nature portfolio

Peer Review File

Redox-Driven Mineral and Organic Associations in Jezero Crater, Mars

Corresponding Author: Dr Joel Hurowitz

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
Summary:

This is an exciting, interesting, and valuable dataset that deserves publication, however there are major problems in the manuscript. The authors document at low and high resolution a range of outcrops exposed in Jezero Crater and provide new geochemical analyses including Raman spectra of possible carbonaceous material and of various minerals, as well as geochemical analyses of major elements. This work is all done robotically and in situ, on the Martian surface, so it represents a great achievement by the team. However, the story of this manuscript is misguided, probably wrong, and will lead to the creation of a new controversy about the search for past life on Mars. This is because the title will attract a tremendous amount of media attention, and it will be interpreted as another claim for evidence of past Martian life in the community. In its essence, this new dataset is very similar to that published by McKay et al. (1996 - Science), but it is also much weaker and much less detailed. The main difference is for the geological context, which, here, is much better documented than the ALH84001 meteorite. McKay et al. (1996) had also concluded on “possible relicts of biogenic activity” in the Martian meteorite ALH84001, an orthopyroxenite rock, with similar observations: 1) polycyclic aromatic hydrocarbons (which are mostly sp2-hybridised), 2) Fe-Mg carbonate concretions, 3) apatite with carbonate and pyrite, 4) greigite with carbonaceous material, 5) chains of magnetite crystals and 6) ovoid-shaped and S-shaped nanoscopic-size dubiofossils. The latter two observations are additional evidence that were not documented in this manuscript. While the astrobiology community has since mostly dismissed the original interpretation, multiple groups have independently reproduced the original observations in the McKay et al paper. Most of them re-interpreted the observations as the signatures of non-biological processes having occurred on Mars.

In this new manuscript, the title and conclusion invoke biosignatures, and therefore it can be foreseen that this manuscript will not escape the fate of the McKay et al. (1996) paper and be dismissed by the community as too controversial. The authors should recall the conclusion of McKay et al. (1996):

“None of these observations is in itself conclusive for the existence of past life. Although there are alternative explanations for each of these phenomena taken individually, when they are considered collectively, particularly in view of their spatial association, we conclude that they are evidence for primitive life on early Mars.”

This paper made news headlines around the world with President Bill Clinton even giving a press conference about it. This new manuscript by Hurowitz et al. takes the same scientific approach used by the McKay et al. team. In fact, the authors report on olivine and pyroxene bearing mudstone rocks that are variably oxidised with 1) sulfate minerals, Fe-Mg carbonate, and sp2 carbonaceous material (as can be interpreted from Raman spectra – PAHs?), 2) nodular structures (which they refer to as leopard spots (!) and poppy seeds (!) enriched in Fe, P and Zn), and 3) mixtures of sulfate (jarosite), carbonate (siderite), sulfide (greigite), and phosphate (vivianite) – with the last two being uncertain and vaguely inferred. Such mineral mixture could be evaporitic, abiotic, and prebiotic which seems more likely than a biological origin.

Now the main problem in the manuscript is that there is no fossil evidence such as microbialites, carbonaceous compressions, or even dubiofossils to suggest a possible biological origin. There are also various non-biological

explanations, which the authors acknowledge and which should be the preferred default interpretation, following the null hypothesis. I would thus strongly urge the authors to re-think their story, for instance in the context of prebiotic chemistry, instead of repeating the same mistakes of the past, which might be damageable to the reputation of the field of astrobiology. However, I maintain that this is a truly remarkable new and valuable dataset from the Martian surface, and I have no negative criticism of the data, besides for the interpretations. Hence, I argue that the science in this manuscript is weak, not original, and absolutely controversial in its current presentation. This manuscript is thus a disappointment, in my view, as it comes from a very strong team that includes several individuals who have strongly criticised the work of others on biosignatures, but who apparently did not use the same level of self-criticism for their own work, on this occasion. Below are more specific comments on the manuscript.

Specific comments:

Lines 47-50: The team's definition of the term biosignature is inadequate, regardless of it having been published before: "a feature that is consistent with biological processes and that, when encountered, challenges the researcher to attribute it either to inanimate or to biological processes, compelling them to gather more data before reaching a conclusion as to the presence or absence of life". The definition by Gillen et al. (2023) is much better although still a bit long: "any phenomenon for which biological processes are a known possible explanation and whose potential abiotic causes have been reasonably explored and ruled out. "

Line 50 and beyond: The terminology used (i.e. "poppy seeds" and "leopard spots") seems to suggest that it remains unclear to the authors what these objects are, hence, this is a major weakness in their interpretation as a potential biosignature. These features also seemingly have no relevant analogues on Earth, which further weakens that interpretation. Besides, I think the Mars 2020 Science Team's systematic use of seemingly humorous expressions to name localities visited by the rover may have spilled over into naming geological features and objects in a similar fashion, which comes across as a bit 'childish' and lacking seriousness. I find this unhelpful, because these are inadequately descriptive terms that prevent easy comparisons.From what I could gather in the observations presented, "poppy seeds" are nodular structures 100-200 um in size with dark blue-green colours, whereas the "leopard spots" are also nodular structures 200 um to 1mm in size with multiple colours. While the colours are argued to relate to mineralogy, which is agreeable and probably correct, it is unclear whether there are specific patterns in the nodular "seeds and spots" – the latter seems to be rimmed by the darker phase, but the photos I have are too poor in their resolution.

Line 84: It is not clearly explained how the BA-MT rock layer could represent the oldest rocks in Jezero crater without presenting clear evidence for overturned beds. Besides, the stratigraphy described in the text is confusing.

Line 108-109: How does "enhanced cementation" lead to the formation of nodules? This is an unsupported interpretation that weakens the argument, as nodules and concretionary features need to be carefully explained and considered in this manuscript.

Lines 217-222: I do not see how the authors can justify these claims about the composition of "poppy seeds". I see some supporting PIXL analyses (which should be cited there, and shown in Fig. S13), but it is unclear how these minerals were identified only from MgO+FeO, CaO+Na2O, and SiO2.

Line 232: The Ca-sulfate nodules shown in Fig. S18 contain "poppy seeds" similarly to the mudstone matrix. Hence, the "seeds" could have formed alongside the nodules and during sedimentary diagenesis. The formation of the sulfate nodules (suggested on lines 238-239) is considered as nodules or detrital clasts, both of which are considered to be non-biological in origin on Earth. Hence, it is unclear what is interpretable as a biosignature here.

Lines 300-315: This explanation is agreeable, but it does not convince that there is a plausible or even a possible biosignature in the new observations. Again, this is more likely to be prebiotic type chemical reactions with non-biological organic matter, known to occur in several places on Mars.

Lines 351-352: "... one would reasonably assume that microbial metabolisms contributed to its formation" (of Bright Angel), however, I do not see how this would be possible even on Earth. Microorganisms do not directly produce nodules, let alone sulfate or vivianite nodules. There is no experimental nor Earth-based observational basis for this claim.

Figure 3: These Raman spectra are all very noisy and while they show what could be a G-band (with sp2 hybridised carbon – PAHs?), that peak is unusually broad, which is unlike what is expected for graphite, and there is also no D-peak, as would be expected for sedimentary organic matter in mudstone. These two observations are left without explanation, which weaken the story. The Raman spectra also show resolved peaks around 1010 cm-1, suggestive of pyroxene and other peaks around 1090 cm-1 suggestive of carbonate. This is similar to observations from ALH84001 orthopyroxenite.

Figure 4: I do not understand why pyrite, greigite or pyrrhotite can even be mentioned in this plot since none of them contains any oxygen. I believe this is a misrepresentation that is both confusing and false.

Figure S9 and S10: Very little context is given to understand what is shown. The plot in S9A only gives "other natural rock surfaces" as a context, but this is not specifically related to a measure of Fe-oxides. Similarly, in Fig. S9B, there should be

relevant standard spectra shown for context and comparison. I also wonder about the meaning of the technicalities mentioned in the caption and whether this belong in the methods sections, possibly in a table (for instance, “offset +0.1” – What does that mean? Offset from what? In what units?). For instance, Table (S1) in the S.I. is a very useful guide to organize the data presentation. The caption should explain what is shown in the images, so that the observations have a scientific value. As currently presented, I find limited scientific value for that spectral data.

Figure S11: The correlation of the measured spectra with the identified “vivianite” spectrum is very poor. The four spectra shown in panel e are much more like hydrated calcium sulfate than vivianite. Besides, even if the author were correct with that interpretation of vivianite (and they are not at all convincing about this), why would vivianite be a plausible biosignature? To my knowledge there is an absence of peer-reviewed publications on this subject and the manuscript cites six poorly relevant references on this topic (refs 60-66). Also, in figure S11d, I do not think most of the community is familiar with “swarm plots” so please explain what the X-axis mean and the mirror patterns imply.

Referee #2

(Remarks to the Author)
see attached file

Referee #3

(Remarks to the Author)
A.Summary of key results
The manuscript “Detection of a potential biosignature by the Perseverance Rover on Mars” reports findings of a discovery from the Mars 2020 mission. One of the goals of the Mars 2020 mission is to search for signs of past life on Mars; if biosignatures are identified, appropriate samples would be returned to Earth for further analyses.

The study reports the analysis of several outcrops in a sedimentary unit in Jezero Crater on Mars. The “potential biosignature” is found in several outcrops of the “Bright Angel” subunit. The biosignatures are features enriched in Fe-phosphate minerals (e.g. vivianite) and in Fe-S minerals (e.g. greigite) that correlate with the presence of organic carbon. The formation of these features can be explained by both abiotic and microbial processes. On Earth, microbial processes would be a likely explanation for the formation of these features. The biological pathways invoked are microbial dissimilatory Fe(III) reduction and microbial sulfate reduction, both coupled with organic carbon oxidation.

B.Originality and significance
This report is significant, as it presents potential biosignatures on Mars. Verifying the biological origin would however require high-resolution analyses on Earth.
The geochemical characteristics of the outcrops are consistent with deposition under anoxic conditions

C.Data & methodology
Overall, the geologic context, outcrops and petrographic descriptions are clear, besides the questions below:
l. 79 What proxies lead to the conclusion that the BA and MT outcrops are from the same deposition unit? It is partially explained later in the text but it would be useful to clarify in this paragraph.
l. 84-85 The BA-MT strata cold have been deposited above or below the Margin Unit. How does this affect the conclusions of the study? Would the environmental conditions be significantly different if deposition happened before or after this of the Margin Unit?
l.95-97 This statement is unclear: what does that mean for deposition or reworking of sediments after deposition?
Figure 3: I cannot see the differences between Fig 3A and Fig 3B (besides the addition of spectral measurements). What do the black dotted line and the pink cross represent on Fig. 3B and Fig. 3C?

D.Appropriate use of statistics

E.Conclusions
The question of whether there is biology involved in the formation of these features cannot be answered until further analyses are performed on return samples (using high-resolution methods). However, this is an intriguing report. The discussion of the results in the last section (l. 288) opens up interesting avenues for experimental work to understand the how these features could form. I have some additional comments:
-The authors could add a paragraph to discuss why these features occur only at Bright Angel and not in the Masonic Temple area, while these two outcrops form a sedimentary unit. Were the MT outcrops also deposited under anoxic conditions, and then more oxidized than the BA area?
-The features that could represent potential biosignatures are concentrated in a few outcrops. How would you explain the limited localization of “potential” microbial activity?
-Recent studies have investigated the role of microorganisms in the formation of iron sulfide minerals. Some of them would actually support the potential involvement of microbial activity in the formation of these features, even though laboratory experiments cannot completely replicate conditions in sedimentary environments. Findings in these experimental studies include the following: 1) under strict anoxic conditions, greigite is only detected when microorganisms (sulfate-reducing bacteria) are present and metabolically active in the experimental medium (freshwater or marine medium containing Fe^{2+}). Greigite formation from mackinawite is a slow process (detectible after several months), but none of the abiotic controls display transformation of mackinawite to greigite. As culture media for bacteria include phosphate (as a source of P),

vivianite formation occurs in certain conditions. 2) physical properties of mackinawite and greigite are influenced by microbes: crystallite domain sizes are larger and aggregation is heavier in biogenic minerals, 3) iron sulfide minerals have a high affinity for microbial organic matter.

F.Suggested improvements
G.References
Studies on the influence of microorganisms on the formation of iron sulfide minerals: Nabeh et al. 2022 Frontiers in Microbiology, Picard et al. 2021 Astrobiology, Duverger et al. 2020 Frontiers in Earth Science, Berg et al. 2020 Scientific reports, Picard et al. Chemical Geology 2019, Mansor et al. 2019 American Mineralogist, Picard et al. 2018 Geochimica et Cosmochimica Acta,
Studies on vivianite: Cosmidis et al. 2014 Geochimica et Cosmochimica Acta, Miot et al. 2015 Minerals, Busigny et al. 2016 in Lake Pavin: History, geology, biogeochemistry, and sedimentology of a deep meromictic maar lake

H.Clarity and context
The paper is overall clear and well-written

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
I thank the authors for considering my comments and for modifying their manuscript accordingly, however, I am still highly dissatisfied by the essence of the story. The biosignature aspects have not been removed properly and the story now discusses evidence for redox reactions between minerals and organic matter. This is not new for Mars and also barely hiding the fact that the authors are still claiming that they discovered biosignatures on Mars... I list below three major points that lead me to recommend rejection at this time, because 1- the authors failed to tone down their main interpretation which is weakly supported and 2- use adequate vocabulary to characterize the features they observe.

1- The authors state in their rebuttal and on line 28-33 that “In fact, nowhere in the manuscript do we go so far as to say that the features observed in Jezero crater are proof positive of life on Mars. However, we are surprised that the reviewer has interpreted our manuscript to have concluded that such proof has been presented.”, which is strange and inconsistent with their previous manuscript title and assertions/arguments on finding biosignatures on Mars. In fact, this revised manuscript still uses the word “biosignature” a total of six times in the main text (in addition to references to microbial metabolism (as “... sulfide-bearing cores suggests that sulfate reducing metabolisms with...”) and “Martian biosphere”, which demonstrates that the authors believe that they found the signatures of life on Mars. I think they are wrong and they need to make better tests of the null hypothesis. In fact, the authors are still claiming a possible signature of life in their revised manuscript in discussing their new data as “Analysis of the core sample ... will enable tests to confirm or refute the biogenicity of the minerals, ...”. So, the authors fail to discuss the default null hypothesis, which is that this organic carbon is abiotic in origin and the greigite and vivanite are also abiotic reaction products. This latter sentence implies that it represents a possible biosignature and throughout the manuscript, the theme of biosignature returns again and again such that the authors have not changed this fundamental implication. I don't believe they are correct. Saying “... to confirm or refute the biogenicity of these features...” further implies that they interpret their observations as potential biosignatures and the case made is so weak with unconvincing results and dubious interpretations, that it will fall apart and create a significant controversy, especially if it is published in Nature.

In their rebuttal, the authors reply “To describe these features solely in the context of abiotic or “prebiotic” chemical processes would ignore the body of literature on terrestrial occurrences of similar features that are formed in association with microbial activity. This would provide the false impression that we are ignorant of these occurrences and/or intentionally not inviting comparisons to them.”. This is also dubious because the supposed “body of literature” on these kinds of objects on Earth does not exist, or rather very limited and mostly hidden as complementary observations in papers focused on other topics. For instance, to my knowledge, there is not one recent paper in the peer-reviewed literature that specifically addresses the association of greigite and vivianite as a potential biosignature (i.e. agnostically evaluating both abiotic and biological pathways of formation). Hence, without further evidence to argue for a potential biosignature in these nodular rocks, I do not support this view by the authors. Also, the identification of both greigite and vivianite is very weakly supported by their data and certainly not an unambiguous detection: the ternary diagram in Fig 4c does not show a single datapoint where vivianite is expected, while for greigite, it is similar in 4d and it is also a polymorph with several other sulfide minerals. The organic carbon remains a real detection, but its nature is not discussed in this work (rather differed to another manuscript), which is unfortunate.

2- The authors are adamant in keeping misleading words in their manuscript such as “poppy seeds” and “leopards spot”, which are figurative at best and a wrong scientific practice. There is no problem in my view with naming localities with such vocabulary (like “Yogi” for a specific rock location, or “Jezero” for a specific crater), however, specific natural objects need to be named according to specific scientific terminology in order to be comparable with other examples. What geological feature from Earth would the authors compare their small textures from Mars to? If they are nodules, then call them “nodules”, and not anything else. Instead of understanding this basic idea, the authors claim that because they have used this terminology in previous press releases (which are not peer-reviewed), they should be allowed to use it again, and in a peer-reviewed context. This is also a wrong point of view and akin to saying that an error cannot be corrected and that press

releases for public outreach supersede scientific peer-review.

Hence, I do not support publication of this manuscript in Nature and recommend rejection. I am not positive about publication on this occasion, as the case for potential biosignatures on Mars rests on very weak evidence, too few observations, and inadequate terminology.

Referee #2

(Remarks to the Author)
Dear Dr. Mossinger,
In my opinion this paper is substantially improved and I am satisfied that the authors have adequately revised the paper in response to my concerns.
I did notice a typo in the revised manuscript that the authors should correct. Ferrihydrite is misspelled on Fig S10
Best regards, Janice

Referee #3

(Remarks to the Author)
In my opinion, the authors have responded convincingly to all inquiries. The new version of the manuscript has improved in clarity and quality.

Whether or not it will be possible to confirm the biogenicity of these structures, this is exciting science! This remarkable report also highlights the importance of exploration and team work for the advancement of knowledge and deserves publication in Nature.

REVIEWER #1

Referee #1 (Remarks to the Author):

Summary:

This is an exciting, interesting, and valuable dataset that deserves publication, however there are major problems in the manuscript. The authors document at low and high resolution a range of outcrops exposed in Jezero Crater and provide new geochemical analyses including Raman spectra of possible carbonaceous material and of various minerals, as well as geochemical analyses of major elements. This work is all done robotically and in situ, on the Martian surface, so it represents a great achievement by the team. However, the story of this manuscript is misguided, probably wrong, and will lead to the creation of a new controversy about the search for past life on Mars. This is because the title will attract a tremendous amount of media attention, and it will be interpreted as another claim for evidence of past Martian life in the community. In its essence, this new dataset is very similar to that published by McKay et al. (1996 - Science), but it is also much weaker and much less detailed. The main difference is for the geological context, which, here, is much better documented than the ALH84001 meteorite. McKay et al. (1996) had also concluded on "possible relicts of biogenic activity" in the Martian meteorite ALH84001, an orthopyroxenite rock, with similar observations: 1) polycyclic aromatic hydrocarbons (which are mostly sp2-hybridised), 2) Fe-Mg carbonate concretions, 3) apatite with carbonate and pyrite, 4) greigite with carbonaceous material, 5) chains of magnetite crystals and 6) ovoid-shaped and S-shaped nanoscopic-size dubiofossils. The latter two observations are additional evidence that were not documented in this manuscript. While the astrobiology community has since mostly dismissed the original interpretation, multiple groups have independently reproduced the original observations in the McKay et al paper. Most of them re-interpreted the observations as the signatures of non-biological processes having occurred on Mars.

In this new manuscript, the title and conclusion invoke biosignatures, and therefore it can be foreseen that this manuscript will not escape the fate of the McKay et al. (1996) paper and be dismissed by the community as too controversial. The authors should recall the conclusion of McKay et al. (1996):

"None of these observations is in itself conclusive for the existence of past life. Although there are alternative explanations for each of these phenomena taken individually, when they are considered collectively, particularly in view of their spatial association, we conclude that they are evidence for primitive life on early Mars."

This paper made news headlines around the world with President Bill Clinton even giving a press conference about it. This new manuscript by Hurowitz et al. takes the same scientific approach used by the McKay et al. team. In fact, the authors report on olivine and pyroxene bearing mudstone rocks that are variably oxidised with 1) sulfate minerals, Fe-Mg carbonate, and sp2 carbonaceous material (as can be interpreted from Raman spectra – PAHs?), 2) nodular structures (which they refer to as leopard spots (!) and poppy seeds (!) enriched in Fe, P and Zn), and 3) mixtures of sulfate (jarosite), carbonate (siderite), sulfide (greigite), and phosphate (vivianite) – with the last two being uncertain and vaguely inferred. Such mineral mixture could be evaporitic, abiotic, and prebiotic which seems more likely than a biological origin.

Now the main problem in the manuscript is that there is no fossil evidence such as microbialites, carbonaceous compressions, or even dubiofossils to suggest a possible biological origin. There are also various non-biological explanations, which the authors acknowledge and which should be the preferred default interpretation, following the null hypothesis. I would thus strongly urge the authors to re-think their story, for instance in the context of prebiotic chemistry, instead of repeating the same mistakes of the past, which might be damageable to the reputation of the field of astrobiology. However, I maintain that this is a truly remarkable new and valuable dataset from the Martian surface, and I have no negative criticism of the data, besides for the interpretations. Hence, I argue that the science in this manuscript is weak, not original, and absolutely controversial in its current presentation. This manuscript is thus a disappointment, in my view, as it comes from a very strong team that includes several individuals who have strongly criticised the work of others on biosignatures, but who apparently did not use the same level of self-criticism for their own work, on this occasion. Below are more specific comments on the manuscript.

We thank the reviewer for this detailed account of the McKay et al controversy and appreciate the cautionary warning to avoid overreaching on the basis of the data that we have in hand. In fact, nowhere in the manuscript do we go so far as to say that the features observed in Jezero crater are proof positive of life on Mars. However, we are surprised that the reviewer has interpreted our manuscript to have concluded that such proof has been presented. The reviewer quotes the McKay et al. (1996) paper (**note bold text**):

"None of these observations is in itself conclusive for the existence of past life. Although there are alternative explanations for each of these phenomena taken individually, when they are considered collectively, particularly in view of their spatial association, **we conclude that they are evidence for primitive life on early Mars.**"

Our own conclusions come far short of any such claim. Here, excerpted from our text, are all the statements that relate to the possible biogenicity of the features we have observed:

Lines 21-22 (Abstract): Here, we report a detailed geological, petrographic, and geochemical survey of these rocks ~~and describe the discovery of a potential biosignature~~.

- As noted below, we have removed the second part of this sentence so that neither the title nor the abstract contains the term "potential biosignature". The text with the strike-through was present in the original submitted manuscript.

Lines 27-32: On Earth, such reactions are often driven by, or associated with, microbial respiration of organic matter. However, abiotic reactions at low temperatures can yield some of the reaction products observed. Analysis of the core sample collected from this unit using high-sensitivity instrumentation on Earth will enable tests to confirm or refute the biogenicity of the minerals, organics, and textures it contains.

Lines 41-46: Here we present *Perseverance* observations on a set of outcrops in Jezero crater that show evidence for chemical and mineral fabrics and structures associated with organic signatures that, together with the geological context in which they reside, meet proposed definitions for "potential biosignatures"[2-4]. In other words: "a feature that is consistent with biological processes and that, when encountered, challenges the researcher to attribute it either to inanimate or to biological processes, compelling them to gather more data before reaching a conclusion as to the presence or absence of life[2]".

Lines 275-277: Within the low-temperature sedimentary-diagenetic setting we have proposed for the Bright Angel formation, we can consider abiotic pathways to produce reduced Fe and S and concentrate them in poppy seeds and leopard spots.

All text between lines 275-301 contains discussion of plausible mechanisms for the abiotic production of the observed "poppy seeds" and "leopard spots", as well as caveats that might make abiotic scenarios less likely, leading us to summarize on lines 301-302:

Lines 301-302: Abiotic scenarios resulting in the production of the phases observed in Bright Angel should be tested by laboratory experimentation under a range of relevant conditions.

In the next section, from lines 302-331, we describe a scenario in which biological processes could have plausibly given rise to the observed features, beginning with:

Lines 302-304: Next, we consider the possibility that biology played a role in the formation of poppy seeds and leopard spots. If a mineral assemblage like the one at Bright Angel was encountered on Earth, one would reasonably assume that microbial metabolisms contributed to its formation.

We conclude this section with a similar statement to the one on Lines 301-302:

Lines 329-331: New research into the pathways and products of heterotrophic biological processes that give rise to Fe-phosphate and Fe-sulfide-bearing redox fronts like those observed in Bright Angel is recommended.

Finally, in the concluding paragraph, we state:

Lines 332-333: In summary, poppy seeds and leopard spots have textures, chemical and mineral characteristics, and organic signatures that warrant consideration as *potential* biosignatures.

Lines 335-338: While many significant questions remain about the origin of the poppy seeds and leopard spots encountered by Perseverance, our analysis of these features provides compelling evidence for low temperature redox reactions that, on Earth, are commonly associated with microbial activity.

Lines 338-342: The return of samples from Mars for study on Earth, including the Sapphire Canyon sample collected from the Bright Angel unit, provides the best opportunity to understand the processes that gave rise to the unique features described here and determine whether they could have been produced by an ancient Martian biosphere.

Given this summary of our text as it relates to the possible biogenicity of the observed "poppy seeds" and "leopard spots", we do not agree that we are inviting controversy akin to that of the McKay et al. (1996) study which did conclude that the ALH84001 meteorite contained "… evidence of primitive life on early Mars". Instead, we have hewed closely to definitions that have been provided by the Astrobiology research community (citations 2-4 in our manuscript) in the wake of the McKay et al controversy for potential biosignatures, e.g.,

De Marais et al. (2003): "A feature that is consistent with biological processes and that, when encountered, challenges the researcher to attribute it either to inanimate or to biological processes, compelling them to gather more data before reaching a conclusion as to the presence or absence of life[2]"

Mustard et al. (2013): “An object, substance and/or pattern that might have a biological origin and thus compels investigators to gather more data before reaching a conclusion as to the presence or absence of life. “

Gillen et al. (2023): “A potential biosignature is any phenomenon for which biological processes are a known possible explanation but whose potential abiotic causes have not yet been reasonably explored and ruled out”

In our manuscript, we conclude that the “poppy seeds” and “leopard spots” represent phenomena for which biological processes are a known possible explanation, and suggest that new research, and ultimately, sample return is required to confirm or refute the potential biogenicity of these features.

Furthermore, we note from the Mustard et al. (2013) study (their Table 3-2) that there are many recognized classes of “potential biosignature”, including: (1) Organic signatures, (2) Stable isotopic patterns, (3) Minerals, (4) Chemicals, (5) Microscale fabrics and structures, and (6) Macroscale fabrics and structures. The absence of “microbialites”, “carbonaceous impressions” or “dubiofossils”, which might all be categorized as “Macroscale fabrics and structures”, are not required for identification of a potential biosignature, but, if present, would certainly be useful additions to the collection of potential biosignature types that we have observed in the Bright Angel deposit.

To summarize, we have taken great care to adhere to the limits imposed by peer-reviewed definitions of the term “potential biosignature” that have emerged within the astrobiology community in the time since the McKay et al. (1996) report and have discussed both abiotic and biotic processes that could explain the origin of features observed in the Bright Angel deposit. We have also suggested that further research, including the analysis of samples returned from Mars, is required to confirm or refute the biogenicity of these features (both in the abstract and the concluding paragraph). To describe these features solely in the context of abiotic or “prebiotic” chemical processes would ignore the body of literature on terrestrial occurrences of similar features that are formed in association with microbial activity. This would provide the false impression that we are ignorant of these occurrences and/or intentionally not inviting comparisons to them.

Accordingly, we maintain our position that these features represent potential biosignatures. However, we also appreciate the concern that the reviewer has raised about having a title and abstract that invites misinterpretation and have modified the title to read “**Redox-Driven Mineral and Organic Associations in Jezero Crater, Mars**” and removed the term “potential biosignature” from the abstract.

Specific comments:

Lines 47-50: The team’s definition of the term biosignature is inadequate, regardless of it having been published before: “a feature that is consistent with biological processes and that, when encountered, challenges the researcher to attribute it either to inanimate or to biological processes, compelling them to gather more data before reaching a conclusion as to the presence or absence of life”. The definition by Gillen et al. (2023) is much better although still a bit long: “any phenomenon for which biological processes are a known possible explanation and whose potential abiotic causes have been reasonably explored and ruled out. “

There is a significant difference between the terms "potential biosignature" and "biosignature". In our manuscript (lines 44-46), we quote the DesMarais et al. (2003) NASA Astrobiology Roadmap's

definition of a potential biosignature "a feature that is consistent with biological processes and that, when encountered, challenges the researcher to attribute it either to inanimate or to biological processes, compelling them to gather more data before reaching a conclusion as to the presence or absence of life". This definition is in accord with a more recent definition for potential biosignature that is given by Gillen et al. (2023): "A potential biosignature is any phenomenon for which biological processes are a known possible explanation but whose potential abiotic causes have not yet been reasonably explored and ruled out." (note the underlined text for comparison to the next sentence).

In their comment, the reviewer quotes Gillen et al. 2023's definition of a biosignature: "any phenomenon for which biological processes are a known possible explanation and whose potential abiotic causes have been reasonably explored and ruled out." (note the underlined text for comparison to the previous sentence).

Throughout the manuscript (as stated previously), we have made every effort to ensure that our interpretation of the observed features in Jezero Crater remains open to both abiotic and biotic possibilities, and that we have described them appropriately as potential biosignatures rather than as definitive biosignatures. That said, if this distinction was not clear to the reviewer, it may indicate that others in the scientific or broader public communities could also find it ambiguous. With that in mind, we have revised the title to "**Redox-Driven Mineral and Organic Associations in Jezero Crater, Mars**" in the interest of clarity and to help avoid misinterpretation or undue controversy. At the same time, we continue to regard a biological interpretation as a plausible explanation for the features observed in the Bright Angel formation—one that is worthy of evaluation alongside the abiotic explanations we also consider. We feel that recasting the discussion to emphasize only abiotic or "prebiotic" chemistry would risk overlooking relevant parallels with well-documented, microbially mediated redox and mineralization processes in marine and lacustrine sediment porewater systems, which we believe are important for a comprehensive and balanced interpretation of the data.

Line 50 and beyond: The terminology used (i.e. "poppy seeds" and "leopard spots") seems to suggest that it remains unclear to the authors what these objects are, hence, this is a major weakness in their interpretation as a potential biosignature. These features also seemingly have no relevant analogues on Earth, which further weakens that interpretation.

We have updated the text to describe the poppy seeds as authigenic nodules on lines 201-203, in accord with standard definitions for the term (e.g., Prothero and Schwab's textbook "Sedimentary Geology"). We draw the reviewer's attention to citation number 53 (Hsu et al., 2014), which contains descriptions of authigenic vivianite-greigite-mackinawite nodules (see esp. their Fig. 4) of the same scale as Bright Angel poppy seeds. Like poppy seeds, these terrestrial examples are formed in Fe-oxide, phosphorous, and organic matter bearing sediment. In the terrestrial example, reactions forming vivianite-greigite-mackinawite nodules occur in anoxic sediment pore water where biological consumption of sedimentary organic matter results in the reduction of dissolved SO_4^{2-} and solid-phase Fe^{3+} and the release of dissolved Fe^{2+}, HS^-, and PO_4^{3-}.

We believe that the existing description of leopard spots as "*in-situ* reaction fronts" lines 218-219 is adequate. We considered the use of the term "reduction spots" or "reduction halos", but, unlike poppy seeds, we are not aware of terrestrial examples of such features that provide an adequate match to the chemistry and mineralogy of leopard spots, and so we elected to use a more generic description. However, on lines 313-317, we do draw comparisons between leopard spots and authigenic reduction halos and reduction spots observed in sediments (new reference, 63) and sedimentary rocks (references 64, 65), respectively. Accordingly, these references provide

relevant terrestrial examples of sediment-hosted, semicircular, trace element enriched, mineralized reaction fronts that may be associated with microbially mediated respiration of organic matter.

Besides, I think the Mars 2020 Science Team's systematic use of seemingly humorous expressions to name localities visited by the rover may have spilled over into naming geological features and objects in a similar fashion, which comes across as a bit 'childish' and lacking seriousness. I find this unhelpful, because these are inadequately descriptive terms that prevent easy comparisons.

While we appreciate the reviewer's perspective on this, the use of informal names for features and investigation targets observed by landed Mars missions has a long history that has become something of an informal convention (e.g., rocks named "Scooby Doo", "Barnacle Bill", and "Yogi" at the Mars Pathfinder landing site, https://www.jpl.nasa.gov/images/pia00780-three-classes-of-martian-rocks/)

The terms "poppy seeds" and "leopard spots" have been used in a 2024 NASA press release:

https://www.jpl.nasa.gov/news/nasas-perseverance-rover-scientists-find-intriguing-mars-rock/

And a more recent Lunar and Planetary Science Conference presentation by the lead author of this manuscript:

https://www.hou.usra.edu/meetings/lpsc2025/pdf/2581.pdf

These terms have garnered attention by the science media and are thus in the public domain, e.g.,

https://www.scientificamerican.com/article/nasas-perseverance-rover-discovers-a-rock-that-may-contain-alien/

https://www.nature.com/articles/d41586-025-00772-2

We believe that, at this point, it would create confusion if we were to retreat from the use of these terms in this publication after having socialized them with the scientific and public community. However, we believe that our more formal descriptions of poppy seeds as authigenic nodules and leopard spots as in-situ reaction fronts (with analogies drawn to reduction halos and reduction spots) should alleviate any confusion and invite comparison to similar occurrences in terrestrial rocks.

From what I could gather in the observations presented, "poppy seeds" are nodular structures 100-200 um in size with dark blue-green colours, whereas the "leopard spots" are also nodular structures 200 um to 1mm in size with multiple colours. While the colours are argued to relate to mineralogy, which is agreeable and probably correct, it is unclear whether there are specific patterns in the nodular "seeds and spots" – the latter seems to be rimmed by the darker phase, but the photos I have are too poor in their resolution.

The reviewer has drawn the correct conclusions regarding the size, color, and mineral properties of poppy seeds and leopard spots. We are uncertain why the resolution of the images in Figs. 3A-C and 5A, S17-S23 came through as low resolution in the reviewer's copy of the manuscript draft. In addition to the lower-resolution jpg images embedded in the manuscript file, we provided high resolution Adobe Illustrator files that should be available to the reviewer. We also provided relevant DOIs in the Data Availability section of the manuscript, which should enable the reviewer to access full resolution versions of all of our images from the NASA Planetary Data System.

Line 84: It is not clearly explained how the BA-MT rock layer could represent the oldest rocks in Jezero crater without presenting clear evidence for overturned beds. Besides, the stratigraphy described in the text is confusing.

The relative age relationships of the Margin unit and the Bright Angel formation remain a point of considerable debate within the Mars 2020 Science Team, with valid arguments on both sides. We feel that it is important to allow for both possibilities at the present time, especially since this issue is not the primary focus of the paper. Detailed follow-on studies focused on the structural relationships between the Bright Angel formation and the Margin unit will work to resolve this debate. We have softened the language related to this issue to make it clearer that, at the present time, there simply isn't a consensus opinion on the Mars 2020 team about the relative stratigraphic placement of these two lithologic units. The updated text can be found on lines 70-73:

"Subsurface structures detected by the RIMFAX ground penetrating radar[13] (GPR, Fig. S2) can be interpreted to indicate that the Bright Angel formation lies stratigraphically above the Margin unit, but at the present time, we cannot rule out the possibility that the Bright Angel formation represents a part of an older unit."

Line 108-109: How does "enhanced cementation" lead to the formation of nodules? This is an unsupported interpretation that weakens the argument, as nodules and concretionary features need to be carefully explained and considered in this manuscript.

We have removed the text "… that may result from enhanced cementation".

Lines 217-222: I do not see how the authors can justify these claims about the composition of "poppy seeds". I see some supporting PIXL analyses (which should be cited there, and shown in Fig. S13), but it is unclear how these minerals were identified only from MgO+FeO, CaO+Na2O, and SiO2.

While the Red-Green-Blue color mapping of the element combination MgO+FeO, CaO+Na_2O, and SiO_2 shown on Fig. S13 E-H provides additional support for the interpretation of the poppy seeds as Fe-enriched masses, the main lines of evidence for their identification come from their enrichment in Fe and P, described on lines 184-187:

"PIXL XRF data indicates that poppy seeds are enriched in Fe, P, and Zn relative to their host mudstone (Figs. 4C, S21A-H). On a ternary diagram of FeO-P-CaO (Fig. 4C), poppy seeds extrapolate towards a molar FeO:P ratio of ~3:2."

And their color properties, described on lines 179-184:

"Dispersed throughout the fine-grained mudstone in the Bright Angel outcrop area, we observe ~100-200 micron circular to irregularly shaped masses that are black to dark blue to dark green colored in daytime and nighttime white LED illumination (Figs. 3A-C, S17A-B to S19A-B). These features are informally named 'Poppy Seeds'. WATSON (Figs. S17C-S19C) and PIXL Micro Context Camera (MCC, Fig. S20) decorrelation stretch (DCS) images enhance the blue to green color of poppy seeds relative to the mudstone they occur in."

And on lines 188-192:

"The color properties of some of the Fe-phosphate masses in this target are distinctive: colorless in daytime WATSON images, tan/orange-toned under nighttime white light LED illumination, and red in DCS images relative to the surrounding white-gray mud (Fig. S22A-C)."

Leading us to conclude on lines 192-197:

"The properties of the poppy seeds indicate that they are accumulations of microcrystalline vivianite ($Fe^{2+}_3(PO_4)_2 \cdot 8H_2O$) or lower hydration state ferrous phosphates (e.g., phosphoferrite: $Fe^{2+}_3(PO_4)_2 \cdot 3H_2O$) and their oxidation products, e.g., metavivianite ($Fe^{2+}_{2.5}Fe^{3+}_{0.5}(PO_4)_2 \cdot 7.5H_2O$), ferrostrunzite ($Fe^{2+}Fe^{3+}_2(PO_4)_2(OH)_2 \cdot 6H_2O$), santabarbaraite ($Fe^{3+}_3(PO_4)_2(OH)_3 \cdot 5H_2O$), which have molar FeO:P ratios of 3:2 and color properties consistent with what we observe in Bright Angel"

Thus, we are unsure why the reviewer suggests that we have inferred the composition of poppy seeds based only on MgO+FeO, CaO+Na_2O, and SiO_2.

Line 232: The Ca-sulfate nodules shown in Fig. S18 contain "poppy seeds" similarly to the mudstone matrix. Hence, the "seeds" could have formed alongside the nodules and during sedimentary diagenesis.

Poppy seed characteristics, abundances, and spatial distribution are completely unmodified by the presence of Ca-sulfate nodules in the Walhalla Glades (Fig S13, S19) and Grapevine Canyon (Fig. S18) targets, suggesting that cm-scale Ca-sulfate nodules encased pre-existing sub-mm poppy seeds that had formed in the host sediment. While we cannot completely discount the possibility that poppy seeds represent a later diagenetic overprint on host sediment that already contained an earlier generation of diagenetic Ca-sulfate nodules, it would seem surprising that an identical distribution of poppy seeds would form inside and outside of the Ca-sulfate nodules given (1) the likely differences in porosity/permeability of the two media (Ca-sulfate nodules and surrounding host sediment) and (2) the more Fe+P poor nature of the parts of the rock that had been mineralized by Ca-sulfate nodules. We have updated the text in this section to make the relationships between (early) poppy seeds, (later) Ca-sulfate nodules, and an additional (even later) generation of Ca-sulfate fracture fill clearer.

The formation of the sulfate nodules (suggested on lines 238-239) is considered as nodules or detrital clasts, both of which are considered to be non-biological in origin on Earth. Hence, it is unclear what is interpretable as a biosignature here.

We did not suggest that the Ca-sulfate nodules are detrital and have updated the text on line 204 to describe them as "diagenetic" Ca-sulfate nodules. Whether detrital or diagenetic, however, we agree with the reviewer that Ca-sulfate is unlikely to be biological in origin and we have not suggested that it is anywhere in the manuscript.

Lines 300-315: This explanation is agreeable, but it does not convince that there is a plausible or even a possible biosignature in the new observations. Again, this is more likely to be prebiotic type chemical reactions with non-biological organic matter, known to occur in several places on Mars.

We appreciate the reviewer's agreement with our description of the chemical reactions that were likely to have taken place to give rise to the poppy seeds and leopard spots in the first 2 paragraphs of the section named "A Potential Biosignature in Bright Angel". The subsequent 2 paragraphs are devoted to possible abiotic explanations for the reaction chemistry described, concluding with: "In summary, available data suggest ferric iron- and phosphate-rich sediment was delivered to Bright Angel in the presence of organic carbon compounds, which could have been

produced on Mars through abiotic synthesis[57,58] or delivered from non-biological exogenic sources[57,59]. Upon burial, interaction of Fe^{3+} and PO_4^{3-} bearing sediment grains with the organic matter and/or dissolved sulfide could have resulted in the reductive dissolution of Fe^{3+} followed by the precipitation of vivianite. The system must also have had sufficient dissolved sulfide to facilitate the precipitation of Fe-sulfide. Abiotic scenarios resulting in the production of the phases observed in Bright Angel should be tested by laboratory experimentation under a range of relevant conditions."

Given the range of chemistries currently being explored for "prebiotic" scenarios, e.g., cyanosulfidic reaction networks (J. Sutherland and others), hydrothermal synthesis in deep seafloor (M. Russell and others) and near surface volcanic-hydrothermal settings (D. Deamer and others), we feel that it is beyond the scope of this report to try to fit our findings into any one specific prebiotic chemical scenario. As such, we have opted to describe potential abiotic chemical reaction scenarios that are agnostic with respect to any one style of "prebiotic" reaction chemistry.

Lines 351-352: "... one would reasonably assume that microbial metabolisms contributed to its formation" (of Bright Angel), however, I do not see how this would be possible even on Earth. Microorganisms do not directly produce nodules, let alone sulfate or vivianite nodules. There is no experimental nor Earth-based observational basis for this claim.

We reiterate that we do not describe Ca-sulfate nodules as potentially biological in origin. Nor do we claim that microorganisms directly produce nodules. However, "direct" microbial production is not required for many structures to be considered biosignatures (e.g., stromatolites, where microorganisms indirectly promote the conditions for the defining processes of lithification and lamination, e.g., Grotzinger and Knoll, 1999). More generally, microbial processes are well-known to promote the formation of concretions through local precipitation of authigenic minerals (e.g., Coleman and Raiswell, 1993; Loyd et al., 2012; Plet et al., 2020; Loyd et al., 2023).

Similarly, vivianite and greigite (which can be found to form nodules) are a byproduct of microbial metabolisms that alter their environment through the use of Fe(III) and S(VI) as terminal electron acceptors for the respiration of organic carbon. These microbially mediated reactions produce energy via the combination of reductants available in the environment with organic carbon, releasing dissolved Fe(II) and S(red.), which combine to precipitate phases such as vivianite and Fe(II)-sulfide. As such, these chemical reactions and their mineral byproducts qualify as chemical, organic, and mineral biosignatures (e.g., Banfield et al., 2001, Mineralogical Biosignatures and the Search for Life on Mars; Mustard et al., 2013, Report of the Mars 2020 Science Definition Team.).

Nevertheless, we appreciate the reviewer's comment that the quoted statement in the manuscript, and the references supporting that statement, were inadequate to demonstrate the possibility of a biologically promoted origin for sub-mm vivianite nodules and reaction fronts containing vivianite rims and bleached, Fe-sulfide bearing cores. We have therefore significantly rewritten the paragraph (lines 302-317) containing this statement and updated the references that support it (using several new references suggested by Reviewer #3). We hope that the reviewer will find our updated arguments more convincing.

Figure 3: These Raman spectra are all very noisy and while they show what could be a G-band (with sp2 hybridised carbon – PAHs?), that peak is unusually broad, which is unlike what is expected for graphite, and there is also no D-peak, as would be expected for sedimentary organic matter in mudstone. These two observations are left without explanation, which weaken the story. The Raman spectra also show resolved peaks around 1010 cm-1, suggestive of pyroxene and

other peaks around 1090 cm-1 suggestive of carbonate. This is similar to observations from ALH84001 orthopyroxenite.

We agree with the reviewer's assessment that we are not seeing graphite in the SHERLOC Raman spectra presented here. Regarding the nature of the organics, as the reviewer no doubt appreciates, the shape of the G-band can be used as a metric for the thermal maturity of the material and for deep-UV Raman measurements, while the D-band (~1350 cm−1) is highly reduced in its intensity relative to the G-band as compared with measurements made using visible laser wavelengths (see: https://www.liebertpub.com/doi/full/10.1089/ast.2021.0135). Detailed analysis of the shape of the G-band, the search for an associated D-band, and the details of additional mineral detections that can be derived from SHERLOC Raman spectra from the Bright Angel formation, are the topic of a forthcoming publication. In this contribution, we report the detection of organics within the Bright Angel formation as a potential driver of reduction chemistry associated with the development of poppy seeds and leopard spots.

Figure 4: I do not understand why pyrite, greigite or pyrrhotite can even be mentioned in this plot since none of them contains any oxygen. I believe this is a misrepresentation that is both confusing and false.

The use of FeO, SO_3, and CaO as apices of the ternary diagram is simply based on standard reporting conventions for the quantification of elements by X-ray fluorescence. Because the diagram plots the molar abundances of each element oxide (as described in the figure caption), the plotted position of the relevant sulfide minerals depends only on their cation stoichiometry. Thus, e.g., pyrite (FeS_2) plots along the FeO-SO_3 axis at 33%FeO: 67%SO_3 because there are 2 S atoms for every Fe atom in pyrite. The plotted positions of our analyses and mineral endmembers would remain the same on this diagram if it were constructed as an Fe-S-Ca ternary. To alleviate any potential confusion on this subject, we have replotted Figure 4D as a diagram of the molar abundances of Fe, S, and Ca.

Figure S9 and S10: Very little context is given to understand what is shown. The plot in S9A only gives "other natural rock surfaces" as a context, but this is not specifically related to a measure of Fe-oxides.

We have added laboratory data of ferric and ferrous minerals to S9A for additional context.

Similarly, in Fig. S9B, there should be relevant standard spectra shown for context and comparison. I also wonder about the meaning of the technicalities mentioned in the caption and whether this belong in the methods sections, possibly in a table (for instance, "offset +0.1" – What does that mean? Offset from what? In what units?). For instance, Table (S1) in the S.I. is a very useful guide to organize the data presentation. The caption should explain what is shown in the images, so that the observations have a scientific value. As currently presented, I find limited scientific value for that spectral data.

We have added laboratory spectra of ferric and ferrous minerals to S9B for additional context. We have moved the technicalities that had been in the figure caption to a table in the SI Text, and we modified the figure captions for S9A and S9B to emphasize the scientific value of these data.

Figure S11: The correlation of the measured spectra with the identified “vivianite” spectrum is very poor. The four spectra shown in panel e are much more like hydrated calcium sulfate than vivianite.

We agree with the reviewer that the mean spectrum of the Apollo Temple abraded patch is likely dominated by absorption features associated with hydrated Ca-sulfate (especially bassanite), which is consistent with the presence of a light-toned vein in the area sampled by the SuperCam infrared spectrometer (Fig. S11b). Kolb_Arch #1 is also a vein (Fig. S11c) and the spectrum is also dominated by Ca-sulfate. The two other spectra – Walhalla Glades mean and Kolb Arch #8 (matrix) – correspond to areas with no discernible veins (Fig. S11a, c) and have distinct shapes compared to Apollo Temple and Kolb Arch #1 (no sharp drop of reflectance at 2.4 μm, in particular), indicating that they are more representative of the matrix material where the Fe-phosphate masses are found. Though we cannot rule out a contribution from small Ca-sulfate grains dispersed in the matrix, these two spectra likely bear signatures of other minerals present in the rock. The spectra plotted in Figure S11f represent some of the candidate species that could explain the observed spectral features. We included vivianite for completeness, given its identification by PIXL, but there are two important caveats to consider. First, owing to the small areal abundance (7% in the Walhalla Glades PIXL scan, see **SI Text**) and the small size of the individual Fe-phosphate masses (~0.03 mm^2 for a 200-μm poppy seed) relative to the analytical spot size of the SuperCam IR measurements (~6 mm^2 for a target 2.4 m away from the instrument), spectral features associated with vivianite are unlikely to dominate over those of other phases present within the analytical area; in other words, the overall shape of the SuperCam IR spectrum is unlikely to match closely the laboratory spectrum of vivianite, given its relatively low abundance. Second, the color properties of the Fe-phosphate indicate that the vivianite nodules have undergone some amount of post-emplacement oxidation, resulting in their dark green to dark blue to black coloration, and thus there may be a range of Fe-phosphate mineral species that provide better IR spectral matches. Unfortunately, ferrous and partially oxidized Fe-phosphate minerals are not well-represented in spectral libraries and so comparisons between mineral reference IR spectra and Bright Angel targets will have to await future studies. With these caveats in mind, it is nonetheless possible that the positive slope between 1.3-1.8 microns in the IR reflectance spectra from the targets Walhalla Glades and Kolb Arch #8 (matrix) has contributions from vivianite, which also exhibits a positive slope in this region.

In any case, the strongest argument for the identification of the Fe-phosphate mineral comes from PIXL XRF analyses and close-up color images collected by the WATSON and PIXL MCC cameras. These instruments have resolution limits (0.120 microns/pixel for XRF and 10’s of microns/pixel for the camera images) that are comparable to the size of the poppy seeds and leopard spot rims. Analyses by these instruments indicate that poppy seeds and leopard spot rims are composed of a 3:2 Fe:P mineral with color properties consistent with vivianite and its related oxidation products.

Besides, even if the author were correct with that interpretation of vivianite (and they are not at all convincing about this), why would vivianite be a plausible biosignature? To my knowledge there is an absence of peer-reviewed publications on this subject and the manuscript cites six poorly relevant references on this topic (refs 60-66).

Please see our previous response to the reviewer’s related comments.

Also, in figure S11d, I do not think most of the community is familiar with “swarm plots” so please explain what the X-axis mean and the mirror patterns imply.

We have updated the caption text to describe the use of a swarm plot. The updated text reads: “A challenge in representing categorical data with a scatter plot is that all of the points belonging to one category would fall on the same position along the axis corresponding to the categorical variable. Swarm plots solve this problem by adjusting the points along the categorical axis using an algorithm that prevents them from overlapping, thus providing a better representation of the distribution of observations. The swarm plot algorithm is part of the Seaborn Python data visualization library (https://seaborn.pydata.org/index.html).”

REVIEWER #2

Review of "**Detection of a Potential Biosignature by the *Perseverance* Rover on Mars**" submitted by Hurowitz et al to Nature

General Comments

This paper synthesizes data collected by multiple instruments on the Perseverance rover to pull together a tight story on the recent detections of some unusual combinations of minerals and organic material. Claiming to detect biosignatures on another planetary body is a big step and should not be accepted lightly, and I admit that at first I was a bit skeptical of this idea. However, after careful reading of this manuscript and scrutiny of the data presented, I support the authors' main conclusions. I have identified a few places in the manuscript where additional clarification or explanations could be beneficial. In particular, it would be helpful to include more information on how common or widespread the poppy seeds and leopard spots are at the different sites studied by the instruments on Perseverance because the chemistry and mineralogy varies across the regions investigated and the paper would be improved with a clearer understanding of these relationships.

I have conducted a number of field and lab studies with similar observations to some parts of this study and I feel confident in accepting that the unique combination of ferrous phosphates, ferrous sulfides, and organic hydrocarbons together are unusual and represent at the least a remarkable occurrence of abiotic chemistry on Mars. These findings also represent the very real possibility that microbes participated in formation of these reduced minerals. Of course we can't know which of these pathways (or both) may have occurred from the data collected by Perseverance and we may never know, even after analyses of the returned samples, but the "Detection of a Potential Biosignature" is a valid conclusion from this data in my opinion. My major disappointment in reading this paper is that only one sample was collected here to be returned to Earth for more detailed analyses.

This paper presents exciting new findings that will have broad implications for our understanding of biogeochemistry and astrobiology on Mars and potentially leading to addressing the bigger concept of life outside of Earth. I support publication of this study in Nature following consideration of a few minor comments and suggestions outlined below.

Should the authors have questions regarding my review, they are welcome to reach out to me directly: Janice Bishop – jbishop@seti.org.

We thank Dr. Bishop for this positive summary, and we are excited to get the results presented in our manuscript out to the scientific and public communities.

Specific Comments

Line 67 I suggest changing “orbiter spectroscopic signatures” to “orbital spectroscopic signatures”.

Changed.

Line 112 does “layered outcrop” here refer to just the rock containing Steamboat Mountain or both rocks in Fig. 2 ?

The layering refers specifically to the rock containing the targets Apollo Temple, Cheyava Falls, and the core sample Sapphire Canyon. We have updated the caption for Fig. 2 to help clarify these relationships as follows:

Figure 2 The Beaver Falls Workspace: Mastcam-Z image mosaic of the Beaver Falls workspace on sol 1217. The light-toned layered block contains the Cheyava Falls natural surface target, the Apollo Temple abrasion, and the Sapphire Canyon core sample location. The Sapphire Canyon sample was collected from approximately the same location as Cheyava Falls after analysis of the target was completed. The darker-toned granular block contains the Steamboat Mountain abrasion. Scale bar is 10 cm, downhill is to the left on this image. Mastcam-Z enhanced color RGB vertical projection mosaic from sol 1217, sequence zcam09264, acquired at 110-mm focal length. [NASA/JPL-Caltech/ASU/MSSS].

Lines 112-114 This is a long sentence and I found it confusing. I suggest breaking this into 2 sentences and more clearly defining where the layered outcrop is located and on what scale the layering is observed.

In concert with the revisions to the Figure 2 caption, we have also modified the paragraph containing this text as follows:

An example of layering in the Bright Angel formation is visible in the “Beaver Falls” workspace (Fig. 2). Here, the rover’s science instruments (PIXL, SHERLOC and WATSON, Supercam and Mastcam-Z) analyzed a rock containing the targets “Cheyava Falls” and “Apollo Temple”, and a core sample, named “Sapphire Canyon”, was collected. In this rock, layering is expressed by cm-scale, reddish- to tan-colored, recessive bands that are separated by thinner, relatively resistant, light-toned bands (Fig. 2, 3A). The layers in this rock dip more steeply and strike at an angle to layers in nearby outcrop, implying that it may have been displaced from its original orientation. In general, macroscopic rock textures in the Bright Angel formation are diverse and complex. Intervals of rock are variously wind-fluted and massive in appearance (Fig. S3A), show large (cm-scale) nodular features (Fig. S3B), and are layered and crosscut by light-toned erosionally resistant and mineralized fractures and veins (Fig. 2, S3A, S3B). These features challenge the identification of primary depositional structures in much of the Bright Angel formation.

Lines 132-133 and Figure S9A-B Please define how you determined the "528 nm band depth" parameter.

We added this definition, in addition to other band parameters shown in Figures S9-11, to a new Table S2 in the SI Text.

Line 136 imparts should likely be impart here. I believe this refers to "abundances" but it could also refer to "features" and "ratios" from earlier in the sentence. Perhaps reword to clarify that.

Sentence has been clarified.

Line 140 I suggest adding "~" before 1.92 μm as the band center and shape varies a bit for this band based on the data shown in S11E.

Changed.

Line 151 I suggest mentioning here that Raman fluorescence is also caused by nanophase clays and iron oxides. This is in contrast to the D and G bands that are only due to C in graphite, diamond or hydrocarbons.

In the caption for Figure S12, we touch on some of the possible causes for this continuum fluorescence:

"Properties that could produce the continuum signal include i) fine-grain scattering; ii) luminescence related to impurities or radiation-induced defects, possibly in sulfates; iii) organic fluorescence."

We suspect that if the fluorescence were due to np-Fe-oxides, then it might express variations that correspond to the variations in the abundance of Fe^{3+} that are displayed in Figures S9A, S9B, and S10. Instead, the target with the strongest Fe^{3+} signature does not exhibit Raman fluorescence, whereas the target with the weakest Fe^{3+} signature shows the strongest Raman fluorescence. This leads us to suspect that Fe-oxide induced fluorescence is less likely. The similar XRF compositional properties displayed by the Malgosa Crest and Apollo Temple targets indicate that they are both Al- and Si-rich. This probably indicates that both are clay-bearing, and yet only Apollo Temple has the Raman fluorescence signature. In Apollo Temple, the fluorescence signal is accompanied by a Raman G-band detection. These relationships seem more consistent with organic matter as a driver of the observed Raman fluorescence, though we cannot rule out the possibility of "impurities or radiation-induced defects, possibly in sulfates". Given these uncertainties, we prefer to leave the rather cautious language that is in the original text and leave a more detailed analysis of the Raman continuum fluorescence to future study.

Lines 145-151 This paragraph describing the Raman spectra does not mention the D bands due to disordered C that is frequently paired with the G (graphitic) bands in Raman spectra including organic C. See for example Ferrari Robertson (2001), Schopf et al. (2002) and Bishop & Murad (2004) for descriptions of the Raman D and G bands and fluorescence in Raman spectra. I don't see the D band in your spectra – is that because of the offset distance or do you think it would not be present in any case? This may have implications for the form of the C in your samples and would be helpful to address here. You may be able to constrain the type of C if only the G band is observed.

In Deep UV Raman measurements, the D-band (~1350 cm−1) is highly reduced in its intensity relative to the G-band as compared with measurements made using visible laser wavelengths (see Osterhaut et al. 2022, Astrobiology). This issue, combined with the focus offset described in the Methods section, may be the reason why the D-band is absent in SHERLOC Raman spectra from Bright Angel targets. The broad nature of the G-band, however, indicates that it is not graphite and is likely more consistent with some form of macromolecular organic matter. The details of the Raman spectra and the inferences that can be drawn regarding the specific nature of the organic matter is the subject of a forthcoming publication.

Line 178 The fluorescence observed in Raman spectra of the Steamboat Mountain mudstone could be due to nanophase iron oxides. I'm guessing that if clays were present to cause the fluorescence then organics would have been present as well since clays are good carriers of organics.

See our response to the comment on this topic above.

Lines 211-212 This sounds to me that XRD did not identify any phosphate minerals where the PIXL XRF observed elevated Fe and P. I'm wondering if some of this P could amorphous, similar to the amorphous P found by Curiosity at Gale crater (see Rampe et al., 2016).

The Rampe et al. (2016) study was focused on the possibility of chemisorbed phosphate and sulfate on allophane and ferrihydrite in sedimentary rocks on Mars. This chemisorbed phosphate is exactly the source of the phosphate we call on to participate in vivianite precipitation after the Fe^{3+}-bearing minerals that it is adsorbed to are reductively dissolved. We have added the Rampe et al. (2016) reference to our manuscript to strengthen this argument and thank the reviewer for pointing us to it. As for the PIXL diffraction results, all we can say is that the Fe-phosphates are smaller than ~40 microns in diameter because this is the grain size at which we lose the ability to detect diffraction. So unfortunately, we are unable to comment on whether the phosphate is crystalline but fine-grained or amorphous.

Lines 217-223 The phosphates mentioned here as possibilities present in the poppy seeds include ferrous and ferric phosphates. The text seems to

suggest that ferrous phosphates were present and then potentially oxidized to ferric phosphates. This is contradictory to the theme expressed elsewhere that ferric minerals were present that were reduced to ferrous minerals as the organics were oxidized. Of course Mars has a long history and much oxidation and reduction could have taken place. However, I suggest deleting the idea that the ferrous phosphates could have oxidized again through alteration as that muddies your premise. Instead, it could be that some ferric phosphates were present initially, and then were reduced to ferrous phosphates through oxidation of the organics.

Direct precipitation of ferric phosphates (as opposed to their formation by oxidation of a ferrous phosphate, such as vivianite) require low pH conditions to mobilize Fe^{3+}(aq) and concentrate it to the point that saturation with respect to a ferric phosphate is achieved. Such conditions would also be expected to mobilize Al^{3+}(aq), which can then precipitate to form phases such as $AlPO_4.2H_2O$ (variscite). As described in the manuscript, we see no evidence for Al-phosphate minerals in the Bright Angel formation (see esp. Fig. S21H) that would suggest low-pH conditions appropriate to the transport of Fe^{3+} and Al^{3+} in solution. This is in spite of the fact that the Bright Angel formation is the most aluminous suite of sedimentary rocks yet encountered by Perseverance in Jezero crater. The observed enrichments in the trace elements Zn^{2+} and Ni^{2+} in the Fe-phosphate-rich poppy seeds and leopard spot rims instead suggest that redox conditions were more appropriate to the mobilization of 2^+ cations (including Fe^{2+}) to the site of Fe-phosphate precipitation. This mobilization apparently occurred without similar mobilization of 3^+ cations, and so moderate pH and low Eh conditions are more probable. Therefore, we suggest that if the Fe-phosphates in the Bright Angel formation contain Fe^{3+}, that this is more likely a result of oxidative weathering of ferrous phosphate in these rocks during more recent exposure. These points are detailed in the manuscript on lines 257-262.

From the SuperCam NIR spectra in Figure S11E the increasing slope noted from ~1.3 to 1.8 μm is consistent with Fe2+ in minerals in mean spectra from Walhalla Glades and the Kolb Arch matrix at spot 8. This slope increase is characteristic of many minerals containing Fe2+, but less common for minerals with Fe3+ (see Bishop, 2019). Fe3+ - minerals generally increase in slope from ~1 to 1.3 μm then plateau from ~1.3 to longer wavelengths. Does the Walhalla Glades site have more poppy seeds than the Apollo Temple site? or are they different? How do you explain a higher abundance of Fe2+ at the Walhalla Glades and Kolb Arch #8 sites?

The SuperCam NIR observation of the Apollo Temple target sampled the edge of a light-toned vein (Fig. S11b), which resulted in the spectra being dominated by features associated with Ca-sulfates, as shown by the comparison with Kolb Arch #1, a vein more directly "hit" by the spectrometer. In contrast, there are no discernible veins near the field of view of the Kolb Arch #8 (Fig. 11c) and Walhalla Glades (Fig. S11a) observations. Therefore, the differences that the reviewer noted in the 1.3-to-1.8-μm slope is likely due to the variable contribution of Ca-sulfates to

the spectra, and not necessarily to differences in the Fe^{2+} content of the matrix.

We have updated the figure and caption to make these points clearer:

[…] b) RMI context mosaic of abraded target Apollo Temple. Note the presence of multiple light-toned veins in this abraded patch, including one in the area where the SuperCam spectra were collected. c) RMI context mosaic of natural target Kolb Arch. This target is located on the same bedrock block as the PIXL target Cheyava Falls. SuperCam spectrum #1 is taken directly on a light-toned vein, whereas spectrum #8 is taken on the matrix material. […]

Lines 213-214 The text here states that Fe-P masses were observed at Apollo Temple but the mean NIR spectrum of Apollo Temple in Figure S11 is not consistent with a ferrous phase. This would indicate that the phosphate mineral is more likely to include Fe3+.
Spectra of strengite and strunzite in this region are similar to the Apollo Temple mean spectrum and would not be inconsistent. Or it could be that the poppy seeds and leopard spots only comprise a small fraction of the Apollo Temple surface or are below the surface rather than at the surface and the ferrous phosphate phase is not spectrally dominant.
However, past studies have shown that only a tiny amount of Fe2+ is needed in carbonates to greatly influence the spectral slope from ~1.3-1.8 μm (Bishop et al., 2013, 2021). If the authors believe that a ferrous phosphate component is present at the Apollo Temple site it would be helpful to include the rationale behind this and how this is consistent with the spectra shown in Figure S11e.

As stated in the response to the previous comment, we believe that the spectra of Apollo Temple are dominated by features associated with Ca-sulfates, which are likely masking contributions from minerals present in the matrix. On the other hand, the Kolb Arch #8 spectrum, which sampled the same block as Apollo Temple (just a few tens of centimeters away, see Fig. 2) but is vein-free (Fig. S11c), does show the same positive 1.3-to-1.8-μm slope as observed in Walhalla Glades (Fig. S11e) and is thus consistent with the presence of a ferrous phase in the matrix of this rock.

We also touch on this issue and the possibility of ferro- or ferristrunzite in one of our responses below, starting on page 9 of this document.

Is there any correlation between this increase in spectral slope and P? The phosphate minerals gormanite-souzalite [$(Fe^{2+},Mg)_3(Al,Fe^{3+})_4(PO_4)_4(OH)_6 \cdot 2H_2O$; with more Fe2+ in gormanite and more Mg in souzalite] includes Fe2+ and Fe3+ and less water than vivianite and has a stronger spectral slope from ~1.3 to 1.8 μm (see Bishop et al. 2024, EGU abs); thus gormanite could be a potential candidate for a ferrous phosphate if present at the Walhalla Glades and Kolb Arch spot 8 sites.

We thank the reviewer for this interesting suggestion. However, because the

SuperCam NIR observations of the Apollo Temple and Walhalla Glades abraded patches do not overlap with the PIXL scans of these targets (Fig. SX), we cannot investigate the correlation between the NIR spectral slope and the P abundance. We also note that the individual spectra of Walhalla Glades are very homogeneous in terms of 1.3-to-1.8-μm slope.

Line 272 Burns and Fisher (1990) would be a better reference for jarosite as an oxidation product of Fe sulfides on Mars.

Changed to the suggested reference.

Line 275 The Mastcam-Z spectra and Super Cam NIR spectra are not consistent with a strong ferrous component that is characteristic of siderite. The Fe2+ band is particularly strong for this mineral (Bishop et al., 2021). Siderite is also usually brownish or tan rather than red. I suggest that ferrihydrite or a similar poorly crystalline ferric hydroxide phase is contributing the red color there.

As shown in figure S25, there is only one small area in the abrasion patch associated with red-brown coloration, low SO_3, Al_2O_3, SO_3 and low analytical totals. This area is likely no larger than ~250x250 microns, with a few isolated spots ≤ 100 microns. Given how small these spots are, and how few of them there are, we suspect that they would have limited ability to influence the reflectance spectrum in Fig. S9B, which represents the spectrum of the entire 5 cm diameter abrasion patch. Nevertheless, we have modified the text slightly in the manuscript and the caption for Figure S25 to indicate that these small areas have a reddish-brown color (which might be more consistent with siderite) and agree that this color could also be due to the ferric oxide/oxyhydroxide minerals mentioned by the reviewer, which themselves could be oxidation products of siderite. To enable the reader to evaluate this possibility, we have added a ferrihydrite spectrum and spectra of other poorly crystalline ferric materials to Fig. S9B as examples of possible contributors to the red-brown color.

Lines 290-291 Please explain how Ca-phosphate was identified in Figure 4C

This line in the manuscript was intended to indicate that we do not identify Ca-phosphate in the Bright Angel formation. The presence of Ca-phosphate would be indicated by XRF data points scattering towards the locations of apatite or merrillite, which are plotted on Fig. 4C. This is not observed, and so it is unlikely that Ca-phosphate is present. We have reworded this sentence to make this clearer.

Line 303 It may not be necessary to oxidize the C in organic matter and reduce the Fe3+ in an aqueous environment. This process was observed by Campbell et al. (1997) through heating ferrihydrite mixed with organic C in a dry environment and replicated by Bishop et al. (2006). When no C was present, hematite was produced by heating ferrihydrite. When C was present in the ferrihydrite sample, magnetite was produced from heating the sample and if at

least a small amount of O2 was present, then the magnetite partially oxidized to maghemite.

In the manuscript, we have made every effort to determine whether the rocks in Bright Angel were heated by contact metamorphism or hydrothermal fluids. Within the limits of our data, we cannot find any evidence for such processes.

Lines 306-308 Sulfate reduction to sulfide was also observed in the anoxic zones of Lake Hoare in Antarctica, where microbes facilitated formation of tiny pyrite deposits on quartz grains (Bishop et al., 2003). No need to cite this study – I just wanted to point out that this has been observed in the cold lakes of Antarctica.

Interesting – thanks for point us to this occurrence!

Lines 344-346 Would it fit your model for the Apollo Temple site measured by the SuperCam NIR spectrometer to have ferric phosphate that was then reduced to ferrous phosphate at the Walhalla Glades and Kolb Arch sites?

See our response above to the comment about Lines 217-223.

Line 397 what excitation wavelength was used for the SHERLOC Raman measurements?

248.6 nm, now stated in the Methods

Line 412 please define DUV

Deep ultraviolet, now stated in the Methods

Supplementary Information
Table I suggest adding the Kolb Arch site to this table and also a column about the relative abundance of poppy seeds and leopard spots for each target.

We have added all natural surface targets to the SI Table, including Kolb Arch, Tanner Canyon, and Bass Camp to the table. A quantitative analysis of the relative abundance of poppy seeds and leopard spots at each target in the table is beyond the scope of this effort especially since the targets have not all been analyzed by the full instrument payload. However, we have added columns to the table indicate whether these features were observed.

Figures S9A&B The Mastcam-Z ratio trends shown in Figure S9A indicate that the Apollo Temple sample contains less Fe3+ than other samples. I agree that the Fe bands are weaker overall for this sample, but the spectral shape of the data shown in Figure S9B all resemble Fe3+ rather than Fe2+. The text does not say that Fe2+ is present but that is often a logical interpretation for samples

containing less Fe3+. I suggest clarifying that in the caption. It is possible that there is some Fe2+ in some samples, particularly #4,5,6 in Figure S9B, but in my opinion there is more Fe3+ than Fe2+. These data could be consistent with some Fe2+ minerals including low-Ca pyroxenes, but high-Ca pyroxenes, olivines, carbonates, ferrous sulfates, and Fe2+ glass all have Fe2+ bands that extend past 1 μm and are not consistent with any of the spectra shown in Figure S9B. I do agree that the Malgosa Crest Abrasion tailings spectra have more Fe3+ character than the other spectra shown in Figure S9B.

A good point, and to address this, we have included example laboratory spectra of several ferric and ferrous minerals for comparison in Figures S9A&B. It is true that the Mastcam-Z spectra of the BA targets do not have the negative NIR slope indicative of Fe^{2+} for olivines and high-Ca pyroxenes. However, their ~900 nm bands and lack of 528 nm bands are consistent with low-Ca pyroxenes, perhaps in mixture with ferric materials and/or spectrally neutral phases. To clarify this, we have added the following to the captions:

The MT spectra from Malgosa Crest (1-2) exhibit deep 528 nm band depths, consistent with a strong ferric contribution; the BA spectra (3-6) have weak to no 528 nm bands, consistent with ferrous minerals or a mixture of phases.

Further, the Apollo Temple sample bands looks more like Fe3+ phosphates to me than Fe2+ phosphates and the Apollo Temple data in Figure S9B look similar to Pancam convolved channels of strunzite [$Mn^{2+}Fe^{3+}_2(PO_4)_2(OH)_2\ 6H_2O$], (see Lane et al., 2007, 7thMars).

Interesting! Yes, the strunzite spectrum looks like decent match. We note, however, that the Bright Angel formation rocks are unusual relative to all other rocks encountered by Perseverance in having little to no detectable manganese as determined by PIXL XRF chemical analysis (SI Tables). This is true both inside and outside of the poppy seeds and leopard spots. Therefore, strunzite, which is a nominally Mn-bearing ferric phosphate, cannot be reconciled with the chemical properties of the Bright Angel formation.

On the next page, we have compiled a list (from Webmineral.com) of the chemical formula and color properties of potentially relevant phosphate minerals, many of which are mentioned by the reviewer. Those highlighted in yellow contain Mn in their nominal chemical formulae and are discounted at present based on the exceedingly low Mn-content of Bright Angel formation phosphates. Those highlighted in orange have Fe:P ratios that are strongly inconsistent with the observed 3Fe: 2P ratio observed in Bright Angel formation phosphates (**Figure 3C**) and are also discounted. We also discount giniite – though it has a nearly appropriate stoichiometry (5Fe:4P) and appropriate color properties, Adcock et al. (2023) American Mineralogist report that it is most frequently found as the product of hydrothermal alteration of pegmatitic and iron ore bodies, and laboratory syntheses indicate it is most readily formed at temperatures >150°C and at exceedingly low pH (see also Hausrath et al., 2024,

Minerals). These conditions of formation are inconsistent with our observations of the Bright Angel formation, and giniite is also discounted as a likely possibility.

The remaining phases, which are not highlighted, include minerals such as ferri- or ferrostrunzite, beraunite, phosphoferrite, metavivianite, and santabarbaraite. These all have the appropriate stoichiometry and most have color properties appropriate to our observations of poppy seeds and leopard spot rims. The unusual color properties of the Fe-phosphates in the Apollo Temple abrasion ("colorless in daytime WATSON images, tan/orange-toned under nighttime white light LED illumination, and red in DCS images relative to the surrounding white-gray mud") might indicate that metavivianite, santabarbarite, or ferri/ferrostrunzite are the best matches. All these phases are possible oxidation products of vivianite (e.g., Nriagu and Dell, 1974, American Mineralogist; Pratesi et al., 2003, European Journal of Mineralogy). However, if the phosphates in Apollo Temple are in fact colorless, then vivianite and/or phosphoferrite might be more appropriate.

We have updated the manuscript text to include the possibility of ferri/ferrostrunzite and thank the reviewer for pointing this possibility out to us. A more detailed comparison of laboratory-derived mineral spectra to ZCAM-based observations of Bright Angel targets will await future publication on this specific topic.

Figure S11-panels A,B,C Most of the white, red, and yellow markings are dilicult to read, even when zoomed in to the max possible in the pdf. I suggest thicker lines, bolder text and more color contrast.

	Fe2+	Mn2+	Fe3+	PO4	OH	H2O	Fe:P	Fe	P	Color
Vivianite	**3**			**2**		**8**	**3:2**	**60.0**	**40.0**	**Colorless, Green, Blue, Dark green, Dark bluish green**
Rockbridgeite*	1		4	3	5		5:3	62.5	37.5	Green, Dark olive green, Black, Brownish green, Reddish brown
Metaviviante	2.5		0.5	2	0.5	3	3:2	60.0	40.0	Leek green, Gray green, Reddish brown
Santabarbaraite			3	2	3	5	3:2	60.0	40.0	Light brown, Brown
Phosphoferrite	3			2		3	3:2	60.0	40.0	Colorless, Light green, Dark reddish brown, Reddish brown.
Ludlamite*	3			2		4	3:2	60.0	40.0	Apple green, Colorless, Green, Greenish white, Light green
Beraunite	1		5	4	5	4	6:4	60.0	40.0	Green, Greenish brown, Gray green, Dark greenish brown, Dark red
Lipscombite*	1		2	2	2		3:2	60.0	40.0	Green gray, Olive green, Black
Ferristrunzite			3	2	3	6	3:2	60.0	40.0	Brownish yellow, Light yellow
Ferrostrunzite	1		2	2	2	6	3:2	60.0	40.0	Yellow brown, Light brown, Colorless
Giniite	1		4	4	2	2	5:4	55.6	44.4	Blackish brown, Black, Blackish green
Strengite			1	1		2	1:1	50.0	50.0	Colorless, Pale violet, Deep violet, Red, Carmine red
Strunzite*		1	2	2	2	6	1:1	50.0	50.0	Brownish yellow, Golden yellow, Straw yellow
Laueite		1	2	2	2	8	1:1	50.0	50.0	Yellow orange, Honey brown, Light brown
Tinticite**			5	5		7	1:1	50.0	50.0	White, Light brown, Pale yellowish green
*Nominal formula also contains Mn2+ in the Fe2+ site										
**Nominal chemical formula contains a VO4 anion in the PO4 site										

We have updated the figure to make the markings thicker/larger as suggested.

Figure caption for S11D I suggest adding "~" before 1.92 μm as the band center and shape varies a bit for this band based on the data shown in S11E.

We agree that the band center varies a bit, however the central wavelength used for the band depth calculation is 1.92 μm for all data points, so we don't think the "~" symbol is necessary here.

Figure caption for S11E I feel that the paper should say a bit more about NIR spectra and mineralogy. I understand that space is limited in the main text so perhaps this figure caption is the best place to add that.

Apollo Temple and Kolb Arch #1 have stronger bands near 1.92 μm and a bigger drop near 2.4 μm, while Walhalla Glades and Kolb Arch #8 include an increase in slope from 1.3-
1.8 μm, consistent with ferrous iron. I have prepared some plots of your data with some mineral spectra that might be useful to you in thinking about these spectra. While you may not feel comfortable identifying the smaller features in the spectra until the calibration is sorted out, these larger trends should be described a bit more to contribute to the mineralogy understanding of the sites investigated.

We thank the reviewer for their helpful suggestions and their detailed examination of the NIR spectra. We have updated the figure caption to incorporate some of these suggestions. However, as the reviewer noted, space is limited in this manuscript, which is focused on the proximity science results. A more comprehensive analysis of the SuperCam observations of these rocks will be the subject of a forthcoming publication.

REVIEWER #3

Referee #3 (Remarks to the Author):

A.Summary of key results
The manuscript “Detection of a potential biosignature by the Perseverance Rover on Mars” reports findings of a discovery from the Mars 2020 mission. One of the goals of the Mars 2020 mission is to search for signs of past life on Mars; if biosignatures are identified, appropriate samples would be returned to Earth for further analyses.

The study reports the analysis of several outcrops in a sedimentary unit in Jezero Crater on Mars. The “potential biosignature” is found in several outcrops of the “Bright Angel” subunit. The biosignatures are features enriched in Fe-phosphate minerals (e.g. vivianite) and in Fe-S minerals (e.g. greigite) that correlate with the presence of organic carbon. The formation of these features can be explained by both abiotic and microbial processes. On Earth, microbial processes would be a likely explanation for the formation of these features. The biological pathways invoked are microbial dissimilatory Fe(III) reduction and microbial sulfate reduction, both coupled with organic carbon oxidation.

B.Originality and significance
This report is significant, as it presents potential biosignatures on Mars. Verifying the biological origin would however require high-resolution analyses on Earth.
The geochemical characteristics of the outcrops are consistent with deposition under anoxic conditions

C.Data & methodology
Overall, the geologic context, outcrops and petrographic descriptions are clear, besides the questions below:
l. 79 What proxies lead to the conclusion that the BA and MT outcrops are from the same deposition unit? It is partially explained later in the text but it would be useful to clarify in this paragraph.

We have simplified the nomenclature associated with these outcrops throughout the manuscript to better reflect the M2020 Science Team’s current interpretation that the BA-MT outcrops are collectively part of the same “Bright Angel formation”. We have also added a line of text to this paragraph that states:

Lines 69-70: “As described below, the outcrops in these areas share many characteristics and are referred to collectively as the Bright Angel formation.”

l. 84-85 The BA-MT strata cold have been deposited above or below the Margin Unit. How does this affect the conclusions of the study? Would the environmental conditions be significantly different if deposition happened before or after this of the Margin Unit?

Regardless of the timing of Bright Angel formation emplacement relative to the Margin unit, the observations supporting the concept of low-temperature redox-mediated mineral transformations remain the same, and we do not believe that our conclusions would change materially with the relative timing of Bright Angel and Margin Unit emplacement. In the case of a young Bright Angel emplacement age, the deposit would be largely confined to Neretva Vallis, which appears to cut into the Margin unit, but the environment of deposition would still be interpretable as a combination of fluvio-lacustrine suspension and conglomeratic deposits. In the case of an older Bright Angel deposit, we would invoke the same depositional setting, but perhaps one that was more laterally extensive, was later covered by the Margin unit, and then both units were incised by Neretva Vallis.

l.95-97 This statement is unclear: what does that mean for deposition or reworking of sediments after deposition?

Here we were referring to the process by which sediments are sculpted into 2D and 3D bedforms by currents acting on the bed that the current is moving over. To alleviate any confusion, we have modified the sentence to read: “Rock textures include layered and structureless intervals with limited indications for transport and deposition by currents, such as cross bedding or plane bed laminations”

Figure 3: I cannot see the differences between Fig 3A and Fig 3B (besides the addition of spectral measurements). What do the black dotted line and the pink cross represent on Fig. 3B and Fig. 3C?

We thank the reviewer for pointing out that some necessary information was missing from the caption for this figure panel. We have amended the text to include all of the information needed to interpret the relationships between the panels. The text now reads as follows:

Figure 3A-D: Layering, poppy seeds, leopard spots, and organic detections. (A) WATSON nighttime image of Cheyava Falls with both white light LED groups on acquired on sol 1188 at a standoff distance of 3.91 cm. Image resolution: 21.0 ± 0.4 µm/pixel. **(B)** Colorized SHERLOC ACI image acquired on sols 1201-1202 outlined with a white box and overlain on the WATSON image from panel (A). The ACI image is a focus merge of 13 ACI images acquired between standoff distances 4.035 cm – 4.335 cm and has an image resolution of ~10 µm/pixel. Three 1x1 mm SHERLOC spectral scans were acquired at the orange square location at standoff distances of 4.01 cm, 4.035 cm, 4.06 cm. One 1x1 mm spectral scan was acquired at the blue square location at a standoff distance of 4.035 cm. The black dotted rectangular box shows the footprint of the PIXL scan acquired from this target. The magenta cross at the corner of the PIXL scan provides a reference point for comparison to panel **(C)**, which is a close up of the colorized SHERLOC ACI

image from panel (B). Image shows the “poppy seeds” and “leopard spot” features as well as the SHERLOC and PIXL scan locations. **(D)** SHERLOC Raman spectra from representative targets in the Bright Angel unit with fits to an instrument Si-O stretching overtone feature from the SHERLOC fused silica optics (light gray, labeled “bknd”) and the G-band signal at ~1600 cm^{-1} associated with organic carbon in the targets Walhalla Glades (blue), Cheyava Falls (red) and Apollo Temple (green fit on gray spectrum). Malgosa Crest (yellow) shows no G-band signal above the instrument background. NASA/JPL-Caltech/MSSS.

D.Appropriate use of statistics

E.Conclusions

The question of whether there is biology involved in the formation of these features cannot be answered until further analyses are performed on return samples (using high-resolution methods). However, this is an intriguing report. The discussion of the results in the last section (l. 288) opens up interesting avenues for experimental work to understand the how these features could form. I have some additional comments:

-The authors could add a paragraph to discuss why these features occur only at Bright Angel and not in the Masonic Temple area, while these two outcrops form a sedimentary unit. Were the MT outcrops also deposited under anoxic conditions, and then more oxidized than the BA area?

At the present time, we lack sufficient data from the Masonic Temple area to understand the exact details of why the poppy seed and leopard spot features are so prevalent in the Bright Angel area but mostly absent in Masonic Temple. The Masonic Temple area is largely composed of matrix-rich conglomeratic rock, while the Bright Angel area is largely composed of mudstone, suggesting a possible facies control on the development of poppy seeds and leopard spots. Perhaps rapid deposition of sediment to form a conglomerate (e.g., in a debris flow) provided insufficient time for the accumulation of organic matter in the sediment, whereas in the Bright Angel area, the slower deposition of mudstone from suspension (having the same composition as the matrix and mudclasts in the Masonic Temple conglomerate), permitted the accumulation of that organic matter and subsequent redox reactions. This picture is complicated, however, by the presence of "blue-green masses" (see Fig. S21A-C) in one abrasion target from this area that were, unfortunately, not analyzed by the Mars 2020 instrument payload. So, we have elected to remain rather conservative in our approach to the differences in redox chemistry apparent from these two areas of the Bright Angel formation but agree with the reviewer that some mention is warranted. Therefore, we have added the following text at lines 262-264:

"The apparent absence of poppy seeds and leopard spots in most of the conglomerate-bearing Masonic Temple area suggests a depositional facies control on the development of these specific features."

-The features that could represent potential biosignatures are concentrated in a few outcrops. How would you explain the limited localization of "potential" microbial activity?

See the response to the previous comment, while noting that poppy seeds were observed from the base (at Grapevine Canyon) to the top (at Cheyava Falls) of the Bright Angel outcrop section. Localization therefore seems to be to mudstones containing organic matter, Fe(III), and phosphate that were deposited from suspension.

-Recent studies have investigated the role of microorganisms in the formation of iron sulfide minerals. Some of them would actually support the potential involvement of microbial activity in the formation of these features, even though laboratory experiments cannot completely replicate conditions in sedimentary environments. Findings in these experimental studies include the following: 1) under strict anoxic conditions, greigite is only detected when microorganisms (sulfate-reducing bacteria) are present and metabolically active in the experimental medium (freshwater or marine medium containing Fe2+). Greigite formation from mackinawite is a slow process (detectible after several months), but none of the abiotic controls display transformation of mackinawite to greigite. As culture media for bacteria include phosphate (as a source of P), vivianite formation occurs in certain conditions. 2) physical properties of mackinawite and greigite are influenced by microbes: crystallite domain sizes are larger and aggregation is heavier in biogenic minerals, 3) iron sulfide minerals have a high affinity for microbial organic matter.

We thank the reviewer for pointing us to these results. We read the suggested additional references provided below and have incorporated as many of them as possible in support of updated text on lines 302-317 where we discuss terrestrial systems where vivianite and greigite form in association with microbially mediated iron- and sulfate-reduction reactions.

F.Suggested improvements

G.References
Studies on the influence of microorganisms on the formation of iron sulfide minerals: Nabeh et al. 2022 Frontiers in Microbiology, Picard et al. 2021 Astrobiology, Duverger et al. 2020 Frontiers in Earth Science, Berg et al. 2020 Scientific reports, Picard et al. Chemical Geology 2019, Mansor et al. 2019 American Mineralogist, Picard et al. 2018 Geochimica et Cosmochimica Acta,

Studies on vivianite: Cosmidis et al. 2014 Geochimica et Cosmochimica Acta, Miot et al. 2015 Minerals, Busigny et al. 2016 in Lake Pavin: History, geology, biogeochemistry, and sedimentology of a deep meromictic maar lake

H.Clarity and context
The paper is overall clear and well-written

[REDACTED]

REVIEWER #1

I thank the authors for considering my comments and for modifying their manuscript accordingly, however, I am still highly dissatisfied by the essence of the story. The biosignature aspects have not been removed properly and the story now discusses evidence for redox reactions between minerals and organic matter.

We are unsure why the reviewer says that "…the story now discusses evidence for redox reactions between minerals and organic matter…" as if this were a new feature of the manuscript. Redox chemistry has always been central to the submitted, revised, and current versions of the manuscript.

This is not new for Mars…

We are unaware of any published reports of low temperature, diagenetic redox reactions involving organic matter that generate reduced Fe- and S-bearing minerals and textures in a Martian context, and so the observations that we present are indeed "new" for Mars.

… and also barely hiding the fact that the authors are still claiming that they discovered biosignatures on Mars…

We refer to our original response to the reviewer that there is a significant difference between a "potential biosignature" and a "biosignature", the latter of which could be accepted as proof positive of the detection of life. Our manuscript only addresses potential biosignatures.

Again, from the DesMarais et al. (2003) NASA Astrobiology Roadmap's definition of a potential biosignature (also quoted directly in our manuscript): "a feature that is consistent with biological processes and that, when encountered, challenges the researcher to attribute it either to inanimate or to biological processes, compelling them to gather more data before reaching a conclusion as to the presence or absence of life".

This definition is in accord with a more recent definition for potential biosignature that is given by Gillen et al. (2023): "A potential biosignature is any phenomenon for which biological processes are a known possible explanation but whose potential abiotic causes have not yet been reasonably explored and ruled out."

These definitions are distinct from the definitions given for the term “biosignature” by both DesMarais et al. (2003): “A biosignature is an object, substance, and/or pattern whose origin specifically requires a biological agent. The usefulness of a biosignature is determined, not only by the probability of life creating it, but also by the improbability of non-biological processes producing it.”

And Gillen et al. (2023): “any phenomenon for which biological processes are a known possible explanation and whose potential abiotic causes have been reasonably explored and ruled out."

We once again reiterate that the paper presents evidence for low-temperature, early diagenetic redox-driven chemical reactions that have produced mineralogical and textural features. These unusual features require further analyses before one can attribute them to inanimate or biological processes. In other words, they fit the definition of a potential biosignature.

I list below three major points that lead me to recommend rejection at this time, because 1- the authors failed to tone down their main interpretation which is weakly supported and 2- use adequate vocabulary to characterize the features they observe.

The authors state in their rebuttal and on line 28-33 that “In fact, nowhere in the manuscript do we go so far as to say that the features observed in Jezero crater are proof positive of life on Mars. However, we are surprised that the reviewer has interpreted our manuscript to have concluded that such proof has been presented.”, which is strange and inconsistent with their previous manuscript title and assertions/arguments on finding biosignatures on Mars. In fact, this revised manuscript still uses the word “biosignature” a total of six times in the main text (in addition to references to microbial metabolism (as “... sulfide-bearing cores suggests that sulfate reducing metabolisms with...”) and “Martian biosphere”, which demonstrates that the authors believe that they found the signatures of life on Mars.

No. Every instance of the use of the term “biosignature” in our manuscript was, and is, preceded by the qualifier “potential”. The peer-reviewed published distinction between the terms “biosignature” and “potential biosignature” was discussed in our response to the previous review. Our reference list provides citations to these publications, and a quoted definition for the term “potential biosignature” appears in our manuscript.

In the manuscript, we explore two possibilities – the null hypothesis (now stated explicitly in those terms) and a scenario in which biotic chemistry is involved, based on direct similarities to terrestrial phenomena. The quoted text “... sulfide-bearing cores suggests that sulfate reducing metabolisms with...” is taken from the latter section, which specifically describes biologically-mediated reactions that are known, on Earth, to produce mineral and textural features similar to those that we observe in the Bright Angel formation in Jezero crater. We prefer to retain the word “metabolisms” in the discussion of this scenario, though we have now limited it to two instances.

Regarding the term “Martian biosphere”, we have removed it from the statement and replaced the final sentence of the manuscript with:

“Ultimately, the return of samples from Mars for study on Earth, including the Sapphire Canyon sample collected from the Bright Angel formation, would provide the best opportunity to understand the processes that gave rise to the unique features described here.”

I think they are wrong and they need to make better tests of the null hypothesis. In fact, the authors are still claiming a possible signature of life in their revised manuscript in discussing their new data as "Analysis of the core sample ... will enable tests to confirm or refute the biogenicity of the minerals, ...".

This sentence containing the quoted text has been removed from the first paragraph and replaced with: "Ultimately, we conclude that analysis of the core sample collected from this unit using high-sensitivity instrumentation on Earth will enable the measurements required to determine the origin of the minerals, organics, and textures it contains."

So, the authors fail to discuss the default null hypothesis, which is that this organic carbon is abiotic in origin and the greigite and vivanite are also abiotic reaction products.

In an effort to further clarify the pathways by which the features observed in the Bright Angel formation could have been formed by abiotic chemical reactions, we have extensively rewritten the section discussing this matter (lines 263-294 when viewing the manuscript with track changes turned off) to clearly state the requirements of the abiotic null hypothesis, and the challenges that are posed to it by our observations of the Bright Angel formation. The references described below were present in the previous version of the manuscript; we hope that our rewriting of this section helps improve their utility in describing the abiotic null hypothesis. In detail, our discussion of the null hypothesis describes the following requirements:

1. An abiotic source of organic matter. We provide references to literature to indicate how such compounds could be generated by abiotic synthesis on Mars or delivered by meteorites. See line 270.
2. An abiotic pathway to the production of Fe^{2+}(aq) via the reduction of Fe^{3+}-bearing sediment at low temperature.
    a. We provide references to literature indicating that redox reactions between organic matter and Fe^{3+} minerals are well known to occur at low temperatures. We suggest that further analysis is required to determine whether the specific organic compounds discovered in the Bright Angel formation can participate in such reactions. See lines 266-274.
    b. We provide references to literature indicating that Fe^{2+} (aq) can be produced through the oxidation of pyrite by Fe^{3+} (aq). This pathway is challenged because there is no evidence for detrital pyrite in the Bright Angel formation, and this pathway requires low pH to enable Fe^{3+} (aq) solubility. The available evidence from the Bright Angel formation is inconsistent with low pH in association with Fe-phosphate precipitation. See lines 274-277.
    c. We provide references to literature indicating that Fe^{3+} mineral reduction can be promoted by dissolved sulfide (S^{2-}(aq)). See lines 278-281.
3. An abiotic pathway to the production of reduced S (aq) to produce Fe-sulfide minerals in the Bright Angel formation.
    a. We provide references to literature indicating that magmatic gases can produce H_2S, which can provide a source of reduced sulfur when dissolved in water. This source of reduced sulfur provides a potential reactant that can both reduce Fe^{3+} minerals and precipitate as Fe-sulfide minerals. This mechanism as a source of dissolved sulfide is challenged by our observations of the geologic units investigated prior to the Bright Angel formation, which show no evidence for the

production of sulfide-rich magmatic gases or their mineral products. See lines 282-287.

b. We provide references to literature indicating that reactions between organic matter and SO_4^{2-} can reduce SO_4^{2-} to produce S^{2-}(aq), which can both reduce Fe^{3+} minerals and precipitate as Fe-sulfide minerals. This Thermochemical Sulfate Reduction (TSR) mechanism is kinetically inhibited at temperatures <150-200°C. Thus, TSR as a source of dissolved sulfide is challenged by our observations of the Bright Angel formation, which provide no clear evidence that these rocks were heated to the temperatures required for TSR (> 150-200°C). See lines 287-294.

The discussion of the null hypothesis, and the partial challenges to it, lead us to discuss the alternative biological hypothesis on lines 295-323. This section of the manuscript is minimally changed from the previous version, but we note that this is now the only section of the manuscript where biological processes are discussed, via analogy to terrestrial occurrences of microbially mediated reactions that yield mineral phases (vivianite, greigite) and textures (nodules, "reduction spots") similar to those that we observe in the Bright Angel formation.

We conclude this portion of the manuscript by stating that the features observed in the Bright Angel formation warrant consideration as potential biosignatures.

This latter sentence implies that it represents a possible biosignature and throughout the manuscript, the theme of biosignature returns again and again such that the authors have not changed this fundamental implication. I don't believe they are correct. Saying "... to confirm or refute the biogenicity of these features..." further implies that they interpret their observations as potential biosignatures

The sentence containing the quoted text that includes the term 'biogenicity' has been removed from the first paragraph and replaced with: "Ultimately, we conclude that analysis of the core sample collected from this unit using high-sensitivity instrumentation on Earth will enable the measurements required to determine the origin of the minerals, organics, and textures it contains."

However, we disagree with the reviewer that the features we have observed can be confidently attributed to purely abiological processes, and that the chemical reactions, minerals, and textures warrant consideration as "potential biosignatures".

and the case made is so weak with unconvincing results and dubious interpretations, that it will fall apart and create a significant controversy, especially if it is published in Nature.

We are surprised to learn that the reviewer now indicates that we have provided "unconvincing results" given statements in the first review that that were provided, including:

"This is an exciting, interesting, and valuable dataset that deserves publication..."

"The authors document at low and high resolution a range of outcrops exposed in Jezero Crater and provide new geochemical analyses including Raman spectra of possible carbonaceous material and of various minerals, as well as geochemical analyses of major elements. This work is all done robotically and in situ, on the Martian surface, so it represents a great achievement by the team."

"… I maintain that this is a truly remarkable new and valuable dataset from the Martian surface, and I have no negative criticism of the data, besides for the interpretations"

Based on the reviewer's own comments, we disagree with the suggestion of a "weak" case, now made by the reviewer in their second round of reviews.

In their rebuttal, the authors reply "To describe these features solely in the context of abiotic or "prebiotic" chemical processes would ignore the body of literature on terrestrial occurrences of similar features that are formed in association with microbial activity. This would provide the false impression that we are ignorant of these occurrences and/or intentionally not inviting comparisons to them.". This is also dubious because the supposed "body of literature" on these kinds of objects on Earth does not exist, or rather very limited and mostly hidden as complementary observations in papers focused on other topics. For instance, to my knowledge, there is not one recent paper in the peer-reviewed literature that specifically addresses the association of greigite and vivianite as a potential biosignature (i.e. agnostically evaluating both abiotic and biological pathways of formation).

We would point the reviewer to our reference #59:

Banfield, J. F., Moreau, J. W., Chan, C. S., Welch, S. A. & Little, B. Mineralogical Biosignatures and the Search for Life on Mars. Astrobiology 1, 447-465 (2001). https://doi.org/Doi 10.1089/153110701753593856

While not recent (we're not sure why this matters), this paper describes biologically driven Fe- and S-redox reactions, and the mineral products of those reactions, as key lines of evidence in the search for life on Mars. Greigite is mentioned specifically in this context. Our reference #65 builds on this theme, and discusses the key role that mineral, textural, and organic biosignatures play in the search for potential biosignatures on Mars. It is the search for exactly these types of potential biosignatures that led to the selection of the Mars 2020 Science Instrument payload (see our reference #1).

Nevertheless, the reviewer is correct to point out that there is no paper (that we are aware of) that describes the specific association of vivianite and greigite as a potential biosignature in the search for life on Mars. For this reason, our manuscript points the reader to literature describing terrestrial observations of microbially mediated redox reactions that yield vivianite (references #48-52), greigite (references #54-55), and both minerals together (reference #51). Because *Nature* has a limited citation count, and this is not a review paper, we have tried to select references that are illustrative of the general point that vivianite and greigite are known products of low temperature microbially mediated Fe- and S-based redox reactions with organic carbon. As we state in the conclusion of our manuscript (lines 332-337), this possibility should motivate additional in-situ, laboratory, modeling, and field analogue research to understand how this assemblage could have formed on Mars, and that the return of the Sapphire Canyon sample from this unit is critical to understanding the origin of the features observed in the Bright Angel formation.

Hence, without further evidence to argue for a potential biosignature in these nodular rocks, I do not support this view by the authors. Also, the identification of both greigite and vivianite is very weakly supported by their data and certainly not an unambiguous detection: the ternary diagram in Fig 4c does not show a single datapoint where vivianite is expected, while for greigite, it is similar in 4d and it is also a polymorph with several other sulfide minerals. The organic carbon

remains a real detection, but its nature is not discussed in this work (rather differed to another manuscript), which is unfortunate.

To address this concern, we have updated the Supplementary Information with a new Figure S26, which demonstrates with high statistical confidence that the sulfide in the Apollo Temple scan is greigite (Fe_3S_4), and not an Fe-sulfide with a different stoichiometry, such as pyrite (FeS_2), troilite (FeS) or pyrrhotite ($Fe_{0.875}S$).

For the Fe-phosphate identification, we note that the focused PIXL instrument beam diameter varies as a function of the XRF line energy detected, ~116 microns for iron, and between 162-171 microns for S and P (see Das et al., 2024, X-Ray Spectrometry). Thus, for small Fe-phosphate nodules (100-200 microns in diameter) and reaction spot rims (10's of microns thick), we do not expect to measure compositions that represent vivianite in its pure state. The even larger effective beam diameters of the surrounding Si-and Al-rich mudstone will also "bleed over" into the Fe- and P-rich areas, further confounding our ability to measure pure mineral chemistries on small features. The PIXL instrument is remarkable, but analysis at the scale required to determine mineral chemistries in isolation will require electron microprobe studies on Earth. Thus, we rely on mixing trajectories towards likely mineral endmembers when dealing with fine-grained phases that are intimately mixed with materials of different chemistries, in a manner similar to our references #19, 21 and 34.

It is worth noting that our Fe-phosphate mineral identification is based not solely on XRF analysis, but also on color properties, as stated on lines 178-182:

"… consistent with accumulations of microcrystalline vivianite ($Fe^{2+}_3(PO_4)_2{\cdot}8H_2O$) or lower hydration state ferrous phosphates (e.g., phosphoferrite: $Fe^{2+}_3(PO_4)2{\cdot}3H^2O$) and their oxidation products, e.g., metavivianite ($Fe^{2+}_{2.5}Fe^{3+}_{0.5}(PO_4)_2{\cdot}7.5H_2O$), ferrostrunzite ($(Fe^{2+}Fe^{3+}_2(PO4)_2(OH)_2{\cdot}6H_2O$), santabarbaraite ($Fe^{3+}_3(PO_4)_2(OH)_3{\cdot}5H_2O$), all of which have molar FeO:P ratios of 3:2 and color properties that match our observations in Bright Angel."

We also note updated text on lines 243-247:

"Fe-phosphate enriched masses are not associated with Al_2O_3 (Fig. S21H), which might otherwise suggest the co-existence of Al-phosphate minerals typical of transport of Al^{3+} and Fe^{3+} under oxidizing, low-pH conditions, such as variscite ($AlPO_4{\cdot}2H_2O$) and strengite ($FePO_4{\cdot}2H_2O$, Fig. 4C)[31]. Instead, transport of Fe^{2+}, Zn^{2+} and PO_4^{3-} likely occurred under non-oxidizing conditions, which, combined with moderate pH, prevented mobilization of Al^{3+}. Such conditions favor the precipitation of vivianite[32]."

Thus, while the reviewer is correct that no single XRF datapoint has a chemistry indicative of pure vivianite, the combination of mixing trajectories, color properties, anticorrelated Al_2O_3-P_2O_5, and correlated Zn-P_2O_5 leads us to conclude that the Fe-phosphate in the Bright Angel formation is (or was) ferrous and that the conditions under which it precipitated are most consistent with those where vivianite is commonly found on Earth.

2- The authors are adamant in keeping misleading words in their manuscript such as "poppy seeds" and "leopards spot", which are figurative at best and a wrong scientific practice. There is no problem in my view with naming localities with such vocabulary (like "Yogi" for a specific rock location, or "Jezero" for a specific crater), however, specific natural objects need to be named according to specific scientific terminology in order to be comparable with other examples. What

geological feature from Earth would the authors compare their small textures from Mars to? If they are nodules, then call them “nodules”, and not anything else. Instead of understanding this basic idea, the authors claim that because they have used this terminology in previous press releases (which are not peer-reviewed), they should be allowed to use it again, and in a peer-reviewed context. This is also a wrong point of view and akin to saying that an error cannot be corrected and that press releases for public outreach supersede scientific peer-review.

[redacted] We have removed all instances of the use of the terms “poppy seeds” and “leopard spots” from the manuscript text, headers, figures, and supplementary information files. The only instances of this term that we have retained are in parentheticals associated with the initial descriptions of these textural features, as noted above.

The parentheticals could also be removed if required. However, the terms are already in the public record, and we note that the usage in our updated text (one instance of parentheticals each) is in accord with the first published description of sedimentary hematite concretions, informally known as “blueberries”, at Meridiani Planum, Mars, in Squyres et al. (2004) *Science*, doi.org/10.1126/science.1106171:

“Post-egress investigation of the soil adjacent to the lander showed it to be sprinkled with small spherules, typically 4 to 6 mm in diameter. (These spherules have become known informally as “blueberries” although their color is gray rather than blue.)”

REVIEWER #2 (Janice Bishop)

Dear Dr. Mossinger,

In my opinion this paper is substantially improved and I am satisfied that the authors have adequately revised the paper in response to my concerns.

I did notice a typo in the revised manuscript that the authors should correct. Ferrihydrite is misspelled on Fig S10

Best regards, Janice

We thank Dr. Bishop for her careful and insightful reviews of both versions of the manuscript. We have fixed the misspelling, which was on Figure S9B.

REVIEWER #3

In my opinion, the authors have responded convincingly to all inquiries. The new version of the manuscript has improved in clarity and quality.

Whether or not it will be possible to confirm the biogenicity of these structures, this is exciting science! This remarkable report also highlights the importance of exploration and team work for the advancement of knowledge and deserves publication in Nature.

We thank this reviewer for their careful and insightful reviews of both versions of the manuscript. We appreciate the acknowledgement of the teamwork that was required to complete this report

and look forward to the return of the Sapphire Canyon sample to Earth so that the questions regarding the origin of the unique features we observe in the Bright Angel formation can be answered.

Review of "**Detection of a Potential Biosignature by the *Perseverance* Rover on Mars**" submitted by Hurowitz et al to Nature

General Comments

This paper synthesizes data collected by multiple instruments on the Perseverance rover to pull together a tight story on the recent detections of some unusual combinations of minerals and organic material. Claiming to detect biosignatures on another planetary body is a big step and should not be accepted lightly, and I admit that at first I was a bit skeptical of this idea. However, after careful reading of this manuscript and scrutiny of the data presented, I support the authors' main conclusions. I have identified a few places in the manuscript where additional clarification or explanations could be beneficial. In particular, it would be helpful to include more information on how common or widespread the poppy seeds and leopard spots are at the different sites studied by the instruments on Perseverance because the chemistry and mineralogy varies across the regions investigated and the paper would be improved with a clearer understanding of these relationships.

I have conducted a number of field and lab studies with similar observations to some parts of this study and I feel confident in accepting that the unique combination of ferrous phosphates, ferrous sulfides, and organic hydrocarbons together are unusual and represent at the least a remarkable occurrence of abiotic chemistry on Mars. These findings also represent the very real possibility that microbes participated in formation of these reduced minerals. Of course we can't know which of these pathways (or both) may have occurred from the data collected by Perseverance and we may never know, even after analyses of the returned samples, but the "Detection of a Potential Biosignature" is a valid conclusion from this data in my opinion. My major disappointment in reading this paper is that only one sample was collected here to be returned to Earth for more detailed analyses.

This paper presents exciting new findings that will have broad implications for our understanding of biogeochemistry and astrobiology on Mars and potentially leading to addressing the bigger concept of life outside of Earth. I support publication of this study in Nature following consideration of a few minor comments and suggestions outlined below.

Should the authors have questions regarding my review, they are welcome to reach out to me directly: Janice Bishop – jbishop@seti.org.

Specific Comments

Line 67 I suggest changing "orbiter spectroscopic signatures" to "orbital spectroscopic signatures".

Line 112 does "layered outcrop" here refer to just the rock containing Steamboat Mountain or both rocks in Fig. 2 ?

Lines 112-114 This is a long sentence and I found it confusing. I suggest breaking this into 2 sentences and more clearly defining where the layered outcrop is located and on what scale the layering is observed.

Lines 132-133 and Figure S9A-B Please define how you determined the “528 nm band depth” parameter.

Line 136 imparts should likely be impart here. I believe this refers to “abundances” but it could also refer to “features” and “ratios” from earlier in the sentence. Perhaps reword to clarify that.

Line 140 I suggest adding “~” before 1.92 µm as the band center and shape varies a bit for this band based on the data shown in S11E.

Line 151 I suggest mentioning here that Raman fluorescence is also caused by nanophase clays and iron oxides. This is in contrast to the D and G bands that are only due to C in graphite, diamond or hydrocarbons.

Lines 145-151 This paragraph describing the Raman spectra does not mention the D bands due to disordered C that is frequently paired with the G (graphitic) bands in Raman spectra including organic C. See for example Ferrari Robertson (2001), Schopf et al. (2002) and Bishop & Murad (2004) for descriptions of the Raman D and G bands and fluorescence in Raman spectra. I don’t see the D band in your spectra – is that because of the offset distance or do you think it would not be present in any case? This may have implications for the form of the C in your samples and would be helpful to address here. You may be able to constrain the type of C if only the G band is observed.

Line 178 The fluorescence observed in Raman spectra of the Steamboat Mountain mudstone could be due to nanophase iron oxides. I’m guessing that if clays were present to cause the fluorescence then organics would have been present as well since clays are good carriers of organics.

Lines 211-212 This sounds to me that XRD did not identify any phosphate minerals where the PIXL XRF observed elevated Fe and P. I’m wondering if some of this P could amorphous, similar to the amorphous P found by Curiosity at Gale crater (see Rampe et al., 2016).

Lines 217-223 The phosphates mentioned here as possibilities present in the poppy seeds include ferrous and ferric phosphates. The text seems to suggest that ferrous phosphates were present and then potentially oxidized to ferric phosphates. This is contradictory to the theme expressed elsewhere that ferric minerals were present that were reduced to ferrous minerals as the organics were oxidized. Of course Mars has a long history and much oxidation and reduction could have taken place. However, I suggest deleting the idea that the ferrous phosphates could have oxidized again through alteration as that muddies your premise. Instead, it could be that some ferric phosphates were present initially, and then were reduced to ferrous phosphates through oxidation of the organics.

From the SuperCam NIR spectra in Figure S11E the increasing slope noted from ~1.3 to 1.8 µm is consistent with Fe2+ in minerals in mean spectra from Walhalla Glades and the Kolb Arch matrix at spot 8. This slope increase is characteristic of many minerals containing Fe2+, but less common for minerals with Fe3+ (see Bishop, 2019). Fe3+ - minerals generally increase in slope from ~1 to 1.3 µm then plateau from ~1.3 to longer wavelengths.

Does the Walhalla Glades site have more poppy seeds than the Apollo Temple site? or are they different? How do you explain a higher abundance of Fe2+ at the Walhalla Glades and Kolb Arch #8 sites?

Lines 213-214 The text here states that Fe-P masses were observed at Apollo Temple but the mean NIR spectrum of Apollo Temple in Figure S11 is not consistent with a ferrous phase. This would indicate that the phosphate mineral is more likely to include Fe3+. Spectra of strengite and strunzite in this region are similar to the Apollo Temple mean spectrum and would not be inconsistent. Or it could be that the poppy seeds and leopard spots only comprise a small fraction of the Apollo Temple surface or are below the surface rather than at the surface and the ferrous phosphate phase is not spectrally dominant. However, past studies have shown that only a tiny amount of Fe2+ is needed in carbonates to greatly influence the spectral slope from ~1.3-1.8 µm (Bishop et al., 2013, 2021). If the authors believe that a ferrous phosphate component is present at the Apollo Temple site it would be helpful to include the rationale behind this and how this is consistent with the spectra shown in Figure S11e.

Is there any correlation between this increase in spectral slope and P? The phosphate minerals gormanite-souzalite [$(Fe^{2+},Mg)_3(Al,Fe^{3+})_4(PO_4)_4(OH)_6 \bullet 2H_2O$; with more Fe2+ in gormanite and more Mg in souzalite] includes Fe2+ and Fe3+ and less water than vivianite and has a stronger spectral slope from ~1.3 to 1.8 µm (see Bishop et al. 2024, EGU abs); thus gormanite could be a potential candidate for a ferrous phosphate if present at the Walhalla Glades and Kolb Arch spot 8 sites.

Line 272 Burns and Fisher (1990) would be a better reference for jarosite as an oxidation product of Fe sulfides on Mars.

Line 275 The Mastcam-Z spectra and Super Cam NIR spectra are not consistent with a strong ferrous component that is characteristic of siderite. The Fe2+ band is particularly strong for this mineral (Bishop et al., 2021). Siderite is also usually brownish or tan rather than red. I suggest that ferrihydrite or a similar poorly crystalline ferric hydroxide phase is contributing the red color there.

Lines 290-291 Please explain how Ca-phosphate was identified in Figure 4C

Line 303 It may not be necessary to oxidize the C in organic matter and reduce the Fe3+ in an aqueous environment. This process was observed by Campbell et al. (1997) through heating ferrihydrite mixed with organic C in a dry environment and replicated by

Bishop et al. (2006). When no C was present, hematite was produced by heating ferrihydrite. When C was present in the ferrihydrite sample, magnetite was produced from heating the sample and if at least a small amount of O2 was present, then the magnetite partially oxidized to maghemite.

Lines 306-308 Sulfate reduction to sulfide was also observed in the anoxic zones of Lake Hoare in Antarctica, where microbes facilitated formation of tiny pyrite deposits on quartz grains (Bishop et al., 2003). No need to cite this study – I just wanted to point out that this has been observed in the cold lakes of Antarctica.

Lines 344-346 Would it fit your model for the Apollo Temple site measured by the SuperCam NIR spectrometer to have ferric phosphate that was then reduced to ferrous phosphate at the Walhalla Glades and Kolb Arch sites?

Line 397 what excitation wavelength was used for the SHERLOC Raman measurements?

Line 412 please define DUV

Supplementary Information
Table I suggest adding the Kolb Arch site to this table and also a column about the relative abundance of poppy seeds and leopard spots for each target.

Figures S9A&B The Mastcam-Z ratio trends shown in Figure S9A indicate that the Apollo Temple sample contains less Fe3+ than other samples. I agree that the Fe bands are weaker overall for this sample, but the spectral shape of the data shown in Figure S9B all resemble Fe3+ rather than Fe2+. The text does not say that Fe2+ is present but that is often a logical interpretation for samples containing less Fe3+. I suggest clarifying that in the caption. It is possible that there is some Fe2+ in some samples, particularly #4,5,6 in Figure S9B, but in my opinion there is more Fe3+ than Fe2+. These data could be consistent with some Fe2+ minerals including low-Ca pyroxenes, but high-Ca pyroxenes, olivines, carbonates, ferrous sulfates, and Fe2+ glass all have Fe2+ bands that extend past 1 μm and are not consistent with any of the spectra shown in Figure S9B. I do agree that the Malgosa Crest Abrasion tailings spectra have more Fe3+ character than the other spectra shown in Figure S9B.

Further, the Apollo Temple sample bands looks more like Fe3+ phosphates to me than Fe2+ phosphates and the Apollo Temple data in Figure S9B look similar to Pancam convolved channels of strunzite [$Mn^{2+}Fe^{3+}_2(PO_4)_2(OH)_2\ 6H_2O$], (see Lane et al., 2007, 7thMars).

Figure S11-panels A,B,C Most of the white, red, and yellow markings are difficult to read, even when zoomed in to the max possible in the pdf. I suggest thicker lines, bolder text and more color contrast.

Figure caption for S11D I suggest adding "~" before 1.92 µm as the band center and shape varies a bit for this band based on the data shown in S11E.

Figure caption for S11E I feel that the paper should say a bit more about NIR spectra and mineralogy. I understand that space is limited in the main text so perhaps this figure caption is the best place to add that.

Apollo Temple and Kolb Arch #1 have stronger bands near 1.92 µm and a bigger drop near 2.4 µm, while Walhalla Glades and Kolb Arch #8 include an increase in slope from 1.3-1.8 µm, consistent with ferrous iron. I have prepared some plots of your data with some mineral spectra that might be useful to you in thinking about these spectra. While you may not feel comfortable identifying the smaller features in the spectra until the calibration is sorted out, these larger trends should be described a bit more to contribute to the mineralogy understanding of the sites investigated.

Figure S11E spectra + sulfates

-To my eye, the Apollo Temple and Kolb Arch #1 spectra could contain some bassanite and gypsum but neither is a perfect match, especially at 1.75-1.77 µm and 2.22 and 2.26 µm.

-starkeyite or other Mg sulfates could be consistent with the shoulder at 1.94 µm in the Kolb Arch #8 and the band near 2.54 µm in most spectra, as well as the shoulder near 2.41 µm.

-Jarosite is not consistent with these spectra, but the jarosite is likely a minor phase so might not be observed in these spectra.

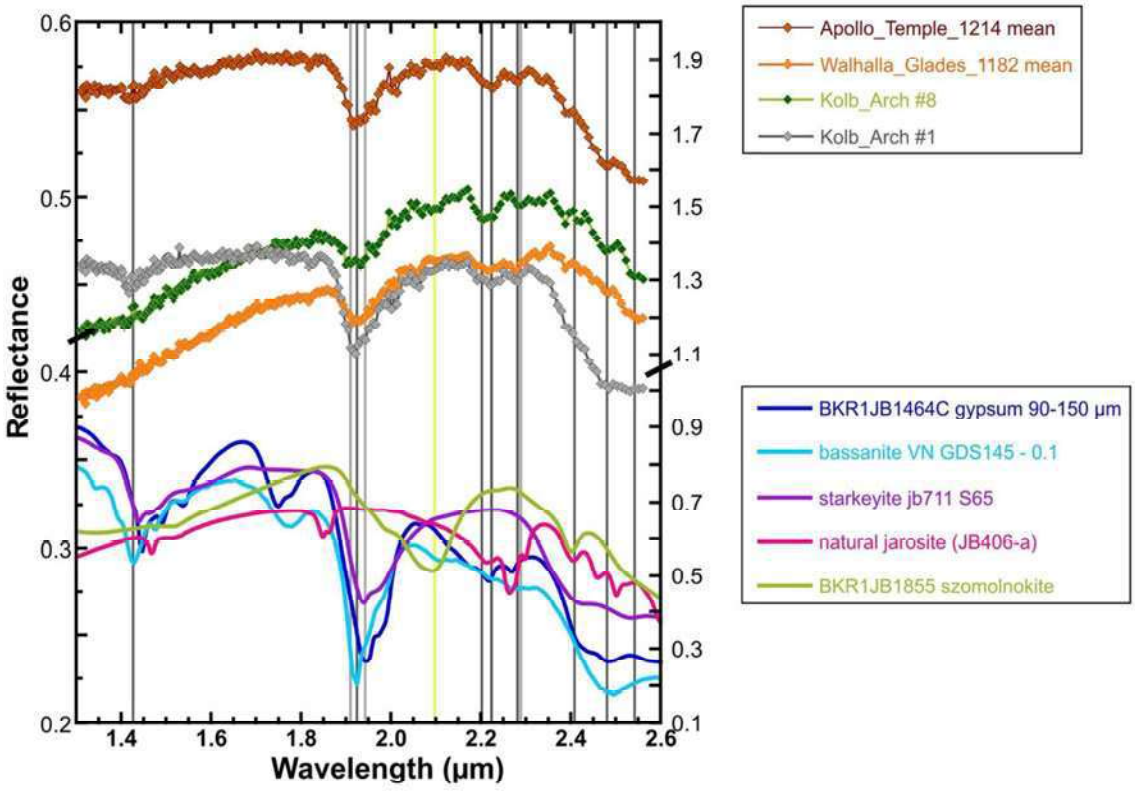


Figure S11E spectra + sulfates

-To my eye, the ~1.3-1.8 µm slope in the Walhalla and Kolb Arch#8 spectra are most consistent with an Fe2+ phosphate such as gormanite/souzalite, but the drop in reflectance near 2.41 and 2.54 µm in the Kolb Arch #1 spectrum is more consistent with strengite and the drop near 2.554 µm in the Apollo Temple spectrum is more consistent with strunzite.

-The shoulder on the ~1.9 water band near 1.95 µm and longer could be consistent with a contribution from vivianite, strunzite or strengite.

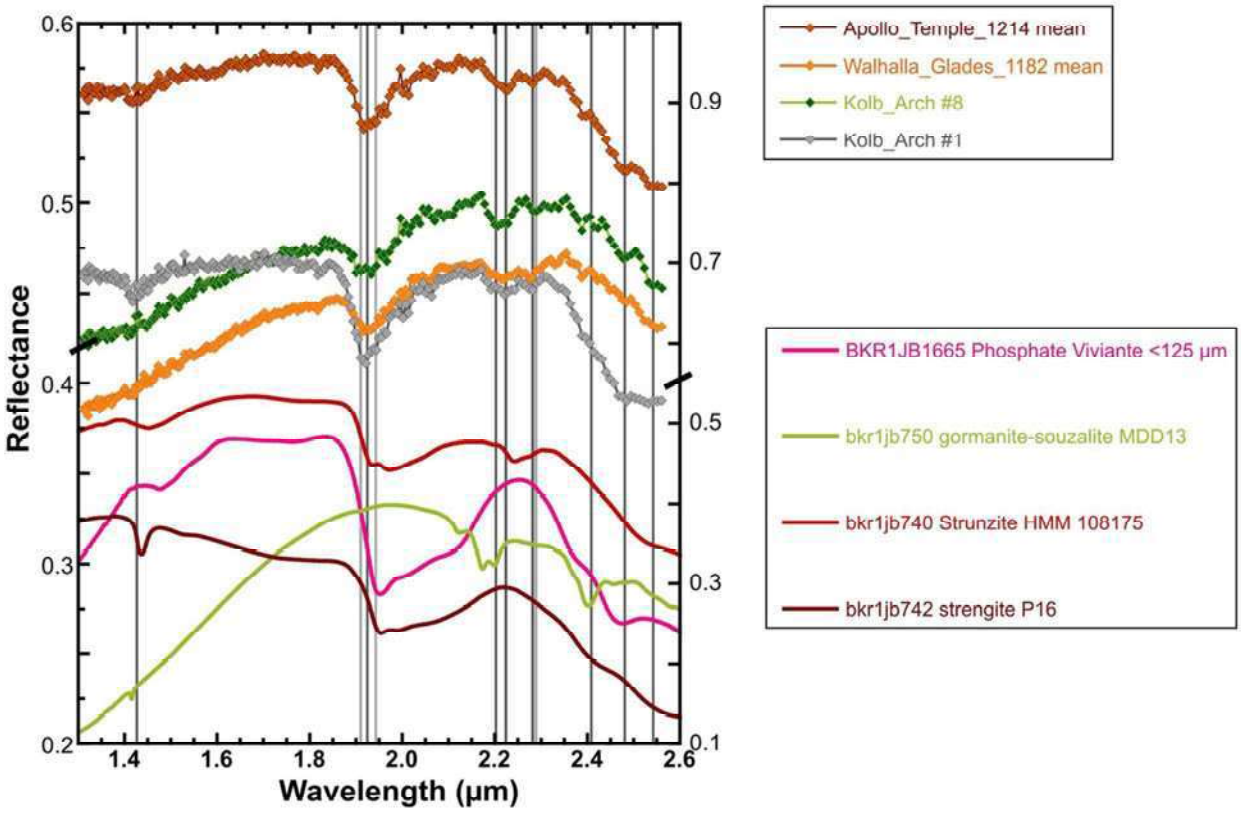


Figure S11E spectra + clays, opal, imogolite, and ferrihydrite

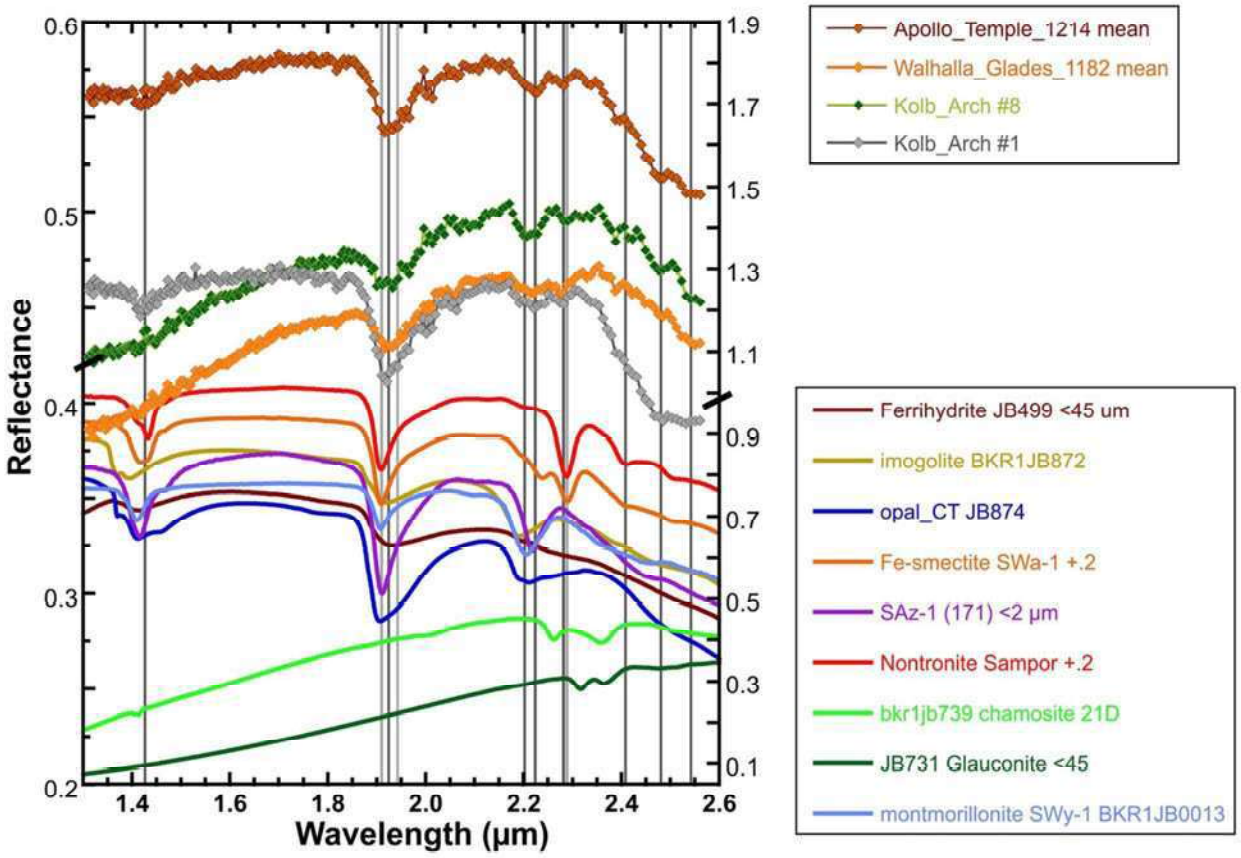
-To my eye, the ~1.3-1.8 µm slope in the Walhalla and Kolb Arch#8 spectra could be consistent with ferrous clays such as chamosite or glauconite.

-The drop in reflectance near 2.48 and 2.54 µm is somewhat similar to opal, but is better explained by the sulfates or phosphates.

-The broadening of the ~1.9 µm band in the Kolb Arch #8 spectrum is somewhat similar to that of opal, but the opal bands near 1.38-1.41 µm are not consistent.

-The broadened band near 2.20-2.22 µm could be explained by a mixture of opal, imogolite, montmorillonite SWy-1 and/or montmorillonite SAz-1.

-The band near 2.28 µm is a better match for nontronite than jarosite or gypsum, but still not perfect.



References that may be useful to the authors in revising this manuscript:

Note – I placed these in a DropBox folder as some are not readily available: https://www.dropbox.com/scl/fo/6yrlrr6xzoachlh3gesra/AJZlXnG6XeFehUrm4dnum8s?rlkey=oe2gf3btvm0egmohkbccc588o&st=highe2cm&dl=0

Note2 – I am not suggesting that all of these papers/abstracts be cited; these are intended to provide input that could be helpful.

Bishop J. L., Anglen B. L., Pratt L. M., Edwards H. G. M., Des Marais D. J. & Doran P. T. (2003) A spectroscopy and isotope study of sediments from the Antarctic Dry Valleys as analogues for potential paleolakes on Mars. *International Journal of Astrobiology*, **2**, 273-287.

Bishop J. L. & Murad E. (2004) Characterization of minerals and biogeochemical markers on Mars: A Raman and IR spectroscopy study of montmorillonite. *J. Raman Spectr.*, **35**, 480-486.

Bishop J. L., Dyar M. D., Parente M., Drief A., Mancinelli R. L., Lane M. D. & Murad E. (2006) Understanding Surface Processes on Mars Through Study of Iron Oxides/Oxyhydroxides: Clues to Surface Alteration and Aqueous Processes. *37th Lunar Planet. Sci. Conf.*, Abstract #1438.

Bishop J. L., Perry K. A., Dyar M. D., Bristow T. F., Blake D. F., Brown A. J. & Peel S. E. (2013) Coordinated spectral and XRD analyses of magnesite-nontronite-forsterite mixtures and implications for carbonates on Mars. *Journal of Geophysical Research*, **118**, 635–650.

Bishop J. L. (2019) Chapter 4: Visible and near-infrared reflectance spectroscopy of geologic materials. Pp. 68-101 in: *Remote Compositional Analysis: Techniques for Understanding Spectroscopy, Mineralogy, and Geochemistry of Planetary Surfaces* (J.L. Bishop, J.F. Bell III & J.E. Moersch, editors). Cambridge University Press, Cambridge, UK.

Bishop J. L., King S. J., Lane M. D., Brown A. J., Lafuente B., Hiroi T., Roberts R., Swayze G. A., Lin J.-F. & Sánchez Román M. (2021) Spectral properties of anhydrous carbonates and nitrates. *Earth and Space Science*, **8**, e2021EA001844.

Burns R. G. & Fisher D. S. (1990) Iron-sulfur mineralogy of Mars: Magmatic evolution and chemical weathering products. *Journal of Geophysical Research*, **95**, 14415-14421.

Campbell A. S., Schwertmann U. & Campbell P. A. (1997) Formation of cubic phases on heating ferrihydrite. *Clay Minerals*, **32**, 615-622.

Ferrari A. C. & Robertson J. (2001) Resonant raman spectroscopy of disordered, amorphous, and diamondlike carbon. *Physical Review B*, **64**, 075414.

Lane M. D., Bishop J. L., Dyar M. D., Parente M., King P. L. & Hyde B. C. (2007) The ferric sulfate and ferric phosphate minerals in the light-toned Paso Robles rover track soils: A multi-instrument analysis. *7th Int'l Mars Conf.*, Abstract #3331.

Rampe E. B., Morris R. V., Archer P. D., Jr., Agresti D. G. & Ming D. W. (2016) Recognizing sulfate and phosphate complexes chemisorbed onto nanophase weathering products on Mars using in-situ and remote observations. *American Mineralogist*, **101**, 678-689.

Schopf J. W., Kudryavtsev A. B., Agresti D. G., Wdowiak T. J. & Czaja A. D. (2002) Laser-Raman imagery of Earth's earliest fossils. *Nature*, **416**, 73-76.