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Sulfur isotopes as biosignatures for Mars and Europa exploration

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Abstract: Sulfur (S) isotopes are used to trace metabolic pathways associated with biological S-cycling in past and present environments on Earth. These pathways (sulfate reduction, sulfur disproportionation and sulfide oxidation) can produce unique S isotope signals that provide insight into biogeochemical S-cycling. The S cycle is also relevant for extraterrestrial environments and processes. On early Mars, sulfur existed in different redox states and was involved in a large range of surface processes (e.g., volcanic, atmospheric, hydrothermal and aqueous brines). Sulfur compounds have also been detected on Europa’s icy moon surface, with the S cycle implicated in Europa’s surface and ocean geochemistry. Given the well-established utility of S isotopes in providing a record for past life on Earth, S isotopes are a valuable tool for identifying biosignatures on Mars and Europa. Here, we review S isotopes as a biosignature, in light of two recent advances in understanding the S cycle in both Mars and Europa: (1) the measurements of $\delta^{34}S$ in situ at Gale Crater and quadruple S isotopes (QSI) in Martian meteorites; (2) the identification of a likely exogenous origin of sulfur on Europa’s surface. We discuss important considerations for unravelling QSI biosignatures in Martian environments, considering high- and low-sulfur environments, atmospheric mass-independent fractionation of sulfur isotopes (S-MIF) signals and metabolic energy-limited niches. For Europa, we describe the potential for S isotopes to probe biogeochemistry, and identify key knowledge gaps to be addressed to unlock S isotopic tools for future life detection efforts. The resulting picture demonstrates how S isotopes will be a valuable tool for Mars sample return, and how future missions can focus on the search for environments where QSI signatures of microbial S-cycling processes have a greater chance of being preserved. For Europa, the first step will be to account for the S isotope composition of the various S pools, to recognize or rule out non-biologically mediated S isotope values, with a focus on experimental examination of potential S isotope signatures from exogenous sulfur sources.

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Sulfur (S) is abundant in the Solar System, and speciates into geochemically extensive reservoirs on Earth, Mars and the Jovian moons Europa, Io, Ganymede and Calisto (Carlson et al. 1999; King et al. 2004; Jessup et al. 2007; King and McLennan 2010). Sulfur is a widely utilized source of energy for chemotrophic microbial metabolisms, owing to its range of redox states. These redox states allow microorganisms to perform (1) dissimilatory microbial sulfate reduction (MSR), (2) microbial sulfur disproportionation of intermediate S compounds (MSD) and (3) oxidation of sulfide and reduced sulfur species (SO) (Fig. 1). Each of these metabolic processes produces a characteristic, but not unique, change in the S isotope values of the associated compounds within the reaction. When these values are preserved in the rock record they can provide evidence for past biological activity. Here, we review S isotope biosignatures within the context of S-rich environments on Mars and Europa, and consider how to distinguish S isotope biosignatures from abiotic signatures concurrently preserved in geological deposits. We identify knowledge gaps to be addressed to interpret future isotopic measurements of sulfur phases from these and similar planetary environments, and evaluate the feasibility of interpreting in situ measurements versus samples from return missions.

$S$ isotope biosignatures on Earth

Sulfur (S) has four stable isotopes, with masses 32, 33, 34 and 36, and is one of the most abundant elements on Earth. The $^{34}S$ isotope composition of sulfur species is presented using the standard delta ($\delta$) notation, expressed in permil ($\permil$):

$$\delta^{34}S = \left[ \frac{^{34}S_{\text{SAMPLE}}}{^{34}S_{\text{VCDT}}} \right] - 1$$

where $3x$ is 32, 33, 34 or 36, and $^{34}S_{\text{SAMPLE}}/^{34}S_{\text{VCDT}}$ is the isotopic ratio of a sample ($^{34}S$) relative to the standard, VCDT. Sulfur isotopes are influenced by biological and abiotic processes, and can be preserved in the rock record (e.g. Canfield 2001a, and references therein; Havig et al. 2011, 2017; Johnston 2011; Fike et al. 2015). Effects on S isotopes produced by microbial processes, notably MSR and oxidative sulfur (re)cycling, have been used to reconstruct the evolution of S-based metabolisms and trace the oxygenation of Earth’s surface through time (Canfield and Teske 1996; Scott et al. 2008; Luo et al. 2016). The largest S isotope fractionation effects are generally produced by MSR, although oxidative recycling of sulfur species can increase these fractionations further (e.g. Canfield 2001a, b).

Enzymatic reactions performed by S-utilizing microorganisms control the S isotope composition of S-bearing biomolecules produced during assimilatory processes (e.g. cysteine and methionine), and inorganic S compounds produced during dissimilatory processes (e.g. sulfate and sulfide). Enzymes perform reactions that happen at faster rates than if they were carried out abiotically by lowering the activation energy. The products (P) of these enzymatic reactions are typically depleted in the heavier $S$ isotopes relative to the reactants (R), with the magnitude of the differences in isotopic
compositions between the reactant and the product the result of the isotopic discrimination that happens during the multistep enzymatic reaction (Chambers et al. 1975; Fry et al. 1985; Canfield and Thamdrup 1994; Canfield 2001a; Brunner and Bernasconi 2005). We express the magnitude of this discrimination, also called a ‘isotope effect’, as

$$34\epsilon_{R,P} = (\alpha_{R,P} - 1) \times 1000$$

where $$\alpha_{R,P} = (\delta_{34}S_P + 1000)/(\delta_{34}S_R + 1000)$$ can help to identify the specific enzymatic reaction mechanisms responsible (Canfield and Teske 1996; Shen and Buick 2004; Johnston 2005; Philippot et al. 2007; Leavitt et al. 2013). Where the S isotope values of both products and reactants can be measured, isotope effects between these species can provide evidence for biological activity (biosignatures) and clues to the biogeochemical S cycle during the time of their formation (Fike et al. 2015, and references therein). MSR and MSD in particular produce H$_2$S depleted in $^{34}$S, captured as pyrite and Fe-monosulfides with distinctively negative $\delta^{34}$S values (e.g.Habicht and Canfield 2001; Fike and Grotzinger 2008; Johnston et al. 2008). (Re)oxidation of H$_2$S generally produces much smaller fractionations in $\delta^{34}$S, but can further alter the minor S isotope composition ($^{33}$S and $^{36}$S) of these S compounds (e.g. Zerkle et al. 2009, 2016). Thus, the S isotope values of sedimentary pyrite and sulfate minerals can preserve evidence for these S-cycling metabolisms in past environments.

Fig. 1. Schematic representation of S redox transformations and resulting fractionations in $\delta^{34}$S by biotic and abiotic process. Fractionations are expressed as $34\epsilon_{R,P}$, where R is reactant and P is product. Green lines reflect microbial S-cycling processes, including microbial sulfate reduction (MSR; Canfield et al. 2010; Sim et al. 2011a), microbial sulfur disproportionation (MSD; Canfield and Thamdrup 1996) and sulfur oxidation (SO; Zerkle et al. 2016; Pellerin et al. 2019). For SO, fractionations are summarized for the range of S oxidation pathways between reduced sulfur species (redS) and oxidized sulfur species (oxS). MSD can utilize a number of intermediate sulfur species ($S^0$, $SO_3^{2-}$ or $S_2O_3^{2-}$) to produce both sulfate and sulfide (e.g. Frederiksen and Finster 2003). Dashed brown lines reflect abiotic S-cycling processes, including abiotic sulfur oxidation (Fry et al. 1988; Eldridge and Fanqhar 2018; Eldridge et al. 2021) and thermochemical sulfate reduction (TSR), which happens at temperatures between 80 and 200°C (Machel et al. 1995). The dashed purple line represents the range of $34\epsilon_{R,P}$ measured at Gale Crater (Franz et al. 2017).
**Microbial sulfate reduction (MSR)**

Organisms that perform microbial sulfate reduction (MSR) are taxonomically and metabolically diverse, encompassing both archaea and bacteria capable of heterotrophy and/or autotrophy under anaerobic conditions (Castro et al. 2000; Plugge et al. 2011; Anantharaman et al. 2018). MSR in pure and enrichment cultures can produce S isotope fractionations larger than ~65% between reactant SO$_4^{2-}$ and product H$_2$S (Canfield et al. 2010; Sim et al. 2011a). Sulfur isotope fractionations produced during MSR are influenced by several environmental parameters, such as SO$_4^{2-}$, Fe and NH$_4^+$ concentrations, type and abundance of electron donors, and temperature (Fig. 2) (e.g. Detmers et al. 2001; Hoek et al. 2006; Sim et al. 2011b, 2012; Sim 2012).

Variations in SO$_4^{2-}$ concentration play a major role in the S isotope fractionations produced during MSR, with larger S isotope effects generally associated with higher SO$_4^{2-}$ concentrations (Habicht and Canfield 1997). Moreover, S isotope fractionations during MSR have been shown to be dependent on strain-specific physiological parameters, such as affinity for SO$_4^{2-}$ and electron donors (Bradley et al. 2016). MSR tends to produce larger S isotope fractionations when growing heterotrophically using organic electron donors than when growing autotrophically with H$_2$ (Fig. 2: 6-44‰, Sim et al. 2011b). In addition, when MSR is limited by low concentrations of electron donors, the S isotope fractionations produced are generally larger (Chambers et al. 1975; Hoek et al. 2006; Sim et al. 2011b).

Concentrations of nutrients such as Fe and NH$_4^+$ can also indirectly control the extent of S isotope fractionation during MSR, through their influence on metabolic rates (Sim et al. 2012). Cell-specific sulfate reduction rates (csSRR, generally expressed as moles of SO$_4^{2-}$ reduced/cell/time) respond to environmental parameters such as SO$_4^{2-}$ concentration and source of electron donors, and the largest S isotope fractionations are generally produced at the lowest csSRR (Kaplan and Rittenberg 1964; Chambers et al. 1975; Habicht and Canfield 1997). Similarly, nutrient limitation can reduce overall growth rates, causing a decrease in csSRR and subsequently larger S isotope fractionations.

**Sulfur oxidation (SO)**

Sulfur oxidation (SO) has a broad taxonomic distribution, being present in members of archaeal and bacterial phyla (Ghosh and Dam 2021). It is not known if pH directly affects S isotope fractionations during MSR. Indirectly, pH and O$_2$ concentrations determine aqueous speciation, and therefore Fe and SO$_4^{2-}$ solubility, which can each affect S isotope fractionations as described above. This mechanism was recently suggested to be important in controlling S isotope fractionations observed in Icelandic hydrothermal systems spanning different pH and Fe concentrations (Moreras-Marti et al. 2021a).

More generally, the combined metabolic energy limitation within an environment can have an impact on S isotope fractionations through changing csSRR. A general decrease in microbial metabolic rates has been observed in environments with limited electron donors, termed energy-limited or low-energy environments (Hoehler and Jørgensen 2013; Bowles et al. 2014; Jørgensen and Marshall 2016; Wenk et al. 2018). These metabolic energy-limited niches are widespread on Earth, and include the subsurface of intertidal mudflats, coastal environments, continental shelves, deep-sea sediments and euvnic water columns. Organisms that inhabit these environments have adapted to use electron carriers with modest negative reduction potentials, requiring less energy to grow (Wenk et al. 2018). The large S isotope fractionations associated with metabolic energy-limited niches have also been observed in cold hypersaline habitats (Moreras-Marti et al. 2021b).

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*Fig. 2. Main environmental controls on S isotope fractionation during MSR, modified from Fike et al. (2009) with updated parameters. Arrows indicate the magnitude of the fractionation effect (larger or smaller) for the indicated parameter. Parameters denoted with an asterisk (*) demonstrate strain-specific behaviour, including SO$_4^{2-}$ concentrations, temperature and cell-specific sulfate reduction rates (csSRR). Based on measured changes in S isotope fractionation effects at differing SO$_4^{2-}$ concentrations (Canfield 2001a, b; Habicht et al. 2002; Bradley et al. 2016), substrate effects (Hoeck et al. 2006; Sim et al. 2011b, 2012), temperature (Canfield et al. 2006; Hoek et al. 2006), increasing csSRR (Chambers et al. 1975; Habicht and Canfield 1997; Sim et al. 2011b; Sim 2012), Fe and NH$_4^+$ limitation (Sim et al. 2012) and metabolic energy limitation (Wenk et al. 2018).*
Organisms that perform SO vary in terms of oxygen tolerance (facultative anaerobes or obligate aerobes) and strategies for carbon and energy acquisition (photoautotrophic, chemoautotrophic or chemoheterotrophic) (Sorokin 2003; Ghosh and Dam 2009). Oxidative S-cycling proceeds via a variety of biological pathways in the environment, along with the abiotic pathways described below. The chemolithotrophic oxidation of reduced H$_2$S or S$^0$ can be coupled to the reduction of O$_2$ or NO$_3^−$ for energy gain (Friedrich et al. 2001) (Fig. 1). Sulfur isotope fractionations produced during chemolithotrophic SO are smaller than MSR and highly variable, ranging from −6 to +12‰ between H$_2$S and SO$_4^{2−}$ or S$^0$ (Fry et al. 1988; Zerkle et al. 2016; Pellerin et al. 2019). The environmental parameters affecting S isotope fractionations during chemolithotrophic SO appear to be related to electron donor and acceptor availability, with larger fractionations linked to lower H$_2$S/ O$_2$ ratios (Zerkle et al. 2016).

Anoxicogenic phototrophy can oxidize reduced S compounds. In addition to sulfide, these organisms can oxidize bioavailable intermediate S compounds including thiosulfate (S$_2$O$_3^{2−}$), sulfite (SO$_3^{2−}$) and elemental sulfur (S$^0$), although only S$^0$ is generally stable enough to build up in natural environments (Troelsen and Jorgensen 1982; Jorgensen 1990). Sulfur isotope effects during phototrophic SO are also small but highly variable, ranging from −5 to +5%, depending on the S compounds reduced and the resulting products (e.g. Zerkle et al. 2009).

**Microbial sulfur disproportionation (MSD)**

In addition to MSR and SO, some S-cycling microorganisms are capable of sulfur disproportionation reactions (MSD) (Bak and Pfennig 1987). MSD can utilize a number of intermediate sulfur species (S$^0$, SO$_3^{2−}$ or S$_2$O$_3^{2−}$) to produce both sulfate and sulfide (e.g. Frederiksen and Finster 2003). The disproportionation of S$_2$O$_3^{2−}$ or S$^0$ is performed anaerobically, assimilating carbon from CO$_2$ or acetate (Widdel and Pfennig 1977). For S$^0$ disproportionation to be thermodynamically feasible in an environment, sulfide concentrations must be less than c. 1 mM (Thamdrup et al. 1993). Moderate S isotope fractions are associated with MSD, related to the recycling of reduced S components during metabolic processes (reviewed by Canfield 2001a). Fractionations up to −34‰ have been measured on H$_2$S and SO$_4^{2−}$ during S$^0$ disproportionation (disproportionation of S$^0$ to H$_2$S and SO$_4^{2−}$) by pure cultures at the cellular level (Habicht 1997).

**Minor S isotope biosignatures: $\Delta^{33}S$ and $\Delta^{36}S$ biosignatures**

Minor S isotopes ($^{33}S$ and $^{36}S$), expressed as $\Delta^{33}S = \delta^{33}S - [(^{34}R_{sample/^{34}R_{CDT})^{0.515}} - 1]$ and $\Delta^{36}S = \delta^{36}S - [(^{34}R_{sample/^{34}R_{CDT})^{1.90}} - 1]$, have further contributed to our understanding of S-cycling processes on the early Earth (Farquhar et al. 2000a, b; Farquhar and Wing 2003). Following this notation, the exponents 0.515 ($^{33}S$) and 1.90 ($^{36}S$) represent reference values that approximate mass-dependent fractionations during thermodynamic equilibrium isotope exchange at low temperature (Hulston and Thode 1965; Farquhar and Wing 2003; Johnston et al. 2007). Deviations from these reference values occur in biological systems owing to a linear dependence of isotope ratios during redistribution of mass at the cellular or ecosystem level (e.g. via mixing or Rayleigh processes), which deviate from the predicted exponential relationship (Farquhar et al. 2003, 2007a; Johnston 2005; Johnston et al. 2007). These deviations result in small magnitude anomalies in $\Delta^{33}S$ and $\Delta^{36}S$, termed mass conservation effects, that can be preserved in rocks and sediments alongside $\delta^{34}S$ (e.g. Johnston et al. 2008).

Experimental studies have shown that each of the biological S metabolisms discussed above produce characteristic minor isotope patterns, resulting from the individual steps controlling the sulfur flowing through the metabolic pathways (Fig. 3; Zerkle et al. 2016). MSR in pure cultures has been observed to produce $^{33}\lambda$ values between 0.5077 and 0.5125, MSD produces higher $^{33}\lambda$ values ranging from 0.5145 to 0.5187 (Johnston 2005; Johnston et al. 2007) and chemolithotrophic SO produces $^{33}\lambda$ values ranging from 0.513 to 0.515 (Pellerin et al. 2015, 2019; Zerkle et al. 2016). These differences in the minor isotopes can help to decipher complex environmental isotope records, where MSR, MSD and SO can co-occur, producing $^{33}\delta$ values that overlap. For example, biological variations have been shown to produce distinctive $^{33}\delta$ values in both hydrothermal and hypersaline Mars analogue environments, even when $^{33}\delta$ values are small or indistinguishable (Moreras-Marti et al. 2021b).

**Fig. 3. $^{33}\delta$ v. $^{33}\Delta$S systematics for H$_2$S and S$^0$ produced by different sulfur cycling metabolisms. MSD pure cultures from Johnston (2005); MSR from Sim et al. (2011b) and phototrophic SO from Zerkle et al. (2009). Natural systems: MSR from euxinic lake from Canfield et al. (2010); MSR + MSD also from euxinic lake from Zerkle et al. (2010).**
Abiotic fractionation of $S$ isotopes

Abiotic processes act alongside biology to cycle sulfur in natural systems. Therefore, to use $S$ isotopes as a biosignature for $S$-cycling metabolisms in extra-terrestrial environments, it is important to examine any abiotic processes that could modify or mask the biogenic $S$ isotopic values. Sulfate can be reduced abiotically by thermochromic sulfate reduction (TSR), requiring high temperatures (80–200°C) and organic matter. TSR produces $\delta^{34}S$ fractionations of up to $\sim20\%$ between reactant sulfate and product sulfide (Machel et al. 1995). Sulfide can also be oxidized abiotically by reacting with Fe (III) or Mn (IV), or by rapid reaction with molecular oxygen, producing mean fractionations in $\delta^{34}S$ of $\sim5.5\%$ between $H_2S$ and intermediate $S$ products (Fry et al. 1988; Eldridge and Farquhar 2018; Eldridge et al. 2021). Small pH-dependent equilibrium isotope fractionations have also been measured between aqueous and gaseous $H_2S$, with a fractionation of $\sim1\%$ at pH $<6$ and $+2\%$ at pH 8 (Sim et al. 2019). Theoretical calculations predict that abiotic equilibrium isotope effects in $^{34}S$-$^{32}S$ between sulfur-bearing species can be as large as 58%. These types of effects are rarely seen at Earth’s surface but have been hypothesized to contribute to the $\delta^{34}S$ values measured at Gale Crater (Franz et al. 2017).

Abiotic $S$-cycling processes generally produce negligible changes in minor $S$ isotopes (e.g. Johnston 2011; Eldridge and Farquhar 2018; Eldridge et al. 2021). Two exceptions are TSR, which can induce large $\Delta^{34}S$ values up to $+13\%$ via magnetic isotope effects (Oduro et al. 2011), and mass-independent fractionation of sulfur isotopes (S-MIF) that occurs via gas-phase reactions during atmospheric sulfur photochemistry. S-MIF signatures are commonly observed in sulfide and sulfate minerals in sedimentary rocks older than 2.4 Ga, and are attributed to the interaction of ultraviolet (UV) photons with $SO_3$ and other sulfur gases in the absence of an ozone layer (e.g. Farquhar et al. 2000b). Biomass burning has also been shown to impart small-magnitude S-MIF, with $\Delta^{34}S$ values as low as $-0.19\%$ observed in combustion experiments and associated aerosols (Lee et al. 2002; Shaheen et al. 2014; Lin et al. 2018); however, organic sulfur was very unlikely have been extensive on early Mars. S-MIF signatures are also associated with large stratospheric eruptions (e.g. Crick et al. 2021) and volatilization of massive amounts of sulfur during the end-Cretaceous meteorite impact (Junium et al. 2022), and have previously been measured in Martian meteorites (e.g. Franz et al. 2014). We explore the implications for these abiotic fractionation mechanisms in altering $S$ isotope biosignatures below.

Sulfur isotope biosignatures on Mars

Sulfur cycling on Mars

Sulfur is one of the most abundant elements on the Martian surface (Baird et al. 1976; King and McLennan 2010; Franz et al. 2019a). The main sulfur oxidation states found on Mars are: $S^{2-}$ (pyrrhotite, $FeS_2$), $S^{2-}$ (e.g. pyrite, $FeS_2$), $S^{4-}$ ($SO_4$-$g$), $SO_3^{2-}$, $SO_2^{2-}$-bisulfite), $S^0$ ($SO_2$, $SO_2^{2-}$-sulfate, $SO_2^{3-}$ sulfone), $S^+$ and $S^{2+}$ ($S_2^+$) (Franz et al. 2019a, and references therein), with $S^{2+}$ likely to be dominant (King and McLennan 2010). Reduced sulfur is found in primary igneous phases from Martian meteorites, but also in the Martian sediments at Gale Crater (McAdam et al. 2014, 2020; Franz et al. 2017; Wong et al. 2020). In meteorites, the reduced S is mainly in the form of sulfides, including some reduced Fe-S minerals (e.g. pyrite and pyrrhotite) (Meyer 2012, and references therein). High concentrations of sulfur on the Martian surface are evident through observations and measurements of high $SO_3$ concentrations in the Martian soil, including 5.12 wt% at Gale Crater (Berger et al. 2016) and 6.16 wt% in average Martian soil (Taylor and McLennan 2009; Berger et al. 2016). This abundance of surface sulfur species on Mars ultimately derives from an S-rich mantle. Measurements of Martian meteorites suggest the presence of high sulfur concentrations in the Martian interior, with average concentrations of 6 wt% $SO_3$ found in shergottites, which are Martian basaltic meteorites (Meyer 2012). The prolific volcanism that extended from the Noachian to Hesperian led to significant amounts of outgassing to the surface (Fig. 4) (Haley et al. 2007; Righter et al. 2009; King and McLennan 2010). The outgassing involved a large injection of sulfur gases ($H_2S$ and $SO_3$) into the atmosphere (Settle 1979; Tian et al. 2010; Gaillard et al. 2013). Mass-independent fractionation of $S$ isotopes (S-MIF) from $SO_2$ and $H_2S$ measured in Martian meteorites suggests that atmospheric photochemistry was a key influence on the early Martian $S$ cycle (Farquhar et al. 2000a; Franz et al. 2014). Volcanic $H_2S$ released into the atmosphere would have photo-oxidized to $SO_2$, where $SO_2$ was then either precipitated or further photo-dissociated, further deposited on the Martian surface with a distinctive S-MIF signal (Franz et al. 2014). It is still unknown how S-MIF signals on the Martian surface were affected by environmental factors, or if they have regional or local variations, providing a target for future analysis. Both hydrothermal circulation and meteorite impacts into S-rich sediments could have provided an active geochemical cycle that could have subsequently homogenized the S-MIF signal on the Martian surface (Fig. 4). The delivery of dust into the atmosphere could have also played a role in homogenizing S-MIF between the atmospheric sulfur species and surface sulfates (Farquhar et al. 2000a).

The Sample Analysis at Mars (SAM) instrument on board the Curiosity rover detected $SO_2$, $H_2S$, OCS and $CS_2$ at Gale Crater. The $SO_2$ is probably thermally derived from $Fe$ sulfates, $Ca$ sulfites, oxidation of sulfide (e.g. pyrite or pyrrhotite) (McAdam et al. 2014), or from Mg sulfates (Sutter et al. 2017; McAdam et al. 2020). The OCS and $CS_2$ are probably a product of a reaction involving reduced sulfur and some C source (McAdam et al. 2014; Wong et al. 2020). Sulfide minerals at Gale Crater have been hypothesized to be the product of hydrothermalism and groundwater transport (Franz et al. 2017; Wong et al. 2020).

Sulfur-rich habitats on early Mars

The presence of sulfur species and suitable electron donors and acceptors, including organic C (Eigenbrode et al. 2018), $NO_3^−$ (Stern et al. 2015), $H_2$ (Feldman et al. 2004b), $Fe^{2+}$ and $Fe^{3+}$ (Bain et al. 1993; Morris et al. 2008), on Mars raises the question of whether a Martian sulfur biogeochemical cycle has ever been viable. Both SO and MSR have been proposed as feasible metabolisms under Martian chemical conditions, based on the chemistry of the Martian regolith (Nixon et al. 2013), Mars simulation experiments (Denson et al. 2009; Oliver et al. 2022), Gibbs free energy calculations (Macey et al. 2020; Ramkissoon et al. 2021) and their detection in a range of Mars analogue environments (Perreault et al. 2007; Lay et al. 2013; Pontefract et al. 2017; Cousins et al. 2018; Singh et al. 2019; Macey et al. 2020; Moreras-Martí et al. 2021a, b). The widespread presence of sulfur species would have created several potentially habitable S-rich environments on the Martian surface with different sulfur species available for S metabolic reactions. Two systems of particular relevance are (1) hydrothermal systems accumulating reduced and oxidized species delivered directly from volcanism and (2) evaporative systems accumulating oxidized species from both volcanism and atmospheric deposition. These two types of environments were widespread on early Mars and could have supported a Martian biogeochemical $S$ cycle in a habitat space conducive to biology with regard to other factors (e.g. water activity and availability of other bio-essential elements). These are discussed below.
During the Noachian–Hesperian transition, large amounts of heat were released through endogenic volcanic activity, directly linked to the formation of localized hydrothermal systems (Gulick 1998; Abramov and Kring 2005; Osinski et al. 2013). Exogenic impacts also formed hydrothermal systems within the impact craters themselves (Rathbun and Squyres 2002; Abramov and Kring 2005; Schwenzer et al. 2012). Evidence for hydrothermal systems can be found both on the surface (e.g. Rathbun and Squyres 2002; Ojha et al. 2021) and within the subsurface (Ehlmann et al. 2011).

One example is the relict surficial hydrothermal system at Home Plate (Columbia Hills, Gusev Crater), explored by the MER-A Spirit rover (Squyres et al. 2008). Here, opaline silica associated with volcanic material indicates past hydrothermal activity (Ruff et al. 2007, 2020; Ruff and Farmer 2016a, b). The nearby Columbia Hills have sulfur-rich soils attributed to fumarolic activity of Home Plate (Squyres et al. 2008). A contrasting example of Martian hydrothermal activity can be found in the Eridania Basin, where the presence of both chloride evaporites and hydrothermal alteration minerals points towards the existence of a seafloor hydrothermal system (Michalski et al. 2017). Endogenic Martian hydrothermalism has also been linked to glacial surface deposits, such as Arista Mons (Scanlon and Head 2014) and Sisyphus Montes (Ackiss et al. 2018).

Mineralogical evidence for evaporitic brines on Mars has been found in many regions, including evaporite outcrops in Valles Marineris, Terra Meridiani, Margaritifer Sinus, Gusev Crater, Meridiani Planum and North Polar regions (Squyres et al. 2004; Gendrin et al. 2005; Langevin et al. 2005), and observations of salt minerals in the equatorial and mid-latitudes (Feldman et al. 2004a; Karunatilake et al. 2014). Evaporites on Mars are commonly associated with Hesperian-aged terrain, a period between c. 3.5 and 3.0 Ga (Hurowitz and McLennan 2007). Sulfates (Mg, Fe and Ca) represent a major component of Martian evaporites, both in outcrops (Bibring et al. 2006) and in the globally distributed dust (Yen et al. 2017). Furthermore, Ca-sulfate deposits are a diagenetic alteration feature identified at both Gale Crater (Schwenzer et al. 2016) and Endeavour crater (Arvidson et al. 2016). These data imply a global aqueous chemistry at the time dominated by varying ratios of Mg²⁺/Fe²⁺/SO₄²⁻/Cl⁻ present in evaporite-forming brines, where local variations allowed (H)CO₃ to accumulate. At a neutral to alkaline pH, the presence of aqueous (H)CO₃ would probably have removed Fe²⁺, Mg²⁺ and Ca²⁺ during evaporation. Therefore, the widespread presence of Mg-, Fe- and Ca-sulfate minerals indicates acidic sulfate-enriched brines.

Current and future prospects for the detection of S isotope biosignatures on Mars

To test the utility of S isotope biosignatures, we consider S isotope values measured on Mars’s surface and in Martian meteorites (Franz et al. 2014, 2017; Chela-Flores 2019), within the context of S isotope measurements from terrestrial Martian analogue environments. We show that biogenicity is difficult to assess from major S isotopes alone and propose an important role for minor S isotopes in future life-seeking missions.

The S isotope values of sulfide and sulfate minerals have been measured from shergottite meteorites (Franz et al. 2014, 2017; Chela-Flores 2019), within the context of S isotope compositions of Martian meteorites (Franz et al. 2014, 2019b), with shergottite meteorites providing the closest available representation of Mars’s mantle composition (Franz et al. 2019b). The δ³⁴S values of both types of meteorites vary from −3.34 to +0.7‰ for sulfides, and from −4.98 to +12.8% for sulfates (±0.15‰ for all δ³⁴S values). In contrast, a significant in situ discovery from the SAM instrument on board the Curiosity rover has been the identification of a large variability in the S isotope compositions measured in the sediments from Gale Crater (Franz et al. 2017). The first δ³⁴S values measured at Gale Crater from mudstones show a large δ³⁴S range of sulfides and sulfates, from −47 ± 14 to +28 ± 7‰. These large S isotope variations are proposed to have resulted from equilibrium fractionation between sulfate and sulfide within an impact-driven hydrothermal system, with related atmospheric processing of sulfur gases during warm periods (Franz et al. 2017). These variations are...
larger than the δ34S values seen in S-phases from Martian meteorites and overlap with variations produced on Earth by microbial sulfur metabolisms. Sulfur isotope fractionations (34εCRS-SO4) in Mars analogue hydrothermal environments have been observed to be small, from −9.1 to −3.7‰ between water sulfate and CRS (chromium reducible sulfur: pyrite and elemental S) from sediments (Szynkiewicz et al. 2012; Cousins et al. 2018; Moreras-Martí et al. 2021b). Conversely, hypersaline Mars analogue environments that support both MSR and MSD have larger 34εCRS-SO4 between −49.5 to −43.5‰ (Moreras-Martí et al. 2021b) (Fig. 5). As described in the introduction, the occurrence of MSR with the addition of an oxidative S cycle, supporting SO and/or MSD, generally increases the S isotope fractionations expressed in natural environments, and could explain the larger fractionations in the hypersaline spring. Another important factor contributing to this disparity is the relative availability of metabolic energy in these environments. Hydrothermal environments provide functionally unlimited energy for MSR, MSD and SO in the form of abundant electron donors or acceptors. Under such energy-unlimited conditions on Earth, MSR uses electron carriers with strongly negative reduction potentials, which have been observed to produce fractionations smaller than −22‰ (Wenk et al. 2018). The opposite has been observed for energy-limited or low-energy niches, where MSR is associated with large S isotope fractionations (Sim et al. 2011a, and references therein).

Minor S isotope data from both types of Mars-analogue sites show small-scale ∆33S and ∆36S variations consistent with mass conservation effects that occurred during biological sulfur transformations. Inclusion of the minor S isotope analyses reveals that complex biological S-cycling, consisting of MSR and further oxidative recycling of sulfur species, is occurring in both environments. Particularly in hydrothermal environments with small variations in δ34S, it is only when the minor S isotope values are included that biological and abiotic hydrothermal processes can be decoupled (Moreras-Martí et al. 2021b). This decoupling is of importance when searching for biosignatures within a highly active abiotic S cycle. Likewise, these values form a small range in comparison with Martian meteorites, which instead show large ∆33S values consistent with an atmospheric S-MIF signal (−1.25 ± 0.01 to 0.260 ± 0.008‰ for sulfates, and −0.538 to 0.093‰ for sulfides), and a smaller range of ∆36S values (−0.24 ± 0.2 to 2.6 ± 1.6‰ ± 1.4‰ for sulfides) (Farquhar et al. 2000a, 2007b; Franz et al. 2014, 2019b). One observation from these meteorites is the coexistence of large ∆33S anomalies with near-zero ∆36S values, which implies that Martian S-MIF formed by different pathways than those that operated on the early Earth, as
Archean S-MIF values show a distinctive covariation between $\Delta^{34}S$ and $\Delta^{36}S$ (Farquhar et al. 2000b; Franz et al. 2014).

Future prospects for the detection of Martian S isotope biosignatures: unravelling abiotic from biotic QSI signatures in Martian materials

As described above, the addition of minor S isotopes ($\Delta^{33}S$ and $\Delta^{36}S$, QSI) can provide a third and fourth dimension for interpreting $\delta^{34}S$ values in natural systems. QSI can help to remove ambiguity between abiotic and biotic S-cycling processes, given that mass conservation effects are distinguishable from abiotically produced QSI values (e.g. Ono 2008). In this section we consider the abiotic processes that could contribute to QSI values in Martian systems, and prospects for unravelling these from microbial S isotope signals.

Theoretical calculations predict that equilibrium isotope effects in $^{33}S–^{35}S$ between sulfur-bearing species can be as large as 58% (Farquhar and Wing 2003; Johnston et al. 2007; Ono et al. 2007). The large range in $\delta^{34}S$ values at Gale Crater (from $-47$ to $+28\%$) has been interpreted to reflect a combination of equilibrium isotope effects and atmospheric processing of S-bearing gases that were incorporated into minerals (Franz et al. 2017). Future QSI analyses could help to distinguish between these scenarios, as changes in $\Delta^{33}S$ values associated with equilibrium fractionations (between $-0.02$ and $0.03\%$) are small in comparison with $\Delta^{33}S$ effects produced during biological transformations ($>0.05\%$), providing a diagnostic biosignature (Johnston 2011). TSR can also contribute to QSI values, particularly in hydrothermal systems; however, TSR requires the interaction of sulfate with organic matter, which does not seem to be prevalent for the majority of Martian history.

Mass-independent fractionation of sulfur isotopes (S-MIF) during atmospheric photochemistry provides the most challenging biogenic signal to untangle from biological QSI values. Martian meteorites (sulfide and sulfate) show a larger range of $\Delta^{33}S$ (from $-1.25$ to $0.260\%$) and $\Delta^{36}S$ values (from $-0.67$ to $2.60\%$), interpreted to reflect S-MIF (Fig. 5a and b). $\Delta^{33}S$ values from Martian meteorites are outside the range of values produced during mass conservation effects and mass-dependent processes. Variations in $\Delta^{33}S$ are considered insignificant for Mars owing to the relatively large uncertainties in the measurements, thus these values appear to show a lack of covariation between $\Delta^{33}S$ and $\Delta^{36}S$ (Farquhar et al. 2000b; Franz et al. 2014). Sulfur species processed by biology form coherent mass fractionation arrays in $\Delta^{33}S$/$\Delta^{34}S$ (Farquhar et al. 2000a). For example, biologically influenced samples from Lost Hammer and the Icelandic hydrothermal pools show co-variation in $\Delta^{33}S$ and $\Delta^{34}S$, with $\Delta^{33}S$/$\Delta^{34}S$ slopes between $-5.6$ and $-7.5$, consistent with mass-dependent processes (Fig. 5). The $\Delta^{33}S$/$\Delta^{34}S$ relationship could therefore provide a powerful tool to help distinguish mass-dependent fractionations from S-MIF. Notably, if large atmospheric S-MIF signals are globally distributed at the Martian surface, these could effectively mask small mass-dependent microbial S isotope signals, unless $\Delta^{33}S$/$\Delta^{34}S$ slopes are discernible. In addition, even if S-MIF is globally distributed at the Martian surface, microbial S-cycling can dilute the S-MIF signal by mixing sulfur reservoirs in the environment, leading to characteristic mixing trends (Ono 2008). This process has already been observed in late Archean rocks, revealing trends that reflect that S-MIF signals were overprinted by S-cycling microorganisms (Ono et al. 2003; Zerkle et al. 2021).

To understand mixing processes and resulting dilution effects, the QSI composition of the different sulfur reservoirs would need to be characterized (e.g. volcanic SO$_2$, S$_2$ and H$_2$SO$_4$ aerosols, SO$_4^{2-}$ minerals, sedimentary Fe$_3$S$_2$, etc.), and considered within the depositional setting of the system (e.g. closed- v. open-system processes).

We suggest four considerations for unravelling biogenic v. abiotic QSI values from sedimentary sulfur species preserved on Mars. First, S isotope signals indicative of biological S-cycling would be more clearly detected in systems largely free of contamination by atmospheric S-MIF signatures. Second, if an S-MIF signal was globally widespread on Mars, microbial mixing of sulfur could dilute the S-MIF signal and produce characteristic mixing arrays (Ono 2008). Third, $\Delta^{33}S$/$\Delta^{34}S$ slopes could be useful in distinguishing between S-MIF and mass-dependent S isotope fractionations. Lastly, S-cycling microorganisms inhabiting extreme environments that exert physiochemical stressors to life are more likely to produce larger S isotope fractionations (see Fig. 2) that can be distinguished from non-MIF-forming abiotic processes. Like any biosignature, QSI can only be used together with other life-detection approaches. Carbon isotope analysis of organic matter (OM), for example, pairs well with S isotopes, in addition to characterization of the organic molecules present (Hays et al. 2017). This combination can further constrain the type of environments that are most likely to preserve microbial biosignatures.

Environmental targets for the preservation of QSI biosignatures

Given the four considerations outlined above, we propose that high-sulfur environments, such as relic hydrothermal systems, impact craters and subsurface brines, would have provided the ideal setting for S-cycling organisms to interact directly with mantle-derived sulfur reservoirs, such as volcanic or hydrothermally sourced sulfur (Fig. 6). For example, sulfur delivered directly from inputs of volcanic sources could have had c. 0% S isotope values, reflecting a high contribution of mantle-derived sulfur and a small S-MIF contribution (Labidi and Cartigny 2016). Further, where high-S environments with large mantle input had a physical barrier to the surface, such as in subglacial hydrothermal environments and deep crustal hydrothermal systems, they would also have had a smaller S-MIF contribution (Fig. 6). The Martian meteorites bearing an S-MIF signal represent only a subset of all surface and subsurface processes from the S cycle on Mars, thus it is reasonable to assume that relatively small fluxes of atmospheric S-MIF could have been mixed with sulfur reservoirs dominated by mantle sulfur sources. Conversely, environments with a relatively low flux of mantle-derived S, such as non-hydrothermal, non-sulfatic lacustrine, flood and groundwater environments, would have been more susceptible to overprinting by deposition of S-MIF-bearing atmospheric sulfur. In addition, hydrothermal and volcanic systems would provide excess electron donors and acceptors, whereas settings such as brines would be energy-limited niches, which could promote larger S isotope fractionations. For settings such as lacustrine habitats and flood plains, it is likely that the subsurface sediments presented energy limitation compared with hydrothermal systems, like the Jezero palaeolake; however the palaeochemistry of this lacustrine habitat is yet to be determined (Fig. 6). We therefore suggest that the subsurface of lakes and floodplains could be systems to prioritize when searching for QSI biosignatures, with the likely challenge of having to unravel these biosignatures from S-MIF signals.

QSI analysis on returned samples

Currently, there are limitations with the analytical technique for measuring the four masses of S isotopes with a lander spacecraft. The process to analyse bulk QSI is intricate and requires a fluorination step, vacuum line to purify samples, gas chromatograph and mass spectrometer to allow simultaneous measurement of the four sulfur isotopic abundances. Alternatively, with secondary ion mass spectrometry (SIMS), a complex sample preparation process is required involving sample polishing, grinding and gold coating, followed by analysis in an ion probe. Spacecraft measurements also involve larger errors owing to the inability to control for measurement conditions; for example, $\delta^{34}S$ measurements at Gale Crater involve errors larger than $\pm 4\%$ and as high as $\pm 14\%$ (Franz...
S isotopes as biosignatures for Mars and Europa

**Fig. 6.** Summary figure with the different environmental factors affecting QSI biosignature preservation in high-sulfur environments and low-sulfur environments on Early Mars. Scenarios consider metabolic energy-unlimited v. metabolic energy-limited subsurface niches. High-sulfur environments feature a significant mantle sulfur signal in volcanically driven systems; low-sulfur environments show the opposite. This scenario is hypothesized on a past Martian atmosphere with continuing S-MIF processes, resulting in high S-MIF signals present in environments with direct atmospheric access. Subsurface or subglacial environments present low S-MIF signal owing to their partial disconnection from the surface.

**Potential sulfur cycling on Europa**

Europa’s ocean chemistry is currently not well constrained. Models for Europa’s formation and differentiation into silicate core, ocean and ice shell have predicted that sulfate should be one of the dominant ions in the ocean (Kargel et al. 2000; Fanale et al. 2001; Zolotov and Shock 2001). Sulfate is a major detected component of Europa’s surface, which displays extensive evidence for resurfacing (Figueroedo and Greeley 2004; Leonard et al. 2018). However, other theoretical approaches have argued that the oxidation of accreted sulfides may not have occurred if rates of H₂ escape were low enough to maintain reducing conditions (McKinnon and Zolensky 2003). Furthermore, it is possible that most (if not all) S on Europa’s surface is exogenously; mapping of sulfate spectral signatures shows that their distribution is centred at the apex of Europa’s trailing hemisphere (Carlson et al. 2005; Brown and Hand 2013; Ligier et al. 2016), closely matching the intense flux of exogenic sulfur ions deposited on Europa from the high-energy plasma environment within Jupiter’s magnetic field (Hendrix et al. 2011). Experimental work has shown that sulfate anions are generated via S ion implantation into ice (Strozzulla 2011), and by radiolysis and thermal processing of water ice in the presence of S (Carlson et al. 2002; Loeffler and Hudson 2010), all conditions that are met on Europa’s trailing hemisphere.

By contrast, spectra from the leading hemisphere, which experiences a far lower flux of S ions (Hendrix et al. 2011), can be satisfactorily explained without contribution from sulfates (Brown and Hand 2013; Ligier et al. 2016; Trumbo et al. 2019). Instead, leading hemisphere non-icy materials appear to be dominated by Na and Mg (and possibly K) chlorides, which must originate from water-rock interaction in the interior. Lack of endogenous sulfates on Europa’s surface need not rule out a sulfate-rich ocean, as sulfate-rich fluids can evolve towards a chloride-dominated endmember during freezing (Zolotov and Shock 2001; Vance et al. 2019). However, this explanation assumes an as-yet unknown mechanism within Europa’s ice shell that efficiently

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**Sulfur isotope biosignatures on Europa**

It is likely that oceans of liquid water exist beneath the icy surfaces of several moons of the gas giant planets, including Europa, Ganymede and Callisto (Jupiter), Enceladus and Titan (Saturn), and potentially elsewhere, including dwarf planets Ceres and Pluto, and Neptune’s moon Triton (Nimmo and Pappalardo 2016). Although both Enceladus and Europa have received significant attention as potential habitats for life, owing in large part to the likelihood of hydrothermal water–rock interaction maintaining redox disequilibria in their oceans, only at Europa have S compounds been detected.

Considerable engineering efforts are needed to develop faster and smaller versions of these processes for inclusion in a future spacecraft payload. Mars sample return efforts circumvent this issue by allowing laboratory QSI measurements. Laboratory analysis of QSI through fluorination and vacuum and mass spectrometer requires only between 0.3 and 0.5 mg of Ag₂S. The SAM instrument analysed for S isotopes around c. 45 to c. 135 mg, of which 1% in wt (0.45–1.35 mg) of the samples comprised pyrrhotite and possible pyrite in the JK sample (Franz et al. 2017). QSI measurements are also possible via grain-scale SIMS analysis, which can be conducted on 6–10 μm spots of sulfate or barite grains (e.g. Grema et al. 2022).

The Perseverance rover landed in Jezero crater in February 2021 to study Jezero’s ancient lake system and to drill and store geological sample cores for future return to Earth. Perseverance has been studying the crater floor units, Maaz and Séítah formations, basal units of the lake identified as igneous in origin and affected by aqueous alteration (Gupta et al. 2022; Mangold et al. 2022; Sun et al. 2022). Hydrated Ca- and Mg-sulfates suggest contact with briny waters (Meslin et al. 2022). Perseverance has already taken core samples from these crater floor units, and will sample the delta units (Mangold et al. 2022). These samples will eventually be recovered and returned to Earth (Muirhead et al. 2020). We argue the importance of performing QSI analysis on these returned samples as a tool to characterize the local S-cycling in Jezero’s palaeolake, in addition to contributing to deciphering the wider S cycle on Mars. For example, analysing QSI on S minerals in the crater floor units can identify S-MIF process captured here, together with the extent of its incorporation into magmatic materials. Where the deltaic environment has captured sedimentary sulfide and sulfate, either transported or formed in situ, QSI measurements can inform about their origin, or any putative biological S-cycling involved. There is a need for QSI studies on Jezero palaeolake analogues on Earth to better understand the microbial signals in such settings. This analysis is important not only for QSI but also to improve understanding of the general processes constraining the preservation of biosignatures in Jezero-like environments with deltaic mudstones, authigenic or detrital clay minerals, hydrated silica and magnesium carbonates (Bosak et al. 2021).
returns precipitated sulfates to the ocean, preventing them from being expressed at the surface.

Regardless, it is reasonable to expect a moderate level of bioavailable S in the ocean (Fig. 7). Europa’s ice surface is thought to be less than 100 myr old, and in many regions significantly younger than this (Figueroed and Greeley 2004). Resurfacing via exhumation and burial of subsurface materials has probably resulted in the delivery of radiolysis products, including sulfates, to the ocean over these timescales (Hand et al. 2007; Greenberg 2010). Resurfacing also serves to expose fresh, unirradiated ice, ensuring the continued radiolytic production of oxidants. Estimates of the flux of oxidized S into the ocean through this route suggest that a minimum of $2 \times 10^9$ moles of $\text{SO}_4^{2-}$ per year could be transferred into the ocean (Hand et al. 2007), with other estimates suggesting that rates of total oxidant flux (including $\text{SO}_4^{2-}$ as well as $\text{H}_2\text{O}_2$, $\text{O}_2$ and other minor compounds) could be as high as $3 \times 10^{11}$ moles a$^{-1}$ (Greenberg 2010). Hydrothermal water–rock interaction predicted to occur at Europa’s core–ocean boundary can supply the ocean with reductants such as $\text{H}_2$ at rates of between $c. 10^8$ and $c. 10^{10}$ mol a$^{-1}$ (Vance et al. 2016), which, when coupled with exogenous $\text{SO}_4^{2-}$, can serve as electron donors for MSR. The delivery through the ice of other oxidants such as $\text{H}_2\text{O}_2$ and molecular oxygen (Hand et al. 2007) can provide further electron acceptors for microbial sulfide oxidation (Fig. 7).

Because the ultimate source of S ions in Jupiter’s magnetic field is Europa’s neighboring moon Io, a plausible biogeochemical S cycle on Europa therefore encompasses reductants and oxidants sourced from two separate planetary bodies (Fig. 7), a significant contrast to S cycles on Earth and Mars. The existence of endogenous salts such as Na- and Mg-chlorides on Europa’s leading hemisphere, which must originate from water–rock interactions in the subsurface, demonstrates that ice shell overturn transports oceanic material upwards as well as downwards. Products of biological metabolisms, such as reduced or oxidized S pools, may therefore be incorporated into surface-accessible materials.

**Future prospects for the detection of S isotope biosignatures on Europa**

Upcoming missions such as the Europa Clipper and the proposed Europa Lander will have the capability to make isotopic measurements (at least $\delta^{34}\text{S}$) to interrogate surficial S compounds for evidence of biological processes. In the latter case, this will occur by directly sampling the surface (Hand et al. 2017), and in the former case by encountering eruptive plumes or micrometeoroid impact ejecta during fly-bys (Postberg et al. 2011). Recognizing or ruling out biologically mediated isotopic signals in these materials requires accounting for the S isotope composition of the various pools of S both on and within Europa and their potential for diluting or overprinting biological processes, as discussed for Mars.

Sulfur on Europa’s surface could originate from three reservoirs: (1) S compounds that have been recently delivered exogenously; (2) exogenous S compounds that were delivered to the ocean via ice shell overturn, processed by (bio)geochemical cycling, and
subsequently returned to the surface; or (3) S compounds that are endogenous to Europa (i.e. sourced from the silicate core) (Fig. 7). Scenarios (1) and (2) represent an exotic case for which new knowledge will be required. Specifically, two distinct processes potentially capable of imparting isotopic fractions should be accounted for: ionization of neutral molecules at Io and radiolytic or thermal production of oxidized S compounds on Europa’s surface. Sulfur ions impacting Europa’s surface originate as neutral S molecules (e.g. SO₂, SO) ejected from Io by volcanic activity, which are then ionized by high-energy electrons in Jupiter’s magnetosphere within tens of hours after ejection (Yoshikoa et al. 2017). The production efficiency of S ions (including S⁰, S²⁻ and S³⁺ as well as O⁻) differs markedly, with S²⁻ at least two orders of magnitude more abundant in Io’s plasma ‘torus’ (Smyth and Marconi 2003; Yoshioka et al. 2017). Ionization of SO₂ by electrons has been demonstrated experimentally (Fletcher et al. 2013), but new experiments are required to measure isotopic distribution across ionic products. In addition, theoretical predictions and new experiments are required to understand S isotope fractionations during the production of oxidized S compounds on Europa’s surface. A review of ice-hosted thermal and radiolytic conversion of S compounds has been given by Mitsud et al. (2021). The radiolytic and thermally induced chemistry on Europa’s surface can cycle S through a range of species, including SO₂, SO₂, HSO₃, S₂O₅²⁻, elemental S, S polymers and even H₂S (Carlson et al. 2002; Loeffler and Hudson 2010; Loeffler et al. 2011; Kaniuchová et al. 2017). If these compounds are delivered to the ocean via ice shell overturn, they could each participate in different biogeochemical processes including MSR, MSD and sulfide oxidation, with different implications for the extent of biological S isotope fractionations (as described in the introduction). It is important therefore to understand the δ³⁴S S isotope composition of each product of surface chemistry, and how this relates to the S isotope composition of incoming S ions.

Discriminating between biological and abiotic S isotope fractionations in Europan surface materials also requires understanding the endogenous S content of the ocean. If Europa’s ocean has low endogenous S, then the oceanic S inventory will be limited by the flux of S compounds from the ice shell, and exogenous S will dominate the S cycle, biological or otherwise. If Europa’s ocean contains abundant endogenous sulfate, as predicted by several models (Kargel et al. 2000; Zolotov and Shock 2001; Melwani Daswani et al. 2021), then exogenous S may represent a very small relative contribution. The contemporary delivery of exogenous S risks overprinting any biological S isotope signal that has been exhumed at the surface. This is particularly relevant for the trailing hemisphere, where the highest abundances of exogenous S compounds are found (Hendrix et al. 2011; Brown and Hand 2013). Landed missions can strategically avoid these regions, and instead target locations such as the leading hemisphere, which experience low exogenous S flux. However, fly-by spacecraft such as NASA’s Europa Clipper, which aim to analyse dust ejected from the surface by micrometeoroid impact, have less ability to target specific regions. Finally, if eruptive plumes are active on Europa, as have been tentatively identified (Sparks et al. 2017; Jia et al. 2018), ejected materials would presumably have no contribution from contemporary exogenous S, and therefore would make a high-priority target for probing subsurface (bio)geochemistry.

Conclusions

(1) Sulfur isotope measurements represent a powerful tool with which to probe both abiotic and putative biological processes on Mars and Europa. QSI has the potential to delineate these processes on Mars by taking into account the four considerations suggested in ‘Current and future prospects for the detection of S isotope biosignatures on Mars’.

(2) Analysis of these measurements needs to be conducted within the context of sulfur reservoir inputs and outputs, many of which are still unconstrained, especially for Europa.

(3) On Mars, there is an apparent trade-off between environments most energetically favourable for S-based microbial metabolisms and those that are more likely to capture detectable S isotope biosignatures through exerting environmental stressors. This has implications for future landing site selection and mission targeting.

(4) QSI analysis on future returned samples from Jezero Crater can play a significant role in deciphering the local, and wider, Martian S cycle.

(5) For Europa, significant knowledge gaps currently exist, which hamper the utility of S isotopes as an effective biosignature for S-cycling microorganisms, despite the likely bioavailability of S in the ocean. This includes (a) understanding the S isotope signatures of space-based processing and (b) establishing the relative contributions of exogenous and endogenous S to the Europan ocean environment.

(6) New experiments are required to measure S isotope distribution across products of ionization of S neutrals in Io’s plasma torus and radiolysis of implanted S compounds on Europa’s surface.

(7) Measuring δ³⁴S on Europa could be feasible on a lander or fly-by mission. Furthermore, measuring δ³⁴S will help untangle the S cycle on Europa, and ultimately differentiate the different S pools.

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S isotopes as biosignatures for Mars and Europa


