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THE SULFUR CYCLE ON THE EARLY EARTH: IMPLICATIONS FOR THE SEARCH OF LIFE ON EUROPA AND ELSEWHERE

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Abstract. The search for life in the universe especially on the Jovian satellite Europa could benefit from our knowledge of the bacterial processing of sulfur on the early Earth. We know that sulfate respiring bacteria reduce sulfur and produce large fractionation between its isotopes, especially ^{32}S and ^{34}S. The presence of sulfur patches on the Europan surface, as revealed by the Galileo mission and confirmed by the New Horizons, may have some astrobiological implications. In principle they could be related to sulfate-reducing bacteria and sulfur disproportionation on the ocean seafloor and its subsurface. The presence of pyrite in the oncolitic and stromatolitic laminae recorded from several Precambrian formations of the world reveal pyrite biomineralization in highly reducing conditions in the Archean and Proterozoic. A review of geological and biogeochemical data from the Precambrian demonstrates that both pyrite and evaporite formed biologically by dissimilatory sulfate reduction. In the present review we maintain that S-isotope analysis is a most valuable tool for the exploration of the Solar System. *In situ* analysis of the Europan surficial icy patches should be targets for the future exploration of the Jovian System by the future worldwide effort to explore the Jovian System.

1. Introduction

The possibility of life on Jovian satellite Europa and on Mars could be tested, based on the occurrence of biogenic chemical elements (C, H, O, N, S) on the early Earth (Chela Flores, 2006). We know that sulfate-respiring bacteria reduce sulfur and produce large fractionations between the ^{32}S and ^{34}S isotopes. The presence of sulfur patches on the surfaces of Europa and Mars (including rich concentration of sulfur in the Martian meteorites) may have implications in our search in our Solar System for biomarkers, both for the Galilean satellite and for the Red Planet. We discuss the role of microbial sulfur on the early Earth and its potential astrobiological significance. In the search for biomarkers new techniques and instrumentation are being developed. They will facilitate the interpretation of biomarkers. Firstly, our capability to infer the presence of past life on terrestrial planets such as Mars, by questioning what amount of rock is needed to distinguish past life from non-life (Schopf *et al.,* 2008). Secondly, new instrumentation are available that aims at distinguishing past life (or extant life) on the icy bodies of the outer solar system such as the Galilean satellites Europa, Ganymede and Enceladus in the Saturn System. The recent S isotope data from the 3.4 billion-year old (Ga) North Pole barite deposit in Australia provides the oldest evidence of microbial sulfate reduction.

It also demonstrates the presence of sulfate-reducing microbes in the early Archaean (Shen and Buick, 2004). S isotopic ratios provide valuable clues regarding the presence of sulfur-dependent metabolic activity on the early Earth. Archaean oceans at 3.5 Ga were sulfate rich and sulfides were formed by sulfate-reducing bacteria. S isotopic compositions of sulfides are enriched in ^{32}S and may be biogenic in origin. In geological history, the major deposits of stromatolitic phosphorites (with pyrite and oncolites) occur in the Precambrian–Cambrian boundary succession of the Asian-Pacific region of the world (Tewari, 1991). The Precambrian–Cambrian phosphorite–stromatolite association from the Tal Formation, Lesser Himalaya, India (and elsewhere) shows the presence of pyrite in the oncolitic and stromatolitic microlaminae. The pyrite follows the original biolamination pattern indicating conditions of a reducing palaeoenvironment (cf., Fig. 1 and Tewari, 1991).

A review of the geological and biochemical data from the Precambrian demonstrates that pyrites and evaporates were formed biologically by dissimilatory sulfate reduction. We conclude that S isotope analysis is most valuable for Solar System exploration. *In situ* analysis of the Europan surficial icy patches of sulfur, together with carbon isotopic signatures, will inevitably be targets for future space missions to the Jovian system, now in their planning stages (the worldwide collaboration for an Europa-Jupiter System Mission, EJSM).

The search for life in the universe, the possibility of life on Jovian satellite Europa and the planet Mars could be related to the occurrence of sulfur on early earth (Seckbach and Chela Flores, 2007, Chela Flores *et al.,* 2008). We know that sulphate respiring bacteria on earth reduce sulfur and produce large fractionations between the ^{32}S and ^{34}S isotopes. The presence of sulfur patches on the surface of Europa as well as on the Martian surface and a rich concentration of sulfur in the Martian meteorites has astrobiological implications regarding the existence of microbial life on these two most promising candidates for the search for extraterrestrial life in our solar system. We are discussing the possible role of microbial sulfur on earth and their significance in the origin of life and astrobiology. The Precambrian – Cambrian phosphorite – stromatolite association from the Tal Formation, Lesser Himalaya , India and elsewhere show the presence of pyrite in the oncolitic and stromatolitic microlaminae. The pyrite follows the original biolamination pattern indicating conditions of reducing palaeoenvironment (Tewari, 1991). A review of the geological and biochemical data from Precambrian demonstrates that pyrites and evaporates were formed biologically by dissimilatory sulfate reduction.

We conclude that S isotope analysis is the most valuable for planetary exploration. *In situ* analysis of the Europan surficial icy patches of sulfur together with carbon isotopic signatures will inevitably be targets for future space missions.

2. Earliest evidence of sulfate-reducing bacteria and their modern analogues

In the previous section we have mentioned the existence of evidence of sulfur isotope data from 3.47 Ga old North Pole Dome, Western Austraia. Microscopic pyrite associated with barite deposits from North Western Australia. This pyrite is the oldest evidence of microbial sulfate reduction on primitive earth. Organic carbon isotope data from the oldest metasediments 3.8 Ga old Isua complex is consistent with the existence of autotrophic CO_2 fiixation in to biomass (Schidlowski, 1983). During microbial sulfate reduction, the stable isotopes ^{32}S and ^{34}S are discriminated so that the daughter sulfides are isotopically fractionated with respect to the parent sulfate with the sulfides being depleted in ^{34}S. The oldest terrestrial S-isotopic records come from highly metamorphosed and deformed ferruginous rocks resembling banded iron formations from the Isua Supracrustal Belt, Greenland (3.8 Ga) which shows narrow range with a mean value of $+ 0.5 \pm 0.9\ {}^{0}/_{00}$ (Schidlowski *et al.,* 1983). However, sedimentary sulfides in the 2.7 Ga old iron formations of Canada are highly depleted in $\delta^{34}S$ values as low as $- 17.5\ {}^{0}/_{00}$. This indicates that microbial sulfate reduction must have evolved by 2.7 Ga. The modrn marine environments, sulfate reduction and pyrite formation occurs near the sediment surface where sulfur reduction rates are highest. The occurrence of pyrite and siderite in Archaean sedimentary rocks from Pilbara, Australia indicate that oxygen was less in the Archaean atmosphere (Rasmussen and Buick, 1999). The evolution of atmospheric oxygenation is linked to the Precambrian sulfur isotopic records. Shen and Buick (2004) have also interpreted that the stromatolites associated with the North Pole barite of Australia must have been formed by green and purple photoautotrophic sulfur oxidizing bacteria of the *Cholorobiaceae* and *Chromatiaceae*. Sulfate reduction is a complex process requiring advanced membrane bound transport enzymes, proton motive force generation through the activities of ATPase and other proteins involved in charge separation, and the genetic synthesis through DNA and RNA. The giant sulfur bacterium *Thiomargarita namibiensis* occurs in high biomass in surface sediments off the coast of Namibia (Schutz *et al.,* 2005). This bacterium gains energy by oxidizing sulfide, which accumulates in anoxic marine sediments as a result of the degradation of organic matter by sulfate reducing bacteria. Modern phosphorite formation has been reported from these sulfate-reducing bacteria.

3. Factors that are needed for the understanding the Precambrian sulfur cycle

A series of topics in Precambrian geology will be needed for gathering the insights that will orient us in the eventual search for other microbilly driven sulfutr cycls in the Solar System: Proterozoic–Cambrian sulfur isotopic ratios, pyrite formation in stromatolitic–Oncolitic–Phosphatic sedimentary environment and sulfide microbial mineralization. On the other hand, we recall that Ediacaran (Neoproterozoic) rifting resulted by the breakup of the supercontinent Rodinia around 750 – 690 Ma (Tewari, 2007, 2008) The development of the Krol basin in the Lesser Himalaya (Fig. 1) is associated with this event and the glacial diamictites (Blaini Formation) succession was deposited at the base of the basin which is correlated with the global Neoproterozoic glaciation (Marinoan/ Blainian, Tewari, 2001a,b, 2004, 2007, 2008). The overlying pink limestone is cap microbial carbonate with highly depleted carbon isotope ratios (Tewari and Sial, 2007). The Krol–Tal Ediacaran–Lower Cambrian carbonate–stromatolitic- phosphatic–oncolitic–pyritic beds are located at the Precambrian–Cambrian boundary in the Lesser

Himalaya as well as in the China, Mongolia, Oman and Iran (Brasier, 2002; Tewari, 1989, 1994, 1993, 1999, 2007; Goldberg *et al.,* 2005).

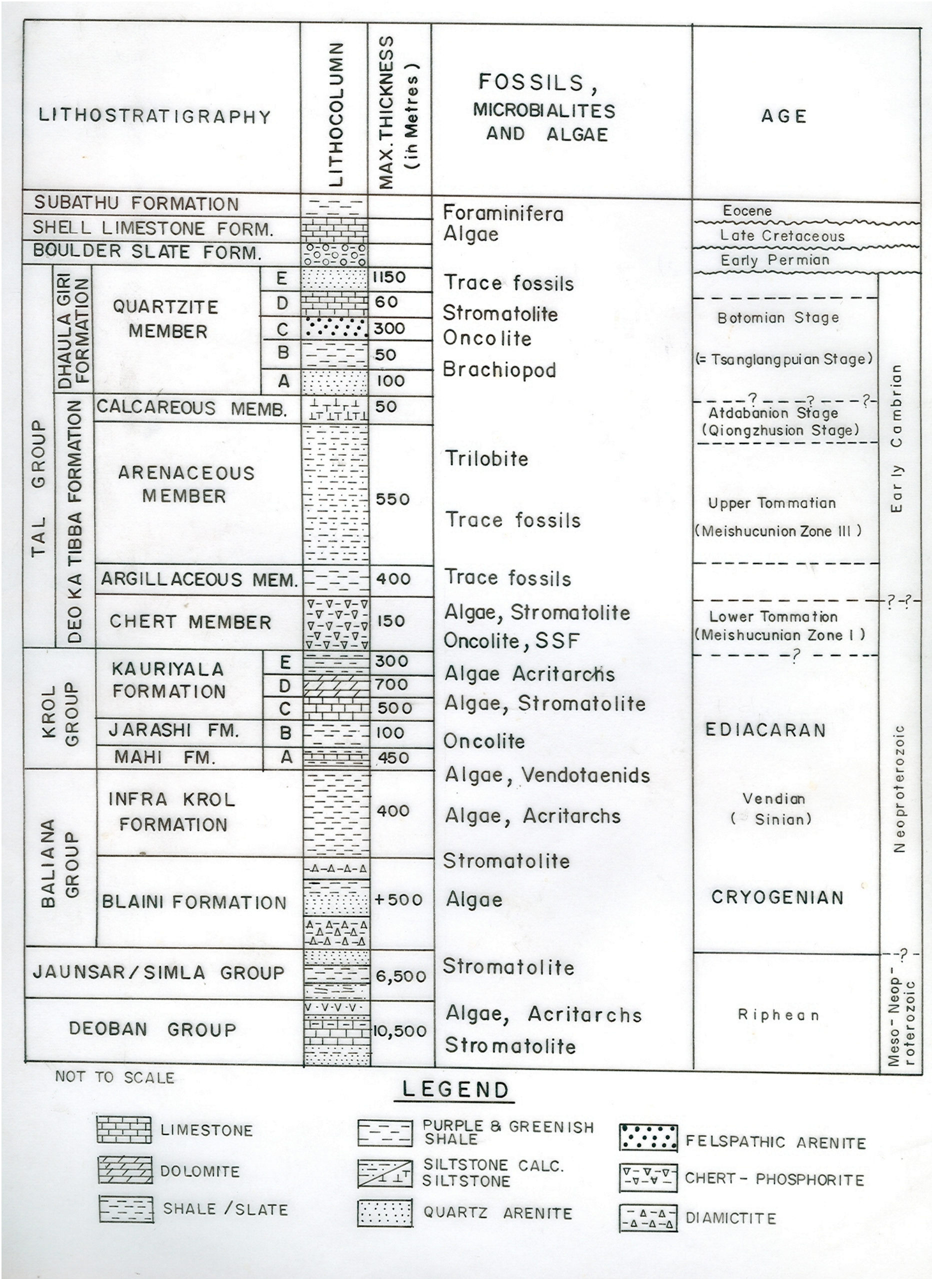

Figure 1. Precambrian – Cambrian Boundary stromatolitic , oncolitic, pyritic phosphatic beds at the contact of the Krol- Tal Formations, Lesser Himalaya, India represents global phosphogenic event (Tewari, 1991, 2007).

There was global oceanic anoxia near the Precambrian/Cambrian boundary. This was also the period when soft-bodied Ediacaran metazoans declined (extinction?) on earth and another biological diversification of Cambrian life took place. It is interesting that small shelly fauna (protoconodonts and conodonts, phosphatised oncolites and stromatolites having pyritic and phosphatic microlaminae and algae) were restricted to the reducing (non photosynthetic) environment, Figs. 2 – 4 and Tewari, 1984, 1989, 1994, 1996, 2004, 2007; Tewari and Qureshy, 1985).

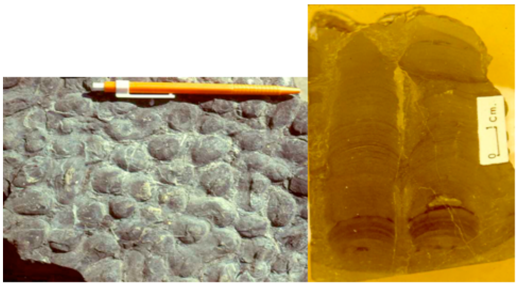

Figure 2. *Collumnaefacta vulgaris* stromatolite, Lower Cambrian, Lower Tal Formation, Lesser Himalaya, India showing the top view heads of the phosphatic pyritic stromatolite (left) and the columnar structure with dark black phosphatic laminae and golden yellow pyritic laminae.(Tewari, 1984, 1989, 1991).

Figure 3. Boxonia stromatolite from the Lower Cambrian Tal Formation, Lesser Himalaya, India showing development of pyrite (golden yellow color) in the stromatolitic laminae as well as in the intercolumnar area and the thick wall between the columns (Tewari, 1989).

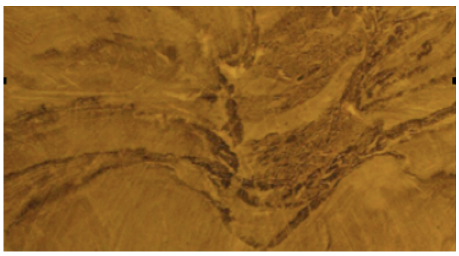

Figure 4. Phosphatic dark black laminae in the stromatolites of Meso- to Neoproterozoic Gangolihat Dolomite, Kumaon Lesser Himalaya, the intraclasts, pyritic pellets and oolites are found in the intercolumnar area (Tewari, 1989).

The global sulfur isotopic trends suggest a major increase in the importance of sulfate reducing bacteria with rising sulfate levels. The decreasing $\delta^{34}S$ values for sedimentary pyrite and increasing $\delta^{34}S$ values for sea water sulfate may be the result of widespread reduction under conditions similar to those of modern oceans (Lambert and Donnelly, 1992). The definitions of $\delta^{34}S$ are given in Section 4.

4. Bacterial sulfate reduction

Sulfur is an important element for all microorganisms, animals and plants on Earth. The main sources of sulfur are bacterial sulfate reduction, plants and soils. Sulfur isotopic ratios provide valuable clues regarding the presence of sulfur-based metabolic activity on the early earth. The redistribution of the primordial isotopic mixtures can be followed up in terms of the appropriate parameter, namely:

$$\delta^{34}S = [(^{34}S/^{32}S)_{sa} / (^{34}S/^{32}S)_{st} - 1] \times 10^{3} \ [^{0}/_{00}, \text{CDT}]$$

For simplicity this function will be referred to as the $\delta^{34}S$ parameter, or simply as the delta parameter. Its value is close to zero when the sample coincides with the corresponding value of the Canyon Diablo meteorite that is a triolite (FeS), abbreviated as CDT. This parameter allows a comparison of a sample (sa) with the standard (st) CDT. The relevant terms are the dominant sulfur isotope (^{32}S) and the next in abundance (^{34}S). In fact, $(^{34}S/^{32}S)_{st}$ coincides with the average terrestrial fraction of the two most abundant isotopes of sulfur. We obtain positive values of the delta-parameter when by comparison we have a larger quantity of the less abundant isotope ^{34}S.

Sulfur isotopic values of $\delta^{34}S$ for sulfide (pyrite) and sulfate (barite) minerals in the early Archaean display a relatively narrow spread around $\delta^{34}S = 0 \pm 3\ ^{0}/_{00}$ for sulfides and delta $^{34}S = 4 \pm 1\ ^{0}/_{00}$ for sulfates (Strauss, 2003). Archaean oceans at 3.5 billion years ago (Ga) were sulfate rich and sulfides were formed by sulfate reducing bacteria (Ohmoto, 1992). The average sulfate content of the mantle is about 300- 400 ppm (Gehlen, 1992). Sulfur isotope ratios in mantle sulfur are close to meteorites (delta $^{34}S = 0.5\ ^{0}/_{00}$). Sulfur isotopic compositions of sulfides are enriched in ^{32}S and may be of biogenic origin.The presence of pyrite in black shales, chert and phosphorite association in Proterozoic and Early Cambrian formations with $\delta^{34}S > +4\ ^{0}/_{00}$ indicate that sulfate and sulfide reducing bacteria were present in these depositional environments (Tewari, 1984, 1996, Krajewski *et al.,* 1994). Precambrian–Cambrian boundary black shale-pyrite, stromatolite and small shelly fauna indicate highly reducing palaeoenvironment in lagoonal facies, where sulfate reducing bacteria must have flourished (Tewari, 1984, 1994, 1996, Tewari and Qureshy, 1985). Neoproterozoic carbonates (1000 - 540 million years before the present) of the world are characterized by positive $\delta^{13}C$ values (Tewari and Sial, 2007, Tewari, 2007), where is defined as follows:

$$\delta^{13}C = [(^{13}C/^{12}C)_{sa} / (^{13}C/^{12}C)_{st} - 1] \times 10^{3} \ [^{0}/_{00}, \text{PDB}]$$

The value of $\delta^{13}C$ is close to zero when the sample coincides with the PeeDee belemnite standard (PDB) in which ($^{13}C/^{12}C$) = 88.99 and $\delta^{13}C$ is defined as equaling 0.00 $^{o}/_{oo}$. This parameter can be used as a good biosignature. On the Earth biota, for instance, there is ample evidence that photosynthetic bacteria, algae and plants have typical significant deviations that yield values of up to –30 and beyond, due to biological processes (Schidlowski *et al.,* 1983). But the main point that we have emphasized in the past is that negative values of the $\boldsymbol{\delta}^{13}C$ parameter do not arise exclusively from biogenic sources. For this reason we have mentioned in the present paper that sulfur is a better bioomarker for the study of possible biosignatures.

5. Sulfur patches on Europa: Is there evidence for biogenicity?

There are significant strategies for identifying those places where future landers could search for the biosignatures, such as the penetrators that are now being tested for the 2014 MoonLITE Mission (Smith *et al.,* 2008), and subsequently for Europa (Gowen *et al.,* 2009). The Jovian satellite Europa is the most appealing site for the discovery of extraterrestrial life in our cosmic neighbourhood. A key factor in this enterprise has already been provided by the discovery of sulphur patches on the icy surface of this satellite by the Galileo mission. The discovery is significant due to several additional measurements that strongly suggest the presence of an internal deep ocean, a potential habitat for extremophilic (cryophilic) microorganisms. The Galileo Near-Infrared Mapping Spectrometer (NIMS) evidence for the presence of sulphur compounds has been discussed in detail in our previous paper (Chela-Flores, 2006). The most likely sites would be where the salt deposits, or organics, are concentrated, as suggested by the NIMS data. For instance, the search for biosignatures could focus on the area north of the equatorial region, between 0 and 30 N and between the longitudes 240 and 270 (cf., McCord *et al.,* 1998, Fig. 2A). But a more intriguing and smaller patch would be the narrow band with high-concentration of non-ice elements that lies east of the Conamara Chaos, between the Belus and Asterius lineae, namely, between 18 - 20 N, and longitudes 198 - 202 (cf., McCord *et al.,* 1998, Fig. 2D).

Definite answers can be searched in situ on the icy surface with GC-MS instrumentation for the corresponding measurements with the help of biogeochemistry, especially with the $\delta^{34}S$ parameter. Measurements by mass spectrometry are needed. In a feasible mission to Europa they are possible as discussed earlier (Chela-Flores, 2006), due to miniaturized equipment that is already in existence.A specific example is provided by mass spectrometry on a possible future lander on Europa. At this stage it is possible to suggest the best possible landing site. We have suggested that at the 'patch' found in the Europan surface coordinates 200W, 20N (longitud and latitude, respectively, there is a scientific valid way of testing biogenicity through isotopic fractionation that may have occurred on sulphur patches on the Europan icy surface (Singer, 2003; Bhattacherjee, and Chela-Flores, 2004).

6. The Antarctic dry valley lakes: possible relevance in the search for biogenicity

The early stages of future missions may be initially tested on Earth, in environments that are similar to Europa, namely the dry valley lakes of southern Victoria Land of Antarctica (Doran *et al.* 1994; Parker *et al.* 1982; Priscu *et al.* 1999). One large lake lies underneath the Vostok Station, the Russian Antarctic base about 1,000 km from the South Pole. A lake, the size of Lake Michigan, was discovered beneath this Station in 1996 (Ellis-Evans and Wynn-Williams, 1996), after having drilled in that area since 1974. The lake lies under some 4 kilometres of ice. Lake Vostok, as it is known, may harbour a unique micro flora. The retrieval of biota from Lake Vostok will serve as a test for handling a larger aquatic medium, such as the proposed Europan submerged ocean that may be teeming with life.

At the time of writing the lake itself has not been sampled, prevented by the bioethical principles of planetary protection. On the other hand, in the dry valley lakes there is already a well-studied biota that consists of abundant microorganisms living underneath their iced surface. The estimated annual sulfur removal is over one hundred kilograms in the case of the Lake Chad in the dry valleys (Parker *et al.,* 1982). Thus, endogenic sulfur and other chemical elements will be, at any time, found on the icy surface of the dry valley lakes. These environments will help us to decide on the experiments that should be performed with the help of the forthcoming Europa missions, such as the above-mentioned EJSM mission that is being considered by the major space agencies (Grassett *et al.,* 2009).

7. Discussion and Conclusion.

Recently the significance of chemoautotrophs (microorganisms using inorganic or organic substances as energy source rather than light) has been discussed with special reference to the Jupiter's satellite Europa (Chela Flores, 2006). The search for presence of sulfur in the core of Europan ocean, its chemistry and sulfur reducing bacteria could be similar to the earth, especially in its early stages of geological evolution. They have emphasized that the sulfur patches on the icy surface of the Europa might contain biomarkers and should be aimed to study in future Europa mission for extraterrestrial life in the universe. We would like to restate the main conclusions of our previous work (Seckbach and Chela Flores, 2007, Tewari and Chela Flores, 2009). S isotope analysis is the most valuable for planetary exploration. *In situ* analysis of the Europan surficial patches of sulfur, together with carbon signatures could yield a clearer interpretation of biosignatures.

In the present paper we have indicated that geological and biogeochemical data from many sources of the Archaean and Proterozoic demonstrate that pyrites and evaporates were formed biologically by dissimilatory sulfate reduction (Shen and Buick, 2004, Schidlowski *et al*., 1983, Konhauser, 2007). Rocks of Archean age [older than 2.5 Gyr BP] provide the best evidence of early metabolic processes. Their study allows reconstruction of the biogeochemical cycle for sulfur since the origin of life on Earth. The remarkable will inevitably be targets for future space missions that are expected to return to Europa in the next decade.

With landers, or low-cost penetrators that could first of all be tried out on the Lunar surface (Smith *et al*., 2008), we would be in a position to test the redox state of the Europan ocean. Alternatively, the imprint of the possibly biogenic signature of the surficial sulfur would be retained in the dust cloud that surrounds this singular Jovian satellite (Kruger *et al.,* 2003). The arguments in the present paper continue to point towards mass spectrometry as the principal instrumentation for future probing of the Europan patches, either in orbit, or with penetrators.

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