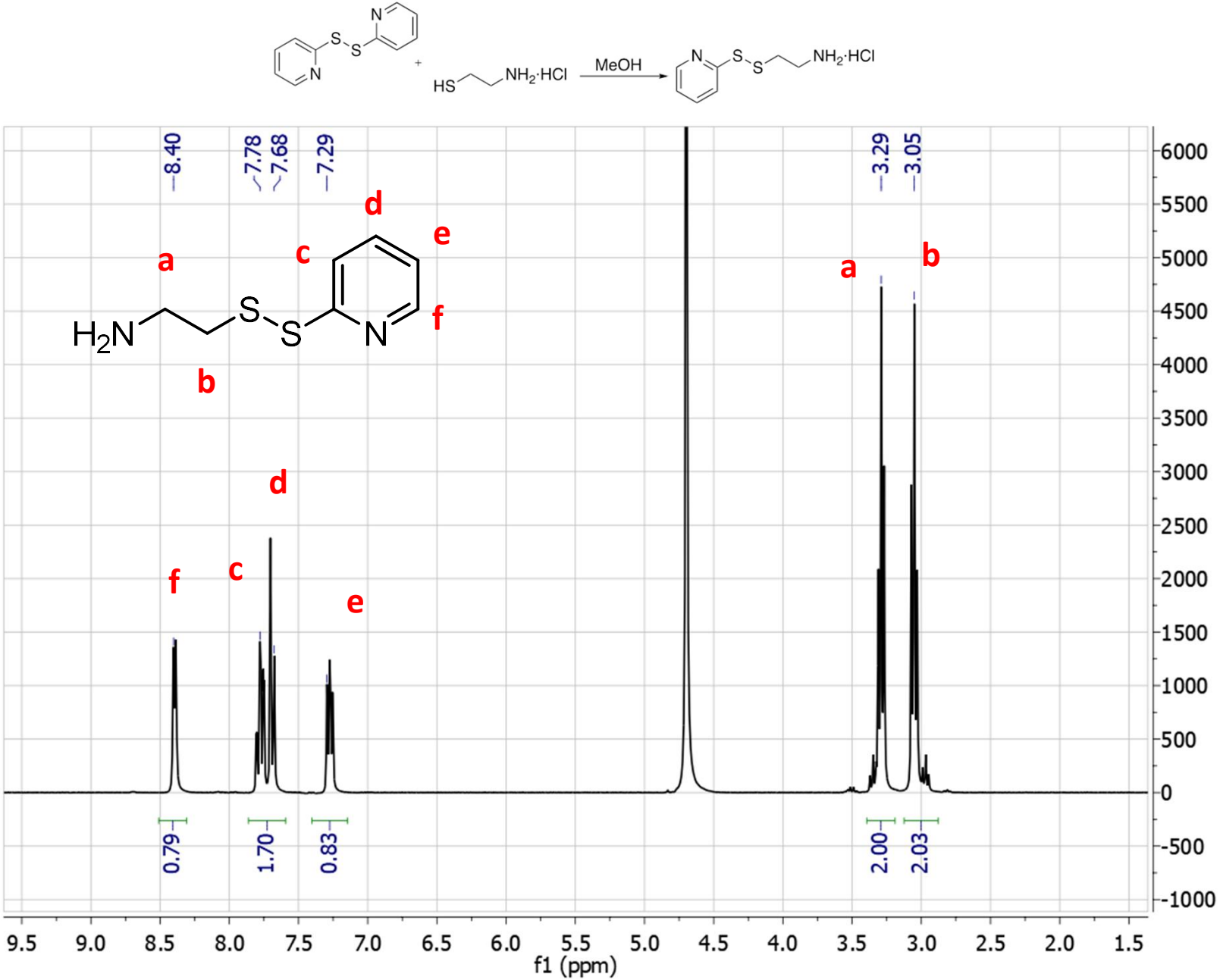
Electronic Supplementary Material (ESI) for Nanoscale.

Figure S1. Synthesis scheme and 1H-NMR analysis of pyridyl dithiol cysteamine (2PD) in DMSO.



		K_D (m^2/s)#	R_h (nm)
PLO	**P**	4,43E-11	556
PLO- 2PD	**P1**	4,96E-11	497
PLO - PEG3000	**P2**	4,08E-11	604
PLO - PEG3000-Fmoc	**P3**	4,11E-11	601
PLO - PEG2000	**P4**	4,12E-11	598
		# DOSY (D_2O)	

Table S1. Calculation of the hydrodynamic radius (Rh) by means of the Stokes-Einstein equation and the D values obtained by DOSY-NMR studies of the PLO derivatives.

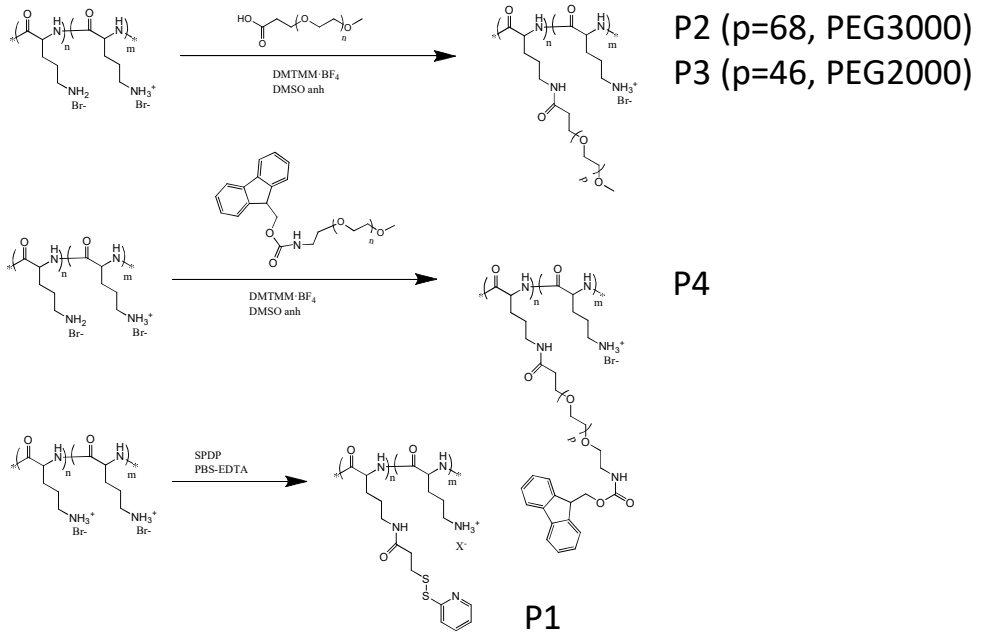


Figure S2. Synthetic Scheme used to obtain PLO derivatives: P1, P2, P3 and P4.

Figure S3. (A) 1H NMR spectrum of compound **P1** (300MHz, D2O).

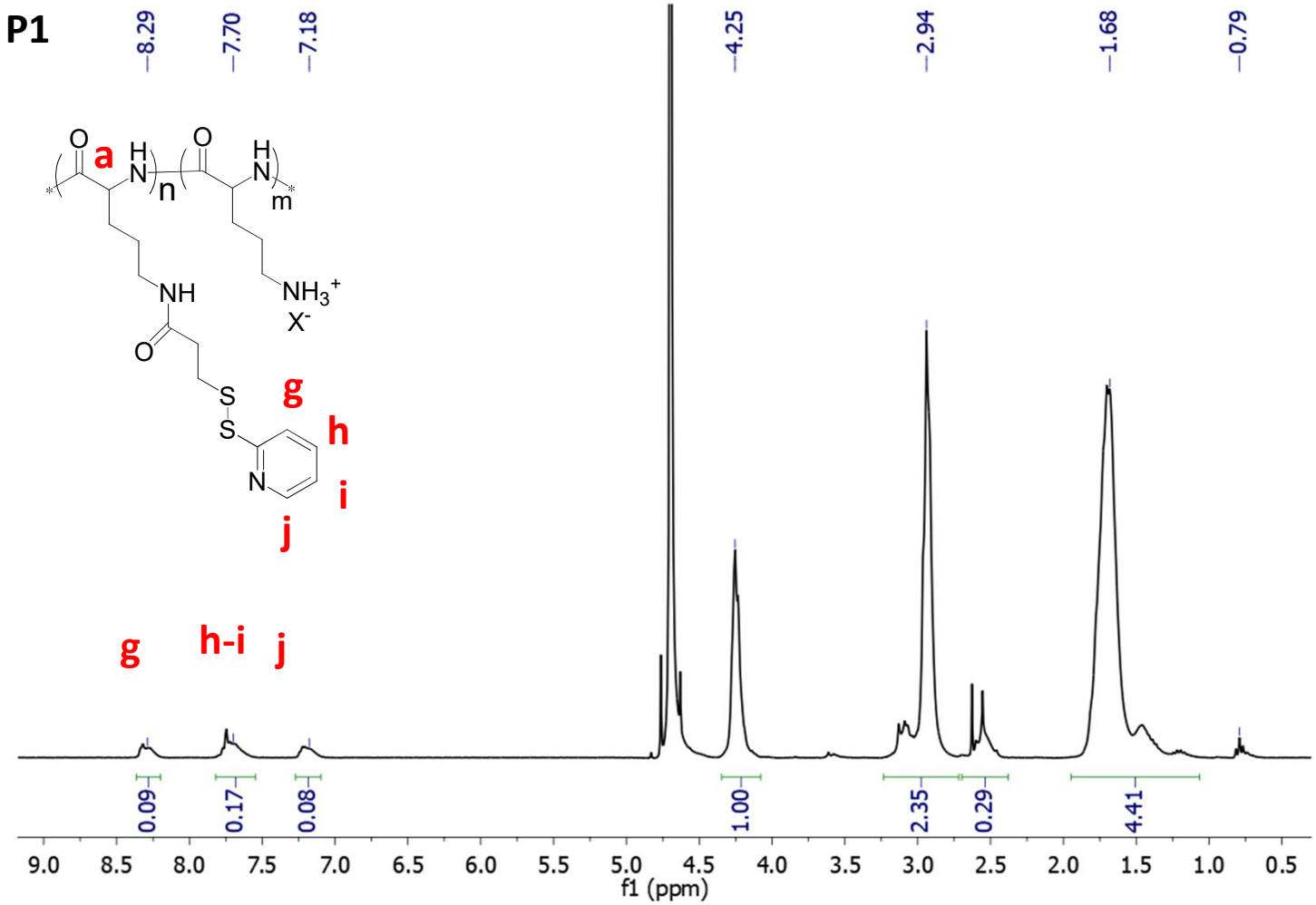


Figure S3. (B) 1H NMR spectrum of compound **P2** (300MHz, D2O).

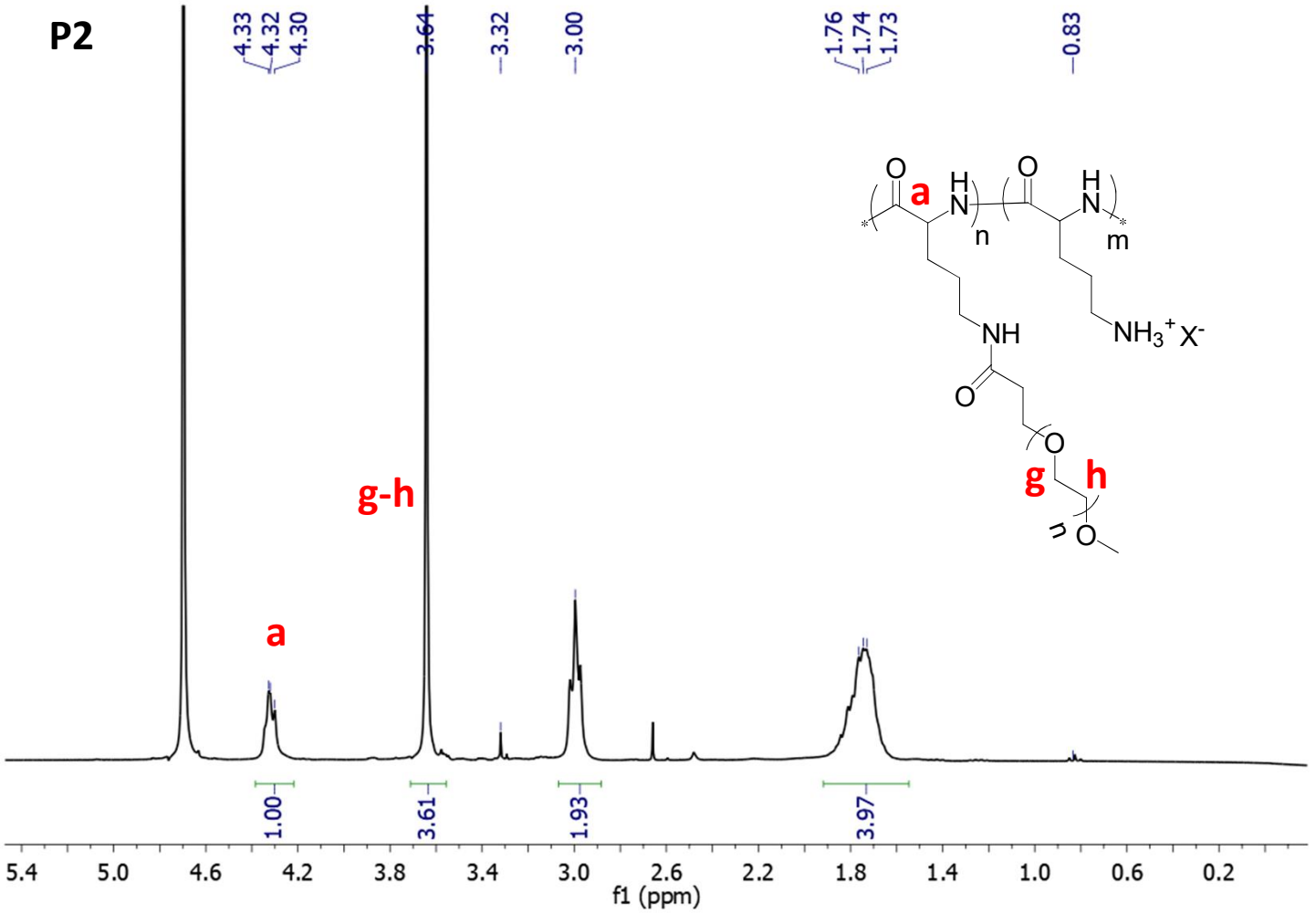


Figure S3. (C) 1H NMR spectrum of compound **P3** (300MHz, D2O).

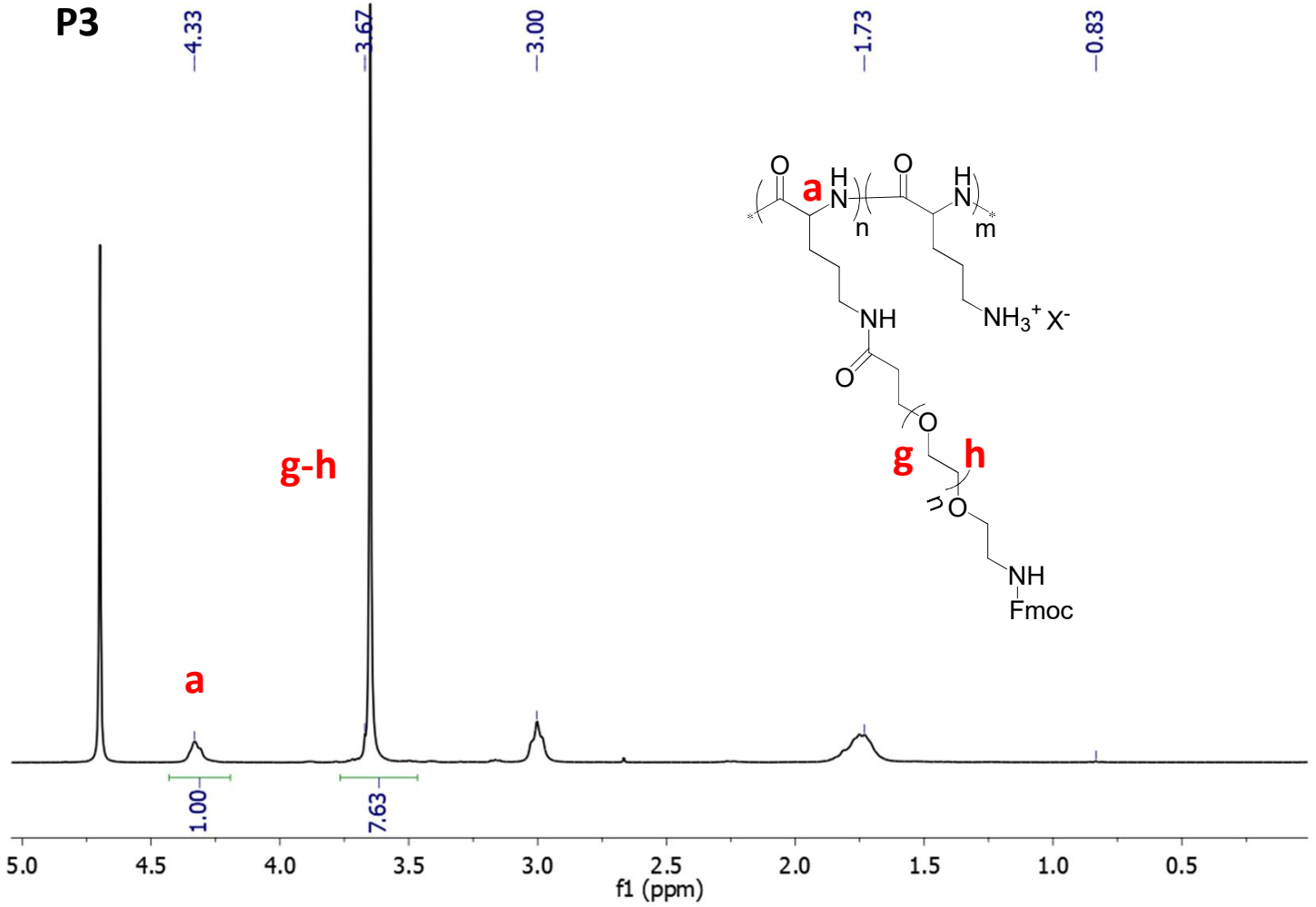
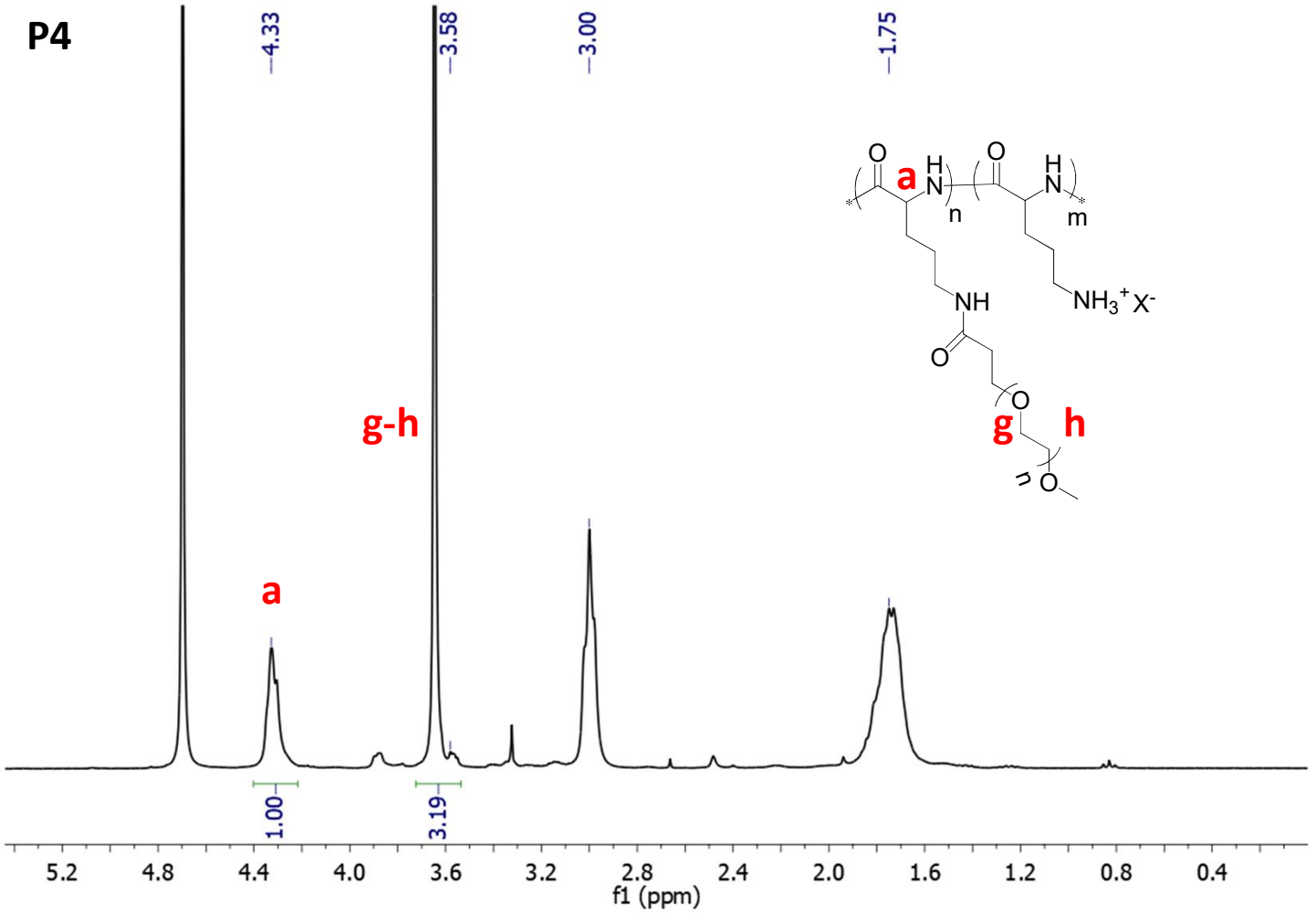


Figure S3. (D) 1H NMR spectrum of compound **P4** (300MHz, D2O).



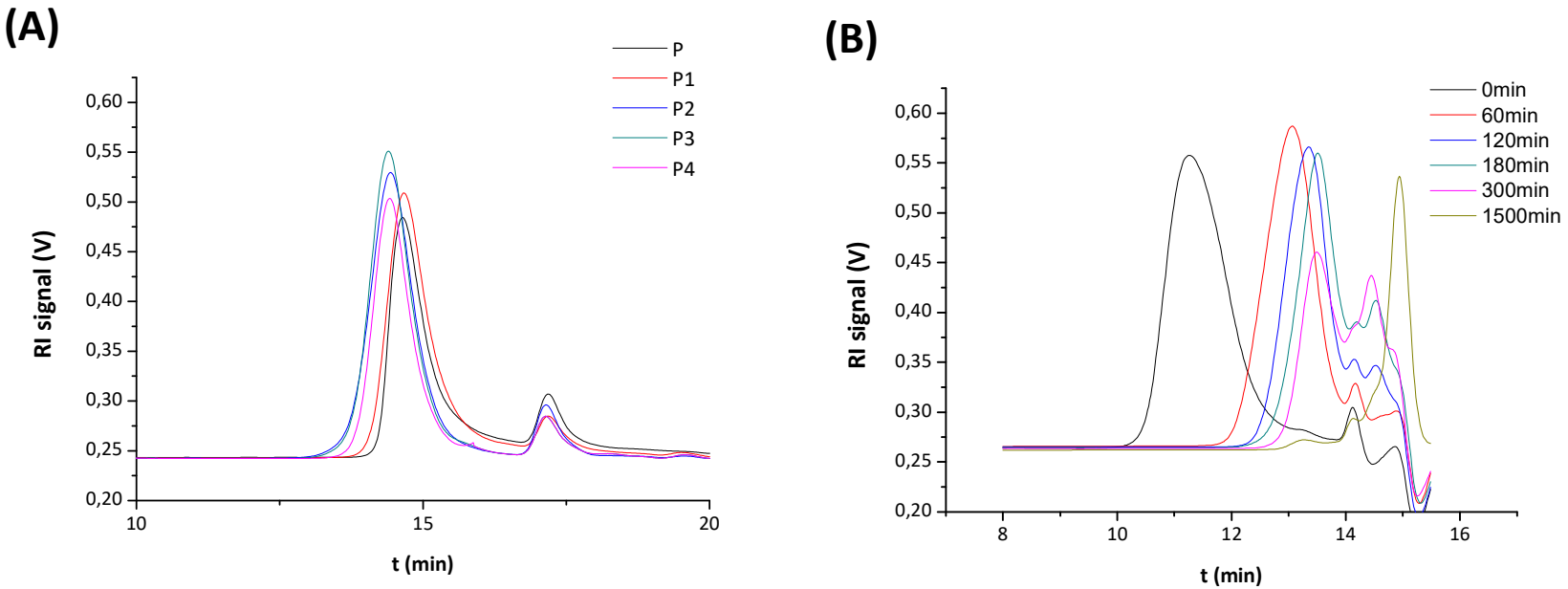


Figure S4. (A) GPC traces of P and P1-P4 derivatives (PLOn, n=52) and (B) enzymatic-degradation profile of PLO_{150}.

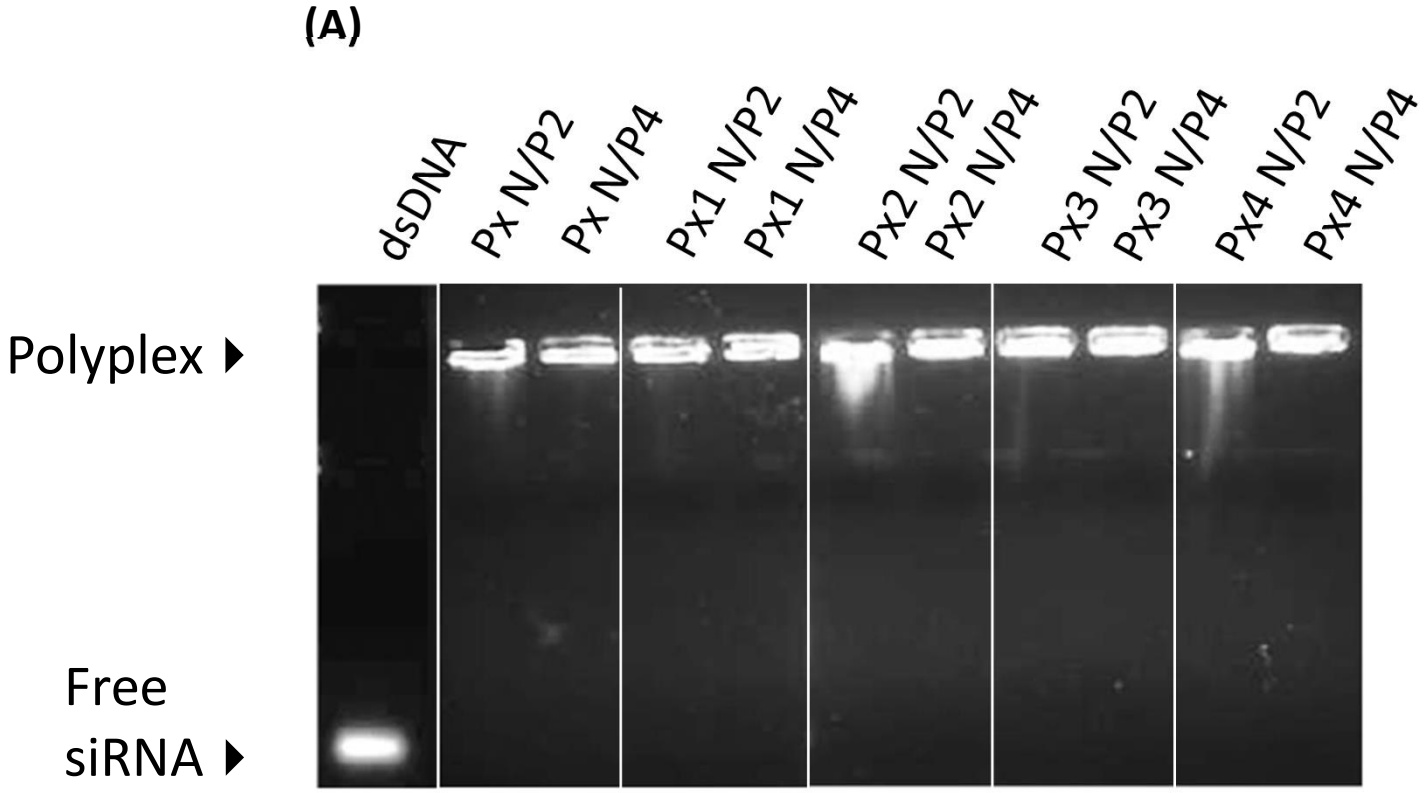


Figure S5. (A) Gel shift assay for PLO-based polyplexes at different N/P ratios (B) N/P ratio optimization study for **Px2** formation in HEPES 20mM pH=7.4.

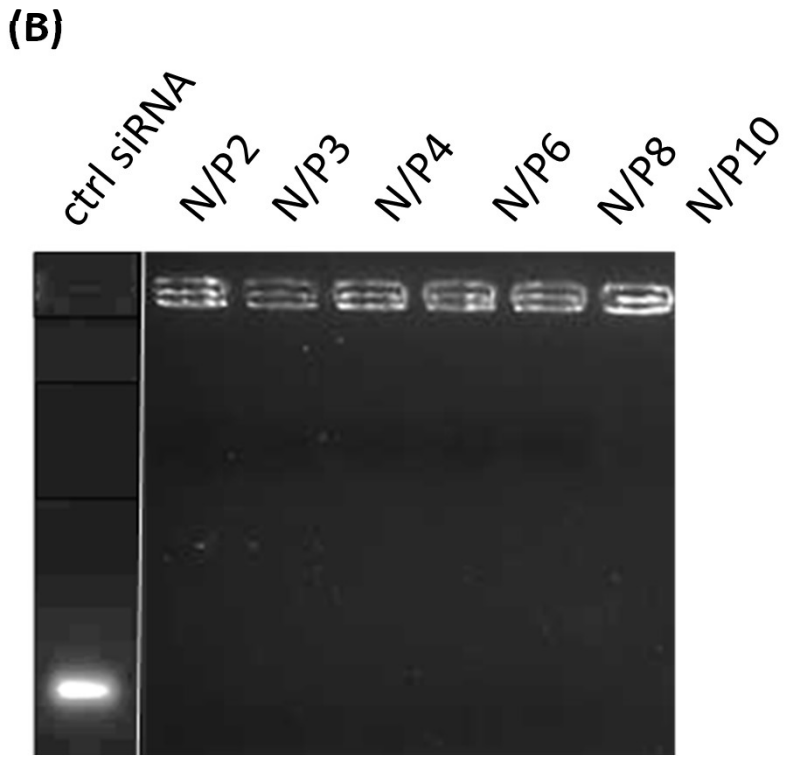


Figure S5. (A) Gel shift assay for PLO-based polyplexes at different N/P ratios (B) N/P ratio optimization study for **Px2** formation in HEPES 20mM pH=7.4.

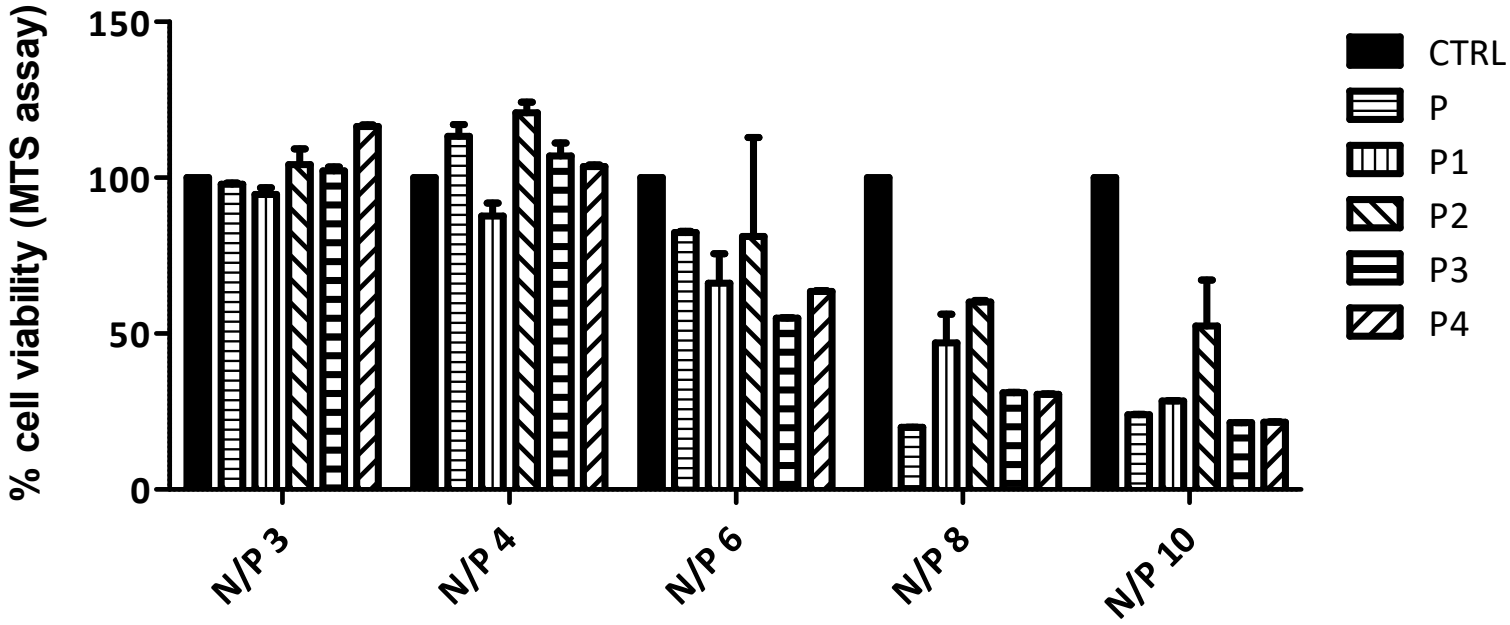


Figure S6. Evaluation of cell viability of the parent polymers without siRNA at different concentrations. MTS assay against B16F10 cell line after 24h incubation.

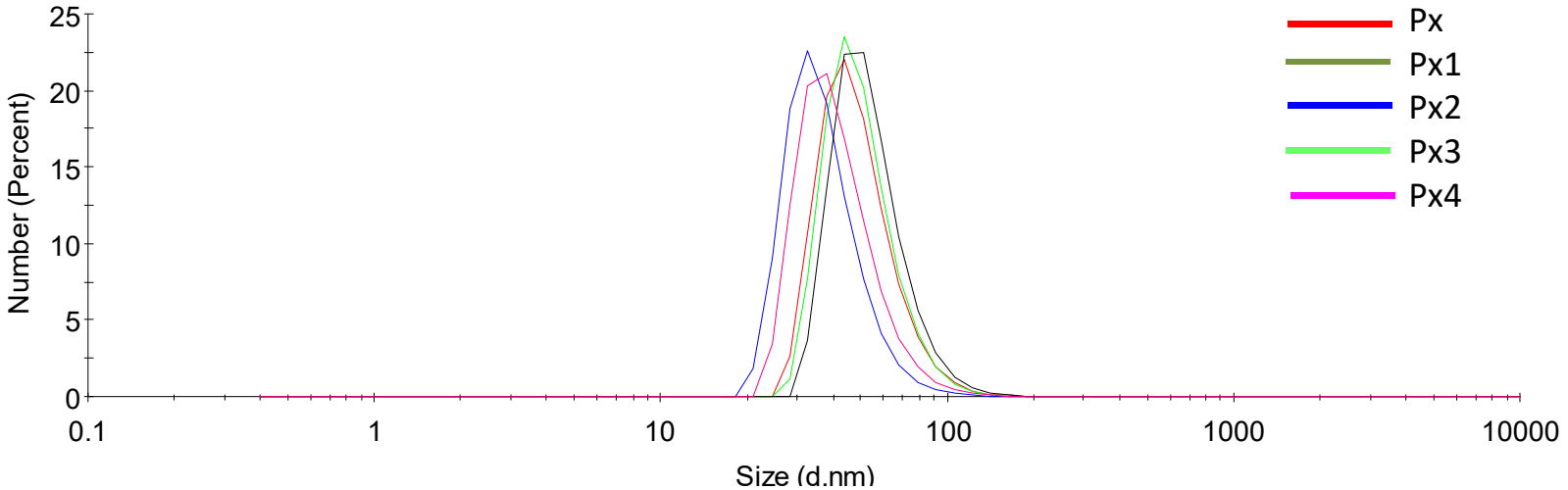


Figure S7. Representative histogram size distribution (number) of the polyplexes obtained by DLS measurement. d=diameter (nm).

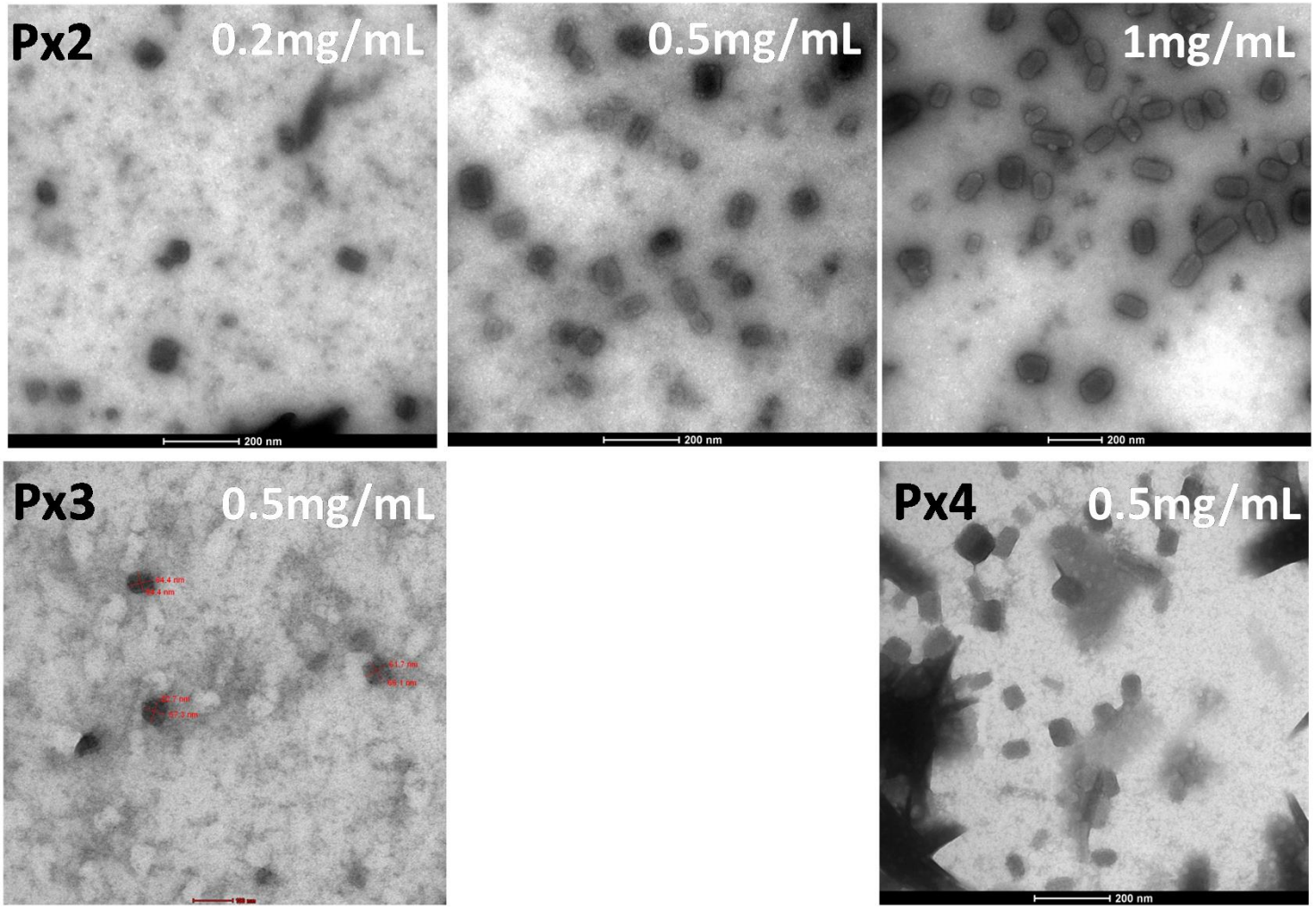
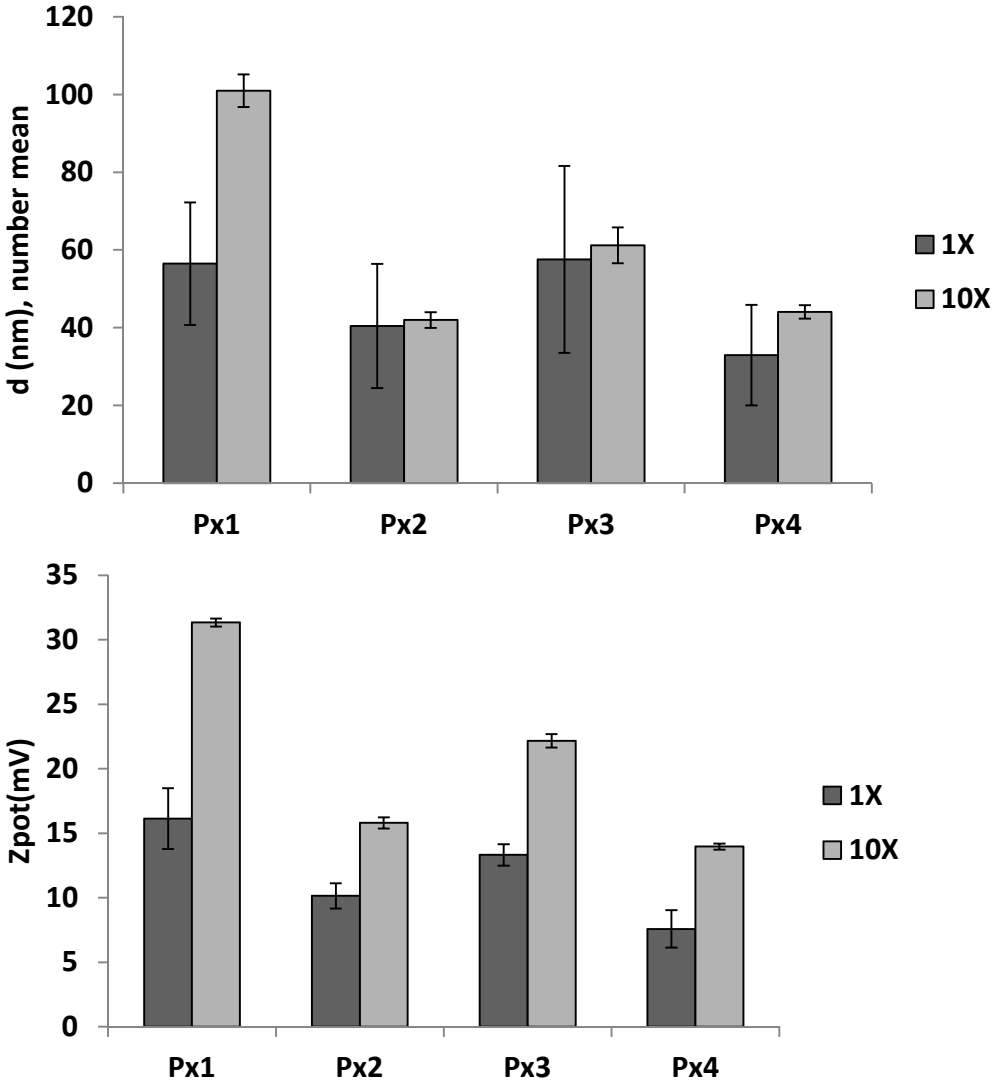


Figure S8. Study of size by TEM of the Px compounds.



	DLS	TEM
	[c] (mg/mL)	[c] (mg/mL)
Px1	0.012	0.117
Px2	0.012	0.121
Px3	0.012	0.123
Px4	0.014	0.141

Figure S9. DLS analysis at TEM concentration. [c] refers to polymer concentration in the sample.

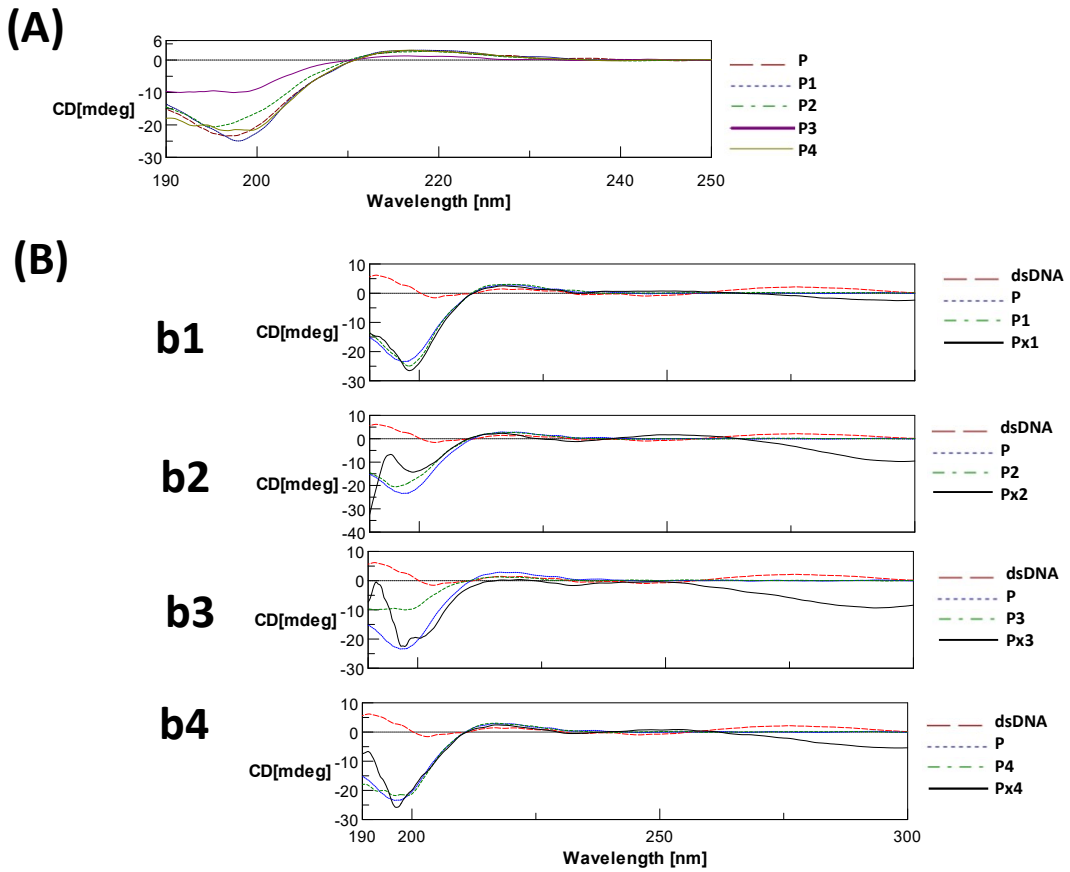
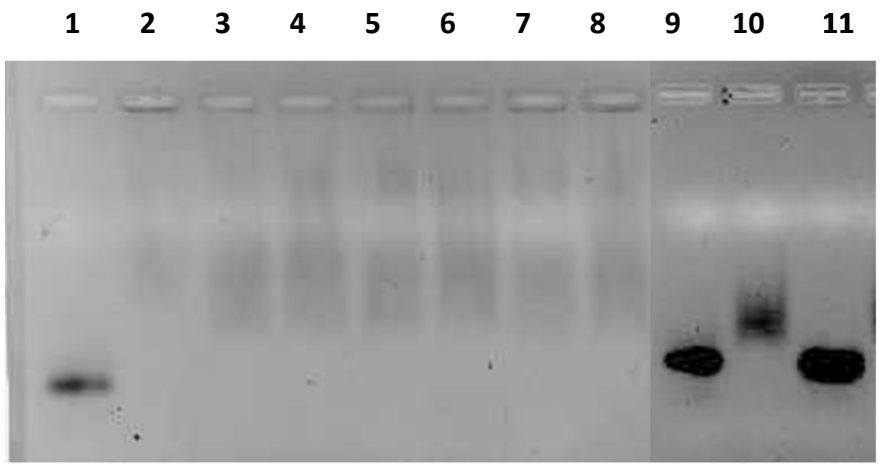


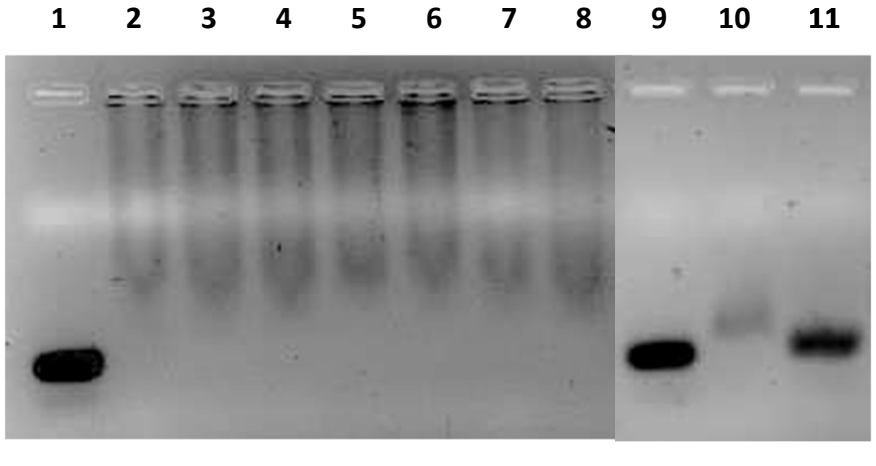
Figure S10. (A) CD spectra of PLO50 (P) and its derivatives. (B) Merged CD spectra of dsDNA, P with P1 and its polyplex Px1 (b2), P2 and its polyplex Px2 (b3), P3 and its polyplex Px3 (b3) and P4 and its polyplex Px4 (b4).

(A)



1. dsDNA control
2. Px2/ HEPES
3. Px2/cell media+FBS t=0
4. Px2/cell media+FBS t=0
5. Px2/cell media+FBS t=1h
6. Px2/cell media+FBS t=1h
7. Px2/cell media+FBS t=5h
8. Px2/cell media+FBS t=5h
9. dsDNA control
10. Px2/cell media+FBS t=24h
11. Px2/cell media+FBS t=24h + heparin

(B)



1. dsDNA control
2. Px2/ HEPES
3. Px2/cell media-FBS t=0
4. Px2/cell media-FBS t=0
5. Px2/cell media-FBS t=1h
6. Px2/cell media-FBS t=1h
7. Px2/cell media-FBS t=5h
8. Px2/cell media-FBS t=5h
9. dsDNA control
10. Px2/cell media-FBS t=24h
11. Px2/cell media-FBS t=24h + heparin

Figure S11. Stability evaluation of **Px2** in B16F10 cell media (A) with and (B) without FBS.

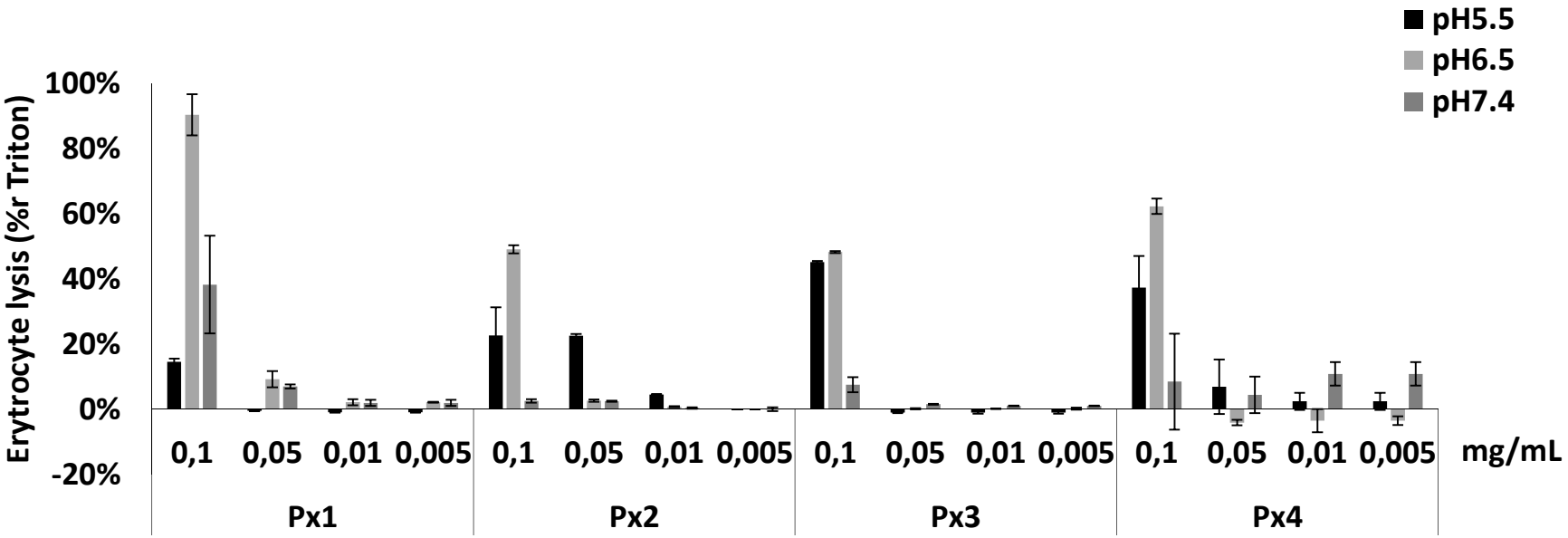
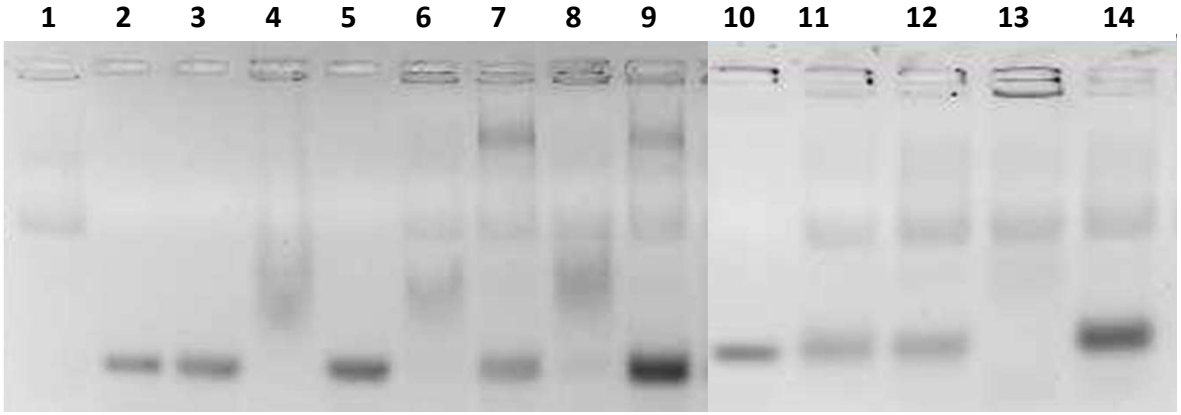


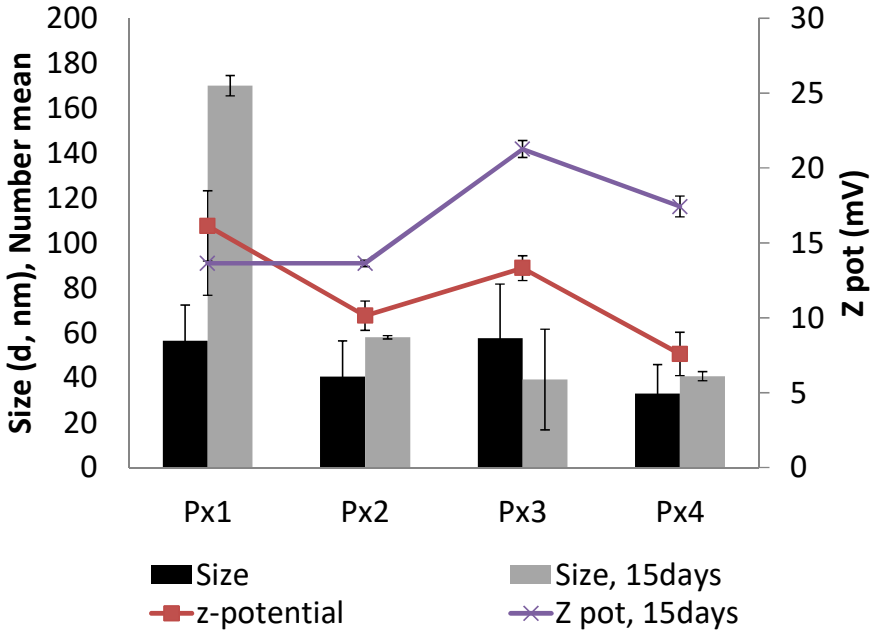
Figure S12. Erythrocyte lysis assays at different pH values: compounds were incubated with erythrocytes at different concentrations at 37°C at physiologic pH and switch-like transition into endo/lyso-somal pHs. Hemoglobin release was measured after 1 h. Data are plotted as mean ± SD, n≥4.



1. Plasma control
2. siRNA control
3. siRNA + heparin
4. Px2 / HEPES
5. Px2 / HEPES + heparin
6. Px2 / plasma t=0h
7. Px2 / plasma t=0h + heparin
8. Px2 / plasma t=2h
9. Px2 / plasma t=2h + heparin
10. siRNA control
11. siRNA control / plasma t=5h
12. siRNA control / plasma t=5h + heparin
13. Px2 / plasma t=5h
14. Px2 / plasma t=5h + heparin

Figure S13. Plasma stability assay of Px2.

(A)



(B)

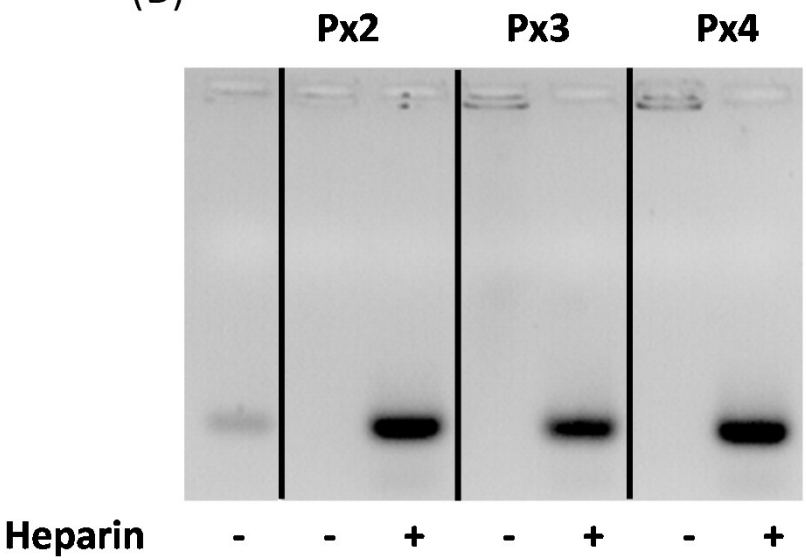


Figure S14. Evaluation of polyplex stability for storage at 4°C for 15 days. N/P 3 (A) size and Z-potential measurement by DLS. (B) Certification of polyplex stability by gel electrophoresis by heparin displacement assay.

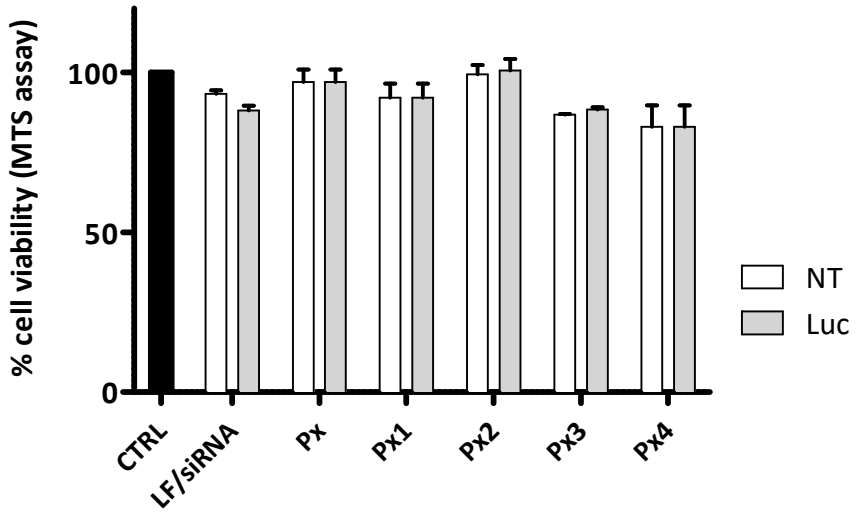


Figure S15. Cell viability assays of Lipofectamine (LF) and polyplexes (Px). (A) MTS assay against B16F10 cell line with Luc-targeted siRNA and non-target siRNA (NT) at N/P3 ratio. n> 3, mean± SEM. Indicated concentration corresponds to siRNA.

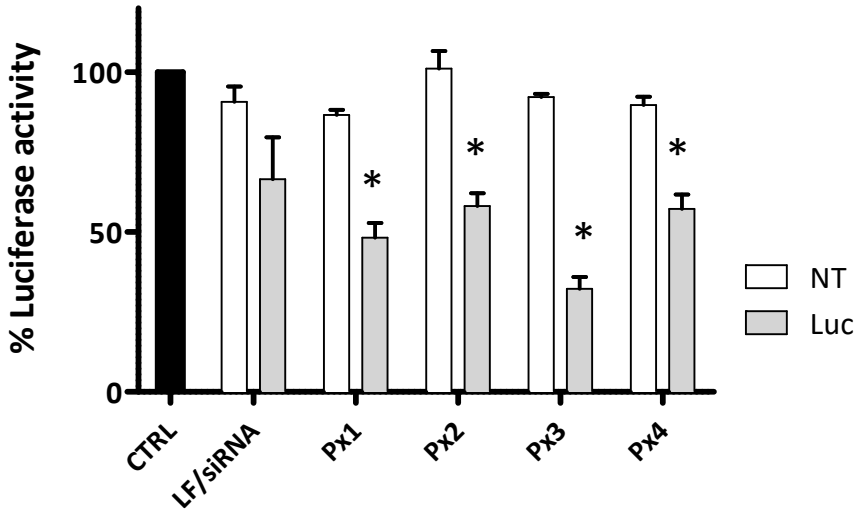


Figure S16. Luciferase activity in B16-F10-luc-G5 cells treated with LF and Px with non-targeted siRNA (NT) and Luciferase siRNA. Data are represented as mean values ± SD, with n = 5 experiments. Significant differences ($p < 0.05$) compared to controls are depicted with an asterisk.

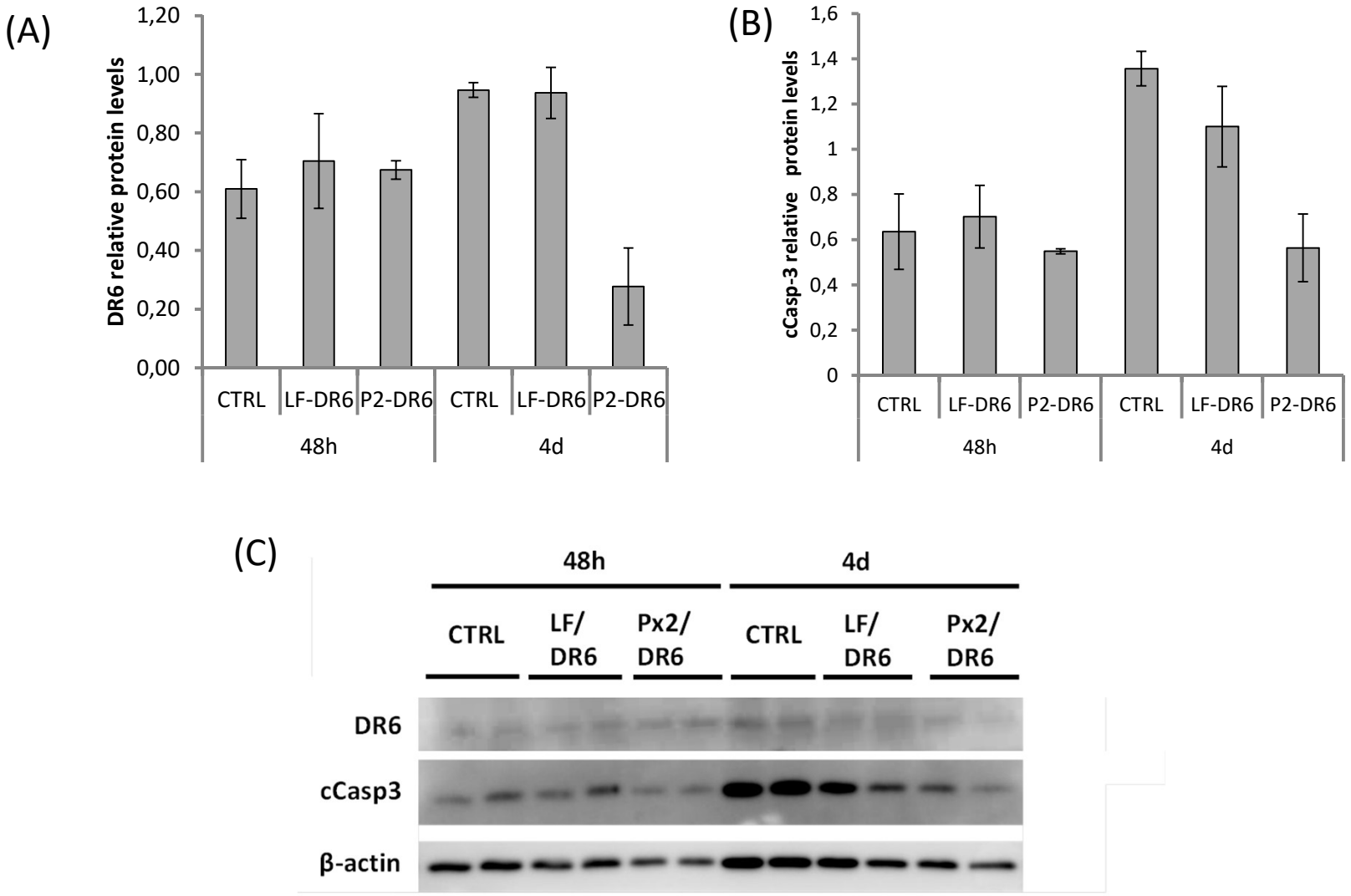


Figure S17. Densitometry-based quantification of (A) DR6 (68kDa) and (B) cleaved Casp3 (17-19KDa) western blots (C), showing siRNA-induced protein knockdown by Px2/DR6 at 48h and 4days after transfection (siRNA conc 100mM). Quantification is expressed relative to β-actin (42kDa).

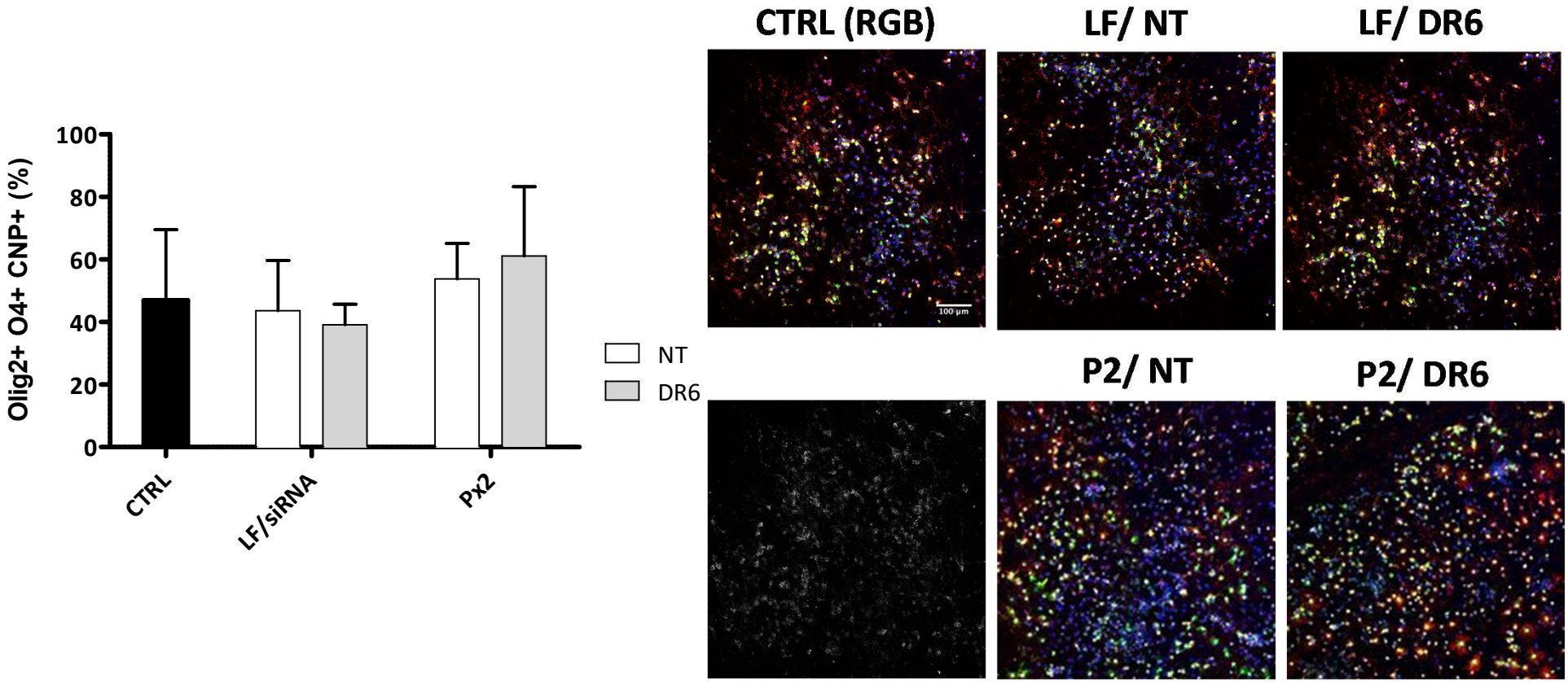


Figure S18. Immunocytochemistry of treated OPCs evaluating CNP expression. Fluorescent images indicate the expression of differentiation CNP (green) and O4 as marker for immature and mature oligodendrocytes (red) ;together with the pan oligodendrocyte lineage marker Olig2 (white). DAPI nuclear staining is shown in all fluorescent images (blue). Quantitative analysis shows the percentage of O4 double-positive cells within the Olig2 expressing cells at 4 days of incubation. Scale bars = 100 µm. Either LF or Px2 with NT or DR6 siRNA displayed non significant effect when compare to control cells. Values are displayed as mean ± SD. Experiments were done in triplicate. Statistics were performed by one-way ANOVA and the Tukey post hoc test.

(A)

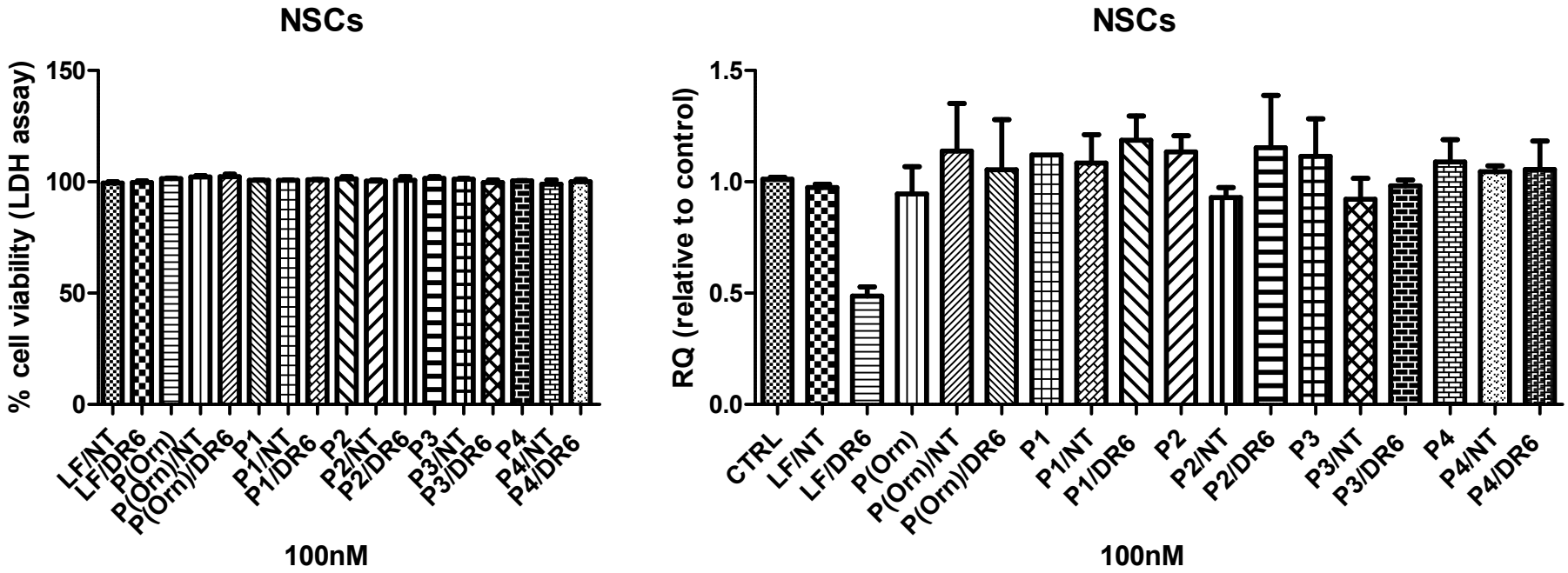
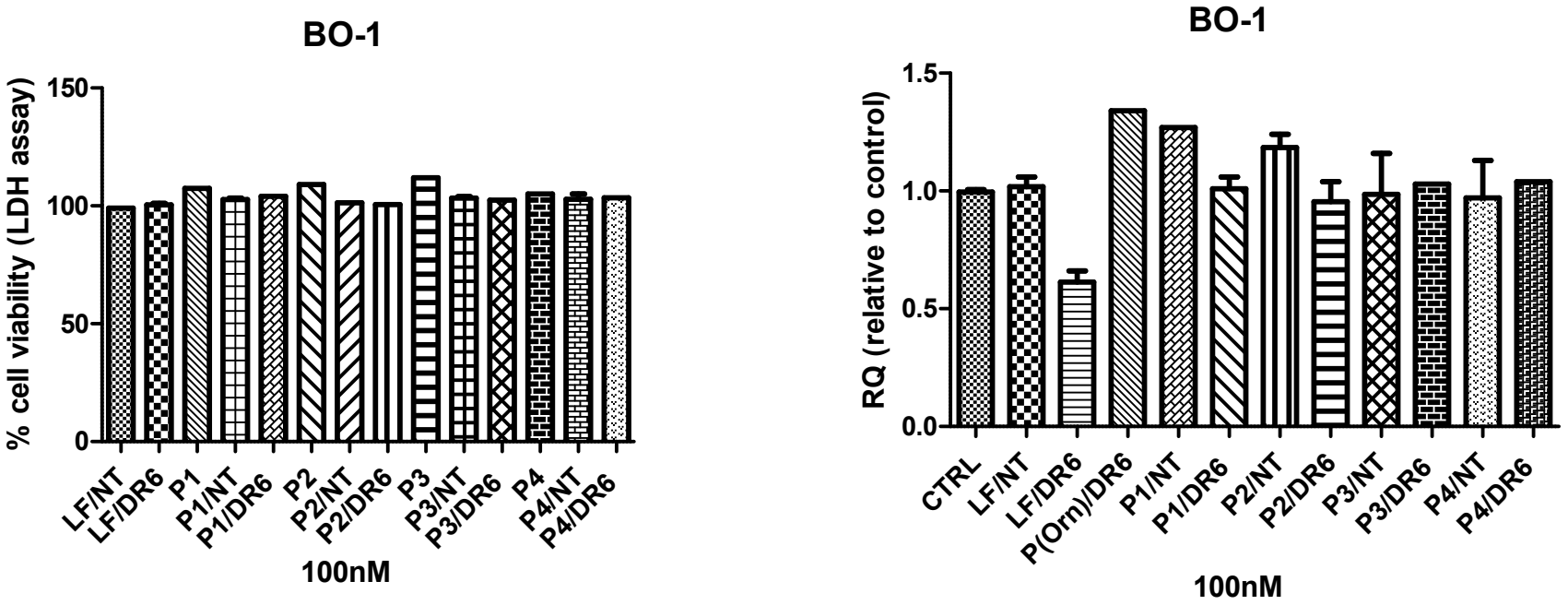


Figure S19. Cell toxicity evaluation by LDH test and qPCR assays showing the knockdown of *tnfrs21* (DR6 gene) target mRNA by polyplexes (both experiments 48h after transfection) against (A) neural stem cells (NSCs), (B) BO-1 and (C) CG-4 cell lines n> 3, mean± SEM. Indicated concentration corresponds to siRNA.

(B)



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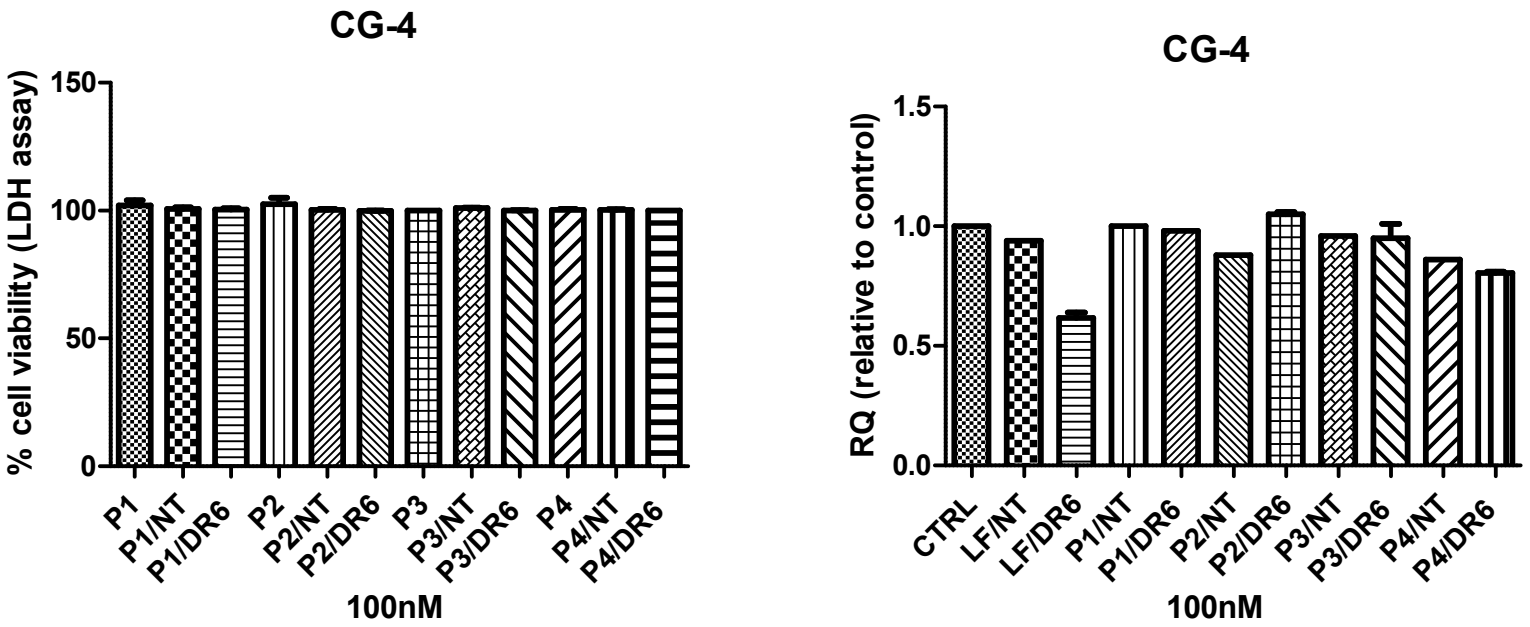
(C)



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