Technische Universität München
Fakultät für Chemie

Chemical evolution of biomolecules under abiotic hydrothermal conditions

Thomas Daniel Geisberger

Vollständiger Abdruck der von der Fakultät für Chemie der Technischen Universität München zur Erlangung eines

Doktors der Naturwissenschaften (Dr. rer. nat.)

genehmigten Dissertation.

Vorsitz: Prof. Dr. Hubert A. Gasteiger

Prüfer*innen der Dissertation:

1. apl. Prof. Dr. Wolfgang Eisenreich
2. Prof. Dr. Corinna Dawid

Die Dissertation wurde am 20.04.2022 bei der Technischen Universität München eingereicht und durch die Fakultät für Chemie am 14.06.2022 angenommen.

Die vorliegende Arbeit entstand im Zeitraum von April 2017 bis März 2022 unter der Anleitung von Herrn Prof. Dr. Wolfgang Eisenreich am Lehrstuhl für Biochemie (2017-2020) und an der Professur für strukturelle Membranbiochemie (ab 2021) der Technischen Universität München.

Für Steffi

Danksagung

Ohne das Mitwirken der folgenden Personen wäre diese Arbeit nicht dieselbe bzw. unmöglich gewesen und deshalb möchte ich mich an dieser Stelle herzlich bedanken:

Meinem Doktorvater Herrn **Prof. Dr. Eisenreich** möchte ich besonders für die freundliche Aufnahme in seine Arbeitsgruppe, die Überlassung des spannenden Themas, für die Betreuung und fachlichen Anregungen sowie sein Vertrauen in mich enorm bedanken.

Ich bedanke mich bei meiner Zweitprüferin Frau **Prof. Dr. Dawid** für die Übernahme der Prüfung. Mit Frau Prof. Dawid verbindet mich eine kurze Zeit während meiner Abschlussarbeit am Lehrstuhl für Lebensmittelchemie.

Zusätzlich möchte ich mich bei Herrn **Prof. Dr. Gasteiger** für die Übernahme des Vorsitzes meiner Dissertationsprüfung bedanken.

Herrn **Prof. Dr. Braun** danke ich für Schaffung und die gute Organisation des SFB (CRC 235). Durch den SFB war es mir möglich auch andere Sichtweisen auf die „Origin-of-Life"-Szene zu gewinnen und Kooperationen mit anderen Arbeitsgruppen zu schließen.

Auch möchte ich mich bei Herrn **Dr. Wächtershäuser** für die zentrale Idee, um die sich meine Laborarbeit dreht, bedanken.

Des Weiteren möchte ich mich herzlich bei Frau **Dr. Huber** bedanken, die mir als mein PI auf meinem SFB-Projekt stets eine große Hilfe für alle Großen und kleinen Probleme in der Arbeit mit präbiotischer Chemie war.

Mein weiterer Dank gilt Herrn **Prof. Dr. Groll** und dem ganzen **Lehrstuhl** für Biochemie mit seinen ehemaligen sowie aktuellen Mitgliedern. Auch bei Herrn **Prof. Dr. Hagn** möchte ich mich für die Aufnahme unsere Arbeitsgruppe bedanken. Vor allem aber möchte ich mich bei den Mitgliedern unserer Arbeitsgruppe bedanken. Bei den ehemaligen **Ina** und **Fan**, danke für Anregungen und Lehrgänge in Isotoplogue Profiling, vor allem aber bei meiner Vorgängerin **Jessy**. Es war sehr schön, dass sich unsere Zeit hier überschnitten hat und was würde ich nur ohne deine riesige Sammlung von Daten und Excel-Tabellen tun. Mein Dank gilt auch **Lena**, vor allem für die Einführungen an diversen NMR-Geräten. Zudem möchte ich den 16 **Studenten** danken, die meinen Weg als Doktorand begleitet haben. Hier möchte ich **Luis** und **Jeany** mit Ihren guten Bachelorarbeiten hervorheben und **Felicia**, deren Laborarbeit zu einem großen Teil in eine Veröffentlichung mit eingeflossen ist. Auch bei meinen neueren Kollegen **Sandra** und **Christian** möchte ich mich für die gute Zusammenarbeit und Laune im Labor bedanken.

Zuletzt möchte ich mich bei zwei besonderen Menschen in der Arbeitsgruppe bedanken, zuerst DANKE für alles **Tine** ohne dich wäre hier nichts möglich und somit hast du auch diese Doktorarbeit möglich gemacht. Ohne dich wären auch mein Wissen über NMR-Geräte nur einen Bruchteil so groß wie es jetzt ist. Mir werden auch die morgendlichen Scrum-Meetings fehlen. **Thomas**, was soll ich sagen, in dir habe ich nicht nur einen guten Kollegen, sondern auch einen guten Freund gefunden. Es waren verdammt gute fünf Jahre mit dir im Labor und später im Büro. Was würden wir nur montags ohne Football oder Basketball machen. Auch will ich mich bei dir bedanken, wenn ich mal wieder deinen Kopf gebraucht habe, weil die Stöchiometrie oder Ähnliches nicht so wollte wie ich.

Mein ganz besonderer Dank gilt meiner Familie, besonders meinen Eltern **Gertraud** und **Robert**, die mir meinen Lebensweg erst ermöglicht haben und immer da waren, wenn ich sie gebraucht habe.

Mein größter Dank geht an meine Frau **Steffi**. Du warst während der gesamten Zeit meiner Doktorarbeit immer mein starker emotionaler Rückhalt und hast mich immer motiviert, wenn ich mal ganz unten war. Wie heißt es so schön, hinter jedem erfolgreichen Mann steht eine starke Frau. Danke, dass du mir immer den Rücken freigehalten hast.

“I would like, if I may, to take you on a strange journey.”

The Narrator, The Rocky Horror Show (by Richard O’Brian)

Zusammenfassung

Selbst die kleinste und einfachste Zelle verfügt über ein komplex strukturiertes Netzwerk von niedermolekularen Stoffen. Unabhängig von der Größe und der Komplexität eines heutigen Lebewesens, bleibt die grundlegende Biochemie der Energieerzeugung und Biosynthese die wichtigsten Biomoleküle im Wesentlichen über alle Lebensformen gleich. Diese Kongruenz lässt auf eine lineare Evolution mit einem gemeinsamen Ursprung schließen. Es erscheint daher möglich, aus dem Regelwerk der heutigen Biochemie auf Reaktionen und Mechanismen der frühen Evolution und im weitesten Sinne sogar auf eine abiotische Entstehung des Lebens rückzuschließen. Eine bekannte Theorie zum Ursprung des Lebens postuliert die Bildung von organischen Molekülen aus einfachen anorganischen Vorläufern wie CO, Cyanid, NH_3 und/oder Acetylen (C_2H_2). Durch Katalyse an Eisenschwefel- und Nickelschwefelmineralien kann aus diesen Stoffen das Atomgerüst vieler organischer Molekülen, wie Aminosäuren, Fettsäuren oder Heterocyclen, aufgebaut werden. In der heutigen Biochemie ist die enzymatische Fixierung einfacher anorganischer Moleküle, wie CO_2 oder N_2, und der Umbau in Biomoleküle gleichermaßen eine zentrale Aufgabe. Bei der Fixierung von Kohlenstoff können dabei in Bakterien und Archaeen unterschiedliche Wege, wie z.B. der reduktive Acetyl-CoA-Weg, der reverse Zitronensäurezyklus, oder der 3-Hydroxypropionat-Bizyklus bestritten werden. Ein gemeinsames Motiv in den meisten Schlüsselschritten dieser Wege ist jedoch die Katalyse an Metallzentren der beteiligten Enzyme, meist an Eisen-Schwefel- oder Eisennickel-Schwefelclustern. Unter den auf der frühen Erde vorherrschenden abiotischen hydrothermalen Bedingungen können ähnliche Mechanismen der Kohlenstofffixierung und Molekülgenese durch Katalyse an Eisenschwefel- oder Nickelschwefelmineralien postuliert werden. Möglicherweise können daher Enzyme und Mechanismen an katalytischen Eisen-Schwefel -oder Nickel-Schwefel-Clustern der heutigen Biochemie ein Relikt aus der frühen Evolution des Lebens darstellen.

In der hier vorliegenden Arbeit wurde die Hypothese eines möglichen Urmetabolismus unter abiotischen hydrothermalen Bedingungen weiter verfeinert und experimentell untermauert. Dabei wurde in wässrigen Reaktionsgemischen in Gegenwart von Nickelsulfid (NiS) die Umwandlung von Kohlenmonoxid (CO) und Acetylen (C_2H_2) in zahlreiche organische Moleküle durch Massenspektrometrie und Isotopenmarkierung nachgewiesen werden. Durch ^{13}C- und ^{15}N-Markierungsexperimente konnte dabei ein komplexes Reaktionsnetzwerk und dabei ablaufende

Mechanismen erkannt werden. Die im Labor eingestellten Reaktionsbedingungen (hohe Temperaturen, wässrige Bedingungen, Metallkatalyse, Gegenwart von vulkanischen Gasen) waren ähnlich zu den Bedingungen an den heutigen aber wahrscheinlich auch den urzeitlichen vulkanischen Hydrothermalquellen auf der frühen Erde. Die in den Laborversuchen gezeigten Reaktionsprodukte umfassten Hunderte von organischen Molekülen mit diverser Funktionalität. Unter den Produkten waren zahlreiche Vertreter, die als biochemische Metabolite, Intermediate und Produkte in den heutigen Stoffwechselwegen auftreten. Als ein Schlüsselergebnis kann die Bildung von Metaboliten bezeichnet werden, die sich auch in den Kohlenstofffixierungswegen (z.B. reverser Citronensäurezyklus, Acetyl-CoA Weg) von genetisch tief verankerten thermophilen Bakterien und Archaeen befinden. Basierend auf dieser Beobachtung könnte das beschriebene abiotische acetyleno/carboxydothrophe Reaktionsnetzwerk als der wahrscheinliche Vorfahr eines Urmetabolismus in den ersten Lebensformen auf der Erde postuliert werden.

Des Weiteren wurde in dieser Arbeit die mögliche Sonderstellung von Thiophen und dessen Folgeprodukten als Anzeichen für einen passenden Ort zur Entstehung von Leben gezeigt. Thiophene entstehen ebenfalls unter den oben genannten Bedingungen ausgehend von Acetylen und Nickelsulfidkatalyse. Die Koordination des fixierten Kohlenstoffs findet hier am Schwefelatom des NiS-Katalysators statt. Vor kurzem konnte, durch die Curiosity-Mission, auf dem Mars Thiophen nachgewiesen werden. Die von uns gezeigte abiotische Synthese von Thiophen unter hydrothermalen Bedingungen könnte somit ein Fingerzeig für eine frühere Umgebung auf dem Mars sein, welche die Entstehung von Leben auf dem Mars ermöglichte.

Es war gewiss ein gewaltiger Schritt in der Evolution von einem abiotischen Reaktionsnetzwerk in wässriger vulkanischer Umgebung an mineralischem Gestein, zu einem umschlossenen zellähnlichen Metabolismus der ersten Lebensformen. In dieser Arbeit wurde daher die Möglichkeit der Bildung von Lipidvesikel unter den oben beschriebenen abiotischen Bedingungen untersucht. Zusätzlich zu den oben genannten Produkten entstanden in den hydrothermalen Reaktionen Fettsäuren. Mittels GC/MS konnten Kettenlängen von bis zu 9 Kohlenstoffatomen und durch FT-ICR-Massenspektrometrie Kettenlängen größer als 20 Kohlenstoffatome gezeigt werden. Diese Fettsäuren konnten in der wässrigen Umgebung sowohl Mizellen als auch Vesikel bilden. Die Konzentration war hier ein bestimmender Faktor. Die Struktur und Art der bipolaren Moleküle war ein weiterer entscheidender Faktor, so unterstützen unter anderem Doppelbindungen oder längere Kohlestoffketten die Bildung von Vesikeln. Es

wurde ein Nass-Trocken-Zyklus nachgestellt, der auch in natürlicher Umgebung von vulkanischen Hydrothermalquellen passieren kann. Ziel dieses Zyklus war die Etablierung einer einschichtigen Lage von Fettsäuren auf einem Trägermaterial. Durch Rehydrierung mit Wasser könnten so bei ausreichender Konzentration Vesikel erzeugt werden. Eine Vergleichsprobe (85 mM Nonansäure) wurde in Wasser aufgenommen und mit Natronlauge titriert, bis sich die Probe komplett gelöst hatte. Im Anschluss wurde mit Salzsäure rücktitriert. Durch die Protonierung der gelösten Fettsäure bilden sich Vesikel in der wässrigen Lösung und es wurde eine opaleszente Trübung erzeugt. Die selbe Prozedur wurde mit dem gewonnen organischen Extrakt einer hydrothermalen Reaktion wiederholt. Es konnte ebenso eine opaleszente Trübung erzeugt werden. Zum Nachweis, ob es sich zweifelsfrei um Vesikel handelt, wird die Probe derzeit mit Transmissionselektronenmikroskopie untersucht.

Die Methoden der Massenspekrometrie sowie der stabilen Isotopenmarkierung waren die wichtigsten Arbeitsmittel in dieser Arbeit. Die Identifizierung von kleinen Molekülen war mittels Massenspektrometrie sehr gut möglich. Moleküle die, selbst bei höheren Temperaturen, nicht flüchtig sind, wurden dabei mit einem Silylierungsmittel derivatisiert. Durch Isotopmarkierung mittels ^{13}CO oder $^{13}C_2$-Acetylen konnte sowohl die Authentizität nachgewiesen werden als auch Mechanismen der Reaktionen untersucht werden. Beispielsweise zeigten die ^{13}C-Profile von Ameisensäure und Pyruvat die Herkunft der C-Atome aus CO oder Acetylen im Verhältnis von ca. 1:1. Die partielle Markierung von Ameisensäure aus $^{13}C_2$-Acetylen konnte als Beweis herangezogen werden, dass Acetylen im Laufe der Reaktionskaskade gespalten wurde und ein $^{13}C_1$-Produkt in Ameisensäure umgewandelt wurde. Mit etwa gleicher Effizienz konnte ^{13}CO in Ameisensäure verwandelt werden. Dies zeigte gleichzeitig die Möglichkeit, auch ungerade Kohlenstoffketten aus geradzahligen Bausteinen von Acetylen aufzubauen. Ausgehend von diesen Beobachtungen wurde ein Mechanismus vorgeschlagen, der die Spaltung von Acetylen an einer NiS-Oberfläche beinhaltet und daraus entstehende C_1-Produkte (zusammen mit CO) weiter zu den organischen Molekülen unter diesen Bedingungen führt. Im Detail wird dabei das Acetylen an zwei aktivierte Nickelzentren koordiniert und durch Reduktion mit H_2 Gespalten und anschließend z.B. in Ameisensäure umgewandelt. Pyruvat wird in diesem Model durch die Kombination von gespaltenem und nicht gespaltenem Acetylen erzeugt. Das Acetylen wird dabei an ein aktiviertes Nickelzentrum angelagert, es erfolgt eine Wasseranlagerung sowie Reduktion in ([Ni]—CO—CH_3). Das gespaltene Acetylen wird oxidiert und Wasser angelagert in ([Ni]—CO). Pyruvat resultiert dann durch Insertion des entstandenen CO in den [Ni]—CO—CH_3 Komplex. Die Freisetzung des Pyruvats erfolgt durch erneute Wasseranlagerung.

Abstract

Even the smallest and simplest cell has a complexly structured network of low molecular weight substances. Regardless of the size and complexity of a living thing today, the basic biochemistry of energy production and biosynthesis of the major biomolecules remains essentially the same across all life forms. This congruence suggests linear evolution with a common origin. It, therefore, seems possible to infer reactions and mechanisms of early evolution and, in the broadest sense, even an abiotic origin of life from the set of rules of contemporary biochemistry. A well-known theory on the origin of life postulates the formation of organic molecules from simple inorganic precursors such as CO, cyanide, NH_3, and/or acetylene (C_2H_2). By catalysis on iron-sulfur and nickel-sulfur minerals, the atomic skeleton of many organic molecules, such as amino acids, fatty acids, or heterocycles, can be built up from these substances. In today's biochemistry, the enzymatic fixation of simple inorganic molecules, such as CO_2 or N_2, and their conversion into biomolecules is equally a central task. In the fixation of carbon, different pathways can be contested in bacteria and archaea, such as the reductive acetyl-CoA pathway, the reverse citric acid cycle, or the 3-hydroxypropionate bicycle. However, a common motif in most key steps of these pathways is catalysis at the metal centers of the enzymes involved, usually at iron-sulfur or iron-nickel-sulfur clusters. Under the abiotic hydrothermal conditions prevalent on the early Earth, similar mechanisms of carbon fixation and molecular genesis can be postulated by catalysis at iron-sulfur or nickel-sulfur minerals. It is possible; therefore, that enzymes and mechanisms at catalytic iron-sulfur or nickel-sulfur clusters of present-day biochemistry may represent a relic from the early evolution of life.

In the present work, the hypothesis of a possible primal metabolism under abiotic hydrothermal conditions was further refined and experimentally substantiated. In aqueous reaction mixtures in the presence of nickel sulfide (NiS), mass spectrometry and isotope labeling detected the conversion of carbon monoxide (CO) and acetylene (C_2H_2) into numerous organic molecules. Through ^{13}C and ^{15}N labeling experiments, a complex reaction network and the mechanisms involved were identified. The reaction conditions set in the laboratory (high temperatures, aqueous conditions, metal catalysis, presence of volcanic gases) were similar to conditions at present-day but probably also prehistoric volcanic hydrothermal vents on early Earth. The reaction products shown in the laboratory experiments included hundreds of organic molecules with diverse functionality. Among the products were numerous representatives that appear as

biochemical metabolites, intermediates, and products in present-day metabolic pathways. A key finding may be the formation of metabolites that are also found in the carbon fixation pathways (e.g., reverse citric acid cycle, acetyl-CoA pathway) of genetically deep thermophilic bacteria and archaea. Based on this observation, the described abiotic acetyleno/carboxydothrophic reaction network could be postulated as the likely ancestor of a primordial metabolism in the first life forms on Earth.

Furthermore, this work has shown the possible special position of thiophene and its derivatives as indications of a suitable site for the origin of life. Thiophenes are also formed under the above conditions starting from acetylene and nickel sulfide catalysis. Here, the coordination of the fixed carbon takes place at the sulfur atom of the NiS catalyst. Recently, through the Curiosity mission, thiophene has been detected on Mars. The abiotic synthesis of thiophene under hydrothermal conditions that we have shown could thus be a pointer to an earlier environment on Mars that enabled the emergence of life on Mars.

It was certainly a giant step in the evolution from an abiotic reaction network in aqueous volcanic environments on mineral rocks, to an enclosed cell-like metabolism of the first life forms. In this work, therefore, the possibility of the formation of lipid vesicles, under the abiotic conditions described above, was investigated. In addition to the above products, fatty acids were formed in the hydrothermal reactions. Chain lengths of up to nine carbon atoms were shown by GC/MS and chain lengths greater than 20 carbon atoms were shown by FT-ICR mass spectrometry. These fatty acids were able to form both micelles and vesicles in the aqueous environment. Concentration was a determining factor here. The structure and nature of the bipolar molecules were other determining factors, including double bonds or longer carbon chains supporting the formation of vesicles. A wet-dry cycle was mimicked, which can also happen in natural environments of volcanic hydrothermal vents. The goal of this cycle was to establish a single layer of fatty acids on a support material. Vesicles could thus be generated by rehydration with water if the concentration was sufficient. A reference sample (85 mM nonanoic acid) was taken up in water and titrated with sodium hydroxide solution until the sample was completely dissolved. Subsequently, back titration was performed with hydrochloric acid. Vesicles formed in the aqueous solution due to protonation of the dissolved fatty acid and opalescent turbidity was produced. The same procedure was repeated with the organic extract obtained from a hydrothermal reaction. Opalescent turbidity was also produced. The sample is currently being examined by transmission electron microscopy to determine whether it is definitely a vesicle.

The methods of mass spectrometry, as well as stable isotope labeling, were the main working tools in this work. The identification of small molecules was very well possible by mass spectrometry. Molecules that are not volatile, even at higher temperatures, were derivatized with a sialylation agent. Isotopic labeling using ^{13}CO or $^{13}C_2$-acetylene allowed both authenticities to be established and mechanisms of the reactions to be investigated. For example, the ^{13}C profiles of formic acid and pyruvate showed the origin of the C atoms from CO or acetylene in a ratio of about 1:1, and the partial labeling of formic acid from $^{13}C_2$-acetylene could be used as evidence that acetylene was cleaved during the reaction cascade and a $^{13}C_1$ product was converted to formic acid. With about the same efficiency, ^{13}CO could be converted to formic acid. This simultaneously demonstrated the possibility of building odd carbon chains from even-numbered building blocks of acetylene.

Based on these observations, a mechanism involving the cleavage of acetylene at a NiS surface and resulting C_1 products (together with CO) was proposed to further lead to the organic molecules under these conditions. In detail, this involves the coordination of acetylene to two activated nickel centers and its cleavage by reduction with H_2 and subsequent conversion to formic acid, for example. Pyruvate is generated in this model by combining cleaved and non-cleaved acetylene. The acetylene is attached to an activated nickel center, and water is added and reduced to ([Ni]-CO-CH_3). The cleaved acetylene is oxidized and water is added in ([Ni]-CO). Pyruvate then results from the insertion of the resulting CO into the [Ni]-CO-CH_3 complex. The pyruvate is released by a water addition.

List of Abbreviations

Molecules

CH_4	methane
N_2	nitrogen
CO_2	carbon dioxide
H_2	hydrogen
H_2O	water
NH_3	ammonia
HCN	cyanide
C_2H_2	acetylene

Definitions

Gly	glycine
Ala	alanine
Asp	aspartic acid
Phe	phenylalanine
Tyr	tyrosine
WLP	warm little pond
rTCA	reductive citric acid cycle
Succ	succinate
2-KG	α-ketoglutarate
Isocit	isocitrate
Cit	citrate
Ac	acetate
Pyr	pyruvate
Oxac	oxaloacetate
Mal	malate
Fum	fumarate
CoA	coenzyme
myr	million years
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
PNA	peptide nucleic acid
LUCA	last universal common ancestor
cbc	critical bilayer concentration
cvc	critical vesicle concentration
cmc	critical micelle concentration
GC/MS	gas chromatography mass spectrometry
NMR	nuclear magnetic resonance
LC-MS	liquid chromatography mass spectrometry
FTICR/MS	Fourier-transform ion cyclotron resonance mass spectrometry
TEM	transition electron microscope

Table of Contents

1 Introduction

1.1 Prebiotic Chemistry or the question, where to start?

With the term prebiotic chemistry, we describe all the chemistry that could have happened during the transition from the Hadean to the Archean period (approx. 4000 myr ago). During this period there have been no complex molecules of life like today, such as DNA or specialized enzymes. However, it can be assumed that the conditions allowed to constitute basic chemistry and simple chemical reactions starting from simple molecules like Reppe-reactions starting from volcanic acetylene exhalations as an example (Reppe, 1949; Reppe, 1953). Unfortunately, a vast amount of traces for this early chemistry potentially leading to life on our planet is lost. Because of that, the philosophical question of "where do we come from?" can be asked.

Fortunately, some traces of environmental conditions have been better conserved than the traces of early life.

There are evidences, found in zircons, that liquid water has been on earth as 4.4 billion years ago (Valley *et al.*, 2002). This water could have served as a solvent for all reactions that needed a liquid state. Unfortunately, there is no unanimous opinion regarding the primitive atmosphere in that times. It could have been strongly reducing, containing gas compositions like CH_4/N_2 or $CO_2/H_2/N_2$. On the other hand, it could have been just neutral $CO_2/N_2/H_2O$ (Lazcano & Miller, 1996; Urey, 1952). The second big system on ancient earth was the ocean. In this primordial times it is said to be strict anoxic and rich in iron-containing minerals (Anbar & Knoll, 2002). The ancient ocean was a divers system with the ingredients to perform a vast variety of chemical reactions. For example, the constituents would make the system able to perform nitrogen fixation. One of the modern nitrogenases needs a Fe-S-cluster and molybdenum to perform the fixation of nitrogen. Iron-sulfur clusters and molybdenum are constituents of the ancient ocean (Anbar & Knoll, 2002; Cheng *et al.*, 2015). A fundamental problem of the two systems, atmosphere and open ocean, was a significant dilution gradient. The probability of the origin of life in this open systems would not have happened. This means not that the systems are not capable of this processes. But it would push up the probability of emergence of life, if the sight is on smaller defined parts of this open systems. There could be many niches

feasible to be the starting point of the emergence of life. There is a way to look at the emergence from two directions to have a look at the transition from an abiotic to biotic earth, namely by using a “Bottom up” or “Top down” approach (Peters & Williams, 2012). The difference lies in the viewpoint. A “Top down” view implicates a look at modern life. Here, the task is to take present biochemistry and phylogeny to create a pathway from current to ancient times. A look into metabolic pathways, processes, and assemblies show common links that connect somewhere in the past to find the first interconnections and, therefore, they give hints to the origin of life. Finally, in a “Bottom up” approach, the perspective changes to the other side. Here the starting point is the chemistry and physics on early earth to create a system that moves from an abiotic “nonliving” to a biotic “living” system. The early earth supports this approach with molecules still present as biomolecules formed in the atmosphere, hydrosphere, and lithosphere. (Kasting, 1993; Mojzsis *et al.*, 1999; Chakraborty *et al.*, 2020).

1.1.1 Different ideas and niches of prebiotic chemistry

The early earth offers many niches containing suitable environments to be the starting point of the origin of living things. Suitable in this consent means an environment, providing conditions that support basic needs like energy. To name some of this suitable environments, there are, submarine vents (Martin *et al.*, 2008; Nitschke & Russell, 2009; Wächtershäuser, 1992), ice (Hao *et al.*, 2018), meteoritic craters (Cockell, 2006) and volcanoes (Bada & Korenaga, 2018; Kitadai & Maruyama, 2018; Miller, 1953).

Experimental work on the origin of life was done by Stanley Miller. He reacted CH_4, NH_3, H_2O, and H_2 through electronic discharging resulting in amino acids like Gly, Ala and Asp (Miller, 1953). His thoughts were encouraged by the theoretical suggestions of Oparin and Urey on this purpose (Oparin, 1924; Urey, 1952). In the 1960s and later, a now proven theory states the presence of organic molecules in meteorites and comets (Anders, 1989; Oró, 1961). Alone the analysis of the carbonous content of the Murchison Meteorite revealed several thousand carbon hydrogen oxygen -and carbon hydrogen oxygen sulfur structures (CHO and CHOS), which showed the extreme chemical diversity in space (Schmitt-Kopplin *et al.*, 2010; Schmitt-Kopplin *et al.*, 2014). Therefore, some theories say

impacts of meteorites in the early years of the earth's existence could have provided precursors molecules for the emergence of life (Oró & Kimball, 1961). Oró went further with Miller's experiments resulting in adenine (Oró, 1960). Adenine is essential in today's biochemistry, as a nucleobase in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Further, it is part of today's energy transfer mechanism as adenosine triphosphate (ATP). Thinking of the elemental composition of adenine $C_5H_5N_5$, Oró suggested that it could be a pentameric HCN, which he stated as a molecule on comets and meteorites (Oró, 1961; Oró & Kimball, 1961).

Miller's experiments suggested a molecular broth, also called primordial soup. Oró's experiments indicated a cometary collision theory. Both theories provide an excellent explanation for the occurrence of biomolecules to enhance the emergence of life. Through the particular location of the creation of these molecules, the emerging biomolecules are likely to be dissolved in the earth's vast oceans. Thus, it isn't very likely to get the needed concentrations of the molecules to enhance any further reaction (Hagmann, 2002; Mojzsis *et al.*, 1999). Because of this fact, supplemental theories were needed that can overcome the concentration factor and take place in niches on the planet.

Important niches to assume would be:

impact craters of meteorites (Cockell, 2006),

warm or cold little ponds on long lasting volcanic hot spot islands (Bada & Korenaga, 2018) and

Hydrothermal vents (Russell, 2018; Russell *et al.*, 2014; Wächtershäuser, 1988b, 2006).

Impact craters

Impact craters give many geochemical conditions for prebiotic reactions. First the impact of the meteorite itself could deliver organic precursor material (Charnley *et al.*, 2002; Chyba & Sagan, 1992). Further the organics could have been created by the impact itself (McKay & Borucki, 1997). After the event there could emerge a hydrothermal system in the crater by flowing of water in the hot rock layer (Cockell, 2006). The stream of water

introduces sediment particles in the system, which contains zeolites and clays. These offer electrically charged surfaces on which reactions for the emergence of life could have occurred (Ferris *et al.*, 1996; Saladino *et al.*, 2001). A step to concentrate the organics takes place at the surface of the crater, induced through the heat of the impact and followed hydrothermal system (Usher, 1977). The conditions for the hydrothermal system in an impact crater can have a very different longevity, varying from several thousand years (Osinski *et al.*, 2001; Versh *et al.*, 2003) up to 2 myr (Abramov & Kring, 2004). The lifetime of those systems is relatively short compared to those emerging in the deep sea. But they offer a more significant temperature gradient starting from around 650°C (Osinski *et al.*, 2001) but they can cool fully down with time. With the loss of heat more complex molecules can occur because lower temperatures support their stability in aqueous solution (Shock & Schulte, 1998).

Warm and cold Ponds

Charles Darwin is famous for his “The Origin of the Species” with was originally printed in 1859 (Darwin, 1909). He is also known for the idea of the “warm little pond” (WLP), as an early imagine of the abiogenesis of life. He never really published this idea in a book, but he mentioned it in a letter to his friend Joseph D. Hooker (Peretó *et al.*, 2009). But also in our time (a century later) the idea of WLP or a cold one, promotes diverse ideas of scientists (Damer, 2016; Follmann & Brownson, 2009). As there were no continents on the young earth, the only bigger landmasses are the islands of so called “hotspot” volcanoes, that are above sea level (Sleep, 1990). The reducing gases from the plume of the volcano island and the energy provided from the electric discharges of this volcanoes smoke columns could hold a system to produce organic compounds for the emergence of life (Bada & Korenaga, 2018; Scheu *et al.*, 2017). The reacted residue can rain in a WLP on one of this islands or is washed in one of those from the flanks of the volcano. In this ponds the further prebiotic synthesis occurred (Bada & Korenaga, 2018). Following wet and dry cycles in these ponds could promote further complexity in the reactions of organic molecules (Bada & Korenaga, 2018; Becker *et al.*, 2018).

(Deep sea) hydrothermal systems

With the discovery of hydrothermal vents, in the 1970s, at the seafloor, these systems got in to sight for a plausible geological location of the emergence of life (Corliss *et al.*, 1981; Holm & Andersson, 2005; Holm & Charlou, 2001; Lonsdale, 1977). The hydrothermal vents are located at the mid oceanic ridges. The water seeps in the basaltic rocks. Here it gets heated by magma chambers, located under the rock layer. Then the water circulates back in the ocean through the vent (Colín-García *et al.*, 2016). This process inheres a high thermal gradient, with temperatures above 300 °C (French, 1970). The stream of the vent contains a large amount of detached minerals from the mantle and a vast quantity of chemicals. This mixture makes up the plume of the vent. At the vent there are several hundred bar of pressure because of the water column. The average depths are between 2000 and 3000 meters below metric sea level (Colín-García *et al.*, 2016). A hydrothermal vent provides hot water at the surface of the sea floor, fluids from the deeper layers are acting with the cold surrounding sea water, which causes a chemical imbalance which further could support the synthesis of organic materials. The out flushed minerals (carbonates, silicates etc.) can now rapidly crystallize into a reactive surface (Pope *et al.*, 2006). On this basis, the first forms of life could be chemoautotrophic and thermophile (Pope *et al.*, 2006; Wächtershäuser, 1988b).

Today's hydrothermal vents found in places of volcanic activity caused by plate tectonic can be divided into two main forms.

One of these is called "black smokers". They are fairly close to the magma containing chambers, so they eject water, with temperatures exceeding 400 °C, at a low pH. By contact with cold sea water, this iron and sulfides rich fluids constitute black-colored turbid clouds which gave them their name (Colín-García *et al.*, 2016).

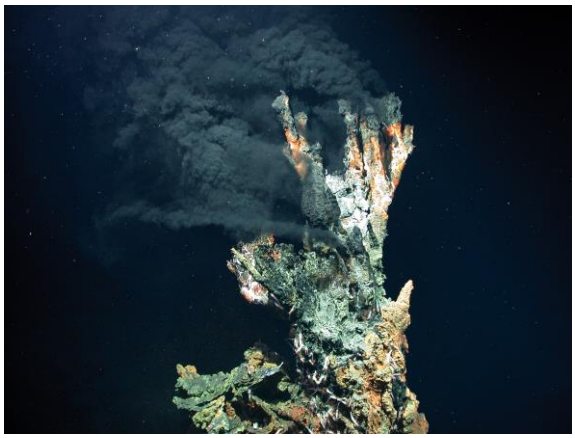


Figure 1: Black smoker (MARUM – Center for Marine Environmental Sciences, University of Bremen (CC-BY 4.0))

The other common type of subaqueous hydrothermal vents is called “white smokers”. The whitish color comes from the difference in the chemical distribution of the vent fluid. Here, the main components are sulfates and calcium ions. The temperature is in the range of 40° to 75 °C due to the larger distances to the magma chambers. The pH of these fluids is typically between 9 and 10 (Kelley *et al.*, 2005).

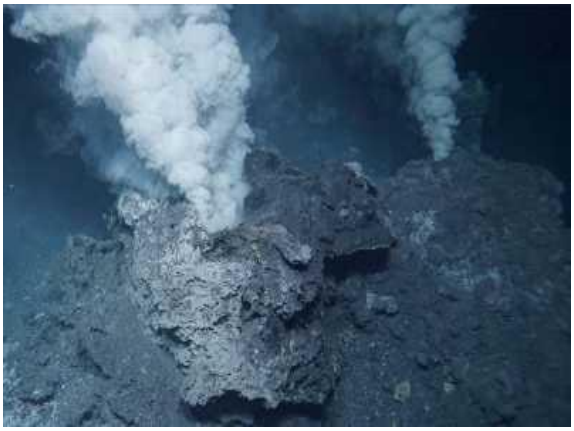


Figure 2: White smoker (MARUM – Center for Marine Environmental Sciences, University of Bremen (CC-BY 4.0))

A special kind of deep sea vents was discovered in 2000 and was called “lost city”. This system is not settled at the mid-Atlantic ridge (region of plate tectonics), but on old oceanic crust (1.5 myr). The heat of the water ranges between 40° to 70 °C sometimes up to 90 °C. The pH is a little more alkaline than sea water (fluids 9-9.5; sea water 8.0). There are low concentrations of magnesium ions, but high concentrations of calcium

ions. The pH and the calcium ions are part of the process of serpentinization (e.g. peridotite minerals into serpentinite minerals) to build up chimneys with 60 m in height (Kelley *et al.*, 2001). Serpentinization leads to the formation of H_2, CH_4 and heat (Boetius, 2005). There is a theory based on serpentinization and methanotrophy to explain the start of the life in such a deep sea environment (Russell *et al.*, 2014; Russell *et al.*, 2010).

Figure 3: Carbonate chimney in the eastern portion of the Lost city field (Kelley *et al.*, 2001)

The problem of energy and substance dilution by the ocean brought Günter Wächtershäuser, 1988, to the postulate of a thermophilic and chemoautotrophic origin of life by pyrite-driven metabolism at volcanic hydrothermal vents (Wächtershäuser, 1988a, 1988b, 1992). In his theory, the hydrothermal vents are freed from plate tectonics, which is said to have evolved after the emergence of life. Rather, these hydrothermal vent systems are based on volcanism, which was highly active on the young earth because of its thin oceanic crust back in that time.

1.1.2 Iron-Sulfur-World designed by Günter Wächtershäuser

During the further and deeper development of his theory, Wächtershäuser created the phrase of “iron sulfur world”, which sets a plausible explanation for a chemoautotrophic emergence of life (Wächtershäuser, 1988a, 1988b, 1992). He suggested that a pioneer

organism evolved at volcanic hydrothermal vents, with the circumstance of surface metabolism (Wächtershäuser, 1988a).

Energy and evolution of the pioneer organism

An early pioneer organism has to be autotrophic and needs an autocatalytic metabolism and is anionic bonded to a positive charged surface (e.g. pyrite) in the surrounding of hot water (Hartman, 1975; Wächtershäuser, 1988a). The pioneer organism is acellular and therefore it cannot divide itself like recent cellular organism can do, but through surface metabolism it can grow (Wächtershäuser, 1988a). A simple state of an autocatalytic system is that small molecules catalyze their own synthesis using external educts and producing themselves and some reactional waste (Peretó, 2012).

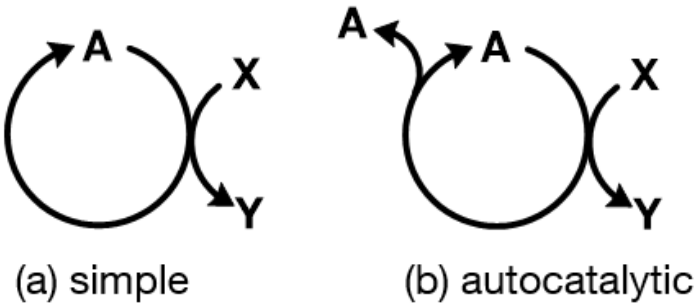


Figure 4: Scheme of two easy metabolically cycles. A simple (a) cycle leading to product A and an autocatalytic (b) cycle. (A = organic reaction product; X = inorganic educt; Y = reactional waste) adapted from (Peretó, 2012)

Today's methanogens get their needed energy through the reduction of CO_2 and H_2 to CH_4 (methane), for example from subaqueous exhalation at hydrothermal vents. Günter Wächtershäuser recognized this circumstance but also stated that an endergonic barrier has be to be overcome for this reaction, so there must be an additional energy source. He concluded that the oxidative formation of FeS_2 (pyrite) forms a reaction of FeS and H_2S (hydrogen sulfide) under the release of H_2 is exergonic and could serve as a continuous energy source for the fixation of CO_2 (Wächtershäuser, 1988b). The transition metal sulfides, iron sulfide (FeS) as well as the similar reactive nickel sulfide (NiS) were both present on the early earth (Allegre *et al.*, 1995). They originate by the precipitation

of water soluble Fe^{2+}-and Ni^{2+}-ions with H_2S forming the not soluble metal sulfides. For example, this reaction occurs in the flows of hydrothermal vents.

The exergonic pyrite formation can support the primordial organism with a Gibbs energy of -38.4 kJ/mol (pH=0; T=25 °C) which gives this one a reducing power (Wächtershäuser, 1990). The calculated exergonic formation is shown in following reaction equation:

$$FeS + H_2S \longrightarrow FeS_2 + 2H + 2e^- \qquad \Delta G° = -38.4 \text{ kJ/mol} \qquad (1)$$

Reactions with the reducing agents FeS and H_2S under primordial conditions are also proven to occur e.g. by the conversion of NO_3^- to NH_3 (ammonia) or alkynes to alkanes (Blöchl *et al.*, 1992). It is also shown that under primordial conditions a system containing FeS and H_2S can reduce nitrogen (N_2) to ammonia (Dörr *et al.*, 2003). Wächtershäuser's theory is supported by nowadays enzyme activities, because metal-sulfur clusters play an important role in today's existing metallo-enzymes (Berg *et al.*, 2010; Span *et al.*, 2012). Their role in biochemistry developed even further in catalyzing gas-based redox reactions, including gases like hydrogen, nitrogen, carbon mono oxide, carbon dioxide, and methane (Fontecilla-Camps *et al.*, 2009; Volbeda & Fontecilla-Camps, 2006). Important enzymes that fall under this category are e.g. the carbon monoxide dehydrogenase, converting CO_2 to CO (Ragsdale, 1994) or the acetyl coenzyme A synthase with which can utilize CO (Darnault *et al.*, 2003). The iron-sulfur clusters are also playing a role in non-redox reactions like the hydration of acetylene to acetaldehyde by the acetylene hydratase (Seiffert *et al.*, 2007; tenBrink *et al.*, 2011). From this coincidence, the connection of the activity of Fe-S-minerals and the enzymatic Fe-S clusters gained support for Wächtershäusers theory of a primordial surface metabolism on transition-metal sulfides (Sobotta, 2018). The core of this FeS-theory is built up by the assumption that some core elements (H, O, C, N, Mg, Fe, S, Ca, Na, Ni and P) make up the biomass for the primordial pioneer organism. These elements have their origin in the mantle of the earth and can form gases as H_2O, CO_2, CO, C_2H_2, H_2S, N_2, NH_3, H_2, HCN and P_4O_{10} (Igari *et al.*, 2000; Oremland & Voytek, 2008; Wächtershäuser, 2007; Wächtershäuser, 2014). When these gases, transported by a flow of hot water in the vents, react with transition-metal sulfide minerals (e.g., FeS_2 or NiS), the products of this reaction promote their

reaction. Therefore, an autocatalytic C-fixation is the result. Hereafter this carbon fixation, low-molecular organic compounds emerge on these autocatalytic surfaces. Through further evolution chemoautotrophic reactions occur, forming more complex biotic molecules and polymers. This approach represents a “metabolism first” approach, the building of molecules/polymers without templates. The following link for this process would be the genetic evolution.

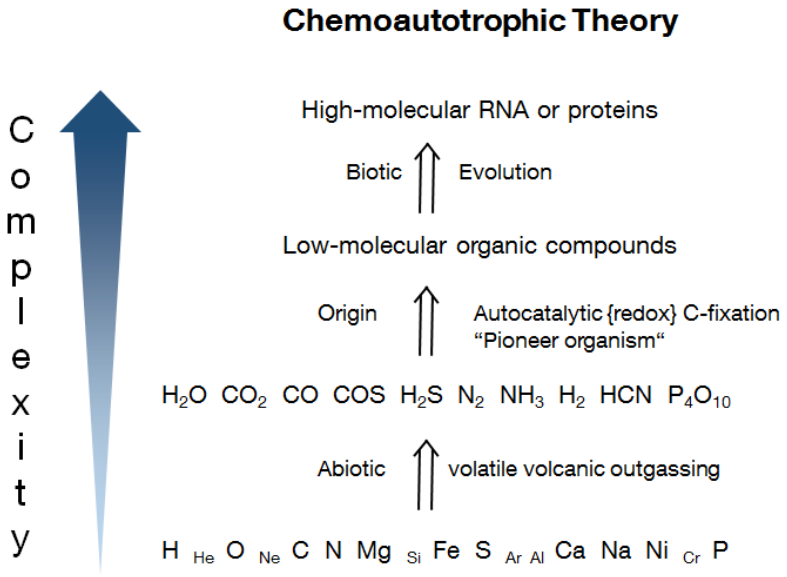


Figure 5: Representation of the various steps of the chemoautotrophic theory formulated by Günter Wächtershäuser. Origin biomolecules from chemical elements present in the earth mantle, followed by an abiotic reaction to special gases. Autocatalytic carbon fixation on transition-metal sulfide minerals promotes the emergence of low-molecular organic compounds (pioneer organism). This further leads to the transition of a biotic evolution and polymerization reactions adapted from Sobotta,2018; Wächtershäuser, 1990.

“Pioneer organism”, a definition

A “pioneer organism” requires an organized being (system) at the start of the evolution. It is to be considered the turn point from an abiotic to a biotic world.

In opposition to this stands the “backwards projection” (Lipmann, 1965). The backward projection describes the process as a one-to-one extrapolation of individual biochemical features between extant- and precursor features. That means more and more projections

lead to more independent features added, which leads to a primordial broth of primordial individuals.

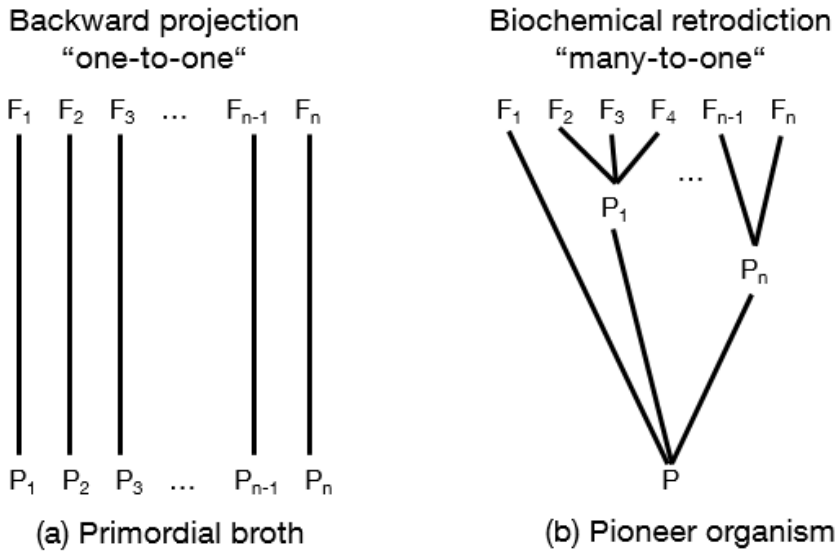


Figure 6: Representation of the heuristics of (a) backward projection and (b) biochemical retrodiction. (a) leads to a sum of primitive cells, called primordial broth, each with their own features. (b) leads back to one pioneer organism from which the diversity begun. (F = extant biological feature; P = precursor feature) (Wächtershäuser, 2014)

The idea of the pioneer organism brings up the biochemical retrodiction (what means many-to-one). Different extant features are resting on lesser primordial elements, which again rest on the pioneer organism. It is no coincidence that the biochemical retrodiction and the phylogenetic tree of life have a similar structure (Wächtershäuser, 2014). Wächtershäuser's idea of the iron sulfur world does not only take its inspiration from this biological-inspired retrodiction, it also uses plausible geochemical circumstances to create a niche for the pioneer organism (Sobotta, 2018). In this context his pioneer organism is based not only on non-metal elements like H, C, N, O and S but also on active and catalyzing transition metals as Fe, Co, Ni, W (Wächtershäuser, 2014).

Surface metabolism and setting of the pioneer organism

The setting the pioneer organism emerged in had a nearly neutral pH. Caused by an offset of orthosilicates (<45% SiO_2), the dominant mineral during Hadean times, an

alkaline pH range (pH 9-12) occurs, and volcanic exhalations, which have a severe acidic pH range, are compensating the alkaline pH (Wächtershäuser, 1992; Wächtershäuser, 2014). Other gases deriving from magmatic plume exhalations are highly reducing due to their high CO/CO_2 ratio (Wächtershäuser, 2007). A relatively near to today's industrial reaction to form cyanide from carbon monoxide and ammonia occurred, and the reduction of nitrogen to ammonia occurred (Elsner *et al.*, 2002; Owen, 1961). The cyanide synthesis is favored by higher temperatures, where in the opposite the ammonia synthesis is driven up by lower temperatures. This brings the equilibrium of HCN/NH_3, at the temperature of a hydrothermal vent (100 °C), nearly completely to the ammonia side. But the equilibrium could be shifted by the formation of highly stable complexes of cyanide with transition metals like nickel (here Ni^{2+}-Ions). By a reaction of CO with H_2S from volcanic exhalations COS is formed, which can be fast hydrolyzed to CO_2 and H_2S. The process can also be activated as a carbon source through a reaction at a transition-metal surface to form methanethiol (Barrault *et al.*, 1987; Wächtershäuser, 2007). The full reaction is summed up in reaction equation (2).

$$CO + H_2S \longrightarrow COS + 2H^+ + 2e^-$$

$$COS + 6H^+ + 6e^- \longrightarrow CH_3SH + H_2O \qquad (2)$$

Surface catalysis on positive charged transition metal surfaces (e.g. pyrite) converts exhaled gases (educts) to organic compounds (products). Further, the catalyzing surfaces have strong interactions with the resulting anions like COO^-, PO_3^{2-} or S^- (Wächtershäuser, 1992). From this functionalized organic compounds, the cell-free pioneer organism evolved. Using metallo-sulfide surfaces ((Fe/Ni)S) under primordial conditions, CO and CH_3SH can be reacted to acetic acid, thioacetic acid or methyl thioacetate. These experiments were performed by Huber and Wächtershäuser (Huber & Wächtershäuser, 1997), a hypothetical mechanism (Cody, 2004) of the experiments is shown in Figure 7.

To enhance the chemical space, reactions with acetylene as acceptor of a carbonylation on nickel sulfide have been done. They showed the creation mono/di- or branched

carboxylic acids of variable lengths (Sobotta *et al.*, 2020). The proposed mechanism for those is shown in Figure 8.

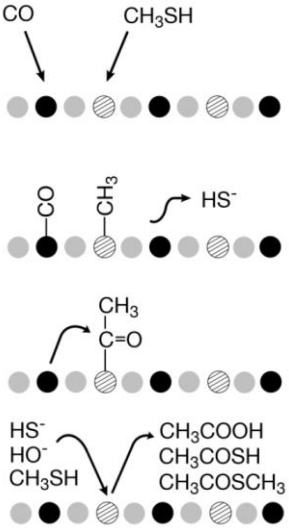


Figure 7: A hypothetic mechanism of the reaction of CO and CH_3SH on a (Fe/Ni)S surface as proposed by Huber and Wächtershäuser. The methyl group of methanethiol is transferred to a nickel atom, a neighboring iron atom is carbonylated by CO. The following transfer and insertion of the carbonyl to the nickel atom leads to an acetyl group. A nucleophilic attack at the carbonyl by a hydroxyl, bisulfide or methanethiol leads to either acetic acid, thioacetic acid or methyl thioacetate. Adapted from Cody, 2004. (black = iron atom, lined = nickel atom, grey = spacer)

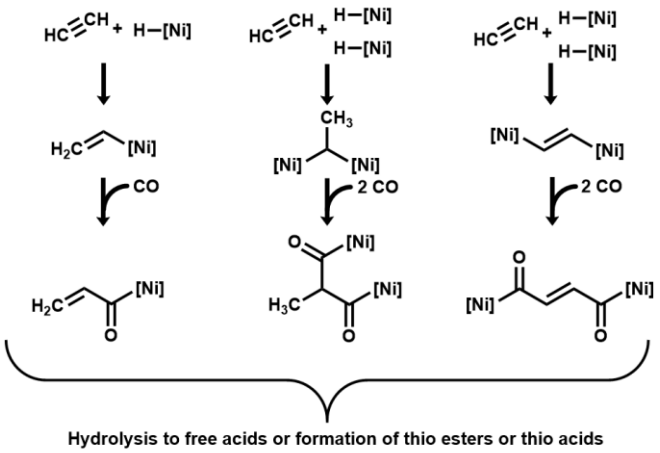


Figure 8: Hypothetical mechanism of acetylene/carboxydotrophic reactions on a catalytic nickel center [Ni]. Leading to either mono carboxylic acids, branched carboxylic acids or dicarboxylic acids by hydrolysis. By the reaction with thiol to the corresponding thioesters/acids. Adapted from Sobotta *et al.*, 2020.

The interaction of the compounds and the surface plays a significant role in the Fe-S-theory, thus in this circumstance, this could overcome the dilution problem of the soup theory. This factor inspired other scientists to have a look on the surface binding and accumulation. They consider that it also plays a role in other scenarios, like the warm/cold pond (Bada & Korenaga, 2018) or ice (Hao *et al.*, 2018), as well as in processes found in thermal pores and cracks, of the mantle rock. These are concentration-dependent by a thermal gradient (Kreysing *et al.*, 2015; Mast *et al.*, 2013; Salditt *et al.*, 2020).

Appearance of the pioneer organism

The pioneer organism has a minimal substructure-superstructure organization. The mineral containing inorganic substructure, a (Fe/Ni)S-mineral, is permeable for gases and water. The organic superstructure consists of low-molecular bioorganic compounds that derived from the fixation of carbon from the gases on the mineral surface (Wächtershäuser, 2006). Figure 9 gives a cross-sectional representation of the organization of the pioneer organism.

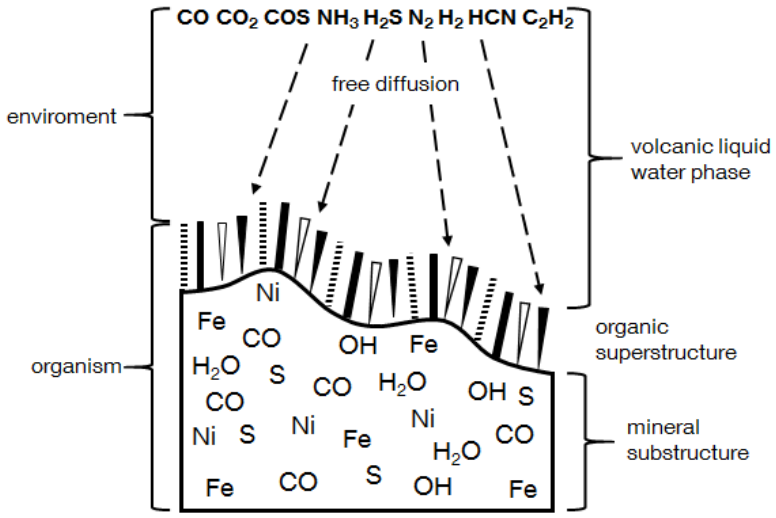


Figure 9: Cross-sectional representation of the pioneer organism. The organic superstructure, obtained by C-fixation, stands on the inorganic mineral substructure, both together build the organism. The organism stays in contrast to the environment, here the volcanic liquid water phase. Through the water phase and the volcanic exhalations there is a permanent supply of educts to ensure the growth of the organism. Due to permanent growth the permeable surface becomes lipophilisated. Adapted from Wächtershäuser, 2006.

Together the mineral substructure and the organic superstructure make up the pioneer organism. The volcanic water phase and the permeable mineral ground are supplying the organism with constant fresh gases to further fixate them through surface catalysis. Further, the carbon chains bound to the mineral (Fe/Ni)S surfaces elongate and become characteristics of fatty acids, which means the surface lipophilisates (Wächtershäuser, 1992, 2006).

By the fixation of carbon from CO into the superstructure, two aspects gain importance. First, the oxidation level of the C-atom is reduced from CO/CO_2 to the one of methane (CH_4), and second, a formation of a C-C-bond takes place. Both aspects lead to a elongating —$(CH_2)_n$— polymeric structure bonded to the surface by an anionic group. Under the anaerobic conditions of a pyrite-pulled metabolism they are chemically inert and have a strong surface bonging moiety. Therefore, they start to accumulate on the pyrite surface, so called lipophilization. This autocatalytic process stepwise coats the whole pyrite surface with generated lipids (Wächtershäuser, 1992). Figure 10 shows how

the lipid layers can interact between themselves (a). The transition from surface lipophilization to a bilayer membrane (b). There is also a stepwise conversion from the surface metabolism to a cellular organism (c).

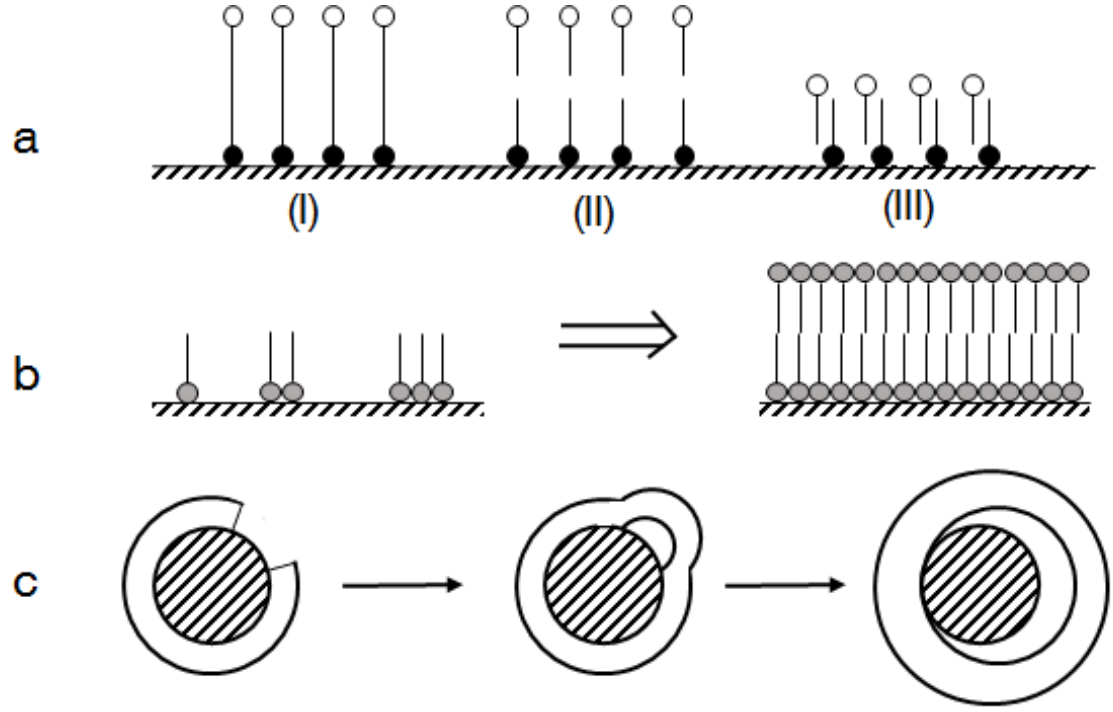


Figure 10: (a) Schematic representation of different membrane cross-sections: monolayer (I), bilayer (II), interdigitated membrane (III); (b) Transition from surface lipophilization to a surface-supported bilayer membrane; (c) Starting with a semi-cell formation, ongoing to a complete cellularization and further growth, lastly the pioneer organism. Adapted from Wächtershäuser, 1992,2006.

The lipids can derive in a complex manner through the autocatalytic synthesis, but the three most accessible types (here called a; b; c) are presented in the following:

(type a) $^{-}OOC—(CH_2)_n—COOH$

(type b) $^{-}OOC—(CH_2)_n—OH$

(type c) $^{-}OOC—(CH_2)_n—H$

The formation of a pre-cellular structure will end with the inclusion of water, saturated with volcanic gases. Further, a separation process starts by the elongation of lipids,

leading to a prototypic membrane. A two-dimensional phase separation can be obtained by three different types of lipids (Figure 10 (a)). The appearance is like any extant lipid with a hydrophilic head and a hydrophobic tail.

Autocatalytic possibilities of the pioneer organism

Another problem in the emergence of life is the establishment of a metabolism further than the upbringing of a membrane layer. The sticking point on this is whether the metabolism is either heterotrophic or autotrophic. Wächtershäuser is a proponent of an autocatalytic self-replicating cycle. Thus to the given conditions on hadean earth, he shifted his focus on the reductive citric acid cycle (rTCA). He set up an archaic form of the rTCA where the hydroxyl groups coexist with thiols and the carbonyls with their corresponding COS-forms (Wächtershäuser, 1990). Wächtershäuser showed, theoretically, that with every turn of the rTCA, starting with succinate, the cycle fixates four molecules of CO_2. This creates a second molecule of succinate. The creation of the second succinate shows that the cycle is autocatalytic, because it doubles with every full turn (Wächtershäuser, 1990).

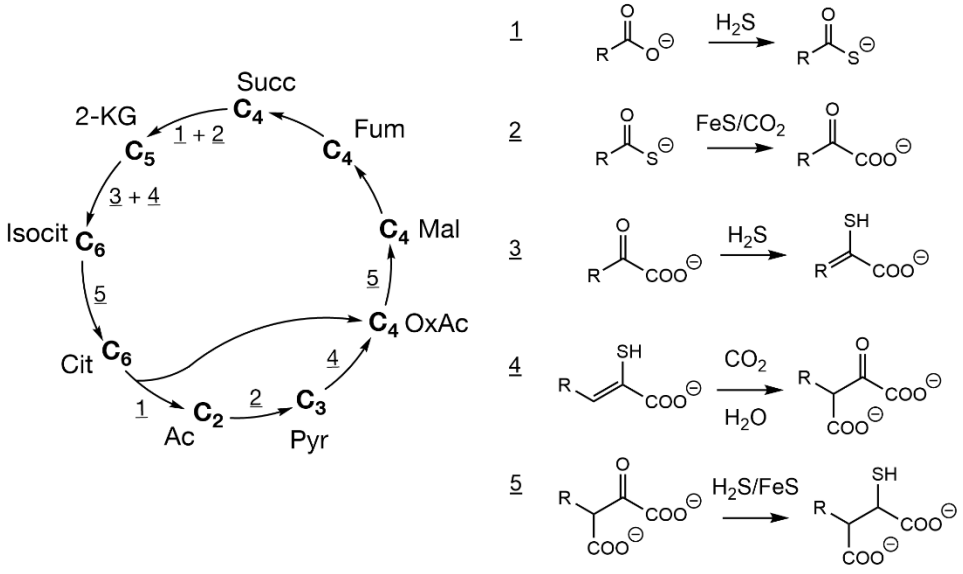


Figure 11: On the left is the simplified, hypothetical rTCA, starting with succinate (Succ). The numbers at the reaction arrows indicate, the associated ideal reaction taking place in the hadean atmosphere. Adapted from Wächtershäuser, 1990.

The CO_2 fixation is available through the reduction potential of the FeS/H_2S-system. Figure 11 shows clearly why the archaic rTCA is autocatalytic, because it generates a small launch molecule like succinate or acetate. Succinate is found again after a full turn of the cycle and the fixation of four CO_2. Further, through the cleavage of citrate to oxaloacetate and acetate this procedure generates two molecules of succinate from one. Theoretically, this succinate can start a new cycle again, launching parallel cycles in neighboring. So the succinate promotes its synthesis over these steps, making the whole procedure autocatalytic. Part (b) of figure 4 shows the point of autocatalysis in a simplicity but also full explanation. In the example of the rTCA, succinate would stand for A. After the cleavage of the citrate molecule, the precursor of acetate, has the following structure: $H_3C—COS^-$. This molecule could be an ancestor molecule of the todays activated acetic acid in form of acetyl-CoA (Wächtershäuser, 1990). Additionally, Wächtershäuser calculated the free energy of this proposed archaic cycle. The data, for reactions like this archaic cycle, had been compiled earlier (Thauer *et al.*, 1977). Wächtershäuser came to the conclusion, that the pyrite-pulled formation of succinic acid is a very exergonic reaction, with a Gibbs energy of -420.0 kJ/mol at pH = 0 (Wächtershäuser, 1990).

$$4CO_2 + 7H_2S + 7FeS \longrightarrow (CH_2—COOH)_2 + 7FeS_2 + 4H_2O \quad \Delta G° = -420.0\ kJ/mol \quad (3)$$

Reaction equation (3) shows the full exergonic reaction from CO_2 to succinate.

The reductive citric acid cycle putatively being one of the first metabolic pathways, and its evolutionary prebiotic origin, is still a great discussion in the scientific world (Berg *et al.*, 2010; Keller *et al.*, 2019; Keller *et al.*, 2017; Kitadai *et al.*, 2017).

By further deepening his theory, Wächtershäuser described synthetic reactions that the pioneer organism is “able” to perform. This means these reactions are still catalyzed by the metal centers of the inorganic substructure of the pioneer organism, but there is progress to complexity observable (Wächtershäuser, 2006). The described reactions will get a short enlightening and discussion in the following.

The first one is the least complex reaction, the formation of ***C_2-structures***. Through the surface catalysis, the pioneer organism can form activated thioacetic acid from CH_3SH and CO (Huber & Wächtershäuser, 1997).

By linking carbon atoms, the pioneer organism is able to create elongated carbon chains, like ***C_3-structures***. But here, arithmetic is not only C1+C1+C1=C3. When CO is used solely at high pressure and temperature (Cody *et al.*, 2000), the arithmetic could also be C2+C1=C3, by taking a C2 body from e.g. the volcanic gases (Sobotta *et al.*, 2020).

With the addition of nitrogen, in the reactive form of ammonia (NH_3), to the reactive system of the pioneer organism, it could perform a ***reductive amination*** (Wächtershäuser, 1990). This reaction was demonstrated at 105 °C under primordial requirements (Huber & Wächtershäuser, 2003). Under these conditions also an ***activation of amino acids and peptide cycles*** is possible (Huber *et al.*, 2003).

There is also the possibility of a ***phosphorylation*** reaction of the pioneer organism. Thus there is P_4O_{10} available in the volcanic gases, it can undergo hydrolyses via poly- to pyrophosphates (Yamagata *et al.*, 1991), which can be used for further COS activated reactions (Leman *et al.*, 2004, 2006).

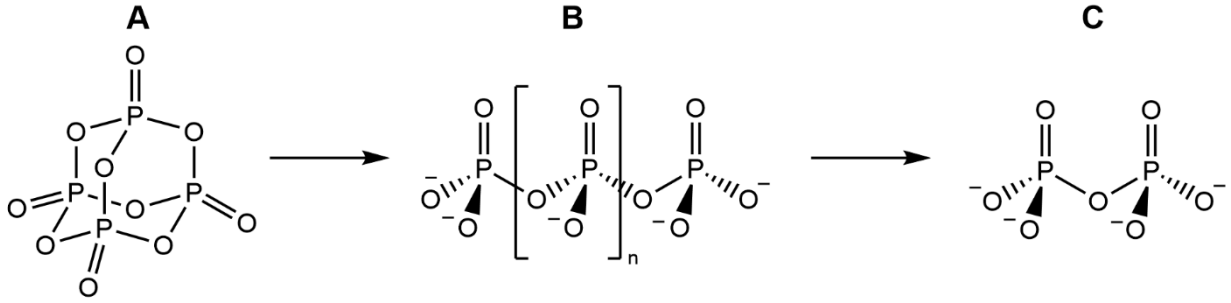


Figure 12: Schematic breakdown of P_4O_{10} (**A**) to first polyphosphate (**B**) and further to pyrophosphate (**C**).

Activated acetic acid (C_2) – Some Experiments with the goal to show a “metabolism first” approach, have been performed by Claudia Huber in the mid-nineties. The theory states that activated thioacetic *S*-acid (CH_3COSH) forms from methyl thiol and carbon monoxide. In contrast, the first was the source for the methyl group, and CO was the provider of the thiocarbonyl moiety. The experiments confirmed this assumption (Huber

& Wächtershäuser, 1997) at 100 °C on a freshly precipitated NiS or (Ni,Fe)S catalyst. Further experiments showed that if there is already an activated C2-body (e.g., C_2H_2) in volcanic exhalations available (Igari *et al.*, 2000), this one favorably used (Sobotta *et al.*, 2020). Sobotta *et al.* showed that methyl thiol acetic ester forms from acetylene with the addition of methyl thiol. They used ^{13}CO, in this case, as part of a 1:1 atmosphere of ^{13}CO and C_2H_2, on a NiS catalyst. There was no increase in molecular weight of the methyl thiol acetic ester, using GC/MS. Sobotta *et al.* concluded that acetylene is the carbon source. This leads to a theoretical precursor analogue of the reductive acetyl-CoA pathway, shown in Figure 13.

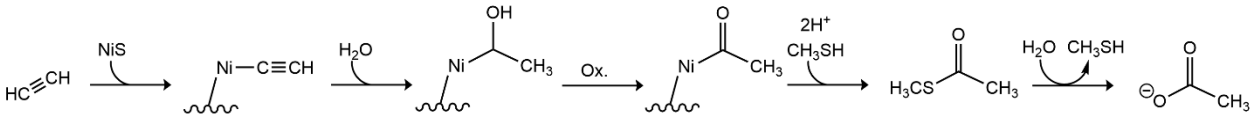


Figure 13: Theoretical pathway of a NiS/C_2H_2/CO system resulting in thio acetate ester, after hydroxylation in acetate. Possible precursor of today's reductive acetyl-CoA pathway. Adapted from Sobotta *et al.*,2020.

Pyruvate (C_3) – The theoretically formation of pyruvate, under primordial conditions, was suggested by Huber & Wächtershäuser (1997). Pyruvate was synthesized using a FeS/H_2S-System with CO as source of carbon (Cody *et al.*, 2000). Thus very harsh conditions were used in this experiment, namely 2000 bar pressure and 250 °C. The educts of the reaction have been FeS (catalyst), alkyl (nonyl) thiol (S source) and formic acid (C source). Through thermal decomposition of formic acid CO and H_2O are formed, in this manner a reactive C_1-body (CO) is formed (Cody *et al.*, 2000). Unfortunately, there is still no evidence if these conditions were present in Hadean times (Wächtershäuser, 2000). Pyruvate was generated in a Wächtershäuser system using NiS/β - $Ni(OH)_2/C_2H_2$/CO at 1 bar/105 °C. The authenticity was checked with labeled carbon monoxide (^{13}CO), prompting in an increase of the molecular mass of one (Sobotta *et al.*, 2020).

α-Hydroxy and α-amino acids – With the finding of the activated acetic acid and the proposal of pyruvate (C_3-body), an elongation seemed as the next logical step in the evolution of reactions. Therefore, the attention shifted to α-hydroxy and α-amino acids

as products of an C_1 extension or an C_1 extension with concurrent NH_3 insertion. It has been showed that a reductive amination can be performed, when treating an α-keto acid with NH_3 to obtain the corresponding amino acid (Huber & Wächtershäuser, 2003). The scheme is shown in equation (4).

$$R–CO–COOH + 2Fe^{2+} + NH_3 + 2H^+ \longrightarrow R–CH(NH_2)–COOH + 2Fe^{3+} + H_2O \quad (4)$$

Further reactions have been carried out, to find a synthesis of α-hydroxy and α-amino acids at a Hadean hydrothermal setting. The reactions had iron- or nickel ions as catalytic centers and the carbon was provided through CO, KCN and CH_3SH (Huber & Wächtershäuser, 2006). For the experiments the heat was variated from 80 °C up to 120 °C with a gas pressure of CO at 1 bar. The reaction time was 10 days. For a shortening of the reaction time and an increase of the net amount of the products, the pressure was increased up to 75 bar. The pH was chosen to be light alkaline to promote the creation of amino acids. Therefore, $(Mg,Ca)(OH)_2$ was added to the reaction system to prevent acidification. As result a series of α-hydroxy- and α-amino acids were found with a chain length up to C_5. More specifically, it showed the formation of pyruvate, glycolate, lactate, glycerate and even 2-hydroxy butanoic acid. When KCN was added, the amino analogues of the just mentioned molecules occurred; glycine, alanine, serin and amino butanoic acid, respectively. Glycinamide was also found in this reactions, implicating that amino acids could be formed by CN-ligands via their corresponding carboxamides. This entails the conclusion that $Ni(CN)_2$ could be an important reactant (Huber & Wächtershäuser, 2006; Wächtershäuser, 2007). The educts have been CO and KCN as described earlier. They classified the products as followed: glycine family e.g., glycine, glycolate, glycinamide; alanine family e.g., alanine, lactate, alaninamide; serine family e.g. serine, glycerate, glyceramide. By stable isotope labeling they confirmed their findings as authentically and showed that KCN is the major carbon and nitrogen donor. The carbon monoxide acts as reductant and minor carbon donor (C. Huber *et al.*, 2010) in this reaction. The usage of high rates of pressure in some experiments gained criticism from other scientists in field, especially the high concentration of cyanide would just hydrolyze above 100 °C (Bada *et al.*, 2007). Against this, Wächtershäuser and Huber showed that there are no detectable free cyanide ions in the reaction solution (author

reply to Bada *et al.* 2007). Furthermore, they answered that all reaction products, found in the increased pressure runs, are also detectable in the 1 bar experiments, albeit at a lower concentration. Additionally, the amount of produced hydroxy/amino acids can be increased when the temperature is increased, too.

Later Huber *et al.* (2010), showed that the amount is increased when starting at 100°C. The reaction comes to its peak at around 180 °C, when the temperature was increased further, the amount of products started to decrease. This brought them to the conclusion that these reactions could take place in flow zones of the hydrothermal vents where the water, coming from the mantle, still has a high temperature (Huber *et al.*, 2010). More investigations were performed on this purpose, ending in a hypothetical mechanistic scheme leading to lactate, alanine, glycolate, glycine, glycerate, serine and isoserine. The reaction was catalyzed by organo-metal complexes (Huber *et al.*, 2012). They proposed that the start of the mechanism is a stable tetracyanonickelate $[Ni[CN]_4]^{2-}$ in the reductive presents of CO or H_2. The reaction keeps going on by multiple insertions of cyanide.

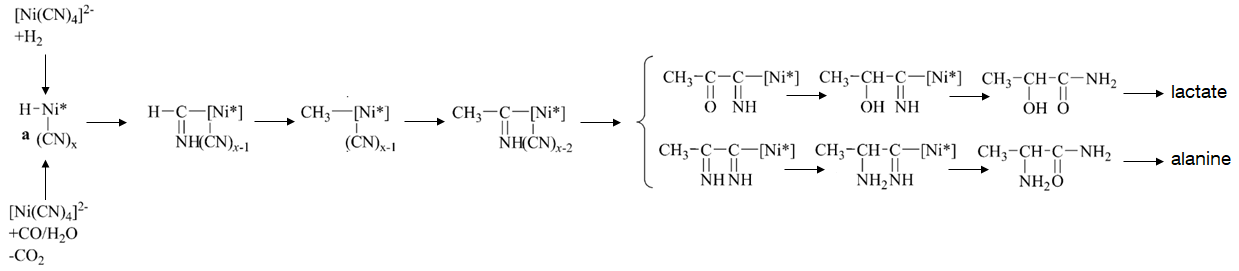


Figure 14: Proposed mechanism of the reaction of tetracyanonickelate to alanine/lactate *via* multiple cyanide insertions. Adapted from Huber *et al.*, 2012.

The hydrated nickel complex, shown in Figure 14, is of unknown geometry, oxidation state, nuclearity and ligand sphere. Therefore, it is indicated as [Ni*]. The first step of this hypothetical reaction is the hydration of the tetracyanonickelate to the activated H–[Ni*]-complex. The next step is an insertion of cyanide as the proton ligand. Therefore, the adhered cyanides of the complex come down from n to n-1. Because of the atmosphere around the complex, CO/H_2, a reduction takes place to a methyl group. Then the insertion step is repeated. From this stage a third insertion takes place. For the α-hydroxy acid product, a subsequent hydration takes place to form an carboxamide. The next step is a

reduction of the keto/imine group at the β-C of the product. Lastly the imine located at the α-C is hydrated to form the corresponding amino- or α-hydroxy acid (Huber *et al.*, 2012).

Activated amino acids and peptides – Finding how to produce amino acids with reaction mechanisms of the pioneer organism, it was just consequent to have a look on the emergence of peptides. Peptides in today's biochemistry are the far most important molecules, because they are building up the proteins. Huber und Wächtershäuser started to add amino acids in reactions to form the thioester of acetic acid. Conditions were (Fe/Ni)S, CO, H_2S (or CH_3SH) at 100 °C with a slight basic pH, buffered by $Mg(OH)_2$. They added phenylalanine (Phe), tyrosine (Tyr) and glycine (Gly). The creation of a peptide bond was observed, this occurred through a condensation reaction, the amino acids formed dipeptides (C. Huber & Wächtershäuser, 1998). In further study's the authors were able to propose a peptide cycle to form dipeptides (Huber & Wächtershäuser, 2003).

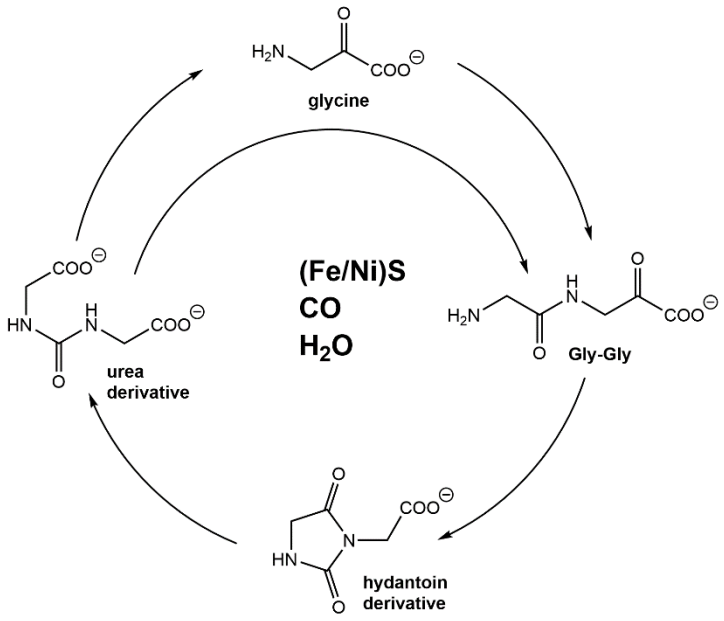


Figure 15: Simplified CO driven peptide cycle of glycine in aqueous (Fe/Ni)S system. Formation of the dipeptide gly–gly *via* hydantoin derivative and urea derivative. Adapted from Huber et al. 2013, Wächtershäuser 2006.

In Figure 15, a simplified version of a CO driven cyclic reaction of glycine to glycylglycin is shown (Huber *et al.*, 2003). The amino acid gets activated by COS to its aminoacyl *N*-carboxanhydride. A second molecule of Gly acts as nucleophile to form the dipeptide. With an activation by COS the dipeptide is transferred to the hydantoin derivative. By the hydrolysation of the intramolecular amide bond, the urea derivative is formed. By cleavage of a carboxyl group and a rearrangement of the molecule, the dipeptide is formed again. This can go into the circle again to form tri- or even tetrapeptides. By creating more pressure *via* CO gas the reaction is supported (Huber *et al.*, 2003; Wächtershäuser, 2006). The mechanism of the nowadays nickel enzyme urease and the proposed peptide cycle share analogies. Further the continuously formed hydantoin could be considered to be a precursor on a pathway to purines (Huber *et al.*, 2003). These different approaches on several important classes of molecules and reactions under volcanic hydrothermal vent conditions show the importance and the potential of the iron-sulfur world from Günter Wächtershäuser.

1.1.3 Further works in prebiotic iron-sulfur-chemistry

Another scientist who worked on the importance of prebiotic iron-sulfur-chemistry is Michael Russel. He suggested that iron monosulfide gels can form spherical shells. These aggregates are found in fossil hydrothermal chimneys. The described shells could have provided a surface for the concentration of polar organic species (Russell & Hall, 1990; Russell *et al.*, 1993). Studies have also shown that if an alkaline solution were injected into light acidic iron-ion solution, a chimney of iron-sulfide was growing (Mielke *et al.*, 2011). Laura Barge et *al.* showed utilization of the iron-sulfide chimneys. By adding pyruvate to the alkaline stream, they showed a conversion from pyruvate to lactate. There were also experiments to generate amino acids by adding ammonia into the solution. Unfortunately, no amino acids (e.g., alanine) were detectable (Barge *et al.*, 2019).

1.2 Chemical evolution

1.2.1 Definition of chemical evolution and life

The name “chemical evolution” was introduced by Melvin Calvin to describe the conversion of simple organic and inorganic molecules into complicated and partly polymeric structures (Calvin, 1956, 1959). Chemical evolution can be considered as an intermediate state between non-living chemistry and fully fledged biological evolution. This means diversity is created through non-directional chemical synthesis instead of mutation and selection of properties (Higgs, 2017). There is a point of view considering that the definition of life has nothing to do with the understanding of its origin or evolution (Szostak, 2012). Life needs a feature of ongoing development and adaptation to new influences, that is what we call evolution (Baross, 2007). But there is the problem of the definition of “what is life” in general. There have been many approaches and opinions over the years (Schrödinger, 1944; Palyi *et al.*, 2002; Gayon *et al.*, 2010;Trifonov, 2011). A recognized definition of life states, that life is “a self-sustaining chemical system capable of undergoing Darwinian evolution (Joyce, 1994). This sentence describes the definition as big picture, where it is not easy to see nuances. Thus, there is no generally applicable definition available, but there are criteria found that are valid in all living systems (Eigen, 1995):

1 Self-reproduction – transfer of information between generations, if not, their information would be lost
2 Metabolism – without it a state of equilibrium would be reached, no further change would be possible
3 Mutation – without it there would be no change of information, therefore mutation implies development for the living system

1.2.2 Metabolism first vs. transcription/genetic first

As described in the pages before, there is an approach to the origin of life, called metabolism first. Which means that the emergence of life started with simple reactions on inorganic surfaces, that went more and more complex over time. Sometimes the products of this reactions catalyze their own building reaction. From this point, the reactions went more and more in the way of biochemical reactions, forming metabolic reaction chains and cycles. Followed by a development to more complex structures like proteins.

The start of the RNA world and central dogma of molecular biology

On the other hand, there is an approach to start the emergence of life from a point of storing information and building structures to catalyze biochemical reactions, mostly called the RNA world theory. This term is very common in publications on the biogenesis of life (Rauchfuss, 2008). The theory, better the hypothesis, was named in 1986 by Harvard scientist Walter Gilbert (Gilbert, 1986). The groundworks for this hypothesis started 60 years ago, with the finding that information is stored in the genetic code. The code is built of the combination of four nucleobases (Gamow, 1954; Nirenberg & Matthaei, 1961; Woese *et al.*, 1966). It was already known that the information is passed further by the replication of deoxyribonucleic acid (DNA) (Watson & Crick, 1953). The important role in the transcription of the naming structure ribonucleic acid (RNA) was found short after the finding of the genetic code (Crick, 1968; Orgel, 1968; Woese, 1967). These insights let Francis Crick to his “central dogma of molecular biology” (Crick, 1970). His dogma describes the flow of genetic information in biological systems. The flow is structured in three steps, namely replication, transcription and translation. The replication is done by the enzyme DNA-polymerase, here starts the flow of information. The information is then transcribed to a mobile form of information called messenger ribonucleic acid (mRNA), this process is done by a RNA-polymerase. RNA and DNA deviate in one nucleic acid. In the RNA, the DNA’s nucleic acid thymine, is substituted

with the nucleic acid uracil. The mRNA gets then translated into proteins by cellular structures called ribosomes (Crick, 1970).

RNA-World-Theory

A central problem of the genetic theory was that DNA and proteins are not present at the same time at the same spot in a living system. Further there is no catalytic function in the DNA molecule, its only purpose is the storage of information. After encoding into protein, these ones unfold their catalytic properties. So it is safe to say, by observing Cricks central dogma (Crick, 1970), that DNA and proteins depend on each other. This leads to a discord. What was first? The RNA steps in this gap. As a polynucleotide it is able to store information like DNA can, but RNA even inheres catalytic functions, e.g. catalyze its own synthesis (Cech, 1986). With this equipment, RNA could perform all vital functions of life, like catalysis, heredity, recombination and evolution, this led to the RNA-World-Theory (Gilbert, 1986; Martin *et al.*, 2008). Seen as transcription first, the RNA-World would have originated from the nothing. This leads to a wall between biochemistry and prebiotic chemistry (Martin *et al.*, 2008). Newer studies state that RNA, of course would not occur *de novo*, more it was a transition from peptide nucleic acids to tetrose nucleic acids to RNA (Bada, 2004; Orgel, 2004). That shows RNA could have been a central point of primordial synthesis. If you look from the evolutionary side, all processes in which RNA is involved in today's biochemistry, are very old (Rauchfuss, 2008). Thus there are quite good approaches in the RNA-World-Theory, it is only a hypothesis, that entailed some interesting models of biogenesis (Rauchfuss, 2008). An example for the ambivalence of the theory is shown by Dworkin et al., stating that, because of its metabolic arguments, RNA genetic material precedes DNA. But on the other side 2'-deoxyribose is more soluble, stable and reactive than ribose, which would favor DNA as the older one (Dworkin *et al.*, 2003). Another point of criticism is the competition to be assembled into a polymer. In prebiotic times there was a vast number of compounds capable to be incorporated into a polymeric structure as RNA is. As example Robert Shapiro showed that if a polymeric chain of L-amino acids is build up from the prebiotic material found on the Murchinson meteorite, the L-amino acids compete with D-amino acids, β-amino acids and hydroxyl acids. Some reactions will bring the elongation to a sudden end, while

reactions containing trifunctional amino acids like aspartic acid will create branches in the polymer chain and increase the complexity. He concluded that the creation of an information transmitting polymer like RNA from a complex mixture is not excluded, but it is very unlikely (Shapiro, 2000a, 2000b). A molecular code, stored in the RNA, of complex molecules like enzymes was not necessarily needed in the origin of life. Further, the formation of the molecule carbamate from CO_2 and furfuryl amine, can occur spontaneous and without an enzyme. This reaction is the first step of biological methanogenesis in today's microorganisms (Vorholt & Tauer, 1997; Bartoschek *et al.*, 2000; Martin *et al.*, 2008). Varma *et al.* showed the formation of pyruvate from CO_2 on a Fe^0 surface, with an enzyme free acetyl-CoA-like pathway (Varma *et al.*, 2018). Further the prebiotic synthesis of RNA is challenging (Powner *et al.*, 2009) and the chain length needed of a ribozyme to replicate RNA sequences is at a minimum of 200 nucleotides, which is quite a big molecule for prebiotic times (Wochner *et al.*, 2011). On the chemical side there are some problems regarding the sugar backbone of RNA, namely the instability of ribose and the implementation of glycosidic bonds between the nucleotides under primordial conditions (Nelson *et al.*, 2000a, 2000b). Because of all these negativities about the hypothesis of the RNA-World there are aspirations to simplify the structure of the primordial molecule. Here mainly the backbone is considered and not the nucleotides to maintain the ability to hold information. A substance inhering this simplicity and function is PNA (peptide nucleic acid). Here the phosphate-ribose backbone is replaced by a polyamide polymer. The normal Watson-Crick base pairing is still in function, as PNA can interact with DNA and RNA (Egholm *et al.*, 1992; Nielsen *et al.*, 1991). Because of its simplicity PNA could be a starter to a "Pre-RNA-World" (Rauchfuss, 2008).

But still there is the question whether metabolism or genetics was first?

The first two outline items laid the foundation for this work. From early experiments of Stanley Miller to today's works of e.g. Laurie Barge had an influence on the experimental work. Especially the work of Günter Wächtershäuser, together with Claudia Huber paved

the way. The thesis is based on the iron-sulfur-world theory. Therefore, the experiments performed in this thesis work were designed to recreate the metabolic options of Wächtershäusers pioneer organism. Because of this it follows that the practical work was based on a metabolism first approach. But the other approach should also be highlighted.

1.3 Emergence of metabolism

1.3.1 Metabolism and its offspring

A definition of metabolism could be read as follows, “the chemical process that occur within a living organism in order to maintain life” (Stevenson, 2010). This concedes that metabolism is the main subject of biochemistry, “the branch of science concerned with the chemical and physio-chemical processes and substances which occur within living organisms (Lazar & Birnbaum, 2012; Stevenson, 2010). This shows the strong interconnection of metabolism and biochemical reactions. The emergence of life can also be seen as the emergence of metabolism. The first conversions of inorganic material to a vast number of organic compounds capable of, early and non-complex, biochemical reactions marks a start in the evolution of metabolism. This conversion is the catabolically source of energy in modern life (Wächtershäuser, 1988a, 2006). A central point of the first metabolic networks, derived from high variety of compounds, was the fixation of carbon as source of molecular growth. This process was driven by the force to reduce entropy in the reaction system. A possible carbon donor of primordial atmosphere could be CO_2 (Kasting, 1993). In extant biological life the fixation of CO_2 is the main carbon source, the reactions, e.g. phosphorylation or cofactor coupling *via* thioester formation, are very energy consuming and therefore catalyzed by enzymes. Primordial fixation of CO_2 could have been done by inorganic catalysis on metal surfaces of minerals (Preiner *et al.*, 2020; Varma *et al.*, 2018). Another source of carbon fixation agents could be volcanic exhalations. For example, carbon monoxide and acetylene can be potential compounds, because of their availability in exhalations, chemical reactivity and high potential of

ligation with transition metals for catalysis (Igari *et al.*, 2000; Mukhin, 1974; Oremland & Voytek, 2008; Sobotta *et al.*, 2020). With the ability to fixate carbon, diversity of compounds further increases, although the probability for more complex reactions is increased.

1.3.2 First enrobed metabolism or who is LUCA?

The term "LUCA" stands for universal last common ancestor. What does this mean for the emergence of life? First, descending from Darwinian evolution, there was a tree of life with two main branches, the eukarya and the bacteria. With the finding of unusual bacteria in 1981 by Woese, the phylogenetic tree grew to its today lookalike with three domains, bacteria, archaea and eukarya, with the last two sitting on a symbiotic branch of the tree (Woese, 1981; Woese *et al.*, 1990).

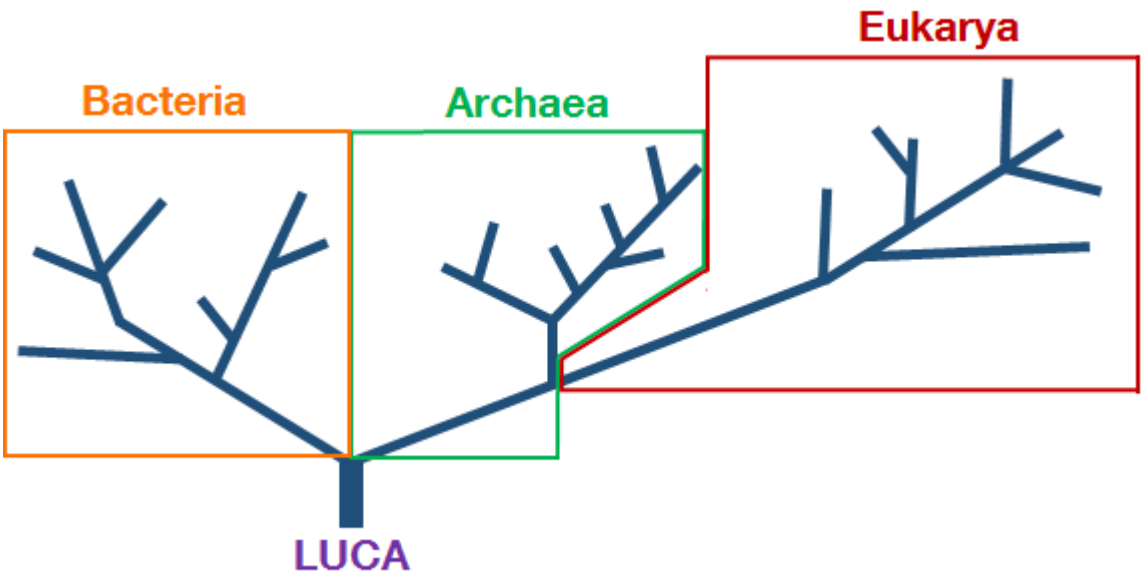


Figure 16: Simplified traditional phylogenetic tree of life. Showing Bacteria (orange) on the left branch, archaea (green) and eukarya (red) on the right branch. LUCA is identified on the bottom of the trunk. Adapted from (Doolittle, 1999; Woese *et al.*, 1990)

The shown traditional phylogenetic tree pictures just a lateral gene transfer from predecessor to successor. It was shown by Doolittle that there is not just lateral gene transfer, furthermore he found that there is also horizontal gene transfer in

microorganisms. Implementing this horizontal gene transfer, the phylogenetic tree looks more woven (Doolittle, 1999).

To be clear, LUCA is a theoretical organism sitting on the down end of the trunk to bring the early biochemistry and the presentation of first cells together (Weiss *et al.*, 2018). A timeline for the emergence of LUCA and the three domains could look as follows (Glansdorff *et al.*, 2008). Firstly, there was prebiotic chemistry on iron-sulfur cluster rich minerals, proceeding the first biochemical reactions. Over time the reaction network assembles into a self-sustaining proto-metabolism, which was followed by a phase of pregenomic nature. This means the catalytic networks producing polynucleotides and peptides, get closed up in vesicles. With the pregenomic phase also an era of community of ancestor cells starts. In this era a RNA-progenote evolved. This means the genetic code as well as *sn 1,2* lipids develop. Further a protonucleus evolves. This is followed by the evolution of various metabolic types. The end of the community era marks a protoeukaryotic LUCA. A promiscuous community of cells with mesophilic features and thermotolerant. These organisms were metabolically and morphological diverse and genetically redundant. From this point followed a transition from RNA to DNA and a diversification into the three domains. The Eukarya emerged from the protoform and showed an increasing tolerance to O_2. The archaea emerged by thermoreduction and further developed *sn 2,3* lipids. The bacteria emerged out of a reductive evolution.

These LUCA communities probably mainly lived under anaerobe conditions, because of the sensitivity of iron-sulfur clusters to O_2, from gases. It is assumed that LUCA used the acetyl-CoA pathway (Wood-Ljungdahl) to fixate CO_2. The reason for the assumption of the acetyl-CoA pathway was because, its chemical simplicity and exergonic nature (Weiss *et al.*, 2016). In the Isua supracrustal belt, located in Greenland, stromatolites were found. These showed microbial structures, which is an indication of biotic CO_2 fixation. The stromatolites were dated back to about 3.7 billion years ago (Nutman *et al.*, 2016). Additionally, in the same geological region, the Canadian shield, microbial structures were found in fossilized hydrothermal vent. This was dated to be 3.8 billion years old (Dodd *et al.*, 2017). These findings support the hypothesis of the emergence of life at hydrothermal systems. Still today, submerged regions with volcanic activity are flowering

environments, hosting for example different classes of archaea. These microorganisms are able to do an autotrophic metabolism (Berg *et al.*, 2010).

1.3.3 Traits in today's biochemistry

The start of any todays intra cellular metabolism, has its traits in cell free metabolic reactions at the emergence of life. Based on the carbon dependency of any living organism on our planet, the fixation of carbon is one of the most important metabolic tasks to be performed. This means that carbon fixation is a main part of the evolution of metabolism, which is an important basis for the emergence of life. A look on today's carbon fixation can help to extract the ancient parts. All present carbon fixation pathways are seen as successors of an ancient fixation pathway. The pathways can be conducted to a phylometabolic tree of carbon fixation (Braakman & Smith, 2012). The extant fixation of carbon is mainly done by the fixation of CO_2. The reaction to activate CO_2 for fixation is chemical and energetically complex. It is an elaborate way to perform just a C_1 extension. Because of this nature other gases at volcanic hydrothermal vents were considered to be involved in a first carbon fixation reaction network (Sobotta *et al.*, 2020). The proposed acetyleno/carboxydotrophic reaction network by Sobotta *et al* works an example for ancient carbon fixation with traits in today's biochemistry.

In their experiments they found several biochemical components, that are members of extant CO_2 fixation pathways. The pathways were the following: reductive acetyl-CoA pathway (purple) (Ljungdahl & Wood, 1969), reductive tricarboxylic acid (rTCA) cycle (orange) (Fuchs *et al.*, 1980), 3-hydroxypropionate-4-hydroxybutyrate (3HP-4HB) cycle (blue) (Kockelkorn & Fuchs, 2009), Dicarboxylate-4-hydroxybutyrate (DC-4HB) cycle (red) (Huber *et al.*, 2008) and 3-hydroxypropionate (3HP) bicycle (green) (Strauss & Fuchs, 1993). These findings can show connection of an ancient transition metal dependent carbon fixation pathway and extant pathways.

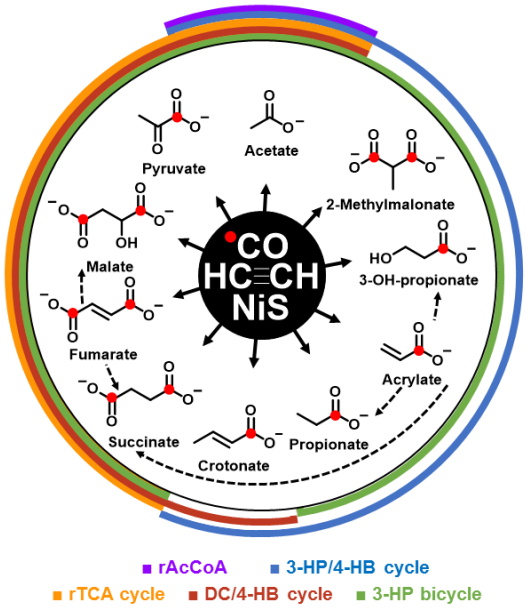


Figure 17: Proposed acetyleno/carboxydotrophic reaction network by Sobotta *et al.*, the different colors indicate connections to extant CO_2 fixation pathways, red dots show labeling with ^{13}C-atom. Adapted from (Sobotta *et al.*, 2020)

As mentioned before, this work has a distinct look on the metabolic options of the pioneer organism. This organism is a cell free structure. Therefore, it is a requirement for all reactions, taking place in this metabolism, to function in an aqueous environment on a transition metal catalyst. With these basic requirements we could show an acetyleno/carboxydotrophic reaction network, which shows parts of a primordial metabolism (Sobotta *et al.*, 2020). In this network we found molecules which are metabolites in today's CO_2 fixation reactions systems. Further we could show metabolic reactions taking place in our network. This highlights a connection of old cell-free metabolic reactions with modern biochemistry in living systems. Furthermore, we showed that substance classes like thiophenes can evolve during these reactions. This shows that they are not only degradation products, on the contrary they can show sites and niches with evolving cell-free metabolism (Geisberger *et al.*, 2021).

1.4 Capsulation of metabolism

1.4.1 Fatty acid vesicles and micelles

To obtain a vesicle or a micelle in an aqueous solution the surface active molecules need to inhere amphiphilic characteristics. For that purpose, both polar and nonpolar functional groups must be present (Walde, 2006). Fatty acids as well as fatty alcohols are typical examples for molecules that are able to self-organization in a spatial alignment to neighboring molecules (Meierhenrich *et al.*, 2010). In extant organisms this function is executed by phospholipids (Menger & Peresypkin, 2001; Zepik *et al.*, 2008). The force that pushes amphiphilic structures into a round shape, are the hydrophobic interactions at the hydrocarbon water interface. This drives the molecules to association. Against this force, there are hydrophilic, sterically and ionic repulsive forces that favor the dissociation. This is called the concept of “Opposing Forces” (Tanford, 1978). The hydrocarbon chains inside a vesicle or a micelle behave like fluids (Lindblom & Wennerström, 1977; Shinitzky *et al.*, 1971).

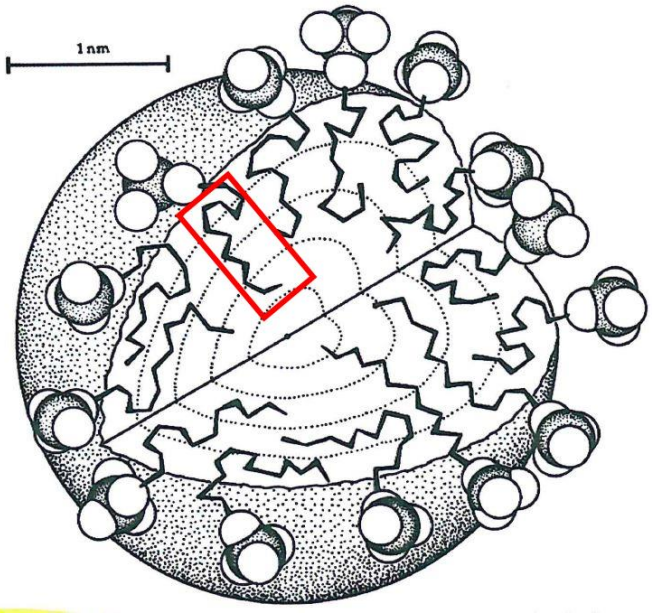


Figure 18: Example micelle, showing hydrocarbon chains (marked in red) in a disordered manner, comparable to a fluid. Adapted from (Gruen & De Lacey, 1984).

Above a critical concentration the amphiphilic material tents to build microscopic structures. These concentrations are called cmc (critical micelle concentration) and cvc/cbc (critical vesicle/bilayer concentration). The cmc is normally extremely lower than the cvc. Additional to the cvc/cmc there is the Krafft-temperature, which is defined as the minimum temperature at which the formation of micelles take place (Krafft & Strutz, 1896). If the system is above the Krafft-temperature and the cmc the amphiphilic molecules will build up spherical micelles (Rasi *et al.*, 2003). Above the cvc the amphiphilic molecules will build up bilayer sheets and vesicles, which are in a dynamically equilibrium with the sole amphiphilic molecules and vesicles (Apel *et al.*, 2002; Namani & Walde, 2005). These lipid vesicles are called liposomes (Berclaz *et al.*, 2001; Cheng & Luisi, 2003; Fredric M. Menger & Gabrielson, 1995); the characteristic is the encapsulation of a water phase in the bilayer construct (Frisken *et al.*, 2000; Hunter & Frisken, 1998; Fredric M. Menger & Gabrielson, 1995). From this base, there can occur uni-laminar or multi-laminar vesicles, with diameters from 20 nm to 100 µm (Segre *et al.*, 2001; Walde, 2006). Following their structural similarity to cell membranes, fatty acid uni-laminar vesicles are in consideration to be a predecessor of modern membranes (Segre *et al.*, 2001). The internal structure of uni-laminar vesicle protocells would be a lot simpler than modern cells, additionally these protocells would have been smaller than today's smallest bacteria (Rasmussen *et al.*, 2004). Some scientists are even going so far to call uni-laminar fatty acid vesicles "protocells", "progenotes" and "proto-/probionts" (Doolittle & Brown, 1994; Oparin & Gladilin, 1980; Woese, 1998).

1.4.2 Fatty acid vesicles as a possible protocell

If there is an additional amount of amphiphilic material available, whether free fatty acids or fatty acid micelles, vesicles are able to absorb this material and grow (Berclaz *et al.*, 2001; Hanczyc *et al.*, 2003). There are three models for the growth of vesicles (Meierhenrich *et al.*, 2010):

1 A direct fusion of vesicles with micelles in one step

2 A disassembly of micelles into fatty acid units and installation into a vesicle bilayer

3 Fusion of vesicles

The enlargement of vesicles and the increase in number is called autopoietic self-reproduction (Walde *et al.*, 1994; Wick *et al.*, 1995). The formation of vesicles can be induced by mineral surfaces. Quartz minerals or minerals with iron-sulfur surfaces enhance the transition of fatty acid micelles to bilayer vesicles (Hanczyc *et al.*, 2007). Bilayer vesicles are dynamic systems. This means that single molecules can be easy assembled into the vesicle structure. The amphiphilic molecules can move between the layers of the vesicle (Mansy *et al.*, 2008; Segre *et al.*, 2001). This movement can be a hint to the cross membrane transportation of metabolites.

Because there are no complex biochemical processes for cell division in a fatty acid vesicle it needs physical/chemical mechanisms to divide a vesicle (Szostak *et al.*, 2001). The division of vesicles takes place by extrusion through pores. The vesicle enters the pore under pressure and changes its shape from spherical to cylindrical. Smaller subsidiary vesicles are cut off with a diameter similar to the pore (Hunter & Frisken, 1998). Hanczyc *et al.* differentiate between two mechanisms of vesicle division. The first mechanism is, the breakdown of the vesicle under pressure into membrane fragments, which assemble back into smaller vesicles. As second mechanism of division it is stated that smaller vesicles are cut off from the parental structure. (Hanczyc *et al.*, 2003). They showed that vesicles can divide, without a molecular machinery, just by a physiochemical mechanism, which can occur also naturally. Further shearing forces can also initiate the division of vesicles (Szostak *et al.*, 2001).

As fatty acid vesicles can grow and divide in some kind of manner, the next step would be the providing of a reaction chamber. This can be achieved through a dehydration and rehydration process. By dehydration a fatty acid mono layer area will be created on a surface. With rehydration, substances solved or transported by water can be capsulated in the fatty acid vesicles, also nanoparticles can get enclosed by vesicles (Apel *et al.*, 2002). Vesicle membranes reforming by hydration on a mineral particle, can merge together with other vesicles formed on this particle. With further growth the vesicles can encapsulate the particle whole. This encapsulation transports the catalytic moiety into the vesicle (Hanczyc *et al.*, 2003; Wächtershäuser, 1992, 2006). Oligonucleotides can be synthesized by encapsulated clay particles (Hanczyc *et al.*, 2003). Chen *et al*. showed

additionally, that oligonucleotides are transported into the vesicle. There occurred a polymerization on a catalytic mineral surface to RNA-molecules. The synthesized RNA increased the osmotic pressure inside the vesicle, which promoted the further growth of the vesicle (Chen *et al.*, 2004). A mechanism to transport metabolites through a fatty acid bilayer is described by the static solubility-diffusion theory. The theory says that the fatty acid bilayer is a liquid phase separating two aqueous phases. Therefore, metabolites can infiltrate this bilayer, get distributed in the hydrophobic phase. They defund through it, and again get resolved in the aqueous phase. This mechanism is primarily driven by a gradient in the concentration. The theory can be applied on uncharged molecules, because it is easier for them to cross the hydrocarbon phase of the vesicle (Meierhenrich *et al.*, 2010).

A big step in the evolution of a living cell was the development of compartments. The easiest way to create a compartment in an aqueous solution is the addition of nonpolar molecules. Substances that meet these requirements are fatty acids. These are products of our reactions. Fatty acids can build up two different compartments in aqueous solution, micelles and vesicles. So the next big step would be to show that the concentration of fatty acids produced in our hydrothermal vent experiments is big enough to form one of these compartments, in the best case vesicles. To show this is the actual task.

2 Motivation

In summary the aim of this work was to further investigate on the first metabolic pathways of the pioneer organism in accordance with the iron-sulfur world and the possible emergence of compartmentation for those pathways. This means to sound out the possibilities of a metabolism suitable for a cell-free pioneer organism. On the base of simple and reactive educt molecules provided by primordial volcanic hydrothermal vents. Further the traceability of this rather old reactions and their products in today's biochemistry. Specific on nowadays CO_2 fixation reactions, as follow up of primordial carbon fixation from e. g. acetylene. Lastly the look on how an encapsulated reaction could take place. Here it focuses on the compartmentation its self. The building of a compartment from, hydrothermal vent reaction provided, fatty acids is the aim.

A first part of this work concerns about the connection of a acetyleno/carboxydotrophic core metabolism of the pioneer organism to extant mechanisms to fixate carbon (mainly as CO_2). This work shows the occurrence of metabolites, still present in today's carbon dioxide fixation pathways, through a primordial mechanistic pathway at simulated volcanic hydrothermal vent conditions. The "metabolites" are synthesized on NiS-surface using acetylene and carbon monoxide as carbon sources. These gases are existing in volcanic exhalations. So this part describes a conversion of inorganic educts to biochemistry suitable products by transition metal catalysis.

Further this work focused on the possibility of niches suitable for the emergence of life. There were findings on Mars showing metabolites and mainly sulfur containing compounds from the family of thiophenes (Heinz & Schulze-Makuch, 2020). Thiophene and its successor molecules can be found in reactions of volcanic gases activated on a NiS-surface. Here a theoretical mechanism was described concerning the attachment of a C-atom at the sulfide and the carbon chain extension. Further the resulting sulfur containing molecules can be seen as predecessors of those containing oxygen or nitrogen at this certain position. With these findings there can be theorized that thiophenes not only show a decay of organic life, they can also show a possible habitable site for the emergence of life on earth or other extraterrestrial bodies.

Lastly this work was on the building of compartments for possible encapsulation of metabolic reactions. A previous work showed the diversity of short chain fatty acids found in reactions simulating volcanic hydrothermal vent conditions (Scheidler *et al.*, 2016). The reaction mixtures pass through conditions to theoretically form fatty acid vesicles. The aim was to find evidences for vesicles in these mixtures. The short chain length, unsaturation and the reaction surface should favor the formation of fatty acid vesicles in the aqueous reaction solution.

3 Results

The articles shown on the following pages are referenced as:

Sobotta, J., Geisberger, T., Moosmann, C., Scheidler, C. M., Eisenreich, W., Wächtershäuser, G., & Huber, C. (2020). A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism. *Life (Basel), 10*(4). doi:10.3390/life10040035

Geisberger, T., Sobotta, J., Eisenreich, W., & Huber, C. (2021). Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth—Implications for Early Life on Extraterrestrial Planets? *Life, 11*(2), 149.

3.1 Summary and Article: A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism

To enhance an evolution of life, there must have been the critical step of carbon fixation, to a further establishment of metabolism. In this work we showed the synthesis of C_{2-4}-products representative for C_{2-4}-segments of all four CO_2-fixation cycles of the domains Archaea and Bacteria. These products were obtained from experiments simulating volcanic hydrothermal vent conditions. For a typical experiment a reactor vessel was charged with an aqueous solution of freshly precipitated NiS with a ratio of C_2H_2:CO_2 (1:1) as reaction gases. The preparation was done at room temperature followed by 7 days at 105 °C at a 1 bar of pressure. The supernatant was analyzed by GC/MS, showing C_{2-4}-products (Figure 19). The evidence of authenticity was done by ^{13}CO labeling experiments. The products were found in concentrations up to 29 mM.

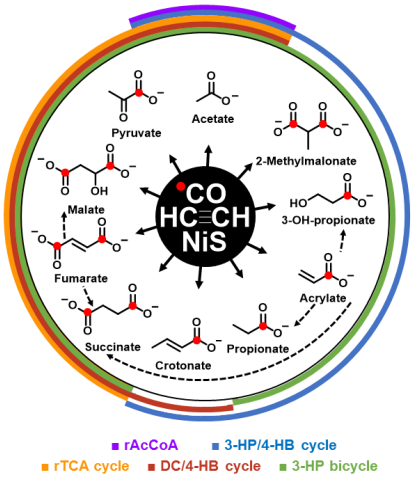


Figure 19: Acetyleno/carboxydotrophic reaction network. NiS-catalyzed reaction network starting from acetylene and carbon monoxide. Observed products are shown with their chemical formula and names. Adapted from (Sobotta *et al.*, 2020).

My individual contribution of this work included the interpretation of available data and performing necessary additional experiments with analytics and interpretation of the data. Additionally, I was involved in the preparation of this published research paper.

life

MDPI

Article

A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism

Jessica Sobotta [1,†], Thomas Geisberger [1,†], Carolin Moosmann [1], Christopher M. Scheidler [1], Wolfgang Eisenreich [1], Günter Wächtershäuser [2] and Claudia Huber [1,*]

[1] Lehrstuhl für Biochemie, Department Chemie, Technische Universität München, Lichtenbergstraße 4, 85748 Garching, Germany; Jessy.Sobotta@web.de (J.S.); thomas.geisberger@tum.de (T.G.); carolin.moosmann@tum.de (C.M.); christopher.scheidler@cup.lmu.de (C.M.S.); wolfgang.eisenreich@mytum.de (W.E.)

[2] 209 Mill Race Drive, Chapel Hill, NC 27514, USA; gwmunich@bellsouth.net

* Correspondence: claudia.huber@tum.de

† These authors contributed equally.

Received: 9 March 2020; Accepted: 4 April 2020; Published: 7 April 2020

Abstract: Carbon fixation, in addition to the evolution of metabolism, is a main requirement for the evolution of life. Here, we report a one-pot carbon fixation of acetylene (C_2H_2) and carbon monoxide (CO) by aqueous nickel sulfide (NiS) under hydrothermal (>100 °C) conditions. A slurry of precipitated NiS converts acetylene and carbon monoxide into a set of $C_{2–4}$-products that are surprisingly representative for $C_{2–4}$-segments of all four central CO_2-fixation cycles of the domains Bacteria and Archaea, whereby some of the products engage in the same interconversions, as seen in the central CO_2-fixation cycles. The results suggest a primordial, chemically predetermined, non-cyclic acetyleno/carboxydotrophic core metabolism. This metabolism is based on aqueous organo–metal chemistry, from which the extant central CO_2-fixation cycles based on thioester chemistry would have evolved by piecemeal modifications.

Keywords: origin of life; chemical evolution; early metabolism; transition metal catalysis; carbon fixation; nickel sulfide; acetylene; carbon monoxide

1. Introduction

All scientific theories concerning the origin and early evolution of life have to consider carbon fixation and the evolution of metabolism. Extant carbon fixation cycles are seen as successors of primordial carbon fixation, and their evolutionary history has been reconstructed as a "phylometabolic" tree [1]. The extant biosphere mainly owes its existence to CO_2-fixation. Scientific theories concerning the origin and early evolution of life are expected to be explanatory for this overarching fact. However, any attempt to project from extant CO_2-fixation back to a primitive CO_2-based core metabolism as wellspring for all biosynthetic pathways faces severe chemical hurdles. Due to its high chemical stability, the conversion of CO_2 into core metabolic constituents mainly requires energy coupling by phosphorylation and thioester formation, as well as a nucleophilic attack by carbanion intermediates, and all that is aggravated by the number of C_1-extensions. Despite recent findings of acetate and pyruvate formation from CO_2 through inorganic catalysis [2,3], alternative geochemically-available carbon sources should be considered. We chose acetylene and CO as primordial carbon nutrients with the following benefits: (a) availability in volcanic-hydrothermal settings [4–7]; (b) high chemical reactivity with the avoidance of energy coupling; (c) low C-oxidation numbers; (d) CO also serving as reducing agent; (e) strong ligation to catalytic transition metal centers, notably of Ni(Fe)S; (f) propensity to engage in organo–nickel reactions instead of carbanion condensations, (g) acyl-nickel activation

instead of thioester activation; and (h) C_2-extensions by acetylene ligands instead of C_1-extensions by CO_2, with the consequence of a lessened number of required reaction steps. Our findings may be seen as a hint to the evolution of extant carbon fixation cycles through the suggestion of replacing them through a linear reaction system with the inherent possibility of evolving cyclic reaction systems.

2. Materials and Methods

All chemicals were purchased from Sigma Aldrich GmbH (D-Steinheim) in the highest purity available. Acetylene was purchased from Linde AG (D-Pullach), carbon monoxide 2.5 and argon 4.6 were purchased from Westfalen AG (D-Münster), and ^{13}CO was purchased from Cambridge Isotopes Laboratories Inc. (Tewksbury, MA, USA).

In a typical run, a 125 mL glass serum bottle was charged with 0.5 or 1.0 mmol $NiSO_4 \bullet 6H_2O$ and closed with a silicon stopper. Additionally, 0.5 mmol β-$Ni(OH)_2$ or 0.5 mmol $FeSO_4 \bullet 7\ H_2O$ was charged in run B or D (Table 1), respectively. To achieve a constant ion strength, run B was supplemented with 0.5 mmol Na_2SO_4. The bottle was evacuated three times and filled with argon, finally ending in a deaerated state. Subsequently, the bottle was charged with argon-saturated water (calculated for a final volume of 5 mL), with 0.5 or 1.0 mL argon-saturated 1M Na_2S solution, with 0.5 mL 1M NaOH solution, and finally with 60 mL of CO and 60 mL of acetylene, using gas-tight syringes for injection. For consecutive reactions, the conditions of run A (see Table 1) were applied, replacing acetylene by 0.5 mmol of the indicated substrates and 60 mL of CO. To confirm the authenticity of the products, ^{13}CO or D_2O were used in otherwise identical experiments. Reactions were carried out at 105 °C. After 7 days, the reaction mixture was allowed to cool down and was centrifuged at 10,000 rpm for 5 minutes. The pH was measured by a glass electrode, and 1 ml of the supernatant was freeze-dried.

For analysis by gas chromatography-mass spectrometry (GC–MS), the residue was dissolved in 250 µL of anhydrous acetonitrile and derivatized with 250 µL of *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) for 30 minutes at 70 °C. For the detection of pyruvate, another ml was freeze dried, and the residue was shaken at 40 °C for 90 min in 250 µL of pyridine containing 5 mg of *O*-methylhydroxylamine hydrochloride. Afterwards, 250 µl of MTBSTFA were added, and the solution was kept at 70 °C for 30 min. The analysis of the silylated products was performed with GC–MS using GC-2010, coupled with MS-QP2010, Plus (Shimadzu GmbH, D-Duisburg) with a 30 m × 0.25 mm × 0.25 µm fused silica capillary column (Equity TM5, Supelco, USA-PA-Bellefonte) and an AOC-20i auto injector. Temperature program and settings:

Program 1 (used for mono silylated products): 0–6 min at 60 °C; 6–25 min at 60–280 °C, 10 °C/min; 25–28 min at 280 °C; injector temperature: 260 °C; detector temperature: 260 °C; column flow rate: 1 mL/min; scan interval: 0.5 sec; and injection volume 0.2 µL.

Program 2 (used for multiple silylated products): 0–6 min at 90 °C; 6–25 min at 90–280 °C, 10 °C/min; and 25–28 min at 280 °C. Otherwise, identical to program 1, with an injection volume of 1 µL. Peak assignment was achieved by a comparison of the retention times and mass spectra of purchased reference compounds, as well as data from the National Institute of Standards and Technology (NIST) spectra library. Quantification was performed by external calibration using known concentrations of commercially-available reference compounds.

In additional experiments for the formation of the thioacetic acid S-methyl ester (methyl thioacetate), a 125 ml serum bottle was charged with 2.0 mmol $NiSO_4 \bullet 6H_2O$, closed with a silicon stopper, and deaerated as described above. Subsequently, 1.5 ml of 1M Na_2S, 0.6 mL of 1M NaOH, 7.9 mL of H_2O, 25 mL of CH_3SH, 90 mL of HC≡CH (Table S2 run A), or 45 mL of HC≡CH plus 45 mL of CO (Table S2 run B) were added. Additional runs were performed with ^{13}CO or deuterated educts. Reactions were carried out at 105 °C. After one day, the reaction mixture was allowed to cool down. For the isolation of methyl thioacetate, 8 ml of the reaction mixture were extracted with 3 mL of ethyl acetate. The organic phase was dried over Na_2SO_4 and analyzed with GC–MS as described above, using an initial oven temperature of 40 °C. The injection volume was 1 µL. Methyl thioacetate showed a retention time of 2.9 min.

3. Results

We reacted acetylene and carbon monoxide under fastidiously anaerobic, aqueous conditions at the hydrothermally plausible temperature of 105 °C in the presence of NiS, which was precipitated in situ from Ni_2SO_4 with Na_2S, in the presence or absence β-$Ni(OH)_2$. We obtained highly functionalized C_2–C_4 products, which are typical for extant carbon fixation cycles (Table 1). Runs with ^{13}CO and D_2O ascertained that the products were genuine reaction products [8]. For these and further quantified products, GC–MS fragmentations and their isotopic labelling indices are listed in Table S1.

Table 1. Metabolic products of the nickel-catalyzed reaction of acetylene with carbon monoxide. Reactions were carried out in 125 mL serum bottles with 5 mL of aqueous liquid phase for 7 days at 105 °C; products were identified by GC–MS as tert-butyldimethylsilyl derivatives. n.d.: not detected.

Runs		**A**	**B**	**C**	**D**
$NiSO_4 \bullet 6\ H_2O$ (mmol)		1.0	0.5	-	0.5
$FeSO_4 \bullet 7\ H_2O$ (mmol)		-	-	-	0.5
β-$Ni(OH)_2$ (mmol)		-	0.5	1.0	-
Na_2SO_4 (mmol)		-	0.5	1.0	-
$Na_2S \bullet 9\ H_2O$ (mmol)		1.0	0.5	-	1.0
NaOH (mmol)		0.5	0.5	0.5	0.5
CO (ml)		60	60	60	60
C_2H_2 (ml)		60	60	60	60
pH end		8.0	8.1	9.8	8.5
Products (μM)	**Chemical formula**				
C1					
formate	$HCOO^-$	**18983**	**24207**	**310**	**434**
C2					
acetate	CH_3COO^-	**4358**	**3434**	**112**	**749**
glycolate	$HOCH_2COO^-$	**32**	**38**	**n.d.**	**11**
C3					
acrylate	CH_2CHCOO^-	**9692**	**16874**	**243**	**763**
propionate	$CH_3CH_2COO^-$	**10368**	**15021**	**171**	**339**
pyruvate	CH_3COCOO^-	**43**	**117**	**n.d.**	**4**
β-lactate	$HOCH_2CH_2COO^-$	**273**	**793**	**n.d.**	**n.d.**
glycerate	$HOCH_2CH_2(OH)COO^-$	**108**	**102**	**n.d.**	**n.d.**
C4					
crotonate	$CH_3CHCHCOO^-$	**226**	**516**	**n.d.**	**22**
2-methylmalonate	$^-OOCCH(CH_3)COO^-$	**48**	**145**	**n.d.**	**n.d.**
maleate	$^-OOCCHCHCOO^-$	**72**	**585**	**n.d.**	**14**
succinate	$^-OOCCH_2CH_2COO^-$	**3964**	**4747**	**3**	**187**
fumarate	$^-OOCCHCHCOO^-$	**358**	**391**	**n.d.**	**12**
malate	$^-OOCCH(OH)CH_2COO^-$	**17**	**85**	**n.d.**	**n.d.**
C5					
(E)-2-methylbut-2-enoate	$CH_3CHC(CH_3)COO^-$	**196**	**411**	**n.d.**	**n.d.**
∑ C2–C5		**29755**	**43259**	**358**	**2101**

The detected C_2-products acetate and glycolate did not show the ^{13}C-label in runs with ^{13}CO and therefore must have been the products of acetylene as the sole carbon source undergoing oxidative addition reactions. Ni^{2+} ions may have served as the required oxidant, as evidenced by Ni^0 particles that have previously been shown to form from NiS with CO as reductants under similar conditions [9]. In agreement with a previous proposal [10], the thioacetic acid S-methyl ester (methyl thioacetate) was formed (2μM in one day) by the reaction of acetylene with methanethiol. A shorter reaction time was

chosen due to the chemical instability of methyl thioacetate, which is readily hydrolyzed into acetic acid. Under the chosen conditions, CO did not operate as carbon source (Figure S1), but it enhanced the conversion of acetylene into methyl thioacetate, perhaps by ligand effects (Table 2). Methyl thioacetate can be seen as precursor of acetyl-CoA, which is formed by the reductive acetyl-CoA pathway in extant organisms [11]. In Figure S2, the extant acetyl-CoA pathway is compared to the here-described acetylene reaction. In earlier experiments, methyl thioacetate was found to form by the reaction of CO with methanethiol by Ni(Fe)S catalysis [12]. Methanethiol has been shown to form from CO with Ni(Fe)S/H_2S [12] or from CO_2 with FeS/H_2S [13].

Table 2. Formation of methyl thioacetate (thioacetic acid S-methyl ester; CH_3COSCH_3) from acetylene and methane thiol with or without carbon monoxide. Reactions were carried out in 125 ml serum bottles with 10 ml of aqueous liquid phase for 1 day at 105 °C; methyl thioacetate was identified by GC–MS after ethyl acetate extraction. Labelling in characteristic fragments is shown for runs with D_2O or ^{13}CO. n^ signifies D-labels, n• signifies n ^{13}C-labels.

Runs	A	B	Labelling in Characteristic Fragments	
			Mass 1	Mass 2
$NiSO_4$ • 6 H_2O (mmol)	2	2		
Na_2S • 9 H_2O (mmol)	1.5	1.5		
NaOH (mmol)	0.6	0.6		
C_2H_2 (ml)	90	45		
CO (ml)	-	45		
CH_3SH (ml)	25	25		
Methyl thioacetate (μM)	**2**	**4**	90_3^0•	43_3^0•

The formation of the detected organic >C_2-products required, not only acetylene but also CO as carbon source, as evidenced by the ^{13}C-labelling (Table S1). If NiS was precipitated in the presence of β-Ni$(OH)_2$, productivity increased significantly, notably from 20 to 32 mM for (acrylate and propionate) or from 4.4 to 5.7 mM for (fumarate, maleate, and succinate) (Table 1, runs A vs. B). The use of β-Ni$(OH)_2$ alone (Table 1, run C) or a mixed use of NiS/FeS (Table 1, run D) showed only minor product formation. The organic >C_2-products had the proper functional groups (COOH, CH=CH, CO, and CHOH) required for core metabolites, from which metabolic pathways could emanate. We detected a set of C_2–C_4 products (acetate, pyruvate, propionate, 3-hydroxy propionate, acrylate, malate, fumarate/maleate, succinate, crotonate, and methyl malonate) that were representative of the (hydrolyzed) constituents of the C_2–C_4-segments of the four extant central CO_2-fixation cycles of the domains Bacteria and Archaea. In Figure 1, the observed molecules are shown as co-radiating NiS-catalyzed products from acetylene and carbon monoxide, including possible interconversions in the same system. Through overlapping semicircles, these products are assigned to extant pathways. Figures S3–S6 show, in detail, the known carbon fixation cycles in which products from our abiotic system are highlighted by red boxes: the reductive tricarboxylic acid (rTCA) cycle [14] (Figure S3), the 3-hydroxypropionate–4-hydroxybutyrate (3HP–4HB) cycle [15] (Figure S4), the dicarboxylate-4–hydroxybutyrate (DC–4HB) cycle [16] (Figure S5), and the 3-hydroxypropionate (3HP) bicycle [17] (Figure S6). The rTCA cycle has been recognized as being autocatalytic for acetyl-CoA production [18]. The other three CO_2-fixation cycles are similarly autocatalytic for acetyl-CoA production and have been recognized for their importance in the evolution of metabolism [19–21]. Remarkably, we also found glycerate and (E)-2-methylbut-2-enoate as entry gates into carbohydrate and isoprenoid metabolisms (Table 1). In total, the here-described C_2–C_5 products summed up to a concentration of 43 mM (run B; Table 1) in the 5 ml setup, which corresponded to about 10% yield based on acetylene.

Concerning the question of the experimental interconversion of cycle constituents [22–24], we performed experiments with the replacement of acetylene by acrylate, fumarate, malate, and succinate as starting materials under otherwise identical conditions. The reaction products

of fumarate were succinate, malate and maleate. Acrylate reacted to propionate, β-lactate, succinate, fumarate, and malate. Malate formed maleate, succinate, and fumarate. Succinate remained mainly unchanged and showed only minor conversion to fumarate and malate (Table 3).

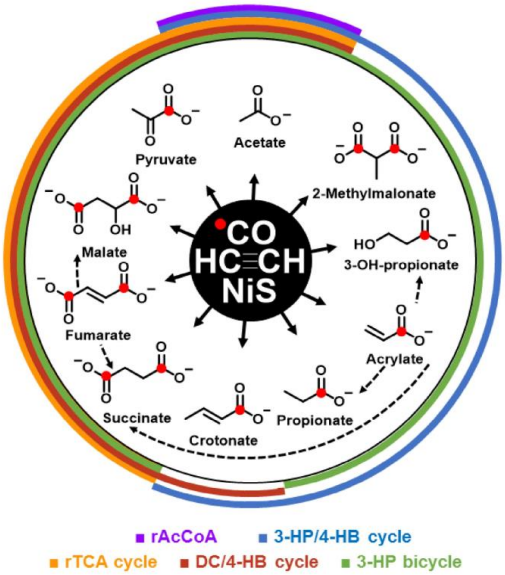


Figure 1. Acetyleno/carboxydotrophic reaction network. NiS-catalyzed reaction network starting from acetylene and carbon monoxide. Observed products are shown with their chemical formula and names; red dots indicate the observed ^{13}C label from ^{13}CO. Colored semi cycles signify the corresponding parts of the indicated carbon fixation pathways. (rAcCoA: reductive acetyl-CoA pathway; 3-HP/4-HB cycle: 3-hydroxpropinate/4-hydroxybutyrate cycle; rTCA: reductive tricarboxylic acid cycle; DC/4-HB cycle: dicarboxylate/4-hydroxbutyrate cycle; and 3-HP bicycle: 3-hydroxypropionate bicycle. Dotted arrows show observed interconversions between products of the co-radiating, linear pathways.

Table 3. Consecutive products from selected acids in the presence of CO. Reactions were carried out in 125 mL serum bottles with 5 mL of aqueous liquid phase and 120 ml of CO as gaseous phase for 7 days at 105 °C; further conditions are as described in run A of Table 1, replacing acetylene by 0.5 mmol of the indicated substrates. Products were identified by GC–MS as tert-butyldimethylsilyl derivatives.

Runs		**D**	**E**	**F**	**G**
Substrate		fumarate	malate	acrylate	succinate
Product (%)	**Chemical formula**				
acrylate	CH_2CHCOO^-	**n.d.**	**n.d.**	**6.37**	**n.d.**
propionate	$CH_3CH_2COO^-$	**n.d.**	**n.d.**	**6.80**	**n.d.**
β-lactate	$HOCH_2CH_2COO^-$	**n.d.**	**n.d.**	**39.21**	**n.d.**
maleate	$^-OOCCHCHCOO^-$	**2.57**	**0.02**	**0.31**	**n.d.**
succinate	$^-OOCCH_2CH_2COO^-$	**71.67**	**1.13**	**43.83**	**99.82**
fumarate	$^-OOCCHCHCOO^-$	**21.94**	**0.24**	**1.14**	**0.14**
malate	$^-OOCCH(OH)CH_2COO^-$	**3.70**	**98.58**	**2.36**	**0.04**

4. Discussion

The chosen reaction conditions (starting materials, catalysts and reaction parameters) are compatible with a variety of scenarios. They fit particularly well to submarine or terrestrial volcanic-

hydrothermal flow scenarios with late Hadean or early Archaean geochemistry. Acetylene is formed by simulating underwater volcanic activities [4], it is found in fumaroles [5] and on solar planets [6], and it is generated by the hydrolysis of calcium carbide (CaC_2), which, in turn, is formed by the magmatic reaction of calcium oxide with graphite [7]. Biochemically speaking, acetylene is mainly known as an inhibitor for enzymatic reactions [25] and as a substrate for acetylene hydratase, an FeS enzyme with a tungstopterin cofactor that functions biosynthetically [26], or as detoxifying enzyme [27], and it may be more widespread than previously suspected [28]. Early on, when acetylene abundance would have been greater than today, a precursor of extant acetylene hydratase may have functioned as enzyme for the oxidative addition of H_2O to acetylene to generate acetyl thioester and glycolate. As evidenced here, these reactions could have proceeded still earlier non-enzymatically in volcanic-hydrothermal vent scenarios. Carbon monoxide is found in volcanic exhalations. At low temperatures, the equilibrium $CO:CO_2$ molar ratio is low, but higher molar equilibrium ratios at high temperatures and pressures, e.g., 1:1 at 1200, °C and 2000, bar [29], could be conserved downstream by quenching [30]. Therefore, a mixture of acetylene and CO can be seen as a geochemically plausible carbon source for the synthesis of biomolecules under primordial conditions. Iron and nickel are the most abundant transition metals in the crust of the Earth [31], and iron–nickel sulfides are formed at the early stages of crustal evolution [32]. Nickel and iron–nickel centers are still widely spread in extant enzymes and catalyze a variety of reactions [33]. The here-described reactions showed a clear preference to NiS as catalyst, but for evolving further reaction cascades, e.g., reductive amination [34], a mixed FeS/NiS catalyst may be advantageous.

As initial interaction in the here-investigated acetylene/CO/NiS system, we suggest the coordination of acetylene and CO as ligands to Ni centers. The oxidation of the CO ligand to CO_2 would generate hydride ligands. As next stages, we propose end-on organo–metal adducts between acetylene and one or two Ni centers, hydride transfer, and carbonyl insertion to form highly energetic acyl-[Ni] intermediates [35], which may hydrolyze to carboxylic acids with a total loss of the organo–metal energy. Instead of hydrolysis to free acids, they may react with a mercaptan (or H_2S) to form thioesters (or thioacids) with partial energy conservation (Figure 2). The unsaturated carboxylates (acrylate and fumarate) that result from hydrolysis may subsequently convert by hydrogenation with CO as reductant to propionate and succinate. The addition of H_2O may lead to the formation of lactate and malate.

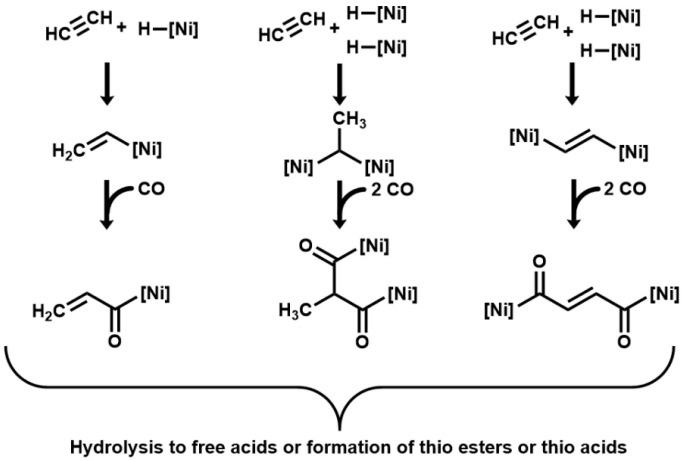


Figure 2. Proposed mechanism of acetylene/carboxydotrophic reactions on catalytic nickel centers. ([Ni] signifies a catalytic nickel center).

In the context of a volcanic hydrothermal flow setting, the continuous supply of starting materials permits a metabolism with linear carbon fixation pathways that co-radiate from the ligand sphere of NiS. The products of these radial, linear pathways would have operated as pre-established stepping stones for later piecemeal cyclization. Subsequently, a scarcity of starting materials would have been compensated for by a conversion to autocatalytic CO_2 fixation cycles, involving nutrient replacement, energy coupling, enzymatization, and the replacement of organo–metal activation by thioester activation without the violation of the principle of continuity. In our opinion, all extant carbon fixation cycles could be seen as successors of this primordial linear reaction system.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-1729/10/4/35/s1. Table S1. Metabolic products of the nickel-catalyzed reaction of acetylene with carbon monoxide. Figure S1. Formation of methyl thioacetate (thioacetic acid S-methyl ester) from HC≡CH and CH3SH in the presence of NiS. Figure S2. Comparison of the reductive acetyl-CoA pathway and the proposed primordial reaction mechanism to thioacetate. Figure S3. Reductive tricarboxylic acid cycle. Figure S4. 3-Hydroxpropinate/4-hydroxybutyrate cycle. Figure S5. Dicarboxylate/4-hydroxbutyrate cycle. Figure S6. 3-Hydroxypropionate bicycle.

Author Contributions: Conceptualization, C.H., W.E., and G.W.; data curation, J.S., T.G., C.M., and C.M.S.; funding acquisition, C.H.; investigation, J.S. and T.G.; methodology, J.S.; supervision, C.H.; visualization, T.G.; writing—original draft, C.H.; writing—review and editing, C.H., W.E. and G.W. All authors read, commented on and jointly approved submission of this article.

Funding: This research was funded by the Hans-Fischer-Gesellschaft (D-Munich) and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Project-ID 364653263—TRR 235.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Braakman, R.; Smith, E. The emergence and early evolution of biological carbon-fixation. *PLoS Comput. Biol.* **2012**, *8*, e1002455. [CrossRef] [PubMed]
2. Varma, S.J.; Muchowska, K.B.; Chatelain, P.; Moran, J. Native iron reduces CO_2 to intermediates and end-products of the acetyl-CoA pathway. *Nat. Ecol. Evol.* **2018**, 1019–1024. [CrossRef] [PubMed]
3. Preiner, M.; Igarashi, K.; Muchowska, K.B.; Yu, M.; Varma, S.J.; Kleinermanns, K.; Nobu, M.K.; Kamagata, Y.; Tüysüz, H.; Moran, J.; et al. A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism. *Nat. Ecol. Evol.* **2020**, *4*, 534–542. [CrossRef] [PubMed]
4. Mukhin, L.M. Volcanic processes and synthesis of simple organic compounds on primitive earth. *Orig. Life Evol. Biosph.* **1976**, *7*, 355–368. [CrossRef] [PubMed]
5. Igari, S.; Maekawa, T.; Sakata, S. Light hydrocarbons in fumarolic gases: A case study in the kakkonda geothermal area. *Chikyukagau* **2000**, *34*, 103–109.
6. Oremland, R.S.; Voytek, M.A. Acetylene as fast food: Implications for development of life on anoxic primordial earth and in the outer solar system. *Astrobiology* **2008**, *8*, 45–58. [CrossRef]
7. Hollemann, A.F.; Wiberg, E.; Wiberg, N. *Lehrbuch der Anorganischen Chemie*, 102nd ed.; Walter de Gryter: Berlin, Germany, 2007; pp. 1243–1247.
8. Geisberger, T.; Diederich, P.; Steiner, T.; Eisenreich, W.; Schmitt-Kopplin, P.; Huber, C. Evolutionary steps in the analytics of primordial metabolic evolution. *Life* **2019**, *9*, 50. [CrossRef]
9. Huber, C.; Kraus, F.; Hanzlik, M.; Eisenreich, W.; Wächtershäuser, G. Elements of metabolic evolution. *Chemistry* **2012**, *18*, 2063–2080. [CrossRef]
10. Scheidler, C.; Sobotta, J.; Eisenreich, W.; Wächtershäuser, G.; Huber, C. Unsaturated C-3,5,7,9-monocarboxylic acids by aqueous, one-pot carbon fixation: Possible relevance for the origin of life. *Sci. Rep.* **2016**, *6*. [CrossRef]
11. Ljungdahl, L.G.; Wood, H.G. Total synthesis of acetate from co_2 by heterotrophic bacteria. *Annu. Rev. Microbiol.* **1969**, *23*, 515–538. [CrossRef]
12. Huber, C.; Wächtershäuser, G. Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. *Science* **1997**, *276*, 245–247. [CrossRef] [PubMed]
13. Heinen, W.; Lauwers, A.M. Organic sulfur compounds resulting from the interaction of iron sulfide, hydrogen sulfide and carbon dioxide in an anaerobic aqueous environment. *Orig. Life Evol. Biosph.* **1996**, *26*, 131–150. [CrossRef] [PubMed]

14. Fuchs, G.; Stupperich, E.; Eden, G. Autotrophic CO_2 fixation in *Chlorobium limicola*. Evidence for the operation of a reductive tricarboxylic acid cycle in growing cells. *Arch. Microbiol.* **1980**, *128*, 64–71. [CrossRef]
15. Kockelkorn, D.; Fuchs, G. Malonic semialdehyde reductase, succinic semialdehyde reductase, and succinyl-coenzyme a reductase from *Metallosphaera sedula*: Enzymes of the autotrophic 3-Hydroxypropionate/4-Hydroxybutyrate cycle in sulfolobales. *J. Bacteriol.* **2009**, *191*, 6352. [CrossRef]
16. Huber, H.; Gallenberger, M.; Jahn, U.; Eylert, E.; Berg, I.A.; Kockelkorn, D.; Eisenreich, W.; Fuchs, G. A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic Archaeum *Ignicoccus hospitalis*. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7851–7856. [CrossRef]
17. Strauss, G.; Fuchs, G. Enzymes of a novel autotrophic CO_2 fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *Eur. J. Biochem.* **1993**, *215*, 633–643. [CrossRef]
18. Wächtershäuser, G. Evolution of the first metabolic cycles. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 200. [CrossRef]
19. Fuchs, G. Alternative pathways of carbon dioxide fixation: Insights into the early evolution of life? *Annu. Rev. Microbiol.* **2011**, *65*, 631–658. [CrossRef]
20. Berg, I.A. Ecological aspects of the distribution of different autotrophic CO_2 fixation pathways. *Appl. Environ. Microbiol.* **2011**, *77*, 1925–1936. [CrossRef]
21. Hügler, M.; Sievert, S.M. Beyond the calvin cycle: Autotrophic carbon fixation in the ocean. *Annu. Rev. Mar. Sci.* **2011**, *3*, 261–289. [CrossRef]
22. Muchowska, K.B.; Varma, S.J.; Chevallot-Beroux, E.; Lethuillier-Karl, L.; Li, G.; Moran, J. Metals promote sequences of the reverse krebs cycle. *Nat. Ecol. Evol.* **2017**, *1*, 1716–1721. [CrossRef]
23. Keller, M.A.; Kampjut, D.; Harrison, S.A.; Ralser, M. Sulfate radicals enable a non-enzymatic krebs cycle precursor. *Nat. Ecol. Evol.* **2017**, *1*, 83–91. [CrossRef] [PubMed]
24. Muchowska, K.B.; Varma, S.J.; Moran, J. Synthesis and breakdown of universal metabolic precursors promoted by iron. *Nature* **2019**, *569*, 104–107. [CrossRef] [PubMed]
25. Jensen, M.M.; Thamdrup, B.; Dalsgaard, T. Effects of specific inhibitors on anammox and denitrification in marine sediments. *Appl. Environ. Microbiol.* **2007**, *73*, 3151–3158. [CrossRef] [PubMed]
26. Schink, B.; Kroneck, P.M. Exploring the active site of the tungsten, iron-sulfur enzyme acetylene hydratase. *J. Bacteriol.* **2011**, *193*, 1229–1236. [CrossRef]
27. Rosner, B.M.; Schink, B. Purification and characterization of acetylene hydratase of *Pelobacter acetylenicus*, a tungsten iron-sulfur protein. *J. Bacteriol.* **1995**, *177*, 5767. [CrossRef]
28. Akob, D.M.; Sutton, J.M.; Fierst, J.L.; Haase, K.B.; Baesman, S.; Luther, G.W., III; Miller, L.G.; Oremland, R.S. Acetylenotrophy: A hidden but ubiquitous microbial metabolism? *FEMS Microbiol. Ecol.* **2018**, *94*, fiy103. [CrossRef]
29. Holloway, J.R.; Blank, J.G. Application of experimental results to coh species in natural melts. *Rev. Mineral.* **1994**, *30*, 187.
30. Wächtershäuser, G. On the chemistry and evolution of the pioneer organism. *Chem. Biodivers.* **2007**, *4*, 584–602. [CrossRef]
31. Cox, P.A. The elements. Their origin, abundance, and distribution. In *The Elements. Their Origin, Abundance, and Distribution*; Cox, P.A., Ed.; Oxford University Press: Oxford, UK, 1989.
32. Hazen, R.M. Evolution of minerals. *Sci. Am.* **2010**, *302*, 58–65. [CrossRef]
33. Boer, J.L.; Mulrooney, S.B.; Hausinger, R.P. Nickel-dependent metalloenzymes. *Arch. Biochem. Biophys.* **2014**, 142–152. [CrossRef] [PubMed]
34. Huber, C.; Wächtershäuser, G. Primordial reductive amination revisited. *Tetrahedron Lett.* **2003**, *44*, 1695–1697. [CrossRef]
35. Bernardi, F.; Bottoni, A.; Nicastro, M.; Rossi, I.; Novoa, J.; Prat, X. Theoretical study of the mechanism of carbonyl insertion reactions catalyzed by nickel complexes. *Organometallics* **2000**, *19*, 2170–2178. [CrossRef]

3.2 Summary and Article: Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth —Implications for Early Life on Extraterrestrial Planets?

The mission of the mars rover Curiosity showed the presence of thiophene on mars. Therefore, it was discussed as an eventual marker for biotic life, as degradation product. In this work we showed that thiophene and its derivatives are formed in abiotic reactions from acetylene and transition metal sulfides. The typical experiment was the same as in 3.1. Additionally, here we tested other transition metal sulfides and performed experiments to show the abundancy to the pH. The detection of thiophenes was performed by GC/MS. These conditions showed in other works of our group a great variety of organic molecules like fatty acids and other molecules of today's metabolic systems.

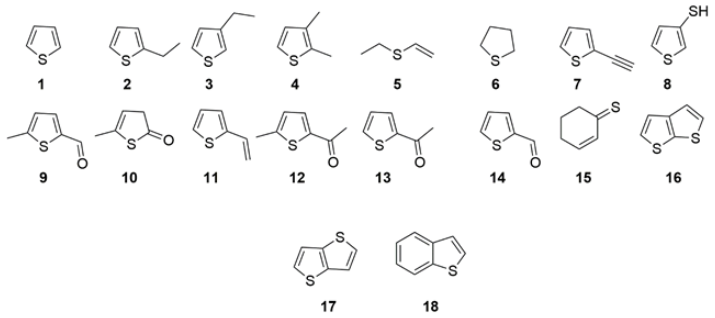


Figure 20: Thiophene (1) and its derivatives obtained from volcanic hydrothermal vent experiments. Adapted from (Geisberger et al., 2021)

Therefore, we suggested to see thiophene not only as a biomarker of degrading life, but also as a marker for sites and conditions well-suited for the emergence of life on earth and other extraterrestrial planets.

My individual contribution to this work included the conception and performance of the experiments. Further the analytics and interpretation of the obtained data, as well as the preparation of the published manuscript.

life

MDPI

Communication

Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth—Implications for Early Life on Extraterrestrial Planets?

Thomas Geisberger, Jessica Sobotta, Wolfgang Eisenreich and Claudia Huber *

Lehrstuhl für Biochemie, Department Chemie, Technische Universität München, Lichtenbergstraße 4, 85748 Garching, Germany; thomas.geisberger@tum.de (T.G.); Jessy.Sobotta@web.de (J.S.); wolfgang.eisenreich@mytum.de (W.E.)
* Correspondence: claudia.huber@tum.de

Abstract: Thiophene was detected on Mars during the Curiosity mission in 2018. The compound was even suggested as a biomarker due to its possible origin from diagenesis or pyrolysis of biological material. In the laboratory, thiophene can be synthesized at 400 °C by reacting acetylene and hydrogen sulfide on alumina. We here show that thiophene and thiophene derivatives are also formed abiotically from acetylene and transition metal sulfides such as NiS, CoS and FeS under simulated volcanic, hydrothermal conditions on Early Earth. Exactly the same conditions were reported earlier to have yielded a plethora of organic molecules including fatty acids and other components of extant metabolism. It is therefore tempting to suggest that thiophenes from abiotic formation could indicate sites and conditions well-suited for the evolution of metabolism and potentially for the origin-of-life on extraterrestrial planets.

Keywords: thiophene; acetylene; transition metal sulfides; hydrothermal conditions; early metabolism; origin-of-life

Citation: Geisberger, T.; Sobotta, J.; Eisenreich, W.; Huber, C. Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth—Implications for Early Life on Extraterrestrial Planets? *Life* **2021**, *11*, 149. https://doi.org/10.3390/life11020149

Received: 16 December 2020
Accepted: 12 February 2021
Published: 16 February 2021

1. Introduction

Recent findings by the Curiosity mission have shown the existence of thiophene and some of its derivatives on Mars [1,2]. Within this context, different possibilities for their abiotic as well as biotic formation were discussed. Thiophene was even suggested as a biomarker in the search for life on Mars [2], whereas a hydrothermal abiotic origin was also considered [1]. Likewise, thiophenes are common pyrolysis products from meteoritic macromolecular materials. For example, these compounds are produced by aqueous and/or thermal alteration of carbonaceous chondrites like the Murchison meteorite [3,4]. On Earth, thiophenes can be detected in volcanic gas discharges and in fluid emissions related to hydrothermal systems [5]. In submarine basins, like the Guaymas basin, thiophene derivatives can be detected after hydrothermal pyrolysis of organic material [6,7]. Furthermore, thiophenes are suggested to be at least a part of the organic sulfur found in the globules of 2.72 Ga years old stromatolites of the Tumbiana Formation. In the Western Australian Dresser Formation, stromatolites are dated back to 3.5 Ga and are considered as one of the most ancient traces of life on Earth [8,9]. On an industrial scale, thiophene is produced from butane and sulfur at 560 °C, from sodium succinate and phosphorous trisulfide, and from acetylene and hydrogen sulfide at 400 °C on alumina [10].

We have shown earlier that acetylene is also an excellent source for primordial carbon fixation, especially in combination with carbon monoxide, e.g., for the synthesis of short chain fatty acids [11] and intermediates of extant carbon fixation cycles [12]. On Earth, acetylene is present in fumarolic exhalations [13]. It can also be found extra-terrestrially, for example, on Saturn's moon Titan [14]. It was also proposed that explosive volcanism may have injected $\sim 6 \times 10^{12}$ g/year of acetylene into the atmosphere of early Mars [15].

The importance of sulfides in an origin-of-life scenario is emphasized by Wächtershäuser's Iron-Sulfur-World hypothesis [16]. Based on this hypothesis, we here report the facile abiotic formation of thiophene and some of its derivatives from acetylene and metallo-sulfides, especially NiS, under aqueous conditions at 105 °C. In context with the formation of potential building units and reaction networks for the emergence of metabolism under the same conditions [11,12] and capitalizing on recent hypotheses [2], the detection of extraterrestrial or terrestrial thiophenes could therefore indeed be indicative of early metabolic evolution under chemoautotrophic conditions.

2. Materials and Methods

All chemicals were purchased from Sigma Aldrich GmbH (Steinheim, Germany) in the highest purity available. Acetylene 2.6 (acetone free) was purchased from Linde AG (Pullach, Germany), and CO 2.5 and argon 4.6 were purchased from Westfalen AG (Münster, Germany). In a typical run (run 1, Table 1), a 125 mL glass serum bottle was charged with 1.0 mmol $NiSO_4 \cdot 6H_2O$ and closed with a silicon stopper. The bottle was evacuated three times and filled with argon, finally resulting in a de-aerated state. Subsequently, the bottle was filled with 3.5 mL argon-saturated water (calculated for a final volume of 5 mL) to dissolve the $NiSO_4$ and with 1.0 mL argon-saturated 1 M Na_2S solution. In this mixture, a precipitate of black NiS is immediately formed due to its low solubility constant of 1×10^{-22} [17,18] in aqueous solution. Furthermore, the bottle was filled with 0.5 mL 1 M NaOH solution, and finally with 120 mL of acetylene gas using gas-tight syringes for injection. The freshly precipitated NiS acted as a putative transition metal catalyst for the reaction and the molar variations of Na_2S to $NiSO_4$ resulted in free sulfide ions in the solution. In runs 10–12, 17, and 20, a mixture of 60 mL CO and 60 mL acetylene was used as gaseous phase. Instead of $NiSO_4 \cdot 6H_2O$, runs 2, 11, and 14 were loaded with 1 mmol $FeSO_4 \cdot 7H_2O$ and runs 3, 12, and 15 were loaded with 1 mmol $CoSO_4 \cdot 7H_2O$. In run 9, $NiSO_4 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ were combined. Otherwise, the settings were identical to the above described procedure. Reactions were carried out at 105 °C. pH-Variations were achieved through the addition of 0.1–1.0 mL 1M H_2SO_4 or NaOH. For safety reasons (danger of explosion) and for technical reasons, the reactions were carried out at low pressure (1 bar) of acetylene. After 1 day (24 h) or 7 days, the reaction mixture was allowed to cool down and, after vigorous shaking, 1 mL was taken out and centrifuged at 10,000 rpm for 10 min. For the isolation of thiophenes, the supernatant and the solid residue were extracted separately with 1 mL ethyl acetate. The organic phases were dried over Na_2SO_4 and directly analyzed with gas chromatography-mass spectrometry (GC-MS). GC-MS analysis was performed with a GC-2010, coupled with MS-QP2010 Ultra (Shimadzu GmbH, Duisburg, Germany) with a 30 m × 0.25 mm × 0.25 µm fused silica capillary column (Equity TM5, Supelco, PA-Bellefonte, USA) and an AOC-20i auto injector. Temperature program and settings: 0–6 min at 40 °C; 6–25 min at 40–280 °C, 10 °C/min; injector temperature: 260 °C; detector temperature: 260 °C; column flow rate: 1 mL/min; scan interval: 0.5 sec; and injection volume 0.1 µL. For detection of thiophene derivatives, a larger injection volume of 3 µL was used. Peak assignment was achieved by comparison with the retention times and mass spectra of purchased reference compounds, as well as with data from the National Institute of Standards and Technology (NIST) spectral library. Thiophene showed a retention time of 3.7 min. Retention times for derivatives are given in Table S2. Quantification was performed by external calibration using known concentrations of thiophene. Runs without a transition metal compound or without acetylene were performed for comparison.

Table 1. Transition metal catalyzed formation of thiophene. Reactions were performed with 120 mL (5.36 mmol) or 60 mL (2.68 mmol) acetylene and freshly precipitated sulfides under aqueous conditions at 105 °C. $NiSO_4$, $CoSO_4$ and $FeSO_4$ were used as hydrates (see method section). Reactions were performed for 24 h (run 1–12) or 7 days (run 13–18). pH-Values were measured at the end of the reaction time. Run 1 was performed three times showing a representative standard deviation of 14%. Thiophene concentrations were given in mM for the separated organic extracts of supernatants and solid sulfides as well as total concentration in the 5 mL setups. Yields are given in mol% conversion based on acetylene.

Run	$NiSO_4$ (mmol)	$FeSO_4$ (mmol)	$CoSO_4$ (mmol)	Na_2S (mmol)	NaOH (mmol)	CO (mL)	C_2H_2 (mL)	pH_{end}	Extract Supernatant (mM)	Extract Solid (mM)	Total Conc. (mM)	Total Yield (%)
1	1			1	0.5	-	120	9.7	0.379	0.709	2.175	0.406
2	-	1	-	1	0.5	-	120	9.0	<0.001	<0.001	<0.001	<0.001
3	-	-	1	1	0.5	-	120	9.0	0.047	0.046	0.185	0.035
4	1	-	-	1	-	-	120	6.5	0.337	1.243	3.160	0.590
5	1	-	-	1.5	-	-	120	11.0	0.393	0.740	2.268	0.423
6	1	-	-	2	-	-	120	13.5	0.333	0.129	0.925	0.173
7	-	1	-	1.5	-	-	120	12.0	0.023	0.066	0.177	0.033
8	-	1	-	2	-	-	120	13.5	0.002	0.002	0.008	0.001
9	0.5	0.5	-	1	0.5	-	120	11.0	0.377	0.503	1.761	0.329
10	1	-	-	1	0.5	60	60	9.5	0.220	0.067	0.574	0.214
11	-	1	-	1	0.5	60	60	9.0	0.002	<0.001	0.004	0.001
12	-	-	1	1	0.5	60	60	9.5	0.075	0.476	1.103	0.412
13	1	-	-	1	0.5	-	120	10.1	0.135	0.618	1.505	0.281
14	-	1	-	1	0.5	-	120	8.5	0.001	0.001	0.003	0.001
15	-	-	1	1	0.5	-	120	8.7	0.023	0.012	0.070	0.013
16	1	-	-	1	-	-	120	7.1	0.040	0.468	1.015	0.190
17	1	-	-	1	0.5	60	60	7.8	0.225	0.213	0.877	0.327
18	-	1	-	1	0.5	60	60	7.6	0.000	0.001	0.002	0.001

3. Results

We reacted acetylene at 105 °C for one day or seven days under strictly anoxic aqueous conditions, with freshly precipitated nickel sulfide, iron sulfide, cobalt sulfide, or mixtures thereof. In some runs, CO was added additionally as another putative reactant. The pH values were measured at the end of the reaction time (Table 1). After the indicated periods, the reaction mixtures were separated by centrifugation into a clear liquid supernatant and a black solid residue. Supernatants and solid residue were extracted separately using ethyl acetate. These extracts were finally analyzed by GC-MS. In a blank run without any addition of transition metal, under otherwise identical conditions to run 1, no formation of thiophene or thiophene derivatives was observed. Run 1, using NiS as the sulfide compound and catalyst at pH 9.7, is defined as standard run and was performed three times showing the formation of 2.2 mM thiophene as a mean concentration with a representative standard deviation of 14%. Under these conditions, thiophene formation was observed in a broad pH range from pH 5 to pH 11. However, yields were pH dependent as shown in Figure 1 and Table S1, with a pH optimum in the neutral range. Up to 3 mM thiophene were detected at pH 6.5 (run 4). Thiophene was also formed in comparable amounts in runs 3 and 10, using CoS or a mixed FeS/NiS catalyst, whereas only low amounts of thiophene were formed in the presence of FeS alone (Table 1, runs 2,7,8, and 14; Figure 2). Interestingly, in one third of the reactions, the amount of thiophene in the residue was up to two times higher than in the corresponding supernatants (Table 1) which reflects a strong binding of thiophene to the metal sulfide surfaces. This led us to the question as to whether the sulfur in thiophene derives from the solid NiS or, alternatively, from free sulfide in the solution. We therefore increased the amount of Na_2S in runs 5–8. In run 5 which contained 0.5 mmol additional free sulfide, the amount of thiophene was not significantly changed (Table 1, run 5 vs. run 1). In run 6 with 1 mmol additional sulfide, the yield was diminished to one half (run 6 vs. run 1). This could again indicate that solid nickel sulfide served as the reacting agent and not the free sulfide, with a possible blockage of catalytic sites through excess sulfide. Otherwise, free sulfide ions shifted the pH to a more alkaline value which is less suited for thiophene formation (Figure 1). In the presence of FeS alone, only traces of thiophene were detected. In the presence of 0.5 mmol additional sulfide (run 7 vs. run 2), the thiophene yield was significantly enhanced, but again lowered in the presence of 1 mmol free sulfide (run 7 vs. 8). This could indicate a different reaction mechanism of FeS catalysis involving

free sulfide ions and, possibly, a different pH dependency compared to NiS catalysis. Next, we chose a longer reaction time of 7 days, in an attempt to estimate reaction kinetics. We observed that the elongation of the reaction time in the NiS/acetylene system from 24h to one week was not favorable for thiophene formation (run 1 vs. 13, 3 vs. 15, 4 vs. 16), whereas thiophene yields increased in the NiS/acetylene/CO system (run 10 vs. 13) and the FeS/acetylene system (run 2 vs. 14). This showed that the formation of thiophene is a complex process influenced by many parameters, involving also consecutive reactions to other products. In Table 1, yields in mol% conversion based on acetylene are given additionally to the measured concentrations. The maximum conversion rate of 0.59% is reached for the NiS experiment at a nearly neutral pH value (run 4). For safety reasons the reactions were carried out at low pressure (1 bar). At a high sub-seafloor pressure (maybe >1000 bar), yields would be increased because of negative volumes of reaction [19,20].

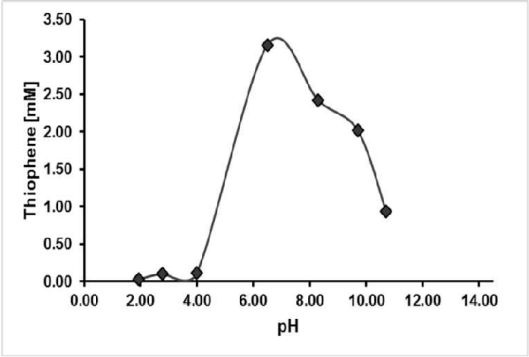


Figure 1. pH-Dependent formation of thiophene in the presence of NiS. Reactions were performed with 5.36 mmol acetylene and 1 mmol freshly precipitated nickel sulfide under aqueous conditions at 105 °C. Reactions were performed for 24 h and pH values were measured at the end of the reaction time.

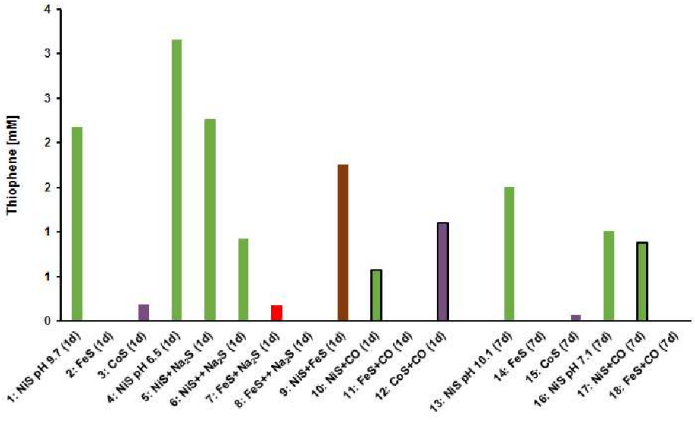


Figure 2. Total yield of thiophene formed in experiments as described in Table 1. Reactions were performed for 24 h (1 d) or 7 days (7 d). $+Na_2S/++Na_2S^{-}$ imply free sulfide ions because of a higher molar ratio of Na_2S to $NiSO_4$. Bars are colored according to the metal sulfide used: NiS: green; FeS: red; CoS: purple; NiS/FeS: brown; Runs with CO show black frames.

Further analysis by GC-MS led to the detection of several thiophene derivatives. Next to thiophene (1, Figure 3), 2-ethylthiophene (2), 3-ethylthiophene (3), 2,3-dimethylthiophene (4), ethyl-vinyl-sulfide (5), tetrahydrothiophene (6), 3-ethynylthiophene (7), 3-thiophenthiol (8), 5-methylthiophen-2-carboxaldehyde (9), 2[5H]-5-methylthiophenon (10), 2-vinylthiop hene (11), 2-acetyl-5 methyl-thiophene (12), 2-acetylthiophene (13), thiophen-2-carboxalde hyde (14), cyclohex-2-enthion (15), cis-1,4-dithiapentalene (16), trans-1,4-dithiapentalene (17) and benzo[b]thiophene (18) were observed in the extracts of the reaction mixtures at estimated concentrations of 0.01–0.03 mM (Figure 3, Table S2). Product identification was performed by comparison with commercially available standards and/or comparison to the NIST14 database (Figure S1 and Figure S2, in the supplementary materials). Individual amounts of each derivative were not calculated, but ratios of thiophene to the total amount of thiophene derivatives are given in Table S2. Reactions performed in the presence of NiS showed ratios from 1.0–17.9, whereas the highest ratio of 25.4 was observed in the reaction setting using a mixed NiS/FeS catalyst. Reactions performed in the presence of CoS showed ratios in the range of 0.3 to 2.8, indicating higher amounts of derivatives in comparison to NiS. The low ratios in FeS settings (<0.8) were due to the low amounts of thiophene found in these settings. The decrease of the ratio thiophene to thiophene derivatives by addition of CO in the presence of NiS (run 1 vs. 10) could indicate follow up reactions initiated by CO. The fact, that no thiophene derivatives were detected after 7 days in the presence of CO could indicate reaction or degradation steps towards products, which are not covered by our experimental setup.

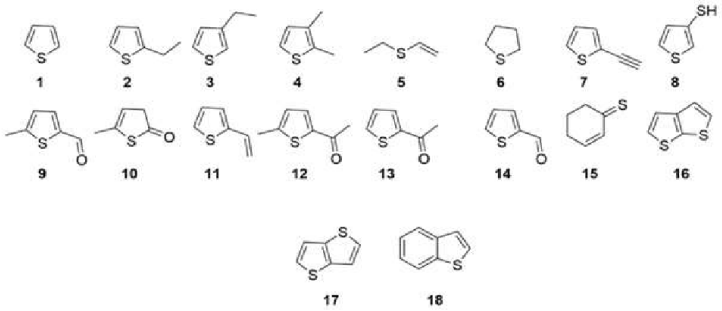


Figure 3. Thiophene and thiophene derivatives as formed from acetylene and nickel sulfide. Structures were verified by analytical standards and/or spectral libraries.

When thiophene was used as a starting material under conditions as described for run 11, tetrahydrothiophene (6), 2/3-ethylthiophene (1,2) and 5-methyl-thiophen-2-carboxaldehyde (9) were observed by GC-MS.

These findings demonstrate the formation of thiophene and its derivatives as products from acetylene and nickel sulfide under relatively mild hydrothermal conditions with the opportunity for further evolution. In Figure 4, a mechanism is proposed in analogy to the reaction of acetylene and hydrogen sulfide in super basic media [21]. In this scheme, the sulfur atom of NiS reacts with two molecules of acetylene in a concerted one-step mechanism. The so formed divinyl sulfide was not detected probably due to rapid conversion into by dehydrogenation to thiophene (1) or reduction to ethyl-vinyl-sulfide (5). Thiophene (1) could then react with further acetylene or sulfide to form 2-vinylthiophene (11), 2-ethylthiophene (2), 3-ethylthiophene (3) or 3-thiophenthiol (8). Further, it could be reduced to tetrahydrothiophene (6) or react with CO to form thiophen-2-carboxaldehyde (14). Additional experiments including stable isotope labelled precursors are required to unravel this mechanism in more detail. However, the various products observed under these conditions clearly imply that products downstream of thiophene are formed in a reaction network that could further evolve.

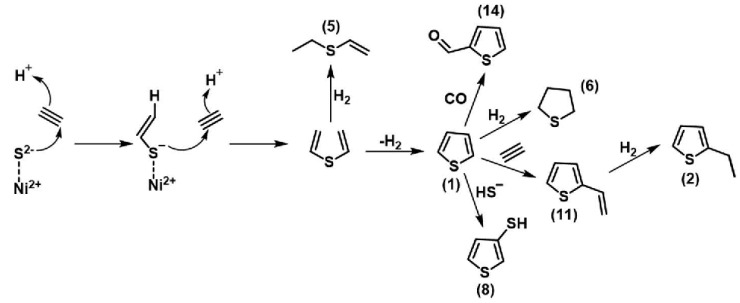


Figure 4. Hypothetical mechanism for the formation of thiophene and its derivatives from acetylene and nickel sulfide. ≡ signifies acetylene (C_2H_2).

4. Discussion

Chemical reactivity on Earth and Mars could be determined by metal sulfide catalysis, e.g., by FeS catalysis, since both planets contain high amounts of iron in their mantles [22]. Nickel and cobalt as members of the iron group are often found together with iron and are also of special interest as catalysts in the "iron–sulfur theory" for the origin-of-life [23]. Earth's core consists of 80–90% Fe-Ni alloys and 2.3wt% sulfur [24]. Fe-Ni sulfides are present on Earth as well as on Mars through ultramafic lava eruptions [25] and additional Ni is deposited on Mars through meteoritic impact [26]. On Earth, the formation of mixed NiFeS minerals, for example, of pentlandite $(FeNi)_9S_8$ and violarite $FeNi_2S_4$ is investigated in the context of serpentinization, a potential key process for metabolic evolution on early Earth [27].

In earlier work starting from acetylene, CO or cyanide under simulated hydrothermal conditions, we showed that nickel, especially NiS, is a potent catalyst for the formation of organic molecules, such as fatty acids, intermediates serving in biological carbon fixation and amino acids [11,12,28]. As we can now show, NiS catalyzes additionally the formation of thiophene from acetylene. The observed low thiophene formation with FeS underlines the importance of nickel minerals in this context.

Organic sulfur compounds in general are indeed essential for life and play an important role in the sulfur cycle on Earth [29]. In addition to thiophene, methanethiol and carbonyl sulfide can be found in terrestrial hydrothermal exhalations and were also used for simulated primordial synthesis of biomolecules [23,30]. In the biological context, thiophene and its derivatives (such as benzo-thiophenes) are sometimes considered as secondary biomarkers, preserving the original n-alkane chain or carbon skeleton of biomolecules in sulfur-bound forms at different lithofacies [31]. Organic sulfur compounds are more stable under sulfide rich geological conditions than their biological precursors (e.g., functionalized lipids), and can therefore be found in ancient sedimentary rocks [32].

In extant biochemistry, thiophene is still conserved as a structural part of biotin, which is a prosthetic group for carboxylase classed enzymes, like the pyruvate carboxylase [33]. Furthermore, thiophenes can be found as structural components in the quinone fractions (e.g., caldariellaquinone—benzo[b]thiophene-4,7-quinone) of extreme thermophilic and acidophilic archaeons, like *Caldariella acidiphila* and *Sulfolobus solfataricus* [34,35].

According to recent literature [7,36], thiophenes *per se* should not be named biomarkers, due to their possible abiotic origin, but the presence of thiophenes, as easily detectable molecules, could indicate samples or sites, which should be investigated in more detail for the presence of additional organic molecules like amino acids or fatty acids. It is also suggested that terrestrial origin-of-life conditions could be used as a guideline in the search for life on extraterrestrial planets [37].

5. Conclusions

We here could show the abiotic formation of thiophenes from acetylene and transition metal sulfides under aqueous conditions, which we consider as a valid simulation of volcanic hydrothermal settings on early Earth, but also on other planets. Under identical conditions, we could previously demonstrate the conversion of acetylene and CO into short chain fatty acids (C_3–C_9) and other C_2–C_4 compounds including metabolic intermediates of carbon fixation in extant life [9,10]. These compounds are also considered as important precursors for a potential chemo-autotrophic origin-of-life. It is therefore tempting to speculate that the detection of thiophenes on planets reflects possible habitats for the early emergence and evolution of metabolism and life.

Supplementary Materials: The following are available online at https://www.mdpi.com/2075-1729/11/2/149/s1, Table S1: pH dependent formation of thiophene in the presence of NiS. Table S2: Identified thiophene derivatives and their retention times. Figure S1: GC/MS chromatograms comparing thiophene and commercially available thiophene derivatives, Figure S2: GC/MS mass spectra comparing reaction products to commercially available thiophene standards and mass spectra from NIST14 library.

Author Contributions: Conceptualization, C.H., W.E; data curation, T.G.; funding acquisition, C.H.; methodology, J.S., T.G.; supervision, C.H., W.E.; visualization, T.G.; writing—original draft, C.H.; writing—review and editing, T.G., C.H. and W.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Project-ID 364653263—TRR 235 and the Hans-Fischer-Gesellschaft (Munich, Germany).

Acknowledgments: We thank Günter Wächtershäuser for continuous support and valuable discussions and we thank Felicia Achatz for practical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Eigenbrode, J.L.; Summons, R.E.; Steele, A.; Freissinet, C.; Millan, M.; Navarro-González, R.; Sutter, B.; McAdam, A.C.; Franz, H.B.; Glavin, D.P. Organic matter preserved in 3-billion-year-old mudstones at Gale crater, Mars. *Science* **2018**, *360*, 1096–1101. [CrossRef]
2. Heinz, J.; Schulze-Makuch, D. Thiophenes on Mars: Biotic or Abiotic Origin? *Astrobiology* **2020**, *20*, 552–561. [CrossRef]
3. Ehrenfreund, P.; Sephton, M.A. Carbon molecules in space: From astrochemistry to astrobiology. *Faraday Discuss.* **2006**, *133*, 277–288. [CrossRef]
4. Sephton, M.A. Organic compounds in carbonaceous meteorites. *Nat. Prod. Rep.* **2002**, *19*, 292–311. [CrossRef] [PubMed]
5. Tassi, F.; Montegrossi, G.; Capecchiacci, F.; Vaselli, O. Origin and distribution of thiophenes and furans in gas discharges from active volcanoes and geothermal systems. *Int. J. Mol. Sci.* **2010**, *11*, 1434–1457. [CrossRef] [PubMed]
6. Kawaka, O.E.; Simoneit, B.R.T. Hydrothermal pyrolysis of organic matter in Guaymas Basin: I. Comparison of hydrocarbon distributions in subsurface sediments and seabed petroleums. *Org. Geochem.* **1994**, *22*, 947–978. [CrossRef]
7. Simoneit, B.R.T. A review of current applications of mass spectrometry for biomarker/molecular tracer elucidation. *Mass Spectrom. Rev.* **2005**, *24*, 719–765. [CrossRef]
8. Lepot, K.; Benzerara, K.; Rividi, N.; Cotte, M.; Brown, G.E.; Philippot, P. Organic matter heterogeneities in 2.72 Ga stromatolites: Alteration versus preservation by sulfur incorporation. *Geochim. Cosmochim. Acta* **2009**, *73*, 6579–6599. [CrossRef]
9. Van Kranendonk, M.J.; Philippot, P.; Lepot, K.; Bodorkos, S.; Pirajno, F. Geological setting of Earth's oldest fossils in the ca. 3.5 Ga Dresser Formation, Pilbara Craton, Western Australia. *Precambrian Res.* **2008**, *167*, 93–124. [CrossRef]
10. Mishra, R.; Jha, K.K.; Kumar, S.; Tomer, I. Synthesis, properties and biological activity of thiophene: A review. *Der Pharma Chem.* **2011**, *3*, 17.
11. Scheidler, C.; Sobotta, J.; Eisenreich, W.; Wächtershäuser, G.; Huber, C. Unsaturated C-3,C-5,C-7,C-9-Monocarboxylic Acids by Aqueous, One-Pot Carbon Fixation: Possible Relevance for the Origin of Life. *Sci. Rep.* **2016**, *6*, 27595. [CrossRef] [PubMed]
12. Sobotta, J.; Geisberger, T.; Moosmann, C.; Scheidler, C.M.; Eisenreich, W.; Wächtershäuser, G.; Huber, C. A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism. *Life* **2020**, *10*, 35. [CrossRef]
13. Igari, S.; Maekawa, T.; Sakata, S. Light hydrocarbons in fumarolic gases: A case study in the Kakkonda geothermal area. *Chikyukagau* **2000**, *34*, 7.

14. Singh, S.; McCord, T.B.; Combe, J.P.; Rodriguez, S.; Cornet, T.; Mouélic, S.L.; Clark, R.N.; Maltagliati, L.; Chevrier, V.F. Acetylene on Titans surface. *Astrophys. J.* **2016**, *828*, 55. [CrossRef]
15. Segura, A.; Navarro-Gonzalez, R. Production of low molecular weight hydrocarbons by volcanic eruptions on early Mars. *Orig. Life Evol. Biosph.* **2005**, *35*, 477–487. [CrossRef] [PubMed]
16. Wächtershäuser, G. Groundworks for an evolutionary biochemistry: The iron-sulphur world. *Prog. Biophys. Mol. Biol.* **1992**, *58*, 85–201.
17. Hollemann, A.F.; Wiberg, N. *Lehrbuch der Anorganischen Chemie*, 101st ed.; Walter de Gruyter: Berlin, Germany, 1995; p. 1582.
18. Sillen, L.G.; Martell, A.E. Stability Constants of Metal-Ion Complexes. In *Lange's Handbook*; Special Publ. No. 17; The Chemical Society: London, UK, 1964; pp. 8-6–8-11.
19. Matsumoto, K.; Sera, A.; Uchida, T. Organic Synthesis under high pressure. *Synthesis* **1985**, *18*, 1–26. [CrossRef]
20. Klärner, F.-G.; Wurche, F. The effect of pressure on organic reactions. *J. Prakt. Chem.* **2000**, *342*, 609–636.
21. Trofimov, B.A. New Reactions and Chemicals Based on Sulfur and Acetylene. *Sulfur. Rep.* **1983**, *3*, 83–114. [CrossRef]
22. Halliday, A.N.; Wänke, H.; Birck, J.-L.; Clayton, R.N. The accretion, composition and early differentiation of Mars. *Space Sci. Rev.* **2001**, *96*, 197–230.
23. Huber, C.; Wächtershäuser, G. Activated Acetic Acid by Carbon Fixation on (Fe,Ni)S Under Primordial Conditions. *Science* **1997**, *276*, 245–247. [CrossRef]
24. Allegre, C.J.; Poirier, J.-P.; Humler, E.; Hofmann, A.W. The chemical composition of the Earth. Earth Planet. *Sci. Lett.* **1995**, *134*, 515–526.
25. Burns, R.G.; Fisher, D.S. Evolution of sulfide mineralization on Mars. *J. Geophys. Res. Solid Earth* **1990**, *95*, 14169–14173. [CrossRef]
26. Yen, A.S.; Mittlefehldt, D.W.; McLennan, S.M.; Gellert, R.; Bell, J.F.; McSween, H.Y.; Ming, D.W.; McCoy, T.J.; Morris, R.V.; Golombek, M. Nickel on Mars: Constraints on meteoritic material at the surface. *J. Geophys. Res.* **2006**, *111*, E12S11. [CrossRef]
27. Klein, F.; Bach, W. Fe–Ni–Co–O–S Phase Relations in Peridotite–Seawater Interactions. *J. Petrol.* **2009**, *50*, 37–59. [CrossRef]
28. Huber, C.; Kraus, F.; Hanzlik, M.; Eisenreich, W.; Wächtershäuser, G. Elements of metabolic evolution. *Chemistry* **2012**, *18*, 2063–2080. [CrossRef] [PubMed]
29. Kertesz, M.A. Riding the sulfur cycle–metabolism of sulfonates and sulfate esters in Gram-negative bacteria. *FEMS Microbiol. Rev.* **2000**, *24*, 135–175.
30. Leman, L.J.; Orgel, L.E.; Ghadiri, M.R. Carbonyl sulfide-mediated prebiotic formation of peptides. *Science* **2004**, *306*, 4. [CrossRef] [PubMed]
31. Van Kaam-Peters, H.M.; Rijpstra, W.I.C.; De Leeuw, J.W.; Damsté, J.S.S. A high resolution biomarker study of different lithofacies of organic sulfur-rich carbonate rocks of a Kimmeridgian lagoon (French southern Jura). *Org. Geochem.* **1998**, *28*, 151–177. [CrossRef]
32. Sinninghe Damste, J.S.; Rijpstra, W.I.C.; Kock-van Dalen, A.C.; de Leeuw, J.W.; Schenck, P.A. Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. *Geochim. Cosmochim. Acta* **1989**, *53*, 13. [CrossRef]
33. Jitrapakdee, S.; Wallace, J.C. Structure, function and regulation of pyruvate carboxylase. *Biochem. J.* **1999**, *340*, 1–16. [CrossRef]
34. Lanzotti, V.; Trincone, A.; Gambacorta, A.; De Rosa, M.; Breitmaier, E. 1H and 13C NMR assignment of benzothiophenquinones from the sulfur-oxidizing archaebacterium Sulfolobus solfataricus. *FEBS J.* **1986**, *160*, 37–40. [CrossRef] [PubMed]
35. Zhou, D.; White, R. Biosynthesis of caldariellaquinone in Sulfolobus spp. *J. Bacteriol.* **1989**, *171*, 6610–6616. [CrossRef] [PubMed]
36. Simoneit, B.R.T. Prebiotic organic synthesis under hydrothermal conditions: An overview. *Adv. Space Res.* **2004**, *33*, 88–94. [CrossRef]
37. Longo, A.; Damer, B. Factoring Origin of Life Hypotheses into the Search for Life in the Solar System and Beyond. *Life* **2020**, *10*, 52. [CrossRef]

4 Conclusion and Outlook

In this work we made one more step towards the understanding of the metabolic carbon fixation of the pioneer organism. By looking on the similarities of extant CO_2-fixation pathways and the gained knowledge of hydrothermal vent experiments. There was considerable process made in the field of the habitability of certain niches for the emergence of life. Experiments looking on thiophene showed, that it is not only a marker for possible decay of organic life. Further it can also be seen as a marker for habitable zones to support the origin of life. In the following chapter unpublished results of ongoing studies and their preliminary results are briefly discussed, but not disclosed.

4.1 Elements of metabolic evolution

Mild amino acid synthesis — The synthesis of α-amino acids was shown by our group before (Huber & Wächtershäuser, 2006). In this reactions CO as well as CN^- are used as carbon source to form amino acids under primordial conditions. Further they showed that it is possible to from amino acids from their corresponding α-hydroxy acids (Huber & Wächtershäuser, 2003). The method of amination was far more mild in contrast to CN^-, by using NH_4Cl or NH_4CH_3. Therefore, the design of the new experiment was to implement the milder amination into the reaction, to form organic molecules. Here we started to add NH_4Cl directly to our reaction mixtures. But the focus was not only find amino acids. We also tried to find the corresponding amines of short chain fatty acids that evolve in our experiments like acrylic acid. The finding of amides where not fully positive to this time, thus with the new system we could show the emergence of alanine, glycine and aspartic acid. Also the formation of peptide bonds is a subject of this reactions with phenylalanine, tyrosine as promising candidates. The finding of serine in this experiments would be a good thing. Following the concepts of the iron sulfur world by Günter Wächtershäuser, serine would be the hydroxylated form of cysteine. This would be promising because it shows the presence of cysteine, which is the step stone for interconnection between different peptide chains.

Mechanisms of building metabolites — A very good way to verify the reaction products resulting in our experiments is the labeling with stable isotopes like ^{13}C. The addition was first only *via* ^{13}CO. This not only verified the reaction products as genuine products of the reaction, it further gives information about the structure and the location of the labeled carbon. Here it showed that CO is a starting point to build up for further elongation of the formed molecules. Solely in uneven fatty acids or di acids a label could be found. Further in the metabolites formic acid and pyruvic acid occurred a specialty. In this molecules the labeling was formal just 0.5, which means that the portion of labeled and unlabeled material was even after the experiment. So, acetylene the second carbon containing molecule gained attention. There were experiments with $^{13}C_2$-acethylene. This verified what was already thought. The carbon chain of the uneven fatty acids and molecules is built of acetylene. More interesting is that the even fatty acids are completely labeled in experiments only using $^{13}C_2H_2$. From this point a hypothetical mechanism can be formulated. The acetylene is coordinated to the NiS-surface *via* the [Ni]—H (activated nickel surface). At this stage an elongation, functionalization or reduction is taking place. Followed by an insertion of CO. By hydroxylation, the molecule is released as a free acid (Sobotta *et al.*, 2020). Still there is the mystery of the 0.5 labeled constituents of the reactions. The labeling with acetylene showed exactly the same picture as the labeling with CO. This shows that both formic acid and pyruvic acid can be build up completely by CO or C_2H_2, respectively for pyruvate with or without CO. But both molecules have an uneven number of carbons, with formic acid being a C_1-body and pyruvic acid a C_3-body. In this case there must be a mechanism to cleave the C—C bond in the acetylene to form the C_1-bodys. This must be a multistep reaction because it involves reduction steps followed by oxidations. A hypothetical mechanism for this reaction is shown in Figure 21:

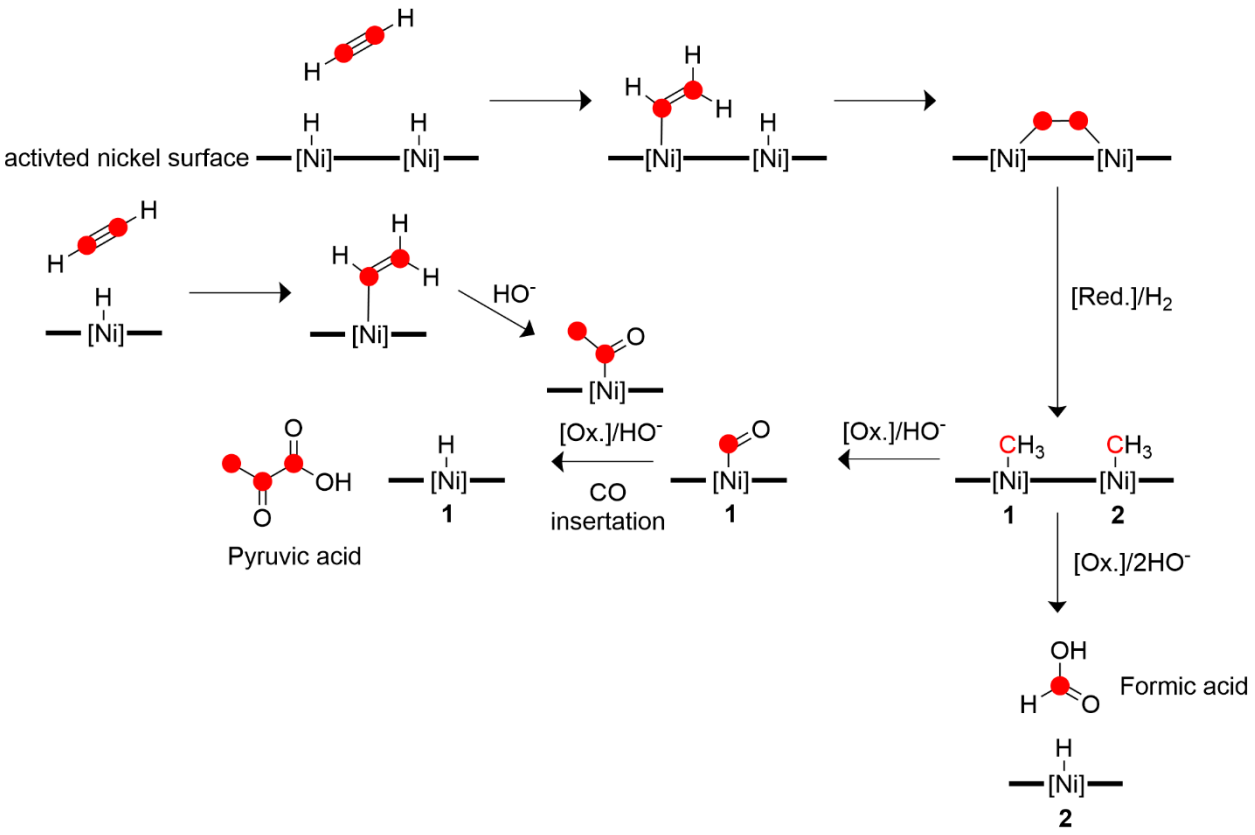


Figure 21: Hypothetical mechanism showing the cleavage of acetylene on a catalytic nickel surface. Further the reaction of the cleaved acetylene to formic acid and pyruvic acid. The numbers 1 and 2 are indicating the two nickel centers of the cleaved acetylene. Red dots and red colored C indicate a ^{13}C isotope labeling. (unpublished data).

Exploring the reaction network — Together with the group of Prof. Schmitt-Kopplin, from the Helmholtz Zentrum Munich, we are investigating in the reaction networks of our volcanic hydrothermal vent experiment. An interesting approach to the network is the timeline of the emergence of molecules in the reaction. A typical reaction vessel for the cooperation experiment was loaded with 262 mg $NiSO_4$. After that the reaction vessel was sealed and it was pumped to vacuum with a refill of argon. This procedure was repeated again for two times. Again it was pumped to vacuum. Then the liquids were added to the reaction mix, 8.5 ml H_2O, 1 ml Na_2S and 0.5 ml of NaOH (1M). The solved nickel ions and the sulfide form fresh precipitated NiS. Then CO and acetylene are filled into the vessel, each 60 ml. The reaction vessel is placed in an oven for seven days at 105 °C. The pressure will level out at 1 bar. The product mixture is analyzed my GC/MS

and NMR (TUM) and by FTICR/MS, LC-MS and NMR (Helmholtz). The resolution over time showed that acrylic acid is one of main components in the product mixture. The shift in time to a later point of time for e.g. propionic acid shows a dependency from acrylic acid as it is the reduced form of it. Another surprise of this timeline is the formation of succinic acid before its more functionalized analogues fumaric acid and maleic acid.

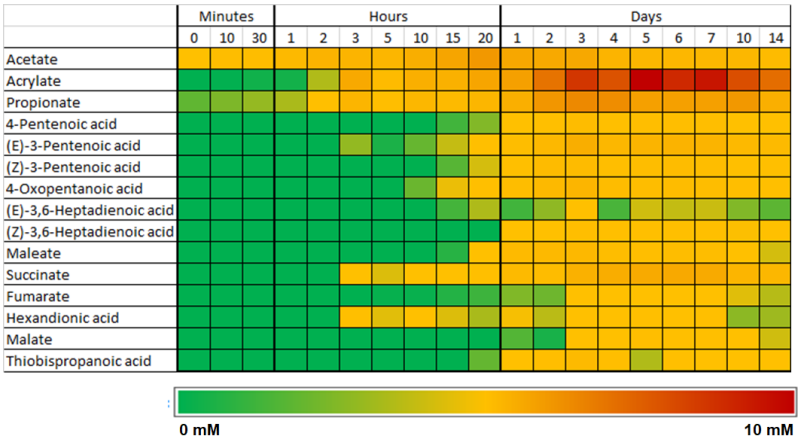


Figure 22: Resolution over time of assorted reaction products from volcanic hydrothermal vent experiments.

The Helmholtz-team works on the chemical space which is build up by a CHOS-system. This means a reaction network containing carbon, hydrogen, oxygen and sulfur. With mathematical features and FTICR/MS they can build a reaction network (Popova *et al.*, 2013; Schmitt-Kopplin *et al.*, 2010; Tziotis *et al.*, 2011).

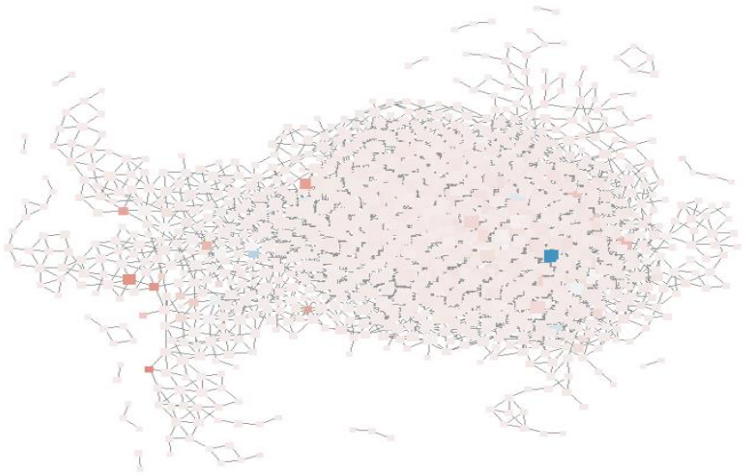


Figure 23: Reaction network of the CHOS space of the reaction mix. Picture in courtesy of Philippe Diederich (unpublished data).

Volcanic lightning — A cooperation with the group of Prof. Scheu has the target to investigate the reactions implemented by volcanic lightning. During a volcanic eruption a high amount of material and gases is released in the plume. The mineral material is a very fine powder. Therefore, it is electrically charged through the friction of the material. This electrically charge is released as lightning (Springsklee *et al.*, 2020). The plume contains both porous material, as reaction chambers, and various gases. Additionally, there is also steam which acts as solvent when cooling. The volcanic lightning should be reproduced in lab scale (Cimarelli *et al.*, 2014; Gaudin & Cimarelli, 2019) to test various gas mixtures on their potential to create organic material. The material getting charged by a “eruption” are cleaned volcanic ashes. The final step of the cooperation would be to use the organic loaded ashes in volcanic hydrothermal vent experiments. In earlier unpublished experiments, there were traces of glycine and alanine detectable using an atmosphere of ammonia and methane. With the actual experiments using nitrogen and CO_2, no organics can be found (unpublished data).

4.2 Encapsulation with fatty acids

Building vesicles from experiments —Previous works of our group showed the tendency to build up fatty acids and di acids (Scheidler *et al.*, 2016; Sobotta *et al.*, 2020).

Therefore, a logic coincidence would be to have a look if there are micelles/vesicles in our experiments or if the organic mixture would be able to form vesicles under special circumstances. In the past there was already an experiment examined with a transmission electron microscope (TEM). Actually hydrophobic drop like compartments could be visualized using α-hemolysin, a hydrophobic protein core. The protein core would embed its self in the hydrophobic bilayer of a vesicle, making it visual in the microscope. Regarding the papers, mentioned above, the main constituents of the reactions are short to medium chained, mostly unsatisfied, fatty acids. Scheidler *et al.* (2016) specify the amount of $C_{3\text{-}9}$ to be around 19 mM with an end pH 8.8. They even obtained up to 24 mM when NiS is 0.5 mmol and 0.5 mmol β-$Ni(OH)_2$ is added, end pH was 8.9. The inside of a vesicle bilayer behaves like a fluid (Lindblom & Wennerström, 1977; Shinitzky *et al.*, 1971). Further, the creation of vesicles is promoted by unsatisfied chains of fatty acids (Meierhenrich *et al.*, 2010). Because of these findings the product mixture of a standard reaction (1 mmol $NiSO_4$, 1 mmol Na_2S, 0.5 mmol NaOH, 3,5 ml H_2O, 60 ml CO/C_2H_2 each) is subject to a deeper investigation on vesicles.

The literature states that octanoic acid is the shortest acid to form vesicles, when prepared (Apel *et al.*, 2002). Following the protocol from Apel *et al.* a comparative sample of fatty acid vesicles from nonanoic acid was produced to have a look at it with a TEM. The 85 mM nonanoic acid in water 5 ml water were alkalized with 1M NaOH to deprotonate the carbonic acid. In this state the acid will accumulate as micelles, showing a clear solution with no abundant fatty acid droplets or foam. After that the solution is acidified with 1M HCl to fully protonate the acid molecules. The solution got opalescent, indicating the emergence of vesicles. Figure 24 shows this control sample (unpublished data).

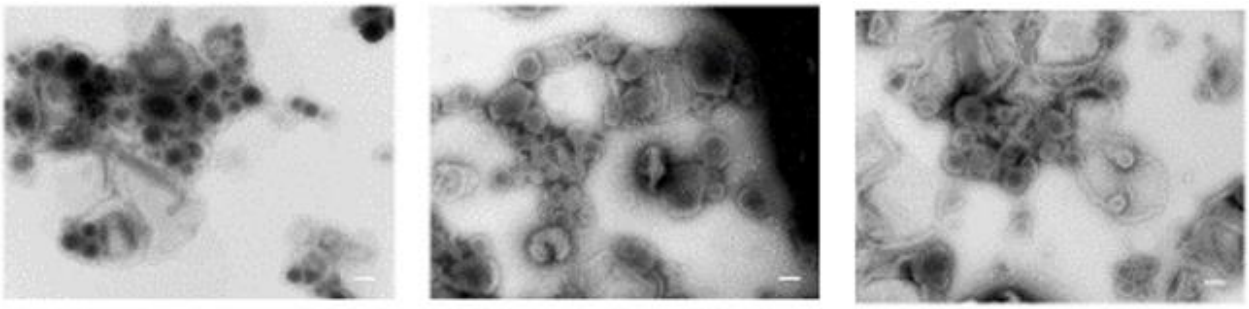


Figure 24: Control experiment (TEM) with 85 mM nonanoic acid, following the protocol from Apel *et al.* (2002). The scale bar represents 100 nm in all micrographs. Pictures in courtesy of Christoph Kaiser (unpublished data).

The pictures from the TEM showed multi-laminar vesicles formed from nonanoic acid. The reaction mixture needs to have at least a concentration of 85 mM for nonanoic acid or 250 mM for octanoic acid. But the cvc can be lowered when there are fatty alcohols and hydrocarbons in the mixture. Further the cvc can be lowered by adding longer chained fatty acids (Apel *et al.*, 2002; Cape *et al.*, 2011; Deamer, 1985). Mostly longer fatty acids as C_9 were not found in detectable amounts by GC/MS. The group of Prof. Schmitt-Kopplin could show *via* FTICR/MS that there are longer fatty acids in the product mixture. Figure 25 shows a van Krevelen diagram of a product mix measured by FTICR/MS (unpublished data).

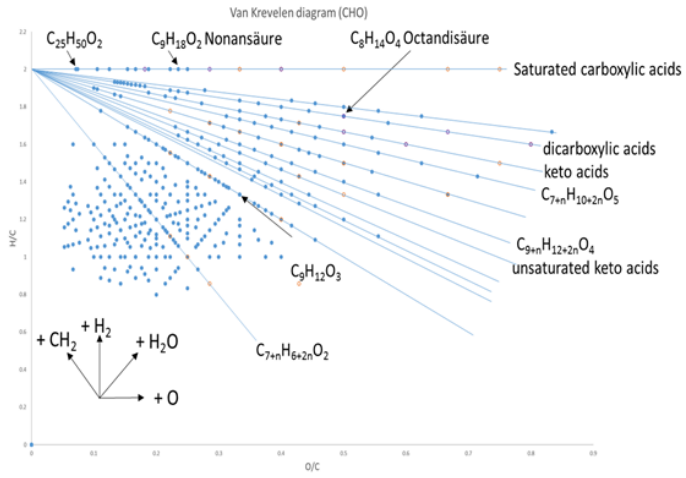


Figure 25: Van Krevelen diagram of a typical reaction run, showing the fatty acid moiety of the sample. Dots on lines indicating the same homologous series. Going left up indicating the addition of a CH_2-group. Picture in courtesy of Philippe Diederich (unpublished data)

The investigation of vesicles to be found free in the reaction solution was not positive by TEM. So the procedure was adapted to focus on the building of vesicles from the organic moiety of the reaction. In a volcanic hydrothermal vent system there is the possibility to get wet and dry cycles in the cavities of the sounding mineral rocks (Ross & Deamer, 2016). During a possible dry phase, the fatty acids will form a monolayer on the surface of the minerals. With a rehydration of the cavity the fatty acids will form vesicles, some of these can encapsulate mineral structures. With this happening a catalytic structure could be transported into a vesicle (Hanczyc *et al.*, 2007). Further there could also be the lipophilization of a catalytic particle, ending in a complete cellularization of the particle (Wächtershäuser, 1992, 2006). Figure 26 shows the hypothetical process of building up a bilayer vesicle around a catalytic NiS particle (unpublished data).

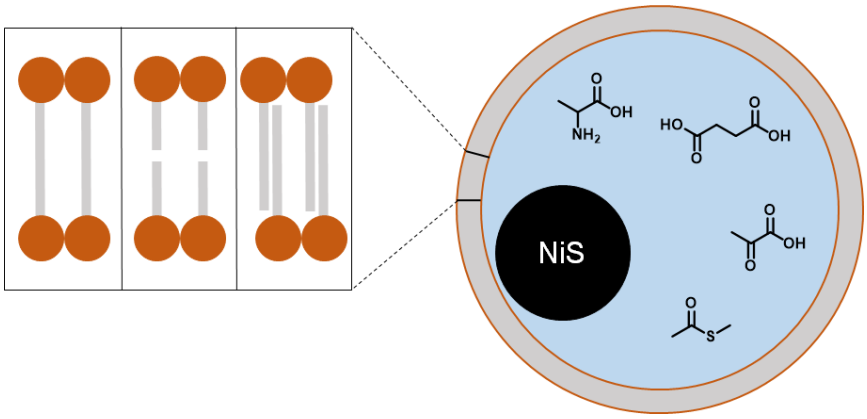


Figure 26: Hypothetical fatty acid vesicle grown around of a nickel sulfide particle. Light blue signifies encapsulated water phase. Chemical structures show metabolites derived from Sobotta *et al.* (2020) that could possibly evolve in this vesicle reaction chamber. Structures on the left clarifying possible structures of the lipid bilayer of the vesicle. (unpublished data)

To obtain this drying process the supernatant of the reaction as well as the precipitate were subject to an organic extraction. The extracted organic phase was dried and then the same protocol was applied as for nonanoic acid, adapted from Apel *et al.* (2002). The sample was transferred into the micelle-form by raising the pH with NaOH 1M. By adding HCl 3M the sample showed opalescence. This sample is now subject to transition electron microscopy.

5 References

Abramov, O., & Kring, D. (2004). *Impact-induced hydrothermal system at the Sudbury crater: Duration, temperatures, mechanics, and biological implications.* Paper presented at the Lunar and Planetary Science Conference.

Anbar, A. D., & Knoll A. H. (2002). *Proterozoic ocean chemistry and evolution: A bioinorganic bridge? Science, 297,* 1137-1142

Allegre, C. J., Poirier, J.-P., Humler, E., & Hofmann, A. W. (1995). The chemical composition of the Earth. *Earth and Planetary Science Letters, 134*(3), 515-526.

Anders, E. (1989). Pre-biotic organic matter from comets and asteroids. *Nature, 342*(6247), 255-257.

Apel, C. L., Deamer, D. W., & Mautner, M. N. (2002). Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1559*(1), 1-9. doi:https://doi.org/10.1016/S0005-2736(01)00400-X

Bada, J. L. (2004). How life began on Earth: a status report. *Earth and Planetary Science Letters, 226*(1-2), 1-15.

Bada, J. L., Fegley, B., Miller, S. L., Lazcano, A., Cleaves, H. J., Hazen, R. M., & Chalmers, J. (2007). Debating evidence for the origin of life on Earth. *Science, 315*(5814), 937-939.

Bada, J. L., & Korenaga, J. (2018). Exposed Areas Above Sea Level on Earth >3.5 Gyr Ago: Implications for Prebiotic and Primitive Biotic Chemistry. *Life (Basel), 8*(4). doi:10.3390/life8040055

Barge, L. M., Flores, E., Baum, M. M., VanderVelde, D. G., & Russell, M. J. (2019). Redox and pH gradients drive amino acid synthesis in iron oxyhydroxide mineral systems. *Proceedings of the National Academy of Sciences, 116*(11), 4828. doi:10.1073/pnas.1812098116

Baross, J. (2007). Evolution: a defining feature of life. *Planets and Life: The Emerging Science of Astrobiology. Ed. by WT Sullivan and JA Baross. Cambridge: Cambridge University Press*.

Barrault, J., Boulinguiez, M., Forquy, C., & Maurel, R. (1987). Synthesis of methyl mercaptan from carbon oxides and H_2S with tungsten—alumina catalysts. *Applied catalysis, 33*(2), 309-330.

Bartoschek, S., Vorholt, J. A., Thauer, R. K., Geierstanger, B. H., & Griesinger, C. (2000). N-Carboxymethanofuran (carbamate) formation from methanofuran and CO2 in methanogenic archaea: Thermodynamics and kinetics of the spontaneous reaction. *European Journal of Biochemistry, 267*(11), 3130-3138.

Becker, S., Schneider, C., Okamura, H., Crisp, A., Amatov, T., Dejmek, M., & Carell, T. (2018). Wet-dry cycles enable the parallel origin of canonical and non-canonical nucleosides by continuous synthesis. *Nature communications, 9*(1), 1-9.

Berclaz, N., Müller, M., Walde, P., & Luisi, P. L. (2001). Growth and Transformation of Vesicles Studied by Ferritin Labeling and Cryotransmission Electron Microscopy. *The Journal of Physical Chemistry B, 105*(5), 1056-1064. doi:10.1021/jp001298i

Berg, I. A., Kockelkorn, D., Ramos-Vera, W. H., Say, R. F., Zarzycki, J., Hügler, M., . . . Fuchs, G. (2010). Autotrophic carbon fixation in archaea. *Nature Reviews Microbiology, 8*(6), 447. Retrieved from https://www.nature.com/articles/nrmicro2365

Blöchl, E., Keller, M., Wächtershäuser, G., & Stetter, K. O. (1992). Reactions depending on iron sulfide and linking geochemistry with biochemistry. *Proceedings of the National Academy of Sciences, 89*(17), 8117-8120.

Boetius, A. (2005). Lost city life. *Science, 307*(5714), 1420-1422.

Braakman, R., & Smith, E. (2012). The emergence and early evolution of biological carbon-fixation. *PLoS Computational Biology, 8*(4), e1002455.

Calvin, M. (1956). Chemical evolution and the origin of life. *American Scientist, 44*(3), 248-263.

Calvin, M. (1959). Evolution of enzymes and the photosynthetic apparatus. *Science, 130*(3383), 1170-1174.

Cape, J. L., Monnard, P.-A., & Boncella, J. M. (2011). Prebiotically relevant mixed fatty acid vesicles support anionic solute encapsulation and photochemically catalyzed trans-membrane charge transport. *Chemical Science, 2*(4), 661-671.

Cech, T. R. (1986). A model for the RNA-catalyzed replication of RNA. *Proceedings of the National Academy of Sciences, 83*(12), 4360-4363.

Chakraborty, P. P., Mukhopadhyay, J., Paul P. P., Banerjee, D. M., & Bera, M. K. (2020) Early atmosphere and hydrosphere oxygenation: Clues from Precambrian paleosols and chemical sedimentary records of India. *Episodes, 43(1)*, 175-186

Charnley, S., Rodgers, S., Kuan, Y.-J., & Huang, H.-C. (2002). Biomolecules in the interstellar medium and in comets. *Advances in Space Research, 30*(6), 1419-1431.

Chen, I. A., Roberts, R. W., & Szostak, J. W. (2004). The Emergence of Competition Between Model Protocells. *Science, 305*(5689), 1474-1476. doi:doi:10.1126/science.1100757

Cheng, M., Chao, L., Zhou, L., & Xie, S. (2015). *Mo marine geoschemistry and reconstruction of ancient ocean redox states. Science China Earth Sciences, 58(12),* 2123-2133

Cheng, Z., & Luisi, P. L. (2003). Coexistence and Mutual Competition of Vesicles with Different Size Distributions. *The Journal of Physical Chemistry B, 107*(39), 10940-10945. doi:10.1021/jp034456p

Chyba, C., & Sagan, C. (1992). Endogenous production, exogenous delivery and impact-shock synthesis of organic molecules: an inventory for the origins of life. *Nature, 355*(6356), 125-132.

Cimarelli, C., Alatorre-Ibargüengoitia, M., Kueppers, U., Scheu, B., & Dingwell, D. B. (2014). Experimental generation of volcanic lightning. *Geology, 42*(1), 79-82.

Cockell, C. S. (2006). The origin and emergence of life under impact bombardment. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*(1474), 1845-1856. doi:10.1098/rstb.2006.1908

Cody, G. D. (2004). Transition metal sulfides and the origins of Metabolism. *Annu. Rev. Earth Planet. Sci., 32*, 29. doi:10.1146/annurev.earth.32.101802.120225

Cody, G. D., Boctor, N. Z., Filley, T. R., Hazen, R. M., Scott, J. H., Sharma, A., & Yoder, H. S. J. (2000). Primordial Carbonylated Iron-Sulfur Compounds and the Synthesis of Pyruvate. *Science, 289*, 5.

Colín-García, M., Heredia, A., Cordero, G., Camprubí, A., Negrón-Mendoza, A., Ortega-Gutiérrez, F., . . . Ramos-Bernal, S. (2016). Hydrothermal vents and prebiotic chemistry: a review. *Boletín de la Sociedad Geológica Mexicana, 68*, 599-620. Retrieved from http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S140533222016000300599&nrm=iso

Corliss, J. B., Baross, J., & Hoffman, S. (1981). An hypothesis concerning the relationships between submarine hot springs and the origin of life on earth. *Oceanologica Acta, Special issue*.

Crick, F. (1970). Central dogma of molecular biology. *Nature, 227*(5258), 561-563.

Crick, F. H. (1968). The origin of the genetic code. *Journal of molecular biology, 38*(3), 367-379.

Damer, B. (2016). A field trip to the Archaean in search of Darwin's warm little pond. *Life, 6*(2), 21.

Darnault, C., Volbeda, A., Kim, E. J., Legrand, P., Vernède, X., Lindahl, P. A., & Fontecilla-Camps, J. C. (2003). Ni-Zn-[Fe 4-S 4] and Ni-Ni-[Fe 4-S 4] clusters in closed and open α subunits of acetyl-CoA synthase/carbon monoxide dehydrogenase. *Nature Structural & Molecular Biology, 10*(4), 271-279.

Darwin, C. (1909). *The origin of species*: PF Collier & son New York.

Deamer, D. W. (1985). Boundary structures are formed by organic components of the Murchison carbonaceous chondrite. *Nature, 317*(6040), 792-794.

Dodd, M. S., Papineau, D., Grenne, T., Slack, J. F., Rittner, M., Pirajno, F., . . . Little, C. T. (2017). Evidence for early life in Earth's oldest hydrothermal vent precipitates. *Nature, 543*(7643), 60-64.

Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science, 284*(5423), 2124-2128.

Doolittle, W. F., & Brown, J. R. (1994). Tempo, mode, the progenote, and the universal root. *Proceedings of the National Academy of Sciences, 91*(15), 6721-6728. doi:10.1073/pnas.91.15.6721

Dörr, M., Käßbohrer, J., Grunert, R., Kreisel, G., Brand, W. A., Werner, R. A., . . . Weigand, W. (2003). A possible prebiotic formation of ammonia from dinitrogen on iron sulfide surfaces. *Angewandte Chemie International Edition, 42*(13), 1540-1543.

Dworkin, J. P., Lazcano, A., & Miller, S. L. (2003). The roads to and from the RNA world. *Journal of Theoretical Biology, 222*(1), 127-134.

Egholm, M., Buchardt, O., Nielsen, P. E., & Berg, R. H. (1992). Peptide nucleic acids (PNA). Oligonucleotide analogs with an achiral peptide backbone. *Journal of the American Chemical Society, 114*(5), 1895-1897.

Eigen, M. (1995). *What Will Endure of 20th Century Biology?* (Vol. 1). Cambridge: Cambridge University Press.

Elsner, M. P., Dittrich, C., & Agar, D. W. (2002). Adsorptive reactors for enhancing equilibrium gas-phase reactions—two case studies. *Chemical engineering science, 57*(9), 1607-1619.

Ferris, J. P., Hill, A. R., Liu, R., & Orgel, L. E. (1996). Synthesis of long prebiotic oligomers on mineral surfaces. *Nature, 381*(6577), 59-61.

Follmann, H., & Brownson, C. (2009). Darwin's warm little pond revisited: from molecules to the origin of life. *Naturwissenschaften, 96*(11), 1265-1292.

Fontecilla-Camps, J. C., Amara, P., Cavazza, C., Nicolet, Y., & Volbeda, A. (2009). Structure–function relationships of anaerobic gas-processing metalloenzymes. *Nature, 460*(7257), 814-822.

French, B. M. (1970). Stability Relations of Siderite (FeCO3), Determined in Controlled fO2 Atmospheres. *NASA, Goddard Space Flight Center, X-63895*.

Frisken, B. J., Asman, C., & Patty, P. J. (2000). Studies of Vesicle Extrusion. *Langmuir, 16*(3), 928-933. doi:10.1021/la9905113

Fuchs, G., Stupperich, E., & Eden, G. (1980). Autotrophic CO2 fixation in Chlorobium limicola. Evidence for the operation of a reductive tricarboxylic acid cycle in growing cells. *Archives of Microbiology, 128*(1), 64-71. doi:10.1007/bf00422307

Gamow, G. (1954). Possible relation between deoxyribonucleic acid and protein structures. *Nature, 173*(4398), 318-318.

Gaudin, D., & Cimarelli, C. (2019). The electrification of volcanic jets and controlling parameters: A laboratory study. *Earth and Planetary Science Letters, 513*, 69-80.

Gayon, J., Malaterre, M., Morange, M., Raulin-Cercau, F., & Tirard S. (2010). Special Issue: Definitions of life. *Origins of life and Evolution of Biosheres, 40*, 119-244

Geisberger, T., Sobotta, J., Eisenreich, W., & Huber, C. (2021). Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth—Implications for Early Life on Extraterrestrial Planets? *Life, 11*(2), 149.

Gilbert, W. (1986). Origin of life: The RNA world. *Nature, 319*(6055), 618-618.

Glansdorff, N., Xu, Y., & Labedan, B. (2008). The last universal common ancestor: emergence, constitution and genetic legacy of an elusive forerunner. *Biology Direct, 3*(1), 1-35.

Gruen, D., & De Lacey, E. (1984). Surfactants in solution. *KL Mittel, B. Lindman (Eds.), 1*, 279.

Hagmann, M. (2002). Between a rock and a hard place. *Science, 295*(5562), 2006-2007.

Hanczyc, M. M., Fujikawa, S. M., & Szostak, J. W. (2003). Experimental Models of Primitive Cellular Compartments: Encapsulation, Growth, and Division. *Science, 302*(5645), 618-622. doi:doi:10.1126/science.1089904

Hanczyc, M. M., Mansy, S. S., & Szostak, J. W. (2007). Mineral Surface Directed Membrane Assembly. *Origins of life and evolution of biospheres, 37*(1), 67-82. doi:10.1007/s11084-006-9018-5

Hao, J., Giovenco, E., Pedreira-Segade, U., Montagnac, G., & Daniel, I. (2018). Compatibility of Amino Acids in Ice Ih: Implications for the Origin of Life. *Astrobiology, 18*(4), 381-392. doi:10.1089/ast.2017.1735

Hartman, H. (1975). Speculations on the origin and evolution of metabolism. *Journal of Molecular Evolution, 4*(4), 359-370.

Heinz, J., & Schulze-Makuch, D. (2020). Thiophenes on Mars: Biotic or Abiotic Origin? *Astrobiology, 20*(4), 552-561. doi:10.1089/ast.2019.2139

Higgs, P. G. (2017). Chemical evolution and the evolutionary definition of life. *Journal of Molecular Evolution, 84*(5), 225-235.

Holm, N. G., & Andersson, E. (2005). Hydrothermal simulation experiments as a tool for studies of the origin of life on earth and other terrestrial planets: a review. *Astrobiology, 5*(4), 444-460.

Holm, N. G., & Charlou, J. L. (2001). Initial indications of abiotic formation of hydrocarbons in the Rainbow ultramafic hydrothermal system, Mid-Atlantic Ridge. *Earth and Planetary Science Letters, 191*(1-2), 1-8.

Huber, C., Eisenreich, W., Hecht, S., & Wächtershäuser, G. (2003). A possible primordial peptide cycle. *Science, 301*(5635), 938-940. doi:10.1126/science.1086501

Huber, C., Eisenreich, W., & Wächtershäuser, G. (2010). Synthesis of a-amino and a-hydroxy acids under volcanic conditions:implications for the origin of life. *Tetrahedron, 51*(7), 3. doi:10.1016/j.tetlet.2009.12.084

Huber, C., Kraus, F., Hanzlik, M., Eisenreich, W., & Wächtershäuser, G. (2012). Elements of metabolic evolution. *Chemistry, 18*(7), 2063-2080. doi:10.1002/chem.201102914

Huber, C., & Wächtershäuser, G. (1997). Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. *Science, 276*(5310), 245-247. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9092471

Huber, C., & Wächtershäuser, G. (1998). Peptides by activation of amino acids with CO on (Ni,Fe)S surfaces: implications for the origin of life. *Science, 281*(5377), 670-672.

Huber, C., & Wächtershäuser, G. (2003). Primordial reductive amination revisited. *Tetrahedron Letters, 44*(8), 1695-1697. doi:https://doi.org/10.1016/S0040-4039(02)02863-0

Huber, C., & Wächtershäuser, G. (2006). alpha-Hydroxy and alpha-amino acids under possible Hadean, volcanic origin-of-life conditions. *Science, 314*(5799), 630-632. doi:10.1126/science.1130895

Huber, H., Gallenberger, M., Jahn, U., Eylert, E., Berg, I. A., Kockelkorn, D., . . . Fuchs, G. (2008). A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic Archaeum Ignicoccus hospitalis</em&gt. *Proceedings of the National Academy of Sciences, 105*(22), 7851. doi:10.1073/pnas.0801043105

Hunter, D. G., & Frisken, B. J. (1998). Effect of Extrusion Pressure and Lipid Properties on the Size and Polydispersity of Lipid Vesicles. *Biophysical Journal, 74*(6), 2996-3002. doi:10.1016/S0006-3495(98)78006-3

Igari, S., Maekawa, T., & Sakata, S. (2000). Light hydrocarbons in fumarolic gases: A case study in the Kakkonda geothermal area. *Chikyukagau 34*, 7.

Joyce, G. F. (1994). Foreword In: DW Deamer and GR Fleischacker. *Origins of Life: The Central Concepts*.

Kasting, J. F. (1993). Earth's early atmosphere. *Science, 259*(5097), 920-926.

Keller, M. A., Kampjut, D., Harrison, S. A., Driscoll, P. C., & Ralser, M. (2019). Reply to 'Do sulfate radicals really enable a non-enzymatic Krebs cycle precursor?'. *Nat Ecol Evol, 3*(2), 139-140. doi:10.1038/s41559-018-0792-z

Keller, M. A., Kampjut, D., Harrison, S. A., & Ralser, M. (2017). Sulfate radicals enable a non-enzymatic Krebs cycle precursor. *Nat Ecol Evol, 1*(4), 83. doi:10.1038/s41559-017-0083

Kelley, D. S., Karson, J. A., Blackman, D. K., FruÈh-Green, G. L., Butterfield, D. A., Lilley, M. D., . . . Lebon, G. T. (2001). An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30 N. *Nature, 412*(6843), 145-149.

Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., . . . Proskurowski, G. (2005). A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science, 307*(5714), 1428-1434.

Kitadai, N., Kameya, M., & Fujishima, K. (2017). Origin of the Reductive Tricarboxylic Acid (rTCA) Cycle-Type CO2 Fixation: A Perspective. *Life (Basel), 7*(3), 16. doi:10.3390/life7040039

Kitadai, N., & Maruyama, S. (2018). Origins of building blocks of life: A review. *Geoscience Frontiers, 9*, 38. doi:10.1016/j.gsf.2017.07.007

Kockelkorn, D., & Fuchs, G. (2009). Malonic Semialdehyde Reductase, Succinic Semialdehyde Reductase, and Succinyl-Coenzyme A Reductase from *Metallosphaera sedula*: Enzymes of the Autotrophic 3-Hydroxypropionate/4-Hydroxybutyrate Cycle in *Sulfolobales*. *Journal of Bacteriology, 191*(20), 6352. doi:10.1128/JB.00794-09

Krafft, F., & Strutz, A. (1896) *Über das Verhalten seifenähnlicher Substanzen gegen Wasser. Ber. Dt. chem. Ges.*,29, 2, S. 1328–1334.

Kreysing, M., Keil, L., Lanzmich, S., & Braun, D. (2015). Heat flux across an open pore enables the continuous replication and selection of oligonucleotides towards increasing length. *Nature Chemistry, 7*(3), 203-208.

Lazar, M. A., & Birnbaum, M. J. (2012). De-Meaning of Metabolism. *Science, 336*(6089), 2.

Lazcano, A., & Miller, S. L. (1996). The Origin and Early Evolution of Life: Prebiotic Chemistry, the Pre-RNA World, and Time. *Cell, 85*, 6.

Leman, L. J., Orgel, L. E., & Ghadiri, M. R. (2004). Carbonyl sulfide-mediated prebiotic formation of peptides;. *Science, 306*, 4. doi:10.1126/science.1102722

Leman, L. J., Orgel, L. E., & Ghadiri, M. R. (2006). Amino acid dependent formation of phosphate anhydrides in water mediated by carbonyl sulfide. *J Am Chem Soc, 128*(1), 20-21. doi:10.1021/ja056036e

Lindblom, G., & Wennerström, H. (1977). Amphiphile diffusion in model membrane systems studied by pulsed NMR. *Biophysical chemistry, 6*(2), 167-171.

Lipmann, F. A. (1965). *The Origin of Prebiological Systems and of Their Molecular Matrices*. New York: Academic Press.

Ljungdahl, L. G., & Wood, H. G. (1969). TOTAL SYNTHESIS OF ACETATE FROM CO2 BY HETEROTROPHIC BACTERIA. *Annual Review of Microbiology, 23*(1), 515-538. doi:10.1146/annurev.mi.23.100169.002503

Lonsdale, P. (1977). Clustering of suspension-feeding macrobenthos near abyssal hydrothermal vents at oceanic spreading centers. *Deep Sea Research, 24*(9), 857-863.

Mansy, S. S., Schrum, J. P., Krishnamurthy, M., Tobé, S., Treco, D. A., & Szostak, J. W. (2008). Template-directed synthesis of a genetic polymer in a model protocell. *Nature, 454*(7200), 122-125. doi:10.1038/nature07018

Martin, W., Baross, J., Kelley, D., & Russell, M. J. (2008). Hydrothermal vents and the origin of life. *Nat Rev Microbiol, 6*(11), 805-814. doi:10.1038/nrmicro1991

Mast, C. B., Schink, S., Gerland, U., & Braun, D. (2013). Escalation of polymerization in a thermal gradient. *Proceedings of the National Academy of Sciences, 110*(20), 8030-8035.

McKay, C. P., & Borucki, W. J. (1997). Organic synthesis in experimental impact shocks. *Science, 276*(5311), 390-392.

Meierhenrich, U. J., Filippi, J. J., Meinert, C., Vierling, P., & Dworkin, J. P. (2010). Die Entstehung erster Zellen–von der Nährstoffaufnahme hin zur Verlängerung eingeschlossener Nucleotide. *Angewandte Chemie, 122*(22), 3826-3839.

Menger, F. M., & Gabrielson, K. D. (1995). Cytomimetic Organic Chemistry: Early Developments. *Angewandte Chemie International Edition in English, 34*(19), 2091-2106. doi:https://doi.org/10.1002/anie.199520911

Menger, F. M., & Peresypkin, A. V. (2001). A combinatorially-derived structural phase diagram for 42 zwitterionic geminis. *Journal of the American Chemical Society, 123*(23), 5614-5615.

Mielke, R. E., Robinson, K. J., White, L. M., McGlynn, S. E., McEachern, K., Bhartia, R., . . . Russell, M. J. (2011). Iron-sulfide-bearing chimneys as potential catalytic energy traps at life's emergence. *Astrobiology, 11*(10), 933-950. doi:10.1089/ast.2011.0667

Miller, S. L. (1953). A production of amino acids under possible primitive earth conditions. *Science, 117*, 1. doi:10.1126/science.117.3046.528, S. 528–529

Mojzsis, S. J., Krishnamurthy, R., & Arrhenius, G. (1999). Before RNA and after: Geophysical and geochemical constraints on molecular evolution. *COLD SPRING HARBOR MONOGRAPH SERIES, 37*, 1-48.

Mukhin, L. E. V. (1974). Evolution of organic compounds in volcanic regions. *Nature, 251*(5470), 50-51. doi:10.1038/251050a0

Namani, T., & Walde, P. (2005). From Decanoate Micelles to Decanoic Acid/Dodecylbenzenesulfonate Vesicles. *Langmuir, 21*(14), 6210-6219. doi:10.1021/la047028z

Nelson, K., Levy, M., & Miller, S. (2000a). Peptide nucleic acids rather than RNA may have been the first genetic molecule. *Proceedings of the National Academy of Sciences, 97*(8), 3868-3871.

Nelson, K., Levy, M., & Miller, S. (2000b). THE PREBIOTIC SYNTHESIS OF THE COMPONENTS OF PEPTIDE NUCLEIC ACID, A POSSIBLE FIRST GENETIC MATERIAL. *Origins of life and evolution of the biosphere, 30*(2/4), 259-259.

Nielsen, P. E., Egholm, M., Berg, R. H., & Buchardt, O. (1991). Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science, 254*(5037), 1497-1500.

Nirenberg, M. W., & Matthaei, J. H. (1961). The dependence of cell-free protein synthesis in E. coli upon naturally occurring or synthetic polyribonucleotides. *Proc. Natl. Acad. Sci. USA, 47*, 14.

Nitschke, W., & Russell, M. J. (2009). Hydrothermal focusing of chemical and chemiosmotic energy, supported by delivery of catalytic Fe, Ni, Mo/W, Co, S and Se, forced life to emerge. *J Mol Evol, 69*(5), 481-496. doi:10.1007/s00239-009-9289-3

Nutman, A. P., Bennett, V. C., Friend, C. R., Van Kranendonk, M. J., & Chivas, A. R. (2016). Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. *Nature, 537*(7621), 535-538.

Oparin, A. (1924). The Origin of Life. *Proiskhozhdenie zhizny, Moscow, Trad.*

Oparin, A. I., & Gladilin, K. L. (1980). Evolution of self-assembly of probionts. *Biosystems, 12*(3), 133-145. doi:https://doi.org/10.1016/0303-2647(80)90011-8

Oremland, R. S., & Voytek, M. A. (2008). Acetylene as fast food: implications for development of life on anoxic primordial Earth and in the outer solar system. *Astrobiology, 8*(1), 45-58. Retrieved from https://www.liebertpub.com/doi/abs/10.1089/ast.2007.0183

Orgel, L. E. (1968). Evolution of the genetic apparatus. *Journal of molecular biology, 38*(3), 381-393.

Orgel, L. E. (2004). Prebiotic chemistry and the origin of the RNA world. *Critical reviews in biochemistry and molecular biology, 39*(2), 99-123.

Oró, J. (1960). Synthesis of adenine from ammonium cyanide. *Biochemical and Biophysical Research Communications, 2*(6), 407-412.

Oró, J. (1961). Comets and the formation of biochemical compounds on the primitive Earth. *Nature, 190*(4774), 389-390.

Oró, J., & Kimball, A. (1961). Synthesis of purines under possible primitive earth conditions. I. Adenine from hydrogen cyanide. *Archives of biochemistry and biophysics, 94*(2), 217-227.

Osinski, G. R., Spray, J. G., & LEE, P. (2001). Impact-induced hydrothermal activity within the Haughton impact structure, arctic Canada: Generation of a transient, warm, wet oasis. *Meteoritics & Planetary Science, 36*(5), 731-745.

Owen, A. (1961). Calcium cyanamide synthesis. Part 1.—Thermodynamic studies. *Transactions of the Faraday Society, 57*, 670-677.

Palyi, G., Zucci, C., & Caglioti L. (2002). *Fundamentals of Life*, Elsevier SAS, Paris

Peretó, J. (2012). Out of fuzzy chemistry: from prebiotic chemistry to metabolic networks. *Chemical Society Reviews, 41*(16), 5394-5403.

Peretó, J., Bada, J. L., & Lazcano, A. (2009). Charles Darwin and the origin of life. *Origins of life and evolution of biospheres, 39*(5), 395-406.

Peters, J. W., & Williams, L. D. (2012). The Origin of Life: Look Up and Look Down. *Astrobiology, 12*(11), 1087-1092. doi:10.1089/ast.2012.0818

Pope, K. O., Kieffer, S. W., & Ames, D. E. (2006). Impact melt sheet formation on Mars and its implication for hydrothermal systems and exobiology. *Icarus, 183*(1), 1-9.

Popova, O. P., Jenniskens, P., Emel'yanenko, V., Kartashova, A., Biryukov, E., Khaibrakhmanov, S., . . . Chelyabinsk Airburst, C. (2013). Chelyabinsk airburst, damage assessment, meteorite recovery, and characterization. *Science, 342*(6162), 1069-1073. doi:10.1126/science.1242642

Powner, M. W., Gerland, B., & Sutherland, J. D. (2009). Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions. *Nature, 459*(7244), 239-242. doi:10.1038/nature08013

Preiner, M., Igarashi, K., Muchowska, K. B., Yu, M., Varma, S. J., Kleinermanns, K., . . . Moran, J. (2020). A hydrogen-dependent geochemical analogue of primordial carbon and energy metabolism. *Nature ecology & evolution, 4*(4), 534-542.

Ragsdale, S. W. (1994). CO Dehydrogenase and the Central Role of This Enzyme in the Fixation of Carbon Dioxide by Anaerobic Bacteria. In H. L. Drake (Ed.), *Acetogenesis* (pp. 88-126). Boston, MA: Springer US.

Rasi, S., Mavelli, F., & Luisi, P. L. (2003). Cooperative micelle binding and matrix effect in oleate vesicle formation. *The Journal of Physical Chemistry B, 107*(50), 14068-14076.

Rasmussen, S., Chen, L., Deamer, D., Krakauer, D. C., Packard, N. H., Stadler, P. F., & Bedau, M. A. (2004). Transitions from Nonliving to Living Matter. *Science, 303*(5660), 963-965. doi:doi:10.1126/science.1093669

Rauchfuss, H. (2008). *Chemical evolution and the origin of life*: Springer Science & Business Media.

Reppe, W. (1949). *Neue Entwicklungen auf dem Gebiet der Chemie des Acetylen und Kohlenoxyds*. Berlin/Göttingen/Heidelberg: Springer Verlag.

Reppe, W. (1953). Carbonylierung I. Über die Umsetzung von Acetylen mit Kohlenoxyd und Verbindungen mit reaktionsfähigen Wasserstoffatomen Synthesen α,β-ungesättigter Carbonsäuren und ihrer Derivate. *Justus Liebigs Annalen der Chemie, 582*(1), 1-37. doi:https://doi.org/10.1002/jlac.19535820102

Ross, D. S., & Deamer, D. (2016). Dry/Wet Cycling and the Thermodynamics and Kinetics of Prebiotic Polymer Synthesis. *Life, 6*(3), 28. Retrieved from https://www.mdpi.com/2075-1729/6/3/28

Russell, M. J. (2018). Green Rust: The Simple Organizing 'Seed' of All Life? *Life (Basel), 8*(35), 29. doi:10.3390/life8030035

Russell, M. J., Barge, L. M., Bhartia, R., Bocanegra, D., Bracher, P. J., Branscomb, E., . . . Kanik, I. (2014). The drive to life on wet and icy worlds. *Astrobiology, 14*(4), 308-343. doi:10.1089/ast.2013.1110

Russell, M. J., & Hall, A. J. (1990). Pyrite and the origin of life. *Nature, 344*, 1.

Russell, M. J., Hall, A. J., & Martin, W. (2010). Serpentinization as a source of energy at the origin of life. *Geobiology, 8*(5), 17. doi:10.1111/j.1472-4669.2010.00249.x

Russell, M. J., Roy, D. M., & Hall, A. J. (1993). On the emergence of life via catalytic iron-sulphide membranes. *Terra Nova, 5*(4), 4. doi:10.1111/j.1365-3121.1993.tb00267.x

Saladino, R., Crestini, C., Costanzo, G., Negri, R., & Di Mauro, E. (2001). A possible prebiotic synthesis of purine, adenine, cytosine, and 4 (3H)-pyrimidinone from formamide: implications for the origin of life. *Bioorganic & medicinal chemistry, 9*(5), 1249-1253.

Salditt, A., Keil, L. M., Horning, D. P., Mast, C. B., Joyce, G. F., & Braun, D. (2020). Thermal habitat for RNA amplification and accumulation. *Physical Review Letters, 125*(4), 048104.

Scheidler, C., Sobotta, J., Eisenreich, W., Wächtershäuser, G., & Huber, C. (2016). Unsaturated C-3,C-5,C-7,C-9-Monocarboxylic Acids by Aqueous, One-Pot Carbon Fixation: Possible Relevance for the Origin of Life. *Scientific Reports, 6*. doi:10.1038/srep27595

Scheu, B., Dingwell, D. B., Cimarelli, C., Bada, J., Chalmers, J. H., & Burton, A. S. (2017). *Prebiotic Synthesis in Volcanic Discharges: Exposing Ash to Volcanic/Primordial Gas Atmospheres.* Paper presented at the AGU Fall Meeting Abstracts.

Schmitt-Kopplin, P., Gabelica, Z., Gougeon, R. D., Fekete, A., Kanawati, B., Harir, M., . . . Hertkorn, N. (2010). High molecular diversity of extraterrestrial organic matter in Murchison meteorite revealed 40 years after its fall. *Proc Natl Acad Sci U S A, 107*(7), 2763-2768. doi:10.1073/pnas.0912157107

Schmitt-Kopplin, P., Harir, M., Kanawati, B., Gougeon, R. D., Moritz, F., Hertkorn, N., . . . Gabelica, Z. (2014). Analysis of Extraterrestial Organic Matter in Murchison meteorite. In V. Kolb (Ed.), *ASTROBIOLOGY - An evolutionary approach* (Vol. 1, pp. 20). Boca Raton: CRC Press.

Schrödinger, E. (1944). *What is Life? The Physicist's approach to the Subject — With an Epilogue on Determinism and Free Will*, Cambridge University Press, The Macmillan Company

Segre, D., Ben-Eli, D., Deamer, D. W., & Lancet, D. (2001). The lipid world. *Orig Life Evol Biosph, 31*(1-2), 119-145. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11296516

Seiffert, G. B., Ullmann, G. M., Messerschmidt, A., Schink, B., Kroneck, P. M. H., & Einsle, O. (2007). Structure of the non-redox-active tungsten/[4Fe:4S] enzyme acetylene hydratase. *Proceedings of the National Academy of Sciences, 104*(9), 3073. doi:10.1073/pnas.0610407104

Shapiro, R. (2000a). The homopolymer problem in the origin of life. *Origins of life and evolution of the biosphere, 30*(2/4), 243-244.

Shapiro, R. (2000b). A replicator was not involved in the origin of life. *IUBMB Life, 49*(3), 173-176.

Shinitzky, M., Dianoux, A. C., Gittler, C., & Weber, G. (1971). *Biochemistry, 10*, 7.

Shock, E. L., & Schulte, M. D. (1998). Organic synthesis during fluid mixing in hydrothermal systems. *Journal of Geophysical Research: Planets, 103*(E12), 28513-28527.

Sleep, N. H. (1990). Hotspots and mantle plumes: Some phenomenology. *Journal of Geophysical Research: Solid Earth, 95*(B5), 6715-6736.

Sobotta, J. (2018). *Investigations of carbon fixation in model organisms and in cell-free prebiotic transition metal-catalyzed reactions.* (PhD Dissertation). Technical University Munich, Munich.

Sobotta, J., Geisberger, T., Moosmann, C., Scheidler, C. M., Eisenreich, W., Wächtershäuser, G., & Huber, C. (2020). A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism. *Life (Basel), 10*(4). doi:10.3390/life10040035

Span, I., Wang, K., Wang, W., Zhang, Y., Bacher, A., Eisenreich, W., Groll, M. (2012). Discovery of acetylene hydratase activity of the iron–sulphur protein IspH. *Nature communications, 3*(1), 1-8.

Springsklee, C., Steiner, T., Geisberger, T., Scheu, B., Huber, C., Eisenreich, W., Dingwell, D. B. (2020). *Prebiotic synthesis in volcanic discharges: lightning, porous ash and volcanic gas atmospheres*, EGU General assembly 2020, Online, 4-8 May 2020, EGU2020-8328

Stevenson, A. (2010). *Oxford dictionary of English*: Oxford University Press, USA.

Strauss, G., & Fuchs, G. (1993). Enzymes of a novel autotrophic CO2 fixation pathway in the phototrophic bacterium Chloroflexus aurantiacus, the 3-hydroxypropionate cycle. *European Journal of Biochemistry, 215*(3), 633-643. doi:10.1111/j.1432-1033.1993.tb18074.x

Szostak, J. W. (2012). Attempts to define life do not help to understand the origin of life. *Journal of Biomolecular Structure and Dynamics, 29*(4), 599-600.

Szostak, J. W., Bartel, D. P., & Luisi, P. L. (2001). Synthesizing life. *Nature, 409*(6818), 387-390. doi:10.1038/35053176

Tanford, C. (1978). The hydrophobic effect and the organization of living matter. *Science, 200*(4345), 1012-1018.

tenBrink, F., Schink, B., & Kroneck, P. M. (2011). Exploring the active site of the tungsten, iron-sulfur enzyme acetylene hydratase. *Journal of Bacteriology, 193*(5), 1229-1236.

Thauer, R. K., Jungermann, K., & Decker, K. (1977). Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological reviews, 41*(1), 100-180.

Trifonov, E. N. (2011). Vocabulary of definitions of life suggests a definition. *Journal of Biomolecular Structure and Dynamics, 29*(2), 259-266.

Tziotis, D., Hertkorn, N., & Schmitt-Kopplin, P. (2011). Kendrick-analogous network visualisation of ion cyclotron resonance Fourier transform mass spectra: improved options for the assignment of elemental compositions and the classification of organic molecular complexity. *European Journal of Mass Spectrometry, 17*(4), 415-421.

Urey, H. C. (1952). The planets, their origin and development. *New Haven: Yale University Press*.

Usher, D. (1977). Early chemical evolution of nucleic acids: a theoretical model. *Science, 196*(4287), 311-313.

Valley, J. W., Peck, W. H., King, E. M., & Wilde, S. A. (2002). A cool early Earth. *Geology, 30*(4), 4.

Vorholt, J.A., & Thauer,R.K. (1997). The active species of 'CO2' utilized by formylmethanofuran dehydrogenase from methanogenic Archaea. *Europrean Journal of Biochemisty, 248*, 919±924.

Varma, S. J., Muchowska, K. B., Chatelain, P., & Moran, J. (2018). Native iron reduces CO2 to intermediates and end-products of the acetyl-CoA pathway. *Nat Ecol Evol, 2*(6), 1019-1024. doi:10.1038/s41559-018-0542-2

Versh, E., Kirsimäe, K., Jõeleht, A., & Plado, J. (2003). Impact Induced Hydrothermal System at Kärdla Impact Crater: Development and Biological Consequences. *Large Meteorite Impacts*, 4120.

Volbeda, A., & Fontecilla-Camps, J. C. (2006). Catalytic nickel–iron–sulfur clusters: from minerals to enzymes. In *Bioorganometallic Chemistry* (pp. 57-82): Springer.

Wächtershäuser, G. (1988a). Before Enzymes and Templates - Theory of Surface Metabolism. *Microbiological Reviews, 52*(4), 452-484. Retrieved from http://mmbr.asm.org/content/52/4/452.full.pdf

Wächtershäuser, G. (1988b). Pyrite Formation, the 1st Energy-Source for Life - a Hypothesis. *Systematic and Applied Microbiology, 10*(3), 207-210. Retrieved from <Go to ISI>://WOS:A1988Q013000001

Wächtershäuser, G. (1990). Evolution of the first metabolic cycles. *Proceedings of the National Academy of Sciences, 87*(1), 200. doi:10.1073/pnas.87.1.200

Wächtershäuser, G. (1992). Groundworks for an evolutionary biochemistry: the iron-sulphur world. *Prog Biophys Mol Biol, 58*(2), 85-201. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1509092

Wächtershäuser, G. (2000). Life as we don't know it. *Science, 289*(5483), 1307-1308.

Wächtershäuser, G. (2006). From volcanic origins of chemoautotrophic life to Bacteria, Archaea and Eukarya. *Philos Trans R Soc Lond B Biol Sci, 361*(1474), 1787-1806; discussion 1806-1788. doi:10.1098/rstb.2006.1904

Wächtershäuser, G. (2007). On the chemistry and evolution of the pioneer organism. *Chem Biodivers, 4*(4), 584-602. doi:10.1002/cbdv.200790052

Wächtershäuser, G. (2014). From chemical invariance to genetic variability. *Bioinspired Catalysis: Metal-Sulfur Complexes*, 1-20.

Walde, P. (2006). Surfactant assemblies and their various possible roles for the origin (s) of life. *Origins of life and evolution of biospheres, 36*(2), 109-150.

Walde, P., Wick, R., Fresta, M., Mangone, A., & Luisi, P. L. (1994). Autopoietic Self-Reproduction of Fatty Acid Vesicles. *Journal of the American Chemical Society, 116*(26), 11649-11654. doi:10.1021/ja00105a004

Watson, J. D., & Crick, F. H. (1953). *The structure of DNA.* Paper presented at the Cold Spring Harbor symposia on quantitative biology.

Weiss, M. C., Preiner, M., Xavier, J. C., Zimorski, V., & Martin, W. (2018). The last universal common ancestor between ancient Earth chemistry and the onset of genetics. *PLoS Genetics, 14*(8), 19. doi:10.1371/journal.pgen.1007518

Weiss, M. C., Sousa, F. L., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S., & Martin, W. F. (2016). The physiology and habitat of the last universal common ancestor. *Nat Microbiol, 1*(9), 16116. doi:10.1038/nmicrobiol.2016.116

Wick, R., Walde, P., & Luisi, P. L. (1995). Light microscopic investigations of the autocatalytic self-reproduction of giant vesicles. *Journal of the American Chemical Society, 117*(4), 1435-1436. doi:10.1021/ja00109a031

Wochner, A., Attwater, J., Coulson, A., & Holliger, P. (2011). Ribozyme-catalyzed transcription of an active ribozyme. *Science, 332*(6026), 209-212.

Woese, C. (1967). The evolution of the genetic code. The Genetic Code. In: Harper & Row, New York.

Woese, C. (1981). Archaebacteria. *SciAm, 244*, 24.

Woese, C. (1998). The universal ancestor. *Proceedings of the National Academy of Sciences, 95*(12), 6854-6859. doi:10.1073/pnas.95.12.6854

Woese, C., Dugre, D., Saxinger, W., & Dugre, S. (1966). The molecular basis for the genetic code. *Proceedings of the National Academy of Sciences of the United States of America, 55*(4), 966.

Woese, C. R., Kandler, O., & Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences, 87*(12), 4576-4579.

Yamagata, Y., Watanabe, H., Saitoh, M., & Namba, T. (1991). Volcanic production of polyphosphates and its relevance to prebiotic evolution. *Nature, 352*(6335), 516-519. doi:10.1038/352516a0

Zepik, H. H., Walde, P., & Ishikawa, T. (2008). Vesikelbildung aus reaktiven Tensiden. *Angewandte Chemie, 120*(7), 1343-1345.

6 Reprint Permission

6.1 Reprint Permission: “A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism” and “Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth —Implications for Early Life on Extraterrestrial Planets?”

Agata Kołomańska / MDPI <agata.kolomanska@mdpi.com>
Mo 30.08, 09:38

Dear Thomas,
Thanks a lot for contacting me.
MDPI is a pioneer in scholarly open access publishing and all the journals we have, are fully open access.
This means that everyone has free and unlimited access to the full-text of all articles published in MDPI journals and that everyone is free to re-use the published material if proper accreditation/citation of the original publication is given.
In other words, with proper citation of your papers, you are free to post them in your theses.
Should you have further questions, please feel free to contact us again.
Best regards and good luck!

Agata Kołomańska
Associate Publisher, MDPI Barcelona
agata.kolomanska@mdpi.com

MDPI Branch Office, Barcelona
Avenida de Madrid 95, 08028 Barcelona, Spain
+34 93 639 7662
---

7 Supporting Materials

7.1 Supporting Material: A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism

Supplementary Information

A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism

Jessica Sobotta[†], Thomas Geisberger[†], Carolin Moosmann, Christopher M. Scheidler, Wolfgang Eisenreich, Günter Wächtershäuser, Claudia Huber[*]

Table S1: Metabolic products of the nickel catalyzed reaction of acetylene with carbon monoxide. Reactions were carried out in 125 ml serum bottles with 5 ml aqueous liquid phase for 7 days at 105 °C; Products were identified by GC-MS as *tert*-butyldimethylsilyl derivatives; Labelling in characteristic fragments is shown for runs with D_2O or ^{13}CO. n^ signifies n D-labels, n• signifies n ^{13}C-labels, 0.5• signifies 50% labelling of the indicated product.

Runs	**A**	**B**	**C**	**D**			
$NiSO_4 \bullet 6\ H_2O$ [mmol]	1	0.5		0.5			
$FeSO_4 \bullet 7\ H_2O$ [mmol]				0.5			
β-$Ni(OH)_2$ [mmol]	-	0.5	1	-			
Na_2SO_4 [mmol]	-	0.5	1	-			
$Na_2S \bullet 9\ H_2O$ [mmol]	1	0.5	-	1.0			
NaOH [mmol]	0.5	0.5	0.5	0.5			
CO [ml]	60	60	60	60			
C_2H_2 [ml]	60	60	60	60			
pH_{end}	8.0	8.1	9.8	8.5			
Products [µM]					labelling in characteristic fragments		
					mass1	mass2	mass3
C1							
formate	**18983**	**24207**	**310**	**434**	145_1^1•	103_1^1•	
C2							
acetate	**4358**	**3434**	**112**	**749**	117_3^0•	99_0^0•	75_1^0•
glycolate	**32**	**38**	**n.d.**	**11**	247_2^0•	219_2^0•	163_2^0•
C3							
acrylate	**9692**	**16874**	**243**	**763**	129_3^1•	99_1^1•	85_3^0•
propionate	**10368**	**15021**	**171**	**339**	131_5^1•	115_0^0•	173_5^1•
pyruvate	**43**	**117**	**n.d.**	**4**	259_2^0.5•	231_0^0•	189_0^0•
β-lactate	**273**	**793**	**n.d.**	**n.d.**	261_4^1•	219_3^0•	163_2^0•
glycerate	**108**	**102**	**n.d.**	**n.d.**	391_3^0.5•	363_3^0•	289_3^0•
C4							
but-3-enoate	**187**	**563**	**n.d.**	**n.d.**	143_5^1•	115_0^0•	99_5^0•
crotonate	**226**	**516**	**n.d.**	**22**	143_5^0.5•	99_5^0•	185_0^1•
2-methylmalonate	**48**	**145**	**n.d.**	**n.d.**	289_3^2•	331_0^2•	133_0^0•
maleate	**72**	**585**	**n.d.**	**14**	287_2^2•	329_2^2•	115_0^1•
succinate	**3964**	**4747**	**3**	**187**	289_4^2•	331_4^2•	215_4^2•
fumarate	**358**	**391**	**n.d.**	**12**	287_2^2•	245_2^2•	329_2^2•
2,3-dihydroxybutyrate	**31**	**41**	**n.d.**	**n.d.**	405_5^1•	377_5^0•	303_5^0•
malate	**17**	**85**	**n.d.**	**n.d.**	419_3^2•	287_2^2•	461_3^0•
C5							
(E)-2-methylbut-2-enoate	**196**	**411**	**n.d.**	**n.d.**	157_7^1•	113_7^0•	83_7^1•

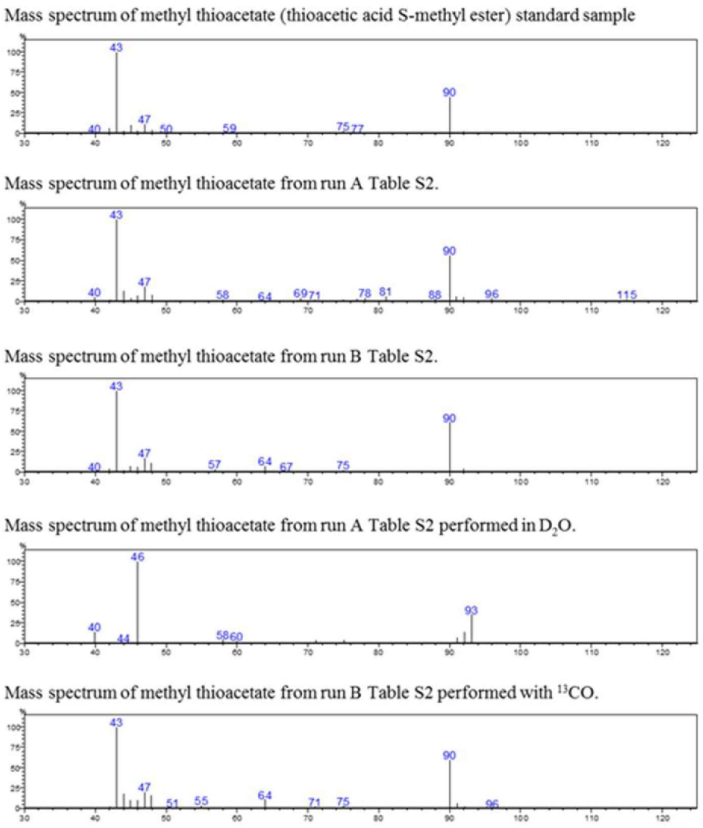


Figure S1: Formation of methyl thioacetate (thioacetic acid S-methyl ester) from HC≡CH and CH_3SH in the presence of NiS. Mass spectra are shown for the acetyl thioester standard sample as well as for experiments with HC≡CH (Table 3 run A), HC≡CH + CO (Table 3 run B), D_2O and ^{13}CO.

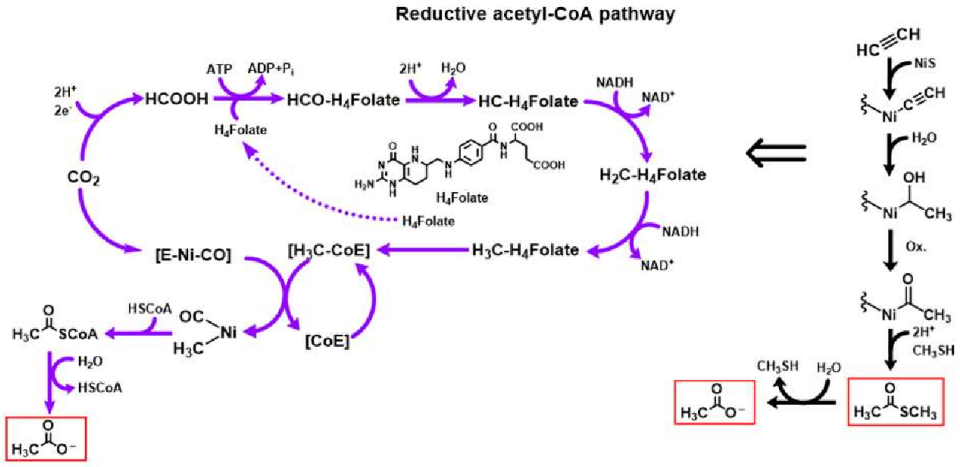


Figure S2: Comparison of the Reductive acetyl-CoA pathway[11] and the proposed primordial reaction mechanism to thio acetate. Metabolites highlighted by red boxes correspond to products of the NiS/HC≡CH/CO system. Pathway is adapted from[11,39]. CoE stands for corrinoid Enzyme, H_3C-CoE for methyl corrinoid protein; E-Ni-CO for CO dehydrogenase complex.

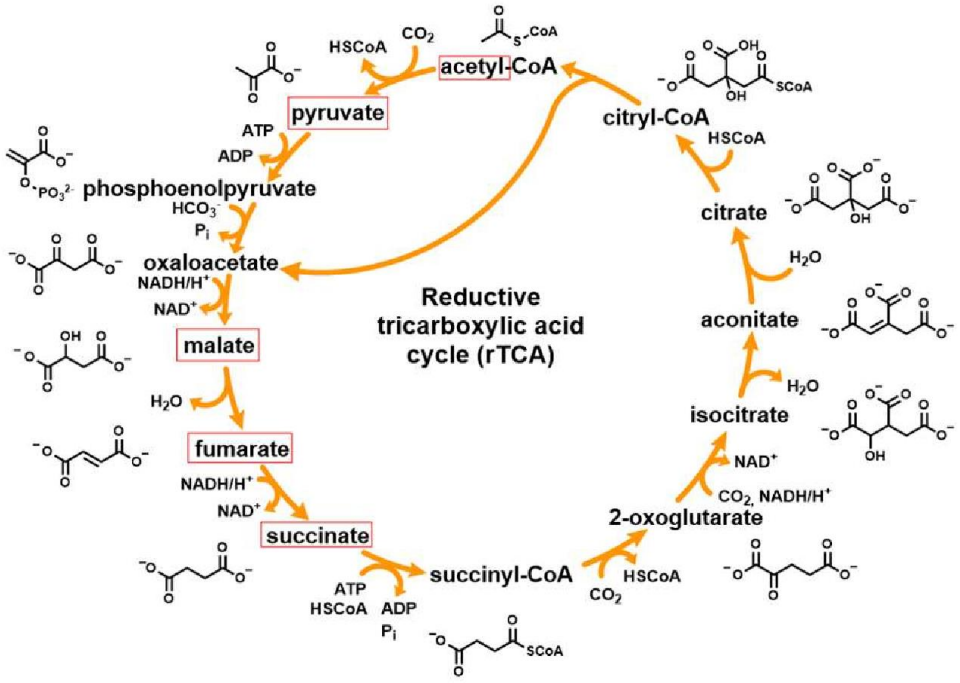


Figure S3: Reductive tricarboxylic acid cycle[14]. Metabolites highlighted by red boxes correspond to products of the NiS/HC≡CH/CO system. Graphics partly adapted from[40,41]

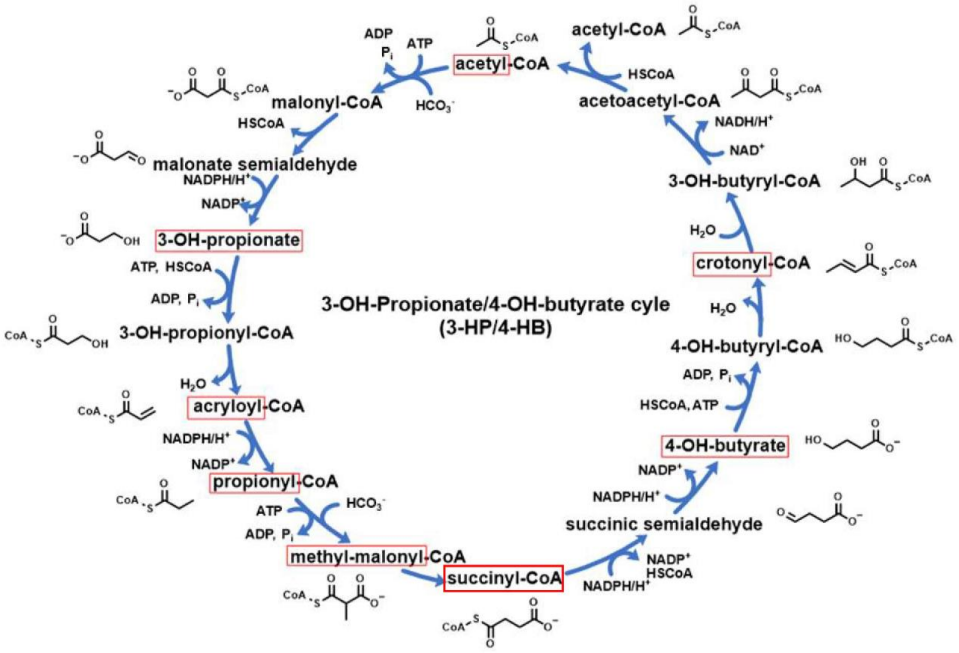


Figure S4: 3-Hydroxypropinate/4-hydroxybutyrate cycle[15]. Metabolites highlighted by red boxes correspond to products of the NiS/HC≡CH/CO system. Graphics partly adapted from[15,42].

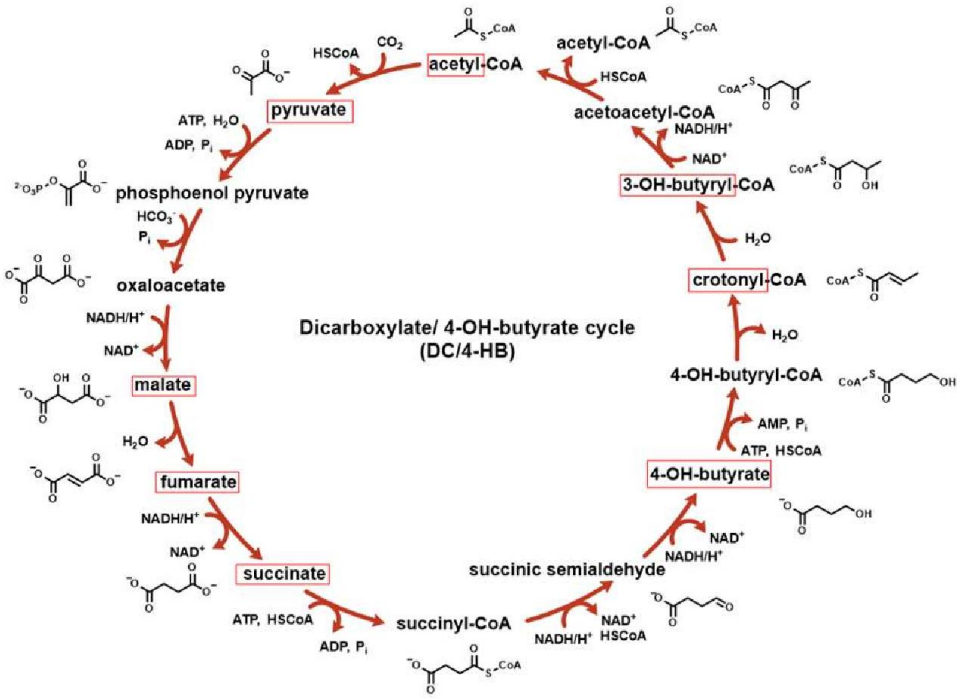


Figure S5: Dicarboxylate/4-Hydroxbutyrate cycle[16]. Metabolites highlighted by red boxes correspond to products of the NiS/HC≡CH/CO system. Graphics partly adapted from [16,43].

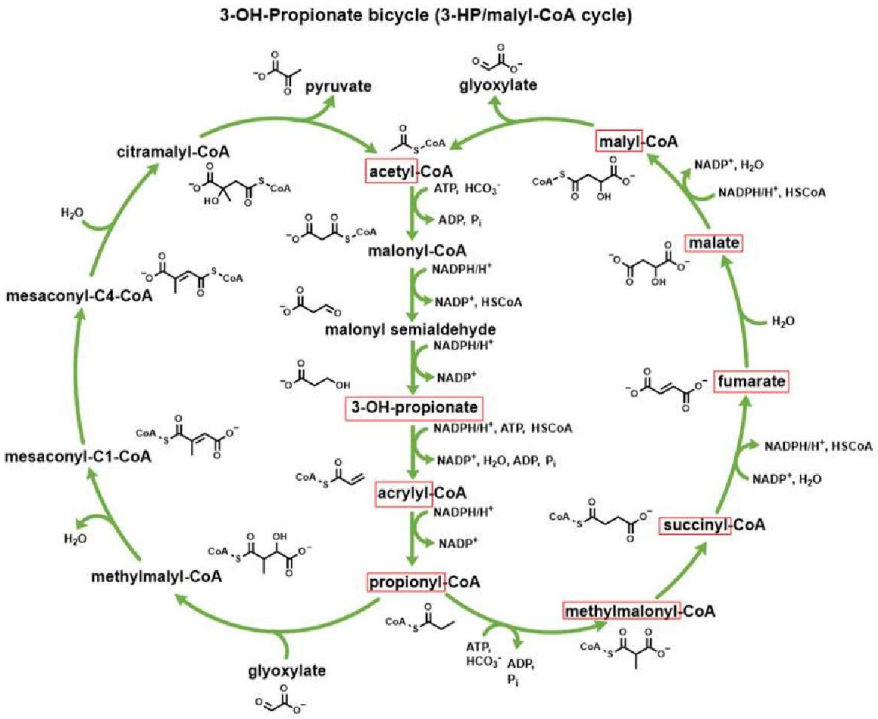


Figure S6: 3-Hydroxypropionate bicycle[17]. Metabolites highlighted by red boxes correspond to products of the NiS/HC≡CH/CO system. Graphics partly adapted from[17,44].

Additional references

39. Ljungdahl, L. G. The Autotrophic Pathway of Acetate Synthesis in Aceto Genic Bacteria. *Ann. Rev. Microbiol.* **1986**, *40*, 415-50.
40. Kitadai, N.; Kameya, M.; Fujishima, K. Origin of the reductive tricarboxylic acid (rtca) cycle-type CO_2 fixation: A perspective. *Life (Basel)* **2017**, *7*, 16.
41. Wahlund, T. M.; Tabita, F. R. The Reductive Tricarboxylic Acid Cycle of Carbon Dioxide Assimilation: Initial Studies and Purification of ATP-Citrate Lyase from the Green Sulfur Bacterium *Chlorobium tepidum*. *J Bacteriol* **1997**, *179*, 4859-4867.
42. Tully, B. J., Nelson, W. C.; Heidelberg, J. F. Metagenomic analysis of a complex marine planktonic thaumarchaeal community from the Gulf of Maine. *Environ. Microbiol.* **2012**, *14*, 254–267.
43. Berg, I. A.; Kockelkorn D.; Ramos-Vera W. H.; Say R. F.; J. Zarzycki; Hügler M.; Alber B. E.; Fuchs G. Autotrophic carbon fixation in archaea. *Nat. Rev. Microbiol.* **2010**, *8*, 447-460.
44. Zarzycki, J., Brecht, V., Müller, M.; Fuchs, G. Identifying the missing steps of the autotrophic 3-hydroxypropionate CO_2 fixation cycle in *Chloroflexus aurantiacus*. *PNAS* **2009**, *106*, 21317-21322.

7.2 Supporting Material: Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth —Implications for Early Life on Extraterrestrial Planets?

Supplemental Materials

Table S1: pH dependent formation of thiophene in the presence of NiS.

Table S2: Identified thiophene derivatives and their retention times.

Figure S1: GC/MS chromatograms comparing thiophene and commercially available thiophene derivatives.

Figure S2: GC/MS mass spectra comparing reaction products to commercially available thiophene standards and mass spectra from NIST14 library.

Table S1. pH dependent formation of thiophene in the presence of NiS. Reactions were performed with 5.36 mmol acetylene and 1 mmol freshly precipitated nickel sulfide under aqueous conditions at 105°C. Reactions were performed for 1d and pH values were measured at the end of the reaction time.

pH	H_2SO_4 [mmol]	NaOH [mmol]	Supernatant [mM]	Solid [mM]	Total [mM]
1.9	1.0	-	0.001	0.013	**0.03**
2.8	0.5	-	0.027	0.025	**0.11**
4.0	0.2	-	0.003	0.051	**0.11**
6.5	-	-	0.336	1.242	**3.16**
8.3	-	0.5	0.060	1.148	**2.42**
9.7	-	1.0	0.091	0.971	**2.02**
10.7	-	1.5	0.212	0.255	**0.94**

Table S2: Identified thiophene derivatives (x) and their retention times (RT, min) from experiments with acetylene and metal sulfides as described in Table 1. Numbers corresponding to Figure 3 are given in brackets. The amounts were not quantified, but indicated by x, if detected. The ratio thiophene to thiophene derivatives is calculated from peak areas.

Run	RT [min]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Product																			
2-Ethylthiophene (2)	7.5	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	x	-	-
3-Ethylthiophene (3)	8.2	x	-	x	x	x	x	-	-	x	x	-	x	-	-	-	-	-	-
2,3-Dimethylthiophene (4)	7.7	x		x	x	x	x	-	-	x	x	-	x						
Ethylvinylsulfide (5)	4.0	-	-	-	-		-	-		-	-	-	-	-	-	-	-	-	-
Tetrahydrothiophene (6)	6.2	x	x	x	-	x	x	x	x	-	x	x	x	-	-	-	-	-	-
3-Ethynylthiophene (7)	8.0	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
3-Thiophene thiol (8)	10.2	x	x	x	x	x	x	x	-	x	x	-	x	x	-	x	x	-	-
5-Methylthiophene-carboxaldehyde (9)	11.5	-	-	x	-		-	-	-	-	x	-	-	x	x	x	-	-	-
2[5H]-5-Methylthiophenon (10)	11.8	-	-	-	-		-	-	-	-	x	-	x	-	-	-	-	-	-
2-Vinylthiophene (11)	8.1	x	-	x	x	x	x	-	-	x	x	-	x	x	-	-	x	-	-
2-Acetyl-5-methylthiophene (12)	12.5	-	-	-	-		-	-	-	-	-	-	x	-	-	-	-	-	-
2-Acetylthiophene (13)	11.5	x	-	-	x	x	-	-	-	-	-	-	x	-	-	-	-	-	-
Thiophene-carboxaldehyde (14)	9.9	-	-	-	-		-	-	-	-	x	-	-	-	-	-	-	-	-
Cyclohex-2-enthion (15)	9.2	x	-	x	x	x	x	-	-	x	x	-	x	-	-	-	-	-	-
cis-1,4-Dithiapentalene (16)	13.5	x	-	-	x	x	x	x	x	x	x	-	x	-	-	-	-	-	-
trans-1,4-Dithiapentalene (17)	13.6	x	-	x	x	x	x	-	-	x	x	-	x	-	-	-	-	-	-
Benzo[*b*]thiophene (18)	13.3	x	-	x	x	x	x	-	-	x	x	-	x	-	-	-	-	-	-
Ratio thiophene : Σthiophene derivatives		**12.5**	**<0.1**	**0.3**	**9.6**	**19.3**	**2.7**	**<0.1**	**0.8**	**25.4**	**1.3**	**0.1**	**3.2**	**17.9**	**0.1**	**2.8**	**1.0**	**-**	**-**

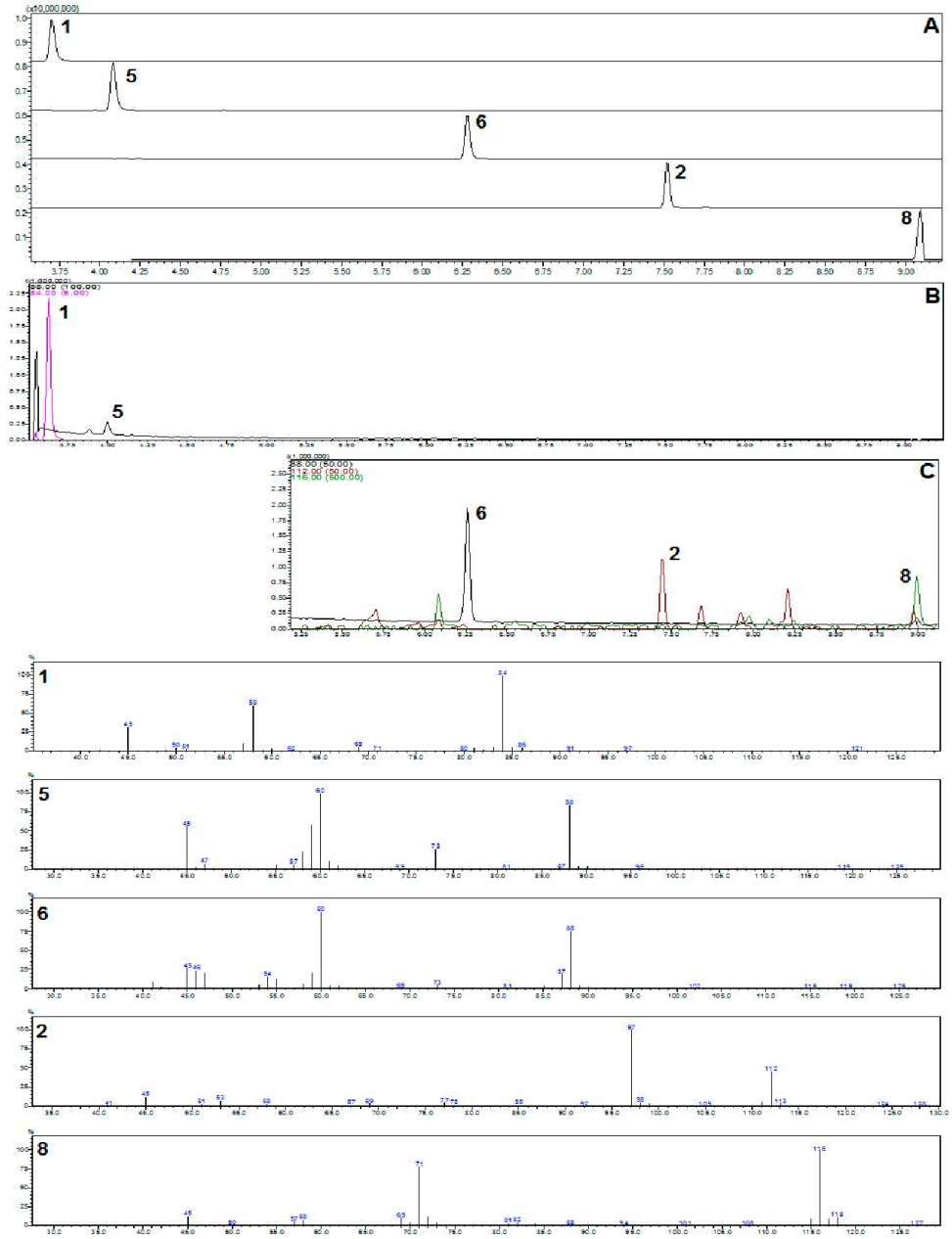
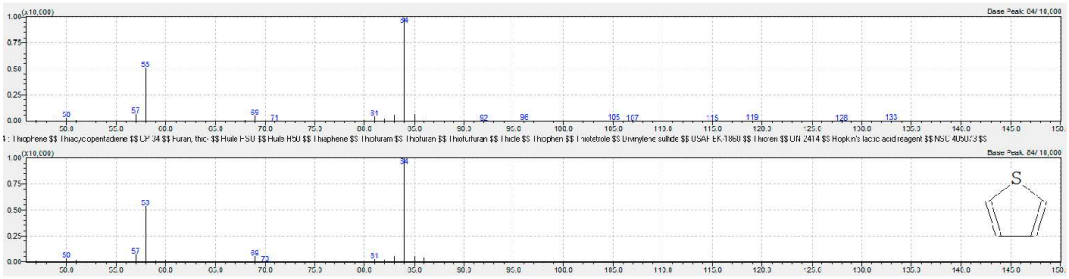
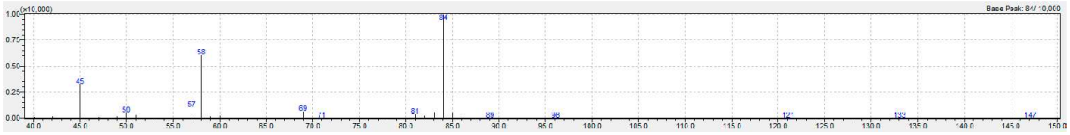


Figure S1: **GC/MS chromatograms comparing thiophene and commercially available thiophene derivatives** (**A**) to reaction products (**B,C**). Numbers correspond to structures in Figure 3

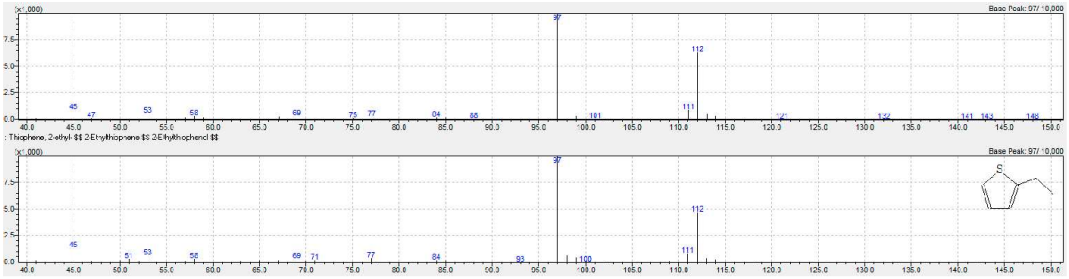
Thiophene (1) **similarity to NIST14 database: 96%**



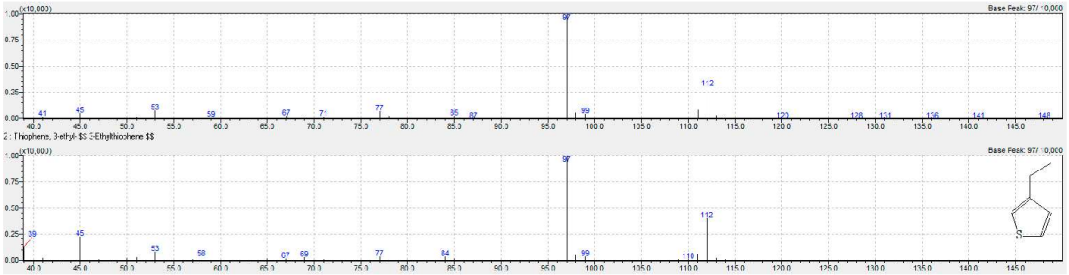
Thiophene analytical standard (Sigma Aldrich)



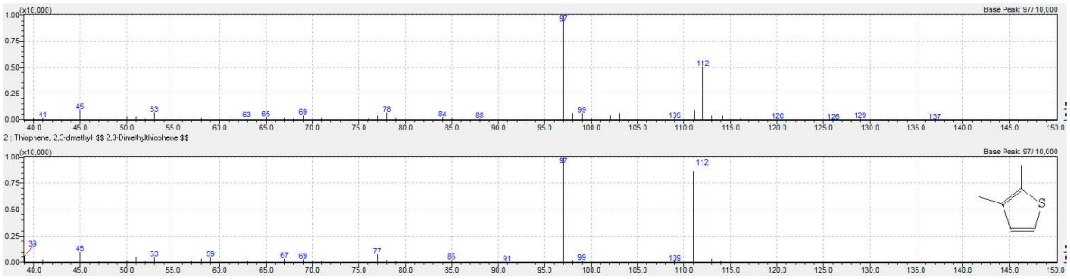
2-Ethylthiophene (2) **similarity to NIST14 database: 93%**



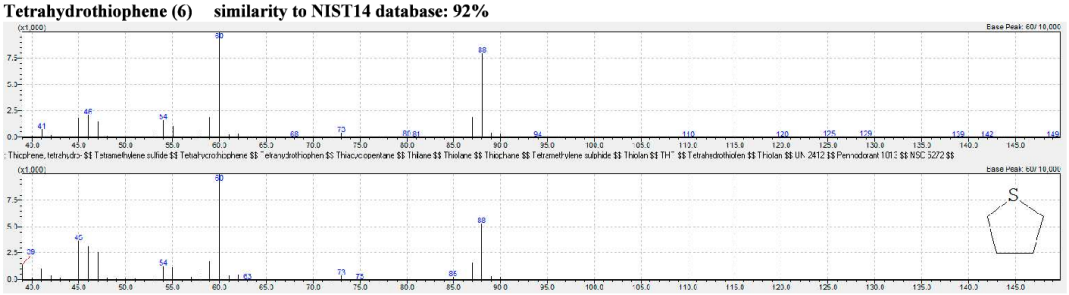
3-Ethylthiophene (3) **similarity to NIST14 database: 91%**



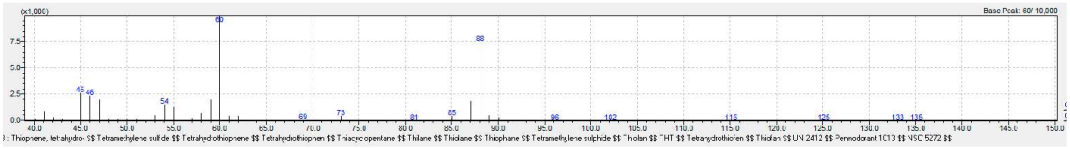
2,3-Dimethylthiophene (4) similarity to NIST14 database: 82%



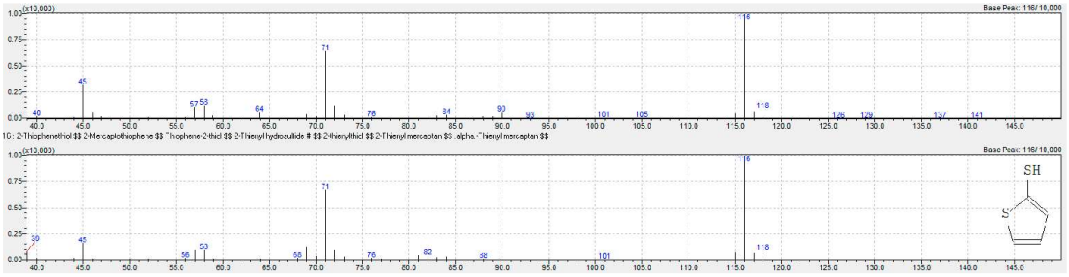
Tetrahydrothiophene (6) similarity to NIST14 database: 92%



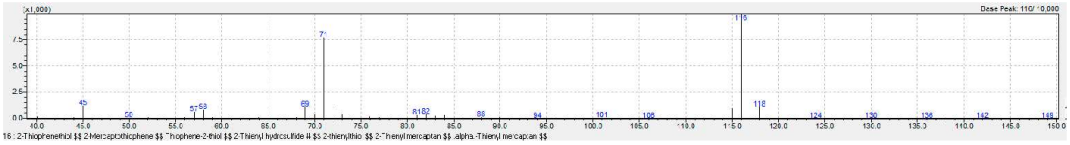
Tetrahydrothiophene analytical standard (Sigma Aldrich)



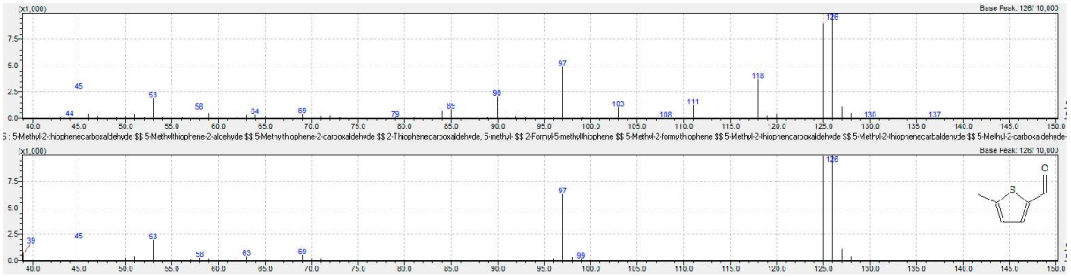
2-Thiophenthiol (8) similarity to NIST14 database: 93%



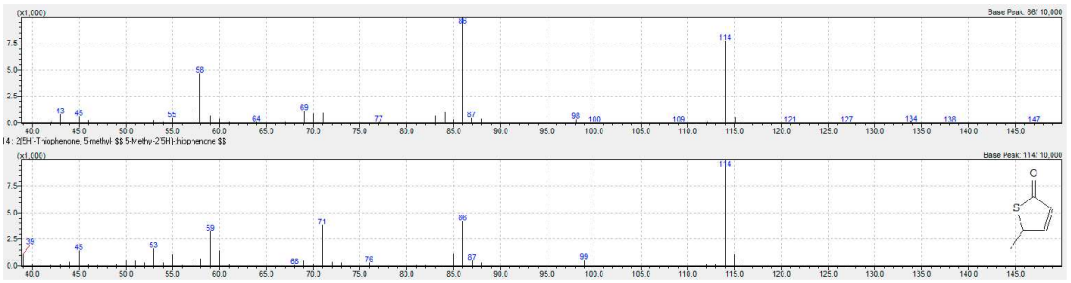
2-Thiophenthiol analytical standard (Sigma Aldrich)



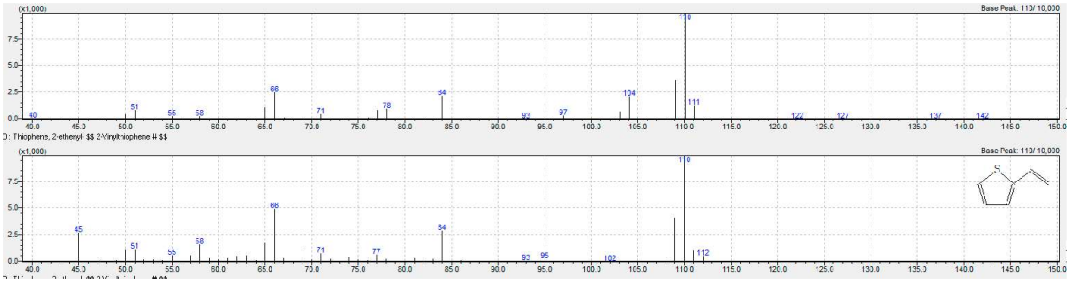
5-Methylthiophene-carboxaldehyde (9) similarity to NIST14 database: 82%



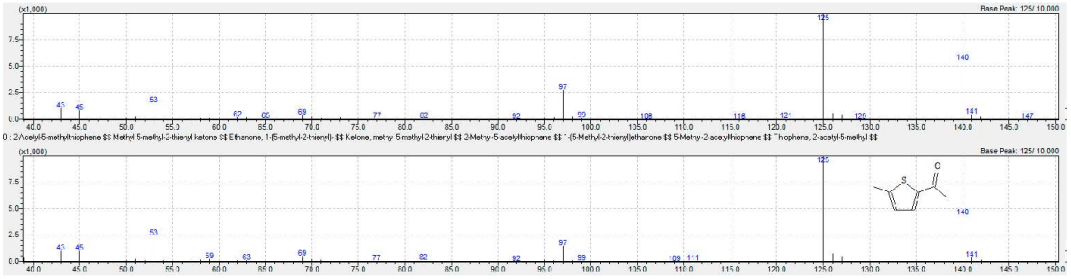
2[5H]-5-Methylthiophenon (10) similarity to NIST14 database: 77%



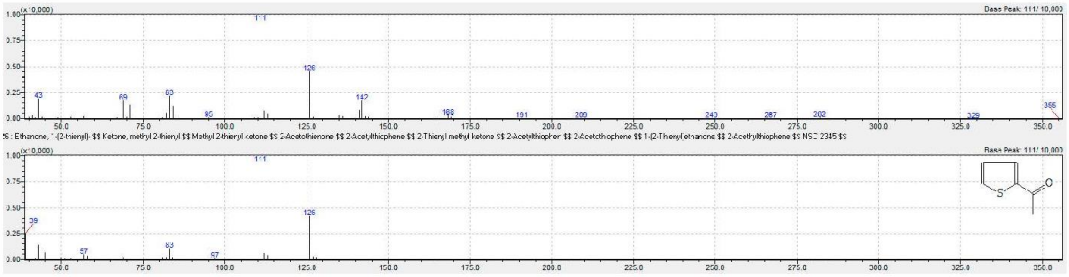
2-Vinylthiophene (11) **similarity to NIST14 database: 81%**



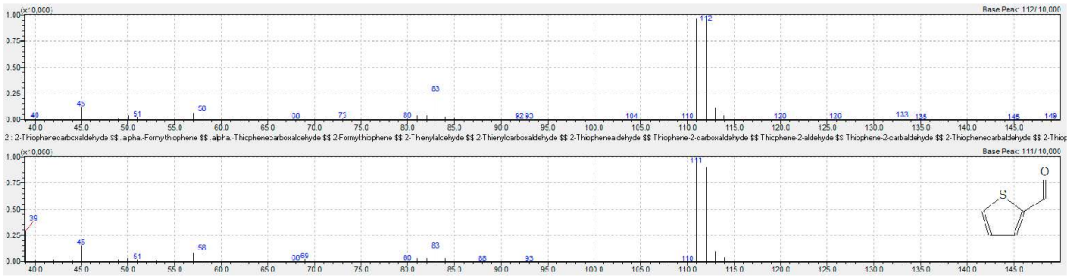
2-Acetyl-5-methylthiophene (12) **similarity to NIST14 database: 93%**



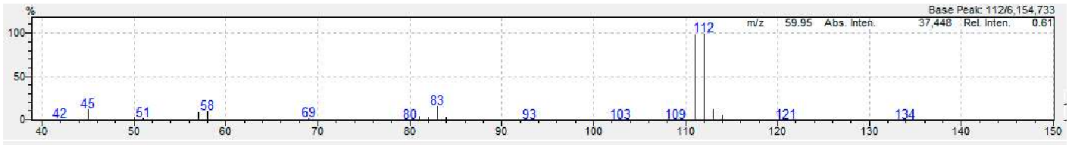
2-Acetylthiophene (13) **similarity to NIST14 database: 78%**



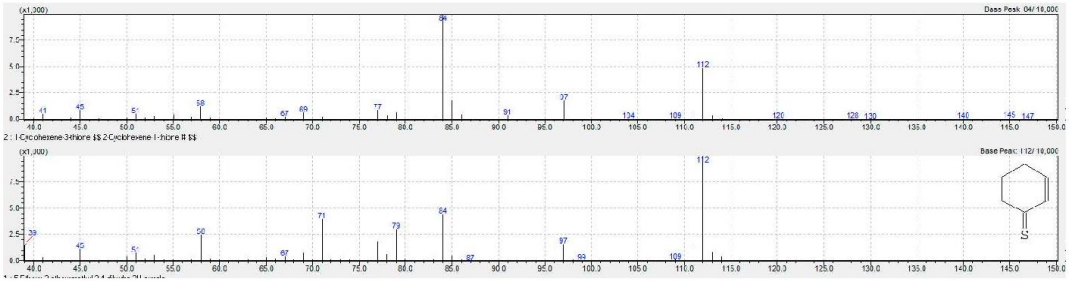
Thiophene-2-carboxaldehyde (14) similarity to NIST14 database: 84%



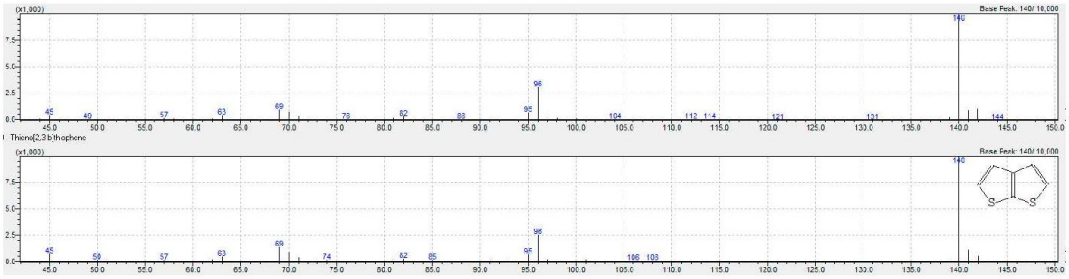
Thiophene-2-carboxaldehyde analytical standard (Sigma Aldrich)



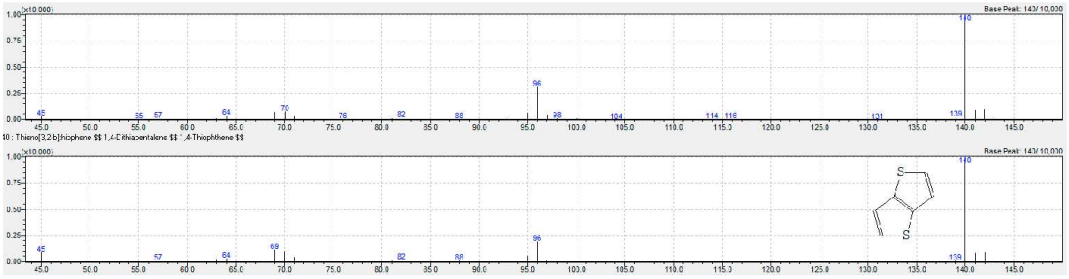
Cyclohex-2-enthion (15) **similarity to NIST14 database: 78%**



cis-1,4-Dithiapentalene (16) **similarity to NIST14 database: 89%**



trans-1,4-Dithiapentalene (17) **similarity to NIST14 database: 92%**



Benzo[*b*]thiophene (18) similarity to NIST14 database: 97%

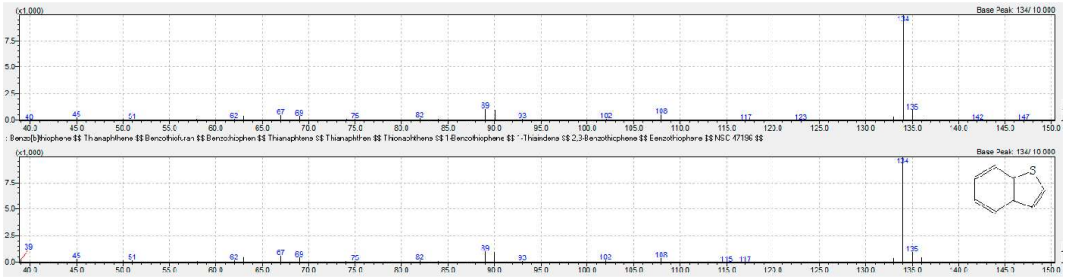


Figure S2: GC/MS mass spectra comparing reaction products to commercially available thiophene standards and mass spectra from NIST14 library. Numbers corresponding to Figure 3 in brackets.

8 Publications & Conference Contributions

8.1 Journal Articles

Geisberger, T.; Sobotta, J.; Eisenreich,W.; Huber, C. Formation of Thiophene under Simulated Volcanic Hydrothermal Conditions on Earth—Implications for Early Life on Extraterrestrial Planets? *Life* **2021**,*11*, 149.

DOI: 10.3390/life11020149

Basiouni, S.; Fayed, M.A.A.; Tarabees, R.; El-Sayed, M.; Elkhatam, A.; Töllner, K.-R.; Hessel, M.; **Geisberger, T.**; Huber, C.; Eisenreich, W.; Shehata, A.A. Characterization of Sunflower Oil Extracts from the Lichen *Usnea barbata*. *Metabolites* **2020**, *10*, 353.

DOI: 10.3390/metabo10090353

Sobotta, J.; **Geisberger, T.**; Moosmann, C.; Scheidler, C.M.; Eisenreich, W.; Wächtershäuser, G.; Huber, C. A Possible Primordial Acetyleno/Carboxydotrophic Core Metabolism. *Life* **2020**, *10*, 35.

DOI: 10.3390/life10040035

Ritter, S., Urmann, C., Herzog, R., Glaser, J., Bieringer, S., **Geisberger, T.**, Eisenreich, W. & Riepl, H. (2020). Where is Bacosine in commercially available Bacopa monnieri? *Planta medica*, *86*(08), 565-570.

DOI: 10.1055/a-1137-4289

Geisberger, T.; Diederich, P.; Steiner, T.; Eisenreich, W.; Schmitt-Kopplin, P.; Huber, C. Evolutionary Steps in the Analytics of Primordial Metabolic Evolution. *Life* **2019**, *9*, 50.

DOI: 10.3390/life9020050

Kuhn, V., **Geisberger, T.**, Huber, C., Beck, A., & Eisenreich, W. (2019). A facile in vivo procedure to analyze metabolic pathways in intact lichens. *New Phytologist*, *224*(4), 1657-1667.

DOI: 10.1111/nph.15968

8.2 Posters

10/2018 Molecular Origins of Life, Munich. Poster on “Formation of biomolecules under volcanic hydrothermal vent conditions and investigation with high-resolving analytical tools”

02/2019 Annual retreat CRC 235, Brixen. Poster on “Chemical evolution of biomolecules formed under volcanic hydrothermal conditions”.

02/2020 Annual retreat CRC 235, Brixen. Poster on “Chemical evolution of biomolecules formed under volcanic hydrothermal conditions”

07/2020 Molecular Origins of Life, Munich. Poster on “Chemical evolution of biomolecules formed under volcanic hydrothermal conditions”