1,4-Butanediol diglycidyl ether (BDE)-crosslinked PEI-g-imidazole nanoparticles as nucleic acid-carriers in vitro and in vivo

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Determination of DNA binding capacity of PNIm 10(6) nanoparticles

The pDNA binding capacity of PNIm 10(6) nanoparticles was determined from the difference between the total amount of pDNA added to the nanoparticles and the residual non-incorporated pDNA collected after filtration from a Millipore filter (Centricon YM-100, 100-kDa cut-off). PNIm 10(6)/pDNA complex was made at a weight ratio of 1:3 (i.e. polymer, 2µg : DNA, 6 µg) in a total volume of 250 µl of 5 % dextrose solution and incubated for 30 min at room temperature. Thereafter, the solution was passed through a Centricon filter (1 hr, 1000 rpm). The residue was washed once with 100 µl dextrose solution. The amount of pDNA present in the collected filtrate was measured spectrophotometrically at 260 nm. It was observed that 1 µg of PNIm 10(6) could carry 1.79 µg of pDNA. Subsequently, the bound DNA was released using heparin (20 U), and it was observed that ~86% of the bound DNA was found to be released within 2h.
**Figure S1.** Cell viability profile of PNIm/DNA nanoplexes, PEI/DNA polyplexes (w/w ratio of 1:1), Superfect™/DNA, GenePORTER 2™/DNA and Lipofectamine™/DNA complexes in HEK293, CHO and HeLa cells. Cells were treated with DNA complexes and cytotoxicity was determined using MTT assay. Percent viability of cells is expressed relative to control cells. Each point represents the mean of three independent experiments performed in triplicates. *P<0.05 vs PEI and commercial transfection reagents.

**Figure S2.** Acid-base titration curve for PNIm particles, PN-2 and PEI.