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Short Communication

Photoenzymatic synthesis of 1-alkenes and hydroxyl fatty acids by cascading a COF photocatalyst and P450 peroxygenases

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Fuels and oleochemicals have been chemically produced from abundant biological oils or fatty acids for more than a century, and modern biotechnology is now accelerating the advances in fatty acid chemistry [1,2]. A number of enzymes responsible for the biosynthesis of hydrocarbons from fatty acids have been discovered, providing a promising strategy for enzymatic synthesis of fatty hydrocarbons [3]. CYP152 peroxygenases have attracted a great deal of attention due to their ability to one-step oxidatively decarboxylate or hydroxylate fatty acids using H_2O_2 as sole oxidant, generating valuable α-olefins or hydroxylated fatty acids [4,5] (Fig. 1a). In addition to α-olefins as biofuels and chemical intermediates, hydroxylated fatty acids are also an important class of compounds with various applications in food, cosmetic, pharmaceutical, and biomaterial industries [6,7] (Fig. 1b). Among them, $P450_{BS\beta}$ is considered as the most promising CYP152 peroxygenase for practical applications due to its high stability and activity to catalyze both *β*-hydroxylation (the preferred reaction) and decarboxylation (the side reaction) of fatty acids that are both mechanistically initiated by C_β-H abstraction. Of note, directed evolution could remodel its catalytic preference on decarboxylation or *β*-hydroxylation (e.g., the $P450_{BS\beta}$-DC mutant with 67% decarboxylation selectivity) [5].

However, peroxygenases suffer from irreversible deactivation by stoichiometric amounts of H_2O_2 [8]. To balance the catalytic efficiency and H_2O_2-induced inactivation of P450 peroxygenases, additional redox catalysts have been adopted to reduce O_2 to H_2O_2 *in situ* at the expense of artificial electron donors or co-substrates [9–12] (Fig. 1c), albeit with limited success (Tables S2 and S3 online). Thus, a simple and efficient redox catalyst for the direct oxidation of water and reduction of O_2 to H_2O_2 *in situ* is highly demanded to solve the problematic issue of P450 peroxygenases.

Recently, several covalent organic framework materials (COFs) have been reported to photo-catalytically produce H_2O_2 through O_2 reduction and water oxidation with production rates at a hundreds μmol L^{-1} h^{-1} level (Table S4 online) [13]. COF-TfpBpy is the first reported COF photocatalyst that can photocatalytically synthesize H_2O_2 without sacrificial reagents, directly from water by reacting it with two holes as well as from O_2 by reacting it with two electrons and two protons over bipyridine via one-step redox reactions with good stability and high activity [14] (Fig. S1 online). These significant advances in the field of photocatalysis make it possible to build a photobiorefinery model that requires only light, water, and molecular oxygen for efficient enzymatic conversion of substrates, as nature does. Therefore, herein, we built an artificial photoenzymatic cascade by combining the H_2O_2-generating activity of the photocatalyst COF-TfpBpy and H_2O_2-dependent activity of selected CYP152 biocatalysts (Fig. 1d), thereby resulting in a concise and efficient pathway for green synthesis of α-olefins and hydroxyl fatty acids from naturally abundant fatty acids.

The COF-TfpBpy photocatalyst was prepared from 1,3,5-triformylphloroglucinol (Tfp) and 2,2′-bipyridine-5,5′-diamine (Bpy) by following the previous report [14], and the structure was confirmed by XRD, ATR-IR, solid state ^{13}C NMR, UV-Vis and XPS analyses (Fig. S2 online). The photosynthetic rates of H_2O_2 from water and air with different concentrations of COF-TfpBpy in biologically friendly aqueous reaction buffer (50 mmol/L Tris-HCl, pH = 8.0) revealed clear linear relationships between H_2O_2 production and irradiation time (Fig. S3 online). Among the tested photocatalyst loading, 1.5 mg/mL COF-TfpBpy gave the highest H_2O_2 production rate of 1071 μmol^{-1} L^{-1} h^{-1}, and water should

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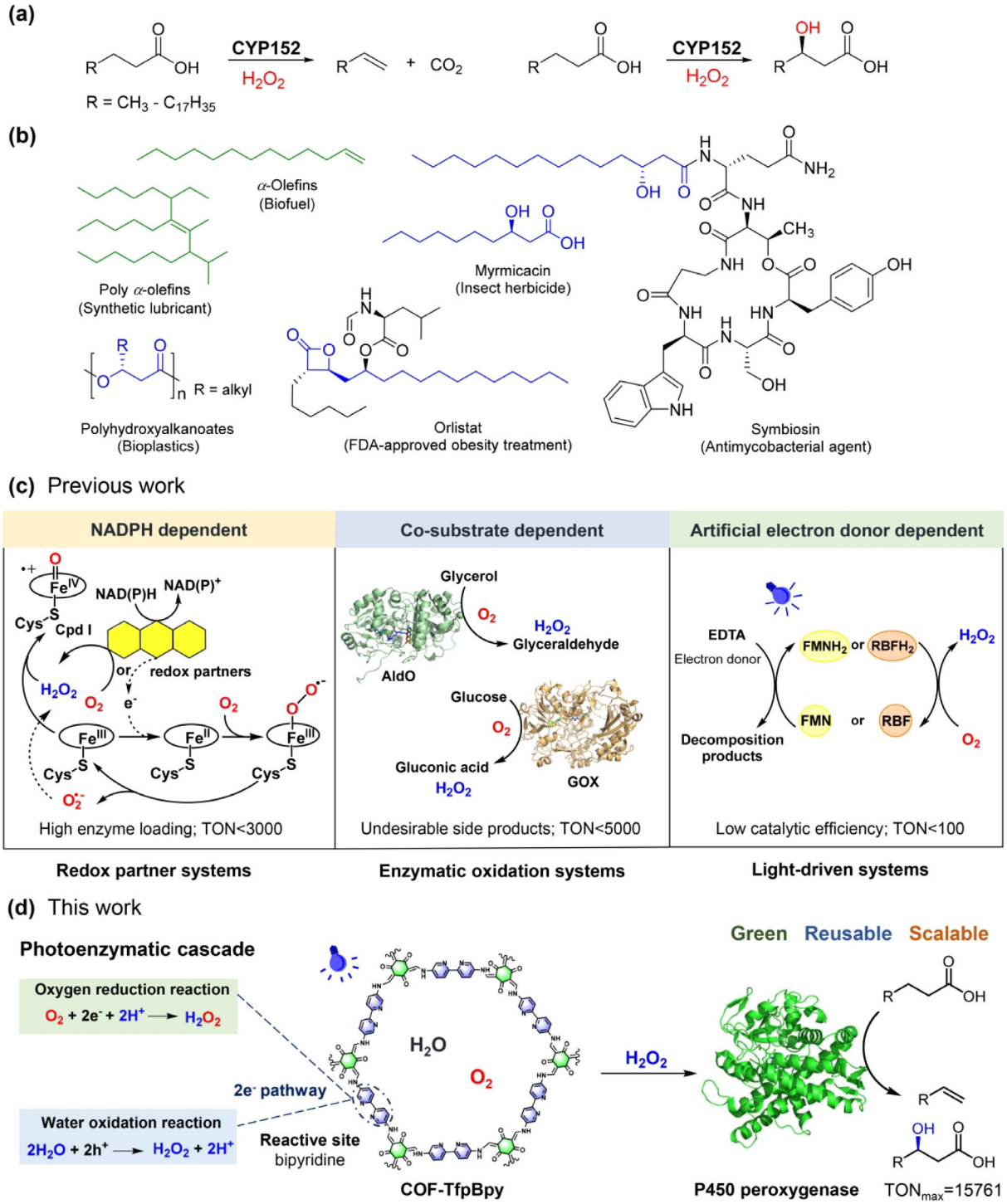

Fig. 1. Enzymatic decarboxylation and β-hydroxylation of fatty acids mediated by P450 peroxygenases. (a) Reaction scheme of CYP152 peroxygenases. (b) Applied fatty acid derivatives bearing an α-olefin (in green) or β-hydroxyl (in blue) moiety. (c) Previously reported cofactor supplying systems to support the catalytic activity of P450 peroxygenases. (d) The artificial photoenzymatic cascade developed in this work.

be the predominant (if not sole) electron donor for the photocatalytic system (Fig. 2a, Fig. S4 online). As a model reaction, 5 mmol/L myristic acid (the preferred substrate) was selected to test the catalytic activity of two representative CYP152 peroxygenases in a 1 mL photocatalytic reaction system. After 6 h of irradiation, $P450_{BS\beta}$ and $P450_{BS\beta}$-DC converted myristic acid (5 mmol/L) into their corresponding products with the substrate conversion ratios of 99.5% and 88.8%, respectively (Fig. 2b, Figs. S5–S7 online). The control reactions in absence of photocatalyst or P450 peroxygenase, or in darkness under the same conditions generated no product. In comparison, the reported exogenous H_2O_2 addition system and AldO-glycerol *in situ* H_2O_2 releasing system exhibited lower supporting activities for both $P450_{BS\beta}$ and $P450_{BS\beta}$-DC towards 5 mmol/L myristic acid, albeit with similar product distributions. We next sought to characterize the substrate preference towards a range of naturally abundant straight-chain saturated

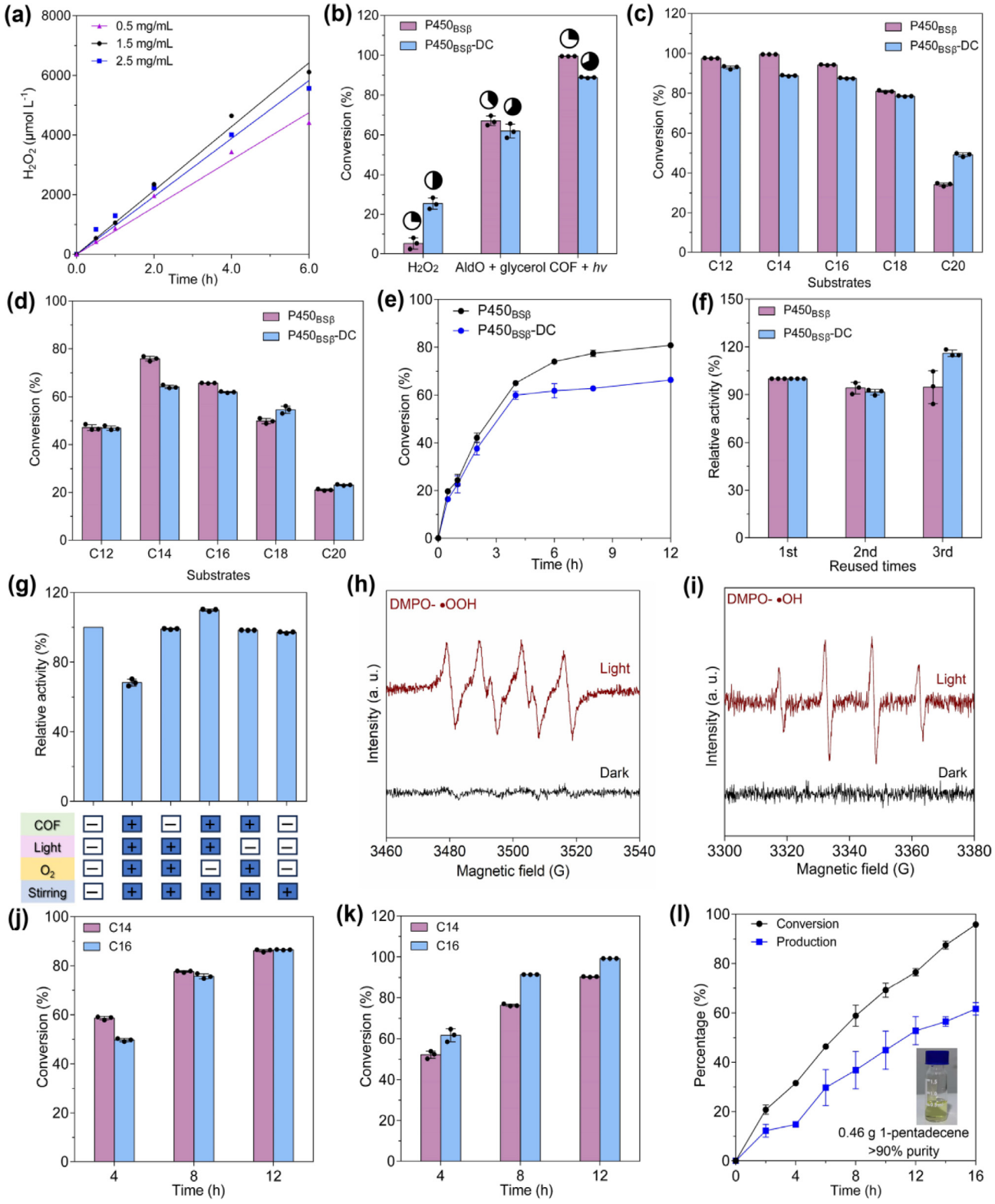

Fig. 2. Photocatalytic performances and optimization of the COF-TfpBpy and P450 peroxygenase cascade. (a) Photocatalytic activities of COF-TfpBpy for H_2O_2 production under different catalyst concentrations in biologically friendly aqueous reaction buffer (50 mmol/L Tris-HCl, pH = 8.0). Irradiation conditions: λ = 440 nm, light intensity: 49 mW/cm^2. Data are presented as mean ± s.d. (n = 3). (b) Catalytic activities of P450$_{BS\beta}$ and P450$_{BS\beta}$-DC supported by three different H_2O_2-supplying systems. Reaction conditions: 5 mmol/L myristic acid, 1 μmol/L P450 at r.t. for 6 h. The relative proportions of decarboxylation (black) and hydroxylation (white) products are shown in the pie charts. (c, d)The substrate preference of P450$_{BS\beta}$ and P450$_{BS\beta}$-DC when supported by the photocatalytic system. Reaction conditions: 5 mmol/L (c) or 10 mmol/L (d) fatty acid substrates, 1.5 mg/mL COF-TfpBpy, 1 μmol/L P450 at r.t. for 6 h, magnetic stirring at 150 r/min. (e) Time-courses of photoenzymatic conversions of 10 mmol/L myristic acid. (f) Reusability of COF-TfpBpy for supporting the catalytic activity of P450 peroxygenases. (g) Stability of P450$_{BS\beta}$-DC under different conditions for 1 h. The DMPO spin trapping EPR spectra of COF-TfpBpy measured by ·OOH (h) and ·OH (i) in dark and visible light. Effects of fresh enzyme supplementation on the conversions of 10 mmol/L fatty acid substrates (C_{14} and C_{16}) by P450$_{BS\beta}$ (j) and P450$_{BS\beta}$-DC (k). (l) Time-courses of palmitic acid conversion and 1-pentadecene production during the photo-biocatalytic cascade reactions at gram scale.

fatty acids (C_{12}-C_{20}). At the substrate concentrations of 5 mmol/L, $P450_{BS\beta}$ and $P450_{BS\beta}$-DC exhibited similar substrate preference profiles with myristic acid (C_{14}) and lauric acid (C_{12}) as their optimal substrate (Fig. 2c, Figs. S8 and S9 online). The conversion ratios of the reported poor substrate arachidic acid (C_{20}) by $P450_{BS\beta}$ and $P450_{BS\beta}$-DC also reached 34.2% and 46.5%, respectively. Myristic acid (C_{14}) became the preferred substrate for both P450 peroxygenases when the concentration of substrates increased from 5 mmol/L to 10 mmol/L, achieving the highest conversion ratio of 75.9% by $P450_{BS\beta}$ (Fig. 2d).

To optimize the photoenzymatic cascade, we selected 10 mmol/L myristic acid as the testing substrate. First, the time-course experiments of the photoenzymatic cascade reactions co-mediated by COF-TfpBpy and $P450_{BS\beta}$/$P450_{BS\beta}$-DC indicated that there was no significant improvement in catalytic activity after 6 h of reaction (Fig. 2e). Next, it was found that the progressing H_2O_2 concentrations of the P450-involving cascade system remained below 465 μmol/L (Table S5 online). The rate of H_2O_2 consumption by P450 peroxygenase was positively correlated with H_2O_2 concentration with the highest consumption rate of 7.5 mmol L^{-1} min^{-1} (Figs. S10 and S11 online). Furthermore, good sustainability, stability (Figs. S12 and S13 online) and reusability (Fig. 2f) of COF-TfpBpy indicated that the concentration of photosynthetic H_2O_2 was not a major constraining factor for catalytic activity. Thus, we reasoned that the stability of P450 biocatalyst in the photoenzymatic cascade system might be a key factor limiting the overall catalytic activity. Supporting this, when $P450_{BS\beta}$-DC was incubated with COF-TfpBpy in dark, only negligible reduction of the enzymatic activity was detected, unlike the significant activity loss in light (Fig. 2g). The light-dependent reduction of the enzymatic activity could be resulted from the deactivation of enzyme by photogenerated reactive oxygen species as reflected by a series of control experiments without photocatalyst or O_2 (Fig. 2g, Fig. S14 online). As expected, the ·OOH and ·OH radicals were detected during the H_2O_2 photosynthesis by COF-TfpBpy through *in situ* electron spin resonance (ESR) spectroscopic analysis using 5,5-dimethyl-pyrroline *N*-oxide (DMPO) as a free-radical spin-trapping agent (Fig. 2h, i). Nonetheless, compared to the almost complete deactivation of biocatalysts by some reported photocatalysts within 1 h [8], $P450_{BS\beta}$-DC retained 68.3% activity in the presence of COF-TfpBpy within 1 h (Fig. S15 online). Thus, we achieved excellent conversions of 10 mmol/L C_{14} and C_{16} substrates by $P450_{BS\beta}$-DC at very low biocatalyst loading (0.015 mol%) through fresh enzyme supplementation every 4 h (Fig. 2j, k).

To evaluate the reactivity and application potential of $P450_{BS\beta}$ and $P450_{BS\beta}$-DC, we compared the turnover numbers (TONs) of these two P450 peroxygenases when supported by five different H_2O_2-supplying systems. Despite largely varied TONs, the catalytic activities supported by the *in situ* H_2O_2 photosynthesis system were unanimously higher than those by the AldO/glycerol-based H_2O_2 releasing system, NADPH-dependent redox partner system to produce H_2O_2 via electron uncoupling, and the exogenous H_2O_2 addition system (either fed-batch or one-time addition) (Tables S6–S8 online). In the case of $P450_{BS\beta}$ plus COF-TfpBpy, the highest TON was achieved by myristic acid (C_{14}, TON = 15,761), 7.5 times higher than that of arachidic acid (C_{20}, TON = 2105). Surprisingly, both $P450_{BS\beta}$ and $P450_{BS\beta}$-DC showed weak activity against 5 mmol/L of the C_{20} substrate in the exogenous H_2O_2 addition system and two enzymatic *in situ* H_2O_2 releasing systems, and the TON only reached 270 when coupled to the AldO/glycerol-based H_2O_2 releasing system, which was far lower than that of the corresponding reaction supported by the photocatalytic system (TON = 2323). Notably, $P450_{BS\beta}$-DC showed the highest TON of 15,380 towards palmitic acid (C_{16}).

To further demonstrate the application potential of the photoenzymatic hybrid tandem fatty acid decarboxylation system, we performed the cascade reaction at a semipreparative scale. The photocatalytic rate of H_2O_2 in a scalable 100 mL reaction system in a 500 mL reactor (Fig. S16 online) was measured to be 700 μmol L^{-1} h^{-1}, and the concentration of H_2O_2 released *in situ* reached 10.2 mmol/L after 16 h of irradiation (Fig. S17 online). To compensate for the inactivation of P450 biocatalyst as mentioned above, $P450_{BS\beta}$-DC (0.5 μmol/L) was replenished every 4 h. In this setup, 10 mmol/L palmitic acid (C_{16}) was almost completely converted into the value-added products 1-pentadecene (61.6%) and β-hydroxy-palmitic acid (32.1%) after 16 h of reaction (Fig. 2l, Figs. S18 and S19 online). Besides, we also observed approximately 7.0% of α-hydroxy palmitic acid and small amounts of over-oxidation products (i.e., 2-pentadecanone and tetradecanal; Fig. S20 online). In a gram-scale preparation, 1 g of palmitic acid was converted into the five products at low catalysts loading (0.02 mol% biocatalyst and 1.5 mg/mL COF-TfpBpy), and 0.46 g of the desired product 1-pentadecene was readily isolated by saponification and extraction to > 90% purity (as calculated by GC and NMR analyses, Fig. 2l, Figs. S21–S25 online). To the best of our knowledge, this represents the most concise and efficient pathway for the synthesis of α-olefin from natural fatty acid.

In this study, we designed and built a photoenzymatic cascade that only requires two catalysts, water, air and light to achieve efficient visible-light-driven oxidation of a range of fatty acids (C_{12}-C_{20}). This "green" process gave a promising TON > 15,000 for two tested P450 peroxygenases and achieved gram scale production of α-olefins. This represents the most efficient H_2O_2-supplying system to support the catalytic activity of P450 peroxygenases so far. Considering the simplicity, productivity and reusability of the photocatalyst COF-TfpBpy and scalability of the established cascade, we envision a more catalytically efficient, environmentally friendly, sustainable cascade reaction will be available upon more optimization and development for large-scale bioproduction of 1-alkenes or hydroxyl fatty acids from abundant fatty acid substrates. Furthermore, COFs have been known as emerging host platforms for enzyme immobilization owing to their unique features in terms of porous and adjustable structures [15]. Thus, we will design a COF material with functional and structural compatibility to realize both H_2O_2 photosynthesis and enzyme immobilization in the future. It is convincing that the present study will pave the way for the following development of more practical photo-biocatalytic cascade reactions.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Author contributions

Yuanyuan Jiang, Biaobiao Zhang and Shengying Li conceived the concept and designed the experiments. Yuanyuan Jiang, Peifeng Li and Zhong Li performed the experiments and analyzed the data. Yuanyuan Jiang and Peifeng Li wrote the original draft. Shengying Li and Biaobiao Zhang reviewed and edited the manuscript. All authors discussed the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2024.04.069.

References

[1] Biermann U, Bornscheuer UT, Feussner I, et al. Fatty acids and their derivatives as renewable platform molecules for the chemical industry. Angew Chem Int Ed 2021;60:20144–65.
[2] Zhou C, Zhang T. Photocatalytic alkane production from fatty acid decarboxylation overhydrogenated catalyst. Sci Bull 2020;65:870–1.
[3] Liu K, Li S. Biosynthesis of fatty acid-derived hydrocarbons: Perspectives on enzymology and enzyme engineering. Curr Opin Biotech 2020;62:7–14.
[4] Munro AW, McLean KJ, Grant JL, et al. Structure and function of the cytochrome P450 peroxygenase enzymes. Biochem Soc T 2018;46:183–96.
[5] Wang S, Jiang S, Chen H, et al. Directed evolution of a hydroxylase into a decarboxylase for synthesis of 1-alkenes from fatty acids. ACS Catal 2020;10:14375–9.
[6] Schildknecht H, Koob K. Myrmicacin, the first insect herbicide. Angew Chem Int Ed 1971;10:124–5.
[7] Sjogren J, Magnusson J, Broberg A, et al. Antifungal 3-hydroxy fatty acids from *Lactobacillus plantarum* MiLAB 14. Appl Environ Microb 2003;69:7554–7.
[8] van Schie MMCH, Zhang W, Tieves F, et al. Cascading g-C_3N_4 and peroxygenases for selective oxyfunctionalization reactions. ACS Catal 2019;9:7409–17.
[9] Dennig A, Kuhn M, Tassoti S, et al. Oxidative decarboxylation of short-chain fatty acids to 1-alkenes. Angew Chem Int Ed 2015;54:8819–22.
[10] Zhang L, Manley OM, Ma D, et al. Enhanced P450 fatty acid decarboxylase catalysis by glucose oxidase coupling and co-assembly for biofuel generation. Bioresource Technol 2020;311:123538.
[11] Zachos I, Gassmeyer SK, Bauer D, et al. Photobiocatalytic decarboxylation for olefin synthesis. Chem Commun 2015;51:1918–21.
[12] Jiang Y, Peng W, Li Z, et al. Unexpected reactions of α, β-unsaturated fatty acids provide insight into the mechanisms of CYP152 peroxygenases. Angew Chem Int Ed 2021;60:24694–701.
[13] Yong Z, Ma T. Solar-to-H_2O_2 catalyzed by covalent organic frameworks. Angew Chem Int Ed 2023;135:e202308980.
[14] Kou M, Wang Y, Xu Y, et al. Molecularly engineered covalent organic frameworks for hydrogen peroxide photosynthesis. Angew Chem Int Ed 2022;61:e202200413.
[15] Gan J, Bagheri AR, Aramesh N, et al. Covalent organic frameworks as emerging host platforms for enzyme immobilization and robust biocatalysis-A review. Int J Biol Macromol 2021;167:502–15.

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