Electronic Supplementary Information

Enzyme immobilization and molecular modeling studies on organic-inorganic polypyrrole-titanium(IV)phosphate nanocomposite

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ESI. 1. Loading Efficiency of varying amount of YADH added to nanocomposite

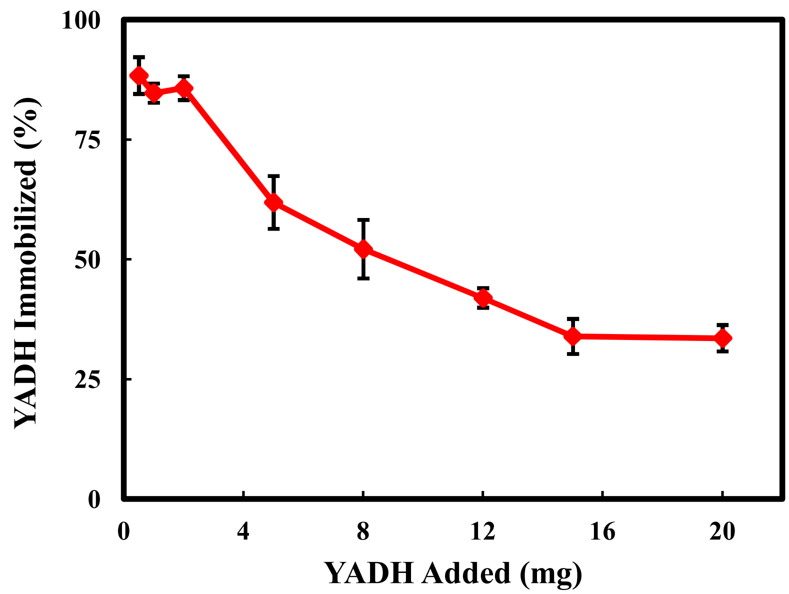

Fig. S1. Binding efficiency of varying amount of YADH added to 20 mg of nanocomposite.

When the amount of PPy and PPy-TiP nanocomposite was kept constant at 20 mg and the concentration of YADH varied from 1-15 mg/mL, it was that the most efficient level of enzyme binding occurred upto 2 mg/mL of YADH as shown in **Fig. S1**. At this level, around 85 % YADH had become immobilized. Beyond 2 mg/mL, the percentage of bound YADH decreases exponentially. So, for further experiments we have used 2 mg/ mL of YADH immobilized on 20 mg of nanocomposite. We have also calculated the weight ratio (mg of bound YADH/mg of nanocomposite) which was 0.335. It was found that when weight ratio increases the residual activity decreases. It has been proposed that when YADH is too heavily saturated on the surface of nanocomposite, it causes steric hindrance and blocks the active site of the YADH. If active site of an enzyme is hindered by any means or substrates are not accessible to the enzyme, the overall activity of that enzyme decreases. As shown in **Table S1** that the point of saturation for the enzyme complex on the nanocomposite surface is 0.08, at which the enzyme attained maximum activity value of 417.08 IU/mg.

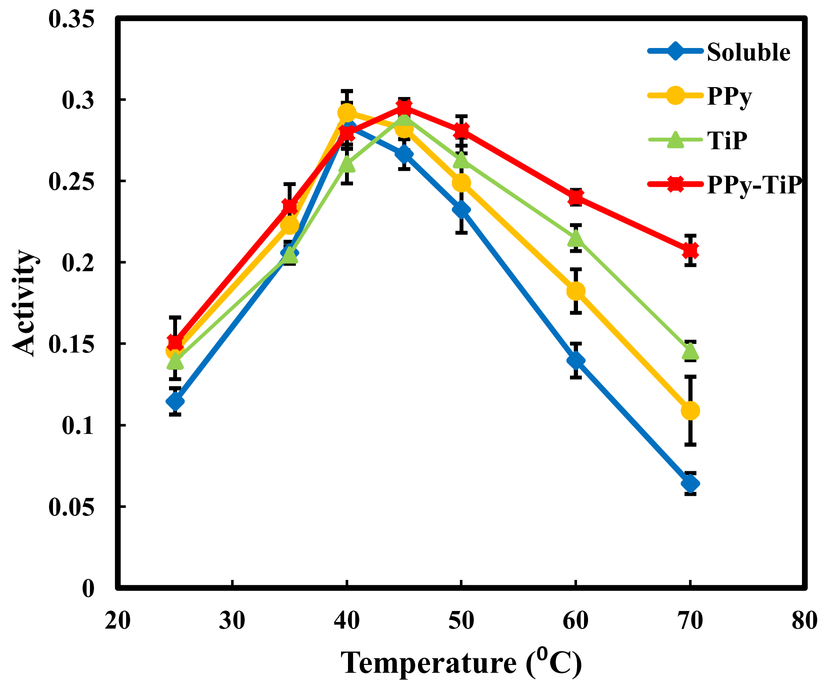

Fig. S2. Effect of temperature on the activity of soluble and immobilized YADH.

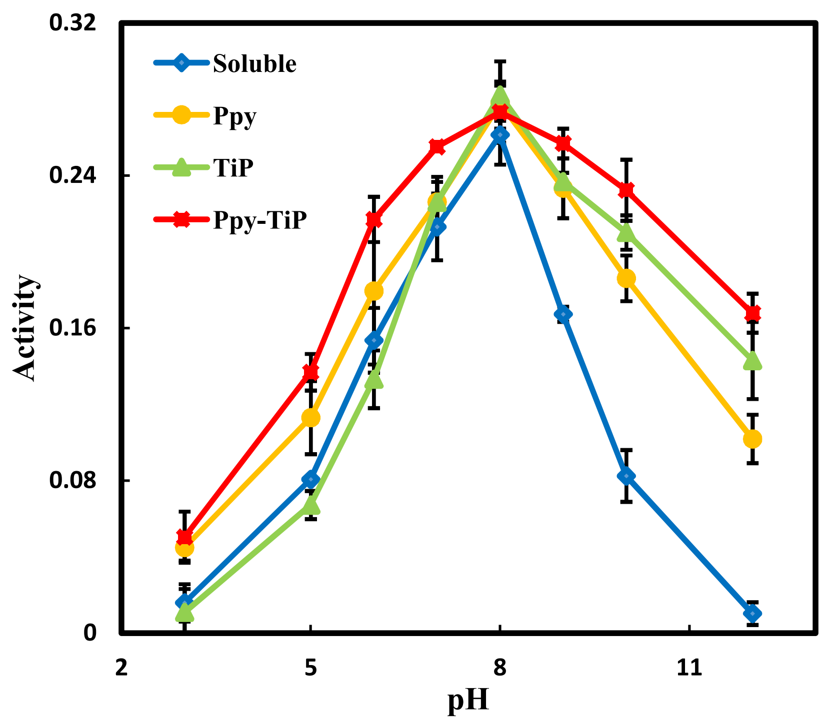

Fig. S3. Effect of pH on the activity of soluble and immobilized YADH.

Table S1. Loading efficiencies for varying ratio of bound YADH to PPy-TiP nanocomposite.

YADH added (mg)	YADH bound (mg)	Weight ratio (mg of bound enzyme/mg of nanoparticles)	Enzyme activity (IU/mg) Theoretical (A)	Enzyme activity (IU/mg) Calculated (B)	Loading efficiency (B/A) x100
0.5	**0.43**	**0.021**	**150**	**81.44 ± 7.95**	**52.49 ± 31**
1	**0.82**	**0.041**	**300**	**196.28 ± 5.53**	**65.81 ± 1.41**
2	**1.70**	**0.084**	**600**	**417.08 ± 16.22**	**68.84 ± 3.12**
5	**3.09**	**0.154**	**1500**	**768.32 ± 42.87**	**50.81 ± 2.44**
8	**4.17**	**0.208**	**2400**	**992.52 ± 38.74**	**40.99 ± 1.20**
12	**5.03**	**0.251**	**3600**	**1065.8 ± 37.99**	**19.97 ± 1.71**
15	**5.09**	**0.254**	**4500**	**1009.79 ± 27.57**	**22.51 ± 1.91**
20	**6.71**	**0.335**	**6000**	**910.49 ± 19.54**	**16.68 ± 1.64**

ESI. 1. Enzyme leakage behavior

The enzyme leakage behavior was calculated during reusability assay. In this assay, immobilized formulations were stored in Tris-HCl buffer of pH 8.0 at 4 ^{0}C for the period of 24 hrs. After 24 h, whole formulations were centrifuged at 4000 rpm for 20 min and collected the supernatant as well as the pallet. In the supernatant, protein concentration as well as the YADH activity was measured. This process was run for 10 successive uses. The leakage ratio was calculated by the following equation:

$$\textbf{Leakage (\%)} = ([\text{YADH}]_{solution} \times V_{solution} / \text{Total YADH immobilized}) \times 100$$

As shown in **Table S2**, 42.58% of immobilized YADH on PPy nanoparticles leaks out during the course of 10 successive uses whereas only 9.54 % leaks out in the case of PPy-TiP nanocomposite. Therefore, the YADH immobilized on PPy-TiP nanocomposite was having maximum reusability. Now this part is added as supplementary information.

Table S2. Leakage of YADH in PPy, TiP and PPy-TiP nanocomposite.

S. No	Source of YADH	Leakage percentage (%)
1	PPy-YADH	42.58±4.45
2	TiP-YADH	21.76±2.87
3	PPy-TiP-YADH	9.54±1.06