Substrate-Triggered Addition of Dioxygen to the Diferrous Cofactor of Aldehyde-Deformylating Oxygenase to form a Diferric-Peroxide Intermediate[†]

Maria E. Pandelia,[a,e] *Ning Li,*[b,c,e] *Hanne Nørgaard,*[a,d] *Douglas M. Warui,*[b] *Lauren J. Rajakovich,*[b] *Wei-chen Chang,*[a] *Squire J. Booker,*[a,b,*] *Carsten Krebs,*[a,b,*] *J. Martin Bollinger, Jr.*[a,b,*]

[a]Department of Chemistry and [b]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

[c]Present address: Joule Unlimited Technologies, Inc., 18 Crosby Drive, Bedford, MA 01730

[d]Present address: Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

[e]Equal contributors

[†]This work was supported by the National Institutes of Health (GM-55365 to JMB and CK) and the National Science Foundation (MCB-1122079 to CK, SJB, and JMB).

AUTHOR EMAIL ADDRESS jmb21@psu.edu, ckrebs@psu.edu, sjb14@psu.edu

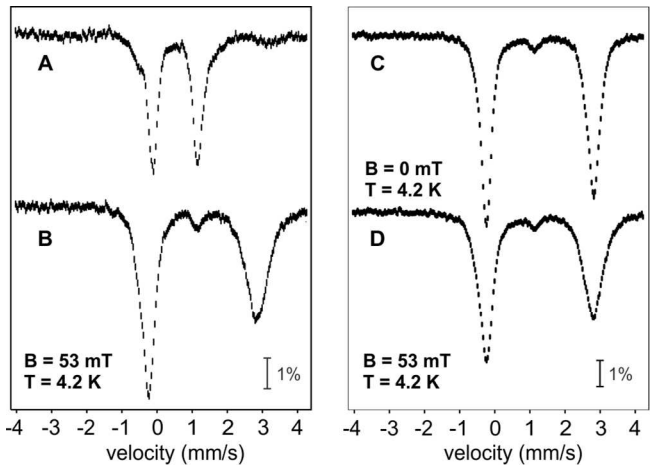

Figure S1: Mössbauer spectra of the aerobically purified ADO (**A**, vertical bars) and anaerobically purified ADO (**B**, vertical bars) recorded at 4.2 K with a magnetic field of 53 mT applied parallel to the γ beam. There is a ~ 6% contribution from the $Fe_2^{III/III}$ complex in the spectrum of the anaerobically purified enzyme. Anaerobically purified ADO with 8 mM decanal at B = 0 mT (**C**, vertical bars) and B = 53 mT (**D**, vertical bars) recorded at 4.2 K.

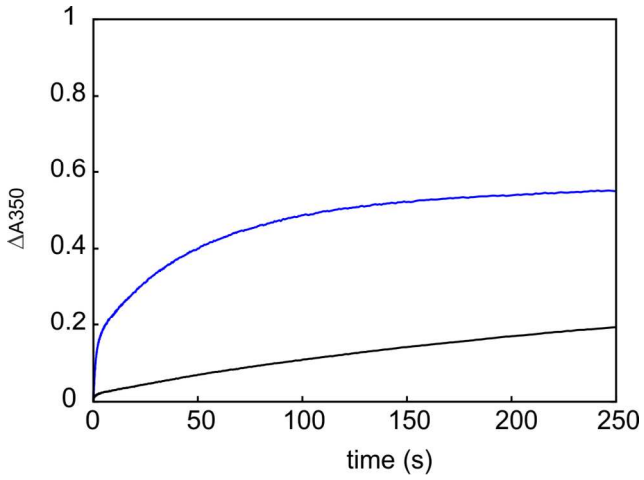

Figure S2: Demonstration of substrate triggering of O_2 addition to the $Fe_2^{II/II}$ cofactor in ADO. An O_2-free solution of $Fe_2^{II/II}$-ADO (prepared by anaerobic purification) either lacking an aldehyde substrate (black trace) or containing 10 mM decanal (blue trace) was mixed in the stopped-flow apparatus at 5 °C with an equal volume of O_2-saturated buffer (50 mM sodium Hepes, pH 7.6, 10% glycerol), and the reaction was monitored by the absorbance at 350 nm.

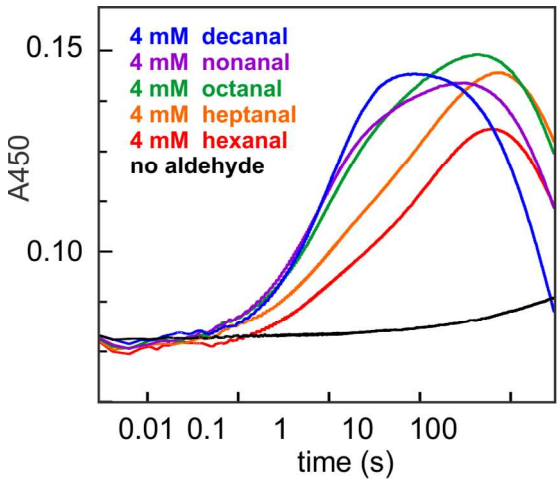

Figure S3: Comparison of the efficacy of substrate triggering by short-chain aldehydes of varying chain length. An O_2-free solution of $Fe_2^{II/II}$-ADO (200 μM; prepared by treatment of the aerobically purified protein with dithionite) containing 4 mM of an aldehyde substrate was mixed in the stopped-flow apparatus at 5 °C with an equal volume of O_2-saturated (1.8 mM) buffer. The polychromatic light source and photodiode array detector were used. Therefore, the intermediate decays more rapidly due to photolysis.

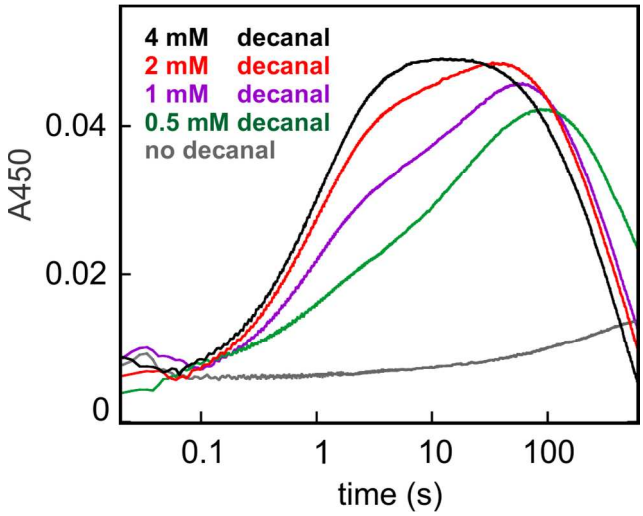

Figure S4: Determination of the concentration dependence for substrate triggering by decanal. An O_2-free solution of $Fe_2^{II/II}$-ADO (200 μM; prepared by treatment of the aerobically purified protein with dithionite) containing the indicated concentration of decanal was mixed in the stopped-flow apparatus at 5 °C with an equal volume of O_2-saturated (1.8 mM) buffer. The polychromatic light source and photodiode array detector were used. Therefore, the intermediate decays more rapidly due to photolysis.

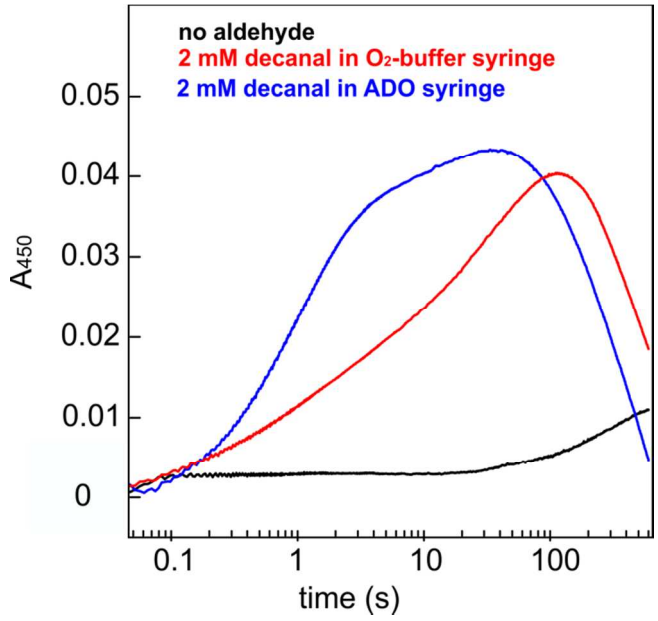

Figure S5: Demonstration of a delay in intermediate formation associated with binding of the substrate, decanal. An O_2-free solution of $Fe_2^{II/II}$-ADO (200 μM; prepared by treatment of the aerobically purified protein with dithionite) either lacking decanal (red and black trace) or containing 2 mM decanal (blue trace) was mixed in the stopped-flow apparatus at 5 °C with an equal volume of O_2-saturated (1.8 mM) buffer either lacking decanal (blue and black traces) or containing 2 mM decanal (red trace). The polychromatic light source and photodiode array detector were used. Therefore, the intermediate decays more rapidly due to photolysis.

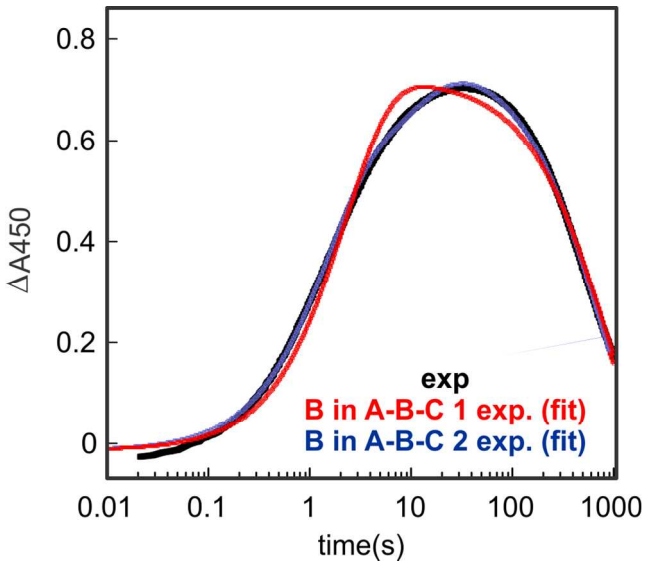

Figure S6: Kinetics of the $Fe_2^{III/III}$-peroxide complex by SF-Abs under the conditions of the FQ Mössbauer experiment in Figure 4. An O_2-free solution of 1.49 mM ADO (cofactor concentration) and 16.6 mM 1-[^{13}C] octanal was mixed in the stopped-flow apparatus at 5 °C with an equal volume of O_2-saturated (1.8 mM) buffer (50 mM Hepes pH 7.6, 10% glycerol), and the reaction was monitored by absorbance at 450 nm with monochromatic incident light and the PMT detector. The data are shown as the solid black trace, and the fit according to Eq. 6 (which considers that the reactant exists in two forms, R and R', that can both convert to intermediate I; see Experimental Section) is shown as the blue solid trace. The fit corresponds to formation rate constants of 0.71 (± 0.005) s^{-1} and 0.10 (± 0.002) s^{-1} and a decay rate constant of 0.0015 (± 0.00004) s^{-1}. The fit obtained by assuming that only one form of the reactant, R, converts to the intermediate, I [which gives a formation rate constant of 0.43 (±0.004) s^{-1} and a decay rate constant of 0.0013 (± 0.00004) s^{-1}], is shown in red to illustrate that this model does not account well for the experimental data.

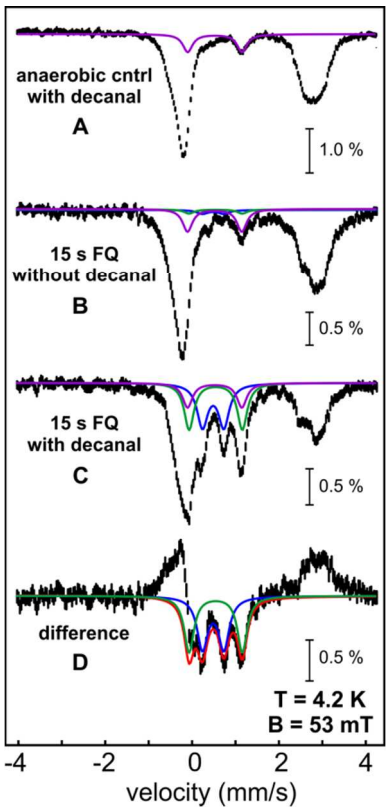

Figure S7: Demonstration by FQ Mössbauer spectroscopy of substrate triggering of O_2 addition to $Fe_2^{II/II}$-ADO. Spectrum **A** is of a sample of the O_2-free $Fe_2^{II/II}$-ADO•decanal reactant (prepared from anaerobically purified enzyme; 1.6 mM diiron cofactor; 10 mM decanal). In **B**, an O_2-free solution of $Fe_2^{II/II}$-ADO (same cofactor concentration) lacking an aldehyde substrate was mixed with an equal volume of O_2-saturated (1.8 mM) buffer, and the reaction was freeze-quenched after a reaction time of 15 s. In **C**, the reactant solution in **A** was mixed with an equal volume of O_2-saturated buffer, and the reaction was freeze-quenched after 15 s. **D** is the difference spectrum, **C**–**B**. Spectrum **A** reflects an ~89% contribution from $Fe_2^{II/II}$-ADO•decanal and an ~11% contribution from the oxidized $Fe_2^{III/III}$ form. Spectrum **B** can accommodate as much as a ~ 4% contribution of the $Fe_2^{III/III}$-peroxide species, whereas the contribution from the $Fe_2^{III/III}$ form remains unchanged from **A** (~ 11%). Spectrum **C** reflects a ~ 44% contribution from the $Fe_2^{III/III}$-peroxide complex.

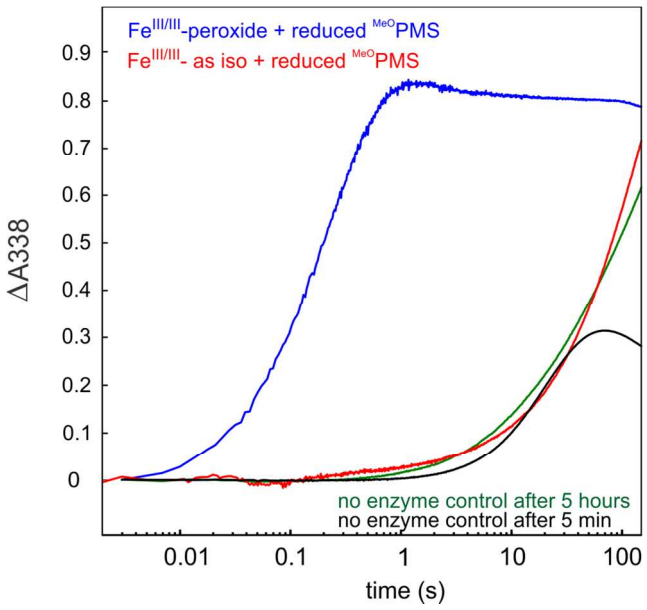

Figure S8: Sequential-mix stopped-flow experiment monitoring oxidation of the reduced 1-methoxy-PMS (^{MeO}PMS) by the peroxide intermediate in ADO, the $Fe_2^{III/III}$ "resting state" of ADO, and O_2 (in the absence of ADO). **Blue trace:** a solution of $Fe_2^{II/II}$-ADO (0.5 mM in cofactor) containing 10 mM decanal was mixed with an equal volume of O_2-saturated (1.8 mM) buffer. The reaction was allowed to proceed for 15 s to accumulate the $Fe_2^{III/III}$-peroxide complex before the solution was mixed with an equal volume of 0.25 mM reduced (by prior treatment with sodium dithionite) ^{MeO}PMS. **Red trace:** a control experiment, in which the aerobically purified $Fe_2^{III/III}$ ADO was used in place of the $Fe_2^{II/II}$ enzyme, demonstrating that the intermediate oxidizes ^{MeO}PMS much more rapidly than the resting, as-aerobically-purified $Fe_2^{III/III}$ ADO. **Green** and **black traces**: control experiments, in which ADO was omitted entirely, showing that the intermediate oxidizes the ^{MeO}PMS much more rapidly than does O_2. In these control experiments, re-reduction of the ^{MeO}PMS by the products of sodium dithionite oxidation (sulfite) complicates interpretation. Although a stoichiometric quantity of sodium

dithionite was used in the initial ^{MeO}PMS reduction, it appears that the sulfite generated by its oxidation is capable of re-reducing the ^{MeO}PMS system, as has been previously described.[1] This behavior was time dependent, as revealed by replicate control experiments done 5 min after the initial reduction of the ^{MeO}PMS (**black**) and then 5 h after (**green**). Regardless, all other events leading to ^{MeO}PMS oxidation and/or re-reduction are much slower than its oxidation by the intermediate (**blue**).

REFERENCE

(1) Dixon, M. *Biochim Biophys Acta* **1971**, *226*, 241.