

Structure of a biohybrid photosystem I-platinum nanoparticle solar fuel catalyst

Corresponding Author: Dr Lisa Utschig

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:

Reviewer #1

(Remarks to the Author)

In their manuscript Gisriel and colleagues describe the solution structure of trimeric cyanobacterial photosystem I with bound Pt nano particles as analyzed by single particle cryo electron microscopy. They claim that this is a first structure determination of a photosystem with a bound man-made catalyst. A claim which to my knowledge is justified. The authors succeeded to pinpoint which subunits and amino acid residues are likely the most important areas for the stabilization of the artificial electron acceptor in tunneling distance to FB. Furthermore, they could also describe a non-productive binding site on the stromal surface of PSI that, if eliminated, should enhance the efficiency of Pt nano-particle usage. Detailed structural insights into the binding sites of Pt nano particles on the surface of photosystem I could inform the design of both protein surface and nano particle shape and size for the improvement of binding affinity and catalytic efficiency, potentially boosting in vitro hydrogen production. Therefore I think this is a straightforward, important and well executed structural study of photosystem I in the field of applied bioenergetics that deserves publication at prominent place.

Before publication, however, I recommend the authors to improve their manuscript in aspects of presentation style in both language and visuals to match their experimental efforts for the execution of this project.

Minor comments:

The last sentence of the abstract is a bit of an overstatement, since side chain densities of interacting amino acids and Pt atom positions are not resolved. Better to tone that down a bit.

Naturally the authors are familiar with the structure of cyanobacterial PSI. But that is not necessarily true for all readers. Please label all shown subunits in Figure 1 in at least one of the visuals. Also label the subunits relevant to the other figures content, e.g. subunit c, d, e, j and a in figure 2. In its current version it looks as if the green Pt nano-particle is binding directly to PsaC, even though it is not but rather to PsaE. The authors used Chimera for making the visuals and perhaps using ChimeraX would benefit the readers of the paper, since it is better at lightening of surfaces.

The side chain conformation is not visualized for most of the likely to interact amino acids. Though I do not think that this impacts the study's conclusions I would like to have this more clearly stated in the main text and figure captions.

I do not request to quote this study, however, I was struck by the fact the position of the Pt A nano-particle is very similar to that of the delta1 density in

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Please check this out.

Figure 4 is not only very hard to understand in terms where is what (subunit labels? surface? PSI stromal surface looks completely flat), but choosing red/green for the indication clashes between artificial and physiological electron acceptors is not fair to a substantial portion of the population. Why did you use the really not so well resolved model 7S3D for the position of Fd? Please use the best resolved one, i.e. 7FIX.

When preparing Pt nano-particle bound PSI the used ratio was 2 to 1, however, the structure indicates 3 to 1. Please discuss.

The postprocessed map was calculated with the nano-particles masked out. Please describe and discuss reasons for doing so.

The image processing might be improved by others. I urge the authors to deposit their image raw data to EMPIAR, which might lead to others informing you how get a better grip on nano-particle image processing for further studies. I am sure you will benefit greatly from a deposition.

Were screened grids transferred to the Krios instrument? I presume yes, please clarify. Same method section: the K3 camera has no slit, but energy filters do have. Selectris I guess? For blotting which BF did you use? Please brush-up this section.

Please check the spelling of Schürmann in reference 60.

The spectrum in FigS3 should also be done for PSI without nano-particles and included for comparison.

The term ladder in FigS4 refers to a molecule weight marker I suppose. Please label accordingly.

Reviewer #2

(Remarks to the Author)
Review comments attached.

Reviewer #3

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)
The authors used cryo-EM technique to interrogate the platinum nanoparticle (NP) binding sites on PSI, a biohybrid solar fuel catalysis model system. Two binding sites of PN per PSI monomer were discovered. This research filled the blank area of structural details underlying the photo-driven H₂ generation and the nature of PtNP interactions with PSI. The high-resolution of cryo-EM biohybrid system also laid the ground work for future research on the fine-tuning of the efficiency of H₂ generation in this biohybrid system.

Minor questions:

1. According to this research there are an average of 400 Pt atoms per PSI monomer. There are total of 2 binding sites per monomer, i.e., 200 Pt atoms per NP. Please cite another reference to support the claim that there are 200 Pt atoms per NP with a size distribution of 1.8-2.0 nm. The cited literature (p276, Ref11) doesn't support the 200 Pt atoms per NP.
2. Include a room temperature absorption spectrum of PSI in Fig. S3, as a control to the isolated PSI-PtNPs.
3. Define TON (p81) the first-time use.
4. Discuss the NP binding stoichiometry per monomer of this research with that of Ref 11, i.e., 2 vs 1.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I am happy to see that the authors took advantage of the reviewers comments to improve their manuscript substantially.

The manuscript should be ready for publication, but before doing so, please try to

take into account red/green color blindness also for figure 2

add a bracket in the caption of figure 4

add the EMPIAR data bank entry to the Data and materials availability section.

Reviewer #2

(Remarks to the Author)

The authors have addressed all of our previous comments in the revised manuscript and supporting information documents. We recommend publication with one minor suggestion below.

In the revised Figure S11 (previous S10) the legend includes a different set of species names than those that are presented in the figure.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

The authors have addressed my comments/suggestions.

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In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

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Reviewer Comments for

Structure of a biohybrid photosystem I-platinum nanoparticle solar fuel catalyst

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Reviewer 1

In their manuscript Gisriel and colleagues describe the solution structure of trimeric cyanobacterial photosystem I with bound Pt nano particles as analyzed by single particle cryo electron microscopy. They claim that this is a first structure determination of a photosystem with a bound man-made catalyst. A claim which to my knowledge is justified. The authors succeeded to pinpoint which subunits and amino acid residues are likely the most important areas for the stabilization of the artificial electron acceptor in tunneling distance to FB. Furthermore, they could also describe a non-productive binding site on the stromal surface of PSI that, if eliminated, should enhance the efficiency of Pt nano-particle usage. Detailed structural insights into the binding sites of Pt nano particles on the surface of photosystem I could inform the design of both protein surface and nano particle shape and size for the improvement of binding affinity and catalytic efficiency, potentially boosting in vitro hydrogen production. Therefore I think this is a straightforward, important and well executed structural study of photosystem I in the field of applied bioenergetics that deserves publication at prominent place.

Before publication, however, I recommend the authors to improve their manuscript in aspects of presentation style in both language and visuals to match their experimental efforts for the execution of this project.

We are pleased to see the reviewer's overall positive assessment of this manuscript and appreciate the thoughtful and helpful suggestions to clarify our presentation of the science. In response to the reviewer's comments, we have inspected the manuscript and made many changes to improve language and visuals and detail these as follows:

Minor comments:

The last sentence of the abstract is a bit of an overstatement, since side chain densities of interacting amino acids and Pt atom positions are not resolved. Better to tone that down a bit.

We have changed this statement to read "Overall, the investigation reveals the first direct structural look at a photon-to-fuels photosynthetic biohybrid system."

Naturally the authors are familiar with the structure of cyanobacterial PSI. But that is not necessarily true for all readers. Please label all shown subunits in Figure 1 in at least one of the visuals.

We think this would make the figure a bit too busy. We have added **Supplementary Fig. 10** which shows a stromal and membrane plane view of a PSI monomer with all 12 subunits clearly labeled.

In addition, we have added **Supplementary Fig. 14** which clearly shows the stromal ridge of PSI, which is comprised of the PsaE, PsaC, and PsaD subunits.

Also label the subunits relevant to the other figures content, e.g. subunit c, d, e, j and a in figure 2.

Thank you for this suggestion. We have labeled the subunits in **Fig. 2**.

In its current version it looks as if the green Pt nano-particle is binding directly to PsaC, even though it is not but rather to PsaE. The authors used Chimera for making the visuals and perhaps using ChimeraX would benefit the readers of the paper, since it is better at lightening of surfaces.

This figure was made in PyMOL. We attempted to remake this figure in ChimeraX, but it did not improve the clarity. We think the concern of the reviewer is better addressed in **Fig. 1c** showing the outlines of the PtNPs from a stromal view. We have added a label on **Fig. 1c** pointing out the location of PsaC so that the reviewer's point is clearer to the reader.

The side chain conformation is not visualized for most of the likely to interact amino acids. Though I do not think that this impacts the study's conclusions I would like to have this more clearly stated in the main text and figure captions.

We have altered the text on page 6 to read the following: "However, we cannot account for whether there is full coverage of mercaptosuccinic acid on the PtNPs, nor how the mercaptosuccinic acid might rearrange upon PtNP-binding to PSI. Furthermore, the sidechains of amino acids that potentially interact with the PtNPs are not well resolved. It should also be noted that surface residues are often the most flexible and therefore difficult to precisely position in a cryo-EM map. Coupled with the lower local resolution at the periphery of the structure, and with the probable positional heterogeneity of the PtNPs, it is therefore unsurprising that interactions between amino acids and PtNPs are challenging to determine. That said, numerous residues are close enough to the PtNP in site A to propose specific interactions."

I do not request to quote this study, however, I was struck by the fact the position of the Pt A nano-particle is very similar to that of the delta1 density in

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Please check this out.

Very interesting. Maybe no matter what binds there, there are dynamics at play, perhaps due to interactions with PsaE or light-induced conformational changes of PSI. Possibly it can be thought of as a landing strip, an initial collision complex wherein, after initial binding, Fd/Flv would readjust into the optimal positions for efficient electron transfer due to specific hydrophilic and hydrophobic interactions to bind tightly, but other things (PtNPs, PGR5?) get only part of the way down the runway due to lack of evolutionarily tuned protein-protein interactions. Something to think about.

Figure 4 is not only very hard to understand in terms where is what (subunit labels? surface? PSI

stromal surface looks completely flat), but choosing red/green for the indication clashes between artificial and physiological electron acceptors is not fair to a substantial portion of the population.

We have updated the figure so that (a) the native acceptor maps are blue and the PtNPs are grey, (b) it states that this is a view from the stromal side, and (c) the stromal subunits are labeled. We hope the figure is now clearer.

Why did you use the really not so well resolved model 7S3D for the position of Fd? Please use the best resolved one, i.e. 7FIX.

We now use 7FIX for **Fig. 3** instead of 7S3D.

When preparing Pt nano-particle bound PSI the used ratio was 2 to 1, however, the structure indicates 3 to 1. Please discuss.

We appreciate the reviewer's comment. The initial ratio of 2 to 1 was estimated for a PtNP size of 2.5 nm. From our more recent STEM imaging the nominal size of PtNP is ~2 nm. This increases the estimate of the PtNP concentration in solution from 3.5 μM to 7.2 μM . In the hybrid preparation, we are mixing ~4.6 mol equivalents of PtNP with 1 mol equivalent of PSI monomer. We are grateful for the reviewer's comment that allowed us to catch this mistake. We updated the **Methods** section with these details. Still, this remains a rough estimate because PtNP preparations yield heterogeneous size populations and are difficult to reproduce exactly between batches. We also think the structure does not necessarily indicate a particularly accurate stoichiometry because it is possible that the PtNP sites are only partially occupied. Please see the changes to the manuscript we have made on page 9 to better describe PtNP binding heterogeneity based on Reviewer 2's comments. Consistent with this, the ICP-AES analysis of the sample used for cryo-EM determined 400 ± 28 Pt per 12 Fe, or roughly one ~2 nm PtNP per PSI monomer.

The postprocessed map was calculated with the nano-particles masked out. Please describe and discuss reasons for doing so.

We have now added the following text to the **Methods**: "Note that in the masked and sharpened map, the PtNPs are masked out. This was to (a) consider only the protein that would result in the best structure of *S. lividus* PSI possible, and (b) avoid oversharpening in the PtNP signal regions that are low resolution."

The image processing might be improved by others. I urge the authors to deposit their image raw data to EMPIAR, which might lead to others informing you how get a better grip on nano-particle image processing for further studies. I am sure you will benefit greatly from a deposition.

As the reviewer suggested, we have deposited these data into EMPIAR. Please note that the EMPIAR web site states that no entries will be annotated or released before August 18th. But, all the files were successfully uploaded on our end.

Were screened grids transferred to the Krios instrument? I presume yes, please clarify.

We now specify that the grids were transferred to the Krios after initial Glacios screening.

Same method section: the K3 camera has no slit, but energy filters do have. Selectris I guess?

We have altered the text to the following: “Grids were transferred to a 300 kV Titan Krios G2 transmission electron microscope (Thermo Fisher/FEI) equipped with a Gatan K3 direct electron detector and a Gatan BioQuantum energy filter with a slit width of 15 eV for high-resolution data collection. The data were collected in super-resolution mode. The nominal magnification was 105,000 ×, the defocus range was set to −0.8 to −2.2 μm, and the super-resolution pixel size was 0.4125 Å. The total dose was 40 e[−] (Å)^{−2} delivered over 1.8 s. EPU was used to collect 13,349 micrograph movies with 40 frames per stack.”

For blotting which BF did you use? Please brush-up this section.

We now state that the blotting force was zero.

Please check the spelling of Schürmann in reference 60.

We checked. It is Schüürmann.

The spectrum in FigS3 should also be done for PSI without nano-particles and included for comparison.

We have added a new panel to this figure showing the spectrum without PtNPs bound.

The term ladder in FigS4 refers to a molecule weight marker I suppose. Please label accordingly.

We have clarified this.

Reviewer 2

The primary purpose of your review is to provide feedback on the soundness of the research reported. This will help authors to improve their manuscript and editors to reach a decision. When composing your report, the following questions might assist you in writing a well-justified review, but please feel free to raise any further questions and concerns about the paper.

• What are the noteworthy results?

The manuscript by C.J. Gisriel and co-authors summarizes the photochemical reactivity and cryo-EM structure of biohybrid complexes formed from platinum (Pt) nanoparticles and cyanobacterial photosystem I (PSI). A motivation for the work was to develop a system that combined the near unity quantum-yield of PSI with a highly reactive catalyst as a model for solar-driven production of H₂. The results are noteworthy in producing a high resolution cryo-EM structure of a functional complex that identifies the locations of Pt binding sites on each monomer of an intact PSI trimer. The structure demonstrates that there are two Pt binding modes, one being proximal to the terminal

electron transfer chain component, FB, of PSI. This site is similar on each monomer and is located nearby and partially overlaps the binding site of the natural electron acceptors ferredoxin and flavodoxin. This binding mode is hypothesized as stabilized by charge-charge interactions. The mercapto-succinate capping ligand on Pt is negatively charged and mimics the surface chemistry of ferredoxin and flavodoxin. Overall, the work makes a substantial advancement to the physical understanding of PSI based hybrid complexes capable of coupling photoexcited electrons to the reaction chemistry of catalysts.

The main outcomes include:

- The structure and resolution allowed the authors to map the Pt binding sites on each PSI monomer, which consist of two locations, one having closer proximity to the terminal [4Fe-4S]_h cluster, FB, that is known to mediate electron transfer to biological carriers. This result accounts for observation of light-driven H₂ production in this work. It also agrees previous EPR studies by this group on similar complexes that demonstrated that the Pt particles function as electron acceptors (Ref 7). Thus, the authors now provide detailed structural information that rationalizes the functional results presented here and in prior work.
- Identified the size distribution of the bound Pt particles, which centered on ~2.0 nm, this size selectivity matches to the binding pockets located on the stromal side of PSI.
- They were able to map Pt binding sites that identified the importance of positively charged amino acid functional groups and detergent in stabilizing the binding interactions.
- Mapped the overlap of Pt binding sites to the binding sites of natural electron carriers (ferredoxin and flavodoxin) on PSI from the structural database. The interaction sites showed both similarities and differences in the binding locations and support the previous studies by these authors that showed Pt particle binding interfered with binding of the natural donors.
- **Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.**

The results and understanding from the work are related to broader efforts to develop model photochemical systems that use biological components to perform photocatalysis. These results provide detailed information on the physical properties of PSI surface sites and Pt chemistry that control binding, and how binding orientation supports electron transfer.

- **Does the work support the conclusions and claims, or is additional evidence needed?**

For the most part yes, there are no additional experiments needed but some additional analysis is warranted. In our opinion, a more complete map of the Pt binding sites relative to the positions of all the FeS clusters in PSI, and the relative distances of the Pt binding modes at A sites to B sites, would be beneficial to understanding the fate of photoexcited electron transfer. The results as presented mainly focus on site A and the proximity to FB, and how this binding mode mimics the natural acceptor interactions. There appears to be a range in the positions of occupancy on site A that may vary in distance to FB, and there is no mention of the distances to the PtNP modeled in red in site A (Figure 2).

Based on the reviewer's comments, we have added **Supplementary Table 3** which shows the distances of the PtNP in each site (A and B for all three monomers) from the corresponding [4Fe-4S] clusters involved in native electron transfer. This table shows that these measurements are similar among monomers, which we think justifies our approach of describing only monomer 1 as a good representative for the sake of simplicity and clarity. We now specifically state this on page 4: "In each monomer, the closest PtNP to a [4Fe-4S] cluster is between the NP in site A and FB, ~14 Å (**Supplementary Table 3**). Because these distances are very similar, we focus hereafter on monomer 1." We also state on page 5 that: "When the PtNP is modeled in the most peripheral site of site A, the nearest distance between a [4Fe-4S] cluster and the PtNP is ~30 Å, which is too long for efficient electron transfer." This is the furthest position that a PtNP could occupy in site A, which is represented by the red PtNP model in site A of **Fig. 2**.

Following on this point, regarding the paragraph starting at line 116 and specifically based on the information given in lines 121-125, have the authors considered the possibility where two PtNPs are bound at once to an NPA site, as an alternative model that accounts for the ellipsoid density structure (which is interpreted as representing a superposition of particles binding on different monomers). If so, can the authors include discussion on this point in the revised manuscript?

Yes, we have considered this and think it is possible; however, if two PtNPs are ~405 Pt atoms each, then we think two are unlikely to bind in site A because they spatially overlap when we fit two PtNP models (**Fig. 2**). But, it should be understood that there is a distribution of PtNP sizes (**Supplementary Fig. 2**), though the 405 atom model seems to be a reasonable model for the structure analysis. Based on the reviewer's comment, we have added the following text on page 5: "It should be noted that these observations seem reasonable based on the fitting of our spherical 405-atom PtNP model, but this does not consider other particle sizes (**Supplementary Fig. 2**) or possible non-spherical PtNPs (e.g., perhaps two smaller PtNPs could bind in site A)."

As well, there is constant reference to the previous work by some of these authors on related complexes (ref 7) much of which is a demonstration of the functional properties of the biohybrid. In that work the authors had shown by EPR that the presence of Pt changes the redox state of the PSI FeS clusters (more oxidized) under photoexcitation. This context is briefly mentioned in the main text, lines 190-192 as ".....observed electron transfer properties observed in EPR.", and in our view warrants more explanation as to what was observed and how the structure is definitive on this aspect.

Electron transfer in PSI is bidirectional. Following photoexcitation, the primary electron donor of PSI, P₇₀₀, becomes oxidized with concurrent electron transfer down either the A-branch or B-branch of cofactors to A₀, which is a chlorophyll molecule, and A₁, a phylloquinone. From A₁ the electron is transferred to the [4Fe-4S] cluster called F_X, then to the terminal [4Fe-4S] clusters called F_A and F_B.

Using a combination of High-Frequency/High Field D-band (130 GHz) time-resolved EPR spectroscopy and deuterated proteins, we are able to resolve the directionality of electron transfer between the A- and B-branches of cofactors. In PSI from cyanobacteria, a Spin-Correlated Radical Pair (SCRPP) signal P⁺A_{1A}⁻ is observed at cryogenic temperature because electron transfer down the A-branch of cofactors cannot proceed to F_X. This is a well-known, characteristic EPR signal of PSI. However, we observed something unexpected with Flv bound to PSI. NO SCRPP is observed! This suggests that both the A- and B-branches of cofactors are open to electron transfer at low temperature. Furthermore, spectral analysis shows a nearly 1:1 formation of oxidized donor P₇₀₀⁺ and reduced Flv⁻ acceptor (near unity quantum yield of charge separation). This is unexpected as PSI has complicated low temperature electron transfer kinetics with low yields (30-40%) of electron transfer to F_A and F_B acceptors in the absence of acceptor protein.

PtNPs mimic this effect that Flv has on charge separation in PSI at 20 K; no SCRPP is observed! In addition, with X-band EPR (9 GHz) we were able to show that electron transfer from PSI to Flv in solution is blocked when PtNPs are bound to PSI. Also, low temperature charge separation P⁺F_A⁻ and P⁺F_B⁻ is limited when PtNPs are bound, indicating low temperature electron transfer to the PtNP.

Based on the reviewer's comment, we have added to the text on page 7: "In addition, binding of PtNPs to PSI mimics the effect that bound Flv has on low temperature PSI electron transfer. Time-resolved High-Frequency EPR studies show that both symmetric branches of cofactors, the A branch and B branch, are open to low temperature electron transfer when either Flv or PtNP are bound to PSI.⁷ Furthermore, spectral analysis showed near 100% quantum yield for the interprotein electron transfer reaction for PSI-Flv complex.²⁴ It was hypothesized that the enhanced electron flow induced by Flv binding to PSI could explain the high rate of H₂ production if PtNP bound to PSI in a similar manner.⁷ To determine the basis for these observations, we superimposed PSI structures with Fd bound (PDB 7FIX²⁶) or Flv bound (PDB 6KIF²⁵) onto the *S. lividus* PSI structure and generated a low-resolution map of the Fd or Flv, comparing those sites to where the PtNPs are bound (**Fig. 4**). The PtNP in site A overlaps strongly with both the Fd- and Flv-binding sites. Thus, although the PtNP (~20 Å in diameter) does not bind within the stromal ridge as do the similarly sized Fd (~30 Å in diameter) and Flv (~35 Å in diameter), the stromal ridge is blocked by the PtNP in site A, providing a definitive structural basis for the observation of blocked electron transfer to Flv in EPR studies as well as revealing a new binding position that can readily accept light-generated electrons from PSI for photocatalysis."

• Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?

For the most part the data analysis presented here is complete and consistent with the results, but there are some areas where clarification in a revised document might improve the impact and accessibility of the work. Much of the results are presented on the properties of Pt particles to monomer A (Table 1 does present distances for all 3 monomers). Additional explanation on

properties of all 3 sites could be included in the SI (it is in the structures), for instance, the side chain interactions, occupancy of sites, etc, as an average for all 3 monomers, since they are presumably all functional, yet may have some differences.

As far as we can tell, there are no differences in protein interactions with the PtNPs among the monomers, which is consistent with the very similar distances shown in **Supplementary Table 3**. However, please be aware that in response to a comment from Reviewer 1, we now write on page 6: “Furthermore, the sidechains of amino acids that potentially interact with the PtNPs are not well resolved. It should also be noted that surface residues are often the most flexible and therefore difficult to precisely position in a cryo-EM map. Coupled with the lower local resolution at the periphery of the structure, and with the probable positional heterogeneity of the PtNPs, it is therefore unsurprising that interactions between amino acids and PtNPs are challenging to determine. That said, numerous residues from subunits PsaA, PsaE, and PsaJ are close enough to the PtNP in site A to propose specific interactions.”

Based on the reviewer’s comment, we have added the following statement to the figure description for **Fig. 3**: “The observations depicted in panels b-h are shown for monomer 1 only, but these are also consistent with monomers 2 and 3.”

We also wish to mention that we are currently collaborating with a computational group to simulate the interactions between the protein surface and the nanoparticles. We hope this will help to better identify specific protein-PtNP interactions.

Based on lines 220-223, can the authors elaborate a bit more on the nature of the observed significant heterogeneity in the PtNP-binding sites? For example, it is unclear whether any of the other structures might include binding interactions on the luminal side, or how large of a population (relative to the selected conformation) some of these other conformations might represent.

Likewise, on lines 120 and 323, the authors reference the PtNP modeled as having 405 Pt atoms, while in the methods on line 285 they state an average of 400 Pt atoms per PSI monomer determined for the cryoEM sample. Can the authors elaborate a bit on this, also given the distribution of sizes displayed in **Fig S2**? Does this indicate only a single NP is ever bound to each monomer, in either the A or B binding site?

We wish we could comment more upon these points, but we just don’t have a way to extract such information from these data. Based on the reviewer’s comments and our observations inspecting images like those presented in **Supplementary Fig. 14**, we have added the following paragraph on page 9: “A challenge in data interpretation herein is that the process of particle classification and reconstruction in the cryo-EM data processing workflow reveals only the most common PtNP binding sites in the sample. Fractional occupancies of PtNPs seem likely, because despite the 405-atom PtNP model fitting reasonably well into the map signals and that size being consistent with STEM HAADF imaging (**Supplementary Fig. 2**), we also measured only 400 ± 28 Pt atoms per PSI monomer (**Methods**) directly with ICP-AES analysis. This suggests that about one PtNP is bound per PSI monomer on average despite two PtNP signals being present per monomer in the cryo-EM data. In other words, each PtNP site may be occupied ~50%. This hypothesis is supported by HAADF STEM imaging in which trigonal and linear arrangements of PtNPs corresponding to one PtNP bound per monomer of PSI were observed for the biohybrid¹¹. It is possible that PSI complexes are present with PtNPs bound in other locations not represented in the final cryo-EM

maps (**Supplementary Fig. 15**), which would probably have very low occupancy. Further insight could be provided in the future by more advanced cryo-EM data processing techniques that are specifically designed to analyze heterogeneity.” For example, an analysis using cryoDRGN (<https://www.nature.com/articles/s41592-020-01049-4#citeas>) might be fruitful, though we think that is beyond the scope of this manuscript.

Note, too, that Reviewer 1 requested we deposit the raw cryo-EM micrograph images into an online database called EMPIAR, which we have completed. This makes the data more accessible for reprocessing by groups more focused on method development. Since this is the first high-resolution single-particle cryo-EM data of a protein with metal nanoparticles bound, we think these data could be a useful resource for the cryo-EM community.

There also appears to be size selective binding of Pt particles centered on ~2 angstroms, where the diameter distribution of the Pt particles spans 1-3 angstroms. Is this due to complementarity to the binding site on PSI? Is this size range similar to the diameters of ferredoxin or flavodoxin? Could larger (or smaller) particles bind, but have lower stabilities and are not captured in the images? Some discussion on this aspect is warranted and is relevant to design.

Yes, we do believe there could be some size selectivity, wherein the ~2 nm particles in the distribution preferentially bind to the sites we see in the cryo-EM structure. In future experiments we hope to address this by synthesizing different sized nanoparticles. We hope that we now address this with our previously mentioned alterations to the text regarding heterogeneity. We have also added the relevant sizes in the following statement on page 7: “Thus, although the PtNP (~20 Å in diameter) does not bind within the stromal ridge as do the similarly sized Fd (~30 Å in diameter) and Flv (~35 Å in diameter), the stromal ridge is blocked by the PtNP in site A, providing a definitive structural basis for the observation of blocked electron transfer to Flv in EPR studies as well as revealing a new binding position that can readily accept light-generated electrons from PSI for photocatalysis.”

The authors might consider including additional callouts in the figures that point out the main structural features discussed in the main text, more clearly. The figures as presented are not as accessible as they could be in conveying the detailed information that is discussed. Further in this vein, given the journal scope, blending of approaches, and relative nascency of cryoEM in the field, it may be helpful if the authors could include a bit more information in the form of background/context for the general reader, particularly in the supplemental figures. We have compiled some specific changes and suggestions along this line for the authors to address.

We have made numerous alterations to the text and figures based on the reviewer’s comments. We appreciate the insightful reading and thoughtful suggestions for improving the manuscript. Thanks!

Figure 1

- Can the authors consider including highlights for the location of other clusters, perhaps make the micelle densities a different color gray, add labels for monomers 2 and 3 to the depiction with the lumen/membrane/stroma labeling?

Now that we have included **Supplementary Table 3** at the reviewer's suggestion, which shows that the F_B cluster is probably the only relevant native electron transfer cofactor, we think that highlighting all the clusters in each monomer is unnecessary. Please note that all the clusters in an example PSI monomer are shown in **Supplementary Fig. 1**. At the reviewer's suggestion, we have added yellow highlighting of the micelle region on **Fig. 1d-g**. For the membrane plane view of **Fig. 1a**, only monomer 1 is easily observed because the other two are faded in the background for clarity. Because we define the nomenclature in the text of, e.g., $NP1_A$ = the PtNP in site A of monomer 1, we think that the current labeling in **Fig. 1a** is appropriate.

Figure 2

- Similar suggestion for labeling as in Figure 1, to include the location of other clusters, but specifically in this figure to demonstrate the possibility that the particle participates in ET with clusters other than F_B . Do the structures suggest ET pathways other than from F_B ? Is there a reasonable distance for transfer to F_A in any one of the monomers? Is it possible to add distances to F_A/F_X in **Table 1**?

We believe this is now resolved by **Supplementary Table 3**. All the clusters in an example PSI monomer are shown in **Supplementary Fig. 1**.

- What is the distance to F_B from the “non-productive” orientation (red sphere) of the particle in the $NPA1$ site? **Table 1** seems to only gives distances to F_B from the productive orientation green sphere), albeit at two different resolutions.

When the PtNP is modeled in the more peripheral position of site A, the nearest distance from that to a $[4Fe-4S]$ cluster (F_B) is ~ 30 Å, which is too long for efficient electron transfer.

- Please define $[4Fe-4S]$ cluster as described in line 137 of the **Figure 2** legend

$[4Fe-4S]$ cluster corresponds to a cluster containing four Fe atoms and four S atoms. We believe this is the proper chemical notation. We now specify in that caption that we are referring specifically to the $[4Fe-4S]$ cluster, F_B .

Figure 3

- Here in **3a**, along with **Figure S1**, can the authors label the stromal ridge region? It is not clear exactly how the NP is not binding at the stromal ridge based on these views (as stated in lines 210-211).

The purpose of **Fig. 2** is to show the locations of the PtNPs, not necessarily relative to the native binding sites, but the purpose of **Fig. 4** directly addresses the reviewer's question. To provide more clarity of what is meant by the stromal ridge, we now include **Supplementary Fig. 14** and label the three subunits that form the stromal ridge in both **Fig. 2** and **Fig. 4**.

- We are confused by the NP1A density map shown in **Fig. 3c** and **3f**, it appears to be just a strand of gray rather than the shape shown in **3d** and **3e**, perhaps this is just an orientation thing that can be commented on?

Yes, shown at a single threshold, the NP appears like a balloon where the inside corresponds to the signal from the Pt atoms. This is why we placed the NP1_A labels within the regions corresponding to the particles.

- In this vein, it might be better to show more of a blown-out view to give the reader a better sense of where these residues are in relation to the NP, to each other, and the nearby relevant PSI cofactors.

We agree and have added a panel, **Fig. 3c**, similar to what was suggested by the reviewer.

- For **3g**, can the authors comment on the possibility (or not) that if the detergent in U1 and U2 was removed the PtNP might bind better in site B (or in a more productive orientation for ET?). Is there functional significance to the density observed in U1 and U2

Because we do not know the identities of UI_A and UI_B, we do not feel comfortable commenting in detail on these molecular interactions, but due to the reviewer's comment, we have altered the text as follows on page 6: "The most likely candidates for these unidentified signals are lipids or detergent. Also note that both PtNP sites exhibit signals near the disordered detergent micelle comprising *n*-dodecyl β -D-maltoside molecules (**Fig. 1d-g**). If the unidentified signals correspond to polar detergent headgroups, they are probably involved in H-bonding interactions with the PtNP in site B."

Figure 4

- What is the NPA1 overlap with Fd/Flv based on the favorable binding location for ET to FB versus the unfavorable. Can the authors either differentiate the overlap that would occur based on the two binding locations identified in **Figure 2**, or comment on this in the text?

Unfortunately, we do not understand the reviewer's question. As described in the section "Comparison of PtNP-binding sites with those of the native electron acceptor proteins", the overlap of the PtNP in site 1 with the known binding sites of Fd and Flv are shown in **Fig. 4**, and it was previously shown that Flv-binding is blocked by PtNPs (Utschig, L. M. *et al.* Photocatalytic hydrogen production from noncovalent biohybrid photosystem I/Pt nanoparticle complexes. *J. Phys. Chem. Lett.* **2**, 236–241 (2011).).

Supplemental Figures

- Image/figure of PtNP binding site added to **Fig. S1**? It would also help to point out the stromal ridge here.

We believe that this is addressed by the updated **Fig. 4** and new **Fig. S14**.

- **Fig S2**, can the authors comment on what the basis is for the average size of the bound particle. Is it possible to have nucleation events where the binding of a first particle in site A leads to a second particle binding, both perhaps of smaller size? This would be in line with the question we have above regarding the possibility of multiple PtNPs binding to site A at once.

Because the PtNPs are stabilized by mercaptosuccinic acid, we think that such nucleation events are unlikely. Otherwise, we believe this comment is now addressed by the changes we have made on page 9 describing particle heterogeneity due to the reviewer's comments above.

- Please include callouts for the peak assignments in **Fig. S3** that identify the absorbance bands as attributable to NP or PSI cofactors.

We now include the statement on page 3 that “The typical mixed chlorophyll spectrum observed for PSI is maintained after PtNP binding (**Supplementary Fig. 3**).” We have also labeled the characteristic absorbance features for PSI in **Supplementary Fig. 3**. Note, too, that we include an additional panel showing the absorbance spectrum with and without PtNPs based on reviewer 1's comment.

- **Fig. S6** could the authors include some guiding markers for the PSI-PtNP particles? And a more explanatory figure legend might be beneficial to the non-expert.

We now mention in the description that “PtNPs are observed as black spots.” Please note that zoomed in images are also shown in **Supplementary Fig. 15**.

- The caption of **Fig. S8** does not explain the content or importance of what the figure is showing to a non-expert in cryoEM data. It would be helpful to include just a short statement sentence for each figure component to explain what purpose they serve, in the context of this work.

Based on the reviewer's comment, we have altered the descriptions of the figure. For example, “Map-to-map Fourier Shell Correlation that determines the reported resolution of the cryo-EM map.” These technical data are similarly depicted in all cryo-EM papers. It is also described in the **Methods** section.

- Labeling on **Fig S9**. Can the authors include labels for pertinent cofactors and subunits in **S9A** that orient with the callouts given in **S9B** and **S9C**.

Done.

- **Fig S10**, with all this data it would be helpful to highlight identities either above or below a certain threshold that the authors deem significant.

This strongly depends upon the subunit. Subunits like PsaA and PsaB that bind the electron transfer cofactors are typically well conserved among organisms and more peripheral subunits (e.g., PsaI) often show less conservation among organisms. We don't feel comfortable assigning a specific cutoff for each subunit, because this would be somewhat arbitrary unless we performed an analysis of many species, which we think is outside the scope of this manuscript. Since the purpose of the

tables are to show that *S. lividus* PSI is very similar to *T. vestitus* PSI, the latter of which is a commonly used model organism for photosystem studies, we think the figure is appropriate in its current form. We have included the following statement in the figure description: “Note that *S. lividus* and *T. vestitus* PSI subunits exhibit high sequence identity.”

- **Fig S12**, it would be helpful to label the location of relevant cofactors involved in ET since this figure is showing areas of full vs. no conservation.

Likewise, the entire structure shows very high conservation, so we think it is unnecessary to include the locations of cofactors. Basically, *S. lividus* PSI and *T. vestitus* PSI are nearly identical.

Line 108 and on in figure caption, do the authors mean to say monomer 1 (rather than trimer 1)?

Yes! Corrected – thanks!

The supplementary figure numbering appears to be incorrect in the **Methods** starting at line 298

Corrected – thanks.

- **Is the methodology sound? Does the work meet the expected standards in your field?**

The methodology is sound and comprehensive datasets.

- **Is there enough detail provided in the methods for the work to be reproduced?**

Yes.

Reviewer 3

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer 4

The authors used cryo-EM technique to interrogate the platinum nanoparticle (NP) binding sites on PSI, a biohybrid solar fuel catalysis model system. Two binding sites of PN per PSI monomer were discovered. This research filled the blank area of structural details underlying the photo-driven H₂ generation and the nature of PtNP interactions with PSI. The high-resolution of cryo-EM biohybrid system also laid the ground work for future research on the fine-tuning of the efficiency of H₂ generation in this biohybrid system.

Minor questions:

1. According to this research there are an average of 400 Pt atoms per PSI monomer. There are total of 2 binding sites per monomer, i.e., 200 Pt atoms per NP. Please cite another reference to support the claim that there are 200 Pt atoms per NP with a size distribution of 1.8-2.0 nm. The cited literature (p276, Ref11) doesn't support the 200 Pt atoms per NP.

We understand the seeming discrepancy which was also raised by Reviewer 2 which we believe has to do with occupancy in the cryo-EM data. Note that Reference 11 shows the size determined from HAADF STEM images of the PtNPs used in this study. Please see the Supplementary Information of Reference 7 which contains the formula for calculating the agglomeration number (i.e. number of Pt atoms per nanoparticle based on radius). From this equation, a 2.2 nm NP contains ~370 Pt atoms, which is in good agreement with the 2.2 nm size (405-atom) PtNP modeled by DFTB in our manuscript.

We have made the following alteration to the manuscript on page 9 to comment upon possible heterogeneity/occupancy: A challenge in data interpretation herein is that the process of particle classification and reconstruction in the cryo-EM data processing workflow reveals only the most common PtNP binding sites in the sample. Fractional occupancies of PtNPs seem likely, because despite the 405-atom PtNP model fitting reasonably well into the map signals and that size being consistent with STEM HAADF imaging (**Supplementary Fig. 2**), we also measured only 400 ± 28 Pt atoms per PSI monomer (**Methods**) directly with ICP-AES analysis. This suggests that about one PtNP is bound per PSI monomer on average despite two PtNP signals being present per monomer in the cryo-EM data. In other words, each PtNP site may be occupied ~50%. This hypothesis is supported by HAADF STEM imaging in which trigonal and linear arrangements of PtNPs corresponding to one PtNP bound per monomer of PSI were observed for the biohybrid¹¹. It is possible that PSI complexes are present with PtNPs bound in other locations not represented in the final cryo-EM maps (**Supplementary Fig. 15**), which would probably have very low occupancy. Further insight could be provided in the future by more advanced cryo-EM data processing techniques that are specifically designed to analyze heterogeneity. Importantly, the revelation of two PtNP binding sites per monomer in the cryo-EM structure prioritizes optimizing PtNP binding site occupancy in further biohybrid development strategies.

2. Include a room temperature absorption spectrum of PSI in Fig. S3, as a control to the isolated PSI-PtNPs.

Done.

3. Define TON (p81) the first-time use.

Done.

4. Discuss the NP binding stoichiometry per monomer of this research with that of Ref 11, i.e., 2 vs 1.

We appreciate the reviewer's comment. In HAADF STEM images of cyanobacterial thylakoids and PSI trimer, we observed trigonal and linear arrangements of the PtNPs corresponding to 1 PtNP bound per monomer of PSI. Inter PtNP spacings ranged from 6-9 nm. Note that these samples were dropcast onto the SiNx support so PSI was in multiple orientations with respect to the scope.

From the cryo-EM structure, the approximate distance between the PtNP site A signal and site B signal is 19 Å, which is resolvable with our Analytical PicoProbe. We did not see any clear arrangements of PtNP in 4, 5, or 6 configurations.

We have amended the text page 9 to include the following statement: “This hypothesis is supported by HAADF STEM imaging in which trigonal and linear arrangements of PtNPs corresponding to 1 PtNP bound per monomer of PSI were observed for the biohybrid.¹¹”

Reviewer Comments for

Structure of a biohybrid photosystem I-platinum nanoparticle solar fuel catalyst

Christopher J. Gisriel, Tirupathi Malavath, Tianyin Qiu, Jan Paul Menzel, Victor S. Batista, Gary W. Brudvig*, Lisa M. Utschig*

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Reviewer #1

I am happy to see that the authors took advantage of the reviewers comments to improve their manuscript substantially.

The manuscript should be ready for publication, but before doing so, please try to take into account red/green color blindness

also for figure 2 add a bracket in the caption of figure 4

add the EMPIAR data bank entry to the Data and materials availability section.

We corrected these and checked for color blind friendly figures by testing them on a color blindness simulator.

Reviewer #2

The authors have addressed all of our previous comments in the revised manuscript and supporting information documents. We recommend publication with one minor suggestion below.

In the revised Figure S11 (previous S10) the legend includes a different set of species names than those that are presented in the figure.

We corrected this.

Reviewer #3

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

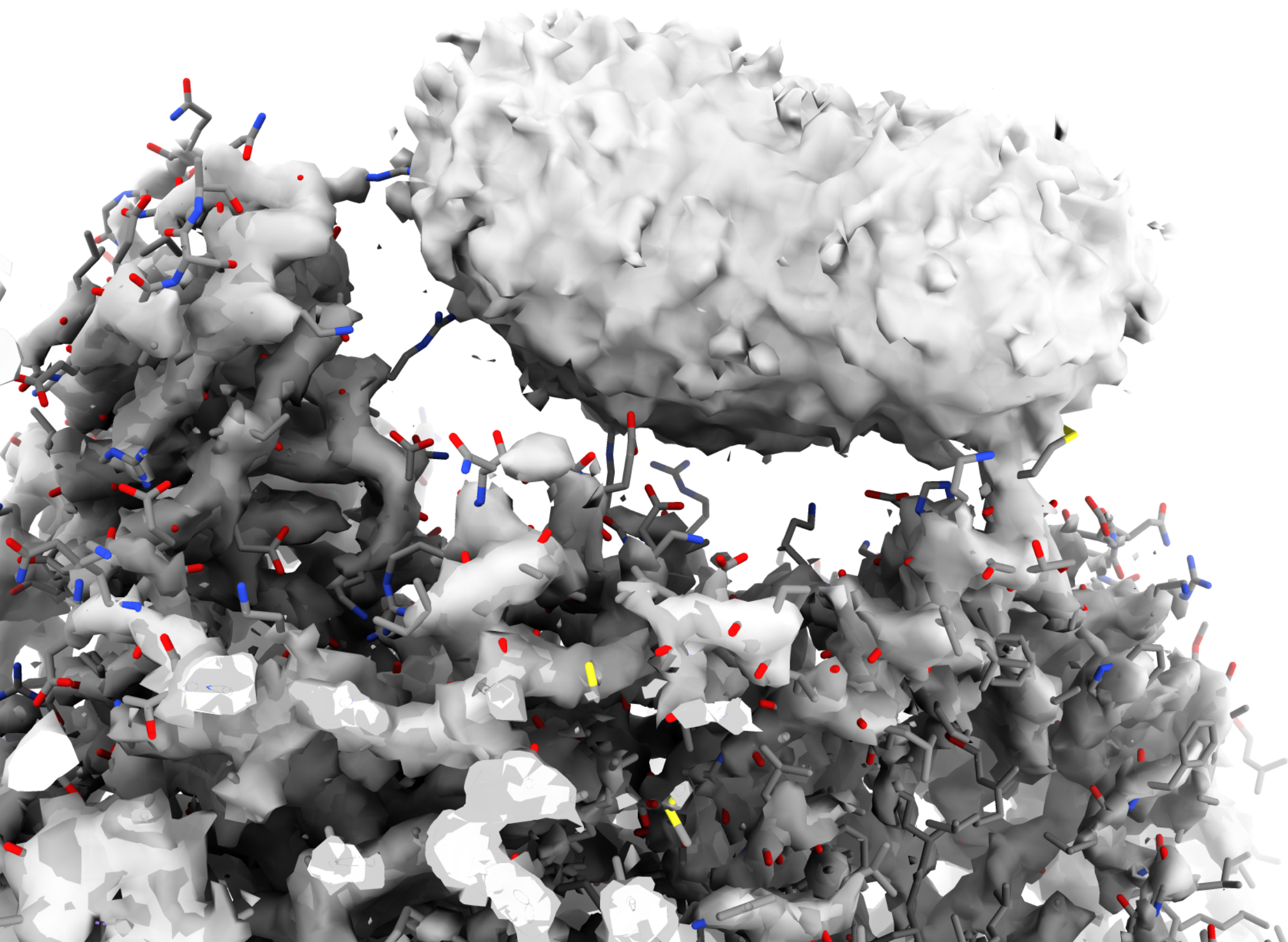
Ok.

Reviewer #4

The authors have addressed my comments/suggestions.

Thank you.

Reviewer #1 attachment:



Reviewer #2 attachment:

The primary purpose of your review is to provide feedback on the soundness of the research reported. This will help authors to improve their manuscript and editors to reach a decision. When composing your report, the following questions might assist you in writing a well-justified review, but please feel free to raise any further questions and concerns about the paper.

- **What are the noteworthy results?**

The manuscript by C.J. Gisriel and co-authors summarizes the photochemical reactivity and cryo-EM structure of biohybrid complexes formed from platinum (Pt) nanoparticles and cyanobacterial photosystem I (PSI). A motivation for the work was to develop a system that combined the near unity quantum-yield of PSI with a highly reactive catalyst as a model for solar-driven production of H₂. The results are noteworthy in producing a high resolution cryo-EM structure of a functional complex that identifies the locations of Pt binding sites on each monomer of an intact PSI trimer. The structure demonstrates that there are two Pt binding modes, one being proximal to the terminal electron transfer chain component, F_B, of PSI. This site is similar on each monomer and is located nearby and partially overlaps the binding site of the natural electron acceptors ferredoxin and flavodoxin. This binding mode is hypothesized as stabilized by charge-charge interactions. The mercapto-succinate capping ligand on Pt is negatively charged and mimics the surface chemistry of ferredoxin and flavodoxin. Overall, the work makes a substantial advancement to the physical understanding of PSI based hybrid complexes capable of coupling photoexcited electrons to the reaction chemistry of catalysts.

The main outcomes include:

- The structure and resolution allowed the authors to map the Pt binding sites on each PSI monomer, which consist of two locations, one having closer proximity to the terminal [4Fe-4S]_h cluster, F_B, that is known to mediate electron transfer to biological carriers. This result accounts for observation of light-driven H₂ production in this work. It also agrees previous EPR studies by this group on similar complexes that demonstrated that the Pt particles function as electron acceptors (Ref 7). Thus, the authors now provide detailed structural information that rationalizes the functional results presented here and in prior work.
 - Identified the size distribution of the bound Pt particles, which centered on ~2.0 nm, this size selectivity matches to the binding pockets located on the stromal side of PSI.
 - They were able to map Pt binding sites that identified the importance of positively charged amino acid functional groups and detergent in stabilizing the binding interactions.
 - Mapped the overlap of Pt binding sites to the binding sites of natural electron carriers (ferredoxin and flavodoxin) on PSI from the structural database. The interaction sites showed both similarities and differences in the binding locations and support the previous studies by these authors that showed Pt particle binding interfered with binding of the natural donors.
- **Will the work be of significance to the field and related fields? How does it compare to the established literature? If the work is not original, please provide relevant references.**

The results and understanding from the work are related to broader efforts to develop model photochemical systems that use biological components to perform photocatalysis. These results

provide detailed information on the physical properties of PSI surface sites and Pt chemistry that control binding, and how binding orientation supports electron transfer.

- **Does the work support the conclusions and claims, or is additional evidence needed?**

For the most part yes, there are no additional experiments needed but some additional analysis is warranted. In our opinion, a more complete map of the Pt binding sites relative to the positions of all the FeS clusters in PSI, and the relative distances of the Pt binding modes at A sites to B sites, would be beneficial to understanding the fate of photoexcited electron transfer. The results as presented mainly focus on site A and the proximity to FB, and how this binding mode mimics the natural acceptor interactions. There appears to be a range in the positions of occupancy on site A that may vary in distance to F_B, and there is no mention of the distances to the PtNP modeled in red in site A (Figure 2).

Following on this point, regarding the paragraph starting at **line 116** and specifically based on the information given in **lines 121-125**, have the authors considered the possibility where two PtNPs are bound at once to an NPA site, as an alternative model that accounts for the ellipsoid density structure (which is interpreted as representing a superposition of particles binding on different monomers). If so, can the authors include discussion on this point in the revised manuscript?

As well, there is constant reference to the previous work by some of these authors on related complexes (ref 7) much of which is a demonstration of the functional properties of the biohybrid. In that work the authors had shown by EPR that the presence of Pt changes the redox state of the PSI FeS clusters (more oxidized) under photoexcitation. This context is briefly mentioned in the main

text, lines 190-192 as " observed electron transfer properties observed in EPR.", and in our view warrants more explanation as to what was observed and how the structure is definitive on this aspect.

- **Are there any flaws in the data analysis, interpretation and conclusions? Do these prohibit publication or require revision?**

For the most part the data analysis presented here is complete and consistent with the results, but there are some areas where clarification in a revised document might improve the impact and accessibility of the work.

Much of the results are presented on the properties of Pt particles to monomer A (Table 1 does present distances for all 3 monomers). Additional explanation on properties of all 3 sites could be included in the SI (it is in the structures), for instance, the side chain interactions, occupancy of sites, etc, as an average for all 3 monomers, since they are presumably all functional, yet may have some differences.

Based on **lines 220-223**, can the authors elaborate a bit more on the nature of the observed significant heterogeneity in the PtNP-binding sites? For example, it is unclear whether any of the other structures might include binding interactions on the luminal side, or how large of a population (relative to the selected conformation) some of these other conformations might represent.

Likewise, on [lines 120](#) and [323](#), the authors reference the PtNP modeled as having 405 Pt atoms, while in the methods on [line 285](#) they state an average of 400 Pt atoms per PSI monomer determined for the cryoEM sample. Can the authors elaborate a bit on this, also given the distribution of sizes displayed in **Fig S2**? Does this indicate only a single NP is ever bound to each monomer, in either the A or B binding site?

There also appears to be size selective binding of Pt particles centered on ~ 2 angstroms, where the diameter distribution of the Pt particles spans 1-3 angstroms. Is this due to complementarity to the binding site on PSI? Is this size range similar to the diameters of ferredoxin or flavodoxin? Could larger (or smaller) particles bind, but have lower stabilities and are not captured in the images? Some discussion on this aspect is warranted and is relevant to design.

The authors might consider including additional callouts in the figures that point out the main structural features discussed in the main text, more clearly. The figures as presented are not as accessible as they could be in conveying the detailed information that is discussed. Further in this vein, given the journal scope, blending of approaches, and relative nascency of cryoEM in the field, it may be helpful if the authors could include a bit more information in the form of background/context for the general reader, particularly in the supplemental figures. We have compiled some specific changes and suggestions along this line for the authors to address.

Figure 1

- Can the authors consider including highlights for the location of other clusters, perhaps make the micelle densities a different color gray, add labels for monomers 2 and 3 to the depiction with the lumen/membrane/stroma labeling?

Figure 2

- Similar suggestion for labeling as in Figure 1, to include the location of other clusters, but specifically in this figure to demonstrate the possibility that the particle participates in ET with clusters other than FB. Do the structures suggest ET pathways other than from FB? Is there a reasonable distance for transfer to FA in any one of the monomers? Is it possible to add distances to FA/FX in **Table 1**?
- What is the distance to FB from the “non-productive” orientation (red sphere) of the particle in the NPA1 site? **Table 1** seems to only gives distances to FB from the productive orientation (green sphere), albeit at two different resolutions.
- Please define [4Fe-4S] cluster as described in [line 137](#) of the **Figure 2** legend

Figure 3

- Here in **3a**, along with **Figure S1**, can the authors label the stromal ridge region? It is not clear exactly how the NP is not binding at the stromal ridge based on these views (as stated in [lines 210-211](#)).
- We are confused by the NP1A density map shown in **Fig. 3c** and **3f**, it appears to be just a strand of gray rather than the shape shown in **3d** and **3e**, perhaps this is just an orientation thing that can be commented on?
- In this vein, it might be better to show more of a blown-out view to give the reader a better sense of where these residues are in relation to the NP, to each other, and the nearby relevant PSI cofactors.
- For **3g**, can the authors comment on the possibility (or not) that if the detergent in U1 and U2 was removed the PtNP might bind better in site B (or in a more productive orientation for ET?). Is there functional significance to the density observed in U1 and U2

Figure 4

- What is the NPA1 overlap with Fd/Flv based on the favorable binding location for ET to FB versus the unfavorable. Can the authors either differentiate the overlap that would occur based on the two binding locations identified in **Figure 2**, or comment on this in the text?

Supplemental Figures

- Image/figure of PtNP binding site added to **Fig. S1**? It would also help to point out the stromal ridge here.
- **Fig S2**, can the authors comment on what the basis is for the average size of the bound particle. Is it possible to have nucleation events where the binding of a first particle in site A leads to a second particle binding, both perhaps of smaller size? This would be in line with the question we have above regarding the possibility of multiple PtNPs binding to site A at once.
- Please include callouts for the peak assignments in **Fig. S3** that identify the absorbance bands as attributable to NP or PSI cofactors.
- **Fig. S6** could the authors include some guiding markers for the PSI-PtNP particles? And a more explanatory figure legend might be beneficial to the non-expert.
- The caption of **Fig. S8** does not explain the content or importance of what the figure is showing to a non-expert in cryoEM data. It would be helpful to include just a short statement sentence for each figure component to explain what purpose they serve, in the context of this work.
- Labeling on **Fig S9**. Can the authors include labels for pertinent cofactors and subunits in **S9A** that orient with the callouts given in **S9B** and **S9C**.
- **Fig S10**, with all this data it would be helpful to highlight identities either above or below a certain threshold that the authors deem significant.
- **Fig S12**, it would be helpful to label the location of relevant cofactors involved in ET since this figure is showing areas of full vs. no conservation.

Line 108 and on in figure caption, do the authors mean to say monomer 1 (rather than trimer 1)? The supplementary figure numbering appears to be incorrect in the **Methods** starting at line 298

- **Is the methodology sound? Does the work meet the expected standards in your field?** The methodology is sound and comprehensive datasets.
- **Is there enough detail provided in the methods for the work to be reproduced?** Yes.