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Experimentální studium chemické evoluce biomolekul v podmínkách rané Země
Experimental study of chemical evolution of biomolecules under early Earth conditions

Diplomová práce

Školitel: RNDr. Martin Ferus, Ph.D.

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**Charles University**

**Faculty of Science**

Physical Chemistry



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Prohlašuji, že jsem vypracoval tuto diplomovou práci samostatně pod vedením svého školitele RNDr. Martina Feruse, Ph.D. a všechny zdroje jsem řádně citoval. Prohlašuji také, že jsem tuto práci ani její podstatnou část nepoužil k získání jiného nebo stejného akademického titulu.

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# Abstract

Origin of life is a still-enduring gap in human knowledge. This work is focused on revealing of several pieces of this puzzle. State of the art scenarios of biomolecules synthesis under prebiotic conditions are presented and discussed.

This thesis presents our recent experiments suggesting a novel idea that neutral planetary atmosphere containing a mixture of neutral volcanic-type gasses ($CO_2$, $N_2$, $H_2O$) can be converted over acidic mineral catalysts upon irradiation by a soft UV-radiation into a relatively reactive mixture of reducing gases ($CH_4$, $CO$), which can be further reprocessed by high-energy chemistry. The resulting mixture ($CH_4$, $CO$ + $N_2$) represents a common reducing atmosphere related e.g. to the chemistry of Titan, the largest moon of Saturn, as well as a possible representation of the secondary atmosphere of our planet. Also, photocatalytic reduction of $CO_2$-rich atmosphere can explain the abiotic origin of methane on current Mars or other terrestrial planets. In our subsequent experiments, corresponding equimolar model mixture of $CH_4$ : $CO$ : $N_2$ in presence of water vapour was subjected to reprocessing by high-power laser plasma simulating an asteroid impact – one of a series of impact events which the young Earth experienced during the first 600 million years of her history. Upon delivery of in total 3250 J of laser shock-wave energy into the sample, formation of organic molecules closely related to prebiotic chemistry was observed. Among those are the nucleic acid bases of RNA (adenine, uracil, guanine and cytosine), glycine and urea. For the first time, a complete scenario of one pot HCN-based synthesis of all the canonical nucleobases, the simplest amino acid glycine and urea is presented. The results are thoroughly discussed, their prebiotic plausibility is evaluated and our explorations are compared to other experiments performed by our team as well as by the other research groups.

**Keywords:** Origin of life, nucleic acid bases, photocatalytic reduction of carbon dioxide, methane, FTIR spectroscopy

# Abstrakt

Vznik života na Zemi je jedním z prázdných míst lidského vědění. Tato práce je zaměřena na odhalení několika dílků této skládačky. Prezentujeme současný stav poznání zejména ve spojení se syntézou biomolekul za prebiotických podmínek.

V této práci jsou předloženy výsledky experimentů, jež naznačují, že neutrální planetární atmosféra obsahující představitele vulkanických plynů ($CO_2$, $N_2$, $H_2O$) může být působením měkkého UV záření v přítomnosti minerálních katalyzátorů přeměněna na relativně reaktivní směs redukovaných plynů ($CH_4$, CO). Ty mohou být dále transformovány vysoce energetickými procesy za vzniku biomolekul. Směs $CH_4$, CO + $N_2$ představuje prototyp běžné redukční atmosféry, jíž podobné lze nalézt např. na Titanu, největším Saturnově měsíci, nebo také v minulosti na naší planetě coby tzv. sekundární atmosféru. V následných experimentech byla ekvimolární směs $CH_4$ : CO : $N_2$ v přítomnosti vodní páry exponována vysoce energetickému plazmatu, jež simuluje dopad asteroidu – jednu ze série impaktních událostí, kterým byla raná Země vystavena během prvních 600 milionů let své existence. Po dodání celkové energie 3250 J v laserových pulzech byla zjištěna přítomnost organických molekul důležitých pro prebiotickou chemii. Mezi nimi jsou např. báze ribonukleové kyseliny (adenin, uracil, guanin, cytosin), glycin a močovina. Ucelený scénář syntézy těchto molekul v jednom systému z HCN je zde předložen vůbec poprvé. Výsledky jsou podrobně rozebrány ve vztahu k současnému stavu poznání, odbornou literaturou a je kriticky posouzen jejich význam ve vztaku k prebiotické chemii. Dále jsou dosažené výsledky dány do kontextu s experimenty provedenými jak naším týmem, tak výzkumnými skupinami z celého světa.

**Klíčová slova:** Vznik života, báze nukleových kyselin, fotokatalytická redukce oxidu uhličitého, methan, FTIR spektroskopie

# Table of Contents

# 1   Introduction

The chemical origin of life has been a topic of research for more than a century and a topic of speculation for more than two millennia. The question of the origin of mankind as well as of all the whole biosphere has prompted in the past many scientific thoughts and influenced many lives, including great thinkers such as Charles Darwin, Alexander I. Oparin, J.B.S. Haldane, Louis Pasteur or even Aristotle. Contemporary science addresses this enigma with the help of physics, mathematics, chemistry, biology, astronomy and geology. In the following chapters, selected state of the art theories and chemical scenarios ranging from formation of the Solar System to evolution of the first living structure – probably molecular life based on replication of the RNA acid – are described.

## 1.1   Formation of the Solar System

The formation of the Solar System started 4.6 billion ($10^{12}$) years ago (Gya) with the creation of a star forming region in a giant molecular cloud (about 20 parsec across). This molecular cloud was created by a supernova explosion more than 6.5 Gya and enriched by the heavy elements during this event. Figure 1 shows an envelope of heavy elements released by supernova exploded around 1680 A.D. and observed now by the satellite Chandra in Cassiopeia A. This explosion dispersed about 10,000 Earth masses of sulfur, 20,000 Earth masses of silicon, 70,000 Earth masses of iron and one million Earth masses worth of oxygen.
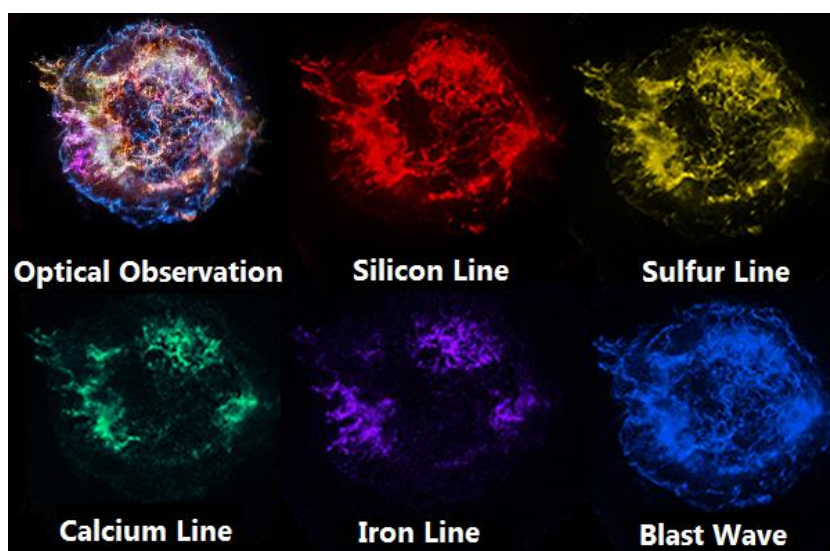


Figure 1. Envelope of the supernova Cassiopeia A as observed by the Chandra X-ray Observatory (http://chandra.si.edu/elements/, credit NASA).

In our parent molecular cloud, the local increase in density, which preceded the formation of our parent "stellar nursery," was triggered probably by an explosion of another nearby supernova [1]. The collapsing and rotating regions in the cloud became increasingly hot and dense in their centres and many protostars were formed. The outer regions around a particular protostar are referred to as protoplanetary discs, which contain regions exhibiting formation of many embryos of protoplanets. Within 50 million years of the beginning of the molecular cloud's collapse, the heat in the protostellar centre triggered a hydrogen fusion, thus creating an internal source of energy strong enough to balance out the gravitational pressure from the collapsing region [2] and the young Tauri-type protostar, our Sun, became a main sequence G2V type star [3]. At the same time, dust particles in the protoplanetary disk began to collide and to form first coarse grains (up to 200 m in diameter) through the process of accretion, then planetesimals (up to 10 km in diameter) and finally planets. The inner Solar System (up to 4 AU from the Sun) was too hot to allow volatile compounds such as water, carbon dioxide, carbon monoxide, ammonia, nitrogen or methane and other organic substances to condense and therefore this region contains rocky planets. Giant gas planets formed in the outer Solar System beyond the frost line [4].

It is assumed that volatiles have been delivered to early rocky planets from outer, colder parts of the Solar System by impacting comets and more probably primitive chondritic asteroids. Recent findings show that this happened relatively soon after the formation of proto-planets and some kind of atmosphere and hydrosphere was present on Earth before and nearly immediately after the Moon forming impact of Theia (4.4 to 4.45 Gya) [5]. However, direct evidence of the existence of some kind of hydrosphere on Earth is provided only by 4.35 Gya zircons [6].

In connection with extraterrestrial delivery of volatile compounds to the atmosphere and hydrosphere, exogenous delivery of organic compounds must also be questioned. In the past decades, many organic molecules have been detected in the Solar System and in other systems throughout the Universe, such as methanol, HNCO or formamide. Their list is shown in Table 1. These molecules have been present in the Solar System since its formation as remnants of material ejected during the parent supernova explosion about 6.5 Gya or synthesized in giant molecular clouds during its 2 Gya long existence before the formation of our Solar System.

Table 1. Important molecules related to prebiotic chemistry detected in space so far.

| Compound | Year of detection | Compound | Year of detection |
|---|---|---|---|
| $C_2$ | 1995 | $NH_2CN$ | 1975 |
| $\cdot CH$ | 1941 | $C_2H_4$ | 1981 |
| $\cdot CN$ | 1941 | $HCONH_2$ | 1989 |
| $CO$ | 1970 | $CH_3CHO$ | 1997 |
| $HCN$ | 1971 | Glycolaldehyde | 2006 |
| $C_2H_2$ | 1976 | Urea | 2014 |
| $HNCO$ | 1984 | Ethanol | 1975 |
| $NH_3$ | 1984 | Acetone | 1984 |
| $CH_3$ | 1991 | Ethylene glycol | 2002 |
| $HCOOH$ | 1971 | Glycine | 2003 |

Many such compounds have been discovered in samples of primitive meteorites (such as Murchinson, Allende, Murray, Paris, Monahans or Zag) and old comets (e.g. Churyumov-Gerasimenko, Halley, Hale-Bopp ISON, P-Borrelly, P/Wild 2 or Lemmon). These compounds may lead even into the production of nucleic acid bases [7]. There are a few possible synthetic pathways of their creation, such as aqueous alteration of a parent body [8] or **Fischer-Tropsch** synthesis in protoplanetary discs [9]. The low D/H ratio in some of these, however, points to low-temperature synthesis [10]. Laboratory experiments show the possibility of the synthesis of organic material from mixtures of simple compounds (such as e.g. $H_2O$, $CO$, $CO_2$, $NH_3$, $CH_3OH$ or $CH_4$) upon UV irradiation [11], laser sparks or electric discharge plasma [12]. The low-temperature UV-driven synthesis is relevant to the outer region of the protostar nebula, where the temperatures are low enough to allow ices to form and where, due to the protoplanetary disc's conical shape, the surface of the disk is exposed to the UV radiation from the parent star or protostar [13]. On the other hand, the high-temperature high-energy density synthesis, such as in the plasma and electric discharges is relevant to the inner parts of the protoplanetary disc, where friction between particles both increases the local temperature and initiates discharges such as in dust storms [12], [14]. Complex organics could therefore be produced in discs around all stars and made available to the surface

of planets forming in the system where they could play a role in the origin of life [13]. Among the observed molecules in the protoplanetary cloud around young stars, such as sun-type triple star IRAS 16293-2422 A1/A2 and B in the Ophiuchus constellation and one of the Orion protostellar cores are dimethylether, $C_2H_3CN$, $C_2H_5CN$, ethanol, $CH_3CN$, methanol, formaldehyde, isocyanic acid, methyl formate, OCS, sulphur oxide, sulphur dioxide, $CH_3CHO$, carbon monoxide, HCN, formic acid, formamide (in Sagittarius B), acetonitrile or methylisocyanate [15]–[17]. Typical representatives of young stars and their protoplanetary discs are shown in Figure 2.
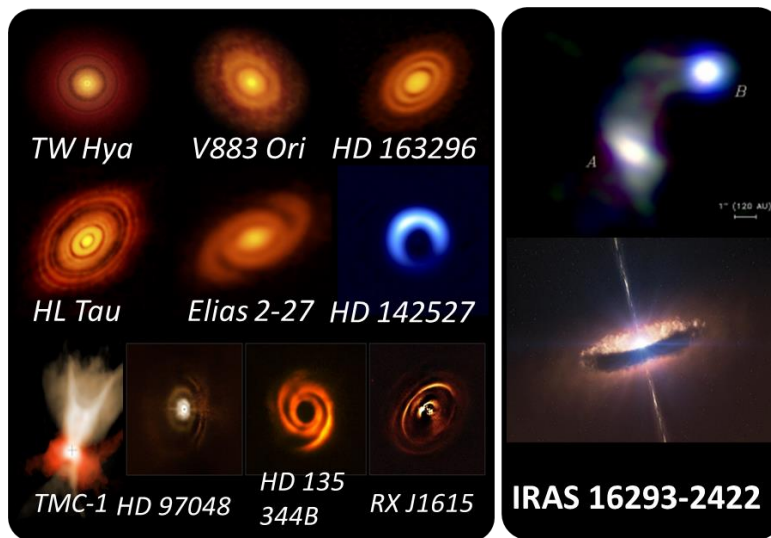


Figure 2. Protoplanetary disks around young stars – formation of planets in chemically rich environments (graphical material for this composition was taken from https://almascience.eso.org/alma-science/planet-forming-disks, credit ALMA).

## 1.2 Heavy bombardment

Apart from the synthesis of molecules during early stages of protoplanetary disk formation, high energy density chemistry occurs during the periods of Early a Late heavy bombardment. The Early heavy bombardment is a period presumably common to all young planetary systems as it is caused by the planet orbiting through the protoplanetary disk full of debris material [18]. The impact flux on the planet's surface since this period generally exponentially decreases. In our Solar System, however, between 4.0 to 3.8 billion years ago, Jupiter and Saturn exhibited orbit frequencies very close to 2:1 ratio and once per every Saturn's orbit they aligned in space creating a gravitational nudge towards Uranus. Uranus was thus ejected to longer orbit and collided with asteroid and cometary belt. Thus, many smaller bodies were ejected from

their trajectories and bombarded the whole of the Solar System for ca 200 million years. This period is referred to as 'Late Heavy Bombardment' (LHB) and was established through the NICE model [18]–[21]. The LHB is documented by the craters on the Moon (on Earth, all craters from this period have already been smeared by plate tectonics and erosion). During this period, the Earth sustained prolonged bombardment by extraterrestrial bodies, which brought water and other volatile compounds, as well as a lot of energy, and initiated a complex chemistry. It is worth noting that the first records of life date back to this period as well [22], which suggests a possible link between this environmental transformation and the prebiotic chemistry.

## 1.3   Evolution of global planetary chemistry

Refractory carbonacesous material detected or expected on terrestrial planets is only a fraction of the organic substances observed throughout space, as most of it is located in interstellar molecular clouds and nebulae. Therefore, not only volatile substances but also organic H-N-C-O-containing compounds may have been delivered from extraterrestrial environment. On the other hand, impact plasma reprocessing is not yet well described in detail and an exploration of balance between *de novo* synthesis and decomposition of organic substances in impact plasma is a great challenge for future research. Whatever their origin, vast quantities of interstellar grains bearing organic molecules enter the Solar System every moment and up to tons of material containing reduced organic carbon enter the Earth's atmosphere every day in form of cosmic dust or small meteoroids [23].

Other expected powerfull source of organic H-N-C-O-containing compounds may include processes of endogenous organic synthesis *in situ* from simple molecular gases or prebiotic feedstock molecules such as HCN and its polymers, formamide, ferrocyanides etc. This approach reflects the original assumptions of Oparin, Miller etc.

The endogenous synthesis of biomolecules from simple precursors is viable to a certain extent in both reduced and oxidized environment. The influence of the composition of the early Earth's atmosphere and its reduction/oxidation state are subject to an ongoing debate. The paleoclimate is a whole field of Earth sciences and most of its results are based on the isotope ratios found in various minerals.

On the early Earth, terrestrial surface water was below the critical point within 100 million years after its formation, so liquid water was present on the young planet [24].

During the first 100 – 500 Myr, the Earth lost its hydrogen envelope of moderate mass, which may have acted as a shield against possible atmospheric deterioration by the Solar wind [25]. Also, the planet was exposed to frequent impacts of extraterrestrial bodies. At the latter stage of the planetary evolution, the impacts became sparser and the collisions larger, able to boil oceans or at least the upper few hundred meters of the ocean [26].

Overall, opinions prevail that a highly reduced global atmosphere is unlikely, while a weakly reduced atmosphere (mainly $CO_2$, $N_2$, small amounts of $H_2$, $CH_4$, $NH_3$) is very likely [27]–[30]. The reduction/oxidation state of the atmosphere is possible to partly determine by the $^{18}O/^{16}O$ ratio. This ratio is well determined for the Earth's mantle and is fairly stable. On the other hand, the ratio in crustal regions is very variable. The best way is to measure the ratio in zircons. Zircon (zirconium silicate, $ZrSiO_4$) is a mineral found primarily in volcanic rocks, which can be readily dated using the uranium-lead isotopic system. Zircons are generally resistant to metamorphism in the crust and are very durable in terms of mechanical qualities [31]. Trail et al. [32] concluded from the analysis of several igneous zircons that the Hadean continental magmatic crust and its outgassing would not lead to overall reduced atmosphere. Yang et al. [33] take this evidence even further, claiming that the Earth's crust during its accretion was strongly reduced and underwent gradual oxidation until the end of the Hadean (up to 3.8 Gya), as is shown in Figure 3.
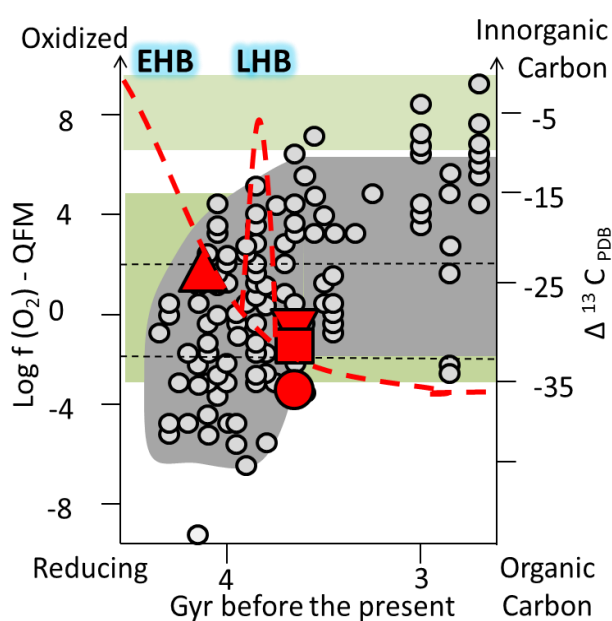


Figure 3. Oxidation state of the continental crust calibrated by the Ce-based oxybarometer by Yang et al. [33]. Results in terms of log of oxygen fugacity of quartz–fayalite–magnetite (QFM) are depicted in circles. The corresponding decrease of impact frequency during Early and Late heavy bombardment (EHB and LHB, red dashed curve) and first evidences of fossilized presumably organic carbon with typical biological $^{13}C$ deficiency are also schematically embedded [34],[35].

During the Hadean, the outgassing of basaltic magmas could have produced local deposits of reduced crust, but their presence would not be enough to transform the atmosphere to globally reduced state once again. Therefore, the second half of the Hadean (4.2-3.8 Gya) saw the Earth's atmosphere overall oxidized ($CO_2$, $N_2$) with admixtures of $H_2$. This complies with the findings of Kasting et al. [36], who claims that the paleotemperature at that era (determined by oxygen isotopic abundance in minerals) points towards an atmospheric mixture of $CO_2$ and $CH_4$.

There are only few locations in the world, which still bear record of ancient geology. The mineralogy of the Hadean and Archean is therefore difficult to determine. Still, meteorites typically contain around 60 different mineral phases and these are taken to be the starting points of planetary geology evolution [37]. This infers that the more than 4400 known minerals on Earth must have been created later, 1500 of which have emerged by the end of the Archean [38]. The first crust on Earth was of mafic composition and the first granitic minerals resulted from its partial melting and cooling down. These are composed mostly of quartz, plagioclase, biotite and K-feldspar. Earth's oldest minerals are detrital zircons from the Jack Hills, some of which have yielded ages up to 4.4 Gya. Their presence infers that the granitic continental crust was produced as early as 200 million years after Earth's accretion. Also, the oxygen isotopes are rather heavier in these minerals, which suggests that early Hadean oceans were involved in the recycling of continental crust during subduction [6]. The presence of granitoid minerals and oceans participating in the subduction is central to the carbon cycle on Earth through water alteration in anoxic environment. The boom in the diversification of minerals came only in the Paleoproterozoic (2.5-1.6 Gya) [39]. Volcanic activity was also larger and the magma hotter [40]. The oceans were anoxic and this resulted in their enrichment in silt, carbonate, chert, iron-rich sediments and evaporite [39]. Therefore, molecules of life must have arisen in much simpler and significantly different environment than we encounter today.

## 1.4   Synthesis of biomolecules on early Earth

Sources of organic molecules on Earth can be divided into two categories: source provided by the exogenous synthesis, i.e. delivery by extraterrestrial objects, and endogenous synthesis in terrestrial conditions, i.e. impact-driven synthesis and organic synthesis driven by energy with terrestrial or extraterrestrial origin (such as electrical discharges, volcanic activity and thermally driven synthesis or UV radiation) [41].

### 1.4.1 Exogenous delivery or organic molecules to Earth

Organic matter is delivered to Earth even today, mostly in the form of interplanetary dust particles small enough to be decelerated by the atmosphere not to ignite or by asteroids large enough not to be ablated completely during the descent [42]. A third minor source is represented by asteroids, which break apart during the descent, because many of the fragments then don't have sufficient energy to heat up their cores [43] as was demonstrated first in the case of the Revelstoke C1 chondrite in Canada in 1965. Table 1 on page 11 shows a list of the most important organic molecules yet detected in space. The specific fluxes of delivery have been concisely compiled and published by Christopher Chyba and Carl Sagan in 1992 [41]. More recently, Zita Martins et al. have embarked on the search for extraterrestrial biomolecules in chondritic meteorites, such as the Murchinson meteorite [7] and Paris meteorite [44], which is a representative of the most primitive CM chondrites. Asteroid and cometary delivery are principally the same, but can be distinguished by their specific H/D, $^{14}N/^{15}N$ or $^{13}C/^{12}C$ ratios. Related explorations show that the early Solar System had already contained a wide range of abiotically formed organic compounds before life was seen on Earth. For example, Martins et al. show that carbon isotope ratios in uracil and xanthine discovered in Murchinson meteorite prove a non-terrestrial origin of these compounds [7]. This refractory material was eventually in most part incorporated into asteroids or planetary bodies and during the accretion and during the heavy bombardment eras. On planets, these compounds were long transformed by tectonics, volcanic activity, erosion and other geological processes. Asteroids and hence meteorites, therefore, present us the only records of the early organic composition and chemistry in the Solar System [45]. The organic synthesis itself in prestellar cores has probably taken place on the cooler outskirts of the prestellar nebula [46]. In the interstellar medium, the synthesis occurs in the gas phases as well as on interstellar grains, which are often composed of, or at least contain, $H_2O$, $CH_4$, $NH_3$, $CO$, $CO_2$, $CH_3OH$, $HCOOH$, $HCONH_2$, etc. [47]. The grains also often contain more complex carbonaceous refractory material. The grains are exposed to varied conditions, such as different temperatures, electromagnetic radiation or proton irradiation during their journey through space, all of which can ignite a complex organic synthesis [13], [48], [49].

The research in our laboratory is mostly focused on the hypothesis concerning the endogenous synthesis conjecture as the most probable scenario of prebiotic synthesis. In

line with our explorations, the rest of this work is largely focused on experiments on the endogenous synthesis of biologically important molecules on Earth, such as nucleic acid bases or amino acids.

## 2   Theory

The first successful synthesis of organic molecules from inorganic substrates was the famous Wöhler synthesis in 1828 [50], shown in figure 4. This reaction is considered the starting point or modern synthetic organic chemistry. The reaction also laid the first foundations of theories connected to scientific explanation of the origin of life.
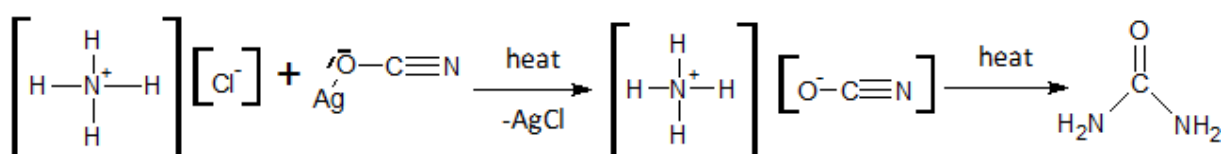


Figure 4. Synthesis of urea by Friedrich Wöhler in 1828. The synthesis starts with heating of ammonium chloride and silver cyanate and proceeds through ammonium cyanate to urea.

In 1924, a Russian biochemist Alexander I. Oparin postulated the theory of coacervates and the origin of life from abiotic environment [51]. He first proposed that the origin of life must first have started with simple chemical reactions, then subsequently pass to biochemical processes and then to biology.

Following the purely theoretical works of A. I. Oparin, the era of experimental prebiotic chemistry began in 1953 with the works of Stanley Miller and his supervisor Harold Urey [52]. They demonstrated experimental synthesis of amino acids from simple gaseous precursors, i.e. methane, CO, water, ammonia and hydrogen [53]. Although delivery of organic matter from space (comets, meteorites, dust particles) contributed to the diversity of organic matter on the early Earth (as mentionded above), there exist serious doubts about the explanation of the origin of life purely by exogeneous impact fluxes of biomolecules ready for prebiotic synthesis [41].  Therefore, an experimental explanation of the chemical origin of prebiotic molecules is neccessary. At the time of Miller, the most popular theory was the theory of the primordial soup (concentrated solution of organic compounds supplemented by atmospheric reactions) [51], [54]. Simultaneously with the Miller's synthesis, the theory of the RNA world was developed

[55]. In this theory, the starting molecules of the emerging life are nucleobases and not amino acids. Moreover, after the 1970s, the emergence of life near deep-sea hydrothermal vents sprouted [56] and became a concurrent of previous assumption of origin of life in primordial soup concentrated in sea coast lagoons. So far, no theory has been confirmed with absolute surety and current theories are unable to determine neither the set of compounds nor their quantities during the chemical evolution. However, original ideas discussing origin of life in lagoons, hydrothermal vents, volcanoes etc. are coming back as fashion waves. What remains clear is that the palette of prebiotically relevant compounds may be different from the palette of biogenic compounds present on Earth today [57].

In laboratory experiments, to achieve detectable levels of compounds, highly reactive and concentrated species are often used. Such concentrations can hardly be expected at the early Earth, but on the other hand, the scale of the reaction on a planet is completely different from that in the laboratory. For example, during the prebiotic synthesis, high concentrations of selected reactants/intermediates/products might have been possible to obtain in evaporating ponds or freezing solutions, but the concentrations in the general environment must have been very low.

Moreover, prebiotic experiments are often conducted with small number of pure compounds, which, again, is not the case of complex chemical environments. The selection of only a small number of reactants mitigates the effect of side reactions, which would potentially occur and therefore artificially raises product levels obtained in experiments. It is therefore important to always consider the relevance of the experiment towards the prebiotic conditions on terrestrial planets. The synthesis of prebiotic compounds, looked upon from the chemists' bottom-up view, may be separated into several specific problems, i.e. the synthesis of amino acids, nucleic acid bases, lipids and their co-evolution.

It is also worth noting that although the aim of prebiotic chemistry is to explain the origin of life on Earth, the explored pathways are viable in any body anywhere in the universe if the desired conditions are met. The origin of life is thus not limited to Earth only, but the chemistry is applicable everywhere.

## 2.1 Synthesis of proteins

Proteins mostly comprise of 22 amino acids (20 common + selenocysteine and pyrrolysine). It is not, however, necessary that these amino acids were the first (possibly glycine, alanine, later valine together with aspartic acid coded by GC respectively GNC code [58] and even later proline, serine, threonine were first and were followed by other canonical amino acids)  or the only that have been present in living structures and the questions on the evolution of amino acids are still open [59].

### 2.1.1 Synthesis of amino acids

The most famous synthesis of amino acids under prebiotic conditions was demonstrated by the experiment of Miller and Urey from 1953 [52]. Their experiment consisted of two vessels, one containing an aqueous solution (the primordial soup) and the other a reduced atmosphere (originally a mixture of $CH_4$, $NH_3$, $H_2O$ and $H_2$), which is depicted in Figure 5.
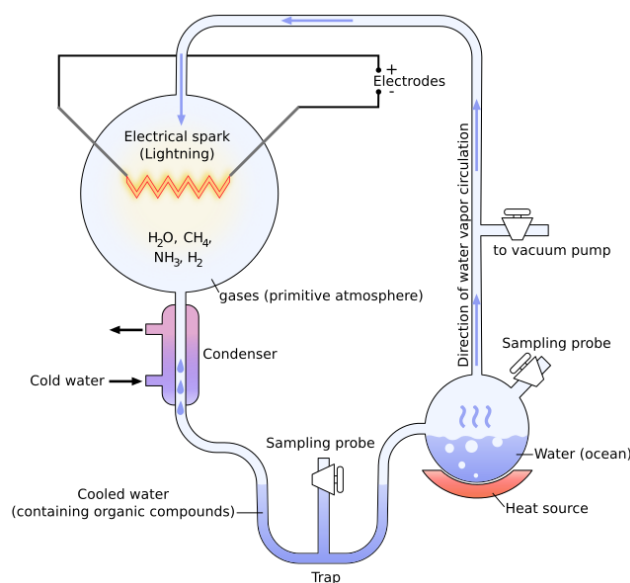


Figure 5. The Miller-Urey experiment set-up. Image courtesy [60]. The boiling water represents early ocean and the gases the early Earth's atmosphere. After several days of electric discharges in the sample, the authors detected amino acids newly formed from the simple mixture.

In the gas phase, electric discharge was mantained and its products were transferred to the other vessel, cooled down (both cells were connected by tubing) and circulated again in the system. The liquid phase was boiled simultaneously. After several days, a yellowish residue accumulated in the vessel. In this residue, Miller detected five amino

acids using thin-layer chromatography (TLC): aspartic acid, glycine, α-amino-n-butyric acid, α- and β-alanine. Modern analyses of the samples stored by Miller demonstrated the presence of a much broader range of amino acids (glycine, α-alanine, β-alanine, aspartic acid, α-aminobutyric acid, serine, isoserine, α-aminoisobutyric acid, β-aminoisobutyric acid, β-aminobutyric acid, γ-aminobutyric acid, valine, isovaline, glutamic acid, norcaline, α-aminoadipic acid, homoserine, 2-methylserine, β-hydroxyaspartic acid, ornithine, 2-methylglutamic acid and phenylalanine) poly-HCN, cyanoacetylene, urea, carboxylic acids and amines [61]–[63]. The mechanism of the formation of these amino acids has never been confirmed, but several theories point to a **Strecker synthesis** [64], [65], whose general mechanism is shown in Figure 6.
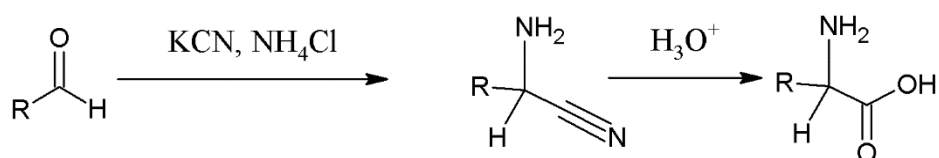


Figure 6. The general mechanism of Strecker synthesis.

Miller and Urey used a reducing atmosphere composed mainly of $NH_3$, $CO_2$, CO, $CH_4$, $H_2O$, etc. In their time, it was assumed that the early Earth had such reduced atmosphere [33], [66], [67]. Models from the early 1990's predicted more neutral conditions (neither reduced nor oxidized) with the majority of the atmosphere being $CO_2$ and $N_2$. Other predicted compounds were $CH_4$, $H_2$ and $O_2$, but their concentrations were low relative to the major components [27], [68], [69]. As was recently documented in analyses of Hadean igneous zircons, strong exogenous delivery on the early Earth may have increased the content of $H_2$, $SO_2$, $CH_4$ and $H_2S$ [70]–[72]. The changing view on the composition of the atmosphere prompted experiments with biomolecules synthesis in less reducing conditions [73]–[79]. In contrast with Miller and Urey, who used high energy inputs (discharge sparks) in their experiments, other experimentalists investigated the effect of milder conditions on the photocatalytic reduction. One of the most prominent approaches is the heating of aqueous solutions of simple carbon- and nitrogen-containing molecules (HCN, formaldehyde, hydroxylamine, ammonia, NaCN, ammonium hydroxide or ammonium cyanide) [80]–[83] These experiments should simulate the origin of life in deep-sea hydrothermal vents [84]–[87]. They usually yielded several amino acids (such as aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine or phenylalanine). Also, it is

expected that the synthesis proceeds through the **Strecker synthesis** or a similar mechanism and therefore the products should be the same.

An alternative proposed mechanism is a variation of the **Fischer-Tropsch synthesis** [88]. The **Fischer-Tropsch** synthesis is a common mechanism in the synthesis of saccharides - under prebiotic conditions; it often produces saccharides and hydrocarbons. Furthermore, upon addition of $NH_3$ along with $CO_2$ and $H_2$, the formation of an assortment of amino acids was observed [89], [90]. Native metal alloys have been used in these pioneering studies as catalysts and the experiments have never been developed further. The general mechanism of the **Fischer-Tropsch** synthesis is shown in Figure 7.
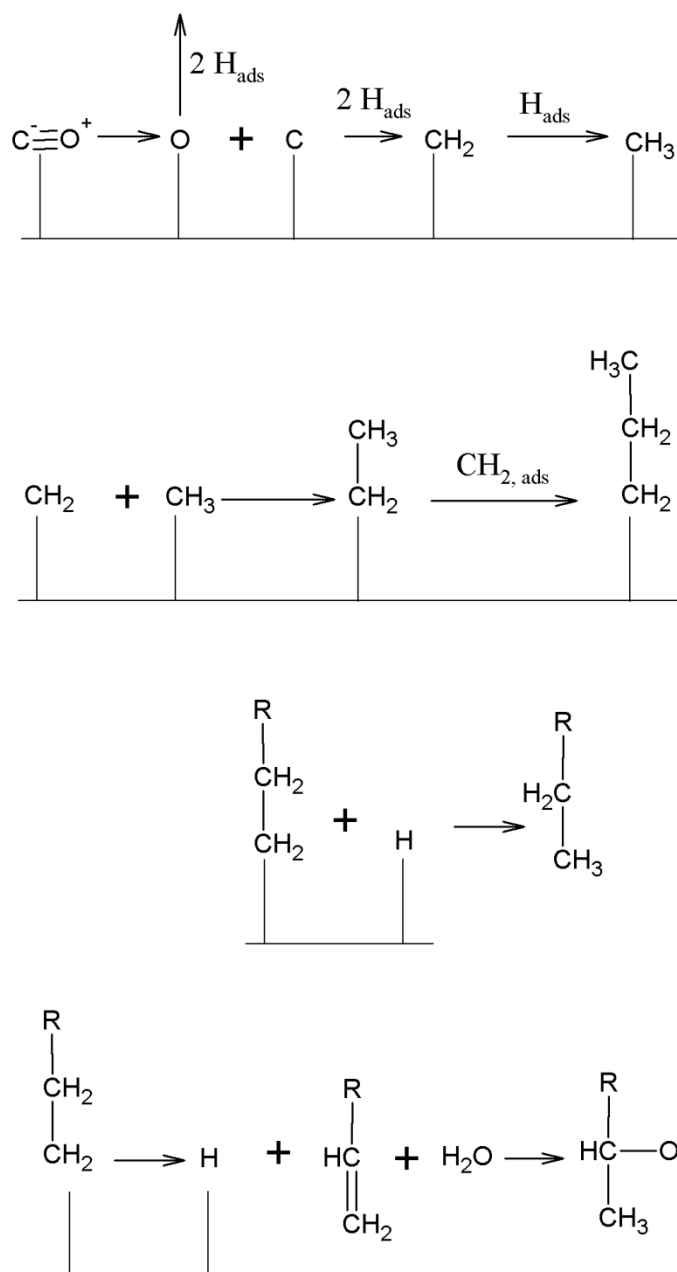
Figure 7. The overall mechanism of the Fischer-Tropsch synthesis on a metal catalyst surface.

Is should be noted that all the above-mentioned experiments produce a variety of amino acids in more or less racemic mixtures and are not limited to the 20 standard amino acids found in living organisms. This suggests that the palette of amino acids found in organisms today has been selected by the mechanisms of evolution through the course of time. What this means is that there must have been more amino acids on the early Earth and therefore should be expected in laboratory experiments [91]. A big issue in the problematic of selection is the fact that organisms use mostly L-amino acids and D-

saccharides. Several works explore the adsorption on mineral surfaces and other processes which could potentially accumulate only one stereoisomer and therefore enrich the mixture in a specific way [92], [93]. The presented works are only proposed mechanisms, however, and it remains uncertain how, when and where the separation of racemic mixtures occurred.

### 2.1.2 Accumulation of amino acids

Another aspect of the synthesis on early Earth is the concentration of the produced amino acids. Amino acids, when heated above laboratory temperature, are relatively unstable and through deamination and decarboxylation give mainly $H_2O$, $NH_3$ and $CO_2$ + $H_2S$ in sulphur-containing amino acids [94]–[97]. The thermal stability of amino acids is variable, but in general, Gly, Cys, Asn, Asp, Glu, Gln, Arg and His are decomposed between 185 °C and 280 °C while the other 12 canonic amino acids are decomposed up to 300 °C [58]. In prebiotic systems, both synthetic and decomposition reactions may occur. The product accumulates only in systems, where synthetic reactions proceed at higher reaction rates than the decomposition reactions, or where the reaction environment protects amino acids against degradation (e.g. by the adsorption on mineral surfaces).

## 2.2 Synthesis of nucleic acids

Nucleic acids are composed of nucleotides. Each nucleotide is made of nucleic acid base (at least in modern life forms by adenine, guanine, cytosine and thymine in DNA and uracil instead of thymine in RNA), pentose sugar (deoxyribose in DNA or ribose in RNA) and phosphate forming phosphate-sugar backbone. The order of nucleotides in nucleic acids is the carrier of genetic information. The presence and the synthesis of nucleic acids are necessary to build up RNA.

The prebiotic laboratory synthesis is typically separated into steps involving the formation of nucleobases and the independent synthesis of pentose, which then combine to give the desired nucleotide. The problem with this approach is the instability of RNA (both the polymer and separate nucleobases) in water or aqueous environment in general. Figure 8 illustrates the two most problematic issures related to the prebiotic synthesis of nucleosides and nucleotides in aqueous environments. Panel A shows the hydrolysis of a nucleotide, while panel B shows spontaneous cleavage of RNA.

**A**

A - nucleobase

**B**

A - nucleobase
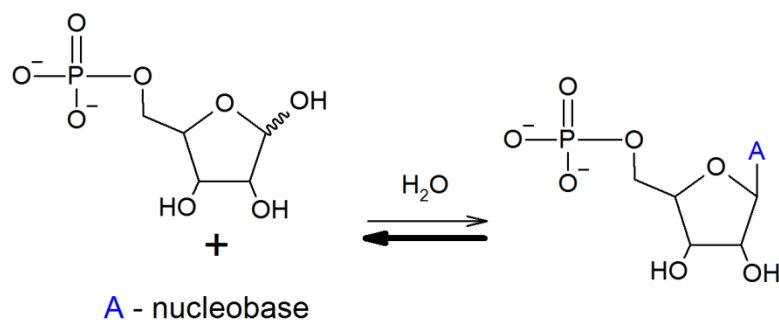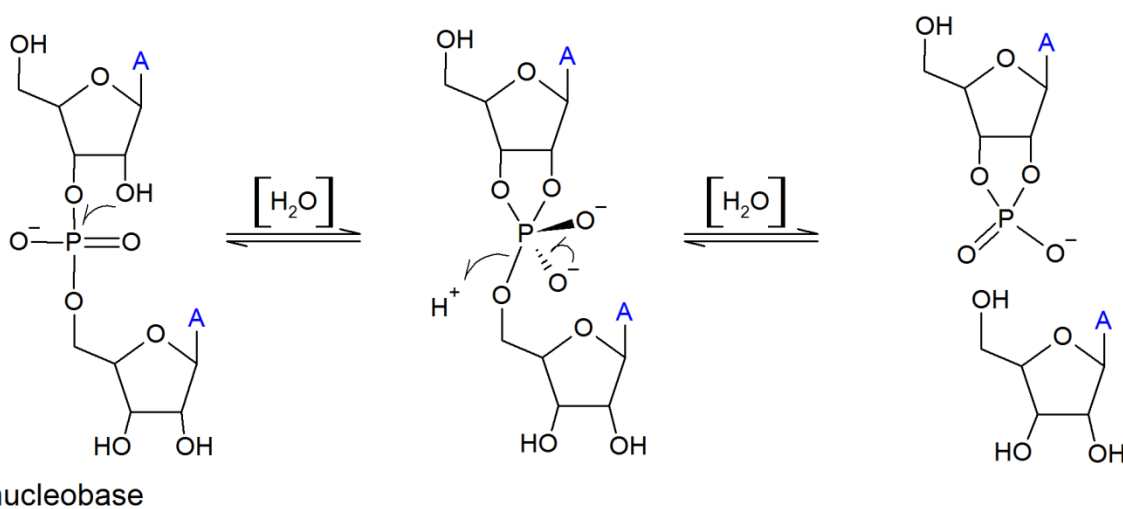
Figure 8. Panel A shows the spontanoues hydrolysis of nucleotides in presence of water. Panel B shows autocatalytic cleavage of RNA in an aqeous environment. Both panels illustrate the problematics of RNA existence in water without catalysts or stabilizing agents and also the implausibility of their direct synthesis in experiments.

### 2.2.1    Synthesis of nucleic acid bases

Prebiotic synthesis of nucleobases and nucleotides became important, more than ever, in the late 1960s, when the theory of the RNA world was first established by Alexander Rich in a book Horizons in Biochemistry [55], [98], [99]. The modern form of this theory follows a discovery of Thomas R. Cech et al. in 1982 [100], who found out that RNA has autocatalytic properties and can replicate itself. He named these molecules as ribozymes, i.e., RNA molecules with enzymatic catalytic properties. Since proteins have no such qualities, the theory of the RNA world proposes that RNA must have been here first and have incorporated proteins later. This theory might be further supported by the

central dogma of molecular biology, which (in a broader sense of view) postulates that proteins are synthesized from nucleic acids and not vice versa [101].

In modern formulation of this theory [58], RNA structures such as ribozymes are assumed to be the most ancient heritage of RNA world in modern cells. Ribozymes still exhibit several independent functions. For example, E. Kejnovský concludes in his book that RNA world still exists on an independent level in modern cells [58]. However, the structure of this small molecular RNA wold in modern cells is still a subject to discussions and explorations.

Autocatalytic properties of DNA have not been reported yet. DNA might have therefore evolved from RNA by a process similar to reverse transcription native to retroviruses, which are amongst the most dangerous to humankind. It was directly the complexity and variability of RNA, which led some scientists to believe that RNA could have been preceded by a simple polymer from which the RNA gradually evolved [102], [103].

The issue of nucleic acids synthesis under prebiotic conditions is usually divided into three separate problems – the synthesis of single components (nucleobases, saccharides and phosphate), the synthesis of nucleotides, origin of nucleosides and the synthesis of the nucleic acid itself from its components (the condensation of nucleotides).

Based on exploration of the most primitive genetic codes common for all the living forms, including the most primitive organisms [58], [104], it is often conjectured that the palette of early RNA bases might have differed from the modern genetic code. Several studies assume an involvement of purine and hypoxanthine and the initial existence of simple code based exclusively on guanine (G) and cytosine (C, i.e. GC system coding glycine and alanine) and later on a code based on triplet system combining guanine (G), the different base in the middle (marked as N), ending with cytosine (C, i.e. GNC system coding glycine, alanine, valine and aspartic acid).

In the early 1960s [83], [105], adenine was the first successfully synthesized nucleobase under prebiotically relevant conditions. In this pioneering experiment, heating of concentrated ammonium cyanide (1-15 M) at 100 °C resulted in the yield of free adenine lower than 1%. The majority of the product was a brown-black polymer similar to refractory organic material called by astronomers "tholines." Owing to more sensitive analytical techniques, guanine was found in up to 40 times lesser concentrations by

Levy et al. [106] in 1999. Both the purine bases have also been reported in spark discharge experiments mimicking the Miller-Urey experiments, where they are supposed to have formed by oligomerization of HCN in the gas phase [107]. It is important to note here, that nucleobases can be regarded as derivatized oligomers of HCN. As metntioned above, from this point of view, e.g. adenine is HCN pentamer, etc. Indeed, experiments by Sanchez et al. and Ferus et al. [12], [107], [108] experimentally confirmed the presence of diaminomaleonitrile (DAMN – a HCN tetramer) in HCN synthesis of adenine. In the proposed reaction mechanism, it is then transformed to 4-aminoimidazole-5-carbonitrile (AICN). The molecule of AICN is a precursor for the synthesis of adenine as well as guanine and other purines (hypoxanthine, etc.). The experiments by Sanchez et al. [102] were performed with HCN in aqueous solution. The reaction scheme of HCN-based purine synthesis proposed by Sanchez et al. [109], [110] is shown in figure 9 on page 26.

In this environment, HCN is hydrolysed to formamide. In combination with the discovery of formamide in Sagittarius A in 1973 with the NRAO Telescope by Gottlieb et al. [111], new theories proposing formamide as the parent molecule emerged in 2003 from Saladino et al. [112]. However, depending on pH and temperature, formamide might be hydrolysed to formic acid and ammonia.
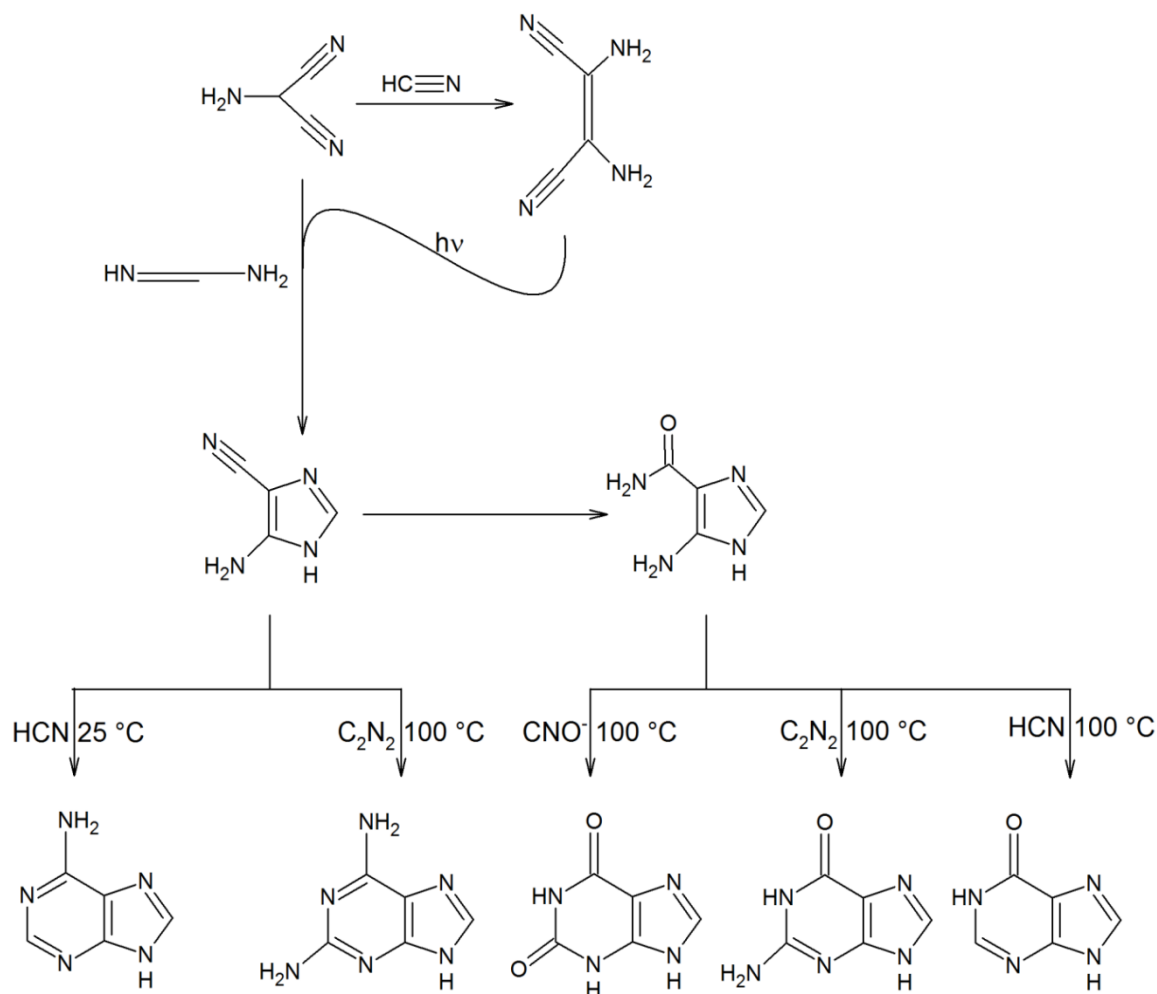
Figure 9. HCN-based synthesis of purines proposed by Sanchez et al. in 1966.

DAMN, as the main intermediate product in HCN-based prebiotic synthesis of nucleobases, is more probably depleted in further HCN polymerization leading eventually to the creation of long and stable HCN polymers (tholines). It has been estimated by Sanchez, Ferris and Orgel in 1966 [110] that the steady state concentration of HCN in the environment on early Earth would be too low for and effective synthesis of purines to take place. Currently, a new model is under development in our laboratory. Therefore, mechanisms to concentrate HCN or formamide in the environment have been proposed in the past. One of them is freezing of HCN solutions leading not only to concentration of HCN, but the low temperature is favourable for the synthesis of DAMN [77], [106], [110]. These studies have also included analyses of 27-year-old samples stored by Miller in 1972. A summarizing review of this problematic has been compiled by Orgel et al. [113]. The authors detected 23 amino acids, 18 of which Miller and Urey did not detect in 1953 due to low sensitivity of their analytical techniques.

Miller in his experiment also synthesized nucleobases, as has been shown by Ferus et al. [114]. In the nucleobase synthesis, DAMN is a key intermediate leading to the synthesis of purines.

The story of formamide-based synthesis of biomolecules (formamide, $HCONH_2$, is assumed as the initial parent molecule in these syntheses) begins with papers of Bredecker et al. published in 1956 [115]. The work did not, however, have any connection to the origin of life. The first proposal that formamide might be the parent molecule of life was discussed by Raffaele Saladino and Leonardo Di Mauro in the second half of 90´s. Their study published in 2001 [116] presents a prebiotically possible synthesis of adenine, cytosine, purine and 4(3H)-pyrimidinone from formamide heated to 160 °C in presence of catalysts such as silica and alumina. The authors argument that formamide is more plausible starting molecule than HCN since it comprises of all four macrobiogenic elements H, N, C and O, whereas to produce biomolecules from HCN requires incorporation of oxygen via hydration or in a complex radical and ion chemistry. From the experimental point of view, formamide is able to directly form biomolecules. Since this pioneering study, chemistry of formamide was explored in many experiments demonstrating synthesis of nucleobases, sugars, carboxylic acids, amino acids and even nucleosides and nucleotides. [14], [117]–[120]. Apart from heating, experiments have been carried out using proton irradiation (simulation of the solar wind), electric discharges (simulating lightning in a planetary atmosphere), UV-driven synthesis or impact plasma synthesis [108].

The first one-step one-pot synthesis (all nucleobases in one experimental procedure) of nucleic acid bases was demonstrated by Saladino et al. [112] in 2003 by heating formamide in presence of meteorites (catalysts). The first one-pot synthesis on nucleobases under plasma conditions was demonstrated by Ferus et al. [108]. This work showed a completed radical chemistry-based origin of the canonical nucleic bases and glycine in laser spark simulations of asteroid-impact-generated plasma. Such harsh asteroid impact plasma conditions have been explored, because they are related to the period of about 500 million years long Early and Late Heavy Bombardment era (EHB and LHB) on the early Earth prior to 4.0 Gya. All the experimental studies and theoretical simulations pointed importance of ·CN radical chemistry.

The radical-based scenario and the classical synthesis scenario from formamide in aqueous solutions are shown in Figures 10 and 11.
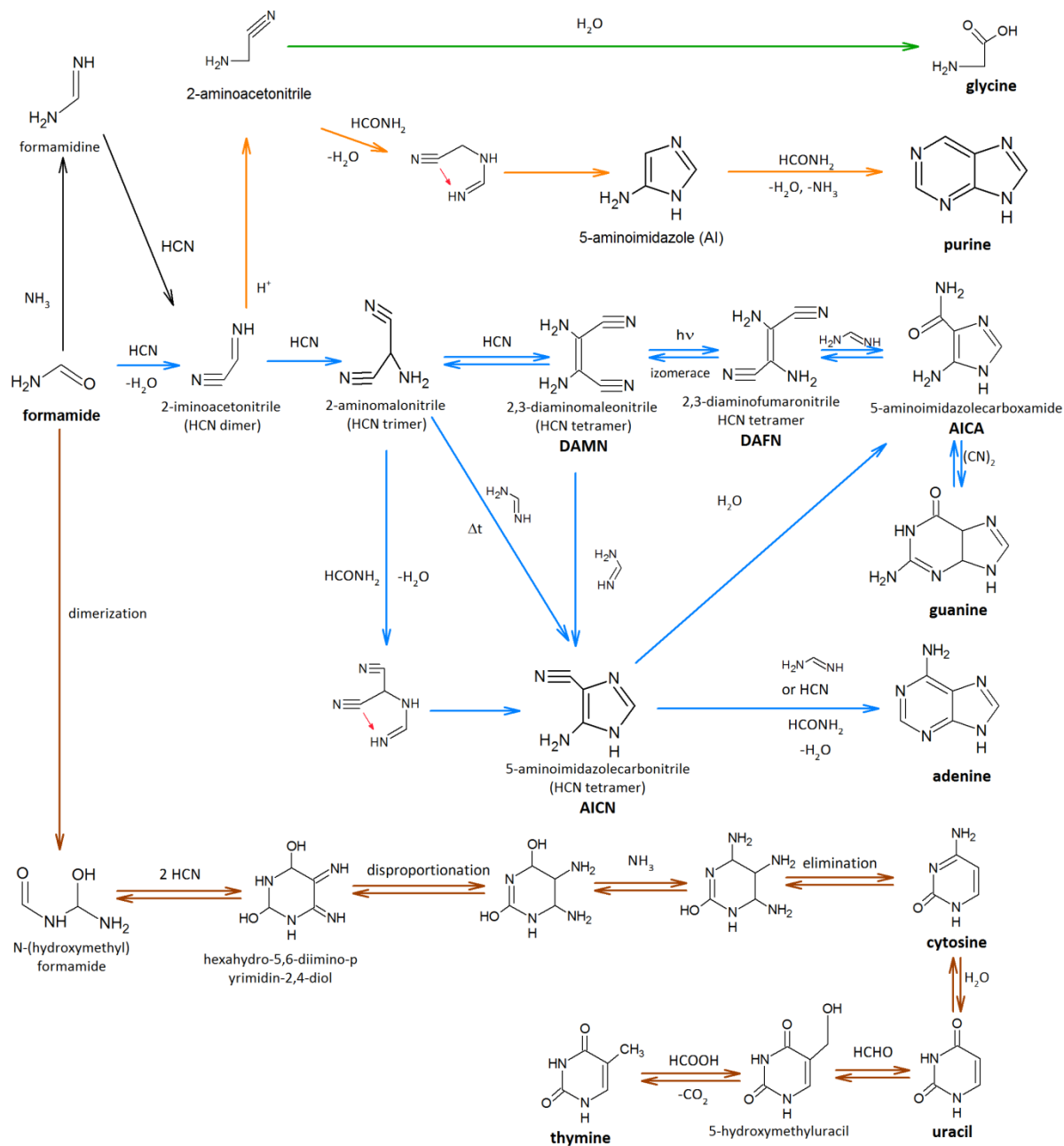


Figure 10. Classical synthesis of nucleic acid bases from formamide in aqueous solution [121].
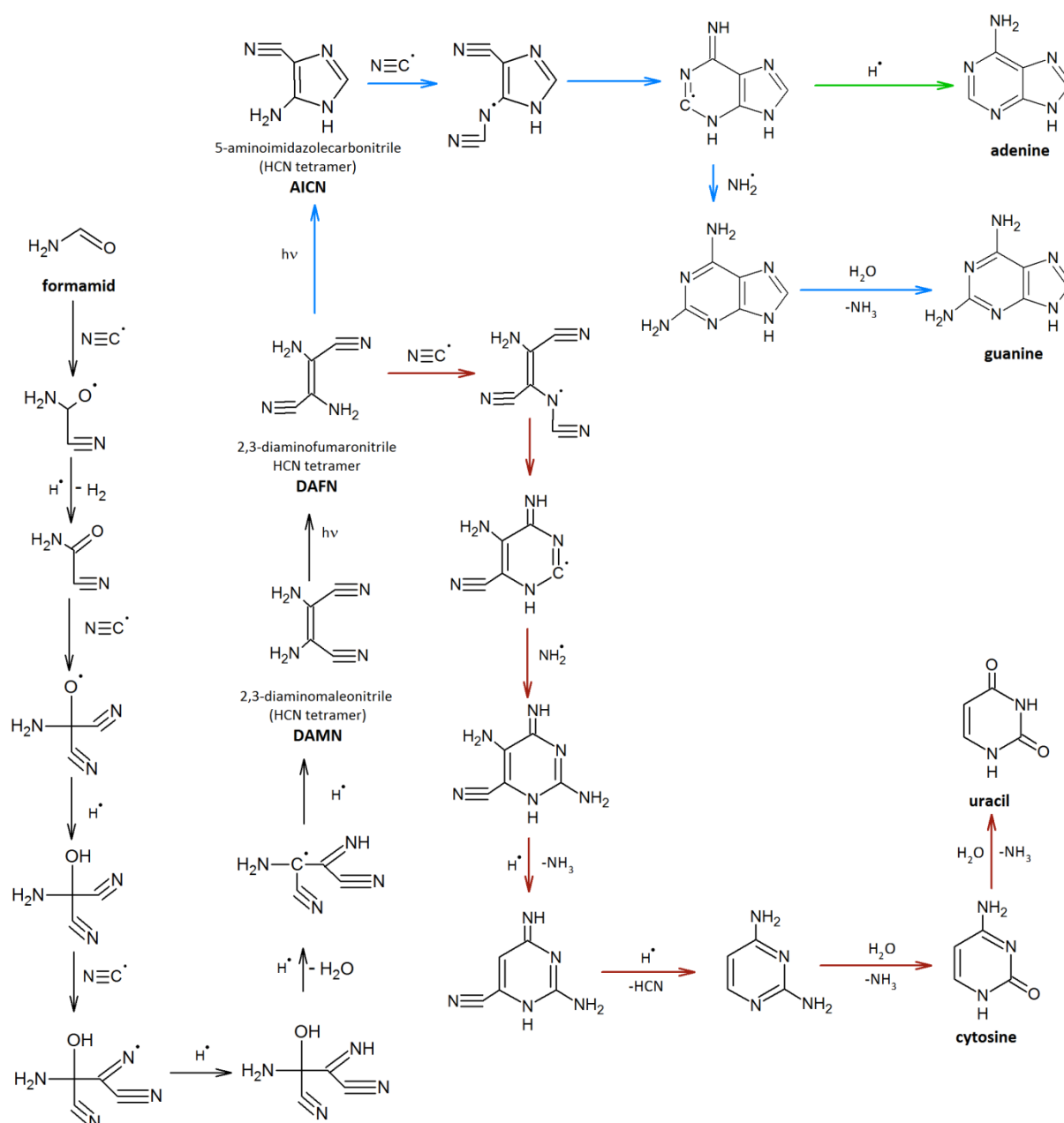
Figure 11. Radical-based synthesis of nucleic acid bases from formamide in high-energy chemistry scenarios according Šponer, Ferus and Civiš [122].

## 2.2.2 Synthesis of nucleotides

A nucleotide is the building block of nucleic acids. Each nucleotide consists of ribose, phosphate and the corresponding nucleobase. Even though each of the constituents has been experimentally prepared under prebiotically relevant conditions, each requires a different environment for its synthesis. These conditions also seem to be incompatible with each other in the case of pH, starting reactions and also due to interfering reactions of intermediates in various synthetic pathways. Several theories assume that each of these components could have been synthesized separately on the early Earth and then all

of them could have been brought together. Nevertheless, it was shown by Sutherland[123], [124] that even if all the constituents are brought together (as created in prebiotic experiments), numerous isomers of the nucleobases and ribose prevent effective assembly of nucleosides or nucleotides. Since the assembly is in fact condensation, then presence of aqueous environment acts in favour of their hydrolysis and not formation, as is shown in figure 8 on page 23.

As a consequence, there have so far been only a few studies on successful experimental syntheses of nucleosides [125], [126] reporting their synthesis by heating of dried mixtures of ribose and purine without the presence of water. Recent studies show prebiotically relevant pathways for the creation of such bonds between the nucleobase and the ribose. However, they are mostly theoretical or showing that experimental achievement of nucleotide synthesis is extremely demanding [127]–[130]. In fact, plausible scenario has never been published as a complete theory answering all the questions. One of the possible solutions is that the bond between the nucleobase and the ribose is formed earlier in the synthesis (i.e. nucleotide is not synthesized from complete base, sugar and phosphate). The nucleobase and the ribose need not be synthesized separately in that way, but can be formed as a complex. The first work on this topic was presented by in 1970 Sanchez and Orgel [131] and their scheme using and oxazoline ring is shown in figure 12.
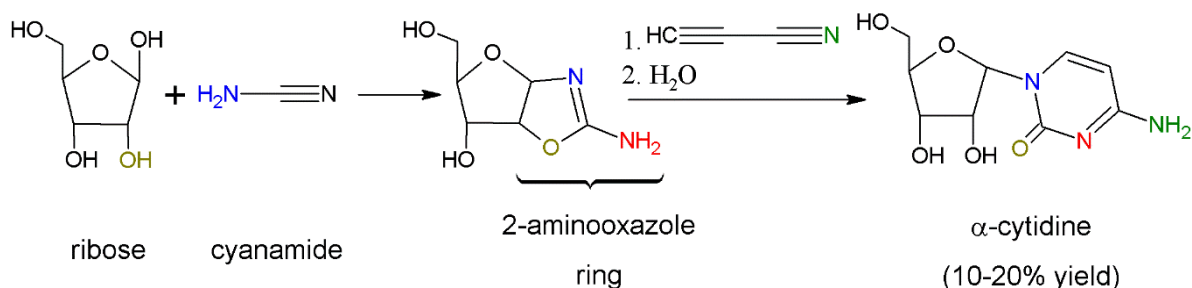


Figure 12. α-cytidine developed by Sanchez and Orgel in 1970 using, for the first time, the oxazoline ring as intermediate.

All these experiments, however, suffer from the already discussed drawback, that they require several steps performed easily in the laboratory but provided hardly in natural environment, such as isolation and purification of intermediates (such as the oxazoles, AICN or DAMN), followed by the subsequent step of the synthesis. Such an approach is less probable under natural conditions. However, many multistep processes occur and

they are well understood by direct observation. In the case of the origin of the first living structures, however, we are not able to observe any step or mechanism in current nature and therefore it is hard to construct complete, plausible and widely accepted scenario.

## 2.3   Synthesis of saccharides

Saccharides are another vital part of living organisms. In the context of prebiotic chemistry, their synthesis is often separated to synthesis of ribose (RNA component) and other saccharides.

### 2.3.1   Synthesis of ribose

The **formose reaction**, which has been discovered towards the middle of the nineteenth century by Butlerow [132], is the usually considered pathway in prebiotic synthesis of ribose. The general mechanism is shown in Figure 13. As described by Breslow [132] in 1959, it is a oligomerization of formaldehyde. The main mechanisms of the oligomerization are aldol, reverse aldol reactions and aldose-ketose isomerizations.
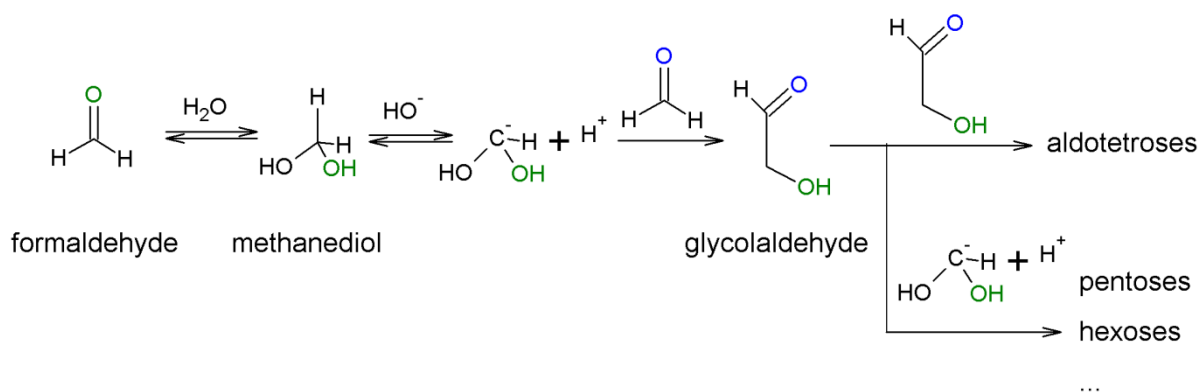


Figure 13. The formose reaction is basically a condensation of aldehydes to form saccharides of carious chain lengths. The reaction was discovered by Butlerow in 1861.

The first condensation of two formaldehyde molecules is the most problematic step of the reaction chain due to the need for a reverse polarization of one of the carbonyl groups. The general effect of reverse polarization is called umpolung. A theoretical description of this first step has been already presented by Ferus, Šponer et al. [133]. The authors propose that high-power laser irradiation might be a plausible mechanism able to overcome the umpolung problem by direct high energy synthesis of glycolaldehyde [133]. Experimentally, the synthesis has also been achieved by heating or mild irradiation of aqueous solutions of formaldehyde on various catalysts ($TiO_2$,

calcite, kaolinite, etc.) [134]–[136]. It has also been observed in all these studies, that addition of glycolaldehyde greatly increases the reactions rate (glycolaldehyde possibly acts as a catalyst). On early Earth, catalytic amounts of glycolaldehyde would suffice to catalyse the oligomerization of the otherwise abundant formaldehyde. A typical product of these prebiotic experiments is a mixture of trioses, tetroses, pentoses and hexoses.

There are, however, drawbacks in the plausibility of the synthetic reaction [137]. Most importantly, in the product mixture, ribose constitutes only about 1 % or less, with the rest being a mixture of other saccharides. Also, ribose is not a terminal product of the reaction and if the reaction is allowed to proceed indefinitely (such as in nature), the created ribose may be transformed consequently into a brown insoluble tar [137]. Also, the reaction in laboratory requires quite concentrated solutions of reactants to begin with and their likelihood on the early Earth is low [138]. What is more, ribose is relatively unstable in aqueous solutions due to hydrolysis. A most favourable prebiotically plausible stabilizing agent are probably borate ions. Not only do borate ions have a potential to stabilize ribose, but they also increase the yield of pentoses in the reaction mixture [139]–[141]. A promising stabilizer are also lead ions, but their efficiency is not as high as that of borates [141].

## 2.4    Synthesis of membrane components

To assess the synthetic pathways of membrane precursor synthesis, it is first important to determine the composition of the membrane. Naturally, amphiphilic compounds tend to form membranes. Depending on the polarity of each constituent of the system, the amphiphilic molecules may encapsulate other molecules, particles or whole chemical compartments. The encapsulation provides a different environment for chemical reactions. Due to the presence of lipids and their natural tendency to form micelles, for example, it is possible to find hydrophobic environment in hydrophilic solutions. The presence of such micelles provides a stable environment required for the emergence of life. Homeostasis in human body is, in fact, a result of the same process – compartmentalization, separation of otherwise interfering reactions and environments and selective effect of membranes. The presence of membranes also prevents the reaction products from being scattered and therefore concentrates the solution [142]–[145].

Contemporary membranes are composed mainly of phospholipids. They are, however, not easily permeable and therefore, in times before the evolution of membrane transport mechanisms, not very probable. Simpler molecules, such as fatty acids or alcohols possess similar qualities and are suitable as precursors. These compounds also spontaneously form micelles or vesicles when hydrated [144]. Also, reduction of $CO_2$ or CO by means of the **Fischer-Tropsch** synthesis is able to yield these molecules effectively [88]. Fatty acids up to $C_{16}$ have been experimentally synthesized in presence of meteoritic material (meteoritic iron, nickel and iron ore) [88]. They have also been identified as products in experiments mimicking Miller's experiments [146], [147]. Many experimental studies have since investigated the mechanism, but mainly intended to simulate the origin of life in deep-sea hydrothermal vents (see [57] and references therein). An interesting feature of the origin of life in hydrothermal vents is the presence of $H_2S$. Upon reaction with FeS, $H_2S$ is transformed into pyrite ($FeS_2$) and $H_2$. $H_2$ could potentially serve as the reducing agent in $CO_2$ reduction to fatty acids [148], [149]. This reaction doesn't have to be fixed to hydrothermal vents, but could proceed on the surface as well. Experimental proof of the whole reaction from $CO_2$ up to organic compounds has been performed by Heinen and Lauwers [150]. The authors heated a mixture of $CO_2$, $H_2S$, $H_2O$ and FeS at 25-90°C and observed the formation of alkylthiols ($C_1$-$C_5$).

## 2.5 Synthesis of amino acids, nucleobases and lipids in one scheme

The chemistry so far presented and discussed is a typical example of the reductionist approach – complicated molecules are divided into logical subunit, which are divided into their respective subunits, etc. These subunits are then synthesized independently on each other and only after that are combined together. Looking at nature, however, we see that the human approach may not be as logical as it seems. For example, the synthesis of nucleotides in human body starts with ribosylpyrophosphate. The nucleobase is synthesized step by step on the 1' position of ribose and so the problematic bond between the ribose and the nucleobase is created first, before it even becomes problematic. Many scientists have noticed this and sparked discussions about the compatibility of the different chemistries of nucleobase, saccharide and lipid formation [151]–[153]. The group of J.D. Sutherland have over the years accepted the premise that a different approach must be taken and in the huge amount of their work have explored synthetic pathways of all the basic constituents of life (amino acids,

saccharides, etc.). All his work is clearly presented in ref [123] and describes all the reactions in detail. Overall, the authors sought for inherently favoured reactions in chemical subsystems. The chemistry of prebiotic mixtures yields complex products. If we look, for example at adenine as a HCN pentamer, then we would expect to synthesize it from HCN. Perform reactions, such as heating HCN whilst adding a catalyst or similar, we obtain not only adenine, but other reaction products (side and by-products) as well. And if we explored each of the reaction in the reaction chain separately, we would find that it very often yields mixtures of products. Furthermore, there are many reactions going on in the system which divert the reaction route to other products, etc. To get to the desired nucleobase, one must seek for inherently favoured reactions. Sequential occurrence of several such reactions may lead to preferred syntheses of important molecules at the expense of others [154]. General aspects aside, Sutherland and his team explored whole myriad of synthetic pathways leading towards ribose.



glyceraldehyde    glycolaldehyde                                                        ribose
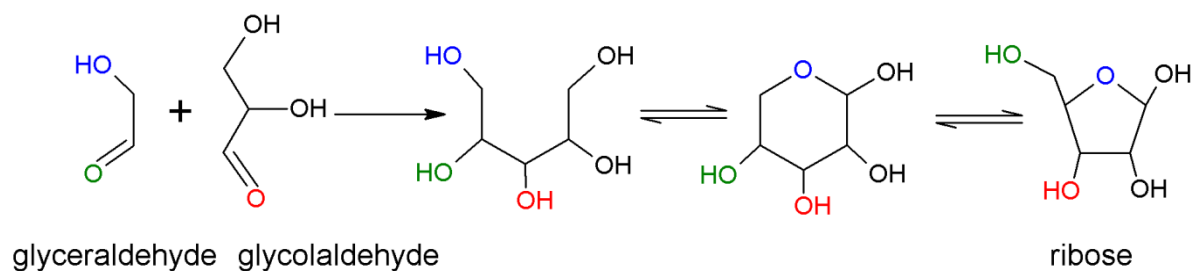
Figure 14. Inherently favoured condensation of glycolaldehyde and glyceraldehyde. Contrary to standard formose reaction, this condensation produces mainly ribose.

Figure 14 shows the inherently favoured reaction of glycolaldehyde and glyceraldehyde, which produces ribose. Contrary to standard **formose reaction**, this condensation is inherently favoured and produces ribose as a major product. The ribose as such is relatively unstable. Ribose may be stabilised in the form of e.g. borate [139] or by inclusion into RNA. What Sutherland found, however, is that it may be well stabilised by forming adduct with cyanamide, which is an important intermediate in nucleobase chemistry. From here on, the authors assumed that there was an inherently favoured route to RNA. According to this, RNA would not be a mere biotic invention, but a

prebiotic product [155], which has just happened to work well as a carrier of information.
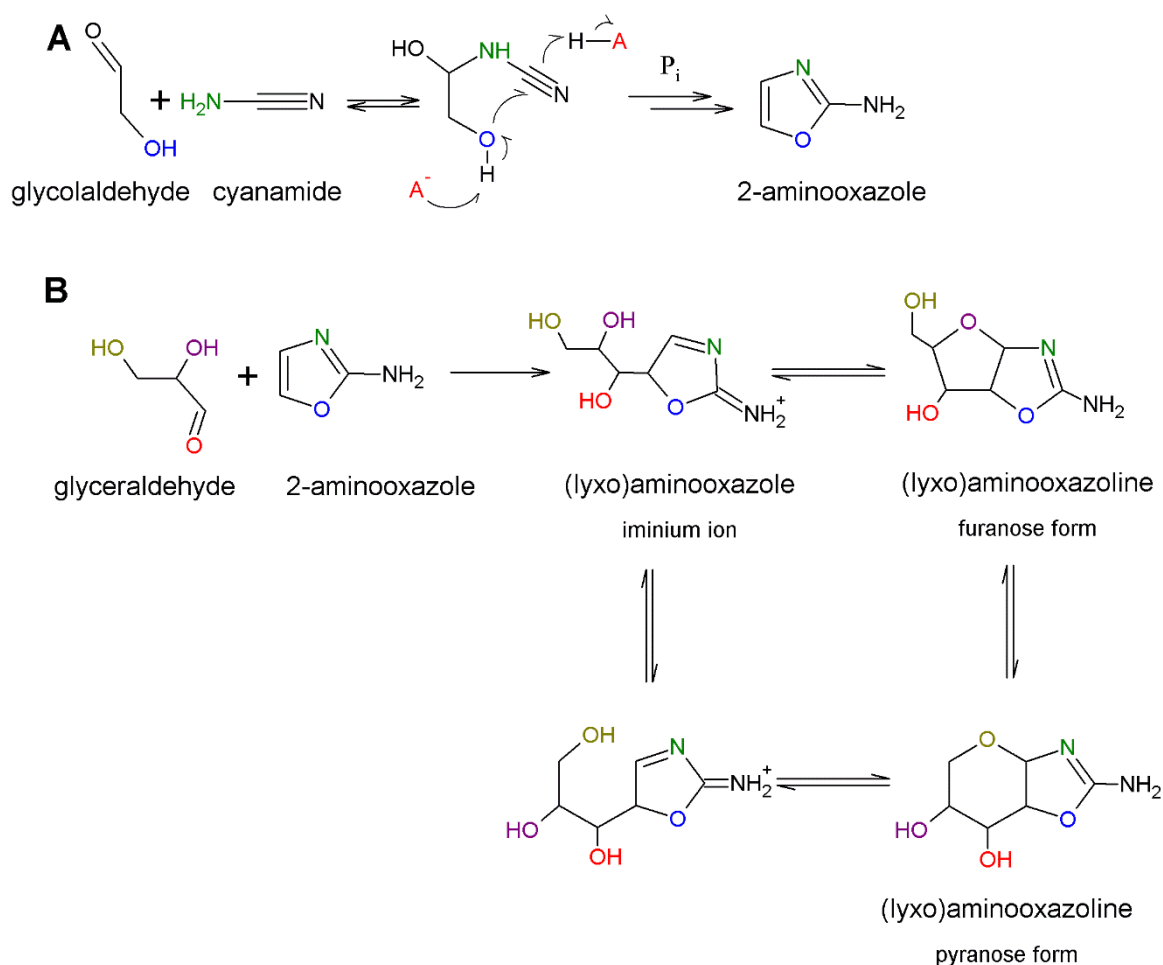


Figure 15. Panel A shows a reaction between glycolaldehyde and cyanamide, which produces 2-aminooxazole. Panel B shows a reaction between glyceraldehyde and 2-aminooxazole, which forms a pentose-aminooxazole. This compound exists in equilibrium between the pyranose and furanose forms.

The chemistry of sugar synthesis and nucleobase synthesis were expected never to be compatible [155]. For example, HCN needed in the synthesis of purines and pyrimidines reacts with aldehydes used in the synthesis of sugars and give cyanohydrins, which are useless for further prebiotic synthesis. Many scenarios have been proposed, that both ribose and a nucleobase could have been synthesized separately and then, by geochemical processes, such as erosion, streams and tectonics,

brought together. Their mutual reaction, however, proceeds only in low yield and with little selectivity (purine nucleobases) or not at all (pyrimidine nucleobases).

Figure 15 shows three reactions which solve the problem, partly. Panel A shows the synthesis of 2-aminooxazoline from glycolaldehyde and cyanamide. The reaction has been reported before [155]. Panel B shows the reaction of 2-aminooxazoline with glyceraldehyde which yields, as an intermediate the iminium ion of lyxoaminooxazoline. Lastly, panel B also shows a spontaneous interconversion between the furanose and pyranose isoforms of lyxoaminooxazoline. This is indeed important, because the observed iminium ion of lyxoaminooxazoline in panel B is the intermediate in panel A as well. Therefore, the reaction in panel B should yield pentoseaminooxazolines. This was observed, as in the end, 44% of riboaminooxazoline, 30% of arabinoaminooxazoline, 13% of lyxoaminooxazoline and 8% of xyloaminooxazoline were found in the resulting product mixture. This creation is not very effective at neutral pH. However, when phosphate is added as a catalyst, the reaction has nearly 90% yield of 2-aminooxazole [156]. The phosphate as catalyst is highly relevant, because it is needed to assemble the RNA polymer. It could, therefore, have been present in the reaction mixture from the very beginning.
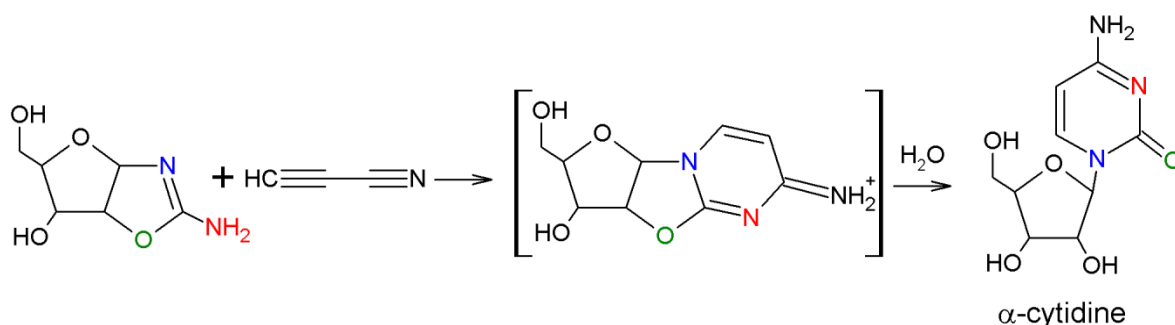


Figure 16. Reaction between ribose aminooxazoline and cyanoacetaldehyde produces α-cytidine.

Figure 16 shows a reaction between riboaminooxazoline and cyanoacetylene. This reaction yields α-cytidine [131]. The reaction is possible with other pentoses, such as arabinose, as well. In the same study [131], Sanchez and Orgel reported that UV irradiation partially converts α-cytidine to β-cytidine as is shown in figure 17 panel A.

As was found out by Powner and Sutherland, however, UV irradiation destroys most of the nucleoside/nucleotide, which is shown in figure 17 panel B.
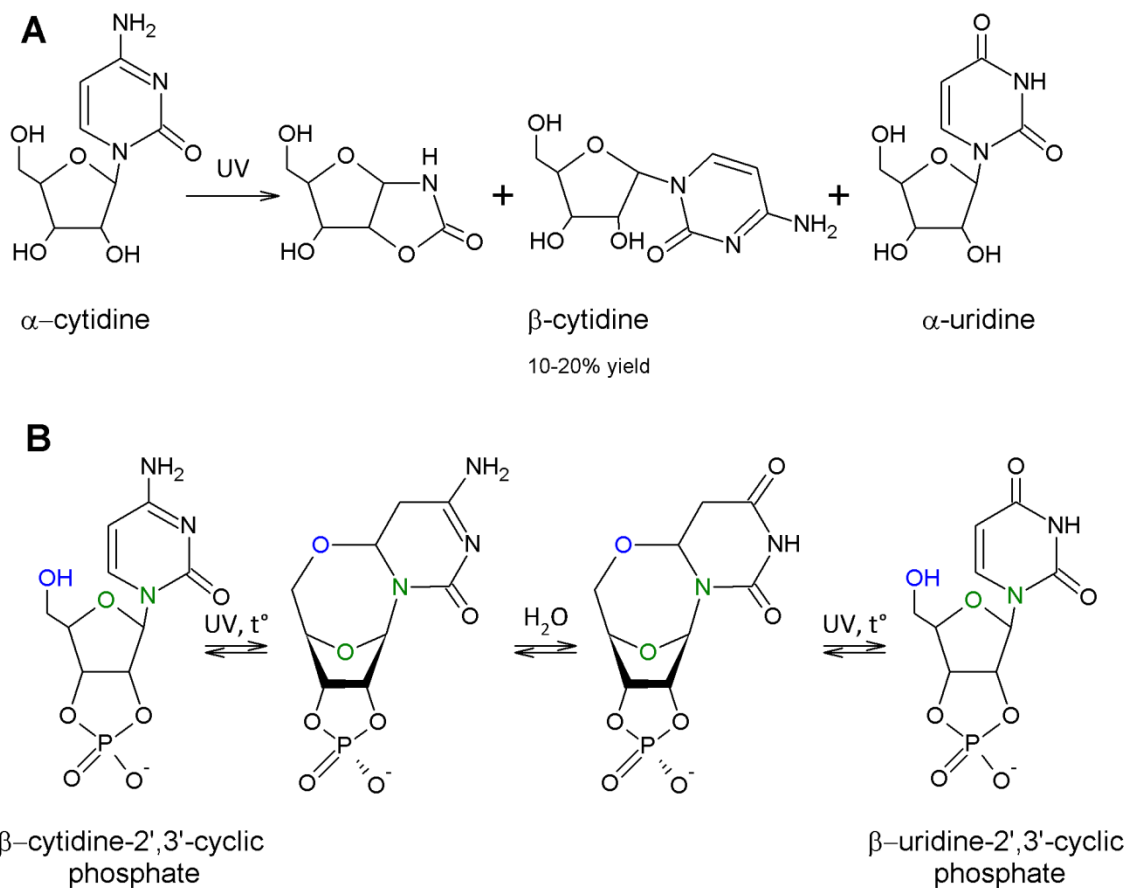


Figure 17. Panel A shows UV induced photolysis of α-cytidine to various products. 10-20% of the compound is transformed into β-cytidine. Panel B shows that if cytidine is phosphorylated in 2',3' positions, is stable and only partly converts to uridine-2',3'-cyclic phosphate, but is not destroyed.

According to the authors, the only intermediate stable under UV irradiation is the β-cytidine-2',3'-cyclic phosphate. UV irradiation can be therefore used to select this product from a mixture of others. As the UV flux was higher on the early Earth (discussion above), this reaction must have very possibly happened.

Encouraged by this finding, Sutherland et al. sought for a prebiotically plausible synthetic pathway towards this intermediate. This they discovered by subjecting the

arabinoaminooxazoline + cyanoacetylene adduct to urea/formamide and phosphate. This reaction was very smooth with high yields and therefore inherently favoured. Also, cytidine can be turned to uracil by UV irradiation. The route to activated pyrimidine nucleotides was thus complete.

The starting materials in this synthesis were glycolaldehyde, glyceraldehyde, cyanamide, cyanoacetylene, phosphate and formamide/urea. Where have these come from?

Considering asteroid impact scenario, Sutherland et al. conceived a model of CN-rich transition metal complexes, such as cyanocuprates and cyanoferrates. HCN itself is abundant in space and may have been delivered, according to their theories, to Earth, or created by reaction of carbonaceous materials with the atmosphere during the impacting body descent [157], as is also discussed by Ferus, Civiš, Knížek and Sutherland [158]. As the result of the CN-rich transition metal chemistry, the authors in the end succeeded in creating a HCN chemistry model of the creation of sugars. Figure 18 shows their suggestion of the synthesis of glycolaldehyde and glyceraldehyde from HCN.
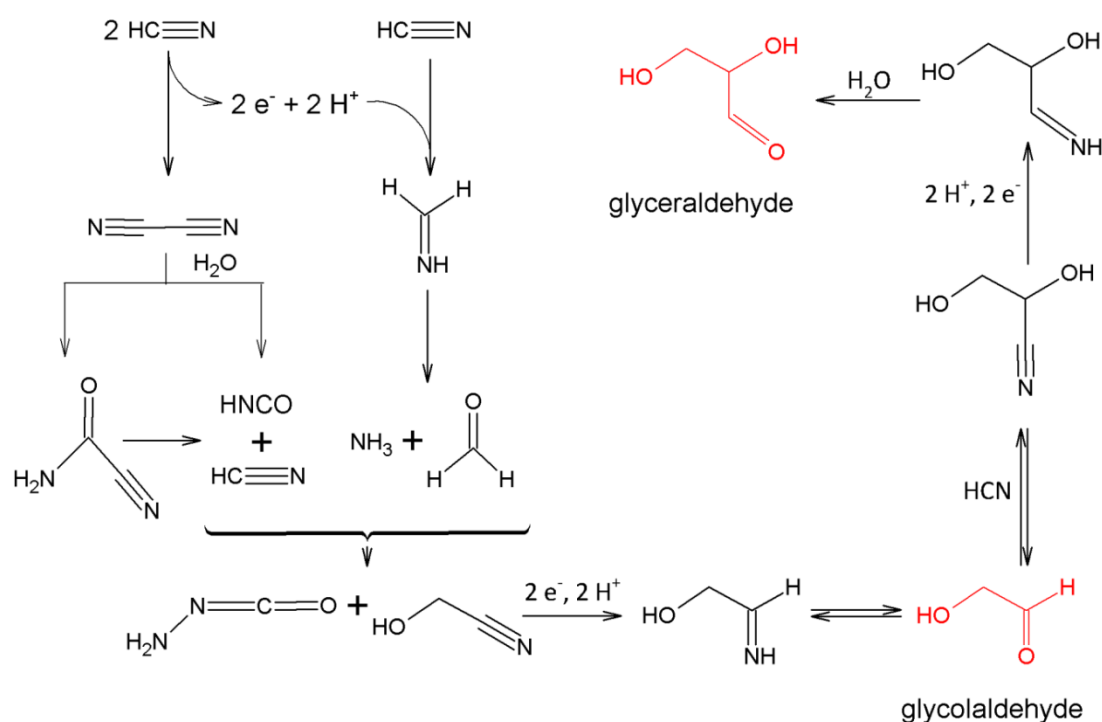


Figure 18. Synthesis of glycolaldehyde and glyceraldehyde from HCN proposed by Powner et al. [156]. The reaction involves glycolonitrile, which is also important in the next reaction step described below.

During this synthesis, the authors also found a way to the problematic condensation of formaldehyde to glycolaldehyde, bypassing the difficult unpolung step by reaction with ammonia and ammonium isocyanate. This is another possible solution alongside with high energy initiated synthesis of glycolaldehyde over titania proposed by Šponer, Ferus and Civiš [159]. The problem of this alternative reaction scheme was that high concentration of HCN was needed in the environment. This led to a suggestion of the involvement of cyanoferrates(II) in the fixation of HCN. HCN could have been fixed in iron containing minerals and upon their thermal metamorphosis released into the reaction cycle. There is one more reactant important for the synthesis and that is a reductant. The reductant turned out to be HS$^-$ (produced during fixation of HCN on FeS or CuS).

The tested chemistry of glycolonitrile (figure 19) yields various aldehydes. These turned out to be amino acid precursors in the **Strecker synthesis**. Overall, HS$^-$ acts as a reductant of glycolonitrile (which arises by reaction of HCN and formaldehyde) and the system yields various aldehydes – formaldehyde, acetaldehyde, 2-hydroxypropanaldehyde and glycolaldehyde, which are precursors to glycine, alanine, threonine and serine, respectively. Upon various intercrossing reactions, it is possible to obtain up to 12 proteinogenic amino acids from the same starting compounds.

**A**



**B**
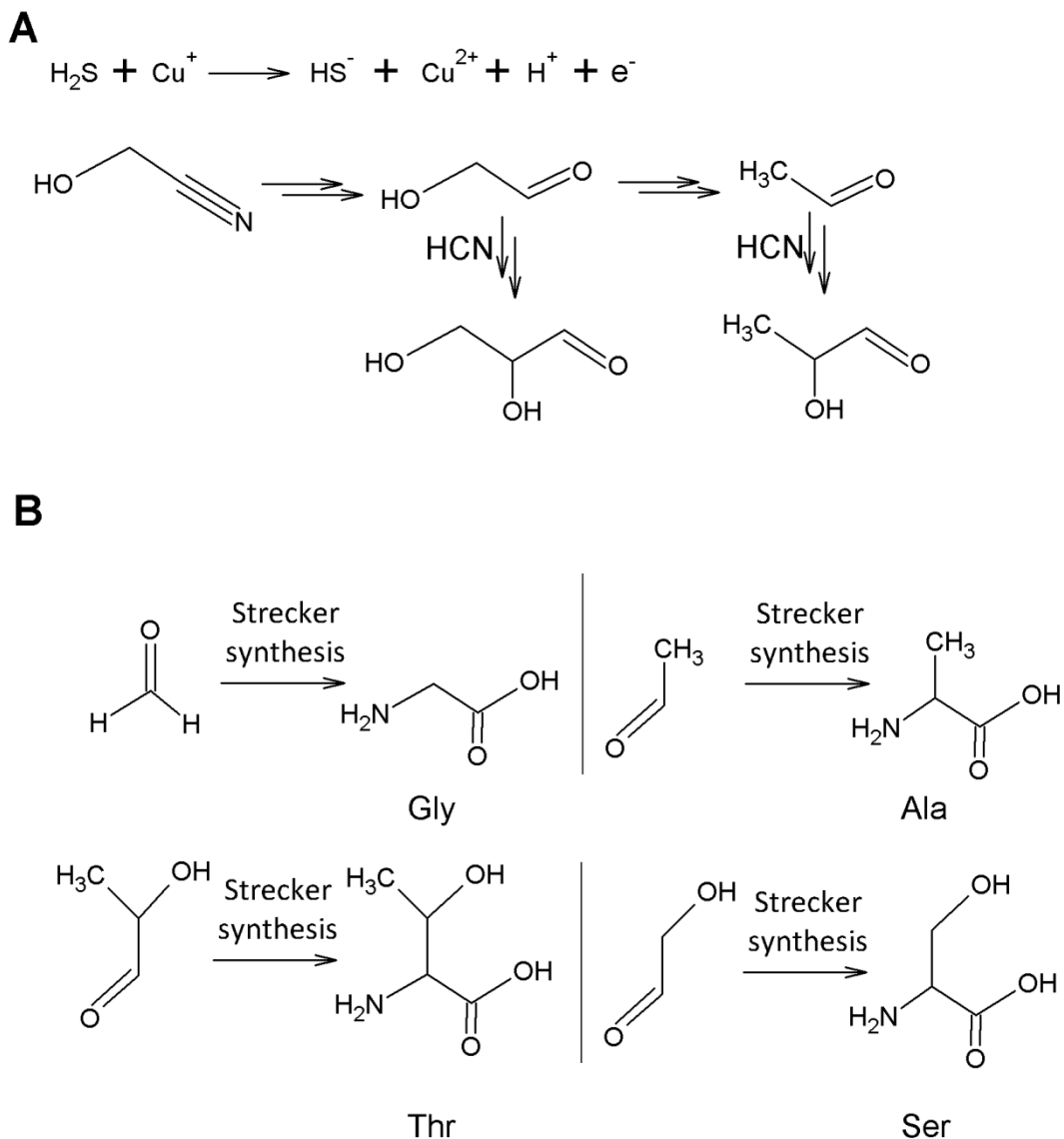


Figure 19. Panel A shows the role of H$_2$S and Cu as reducing agents, which provide an electron for the reduction of glycolonitrile. Panel B shows several aldehydes, which are produced from HCN-based chemistry of glycolonitrile.

The results so far connect nucleic acids and protein chemistries together, while also showing that molecules such as ribose and proteinogenic amino acids might be inherently favoured by the synthetic routes at the cost of the rest, which are disfavoured in similar fashion.

Components of membranes are much more varied throughout the history of life. Often, however, they are based on glycerol or its derivatives. Upon reacting glyceraldehyde

with phosphate (which are both to be found in our reaction system), one gets glycerol (upon UV irradiation). Its reaction with phosphate then easily produces glycerol phosphates used in membrane components. The important point here is that this synthesis proceeds in hydrophilic environment. Upon connection with long aliphatic chains, the route to lipids lies open. Summarizing the chemistry proposed by J. D. Sutherland and his team in a single statement, one should say that HCN is the prebiotic precursor to all necessary living systems chemistry.

The state of the art of prebiotic synthesis may be summarized in the following statement. The synthesis presented above developed by Sutherland et al. [123], [124], [156], [160], [161] regarding HCN chemistry and approaches explored by our team and Saladino, Di Mauro et al. [14], [108], [112], [120], [122], [162]–[164] represent two major concepts in prebiotic chemistry. Sutherland et al. developed multistep high-yield scenario, which requires different conditions and procedures during the synthesis. Our team (for review see papers by Ferus & Šponer et. al., e.g. [122], [165]) follows the logic of one-pot synthesis, which is more probable but exhibits low yield and produces a variety of compounds, not all of which are favourable towards prebiotic synthesis. However, it is obvious that HCN and formamide theories are interconnected and maybe forthcoming explorations will show a general and unifided paradigm.

# 3 Aim of the work

The aim of this work was to study a UV-induced transformation of a $CO_2$-rich planetary atmosphere and its subsequent reprocessing with high-power laser, which simulated and asteroid impact on a young planet's surface. The expected result was the creation of organic molecules from such a simple gaseous mixture.

# 4 Methods

## 4.1 Sample preparation

### 4.1.1 Photocatalytic reduction of $CO_2$

The photocatalytic reduction was studied using several mineral catalysts. Most notably, the first experiments were performed using $TiO_2$ anatase (crystalline form of $TiO_2$ CAS 1317−70−0), which was synthesized in the laboratory of prof. Ladislav Kavan [8], [166]–[171]). Other catalysts include rutile and graphene-doped anatase (also synthesized by prof. Kavan), montmorillonite (CAS 1318−93−0, type K10), $Al_2O_3$, kaolinite, MgO, $CaCO_3$ (all from Sigma Aldrich), natural rutile from Golčův Jeníkov, siderite from Příbram, basalt from Děpoltovice, ilmenite from Jizerská Louka and H-, Fe- and Na-doped clays of montmorillonitic type and clays minerals from SOOS natural reservation, which are natural samples collected by prof. Svatopluk Civiš. The last material was a sample of the Nakhla meteorite, which was provided by Dr. Martin Ferus in cooperation with Prof. Elias Chatzitheodoridis. The Nakhla meteorite is a nakhlite-type meteorite, which has a Martian origin, and fell in Egypt in 1911.

All samples were degassed in $10^{-3}$ Torr vacuum and annealed at 150 °C. In each experiment, 0.5 g of each sample was loaded into a special sample cuvette made of a 15-cm quartz finger and a ca 400 ml borosilicate glass sphere, which served as gas reservoir. The cuvette is shown in the results and discussion section.

After the annealing, the samples (except $TiO_2$ anatase) were connected to a 36 (w/v) % HCl reservoir. The HCl was frozen in liquid $N_2$ and the system was evacuated. After the evacuation, the system was closed and the HCl was left to melt, evaporate and freely transfer to the sample. Each sample was connected to the HCl reservoir. After the night, the pressure in the sample was regulated to 8 Torr, which means 7.98 Torr HCl and 0.02 Torr $H_2O$. The $TiO_2$ anatase was synthesized from $TiCl_4$ by hydrolysis. The synthesized anatase therefore contains 14 (w/w) % of HCl as a leftover from synthesis (determined by titration), therefore the HCl addition step was skipped.

Subsequently, each sample was filled with 10 Torr $CO_2$ (Linde, Germany) and 2 Torr $N_2$ (Linde, Germany).

## 4.2 Methods & Experimental procedures

### 4.2.1 UV Irradiation

The irradiation of samples was performed using a 365 nm narrow band lamp (Hönle, Germany). The lamp output was 3000 mW.cm$^{-2}$ and the sample was 20 cm distant from the light source. The composition of the gas phase was analysed by FTIR at regular intervals during the experiment. We assume that the mixture is stable when the irradiation is turned off.

### 4.2.2 FTIR Spectroscopy

The gas phase during the experiments was monitored using a Bruker IFS 125HR spectrometer (Bruker Optics, GmbH, Germany). The spectrometer was equipped with a KBr beamsplitter, NIR infrared source and a liquid nitrogen-cooled InSb semiconductor detector. The measurement was performed in 1800-6000 cm$^{-1}$ interval with the resolution 0.02 cm$^{-1}$. 25 scans of each sample were taken and averaged in order to obtain better signal-to-noise ratio. The interferograms were apodized in the OPUS software package using a Blackmann-Harris apodization function. After the measurement, each spectrum was post-zerofilled (spline function, factor 4) and the area of three randomly selected peaks for each substance (CO, $CO_2$, $CH_4$, HCl, $H_2O$) was integrated. Separately, calibration measurements using pure gases were performed (Linde gas, Germany). The intensities of the peaks were then matched with partial pressures in the sample cuvette. The post-measurement analyses were performed in the OPUS Software package 6.0 (Bruker, Germany) and Origin 8.0 (OriginLab, USA).

### 4.2.3 High-power laser irradiation

The mixture obtained from the irradiation experiment was taken and exposed to 25 laser shock waves on the Prague Asterix Laser System (PALS) in Prague. Each pulse had energy 150 J (PALS max energy per pulse is 1000 J) and lasted 350 ps. PALS is an infrared laser and has 1315 nm wavelength. A pulse was delivered into the sample every 25 minutes (the time it takes for the laser to cool down). The beam (ca 15 cm in diameter) was focused into the sample by a 15 cm in diameter plano-convex lens with a focal length 25 cm. The energy losses at the lens and sample cell window did not exceed 15 %.

The irradiated samples were analysed separately after the experiment. The gas phase was analysed using the FTIR instrument described above. The sample gas phase was transferred to a White multipass cell (optical path 10 m). The spectra were recorded

using mercury-tellur-cadmium (MCT) detector, KBr beamsplitter and a globar lamp. Data were saved in the region from 1200 to 7800 cm$^{-1}$ and with the resolution 0.02 cm$^{-1}$. 100 scans were accumulated for each sample and averaged.

### 4.2.4 GC−MS analysis of the non-volatile part of the sample

The non-volatile phases of the sample were analysed using gas chromatography-mass spectroscopy. The sample cuvette contents were diluted in methanol (200 µl) and the solution was transferred to separate vials. The vials were dried under vacuum at room temperature. The dried samples were derivatized by a technique developed in our laboratory. 50 µL of the derivatization agent N−(tert−butyldimethylsilyl)−N−methyl−trifluoroacetamide (Sigma Aldrich; 99 % (wt./wt.) MTBSTFA, CAS 77377−52−7) and 150 µL of pyridine aprotic solvent (Scharlau; 99.5 % (wt./wt.) anhydrous) were added to the dried sample. Then, the sample was kept at 110 °C for 2 h. The measurement was performed using Trace 1300 Gas chromatograph (GC) coupled with an Ion−Trap Mass Spectrometer (MS) 1100 (Thermo Scientific, USA). The chromatograph was equipped with a nonpolar Trace GOLD TG−5 MS capillary column (Thermo Scientific, USA; 95 % (wt./wt.) dimethylpolysiloxane and 5 % (wt./wt.) phenylmethylpolysiloxane). 1 µl of the derivatized sample was injected to the system. The separation was performed using a 10 °C.min$^{-1}$ gradient from 180 °C to 280 °C. The samples were scanned for the presence of nucleic acid bases, urea, purine and glycine. The detection of the products was confirmed by comparison of the retention times and mass spectra of the sample with standards of the detected compounds treated in the same manner as the sample. Blank runs were also performed to rule out any external contamination.

## 4.3 Design of the experiment with CO$_2$-rich atmosphere reduction

As was discussed above, the geological records [27]–[32] point most likely towards and atmosphere during the Hadean, which consisted mainly of carbon dioxide, water vapour and molecular nitrogen, however, very probably more reduced than today, with significant content of several reducing compounds such as methane, ammonia, hydrogen cyanide, hydrogen sulphide, formaldehyde, carbon monoxide molecular hydrogen, of varied concentrations. This composition is a compromise between the all-reducing atmosphere proposed by Oparin and explored by Miller and Urey, which is probably unstable and the neutral atmosphere. However, the reducing atmosphere might have been continuously replenished by frequent impacts of extraterrestrial bodies rich in

reduced carbon and nitrogen refractory material, active tectonics and increased volcanism. The neutral atmosphere, which carries lesser potential for successful prebiotic synthesis and is also depleted in $CO_2$ by its dilution in oceans and sedimentation as carbonate minerals. Also, outgassing from organic material contained in carbonaceous asteroids and comets impacting on early Earth as well as **Fischer-Tropsch** processes, hydrothermal vents and volcanic activity supplied reducing gases material into the atmosphere and its contribution in the Hadean was non-negligible. In the first part, a novel additional source of reducing gases in early Earth's secondary atmosphere was explored: photoreduction processes over acidic minerals.

The efficiency of the photocatalytic reduction of carbon dioxide was explored in this study on several mineral surfaces, of which two are most important: the widely used photocatalyst titania (in anatase form) and also the newly recognized photocatalyst montmorillonite, which exhibited photocatalytic properties comparable with titania, according to the results described in following chapters. Titanium dioxide was chosen, because it is a well-described mineral, which is often used in photocatalytic reactions of $CO_2$ related to environmental chemistry. Other minerals were chosen as minerals, which are present on contemporary Earth and possibly on the early Earth as well [37], [172] The activity of some of these minerals as catalysts in this reaction has not been documented so far, most notably in the case of montmorillonite, which represents the family of clays, which is very common on Earth. Also, our team has shown, that the photocatalytic reduction of $CO_2$ is boosted by acidic environment[8]. In our experiments, HCl has been chosen, because the data have been interpreted in connection to Mars as well as Earth and on Mars, HCl may play a role in the creation of perchlorate salts, whose origin has not been explained yet.
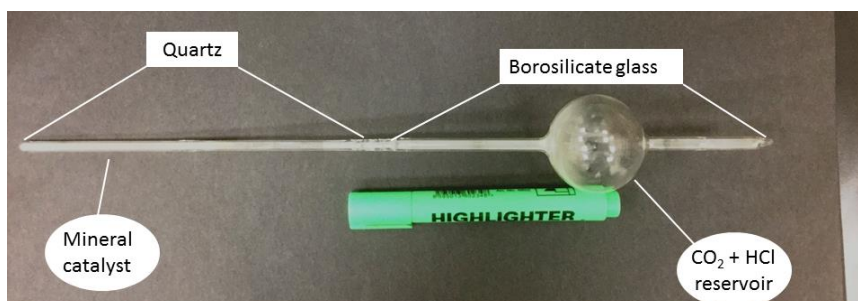


Figure 20. The sample cuvette created for the UV irradiation of mineral catalysts. The left part serves as a space for the mineral catalyst and the right part as a reservoir for the gases.

As is shown in figure 20, special sample cells were created for the irradiation. The left part of the sample is made of quartz glass. This part serves as a space for the mineral catalyst and is transparent for UV radiation in the 250-400 nm region, which our lamp predominantly produces. The reaction cascade takes place on the surface of the catalyst and is initiated by the UV photons. The right part is made of borosilicate glass, which is transparent for IR radiation in 2-5 μm spectral range and therefore is suitable for infrared detection. The glass ball serves as reservoir for the reaction gases.

Each experiment, the cell was filled with a mineral catalyst (typically 0.5 g). The catalyst was annealed for 2h at 150 °C under vacuum ($10^{-2}$ Torr). This process released water from the catalyst. Then, the cell was connected to an HCl reservoir. The HCl was frozen, the air was evacuated from the sample cell and then the HCl was left to melt and its vapours transferred to the sample cell. After 12 hours, the pressure in the sample cell was regulated, typically to 10 Torr HCl. Subsequently, the cell was filled with 10 Torr of $CO_2$, 2 Torr $N_2$ and sealed off. This closed system represented a planetary surface in contact with a neutral atmosphere under the effect of UV radiation. The pressures and amounts of substances in each sample were either typical (as described above) or varied to mimic specific circumstances. In the case of mimicking Mars, the $CO_2$ pressure was 6 Torr.

## 5   Results and Discussion

Samples of $CO_2$ + HCl with various mineral catalysts were irradiated with soft UV radiation. The experiment represented UV-induced transformation of early atmospheres of young terrestrial planets.

### 5.1   Transformation of early atmospheres

After as early as 48 hours of irradiation with a 160 W Hg lamp (peak intensity 360 nm, distance from the cell 20 cm), several samples, representing approach to mineralogy of the early Earth or Mars surface, showed a partial transformation of $CO_2$ to $CH_4$. From the qualitative point of view, some minerals are active catalysts and some are not, which is summarized in table 2. Samples, which showed no presence of methane or no decrease of $CO_2$ after 3500 hours of irradiation were proclaimed as inactive.

Table 2. List of tested minerals and an indication, whether they are active in the photocatalytic reduction. Samples, whose spectra showed no presence of methane or no decrease of $CO_2$ after 3500 hours of irradiation were proclaimed as inactive.

| | |
|---|---|
| $TiO_2$ Anatase A200 (containing HCl) | ✓ |
| $TiO_2$ Anatase from isopropoxide | ✓ |
| $TiO_2$ Anatasa from isopropoxide + graphene | ✓ |
| $TiO_2$ rutile (synthetic, Bayer 5556) | ✓ |
| Montmorillonite K10 (Sigma Aldrich) | ✓ |
| $Al_2O_3$ (Sigma Aldrich) | ✓ |
| Kaolinite (Sigma Aldrich) | ✓ |
| $TiO_2$ rutile (natural, Golčův Jeníkov) | ✓ |
| Natural clay (vz. 5, Sokolovská pánev) | ✓ |
| MgO | ✗ |
| $CaCO_3$ | ✗ |
| Siderite ($FeCO_3$, natural, Příbram) | ✗ |
| Basalt (natural, Děpoltovice) | ✗ |
| Ilmenite ($FeTiO_3$, natural, Jizerská louka) | ✗ |
| H-clay | ✗ |
| Na-clay | ✗ |
| Fe-clay | ✓ |

The composition of the gas phase of the samples was observed by high-resolution infrared spectrometer Bruker. The typical spectra representing both extremes are shown in figure 21.
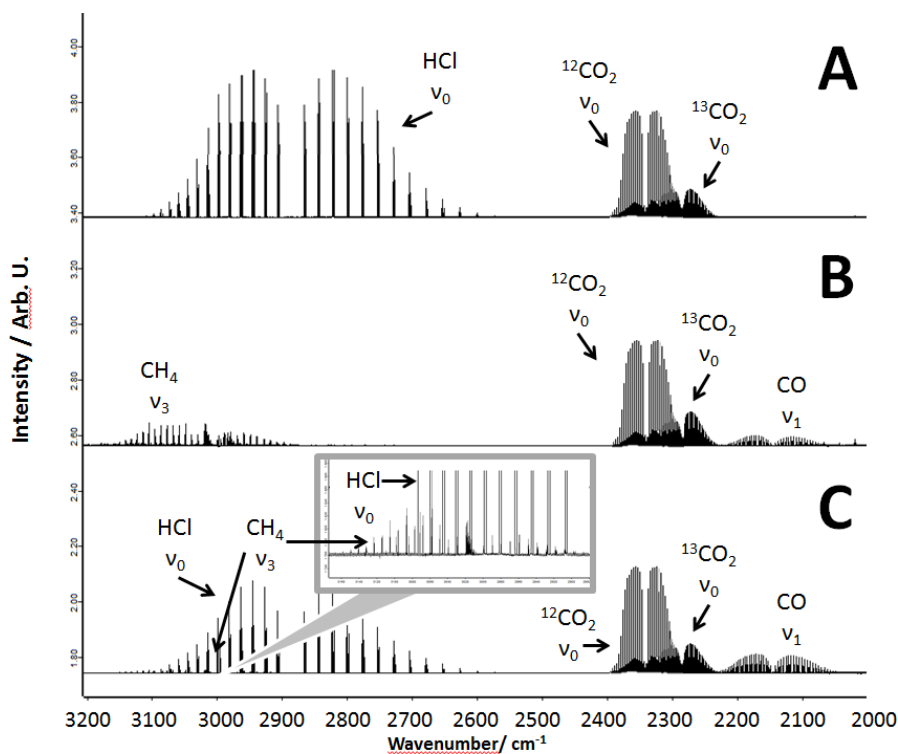
Figure 21. Spectra of the gas phase before and after UV irradiation. Panel A shows the gas phase composition before the irradiation. Panel B shows the gas phase spectrum of the sample with $TiO_2$ anatase (containing 14% of HCl) + $CO_2$ after irradiation for 650 h. Panel C analogically shows the sample of montmorillonite with HCl (g) + $CO_2$ after 650 h irradiation.

Panel A shows the spectra before irradiation. If the spectra did not change for 3500 hours, the sample was inactive. Panel B shows the spectra of titanium dioxide sample after 650 hours of irradiation. HCl is not visible, because in the case of $TiO_2$, it has not been added to the sample as vapour, because the mineral contained 14 % (w/w) of HCl embedded in its structure. Panel C shows the spectra of the sample containing montmorillonite whose catalytic effect in the photocatalytic reduction of $CO_2$ has never been recognized before and which is a representant of a very common mineral on Earth. No other gases were detected during these experiments. Note also that the spectra show $^{13}CO_2$, which naturally constitutes about 1% of $CO_2$.

## 5.1.1 Kinetics of the photocatalytic reduction

The kinetic aspect of the photocatalytic reduction was also analysed. Three signal peaks of each compound in each sample were randomly selected and their area was integrated

50

using the OPUS software package. The intensities were transformed to partial pressures using independent calibration measurements of pure gases at laboratory temperature. The resulting plots are shown in figure 22 and figure 23.



Figure 22. Concentration of methane and CO in the sample containing montmorillonite as catalyst.



Figure 23. Concentration of methane and CO in the sample containing titanium dioxide anatase as catalyst.

The photocatalytic reduction of $CO_2$ to $CH_4$ and CO can be summarized by the following equation [173] (1):

$$2CO_2 + 10[H^+] = CO + CH_4 + 3H_2O \tag{1}$$

The mechanism of the photocatalytic reduction has not been determined yet with definitive validity, but this summarizing reaction holds whatever the proposed mec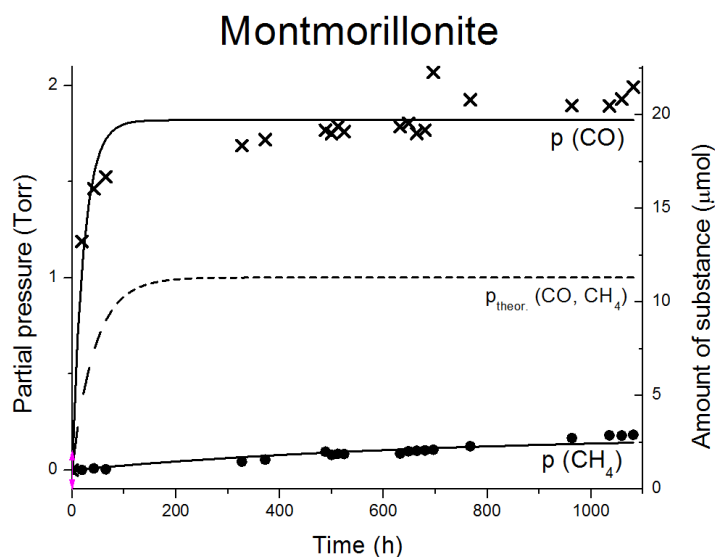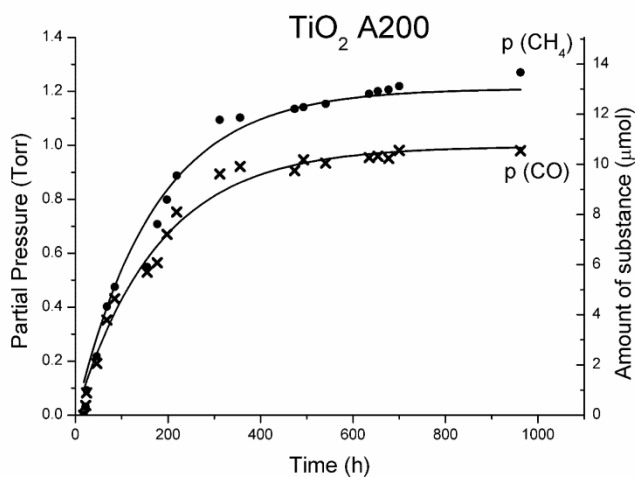hanism. This fact was used to determine the first order rate constants $k_{I(eff)}$, while considering the $H^+$ as a reactant coming into play at the surface of the catalyst and always in excess. The reactant whose concentration actually decreased was the $CO_2$. The constants are henceforth called pseudo-first order, because the reaction mechanism is presumably much more complicated. They, however, can be used to compare the efficiencies of various catalysts. It is worth mentioning that the proton in this reaction comes from HCl. Other acids can be used as well (e.g. $H_2SO_4$ [174]).

Applying the simplification (i.e. the first order kinetics) and studying the proposed mechanisms (as discussed below), the reaction can be separated to two reactions: the formation of methane and CO (2), (3).

$$CO_2 + 8H^+ = CH_4 + 2H_2O \tag{2}$$
$$CO_2 + 2H^+ = CO + H_2O \tag{3}$$

Both the equations can be described from the kinetics point of view by (4):

$$\frac{-dp_{CO_2}(t)}{dt} = k_{I_{eff}} \times p_{CO_2}(t) \tag{4}$$

where $p_{(CO2)}$ is the partial pressure of $CO_2$.

The product concentration can be described by (5):

$$p_{prod}(t) = \left(p_{CO_2}(t_0) - p_{CO_2}(t_0) \times e^{-k_{I_{eff}}t}\right) \times \frac{p_{prod}}{\Delta p_{CO_2}} \tag{5}$$

where $p_{(prod)}$ is the partial pressure of the monitored gas (either carbon monoxide or methane) and $p_{(CO2)}(t=0)$ is the total pressure in the system at the beginning (to exclude desorption effects which cause the total pressure in the system to rise regardless of the reaction progress). The term $p_{(prod)}/\Delta p_{(CO2)}$, the ratio of the partial pressure of the product $p_{(prod)}$ formed during the reaction and the decrease in the pressure of the reactant in the gas phase $\Delta p_{(CO2)}$, is present in the equation to consider the fact that some of the products remain adsorbed on the surface of the catalyst.

Rate constants, $k_{I(eff)}$ were determined for each product in each experiment and in the case of titania and montmorillonite are shown in table 3.

Table 3. Effective rate constants and a theoretical rate constant for the production of $CH_4$ and CO over titanium dioxide and montmorillonite K10.

| Compound | $k_{eff}(h^{-1})$ | $k_{eff}(h^{-1})$ | $k_{theor.}(h^{-1})$ |
| --- | --- | --- | --- |
| | TiO$_2$ A200 | montmorillonite | montmorillonite |
| CH$_4$ | 0.0059 | 0.0014 | 0.0231 |
| CO | 0.0056 | 0.0447 | 0.0231 |

### 5.1.2    External quantum efficiency of the photocatalytic reduction

Apart from the kinetic aspect, the reactions on the discussed catalysts can be compared in terms of the number of photons generated and delivered to each sample per molecule of product. In this case only methane was considered. By combining some basic formulae, the number of photons may be calculated (6-10):

$$E_{photon} = \frac{hc}{\lambda} \tag{6}$$

$$E_{tot} = p_{lamp} \times t \tag{7}$$

$$N_{photons} = \frac{E_{tot}}{E_{photon}} \tag{8}$$

$$N_{methane} = \frac{pV}{RT} \times N_A \tag{9}$$

$$N_{\frac{photons}{molecule}} = \frac{N_{photons}}{N_{methane}} \tag{10}$$

where $E_{photon}$ is the energy of a single photon, $h$ is Planck's constant, $c$ is the speed of light, $\lambda$ is the photon wavelength, $E_{tot}$ is the total energy delivered to the sample by a UV lamp with power $P_{lamp} = 3000$ mW.cm$^{-2}$ on the surface of 1 cm$^2$ over time $t$. $N_{photons}$ represents the total number of photons delivered to the sample, $p$ represents the methane pressure in the system, $V$ is the sample chamber volume, $R$ is the universal gas constant, $T$ is the temperature (298.15 K), $N_A$ is the Avogadro's constant and $N_{photons/molecule}$ is the total number of photons delivered to the sample per molecule of methane. The results are shown in table 4. The total amount of generated photons per molecule is called the 'external quantum efficiency' (EQE) and is not related with the actual amount of absorbed photons. The exact EQE is a function of the actual experiment geometry and set-up (i.e. the UV source, sample angle, source distance from the sample, etc.). Table 4

also shows total amounts of each reactant in the sample. The amounts were calculated using the ideal gas law. Each number represents the amount of molecules at the end of the experiment (except for $CO_2$ as the reactant). It is obvious from the table that HCl concentration varies greatly in each sample. This is because the in the case of titanium dioxide (as is described in the sample preparation section), the catalyst contains 14 (w/w) % of HCl embedded in the internal structure as a leftover from synthesis. Also, this is why the gas phase contains no HCl. Montmorillonite, on the other hand, is initially HCl-free and the acid is added after the catalyst annealing.

Table 4. Amounts of substances in the reaction mixture, and the number of photons delivered to each sample to generate one molecule of $CH_4$.

| | $CH_4$ /µmol | CO /µmol | HCl/µmol | $CO_2$(initial)/µmol | $N_2$ /µmol | $N_{photons/molecule}$ |
|---|---|---|---|---|---|---|
| montmorillonite | 1.80 | 21.38 | 46.74 | 66.68 | 11.73 | $9.60 \times 10^6$ |
| $TiO_2$ anatase | 11.81 | 10.48 | 1333.19 | 66.66 | 5.24 | $5.00 \times 10^5$ |

### 5.1.3 The reaction mechanism

The results dealing with the kinetic aspect of the photocatalytic reduction show that $CH_4$ and CO are formed at similar rates in the case of titanium dioxide. On the other hand, on montmorillonite, the observed ratios are vastly different. This disparity points to a different reaction mechanism or to the existence of a side reaction or a reverse reaction.

There exist so far three theories on the possible reaction mechanism of the photocatalytic reduction in the literature. They are called the glyoxal pathway [175], the carbene pathway [176] and the formaldehyde pathway [177]. They have been named after their unique intermediates. The three theories are summarized in the figure 24.
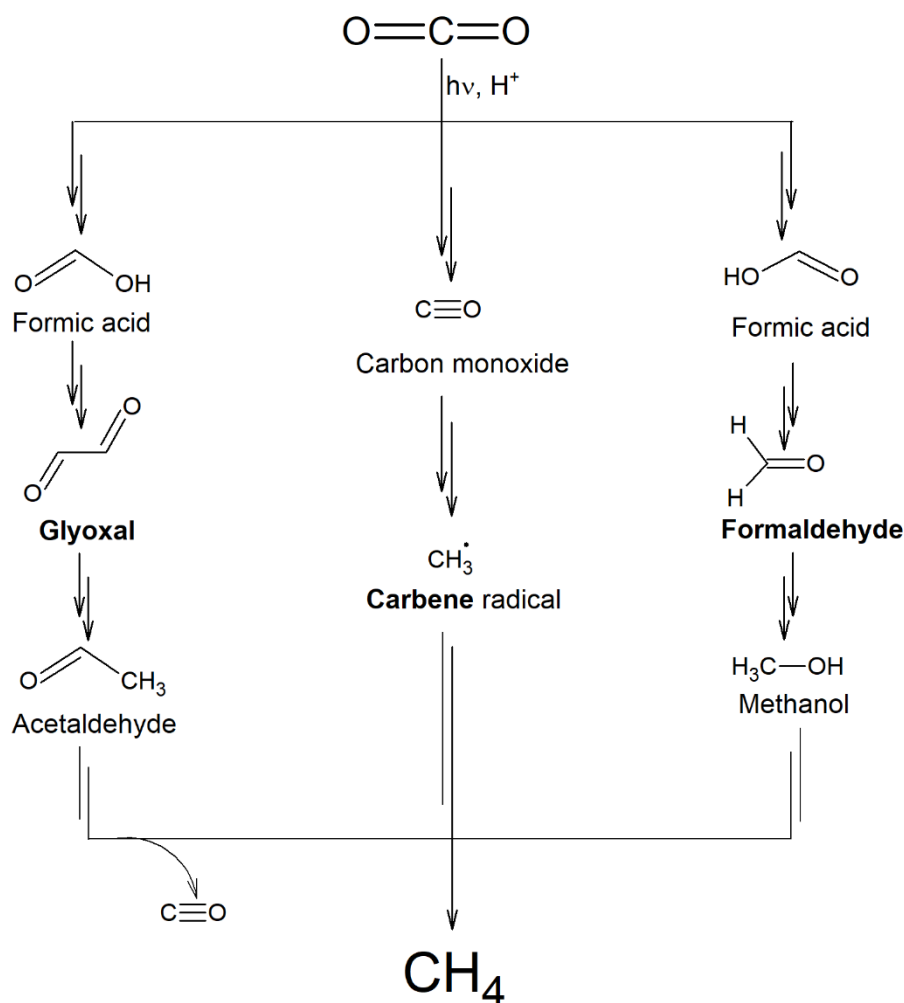
Figure 24. A simplified scheme of the three theories of the photocatalytic reduction of $CO_2$. The left pathway is called Glyoxal pathway, the central pathway the Carbene pathway and the right-side the Formaldehyde pathway, according to their unique intermediates.

All the pathways are briefly discussed in a recent paper by our team [178] and are thoroughly discussed in a review by Habisreutinger et al. [173] None of these theories has been definitively confirmed and experiments in the literature show evidence for all three pathways under certain conditions and with the use of certain techniques [179]. The verification or disproving of these theories will be subject to future work in our laboratory.

In order to identify the reason for the relative disparity in production rates of methane and CO on both catalysts, likenesses and differences between the two catalysts should be assessed. $TiO_2$ is a crystalline compound with well-defined crystal structure (spatially homogenous). The used $TiO_2$ has the crystal size of about 100 nm (general

size of crystals created by this procedure in Prof. Kavan's laboratory, the size has not been determined for this sample). Montmorillonite, on the other hand, has a variable composition $(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$ without a clearly defined spatial structure. Also, both materials are semiconductors, but their band gap is different. $TiO_2$ has a band gap of $3.1 \pm 0.1$ eV [173], whereas montmorillonite possesses a band gap 5.35 eV [180]. Therefore, soft UV radiation would be insufficient to excite and electron from the valence to the conduction band in montmorillonite. It is probably the effect of the acidic environment that this catalyst is active in the reduction. All the mechanisms defined in the literature, however, work with photocatalysts with band gaps 3.5 eV and lesser (in order to utilize solar radiation) [173], [175].

The reason for the production rate disparity might also lie in the presence of a side reaction, where methane is oxidized back to $CH_4$. This reaction is photochemically driven as well and is a reaction, which is common in atmospheric chemistry and planetary atmospheres. The reaction begins with photolysis of water [181] (eqs. 11&12):

$$H_2O = H\cdot + \cdot OH \qquad\qquad (11)$$

$$H_2O = H_2 + O: \qquad\qquad (12)$$

Photolysis of water is usually effective at wavelengths <186 nm (i.e. the vacuum ultra violet radiation) [182]. Soft UV radiation (around 350 nm) has reportedly been effective in the photolysis only at high pressure [183] and only according to reaction (11). It is possible that in the case of water adsorbed on montmorillonite, the molecule can be photolyzed due to a catalytic effect, which has not yet been explained.

Following the photolysis of water (13), $\cdot OH$ reacts with methane, which gives rise to CO, $H_2$ and $H_2O$.

$$CH_4 + 2\cdot OH = CO + 2H_2 + H_2O \qquad\qquad (13)$$
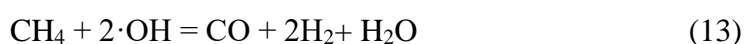
For this reason, a theoretical rate constant has been introduced in the kinetic analysis of the reaction. This theoretical constant shows the production rate of methane and CO over montmorillonite, were there no side reaction to the process. In this way, we see the montmorillonite as about half as efficient a photocatalyst in this process as $TiO_2$. This

theoretical constant is a pure assumption and the existence of this side reaction of methane photolysis, although probable, has not been demonstrated experimentally yet.

Direct photolysis of methane is possible as well, since the molecule exhibits an electronic absorption spectrum at wavelengths <140 nm (VUV region). The hydrogen Lyman $\alpha$ line (121.567 nm) is the most prominent wavelength in the solar spectrum. Photolysis induced by this line proceed in the ratio 3:1 (reactions (11):(12)). For longer wavelengths, however, the contribution of the second reaction is negligible [184]. The photolysis of water might occur on titania as well, but since only a small disparity in the product ratio was observed, its effect is significantly smaller than in the case of montmorillonite. A possible reason for this might be that after annealing at 150°C, surfaces of both $TiO_2$ and montmorillonite are water-free. However, montmorillonite contains water embedded in its structure (the mineral is a hydrate), which cannot be evaporated by annealing.

The reaction exhibits equilibrium, which may be a direct consequence of the reaction mechanism or some other unconnected reason. The yield of each product (CO, $CH_4$) over each catalyst ($TiO_2$, montmorillonite) generally depends on the availability of reactants ($CO_2$, $H^+$). As is clear from table 4 on page 54, the sample contained a large excess of $CO_2$ compared to the products. However, as 8 $H^+$ are needed for the creation of one $CH_4$ molecule[173], it is the $H^+$ concentration, which is the limiting factor in our case. In the case of montmorillonite, by multiplying the amount of $CH_4$ created in the reduction process (in moles, without the dissociation to CO that is, approximately 11.59 moles) by the amount of hydrogens in the molecule, 4, the amount of converted HCl is approximately obtained (46.36 moles, compared to 46.74 moles in the initial mixture). On titania, the HCl amount was in great excess, since the catalyst contained 14 (w/w) % of HCl as a leftover from synthesis. This means, that only a small fraction of the HCl was exposed on the crystal surface and therefore available for the reaction. The effective amount of HCl is difficult to estimate, but the presence of the reaction equilibrium implies that there was a limited amount of protons available and the fact that the final concentration of products was comparable suggests that the amounts of available HCl were similar on both catalysts.

Overall, the photocatalytic reduction is an important process, which must be included in future planetary models of both early Earth and other terrestrial planets. It is also

apparent that on the early Earth, an atmosphere containing methane and carbon monoxide has been created, although it probably was only locally and temporarily. The emergence of life [35] coincides with the period of Late heavy bombardment on Earth [19]. It is therefore important to assess what happens when an extraterrestrial body impacts into a planetary atmosphere of such composition.

### 5.1.4    UV flux on early Earth and formation of methane in photochemical processes

The reactions studied in this work are photochemically driven, or at least the sequence of reactions is photochemically initiated by UV radiation. If this reaction is to be applied to models of early Earth and planetary chemistry in general, the UV fluxes on the surface must be determined. So far, there exist ways how to model surface UV fluxes for planets, but due to lack of data and high amount of calculations involved are very complicated and often inaccurate. Nevertheless, attempts have been made to surface UV flux models on the early Earth in the literature. The exact values of the UV flux on early Earth, or rather its surface, are highly disputable and are highly dependent on the current atmosphere. For example, larger amounts of hydrocarbons create hazes in the atmosphere, which effectively shield the surface from the UV radiation. Also, dust swirling, the presence of ozone and the overall chemical composition are important, but very difficult to generalize and define on a global scale.

Currently, the mean soft UV spectral irradiance of the Earth's surface is in the order of 0.1 W. m$^{-2}$. This is also comparable to Mars [185]. On the early Earth, the atmosphere was presumably denser and therefore the UV flux at the surface lesser. On the other hand, young parent stars emit stronger UV/XUV/X-ray fluxes than older matured stars (such as the present Sun). [186] Also, photochemical processes certainly influence upper parts of even dusty and cloudy atmospheres producing hazes, which exhibit rich photochemistry, such as for instance on Titan or Venus. On current Earth, shorter wavelengths, i.e. <300 nm, the spectral irradiation decreases dramatically to 10$^{-3}$ W.m$^{-2}$, mainly due to the presence of the ozone layer [187]. On the early Earth, up to some 2.5 Gyr ago, there was no ozone layer. Ozone is created from free oxygen which was first present in significant amounts in the Earth's atmosphere only after the photosynthetic forms of life emerged. This also has implications for the origin of life. It seems probable, that cellular life first emerged in oceans and one of the arguments in favour of this claim is the shielding, which water efficiently provides. According to models, radiation in the region 260 – 315 nm was about 4 times higher than on present-day

Earth. Specifically, the intensity increases with the increasing wavelength, at 260 nm being $10^{-2}$ W.m$^{-2}$ while at 315 nm, it is already $10^{-1}$ W.m$^{-2}$. Other models predict that before the formation of the ozone layer, the Earth was transparent to hard UV (<280 nm) radiation and the irradiance varied from $1\times10^{-3}$ W.m$^{-2}$ at 180 nm to $1\times10^{-2}$ W.m$^{-2}$ at 280 nm [188], [189].

The presence of UV near the surface of our young planet has been considered before, but only as a destructive agent in the photolysis on methane and ammonia, from which the study inferred that the atmosphere would have been neutral or oxidized. Our study shows, that the presence of UV light may generate reduced compounds, thus rendering the argument for the oxidized state of the atmosphere unnecessary [190].

It is obvious that photochemical transformation of early Earth atmosphere in terms of the formation of $CH_4$ and CO from the atmospheric $CO_2$ must be carefully investigated and at this stage of knowledge, when the effect itself was very recently discovered and published in relation with early Earth chemistry for the first time by our team, it is difficult to answer this question without the corresponding model of global chemistry. Every such study will be complicated by the unknown UV flux on the surface, because there is lack of knowledge of several important aspects of early environment, such as what part the atmospheric aeorosols, hazes and dust played, understanding the climate, surface mineralogy and atmospheric composition.

Nevertheless, what was theoretically possible on early Earth, presumably occurs on the current Mars. Although this relation to Martian chemistry is beyond the scope of this thesis, it should be noted that rovers sent to Mars (where $CO_2$ is the main constituent of the atmosphere) detected methane, perchlorates and chlorinated alkanes. The origin of these species has not yet been successfully explained. It was already shown in the Results section that UV-initiated chemical reactions of carbon dioxide with HCl in presence of mineral catalysts (including the Nakhla meteorite from Mars) are able to produce all the mentioned compounds (perchlorates, CO, $CH_4$, chlorinated hydrocarbons) detected on Mars. It can be presumed that similar chemistry occurs or occurred of the early Earth and exploration and planetary modelling of Earth and Mars can both be used for one purpose, which is the answer to the state of the atmosphere of young terrestrial planets. Figure 25 shows UV flux on the surface of present-day Mars [191]. Contrary to Earth, Mars has no ozone layer and is not shielded against hard UV

radiation (<300 nm). It should be noted, however, that Earth did not have its ozone layer until the first forms of life were present.
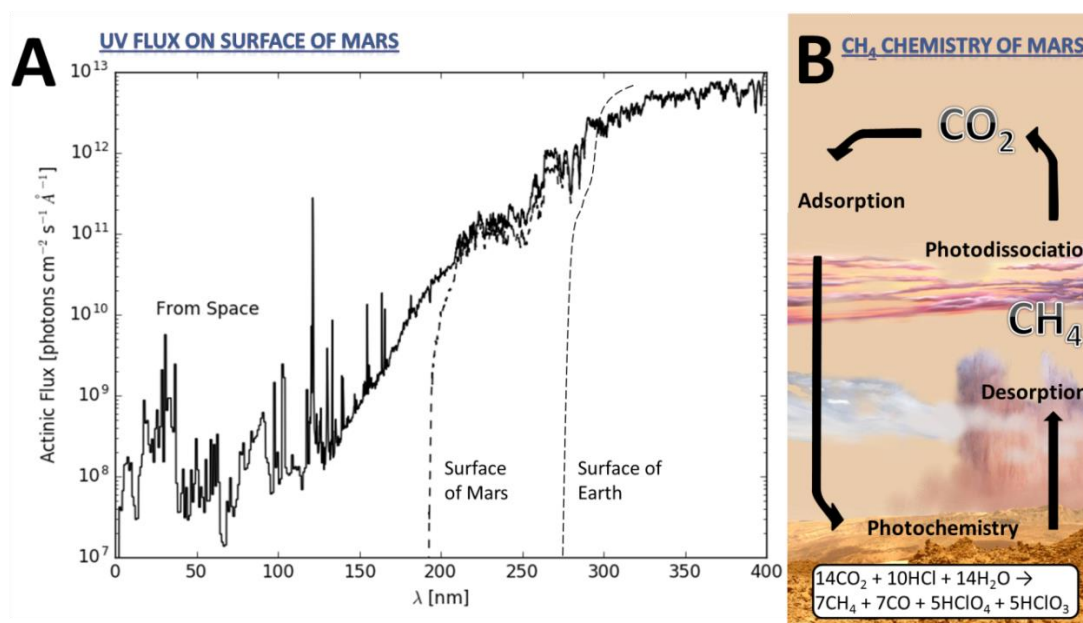


Figure 25. Panel A shows the UV flux on current Mars – a planet with a mostly photochemically driven atmospheric chemistry. The graph shows a high-resolution spectrum incident on the upper atmosphere of Mars and projections of incident UV flux on the surface of Mars and Earth at zenith angles 0°. The spectrum was created by Dr. Paul B. Rimmer for our future publication currently under preparation. Panel B shows a proposed carbon cycle on past and present Mars. Carbon dioxide from the atmosphere is adsorbed on the surface of the planet, where it is photochemically reduced to methane and CO. $CH_4$ is released from the surface and photolyzed in the atmosphere back to $CO_2$. HCl is transformed to perchlorates and chlorates during this process.

## 5.2 Reprocessing of the partially reduced atmosphere to a HCN rich atmosphere

Previously published studies [12], [79], [108], [192], [193] on high-density plasma reprocessing of methane + nitrogen mixtures in presence of water report the production of significant amounts of acetylene, hydrogen cyanide, propadiene, ethene, methanol, propane, butadiene, ethanol, cyanoacetylene, acetone, propanol and propene. These samples were typically treated with 25 plasma shots from a high-power infrared laser with the total energy 3250 J. Each shot has 350 ps, so each sample was exposed in total

to 5.25 ns of high-density discharge plasma. Each laser shot creates a frozen equilibrium in the sample, which is increasingly shifted in favour of the products which are created by reactions which typically take microseconds and predominantly occur in the afterglow of the laser shot. Following the same ideology, the partially converted atmospheres now containing $CO_2$, $N_2$, HCl, CO, $CH_4$ and a mineral catalyst were exposed to the same environment.
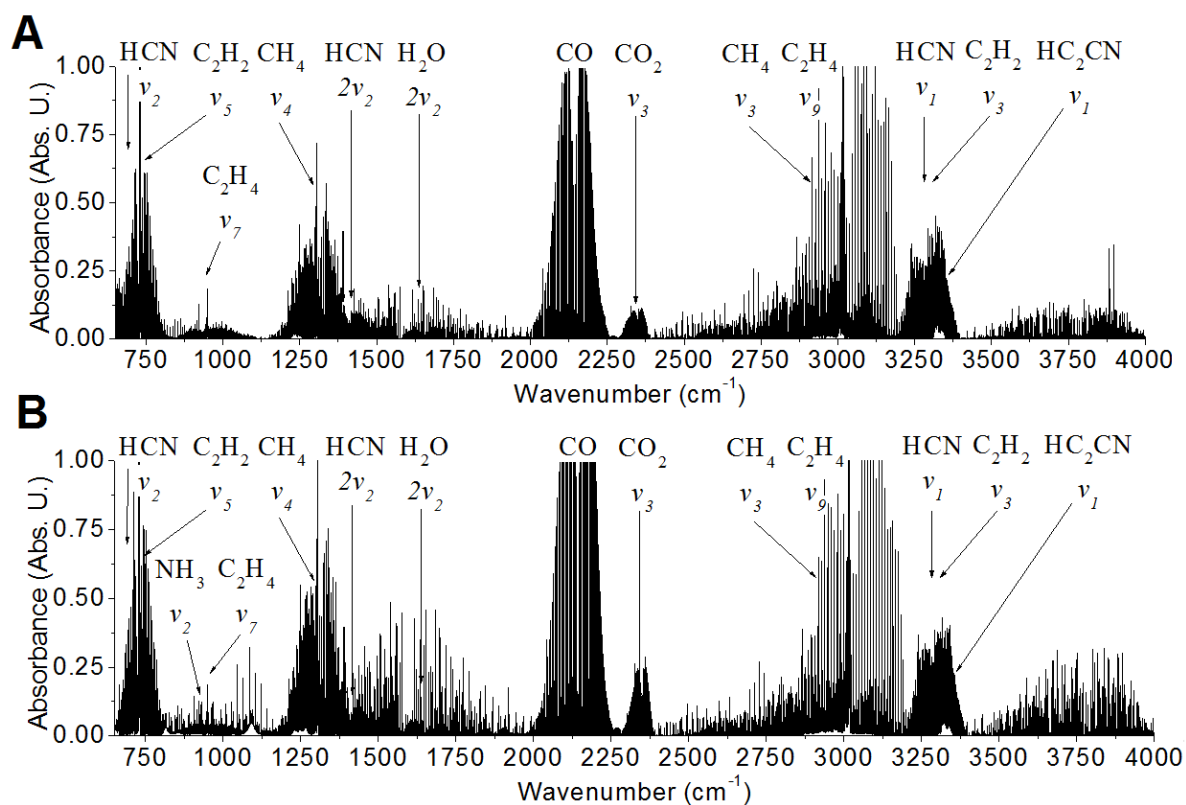


Figure 26. Gas phase spectra of samples without montmorillonite (A) and with montmorillonite (B) + $CO_2$, CO, $CH_4$, $N_2$ and HCl exposed to 20 laser 150 J shots on the Prague Asterix laser facility. The spectrum contains signals of acetylene, hydrogen cyanide, cyanoacetylene, water, methane, CO and $CO_2$.

The gas phase spectra of the plasma treated samples (after 20 laser shots; figure 26) show that CO and $CH_4$ remain the main constituents of the mixture. The spectra of the sample containing anatase as catalyst also show prominent bands of HCN, acetylene, cyanoacetylene and ethene. The total yield of substances other than the starting ones was approximately 5%. As before, however, it is important to bear in mind the presence of the frozen equilibrium. In the course of the total of 20 laser shots, the samples were

exposed to a total of 7 ns of the laser plasma and the respective afterglows, i.e. in total approx. 40 μs of plasma exposure, while the yield of the products increased after every shot. It is only possible to suppose that more laser shots would produce higher yields of these compounds, but this was not tested due to the limited experimental time at the high-power laser.

With the intention to further investigate the high-energetic chemistry of prebiotic relevance, different mixtures were treated by the high-power laser or electric discharge. The gas compositions were chosen to represent possible neutral or reduced atmospheres on the early Earth. High-power laser irradiation represented impacts of an asteroid and the electric discharge represented lightning in dust storms or volcanic debris clouds (static energy lightning) or regular storms. The reactive intermediates and products were monitored using time-resolved high-resolution emission infrared spectroscopy (in the case of the discharges) and by optical emission spectroscopy and high-resolution absorption infrared spectroscopy (in the case of the shock wave).

### 5.2.1 Shock-wave decomposition of simple gaseous atmospheres containing H, N, C and O atoms

Upon exposure of simple carbon bearing gases (CO, $CO_2$, $CH_4$) together with ammonia or nitrogen and water to high-power laser plasma, we have observed formation of various products, as is shown in figure 28. A common feature of all these experiments was the formation of HCN. The same was also observed during shock-wave decomposition of formamide as the only reactant.
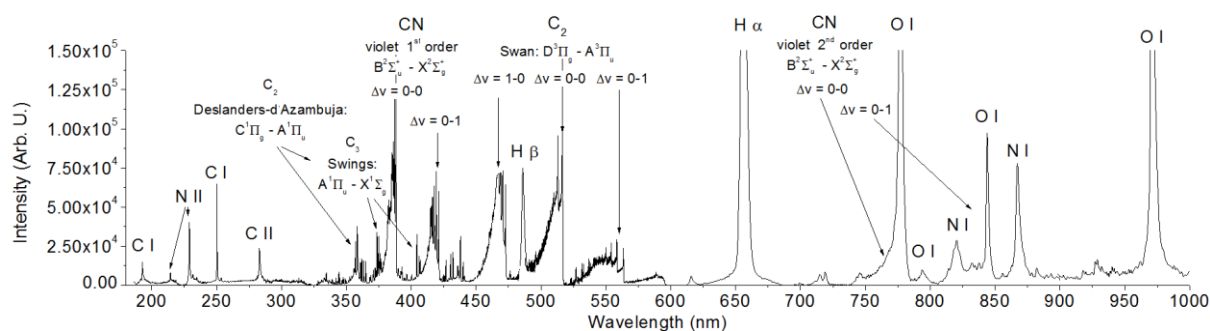


Figure 27. Compiled emission spectrum of laser induced breakdown in mixture of C, H, O & N bearing molecules [79], [192], [194], [195].

Very prominent emission molecular band in all the observed spectra is the ·CN transition between $X^2\Sigma^+_g$ and $B^1\Sigma^+_g$ states. Figure 27 shows a spectrum compiled from [79], [192], [194], [195] and represents a typical spectrum observed in all our experiments. The ·CN radical is a presumed precursor of HCN [192], [196]–[200], which has also been detected. It is also important to note that the highest yields of HCN were obtained from mixtures which contained high amounts of $CH_4$ or $HCONH_2$. This is in accordance with the previously stated fact that the reduced atmosphere carries
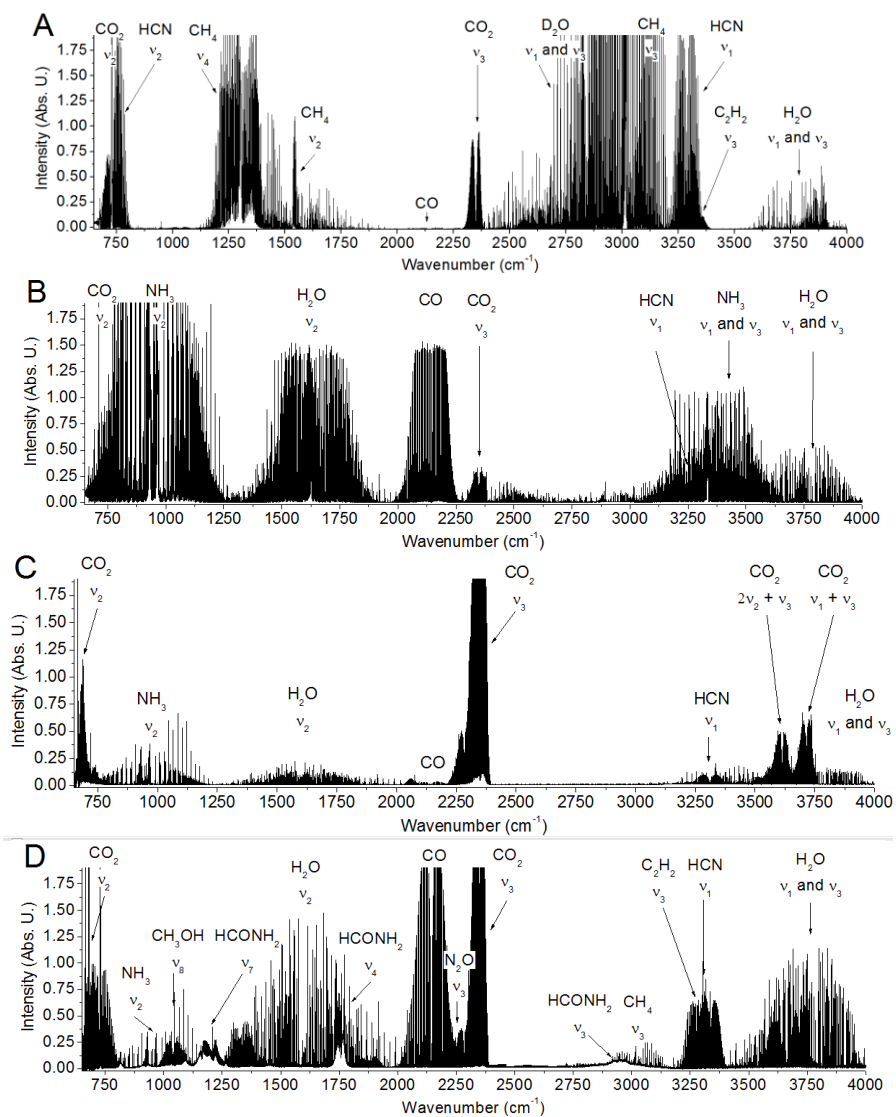


Figure 28. Absorption spectra of gaseous mixtures exposed to high energy laser shock waves. The mixtures are as follows: (A) $CH_4$, $N_2$ and $D_2O$, (B) CO and $NH_3$, (C) $CO_2$, $N_2$ and $H_2O$ and (D) $HCONH_2$ and $N_2$. All spectra share a common feature, which is the presence of HCN.

larger potential for prebiotic synthesis. It is even more important, that HCN was produced in mixtures of neutral atmospheric gases, albeit in smaller concentrations. The decomposition pathways of these intermediates are discussed in the sections below.

### 5.2.2 Electric discharge decomposition of simple gaseous atmospheres containing H, N, C and O atoms

Similar experiments were performed using discharge plasma treatment of these samples. The products were monitored using time-resolved high-resolution infrared spectroscopy, which allows monitoring the reaction on microsecond time scales with maximum time resolution 1 µs [201]. The resulting spectra are shown in figure 29. Surprisingly and similarly to the shock-wave experiments, the ·CN radical is the main reactive intermediate of these decompositions with the HCN being the most prominent product and also present in all these experiments. Other radicals present include ·$C_2$, ·CH and others.
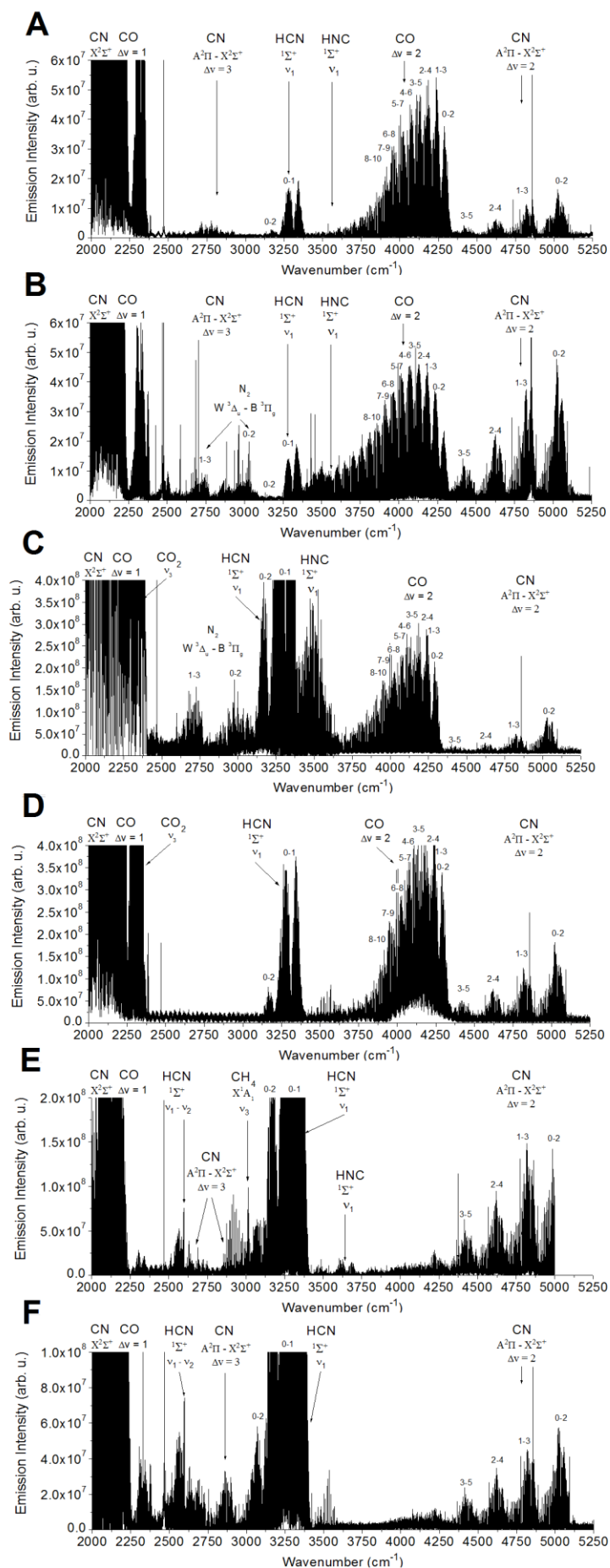
Figure 29. Electric discharge plasma emission spectra of (A) NH$_3$, CO and H$_2$O), (B) HCONH$_2$, N$_2$ and H$_2$O, (C) HCHO, N$_2$, H$_2$O, (D) CH$_3$OH, N$_2$ and H$_2$O (E) CH$_4$, N$_2$ and H$_2$O and (F) CH$_3$CN, N$_2$ and H$_2$O observed using time-resolved Fourier transform infrared spectroscopy between 1 and 5 µs after the discharge plasma pulse.

The discharge experiment was performed in a more varied array of compounds, including formamide, formaldehyde, methanol, methane or acetonitrile, which can be found throughout space and could be present during impacts as molecules delivered by the impacting body (a comet or an asteroid) or as compounds produced by atmospheric chemistry. In all the spectra, CO, HCN and ·CN are the most prominent species.

### 5.2.3    Chemistry of the high-energy plasma of simple atmospheres

The ·CN radical is the most prominent feature of all the performed experiments. This implies that the radical chemistry plays an important role in these processes. The ·CN radical is very rigid (triple bond with 7.77 eV dissociation energy [202]) and a complicated rovibrational spectrum). Among the allowed transitions are $A^2\Pi - X^2\Sigma^+$ $\Delta v$ = 2, 3; the ground state $X^2\Sigma^+$; $B^1\Sigma^+_u$ - $X^2\Sigma^+_g$ etc. In effect, this means that this radical is very efficient as energy mediator or very plausible reactant as it can be excited to various energy levels.

The potential energy diagram is shown in figure 30.
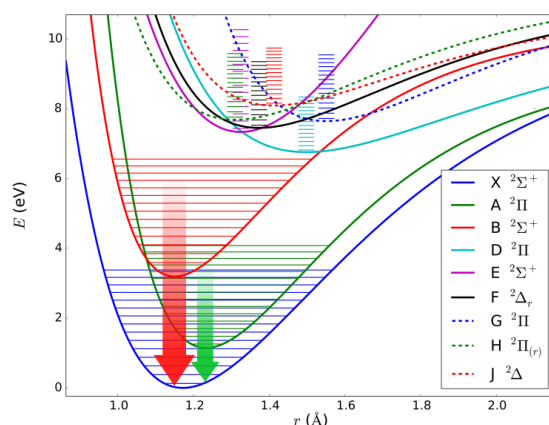


Figure 30. Potential energy surfaces for the 9 lowest excited electronic states of ·CN. Some electronic and rovibrational transitions were observed using time-resolved Fourier transform infrared spectroscopy and are marked in green and red, respectively. The figure was adopted from [158], where it has been compiled using constants from [203].

The diagram shows the first 9 energy levels and the possible transitions of the ·CN radical. The diagram was adopted from [158], where it has been compiled using constants from [203]. Due to favourable Frank-Condon factors for so large an amount

of states, a vast range of transitions can be observed [108], [204]–[209] from the visible to the microwave region.

Another similar molecule is the CO. Most of the spectra in our experiments show its presence as well. Figure 31 shows the potential energy diagram of CO. The figure was also adopted from [158].
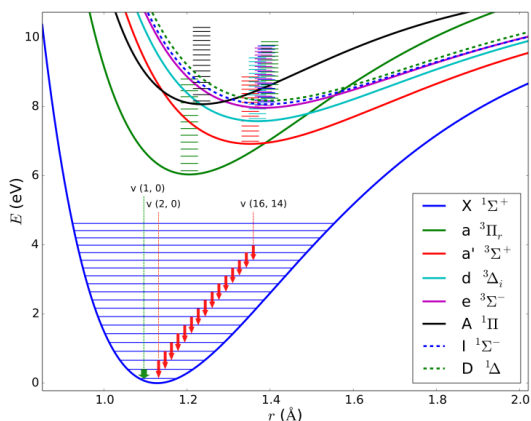


Figure 31. Potential energy surfaces of CO in its electronic states. Some observed rovibrational transitions up to $v = 16$ within the $X^1\Sigma^+$ electronic state and a second harmonic band transition were observed using time-resolved Fourier transform infrared spectroscopy and are marked in green and red.

Together with ·CN, CO controls the flow of energy in the hot plasma, where it can absorb, store and give off energy in a range of options. It can be assumed that larger molecules, such as acetylene, formamide, ethene, etc. are created by reactions of either CO or ·CN with other species in the plasma, such as ·NH or ·OH.

As proposed in [158] with additional data taken from [210], the mechanism of the production of HCN is the following:

A molecule of $N_2$ is first dissociated to two radicals (14):

$$N_2 \rightarrow N\cdot + N\cdot \tag{14}$$

which is energetically the most favourable way to split a nitrogen molecule [196]. If excess energy is present, a radical may be split into a cation and an electron (15):

$$N\cdot \rightarrow N^+ + e^- \tag{15}$$

It should be noted that both species (in spectroscopic notation N I and N II) were observed during our experiments (as shown in figure 27 on page 62). It can be assumed

that since the N· radical is the dominant species of $N_2$ decomposition, the following reaction takes place (16,17):

$$·CH_3 + N· \rightarrow HCN + H_2 \tag{16}$$

or

$$CH_4 + N· \rightarrow HCN + H_2 + H· \tag{17}$$

Both reactions directly produce HCN. Therefore, HCN can be considered a terminal product in the $CH_4 + N_2$ high-energy chemistry.

HCN can also be produced from reactions between CO and $NH_3$. This pathway proceeds through synthesis of formamide and its subsequent dehydration (18,19):

$$NH_3 + CO \rightarrow HCONH_2 \tag{18}$$

$$HCONH_2 \rightarrow H_2O + HCN \tag{19}$$
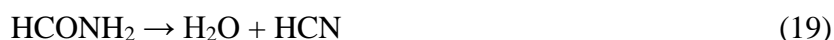
Another route was suggested in [211]. The authors observed formation of diazirinone $N_2CO$, ·OCN and ·NCN. Under our conditions, the formation of these species was not observed. Our experiments, however, also showed production of some $N_2O$. If we assume that CO reacts with an N·, the following may be inferred (20&21):

$$CO + N· \rightarrow ·OCN \tag{20}$$

$$·OCN \rightarrow CO \text{ or } ·CN + O· \text{ or } N· \text{ (fast)} \tag{21}$$

The ·CN radical then reacts with H· to form HCN (22).

$$·CN + H· \rightarrow HCN \tag{22}$$

Now the reactions leave a free O· radical. The following reactions (23-26) may explain the sink of these radicals as well as the origin of the observed $N_2O$[211][212].

$$N + O \rightarrow NO \tag{23}$$

$$O + N2 \rightarrow NO + N \tag{24}$$

$$NO + O \rightarrow NO_2 \tag{25}$$

$$NO + NO \rightarrow N_2O + O \tag{26}$$

In our experiments, NO and $NO_2$ are usually only trace species, while $N_2O$ is the most stable oxide of nitrogen under these conditions.

These experiments suggest that as HCN is the most abundant terminal product of these syntheses, it had been produced continually on the early Earth and was stable after the impact was over. If no other sinks of HCN were present, its feedstock would have been depleted by atmospheric chemical erosion. A possible sink might be the sequestration of HCN in aqueous solution by reaction with ferrous ions, which results in the creation of

ferrocyanide [123]. Either through this process or directly, further reactions leading to the creation of biomolecules may take place.

## 5.3  Model of planetary chemistry

Chemistry over mineral surfaces explored within this thesis supplies CO, CH₄, (mixed with N₂, and possibly other molecules) and produces HCN as the main reaction product of plasma chemistry. However, if the reaction plays an important role in planetary chemistry, it must be scaled to planetary sized experiments. This is possible using a planetary chemistry model. The first thing to be considered is the total energy delivered to a planet by an impacting body. Collins et al. [213] have created a web-page, which, using their algorithm, computes effects of impact to Earth. Data from this web-page have been compiled into figure 32.



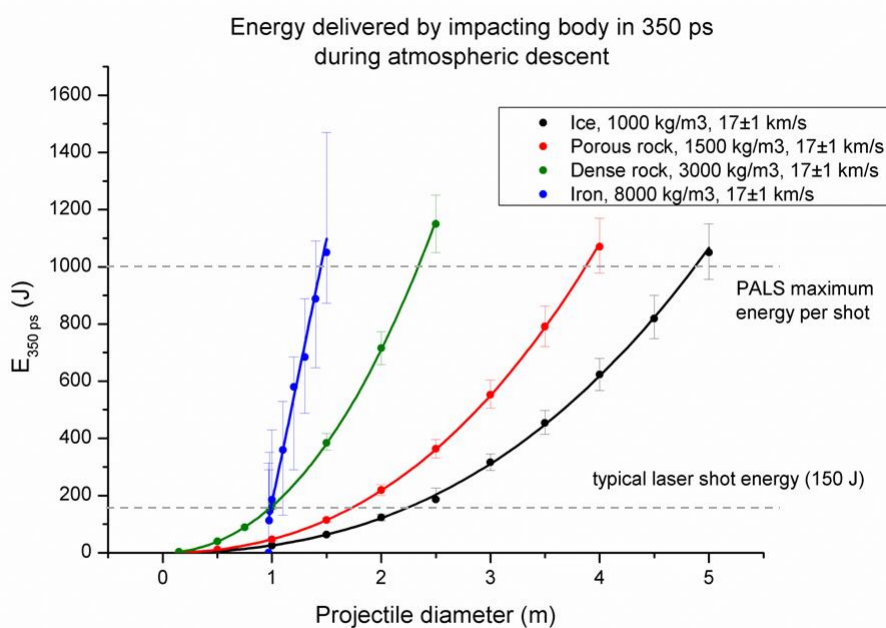Figure 32. The energy of an impacting body on Earth's surface.

The plot shows the energy of an impacting body (projectile) which is dissipated into the surrounding atmosphere during 350 ps of atmospheric descent. The energy was subtracted from the total energy of the impacting body. The other part of the energy is the energy of the impact itself. As the body passes through the atmosphere, it ignites

and creates plasma around itself. The interval 350 ps was chosen, because the Prague Asterix laser system produces 350 ps laser pulses. The typical energy used in our experiments (150 J per shot) and the maximum energy (1000 J per shot) are indicated as well. The data were obtained for four types of asteroids. Icy bodies have typical specific mass 1000 $kg.m^{-3}$. Typical specific masses of porous rocks, dense rocks and iron meteorites are also indicated. The impacting velocity 17 $km.s^{-1}$ is typical velocity of an asteroid impacting on Earth. The average velocity for comets is 72 $km.s^{-1}$ (not indicated). Deviation $\pm$ 1 $km.s^{-1}$ was introduced purportedly in order to show the relative effect of temperature on the calculation. The impacting angle was chosen to be 45°, which is the average impacting angle. In the case of ice and porous and dense rocks, the energy of the projectiles starts with 0 and smoothly progresses. In the case of iron bodies, projectiles less than 94.75 cm in diameter (at 17 $km.s^{-1}$) are slowed down in the atmosphere and gently hit the surface eventually. Their kinetic energy is insufficient to ignite the ablation process.

Therefore, in terms of energy, the 150 J PALS laser shot well-simulates the impact of 1 m iron or dense rock asteroid, 1.7 m porous rock asteroid or 2.3 m icy body. The frequency of impacts of such bodies on the Earth is nowadays below 0.7 years and must have been therefore much higher during the Late Heavy Bombardment when life first arose. In terms of temperature (about 5000 K), the laser plasma generated by PALS simulates the plasma plume surrounding an impacting asteroid accurately. We should note, that plasma temperature around descending bodies seems to be independent on theirs size [214].

In our experiments, the total yield of HCN was about 5%. Therefore out of 1 bar of starting compounds, 0.05 bar of HCN is produced after 15 shots. A planetary model was proposed and is currently constructed by Dr. Paul B. Rimmer from Cambridge. This model suggests, that even if we begin with a 10% $CH_4$, 10% CO, 78% $N_2$ and 2% $H_2O$, a 5% conversion to HCN is possible. Also, in a 1 litre vessel containing such gaseous composition and 1 g of montmorillonite clay, up to 7 ppm of adenine, 4 ppm of guanine, 0.5 ppm of cytosine and uracil and 60 pm of glycine can be produced in laser shock wave plasma reprocessing.

Furthermore, methane is photolyzed on planets such as Earth and Mars. Under early Earth's conditions, methane reacts with water and produces $CO_2$ and $H_2$. Therefore

under these conditions, $CH_4$-rich atmosphere is unstable, but sufficient to produce biomolecules if it is present at the time of the impact.

## 5.4 Shock-wave induced HCN-based synthesis of biomolecules on early Earth

Previous assumptions [71] predict a limited lifetime of methane, CO and other reduction gases ($NH_3$, $H_2S$, $H_2$, HCN, $H_2CO$, $HCONH_2$). This limit is due to escape from the atmosphere or photolysis [190]. There are, however, also sources of these compounds, such as the impacts, delivery by asteroids [72] and comets [215] or endogenous synthesis [41], [92]. Even though the exact composition remains yet unknown, we selected a representative CO, $CO_2$, $CH_4$ and $N_2$ mixture (which represents the general degree of the oxidation/reduction of early Earth's crust). This mixture was, as was said before, used for discharge experiments as well as shock-wave experiments. The gas phase from the shock-wave was analysed using our Bruker IFS 125 HR spectrometer, as is described in section 6.2.

After the gas phase analysis, non-volatile parts of the sample were analysed by gas chromatography-mass spectrometry. In each sample, various organic molecules were detected, among them most notably adenine, guanine, cytosine, uracil, urea and glycine. The data are shown in table 5. The full GC-MS spectra were published in [178] – Supplementary Information. These results are in agreement with the work of Hoesrt et al. [216]. These results are important, because they show that nucleic acid bases may be produced by treatment of HCN and not formamide as the parent molecule. There exists a large disagreement in the prebiotic community as to whether HCN [217]–[219] or formamide [165] were the starting molecule. Compared to our previously performed experiments with shock wave synthesis in pure formamide [122], the overall yield was about 10 % in our current experiment with HCN-based chemistry. On the other hand, our expriments demonstrates *de novo* synthesis starting with simple gases ($CH_4$ + CO + $N_2$ + $H_2O$), leading to HCN and ending with biomolecules. Formamide-based synthesis is one step further and therefore the energy is consumed for direct synthesis. It should be noted that formamide was not observed in this experiment.

It is important to bear in mind that if there existed a HCN-rich atmosphere, not only could HCN have been adsorbed on mineral surfaces [123], but also in contact with liquid water could have been hydrolysed to produce formamide [220]. Therefore, one-pot HCN-based synthesis of nucleobases may be an alternative route to formamide

driven synthesis, or it may be just its predecessor and formamide the reaction intermediate. However, experiments with pure formamide as the starting compound produce ca 10x more nucleobases.

Table 5. Nucleobase content in samples of $CO_2$ + CO + $N_2$ + $CH_4$ mixtures in presence of $TiO_2$ anatase and montmorillonite detected by GC-MS.

| $N_2$+CO+$CH_4$ + $CO_2$ in presence of: | Biomolecule content (ppmv) | | | | |
|---|---|---|---|---|---|
| | Adenine | Guanine | Uracil | Cytosine | Glycine |
| Anatase A200 | 7 | 4 | 0.2 | 0.5 | 60 |
| Montmorillonite + HCl | 4 | 4 | 0.2 | 0.5 | 20 |

It should be noted that concentrations of uracil and cytosine are near the detection limit of our spectrometer and the data may not be quantitatively accurate.

Using already published literature [12], [108], [122], [165], [192], [196], [208], [209], [221]–[224], a HCN-based synthesis may be explained. Figure 33 was compiled by Dr. Ferus and published in [178].
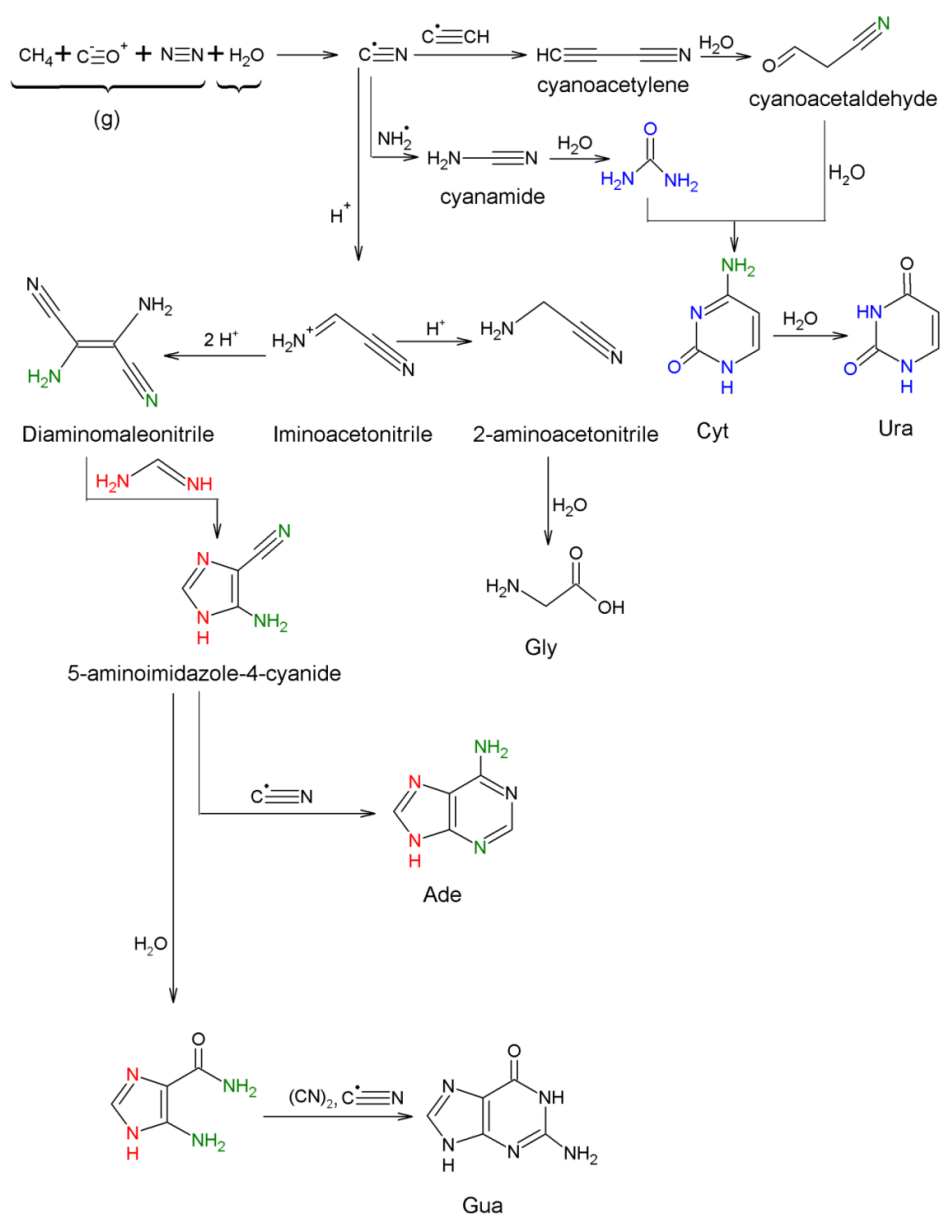
Figure 33. HCN-based synthesis of nucleic acid bases in our experiments. The figure was adapted from [178].

As is shown in figure 33 the synthesis proceeds in two directions. One eventually leads to the synthesis of pyrimidine nucleobases and the other to the synthesis of purine nucleobases and glycine, the simplest amino acid.

Both routes start with the mixture we used in our experiments, i.e. CO, $CH_4$ and $N_2$ in presence of montmorillonite as a catalyst and low amounts of water. The synthesis in presence of this catalyst has never been described before. In our shock-wave experiments and discharge experiments, HCN is created as the main product in the gas phase. Its yield in our experiments was about 5 %. Following the route A in figure 33,

HCN reacts to produce iminoacetonitrile. This then reacts with $H_2$, or 2 H· and water to give glycine. Iminoacetonitrile may also polymerize to give the purine nucleobases. A possible way is its acidic dimerization to diaminoacetonitrile, which then reacts with another molecule of iminoacetonitrile to give 5-aminoimidazole-4-cyanide (AICN) [222]. AICN may either directly react with iminoacetonitrile to give adenine or hydrate to 5-aminoimidazoleacarboxamide (AICA) and the react with cyanogen or 2 ·CN to give guanine [223], [224]. This scheme may exist as both thermally-driven [222] or radical-based [122] chemical pathway, as is shown in alternate routes in various reaction steps.

Another pathway leads directly to the creation of pyrimidine nucleobases. The pathway starts with the production of hydrogen cyanide as well. From HCN, there are two pathways, which lead to two important intermediates, cyanoacetaldehyde and urea. One route starts with reaction between HCN and acetylene (a well-known product of the shock-wave chemistry) to give cyanoacetylene [109], [225] (detected in our experiments). Upon hydrolysis, cyanoacetylene spontaneously gives cyanoacetaldehyde, which is a thermodynamically favoured reaction. The other pathway involves HCN, ·CN and $·NH_2$ in the first step. These three compounds produce cyanamide, which has not been detected in our spectra. Upon hydrolysis, however, cyanamide produces urea which has been detected in 0.1 ppm amounts in our product mixtures. Urea and cyanoacetaldehyde together react to produce cytosine. Its further hydrolysis results in the formation of uracil.

This experiment and reaction scheme shows formation of nucleic acid bases and glycine from simple gaseous precursors, such as CO and $CH_4$ which represent a reduced planetary atmosphere. The only energy impulse inserted into the system is a shock-wave, which simulates an asteroid impact and which raises the temperature to 4500 K and produces a shock wave and XUV radiation, which means that both thermal and radical processes may play a role in the reaction. In one some steps, acidic environment is present which is in this case provided by the acidic surface of montmorillonite.

This scenario does not involve formamide, contrary to many opinions in the literature [220] that formamide is a parent molecule of life. This scheme does not disprove the work published before, but offers and alternative route to already existing experimentally proven scenarios. What it contests, however, is the claim the formamide-based chemistry was the only possible source of biomolecules on early Earth. The scenario, however, must be investigated theoratically on a quantum

mechanical level, before it is compared to other theoretically proven scenarios from the literature.

## 5.5    Synthesis of biomolecules from $NH_3$ + CO atmosphere

Experiments with the $CH_4$ + CO + $N_2$ mixture were also conducted with a mixture of $NH_3$ + CO + $H_2O$ in both the electric discharge and the laser shock-wave plasma.

### 5.5.1    Discharge reprocessing of $NH_3$ + CO atmosphere

During the discharge experiments, unstable products were monitored using high-resolution time-resolved Fourier transform infrared spectroscopy [196], [201]. The spectra, shown in figure 29 on page 65 above, exhibit bands belonging to CO, $CO_2$, HCN, HNC and ·CN. The experiment was performed in a dynamic flow system and the product mixture was continuously trapped in a liquid nitrogen trap. After the experiment, the trapped products were heated to room temperature and their gas phase absorption spectra were measured. The stable reaction products were ammonia, carbon monoxide, acetylene, nitrous oxide, hydrogen cyanide and formamide. The absorption spectra are shown in figure 26 on page 61 above.

### 5.5.2    Shock-wave reprocessing of $NH_3$ + CO atmosphere

In this series of experiments, mixtures of $NH_3$ and CO were exposed to laser shock-wave plasma both with and without the presence of montmorillonite. The resulting spectra are shown in figure 28 on page 63 above and exhibit a similar pattern, i.e. HCN is the main synthesis product.

### 5.5.3    Detection of nucleic acid bases

Both sections above describe reprocessing of reduced planetary atmospheres to HCN. Non-volatile parts of these samples were taken and analysed by GC-MS, as is described in the methods section. Following the analysis, all four canonical RNA nucleic acid bases were detected, as well as urea and glycine. The results are shown in table 6, which was adapted from [114]. These results show that mixture $NH_3$ + CO + $H_2O$, which represents another prototype of a reducing atmosphere, and which is related to the Miller-Urey atmosphere, is capable of producing nucleobases. This means, as was stated in the article, that Miller in his famous experiments in 1953 probably managed to produce nucleic acid bases, but that due to the limited analytical techniques available at that time was unable to detect them and only the later developed sensitive spectrometers and chromatographs were able to confirm their presence.

Table 6. Compounds detected after laser and electric discharge plasma treatment of reducing atmosheres.

| Composition | Formamide in N₂ with clay* | | NH₃+CO** | | NH₃+CO+H₂O*** | | NH₃+CO+H₂O** | | Adenine**** | Cytosine***** |
|---|---|---|---|---|---|---|---|---|---|---|
| | Blank | LIDB | Blank | LIDB | Blank | LIDB | Blank | Discharge | Discharge | Discharge |
| $NH_3$ | 10 ppm | 3 | 5 | 41 | 40 | 39 | 33 | 1 | - | 35 |
| $CO$ | - | 36 | 50 | 41 | 40 | 36 | 33 | 1 | - | 9 |
| $H_2O$ | - | 7 | - | 10 | 20 | 22 | 33 | 9 | - | 1 |
| $HCN$ | - | 40 | - | 8 | - | 3 | - | 30 | - | 41 |
| $CO_2$ | 25 ppm | 12 | - | 200 ppm | - | 100 ppm | - | 57 | - | 1 |
| $C_2H_2$ | - | - | - | 800 ppm | - | - | - | 1 | - | 14 |
| Formamide | - | 2 | - | - | - | 5 ppm | - | 200 ppm | - | - |
| Adenine | - | 37 ppmV | - | 2 ppmV | - | 10 ppmV | - | 4 ppmV | 4800 ppmV | 24 ppmV |
| Guanine | - | 2 ppmV | - | 1 ppmV | - | 1 ppmV | - | 2 ppmV | 20 ppmV | 4 ppmV |
| Uracil | - | 1 ppmV | - | 0.2 ppmV | - | 0.5 ppmV | - | 1 ppmV | 10 ppmV | 16 ppmV |
| Cytosine | - | 6 ppmV | - | 0.1 ppmV | - | 0.5 ppmV | - | 8 ppmV | 14 ppmV | 3600 ppmV |
| Glycine | - | 35 ppmV | - | 25 ppmV | - | 80 ppmV | - | 40 ppmV | 40 ppmV | 35 ppmV |

Blank, discharge and LIDB (laser-induced dielectric breakdown) represent the energy source used for each experiment. The chemical composition is in %V, or ppmv, where indicated

*Mixture of 1 mL of liquid formamide and 760 Torr of $N_2$

**Mixture of 350 Torr $NH_3$ and 350 Torr CO

***Mixture of 350 Torr $NH_3$, 350 Torr CO, 0.2 mL $H_2O$ and 1 g of clay

****Mixture of $NH_3$, CO and $H_2O$, 1.5 kV, 500 mA, products trapped in liquid $N_2$

*****Solid sample in the discharge tube in 0.3 Torr $H_2O$ and 2 Torr $N_2$

The results of this section were also supported by ab initio molecular dynamics simulations performed by Fabio Pietrucci and Antonino Marco Saitta from the Sorbonne University, but are out of the scope of this work [114].

## 5.6 Discharge decomposition of nucleic acid bases

Apart from the 'synthetic' experiments, which begin with simple gaseous reactants and result in the formation of more complex molecules, experiments were also performed with decomposition of nucleic acid bases and other products of our synthetic chemistry. In two consequent experiments, adenine and cytosine bases in presence of water vapour were inserted in the discharge tube and treated with nitrogen discharge plasma. Yet again, the main decomposition product was HCN. Other products were also present, but their ratio was varied in comparison to the synthetic reactions. All the recorded spectra were published by Ferus et al. [114].

As before, the reprocessing of this atmosphere to nucleobases is not contradictory to the popular formamide based scenarios, but offers an alternative pathway, which leads to the same results, possibly, because formamide plays the role of a reactive intermediate.

## 5.7 Synthesis of nucleotides and polymerization

So far, the results presented in this thesis show several pathways leading from for novel scenario of reducing atmosphere formation to synthesis of basic precursors of biomolecules to their major building blocks. In the framework of the discussion, state of the art scenarios of further formation of informational biopolymers (starting with the molecules investigated in this study or alternatively, with other parent substances) should be contested as well.

Successful synthesis on nucleotides has been reported in literature only in recent years, which is why the topic has not been covered in the Introduction section of this thesis. The main obstacle in successful synthesis of both purine and pyrimidine nucleosides and nucleotides is the creation of the N-glycosidic bond, which from organic chemists' point of view is formed by dehydration. As the bond is liable to hydrolyse in aqueous environment, where life exists, its formation is not favourable under prebiotically relevant conditions through direct reaction. Also, living organisms usually exhibit a strong tendency towards one of the enantiomers of nucleotides (as well as saccharides and amino acids). A general reaction (under prebiotically not plausible conditions) provides only a racemic mixture and therefore a mechanism of selection is needed.

A question remains whether this mechanism is on the basis of enantioselectvie synthesis or evolution at higher stages of organizational structure.

### 5.7.1 Oxazoline chemistry

One of the recent ideas in the field on the formation of the N-glycosidic bond is that the bond is not the terminal synthetic step and that nucleoside is not synthesised per partes but as a whole. The idea comes from Powner et al. [156], who developed oxazoline-based chemistry, which leads to the formation of both purine and pyrimidine nucleosides.

The reaction of N-glycosidic bond synthesis from oxazoline-based compounds as shown in figure 34 was published in 1966 by Wolfrom and Winkley [226].
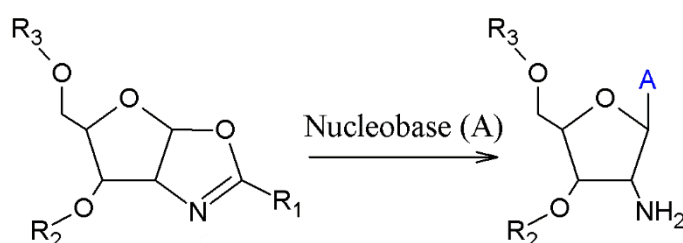


Figure 34. N-glycosidic bond synthesis first developed by Wolfrom and Winkley in 1966 using oxazoline-based compounds.

In this reaction, oxazoline reacts with a nucleobase, which breaks the double bond in the oxazoline cycle and creates an N-glycosidic bond. This reaction was taken and used by Powner et al. [156], who developed the a scheme presented in figure 35.
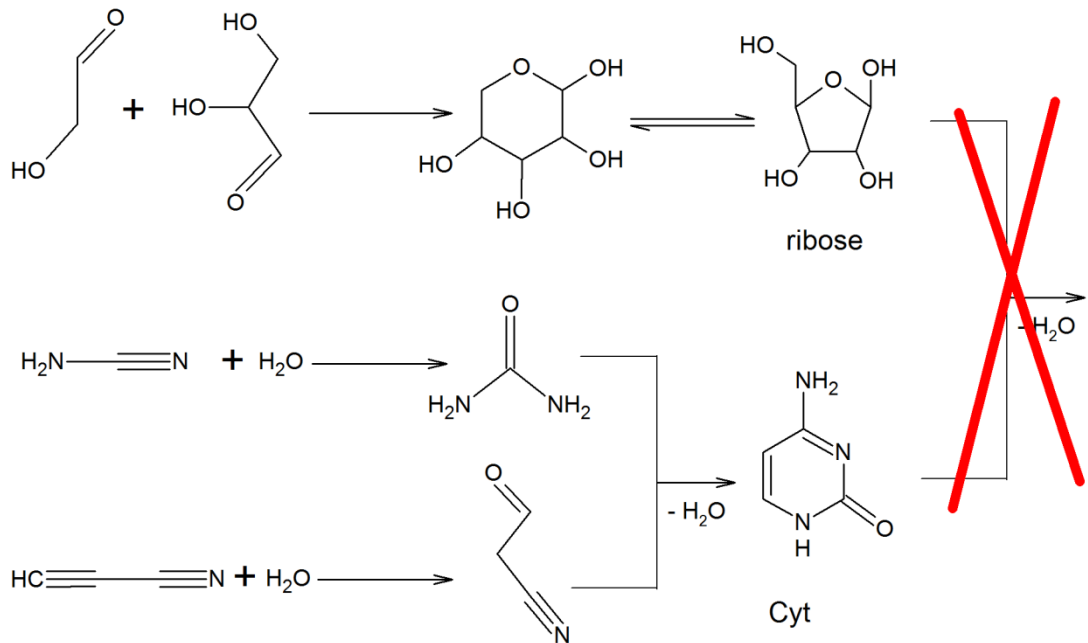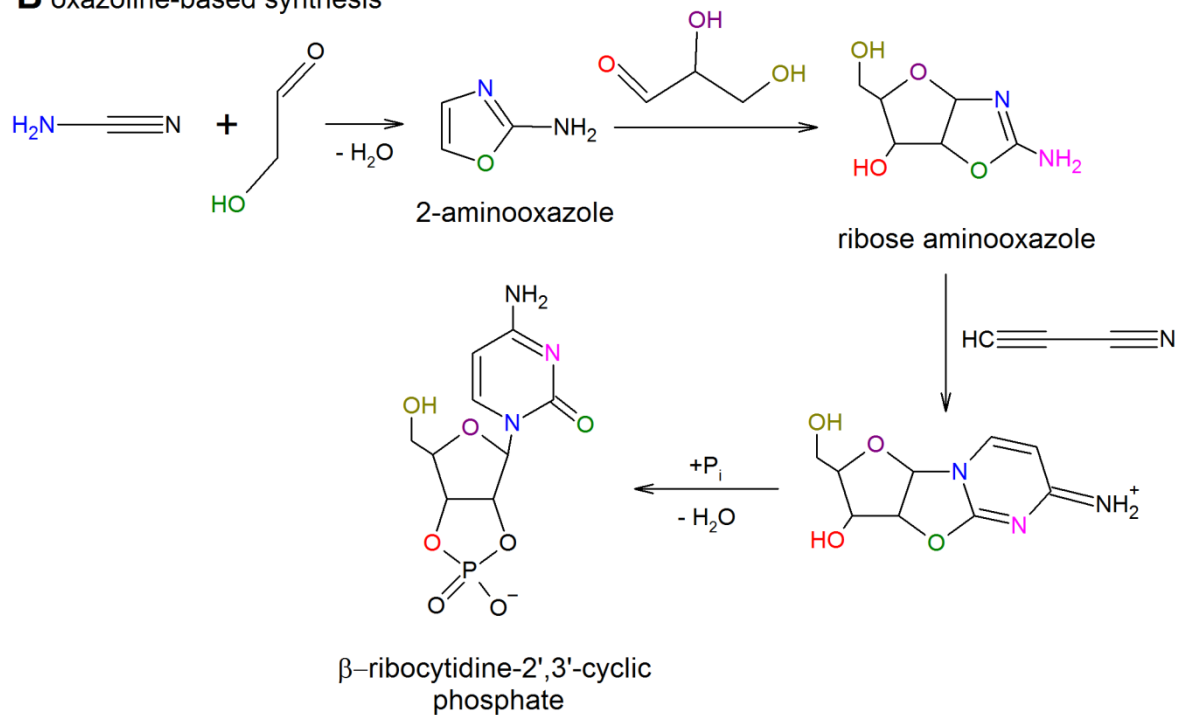
**Figure 35.** Panel A shows attempted classical synthesis of nucleotides – first the synthesis of ribose and nucleobase separately. Their condensation is implausible in prebiotically relevant conditions. Panel B shows oxazoline-based chemistry, which leads to stable β-ribocytidine-2',3'-cyclic phosphate.

In this scheme, panel A shows the already known synthesis of nucleic acid bases and shows that their direct condensation with pentoses is prebiotically implausible. Panel B shows a newly developed route, which starts with cyanamide and glycolaldehyde. These compounds react together to give aminooxazoline. This reacts with propiolonitrile and subsequently a phosphate to give a nucleotide. The important feature of this reaction is that the to-be N-glycosidic bond is formed in the first reaction as a part of the oxazoline and the rest of the saccharide and the nucleobase are synthesized afterwards.

### 5.7.2 Phosphorylated carbohydrates

If we wish to retain the direct synthesis scenario of a reaction between a nucleobase and a carbohydrate, a catalytic way must be found, which would overcome the difficulty. Kim&Benner [227] propose that phosphorylation in 1' and 2' positions of the respective carbohydrate might be the key and have developed the reaction scheme shown in figure 36 on page 82.
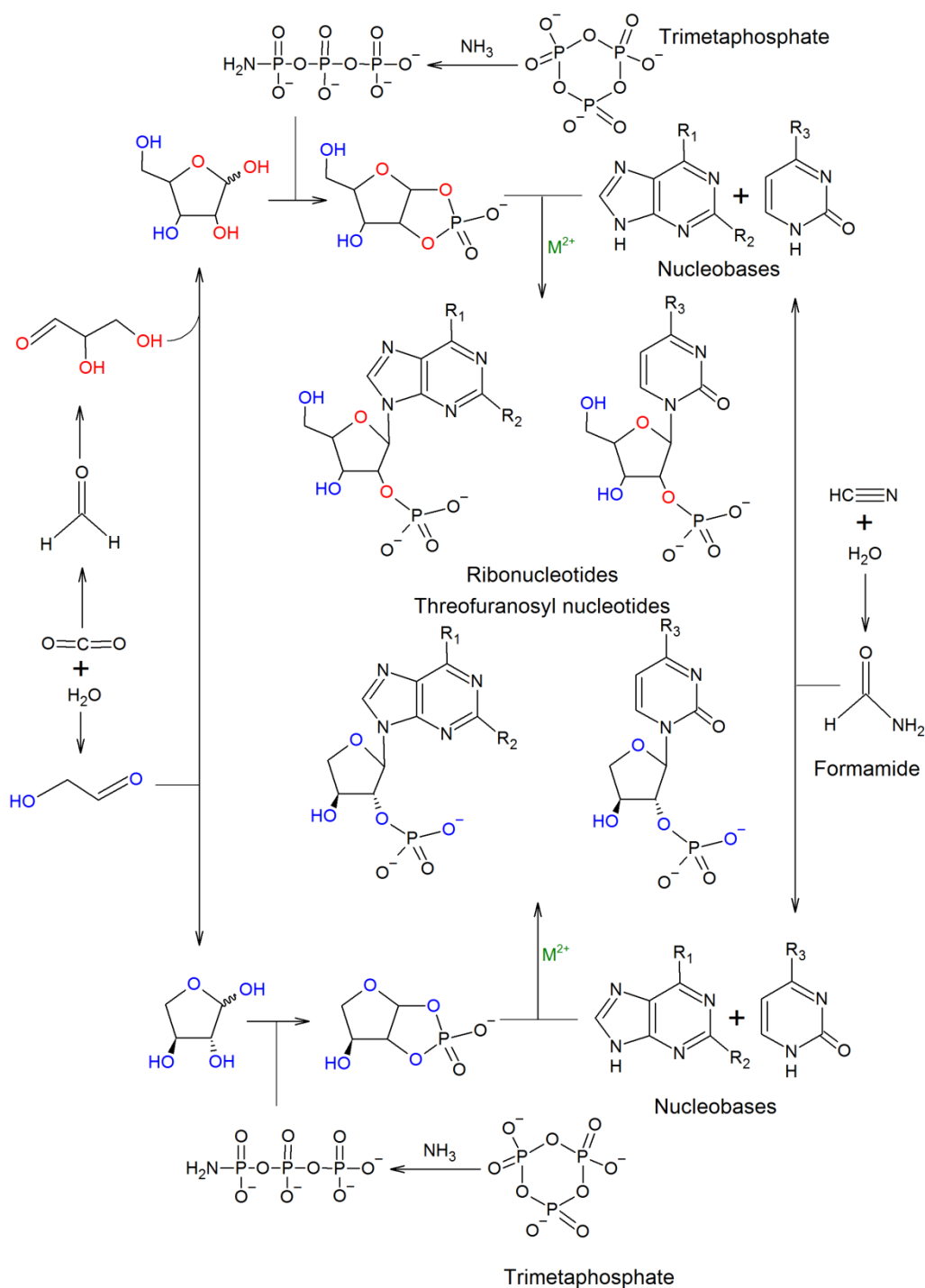
Figure 36. Phosphate-mediated synthesis of nucleotides. This step bypasses the water paradox and allows a direct condensation of nucleobase and ribose-phosphate [227].

In the scheme, HCN or formamide are used in the synthesis of nucleic acid bases, which has been demonstrated before. Formaldehyde, on the other hand, reacts with glyceraldehyde to give a pentose. Alternatively, glycolaldehyde dimerizes to give a tetrose. Both these carbohydrates (the pentose and the tetrose) then react with

1-aminotriphosphate (created by a reaction between trimetaphosphate and ammonia) to give 1',2'-phosphocarbohydrate. This phosphorylated precursor then condenses with a nucleobase in presence of metal cations $M^{2+}$. One phosphodiester bond hydrolyses and gives way to the N-glycosidic bond while also providing energy for the reaction. In this way, 2'-phospho-nucleotides are created. This pathway suggests that in favourable conditions, such as the presence of a mineral catalyst, nucleotides could be created on the early Earth. The phosphorylation, however, is in the position 2', which is not favourable towards the standard 3'-5'- polymerization into nucleic acids. Therefore, either this pathway did not lead into current RNA and DNA, or an alternative way for shifting the phosphate took place on our planet.

### 5.7.3   High-energy radical synthesis of nucleosides

A third option of obtaining nucleosides or nucleotides is high-energy radical chemistry. It has been shown in previous sections that high-energy reprocessing of HCN rich atmospheres proceeds through radical chemistry and leads under certain conditions to nucleobases. A possible intermediate in these syntheses is formamide. If high-energy sources are employed to chemical transformation of HCN or formamide, the yields of products are similar (even though ratios may vary), which suggests a similar reaction network. It has been shown, in the case of formamide, that several radical species play an important role in the synthesis. These radicals are shown in figure 37.
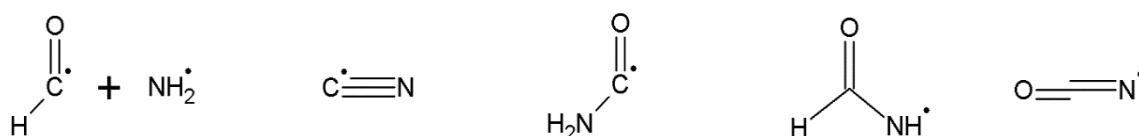
Figure 37. Important radicals in the synthesis of biologically important molecules from formamide.

These radicals may either be quenched by hydrogen radicals or others, or they may undergo further reactions with each other and with molecular species present in the respective system, such as CO, $NH_3$, HCN, HNCO or other. The mechanism of this synthesis has not been thoroughly described yet. Nevertheless, Saladino et al. [228] performed experiments, where they irradiated formamide and water in presence of meteoritic catalysts (most successfully meteorite NWA 1465, which is a petrologic type 3 carbonaceous chondrite with anomalous signs [229]. Upon exposure to proton irradiation, radicals were created in the formamide sample and complex chemistry took

place. The authors analysed their sample after the irradiation on high-performance liquid chromatography and detected a range of products, such as amino acids, carboxylic acids, heterocycles, saccharides, nucleobases and also nucleosides. The products detected by the authors are listed in table 7.

Table 7. List of biomolecules produced by proton irradiation of formamide and water in presence of meteoritic catalysts by Saladino et al.

| Name | Formula | Name | Formula |
|---|---|---|---|
| Glycine | $C_2H_5NO_2$ | Arachidic acid | $C_{20}H_{40}O_2$ |
| Formyl glycine | $C_3H_5NO_3$ | Oxalic acid | $C_2H_2O_4$ |
| Alanine | $C_3H_7NO_2$ | Glycolic acid | $C_2H_4O_3$ |
| Formyl alanine | $C_4H_7NO_3$ | Pyruvic acid | $C_3H_4O_3$ |
| 2(Me)alanine | $C_5H_{11}NO_2$ | Lactic acid | $C_3H_6O_3$ |
| Proline | $C_5H_9NO_2$ | Malonic acid | $C_4H_4O_4$ |
| Pyroglutamic acid | $C_5H_7NO_3$ | Succinic acid | $C_4H_6O_4$ |
| 2-Pyrrolidone | $C_4H_7NO$ | Oxaloacetic acid | $C_4H_4O_5$ |
| β-amino isobutyric acid | $C_4H_9NO_2$ | Ketoglutaric acid | $C_5H_6O_5$ |
| Urea | $CH_4N_2O$ | Uridine | $C_9H_{12}N_2O_6$ |
| Guanosine | $C_{10}H_{13}N_5O_5$ | Cytidine | $C_9H_{13}N_3O_5$ |
| Glycerol | $C_3H_8O_3$ | Adenosine | $C_{10}H_{13}N_5O_4$ |
| Nonanoic acid | $C_9H_{18}O_2$ | Thymidine | $C_{10}H_{14}N_2O_5$ |
| Citric acid | $C_6H_8O_7$ | Ribose | $C_5H_{10}O_5$ |
| Ketoisocaproic acid | $C_6H_{10}O_3$ | 2'-deoxyribose | $C_5H_{10}O_4$ |
| Hexanoic acid | $C_6H_{12}O_2$ | Glucose | $C_6H_{12}O_6$ |
| Pimelic acid | $C_7H_{12}O_4$ | 2'-deoxyglucose | $C_6H_{12}O_5$ |
| Octanoic acid | $C_8H_{16}O_2$ | Galactose | $C_6H_{12}O_6$ |
| 4-hydroxypyrimidine | $C_4H_4N_2O$ | Mannose | $C_6H_{12}O_6$ |
| Lauric acid | $C_{12}H_{24}O_2$ | Inositol | $C_6H_{12}O_6$ |
| Myrystic acid | $C_{14}H_{28}O_2$ | Arabitol | $C_5H_{12}O_5$ |
| Palmitic acid | $C_{16}H_{32}O_2$ | Uracil | $C_4H_4N_2O_2$ |
| | | The table continues on the next page. | |

| | | | |
|---|---|---|---|
| Heptadecanoic acid | $C_{17}H_{34}O_2$ | Thymine | $C_5H_6N_2O_2$ |
| Stearic acid | $C_{18}H_{36}O_2$ | Orotic acid | $C_5H_4N_2O_4$ |
| Cytosine | $C_4H_5N_3O$ | Purine | $C_5H_4N_4$ |
| Adenine | $C_5H_5N_5$ | Hypoxanthine | $C_5H_4N_4O$ |
| 3-hydroxy pyridine | $C_5H_5NO$ | Isocytosine | $C_4H_5N_3O$ |
| 4(3H)pyrimidone | $C_4H_4N_2O$ | 4-aminoimidazole | $C_3H_5N_3$ |
| Guanine | $C_5H_5N_5O$ | 2,6-diaminopurine | $C_5H_6N_6$ |
| 2-amino-2-hydroxymalonitrile | $C_3H_3N_3O$ | diaminomaleonitrile | $C_4H_4N_4$ |
| 4,6-dihydroxypyrimidine | $C_4H_4N_2O_2$ | | |

The authors tested a variety of meteorites, including the Campo del Cielo or the Orgueil meteorite. The resulting mixture was analysed with high-performance liquid chromatography, which clearly showed the presence of nucleosides in the reaction mixture. It is important to note that since the reaction started from pure formamide + catalyst, the formamide, which did not transform in the reaction, became a solvent for the created biomolecules. This reaction therefore explains the creation of N-glycosidic bonds, but the solvent is not water, but formamide. It is questionable, whether pool of formamide could be found on early Earth, which would serve as the reactant mixture for this process. The authors then assume that only after the synthesis did the biomolecules transfer to water as the main solvent. A recent article by Cassone et al. [228] also shows that in aqueous environment, 2'-3'-cyclic nucleotides are more stable than their 3'-5'-counterparts. In formamide above 400 K, however, the stability is reversed. This suggests that either nucleotides formed and polymerized in their 2'-3'-cyclic forms in aqueous solution at mild temperature after their activation by amines and alcohols or that the prebiotic chemistry began at temperatures above 400 K in formamide as a solvent, where the 3'-5'-cyclic nucleotides accumulated, and 3'-5'-cyclic nucleotides oligomerized spontaneously upon cooling below 400 K.

Last but not least, the question of stereoselectivity arises. So far, the only proposed scenarios employ mineral catalysts with double charged metal cations ($M^{2+}$). Using these catalysts, the emerging β-stereomer is stabilized by two H-bonds, while the

α-stereomer is stabilized only by one H-bond. If these catalysts, which are very common, played a role in the emergence of life, then β-stereomers would predominantly form in the product mixture. In this effect may lie the origin of the use of β-stereomers in living structures. The topic, however, remains largely unresolved pending further research.


# 6 Conclusion

The experimental part of this work shows that a prototype of neutral $CO_2$-rich planetary atmosphere of any terrestrial planet (such as that of early Earth) can be under sufficient UV flux in presence of acidic minerals such as metal oxides or clays partially converted to a reduced atmosphere containing methane and carbon monoxide – the mixture representing a productive chemical environment plausible for prebiotic synthesis. We should note that the same process possibly still produces methane on current Mars.

It is also shown that subsequent reprocessing of thus created partly reduced atmosphere by impact plasma generated by asteroid or comet (simulated by high-power laser dielectric breakdown in this work) initiates synthesis of basic components of biomolecules: the nucleic acid bases, which are the building blocks of RNA (adenine, uracil, guanine and cytosine), and glycine.

Therefore, in this work and in the related scientific papers co-authored by the author of this thesis, a novel scenario starting with the transformation of planetary atmosphere involving novel photocatalytic processes, and including impact plasma reprocessing or synthesis of the major building blocks of biopolymers, is presented. Several plausible theories concerning further formation of biopolymers starting with the molecules produced in our experiments are discussed.

The work therefore shows a prebiotically plausible pathway for the creation of biomolecules from simple gaseous atmospheres containing at first only carbon dioxide and nitrogen in acidic environment and in presence of a mineral catalyst. Furthermore, a thorough discussion was provided on the relevance and applicability of these experimental results.

# 7 Selected scientific papers attached to this thesis as Supplementary material

## Meteorite-catalyzed synthesis of nucleosides and other prebiotic compounds

**Martin Ferus[1], Antonín Knížek, and Svatopluk Civiš**

*Department of Spectroscopy, J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, 18223 Prague 8, Czech Republic*

## Formation of nucleobases in a Miller–Urey reducing atmosphere

**Martin Ferus[a], Fabio Pietrucci[b], Antonino Marco Saitta[b], Antonín Knížek[a,c], Petr Kubelík[a], Ondřej Ivanek[a], Violetta Shestivska[a], and Svatopluk Civiš[a,1]**

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The Miller–Urey experiments pioneered modern research on the molecular origins of life, but their actual relevance in this field was later questioned because the gas mixture used in their research is considered too reducing with respect to the most accepted hypotheses for the conditions on primordial Earth. In particular, the production of only amino acids has been taken as evidence of the limited relevance of the results. Here, we report an experimental work, combined with state-of-the-art computational methods, in which both electric discharge and laser-driven plasma impact simulations were carried out in a reducing atmosphere containing $NH_3$ + CO. We show that RNA nucleobases are synthesized in these experiments, strongly supporting the possibility of the emergence of biologically relevant molecules in a reducing atmosphere. The reconstructed synthetic pathways indicate that small radicals and formamide play a crucial role, in agreement with a number of recent experimental and theoretical results.

## The origin of methane and biomolecules from a $CO_2$ cycle on terrestrial planets

**Svatopluk Civiš[1]\*, Antonín Knížek[1,2], Ondřej Ivanek[1], Petr Kubelík[1,3], Markéta Zukalová[1], Ladislav Kavan[1] and Martin Ferus[1]**

Understanding the chemical evolution of newly formed terrestrial planets involves uncertainties in atmospheric chemical composition and assessing the plausibility of biomolecule synthesis. In this study, an original scenario for the origin of methane on Mars and terrestrial planets is suggested. Carbon dioxide in Martian and other planetary atmospheres can be abiotically converted into a mixture of methane and carbon monoxide by 'methanogenesis' on porous mineral photoactive surfaces under soft ultraviolet irradiation. On young planets exposed to heavy bombardment by interplanetary matter, this process can be followed by biomolecule synthesis through the reprocessing of reactive reducing atmospheres by impact-induced shock waves. The proposed mechanism of methanogenesis may help to answer the question concerning the formation of methane and carbon monoxide by photochemical processes, the formation of biomolecules on early Earth and other terrestrial planets, and the source and seasonal variation of methane concentrations on Mars.

# 8   List of abbreviations

FT-IR                  Fourier transform infrared spectroscopy

GC-MS                  Gas chromatography – mass spectrometry

LHB                    Late heavy bombardment

DAMN                   diaminomaleonitrile

AICN                   4-aminoimidazole-5-carbonitrile

PALS                   Prague Asterix laser system

EQE                    External quantum efficiency

AICA                   5-aminoimidazoleacarboxamide

TLC                    Thin layer chromatography

# 9 Literature

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