

BAO001-01

Room:301B

Time:May 23 08:30-08:55

## Alteration of interstellar complex organics in Solar system environments and its relevance to origins of life

Kensei Kobayashi<sup>1\*</sup>

<sup>1</sup>Yokohama National University

A wide variety of organic compounds including amino acid precursors were detected in extraterrestrial bodies such as meteorites and comets, and their relevance to the generation of life on the primitive Earth has been discussed. Laboratory experiments simulating interstellar dusts in molecular clouds have suggested that complex amino acid precursors could be formed in interstellar environments. We can draw as follows: (1) Complex interstellar organics were introduced to the primitive Solar system; (2) further alteration of organics occurred in meteorite parent bodies, comets and interplanetary dust particles (IDPs); (3) the organics were delivered to the primitive Earth by meteorites, comets and IDPs. It is suggested that IDPs brought much more organics to the Earth than meteorites and comets. We, however, have very little information about organics in IDPs. We are performing laboratory simulation experiments how organic compounds are altered in the solar system environments by using accelerators: Both particles accelerators simulating actions of cosmic rays and solar flares and cyclotrons simulating actions of solar radiation. In addition to them, we are planning the Tanpopo Mission by utilizing the Exposed Facility of the Japanese Experiment Module, the International Space Station: Capture of interplanetary dusts in space and direct exposure of organic compounds to space environments would be done.

Keywords: amino acid precursors, interstellar complex organics, origins of life, interplanetary dust particles, Tanpopo mission, accelerator experiments

BAO001-02

Room:301B

Time:May 23 08:55-09:10

## Valine peptide formation under high temperature and high pressure conditions

Yoshihiro Furukawa<sup>1\*</sup>, Takato Ishiguro<sup>1</sup>, Tsubasa Otake<sup>1</sup>, Hiromoto Nakazawa<sup>2</sup>, Takeshi Kakegawa<sup>1</sup>

<sup>1</sup>Tohoku University, <sup>2</sup>NIMS

Peptide formation on the early Earth is an essential process for the origin of life. Heating experiments of compressed solid valine, one of the simplest amino acid having an alkyl side chain, were performed under various temperature (150-200°C) and pressure (50-150 MPa) conditions up to 384 hours, in order to investigate how temperature and pressure affect the stability of valine and reaction rates of the peptide formation from valine monomers. The samples were enclosed in a gold tube and pressurized with a test-tube type autoclave using water as pressure medium. Produced peptides were analyzed by liquid chromatography-mass spectrometry (LC/MS). The recovered valine and decomposition products having amino groups were analyzed with a high performance liquid chromatography (HPLC) after the derivatization with a fluorescent reagent.

The run products contained linear peptides from dimer to hexamer, cyclic dimer, other amino acids, ammonia, and amines. The decomposition rates of starting valine at three different temperatures showed that the decomposition of the starting valine was very sensitive to the temperature change. Increasing temperature also accelerate the rates of both formation and decomposition of the linear peptides. On the other hand, the decomposition rates of valine and its peptides decreased with increasing pressure. The effect of pressure on production rates of valine peptides were very small, compared to that of temperature. Because the major decomposition products were ammonia and carbon dioxide, which were vapor or supercritical phase at the experimental conditions, pressure could suppress the degradation of valine and peptides by inhibiting their degassing reactions. The results of our experiments support a hypothesis that peptides were formed through diagenesis and suggest that pressure expand the stability of valine and the peptides under high temperature conditions. The present study also suggests that the typical diagenetic condition (up to 100°C) is suitable for the high yield peptide formation in geological time scale. Polymerization of other amino acids, such as glycine and alanine, were also confirmed at different series of anhydrous experiments, suggesting a general importance of pressurized deep sediments for prebiotic peptide formations.

Keywords: amino acid, polymerization, protein, origin of life, early Earth, pressure

BAO001-03

Room:301B

Time:May 23 09:10-09:35

## Experimental and geological link for prebiotic peptide and ribose formation

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

<sup>1</sup>Tohoku University

Controversy exists as to which geological environments were suitable for prebiotic organic formation. In this presentation, potential geological environments to form peptide and ribose will be discussed. Heat energy is necessary to promote polymerization of amino acids and then to form peptides. However, once-formed peptides easily break if heat energy suppresses and amounts of water exceed the peptide-equilibrated amounts. During diagenesis of deep marine sediments, where dehydration proceeds under high P and T conditions, may provide ideal environments for the peptide formation.

High pressure (150MPa) and temperature (up to 180°C) experiments were performed in order to examine if diagenetic conditions are ideal for peptide formations. A mixture of glycine and alanine or a mixture of methionine and glycine was used as a starting material. Amounts of ammonia in reaction system increased with time, suggesting broke down of amino acids. On the other hand, amounts of glycylalanine, glycylglycylalanine, glycylmethionine and methiolmethionine were high and exceed the amounts of glycine-5mers and alanine-4mers. The results of the present study suggest that peptides composed of different amino acids have easily formed with high yields under high P and T conditions accompanied with high ammonia concentrations. Presence of ammonium-mica in Isua Supracrustal Belt in Greenland may suggest ammonia-rich diagenesis in ancient marine sediments, supporting the present experimental results.

For prebiotic ribose formation, stepwise reactions between borates and formaldehyde are suggested. Such interaction happens only under high borate concentrations. Borate-rich environments are often considered as unrealistic on the early Earth. However, tourmaline-rich garnets in sediment-protolith were found in Isua Supracrustal Belt. This finding suggests that borate-rich conditions were present during diagenesis of ancient marine sediments, and promises ribose formation during diagenesis.

Keywords: prebiotic, peptide, ribose, Isua

BAO001-04

Room:301B

Time:May 23 09:35-09:50

## On the formation environment of the nano-bacteria fossil-like texture

Yasunori Miura<sup>1\*</sup>

<sup>1</sup>Yamaguchi University

A characteristic formation environment of a fossil-like material related to life activity are summarized as follows.

- 1) The shape of the fossil solidified material shows a curved nano-texture at the time of the solidification from a fluid phase.
- 2) The compositions of fossil solidified materials are carbon-bearing minerals with cations (Ca, Fe, Mg) remained in seawater fluid phase environment. When it is formed with the surface crust rocks, it contains Si from silicate rocks with complex formation.
- 3) Impacted nano-texture with fossil-like curved features can be found in the air environment with a fluid phase compared with vacuum condition, though both textures are irregular crack textures.
- 4) The composition of the nanobacteria-like texture organization in the of the fusion crusts of the Kuga iron meteorite found in Yamaguchi, Japan is Akaganeite composition in minor size.
- 5) The present results indicate that the nano-bacteria textures of Martian meteorites with magnetite and carbonates separately are not formed at vacuum collision process, but carbonate formations with changes of positive ions (Ca, Mg, Fe) under fluid phase.

Keywords: nano-texture, fossil-like, formation environment, fluid phase, carbonate, irregular cracking

BAO001-05

Room:301B

Time:May 23 09:50-10:25

## Life detection in Archean rocks: are stable isotopes reliable?

Daniele Pinti<sup>1\*</sup>, Ko Hashizume<sup>2</sup>

<sup>1</sup>Universite du Quebec, <sup>2</sup>Osaka University

Among signatures of ancient biological activity, stable isotopes of C, S, Fe and N hold an important place. Indeed, metabolic processes tend to produce different, and sometimes diagnostic, enrichment or depletion in certain isotopes. Large environmental and biological changes at the surface of the Earth, as those provoked or provoking the oxygenation of the primitive atmosphere are clearly imprinted in the C, S, N and Fe geological record. Yet, the reliability of stable isotopes as biological and environmental proxies has been recently questioned. Short-chain hydrocarbons synthesized via Fischer-Tropsch-type reactions in hydrothermal environments are depleted in <sup>13</sup>C in a way typically ascribed to metabolic processes such as photosynthesis and methanogenesis (small  $\delta^{13}\text{CPDB} = [(^{13}\text{C}/^{12}\text{C})_{\text{sample}}/(^{13}\text{C}/^{12}\text{C})_{\text{std}} - 1 \times 1000] = -30$  to  $-60$  permil). This suggests that carbon isotopic composition might not be an effective discriminant between biologic and non-biologic sources. Sulfur isotopes, and particularly the <sup>33</sup>S/<sup>34</sup>S ratios show variations in the geological record usually interpreted as reflecting changes in the redox state of the atmosphere and in the biologically related sulfur cycle. Yet, thermochemical reactions might produce similar isotopic fractionations. Nitrogen has been longtime ignored as biosignature because being extremely fragile compared to the more stable graphitic forms of C. Indeed, it can be easily fractionated by metamorphic or hydrothermal-driven reactions. However, N has an advantage over other isotopic systems such as those of C and S. The dominant source of N at the surface of the Earth, that is, the atmospheric triple-bonded N<sub>2</sub>, is so stable that only a very limited number of metabolic processes can bridge the abiotic and biotic world. Finally Fe (small  $\delta^{56}\text{Fe} = (^{56}\text{Fe}/^{54}\text{Fe})_{\text{sample}}/(^{56}\text{Fe}/^{54}\text{Fe})_{\text{std}} - 1 \times 1000$ ) has very little isotopic fractionation ( $\pm 1$  permil) and numerous studies shown that the biological-induced fractionation is not completely understood or yet measured. Here we present new data on N isotopes and their behavior in cherts and banded iron formations of South Africa (3.45 Ga Hooggenoeg Fm., Barberton Greenstone Belt) and India (2.9-2.7 Ga Bababudan Group, Dharwar Craton). Combination of two or more isotope markers (N, C and Fe) with largely different geochemical natures may help us to discriminate between possible fractionation pathways, biotic or abiotic, and/or rule out part of the anticipated post-depositional fractionation events. This is the case of the India Banded Iron Formations, where N isotopes have been coupled with Fe and C isotopes. Observed Fe, C and N isotopic co-variations in cherty and iron-rich layers have been related to the appearance of denitrification and dissimilatory iron reduction in the water column at the onset of the Great Oxygenation Event. Organic nitrogen was trapped as ammonium (NH<sub>4</sub><sup>+</sup>) in hydromuscovite and feldspars preserved in cherty formations of the Hooggenoeg Fm. at the Komati River, South Africa. Here nitrogen isotopes have been coupled with argon isotopes (<sup>40</sup>Ar/<sup>36</sup>Ar). Indeed, an indirect relation relates NH<sub>4</sub><sup>+</sup> which replace K<sup>+</sup> ions in the structure of K-bearing silicates and radiogenic <sup>40</sup>Ar\*, which is produced by electron capture of K<sup>+</sup>. These formations show small  $\delta^{15}\text{N}$  values of  $+7.1 \pm 0.5$  to  $+12.6 \pm 0.4$  permil, higher than those usually found in Early Archean ammonium ( $-5$  to  $+2$  permil). K-Ar dating of mica and feldspars give younger Proterozoic ages of  $2137 \pm 15$  Ma and  $1191 \pm 27$  Ma, respectively. This suggests that the mineral phase preserving ammonium is not a closed system and post-depositional metamorphic events likely reset the K-Ar clock. The same phenomenon possibly caused 1) partial devolatilization of the pristine organic N with preferential loss of <sup>14</sup>N and increase of the small  $\delta^{15}\text{N}$  values; or 2) isotopic exchange with metasomatic fluids which usually contain <sup>15</sup>N-enriched nitrogen.

Keywords: life, Archean, stable isotope

BAO001-06

Room:301B

Time:May 23 10:45-11:20

## Geochemical constraints on the partial pressure of carbon dioxide in the Archaean atmosphere from Banded Iron Formations

Minik T. Rosing<sup>1\*</sup>, Dennis K. Bird<sup>2</sup>, Norman H. Sleep<sup>3</sup>, Christian J Bjerrum<sup>1</sup>

<sup>1</sup>University of Copenhagen, Nordic Center, <sup>2</sup>Stanford Univ., Dept.Earth/Env., <sup>3</sup>Stanford Univ., Dept.Geophy.

There is geological evidence from the widespread preservation of waterlain sediments that Earth's climate resembled the present during the Archean, despite a much lower solar luminosity. This was cast as a paradox by Sagan and Mullen in 1972. Kasting (1993) suggested a solution to the paradox by increased mixing ratios of greenhouse gasses, notably CO<sub>2</sub> in the early atmosphere. However geochemical evidence for high partial pressures of CO<sub>2</sub> are absent in marine sediments as well as in paleosols. We have used banded iron formation (BIF) to characterize the composition of the atmosphere. BIFs originated as chemical sediments precipitated from the Archaean ocean and sedimented as particles to the seafloor. Magnetite is ubiquitous in Archaean BIFs which indicates that it was thermodynamically stable during exposure of the primary sediment to ocean water and during subsequent diagenesis and compaction of the sediment. The involvement of biologic processes in the original precipitation of iron-rich minerals and/or sediment diagenesis does not alter the constraint of magnetite saturation. The stability relations of magnetite preclude CO<sub>2</sub> mixing ratios much higher than the present atmospheric level (~3-5 times PAL). At higher partial pressures of CO<sub>2</sub> siderite would replace magnetite as the stable iron bearing phase. The CO<sub>2</sub> pressure of the atmosphere is expressed in the CO<sub>2</sub> concentration of seawater through the water column and well into the sediment because CO<sub>2</sub> is highly soluble in water. In the absence of substantial compensation for the lower solar irradiance by greenhouse gasses in the atmosphere, we have examined the factors that controlled Earth's albedo. These are primarily the surface albedo of Earth and the abundance and properties of clouds. We have applied a model that takes into account the apparent growth of Earth continents (Collerson and Kamber 1999) and the absence of land vegetation during the Precambrian for the evolution of the surface albedo, and a model for the abundance and properties of clouds that takes into account the lower abundance of biogenic cloud condensation nuclei in a less productive prokaryotic world. The higher transparency of the atmosphere for short wave incoming solar radiation and the lower surface albedo on an early Earth dominated by oceans, provided significant compensation for the lower solar irradiance which allow the presence of liquid oceans, even at greenhouse gas concentrations broadly similar to the present day values.

We therefore suggest that the thermostasis during Earth geologic record, is not paradoxical, but is the combined effect of many factors, which are to a large part biologically controlled.

### References

- Collerson, K. D. and B. S. Kamber (1999). "Evolution of the continents and the atmosphere inferred from Th-U-Nb systematics of the depleted mantle." *Science* 283(5407): 1519-1522.  
Kasting, J. F. (1993). "Earth's Early Atmosphere." *Science* 259(5097): 920-926.  
Sagan, C. and G. Mullen (1972). "Earth and Mars - Evolution of Atmospheres and Surface Temperatures." *Science* 177(4043)

Keywords: carbon dioxide, Archaean, Faint early sun, BIF

BAO001-07

Room:301B

Time:May 23 11:20-11:45

## Microbe space exposure experiments at International Space Station (ISS) in the mission "Tanpopo"

Shin-ichi Yokobori<sup>1\*</sup>, Yinjie Yang<sup>1</sup>, Tomohiro Sugino<sup>1</sup>, Yuko Kawaguchi<sup>1</sup>, Yuta Takahashi<sup>1</sup>, Issay Narumi<sup>2</sup>, Hirofumi Hashimoto<sup>3</sup>, Nobuhiro Hayashi<sup>4</sup>, Eiichi Imai<sup>5</sup>, Hideyuki Kawai<sup>6</sup>, Kensei Kobayashi<sup>7</sup>, Katsumi Marumo<sup>8</sup>, Hajime Mita<sup>9</sup>, Kazumichi Nakagawa<sup>10</sup>, Kyoko Okudaira<sup>11</sup>, Makoto Tabata<sup>3</sup>, Yuichi Takahashi<sup>12</sup>, Kaori Tomita-Yokotani<sup>13</sup>, Masamichi Yamashita<sup>3</sup>, Hajime Yano<sup>3</sup>, Yoshitaka Yoshimura<sup>14</sup>, Akihiko Yamagishi<sup>1</sup>

<sup>1</sup>Tokyo Univ. Pharm. Life Sci., <sup>2</sup>JAEA, <sup>3</sup>JAXA/ISAS, <sup>4</sup>Tokyo Inst. Tech., <sup>5</sup>Nagaoka Univ. Tech., <sup>6</sup>Chiba Univ., <sup>7</sup>Yokohama Natl. Univ., <sup>8</sup>AIST, <sup>9</sup>Fukuoka Inst. Tech., <sup>10</sup>Kobe Univ., <sup>11</sup>Univ. Aizu, <sup>12</sup>Yamagata Univ., <sup>13</sup>Univ. Tsukuba, <sup>14</sup>Tamagawa Univ.

To explain how organisms on the Earth were originated at the quite early stage of the history of Earth, Panspermia hypothesis was proposed [1, 2]. Recent findings of the Martian meteorite suggested possible existence of extraterrestrial life, and interplanetary migration of life as well. On the other hand, microbes have been collected from high altitude using balloons, aircraft and meteorological rockets since 1936, though it is not clear how could those microbes be ejected up to such high altitude [3]. Indeed, we have also collected microorganisms at high altitude by using airplanes and balloons. Spore forming fungi and Bacilli, and Deinococci have been isolated in these experiments. We also collected two novel species of the genus *Deinococcus*, one from top of troposphere (*D. aerius*) and the other from bottom of stratosphere (*D. aetherius*) [4-6]. In addition, we collected various spore-forming bacilli and their related species. Spores and Deinococci are known by their extremely high resistance against UV, gamma ray, and other radiation [4]. *D. aerius* and *D. aetherius* showed high resistance comparable with *D. radiodurans* R1 to the UV and radiation such as gamma ray. If microbes could be found present even at the higher altitude of low earth orbit (400km), the fact would endorse the possible interplanetary migration of terrestrial life.

We proposed the "Tanpopo" mission to examine possible interplanetary migration of microbes, and organic compounds on Japan Experimental Module (JEM) of the International Space Station (ISS) [7]. Tanpopo consists of six subthemes. Two of them are on the possible interplanetary migration of microbes ? capture experiment of microbes at the ISS orbit and space exposure experiment of microbes. In this paper, we focus on the space exposure experiment of microbes.

Microbes in space are assumed be exposed to the space environment with a kind of clay materials that might protect microbes from vacuum UV and cosmic rays, or exposed as the aggregates of which outer cells might protect inner cells from vacuum UV and cosmic rays. Dried vegetative cells of *D. radiodurans* and our novel deinococcal species isolated from high altitude are candidates for the exposure experiment. In addition, we are planning to perform another space exposure experiments of microbes. In this paper, we discuss current status of exposure experiment of microorganisms defined for the Tanpopo mission and others.

### References

- [1] Arrhenius, S. (1908) *Worlds in the Making-the Evolution of the Universe* (translation to English by H. Borns) Harper and Brothers Publishers, New York. [2] Crick, F. (1981) *Life Itself*. Simon & Schuster, New York. [3] Yang Y. et al. (2009) *Biol. Sci. Space*, 23, 151-163. [4] Yang, Y. et al. (2008) *Biol. Sci. Space*, 22, 18-25. [5] Yang, Y. et al. (2009) *Int. J. Syst. Evol. Microbiol.*, 55, 105-115. [6] Yang, Y. et al. (2010) *Int. J. Syst. Evol. Microbiol.*, 60, 776-779. [7] Yamagishi, A. et al. (2008) *Int. Symp. Space Tech. & Sci. (ISTS) W* 2008-k-05.

Keywords: International Space Station, Space exposure, Microbes, Deinococcus



BAO001-08

Room:301B

Time:May 23 11:45-12:00

## Prolonged survival of multilayer bacteria under UV radiation and vacuum

Yinjie Yang<sup>1\*</sup>, Kazumichi Nakagawa<sup>2</sup>, Tanabe Maiko<sup>2</sup>, Momoki Yohei<sup>2</sup>, Hirofumi Hashimoto<sup>3</sup>, Shin-ichi Yokobori<sup>1</sup>, Akihiko Yamagishi<sup>1</sup>

<sup>1</sup>Tokyo Univ. of Pharmacy and Life Science, <sup>2</sup>Kobe University, <sup>3</sup>Japan Aerospace Exploration Agency

In early 20th century, Arrhenius proposed the possible migration of life through space. The Hypothesis is called Panspermia Hypothesis (1908). In the hypothesis, the interplanetary transfer of single spores is propelled by radiation pressure. However, the solar UV has been proven to be lethal for unshielded microorganisms (Nicholson et al., 2000; Horneck et al., 2010), which invalidated his Hypothesis.

Another possible form of the interplanetary transfer of life, micro-aggregate or micro-clump, has just emerged from recent studies. Space environment exposure experiments evidenced that microorganisms in thick layers can survive larger UV doses than single cells. Some bacterial spores in multilayer-spore samples survived intense solar UV radiation, while all the spores in monolayer were killed (Horneck et al., 1994, 1995; Mancinelli and Klovstad, 2000). Terrestrial microorganisms may be transported into the upper atmosphere and space by human activities (e.g., spacecraft launch) and natural mechanisms (e.g., electric field, meteorite impact). Based on the microbiological studies in the upper atmosphere, we have roughly estimated the altitude-dependent distribution of microorganisms, suggesting the extended distribution of microorganisms into space (Yang et al., 2009). Bacterial cell clumps have been found in the upper atmosphere (about 40-km altitude) (Wainwright et al., 2003). The cells of the *Deinococcus* strains (ST0316 and TR0125) we isolated from the upper atmosphere (about 10-km altitude) multiply and grow in aggregated form (Yang et al., 2009).

However, there has no study to quantitatively examine the relationships between microbial survival, size of micro-aggregate and UV doses. It is unknown what size of micro-aggregate may protect some cells inside it from long-term space UV radiation. Our current study investigates quantitatively the survival of bacteria against extraterrestrial UV radiation in dependence of sizes of cell aggregates, assessing the possibility of viable transfer of microorganisms in aggregated form.

We have obtained preliminary data on the survival of *D. radiodurans* against UV<sub>172nm</sub> radiation under vacuum in dependence of the cell aggregate thickness. At the same UV<sub>172nm</sub> dose, larger cell aggregate exhibited higher survival rate. The preliminary results suggest that upper layers of cells protected cells underneath from the UV<sub>172nm</sub> inactivation, and that 20 micrometer of thickness was enough for protecting a high percent of cells at lower layers alive under UV<sub>172nm</sub> and vacuum conditions.

### Reference

- Arrhenius, S. (1908) The Spreading of Life Throughout the Universe. In: Arrhenius, S. (Ed.), Worlds in the Making: The Evolution of the Universe. Harper and Brothers, New York.
- Horneck, G., Bucker, H. and Reitz, G. (1994) Long-term survival of bacterial spores in space. Adv Space Res, 14, 41-45.
- Horneck, G., Eschweiler, U., Reitz, G., Wehner, J., Willimek, R. and Strauch, K. (1995) Biological responses to space: results of the experiment "Exobiological Unit" of ERA on EURECA I. Adv Space Res, 16, 105-118.
- Horneck, G., Klaus, D.M. and Mancinelli, R.L. (2010) Space microbiology. Microbiol Mol Biol Rev, 74, 121-156.
- Mancinelli, R.L. and Klovstad, M. (2000) Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. Planet Space Sci, 48, 1093-1097.
- Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J. and Setlow, P. (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev, 64, 548-572.
- Wainwright, M., Wickramasinghe, N.C., Narlikar, J.V. and Rajaratnam, P. (2003) Microorganisms cultured from stratospheric air samples obtained at 41 km. FEMS Microbiol Lett, 218, 161-165.
- Yang, Y., Yokobori, S. and Yamagishi, A. (2009) Assessing Panspermia Hypothesis by microorganisms collected from the high altitude atmosphere. Biol Sci Space, 23, 151-163.

Keywords: microorganisms, survival, ultraviolet, multilayer, vacuum, panspermia



BAO001-09

Room:301B

Time:May 23 12:00-12:35

## Japan Astrobiology Mars Project (JAMP)

Akihiko Yamagishi<sup>1\*</sup>, Yoshitaka Yoshimura<sup>2</sup>, Atsuo Miyakawa<sup>3</sup>, Hajime Honda<sup>4</sup>, Kensei Kobayashi<sup>5</sup>, Takeshi Naganuma<sup>6</sup>, Hajime Mita<sup>7</sup>, Sohsuke Ohno<sup>8</sup>, Hideaki Miyamoto<sup>9</sup>, MELOS Life Search Subgroup<sup>1</sup>

<sup>1</sup>Tokyo Univ. Pharm. Life Scie, <sup>2</sup>Tamagawa Univ., <sup>3</sup>Shizuoka Univ., <sup>4</sup>Nagaoka University of Technology., <sup>5</sup>Yokohama National Univ., <sup>6</sup>Hiroshima Univ., <sup>7</sup>Fukuoka Inst. Tech., <sup>8</sup>Chiba Inst. Tech., <sup>9</sup>The Univ. Tokyo

The liquid water is considered to be an critical factor for life. Gibbs free energy is another factor that should be counted to sustain life for long duration. The Gibbs free energy is obtained by reaction between reductant and oxidant, or from any other non-equilibrium state of matter. As an example, aerobic organisms use carbohydrate and oxygen for getting Gibbs free energy. Many types of chemoautotrophic mechanisms are known for the process as well. On Mars surface, methane and oxidative compound such as ferric oxide or sulfate are found, and they can be a source of Gibbs free energy. Iron-dependent methane oxidizing bacteria was found in marine environment on Earth (1). This finding suggests possible presence of methane-oxidizing bacteria on Mars surface, if local thermal environment and other resources permit proliferation and metabolism of the bacteria during limited portion of time period.

Our project aims to search for the methane-oxidizing microbes on Mars surface. Martian soil will be sampled from a depth of about 5 or 10 cm below the surface, where organisms are supposed to be protected from harsh hyper-oxidative environment of Mars surface. Small particles less than 0.1 mm are sieved from the sample, before transferred to analysis section by a micro-actuator. The particles are stained by cocktail of fluorescence reagents, and examined by a fluorescence microscope.

Combination of fluorescence dyes is selected to identify life forms from the soil sample. Intercalating fluorescence dye such as SyberGreen is used to detect genetic compounds such as DNA. Membrane specific dye or the combination of dyes is used to detect membrane surrounding the cell. Substrate dye that emits fluorescence upon cleavage by the catalytic reaction is used to detect the catalytic activity of the cell. A combination of staining reagents is chosen based on the definition of life. DNA or genetic material is required for replication of life form. Membrane separating cell from ambient leads to identification of individual. Catalytic reaction of enzymes drives metabolism. The combination is useful also for detecting pre-biotic organic material as well as remnant of ancient life.

Hydrolysis of the polymers in the cell followed by HPLC or soft ionization MS for amino acid analysis is effective in examining whether Martian life is identical or different from terrestrial life. The number and type of the amino acids as well as chirality will be analyzed to distinguish if the polymers are contamination made by Earth-related life form.

Reference: (1) E. J. Beal, et al (2009) Science 325, 184-187

Keywords: Life search, Mars, microbe, methane oxidizing bacteria, fluorescence microscope