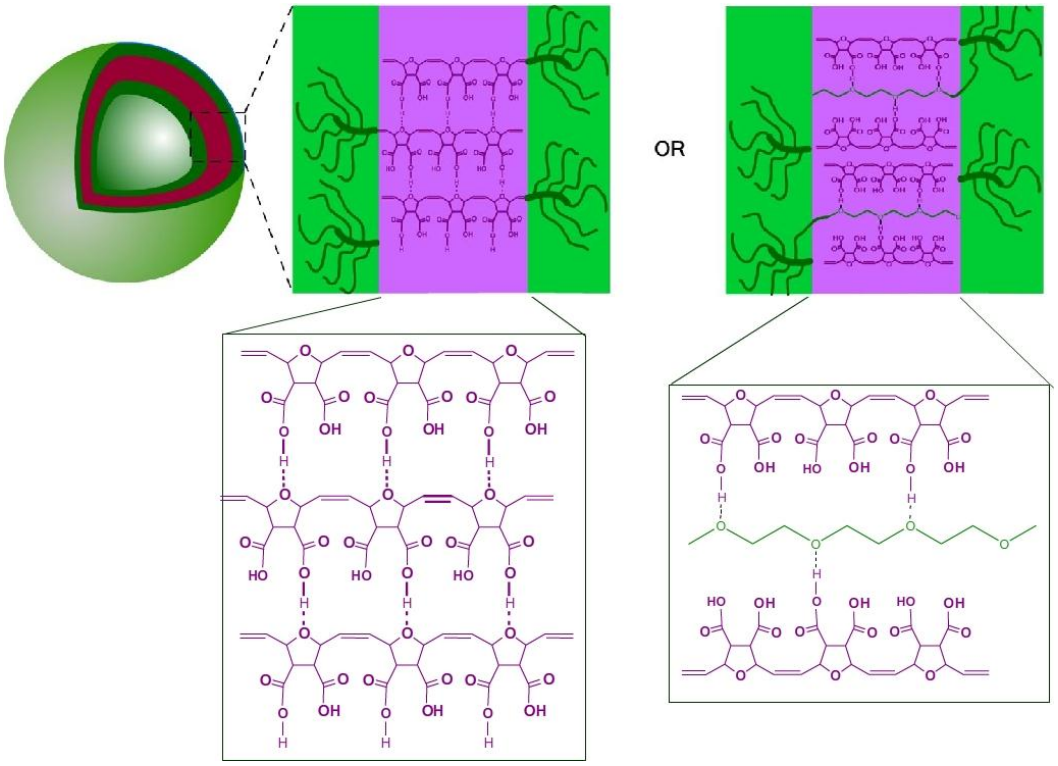


Fig. S5 Hydrodynamic particle size distributions of linear-brush diblock copolymer P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (DP25) at different pH. The concentration was 3 mg/mL. The D_h values were measured 10 min after the solutions preparation.



Scheme S2 The proposed chemical structures of interpolymer complexation (IPC) between poly(carboxylic acid) and polyoxanorbornene ether backbone, PEG brush or both for the self-assembly

of block copolymer P(ONB-diacid)$_m$-*b*-P(ONB-PEG2K)$_n$,**4**, in acidic solutions. As shown in the scheme, the polymer vesicle structure would not be destroyed at all even if all of the protonated acid functionalities form complex only with PEG brush, because the amounts of ether groups in PEG brush are far more than protonated acid functionalities in the used pH range.

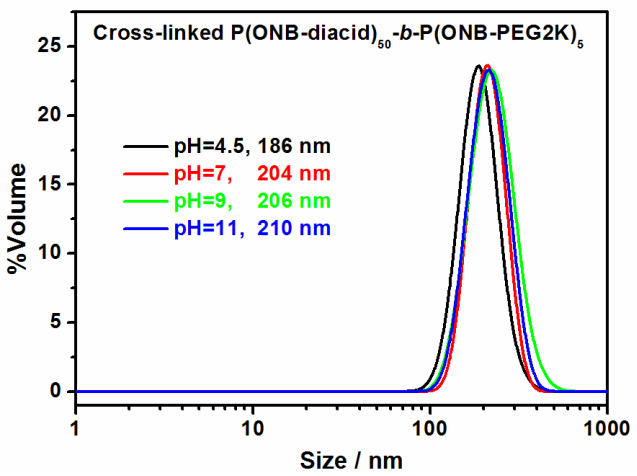


Fig. S6 Particle sizes and size distributions of self-assembled diblock copolymer P(ONB-diacid)$_{50}$-*b*-P(ONB-PEG2K)$_5$ (DP50) at different pH conditions after cross-linking.

SLS Measurements

For small sized particles (qR < 1, where q = scattering vector and R is the aggregate radius), the radius of gyration R_g can be calculated from a Zimm plot or Guinier approximation. When the ratio of R_g/R_h is close to unity it indicates that a hollow particle (vesicle) is present, as opposed to a value of 0.77, which corresponds to a solid sphere or micelle.[11,12] However, in this case the qR values are greater than 1 and R_g cannot be easily measured using this technique.

Instead the intensity as a function of scattering angle of incident light was measured and fitted with mathematical models for both spherical hollow spheres (vesicles) and solid spheres (micelles) in order to confirm whether or not the self-assemblies truly are vesicles, and determine particle sizes and

shell thickness.[13] The scattering factor, P(q), for hollow spheres with a shell of thickness t can be expressed as follows:

$$\mathrm{P}(q) = \left(\frac{3}{R_0^3 - R_i^3}\right)^2 \times \left[R_0^3 \frac{j_1(qR_0)}{qR_0} - R_i^3 \frac{j_1(qR_i)}{qR_i}\right]^2 \quad \text{(S1)}$$

Where R is the average radius, $R_0 = R + t/2$ is the outer radius, $R_i = R - t/2$ is the inner radius and $j_1(x)$ is the first-order spherical Bessel function given by:

$$j_1(x) = \frac{\sin x}{x^2} - \frac{\cos x}{x} \quad \text{(S2)}$$

As a comparison, a solid sphere model (as would be the case for a micelle) is also used:

$$\mathrm{P}(q) = \frac{4}{3}\pi R^3 \times \frac{3[\sin(qR) - qR\cos(qR)]}{(qR)^3} \quad \text{(S3)}$$

In a polydisperse suspension of particles, the total scattering intensity as a function of angle will be given by:

$$I(q) = \int_0^\infty R^6 P(q,R) G(R) dR$$

where G(R) is the particle size distribution. Both log-normal and Schultz distributions were tried, each characterized by a mean radius, R, and a polydispersity, σ. The details of the analysis and fitting methods are described in previous publications.[14,15]

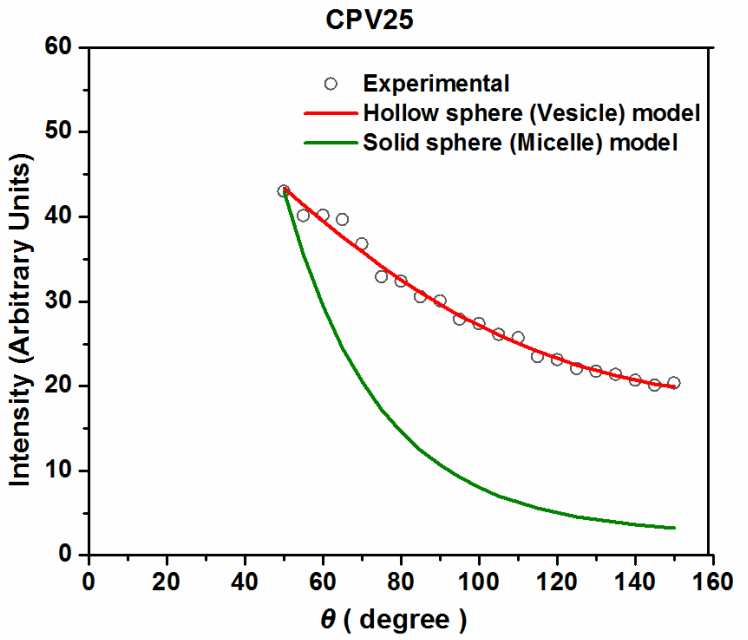


Fig. S7 Scattered intensity correlation as a function of the angle of incident light for the cross-linked polymer vesicle from P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (CPV25). The lines correspond to the fits generated using the hollow sphere (vesicle) (red line) and solid sphere (micelle) model (green line). Particle size D = 132 nm; Shell thickness t = 27 nm, log Normal distribution with σ = 43%. No good fit could be obtained with the solid sphere model: for comparison, the calculated data for a solid sphere with the same parameters is shown (green line).

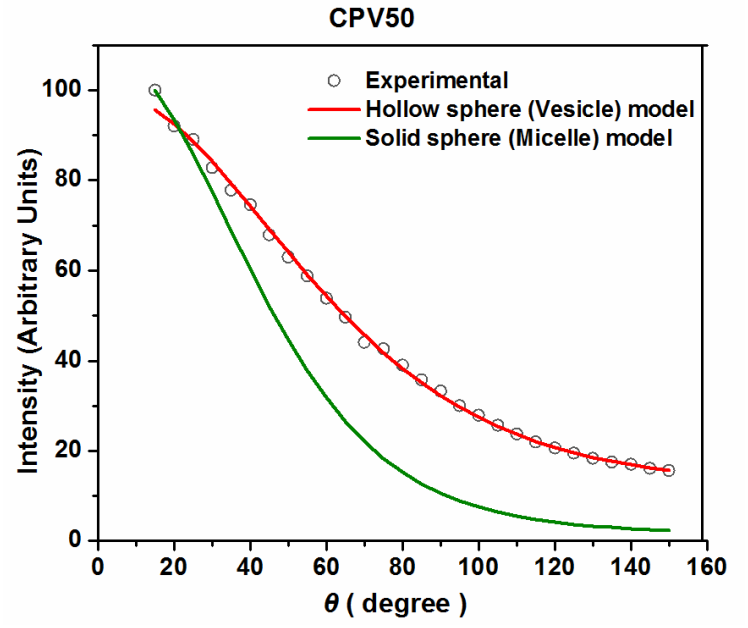


Fig. S8 Scattered intensity correlation as a function of the angle of incident light for the cross-linked polymer vesicle from P(ONB-diacid)$_{50}$-*b*-P(ONB-PEG2K)$_5$ (CPV50). The lines correspond to the fits

generated using the hollow sphere (vesicle) (red line) and solid sphere (micelle) model (green line). Particle size D = 188 nm; Shell thickness t = 37 nm, Schultz distribution with σ = 31%. No good fit could be obtained with the solid sphere model: for comparison, the calculated data for a solid sphere with the same parameters is shown (green line).

The two different samples were fitted with both log-normal distributions and Schultz distributions, assuming both solid sphere (micelle) and hollow sphere (vesicle) models, and the best fit results are shown in the figures above (Fig. S7 & S8). The size distributions of the vesicle fits are shown in Fig. S9, which shows that CPV25 has a smaller average size, but a larger polydispersity (distribution width) than CPV50. The large polydispersities are consistent with the results from Cryo-TEM.

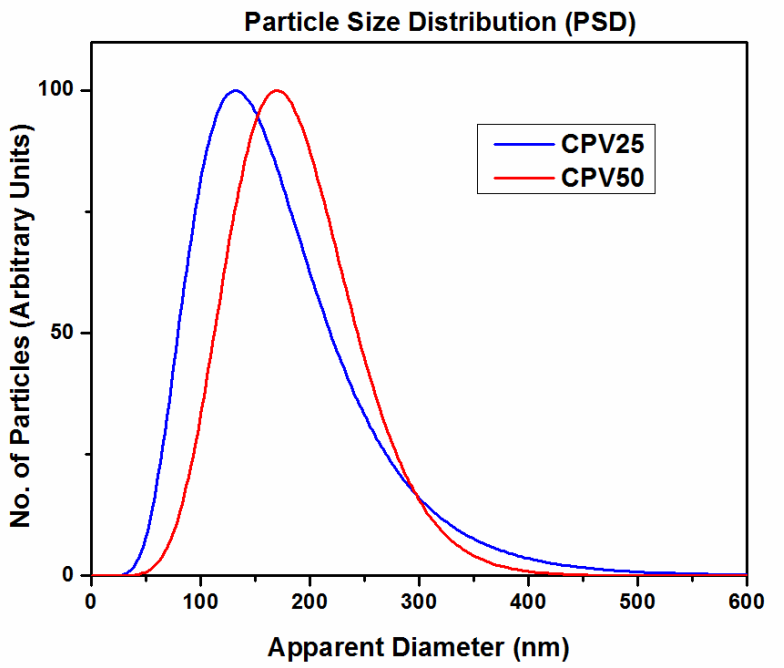


Fig. S9 Particle size distributions for samples of cross-linked polymer vesicles from diblock copolymer P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (CPV25) (blue line) and P(ONB-diacid)$_{50}$-*b*-P(ONB-PEG2K)$_5$ (CPV50) (red line). It is worthy to note that CPV25 has a smaller average size, but a wider particle distribution than CPV50.

Transmission Electron Microscopy (TEM) and Cryo-TEM Measurements

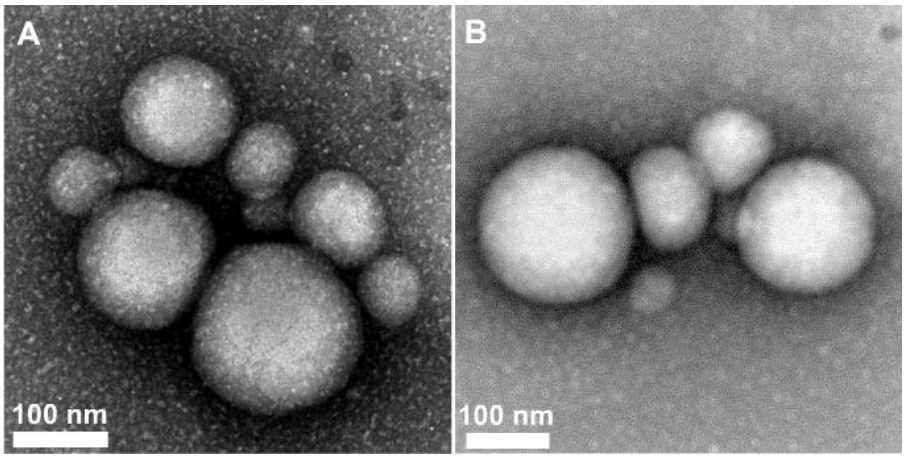


Fig. S10 TEM images with negative staining for the dried sample of cross-linked polymer vesicle from P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (CPV25).

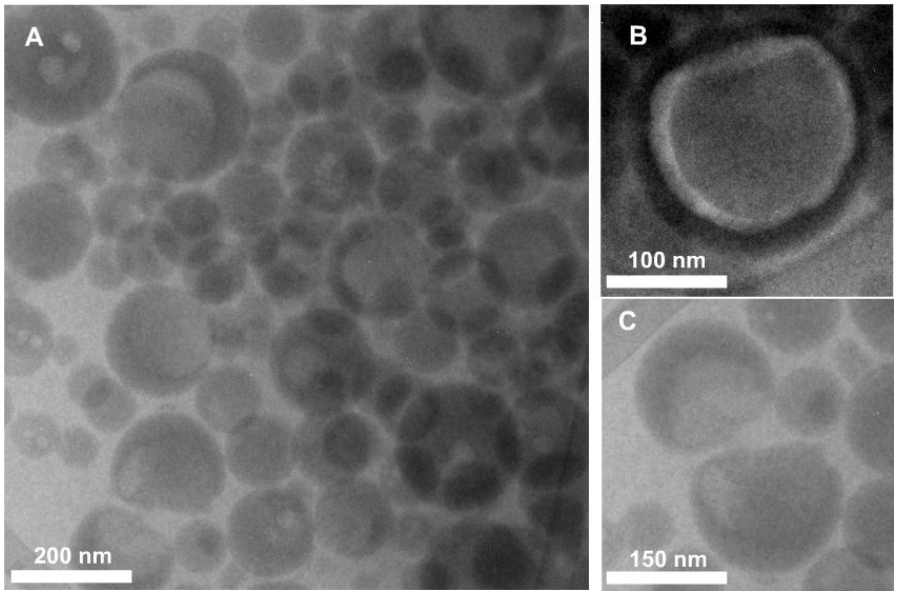


Fig. S11 Cryo-TEM images for the sample of cross-linked polymer vesicle from P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (CPV25) with positive staining after *cis*-platinum loading.

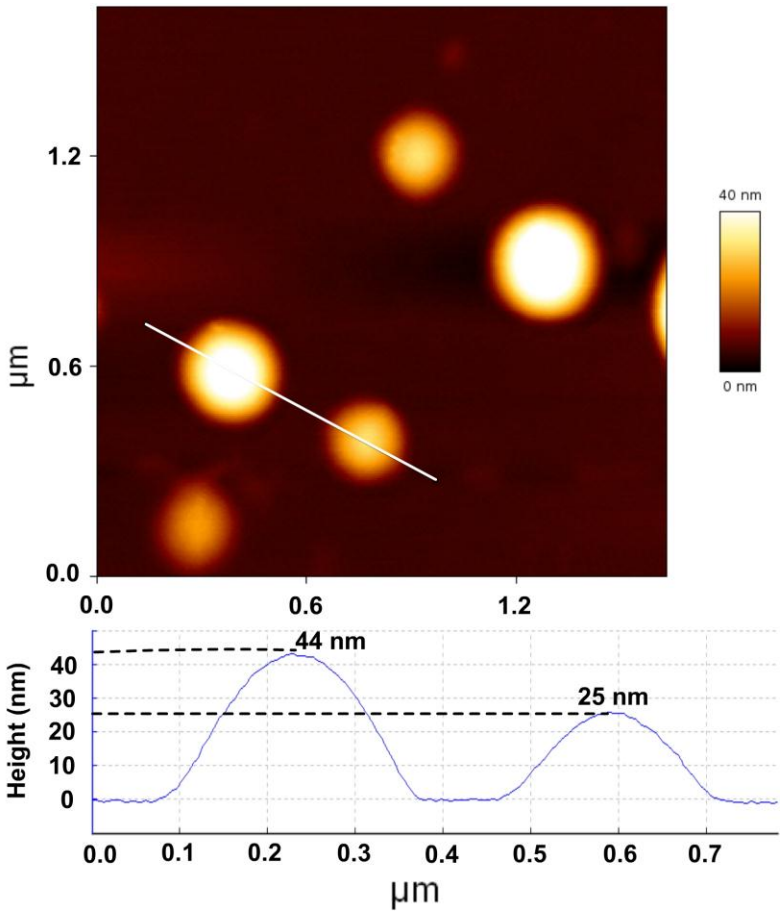


Fig. S12 Atomic Force Microscopy (AFM) image for the sample of cross-linked polymer vesicle from P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (CPV25). The different thickness of two particles (44 nm and 25 nm) as indicated was originated from two distinguished particle shapes: fully enclosed (doubled shell thickness) and partially enclosed vesicles (singled shell thickness).

This journal is © The Royal Society of Chemistry 2012

Cis-Platinum Drug Loading and Drug Release

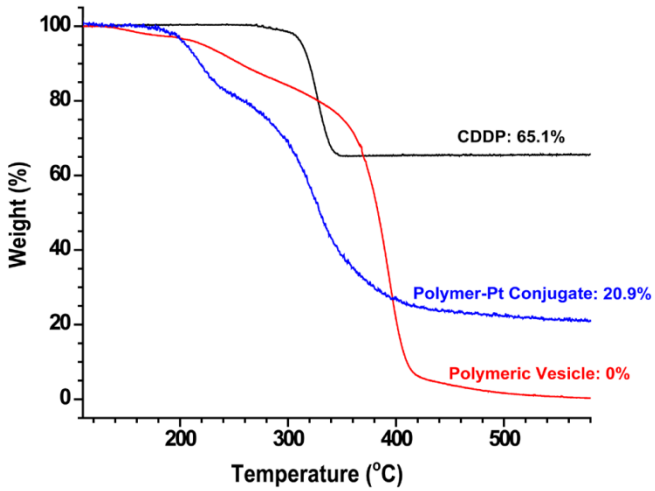


Fig. S13 TGA traces for CDDP (black), cross-linked polymer vesicle from P(ONB-diacid)$_{25}$-*b*-P(ONB-PEG2K)$_5$ (CPV25, red) and polymer-platinum conjugate (blue, drug loaded polymer vesicles).

Calculation of drug conjugation efficiency based on TGA data:

$$f = \frac{m_{Pt,exp}}{m_{Pt,theo}} \times 100\% = \frac{W_{Pt}/M_{Pt}}{W_{di-acid}/M_{di-acid}} \times 100\%$$

$$= \frac{20.9\%/195}{\left[\left(79.1\% - \frac{20.9\% \times 34.9}{65.1}\right) \times \frac{4100}{4100+10000}\right]/164} \times 100\%$$

$$= 88.4\%$$

Calculation of drug loading content based on conjugation efficiency:

$$\text{Loading content} = \frac{\text{amount of loaded CDDP}}{\text{amount of polymer carrier} + \text{amount of loaded CDDP}} \times 100\%$$

$$= \frac{10\ \text{mg} \times 88.4\%}{38\ \text{mg} + 10\ \text{mg} \times 88.4\ \%} \times 100\% = 18.9\ \%$$

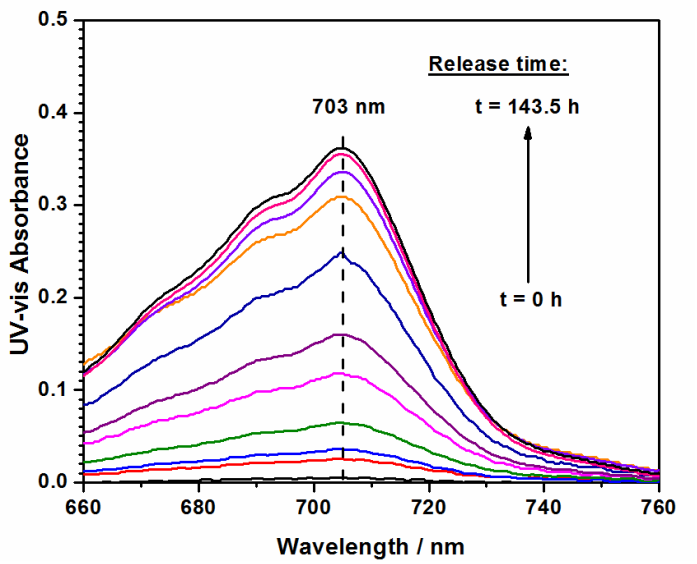


Fig. S14 UV-vis spectra for the samples withdrawn at different time intervals during drug release study.

References

1. R. B. Woodward, H. Baer, *J. Am. Chem. Soc.* **1948**, *70*, 1161–1166.

2. H. Gao, K. Matyjaszewski, *J. Am. Chem. Soc.* **2007**, *129*, 6633–6639.

3. J.-M. Ren, J. Wiltshire, A. Blencowe, G. G. Qiao, *Macromolecule* **2011**, *44*, 3189–3202.

4. J. A. Love, J. P. Morgan, T. M. Trnka, R. H. Grubbs, *Angew. Chem. Int. Ed.* **2002**, *41*, 4035–4037.

5. S. Yu, T. Azzam, I. Rouiller, A. Eisenberg, *J. Am. Chem. Soc.* **2009**, *131*, 10557-10566.

6. D. Le, V. Montembault, J.-C. Soutif, M. Rutnakornpituk, L. Fontaine, *Macromolecule* **2010,** *43*, 5611–5617.

7. H. Ye, L. Jin, R. Hu, Z. Yi, J. Li, Y. Wu, X. Xi, Z. Wu, *Biomaterials* **2006**, *27*, 5958–5965.

8. V. T. Huynh, G. Chen, P. de Souza, M. H. Stenzel, *Biomacromolecules* **2011**, *12*, 1738-1751.

9. E. Gianasi, M. Wasil, E. G. Evagorou, A. Keddle, G. Wilson, R. Duncan, *Eur. J. Cancer* **1999**, *35*, 994–1002.

10. B. Schechter, A. Neumann, M. Wilchek, R. Arnon, *J. Controlled Release* **1989**, *10*, 75–87.

11. H. Huang, B. Chung, J. Jung, H. W. Park, T. Chang, *Angew. Chem. Int. Ed* **2009**, *121*, 4594-4597.

12. W. Burchard, *Adv. Polym. Sci.* **1983**, *48*, 1.-124

13. J. Pencer, F. R. Hallet, *Langmuir*, **2003**, *19*, 7488-7497.

14. G. Bryant, S. Martin, A. Budi, W. van Megen, *Langmuir*, **2003**, *19*, 616-621.

15. H. J. Schöpe, O. Marnette, W. van Megen, G. Bryant, *Langmuir*, **2007**, *23*, 11534-11539.