nanomaterials

MDPI

Supplementary Materials

The Impact of Nylon-3 Copolymer Composition on the Efficiency of siRNA Delivery to Glioblastoma Cells

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1. Polymer synthesis and characterization

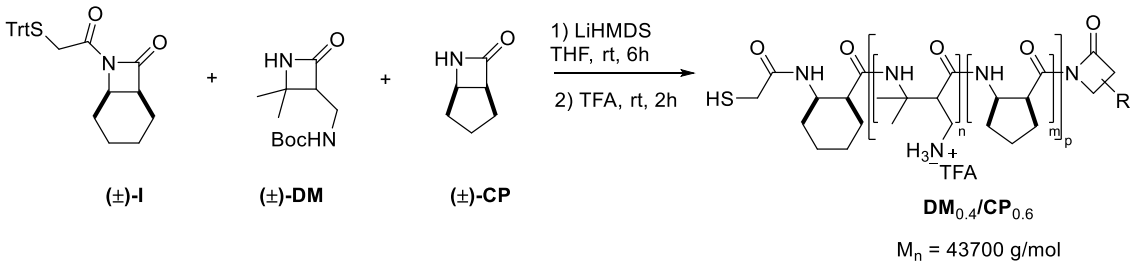

Figure S1. Synthesis of TFA-salt of Nylon-3 polymer $DM_{0.4}/CP_{0.6}$ in presence of a co-initiator I and a base LiHMDS. DM = dimethyl, CP = cyclopentyl, R = side chain groups of DM or CP. Reproduced with permission from [1]. Copyright American Chemical Society, 2014

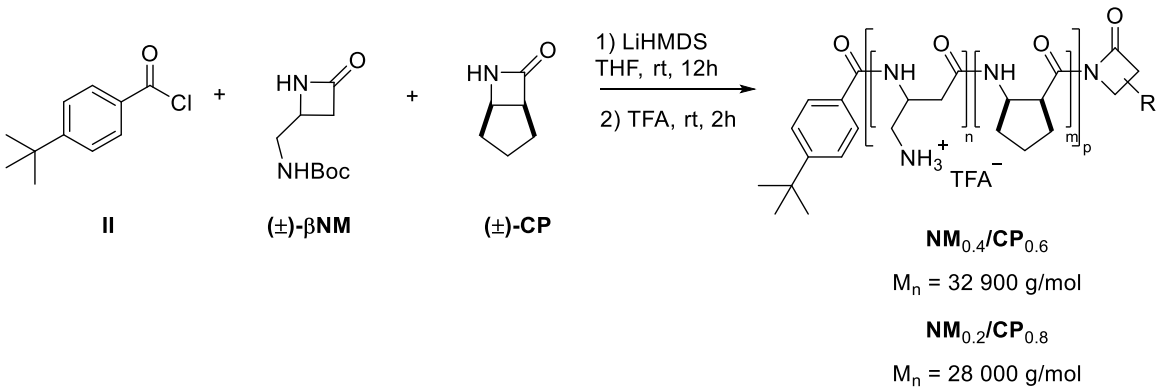

Figure S2. Synthesis of TFA-salts of gene delivery Nylon-3 polymers (NM/CP) in presence of a co-initiator II and a base LiHMDS. R = side chain groups of NM or CP. Reproduced with permission from [1]. Copyright American Chemical Society, 2014

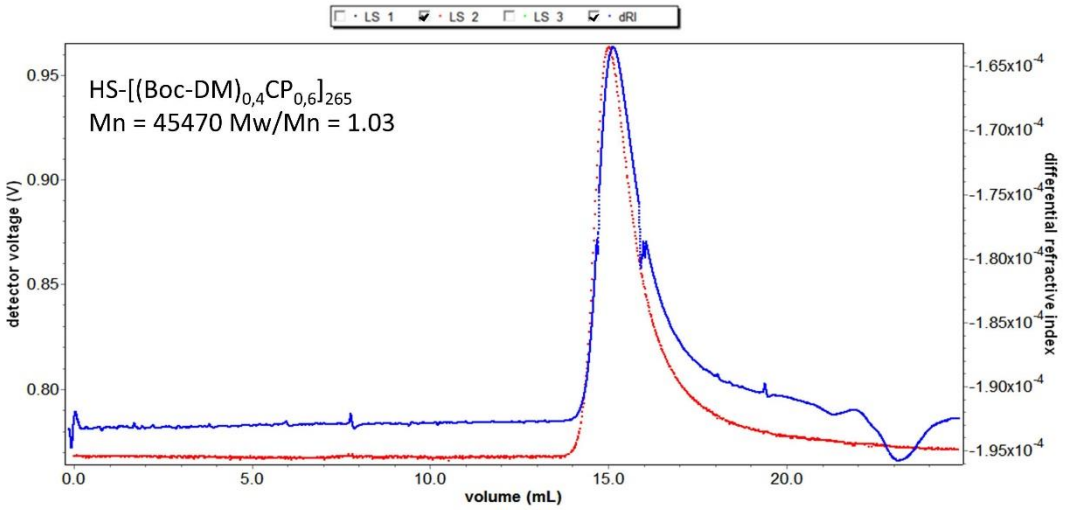

Figure S3. GPC chromatograph of Boc-protected HS-[(Boc-DM)$_{0.4}$CP$_{0.6}$]$_{265}$ copolymer measured with light-scattering (red) and refractive index (blue) detectors, mobile phase: THF.

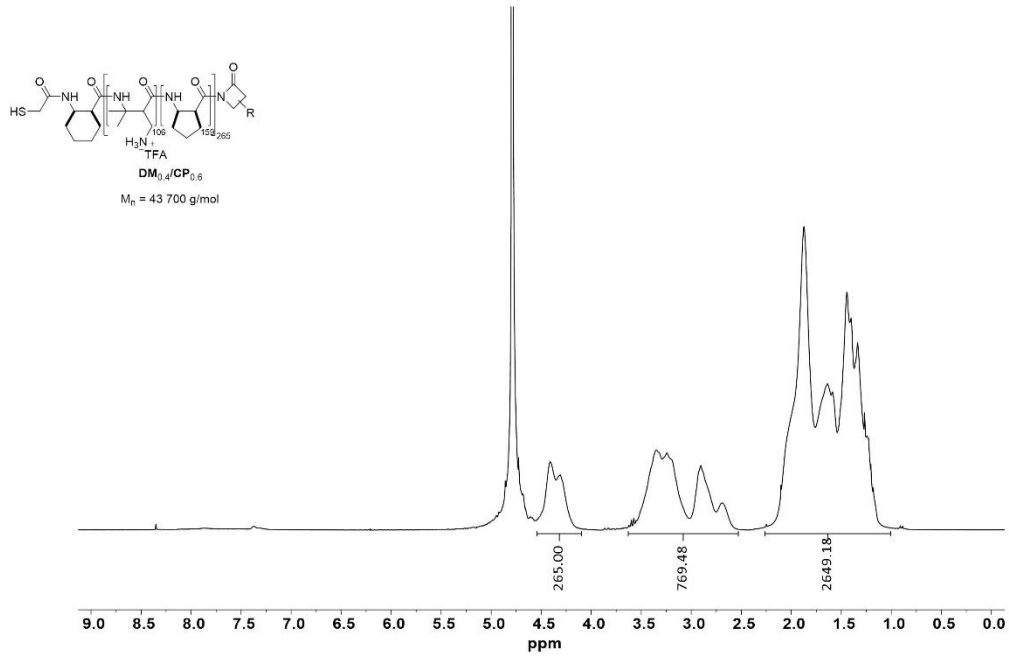

Figure S4. ^{1}H-NMR spectrum of unprotected DM$_{0.4}$/CP$_{0.6}$ polymer measured in D$_2$O (300 MHz, 512 scans).

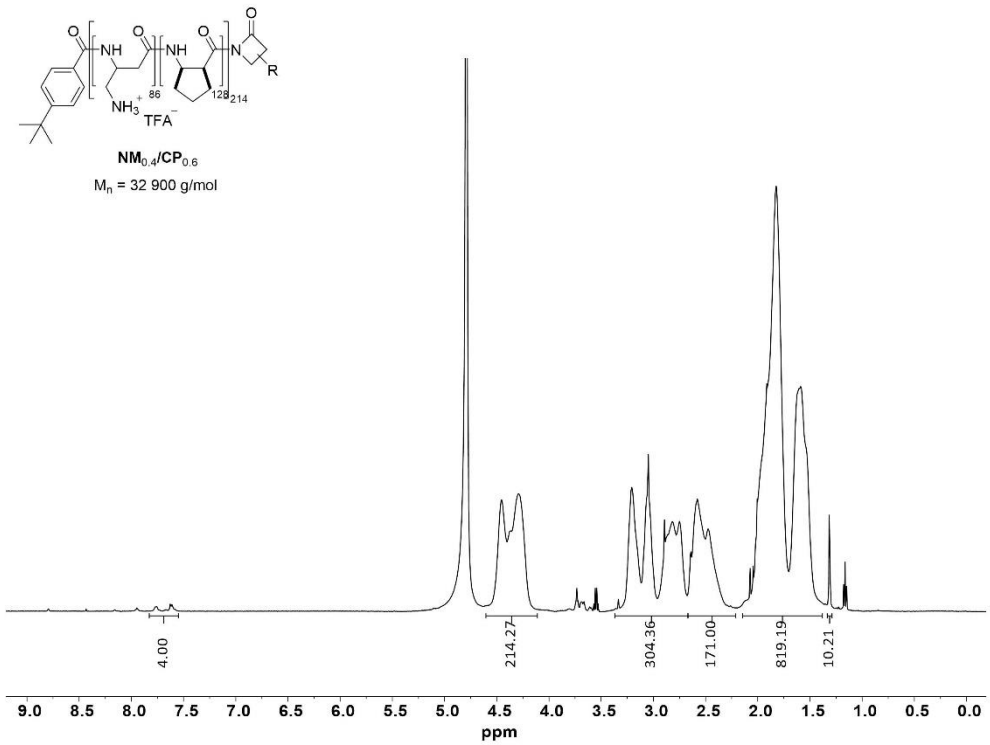

Figure S5. ^{1}H-NMR spectrum in D_2O of unprotected $NM_{0.4}/CP_{0.6}$ polymer measured in D_2O (500 MHz, 126 scans).

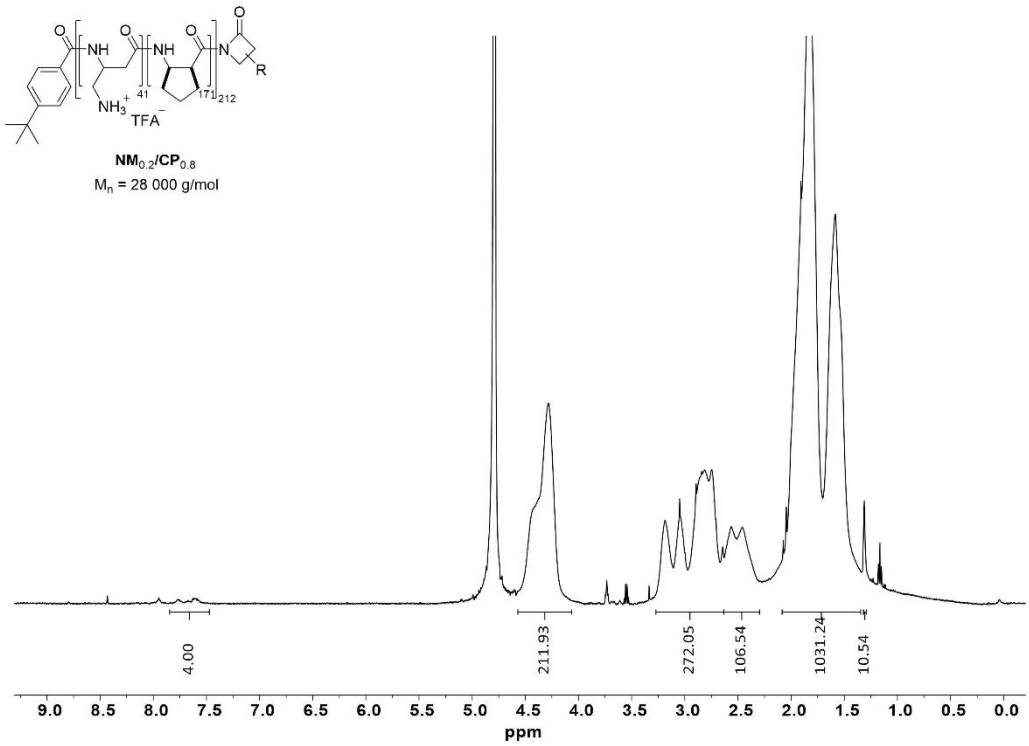

Figure S6. ^{1}H-NMR spectrum of unprotected $NM_{0.2}/CP_{0.8}$ polymer measured in D_2O (500 MHz, 126 scans).

2. Polyplex characterization

2.1. N/P ratio optimization

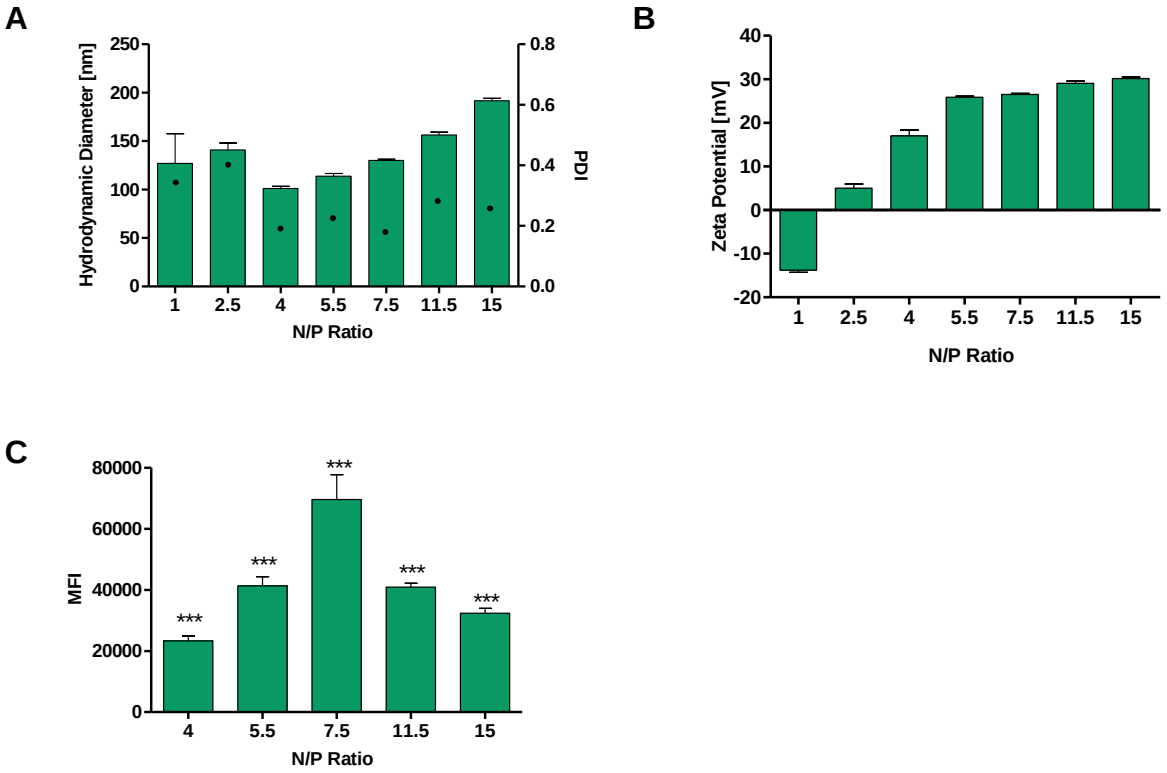

Figure S7. (**A**) Hydrodynamic diameters investigated by DLS (left y-axis) and polydispersity indices (PDI, right y-axis), (**B**) zeta potentials measured by LDA and (**C**) MFIs of $NM_{0.2}/CP_{0.8}$ cells treated with respective polyplexes determined by flow cytometry. (Data points indicate mean ± SD, $n = 3$).

2.2. Size measurements by TRPS

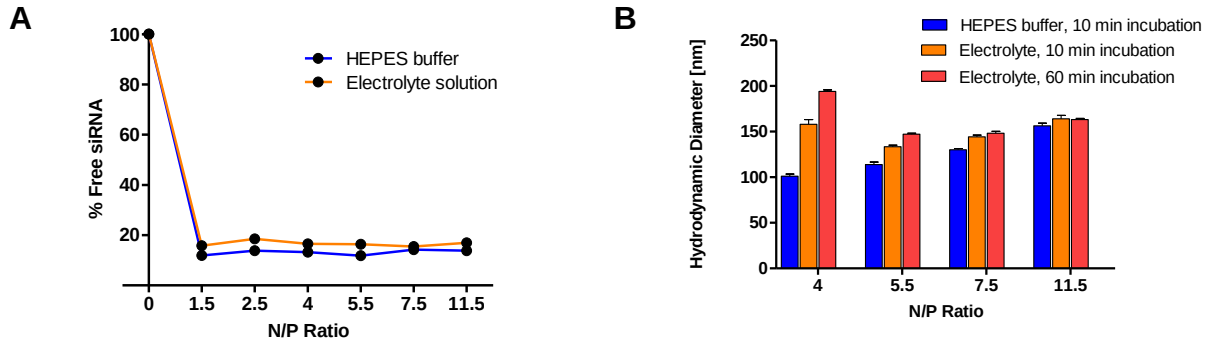

Figure S8. (**A**) siRNA encapsulation profiles of $NM_{0.2}/CP_{0.8}$ polyplexes prepared in 10 mM HEPES buffer at N/P 4 and 1X diluted with either 10 mM HEPES buffer or TRPS electrolyte solution (30 mM HEPES, 100 mM potassium chloride, 2 mM EDTA and 0.03% Tween®20). 100% values (N/P = 0) are determined by fluorescence of uncondensed siRNA. (Data points indicate mean, $n = 3$). (**B**) DLS measurements of $NM_{0.2}/CP_{0.8}$ polyplexes prepared in 10 mM HEPES buffer at N/P 4 and 1X diluted with either 10 mM HEPES buffer or TRPS electrolyte solution, measured after 10 and 60 min incubation period (Data points indicate mean ± SD, $n = 3$).

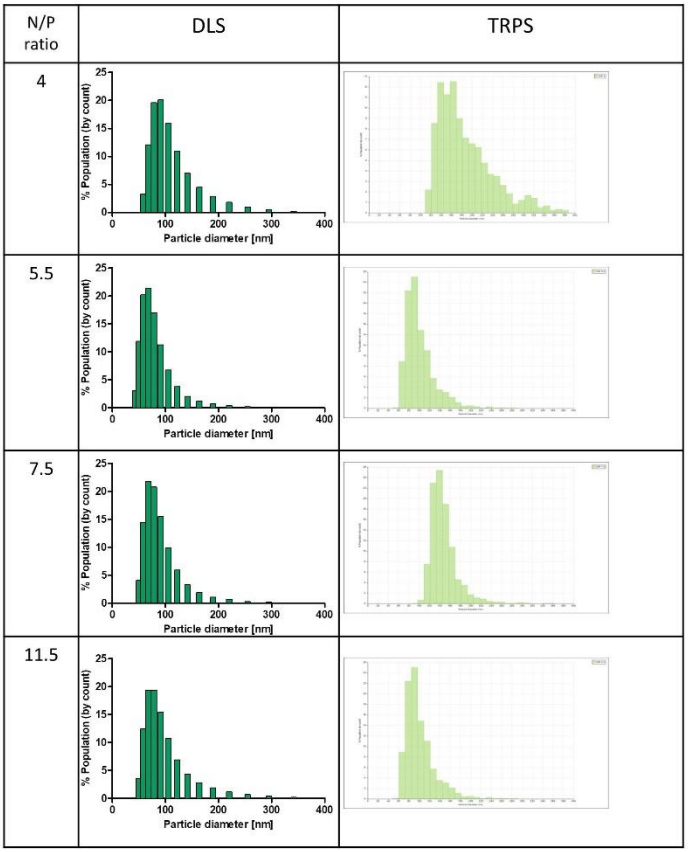

Figure S9. Number-weighted size distributions of $NM_{0.2}/CP_{0.8}$ polyplexes at N/P ratios 5, 5.5, 7.5 and 11.5. 1:1 diluted with electrolyte solution and measured by DLS and TRPS, respectively.

3. Quantification of Cellular Uptake

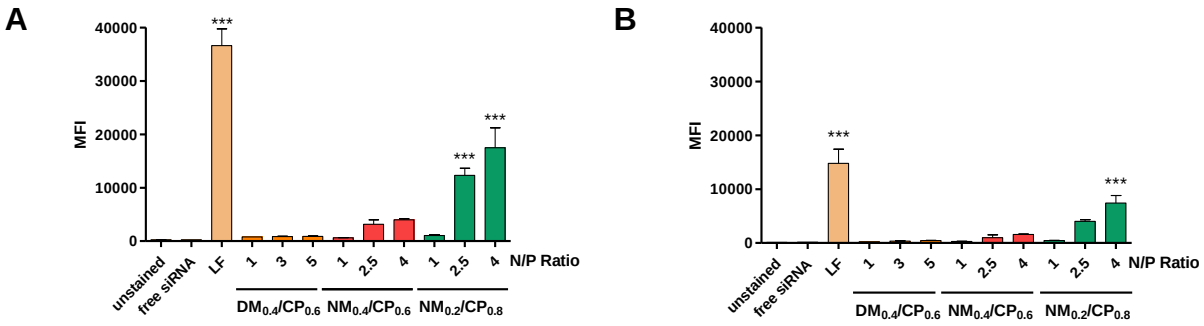

Figure S10. Cellular uptake of polyplexes performed at various N/P ratios in (**A**) H1299 cells and (**B**) U87 cells after 5 h incubation as determined by flow cytometry presented as median fluorescence intensity. Negative control: untreated cells and cells treated with free siRNA, positive control: cells transfected with Lipofectamin (LF) lipoplexes. (Data points indicate mean ± SD, n = 3, two-way ANOVA with Bonferroni post-hoc test, ***$p < 0.005$).

4. Route of cellular Uptake

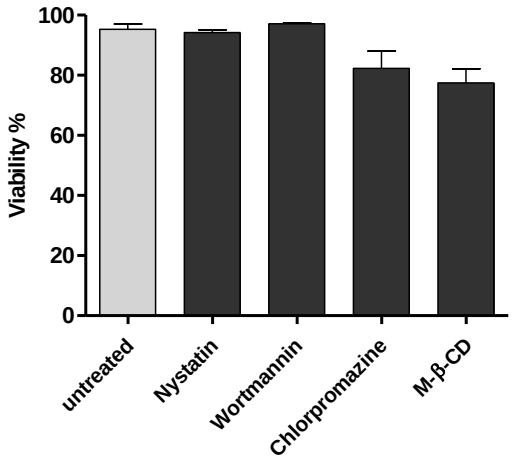

Figure S11. U87 cell viabilities after treatment with nystatin (10 μg/mL), wortmannin (12ng/mL), chlorpromazine (10 μg/mL) and methyl-beta-cyclodextrin (3 mg/mL); determined by trypan blue staining. Number of living and dead cells was counted in a Neubauer chamber using an Axio Vert.A1 microscope. The percentage of viable cells was calculated. (Results are given as mean ± SD, n = 3)

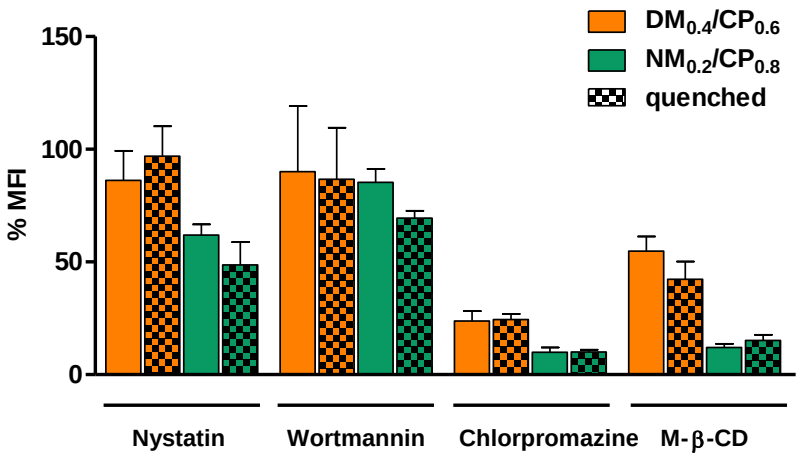

Figure S12. Cellular uptake of polyplexes ($DM_{0.4}/CP_{0.6}$ polyplexes: N/P ratio= 5 and $NM_{0.2}/CP_{0.8}$ polyplexes: N/P ratio = 4) after treatment with nystatin (10 μg/ml), wortmannin (12 ng/mL), chlorpromazine (10 μg/mL) and methyl-β-cyclodextrin (M-β-CD) (3 mg/mL) conducted with and without trypan quenching as evaluated by flow cytometry and presented as MFI. (Results are shown as mean ± SD as percentage of median fluorescence intensity related to not inhibited samples, n = 3).

References

1. Liu, R.; Masters, K.S. Polymer chain length effects on fibroblast attachment on nylon-3-modified surfaces. *Biomacromolecules*, **2012**, *13*, 1100–1105,.
2. Liu, R.; Chen, X. Structure-activity relationships among antifungal nylon-3 polymers: Identification of materials active against drug-resistant strains of Candida albicans. *Journal of the American Chemical Society*, **2014**, *136*, 4333–4342.