Supporting Information

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SI Materials and Methods

Aldehyde Synthesis. When not commercially available, fatty aldehydes were synthesized by methylation, successive reduction of corresponding fatty acids to alcohol, and then chemoselective oxidation to the aldehyde. Briefly, fatty acids were methylated by dissolving fatty acid (~100 mg) in sulfuric acid in methanol [2% (vol/vol), 10 mL]. After 15 min of stirring at room temperature, essentially all of the fatty acid was converted to the corresponding fatty acid methyl ester (FAME) as determined by GC/MS. The FAME was isolated by biphasic extraction by adding water (10 mL) and hexane (20 mL) to the reaction mixture. The hexane layer was recovered, and the hexane was removed under a stream of nitrogen. The dried FAME was then reduced to the corresponding alcohol by first dissolving in tetrahydrofuran (THF, 3 mL) and adding lithium aluminum hydride (LAH, 30 mg) suspended in THF (1 mL). The reaction was stirred at 25 °C for 3 h at which time LAH (10 mg) was added and the reaction was stirred for 30 min. Then, the reaction was quenched by adding water (100 μL), aqueous sodium hydroxide solution [15% (wt/vol), 300 μL], and then more water (100 μL). The resulting alcohol was then isolated by biphasic extraction by adding 4 mL water and 8 mL hexane. Conversion to the alcohol was essentially complete as determined by GC/MS. The hexane phase containing the alcohol was separated and evaporated under nitrogen The residual alcohol was dissolved in chloroform (3 mL) and oxidized to the aldehyde by adding pyridinum chlorochromate (50 mg). The reaction mixture was stirred at 25 °C for 3 h, and the solvent was removed in vacuo. The residue was suspended in hexane and filtered. The filtrate was finally purified by flash chromatography using silica gel to give desired aldehyde. Purity of aldehydes was assessed by GC/MS.

Kinetic Calculations. The K_m and V_{max} values for O_2 and long-chain aldehydes (C14–C18), which followed saturable Michaelis–Menten kinetics, were calculated with Eq. **S1**.

$$v = \frac{V_{max} \times [S]}{K_m + [S]}. \quad \textbf{[S1]}$$

The IC_{50} of H_2O_2 at three different O_2 concentrations was determined by fitting the data to Eq. **S1**, substituting H_2O_2 concentration for [S], % inhibition for V_{max}, and IC_{50} for K_m. The K_i for H_2O_2 was then calculated for each IC_{50} value using Eq. **S2**, where the K_m is that of O_2 and [S] the concentration of O_2 at which a particular IC_{50} value was determined. The reported K_i for H_2O_2 is the average of the three values.

$$IC_{50} = K_i\left(1 + \frac{[S]}{K_m}\right). \quad \textbf{[S2]}$$

Kinetic constants for aldehydes that caused substrate inhibition (C8–C12) were calculated using Eq. **S3**:

$$v = \frac{V_{max}}{1 + \frac{K_m}{[S]} + \frac{[S]}{K_i}}. \quad \textbf{[S3]}$$

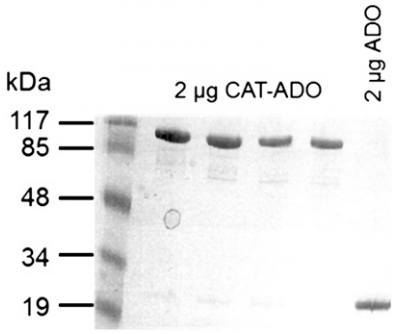

Fig. S1. SDS/PAGE gel showing purified CAT–ADO and ADO. Each lane contains ~2 μg of purified protein. Shown are four independent preparations of CAT–ADO compared with a single preparation of ADO.

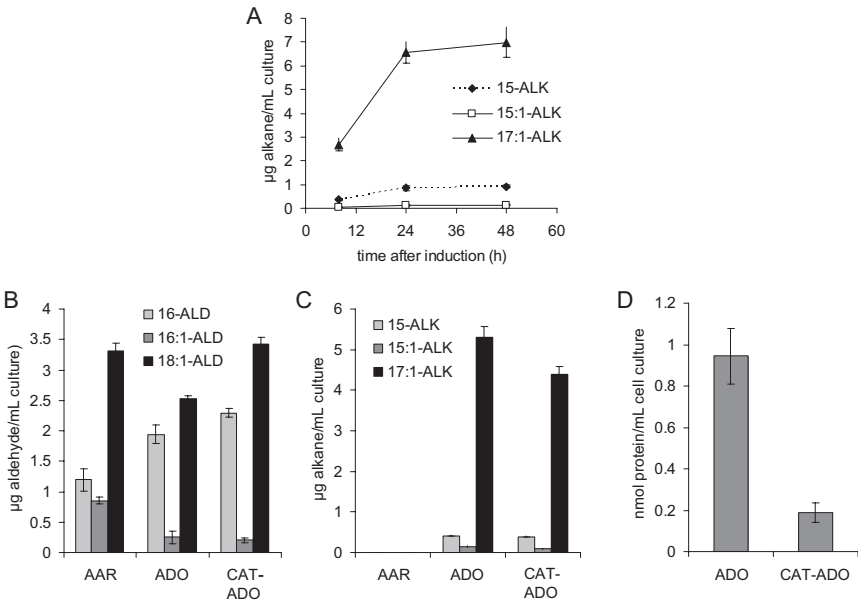

Fig. S2. In vivo alkane production by ADO and CAT–ADO in *Escherichia coli*. (*A*) Time course of alkane accumulation in *E. coli* cultures expressing acyl–ACP reductase (AAR) and ADO. Cell culture conditions and induction of AAR and ADO expression was performed as described in *Materials and Methods*. (*B*) Aldehyde production by AAR after 16 h of induction in cultures containing AAR alone (AAR), AAR and ADO (ADO), or AAR and CAT–ADO (CAT–ADO). (*C*) Alkane production after 16 h of induction in *E. coli* cultures containing AAR alone (AAR), AAR and ADO (ADO), or AAR and CAT–ADO (CAT–ADO). (*D*) ADO active sites in *E. coli* cultures producing alkanes 16 h after induction. Data are the mean ± SD (n = 3).

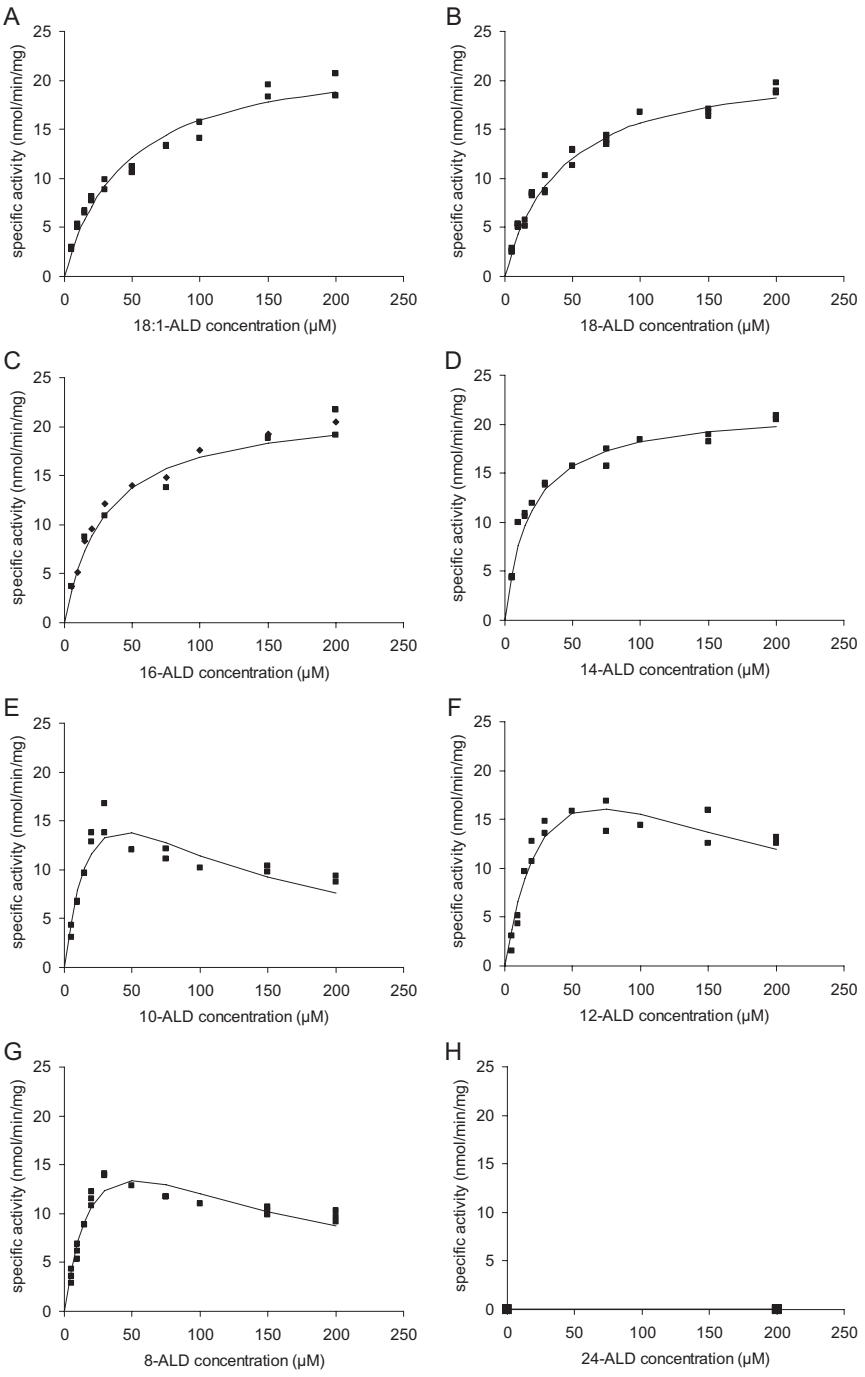

Fig. S3. Kinetic analysis of ADO with respect to various aldehyde substrates. ADO activity assays were performed with (*A*) 9-octadecenal or 18:1-ALD, (*B*) octadecanal or 18-ALD, (*C*) hexadecanal or 16-ALD, (*D*) tetradecanal or 14-ALD, (*E*) dodecanal or 12-ALD, (*F*) decanal or 10-ALD, (*G*) octanal or 8-ALD, and (*H*) tetracosanal or 24-ALD. For each curve, all data points are shown along with a best fit curve. Regression analysis was performed with Grafit software and either used Michaelis–Menton (*A*–*D*) or the substrate inhibition equation (*E*–*G*) described in *Materials and Methods*.

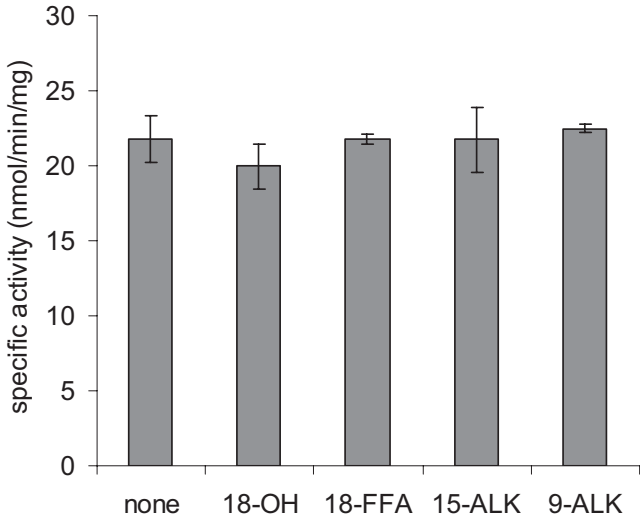

Fig. S4. ADO activity is not inhibited by 100 μM of either octadecanol (18-OH), stearic acid (18-FFA), pentadecane (15-ALK), or nonane (9-ALK) when added as 10% (vol/vol) Triton X-100 solubilized solutions. Data are the mean ± SD (n = 3).