Supporting Information for:

**Nonspherical Polysaccharide Vesicles and Their Shape and Volume Regulation via Osmotically Sensitive Channels**

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1. **Materials and Method**

2. **Results and Analysis**

1. **Materials and Methods:**

Ethyl (hydroxyethyl) cellulose (EHEC) was purchased from Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden and degraded before use. The number–average molecular weight (Mn) of EHEC treated is 30 kDa and the degrees of substitution with ethyl and hydroxyethyl substituents of EHEC were 0.8 and 1.2, respectively. Acrylic acid (AA) (Shanghai Guanghua Chemical Company) was distilled under reduced pressure in nitrogen atmosphere. Potassium persulfate (K_2S_2O_8, Shanghai Guanghua Chemical Company) was recrystallized from distilled water. Hydrogen peroxide (H_2O_2, 30% wt/wt) was purchased from Shanghai Jinglu Chemical Company. Dye Rhodamine B isothiocyanate (RBITC) and Fluoresamine was purchased from AMRESCO Company. Dextran was purchased from Pharmacia Uppsala, Sweden. 2-Isocyanatoethyl methacrylate was purchased from ACROS, USA. All other chemicals were reagent grade.

1) **Preparation of EHEC-PAA Vesicles**

EHEC were firstly pretreated to reduce its molecular weight to 30 kDa by degradation reaction using hydrogen peroxide (H_2O_2, 30% wt/wt), for escaping the influence on the polymerization with the high viscosity of the EHEC solution. In a typical vesicle preparation, EHEC (25 mg, 30 kDa) were dissolved in 35 ml deionized water (0.07%, w/v) in a three-neck flask under magnetic stirring. When the solution became homogeneous and clear, the temperature was raised to 70 °C and monomer AA (200 mg) was dropped into the system. Then 200 mg amount of potassium persulfate (K_2S_2O_8) was added to above...
mixture under a nitrogen stream to initiate polymerization of AA in EHEC solution. When the opalescent suspension was observed, reaction system was cooled quickly to ambient temperature and deposited for one night.

2) **Fluorescence Labeling of EHEC and Dextran**

EHEC and Dextran are labeled by RBITC and FITC, respectively. The labeling method refers to a technique published by Schreiber et al. (1). Briefly, the fluorescent dye RBITC or FITC was dissolved in DMSO at a concentration of 1 mg/ml. Subsequently, 1 ml of the dye solution was added to the 50 ml of 1% (w/v) EHEC or dextran (40000 Da) aqueous solution and stirred for 2 hours at 40 °C. The reaction was stopped by adding 50 μl thamolamine. Labeled EHEC or Dextran was precipitated in dehydrated ethanol and then was dialyzed against water. Finally, labeled EHEC or dextran was dried at 60 °C in vacuum.

3) **Fluorescence Labeling of PAA**

1mg amount of fluorescamine and 30 μl 2-isocyanatoethyl methacrylate were mixed in 1ml of acetone containing trace amount of water to react for one night. The solution transformed from colorless into yellow illustrated that 2-isocyanatoethyl methacrylate was labeled by fluorescamine successfully. Subsequently, the resulted yellow solution was added to the degraded EHEC solution with acrylic acid to take part in polymerization of AA in the process of preparing EHEC-PAA vesicles. Therefore, PAA in the vesicles was also labeled.

4) **Fourier Transfer Infrared Spectroscopy (FT-IR) Analysis**

FT-IR spectra were collected using a Bruke IFS 66V vacuum-type spectrometer. The EHEC-PAA vesicles were frozen by liquid nitrogen and lyophilized to obtain dried EHEC-PAA vesicles. These gained vesicles were mixed with KBr and pressed to a plate for measurement.

5) **Transmission Electron Microscopy (TEM)**

TEM experiments were performed on a JEOL 100 transmission electron microscope at 80 keV and ambient temperatures without being negative stained. TEM samples were prepared by applying one drop of EHEC-PAA vesicle suspensions on a copper grill covered with nitrocellulose and allowing the solution to evaporate under ambient conditions for 90s. Filter paper was then used to wick away residual sample and liquid.

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6) Scanning Electron Microscopy (SEM)

SEM observations were performed on a LEO1530VP field emission SEM (Zeiss, Germany). SEM samples were prepared by placing a drop of EHEC-PAA vesicle suspensions on a clean silicon wafer and air dried. The sample surface was coated with a thin layer of gold prior to observation.

7) Differential Interference Contrast (DIC) Microscope Characterization

Osmotic experiments: 150 μl of EHEC-PAA vesicles solution was dropped onto the slide for the DIC observation. Select one vesicle as the observation object for the following step. Then 150 μl of 300 mM sucrose solution was added to the drop of EHEC-PAA vesicles solution as carefully as possible. The shape of vesicles was recorded by capture photographs every minute until the recovery of vesicles.

8) Static and Dynamic Light Scattering Characterization (SLS and DLS)

SLS measurements were performed using a Brookheaven spectrometer (BI-200SM). Angle dependence of scattered light intensity of EHEC-PAA vesicle suspension was measured. Scattering angle ranged from 40° to 130°. For the vesicle size less than 3 μm, average diameter and size distribution of the EHEC-PAA vesicles were determined by DLS using Brookheaven BI900AT system (Brookheaven Instruments Corporation, USA). For vesicle size larger than 3 μm, average diameter and size distribution of the samples were determined by DLS using Mastersizer 2000 (Malvern, UK). All DLS measurements were taken with a wavelength of 633.0 nm at 25 °C with a detection angle of 90°.

9) Axial ratio of the EHEC-PAA vesicle measured by static and dynamic light scattering

As proposed in literature (28), static and dynamic light scattering measurements were used to measure the axial ratio of the EHEC-PAA vesicle. A series of number distributions with varying axial ratio were calculated. Theoretical SLS curves were generated by these distributions, which were plotted against measured SLS data as shown in Figure 2F.

a. Theoretical static light scattering (SLS) curves for each value of axial ratio (a/b)

SLS data gives the time-averaged scattered intensity from dilute vesicles in suspension as a function of scattering vector q, given by

\[
\frac{I(q)}{I_0} \propto K^4 N M^2 \left(\frac{m^2 - 1}{m^2 + 2}\right)^2 P(q),
\]
Where $k=\frac{2m\pi}{\lambda}$, $m = n_1/n_0$, $n_0$ is the medium refractive index, $n_1$ is the refractive index of the particles, $M$ is the particle mass, and the particle scattering factor $P(q)$ depends on particle size and shape.

We have simulated SLS data using Rayleigh-Gans-Debye (RGD) scattering factors for spherical and prolate vesicles. The normalized RGD scattering factor for spherical vesicles is given by

$$p(q) = \left[\frac{3}{u^3 - v^3}\right]^2 \left[u^3 \frac{j_1(qu)}{qu} - v^3 \frac{j_1(qv)}{qv}\right]^2,$$

Where, $u = R + t/2$, $v = R - t/2$, $R$ is the radius, $t$ is the membrane thickness, and $j_1(x)$ is the first-order spherical Bessel function given by

$$j_1(x) = \frac{\sin x}{x^2} - \frac{\cos x}{x},$$

The RGD scattering factor for prolate vesicles is given by

$$p(q) = \left[\frac{3}{u^3 - v^3}\right]^2 \int_0^1 \left[u \frac{j_1(qU)}{qU} - v \frac{j_1(qV)}{qV}\right]^2 dx,$$

Where

$$U = \sqrt{a_0^2 x^2 + b_0^2 (1 - x^2)},$$
$$V = \sqrt{a^2 x^2 + b^2 (1 - x^2)},$$
$$u = a_0 b_0^2, \quad v = a b_0^2, \quad a_0 = a + t/2, \quad b_0 = b + t/2, \quad a = a - t/2, \quad b = b - t/2,$$

$t$ is the membrane thickness, $a$ is the length of the vesicle long axis, and $b$ is the length of the vesicles short axis.

In our system, the vesicles displayed a wider size distribution. Therefore SLS measured from polydisperse vesicles is equal to the mass-squared weighted average of the scattering factor $P(q, Ra, e_{pro})$ given by

$$< P(q, Ra, e_{pro}) > = \int_0^\infty m^2 \omega P(q, Ra, e_{pro}) G(Ra)dRa$$
$$\times \left(\int_0^\infty m^2 \omega G(Ra)dRa\right)^{-1},$$

Where $e_{pro} = (1 - b^2/a^2)^{1/2}$, $m_{Ra}$ is the mass of vesicles with equivalent radius $Ra$, and $Ra$ has been corrected for the vesicle thickness $t$, which is assumed to be 6 nm estimated by TEM measurement. The number distribution $G(Ra)$ is modeled using the Weibull distribution (5) given by
\[ G(R_a) = \frac{\delta}{\eta} \left( \frac{R_a - R_0}{\eta} \right)^{\delta-1} \exp\left(-\frac{R_a - R_0}{\eta}^\delta \right). \] 

Where the value of \( R_0, \eta, \delta \) could be obtained by DLS measurement, which is assumed to be 1070.6 nm, 0.89, 0.58677, respectively.

For each value of axial ratio \( a/b \), theoretical SLS curves (Fig S2A) are calculated using the number distributions determined above and Eqs. (1) and (2).

b. Comparison of simulated and measured SLS data for EHEC-PAA vesicles

Based on our comparison of simulated and measured SLS data, EHEC-PAA have an axial ratio closed to 1.8 (Figure 2F).

10) Laser Scanning Confocal Microscopy (LSCM)

Confocal fluorescence images of aqueous-phase vesicles were taken on an Olympus TCS-SP Mp confocal equipped with an argon laser (488 nm blue excitation: IDS Uniphase) and a 561 nm (green) diode laser (DPSS: Melles Griot). The laser was adjusted in the green/red fluorescence mode which yielded two excitation wavelengths at 488 and 514 nm. The emission filter blocks VHS/A1 and A2 were used. Green and red fluorescence images were obtained from two separate channels. All confocal fluorescence pictures were taken with a 63× objective (oil immersion).

11) Gel Permeation Chromatography (GPC)

Molecular weight and composition ratio of EHEC and PAA were measured by a GPC equipped with a static light scattering detector (DAWN HELEOS, Wyatt Technology Corporation).
3. Results and Analysis

The thickness of the vesicle membrane

Figure S1. TEM images for membrane thicknesses measurement of small (A) and large (B) EHEC-PAA vesicles. Inserts are the edges of the vesicle membrane with high magnification.

Calculation of the ration of PAA chain to a single EHEC chain in vesicles

The GPC results provided two key data:

1. The molar mass of generated PAA in the EHEC-PAA vesicles is 1.9 kDa.
2. The composition of EHEC-PAA vesicle is 85% for EHEC and 15% for PAA.

In addition, the molar mass of EHEC using in this study is 30 kDa. So we can calculate the molar ratio of EHEC to PAA in the vesicles, \( R \), according to following equation:

\[
\frac{(M_{EHEC} \times M_{wEHEC})}{(M_{PAA} \times M_{wPAA})} = \frac{85\%}{15\%},
\]

Thus, \( R = M_{EHEC}/M_{PAA} \approx 1/3 \)

Where \( M_{EHEC} \) = the mole of EHEC in vesicles, \( M_{PAA} \) = the mole of PAA in vesicles, \( M_{wEHEC} \) = weight-averaged molecular weight of EHEC, \( M_{wPAA} \) = weight-averaged molecular weight of PAA.

Volume change of vesicle when addition of sucrose solution to system

Shape self-recovery and volume change of vesicle were observed when addition of sucrose solution to EHEC-PAA vesicle system. We also found that this interesting phenomenon would reappear when sucrose solution was again added to recovered vesicle system. Figure S1 shows the statistically time-varying in radius of the vesicles in sucrose solution. Round 1 recorded the variation process when the
vesicle system was subjected to 150 mM sucrose solution for the first time. After the vesicles completely recovering, 300 mM sucrose solution was again added to the system and Round 2 exhibited the same change tendency of vesicle volume as that in the Round 1.

**Figure S2.** Statistically time-elapsing lateral dimensions of field vesicles in sucrose solution twice. $R_0$ and $R$ are the lateral dimensions of the vesicle at the beginning and at the given time.