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Graphitic carbons and biosignatures

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Box 1: Glossary of terms

Aliphaticity: Proportion of aliphatics in a molecule. Aliphatic groups are groups of carbon atoms joined together in straight chains, branched chains, or non-aromatic rings.
Archean: The Archean Eon is one of the five eons of Earth history. Its starts after the Headean eon (4 Ga) and ends with the beginning of the Proterozoic Eon (2.5 Ga). It is subdivided chronologically into four eras: the Eoarchean era, the Paleoarchean era, the Mesoarchean era and the Neoarchean era.
Aromaticity: Proportion of aromatics in a molecule. Aromatic groups are groups of carbon atoms arranged in rings with saturated and unsaturated bonds alternating in a particular way so that they follow Huckel's aromaticity law.
Biosignature: Morphological, elemental, molecular or isotopic feature inherited from life and preserved in rocks. Note that some biosignatures can potentially be mimicked by non-biological processes and should thus be considered less reliable and/or be supported by independent biosignatures.
Diagenesis: Collection of chemical, biological and physical post-depositional processes involved in the lithification of unconsolidated sediments into rocks at shallow depths and low temperatures (<200°C).
Fischer-Tropsch Type synthesis: Chemical reactions that convert a mixture of gaseous carbon oxides (e.g. CO, CO_2, HCO_3^-, etc) and di-hydrogen into hydrocarbons through the catalytic effect of some transition metals (e.g. Ni, Cr, Fe, etc.).
Indigenicity/Syngenicity (or Indigeneity/Syngeneity): quality for graphitic carbons having been incorporated into sediments at the time these sediments were forming (syngenetic) and still present in the rock that has formed from these sediments (indigenous).
Kerogen: After having long been used to describe all organic molecules capable of oil generation, the term kerogen is now defined as the insoluble macromolecular organic matter that can be found in sedimentary rocks, regardless its biogenic or abiotic origin.
Organic biomarker: Class of biological organic molecules, typically from cellular membranes of living organisms, and their geological equivalents found in the rock record.

Box 2: Glossary of techniques

CLSM: Confocal Laser Scanning Microscopy is a non-destructive high spatial resolution optical microscopy technique used on a selected depth in the specimen and which exploits the fluorescent response of minerals and organic molecules to a given laser wavelength to provide imaging contrast.

EA: Elemental Analysis is a destructive analytical technique that allows the quantitative determination of light element abundances (H, C, N, O, S) using the combustion or thermal conversion pyrolysis of a solid (e.g. rock powder) into a gas. When coupled to IRMS, this technique also allows the determination of isotope compositions.

EDS: Energy Dispersive Spectroscopy is a non-destructive high spatial resolution technique used to determine chemical compositions using characteristic X-ray emitted from the impact of high-energy electrons on a solid surface. Electron microscopes and microprobes are generally fitted with an EDS detector.

FIB-SEM: Focused Ion Beam – Scanning Electron Microscopy is a minimally-destructive high spatial resolution technique typically used to mill through a target for 3-D reconstructions or micro-fabrication of ultrathin foils for TEM or STXM imaging.

FTIR: Fourier Transform InfraRed microscopy is a non-destructive high spatial resolution technique used to spectroscopically map chemical bonds in minerals and organic molecules.

GC-MS: Gas chromatography – mass spectrometry is a destructive and highly sensitive technique that uses a capillary column to separate gaseous molecules and then measures their molecular weight for identification.

IRMS: Isotope Ratio Mass Spectrometry is a destructive technique that quantitatively measures the relative abundance of stable and radiogenic isotopes to trace fractionation processes or dating.

NanoSIMS: Nanoscale Secondary Ion Mass Spectrometry is a minimally-destructive high spatial resolution technique that quantitatively measures the abundance of isotopes.

NMR: Nuclear Magnetic Resonance is a non-destructive radio frequency spectroscopy technique that quantitatively measures the chemical shift of isotopes with an odd number of protons or neutrons which depends on its chemical bonding environment.

Raman: Laser Raman microspectroscopy is a non-destructive high spatial resolution technique used to acquire diagnostic spectra from minerals and organic compounds that have a Raman response to an incident beam of monochromatic photons.

SEM: Scanning Electron Microscopy is a non-destructive high spatial resolution technique that allows imaging of solids using a range of quantum effects under high-energy electron bombardment. SEM imaging is generally performed using secondary electrons, which give a depth of field created by the relative number of secondary electrons detected as a function of the angle between the surface and the electron beam, or using back-scattered electrons, where increased contrast is given by increasing atomic numbers of the atoms composing the target.

SIMS: Secondary Ion Mass Spectrometry is a minimally-destructive high spatial resolution technique that quantitatively measures the abundance of isotopes.

STXM: Scanning Transmission X-ray Microscopy is a non-destructive, high spatial resolution, and synchrotron-based microscopy technique that allows collecting images with a contrast based on atomic speciation and spectroscopy. Imaging is performed using the X-ray absorption near edge structure (XANES) spectra of an element of interest, which offer insights into the local electronic structure of atoms and allows the identification of chemical bonds.

TEM: Transmission Electron Microscopy is a non-destructive high spatial resolution technique that allows imaging of ultrathin electron-transparent specimens. Contrast is given by the variable absorption of electrons transmitted through the specimen caused by its chemical composition or thickness. TEM allows bright-field imaging, High-Angle Annular Dark Field (HAADF) imaging, selected area electron diffraction and EDS analyses.

ToF-SIMS: Time-of-Flight Secondary Ion Mass Spectrometry is a minimally-destructive high spatial resolution technique that quantitatively measures the abundance of isotopes and molecule (with up to 10,000 amu).

Abstract (<100 words)

Unambiguous identification of graphitic carbons as remains of life in ancient rocks is challenging because fossilized biogenic molecules are inevitably altered and degraded during diagenesis and metamorphism of the host rocks. Yet, recent studies have highlighted the possible preservation of biosignatures carried by some of the oldest graphitic carbons. Laboratory simulations are also increasingly used to better constrain the transformations of organic molecules into graphitic carbons induced by sedimentation and burial processes. These recent research advances collectively justify a reevaluation of the putative biogenicity of numerous ancient graphitic carbons, including the presumed oldest traces of life on Earth.

Keywords:

- Microfossils
- Degradation/Preservation
- Diagenesis/Metamorphism
- Experimental Fossilization
- Abiotic Processes
- Archean/Early Life

Introduction

The search for evidence of early life in the geological record has often involved graphitic carbons and has been a subject of intense debates and dividing controversies (e.g., Mojzsis et al. 1996; Rosing 1999; Schopf et al. 2002; Brasier et al. 2002; van Zuilen et al. 2003). Numerous challenges exist such as contamination and consideration of abiotic pathways that may lead to the formation of graphitic carbons, which can be difficult to distinguish from fossilized biological remains (Pasteris and Wopenka 2003; McCollom and Seewald 2006). The main complication resides in the generally poor preservation of biosignatures in the oldest metasedimentary rocks. Bio-alteration processes, as well as the increase of temperature and pressure conditions during burial inevitably alter the original biochemical signatures of organic molecules. As a result, the general paleobiological perception has long been that burial processes are detrimental to the preservation of signatures of life by graphitic carbons (see also Schiffbauer et al. 2007). However, although numerous challenges still remain, newly available micro-analytical techniques create innovative opportunities for the assessment of the biogenicity of graphitic carbons.

This contribution begins with an overview of the processes involved in the conversion of biogenic organic molecules into graphitic carbons. Remarkable examples of exceptional preservation of biosignatures by graphitic carbons in metasedimentary rocks are then described. Possible solutions and new approaches to evaluate the biogenicity of graphitic carbons are then explored. Finally, the question of the putative biogenicity of some Eo- and Paleoarchean graphitic carbons is discussed in light of a selection of emblematic examples from the recent literature.

1. Conversion of organic biological remains into graphitic carbons

When living organisms die, their organic molecules are usually rapidly biodegraded. Most biomolecules released to the ambient environment are either oxidized or assimilated

by living microbes. Yet, some organic macromolecules resistant to biodegradation may escape the biological cycle and get incorporated in sediments by selective preservation. For instance, some labile organic molecules may bypass biodegradation through degradation/recondensation reactions (e.g., Vandenbroucke and Largeau 2007). Adsorption on inorganic surfaces as well as encapsulation within mineral microcavities or larger molecules have also been reported as processes permitting the incorporation of organic molecules into sediments (e.g., Vandenbroucke and Largeau 2007). The respective contribution of each of these processes remains debated. Of note, organic molecules incorporated into sediments through selective preservation, adsorption, or encapsulation have likely only been slightly degraded and may still thus be identified. In contrast, labile molecules incorporated into the sediments through degradation/recondensation reactions have become geopolymers that may exhibit chemical signatures distinct from those of the initial biomolecules. All these processes lead to the formation of insoluble polymeric sedimentary organic macromolecules, collectively called kerogen (Vandenbroucke and Largeau 2007).

Subsequent diagenetic processes may further complicate the recognition of biosignatures (Figure 1). Partially preserved biosignatures carried by kerogen may suffer from compaction and fluid migration. As fluid migration is limited within low permeability sediments, they tend to favor the preservation of isotopic, elemental and molecular biosignatures, whereas with high permeability sediments are unlikely to preserve indigenous biosignatures (e.g., Vandenbroucke and Largeau 2007). During burial, reducing sediment may minimize redox reactions with biogenic organic matter and improve its preservation potential, through kerogen sulfurization for example (e.g., Vandenbroucke and Largeau 2007).

When burial increases, sedimentary rocks experience metamorphism at increasing temperature and pressure conditions. The general paleobiological perception has long been that burial-induced biomolecule degradation processes (carbonization/graphitization processes) are detrimental to the morphological and chemical preservation of life remains in rocks (Figure 1).

With increasing temperature conditions, the weaker bonds in kerogen molecules are thermally broken and dehydrogenation reactions become predominant, leading to the formation of graphitic carbons. Metamorphic degradation of kerogen is associated with an increase in aromaticity and the release of heteroatoms such as O, N or S. By promoting structural reorganization, thermal metamorphism may ultimately lead to pure graphite that may have completely lost its original biosignatures Finally, graphitic carbons remain susceptible to late oxidation and hydrogenation, as well as to alteration or contamination by younger biological carbon from the subsurface (particularly if carried by groundwater) during exhumation processes.

Biogenic organic molecules may thus experience multiple stages of degradation induced by a combination of biological, chemical, and physical factors during the geological history of their host rocks. Yet, it has been demonstrated that products released by kerogen degradation (i.e., thermally matured bitumen, molecular fossils and organic biomarkers) may sometimes preserve structural information diagnostic of biological and environmental provenance (e.g., Vandenbroucke and Largeau 2007). This can also be the case for graphitic carbons, as detailed in the following section.

2. Preservation of biosignatures by natural graphitic carbons

In contrast to the general belief, graphitic fossil occurrences in metamorphic rocks are relatively common. The literature conceals a number of examples of graphitic fossils that can be found in rocks of various ages and metamorphic facies, including the greenschist facies (e.g., Butterfield et al. 2007), the amphibolite facies (e.g., Schiffbauer et al. 2007) and the blueschist facies (e.g., Galvez et al. 2012). Notably, most of the metamorphic fossils reported in the literature are organic-walled microfossils such as spores, dynocysts or ‘acritarchs’, the latter being an emblematic graphitic fossil from the Proterozoic.

Numerous ‘soft-bodied’ fossils made of graphitic carbons have been described. Some of the most illustrative examples undoubtedly include the graphitic fossils found in the Middle Cambrian Burgess Shale Formation in the Rocky Mountains of southern British Columbia, Canada (Figure 2). This formation is composed of calcareous mudstones and limestones, and has experienced greenschist facies metamorphism at burial depths around 10 kilometers (Powell 2003). Yet, the Burgess fossil assemblage constitutes one of the most diverse records of Cambrian animal life. Soft-bodied fossils from the Burgess Shale that are preserved as graphitic films or graphitic carbonaceous compressions are reliably interpreted as the remains of relatively recalcitrant organic exoskeletons, whereas those preserved in three dimensions, via early diagenetic mineralization, typically represent more labile cellular tissues (Butterfield et al. 2007). Although the mechanisms that allowed their exceptional preservation remain debated, recent investigations have evidenced the preservation of chitin molecular signatures in some of these fossils (Ehrlich et al. 2013).

Also remarkable is the reported morphological preservation of graphitic plant macrofossils in Mesozoic blueschist metamorphic rocks from the Marybank formation in New Zealand (Galvez et al. 2012). Although these rocks have experienced burial to a minimum depth of 15 km, fossil leaves and stems can be unambiguously identified. Graphitic polygonal structures coated with micas may represent original cellular ultrastructure. Yet, despite the excellent morphological preservation, organic biomarkers and molecular functional groups of these graphitic carbons have been completely lost during metamorphism.

Other spectacular, morphologically preserved graphitic fossils are lycophyte megaspores from Triassic metasedimentary carbonate concretions from Vanoise in the western Alps, France (Figure 3 - Bernard et al. 2007). These partially mineralized microfossils experienced blueschist facies metamorphism (~360°C, ~14kbars, i.e. a burial depth of about 40 km) during the late Mesozoic and Cenozoic Alpine Orogeny. While mainly composed of graphitic carbons, these microfossils exhibit chemical and structural heterogeneities, which have been interpreted as remnants of original biochemical signals.

In particular, the chemical signature of degraded sporopollenin, the resistant biopolymer composing modern spore and pollen grain walls, has been identified within the fossilized megaspore walls using synchrotron-based STXM and XANES spectroscopy (Bernard et al. 2007).

Thus, the preservation of micro- to macroscale morphologies and of some molecular information in graphitic microfossils may not be as extraordinary as generally believed. Still, original biosignatures carried by organic molecules are degraded at least partially, if not completely, during bio-alteration and burial processes. Therefore, unambiguously identifying fossils of microorganisms in ancient rocks remains a challenging goal. As detailed in the following section, the next step to adequately interpret the ancient organic fossil record lies in quantitatively constraining the impact of bio-alteration and burial processes on organic molecule degradation.

3. Experimental fossilization

Although laboratory simulations do not perfectly simulate natural diagenesis, experimental investigations are increasingly seen as a promising route towards quantitative analyses of the transformations of organic molecules induced by burial processes. Most of the recent attempts of experimental fossilization have aimed at reproducing and better constraining early diagenetic processes that contribute to the morphological preservation of organic structures (e.g., Li et al. 2013). Yet, numerous studies are now also aimed at exploring the molecular evolution of organic molecules during artificial maturation (e.g., Li et al. 2013; Figure 4).

The use of isotope compositions to evaluate the biogenicity of graphitic carbons appears necessary but insufficient. Abiotic pathways such as the decarbonation of siderite and Fischer-Tropsch Type synthesis (FTT) are known to produce organic molecules with isotopic signatures similar to those of biological carbons, *i.e.* depleted in ^{13}C (McCollom and Seewald 2006). Recent hydrous pyrolysis experiments on kerogen in isotopically-

labeled aqueous solutions have shown that temperature increase promotes isotopic exchange between macromolecular organic matter and reactive inorganic compounds (Schimmelman and Lis 2010). Kinetic extrapolation from long experiments performed at 100°C suggests that isotopic exchange may even be significant in natural settings at temperatures as low as 40-50°C. Although, isotopic re-equilibration is not ineluctable, such modifications of isotopic signatures may lead to erroneous interpretations that would jeopardize the search for early life in the geological record.

Structural and molecular biosignatures appear a bit more robust. For instance, using heating experiments to simulate organic matter thermal alteration, Schopf et al. (2005) showed that Raman spectroscopy may provide sensitive indicators of the low-temperature alteration of graphitic carbons. More, recently, Schiffbauer et al. (2012) exposed natural fossilized acritarchs from the Mesoproterozoic Ruyang Group to experimental heating at approximately 500°C for up to 250 days, in both oxic and anoxic conditions. Notably, anoxic replicates retain biological morphologies despite an increasing degree of carbonization with continuous heating. In contrast, oxic replicates experienced more aggressive degradation when submitted to the same time/temperature conditions. Following a similar approach, Li et al. (2013) have investigated the structural and chemical transformations of bacterial cells during thermal aging experiments performed under anoxic conditions. Combined microscopy and spectroscopy data demonstrate partial morphological and chemical preservation of bacterial cells completely encrusted by iron phosphates despite heating treatments at hundreds of degrees for several hours under argon atmosphere. This study illustrates that inorganic phases may favor morphological and chemical preservation during carbonization processes.

Altogether, the trends in chemical, structural and isotopic changes reported in experimental fossilization studies provide new milestones towards a generalized mechanistic model of organic molecule degradation processes occurring during diagenesis and metamorphism. In addition to simulating biodegradation and taphonomic processes in the laboratory, future studies should investigate the impact of key parameters such as the geochemical nature of the fluid and the mineral matrix on the extent of

biomolecule degradation occurring during burial in natural settings. Artificial maturation experiments performed in open or closed systems under well-constrained physical and chemical conditions have been extensively used for decades to better constrain kerogen degradation processes (e.g., Vandenbroucke and Largeau 2007). In addition, the recent development of advanced characterization techniques allow the evolution of chemical, structural and isotopic signatures of graphitic carbons within natural maturation series to be precisely documented (Bernard et al. 2014).

4. Controversies over the earliest traces of life

Although many claims for Eoarchean and Paleoarchean life have been made on the basis of graphitic carbons, many controversies have arisen regarding their origin (e.g., Mojzsis et al. 1996; Rosing 1999; Schopf et al. 2002; Brasier et al. 2002; van Zuilen et al. 2003). Because of the inevitable alteration of organic molecules that occurs during burial, biogenic graphitic carbons can be difficult to differentiate from carbons coming from contamination. Contamination in geological specimens may have several origins: (1) an experimental origin where samples are contaminated during sampling, handling, and/or preparation; and (2) a natural origin where samples may be contaminated by natural abiotic carbons or younger biogenic carbons during fluid migration or biodegradation. Because of these difficulties, the distinction of biogenic from non-biogenic graphitic carbons in Archean rocks remains an open issue.

Here we propose a series of criteria, listed in Table 1, to be considered when investigating the possible biological origin of Eo- and Paleoarchean graphitic carbons in order to prevent misinterpretations. For instance, the occurrence of ^{13}C-depleted diamonds and graphitic carbons included in Hadean zircons from the Jack Hills in Western Australia has been interpreted as possible evidence of the earliest carbon cycle and/or early life (Menneken et al. 2007). Yet, nanoscale TEM observations performed on foils micro-fabricated by focused ion beam (FIB) have recently revealed that the observed graphite-diamond mixtures included in the Hadean zircons are embedded in

316 epoxy, leading to the conclusion that they are contaminants from polishing compounds
317 during sample preparation (Dobrzhinetskaya et al. 2014).
318
319 Among other controversial findings is the isotopically light graphite (with $\delta^{13}C$ values
320 between -21 and -49‰) reported as inclusions within apatite crystals and interpreted to
321 be the oldest signs of life from ca. 3.83 Ga granulite facies quartz-pyroxene (Qp) rock on
322 the Island of Akilia, Southwest Greenland (Mojzsis et al. 1996). It has been argued that
323 because these graphitic particles are completely included within apatite crystals, their
324 isotopic signatures could not have resulted from isotope exchanges with fluids during
325 long crustal residence times (Mojzsis et al. 1996). Yet, laboratory demonstration that
326 abiotic hydrothermal pathways, such as siderite decarbonation and Fischer-Tropsch–Type
327 (FTT) synthesis, may lead to the formation of carbonaceous compounds depleted in ^{13}C
328 (McCollom and Seewald 2006) have led to question the biogenic origin of these graphite
329 particles. In addition, recent nanoscale investigations performed on FIB foils have
330 revealed clear evidence for fluid-deposited graphite particles in the Akilia Qp rocks
331 (Figure 5a), rather present as coatings on apatite grains and not demonstrably as
332 inclusions (Figure 5b - Papineau et al. 2010a). The graphite from this rock has been
333 further shown by two other independent analytical methods to have $\delta^{13}C$ values between -
334 4.1 and -23.6‰ and thus are not as fractionated as previously reported (Papineau et al.
335 2010b). Although H, O, N, S and P have all been detected in this graphite, recent
336 nanoscale observations by FIB of graphite coatings on apatite grains within the banded
337 iron formations from the ca. 3.75 Ga old Nuvvuagittuq Supracrustal Belt have shown that
338 such mineral association can be fluid-deposited during the late history of the rock
339 (Papineau et al. 2011). This example illustrates the importance of unambiguously
340 demonstrating indigenicity before discussing the age and biogenicity of graphitic
341 carbons.
342
343 Graphite particles from the ca. 3.77 Ga turbiditic schists from the Isua Supracrustal Belt
344 have also been interpreted as biogenic remains based on their abundance and isotopic
345 signatures (Rosing 1999). Yet, additional measurements showing less ^{13}C-depleted
346 graphite particles in Isua metacarbonate rocks have led to an interpretation of these

particles as resulting from the abiotic thermal decarbonation of siderite (van Zuilen et al. 2003). Since then, turbiditic schists from Isua have been reported to contain graphitic layers with highly variable graphite content (from 5 up to 10 wt %) and $\delta^{13}C$ values (from -15‰ down to -24‰). Such values have been interpreted as consistent with a biogenic origin (Ohtomo et al. 2014). However, these recent interpretations remain debated, notably because they rely on samples from a unique formation.

Perhaps the most famous report of graphitic microfossil evidence for earliest life is the discovery of microbial-like structures composed of graphitic carbon from the ca. 3.46 Ga Apex chert in the Warrawoona Group (Schopf et al. 2002). While these microscopic objects were originally interpreted as cyanobacterial microfossils and subsequently became the benchmark for *bona fide* Paleoarchean microfossils, their biogenic origin has come into question after re-examination of their morphology and their geological and geochemical context (Brasier et al. 2002). Recent investigations have demonstrated that these objects are composed of variably graphitized carbons that contain molecular functional groups and heteroatoms of N, S, and P that would suggest a biological origin (De Gregorio et al. 2009). Yet, the origin of this graphitic carbon is still debated, with interpretations for their origin varying from Fischer-Tropsch Type (FTT) synthesis (Brasier et al. 2011) to contamination by younger organic matter (Olcott Marshall et al. 2012). Additional support to better assess the origin of these microscopic objects may come from similar analyses on graphitic carbons from other rocks of similar age (e.g. Table 1). For instance, Wacey et al. (2012) have recently concluded on the biogenicity of microfossil-like structures containing spheroidal nano-grains of silica embedded in graphitic carbon from the 3.35 Ga Strelley Pool formation. Yet, their conclusions mostly rely on the morphological similarities that these objects share with Gunflint microfossils as observed using FIB-SEM and TEM. The Strelley Pool formation is the target of a number of investigations as it hosts different types of graphitic carbons (Figure 5c) as well as stromatolites (Figure 5d).

4. Remaining questions and concluding remarks

The controversies detailed above highlight the need for a great deal of caution to be implemented to gather the multiple lines of evidence that may allow the unambiguous identification of ancient graphitic carbons as biological remains. Yet, a series of fundamental questions still needs to be addressed.

For instance, almost nothing is known regarding the possibility that abiotic carbons accumulate as aromatic compounds and polymerize within sediments or sedimentary rocks. FTT synthesis generally proposed as the source of controversial graphitic carbons dominantly produces short aliphatic hydrocarbons (McCollom and Seewald 2006). The fate of such abiotic organic molecules in sedimentary rocks over geological time-scales indeed remains totally unknown. Under what conditions can these hydrocarbon chains become aromatic macromolecules, difficult to distinguish from graphitic carbons resulting from the degradation of organic molecules of biogenic origin? What geological mechanisms and conditions would have to work in concert to yield accumulations of abiotic poorly crystalline graphitized carbon from such aliphatic precursors? What type of FTT synthesis or diagenetic pathways could produce poorly graphitic microbial-like features?

Galvez et al. (2013) reported graphite formation from abiotic calcite reduction at low temperature in an exhumed serpentinite-sediment contact in Alpine Corsica (France). Based on isotopic and spectroscopic measurements, these authors argued that this graphite has formed from an abiotic source of carbon and could be distinguished from the biogenic graphitic carbons observed in the same rocks. Yet, such reports of robust geochemical evidence for geological deposits of abiotic graphitic carbons remain quite scarce, in contrast to what should be expected based on the null hypothesis. In the present context, the null hypothesis stipulates that all possible abiotic pathways need to be disproven before a biogenic origin can be accepted. This cautionary approach certainly deserves merits for the oldest sedimentary rocks and in an exobiological context. However the null hypothesis could likewise be used in the opposite way as far as

terrestrial organic matter is concerned, from a uniformitarian perspective. Ultimately, such debates are healthy for this field of science as they stimulate additional research and can only be solved by using new analytical approaches and sample suites.

Altogether, all these questions can be summarized in a single question: "*How can ancient graphitic carbon be unambiguously assigned an origin?*". This apparently trivial question is full of hidden fundamental and methodological issues. Table 1 is an attempt at listing criteria that should be fulfilled in order to unambiguously assign a biogenic origin to graphitic carbons of Eoarchean or Paleoarchean age. Undoubtedly, a great deal of caution is required to prepare samples for micro-analyses and to gather the multiple lines of evidence that may eventually lead to the identification of ancient graphitic carbons as biological remains. In addition, we suggest here that identification of biogenic graphitic carbons in ancient rocks should not be based on results obtained from a single locality. Most importantly, the determination of syngenicity and indigenicity of putative microfossils and/or graphitic carbons within metasedimentary rocks requires a precise assessment of the thermal history that they have experienced, as well as quantitative constraints on the biosignatures that may be preserved by these graphitic carbons in light of their thermal history. Development of experimental fossilization shall allow substantial progress in that regard in the future.

As described here, the assessment of the origin of graphitic carbons in ancient rocks remains challenging and debatable since the behaviors of biogenic and abiotic carbons during diagenesis and metamorphism have not yet been precisely constrained. Likewise, while there remain uncertainties about the possible biogenic origin of graphitic carbons in Eo- and Paleoarchean metasedimentary rocks, significant advances have recently been made with the introduction of new micro-analytical techniques. Such tools have helped to expand databases and generate reproducible observations of samples from ancient sedimentary rocks. Such progress opens up new sub-fields in Precambrian micro-paleontology and nanobiogeochemistry and holds great promise to contribute to the current challenges of addressing the origin of graphitic carbons.

439 Table 1: list of suggested criteria for graphitic carbons that need to be satisfied in order to
440 assign a biogenic origin to graphitic carbons.

	Required criteria	**Cautionary notes**	**Techniques useful for assessment***
Geological context	Sedimentary nature of the protolith	Chemical sediments might have formed in multiple generations and under the seafloor or in the crust.	Field studies, geological, petrological and/or geochemical studies.
Petrographic context	Occurrences of graphitic carbons should be numerous within a single thin section and demonstrably indigenous. Morphological and textural structures may be important clues but may not be unambiguous.	Possible artefacts resulting from sample preparation and other sources of contamination should be identified and avoided.	Macroscopic observations, Optical microscopy, CLSM, Raman, SEM, EA.
Mineralogical context	Graphitic carbon occurrences should be included within the crystalline mineral matrix and not in pore spaces.	Occurrences associated with neoformed minerals, alteration and skarn phases should be investigated with special care.	Optical microscopy, Raman, FTIR, FIB-SEM, TEM-EDS.
Structural and microstructural characteristics	Proofs of syngeneity: the crystalline structure of the graphitic carbon is consistent with the peak metamorphic conditions experienced by the host rock.	Care is need for host rocks that have experienced a multi-phase geological history.	Raman, FTIR, STXM, TEM-based electron diffraction.
Isotopic composition	^{13}C-depleted carbon isotopic compositions consistent with biological origin, taking into account the possible metamorphic fractionation and re-equilibration of	Adequate standards should be used, and artefacts should be identified and avoided. Care is also needed as non-biogenic carbons	IRMS, SIMS, NanoSIMS.

	inorganic carbon phases.	may exhibit similar values.	
Elementary composition	Graphitic carbons of biogenic origin might still contain heteroatoms (O, N, P, S) as traces.	Contamination by recent carbon, if any, should be identified.	EA-IRMS, SIMS, NanoSIMS, ToF-SIMS.
Molecular Structure	Trace levels of molecular functional groups might remain present and be consistent with heteroatom content.	Their amount should be consistent with the thermal history of the host rock	STXM-based XANES, ToF-SIMS, GC-MS, NMR.
Reproducible Observations	Reproducible observations in other samples from other localities appear necessary.	Absence of evidence is not evidence of absence	

*List of abbreviations: IRMS is Isotope Ratio Mass Spectrometry, EA is Elemental Analysis, CLSM is Confocal Laser Scanning Microscopy, FIB is Focused Ion Beam, TEM is Transmission Electron Microscopy, EDS is Energy Dispersive Spectroscopy, SIMS is Secondary Ion Mass Spectrometry, ToF-SIMS is Time-of-Flight Secondary Ion Mass Spectrometry, NanoSIMS is Nanoscale Secondary Ion Mass Spectrometry, STXM is Scanning Transmission X-ray Microspectroscopy, GC-MS is Gas Chromatography – Mass Spectrometry, NMR is Nuclear Magnetic Resonance.

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FIGURE CAPTION

Figure 1*: Illustration of the possible evolution of a living organism after its death during diagenesis and metamorphism.*

Figure 2*: Photographs of Burgess shale graphitic soft-bodied fossils: a 5-centimeter long Opabinia specimen (**A**) and a 2-centimeter long Hallucigenia specimen (**B**). Source: Museum of Natural History, Smithsonian Institution (www.mnh.si.edu)*

Figure 3*: **A**: Reflected light photomicrograph of a fossilized megaspore found in Triassic metamorphic rocks from the western French Alps (blueschist facies - 360°C - 14 kbars). **B**: Corresponding Raman map showing the spatial distribution of graphitic carbon (blue), calcite (red) and ankerite (yellow). **C**: XANES spectra of graphitic carbons composing the wall of these objects and reference spectra of sporopollenin (the biopolymer that constitutes modern spores and pollen grains walls), lignin and graphite. The graphitic carbon composing the Vanoise megaspore wall is identified as degraded sporopollenin (modified from Bernard et al. 2007).*

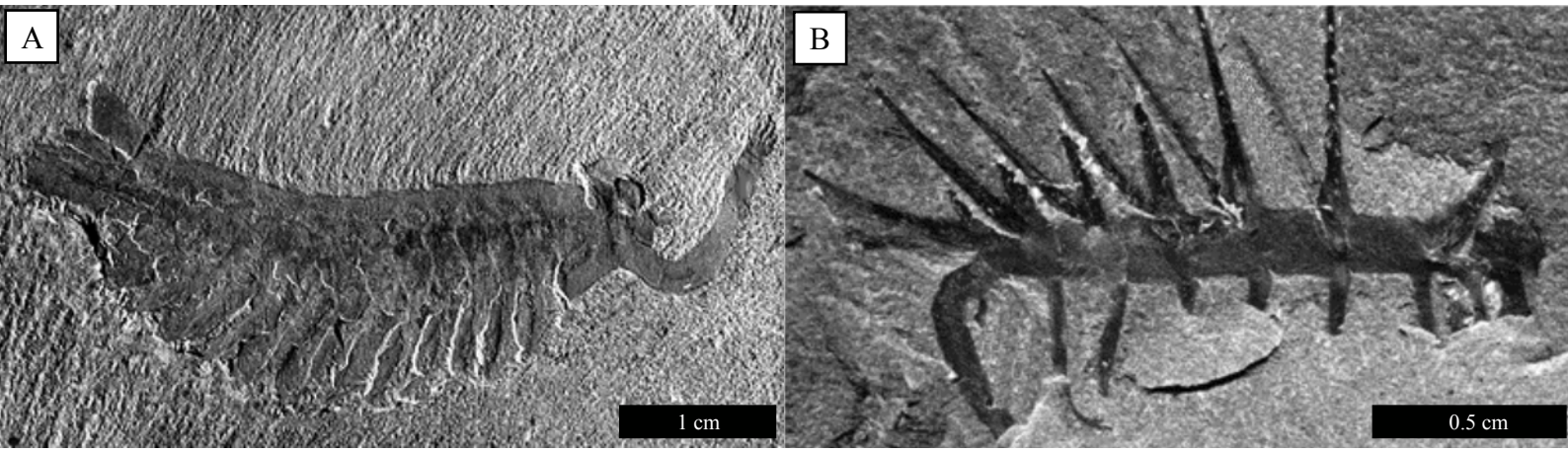
Figure 4*: Evolution of the XANES signal of lignin experimentally heat-treated under an inert Argon atmosphere at different temperature up to 1000°C for 90 minutes (**A**) and for different durations up to 900 minutes at 360°C (**B**). These data show a significant increase of aromaticity and a net loss of heteroatom-containing functional groups with increasing temperature and experimental duration.*

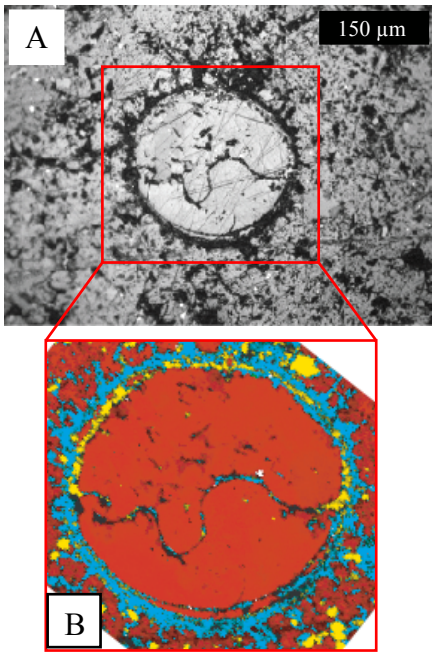
Figure 5*: **A-B**: Back-Scattered Electron images of graphite from the Eoarchean Akilia Quartz-pyroxene rock showing fluid-deposited graphite (gra)indicated by mineral associations with calcite (cal), magnetite (mag), chalcopyrite (ccp), pyrrhotite (po), and enveloped in hornblende (**A**) and graphite coating on apatite (ap), also possibly fluid-deposited (**B**). **C**: Crossed polar image of a globule of black graphitic carbon surrounded by a halo of finely disseminated brown organic matter and euhedral dolomite (red arrow) from a Paleoarchean chert in the Strelley Pool Formation. **D**: Small domal laminated stromatolites from the Strelley Pool Formation (coin is 28.5 mm for scale).*

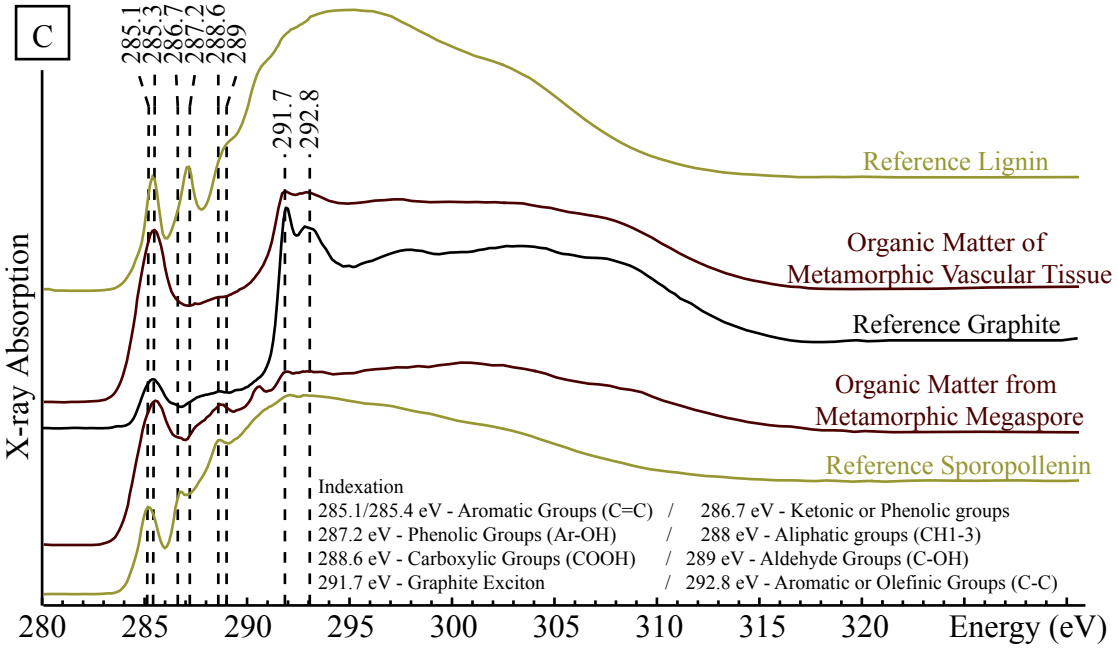
Living Organism

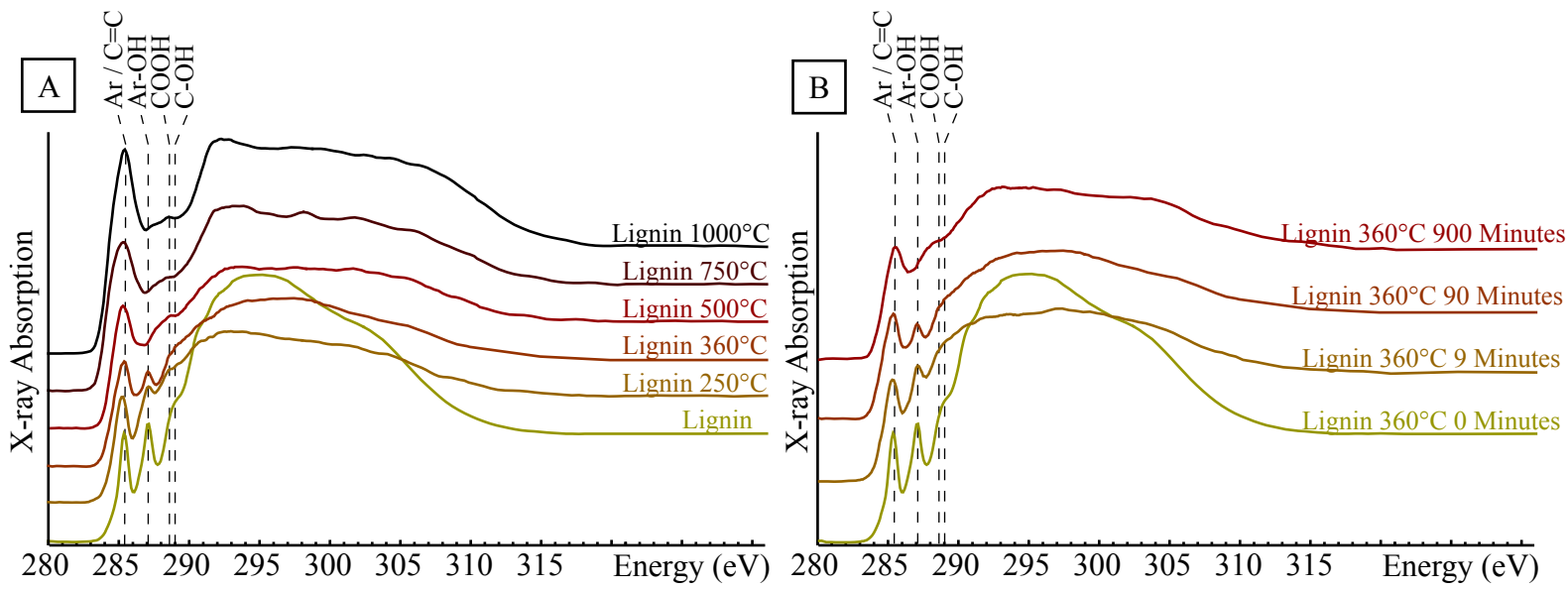
Sedimentary Rock

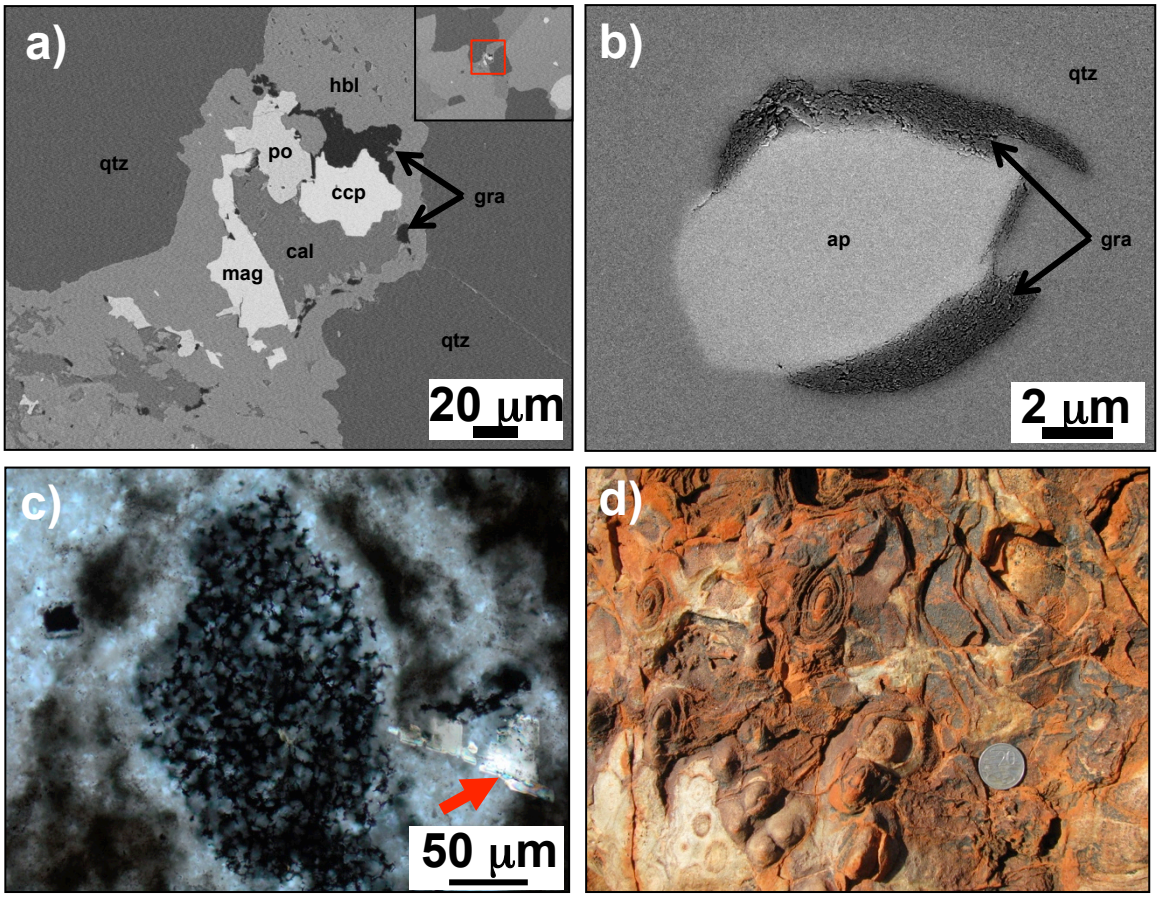
Metasedimentary Rock


A
1 cm
B
0.5 cm


A
150 µm
B


C
285.1
285.3
286.7
287.2
288.6
289
291.7
292.8
Reference Lignin
Organic Matter of
Metamorphic Vascular Tissue
Reference Graphite
Organic Matter from
Metamorphic Megaspore
Reference Sporopollenin
X-ray Absorption
Indexation
285.1/285.4 eV - Aromatic Groups (C=C) / 286.7 eV - Ketonic or Phenolic groups
287.2 eV - Phenolic Groups (Ar-OH) / 288 eV - Aliphatic groups (CH1-3)
288.6 eV - Carboxylic Groups (COOH) / 289 eV - Aldehyde Groups (C-OH)
291.7 eV - Graphite Exciton / 292.8 eV - Aromatic or Olefinic Groups (C-C)
280
285
290
295
300
305
310
315
320
Energy (eV)


A
Ar / C=C
Ar-OH
COOH
C-OH
X-ray Absorption
Lignin 1000°C
Lignin 750°C
Lignin 500°C
Lignin 360°C
Lignin 250°C
Lignin
280
285
290
295
300
305
310
315
Energy (eV)
B
Ar / C=C
Ar-OH
COOH
C-OH
X-ray Absorption
Lignin 360°C 900 Minutes
Lignin 360°C 90 Minutes
Lignin 360°C 9 Minutes
Lignin 360°C 0 Minutes
280
285
290
295
300
305
310
315
Energy (eV)



Revised caption: a-b) Back-Scattered Electron images of graphite from the Eoarchean Akilia Quartz-pyroxene rock showing a) fluid-deposited graphite (gra) indicated by mineral associations with calcite (cal), magnetite (mag), chalcopyrite (ccp), pyrrhotite (po), and enveloped in hornblende and b) graphite coating on apatite (ap), also possibly fluid-deposited. c) Crossed polar image of a globule of black graphitic carbon surrounded by a halo of finely disseminated brown organic matter and euhedral dolomite (red arrow) from a Paleoarchean chert in the Strelley Pool Formation. d) Small domal laminated stromatolites from the Strelley Pool Formation (coin is 28.5 mm for scale).