

## Estimated Abundances of a Precursor of Glycine, $\text{CH}_3\text{NH}_2$

Masatoshi Ohishi<sup>1\*</sup>

<sup>1</sup>National Astronomical Observatory of Japan

It is widely accepted that prebiotic chemical evolution from small to large and complex molecules would have resulted in the Origin of Life. On the other hand there have been conflicting two views regarding where inorganic formation of organic molecules (hereafter OMs) occurred in the early Earth, in the Earth or out of the Earth. Ehrenfreund et al. (2002) indicated that exogenous delivery of OMs to the early Earth could be larger than their terrestrial formation by three orders of magnitude. If amino acids are formed in interstellar clouds, significant amount of them may be delivered by comets and/or asteroids to (extra-solar) planets. Detection of amino acids would accelerate the discussion concerning the universality of “life”.

So far, many trials to detect simplest amino acid, glycine ( $\text{CH}_2\text{NH}_2\text{COOH}$ ), were made towards Sgr B2 and other high-mass forming regions, but none of them were successful due to insufficient sensitivities and spatial resolution of telescopes. This is the background that detection of amino acids and other prebiotic in the Universe is one of the key science targets for ALMA. However we need to have carefully selected good candidate sources for amino acids before conducting searches for amino acids by ALMA since lines could be contaminated by other molecular lines. One idea would be to survey precursors of amino acids; higher abundances sources of precursors would be amino acid rich sources.

Although the chemical evolution of interstellar N-bearing OMs is poorly known, methylamine ( $\text{CH}_3\text{NH}_2$ ) is proposed as a precursor to glycine; theoretical and laboratory studies have indicated that glycine is formed on icy grain surface from methylamine and  $\text{CO}_2$  through UV irradiation (Holtom et al. *ApJ*, 626, 940 (2005), Kim and Kaiser et al., *ApJ*, 729:68 (2011)). These studies also suggest that methylamine can be formed from abundant species,  $\text{CH}_4$  and  $\text{NH}_3$ , on icy dust surface. Although  $\text{CO}_2$  is widely known in molecular clouds, distribution of methylamine is poorly known. Further methylene imine ( $\text{CH}_2\text{NH}$ ) would be related to  $\text{CH}_3\text{NH}_2$ . These species are thought to be formed through hydrogenation (addition of hydrogen) to HCN on dust surface (Dickens et al., 1997, Kim and Kaiser et al. 2011):  $\text{HCN} \rightarrow \text{CH}_2\text{NH} \rightarrow \text{CH}_3\text{NH}_2$ . This is similar to the hydrogenation of CO to form  $\text{CH}_3\text{OH}$ :  $\text{CO} \rightarrow \text{H}_2\text{CO} \rightarrow \text{CH}_3\text{OH}$ .

The first detection of methylamine was made by Kaifu et al. (1974). But even now,  $\text{CH}_3\text{NH}_2$  is known toward two objects (Orion KL and Sgr B2) only.  $\text{CH}_2\text{NH}$  has been reported only in Sgr B2, W51, Orion KL, and G34.3 (Dickens et al. 1997).

Therefore it would be crucial to estimate abundances of  $\text{CH}_3\text{NH}_2$  and  $\text{CH}_2\text{NH}$  by using a chemical reaction network, which may be compared with observations. In this paper we present preliminary results of estimated abundances of these species, which would provide good guidance in finding good glycine sources and in understanding poorly known chemistry of N-bearing organic molecules in the Universe.

Keywords: Organic Molecules in Space, Chemical Evolution, Planetary Formation

## Near-Infrared Circular Polarization Images of NGC 6334-V: Implication for Astrobiology

Jungmi Kwon<sup>1\*</sup>, Motohide Tamura<sup>1</sup>

<sup>1</sup>The Graduate University for Advanced Studies, National Astronomical Observatory of Japan

We present results from deep imaging linear and circular polarimetry of the massive star-forming region NGC 6334-V. These observations show high degrees of circular polarization (CP), as much as 22% in the Ks band, in the infrared nebula associated with the outflow. The CP has an asymmetric positive/negative pattern and is very extended (~80" or 0.65 pc). Both the high CP and its extended size are larger than those seen in the Orion CP region. Three-dimensional Monte Carlo light-scattering models are used to show that the high CP may be produced by scattering from the infrared nebula followed by dichroic extinction by an optically thick foreground cloud containing aligned dust grains. Our results show not only the magnetic field orientation of around young stellar objects, but also the structure of circumstellar matter such as outflow regions and their parent molecular cloud along the line of sight. This is the second case to support the large CP in scattering protostellar nebulae as a possible explanation for the extraterrestrial origin of homochirality of life on Earth.

## The energy transfer calculation of light harvesting systems for detecting biomarker on extrasolar planets

Yu Komatsu<sup>1\*</sup>, Masayuki Umemura<sup>1</sup>, Mitsuo Shoji<sup>1</sup>, Kazuhiro Yabana<sup>1</sup>, Kenji Shiraishi<sup>1</sup>, Katsumasa Kamiya<sup>1</sup>, Megumi Kayanuma<sup>1</sup>, Masahiko Taguchi<sup>1</sup>, Akimasa Sato<sup>1</sup>

<sup>1</sup>University of Tsukuba

A large number of extrasolar planet candidates have been detected by Kepler. Moreover the planets in habitable zone have already been detected, and ELP (Earth-like planet) is expected to be discovered. As detecting signs of life from the spectrum of ELPs in future, several indicators called as biomarkers or biosignatures were proposed [1]: (1) molecule absorption line, such as O<sub>2</sub>, CH<sub>4</sub>, and (2) red edge, which is a strong contrast in NIR (~700-750 nm) that derives from plant's feature. Red edge comes from absorption in visible by the chlorophyll, which is one of the photosynthetic pigments, and reflectance in NIR due to the structural features such as cell wall, air space of leaf and so on. The spectrum considered as red edge is observed by remote sensing or earthshine [2].

However, it is not guaranteed that red edge on extrasolar planets is detected as same wavelength on Earth. In fact, the photosynthetic organisms on Earth harvest light according to the surrounding environments to efficiently use light having a variety of wavelength that reaches, and of course their spectrum varies. In case of photosynthesis in extrasolar planets, photosynthetic organisms should evolve as optimized to utilize their principal star. We focus on the fundamental light harvesting mechanism and aim to propose how to detect the spectrum of the planet orbiting the different spectrum types of star otherwise Sun. At first, we adopted our models to photosynthetic organisms on Earth and compared with the experimental data. The light harvesting antenna in these organisms differs from kinds of pigments and their conformations.

We investigated the mechanism how the organisms harvest light by quantum mechanical calculation. However, because of the cost difficulty, we introduced an approximation instead of calculating all the electrons in the system. First, we calculated the excitation energies and the transition moments from the ground state to the excited states in the pigments by TDDFT (time-dependent density functional theory) [3]. Then, by introducing the transition moments of each pigment to the antenna, which consists of several kinds of pigments (other environment: proteins, solvent,...), we assume as an approximation that one pigment has the excited energy and interacts with the other pigments by the dipole-dipole interaction. When the light, as it seems to be considered as traveled from a star to a surface of an extrasolar planet, induces the system, we traced the time evolution of the energy transfer by solving Liouville equation. We dealt the light with an external potential. By this method, we can calculate the spectral intensity and energy efficiency.

In certain types of bacteria, the contrast like red edge can be detected, although the contrast is weaker than that of plants. In purple bacteria, red edge is not detected or emerges in longer wavelength (~1013-1025 nm). In addition, its structure of the antenna is simple so that we adopted easily our model to the bacteria. The calculated spectrum has a good agreement with the experimental result from purple bacteria. We will extend our model to the other species. By comparing the light harvesting mechanisms showing red edge and no red edge, we can examine how red edge emerges in photosynthetic organisms. For the other inducing light condition, we will survey the light harvesting mechanism optimized to extrasolar planets.

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Keywords: extrasolar planet, biomarker, quantum mechanical calculation

## Organic compounds in interplanetary dust particles and their relevance to origins of life

Kensei Kobayashi<sup>1\*</sup>, Yukinori Kawamoto<sup>1</sup>, Takuto Okabe<sup>1</sup>, Takeo Kaneko<sup>1</sup>, Yumiko Obayashi<sup>1</sup>, Kazuhiro Kanda<sup>2</sup>, Hajime Mita<sup>3</sup>, Hikaru Yabuta<sup>4</sup>, Kazumichi Nakagawa<sup>5</sup>, Kyoko Okudaira<sup>6</sup>, Hajime Yano<sup>7</sup>, Hirofumi Hashimoto<sup>7</sup>, Shin-ichi Yokobori<sup>8</sup>, Akihiko Yamagishi<sup>8</sup>

<sup>1</sup>Yokohama National University, <sup>2</sup>University of Hyogo, <sup>3</sup>Fukuoka Institute of Technology, <sup>4</sup>Osaka University, <sup>5</sup>Kobe University, <sup>6</sup>Aizu University, <sup>7</sup>JAXA/ISAS, <sup>8</sup>Tokyo University of Pharmacy and Life Science

Organic compounds including amino acid precursors and nucleic acid bases have been detected in carbonaceous chondrites, and glycine was detected from the captured cometary dust. These organics have been discussed in the context of possible organic sources for the first terrestrial life. It was suggested that more organic carbons were delivered to the early Earth by interplanetary dust particles (IDPs) than by meteorites or comets [1]. A demerit of IDPs for the carriers of extraterrestrial organics is that IDPs are so small that they are directly exposed to solar radiation that might decompose organics. Thus the presence of bioorganics in IDPs is expected, but it is difficult to judge it since IDPs (or micrometeorites) are so small and they have been collected in the terrestrial biosphere. Thus it would be of importance to study possible alteration of IDP organics in space environments, and to collect pristine IDPs out of the terrestrial biosphere.

Since carbonaceous chondrites and comets contain such organic compounds as amino acids or their precursors, IDPs that seem to be formed from meteorites and comets can also contain them. In order to study possible alteration of bioorganic compounds in IDPs, amino acids, amino acid precursors and nucleic acid bases were irradiated with high-energy particles and high-energy photons to simulate the actions of cosmic rays and solar radiation, respectively. We used glycine and isovaline as free amino acids since they are found abundant in carbonaceous chondrites. Their possible precursors, hydantoin and 5-ethyl-4-methyl hydantoin, and complex amino acid precursors synthesized from a mixture of CO, NH<sub>3</sub> and H<sub>2</sub>O (hereafter referred to as CAW) [2] were also used as irradiation targets. These molecules were irradiated with continuous light from soft X-rays to IR (hereafter referred to as soft X-rays) at the beam line 6 of NewSUBARU (the synchrotron facility of University of Hyogo). They were also irradiated with heavy ions from HIMAC (NIRS, Chiba). Irradiated samples were evaluated by amino acid analysis after acid-hydrolysis and/or by C-XANES by using the beam line 5 of NewSUBARU. Nucleic acid bases (adenine etc.) were also irradiated with soft X-rays and with heavy ions, and recovery was also determined by HPLC.

Amino acid precursors were more stable against soft X-rays than free amino acids. Water-insoluble products were formed after soft X-rays irradiation. Nucleic acid bases were more stable than amino acids and their precursors against the irradiation. Heavy ions were generally less effective than soft X-rays for decomposition or alteration of the molecules examined.

We are planning a novel astrobiology mission named Tanpopo by utilizing the Exposed Facility of Japan Experimental Module (JEM/EF) of the International Space Station (ISS). Two types of experiments will be done in the Tanpopo Mission: Capture experiments and exposure experiments.

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Keywords: interplanetary dust particles, amino acids, origins of life, complex organic compounds, soft X-rays, the Tanpopo Mission

## Interdisciplinary Perspectives on Abiogenesis

Piet Hut<sup>1\*</sup>

<sup>1</sup>Institute for Advanced Study, Earth-Life Science Institute, Tokyo, Japan

I will report on new interdisciplinary activities at the Institute for Advanced Study, in Princeton, NJ, USA, focused on the origins of life. We have recently started a series of weekly meetings, involving mathematicians, physicists, chemists, biologists, astrophysicists and others. Our discussions range from important technical details to more general meta-level discussions concerning the flow of information through biological networks and the characteristics of life in general, on Earth and possibly elsewhere in the Universe. We include scholars in humanities and linguistics, since there are interesting parallels between the origins of culture and the origins of life, and in general with the origins of "systems", where unreliable parts are used to create reliable systems. My work is inspired by my affiliation with ELSI, the new Earth-Life Science Institute at Tokyo Tech, under the WPI program of MEXT.

Keywords: origins of life, flow of information, biological networks, characteristics of life, systems

## Exposure experiments of organic compounds in space environments in the TANPOPO mission

Hajime Mita<sup>1\*</sup>, Shin-ichi Yokobori<sup>2</sup>, Hirofumi Hashimoto<sup>3</sup>, Masumi Higashide<sup>4</sup>, Eiichi Imai<sup>5</sup>, Yuko Kawaguchi<sup>2</sup>, Hideyuki Kawai<sup>6</sup>, Yukinori Kawamoto<sup>7</sup>, Kazuhiro Knada<sup>8</sup>, Kensei Kobayashi<sup>7</sup>, Kazumichi Nakagawa<sup>9</sup>, Issay Narumi<sup>10</sup>, Kyoko Okudaira<sup>11</sup>, Makoto Tabata<sup>3</sup>, Hikaru Yabuta<sup>12</sup>, Masamichi Yamashita<sup>3</sup>, Hajime Yano<sup>3</sup>, Satoshi Yoshida<sup>13</sup>, Akihiko Yamagishi<sup>2</sup>, Tanpopo WG<sup>3</sup>

<sup>1</sup>Fukuoka Inst. Technol., <sup>2</sup>Sch. Life Sci., Tokyo Univ. Pharm. Life Sci., <sup>3</sup>JAXA/ISAS, <sup>4</sup>Innovative Tech. Res. Cen., JAXA, <sup>5</sup>Nagaoka Univ. Tech., <sup>6</sup>Grad. Sch. Sci., Chiba Univ., <sup>7</sup>Grad. Sch. Eng., Yokohama Natl. Univ., <sup>8</sup>LASTI, Univ. Hyogo, <sup>9</sup>Grad. Sch. Human Develop. Environ., Kobe Univ., <sup>10</sup>Quantum Beam, JAEA, <sup>11</sup>Univ. Aizu, <sup>12</sup>Grad. Sch. Sci., Osaka Univ., <sup>13</sup>NIRS

The Tanpopo mission is a Japanese astrobiological experiment which will be conducted on the Japanese Experiment Module (JEM) of the International Space Station (ISS). The Tanpopo mission consists of six subthemes: 1) capture of microbes in space, 2) exposure of microbes in space, 3) exposure of organic compounds in space, 4) capture of organic compounds (in micrometeoroids) in space, 5) evaluation of ultra low-density aerogel developed for the Tanpopo mission, and 6) capture of space debris at the ISS orbit (approximately 400 km altitude).

Here, we overview the exposure experiment of organic compound in space environment. Since many kinds of organic compounds, especially, amino acids which are ones of most important organic compounds in living organisms, are found from extraterrestrial materials, extraterrestrial and outer-solar environments are thought as the place for the prebiotic organic compound synthesis. Then, it is proposed that the first organisms on the earth was born from the prebiotic organic compounds delivered into the early earth on meteorites, micrometeorites and/or comets. In order to discuss the possibility of the hypothesis, alteration of prebiotic compounds in space environments should be clear. Therefore, we will expose some prebiotic organic compounds on the exposure facility at ISS-JEM.

Glycine, isovaline, hydantoin, ethylmethylhydantoin and complex organics (CAW) are chosen for the exposure. Hydantoin and ethylmethylhydantoin are plausible low molecular weight precursors for glycine and isovaline, respectively. CAW which is a simulated material of interstellar medium prepared by proton radiation into mixture of CO, NH<sub>3</sub> and H<sub>2</sub>O is a different type of plausible precursors for amino acids. In the space environments, uv-light and cosmic rays (heavy ions and gamma-rays) will cause the alteration of organic compounds. Therefore, simulation experiments were studied using Xe-excimer lamp (uv 172 nm), synchrotron radiation at NewSUBARU BL06 (uv > 130 nm), <sup>60</sup>Co gamma-ray radiation (JAEA Takasaki) and carbon ion beams (290MeV, NIRS). gamma-Ray and heavy ion beam irradiation with dose of ISS environment for one year induced little decomposition of organic compounds. However, uv irradiation was critical for organic compounds. Although almost all glycine and isovaline were decomposed, remains of hydantoin and ethylmethyl hydantoin were approximately 29% and 72%, respectively, with uv dose of ISS environment for one year. Furthermore, CAW was more stable than hydantoin. Amino acids precursors, especially, complex organics were more stable than free amino acids. Therefore, extraterrestrial amino acids precursors would be effective source for origins of life on the earth. We will demonstrate this conclusion on the ISS-JEM.

In addition, Nakagawa and his colleagues were found that dialanine was formed from alanine films by uv-irradiation. We will demonstrate a peptide synthesis with uv-irradiation in the space environment. Furthermore, piece of meteorite will be also exposed in order to examine the weathering effect in the ISS environment.

Keywords: Tanpopo, exposure of organics, ISS, amino acids

## Effects of borate on the stability of ribose

Yoshihiro Furukawa<sup>1\*</sup>, Mana Horiuchi<sup>1</sup>, Takeshi Kakegawa<sup>1</sup>

<sup>1</sup>Graduate School of Science, Tohoku University

RNA is considered an important biomolecule for the origin of life, with its abilities as the carrier of genetic information and catalysts for biological reactions. RNA is composed of phosphoric acid, nucleic acid bases, and ribose. Among them, ribose is the most unstable component. Therefore, accumulation of ribose on the early Earth is an important step for the origin of RNA. Ribose as well as other aldopentoses can be abiotically synthesized by formose reaction in which formaldehyde oligomerize under alkaline conditions. Previous studies showed a stabilization of pentoses in the formose reaction by borate. In this study, we have investigated the effects of borate on the stability of aldopentoses.

Incubation experiments of each four aldopentoses were conducted at approximately 45 degree C with sodium tetraborate decahydrate of three concentrations. The pH of the experimental solution was buffered with calcium hydroxide. The experimental solution was collected at a fixed interval and analyzed with liquid chromatography-mass spectrometry.

In the borate-free experiments, all aldopentoses showed high decrease rates. In borate-containing experiments, formations of pentose-borate complexes were observed. The decrease rates for all aldopentoses were reduced with the concentration of borate. These results indicate that borate can stabilize all aldopentoses. Therefore, borate might have stabilized aldopentoses including ribose and contributed to the formation of primordial RNA on the early Earth.

Keywords: ribose, RNA, borate, origin of life

## A Possible Pathway of Homochirality Accumulation by Oligopeptides

Toratane Munegumi<sup>1\*</sup>

<sup>1</sup>Department of Science Education, Naruto University of Education

Although numerous hypotheses have been proposed for chemical-evolutionary explaining the homochirality of biomolecules, most of them have discussed the homochirality of only monomeric compounds like amino acids or monosaccharides. However, the homochirality of monomeric compounds do not always guarantee the homochirality of polymers, because there had been many energy sources which made the chiral compounds decompose in the primitive earth or extraterrestrial environments. The processes or environments to conserve the homochirality must have existed.

This research focuses the homochirality accumulation in polypeptides by oligopeptides. Dimerization of racemic amino acids usually produces diastereomeric dipeptides. If the amino acid is DL-alanine, the condensation reaction gives cyclic and acyclic LL-, LD-, DL-, and DD-diastereomers of Ala-Ala. The total seven diastereomers can be yielded, because cyclic D-Ala-L-Ala and cyclic L-Ala-D-Ala are same compounds (meso-type isomers). The diastereomers are different in the hydrophobicity, decomposition rate, epimerization rate, as well as stereochemistry. Hydrophobicity difference reflects a difference in water solubility to lead the segmentation of the diastereomers in aqueous media. Acyclic heterochiral (LD- and DL-) oligopeptides are usually more hydrophobic than acyclic homochiral (LL- and DD-) oligopeptides.

In the presentation, a scheme of the homochirality accumulation in polypeptides by oligopeptides will be shown with the processes of the segmentation, epimerization, decomposition, and stereospecific condensation of oligopeptides.

Keywords: homochirality, condensation, oligopeptides



## GADV peptide/aggregate synthesis using a hydrothermal simulator at elevated temperature

Kuhan Chandru<sup>1\*</sup>

<sup>1</sup>Faculty / Graduate School of Engineering Yokohama National University

GADV peptide/aggregate synthesis using a hydrothermal simulator at elevated temperature

Kuhan Chandru, Eiichi Imai, Yumiko Obayashi, Takeo Kaneko and Kensei Kobayashi

GADV peptide hypothesis was proposed by Ikehara (2009) as a possible alternative that precedes the RNA world hypothesis (Gilbert, 1986) due to many limitations. The hypothesis suggest a very plausible explanation, but has only been experimentally tested by Oba and co workers (2012), in terms of possible peptide formation using heat-drying cycles and their catalytic activities. We would want to examine the formation of GADV aggregates (or peptides) in an simulated hydrothermal system to represent a more realistic prebiotic environment. We have used Gly, L-Asp, L-Asp and L-Val into our Supercritical Water Flow Reactor (SCWFR) at a temperature range of 100-300 Celsius . Our initial results suggest that no visible aggregates (bigger than 0.2micrometer) were seen after the heating of 2 min in the mentioned temperature range. Initial MALDI-TOF-MS are also suggesting that we only obtained a small peaks about m/z 410 within the spectrum lesser what Oba et al (2012) which was 525, 539, 657. Although many hydrothermal simulation experiments has shown the recovery of amino acids (Islam et al 2002; Kohara et al; Kobayashi et al, 1997) and the formation of oligomers (Imai, 1999; Goto et al , 2005) we only believe, that only small aggregation occur and cannot promote bigger oligomers or polymers due to heat and pressure stress. Hence, based on our initial findings, we are very uncertain about the formation of GADV aggregates or peptides in a hydrothermal system if we use free-form amino acids. It would be of interest to investigate the hypothesis by using bound amino acids or amino acid precursors.

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Keywords: GADV, Hydrothermal, prebiotic

## Modeling the rise of oxygen after the Snowball earth: implications for the Paleoproterozoic manganese and iron formation

Mariko Harada<sup>1\*</sup>, Eiichi Tajika<sup>2</sup>, Yasuhito Sekine<sup>2</sup>, Kazumi Ozaki<sup>3</sup>

<sup>1</sup>Dept. of Earth and Planetary Science, The University of Tokyo, <sup>2</sup>Dept. of Complexity Science & Engineering, The University of Tokyo, <sup>3</sup>Atmosphere and Ocean Research Institute, The University of Tokyo

Earth's atmosphere and oceans are considered to have experienced stepwise and irreversible oxidation through its history (e.g. [1]), which may have paved the way to the complicated life such as eukaryotes and metazoans [2]. It is interesting to note that geological evidence suggests that a remarkable increase of oxygen concentration has occurred shortly after the Paleoproterozoic Snowball Earth event, based on widespread depositions of manganese and iron oxides immediately above the glacial diamictites found in the Paleoproterozoic sedimentary sequences from the Transvaal Supergroup, South Africa, and the Huronian Supergroup, Canada [3,4]. Carbonate precipitation occurs above the deposition of manganese and iron in both the Transvaal and Huronian Supergroups [3,5], which may represent a climate recovery from the greenhouse condition in the Snowball earth aftermath [3]. In this study we numerically investigated the linkage between a global-scale glaciation and an oxygenation of the atmosphere-ocean system, in the aim of comparing our results to the geological records.

The results of our numerical experiments with a biogeochemical cycle model indicate that the super greenhouse conditions ( $p\text{CO}_2 \sim 0.7 \text{ atm}$  and  $T \sim 320 \text{ K}$ ) in the aftermath of the Paleoproterozoic Snowball Earth event significantly enhance the chemical weathering of continents, causing  $\sim 10$  times as high as the present levels of nutrient input and the biological productivity. In the consequence of high levels of biological productivity together with a positive feedback in the atmosphere among a rise in oxygen, ozone formation, and UV shielding of methane, the atmospheric oxygen levels rapidly rise from  $< 10^{-5} \text{ PAL}$  to  $0.01 \text{ PAL}$  (PAL: the present atmospheric level) after the glaciation. The oxygen levels then overshoot to  $\sim 1 \text{ PAL}$  in  $\sim 10^6$  years after the glaciation due to the high levels of biological productivity sustained by a long-term global warming. Atmospheric oxygen then gradually decreases by oxidizing reducing materials from Earth's interior. Eventually, a steady state of atmospheric oxygen of  $\sim 0.01 \text{ PAL}$  is achieved in  $10^8$  years. Such an irreversible rise in atmospheric oxygen (i.e., from  $< 10^{-5} \text{ PAL}$  to  $\sim 0.01 \text{ PAL}$ ) is explained by a transition between different steady state of atmospheric oxygen levels [6] caused by a Snowball Earth glaciation and the subsequent perturbations of biogeochemical cycles.

We found that the rapid oxygenation causes the deposition of manganese and iron in the shallow marine environments. Manganese and ferrous iron in the anoxic deep water are driven by thermohaline circulation, immediately oxidized in the shallow marine water within  $10^4$  years after the glaciation. Assuming deep water is initially saturated with respect to Mn-carbonates, we derive  $10^{15} \text{ mol}$  of manganese deposition, which would be sufficient to form manganese ore in the Hotazel formation, Transvaal Supergroup ( $\sim 8 \text{ Gt}$ ,  $\sim 10^{14} \text{ mol}$  of manganese [7]). We also found that calcite precipitation is prevented in the ocean during  $10^5$  years after the glaciation owing to high atmospheric  $p\text{CO}_2$ . Our results imply that manganese and iron oxides deposition might precede the carbonate deposition, which is consistent with the geological records found both in the Transvaal and Huronian supergroups [3,4].

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## A Simple Astrobiological Scenario to go from the Great Oxygenation Event to the Origination of Eukaryotes

Joseph Kirschvink<sup>1\*</sup>

<sup>1</sup>Geological and Planetary Sciences, California Institute of Technology, Pasadena CA, USA and, <sup>2</sup>Earth/Life Science Institute, Tokyo Institute of Technology, Tokyo, Japan

Taken in the context of Earth History, it is now clear that all previously reported Archean biomarkers indicative of oxygenic photosynthesis are most likely in error, a result of contamination during sampling. Reported geochemical whiffs of oxygen from ABDP-9 are also tightly associated with hydrothermal veins of highly crystalline clinochlore, and are thus metasomatic artifacts from hydrothermal vein deposits. Firm evidence for Archean oxygen simply does not exist.

A much simpler scenario will be presented here in which photosystem-II evolves at  $\sim 3.35$  Ga, causing the radiation of the Cyanobacterial clade during the Gowganda glaciation. The resulting drawdown of CO<sub>2</sub>, and destruction of CH<sub>4</sub>, triggered the Makganyene global (snowball) glaciation, freezing the ocean surfaces for  $\sim 50$  to 100 Myr, ending at 2.22 Ga. This is immediately followed by the deposition of the Kalahari Manganese Field, which is the oldest firm geological constraint on copious quantities of free O<sub>2</sub> in Earth's environment. As there must be a time gap between the evolution of Photosystem-II and the origin of oxidative phosphorylation (aerobic respiration), Earth's carbon cycle must have been extremely out of balance. We interpret the Lomagundi/Jatuli Carbon Isotope excursion (about 2.2 to 2.06 Ga) as precisely this imbalance, where the oxygen produced by photosynthesis, and organic matter produced, could not be easily recycled. The fraction of organic carbon buried increased from the long-term average of about 20% up to about  $\sim 75\%$ , yielding isotopic compositions of carbonate as heavy as +15 per mil. Oxygen in the atmosphere is known to have increased to high levels ? perhaps several bars - resulting in the supergene alteration of the  $\sim 2.1$  Ga iron ores in the Sishen deposit of South Africa during formation of the Hekpoort Paleosol. During this Lomagundi event, the high oxygen levels would be limited by the inability of cyanobacteria to release oxygen against high back pressures, and the remineralization of organic carbon would be accomplished by oxidative weathering of sulfide minerals releasing sulfate, and by the subsequent action of sulfate reducing bacteria to break down the organic carbon.

As the radiation of the Proteobacteria post-dates the radiation of the Cyanobacteria, and the ancestor of the Eukaryotic mitochondria was once a free-living alpha-Proteobacterium, it also follows that the first organelle-bearing eukaryotes must post-date the Makganyene Snowball Earth event. Numerous eukaryotic organelles have ancestry in this bacterial endosymbiont, as do cellular components such as the hydrogenosome in basal Protists.

A puzzling organelle present in both Bacteria and Eukaryotes (including many Protists) is the magnetosome, a membrane-bound, single crystal of biological magnetite (Fe<sub>3</sub>O<sub>4</sub>) or greigite (Fe<sub>3</sub>S<sub>4</sub>). Magnetosome chains orient single cells in the geomagnetic field and allow environmental positioning. Recent work on the genetics of biomineralization in magnetotactic bacteria (MB) has shown that both the greigite and magnetite genetic pathways share a common origin, supporting an earlier suggestion that the biochemistry of magnetotaxis is monophyletic.

Small subunit RNA sequence data from hundreds of extant MB are consistent with the origin of magnetotaxis within the Bacterial domain postdating the separation of stem-group cyanobacteria, but preceding radiation of the Proteobacteria (including the mitochondrial ancestor). We propose that the free-living mitochondrion ancestor was a magnetotactic bacterium. This hypothesis presents attractive implications for adopting cytoskeletal framework throughout the eukaryotic cell, for explaining the presence of magnetosomes throughout the Eukaryotes, and for justifying the demonstrated ability of the bacterial MagA protein to induce magnetite biomineralization in mammalian cells. The radiation of the Eukaryotic Domain of Life would therefore restore balance to the carbon cycle.

Keywords: Great Oxygenation Event, Cyanobacterial Evolution, Lomagundi Event, Mitochondrial Evolution, Magnetotactic Bacteria, Sedimentary Manganese

## STEM observation of graphite from 3.8 Ga Isua Supracrustal Belt

Takeshi Kakegawa<sup>1\*</sup>

<sup>1</sup>Tohoku University

Graphite is known to occur in the 3.8 billion years old Isua Supracrustal Belt (ISB) of western Greenland, and <sup>13</sup>C-depleted graphite from sedimentary rocks of the ISB have been interpreted as traces of early life. The unequivocal documentation of biogenicity for graphite is complicated by the possibility of secondary graphite precipitation from metamorphic or igneous fluids and the difficulties in distinguishing biogenic from secondary graphite.

Graphite in meta-sedimentary rocks and carbonate veins in ISB is studied by STEM and Laser-Raman spectroscopy. Previous studies proposed that graphite in meta-sedimentary rocks are biogenic in origin, and others are chemical precipitates from CO<sub>2</sub>-rich fluids. STEM observation of graphite in meta-sedimentary rocks indicated many unique textures, resemble to carbon nano-tube. On the other hand, graphite in veins show sheet-structure-dominated features. Laser-Raman analyses indicate that sedimentary graphite recorded the peak metamorphic temperature. Graphite in veins showed lower temperature than that of the peak metamorphism. Those data comprehensively provide more evidence of biogenic graphite that differ from non-biogenic graphite in ISB.

Keywords: STEM, Raman

## Origin of life: Source rocks for the origin and evolution of life

Shigenori Maruyama<sup>1\*</sup>, Toshikazu Ebisuzaki<sup>2</sup>

<sup>1</sup>Earth-Life Science Institute, Tokyo Institute of Technology, <sup>2</sup>RIKEN

One of the key factors for the origin and evolution of life is a nutrient supply which is derived from rocks. Life cannot be synthesized under nutrient-free conditions in an atmosphere as shown in the famous experiments of Miller (1953). For the beginning of life, three components are essential; (1) C (carbon)-centered sugar for fuel, (2) P (phosphorus)-centered metabolism, and (3) N (nitrogen)-centered information coded by basic pairs (DNA). Among them, P (negative ion), which is a centered nutrient coupled with K (positive ion), plays a critical role in metabolic activity. Can those nutrients be derived from any kinds of rocks on the Earth? The answer is, yes, but only three kinds of rocks as follows

(1) Granite: Granite can be formed by two-step extractions of nutrients: partial melting of mantle peridotite at a mid-oceanic ridge followed by partial melting at a subduction zone, either by slab melting or re-melting of lower mafic crust generated by partial melting of a mantle wedge. The major nutrient elements, such as P and K, are large ion lithophile elements (LILE), and hence, difficult to be bound into major mantle minerals. Plate tectonics increases the volume of calc-alkaline rocks such as TTG (tonalite-trondhjemite-granodiorite) or andesite and dacite at subduction zones through time.

Granitic rock was absent when a magma ocean was consolidated at 4.5Ga. In the Hadean, the major rock source for nutrient supply was not granite, but rather presumably the primordial continents.

(2) Primordial continents (Anorthosite with KREEP basalts): Primordial continental material is the second candidate for the source of nutrients. No remnants of those continents remain on the modern Earth (i.e., no Hadean rocks are left). Understanding of the primordial continent is developed through the geology of the Moon, and also from the concept of giant impact. The surface of Moon is covered by 50 to 70 km-thick anorthositic crust with local cover and dikes of KREEP (Potassium, Rare Earth Elements, and Phosphorus) basalt composition, and presumably underplated by KREEP-like rock types beneath the anorthositic continent. Both rock units have been interpreted as the final residue of magma ocean when the Moon was formed by the giant impact that led to the formation of the early Earth, where Mars-sized protoplanets collided with each other. If the giant impact theory is correct, the Earth must have been completely molten even up to the core. During the gradual cooling of the Moon and the Earth, the final liquid remained near the surface, forming the buoyant anorthositic crust, covered or underplated by KREEP magma similar to that observed on the Moon.

(3) Carbonatite: Carbonatite has only a single step of fractional melting of mantle peridotites under the cratons with an extremely small degree of melting. The selective removal of melt to form considerable amounts of nutrients under the sub-cratonic mantle creates carbonatite magma enriched in nutrients with highly volatile incompatible elements such as H<sub>2</sub>O and CO<sub>2</sub> (more than 80% are volatiles). Nutrients concentrate into melt, depending on the degree of melting. Peridotite contains P=50 and K=240 (all values in ppm hereafter), but 100 times concentration of P=4495-3273 and 200-300 times of K=56118-68902 are seen in carbonatite. In general, the nutrient abundance is ideal for carbonatite, except for the U abundance. Carbonatite plays the role of milk-like materials to grow life. However, it may also function like an atomic-bomb magma to cause local mass extinctions, as well as resultant promotion of genome mutation by internal radiation through food chains.

## JAMP (Japan Astrobiology Mars Project): Search for Microbes on the Mars Surface with a Fluorescent Microscope.

Akihiko Yamagishi<sup>1\*</sup>, Yoshitaka Yoshimura<sup>2</sup>, Hajime Honda<sup>3</sup>, Atsuo Miyakawa<sup>1</sup>, Takehiko Satoh<sup>4</sup>, Genya Ishigami<sup>4</sup>, Junichi Haruyama<sup>4</sup>, Kensei Kobayashi<sup>5</sup>, Takeshi Naganuma<sup>6</sup>, Hirohide Demura<sup>7</sup>, Sho Sasaki<sup>8</sup>, Hideaki Miyamoto<sup>9</sup>

<sup>1</sup>Tokyo Univ. Pharm. Life Scie., <sup>2</sup>Tamagawa University, <sup>3</sup>Nagaoka University of Technology, <sup>4</sup>ISAS/JAXA, <sup>5</sup>Yokohama National Univ., <sup>6</sup>Hiroshima Univ., <sup>7</sup>Aizu Univ., <sup>8</sup>NAOJ, <sup>9</sup>The Univ. Tokyo

**Introduction:** Among the planets and giant satellites in our solar system, the characteristics of Mars are most similar to those of Earth. This suggests that the life similar to terrestrial life may arise and survive on Mars.

Here we propose a new life detection project on Mars within the scope of MELOS (Mars Exploration with Lander Orbiter Synergy), to search for methane-oxidizing microbes by fluorescence microscopy [1]. We propose to search for cells from a depth of about 5 - 10 cm below the surface, which is feasible with current technology. Microscopic observation has the potential to detect single cells. The subsequent analysis of amino acids will provide the information needed to define the origin of the cell.

**Survivability of Life in the Mars Environment:** Physical and chemical limits for terrestrial life have been major foci in astrobiology [2], and are summarized in ref. [1]. Combining the environmental factors, anywhere in the Martian environment where we can find the three components, water molecules, reducing compounds and oxidative compounds could be an environment where life can be sustained for long periods of time, if other factors such as temperature, pressure, UV and other radiations permit.

**Methane Oxidizing Bacteria on Earth:** Recently, a microbial consortium that is capable of using manganese (birnessite) and iron (ferrihydrite) to oxidize methane has been predicted in marine methane-seep sediments in the Eel River Basin in California [3]. Thus, there are several mechanisms of methane oxidation carried out by Bacteria and Archaea on Earth, and possibly on Mars.

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**Keywords:** Mars, microbes, fluorescence microscope, Mars exploration, Astrobiology

## Amazonis and Elysium basins and Their Link, Marte Vallis (AME), Tharsis/Elysium Corridor, Mars

James Dohm<sup>1\*</sup>, Shigenori Maruyama<sup>1</sup>, Hideaki Miyamoto<sup>2</sup>

<sup>1</sup>Earth-Life Science Institute, Tokyo Institute of Technology, <sup>2</sup>The University Museum, The University of Tokyo

The geologic provinces of Mars, as identified through a synthesis of geologic, paleohydro-logic, topographic, geophysical, spectral, and elemental information [1], are windows into its evolution, with the youthful province, the Tharsis/Elysium corridor [2-4], recording hydrologic, tectonic, and volcanic activity, including fissure-fed eruptions and shield-volcano field development, within at least tens of millions of years.

Geologically recent activity in the Tharsis/Elysium corridor region is particularly high-lighted in the Amazonis and Elysium basins and their link, Marte Vallis (hereafter referred to as AME). A youthful shield-volcano field with at least seven gentle sloping shield volcanoes and fissure-fed vent structures have been identified, mapped, and characterized in the western part of the Elysium. In addition, the lava flows on and near the margins of the shield volcanoes display crenulated lava flow margins, possibly marking lava-water-ice interactions. Both the shield volcanoes and pristine lavas located within AME, which are marked by a paucity of relatively small (~few-km-diameter) superposed impact craters and modified by faults and fractures and valleys, point to geological and hydrological activity on Mars in recent geologic time, making AME a significant target for future reconnaissance, including testing the hypotheses of whether Mars is geologically, hydrologically, and biologically active.

Specifically, the following questions might be addressed through international reconnaissance missions to AME, which would include instrument suites with optimal geologic, geochemical, geophysical (including seismic), environmental, and biological capabilities, including whether Mars: (1) is geologically and hydrologically active, (2) contains salty groundwater and magma at relatively shallow depths, (3) has sustained elevated heat flow, (4) records seismic activity, and (5) comprises fossilized and/or extant life.

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## Current status of preparation of TANPOPO mission and investigation of survivability of microbes in space

Shin-ichi Yokobori<sup>1\*</sup>, Yuko Kawaguchi<sup>1</sup>, Hirofumi Hashimoto<sup>7</sup>, HAYASHI, Nobuhiro<sup>6</sup>, HIGASHIDE, Masumi<sup>7</sup>, Eiichi Imai<sup>8</sup>, KAWAI, Hideyuki<sup>9</sup>, Kensei Kobayashi<sup>10</sup>, Hajime Mita<sup>11</sup>, NAKAGAWA, Kazumichi<sup>2</sup>, Issay Narumi<sup>3</sup>, Kyoko Okudaira<sup>12</sup>, SHIMIZU, Yasuyuki<sup>1</sup>, TABATA, Makoto<sup>9</sup>, Hikaru Yabuta<sup>14</sup>, TACHIKAWA, Sumitaka<sup>4</sup>, Kaori Tomita-Yokotani<sup>13</sup>, YAMASHITA, Masamichi<sup>4</sup>, Yinjie Yang<sup>1</sup>, Hajime Yano<sup>4</sup>, YOSHIDA, Satoshi<sup>5</sup>, Akihiko Yamagishi<sup>1</sup>

<sup>1</sup>Sch. Life Sci., Tokyo Univ. Pharm. Life Sci., <sup>2</sup>Grad. Sch. Human Develop. Environ., Kobe Univ., <sup>3</sup>Quantum Beam, JAEA, <sup>4</sup>JAXA/ISAS, <sup>5</sup>NIRS, <sup>6</sup>Grad. Sch. Biosci. Biotech., Tokyo Inst. Tech., <sup>7</sup>JAXA, <sup>8</sup>Nagaoka Univ. Tech., <sup>9</sup>Grad. Sch. Sci., Chiba Univ., <sup>10</sup>Grad. Sch. Eng. Yokohama Natl. Univ., <sup>11</sup>Fac. Eng., Fukuoka Inst. Tech., <sup>12</sup>Univ. Aizu, <sup>13</sup>Grad. Sch. Life Environ. Sci., Univ. Tsukuba, <sup>14</sup>Grad. Sch. Sci., Osaka Univ.

Microbes have been collected from high altitude using balloons, aircraft and meteorological rockets since 1936, even it is not clear how those microbes could be ejected up to such high altitude. Spore forming fungi, spore-forming Bacilli, and Deinococci (e.g. *Deinococcus aerius* and *Deinococcus aetherius*) have been isolated in these experiments. If microbes could be found even at the higher altitude of low earth orbit (400 km), the fact would endorse the possibility of interplanetary migration of terrestrial life.

For the origin of life on Earth emerged within a short period after the end of heavy bombardment, Panspermia hypothesis was proposed. Recent the reports on the possible fossils of microbes in the Martian meteorite promote the debate on the possible existence of extraterrestrial life, and interplanetary migration of life as well.

On the other hand, it is the question where precursors of materials such as protein and nucleic acids came from in the era of "chemical evolution" on the Earth? Recent studies suggest that the some of such organic compounds were created in space. Then, they reached the surface of Earth via meteorites, cosmic dusts, and so on. Avoiding contamination of terrestrial materials from the extraterrestrial materials is quite important issues for the analysis of extraterrestrial materials. Capturing such extraterrestrial materials before falling down on the surface of Earth might be one of possible solutions.

We have proposed a mission, named TANPOPO, to examine possible interplanetary migration of microbes, and organic compounds at the Exposure Facility of Japan Experimental Module (JEM) of the International Space Station (ISS). The Tanpopo mission consists of six subthemes ? capture of microbes in space, exposure of microbes in space, capture of organic compounds in space, exposure of organic compounds in space, measurement of space debris at the ISS orbit, and evaluation of ultra low-density aerogel special for the TANPOPO mission.

Ultra low-density aerogel will capture micrometeoroid and space debris. Particles captured by aerogel will be analyzed from biological, chemical, and meteorological aspects.

In addition to particle-capture on ISS, we also proposed direct exposure experiments of microbial cell aggregates that might protect the microbes themselves from UV and cosmic rays. *Deinococci* (*Deinococcus radiodurans*, *D. aerius*, and *D. aetherius*), terrestrial cyanobacteria, and fungi are under consideration for space exposure. Amino acids and complex organic compounds that can be formed in space are also planned for space exposure.

In this paper, we overview the TANPOPO mission and discuss the current status of experiments related to the microbe existence/survival set for this mission.

Keywords: Panspermia, Space exposure, Origin of life

## The sub-millimeter-sized aggregated deinococcal cells could be shield from solar UV

Yuko Kawaguchi<sup>1\*</sup>, Yinjie Yang<sup>1</sup>, Narutoshi Kawashiri<sup>1</sup>, Keisuke Shiraishi<sup>1</sup>, Masako Takasu<sup>1</sup>, Issay Narumi<sup>2</sup>, Katsuya Satoh<sup>2</sup>, Hirofumi Hashimoto<sup>3</sup>, Kazumichi Nakagawa<sup>4</sup>, Yoshiaki Tanigawa<sup>4</sup>, Yoh-hei Momoki<sup>4</sup>, Maiko Tanabe<sup>4</sup>, Tomohiro Sugino<sup>1</sup>, Yuta Takahashi<sup>1</sup>, Yasuyuki Shimizu<sup>1</sup>, Satoshi Yoshida<sup>5</sup>, Kensei Kobayashi<sup>6</sup>, Shin-ichi Yokobori<sup>1</sup>, Akihiko Yamagishi<sup>1</sup>

<sup>1</sup>Department of Applied Molecular Biology, School of Life Sciences, Tokyo University of Pharmacy and L, <sup>2</sup>Ion Beam Mutagenesis Research Group, Quantum Beam Science Directorate, <sup>3</sup>Institute of Space and Astronautical Science, JAXA, <sup>4</sup>Graduate School of Human Development and Environment, Kobe University, <sup>5</sup>National Institute of Radiological Sciences, <sup>6</sup>Graduate School of Engineering, Yokohama National University, <sup>7</sup>Protein Metabolism Project, Tokyo Metropolitan Institute of Medical Science

To investigate the interplanetary transfer of life, numerous exposure experiments have been carried out on various microbes in space since 1960s. The results suggested that microbe spores might survive for a long period if the spores are shielded from intense solar radiation [1]. In the Tanpopo mission, we have proposed to carry out the experiments on capture and space exposure of microbes at International Space Station (ISS) [2]. Microbial candidates for the exposure experiments in space include *Deinococcus radiodurans*, *D. aerius* and *D. aetherius*. We have examined the survivability of *Deinococcus* spp. under the environmental conditions on ISS in orbit (i.e., long exposure to heavy-ion beams, temperature cycles, vacuum and UV irradiation). Among the space environmental factors, solar UV is most lethal to microbes, and damage is caused by the absorption of UV by DNA [3].

In this report, we examined the effect of solar UV radiation (172 nm, 254 nm and 280-315 nm respectively) on the deinococcal cell aggregates with different thicknesses to determine whether the size of the cell aggregate influences the cell survivability. Though the cells in thin layers of aggregates were killed by UV radiation, large number of cells survived the radiation when the cell layer was thick. The similar trend of survivability was observed for other UV range. Supposing that the aggregates are sphere, the diameter of the aggregate that is sufficient to shield the cells in the inner layer from solar UV radiation is 200 micrometer for *D. radiodurans*, 850 micrometer for *D. aerius*, and 700 micrometer for *D. aetherius*. We propose the microbial cell aggregate as an ark for the interplanetary transfer of microbes, and name it the 'masspanspermia' hypothesis.

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Keywords: Panspermia hypothesis, Space exposure experiments, Deinococcus, Cell aggregation, Massapanspermia hypothesis

## Restricting the amino acid usage of a resurrected ancestral protein

Satoshi Akanuma<sup>1\*</sup>, Masami Shimada<sup>1</sup>, Yoshiki Nakajima<sup>1</sup>, Shin-ichi Yokobori<sup>1</sup>, Akihiko Yamagishi<sup>1</sup>

<sup>1</sup>Department of Applied Bioscience, Tokyo University of Pharmacy and Life Sciences

It is common among life on Earth that proteins are composed of 20 or nearly 20 kinds of amino acids. However, it is uncertain that, from the standpoint of astrobiology, proteins are always composed of the 20 kinds of amino acids. Even for the evolution of proteins on Earth, it has been argued that primordial proteins that existed before the last universal common ancestor involved less than 20 kinds of amino acids (Crick, 1968). Given simpler protein synthesis system, the primitive proteins, which might have comprised a reduced set of amino acids, must have had a sufficiently adequate structure for functional interactions and catalysis. To address this issue experimentally, we used the protein simplification engineering (Akanuma et al., 2002) to examine whether a protein composed of less than 20 types of amino acids can retain its stable structure and biological function. To this end, we first resurrected several ancestral proteins and then restricted the amino acid usage of a resurrected protein to a reduced amino acid set.

As the model protein, we chose a house keeping enzyme, nucleoside diphosphate kinase (NDK). Because extant genes are evolutionary descendants of ancient genes, ancestral sequences of a particular protein can be inferred by comparing extant homologous protein sequences. Along this line, we first inferred several ancestral amino acid sequences of NDK by phylogenetic analysis of the extant homologous sequences. The inferred sequences were then genetically reconstructed. One of the reconstructed protein, Arc1, is very thermally stable (unfolding temperature = 113°C) and shows catalytic efficiency similar to those of the modern NDKs.

It is currently impossible to infer amino acid sequences that existed before the last universal common ancestor. Therefore, using Arc1 as the starting molecule, we reconstructed a reduced amino acid set variant, Arc1-s2, in which Met, Gln, Lys, Tyr and Asn were replaced by other amino acids. Because cysteine is absent from Arc1, Arc1-s2 consists of only 14 amino acid letters. Arc1-s2 retains thermal stability similar to that of Arc1; whereas, no detectable level of catalytic activity was observed for Arc1-s2. Therefore, the fourteen amino acid types are sufficient to encode a thermally stable protein but more amino acid types would be required for its function. To reevaluate the individual contributions of the 20 amino acid types to the stability and activity of Arc1, we reconstructed 19 Arc1 variants in which one of the 19 amino acid types was all replaced by other amino acids. The result will be also present in the meeting.

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## Theoretical investigation on the absorption spectrum of photosystem for the biomarker of extrasolar planets

Masahiko Taguchi<sup>1</sup>, Yu Komatsu<sup>1</sup>, Akimasa Sato<sup>1</sup>, Mitsuo Shoji<sup>1\*</sup>, Megumi Kayanuma<sup>2</sup>, Katsumasa Kamiya<sup>1</sup>, Kenji Shiraishi<sup>1</sup>, Kazuhiro Yabana<sup>1</sup>, Masayuki Umemura<sup>1</sup>

<sup>1</sup>Graduate School of Pure and Applied Science, University of Tsukuba, <sup>2</sup>Department of Computer Science, University of Tsukuba

Over 800 extrasolar planets have been discovered, and more than 20,000 candidates have been detected. Planets in habitable zone have been observed, and the discovery of Earth-like planets is expected. Great attentions have been paid to the detection of life in extrasolar planets. For the detection, various indices have been proposed as biomarkers. One of the indicators is red edge[1], which is a characteristic steep gradient observed in the near-infrared region of around 750 nm in plant's reflection spectra [2]. In fact, red edge can be observed in the reflection spectrum of the Earth via the Moon (earthshine [3]). Since red edge is affected by many factors, its precise predictions is not simple. However, a leaf chlorophyll absorption is thought to be the major factor of red edge [2].

On Earth, photosynthetic organisms have evolved through the collection of sunlight. On the other hand for the extrasolar planets, whose surrounding space environment has different spectrum from their primary star, photosystems should be different in many parts, such as pigment types and arrangements even though the environment is similar to the Earth.

Before predictions of biomarkers of extrasolar planets, we examine the basic characters of chlorophylls in photosystems. It is also important for the study of the diverse photosystems on Earth. Chlorophylls are concentrated in a chloroplast, and form pigments-protein complexes in the photochemical systems.

The purpose of this research is to characterize the absorption spectrum of chlorophylls in a photosystem. First of all, we calculated the absorption spectrum of the pigment in methanol using DFT based polarization continuum model (PCM) method, and confirmed the validity of our calculation method. Then, quantum mechanics/molecular mechanics (QM/MM) calculations were performed for the absorption spectra of the photosystem. Each chlorophyll was included in the QM region. We found that the absorption wavelengths are shifted about +10 nm by the effects of the protein environment. Similar influence was observed by the effect of amino acid coordination to the central Mg ion in the chlorophyll. These calculated results indicate a fine modulation character of the adsorption wavelength for the photosystem. This character is important for photosystems in extrasolar planets as well as in extreme conditions on Earth.

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Keywords: biomarker, extrasolar planet, photosystem, QM/MM, absorption spectrum

## Experimental studies on abiotic formation of amino acid precursors from interstellar media by cosmic rays

Midori Eto<sup>1\*</sup>, Takuto Okabe<sup>1</sup>, Takeo Kaneko<sup>1</sup>, Yumiko Obayashi<sup>1</sup>, Hitoshi Fukuda<sup>2</sup>, Yoshiyuki Oguri<sup>2</sup>, Satoshi Yoshida<sup>3</sup>, Ken-  
sei Kobayashi<sup>1</sup>

<sup>1</sup>Yokohama National University, <sup>2</sup>Tokyo Institute of Technology, <sup>3</sup>National Institute of Radiological Sciences

Various organic compounds including amino acid precursors have been found in extraterrestrial bodies such as meteorites and comets, and their relevance to the origin of life are discussed. It has been suggested that these extraterrestrial organics were formed in interstellar media in dense clouds. In the present study, we examined possible formation of amino acid precursors from interstellar media by irradiation of high-energy protons or heavy ions.

Carbon monoxide (350 Torr), ammonia (350 Torr) and liquid water (5 mL) was put in a Pyrex tube, and the gas mixture was irradiated with 2.5 MeV protons from a Tandem accelerator (Tokyo Institute of Technology). Total electric quantity irradiated was 1 - 4 mC, and the products were hereafter referred to as CAW. Gas mixtures of carbon monoxide (350 Torr) and ammonia (87.5 - 350 Torr) were also irradiated with 2 mC of 2.5 MeV protons, and the products were referred to as CA. A mixture of methanol, ammonia and water (molar ratio was 1:1:2.8) was irradiated with heavy ions (290 MeV/u carbon ions, or 500 MeV/u argon ions) from HIMAC, NIRS, Japan. Total irradiation dose was 1.5 - 15 kGy, and the products were referred to as MeAW. All the irradiated products were acid-hydrolyzed and then were subjected to amino acid analysis by HPLC and/or GC/MS.

When the gas mixture was irradiated with protons, white mist was formed in the gas phase that suggested that high molecular weight organic compounds were produced in the gas phase by the action of high-energy protons. The products dissolved water yielded a wide variety of amino acids after acid-hydrolysis. In all the CAW and CA products, glycine was predominant, followed by aspartic acid, serine, alpha-aminobutyric acid and beta-alanine. Yield of each amino acid was proportionate to the total electric quantity (or the total dose to the gas mixture). Heterocyclic compounds including uracil were also identified in CAW. MeAW also yielded glycine and other amino acids after acid-hydrolysis, but the yield of amino acids was not proportionate to the total dose.

The facts that solid products (mist) were formed in the gas mixture by proton irradiation and that the amino acid yield were proportionate to the dose showed that high molecular weight amino acid precursors were formed directly from the gas mixtures. Further study including irradiation to simulated interstellar ices will be done to examine possible formation mechanisms of amino acid precursors in space.

Keywords: cosmic rays, interstellar media, amino acid precursors, proton irradiation, origins of life, heavy ions

## Preliminary examination plan and subsequent analytical procedure of captured samples by aerogel in the Tanpopo mission

Kyoko Okudaira<sup>1\*</sup>, Akihiko Yamagishi<sup>2</sup>, Hajime Yano<sup>3</sup>, Hikaru Yabuta<sup>4</sup>, Makoto Tabata<sup>3</sup>, Shin-ichi Yokobori<sup>2</sup>, Eiichi Imai<sup>5</sup>, Kensei Kobayashi<sup>6</sup>, Hajime Mita<sup>7</sup>, Hideyuki Kawai<sup>8</sup>, Masumi Higashide<sup>3</sup>, Satoshi Sasaki<sup>9</sup>, Tanpopo WG<sup>3</sup>

<sup>1</sup>The University of Aizu, <sup>2</sup>Tokyo University of Pharmacy and Life Science, <sup>3</sup>JAXA, <sup>4</sup>Osaka University, <sup>5</sup>Nagaoka University of Technology, <sup>6</sup>Yokohama National University, <sup>7</sup>Fukuoka Institute of Technology, <sup>8</sup>Chiba University, <sup>9</sup>Tokyo University of Technology

The Tanpopo mission is a Japanese astrobiological experiment which will be conducted on the Japanese Experiment Module (JEM) of the International Space Station (ISS) [1]. The Tanpopo mission consists of several subthemes: 1) capture of microbes in space, 2) exposure of microbes in space, 3) exposure of organic compounds in space, 4) capture of organic compounds (in micrometeoroids) in space, 5) evaluation of ultra low-density aerogel developed for the Tanpopo mission, and 6) capture of space debris at the ISS orbit (approximately 400 km altitude).

Here, we overview Preliminary Examination Team (PET) analysis and subsequent analytical procedure of aerogel samples for the mission, i.e. analyses for the subthemes 1, 3, 5, and 6 described above. Silica aerogel with  $0.01 \text{ g cm}^{-3}$  density supported by higher density aerogel [2] will be used to capture micrometeoroid and space debris at LEO. Captured particles and their penetration tracks will be offered for various analyses after retrieval to Earth. These samples will be analyzed for mineralogical, organic and microbiological characteristics.

In this paper, current status of Tanpopo-Aerogel-PET preparation will be introduced. In Preliminary Examination (PE), Curation team covers the receipt of retrieved samples (Sample Aerogel Panels), sample catalog preparation for data archiving and sample storage. Whole documentation team deals with penetration track mapping, penetration track measurement (e.g. incoming angle, track depth and track volume) and evaluation of aerogel as a capture medium. Processing team prepares keystones and quickstones (small pieces of aerogel) containing particles and their penetration tracks for allocation to researchers. After preliminary characterization, the samples (tracks and/or particles in keystones/quickstones) will be properly processed in accordance with a request by each sub team for the subsequent detailed analyses.

Aerogel panels attached to zenith (space)-facing side will be allocated mainly to Organic and Inorganic Sub-Teams, and ones attached to ram-facing side (facing east) to both Debris and Microbe (terrestrial origin) Sub-Teams, while ones facing north to all Sub-Teams. We plan to preserve basically one of each aerogel panel for storage in the scope of future analyses and possible provision to researchers.

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Keywords: Tanpopo mission, International Space Station, Silica aerogel, Micrometeoroid, Space debris, Curation

## Analysis of amino acids in small particles captured with aerogel

Taka Kogawa<sup>1\*</sup>, Yuko Kawaguchi<sup>2</sup>, Kyoko Okudaira<sup>3</sup>, Hajime Mita<sup>4</sup>, Makoto Tabata<sup>5</sup>, Eiichi Imai<sup>6</sup>, Sunao Hasegawa<sup>5</sup>, Hideyuki Kawai<sup>7</sup>, Hajime Yano<sup>5</sup>, Hirofumi Hashimoto<sup>5</sup>, Shin-ichi Yokobori<sup>2</sup>, Akihiko Yamagishi<sup>2</sup>, Yumiko Obayashi<sup>1</sup>, Takeo Kaneko<sup>1</sup>, Kensei Kobayashi<sup>1</sup>

<sup>1</sup>Yokohama National University, <sup>2</sup>Tokyo University of Pharmacy and Life Science, <sup>3</sup>Aizu University, <sup>4</sup>Fukuoka Institute of Technology, <sup>5</sup>JAXA/ISAS, <sup>6</sup>Nagaoka University of Technology, <sup>7</sup>Chiba University

Amino acids have been detected in such extraterrestrial bodies as carbonaceous chondrites and comets, and their relevance to the origin of life on the Earth is discussed. We are planning a space experiment named the Tanpopo Mission, where several experiments including capture of space dusts and exposure of organic compounds and microorganisms. As to the capture experiments, several aerogel blocks will be attached on several faces of an integrated experimental rack that will be placed on JEM/EF of ISS. High-speed dusts will make tracks in the aerogel. After recovering them to the Earth, we will separate each track with a terminal grain, and will apply to chemical analysis, including microscopic techniques (FT-IR, STXM-XANES, etc.) and amino acid enantiomers analysis after acid hydrolysis.

Amino acid is one of the main target molecules to be found in the capture experiments. We have tested whether hypervelocity dusts can be trapped in aerogel by using a two-stage light gas gun equipped in JAXA/ISAS. Samples such as amino acids adsorbed to porous silica gel and powder of Murchison meteorite were shot out at 4 - 6 km/s, and were captured in an aerogel to see whether organics could be recovered in the terminal grains or tracks. The aerogel block containing tracks of high-velocity particles was digested with HF-HNO<sub>3</sub> in a Teflon container. The digested solution was then acid-hydrolyzed with 6 M HCl, was desalted with a solid-state extraction column (MonoSpin SCX), and amino acids were determined by cation-exchange HPLC after post-column derivatization for fluorometric detection.

It is of quite importance to reduce amino acids in a procedural blank. The current situation in reducing the procedural blank will be presented.

Keywords: the Tanpopo Mission, interplanetary dust particles, small particles, microorganisms, aerogel, amino acids

## Synthesis of an amino acid from carboxylic acid and ammonia with shock wave

Chizuka Suzuki<sup>1\*</sup>, Yoshihiro Furukawa<sup>1</sup>, Takamichi Kobayashi<sup>2</sup>, Toshimori Sekine<sup>3</sup>, Takeshi Kakegawa<sup>1</sup>

<sup>1</sup>Graduate School of Science, Tohoku University, <sup>2</sup>National Institute for Materials Science, <sup>3</sup>Graduate School of Science, Hiroshima University

Proteins are composed of twenty kinds of amino acids and are essential biomolecules for life on the Earth. Therefore, origins of amino acids on the early Earth have been an important concern. Many previous studies indicate that the late heavy bombardment (LHB) of extraterrestrial objects had occurred during 3.8-4.0 billion years ago. These impacts might have delivered and produced prebiotic organic compounds including amino acids, amines, and carboxylic acids as well as ammonia (Cronin and Pizzarello et al., 1988; Furukawa et al. 2009). However, the number of biomolecule by these processes was limited number of amino acids among protein-constituent amino acids. The organic compounds supplied by the impacts of extraterrestrial objects to the oceans must have experienced further impacts, because the LHB is a successive impact event. In this study, we demonstrated shock-recovery experiments on a solution of formic acid and ammonia to investigate whether amino acids form from low molecular weight organic compounds by oceanic impacts on the early Earth.

Shock-recovery experiments were performed with a single-stage propellant gun using an improved sample container. Starting material is a mixture of <sup>13</sup>C-formic acid and ammonia. After the impact experiments, soluble organic compounds were extracted into water and then amines and amino acids were analyzed with liquid chromatography-mass spectrometer (LC/MS). Glycine, methylamine and ethylamine whose carbons are composed of <sup>13</sup>C were identified in all of the samples. The amounts of glycine were almost constant regardless of the impact velocity (0.7-0.8 km/s). The amounts of produced amines increased depending on the impact velocity. The present results suggest that shock wave converts a low molecular weight organic compound to larger molecular weight organic compounds. The successive impacts might have contributed to chemical evolution providing variety in biomolecules on the prebiotic Earth.



## Enantiomer-specific isotope analysis (ESIA): D- and L-amino acids by biotic and abiotic processes

Yoshinori Takano<sup>1\*</sup>

<sup>1</sup>JAMSTEC

### Introduction

The one-handedness of terrestrial L-amino acids in proteins and in D-sugars of DNA and RNA are primary formation, structure and function of biopolymers for life on the Earth. Recently D-alanine has been recognized as a physiologically essential enantiomer for microbial growth and metabolic maintenance. The cell wall of domain Bacteria, especially for Gram-positive Bacteria, consists of a thick and uniform peptidoglycan layer that includes D-amino acids. Laboratory studies of the degradation of peptidoglycan showed it to decompose more slowly than proteins, indicating semi-labile compounds in nature. We have developed an analytical method to determine the ESIA of individual amino acid enantiomers and revealed nitrogen isotopic hetero- and homogeneity for D-alanine and L-alanine in terms of microbial processes in domain Bacteria and chemical processes in organic symmetric synthesis.

### Experimental

The nitrogen isotopic composition of the individual amino acids was determined using a gas chromatograph/combustion/isotope ratio mass spectrometer (GC/C/IRMS) with a ThermoFinnigan Delta Plus XP combined with an Agilent Technologies 6890N GC and an Ultra-2 capillary column. Novel derivatization of amino acid diastereomers by optically active (R)-(-)-2-butanol or (S)-(+)-2-butanol with pivaloyl chloride produces N-pivaloyl-(R,S)-2-butyl esters (NP/2Bu) of the amino acid diastereomers. The elution order of these compounds on the chromatogram can be switched by a designated esterification reaction. We used purified peptidoglycans from domain Bacteria (phylum Firmicutes and Actinobacteria; *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus staphylolyticus*, *Lactobacillus acidophilus*, *Bacillus subtilis*, *Micrococcus luteus* and *Streptomyces* sp.), (pseudo)-peptidoglycan from domain Archaea (*Methanobacterium* sp.), cell walls from domain Eukarya (*Saccharomyces cerevisiae*). Racemic D- and L-alanine were synthesized by a nucleophilic substitution 1 (SN1) reaction via an intermediate carbocation formed between alpha-bromopropionic acid (as amino acid racemic precursors) and aqueous ammonia.

### Results and Discussion

The nitrogen isotopic difference of peptidoglycan defined as Delta15ND-L in bacteria, representative gram-positive phylum Firmicutes and Actinobacteria, tended to be 15N-depleted in D-alanine, suggesting that heterogeneous components are mainly controlled by enzymatic pathways prior to formation of the bacterial cell wall. Alanine racemase (Enzyme Commission, EC; 5.1.1.1) that interconvert L-alanine to D-alanine, one of isomerases for chiral amino acids, previously identified in a biosynthetic pathway, participates in crucial enzymatic reaction to form D-alanine before D-alanine-D-alanine ligase (EC; 6.3.2.4) pathway in peptidoglycan metabolism. In contrast, the Delta15ND-L of racemic alanine in the chemical pathway during the nucleophilic substitution reaction between 2-bromopropionic acid and ammonia showed infinitely homogeneous components for each enantiomers. We present recent preliminary results in terms of abiotic geochemical samples for ESIA.

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## Effects of glycine and its decomposition products on polymerization of methionine under high temperature and pressure

Rui Huang<sup>1\*</sup>, Yoshihiro Furukawa<sup>1</sup>, Takeshi Kakegawa<sup>1</sup>

<sup>1</sup>Graduate school of Science, Tohoku University

It is widely believed that abiotic polymerization of amino acids is an important process for the formation of the first life. Several geological settings have been proposed as the place for the polymerization: sub-marine hydrothermal vents (Imai et al., 1999), tidal flats (Lahav et al., 1978), and marine sediments (Nakazawa et al., 1993). A unique point of the marine sediment is its pressurized conditions. Previous studies have suggested the importance of pressurized conditions for the production of longer peptides (Ohara et al., 2007; Otake et al., 2011; Furukawa et al., 2012). These previous studies also indicate that the reactivity of each amino acid is widely different, leading to skepticism about the formation of peptides composed of plural amino acids. In this study, we investigated oligomerization of methionine and glycine under the conditions of high temperature and high pressure (at 175°C, 150 MPa, and 0-96 hours).

Methionine and glycine were used for representatives of each low and high reactive amino acid, respectively. Starting materials were solid methionine or solid methionine mixed with solid glycine, water, aqueous ammonia, or ammonium hydrogen carbonate. The additives other than glycine (water, aqueous ammonia, and ammonium hydrogen carbonate) are simulated decomposition products of glycine. Ammonium hydrogen carbonate decompose at about 60°C and yields ammonia, carbon dioxide, and water. For each starting material, 0.43 mmol of methionine were used. The amounts of each additive were 0.43 mmol. Each starting material was sealed into a gold tube of 25 mm length and 5.5mm diameter. Then, high temperature and pressure conditions were applied using a test-tube-type autoclave system. After these experiments, amino acids and their oligomers were extracted into aqueous solution from the experimental products and analyzed with a high performance liquid chromatograph connected to a mass spectrometer (LC/MS).

In all experiments, methionine decomposed with elapsed time. Peptides longer than di-methionine were not formed in experiments without the additives. On the other hand, methionine was oligomerized to di-methionine, tri-methionine and methionine diketopiperazine in the experiments with additives. Methionyl-glycine and glycy-methionine were also produced in experiments containing such additives. The rates of methionine decomposition and methionine-peptide formation were increased in experiments with additives. These rates were especially increased in samples containing aqueous ammonia, and ammonium hydrogen carbonate, suggesting that ammonia promote both the production rates of peptides and the decomposition reactions of methionine. The difference in reaction rates might have been caused by the difference in pH as suggested in a previous study (Sakata et al., 2010). When these results are applied to diagenesis in Hadean marine sediments, these results suggest that amino acids of lower reactivity may have been activated by amino acids of higher reactivity and might have produced peptides composed of plural amino acids.

## Open system incubation experiments of glycine-montmorillonite-water mixture at high temperature and high pressure

Kentaro Oguchi<sup>1\*</sup>, FURUKAWA, Yoshihiro<sup>1</sup>, KAKEGAWA, Takeshi<sup>1</sup>

<sup>1</sup>Graduate School of Science, Tohoku University

There are several theories as to the place where primordial protein formed. Most of studies assume that polymerization of amino acids occurred in oceanic environments. However, there is a difficulty in the oligomerization of amino acids in oceans where huge amount of water exit, because the oligomerization of amino acid is a dehydration reaction. To address this contradiction, a model that hypnotizes the oligomerization proceeded in oceanic sediments was proposed. The effects of pressure in this model have been investigated previously. Clay minerals play an important role for the accumulation of amino acids in this model. However, the effect of clay minerals remains unclear. Therefore, this study investigated the effects of a typical clay mineral on the oligomerization of amino acids in a simulated diagenetic condition.

Glycine (Gly) adsorbed on montmorillonite was compressed and heated at 90°C and 9 MPa with a piston cylinder for 7 days using silica powder as a pressure medium. Samples were collected and divided into three sections (S1, S2, and C1). The S1 was the outermost part of the sample, which was composed of mainly silica. S2 was the part between S1 and C1. Most of the S2 sample was silica. C1, the part of the center of the sample, was composed of mainly montmorillonite. Gly and peptides in these three samples were extracted with ammonia water. The extracted solution were filtered and concentrated to analyze the amount of Gly and peptides with LC/MS. Results show that Gly, diketopiperazine of Gly (Gly<sub>DKP</sub>), and Gly dimer (Gly<sub>2</sub>) were detected from three samples. The amount of Gly<sub>2</sub> and Gly<sub>DKP</sub> were higher in C1 than in S1 and S2. Therefore, montmorillonite was considered to be effective to form peptides.

## Effect of oxygen fugacity in chemical processes of alanine during oceanic impacts of meteorites

Yuhei Umeda<sup>1\*</sup>, Toshimori Sekine<sup>1</sup>, Yoshihiro Furukawa<sup>2</sup>, Takeshi Kakegawa<sup>2</sup>, KOBAYASHI, Takamichi<sup>3</sup>

<sup>1</sup>Graduate School of Science, Hiroshima University, <sup>2</sup>Graduate School of Science, Tohoku University, <sup>3</sup>National Institute for Materials Science

The biomolecules on the Earth are thought either to have come from the extraterrestrial parts carried with flying meteorites or to have been formed on the Earth from the inorganic materials through given energy. From the standpoint to address the importance of impact energy, it is required to simulate experimentally the chemical reactions during impacts, because violent impacts may have occurred 3.8-4.0 Gyr ago to create biomolecules initially. It has been demonstrated that shock reactions between ocean and meteoritic constitutions can induce locally reduction environment to form bioorganic molecules such as amino acid (Nakazawa et al., 2005; Furukawa et al., 2009). We need to know possible processes how further chemical evolutions proceed by repeated impacts and how more complicated biomolecules are formed.

In this study, we carried out shock recovery experiments to investigate the chemical reactions of alanine in aqueous solutions and the effect of oxygen fugacity. Experiments were carried out with a propellant gun. We used alanine labeled by <sup>13</sup>C to distinguish products from contaminants. Sample of aqueous solution immersed in olivine or hematite powders, sealed in a stainless steel container, was used as a target. The sample space has air gap behind the sample. The powder, solution, and air correspond to meteorite, ocean, and atmosphere on early Earth, respectively. Two powders of olivine and hematite can keep the oxygen fugacity low and high during experiments, respectively. After shots, the steel containers, after cleaned, were immersed into liquid nitrogen for sample solution to be frozen and then we drilled on the impact surface to extract water-soluble components from the sample using pure water in a beaker. After that, water-soluble components were analyzed by LC/MS for four amino acids (glycine, alanine, valine, and phenylalanine) and four amines (methylamine, ethylamine, propylamine, and butylamine).

The results indicate the formation of decomposition products (glycine, methylamine, ethylamine, and propylamine) of alanine and butylamine as a new biomolecule. However, the results did not detect any formation of valine and phenylalanine those could be expected to form by reactions. Glycine and some amines were detected in samples under low oxygen fugacity, while these molecules were hardly detected in samples under high oxygen fugacity. Therefore, oxidative conditions are not preferable to the formation of biomolecules. On the other hand, the present experimental results suggest that the survival rate of alanine depends on pressure and temperature but that it is not dependent on oxygen fugacity. In applying the present results to actual meteorite impacts, the physical condition during impact is a key factor in chemical reactions, although it also must be taken into account the heterogeneous distribution of impact energy in an impact that may cause a significant effect on the chemical reactions.

## Diastereoisomeric excess of Ala-DKP during condensation of racemic-Ala on olivine under hydrothermal condition

Shigeshi Fuchida<sup>1\*</sup>, Hiroshi Naraoka<sup>2</sup>, Harue Masuda<sup>1</sup>

<sup>1</sup>Faculty of Science, Osaka City University, <sup>2</sup>Department of Earth and Planetary Science, Kyusyu University

### <Introduction>

When peptides are abiotically formed from amino acid monomers under thermal condition, cyclic dipeptides, diketopiperazine (DKP), are intensely dominant. DKP was defined as an obstacle for peptide elongation (Basiuk et al., 1990), while, Nagayama et al. (1990) supposed that the DKP was an effective intermediate phase to provide internal free energy necessary to form additional peptide bond. If DKP play as an intermediate phase, DKP formation must be important as the first step of chemical evolution of peptides continuing to life. Minerals promote the DKP formation under laboratory thermally condition (e.g., Bujdak and Rode, 1996; Meng et al., 2004); e.g., DKP formation would be promoted on olivine surface, where amino acid monomers are dehydrated and the olivine is hydrated (serpentinization). Diastereoisomeric DKPs (*cis/trans*) are formed, when chiral amino acids are dimerized. In this study, the diastereoisomeric excess (*de*) of DKP formed from the simplest chiral amino acid, alanine (Ala), was observed on the surface of olivine (during aqueous reaction with olivine) at 120 degree C for 8 days.

### <Experiment>

DL-Ala powder was reacted with/without powdered olivine and a small amount of ultrapure water in sealed glass ampoules under Ar atmosphere. The ampoules were heated in a drying oven at 120 degree C for 1-8 days. After cooling at room temperature, the reacted product was suspended in 5 mL ultrapure water and the dissolved diastereoisomers of DKP were quantified using a high performance liquid chromatograph with UV detection.

### <Result and Discussion>

When the DL-Ala was heated without olivine, 3.0 % DL-Ala transformed into DKPs. On the other hand, 12.2 % of Ala changed to DKPs when the olivine coexisted. Olivine would be a good catalyst for DKPs formation. The DKPs were not detected even if the olivine coexisted after heating for 8 days, when a small amount of water was not added. A small amount of water would play a role to break the strong bonds of Ala crystals and promote the DKP formation reaction.

When  $de = \frac{[cis\ DKP] - [trans\ DKP]}{[cis\ DKP] + [trans\ DKP]}$  is defined, positive *de* means *cis* DKP excess. The *de* of DKP formed from the reaction without olivine heating for 8 days was +7.3 %. On the contrast, it was +16.3 % when reacted with olivine. It was reported that *trans* DKP is preferentially formed relative to *cis* DKP during racemic amino acid condensation, then the *de* gradually decreased with increasing reaction time (Naraoka and Harada, 1986). In this study, the *de* of DKP considerably increased when reacted with olivine. Olivine would be not only an efficient catalyst to promote the DKPs formation but also a determining factor on the selectivity of diastereoisomeric DKPs. Thus, serpentinization of ultramafic rock would have connection to DKP formation with regulation of peptide stereoisomers in the primitive ocean on planets.

Keywords: diketopiperazine, diastereoisomeric excess, olivine, alanine

## Effects of silicate on the decomposition rates of pentoses

Sakiko Nitta<sup>1\*</sup>, Yoshihiro Furukawa<sup>1</sup>, Takeshi Kakegawa<sup>1</sup>

<sup>1</sup>Graduate School of Science, Tohoku University

RNA is considered as a very important molecule for the origin of life because RNA carries genetic information and several RNA catalyze biological reactions. Ribose is an essential constituent of RNA. Ribose as well as the other pentoses can be produced abiotically through formose reaction. However, ribose is the most unstable pentose among of the pentoses produced by the formose reaction. Therefore, stabilization of ribose has been very important issue. For the solution of this problem, a previous study proposed that pentoses including ribose are stabilized forming complexes with silicate. Because of technical difficulties, it has not been clear which pentoses are stabilized by silicate. This study adopted a new application of liquid chromatography-mass spectrometry for the pentose analysis. The method made it possible to determine the concentration of each uncomplexed pentose. Incubation experiments of aldopentoses, ribose, lyxose, xylose, and arabinose, with three concentration of silicate have conducted in this study. In silicate-free solution, ribose had the highest rate of decrease. The rate of decrease for all aldopentoses became smaller with the concentration of silicate. In particular, the rate of decrease for ribose was significantly decreased. This result shows that silicate stabilize aldopentoses, especially ribose. Silicate is common in all over the world as silicate minerals and might have been common on the early Earth. Therefore, the selective stabilization of ribose by silicate might have provided a mechanism for the selection of ribose as the sugar in RNA on the early Earth.

Keywords: RNA, silicate, ribose, pentose, liquid chromatography-mass spectrometry

## The mechanism that had formed the primitive liposome in the early Earth

Shinji Karasawa<sup>1\*</sup>

<sup>1</sup>Miyagi National College of Technology: Professor emeritus

### [Introduction]

Materials used for the experiment for investigation of first primitive liposome had existed in the early Earth. Bubbles were generated by injection of iron powder into carbonated water. The side chain of appropriate amino acid adsorbs on the surface of the membrane. So, the life of bubble became longer by injection of the appropriate amino acid, because thermal motion of the adsorbed amino acid is suppressed and degradation of peptide bond of the amino acid is suppressed. A primitive protein is synthesized by incorporating into the membrane. The bubble rise to surfaces of water, and it will burst at the surface. As the result of repeating of the bursts, the surface of water was covered with similar membrane. After stirring that water, the bubbles were generated again under atmosphere of CO<sub>2</sub>. After some time, the vesicles that stayed long time at middle portion of the water between surface and bottom were generated. The vesicle must be produced at burst of the bubble. It is able to include the membrane and the water inside of it. This special vesicle with long life was made from the membrane and the amino acids.

### [Experiments on effects of amino acid to the bubble made from carbonated water and iron]

By addition of amino acid, the life of bubble generated in carbonated water mixed with iron powder becomes longer and its number increases. Here, materials for these experiments are (a)carbonated water: 75 cc, (b)iron powder: 5g and (c)amino acid (glutamine: 143mg, valine: 36mg, leucine: 71mg, isoleucine: 36mg). After several days from the mixing, the bubbles and the substance that had floated on the surface of the water were dissolved in the water by stirring of the water. After this stirring, bubbles were generated again. Initially, the rises-up and the fall-down were repeated. After some time, there emerged the vesicle that stayed in middle portion of the water. At this case, the atmosphere was filled with CO<sub>2</sub>.

### [Theoretical understandings]

The reason why bubbles are generated by injection of iron powder into carbonated water is as follows. The iron atom reacts with the oxygen atom of the carbon dioxide because the electronegativity of carbon atom is larger than that of hydrogen atom. The free carbon atom released from oxygen reacts with the iron atom. The iron carbide that has been produced reacts with the water. As the result, the free carbon atoms and the free hydrogen atoms form the membrane of the bubble. If insoluble gas is generated in the water where suitable organic molecules exist, the appropriate molecules will be arranged at the interface of the gas. The bubble made of the membrane rises up to the surface. The bubble will soon burst at the surface. The organic molecule that had been organized membrane of the bubble will cover the surface of water. After that, following phenomenon takes place at the burst of the bubble. The closed vesicle that includes the membrane and the water inside is produced by the mechanism to form the bubble. The reason why a liposome is generated is as follows. Amino acid is soluble in water but not soluble in oil. Organic molecules exist in the membrane of the bubbles generated in carbonated water mixed with iron powder. Appropriate side chain of amino acid is able to adsorb on surface of the membrane. Thermal motion of the adsorbed amino acid is suppressed and degradation of the peptide bond of amino acids is suppressed. The amino acid molecules adsorbed to the membrane will be mutually linked by the peptide bond. Although the association of amino acid is sensitive to the environment, the bubble of which membrane incorporated with linked amino acids becomes robust. The number of vesicles with long life will be increased. The large number of vesicles makes possible to form complicate cell by using broken parts of the membrane as units of the organization. The closed vesicle is able to include plural of small liposome.

Keywords: bubble, membrane, amino acid, peptide bond, protein, liposome

## Stratospheric Microorganisms Collection Using a Balloon

Sohsuke Ohno<sup>1\*</sup>, Ko Ishibashi<sup>1</sup>, Akihiko Yamagishi<sup>2</sup>, Yuko Kawaguchi<sup>2</sup>, Shota Nitahara<sup>2</sup>, Takahiro Segawa<sup>3</sup>, Gensuke Tokoro<sup>4</sup>, Kazuya Yamanouchi<sup>4</sup>, Yuko Ishikawa<sup>5</sup>, Norimune Miyake<sup>1</sup>, Masanori Kobayashi<sup>1</sup>, Takafumi Matsui<sup>1</sup>

<sup>1</sup>Planetary Exploration Research Center, Chiba Institute of Technology, <sup>2</sup>Tokyo University of Pharmacy and Life Sciences, <sup>3</sup>National Institute of Polar Research, <sup>4</sup>aRigen Pharmaceuticals, Inc., <sup>5</sup>Nippon Biologicals, Inc.

The subject of this study is focused on the stratospheric microorganisms, which are important for assessing the chances and flux of biological transfer escaping from and/or entering into the earth. Some previous studies reported the presence of microbes captured in the stratosphere by balloon, aircraft or rocket experiments. The presence of radiation- and desiccation resistant microbes, such as Bacillus and Deinococcus, have been accepted, although stratospheric environment is harsh against the microorganisms. However, it is difficult to discuss about the dynamic behavior of the stratospheric microorganisms, the mechanism of its vertical transportation, and its lifetime, because the time of observations and the number of detected microorganisms are not sufficient. In this talk, we introduce the plan of the balloon experiment to collect stratospheric microorganisms using a newly developed capturing system.

Keywords: Astrobiology, Panspermia, Stratosphere, Balloon, Extremophile



## Evaluation of biological activity in extreme environments by phosphatase activity

Taihei Kurizuka<sup>1\*</sup>, Taka Kogawa<sup>1</sup>, Yumiko Obayashi<sup>1</sup>, Takeo Kaneko<sup>1</sup>, Hajime Mita<sup>2</sup>, Yoshitaka Yoshimura<sup>3</sup>, Yoshinori Takano<sup>4</sup>, Mari Ogawa<sup>5</sup>, Kensei Kobayashi<sup>1</sup>

<sup>1</sup>Yokohama National University, <sup>2</sup>Fukuoka Institute of Technology, <sup>3</sup>Tamagawa University, <sup>4</sup>JAMSTEC, <sup>5</sup>Yasuda Women's University

Terrestrial organisms are widely distributed even in extreme environments such as submarine hydrothermal systems and Antarctica soils. In order to evaluate biological activities in such extreme environments, we analyzed phosphatase activity and amino acids in soils and rocks in extreme environments.

Antarctic soil samples were collected near Showa Station during the 49th Japanese Antarctic exploration mission in 2006-7. Reference samples used were surface soil collected in the campus of Yokohama National University. Sea sand after heated at 773 K was used as blank. Chimney samples were collected in South Mariana hydrothermal systems, the Pacific Ocean in 2003 in a part of the Archaean Park Project.

Phosphatase was extracted from solid samples with Tris buffer solution, and the enzymatic activity in the extract was fluorometrically assayed with 4-methylumbonylferyl phosphate as a substrate. Thermal stability and temperature-dependence of phosphatase were examined. Amino acid concentration in the same sample was also determined.

Outer part of the chimney samples faced to cold seawater, while inner part of them faced to superheated hydrothermal fluid. The former showed phosphatase activity, but the latter did not. Phosphatase extracted from the outer chimney, however, showed higher optimum temperature than E. coli. Thus, it is suggested that phosphatase found in the inner chimney was of thermophiles origin. Some Antarctica soils showed phosphatase activity, and some of them showed poorer thermal stability than E. coli alkaline phosphatase. Soil sampled at Langhovde penguins rookeries showed high phosphatase activity, and their thermal stability was close to E. coli's. This suggested that major source of phosphatase found in the soil was of microorganisms living in penguins' bodies.

Various enzymes have been detected in extreme environments. Characterization of such enzymes would give us further information on organisms living there. It can be said that phosphatase activity is a good biosignature for extant life in extreme environments.

Keywords: phosphatase activity, biological activity, extreme environments, amino acids, Antarctica, submarine hydrothermal systems

## Analysis of C<sub>20</sub>-C<sub>25</sub> isoprenoidal diether of halophilic archaea-lipid content changes in the incubation

TANOUE, Ryo<sup>1</sup>, Noriaki YAMAUCHI<sup>2\*</sup>

<sup>1</sup>Dept. Earth and Planetari Sci., Grad. School Sci., Kyushu Univ., <sup>2</sup>Dept. Earth and Planetari Sci., Fac. Sci., Kyushu Univ.

Archaea lives in a relatively harsh condition such as submarine hydrothermal vents (high temperature, low pH), swamp mud (anaerobic), and salt marsh (high salt concentration). Their living condition, especially the high temperature and low pH may be thought to resemble to the environment of primitive earth. It is settled for the third domain of life for 16SrRNA phylogenetic tree. So, it is important microorganisms for the tools to imagine the first living organisms on the earth. Archaea has a characteristic lipid which core is consisted from the glycerol and saturated isoprenoid with ether linkage. Among them, halophilic archaea lives in a salt marsh, saltpan and the environment at the high salt concentration. The main lipid core is the C<sub>20</sub>-C<sub>20</sub> isoprenoid diether (called archaeol, **1**). And characteristic C<sub>20</sub>-C<sub>25</sub> diether (**2**), rarely seen in other archaea, is also existed at the core. **2** have a possibility of the biomarker for hyper saline environment. Thus, the easy analytical method of **1** and **2** were investigated toward the possibility of biomarker for hypersaline environment to the evaluation of living halophilic archaea around coastal region. For the reevaluation of the C<sub>20</sub>-C<sub>25</sub> diether as a biomarker for a hypersaline environment, analytical methods of isoprenoidal lipid-core produced by the halophilic archaea by ESI-MS was conducted. Further, the compositional change for **1** and **2**, which simultaneously produced by the microorganism were observed by the two halophilic archaea species.

The lipid content of thermophilic archaea adopt the harsh condition to make the single-layer C<sub>40</sub> tetraether lipid and, further, the five membered ring structure were formed inside of the isoprenoidal lipid core with higher temperature. (This phenomenon has been applied to create a new index, TEX<sub>86</sub>.) Further, cryophilic archaea, which lives in an Antarctic salt lake, changes the lipid content toward increasing the number of unsaturation in archaea with low temperature. For our preliminary experiment, lipid content changes with the incubation condition were observed from hypersaline neutral *N. pallidum* JCM 8980. Thus, content change with the incubation condition (temperature, pH, salt concentration) for other species were observed.

Alkaline hypersaline archaea *Natronomonas pharaonis* JCM 8858 has similar optimum condition other than optimum pH (8.5). Lipid core changes about the several pH, salt concentration, and temperature (normal condition, 300 ml medium contains casamino acids 4.5g, sodium citrate 0.9g, glutamate 0.75g, magnesium sulfate 0.75 g, potassium chloride 0.6 g, sodium chloride 60 g) were observed. The microorganism was incubated for ordinal 12 days (at the beginning of the stationary phase) and lipid were extracted, hydrolyzed, and purified to give the lipid core. The ratio of **1** and **2** were determined with the ESI-MS at the negative ion mode with the CHCl<sub>3</sub>-containing solvent system for the observation of chloride-adduct ion.

The growth ratio was almost the same without the low temperature condition observed significant growth bunting. The lipid content of *N. pharaonis* previously reported was consisted to our result. Further, temperature increasing caused increasing of the content of **2** with the hypersaline archaea. For the pH change, optimal pH tends to increase **2**, and other pH decrease the content of **2**. Salt concentration is not seemed to affect to the content of the lipid core. Usually, Salt tolerance of halophilic archaea has been secured and the nature of the protein, high potassium concentration in the cytoplasm. However, a change of the contents of the lipid core with the temperature change is the same as *N. pallidum*, which is considered to the adaptation to temperature change in halophilic archaea. Similar experiment for alkaline halophilic archaea *Natronobacterium gregoryi* will be reported.

Keywords: halophilic, archaea, biomarker, hypersaline environment

## Mineralogical study of clastic sedimentary rocks in the 3.2 Ga Moodies Group, South Africa

Shohji Kawai<sup>1\*</sup>, Takeshi Kakegawa<sup>1</sup>

<sup>1</sup>Graduate School of Science, Tohoku University

It is important to investigate the timing of emergency of oxygenic phototrophs (i.e., cyanobacteria) on the early Earth. To approach this problem, Sakamoto (2012) studied chemical sedimentary rocks (Banded Iron Formation:BIF) deposited in shallow ocean environments in Moodies Group in the Barberton Greenstone Belt, South Africa (ca.3.2Ga). Sakamoto (2012) concluded that chromite in Moodies BIFs is a chemical precipitates from oxygenated 3.2 Ga ocean water. However, absence of knowledge of a clastic chromite creates ambiguity if Sakamoto's chromite was a real chemical precipitate. Therefore the objectives of this study is set: (1) to constrain paragenesis and find minerals formed under oxic environments in shallow water clastic sediments, (2) to determine the chemical compositions and occurrence of clastic chromite, and (3) to discuss microbial ecosystem through stable carbon isotopic compositions.

We collected of the Moodies Group from the under-ground mining site (Sheba mine). Chromite in the examined samples is rounded or angular and surrounded by fuchsite and Cr-bearing biotite, contrasting chemical precipitated euhedral chromite surrounded by magnetite. Mg# of clastic chromite is 0.012 to 0.043, which differs from Mg# of chemical precipitated chromite (Mg# = 0.000). Such contrast suggests that both detrital and chemical precipitated chromites are present in Moodies sedimentary rocks. Additionally, stable carbon isotopic compositions are within a range of organic matter produced by cyanobacteria. Overall results of this study indicated that presence of 3.2 Ga oxygenic shallow oceans in where cyanobacteria were active.