

**THE ROLE OF IRON IN PROTOCELL FORMATION; IMPLICATIONS FOR ORIGIN OF LIFE**

**PROCESSES ON EARLY MARS.** F. Cary<sup>1</sup>, D. Deamer<sup>2</sup>, B. Damer<sup>2</sup>, S. Fagents<sup>1</sup>, <sup>1</sup>Hawaii Institute of Geophysics and Planetology ([fcary@hawaii.edu](mailto:fcary@hawaii.edu)), <sup>2</sup>Department of Biomolecular Engineering University of California, Santa Cruz Santa Cruz CA 95060 ([deamer@soe.ucsc.edu](mailto:deamer@soe.ucsc.edu)).

**Introduction:** The origin of cellular life on Earth required the formation of membranous boundary compartments, a requirement that is evident in modern organisms as an echo of primitive cellular life. Membrane-forming compounds such as amphiphiles are readily available through meteoritic infall and geochemical synthesis in hot spring settings [1-4]. Amphiphiles can self-assemble into vesicles that are able to encapsulate nucleic acids and polymers thought to be precursors for RNA, as well as maintaining a permeability barrier controlling access of nutrients and ionic solutes to the cell's interior volume. The resulting structures of membranous vesicles with encapsulated polymers are referred to as protocells, which can aggregate into proto-cellular networks that are collectively capable of primitive evolution [4]. Protocells represent the earliest step in the path towards evolving cellular life on Earth, and potentially on other rocky water-bearing worlds with similar chemicals available such as Mars.

Mars and Earth have diverged in terms of their surface and geochemical conditions over the past 4 billion years. Little is known about the effect of specific geological and chemical differences on the process of protocell formation under early martian versus early terrestrial conditions. One globally evident difference on Mars is the higher iron content in the crust and minerals compared to Earth [5-8]. Ferrous iron ( $\text{Fe}^{2+}$ ) likely dominated early Mars before being oxidized to ferric ( $\text{Fe}^{3+}$ ) iron [9]. Dissolved iron could potentially impact the self-assembly of amphiphilic membrane vesicles because amphiphiles contain charged hydrophilic groups sensitive to divalent cations such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  [1], and potentially  $\text{Fe}^{2+}$  as well. In this work, we are investigating the effect of iron on protocell self-assembly and performance.

Iron is known to play a key role in biological functions such as metabolic systems and enzyme activity. In a primitive world under reducing and anoxic conditions,  $\text{Fe}^{2+}$  preceded the role of  $\text{Mg}^{2+}$  in catalysing RNA synthesis [10-12]. Iron also works to expand the catalytic repertoire of RNA [10], act as an ideal redox active co-factor [13], and catalyse the synthesis of RNA [11] and other organic compounds [1]. Heretofore, high iron content could have impacted emerging proto-cellular networks on Mars beyond membrane stability, given it was a useful catalyst for biologically relevant reactions for life on Earth.

**Objectives:** This work is investigating protocell formation in simulated hot spring water with  $\text{Fe}^{2+}$  present in varying concentrations to represent a plausible key difference in hydrothermal settings on Mars compared to the early Earth. The effect of  $\text{Fe}^{2+}$  relative to other divalent cations ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) and salts on the self-assembly of fatty acid vesicles are being investigated first, and will be followed by the effect of  $\text{Fe}^{2+}$  on nucleic acid encapsulation, and overall protocell functions. The samples are subjected to a series of wet-dry cycling processes under anaerobic and plausible pH and temperature conditions in order to simulate early Martian hydrothermal environments on volcanic landscapes.

*Key questions to be addressed.* 1) What is the  $\text{Fe}^{2+}$  tolerance or "fatal" concentration of  $\text{Fe}^{2+}$  for membrane vesicle self-assembly? 2) How does the effect of ferrous iron cations compare to the effect of calcium and magnesium cations on fatty acid vesicle self-assembly? 3) Can nucleic acids be encapsulated in vesicles in the presence of  $\text{Fe}^{2+}$ ?

**Experimental Approach:** Vesicles are prepared from a mixture of capric (C10) and lauric (C12) fatty acids, which are simple, medium length fatty acids that readily self-assemble into membranes and are plausible precursors for modern phospholipid membranes [1]. Vesicles are prepared for the purposes of this work in solutions containing  $\text{Fe}^{2+}$ , supplied in the form of  $\text{FeCl}_2$ . Samples with  $\text{Fe}^{2+}$  concentrations between 1 and 45 mM will be set up, a reasonable range for investigation given that "fatal" concentrations established for  $\text{Mg}^{2+}$  are 25–30 mM [14]. Stock solutions of capric and lauric fatty acids (1:1 mole ratio, 10 mg/ml) are prepared using chloroform as a solvent. The solvent is allowed to evaporate on a slide, leaving a dry phase fatty acid film. This film is then rehydrated with deionized water (Milli-Q 18 M $\Omega$ ) and a non-interacting buffer (triethanolamine 10 mM) to control the pH to 7-7.5 and deprotonate the fatty acid carboxyl group. Glycerol monolaureate (GML) is included in a 1:1 mix with the 10 mg/mL fatty acid solution to stabilize the membranes. The ionic content can be altered to include  $\text{FeCl}_2$ ,  $\text{MgCl}_2$ , and  $\text{CaCl}_2$  at varying concentrations between 1 and 45 mM. This solution is pipetted onto a slide to undergo wet-dry cycling, using a hot plate to heat samples to  $\sim 70^\circ\text{C}$ . In order to simulate evaporation and precipitation cycles in natural hot spring pools, deionized  $\text{H}_2\text{O}$  is used for rehydration. Yeast RNA (1 mg/mL dissolved in water) is included

during rehydration as a proxy for locally synthesised nucleic acids that are captured by the vesicles during cycling. Instead of yeast RNA, individual nucleotides can be included which polymerize in condensation reactions during wet dry cycling. Fluorescent dye (10 mM 6-carboxyfluorescein) is also included at this step to observe the encapsulated RNA by phase and fluorescence microscopy. UV absorbance at 260 nm is used to quantitatively analyse the encapsulation efficiency of membranous vesicles under various ionic conditions. Membrane stability is inversely related to permeability, hence relative stability can be measured by the rate at which 6-carboxyfluorescein leaks through the membranous vesicles, using absorbance at 497 and 520 nm with a spectrofluorometer.

**Implications and Expected Outcomes:** It is possible that iron inhibits the assembly and stability of protocell membranes. However, given the biological importance of iron, exploring the balance between inhibition and the other aspects of protocell functions that could be enhanced by iron will inform the role iron plays in hydrothermal environments. A noticeable effect of ferrous iron on protocell assembly processes would help us contrast early Mars to early Earth in terms of how conducive each were to the origin of life. If, as expected, the proposed experiments show that iron adversely affects protocell stability, they may provide one variable that helps form the basis for accounting for divergent evolutionary trajectories in the resultant protocell networks on Mars vs. on Earth.

Future experiments could investigate the effect of iron on amino acid and RNA polymerization under similar experimental conditions of wet-dry cycling that support peptide and ester bond formation, and explore whether  $\text{Fe}^{2+}$ , in comparison to other metal ions [15], could act as a catalyst for early polymer synthesis.

Understanding the relationship between geochemistry and keystone organic molecules in protocell formation processes is essential in order to relate the different environmental conditions of Mars to Earth and the possibility of originating life there. Life in the universe may be more diverse than we currently understand, with molecular characteristics specific to the chemical environment in which it emerges, making it necessary to explore the effects of environmental chemical variability on prebiotic processes as part of our search for life on other planetary bodies.

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