

## Prebiotic Organic Microstructures

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### Abstract

Micro- and sub-micrometer spheres, tubules and fiber-filament soft structures have been synthesized in our experiments conducted with 3 MeV proton irradiations of a mixture of simple inorganic constituents, CO, N<sub>2</sub> and H<sub>2</sub>O. We analysed the irradiation products, with scanning electron microscopy (SEM) and atomic force microscopy (AFM). These laboratory organic structures produced wide variety of proteinous and non-proteinous amino acids after HCl hydrolysis. The enantiomer analysis for D-, L- alanine confirmed that the amino acids were abiotically synthesized during the laboratory experiment. Considering hydrothermal activity, the presence of CO<sub>2</sub> and H<sub>2</sub>, of a ferromagnesian silicate mineral environment, of an Earth magnetic field which was much less intense during Archean times than nowadays and consequently of a proton excitation source which was much more abundant, we propose that our laboratory organic microstructures might be synthesized during Archean times. We show similarities in morphology and in formation with some terrestrial Archean microstructures and we suggest that some of the observed Archean carbon spherical and filamentous microstructures might be composed of abiogenic organic molecules. We further propose a search for such prebiotic organic signatures on Mars. This article has been posted on Nature precedings on 21

July 2010 [1]. Extinct radionuclides as source of excitation have been replaced by cosmic radiations which were much more intense 3.5 Ga ago because of a much less intense Earth magnetic field. The new version of the article has been presented at the ORIGINS conference in Montpellier in July 2011 [2] and is waiting to be published.

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**Keywords:** Origin of Life; prebiotic signatures; geobiology; analytical chemistry.

## 1. Introduction

40 Experiments simulating the primitive Earth atmosphere were conducted on gaseous mixtures of CO, N<sub>2</sub>/NH<sub>3</sub> above liquid water irradiated with protons, helium ions, electrons, heavy ions, gamma and X and UV -rays, in a glass tube. Most of them led to proteinous and nonproteinous amino acids [3]. The first Kobayashi experiment irradiating with protons a gaseous mixture of CO/(CO+CO<sub>2</sub>) and N<sub>2</sub> over liquid H<sub>2</sub>O was performed in 1989 [4,5]. The resulting liquid aqueous solution was filtered  
45 through a membrane filter (pore size: 0.2 μm). The analysis of the remainder of the solution led to amino acids. Mixtures of CO/(CO+CO<sub>2</sub>), N<sub>2</sub>, H<sub>2</sub>O irradiated with protons, helium ions and electrons, also produce amino acids after HCl hydrolysis of the resulting aqueous solution [6]. Mixtures of CO and N<sub>2</sub> over liquid H<sub>2</sub>O, irradiated with X-rays, led to amino acids after freeze drying and HCl hydrolysis of the product aqueous solution [7]. Mixtures of CO and NH<sub>3</sub> over liquid water irradiated  
50 with protons also led to amino acids after HCl hydrolysis of the irradiation products [8]. Asymmetric syntheses of amino acid precursors have also been performed after proton irradiation of a CO, NH<sub>3</sub>, H<sub>2</sub>O mixture, followed by irradiation with right and left ultraviolet circularly polarized light [9]. None of the above cited experiments gave informations on the morphology of the synthesized compounds.

Envisioning a laboratory synthesis of amino acids as a consequence of the process of  
55 serpentinization, with as reactant a solid phase such as mafic or ultramafic rocks or their mineral constituents, olivine and pyroxenes [10-12], we first irradiated with protons, a gaseous mixture of CO, N<sub>2</sub> and water and we analysed the 3D-morphology of the products. We chose CO instead of CO<sub>2</sub> since an earlier experiment irradiating with protons a mixture of CO<sub>2</sub>, N<sub>2</sub> and H<sub>2</sub>O did not produce amino acids [4]. And also, we considered that CO<sub>2</sub> may be transformed into CO in a natural  
60 hydrothermal process of serpentinization [13]. Discussions of abiotic synthesis of organic molecules in hydrothermal systems have focused mainly on methane and hydrocarbons [14-15]. The abiotic synthesis of amino-acids in hydrothermal systems has been suggested but is not yet demonstrated.

Here we analyse for the first time the 3D-morphology of the products synthesized during proton irradiation of a gaseous mixture of CO, N<sub>2</sub> and H<sub>2</sub>O. We observe filamentous and spherical

65 micro and sub-micrometer structures which produce amino acids after HCl hydrolysis. As criteria to  
differentiate abiotic synthesis from contamination of biogenic origin, we used the concept of chirality  
and we proceeded to enantiomer analysis after derivatization of the hydrolyzed product. We observed a  
racemic mixture of the most abundant chiral amino acid synthesized in this study, D-, L- alanine, thus  
eliminating a biogenic contamination. Considering geology with the presence of mafic and ultramafic  
70 ferromagnesian rocks, hydrothermal chemistry with the exothermic natural process of serpentinization  
and the release of H<sub>2</sub>, the high abundance of atmospheric CO<sub>2</sub>, energy arising from cosmic protons or  
cosmic gamma rays irradiating water, we propose that these laboratory organic microstructures may  
have been synthesized during Archean times. The results and discussions written in the present article  
have been posted on Nature preceedings on 21 July 2010 [1] and presented on a poster at the ORIGINS  
75 conference in Montpellier in July 2011 [2].

## 2. Experimental section

High-energy proton irradiation (3 MeV) was performed for two hours, at the Tokyo Institute  
of Technology using a Van de Graaff accelerator, in collaboration with Kensei Kobayashi of Yokohama  
80 National University [3-9]. A Pyrex glass tube was filled with inorganic gas components consisting of  
350 Torr carbon monoxide (CO) and 350 Torr nitrogen (N<sub>2</sub>) over 5 mL of distilled liquid water (H<sub>2</sub>O)  
which provided 20 Torr of water vapor at room temperature.

Ultra-pure grade carbon monoxide and dinitrogen gases were purchased from Nihon Sanso  
Co.. All glassware was heated in a high temperature oven (DR-22, Yamato Co., Tokyo, Japan) at  
85 500°C to eliminate any possible contaminants prior to use. Deionized water was further purified with a  
Millipore Milli-Q LaboSystem™ and a Millipore Simpli Lab-UV (Japan Millipore Ltd., Tokyo, Japan)  
to remove inorganic ions and organic contaminants.

The irradiation product analysis was conducted in the Institute of Biogeosciences, Japan  
Agency for Marine-Earth Science and Technology, JAMSTEC, in Yokosuka. Morphological analysis  
90 was performed with scanning electron microscopy (SEM, JSM-6700F, JEOL) and atomic force  
microscopy (AFM, Seico Instruments Inc., SII SPA 400 unit, Japan) conducted in non-contact mode.  
The gel filtration chromatograph (GFC) was composed of a high performance liquid chromatography  
(HPLC) pump (TOSOH DP-8020) and a UV detector (TOSOH UV-8020). The separation columns  
used were TSKgel G2000 SWxL (7.8 mm i.d. x 300 mm) for gel filtration, and Inertsil ODS-3 (4.6  
95 mm i.d. x 250 mm) for reversed-phase chromatography [9]. The mobile phase was a mixture of 25 mM  
acetonitrile (25%) and 0.1% trifluoroacetic acid (75%). Molecular weights were calibrated using  
several molecular weights of polyethylene glycol (PEG) and human serum albumin [8]. The aqueous  
solution containing the irradiation products was not filtered and an aliquot was hydrolyzed with 6 M

HCl at 110 °C for 24 hours. Amino acids in the hydrolyzed fraction were analysed with an ion-exchanged HPLC system with analytical methods improved since the analysis of lunar samples [16,5,17,8]. The HPLC system used was composed of two high performance liquid chromatograph pumps (Shimadzu LC-6A), a cation exchange column (Shimpak ISC-07/S1504, 4 mm i.d.×150 mm), a post-column derivatization system with o-phthalaldehyde and N-acetyl-L-cystein, and a Shimadzu RF-535 fluorometric detector [18].

We also proceeded to enantiomer analysis after derivatization procedures to yield N-pivaloyl-(S)-2-butyl esters (NP/S2Bu) of the amino acid diastereoisomers [19]. The NP/S2Bu esters were identified by a gas chromatograph/mass spectrometry (GC/MS; Agilent Technologies 6890N/5973MSD). The capillary column used for GC was an HP-5ms (30 m x 0.32 mm i.d., 0.52 µm film thickness; Agilent Technologies). The GC oven temperature was programmed as follows: initial temperature 40 °C for 4 min, ramped up at 10 °C min<sup>-1</sup> to 90 °C, and ramped up at 5 °C min<sup>-1</sup> to 220 °C, where it was maintained for 10 min. The MS was scanned over *m/z* of 50–550 with the electron-impact mode set at 70 eV.

In order to obtain the yield of amino acids, we used the G-value (the number of formed molecules per 100 eV) of glycine in the hydrolyzed products [6,20], because (i) glycine is the most abundant amino acid and (ii) it was demonstrated that glycine was formed in proportion to total energy deposit including particle and photon irradiation [3].

### 3. Results and Discussion

#### 3.1 Results

After proton irradiation, an aliquot of the unfiltered solution containing the irradiation products was gently dried at ambient temperature and pressure in clean bench to obtain involatile organic matter. SEM (Figs. 1a, 1b) and AFM (Figs. 2a, 2b) were performed to observe three-dimensional morphological characteristics of the yellow-colored microstructures synthesized during the irradiation. SEM images show micro- and sub-micrometer spheres, tubules and fiber-filament soft tissues. AFM was used to observe the surface of these micro- and sub-microstructures. Figures 2a and 2b show AFM images of the same kind of structure. On Fig. 2a is observed a depression, 140 nm in depth and 1 µm in width. On Fig. 2b is observed a depression, 125 nm in depth and 0.5 µm in width. Fig 2a shows the edges of the depression covered with protuberances which are irregular in shape. The striations observed on the white prominent parts of the depression edges (Fig. 2b) result most probably from an image of the probe tip on the depression slope and not from an image of the structure surface. However, the depression is wide enough to say that the AFM images show the surface of the structures and are not an artifact image of the probe tip. The molecular weights of these organic microstructures,

determined with GFC, are distributed between several hundred and a maximum of 3000 Da. A wide variety of amino acids were detected after HCl acid-hydrolysis of this dried aliquot (Figs. 3a, 3b). To eliminate possible contamination results, we conducted chiral analysis after derivatization of the hydrolyzed fraction [19]. Figure 4 shows GC separation of N-pivaloyl-(S)-2-butyl esters of D-, L-alanine and glycine. The most abundant chiral amino acid, D-, L- alanine, shows a racemic mixture produced by pristine abiotic chemical synthesis. Therefore, we exclude potential contamination on our organic analysis and we may conclude that the dried irradiation products are composed of abiogenic organic nano and microstructures.

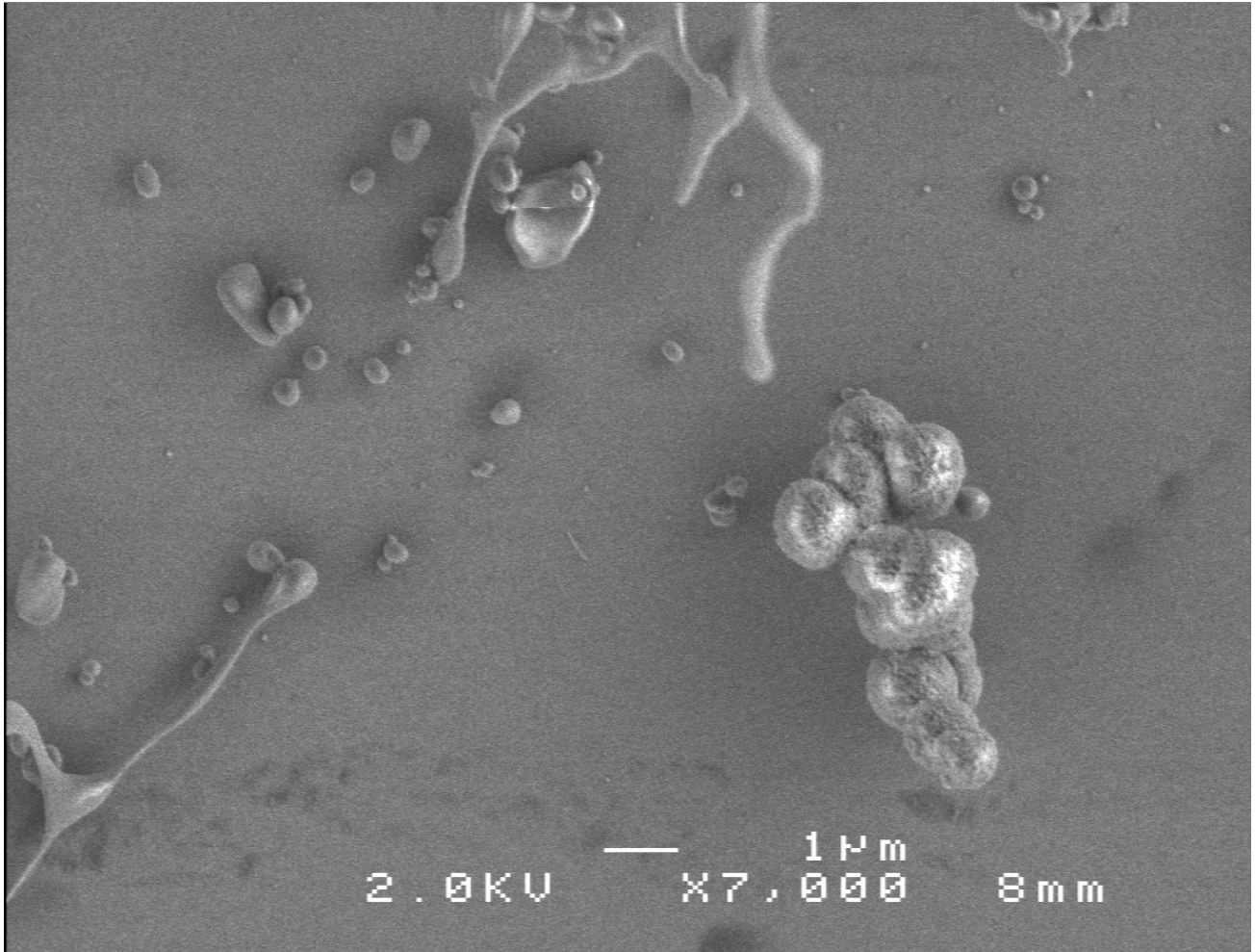
It is to be noticed that we conducted earlier same analytical procedures for analyses of peridotite rocks which were dredged on the ocean floor of the mid-atlantic ridge (MAR) [21]. Non racemic mixtures of amino acids were obtained leading to the conclusion of sedimentary biological origin for the observed amino acids. These two opposite conclusions for similar analytical procedures applied to abiotic synthesis and rock analysis, also prove that our analytical procedures are exempt of biological contamination. The amino acids synthesized in this study include glycine, alanine, aspartic acid, serine and the non-proteinous amino acids  $\beta$ -alanine (BALA),  $\alpha$ -aminobutyric (ABA) acid and  $\gamma$ -aminobutyric acid (GABA). Glycine was most abundant followed by D-, L- alanine, D,L-  $\alpha$ -aminobutyric acid, D-, L- aspartic acid,  $\beta$ -alanine and D-, L- serine, in logarithmic decrease.

The energetic yield of glycine normalized by G-value (number of synthesized molecules per 100 eV absorbed) in the present proton irradiation experiment was 0.02 (cf. supporting data [3]).

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**Figure 1a.** Three-Dimensional Scanning Electron Microscopy, 3D-SEM, images of the dried product, abiotically synthesized from a gas mixture of CO-N<sub>2</sub>-H<sub>2</sub>O excited with 3 MeV proton irradiation; bar is 1  $\mu$ m, acceleration voltage 2.0 kV, magnification x7,000, working distance 8mm.

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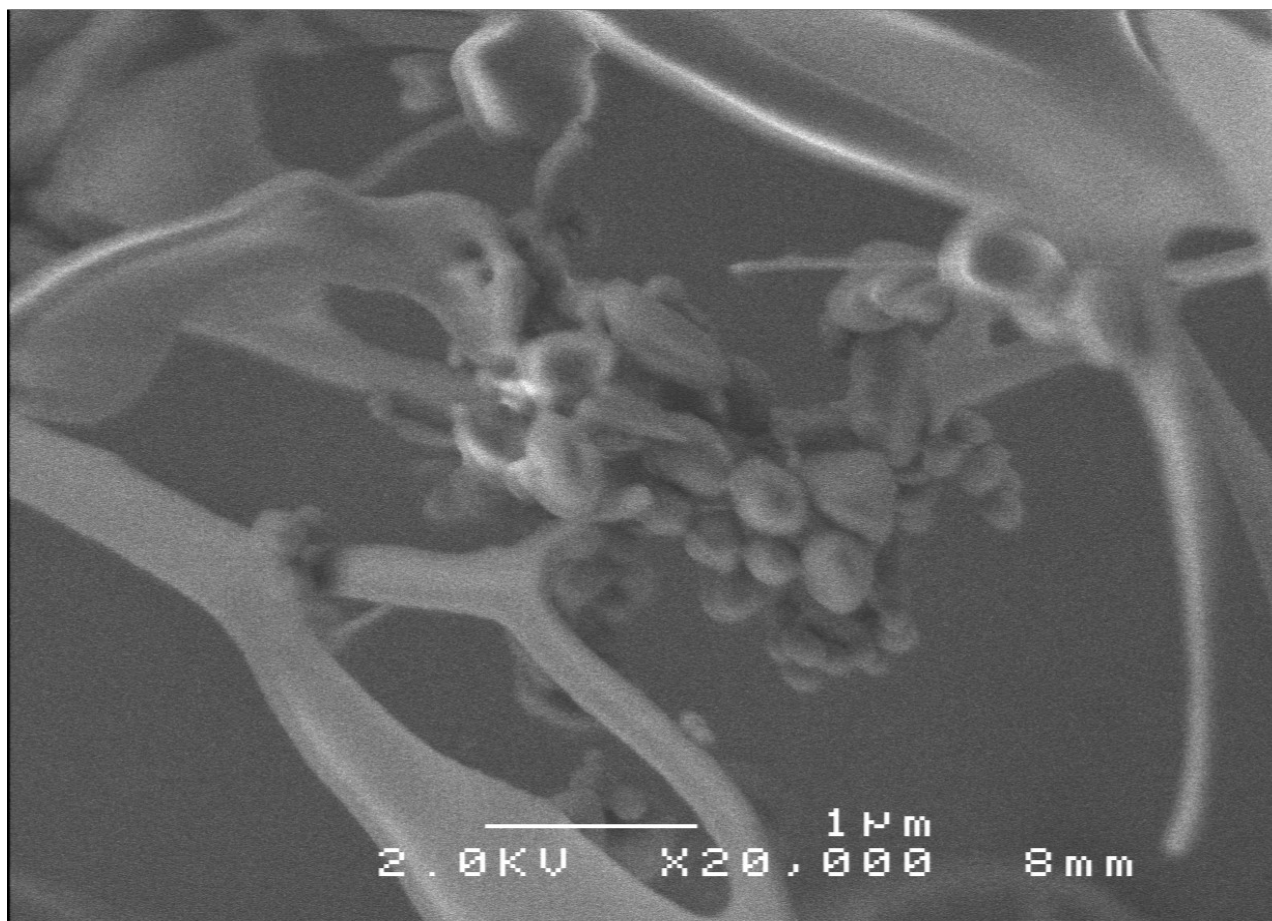
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**Figure 1b.** 3D-SEM, image of the dried proton irradiation product; bar is 1  $\mu\text{m}$ , acceleration voltage 2.0 kV, magnification x20,000, working distance 8mm.

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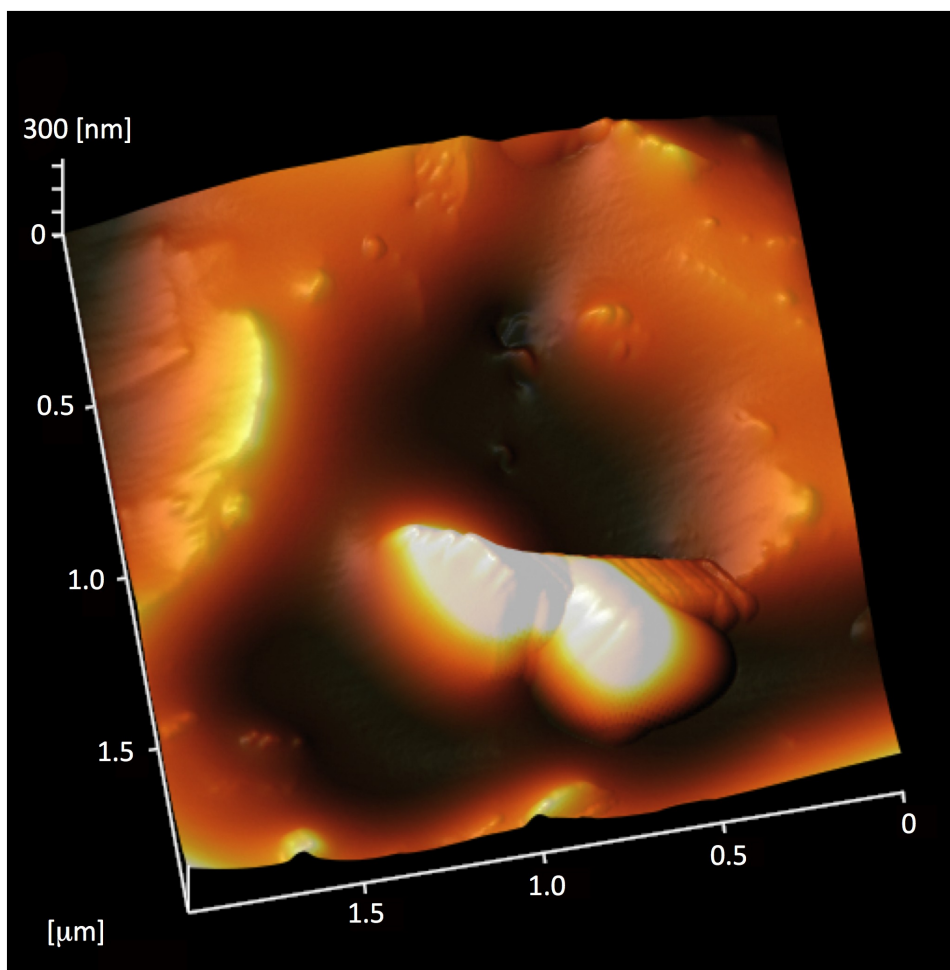
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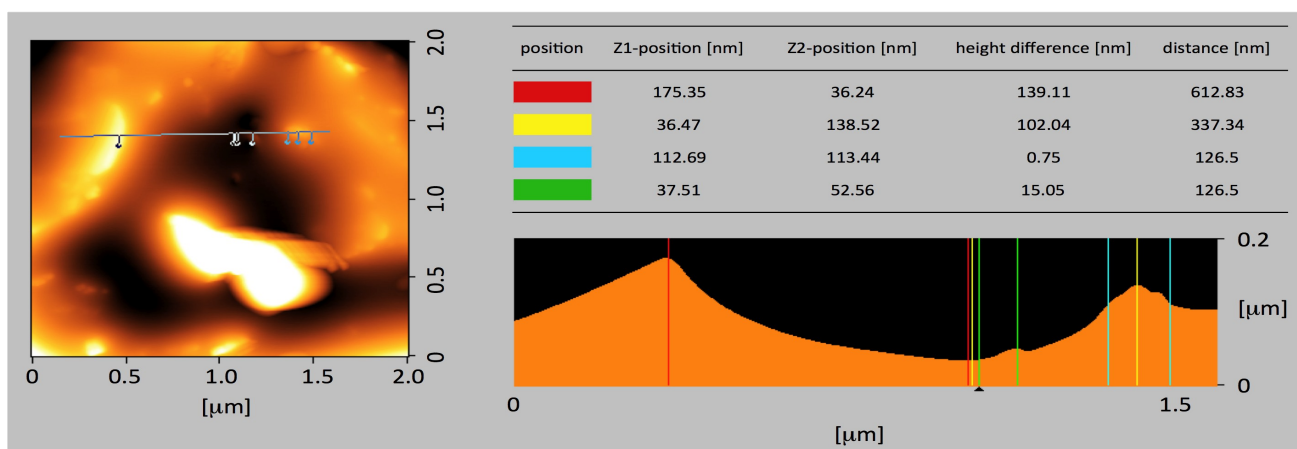
**Figure 2a.** Three-Dimensional Atomic Force Microscopy, 3D-AFM, images of the dried product, abiotically synthesized from a gas mixture of CO-N<sub>2</sub>-H<sub>2</sub>O, excited with 3 MeV proton irradiation.

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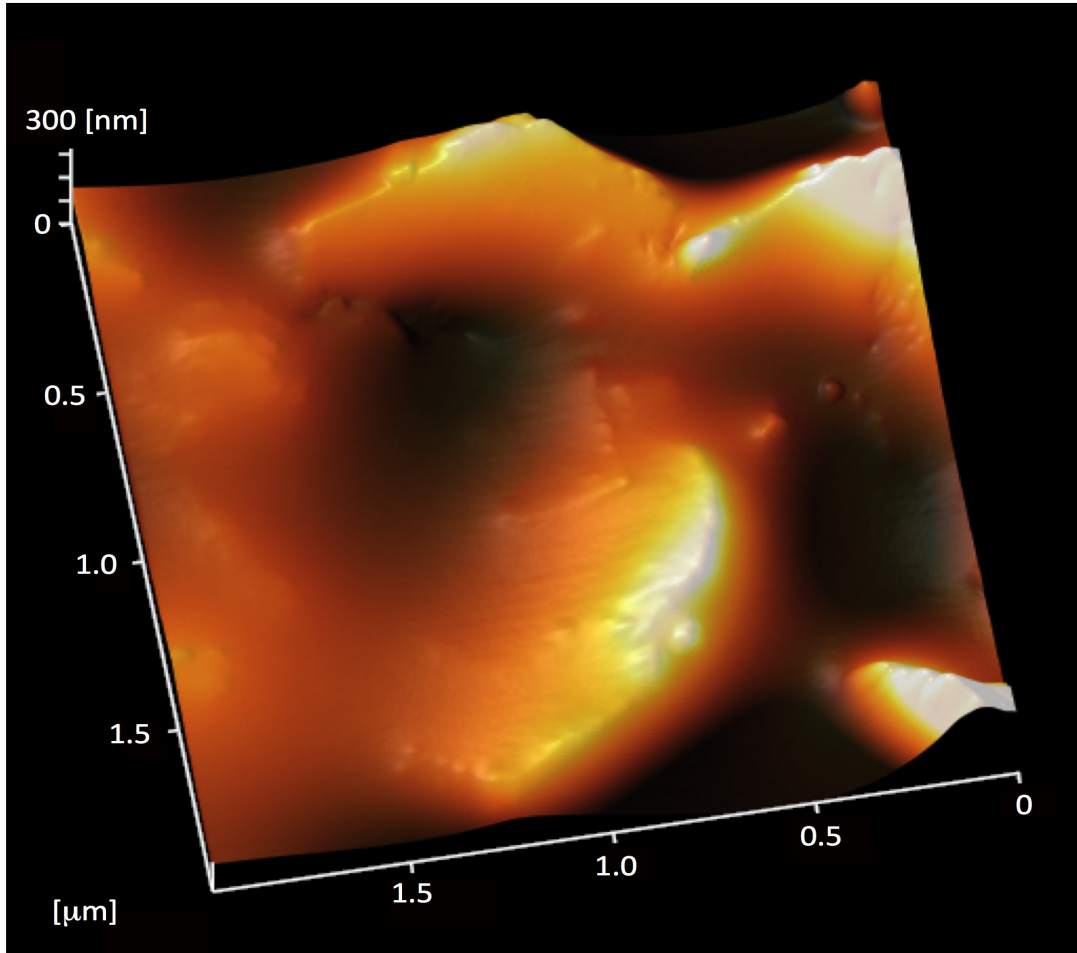
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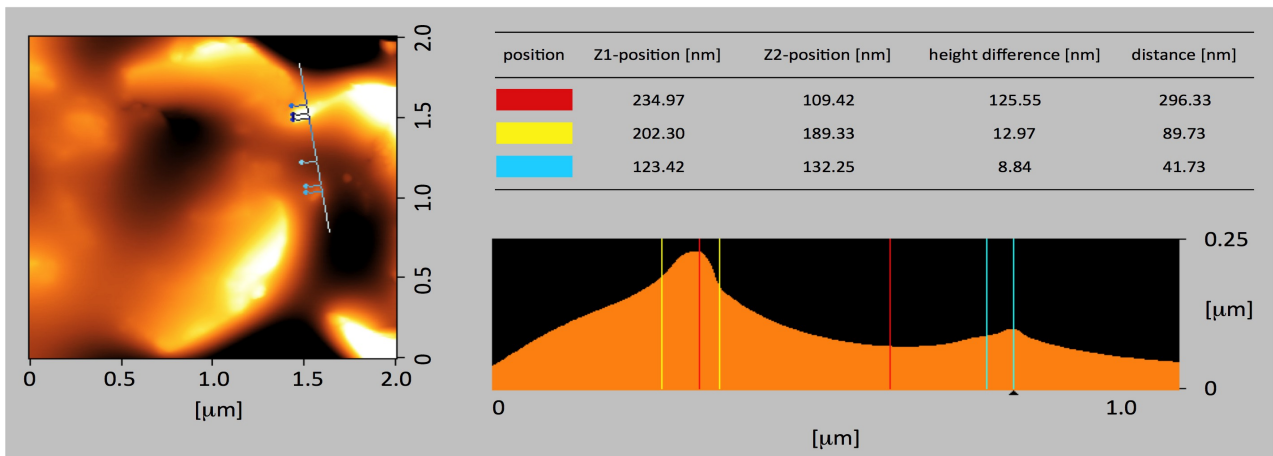
**Figure 2b.** 3D-AFM images of the same structure.



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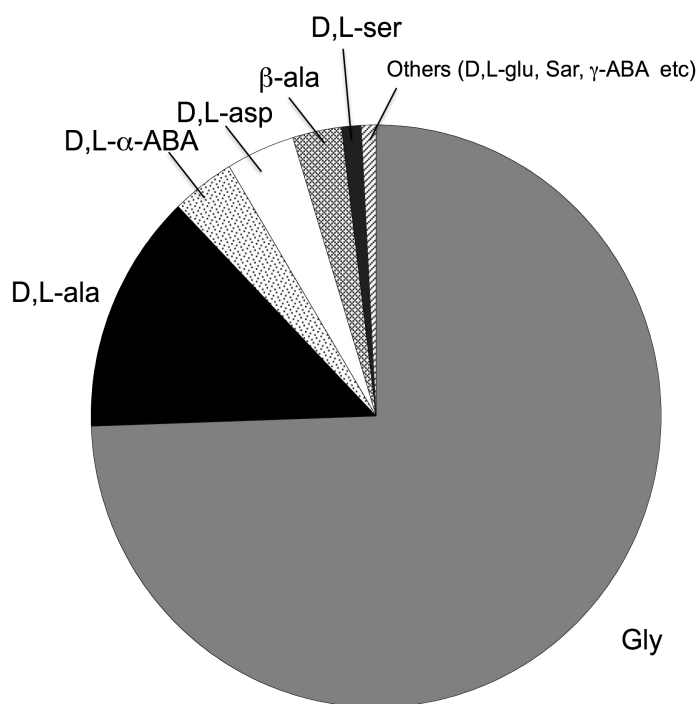
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**Figure 3a.** Relative abundance of amino acids detected after acid hydrolysis of the dried irradiation product. Abbreviations. Gly, glycine; D,L-ala, D,L-alanine; D,L- $\alpha$ -ABA, D,L- $\alpha$ -aminobutyric acid; D,L-asp, D,L-aspartic acid;  $\beta$ -ala,  $\beta$ -alanine; D,L ser, D,L-serine; others, including very minor amino acids.



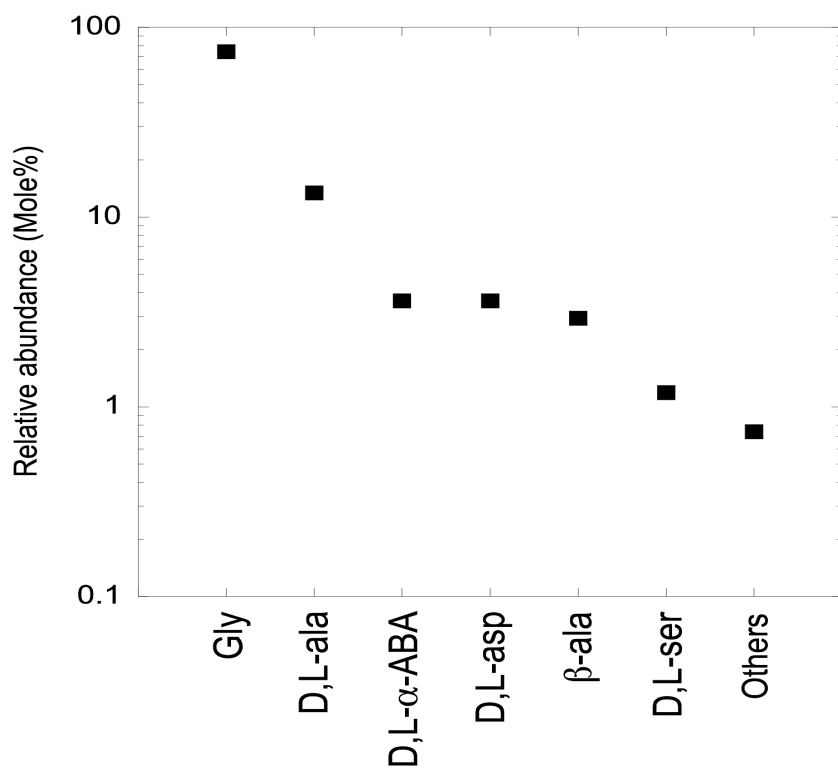
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**Figure 3b.** Relative abundance of amino acids on a logarithmic scale.

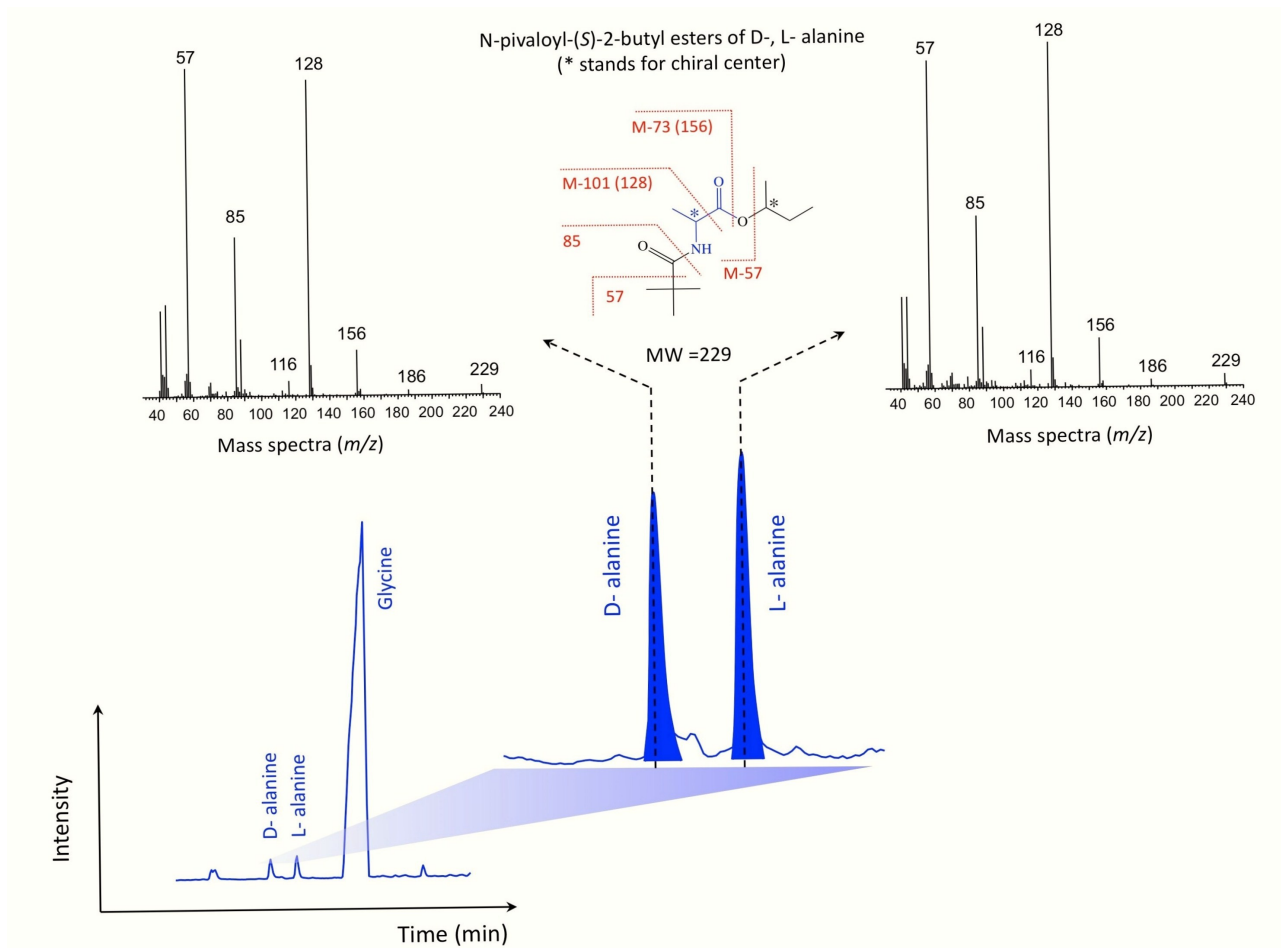


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**Figure 4.** Gas chromatograph (GC) separation and its mass fragment pattern of the N-pivaloyl-(S)-2-butyl esters of D-, L- alanine.



290 3.2 Discussion

Our structures are synthesized when gaseous CO and N<sub>2</sub> are present over liquid water. On Earth, the source of CO could be hydrothermal, arising from the transformation of CO<sub>2</sub> into CO (CO<sub>2</sub> + H<sub>2</sub> ↔ CO + H<sub>2</sub>O). The temperature of the experiment which led to the formation of CO and CH<sub>4</sub> from a mixture of CO<sub>2</sub> dissolved in flowing seawater, of gaseous H<sub>2</sub> and of magnetite was conducted at 295 250°C-300°C and 250 bar [13]. Theoretical calculations showed that at 35 MPa, H<sub>2</sub> production occurred during serpentinization of ultramafic rocks, between 200 and 315°C [22] and that serpentinization may occur at temperatures below 300°C [23]. H<sub>2</sub> was also generated in a recent experiment conducted at 300°C and 500 bars on hydrolysis of komatiite glass [24]. At those temperatures, CO is present in both aqueous and gaseous phases. Consequently, CO is available in the gaseous phase in terrestrial hydrothermal environments where olivine encounters serpentinization, producing H<sub>2</sub> and magnetite. Olivine and pyroxenes minerals such as those found in mafic and ultramafic rocks, are iron and magnesium silicates. Exothermic reactions of olivine (Mg,Fe)<sub>2</sub>SiO<sub>4</sub> and pyroxene (Mg,Fe)SiO<sub>3</sub> with H<sub>2</sub>O and CO<sub>2</sub> lead to the products, quartz, magnetite, serpentine, calcium carbonate, H<sub>2</sub> and recently CO [13]. Geological sites where exothermic mineral transformation occurs with a release of H<sub>2</sub> are consequently appropriate sites for the synthesis of abiotic organic microstructures.

A recent article shows that release of H<sub>2</sub> occurs at low temperature, 30 to 70 °C, when olivine containing magnetite and chromite is hydrolysed [25 ]. However at these temperatures, the formation of CO from CO<sub>2</sub> is not thermodynamically favorable. Indeed, earlier experimental investigations of the CO transformation showed that substantially higher CO concentrations occur at 350°C rather than at 150°C [26].

It is consequently plausible to propose that dinitrogen embedded in a ferromagnesian geological terrestrial environment may react with CO and H<sub>2</sub>O to form molecules which may assemble during a dryness period into microspheres, filaments and tubules containing organic structures such as those synthesized in our laboratory experiment. The abundance of CO<sub>2</sub> was higher during Archean times. The atmospheric partial pressure of CO<sub>2</sub> was several times higher in early Archean times than present-day values [27]. The source of excitation, protons, was also higher. Protons arise from cosmic radiation or from gamma rays included in cosmic radiation which induce protons through water radiolysis. In the Archean times, 3.5 Ga ago, the Earth magnetic field was much lower than nowadays. A very low equatorial paleointensity of 5 μT at c.a. 3.5 Ga was reported [28, 29] which corresponds to 17% of the present day value. Cosmic radiation could consequently easily reach the surface of the Earth. Little is known about coronal mass ejection of the paleoarchean Sun. However, a proton source from cosmic radiation reaching the surface of the Earth seems more probable than a proton source

arising from extinct radionuclides. Indeed, the amount of radioactivity brought by the late heavy  
325 bombardment has been recently controversial.

Our laboratory organic microstructures are synthesized in presence of silicates since proton  
irradiation was conducted in a glass tube. It is to be noticed that microspheres,  $\sim 0.5$  to  $2.5 \mu\text{m}$ , formed  
experimentally from amino acids in aqueous solutions which were consecutively dried [30] were  
produced in a glass tube and not in a stainless steel tube [31]. It is also to be noticed that a correlation  
330 is observed between a release of Si and a local accumulation of  $\text{CH}_4$  during olivine hydrolysis at low  
temperatures [25].

Consequently, our laboratory organic microstructures might form on Earth, in silicate  
mineral environments where ferromagnesian minerals evolve during exothermic reactions with a  
release of  $\text{H}_2$ , where carbon monoxide can form from carbon dioxide in hydrothermal conditions and  
335 when the intensity of cosmic radiation on the Earth surface was more intense than nowadays, i.e. 3.5  
Ga ago.

The Australian 3.446 Ga Kitty's Gap chert, Warrawoona Group, Pilbara, is composed of a  
crystal structure of quartz, is located near mafic and ultramafic environments containing olivine and  
has been submitted to hydrothermal activity through a hydrothermal vein [32].

Spherical, cylindrical and filamentous micro and sub-microstructures were observed in  
Kitty's Gap chert. SEM images of the unprocessed rock [32 Figs. 4, 7-12) show filaments with  
cylindrical form ( $\sim 0.3$ - $0.45 \mu\text{m}$  in diameter and up to  $40 \mu\text{m}$  in length), rods ( $\sim 0.4 \mu\text{m}$  in width and  
 $\sim 0.8 \mu\text{m}$  in length) and spheres ( $\sim 0.5$ - $0.8 \mu\text{m}$  in diameter). These Archean carbon microstructures were  
considered with a high probability of biogenicity and were interpreted as fossils of microorganisms.  
345 The filaments were considered as extracellular polymeric substances EPS, secreted by  
microorganisms. These Archean microstructures resemble in shapes and in sizes our laboratory organic  
microstructures. This is supported by the fact that microspheres may have variable geometries  
depending on temperature, pressure, proportion of reactants and speed of rotation of the reacting  
medium in which they are synthesized [33, for a reacting medium made of aqueous and organic  
350 phases; 34, for a reacting medium made of supercritical water]. SEM images of Kitty's Gap particles  
show very distinctly that the spheres and filaments are located near quartz surfaces [32, Figs. 11C,  
12B-D]. They may require the presence of silicates to be synthesized. These Archean carbon  
microstructures were considered as morphological biosignatures [35].

Other carbon microstructures observed in the Apex chert, Warrawoona group, Pilbara, were  
355 also interpreted as fossils of microorganisms [36]. They were also identified as carbon compounds  
obtained through Fischer-Tropsch reactions with volcanogenic CO in an hydrothermal setting ( $T \sim 250$ -  
 $350^\circ\text{C}$ ) [37] and as haematite pseudomicrofossils [38]. SEM observations of the mineralogy of Apex

cherts from the vein where the microfossils were found, show assemblage of sulphates, quartz and silicates with micrometer and submicrometer silica filaments anchored to the surface of quartz [39].

360 The experimental fossilization of prokaryotes [40,41] demonstrates that some Archaea can be fossilized and others cannot and that, considering cell lysis during silicification, a strict identification of fossils of microorganisms cannot be based on morphology.

The microfossil identification of Kitty's Gap was based on a micro-environment which could have hosted life, on a morphological resemblance with prokaryotes and on a difficult analysis of the carbon isotopic composition ( $^{13}\text{C}$  delta values range for most cases from -22 permil to -30 permil) which was similar for the contaminating carbon and for the carbon intrinsic to the sample [32]. An alternative explanation for the carbon isotope values is abiogenic fractionation of carbon by Fisher-Tropsch synthesis in hydrothermal systems [32 p.127]. Laboratory experiments conducted to evaluate the isotopic composition of organic compounds synthesized abiotically under hydrothermal conditions (250°C, 325 bar) showed that the organic products were depleted in  $^{13}\text{C}$  to a degree typically ascribed to biological processes [42]. Raman spectra were used to infer a biological origin to Archean carbon structures called microfossils. However, it seems highly difficult to determine the biogenicity of disordered  $\text{sp}^2$  carbonaceous material by Raman spectroscopy [43]. Consequently, a biogenic assignment of early Archean microstructures is not ascertained and other interpretations may be considered.

375 On the basis of morphology, of mineralogy with the presence of ferromagnesian minerals and quartz, of hydrothermal activity with the plausible transformation of  $\text{CO}_2$  into  $\text{CO}$ , and of an energy source, protons, which was much more intense in the Archean times than nowadays, we suggest that the organic micro and sub-microstructures that we observed in our proton irradiation experiments may be considered for the identification of some Archean carbon microstructures. These prebiotic signatures could be mentioned in a database of morphological signatures for fossils and pseudofossils such as the one proposed in 2003 [44].

We further suggest that these abiotically synthesized microstructures may be considered for the identification of molecular prebiotic signatures on Earth and also on Mars. Olivine, serpentine and carbonates were discovered in and near Nili Fossae, west of the Isidis basin on Mars [45]. The images were obtained with the spectrometer CRISM of the Mars Reconnaissance Orbiter (MRO). The observation was conducted with the detector working in the near infrared region, from 1.0 to 2.6  $\mu\text{m}$  which allows mapping of mafic minerals and hydrated or hydroxylated minerals. This assemblage of minerals seems typical of serpentinized ultramafic rocks found on Earth. Hydrothermal activity has consequently occurred in this region and  $\text{CO}$  might have been produced from  $\text{CO}_2$ . The same process of organic molecules synthesis than our laboratory synthesis from a mixture of  $\text{CO}$ ,  $\text{N}_2$  and  $\text{H}_2\text{O}$ ,

irradiated with protons, might have produced molecular precursors of life. Consequently, a search for molecular prebiotic signatures may be envisioned in the Nili Fossae region.

#### 395 4. Conclusion

We demonstrate that organic micro and sub-microstructures are synthesized during proton irradiation of a gaseous mixture of CO, N<sub>2</sub>, H<sub>2</sub>O. Their shapes vary from spheres to filaments and they produce amino acids after HCl hydrolysis. The enantiomer analysis for D-, L- alanine confirmed that the amino acids were abiotically synthesized during the laboratory experiment. Analysing hydrothermal, chemical and mineral conditions of natural formation on Earth, we show that these organic microstructures might be synthesized during Archean times from a mixture of CO, N<sub>2</sub> and H<sub>2</sub>O, in hydrothermal silicates environments and under a proton excitation source which existed in higher intensity 3.5 Ga ago than nowadays. We show that these prebiotic organic microstructures should be considered for the identification of microstructures observed in Archean geological sites.

Amino acid precursors were first obtained from proton irradiation of CO, N<sub>2</sub>, H<sub>2</sub>O in 1989 [4]. Kensei Kobayashi considered the formation of these organic molecules in a primitive Earth atmosphere rich in CO<sub>2</sub>. We advanced a step further, considering hydrothermal formation of CO as a product of the transformation of CO<sub>2</sub> in geological sites where ferromagnesian silicate minerals encounter the process of serpentinisation with the hydrothermal release of H<sub>2</sub>.

We suggest that a search for such organic micro and sub-microstructures, inside or nearby serpentinized rocks on Earth and on Mars, could be envisioned. The organic geochemistry of these rocks has been very little studied [21]. A discovery of such structures would confirm the hypothesis concerning prebiotic formation of amino acids near hydrothermal sites where olivine encounters serpentinization and considering a proton excitation source from cosmic radiation or as a product of water radiolysis [10-12]. Consequently, the organic microstructures that we observed may be considered for the identification of molecular prebiotic signatures on Earth and also on Mars.

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