Electronic Supplementary Information

Anion-Induced Thermoresponsiveness in Cationic Polycysteine and DNA Binding

Mahammad Anas, Priyanka Dinda, Mahuya Kar and Tarun K. Mandal*

School of Chemical Sciences, Indian Association for the Cultivation of Science, Jadavpur, Kolkata 700032, India

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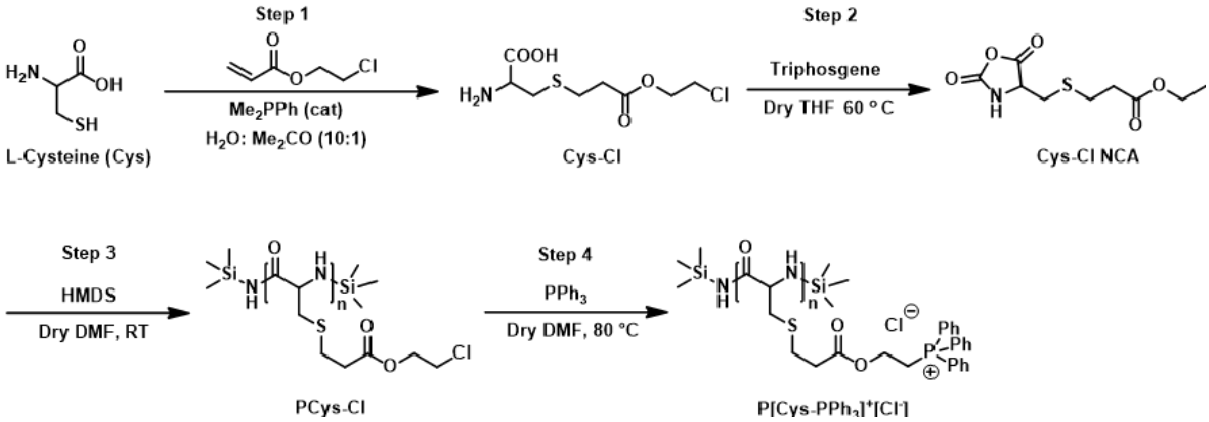


Scheme S1. Synthesis scheme for PCys-Cl and its cationization to P[Cys-PPh_3]$^+$[Cl^-]

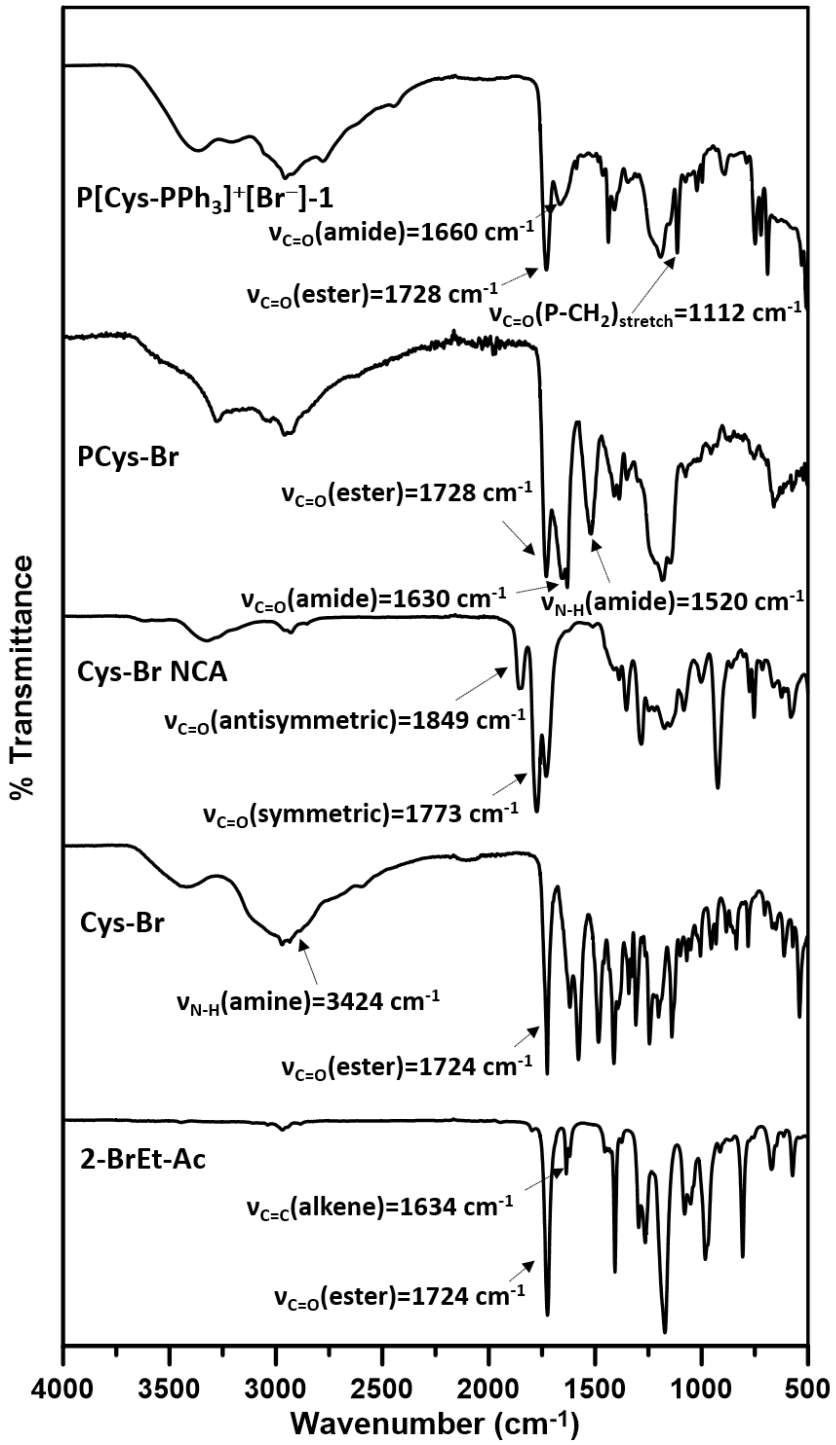


Figure S1. FT-IR spectra of all the as-synthesized compounds.

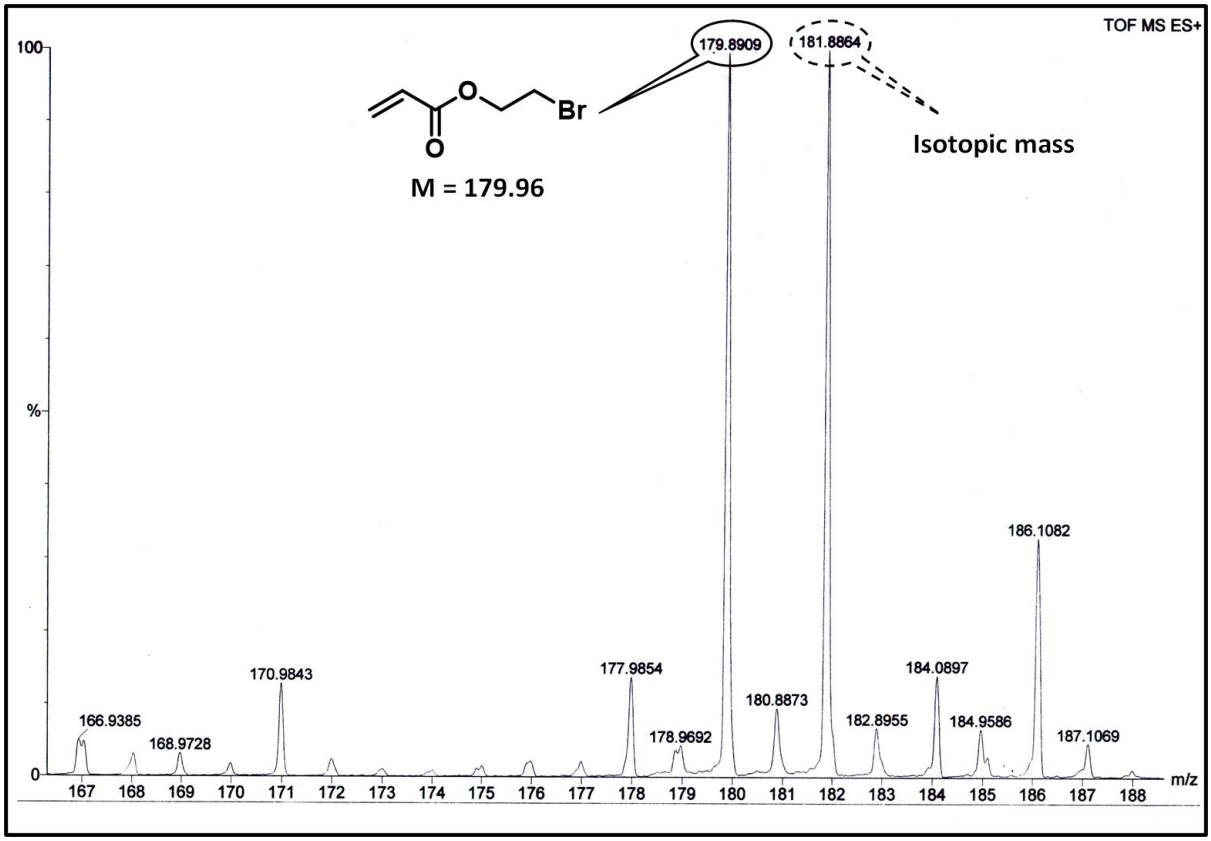


Figure S2. ESI-MS spectrum of 2-BrEt-Ac in DCM.

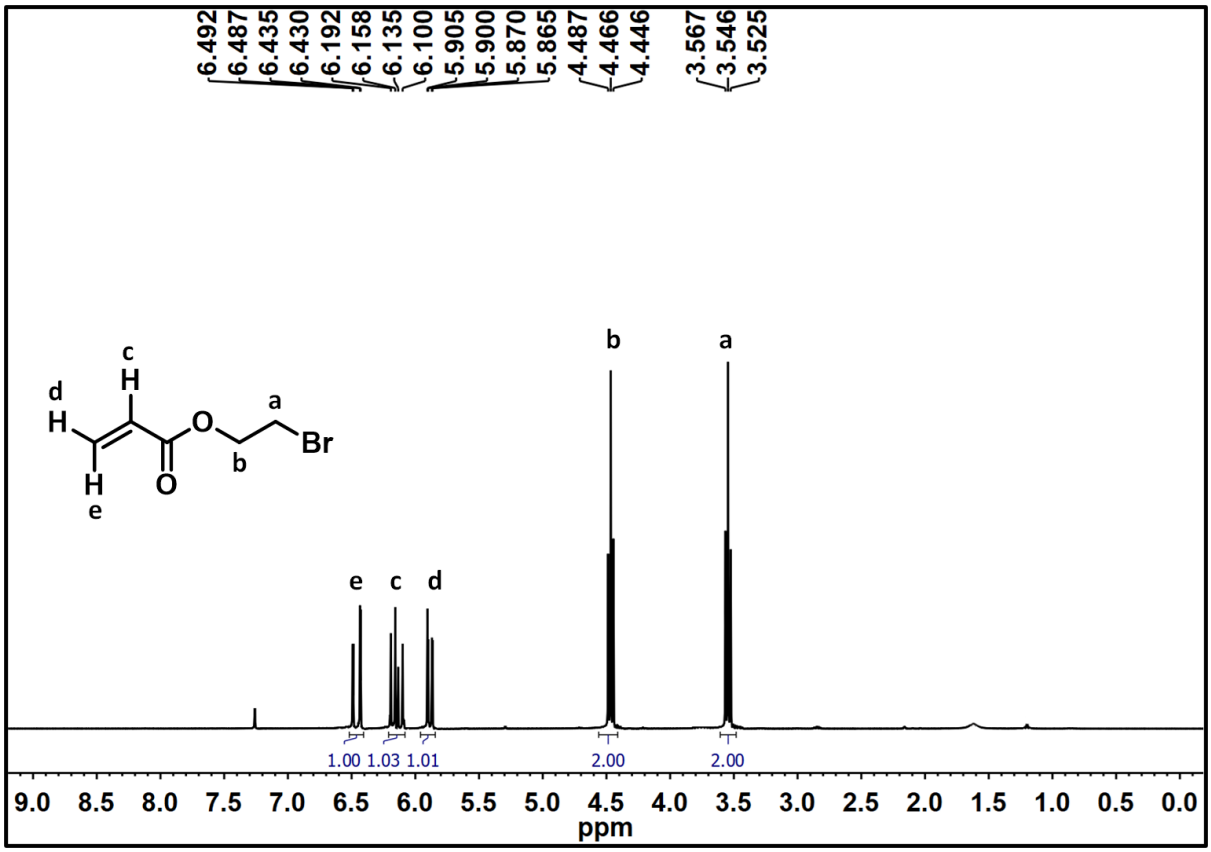


Figure S3. ^{1}H-NMR spectrum of 2-BrEt-Ac in $CDCl_3$.

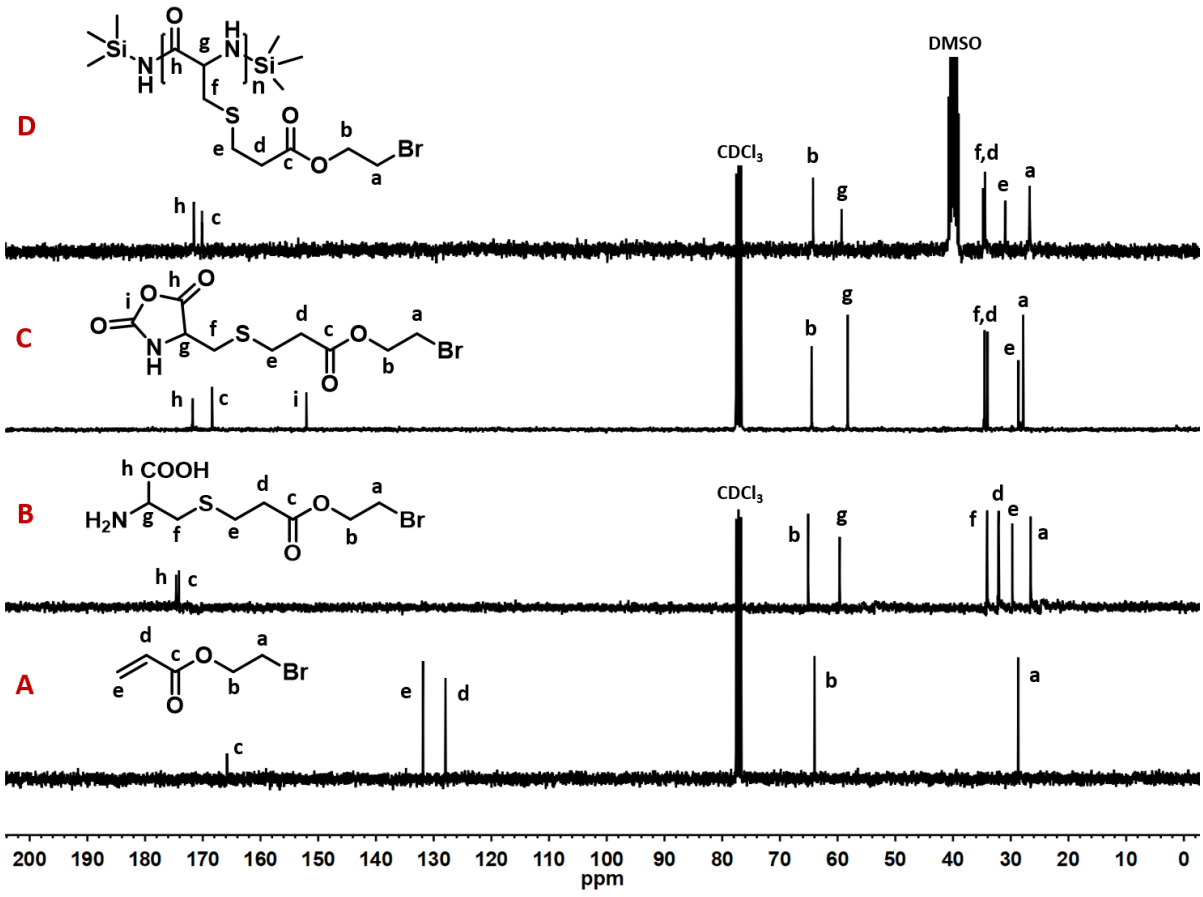


Figure S4. ^{13}C-NMR spectra of 2-BrEt-Ac in $CDCl_3$ (A), Cys-Br in D_2O (B), Cys-Br NCA in $CDCl_3$ (C) and PCys-Br-1 in DMSO-d_6 (D).

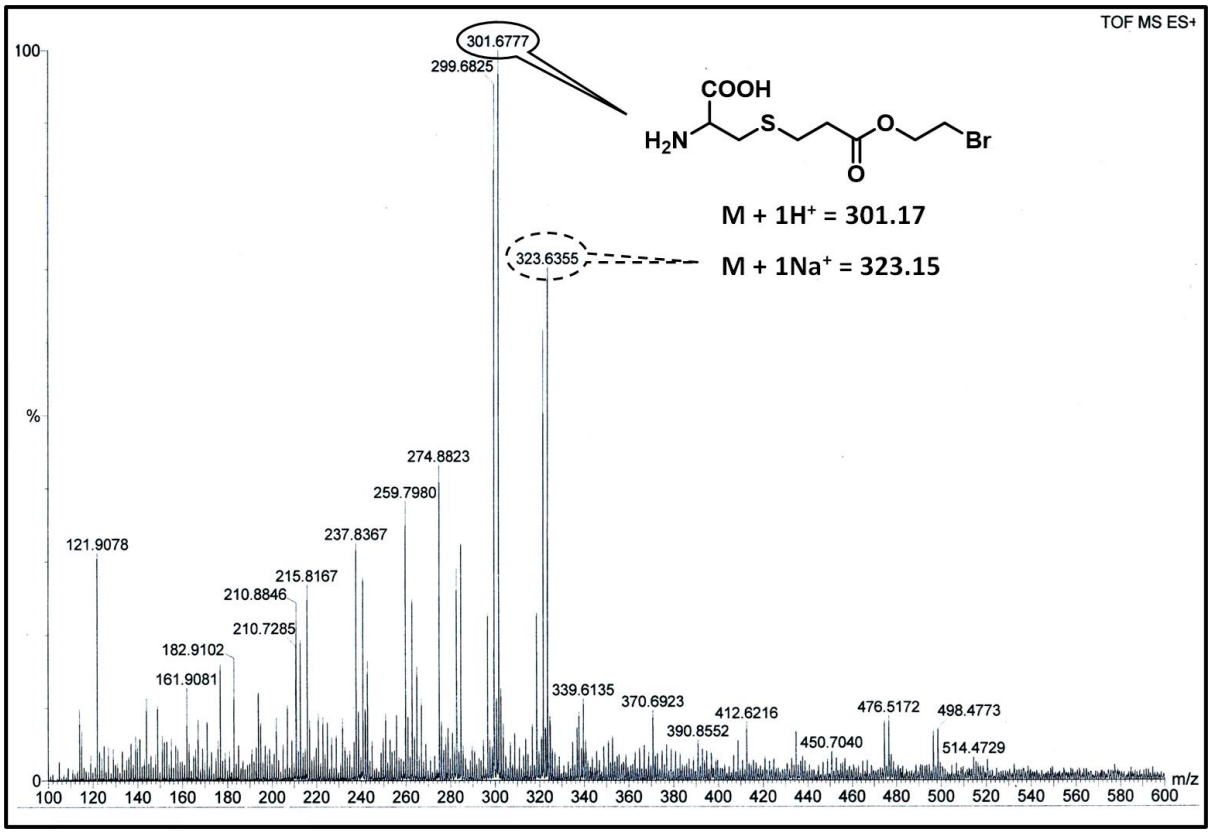


Figure S5. ESI-MS spectrum of Cys-Br in H_2O: MeOH (1:1) mixture.

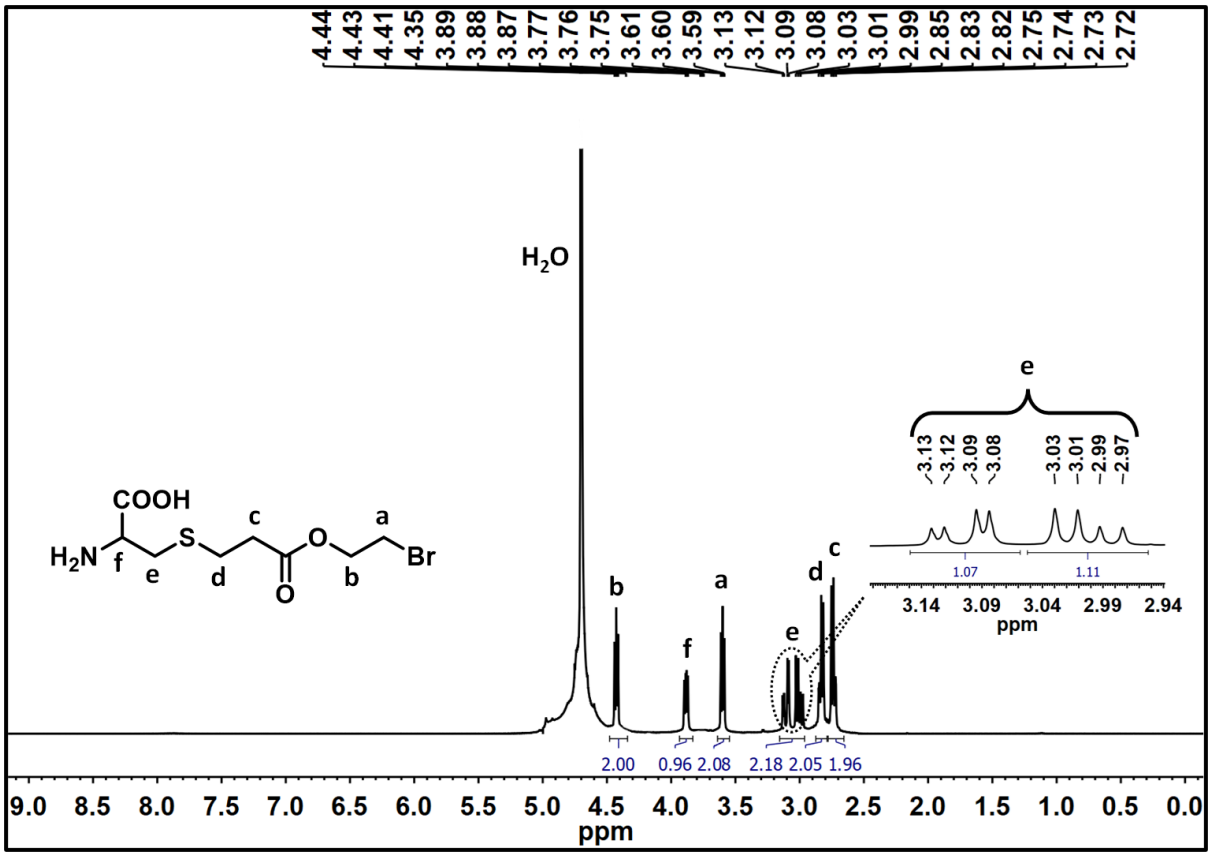


Figure S6. ^{1}H-NMR spectrum of Cys-Br in D_2O.

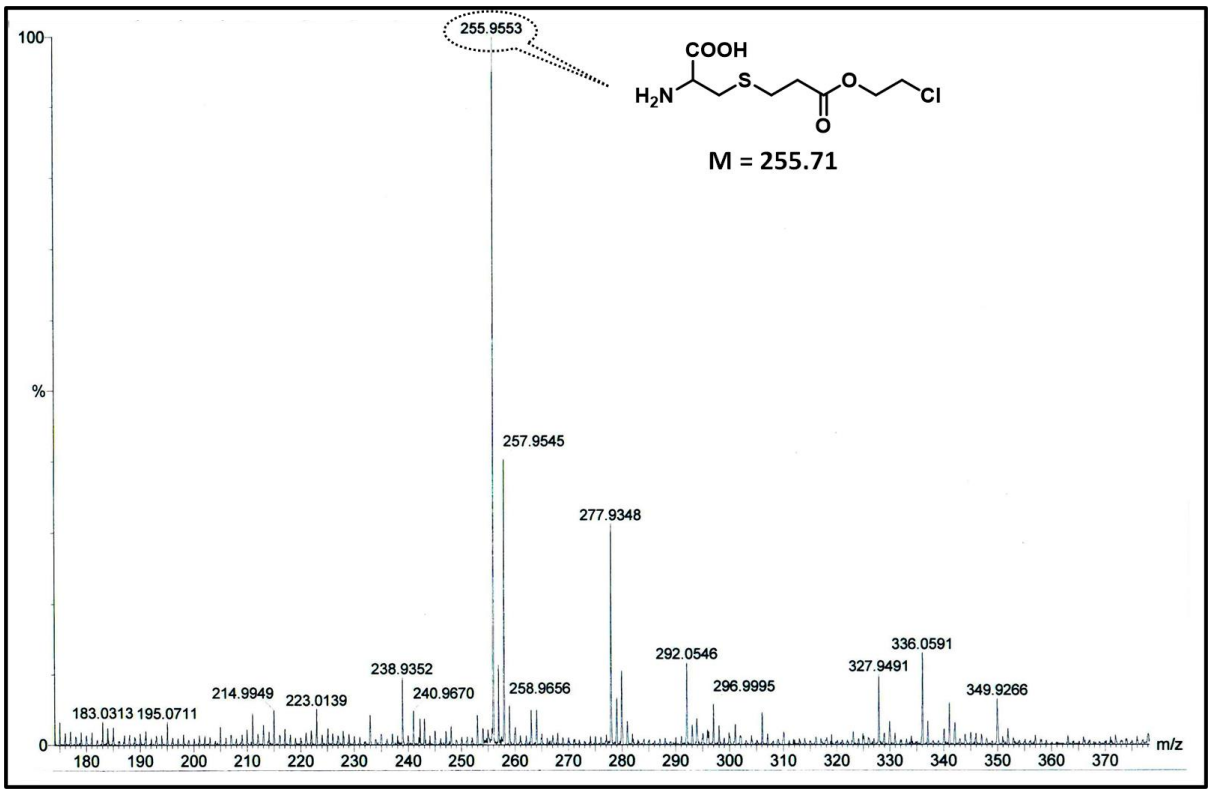


Figure S7. ESI-MS spectrum of Cys-Cl in H_2O: MeOH (1:1) mixture.

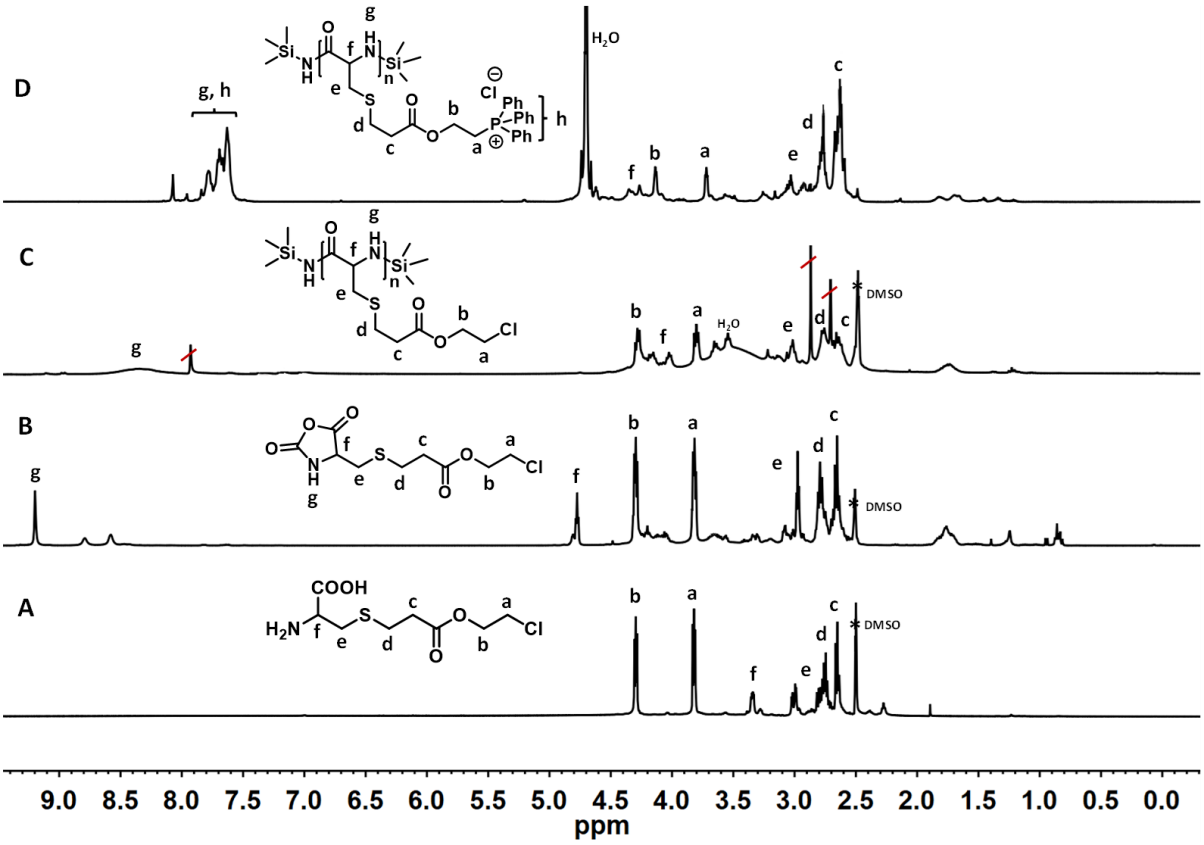


Figure S8. ^{1}H-NMR spectra of Cys-Cl (A), Cys-Cl NCA (B), PCys-Cl (C) in DMSO-d_6 and P[Cys-PPh_3]$^{+}$[Cl^{-}] (D) in D_2O.

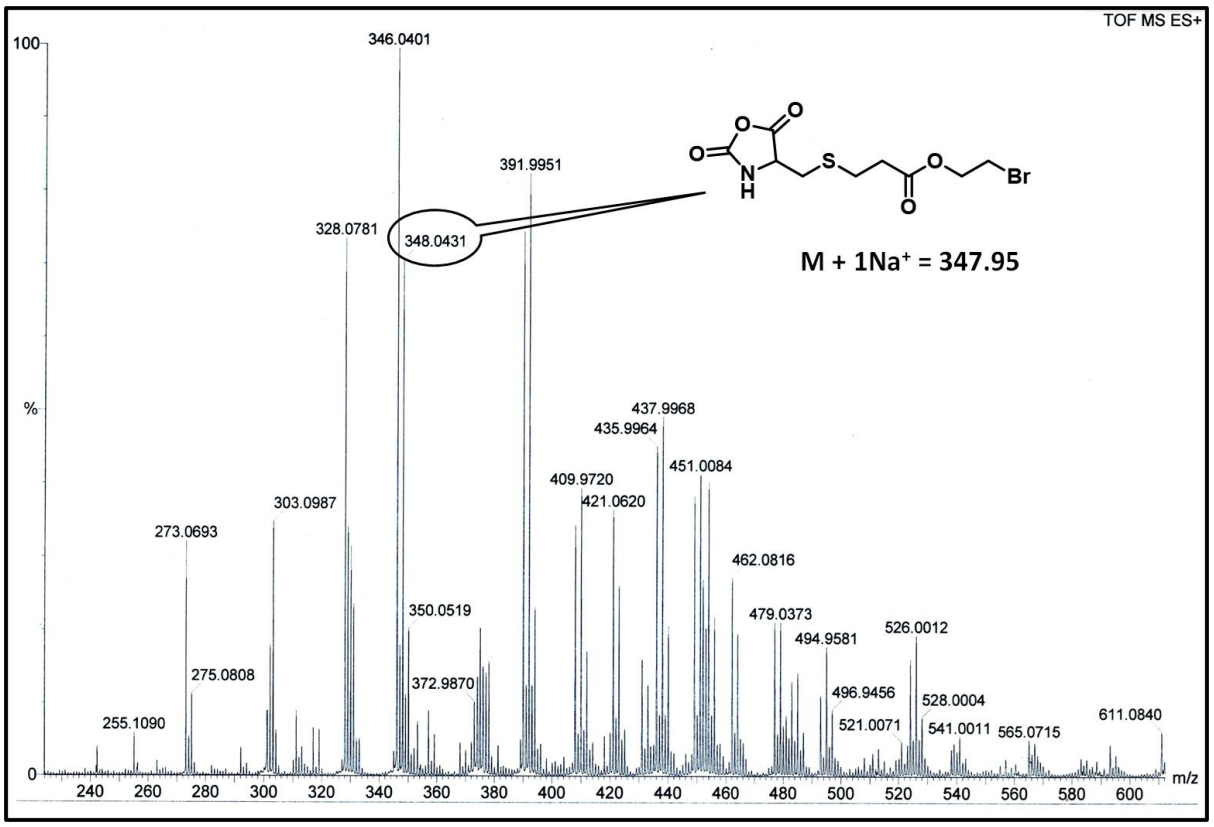


Figure S9. ESI-MS spectrum of Cys-Br NCA in DCM.

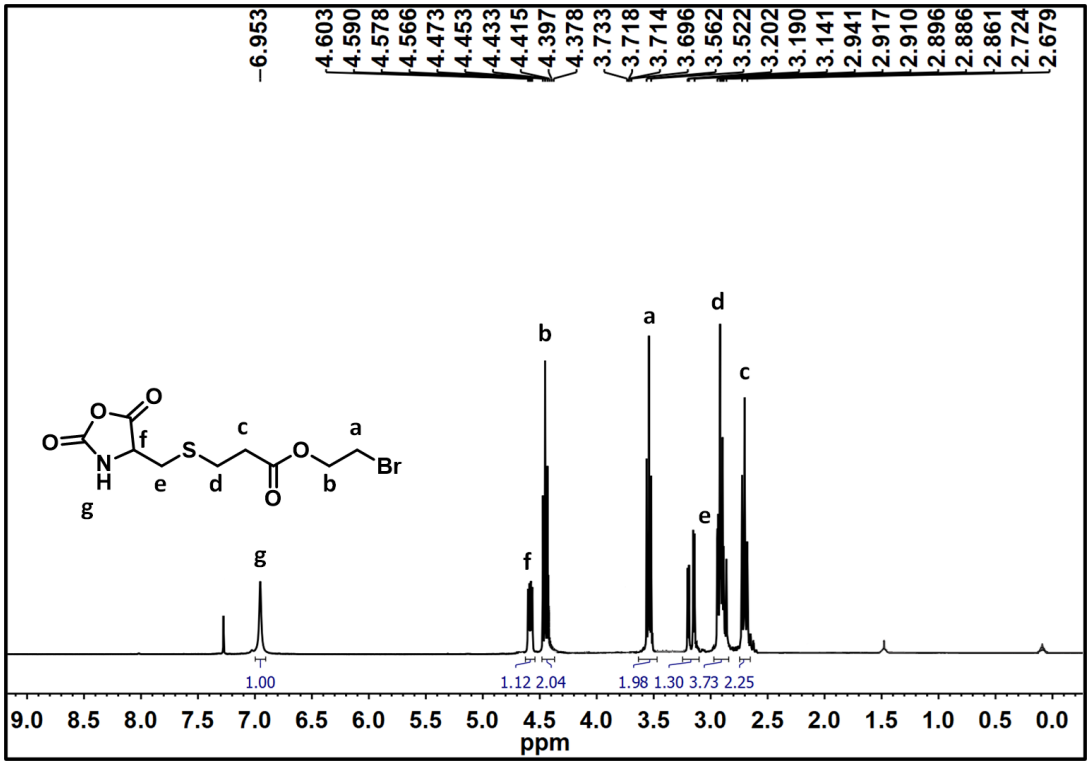


Figure S10. ^{1}H-NMR spectrum of Cys-Br NCA in $CDCl_3$.

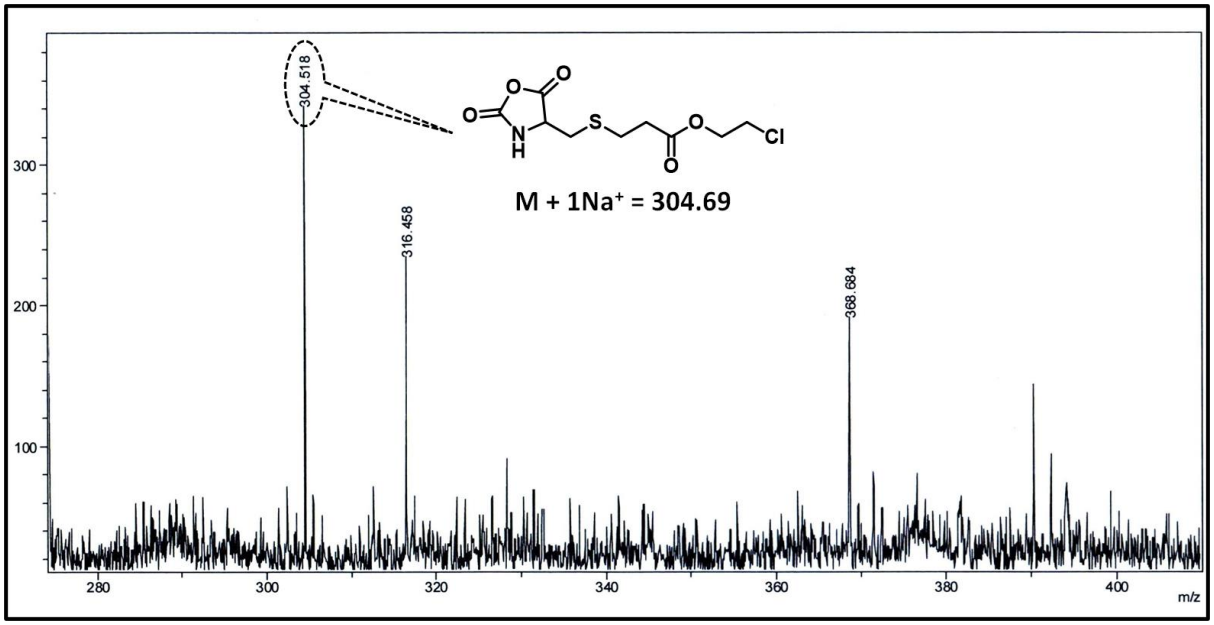


Figure S11. MALDI-TOF-MS of Cys-Cl NCA in THF using DCTB matrix and NaI.

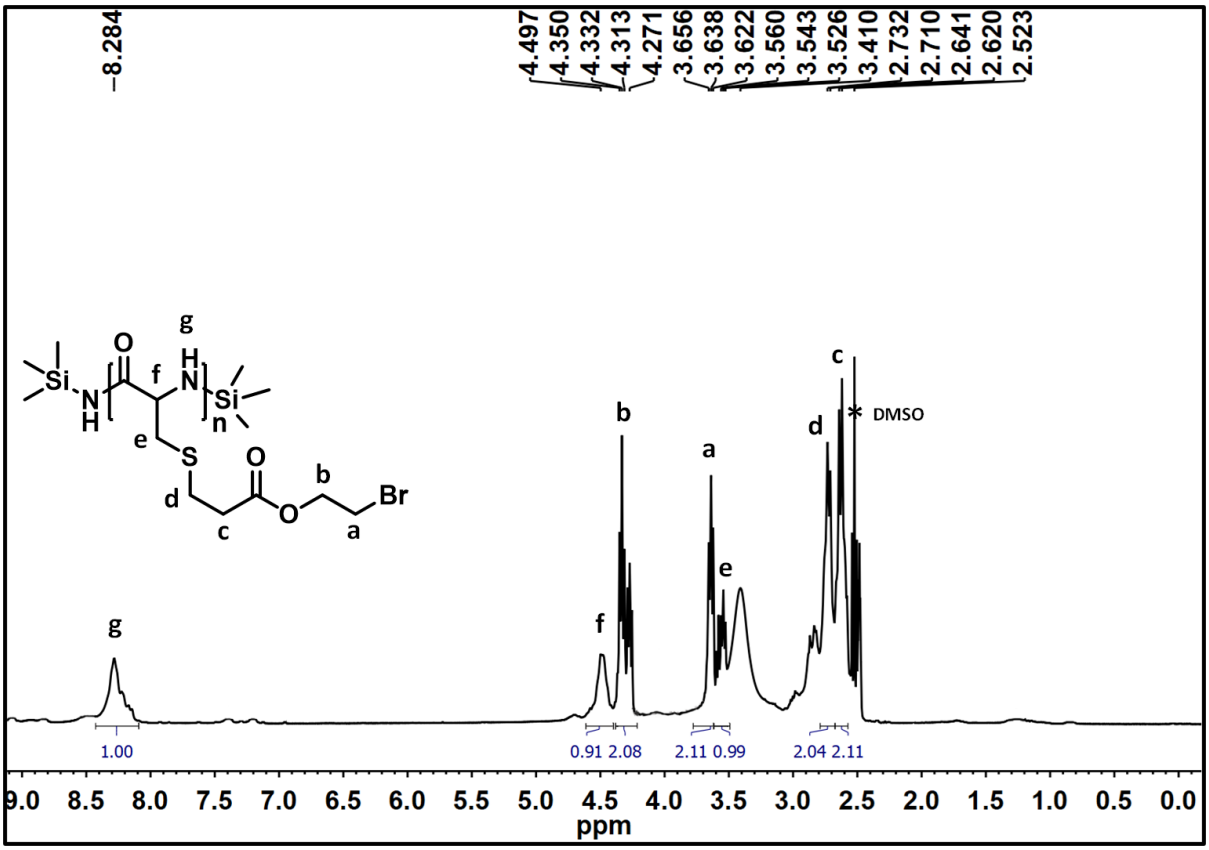


Figure S12. [1]H-NMR spectrum of PCys-Br-1in DMSO-d_6.

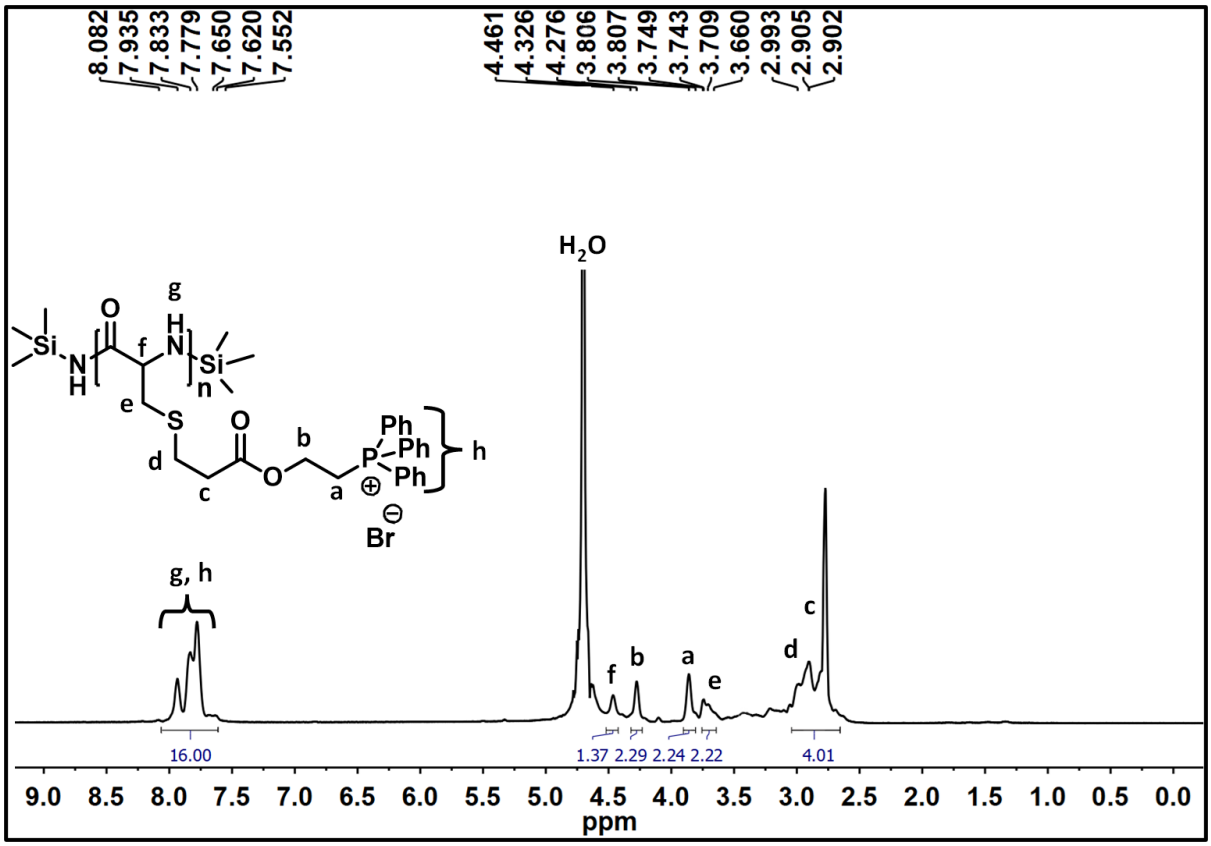


Figure S13. ^{1}H-NMR spectrum of P[Cys-PPh$_3$]$^{+}$[Br^{-}]-1in D_2O.

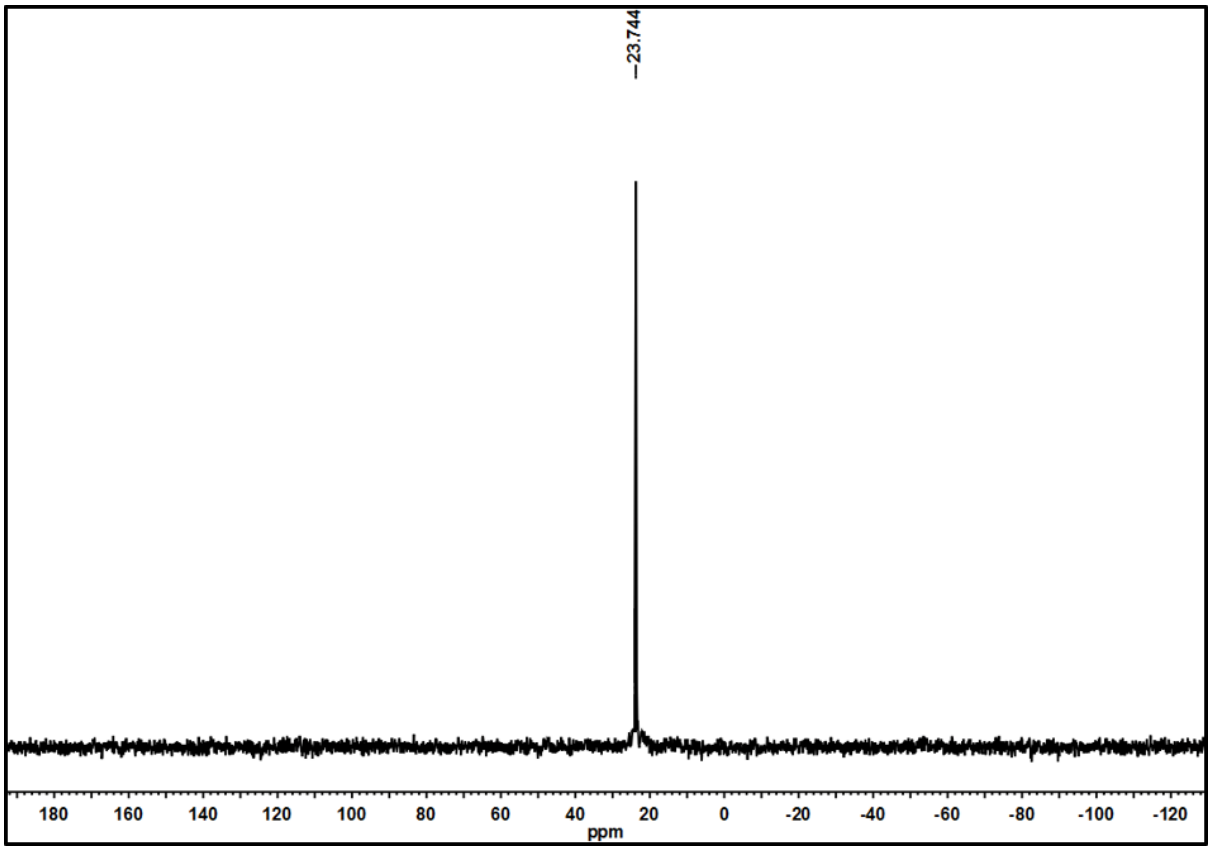


Figure S14. ^{31}P-NMR spectrum of P[Cys-PPh$_3$]$^+$[Br$^-$]-1 in D_2O.

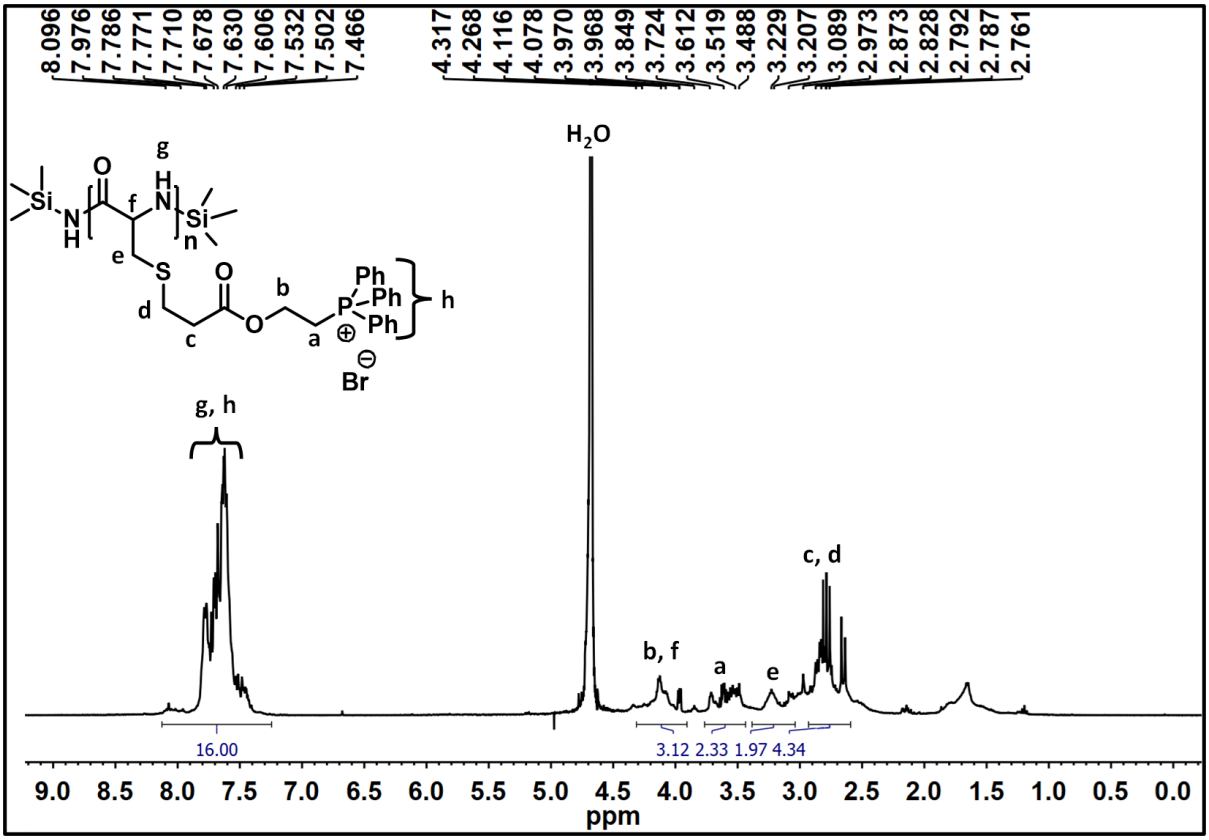


Figure S15. ^{1}H-NMR spectrum of P[Cys-PPh$_3$]$^{+}$[Br^{-}]-2 in D_2O.

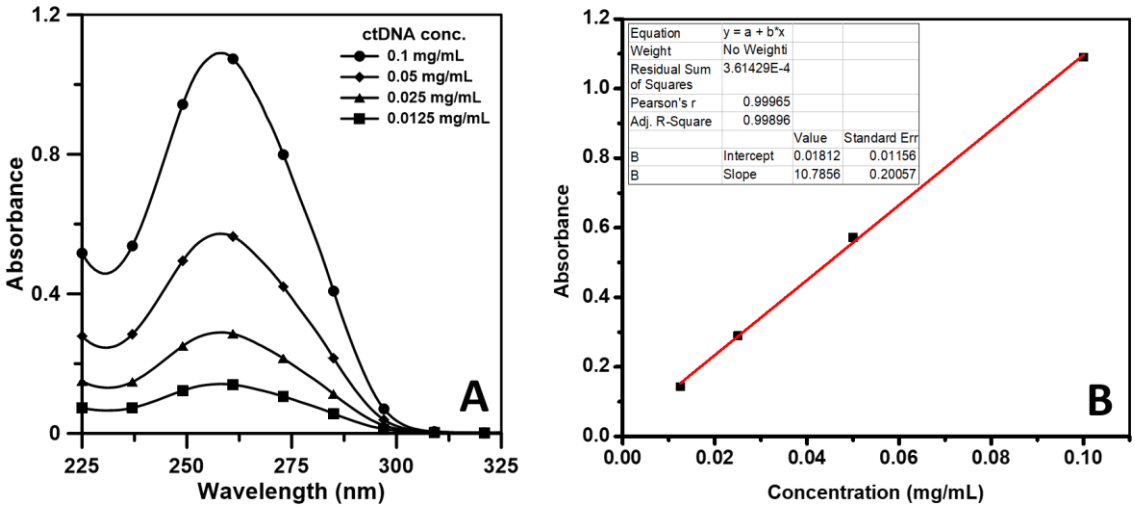


Figure S16. UV-Vis absorption spectra of neat ctDNA in Tris-HCl buffer against the variation of its concentration (A). Calibration curve of neat ctDNA in Tris-HCl buffer (B).

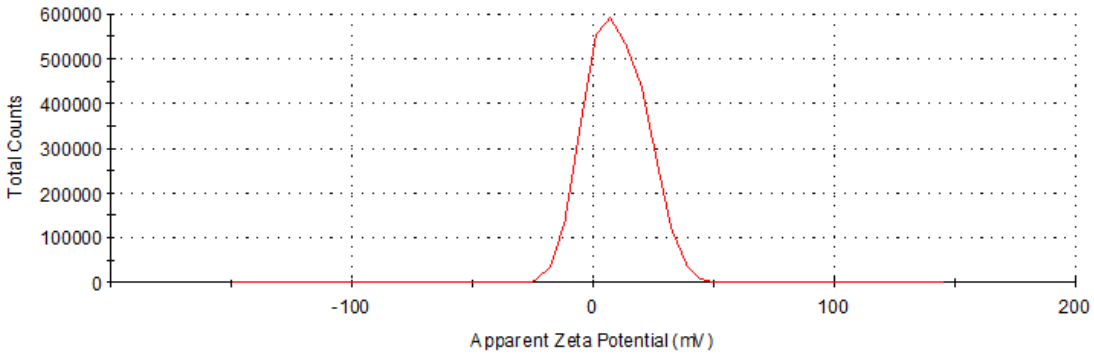


Figure S17. Zeta potential (ξ) curve of 0.1 wt% P[Cys-PPh_3]$^+$[Br^-]-1 in H_2O.

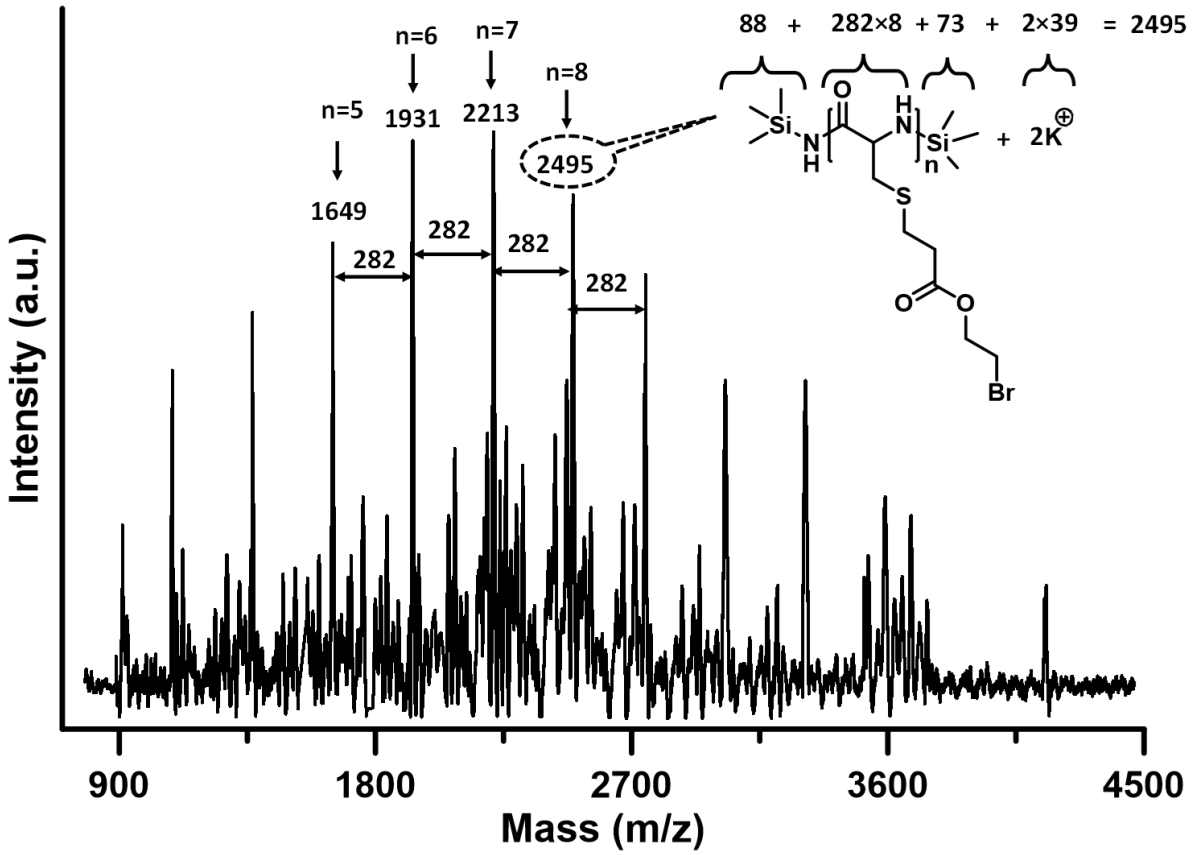


Figure S18. MALDI-TOF-MS of PCys-Br-1 in DMF using DHB matrix and NaI.

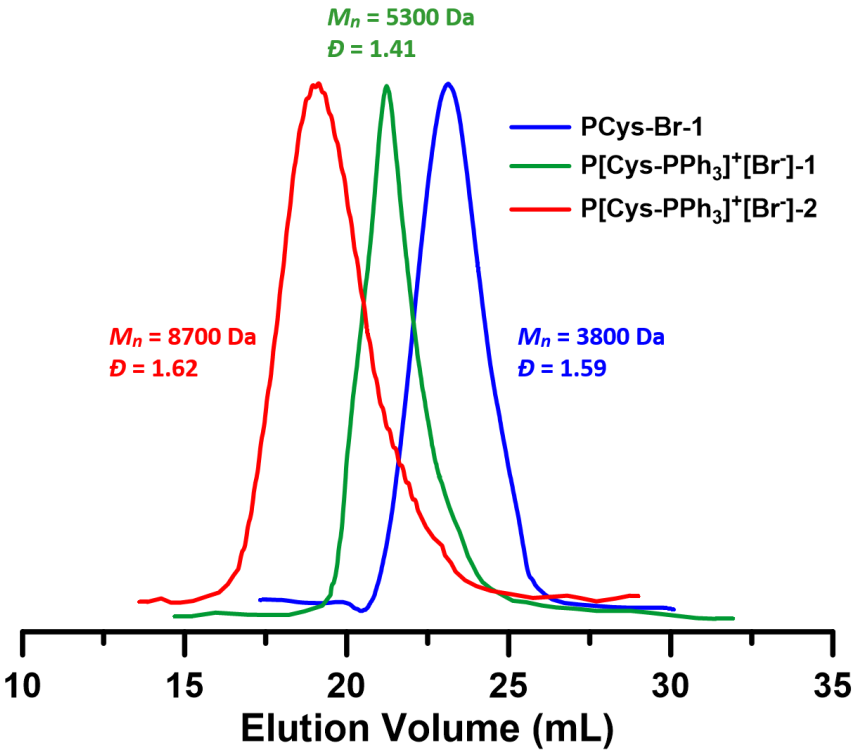


Figure S19. SEC chromatograms of PCys-Br-1, P[Cys-PPh_3]$^+$[Br^-]-1 and P[Cys-PPh_3]$^+$[Br^-]-2.

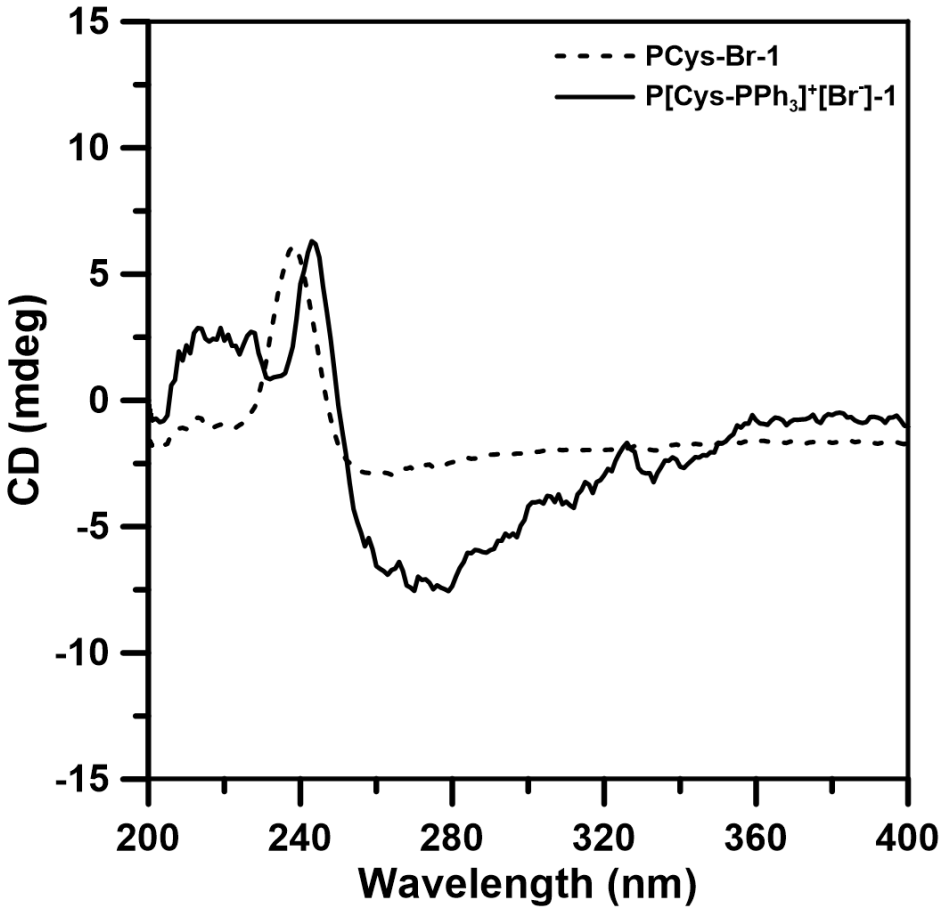


Figure S20. CD spectra of PCys-Br-1 and P[Cys-PPh_3]$^+$[Br^-]-1 showing antiparallel β-sheet like secondary structure.

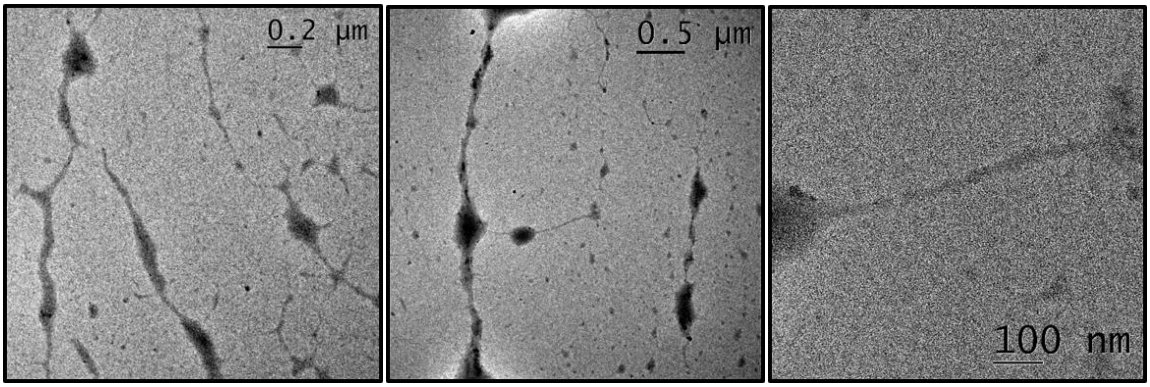


Figure S21. TEM image of P[Cys-PPh_3]$^+$[Br^-]-1(1 mg/mL) in water.

Table S1. Sizes of aggregates of P[Cys-PPh_3]$^+$[Br^-]-1 (0.5 wt%) in the presence of anions in water obtained from DLS experiment

Polypeptide	**Anion**	**[Anion]$_{min}$ (mM)**	**[a] D_h at 25 °C (nm)**	**[b] T_{cp} (°C) (DLS) (Heating)**	**T_{cp} (°C) (Turbidity) (Heating)**	**T_{cp} (°C) (Turbidity) (Cooling)**
P[Cys-PPh_3]$^+$[Br^-]-1 (0.5 wt%)	BF_4^-	115	1700	36	39	37
	I^-	65	1200	40	45	47
	ClO_4^-	35	850	49	57	57.4
	SCN^-	15	800	58	66	67

[a] D_h – Hydrodynamic diameter

[b] T_{cp} – Cloud point (data obtained from the temperature-dependent DLS study as shown in Figure S13)

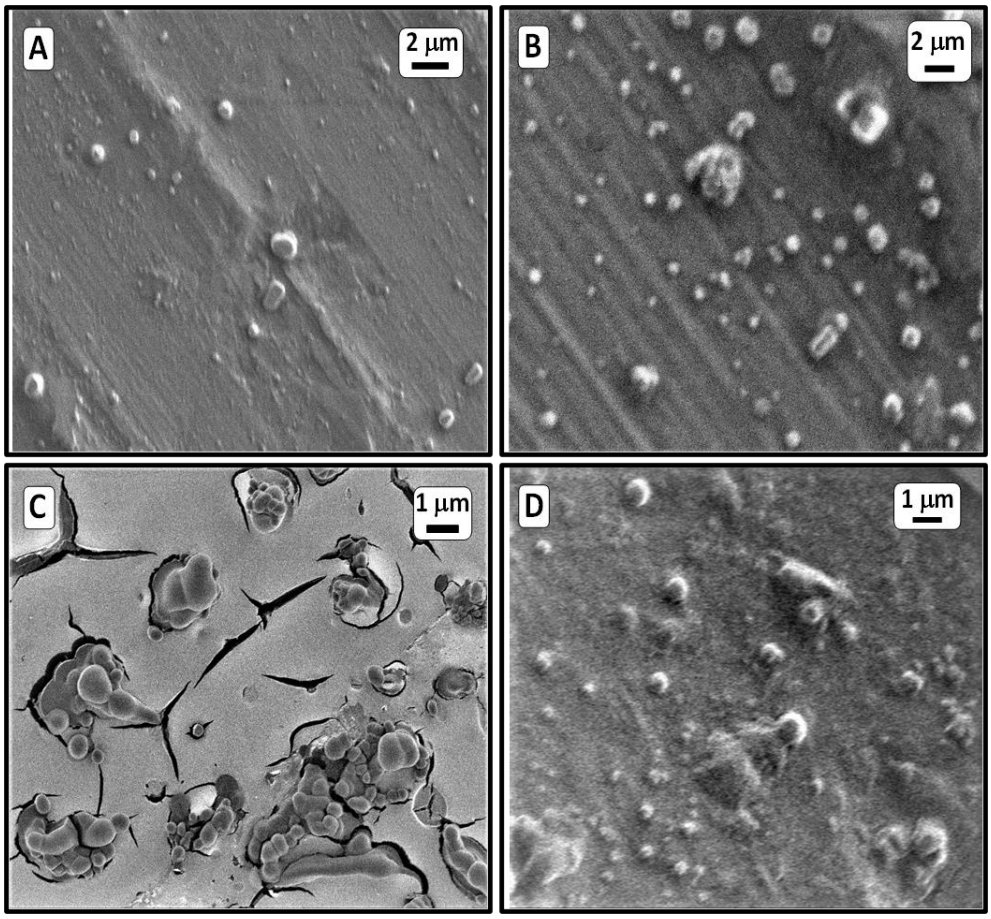


Figure S22. FESEM images of P[Cys-PPh_3]$^+$[Br^-]-1 (0.5 wt%) aggregates in water in the presence of 115 mM BF_4^- (A), 65 mM I^- (B), 35 mM ClO_4^- (C) and 15 mM SCN^- (D), showing spherical morphologies.

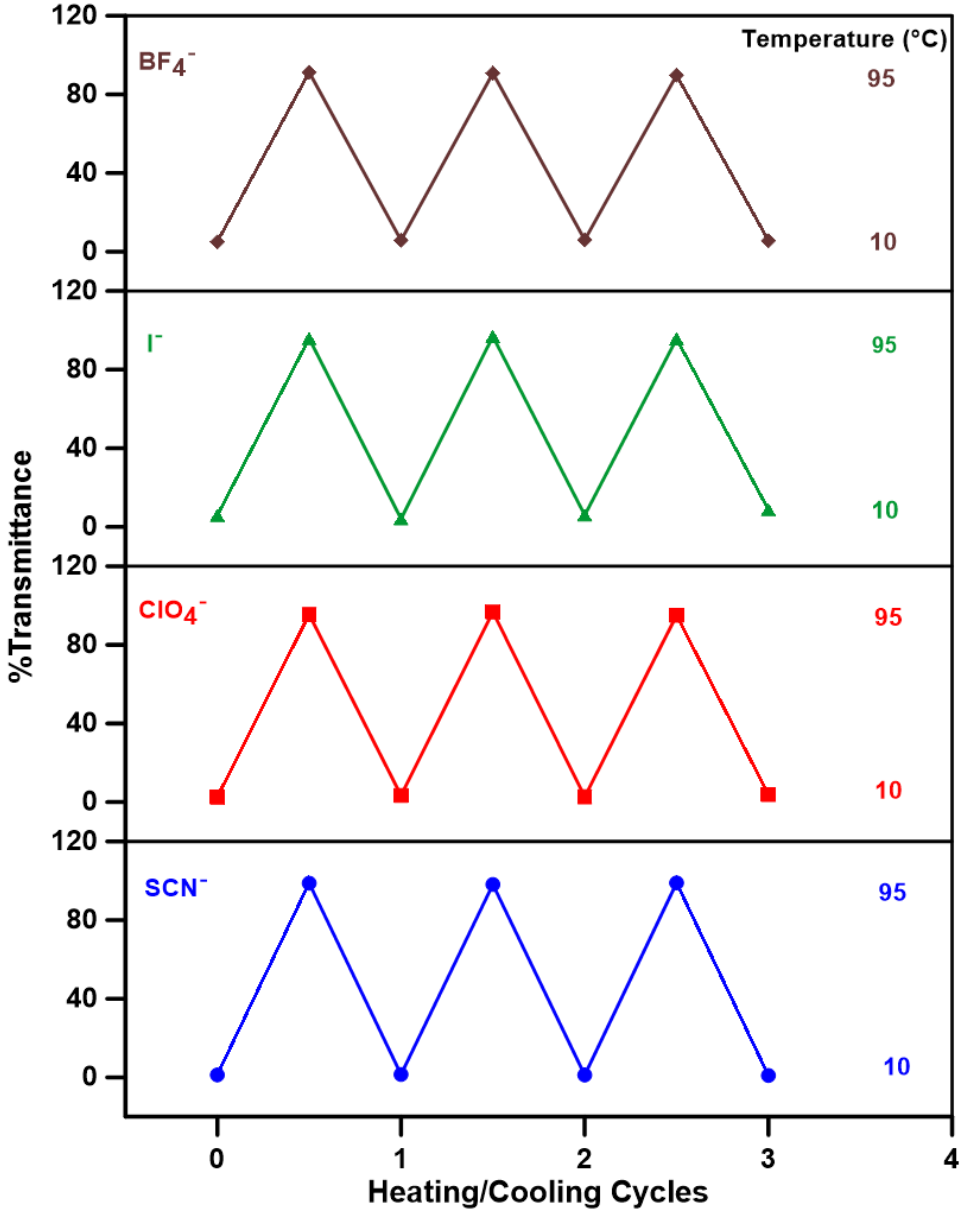


Figure S23. Temperature-dependent % transmittance of 0.5 wt% aqueous P[Cys-PPh_3]$^+$[Br^-]-1 solutions in the presence of different chaotropic anions. Each data point was obtained after equilibrating the corresponding sample solution at the particular temperature for 5 min.

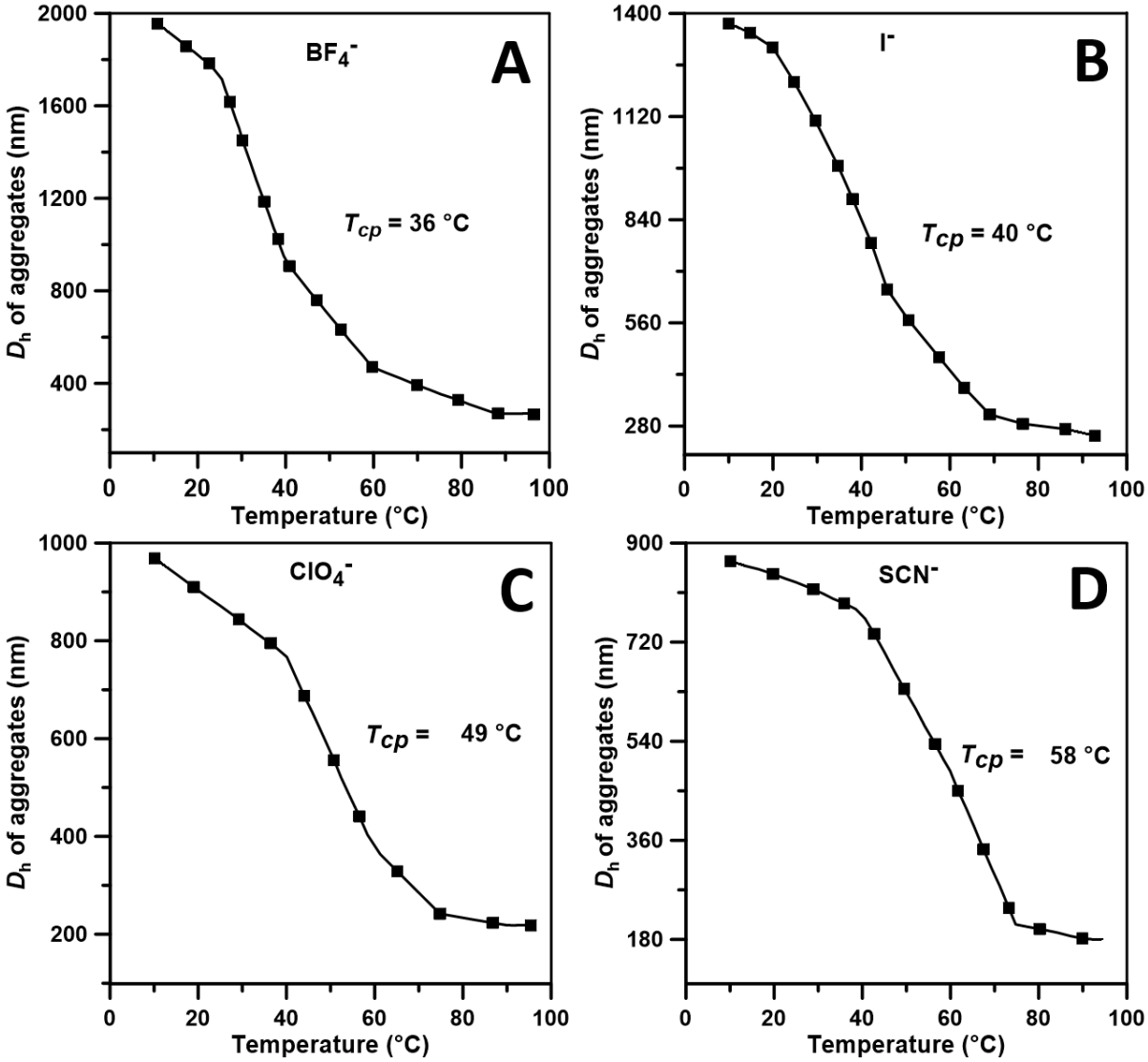


Figure S24. Variation of the D_hs of aggregates of P[Cys-PPh_3]$^+$[Br^-]-1 (0.5 wt%) in the presence of 115 mM BF_4^- (A), 65 mM I^- (B), 35 mM ClO_4^- (C) and 15 mM SCN^- (D) at different temperatures.

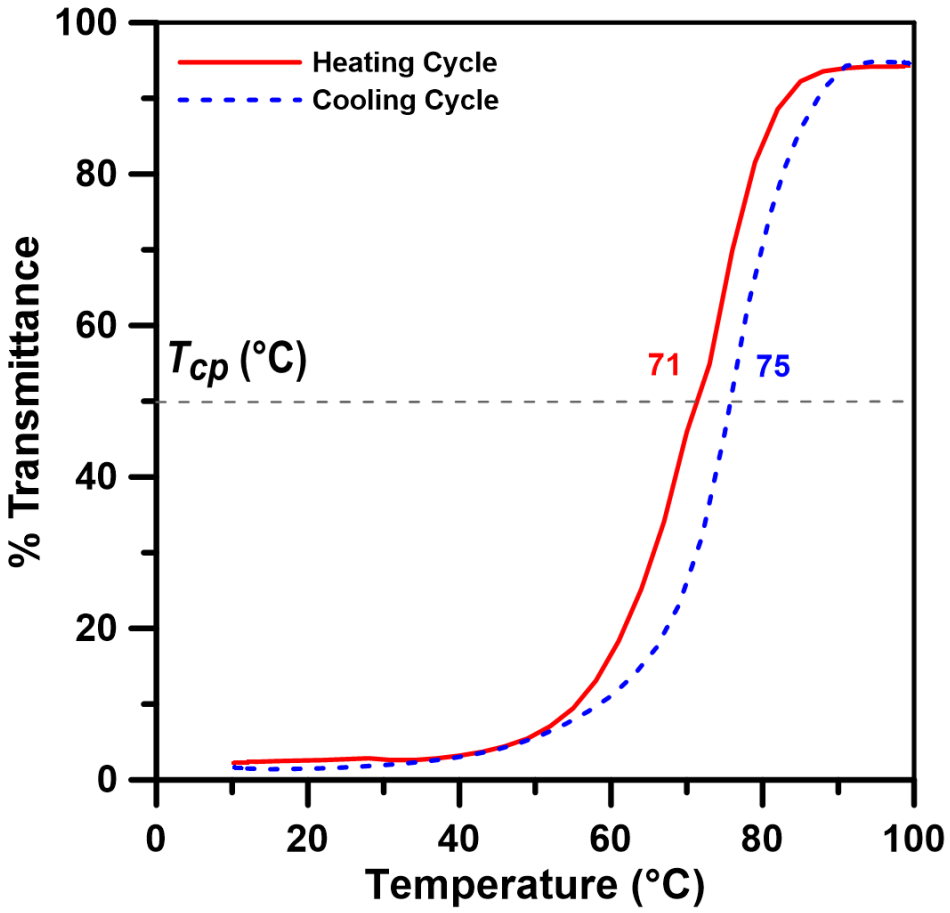


Figure S25. Turbidity plot (λ= 500 nm) of aqueous P[Cys-PPh_3]$^+$[Br^-]-2 (0.5 wt%) solution in the presence of 22 mM SCN^-.

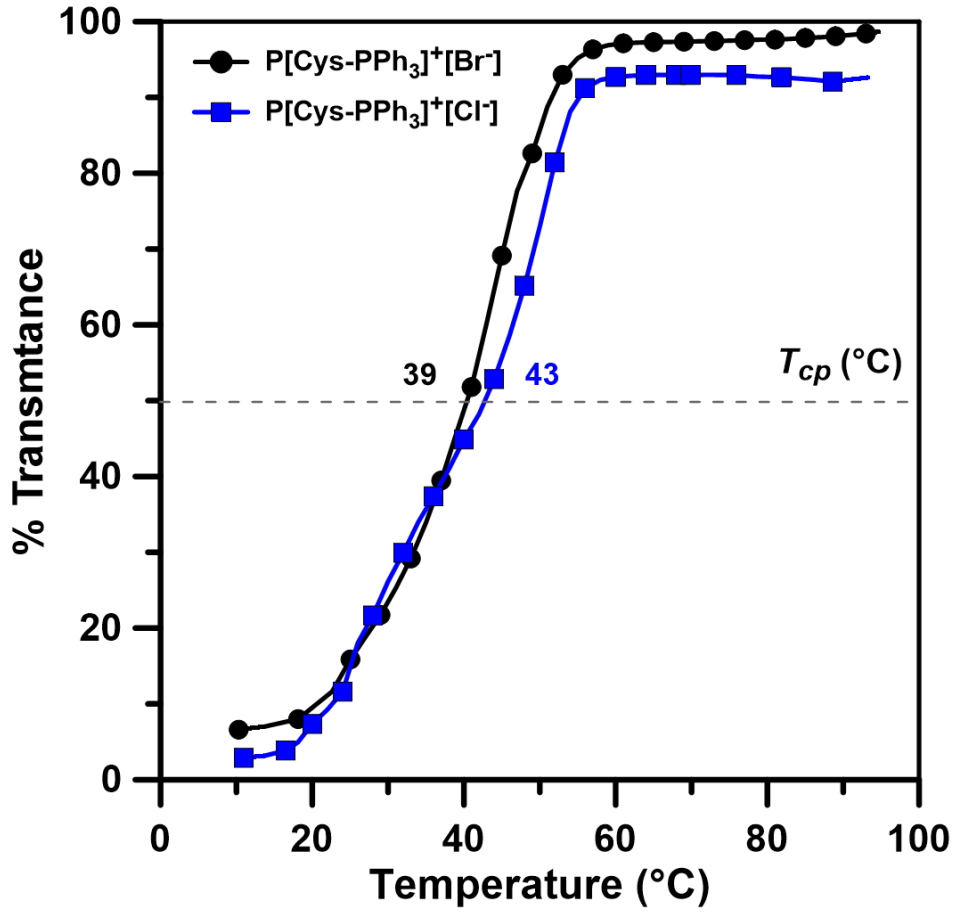


Figure S26. Turbidity plot (λ= 500 nm) of aqueous P[Cys-PPh_3]$^+$[Br^-] and P[Cys-PPh_3]$^+$[Cl^-] (0.5 wt%) solution in the presence of 115 mM BF_4^-.

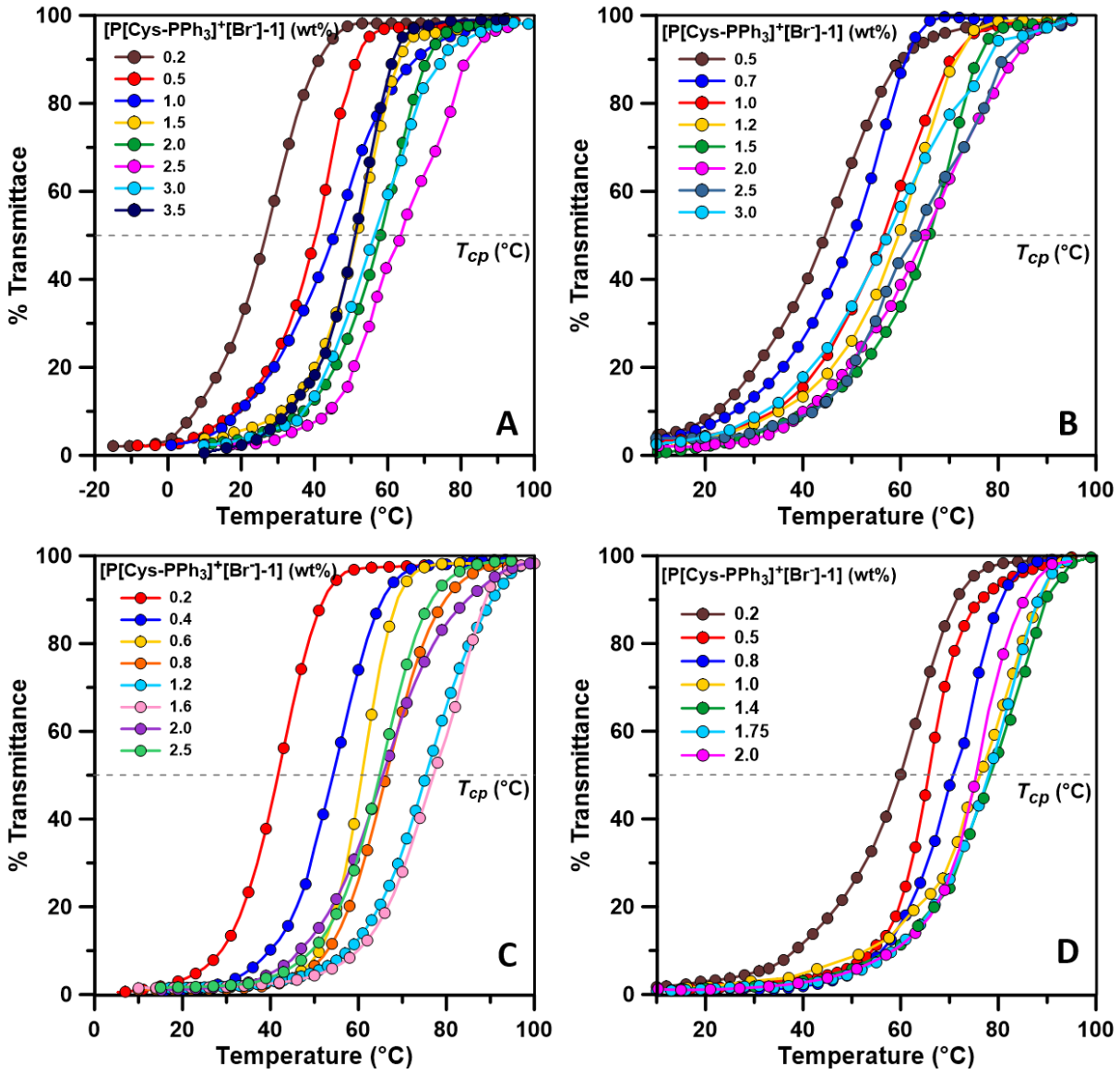


Figure S27. Turbidity plots (λ= 500 nm) of aqueous P[Cys-PPh_3]$^+$[Br^-]-1 solutions with varying polypeptide concentration in the presence of 115 mM BF_4^- (A), 65 mM I^- (B), 35 mM ClO_4^- (C) and 15 mM SCN^- (D).

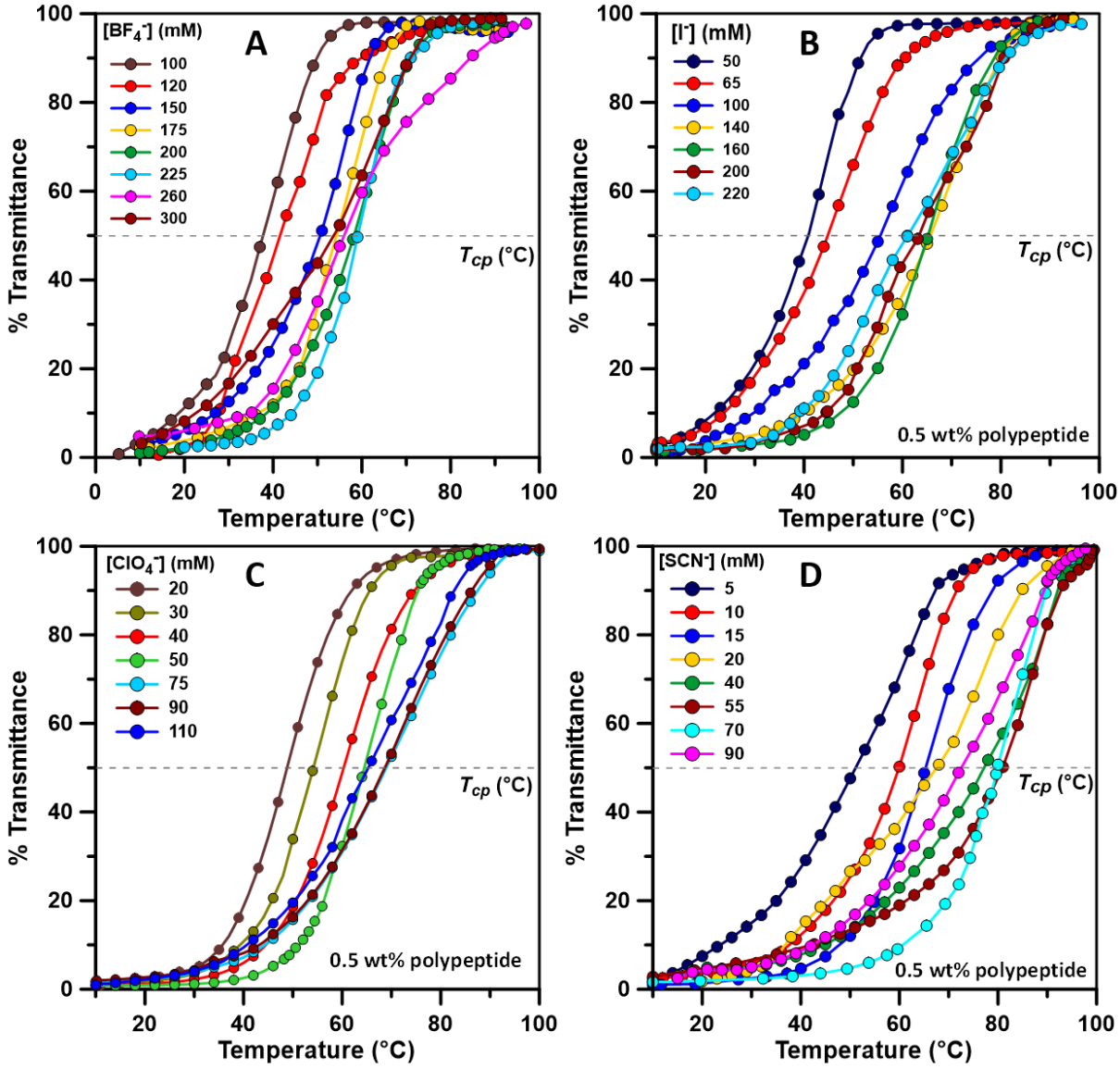


Figure S28. Turbidity plots (λ= 500 nm) of 0.5 wt% aqueous P[Cys-PPh_3]$^+$[Br^-]-1 solutions with varying concentration of BF_4^- (A), I^- (B), ClO_4^- (C) and SCN^- (D).

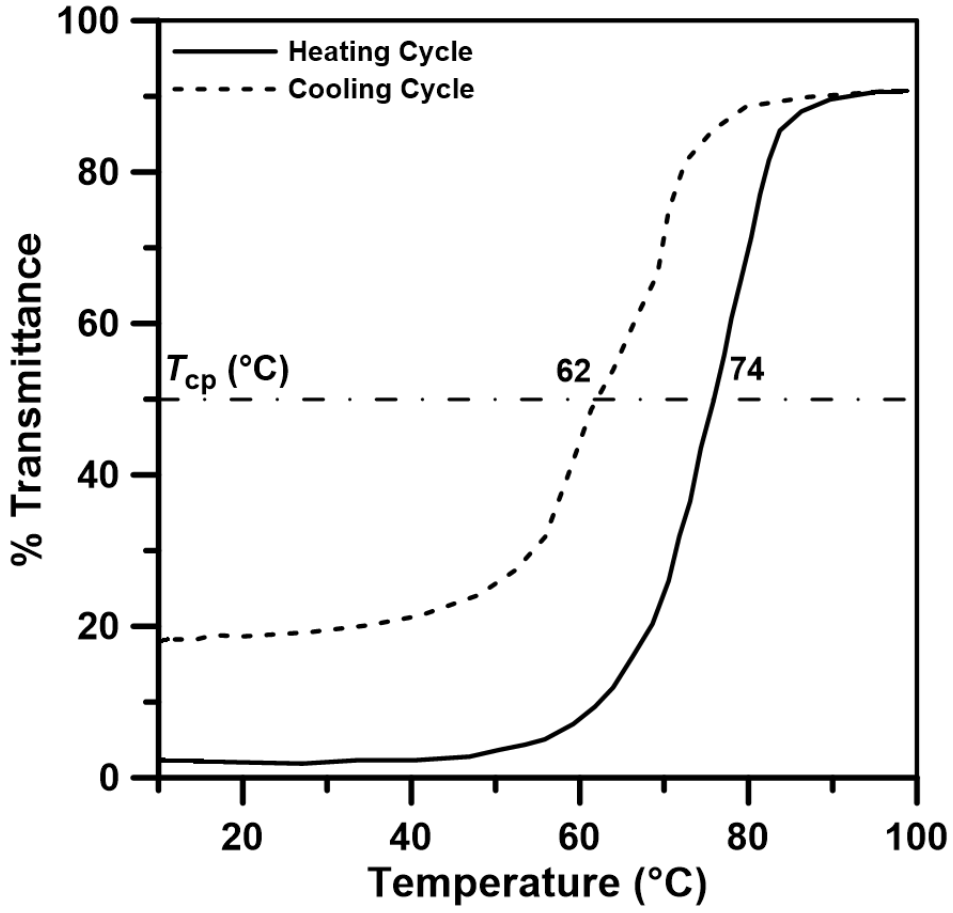


Figure S29. UCST-type turbidity curve (λ= 500 nm) of 0.5 wt% aqueous solution of P[Cys-PPh_3]$^+$[Br^-]-1 in the presence of 500 μM of SDS; heating and cooling run showed huge hysteresis.

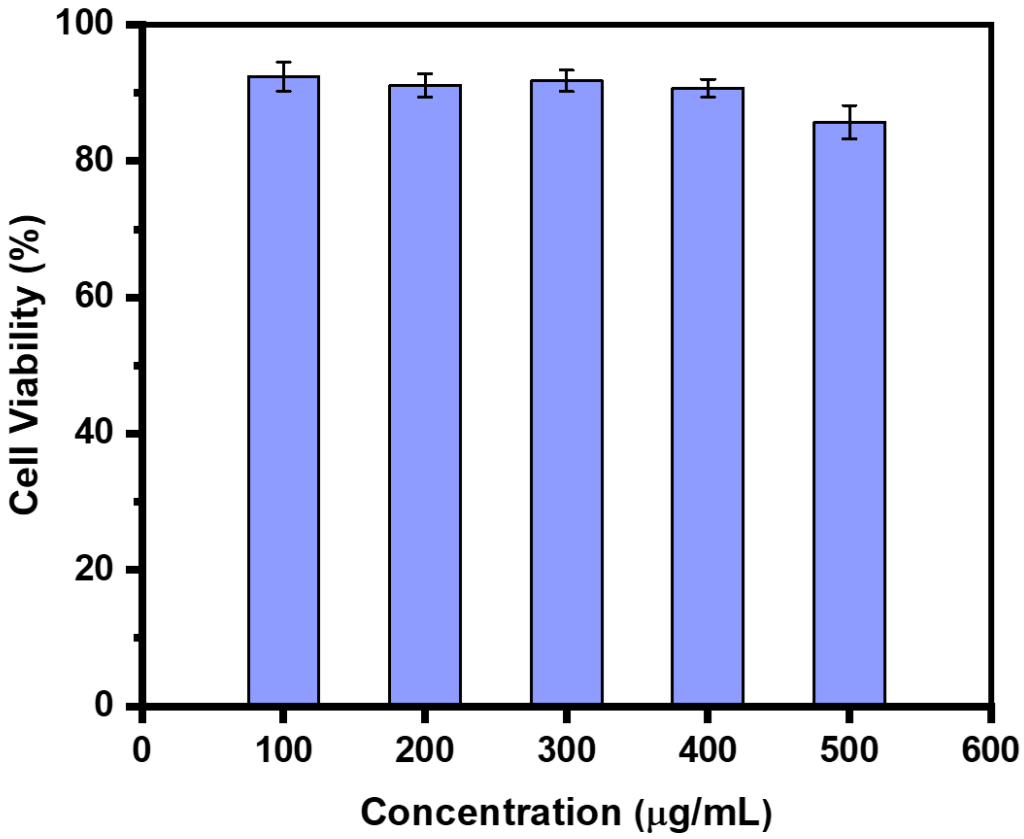
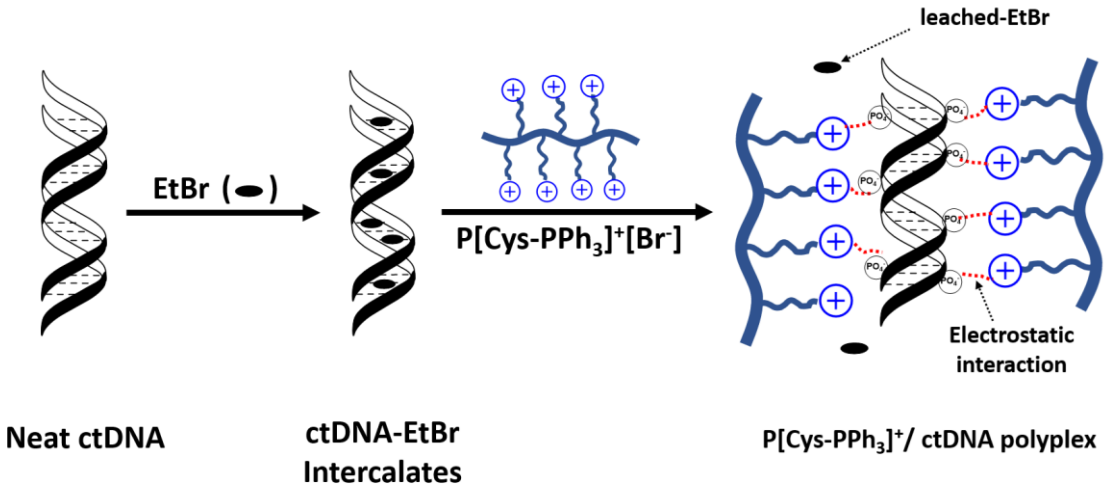


Figure S30. The cell viability of KB cells with different concentrations of P[Cys-PPh_3]$^+$[Br^-]-1 with after 24 h of incubation.



Scheme S2. Schematics of polyplex formation of $P[Cys\text{-}PPh_3]^+[Br^-]$ with ctDNA in aqueous solution.

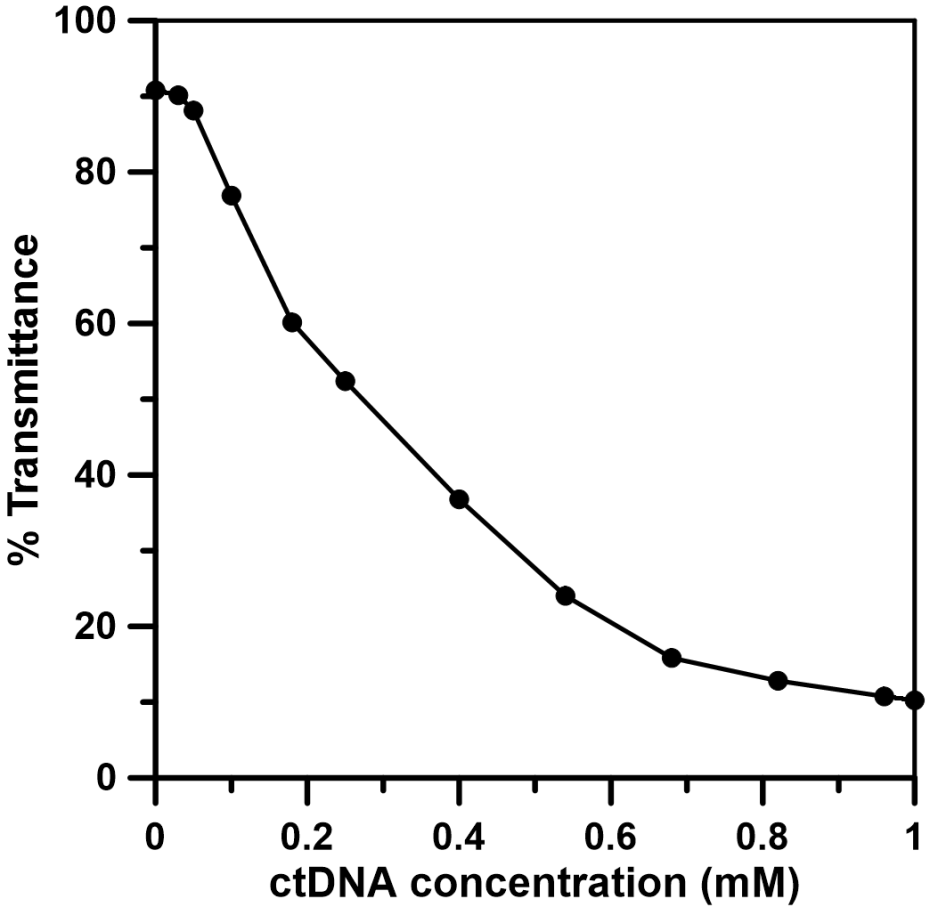


Figure S31. Titration plot, showing the % transmittance of 0.5 wt% aqueous P[Cys-PPh_3]$^+$[Br^-]-1 solution in the presence of different concentration of ctDNA.

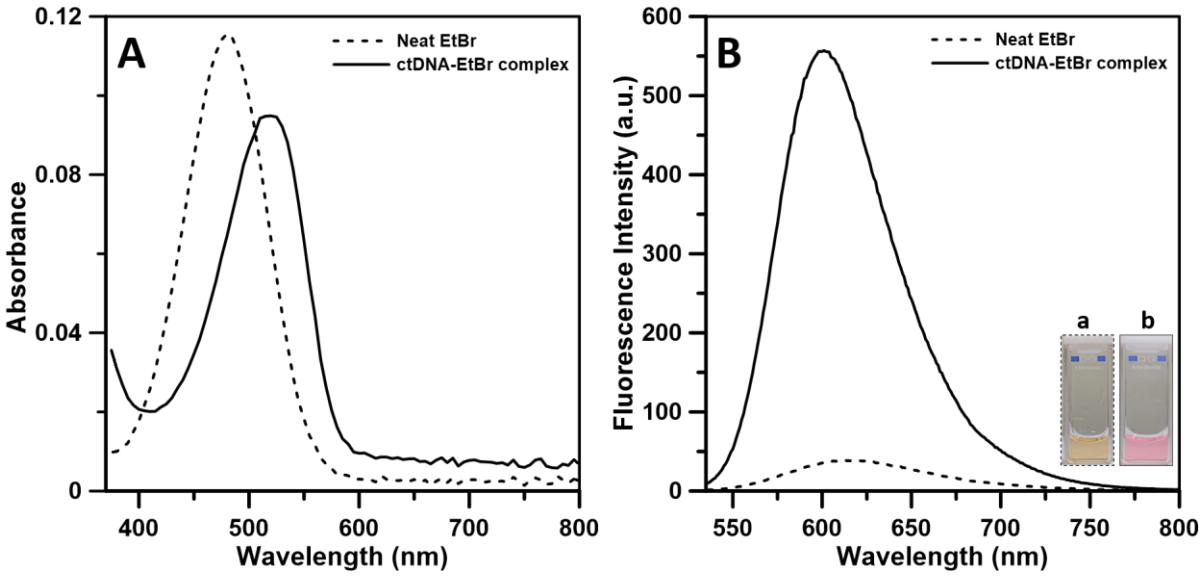


Figure S32. Absorption (A) and emission (B) spectra of neat EtBr in water and EtBr-ctDNA complex in Tris-HCl buffer. Inset of the Figure B showed the change in colour of EtBr solution before (a) and after (b) the intercalation with ctDNA.

Figure S33. Photographs showing the decrease in colour intensity of the ctDNA-EtBr complex with increase in P[Cys-PPh_3]$^+$[Br^-]-1 concentration in Tris-HCl buffer, indicating the leaching out of EtBr from its intercalated state into the bulk solvent.

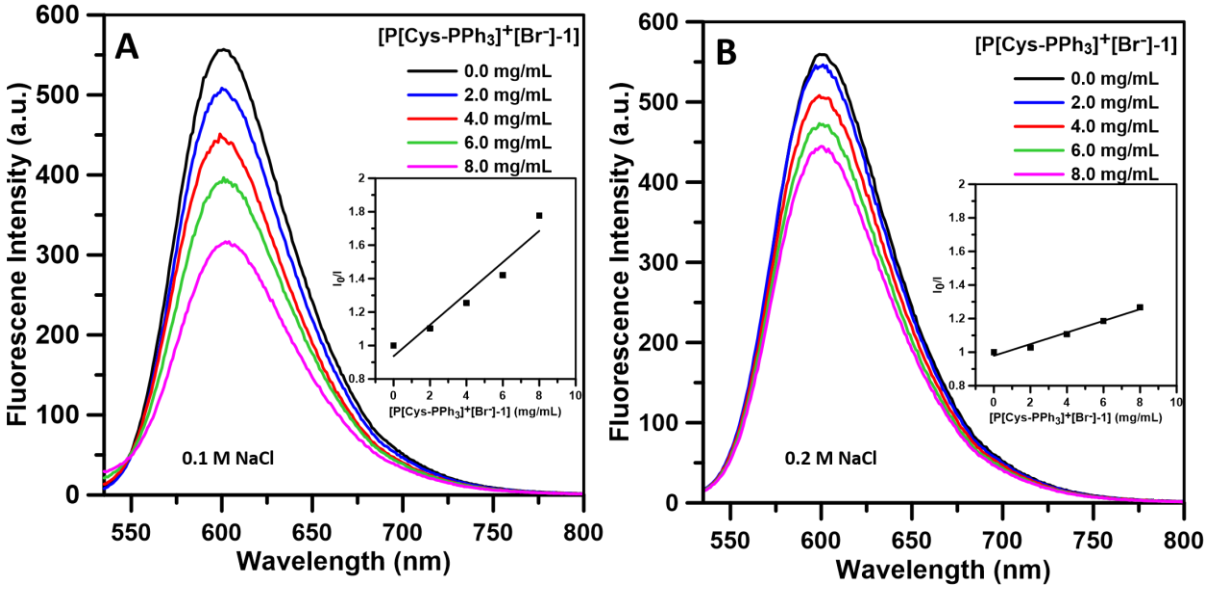


Figure S34. Emission spectra of EtBr-ctDNA complex in the presence of different amount of P[Cys-PPh_3]$^+$[Br^-]-1 with 0.1 M NaCl (A) and 0.2 M NaCl (B) showing the decrease in extent of fluorescence quenching with increase of the ionic strength. The insets of the both figures showed the plot of I_0/I vs respective [P[Cys-PPh_3]$^+$[Br^-]-1], revealing the decrease in fluorescence quenching of EtBr-ctDNA complex by P[Cys-PPh_3]$^+$[Br^-]-1.