

Bone mineral platelets are mesocrystals formed by monoclinic nanocrystals

Corresponding Author: Dr Viktoria K.Kis

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Version 0:

Decision Letter:

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Dear Dr K.Kis,

Thank you for submitting your manuscript, "Bone mineral platelets are mesocrystals formed by monoclinic nanocrystals", to Communications Materials. It has now been seen by 2 referees, whose comments are appended below. You will see that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Communications Materials, but would like to consider your response to these concerns in the form of a revised manuscript before we make a decision on publication.

In particular, both Reviewers have further comments about the crystal structure sample data, which could benefit from additional measurements.

We therefore invite you to revise and resubmit your manuscript, taking into account the points raised.

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When submitting your revised manuscript, please include the following:

-A response letter with a point-by-point reply to each of the referee comments and a description of changes made. Please include the complete referee report in the response letter. Please note that the response letter must be separate to the cover letter to the editors.

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In particular, the Data availability statement should include:

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We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we will close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Materials or published elsewhere in the meantime.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

Dr Jet-Sing Lee
Senior Editor
Communications Materials
orcid.org/0000-0002-6740-8700

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The crystal structure of bone minerals remained as controversy due to technical difficulties including sample size, irradiation sensitivity etc. In this work, the bone mineral platelets were characterized by high-resolution TEM and related FFT patterns. It is confirmed that the minerals exhibit monoclinic structure rather than commonly believed hexagonal structure. This explains the morphology of the bone minerals. In my opinion, this is a very significant achievement, which provides solid evidences proving bone minerals are not hexagonal apatite. However, before it can be accepted for publication, the authors need to further clarify or discuss whether it could be octacalcium phosphate as this is another important view in literature. Please explain in detail why bone mineral is monoclinic rather than triclinic based on the experimental data.

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The study is well conducted, and the manuscript is well written. It challenges the common belief that the mineral particles are hexagonal in structure and suggests a monoclinic structure based on high resolution TEM and indexing the corresponding

FFT diffractogram.

I'm not an expert in diffraction and cannot judge the interpretations *per se*, however, I want to know the risks in altering the native structure during the sample preparations, a rather tedious process in isolating the mineral particles? Is there a way to confirm the crystal structure on non-isolated particles, still remaining in the collagen structure, or to reject that the phase could be hexagonal? It is unclear why the mineral particles were analyzed from sheep bone while the bone structure was evaluated on human bone.

The overviews of the isolated and analyzed mineral particles are only as inserts in the figures and difficult to judge if overlapped, while to me figure S2:4a could potentially be overlapping particles? Or at least it looks to be a variation in thickness.

Overall, an interesting paper shedding light on the ultrastructure of bone. Well done!

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Version 1:

Decision Letter:

** Please ensure you delete the link to your author homepage in this e-mail if you wish to forward it to your coauthors **

Dear Dr K.Kis,

Your manuscript titled "Bone mineral platelets are mesocrystals formed by monoclinic nanocrystals" has now been seen again by our referees, whose comments appear below. In light of their advice I am delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Materials.

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our journal policies and formatting style in order to maximise the accessibility and therefore the impact of your work.

EDITORIAL REQUESTS

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We hope to hear from you within two weeks; please let us know if the process may take longer.

Best regards,

Dr Jet-Sing Lee
Senior Editor
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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

It is well known that correctly identifying the symmetry of a nano-crystal and its space group relies on convergent beam electron diffraction or a series of electron diffraction. I don't think the bone mineral crystal has been identified as monoclinic crystal without any doubt in this work. Instead, I strongly recommend other possibilities should be discussed in detail in this paper, which help authors have an complete view of this topic. In the current manuscript, relevant discussion has not been included.

Reviewer #2 (Remarks to the Author):

No more comments! Congratulations for a great paper!

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Response: In [001] zone axis orientation triclinic OCP can be clearly distinguished from both hexagonal and monoclinic HAP, as seen on the simulated SAED patterns shown on Figure 1A in this response letter. The angle between [100]* and [010]* of HAP (hexagonal and monoclinic) is 60°, while for OCP, the angle is approx. 71°, and accordingly the diffraction pattern has different geometry.

We have obtained [001] zone axis patterns, both from a suspension sample and from a cryo Ar-milled sample. The angles measured on the FFTs of these images support a HAP crystal structure and contradict a triclinic OCP crystal structure. We plan to further analyze these edge view images and therefore they are not included in the present manuscript, which focuses on the plane view analysis of HRTEM images of the mineral platelets.

Based on these data, we believe that a monoclinic structure provides a more feasible explanation for the observed FFT features. At the same time, for the complete crystal structure model for bone mineral including space group, common structure motifs of OCP and HAP might serve as good starting points.

The unequal development of all the hexagonal facets of apatite crystals was previously interpreted as a result of pseudomorphism after octacalcium phosphate (OCP) by several authors (Brown, Chow 1976, Davies et al. 2014). However, formation of OCP during synthesis of biomimetic apatites was not observed by ³¹P solid-state NMR without (Wang et al. 2013) or in the presence of type I collagen (Nudelman et al. 2010, Wang et al. 2012).

OCP has also a different stoichiometry; its formula $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ requires that the Ca/P ratio should be "1.33 whereas in apatite it is "1.66 (5/3), while human bone has a typical value of 1.60 (Shah, 2025). In addition, there are characteristic x-ray diffraction peaks from OCP, which are missing in the XRD of bone mineral. Only a few bone researchers (Davies et al., 2014) believe that bone is made of OCP, but they do not appear to have considered these issues.

References:

- Brown, W.E., Chow, L.C. Chemical Properties of Bone Mineral. *Annu. Rev. Mater. Sci.* **6**, 213–236 (1976).
- Davies, E., Müller, K.H., Wong, W.C., Pickard, C.J., Reid, D.G., Skepper, J.N., Duer, M.J., 2014. Citrate bridges between mineral platelets in bone. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E1354–E1363 (2014).
- Nudelman, F., Pieterse, K., George, A., Bomans, P.H.H., Friedrich, H., Brylka, L.J., Hilbers, P.A.J., De With, G., Sommerdijk, N.A.J.M. The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. *Nat. Mater.* **9**, 1004–1009 (2010).

Shah, F Revisiting the physical and chemical nature of the mineral component. *Acta Biomaterialia* (2025) 196: 1-16

Wang, Y., Azaïs, T., Robin, M., Vallée, A., Catania, C., Legriel, P., Pehau-Arnaudet, G., Babonneau, F., Giraud-Guille, M.M., Nassif, N. The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite. *Nat. Mater.* **11**, 724–733 (2012).

Wang, Y., Von Euw, S., Fernandes, F.M., Cassaignon, S., Selmane, M., Laurent, G., Pehau-Arnaudet, G., Coelho, C., Bonhomme-Coury, L., Giraud-Guille, M.M., Babonneau, F., Azaïs, T., Nassif, N. Water-mediated structuring of bone apatite. *Nat. Mater.* **12**, 1144–1153 (2013).

Unpublished data redacted

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Reviewer #2 (Remarks to the Author):

The submitted manuscript to communications materials entitled "Bone mineral platelets are mesocrystals formed by monoclinic nanocrystals" by Kis et al. uses high end microscopy to resolve the phase and structure of bone apatite.

The study is well conducted, and the manuscript is well written. It challenges the common belief that the mineral particles are hexagonal in structure and suggests a monoclinic structure based on high resolution TEM and indexing the corresponding FFT diffractogram.

I'm not an expert in diffraction and cannot judge the interpretations *per se*, however, I want to know the risks in altering the native structure during the sample preparations, a rather tedious process in isolating the mineral particles?

Response: Isolating mineral particles is done by using NaOCl as a solvent, which removes the collagen from the bone without changing the composition and structure of the mineral component, as shown by Weiner and Price (1986). They say: *"The procedures used for removing the organic matrix, dispersing the resulting particles, and separating them by size are relatively mild and safe to use. Evidence is presented to show that the crystals are unlikely to have been severely altered."*

Thus, we believe that there is no significant risk in altering the native structure when we apply NaOCl treatment.

At the same time, we have also studied the bone mineral inside the bone tissue, using cryo-Ar milled samples. In this case, no NaOCl treatment was applied. In these samples, it is extremely difficult to observe isolated mineral platelets in plane view, at crystal lattice resolution, due to the approx. 5 nm thickness of individual platelets and the very close intergrowth with collagen in a few tens of nm thick TEM specimen (at least). However, in one case, we could record a stack of mineral platelets in $[110]_{\text{mon}}$ orientation, and both the crystallographic orientation with respect to collagen fibril elongation direction and the doubling of the unit cell along b-axis was observed (see Figure 2 below).

Last, physico-chemical characterizations of biomimetic apatite and extracted bone MPs were shown to be similar, see Wang, Y., Von Euw, S., Fernandes, F.M., Cassaignon, S., Selmane, M., Laurent, G., Pehau-Arnaudet, G., Coelho, C., Bonhomme-Courty, L., Giraud-Guille, M.M., Babonneau, F., Azaïs, T., Nassif, N. Water-mediated structuring of bone apatite. *Nat. Mater.* **12**, 1144–1153 (2013).

Reviewer: Is there a way to confirm the crystal structure on non-isolated particles, still remaining in the collagen structure, or to reject that the phase could be hexagonal?

Response: On Figure 2, the mineral platelet is in the same orientation as on the plane view images from suspension samples in the manuscript and the FFT shows the same set of extra reflections with respect to hexagonal apatite (green arrows). These extra reflections can be indexed supposing monoclinic unit cell with b axis doubled, in the same way as shown in Fig3 in the submitted manuscript.

Due to space limitations in this submission, we decided to exclude these images (Figure 2 in this response letter) and associated discussion, which would have made this point quite clear. With the permission of the editor, we could include them and extend the length of the paper beyond the stipulated limit.

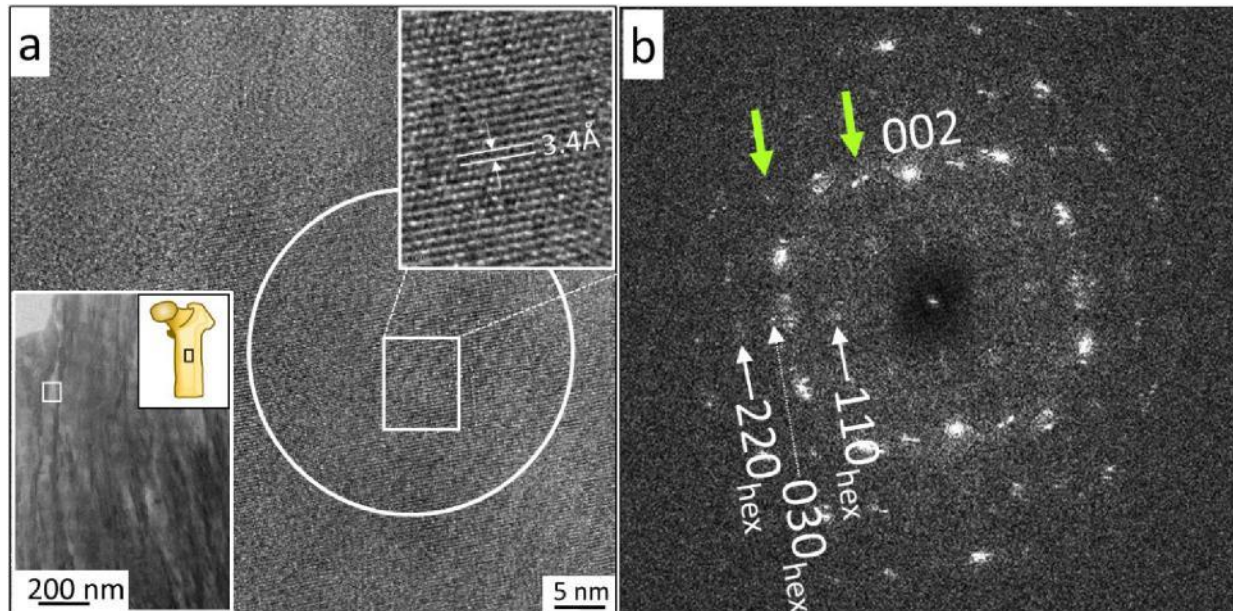


Figure 2

Correlation of bone morphology with crystallographic directions. (a) HRTEM image of region between two stacks of MPs in a cryo-milled human femur parallel section; bottom left insert: low magnification BF image; upper right insert: magnified image of HRTEM showing lattice fringes normal to c -axis, with 3.4 Å spacing corresponding to (002) interplanar distance. (b) FFT taken from the area indicated with white circle on (a). The slight arc shape of the reflections is due to the mosaic character of the MPs as explained earlier. The average orientation of the MP is $[110]_{\text{mon}}$. Green arrows indicate extra reflection rows with respect to hexagonal structure, characteristic of monoclinic apatite. The weak reflection indicated as 030_{hex} comes from another crystallite in $[100]$ orientation ($[100]_{\text{mon}}$ and $[100]_{\text{hex}}$ projections coincide).

2.6. Organization of monoclinic bone apatite crystals inside bone tissue

To complement our new understanding of the crystal morphology and their monoclinic symmetry inside MPs, it is also useful to identify these characteristics in a TEM section of bone. Insert of Figure 2a in this reply letter shows a FIB section of human femoral cortical bone oriented so that the CF axes lie in the plane of the section. Stacks of co-aligned MPs are spaced about 40 nm apart, perpendicular to the fainter 67-nm spaced gap zones of the CFs. The CF axis must be perpendicular to these lines and therefore parallel to the MP stacks, lying in the plane of the image. Between each stack of MPs in a parallel section, we are looking through a CF, which is continuously surrounded by MPs, as logically follows from cross sectional views (Fig 1a of the original submission). In BF images of parallel sections, the surrounding MPs, which lie in the plane of the section, are unresolved (Fig 1b original submission, Fig 2a insert). We are viewing them normal to the (010) plane; as confirmed by an HRTEM image of a region between two MPs stacks (Fig 2a insert) and the corresponding FFT showing $[110]_{\text{mon}}$ zone axis

orientation (Fig 2b). The HRTEM image shows 0.34 nm-spaced layer lines oriented normal to the **c**-axis of the apatite crystals. On the FFT of the area inside the white circle the additional reflection rows parallel to the [001]* axis, indicative of monoclinic symmetry, are clearly distinguished. This image confirms that the monoclinic apatite crystals of the MPs are oriented in mineralized bone with their **c**-axis aligned parallel to the collagen fibrils.

[Note: this whole text must be included as the images + caption are not sufficiently explanatory]

Reviewer: It is unclear why the mineral particles were analyzed from sheep bone while the bone structure was evaluated on human bone.

Response: It is widely accepted that, at the ultrastructural level examined in this study, bone is constructed in an equivalent manner throughout the skeleton and across all vertebrate species. This simply reflects the conservation of fundamental morphological features that is characteristic of all evolving systems. For a similar reason, the structure of mitochondria is essentially conserved across all organisms.

Notably, sheep shares substantial similarities with human in weight, bone and joint structure and in bone regeneration (Nunamaker, 1998). Therefore, this is the preferred orthopedic model for human (Robin et al. Mineralized collagen plywood contributes to bone autograft performance. Nature, 636, 100-107 (2024)).

In any case, it would have been ethically challenging to obtain a sample of human bone to use for all the aspects of this study.

The cryo-Ar milled bone sample (Figure 2 of the response letter) is human cortical bone (femur).

Reviewer: The overviews of the isolated and analyzed mineral particles are only as inserts in the figures and difficult to judge if overlapped, while to me figure S2:4a could potentially be overlapping particles? Or, at least it looks to be a variation in thickness.

Response: The reviewer is right: sometimes it is very challenging to distinguish overlaps and variations of thickness in plane view images. From edge view TEM studies (either dark field or STEM, e.g. (Schwarcz et al., 2020)) we can conclude that the thickness of the mineral platelets is quite uniformly 3-6 nm. At the same time, we cannot exclude that during sample preparation, the organics from the gap between individual mineral platelets has not been completely removed. In this case, we can find two platelets overlapping, still glued together with the organics in between them (largely citrate: see Schwarcz HP and Jasiuk I, J. Mech. Behavior Biomed. Mat. 170: 107077 (2025). That is why in the Supplementary Material of the original manuscript we highlight that

„However, in case of reflection splitting in suspension samples, incidental overlap formed during sample preparation can never be excluded for sure.” (discussion of Fig S2.4 of the submitted manuscript)

Reviewer: Overall, an interesting paper shedding light on the ultrastructure of bone. Well done!

Response: Thank you

References:

Weiner, S., Price, P.A. Disaggregation of bone into crystals. *Calcif. Tissue Int.* **39**, 365–375 (1986).

Wang, Y., Von Euw, S., Fernandes, F.M., Cassaignon, S., Selmane, M., Laurent, G., Pehau-Arnaudet, G., Coelho, C., Bonhomme-Coury, L., Giraud-Guille, M.M., Babonneau, F., Azaïs, T., Nassif, N. Water-mediated structuring of bone apatite. *Nat. Mater.* **12**, 1144–1153 (2013).

Nunamaker, D. M. Experimental models of fracture repair. *Clin. Orthop. Relat. Res.* 355, S56–S65 (1998).

Robin et al. Mineralized collagen plywood contributes to bone autograft performance. *Nature*, 636, 100107 (2024)

Schwarcz, H.P., Binkley, D.M., Luo, L., Grandfield, K., 2020. A search for apatite crystals in the gap zone of collagen fibrils in bone using dark-field illumination. *Bone*.

<https://doi.org/10.1016/j.bone.2020.115304>

Schwarcz, H.P. and Jasiuk, I, 2025, The function of citrate in bone: platelet adhesion and mineral nucleation. *Journal of the Mechanical Behavior of Biomedical Materials*, in press

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

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Answer: The Reviewer has raised doubts on our proposal of monoclinic symmetry. We believe that it is a good solution because it explains and relates two features of the mineral of bone: (1) the extra rows of spots in the FFTs and (2) the existence of flat crystals, which would be characterized by large (010) facets. In addition, Elliot et al. (1973) had shown a monoclinic structure in hydroxyapatite, which we believe the mineral of bone resembles. We have no reason to suggest other symmetries based on the evidence at our disposal. Other crystal symmetries are indeed possible (triclinic, orthorhombic) but there is no basis in the data at our disposal for suggesting these.

In this manuscript we focus on the analysis and interpretation of the highest resolution HRTEMs of bone mineral platelets, obtained until now, according to our knowledge. The exact space group determination of bone apatite single crystals is, however, still open for future work. We have added a discussion of this fact in the revised paper.

We believe that convergent beam electron diffraction (CBED) is not suitable for this purpose, because of the very low thickness (maximum 6 nm) of the mineral platelets. CBED symmetry determination (both point group and space group) relies on the analysis of contrast features measured in the diffraction discs (see e.g. Tanaka, Terauchi, Convergent-Beam Electron Diffraction, published by JEOL Ltd, 1985, and also the textbook by Williams and Carter: Transmission Electron Microscopy, Springer, 2009). These contrasting features are result of dynamical interaction of electrons with the crystal potential. For their formation, a sample thickness of at least, 50-100 nm is needed. Because the maximum thickness of bone apatite mineral platelets is 6 nm, no intensity modulation is expected inside the diffraction disks.

According to the Reviewer's suggestion, we have included a paragraph in the revised manuscript, discussing other possibilities of interpretation. This paragraph is based on our reply to critical comments that the Referee had raised in his/her first review, which we regrettably did not adequately included into the first revision of the paper.

This is the new text inserted in section 2.4, highlighted in yellow:

"Unequal development of all the hexagonal facets of apatite crystals was previously interpreted as a result of pseudomorphism after octacalcium phosphate (OCP), a hypothetical crystalline precursor phase of bone apatite^{39,40} of triclinic symmetry. However, the presence of OCP can be easily rejected in bone mineral samples⁴¹ nor does it form during synthesis of biomimetic apatites in the presence of type I collagen^{42,43}. Also, OCP has a different stoichiometry; its formula $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ requires that the Ca/P ratio should be ~ 1.33 whereas in apatite it is ~ 1.66 (5/3), while human bone has a typical value of 1.60⁴⁴. Our proposed monoclinic unit cell is inspired by the monoclinic hydroxyapatite structure proposed by

Elliott and coworkers²⁷. It yields a common indexing solution for all FFTs and a simple and plausible explanation for the typical (010) flat shape of the nanocrystals.

Hydroxyapatite crystallizes in monoclinic symmetry ($P2_1/b^{27}$), by ordering of the asymmetric OH^- groups in neighbouring channels parallel to the c -axis²⁷. However, this model for OH^- group ordering cannot apply to BA because: (1) $P2_1/b$ symmetry would require systematic absences of reflections along $(001)_{\text{mon}}^*$ and $(1-10)_{\text{mon}}^*$, but in our FFT images absences are only observed along $(001)_{\text{mon}}^*$; (2) this would require stoichiometric amounts of OH^- , whereas only minor amounts exist in BA^{18,19}. The complete crystal structure of bone mineral including its space group is still unresolved. The possible role of twinning⁴⁵ must be examined. Common structure motifs seen in OCP and HAP might also serve as good starting points.”

Accordingly, the following new references have been added to the reference list:

39. Brown, W.E. & Chow, L.C. Chemical properties of bone mineral. *Annu. Rev. Mater. Sci.* 6, 213–236 (1976).
40. Davies, E. et al. 2014. Citrate bridges between mineral platelets in bone. *Proc. Natl. Acad. Sci. U. S. A.* 111, E1354–E1363 (2014).
41. Rey, C., Combes, C., Drouet, C. & Glimcher, M.J. Bone mineral: update on chemical composition and structure. *Osteoporosis Int.* 20, 1013–1021 (2009).
42. Nudelman, F. et al. The role of collagen in bone apatite formation in the presence of hydroxyapatite nucleation inhibitors. *Nature Mater.* 9, 1004–1009 (2010).
43. Wang, Y. et al. The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite. *Nature Mater.* 11, 724–733 (2012).
44. Shah, F. Revisiting the physical and chemical nature of the mineral component. *Acta Biomaterialia* 196, 1-16 (2025).
45. Aquilano, D., Bruno, M., Rubbo, M., Massaro, & F.R., Pastero, L. Low symmetry polymorph of hydroxyapatite. Theoretical equilibrium morphology of the monoclinic $\text{Ca}_5(\text{OH})(\text{PO}_4)_3$. *Cryst. Growth Des.* 14, 2846–2852 (2014).

Reviewer #2 (Remarks to the Author):

No more comments! Congratulations for a great paper!

Answer: Thank you!