# Development of a High-Pressure Reactor Based on Liquid-Flow Pressurisation to Facilitate Enzymatic Hydroxylation of Gaseous Alkanes

Shinya Ariyasu,[+] Yusaku Kodama,[+] Chie Kasai, Zhiqi Cong, Joshua Kyle Stanfield, Yuichiro Aiba, Yoshihito Watanabe,* and Osami Shoji*

**Abstract:** A new type of high-pressure reactor based on liquid-flow pressurisation using a HPLC pump has been developed. This high-pressure reactor allows the easy and safe performance of reactions with gaseous alkanes under high-pressures up to 10 MPa (100 atm), without the need for high-pressure gas cylinders. The amount of substrate gas required for a single reaction is very small compared with reactions using a conventional autoclave, which, when using expensive substrate gasses, such as $^{13}$C-labelled ethane, becomes critical. Employing this high-pressure reactor in conjunction with cytochrome P450BM3 and the assistance of decoy molecules, the direct hydroxylation of gaseous alkanes was drastically improved. At 5 MPa the TOF of propane hydroxylation increased 10-fold, reaching 2200 min$^{-1}$. Hydroxylation of ethane was also substantially accelerated at 5 MPa, reaching a TOF of 28 min$^{-1}$.

Methane and ethane are the main components of natural gas and represent promising starting materials for industrial chemistry.[1] The catalytic hydroxylation of gaseous alkanes still remains a challenging task in the expanding field of catalysis, due to inertness of their C-H bonds.[2-6] In addition, the C-H bonds of the oxidized products are significantly more reactive than those of the initial substrates, resulting in overoxidation to give undesired side-products, such as $CO_2$. Harsh reaction conditions are required for the activation of gaseous alkanes, making it especially difficult to avoid overoxidation, whilst also maintaining high catalytic activity. In contrast, monooxygenases found in nature, such as cytochrome P450s, catalyse a variety of oxyfunctionalisations of C-H bonds, including non-activated C-H bonds under ambient conditions, and have thus been regarded as promising candidates as biocatalysts for the direct hydroxylation of alkanes.[7,8] Although cytochrome P450BM3 (P450BM3) isolated from *Bacillus megaterium* is a highly specific long-alkyl-chain fatty acid hydroxylase (Figure 1a),[9-11] we have previously achieved the hydroxylation of non-native substrates, by utilising perfluorocarboxylic acids (PFCs) and their derivatives (Figure 1c) possessing shortened alkyl chains as "decoy molecules". Decoy molecules are conveniently misrecognised as substrates by P450BM3 and trick P450BM3 into forming the active species, namely "compound I" (Figure 1b).[12-14] Decoy molecules themselves are not-easily oxidised and only support the generation of the active species. Non-native substrates bound to the active site of P450BM3 together with decoy molecules can react with compound I to afford hydroxylated products otherwise not obtained. Recently, the decoy molecule approach has been applied to other enzyme systems to facilitate the biotransformation of non-native substrates.[15,16] Previously, we successfully hydroxylated propane by P450BM3 in the presence of 2$^{nd}$ generation decoys ,which are amino acid-functionalized PFCs (Figure 1c).[17]

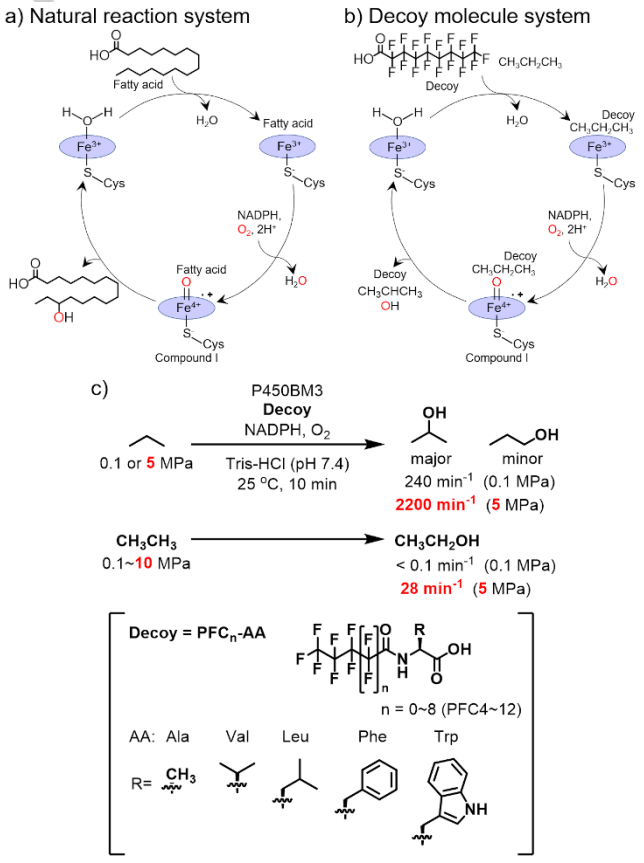



**Figure 1.** Reaction mechanisms of (a) the natural reaction system and (b) the decoy molecule system of P450BM3. (c) Hydroxylation of propane and ethane by P450BM3 with decoy molecules and chemical structures of decoy molecules employed in this work.

[*] Dr. S. Ariyasu,[+] Y. Kodama,[+] C. Kasai, Z. Cong, J. K. Stanfield, Dr. Y. Aiba, Prof. Dr. O. Shoji
Department of Chemistry, Graduate School of Science
Nagoya University
Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8602 (Japan)
E-mail: shoji.osami@a.mbox.nagoya-u.ac.jp
Prof. Dr. O. Shoji
Core Research for Evolutional Science and Technology (Japan)
Science and Technology Agency
5 Sanbancho, Chiyoda-ku, Tokyo, 102-0075 (Japan)
Prof. Dr. Y. Watanabe
Research Center for Material Science
Nagoya University
Furo-cho, Chikusa-ku, Nagoya, Aichi, 464-8602 (Japan)
E-mail: p47298a@nucc.cc.nagoya-u.ac.jp
[+] These authors contributed equally to this work.
Supporting information for this article can be found under: https://doi. Org./10.1002/cctc.XXXXX.

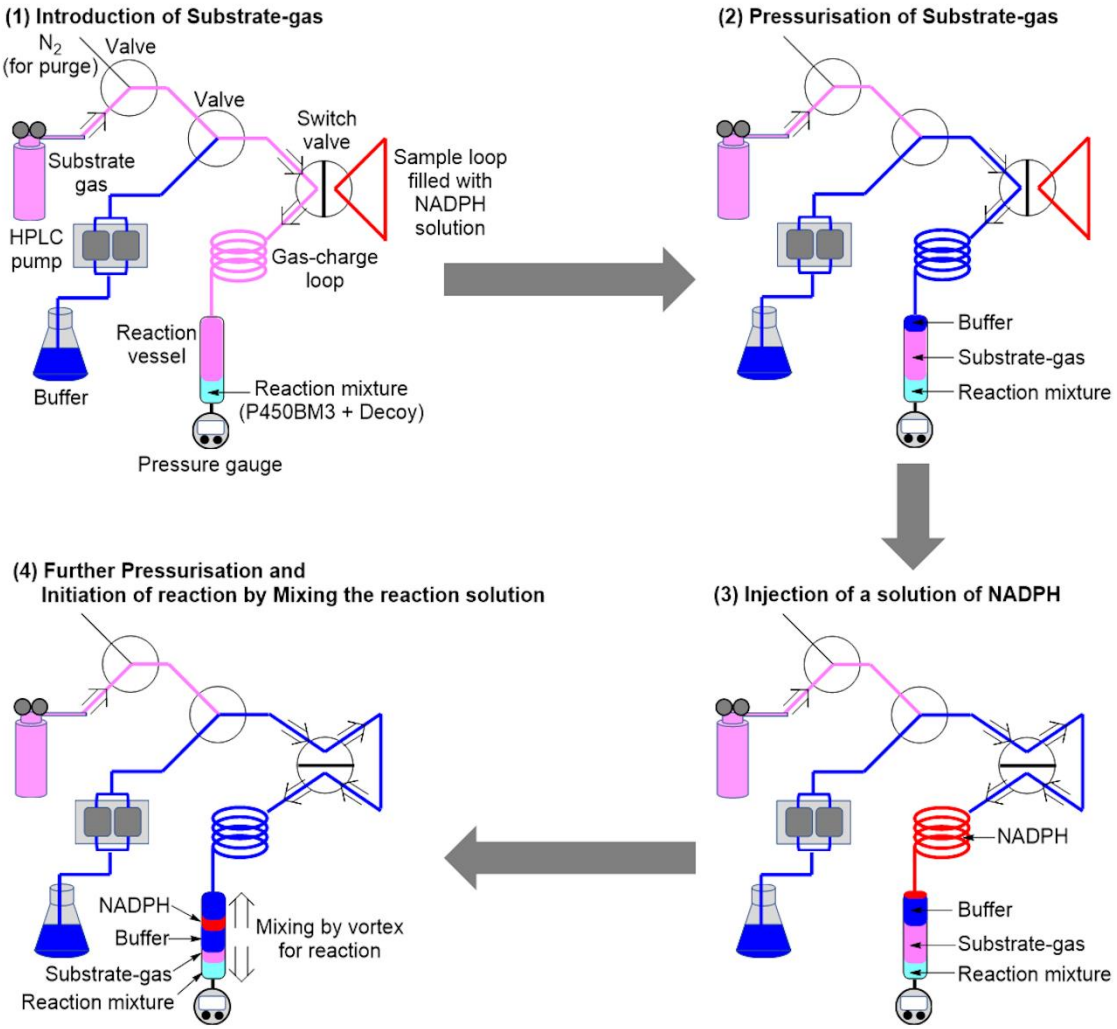



**Figure 2.** Schematic images of the HPLC-based high-pressure reactor. Substrate gas, buffer for pressurisation, NADPH solution and reaction mixture containing P450BM3 and decoy molecules are shown in pink, blue, red and light blue, respectively.

However, their turnover frequencies (TOFs) are not as high as for natural substrate hydroxylation, and the coupling efficiencies (the ratio of product against consumed NADPH) did not exceed 40%, indicating that 60% of NADPH was not used for the formation of alcohol. A plausible explanation for the observed low coupling efficiencies may be due to a low binding affinity of small gaseous alkanes for the active site of P450BM3. Consequently, compound I would be reduced to water before a molecule of gas can bind productively to the active site for hydroxylation.

As hydroxylation of gaseous alkanes and uncoupling seemingly compete, the coupling efficiency is expected to improve by enriching the solvent with gaseous alkanes to compensate for their low binding affinity in the active site of the P450BM3–decoy molecule binary complex. Enhanced binding of gaseous alkanes to P450BM3 is also expected to facilitate generation of compound I with the support of decoy molecules, resulting in acceleration of hydroxylation of gaseous alkanes.

Although decreasing reaction temperature is one of the most conventional approach to increase the concentration of gaseous substrate in reaction system, natural enzymes frequently lost their catalytic abilities under such condition. Thus, pressurization of gaseous substrates should be promising strategy to enrich them. In fact, we have previously reported the hydroxylation of propane and ethane at an elevated pressure of 0.5 MPa, using a conventional autoclave-based high-pressure reactor, and succeeded in improving the total turnover number (TON) of both gasses. Nevertheless, the TON of ethane hydroxylation was estimated to be only 0.67.[13] One possible reason for the apparently low oxidation efficiency is the suspected low concentration of ethane in the reaction mixture discussed earlier, suggesting that further pressurization is required.

Herein we report the development of a new type of high-pressure reactor based on liquid-flow pressurisation, employing a high-performance liquid chromatography (HPLC) pump (Figure 2), which enables us to accomplish the hydroxylation of gaseous alkanes at high pressures up to 10 MPa (100 atm). We have succeeded in improving the TOF and NADPH-coupling efficiency of both propane and ethane hydroxylation reactions by using this high-pressure reactor.

In the case of conventional high-pressure reactors (autoclave), pressure needs to be applied using gaseous alkanes from a high-pressure gas cylinder. However, most facilities generally do not authorise the application of highly pressurised flammable gasses as they present a safety hazard if not handled appropriately. Moreover, technical limitations regarding the addition of compounds after pressurisation of the reaction vessel have needlessly increased the complexity of high-pressure reactions. In the case of P450BM3, reactions are initiated upon the addition of an NADPH solution, which is preferentially done following pressurisation of the reaction vessel. To overcome aforementioned technical limitations, we have developed a system where pressure is applied by flowing of a buffer solution using a HPLC pump as shown in Figure 2. This equipment consists of a HPLC pump, a reaction vessel (an empty HPLC column), a loop for gas charge, a sample loop for injection of the NADPH solution, and three valves. We envisioned that gaseous alkanes can be transferred into and confined in the reaction vessel, whilst pressure can be controlled and increased by the continuous flow of a buffer solution (Figure 2 (1) and (2)). Furthermore, use of a sample injection loop enables the quick and easy addition of an aqueous NADPH solution even after pressurisation (Figure 2 (3)).

After loading the reaction vessel (0.7 mL) and gas charge loop (1 mL) with gaseous alkane (Figure 2 (1)), the substrate gas in the gas charge-loop can be transferred to the reaction vessel by solvent flow, resulting in the build-up of pressure in the reaction vessel (Figure 2(2)). The amount of substrate gas transferred to the reaction vessel can be determined from the length of the gas charge-loop (Figure 2 (1)). For example, for reactions at 10 MPa of ethane or propane, a maximum of 0.2 mmol of gaseous alkane can be confined into the reaction vessel, when a 0.7 mL reaction vessel and 1 mL gas charge-loop were employed. The pressure of the reaction vessel can be monitored both directly with a pressure gauge equipped to the reaction vessel and indirectly by the back pressure-monitoring system of the HPLC pump (Figure S1). By using a switch-valve commonly used with HPLC for sample-injection, a solution of NADPH, which initiates the reaction, can be injected into the reaction vessel after pressurisation (Figure 2 (3)). We confirmed that by flowing of the buffer solution pressures can reach more than 10 MPa and be maintained for over 12 h.

Initially, we investigated the effect of pressure on the hydroxylation of ethane by P450BM3 with either PFC9-Trp or PFC9-Phe (Figure 1c and Figure 3a). In the reactions under 0.1 (atmospheric pressure), 0.5 and 1 MPa of ethane, its pressure was controlled by conventional pressure-regulator equipped with ethane-cylinder (See Supporting Information). On the other hand, in the reaction under 5 or 10 MPa of ethane, 1 mL of ethane gas at 0.5 MPa in gas-charge loop was compressed into reaction vessel by solvent-flow to achieve desired pressures (Figure 2). After enzymatic reaction for 10 min, the reaction mixtures were directly injected to GCMS, and the concentration of ethanol generated in enzymatic reaction was calculated based on the peak areas and standard curve. Although under atmospheric pressure, detectable quantities of ethanol were not observed, relatively large amounts of ethanol were detected after 10 min under pressurised condition, resulting in a significant improvement of TOF values, and TOF values were increased as increasing ethane-pressure. At 5 MPa, the hydroxylation of ethane reached its maximum with a TOF of 28 $min^{-1}$ $P450BM3^{-1}$, and no further improvement could be detected at 10 MPa, most likely due to saturation of ethane in the aqueous media. It has been reported that saturation of ethane in aqueous solutions is reached at approximately 4 MPa at room temperature.[18] Based on the trend of TOF values in Figure 3a, there are threshold of pressure for ethane-hydroxylation in our enzymatic reaction. It might be corresponded to $K_d$ value of ethane against P450BM3 in the presence of decoy molecules. Spectroscopic analyses for evaluation of affinity of ethane under high pressure condition are ongoing task in our group. A possible hypothesis that structural perturbations of P450BM3 induced by high-pressures may be affecting the system can be discarded, as the catalytic activity of benzene-hydroxylation was not significantly affected by nitrogen gas at 10 MPa (Figure S4). Thus, the improvement in catalytic efficiency of ethane hydroxylation shown in Figure 3a can be attributed entirely to an increase in concentration of ethane in the reaction system. We would like to point out that reproducibility using this reaction system was superior to conventional high-pressure reactors in previous reports, and improved standard deviations of TOFs were obtained. This enhanced reproducibility of measurements may be due to the possibility to initiate the reaction directly after pressurisation. Furthermore, because the enzymatic reaction can be initiated after pressurisation by adding an aqueous solution of NADPH, uncoupling can be suppressed and coupling efficiencies improved. Indeed, when an aqueous solution of NADPH was added to the reaction vessel before pressurisation, 20~40% lower coupling efficiencies and 50~60% lower TOFs were observed, compared with the addition of NADPH after pressurisation. (Figure S5).

To confirm that ethanol originated from the enzymatic hydroxylation of ethane, $^{13}$C-labelled ethane gas was used as substrate (Figure 3b). $^{13}C_2H_5OH$ was only detected when $^{13}$C-labelled ethane was used, indicating that ethanol originated from the enzymatic hydroxylation of ethane and not from other impurities. It is worthwhile to address here that the amount of gaseous alkane required for a single reaction is very low when compared with reactions using a conventional autoclave. This economical use of gas becomes especially important in reactions where expensive $^{13}$C-labelled alkane gasses, such as $^{13}$C-labelled ethane and methane, are employed.

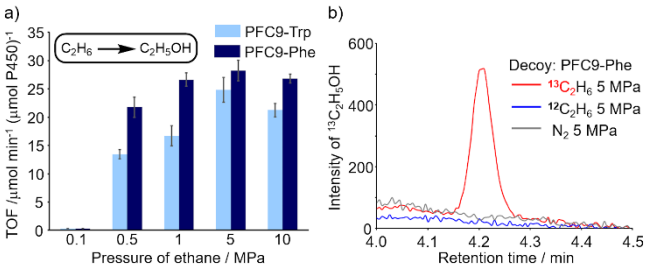



**Figure 3.** Hydroxylation of ethane by P450BM3 under high pressure condition. a) Hydroxylation of ethane by P450BM3 with PFC9-Trp or PFC9-Phe under different pressure of ethane. TOF values of ethane hydroxylation by P450BM3 with PFC9-Trp or PFC9-Phe are shown as light blue bars or dark blue bars, respectively. b) GCMS charts of hydroxylation of $^{13}$C-labelled ethane by P450BM3 with PFC9-Phe at 5 MPa. GCMS signal at Mw. 48 was assigned to $^{13}C_2H_5OH$ in the enzymatic reactions at 5 MPa of $^{13}C_2H_6$. $^{13}C_2H_6$, $^{12}C_2H_6$, and $N_2$ are shown in red, blue, and black, respectively. Standard conditions: Gas ($^{13}C_2H_6$, $^{12}C_2H_6$ or $N_2$)-saturated 20 mM Tris-HCl buffer (100 mM KCl, pH = 7.4), P450BM3 (0.2 μM), decoy molecules (20 μM), NADPH (5 mM), Gas ($^{13}C_2H_6$, $^{12}C_2H_6$ or $N_2$)-pressure (0.1, 0.5, 1, 5 or 10 MPa), room temperature, 10 min.

The effect of decoy molecules on ethane hydroxylation was examined at 5 MPa (Table 1). Amongst a series of PFCn-Trp (n = 4, 5, 6, 7, 8, 9, 10 or 12) examined, PFC9-Trp displayed the highest NADPH-consumption and TOF values, suggesting that the alkyl chain length of PFC9-Trp is particularly suited for stimulation of compound I formation in the presence of ethane. Further screening of the amino acid side chain revealed PFC9-Phe to induce the highest catalytic activity. The TOF and coupling efficiency reached 28.2 $min^{-1}$ P450BM3$^{-1}$ (TON = 282) and 1.4%, respectively. Decoy molecules with hydrophobic and aromatic amino acid side chains, such as phenylalanine and tryptophan, displayed a tendency to yield better catalytic activities for ethane hydroxylation. We reasoned that this was due to comparatively high binding affinities exhibited by PFC9-Phe and PFC9-Trp ($K_d$ of PFC9-Phe and PFC9-Trp were estimated at 3.4 μM and 1.6 μM, respectively) toward P450BM3, compared with other 2nd generation decoys such as PFC9-Ala ($K_d$ = 26 μM) and PFC9-Leu ($K_d$ = 39 μM).[17]

**Table 1.** Hydroxylation of ethane by P450BM3 with 2nd generation decoys at 5 MPa of ethane.[a]

| Decoy | TOF [$min^{-1}$] | NADPH Consumption [mM] | Coupling Efficiency [%][b] |
|---|---|---|---|
| No decoy | 0 | 0.55 ± 0.18 | 0 |
| PFC4-Trp | 2.5 ± 1.2 | 0.22 ± 0.25 | 2.4 ± 1.0 |
| PFC5-Trp | 4.7 ± 0.8 | 0.09 ± 0.10 | 11.2 ± 6.2 |
| PFC6-Trp | 9.1 ± 0.2 | 0.58 ± 0.01 | 3.1 ± 0.1 |
| PFC7-Trp | 19.1 ± 1.4 | 0.68 ± 0.05 | 5.6 ± 0.3 |
| PFC8-Trp | 19.5 ± 2.8 | 2.5 ± 0.3 | 1.6 ± 0.1 |
| PFC9-Trp | 24.8 ± 2.2 | 3.4 ± 0.3 | 1.5 ± 0.1 |
| PFC10-Trp | 6.1 ± 0.6 | 1.7 ± 0.2 | 0.71 ± 0.04 |
| PFC12-Trp | 1.5 ± 0.3 | 2.0 ± 0.2 | 0.15 ± 0.01 |
| PFC9 | 5.4 ± 0.5 | 0.77 ± 0.11 | 1.5 ± 0.3 |
| PFC9-Ala | 5.5 ± 1.0 | 1.2 ± 0.2 | 0.93 ± 0.04 |
| PFC9-Val | 11.8 ± 0.8 | 1.9 ± 0.1 | 1.2 ± 0.1 |
| PFC9-Leu | 13.3 ± 0.8 | 1.8 ± 0.2 | 1.5 ± 0.1 |
| PFC9-Phe | 28.2 ± 1.8 | 4.0 ± 0.2 | 1.4 ± 0.1 |

[a] Standard conditions: ethane-saturated 20 mM Tris-HCl buffer (100 mM KCl, pH = 7.4), P450BM3 (0.2 μM), Decoy molecules (20 μM), NADPH (5 mM), ethane-pressure (5 MPa), room temperature, 10 min. [b] Coupling efficiency = [product]/[NADPH consumption]×100

Propane hydroxylation was also drastically improved under high-pressure conditions (Table S1 and Figure S6). The TOF value for a 10 min reaction at 5 MPa reached 500 $min^{-1}$ P450BM3$^{-1}$ when PFC9-Phe was used as a decoy. We noticed that NADPH (5 mM) was completely consumed within 10 min (Figure 4), likely skewing the results. Thus the reaction time was shortened, but even within 3 min NADPH was completely consumed. Accordingly, the TOF was estimated for 1 min reactions. Remeasuring the value of PFC9-Phe for a 1 min reaction gave a TOF of 2200 $min^{-1}$ P450BM3$^{-1}$, which represents the highest TOF for all monooxygenases reported so far.[19] Furthermore, the coupling efficiency also improved to 50%.

Finally, we attempted methane oxidation by P450BM3 with PFC9-Phe and PFC9-Trp at 5 and 10 MPa of methane, but methanol, or any other oxidation products, could not be detected. The C-H bond-dissociation energy of methane (104.9 kcal $mol^{-1}$) is considerably higher than that of ethane (101.1 kcal $mol^{-1}$), which was expected to cause a decrease in activity. Even for propane and ethane hydroxylation reactions at high pressures, NADPH coupling efficiencies were still lower than for native substrates, fatty acids. Considering the crystal structure of wild-type P450BM3 with PFC9-Trp,[17] the space around the haem prosthetic group seems rather large and therefore unsuitable for methane binding. It is expected, that a reduction of the cavity size to more appropriately accommodate methane by introducing point mutations in amino acid residues lining the haem-binding pocket of P450BM3 will facilitate the suitable incorporation of methane molecules in the active site, resulting in more efficient methane hydroxylation.

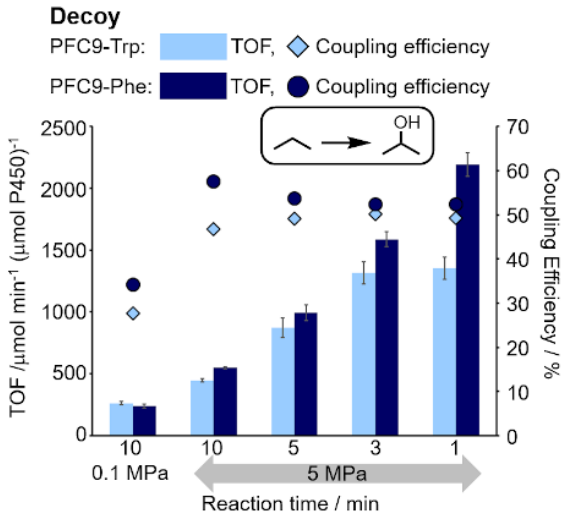



**Figure 4.** Hydroxylation of propane by P450BM3 with 2nd generation decoys at 5 MPa of propane. TOF values of propane hydroxylation by P450BM3 with PFC9-Trp or PFC9-Phe are shown as light blue bars or dark blue bars, respectively. The coupling efficiencies of PFC9-Trp and PFC9-Phe are shown as light blue squares and dark blue circles, respectively. Standard conditions: propane-saturated 20 mM Tris-HCl buffer (100 mM KCl, pH = 7.4), P450BM3 (0.5 μM), decoy molecules (100 μM), NADPH (5 mM), propane-pressure (0.1 or 5 MPa), room temperature. Coupling efficiency = [product]/[NADPH consumption]×100

In conclusion, we have developed a HPLC-based high-pressure reactor wherewith reactions under high gaseous alkane pressure (maximum 10 MPa) can be safely and easily performed. We have demonstrated that the TOF and NADPH-coupling efficiency for the hydroxylation of propane and ethane were significantly improved compared to reactions under atmospheric pressure, indicating that our reaction system is a useful tool to evaluate the catalytic efficiencies of gaseous alkanes. As the high-pressure reactor reported herein can also be adapted for use with a variety of gaseous molecules, such as hydrogen, this system is expected to open the door to new chemical conversions involving gaseous molecules.

## Acknowledgements

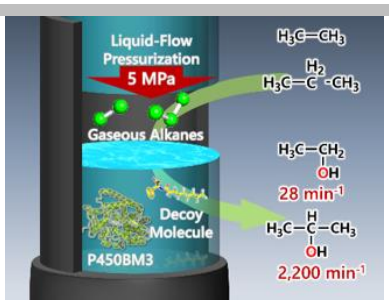
This work was supported by Grants-in-Aid for Scientific Research (S) to Y.W. (24225004) from the Ministry of Education, Culture, Sports, Science, and Technology (Japan) and JST CREST Grant Number JPMJCR15P3, Japan. And this work was supported by JSPS KAKENHI Grant Number JP15H05806 in Precisely Designed Catalysts with Customized Scaffolding to O.S. This work was also supported by Iwadare Scholarship from Iwadare Scholarship Foundation to Y.K.

**Keywords:** high pressure • cytochrome P450 • hydroxylation • ethane • propane

[1] E. McFarland, *Science* **2012**, *338*, 340-342.
[2] S. I. Chan, Y. J. Lu, P. Nagababu, S. Maji, M. C. Hung, M. M. Lee, I. J. Hsu, P. D. Minh, J. C. Lai, K. Y. Ng, S. Ramalingam, S. S. Yu, M. K. Chan, *Angew. Chem. Int. Ed.* **2013**, *52*, 3731-3735.
[3] K. T. Smith, S. Berritt, M. González-Moreiras, S. Ahn, M. R. Smith. III, M.-H. Baik, D. J. Mindiola, *Science* **2013**, *351*, 1424-1427.
[4] S. Grundner, M. A. Markovits, G. Li, M. Tromp, E. A. Pidko, E. J. Hensen, A. Jentys, M. Sanchez-Sanchez, J. A. Lercher, *Nat. Commun.* **2015**, *6*, 7546.
[5] R. A. Periana, D. J. Taube, S. Gamble, H. Taube, T. Satoh, H. Fujii, *Science* **1998**, *280*, 560-564.
[6] C. Hammond, M. M. Forde, M. H. Ab Rahim, A. Thetford, Q. He, R. L. Jenkins, N. Dimitratos, J. A. Lopez-Sanchez, N. F. Dummer, D. M. Murphy, A. F. Carley, S. H. Taylor, D. J. Willock, E. E. Stangland, J. Kang, H. Hagen, C. J. Kiely, G. J. Hutchings, *Angew. Chem. Int. Ed.* **2012**, *51*, 5129-5133.
[7] J. Rittle, M. T. Green, *Science* **2010**, *330*, 933-937.
[8] T. H. Yosca, J. Rittle, C. M. Krest, E. L. Onderko, A. Silakov, J. C. Calixto, R. K. Behan, M. T. Green, *Science* **2013**, *342*, 825-829.
[9] S. S. Boddupalli, R. W. Estabrook, J. A. Peterson, *J. Biol. Chem.* **1990**, *265*, 4233-4239.
[10] A. W. Munro, D. G. Leys, K. J. McLean, K. R. Marshall, T. W. B. Ost, S. Daff, C. S. Miles, S. K. Chapman, D. A. Lysek, C. C. Moser, C. C. Page, P. L. Dutton, *Trends Biochem. Sci.* **2002**, *27*, 250-257.
[11] C. J. Whitehouse, S. G. Bell, L. L. Wong, *Chem. Soc. Rev.* **2012**, *41*, 1218-1260.
[12] N. Kawakami, O. Shoji, Y. Watanabe, *Angew. Chem. Int. Ed.* **2011**, *50*, 5315-5318.
[13] N. Kawakami, O. Shoji, Y. Watanabe, *Chem. Sci.* **2013**, *4*, 2344-2348.
[14] O. Shoji, T. Kunimatsu, N. Kawakami, Y. Watanabe, *Angew. Chem. Int. Ed.* **2013**, *52*, 6606-6610.
[15] W. Zhang, M. Ma, M. M. E. Huijbers, G. A. Filonenko, E. A. Pidko, M. van Schie, S. de Boer, B. O. Burek, J. Z. Bloh, W. J. H. van Berkel, W. A. Smith, F. Hollmann, *J. Am. Chem. Soc.* **2019**, *141*, 3116-3120.
[16] R. M. Demming, S. C. Hammer, B. M. Nestl, S. Gergel, S. Fademrecht, J. Pleiss, B. Hauer, *Angew. Chem. Int. Ed.* **2019**, *58*, 173-177.
[17] Z. Cong, O. Shoji, C. Kasai, N. Kawakami, H. Sugimoto, Y. Shiro, Y. Watanabe, *ACS Catal.* **2014**, *5*, 150-156.
[18] A. Chapoy in *Phase behaviour in water/hydrocarbon mixtures involved in gas production systems. Engineering Sciences*. École Nationale Supérieure des Mines de Paris, **2004**, pp. 175-176.
[19] R. Fasan, M. M. Chen, N. C. Crook, F. H. Arnold, *Angew. Chem. Int. Ed.* **2007**, *46*, 8414-8418.

# COMMUNICATION

A novel high-pressure reactor based on liquid-flow pressurisation using HPLC pump has been developed. Direct hydroxylation of gaseous alkanes by cytochrome P450BM3 with assistance of decoy molecules was drastically improved using this high-pressure reactor. The TOF of propane-hydroxylation was reached 2200 /min under 5 MPa of propane. Ethane hydroxylation was also greatly accelerated and the TOF reached 28 /min.




*Dr. Shinya Ariyasu, Yusaku Kodama, Chie Kasai, Dr. Zhiqi Cong, Joshua Kyle Stanfield, Dr. Yuichiro Aiba, Prof. Dr. Yoshihito Watanabe,* Prof. Dr. Osami Shoji**

***Page No. – Page No.***

**Development of High-Pressure Reactor Based on Liquid-Flow Pressurisation to Facilitate Enzymatic Hydroxylation of Gaseous Alkanes**