

Report for the Joint Use/Research of the Institute for Planetary Materials, Okayama University for FY2023

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Category: International Joint Research General Joint Research Joint Use of Facility
Workshop

Name of the research project: Creation of a membraneless protocell with Earth-abundant transition-metal catalysts

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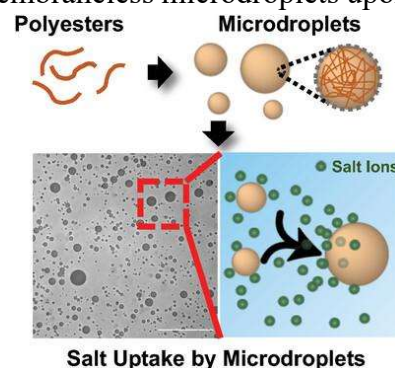
Affiliated institution and department: IPM , Okayama University

Research report:

Research Background

α -hydroxyacids (α HAs), molecules similar in structure to α -amino acids, which are one type of abundant abiotic monomers likely present on early Earth, possibly formed through atmospheric discharge, hydrothermal reactions, ultraviolet photochemistry, or even delivered by meteorites [1,2]. Previously, α HAs can undergo dehydration synthesis to form gel-like polyesters in conditions that

mimic aqueous environments on early Earth, which assemble into membraneless microdroplets upon aqueous rehydration. These microdroplets are proposed as protocells that can segregate and compartmentalize biomolecules such as proteins and nucleic acids [3,4]. Moreover, early aqueous environments (*e.g.*, oceans) have shown a much higher level of salt compared to the salinity today and even many kinds of salt ions still play essential roles in regulating many biochemical reactions in modern life.



Supported by IPM joint funding, the applicant went to Misasa during FY 2022 and did cooperation with researchers at the Pheasant Memorial Lab from IPM in Okayama University. **We employed ICP-MS, one typical technique for analysis on extraterrestrial samples (*e.g.*, Ryugu), to analyze the amount of salt cations possibly abundant in early oceans such as NaCl, KCl, MgCl₂ and CaCl₂.** Combined with other spectroscopic and biophysical methods, we found that polyester microdroplet could uptake salts and different salts are taken up at different rates. Moreover, the salts tended to accumulate near the charged surfaces of the droplets, resulting in an overall neutralization of the droplets' surface charge and thus high coalescence possibility for droplets to grow [5]. This resulted in one first-author paper with IPM researchers published in *Small Methods* (2023) titled **Spectroscopic and Biophysical Methods to Determine Differential Salt-Uptake by Primitive Membraneless Polyester Microdroplets**. In addition, the applicant also did conference presentations four times, including ELSI workshop, one invited presentation in China and the 103rd CSJ Annual Meeting held at Tokyo University of Japan and invited presentation in RIKEN in 2023.

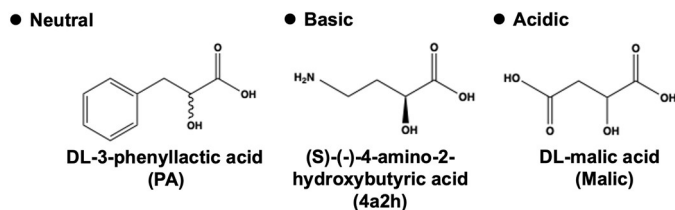
Research Purpose

However, **one major unanswered question is how can protocells obtain energy from the surrounding geological environment, which is abundant in naturally Earth-abundant transition metal salts, so that they can perform essential life-like properties including catalysis and metabolism?** Thus, the applicant applied for IPM joint funding in both FY 2023 and 2024 as PI and was supported to went Misasa in October 2023 to collaborate with Prof. Eizo Nakamura, Prof. Katsura Kobayashi, Prof. Tak Kunihiro and Ms. Chie Sakaguchi on initial test experiments related to the uptake of transition metals by polyester microdroplets, with some results listed out below.

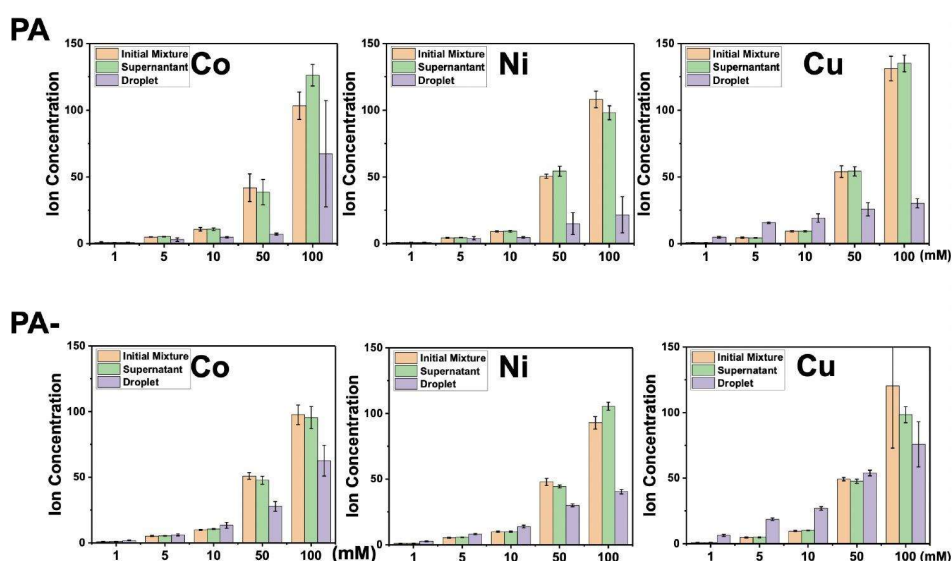
Conducted Research and Outcomes

Microdroplets have been assembled by frist drying α HA solution mixtures (see on the pic for potential α HA structures) in Eppendorf tubes at 80°C for 1 week to produce homo- and heteropolyester gels,

followed by rehydration in the appropriate aqueous medium, forming droplets. The transition-metal salts solutions like MnCl_2 , CoCl_2 , NiCl_2 and CuCl_2 (ranging from 2, 10, 20, 100 to 200 mM) will be added to fresh droplet solutions at 1 to 1 volume ratio, respectively.



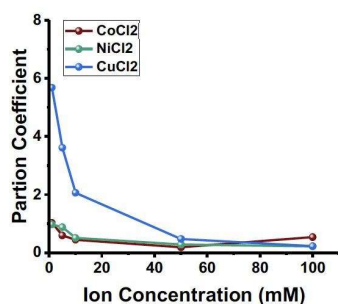
First, we did mixing and then conducted centrifugation for each group. This will allow all droplets to coalesce to grow into large one and also can separate droplets and supernatant solutions. Then, the specific volume of large droplet was pipetted out to dissolve in the appropriate solvent and subjected to ICP-MS analysis, along with concentration standards for the external solution (depending on the salt used). ICP-MS spectra could give us information on the spatial concentration of the compartmentalized salt analytes; in particular, we could understand the degree of uptake of salts into polyester droplets (which are known to cause structural changes and coalescence of the droplets). For the initial test experiments last year, the applicant prepared for two kinds of polyester microdroplets: homopolymer PA and heteropolymer PA-malic (PA-). Salts solutions included CoCl_2 , NiCl_2 and CuCl_2 (ranging from 2, 10, 20, 100 to 200 mM) to mix with each droplet solutions at 1 to 1 volume ratio. Each group was repeated three times for ICP-MS measurements and the selective uptake on cations can be observed below:



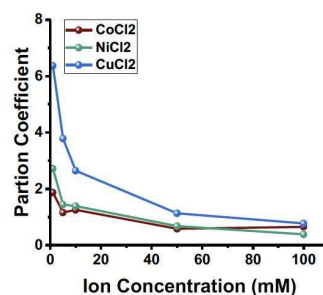
Uptake on Co^{2+} , Ni^{2+} and Cu^{2+} by polyester microdroplets PA and PA-.

In order to compare the difference on uptake efficiency, we calculated the partition coefficient defined by using the cation concentration inside droplet to divide that in supernatant solution.

PA:



PA-



Partition coefficient of polyester microdroplets PA and PA- on cations.

As a result, we have found that polyester droplets have the ability to selectively partition Cu^{2+} compared with Co^{2+} and Ni^{2+} ; $\text{Cu}^{2+} > \text{Co}^{2+} \approx \text{Ni}^{2+}$. The higher ion concentration is, the lower partition coefficient of droplets will have, which means our droplets have the limitation to uptake cations; Compared PA droplets, PA- tends to have higher possibility to partition cations, which means the hydrophilic modification can change droplets physical or chemical properties.

Research Plan and Expectations

Since the applicant was accepted this year to do continuous joint research in IPM with same collaborators, he will do more exploration next on this project in Misasa. For example, some previous experimental groups have low reproductivity, possibly due to manual factors or material problems (*e.g.*, unstability at high concentration). In addition, the applicant will also try the measurements on other kinds of transition metal salts, such as Mn^{2+} , which are abundant geologically and have possible catalytic functions.

In addition, the applicant will use Micro-FTIR to detect the small chemical differences for droplets mixed with different salts at different concentrations. Due to the existence of salts, the intermolecular interactions inside the droplets will be changed. This strategy will help to reveal the structure of droplets, *e.g.*, the hydrophobicity and water arrangement, at different areas, which may affect the efficacy of catalytic and metabolism reactions in the droplets.

As for expectations, **on one hand, new paper publications, conference presentations and joint grant applications will be conducted sequentially. On other hand, it will also help the applicant, one young researcher, to take the first step in Japan to lead independent joint research combining complementary fields, including biophysics, biochemistry, geochemistry and electrochemistry.**