SUPPORTING INFORMATION

Light-Enhanced Catalysis by Pyridoxal Phosphate Dependent Aspartate Aminotransferase

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Free PLP and Schiff bases in Water

Power dependent rate constants for the conversion of PLP-SB to PMP are reported for PLP-Asp in solution (Figure S1). HPLC analyses confirmed that the PLP-Schiff base photoproduct that absorbs at 325 nm is PMP (pyridoxamine 5'-phosphate) (Figure S3). Standards of PMP and PLP have retention times at 6.2 min and 9.2 min, respectively (Figure S3: solid). A PLP-valine Schiff base sample that had been irradiated for 30 minutes at pH 7.5, 25°C under a stream of N_2 gas showed a single peak that corresponded to the PMP standard (Figure S3: dotted).

UV-Visible spectra were collected over time for the PLP-valine (Figure S2) and PLP-AIB (α-aminoisobutyric acid; Figure S4) Schiff bases. The absorbance spectrum of PLP-valine in the dark at pH 7.0 exhibits peaks at 412 nm and 280 nm. PLP-Schiff base samples exhibit two

clear isosbestic points at 290 nm and 350 nm during reaction. The photoproduct was identified as PMP.[1,2] PLP-valine kinetics were measured in μs-transient absorption experiments following pulsed 400 nm excitation. The kinetic traces were wavelength independent over the spectral range collected (Figure S5 and S6).

Enzyme Photodynamics

To provide evidence for the existence of the triplet in AAT reactions, spectra were calculated (Figure S7), by subtracting the ground state (scaled to the peak of the bleach) and solvated electron spectra from the 6 ns spectrum for PLP-Asp and AAT-α-methylaspartate samples. The 6 ns spectra were chosen since this time period represent ~8 half-lives for excited state decay and an insignificant amount of triplet decay has occurred. Thus, the 6 ns spectra should be well representative of pure triplet state. The external and internal aldimine in AAT and PLP-Asp exhibit similar absorbance spectra (Figure S10), providing evidence that the AAT reaction indeed goes through a reactive triplet state as do PLP Schiff bases in solution.

A control experiment with MDH was performed to ensure light enhancement of AAT was not due to an effect on the coupling enzyme. No increase in activity was observed after exposure to 440 nm light (Figure S8). This also confirmed that the increase in activity is not due to heating of the sample by the LED array used as actinic light source. An additional control was performed to investigate the possibility that the increase in activity with light is due to active sight heating from vibronic relaxation of the chromophore. The FAD dependent enzyme glutathione reductase absorbs similar wavelengths compared to the PLP dependent enzyme and did not show any increase in activity during exposure to blue light (Figure S9).

The activity of the K258A mutant, where the catalytic lysine base in replaced with an alanine, was enhanced 1000-fold in the presence of 1W of blue light (Figure S11) at pH 8.5, 25 °C. The power dependence of the k_{max} data is shown in Figure S12.

Kinetics Simulations

COPASI v.4.5 (COmplex PAthway SImulator) was used to simulate the rate of product formation in AAT reactions at various light intensities using the rate constants from ultrafast transient absorption or μs-transient absorption experiments, and from previously published rate constants.[3,4] Values not explicitly reported were estimated based on the percentage population present under steady state conditions.[3] The rate constant for the ground to excited state transition (k*) was obtained as follows:

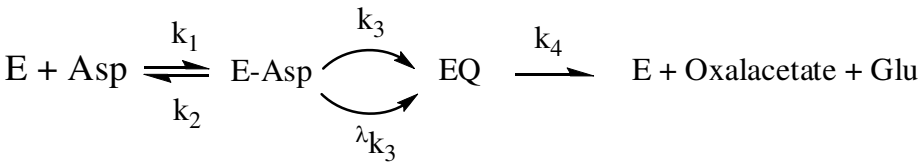
$k^* = \frac{B_{13} I}{c}$ × spectral overlap between LED and sample

B_{13} is the Einstein B coefficient: $B = \int \varepsilon \, d\omega \,/\, \frac{2\pi N_A \hbar \omega}{10^3 c \ln 10}$ in $m^3\ J^{-1}\ s^{-2}$

I is the irradiance per unit frequency interval in $W\ m^{-2}\ Hz^{-1}$

$\int \varepsilon \, d\omega$ is the integral of the extinction coefficient associated with the singlet transition of PLP. The linewidth of this transition was obtained after fitting the sample absorbance spectrum in angular frequency to a Gaussian curve. The quantum yield of the triplet state (64%) was estimated from the ultrafast transient absorption data based on the following equation: $\Phi_{ISC} = \frac{k_{ISC}}{k_{IC} + k_{ISC}}$.

The equation describing the power dependence of the rate enhancement was obtained as follows. The simplified mechanism describing the reaction is:



$$k_{cat} = \frac{k_3 k_4}{k_3 + k_4} \left(= k_{cat}^{dark} \right)$$

$$k_3' = k_3 + {}^{\lambda}k_3$$

$${}^{\lambda}k_{cat} = \frac{k_3' k_4}{k_3' + k_4} \left(= k_{cat}^{light} \right)$$

$$\frac{{}^{\lambda}k_{cat}}{k_{cat}} = \frac{\left(\frac{k_3' k_4}{k_3' + k_4} \right)}{\left(\frac{k_3 k_4}{k_3 + k_4} \right)} = \left(\frac{k_3'}{k_3} \right) \left(\frac{k_3 + k_4}{k_3 k_4} \right) = \frac{k_3' k_3 + k_3' k_4}{k_3 k_3' + k_3 k_4} = \frac{k_3'/k_3 + k_3'/k_4}{1 + k_3'/k_4}$$

$$\frac{{}^{\lambda}k_{cat}}{k_{cat}} = \frac{\frac{k_3 + {}^{\lambda}k_3}{k_3} + \frac{k_3 + {}^{\lambda}k_3}{k_4}}{1 + \frac{k_3 + {}^{\lambda}k_3}{k_4}} = \frac{1 + \frac{{}^{\lambda}k_3}{k_3} + \frac{k_3}{k_4} + \frac{{}^{\lambda}k_3}{k_4}}{1 + \frac{k_3}{k_4} + \frac{{}^{\lambda}k_3}{k_4}}$$

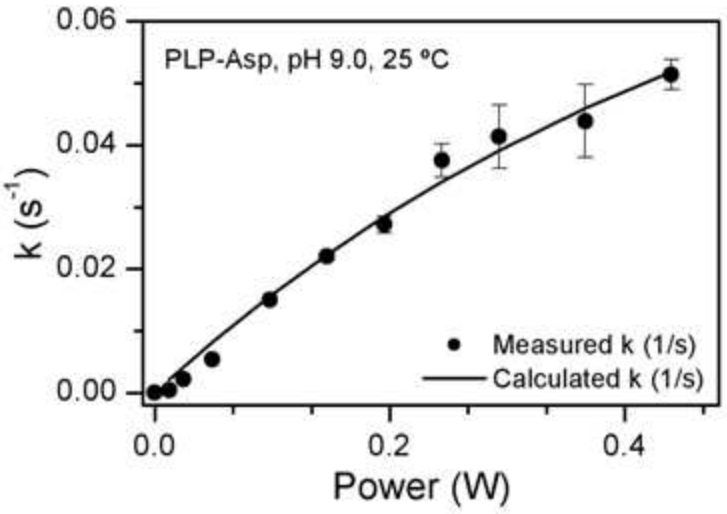


Figure S1. Power dependence of the PLP-Asp Schiff base k in solution, pH 9.0, 25 ºC.

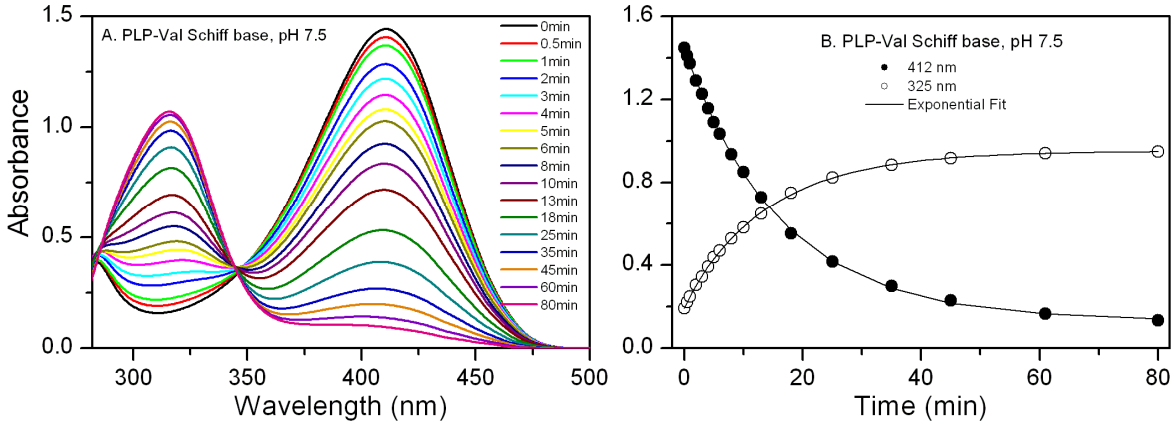


Figure S2. PLP-Val reactions under 440 nm irradiation. A) Absorption spectra of PLP-valine exposed to 50 mW of light. B) Traces at 412 nm (closed circles) and 325 nm (open circles) with exponential fits (solid).

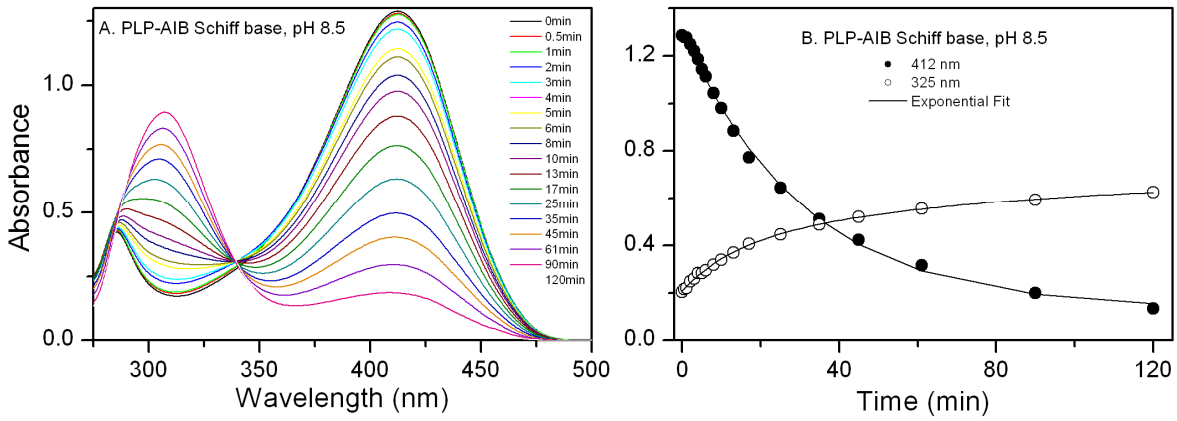


Figure S3. PLP-AIB reactions under 440-nm irradiation. A) Absorption spectra of PLP-AIB exposed to 50 mW of light. B) Traces at 412 nm (closed circles) and 325 nm (open circles) with exponential fits (solid).

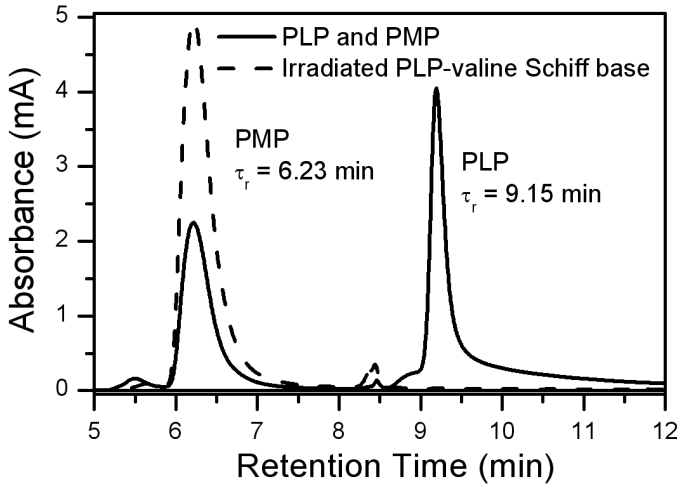


Figure S4. HPLC of standards PLP and PMP (solid) compared with the irradiated PLP-valine (dotted). Chromatograms were monitored at 330 nm.

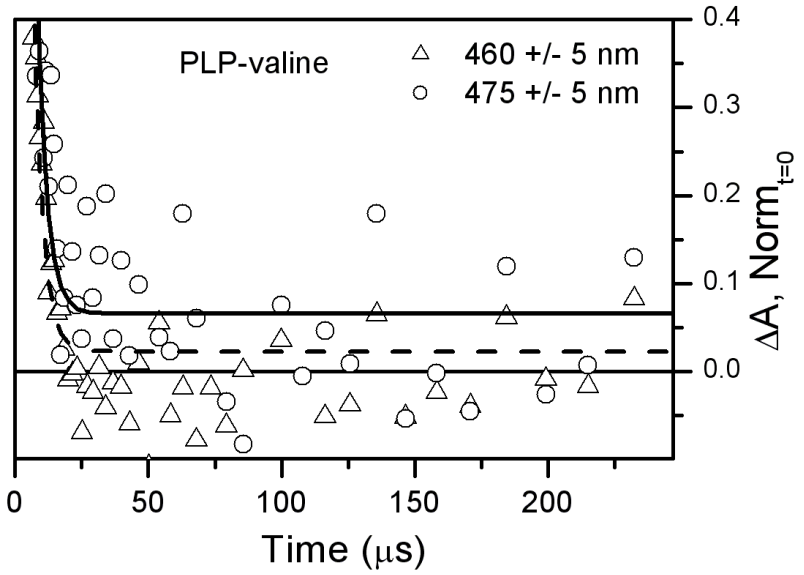


Figure S5. Wavelength independence of μs pump-probe kinetics with PLP valine.

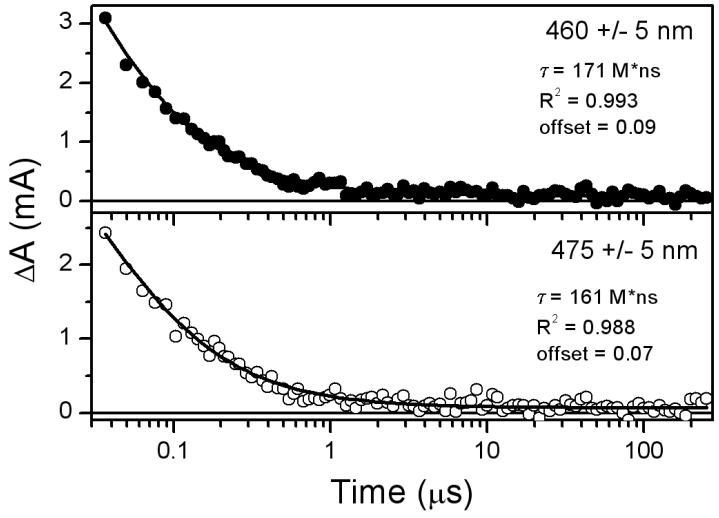


Figure S6. μs-Transient absorption traces of PLP-valine with KI (circles) with exponential fits (line).

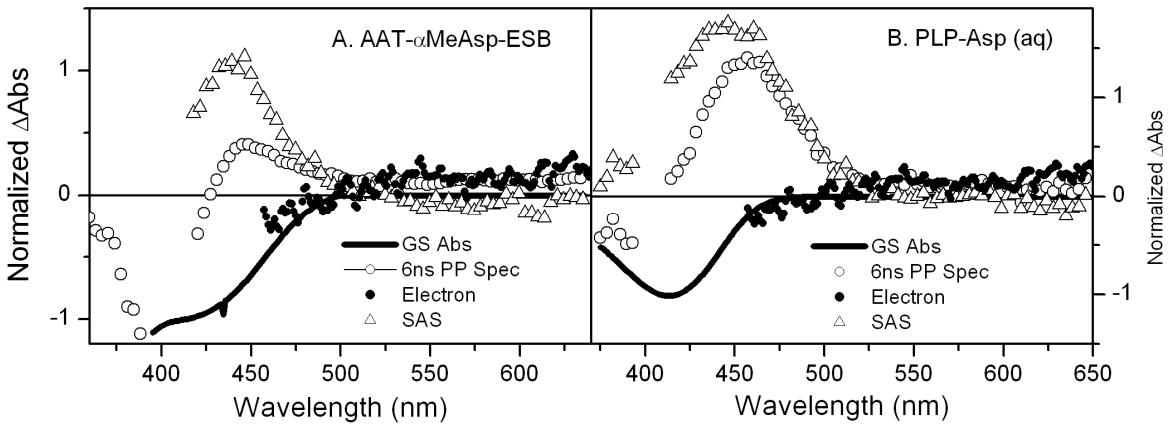


Figure S7. Construction of SAS for external aldimine in AAT (A) and PLP-Asp in water (B).

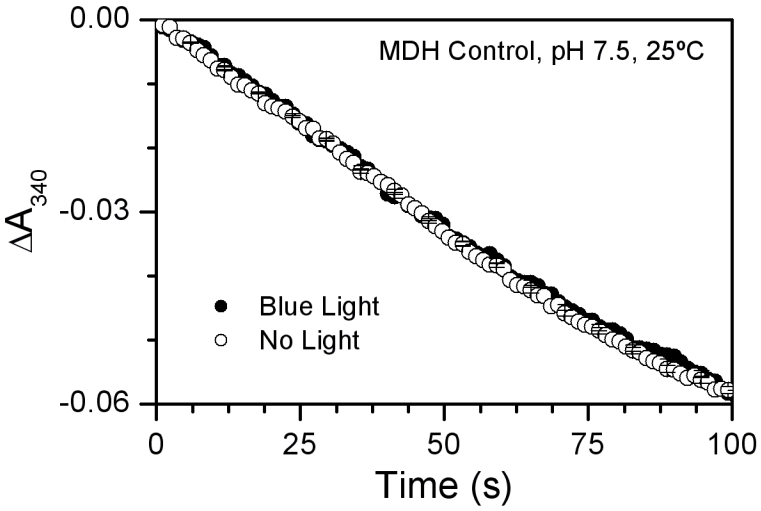


Figure S8. Catalytic activity (k_{cat}) of MDH with (closed circles) and without (open circles) 250 mW of 440-nm irradiation. Error bars indicate one standard deviation calculated from three trials.

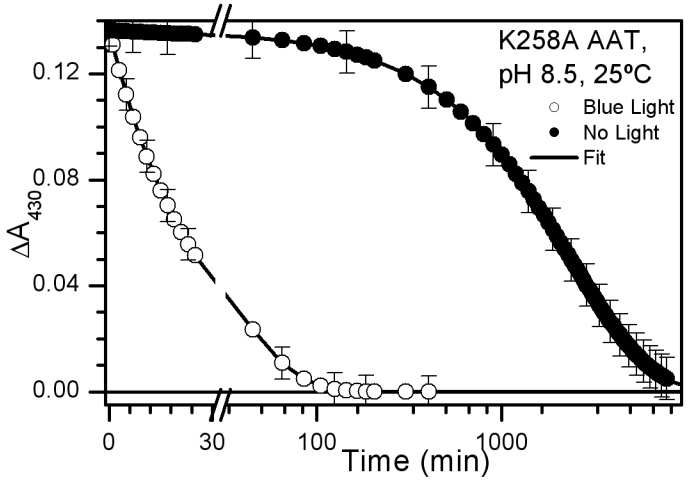


Figure S9. Reaction of the K258A mutant with and without 1 W of 450 nm irradiation: closed circles, light off; open circles, light on. Error bars indicate one standard deviation calculated from three trials.

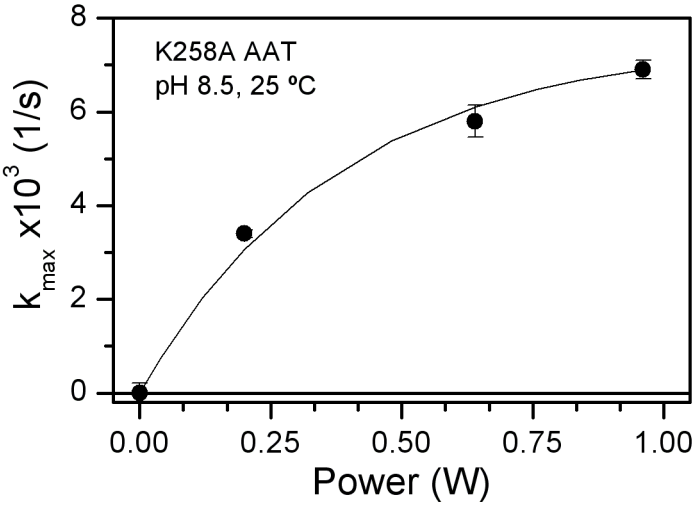


Figure S10. Power dependence of the K258A AAT k_{max}, pH 8.5, 25 ºC.

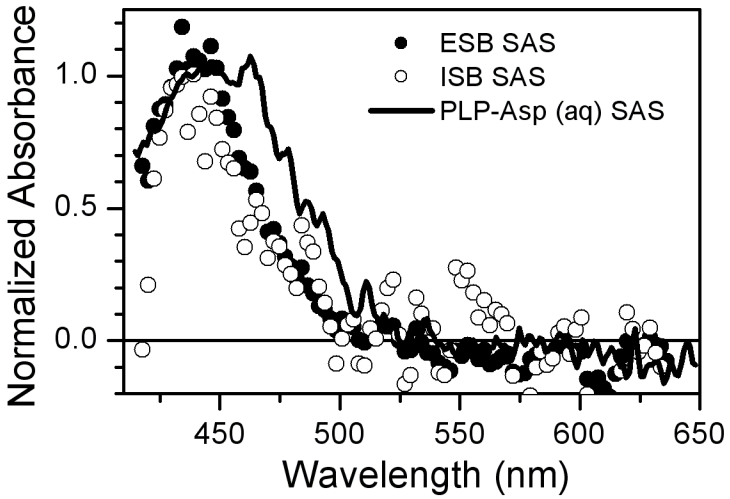


Figure S11. Species Associated Spectra (SAS) for external aldimine in AAT, internal adlimine in AAT, and PLP-Asp in water.

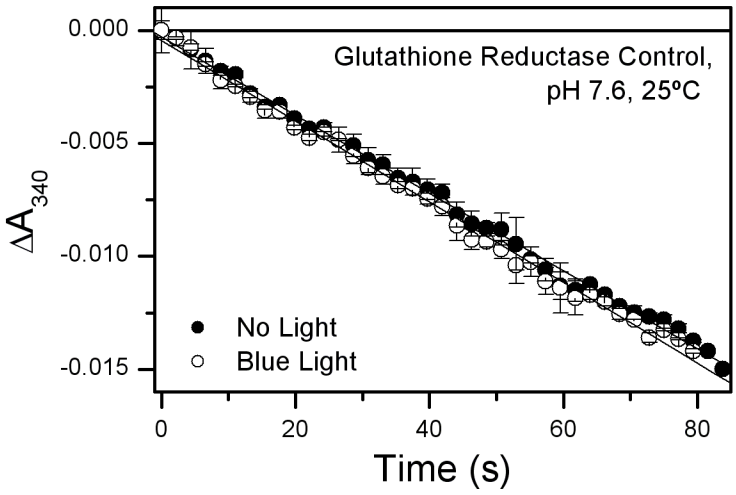


Figure S12. Catalytic activity (k_{cat}) of glutathione reductase under 250 mW of 440 nm irradiation: closed circles, light off; open circles, light on. Error bars indicate one standard deviation calculated from three trials.

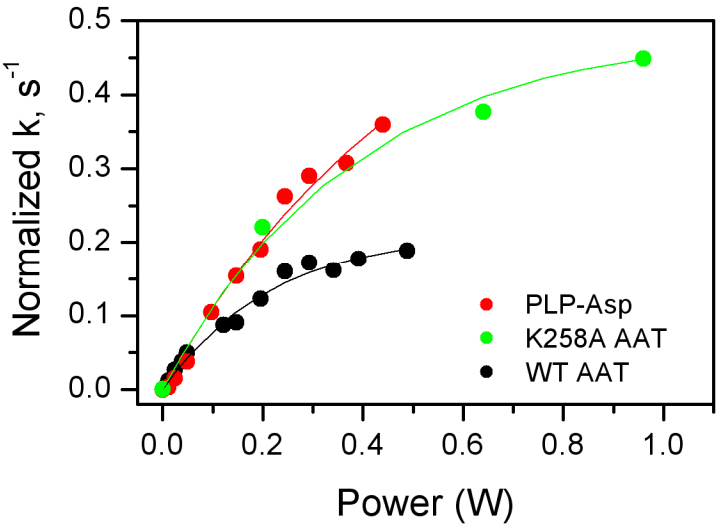


Figure S13. Comparison of power dependence of all PLP Schiff base and AAT enzyme samples.

All data was normalized to have the same initial slope. Fit lines represent COPASI modeling.

References

1) Reiber, H. *Biochimica et Biophysica Acta* **1972**, *279*, 310.
2) Bazhulina, N. P.; Kirpichnikov, M. P.; Morozov, Y. V.; Savin, F. A.; Sinyavina, L. B.; Florentiev, V. L. *Molecular Photochemistry* **1974**, *6*, 367.
3) Goldberg, J. M.; Kirsch, J. F. *Biochemistry* **1996**, *35*, 5280.
4) Kuramitsu, S.; Hiromi, K.; Hayashi, H.; Morino, Y.; Kagamiyama, H. *Biochemistry* **1990**, *29*, 5469.