



## Commentary and Perspective

### Increasing complexity of primitive compartments

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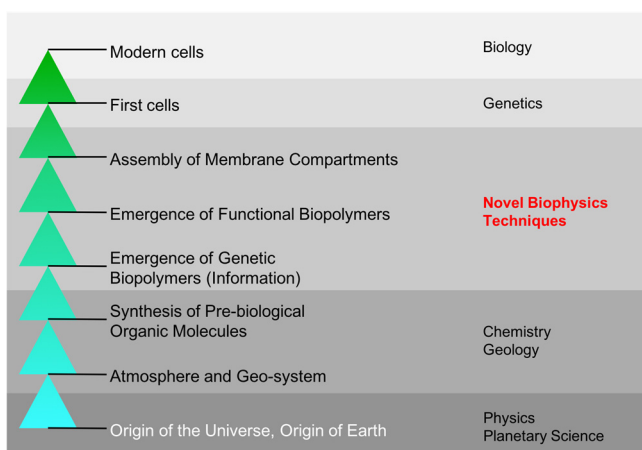
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One of the major questions in ancient and modern science is the question of our own creation: how did life emerge on Earth? In particular, what were the structures and functions of the first cells? Answering such questions requires input from researchers in a variety of disciplines, such as planetary science, geology, chemistry, and biology, to name a few. Recent advances in biophysics are now allowing biophysics researchers and those in many related fields such as synthetic biology, evolutionary biology, and biochemistry to finally begin to contribute to solving such fundamental mysteries of the origins of life [1] (**Figure 1**). To highlight recent progress in this endeavor by biophysicists in Japan and around the world, a symposium was organized during the 59<sup>th</sup> Annual Meeting of the Biophysical Society of Japan held in November, 2021 titled “Recent Advances in Origins of Life and Proto-cell Research”, organized by Tony Z. Jia (Earth-Life Science Institute (ELSI), Tokyo Institute of Technology and Blue Marble Space Institute of Science (BMSIS)) and Yutetsu Kuruma (Extra-cutting-edge Science and Technology Avant-garde Research Program (X-Star), Japan Agency for Marine-Earth Science and Technology (JAMSTEC) and Japan Science and Technology Agency (JST), PRESTO). In particular, speakers focused wide variety of primitive compartment systems with increasing structural and functional complexity, ranging from simple phase-separated membraneless droplets with limited function [2] to complex artificial cells with biology-like functions [3], suggesting that exploration of a breadth of structures and functions of potential primitive compartments is necessary to understand how the first cells emerged and evolved.

It has been postulated that perhaps before the advent of membrane-bound compartments (such as lipid vesicles), membraneless droplets may have been the form that early compartments took on [4]. In particular, the assembly, structure,



**Figure 1.** Where biophysics lies in the quest to understand the origins of life. Reprinted with permission from Jia, T. Z., Kuruma, Y. Recent Advances in Origins of Life Research by Biophysicists in Japan. *Challenges*. 10(1), 28 (2019) [1] under a Creative Commons License.

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and basic function of such membraneless compartments (often through phase separation) has been a key unanswered question for many researchers in the origins of life field. Building such structures from the bottom up, such as through assembly of polymerization products generated from primitive wet-dry cycles [5], remains one mechanism to study the basic characteristics of such systems. Recently, polyesters generated from drying of simple prebiotic monomers can assemble to form membraneless droplets which can perform simple functions such as nucleic acid encapsulation [6,7]. As other “non-biological” polymers [8], along with polyesters [9], have shown the ability to form such membraneless compartments, focus should not be placed only on “biological” polymers such as nucleic acids and peptides from an origins of life perspective. Nevertheless, it has been shown that incorporating amino acid residues into such primitive polymers, forming deipeptides through wet-dry cycling, can lead to formation of peptide polymers [10].

One such function of peptides is the assembly of membraneless coacervates through binding with primitive nucleic acids followed by phase-separation. Such membraneless coacervates have been investigated and could contribute functions such as biopolymer segregation and exchange [11], diffusion [12], growth and division [13], enhancement of compartmentalized reactions [14,15], and even scaffolding of other self-assembled structures [16-18] to an evolving prebiotic chemical system. Recent studies have found that more higher-order structures can assemble within peptide-DNA coacervates, leading to potential emergent functions with greater complexity depending on the environmental conditions and identity of the coacervate-forming polymers. For example, DNA liquid crystals can form within peptide-DNA coacervates upon sufficient concentration increases of the DNA and peptide components [19], potentially through environmental dehydration [20]. DNA quadruplex structures have also been found to promote phase separation and droplet formation for protein-DNA coacervates [21]. Such structures and other functions may be affected by physical properties such as molecular crowding within the droplets [22-24], and as such, further demonstration of life-like functions within or by primitive membraneless droplets, such as catalytic cascades [25], primitive gene expression [26,27], or speciation [28] could lead to more understanding of the suitability of phase-separation as a relevant prebiotic compartmentalization method. To highlight recent work in the area of membraneless droplet assembly and function in our recent symposium, invited symposium speaker Tony Z. Jia (ELSI, Tokyo Institute of Technology and BMSIS) began with a discussion of a novel DNA liquid crystal coacervate droplets [19,20]. Invited speaker Shunsuke Tomita (National Institute of Advanced Industrial Science and Technology) then followed with a presentation about primitive DNA liquid crystal coacervates and nucleic acid scaffolds that undergo phase separation into liquid droplets [21].

Although the first primitive compartments on Earth may have indeed been membraneless, and many membraneless organelles provide important biological functions within extant cells (suggesting the importance of phase separation throughout evolution) [1,29], at some point in history, a membrane-bound cell-like compartment must have preceded modern cells. Such a membrane-bound protocell could have taken the form of lipid-bound liposomes or vesicles [30-32], perhaps initially composed of simpler fatty acids [33,34] with subsequent evolution first into mixed amphiphile membranes [35-37] and finally into the phospholipid-containing form reminiscent of modern membranes [38]. As such, mechanisms of membrane assembly, dynamics, and evolution are key to understanding the emergence of primitive membranes. For example, modulation of membrane tension and lipid diffusion of liposomes membranes may allow interact with substrates other liposomes *via* electrostatic interactions and deflation, potentially allowing membrane-bound protocells to interact selectively with other prebiotic materials [39]. Similarly, vesicle growth could be caused by various effects including lipid concentration increases [40] or encapsulated catalysts [41], while vesicle division can be driven by encapsulated components [42] or external stressors such as light [43]. Finally, such could inevitably result in modulations in diffusion rate of encapsulated components, such as genetic materials, due to membrane surface effects and/or small volume confinement effects, which may affect efficacy or rates of primitive genetic evolution [44]. Incorporation of newly acquired knowledge could help to support further research demonstrating dynamic functions of evolving lipid vesicle-based protocell models, such as recently demonstrated primitive growth and division cycles [45,46] and recombination driven by reversible membrane phase transitions [47]. To address issues related to understanding of growth of membrane-bound protocells, Akiko Baba (Tohoku University) contributed a presentation discussing a mechanism of primitive membrane growth coupled with amino acid sequences, while invited speaker Anna Wang (University of New South Wales) discussed the various barriers to fusion of fatty acid-based protocell compartments [34,46].

Eventually, such simple membrane-bound protocells must have evolved more complex life-like functions, eventually leading to the emergence of the last universal common ancestor (LUCA), which eventually begat modern cells [48]. As such, synthetic or artificial cells have been developed as laboratory models in order to test the limits of what functions can be demonstrated in not-quite-living cell-like constructions. For example, artificial cells demonstrating self-organization and reproduction [49], gene expression [50], communication [51], or symmetry emergence [52] give insights into the requirements that would have allowed primitive cells to accomplish such life-like functions. The recent demonstration of an artificial cell capable of photosynthesis may even suggest that such important functions in extant autotrophs could have even been more ancient than imagined [3]. To present recent research that sheds light onto these more-advanced functions of protocells and artificial cells, invited speaker Chiho Watanabe (Hiroshima University) discovered that cell size is a key determinant of macromolecular crowding within artificial cells [22]. Yusuke Maeda

(Kyushu University) then discussed how synthetic cells can intracellularly organized, along with the instability that comes along with it [52]. Finally, Kanji Tomohara contributed a presentation describing the development of an intra-artificial cell transcription field.

As we have explored the gambit of structural and functional complexity through this symposium, it is quite apparent that there is no clear answer now as to what shape the first cells took on or what structures or functions were required for protocell assembly and subsequent evolution. As such, continued investigation into all facets and categories of protocells, ranging from simple geologically-influenced protocell assemblies to artificial cell models assembled in modern laboratories is essential. We suggest that a holistic collaborative approach incorporating interdisciplinary studies of many researchers from a variety of fields and with research focusing a variety of different primitive compartment systems may be the key to understanding such difficult questions and invite biophysicists in particular, especially young researchers, to work together to challenge these very difficult (but important) unanswered questions.

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