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Potassium at the origins of life: Did biology emerge from biotite in micaceous clay?

Helen Greenwood Hansma[1]*

Physics Department, University of California, Santa Barbara, CA; hhansma@physics.ucsb.edu

[1] Physics Department, University of California, Santa Barbara, CA; hhansma@physics.ucsb.edu
* Correspondence: hhansma@physics.ucsb.edu

Abstract
Intracellular potassium concentrations, [K^+], are high in all types of living cells, but the origins of this K^+ are unknown. The simplest hypothesis is that life emerged in an environment that was high in K^+. One such environment is the spaces between the sheets of the clay mineral, mica. The best mica for life's origins is the black mica, biotite, because it has a high content of Mg^{++} and it has iron in various oxidation states. Life also has many of the characteristics of the environment between mica sheets, giving further support for the possibility that mica was the substrate on and within which life emerged.

Keywords: clay, mica, biotite, muscovite, origin of life, abiogenesis, mechanical energy, work, wet-dry cycles

1. Introduction

All types of living cells have high intracellular potassium concentrations, [K^+] [1]. When and how did this high [K^+] appear? There are two options for when high intracellular [K^+] might have appeared in living systems: before or after the origins of life. The strongest hypothesis is arguably that life originated in a high-K^+ environment, because maintaining the K^+ gradient across the cell membrane is energetically expensive [2-5]. The earliest membrane-bound cells would also have had leaky membranes, causing them to be in equilibrium with the extracellular ionic environment [6].

As Morowitz and others have noted, features that are ubiquitous in biology are likely to have evolved early in life's origins [7-9]. This also argues for the origin of life in a high [K^+] environment. Why does life have intracellular K^+ when it could seemingly use intracellular Na^+ for the same purpose?, Bracher asks [8].

In living systems, K^+-dependent enzymes are typically intracellular, and Na^+-dependent enzymes are typically extracellular [10,11]. Ribosomes require K^+ and are essential for life [12]. Many other key cellular processes also require K^+ [13].

Most research on the origins of life ignores potassium K^+ or mentions it only superficially. The question of K^+ at life's origins is, arguably, an elephant in the room of research on the origin of life. Dubina and colleagues propose that life emerged in an environment high in [K^+] and Dubina has shown that potassium ions are better than sodium ions for polymerizing glutamic acid [14-16]. Bracher's group also has research on the advantages of K^+ at life's origins [8,17]. For example, K^+ stabilizes linear dipeptides against hydrolysis, while Na^+ stabilizes cyclic dipeptides, consistent with the predominance of linear peptides in living systems.

Where was there a prebiotic environment high in [K^+]? The ocean is not high in [K^+]. Concentrations of Na^+, [Na^+], are 40 times as high as [K^+] in the ocean. Similarly, river water is not high in [K^+] [18].

Two main possibilities have been published, for the origins of life in high [K^+]: in geothermal fields [1] and between the sheets of mica, perhaps in micaceous clay [19,20]. Both of these options might have been true, if micaceous clay was present in geothermal fields. Some advantages of mica are the partial confinement provided by mica sheets and the hexagonal grid of K^+ holding mica's anionic mineral sheets together. This grid has a periodicity of 0.5 nm, which is also the spacing of anionic phosphate groups in extended single-stranded nucleic acids, DNA and RNA.

2. K+ between mica sheets.

There are high concentrations of K^+ between mica sheets (Fig. 1). Fig. 1A shows 3 sheets of the black mica, biotite, bridged by K^+ (purple) between adjacent sheets. K^+ are at the sites of partial negative charges from recessed hydroxyl groups on the adjacent sheets.

With an 0.5-nm hexagonal grid of K^+, there are 6 K^+ per nm^2 between pairs of mica sheets (Fig. 1B), giving a concentration of 10 M K^+ when the mica sheets are separated by 1 nm (Fig. 1C). The sheets need to be separated to a distance of 100 nm to give a 100 mM concentration of K^+, comparable to [K^+] in living cells (Fig. 1D). 100 mM is

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the initial [K^+] when sheets are separated; the [K^+] will be decreasing at the edges of the sheets in contact with the external environment, and the [K^+] will also be increasing on the inside regions of the split mica, where the sheets are separated by less than 100 nm.

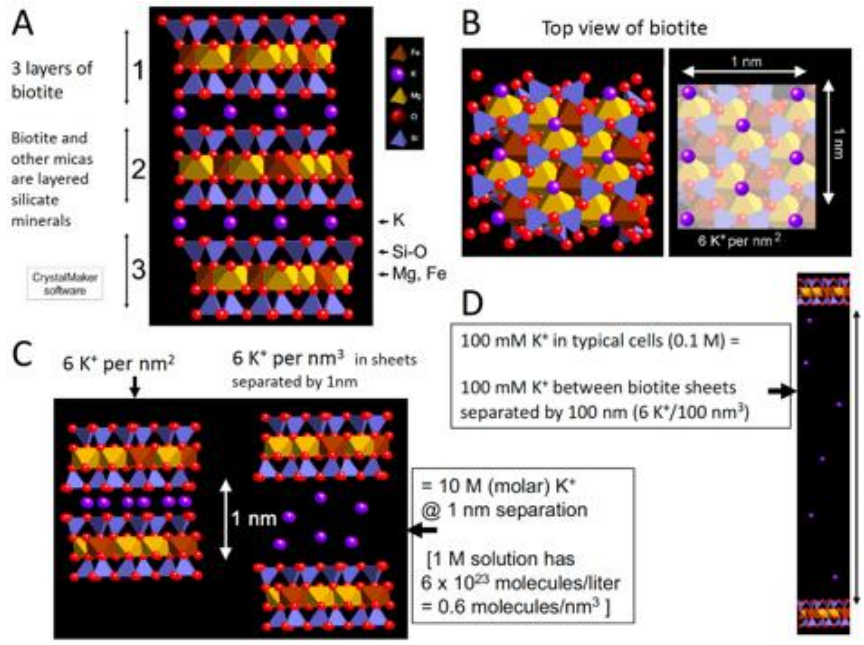

Figure 1. [K^+] between mica sheets. Structure of the black mica, biotite. **(A)** Side view of 3 biotite sheets. **(B)** Top view of 1 nm^2 biotite, with K^+ highlighted in the right-hand image, showing that there are 6 K^+ per nm^2 between mica sheets. **(C)** Side view of 2 mica sheets, not separated and separated to a distance of 1 nm, where [K^+] = 10 M between the sheets. **(D)** Scale model of biotite sheets at a separation of 100 nm, where [K^+] = 100 mM. (CrystalMaker X software, version 10.6.4, CrystalMaker Software Ltd.)

3. A scenario for life's origins between mica sheets.

The spaces between mica sheets provide a semi-enclosed environment for life's emergence (Fig. 2). According to de Duve, in the early stages of life's origins, the need for free exchanges would have given an advantage to open systems, due to the constraints of encapsulation [21].The spaces between mica sheets have the advantages of open exchanges at the outer edges and also the advantages of partial isolation farther within the spaces between the sheets. This might be ideal, for example, for processes such as the evolution of ribozymes, where isolated niches prevent easily replicated ribozymes from dominating the entire population; and interactions such as ligations can occur in other niches and in the space beyond the sheets, to allow ribozymes to change and evolve [19,22,23].

Niches between biotite sheets could also provide spaces where auto-catalytic cycles and proto-metabolic cycles were forming and evolving (e.g., [24-26]). In a beautiful piece of work showing the possibilities of prebiotic syntheses, Muchowska et al. have synthesized 9 of the 11 main components of the TCA cycle, from glyoxylate and pyruvate, with Fe(II), in a test tube at 70°C in only hours [25,27]. Vast numbers of niches exist between mica sheets, providing spaces also for the evolution of genetic coding and ribosomes.

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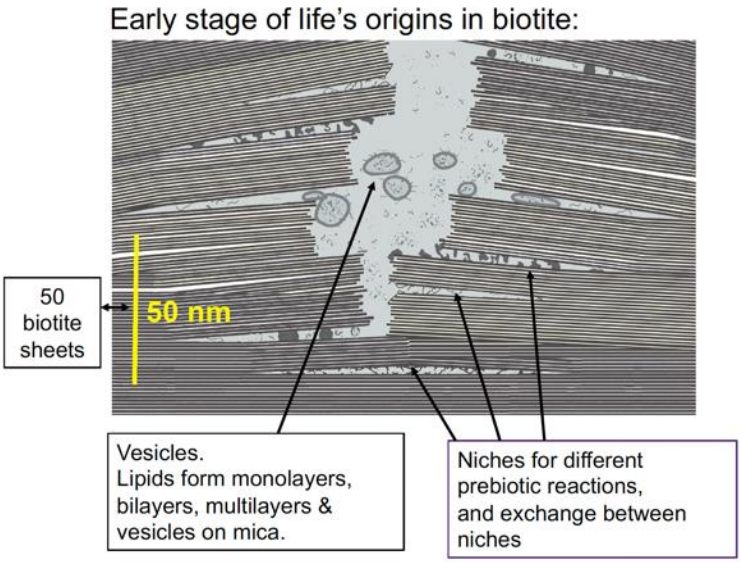

Figure 2. Nanometer-scale diagram of how the early stages of life might have originated between biotite mica sheets. Niches within the biotite sheets provide partially enclosed spaces for molecular evolution of different processes essential for life. Vesicles form, encapsulating molecules and molecular complexes from the niches.

Membranes would be forming and encapsulating molecular complexes that were accumulating between the biotite sheets, forming vesicles and protocells. These would tend to aggregate and fuse, bringing together the molecular complexes for metabolism, self-replication, protein synthesis and other necessary processes for life. This would be a slow, gradual, complex process, occurring at many locations in the mica (Fig. 3). After a long long time, membranes would occasionally encapsulate *everything* needed for self-reproducing living cells. Some of these living cells would survive, while others would die after a few generations or more. Life has indeed emerged on Earth, providing conclusive evidence that some living cells survived.

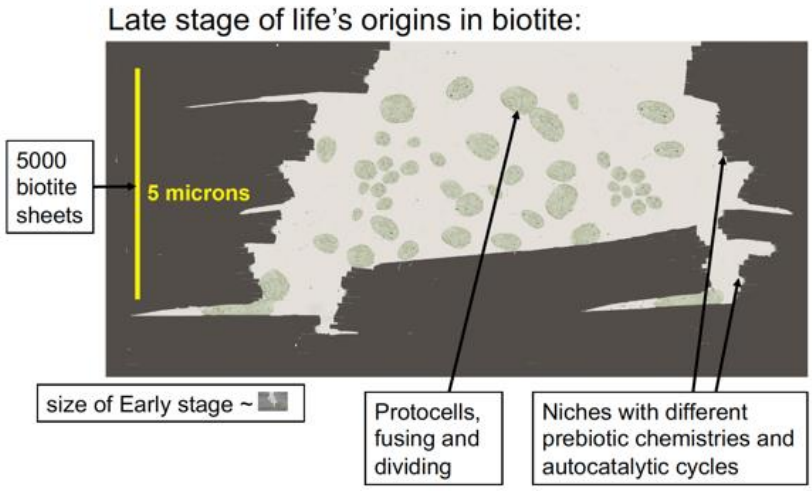

Figure 3. Micron-scale diagram of how life might have originated between biotite mica sheets. Protocells in the aqueous environment encapsulate prebiotic molecular aggregates in the niches between mica sheets. Mechanical energy from moving mica sheets can bleb off protocells, as seen in the lower left corner of the figure. Eventually, occasionally, a living cell will be produced, capable of self-reproduction.

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Mica is old enough to be a site for the origins of life [28]. Muscovite and biotite are among the major minerals found in zircon grains from the Hadean [29]. Most of the mica would not have been in clays as early as the Hadean, but, as Hazen says, even traces of a mineral could have been sufficient for the mineral to be involved in life's origins [30,31]. Borates, for example, were not present in large quantities at life's origins [28]. Borates, however, are valuable for stabilizing ribose; and even traces of borate on the early Earth might have served this function [32,33]. Similarly, even traces of micaceous clay might have been the site of life's origins on Earth.

Biotite mica has advantages over muscovite mica. Biotite is rich in iron (Fe) and magnesium (Mg). The iron is predominately Fe(II) [34]. Especially in the Hadean reducing environment, Fe(II) predominated over Fe(III). Mg(II) is a major inorganic divalent cation in living systems, where it stabilizes DNA and RNA structures and provides the counterions for ATP, among other things. [[[[Biotite is the most conductive mica, because of its iron content. Electrical conductivity increases exponentially with the iron content of micas [35,36]. Biotite's iron may have been useful for redox reactions [37] at life's origins, in the redox-active and conducting environment of clay [38,39] and the reducing atmosphere of the Hadean [40]. Acid accelerates the dissolution of biotite, acting primarily at step edges of biotite sheets and at etch pits [41]. Biotite is also found on Mars, which may have been the original source of life in the Solar System, seeding life on earth [42].

4. Energy from mica

4.1 Mechanical energy. (Fig. 4) Moving mica sheets can produce endless energy for the origins of life [43]. This energy of moving mica sheets is mechanical energy that can be used for mechanochemistry, to make and break chemical bonds, when stacks of mica sheets move, open and shut. Mechanochemistry is a growing research field, in which biomolecules are synthesized with mechanical forces [44]. Mechanochemistry has been used in possible prebiotic syntheses [45,46] and nucleobase pairing [47]. Glycine polymerizes by mechanochemistry in mica, by ball milling [48].

Both mica sheets and enzymes have open and shut motions that do work on the molecules between them. As the title of a recent article says, 'Enzymes at work are enzymes in motion' [49]. Experimental results support this statement [50,51]. Further evidence for the importance of mechanical energy in biology is that *Molecular Biology of the Cell* is soliciting submissions for its Sixth Special Issue on Forces on and Within Cells" [52].

How much energy can moving mica sheets provide? If the mica sheets move even 0.1 nanometer (nm) closer together, in air, they can push together 2 molecules to form a covalent bond, if the mica has a spring constant stiff enough to provide 170 piconewtons (pN) of force [19]. The equation for a spring constant, $F=kx$, with $x = 0.1$ nm, shows that a spring constant (k) of 1.7 N/m (Newtons/meter) is stiff enough. The spring constant of the mica depends on the number of mica sheets in the layer that is moving open and shut. Each mica sheet is ~1 nm thick. Only about 7 mica sheets are needed to provide this spring constant, in air [53]. In practice, the layers of moving mica sheets will often have thickness of microns, not nanometers, due to the fragility and consequent damage to nanometers-thick layers of mica sheets.

Mechanochemical polymerizations can create oligomers and longer polymers that can bind to the mica surface more strongly than monomers and short oligomers. Monomers and short oligomers can be preferentially washed off the mica sheets, favoring polymerization by mechanochemistry over polymer breakdown.

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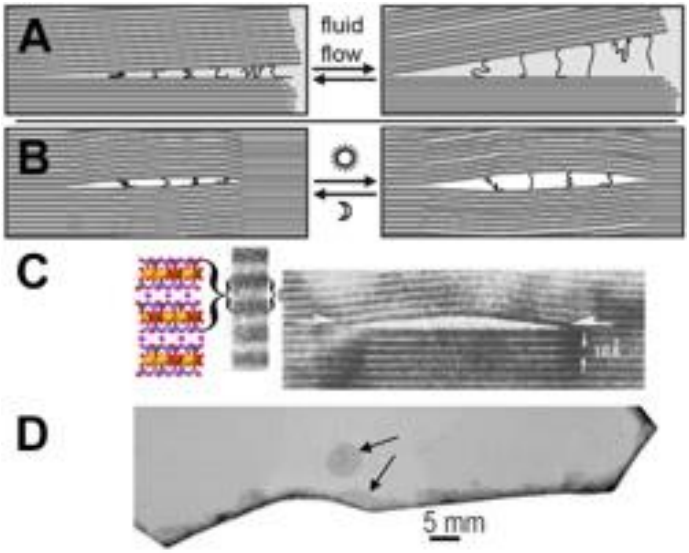

Figure 4. Mica and Mechanical energy. A. Diagram of mechanical forces between biotite mica sheets, stretching and compressing polymers, due to water flow at the edges of the biotite sheets. **B.** Diagram of mechanical forces between biotite mica sheets due to heat pumps in a biotite bubble. This mechanical energy can be used to synthesize prebiotic molecules, stretch and compress polymers (as shown in the diagram), or bleb off protocells [19]. Seven mica sheets, as shown in **A,** provides enough force to form a covalent bond in air, when moved a distance of 0.1 nm. **C.** Biotite bubble imaged by HRTEM (high-resolution transmission electron microscopy) [54], with expanded view of HRTEM image and CrystalMaker model of biotite on left. "{" or "}" = 2 biotite layers. The thickness of a single biotite sheet is 1 nm (10 Angstroms). **D.** Photograph of muscovite mica, showing a bubble (upper arrow) and separation at the edges of the mica sheet (lower arrow). Bubbles are common even in 'high grade' micas such as this one.

4.2 Wet-dry cycles. Entropy drives polymerization during wet-dry cycles [55]. Polymers of amino acids and nucleotides form by dehydration, but polymers hydrolyze in the presence of water [56-59]. In mica, wet-dry cycles occur at split edges of mica sheets, as in Fig. 4A and 4D (lower arrow). The slow wet-dry cycles that can occur at the edges of mica sheets will generate longer polymers during the longer drying cycles, before hydrolysis occurs during the wet phase. These longer polymers will bind to mica better than short polymers and will, consequently, be more likely to remain bound to the surface. This is seen in Atomic Force Microscopy (AFM), where long DNA molecules bind to mica strongly enough for AFM imaging, but short DNA molecules do not bind to mica well enough to be imaged [60]. In contrast, rapid wet-dry cycles in small clay particles will cause polymerization to be followed more quickly by depolymerization, resulting in shorter polymers that will more easily detach from the surface.

How much water was on the Hadean Earth? The question has been reviewed recently, giving evidence that water may have covered up to 80% of Earth in the Hadean and more in the Archaean [61].

5. How big do the mica sheets need to be?

One needs only tiny pieces of mica for mechanochemistry. The mica fragments in micaceous clays are large enough. The 'mica world' diagram in Fig. 3 is lengthened, in Fig. 5A, to show that even sub-millimeter-sized mica fragments are big enough to generate mechanical energy for life's emergence. A submillimeter mica fragment of the same length is highlighted in Fig. 5C. Therefore, life may have emerged in micaceous clay, as opposed to larger pieces of mica. The swelling clay particles surrounding the mica fragments would also be advantageous for life's emergence. For example, polymer syntheses might occur during the drying phase of wet-dry cycles in clay, and solutes in the surrounding fluid would be concentrated during drying. Some of these molecules and other solutes would move between the mica sheets.

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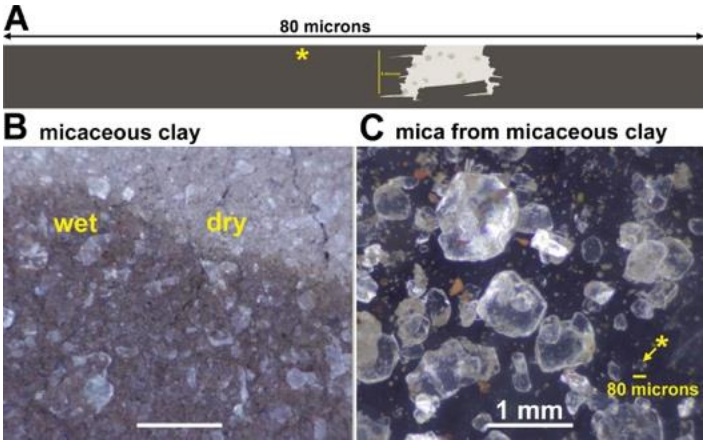

Figure 5. Micaceous clay and the origin of life between mica sheets. A. Mica origins diagram of Fig. 3, a late stage in the origin of life, extended to a length of 80 microns. **B.** Mica Red Micaceous Clay, from New Mexico Clay Store, containing pale reflecting pieces of mica, in the middle of a wet-dry cycle. **C.** Mica and a few clay particles, washed from the micaceous clay. Yellow asterisk and arrow point to a mica fragment with a diameter of ~80 microns.

6. Origin of life in micaceous clay?

Micas are non-swelling clay minerals, unlike smectite clays such as montmorillonite (Fig. 6), which swells and shrinks during wet-dry cycles [62]. Typical clay particles, ~1-2 microns in size, are also much smaller than mica particles. The swelling and shrinking of these clays is as if they were tiny sandwiches whose filling were growing thicker and thinner, and wetter and dryer. Non-swelling mica provides a more stable environment for life's origins than swelling clays. Wet-dry cycles do occur, however, at the split edges of mica sheets, where water seeps slowly in and out (Fig. 4D), leaving dry and nearly dry regions beyond the wet edges. Experimentally, water seeped a few millimeters between the sheets of mica pieces that were cycled daily between 22ºC and 4ºC for 2 weeks [19].

Why does montmorillonite clay swell, while micas do not swell? Anhydrous K^+ is larger than anhydrous Na^+ (Figure 6 caption). The larger ions of K^+ fill the spaces at the recessed hydroxyls between mica sheets, while the smaller ions of Na^+ are hydrated at the recessed hydroxyls between the sheets of clays such as montmoillonite.

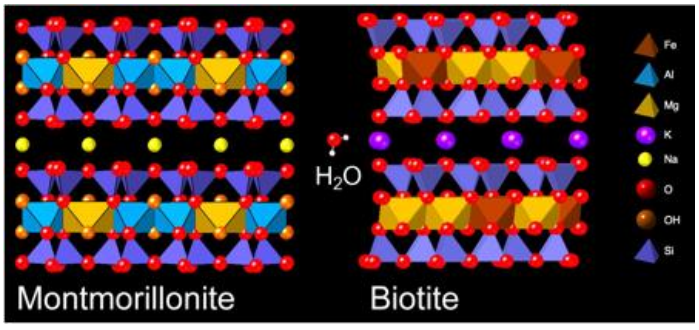

Figure 6. Swelling clay [montmorillonite] and non-swelling clay [biotite]. Molecular models showing 2 sheets of montmorillonite clay (left) and biotite mica (right); water molecule (center). Surfaces of the sheets are tetrahedral silicon-oxygen (Si-O) layers; see Fig. 1B for top view. A major difference between these clays is that Na^+ [yellow] bridges sheets of montmorillonite and K^+ [purple] bridges sheets of biotite. Ionic radii are 0.095 nm for Na^+ and 0.133 nm for K^+ [63]. The smaller Na^+ are hydrated between montmorillonite sheets, which causes montmorillonite to swell and shrink with wetting and drying. The larger K^+ between biotite sheets are not hydrated; biotite does not swell and shrink with wetting and drying. (CrystalMaker X software, version 10.6.4, CrystalMaker Software Ltd.)

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Why clay? Hyman Hartman explains it thus: "The genetic code drives all biological life. But even a mechanism this fundamental rests on still more ancient biochemical processes, as well as the intriguing chemical properties of a seemingly nondescript material—clay. … Formed through the reaction of silicates with water, clay minerals have layered crystal structures that provide ideal surfaces for molecules to bind to and interact with each other in close proximity. In fact, we have long used these very properties of clay to speed up chemical reactions in oil refineries and in the catalytic converters found in cars." [64].

Clay mineral surfaces catalyze or support syntheses of amino acids from simple precursors and polymerizations of amino acids and nucleotides into oligopeptides and oligonucleotides, e.g., [57,65-69]. Nucleotides on clay polymerize preferentially in the 3'-5' orientation, as in life, and not in the non-biological 2'-5' orientation [70]. A Molecular Dynamics study [71] of montmorillonite clay indicates that polymerization in the 3'-5' direction occurs fastest in clay sheets that are closer together, compared with the non-biological 2'-5'direction, which occurs fastest at greater separations of the clay sheets. The mineral sheets in mica are more often close together than the mineral sheets in clay, which swells and shrinks. Polymerizations occur preferentially in the clay interlayer, as opposed to the edges of the sheets. Homochiral polymerizations are favored over achiral polymerizations on clay. Clays form in association with the water needed for life [72], which is another advantage of clay over other rocks and minerals. The mica in micaceous clay might have been the site where life originated.

7. Biology and biotite

Biotite and other micas have similarities with life, as would be expected for places where life might have originated (Table 1) [19,73].

Table 1. Characteristics of life and mica.

Life:	**Mica:**
Cellular compartments	Compartments between mica sheets
High intracellular potassium, [K^+]	High [K^+]; potassium ions bridge mica sheets
Nucleotides polymerize to DNA & RNA	Nucleotides polymerize to RNA in wet/dry cycles [74]
0.5 nm spacing of anionic phosphates in ssDNA	0.5 nm anionic crystal lattice on mica surface
Exchangeable inorganic cations bridge anionic sites on molecules such as DNA	Exchangeable inorganic cations bridge anionic sites between mica sheets
Water-rich; aqueous	Hydrophilic
Forms H-bonds	Forms H-bonds [75]
Mechanical energy of enzyme motion[1]	Mechanical energy from moving mica sheets[1]
Synthesis of biomolecules in confined spaces	Supports chemistry of confinement
Filled and covered with lipid membranes	Supports lipid membranes & vesicles

[1]The mechanical energy of enzymes is powered by chemical energy, primarily ATP. The mechanical energy of moving mica sheets is powered primarily by thermal disequilibria.

7.1 RNA and DNA on mica. RNA polymers form spontaneously on mica [74]. RNA monomers polymerize, non-enzymatically, on a mica surface during wet-dry cycles. Nucleotide monophosphates of Adenine (A), Guanine (G), Cytosine (C) and Uracil (U) on mica were cycled through wet-dry cycles at 80ºC and imaged by Atomic Force Microscopy (AFM) [74]. This simple process, with no enzymes or activated nucleotides, produced RNA on bare mica. RNA lengths were ~100-1000 nucleotides, which is about an order of magnitude longer than the RNA lengths obtained when polymerization occurred in the presence of lipids, without mica [76]. It is reasonable that mica's anionic crystal lattice is a better substrate than lipids, for polymerizing RNA, because RNA has the same periodicity – 0.5 nm – as mica's crystal lattice.

Mica may have been a template for RNA polymerization at life's origins. Perhaps nucleic acid linkages are 3'-5' and not 2'-5' because mica sheets served as a template that favored 3'-5' linkages. Perhaps nucleotide templating on mica's crystal lattice prevented diphosphate linkages, which form a bent polymer, and other irregularities of nucleotide polymerization.

DNA binds reversibly to mica in the presence of various divalent inorganic cations. For example, freshly cleaved mica was soaked in 33 mM magnesium acetate to bind DNA to mica for early AFM imaging (Fig. 7) [77,78]. With AFM in aqueous fluid, stable DNA imaging on mica was observed when Ni^{++}, Co^{++}, and Zn^{++} salts were present; in contrast, DNA binding was not strong enough for AFM imaging when salts of Mn^{++}, Cd^{++}, Hg^{++}, or

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K^+ were used [79]. DNA transcription by RNA polymerase was observed by AFM when Zn^{++} was alternately added, to bind the DNA to mica, and removed, to allow polymerase activity [80].

If polymers that have an affinity for a mineral surface, longer polymers will be more firmly bound to the surface than shorter polymers, facilitating the accumulation of long polymers [81]. This has been observed for AFM of DNA [60].

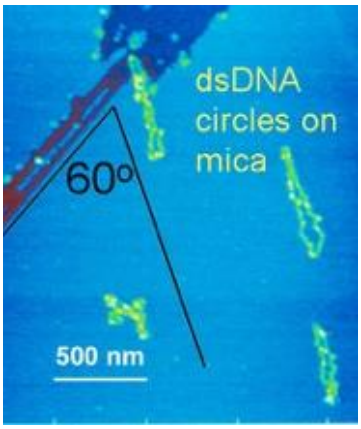

Figure 7. Atomic Force Microscopy of circular double-stranded DNA (dsDNA) on mica with cracks. (Cracks are dark streaks at the upper left). Three of the 4 dsDNA circles form a 60-degree angle with the mica crack, consistent with alignment on mica's hexagonal crystal lattice.

7.2 Sugars. Sugars, especially ribose, are a major biomolecule in living systems. A plausible prebiotic reaction for forming sugars is the formose reaction, in which formaldehyde reacts to form sugars [82]. In a test tube, the end products become increasingly large polymers of sugars, branched sugar polymers, and eventually a tarry mess. Monosaccharides, especially ribose for RNA, are a desired product, at the origins of life [83]. The spacing of sugars in oligosaccharides is 0.5 nm, like the periodicity of the mica lattice.

If the formose reaction is tightly confined between mica sheets, simpler sugars might predominate. Mica's anionic hexagonal lattice may also favor linear oligosaccharides over branched or bent ones. The formose reaction produces a simple sugar when the reactants are confined in vesicles [84]. This is an example of the advantage that confinement gives for limiting the products of the formose reaction.

7.3 Peptides. Peptide amyloids are proposed, in an intriguing new hypothesis for the functional molecules at life's origins [85]. Beta-sheets are the simple linear structure for peptides that can form amyloids, which form into stable ordered structures capable of various catalytic activities.

Peptide bond formation varies in difficulty, depending on whether the reactants are amino acids or peptides. Bond formation is easiest when it forms between 2 polypeptides. Intermediate in difficulty is bond formation between an amino acid and a polypeptide. Bond formation is most difficult between 2 amino acids [86]. This favors the formation of longer polypeptides at life's origins.

7.4 Membranes and the origins of life. Membranes on mica have been observed by Atomic Force Microscopy [87-89]. Vesicles on mica fuse to form extended bilayers and multilayers. Even without lipids, however, mica sheets could have provided partially enclosed spaces for emerging life, before the molecules of emerging life were enclosed in membranes. Membranes can be fragile. They leak, acquire and lose molecules, swell, and rupture. Membranes of living cells are highly evolved structures that provide more extensive support, and selective permeabilities, for their contents than primitive vesicles and membranes.

Lipid membranes might not have been essential at the early stages of the origins of life. Root-Bernstein et al. say that the evolution of membranes would be a late development, in their paper about 'prebiotic ecology [90]. An 'ecosystems first' perspective is proposed by Baum and others, based on their intriguing research involving chemical selection on mineral surfaces [91].

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Perhaps, instead of membranes, protolife evolved as an acellular ecosystem, sharing all the necessary enzymes in an open system. Imagine pieces of this ecosystem periodically encapsulating in membranes. Nearly all of these membrane-encapsulated protocells would lack some essential component of life or enzyme. Occasionally, membrane-encapsulated protocells would contain all the essential components of life and became alive. Occasionally some of these living protocells would reproduce successfully and begin seeding Earth with Life.

On the other hand, there is also a school of thought in which membranes are the enclosed spaces where proto-life first evolved, e.g., [92].

7.5 Coacervates and Membraneless organelles. There is an increasingly popular alternative to membranes at the origins of life – 'membraneless organelles' or 'membraneless biomolecular condensates' also known as 'coacervates'. Peptides/proteins and RNA interact in membraneless organelles in living cells, as nucleoli and other particles [93-95]. Nucleoli, the membraneless organelles in cell nuclei where ribosomes are formed, are now known to contain other membraneless organelles inside them [96,97]. Membraneless organelles form by liquid-in-liquid phase separation (LLPS) [98]. Membraneless organelles are increasingly of interest to origins-of-life researchers [99-106].

Ribosomes are ancient biomolecular condensates, composed of proteins and RNA, and are now necessary for translating nucleic acids into proteins. Ribosomes were present in the Last Universal Common Ancestor of life (LUCA) [107]. When life was coming into being, in the pre-LUCA stages, ribosomes and their precursors may have been early 'membraneless organelles', protected within mica sheets [101]. Prokaryotic ribosomes are ~20 nm in diameter, comparable to the thickness of 20 mica sheets (see Figs. 1 and 2) and much smaller than the 100-nm separation of mica sheets at which the $[K^+]$ is 100 mM (Fig. 1D).

8. Dielectric constant at surfaces

The Dielectric constant, or permissivity, of water is 80 for bulk water but only ~2 for the first 2 or 3 water layers above a surface (~2 nm) [108,109]. This means that the charges on charged molecules will become progressively unscreened as the charged molecules approach the mica surface. Electrostatic forces will be stronger, resulting in stronger interactions between charged organic molecules, the anionic mica surfaces, and inorganic cations.

9. Crowding at the origins of life

"There is a growing consensus that confinement may have facilitated the transformation of inanimate matter into living organisms" [110]. Confinement exists within compartments of various sizes between the sheets of mica; these compartments may have provided confined spaces for the isolation and stabilization of supramolecular assemblies and protocells.

Molecules in cells are crowded. The space between protein molecules in cells is typically only 10 nm [111]. Crowding speeds up the rates of reactions that are diffusion limited [112]. Crowding may even be the origin of homochirality [113]. Given the molecular crowding in living cells, molecular crowding at life's origins is a desirable scenario for hypotheses about the origins of life. Molecules in wet-dry cycles become crowded during the drying phase. Molecules that bind to a surface, e.g., the mica surface, will become concentrated and crowded. Molecules in narrow spaces between mica sheets will typically be crowded, by the mica sheets above and below, in addition to crowding by other molecules. Clays will also crowd molecules between their sheets, but clay's swelling will then dilute molecules. Swelling to even 2 layers of water molecules between Na-montmorillonite clay sheets reduces the interaction energy between sheets to near zero, according to molecular modeling [114]. Thermophoresis is another way to concentrate molecules, in a spatially confined thermal gradient, and even to escalate nucleotide polymerization [115,116].

Confinement chemistry would occur between mica's sheets during drying and during the compression stage of mechanochemistry. Chemistry in confined spaces produces fewer different molecules and simpler molecules [117,118]. Confined spaces also help proteins fold [119,120]. Enzymes confine their substrates to facilitate the enzymatic reactions. Zeolites mimic enzymes in some respects [121]. Nano-confined liquids have very different properties from bulk liquids [122]. Crowding is also proposed to have enhanced evolutionary capabilities through the networks created by the proximity of components in crowded environments [123]. Confinement chemistry is likely to be a characteristic of any good hypothesis for the origins of life.

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10. Heirarchy, Complexification, and Error Tolerance

Herbert Simon describes the need for a hierarchy of structures in abiogenesis. [124] He uses the watchmaker as an example. The watchmaker needs stable intermediates in the watchmaking process. If no intermediates were stable, the partially assembled watch would disassemble whenever the watchmaker set it down – to answer the phone, for example, for getting a new order for a watch, to use Herbert Simon's example. Stable intermediates in abiogenesis can assemble on the hexagonal 0.5-nm anionic grids on and between the 'ceilings' and 'floors' of the spaces between mica sheets. As Pross and Paschal say, complexification is also an often-ignored but necessary aspect of abiogenesis [125]. Molecular interactions with mica sheets will stabilize intermediates and enable further complexifications.

Freeman Dyson says error tolerance is essential for life's origins [126]. With the redundancy of the vast areas between biotite mica sheets, in micaceous clay, almost everything can go wrong, and life can still emerge. If not from micaceous clay, life emerged from some other habitat with vast error tolerance.

11. Conclusions

Somewhere in the universe there was a hospitable habitat, with everything needed for the origins of life. We know this because life now exists on Earth. The habitat may have been on Earth, ~4 billion years ago [127]. In this habitat, the components and processes of life were evolving, resulting, eventually, in LUCA, a Last Universal Common Ancestor [128,129], which may have been a primitive acellular community of some type [90,91,130,131]. Among these components and processes, RNA-peptide 'worlds' [132-140] were evolving to create and replicate genetic information [141]; proto-metabolic cycles were evolving into early metabolism [142,143], and ribosomes were evolving to synthesize proteins [144,145]. This may have occurred in 'lipid worlds' [76,146,147]; hydrothermal vents have also been proposed [148-151]. Vast numbers of interconnections were needed, for bringing the precursors to Earth or synthesizing them on Earth and bringing them, eventually, to the hospitable habitat from which 'biology' emerged. The habitat was likely clay [152,153], the stuff of life.

Biology may have emerged from the spaces between biotite sheets in micaceous clay. The spaces between the sheets in these mica 'books' might have been ancient pre-cellular habitats where prebiotic molecules were confined, concentrated, and synthesized, before membrane-bound cells emerged (Fig. 2) [19,43,73,101,113,154]. These pre-cellular spaces have an anionic crystal lattice, with 'ceilings' and 'floors' that could have templated molecular syntheses and polymerizations. The mineral sheets of biotite and muscovite micas have a layered silicate structure, like montmorillonite and other clays, which have been used successfully to catalyze reactions non-enzymatically, such as the synthesis of biopolymers. [65-67,155]. Mica sheets move, open and shut, at their edges, as fluids flow and temperature changes; they are thus in a constant state of the thermodynamic non-equilibrium necessary for life.

We will never know for certain whether this, or any origins hypothesis, is completely true. The experimental method traditionally starts with a hypothesis, followed by experimentally testing the hypothesis. Testable hypotheses are presented here. Experimental results will show what is possible today, but these results may, in fact, be false positives or false negatives. The origins of life are partly an ahistorical science in which much will remain hypothetical, both experiments and ideas [125]. Experimental results give us ideas about how life might have originated, but they cannot absolutely prove how life originated, though strong experimental results are seen as convincing evidence [27].

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