

Benthic Habitat Mapping in the Iheya North Hydrothermal Field

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Deep-sea hydrothermal systems can support large and diverse populations of vent-associated organisms. In this paper, we describe a practical method to rapidly assess the distribution and diversity of megabenthos over wide areas based on a two-phase multi-resolution visual mapping technique. The technique is applied to two areas in the Iheya North Field of the Okinawa trough, in regions that were drilled to varying extents during the IODP 331 expedition. A total area of more than 30,000m² was mapped in a single dive with a remotely operated vehicle (ROV) and more than 80,000 organisms were identified from six different species. The results give insight into the effects that drilling activity has had on the distribution of megabenthos in this area. The method described forms a practical way to quantitatively assess the distribution of megabenthos over statistically meaningful spatial scales in a way that is repeatable and is suitable for comparison between sites or for monitoring sites over time.

Keywords: 3d visual reconstruction, Habitat mapping, Hydrothermal vent

Niche separation of nitrifiers from the sea surface to the hadal ocean

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Ammonium (ammonia) and nitrite are important intermediates of oceanic nitrogen cycle, but these are depleted in most of the oceanic waters. In contrast, availability of ammonia most likely influence on niche separation of nitrifiers, and thus the niche separation would be a signature of geochemical interface in oceanic environments. In fact, niche separation of nitrifiers has been observed in Arctic to tropical oceans, and sea surface to hadal ocean (Sintes et al. 2013, Nunoura et al. 2015 and references therein). In this study, we analyzed single amplified genomes (SAGs) to know genomic backgrounds of niche separation of ammonia-oxidizing thaumarchaeotes from sea surface to hadal oceans.

Keywords: nitrification, ocean, nitrogen cycle

Technological breakthroughs in search of the deep seafloor biosphere

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During the first microbiology-dedicated scientific ocean drilling, the Ocean Drilling Program (ODP) Leg 201 off Peru and Eastern Equatorial Pacific in 2002, the number of microbial cells was evaluated by direct counting of acridine orange-stained cells under fluorescent microscopy, and the minimum quantification limit (MQL) of cell number was approximately 10^5 cells/cm³ of sediment. Although this technique is still applicable to high-biomass sedimentary habitats such as shallow organic-rich sediments near the seafloor, some innovative technological breakthroughs have been long required in order to explore low-biomass habitats close to the limit of biosphere. A decade later since Leg 201, we developed a computer image-based cell detection and enumeration method for deep sedimentary microbes. It enabled discriminable cell recognition based on the difference of fluorescence color between intracellular DNA and non-biological mineral particles after DNA stain with SYBR Green I, and resulted in objective and statistically mean cell numbers with higher reproducibility. In addition, we standardized a new protocol for effective cell separation from sedimentary mineral grains using a multi-layer density centrifugation. The combined use of this cell separation technique with flow cytometry or cell sorter opened the way to more fast, sensitive, and precise cell counting than before, even for very low-biomass sediment samples. For example, under the strictly controlled ultra-clean lab condition, our current minimum quantification limit approaches to less than 10 cells/cm³ of sediment, at least 4 orders of magnitude lower than that during Leg 201. The sorted cells in each well are applicable for single cell-genomic study using the genome amplification techniques. Moreover, the separated cells can be concentrated and placed at one place on the membrane filter, and then isotopic ratios (i.e., ¹³C/¹²C, ¹⁵N/¹⁴N) and elemental abundances of each single cell can be analyzed on rastered ion imaging with nano-scale secondary ion mass spectrometry (NanoSIMS). To date, based on these technological breakthroughs, we are finally ready for exploring the limits of seafloor life and the biosphere through scientific ocean drilling.

Keywords: Seafloor biosphere, Life Detection

Coenzyme F430 as a biomarker for methanogenesis and anoxic methane oxidation

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Coenzyme factor (F430) is a prosthetic group of a key enzyme for methanogenesis, methyl coenzyme M reductase (MCR) [e.g. Ellefson et al., 1982]. Coenzyme F430 should be a practical biomarker to investigate distribution of methanogens and methanogenic potential in natural environments for the following reasons: 1) it should be common in all methanogens, 2) it has a potential to reflect only modern methanogenic activity due to its unstable nature, 3) it is clear proxy because other source organisms are highly restricted (only anaerobic methane oxidizing archaea [Krüger et al., 2003; Mayr et al., 2008]).

Recently we developed quantitative analysis of coenzyme F430 by triple quadrupole mass spectrometry coupled with liquid chromatography, which allow to detect coenzyme F430 in environmental samples including marine sediment with fmol level concentration [Kaneko et al., 2014].

The major concerns in application of the coenzyme F430 analysis as a biomarker tool are stability of coenzyme F430 and discrimination of source archaea (methanogens vs. ANMEs). Previous studies reported that free (not bound to MCR) coenzyme F430 changed to epimers in hour scale at 200C and hour to day scale at room temperature [e.g. Diekert et al., 1981]. However, it is still ambiguous how the epimerization is observed in environmental conditions. In general marine setting, methanogenesis occurs after sulfate reduction and the habitats of methanogens and ANME are clearly controlled by sulfate concentrations. On the other hands, these archaeal sources should be discriminable by compound specific isotope analysis of coenzyme F430 because isotope effects involved with their metabolic pathways are quite deferent [Hinrichs et al., 1999].

In this talk, we will show distribution of coenzyme F430 in environmental samples including paddy soils, ANME microbial mats and marine sediments, and carbon isotopic composition of coenzyme F430 from ANME archaea to address stability of coenzyme F430 and discrimination of source archaea.

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Keywords: Coenzyme F430, function specific biomarker, methanogenesis

Electrochemical Carbon fixation

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The emergence and evolution of proto-metabolic networks have recently attracted much interest as an essential initial step for the origin of life (Braakman and Smith, 2013). Alkaline hydrothermal systems have been proposed as a plausible site to drive proto-metabolism (Russell et al., 2010), where reduction and fixation of CO₂ could have proceeded with the aid of ample and continuous supplies of reductive chemicals such as H₂, H₂S, and FeS, together with active mineral catalysts (Huber and Wächtershäuser, 1997). Recently, a direct electrochemical measurement of a deep-sea hydrothermal vent in the Okinawa Trough demonstrated that the geochemical redox potential between hydrothermal fluid and seawater generates electrical current through the vent structure, and electrons are concentrated at the vent-seawater interface (Yamamoto et al., unpublished).

Electrochemistry is an effective means for CO₂ reduction and fixation. It has been experimentally shown that electrocatalytic reduction of CO₂ on metal sulfide deposits produces CO and CH₄ with excellent efficiencies under naturally plausible electrochemical conditions (from -0.4 to -1.3V; Yamamoto et al., 2014). There is a good probability that the geo-electrochemical systems occurring at alkaline hydrothermal vents served as a source of energy and reducing power to drive proto-metabolic reactions. Following these geological and experimental findings, we have been conducting electrochemical experiments in ELSI. Here, we will introduce our research progress and its implication for the origin and early evolution of life.

Keywords: Origin of Life, Alkaline hydrothermal vent, Metabolism

HADEAN EVOLUTIONAL HISTORY OF ROCKY PLANET IN SOLAR SYSTEM

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Planetary formation theory based on meteorites and numerical simulation model is now entering a phase of major change, due to the discovery of hot Jupiter and super-Earth through exploration of exo-solar planets. The problem is typically seen in two extremes that is classic Kyoto model and Grand Tack Model. The key to advance from current level of debates to next stage is to conduct systematic material science including sample return missions from asteroid belt. To proceed it, I review previous researches for asteroid belt, and summarize the origin of the Earth, early evolution, and whole Earth history. Based on those reviews, I define next targets of the research as follows. 1) To confirm the chemical zoning of asteroid belt (2-5AU) from most inner part to outer part, and reveal the cause of chemical zoning. 2) To reveal the material differentiation to form a parental body of meteorite and the time to spend for it. 3) Surface geology and chronology of Moon and Mars, particularly the age of solidification and late heavy bombardment. 4) Early evolution of the Earth and its surface environment based on above three, particularly the reconstruction of Hadean Earth.

For next step, I suggest to verify currently provided formation theory of proto-solar system during Hadean time, based on above 4 researches. Details are as follows. 1) Earth-Moon system was formed from enstatite chondrite-like materials at 4,567Ma, which did not have atmospheric nor oceanic component. 2) Giant Impact event occurred around 4.4Ga, but inner core of the Earth did not melt. Moon was solidified by 4.3Ga. 3) Magma ocean of Moon and Earth was solidified by approximately 4.34Ga. At around 4.3Ga, the late heavy bombardment reached a peak. This event gave both atmosphere and ocean on the Earth. 4km thick ocean triggered the operation of plate tectonics since 4.266Ga and tectonic erosion to carry primordial continent into deep mantle. By 4.1Ga, first life was born through 3 steps.

Keywords: origin of Earth, Planetary formation theory, Chemical zoning in asteroid belt

Organism-resolved Metagenomics using ggKbase: Recovering and analyzing thousands of genomes from metagenomic samples

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Metagenomics has the potential to recover near complete and complete genomes for the majority of microbial members present at > 0.2 % abundance level in natural communities, to track changes in community composition across space and time, and to document evolution *in situ*. The field has advanced tremendously since the first community metagenomic study of an acid mine drainage biofilm, more than a decade ago. Metagenomics is now a tool widely used by microbial ecologists. Thousands of research groups worldwide are now collecting complex metagenomic datasets, yet expertise in how to effectively and efficiently use the information is lacking. Currently most metagenomic data analysis methods yield only fragmented, partial genomes, or worse, they attempt to analyze only the read data from a sample and forego any genome assembly. Although such studies provide information about the representation of genes in an environment, much information is also lost. The result is often unsuitable for the development of useful, organism-resolved metabolic models.

Here we describe a multi-faceted approach for genomic analyses using ggKbase. ggKbase is a platform for storing, integrating, managing and analyzing metagenomic data. ggKbase provides intuitive visual binning tools which display key binning traits in a dynamic fashion allowing for the quick creation and assessment of organism bins. ggKbase provides a rapid functional profiling of genomes from a community and generates a detailed analysis of the overall community composition. ggKbase integrates multiple sources of annotation (e.g. KEGG, UniRef etc.) and provides high quality metabolic pathway information. Using ggKbase we have analyzed tens of thousands of genomes from almost 1000 metagenome samples. We will present the ggKbase framework and highlight several use cases.

Keywords: metagenomics

Studies on the functions of organic matrices to make the molluscan shell microstructures.

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Biomaterials are biogenic mineralized tissues containing not only inorganic compounds, but also a small amount of organic matrices that play an important role in biomaterial formation. The mollusk shells which are typical biomaterials consisting of calcium carbonate and organic matrices, have various microstructures. The organic matrices promote the nano-cluster formation of minerals to regulate the nucleation, crystal growth, crystal orientation and crystal morphology. We want to introduce two molecular formation mechanisms of shell microstructures in two molluscan species using organic matrices.

The shells of gastropods have spiral in shape around the central axis. As the living body grows up, the shell thickness in the internal side of spiral becomes thin to expand interior space. These observations suggested that a dissolution process, working as the remodeling mechanism, changes the shell shape in molluscan shells. The molecular mechanisms of remodeling processes in the vertebrate bone have been studied well. The function of both osteoblast and osteoclast cells orchestrate the bone formation and remodeling. Although various proteins associated with shell calcification have been identified in molluscs, no organic molecules related to the dissolution of molluscan shells have been identified and the remodeling mechanism of molluscan shells is unclear.

To reveal the dissolution mechanism for the remodeling of the spiral shells in gastropod, we used the fresh water snail, *Lymnaea stagnalis*, as an experiment material and focused on chitinases of the organism. Chitinase activity was observed in the acetic acid-soluble fraction from the shell and the buffer extract from the mantle, indicating that the shell and mantle may have a function to degrade the chitin scaffold in the shell. The chitinase activity in both fractions was disappeared by the heat treatment. Allosamidin, a specific inhibitor of family 18 chitinases completely inhibited the chitinase activity of both fractions indicating that the enzyme activities in the shell and mantle were from only the family 18 chitinases. Homology cloning and transcriptome analyses from the mantle revealed five genes encoding family 18 chitinases (*chi-I*, *chi-II*, *chi-III*, *chi-IV* and *chi-V*). GH18 domain for the activity of chitin degradation was conserved in all chitinases. All chitinases were expressed not only in the mantle, but also in other tissues, suggesting that chitinases in the mantle have multiple-functions. We injected the allosamidin into living snails to inhibit the chitinase activity in the mantle. Although the chitinase activity in the mantle was strongly suppressed by allosamidin injection, the shell microstructure before and after injections was not changed. However, treatment of chitinase from *Trichoderma viride* by a commercially available altered the shell microstructure of *L. stagnalis* suggesting that the chitinase was associated with the shell dissolution process.

On the other hand, the "scaly foot" gastropods (*Chrysomallon squamiferum*) use the organic matrices to make the scales of iron sulfide on the foot and shell surface. The "scaly foot" was discovered in the Kairei deep-sea hydrothermal field of the Central Indian Ridge. The black scales consist of nano-crystal of iron sulfide minerals within a laminated organic matrix. Although iron sulfide mineralization is known in the metabolic sulfate reduction from prokaryotes, the formation mechanism of iron sulfide nano-crystal is unclear. In this study, we tried to extract the key organic components that interact with iron in the scale. Such organic components may keep the small size of iron sulfide crystals on the foot and shell surface.

Keywords: biomineralization, molluscan shell, organic matrices

Distribution of bacterial magnetites in deep-sea surface sediments and variations of magnetosome morphology with chemical conditions

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Magnetotactic bacteria (MTB) have attracted interest of paleo- and rock magnetists as a source of magnetic minerals in sediments and from a viewpoint of remanent magnetization acquisition processes. Furthermore, MTB should also play an important role for biogeochemical cycles of iron. MTB are considered to be microaerophilic and most commonly live near or below the Fe-redox boundary. However, their actual distribution within natural deep-sea sediments was little studied. Recent progress in rock magnetic techniques has enabled semi-quantitative detection of fossil biogenic magnetites (magnetofossils) in sediments. Common occurrence of magnetofossils in Pacific red clay (Yamazaki and Shimono, 2013), which contains abundant dissolved oxygen and does not have a Fe-redox boundary, may conflict with the widespread interpretations of MTB ecology mentioned above. For better understanding of the ecology of MTB in deep-sea sediments, we have conducted rock-magnetic, biogeochemical, and microbiological analyses of surface sediments taken from the Japan Sea with a multiple corer. From dissolved oxygen and Fe (II) contents of interstitial water and color reflectance of the sediments, the Fe-redox boundary was clearly detected at 7 to 25 cm below the seafloor at three sites (1770 to 2710m in water depth). Rock magnetic proxies and TEM observations indicate that magnetofossils occur throughout the sediment columns regardless of the distance from the Fe-redox boundary, even at the sediment-water interface. We found that the proportion of magnetofossils with tear-drop morphology increases near the Fe-redox boundary. On the other hand, the morphology of magnetofossils in oxic red clay is dominantly (>90%) octahedral. These results suggest that some species of MTB that produce magnetosomes of tear-drop morphology prefer a chemical condition near the Fe-redox boundary, whereas other species may live in microaerophilic microenvironments around organic particles near the water-sediment interface. Even some species of MTB that yield octahedral magnetosomes might be aerotolerant and prefer oxic environments. To strengthen the notion above, pyrosequencing of 16S rRNA gene sequences was conducted for the corresponding sediments. Among diverse bacterial lineages known to produce magnetosomes, 16S rRNA gene sequences phylogenetically affiliated within the lineages of Nitrospirae known to produce magnetosomes with tear-drop morphology were distributed only around the Fe-redox boundary, whereas those affiliated within the family Rhodospirillaceae (Alphaproteobacteria) and known to produce octahedral magnetosomes were distributed in all investigated sediments regardless of the Fe-redox boundary. Taken together, it is strongly suggested that the dependency on the Fe-redox boundary is different among phylogenetically and morphologically diverse magnetotactic bacteria.

Keywords: magnetotactic bacteria, magnetofossil, rock magnetism

Stable isotope signature of Fe to understand the Fe-biocytle in the hydrothermal-vent

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Recent progresses in isotope analysis based on the mass spectrometry technique enabled us to detect small changes of the many elements in the periodic table. Among the elements, stable isotope studies using Fe has been widely adopted to understand both the mechanism of Fe metabolism in marine organisms and the bio-cycling of Fe in marine environment. Iron is typical essential inorganic nutrients for all plants and animals. For the land organisms, the Fe isotope ratios varies significantly with increase of the trophic level (Walczyk and Blanckenburg, 2002, 2005). In strike contrast, for marine organisms, because of very limited availability of Fe in seawater, the intake efficiency for Fe could be higher than those for the terrestrial animals. Higher intake efficiency of Fe can results in smaller magnitude of isotopic fractionation through dietary process, and therefore, the difference in the Fe isotope ratios for marine organisms of lower trophic levels were close to the seawater sample (Jong et al., 2007; Bergquist and Boyle, 2006). Moreover, there were no significant difference in the Fe isotope ratios (<0.5‰) for high trophic level marine organisms between muscle and liver (Yamagata, in prep). These studies revealed that magnitude of the isotope effects on Fe can reflect both the nutritional status of Fe in animals and the availability of Fe in marine environments. To investigate this, we have measured the ⁵⁶Fe/⁵⁴Fe and ⁵⁷Fe/⁵⁴Fe for deep-sea organisms in hydrothermal field for understanding Fe bio-cycle which has both characteristics of environment, terrestrial and marine.

In this study, *Chrysomallon squamiferum* called "Scaly-foot" gastropod (n=5) and *Gigantopelta aegis* (n=5) from a deep-sea hydrothermal field at the Longqi vent field, Southwest Indian Ridge, were subsidized to the Fe isotope ratio analysis. The *Chrysomallon squamiferum* has unique scale made of iron sulphide on its foot (Suzuki et al., 2006). The *Gigantopelta aegis*, collected in the identical locations for the *Chrysomallon squamiferum*, has a thick iron oxide coating on the shell. Both the *Chrysomallon squamiferum* and *Gigantopelta aegis* has sulphur-oxidizing bacteria in oesophageal gland to form a symbiotic relation. Sclerite samples and soft body samples of muscle, ctnidium, blood, heart, and oesophageal gland were decomposed, and the Fe was extracted by anion-exchange chromatography. The Fe isotopic ratios were analyzed by a multiple collector-ICP-mass spectrometer (MC-ICP-MS) technique (Nu Plasma II) equipped with a desolvating sample introduction system and pseudo high resolution mode.

The resulting Fe isotope ratios demonstrated the distinct variations in the ⁵⁶Fe/⁵⁴Fe and ⁵⁷Fe/⁵⁴Fe ratios for *Chrysomallon squamiferum* and *Gigantopelta aegis* samples. The resulting $\delta^{56}\text{Fe}$ values for all soft body samples collected from the *Chrysomallon squamiferum* were systematically higher than those for the *Gigantopelta aegis*, whereas no significant difference in the $\delta^{56}\text{Fe}$ values could be found for oesophageal gland samples collected from *Chrysomallon squamiferum* and *Gigantopelta aegis* samples. It should be noted that the $\delta^{56}\text{Fe}$ for all the soft body samples from the *Chrysomallon squamiferum* was rather higher than those for symbiotic bacteria. This can reflect both very high intake efficiency of Fe from marine environments and the small contribution of Fe intake through the symbiotic bacterial for the *Chrysomallon squamiferum*. The Fe isotope signature obtained here demonstrate the clear difference in the Fe metabolism between *Chrysomallon squamiferum* and sulphur-oxidizing bacteria. The details of the mechanism why separate the $\delta^{56}\text{Fe}$ values of these two samples will be discussed in this presentation.

Keywords: Iron stable isotope, Fe bio-cycle, deep-sea organisms, multiple collector-ICP-mass spectrometer

Coupling of multi-range imaging mass spectrometry and isotope chronology for multidisciplinary study on life and environmental sciences

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Images of trace-elements or isotopes can provide key information to evaluate the contribution of metamorphic events or movements of elements through secondary heating events. The LA-ICPMS technique is a useful tool to obtain image data of major to trace-elements from samples. Elemental and isotope images can be obtained by repeated analysis of line-scanning measurements using the LA-ICPMS technique. Time resolved-signal intensity profile obtained by laser rastering can be converted to a position based-signal intensity profile via the relationship between the rastering rate and the elapsed time. The resulting spatial resolution and the elemental sensitivity is dependent upon several key operational parameters such as, size of laser beam, raster rate as well as time slice of the data acquisitions (ie., dwell time for each analytes). The LA-ICPMS technique is highly sensitive to determine the abundance of the trace elements. One of the main advantages to use the LA-ICPMS technique for imaging analysis is that the sample is placed under atmospheric pressure, and neither evacuation of the sample housing nor coating with conductive materials is required. Moreover, because of both the minimum sample preparation and the post-ionization system configurations, the LA-ICPMS technique represents a fast and accurate method for quantitative imaging technique for trace-elements from biochemical or geochemical samples.

Another important feature of the elemental imaging using the LA-ICPMS technique is the analytical capability for large-sized samples (>10 mm). This is of crucial importance to secure a bridge between the microscopic and macroscopic realm in geochemical studies. The elemental imaging for trace-elements is an essential tool to derive inherent information from samples. Difference in the distribution pattern of the isotopes can reflect the geochemical and cosmochemical features of the elements. Moreover, the images suggests possible contribution of a re-distribution or secondary movement of these elements through heating or weathering. In addition, difference or similarity in the distribution pattern of the elements may provide key information concerning the status of system closure for chronologies. Thus, the coupling of the elemental imaging and the isotope chronology can become a major analytical tool to obtain reliable and precise age data from the geochemical samples, including biochemical or even micro-fossil samples. Analytical capability of the imaging mass spectrometer using the LA-ICPMS technique will be demonstrated in this presentation.

Keywords: Elemental imaging, Elemental Metabolism, Isotope Chronology