

## **Supporting Information**

### **Loading of Single-walled Carbon Nanotubes in Cationic Cholesterol Suspensions Significantly Improve Gene Transfection Efficiency in Serum**

*Santosh K. Misra,<sup>a</sup> Parikshit Moitra,<sup>a</sup> Bhupender S. Chhikara,<sup>a</sup> Pataru Kondaiah<sup>b</sup> and  
Santanu Bhattacharya<sup>a,c,\*</sup>*

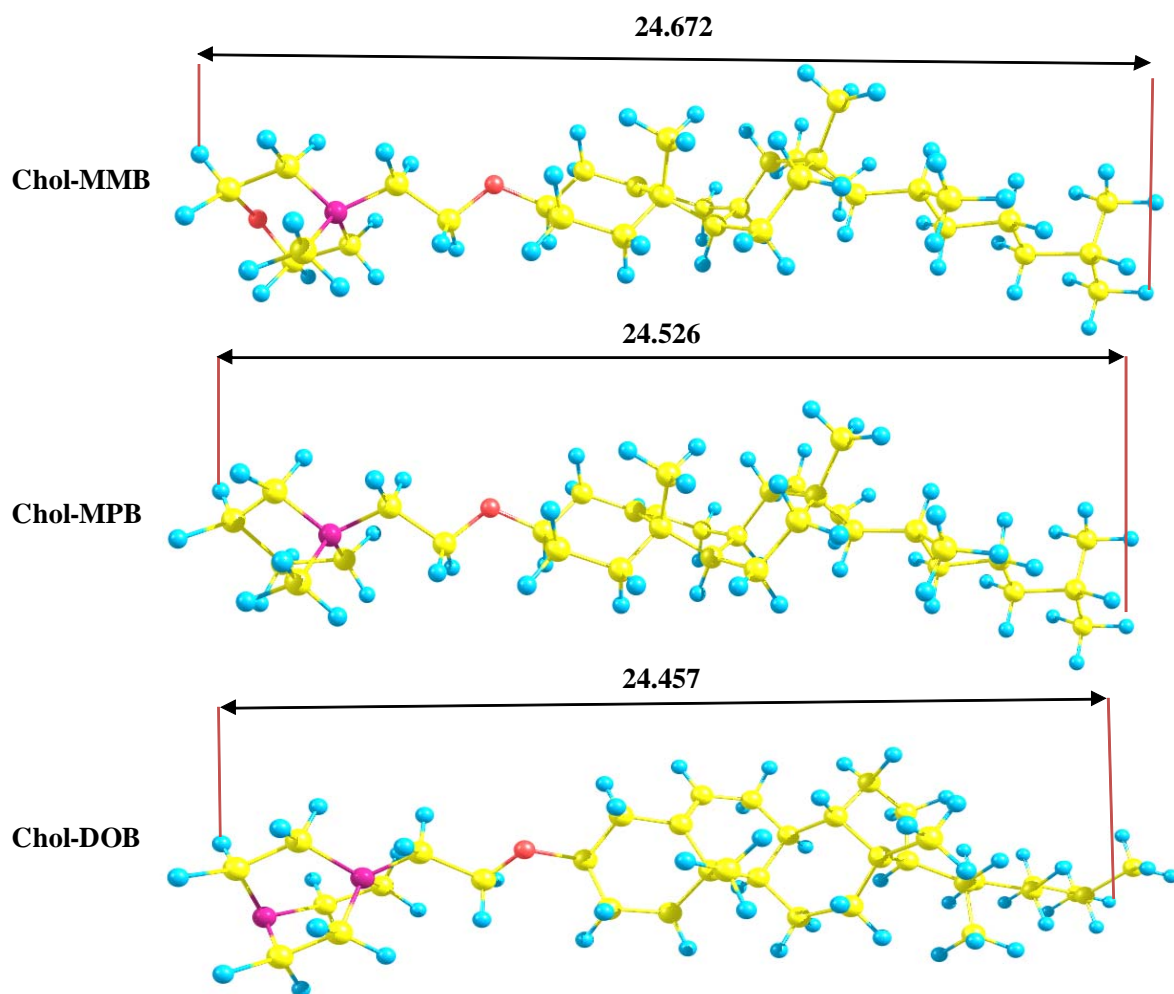
<sup>a</sup>Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India.

<sup>b</sup>Department of Molecular Reproduction, Development and Genetics, Indian Institute of  
Science, Bangalore 560 012, India.

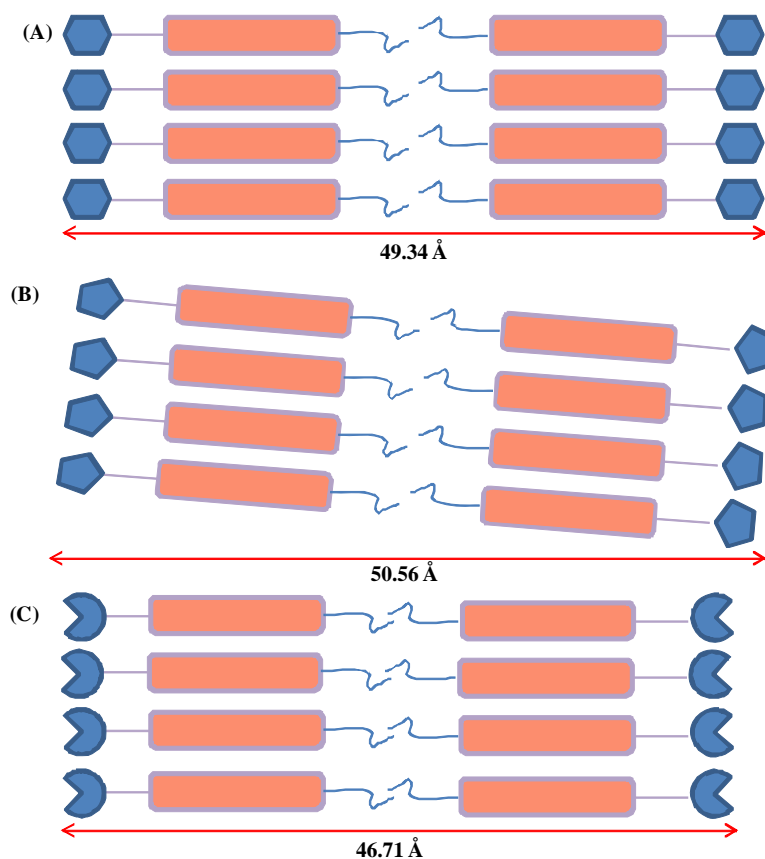
<sup>c</sup>Chemical Biology Unit of JNCASR, Bangalore 560 064, India.

\*Corresponding author. Email: [sb@orgchem.iisc.ernet.in](mailto:sb@orgchem.iisc.ernet.in) Phone: (91)-80-2293-2664; Fax:  
(91)-80-2360-0529.

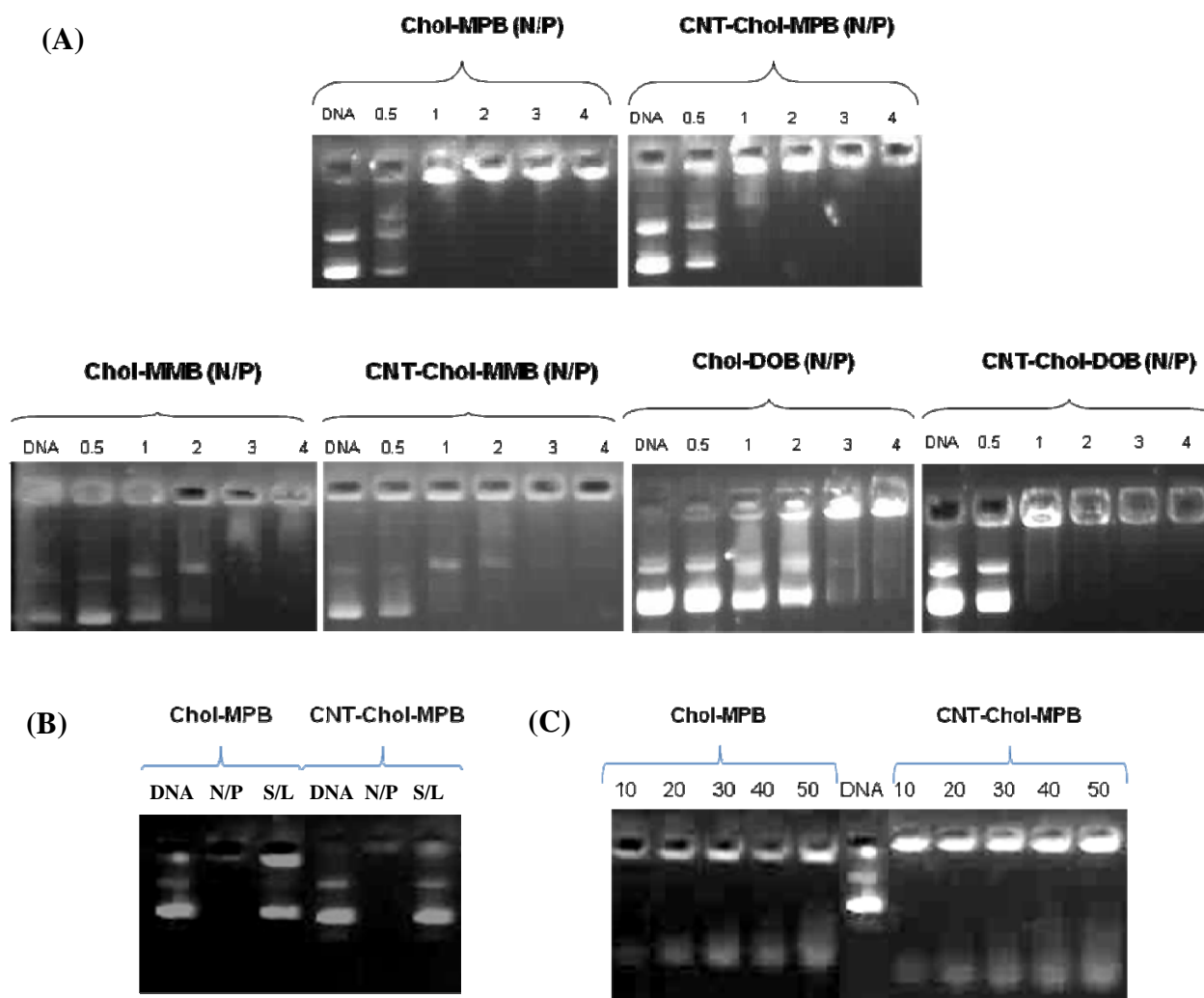
<b>Figure No.</b>	<b>Content</b>	<b>Page No.</b>
<b>S1.</b>	Energy-minimized structure of the cationic cholesterol	<b>3</b>
<b>S2.</b>	Probable model for bilayer arrangements	<b>4</b>
<b>S3.</b>	DNA binding ability of formulations	<b>5</b>
<b>S4.</b>	Extent of DNA binding	<b>6</b>
<b>S5.</b>	Scanning electron microscopy images	<b>7</b>
<b>S6.</b>	Optimization of the amount of SWCNT in CNT-Chol-MPB-DNA complex	<b>8</b>
<b>S7.</b>	Histogram showing an increase in transfected cell population	<b>9</b>
<b>S8.</b>	Effect of cationic Chol-MPB amount on the gene transfection efficiency	<b>10</b>
<b>S9.</b>	Effect of the variation of the amount of DNA on gene transfection	<b>11</b>
<b>S10.</b>	Shift in the FACS peak, on pEGFP-C3 reporter gene transfection	<b>12</b>



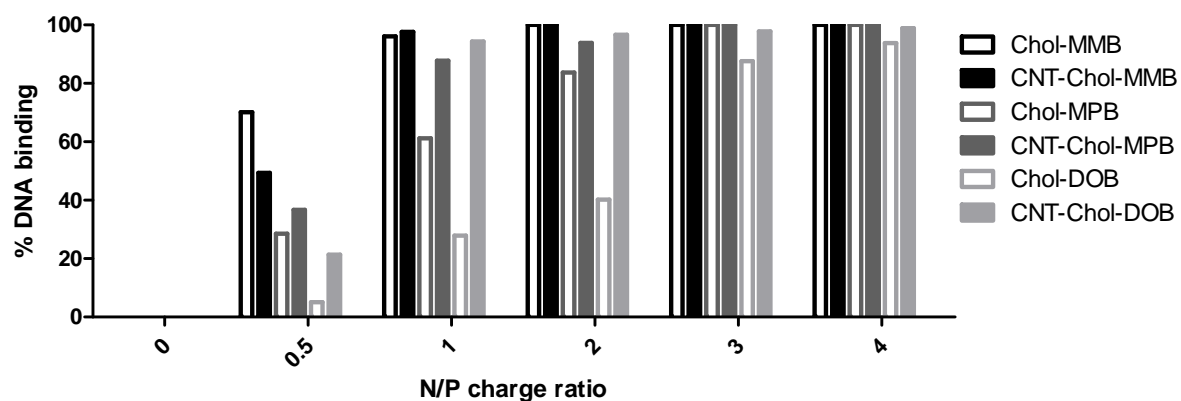
**Figure S1.** Energy-minimized structure of the cationic cholesterol, Chol-MMB, Chol-MPB and Chol-DOB where 'yellow' atom represents the Carbon atom, light blue: the Hydrogen, purple: Nitrogen and red represents Oxygen.



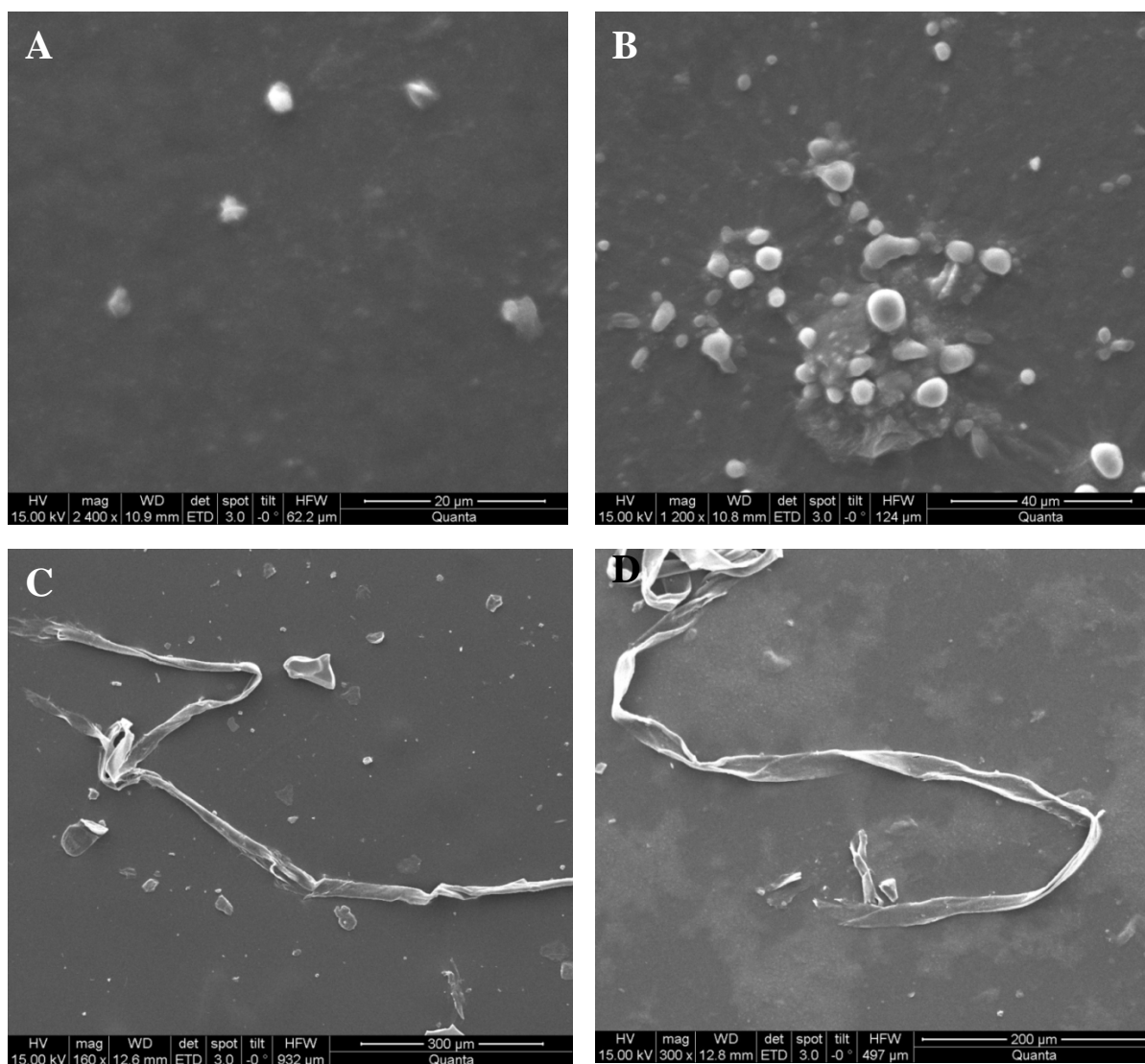
**Figure S2.** Probable model for bilayer arrangements of (A) Chol-MMB; (B) Chol-MPB and (C) Chol-DOB aggregates as observed from the XRD measurements.



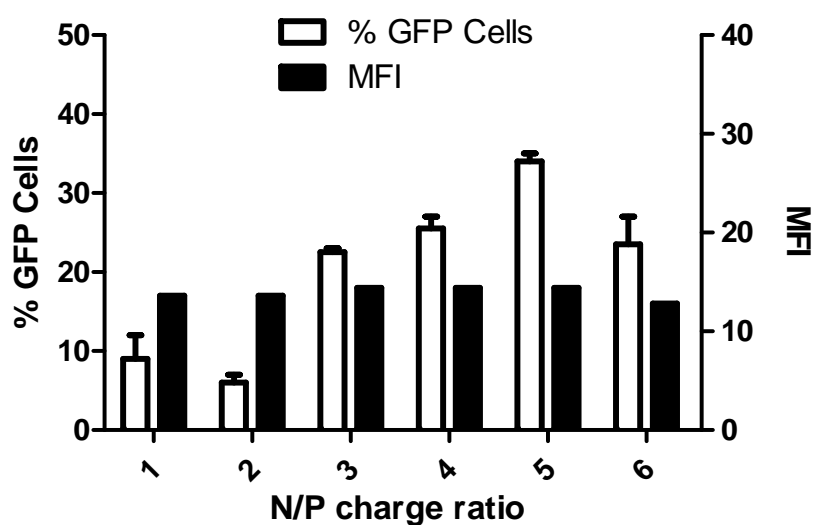
**Figure S3.** DNA binding ability of formulations based on Chol-lipid alone and CNT-Chol-lipid composites having identical amount of lipid (A). SDS mediated DNA release efficiency of Chol-MPB-DNA and CNT-Chol-MPB-DNA complex, where N/P represents Lipid/DNA charge ratio = 3/2 and S/L represents SDS/Lipid molar ratio = 1 (B). Comparison of the stability of Chol-MPB-DNA and CNT-Chol-MPB-DNA complexes in various % of FBS (10 – 50%) added to the complexes (C). Each well has 0.2  $\mu$ g of DNA.



**Figure S4.** Extent of DNA binding with different cationic Chol lipid suspensions, with or without CNT as obtained from gel electrophoresis. Experiment was performed using 0.2  $\mu$ g DNA/well.

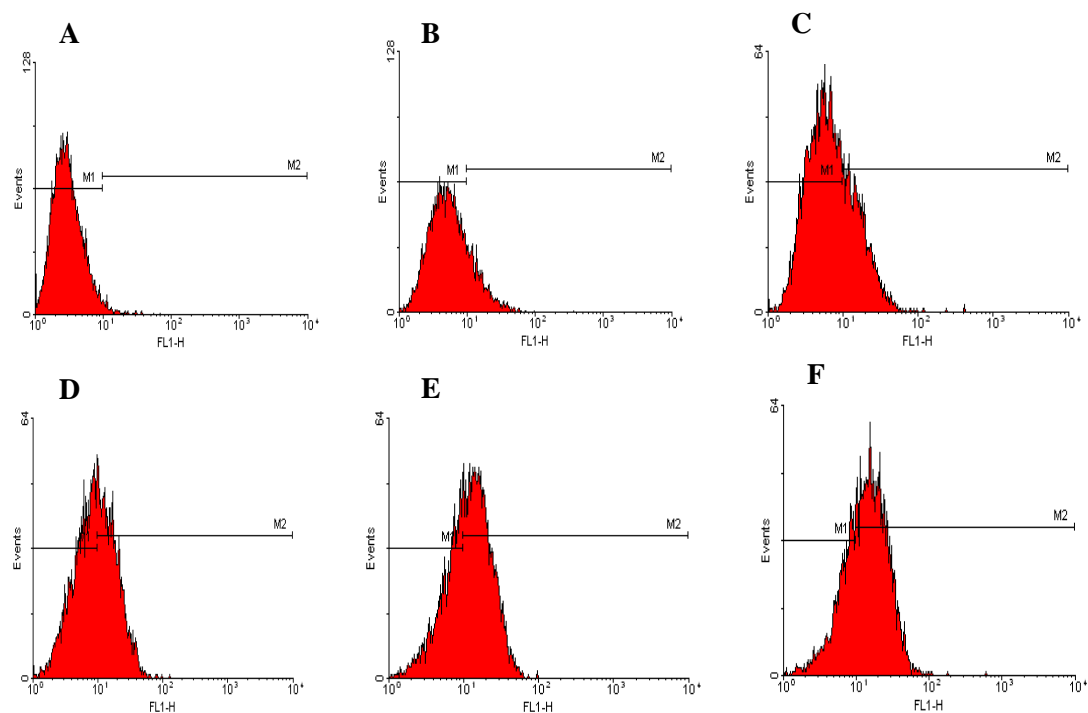


**Figure S5.** Scanning electron microscopy images of samples of aqueous suspensions of (A) Chol-MPB; (B) Chol-MPB-DNA; (C) CNT-Chol-MPB and CNT-Chol-MPB-DNA.

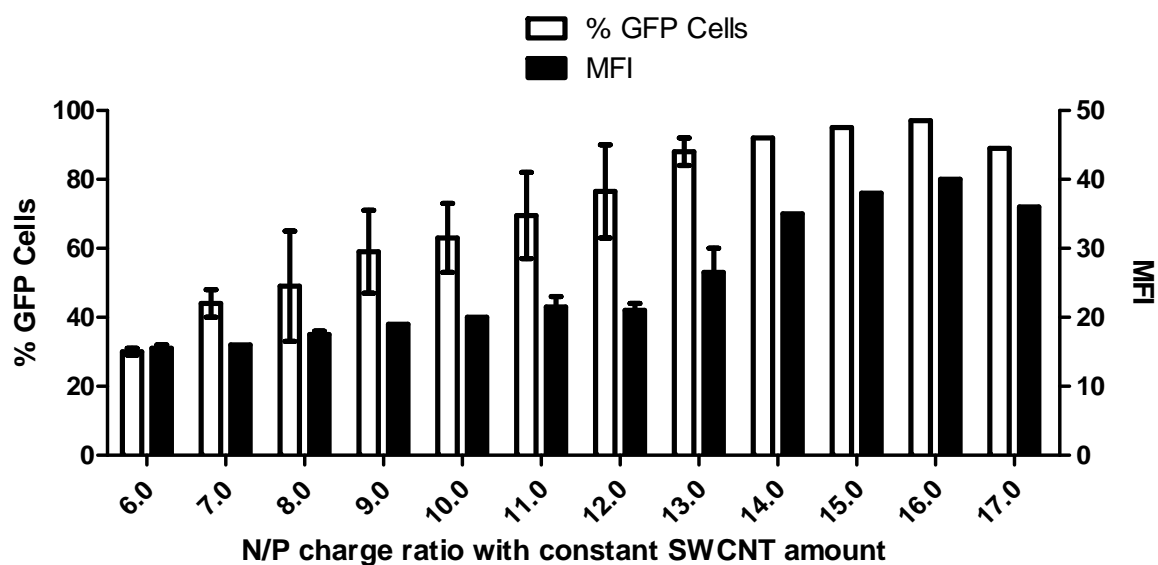


**Figure S6.** Optimization of the amount of SWCNT in CNT-Chol-MPB-DNA complex. N/P charge ratio represents the ratio of the cationic chol and DNA base molarity present in the complex. Experiment was performed using 0.8  $\mu$ g DNA/well in A549 cells.

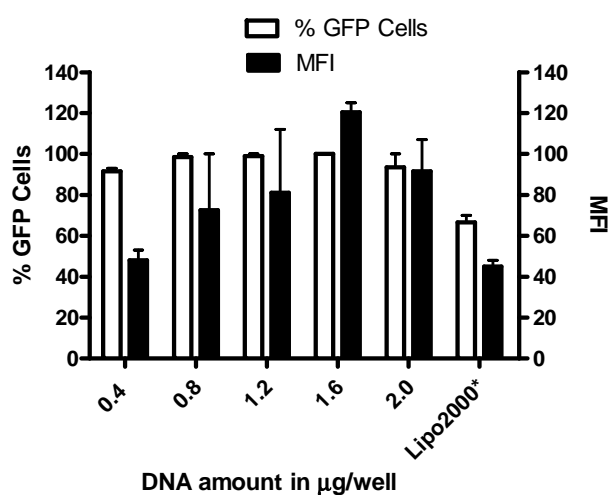




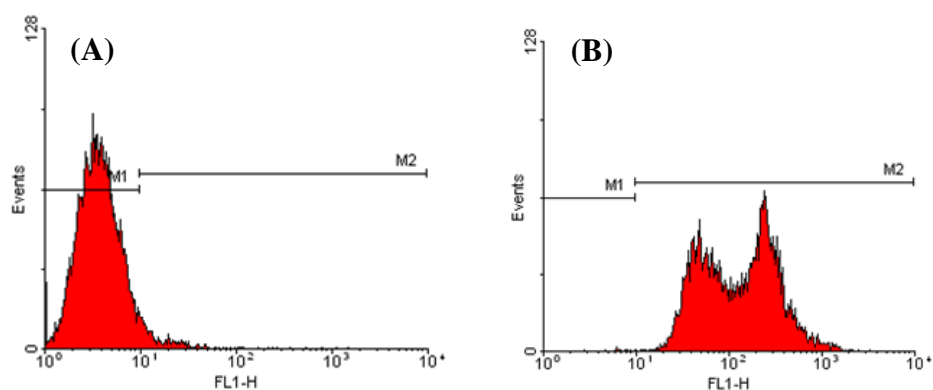
**Figure S7.** Histogram showing an increase in M2 cell population (cells with GFP expression) with increases in N/P charge ratio from 0-to-5 (A-F). Experiment was performed using 0.8  $\mu$ g DNA/well in A549 cells.



**Figure S8.** Effect of gradual increases of cationic Chol-MPB in pre-formed CNT-Chol-MPB-DNA complex on the gene transfection efficiency. Experiment was performed using 0.8  $\mu$ g DNA/well in A549 cells.



**Figure S9.** Effect of the variation of the amount of DNA on pEGFP-C3 gene transfection. Aqueous suspension of SWCNT-Chol lipid was used for experiment in presence of different percentage of serum. 1.6  $\mu\text{g}$  of DNA per well for 60,000 cells is best optimized for the reporter gene feed.



**Figure S10.** Shift in the FACS peak, on pEGFP-C3 reporter gene transfection in A549 cells in absence of serum. FACS histogram shows population of (A) un-transfected cells and (B) transfected cells. CNT-Chol-MPB suspension at N/P charge ratio = 15 with DNA = 1.6  $\mu\text{g}/\text{well}$  was used in the transfection experiment. The region 'M1' shows the residual fluorescence which belongs to non-specific proteins present in cells while 'M2' region shows the fluorescence originated from the GFP alone, expressed from reporter gene introduced in A549 cells via CNT-lipid mediated transfection.