Electronic Supplementary Information

Structural variations in hyperbranched polymers prepared via thermal polycondensation of lysine and histidine and their effects on DNA delivery

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1 Synthesis and characterization of hyperbranched polymers

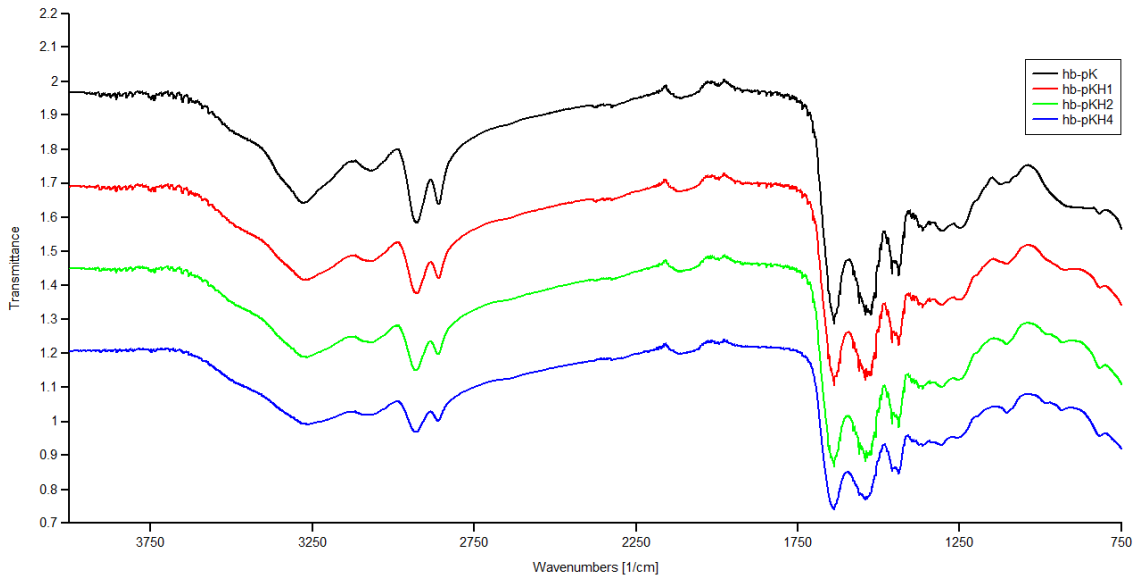


Figure S1: FT-IR of hyperbranched polymers

Vibrational absorptions at (3283, 3063, 2925, 2862, 1644, 1529, 1439, 1369, 1309, 1253 cm^{-1}) and strong peaks at 1640 cm-1 and 1529 cm-1 (amide bands I and II) confirm the formation of the peptide bonds.

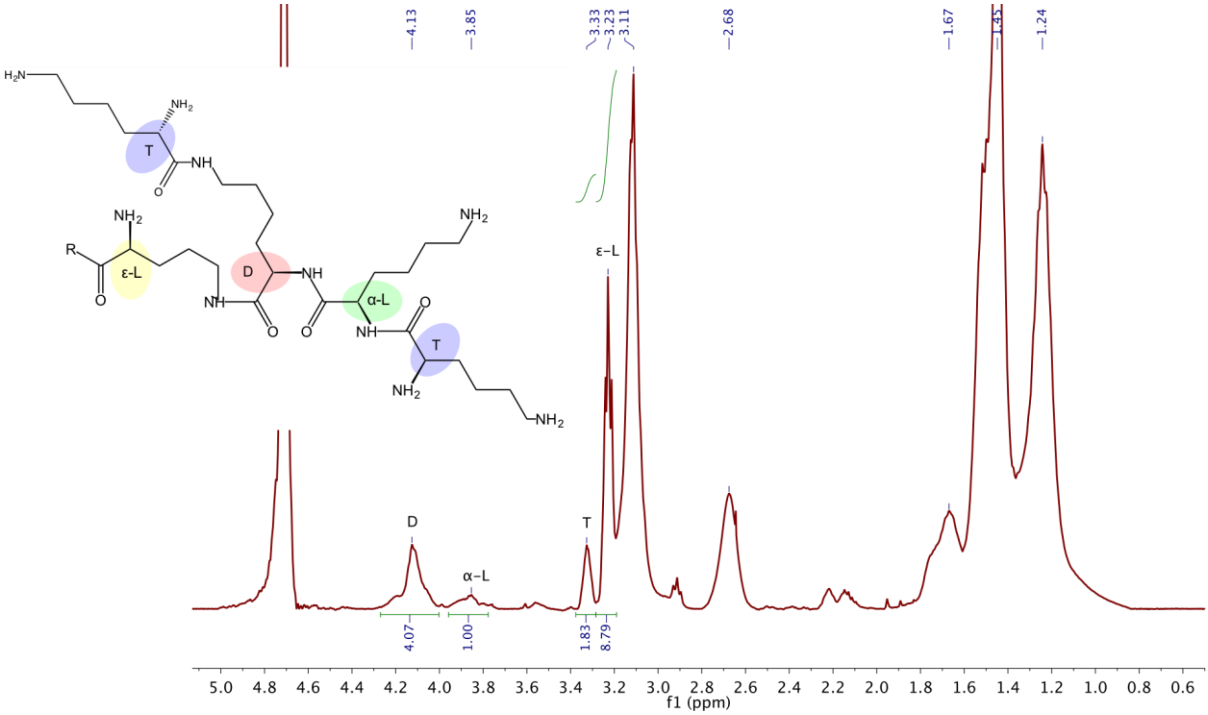


Figure S2: ^{1}H-NMR spectrum of *hb*-polyK, shows the structural units of the polymers, (400 MHz, D_2O) δ = 4.13 (b, 1H, COC<u>H</u>(R)NαH, dendritic unit), 3.85 (br, 1H, COC<u>H</u>(R)NαH, α-linear unit), 3.33 (br, 1H, COC<u>H</u>(R)NαH_2, terminal unit), 3.23 (br, 1H, COC<u>H</u>(R)NαH, ε-linear unit), 3.11 (br, 2H, -C<u>H_2</u>-NεH), 2.68 (br, 2H, -C<u>H_2</u>-NεH_2), 1.8–1.2 (br, 6H, -C<u>H_2</u>-).

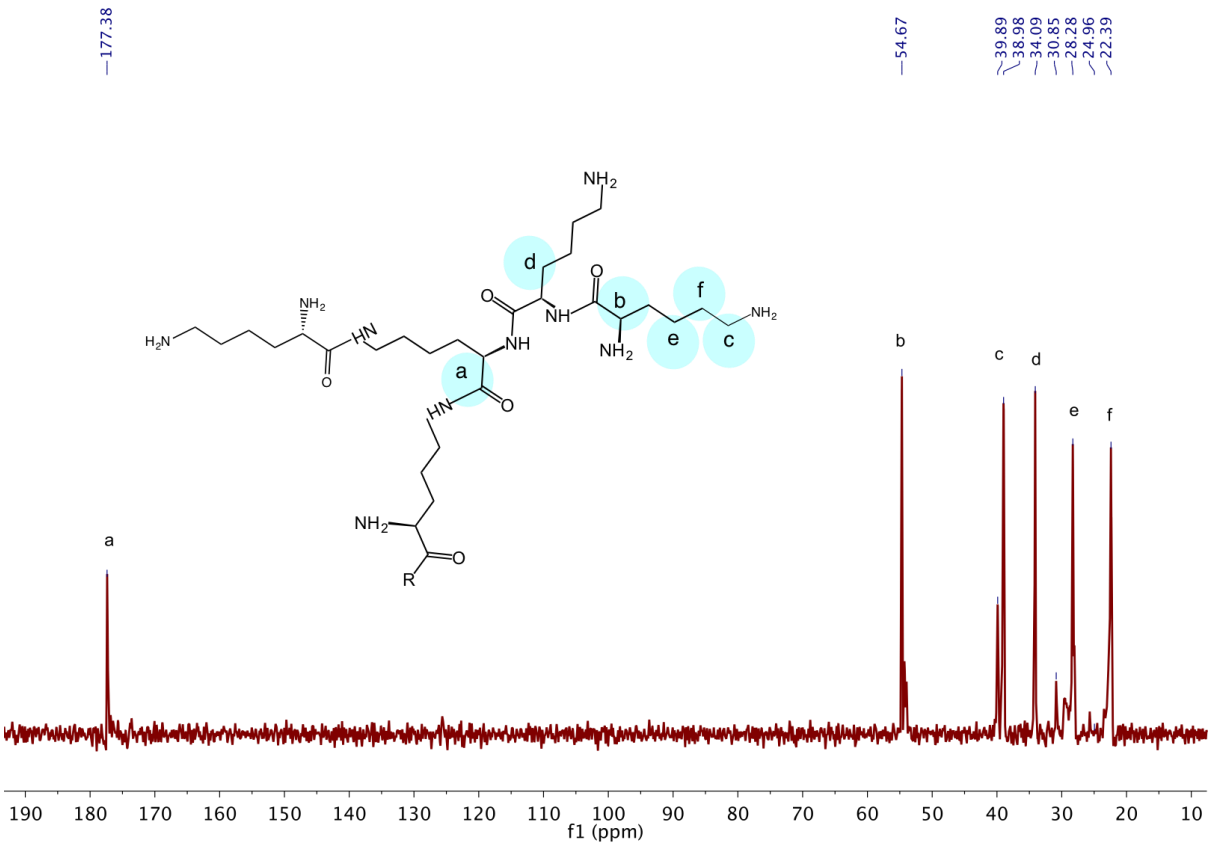


Figure S3: ^{13}C-NMR spectrum of *hb*-polyK, (100 MHz, D_2O) δ = 177.3 (-C(O)-NH), 54.6 (-COCH(R)NαH), 38.9 (-CH_2-NεH_2), 34.0 (-CH_2-CH-NαH_2), 28.2 (-CH_2-CH_2–NεH), 22.3 (-CH_2-CH_2-CH_2-NεH_2).

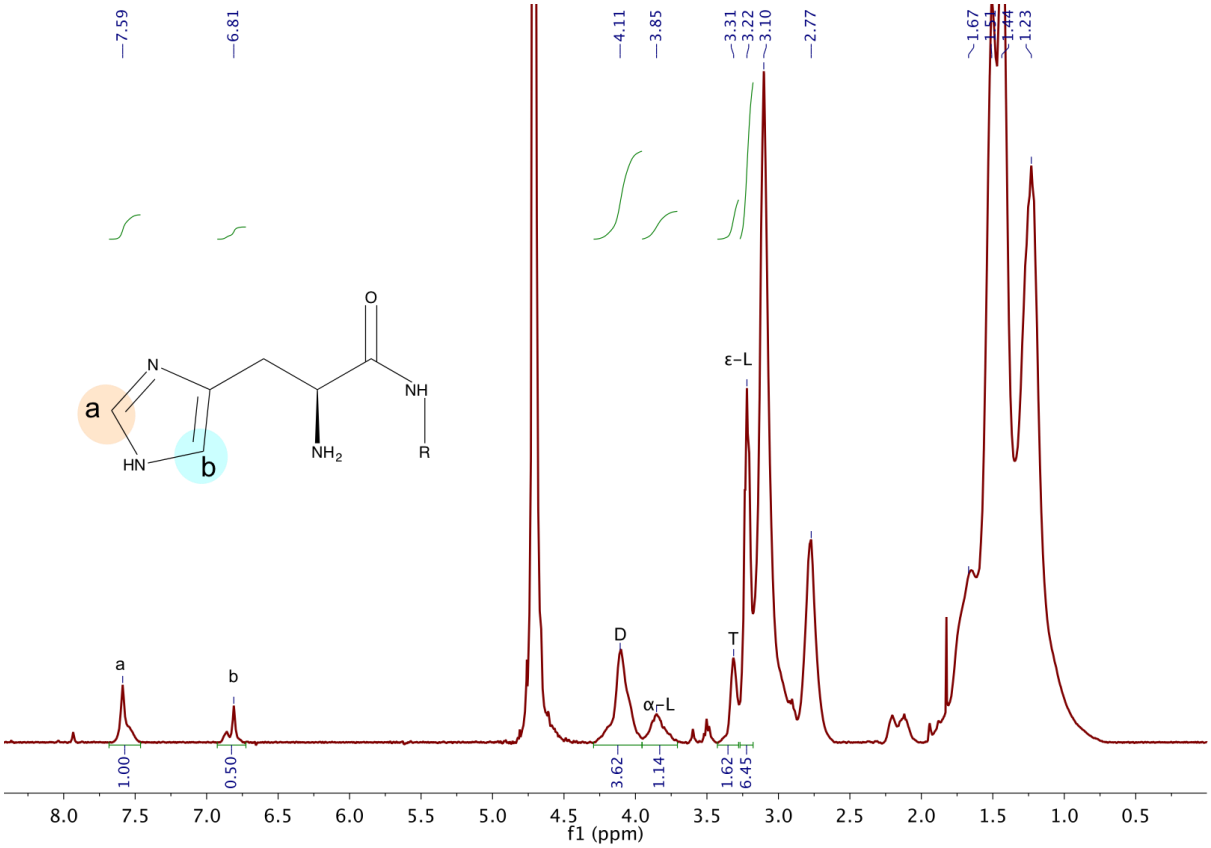


Figure S4: ^{1}H-NMR spectrum of *hb*-polyKH, (400 MHz, D_2O) δ = 7.59 (br, 1H, C<u>H</u> of imidazole ring), 6.81 (br, 1H, C<u>H</u> of imidazole ring), 4.11 (br, 1H, COC<u>H</u>(R)NαH, dendritic unit), 3.85 (br, 1H, COC<u>H</u>(R)NαH, α-linear unit), 3.31 (br, 1H, COC<u>H</u>(R)NαH_2, terminal unit), 3.22 (br, 1H, COC<u>H</u>(R)NαH, ε-linear unit), 3.10 (br, 2H, -C<u>H_2</u>-NεH), 2.77 (br, 2H, -C<u>H_2</u>-NεH_2), 1.8−1.2 (br , 6H, -C<u>H_2</u>-).

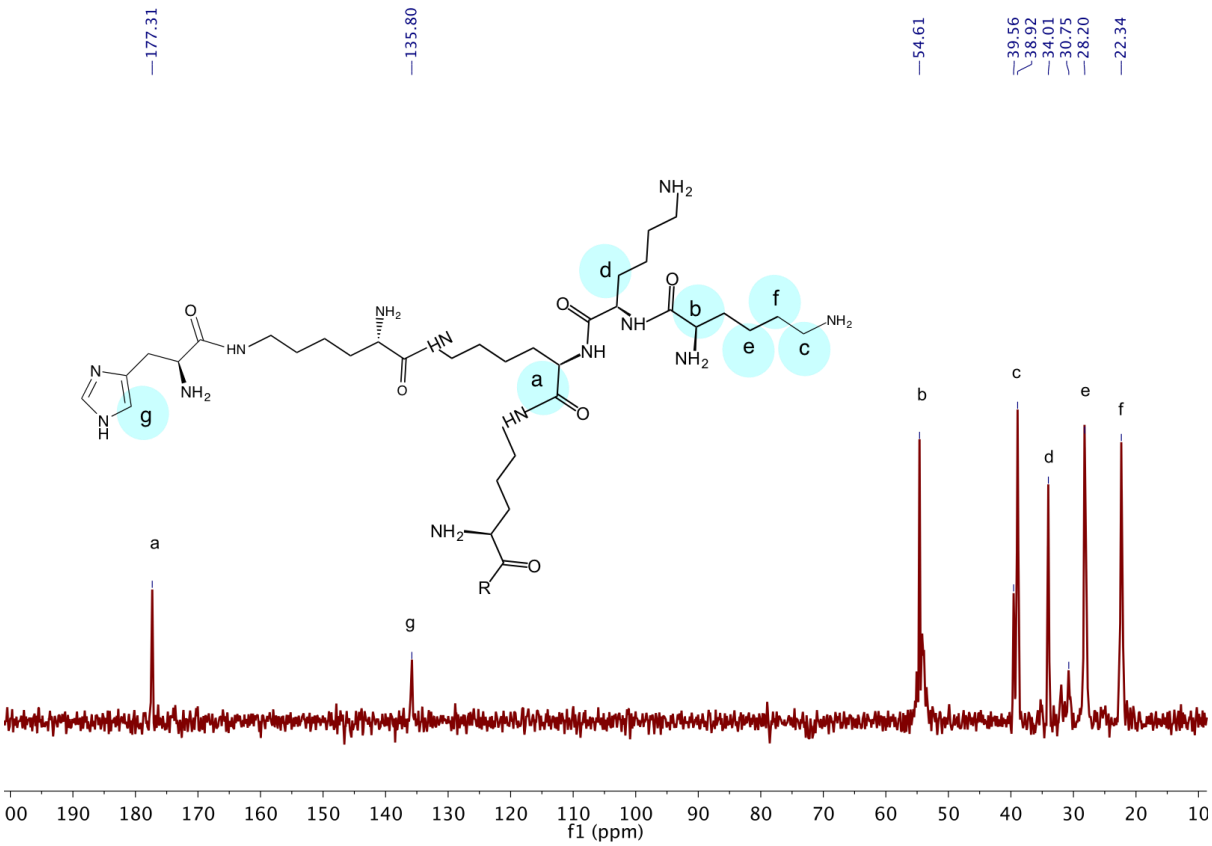


Figure S5: ^{13}C-NMR spectrum of *hb*-polyKH, (100 MHz, D_2O) δ = 177.3 (C(O)-NH), 135.8 (C- OF IMIDAZOLE RING), 54.6 (COCH(R)NαH), 38.9 (-CH_2-NεH$_2$), 34.0 (-CH_2-CH_2-NαH$_2$), 28.28 (-CH_2-CH_2–NεH), 22.38 (-CH_2-CH_2-CH_2-NεH$_2$).

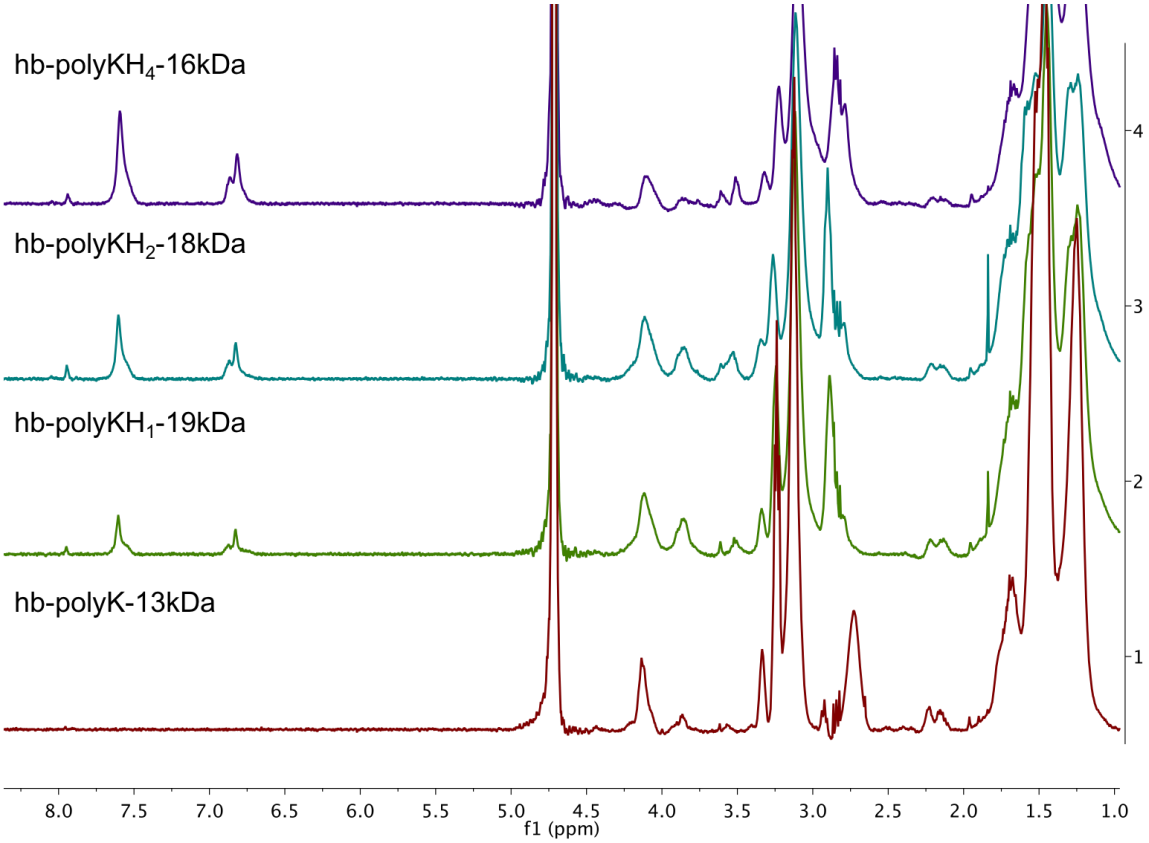


Figure S6: ^{1}H-NMR spectra of polymers in Group-A. The integrals corresponding to the protons of the imidazole ring (7.59 ppm and 6.81 ppm) increase with the increase in molar ratio of histidine.

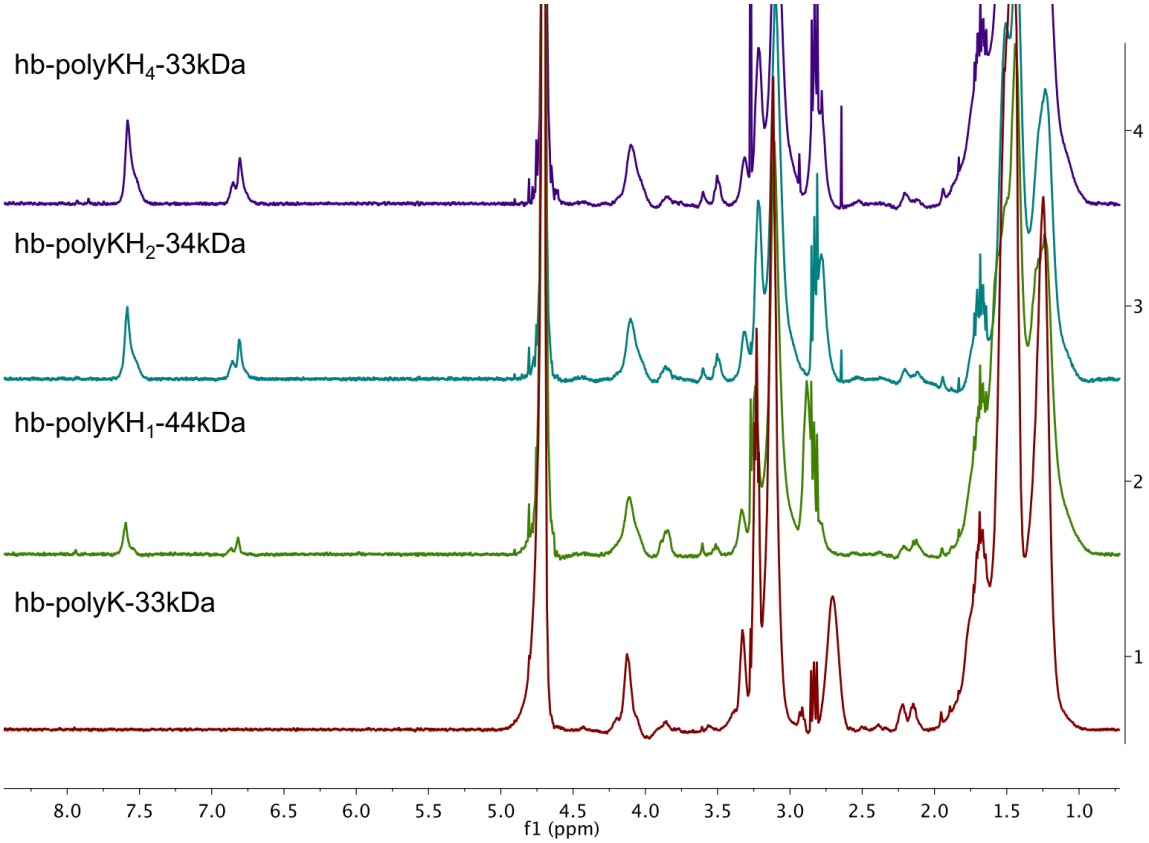


Figure S7: ^{1}H-NMR spectra of polymers in Group-B. The integrals corresponding to the protons of the imidazole ring (7.59 ppm and 6.81 ppm) increases with the increase in molar ratio of histidine.

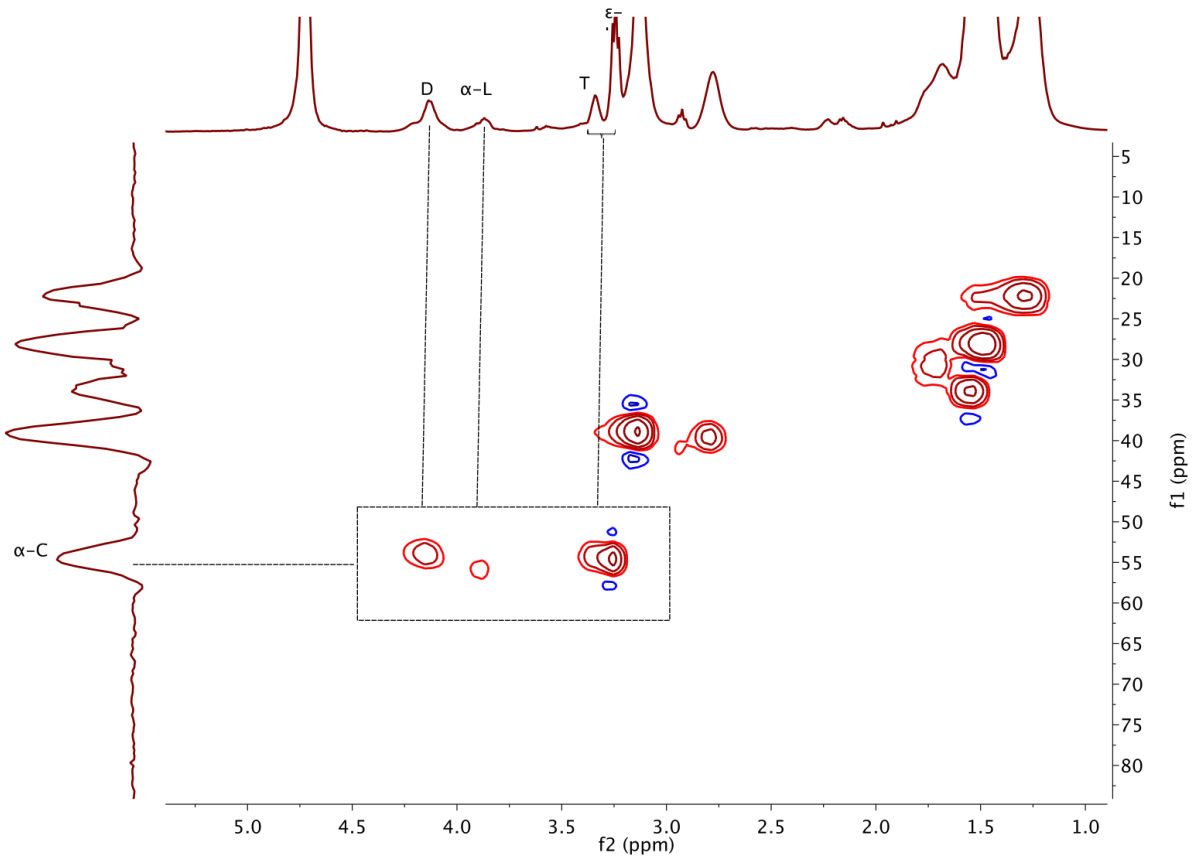


Figure S8: HSQC spectrum of *hb*-polyK.

The spectrum shows the resonances of the various α-CH protons linked to those for the α-C of ^{13}C-NMR spectrum, which confirms that there are several environments for these protons dependent on the different branch units.

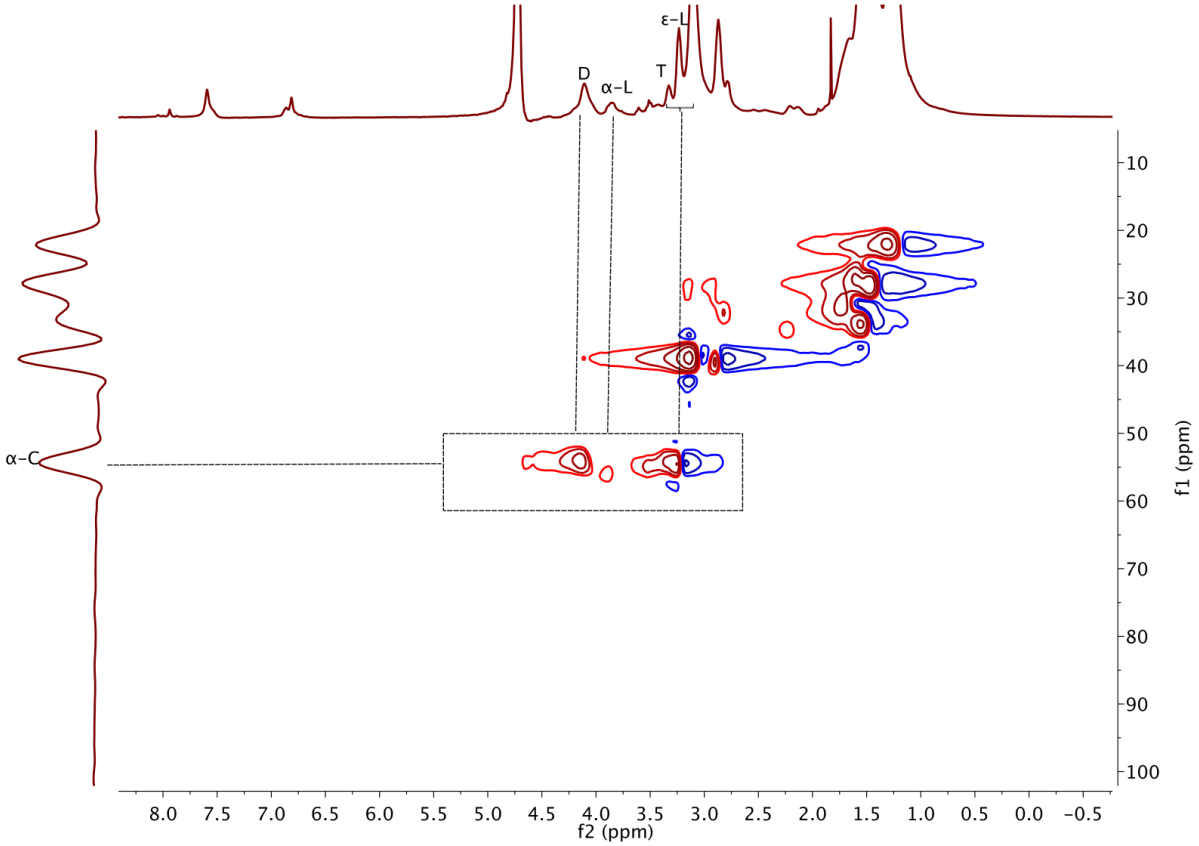


Figure S9: HSQC spectrum of *hb*-polyKH, confirming the incorporation of histidine in the polymers

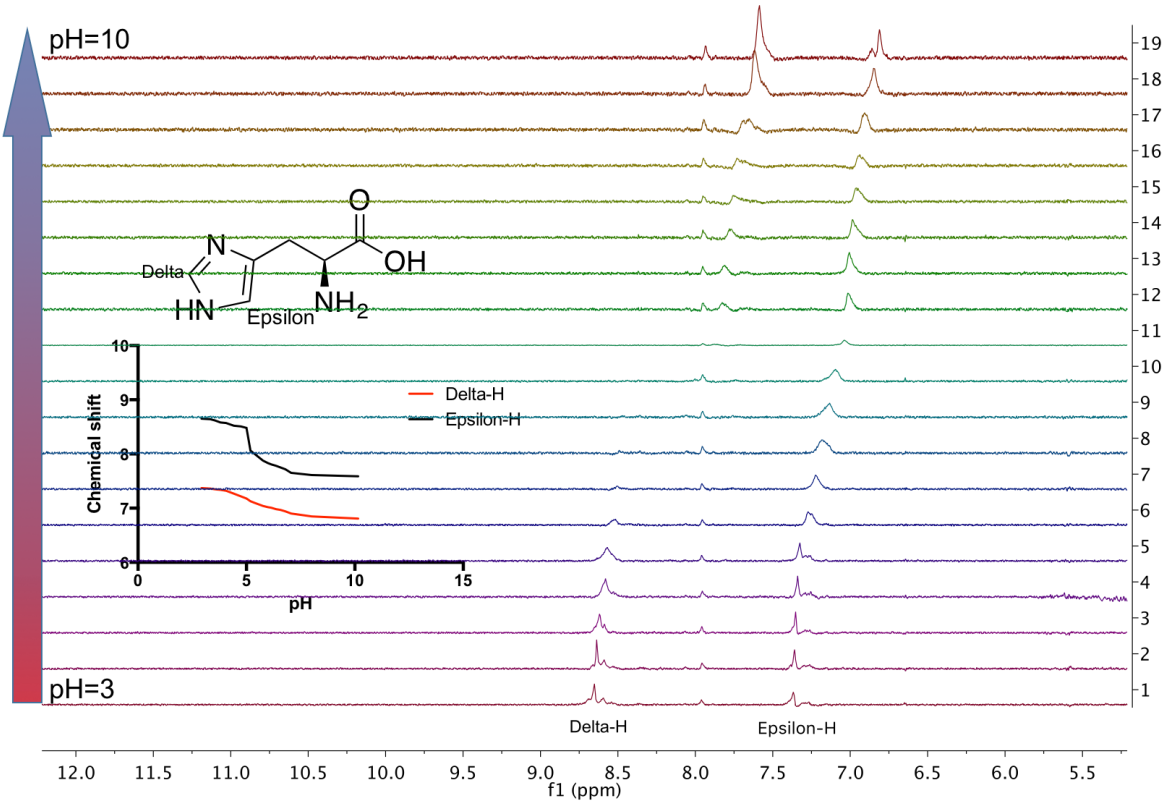
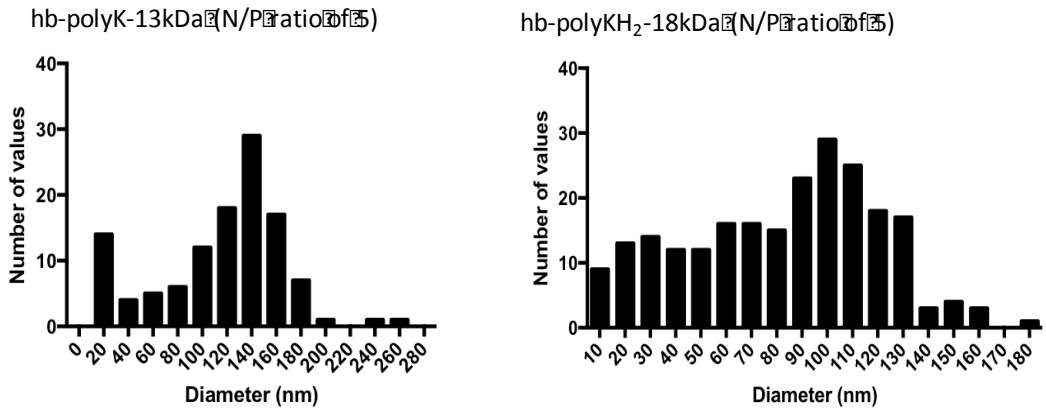


Figure S10: Chemical shifts of imidazole protons of hyperbranched polymers at different pH values, which were used to calculate the pKa of imidazole amine.

2 Polyplexes preparation and characterization.

A



B

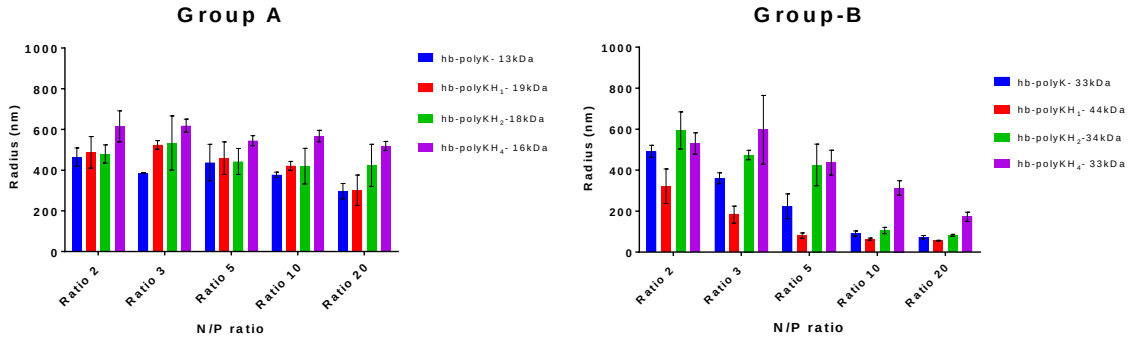
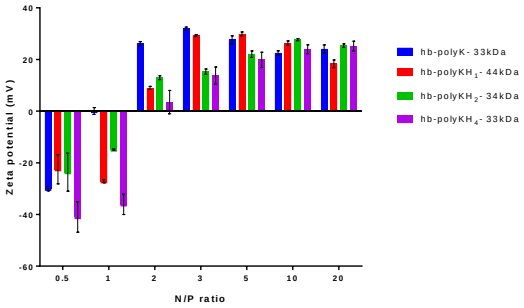
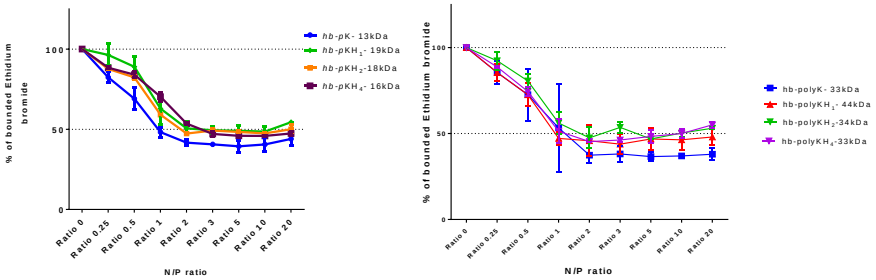


Figure S11: (A) the histograms of AFM images, analysed by Image-J, show the size distribution of polyplexes. (B) Hydrodynamic radii of polyplexes prepared in PBS, pH 7.4 at different N/P ratios using polymers of Group-A and Group-B

A



B



C

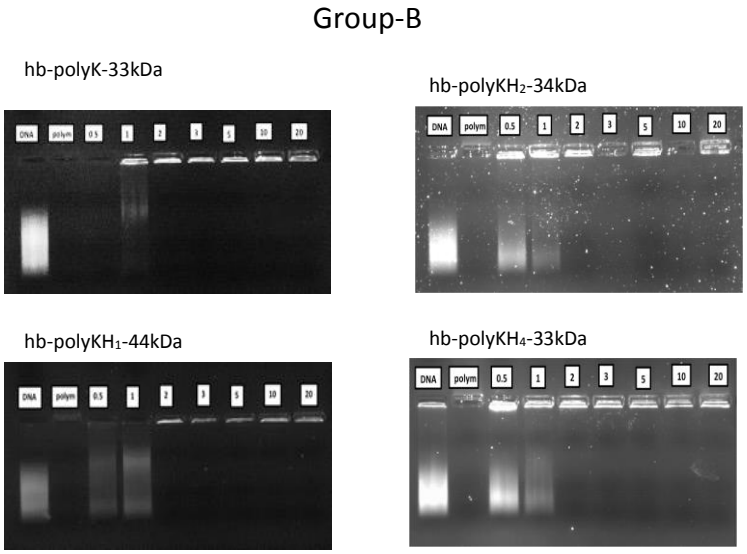


Figure S12: (A) Zeta potential measurements, (B) ethidium bromide displacement assay and (C) agarose gel electrophoresis of hyperbranched polymers/DNA polyplexes prepared in PBS, pH 7.4 at different N/P ratios.

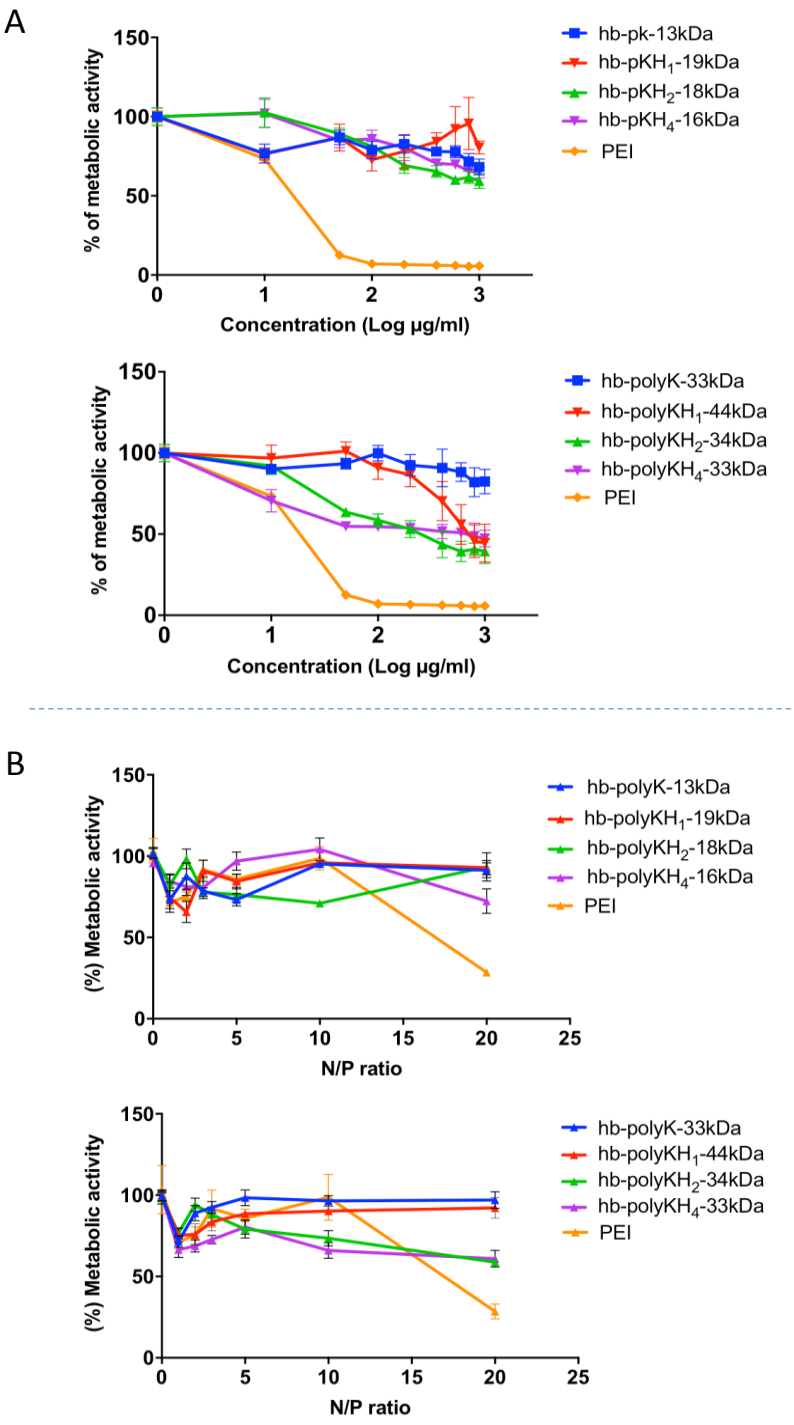
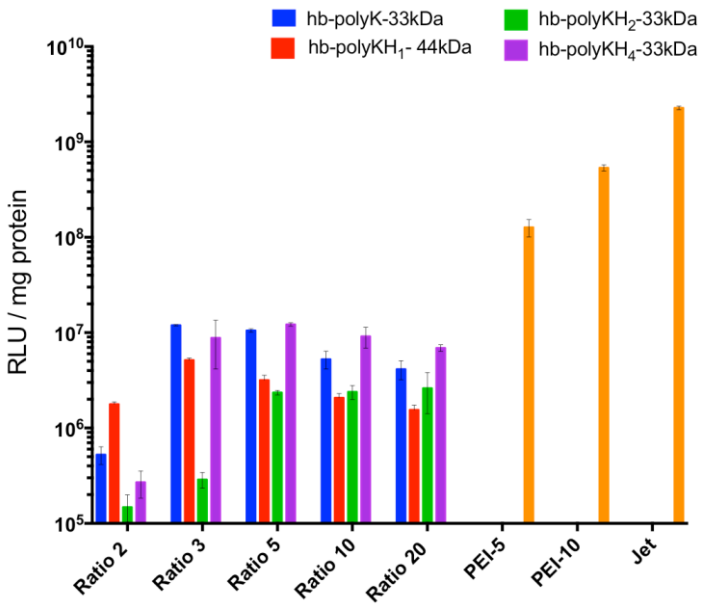


Figure S13: Metabolic activity of A549 cells treated with (A) the hyperbranched polymers and (B) their polyplexes.

(A) A549



(B) H1299

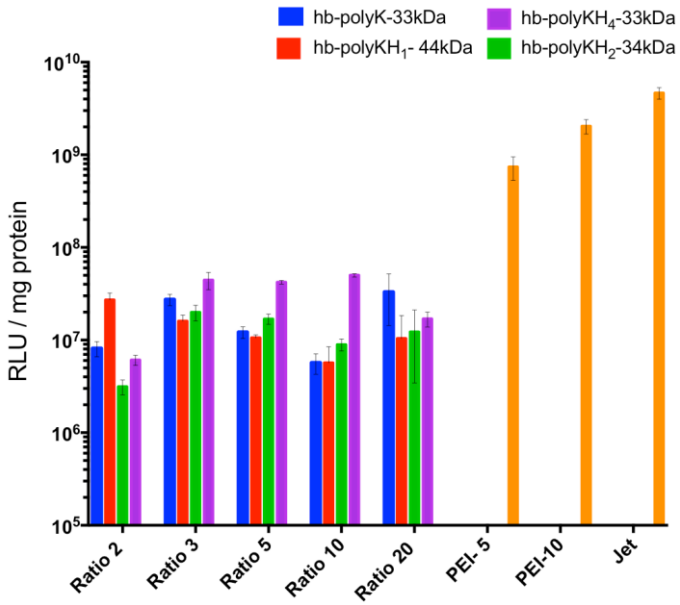


Figure S14: Luciferase activity of polyplexes prepared in PBS, pH 7.4 with polymers of high molecular weight.

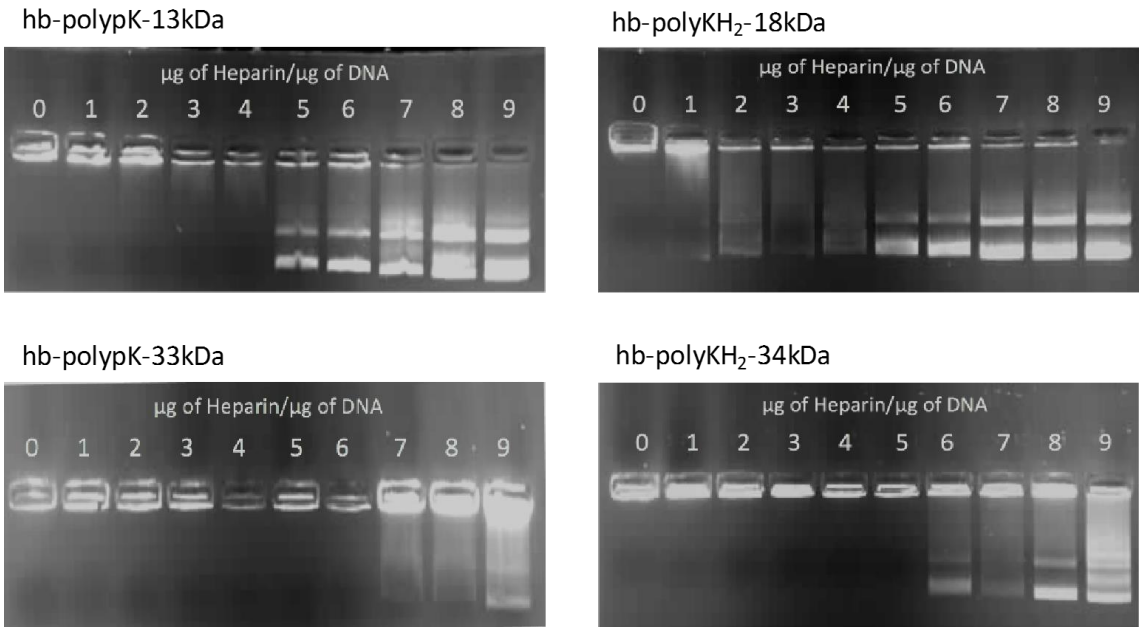


Figure S15: Heparin competition assay, the polyplexes of hb-polyK-13kDa, hb-polyKH$_2$-18kDa, hb-polyK-33kDa and hb-polyKH$_2$-34kDa at N/P ratio of 10 were incubated with increased amount of heparin sulfate for 30 min and observed by gel electrophoresis.

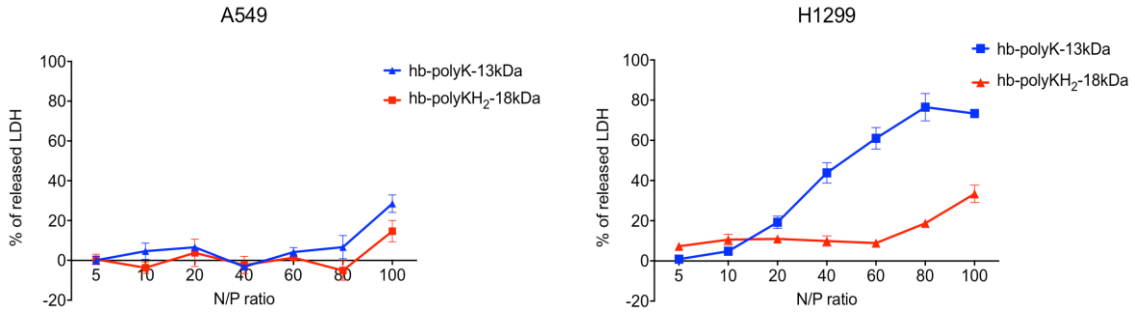


Figure S16: LDH assay of hb-polyK-13kDa and hb-polyKH$_2$-18kDa polyplexes of N/P ratio of 10 in A549 and H1299 cell lines, which reflect the stable nature of A549 membrane in comparison with H1299 where the difference between hb-polyK-13kDa and hb-polyKH$_2$-18kDa in their ability to interact and permeablise the membranes can be seen clearly in H1299 but not in A549.