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Enhancing Enantioselectivity and Productivity of P450-Catalyzed Asymmetric Sulfoxidation with an Aqueous/Ionic Liquid Biphasic System

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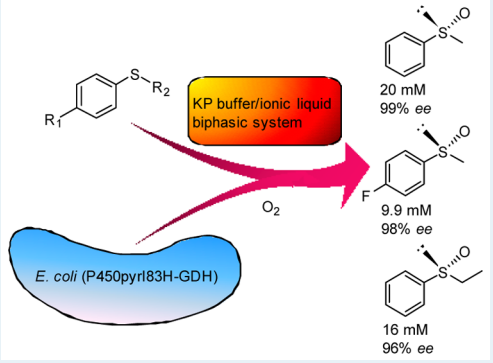
Supporting Information

ABSTRACT: Biocatalytic asymmetric sulfoxidation represents a green method to prepare the useful and valuable enantiopure sulfoxides, but this method sometimes suffers from unsatisfied enantioselectivity and low productivity due to substrate and product inhibitions. Here we developed an aqueous/ionic liquid (IL) biphasic system for simultaneously enhancing the enantioselectivity and productivity of P450 monooxygenase-catalyzed asymmetric sulfoxidations of sulfides **1**, **3**, **5**, **7**, and **9**, as the first example of this kind for a biooxidation. *Escherichia coli* (P450pyrI83H-GDH) coexpressing P450pyrI83H monooxygenase and glucose dehydrogenase was engineered for the asymmetric sulfoxidations with cofactor recycling, giving higher *R*-enantioselectivity than any other known P450 monooxygenases and showing high specific activities. The inhibition to the reactions and the toxicity to the cells of the substrates and products were investigated and mostly avoided by using a KP buffer/$[P_{6,6,6,14}][NTf_2]$ biphasic reaction system, in which the IL showed excellent biocompatibility to the cells and high solubility to the substrates and products. Sulfoxidations of **1**, **3**, **5**, **7**, and **9** with the resting *E. coli* cells in the biphasic system increased the product concentration from 9.4 to 20 mM for (*R*)-phenyl methyl sulfoxide **2**, from 1.9 to 9.9 mM for (*R*)-4-fluorophenyl methyl sulfoxide **4**, from 5.4 to 16 mM for (*R*)-ethyl phenyl sulfoxide **6**, from 4.2 to 22 mM for (*R*)-methyl *p*-tolyl sulfoxide **8**, and from 5.7 to 24 mM for (*R*)-methyl *p*-methoxyphenyl sulfoxide **10**, respectively, and improved the product ee from 85 to 99% for (*R*)-**2**, from 80 to 98% for (*R*)-**4**, from 88 to 96% for (*R*)-**6**, from 35 to 62% for (*R*)-**8**, and from 53 to 67% for (*R*)-**10**, respectively. The enhancements in enantioselectivity are possibly caused by the low substrate concentrations in the aqueous phase of the biphasic system. Preparative sulfoxidations to produce the useful and valuable sulfoxides (*R*)-**2**, (*R*)-**4**, and (*R*)-**6** in 99%, 98%, and 96% ee, respectively, were demonstrated.



KEYWORDS: *P450 monooxygease, sulfoxidation, enantioselectivity, productivity, ionic liquid, biphasic system*

INTRODUCTION

Biocatalysis is a useful and green tool to produce enantiopure compounds for pharmaceutical manufacture.[1−5] Despite the great achievements in biocatalysis, many biotransformations such as some asymmetric oxidations suffer from unsatisfied enantioselectivity and/or low productivity due to the inhibition of substrate and/or product.[6−9] Directed evolution has been proven as a useful method[10−15] to create new enzymes with enhanced selectivity,[16] productivity,[17,18] and tolerance.[19] However, such an approach is still labor-intensive, time-consuming, and costly. On the other hand, solvent engineering using a two-liquid-phase system represents a simple way to enhance the productivity by dissolving substrate and/or product in an organic solvent and thus reducing the inhibition on enzyme in aqueous phase.[6,7] Several organic solvents with high log P values showing less toxicity to the biocatalysts are widely used in the two-liquid-phase system. However, they also suffer from low solubility of many types of substrates and products, and they are not environmentally benign. Recently, ionic liquids (ILs) have received increasing attention as greener and promising solvents for multiphase biocatalysis,[6,20−24] providing with less toxicity to the biocatalysts and high solubility. Thus far, ILs have been used as cosolvents for several types of reactions to enhance the productivity.[6,21,23−26] It is our great interest to explore aqueous/IL biphasic systems,

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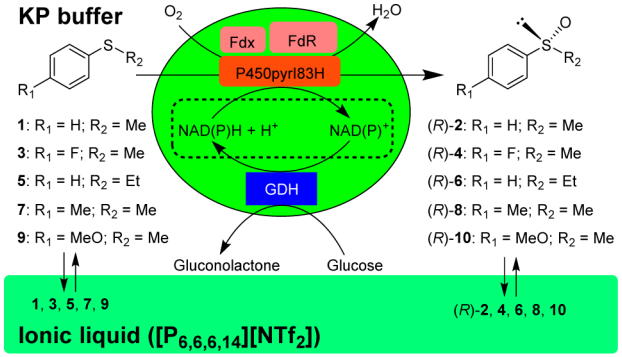
as the first example, for simultaneously enhancing the enantioselectivity and productivity for a biooxidation.

Biocatalytic asymmetric sulfoxidation was selected for such investigation, because it could produce chiral sulfoxides that are important auxiliaries or synthetic intermediates[27] and it often suffers from unsatisfactory enantioselectivity and low productivity. Several sulfoxidation reactions and chiral sulfoxides were chosen as the target reactions and products, respectively (Scheme 1). (*R*)-Phenyl methyl sulfoxide **2** is an intermediate

Scheme 1. Asymmetric Sulfoxidations with *E. coli* (P450pyrI83H-GDH) in a KP Buffer/IL Biphasic System



for the synthesis of antiobesity drug Tetrahydrolipstatin;[28] (*R*)-ethyl phenyl sulfoxide **6** is useful for preparing luminescent materials;[29−31] (*R*)-methyl *p*-tolyl sulfoxide **8** is used for synthesizing glucocorticoid receptor ligands[32] and the drugs such as Compactin, Mevinolin and Provastatin;[33,34] and (*R*)-methyl *p*-methoxyphenyl sulfoxide **10** is an intermediate to produce antihypertensive agent for lowing blood pressure.[35] Cyclohexanone monooxygenase (CHMO) was reported to catalyze the sulfoxidation of thioanisole **1** to give (*R*)-**2** in 99% ee by using an in vitro oxidation system with two coupled enzymes;[27] however, no product concentration was mentioned, and the number of NADPH recycling was low. Sulfoxidations of **1** with toluene dioxygenase[36] and chloroperoxidase[37] produced (*R*)-**2** in 92% ee and 17.8 mM and in 99% ee and 9.3 mM, respectively. Sulfoxidation of other substrates **3**, **5**, **7**, and **9** with CHMO gave unsatisfied product ee [(*R*)-**4**: 92%; (*R*)-**6**: 47%; (*R*)-**8**: 37%; and (*R*)-**10**: 51%].[27] P450 monooxygenases were known for the sulfoxidations of **1**, **3**, **5**, **7**, and **9**, giving either (*S*)-enantiomers in low concentrations (≤1 mM) or (*R*)-enantiomers in low ee (11−56%).[38−42] Herein, we report a novel P450 monooxygenase-catalyzed *R*-enantioselective sulfoxidations of **1**, **3**, **5**, **7**, and **9** by using an aqueous/IL biphasic system to enhance both enantioselectivity and productivity to produce the corresponding products (*R*)-**2**, **4**, **6**, **8**, and **10**, with better performance than other known biosystems for the same reactions.

RESULTS AND DISCUSSION

Asymmetric Sulfoxidation of Thioanisole 1 with Recombinant *E. coli* Strains Coexpressing P450pyr or Its Mutant and Glucose Dehydrogenase. Recently, a novel three-component P450pyr monooxygenase from *Sphingomonas* sp. HXN-200 was discovered and engineered in our laboratory for regio- and stereoselective biohydroxylations and asymmetric epoxidations.[16,43−50] As some P450 enzymes are known to catalyze sulfoxidations,[38−42] we decided to examine the possibility of using P450pyr and several P450pyr mutants generated in our previous work for asymmetric sufoxidations. At first, the three-component monooxygenase of P450pyr and other P450pyr mutants[45] (P450pyrI83H, P450pyrM305Q, and P450pyrA77S) was coexpressed, respectively, with a glucose dehydrogenase (GDH) in *Escherichia coli*. As a representative example, *E. coli* (P450pyrI83H-GDH) was engineered by using double plasmids: pETDuet-1-P_{I83H}R carrying P450pyrI83H and ferredoxin reductase genes and pRSFDuet1-GDH-Fdx[44] carrying GDH and ferredoxin genes (Figure 1a). Biotransformation with these *E. coli* cells could give the desired oxidation with the recycling of the necessary cofactor NAD(P)H via GDH-catalyzed oxidation of glucose. The four newly engineered strains and *E. coli* (P450pyrTM-GDH)[44] were

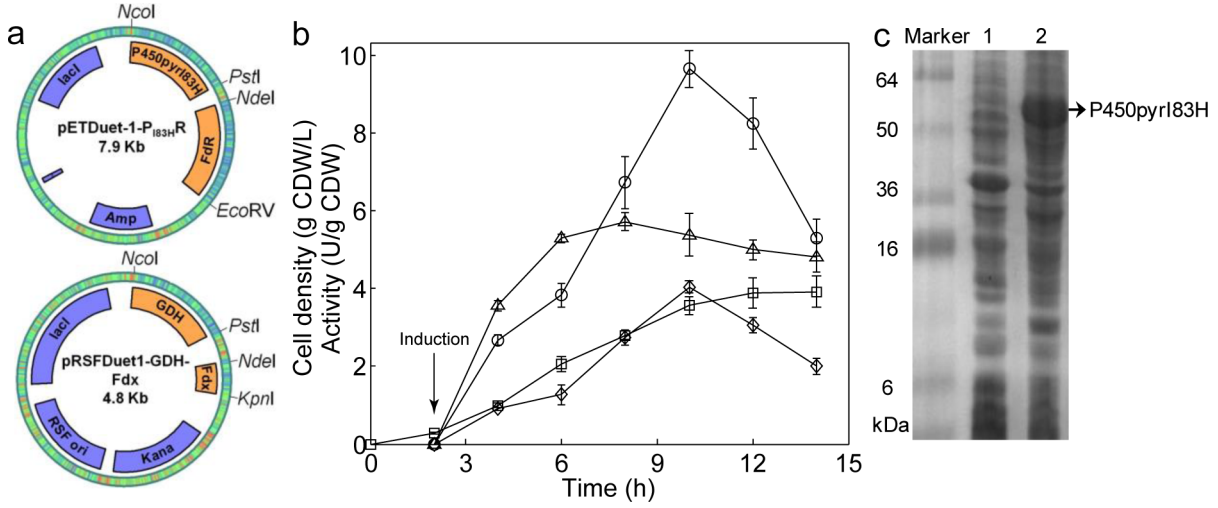


Figure 1. (a) Engineering of *E. coli* (P450pyrI83H-GDH) with double plasmids. (b) Cell growth and specific activity curves for the asymmetric sulfoxidations with *E. coli* (P450pyrI83H-GDH). Cell density (□); activity for thioanisole **1** (○), 4-fluorothioanisole **3** (◇), and ethyl phenyl sulfide **5** (Δ). Cells were cultivated at 37 °C and 250 rpm in TB medium and induced at 2 h at 25 °C by adding isopropyl *β*-D-1-thiogalactopyranoside and *δ*-aminolevulinic acid hydrochloride to the final concentrations of 0.25 mM and 0.5 mM, respectively. Data are the mean values with standard deviations of three replicates. (c) SDS-PAGE. Lane 1, cell-free extract of *E. coli* (P450pyrI83H-GDH) before induction; lane 2, cell-free extract of *E. coli* (P450pyrI83H-GDH) after 6 h induction.

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Table 1. Asymmetric Sulfoxidations with Resting Cells of *E. coli* (P450pyrI83H-GDH) in a KP Buffer/$[P_{6,6,6,14}][NTf_2]$ Biphasic System and Monophasic KP Buffer

		KP buffer[a]			KP buffer/$[P_{6,6,6,14}][NTf_2]$[b]					
sub	prod	$C_{sub,\ initial}$ (mM)	C_{prod} (mM)[c]	prod ee (%)[d]	C_{sub} initial (mM)[e]	C_{sub} buffer (mM)[f]	C_{prod} buffer (mM)[f]	C_{prod}IL (mM)[f]	C_{prod} total (mM)[g]	prod ee (%)[d]
1	(*R*)-**2**	10.0	9.38 ± 0.66	85.4 ± 0.4	50.0	0.19 ± 0.01	7.42 ± 0.37	12.1 ± 1.1	11.4 ± 0.7	99.2 ± 0.0
1	(*R*)-**2**	20.0	4.36 ± 0.37	85.5 ± 0.0	60.0	0.34 ± 0.01	12.9 ± 1.2	21.1 ± 2.1	20.0 ± 1.9	99.5 ± 0.0
1	(*R*)-**2**	30.0	4.24 ± 0.36	85.9 ± 0.2	70.0	0.34 ± 0.02	7.93 ± 0.51	13.4 ± 0.8	12.4 ± 0.7	99.5 ± 0.0
3	(*R*)-**4**	10.0	1.93 ± 0.14	80.2 ± 0.4	30.0	0.10 ± 0.01	5.83 ± 0.35	12.2 ± 0.4	9.91 ± 0.48	98.2 ± 0.5
5	(*R*)-**6**	10.0	5.44 ± 0.19	88.3 ± 0.6	40.0	0.04 ± 0.00	6.41 ± 0.23	28.4 ± 1.4	15.8 ± 0.6	96.0 ± 1.6
7	(*R*)-**8**	10.0	4.17 ± 0.12	35.2 ± 0.0	40.0	0.03 ± 0.00	9.84 ± 0.52	35.3 ± 1.3	21.6 ± 0.9	61.8 ± 0.0
9	(*R*)-**10**	10.0	5.67 ± 0.23	52.8 ± 0.1	40.0	0.04 ± 0.00	15.5 ± 0.5	26.8 ± 1.0	24.4 ± 0.8	66.9 ± 0.0

[a]Biotransformation was conducted at 30 °C and 250 rpm in 10 mL KP buffer (100 mM; pH 8.0) containing 2% (w/v) glucose at a cell density of 10 g CDW/L for 24 h; product formation was determined by HPLC. [b]Biotransformation was conducted in KP buffer (10 mL; 100 mM; pH 8.0)/$[P_{6,6,6,14}][NTf_2]$ (3.3 mL) at 30 °C and 250 rpm for 24 h; KP buffer (10 mL) contained 2% (w/v) glucose and the *E. coli* cells (10 g CDW/L); product formation was determined by HPLC. [c]Concentrations at 24 h. [d]ee was determined by chiral HPLC. [e]Concentrations were normalized on the basis of the volume of aqueous phase. [f]Concentrations (at 24 h) without normalization. [g]Concentrations (at 24 h) were normalized on the basis of the volume of aqueous phase. Data are the mean values with standard deviations of three replicates.

Table 2. Kinetic Data of the Asymmetric Sulfoxidations of 1, 3, 5, 7, and 9 with the Resting Cells of *E. coli* (P450pyrI83H-GDH)

sulfoxidation[a]	cell density (g CDW/L)	K_m, app (mM)	V_{max}, app (μmol min^{-1} g CDW^{-1})	K_i, app[b] (mM)	V_{max}/K_m, app (μmol min^{-1} g CDW^{-1} mM^{-1})
1 to **2**	1.50	0.58 ± 0.02	27.0 ± 0.7	1.95 ± 0.07	46.5 ± 0.4
3 to **4**	1.50	0.37 ± 0.01	10.5 ± 0.1	1.18 ± 0.02	28.5 ± 0.6
5 to **6**	1.50	0.75 ± 0.00	15.4 ± 0.1	1.52 ± 0.01	20.6 ± 0.7
7 to **8**	1.50	2.23 ± 0.02	48.9 ± 0.2	3.71 ± 0.11	21.9 ± 0.1
9 to **10**	1.50	0.95 ± 0.01	41.5 ± 1.0	7.26 ± 0.38	43.5 ± 0.8

[a]The reactions were carried out at 30 °C with cell suspension (1.5 g CDW/L) in 0.5 mL KP buffer (50 mM; pH 8.0) containing 2% (w/v) glucose for 15 min; product formation was determined by HPLC; data are the mean values with standard deviations of three replicates. [b]K_i is for the sulfoxide product (*R*)-**2**, **4**, **6**, **8**, or **10** as an inhibitor of the corresponding sulfoxidation.

then used separately as resting cells (10 g CDW/L; CDW: cell dry weight) to catalyze the sulfoxidation of thioanisole **1** (10 mM) in potassium phosphate (KP) buffer at 30 °C and 250 rpm for 24 h. All these P450s are able to catalyze the asymmetric sulfoxidation with *R*-enantioselectivity. *E. coli* (P450pyrI83H-GDH), *E. coli* (P450pyrM305Q-GDH), *E. coli* (P450pyrA77S-GDH), *E. coli* (P450pyrTM-GDH),[44] and *E. coli* (P450pyr-GDH) produced (*R*)-phenyl methyl sulfoxide **2** in 85%, 62%, 83%, 63%, and 82% ee, respectively (Supporting Information). These P450s demonstrated much higher *R*-enantioselectivity for the sulfoxidation than other P450s.[38−42] Among them, *E. coli* (P450pyrI83H-GDH) gave the highest product ee, thus being selected for further study.

Asymmetric Sulfoxidation of Thioanisole 1, 4-Fluorothioanisole 3, Ethyl Phenyl Sulfide 5, Methyl *p*-Tolyl Sulfide 7, and 4-Methoxythioanisole 9 with Resting Cells of *E. coli* (P450pyrI83H-GDH) in Aqueous Buffer. Cell growth curves and specific sulfoxidation activities of *E. coli* (P450pyrI83H-GDH) are given in Figure 1b. Cells were cultivated in Terrific Broth (TB)[44] medium at 37 °C and 250 rpm, following by induction at 2 h with isopropyl β-D-1-thiogalactopyranoside and δ-aminolevulinic acid hydrochloride. The expression of the P450 enzyme was evidenced by the SDS-PAGE (Figure 1c) of the cell-free extracts of the cells taken at 8 h growth. At 8−12 h, the cells reached a density of 2.8−3.8 g CDW/L in the late exponential or early stationery growth phase and gave higher specific sulfoxidation activities (Figure 1b and Figure S2 in Supporting Information). The highest specific activities for the sulfoxidation of **1**, **3**, **5**, **7**, and **9** were 9.6, 4.0, 5.7, 11, and 16 U/g CDW, respectively.

The cells harvested after 8−10 h growth were used as resting cells (10 g CDW/L) for the asymmetric sulfoxidation of 10 mM sulfides **3**, **5**, **7**, and **9**, respectively, in a monophasic KP buffer system for 24 h. The corresponding sulfoxides (*R*)-**4**, **6**, **8**, and **10** were obtained in 80%, 88%, 35%, and 53% ee, respectively (Table 1). Accordingly, P450pyrI83H showed much higher *R*-enantioselectivity for the sulfoxidation of **3**, **5**, **7**, and **9** than other P450s.[38−42] The final concentrations of (*R*)-**4**, **6**, **8**, and **10** were 1.9, 5.4, 4.2, and 5.7 mM, respectively. These values were much lower than the expected product concentrations based on the specific activities, indicating inhibition during the biotransformations. The sulfoxidation of **1** was then performed with the resting cells under the same conditions at a substrate concentration of 10, 20, and 30 mM, respectively. (*R*)-**2** was formed in 9.4, 4.4, and 4.2 mM, respectively (Table 1). The decrease of product concentration with the increase of substrate concentration clearly suggests the substrate inhibition.

Toxicity to the *E. coli* (P450pyrI83H-GDH) Cells and Inhibition to the Sulfoxidation with the Sulfide Substrates and Sulfoxide Products. The toxicity and inhibition were further investigated by adding 20 mM sulfides (**1**, **3**, **5**, **7**, or **9**) and sulfoxides (**2**, **4**, **6**, **8**, or **10**), respectively, to the cell suspension (10 g CDW/L) of *E. coli* (P450pyrI83H-GDH) in KP buffer and incubating at 30 °C and 1000 rpm for 6 h. Only 3−5% of the cells were viable after the pretreatment with the sulfides **1**, **3**, **5**, **7**, or **9**, while 40−75% of the cells could survive the pretreatment with the sulfoxides **2**, **4**, **6**, **8**, or **10** (Figure S3 in Supporting Information). *E. coli* cells pretreated by the sulfides **1**, **3**, **5**, **7**, or **9** showed 0.5−2% specific activity for the sulfoxidation of **1**, **3**, **5**, **7**, or **9**, respectively, while the cells pretreated by the sulfoxides **2**, **4**, **6**, **8**, or **10** retained 42%−91% specific activity for the sulfoxidation of **1**, **3**, **5**, **7**, or **9**, respectively (Figure S3 in

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Supporting Information). These results clearly demonstrated that both sulfides and sulfoxides were toxic to the cells and inhibitory to the sulfoxidation, and sulfides were much more toxic and inhibitory than the corresponding sulfoxides.

The loss of cell viability could be caused by the damages to membrane integrity with the high concentrations of substrate and product. To investigate the inhibition, the apparent kinetics of the whole cell-catalyzed asymmetric sulfoxidations was measured. Only 2 mM sulfoxides were used for 15 min to determine the kinetics, thus the toxicity of sulfoxides to the cells could be negligible. Sulfoxides were found to show competitive inhibitions, with apparent K_i values of 2.0 mM for (*R*)-**2**, 1.2 mM for (*R*)-**4**, 1.5 mM for (*R*)-**6**, 3.7 mM for (*R*)-**8**, and 7.3 mM for (*R*)-**10**, respectively (Table 2). Apparent V_{max} values for the sulfoxidations of **1**, **3**, **5**, **7**, and **9** were 27, 11, 15, 49, and 41 μmol/min/g CDW, respectively, and apparent K_m values for the sulfoxidations of **1**, **3**, **5**, **7**, and **9** were 0.6, 0.4, 0.7, 2.2, and 1.0 mM, respectively.

Selection of Ionic Liquid for Establishing a KP Buffer/IL Biphasic System for Efficient Sulfoxidation. To select a suitable IL for establishing the efficient KP buffer/IL biphasic system, the biocompatibility of four hydrophobic ILs, $[P_{6,6,6,14}][NTf_2]$, $[BMIM][NTf_2]$, $[BMIM][Pf_6]$, and $[NMeOct_3][NTf_2]$ (Table S1 in Supporting Information), to the *E. coli* cells were examined on the basis of cell growth rate. ILs with better biocompatibility should have less toxic effects on the cell growth, thus giving higher growth rate, and vice versa.[6] With this criterion, *E. coli* (P450pyrI83H-GDH) cells were first cultivated in aqueous medium, followed by the addition of individual IL at 2 h to a phase ratio of 10:1 (v/v; aq/IL). As shown in Figure 2a, the biphasic system of KP buffer/$[P_{6,6,6,14}][NTf_2]$ remained the highest growth rate of 1.02 h^{-1}, similar to the cell cultivation without adding IL. The cells ceased to grow in all other three biphasic systems. These results indicated that $[P_{6,6,6,14}][NTf_2]$ is compatible with the *E. coli* cells, while the other three ILs imposed toxic effects to the cells. Although $[BMIM][Pf_6]$ was reported as cosolvent for biohydroxylation[21] and $[NMeOct_3][NTf_2]$ was reported with biocompatibility to other *E. coli* cells deprived of plasmids,[6] the hazardous effects brought by the two ILs to the cells of our *E. coli* strain containing dual plasmids carrying four different genes were observed in this work.

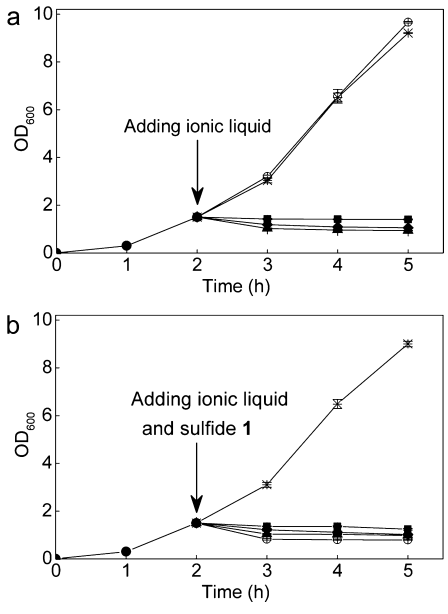

Figure 2. Growth curves of *E. coli* (P450pyrI83H-GDH) in LB medium at 37 °C and 250 rpm with the addition of ILs at 2 h to a phase ratio of 10:1 (aqueous to IL, v/v). (a) Without adding sulfide **1** at 2 h. (b) With the addition of 10 mM sulfide **1** at 2 h. ●: Before adding ILs; ○: control (without adding IL); *: KP buffer/$[P_{6,6,6,14}][NTf_2]$; ■: KP buffer/$[BMIM][NTf_2]$; ◆: KP buffer/$[BMIM][Pf_6]$; ▲: KP buffer/$[NMeOct_3][NTf_2]$. Data are the mean values with standard deviations of three replicates.

To test the capability of ILs in alleviating the toxicity of substrate during cell growth, 10 mM sulfide **1** was also added, together with the IL, at 2 h. The results in Figure 2b indicated that sulfide **1** has no toxic effect on the cell growth in KP buffer/$[P_{6,6,6,14}][NTf_2]$ biphasic system. This indicated that $[P_{6,6,6,14}][NTf_2]$ could shield much of the substrate toxicity to the cells and hence protect the cells. Remarkably, cell growth in control (i.e., in monoaqueous system) was most severely inhibited by sulfide **1** (Figure 2b). As for the other three KP buffer/IL biphasic systems, cell growths were inhibited but better than that from the control (Figure 2b).

Simple tests for the sulfoxidation of **1** (10 mM) with resting cells of *E. coli* (P450pyrI83H-GDH) in the four buffer/IL biphasic systems (phase ratio 10:1, v/v) gave (*R*)-**2** in 98% ee in KP buffer/$[P_{6,6,6,14}][NTf_2]$, much higher than the 85% ee from the control, (*R*)-**2** in 88% ee in KP buffer/$[BMIM][Pf_6]$, (*R*)-**2** in 87% ee in KP buffer/$[BMIM][NTf_2]$, and no product in KP buffer/$[NMeOct_3][NTf_2]$ (Table S2 in Supporting Information), respectively.

On the basis of these results, $[P_{6,6,6,14}][NTf_2]$ was chosen as the cosolvent in the KP buffer/IL biphasic system for efficient asymmetric sulfoxidations.

Enhancements of Productivities of Asymmetric Sulfoxidations with *E. coli* (P450pyrI83H-GDH) Cells in KP Buffer/$[P_{6,6,6,14}][NTf_2]$ Biphasic System. To perform the asymmetric sulfoxidations in the biphasic system, the cells of *E. coli* (P450pyrI83H-GDH) that were harvested at exponential phase were first resuspended in KP buffer containing 2% (w/v) glucose to a density of 10 g CDW/L. $[P_{6,6,6,14}][NTf_2]$ containing sulfides **1**, **3**, **5**, **7**, or **9**, respectively, was then added to the cell suspension to form a biphasic system with a 3:1 ratio of aqueous phase to IL to start the biotransformation. In such a biphasic system, partition coefficients ([Conc in IL]/[Conc in aq]) for sulfide **1**, **3**, **5**, **7**, and **9** were determined to be 855, 670, 1144, 364, and 324, respectively. More than 99% of the substrates **1**, **3**, **5**, **7**, and **9** remained in IL (Table S3 in Supporting Information), thus greatly reducing the substrate toxicities and inhibitions. Due to the hydrophilicity of the sulfoxides, 40−65% of the products **2**, **4**, **6**, **8**, and **10** existed in aqueous buffer, and the remaining 60−35% were in $[P_{6,6,6,14}][NTf_2]$ (Table 1). Thus, the biphasic system can only partially reduce the product toxicity and inhibition. Nevertheless, because the product is less toxic and inhibitory than the substrate, biotransformation in such a system could still significantly enhance the product concentration and thus the productivity. As shown in Table 1, sulfoxidation of **1** in the biphasic system for 24 h gave (*R*)-**2** in 20 mM, being 2-fold higher than the 9.4 mM obtained from the monophasic control. Similarly, sulfoxidations of **3**, **5**, **7**, and **9** in the biphasic system for 24 h enhanced the productivities and gave (*R*)-**4** in 9.9 mM, (*R*)-**6** in 16 mM, (*R*)-**8** in 22 mM, and (*R*)-**10** in 24 mM, respectively. The product concentrations are 3−5-fold higher than those achieved in the monophasic control (1.9−5.7 mM)

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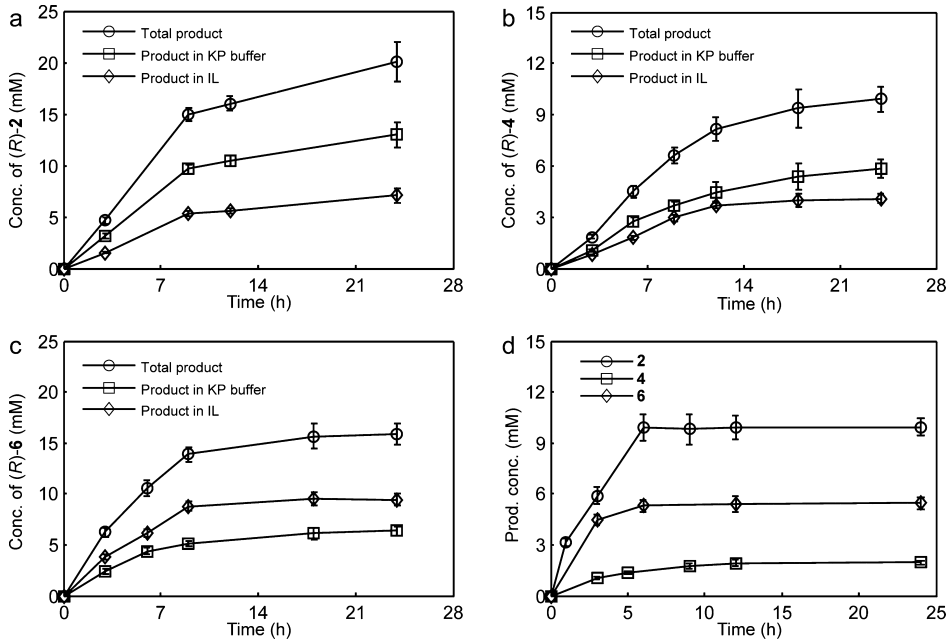

Figure 3. (a–c) Time courses of asymmetric sulfoxidations with resting cells of *E. coli* (P450pyrI83H-GDH) (10 g CDW/L) in KP buffer (100 mM, pH 8.0)/[$P_{6,6,6,14}$][NTf_2] biphasic system (3:1, v/v) containing 2% (w/v) glucose at 30 °C and 250 rpm. All concentrations were normalized on the basis of the volume of aqueous phase. (a) Sulfoxidation of **1** (60 mM). (b) Sulfoxidation of **3** (30 mM). (c) Sulfoxidation of **5** (40 mM). (d) Time courses of asymmetric sulfoxidations of 10 mM **1**, **3**, and **5** with resting cells of *E. coli* (P450pyrI83H-GDH) (10 g CDW/L) in monophasic KP buffer (100 mM, pH 8.0) containing 2% (w/v) glucose at 30 °C and 250 rpm, respectively. Data are the mean values with standard deviations of three replicates.

(Table 1). No further oxidations of the sulfoxides to the corresponding sulfons were observed at 24 h.

The reaction courses for the sulfoxidations in the biphasic system were demonstrated in Figure 3a–c. The product concentrations in aqueous buffer, IL, and total were normalized on the basis of the volume of aqueous phase and given at several selected time points. During biotransformations of sulfide **1**, **3**, and **5**, the corresponding products **2**, **4**, and **6** in aqueous phase amounted to 65–67%, 55–60%, and 37–41%, respectively, while over 99% of the substrate was maintained in IL phase. The sulfoxidations of **1**, **3**, and **5** were faster in the first 9, 12, and 9 h, respectively, and became slower afterward. The time courses of the sulfoxidations in the monophasic buffer are given in Figure 3d. In comparison, the biphasic system demonstrated a much longer period for faster reaction and gave higher product concentration at late time points than the monophasic one. The enhancement was mainly caused by the reduction of the substrate inhibition via keeping a very low substrate concentration in aqueous buffer (initial concentration of 0.15, 0.10, and 0.08 mM for sulfide **1**, **3**, and **5**, respectively) of the biphasic system. Moreover, the product inhibition was also reduced by in situ partial extraction of the product into IL during reaction. As the apparent K_i values for (*R*)-**2**, (*R*)-**4**, and (*R*)-**6** were 2.0, 1.2, and 1.5 mM, respectively, biotransformation of **1** to (*R*)-**2** showed better tolerance to higher product concentration in aqueous buffer of the biphasic system than other two biotransformations. Also a higher value of V_{max}/K_m (47 μmol min^{-1} g CDW^{-1} mM^{-1}) for the sulfoxidation of **1** contributed to the higher product concentration of (*R*)-**2**, in comparison with the sulfoxidation of **3** or **5**.

Enhancements of Enantioselectivities of Asymmetric Sulfoxidations with *E. coli* (P450pyrI83H-GDH) Cells in KP Buffer/[$P_{6,6,6,14}$][NTf_2] Biphasic System. The biphasic system was found to enhance the enantioselectivities for the asymmetric sulfoxidations. As shown in Table 1, sulfoxidation of **1**, **3**, and **5** in the biphasic system gave (*R*)-**2** in 99% ee, (*R*)-**4** in 98% ee, and (*R*)-**6** in 96% ee, respectively. These values are much higher than the corresponding product ee of 85%, 80%, and 88% from the monophasic control experiments (Table 1). Similar enhancement of chemoselectivity in biohydroxylation of camphor in aqueous–organic two-phase system was observed through the gradually supply of the substrate to aqueous phase from the organic phase.[51] The enhancements in enantioselectivity in our cases are also possibly caused by the low substrate concentrations in the aqueous phase of the biphasic system. This was further evidenced by the sulfoxidation of 1 mM sulfide **1** with the resting cells of *E. coli* (P450pyrI83H-GDH) (10 g CDW/L) in monophasic KP buffer to give (*R*)-**2** in 97% ee. Moreover, lower ratio of aqueous buffer to [$P_{6,6,6,14}$][NTf_2] (v/v) in the biphasic system tended to give higher product ee in the asymmetric sulfoxidation (Tables S3 and S4 in Supporting Information). For example, sulfoxidation of 40 mM sulfide **1** in KP buffer/[$P_{6,6,6,14}$][NTf_2] gave (*R*)-**2** in 96%, 98%, and 99% ee at phase ratios (aq to IL, v/v) of 20:1, 10:1, and 5:1, respectively. Here again, the enhancements in enantioselectivity are possibly caused by the low substrate concentrations in the aqueous phase of the biphasic system, because the sulfide concentration in the aqueous phase at the three phase ratios was 0.32, 0.19, and 0.17 mM, respectively (Tables S3 and S4 in Supporting Information). Similarly, sulfoxidation of sulfide **3** (30 mM), **5** (40 mM), **7** (40 mM), or **9** (40 mM) at a phase ratio (aq to IL, v/v) of 5:1 and 3:1 gave product (*R*)-**4** in 96% and 98% ee, (*R*)-**6** in 94% and 96% ee, (*R*)-**8** in 44% and 62% ee, or (*R*)-**10** in 54% and 67% ee, respectively (Tables S3 and S4 in Supporting Information). The change of enantioselectivity at different substrate concentration might be explained as follows: higher substrate

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concentrations might change the conformation of enzyme and hence alter the enzyme enantioselectivity.

Preparation of Sulfoxides (*R*)-2, (*R*)-4, and (*R*)-6 by Asymmetric Sulfoxidations with Resting Cells of *E. coli* (P450pyrI83H-GDH) Cells in KP Buffer/[$P_{6,6,6,14}$][NTf_2] Biphasic System. The potential of using the KP buffer/[$P_{6,6,6,14}$][NTf_2] biphasic system for asymmetric biosulfoxidations to prepare chiral sulfoxides in high ee was explored. Oxidations of sulfides **1** (60 mM), **3** (30 mM), and **5** (40 mM) were performed with resting cells of *E. coli* (P450pyrI83H-GDH) on a 50 mL-scale for 24 h, respectively. After separation of the two phases, the aqueous phase contains mainly the sulfoxides, and the IL phase contains both unreacted sulfides and the sulfoxides products. Water was added to IL to extract the sulfoxides, and the aqueous solutions were combined and then extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, the solvent was removed by evaporation, and the crude sulfoxides were purified by flash chromatography on a silica gel column. The useful and valuable sulfoxides (*R*)-**2**, (*R*)-**4**, and (*R*)-**6** were obtained in 99%, 98%, and 96% ee, respectively, and in 89, 52, and 59 mg, respectively. Because high substrate concentration was used, the yields amounted to 21−19% for (*R*)-**2**, **4**, and **6**. Nevertheless, 119 mg (*R*)-**2**, 69 mg (*R*)-**4**, and 79 mg (*R*)-**6** were produced by using 1 g of dry cells, respectively. In another preparative experiment, (*R*)-phenyl methyl sulfoxide **4** was obtained in 98% ee and 68% isolated yield from the sufoxidation of **3** (10 mM).

CONCLUSION

Recombinant *E. coli* (P450pyrI83H-GDH) coexpressing three-component P450pyrI83H monooxygenase and glucose dehydrogenase (GDH) was engineered for asymmetric sulfoxidations of thioanisole **1**, 4-fluorothioanisole **3**, ethyl phenyl sulfide **5**, methyl *p*-tolyl sulfide **7**, and 4-methoxythioanisole **9**, respectively, with cofactor recycling, giving higher *R*-enantioselectivity and higher specific activity than any other known P450 monooxygenases for this type of reaction. Although the substrates and the corresponding products demonstrated strong toxicity to the cells and inhibition to the sulfoxidations, these problems were solved by developing a suitable KP buffer/IL biphasic system for the biotransformations. [$P_{6,6,6,14}$][NTf_2] showed excellent biocompatibility for the growth of *E. coli* (P450pyrI83H-GDH) cells and ability to shield much of the substrate toxicity to the cells, being an ideal cosolvent in the KP buffer/IL biphasic system for efficient biooxidations. Sulfoxidations of **1**, **3**, **5**, **7**, and **9** with resting cells of *E. coli* (P450pyrI83H-GDH) in KP buffer/[$P_{6,6,6,14}$][NTf_2] at a ratio of 3:1 (v/v) significantly enhanced both enantioselectivity and productivity. In comparison with the monophasic KP buffer system, the product concentration was increased from 9.4 mM to 20 mM for (*R*)-phenyl methyl sulfoxide **2**, from 1.9 mM to 9.9 mM for (*R*)-4-fluorophenyl methyl sulfoxide **4**, from 5.4 mM to 16 mM for (*R*)-ethyl phenyl sulfoxide **6**, from 4.2 mM to 22 mM for (*R*)-methyl *p*-tolyl sulfoxide **8**, and from 5.7 mM to 24 mM for (*R*)-methyl *p*-methoxyphenyl sulfoxide **10**, respectively; the product ee was enhanced from 85% to 99% for (*R*)-**2**, from 80% to 98% for (*R*)-**4**, from 88% to 96% for (*R*)-**6**, from 35% to 62% for (*R*)-**8**, and from 53% to 67% for (*R*)-**10**, respectively. In such a biphasic system, >99% of the substrates and 35−60% of the products remained in IL, thus greatly reducing the substrate toxicity and inhibition and partially reducing the product toxicity and inhibition. Accordingly, biotransformation in such a biphasic system significantly enhanced the product concentration. The enhancements in enantioselectivity are possibly caused by the low substrate concentrations in the aqueous phase of the biphasic system, evidenced by the higher enantioselectivity observed in the sulfoxidation of **1** at a smaller substrate concentration in aqueous system and the sulfoxidations of **1**, **3**, **5**, **7**, and **9** in the biphasic system at a lower ratio of aqueous phase to IL (v/v). Preparative sulfoxidations of **1**, **3**, and **5** in the biphasic system gave a green method to produce the useful and valuable sulfoxides (*R*)-**2**, (*R*)-**4**, and (*R*)-**6** in 99%, 98%, and 96% ee, respectively. To our knowledge, it is the first report on the engineering of a biphasic system with IL to enhance both the enantioselectivity and productivity for a bio-oxidation reaction. For the first time, IL is successfully used as cosolvent in a biphasic system for a P450 monooxygenase-catalyzed oxidation. Our success shows that solvent engineering with IL as a cosolvent is a simple and useful way for improving the catalytic performances of a bio-oxidation. The biphasic system with IL provides with a green and potent reaction platform for efficient bio-oxidation with better compatibility to cells and enzymes.

EXPERIMENTAL SECTION

Engineering of *E. coli* (P450pyrI83H-GDH), *E. coli* (P450pyrM305Q-GDH), *E. coli* (P450pyrA77S-GDH), and *E. coli* (P450pyr-GDH) Coexpressing the Corresponding P450 Monooxygenase and GDH. The four target P450 genes[45] (i.e., P450pyrI83H, P450pyrM305Q, P450pyrA77S, and P450pyr genes) were amplified with four plasmids[45] of pRSFDuet1-P450pyrI83H, pRSFDuet1-P450pyrM305Q, pRSFDuet1-P450pyrA77S, and pRSFDuet1-P450pyr as the templates, respectively. Ferredoxin reductase gene was amplified with plasmid pETDuet-P450pyrTM-FdR[44] as template. The forward and reverse primers for the P450 genes were 5′-GAATTCCCATGGAACATACAGGACAAAGCG-3′ (underlined is the *Nco*I restriction site) and 5′-GAATTCCTGCAGCTACGCGTGGACGCGAACC-3′ (underlined is the *Pst*I restriction site), respectively. The forward and reverse primers for ferredoxin reductase gene were 5′-GGATTCCATATGATCCACACCGGCGTGACCG-3′ (underlined is the *Nde*I restriction site) and 5′- GAATTCGATATCTTAGAGGGAGGTTGGGGACG-3′ (underlined is the *Eco*RV restriction site), respectively. The digested ferredoxin reductase gene fragment was ligated into the multiple cloning site 2 of the pETDuet-1 vector digested by restriction enzymes *Nde*I and *Eco*RV, which gave plasmid pETDuet-1-R. The fragment of the digested P450 gene was ligated into the multiple cloning site 1 of pETDuet-1-R (digested by restriction enzymes of *Nco*I and *Pst*I), giving the plasmid pETDuet-1-P_{I83H}R, pETDuet-1-P_{M305Q}R, pETDuet-1-P_{A77S}R, and pETDuet-1-PR, respectively. Each of these plasmids was cotransformed together with Plasmid pRSFDuet1-GDH-Fdx[44] into competent cell *E. coli* BL21-(DE3) to give *E. coli* (P450pyrI83H-GDH), *E. coli* (P450pyrM305Q-GDH), *E. coli* (P450pyrA77S-GDH), and *E. coli* (P450pyr-GDH), respectively.

Cell Growth and Specific Activity for Asymmetric Sulfoxidation of *E. coli* (P450pyrI83H-GDH). *E. coli* (P450pyrI83H-GDH) cells (preserved at −80 °C) were inoculated into 3 mL of LB medium containing 50 μg/mL kanamycin and 100 μg/mL ampicillin. Incubation was conducted at 37 °C and 250 rpm for 12 h. The seeds (3−5%, v/v) were then introduced to 50 mL TB medium containing 100 μg/mL ampicillin and 50 μg/mL kanamycin.

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Incubation was conducted at 37 °C and 250 rpm for 2 h to reach an OD_{600} of 0.6 to 0.8. Isopropyl β-D-1-thiogalactopyranoside and δ-aminolevulinic acid hydrochloride were added to a final concentration of 0.25 mM and 0.5 mM for induction, respectively, followed by incubation at 25 °C. Samples at different time points were taken for the analysis of the cell density and the specific activity for sulfoxidations of **1**, **3**, **5**, **7**, and **9**, respectively. For biotransformation with resting cells, the *E. coli* cells were harvested at late exponential phase at 10 h with a cell density of 3.6 CDW/L and used as the fresh biocatalyst.

Activity test: Cell pellets were suspended to a cell density of 1.5 g CDW/L in 0.5 mL KP buffer (50 mM; pH 8.0) containing 2% (w/v) glucose and 2 mM substrates of **1**, **3**, **5**, **7**, or **9**. The reactions were carried out at 30 °C and 1000 rpm for 30 min. Aliquots (0.3 mL) were taken and mixed thoroughly with 0.3 mL ethanol containing 2 mM benzylacetone as internal standard. After centrifugation of the mixture at 13 000*g* for 5 min, the supernatant was separated and analyzed by HPLC to quantify the product concentration. The specific activity was then calculated in U/g CDW. One U is defined as 1 μmol product formed per minute.

Toxicity of the Sulfides 1, 3, 5, 7, and 9 and Sulfoxides 2, 4, 6, 8, and 10 on the Cells of *E. coli* (P450pyrI83H-GDH). Freshly prepared *E. coli* (P450pyrI83H-GDH) cells were resuspended to a cell density of 10 g CDW/L in 15 mL KP buffer (50 mM; pH 8.0). The suspension was divided equally into 15 tubes, and each tube contains a 1 mL cell suspension. Sulfides (**1**, **3**, **5**, **7**, and **9**) and sulfoxides (**2**, **4**, **6**, **8**, and **10**) were added into 10 tubes to a final concentration of 20 mM, respectively. The remaining five tubes served as the control without the addition of sulfide or sulfoxide. The mixtures in the 15 tubes were shaken at 30 °C and 1000 rpm for 6 h, followed by washing with KP buffer (50 mM; pH 8.0) for eight times to totally remove the sulfide and sulfoxide.

The cells pretreated with the sulfide and corresponding sulfoxide (i.e., **1** and **2**; **3** and **4**; **5** and **6**; **7** and **8**; **9** and **10**) were examined for sulfoxidation activities of the corresponding sulfides **1**, **3**, **5**, **7**, and **9**, respectively, by using the procedures described above. The control cells in the five tubes were also tested for sulfoxidation activities of the sulfides **1**, **3**, **5**, **7**, and **9**, respectively. The residual activity was defined as the ratio of the specific activity of pretreated cells to that of the control cells.

The viability of the pretreated cells was checked via inoculation of diluted cells onto the LB agar plate at 37 °C for 12 h, followed by counting the number of the colonies.

Apparent Kinetics of Asymmetric Sulfoxidations of Sulfides 1, 3, 5, 7, and 9 with the Resting Cells of *E. coli* (P450pyrI83H-GDH). *E. coli* (P450pyrI83H-GDH)-catalyzed asymmetric sulfoxidations of **1**, **3**, **5**, **7**, and **9** were carried out at 30 °C and 250 rpm with cell suspension (1.5 g CDW/L) in 0.5 mL KP buffer (50 mM; pH 8.0) containing 2% (w/v) glucose at substrate concentrations ranging from 0.5 to 5 mM for 15 min. Product formation was determined by HPLC. The initial rates were measured, and the apparent V_{max} and K_m were calculated from the Lineweaver−Burk plot.[43] To determine the apparent dissociate constant K_i, sulfoxides (*R*)-**2**, **4**, **6**, **8**, and **10** were added to a final concentration of 2 mM, respectively, and the reactions were carried out at the same range of substrate concentrations under the same conditions as described above. K_i was then calculated accordingly.[52]

Examination of the Effects of ILs and Sulfide on the Cell Growth of *E. coli* (P450pyrI83H-GDH). Seeds (3−5%, v/v) of the *E. coli* cells were introduced to 50 mL LB medium containing 50 μg/mL kanamycin and 100 μg/mL ampicillin, and the cultivation was carried out at 37 °C and 250 rpm for 2 h to reach an OD_{600} of 1.5. Cell culture broth (10 mL) was then divided equally into 10 sterile tubes with each tube containing 1 mL cell culture broth. One hundred microliters of IL $[P_{6,6,6,14}][NTf_2]$, $[BMIM][NTf_2]$, $[BMIM][Pf_6]$, or $[NMeOct_3][NTf_2]$ was added into four tubes, respectively. A mixture of IL $[P_{6,6,6,14}][NTf_2]$, $[BMIM][NTf_2]$, $[BMIM][Pf_6]$, or $[NMeOct_3][NTf_2]$ (100 μL), and sulfide **1** (1.2 μL) was added into another four tubes, respectively. The last two tubes without adding any IL served as the controls, one containing 1.2 μL sulfide **1** and the other containing no sulfide. *E. coli* cells in the 10 tubes were then grown at 37 °C and 1000 rpm, and the cell densities were recorded.

Asymmetric Sulfoxidations of Sulfides 1, 3, 5, 7, and 9 with Resting Cells of *E. coli* (P450pyrI83H-GDH) in KP Buffer/$[P_{6,6,6,14}][NTf_2]$ Biphasic System. $[P_{6,6,6,14}][NTf_2]$ (3.3 mL) containing sulfide **1** (71 μL), **3** (37 μL), **5** (54 μL), **7** (54 μL), or **9** (56 μL) was added to the freshly prepared 10 mL cell suspension (10 g CDW/L) in KP buffer (100 mM; pH 8.0) containing 2% (w/v) glucose to give an initial substrate concentration of 60, 30, 40, 40, or 40 mM (concentrations were based on the volume of aqueous phase), respectively. The biotransformations were carried out at 30 °C and 250 rpm in multiple flasks. Samples were taken at different time points. For each sampling, a whole flask (containing 3.3 mL $[P_{6,6,6,14}][NTf_2]$ and 10 mL cell suspension) was withdrawn for analysis. For assaying asymmetric sulfoxidation of **1**, samples were taken at 3, 9, 12, and 24 h; for determining asymmetric sulfoxidation of **3**, samples were taken at 3, 6, 9, 12, 18, and 24 h; for monitoring asymmetric sulfoxidation of **5**, samples was taken at 3, 6, 9, 18, and 24 h; for measuring asymmetric sulfoxidation of **7** and **9**, samples were taken at 24 h.

The concentrations of products **2**, **4**, **6**, **8**, and **10** in both aqueous and IL phases were analyzed by HPLC. Analytical samples of aqueous phase were prepared as follows: after separating from the IL phase by centrifugation at 1000*g* for 1 min, the aqueous phase of the cell suspension (0.1 mL) was combined with 0.4 mL water and mixed thoroughly with 0.5 mL ethanol containing 2 mM benzylacetone as internal standard; after centrifugation of the mixture at 13 000*g* for 5 min, the supernatant was analyzed by HPLC. Analytical samples of IL phase were prepared as follows: after separating from aqueous phase by centrifugation at 1000 *g* for 1 min, $[P_{6,6,6,14}][NTf_2]$ (20 μL) was dissolved in the 1 mL ethanol containing 1 mM benzylacetone as internal standard; after centrifugation of the mixture at 13 000 *g* for 5 min, the supernatant was analyzed by HPLC.

The ee values of products (*R*)-**2**, **4**, **6**, **8**, and **10** at 24 h were determined by chiral HPLC. The analytical samples were prepared as follows: the aqueous phase was first separated from the IL phase by centrifugation at 1000 *g* for 1 min; aqueous phase (1 mL) was subjected to centrifugation at 13 000 *g* for 5 min to remove the cell pellets; supernatant (600 μL) was extracted by ethyl acetate (600 μL); after separation from the aqueous phase by centrifugation at 10 000*g* for 1 min, ethyl acetate phase was dried over Na_2SO_4, and the solvent was then removed by evaporation at reduced pressure; *n*-hexane (60−100 μL) was added to dissolve the extractives by vortexing; after centrifugation at 13 000*g* for 5 min, the supernatant of *n*-hexane (containing the product) was subjected to chiral HPLC analysis

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Preparation of (*R*)-2, 4, and 6 by Asymmetric Sulfoxidations of 1, 3, and 5 with Resting Cells of *E. coli* (P450pyrI83H-GDH), Respectively. $[P_{6,6,6,14}][NTf_2]$ (17 mL) containing **1** (353 μL), **3** (183 μL), or **5** (271 μL) was added to the freshly prepared 50 mL cell suspension (15 g CDW/L) in KP buffer (100 mM; pH 8) containing 2% (w/v) glucose to give an initial substrate concentration of 60, 30, or 40 mM (concentrations based on the volume of the aqueous phase), respectively. The asymmetric sulfoxidations were conducted at 30 °C and 250 rpm for 24 h. The biotransformation solution was centrifuged at 1000*g* for 1 min to separate aqueous phase of cell suspension from IL phase. The aqueous phase (50 mL) was then centrifuged at 5000*g* for 10 min to remove the cell pellets, and the supernatant was extracted with ethyl acetate (3 × 50 mL). $[P_{6,6,6,14}][NTf_2]$ phase (17 mL) was first washed with water (3 × 50 mL) to extract the sulfoxide; the collected aqueous phase was extracted with ethyl acetate (2 × 150 mL). The separated ethyl acetate phase was combined and dried over Na_2SO_4, followed by evaporation at reduced pressure. The crude sulfoxides were purified by flash chromatography on a silica gel with *n*-hexane/ethyl acetate (2:1−2) as eluent ($R_f \approx 0.4$). (*R*)-phenyl methyl sulfoxide **2**: 89 mg, colorless liquid; 99% ee and 21% isolated yield. ^{1}H NMR ($CDCl_3$) δ: 2.72 (3H, s), 7.47−7.55 (3H, m), 7.63−7.66 (2H, m). (*R*)-4-fluorophenyl methyl sulfoxide **4**: 52 mg, colorless liquid; 98% ee and 22% isolated yield. ^{1}H NMR ($CDCl_3$) δ: 2.71 (3H, s), 7.19−7.25 (2H, m), 7.62−7.67 (2H, m). (*R*)-ethyl phenyl sulfoxide **6**: 59 mg, colorless liquid; 96% ee and 19% isolated yield. ^{1}H NMR ($CDCl_3$) δ: 1.19 (3H, t, J = 7.6), 2.71−2.94 (2H, m), 7.48−7.53 (3H, m), 7.59−7.61 (2H, m).

In another representative experiment, $[P_{6,6,6,14}][NTf_2]$ (17 mL) containing sulfide **3** (61 μL) was added to the freshly prepared 50 mL cell suspension (15 g CDW/L) in KP buffer (100 mM; pH 8) containing 2% (w/v) glucose to give an initial substrate concentration of 10 mM (concentrations based on the volume of the aqueous phase). The reaction was performed for 24 h and then subjected to purification, according to the same process described above. 53.7 mg (*R*)-phenyl methyl sulfoxide **4** was obtained as colorless liquid in 98% ee and 68% isolated yield.

■ ASSOCIATED CONTENT

Supporting Information

Chemicals and strains; analytical methods; procedures including screening, sulfoxidations in monophasic KP buffer system, and product recovery; growth/activity curves; substrate/product toxicity tests; additional data for the sulfoxidations in KP buffer/IL biphasic systems; chiral HPLC chromatograms; ^{1}HNMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Mugford, P. F.; Wagner, U. G.; Jiang, Y.; Faber, K.; Kazlauskas, R. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 8782−8793.
(2) Faber, K.; Kroutil, W. *Curr. Opin. Chem. Biol.* **2005**, *9*, 181−187.
(3) Panke, S.; Held, M.; Wubbolts, M. *Curr. Opin. Biotechnol.* **2004**, *15*, 272−279.
(4) Weiss, R.; Panke, S. *Curr. Opin. Biotechnol.* **2009**, *20*, 447−448.
(5) Meunier, B.; de Visser, S. P.; Shaik, S. *Chem. Rev.* **2004**, *104*, 3947−3980.
(6) Cornmell, R. J.; Winder, C. L.; Schuler, S.; Goodacre, R.; Stephens, G. *Green Chem.* **2008**, *10*, 685−691.
(7) Li, A.; Zhang, J.; Yu, H.; Pan, J.; Xu, J. *Process Biochem.* **2011**, *46*, 689−694.
(8) Boyd, D. R.; Sharma, N. D.; Byrne, B.; Hand, M. V.; Malone, J. F.; Sheldrake, G. N.; Blacker, J.; Dalton, H. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1935−1943.
(9) Jenkins, R. O.; Stephens, G. M.; Dalton, H. *Biotechnol. Bioeng.* **1987**, *29*, 873−883.
(10) Lauchli, R.; Rabe, K. S.; Kalbarczyk, K. Z.; Tata, A.; Heel, T.; Kitto, R. Z.; Arnold, F. H. *Angew. Chem., Int. Ed.* **2013**, *52*, 5571−5574.
(11) Zhao, H.; Giver, L.; Shao, Z.; Affholter, J. A.; Arnold, F. H. *Nat. Biotechnol.* **1998**, *16*, 258−261.
(12) McLachlan, M.; Chockalingam, K.; Lai, K.; Zhao, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 7783−7786.
(13) Cobb, R. E.; Si, T.; Zhao, H. *Curr. Opin. Chem. Biol.* **2012**, *16*, 285−291.
(14) Reetz, M. T. *J. Am. Chem. Soc.* **2013**, *135*, 12480−12496.
(15) Reetz, M. T.; Carballeira, J. D. *Nat. Protoc.* **2007**, *2*, 891−903.
(16) Yang, Y.; Liu, J.; Li, Z. *Angew. Chem., Int. Ed.* **2014**, *53*, 3120−3124.
(17) Turner, N. J. *Nat. Chem. Biol.* **2009**, *5*, 567−573.
(18) Zha, W.; Rubin-Pitel, S. B.; Zhao, H. *Mol. BioSyst.* **2008**, *4*, 246−248.
(19) Korman, T. P.; Sahachartsiri, B.; Charbonneau, D. M.; Huang, G. L.; Beauregard, M.; Bowie, J. U. *Biotechnol. Biofuels* **2013**, *6*, 1−13.
(20) Pfrueder, H.; Amidjojo, M.; Kragl, U.; Weuster-Botz, D. *Angew. Chem., Int. Ed.* **2004**, *43*, 4529−4531.
(21) Wu, D.; Guan, Y.; Wang, H.; Yao, S. *Bioresour. Technol.* **2011**, *102*, 9368−9373.
(22) Sureshkumar, M.; Lee, C. *J. Mol. Catal. B: Enzym.* **2009**, *60*, 1−12.
(23) van Rantwijk, F.; Sheldon, R. A. *Chem. Rev.* **2007**, *107*, 2757−2785.
(24) van Rantwijk, F.; Lau, R. M.; Sheldon, R. A. *Trends Biotechol.* **2003**, *21*, 131−138.
(25) Sanfilippo, C.; D'Antona, N.; Nicolosi, G. *Biotechnol. Lett.* **2004**, *26*, 1815−1819.
(26) Lichtenecker, R. J.; Schmid, W. *Monatsh. Chem.* **2009**, *140*, 509−512.
(27) Carrea, G.; Redigolo, B.; Riva, S.; Colonna, S.; Gaggero, N.; Battistel, E.; Bianchi, D. *Tetrahedron: Asymmetry* **1992**, *3*, 1063−1068.
(28) Raghavan, S.; Rathore, K. *Tetrahedron* **2009**, *65*, 10083−10092.
(29) Buese, M. A.; Hogen-Esch, T. E. *J. Am. Chem. Soc.* **1985**, *107*, 4509−4513.
(30) Li, J.; Bu, X.; Zhang, R.; Duan, C.; Wong, K. M.; Yam, V. W. *New. J. Chem.* **2004**, *28*, 261−265.
(31) Bu, X.; Weng, W.; Du, M.; Chen, W.; Li, J.; Zhang, R.; Zhao, L. *Inorg. Chem.* **2002**, *41*, 1007−1010.
(32) Han, Z.; Song, J. J.; Yee, N. K.; Xu, Y.; Tang, W.; Reeves, J. T.; Tan, Z.; Wang, X.; Lu, B.; Krishnamurthy, D.; Senanayake, C. H. *Org. Process Res. Dev.* **2007**, *11*, 605−608.
(33) Kohlmann, C.; Lütz, S. *Eng. Life Sci.* **2006**, *6*, 170−174.
(34) Tang, J.; Brackenridge, I.; Roberts, S. M.; Beecher, J.; Willetts, A. J. *Tetrahedron* **1995**, *51*, 13217−13238.

(35) Davis, R.; Kern, J. R.; Kurz, L. J.; Pfister, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 7873−7874.
(36) Finn, K. J.; Cankar, P.; Jones, T. R. B.; Hudlicky, T. *Tetrahedron: Asymmetry* **2004**, *15*, 2833−2836.
(37) Spreti, N.; Germani, R.; Incani, A.; Savelli, G. *Biotechnol. Prog.* **2004**, *20*, 96−101.
(38) Zhang, J.; Li, A.; Xu, J. *Bioprocess Biosyst. Eng.* **2010**, *33*, 1043−1049.
(39) O'Reilly, E.; Corbett, M.; Hussain, S.; Kelly, P. P.; Richardson, D.; Flitsch, S. L.; Turner, N. J. *Catal. Sci. Technol.* **2013**, *3*, 1490−1492.
(40) Fruetel, J.; Chang, Y.; Collins, J.; Loew, G.; de Montellano, P. R. O. *J. Am. Chem. Soc.* **1994**, *116*, 11643−11648.
(41) Schallmey, A.; den Besten, G.; Teune, I. G. P.; Kembaren, R. F.; Janssen, D. B. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 1475−1485.
(42) Fujishiro, T.; Shoji, O.; Watanabe, Y. *Tetrahedron Lett.* **2011**, *52*, 395−397.
(43) Li, A.; Pham, Q. S.; Li, Z. *Chem. Commun.* **2013**, *49*, 11572−11574.
(44) Pham, S. Q.; Gao, P.; Li, Z. *Biotechnol. Bioeng.* **2013**, *110*, 363−373.
(45) Pham, S. Q.; Pompidor, G.; Liu, J.; Li, X.; Li, Z. *Chem. Commun.* **2012**, *48*, 4618−4620.
(46) Chang, D.; Feiten, H. J.; Witholt, B.; Li, Z. *Tetrahedron: Asymmetry* **2002**, *13*, 2141−2147.
(47) Chang, D.; Feiten, H. J.; Engesser, K. H.; van Beilen, J. B.; Witholt, B.; Li, Z. *Org. Lett.* **2002**, *4*, 1859−1862.
(48) Chang, D.; Witholt, B.; Li, Z. *Org. Lett.* **2000**, *2*, 3949−3952.
(49) Li, Z.; Feiten, H. J.; van Beilen, J. B.; Duetz, W.; Witholt, B. *Tetrahedron: Asymmetry* **1999**, *10*, 1323−1333.
(50) Tang, W. L.; Li, Z.; Zhao, H. *Chem. Commun.* **2010**, *46*, 5461−5463.
(51) Mouri, T.; Michizoe, J.; Ichinose, H.; Kamiya, N.; Goto, M. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 514−520.
(52) Li, A.; Yu, H.; Pan, J.; Zhang, J.; Xu, J.; Lin, G. *Bioresour. Technol.* **2011**, *102*, 1537−1542.