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Electronic Supplementary Information

An HASApf-Redoxin Complex causing Asymmetric Catalytic Oxidation via the Regenerative Formation of a Reactive Oxygen Species

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Abbreviations: HasApf: heme acquisition system A from *Pseudomonas fluorescens* Pf-5, ME: membrane-bound enzymes, PP: pea protein, DMSO: dimethyl sulfoxide, PP-gel: calcium-alginate gel containing PP, GA: glutaraldehyde, CMME: compound-modified ME, AGME: PEG (MW: 4000/1000 = 1/2)-aggregated ME, *S*-**1**: *S*-(+)-1-(6-methoxynaphthalen-2-yl)ethanol, *S*-**2**: *S*-(+)-1-(2-naphthyl)ethanol, ICP-AES: inductively coupled plasma-atomic emission spectroscopy, IC: ion chromatography. ESR: electron spin resonance, FTIR: Fourier-transform infrared spectroscopy, Fdx: ferredoxin.

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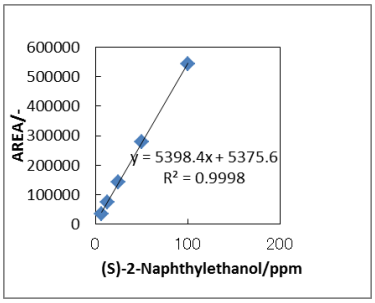
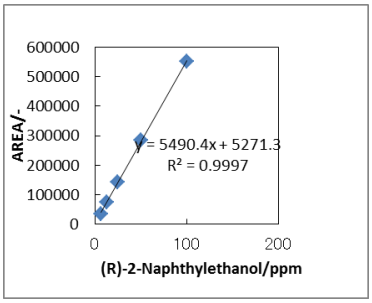
1. General procedure for reactions using CMME and AGME

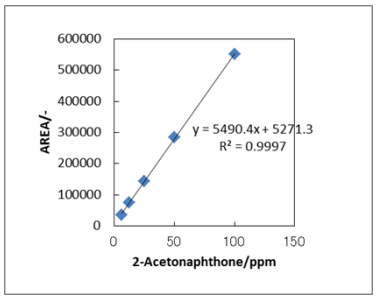
A calibration curve configured, the chromatograms of the *rac*-**1** biotransformation with CMME, and the raw data of the *rac*-**1** biotransformation with CMME/ME suspension were shown. Enantiomeric excess (ee) values, chemical yield ratio (% yield)/convertion ratio of all compounds were obtained from chiral HPLC analysis.

1-1. Calibration curve configured using HPLC.

The ee was calculated for either *rac*-**1** (0.8 mM or 1.2 mM) or *rac*-**2** (0.8 mM or 1.2 mM), which were separated with either a Daicel Chiralcel OB-H column ((S)-isomer/(R)-isomer/product ketone = 7.8/8.8/11.6 min) or a Daicel Chiralpak AS-H column ((S)-isomer/(R)-isomer/product ketone = 7.5/8.25/9.5 min) connected to an HPLC LC-10A system (Shimadzu). Analytical conditions were as follows: mobile phase, n-hexane/IPA: 9/1, flow rate: 1.0 mL/min, temperature: 30 °C, wavelength: UV 254 nm. The stereochemistry of the isolated optically active alcohol was identified by comparing the values (+ or –) for the specific rotation detected using a polarimeter, as done previously.[1]





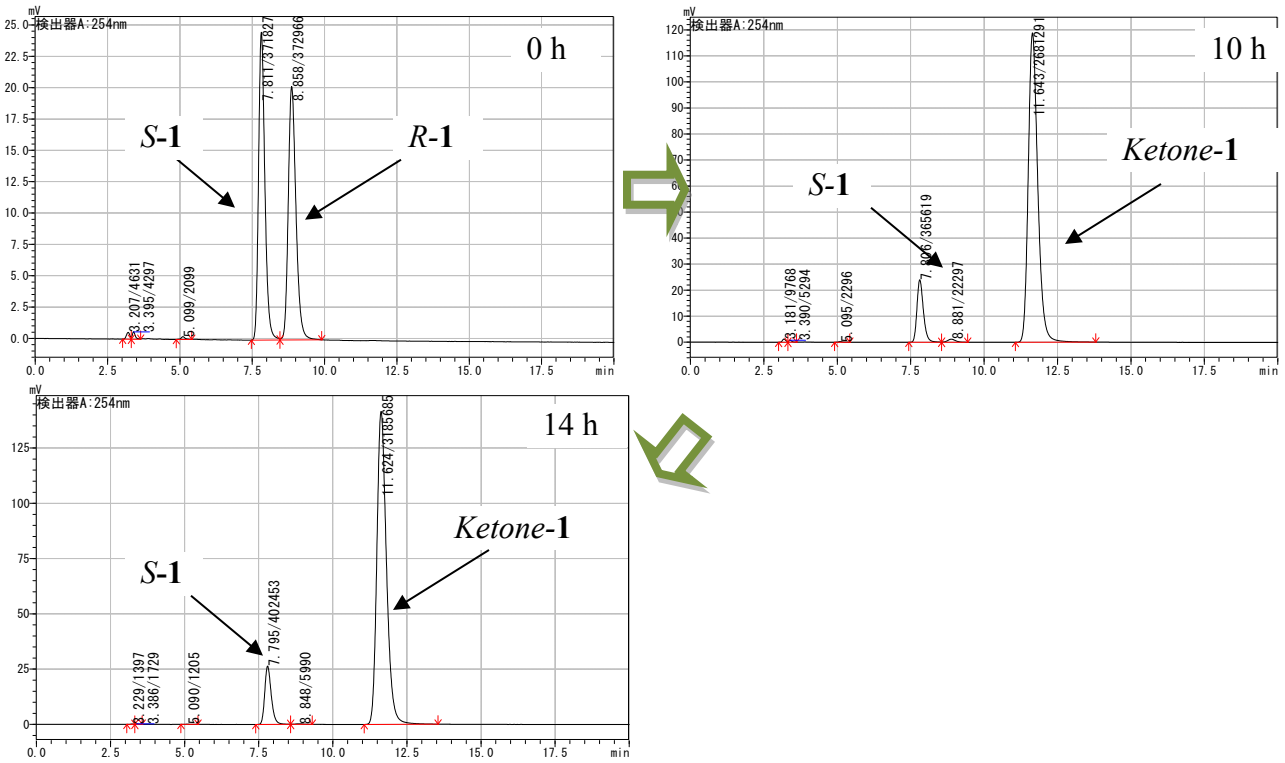


Conc./ppm	(*R*)-2/abs‡	(S)-2/abs‡	ketone/abs†
3.125	18408	18403	-
6.25	34455	34671	64884
12.5	69207	69837	129479
25	133042	134274	256966
50	263249	267577	509690
100	515506	524152	983200

‡Absorbance of HPLC analysis, †Average

1-2. Data of chromatograms

The time course/chromatograms of the asymmetric oxidation of *rac*-**1** (1.2 mM) using a CMME (20 mg) was monitored and quantitatively analyzed under the suggested conditions.[1]



Time/h	*S*-**1**/Abs‡	*R*-**1**/abs‡	Ketone/Abs‡	%ee	Avg† (%ee)
0	384592 371827	386537 372966	- -	0.252 0.152	0.20
10	341321 365619	27703 22297	2437138 2681291	84.985 88.50	86.74
14	421760 402453	2286 1190	3429781 3185685	98.92 99.41	99.17

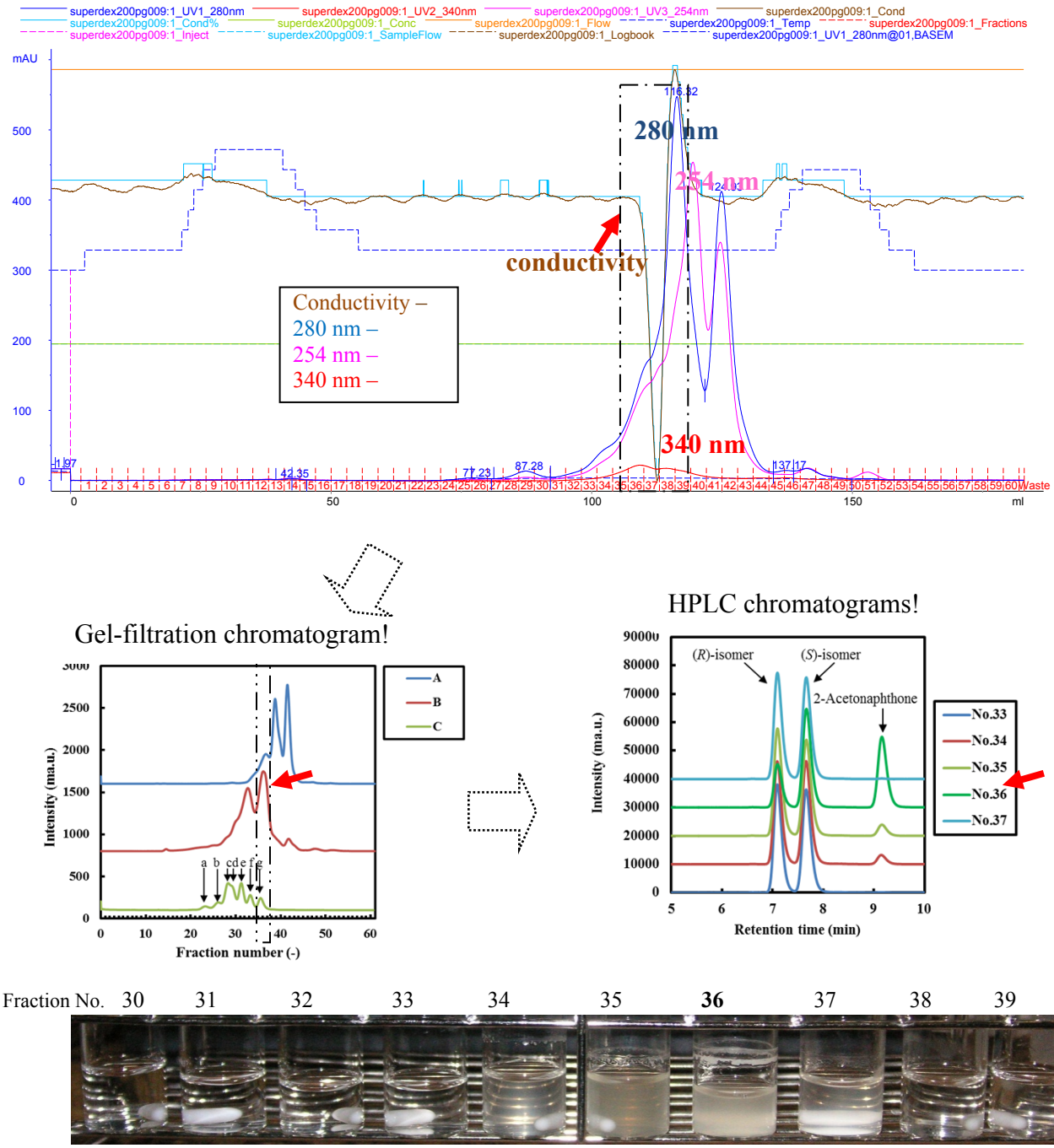
‡Absorbance of HPLC analysis, †Average

2. Gel-filtration for given a single band-F from many band-A in SDS-PAGE

To determine the nature of the redox protein catalyzing asymmetric oxidation, the PP gel-suspension (10 mL) eluted from the PP-gel, which was incubated for 48 h, was first separated into 60 fractions (acquired in 18 mm test tubes with 3.0 mL portions in each tube) using a gel-filtration system. These were determined using a ÄKTA explorer 10S system. Sample (10 mL or 2 mL) was injected onto a

HiLoad16/60 Superdex 200 pg column at 4 °C. Conductivity-**brown**, 280 nm-**blue**, 254 nm-**pink**, and 340 mn-**red**.

2-1. Data of Gel-filtration chromatograms [S1,2]



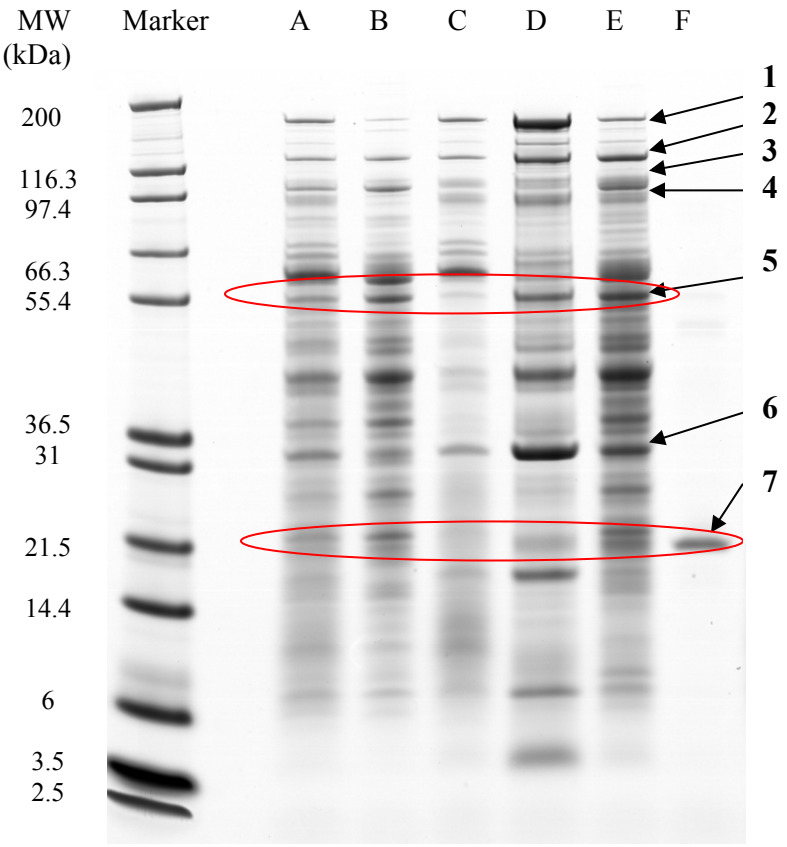
Gel-filtration chromatogram: (–A) the supernatant of the ME suspension acquired by centrifugation, (–B) the supernatant of the 5% PP aqueous suspension acquired by centrifugation, (–C) molecular standards: a, ferritin (440 kDa); b, aldolase (158 kDa); c, conalbumin (75 kDa); d, ovalbumin (44 kDa); e, carbonic anhydrase (29 kDa); f, ribonuclease A (13.7 kDa); g, aprotinin (6.5 kDa).

HPLC chromatograms of each fraction (3.0 mL) after the addition of 0.48 mL of a substrate solution containing rac-**2** (0.8 mM) and DMSO (1.03% (v/v)) and incubation of the resulting mixture at 40 °C for 48 h with magnetic stirring at 700 rpm. After reactions, the mixture was extracted by Hexame due to measure both chemical yield and % ee.

Figure S1. The gel-filtration system was used as PP-HasA purification: The PP gel-suspension (10 mL) purified applied by filtration using Vivaspin 2-10K (GE, MWCO =10 kDa) was significantly monitored

for line in conductivity: the target PP-HasA fraction can be collected in red between 110 ml and 120 ml (namely fraction 34-37, especially fraction 36).

2-1. Data of SDS-PAGE



Labeling bands:

1. Na^+-type flagellar protein MotY precursor

2. unnamed protein product [Clostridium ljungdahlii DSM 13528]

3. GTP diphosphokinase [Phascolarctobacterium succinatutens YIT 12067]

4. extracellular ligand-binding receptor [Desulfovibrio africanus str. Walvis Bay]

5. oligopeptide ABC transporter substrate-binding protein [Brevibacillus brevis NBRC 100599]

6. conserved hypothetical protein [Wolbachia endosymbiont of Drosophila ananassae]

7. HasAp gene product [*Pseudomonas fluorescens Pf-5*] from plant commensal bacteria

Figure S2. SDS-PAGE of samples[S1,S2]: (A) Eluant from the supernatant 1 of the PP gel (10 µL), (B) Aqueous suspension of the sample A precipitate acquired via centrifugation (10 µL), (C) Sample A supernatant acquired via centrifugation (10 µL), (D) Aqueous suspension acquired via the centrifugation of the sample C precipitate generated using 30% (w/v) saturated $(NH_4)_2SO_4$ (10 µL), (E) Aqueous suspension acquired via the centrifugation of the sample A precipitate generated using 30% (w/v) saturated $(NH_4)_2SO_4$ (10 µL), (F) Fraction 36 obtained via gel-filtration chromatography using a HITEC-CR20G (Hitachi) system at 10,000 rpm (10 min).

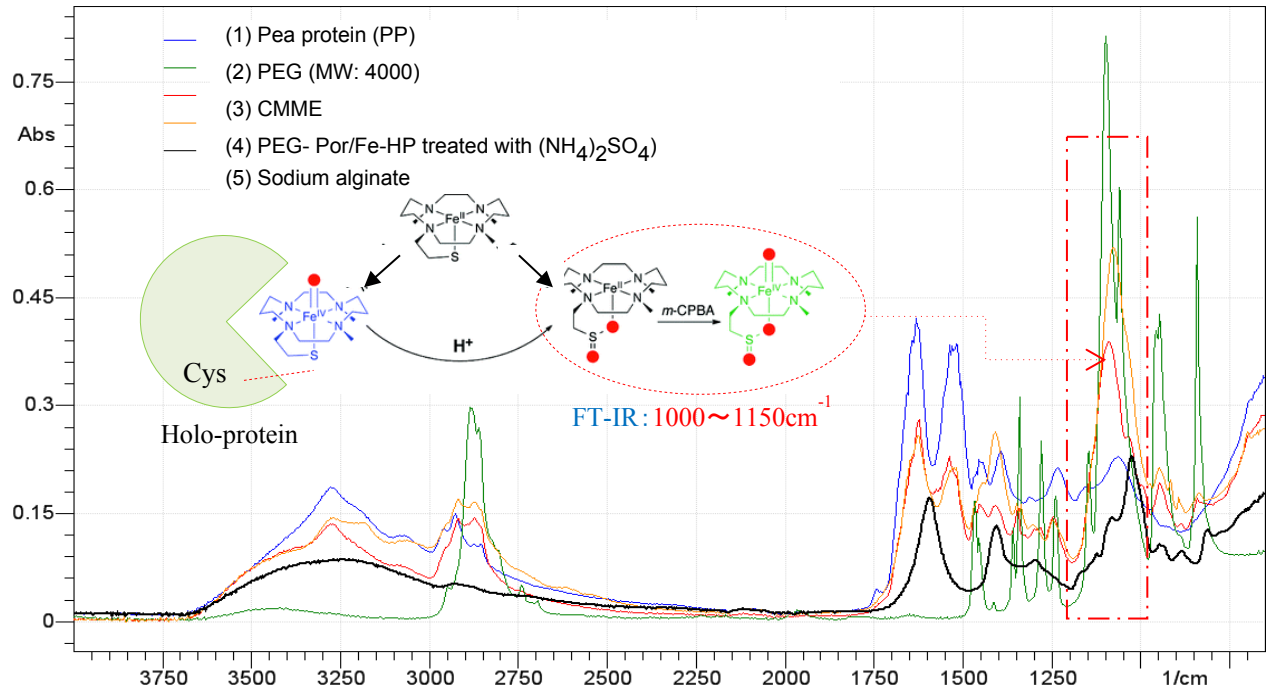
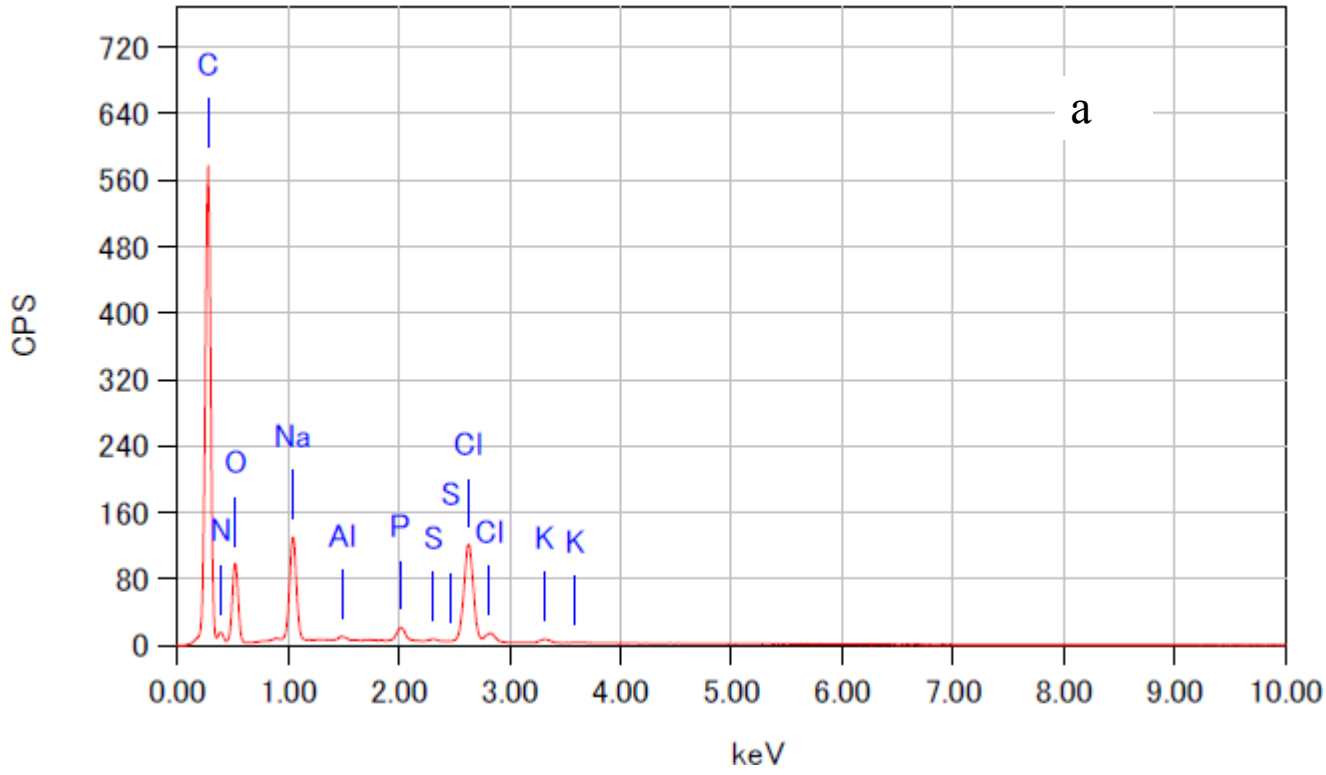
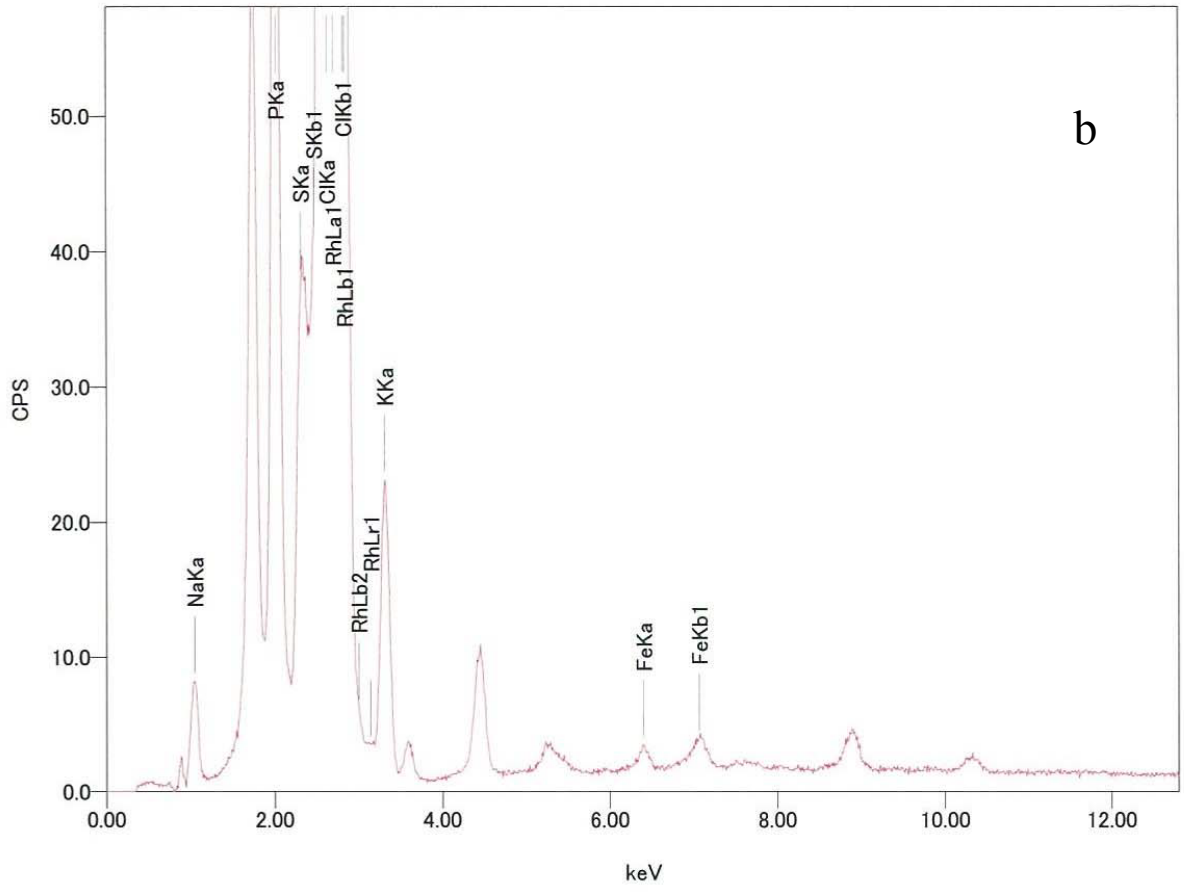


Figure S3. Differences in the content of functional groups among four samples (1) pea protein, (2) PEG (MW: 4000), (3) CMME, (4) PEG-ME treated with 30% (w/v) aqueous $(NH_4)_2SO_4$), and (5) sodium alginate: The red circles indicate oxygen atoms. The peak around 1000–1250 cm^{-1} is attributed to a sulfate ion, which is thought to be the absorption in the PP gel under aeration.



Elements	KeV	wt%	σ	atom%	K
C K	0.277	83.09	0.07	89.67	75.7112
N K	0.392	1.55	0.01	1.43	0.8009
O K	0.525	5.60	0.02	4.54	4.0924
Na K	1.041	3.78	0.02	2.13	6.6494
Al K	1.486	0.12	0.00	0.06	0.2053
P K	2.013	0.59	0.01	0.25	1.2138
S K	2.307	0.09	0.00	0.04	0.1881
Cl K	2.621	4.91	0.02	1.80	10.5824
K K	3.312	0.27	0.01	0.09	0.5565



Minerals	wt%	mol%	3σ	Intensity	K ratio	K
15 P	5.6710	5.9647	0.6176	129802	0.0047077	K
16 S	0.8657	0.8796	0.2266	28703	0.0008160	K
17 Cl	70.6465	64.9184	0.4726	1064404	0.0405031	K
19 K	6.9008	5.7501	1.2482	35533	0.0017630	K
26 Fe	0.0802	0.0468	0.1366	3227	0.0000470	K

Figure 4 (a) Element analysis for HasApf dried by using energy dispersive X-ray spectroscopy (JSM-6610LA; Voltage (15 kV), Current (1.0 nA), and Live time (114 sec)), and (b) Mineral analysis for HasApf dried by using radiation induced X-ray emission (JSX-3100R2; Voltage (50 kV), Current (1.0 mA), Live time (100 sec), and Atomosphere (Vacuum))

3. Chromatograms of the N-terminal amino-acid sequence of band-F

Precise analysis of the N-terminal amino acid sequence (protein sequencing) was accomplished using the protein sequencer PPSQ-21A (Shimadzu) with single band 7 of the PX-redox protein in sample F monitored by SDS-Page.

3-1. Results of both N-terminal amino-acid sequence and its BLAST query sequence analysis[S1,2]

Table S1. Results of a BLAST query sequence analysis based on the N-terminal amino-acid sequence identified from fraction 36 (band 7)

Cycle No. for fraction 36 (band 7)
N-terminal amino-acid sequence identified (33 residues)

1. M S X^a S I S Y S T X^b Y A T N T V A Q Y L X^a D W X^b A Y F G D L
30. N H R E

Cycle No. for YP 262445.1[c]
Full length gene and Protein sequence based on a BLAST query sequence analysis

1.	M S I S I S Y S A T atg agc att tcg atc tct tac agc gct acc	Y G G N T V A G Y L tac ggc ggt aat act gtt gcg caa tac ctg	T D W S A Y F G D V act gac tgg tcg gcc tac ttc ggc gac gtc
30.	N H R P G E V V D G aac cac cgc cca ggc gaa gtg gtc gac ggc	T N T G G F N P G P acc aac acc ggt ggc ttc aac ccg ggc ccg	F D G T Q Y A I K S ttc gac ggc acc cag tac gcc atc aag agc
60.	T A S D A A F V A D acc gcc agt gac gcg gcc ttc gtc gcc gac	G N L H Y T L F S N ggc aac ctg cac tac acc ctg ttc agc aac	P S H T L W G S V D ccg agc cac acc ctg tgg ggc tcg gtg gac
90.	T I S L G D T L A G act atc tcc ctg ggc gac acc ctc gcc ggt	G S G S N Y N L V S ggt tcg ggc agc aac tac aac ctg gtc agc	Q E V S F T N L G L cag gaa gtc agc ttc acc aac ctg ggc ctc
120.	N S L K E E G R A G aac agc ctg aag gaa gaa ggc cgt gca ggc	E V H K V V Y G L M gaa gtg cac aag gtg gtc tac ggc ctg atg	S G D S S A L A G E agt ggc gac agc tcg gcg ctg gcc ggc gag
150.	I D A L L K A I D P atc gat gcc ctg ctc aag gcg atc gac cca	S L S V N S T F D D agc ctg tcg gtg aac tcc acc ttc gac gac	L A A A G V A H V N ctg gcc gct gct ggc gtt gct cac gtc aac
180.	P A A A A A A D V G ccg gct gcc gca gcc gct gcc gat gtt ggc	L V G V Q D V A Q D ctg gtg ggt gtg cag gac gtg gcc cag gac	W A L A A tgg gcg ctg gcc gcc

X^a: may be Cys (C) but not detected, X^b: many amino acids were detected.
[c]YP 262445.1: the accession hit on the query sequence was limited between the query coverage (>93%) and E value (2e-11), a 20.853 Da HasAp gene product [hemophore: *Pseudomonas fluorescens Pf-5*] from plant commensal bacteria, which can inhibit the rhizosphere and produce secondary metabolites that suppress soil-borne plant pathogens.
Red amino acids indicate "hits" between fraction 36 and YP 262445.1[c].
Squares indicate the heme-binding site: His-32 (bearing loop), Tyr-75 (axial heme ligand), and His-83 (hydrogen ligand).
Protein sequence based on a BLAST query sequence analysis of Cycle No. for YP 262445.1 is available on NCBI resource: http://www.ncbi.nlm.nih.gov/protein/70732682 and the Full length gene sequences on PATRIC: VBIPseFlu72549_5489: http://patricbrc.vbi.vt.edu/portal/portal/patric/Feature?cType=feature&cId=19880237.

References

(S1) Nagaoka, H. *ACS Catal.* **2014**, 4, 553–565.

(S2) Nagaoka, H. *RSC Adv.* **2014**, 4, 16333–16344.