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DOI

[10.1002/cctc.202000512](https://doi.org/10.1002/cctc.202000512)

Publication date

2020

Document Version

Final published version

Published in

ChemCatChem

Citation (APA)

Hobisch, M., van Schie, M. M. C. H., Kim, J., Røjkjær Andersen, K., Alcalde, M., Kourist, R., Park, C. B., Hollmann, F., & Kara, S. (2020). Solvent-Free Photobiocatalytic Hydroxylation of Cyclohexane. *ChemCatChem*, 12(16), 4009-4013. <https://doi.org/10.1002/cctc.202000512>

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Solvent-Free Photobiocatalytic Hydroxylation of Cyclohexane

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The use of neat reaction media, that is the avoidance of additional solvents, is the simplest and the most efficient approach to follow in biocatalysis. Here, we show that unspecific peroxygenase from *Agrocybe aegerita* (AaeUPO) can hydroxylate the neat model substrate cyclohexane. H₂O₂ was photocatalytically generated in situ by nitrogen-doped carbon nanodots (N-CNDs) and UV LED illumination. AaeUPO entrapment in alginate beads increased enzyme stability and facilitated the reaction in neat cyclohexane. N-CNDs absorption in beads containing AaeUPO created a 2-in-1 heterogeneous photobiocatalyst that was active for up to seven days under reaction conditions and produced cyclohexanol, 2.5 mM. To increase productivity, the bead size and the photocatalyst-to-enzyme ratio have been identified as promising targets for optimisation.

Selective oxyfunctionalisation still represents a major challenge for preparative organic synthesis.^[1] A biocatalytic alternative to usually metal based chemocatalysts^[2] was found in P450 monooxygenases, which have been reported to catalyse a broad range of selective oxidation and oxyfunctionalisation reactions.^[3] Their use however, has not yet exceeded the synthesis of pharmaceutical ingredients. This may be due to their dependency on nicotinamide cofactors (NAD(P)H). These cofactors are exclusively water-soluble thereby confining P450 monooxygenase catalysis to aqueous reaction media. The majority of reagents of interest, however, is rather hydrophobic and therefore incompatible with the requirements of P450 monooxygenases for mostly aqueous media. One major drawback of aqueous reaction media is the low solubility of hydrophobic substrates and products.^[4]

More recently, peroxygenases have gained increased attention as alternatives to P450 monooxygenases.^[5] As heme-thiolate enzymes, their reaction scope is comparable to that of P450 monooxygenases, but unlike P450s, peroxygenases utilise simple H₂O₂ or organic hydroperoxides as oxidants, which makes them independent from NAD(P)H and principally enables their application in non-aqueous media.

As early as the 1980s, Klibanov and coworkers^[6] reported the application of peroxidases in non-aqueous media. However, it has not yet received much attention. Recently, some examples of the use of an immobilised peroxygenase under non-aqueous conditions for selective hydroxylation and epoxidation reactions have been reported.^[7] To drive the reaction stoichiometric amounts of ^{tert}BuOOH may be used, leaving equimolar amounts of ^{tert}BuOOH waste behind. More elegantly, the stoichiometric oxidant would be generated *in situ* from O₂ (Scheme 1).^[8]

The photocatalytic production of H₂O₂ was achieved using flavin,^[9] different water oxidation catalysts^[10] and carbon nanodots (CNDs), which were reported to be most efficient. Use of CNDs gave five-times higher reaction rates compared to gold nanoparticles deposited on titanium dioxide (Au-TiO₂).^[10] Those results drew our attention to CNDs for *in situ* H₂O₂ generation.

In this study, we focused on medium engineering to explore photobiocatalytic UPO-catalysed reactions in non-aqueous media to alleviate aforementioned water-related limitations. Cyclohexane was chosen as poorly water-soluble

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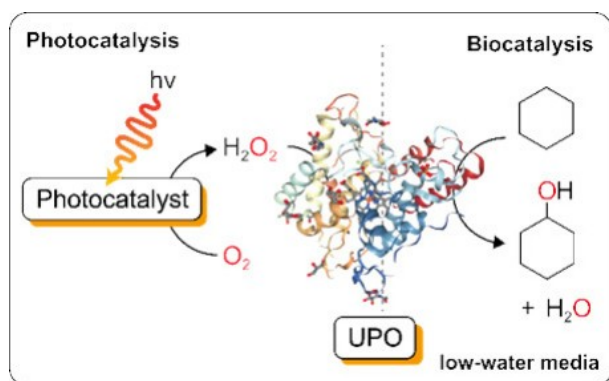
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Supporting information for this article is available on the WWW under <https://doi.org/10.1002/cctc.202000512>

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Scheme 1. UPO-mediated photobiocatalytic hydroxylation of cyclohexane in non-conventional media.

(0.06 gL⁻¹, 0.7 mM at 20 °C) model substrate, whereas the work dedicated to the evaluation of the photocatalyst was based on ethylbenzene (water solubility of 0.2 gL⁻¹, 1.4 mM at 20 °C), a standard substrate for UPO. PaDa-I is a laboratory-evolved variant of UPO from *Agroclybe aegerita*^[11] with strong heterologous expression, high activity and stability. Hence, *AaeUPO* PaDa-I became the enzyme of choice for the here presented study.

For the first experiment we examined the hydroxylation of cyclohexane using free enzyme in six organic solvents: Ethyl acetate (EtOAc), 2-methyl tetrahydrofuran (2-MeTHF), diisopropyl ether (DIPE), cyclopentyl methyl ether (CPME), heptane, and octane. In addition to those, a neat substrate system using only cyclohexane was evaluated. They were all brought to a uniform water activity (a_w) of 0.53 by incubation with a saturated solution of Mg(NO₃)₂ in a desiccator. The water activity was chosen based on a literature survey by Adlercreutz, suggesting that oxidoreductases require an optimal a_w value of 0.1–0.7.^[12] Reactions (1 mL, 0.15 μM PaDa-I either in organic solvents with 50 mM cyclohexane or in neat substrate cyclohexane) were incubated at 30 °C at 1200 rpm for 5 h and started with the addition of 1 μL 3.5% H₂O₂, which was repeated every hour, resulting in a final H₂O₂ concentration of 5.8 mM. After 5 h, no cyclohexanol could be detected, which was most probably due to the deactivation of the enzyme exposed to high amounts of organic media.

In an attempt to stabilise PaDa-I in organic media, enzyme immobilisation was employed. Gel entrapment was the method of choice owing to its simplicity in preparation and the promising results documented in the literature by the research groups of Hofrichter^[13] and of Plou, independently.^[14] In the here presented study *AaeUPO* PaDa-I was entrapped in calcium alginate beads. During the immobilisation process, a 19.2% loss of enzyme amount was observed and the beads were highly uniform regarding size (2.77 ± 0.09 mm) and weight (11.6 ± 0.8 mg).

For a first assessment whether the entrapment provided stability in organic media, 1 g of alginate beads (37.9 U) was incubated in a stirred tank reactor (30 °C, 500 rpm) with

5 mL cyclohexane and hourly addition of H₂O₂ over 7 h, resulting in a H₂O₂ concentration of 5.6 mM. The reaction was then left to run overnight and a sample was taken after 24 h, dried and used for GC analysis. A distinct cyclohexanol peak in the chromatogram indicated that the enzyme entrapment had a positive effect on enzyme stability (Figure S2). The manual addition of aqueous H₂O₂ to the organic media however, was less than ideal.

An alternative strategy for H₂O₂ supply was found using nitrogen-doped carbon nanodots (N-CNDs).^[15] Their ability for light-driven H₂O₂ production was first evaluated in the hydroxylation of ethylbenzene (50 mM) with PaDa-I (100 nM) and N-CNDs (5 mg mL⁻¹) in potassium phosphate buffer (KPi, 100 mM, pH 7) under white light illumination at 30 °C and magnetic stirring (600 rpm). After 47 h 9.0 mM (18% theoretical yield) (*R*)-1-phenyl ethanol in excellent enantiomeric excess (*ee*) (Figure S11) and 0.5 mM overoxidation product acetophenone were produced (Figure 1). This corresponds to a productivity of 1.91 mM_{product} μM_{enzyme}⁻¹ h⁻¹. A previously reported application of CNDs and FMN to run the UPO driven hydroxylation of ethylbenzene in the same light reactor gave 1.39 mM_{product} μM_{enzyme}⁻¹ h⁻¹.^[10] The light intensity of the light reactor setup used for this experiment is shown in Figure S3.

The reaction rate and low overoxidation looked promising, but the mechanism of the light-driven H₂O₂ formation was still unclear at this stage. Experiments conducted to understand the mechanism showed: (i) no product formation without O₂ (Figure S4), (ii) no O¹⁸-labelled product detected when O¹⁸-labelled water was used as the reaction medium (Figure S5), (iii) when illuminating N-CNDs hydroxyl radicals (HO•) were formed (Figure S6) and (iv) N-CNDs were degraded under illumination (Figure S8). Even though radicals were produced, the addition of formate or methanol as radical scavengers did not further improve the product formation rates. The experiments (i) and (ii) show that O₂ is incorporated into the product and therefore essential for the reaction, and the presence of hydroxyl

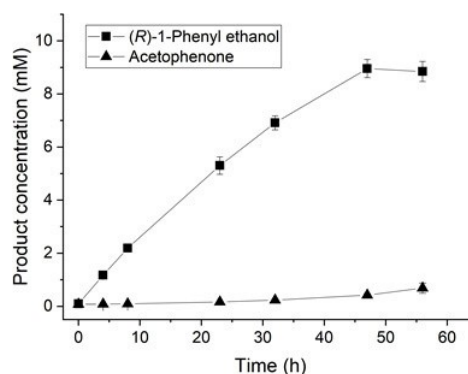


Figure 1. Light driven conversion of ethylbenzene (50 mM) to (*R*)-1-phenyl ethanol (squares) and acetophenone (triangles, overoxidation product) in potassium phosphate buffer (100 mM, pH 7) with N-CNDs (5 mg mL⁻¹) and UPO (100 nM) at 30 °C, magnetic stirring (600 rpm) and white light. Results are average values of duplicates. The data points are connected by a solid line to guide the eye.

radicals (iii) is to be expected in a light-driven reaction involving H₂O₂. However, the degradation of N–CNDs under illumination (iv) suggests some kind of light-induced self-oxidation. Those results did not lead us to a clear conclusion. Therefore, we must conclude that the complete elucidation of the light induced H₂O₂ formation mechanism is beyond the scope of this communication.

With the right tools to stabilise the enzyme and to continuously provide H₂O₂ *in situ*, the next step was to select a solvent for the organic reaction medium to increase cyclohexane availability to the enzyme. Octane, heptane, cyclopentylmethylether (CPME), diisopropyl ether (DIPE), methyl *tert*-butylether (MTBE), 2-methyl tetrahydrofuran (2-MeTHF) and neat substrate were the possible candidates. First, enzyme stability was evaluated. Solutions of *Aae*UPO PaDa–I (0.5 mg mL^{−1}) in KPi buffer (100 mM, pH 7) saturated with organic solvents were prepared and subjected to NanoDSF measurements to assess the solvents effect on enzyme stability by determination of the inflection temperature (T_i , see Supporting Information). While the enzyme showed a T_i of 72.0 °C in untreated KPi buffer, and barely any stability loss with the hydrocarbon solvents, its stability and therefore T_i steadily decreased with increasing water solubility of the solvents, reaching 54.6 °C for 2-Me-THF (Table 1).

The analysis of T_i values showed a clear trend where the T_i value decreases with decreasing $\log P$ values and therefore increasing hydrophilicity (Table 1). This can be explained by the enzyme's stability loss due to the molecular toxicity of the applied organic solvents dissolved in the aqueous medium.

For example, the water solubility of 2-MeTHF is >2,000-fold higher than that of cyclohexane. However, the decrease in the T_i value from buffer to cyclohexane was only 0.6 °C, which makes it difficult to explain why no product formation was observed with free enzyme in neat cyclohexane. Regardless, it showed that the use of cyclohexane as a neat substrate system is favoured compared to the other solvents screened.

To conclude the solvent screening, the previously investigated solvents were used in combination with the

PaDa–I alginate beads and N–CNDs in a small-scale photoreactor. As the N–CNDs (Figure S9), mainly absorb light in the UV range, with only a slight tailing into the visible wavelengths, UV LEDs with an emission peak at 391 nm were chosen.

For the photocatalytic reactions, the reaction media were the organic solvents from Table 1, supplemented with 10 mM cyclohexane or the neat substrate cyclohexane. All organic solvents were saturated with water ($a_w=1$). While heptane and octane were part of the NanoDSF analysis and gave valuable information about the relation of water solubility and enzyme stability, they are also potential substrates for UPOs^[16] and were therefore excluded from the following experiments. *Aae*UPO PaDa–I alginate beads (0.8 g, 13.5 U) and N–CNDs (4 mg) were mixed in the 4 mL glass vials by brief shaking, so the hydrophilic N–CNDs could spread throughout the water-rich beads. After addition of organic solvents and substrate the brown N–CNDs were clearly confined to the beads and did not mix with the organic phase surrounding them (Figure S10a). The samples were then incubated in the photoreactor (Figure S1) at 30 °C and 200 rpm for 44 h. The GC analysis of the samples showed that cyclohexanol was only produced in the neat cyclohexane samples. Based on these results, neat cyclohexane was selected as the reaction medium of choice for further experiments.

After this proof-of-principle, an experiment to investigate the time course of the reaction and the effect of varying N–CND amounts was conducted. Three ratios of N–CNDs to enzyme (mg:nmol) were applied in the light-driven hydroxylation of neat cyclohexane: 4:1, 8:1 and 24:1. PaDa–I alginate beads (1 g, 28 U, 0.696 nmol enzyme) were mixed with the appropriate amount of N–CNDs and incubated with 1 mL cyclohexane for seven days under UV illumination at 200 rpm and 30 °C. 50 μ L samples were taken every 24 h. While reactions with 4:1 and 8:1 ratio showed similar productivity up to three days, the one with 4:1 ratio stopped thereafter, whereas the 8:1 reaction continued until day five at the same rate (Figure 2).

The 24:1 reaction however, showed a much lower product formation rate, only reaching 0.5 mM after three days (half the rate as compared to 4:1 and 8:1 reactions). It steadily continued at this rate and even after seven days showed no sign of slowing down (Figure 2). The low reaction rate of the 24:1 samples might be due to the excess amounts of N–CNDs having a shading effect thereby hindering the efficient use of light. Overoxidation in form of cyclohexanone production was not detected. Negative controls with alginate beads without *Aae*UPO PaDa–I, but otherwise under identical conditions did not yield any product, ruling out hydroxylation of cyclohexane by N–CNDs. This also confirms the excellent *ee* value of (*R*)-1-phenyl ethanol by UPO catalysis. A regular sample incubated in the dark showed only trace amounts of product formation, demonstrating the reaction's dependence on light.

Table 1. The list of solvents used to saturate KPi (50 mM, pH 7) buffer and the inflection temperature (T_i) of *Aae*UPO PaDa–I measured in solvent-treated aqueous media.

Organic solvent	$\log P$ (–)	Solubility of the solvent in water at 20 °C [g L ^{−1}]	Inflection Temperature [T_i , °C] ^[a]
None	–	–	72.0
Octane	5.15	0.007	71.2
Heptane	4.66	0.02	71.7
Cyclohexane	3.4	0.06	71.4
DIPE	1.5	12	69.8
CPME	1.6	11	66.8
MTBE	0.9	42	66.5
2-MeTHF	1.1	140	54.6

[a] T_i values measured with Tycho NT.6 (NanoTemper Technologies, Germany) over T increase from 35 °C to 95 °C.

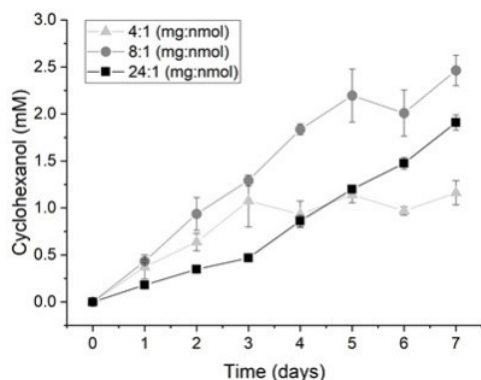


Figure 2. Hydroxylation of neat cyclohexane with PaDa-I alginate beads and varying ratios of N-CNDs to enzyme (mg:nmol): 4:1 (light-grey triangles), 8:1 (grey circles), 24:1 (black squares). Reactions run at 30 °C, shaking at 200 rpm, UV-LED illumination. The data points are connected by a solid line to guide the eye.

We also set up two reactions with a photocatalyst-to-enzyme ratio of 4:1 under the same conditions applied in Figure 2, where one reaction was resupplied with N-CNDs after three and five days. No increase in productivity upon fed-batch photocatalyst addition was detected (data not shown). Hence, enzyme stability rather than photocatalyst stability is a limiting factor in this reaction.

To investigate whether mass transfer limitations were also involved, smaller alginate beads (ϕ_{bead} of 0.9 mm instead of 2.7 mm) were prepared and used for light driven cyclohexane hydroxylation with N-CNDs. The beads were only one third in diameter compared to the previously used ones and therefore had three times the specific surface area. However, this also increased enzyme loss during immobilisation. When used in the reaction with a 8:1 (mg:nmol) N-CND:enzyme ratio under UV illumination as described above, the smaller beads yielded similar reaction rates as the big ones, with only a third of the enzyme amount (Figure S13). Producing 2.25 mM cyclohexanol in four days they showed a productivity of $0.08 \text{ mM}_{\text{product}} \mu\text{M}_{\text{UPO}}^{-1} \times \text{h}^{-1}$ with 7,806 turnovers per enzyme.

In order to show the applicability for another substrate, the small alginate beads were used for the hydroxylation of cyclopentane (Figure S14). Applying the same conditions as in Figure 2 with an 8:1 N-CND-to-enzyme ratio, the reaction only produced 1 mM cyclopentanol after three days, exhibiting a much lower performance than for cyclohexane.

In conclusion, we demonstrated the application of UPO-catalysed hydroxylation of neat alkane model substrate coupled with a photocatalyst. While the productivity of the photobiocatalytic system represented here is still lacking, it demonstrates a proof-of-principle for the application of photobiocatalysis in organic media. Finally, the fact that the 2-in-1 heterogeneous photobiocatalyst works for several days and the possibility to optimise the enzyme-to-photocatalyst ratio, are promising starting points for further developments towards higher volumetric productivities.

These principles demonstrated here can be used for future hydroxylations of hydrophobic value added compounds in neat conditions.

Acknowledgements

Kristian Graf and Henrik Hartvig Kolmos, Department of Molecular Biology of Genetics, are gratefully acknowledged for the construction of screening scale photoreactor. Kim Møller Johansen and Bekir Engin Eser, Department of Engineering, are acknowledged for their technical support in GC analytics. Klaus Koren, Department of Bioscience, is gratefully acknowledged for spectroscopic measurements with Gigahertz-Optik MSC15. Peter Leon Hagedoorn, Department of Biotechnology, TU Delft, is acknowledged for his technical support in EPR analytics. This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 764920.

Conflict of Interest

The authors declare no conflict of interest.

Keywords: Photobiocatalysis · non-conventional media · carbon nanodots · organic media · hydroxylation

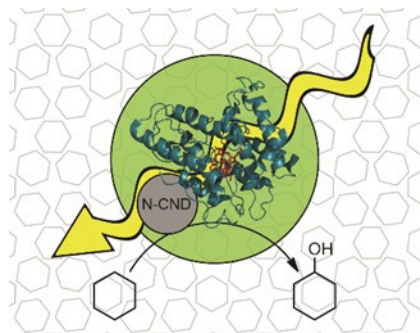
- [1] a) T. Punniamurthy, S. Velusamy, J. Iqbal, *Chem. Rev.* **2005**, *105*, 2329–2363; b) E. Roduner, W. Kaim, B. Sarkar, V. B. Urlacher, J. Pleiss, R. Gläser, W.-D. Einicke, G. A. Sprenger, U. Beifuß, E. Klemm, C. Liebner, H. Hieronymus, S.-F. Hsu, B. Plietker, S. Laschat, *ChemCatChem* **2013**, *5*, 82–112.
- [2] J. Dong, E. Fernandez-Fueyo, F. Hollmann, C. E. Paul, M. Pesic, S. Schmidt, Y. Wang, S. Younes, W. Zhang, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 9238–9261.
- [3] a) R. Fasan, *ACS Catal.* **2012**, *2*, 647–666; b) V. B. Urlacher, M. Girhard, *Trends Biotechnol.* **2019**, *37*, 882–897.
- [4] a) M. Patzold, S. Siebenhaller, S. Kara, A. Liese, C. Syldatk, D. Holtmann, *Trends Biotechnol.* **2019**, *1768*, 1–17; b) S. Kara, A. Liese, *Industrial Enzyme Applications*, 1 ed. (Ed.: O. M. A. Vogel), Wiley-VCH Verlag GmbH & Co. KGaA **2019**.
- [5] Y. Wang, D. Lan, R. Durrani, F. Hollmann, *Curr. Opin. Chem. Biol.* **2017**, *37*, 1–9.
- [6] a) J. S. Dordick, M. A. Marletta, A. M. Klibanov, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 6255–6257; b) J. S. Dordick, M. A. Marletta, A. M. Klibanov, *Biotechnol. Bioeng.* **1987**, *30*, 31–36.
- [7] a) E. Fernández-Fueyo, Y. Ni, A. Gomez Baraibar, M. Alcalde, L. M. van Langen, F. Hollmann, *J. Mol. Catal. B* **2016**, *134*, 347–352; b) M. C. R. Rauch, F. Tieves, C. E. Paul, I. W. C. E. Arends, M. Alcalde, F. Hollmann, *ChemCatChem* **2019**, *11*, 4519–4523; c) P. Molina-Espeja, P. Santos-Moriano, E. Garcia-Ruiz, A. Ballesteros, F. J. Plou, M. Alcalde, *Int. J. Mol. Sci.* **2019**, *20*.
- [8] B. O. Burek, S. Bormann, F. Hollmann, J. Z. Bloh, D. Holtmann, *Green Chem.* **2019**, *21*, 3232–3249.
- [9] a) E. Churakova, M. Kluge, R. Ullrich, I. Arends, M. Hofrichter, F. Hollmann, *Angew. Chem. Int. Ed.* **2011**, *50*, 10716–10719; *Angew. Chem.* **2011**, *123*, 10904–10907; b) D. I. Perez, M. M. Grau, I. W. Arends, F. Hollmann, *Chem. Commun. (Camb.)* **2009**, 6848–6850; c) E. Churakova, I. W. C. E. Arends, F. Hollmann, *ChemCatChem* **2013**, *5*, 565–568; d) I. Zachos, S. K. Gassmeyer, D. Bauer, V. Sieber, F. Hollmann, R. Kourist, *Chem. Commun. (Camb.)* **2015**, *51*, 1918–1921.

- [10] W. Zhang, E. Fernandez-Fueyo, Y. Ni, M. van Schie, J. Gacs, R. Renirie, R. Wever, F. G. Mutti, D. Rother, M. Alcalde, F. Hollmann, *Nat. Can.* **2018**, *1*, 55–62.
- [11] M. Ramirez-Escudero, P. Molina-Espeja, P. Gomez de Santos, M. Hofrichter, J. Sanz-Aparicio, M. Alcalde, *ACS Chem. Biol.* **2018**, *13*, 3259–3268.
- [12] P. Adlercreutz, *Organic Synthesis with Enzymes in Non-Aqueous Media* (Eds.: G. Carrea, S. Riva), Wiley-VCH Verlag GmbH & Co. KGaA **2008**.
- [13] M. Poraj-Kobielska, S. Peter, S. Leonhardt, R. Ullrich, K. Scheibner, M. Hofrichter, *Biochem. Eng. J.* **2015**, *98*, 144–150.
- [14] L. Fernandez-Arrojo, B. Rodriguez-Colinas, P. Gutierrez-Alonso, M. Fernandez-Lobato, M. Alcalde, A. O. Ballesteros, F. J. Plou, *Process Biochem.* **2013**, *48*, 677–682.
- [15] J. Kim, S. H. Lee, F. Tieves, D. S. Choi, F. Hollmann, C. E. Paul, C. B. Park, *Angew. Chem. Int. Ed. Engl.* **2018**, *57*, 13825–13828.
- [16] M. Hofrichter, R. Ullrich, *Curr. Opin. Chem. Biol.* **2014**, *19*, 116–125.

Manuscript received: March 24, 2020
Revised manuscript received: April 21, 2020
Accepted manuscript online: April 24, 2020
Version of record online: ■■■, ■■■■

COMMUNICATIONS

Photobiocatalysis: In this communication we demonstrate the potential of peroxygenase for light driven hydroxylation of neat cyclohexane. The new generation easy-to-prepare carbon nanodots and gel-entrapped peroxygenase were applied as 2-in-1 photobiocatalyst in a neat substrate system.



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