Supporting Information for Polymer Chemistry manuscript

Coupling of RAFT Polymerization and Chemoselective Post-modifications of Elastin-like Polypeptides for the Synthesis of Gene Delivery Hybrid Vectors

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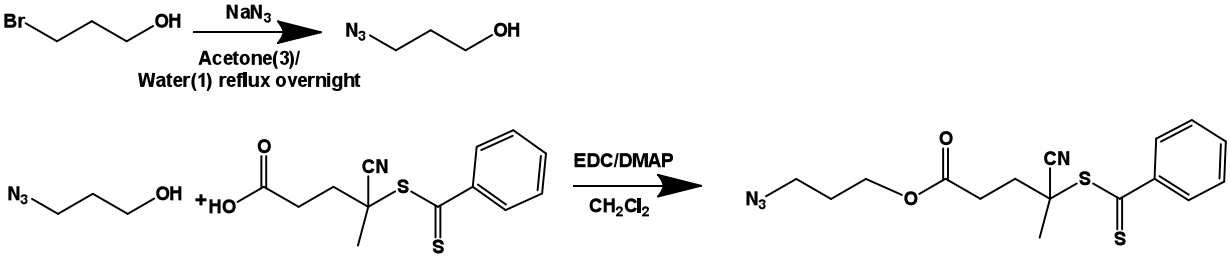
Synthesis of azido-RAFT agent



Scheme S1.- Synthesis of the azido-RAFT agent [1].

3-bromo-1-propanol (15.0 g, 1 eq, 108 mmol) and sodium azide (12.3 g, 1.75 eq, 189 mmol) were dissolved in a mixture of acetone (180 mL) and water (60 mL) and the resulting solution was refluxed overnight. Acetone was then removed under reduced pressure, 100 mL of water were added and the mixture was extracted with diethyl ether (3 x 100 mL). The organic layers collected were dried over $MgSO_4$ and, after removal of the solvent under reduced pressure; 3-azido-1-propanol was isolated as colorless oil (8.32 g, 76%).

A solution of (4-cyanopentanoic acid)-4-dithiobenzoate (3.26 g, 1 eq, 11.6 mmol) and 3-azido-1-propanol (5.90 g, 5 eq, 17 mmol) in dichloromethane (200 mL) was cooled to 0 ºC and *N*-(3-dimethylaminopropyl)-N'ethylcarbodiimide hydrochloride (6.71 g, 3 eq, 35.0 mmol) and 4-dimethylaminopyridine (71.2 mg, 0.05 eq, 0.58 mmol) were subsequently added. The orange

solution was stirred at 0 ºC for 2h, then at ambient temperature overnight. The reaction mixture was washed with water (2x200mL) and dried over $MgSO_4$. The volatiles were removed under reduced pressure and the crude product purified by flash chromatography (SiO_2, 1) 100% CH_2Cl_2; 2) Hexane/Ethyl acetate 4/1) (2.81 g, 67%). Figure S1 represents step by step the procedure to obtain the azido-RAFT agent followed by ^{1}H NMR.

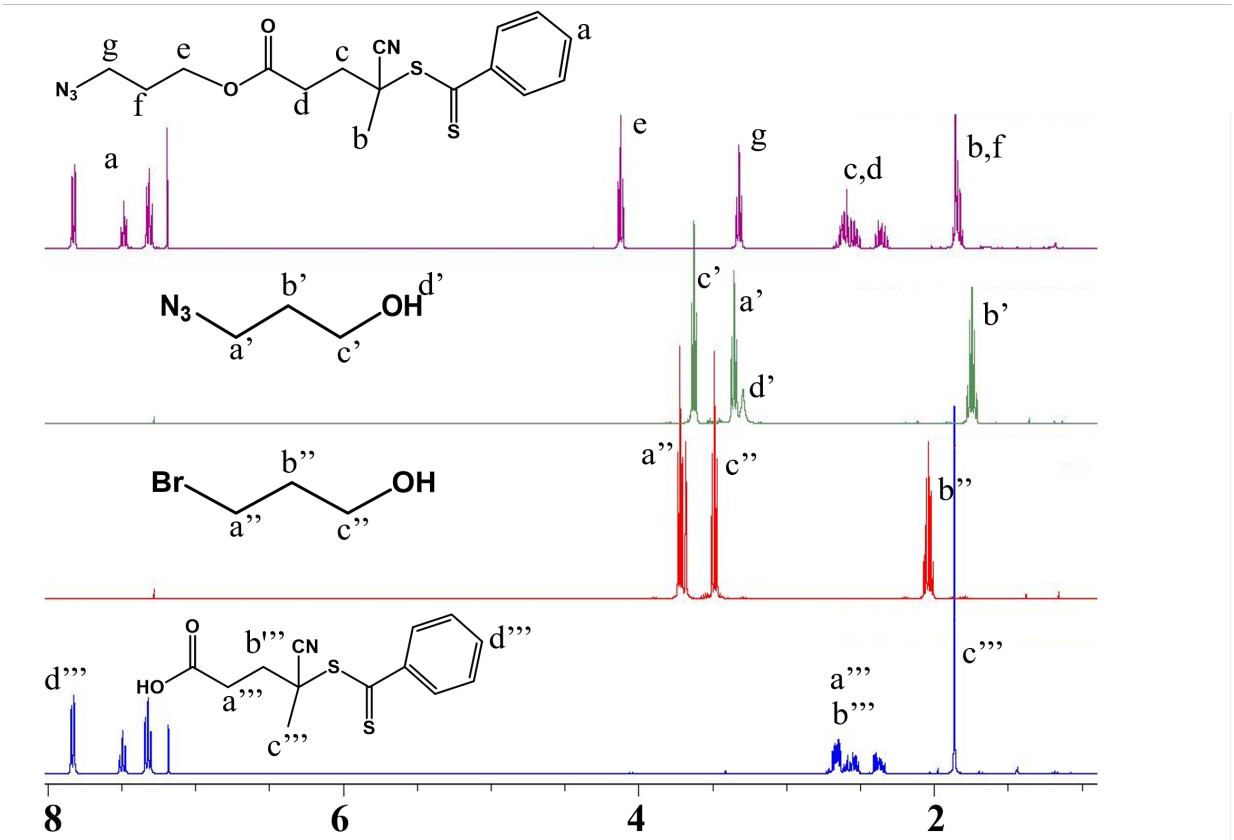


Figure S1.- ^{1}H-NMR spectrum of azido-RAFT agent and its precursors in $CDCl_3$ 25 °C, 400.2 MHz).

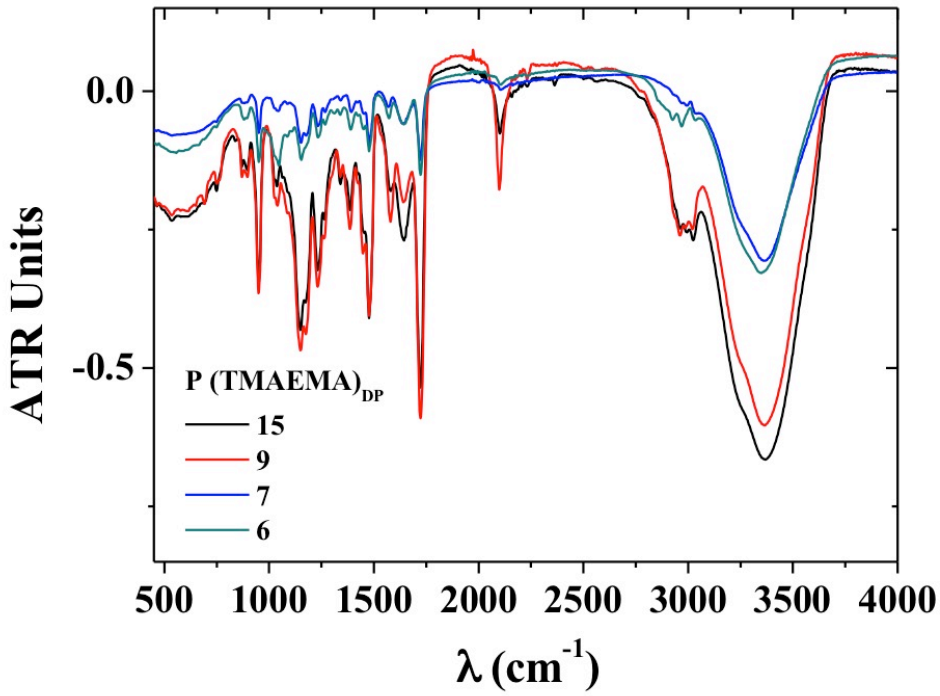


Figure S2.- Infra-red spectrum of $P(TMAEMA)_{DP}$ oligomers, DP = 15, 9, 7, 6.

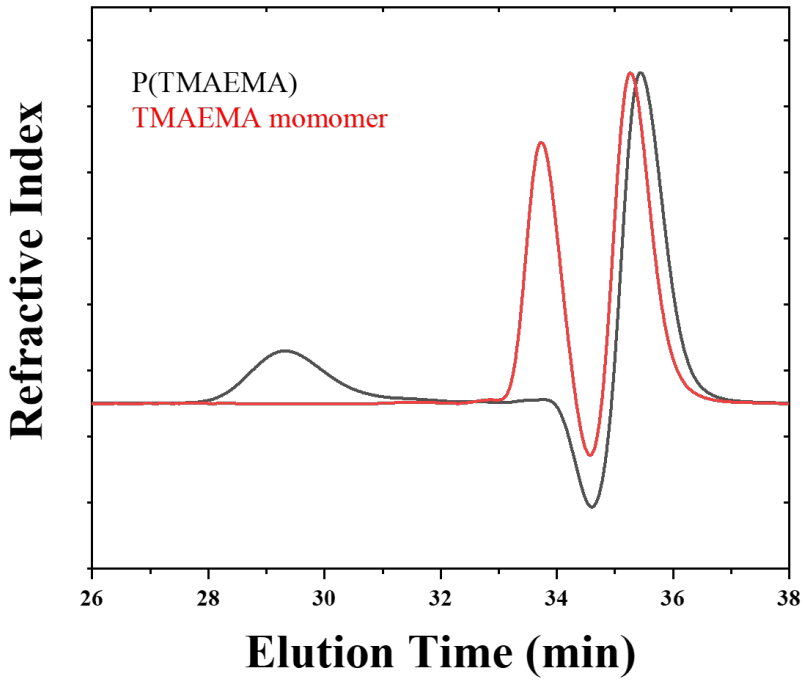
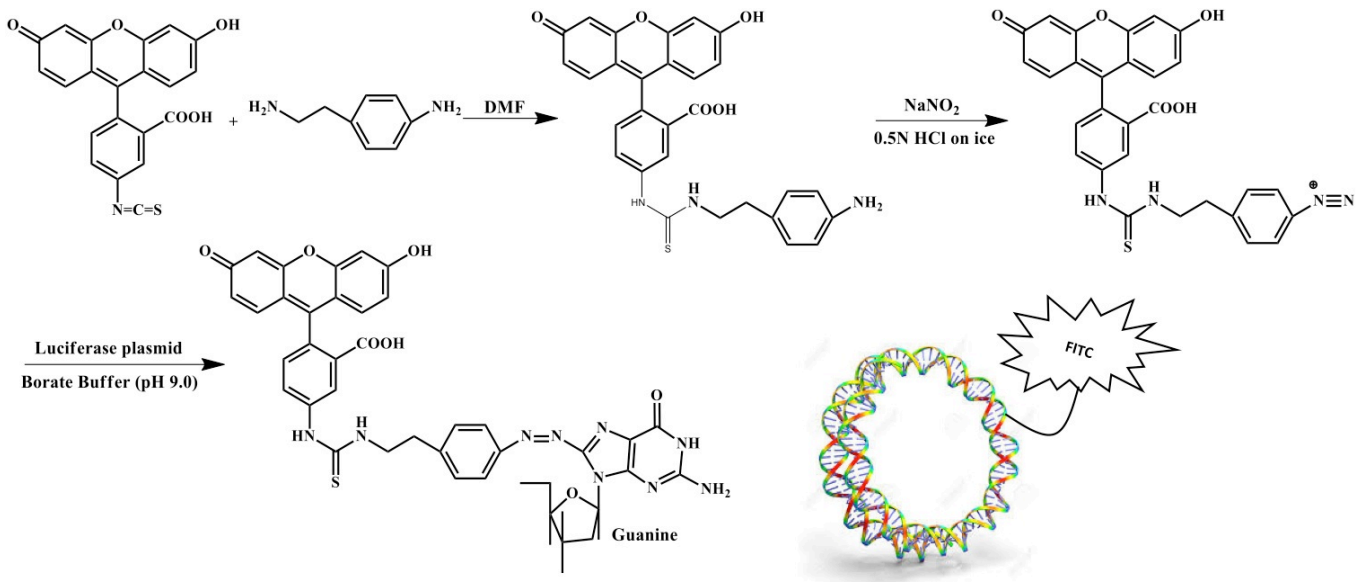


Figure S3.- SEC traces in AcOH/Ammonium Acetate/ACN of $P(TMAEMA)_{15}$ and the TMAEMA monomer using a RI detector.



Scheme S2.- Synthesis of the fluorescent plasmid, pDNA-FTIC. Reproduced from [2] with permission.

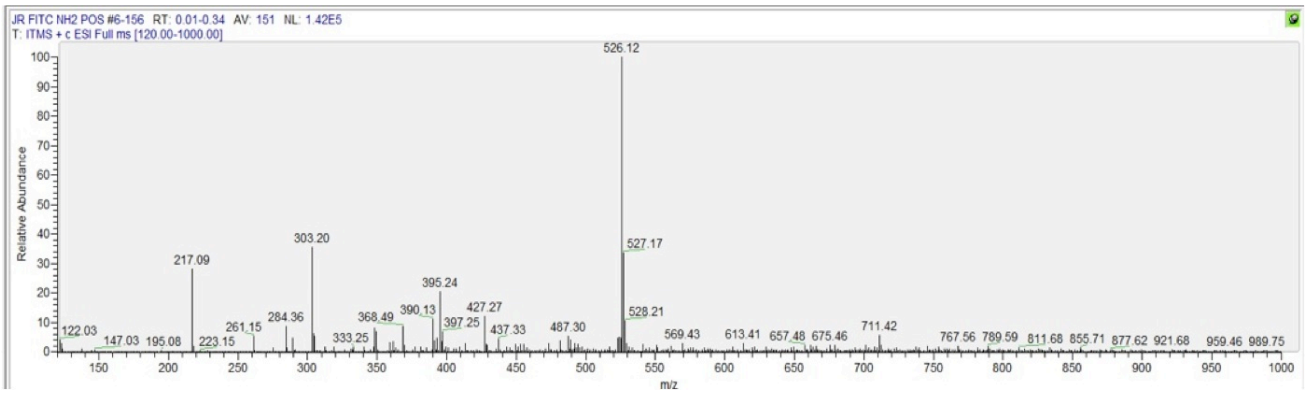


Figure S4. – Mass spectroscopy spectra of the pDNA-FITC intermediate.

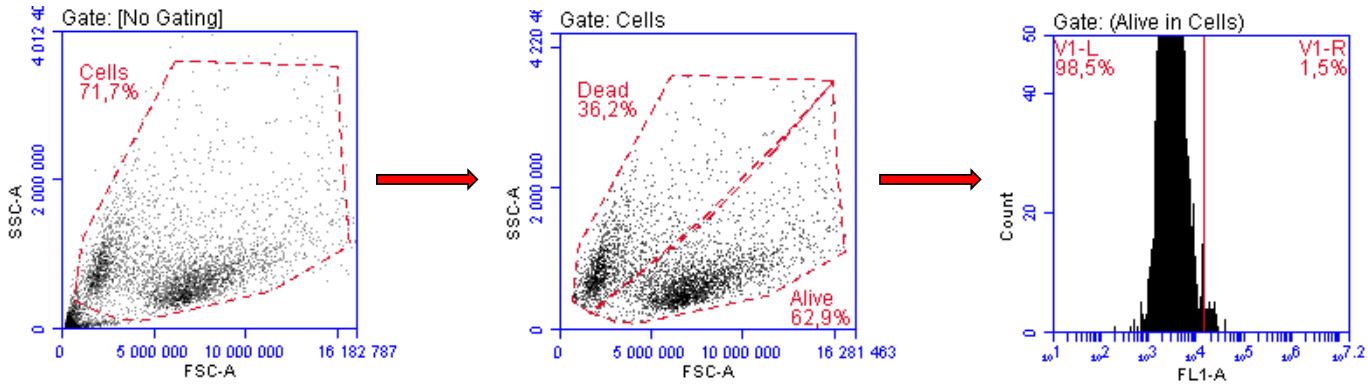


Figure S5.- Processing procedure of the cytometry events. On the negative control assay (untransfected cells) the population of cells was selected in order to discard small debris and artefacts. Thereafter two gates were defined to sort "Alive" or "Dead" cells. Finally the "Alive" gated cells were analyzed according to their fluorescence intensity. The negative control sample was the reference to set limit for non-fluorescent cells (left hand) or fluorescent cells (right hand). Gates and limits defined on negative control sample were applied for all samples.

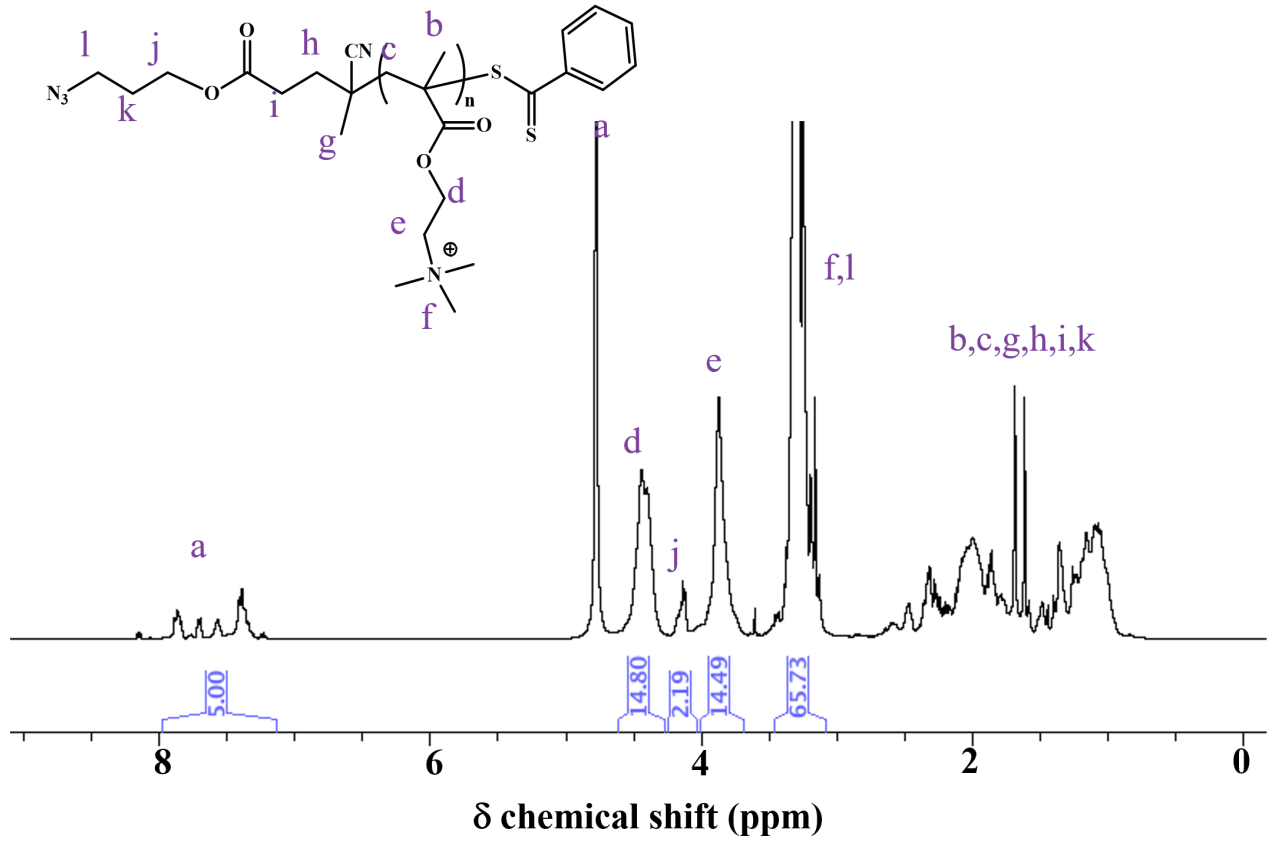


Figure S6. ^{1}H NMR spectrum of the P(TMAEMA)$_7$ in D_2O (25 °C, 400.2 MHz).

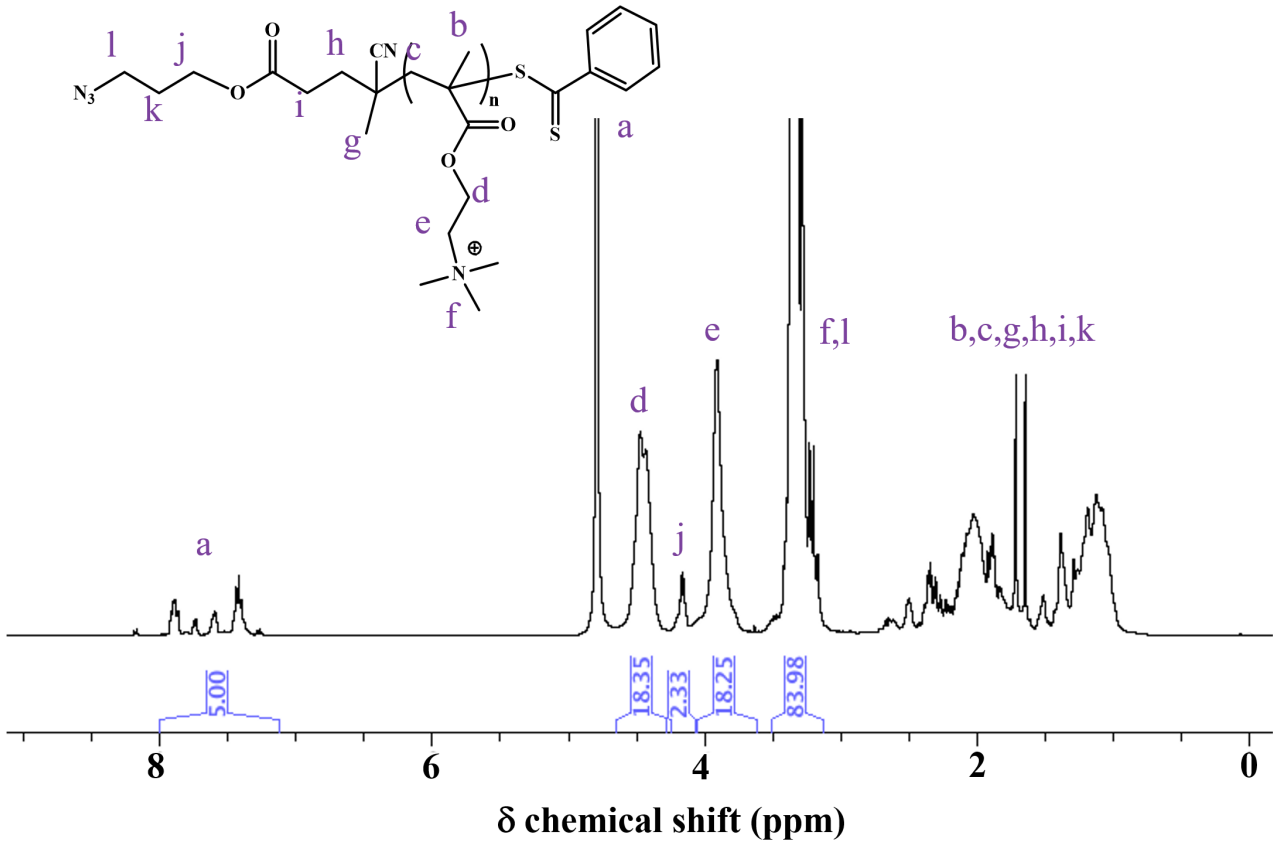


Figure S7. ^{1}H NMR spectrum P(TMAEMA)$_9$ in D_2O (25 °C, 400.2 MHz).

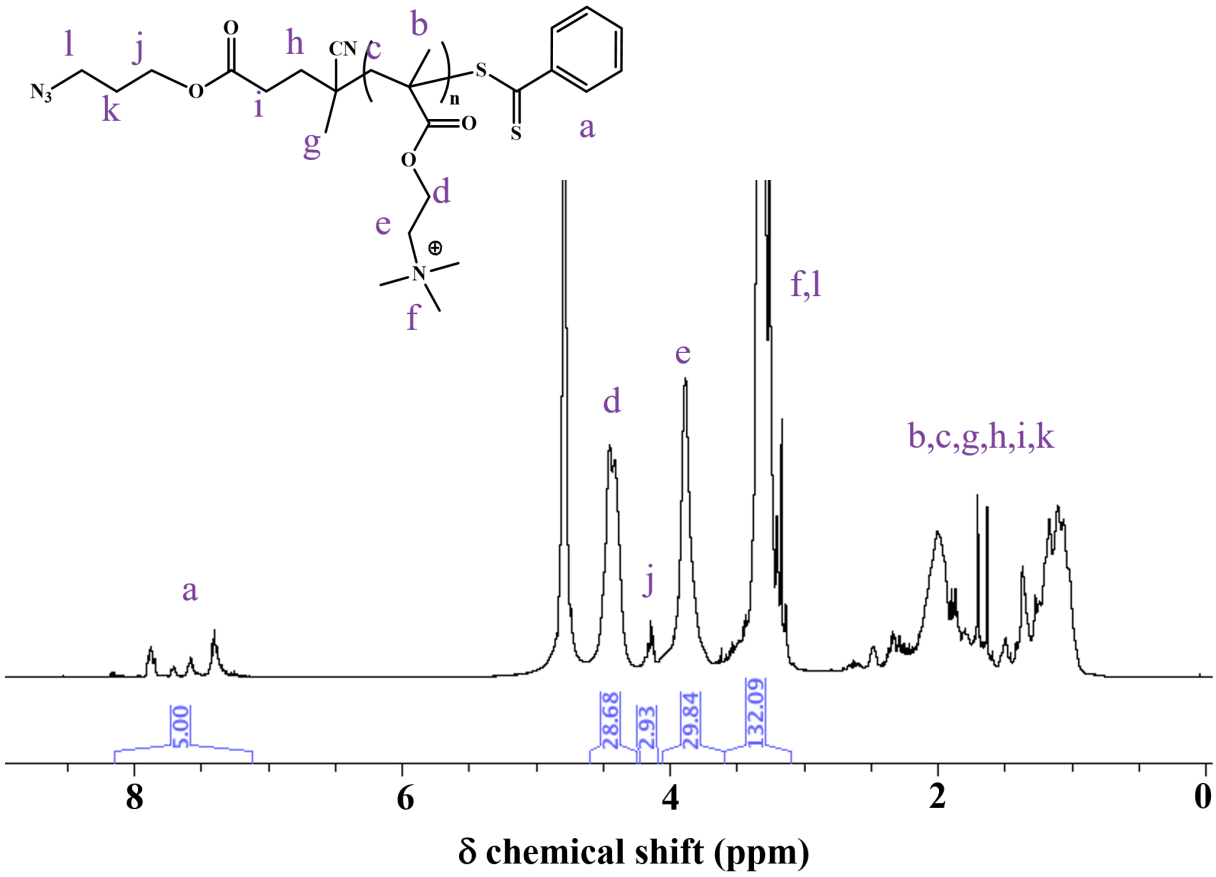


Figure S8. ^{1}H NMR spectrum in D_2O of $P(TMAEMA)_{15}$ in D_2O (25 °C, 400.2 MHz).

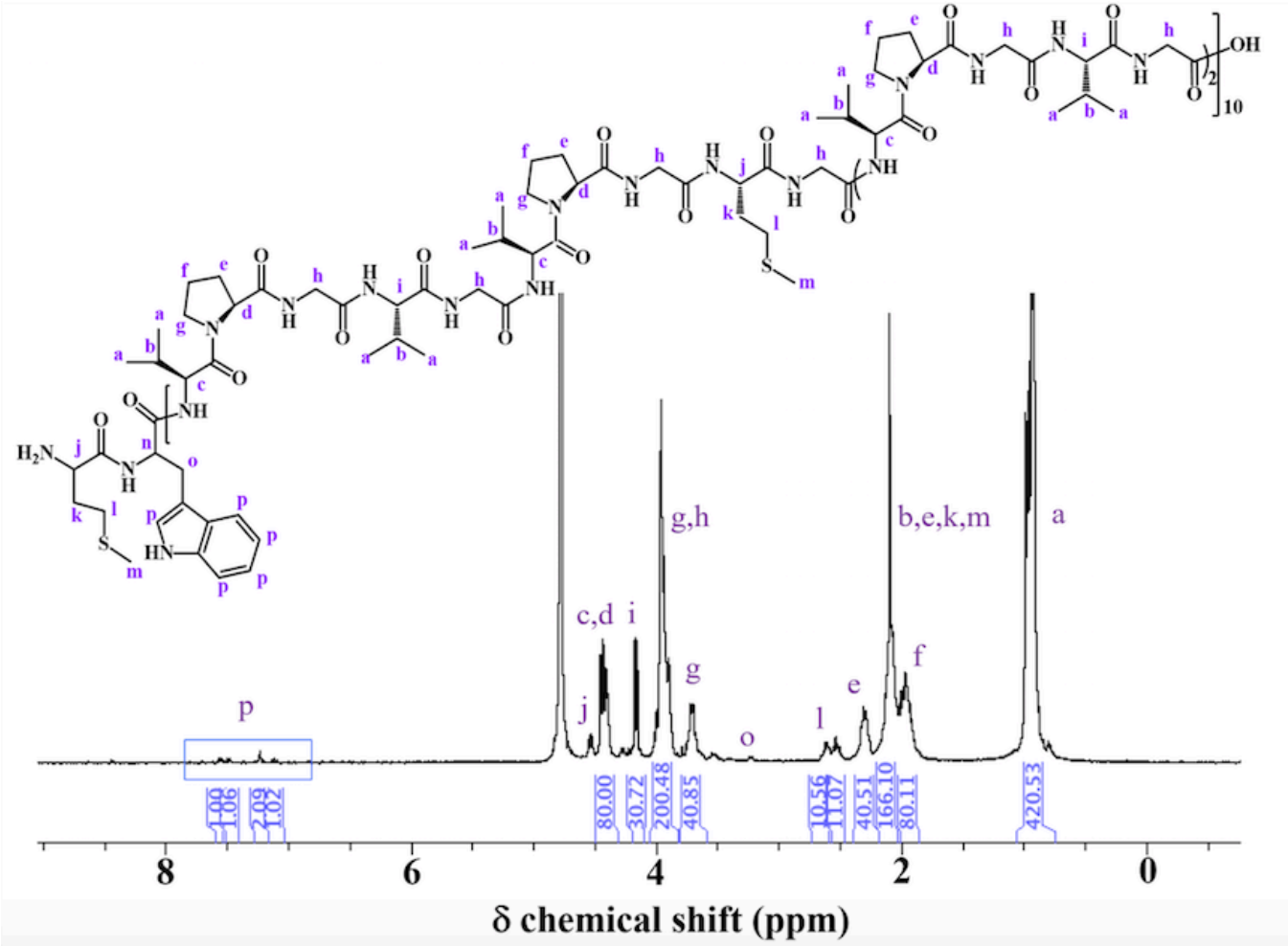


Figure S9.- ^{1}H-NMR spectra of ELP in D_2O (25 °C, 400.2 MHz).

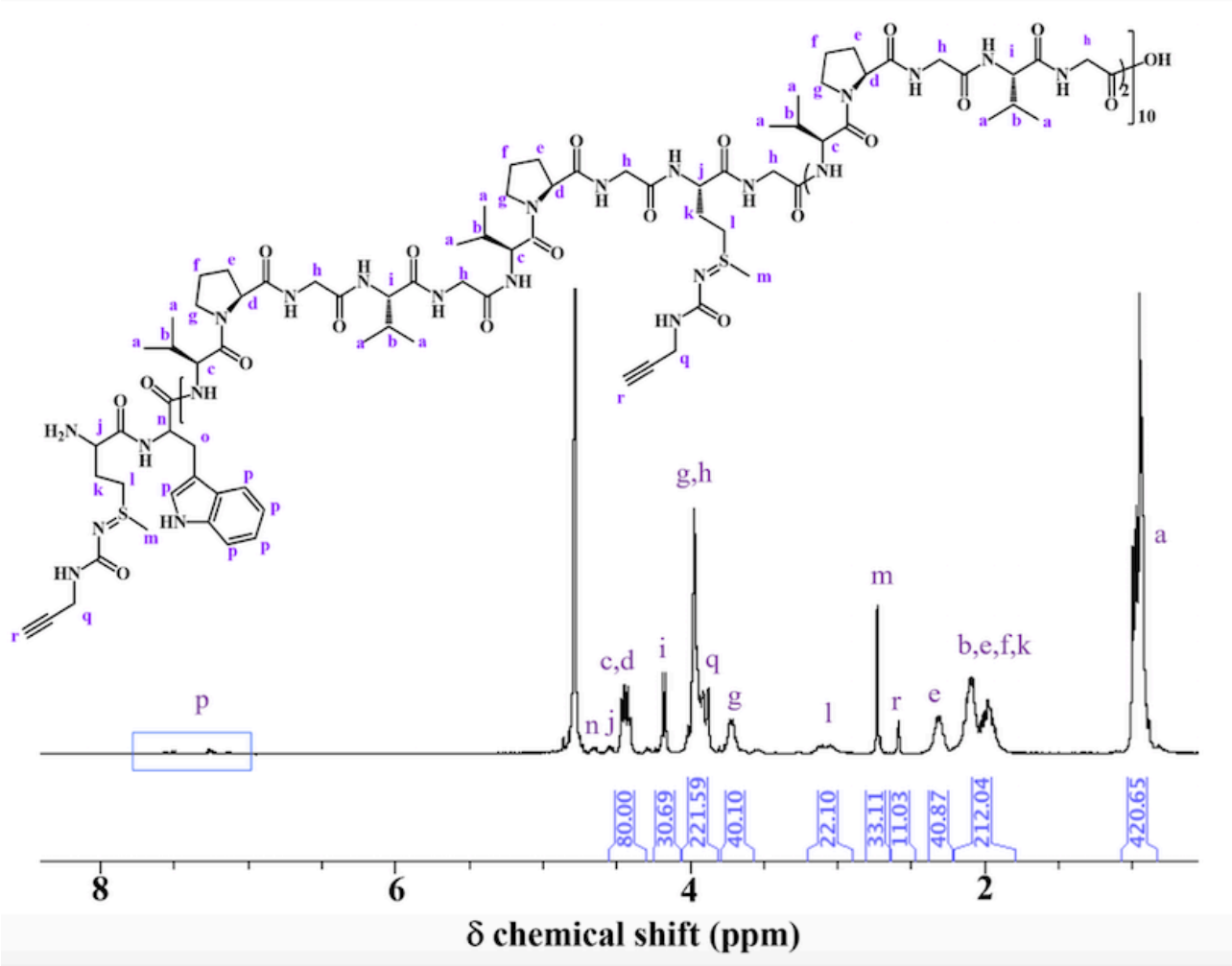


Figure S10.- ^{1}H-NMR spectra of alkyne-modified ELP in D_2O (25 °C, 400.2 MHz).

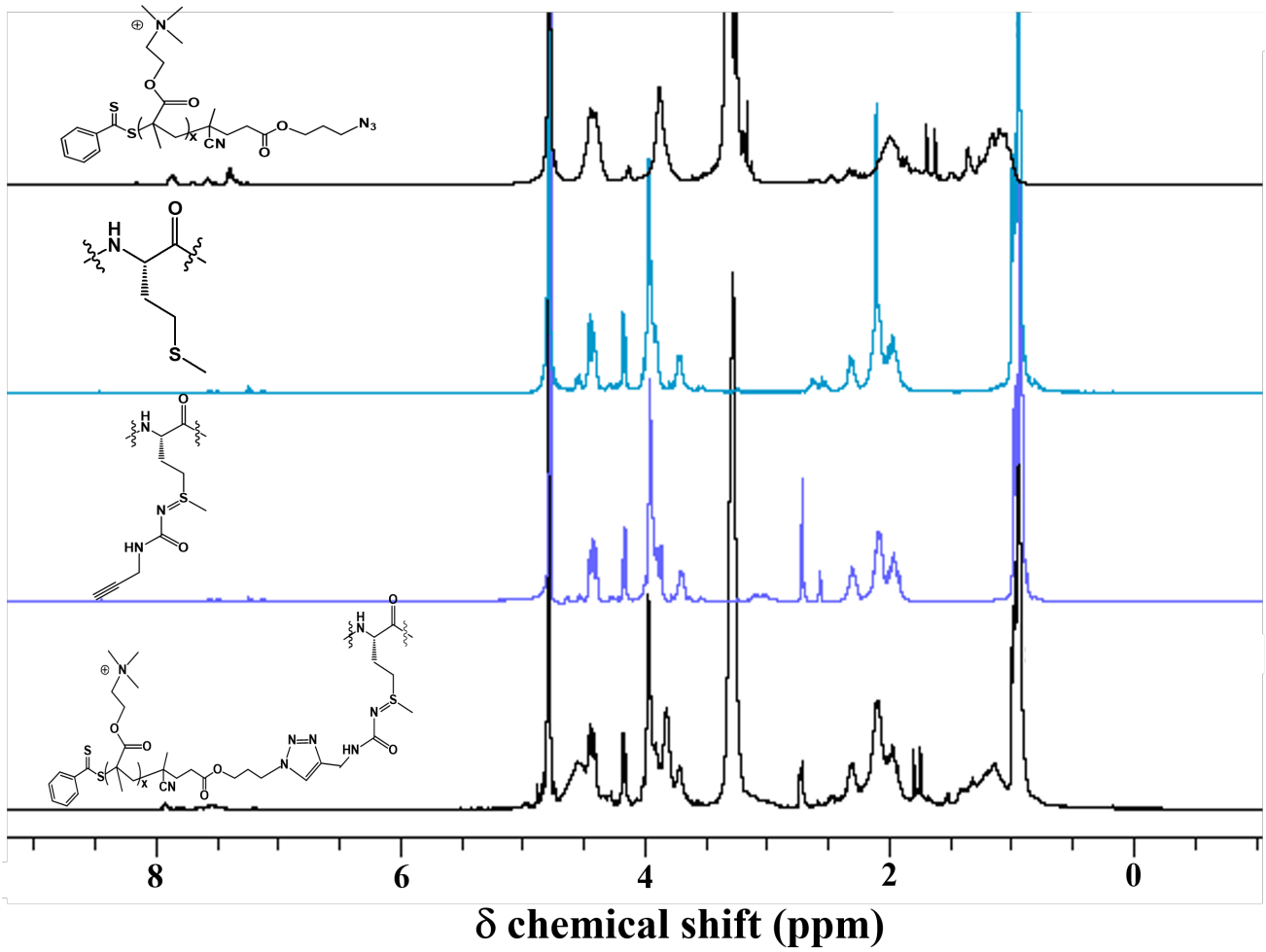


Figure S11. Superimposed ^{1}H NMR spectra of a) cationic oligomer P(TMAEMA)$_6$, b) ELP, c) ELP(alkyne) and d) ELP-g-P(TMAEMA)$_6$ in D_2O (25 °C, 400.2 MHz). Resonance # corresponds to *Val* αCH of the guest residue in VPGVG repeat units, and resonance corresponds to *Val* αCH and *Pro* αCH of VPGXG repeats.

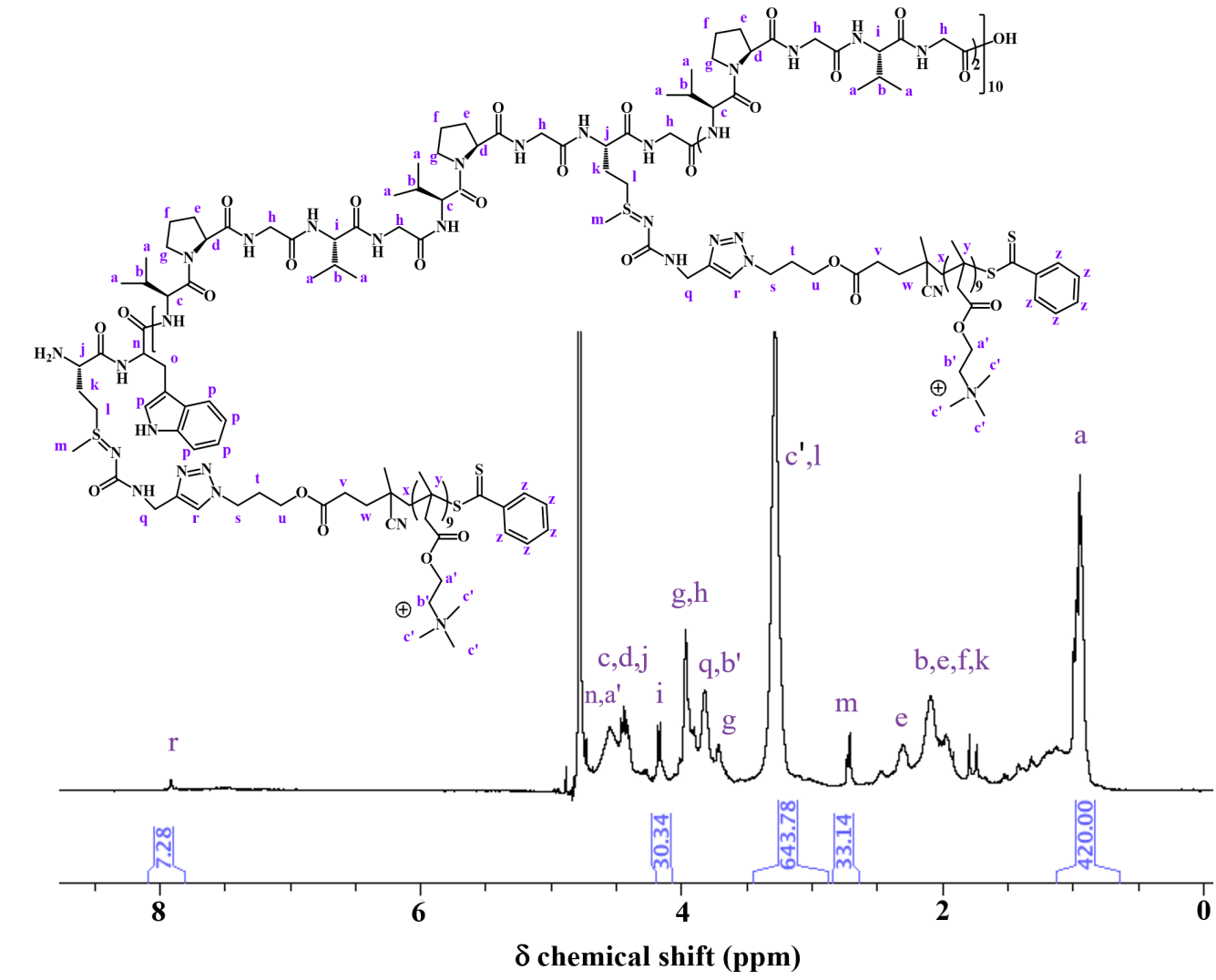


Figure S12.- ^{1}H-NMR spectra of ELP-*g*-P(TMAEMA)$_9$ in D_2O (25 °C, 400.2 MHz).

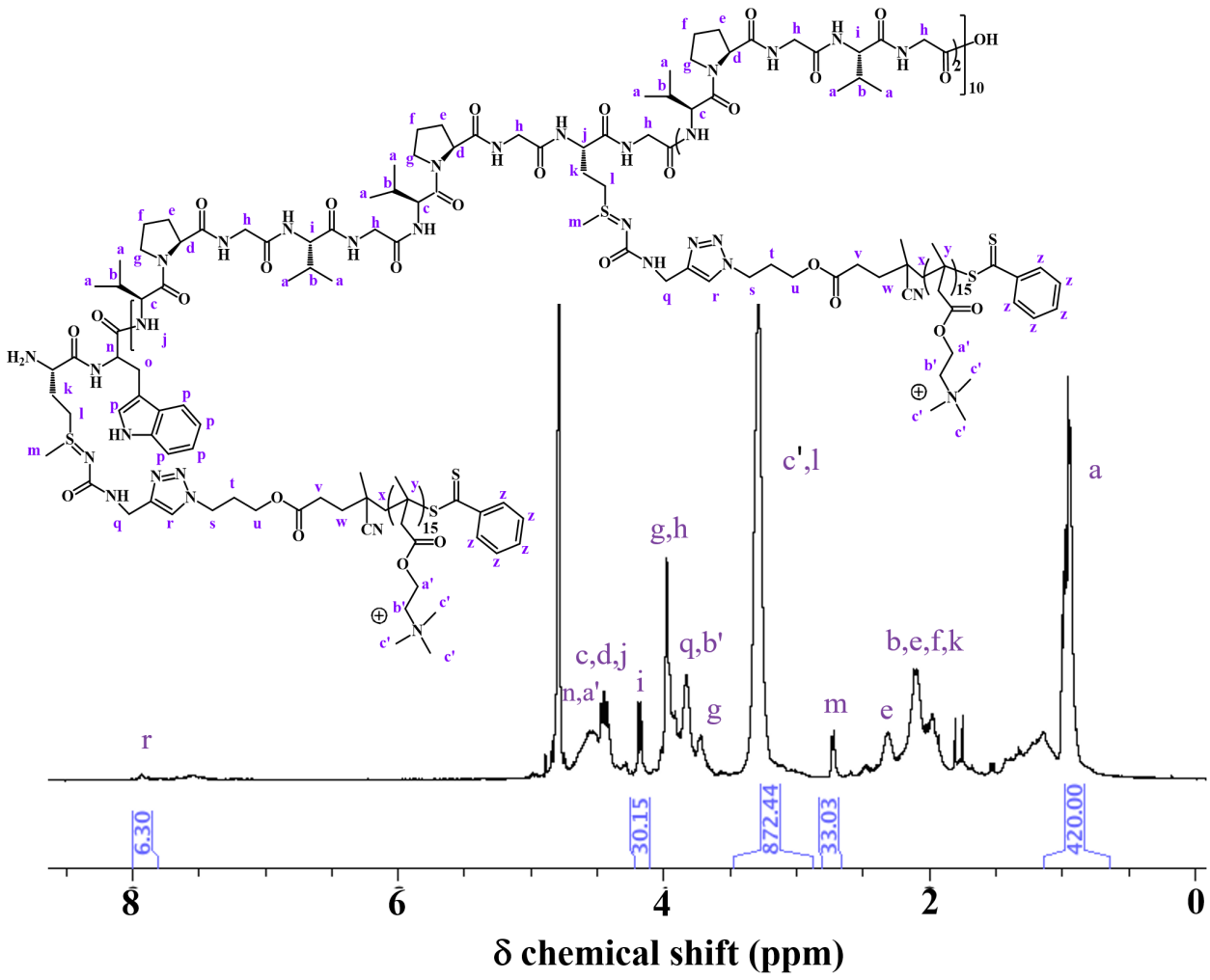


Figure S13.- ^{1}H-NMR spectra of ELP-*g*-P(TMAEMA)$_{15}$ in D_2O (25 °C, 400.2 MHz).

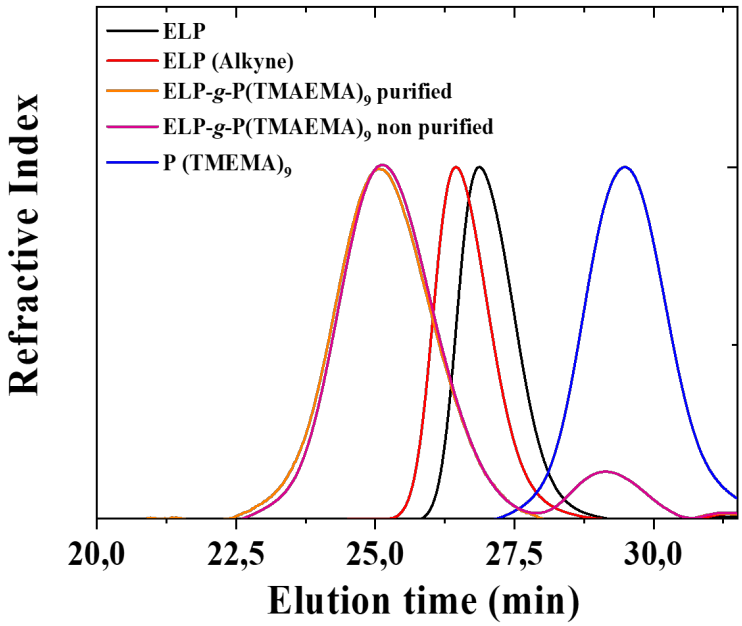


Figure S14.- SEC traces in AcOH/Ammonium Acetate/ACN of ELP (black), ELP(Alkyne) (red), ELP-*g*-P(TMAEMA)$_9$ purified (orange), ELP-*g*-P(TMAEMA)$_9$ non purified (pink) and P(TMAEMA)$_9$ (blue) using a RI detector.

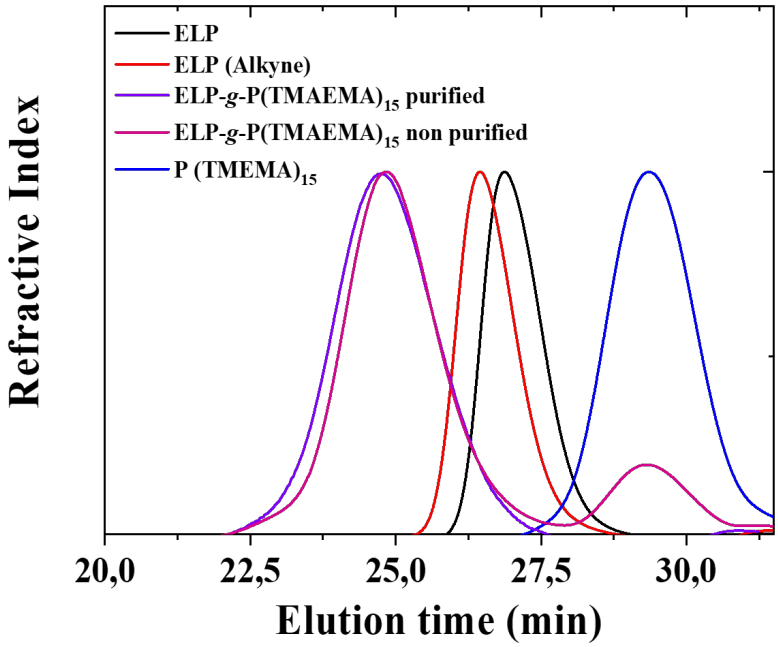


Figure S15.- SEC traces in AcOH/Ammonium Acetate/ACN of ELP (black), ELP(Alkyne) (red), ELP-*g*-P(TMAEMA)$_{15}$ purified (purple), ELP-*g*-P(TMAEMA)$_{15}$ non purified (pink) and P(TMAEMA)$_{15}$ (blue) using a RI detector.

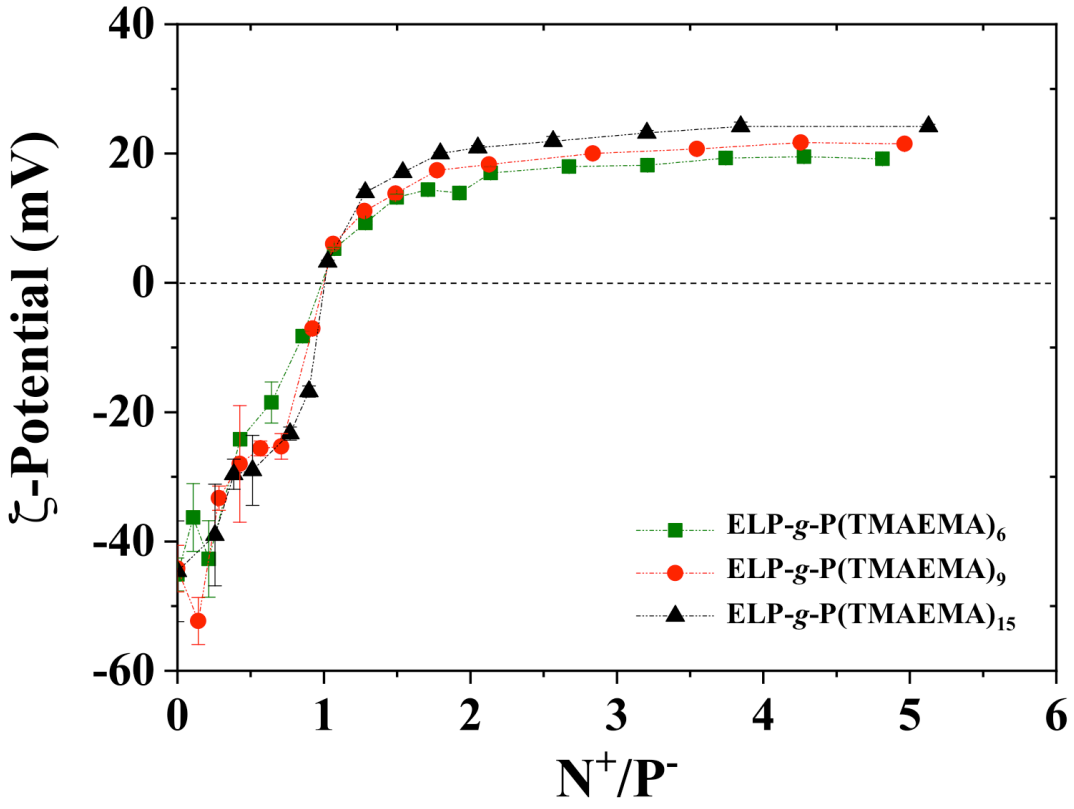


Figure S16.- ζ-potential as a function of N^+/P^- ratio during ELP-*g*-P(TMAEMA)$_X$ complex formation. Conventional slow dropwise mixing of ELP-*g*-P(TMAEMA)$_X$ to a pDNA solution was used for these measurements. Temperature measurement: 37 ºC. C_{pDNA} = 0.005 mg/mL prepared in Tris 10 mM buffer at a pH=7.4, $C_{ELP\text{-}P(PTMAEMA)6,9\ or15}$= 0.2 g/L prepared in water at pH = 6.0.

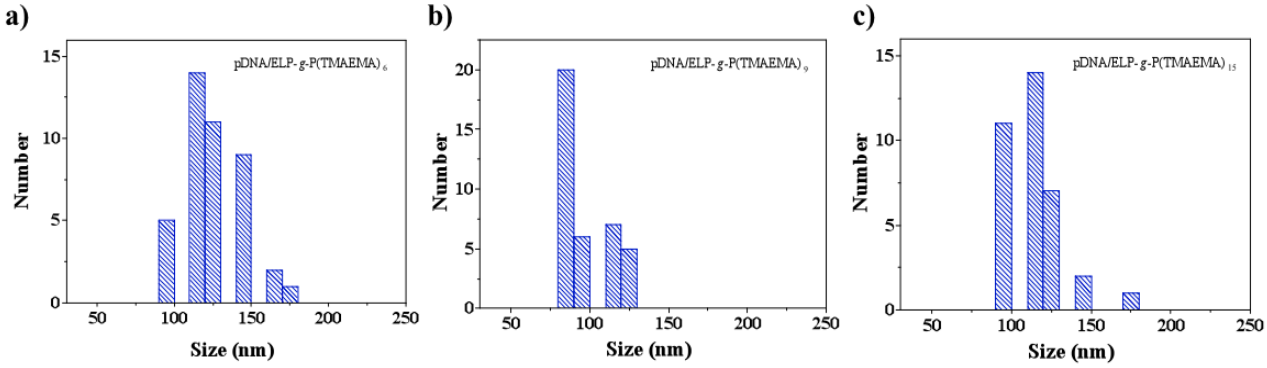


Figure S17.- Particle size distribution from AFM measurements for a) pDNA/ELP-*g*-P(TMAEMA)$_6$, b) pDNA /ELP-*g*-P(TMAEMA)$_9$ and pDNA /ELP-*g*-P(TMAEMA)$_{15}$ complexes prepared at the charge ratios N^+/P^-=10. C_{pDNA}= 0.03 mg/mL prepared in Tris 10 mM buffer at a pH=7.4 $C_{ELP\text{-}P(PTMAEMA)6,\ 9\ or15}$= 0.2 mg/mL prepared in water at pH = 6.0.

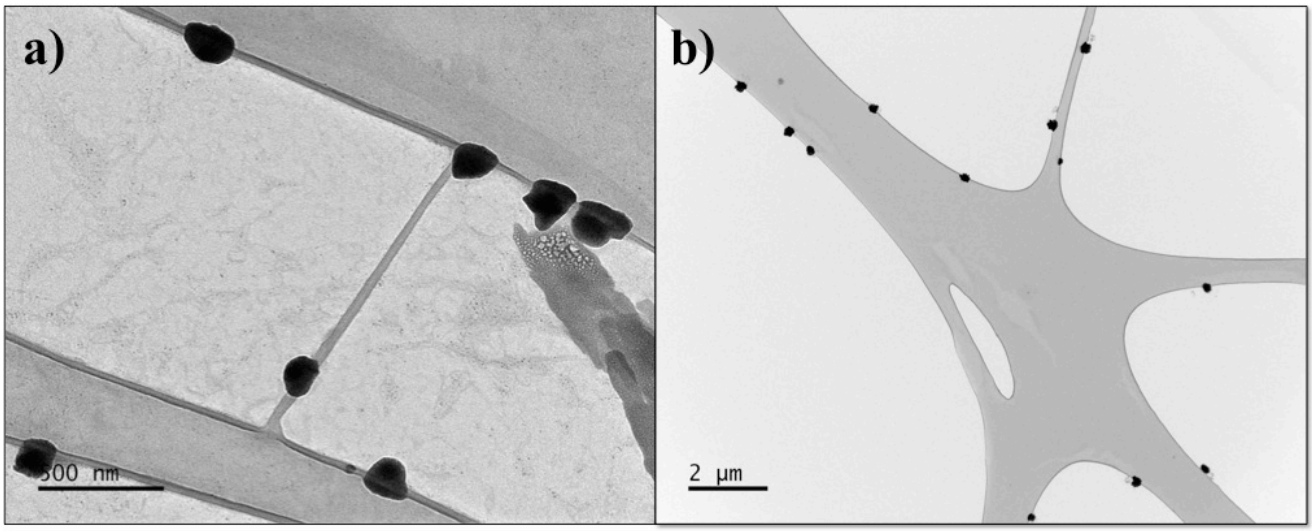


Figure S18.- TEM images of a) pDNA/ELP-*g*-P(TMAEMEA)$_6$ and b) pDNA/ELP-*g*-P(TMAEMEA)$_9$, prepared at a charge ratio N^+/P^- =10. C_{pDNA} = 0.03 mg/mL prepared in Tris 10 mM buffer at a pH=7.4, $C_{ELP\text{-}P(PTMAEMA)6,9\ or15}$= 0.2 g/L prepared in water at pH = 6.0.

Internet link

https://www.dropbox.com/s/9ofjg94i9pruqg1/2-A1-48h.avi?dl=0

Video S1.- Movie made from a 3D reconstruction of a typical cell after 48 hours of post-transfection

References supporting information

[1] Quemener Damien; Davis Thomas P; Barner-Kowollik Christopher; Stenzel Martina H. *Chemical communications* (**2006**), (48), 5051-3.

[2] Ishii, Tsuyoshi; Okahata, Yoshio; Sato, Toshinori. *Chemistry Letters* (**2000**), (4), 386-387.