ACS Catalysis

Letter

pubs.acs.org/acscatalysis

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

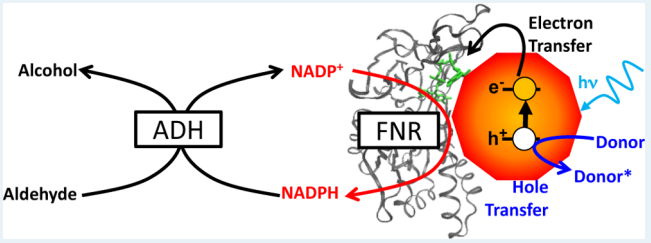
Katherine A. Brown,*[,†] Molly B. Wilker,[‡] Marko Boehm,[†] Hayden Hamby,[‡] Gordana Dukovic,[‡] and Paul W. King[†]

[†]Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401, United States

[‡]Department of Chemistry and Biochemistry, University of Colorado Boulder, Boulder, Colorado 80309, United States

Supporting Information

ABSTRACT: We report the characterization of biohybrid complexes of CdSe quantum dots and ferredoxin $NADP^+$-reductase for photocatalytic regeneration of NADPH. Illumination with visible light led to reduction of $NADP^+$ to NADPH, with an apparent k_{cat} of 1400 h^{-1}. Regeneration of NADPH was coupled to reduction of aldehydes to alcohols catalyzed by a NADPH-dependent alcohol dehydrogenase, with each NADPH molecule recycled an average of 7.5 times. The quantum yield both of NADPH and alcohol production were 5–6% for both products. Light-driven NADPH regeneration was also demonstrated in a multienzyme system, showing the capacity of QD-FNR complexes to drive continuous NADPH-dependent transformations.



KEYWORDS: *biohybrid, NADPH regeneration, photocatalysis, biocatalysis, quantum dots*

Regeneration of enzyme cofactors is essential for *in vitro* biocatalytic synthesis. Oxidoreductase enzymes can catalyze a wide range of industrially relevant reactions with a high degree of substrate specificity and regioselectivity.[1,2] The majority of these enzymes are dependent on nicotinomide cofactors (e.g., NADH or NADPH) for activity. The high cost of pyridine cofactors makes the use of stoichiometric amounts cost prohibitive, and efficient systems for cofactor regeneration are essential for the use of these enzymes in industrial applications. Traditionally, cofactor regeneration has been accomplished using enzymatic recycling by whole cell extracts or complementary NAD^+- or $NADP^+$-dependent enzymes.[3−5] These systems most often rely on multiple components catalyzing a series of redox reactions to regenerate the cofactors. Electrochemical regeneration of mediators and/or organometallic complexes as $NAD(P)^+$ cofactor redox partners have also been developed.[6−9] A simpler, potentially more efficient method is the use electrochemical or photochemical reduction for cofactor regeneration. Direct photochemical reduction of NAD^+ by stepwise single-electron transfers (ET) has been demonstrated using semiconductor quantum dots,[10,11] metallic nanoparticles,[12−14] and carbon nitride[15,16] These systems show promise, but they face the challenge of a high-energy requirement for the second ET step required to complete the NAD^+ reduction, and can result in non-biologically relevant products.[15]

An alternative approach is to combine light-generated reducing potential with an enzyme catalyst to create a biohybrid system for cofactor photoregeneration.[17−19] Enzymes such as ferredoxin $NADP^+$-reductase (FNR) catalyze cofactor reduction via a hydride transfer mechanism that minimizes the high energy steps that limit the direct reduction approach.[20] FNR contains a bound flavin adenine dinucleotide (FAD) which, when fully reduced, transfers a hydride to the $NADP^+$ molecule, delivering two electrons in a single step.[21]

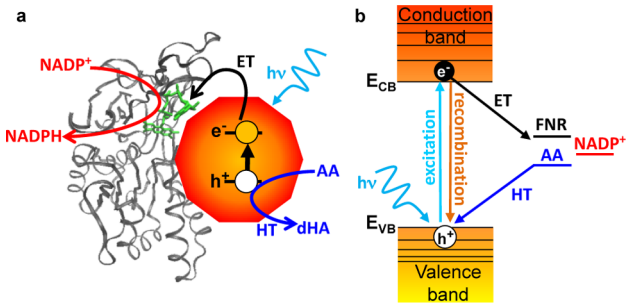
In this manuscript, we describe biohybrid complexes of the NADPH-specific FNR from *Chlamydomonas reinhardtii* and a CdSe quantum dot (QD) capable of photocatalytic regeneration of NADPH under illumination. Scheme 1 shows the proposed structure of the biohybrid complexes and the energy diagram for charge transfer in this system. The CdSe QD absorbs visible light and undergoes charge separation to generate an electron-hole pair. The electron can transfer to the FAD in the bound FNR, while the hole is quenched by a sacrificial electron donor, ascorbic acid (AA), to regenerate the QD ground state. Transfer of two electrons to FAD drives reduction of $NADP^+$ to NADPH via the enzymatic hydride transfer mechanism. These complexes can recycle NADPH used in enzymatic reduction of aldehydes to alcohols by NADPH-dependent alcohol dehydrogenase, with a quantum yield of 5.8%.

FNR is a ferredoxin (Fd)-dependent enzyme *in vivo*. Based on our previous work with Clostridial [FeFe]-hydrogenase,[22,23] QDs capped with mercaptopropionic acid (MPA) as their solubilizing ligand show a preference for site-specific adsorption at Fd binding sites. This preferential binding is the result of electrostatic interactions between the negatively charged QD

Received: December 14, 2015
Revised: February 11, 2016

DOI: 10.1021/acscatal.5b02850
ACS Catal. 2016, 6, 2201−2204

Scheme 1. (a) Proposed Structure of QD-FNR Complex (to scale), with QD Binding at Fd Binding Site (FAD shown in green); (b) Energy-Level Diagram of $NADP^+$ Reduction by QD-FNR Biohybrid Complexes[a]



[a]Abbreviations: FNR, ferredoxin $NADP^+$ reductase; ET, electron transfer; E_{CB}, QD conduction band energy; E_{VB}, QD valence band energy; AA, ascorbic acid; dHA, ascorbyl radical; $NADP^+$, nicotinamide adenine dinucleotide phosphate.

ligands and the positively charged Fd binding site. We predict that in QD-FNR complexes, the negatively charged QDs will bind similarly at the FNR Fd binding site, which is located at the binding pocket for the bound FAD. The QD is size-compatible with this binding pocket due to its small diameter (2.8 nm). This configuration will lead to an estimated electron transfer distance from the QD to the FAD of ~1 nm, based on the FNR-Fd complex crystal structure.[21]

The ability of QD-FNR complexes to reduce $NADP^+$ was assessed using a fluorescence based detection kit for $NADP^+$/NADPH (eNZYME, $\lambda_{emission}$ = 590 nm). Treatment with base and heat degraded unreduced $NADP^+$ selectively and precipitated the QDs, removing their background photoluminescence signal. Figure 1 shows the reduction of $NADP^+$ by QD- FNR complexes under illumination with 405 nm light and in the presence of AA as a sacrificial donor. The NADPH concentration increased linearly with illumination time up to 2 h, when all the available $NADP^+$ was reduced. Control samples of QDs alone, QD-FNR in the dark, and QD-FNR without AA showed no detectable NADPH production. The average turnover frequency (TOF) of FNR over the linear range of NADPH production (2 h) for these complexes was 1440 h^{-1}. This TOF by QD-FNR is lower compared to a TOF of 3.2 × 10^5 h^{-1} for FNR with its native redox partner Fd. This low photocatalytic TOF (~0.4% of biochemical TOF) is likely the result of low electron injection efficiency from the QD to FNR. In similar previously studied nanoparticle-enzyme systems, the electron flux through the enzyme is limited by both the light intensity and the internal recombination processes of the nanoparticle.[23−25]

The ability of QD-FNR complexes to recycle NADPH under enzymatic turnover was investigated using *Thermoanaerobium brockii* alcohol dehydrogenase (*tb*ADH, Sigma). *tb*ADH catalyzes NADPH-dependent reduction of a wide range of aldehydes to alcohols.[26] Isobutyraldehyde was added to a solution of *tb*ADH, $NADP^+$, QD-FNR, and AA. The reaction mixture was illuminated for 4 h and the concentration of isobutyraldehyde and isobutanol were measured by GC (Figure 2a) at various time points. Figure 2b shows the rate of isobutanol production in illuminated and dark solutions. In

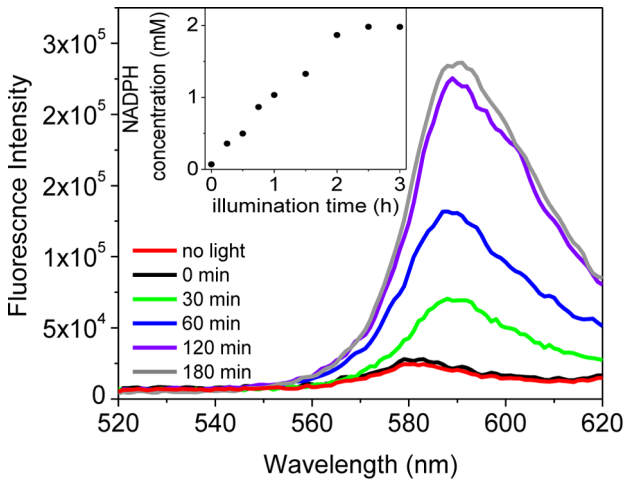


Figure 1. Reduction of $NADP^+$ to NADPH by QD-FNR complexes upon illumination with 405 nm light. Fluorescence detection of NADPH production (Elite NADPH assay kit, eNzyme. Excitation = 540 nm) (1.2 uM QD, 0.6 uM FNR, 2 mM $NADP^+$, 100 mM AA). Selected illumination time points, 0 min, 30 min, 1, 2, and 3 h, and an unilluminated control. Inset: NADPH concentration in solution over the full illumination time timecourse.

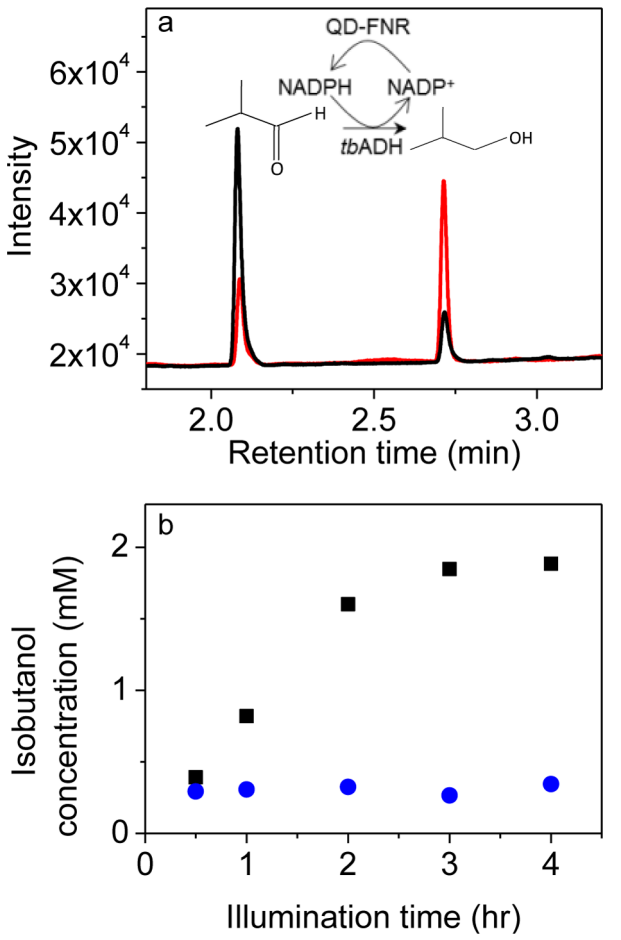


Figure 2. Isobutanol production by *tb*ADH/QD-FNR solution with 10 mM isobutyraldehyde upon illumination with 405 nm light. (a) Chromatogram of solution after 1 h illumination (red), or reaction in the dark (black). (b) Isobutanol concentration with illumination time (1.2 uM QD, 0.6 uM FNR, 0.25 mM $NADP^+$, 10 mM isobutyraldehyde, 1 unit of *tb*ADH, 100 mM AA). Illuminated sample (black squares) and dark sample (blue circles).

DOI: 10.1021/acscatal.5b02850
ACS Catal. 2016, 6, 2201−2204

Table 1. Alcohol Production by *tb*ADH/QD-FNR Mixtures with Various Aldehyde Substrates with 1 h Illumination

substrate	product	product (uM)	TOF[a] (mol NADPH mol FNR^{-1} h^{-1})	ratio $\frac{[\text{product}]}{[\text{NADP}^+]}$
isobutyraldehyde	isobutanol	0.89 ± 0.03	1488	3.6
butyraldehyde	butanol	0.87 ± 0.05	1446	3.5
2-methylbutyraldehyde	2-methylbutanol	0.83 ± 0.06	1386	3.3
isovaleraldehyde	3-methylbutanol	0.69 ± 0.08	1158	2.8

[a]mole NADPH calculated as the concentration required to produced the mole alcohol measured.

samples kept in the dark, a small amount of isobutanol is detected at all illumination times (Figure 2a, black line; Figure 2b, blue circles). This is the result of intrinsic activity in the *tb*ADH, which produced small amounts of alcohol upon addition of aldehyde. Alcohol was detected even in solutions with no $NADP^+$ added, and equal concentrations were observed in dark samples and all control samples except those lacking *tb*ADH (Figure S4).

Alcohol production increased linearly for 2 h, then the rate of production plateaued, and no further aldehyde reduction occurred after 3 h of illumination. Based on the initial reaction rate shown in Figure 2b, the concentration of isobutanol is equal to the total $NADP^+$ added to the solution (0.25 mM) at 30 min illumination. At the end of 3 h of illumination, the isobutanol concentration is 1.6 mM. These results indicate that the QD-FNR complexes are regenerating NADPH after each aldehyde reduction reaction and that each molecule of $NADP^+$ initially added has been recycled an average of 7.5 times.

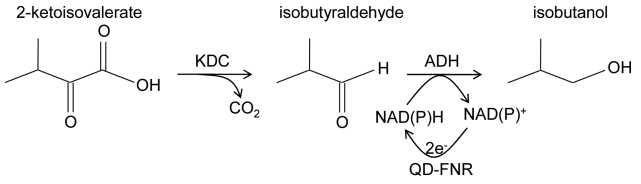
The loss of alcohol production at longer illumination times was investigated. The *tb*ADH activity was unaffected, and after 4 h of illumination, the addition of NADPH produced a stoichiometric increase in the isobutanol concentration. From this we conclude that the plateau in alcohol concentration after 2 h is due to the lack of NADPH in solution. This is likely the result of the loss of QD-FNR activity with prolonged illumination time. A combination of two factors are likely influencing this change in rate. First, the QDs gradually precipitate during illumination due to the oxidation and loss of MPA ligands, an effect previously observed in QD-hydrogenase systems.[23] Second, precipitation of the CdSe QDs in the assay may release Cd^{2+}, and FNR activity is sensitive to the presence of cadmium ions in solution. Cd^{2+} ions are thought to chelate the amino acids at both the Fd and quinone binding sites.[27] These two effects combine to limit the active lifetime of the QD-FNR complexes under illumination to ∼3 h.

The activity of *tb*ADH/QD-FNR solutions toward other aldehyde substrates was also tested. Table 1 shows the concentration of alcohol produced after 1 h of illumination for 4 aldehyde substrates, as well as the calculated turnover frequencies of the solution at the start of the experiment. The *tb*ADH/QD-FNR mixtures are active and produce alcohol at levels that yield similar ratios of product to the [$NADP^+$] present levels for three of the four substrates. The isovaleraldehyde turnover and alcohol/$NADP^+$ ratio are slightly lower, which we attribute to the lower activity of *tb*ADH for this substrate. In control experiments with NADPH, *tb*ADH showed a ∼25% slower rate toward isovaleraldehyde compared to the other three substrates.

The efficiency of QD-FNR complexes was measured as the quantum yield of product formation, that is, the percent of absorbed photons that were converted to either NADPH or alcohol (2 photons per product molecule). At the concentrations investigated, the QD-FNR complexes absorbed 9% of the incident 405 nm photons. In solutions composed of QD-FNR, $NADP^+$, and AA, the conversion of absorbed photons to NADPH was 5.8 ± 1.2%. Solutions of *tb*ADH/QD-FNR with $NADP^+$ and AA showed a quantum yield of 4.8 ± 1.2% absorbed photons converted to isobutanol (Table S1).

The capacity for a cofactor-regenerating complex to operate effectively as a component of a multienzyme reaction system is important for driving a complete reaction pathway. To test the capacity of QD-FNR to function in photoregeneration of NADPH in more complex enzymatic transformations, we developed a two-step, NADPH-dependent reduction reaction (Scheme 2). Keto acid decarboxylase (KDC) from *Lactococcus lactis*[28] was added to solutions of *tb*ADH/QD-FNR, and 2-ketoisovalerate was used as a starting material for isobutanol production. Isobutyraldehyde is produced by decarboxylation of 2-ketoisovalerate by KDC, and the aldehyde is then reduced by *tb*ADH to isobutanol. Illumination of the KDC/*tb*ADH/QD-FNR solutions for 1 h produced an isobutanol concentration 3.7-fold higher than the initial $NADP^+$ concentration. This ratio is consistent with the ratio seen for the one step aldehyde reduction reaction (Table 1), and demonstrates that QD-FNR can be used to regenerate NADPH pools to drive continuous NADPH-dependent transformations as shown in Scheme 2.

Scheme 2. Two-Step Enzymatic Production of Isobutanol from 2-Ketoisovalerate by Keto Acid Decarboxylase (KDC), Alcohol Dehydrogenase (ADH), and QD-FNR



In summary, we present evidence that QD-FNR biohybrid complexes can effectively regenerate nicotinamide cofactors under turnover conditions. In the presence of 100 mM AA, illumination of QD-FNR produces NADPH at an average TOF of 1440 h^{-1} and a quantum yield of 5.8%. While this TOF is lower than the values obtained by other enzymatic cofactor recycling systems (≥10 000 h^{-1}),[4] it is equivalent to other published systems for photodriven cofactor regeneration.[29,30] The rate of photogenerated NADPH is maintained under enzymatic turnover, and the reduction of aldehydes was accomplished with high efficiency. Each $NADP^+$ molecule can be used an average of 7−8 times for isobutanol production before inactivation of the QD-FNR due to prolonged illumination. In a multienzyme system, photogenerated NADPH by QD-FNR can drive a two-step conversion of keto acids to alcohols with equivalent efficiency. The utility of these complexes is limited by their active lifetime, which is impacted by QD stability and QD degredation effects on

DOI: 10.1021/acscatal.5b02850
ACS Catal. 2016, 6, 2201−2204

enzyme activity. Despite these limitations, these biohybrid complexes show promise for efficient photocatalytic cofactor regeneration. One means to address these limitations in the future is by the selection of more photostable QD materials.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b02850.

FNR expression and isolation; QD synthesis; light-driven NADPH production; alcohol production by illuminated QD-FNR; keto acid to alcohol conversion; quantum yield measurement; QD spectra; calibration curves for NADPH and alcohol detection; alcohol production control sample GC traces; quantum yield measurement raw data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kate.brown@nrel.gov.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Laboratory Directed Research and Development (LDRD) Program at the National Renewable Energy Laboratory (to K.A.B. and P.W.K.). FNR expression was supported by the U.S. Department of Energy, Office of Biological and Environmental Research (BER) (to M.B.). NREL is a national laboratory of the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy operated by the Alliance for Sustainable Energy, LLC. Nanocrystal synthesis and ligand exchange were supported by U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering under Award DE-SC0010334 (to M.B.W., H.H, and G.D.).

■ REFERENCES

(1) Hollmann, F.; Schmid, A. *Biocatal. Biotransform.* **2004**, *22* (2), 63−88.
(2) Siu, E.; Won, K.; Park, C. B. *Biotechnol. Prog.* **2007**, *23* (1), 293−296.
(3) Hollmann, F.; Arends, I. W. C. E.; Buehler, K. *ChemCatChem* **2010**, *2* (7), 762−782.
(4) Uppada, V.; Bhaduri, S.; Noronha, S. B. *Curr. Sci. India* **2014**, *106* (7), 946.
(5) Wu, H.; Tian, C.; Song, X.; Liu, C.; Yang, D.; Jiang, Z. *Green Chem.* **2013**, *15* (7), 1773−1789.
(6) Ali, I.; Gill, A.; Omanovic, S. *Chem. Eng. J.* **2012**, *188*, 173−180.
(7) Ali, I.; Khan, T.; Omanovic, S. *J. Mol. Catal. A: Chem.* **2014**, *387*, 86−91.
(8) Kim, S.; Lee, G. Y.; Lee, J.; Rajkumar, E.; Baeg, J.-O.; Kim, J. *Electrochim. Acta* **2013**, *96*, 141−146.
(9) Song, H. K.; Lee, S. H.; Won, K.; Park, J. H.; Kim, J. K.; Lee, H.; Moon, S. J.; Kim, D. K.; Park, C. B. *Angew. Chem.* **2008**, *120* (9), 1773−1776.
(10) Nam, D. H.; Lee, S. H.; Park, C. B. *Small* **2010**, *6* (8), 922−926.
(11) Shi, Q.; Yang, D.; Jiang, Z.; Li, J. *J. Mol. Catal. B: Enzym.* **2006**, *43* (1), 44−48.
(12) Bhoware, S. S.; Kim, K. Y.; Kim, J. A.; Wu, Q.; Kim, J. *J. Phys. Chem. C* **2011**, *115* (5), 2553−2557.
(13) Kim, E.; Jeon, M.; Kim, S.; Yadav, P. N.; Jeong, K.-D.; Kim, J. *Rapid Commun. Photosci.* **2013**, *2* (2), 42−45.
(14) Sánchez-Iglesias, A.; Chuvilin, A.; Grzelczak, M. *Chem. Commun.* **2015**, *51*, 5330−5333.
(15) Liu, J.; Antonietti, M. *Energy Environ. Sci.* **2013**, *6* (5), 1486−1493.
(16) Liu, J.; Cazelles, R.; Zhou, H.; Galarneau, A.; Antonietti, M. *Phys. Chem. Chem. Phys.* **2014**, *16* (28), 14699−14705.
(17) Shumilin, I. A.; Nikandrov, V. V.; Popov, V. O.; Krasnovsky, A. A. *FEBS Lett.* **1992**, *306* (2−3), 125−128.
(18) Szczepaniak, K.; Worch, R.; Grzyb, J. *J. Phys.: Condens. Matter* **2013**, *25* (19), 194102.
(19) Burai, T. N.; Panay, A. J.; Zhu, H.; Lian, T.; Lutz, S. *ACS Catal.* **2012**, *2* (4), 667−670.
(20) Pueyo, J. J.; Gomez-Moreno, C.; Mayhew, S. G. *Eur. J. Biochem.* **1991**, *202* (3), 1065−1071.
(21) Kurisu, G.; Kusunoki, M.; Katoh, E.; Yamazaki, T.; Teshima, K.; Onda, Y.; Kimata-Ariga, Y.; Hase, T. *Nat. Struct. Biol.* **2001**, *8* (2), 117−121.
(22) Brown, K. A.; Dayal, S.; Ai, X.; Rumbles, G.; King, P. W. *J. Am. Chem. Soc.* **2010**, *132* (28), 9672−9680.
(23) Brown, K. A.; Wilker, M. B.; Boehm, M.; Dukovic, G.; King, P. W. *J. Am. Chem. Soc.* **2012**, *134* (12), 5627−5636.
(24) Brown, K. A.; Song, Q.; Mulder, D. W.; King, P. W. *ACS Nano* **2014**, *8* (10), 10790−10798.
(25) Utterback, J.; Wilker, M.; Brown, K.; King, P.; Eaves, J.; Dukovic, G. *Phys. Chem. Chem. Phys.* **2015**, *17*, 5538.
(26) Lamed, R. J.; Zeikus, J. G. *Biochem. J.* **1981**, *195* (1), 183−190.
(27) Grzyb, J.; Bojko, M.; Waloszek, A.; Strzałka, K. *Phytochemistry* **2011**, *72* (1), 14−20.
(28) Gocke, D.; Nguyen, C. L.; Pohl, M.; Stillger, T.; Walter, L.; Müller, M. *Adv. Synth. Catal.* **2007**, *349* (8−9), 1425−1435.
(29) Lee, S. H.; Nam, D. H.; Kim, J. H.; Baeg, J.-O.; Park, C. B. *ChemBioChem* **2009**, *10* (10), 1621−1624.
(30) Lee, S. H.; Nam, D. H.; Park, C. B. *Adv. Synth. Catal.* **2009**, *351* (16), 2589−2594.

DOI: 10.1021/acscatal.5b02850
ACS Catal. 2016, 6, 2201−2204