*Electronic Supplementary Information*

# pH-Dependent Permeability Change and Reversible Structural Transition of PEGylated Polyion Complex Vesicles (PICsomes) in Aqueous Media

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## Experimental Procedures

### Materials

1,5-Diaminopentane (DAP) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and distilled over $CaH_2$ under reduced pressure. Dichloromethane ($CH_2Cl_2$) was purchased from Wako Pure Chemical Industries (Osaka, Japan) and distilled by a general method before use. 1M hydrochloric acid and 1 M Sodium Hydroxide were purchased from Wako Pure Chemical Industries (Osaka, Japan) and used without further purification. Fluorescein isothiocyanate-labeled dextran (FITC-Dex: $M_n$=2,000,000) and tetramethylrhodamine isothiocyanate labeled-dextran (TRITC-Dex: $M_n$=10,000, 40,000 and 70,000) were purchased from Sigma (St. Louis, MO) and used without further purification. Charged block copolymers, poly(ethylene glycol)-*b*-poly ([5-aminopentyl]-*α*,*β*- aspartamide) (PEG-P(Asp-AP); degree of polymerization (DP) = 45-75) and poly(ethylene glycol)-*b*-poly(*α*,*β*-aspartic acid) (PEG-P(Asp); DP = 45-75), were synthsized and fully characterized as previously reported.[1,2,4] Typically, charged polymers were purified by preparative size-exclusion chromatography performed at room temperature on a preparative HPLC system (Gilson Inc., USA) equipped with a Superdex 200, Prep grade (GE Healthcare UK Ltd., England) column using 50 mM phosphate buffer containing 150 mM NaCl (pH 7.4) as an eluent at a flow rate of 10 mL/min.

### Titration of Block Copolymers

Potentiometric titration of PEG-P(Asp-AP) was performed on an automatic titrator (TS-2000, Hiranuma, Kyoto, Japan). Determinaton of p$K_a$ of PEG-P(Asp-AP) was carried out by a previously described method.[3,4] 70 mg of PEG-P(Asp-AP) was dissolved in 30 mL of 0.01 N HC1 and titrated with 0.01 N NaOH. The titrant was added in 0.01 mL quantities at 12-120 s intervals. The dissociation degree at a given pH was calculated from the resulted titration curves†.

### Preparation of PICsomes

PICsomes were prepared by mixing an aqueous solution of PEG-P(Asp-AP) (1 mg/mL, 50 mM phospate buffer, pH 7.4, 150 mM NaCl) with an aqueous solution of PEG-P(Asp) (1 mg/mL, 50 mM phospate buffer, pH 7.4, 150 mM NaCl)[1,2].
pH adjustment of resulted mixture was carried out by adding an appropriate amount of 1 M HCl to the solution. Neutralization was also carried out by adding a specific amount of 1 M NaOH to the acidic solution.

### Dark field microscopic observation

Dark-field microscopic (DFM) observations were carried out using an Olympus model BX51 equipped with a 100× Oil-immersed objective (UPlanApo, Olympus, Japan) and a digital camera (VB-7000, Keyence, Japan) at ambient temperature.

### Dynamic light scattering (DLS) measurements of PICsomes

DLS measurements were carried out using a DLS-7000 instrument (Otsuka Electronics Co., Ltd., Japan). Vertically polarized light of 488 nm wavelength from an Ar ion laser was used as an incident beam. Detection angle was 90°. All measurements were

performed at 28 ºC. All free polymer samples were purified by passing through a 0.45μm filter (Minisart RC4, Sartorius, Germany) before formation of PICsomes.

**Confocal laser scanning micrsoscopic observations**

Permeablity and morphology of PICsomes were evaluated using a confocal laser scanning microscope (CLSM) (LSM510 META, Carl Zeiss, Germany) equipped with a 63×objective (C-Apochromat, Carl Zeiss, Germany) at excitation wavelengths of both 488 nm (Ar laser) and 543 nm (He-Ne laser) in parallel.
After addition of 2 mg/mL buffer solution of TRITC-Dex or FITC-Dex to the solution of PICsomes, the relative fluorescence intensity profiles of the cross-section passing through the center of spherical particles whose size were approximately 2 μm were collected spectrophotometrically as shown in Fig. S5.
% Relative fluorescence intensity was defined by:
**[% Relative intensity] = [Relative intensity inside particle]/ [Relative intensity of outer medium]**
Plots shown in Fig. S5 were prepared based on the mean value of % relative intensity at the center of the particle (n=5).

**Time-resolved laser diffraction measurements**

Laser diffraction measurements were performed at room temperature on a SALD-7100 (Shimadzu, Kyoto, Japan) instrument. Time-resolved measurement was carried out with a minimal time interval of 10 sec. Size distributions were calculated using a value of 1.35 as the reflective index of the particles.

**Table S1** Characteristics of PICsomes 4 h and 24 h after preparation determined by DLS.

| Time (h) | Cumulant Diameter ($\mu$m) | Polydispersity ($\mu_2/\Gamma^2$) | Diffusion Coefficient (cm$^2$/sec) |
|---|---|---|---|
| 4 | 1.94 | 0.17 | $2.73 \times 10^{-9}$ |
| 24 | 1.92 | 0.20 | $2.76 \times 10^{-9}$ |

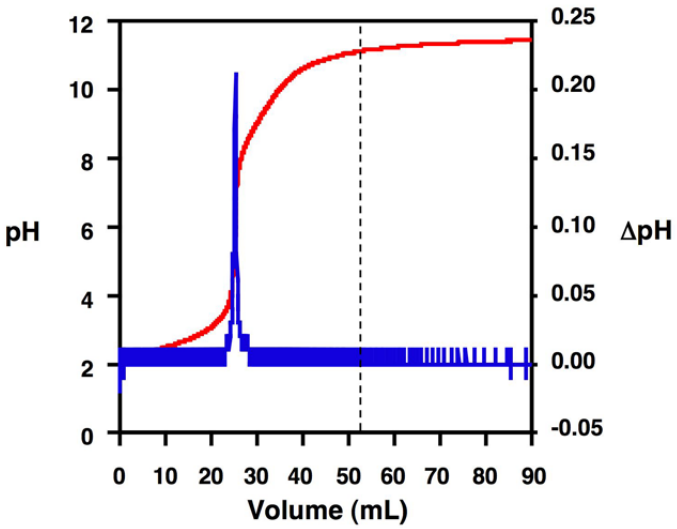


***Fig. S1*** **The titration curves of PEG-P(Asp-AP) (DP: 45-75).** The spike on the titrated volume-ΔpH curve (blue) shows the start point of the titration of PEG-P(Asp-AP). Dashed line shows the end point of the titration of PEG-P(Asp-AP) which was calculated assuming that all amino groups were deprotonated at that point. The red plot shows the titrated volume-pH curve that is used for creation of pH-Ionization degree curve shown in Fig. S2. The left axis corresponds to red curve; the right axis corresponds to blue curve.

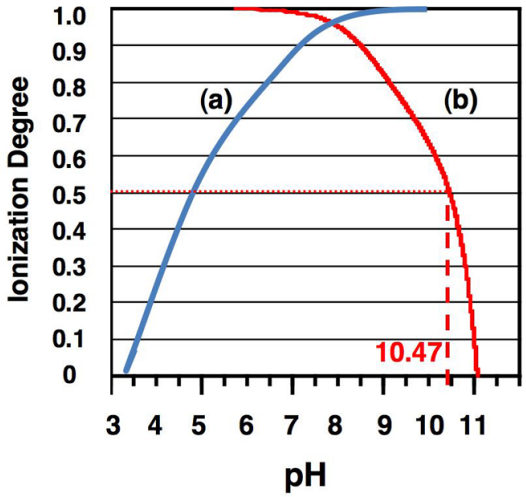


***Fig. S2*** **Change in dissociation degree with pH for PEG-PAsp (a; blue; the curve was taken from ref. 3) and PEG-P(Asp-AP) (b; red).** The red curve was created using the titrated volume-pH curve shown in Fig. S1 and the following equation: [Ionization Degree] = 1.0 – ([$V$]– [$V_s$])/([$V_e$] – [$V_s$]), [$V_s$] = 25.39, [$V_e$] = 52.16, where $V$ is titrated volume, $V_s$ is titrated volume at the start point, and $V_e$ is titrated volume at the end point.

p$K_a$ value was defined as a pH value on the titration curve at the ionization degree of 0.5 (dashed line).

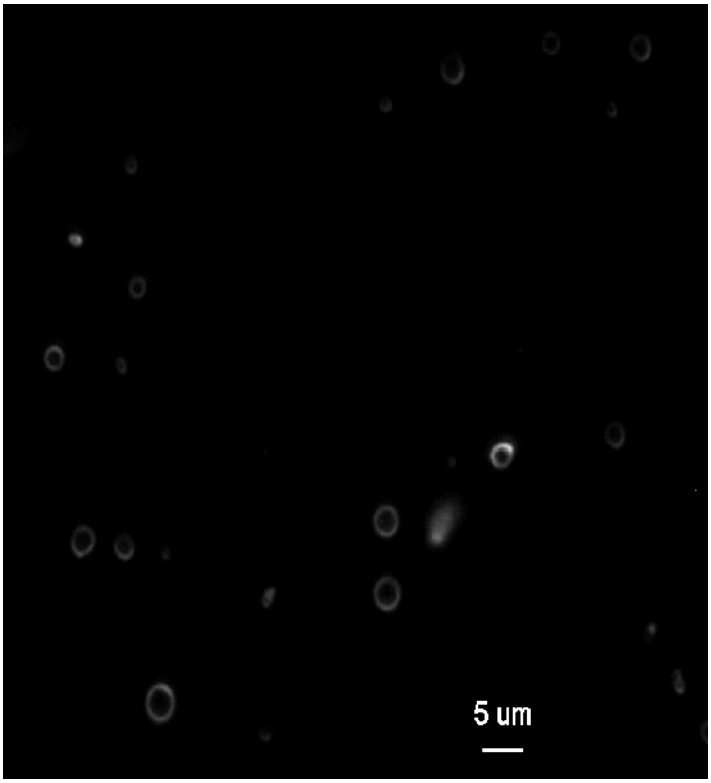


***Fig. S3*** **Dark field microscopic image of PICsomes prepared at pH 7.4.**

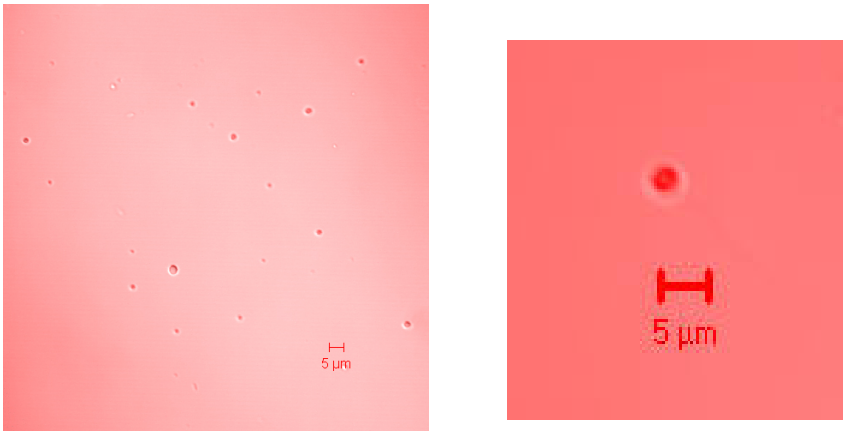


***Fig. S4*** **Microscopic pictures of PICsomes which were regenerated in the presence of TRITC-Dex$_{70K}$.** In both pictures, the confocal image and the optical transmission image were merged, indicating that TRITC-Dex$_{70K}$ was loaded into the regenerated PICsomes.

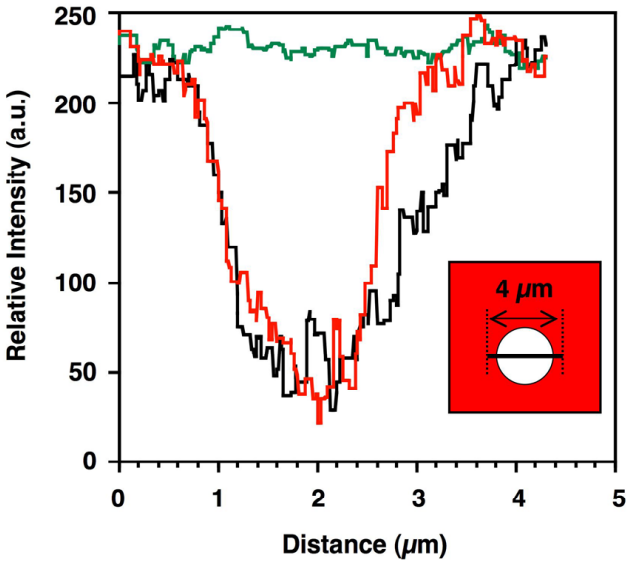


***Fig. S5*** **Cross-sectional fluorescence intensity profiles of PICsome at pH 6.2 just after addition of the TRITC-Dex$_{10K}$ (green), TRITC-Dex$_{40K}$ (black), TRITC-Dex$_{70K}$ (red).** Each cross-section passes through the center of the particle (inset). The mean values of relative fluorescence intensity of particles in similar size (n=5) were plotted. These results show that only TRITC-Dex$_{10K}$ can pass through the PIC membrane immediately.

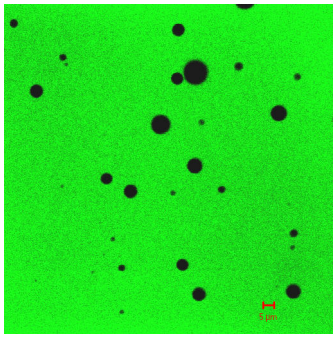

***Fig. S6*** **CLSM image of PICsomes 48 h after the addition of FITC-Dex$_{2M}$ at pH 7.4.** This result indicates that FITC-Dex$_{2M}$ cannot permeate PIC-membrane of the PICsome.

**Real-time observation of the structural transition of PICsomes**

Real-time observation movie is available in the electronic file "real_time.avi".

**References**

1. A. Koide, A. Kishimura, W.-D. Jang, K. Osada, Y. Yamasaki and K. Kataoka, *J. Am. Chem. Soc.*, 2006, **128**, 59885989.
2 A. Kishimura, A. Koide, K. Osada, Y. Yamasaki and K. Kataoka, *Angew. Chem. Int.Ed.*, 2007, **46**, 6085–6088.
3 A.Harada and K.Kataoka, *Macromolecules*, 1996, **28**, 52945299.
4 M. Nakanishi, J.-S. Park, W.-D. Jang, M. Oba and K. Kataoka, *React. Funct. Polym.,* 2007, **67**, 1361–1372.