Supporting information

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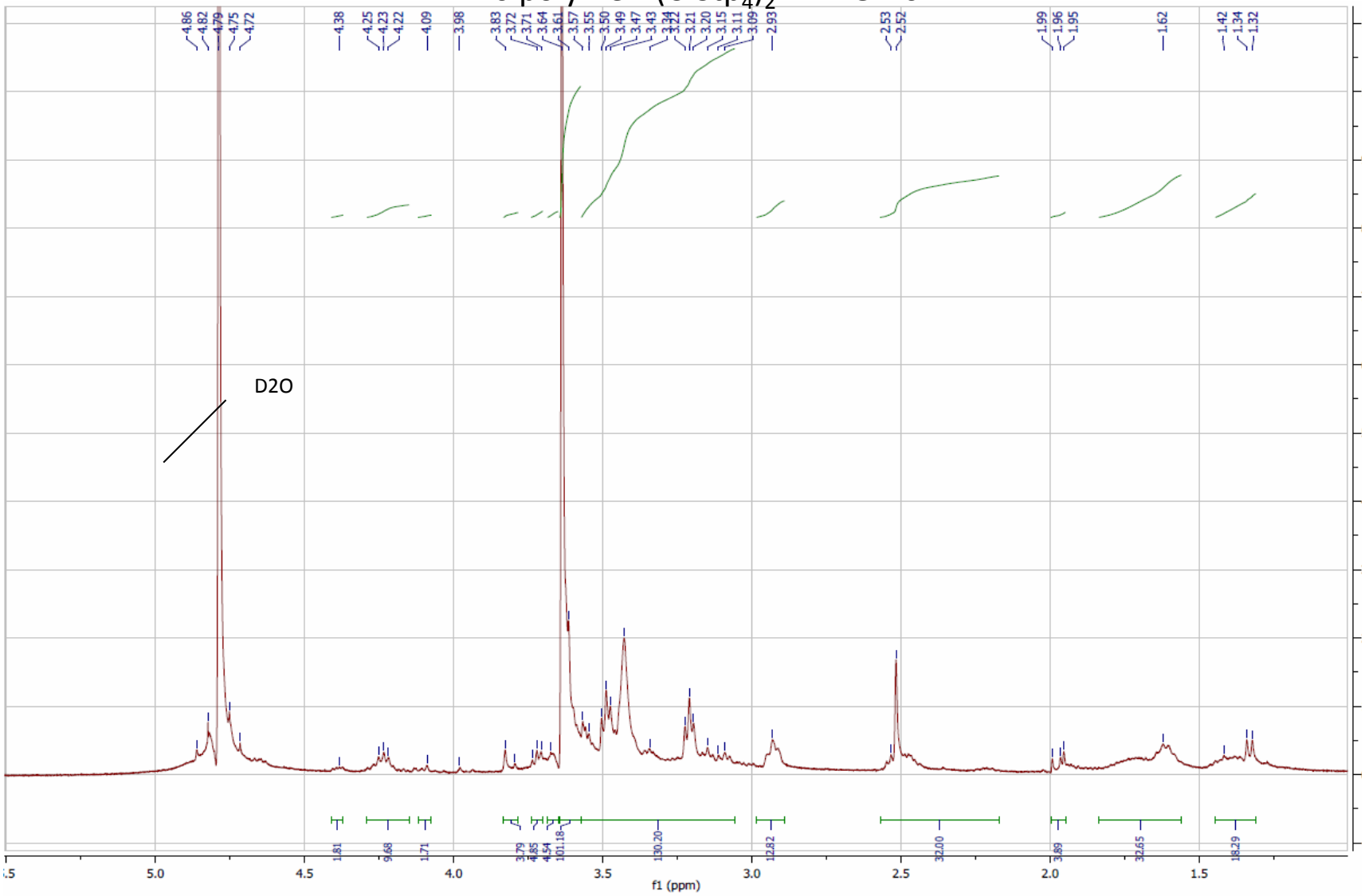
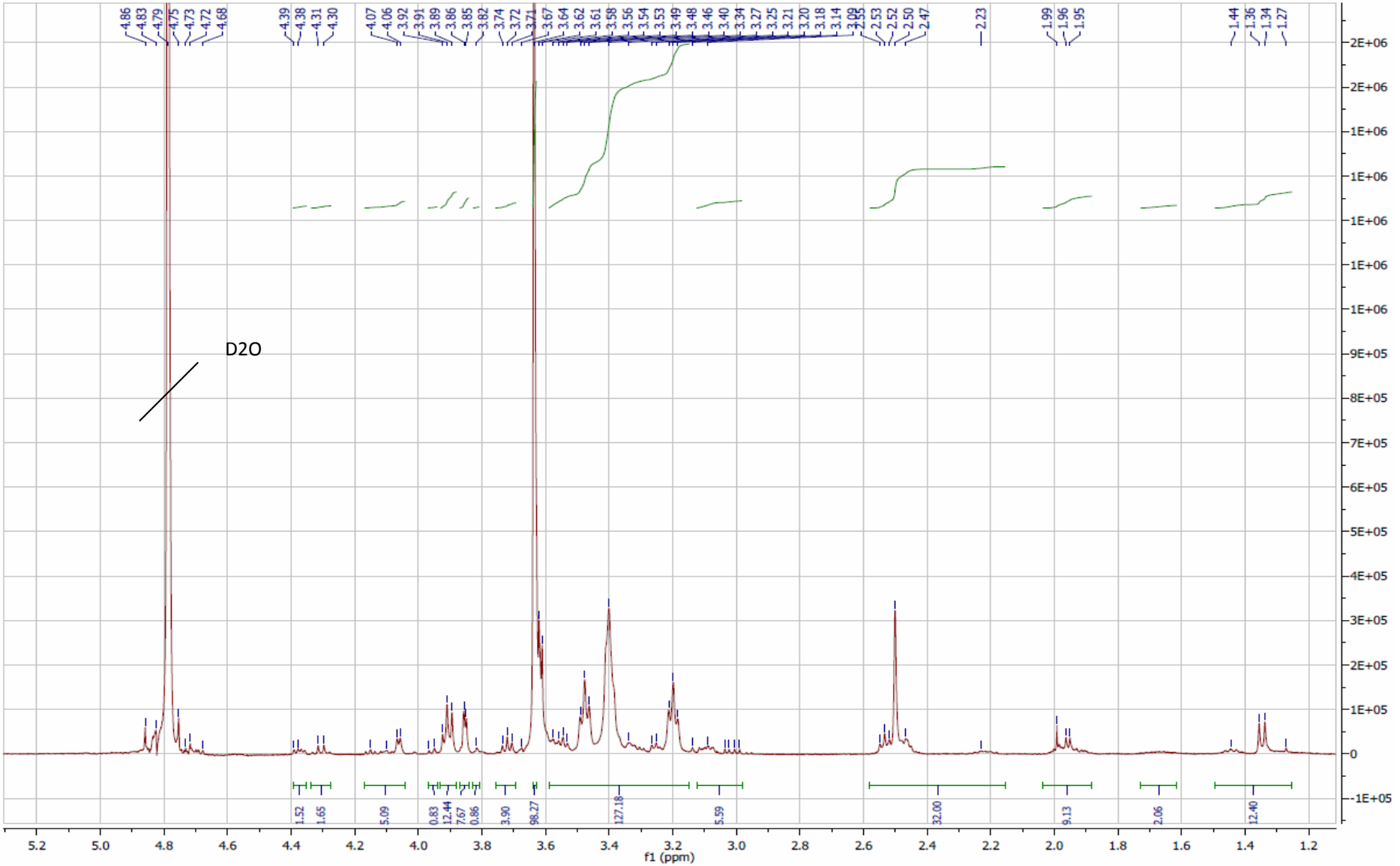
Figure S13. Erythrocyte lysis assay.

B6 polymer: $(C\text{-}Stp_4)_2$-K-PEG-B6



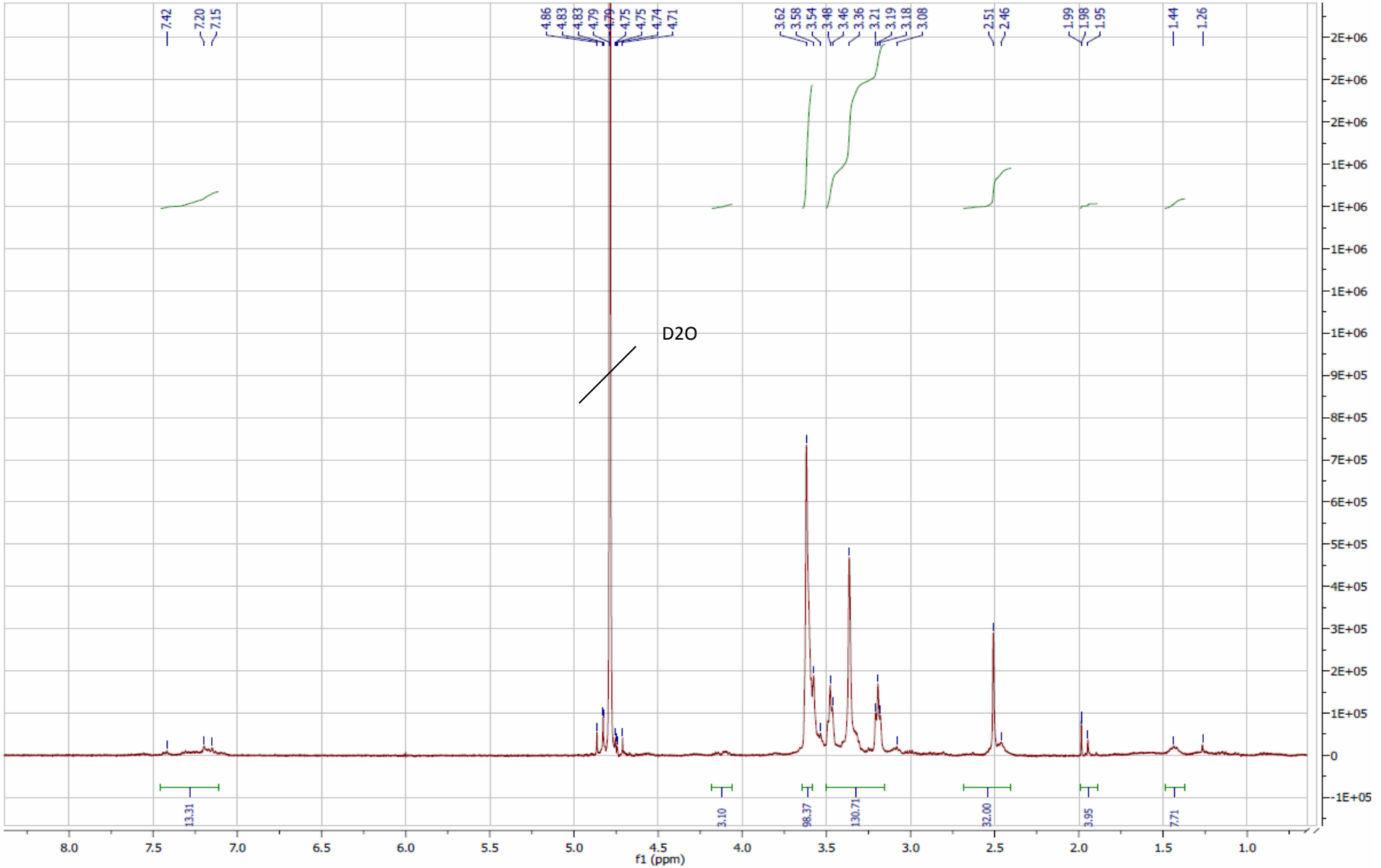
1H-NMR spectrum in D2O. δ (ppm) = 1.32-2.0 (m, 55 H, βH lysine, arginine and proline, γH lysine, proline and arginine, δH lysine), 2.0-2.53 (m, 32 H, -CO-CH2-CH2- CO-), 2.8-3.0 (m, 12 H, βH histidine, εH lysine, proline and arginine), 3.09-3.55 (m, 128 H, -CH2- Tp), 3.57-3.64 (m, 96 H, PEG), 3.71-4.0 (m, 10 H, αH glycine and cysteine, δH proline), 4.09-4.38 (m, 12 H, αH histidine, lysine, alanine, glycine, proline, arginine and cysteine).

B6mod polymer: $(C\text{-}Stp_4)_2$-K-PEG-B6mod



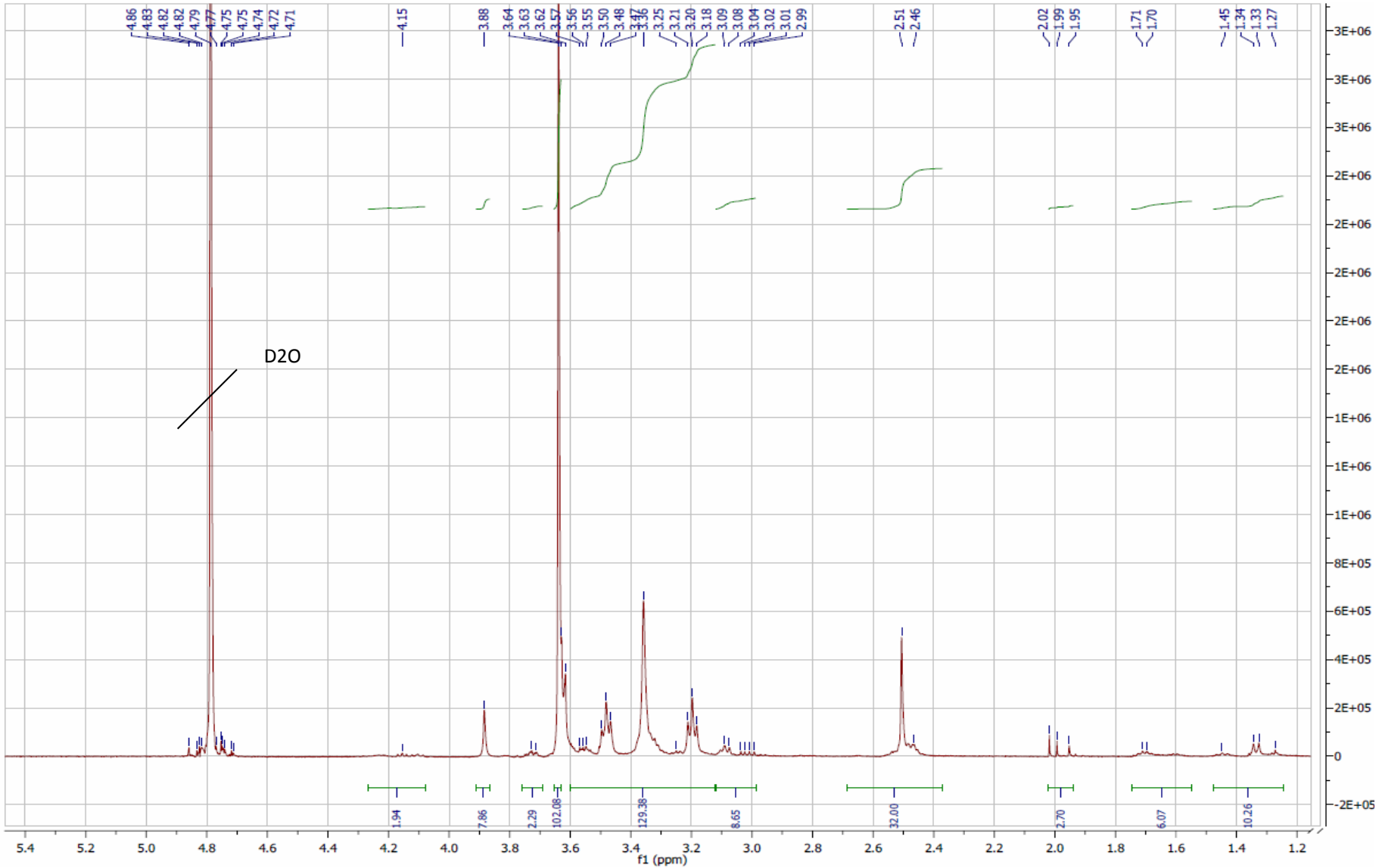
1H-NMR spectrum in D2O. δ (ppm) = 1.27-1.99 (m, 10 H, βγδH lysine, γH proline), 2.23-2.55 (m, 32 H, -CO-CH2-CH2- CO-), 3.09-3.46 (m, 4 H, γH lysine, εH proline), 3.48-3.64 (m, 128 H, -CH2- Tp), 3.67-3.71 (m, 96 H, PEG), 3.72-4.07 (m, 22 H, αH glycine and histidine, βH cysteine, γH histidine, δH proline), 4.30-4.85 (m, 5 H, αH histidine, lysine, alanine, proline, and cysteine).

c(RGDfK) polymer: $(C\text{-}Stp_4)_2$-K-PEG-A-c(RGDfK)

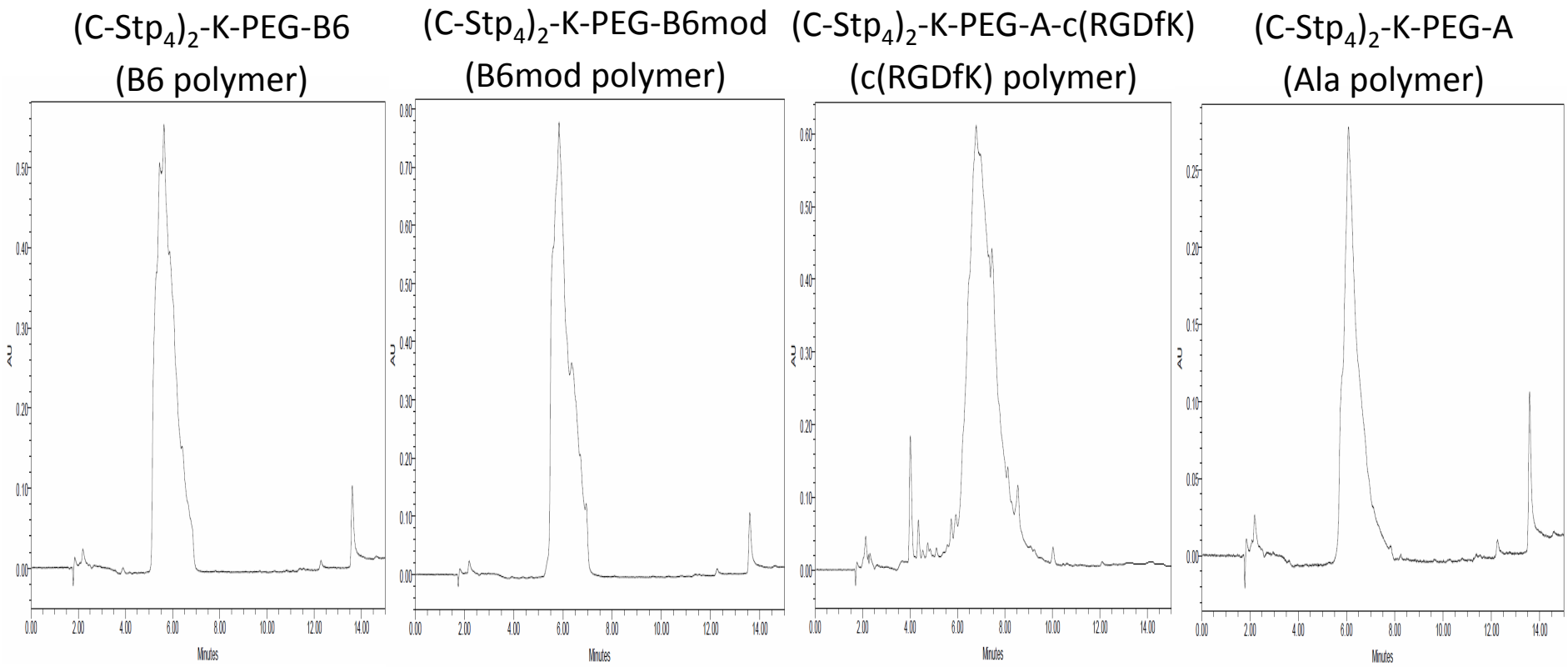


1H-NMR spectrum in D2O. δ (ppm) = 1.26-1.99 (m, 19 H, βH lysine and alanine, γδH lysine), 2.46-2.51 (m, 32 H, -CO-CH2-CH2- CO-), 3.08-3.48 (m, 128 H, -CH2- Tp), 3.36-3.62 (m, 96 H, PEG), 4.0-4.70 (m, 7 H, αH aminoacids), 7.15-7.42 (m, 11H, βγδH phenilalanine and triptophan).

Ala polymer: $(C\text{-}Stp_4)_2$-K-PEG-A



1H-NMR spectrum in D2O. δ (ppm) = 1.27-2.02 (m, 9 H, βγδH lysine, βH alanine), 2.46-2.51 (m, 32 H, -CO-CH2-CH2- CO-), 2.99-3.3.09 (m, 6 H, βH cysteine, δH lysine), 3.18-3.55 (m, 128 H, -CH2- Tp), 3.57-3.62 (m, 96 H, PEG), 3.63-4.15 (m, 4H, αH aminoacids).



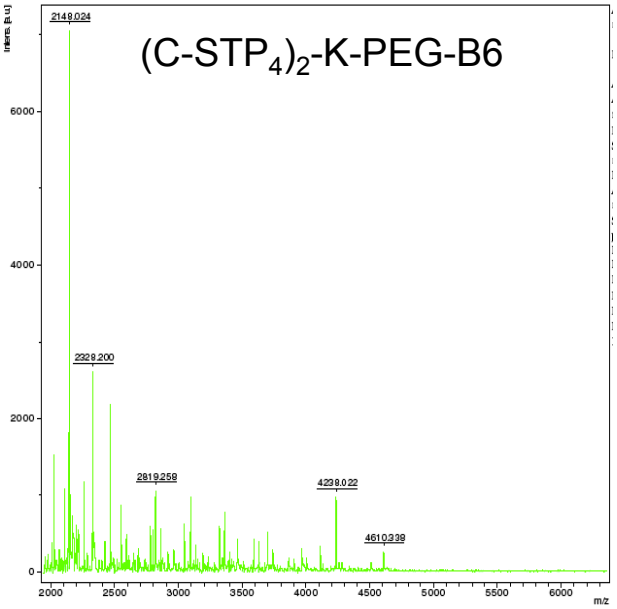
RP-HPLC profiles at λ=220 nm in a C_4 column (gradient 0–100% ACN in 15 min) of B6, B6mod, c(RGDfK) and Ala polymers.

Table 1. Mass spectrometry data of the polymers employed

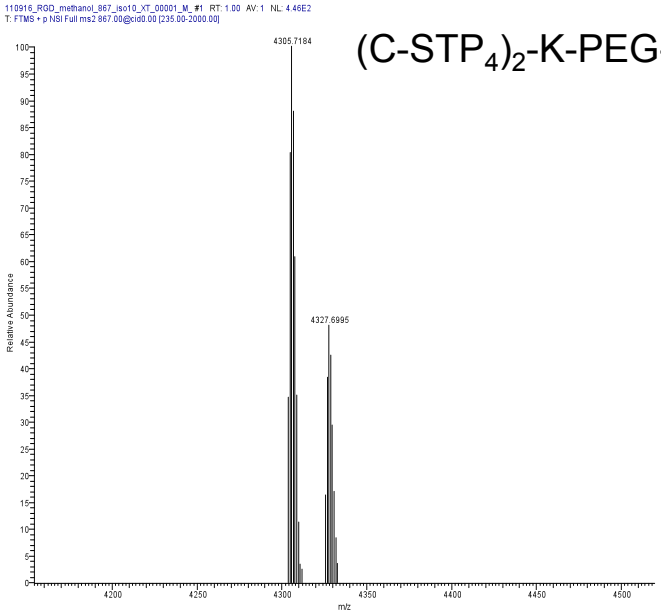
Polymer	Calculated mass [M+H$^+$]	Detected mass [M+H$^+$]
(C-STP$_4$)$_2$-K-PEG-B6	4611.8	4610.3
(C-STP$_4$)$_2$-K-PEG-B6mod	4298.3	4295.7
(C-Stp$_4$)$_2$-K-PEG-A-c(RGDfK)	4308.4	4305.7
(C-STP$_4$)$_2$-K-PEG-A	3722.7	3724.4
(C-STP$_4$)$_2$-K-A	2594,4	2592.7

MS spectra

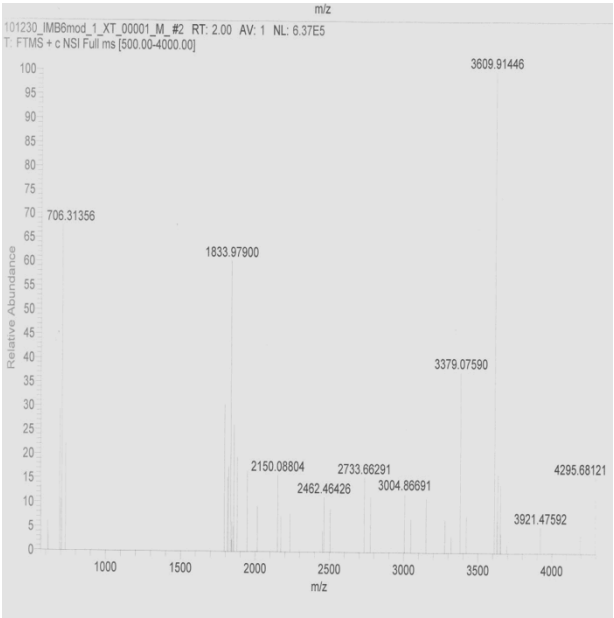
$(C\text{-}STP_4)_2$-K-PEG-B6



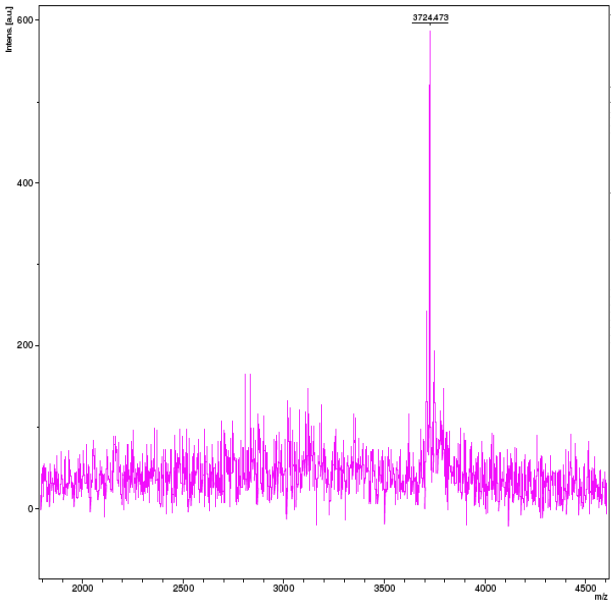
$(C\text{-}STP_4)_2$-K-PEG-A-c(RGDfK)



$(C\text{-}STP_4)_2$-K-PEG-B6mod



$(C\text{-}STP_4)_2$-K-PEG-A



$(C\text{-}STP_4)_2$-K-A

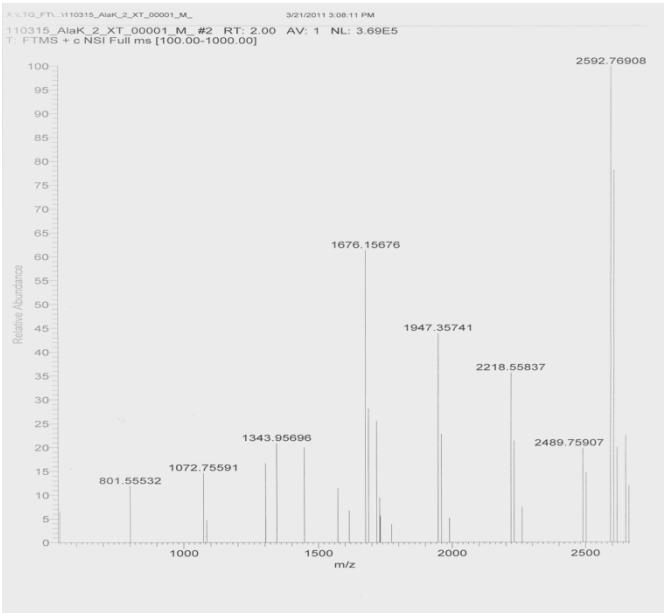
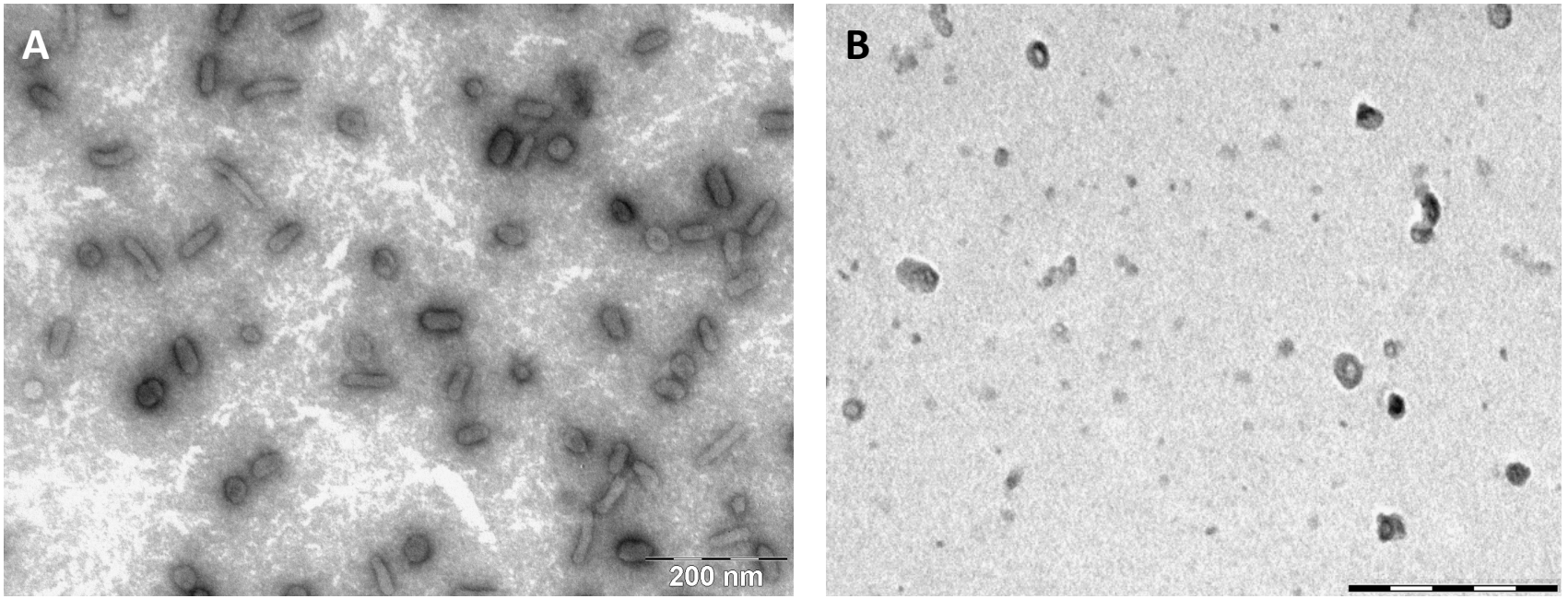
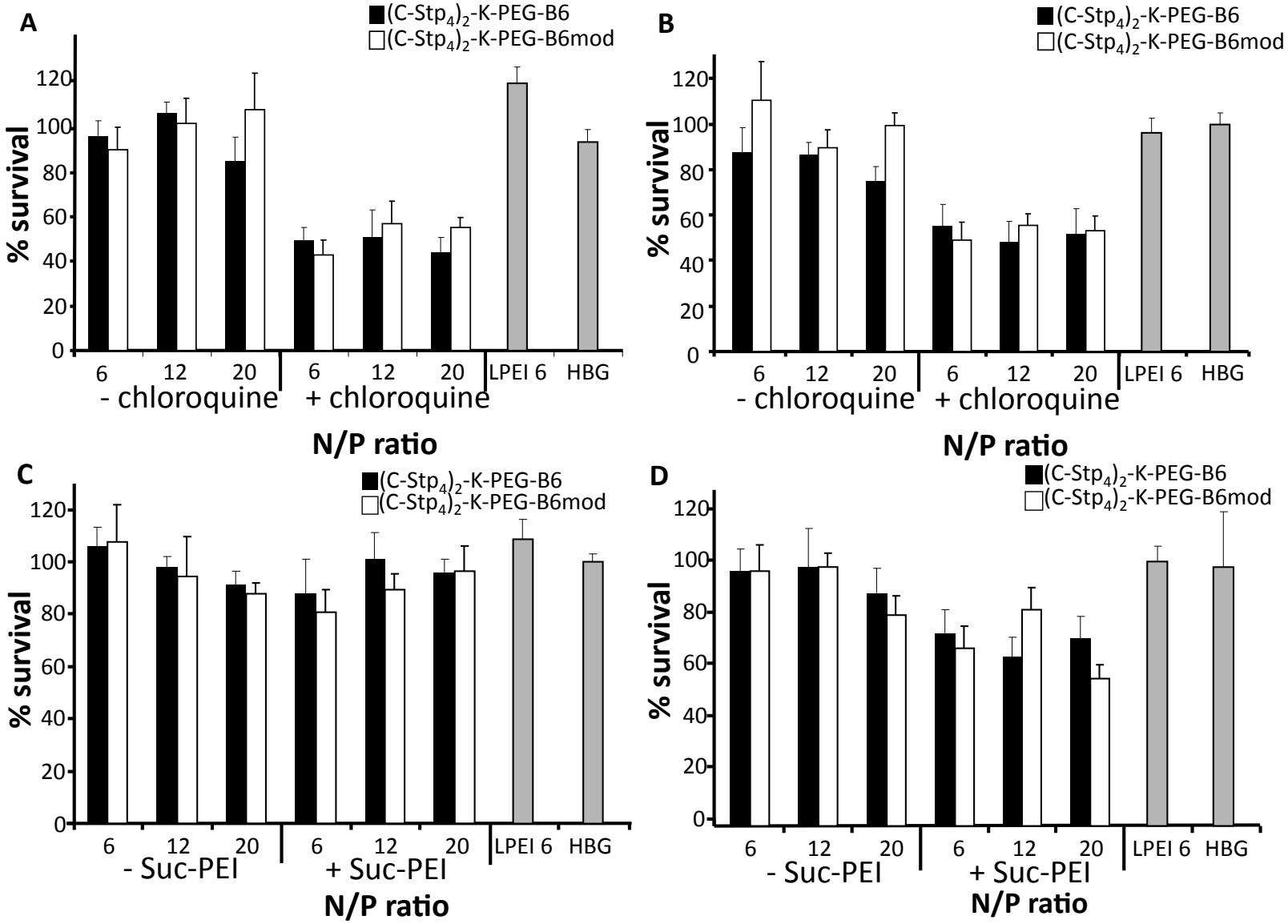


Table 2. Size and zeta potential data from dynamic light scattering (DLS).

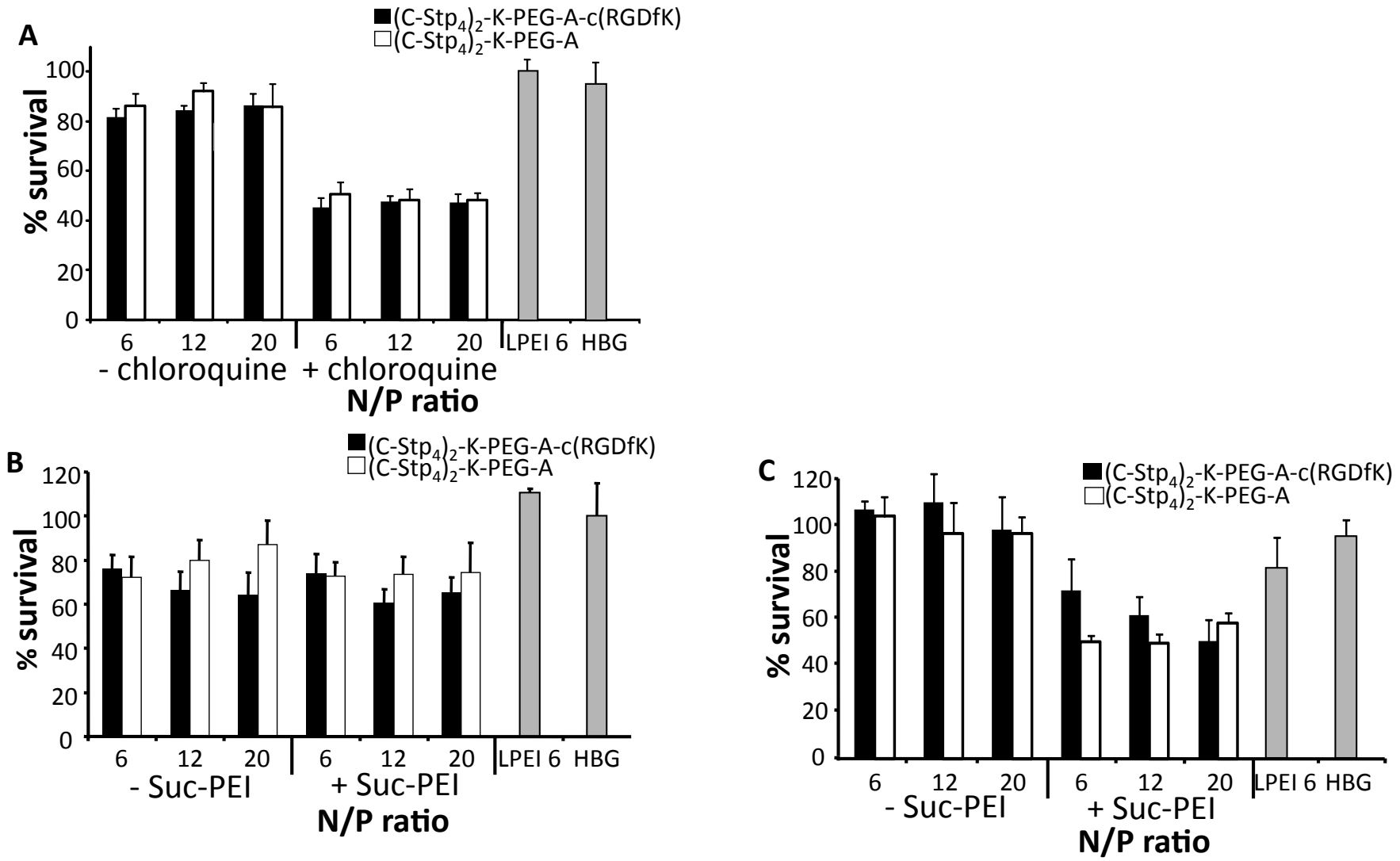
$(C\text{-}STP_4)_2$-K-PEG-B6	**Size**	**PDI**	**Zeta potential**
N/P 6	167.1 ± 3.0	0.34 ± 0.047	12.1 ± 0.9
N/P 12	183.4 ± 39.1	0.43 ± 0.14	15.0 ± 2.5
N/P 20	200.5 ± 46.4	0.26 ± 0.029	13.6 ± 2.0
$(C\text{-}STP_4)_2$-K-PEG-B6mod			
N/P 6	241.4 ± 69.9	0.49 ± 0.21	1.1 ± 1.9
N/P 12	174.1 ± 23.6	0.35 ± 0.088	-0.1 ± 0.3
N/P 20	156.3 ± 16.1	0.24 ± 0.022	0.02 ± 0.2
$(C\text{-}STP_4)_2$-K-PEG-A-c(RGDfK)			
N/P 6	153.2 ± 30.3	0.45 ± 0.15	0.1 ± 0.1
N/P 12	186.1 ± 6.2	0.26 ± 0.030	0.09 ± 0.1
N/P 20	137.3 ± 1.5	0.32 ± 0.0026	0.3 ± 0.2
$(C\text{-}STP_4)_2$-K-PEG-A			
N/P 6	283.5 ± 8.7	0.44 ± 0.019	0.1 ± 0.08
N/P 12	94.6 ± 1.2	0.32 ± 0.015	-0.07 ± 0.1
N/P 20	128.7 ± 11.5	0.31 ± 0.10	0.08 ± 0.06



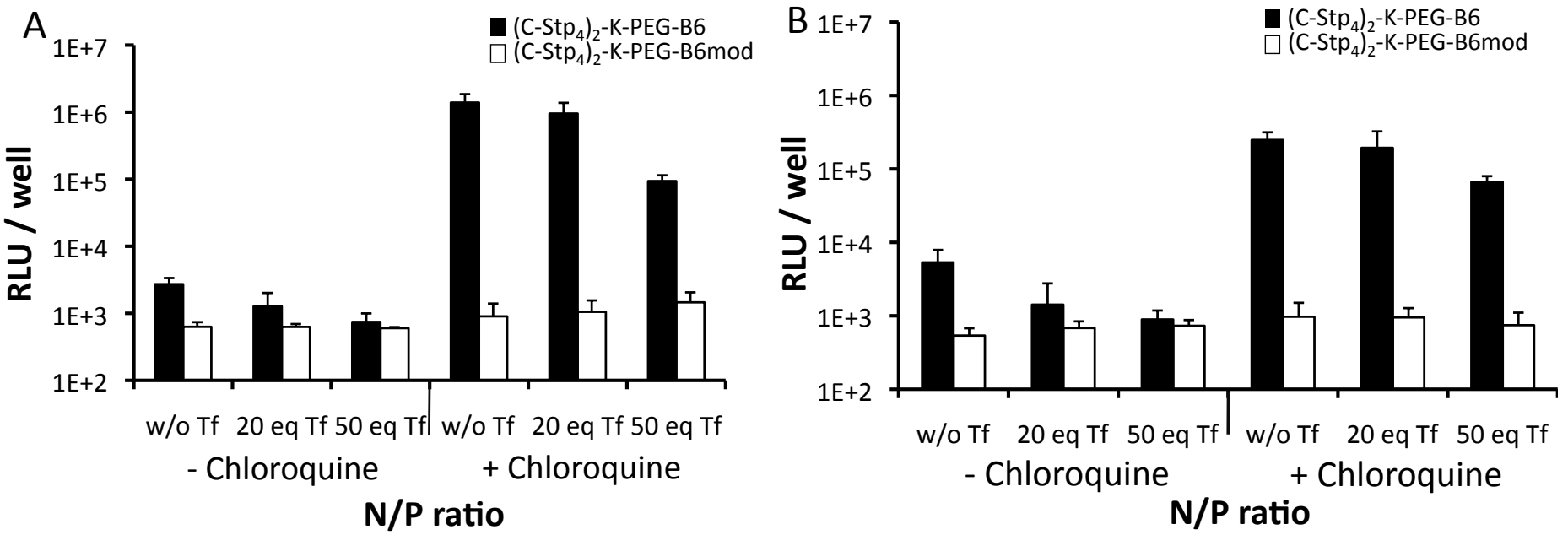
TEM images of Ala-K ((C-Stp$_4$)$_2$-K-A) polyplex (N/P ratio of 20:1) placed on a carbon film-coated copper grid and stained with 2% uranyl acetate (A) or the replica obtained after freeze-fixation and freeze-drying of a 50 mm aqueous solution (B). Scale bar = 200 nm.



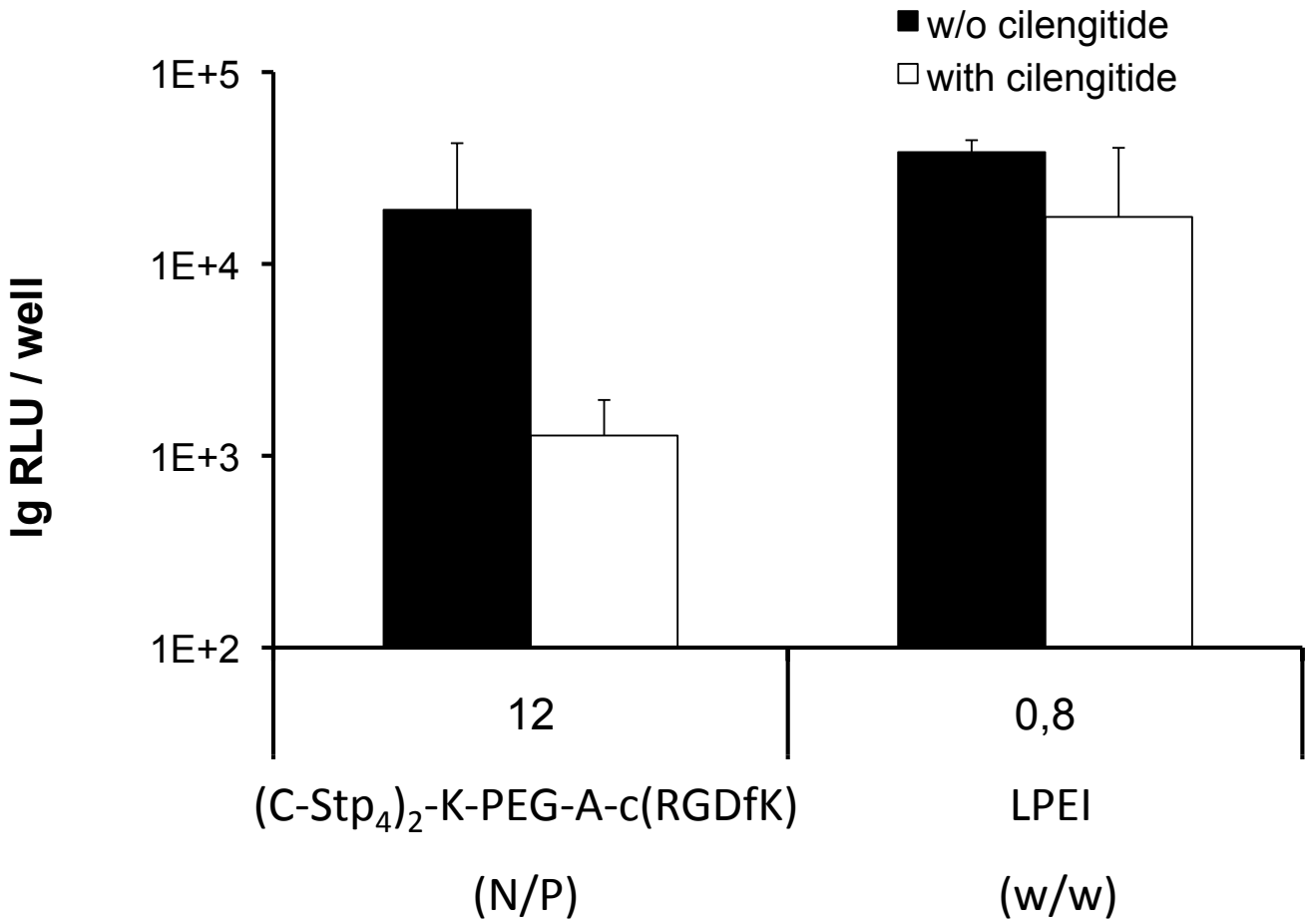
N2A (A, C) and DU-145 (B, D) cell viability assays after 24h incubation with pDNA-B6 and B6mod polyplexes at 6, 12 and 20 N/P ratios. After 1-h incubation at 37 ºC and 5% CO_2, medium was removed and replaced by fresh one. Where indicated, 100 µM chloroquine was added in (A) and (B) or 0.8 µg suc-PEI/well was added in (C) and (D) as described in Materials and Methods. Linear polyethyleneimine (LPEI) and HBG were used as controls.



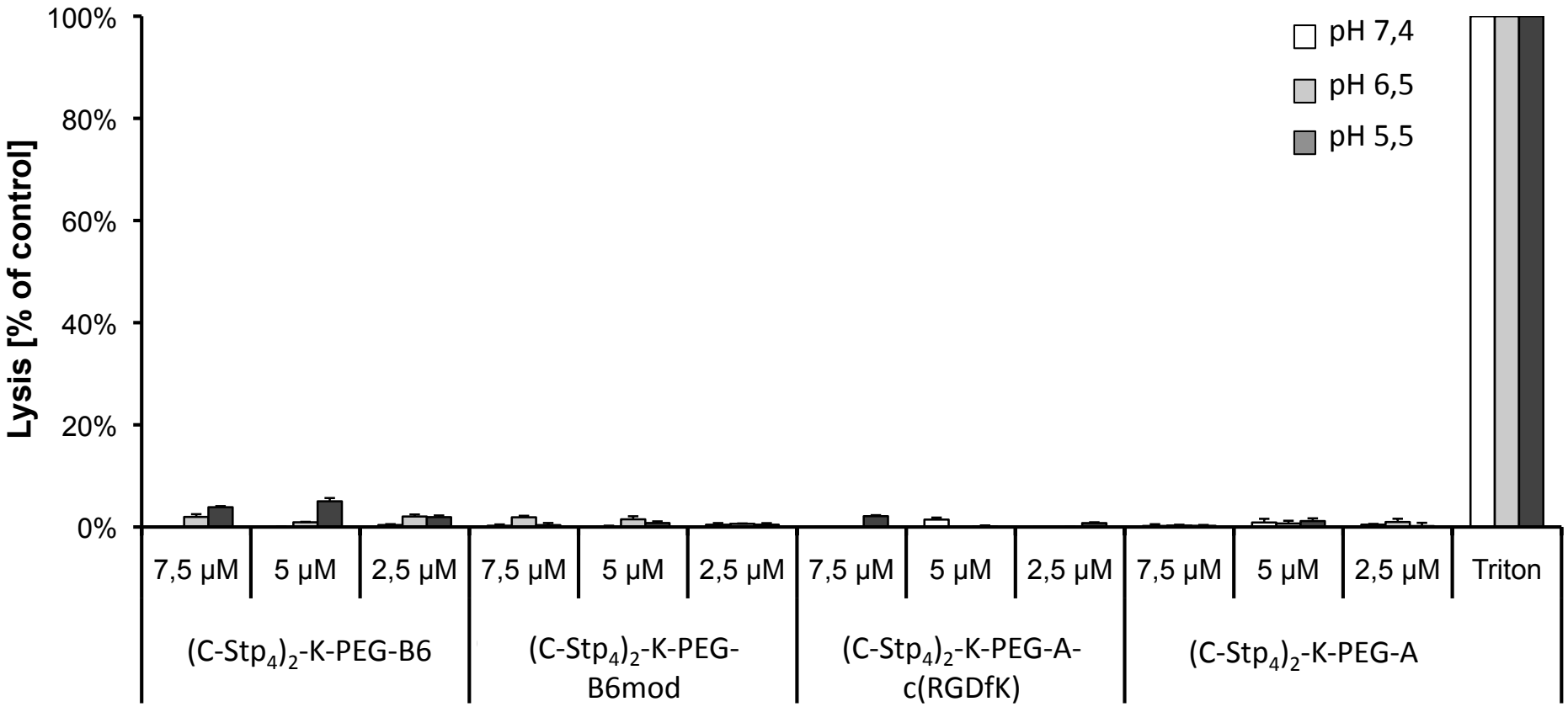
DU-145 (A, C) or N2A (B) cell viability assays with c(RGDfK) or Ala polyplexes. After 1-h incubation at 37ºC and 5% CO2, medium was removed and replaced by fresh one. Where indicated, 100 µM chloroquine was added in (A) or 0.8 µg suc-PEI/well was added in (B, C) as described in Materials and Methods. LPEI and HBG were used as controls.



Competitive assays between the transferrin (Tf) peptide with B6 polyplex in N2A (A) and DU-145 (B) cells. Cells were treated where indicated with the free molecule at the indicated concentration for 10 min at 4 ºC to allow binding on the transferrin receptor (TfR). Polyplexes at a N/P ratio of 6 were then added to the cells. After 1h of polyplex incubation at 37 ºC under 5% CO2, medium was removed and replaced by a fresh one. Where indicated, 100 µM chloroquine was added



Competitive assays between cilengitide and RGD polymer or LPEI in DU-145 cells. Cells were treated where indicated with the free molecule at the indicated concentration for 10 min at 4 ºC to allow binding on αvβ3. Polyplexes at a N/P ratio of 12 or w/w ratio of 0,8 were then added to the cells. After 1h of polyplex incubation at 37 ºC under 5% CO2, medium was removed and replaced by a fresh one. In all the cases, 100 µM chloroquine was added.



Erythrocyte lysis assay. Erythrocytes were incubated with 2.5 µM polymer solutions at 37°C and indicated pH. Hemoglobin release was measured after 1 h.