

jz-2023-007649.R1

Name: Peer Review Information for "Excited-State Dynamics in a DNA-Stabilized Ag16 Cluster with Near-Infrared Emission"

First Round of Reviewer Comments

Reviewer: 1

Comments to the Author

In this work, Chen et al. probed the early time excited state dynamics of DNA stabilized Ag16 NCs using ultrafast transient absorption spectroscopy and 2DES spectroscopy. The excited state properties of metal NCs is very important for understanding their energy dissipation mechanism for their real applications in biosensing and photoluminescence. This work is carefully performed and the time-resolved data is of very high quality. They picture a relaxation model based on the time-resolved data, which clearly show how the long-lived photoluminescence occurs. I therefore recommend it accepted by JPCL after the following minor revisions.

(1) In Figure 1, it is suggested to provide the structure of metal NCs to show how the DNA molecules and metal NCs are arranged. Even if there is no accurate structure, a cartoon figure will help the readers to understand the structure of metal clusters look like.

(2) In Figure 3, global analysis including EADS and DAS were shown, while there is not TA kinetic traces and fitting results, which is helpful for evaluating the quality of the fitting.

(3) In Figure 2, the label of time delay in the figure is misleading, I suggested to add color to the time delay "100 fs" , "500 fs"

Reviewer: 2

Comments to the Author

An in-depth understanding of the excited-state dynamics of fluorescent silver nanoclusters is still lacking which is beneficial to modify the PL properties of nanoclusters. In this work, the authors claim that the kinetic model of the photoinduced dynamics has been clarified of DNA-protected Ag16 nanocluster via ultrafast optical spectroscopies. I think this work is meaningful and interesting for nanocluster science. However, some major issues should be addressed before publication.

1) Page 2, the radiative rate (7.9×10^8) seems to be wrong. According to the equation: radiative rate $k_r = \Phi_{em} / \tau_{av} = 0.26 / (3.3 \times 10^{-9}) = 7.9 \times 10^7 \text{ s}^{-1}$. Although this rate is still large, the authors must check whether it has any influence on your viewpoint.

- 2) I noticed that the photophysical data was measured at room temperature. In this context, many energy was dissipated such as the vibration of ligands as the nonradiative process. How does the author think about the effect of such nonradiative energy on the photoinduced dynamics of DNA-Ag16 nanocluster?
- 3) Page 3, “the Stokes shift 2λ from the standard deviation σ of the absorption line using the expression”. Please give relevant references.
- 4) Page 3, “Instead, we must conclude that relaxation involves fundamental structural and/or electronic reorganization”. What has this led to? And, “This is in line with previous observations that suggest that the absorbing and emitting states are different in these systems”, what are the previous observations? A brief description of these results should be mentioned.
- 5) Page 5, “The assignment of these subtle spectral changes to the vibrational cooling of a “hot” excited state is in good agreement with the observations from transient infrared spectroscopy experiments on a green emitting DNA-AgNC”, please give more details of the experiment’s results.
- 6) The elucidation of electronic structure by theoretical calculations is necessary to understand not only the whole photoinduced dynamics but in verify the validity of the results of ultrafast time-resolved optical spectroscopy in experiments.

Author's Response to Peer Review Comments:

Dear editor and reviewers

We thank the reviewers for their positive and constructive feedback. In the following we detail our response to the reviewer queries, and outline the explicit changes done to the manuscript based on the reviewer recommendations. We believe we have carefully addressed all the comments to the best of our ability, and hope that the text is now satisfactorily amended.

We have provided the following updated documents:

- A revised manuscript with all changes tracked: "Photoinduced_Dynamics_Revised_TrackChanges.docx"
- A revised manuscript without tracked changes: "Photoinduced_Dynamics_Revised.docx"
- A revised Supporting information: "SI_Photoinduced_Dynamics_Revised.docx"
- Revised figures in .pdf format, at correct size and resolution.

In order to aid in the organization and readability of this reply, the text is color coded. Our responses are written in **blue font**, and changes to the manuscript are highlighted with **red font**. Direct quotes from the text are in "*black italics*".

Changes not related to reviewer queries

The affiliation of Ajeet Kumar has been corrected.

Corrections to minor issues in punctuation and formatting of the references have been made throughout the text.

All changes can be found in the manuscript file "Photoinduced_Dynamics_Revised_TrackChanges.docx"

Reviewer 1:

In this work, Chen et al. probed the early time excited state dynamics of DNA stabilized Ag16 NCs using ultrafast transient absorption spectroscopy and 2DES spectroscopy. The excited state properties of metal NCs is very important for understanding their energy dissipation mechanism for their real applications in biosensing and photoluminescence. This work is carefully performed and the time-resolved data is of very high quality. They picture a relaxation model based on the time-resolved data, which clearly show how the long-lived photoluminescence occurs. I therefore recommend it accepted by JPCL after the following minor revisions.

We thank the reviewer for the positive reception of our work.

Comment #1:

In Figure 1, it is suggested to provide the structure of metal NCs to show how the DNA molecules and metal NCs are arranged. Even if there is no accurate structure, a cartoon figure will help the readers to understand the structure of metal clusters look like.

Author Response:

We thank the reviewer for pointing this out. One of the reasons we study this cluster in particular is that it is in fact one of very few DNA-stabilized silver clusters with a known structure. As such, it is of course valuable to show the structure in the text. We have modified manuscript Figure 1 to include the cluster structure. We have also added the reference to the structure in the Protein Data Bank to the figure caption.

Comment #2:

In Figure 3, global analysis including EADS and DAS were shown, while there is not TA kinetic traces and fitting results, which is helpful for evaluating the quality of the fitting.

Author Response:

We have added new figures **Figure S6** and **Figure S7** to the supporting information, showing representative kinetic traces from the two TA experiments, the corresponding fits, and the residuals.

In the main text we have updated the “Associated Content. Supporting Information” section to reflect this.

Comment #3:

In Figure 2, the label of time delay in the figure is misleading, I suggested to add color to the time delay “100 fs”, “500 fs”

Author Response:

The time-delays of the spectra in Figure 2c are explicitly given in the figure text. The spectra at earliest (latest) time-delays in each panel are colored blue (orange). The font color in the inset figure text has been changed to make this more explicit.

Reviewer 2:

An in-depth understanding of the excited-state dynamics of fluorescent silver nanoclusters is still lacking which is beneficial to modify the PL properties of nanoclusters. In this work, the authors claim that the kinetic model of the photoinduced dynamics has been clarified of DNA-protected Ag16 nanocluster via ultrafast optical spectroscopies. I think this work is meaningful and interesting for nanocluster science. However, some major issues should be addressed before publication.

We thank the reviewer for the constructive feedback, and largely positive reception to our work.

Comment #1:

Page 2, the radiative rate (7.9×10^8) seems to be wrong. According to the equation: radiative rate $k_r = \Phi_{em} / \tau_{av} = 0.26 / (3.3 \times 10^{-9}) = 7.9 \times 10^7 \text{ s}^{-1}$. Although this rate is still large, the authors must check whether it has any influence on your viewpoint.

Author Response:

The reviewer is correct in pointing out this unfortunate error. We have updated the rate constant to the correct value. The value $\sim 8 \times 10^7 \text{ s}^{-1}$ of course remains well within the range of “strongly allowed” transitions, where one by the Strickler-Berg relationship would expect easily observable features in the UV/Vis absorption spectra – typically with molar absorption coefficients in the range of a few tens of thousand $\text{cm}^{-1}\text{M}^{-1}$. As such, this change has no effect on quantitative or qualitative interpretation of the data.

Comment #2:

I noticed that the photophysical data was measured at room temperature. In this context, many energy was dissipated such as the vibration of ligands as the nonradiative process. How does the author think about the effect of such nonradiative energy on the photoinduced dynamics of DNA-Ag16 nanocluster?

Author Response:

On the basis of our observations of similar fast relaxation dynamics in experiments on a cluster at 77K (Ref 10 in the manuscript), we do not expect the optical properties to be qualitatively altered within a reasonable range of temperatures. Of course, at sufficiently high temperatures one would expect significant changes to the DNA folding and its ability to ligate the silver cluster. As the DNA-Ag clusters we have investigated so far (including the one investigated in this work) are robust at biological temperatures, however, we appear to be well below temperatures where such overall structural changes are a concern.

Beyond this, as noted throughout the text, we expect the energy dissipation into the environment after photoexcitation to be crucial in the excited-state behavior of these clusters. The specifics of these thermalization processes are at this point an open question, and it is e.g. not clear which vibrational modes are most strongly involved in the relaxation dynamics. That is; it is not clear to which extent specific discrete modes of the nucleobases contribute, relative to discrete high-frequency modes or the low-frequency continuum of the solvent.

That said, we do not expect the thermal energy present in the bath to strongly influence the initial relaxation dynamics. The cluster dissipates $\sim 5500 \text{ cm}^{-1}$ of energy into the bath within the first 100 fs –

this is some 25 times $k_B T$ at room temperature, and while we expect that this substantially disturbs the environment, it seems unlikely that the thermal population of vibrations significantly influence the relaxation dynamics per se. In terms of relaxation within the fluorescent state, we comment on the relatively minor contribution from vibrational relaxation in the manuscript:

“This is consistent with the 26% fluorescence quantum yield, and suggests that the major non-radiative relaxation pathway from the fluorescent state is via a μs -lived excited state¹⁸ as opposed to direct internal conversion to the ground state. The lack of apparent internal conversion from the fluorescent state might be a beneficial property of this class of emitters, allowing potential design of NIR emitters with high fluorescence quantum yield. That this is a reasonable assumption was demonstrated by a DNA-AgNC with an emission maximum 721 nm that had a near unity fluorescence quantum yield in D2O at 5°C.”

Comment #3:

Page 3, “the Stokes shift 2λ from the standard deviation σ of the absorption line using the expression”. Please give relevant references.

Author Response:

This relation between lineshape parameters appears in the high-temperature and slow-fluctuation limit of the Brownian oscillator model (e.g. a room temperature ensemble, where the transition frequencies of the individual members are static on the timescale of the experiment), as referred to in the preceding paragraph in the text and referenced to a standard textbook on the subject - Shaul Mukamel’s monograph on non-linear spectroscopy (Ref 22). We have updated the text to make this more explicit:

“... 2λ from the standard deviation σ of the absorption line using the expression...”

Now reads:

“In this limit, the Stokes shift 2λ can be predicted from the standard deviation σ of the absorption line with the expression $\sigma^2 = 2\lambda (k_B T) / \hbar$, which yields a value of only $\approx 800 \text{ cm}^{-1}$ – about one seventh of the observed value”

Comment #4:

Page 3, “Instead, we must conclude that relaxation involves fundamental structural and/or electronic reorganization”. What has this led to? And, “This is in line with previous observations that suggest that the absorbing and emitting states are different in these systems”, what are the previous observations? A brief description of these results should be mentioned.

Author Response:

To the first comment:

In this paragraph of the text, we demonstrate that the observed Stokes shift is completely incompatible with a model of an electronic two-level system embedded in a slowly fluctuating bath. That is, the solvent (or more generally, the environment) reorganization energy λ predicted by the absorption linewidth in this model is much smaller (by \sim a factor 7) than the observed value. As the observed data

cannot be explained by simple solvent/environment reorganization, we can directly conclude that relaxation from the Franck-Condon point involves either substantial reorganization (of nuclear and/or electronic structure) or transfer into another lower-energy state.

Regarding the second comment:

We have slightly expanded on this in order to clarify the conclusions of multiple states drawn from earlier work. **We have updated the relevant statement:**

“This is in line with previous observations that suggest that the absorbing and emitting states are different in these systems”

To now read:

“In earlier work on similar clusters, the absorption and emission were assigned to different distinct states: in one case based on multiple distinct spectral features in the ultrafast optical spectra¹⁰, and in a second based on bi-modal ground-state recovery kinetics¹¹. These assignments are in line with our observations here.”

Comment #5:

Page 5, *“The assignment of these subtle spectral changes to the vibrational cooling of a “hot” excited state is in good agreement with the observations from transient infrared spectroscopy experiments on a green emitting DNA-AgNC”, please give more details of the experiment’s results.*

Author Response:

We have modified the text to more explicitly note the observations from the TR-IR experiment of the Kohler group (ref 24). The above sentence now reads:

“... is in good agreement with the observation of picosecond lineshape dynamics in transient infrared spectroscopy experiments on a green emitting DNA-AgNC²⁴.”

Similarly, in the discussion of quantitative dynamics on page 7, we have modified a reference to the same TR-IR work from

“Both the timescale and the observed spectral changes are consistent with vibrational cooling of “hot” excited states, as also by earlier ultrafast vibrational experiments²⁴”

To now read:

“...“hot” excited states, and consequently also corresponds well to subtle lineshape-dynamics observed on the same timescales in earlier ultrafast vibrational experiments²⁴”

Comment #6:

The elucidation of electronic structure by theoretical calculations is necessary to understand not only the whole photoinduced dynamics but in verify the validity of the results of ultrafast time-resolved optical spectroscopy in experiments.

Author Response:

As we note in the manuscript, we agree that significant efforts from the theory side are needed to elucidate the excited state behavior and the detailed electronic structure of these clusters. While some progress on ground-state structure and electronic properties have been made, *e.g.* by the groups of Christine Aikens and Stacy Copp (as well as the work of some of us in the recent preprint in Ref 15), we are not aware of any detailed dynamics calculations at this point.

While our experimental data stands on itself, we acknowledge that theoretical efforts are necessary in order to form a robust and detailed interpretation of the experimental observations.

jz-2023-007649.R2

Name: Peer Review Information for "Excited-State Dynamics in a DNA-Stabilized Ag₁₆ Cluster with Near-Infrared Emission"

Second Round of Reviewer Comments

Reviewer: 1

Comments to the Author

The authors have addressed all of my concerns and the manuscript should be accepted by JPCL. It provides new insight to the photophysics of metal NCs.

Reviewer: 2

Comments to the Author

This revised manuscript has addressed all reviewers' concerns. I think the manuscript is acceptable as it.

Author's Response to Peer Review Comments:

Dear Reviewers, Editor and Editorial staff

First of all, it should be noted that the fast and positive action & response from both JPCL editorial and reviewers in the handling of our manuscript is very much appreciated.

We have submitted an (we believe) appropriately edited manuscript and related files. Our comments to the editorial queries are as follows:

TOC GRAPHIC

We have uploaded a figure called "TOC GRAPHIC.tif" as a figure graphic, as there does not appear to be an option to label any submitted file as "TOC GRAPHIC" specifically in the roll-down menus.

GRAPHICS

As far as we can tell, this comment refers to Figure 4 in the manuscript. This figure has not been reproduced from any other publication

- it is simply a combined energy-level diagram and "graphic kinetic scheme". Such figures are, by their nature, fairly similar looking for many systems, so perhaps this is where this confusion stems from.

Beyond this: all other graphics in the paper are naturally also produced uniquely for this manuscript. If editorial have specific concerns about similarity between figures in this manuscript and previously

published work, we would very much like to be informed of this. The original data & scripts used to produce the figures here can of course be provided if necessary.

REFERENCES

- Author with >10 authors have been shortened to "10 authors, + et al."
- Reference numbers now encased in ().
- All article titles now in Title Case.
- We have amended the incomplete citations in manuscript and SI.

Note, that reference 15 is to a pre-print (on ChemRxiv) rather than a printed journal. It is not entirely clear to us from the ACS style guide how you wish these citations to be formatted. In our revised manuscript, reference 15 reads as follows:

Gonzalez-Rosell, A.; Malola, S.; Guha, R.; Arevalos, N.; Matus, M.; Goulet, M.; Haapaniemi, E.; Katz, B.; Vosch, T.; Kondo, J.; et al., Chloride ligands on DNA-stabilized silver nanoclusters. ChemRxiv (Materials Chemistry) 2023, DOI: 10.26434/chemrxiv-2023-m9txt (accessed 2023-04-17).

If a different format is preferred, please advise.

SUPPORTING INFORMATION

- Slight update to author affiliations to match those in the manuscript proper
- We have amended the incomplete citation and corrected the reference format.
- Pages are now numbered S1-S6