Supporting Information

Quantitative analysis of coenzyme F430 in environmental samples: a new diagnostic tool for methanogenesis and anaerobic methane oxidation

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Microbial cell staining and counting

Microbial cell staining and counting were conducted based on Kimura et al.[1] The groundwater samples for total cell counts were fixed with formaldehyde (final concentration 2%). Exactly 10ml of groundwater sample was filtered using pre-blackened polycarbonate filters (pore size, 0.22 mm; diameter, 25mm; Millipore, Bedford, MA, USA). Microbial cells collected on the filterwere stained with SYBR Green I (1:100 dilution, Molecular Probes, Eugene, OR, USA). The microbial cells were observed under a model BX51 epifluorescence microscope (Olympus, Tokyo, Japan), and over 50 microscopic fields were counted for each sample.

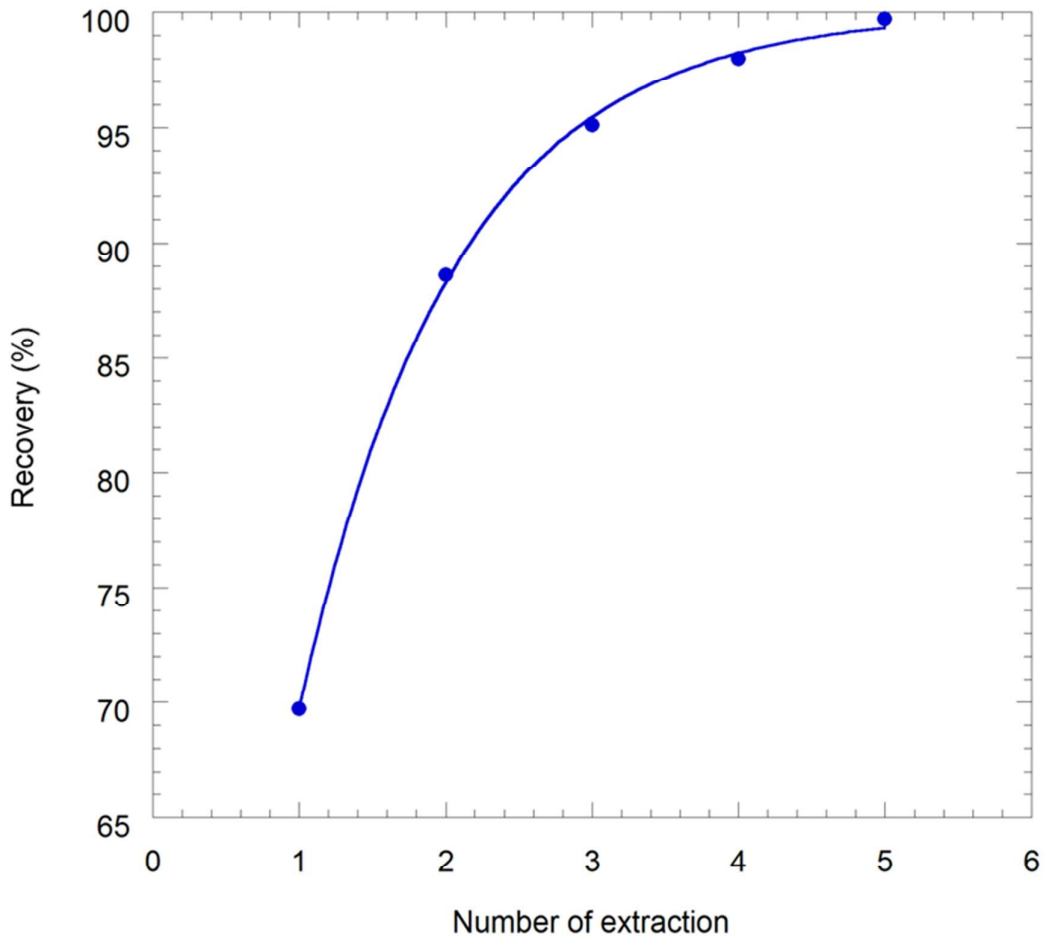

Figure S-1. Extraction efficiency of entire procedure from extraction to derivatization to F430M. Recovery is represented an accumulated value. In this study, samples were extracted three times by sonication, indicating more than 95% of recovery based on asymptotic of F430M concentration.

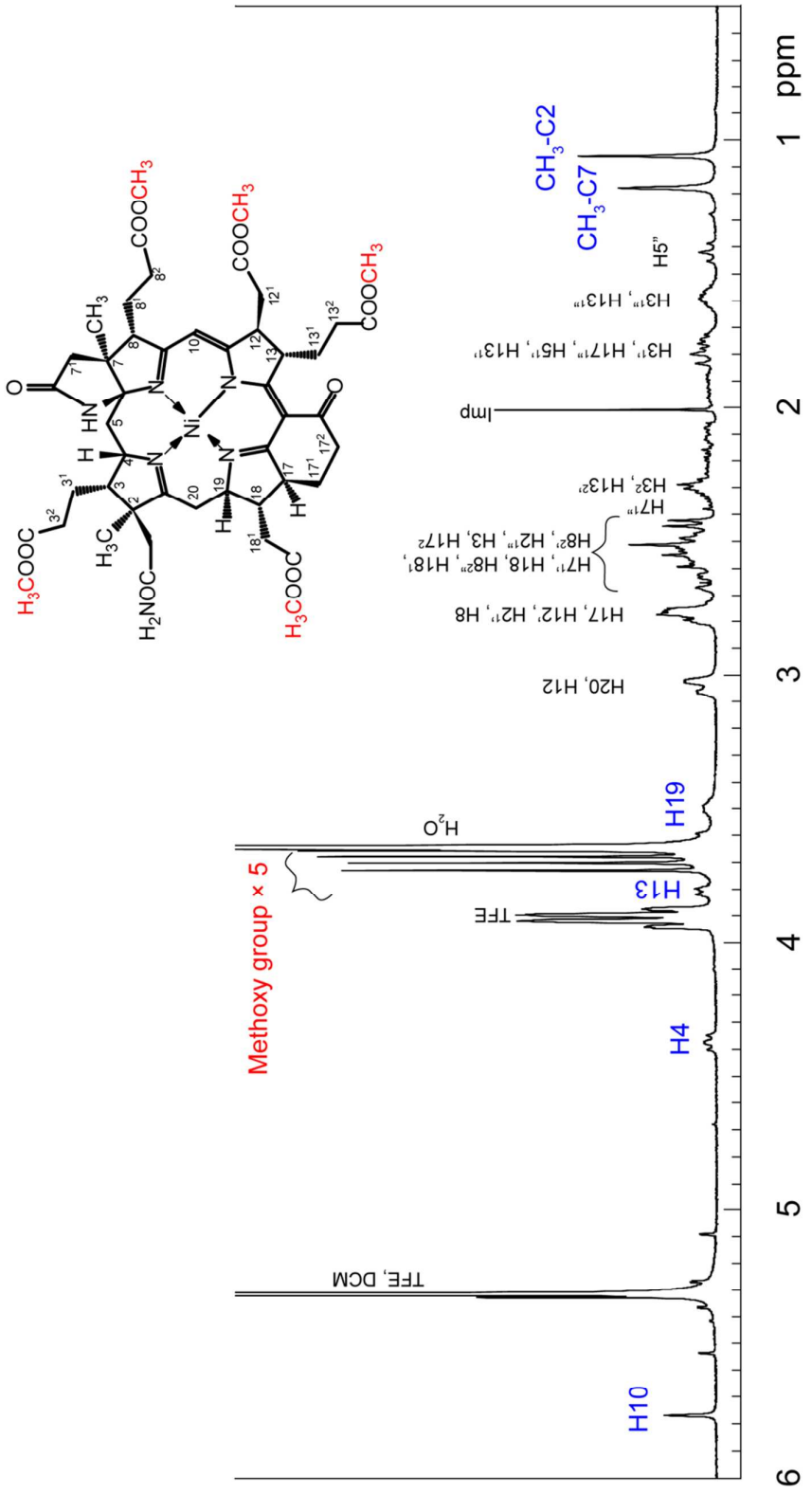

Figure S-2. ^{1}H-NMR spectrum of F430M.

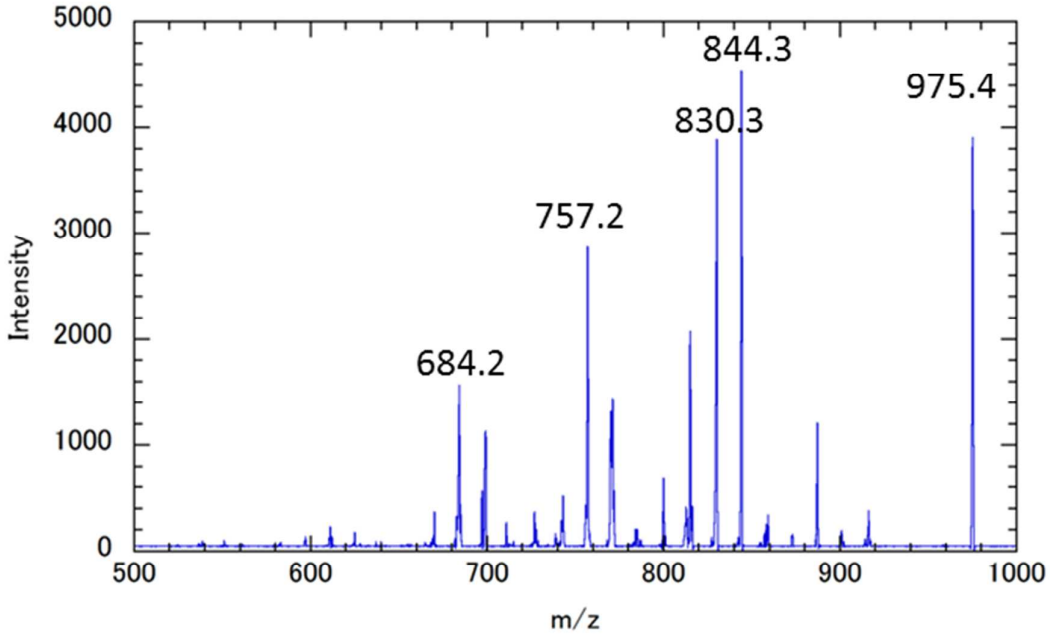

Figure S-3. A mass fragment pattern of F430M. Fragmentor voltage: 250V; Collision Energy: 66V.

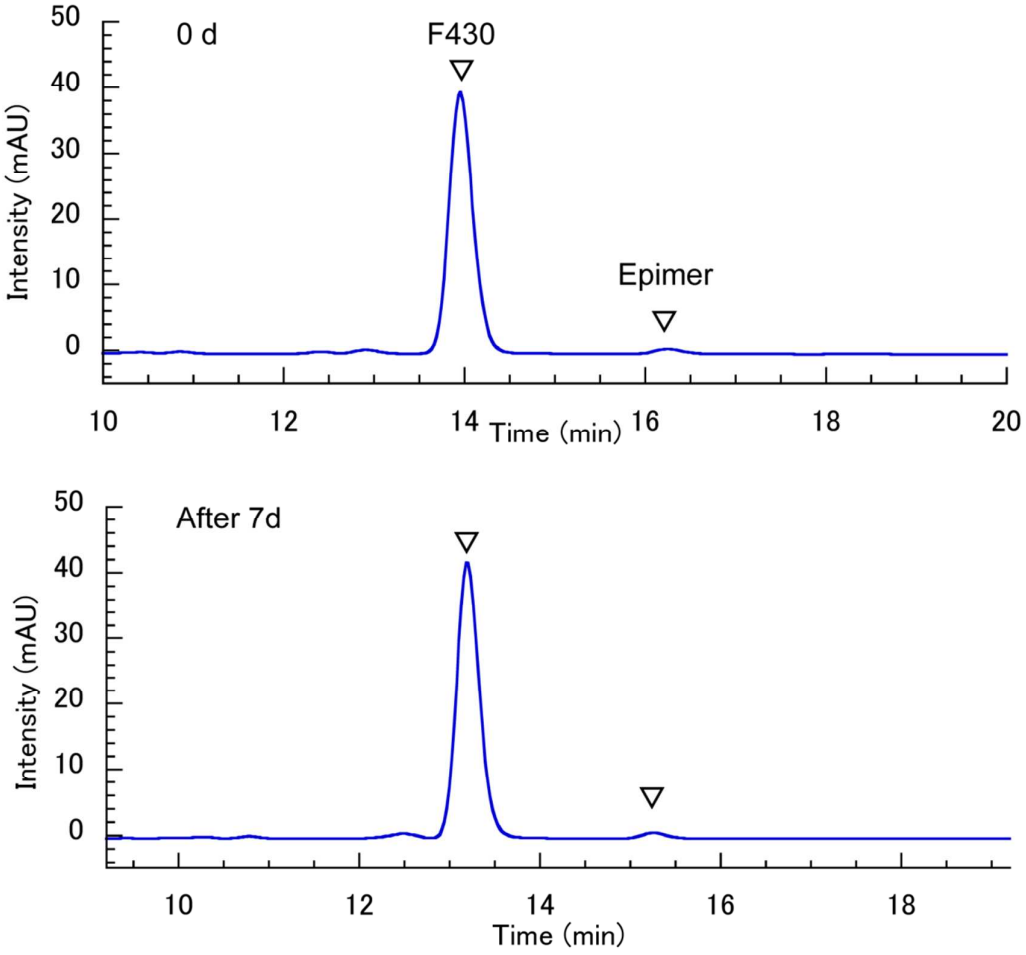

Figure S-4. DAD chromatogram (430 nm) of F430 purified from MBK sample. The F430 solution (pH 2) ws left for 7 d on ice (1°C).

Table S-1. Time course variation of relative abundance of F430 under 1°C.

Time (h)	Relative abundance (%)	
	F430	Epimer
0	97.9	2.1
1	97.5	2.5
4	97.9	2.1
22	97.7	2.3
77	97.6	2.4
192	97.9	2.1

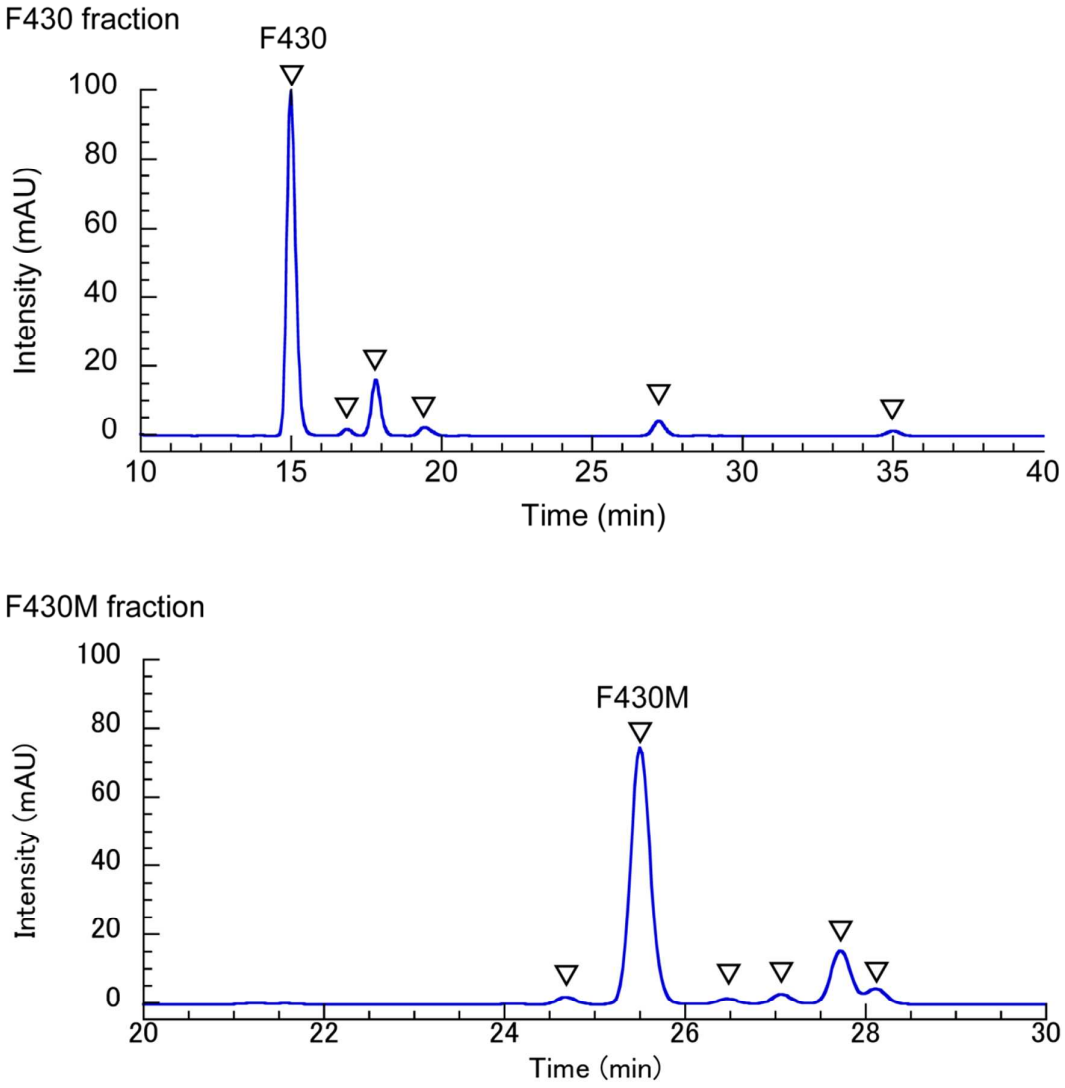

Figure S-5. DAD chromatograms (430 nm) of F430 fraction from MBK sample. Upper: before methyl esterification, lower: after methylesterification.

Table S-2. Relative abundance of F430 and F430M on chromatograms in Figure S-5

		% of intact F430 or F430M
F430 fraction		76.8
F430M fraction	n=1	73.8
	n=2	73.3
	n=3	73.4
	n=4	72.9

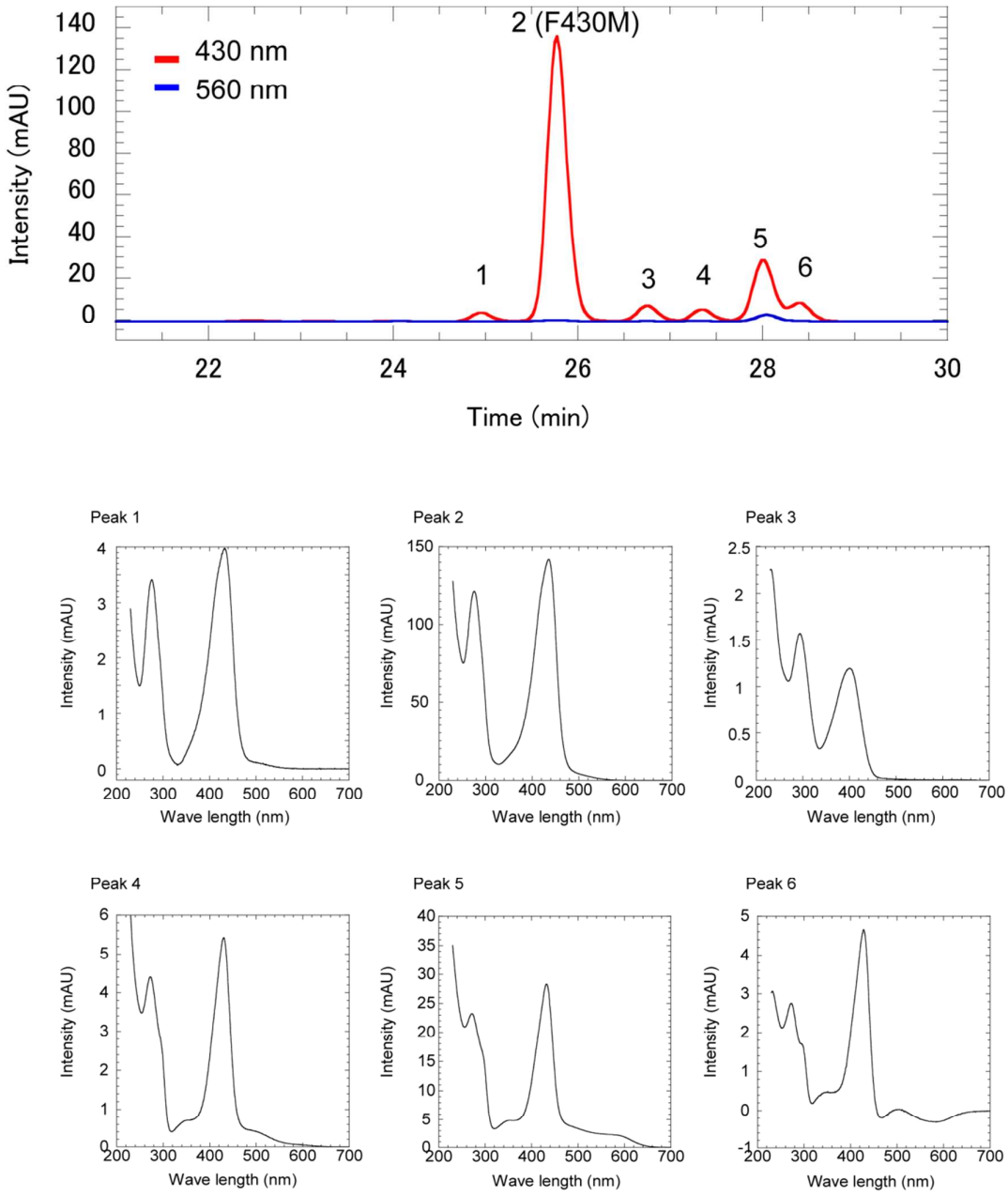

Figure S-6. DAD chromatograms (430 and 560 nm) of F430M fraction from MBK samples and photoabsorption spectrum of each peak.

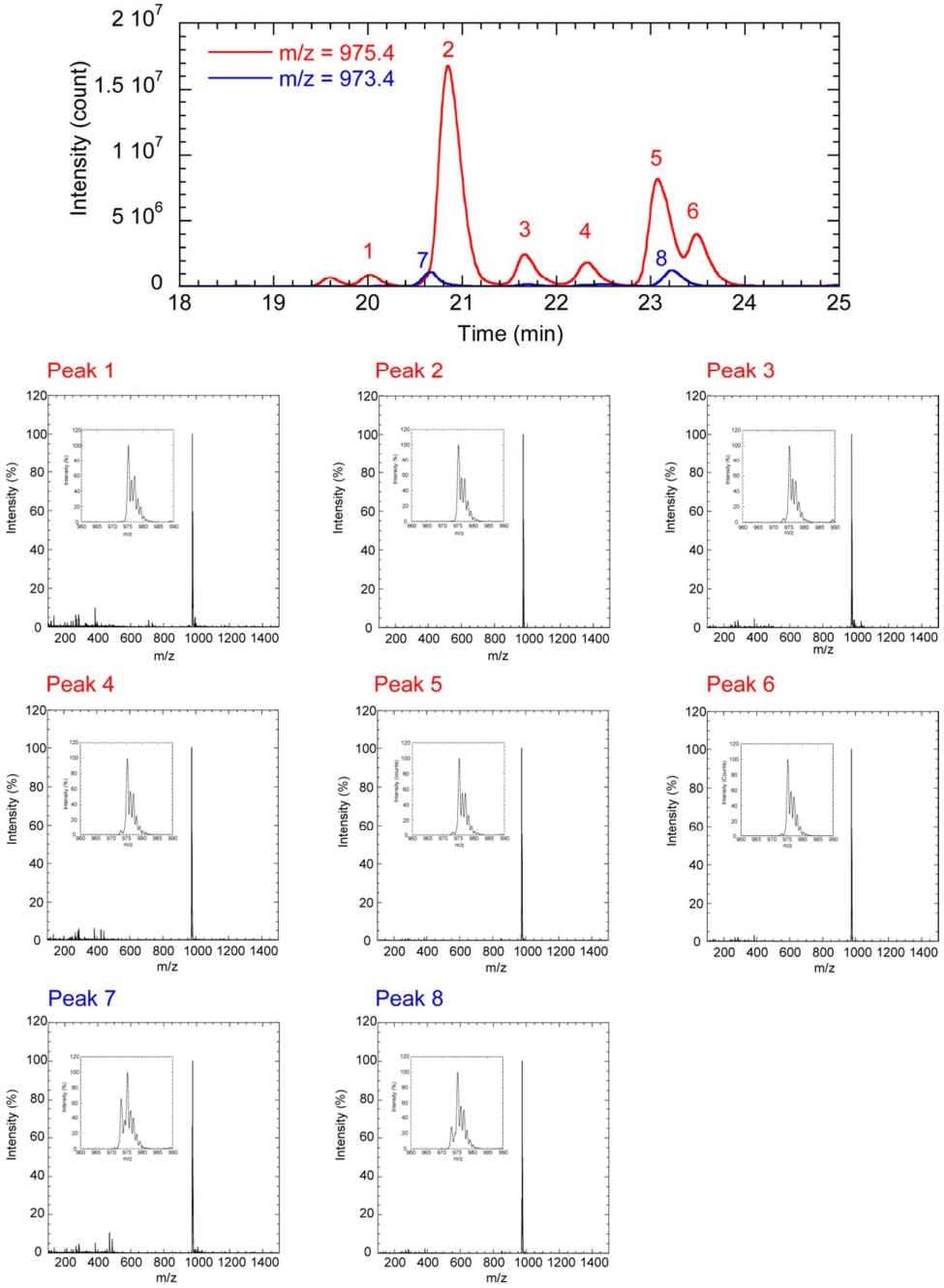

Figure S-7. Extracted ion chromatograms of m/z = 975.4 and 973.4 and mass spectra of each peaks of F430M fraction of MBK sample. LC-MS/MS analysis was performed by a scan mode.

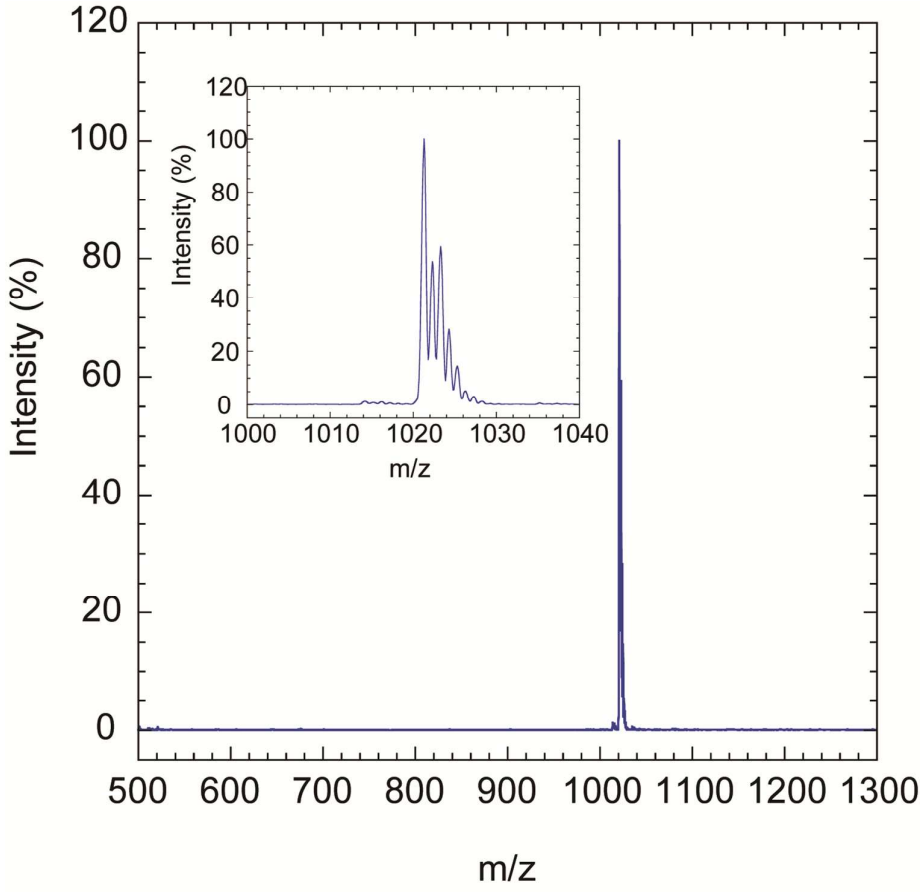

Figure S-8. Mass spectrum of methylthio-F430 from Pink mat.

Reference

(1) Kimura, H.; Nashimoto, H.; Shimizu, M.; Hattori, S.; Yamada, K.; Koba, K.; Yoshida, N.; Kato, K. *ISME J.* **2010**, *4*, 531-541.