Supplemental data

Mechanism of Radical-Based Catalysis in the Reaction Catalyzed by Adenosylcobalamin-Dependent Ornithine 4,5 Aminomutase.

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Deconvoluting spectral changes associated with holo-OAM homolysis. The overlapping of the PLP and AdoCbl absorbance spectra makes it difficult to assign small spectral shifts induced by substrate binding to actual catalytic events, as is the case with D-ornithine binding to the holo-OAM. The UV-visible spectra shows a small decrease in absorbance at 528 nm, reflecting homolysis of the AdoCbl C-Co bond, which is accompanied by and even smaller increase in absorbance at 470 and 311 nm (Figure 2A, main manuscript). To identify if this absorbance change is due to accumulation of cob(II)alamin or formation of the external aldimine, the spectra of the internal and external aldimine form of PLP were subtracted from holo-OAM before and after the addition of D-ornithine, respectively (Figure S1). The resulting difference spectrum reveals no absorbance change at 470 nm and only a small increase at 311 nm. In contrast, the corresponding difference spectrum for D,L 2,4-diaminobutryic acid shows a prominent peak at 311 and 470 nm, signifying clearly the accumulation of cob(II)alamin.

Anaerobic single wavelength stopped-flow traces following rapid mixing of holo-OAM with D-ornithine showed a rapid increase in absorbance at 470 nm (Figure 4B). This stopped-flow experiment was also performed at lower temperature to determine if the small, rapid absorbance changes are attributed to a true catalytic event and not stopped-flow mixing artefacts. At 10 °C, the absorbance change occurs over a longer time frame, consistent with the absorbance change being attributed to cob(II)alamin formation and decay (Figure S2) rather than a mixing artefact.

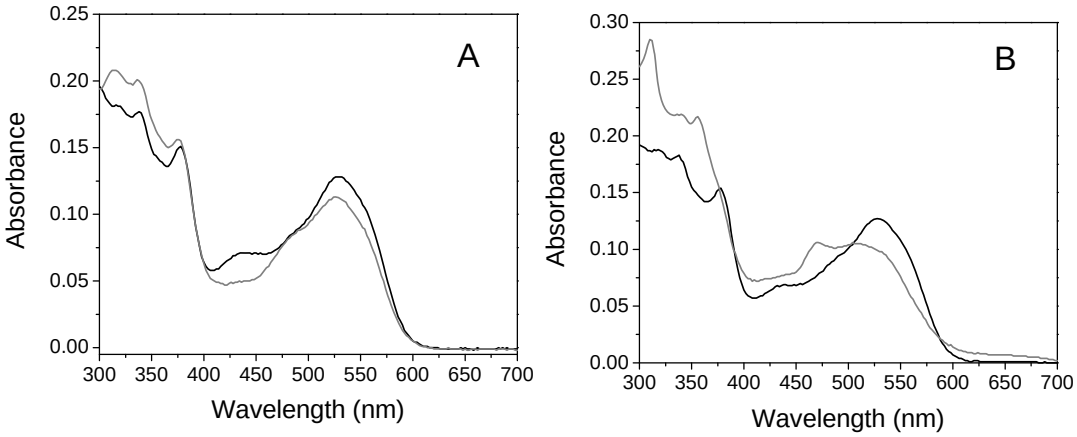

Figure S1. Subtraction spectra of holo-OAM following mixing of the enzyme with (A) D-ornithine and (B) D,L 2, 4 diaminobutryic acid in an anaerobic environment at 25 °C. The holoenzmye solution contained 100 mM NH_4-EPPS, pH 8.5, 30 μM OAM, 30 μM PLP, and 30 μM AdoCbl in a total volume 1 mL. To separate spectral changes associated with AdoCbl homolysis, the spectral component of PLP was subtracted from the holoenzyme prior to and after the addition of 5 mM substrate or inhibitor to the reaction mixture. The internal aldimine component was subtracted from the 'resting' holo-OAM spectraum (black line) and the external aldimine component was subtracted from the holo-OAM in the presence of D-ornithine or DAB (grey line).

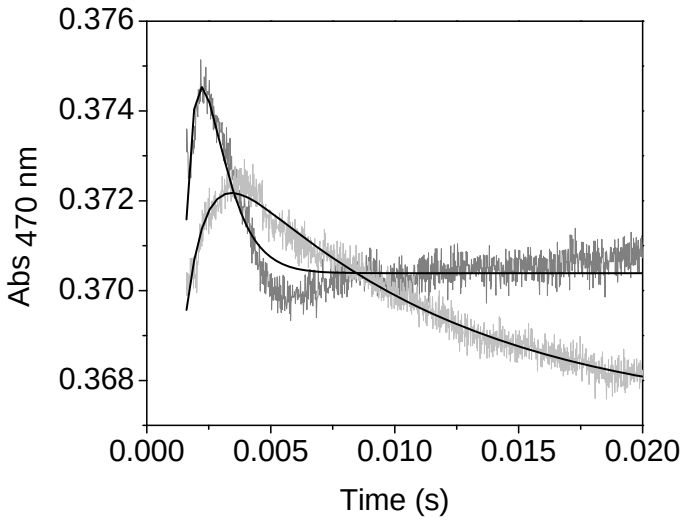

Figure S2. Stopped-flow absorbance changes at 470 nm following mixing of holo-OAM (50 μM; before mixing) with D-ornithine (5 mM; before mixing) at 25 °C (dark grey) and 10 °C (light grey). The kinetic traces were fitted to eq 1 (Materials and Methods, main manuscript).