Supporting Information

Photocatalytic Regeneration of Nicotinamide Cofactors by Quantum Dot-Enzyme Biohybrid Complexes

Katherine A. Brown,[1]* Molly B. Wilker,[2] Marko Boehm,[1] Hayden Hamby,[2] Gordana Dukovic,[2] and Paul W. King[1]

[1]Biosciences Center, National Renewable Energy Laboratory, Golden CO 80401

[2]Department of Chemistry and Biochemistry, University of Colorado Boulder, Boulder, CO 80309

* Address correspondence to kate.brown@nrel.gov

Methods

FNR expression and purification. Expression and purification of Ferredoxin $NADP^+$-reductase (FNR) from *Chlamydomonas reinhardtii* was adapted from previously published procedures.[1] The His-tagged FNR was expressed in *Escherichia coli* (KRX cells, Promega) grown in Terrific Broth (VWR). 1-liter cultures at A_{600} of ~0.6 were induced with L-rhamnose to final concentration of 0.05% (w/v), and ferric ammonium citrate was added to final concentration of 2.5 mM. After overnight induction pelleted cell pysate was incubated for 1 h at 4 °C with 5 ml of Talon metal affinity resin (Clontech) and eluted with imidazole. Concentrated eluent was loaded on a HiLoad™ 26/60 Superdex™ 75 prep grade (GE Healthcare) size exclusion column. Purified FNR was eluted in 25 mM $NaPO_4$, pH 7.0, 100 mMNaCl, and 5% (w/v) glycerol. Protein concentration was measured by Bradford assay, and the activity of purified FNR was measured by NADPH photo-production by *Chlamydomonas* thylakoids.

CdSe quantum dot synthesis. The synthesis was adapted from previously published procedures.[2, 3] For the preparation of CdSe nanocrystals capped with organic ligands, 4 g TOPO, 2.5 g HDA and 0.075 g TDPA were dried and degassed under vacuum at 120 °C in a 25-mL three-neck flask. Under Ar, 1 mL of a stock solution of Se precursor (0.79 g of selenium in 8.3 g of TOP) was added and the mixture was again dried and degassed under vacuum at 110 °C. With the reaction temperature stabilized at 300 °C under Ar, 1.5 mL of Cd precursor stock solution (0.12g of $Cd(Ac)_2$ in 2.5 g of TOP) was quickly injected under vigorous stirring, resulting in nucleation of CdSe nanocrystals. The temperature was set to 260 °C for nanocrystal growth, after which the reaction mixture was cooled to 90 °C. The mixture was added to a 20 % (v/v) ethanol in chloroform solution and centrifuged to precipitate the nanocrystals. Under an inert atmosphere in a glovebox, the supernatant was discarded and the nanocrystals were redissolved in toluene. The solution was centrifuged to precipitate excess HDA. The resulting nanocrystals were washed with a 1:2 mixture of isopropanol:ethanol and redispersed in toluene.

The native organic ligands were replaced with MPA following a previously reported procedure.[4, 5] 50 µL of the nanocrystal solution was added to 1 mL of toluene followed by 1 mL of methanol to produce a cloudy suspension of the nanocrystals. The nanocrystals were redispersed upon addition of 3 mL of MPA stock solution (prepared by dissolving MPA (0.133 g) in methanol (20 mL) and adjusting to pH 12 with TMAH (1.35 g)). 30 mL of toluene and 2 mL of isopropanol were added to the mixture. The nanocrystals were precipitated by centrifugation and redispersed in 1 mL of methanol. The particles were washed with 15 mL of toluene and dried under reduced pressure. The clean, dry nanocrystals were redissolved in Tris buffer, pH 7. A nanoparticle diameter of 2.8 nm was determined from the determined from the first excited state $1S_{3/2}(h) \rightarrow 1S(e)$ transition peak wavelength (537 nm) as described in Yu *et al.*[6]

(Abbreviations: TOPO, Trioctylphosphine oxide (TOPO); HDA, hexadecylamine; TDPA, tetradecylphosphonic acid; TOP, Trioctylphosphine; MPA, 3-mercaptopropionic acid)

Light-driven NADPH production. Samples of QD-FNR were prepared under anaerobic conditions by mixing a molar ratio of 2:1 QD:FNR for a final concentration of 1.2 µM QD and 0.6 µM FNR, in buffer (50mM Tris, 5mM NaCl, 5% glycerol, pH 7). The QD and FNR were mixed at high concentration prior to dilution to ensure self-assembly of active complexes. Solution also contained 2 mM $NADP^+$ (Sigma) and 100mM Ascorbic Acid (AA, Fluka). Samples were illuminated with stirring using an Ocean Optics 405 nm LED light source (750 mW, 500 mA) at 2500 µE m^{-2} s^{-1} (light meter LI-250, LI-COR Biosciences). Aliquots of 100 µl QD-FNR solution were removed from the sample at specific illumination times and 10 µl 1 N NaOH added. Samples were heated to 90 °C for 10 min to degrade any $NADP^+$ present, placed on ice, and neutralized with 10 µl 1 N HCl. Samples were centrifuged to remove precipitated QDs and the NADPH concentration was measured by using a red fluorescence NADH/NADPH detection kit (eNZYME) in a 1:1 mixture of treated sample:kit mix. This mixture was incubated at room temperature for 20 min in the dark, and the fluorescence signal at 594 nm was measured. NADPH calibration curves were prepared using known concentration of NADPH also treated with base/heat (figure S2). Control samples of $NADP^+$ showed no signal after base/heat treatment, while NADPH controls showed the fluorescence signal with and without base/heat treatment.

Alcohol production by illuminated QD-FNR. Samples of tbADH/QD-FNR were prepared under anaerobic conditions by mixing a molar ratio of 2:1 QD:FNR for a final concentration as follows; 1.2 uM QD, 0.6 uM FNR, 0.25 mM $NADP^+$, 10 mM aldehyde, 1 u of *tb*ADH, and 100 mM AA in buffer (50 mM Tris, 5 mM NaCl, 5% glycerol, pH 7). The QD and FNR were mixed at high concentration prior to dilution to ensure self-assembly of active complexes. Samples were illuminated with stirring using an Ocean Optics 405 nm LED light source (750 mW, 500 mA) at 2500 µE m^{-2} s^{-1} (light meter LI-250, LI-COR Biosciences). Aliquots of 100 µl were removed from the sample at specific illumination times and the aldehyde and alcohol concentrations measured using Gas Chromatography

(Aglient technologies 7890 A with FID detector, DB-5 column). The calibration curves for the aldehydes and alcohols used in this study are shown in figure S3.

Keto acid to alcohol conversion. Keto acid decarboxylase was expressed and isolated as previously reported.[7] Solutions of KDC/tbADH/QD-FNR were prepared under anaerobic conditions by mixing a molar ratio of 2:1 QD:FNR for a final concentration as follows; 1.2 µM QD, 0.6 µM FNR, 0.25 mM $NADP^+$, 10 mM aldehyde, 1 u of *tb*ADH, 0.1 ug KDC, and 100 mM AA in buffer (50 mM Tris, 5 mM NaCl, 5% glycerol, pH 7). The QD and FNR were mixed at high concentration prior to dilution to ensure self-assembly of active complexes. Samples were illuminated with stirring using an Ocean Optics 405 nm LED light source (750 mW, 500 mA) at 2500 µE m^{-2} s^{-1} (light meter LI-250, LI-COR Biosciences). Aliquots of 100 µl were removed from the sample after 1 h of illumination and the aldehyde and alcohol concentrations measured using gas chromatography.

Quantum Yield Measurement: Samples of QD-FNR were prepared under anaerobic conditions by mixing a molar ratio of 2:1 QD:FNR for a final concentration of 1.2 µM QD and 0.6 µM FNR. NADPH production assay samples contained 2 mM $NADP^+$ (Sigma) and 100mM Ascorbic Acid (AA, Fluka) in buffer (50 mM Tris, 5 mM NaCl, 5% glycerol, pH 7). The QD and FNR were mixed at high concentration prior to dilution to ensure self-assembly of active complexes. Isobutanol production samples contained 0.25 mM $NADP^+$, 10 mM aldehyde, 1 u of *tb*ADH, and 100 mM AA in buffer (50 mM Tris, 5 mM NaCl, 5% glycerol, pH 7). Solutions were illuminated in an anaerobic cuvette with a pathlength of 3 mm, using a 405 nm laser pointer with an output power of 1.2 mW. The total light absorption by the samples was determined by comparison of light transmission through QD-FNR samples and control samples without QD. The QD-FNR samples absorbed ~9% of incident photons (average transmission through control samples = 1.07 mW; average transmission through QD-FNR samples = 0.98 mW).

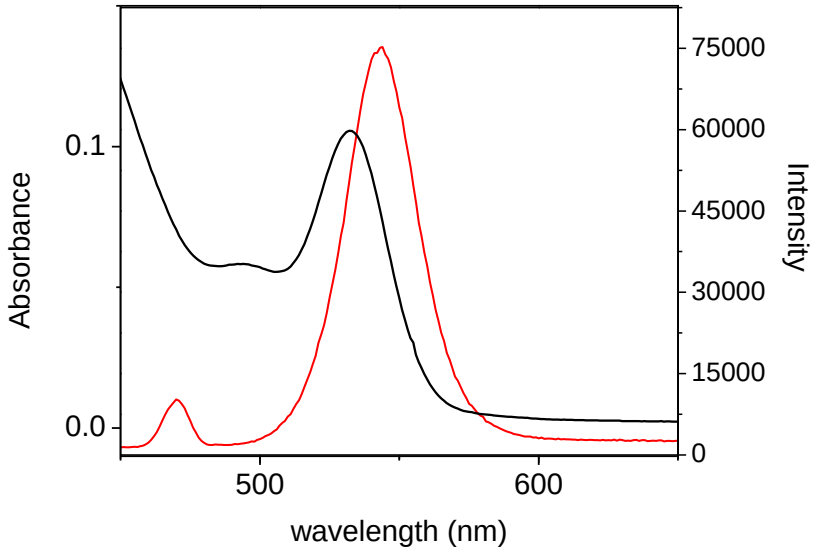

Figure S1. CdSe optical properties. Absorbance spectra (black line) and photoluminescence spectra (red line).

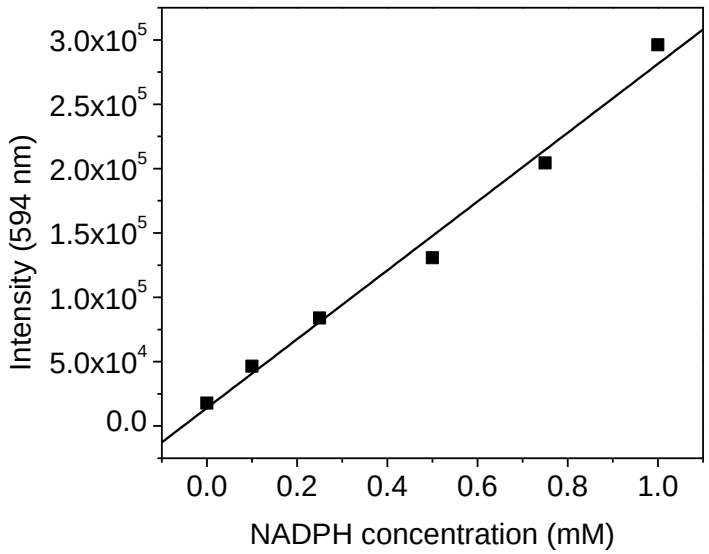

Figure S2. NADPH detection calibration curve (20 min incubation with kit components).

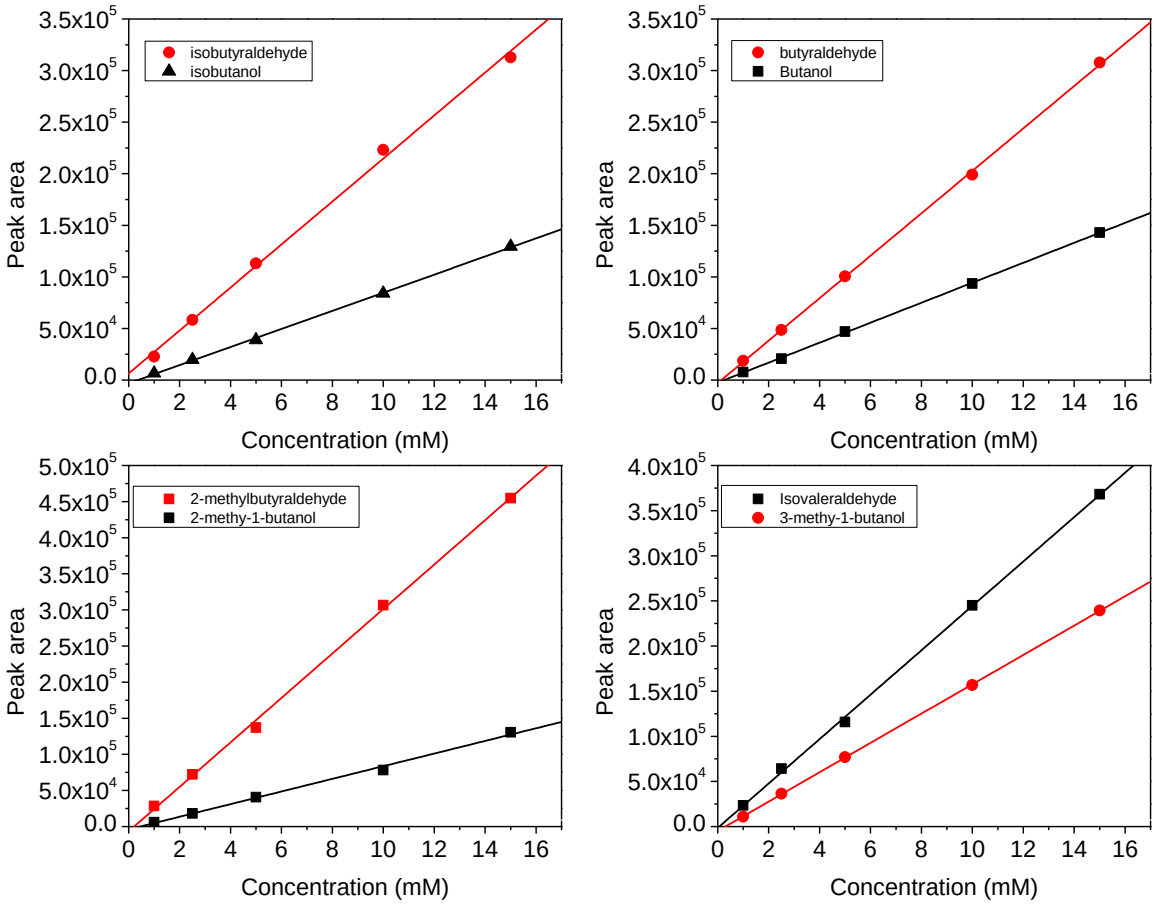

Figure S3. GC-FID alcohol and aldehyde calibration.

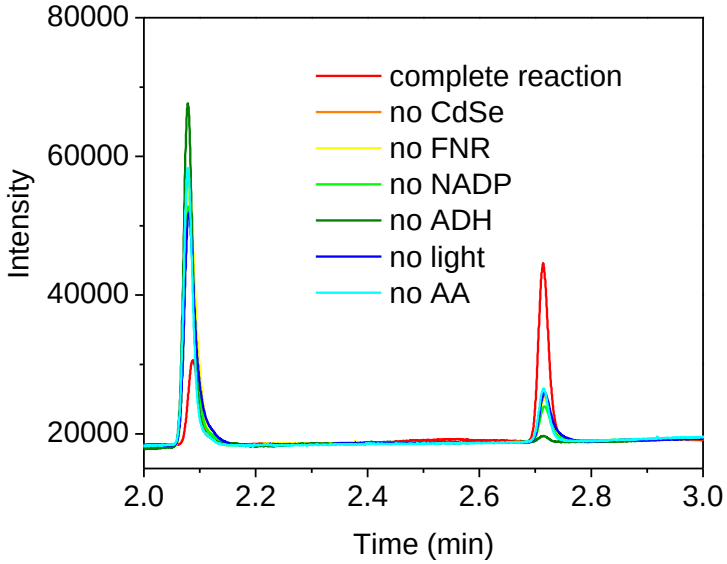

Figure S4. Isobutanol production control samples.

Table S1. Quantum Yield measurements of QD-FNR light driven NADPH and isobutanol production.

Product molecule	Power absorbed (μW)	Photons absorbed (μmol)	Product concentration (mM)	Quantum yield (%)
NADPH	104 ± 19	1.3 ± 0.3	0.12 ± 0.02	5.7 ± 1.4
Isobutanol	94 ± 16	1.15 ± 0.2	0.11 ± 0.03	5.8 ± 1.7

1. Peden, E. A.; Boehm, M.; Mulder, D. W.; Davis, R.; Old, W. M.; King, P. W.; Ghirardi, M. L.; Dubini, A. *J. Biol. Chem.* **2013,** 288, (49), 35192-35209.
2. Han, Z.; Qiu, F.; Eisenberg, R.; Holland, P. L.; Krauss, T. D. *Science* **2012,** 338, (6112), 1321-1324.
3. Mekis, I.; Talapin, D. V.; Kornowski, A.; Haase, M.; Weller, H. *J. Phys. Chem. B* **2003,** 107, (30), 7454-7462.
4. Amirav, L.; Alivisatos, A. P. *J. Phys. Chem. Lett.* **2010,** 1, (7), 1051-1054.
5. Brown, K. A.; Wilker, M. B.; Boehm, M.; Dukovic, G.; King, P. W. *JACS* **2012,** 134, (12), 5627-5636.
6. Yu, W. W.; Qu, L.; Guo, W.; Peng, X. *Chem. Mater.* **2003,** 15, (14), 2854-2860.
7. Smit, B. A.; van Hylckama Vlieg, J. E. T.; Engels, W. J. M.; Meijer, L.; Wouters, J. T. M.; Smit, G. *Appl. Environ. Microbiol.* **2005,** 71, (1), 303-311.