**Detection of lipid biomarkers in oxidative environments by the Exomars 2020/RLS instrument**

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Running title: Raman detection of lipid biomarkers

**Abstract**

Establishing if life ever existed on Mars is one of the outstanding scientific questions of our time. To address this, the European Space Agency (ESA) established the ExoMars mission to investigate the Martian environment searching for potential traces of present or past life. To be launched in 2021, the ExoMars rover mission relies on a suite of analytical instruments envisioned to identify organic compounds with biological value (biomarkers) among the Martian mineralogical matrix. Here we investigate the feasibility of detecting basic organics (linear lipid molecules) by the Raman Laser Spectroscopy (RLS), one of the Pasteur Payload instruments onboard the ExoMars rover. We compared the detectability of six lipid molecules (alkanes, alkanols, fatty acid, and isoprenoid) before and after an oxidation treatment (15 days with H2O2), with and without mineral matrix support (amorphous silica rich versus iron rich). Raman and IR spectrometry was combined with gas chromatography-mass spectrometry to determine detection limits and technical constrains. We observed different responses to degradation depending on the lipid molecule and mineral substrate, with the silica-rich material showing better preservation of organic signals. These findings have implications for the ExoMars mission plans of drilling on the hydrated silica-enriched delta fan on Cogoon Vallis (Oxia Planum).

**Keywords**

Lipid biomarkers, Raman Laser Spectrometer, Mars exploration, ExoMars 2020, Astrobiology.

1. **Introduction**

The ExoMars rover mission (to be launched in 2021) has as a priority objective the search for potential biosignatures on Mars (ESA, 2013). To achieve this, the mission will have to address the issue of the role played by mineralogy in preserving organics at the recently selected landing site, Oxia Planum. ExoMars 2020 relies on a set of integral instruments dedicated to exobiology and geochemistry research in the Martian subsurface. The ExoMars rover is equipped with a drill capable of acquiring subsurface samples down to two meters depth. Samples will be delivered to the internal rover laboratory (Pasteur instrument suite), grounded inside, and then analyzed by a number of geochemical techniques. One of the Pasteur Payload instruments onboard ExoMars, the Raman Laser Spectrometer (RLS), will provide context information for the identification and characterization of potential organic compounds linkable with present or past signatures of life. Among other things, RLS was conceived to identify organic compounds and mineral products attributable to biologic activities, as well as characterize mineral phases produced by water-related processes. Complementarily, a visual/infrared micro-imaging spectrometer (MicrOmega) and a mast-mounted IR spectrometer (ISEM) will assist in deciding which surface targets to investigate by determining mineralogical and molecular composition of Martian samples more or less distant (rocks, outcrops, and regolith). Ultimately, a laser desorption-mass spectrometry coupled to a gas chromatography-mass spectrometer (i.e., the Mars Organic Molecule Analyzer, MOMA) will be in charge of pursuing organics that allow answering questions about potential origin, evolution and distribution of life on Mars. The ExoMars mission is envisioned for achieving an unequivocal detection of biomarkers based on the synergic work of a complete set of detection instruments that enable to establish if life ever existed on Mars.

Mars is an extremely oxidative environment, which surface receives high penetration-galactic cosmic rays and solar energy particles due to the thin atmosphere and lack of magnetic field on the planet (Dartnell et al., 2007). The high irradiation energy reacts with water molecules and can generate hydroxyl radicals, which eventually may produce peroxides and other oxidizing species by reacting with other elements present in the Martian surface (ten Kate et al., 2005). Indeed, the hydrogen peroxide (H2O2) present in the Martian atmosphere is thought to be formed by water photolysis and by electrostatic fields generated during dust devils and storms (Clancy et al., 2004; Atreya et al., 2006; Encrenaz et al., 2010). In addition, H2O2 could be directly formed in the regolith by the interaction between minerals and water (Hurowitz et al., 2007; Davila et al., 2008; Gil-Lozano et al., 2016). Despite the strong oxidizing character of H2O2 and its relative abundance on Mars, little is known about its effects on the Martian regolith components.

Evidence of the global oxidizing conditions on Mars is the red coloration of the Martian regolith, which is caused by the dominance of the oxidized state of iron forming minerals such as goethite, hematite or magnetite (Morris et al., 2000). The oxidized form of iron has been observed at high concentration in different Martian locations in Gale crater by the MSL Curiosity (Blake et al., 2013) or in Meridiani Planum by the MER Opportunity (Hurowitz et al., 2010). The intense radiation received by the Martian surface over the past thousand million years is considered to be enough to destroy any potential remnant of life (Pavlov et al., 2013), such as cells or spores potentially lying on the top few meters of the Martian regolith (ten Kate et al.,2005; Schuerger et al., 2006).

Finding well-preserved organic matter in the subsurface of Mars is a key point for the success of the ExoMars 2020 mission. Oxia Planum landing site is located at the east margin of Chryse Planitia, where several valley systems converge and signatures of abundant Fe/Mg phyllosilicates along the area are reported. Interestingly for the search of biomarkers, fine grain materials accumulate at the end of Cogoon Vallis, a valley forming delta-fan deposits enriched in silica. If organic matter is present and accessible in the Martian sediments, it is very likely that it has experienced some degree of degradation. Hence, knowing about the oxidability of organics is fundamental when searching for signs of life on Mars. As acquired from down to 2 m depth, subsurface samples to be analyzed by the ExoMars rover instruments are somehow expected to come from beneath the most oxidizing zone at the Martian surface (Vago et al., 2006), thus potentially preserving any existing biomarker. Assuming a very low concentration of any potential residue of biomass on Mars, a deep understanding of organics preservation and their detection limits in a highly oxidizing environment is essential for tuning the ExoMars rover instruments.

The aim of this work is to investigate the feasibility of detecting basic organic molecules (e.g., hydrocarbons, *n*-carboxylic acids and *n*-alkanols) using Raman spectroscopy in synergy with other detection techniques such as IR spectrometry and gas chromatography-mass spectrometry (GC-MS), all instruments loaded on-board the ExoMars 2020 rover mission. Experiments were conducted to test the oxidability of lipid biomarkers exposed to a highly oxidizing agent (H2O2) alone and supported on two different substrates (rich in amorphous silica, opal-A, or rich in iron oxides). Parameters affecting the lipids degradability (e.g., matrix effects) and their detection by RLS (i.e., detection limits) were assessed.

1. **Materials and Methods**
   1. **Samples Preparation**

A number of lipid congeners was selected from the three major families of linear and saturated (i.e., *normal*) lipids (*n-*hydrocarbons, *n-*carboxylic acids, and *n-*alkanols). Assuming that potential life on Mars would be expected to be of prokaryotic nature, we chose a range of even numbered carbons (C16-C30) as the closest lipid proxies of microbial life (Grimalt and Albaigés, 1987; Meyers and Ishiwatari 1993). As microbial markers, we employed pure standards of two *n*-alkanes (*n*-hexadecane and *n*-triacontane), one isoprenoid (2,6,10,14-tetramethyl-pentadecane or pristane), one *n*-carboxylic acid (*n*-hexadecanoic acid or palmitic acid), and two *n*-alkanols (1-decanol and 1-docosanol). All lipid standards were purchased from Sigma-Aldrich (Madrid, Spain), as well as the oxidizing agent hydrogen peroxide (37% v/v) and the organic solvents used for lipid extraction dichloromethane and methanol, all of analytical grade. Ultrapure water (Milli-Q) used for rinsing was provided by an in-house Millipore system (Merck Millipore, USA).

Mineral matrices rich in silica and iron oxide were employed as supporting substrates of the lipid biomarkers. A matrix rich in amorphous silica or opal was selected as primary substrate (#1) by two reasons; first, its amorphous structure does not give well-defined spectroscopic signatures thus permitting/facilitating the detection of lipid biomarkers and second, it does not seem to suffer any alteration after being exposed to H2O2. Thus, all the signals observed in the opal spectra can be attributed to the organics to test, and their alteration products upon oxidation. Iron-rich mineral samples were selected as secondary substrate in order to check whether iron acts as catalytic ion and enhances the oxidation reaction within organics molecules. A sinter sample rich in amorphous silica from El Tatio geothermal field (northern Chile) was considered as substrate #1, whereas a soil sample rich in iron oxide (mostly hematite) and oxyhydroxide (mostly goethite) from Río Tinto (southwest Spain) was regarded as substrate #2. Both solid substrates were prepared extracting them with organic solvents (dichloromethane: methanol, 3:1 v/v; Soxhlet, overnight) and furnace them (550 ºC for 8 hs) to remove any presence of the target lipids.

**2.1.1. Experimental set-up**

The experimental set-up is divided in four steps (Figure 1). First, pure lipid standards were directly analyzed by three detection techniques (i.e., Raman, IR and GC-MS) without previous treatment. Second, a mixture of the six lipid biomarkers (1 mg·ml-1 each) was subjected to the oxidizing effect of H2O2 (0.5 ml), letting them to react at room temperature for 15 days. Third, the same oxidation experiment was performed but with the lipid biomarkers (lipids biomarkers were added to the substrates, as a dissolution with organic solvents: hexane or methanol) being supported on 1 g of amorphous silica (substrate #1), leaving them to react with the H2O2 at room temperature for 15 days. Fourth, identical oxidation experiment as in the third step was but using the iron-rich substrate (#2) (Figure 1). After the oxidation period in the second, third and fourth experiments, samples were analyzed with Raman, IR and GC-MS.

* 1. **Raman spectroscopy analysis**

Taking into account the features of the Raman spectrometer on board the ExoMars rover (Rull et al. 2017), in this work we try to constrain the parameters to take the best measurements possible, and be of assistance to avoid false interpretations. Main issue to confront when measuring organics with Raman spectroscopy is the lack of detection due to wrong accuracy of the measurements parameters chosen, which strongly depend on the type of sample.

The Raman spectrometer Horiba JobinYvon Hri550, with a Charge Coupled Device (CCD) with 1024x256 pixels cooled to 203 K for thermal-noise reduction, is conceived to work with four diffraction gratings of 600, 1200, 1800 and 2400 grooves/mm, which provides a wide range of intensity signal/resolution ratio. Excitation of the sample is done by an intensity-modulated (0 – 200 mW) Nd:YAG solid state laser with a wavelength of 532 nm non polarized. Fiber optics can connect the spectrometer to different optical devices, which are two cryogenic probes with focal lens of 10 and 22.6 mm (Microbeam S. A.), and to a B&W Tek microscope with 20X objective (Microbeam S. A.), with spots size of 105, 175 and 355 µm respectively.

Using the CCD in High Gain mode, the diffraction grating of 1200 grooves/mm and 20X objective, our spectral resolution with a width slit of 200 µm results better than 5 cm-1. Cyclohexane (PAI-ACS Panreac, Spain) is used for Raman shift wavenumber calibration. The detection limits in which we have focused are related to the power of laser (for solids and liquids), the size of the laser beam spot (for solids) and the biomarkers concentration (for liquids). Additionally, the effect of H2O2 on the samples is also evaluated.

* 1. **IR spectroscopy analysis**

Diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) was performed in a thermo-Nicolet spectrometer. Spectra (2 cm-1 of resolution and 64 scans) were collected in the near infrared region (0.9 to 3 microns), using a DTGS-KBr detector and a Quartz beamsplitter

* 1. **Gas chromatography–mass spectrometry** **analysis**

Pure lipid standards and experimental samples (lipid biomarkers plus H2O2 with or without solid substrates) were analyzed by GC-MS. Lipid biomarkers extraction were performed using an ultrasonic bath with a mixture of organic solvents extraction follow previous publish protocols in Sanchez-Garcia et al., 2018 and Carrizo et al., 2019. The acid (palmitic acid) and alcohol (1-decanol, and 1-docosanol) congeners need to be trans-esterified with BF3 in methanol and trimethylsilylated with N,O-bis (trimethylsilyl) trifuoroacetamide (BSTFA), respectively, prior to analysis by GC-MS. The GC-MS analysis was conducted by using a 6850 GC system coupled to a 5975 VL MSD with a triple axis detector (Agilent Technologies) operating with electron ionization at 70 eV and scanning from *m/z* 50 to 650. The analytes were injected in splitless mode (1 µl) and separated on a HP-5MS column (30 m x 0.25 mm i.d. x 0.25 um film thickness) using He as a carrier gas at 1.1 ml min-1. Further analytical details have been previously described elsewhere (Sánchez-García et al., 2018).

1. **Results** 
   1. **Raman spectra**

The C-H stretching of 1-docosanol (Reichenbacher and Popp, 2012) on substrate #1 (amorphous silica-rich) was used to determine the suitability of the laser power. The characteristic signal of the organic starts to disappear above 30 mW of laser power (Fig. 2a). Previous tests in the laboratory showed us the robustness of this organic molecule, which is insoluble in water and H2O2, as well as resistant to oxidant conditions. According to this and to avoid damage of weaker organic molecules, all measurements of organics was set as a maximum laser power of 20 mW.

When looking for organics in matrices with small diameter grains, such as the case of clays, the size of the laser spot is a crucial parameter to bear in mind (Foucher et al. 2013). During the mapping study, we confirmed that the Raman signal minimized when the laser spot was larger than the organic molecule (Fig. 2b, spots 1 and 2). This feature could be interpreted as an absence of organic in a place where actually there is.

The rest of lipid biomarkers (palmitic acid, 1-decanol, pristane, hexadecane and triacontane) were measured before and after being immersed in H2O2 for 15 days (Fig. 3). Initial concentrations were 1 mg·mL-1. After being subjected to the oxidizing agent, the simplest molecules (i.e., aliphatic chains without branches and functional groups: hexadecane and triacontane) resulted the most altered, with their strongest Raman signature (C-Hs stretching) barely disappeared, mostly in the hexadecane case. Gas chromatography-Mass spectrometry analyses reveal that their concentrations fell to 0.49 and 0.62 mg mL-1 respectively. Thus, we can fix our detection limit, at the measurements conditions selected for this study, in liquids at 0.5 mg mL-1. Palmitic acid showed also certain alteration when supported on the amorphous-rich substrate (#1), although its characteristic peak at ca. 2850-2900 cm-1 was still recognizable after the oxidation treatment (Fig. 4b). As for the 1-decanol, its Raman signal vanished completed upon oxidation from the substrate #1-supported sample (in two laser beam spots). In contrast, it remained intact when measured (two laser beam spots) on the residual oxidizing agent (liquid fase) (Fig. 4c).

When supported on the iron-rich substrate (#2), the lipid biomarkers showed a different behavior upon oxidation, with no rest of organics detectable in the Raman spectra after the 15 days of treatment (Figure 5). The Raman spectra of pure goethite (FeO(OH)) and hematite (Fe2O3) minerals taken from the RRUFF project (<http://rruff.info/>) are shown for comparison.

* 1. **IR spectra**

The near infrared (NIR) spectra of both substrates doped with lipid biomarkers did not show perceptible differences after H2O2 treatment (Figure 6). However, in the amorphous silica-rich substrate small signals attributable to the organics were observed at 2.26 and 2.30 microns (4424.78 and 4347.83 cm-1, respectively), which were not perceptible on the original soil (i.e., not spiked with lipids). These vibration frequencies are tentatively assigned to the first combination bands of the C-H stretching (2.2 to 2.5 microns) with various bending and stretching vibrations from the mid-infrared region (Salzer, 2008). Unfortunately, this region matches with the vibration frequencies of cations in octahedral sites of phyllosilicates, so they cannot be conclusive on the field. In contrast, the NIR spectra do not show any signal of the organics after the H2O2 treatment when the lipid biomarkers were supported on substrate #2.

* 1. **GC-MS analysis**

The GC-MS analysis showed a general decrease of signal after treatment with H2O2 in all tested lipids, hexadecane, triacontane and pristane (Fig. 6) as well as palmitic acid, 1-decanol and 1-docosanol (Fig. 7). The oxidation effect yielded signal losses from 40 to 60 % (calculated by comparison of peak areas before and after H2O2 treatment, corrected relative to internal standards) for both the *n*-alkanes (Fig. 6), palmitic acid and *n*-alkanols (Fig. 7). Interestingly, the straight chain hydrocarbons (hexadecane and triacontane) showed larger decreases of signal than the branched pristane upon oxidation (Fig. 6 a-b). No further decrease of the GC-MS signal was observed when the lipids were supported on amorphous silica-rich substrate (Fig. 6c and 7c). In contrast, when they were supported on the iron-rich substrate the signal decreased to barely disappear for the three hydrocarbons (Fig. 6d).

1. **Discussion**
   1. **Oxidation patterns and substrate interaction**

Results indicated that the effect of oxidation on the organic biomarkers was different depending on the lipid compound, mineral substrate, and physical property (spectral or mass signature). According to their Raman spectra, the linear alkanes showed the highest lability upon oxidation, with the characteristic organic signal at ca. 2850-2900 cm-1 virtually disappearing after 15 days in contact with H2O2. In contrast, decanol appeared to be more resistant to H2O2, being even dissolved in it without chemically reacting when the effect of H2O2 was tested on the lipid supported on mineral substrates. At the torsion modes region (low frequencies), broad bands at 450 and 800 cm-1 appeared. The methyl bending modes at 890 and 900 cm-1 shifted to 875 cm-1 and overlapped in just one band. At higher frequencies, there is an alteration of the baseline to higher frequencies (Sousa et al. 2016). Palmitic acid and pristane experienced only partial degradation.

The matrix effect was tested by repeating the oxidation experiments with the target lipids supported on two different substrates rich in either amorphous silica (#1) or iron oxides and oxyhydroxides (#2), both known to preserves biosignatures on Earth. Amorphous silica (Opal)-rich deposits have been discovered in multiples areas of Mars by remote sensing and in situ measurements, including Hellas basin (Bandfield, 2008), Valles Marineris (Millken et al., 2008), Gusev Crater (Squyres et al., 2008) and Gale crater (Morris et al., 2016), whereas hematite is the most stable iron oxide on Mars widespread across its surface (Christensen et al., 2001). At room conditions (293 K and 1 bar), it is observed that amorphous silica-rich is stable upon oxidation, in contrast to the iron-rich substrate increasing the relative Raman signal of hematite over goethite (Fig. 5). Composed mainly by hematite and goethite, the Raman spectra of substrate #2 after treatment showed an increase of the characteristic peak of hematite (i.e. 1320 cm-1), thus denoting an oxidative effect of H2O2 on the very matrix (i.e., oxidizing part of the goethite oxyhydroxide groups to hematite oxide; Faria and Lopes 2007; Hanesch 2008; Jubb and Allen, 2010; Buzgar et al. 2013). The increase of hematite was not noticeable in the NIR spectra, suggesting Raman spectroscopy as a more suitable technique to identify oxidation signals in Fe-rich substrate. It is well know that Fe(II) catalyze the H2O2 decomposition forming hydroxyl free radicals (OH•) by the Fenton reaction. Although more slowly, Fe(III) can also generate oxygen reactive species (ROS) by the so-called Fenton-Like reaction. Indeed, the heterogeneous Fenton-like reaction catalyzed by hydroxide and iron oxide minerals have proved to be a promise strategy for the treatment of wastewaters and soils contaminated with organic compounds (see Pereira et al., 2012 and references therein). Thus, this reaction might explain the rapid degradation of organics observed on iron-rich substrate in the present study.

The XRD spectra revealed lower alteration of the goethite component on substrate #2 when the lipid biomarkers were present relative to the pure substrate (Fig. 9). This observation may be explained by the fact that part of the ROS generated by the iron species are consumed in the oxidation of organics thus, reducing the amount of ROS available for oxidazing goethite to hematite.

* 1. **Searching for Martian biomarkers**

Analytical measurements at the field are more restricted and unstable than conditions when working in the laboratory. In this work, our aim was determining detection limits and technical constrains that the ExoMars instruments may encounter when simulating *in situ* prospection and measuring on the extreme Martian environment. Based on GC-MS measurements, the detection limit for a set of six straight-chained lipid molecules by Raman spectroscopy defined by the size of the laser spot in solids was determined at 0.5 mg·mL-1 for liquids.

Considering the intense radiation and highly oxidative environment on Mars, understanding chemical degradation mechanisms is essential to assess potential preservation of biosignatures on the Martian subsurface. In this regard, there are at least two key variables to consider; the organic molecule to preserve and the mineral matrix that contains it. The mineral matrix may play a key role on the preservation of organics, with active surfaces affecting the reactivity of present organics by either catalyzing their degradation or rather favoring their preservation (e.g., Fornaro et al., 2018). Here, we provide Raman observations that silica-rich substrates preserve organic signals better than iron-rich substrates upon exposition to strongly oxidizing conditions. This is relevant information to take into account for the future ExoMars 2020 mission planning to probe on the hydrated silica-enriched samples from delta fan on Cogoon Vallis. Regarding the organics, the type of molecule is also an important factor to consider when assessing degradability. Here, we observed that certain molecules (docosanol, pristane, and palmitic acid) resisted better than other an oxidation treatment of 15 days with H2O2. The two *n*-alkanes (mostly hexadecane) showed the lowest resistance upon oxidation with H2O2. 1-decanol, in contrast, was not oxidized by the H2O2 treatment but rather dissolved in the oxidation agent. The observed differences in the chemical behavior (degradability) and affinity (solubility) upon the oxidizing agent are of interest on a Martian scenario where potential organic molecules could have been dragged to different depth during diverse weathering events involving liquids (e.g., H2O or H2O2) and gases (e.g., CO2). This is an interesting hypothesis that the ExoMars drill mission will be able to test by targeting depths of up to 2 m on.

In this work, we invested degradability of organics under certain limited and controlled conditions (H2O2, 15 days of treatment, room temperature and ambient pressure). However, there is a variety of conditions and/or oxidizing agents of interest in a Martian scenario that may considerably change the organics response upon degradation. For example, chemical species such as perchlorates and chlorates, or even cosmic or UV radiation may be rather representative oxidizing alternatives (versus H2O2) of the Martian environment. Further work must be devoted to explore other degradation scenarios, including oxidizing agents and environmental conditions or relevance in the Martian environment.

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**Author Disclosure Statement**

No competing financial interests exist.

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**Figure captions**

**Figure 1**. Experimental set-up scheme consisting on oxidation tests of a lipid biomarkers

mixture (*n*-hexadecane, *n*-triacontane, pristane, palmitic acid, 1-dodecanol and1-

hexadecanol) with H2O2, with and without support of a solid substrate of either

amorphous silica (#1) or iron oxide and oxyhydroxide (#2). Detection techniques after

oxidation include Raman and IR spectrometry, as well as gas chromatography coupled to

mass spectrometry (GC-MS).

**Figure 2**. a) Raman spectra of the C-H stretching bond of docosanol measured changing

the laser power. b) Raman spectra and pictures of a sample of soil OMFT with docosanol.

Note the importance of the size of the laser spot to detect the organics immersed in the

soil (picture 3 and 4).

**Figure 3.** Raman spectra of C-H stretching bond of the pure organics before (a) and after

having been immersed in H2O2 during 15 days (b).

**Figure 4**. a) FTIR (left) and Raman (right) spectra of the palmitic acid before and after being subjected to the oxidant during 15 days. The Raman spectra of the H2O2 just when it was added to the organic is also shown (red line). Variations in the vibrational signatures indicate that the molecule, although still identifiable, have suffered changes in its structure. b) Raman spectra and pictures of solid palmitic acid (left) and liquid decanol (right) detected at the soil OMFT after being immersed in H2O2 15 days.

**Figure 5.** FTIR (a) and Raman spectra (b) of the soil RT21 before and after being

spike with alkanes and immersed in H2O2 15 days.

**Figure 6**: NIR spectra of the soils with the organic compounds after being attacked with H2O2 during 15 days: a) substrate 1 (amorphous silica rich) and b) RT21 soils (iron reach).

**Figure 7.** GC-MS analysis of alkanes in the samples following the experimental scheme in figure 1.

**Figure 8.** GC-MS analysis of carboxylic acids and alcohols in the samples following the experimental scheme in figure 1.

**Figure 9**. XRD diffractograms compering five samples of RT21. 1: Original soil, 2: Soil + H2O2, 3: Soil + H2O2 + decane, 4: Soil + H2O2 + pristane, 5: Soil + H2O2 + alkane mixture (C8 to C40). H: hematite, G: Goethite.

**Figure captions**

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**Figure 1**. Experimental set-up scheme consisting on oxidation tests of a lipid biomarkers

mixture (*n*-hexadecane, *n*-triacontane, pristane, palmitic acid, 1-dodecanol and1-

hexadecanol) with H2O2, with and without support of a solid substrate of either

amorphous silica (#1) or iron oxide and oxyhydroxide (#2). Detection techniques after

oxidation include Raman and IR spectrometry, as well as gas chromatography coupled to

mass spectrometry (GC-MS).



**Figure 2**. Raman spectra of the C-H stretching bond in 1-docosanol (1 mg·ml-1) measured

as a) pure lipid standard (solid) along an increasing laser power, or b) as lipid supported

on substrate #1 (amorphous silica-rich). In b, pictures of the measured surface are also

shown. Please note the importance of the size of the laser spot to detect the organics

immersed in the mineral matrix (picture 3 and 4).



**Figure 3.** . Raman spectra of C-H stretching bond of the pure lipid standards (1 mg·ml-1)

before (a) and after being immersed in H2O2 during 15 days (b). Raman spectra were

performed in the glass tubes were the reaction with H2O2 were performed.

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**Figure 4**. Raman spectra of 1 mg·ml-1 of palmitic acid (a-b) and 1-decanol (c) upon

oxidation. In a, Raman spectra between 200 and 4000 cm-1 are shown for the palmitic

acid alone (black), with the oxidizing agent (red), and with the oxidizing agent and

supported on the substrate #1 (blue). In b and c, Raman spectra close-ups (2500-3270 cm-

1) of the palmitic acid (b) and decanol (c) after the 15 days oxidation treatment on the

amorphous silica-rich substrate (#1) are shown, with 3 analytical replicates (i.e., three or

four measurement spots of the laser beam). In c, two laser beam spots were considered

for each the substrate oxidation-residue (solid zone) and the oxidizing agent residue

(liquid zone).

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**Figure 5.** Raman spectra of substrate 2 before (blue) and after being spiked with 1 mg·ml-

1 of alkanes biomarkers (red). and immersed in H2O2 15 days.



**Figure 6**: NIR spectra of the amorphous silica- (a) and iron-rich substrates (b) doped with four lipid biomarkers (1 mg·ml-1) after oxidation treatment with H2O2 for 15 days.

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**Figure 7.** GC-MS signal of the n-alkanes (hexadecane and triacontane) and isoprenoid

(pristane) biomarkers alone (a), with the oxidizing agent (b), and with oxidizing agent

plus mineral substrate (c and d). Please note that scale on Y axis is the same in the four

plots.



**Figure 8.** GC-MS signal of the n-carboxylic acid (palmitic acid) and n-alkanols (1-decanol and 1-docosanol) biomarkers alone (a), with the oxidizing agent (b), and with oxidizing agent plus mineral substrate (c and d). Please note that scale on Y axis is the same in the four plots.

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**Figure 9**. XRD diffractograms comparing five samples of substrate #2 containing pure

substrate (black), and different combination of oxidizing agent and lipid biomarkers

(red, blue, pink and green). Legend: 1) pure substrate #2, 2) substrate #2+ H2O2, 3)

substrate #2+ H2O2 + decane, 4) substrate #2 + H2O2 + pristane and 5) substrate #2 +

H2O2 + *n*-alkane mixture (C8 to C40). H: hematite, G: Goethite.