Role of Cellular Retention and Intracellular State in Controlling Gene Delivery Efficiency of Multiple Nonviral Carriers

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Supplementary Information:

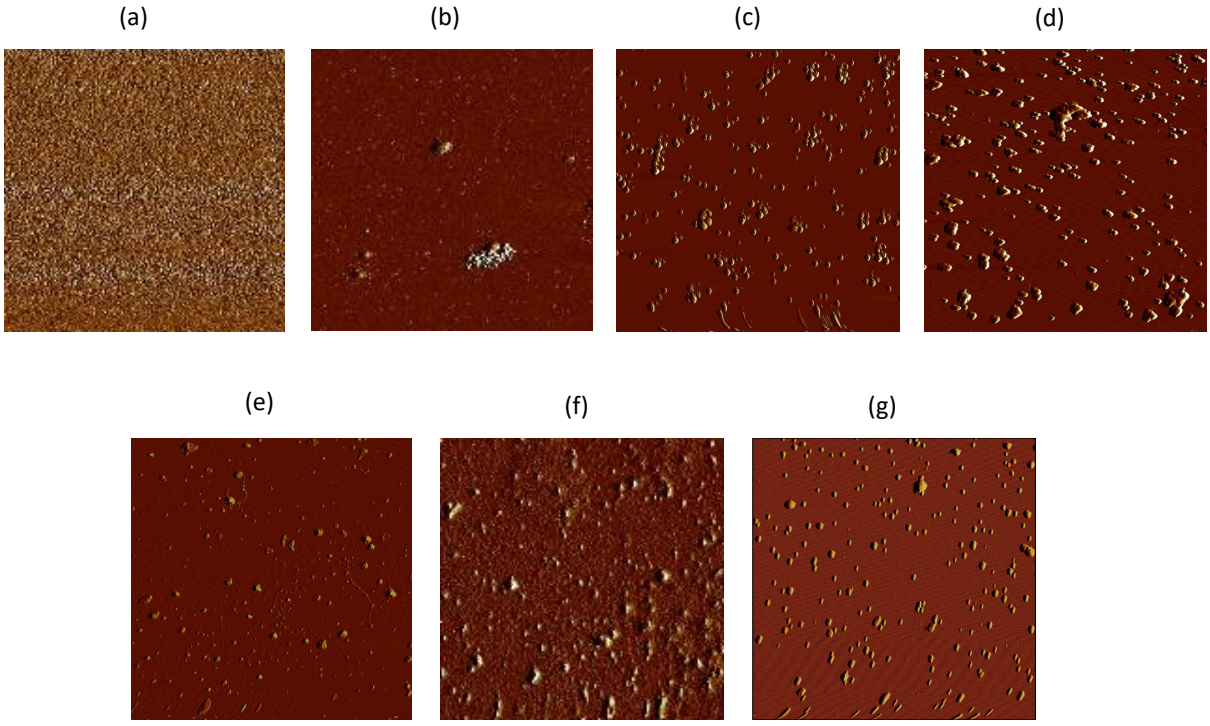


Figure S1: Characterization of shape and size of different nanocomplexes prepared at charge ratio Z (+/-) 5 using AFM (Atomic force microscopy): (a) control (bare mica) (b) M1 (c) M3 (d) M4 (e) Lipofectamine (f) M9 and (g) PEI nanocomplexes. Amplitude images of area 10μm x 10μm are shown in panels for different nanocomplexes.

Primer	Sequences
Forward primer	AGTCCCGTTGATTTTGGTGC
Reverse primer	CAGTACATCAATGGGCGTGG

Table S1: Primer sequences designed for qPCR, against backbone of pMIR plasmid DNA.

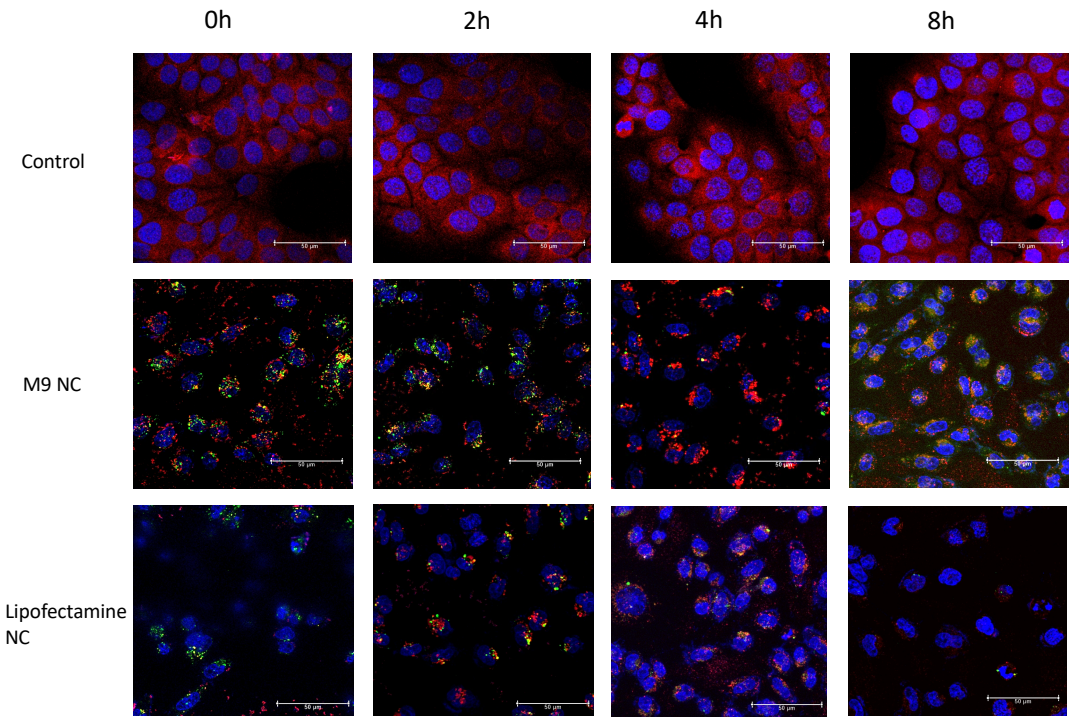
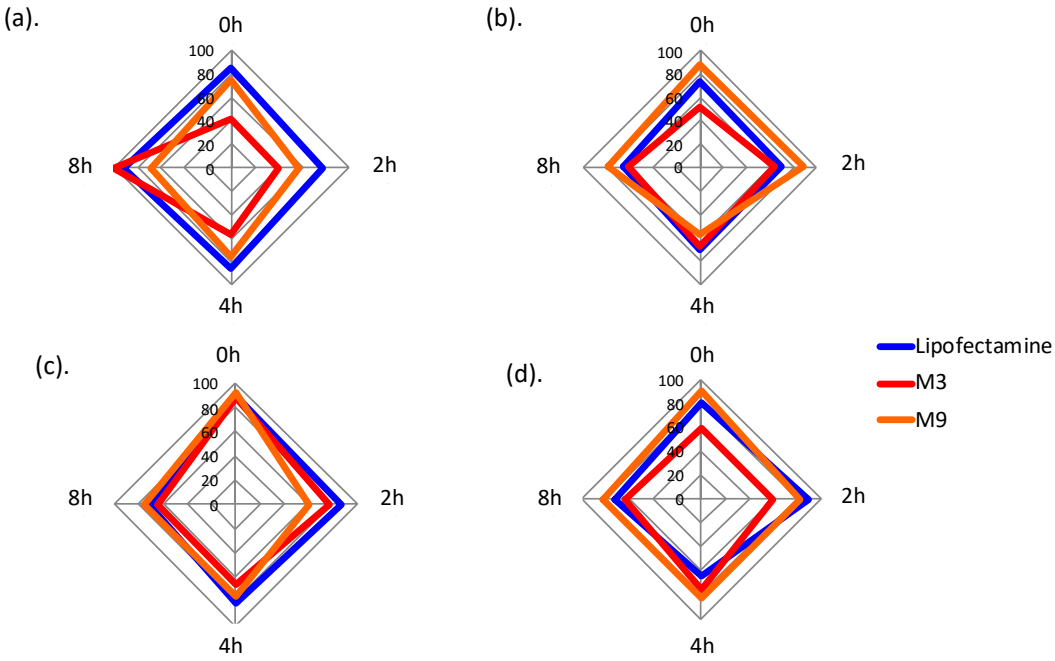
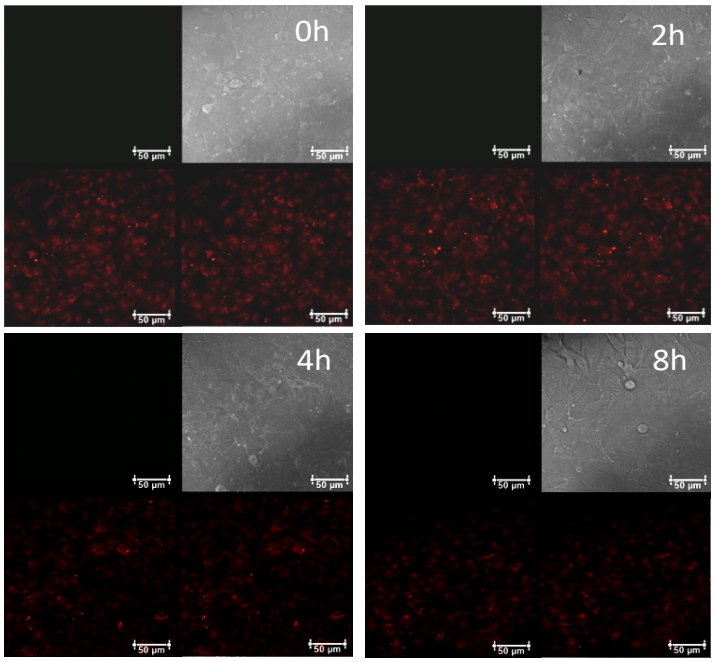


Figure S2: Confocal Imaging for tracking nanocomplex retention with time: B16-F10 cells were incubated with FITC labeled M9 and Lipofectamine nanocomplexes (NC) for 4h, followed by media change and fixing cells at 0, 2, 4 and 8h. Nucleus was stained with Hoechst and plasma membrane with Cell Mask orange plasma.

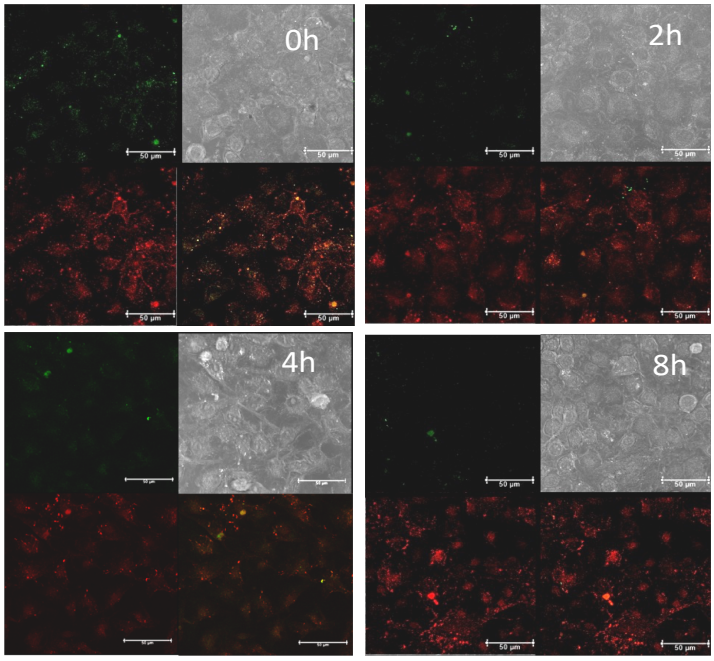


Figures S3: qPCR-based plasmid DNA release study: Amount of complexed plasmid DNA as percentage of total plasmid DNA at each time point is plotted in quadrilateral plots: (a) CHOK-1 (b) HaCaT (c) B16-F10 and (d) HEK-293 cells.

(a)



(b)



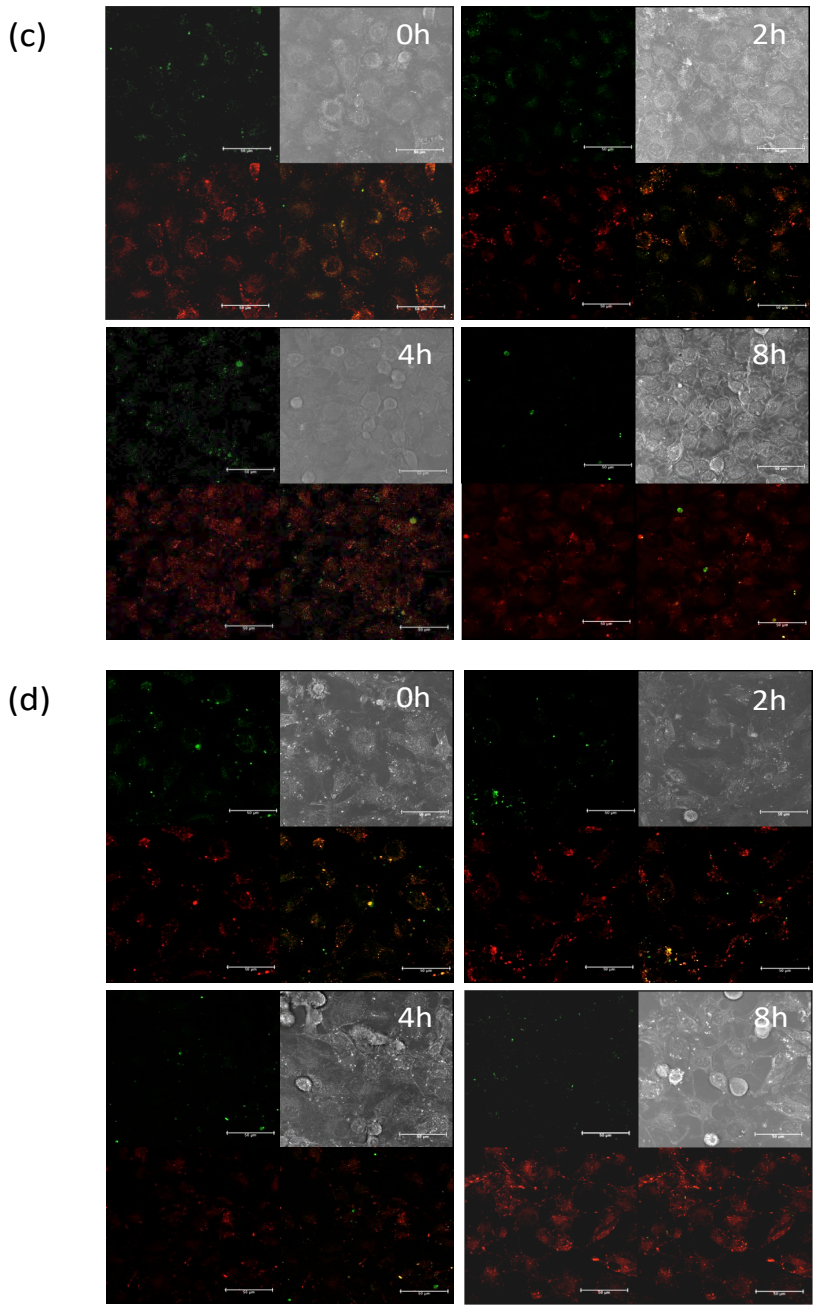


Figure S4: Lysosomal co-localization of nanocomplexes (NCs) with time: NCs formed using FITC-labeled DNA (green) were studied for co-localization with Lysosome (red labeled with Lysotracker Red) in B16-F10 cell line at 0, 2, 4 and 8h: (a) Control, (b) M3-NC, (c) M9-NC and (d) Lipofectamine NC.

Nanocomplexes	0h	2h	4h	8h
M3 NC	0.51 ± 0.05	0.35 ± 0.05	0.30 ± 0.04	0.17 ± 0.04
M9 NC	0.29 ± 0.04	0.31 ± 0.03	0.28 ± 0.06	0.22 ± 0.00
Lipofectamine NC	0.38 ± 0.08	0.40 ± 0.04	0.18 ± 0.02	0.34 ± 0.05

Table S2: Lysosomal co-localization of NCs with time was studied using confocal microscopy and respective Pearson's correlation coefficient estimated from three fields (~50 cells) is reported in Table (Experiments were performed in two independent sets. Data is represented in mean ± standard deviation).

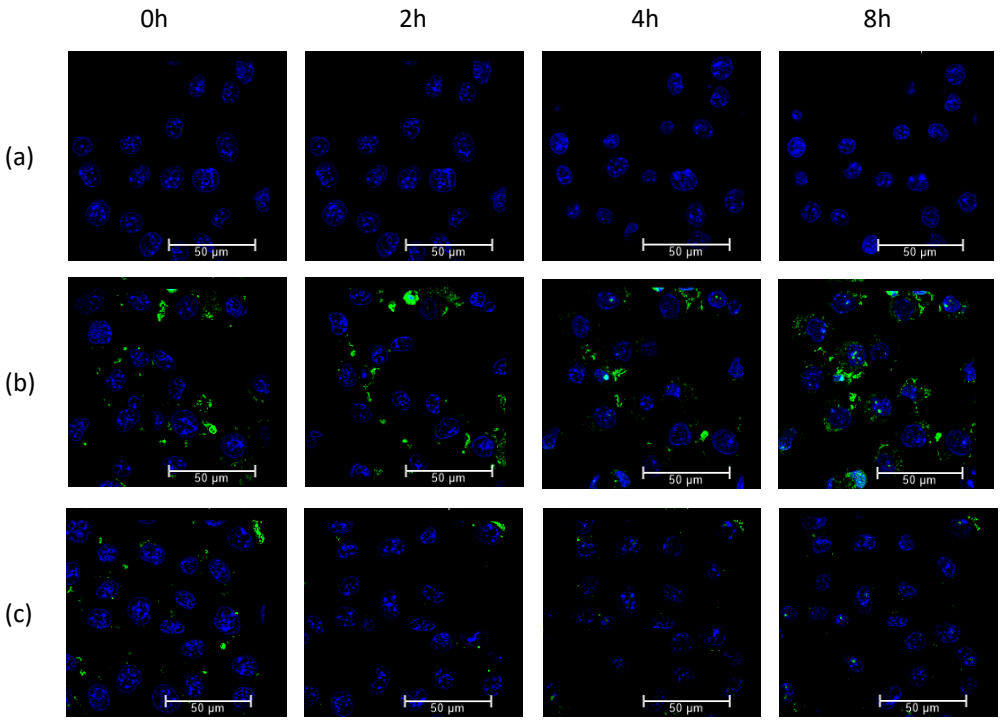


Figure S5: Nuclear co-localization of M9 and Lipofectamine nanocomplexes: Live cells confocal imaging was performed for studying nuclear localization of nanocomplexes for 8h. FITC-labeled nanocomplexes were incubated for 4h and imaging was done after media change: (a) control (b) M9 nanocomplexes (c) Lipofectamine nanocomplexes. B16-F10 Cells were imaged with same field in focus for 8h.

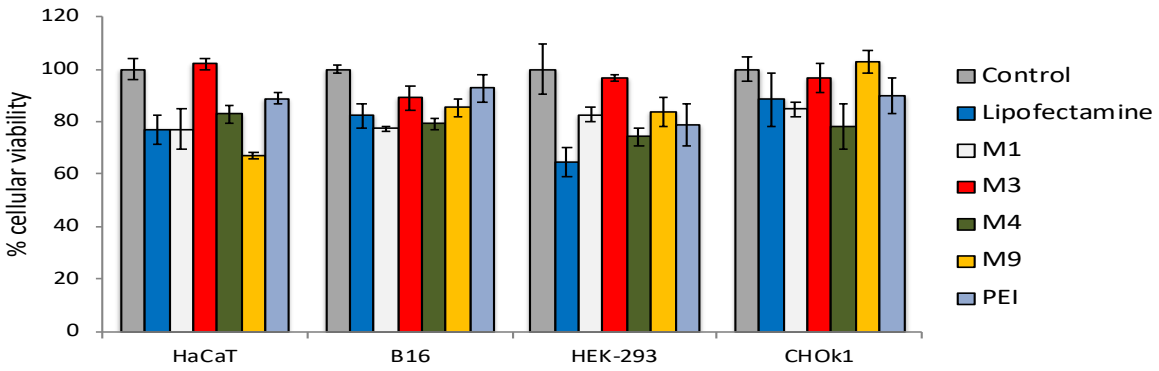


Figure S6: Cellular viability 24h after incubating the cells with the nanocomplexes. MTT assay was performed to assess the percentage viable cells with respect to control.