

## Carbon isotopic composition of Coenzyme F430 from anaerobic methane oxidizing archaea

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Coenzyme F430 is the hydrocorphinoid nickel complex which acts as active sites in methyl-coenzyme M reductase (MCR). The MCR-F430 complex catalyzes the last step of methanogenesis: reduction of methyl-coenzyme M to methane. Since F430 is a common function-specific compound in methanogenic pathways including hydrogenotrophic, acetoclastic and methylotrophic methanogenesis, all methanogens including uncultured methanogens should utilize F430 for methanogenesis. Recent studies suggested that anaerobic methane oxidizing archaea (ANME) also utilize F430 and its homologue for the reversed methanogenesis [1] [2]. Thus, F430 is a function-specific compound for both methanogenesis and anaerobic methane oxidation, which has a potential to be a practical biomarker compound for estimation biomass and activities of methanogens and ANME in subsurface environments.

In the Black Sea, a chimneylike structure of a microbial mat is developed on the seafloor in which ANME is predominant. In this study, we extracted F430 from the inner part of the structure (pink mat, ANME-1 dominated) and the exterior (black mat, ANME-2 dominated). The concentrations were 0.83 nmol g<sup>-1</sup> for the pink mat and 44 nmol g<sup>-1</sup> for the black mat. Only in the pink mat, F430 homologue, (17<sup>2</sup>S)-17<sup>2</sup>-methylthio-F430 was detected in 2.3 nmol g<sup>-1</sup> [3].

We also investigated carbon isotopic composition of F430 from the black mat. Extracted F430 was further purified by silica gel chromatography to remove organic matrices and collected by HPLC coupled with a fraction collector. Carbon isotopic composition of purified F430 was analyzed by high sensitive EA-IRMS. The  $\delta^{13}\text{C}$  value of F430 exhibited a extremely low value (-83.3‰), suggesting that ANME assimilate methane-derived carbon.

[1] Krüger *et al.*, (2003), *Nature*. **426**, 878-881. [2] Mayr *et al.*, (2008), *J. Am. Chem. Soc.* **130**, 10758-10767. [3] Kaneko *et al.*, (2014), *Anal. Chem.* **86**, 3633-3638.