Electronic Supplementary Material (ESI) for Analyst.

Reversible Fluorescence Modulation of BSA Stabilised Copper Nanoclusters for the Selective Detection of Protamine and Heparin

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Supporting Information

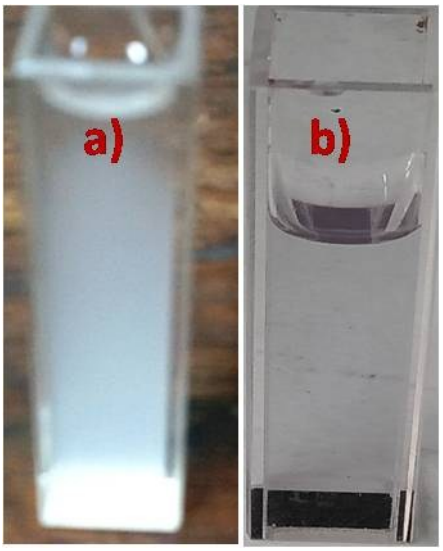

Fig.S.1 Photographs showing (a) BSA Copper complex (b) BSA-CuNCs under day light.

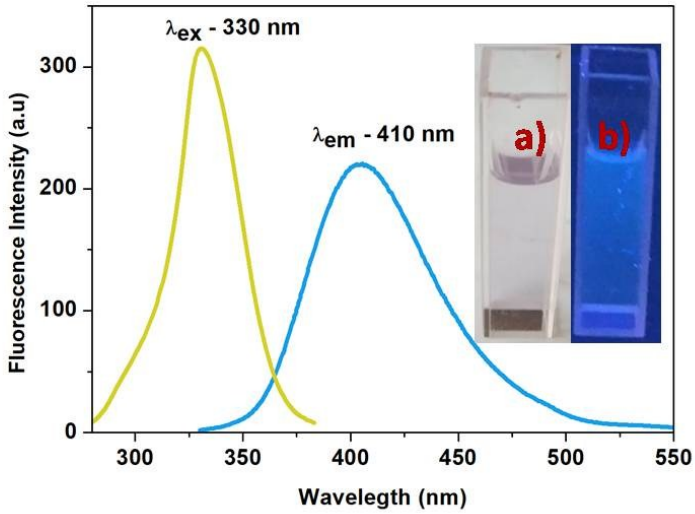

Fig.S.2 The excitation (yellow line) and emission spectra (blue line) of BSA-CuNCs at room temperature (λ_{ex} 330 nm, λ_{em} 410 nm). The inset photographs show BSA-CuNCs **(a)** under daylight **(b)** under UV light (365 nm).

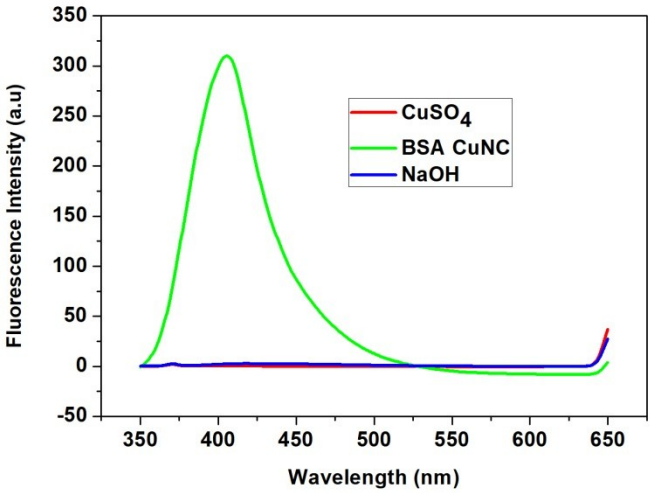

Fig.S.3 Fluorescence emission spectra of $CuSO_4$, BSA-CuNCs and NaOH in aqueous solution at room temperature (λ_{ex} 330 nm, λ_{em} 410 nm).

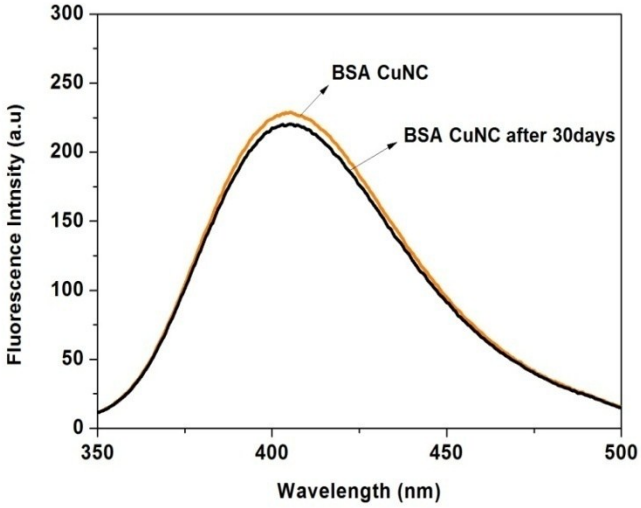

Fig.S.4 Fluorescence stability of BSA CuNCs after 30 days of storage at 4^0C (λ_{ex} 330 nm, λ_{em} 410 nm).

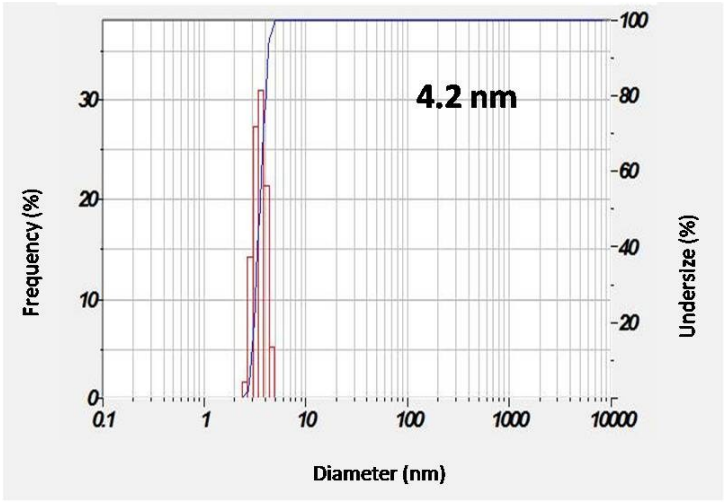

Fig.S.5 The DLS size distribution of BSA-CuNCs. Size obtained is 4.2 nm.

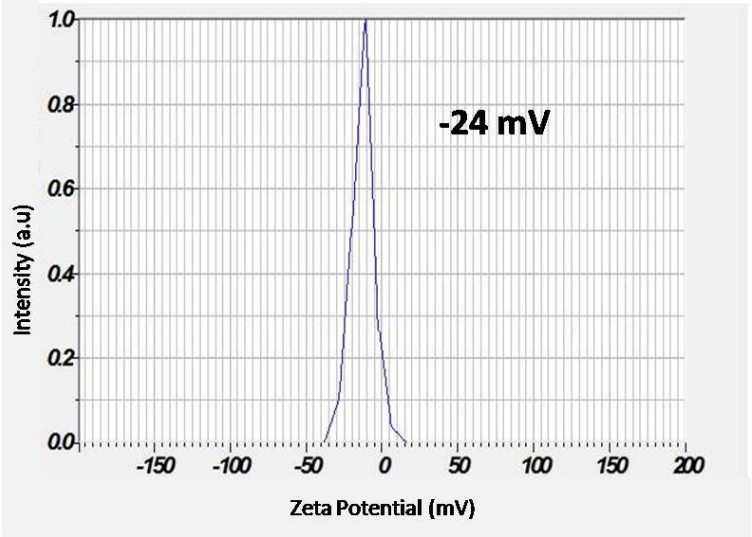

Fig.S.6 Zeta potential analysis of BSA-CuNCs. Surface charge of BSA-CuNCs obtained is -24 mV.

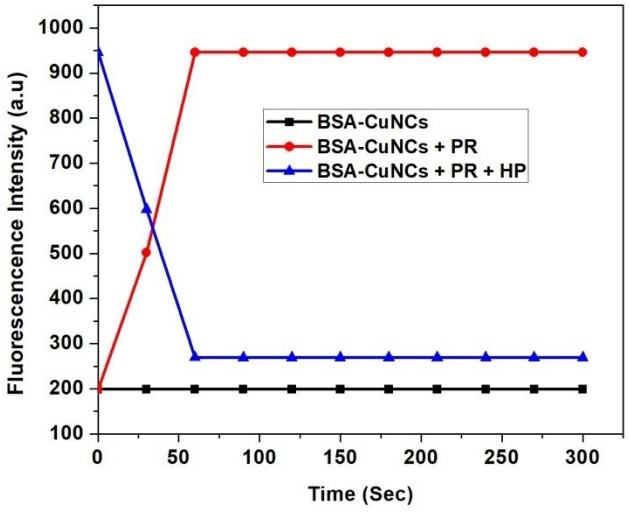

Fig.S.7 The time response curve of BSA- CuNCs, protamine added BSA-CuNCs and heparin added BSA-CuNCs/PR system.

Table.S.1 Comparison of the reported fluorescence probe for the selective detection of protamine.

Sensor	Detection limit	Reference
Carbon quantum dot and gold nanoparticle	1.2 ng/mL	1
Glutathione capped CdTe quantum dot	1.0 ng/mL	2
Gold nanoparticle coupled with fluorophore	0.0067 µg/mL	3
Silicon dot coupled with gold nanoparticle	6.7 ng/mL	4
FITC labelled DNA	2.2 ng/mL	5
BSA-CuNCs	0.12 ng/mL	Present method

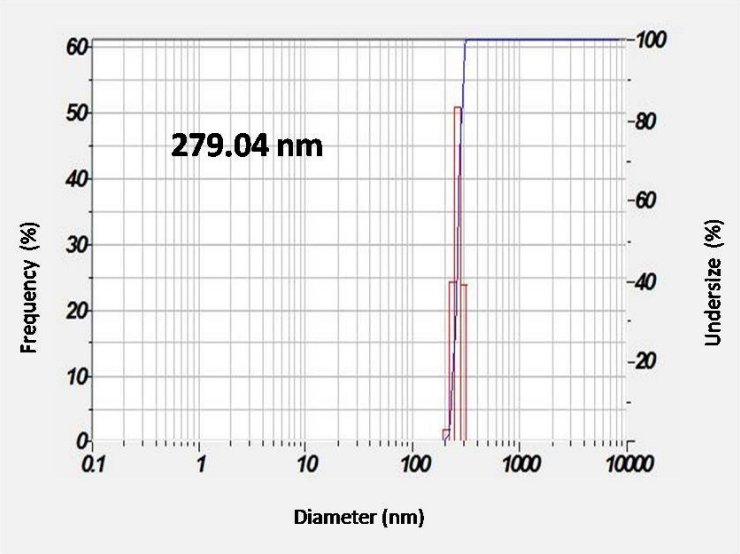

Fig.S.8 The hydrodynamic size of protamine added BSA-CuNCs. Size obtained is 279.04 nm.

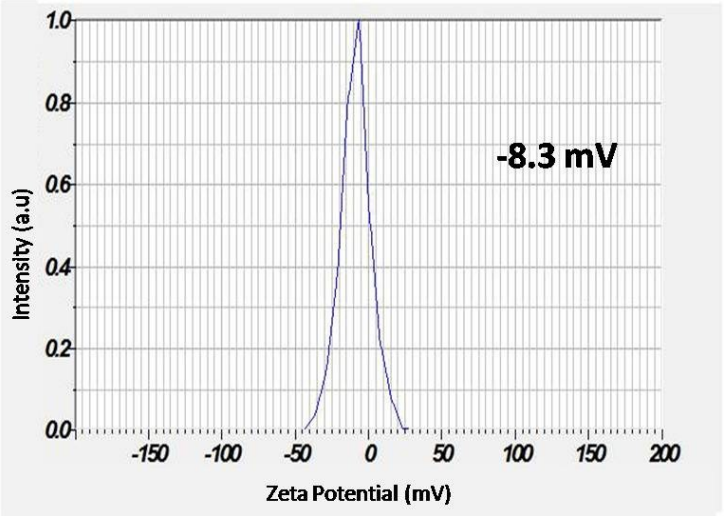

Fig.S.9 The zeta potential analysis of protamine added BSA-CuNCs. Surface charge obtained is -8.3 mV.

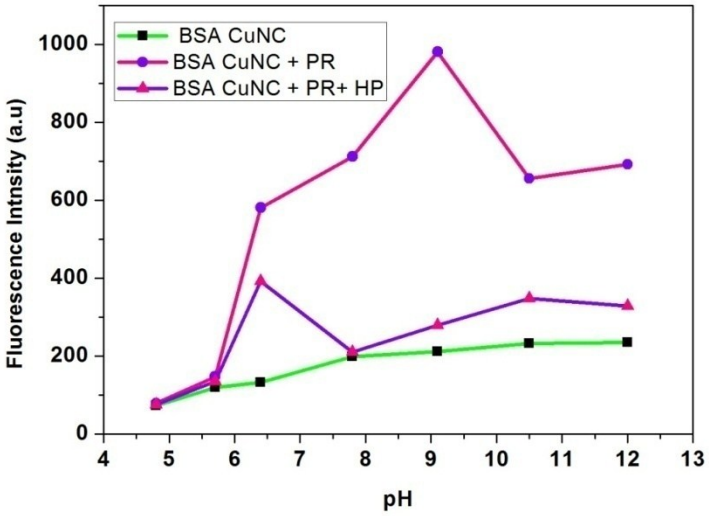

Fig.S.10 Effect of pH on the fluorescence intensity of BSA-CuNCs, Protamine added BSA-CuNCs and Heparin added BSA-CuNCs/PR system at room temperature (λ_{ex} 330 nm, λ_{em} 410 nm).

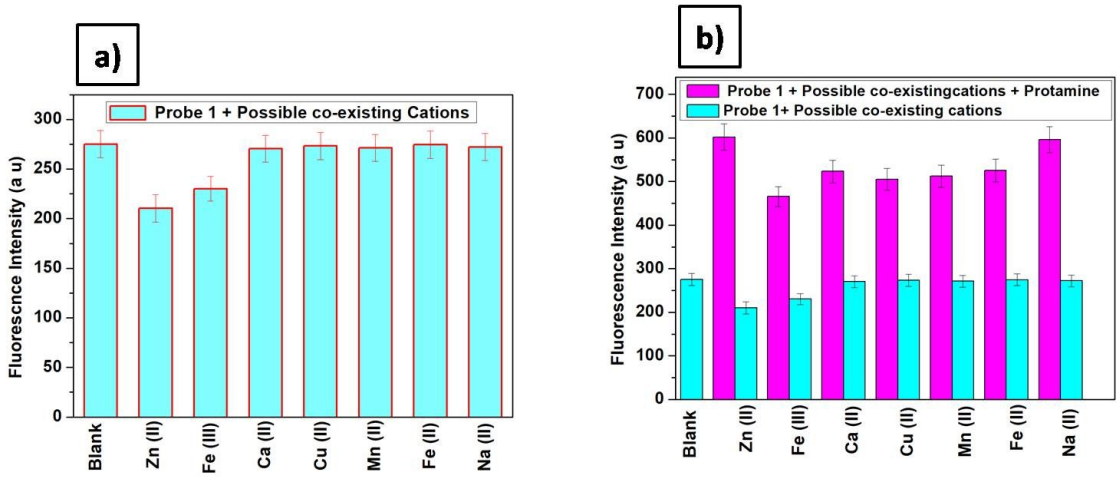

Fig.S.11 (a) Selectivity of BSA-CuNCs over other co-existing cations such as Zn(II), Fe(III), Ca(II), Cu(II), Mn(II), Fe(II) and Na(II) **(b)** Sensitivity of BSA-CuNCs/ PR system over other co-existing cations. (λ_{ex} 330 nm, λ_{em} 410 nm). Probe 1 represents BSA-CuNCs. Concentration of all cations was kept as 100 μM.

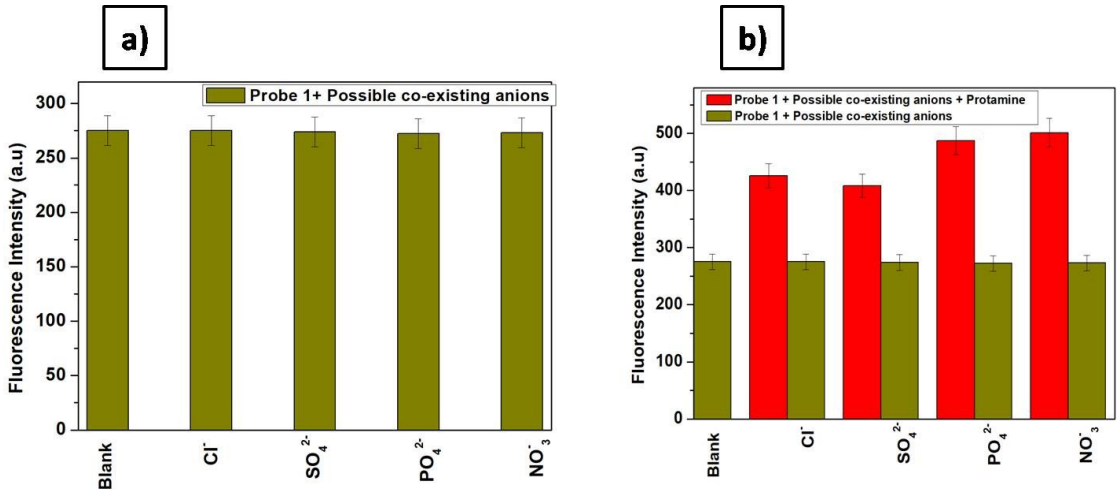

Fig.S.12 (a) Selectivity of BSA-CuNCs over other co-existing anions **(b)** Sensitivity of BSA-CuNCs/ PR system over other co-existing anions such as Cl^-, SO_4^{2-}, PO_4^{2-} and NO_3^- (λ_{ex} 330 nm, λ_{em} 410 nm). Probe 1 represents BSA-CuNCs. Concentration of all anions was kept as 100 µM.

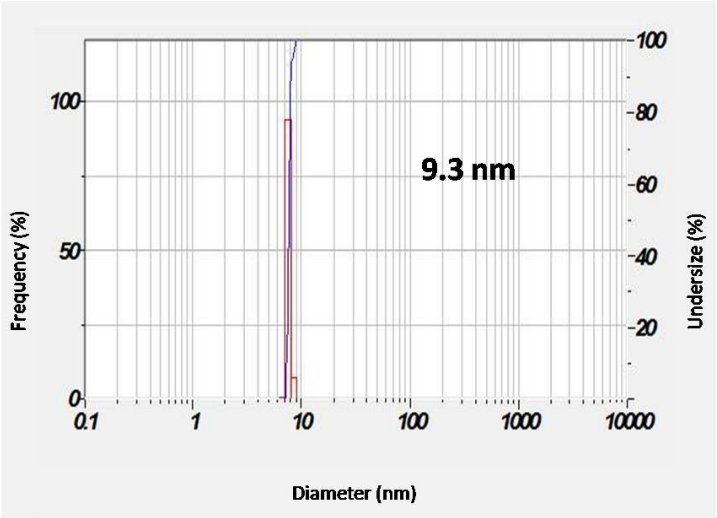

Fig.S.13 The hydrodynamic size of heparin added BSA-CuNCs/PR system. Size obtained is 9.3 nm.

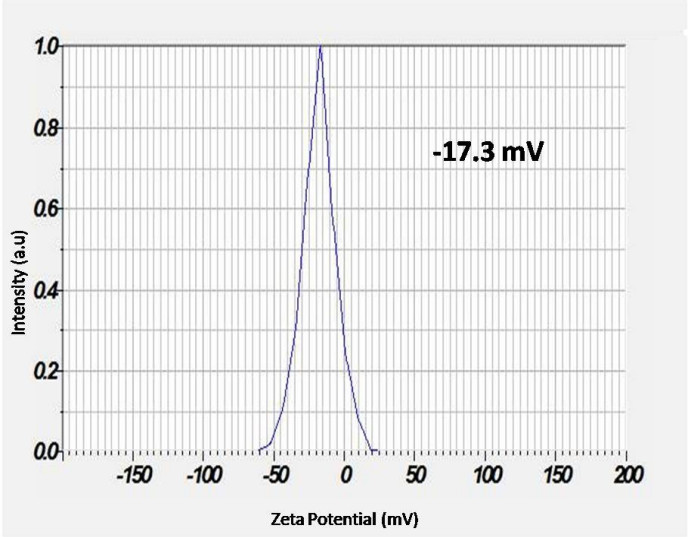

Fig.S.14 The zeta potential analysis of heparin added BSA-CuNCs/PR system. Surface charge obtained is -17.3 mV.

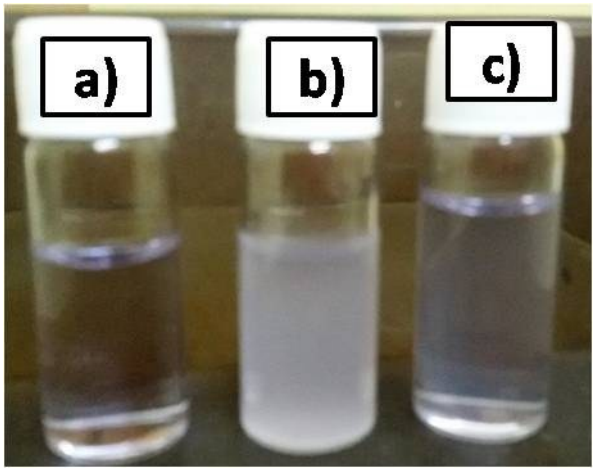

Fig.S.15 (a) Photographs of BSA-CuNCs, **(b)** BSA- CuNCs/PR system, **(c)** BSA-CuNCs/PR after addition of heparin under day light.

Table.S.2 Comparison of the reported detection methods for the selective detection of heparin.

Sensor	Detection limit	Reference
Silicon quantum dot coupled with gold nanoparticle	0.67 ng/mL	4
Cysteamine capped gold nanoparticle	0.03 μg/mL	6
Thioglycolic acid capped CdTe quantum dot	0.033 μg/mL	7
Gold nanoparticle on grapheme oxide	3.0 ng/mL	8
Emissive H aggregate of Thioflavin T	18 nM	9
4-MPY functionalized silver nanoparticles	0.5 ng/mL	10
BSA CuNC/PR system	0.041 ng/mL	Present method

Table.S.3 Nanosecond time resolved luminescent transients of BSA CuNCs, BSA CuNCs/PR system, heparin added BSA CuNCs/PR system. Pulsed excitation of 330 nm is used to measure the decay profile.

System	τ_1 (ns)	τ_2 (ns)	τ_{av} (ns)
BSA CuNC	1.49 (24.10%)	7.70 (75.90%)	7.28
BSA CuNC + PR	1.47 (24.72%)	7.87 (75.28%)	7.50
BSA CuNC + PR + HP	1.45 (23.95%)	7.56 (76.05%)	7.21

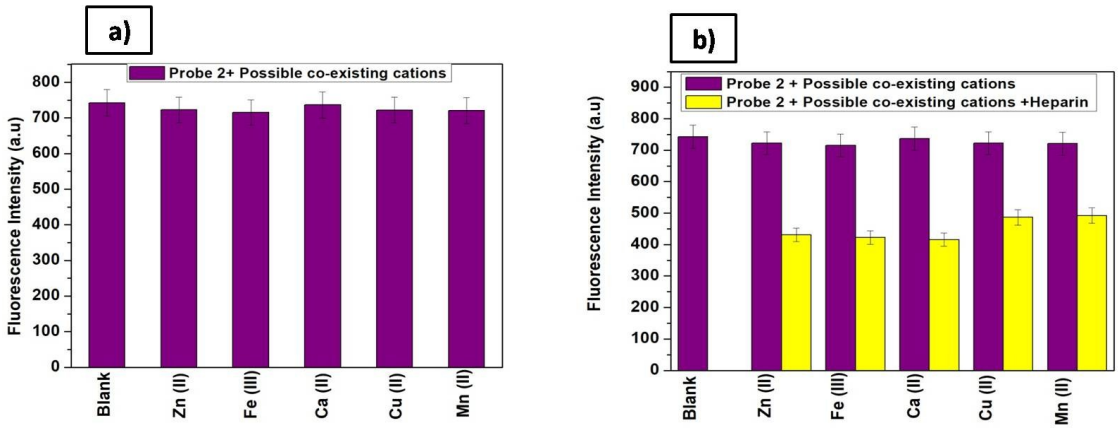

Fig.S.16 (a) Selectivity of BSA-CuNCs/PR system over other co-existing cations such as Zn(II), Fe(III), Ca(II), Cu(II), and Mn(II) **(b)** Sensitivity of heparin added BSA-CuNCs/ PR system over other co-existing cations (λ_{ex} 330 nm, λ_{em} 410 nm). Probe 2 represents BSA-CuNCs/PR. Concentration of all cations was kept as 100 µM.

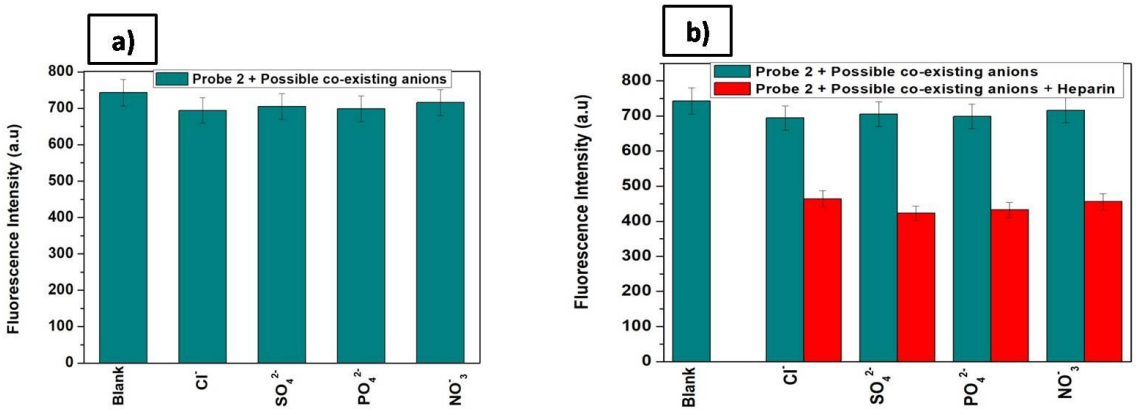

Fig.S.17 (a) Selectivity of BSA-CuNCs/PR system over other co-existing anions **(b)** Sensitivity of heparin added BSA-CuNCs/ PR system over other co-existing anions such as Cl^-, SO_4^{2-}, PO_4^{2-} and NO_3^- (λ_{ex} 330 nm, λ_{em} 410 nm). Probe 2 represents BSA-CuNCs/PR. Concentration of all anions was kept as 100 µM.

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