3-3-2022

Aerosolization of Catalytic RNA for Prebiotic Transport and In Situ Reactivity

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by

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A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Chemistry

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Portland State University
2022

Abstract

Recent theoretical and experimental work suggests that aqueous aerosols in the early Earth's atmosphere might have been an essential component to the development of life. These complex droplets would have served to compartmentalize emerging biomolecules, thereby concentrating them, increasing reactivity, and facilitating transport and exchange between ocean and atmosphere. This project tests an underexplored but potentially important environmental paradigm for the RNA world hypothesis of prebiotic evolution, probing the effects of aerosolization on catalytic RNA using a model ribozyme. Here we demonstrate the successful transport and *in situ* self-assembly of the *Azoarcus* ribozyme from multiple independent fragments via a laboratory-constructed aerosolization system.

Acknowledgments

I'd like to thank my principal advisor Dr. Dirk Iwata-Reuyl, along with my committee members Dr. Dean Atkinson and Dr. Todd Rosenstiel, for their guidance, encouragement, and expert analysis throughout the latter half of what has been a long and tumultuous project. Thank you for sticking with me.

Special thanks to my former advisor Dr. Niles Lehman, who set me on the path of Origins of Life research, and whose expertise built the foundations of this project. And also to former committee members Dr. Steve Reichow and Dr. Aslam Khalil, whose teaching and counsel elevated my graduate studies.

Thanks to my loving parents, my mother Riva and stepfather Steve, my father David and stepmother Shannon, for your constant unconditional support in this endeavor. I love you all.

And lastly to my incredibly supportive fiancée, Kacy, without whom I could not have completed this journey. I cannot wait to start the next chapter of our lives together.

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Background and Significance

Atmospheric Aerosols

Aerosols are colloidal suspensions of solid or liquid particles in air or other gases, formed and emitted into the atmosphere by both natural and anthropogenic processes. Sea Spray Aerosol (SSA) is generated at the air-water interface of the Earth's oceans, while other natural aerosols such as smoke particles are released into the atmosphere via volcanic eruptions and forest fires. In contrast, combustion of fossil fuels in man-made engines, manufacturing plants, and other industrial processes emit synthetic aerosol pollution. Colloquial classifications of aerosols such as dust, smoke, fog, mist, and others are loosely defined and are often used interchangeably. More specific classification divides aerosols into "primary" and "secondary" categories. Primary aerosols are formed and emitted directly into the atmosphere as particles, generated from the sources mentioned above. Secondary aerosols, however, aggregate in the atmosphere via condensation and nucleation of supersaturated gases, driven by concentration and vapor pressure differentials, and primary aerosols often serve as condensation nuclei for these processes, as well as for cloud formation.[1]

The chemical composition of aerosols can vary considerably, often containing water, oceanic salts, trace metals and even organic material, as well as sulfates, nitrates, or ammonium, depending on their origin and where they are ultimately found in the atmosphere. Equally variable is the size distribution of atmospheric aerosol particles, the

diameters of which can span over multiple orders of magnitude, between nanometer and micrometer scales (such estimates assume that aerosols are isometric and spherical, characteristics that are not always present, but which correlate to higher water content). Particles can be separated into 'fine' and 'coarse' based on their diameter distributions (below and above 2.5 μm, respectively; 'ultrafine' distributions exist below 100 nm), although this is an operational definition, and distributions often show overlapping modes between the two. Growth and evolution of atmospheric aerosol particles depend on condensation, evaporation, and coagulation (Fig. 1), governed by heterogeneous or homogeneous nucleation (with or without the use of an existing particle surface, respectively) of atmospheric gases, and aerosols can persist in the atmosphere on timescales of mere minutes to days or weeks.

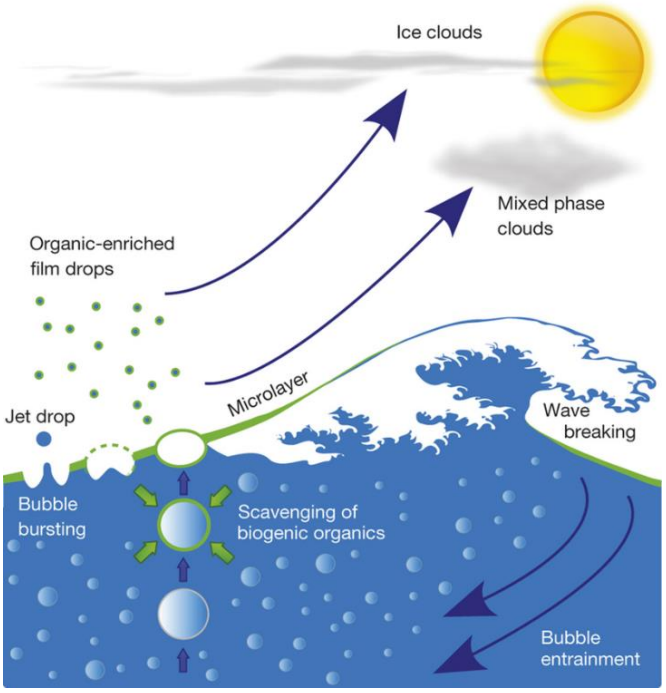

Figure 1. Sea spray aerosol (SSA) generation at the air-water interface. (Figure taken from Wilson, et al. "A marine biogenic source of atmospheric Ice-nucleating particles." (2015) *Nature*.)[51]

SSA, in particular, is emitted as a primary aerosol, largely existing as coarse droplets. Sea spray exhibits diameter distributions from hundreds of micrometers at their largest, to, in some cases, tens of nanometers at their smallest. SSA varies considerably in atmospheric lifetimes, and has shown chemical compositions not only containing sodium chloride and other common seawater ions but also substantial amounts of organic material—as high as 50% by mass—the concentrations of which tend to scale inversely with particle size.[1] SSA is generated from breaking waves and bursting bubble films formed at air-water interfaces, and emitted as the result of two mechanisms known as jet drops and film drops or film cap drops. As bubbles burst at the sea surface, which is coated in a hydrophobic microlayer up to hundreds of micrometers thick, several film drops are emitted, while a jet drop emerges upward from the center of the collapsing bubble cavity.[2] These processes are well-characterized, and largely depend on bubble size and composition as well as surfactant coverage.[3]

SSA is often collected in the field for analysis but has also been consistently produced through several models in a lab setting. Atomizers and humidifiers are commercially available and commonly used to generate aqueous droplets but fail to favorably replicate field measurements of sea spray. In contrast, sintered glass filters, plunging water jets, and wave breaking simulations have all been demonstrated as more reliable systems for SSA-analogous aerosol generation in the laboratory, the latter most closely replicating the size distributions observed at oceanic whitecaps and bubble plumes.[4] The Scripps Institution of Oceanography hydraulics laboratory has developed a walled glass wave

channel, 33 m in length, with which to generate SSA from wave breaking of directly imported ocean water.[5] A similar, scaled down, approach has been used in the Marine Aerosol Reference Tank (MART) system which uses a plunging waterfall method of SSA production within a rectangular plexiglass tank apparatus approximately a meter or less in each dimension.[6]

Aerosols on the Early Earth

Collectively, marine aerosols have a large surface area and a large surface-associated organic content.[7] Louis Lerman has stressed the central role of such aerosols in the living process, and perhaps in the origins of life itself, with his model of the Bubble-Aerosol-Droplet supercycle or "Bubblesol" cycle.[8] The self-sustaining cycle of aerosol production via wave breaking and bubble bursting, transport in the atmosphere, and gravitational deposition and recycling of particles back into the ocean (Fig. 1) would likely have been active on the early Earth once primordial oceans had developed. The unique properties of organic films at the air-water interface of an early ocean would have provided an advantageous platform for proto-biochemical reactions to occur. It has long been recognized that organic molecules partition to air-water interfaces, generating a sea surface microlayer film that provides a relatively hydrophobic phase. This promotes concentration by means of a kind of liquid-liquid phase separation and can even facilitate polymeric folding into primitive enclosed structures.[9] It has been proposed that, as a result of these properties, gravitational deposition of large aerosols, themselves coated

with organic surfactants, into the early ocean might have allowed primitive bilayers to develop at their surfaces.[10] It has also long been known that amide bond formation and spontaneous condensation of amino acid esters can preferentially occur at water surfaces.[9] Hydrophobic interaction among peptides and lipid membranes could result in condensation and polymerization. Possible selection for longer peptides within the hydrophobic phases of the sea surface and of SSA has been suggested, and thus could provide a forward mechanism toward increasing complexity. Recently, the enrichment of extracellular DNA in the outer surfactant layer of marine aerosols has also been experimentally demonstrated, along with lipids, proteins, viruses and prokaryotes.[11]

Aerosols and the Origins of Life

In 1979, Carl Woese, who was notably the first to put forth a new taxonomy of life which included Archaea as a separate prokaryotic domain, submitted a critique of the commonly accepted Oparin-Haldane "Primordial Soup" model of the origin of life.[12] This critique proposed a greater role than previously imagined for atmospheric aerosols in the transition from prebiotic to biological systems. Woese points out that many essential biological reactions are dehydrations, unlikely to occur without catalysis in a bulk aqueous environment, and thus requiring a phase separation. He suggested that the atmospheric droplet phase would have been a potential medium to constrain otherwise diffuse molecules and promote biochemical evolution. These droplets, as suggested above, possess characteristics of "membrane" chemistry, where a relatively hydrophobic

phase develops as macromolecules partition at the surface, thus increasing reactivity that would lead to more efficient chemical development. Early life, according to Woese, thus evolved not within an Oparin ocean, but in the lower atmosphere as a function of interactions with the ocean.[12]

Concentrated aqueous solutions are the preferred reaction medium in extant biology, but the oceans on early Earth would have been dilute with relevant molecules. Progression from simple monomers to complex biomolecules would rely on condensation reactions (such as the formation of peptide and phosphodiester bonds), a challenge in the absence of enzymes and in a milieu of high water activity. Polymerizations such as these under prebiotic conditions have been demonstrated primarily in anhydrous environments and/or on inorganic clay surfaces.[13, 14, 15] Woese's proposal was responded to by Scherer, and counter-responded to by Oberbeck, who noted that clays and salts can act as cloud condensation nuclei, and estimated that concentrations of amino acids, for example, could reach millimolar amounts in mere days, which would take millions of years to amass in a bulk ocean.[16, 17]

Concentration, alignment, and orientation of organic molecules would have been possible at the aerosol-generating surface interface, alleviating the thermodynamic and kinetic obstacles that a dilute aqueous environment would create. In fact, recent studies have shown the efficacy of the protocell environment to aid polymerization.[18] For aerosols, the concentrating function of the surface microlayer in particular might allow hydrophobic

enclosures to form in the bulk aqueous solution and not rapidly disassemble, processes that would otherwise require an exceptionally high lipid monomer content.

The air-sea interface would have been the primary generator—and recycler—of aerosols and their chemical content and would be a central focal point of many contributing forces directed from the upper atmosphere, from the output of deep hydrothermal vents, and from meteoric infall. Organic molecules generated and/or retained by the system of ocean-atmosphere interaction tend to stay within this overarching cycle once they enter and would be exposed to widely fluctuating conditions of solar irradiation, temperature, pressure, and humidity, far from equilibrium. Transport of these materials within an aerosol phase would also have been potentially significant to prebiotic evolution and might have been considerably faster on the early Earth some 4+ billion years ago given the Earth's faster rotational speed.[9] The distributions of sizes of atmospheric aerosols of any kind generally obey a power law, but can show multimodal distributions, determined by competition between coagulation, division, and gravitational deposition (of larger particles, with diameters >5 microns), leading, in the case of SSA, to a median diameter of a few microns.[19] A vast population of submicron- to micron-sized particles generated at the surface of an early ocean would act as a ready-made medium for containing emerging biomolecules.[1] Indeed, the simultaneous generation of biopolymers and primitive enclosures for them would have been essential to the emergence of any kind of proto-cell, much less one with the ability to evolve and maintain a self-reproducing genome. Prebiotic production of long-chain hydrocarbons and other amphiphilic

molecules has been demonstrated, and the self-assembly of functional primitive enclosures is well-characterized.[9]

Atmospheric aerosols with high organic surface content, organized in an "inverted micelle" morphology, would be similar in size and structure to single cell bacteria.[1] Furthermore, synthetically generated enclosures have been shown to exhibit characteristics and behavior analogous to those that sustain modern life. Vesicles suspended in solution spontaneously grow and divide with the addition of lipid micelles, while the aerosol model, often imperfectly coated with surfactant, allows for division and growth through several mechanisms, including fission of single droplets, coagulation between droplets (increasing overall surfactant coverage), and selective evaporation of water.[9] Permeability and exchange of materials for these kinds of early cell-like structures would have been a challenge for prebiotic membranes without the pumps and channels that characterize modern cellular chemistry—early enclosures would likely have been less stable and more dynamic. Regardless, aerosol formation at the air-water interface of Earth's nascent oceans would likely have provided an avenue for the partitioning and concentration of organic material and rate enhancement of relevant prebiotic reactions within an essentially closed phase. Empirical support for biologically relevant reactions occurring in aerosols is scarce, although it has been demonstrated that the presence of aqueous aerosol improves the synthesis of a diverse array of organic molecules.[20] Under conditions highly similar to the original Urey-Miller experiments, aerosolized water and a simple gas mixture of CH4, N2, and H2 subjected to electric discharge showed vastly

increased yields of amino-, hydroxy-, and carboxylic acids, purines, and other heterocycles compared to a non-aerosolized control and previous data. Several species were produced that were absent in the control, such as the dihydroxy acids tartaric and glyceric acid, suggesting that the presence of aqueous aerosol promotes synthesis and makes alternative pathways possible. Also synthesized were malic acid and succinic acid, members of the Krebs cycle, and related precursors to citric and isocitric acid, potentially linking the aerosol droplet phase to the development of primordial metabolism.[21] Hydroxy acids and related monomers have even been suggested as potential backbone precursors for the genetic material of a pre-RNA world.[22]

The RNA World

The RNA World Hypothesis proposes that self-reproducing RNA molecules were crucial precursors to modern biology, possessing both information heritability and catalytic activity—both genotype and phenotype—but the conditions that led to their development and proliferation are unclear.[23] While such a pre-biological paradigm had long been theorized, the first evidence for an RNA world came in the early 1980s with the discovery of catalytic RNA molecules, dubbed ribozymes, by Thomas Cech and others, with the self-splicing *Tetrahymena* ribozyme being the first of these discovered in 1982.[24] Twenty years later, the elucidation of the crystal structure of the ribosome provided what has been deemed the RNA world's "smoking gun": the catalytic core of the ribosome,

including the active site at which peptide polymerization occurs, is constructed purely of RNA, with the organelle's protein components acting largely as outer scaffolding.[25]

These and other discoveries would seem to support a prebiotic paradigm wherein protein enzymes evolved to supplant--or incorporate--established catalytic RNA precursors, but the nature of this transformation and what came before it is still under investigation. As a general model for these precursors, the Lehman lab uses an established catalytic RNA system with novel self-assembly functionality. The *Azoarcus* ribozyme (Fig. 2) is, like

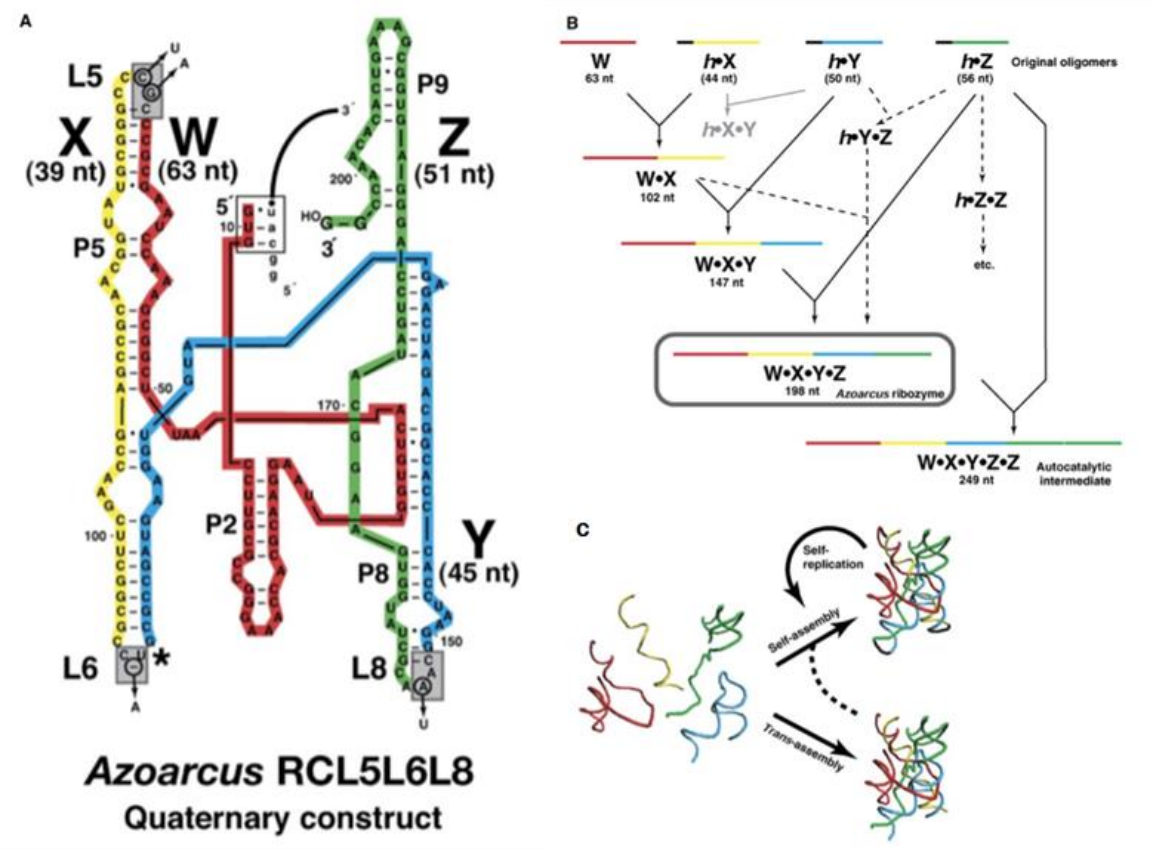

Figure 2. Structure and assembly of *Azoarcus* ribozyme. (a.) Sequence and secondary structure of *Azoarcus.* (b.) Self-assembly from 4 fragments. (c.) Formation of *trans*-complex and further autocatalysis of self-assembly of fully covalent ribozyme. (Figure taken from Hayden, et al. "Self-Assembly of a Group I Intron from Inactive Oligonucleotide Fragments" (2006) *Chem Biol.*)

the *Tetrahymena* ribozyme, a self-splicing group I intron. Found in the isoleucine pre-tRNA of the diazotrophic endophyte *Azoarcus sp*. BH72, it can catalyze its own covalent formation from multiple independent fragments.[26, 27, 28] Directed by an internal guide sequence of three nucleotides, oligomer fragments from this intron can self-assemble, proceeding from a non-covalently associated *trans* complex via recombination into the fully covalent ribozyme sequence, which in turn catalyzes further recombination of itself or other RNA substrates.

The ribozyme is approximately 200 nucleotides (nt) in length, the smallest naturally-occurring group I intron, and assembles from four fragments between 39-63 nucleotides long (termed **W**, **X**, **Y** & **Z**) into its full-length sequence (**WXYZ**). This dual activity of both substrate and catalyst makes the *Azoarcus* ribozyme system an ideal model for testing prebiotic reactions in simulating an early RNA world. In the case of the *Azoarcus* ribozyme, it can be broken into 2, 3, or 4 pieces and spontaneously self-assemble from its fragments in 20–100 mM $MgCl_2$ and at 25–60˚C.[27, 29, 30] In fact, recent work in the Lehman lab suggests that it can be fragmented into 5 or 6 smaller RNAs that can autocatalytically re-assemble without the prior presence of a full-length ribozyme.[31] The *Azoarcus* ribozyme depends on relatively high concentrations of magnesium in solution to properly fold and enact catalysis, although prior work has suggested that the use of other divalent cations such as calcium and manganese can support its activity as well.[32] In addition, compartmentalization via a liquid-liquid phase separation has demonstrated the ability to concentrate ribozymes and enhance the rates of their reactions.[33] The

advantages for compartmentalization are clear, and the constant output of the bubble-aerosol supercycle might have been an ideal medium for supporting prebiotic evolution in the RNA World.

Acting as an empirical test of the hypotheses expounded upon by Woese, Lerman, Tuck, and others, oriented around an RNA World context, we have aimed to provide fundamental insights into the role of aerosol particles as an environmentally relevant aqueous compartment for facilitating prebiotic chemistry. This thesis acts as a preliminary demonstration of RNA transport and reactivity in an aqueous aerosol phase and proposes future directions for investigation based on these findings.

Experimental Methods

General: All reagents (buffers, salts, nucleotides or oligonucleotide sequences) were purchased in the highest purity or quality grade commercially available. 1M $MgCl_2$, 1M Tris-HCL (pH 7.5), 0.5M Ethylenediaminetetraacetic acid (EDTA) (pH 8.0), 3M sodium acetate (NaOAc) (pH 5.5), and all individual NTP and dNTPs (100mM) were purchased from Thermo Fisher Scientific. Primer sequences and VATR (a method of recursive gene synthesis, also known as Vent (DNA polymerase, isolated from *Thermococcus litoralis*)-Assisted Template Reconstruction) template oligos were purchased either from Integrated DNA Technologies (IDT) or TriLink. Enzymes including Taq polymerase, Vent polymerase, AMV and MMLV reverse transcriptases and T7 RNA polymerase were purchased from New England Biolabs or Invitrogen via Thermo Fisher Scientific. All reagents for agarose and polyacrylamide gel electrophoresis, including casting plates and gel chambers, were purchased from Bio-Rad. PAGE analysis was carried out using 8% denaturing gels with 8M urea and visualized via SYBR Green staining, while agarose gels used ethidium bromide (EtBr).

Aerosol chamber design: To empirically test the ability for aqueous aerosols to perform these functions, we required an apparatus for generating, containing and sampling droplets in a lab setting. Design for such an aerosolization chamber, shown in Figure 3, was initially imagined as a closed rectangular apparatus, wherein a unidirectional aerosolization scheme would generate particles on one end to be collected on the other. Implementation of this design involved the modification of a sealed rectangular

aluminum toolbox (purchased from Northern Tool + Equipment, model #36012747), approximately 400L in volume (1.4 m X 0.6 m X 0.5 m) with a single, vertically-opening lid hinged along its long edge (Figure 4). Additions of open ports were made to several faces of the box, including the lid, for the introduction of aerosol-generating and sampling equipment. In addition, openable ports were added for sampling via particle counter, as well as a meter to determine relative humidity (RH) and average temperature within the chamber.

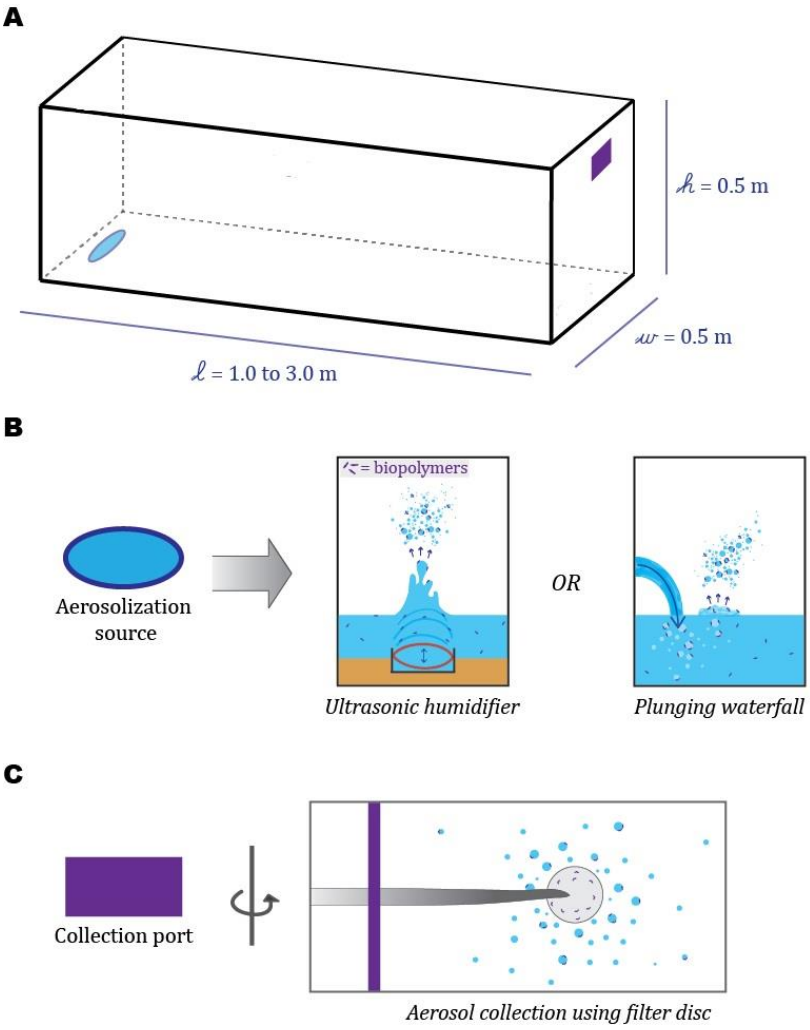

Figure 3. Preliminary schematic of a laboratory aerosol chamber. (A) Chamber dimensions & design. Blue ellipsis indicates an aerosolization source; purple rectangle indicates a collection port at the opposite end. (B) Potential aerosolization methods. (C) Collection method (adsorption/impaction via filter disc).

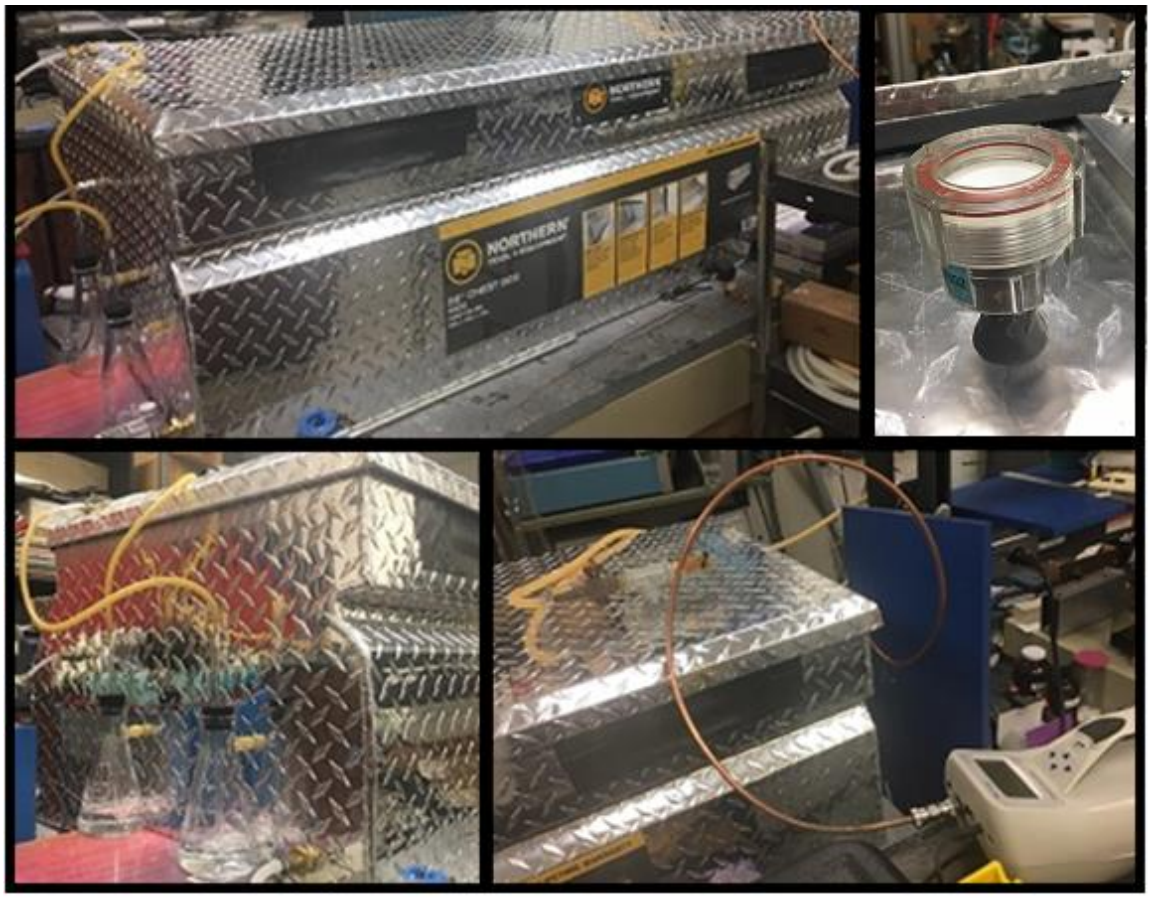

Figure 4. In-house aerosol chamber. (Top Left) a front view of the metal chamber with lid closed. The dimensions are 1.4 m X 0.6 m X 0.5 m, for 420 L total volume. (Bottom Left) a side view of the chamber showcasing the two aerosolization flasks attached by tubing to a nitrogen tank. Aerosols generated through bubbling escape into the chamber. (Top Right) Inner view of filter port through the chamber lid. (Bottom Right) Side output port attached to a CPC, an RH meter, and a temperature sensor.

Aerosol generation: Pure nitrogen was bubbled from an industrial grade steel gas cylinder (size 300, purchased via Industrial Source and Matheson Tri-Gas) through 40-60 μm pore sintered filters into solutions within threaded side-arm Erlenmeyer flasks (seen in Figure 4), attached via rubber tubing to ports on one face of the aerosol chamber. Nitrogen was pumped from an enclosed tank at approximately 10 psi, attached to the filters via polyethylene tubing, the glass stems of which were inserted into a sealed rubber stopper

which plugs the Erlenmeyer flask. Submerged approximately 6.5cm below the surface of a solution within the flasks, the filters generated a bubble foam at the interface which bursts to generate aerosol particles which enter the chamber via the side-arm openings. The interior of the chamber's lid, at the other end from the attached flasks, was fitted with a removable mount compatible with circular Whatman QMA grade 47mm diameter quartz filter discs, through which the entirety of the box's aerosol content was pulled via an external air pump to collect aerosol by adsorption and impaction. Between uses, the aerosol chamber was thoroughly aerated by leaving the lid open overnight and then followed by cleaning with a bleach solution and an anti-nuclease formula.

RNA aerosolization: All source solutions for RNA aerosolization contained 50nM of the *Azoarcus* fragment in question, 100mM $MgCl_2$, and 30mM of Tris HCL (pH 7.5) – this maintained a pH range that allows for the solubility and reactivity of the *Azoarcus* ribozyme and its catalytic salt. Each fragment solution (100mL) was aerosolized for two hours under standardized conditions—the aerosol chamber was flushed with filtered air to 0 pt/cc prior to use; N_2 flow was set at 10 psi; a heater applied to the chamber kept the average temperature at approximately 30°C; relative humidity remained between 50-60% throughout.

Aerosol collection: All air was pumped out of the chamber through an internally mounted Whatman QMA grade 47mm quartz filter disc (approximately 30 min to sample entire volume) following aerosolization. The chamber was then opened to allow removal of the filter disc, which was carefully sliced with a sterilized razor into 2-4mm sized pieces, and

submerged in a 600 μL of solution containing 0.3 M NaOAc and 5 mM EDTA (pH 8.0). Following a soaking period of approximately 12-16 hours (greatly reduced in later trials) at 4°C, the solution was recovered and 20 μL was removed, while the remaining eluent was treated with 2.5x volume of 100% ethanol to precipitate RNA. After centrifugation, the supernatant was removed, and the pellet vacuum-dried and rehydrated in reaction-grade Ambion nuclease-free water.

RNA sample preparation: All lengths of the *Azoarcus* ribozyme sequence above 100 nucleotides (nt) in length– WX (102 nt), WXY (147 nt) and WXYZ (198 nt) – were prepared from a DNA template constructed via recursive gene synthesis (VATR) as previously described by our lab.[39] Template oligomer sequences are shown in Table 2. The resulting dsDNA sequences were amplified via the polymerase chain reaction (PCR) using standard protocols, followed by T7 (RNA polymerase)-mediated runoff transcription (6-8 hours at 48°C) as previously described.[39] This was followed by separation and organic extraction via a dual-phase phenol and chloroform mixture, and purification by gel electrophoresis using 8% polyacrylamide/8M urea gels.[27] The independent Z oligomer (51 nt), noted as hZpch, as well as all *Azoarcus*-associated primers, were purchased separately from Integrated DNA Technologies (IDT).

Table 1. *Azoarcus* fragment and primer sequences.

Name	Sequence (5' – 3')
Azoarcus – WXYZ	5' – GUGCCUUGCGCCGGGAAACCACGCAAGGAA UGGUGUCAAAUUCGGCGAAACCUAAGCGCC CAUCCGGGCGUAUGGCAACGCCGAGCCAAG CUUCGGCGCCAUGCGCCGAUGAAGGUGUAG AGACUAGACGGCACCCACCUAAGGCAUACG CUAUGGUGAAGGCAUAGUCCAGGGAGUGGC GAAAGUCACACAAACCGG – 3'
Azoarcus – WXY	5' – GUGCCUUGCGCCGGGAAACCACGCAAGGAA UGGUGUCAAAUUCGGCGAAACCUAAGCGCC CAUCCGGGCGUAUGGCAACGCCGAGCCAAG CUUCGGCGCCAUGCGCCGAUGAAGGUGUAG AGACUAGACGGCACCCACCUAAGGCAU – 3'
Azoarcus – WX	5' – GUGCCUUGCGCCGGGAAACCACGCAAGGAA UGGUGUCAAAUUCGGCGAAACCUAAGCGCC CAUCCGGGCGUAUGGCAACGCCGAGCCAAG CUUCGGCGCCAU – 3'
Azoarcus – hZpch	5' – GGCAUCGCUAUGGUGAAGGCAUAGUCCAGG GAGUGGCGAAAGUCACACAAACCGG – 3'
Tas2.1a (W forward primer)	5' – CTGCAGAATTCTAATACGACTCACTATAGTGC CTTGCGCCGGG – 3'
Wfwd (W forward primer)	5' – GATAGTACCTTGCGCCGGG – 3'
T20a (Z reverse primer)	5' – CCGGTTTGTGTGACTTTCGCC – 3'
Y'Rpch (Y reverse primer)	5' – AUGTGCCTTAGGTGGGTGC – 3'
X'Rpch (X reverse primer)	5' – AUGGCGCCGAAGCTTGGC – 3'

Table 2. VATR DNA oligomer sequences for generation of full length *Azoarcus* ribozyme template.

Name	Sequence (5’ – 3’)
ONL1000	5’ – CCGGTTTGTGTGACTTTCGCCACTCCCTGGACTATGCCTTCACCA – 3’
ONL1001	5’ – GAGACTAGACGGCACCCACCTAAGGCAAACGC-TATGGTGAAGGCATAGTCCAGG – 3’
ONL1002	5’ – GGGTGCCGTCTAGTCTCTACACCTTCATCGGCGCTGGCGCCGAA-GCTTGG – 3’
ONL1003	5’ – CCTAAGCGCCCGCCCGGGCGTATGGCAACGCCGAGCCAA-GCTTCGGCGC– 3’
ONL1004	5’ – GGCGGGCGCTTAGGTTTCGCCGAATTTGACACCATTCCTT-GCGTGGTTTCCC – 3’
ONL1005	5’ – TAATACGACTCACTATAGTGCCTTGCGCCGGGAAACCAC-GCAAGGGATG – 3’

RNA Detection Methods: Detection of RNA was accomplished via directed reverse transcription-polymerase chain reaction (RT-PCR), following standard protocols. Reverse transcription used the Avian Myeloblastosis Virus (AMV) or Moloney Murine Leukemia Virus (M-MLV) transcriptases (purchased from Invitrogen), incubated for 1 hour at 42°C, with the accompanying buffer, a reverse primer tailored to the target RNA sequence (T20a, Y'Rpch or X'Rpch), and an equimolar mixture of the four base ribonucleotides (A, U, C, G). The RT was heat inactivated for 5 minutes at 80°C prior to use as PCR template. PCR employed the *Thermus aquaticus* (Taq) polymerase and associated buffer, along with forward and reverse primers, and an equimolar mixture of the four base deoxynucleotides (dA, dT, dC, dG) thermocycled as follows (previously optimized):

(Lid 102°C. Volume 100 μL)

1. 94°C, 5min
2. 92°C, 1min
3. 57°C, 1min
4. 72°C, 1min
5. Go to step 2, 24x
6. 72°C, 5min
7. 4°C, hold

A portion of each RT-PCR product (10 μL) was mixed with 2 μL of a 6X bromophenol blue/xylene cyanol loading dye and visualized, alongside a double-stranded DNA ladder, via electrophoresis on a 2% agarose gel mixed with ethidium bromide (EtBr) to allow fluorescent imaging.

Experimental Results

Aerosol chamber functionality: In determining the principal method we would use for aerosol generation, several factors were considered. The architecture of the chamber—with additional modifications for this purpose—could accommodate a standing pool of solution a few inches deep, which could then be acted on to generate aerosols by a simulated wave breaking or plunging waterfall similar to the MART setup developed by Stokes.[6] In order to make efficient use of such a setup for the purposes of RNA aerosolization, however, this would necessitate the transcription-based production of prohibitively large quantities of *Azoarcus* in order to maintain a relevant concentration for experiments, which could not be recovered for re-use. This approach would also be inherently problematic for investigating the separate aerosolization of multiple different RNA oligomers (i.e. *Azoarcus* fragments) and studying their interaction via particle coagulation in the aerosol phase, as the oligomers would necessarily interact in solution prior to aerosol encapsulation.

As such, we chose to bubble gas through submerged sintered glass filters—with pore sizes between 40-60 microns in diameter, attached to thin glass tubing to form an L-shape—as our primary method of aerosol generation. Ports on the furthest face of the chamber would allow solutions to be aerosolized within an external apparatus attached on that end, travel a short distance, and be recovered on the other end of the chamber.

As a preliminary test of our aerosol chamber setup's functionality, 50mL of solution was aerosolized over 1 or 2 hours, containing either 10mM or 100mM of $MgCl_2$ or NaCl diluted in Milli-Q filtered ultrapure water, or simply water alone. The aerosol chamber was flushed prior to aerosolization with filtered air via a peristaltic pump to bring the initial measured particle count (expressed in particles per cubic centimeter, or pt/cc) to zero, as measured by a condensation particle counter (CPC) attached to an outflow port. This was continued at a low rate during aerosolization to act as turbulent mixing and keep aerosol populations airborne. Particle counts for each solution at the respective time point are demonstrated below (Figure 5), with the measurements taken over the course of a 30-minute period after aerosolization had ended.

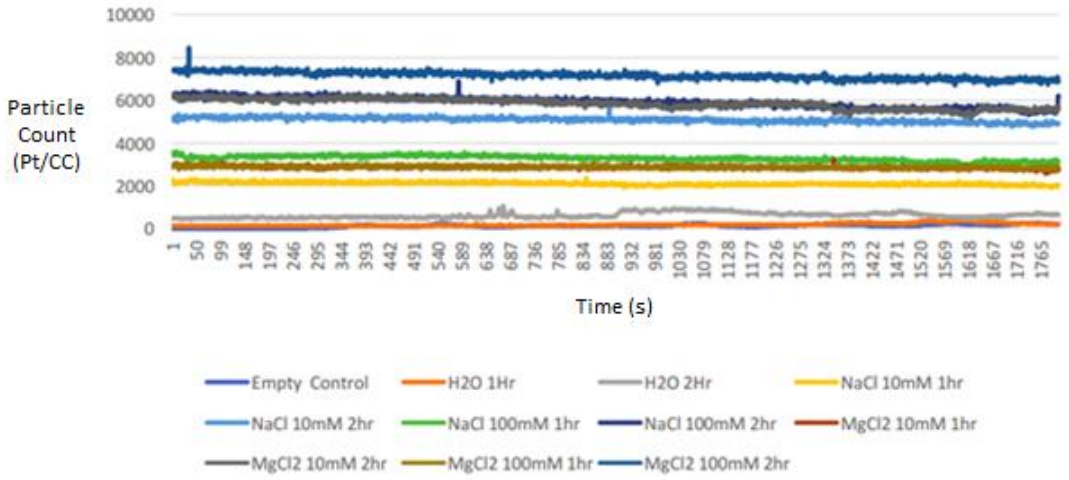

Figure 5. Effects of salt concentration and time on particle generation. Particle count (pt/CC) vs time (s). Solution composition as well as aerosolization time is noted by color in the legend.

CPC measurements of particles within the chamber showed a marked increase of pt/cc with solutions containing either NaCl or $MgCl_2$ as compared to pure water (Figure 5). It has been previously noted that aerosols generated from $MgCl_2$, and NaCl-$MgCl_2$ mixtures in particular, exhibit more favorable hygroscopic behavior and adhere more closely to the properties of naturally generated SSA (which may encourage the use of more complex salt mixtures for improved experimental relevance).[34, 35] Between the 1- and 2-hour generation times for each solution, the particle concentration more than doubled (Figure 5). CPC measurements were also taken over the same period of a sealed chamber previously flushed with filtered air to act as a blank measurement control. These results indicate the time-dependent nature of aerosolization, and the relative stability of an aerosol population in the chamber once generated.

Aerosolization of RNA: Because it is known that entire virus particles can enter the aerosol phase (e.g., the human influenza virus)[37], we had good reason to believe that large macromolecules such as naked RNAs could be aerosolized. Indeed, analysis of the crystal structure of the *Azoarcus* ribozyme suggests that all but the smallest droplets produced would easily take up a molecule its size.[38] In order to test the capacity for multiple lengths of RNA to be encapsulated via aerosols, we used solutions containing a single fragment of the *Azoarcus* ribozyme: WX, WXY or WXYZ. A 50nM concentration of the respective fragment was used to simulate a low but accessible concentration that may be prebiotically relevant, and well within the range of concentrations that can promote ribozyme catalysis.[28] Each solution contained 100mM of $MgCl_2$ to facilitate the ribozyme's optimal conditions for structure and function, although the effective salt

concentration in a droplet phase is not known and could be much higher than the solution it is generated from.[8]

In addition to previous measurements of particle counts following our aerosolization protocol, we wanted to measure the size distribution of generated particles, with and without aerosolized RNA. Using a Scanning Mobility Particle Sizer (SMPS) spectrometer (TSI, specifically a joint SMPS with Optical Particle Sizer (OPS) package, Nanoscan 3910-3330), we were able to accurately sample and determine the diameter of particles between 10nm to 10µm and log distributions directly via instrument software. Figure 6 shows the size distribution of aerosols generated over a 2-hour period from solutions of 100mM $MgCl_2$, with 50nM of full-length *Azoarcus* (WXYZ) included (A) or without (B). These results are typical of sintered filter or bubbler based aerosolization schemes, which tend to show a single diameter mode near 100nm in distributions

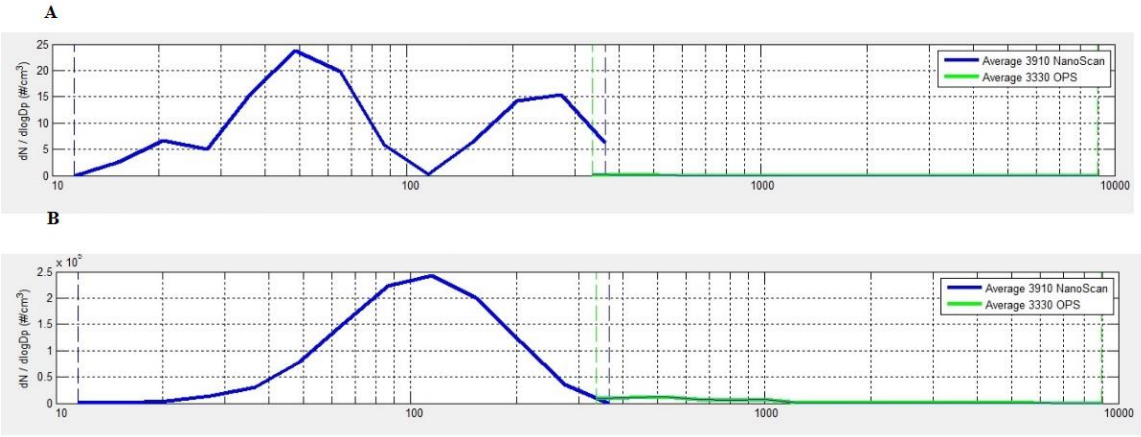

Figure 6. Particle diameter size distributions. Aerosols generated over 2 hours from solutions of (A) 50nM *Azoarcus* full length WXYZ RNA and 100mM $MgCl_2$ or (B) 100mM $MgCl_2$ only. Measurements taken over 30 minutes after ceasing aerosolization via a TSI 3910 Nanoscan Scanning Mobility Particle Sizer (SMPS) and 3330 Optical Particle Sizer (OPS) and logged via instrument software. 3910 SMPS size range includes 10nm-420nm and results are indicated in blue. 3330 OPS size range includes 300nm-10um and results are indicated in green.

containing $NaCl_2$ or seawater solutions.[4] The presence of *Azoarcus* RNA converts the unimodal diameter distribution into a bimodal one, indicating that production of larger particles, and coagulation of smaller ones, are likely factors in successful *Azoarcus* RNA uptake into aerosols generated by our system. Particle count values, however, appeared to drop with the inclusion of RNA by a factor of approximately 10^4—this is contrary to earlier assumptions and may warrant further investigation.

Detection of RNA: The next step was to confirm transport and successful filter capture via detection of the captured RNA. Detection and quantitation of the *Azoarcus* ribozyme and other RNA oligomers has been long accomplished successfully by our lab via 5' P32 radioactive labeling.[26] If radiolabeled *Azoarcus* fragments are aerosolized, however, proper containment could quickly become a problem. In tandem with a collection method such as a cold finger apparatus, which condenses atmospherically suspended droplets, direct detection of radiolabeled RNA via liquid scintillation counting would be possible. This would not, however, allow us to determine the identity of the collected RNA, and the necessary collection process could once again impede the investigation of separately aerosolized RNA fragments by allowing their interaction in a liquid phase.

We chose to sidestep radiolabeling altogether and instead use a reverse transcription/polymerase chain reaction (RT-PCR) technique as the principal method of detection. Once recovered on the filter disc, an aerosolized sample can be eluted from the filter, followed with optional ethanol precipitation for sample concentration, and probed

for a target RNA sequence via its complementary reverse primer. Using reverse transcription, a sequence of complementary DNA (cDNA) can be generated from the target RNA sequence found in the eluted sample. This cDNA can then serve as the template for PCR, where *Thermus aquaticus* (Taq) polymerase acts to amplify the sequence in scale to millions of times the original concentration. Previous data shows robust RT-PCR detection of the full-length *Azoarcus* sequence with diluted template concentrations as low as attomole (10-18 M) scale.[40]

Inhibition of Self-Assembly on Filter Paper Discs: In experiments where more than one *Azoarcus* fragment is aerosolized, the potential exists for self-assembly to occur after adsorption of the fragments onto the filter paper in addition to self-assembly as a result of particle coagulation in the aerosol phase, thus undermining conclusions about aerosol-based self-assembly arising from detection of the full-length WXYZ sequence. Thus, conditions were investigated that would prevent self-assembly on filter paper without interfering with downstream RT-PCR detection.

Filter discs were either untreated or pre-soaked with solutions containing EDTA (pH 8.0), which chelates magnesium and inhibits *Azoarcus* self-assembly, and dried. To find a "sweet spot" concentration of EDTA which inhibited *Azoarcus* self-assembly on the surface of the filter matrix, but which did not inhibit downstream RT-PCR to the point that detection of either filter-incubated or *in vitro* RNA was impossible, we tested EDTA concentrations between 0.01 and 0.5M (Figures 7-9). After soaking with 1mL of the

target concentration of EDTA, the filters were dried in sterile petri dishes. Once dry, 200µL containing 50nM of WXY and Z RNA fragments (separated until the last moment), or containing 50nM of full length WXYZ RNA, in 100mM $MgCl_2$ and 30mM of Tris HCL (pH 7.5) was added and the filters were incubated at 37°C for two hours, and subjected to the same processing for RT-PCR detection. An *in vitro* incubation of equivalent WXY + Z concentrations in the same buffer was conducted in tandem and also subjected to RT-PCR. Agarose gel results are shown in Figures 7-9.

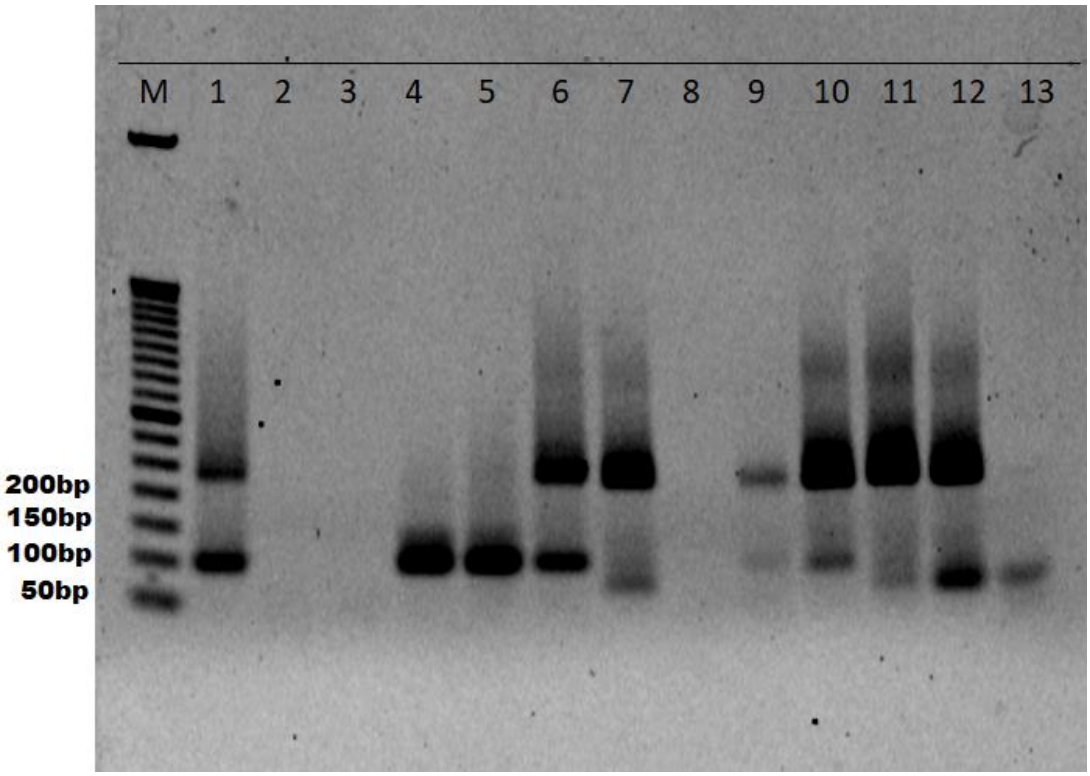

Figure 7. RT-PCR results for filter paper incubation of *Azoarcus* RNA. Filter discs soaked and dried in concentrations of EDTA (0.5M, 0.1M, 0.05M, 0.025M, 0.01M), or not modified. M = marker (50 bp ladder). **Lanes 1-6 (Self-assembly):** Filter either untreated or soaked with EDTA, dried, then incubated at 37°C for 2 hrs with 50nM WXY + Z RNA, along with 100mM $MgCl_2$ and 30mM Tris HCL buffer. (left to right: untreated filter, 0.5M EDTA, 0.1M, 0.05M, 0.025M, 0.01M)
Lanes 7-12: Filters soaked, dried and incubated as in 1-6 but with 50nM of full length WXYZ RNA instead of fragments, to assess effect on RT-PCR of filter-incubated RNA. (Same order as 1-6.)
Lane 13: PCR negative control.

In these results, bands seen approximately at the 200 nt position indicate the presence of full length WXYZ RNA; bands near or below the 100 nt position correspond to known associations between, or partial extensions of, PCR primers.

EDTA concentrations of 0.5M, 0.25M and 0.1M were consistently too high for our purposes, not only inhibiting self-assembly on the filter surface, but also appearing to prevent downstream RT-PCR and even inhibiting primer dimer associations seen in the negative control (Figure 8). Further investigation of 0.1M compared to 0.01M and an untreated filter disc (Figure 9) showed that the lower concentration was insufficient to inhibit filter paper self-assembly, although when compared alongside a suite of concentrations between 0.01M and 0.5M (Figure 7), both concentrations exhibited somewhat inconsistent behavior. After several trials, 0.025M EDTA was chosen as the lowest concentration which consistently inhibited filter paper self-assembly without affecting RT-PCR detection of filter-incubated full length WXYZ.

A 2-hour in vitro assembly time course demonstrating the progression of the WXY + Z reaction, alongside an equally long incubation of full length WXYZ, is shown in Figure 10 to demonstrate rapid self-assembly between *Azoarcus* fragments under the conditions of aerosolization.[26]

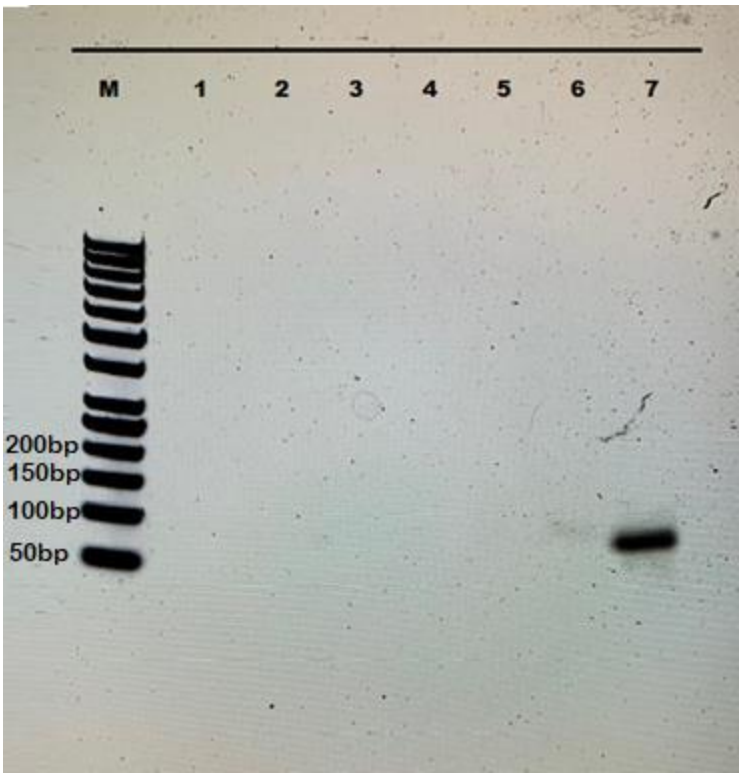

Figure 8. RT-PCR results for filter paper incubation of *Azoarcus* RNA (Higher EDTA Concentrations). Filter discs soaked and dried in either 0.5M, 0.25M or 0.1M EDTA. M = marker (50 bp ladder). **Lanes 1-3:** Filter soaked with (1) 0.5M, (2) 0.25M or (3) 0.1M EDTA, dried, then incubated at 37°C for 2 hrs with 50nM WXY + Z RNA, along with 100mM $MgCl_2$ and 30mM Tris HCL (pH 7.5) buffer. **Lanes 4-6:** Filters treated as above with (4) 0.5M, (5) 0.25M or (6) 0.1M EDTA, incubated as above but instead with 50nM of full length WXYZ RNA. **Lane 7:** PCR negative control.

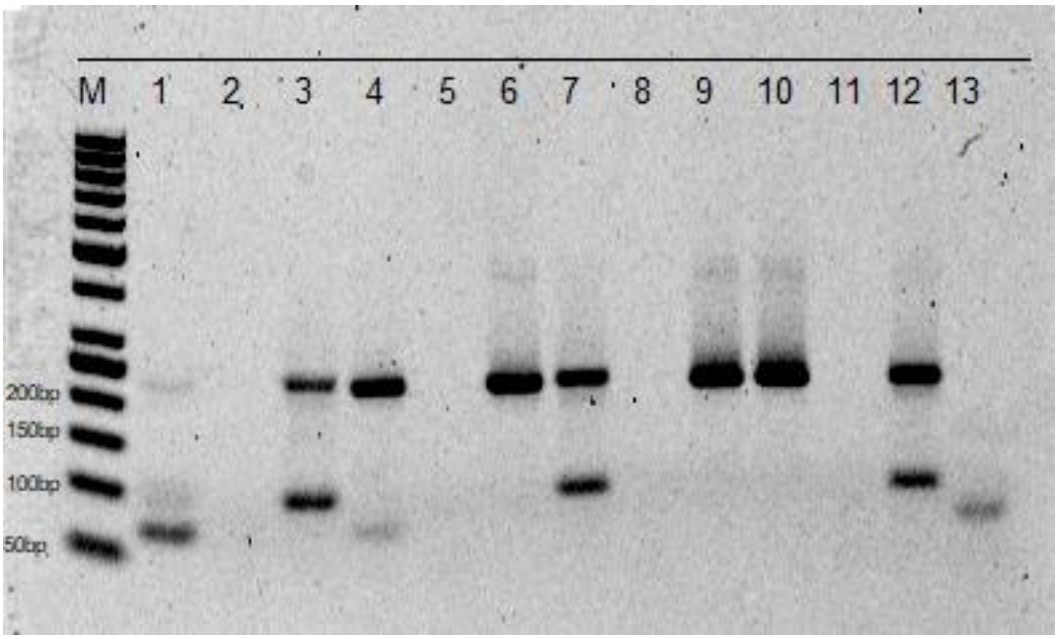

Figure 9. RT-PCR results for filter paper and *in vitro* incubation of *Azoarcus* RNA (EDTA Concentrations of 0M, 0.1M and 0.01M). M = marker (50 bp ladder). All *in vitro* reaction incubated at 37°C with 100mM $MgCl_2$ and 30mM Tris HCL (pH 7.5) buffer.
Lanes 1-3: Filter discs soaked in either (1) no EDTA (untreated), (2) 0.1M or (3) 0.01M EDTA, dried, then incubated as described with 50nM WXY + Z RNA. **Lanes 4-6:** Filters treated as above (same order) but with 50nM of full length WXYZ RNA instead of fragments. **Lanes 7-9:** 50nM concentrations of both WXY and hZpch fragments with either (7) no EDTA added, (8) EDTA added to 0.1M in solution, or (9) EDTA added to 0.01M in solution. **Lanes 10-12:** 50nM of WXYZ RNA incubated with either (10) no EDTA added, (11) EDTA added to 0.1M in solution, or (12) EDTA added to 0.01M in solution. **Lane 13:** PCR negative control.

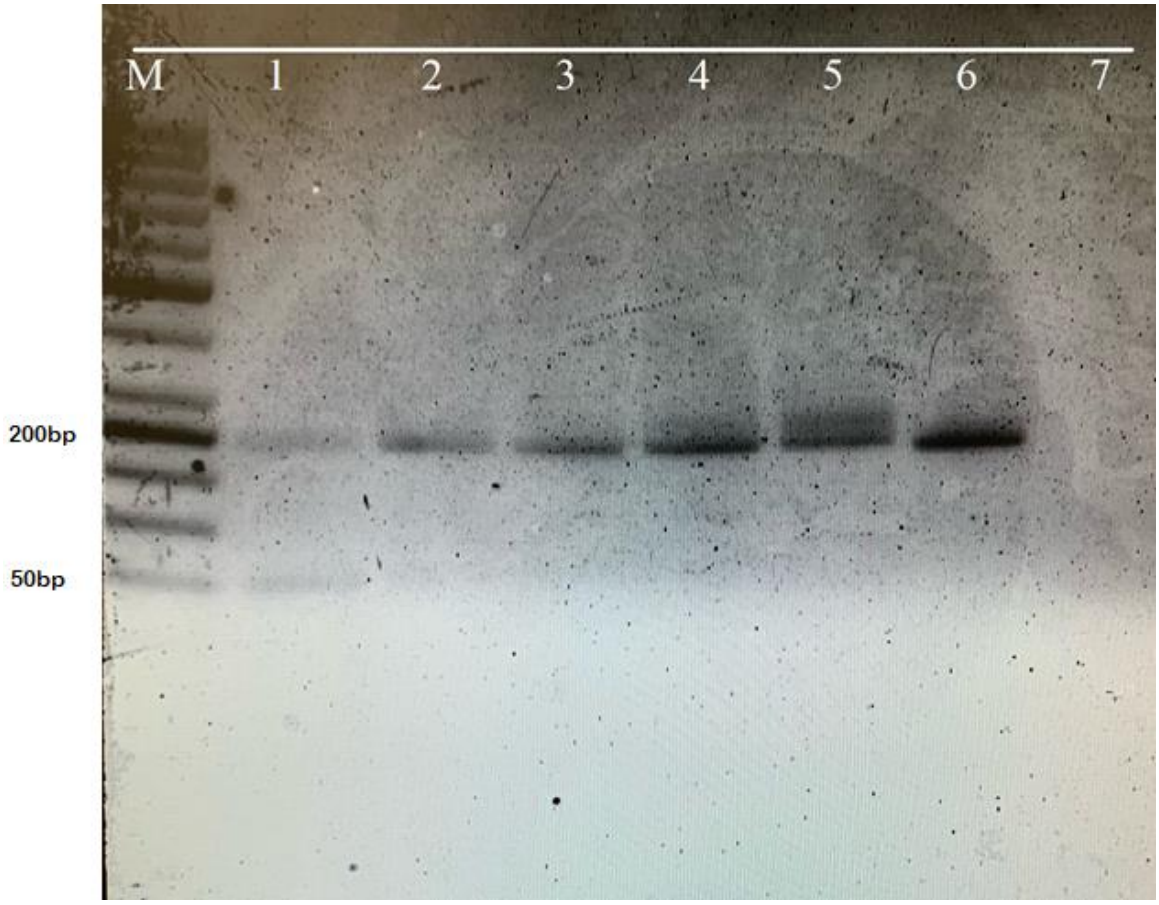

Figure 10. Time course of *Azoarcus* WXY + Z *in vitro* self-assembly. M = marker (50 bp dsDNA ladder). **Lanes 1-5:** 50nM concentrations of both WXY and hZpch fragments incubated at 37°C with 100mM $MgCl_2$ and 30mM Tris HCL buffer. Samples (in lane order) were taken at 3 min (1), 10 min (2), 30 min (3), 1 hr (4) and 2 hrs (5). **Lane 6:** full length WXYZ in the same buffer conditions, incubated for 2 hours at the same temperature. **Lane 7:** PCR negative control.

Aerosolization and Detection of RNA Fragments: Following the described aerosolization and detection methods, detection of three lengths of Azoarcus RNA—WX, WXY and WXYZ—was demonstrated (Figure 11). These results indicate that RNA of multiple sequence lengths, which become more structurally complex with increased length, can be aerosolized, transported at least 1 m (the approximate distance between the bubbler and the collection port) and be subsequently detected by RT-PCR. Ethanol precipitation of the eluent from the soaked collection filter paper seems to help improve detection, though not in all cases, as seen in the comparison between fragment data in Figure 10.

Preliminary Detection of RNA Self-Assembly in the Aerosol Phase: Separate aerosolization of the WXY and Z fragments was carried out in the same manner via a dual flask setup, the results of which are shown in Figure 12. Filter discs were soaked with 0.025M EDTA as previously determined and dried prior to use. RT-PCR of the separate aerosol source solutions post-aerosolization was performed, as well, to ensure that the self-assembly of Azoarcus was not taking place *in vitro* as a result of backflow of aerosol particles between the flasks. While precipitation of eluted RNA was ineffective in this case, full length *Azoarcus* WXYZ RNA was successfully detected following aerosolization (Figure 11, lanes 1-2), while remaining absent from either fragment source solution, or from a control combining equal amounts of the fragment solutions at room temperature. (Figure 11, lanes 5-7.)

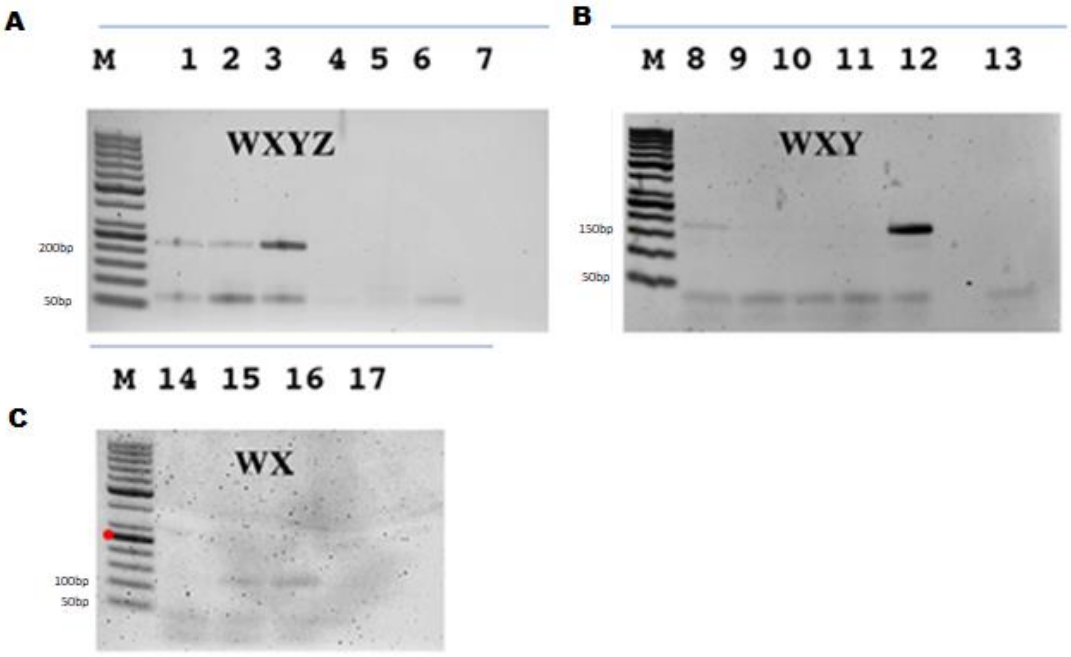

Figure 11. RT-PCR detection of *Azoarcus* ribozyme fragments. (**A**) full length *Azoarcus* **WXYZ**, ~200 nt; (**B**) **WXY** fragment, ~150 nt; and (**C**) **WX** fragment, ~100 nt. M = marker (50 bp dsDNA ladder). (**A**) **Lanes 1-2:** RT-PCR products of **WXYZ** from eluent, without (1) or with (2) ethanol precipitation. **Lane 3:** non-aerosolized positive control. **Lanes 4 & 5:** RT-PCR products of aerosol source solutions containing only 100mM $MgCl_2$., without (4) or with (5) ethanol precipitation. **Lane 6:** non-aerosolized $MgCl_2$ solution. **Lane 7:** PCR negative control. (**B**) **Lanes 8-11:** RT-PCR products of **WXY** from eluent, without (8-9) or with (10-11) ethanol precipitation. **Lane 12:** Non-aerosolized positive control. **Lane 13:** PCR negative control. (**C**) **Lanes 14-15:** RT-PCR products of **WX** from eluent, without (14) or with (15) ethanol precipitation. **Lane 16:** Non-aerosolized positive control. **Lane 17:** PCR negative control.

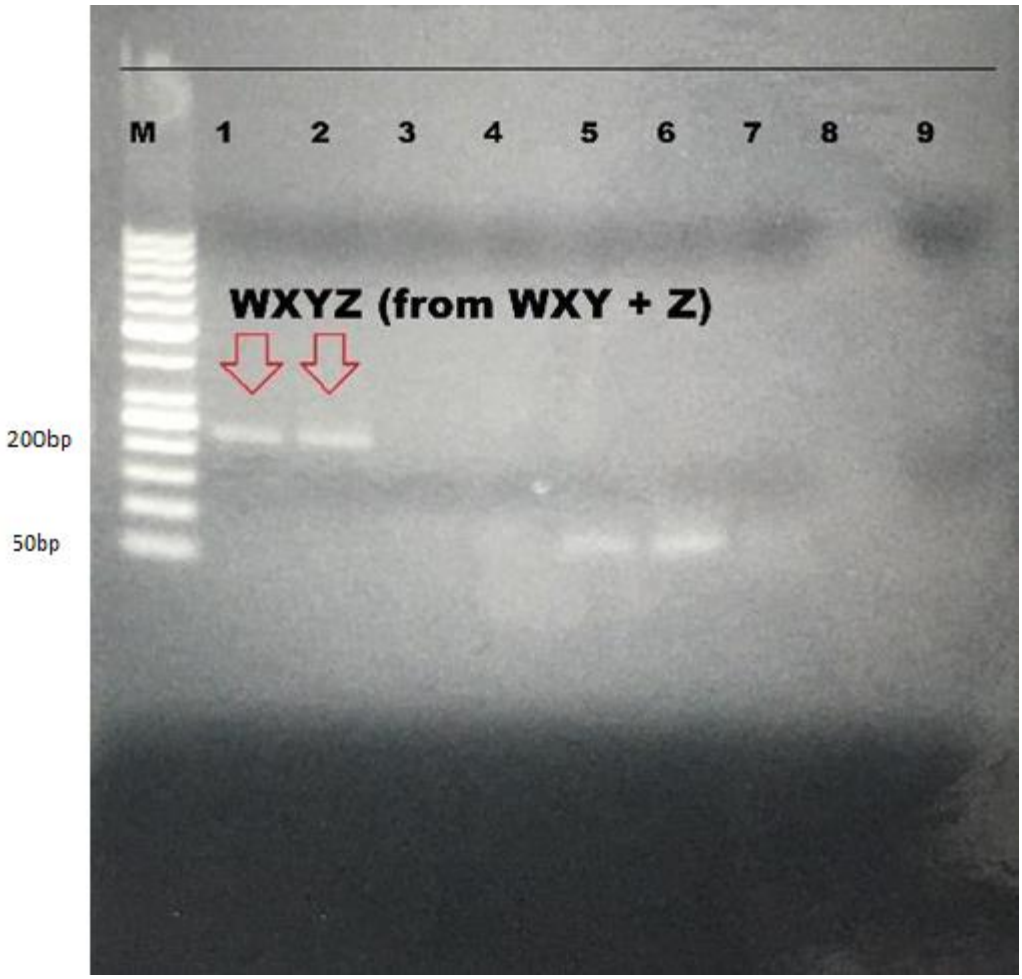

Figure 12. RT-PCR detection of full length *Azoarcus* ribozyme from separately aerosolized **WXY** and **Z** fragments. (Image colors inverted for clarity.) M = marker (50 bp dsDNA ladder**). Lanes 1-4:** RT-PCR products of **WXYZ (**~200 nt) from eluent, with (1 & 2) or without (3 & 4, unsuccessful) precipitation. **Lanes 5-7:** RT-PCR products of aerosol source solutions; respectively, **WXY**, **Z**, and a control solution of equal amounts of both, incubated at room temperature overnight. Lane 8: blank. Lane 9: PCR negative control.

Discussion

The work described in this thesis served as an initial foray into the aerosolization of RNA, using an established ribozyme system to apply aerosol chemistry in the context of a prebiotic RNA world. We demonstrated aerosol encapsulation of RNA molecules up to 200 nucleotides in length, and the self-assembly of multiple RNA fragments into a fully covalent ribozyme within an aqueous droplet medium, taking multiple steps to verify that this is where assembly was occurring. These results serve as proof-of-concept for the capability of RNA self-assembly reactions occurring in the aerosol phase, which represents a significant new avenue for the emergence of prebiotic chemistry without needing to invoke more traditional membrane enclosures, coacervates, or aggregate-based protobionts. However, the results proved difficult to consistently replicate with our system, and questions remain as to whether the model used in this project is generally applicable under various parameters, and whether our conclusions regarding aqueous aerosol as a medium for ribozyme reactivity can be extrapolated to the point of broad prebiotic viability.

Aerosolization Trial Design: Alternative chamber architectures to test RNA aerosolization and transport across various distances can be explored to address the first of these points. At its most basic, our design aerosolizes on one end of a chamber, collects generated particles on the opposite side, and then detects RNA transport, though this could be altered in a variety of ways to answer additional questions. Other

architectures would likely need to address several shortcomings inherent to our experimental design, i.e. the inability to collect aerosol from the same experiment at different time points without opening the chamber and ending the procedure, a solution to which would potentially allow exploration of the kinetics of RNA assembly in the aerosol phase. Parallel studies could include a closed-flask Urey-Miller-like apparatus, for example, constructed in the spirit of the previously described Ruiz-Bermejo experiments,[20] or a design similar to the MART plunging waterfall chamber as constructed by Stokes, et al.[6] Designs like these would serve to more directly test the effects of a simulated aerosol-to-ocean cycle on RNA self-assembly or other reactivity within a closed environment, as material is recycled into an evolving aerosol population.

To a similar end, the possibility of "scaling up" aerosol studies could serve to better represent a comprehensive prebiotic environment—utilizing facilities such as those available at the Pacific Northwest National Laboratory's (PNNL) Atmospheric Measurement Laboratory in Richland, WA, on a scale multiple orders of magnitude larger than our model, would ensure that our results are generalizable to multiple environments and not a function of the particular dimensions of our lab-based chamber.

Other aerosol generation methods not utilized in this project could be employed, as well as sintered filter setups that more closely consider depth submerged in addition to pore size—several setups make use of multiple bubbling filters of different pore sizes in order to produce a more disperse particle size distribution,[4, 5] which could provide insights into

the relationship between particle size and the accessibility and uptake of aerosolized RNA.

Improved methods of collection of aerosolized RNA could also greatly impact the quality and consistency of results. Alternative collection schemes could include the use of commercially available instruments such as a Microorifice Uniform Deposition Impactor (MOUDI), which sorts collected particles by size onto a series of quartz filters, potentially further illuminating the role of particle size in successful RNA aerosolization and addressing assumptions about coagulation-based self-assembly. Additionally, the use of a cold finger apparatus to collect aqueous aerosol by condensation would enable a level of direct quantification which was not possible within the studies described. Additionally, a cold finger-based approach could potentially shut down self-assembly outside of the aerosol phase, alleviating the need for the use of EDTA as in our filter approach. (It is also noted that other potential organic chelators like citric acid or oxalic acid, which were not considered in this work, may perform better than EDTA to prevent filter-associated self-assembly without inhibiting downstream RT-PCR, and should be investigated in future instances of filter capture).

More inherently quantitative detection approaches could improve our understanding of how RNA self-assembly proceeds kinetically in the aerosol phase and how likely the reaction is to occur within an aerosol population. While the nature of LSC is not necessarily suited to distinguishing successful self-assembly from labeled fragments

(though gel-based analysis is possible), Raman spectroscopy of macromolecules in aqueous aerosol has been successfully demonstrated—although detection of nucleic acids in suitably low concentration would likely be difficult and require a Surface Enhanced Raman spectroscopy (SERS) technique, involving costly gold and silver nanoparticles equipped with specific nucleic acid probes.[44, 45] The RT-PCR approach utilized in this project gives a qualitative answer to the question of whether RNA can be aerosolized and transported from the source to the detection port, while a Raman approach could provide a more quantitative answer, with the detection caveat.

Prebiotic Applicability: To expand the scope of future investigations in this realm, the use of other ribozymes besides *Azoarcus* for studies on transport and reactivity would allow a more multidimensional exploration of RNA activity in aerosols. Hammerhead, hairpin, and HDV ribozymes have all been utilized previously by our lab, and could serve as prime candidates for investigation alongside well-characterized ribozymes like Tetrahymena or in vitro-selected artificial ribozymes such as the L1 ligase.[39] Experiments could include a particular RNA and its exogenous substrate (e.g., the hammerhead ribozyme and its substrate) being aerosolized separately, especially in cases where the RNA oligomers involved are closer in size than the *Azoarcus* fragments used in this work, or the exploration of reactions involving more than two reactants or incorporating non-RNA components (peptides, for example, such as the 23-mer L23ββ, which associates with the LSU rRNA in the core of the ribosome, could allow a small foray into the peptide-RNA world concept.[41]) This avenue of investigation would provide greater

insight into how catalytic RNA of multiple sizes, sequences and activities or reaction types fare in an encapsulated aerosol phase.

Additional exploration of the aerosol encapsulation model in general would shed some light on the viability of aqueous droplets as pre-cellular enclosures for emerging biomolecules. While lacking strict membranes, organic molecules such as lipids, carboxylic acids and hydroxy acids are often incorporated by SSA from the ocean surface; measurements taken from the organic coatings of marine aerosols indicate that long single-chain fatty acids including palmitic, stearic and oleic acids are most common, though these are unlikely to be abiotic in origin.[7] The prebiotically relevant malic acid[20] is also a common component of atmospheric SSA and has previously been used in generating aerosols for analysis.[42] Aerosol generation could be attempted with and without appropriate concentrations of malic acid and other short chain organics, assessing whether their presence increases the success of RNA transport by allowing phase separation and some level of enclosure within a surfactant microlayer, and at the same time more closely emulating naturally produced SSA. To the latter end, artificial seawater mixtures with varied salt compositions could also be used in aerosol source solutions to emulate primordial ocean conditions (the compositions of which are not fully known).[43]

Conclusions

Carl Woese stated, "…Venus today serves as a partial model for the state of the Earth during its first several hundred million years of existence… In such an atmosphere the primary chemistry is 'membrane' (interface associated) chemistry. Solution chemistry would be the byproduct of 'membrane' chemistry, not the reverse."[12] This idea with respect to the atmospheric droplet phase has been around for nearly 40 years, but it remains necessary to further test its empirical feasibility. Coincidentally, the RNA World hypothesis has been evolving for roughly that same length of time. While it is not without its challenges, the RNA World hypothesis still serves as a very powerful scaffold onto which testable hypotheses concerning the chemistry-to-biology transition can be framed.[46, 47, 48, 49]

The use of an RNA-based encapsulation system to test some of the fundamental constituents of Woese's hypothesis is a logical step which this project aimed to begin to address. Taking a broad systems chemistry viewpoint which integrates the molecular and environmental aspects of the primordial Earth, we can envision a "discontinuous synthesis model" of prebiotic chemistry in which molecules are made in one location and then transported to another for further reaction; aerosols, which today can circumnavigate the Earth in about two weeks, provide a candidate vessel for such transport.[50] Alternatively, Louis Lerman's Bubble-Aerosol-Droplet supercycle, which "had to have" been in place on the early Earth, may provide a "continuous" synthesis model (albeit an

alternative one from the prebiotic soup of Oparin and Haldane) if it could, for example, incorporate clays and salts as condensation nuclei—not to mention the partitioning effects of a sea-surface microlayer and the vast surface area of surfactant-coated aerosols, which would be exposed to fluctuating conditions of temperature, humidity, and ultraviolet radiation during their lifetimes.[8] In either case, aqueous aerosol acts as a crucial, fundamental aspect of any developing proto-biome, and interaction between prebiotic chemistry and an aerosol phase would be seemingly inevitable.

The ability to aerosolize catalytic RNA within a controlled system lends support to the potential use of the aerosol medium (as previously theorized by Woese, Lerman, and Tuck, and others) to concentrate and transport developing prebiotic molecules in the RNA world. The recovery of fully assembled *Azoarcus* ribozyme from separately aerosolized fragments further indicates an inherent capability for early Earth aerosols to support prebiotic reactions, reinforcing the feasibility of aerosolization, transport, and *in situ* reactivity of macromolecules in general. While further exploration of the aqueous aerosol medium and its relevance to the emergence of life is necessary, the preliminary demonstration of ribozyme reactivity within the aerosol phase is a promising step towards a comprehensive model of the prebiotic world.

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