Electronic Supplementary Information

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

Sameer Hussain,\textsuperscript{a} Akhtar Hussain Malik,\textsuperscript{a} and Parameswar Krishnan Iyer\textsuperscript{a,b}*

\textsuperscript{a}Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati-781039, India
\textsuperscript{b}Centre for Nanotechnology, Indian Institute of Technology Guwahati, Guwahati, 781039, India

AUTHOR EMAIL ADDRESS: pki@iitg.ernet.in

AUTHOR FAX: +91 361 258 2349
Scheme S1. (a) FeCl₃, 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, 1 eq.) and excess 1-methyl imidazole were transferred to a 100mL round-bottom flask and kept for stirring at 80°C in an oil bath. After 36 h, the reaction mixture was poured into excess CHCl₃ 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-$d_6$, $\delta$): 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-$d_6$, $\delta$): 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.

$$y = -4.58 \times 10^x + 951483$$

$$R^2 = 0.9915$$

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)
Fig. S7 Fitting parameters and IRF values correspond to lifetime measurements.
Fig. S8 Emission spectra of PMI, RF and the PMI/RF mixture at $\lambda_{ex}=325$ nm.

Fig. S9 Emission spectra of PMI, FMN and the PMI/FMN mixture at $\lambda_{ex}=325$ 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 efficiency for RF detection. Concentration of PMI and RF were 10 µM and 25 µM, respectively.

**Fig. S15** Effect of ionic strength on FRET efficiency for FMN detection. Concentration of PMI and FMN were 10 µM and 10.33 µM, respectively.
**Fig. S16** Effect of ionic strength on FRET efficiency 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 × $\sigma$/k

LOD = $3 \times 2100.54/1.12 \times 10^{10}$

= $5.62 \times 10^{-7}$ M (0.56 µM)
Fig. S18 Detection limit plot for Cu$^{2+}$ using PMI/FAD complex.

$\text{LOD} = 3 \times \sigma/k$

$\text{LOD} = 3 \times 2802.44/9.76 \times 10^{10}$

$= 8.61 \times 10^{-7}$ M (0.86 µM)

Fig. S19 Change in emission spectra on adding Cu$^{2+}$ ($6.6 \times 10^{-5}$ 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.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Material used</th>
<th>Sensing analytes</th>
<th>Detection Method</th>
<th>Discrimination</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current Work</strong></td>
<td><strong>Conjugated Polyelectrolyte</strong></td>
<td><strong>RF, FMN, FAD and Cu^{2+}</strong></td>
<td><strong>FRET</strong></td>
<td><strong>RF/FMN and RF/FAD</strong></td>
<td><strong>RF-0.69 µM, FMN-19.68 nM and FAD-13.40 nM</strong></td>
</tr>
<tr>
<td><em>J. Am. Chem. Soc.</em> <strong>2007</strong>, 129, 4524-4525</td>
<td>Bis(Zn^{2+}-dipicolylamine complex)</td>
<td>FAD</td>
<td>Direct fluorescence method</td>
<td>Not shown</td>
<td>Not determined (Detection in µM range)</td>
</tr>
<tr>
<td><em>ACS Appl. Mater. Interfaces</em> <strong>2013</strong>, 5, 7392-7399</td>
<td>Sulfonated Graphene</td>
<td>RF and FMN</td>
<td>FRET</td>
<td>Not shown</td>
<td>0.6 µg/mL (1.6 µM)</td>
</tr>
<tr>
<td><em>Organic Letters,</em> <strong>2013</strong>, 15, 1210-1213</td>
<td>Pyrene bound Zn^{2+}-dipicolylamine</td>
<td>FMN and FAD</td>
<td>Ratiometric method</td>
<td>FMN/FAD</td>
<td>Not determined (Detection in µM range)</td>
</tr>
</tbody>
</table>
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:

