# Supplementary Information

## Structural and kinetic studies on native intermediates and an intermediate analogue in benzoylformate decarboxylase reveal a least-motion mechanism with an unprecedented short-lived pre-decarboxylation intermediate

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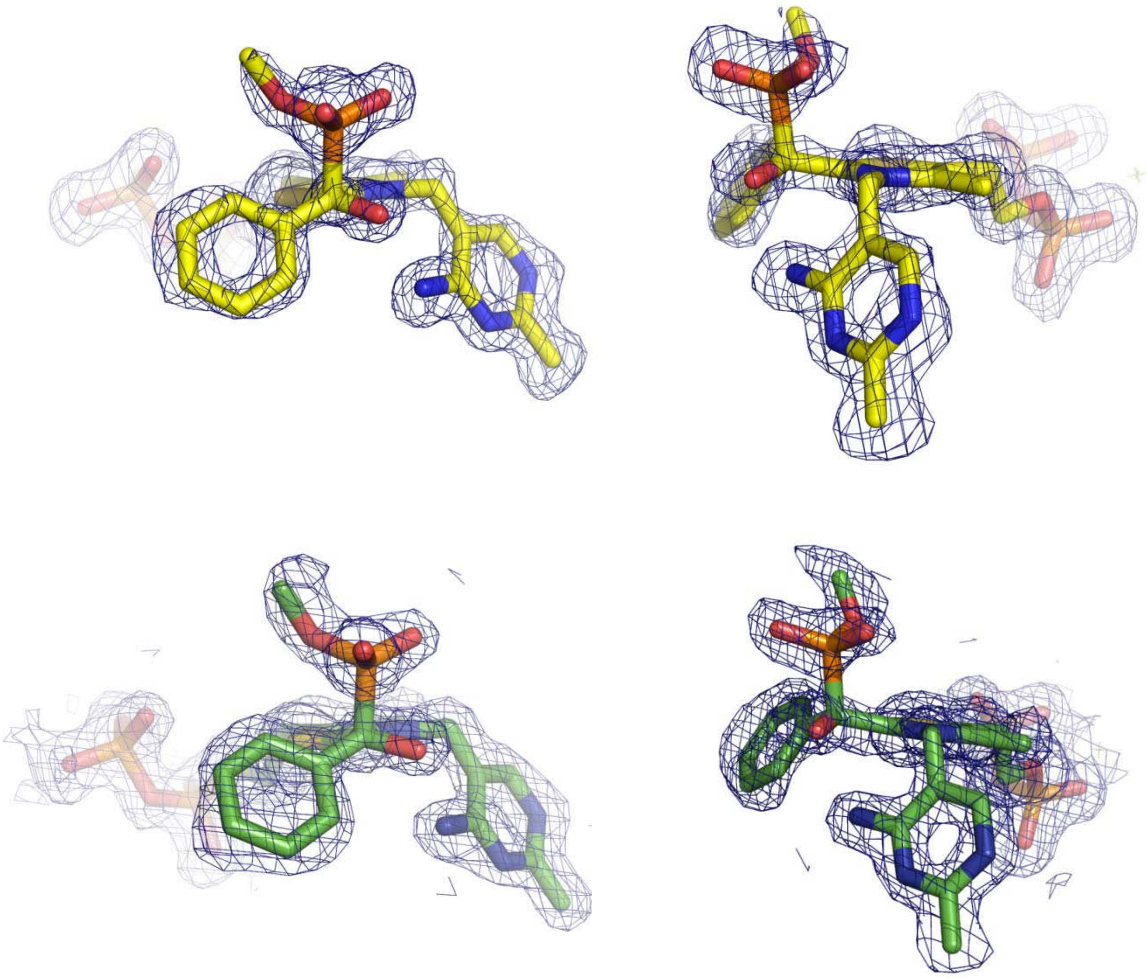
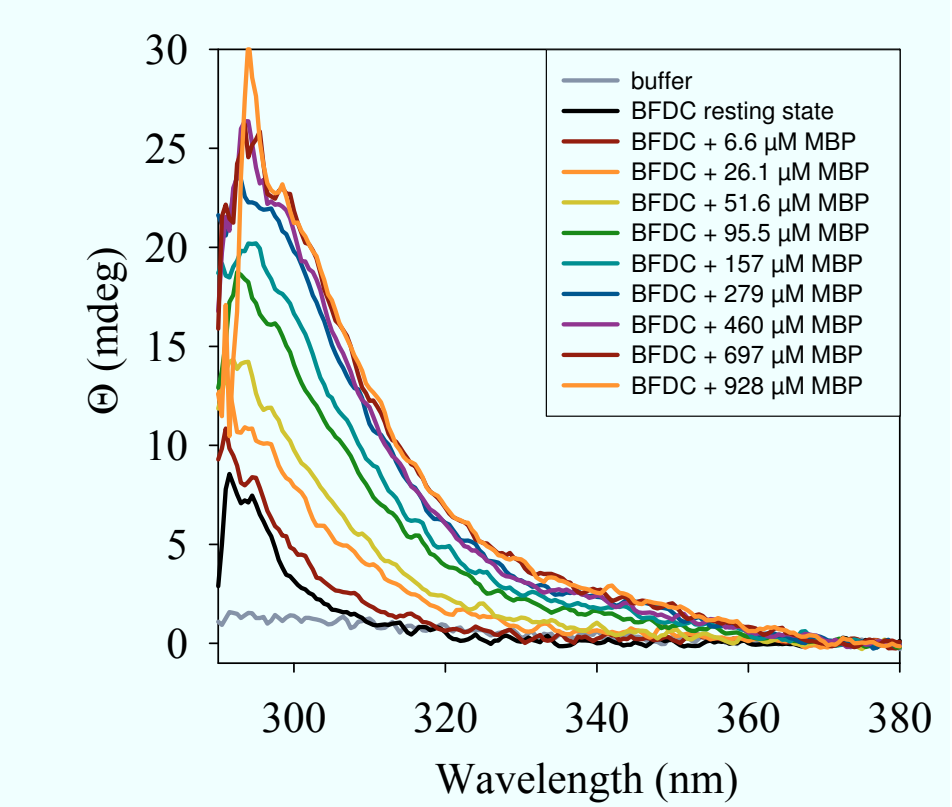
# Supp. Fig. 1



Initial $F_o$ - $F_c$ omit map (top row, contoured at 3σ) and simulated-annealing $2F_o$ - $F_c$ omit map (bottom row, contoured at 1σ) of the PMThDP adduct bound to BFDC from *P. putida*. The electron density maps were calculated from model phases of a model prior to inclusion of the PMThDP atoms. For calculation of the simulated-annealing omit map, a slow cooling protocol (1000 K start temperature, 25 K cooling per cycle, 0.004 ps time steps, 6 steps per cycle) with torsion angle dynamics was applied using CNS (*1*).

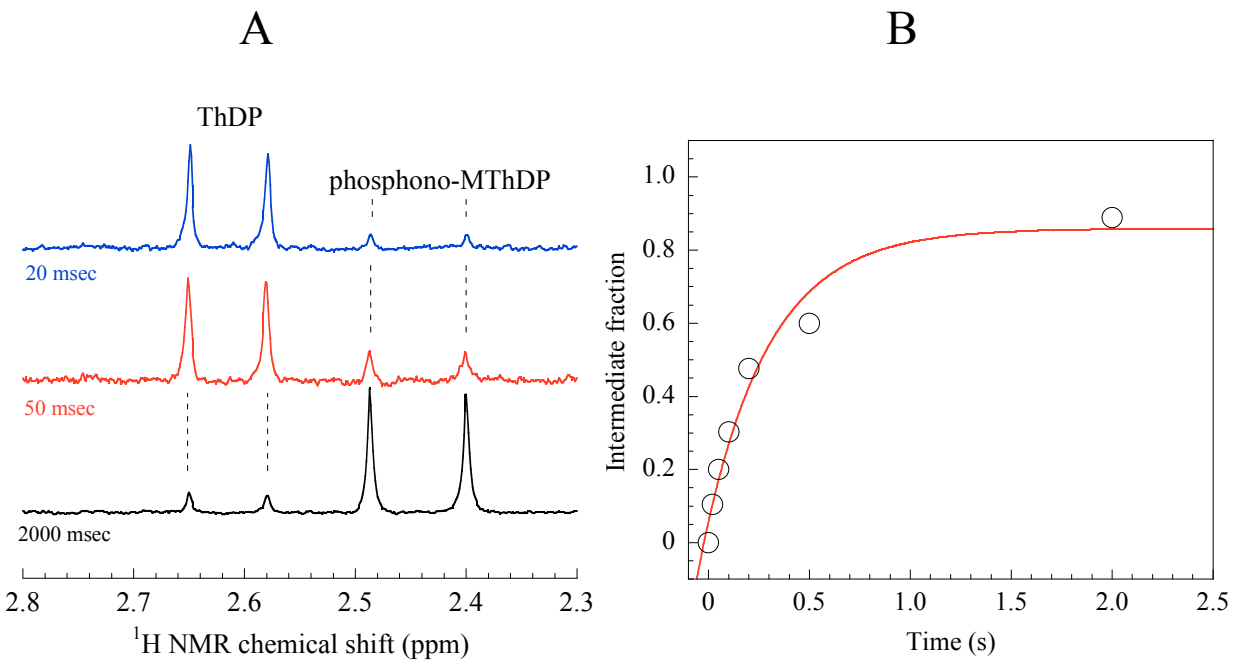
1.) Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination, *Acta Crystallographica Section D-Biological Crystallography 54*, 905-921.

# Supp. Fig. 2




Circular dichroism spectra obtained after addition of increasing amounts of substrate analog methylbenzoylphosphonate to 2 mg/mL BFDC in 50 mM potassium phosphate buffer, pH 6.5 supplemented with 2.5 mM $MgSO_4$ and 200 µM ThDP at 20 °C.

# Supp. Fig. 3

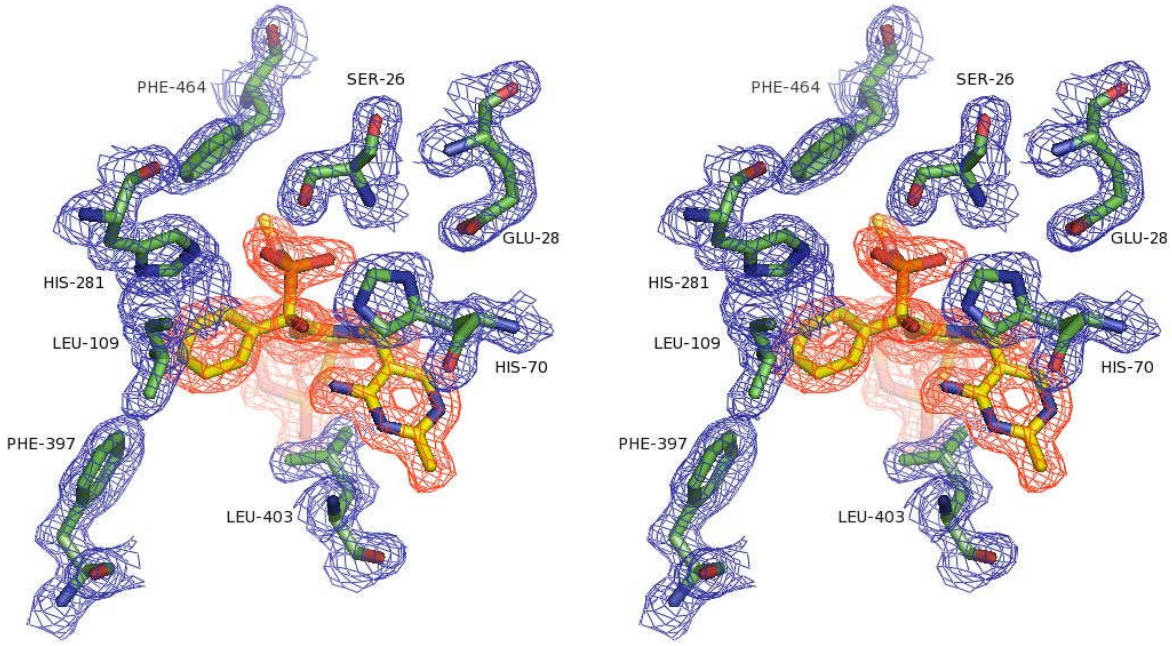



Kinetics of PMThDP formation after addition of 2.5 mM MBP to 7.5 mg/mL in 50 mM potassium phosphate buffer, pH 6.5 with 2.5 mM $MgSO_4$ at 30 °C as analyzed by 1H NMR spectroscopy after acid quench isolation of reaction intermediates.

(**A**) Sections of selected 1H NMR spectra showing the 2'-$CH_3$ and 4-$CH_3$ signals of quench-isolated ThDP and PMThDP after different reaction times.

(**B**) The kinetic analysis reveals a pseudo-first-order rate constant of $k_{obs}$ = 3.1 (± 0.7) $s^{-1}$ being in fair agreement with the stopped-flow results (forward rate constant of PMThDP formation $k_{+2}$ = 2.33 ± 0.17 $s^{-1}$).

# Supp. Fig. 4




Stereo view of the X-ray structure of PMThDP trapped in the active site of BFDC with selected amino acid side chains shown. The electron density is contoured at 1.0σ in a $2F_o$ - $F_c$ map. For clarity, the electron density of the intermediate is depicted in red and that of the protein in blue. The electron density map was calculated from model phases of a model after inclusion of the MBP moiety of the PMThDP adduct.