Electronic Supplementary Information

Sensitive Detection of Acid Phosphatase Enzyme and Screening of Inhibitors Using an Anionic Polyfluorene Derivative

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Reagents and materials

All the reagents and solvents were purchased from Aldrich Chemicals (India), Merck (India) or Ranbaxy (India) and were used as received. Milli-Q water was used in all the experiments. Acid phosphatase (ACP) from potato lyophilized (3.0-10 units/mg solid) powder was obtained from Sigma.

Instrumentation

Fluorescence spectra were carried out on a Varian Cary Eclipse Spectrometer. A 10 mm X 10 mm quartz cuvette was used for solution spectra and emission was collected at 90˚ relative to the excitation beam. Deionized water obtained by Milli-Q system (Millipore) was used.

Synthesis of P1:

The synthesis of polymer P1 was performed as per our earlier method. (A. K. Dwivedi, G. Saikia, P. K. Iyer, *J. Mater. Chem.,* **2011**, *21*, 2502–2507.)

Fluorescence quenching and dequenching experiments:

The experiments were carried out in 15 mM Tris-HCl buffer solution of acidic pH 6.0. The addition of 3 μM Fe^{3+} solution was able to quench the fluorescence of P1 (0.6 μM) solution. For the dequenching experiment two samples containing 40 μM of P_i and 40 μM of pNPP were prepared and incubated with P1/Fe^{3+} solution and fluorescence intensity changes were observed immediately and after 8 hours.

Real Time acid phosphatase assay:

The experiments were carried out in 15 mM Tris-HCl buffer solution of acidic pH 6.0. Fluorescence quenching experiment was performed as described above. Six samples containing P1-Fe^{3+} and 40 μM pNPP in 15 mM Tris-HCl buffer solution at pH 6.0 were prepared followed by the addition of different concentration of enzyme, say 0 nM, 4 nM, 8 nM, 14 nM, 20 nM and 28 nM and fluorescence intensity changes were monitored at the emission intensity 411 nm with increasing concentration of ACP as a function of time.

Detection Limit:

Assay was used to monitor the ACP catalyzed pNPP hydrolysis as a function of time upon incubation with different concentration of ACP (0-28 nM). Limit of Detection (LOD) was calculated by using the following equation:

$$LOD = 3\sigma/K$$

Where σ is the standard deviation and K is the slope of linear plot between fluorescence intensity and ACP concentration.

Calculation of product p-nitro phenol (pNP) concentration:

First, unhydrolyzed pNPP concentration was determined at different times from the obtained fluorescence intensity curves by using the following equation.

$$[S]_t = [S]_0 [(I_o/I_t - 1) / (I_o/I_q - 1)]$$

Where $[S]_t$ is the substrate concentration at a time t, $[S]_0$ is the initial substrate concentration, Io is the initial fluorescence intensity of P1, I_t is the fluorescence intensity at time t and I_q is quenched fluorescence intensity of P1. Product concentration was then calculated by the following equation

$$[pNP] = [S]_0 - [S]_t$$

Calculation of Kinetic Parameters:

Different initial concentrations of substrate from 0 to 40 μM were chosen and fluorescence intensity changes were recorded as a function of time to calculate the kinetics parameters.

Lineweaver-Burk plot was obtained by using the double reciprocal of initial rate vs substrate concentration.

$$1/V = K_m/V_{max} [S]_0 + 1/V_{max}$$

Where, V is the initial rate and calculated from the slopes of the plots $[S]_0$ is the initial substrate concentration, K_m is the Michaelis constant and V_{max} is the maximal velocity.

Real Time acid phosphatase assay in the presence of inhibitor:

The experiments were carried out in 15 mM Tris-HCl buffer solution of acidic pH 6.0. Fluorescence quenching experiments were performed as described above. Six samples containing P1-Fe^{3+} and 40 μM pNPP and 28 nM in 15 mM Tris - HCl buffer solution at pH 6.0 were prepared followed by the addition of different concentration of inhibitor, say 0 nM, 10 nM, 50 nM, 100 nM, 200 nM and 500 nM and fluorescence intensity changes were monitored at the emission intensity 411 nm with increasing concentration of inhibitor as a function of time.