Electronic Supplementary Information

FRET-assisted selective detection of flavins *via* cationic conjugated polyelectrolyte under physiological conditions

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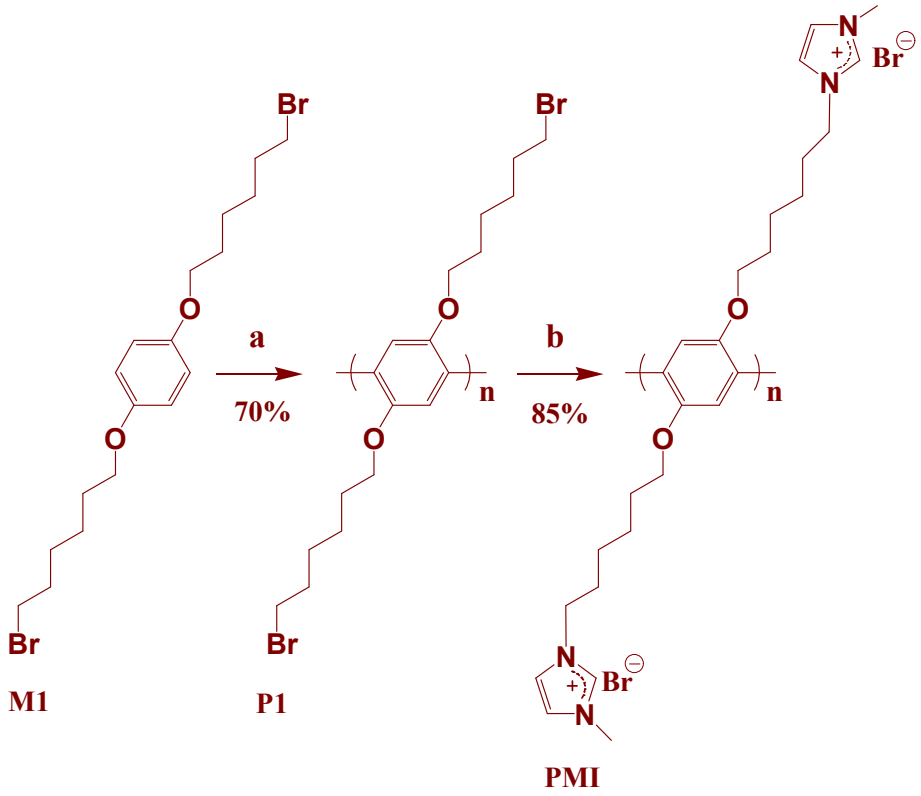

Scheme S1. (a) $FeCl_3$, nitrobenzene, RT, 2 days (b) excess 1-methyl imidazole, reflux, 36 h.

Synthesis of monomer and brominated polymer

Synthesis of monomer M1 and polymer P1 was performed using established method.[1-2]

Synthesis of cationic polymer poly(1,4-bis-(6-(1-methylimidazolium)-hexyloxy)-benzene bromide) (PMI)

The cationic polymer poly(1,4-bis-(6-(1-methylimidazolium)-hexyloxy)-benzene bromide) (PMI) was synthesized using our earlier reported method.[3] In brief, P1 (0.12 mmol, 1eq.) and excess 1-methyl imidazole were transferred to a 100mL round-bottom flask and kept for stirring at 80ºC in an oil bath. After 36 h, the reaction mixture was poured into excess $CHCl_3$ followed by stirring for about 1 h to obtain brownish color precipitate. The process was repeated to remove excess P1 and 1-methyl imidazole. The precipitate was then dried and filtered out to get pure polymer PMI with 85% yield.

^{1}H NMR (600 MHz, DMSO-*d6*, δ): 9.38 (b, 2H), 7.75 (b, 2H), 7.69 (b, 2H), 7.02 (b, 2H), 4.18 (b, 2H), 3.87 (b, 10H), 1.90 (b, 4H), 1.76 (b, 4H), 1.29 (b, 4H), 1.24 (b, 4H).

^{13}C NMR (150 MHz, DMSO-*d6*, δ): 149.47, 136.50, 128.64, 123.50, 122.25, 114.20, 68.73, 48.69, 35.78, 29.79, 28.70, 28.49, 25.53.

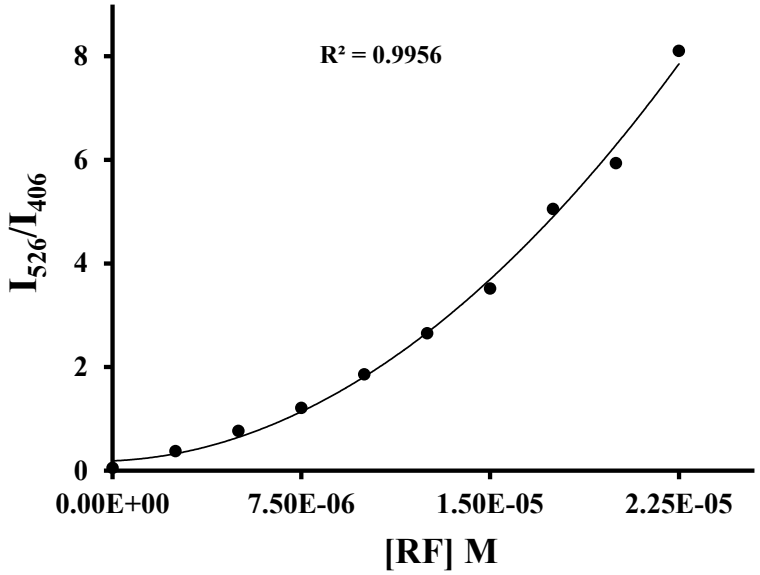

Fig. S1 Calibration plot for RF in 10 mM HEPES buffer (pH=7.4) at room temperature.

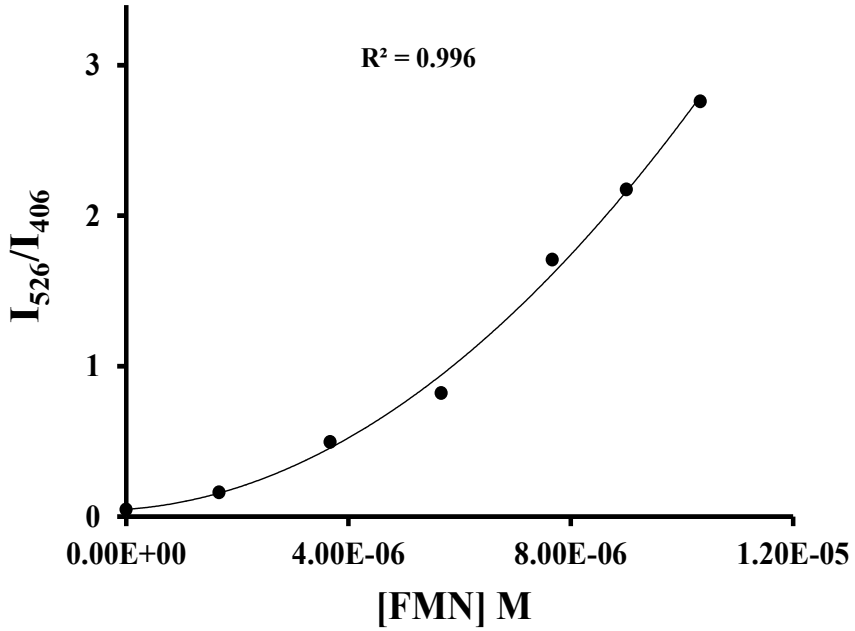

Fig. S2 Calibration plot for FMN in 10 mM HEPES buffer (pH=7.4) at room temperature.

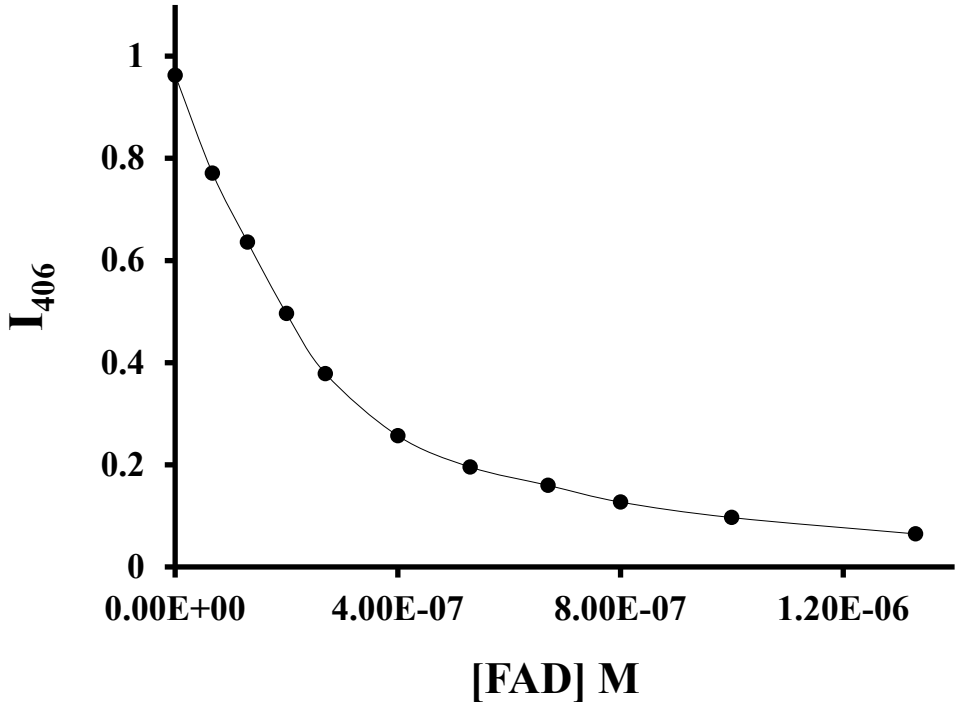

Fig. S3 Calibration plot for FAD in 10 mM HEPES buffer (pH=7.4) at room temperature.

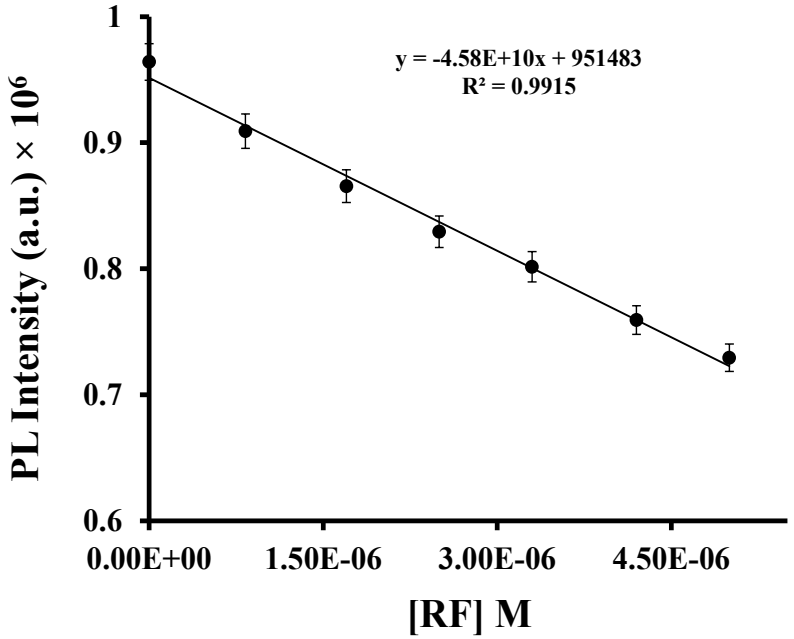

Fig. S4 Detection limit limit plot for RF.

LOD = 3 × σ/k

LOD = 3 × 10548.54/4.58 × 10^{10}

= 6.9 × 10^{-7} M (690 nM)

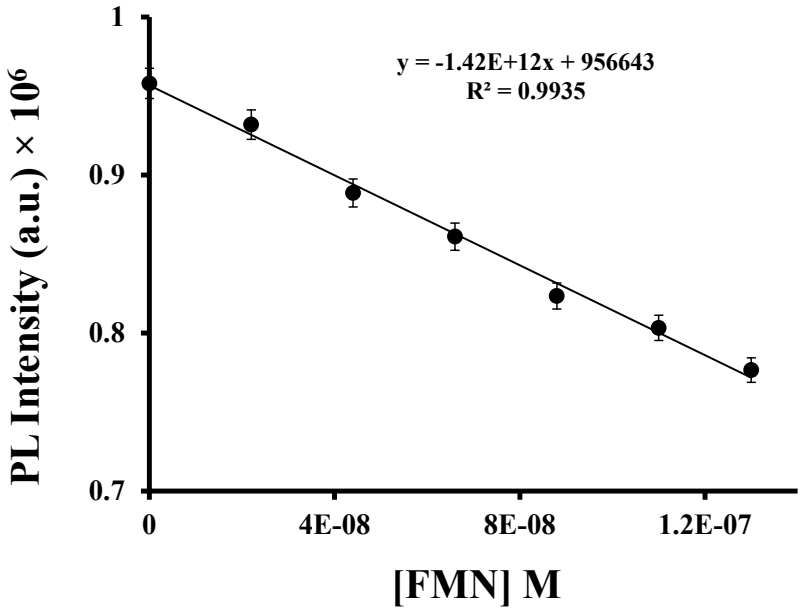

Fig. S5 Detection limit plot for FMN.

LOD = 3 × σ/k

LOD = 3 × 9313.75/1.42 × 10^{12}

= 1.97 × 10^{-8} M (19.7 nM)

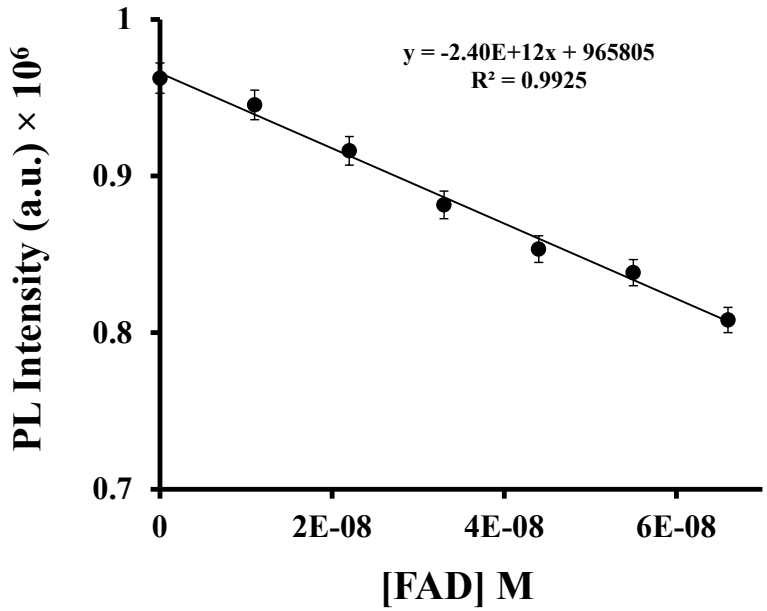

Fig. S6 Detection limit plot for FAD.

LOD = 3 × σ/k

LOD = 3 × 10721.55/2.40 × 10^{12}

= 1.34 × 10^{-8} M (13.4 nM)

File: PMIex336em406cnt2500ns50.FL

v **Discrete Components Analysis (Reconvolution)**

Fitting range : [550; 1500] channels

c^2 : 1.055

Exp Num	B	err_B	f	err_f	t (ns)	err_t
1	0.081	0.017	100.0	15.96	1.190	0.003

Background : 2.613 Background error : 0.117

Shift : -1.474 ns Shift error : 20.53

IRF Background : 0.115 fixed

File: PMI+RFex336em406cnt2500ns50.FL

v **Discrete Components Analysis (Reconvolution)**

Fitting range : [550; 1600] channels

c^2 : 0.985

Exp Num	B	err_B	f	err_f	t (ns)	err_t
1	0.060	0.017	70.07	18.08	0.405	0.061
2	0.007	6.7e-4	29.93	2.971	1.186	0.009

Background : 1.978 Background error : 0.086

Shift : -0.415 ns Shift error : 10.04

IRF Background : 0.115 fixed

File: PMI+FMN336em406cnt2500ns50.FL

v **Discrete Components Analysis (Reconvolution)**

Fitting range : [550; 1450] channels

c^2 : 1.044

Exp Num	B	err_B	f	err_f	t (ns)	err_t
1	0.039	0.006	65.14	15.38	0.589	0.054
2	0.009	8.1e-4	34.86	3.579	1.182	0.017

Background : 1.983 Background error : 0.146

Shift : -0.354 ns Shift error : 8.275

IRF Background : 0.115 fixed

File: PMI+FADex336em406cnt2500ns50.FL

v **Discrete Components Analysis (Reconvolution)**

Fitting range : [550; 1500] channels

c^2 : 1.015

Exp Num	B	err_B	f	err_f	t (ns)	err_t
1	0.030	0.003	63.68	11.53	0.593	0.043
2	0.008	5.6e-4	36.32	3.025	1.206	0.010

Background : 1.891 Background error : 0.123

Shift : -0.219 ns Shift error : 5.530

IRF Background : 0.115 fixed

Fig. S7 Fitting parameters and IRF values correspond to lifetime measurements.

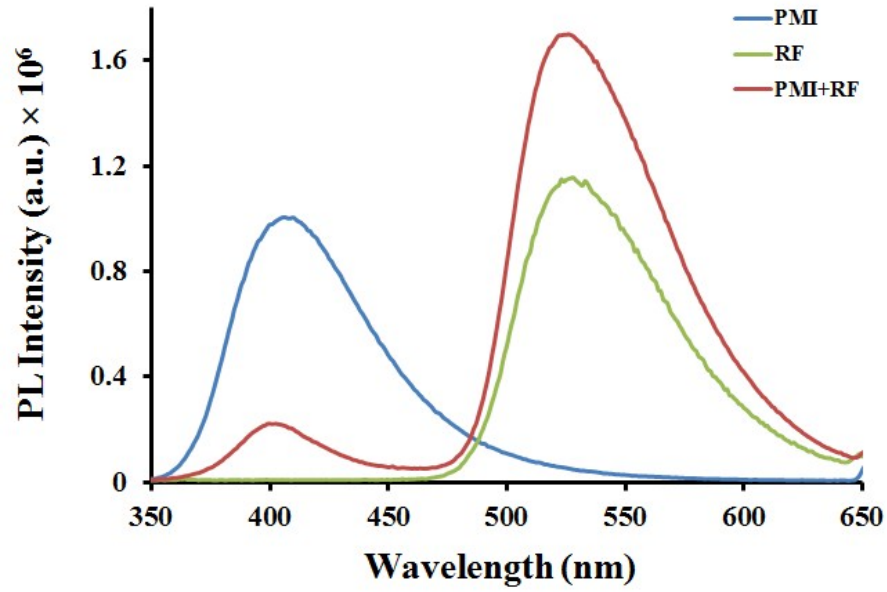

Fig. S8 Emission spectra of PMI, RF and the PMI/RF mixture at λ_{ex}=325 nm.

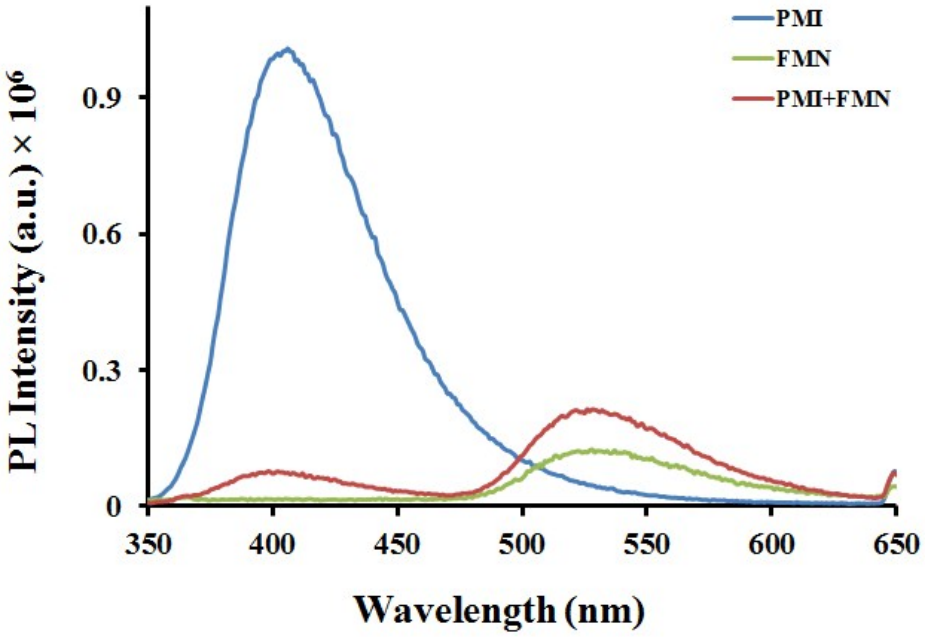

Fig. S9 Emission spectra of PMI, FMN and the PMI/FMN mixture at λ_{ex}=325 nm.

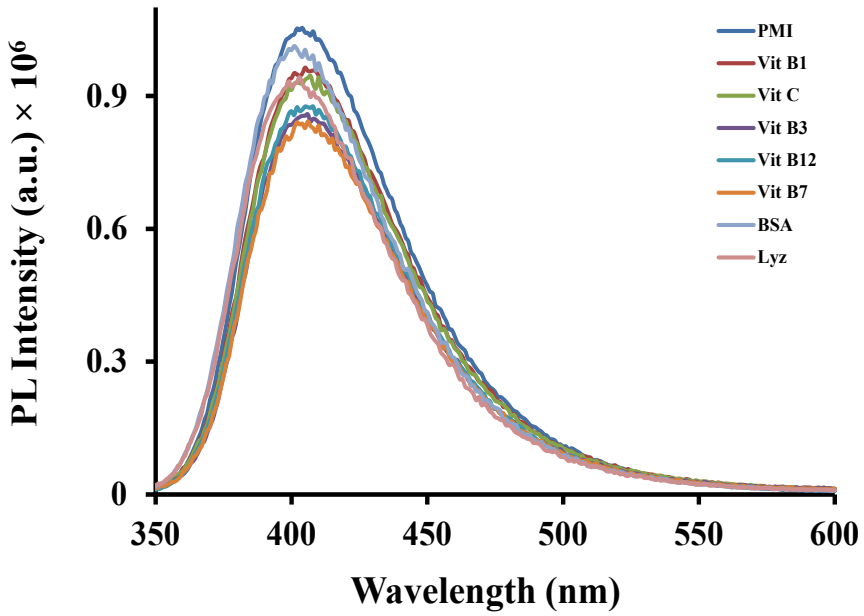

Fig. S10 Effect of various other vitamins, protein (BSA) and enzyme (lysozyme) on the emission of PMI in 10 mM HEPES buffer (pH=7.4) at room temperature. Concentration of PMI and each analyte were 10 μM and 25 μM, respectively.

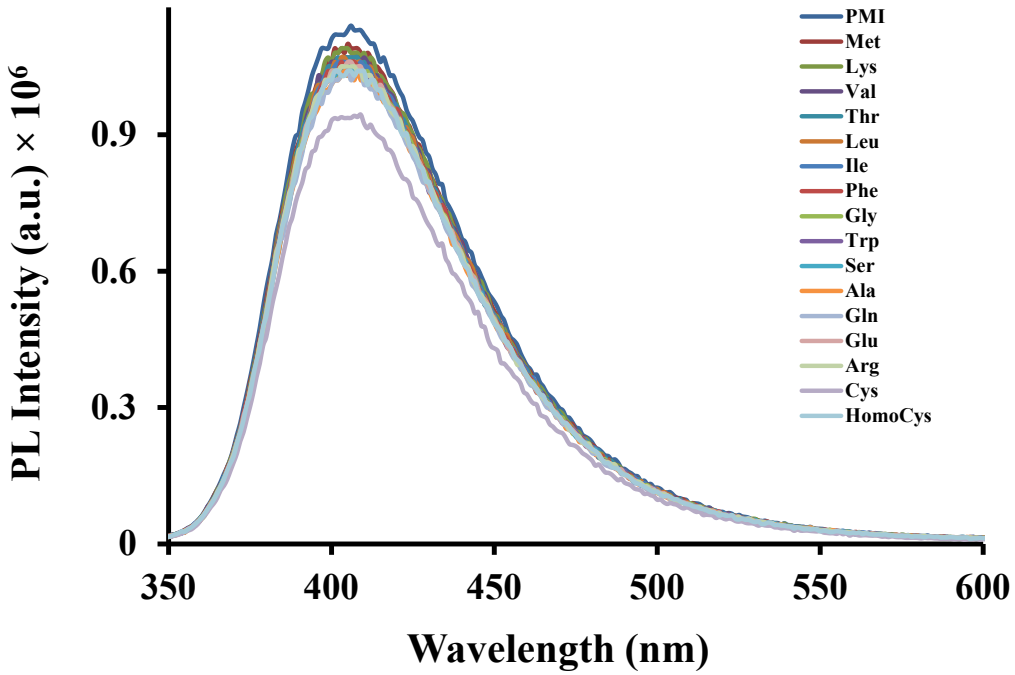

Fig. S11 Effect of various amino acids (25 μM) on the emission of PMI (10 μM) in 10 mM HEPES buffer (pH=7.4) at room temperature.

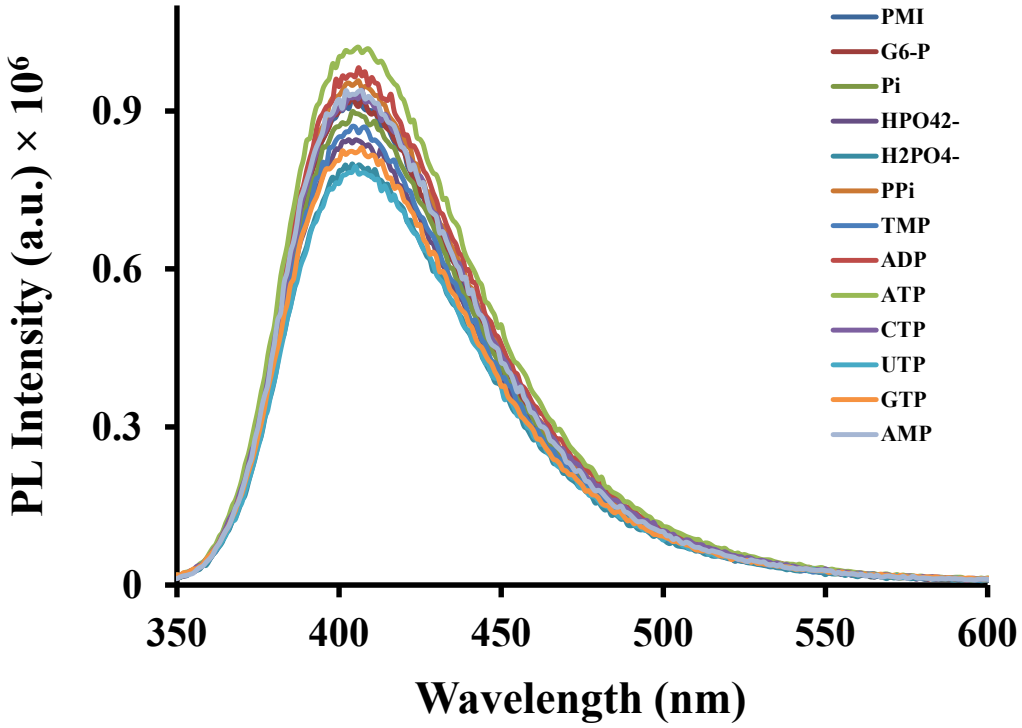

Fig. S12 Effect of various inorganic and organinc phosphates on the emission of PMI in 10 mM HEPES buffer (pH=7.4) at room temperature. Concentration of PMI and each analyte were 10 μM and 25 μM, respectively.

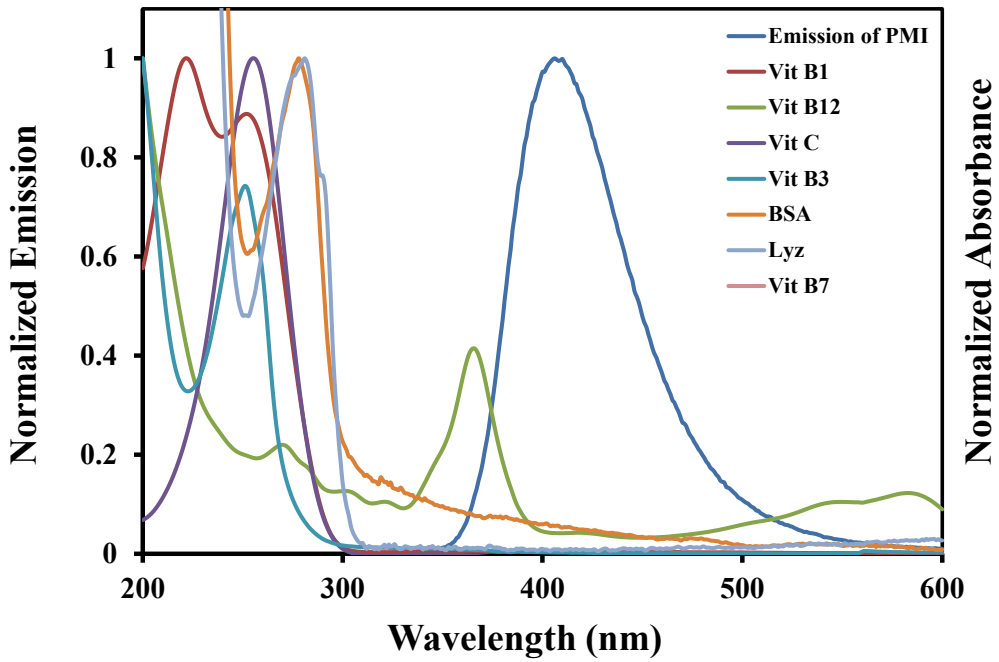

Fig. S13 Spectral overlap between emission spectrum of PMI and absorbtion spectra of various other vitamins, protein (BSA) and enzyme (lysozyme).

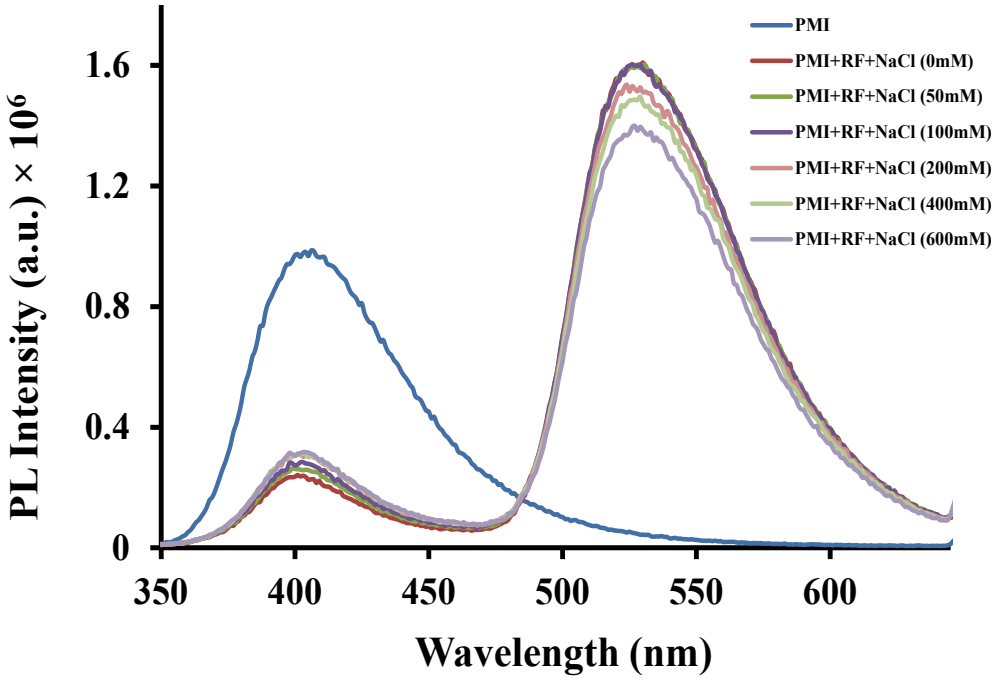

Fig. S14 Effect of ionic strength on FRET efficency for RF detection. Concentration of PMI and RF were 10 μM and 25 μM, respectively.

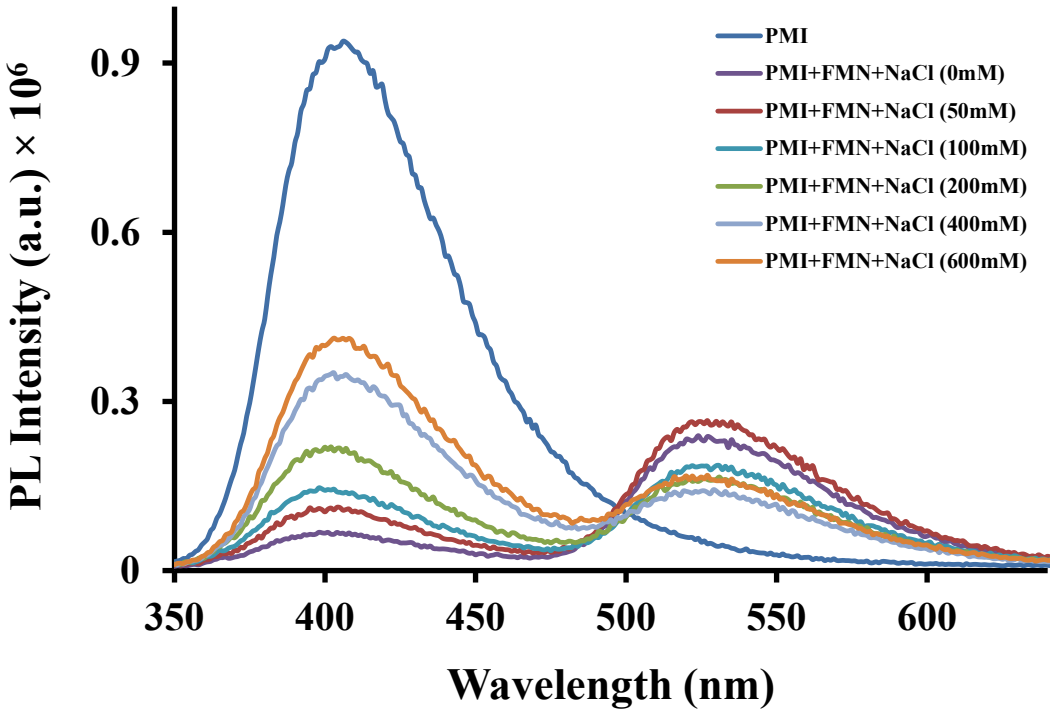

Fig. S15 Effect of ionic strength on FRET efficency for FMN detection. Concentration of PMI and FMN were 10 μM and 10.33 μM, respectively.

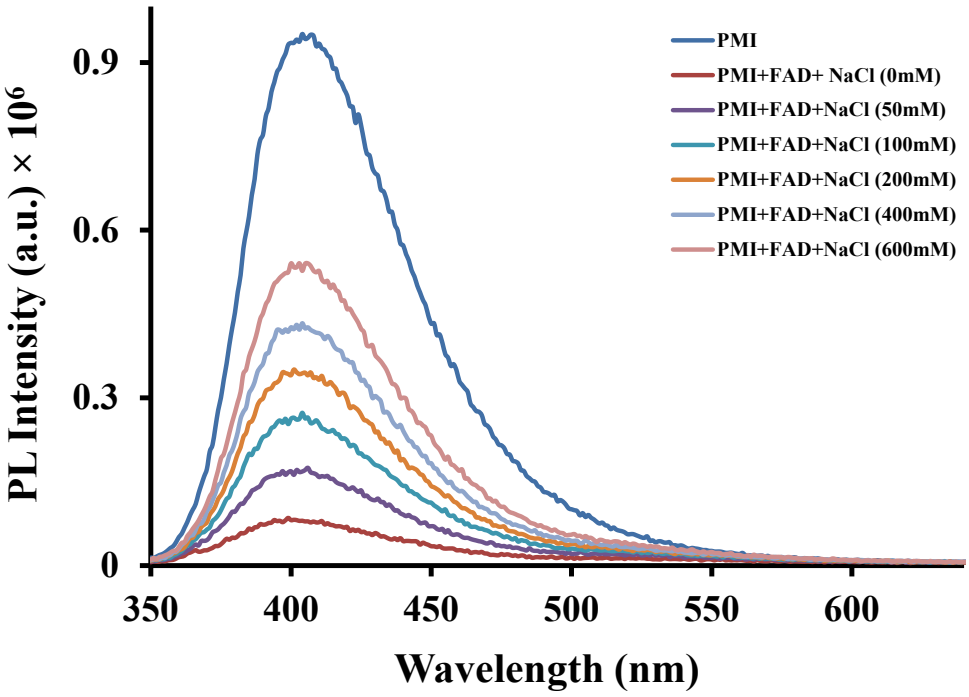

Fig. S16 Effect of ionic strength on FRET efficency for FAD detection. Concentration of PMI and FAD were 10 µM and 1.33 µM, respectively.

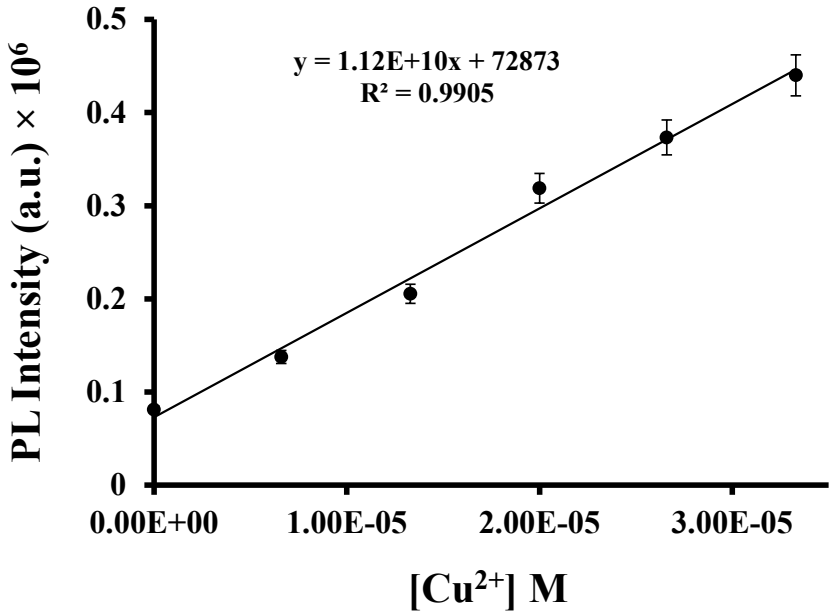

Fig. S17 Detection limit plot for Cu^{2+} using PMI/FMN complex.

LOD = 3 × σ/k

LOD = 3 × 2100.54/1.12 × 10^{10}

= 5.62 × 10^{-7} M (0.56 µM)

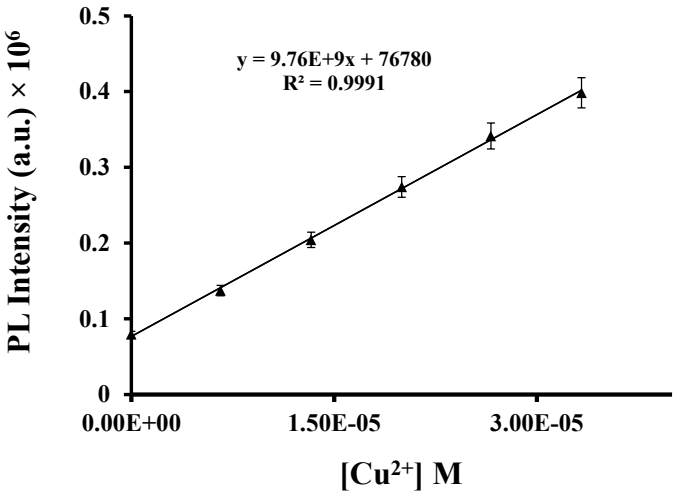

Fig. S18 Detection limit plot for Cu^{2+} using PMI/FAD complex.

LOD = 3 × σ/k

LOD = 3 × 2802.44/9.76 × 10^{10}

= 8.61 × 10^{-7} M (0.86 µM)

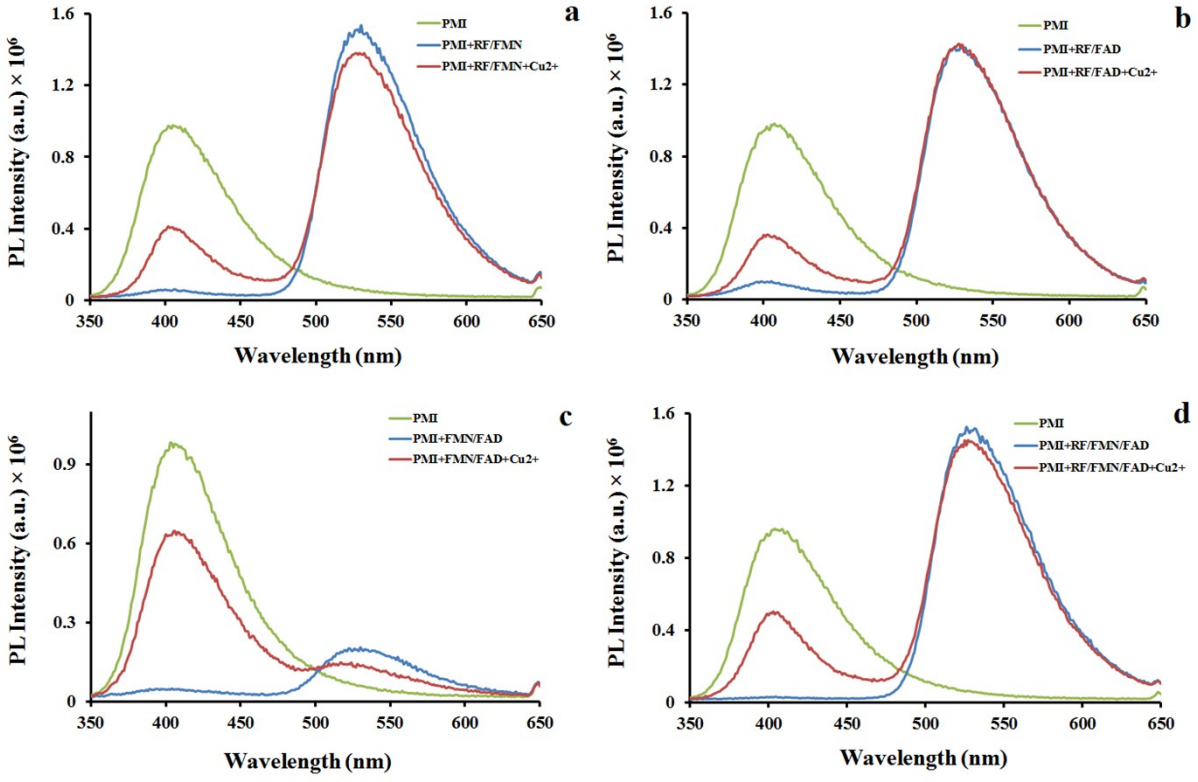

Fig. S19 Change in emission spectra on adding Cu^{2+} (6.6 × 10^{-5} M) to the solution of PMI (10 µM) containing mixture of (a) RF/FMN (25µM/10.33µM), (b) RF/FAD (25µM/1.33µM), (c) FMN/FAD (10.33µM/1.33µM) and (d) RF/FMN/FAD (25 µM/10.33µM/1.33µM) in 10 mM HEPES buffer (pH=7.4).

Table S1. A comparative study of the some best fluorometric based probes for the detection of flavins.

Publication	Material used	Sensing analytes	Detection Method	Discrimination	LOD
Current Work	***Conjugated Polyelectrolyte***	***RF, FMN, FAD and Cu^{2+}***	***FRET***	***RF/FMN and RF/FAD***	***RF-0.69 µM, FMN-19.68 nM and FAD-13.40 nM)***
J. Am. Chem. Soc. **2007**, 129, 4524-4525	Bis(Zn^{2+}-dipicolylamine complex	FAD	Direct fluorescence method	Not shown	Not determined (Detection in µM range)
ACS Appl. Mater. Interfaces **2013**, 5, 7392-7399	Sulfonated Graphene	RF and FMN	FRET	Not shown	0.6 µg/mL (1.6 µM)
Angew. Chem. Int. Ed. **2006**, 45, 1563-1568	DNA-duplex Aptamer	RF	Direct fluorescence method	Not shown	Not determined
Organic Letters, **2013,** 15, 1210-1213	Pyrene bound Zn^{2+}-dipicolylamine	FMN and FAD	Ratiometric method	FMN/FAD	Not determined (Detection in µM range)
Org. Biomol. Chem. **2016**, 14, 447-450	RNA Aptamer	FAD	Direct fluorescence method	Not shown	Not determined
J. Phys. Chem. B **2010**, 114, 10717-10727	Cucurbit[7]uril	RF and FAD	Direct fluorescence method	RF/FAD	Not determined
J. Am. Chem. Soc. **1995**, 117, 1246-1257	RNA Aptamer	RF	Direct fluorescence method	Not shown	Not determined

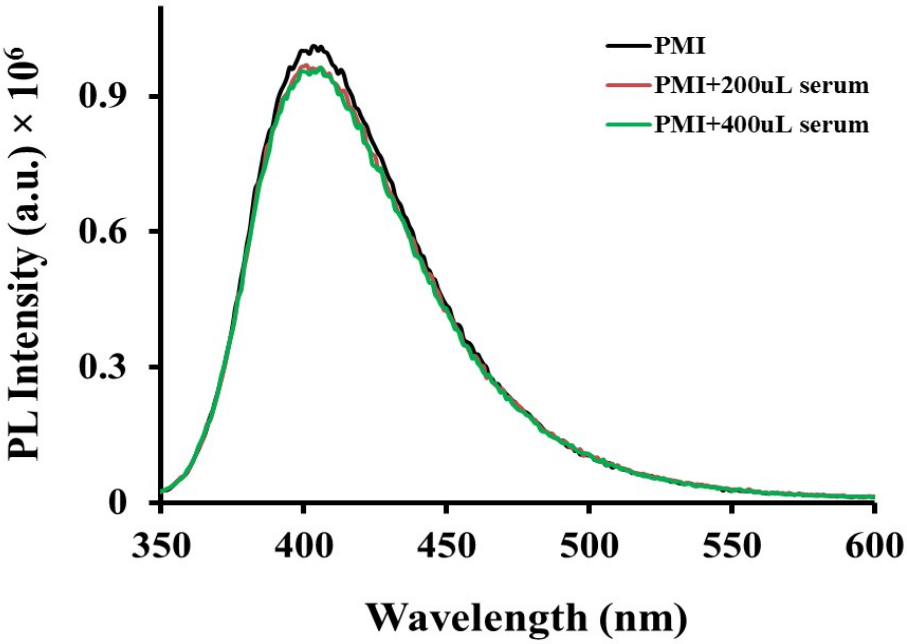

Fig. S20 Change in emission of PMI after adding undoped serum samples.

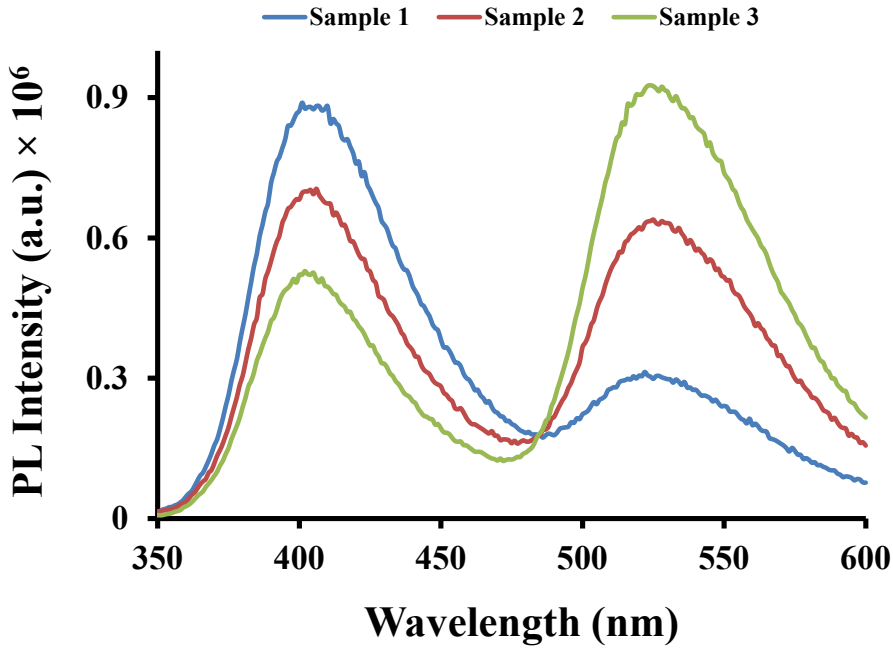

Fig. S21 Change in emission of PMI after adding RF-doped serum samples.

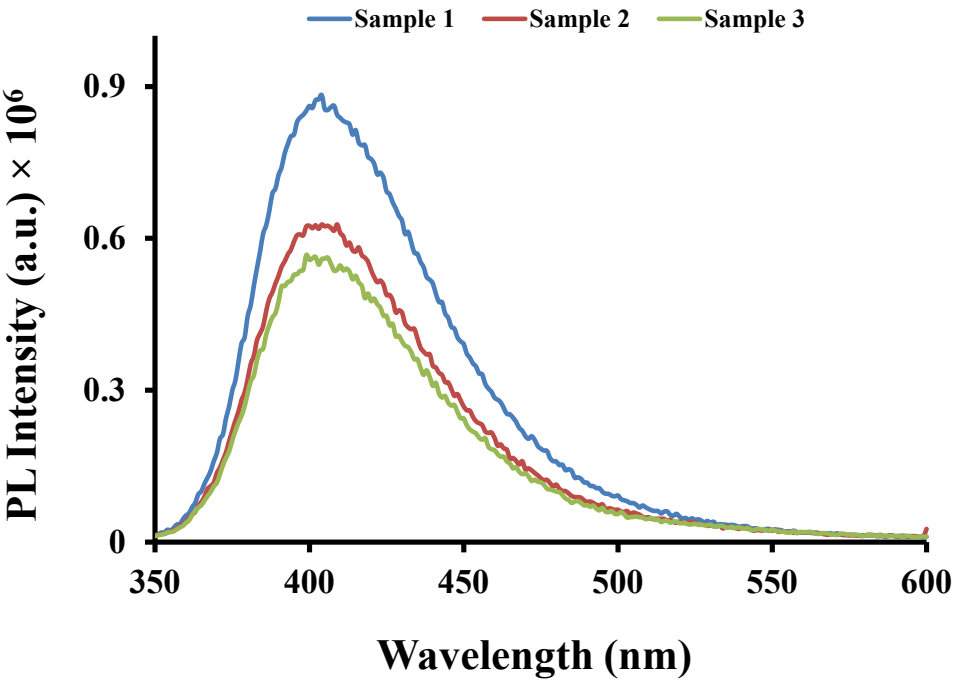

Fig. S22 Change in emission of PMI after adding FMN-doped serum samples.

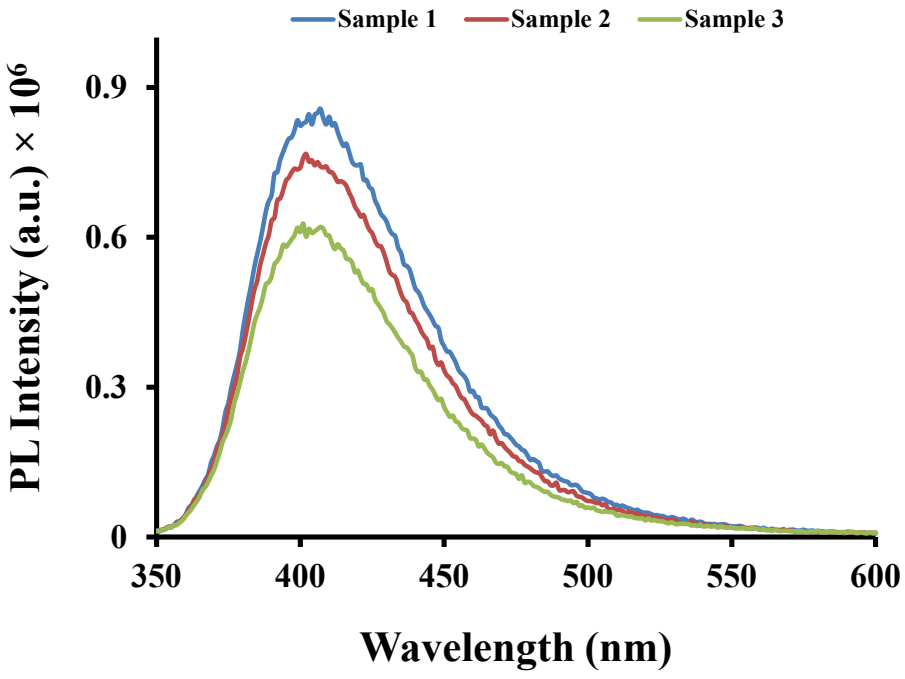

Fig. S23 Change in emission of PMI after adding FAD-doped serum samples.

References:

1. A. J. J. M. van Breemen, P. T. Herwig, C. H. T. Chlon, J. Sweelssen, H. F. M. Schoo, E. M. Benito, D. M. de Leeuw, C. Tanase, J. Wildeman and P. W. M. Blom, *Adv. Funct. Mater.* 2005, **15**, 872-876.
2. G. Saikia, R. Singh, P. J. Sarmah, M. W. Akhtar, J. Sinha, M. Katiyar and P. K. Iyer, *Macromol. Chem. Phys*. 2009, **210***,* 2153-2159.
3. S. Hussain, A. H. Malik and P. K. Iyer, *ACS Appl. Mater. Interfaces,* 2015, **7**, 3189-3198.