Supporting information

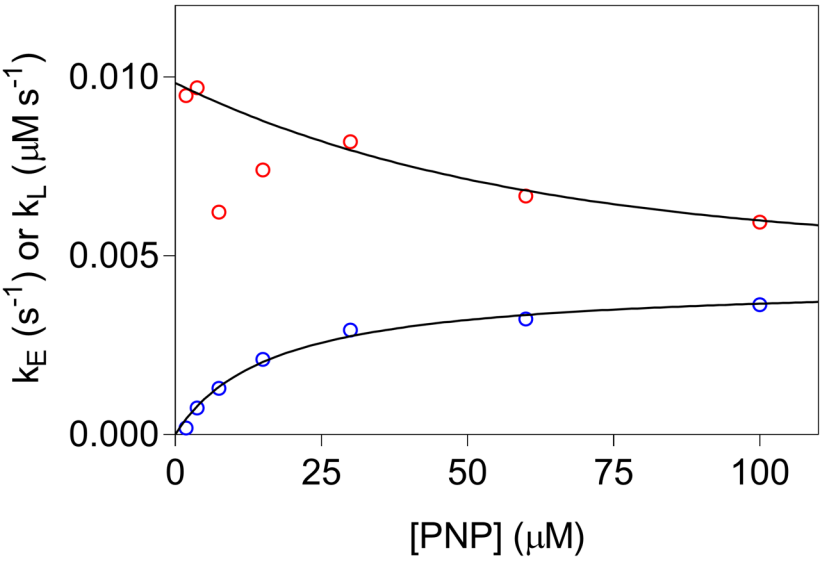

Figure 1S. Parameters estimated from fitting of data shown in Fig 2A. Fitting was carried out using Eq. 1, obtaining values of k_E (red symbols) and k_L (blue symbols) as a function of PNP concentration. Lines through parameter values were obtained by fitting to a hyperbolic saturation equation. Interestingly, fitting of k_L returned a dissociation constant (16.2 ± 0.01 μM) that is comparable to K_{Dp} obtained from PLP inhibition kinetics (see Table 1). This is expected, since the rate of the constant slow phase depends on substrate binding to the enzyme-PLP complex (see Scheme 1A).

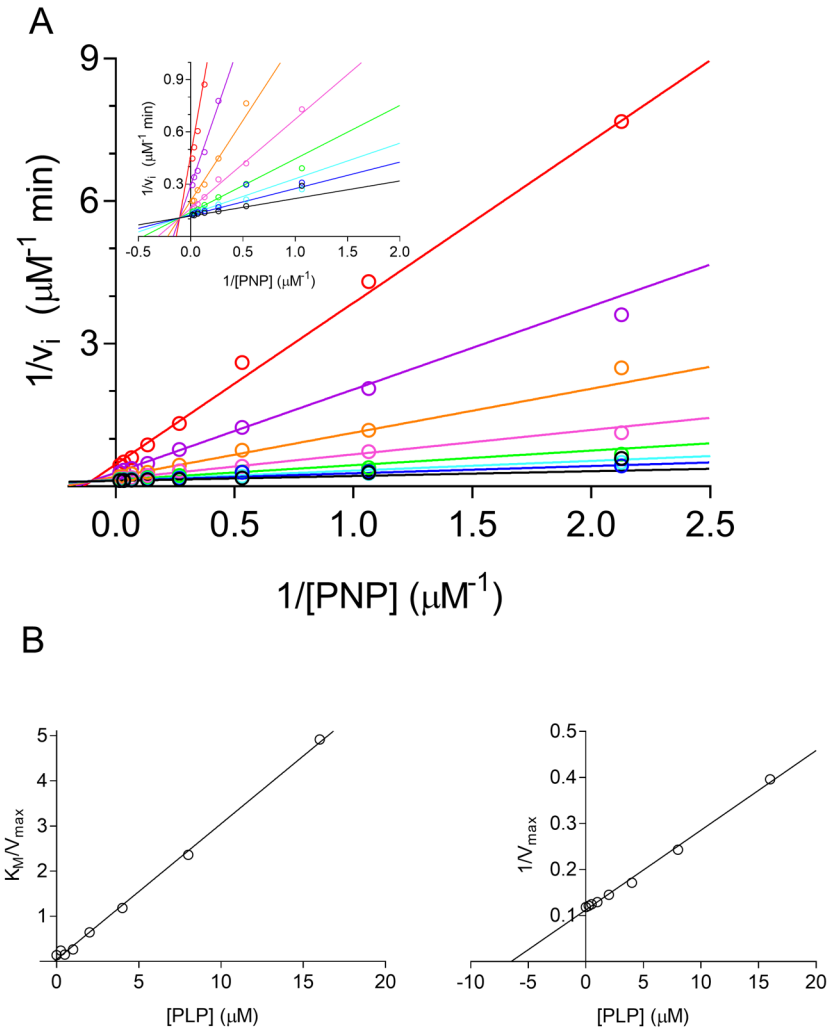

Figure 2S. Analysis of PLP inhibition kinetics. **A)** Double reciprocal plot of data represented in Fig. 3A fitted to a linear equation. Intercepts of straight lines on the Y- and X-axes gave estimates of apparent V_{max} and apparent K_M. It is clear that lines intercept in the fourth quadrant of the plot (inset), revealing the mixed-type nature of PLP inhibition. **B)** Secondary plots of K_M/V_{max} (left panel) and $1/V_{max}$ (right panel), corresponding to the slopes and intercepts of the straight lines shown in Fig. 2SA, respectively, gave estimates of K_I (0.15 ± 0.13 μM) and K_{Is} (6.5 ± 0.3 μM), which are comparable to those obtained by fitting apparent V_{max} and K_D to Eqs. 3, 4 and 5, as explained in the text.

Derivation of the quadratic equation (Eq. 2) used to fit initial velocity data shown in Fig. 3A and 2B.

The model used to describe the substrate binding equilibrium is $E + PNP \leftrightharpoons E \cdot PNP$, whose dissociation constant is $K_D = \frac{[E][PNP]}{[E \cdot PNP]} = \frac{([E_0]-[E \cdot PNP])([PNP_0]-[E \cdot PNP])}{[E \cdot PNP]}$, where $[E_0]$ and $[PNP_0]$ are the total or initial enzyme and substrate concentrations, respectively, and [E·PNP] is the concentration of the enzyme-PNP complex. Solving for [E·PNP], the following equation is obtained:

$[E \cdot PNP]^2 - ([PNP_0] + [E_0] + K_D)[E \cdot PNP] + [PNP_0][E_0] = 0$, which is in the form of $ax^2 + bx + c = 0$,

thus $[E \cdot PNP] = \frac{([PNP_0]+[E_0]+K_D)-\sqrt{([PNP_0]+[E_0]+K_D)^2-4[PNP_0][E_0]}}{2}$. Considering that the initial velocity of the reaction is $v_i = V_{MAX}\frac{[E \cdot PNP]}{[E_0]}$, then $v_i = k_{CAT}[E_0]\frac{([PNP_0]+[E_0]+K_D)-\sqrt{([PNP_0]+[E_0]+K_D)^2-4[PNP_0][E_0]}}{2\,[E_0]}$ (Eq. 2), in which k_{CAT}·$[E_0]$ corresponds to V_{MAX}, the maximum velocity of the reaction.

Derivation of the quadratic equation (Eq. 6) used to fit equilibrium binding data shown in Fig. 4 and Fig. 6.

The dissociation constant of the binding equilibrium $E + P \leftrightharpoons E \cdot P$, between enzyme (E) and PLP (P), is $K_D = \frac{[E][P]}{[E \cdot P]} = \frac{([E_0]-[E \cdot P])([P_0]-[E \cdot P])}{[E \cdot P]}$, where $[E_0]$ and $[P_0]$ are the total or initial enzyme and PLP concentrations, respectively, and [E·P] is the concentration of the enzyme-PLP complex. Solving for [E·P], the following equation is obtained:

$[E \cdot P]^2 - ([P_0] + [E_0] + K_D)[E \cdot P] + [P_0][E_0] = 0$, which is in the form of $ax^2 + bx + c = 0$;

thus $[E \cdot P] = \frac{([P_0]+[E_0]+K_D)-\sqrt{([P_0]+[E_0]+K_D)^2-4[P_0][E_0]}}{2}$. During our titration experiments, we measured the fluorescence increase of FMN due to binding of PLP to the enzyme, which is proportional to the fractional saturation expressed as the ratio between [E·P] and $[E_0]$. Considering that the measured relative fluorescence is $F_{rel} = \Delta F_{MAX}\frac{[E \cdot P]}{[E_0]}$, where the fluorescence change (ΔF_{MAX}) is the difference between fluorescence measured in the absence of ligand (F_0) and fluorescence at infinite ligand concentration (F_{inf}), then

$F_{rel} = F_0 + (F_{inf} - F_0) \times \frac{[P_0]+[E_0]+K_I-\sqrt{([P_0]+[E_0]+K_I)^2-4\,[P_0][E_0]}}{2\,E_0}$ (Eq. 6).

Equilibrium binding data relative to the apoenzyme were fitted to a modified version of the previous equation, since in this case PLP binding to the protein caused an increase of fluorescence:

$$F_{rel} = F_{inf} + (F_0 - F_{inf}) \times \left(1 - \frac{[P_0] + [E_0] + K_I - \sqrt{([P_0] + [E_0] + K_I)^2 - 4\,[P_0][E_0]}}{2\,E_0}\right)$$