Supplementary material

A cationic AIEgen and hexametaphosphate based simple and convenient fluorometric assay for alkaline phosphatase and its inhibitor

Jasvir Kaur, ^a Harshad A. Mirgane,^b Sheshanath V. Bhosale^b and

Prabhat K. Singh^{a,c}

^aRadiation& Photochemistry Division, Bhabha Atomic Research Centre, Mumbai 400 085, INDIA

^bSchool of Chemical Sciences, Goa University, Taleigao Plateau, Goa 403 206, INDIA

°Homi Bhabha National Institute, Anushaktinagar, Mumbai-400085, INDIA

*Authors for correspondence: Email: prabhatk@barc.gov.in; prabhatsingh988@gmail.com

Tel. 91-22-25590894, Fax: 91-22-25505151



Figure S1. Steady-state fluorescence spectra displaying the changes in emission of TPy-TPE ([TPy-TPE] ~ 2 μ M, $\lambda_{ex} = 350$ nm) in the presence of NaCl solutions of different concentrations [mM] (1) 0 (2) 3.0 (3) 5.0 (4) 8.0 (5) 12.0 where, red solid line denotes free TPy-TPE in solution. Inset: Variation in emission intensity at 550 nm at different NaCl concentrations. The blue circles correspond to the data points and the error bar shows the standard deviation (n=3).



Figure S2. Ground-state absorption spectra displaying the changes in absorbance of TPy-TPE-HMP ([TPy-TPE] ~ 2 μ M, [HMP] ~ 26 μ M) in the absence and presence of [NaCl] /12

mM. Inset: Changes in absorbance at 350 nm versus NaCl concentration. The error bar represents the standard deviation value calculated from three successive measurements.



Figure S3. Change in fluorescence intensity of TPy-TPE-HMP complex at 30 °C as a function of incubation time.



Figure S4. Fluorescence decay profiles for TPy-TPE-HMP complex ([TPy-TPE] = $\sim 2 \mu M$, [HMP] = $\sim 12 \mu M$, $\lambda_{ex} = 374 \text{ nm}$, $\lambda_{em} = 550 \text{ nm}$) in aqueous medium at different temperatures (°C) (1) 25 (2) 30 (3) 35 (4) 50 (5) 70 where, the black colour dotted line denotes the instrument response function (IRF). Inset: Variation in average excited state lifetime (τ_{avg}) of

TPy-TPE-HMP versus temperature. The error bar represents the standard deviation value calculated from three successive measurements.



Figure S5. The change in emission intensities at 550 nm for TPy-TPE-HMP complex ([TPy-TPE] ~ 2 μ M, [HMP] ~26 μ M) at different solution pH values. The error bar represents the standard deviation value calculated from three successive measurements.



Figure S6. The change in absorbance at 350 nm for TPy-TPE-HMP complex ([TPy-TPE] $\sim 2 \mu$ M, [HMP] $\sim 26 \mu$ M) at different solution pH values. The error bar represents the standard deviation value calculated from three successive measurements.



Figure S7. Lineweaver-Burk plot for the digestion of HMP by ALP [990 mU/mL] (λ_{ex} = 350 nm, λ_{em} = 550 nm) in 10 mM Tris-HCl, pH 8.2 at 30^oC at different concentrations of HMP (μ M) (1) 1.6 (2) 6.5 (3) 11.0. The error bar represents the standard deviation value calculated from three successive measurements.



Figure S8. Selectivity of ALP measured as normalised fluorescence decay of the TPy TPy-TPE-HMP complex in 1% human serum prepared in 10 mM Tris-HCl, pH 8.2 at 30°C in the presence of various concentrations of ALP (mU/ml) (1) 0 (2) 495 (3) 865 (4) 1237 (5) 1484 (6) 1855.