Supporting Information

Multicatalytic, Light-Driven Upgrading of Butanol to 2-Ethylhexenal and Hydrogen under Mild Aqueous Conditions

Glenn R. Hafenstine,[†] Ke Ma,[†] Alexander W. Harris,[†] Omer Yehezkeli,[†] Eunsol Park,[‡] Dylan W. Domaille,[†] Jennifer N. Cha,[†,§,*] and Andrew P. Goodwin[†,§,*]

† Dept. of Chemical and Biological Engineering, University of Colorado Boulder, 3145 Colorado Ave., 596 UCB Boulder, CO 80303

‡ Dept. of Chemistry and Biochemistry

§ Materials Science and Engineering Program

*Corresponding Author

Email: andrew.goodwin@colorado.edu, jennifer.cha@colorado.edu

General Procedures and Materials

Alcohol dehydrogenase (ADH) from *Saccharomyces cerevisiae* (ADH), β-nicotinamide adenine dinucleotide sodium salt (NAD^+), phosphate buffer solution (PB), β-alanine, triethanolamine, 2-mercaptoethanol, deuterated chloroform ($CDCl_3$) containing trimethylsilane (TMS), octadecylphosphonic acid (ODPA), trioctylphosphine oxide (TOPO), trioctylphoshine (TOP), octanoic acid, and butyraldehyde (BA) were purchased from Sigma-Aldrich. ADH was reported to have 369 units/mg on the basis that one unit will convert 1.0 μmole of ethanol to acetaldehyde per min at pH 8.8 and 25 °C.[1] (1,5-Cyclooctadiene)dimethylplatinum (II), cadmium oxide, and sulfur were purchased from Alfa Aesar. β-Nicotinamide adenine dinucleotide disodium salt hydrate (NADH) and 2-ethylhexenal (2-EH) were purchased from TCI Chemicals. Butanol (BuOH), 2-propanol, acetone, and toluene were purchased from Fisher Scientific. Ethanol (EtOH) was purchased from Decon Labs, Inc. Deuterium oxide (D_2O) and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Cambridge Isotope Laboratories, Inc. Water (dd-H_2O) used as a reaction solvent was deionized using a Milli-Q Advantage A-10 water purification system (Millipore, USA). ^1H NMR spectra were acquired with a 400 MHz Bruker AV-III spectrometer with a Sample Xpress Automatic Sample Changer. Spectra acquired in $CDCl_3$ were referenced to residual $CHCl_3$ (7.27 ppm). Spectra were analyzed with MestreNova 10.0.2 software. UV-vis spectra were acquired on a DU 730 spectrophotometer (Beckman Coulter, USA). Gas chromatography (GC) measurements were performed on an HP/Agilent 6890 (G1540A) system equipped with a 5-A° column and a thermal conductivity detector. Samples were irradiated in sealed gas-tight cuvettes purchased from Starna Cells under approximately 1 sun of illumination from a PV Measurements, Inc. Small-Area Class-BBA Solar Simulator. Electron microscopy images were obtained with a FEI TECNAI T12 Spirit Biotwin TEM.

Synthesis of Photocatalyst

Photocatalytic CdS nanocrystals were produced as previously reported by Robinson et al[2] (Sample A synthesis procedure). Briefly, trioctylphosphine sulfide (TOPS) was produced by mixing TOP with an equimolar amount of sulfur for 48 h at room temperature while bubbling with argon. Then, cadmium oxide (210 mg) and ODPA (1.06 g) were mixed in TOPO (2.75 g) and evacuated for 1 h at 120°C. The solution was heated to 320°C under argon and maintained for 15 min to allow for cadmium complexation. The reaction mixture was then cooled to 120°C and evacuated again for 1.5 h to remove trace amounts of water. Next, the solution was reheated to 320°C and TOP (2 g) was added to act as a stabilizing ligand. TOPS (1.95 g) was then injected and the reaction was allowed to proceed at 315°C for 85 min to form nanorods (NRs). The NRs were washed with equal amounts of 2-propanol and octanoic acid before being dispersed in toluene.

Pt nanoparticles were photodeposited onto the CdS NRs using the process developed by Dukovic et al.[2] Briefly, 33 nM CdS NRs, 13.3 mM (1,5-cyclooctadiene)dimethylplatinum (II), and 50 μL triethanolamine were mixed in 600 μL toluene and purged with argon for 1 h. The solution was then irradiated with a 9 W LED lamp emitting 460 nm light for approximately 30 min until a slight brown color developed. Large aggregates were removed with a 220 nm cutoff syringe filter (PTFE, VWR International) and the solution was stored at 4°C in toluene until immediately before use. The catalysts were transferred into dd-H_2O by ligand exchange with 25 uL of 2-mercaptoethanol in 1 mL of dd-H_2O for 20 min and impurities were removed by microcentrifugation with 30k MWCO filters (Pall Life Sciences) by three washes of 500 μL dd-H_2O at 800 rcf.

Enzyme Kinetics Measurements

590 uL of an aqueous solution containing 1 M phosphate (pH 8.8), 0.15 mM NAD^+, 550 mM β-Alanine, and 50 mM BuOH were added to a cuvette. The UV-vis spectrophotometer was zeroed at 339 nm using this solution and then 0.1 units of ADH in 10 uL PB was added. The absorbance of the solution at 339 nm was measured periodically for 30 min to determine the conversion of NAD^+ into NADH due to enzyme activity.

Partition Coefficient Measurements

Solutions containing BA or 2-EH (5-25 mM) in 1 mL of 50 M PB (pH 7.4) were stirred at room temperature for 24 h. The solutions were then extracted with $CDCl_3$ (525 μL) containing 1% (v/v) tetramethylsilane (TMS) and assessed by ^1H NMR. Product concentrations were determined by comparing peak integrations of aldehyde peaks (9.78 ppm for BA, 9.38 ppm and 10.16 ppm for both isomers of 2-EH) and α-proton peaks (6.88 ppm and 6.45 ppm for both isomers of 2-EH) with TMS (0.13 ppm). The measured concentrations were normalized to an average value of 100% recovery by minimizing the root mean square error between all measurements and the normalization factors were utilized for correcting concentrations of extractions from reaction mixtures (Figure S3).

Photocatalysis Tests

For NADH oxidation tests, 600 μL of 1 M PB (pH 8.8), Pt@CdS (25 nM), and NADH (3 mM) were added to a sealed cuvette. The solution was degassed with argon for 1 h before irradiation under the solar simulator with periodic GC measurements of the gas in the headspace. After 3 h, 450 μL aliquots of the reaction mixtures were removed and mixed with 50μL D_2O containing 10 mM DSS and assessed by ^1H NMR under parameters for water suppression.

Similarly, for the composite reaction tests, 550 µL of 1 M PB (pH 8.8), Pt@CdS (25 nM) NAD^+ (0-3 mM), β-Alanine (550 mM, when applicable) and BuOH (50 mM) or simulated ABE feedstock (30 mM BuOH, 15 mM acetone, and 5 mM EtOH) were added to a sealed cuvette. The solution was degassed with argon for 1 h. Alcohol dehydrogenase was injected into the sealed cuvette (0-100 units in 50 µL of PB) and the reaction was stored in the dark for 15 min to allow for induction of NADH. The solution was then irradiated by the solar simulator with periodic GC measurements of the gas in the headspace. After 3 h, the reactions were extracted with $CDCl_3$ (525 µL) containing 1% or 0.03% (v/v) TMS and assessed by ^1H NMR. Product concentrations were determined by comparison with TMS standard peak integrations and adjusted by the partition coefficient values described above.

Aldol Condensation Tests

Solutions containing β-alanine (550 mM) and butyraldehyde (1-5 mM) in 1 mL of 1 M PB (pH 8.8) were stirred at room temperature for 3 h. The reactions were then extracted with $CDCl_3$ (525 uL) containing 1% (v/v) TMS and assessed by ^1H NMR. Product concentrations were determined by comparison with TMS standard peak integrations and adjusted by the partition coefficient values described above.

Turnover Frequency Calculations

The turnover frequency of the beta-alanine catalyst was determined by performing an aldol condensation test as described above for 1h with a measured yield of 51.7%. For the enzyme catalyst, the results from the photocatalysis tests for BA production were utilized (Figure 2) with a 2.2 mM yield of BA after 3h. For the photocatalyst, the production of H_2 during the NADH oxidation studies was utilized (Figure 1) after subtracting out the H_2 yielded in the absence of NADH to eliminate the water oxidation side reaction. A few approximations were used: the Pt nanocrystals had a diameter of 3 nm, each CdS NR had 3 Pt NP's attached, and the Pt had a surface site density of $1.5 * 10^{15}$ cm^{-1}.[3]

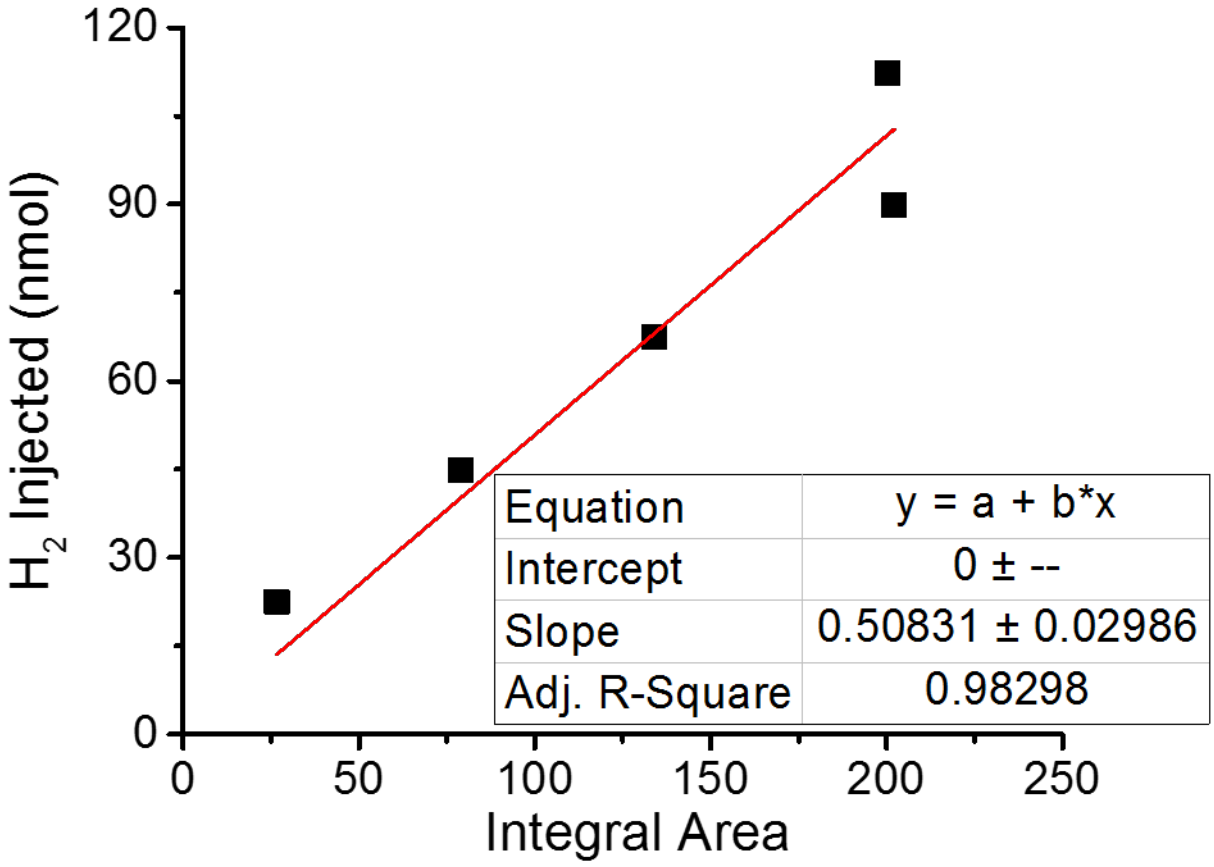

Figure S1: Gas chromatograph calibration curve relating area of integration of TCD output to moles of hydrogen gas.

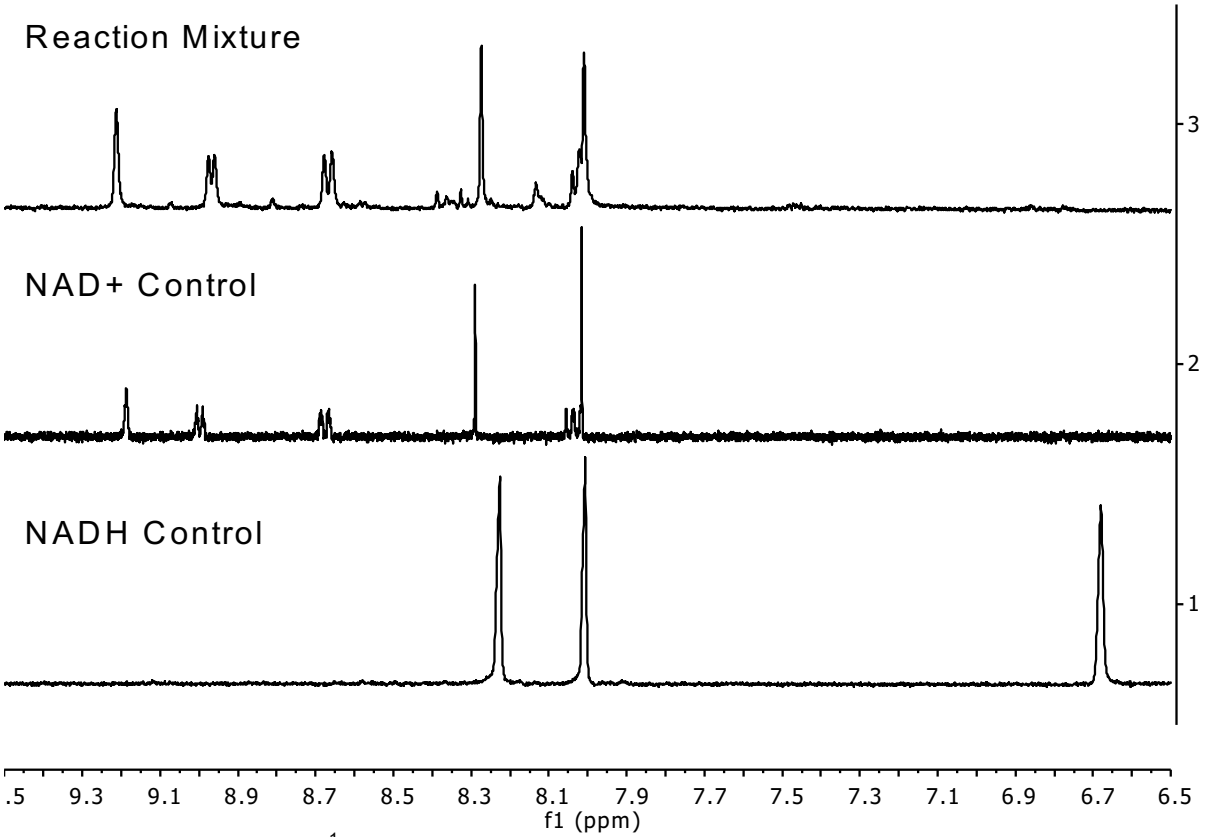

Figure S2: Compilation of ^{1}H NMR spectra in H_2O/D_2O of NADH oxidation reaction by Pt@CdS and standards (controls) of NADH and NAD^+. Relevant NMR signals include the NADH peak at 6.68 ppm and the NAD^+ peaks at 9.19, 8.98, and 8.67 ppm. Reactions were carried out in 1 M PB (pH 8.8) with 25 nM Pt@CdS and 3mM NADH. The reactions were irradiated for 3 h and mixed with 10% D_2O (v/v) containing 10 mM DSS.

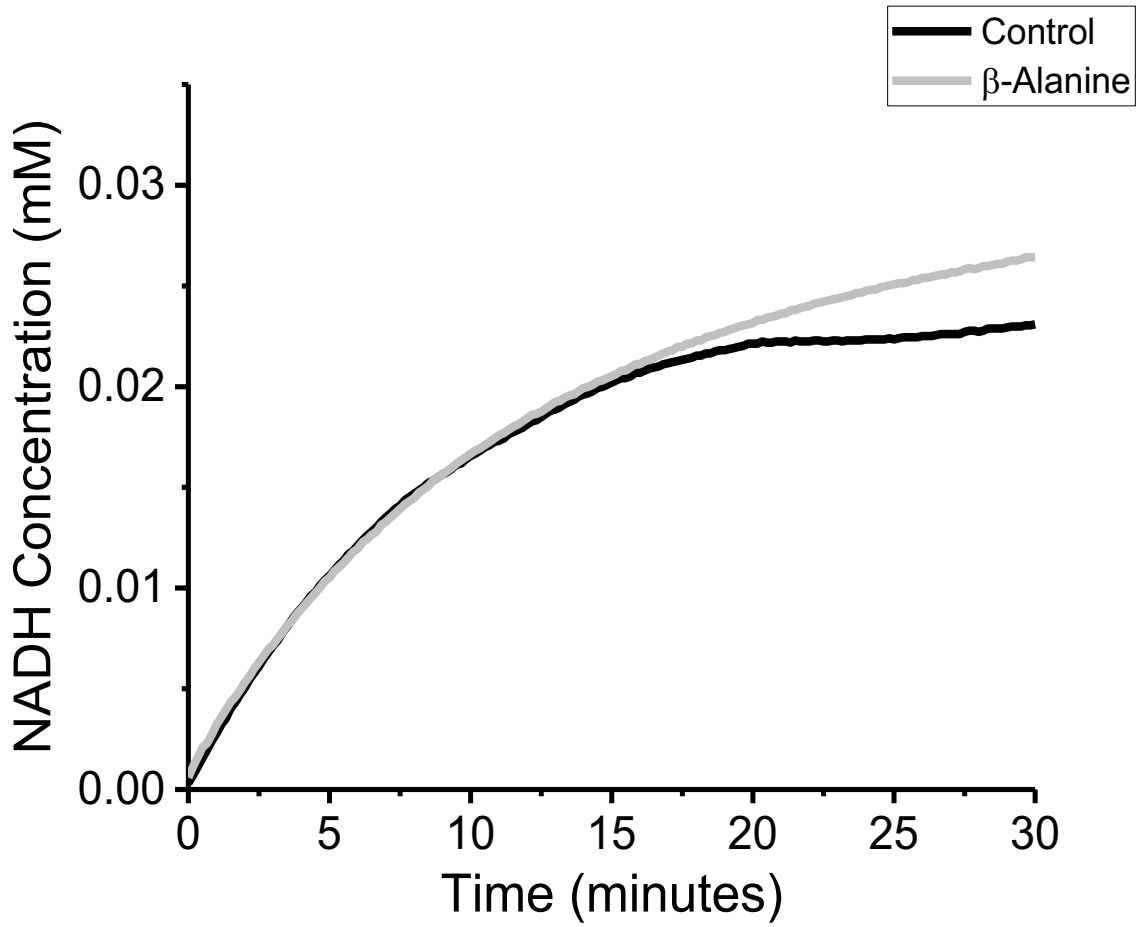

Figure S3: Enzyme inhibition testing of the organocatalyst by measuring production of the NADH cofactor in the presence and absence of β-alanine. Reaction conditions: 600 µL of 50 mM PB (pH 7.4), 0.1 units of ADH, 0.15 mM NAD^+, 50 mM BuOH substrate, 550 mM β-alanine, room temperature. NADH concentration monitored by absorption at 339 nm via UV-Vis spectroscopy.

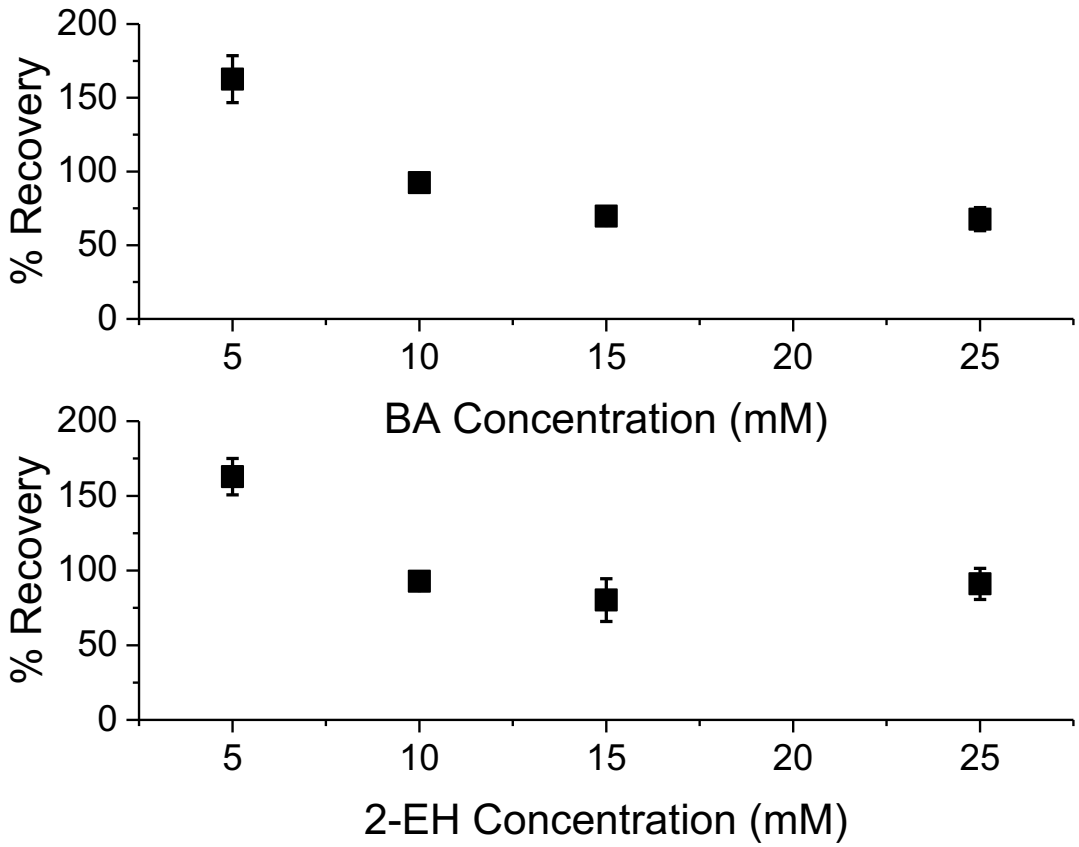

Figure S4: Recovery of BA and 2-EH from standard solutions in 1 mL of 50 mM PB after extraction with $CDCl_3$ (525 µL) containing 1% (v/v) TMS. Concentrations were calculated by comparing integrations of BA and 2-EH to TMS and normalizing the values to 100% recovery by minimizing the root mean square error.

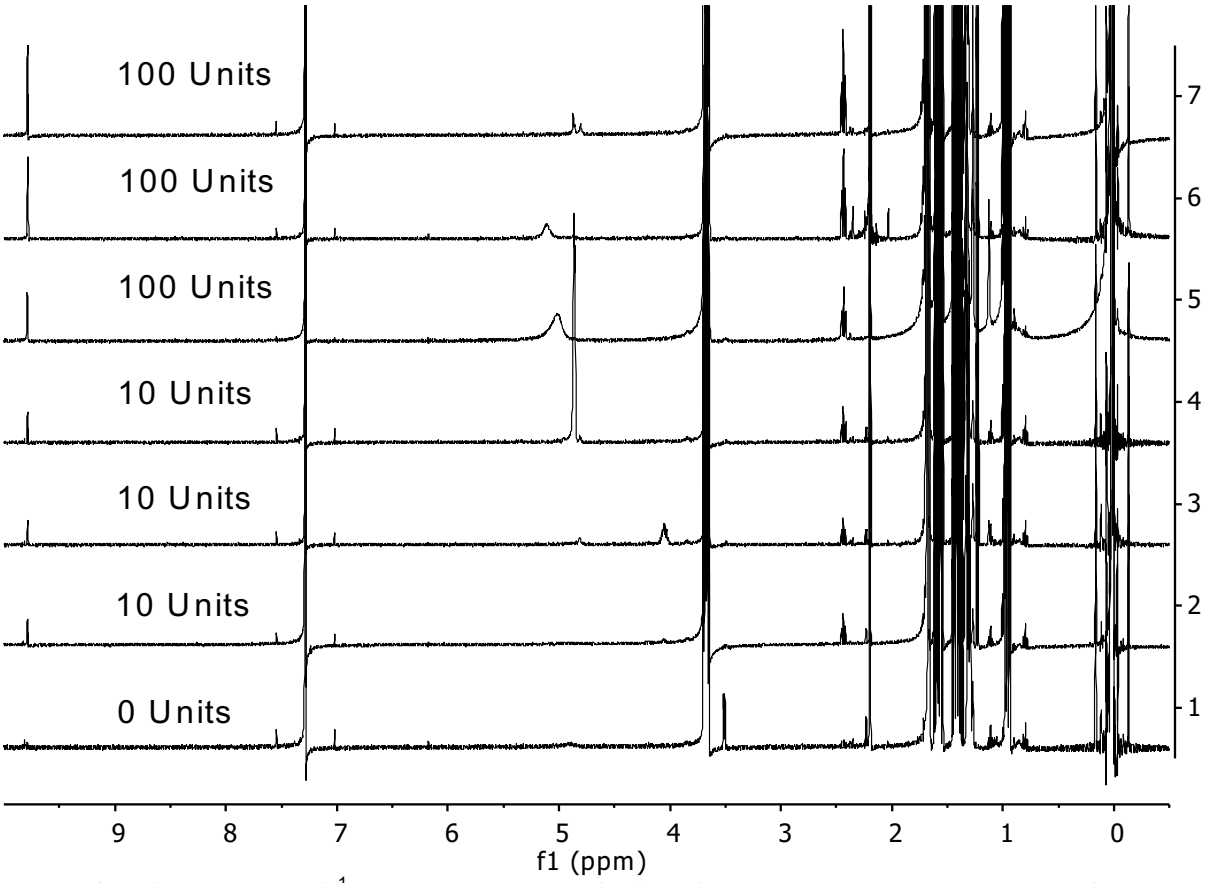

Figure S5: Compilation of ^{1}H NMR spectra in $CDCl_3$ of extracted products generated from enzymatic and photocatalysis composite reactions with diverse concentrations of ADH and NAD^+ (Figure 2). Relevant NMR signals include the BuOH peaks at 3.65, 1.56, 1.39, and 0.94 ppm and the distinguishable BA peaks at 9.77 and 2.42 ppm. Reactions were carried out in 1 M PB (pH 8.8) with 25 nM Pt@CdS, differing units of ADH (0, 10 and 100), various concentrations of NAD^+ (0, 0.3, 3 mM, respectively) and 50 mM BuOH. The reactions were irradiated for 3 h and extracted with $CDCl_3$ (525 µL) containing 1% (v/v) TMS.

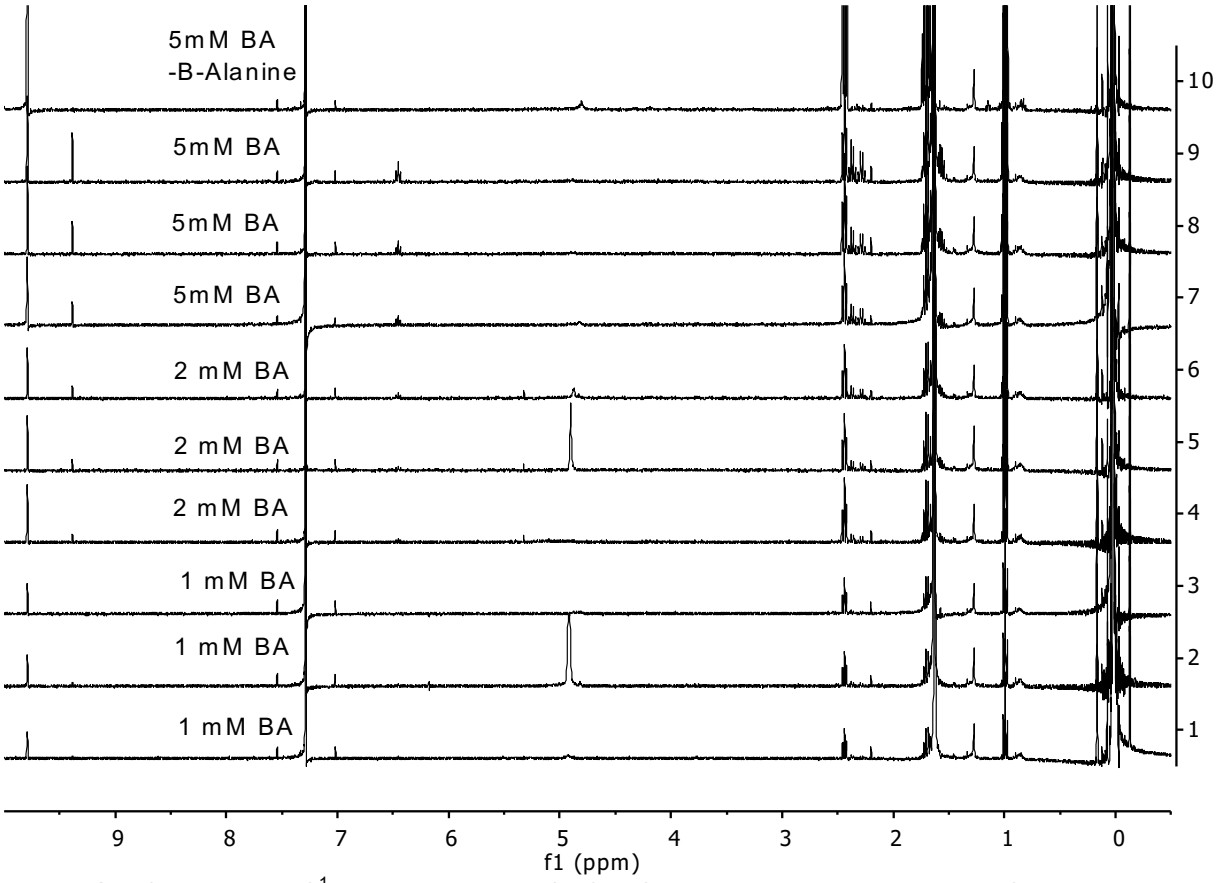

Figure S6: Compilation of ^{1}H NMR spectra in $CDCl_3$ of extracted products generated from BA condensation via β-alanine catalysis. Relevant ^{1}H NMR signals include the BA peaks at 9.77, 2.42, 1.69 and 0.99 ppm and the 2-EH peaks at 9.38, 6.56, 2.35, 2.26, 1.57, and 0.99 ppm. Reactions were carried out in 1 M PB (pH 8.8) with 550 mM β-alanine and varied concentrations of BA. The top spectrum is a control measurement showing the lack of condensation without organocatalyst in solution.

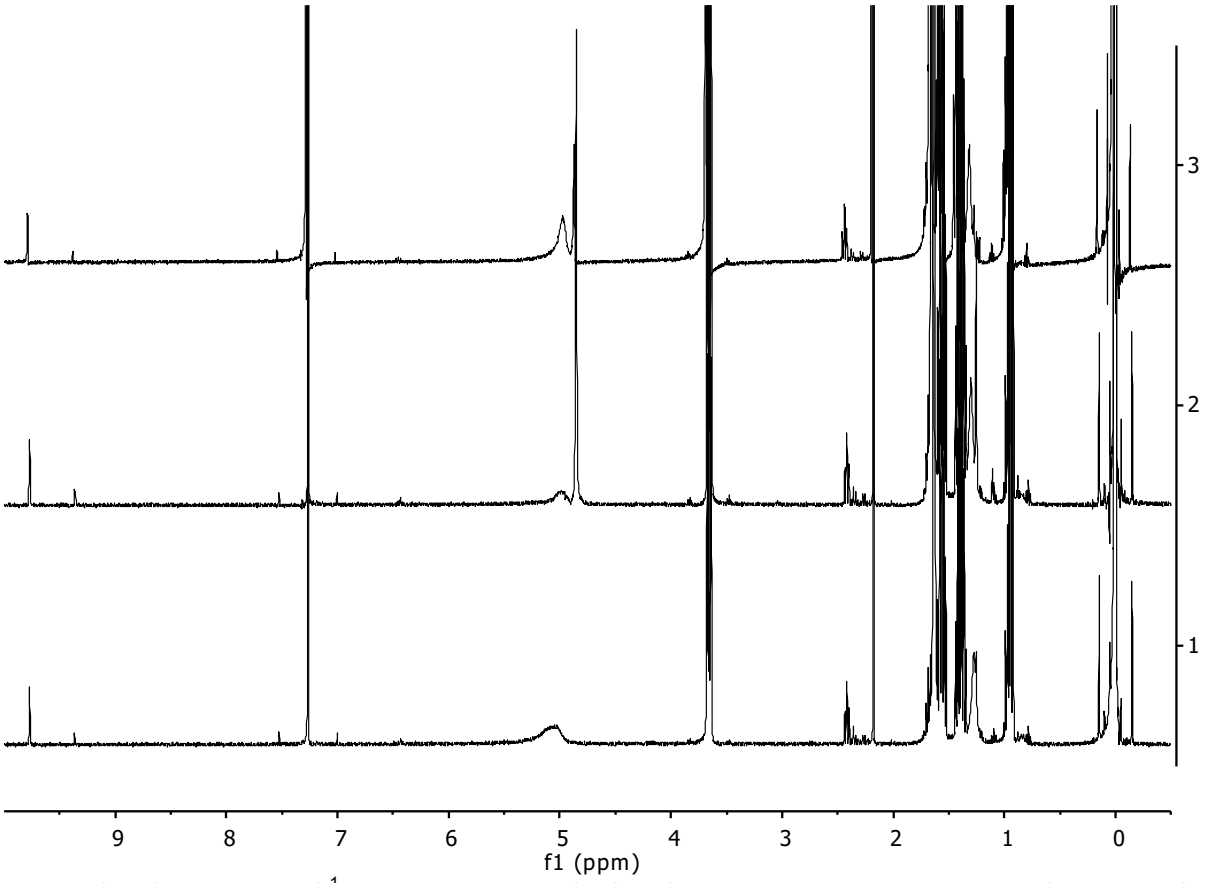

Figure S7: Compilation of ^{1}H NMR spectra in $CDCl_3$ of extracted products generated from three full composite reactions (Figure 4, a and b). Relevant NMR signals include the BuOH peaks at 3.65, 1.56, 1.39, and 0.94 ppm, the distinguishable BA peaks at 9.77 and 2.42 ppm and the distinguishable 2-EH peaks at 9.38, 6.56, 2.35, and 2.26 ppm. Reactions were carried out in 1 M PB (pH 8.8) with 25 nM Pt@CdS, 100 units of ADH, 3 mM NAD^+, 550 mM β-alanine, and 50 mM BuOH. The reactions were irradiated for 3 h and extracted with $CDCl_3$ (525 µL) containing 1% (v/v) TMS.

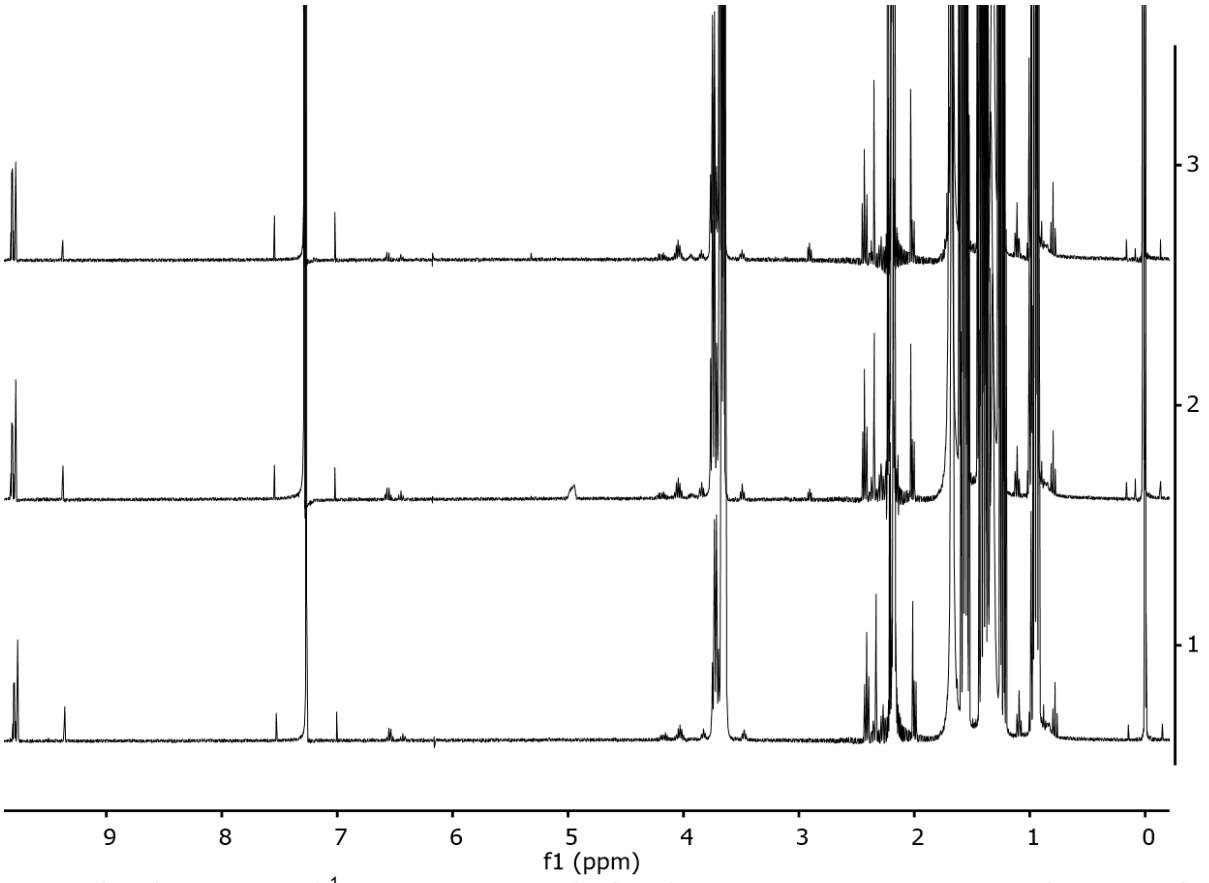

Figure S8: Compilation of ^{1}H NMR spectra in $CDCl_3$ of extracted products generated from three full composite reactions with simulated ABE feedstock (Figure 4, c and d). Relevant NMR signals include peaks attributed to BuOH (3.65 ppm), EtOH (3.72 ppm), acetone (2.19 ppm), BA (9.77 ppm), AA (9.81 ppm), and 2-EH (9.38 and 6.56 ppm). Reactions were carried out in 1 M PB (pH 8.8) with 25 nM Pt@CdS, 100 units of ADH, 3 mM NAD^+, 550 mM β-alanine, 30 mM BuOH, 15 mM acetone, and 5 mM EtOH. The reactions were irradiated for 3 h and extracted with $CDCl_3$ (525 µL) containing 0.03% (v/v) TMS.

References.

(1) Racker, E. *J. Biol. Chem*. **1950**, *184*, 313–320.
(2) Robinson, R. D.; Sadtler, B.; Demchenko, D. O.; Erdonmez, C. K.; Wang, L.-W.; Alivisatos, A. P. *Science* **2007**, *317*, 355–358.
(3) Jaramillo, T. F., Jorgensen, K. P., Bonde, J., Nielsen, J. H., Horch, S., Chorkendorff, I. *Science* **2007,** *317*, 100–102.