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Electronic Supplementary Information

Effective design for PEGylated polyion complex (PIC) nanoparticles to enhance cell–PIC interaction utilising block copolymer combinations of mismatching ionic chain lengths

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1. Characterization of polymers

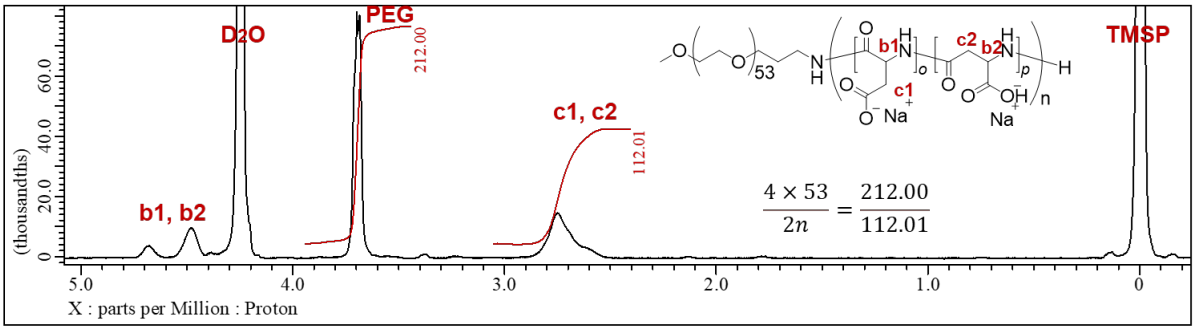


Figure S1. A ^{1}H-NMR spectrum of PEG$_{2k}$-PAsp (10 mg mL^{-1}, D$_2$O) at 80 °C with TMSP as an internal standard. DP was calculated to be 56.

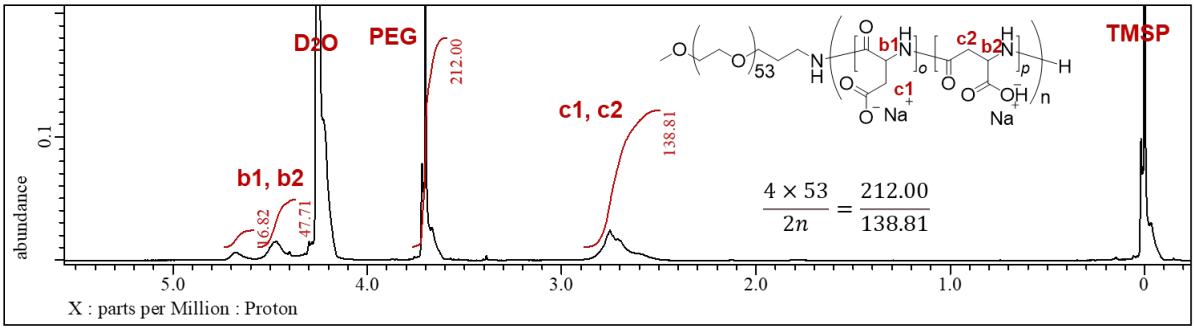


Figure S2. A ^{1}H-NMR spectrum of PEG$_{2k}$-PAsp (10 mg mL^{-1}, D$_2$O) at 80 °C with TMSP as an internal standard. DP was calculated to be 69.

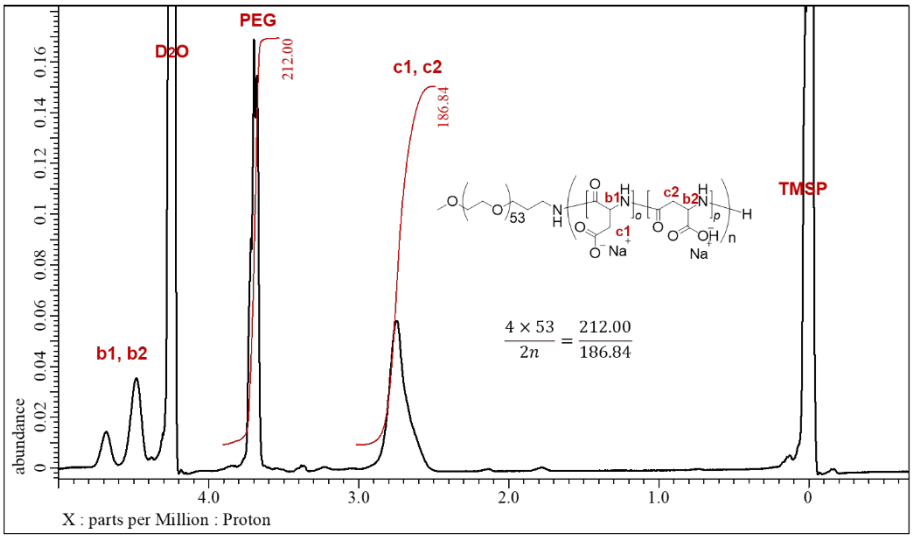


Figure S3. A ^{1}H-NMR spectrum of PEG$_{2k}$-PAsp (10 mg mL^{-1}, D$_2$O) at 80 °C with TMSP as an internal standard. DP was calculated to be 93.

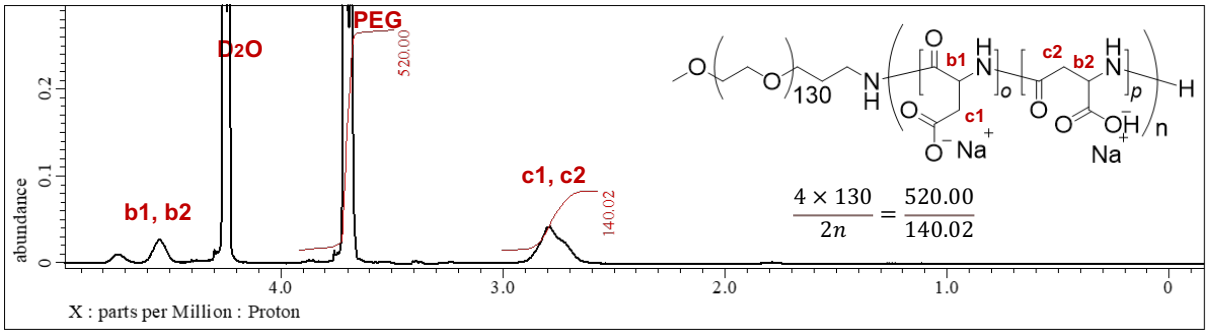


Figure S4. A ^{1}H-NMR spectrum of PEG_{5k}-PAsp (10 mg mL^{-1}, D_2O) at 80 °C. DP was calculated to be 70.

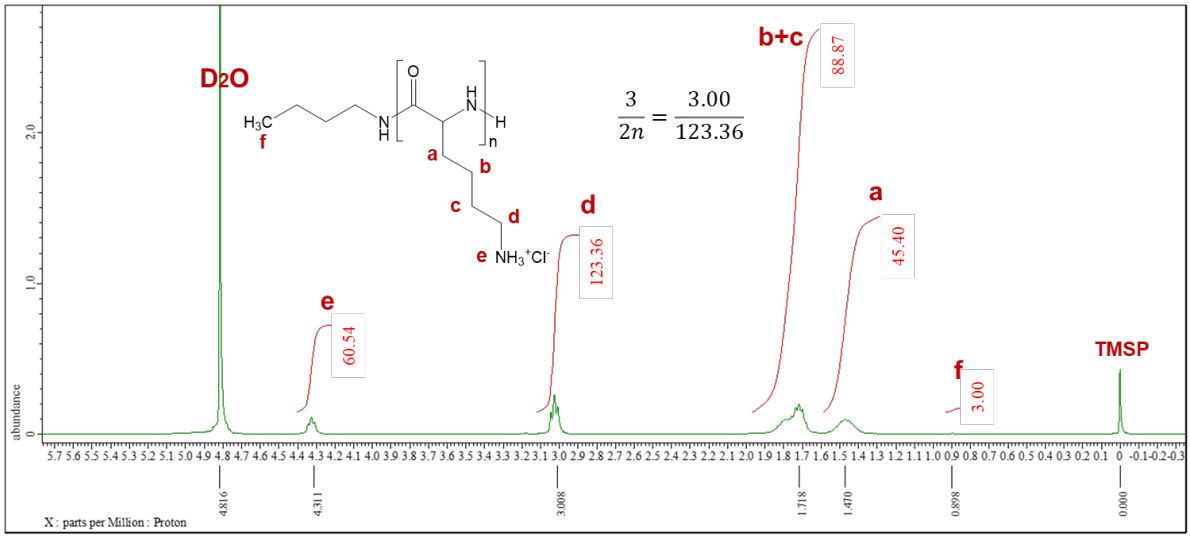


Figure S5. A ^{1}H-NMR spectrum of homo-PLL (10 mg mL^{-1}, D_2O) at 25 °C with TMSP as an internal standard. DP was calculated to be 64.

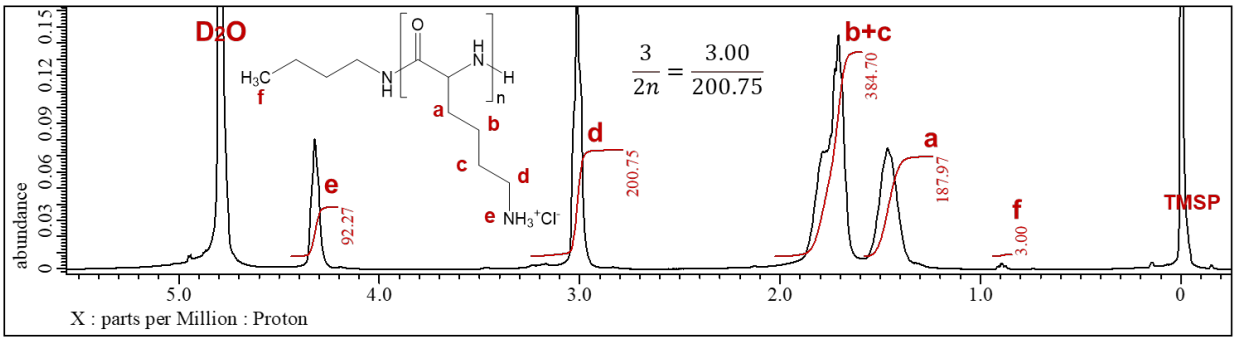


Figure S6. A ^{1}H-NMR spectrum of homo-PLL (10 mg mL^{-1}, D_2O) at 25 °C with TMSP as an internal standard. DP was calculated to be 100.

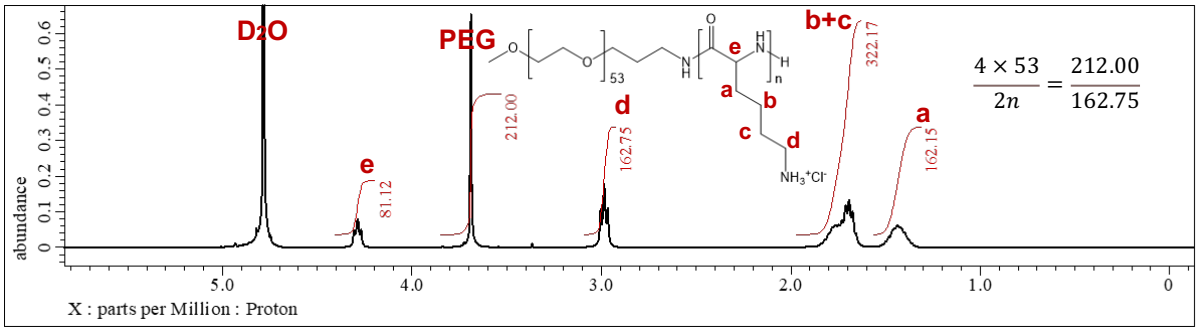


Figure S7. A ^{1}H-NMR spectrum of PEG_{2k}–PLL (10 mg mL^{-1}, D_2O) at 25 °C. DP was calculated to be 81.

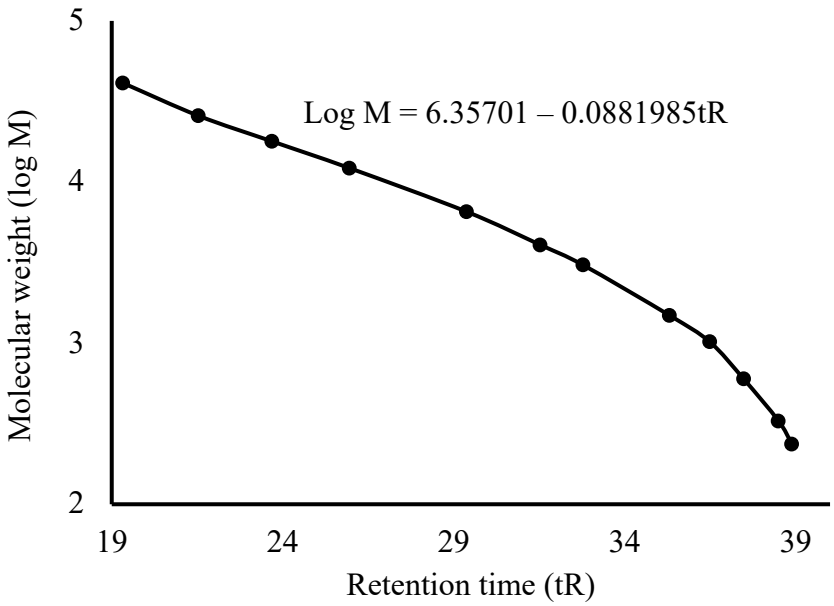


Figure S8. An SEC calibration curve of PEO standards

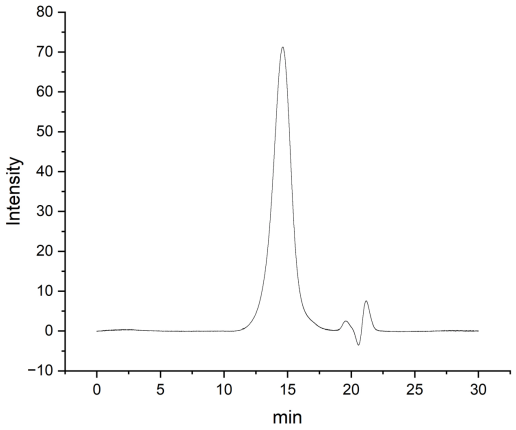


Figure S9. A size-exclusion chromatogram of PEG_{2k}–$PAsp_{56}$.

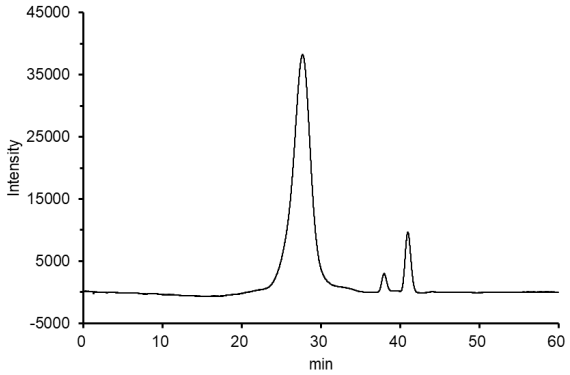


Figure S10. A size-exclusion chromatogram of PEG_{2k}–$PAsp_{69}$.

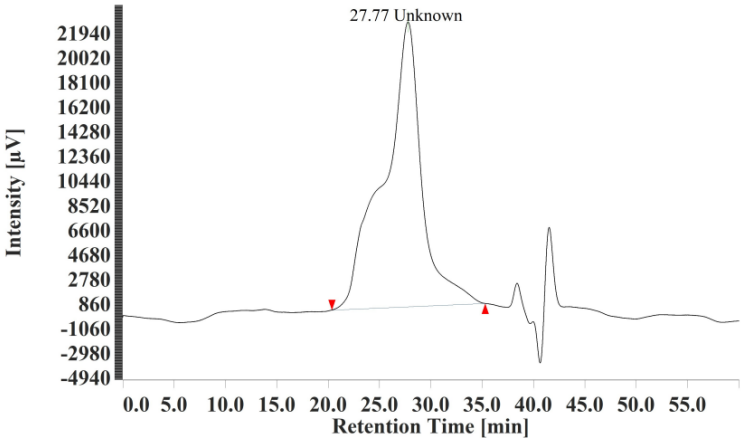


Figure S11. A size-exclusion chromatogram of PEG_{2k}–$PAsp_{93}$.

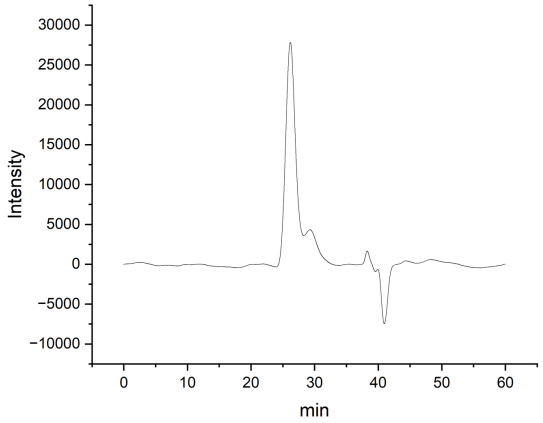


Figure S12. A size-exclusion chromatogram of PEG_{5k}–PAsp.

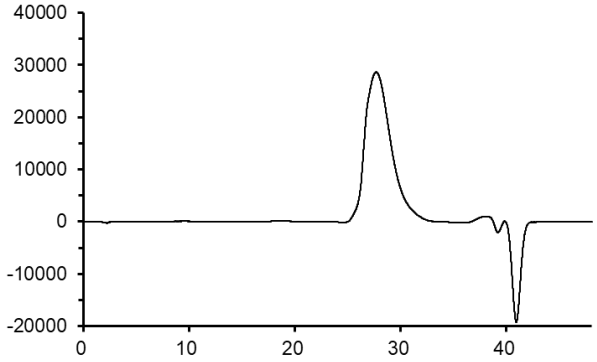


Figure S13. A size-exclusion chromatogram of homo-PLL_{64}.

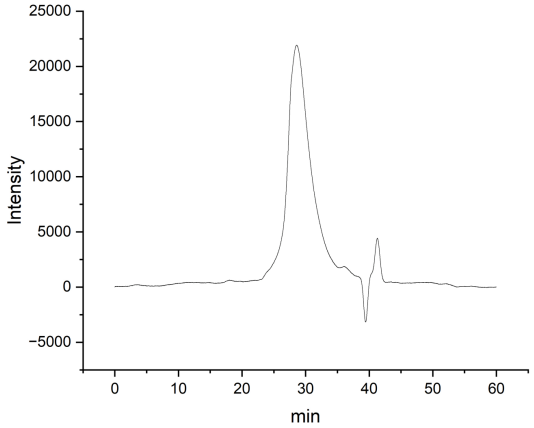


Figure S14. A size-exclusion chromatogram of homo-PLL_{100}.

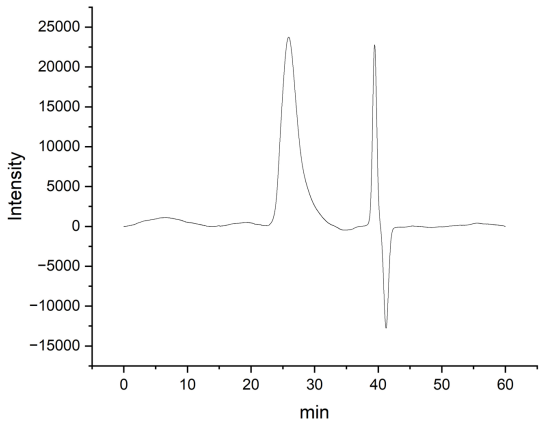


Figure S15. A size-exclusion chromatogram of PEG_{2k}–PLL.

2. Characterization of PICs

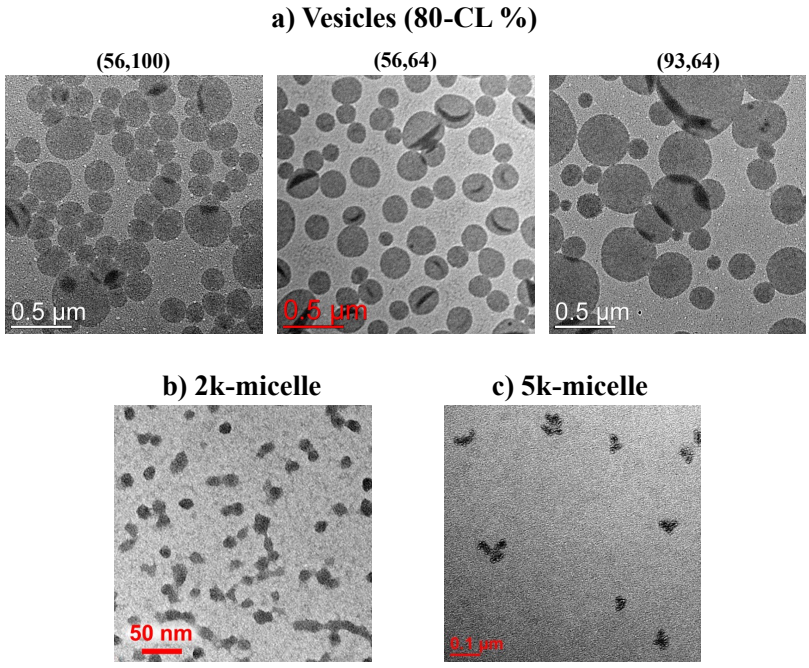


Figure S16. TEM images of the PICs: (a) vesicles, (b) 2k-micelle, and (c) 5k-micelle.

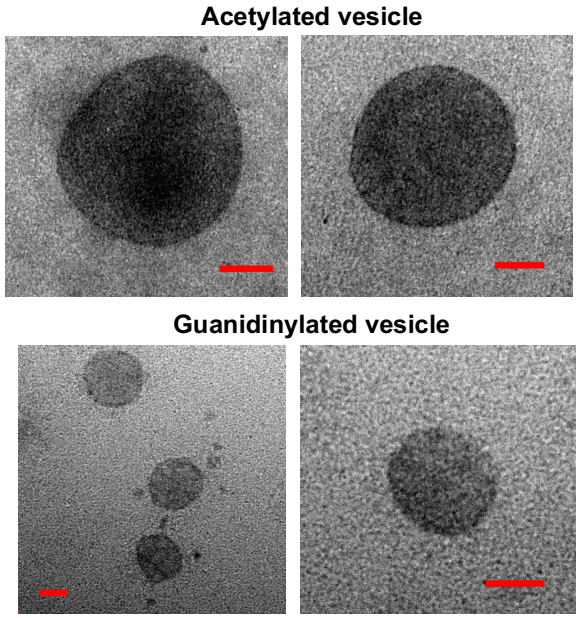


Figure S17. TEM images of Acetylated and Guanidinylated vesicles

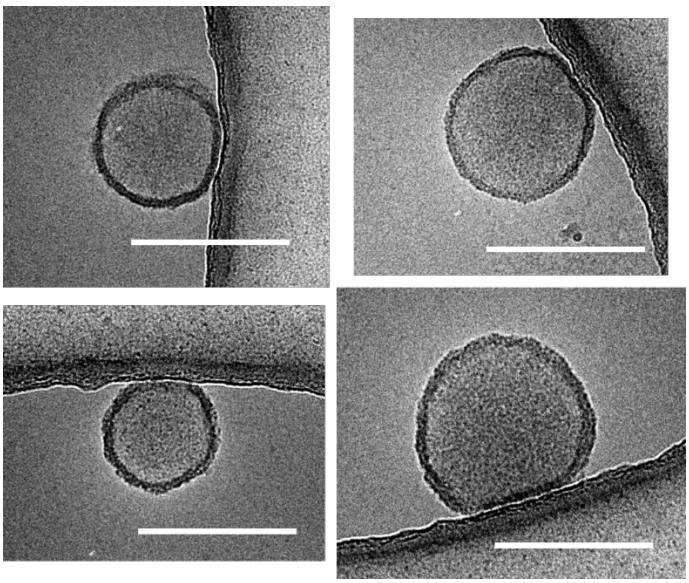


Figure S18. Cryo-TEM images of vesicle PIC (56,100) with 50%-CL (Scale bar: 200 nm). The thickness of the PIC membrane was estimated to be 12.0 ± 2.5 nm (N = 28).

Equation S1. The equation to determine the mixing ratio of polyanion and polycation solutions for the PIC fabrication

$$\frac{(conc.\,of\ polyanion) \times (polyanion\ solution\ volume) \times (DP\ of\ polyanion) \times (ionization\ degree\ of\ carboxylate)}{MW\ of\ polyanion}$$

$$= \frac{(conc.\,of\ polycation) \times (polycation\ solution\ volume) \times (DP\ of\ polycation) \times (ionization\ degree\ of\ amine)}{MW\ of\ polycation}$$

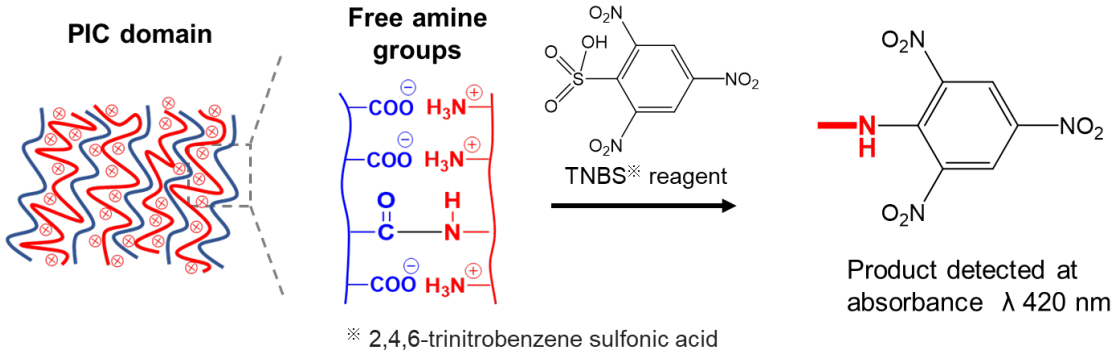
Note:

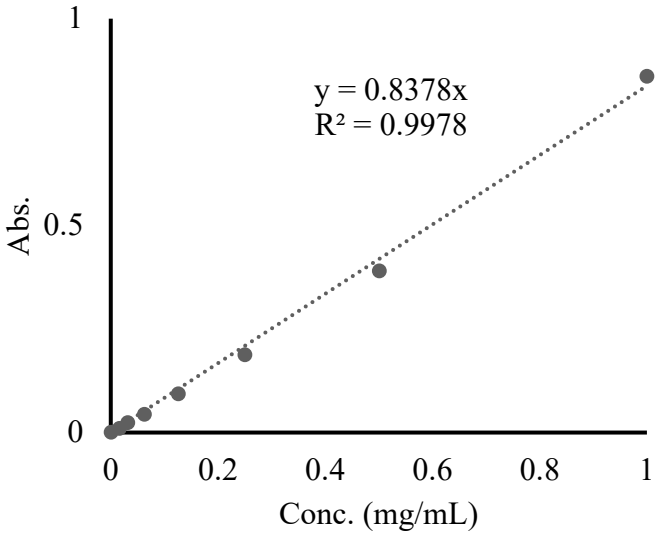
Conc. Of polyanion and polycation are set to be 1 mg/mL.

Ionization rate of carboxyl at pH 7.4 is 0.92.[31]

Ionization rate of amine at pH 7.4 is 0.95.[31]

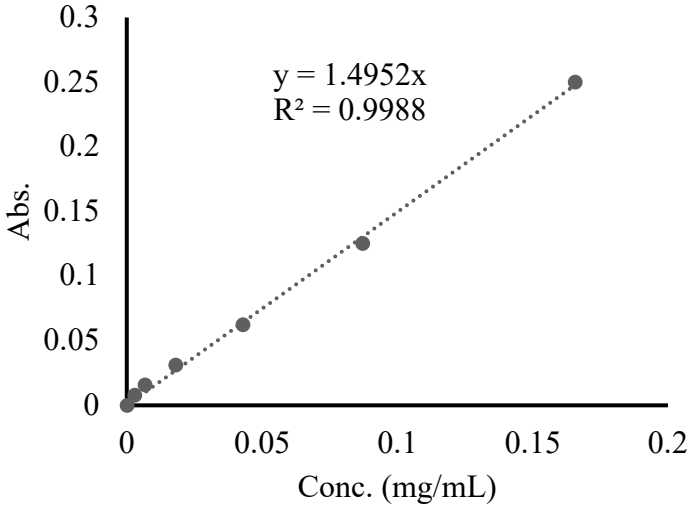
Scheme S1. Schematic drawing of TNBS assay.





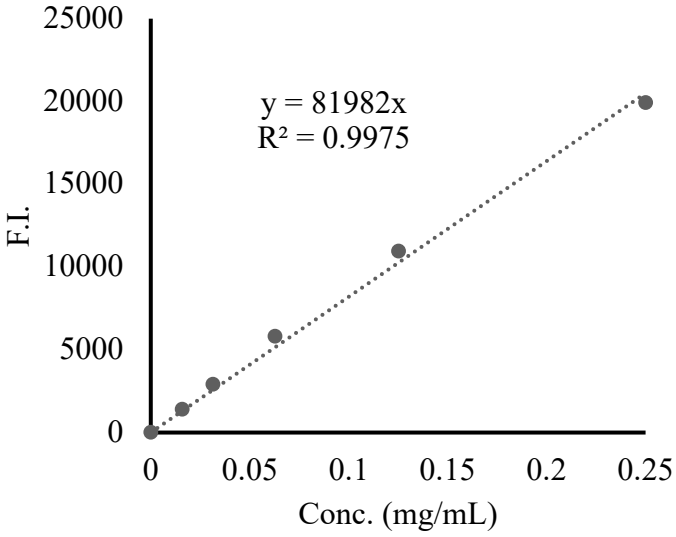
Conc. (mg/mL)	Abs.
1	0.8598
0.5	0.3904
0.25	0.1872
0.125	0.0932
0.0625	0.0429
0.03125	0.0229
0.015625	0.0088
0	0

Figure S19. A TNBS assay calibration curve obtained using homo-PLL



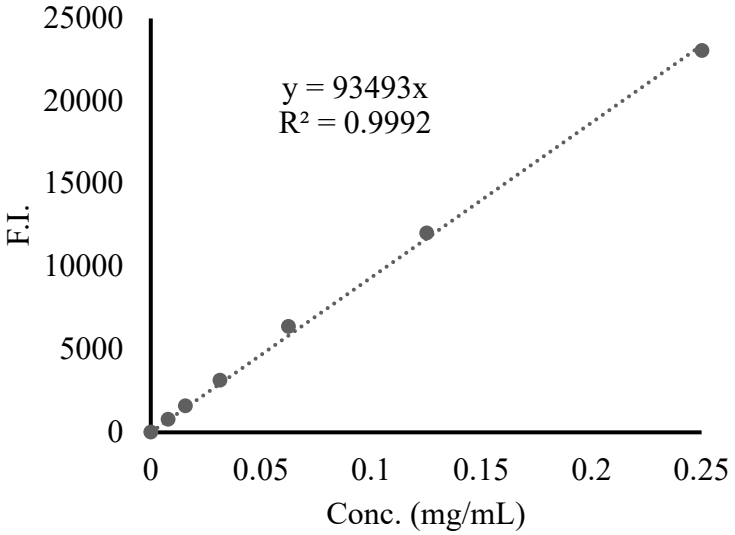
Conc. (mg/mL)	Abs.
0.1656	0.25
0.0871	0.125
0.0429	0.0625
0.0179	0.03125
0.0067	0.015625
0.0028	0.007813
0	0

Figure S20. A TNBS assay calibration curve obtained using PEG_{2k}-PLL



Conc. (mg/mL)	F.I.
0.25	19930
0.125	10933
0.0625	5814
0.03125	2899
0.015625	1412
0	0

Figure S21. A Cy3-fluorescence calibration curve obtained using PEG_{2k}-PAsp-Cy3



Conc. (mg/mL)	F.I.
0.25	23032
0.125	12035
0.0625	6370
0.03125	3136
0.015625	1569
0.007813	789
0	0

Figure S22. A Cy3-fluorescence calibration curve obtained using PEG_{5k}-PAsp-Cy3

Equation S2. The equation to determine the crosslinking degree

$$Remaining\ amine\ ratio\ (\%) = \frac{(DP\ of\ polycation) \times (ionization\ degree\ of\ amino\ groups) \times (apparent\ conc.\ of\ polycation\ from\ TNBS\ assay)}{(MW\ of\ polycation)} \times \frac{(MW\ of\ polyanion)}{(conc.\ of\ polyanion) \times (DP\ of\ polyanion) \times (ionization\ degree\ of\ carboxylate)} \times 100$$

$$Crosslinking\ degree\ (\%) = 100 - [amount\ of\ free\ amino\ groups\ (\%)]$$

Equation S3. The equation for calculation of PEG weight fraction, f_{PEG}, of PICs

$$f_{PEG} = \frac{(PEG\ MW) \times (mole\ fraction\ of\ PEGylated\ ionomer)}{total\ molecular\ weight\ of\ charge\ neutralized\ complex\ in\ mole}$$

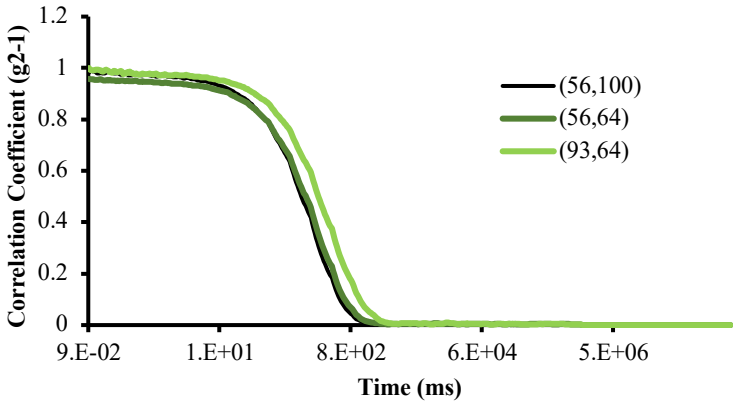


Figure S23. Autocorrelation functions of PIC vesicles

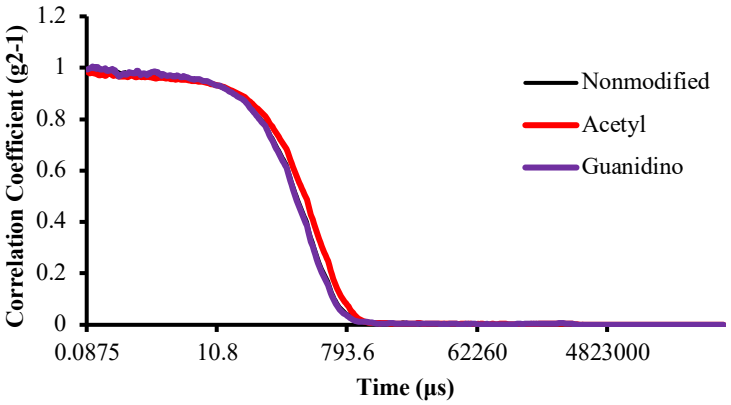


Figure S24. Autocorrelation functions of non- and modified- PIC vesicles

3. Cell experiment results

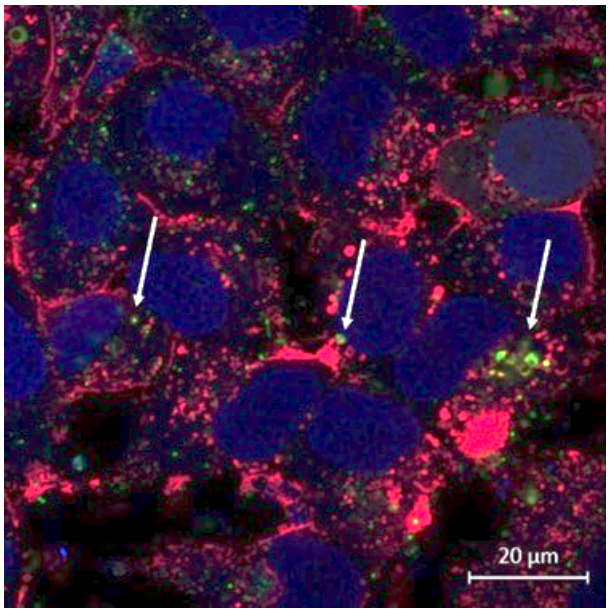


Fig. S25 The result of calcein leakage assay of PIC vesicle in HeLa cells obtained by CLSM. Blue: nuclei; Red: PIC; Green: calcein; Yellow: PIC+calcein colocalization. Briefly, HeLa was incubated with medium containing PIC (56, 100) (equal to final PAsp conc. 0.02 mg/mL) and calcein (final conc. 0.1 mg/mL) for 24 hours. Calcein leakage indicating endosomal escape, which will dye the cell with green color of whole cell body upon calcein dissolution into cytosol.

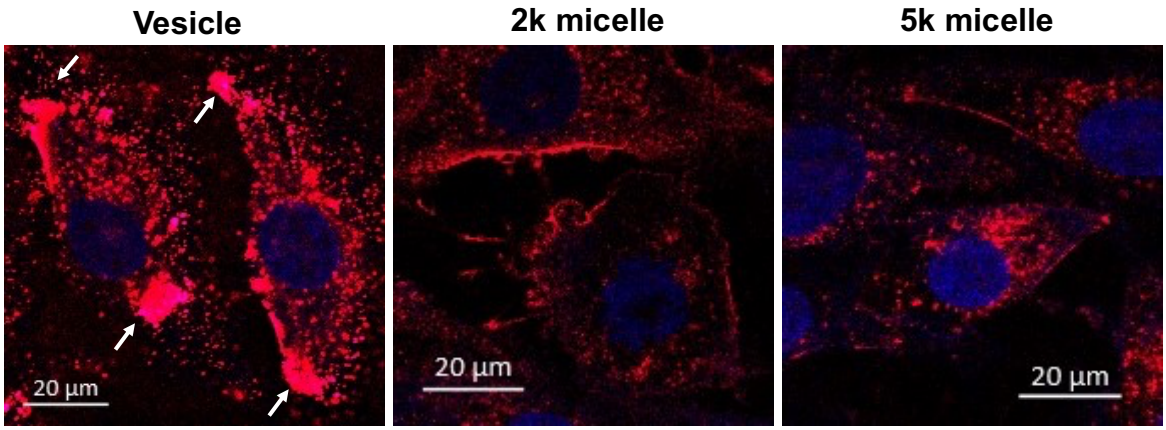


Figure S26. PIC retention on the HeLa cell surface observed after 24 h of incubation. (Blue: nucleus; Red: PICs). Aggregates are indicated by white arrows. The contrast of pictures was adjusted to yield a clear appearance of the PIC position.

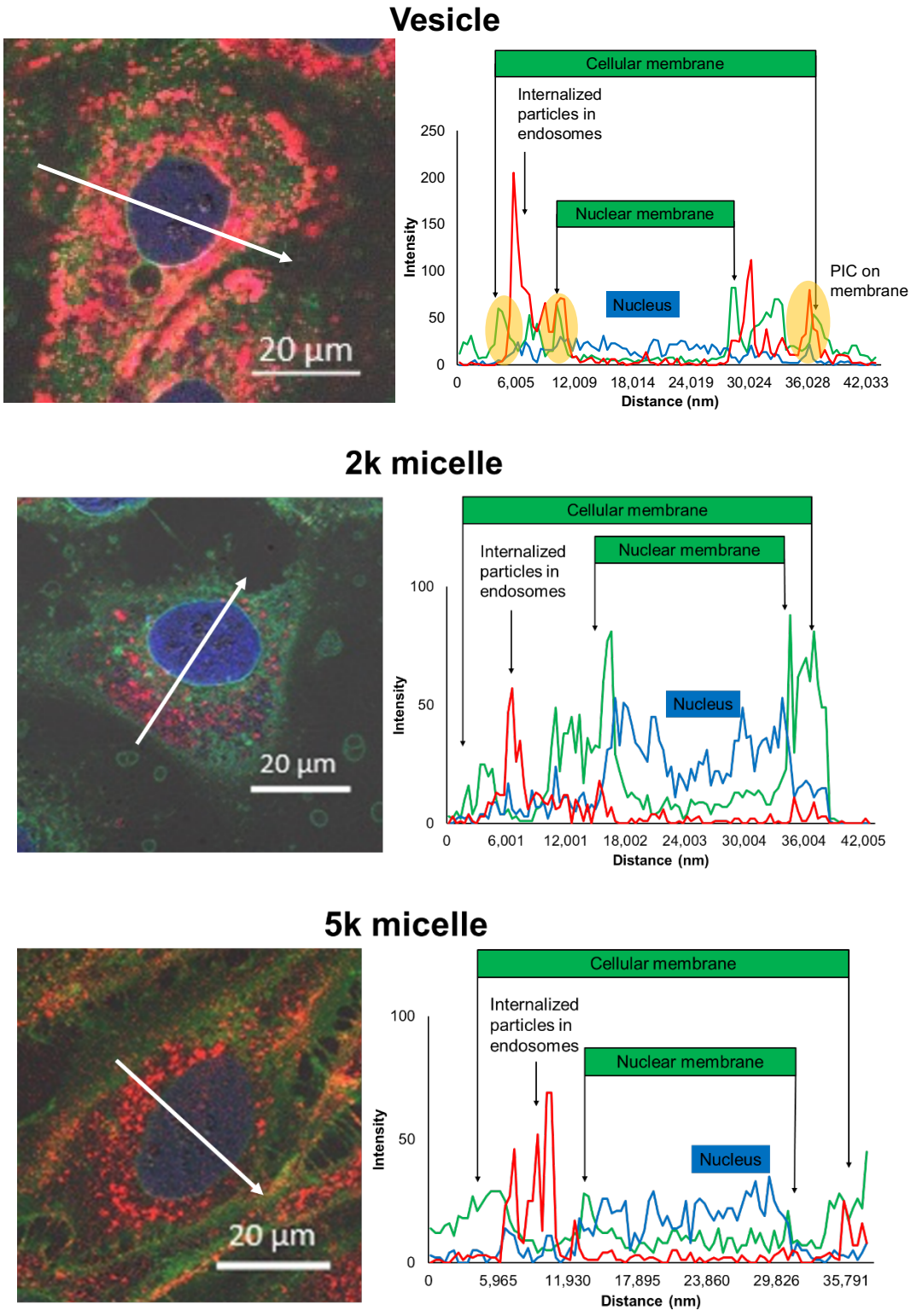


Figure S27. Line profilings of fluorescence intensity across HeLa cells treated with (a) PIC vesicles, (b) 2k-micelle, and (c) 5k-micelle. Left: CLSM images of HeLa cells used for line profiling analysis (scan lines are shown in each image as a white arrow). Right: Line profiling results (Green line: An intensity profile of the green

fluorescence from Cellbrite® (Biotium, Fremont, CA, a cellular membrane staining dye with green fluorescence, used according to manufacturer's protocol). Blue line: An intensity profile of blue fluorescence from Hoecsht3324; Red line: An intensity profile of red fluorescence from Cy3-labelled PICs.)

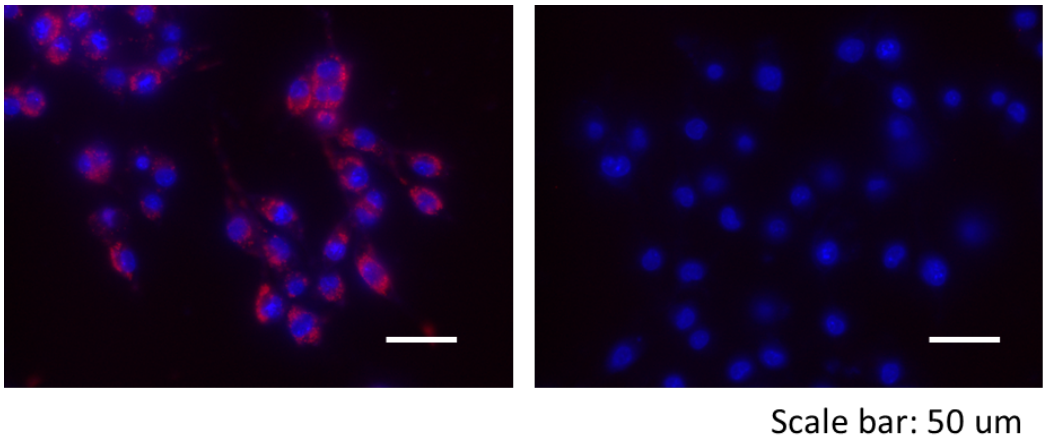
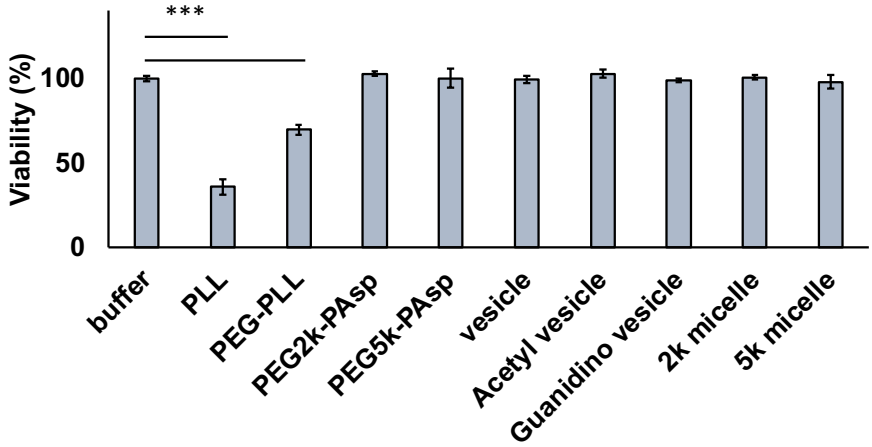
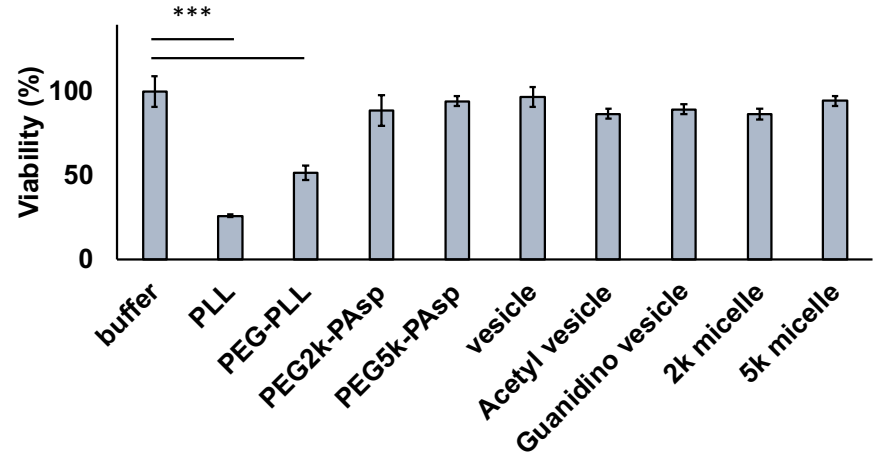


Figure S28. Fluorescence microscopy images of RAW 264.7 cells after 24 h of incubation with the acetylated PIC vesicle in the absence (left) and presence (right) of dextran sulphate ($DexSO_4$) 0.5 mg/mL. Blue: Nucleus (Hoechst 33342); red: acetylated vesicles.

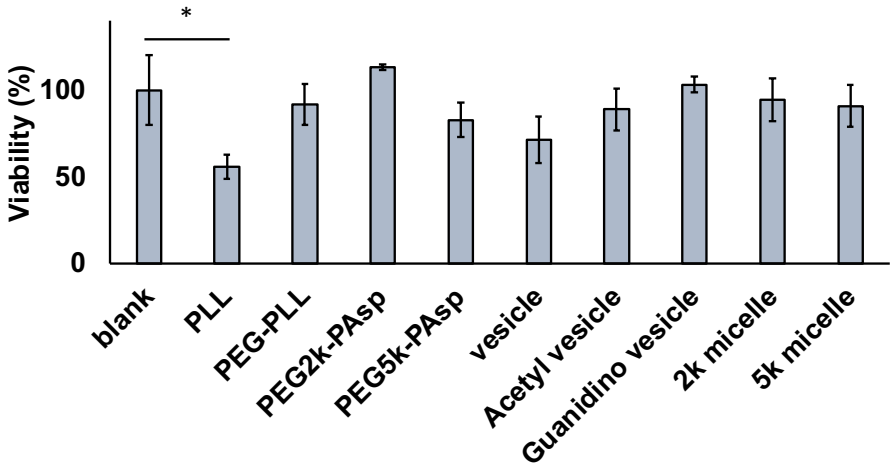
(a) HeLa



(b) RAW 264.7



(c) Caco2



(d) DC2.4

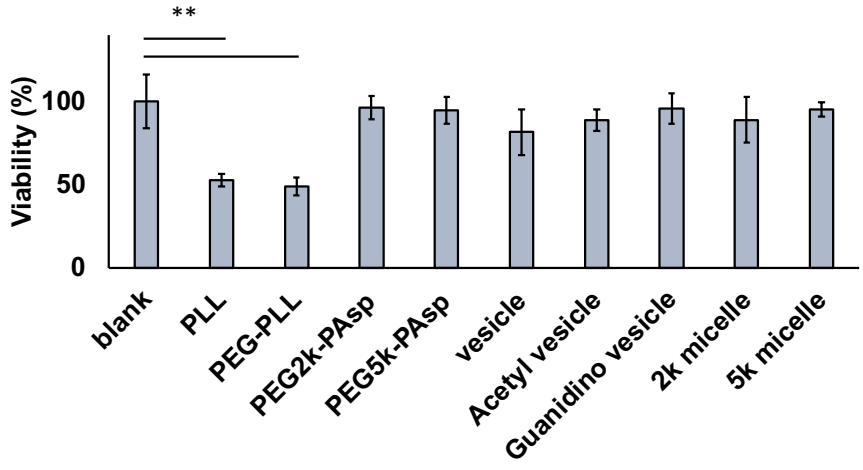


Figure S29. Cytotoxicity assays of PICs in different cell lines. (a) HeLa, (b) RAW 264.7, (c) Caco2 and (d) DC2.4.