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

nature portfolio

Peer Review File

Single polyoxometalate-based nanoclusters characterized by infrared absorption nanospectroscopy

Corresponding Author: Dr Florence Volatron

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This manuscript reports a characterization of molecular self-assemble aggregates with AFM-IR technique. This AFM-IR characterization provide insightful observations of the different aggregates on surface with good chemistry resolution, which could be a value technique for similar system. The manuscript is well organized and can be accepted after certain improvements.

1. Figure S8 the label a and b are missing in the pictures
2. According to the AFM-IR results of POM-ZnPc, these are big and small nano-objects. Sounds like some of these nano-objects is pure POM, while some are the POM-ZnPc. It is possible to further clarify these two types?
3. To clearly demonstrate the results, it is better to provide some schematic diagrams of those molecular assemble aggregates.

Reviewer #2

(Remarks to the Author)
The authors of "Single polyoxometalate-based nanoclusters characterized by infrared absorption nanospectroscopy" have demonstrated the use of AFM-IR for chemical imaging a very challenging sample. This work not only will be of interest to the AFM(-IR) community but also introduces the AFM-IR technique to the field of bottom-up engineering, where it no doubt will find much further interest. I have some minor questions on the experimental part of the work and I have some purely cosmetic suggestions for improvements to the content of the manuscript. But these questions notwithstanding I recommend publication of this manuscript in Communications Chemistry.

Questions on content, experiments and execution:

The authors use HOPG as substrate for their measurements. HOPG is commonly used as substrate for AFM but - to my understanding - it has a significant draw back as an AFM-IR substrate: its high lateral thermal conductivity decreases the signal strength and the achievable contrast. Did the authors observe this effect? Does it affect their results?

The tip radius of the PPP-NCHAu (the designation PPP-NCHAu-10 stated in the manuscript is the order number for pack of 10) is not given int he manuscript. The vendor states the radius to be less than 50 nm. What are the effects of a tip radius that is much larger than the size of measured features on the AFM images? Are the presented height profiles an accurate depiction of the shape are are they distorted in some way by the shape of the tip?

I recommend the authors bring their data availability in line with the policy of Comms Chem as stated on https://www.nature.com/commschem/editorial-policies/reporting-standards

"The data availability statement must make the conditions of access to the “minimum dataset” that are necessary to interpret, verify and extend the research in the article, transparent to readers. This minimum dataset may be provided through deposition in public community/discipline-specific repositories, custom proprietary repositories for certain types of datasets, or general repositories like Figshare, Zenodo and Dryad." Whereas the authors only promise: "The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."

Presentation and minor details:

Fig. 3: the presentation of spectra in Fig 3c is a bit confusing. Maybe a legend would help?

In traditional mid-IR spectroscopy wavenumbers are displayed in descending order. I think that other spectroscopists would if fig.3c, fig 4 d&e, was using this format as well.

Page 18, line 315: "ebullition point" seems to be a translation of "le point d'ébullition". The more common English term is "boiling point".

The use of an asterisk "*" as a sign for multiplication is very uncommon. I believe the Times Operator "╳" should be used instead. Likewise, the "." in units should be replaced by the Bullet Operator "·".

For Raman spectra, integration times and averages should be mentioned.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have made proper revision. It can be accepted currently.

Reviewer #2

(Remarks to the Author)
Thank you for further improving the manuscript. I believe this work can be published as is.

Response to reviewers

We really appreciate the reviewer for taking the time to evaluate our work. Herein, we will provide a point-by-point answer to address the reviewer's comments

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This manuscript reports a characterization of molecular self-assemble aggregates with AFM-IR technique. This AFM-IR characterization provide insightful observations of the different aggregates on surface with good chemistry resolution, which could be a value technique for similar system. The manuscript is well organized and can be accepted after certain improvements.
We thank the reviewer for its positive feedback, acknowledging the novelty of our work.

1. Figure S8 the label a and b are missing in the pictures
We have corrected the picture accordingly.

2. According to the AFM-IR results of POM-ZnPc, these are big and small nano-objects. Sounds like some of these nano-objects is pure POM, while some are the POM-ZnPc. It is possible to further clarify these two types?
We thank the referee for asking this important clarification. AFM-IR, by IR characterization of all types of nano-objects on the surface allows to clarify the nature of the aggregates: pure POMs, pure ZnPc, or a mixing of POM and ZnPc. However, a quantitative analysis of the components proportions in the aggregates is not possible, due to the measurement uncertainty, and out of the scope of this study. A thorough analysis of images 4b) (AFM-IR at a typical wavenumber of the POMs), 4c) (AFM-IR image at a typical wavenumber of the ZnPc) and 5a (combination of topographic and IR images) allows to clarify the nature of the nano-aggregates in the whole image: on 27 big nano-objects, one is made of pure POMs (1% of the total amount of nano-objects) and 26 are made of POM-ZnPc complexes (28%) ; on 6 flat nano-objects, 6 are made of pure ZnPc (6% of the total amount of nano-objects) ; on 61 small nano-objects, 52 correspond to the POM-ZnPc complex (55% of the total amount of nano-objects) and 9 correspond to small aggregates of pure ZnPc (10%). The isolated POM-ZnPc complexes constitutes the majority of nano-objects observed in this image. This more detailed study has been added in the main text (p. 14) and refers to the Supplementary Fig.12, proposing some schemes of the various nano-objects (see remark 3) : "Overall, on the image, on the total amount of nano-objects, 28% corresponded to big aggregates made of POM-ZnPc complexes, 1% corresponded to big aggregates made of pure POMs, 6% corresponded to flat aggregates made of pure ZnPc and 65% were small nano-objects. Most of the small nano-objects (55%) appeared in purple in the 3D image. The others (10%) appeared in red, showing the presence of small nano-objects made of limited aggregation of pure ZnPc (Supplementary Fig.12)"

3. To learly demonstrate the results, it is better to provide some schematic diagrams of those molecular assemble aggregates.
We thank the reviewer for this relevant suggestion. We added a Supplementary Figure (Supplementary Fig. 12) in the supplementary information file showing the supposed structure of the formed aggregates.

Reviewer #2 (Remarks to the Author):

The authors of "Single polyoxometalate-based nanoclusters characterized by infrared absorption nanospectroscopy" have demonstrated the use of AFM-IR for chemical imaging a very challenging sample. This work not only will be of interest to the AFM(-IR) community but also introduces the AFM-IR technique to the field of bottom-up engineering, where it no doubt will find much further interest. I have some minor questions on the experimental part of the work and I have some purely cosmetic suggestions for improvements to the content of the manuscript. But these questions notwithstanding I recommend publication of this manuscript in Communications Chemistry.
We really thank the reviewer for its positive feedback. The field of surface's modification of surface chemistry really lacks of powerful techniques that combine imaging with chemical sensitivity at these reduced scales.

Questions on content, experiments and execution:

The authors use HOPG as substrate for their measurements. HOPG is commonly used as substrate for AFM but - to my understanding - it has a significant draw back as an AFM-IR substrate: its high lateral thermal conductivity decreases the signal strength and the achievable contrast. Did the authors observe this effect? Does it affect their results?
We did not observe any effect of the lateral thermal conductivity during our measurements. Indeed, thermal conductivity does not intervene in this measurement: the device is sensitive to the dilatation of the nano-objects (thermal expansion), which is a much faster process (femtosecond scale compared to the 10-100 ns scale of the thermal diffusion).

The tip radius of the PPP-NCHAu (the designation PPP-NCHAu-10 stated in the manuscript is the order number for pack of 10) is not given int he manuscript. The vendor states the radius to be less than 50 nm. What are the effects of a tip radius that is much larger than the size of measured features on the AFM images? Are the presented height profiles an accurate depiction of the shape are are they distorted in some way by the shape of the tip?
We specified in the main text (“Materials and Methods, 4. Characterization techniques, Infrared absorption nanospectroscopy”) the value of the tip radius informed by the supplier (less than 50 nm). With AFM-IR, the measurement of the height of the nano-objects is very accurate. Nevertheless, a lateral distortion is expected when the size of the tip is much larger than the size of the nano-object due to a deconvolution effect with the tip. The value for the FWHM (50 nm) is coherent with a deconvolution between a nano-object of around 1.5-2 nm and a tip of a radius below 50 nm. In the main text p.15, the unclear sentence p.15 “(the radius of curvature of the AFM tip is around 25 nm)” has been removed and a small paragraph has been added to clarify that point (p.15) :
Moreover, if the measurement of the height of the nano-objects is very accurate with AFM-IR, a lateral distortion is expected due to a deconvolution effect with the tip. A FWHM of around 50 nm is coherent with a tip radius below 50 nm and a lateral size of nano-objects around 1.5-2 nm.

I recommend the authors bring their data availability in line with the policy of Comms Chem as stated on https://www.nature.com/commschem/editorial-policies/reporting-standards

"The data availability statement must make the conditions of access to the “minimum dataset” that are necessary to interpret, verify and extend the research in the article, transparent to readers. This minimum dataset may be provided through deposition in public community/discipline-specific repositories, custom proprietary repositories for certain types of datasets, or general repositories like Figshare, Zenodo and Dryad." Whereas the authors only promise: "The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request."
According to the recommendation of the referee, we will add the minimum dataset in a public repository if the article is accepted (Dryad).

Presentation and minor details

Fig. 3: the presentation of spectra in Fig 3c is a bit confusing. Maybe a legend would help?
We have added a legend in Fig 3c

In traditional mid-IR spectroscopy wavenumbers are displayed in descending order. I think that other spectroscopists would if fig.3c, fig 4 d&e, was using this format as well.
All the figures with AFM-IR spectra (Fig. 3, 4 and 5 in the main text and Supplementary Fig. 6, 8, 9, 10 and 11) have been correctly drawn with the wavenumbers in descending order.

Page 18, line 315: "ebullition point" seems to be a translation of "le point d'ébullition". The more common English term is "boiling point".
We have corrected this typo.

The use of an asterisk "*" as a sign for multiplication is very uncommon. I believe the Times Operator "×" should be used instead. Likewise, the "." in units should be replaced by the Bullet Operator "·".
Thanks for the comment, we have promptly addressed this issue.

For Raman spectra, integration times and averages should be mentioned.
The time of integration was between 30 and 60 seconds and an average of 3 spectra was done for each measurement. We have specified that information in the part “Materials and Methods, 4. Characterization techniques, Raman Spectroscopy”.

Response to reviewers

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors have made proper revision. It can be accepted currently.

Reviewer #2 (Remarks to the Author):

Thank you for further improving the manuscript. I believe this work can be published as is.

We thank both referees for their additional review and positive feedback.