

(Electronic Supplementary Information)

## Complex Formation Between Cationic beta-1,3-Glucan and Hetero-Sequence Oligodeoxynucleotide and Its Delivery into Macrophage-like Cell to Induce Cytokine Secretion

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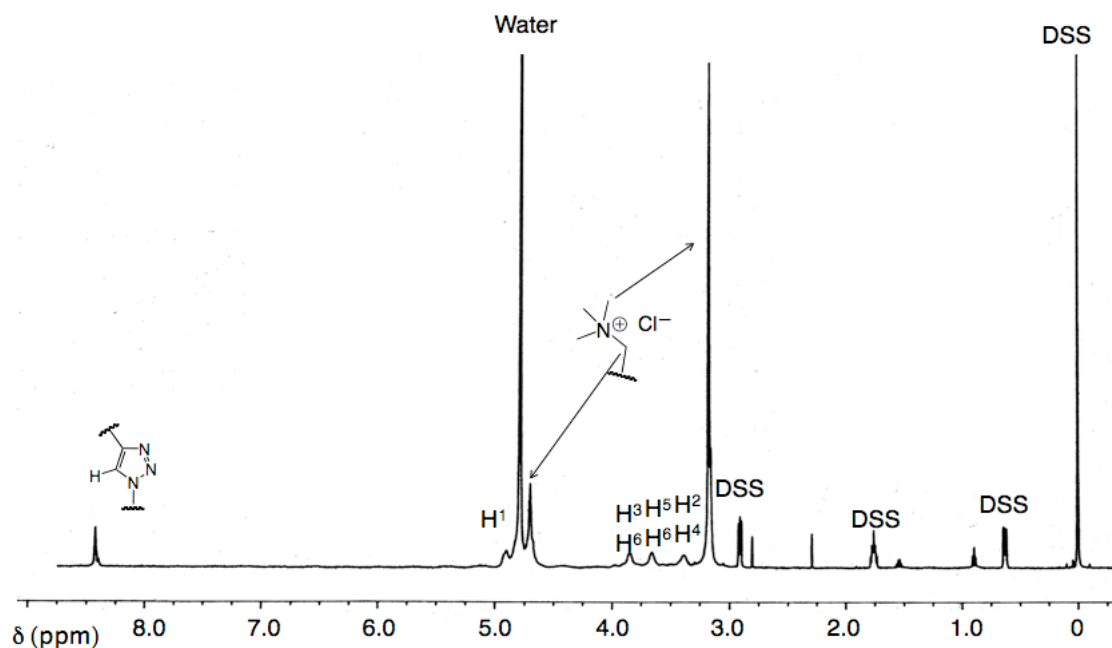
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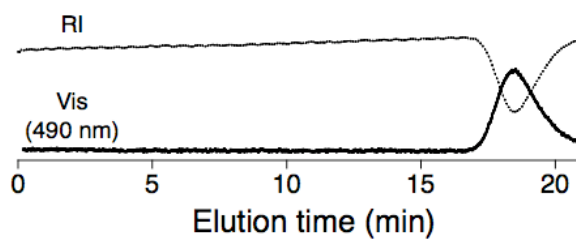
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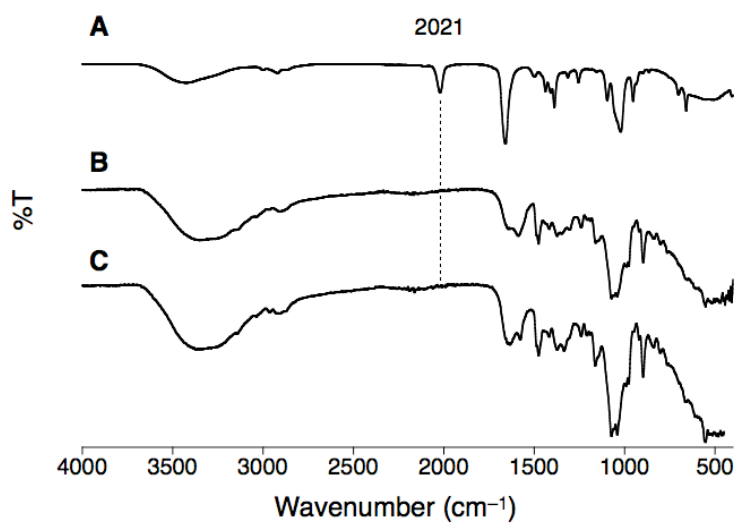
### 1. Characterization of FCUR-N<sup>+</sup>



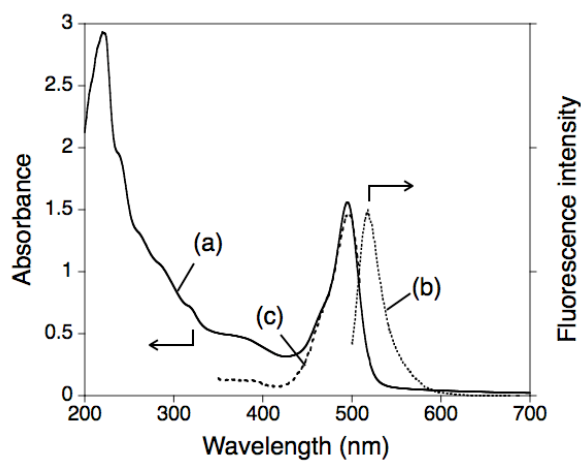
**Figure S1.** <sup>1</sup>H NMR (600 MHz) spectrum of FCUR-N<sup>+</sup> (2.0 mg/mL) in D<sub>2</sub>O (DSS standard) at 25 °C.



**Figure S2.** SEC chromatograms of FCUR-N<sup>+</sup> detected by RI and visible absorption at 490 nm (solid line: Vis at 490 nm, dotted line: RI).



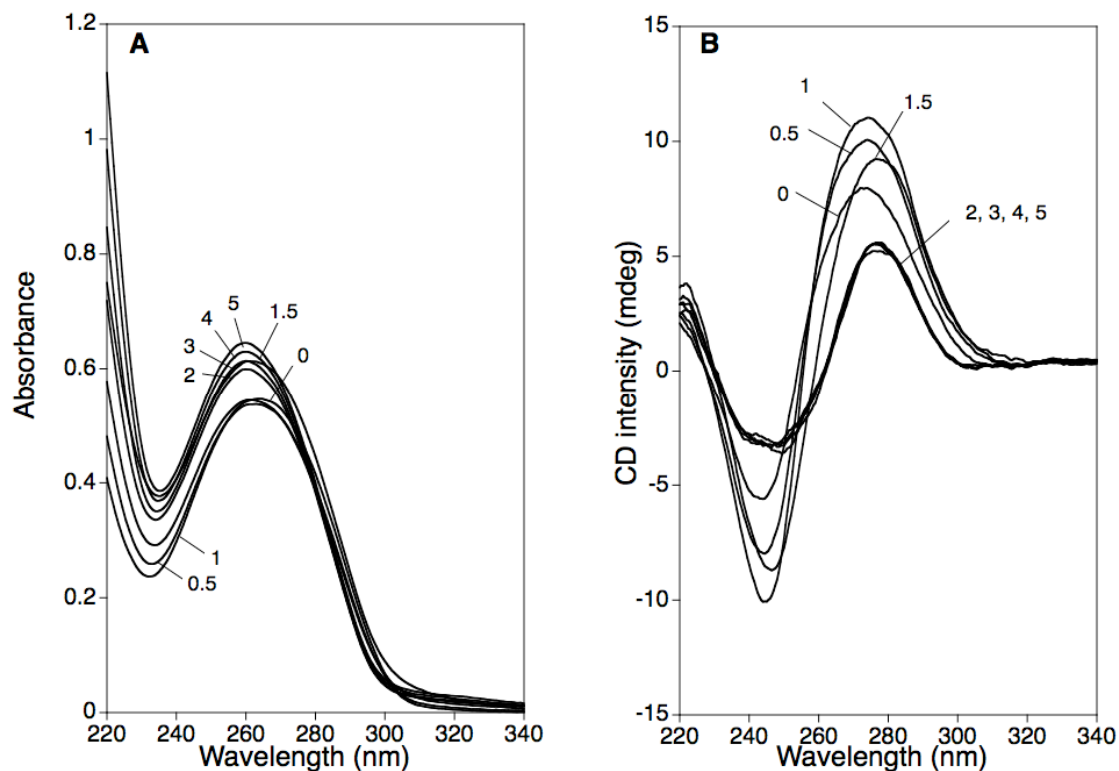
**Figure S3.** IR spectra (ATR method) of (A) CUR-N<sub>3</sub>, (B) CUR-N<sup>+</sup> and (C) FCUR-N<sup>+</sup>.



**Figure S4.** (a) Absorption (0.5 mg/mL), (b) fluorescence (0.05 mg/mL,  $\lambda_{\text{ex}} = 495 \text{ nm}$ ) and (c) excitation spectra (0.05 mg/mL,  $\lambda_{\text{em}} = 518 \text{ nm}$ ) of FCUR-N<sup>+</sup> in water at ambient temperature.

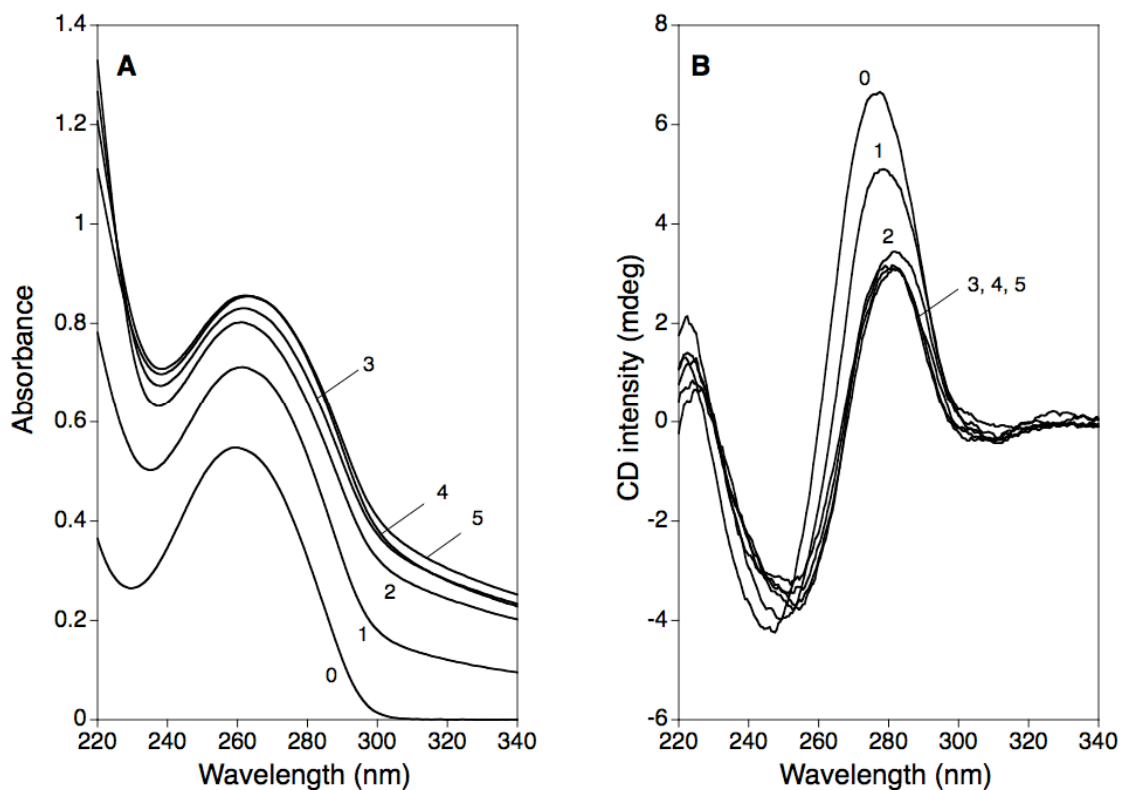
## 2. Results and discussion.

### 2-1. UV-vis and CD spectra of CUR-N<sup>+</sup>-CpG ODN complex in aqueous solution.

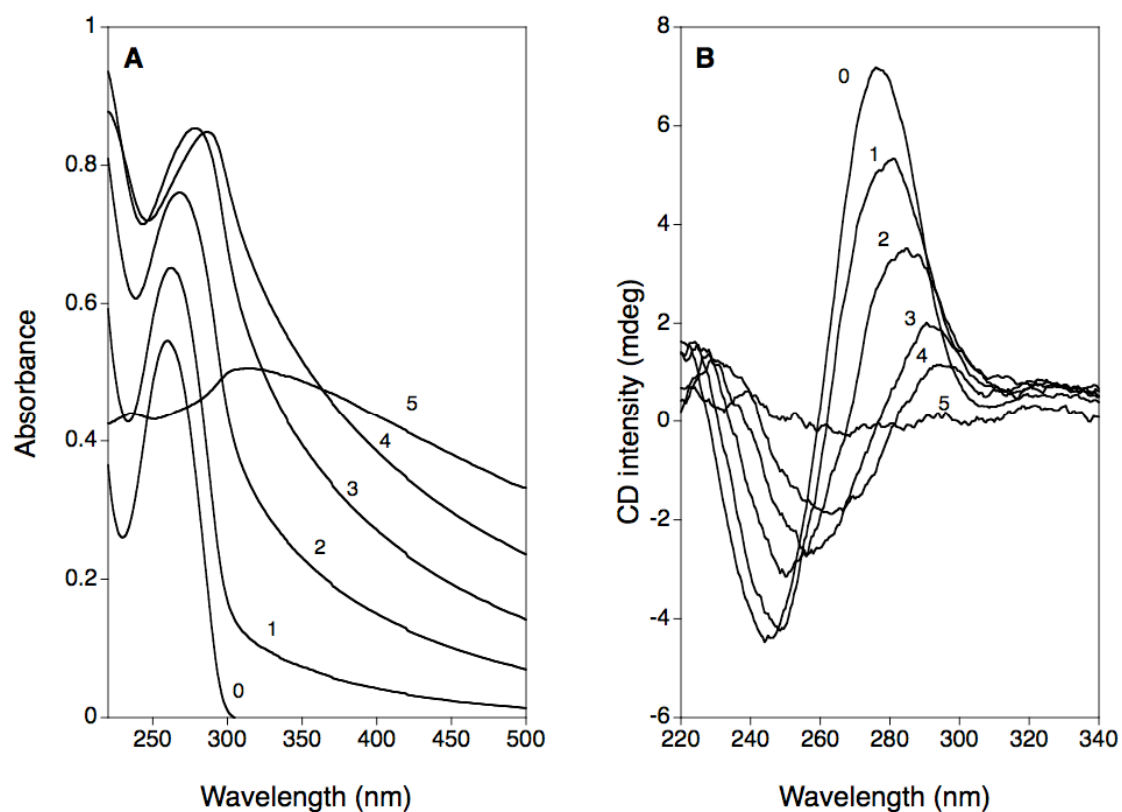


**Figure S5.** (A) UV-vis and (B) CD spectra of [CUR-N<sup>+</sup>]/[CpG ODN] = 0, 0.5, 1, 1.5, 2, 3, 4 and 5 in aqueous solution at 25 °C with 1-cm cell. ([CpG ODN] and [CUR-N<sup>+</sup>] denote the concentration of CpG ODN and CUR-N<sup>+</sup> in monomer unit (base unit), respectively. [CpG ODN] =  $6.3 \times 10^{-5}$  M)

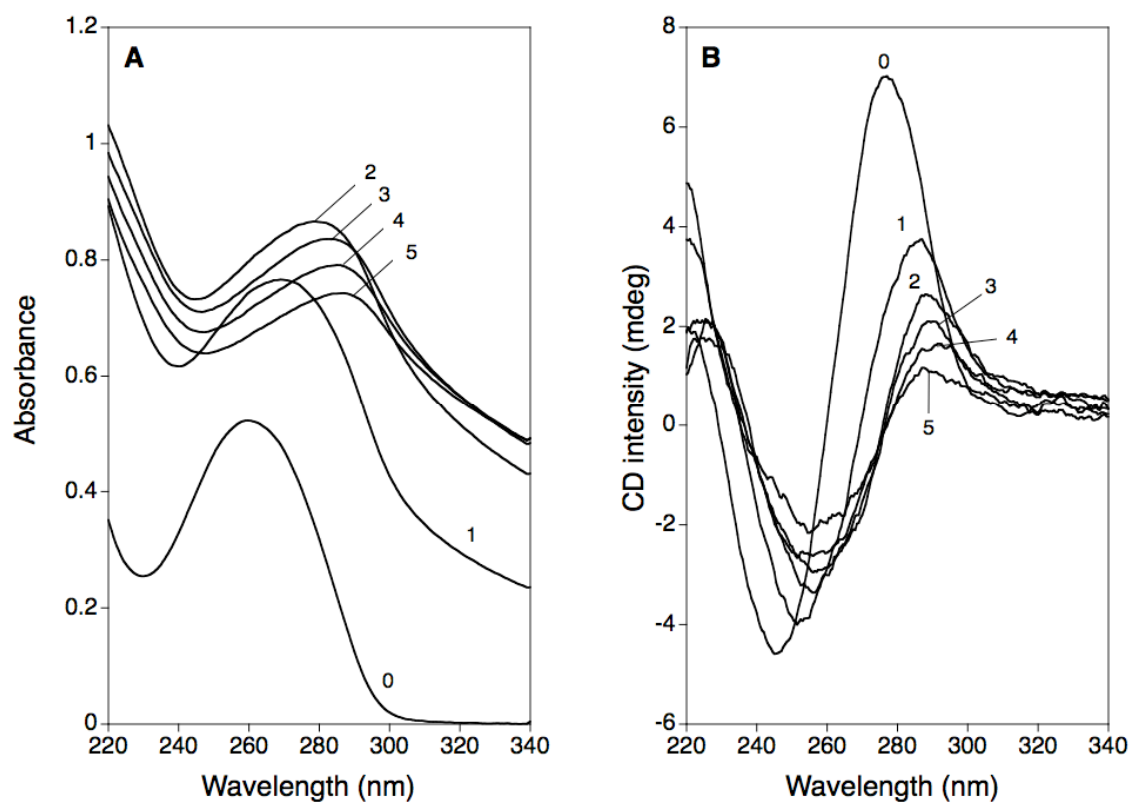
**2-2. UV-vis and CD spectra of CUR-N<sup>+</sup>-CpG ODN, PEI-CpG ODN and PLL-CpG ODN complex in PBS aqueous solution.**



**Figure S6.** (A) UV-vis and (B) CD spectra of  $[\text{CUR-N}^+]/[\text{CpG ODN}] = 0, 1, 2, 3, 4$  and  $5$  in PBS aqueous solution at  $25\text{ }^\circ\text{C}$  with  $1\text{-cm}$  cell.  $[\text{CpG ODN}]$  and  $[\text{CUR-N}^+]$  denote the concentration of CpG ODN and CUR-N<sup>+</sup> in monomer unit (base unit), respectively.  $[\text{CpG ODN}] = 6.3 \times 10^{-5}\text{ M}$

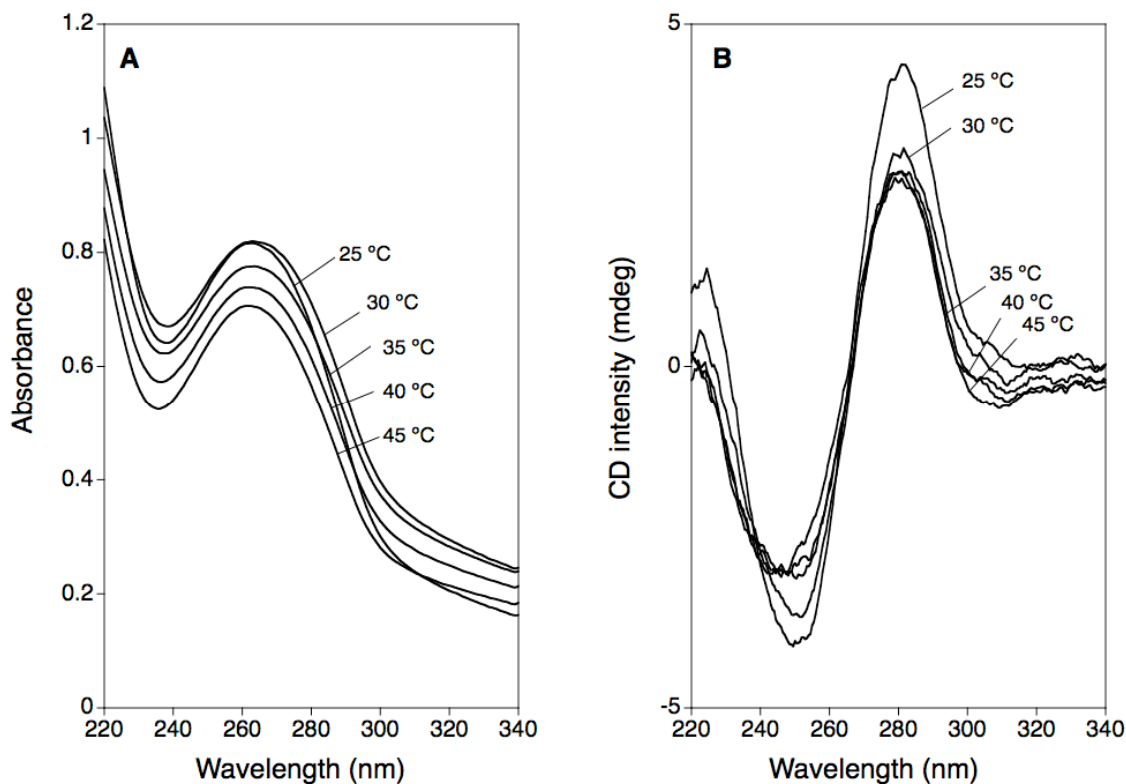


**Figure S7.** (A) UV-vis and (B) CD spectra of [PEI]/[CpG ODN] = 0, 1, 2, 3, 4 and 5 in PBS aqueous solution at 25 °C with 1-cm cell. ([CpG ODN] and [PEI] denote the concentration of CpG ODN and PEI in monomer unit (base unit), respectively. [CpG ODN] =  $6.3 \times 10^{-5}$  M)



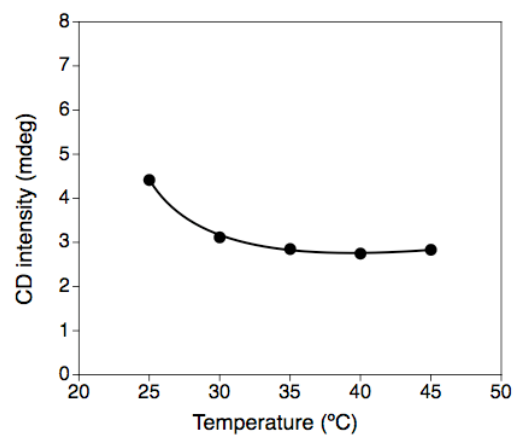
**Figure S8.** (A) UV-vis and (B) CD spectra of [PLL]/[CpG ODN] = 0, 1, 2, 3, 4 and 5 in PBS aqueous solution at 25 °C with 1-cm cell. ([CpG ODN] and [PLL] denote the concentration of CpG ODN and PLL in monomer unit (base unit), respectively. [CpG ODN] =  $6.3 \times 10^{-5}$  M)

**2-3. Temperature dependence of UV-vis and CD spectra of CUR-N<sup>+</sup>-CpG ODN complex in PBS aqueous solution.**



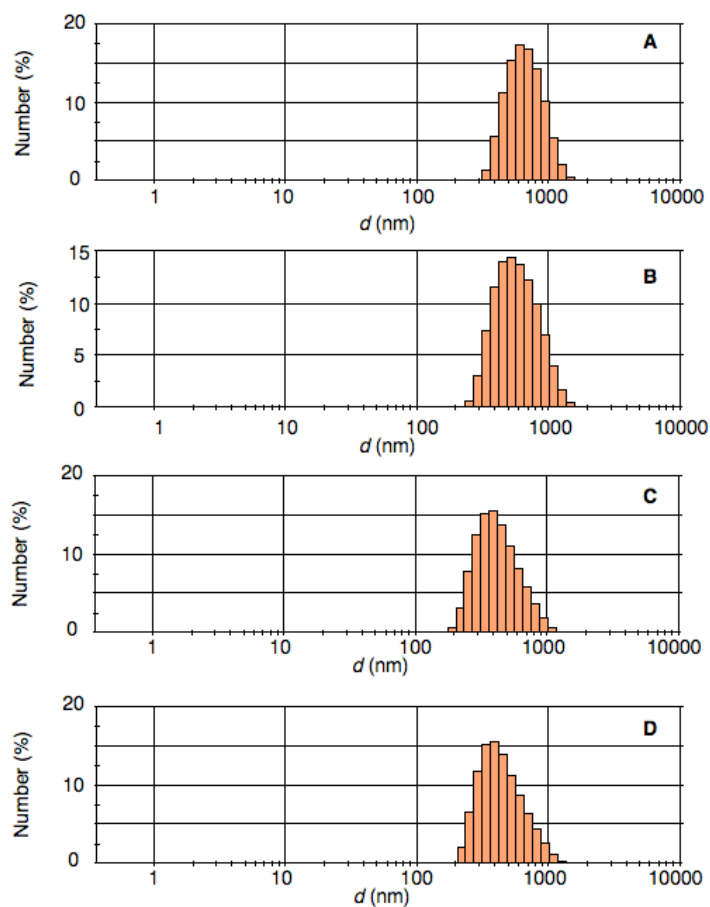
**Figure S9.** Temperature dependence of (A) UV-vis and (B) CD spectra of [CUR-N<sup>+</sup>]/[CpG ODN] = 2 in PBS aqueous solution with 1-cm cell. ([CpG ODN] and [CUR-N<sup>+</sup>] denote the concentration of CpG ODN and CUR-N<sup>+</sup> in monomer unit (base unit), respectively. [CpG ODN] =  $6.3 \times 10^{-5}$  M). The UV-vis and CD spectra of the CUR-N<sup>+</sup>-CpG ODN complex ([CUR-N<sup>+</sup>]/[CpG ODN] = 2) showed slight change in the temperature range of 25 to 45 °C.





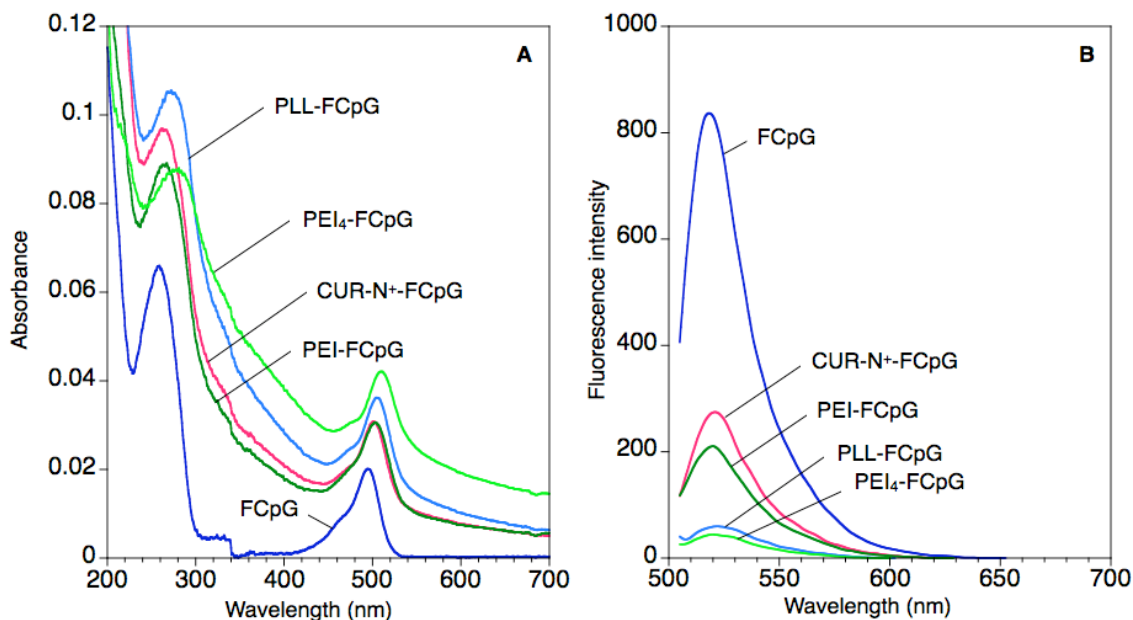
**Figure S10.** Plot of CD intensity at 281 nm of CUR-N<sup>+</sup>-CpG ODN complex ([CUR-N<sup>+</sup>]/[CpG ODN] = 2) against temperature in PBS aqueous solution (pH 7.4) (Plot of Figure S9).

**2-4. DLS analyses of CUR-N<sup>+</sup>-CpG ODN, PEI-CpG ODN and PLL-CpG ODN complex in PBS aqueous solution.**

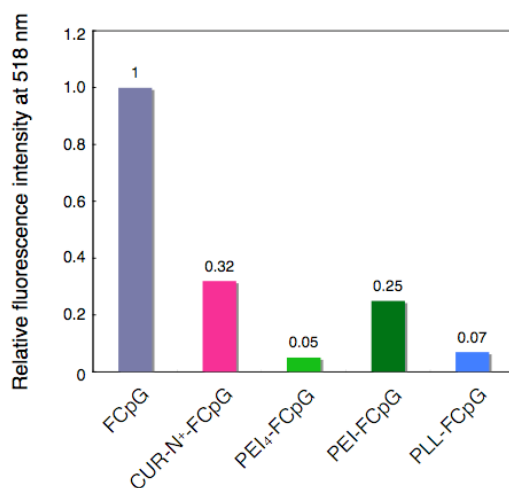


**Figure S11.** Histogram analyses of DLS for (A) CUR-N<sup>+</sup>-CpG ODN ([CUR-N<sup>+</sup>]/[CpG ODN] = 2), (B) PEI-CpG ODN ([PEI]/[CpG ODN] = 2), (C) PEI<sub>4</sub>-CpG ODN ([PEI]/[CpG ODN] = 4) and (D) PLL-CpG ODN ([PLL]/[CpG ODN] = 2) at 25 °C in PBS aqueous solution ([CpG ODN] =  $6.3 \times 10^{-5}$  M).

**2-5. Absorption and fluorescence spectra of CUR-N<sup>+</sup>-FCpG ODN, PEI-FCpG ODN and PLL-FCpG ODN complex in PBS aqueous solution.**

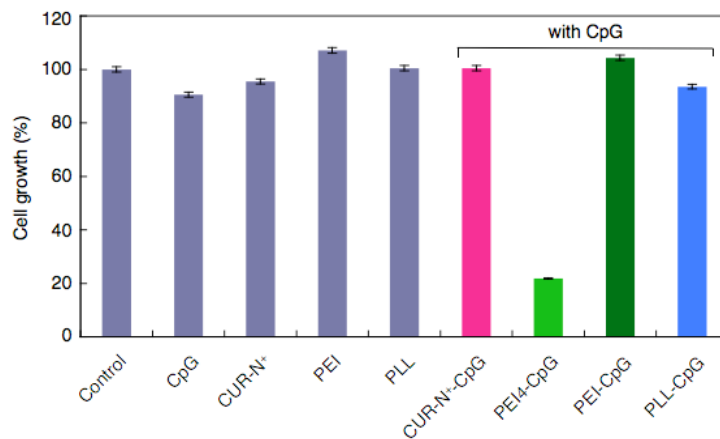


**Figure S12.** (A) UV-vis and (B) fluorescence ( $\lambda_{\text{ex}} = 505 \text{ nm}$ ) spectra of the polycation-FCpG ODN complexes ( $[\text{polycation}]/[\text{FCpG ODN}] = 2$  (and 4 for PEI<sub>4</sub>-FCpG)) in PBS aqueous solution (pH = 7.4) at 25 °C with 0.1-cm cell. ([FCpG ODN], [CUR-N<sup>+</sup>], [PEI] and [PLL] denote the concentration of FCpG ODN, CUR-N<sup>+</sup>, PEI and PLL in monomer unit (base unit), respectively. [FCpG ODN] =  $6.3 \times 10^{-5} \text{ M}$ )



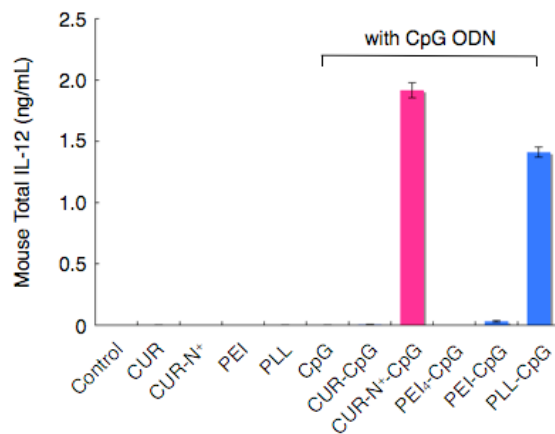
**Figure S13.** Relative fluorescence intensity of polycation-FCpG ODN complexes.

**2-6. Cytotoxicity of CUR-N<sup>+</sup>-CpG ODN, PEI-CpG ODN and PLL-CpG ODN complex assayed by WST-8.**



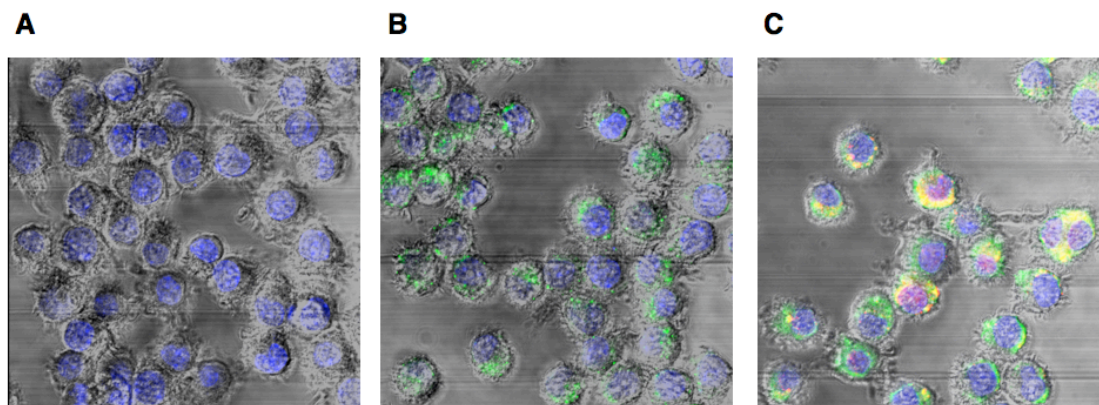
**Figure S14.** Cytotoxicity assessment of the polycations and the polycation-CpG ODN complexes after incubation for 24 h. Cell growth was determined by the MTT assay (WST-8). (see Experimental for the detail condition)

2-7. Cytokine secretion (IL-12) evaluated by ELISA assay.



**Figure S15.** Effect of polycation complexation on CpG ODN-mediated cytokine secretion. The murine macrophage-like cell J744.A1 ( $1 \times 10^6$  cells/mL, 100  $\mu$ L/well) was stimulated with 35  $\mu$ g/mL of the CpG ODN for IL-12. The amount of secretions was determined using an ELISA kit, after incubating the cells in the presence of the CpG ODN or the polymer-polycation complexes for 24 h. The molar ratio ([polycation]/[CpG ODN]) is fixed at 2 (and 4 for PEI<sub>4</sub>-CpG).

**2-8. Confocal microscopic projection images of RCpG ODN, FCUR-N<sup>+</sup> and FCUR-N<sup>+</sup>-RCpG ODN complex treated J774.A1 cells.**



**Figure S16.** Confocal microscopic images of J774.A1 cells treated with (A) ROX-labeled CpG ODN (RCpG ODN), (B) FITC-labeled CUR-N<sup>+</sup> (FCUR-N<sup>+</sup>) and (C) the complex between ROX-labeled CpG ODN and FITC-labeled CUR-N<sup>+</sup> (FCUR-N<sup>+</sup>-RCpG ODN complex) for 6 h. The blue, green and red colors correspond to DAPI (nucleus), FITC (CUR-N<sup>+</sup>) and ROX (CpG), respectively. The yellow color is the outcome of the merge between FITC and ROX. The image A indicates that the RCpG ODN can hardly enter the cells. The presence of green dots in image B in a spotty fashion, which is excluded from nucleus (blue dots), indicates that FCUR-N<sup>+</sup> transfects into the cells efficiently.