Supplementary Information

Chemical modification of biogenous iron oxide to create an excellent enzyme scaffold

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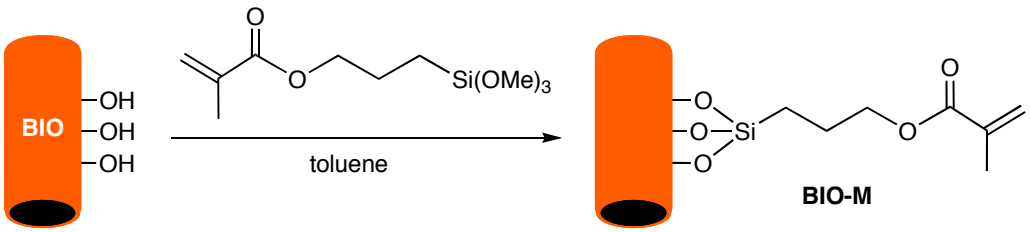
Isolation of biogenous iron oxide (BIO).

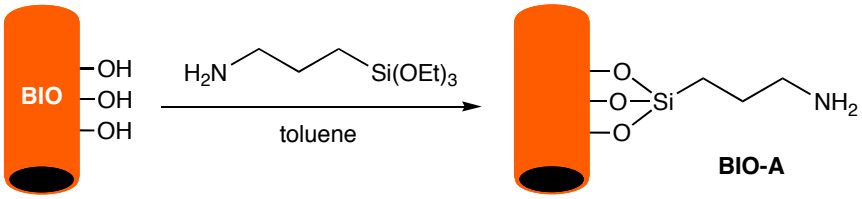
Iron-oxidizing bacteria have been employed to remove iron and manganese ions from groundwater at a water purification plant in Joyo City in Kyoto in Japan. Sediment produced by iron-oxidizing bacteria gets accumulated on a filtration device. The yellow-red turbid water containing the biogenous sediment (20 L) was put in a tank and left undisturbed overnight. After the supernatant solution had been removed carefully by decantation, ion-exchange water was added. The solution in the tank was shaken up and left undisturbed overnight. This procedure was repeated several times. The solution was transferred to centrifuge tubes (800 mL) and sonicated for 5 min. Centrifugation at 9,000 rpm for 10 min followed by the decantation of the supernatant solution gave BIO as a precipitate. The whole solution in the tank was transferred to the four centrifuge tubes by repeating this procedure. The yellow-red precipitate (BIO) was taken up and put in a centrifuge tube, leaving sands behind. To the precipitate was added EtOH (2 L), and the mixture was transferred to a flask and stirred for 1 h. Evaporation of EtOH followed by vacuum drying gave BIO as a reddish brown powder (75 g). Elemental analysis (combustion analysis) indicated C, 1.87%; N, 0.00%.

Chemical modification of BIO.

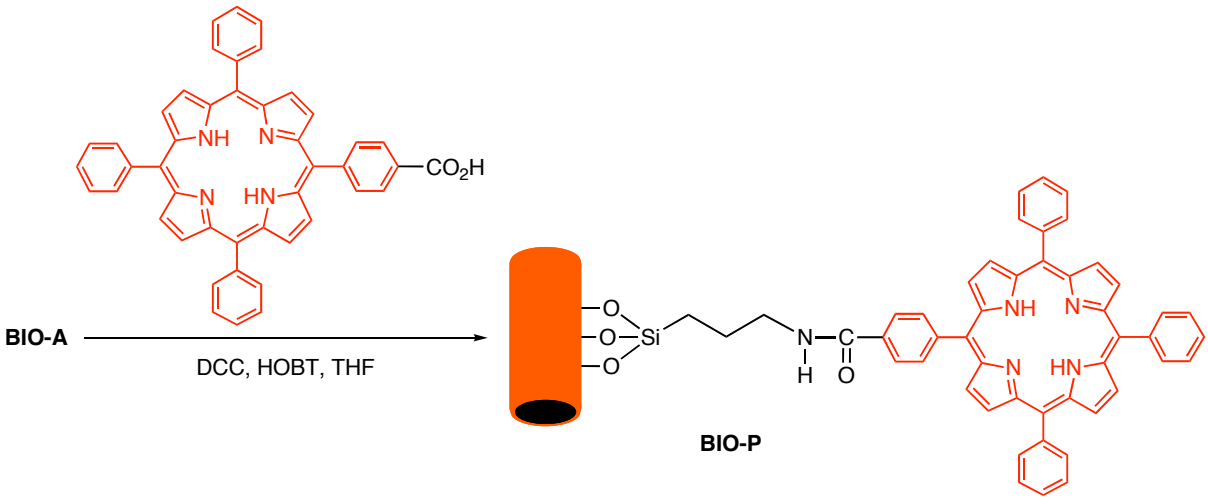
General procedure. BIO isolated as described above was dried in vacuo at 150 ºC for 4 h. A mixture of BIO (300 mg) and silane coupling agent (1.0 mmol) in dry toluene (3 mL) under N_2 was stirred at 100 ºC for 24 h. Toluene was removed by rotary evaporator. The residue was suspended in EtOAc and transferred to a centrifuge tube. The precipitate was washed by centrifugation at 9,000 rpm for 10 min followed by the decantation of the supernatant solution (5 times). The precipitate was dried in vacuo to give chemically modified BIO as a yellow-red powder. The coverage of the organic group on BIO was determined by elemental analysis. Solid-state NMR such as ^{13}C CP/MAS NMR and ^{29}Si CP/MAS NMR exhibited no signals due to the existence of paramagnetic Fe^{3+} in BIO. The chemically modified BIOs were characterized by elemental analysis, IR spectroscopy, SEM, TEM, and in a certain case, UV–Vis spectroscopy, and catalytic activity resulting from the catalyst moiety attached to BIO.



BIO-M. BIO-M was prepared by using 3-methacryloxypropyltrimethoxysilane according to the general procedure. Elemental analysis indicated C, 9.54%; N, 0.10%. Therefore, the coverage of the organic group on BIO can be calculated to be 14.7% (w/w) (0.94 mmol/g). FT-IR (pellet): 1717 cm^{-1}.



BIO-A. BIO-A was prepared by using 3-aminopropyltriethoxysilane according to the general procedure. Elemental analysis indicated C, 7.85%; N, 1.93%. Therefore, the coverage of the organic group on BIO can be calculated to be 15.0% (w/w) (1.74 mmol/g).



BIO-P. BIO-A was dried in vacuo at room temperature for 4 h. A mixture of **BIO-A** (300 mg), 5-(4-carboxyphenyl)-10,15,20-triphenylporphyrin (100 mg, 0.152 mmol), DCC (78.5 mg, 0.380 mmol), and HOBT (51 mg, 0.377 mmol) in dry THF (4 mL) under N_2 was stirred at room temperature for 72 h. The mixture was filtered and washed with THF, EtOH, hot EtOH, and then hexane. The resulting **BIO-P** was dried in vacuo. Elemental analysis

indicated C, 10.41%; N, 2.10%. Therefore, the coverage of the porphyrin moiety on BIO can be calculated to be 11.1% (w/w) (0.17 mmol/g) using the N content. The attachment of the porphyrin moiety to **BIO-A** was further confirmed by UV–Vis spectroscopy and fluorescence microscopy as described in the text.

Typical procedure for the immobilization of lipase on chemically modified BIO.

A mixture of *Burkholderia cepacia* lipase (Wako, lipase PS IM Amano, immobilized on diatomaceous earth, 31.3 g) in 10 mM phosphate buffer (pH 7.0, 125 mL) was stirred at room temperature for 3 h. The mixture was filtered to remove the diatomaceous earth, and the filtrate was dialyzed against 10 mM phosphate buffer (pH 7.0, 500 mL × 5). This enzyme solution (106 mL) and **BIO-M** (500 mg) were put in a centrifuge tube, which was shaken at 125 rpm at 25 ºC for 24 h. Centrifugation (10,000 rpm, 10 min) and drying in vacuo afforded BCL immobilized on **BIO-M** (500 mg). The amounts of enzyme in the solutions before and after immobilization were determined by the method of Bradford[1] to calculate the content of enzyme bound to **BIO-M** (4.6% (w/w)).

Kinetic resolution of secondary alcohols with lipase immobilized on chemically modified BIO.

Typical procedure. A mixture of **1** (1.00 mmol), **BIO-M**-immobilized lipase (10.0 mg), and molecular sieves 3A (three pieces) in dry *i*-Pr_2O (5 mL) was stirred at 30 ºC for 30 min. The reaction was started by adding vinyl acetate (185 μL, 2.00 mmol). The progress of the reaction was monitored by TLC. The mixture was filtered and concentrated. Alcohol (*S*)-**1** and ester (*R*)-**2** were separated by silica gel column chromatography. The enantiomeric purities were determined by chiral GC or HPLC.

Kinetic resolution of 1a with BCL immobilized on BIO-M. The products were characterized according to the literature.[2] (*S*)-**1a**: 31% yield; 83% ee; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.51 (d, J = 6.3 Hz, 3H), 1.77 (d, J = 3.0 Hz, 1H), 4.87–4.95 (m, 1H), 7.28–7.41 (m, 5H); GC: CP-cyclodextrin-β-2,3,6-M-19, Inj. 250 ºC, Col. 95 ºC, Det. 220 ºC, (*R*) 30.0 min, (*S*) 32.6 min. (*R*)-**2a**: 23% yield; 98% ee; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.54 (d, J = 6.8 Hz, 3H), 2.07 (s, 3H), 5.88 (q, J = 6.8 Hz, 1H), 7.27–7.36 (m, 5H); GC: CP-cyclodextrin-β-2,3,6-M-19, Inj. 250 ºC, Col. 95 ºC, Det. 220 ºC, (*S*) 24.6 min, (*R*) 27.5 min.

Kinetic resolution of 1b with BCL immobilized on BIO-M. (*S*)-**1b**: 56% yield; 69% ee; $[\alpha]^{19}_{D}$ –32.6 (*c* 0.96, $CHCl_3$); lit.[3] $[\alpha]^{26}_{D}$ –11.5 (*c* 1.03, $CHCl_3$) for (*S*)-**1b** with 25% ee; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.59 (d, *J* = 6.0 Hz, 3H), 1.88 (s, 1H), 5.08 (q, *J* = 6.0 Hz, 1H), 7.45–7.53 (m, 3H), 7.82–7.85 (m, 4H); HPLC: Chiralcel OJ-H, hexane/*i*-PrOH (9:1), flow rate 0.5 mL/min, detection 254 nm, (*S*) 30.2 min, (*R*) 39.8 min. (*R*)-**2b**: 40% yield; >99% ee; $[\alpha]^{20}_{D}$ +117 (*c* 1.04, $CHCl_3$); lit.[3] $[\alpha]^{25}_{D}$ +88.1 (*c* 1.18, $CHCl_3$) for (*R*)-**2b** with >99% ee; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.63 (d, *J* = 8.3 Hz, 3H), 2.10 (s, 3H), 6.05 (q, *J* = 8.3 Hz, 1H), 7.48–7.49 (m, 3H), 7.81–7.85 (m, 4H); HPLC: Chiralcel OJ-H, hexane/*i*-PrOH (9:1), flow rate 0.5 mL/min, detection 254 nm, (*R*) 25.1 min, (*S*) 28.9 min.

Kinetic resolution of 1c with BCL immobilized on BIO-M. (*S*)-**1c**: 59% yield; 77% ee; $[\alpha]^{22}_{D}$ –28.8 (*c* 1.03, $CHCl_3$); lit.[3] $[\alpha]^{28}_{D}$ –4.14 (*c* 1.11, $CHCl_3$) for (*S*)-**1c** with 7% ee; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.95 (t, *J* = 7.0 Hz, 3H), 1.83–1.95 (m, 3H), 4.78 (t, *J* = 5.3 Hz, 1H), 7.45–7.49 (m, 3H), 7.79–7.85 (m, 4H); HPLC: Chiralcel OJ-H, hexane/*i*-PrOH (9:1), flow rate 0.5 mL/min, detection 254 nm, (*S*) 24.4 min, (*R*) 33.5 min. (*R*)-**2c**: 40% yield; 97% ee; $[\alpha]^{22}_{D}$ +96.8 (*c* 1.11, $CHCl_3$); lit.[3] $[\alpha]^{29}_{D}$ +43.7 (*c* 0.65, $CHCl_3$) for (*R*)-**2c** with >99% ee; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.91 (t, *J* = 7.5 Hz, 3H), 1.91–2.03 (m, 2H), 2.10 (s, 3H), 5.83 (t, *J* = 7.0 Hz, 1H), 7.45–7.48 (m, 3H), 7.78–7.84 (m, 4H); HPLC: Chiralcel OJ-H, hexane/*i*-PrOH (9:1), flow rate 0.5 mL/min, detection 254 nm, (*R*) 18.7 min, (*S*) 25.7 min.

Table S1 Kinetic resolution of secondary alcohols with BCL immobilized on chemically modified BIOs[a]

					% Yield[c] (% ee)			
Entry	Support	**1**	Time/h	c[b]	(*R*)-**2**	(*S*)-**1**	*E* value[d]	TTN[e]
1	**BIO-M**	**1a**	1	46	23 (98)	31 (83)	259	33,000
2	**BIO-A**	**1a**	7	9	7 (99)	75 (10)	220	6,500
3	**BIO-P**	**1a**	1.5	43	32 (99)	44 (74)	444	31,000
4	**BIO-M**	**1b**	1	41	40 (>99)	56 (69)	>413	29,000
5	**BIO-M**	**1c**	12	44	40 (97)	59 (77)	154	32,000

[a] Conditions: immobilized lipase (10.0 mg of 4.6% (w/w) BCL/**BIO-M**, 10.6 mg of 4.3% (w/w) BCL/**BIO-A**, or 13.4 mg of 3.4% (w/w) BCL/**BIO-P**), **1** (1.00 mmol), vinyl acetate (2.00 mmol), molecular sieves 3A (three pieces), dry *i*-Pr_2O (5 mL), 30 ºC. [b] Conversion calculated from c = ee(**1**)/(ee(**1**) + ee(**2**)). [c] Isolated yield. [d] Calculated from $E = \ln[1 - c(1 + \text{ee}(\mathbf{2}))]/\ln[1 - c(1 - \text{ee}(\mathbf{2}))]$. [e] Total turnover number of enzyme.

References

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3 T. Itoh, Y. Matsushita, Y. Abe, S. Han, S. Wada, S. Hayase, M. Kawatsura, S. Takai, M. Morimoto and Y. Hirose, *Chem. Eur. J.*, 2006, **12**, 9228–9237.