Electronic Supplementary Material (ESI) for Soft Matter.

Supplementary data

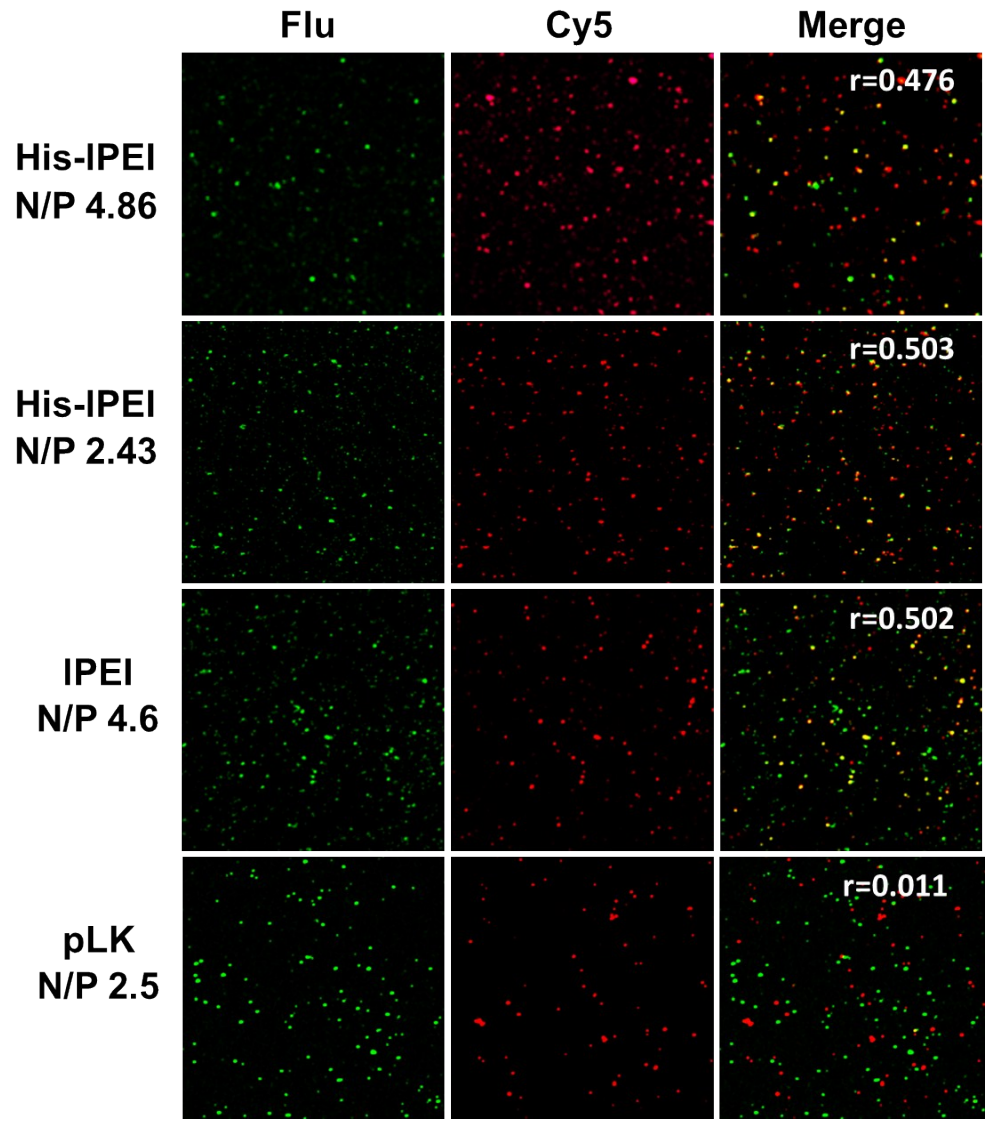


Fig. S1: (A) Fluorescence microscopy analysis of His-lPEI, lPEI and pLK polyplexes interaction. Flu-His-lPEI, Cy5-His-lPEI, Flu-lPEI, Cy5-lPEI, Flu-pLK and Cy5-pLK polyplexes were made either with Flu-labelled pDNA or Cy5-labelled pDNA at different polymer/DNA charge ratio (N/P). Then, Flu-His-lPEI and Cy5-His-lPEI polyplexes; Flu-lPEI and Cy5-lPEI polyplexes; Flu-pLK and Cy5-pLK polyplexes were equally mixed and Flu- and Cy5-fluorescences were analysed by fluorescence confocal microscopy. Flu stands for fluorescence image of mixed polyplexes acquired at λex = 488 nm and λem = 520 nm. Cy5 stands for fluorescence image of mixed polyplexes at λex = 633 nm and λem = 650-690 nm; Merge corresponds to the overlay of Flu and Cy5 images. Cy5 fluorescence is coloured in red for an optimal viewing of the merged images r is the Pearson coefficient.

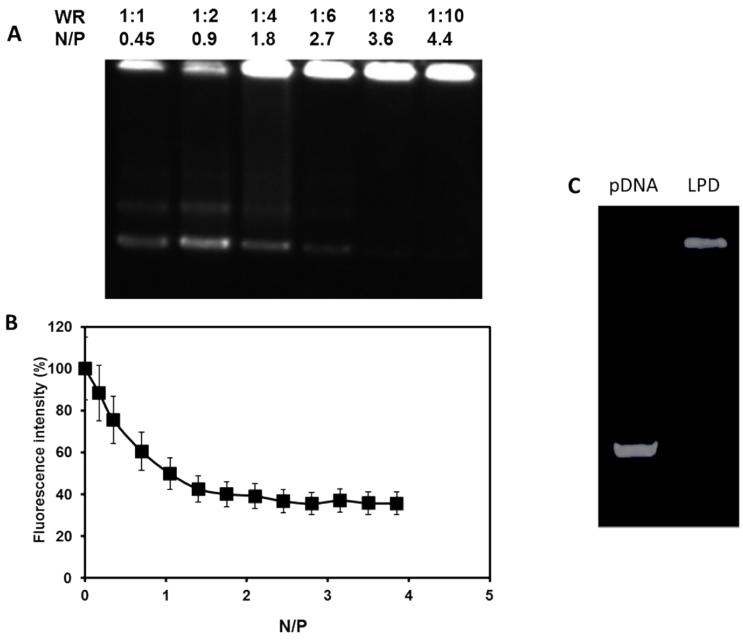
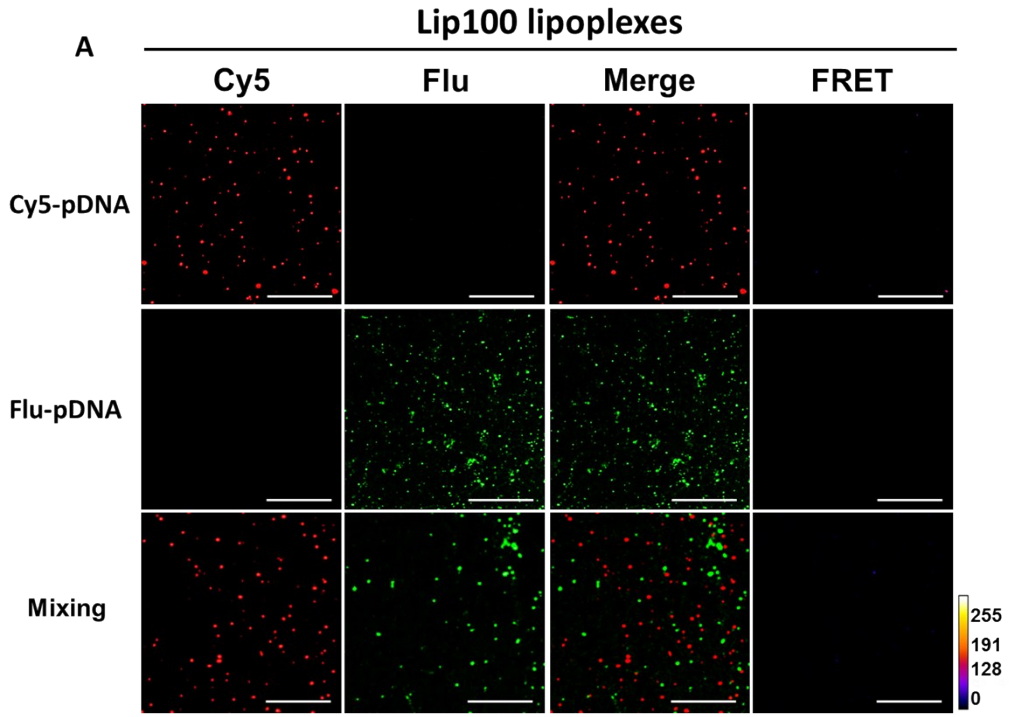


Figure S2: Interaction strength between DNA and cationic liposomes. (A) Agarose gel electrophoresis shift assay of pDNA in the presence of various quantities of KLN24/MM27 liposomes. (B) Dye exclusion assay: the fluorescence intensity of EtBr with 1 µg/mL pDNA in HEPES buffer was measured in the presence of various quantities of KLN24/MM27 liposomes. The fluorescence intensity is expressed as the percent of that in the absence of liposomes. WR is the DNA/liposomes weight ratio. N/P is the lipid/DNA charge ratio. (C) Agarose gel electrophoresis shift assay of pDNA and LPD100.

1. Lipoplexes

Similar experiments were performed with lipoplexes made with KLN25/MM27 liposomes (Lip100) (Scheme 1b). In lipoplexes, pDNA was less condensed than in polyplexes. Agarose gel electrophoresis shift assay showed that N/P > 2.7 was required to fully complex pDNA as shown by the absence of free pDNA migration (Fig. S2A). Dye exclusion experiment indicated that the maximum quenching of EtBr fluorescence of pDNA complexed with Lip100 was 65 % (Fig. S2B). When lipoplexes containing Flu-pDNA were mixed with lipoplexes containing Cy5-pDNA, there were no lipoplexes emitting a dual fluorescence suggesting that there was no mixing and pDNA exchange after their interaction (Fig. S3A). This would imply that lipoplexes comprised only one pDNA molecule or several pDNA molecules were packed inside lipid vesicles. The second hypothesis was supported by known supramolecular organisation of lipoplexes. Indeed, it is known that pDNA molecules are

sandwiched between two lipid layers. Thus, pDNA that belongs to one lipoplex cannot be exchanged with another pDNA from another lipoplex. We conducted flow cytometry experiments with 2 populations of lipoplexes, one made with Cy5-pDNA complexed with unlabelled Lip100 and the second with Flu-Lip100 complexed with unlabelled pDNA (Fig. S3B). No lipoplexes co-emitting blue and green fluorescence were observed indicating the absence of lipoplexes comprising green liposomes and blue pDNA as expected if pDNA exchange occurred and even if one single pDNA molecule was packed *per* lipoplex.



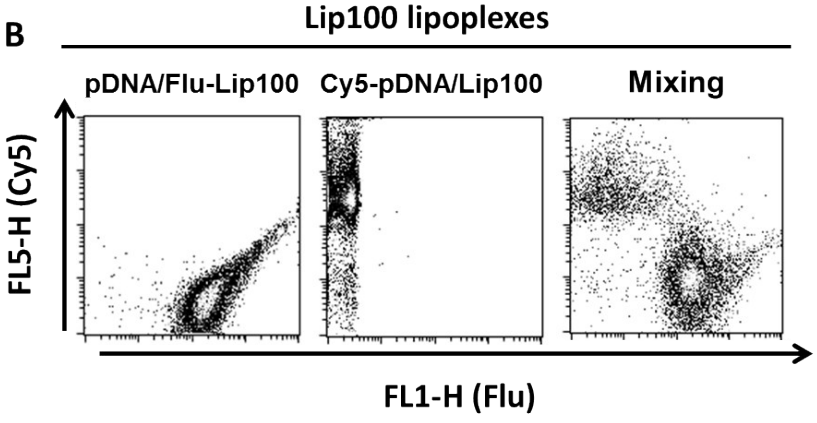


Figure S3: (A) Fluorescence microscopy analysis of lipoplex interaction. Lip 100 lipoplexes were made either with Flu-pDNA or Cy5-pDNA. Then Flu-lipoplexes and Cy5-lipoplexes were equally mixed and their fluorescence and FRET were analysed by fluorescence confocal microscopy. Cy5 stands for fluorescence measured at λex = 663 nm and λem = 650-690 nm; Flu for fluorescence at λex = 488 nm and λem = 500–542 nm; Merge corresponds to the overlay of Flu and Cy5 images; FRET analysis was performed at λex = 488 nm and λem = 650-690 nm. Cy5-pDNA and Flu-pDNA correspond to fluorescence of unmixed Cy5 lipoplexes and unmixed Flu lipoplexes, respectively; Mixing corresponds to fluorescence of the mixture of Cy5-lipoplexes and Flu-lipoplexes. Sidebar: colour pixels scoring for FRET level from 0 (dark blue) to 255 (white). Scale bar: 10 µm. For an optimal merge view, Cy5 fluorescence is coloured red. **(B) Flow cytometry analysis of lipoplex interaction.** Flu-lipoplexes (pDNA/Flu-Lip100) were made with Flu-Lip100 liposomes complexed with unlabelled pDNA. Cy5-lipoplexes (Cy5-pDNA/Lip100) were made with Lip 100 liposomes complexed with Cy5-pDNA. Then Flu-lipoplexes and Cy5-lipoplexes (Mix) were equally mixed and their fluorescence was analysed by flow cytometry. Cy5-pDNA, Flu-pDNA and "Mixing" stand for Cy5-polyplexes, Flu-polyplexes and their mixture at equal volumes, respectively. FL1-H(Flu) (λex = 488 nm and λem = 520 nm) and FL5-H(Cy5) (λex = 633 nm and λem = 660 nm) are the fluorescence intensity of Flu and Cy5, respectively.

2. Polyplex/lipoplex interaction

Cy5-His-lPEI polyplexes and Flu-lipoplexes made with Flu-pDNA and Lip100 liposomes were equally mixed and their fluorescence intensity was observed under fluorescence confocal microscope. As shown in Figure S4, one can observe green fluorescence corresponding to lipoplexes and red fluorescence corresponding to polyplexes. No particles emitting a dual fluorescence were detected indicative of no pDNA exchange between a polyplex and a lipoplex.

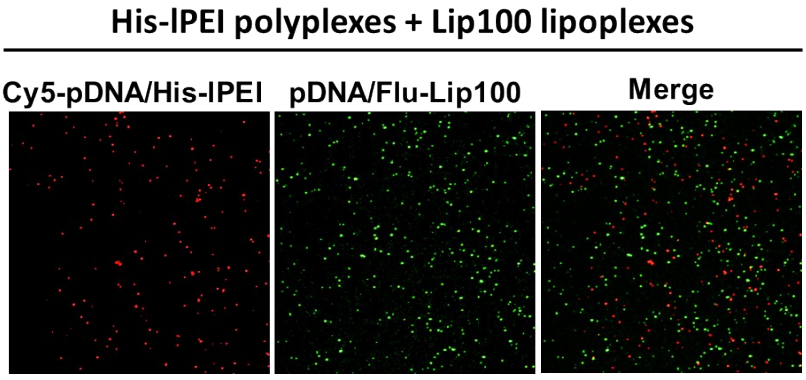


Figure S4: Fluorescence microscopy analysis of polyplex + lipoplex interaction. His-lPEI polyplexes were made with Cy5-pDNA and His-lPEI at N/P of 4.86. Lip100 lipoplexes were made with Flu-pDNA and Lip100 liposomes. After mixing, the fluorescence and FRET were analyzed by fluorescence confocal microscopy. Cy5 fluorescence was measured at λex = 663 nm and λem = 650-690 nm; Flu fluorescence at λex = 488 nm and λem = 500–542 nm; Merge corresponds to the overlay of Cy5 and Flu images; Scale bar: 10 µm. For an optimal merge view, Cy5 fluorescence is coloured in red.

3. Lipopolyplexes

The DNA exchange capacity between ternary complexes made with pDNA/His-lPEI/Lip100 (lipopolyplexes, LPD100) was also assessed (Scheme 1c). Agarose gel electrophoresis shift assay showed that no free pDNA did migrate indicating that it was totally associated with LPD100 (Fig. S2C). When LPD100 made with Flu-pDNA were mixed with LPD100 made with Cy5-pDNA, no LPD100 emitting a dual fluorescence was observed by fluorescence confocal microscopy suggesting that LPD100 interaction did not promote pDNA exchange (Fig. S5). This result suggests that polyplexes were hindered inside the lipid vesicle preventing polyplex mixing and pDNA exchange.

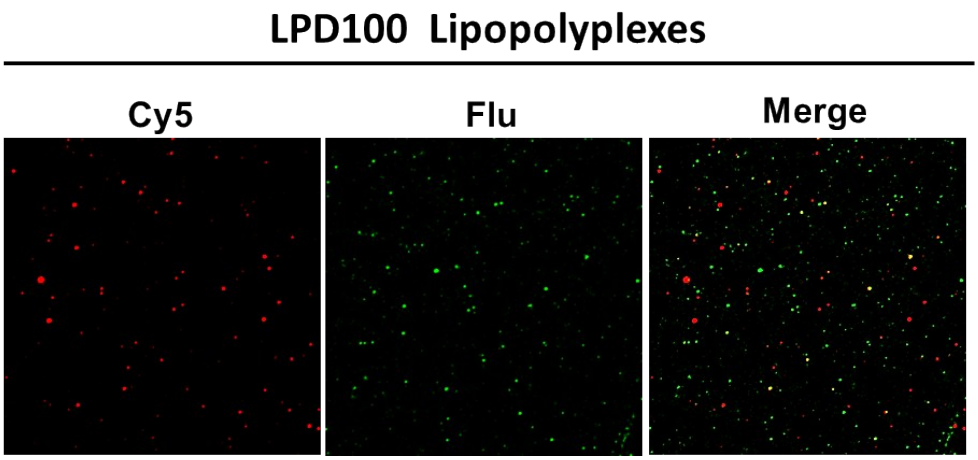


Figure S5: Fluorescence microscopy analysis of lipopolylex interaction. His-lPEI polyplexes were made either with Flu-pDNA or Cy5-pDNA. Then Lip 100 liposomes were added to form lipopolyplexes (LPD100). Flu-LPD100 and Cy5-LPD100 were equally mixed and their fluorescence and FRET were analyzed by fluorescence confocal microscopy. Cy5 stands for fluorescence at λex = 663 nm and λem = 650-690 nm; Flu stands for fluorescence at λex = 488 nm and λem = 500–542 nm; Merge corresponds to the overlay of Cy5 and Flu images. Scale bar: 10 µm. For an optimal merge view, Cy5 fluorescence is coloured in red.