nature portfolio

Peer Review File

A complete set of canonical nucleobases in the carbonaceous asteroid 162173 Ryugu

Corresponding Author: Dr Toshiki Koga

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

Version 0:

Reviewer comments:

Reviewer #1

(Comments for the Author)
Overview
The authors present a robust and impressive analysis of nucleobases from meteorite samples and returned extraterrestrial materials. Moreover, improved analytical techniques enabled the identification of relationships between purine/pyrimidine relative abundances and ammonia concentrations providing new insights into their formation within parent bodies. However, there are a few minor points that need to be revised. In particular, the authors hastily rationalize the importance of nucleobases before proceeding to discuss analytical capabilities/improvements and formation mechanisms. Further elaboration in the introductory section will help the reader better appreciate the significance and impact of the results presented in this study. If properly revised and published eventually, this work will surely attract not only astrochemists but also a wide range of scientists studying prebiotic chemistry and the origins of life.

Minor
The intro paragraph would benefit from a few more sentences explaining the significance of nucleobases. The current version has two sentences which does not fully elaborate their function and significance in prebiotic chemistry (origins of life on Earth) and modern biological systems (adenine is the core molecule in ATP, why is that so? Is it a relic of something?). The remainder of the paper is focused on detection and formation mechanisms (within parent bodies). It is therefore critical to first establish why their detection and ubiquity discussed later in the paper is important and not rush through this opening paragraph.

Line comment:
33 – 36: The introductory sentence is long and provides a lot of information. This sentence could benefit from a break at "… encoding genetic information in DNA and RNA [period]. [New sentence intro]…and also function as components of energy…"
36: Please provide example of energy carriers and coenzymes and their respective citations.
37: "RNA likely played a central role in early life as both genetic material and a catalyst". "early life" should be 'the origins of life'. The authors could briefly acknowledge the RNA world hypothesis rather than deferring to the citations.
37-38: "making its nucleobases particularly relevant to prebiotic chemistry". What about the sugar and phosphate group? This question is not meant to be answered, but to emphasize the importance of taking the time to elaborate the importance of nucleobases in the first paragraph.
46: "implications for extraterrestrial habitability". Please elaborate on 'extraterrestrial habitability' in this statement and provide a citation. Do impacts provide refugia? Facilitate favorable conditions for prebiotic chemistry? On Mars?
49: "freshest" → pristine or unaltered (?)
58 – 59: "likely due to sample limitations in the initial analytical procedures". Please elaborate. Was it due to low sample masses? "extraction efficiency" is mentioned in the following line.
67: "binding". Adsorption?
72: "as well as uncommon nucleobase analogs". Non-biological isomers?
125: Define CE-HRMS
129: "vitamer" → vitamin

Reviewer #2

(Comments for the Author)
The manuscript by Koga et al. presents the detection of nucleobases in samples from the asteroid Ryugu. Two samples were analyzed with an improved extraction protocol compared to Oba et al. 2023 and compared to the Bennu samples and the Orgueil meteorite. All analyses were performed by HPLC coupled with high-resolution mass spectrometry (HRMS) and duplicated by CE-HRMS analyses to strengthen the identification of the targeted molecules. HRMS allows for the precise selection of the mass corresponding to the targeted compound, while the retention time of the targeted molecule in the sample is compared to a standard. For two nucleobases, MS/MS analyses were performed to strengthen the identification. The detection of molecules other than nucleobases is also present and briefly discussed in the discussion section. Interestingly, the authors demonstrate a correlation between purine/pyrimidine ratios and the amount of ammonia present in the samples.

General comments

This work is important for the astrophysics community because the Ryugu samples are unique and knowledge of their organic content is crucial. However, the same group described the detection of a first nucleobase in Nat Com in 2023. After reading the introduction, I had the feeling that this work presented itself more as results from an optimization of the extraction protocol, which is a significant challenge, but seems to me to be outside the scope of Nat Astro. I noted these different sentences in the introduction:

“Based on the results of the initial analysis of the Ryugu samples, we modified the extraction procedure to maximize efficiency by sequentially using water and acid extractions.”

“Here, we applied the improved extraction method to two Ryugu aggregates (A0480 and C0370; Extended Data Fig. 1), which were specifically allocated for nucleobase analysis, to re-examine the distribution of nucleobases and other nitrogen bearing heterocyclic molecules in these samples.”

I suggest the authors rewrite the introduction, clearly explaining the importance of publishing these new results in a high-level journal. They have already published the detection of the first nucleobase in 2023 in Nat Com. If the only innovative aspect lies in the detection of new nucleobases, in my opinion, this work should be considered complementary and does not deserve to be published in Nat Astro.

Authors should better explain in the introduction why detecting in a same object purine and pyrimidine could be interesting and give invaluable information on the history of the body. This aspect is described in the discussion, but has to much more describe in the introduction, than spending half of the introduction in explaining why the new protocol was used.

Major Comments.

1. Page 4 line 69, “The application of the acid extraction procedure to a 17.75 mg sample from the B-type asteroid (101955) Bennu”. Can you clarify if the exact same procedure of extraction was used for Bennu and Ryugu samples?
Because in Glavin Nat Astro 2025 where the detection of nucleobases in Bennu is described, no detail on the HCl procedure are given (Temperature, duration): “A separate 17.75 mg aggregate sample (OREX-800044-101), sub-sampled from Bennu OREX-800044-0, was extracted in HCl (Tama Chemicals Co., Ltd.) and analysed for N-heterocycles using HPLC/ ESI-HRMS at Kyushu University in Japan.”
It seems that the same group as performed the analysis on Benu, but can you be more precise on this aspect?

2. For the Xanthine, it seems from Fig 2 that it is not observed in sample A080 water extract. As written lines 102-103 it suggests that his compound in present in both extracts “adenine (~14.0 min), hypoxanthine (~5.1 min), guanine 101 (~8.2 min), and xanthine (~4.9 min) in the H2O and HCl extracts of the A0480 and C0370 102 samples were observed at retention times consistent with those of authentic standards”

3. Regarding the identification by comparison of retention times between Ryugu extracts and standards, for certain nucleobases such as adenine (Fig. 2), variations in retention times are observed in the different samples. It would be important to add an error to the retention times to estimate the acceptable variability. It might be interesting to include a table in the extended data showing all retention times and their associated error. Given that no fragmentation was performed for most of the detected compounds, this aspect seems important.

4. Only two compound identifications were confirmed by MS/MS: guanine and cytosine. I suggest the authors clarify that only two compounds were confirmed by MS/MS due to the low mass of the samples. This is completely understandable, but should be clearly stated. I suggest merging the information noted in lines 107-110 for guanine with that in lines 123-124 for cytosine, and clearly stating that due to the small amount of material available, only two compounds were in sufficient quantity to perform MS/MS.

5. Lines 2010-215, HCl extraction on the remaining residues is shown to provide better extraction than water alone. After water extraction, is it possible that there is still enough organic matter left that could react in 6 M HCl at 110°C for 12h and give the observed molecules, besides a better extraction process? Can you add a comment on that?

6. Line 264-265, Can you explain what types of high-energy irradiation could affect the reactivity inside parent bodies of meteorites once accreted?

Minor comments

1. Page 6 line 101. Just introduce your sentence by explaining which technique you use to obtain these chromatograms. I guess they are related to HPLC/ESI-HRMS, but this only appears at line 114.

2. I would suggest placing CE-HRMS analyses after LC-HRMS to better reinforce that this is an additional method to confirm identifications, in the absence of MS/MS.

3. Page 7 line 140 just add “of this work” at the end to clarify.

4. Lines 158-180 other compounds were identified. There is no mention of this results in the abstract or in the introduction. It seems that they are present only for the discussion. Wouldn't it be better to add this paragraph to the extended data and refer to it in the discussion section?

Version 1:

Reviewer comments:

Reviewer #1

(Comments for the Author)
The authors have provided and excellent introductory review on nucleobases and relevant origins of life hypotheses that better contextualize the significance of their detections.

No further comments.

Reviewer #2

(Comments for the Author)
Dear authors,
You have fully addressed my main comments, and I now find your manuscript to be of a standard that reflects the importance of your scientific results.
I am delighted to recommend your work for publication in Nature Astronomy.

Reply to reviewers' comments

We deeply appreciate the evaluation and comments from the reviewers. The responses are shown point-by-point below.

Reviewer #1 (Comments for the Author):

Overview

The authors present a robust and impressive analysis of nucleobases from meteorite samples and returned extraterrestrial materials. Moreover, improved analytical techniques enabled the identification of relationships between purine/pyrimidine relative abundances and ammonia concentrations providing new insights into their formation within parent bodies. However, there are a few minor points that need to be revised. In particular, the authors hastily rationalize the importance of nucleobases before proceeding to discuss analytical capabilities/improvements and formation mechanisms. Further elaboration in the introductory section will help the reader better appreciate the significance and impact of the results presented in this study. If properly revised and published eventually, this work will surely attract not only astrochemists but also a wide range of scientists studying prebiotic chemistry and the origins of life.

Response – We are grateful to the reviewer for the constructive and encouraging feedback. We agree with the assessment that the original Introduction devoted excessive attention to methodological aspects before adequately establishing the broader context and significance of nucleobases in extraterrestrial materials. In the revised manuscript, we have streamlined the methodological discussion and expanded the introductory section to better highlight the importance of nucleobases in the context of prebiotic chemistry and the origins of life. These revisions are intended to make the significance of our study clearer and more accessible to readers with diverse scientific backgrounds.

Minor

The intro paragraph would benefit from a few more sentences explaining the significance of nucleobases. The current version has two sentences which does not fully elaborate their function and significance in prebiotic chemistry (origins of life on Earth) and modern biological systems (adenine is the core molecule in ATP, why is that so? Is it a relic of something?). The remainder of the paper is focused on detection and formation mechanisms (within parent bodies). It is therefore critical to first establish why their detection and ubiquity discussed later in the paper is important and not rush through this opening paragraph.

Response – We thank the reviewer for highlighting the need to more fully elaborate the significance of nucleobases in both modern biological systems and prebiotic chemistry. In the revised Introduction, we have substantially expanded the opening section. Specifically, we now describe the central roles of nucleobases not only as components of genetic polymers but also as indispensable constituents of energy carriers (ATP) and cofactors (NAD^+/NADH), and we further emphasize the universal reliance of modern metabolism on adenine-based molecules as a possible relic of prebiotic chemical constraints. In addition, we discuss their essential role in enabling nucleotide formation and polymerization, as well as their importance within the context of the "RNA world" hypothesis. These revisions aim to establish from the outset why the detection and distribution of nucleobases in extraterrestrial materials are of broad significance, thereby addressing the reviewer's concern.

Line comment:

33 – 36: The introductory sentence is long and provides a lot of information. This sentence could benefit from a break at "…encoding genetic information in DNA and RNA [period]. [New sentence intro]…and also function as components of energy…"

Response – We have modified the sentence as suggested in the revised manuscript (lines 43–49) by dividing it into two shorter sentences to improve clarity.

36: Please provide example of energy carriers and coenzymes and their respective citations.

Response – We have provided examples of energy carriers and coenzymes, specifically adenosine triphosphate (ATP, ref. 2: Pinna, S. et al., 2022) and nicotinamide adenine dinucleotide (NAD^+/NADH, ref. 3: Kirschning, A., 2021), along with appropriate citations.

37: "RNA likely played a central role in early life as both genetic material and a catalyst". "early life" should be 'the origins of life'. The authors could briefly acknowledge the RNA world hypothesis rather than deferring to the citations.

Response – We have replaced "early life" with "the origins of life" and briefly acknowledged the RNA world hypothesis by describing that ribonucleic acids are considered to have functioned both as genetic carriers and as catalytic molecules, rather than deferring solely to citations (lines 50–52).

37-38: “making its nucleobases particularly relevant to prebiotic chemistry”. What about the sugar and phosphate group? This question is not meant to be answered, but to emphasize the importance of taking the time to elaborate the importance of nucleobases in the first paragraph.

Response – The phrase “making its nucleobases particularly relevant to prebiotic chemistry” has been removed in the revised manuscript, as it could unintentionally overemphasize nucleobases without acknowledging the roles of sugars and phosphate groups. The revised text now highlights the prebiotic significance of nucleobases in the context of origins-of-life research.

46: “implications for extraterrestrial habitability”. Please elaborate on ‘extraterrestrial habitability’ in this statement and provide a citation. Do impacts provide refugia? Facilitate favorable conditions for prebiotic chemistry? On Mars?

Response – In the original version of our manuscript, we intended to emphasize that the delivery of organic molecules by carbonaceous asteroids could have implications not only for the early Earth but also for other potentially habitable environments in the Solar System, such as Mars and the icy moons. However, during the revision of the Introduction we decided to streamline the text, and therefore this broader discussion of “extraterrestrial habitability” was removed to maintain conciseness.

49: “freshest” → pristine or unaltered (?)

Response – The term “freshest” has been replaced with “pristine” in the revised manuscript (line 69).

58 – 59: “likely due to sample limitations in the initial analytical procedures”. Please elaborate. Was it due to low sample masses? “extraction efficiency” is mentioned in the following line.

Response – The limited characterization in the initial analyses was primarily due to low sample masses. This has been clarified in the revised manuscript (lines 80–82).

67: “binding”. Adsorption?

Response – The phrase containing “binding” has been removed in the revised manuscript during the Introduction revision.

72: “as well as uncommon nucleobase analogs”. Non-biological isomers?

Response – The phrase “uncommon nucleobase analogs” has been rephrased to “non-biological structural isomers” in the revised manuscript (line 77).

125: Define CE-HRMS

Response – Capillary electrophoresis–high-resolution mass spectrometry (CE-HRMS) is now defined in the revised manuscript (lines 138–139).

129: “vitamer” → vitamin

Response – The term “vitamer” has been replaced with “vitamin” in the revised manuscript.

Reviewer #2 (Comments for the Author):

The manuscript by Koga et al. presents the detection of nucleobases in samples from the asteroid Ryugu. Two samples were analyzed with an improved extraction protocol compared to Oba et al. 2023 and compared to the Bennu samples and the Orgueil meteorite. All analyses were performed by HPLC coupled with high-resolution mass spectrometry (HRMS) and duplicated by CE-HRMS analyses to strengthen the identification of the targeted molecules. HRMS allows for the precise selection of the mass corresponding to the targeted compound, while the retention time of the targeted molecule in the sample is compared to a standard. For two nucleobases, MS/MS analyses were performed to strengthen the identification. The detection of molecules other than nucleobases is also present and briefly discussed in the discussion section. Interestingly, the authors demonstrate a correlation between purine/pyrimidine ratios and the amount of ammonia present in the samples.

General comments

This work is important for the astrophysics community because the Ryugu samples are unique and knowledge of their organic content is crucial. However, the same group described the detection of a first nucleobase in Nat Com in 2023. After reading the introduction, I had the feeling that this work presented itself more as results from an optimization of the extraction protocol, which is a significant challenge, but seems to me to be outside the scope of Nat Astro. I noted these different sentences in the introduction:

"Based on the results of the initial analysis of the Ryugu samples, we modified the extraction procedure to maximize efficiency by sequentially using water and acid extractions."
"Here, we applied the improved extraction method to two Ryugu aggregates (A0480 and C0370; Extended Data Fig. 1), which were specifically allocated for nucleobase analysis, to re-examine the distribution of nucleobases and other nitrogen bearing heterocyclic molecules in these samples."

I suggest the authors rewrite the introduction, clearly explaining the importance of publishing these new results in a high-level journal. They have already published the detection of the first nucleobase in 2023 in Nat Com. If the only innovative aspect lies in the detection of new nucleobases, in my opinion, this work should be considered complementary and does not deserve to be published in Nat Astro.

Authors should better explain in the introduction why detecting in a same object purine and pyrimidine could be interesting and give invaluable information on the history of the body. This aspect is described in the discussion, but has to much more describe in the introduction, than spending half of the introduction in explaining why the new protocol was used.

Response – Thank you for your thoughtful comments. In response to your suggestions, we have substantially revised the Introduction to clarify the scientific significance of this work beyond methodological improvements. In the revised manuscript, we now emphasize the importance of analyzing both purines and pyrimidines within the same Ryugu sample. This comparative analysis provides critical insights into nucleobase origins, formation mechanisms, and parent body chemical histories, which cannot be inferred from initial analyses of limited sample amounts.

Specifically, by detecting both purines and pyrimidines in Ryugu and determining their relative abundances, we found that the purine-to-pyrimidine ratios correlate with free ammonia concentrations in Ryugu, Bennu, and Orgueil meteorite. We consider this finding to represent a significant novelty, as it provides a key hint toward elucidating the formation mechanisms of extraterrestrial nucleobases.

The description of the optimized extraction and analytical procedures has been streamlined so that the focus of the Introduction is on the broader scientific implications rather than methodological details. We believe these changes address your concerns regarding the novelty and relevance of the study for a high-impact journal and clearly convey why the results presented here represent an advance over previous work.

Major Comments.

1. Page 4 line 69, “The application of the acid extraction procedure to a 17.75 mg sample from the B-type asteroid (101955) Bennu”. Can you clarify if the exact same procedure of extraction was used for Bennu and Ryugu samples? Because in Glavin Nat Astro 2025 where the detection of nucleobases in Bennu is described, no detail on the HCl procedure are given (Temperature, duration): “A separate 17.75 mg aggregate sample (OREX-800044-101), sub-sampled from Bennu OREX-800044-0, was extracted in HCl (Tama Chemicals Co., Ltd.) and analysed for N-heterocycles using HPLC/ ESI-HRMS at Kyushu University in Japan.” It seems that the same group as performed the analysis on Bennu, but can you be more precise on this aspect?

Response – Thank you for your comment. In this study, the HCl extraction procedure (temperature and duration) was identical to that previously applied to the Bennu sample (as described in the Supplementary Information of Glavin et al., 2025). However, prior to the acid treatment, we performed a room-temperature, water-based ultrasonic extraction to selectively isolate molecules that might otherwise decompose during the HCl extraction. This additional step ensures the preservation of labile organic molecules for subsequent analysis. The details of this procedure, including the sequential water extraction and its integration with the acid extraction, have been added to the Methods section (lines 294–297) for clarity.

2. For the Xanthine, it seems from Fig 2 that it is not observed in sample A080 water extract. As written lines 102-103 it suggests that his compound in present in both extracts "adenine (~14.0 min), hypoxanthine (~5.1 min), guanine 101 (~8.2 min), and xanthine (~4.9 min) in the H2O and HCl extracts of the A0480 and C0370 102 samples were observed at retention times consistent with those of authentic standards"

Response – Thank you for pointing out the typographical error. Adenine was also not detected in the H_2O extract of the C0370 sample. To clarify that xanthine and adenine were absent in the H_2O extracts of A0480 and C0370, respectively, we have added an explanatory sentence at lines 116–117 in the revised manuscript.

3. Regarding the identification by comparison of retention times between Ryugu extracts and standards, for certain nucleobases such as adenine (Fig. 2), variations in retention times are observed in the different samples. It would be important to add an error to the retention times to estimate the acceptable variability. It might be interesting to include a table in the extended data showing all retention times and their associated error. Given that no fragmentation was performed for most of the detected compounds, this aspect seems important.

Response – Thank you for the comment. We have summarized the retention times of all purine and pyrimidine nucleobases in Supplementary Table 1. As shown, analyses using the PFP column occasionally exhibited slight shifts in retention times for adenine and guanine; importantly, these shifts occurred in a coordinated manner, such that when the retention time of adenine increased or decreased, guanine's retention time shifted in parallel. This coordinated behavior allows us to robustly estimate the retention time of adenine based on the MS/MS-validated retention time of guanine. Despite these minor coordinated variations, the retention time ranges for the samples remained consistent with those of the standards, ensuring reliable identification of each nucleobase. This explanation is also provided as a footnote in Supplementary Table 1.

4. Only two compound identifications were confirmed by MS/MS: guanine and cytosine. I suggest the authors clarify that only two compounds were confirmed by MS/MS due to the low mass of the samples. This is completely understandable, but should be clearly stated. I suggest merging the information noted in lines 107-110 for guanine with that in lines 123-124 for cytosine, and clearly stating that due to the small amount of material available, only two compounds were in sufficient quantity to perform MS/MS.

Response – As suggested, we have clarified the corresponding sentences at lines 132–137 in the revised manuscript.

5. Lines 210-215, HCl extraction on the remaining residues is shown to provide better extraction than water alone. After water extraction, is it possible that there is still enough organic matter left that could react in 6 M HCl at 110°C for 12h and give the observed molecules, besides a better extraction process? Can you add a comment on that?

Response – We consider that performing the water-based extraction prior to HCl treatment helps to remove most reactive molecules that could otherwise generate the observed compounds under acidic conditions. However, if such reactive molecules were also trapped within mineral matrices and/or insoluble organic matter (IOM), we cannot fully exclude the possibility of their formation during the HCl extraction. Due to space limitations in the main text, the corresponding sentences have been moved to the Supplementary Text, and this explanation has also been added there.

6. Line 264-265, Can you explain what types of high-energy irradiation could affect the reactivity inside parent bodies of meteorites once accreted?

Response – Thank you for pointing this out. We consider that the relevant processes could involve γ-ray irradiation resulting from the decay of short-lived radionuclides such as ^{26}Al, which has been experimentally shown to induce the formation of amino acids (Kebukawa et al., 2022) and sugars (Abe et al., 2024), and may likewise have contributed to nucleobase synthesis. Accordingly, we have revised the sentence at lines 233–238 to reflect this clarification.

Minor comments

1. Page 6 line 101. Just introduce your sentence by explaining which technique you use to obtain these chromatograms. I guess they are related to HPLC/ESI-HRMS, but this only appears at line 114.

Response – We have modified the sentence at line 111 as suggested to clearly introduce the technique used to obtain the chromatograms: "Our high-performance liquid chromatography with electrospray ionization high-resolution mass spectrometry (HPLC/ESI-HRMS) analysis detected …".

2. I would suggest placing CE-HRMS analyses after LC-HRMS to better reinforce that this is an additional method to confirm identifications, in the absence of MS/MS.

Response – As suggested, we have moved the description of CE-HRMS analyses (lines 137–141) to follow the HPLC/ESI-HRMS section and emphasized that CE-HRMS serves as an additional method to confirm compound identifications in the absence of MS/MS.

3. Page 7 line 140 just add "of this work" at the end to clarify.

Response – As suggested, we have added "of this work" at the end of the sentence (line 159) to clarify.

4. Lines 158-180 other compounds were identified. There is no mention of this results in the abstract or in the introduction. It seems that they are present only for the discussion. Wouldn't it be better to add this paragraph to the extended data and refer to it in the discussion section?

Response – Thank you for the comment. Due to space limitations, the description of these additional compounds has been transferred to the Supplementary Text, which is referenced to ensure readers can access the detailed information if desired (lines 148–150).

That's all, thank you.
