*a.*

*Supplementary Information*

**Diffusive Delivery of Plasmid DNA by Using Zwitterionic Carboxyalkyl Poly(1-vinylimidazole) into Skeletal Muscle**

Ren Misaizu,[a] Yoko Endo-Takahashi,[b] Kei Nirasawa,[b] Yoichi Negishi,[b] Shoichiro Asayama*[a]

[a]Department of Applied Chemistry, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192-0397, Japan.

Phone: +81426771111(ext.)4976.

E-mail: asayama-shoichiro@tmu.ac.jp

[b]School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan.

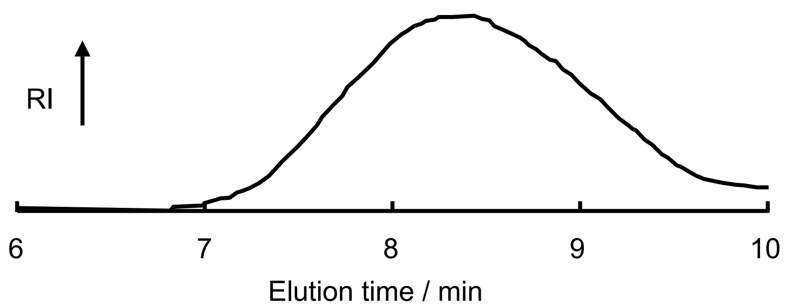



**Fig. S1.** Gel filtration chromatogram of a representative PVIm backbone: column, Shodex OHpak SB-804 HQ; eluent, 0.5 M $CH_3COOH$ and 0.2 M $NaNO_3$; flow rate, 1.0 mL/min; detection, refractive index (RI).

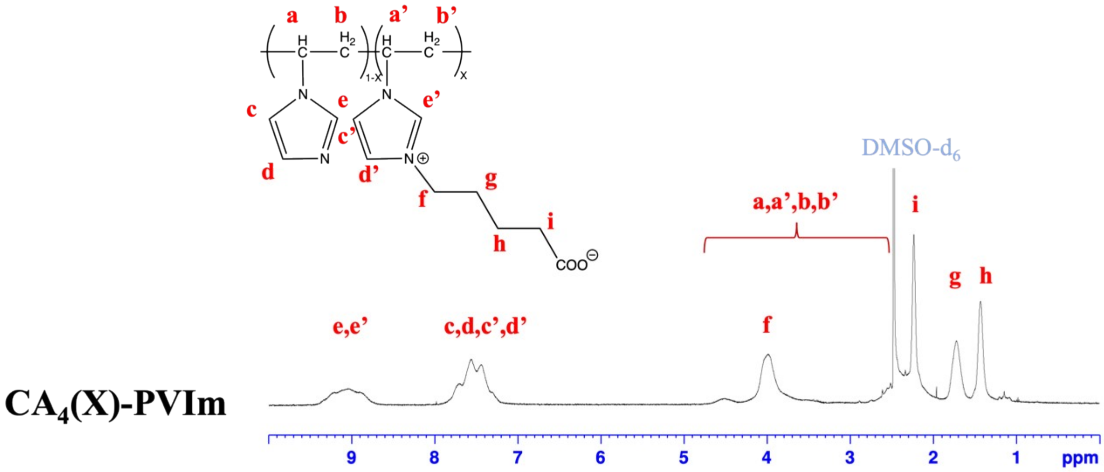
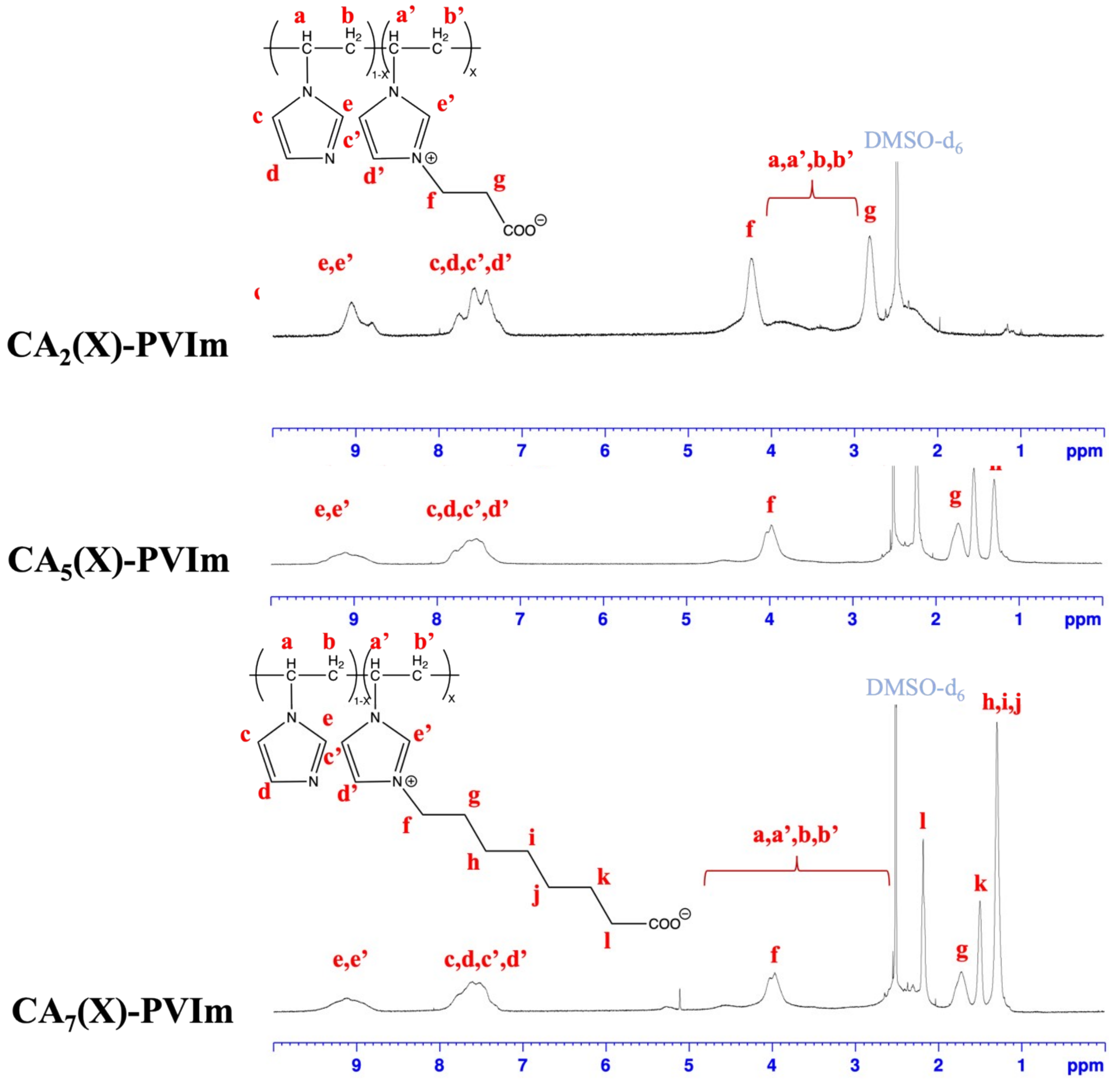



**Fig. S2.** [1]H NMR spectra of PVIm and CA-PVIm in DMSO-$d_6$.



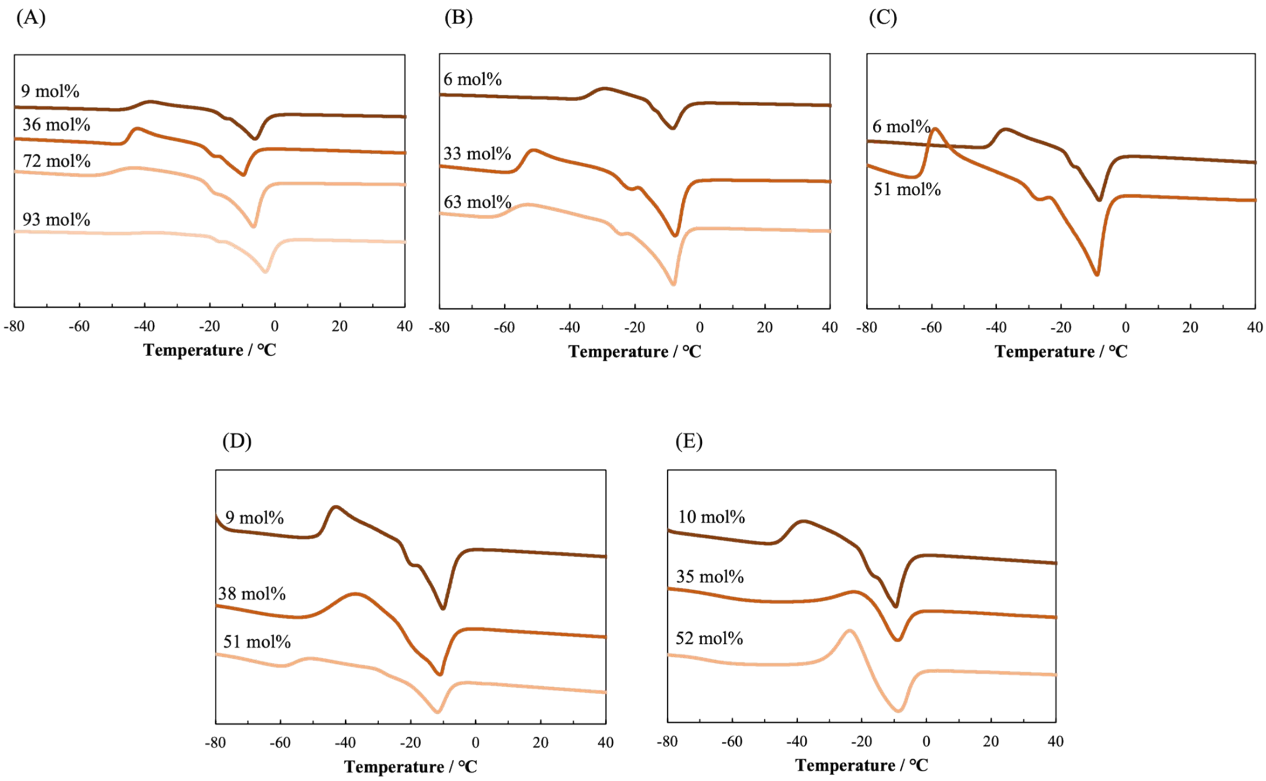



**Fig. S3.** Analysis for intermediate water in CA-PVIm by DSC. (A) $CA_1$-PVIm, (B) $CA_2$-PVIm, (C) $CA_4$-PVIm/pDNA, (D) $CA_5$-PVIm, and (E) $CA_7$-PVIm.

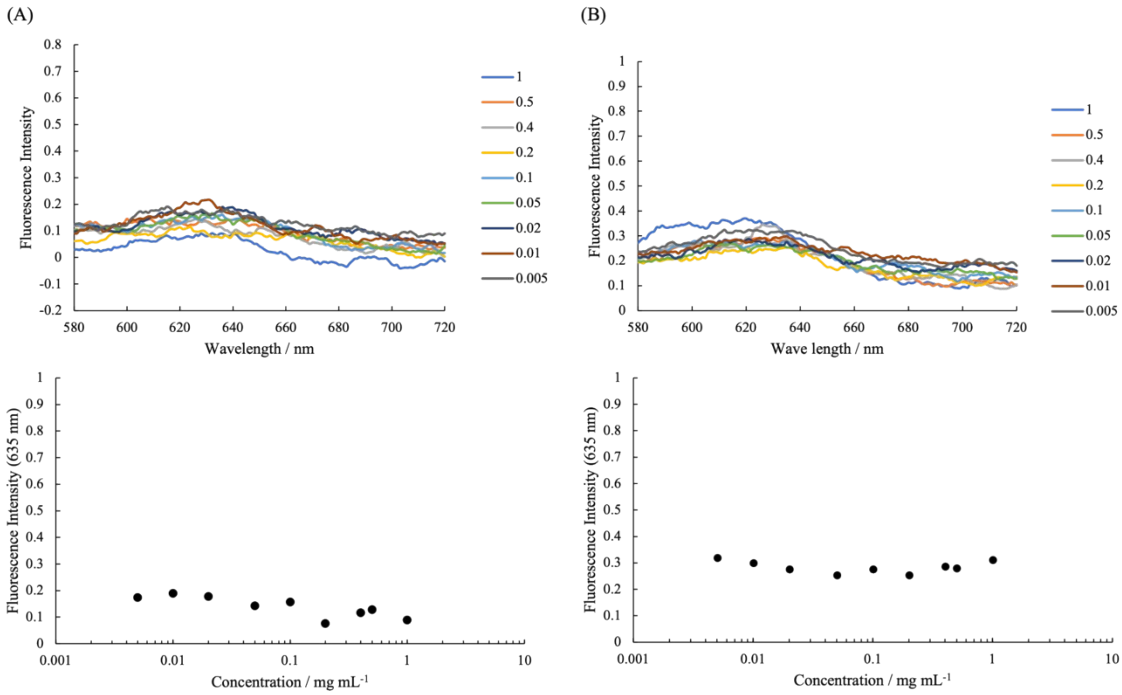



**Fig. S4.** Analysis for critical aggregation concentration (CAC) of $CA_n$-PVIm: (A) $CA_1$(76)-PVIm. (B) $CA_7$(52)-PVIm. Each color bar indicates polymer concentration.

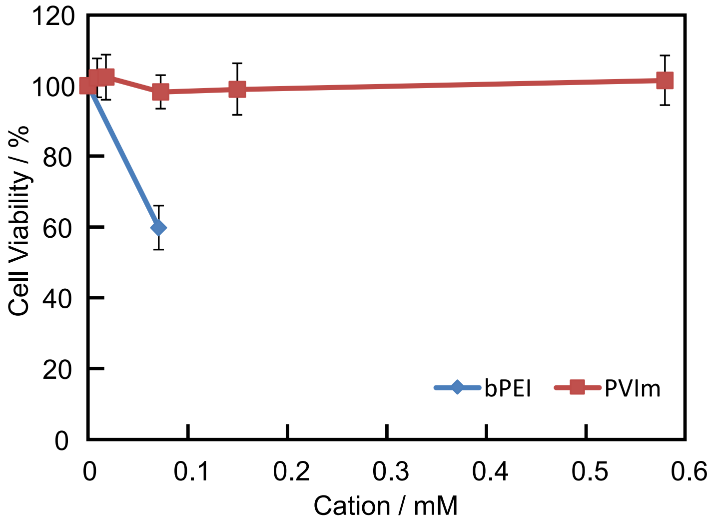
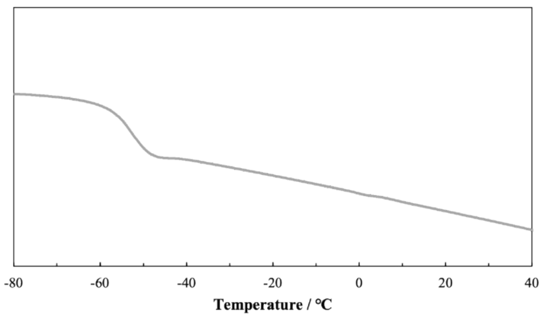



**Fig. S5.** Effect of unmodified PVIm on the cell (C2C12) viability. Symbols and error bars represent the mean and standard deviation of the measurements made in triplicate wells.

(A)




(B)

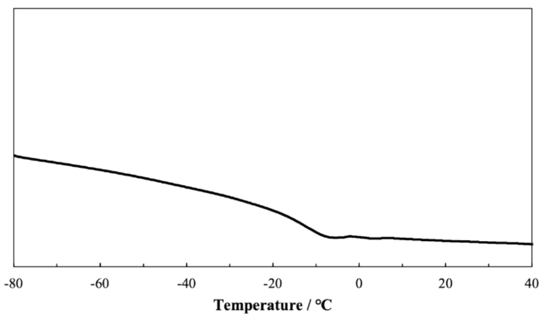



**Fig. S6.** Analysis for intermediate water in (A) bPEI and (B) unmodified PVIm by DSC.

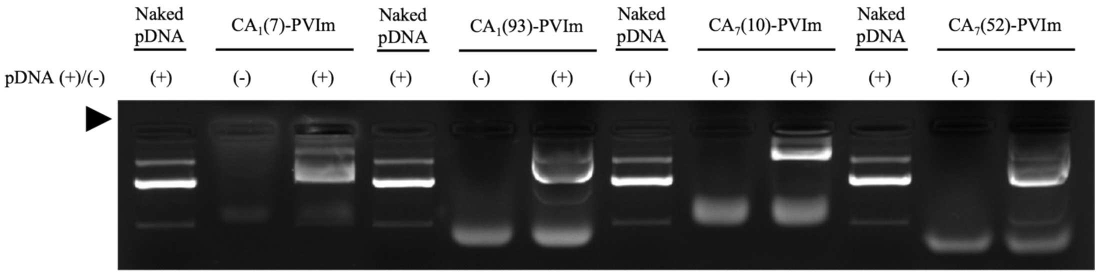



**Fig. S7.** Agarose gel electrophoresis of each CA-PVIm in the absence (-) or presence (+) of pDNA. The mixing charge ratios of the imidazolium group of CA-PVIm to the phosphate group of pDNA are 64. The solid arrowhead indicates the well where each sample was loaded.

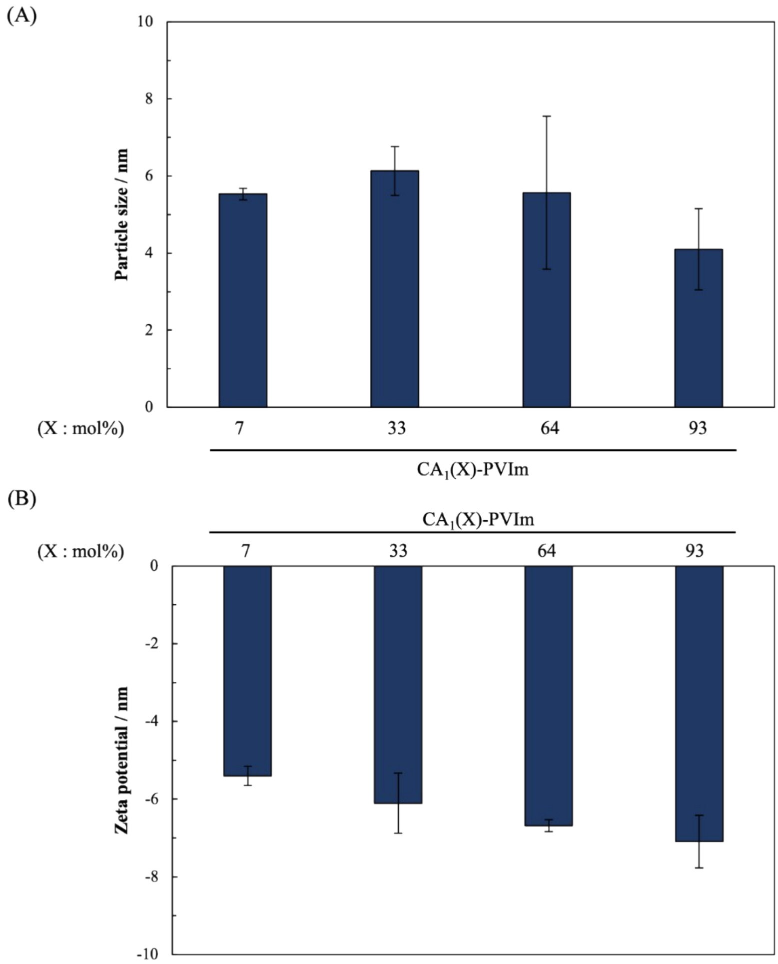



**Fig. S8.** Particle size and ζ-potential of $CA_1$(X)-PVIm alone: (A) Particle size and (B) ζ-potential.

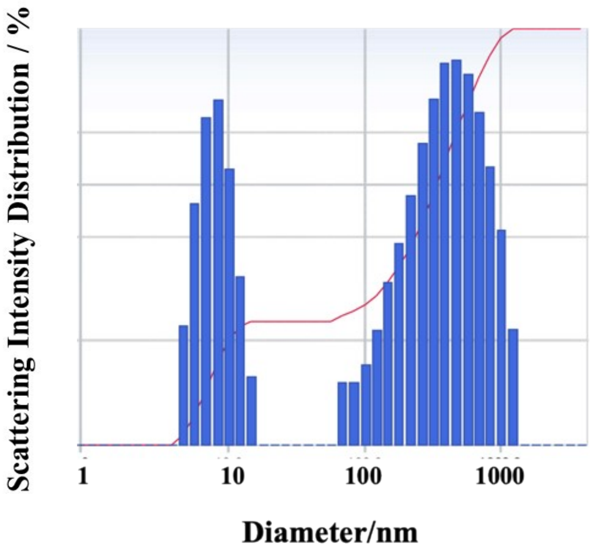



**Fig. S9.** Representative scattering intensity distribution of $CA_1$(7)-PVIm/pDNA PICs.

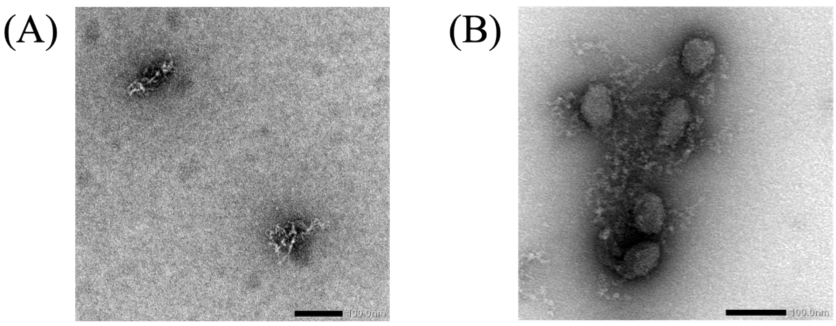



**Fig. S10.** Representative TEM images of the pDNA and CA-PVIm/pDNA PICs: (A) pDNA. (B) $CA_1$(93)-PVIm/pDNA PIC. Each acale bar represents 100 nm.

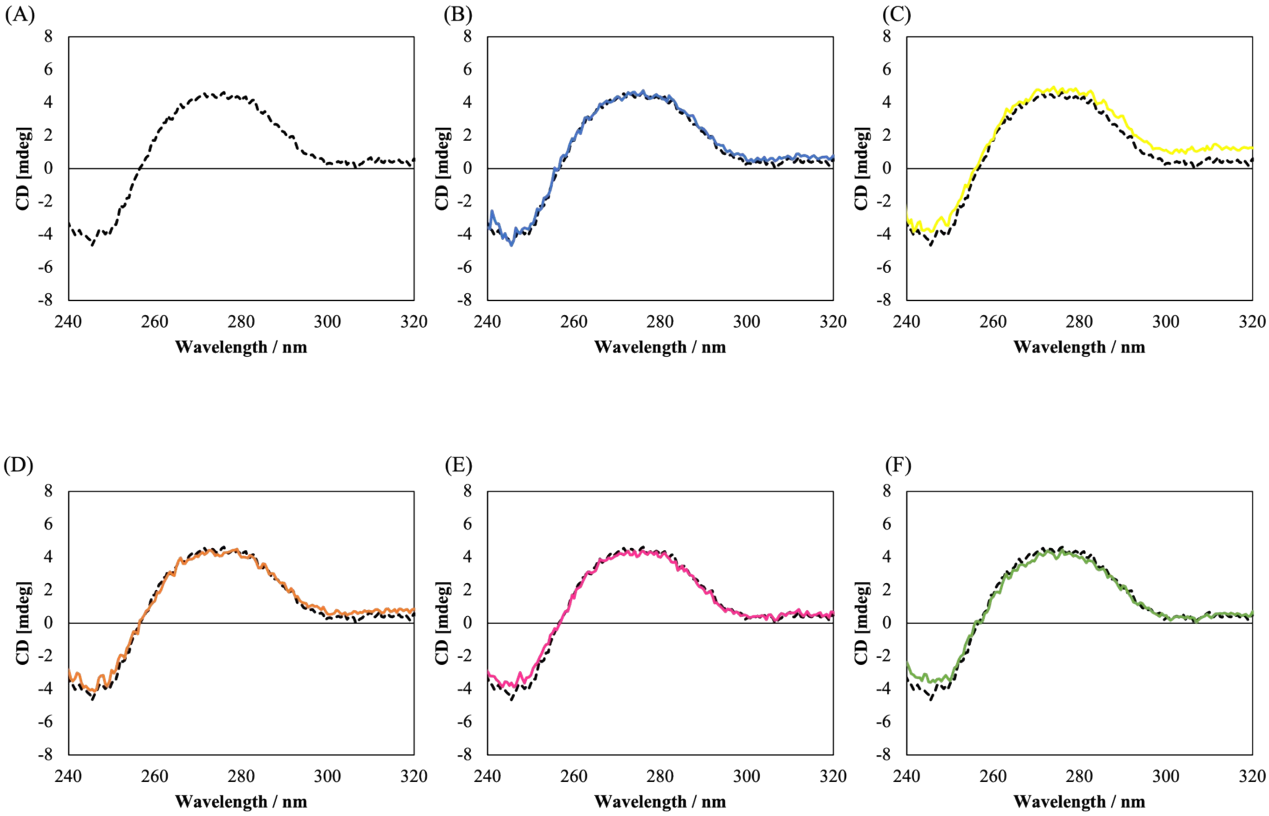



**Fig. S11.** CD spectrum of each CA-PVIm (High CA groups modification degree)/pDNA PICs. (A) Naked pDNA, (B) $CA_1$(93)-PVIm/pDNA complex, (C) $CA_2$(64)-PVIm/pDNA complex, (D) $CA_4$(51)-PVIm/pDNA complex, (E) $CA_5$(59)-PVIm/pDNA complex, and (F) $CA_7$(52)-PVIm/pDNAcomplex.

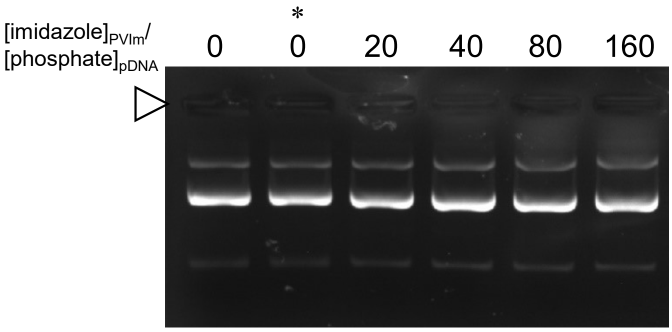



**Fig. S12.** Analysis for the pDNA PIC formation with ummodified PVIm by agarose gel electrophoresis. The mixing charge ratios of the imidazole group of PVIm to the phosphate group of pDNA are indicated as $[\mathrm{Imidazole}]_{\mathrm{PVIm}}/[\mathrm{phosphate}]_{\mathrm{pDNA}}$. The solid arrowhead indicates the well where each sample was loaded. * indicate no scope of the present study (in the presence of $Zn^{2+}$).

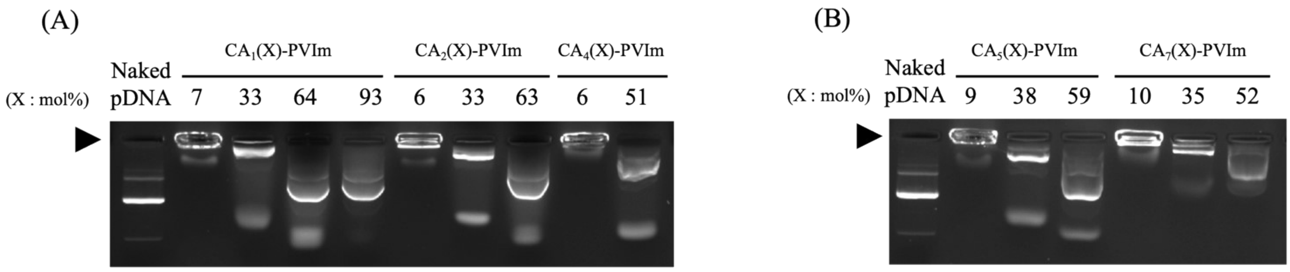



**Fig. S13.** Analysis for pH dependence of CA-PVIm/pDNA PICs at pH 6.0 by agarose gel electrophoresis. The mixing charge ratios of the imidazolium group of CA-PVIm to the phosphate group of pDNA are 64. The solid arrowhead indicates the well where each sample was loaded.

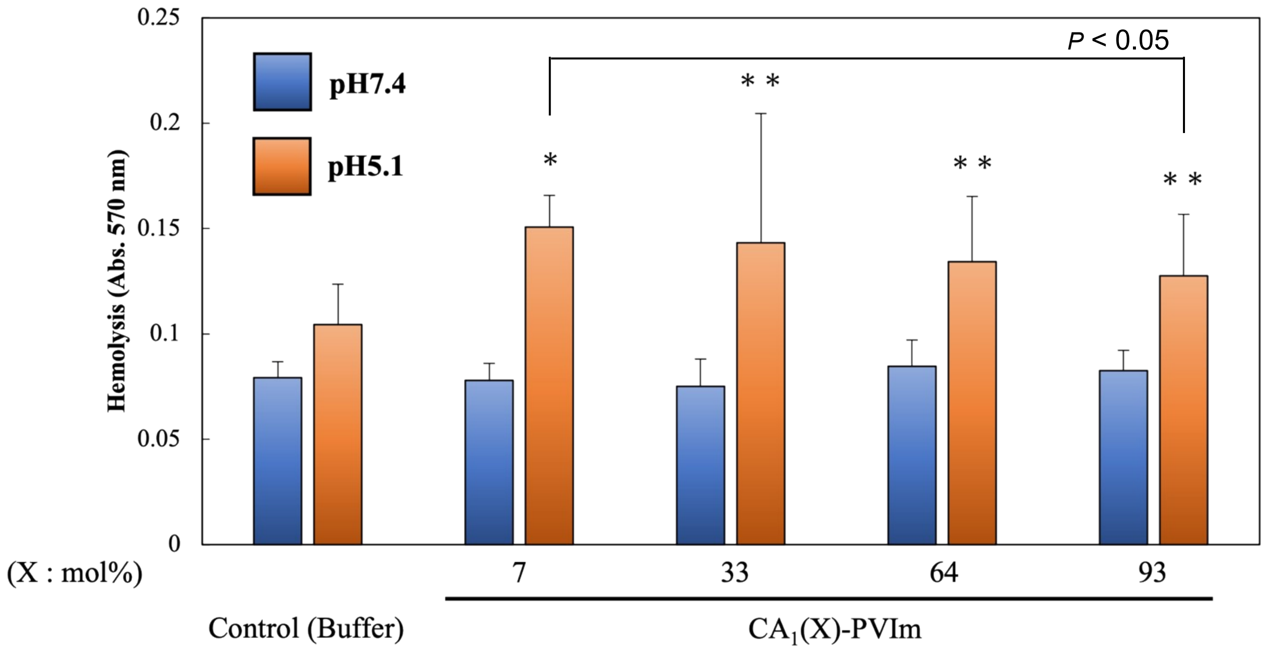



**Fig. S14.** Hemolysis activity against red blood cell by $CA_1$(X)-PVIm at pH5.1 and pH7.4. *P*-values are indicated: **P*<0.01, ***P*<0.1 (n=6-10) vs. buffer. All data are represented as the mean ±SD (n=10-18).

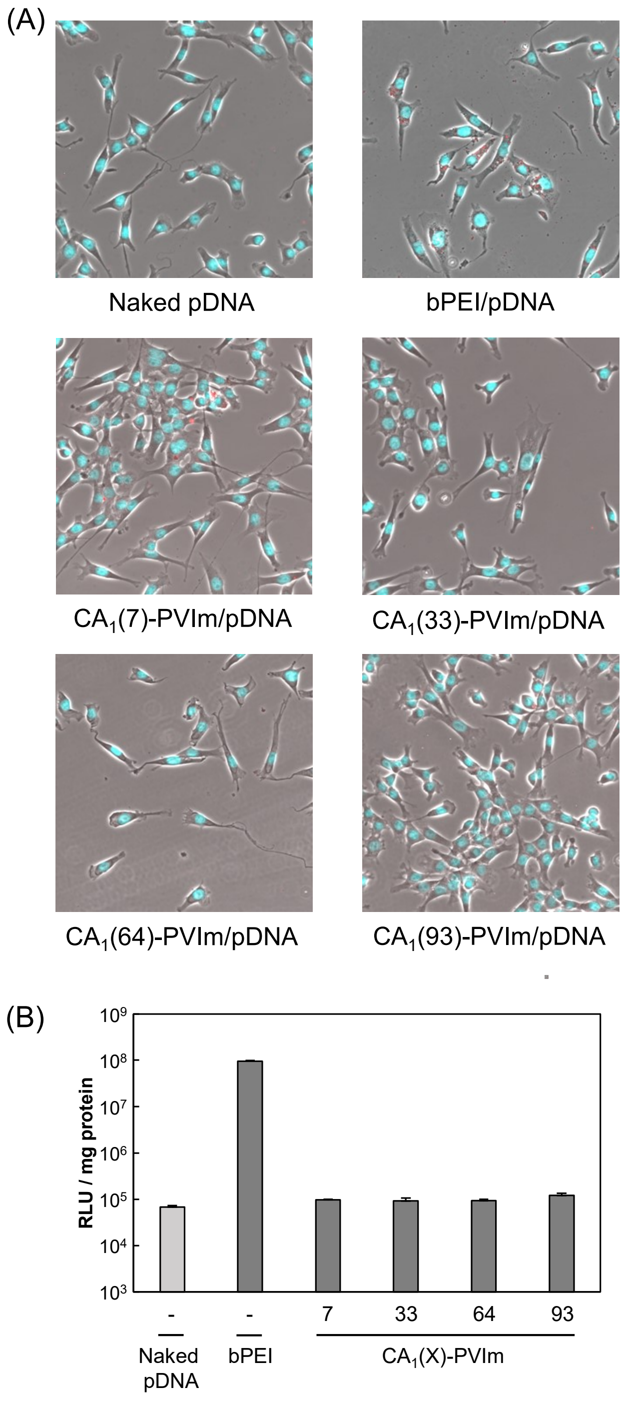



**Fig. S15.** (A) Confocal laser scanning microscopy images of C2C12 cells treated with naked pDNA, bPEI/pDNA, $CA_1$(X)-PVIm/pDNA complexes. The cell morphology was observed using a differential interference contrast (DIC) microscope. The pDNA was labeled with rhodamine (red). The cell nuclei were stainted with 4′,6-Diamidino-2-phenylindole, dihydro-chloride (DAPI: blue). The maerged images are represented. (B) Transfection activity of luciferase gene into C2C12 cells mediated by the corresponding pDNA complexes. Gene expression was determined as RLU normalized by the protein concentration. Symbols and error bars represent the mean and standard deviation in triplicate.

**Table S1.** Polydispersity index (PDI) of CA-PVIm/pDNA PICs

| Sample | PDI |
|---|---|
| Naked pDNA | $0.358 \pm 0.04$ |
| $CA_1$(7)-PVIm/pDNA | $0.344 \pm 0.03$ |
| $CA_1$(33)-PVIm/pDNA | $0.241 \pm 0.03$ |
| $CA_1$(64)-PVIm/pDNA | $0.297 \pm 0.03$ |
| $CA_1$(93)-PVIm/pDNA | $0.247 \pm 0.02$ |
| $CA_2$(6)-PVIm/pDNA | $0.305 \pm 0.15$ |
| $CA_2$(33)-PVIm/pDNA | $0.199 \pm 0.03$ |
| $CA_2$(63)-PVIm/pDNA | $0.217 \pm 0.08$ |
| $CA_4$(6)-PVIm/pDNA | $0.362 \pm 0.01$ |
| $CA_4$(51)-PVIm/pDNA | $0.280 \pm 0.03$ |
| $CA_5$(9)-PVIm/pDNA | $0.386 \pm 0.01$ |
| $CA_5$(59)-PVIm/pDNA | $0.353 \pm 0.03$ |
| $CA_7$(10)-PVIm/pDNA | $0.228 \pm 0.07$ |
| $CA_7$(52)-PVIm/pDNA | $0.275 \pm 0.08$ |