Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Title: *Triple Action Polymer Probe: Carboxylic Distilbene Fluorescent Polymer Chemosensor for Temperature, Metal-ion and Biomolecule*

Authors: A. Balamurugan, Vikash Kumar and M. Jayakannan*

Experimental section

Materials: All the metal perchlorate salts and PIPES diasodium salt were purchased from Aldrich chemicals. Sodium hydroxide, methanol and tetrahydrafuran were locally purchased and purified by using standard protocols. The monomers of 3-(3,5-bis ((diethoxyphosphoryl) methyl)-4-(2-ethylhexyloxy) phenyl) propanoic acid, 4,4'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)dibenzaldehyde and 4,4'-(3,6,9,12,15-pentaoxaheptadecane-1,17-diylbis(oxy))dibenzaldehyde were synthesised as described in our earlier reports.[1]

General procedures: ^{1}H-NMR and ^{13}C-NMR spectra of the polymers were recorded using 400-MHz Joel NMR spectrophotometer in $CDCl_3$ containing small amount of TMS as internal standard. Infra-red spectra of the polymers were recorded using a Thermo-Scientific Nicolet 6700 FT-IR spectrometer with the solid state in KBr. The purity and molecular weight of the polymers were determined by gel permeation chromatographic (GPC) analysis, which was performed using a Viscotek VE 1122 pump, Viscotek VE 3580 RI detector, and Viscotek VE 3210 UV/vis detector in tetrahydrofuran (THF) using polystyrene as standards. The absorption and emission studies were done by a Perkin-Elmer Lambda 35 UV-Visible spectrophotometer and SPEX Flurolog DM3000Fspectrofluorimeter with a double-grating 0.22 m Spex1680 monochromator and a 450W Xe lamp as the excitation source using front face mode at room temperature. The emission spectra were recorded in water. The fluorescence quantum yields of the polymers were determined using quinine sulfate in 0.1N Conc. H_2SO_4 (ϕ = 0.53) as the standard by exciting at 310 nm respectively. The concentration of the polymer and oligomer solutions were adjusted in such a way to obtain the absorbance equal to 0.1 at 415 nm respectively. The quantum yields of the samples are calculated by following the reported procedure.[1,2]

Temperature sensing studies:
The temperature sensing studies of the polymer-Eu^{3+} complexes were carried out in various solvents tetrahydrofuran, chlorobenzene dimethyl sulfoxide and xylene. The concentration of the polymer-Eu^{3+} complexes were fixed at $1.8x10^{-6}$ M (0.1OD absorbance) and emission spectra of the complexes were recoded by exciting at 350 nm with various temperatures from 20- 100 ºC.

Metal ion Sensing studies: The sensing studies were carried out in 3 mL cuvette and the 5 µL of polymer solutions (stock solution = 3.2 x 10^{-3} M) were diluted in the buffer solution. The stock solution of metal perchlorate salts were prepared with $2x10^{-2}$ M concentrations. The absorbance and fluorescence titration was carried out by adding of 3µM metal solutions into the polymer + PIPES buffer solutions.

Anion Sensing studies:
The completely quenched Cu^{2+} bound polymer solutions were prepared by adding the mixture of polymer solutions with 24 µM concentration of Cu^{2+} ions in PIPES buffer soltuions at pH =7.4. The stock solutions of all the amino acids and phosphate ion were prepared with $2x10^{-2}$ M concentrations. The fluorescence turn on experiment was carried out by successive additions of

amino acids to Cu^{2+} bound polymers solutions. The fluorescence spectra of the polymers were recorded for each concentration of amino acid by exciting at 305 nm.

Synthesis of Polymers:

Typical procedure for synthesis of polymers is descrbed here as 3-(3,5-bis ((diethoxyphosphoryl) methyl)-4-(2-ethylhexyloxy) phenyl) propanoic acid (1.0 g, 1.7 mmol) and 4,4'-(2,2'-(ethane-1,2-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy)dibenzaldehyde or 4,4'-(3,6,9,12,15-pentaoxaheptadecane-1,17-diylbis(oxy))dibenzaldehyde (0.23 g, 1.7 mmol) were dissolved in dry THF (15 mL) and the mixture was stirred well under nitrogen atmosphere about 15 minutes at room temperature. Potassium tert-butoxide (10.4 mL in 1 M THF) was added drop wise and stirred for further 12 h at room temperature under nitrogen atmosphere. After 5 minutes the yellow precipitates were formed and accumulated as viscous beads. It was poured into water and neutralised with 10 % HCL, the product was washed with excess of water. The polymer was purified by re-dissolving in THF and precipitated methanol. It was dried in a vacuum oven at 40 ^{o}C for 5 h prior to further analysis.

PTEG: ^{1}HNMR (in $CDCl_3$) δ: 7.47-6.85 (m, 14H, Ar-H and vinylic H), 4.18 (t, 4H, Ar-OCH_2CH_2), 3.90 (t, 4H, Ar-OCH_2CH_2), 3.81 (s, 4H, Ar-OCH_2CH_2-OCH_2), 2.97 (t, 2H, Ar-CH_2CH_2), 2.74 (t, 2H, CH_2-CH_2COOH), 1.90-0.90 (m, 32H, aliphatic H).

PHEG: ^{1}H-NMR (in $CDCl_3$) δ: 7.41-6.87 (m, 14H, Ar-H and vinylic H), 4.10 (t, 4H, Ar-OCH_2CH_2), 3.82 (t, 4H, Ar-OCH_2CH_2), 3.73-3.63 (m, 16H, Ar-OCH_2CH_2-OCH_2), 2.90 (t, 2H, Ar-CH_2CH_2), 2.68 (t,2H, CH_2-CH_2COOH), 1.70-0.83 (m, 32H, aliphatic H).

References:

1). A. Balamurugan, M. L. P. Reddy, M. Jayakannan, *J. Mater. Chem. A*, 2013, **1**, 2256.

2). A. Balamurugan, M. L. P. Reddy, M. Jayakannan, *J. Polym.Sci. Polym. Chem.*, 2009, **47**, 5144.

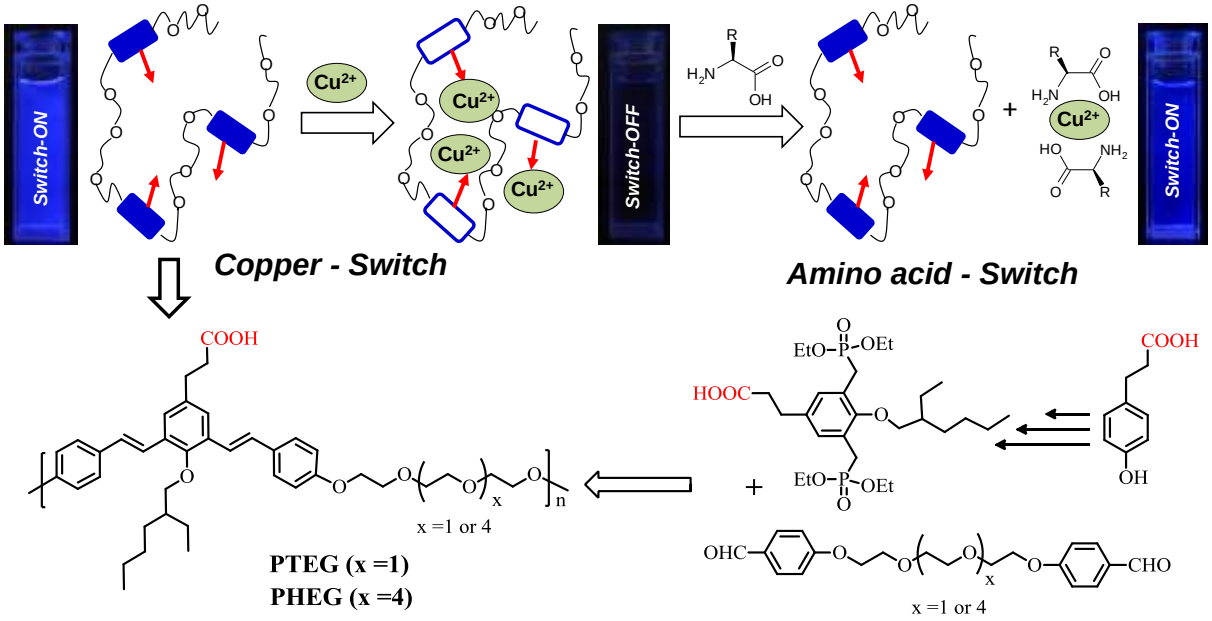

Figure SF1. *Schematic diagram for dual fluorescent turn On and turn-Off probes for selectively detecting of* Cu^{2+} *ion and amino acid and synthesis of segmented polymers.*

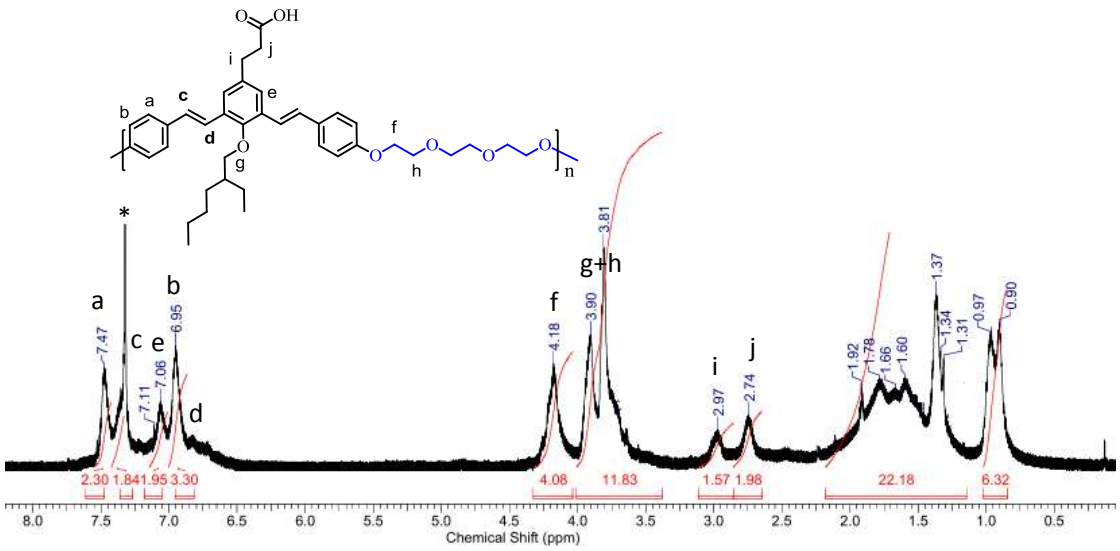

Figure SF2. *^{1}H-NMR spectra of PTEG*

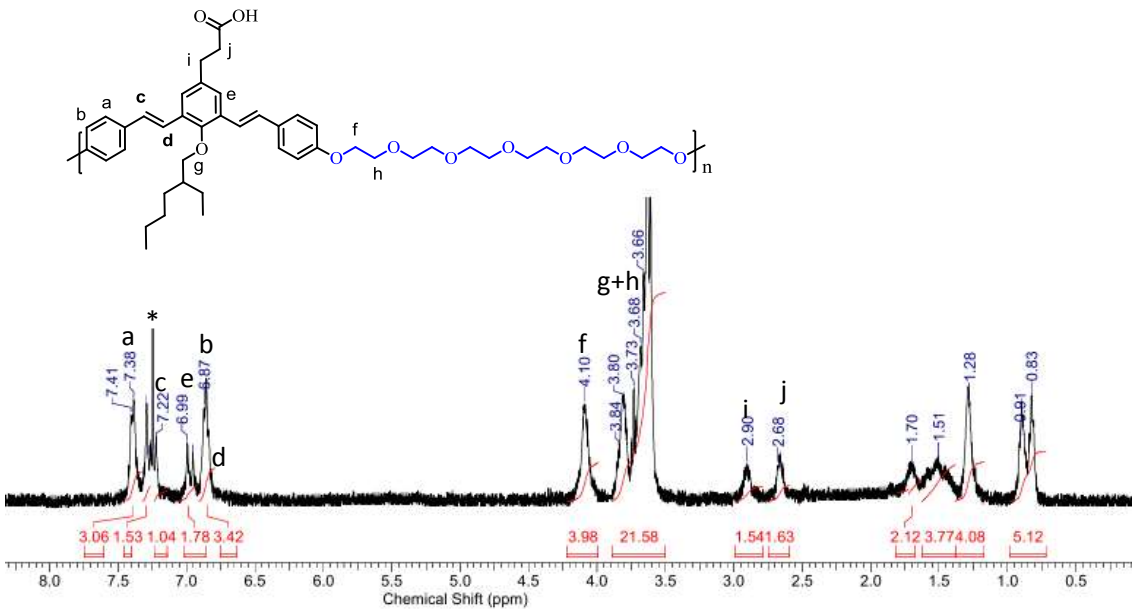

Figure SF3. *^{1}H-NMR spectra of PHEG*

Table ST1. *M_n M_w, quantum yields and Stern-Volmer quenching constant of PTEG and PHEG polymers*

Polymers	**M_n**	**M_w**	**Quantum yield (Φ)**		**Stern-Volmer (K_{sv}) for Cu^{2+} ion**
			THF	**Water**	
PTEG	8300	24200	0.13	0.08	6.1×10^5 M^{-1}
PHEG	8000	16600	0.18	0.11	4.5×10^5 M^{-1}

Note:

Quantum yield of the polymers were calculated using quinine sulfate (ϕ = 0.53 in 0.1 N conc. H_2SO_4) as standard by following equation : $\phi_s = \phi_r (F_s A_r / F_r A_s) (nr/n_s)^2$ where ϕ is the fluorescent quantum yield, F is the area of the emission, n is the refractive index of the solvent, A is the absorbance of the solution at the exciting wavelength and r and s are denoted as reference and sample respectively. The calculated quantum yields of the PTEG and PHEG were obtained in the range of 0.08 and 0.11 respectively in water solutions, and these values are comparable to earlier reported literatures. The obtained quantum yields of the polymers in water are less as compared to THF and chlorobenzene. It is well known that quantum yield of conjugated polymers in water is always low as compared to organic solvents because of polymers forced to form aggregate in water solutions.

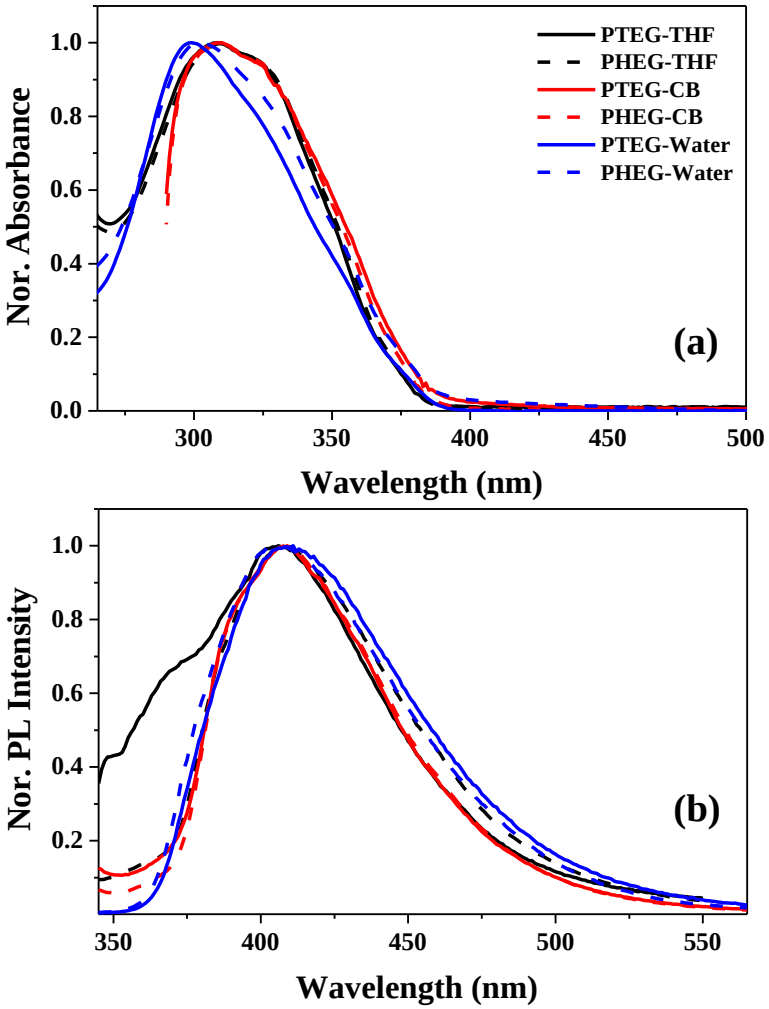

Figure SF4. *Absorption (**a**) and emission(**b**) spectra of segmented polymers PTEG (Solid line) and PHEG (dotted line).* The emission spectra were recorded by exciting at 310 nm.

Note:

The absorption and emission spectra of the polymers were recorded in tetrahydrofuran (THF), chlrobenzene(CB) and water solutions. Both poloymers showed almost same absorption and emission maxima in THF, chlorobenzene and water solutions and also there no aggregation peaks were observed. It indicate that both polymers are existed in completely dissolved state irrespective of the solvents.

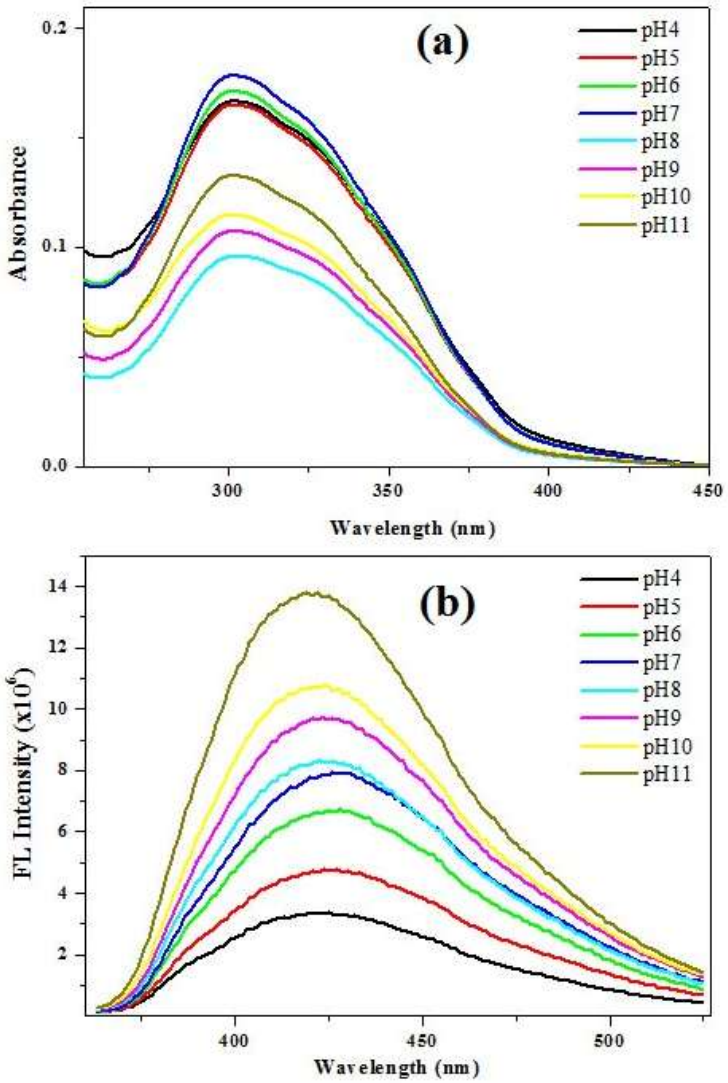

Figure SF5. *pH dependent absorbance (a) and emission (b) spectra of PTEG polymer (excitation wavelength = 310 nm).*

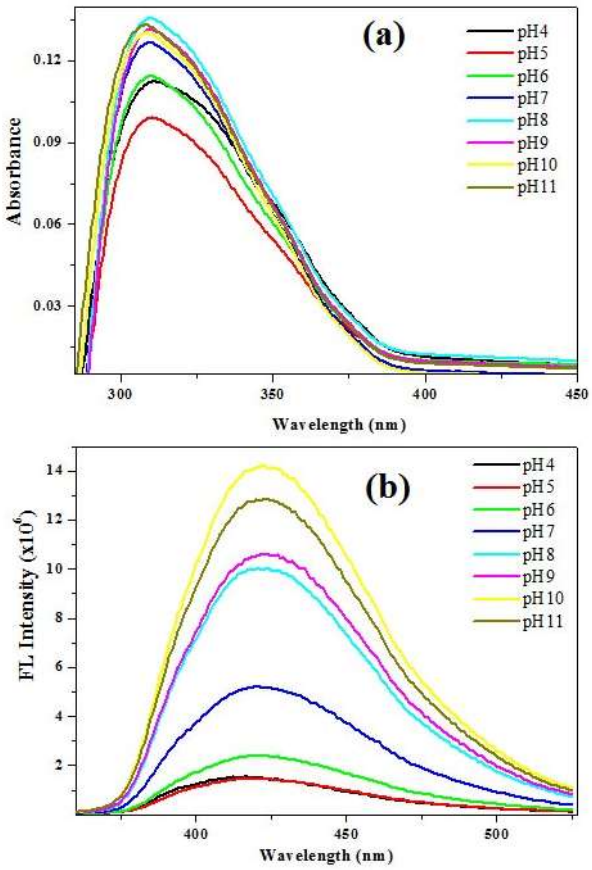

Figure SF6. *pH dependent absorbance (a) and emission (b) spectra of PHEG polymer (excitation wavelength = 310 nm).*

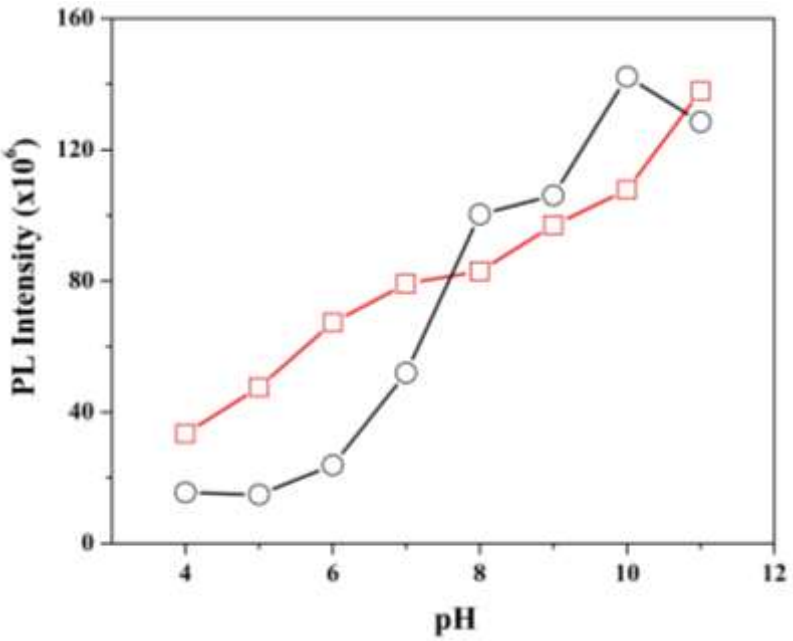

Figure SF7. *The plot of PL maxima versus various pH for both PTEG(open quare) and PHEG (open circle) polymers.*

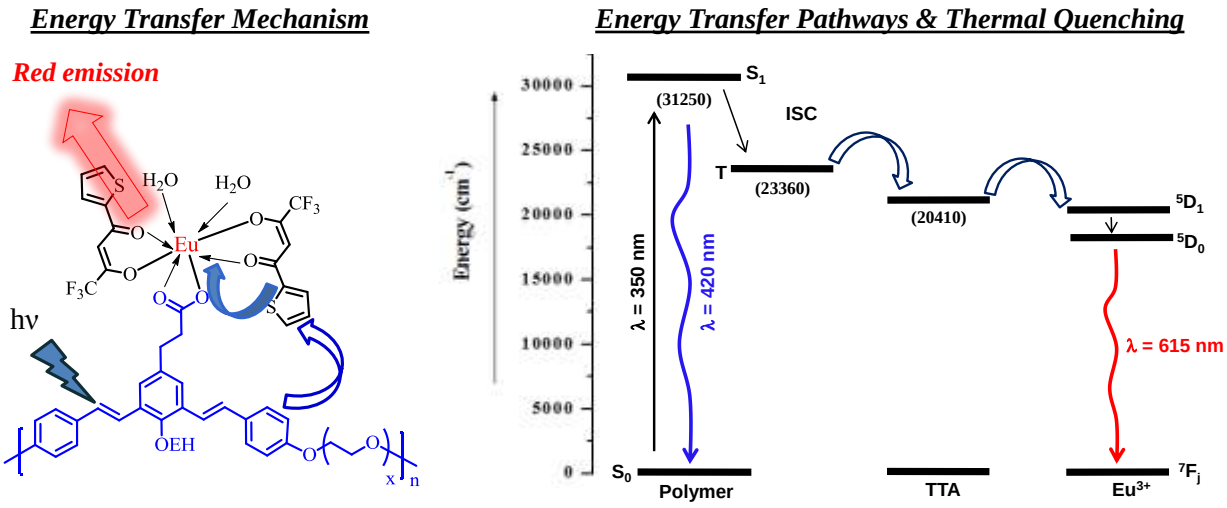

Figure SF8. *Schematic diagram for the energy transfer pathways in segmented polymer-Eu^{3+} complexes.*

Note: The polymer-Eu^{3+} complexes were synthesised by using (theonyltrifluoroacetylacetone)TTA as co ligand in the presence of base in THF/Methanol mixtuxe. The singlet and triplet energy of polymers were calculated using standard procedure. According to Latva's empirical rule[1], energy transfer from ligand to excited state of Eu^{3+} metal ion is more effective when ΔE ($^3\pi\pi^*$-$^5D_0$) is equal to 2500 - 4000 cm^{-1}. As shown in figure (right), energy gap ΔE ($^3\pi\pi^*$-$^5D_0$) between the triplet excited state of the polymers or oligomer and excited state of Eu^{3+} ion is obtained as approximately 5755-5880 cm^{-1}. The triplet energy level of the polymer is higher than TTA and triplet state of TTA is lies at optimum level to excited state of the Eu^{3+} ion. Therefore, according to Latva's rule, the direct energy transfer from the polymer ligand to Eu^{3+} ion is not possible. Upon excitation at 310 nm, the energy was initially transferred from triplet state of polymers (or oligomer) to triplet state of the TTA and subsequently it was transferred to Eu^{3+} ion metal centre for the luminescence.

The temperature dependent fluorescence quenching of the polymer-Eu^{3+} complexes is due to i) the deactivation of the Eu^{3+} ion $^5D_0$ excited state and non-radiative decay of the excitation energy and (ii) less excitation energy transfer from the ligand to the europium excited states ($^5D_0$and$^5D_1$)[2].

1) (a) M. Latva, H. Takalo, V. M. Mukkala, C. Matachescu, J. C. Rodriguez-Ubis, J. Kanakare, *J. Lumin.* 1997, **75**, 149; (b) B. You, H. J. Kim, N. G. Park, Y. S. Kim, *Bull.Korean. Chem. Soc.* 2007, **22**, 1005.

2) (a) G. E. Khalil, K. Lau, G. D. Phelan, B. Carlson, M. Gouterman, *Rev. Sci. Instrum*. 2004, **75**, 192. (b) M. Mitsuishi, S. Kikuchi, T. Miyashita, Y. Amao, *J. Mater. Chem.* 2003, **13**, 2875. (c) M. T. Berry, P. S.May, H. Xu, *J. Phys. Chem.* 1996, **100**, 9216. (d) L. N. Sun, J. Yu, H. Peng, J. Z. Zhang, L. Y. Shi, O. S. Wolfbeis, *J. Phys. Chem. C* 2010**, 114**, 12642. (e) X. D. Wang, X. H. Song, C. Y. He, C. J. Yang, G. Chen, X. Chen, *Anal. Chem*. 2011, **83,** 2434.

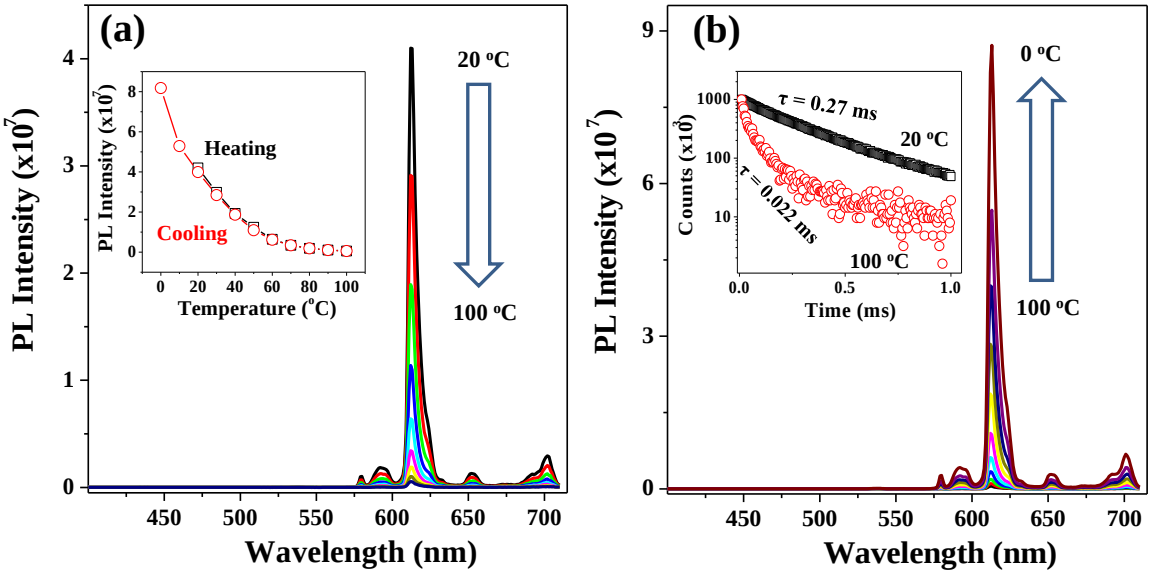

Figure SF9. *Temperature probe of PTEG-Eu^{3+} ions in the heating (a) and cooling (b) cyles. The in-set in (a) showed the plots of PL intesity variation with tempetaures. The in-set in (b) showed the TCSPC decay profiles of polymer-Eu^{3+} at 20° and 100 °C. Concentration of polymer is fixed 2x10^{-6}M. The PL spectra were recorded by using excitation wavelength 350 nm.*

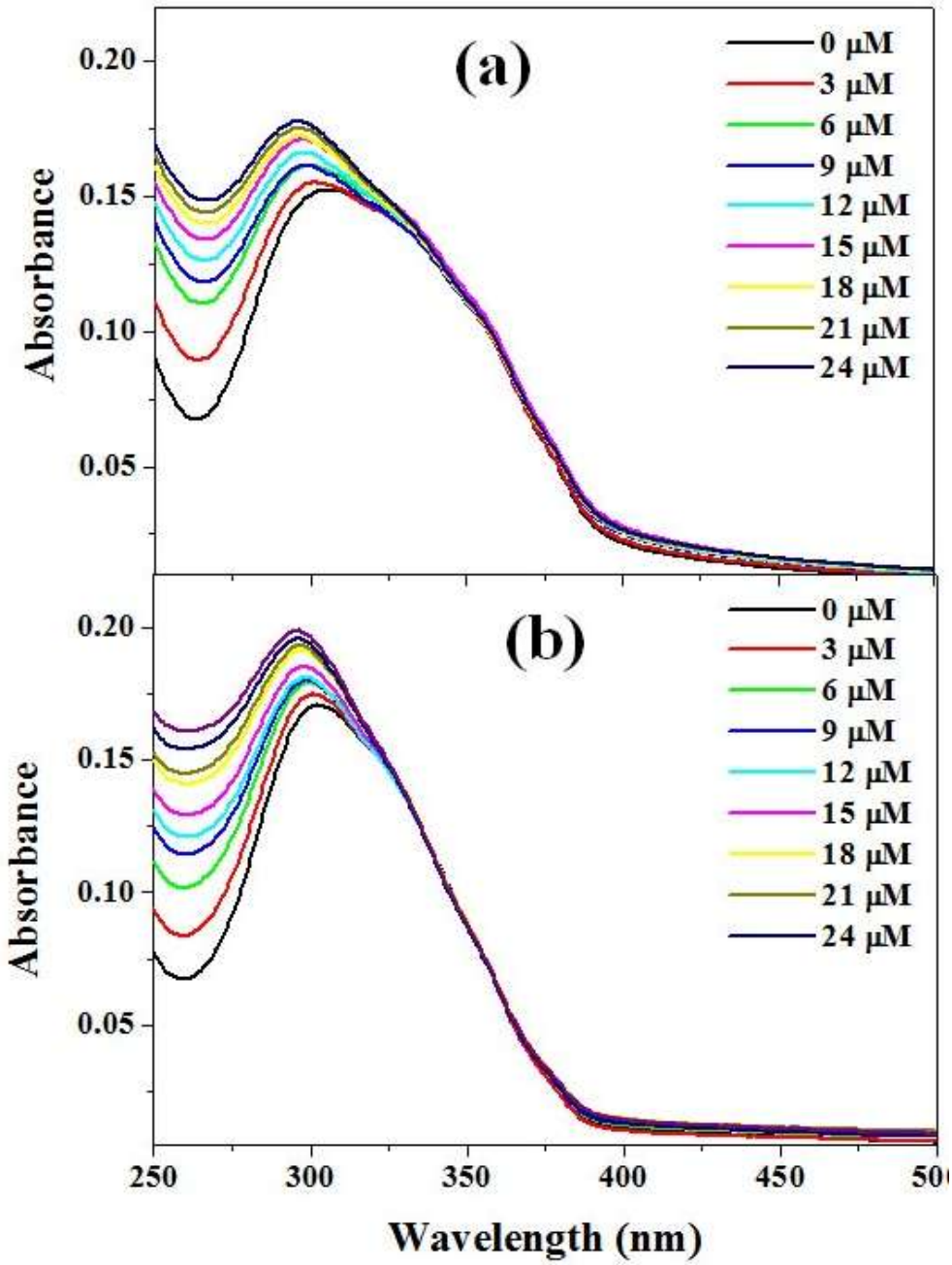

Figure SF10. *UV-Vis Absorption spectra of PTEG (a) and PHEG (b) with various concentrations of* Cu^{2+} *ion in PIPES buffer at pH 7.4. Concentration of polymer is fixed* $5x10^{-6}$ *M and the metal ion concentration is varied.*

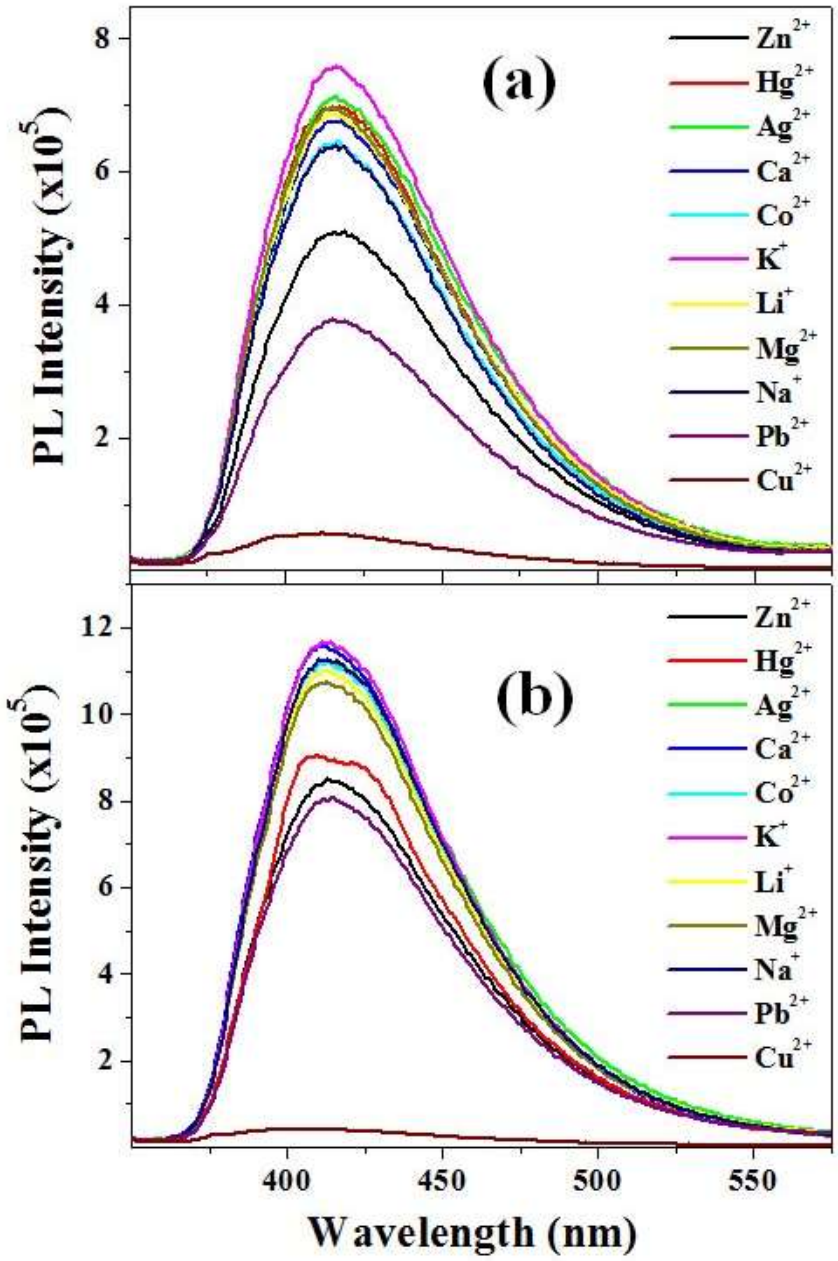

Figure SF11. *Fluorescence responses of PTEG (a) and PHEG (b) for various metal ions in PIPES buffer at pH 7.4 (* λ_{ex} =310 nm). *Concentration of polymer is fixed* $5x10^{-6}$ *M and the metal ion concentration is 24* μ*M.*

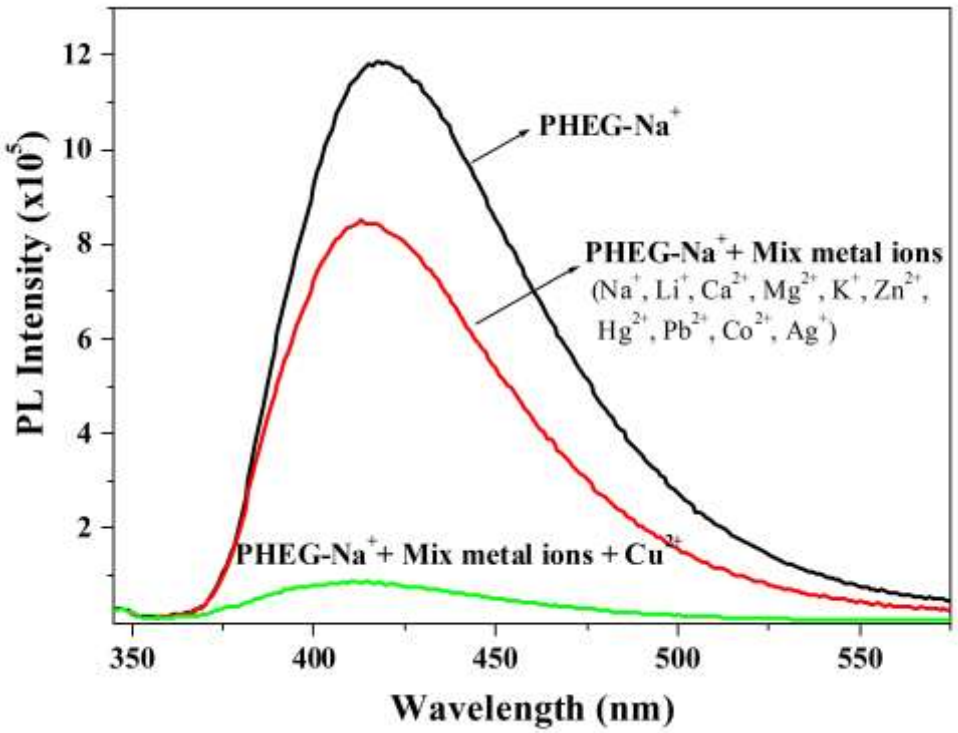

Figure SF12. *Fluorescence spectra of PHEG-Na^+ with addition of mixed metal ions and Cu^{2+} ion PIPES buffer at pH 7.4* (λ_{ex} =310 nm). *Concentration of polymer is fixed $5x10^{-6}$ M and the metal ion concentration is 24 μM..*

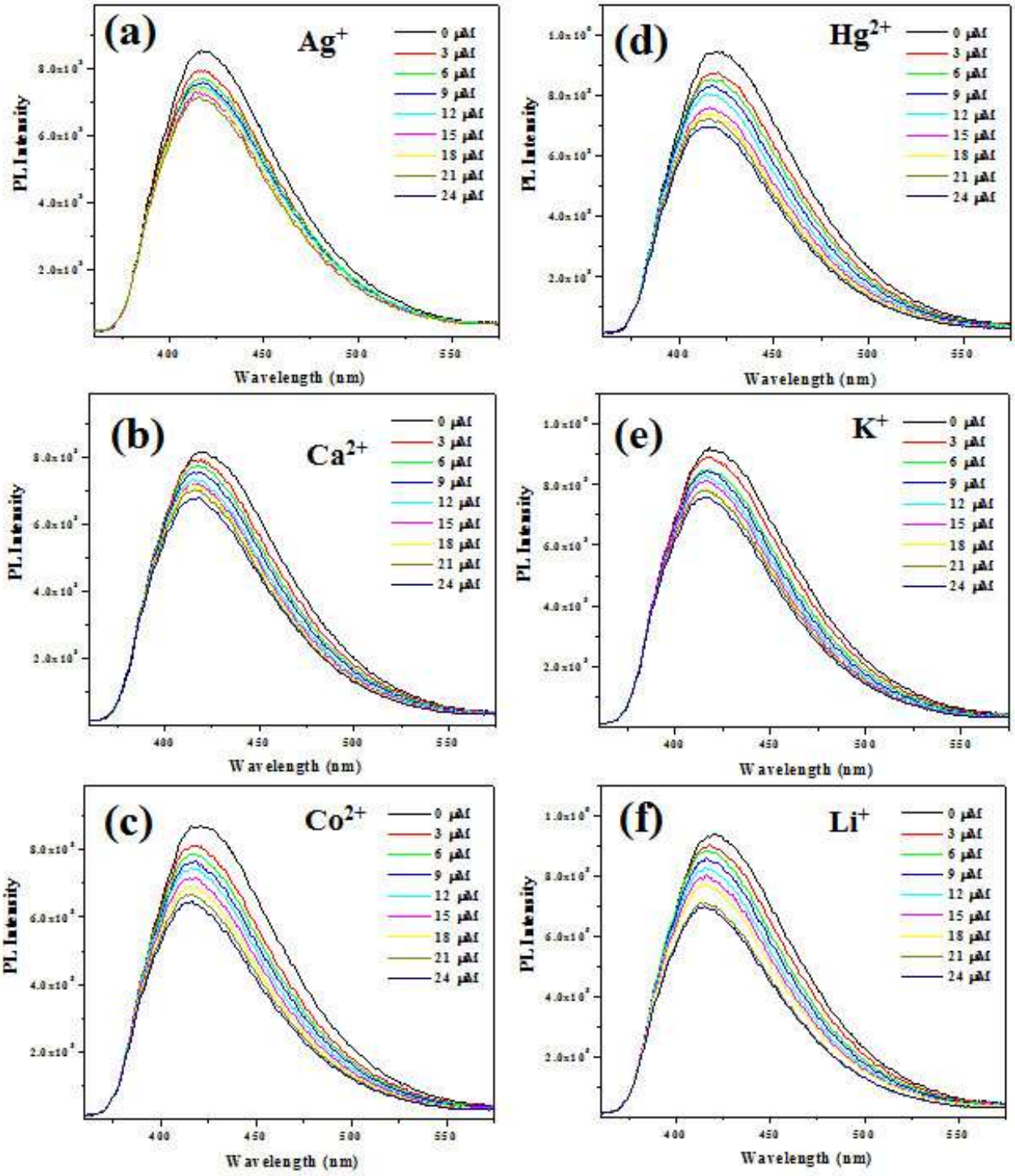

***Figure SF13**: Fluorescence responses of PTEG for various concentrations of Ag^{+} (a), Ca^{2+} (b) Co^{2+}(c), Hg^{2+} (d), K^{+}(e) and Li^{+} (f) ions in PIPES buffer at pH 7.4 (λ_{ex} =310 nm). Concentration of polymer is fixed $5x10^{-6}$ M and the metal ion concentration is varied.*

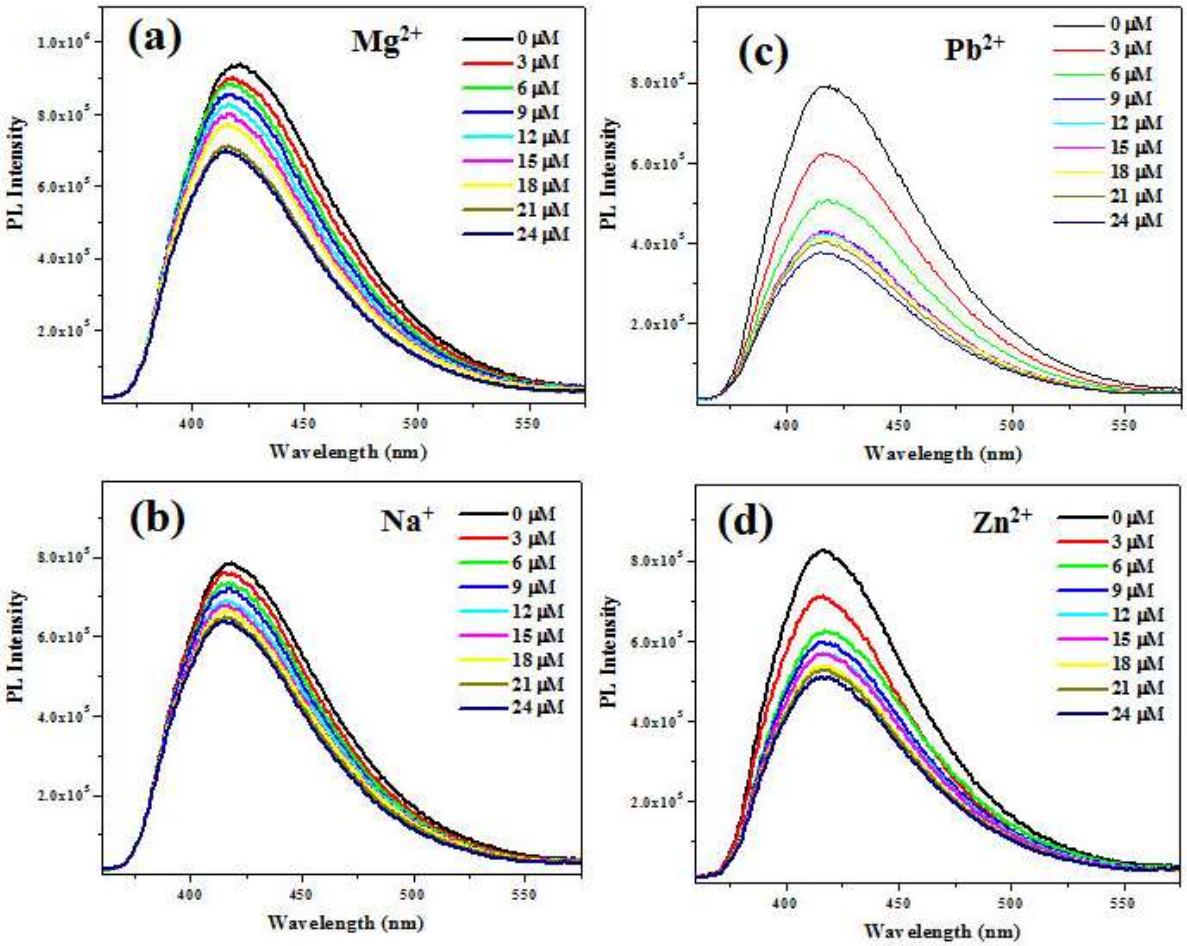

Figure SF14: *Fluorescence responses of PTEG for various concentrations of* Mg^{2+} *(a),* Na^{+} *(b)* Pb^{2+}*(c), and* Zn^{2+} *(d) ions in PIPES buffer at pH 7.4 (* λ_{ex} =310 nm*). Concentration of polymer is fixed* $5x10^{-6}$ *M and the metal ion concentration is varied.*

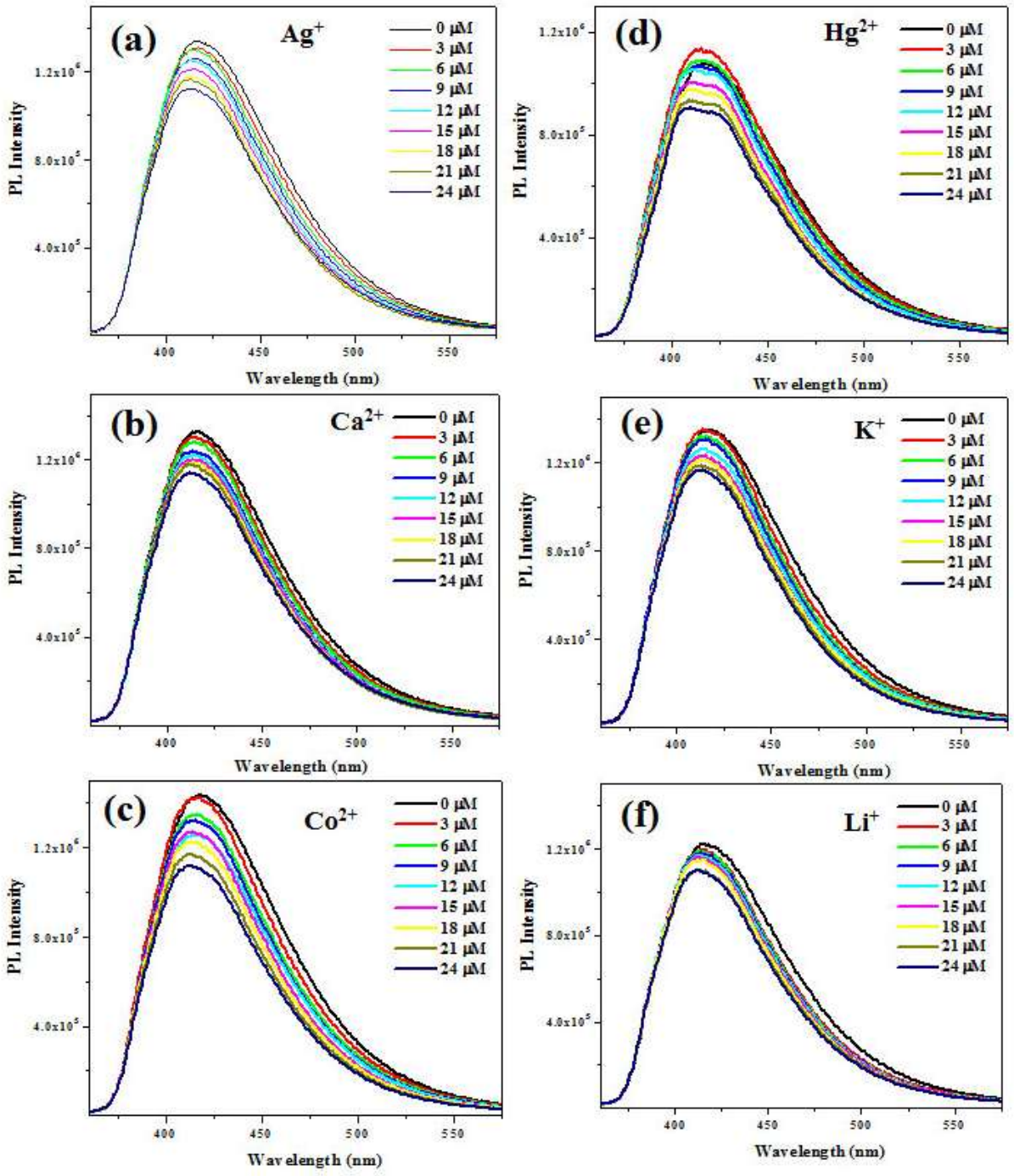

***Figure SF15**: Fluorescence responses of PHEG for various concentrations of Ag^{+} (a), Ca^{2+} (b) Co^{2+}(c), Hg^{2+} (d), K^{+}(e) and Li^{+} (f) ions in PIPES buffer at pH 7.4 (λ_{ex} =310 nm). Concentration of polymer is fixed $5x10^{-6}$ M and the metal ion concentration is varied.*

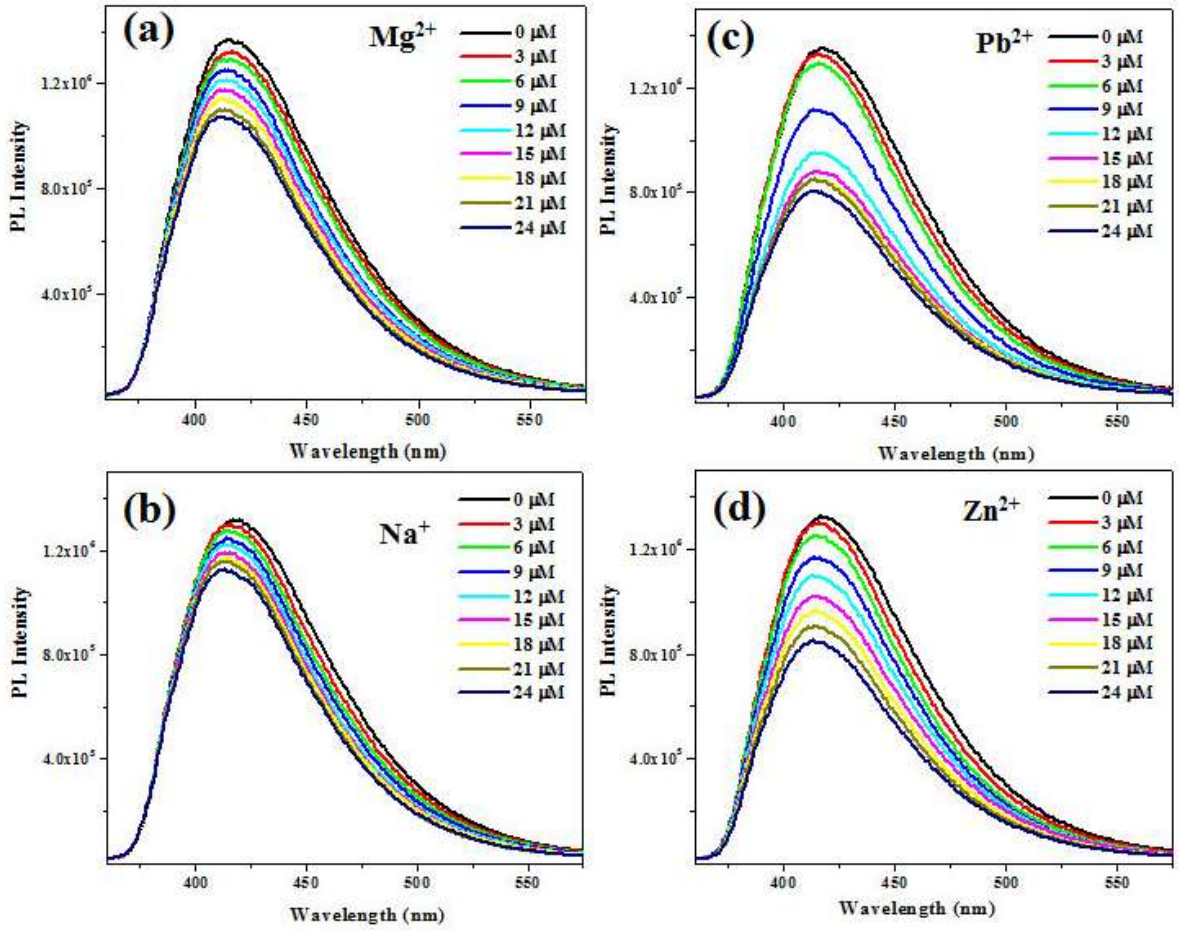

Figure SF1*6: Fluorescence responses of PHEG for various concentrations of* Mg^{2+} *(a),* Na^{+} *(b)* Pb^{2+}*(c), and* Zn^{2+} *(d) ions in PIPES buffer at pH 7.4 (* λ_{ex} =310 nm). *Concentration of polymer is fixed* $5x10^{-6}$ *M and the metal ion concentration is varied.*

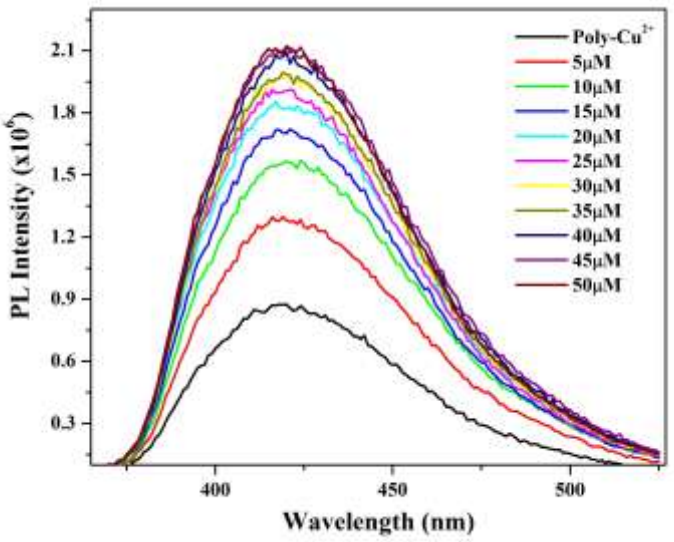

Figure SF17. *Fluorescence spectra of Cu^{2+} ion containing PTEG polymer titration with various concentration of histidine in PIPES buffer at pH 7.4 (excitation wavelength = 310 nm). Concentration of polymer-Cu(II) is fixed [($5x10^{-6}$ M polymer + 30 μm Cu(II)] and the amino acid concentration is varied.*

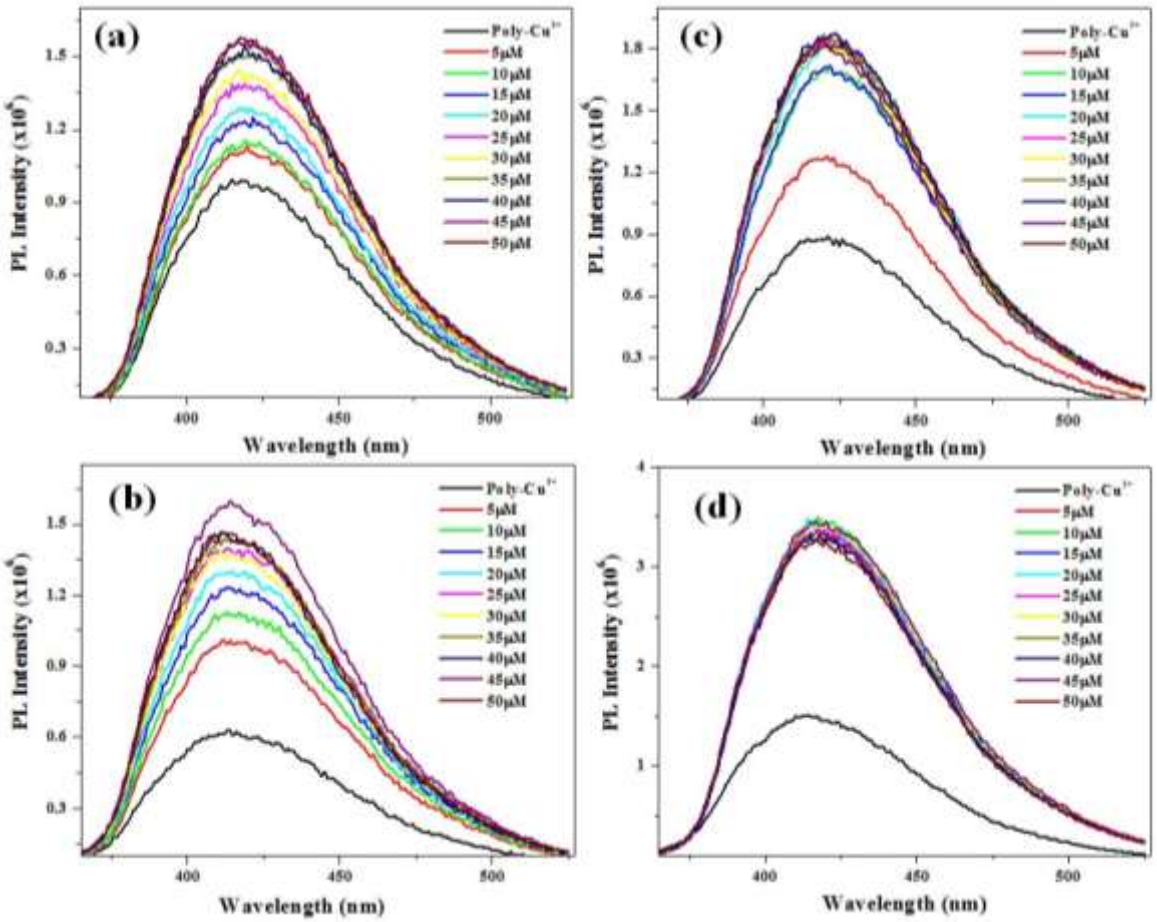

Figure SF18: *Fluorescence titration of Cu^{2+} bound PTEG and PHEG polymers with various concentration of alanine (**a** and **b**) and cysteine (**c** and **d**) in PIPES buffer at pH 7.4 (excitation wavelength = 310 nm). Concentration of polymer-Cu(II) is fixed [($5x10^{-6}$ M polymer + 30 μm Cu(II)] and the amino acid concentration is varied.*

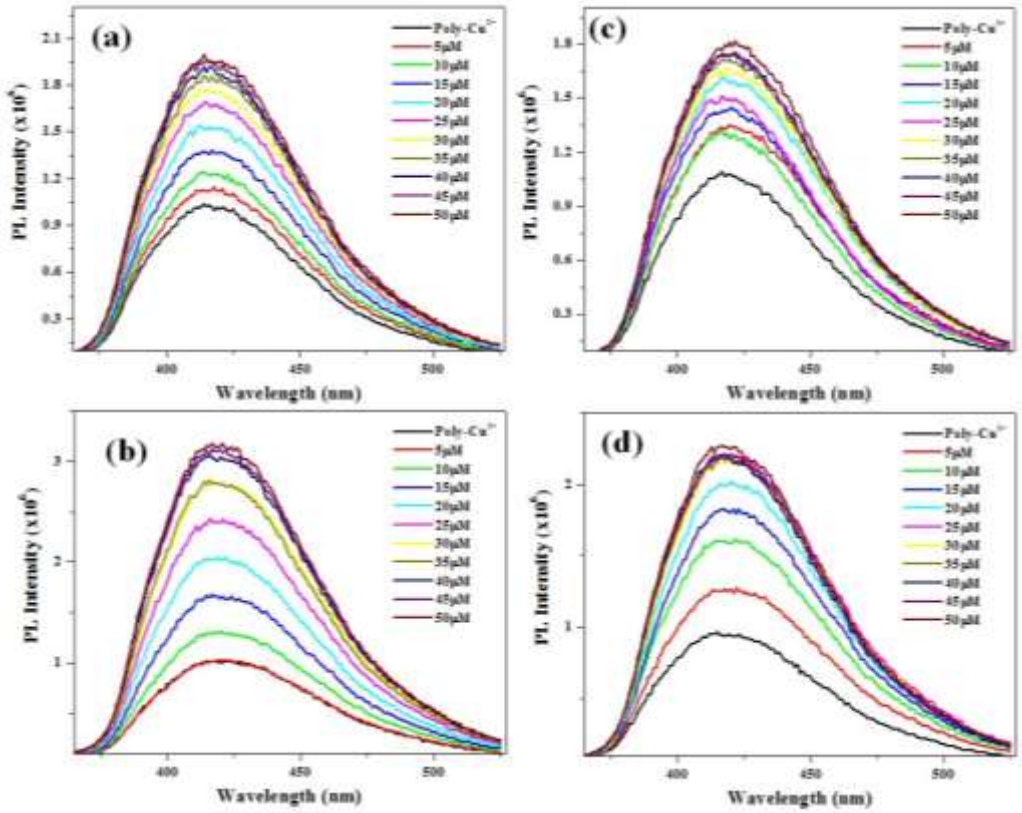

Figure SF19: *Fluorescence titration of Cu^{2+} bound PTEG and PHEG polymers with various concentration of glutamine (**a** and **b**) and serine (**c** and **d**) in PIPES buffer at pH 7.4 (excitation wavelength = 310 nm). Concentration of polymer-Cu(II) is fixed [($5x10^{-6}$ M polymer + 30 μm Cu(II)] and the amino acid concentration is varied.*

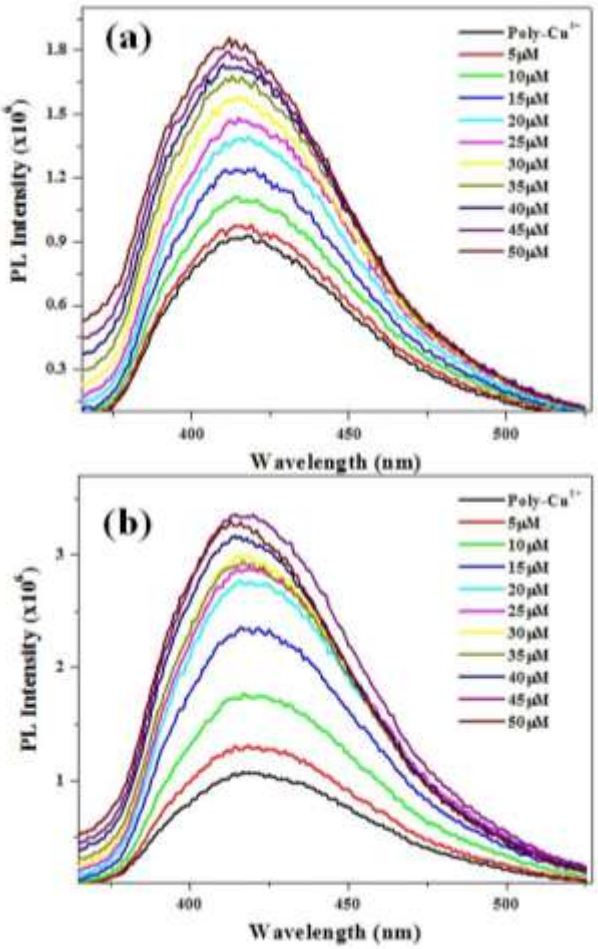

Figure SF20: *Fluorescence titration of Cu^{2+} bound PTEG and PHEG polymers (**a** and **b**) with various concentration of tryptophan in PIPES buffer at pH 7.4 (excitation wavelength = 310 nm). Concentration of polymer-Cu(II) is fixed [($5x10^{-6}$ M polymer + 30 μm Cu(II)] and the amino acid concentration is varied.*

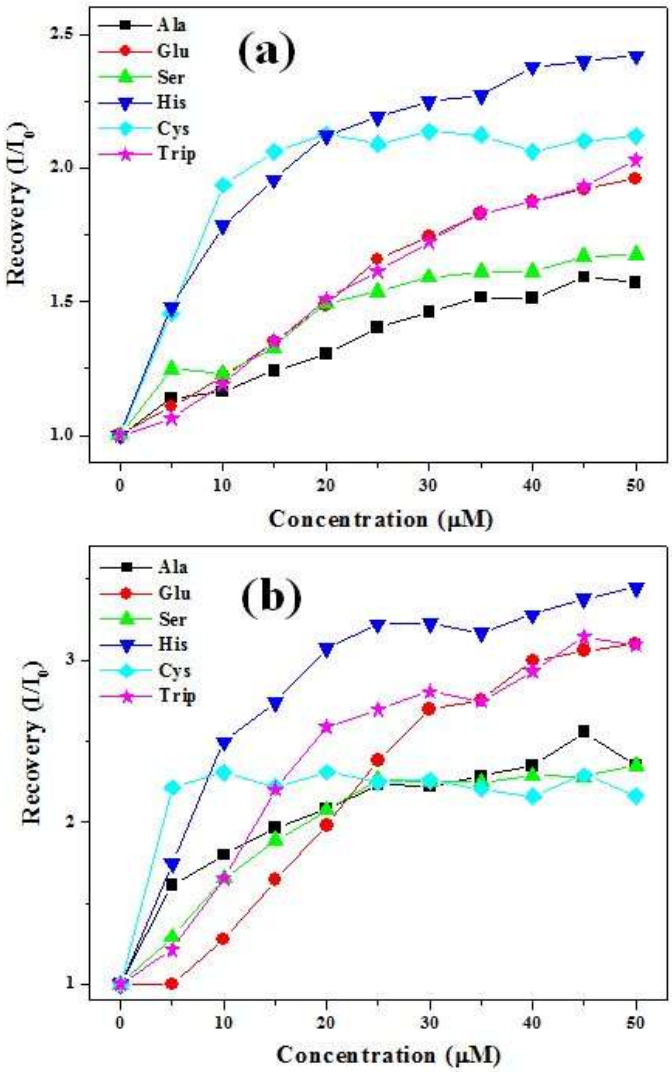

Figure SF21: *The ratio of I/I_0 values for Cu^{2+} bound PTEG and PHEG polymers (**a** and **b**) with various concentration of amino acids in PIPES buffer at pH 7.4.*

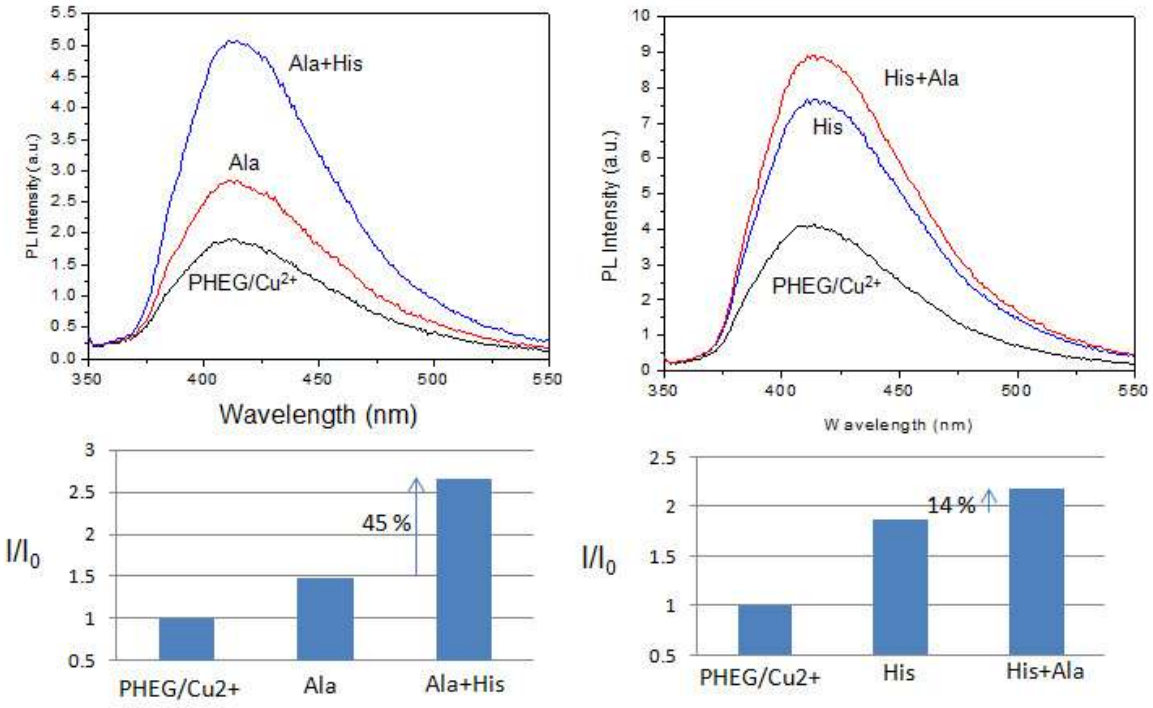

Figure SF22: *PHEG/Cu^{2+} probe (30 μM): (Left) Addition of alanine (30μM) followed by histidine (30μM) at 25 ℃ in PIPES buffer at pH 7.4. (Right) Addition of histidine (30μM) followed by alanine (30μM) at 25 ℃ in PIPES buffer at pH 7.4.*

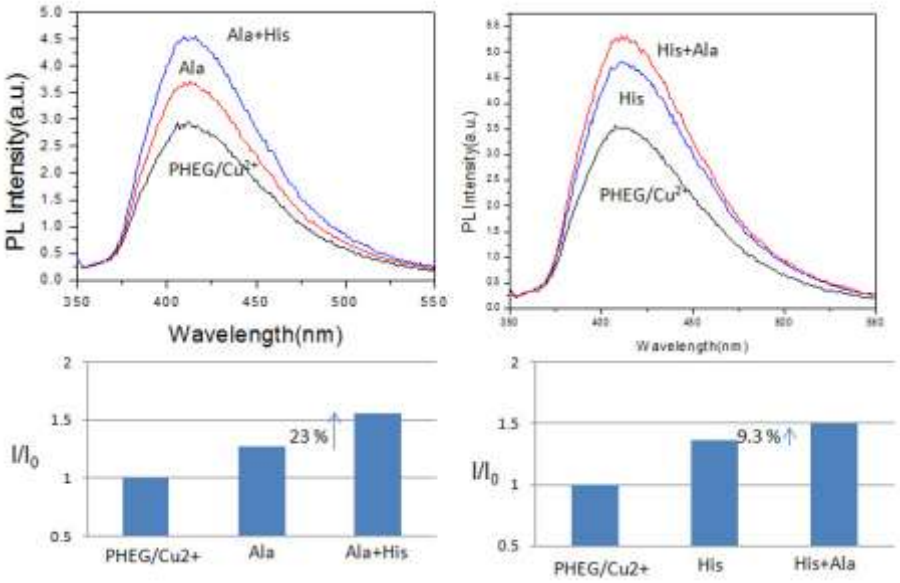

Figure SF23: *PHEG/Cu^{2+} probe (30 μM): (Left) Addition of alanine (30μM) followed by histidine (30μM) at 60 ℃ in PIPES buffer at pH 7.4. (Right) Addition of histidine (30μM) followed by alanine (30μM) at 60 ℃ in PIPES buffer at pH 7.4.*

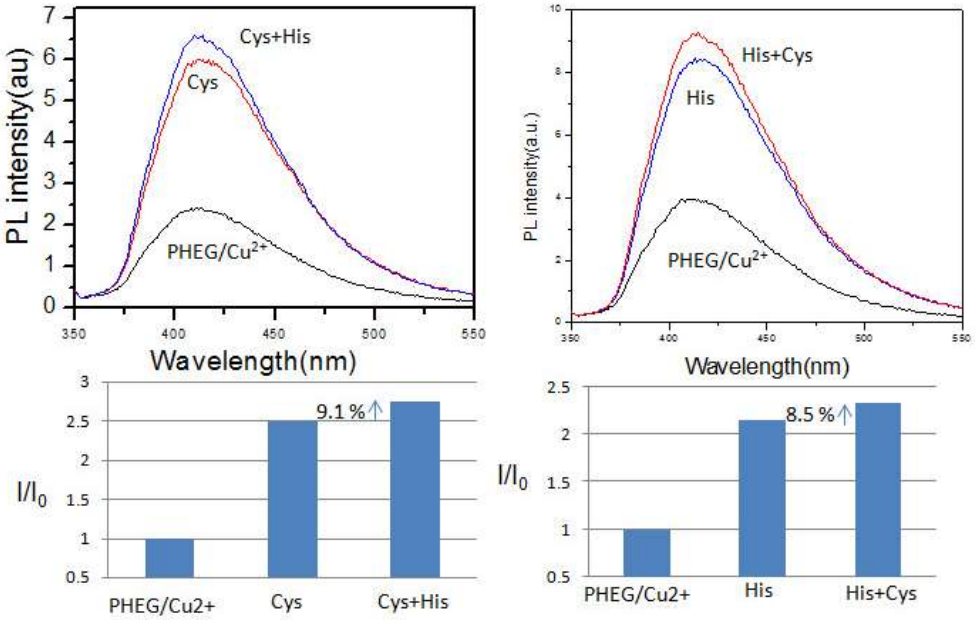

Figure SF24: *PHEG/Cu^{2+} probe (30 μM): (Left) Addition of cysteine (30μM) followed by histidine (30μM) at 25 ℃ in PIPES buffer at pH 7.4. (Right) Addition of histidine (30μM) followed by cysteine (30μM) at 25 ℃ in PIPES buffer at pH 7.4.*

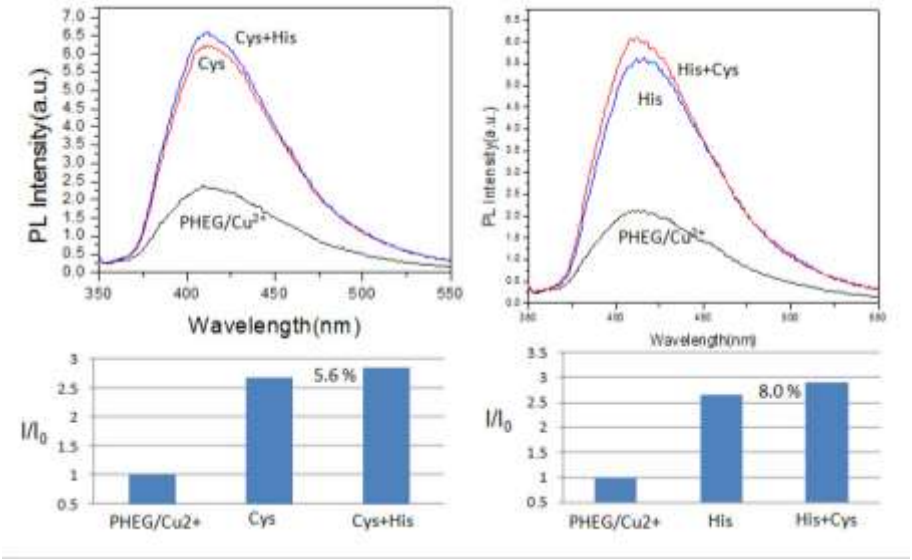

Figure SF25: *PHEG/Cu^{2+} probe (30 μM): (Left) Addition of cysteine (30μM) followed by histidine (30μM) at 60 ℃ in PIPES buffer at pH 7.4. (Right) Addition of histidine (30μM) followed by cysteine (30μM) at 60 ℃ in PIPES buffer at pH 7.4.*