Yucel, Jennifer

From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, July 26, 2016 5:11 PM
To: Yucel, Jennifer
Cc: Hart, Rich; Moses, Randolph
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct
Attachments: Author letter 1.pdf; Author letter 2.pdf; Author letter 3.pdf

Dear Dr. Yucel,

I've attached the three letters that Dr. Zhang provided in response to the reviewer comments. Author letter 1 is the response provided with the first resubmission (2016-00406).

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Tuesday, July 26, 2016 12:00 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. Would it be possible to also get copies of what Dr. Zhang submitted with his revised manuscripts? Of particular importance to our review would be his response to reviewers comments and to the editor accompanying the first resubmission [manuscript # 2016-00406] in which he addressed the identity of IAGP with phylogenic analysis and mass spec.
Would it be possible to get this from you? Again, we greatly appreciate your assistance with this matter.
Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Monday, July 25, 2016 5:20 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've pasted below the four decision letters that our office sent [redacted] The manuscript was originally submitted in early 2015. It was rejected, but the authors were invited to resubmit it. As far as we can tell, these are the only correspondence that our office had with Dr. Zhang.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Decision letter for 2015-01638:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2015-01638 Decision Notification
Message: March 18, 2015

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2015-01638
Authors: [redacted]

Dear Dr. Zhang,

I apologize for the delay and regret to inform you that the PNAS Editorial Board has rejected your manuscript [MS# 2015-01638]. The expert who served as editor obtained 3 reviews, which are included below. After careful consideration, the editor decided that we cannot accept your manuscript.

Note that the PNAS License to Publish conveyed at initial submission is terminated.

However, because the reviewers think the work is of interest and the editor concurs, we are willing to consider one resubmission that constructively addresses all of the concerns raised in the critiques. The paper would have to satisfy both the reviewers and the editor, and new criticisms could arise upon re-evaluation. We cannot guarantee success and will be unable to consider further resubmissions.

Thank you for submitting your work to PNAS.

Sincerely yours,
Inder M. Verma
Editor-in-Chief

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Editor's Remarks to Author:

The manuscript cannot be published in PNAS in the current form. The reviewers raise several concerns regarding the isolated AGP, the glycosyl composition on silicon wafers, the nature of nanoparticle shown in the AFM images, and other technical problems. If the authors can address ALL of the reviewers' concerns, a resubmission could be possible.

The manuscript requires a substantial major revision accompanied by additional experiments to confirm the results presented. All comments of the reviewers need to be carefully considered. Specifically, all of the following matters should be expanded:

(1) In particular, it is questioned if the correct gene was isolated since important domains are lacking. There is convincing doubt that the isolated IAGP represents an AGP at all.

Therefore, I request a molecular phylogenetic tree including AGPs and Cytochrome Oxidase subunits to demonstrate the correct placement of IAGP within the AGP clade. In addition, as suggested by a reviewer, the degree of AG-glycosylation should be estimated as indicator that the cloned sequence is the correct AGP. In this respect, Mass Spectrometry also needs to be performed to corroborate that IAGP peptides are indeed present in abundance in purified particles.

(2) Further evidence that IAGP is in fact the Ivy adhesion molecule is required.

(3) The issue whether nanoparticles in AFM images are individual molecules or aggregates should be clarified.

(4) The nature of adhering residues on silicon wafers (Table 2) needs to be illuminated - can these represent cellulose or hemicellulose impurities?

(5) Stress strain data to show the rheological behavior need to be included.

(6) Methods descriptions need to be more detailed.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: No
Sufficient General Interest?: No
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
The paper investigates the molecular and bio-physical basis of the adhesive secreted by Ivy. A fraction of apparently spherical particles is isolated from exudate and is analysed biochemically and biophysically. It is suggested that the particles mainly consist of an arabinogalactan protein (AGP) that is termed IAGP. Based on a N-terminal peptide sequence of the deglycosylated protein, a putative cDNA for IAGP is cloned. The purified particles display low inherent viscosity and can form adhesive glues when combined with pectin and calcium. From a biophysical perspective the case looks more convincing than from a molecular biological one.

Comments on scientific content:

• AFM: It is not clear what is actually shown with the AFM images. Do the authors suggest that we are looking at individual AGP molecules or aggregates of many AGP and e.g. pectin molecules? Even though the texture of the mages suggests spherical particles the individual particles i.e. connected shapes are rarely ever spherical but are clusters. I am not an AFM expert but it seems helpful to image the particles at increasingly lower density so that individual particles might become the predominant structures. Otherwise it might mean that the spheres are not spheres after all.

• Fig. 1D and 1E. I don't know whether the methods applied for these figures are commonly known among physicists. As a molecular biologist I would find it helpful to get a better explanation of the method and why it should be applied.

• Tables 1 and 2: While the meaning of table 1 is relatively well explained and is interpreted that the linkages probably represent both AGII and pectic structures, the presence Glc, Xyl and Man (together >12 Mol%) is not explained. The sugars are reminiscent of hemicellulose but the terminal Glc is not. Is this an impurity or a component of the AGP/pectin particles? Table 2 describes the sugars found in adhering residues and the text states that "pectin is one of the main components ...". However the predominant monosaccharide in the remnant is glucose. Together with xylose and mannose it accounts for >65% meaning the main constituent is likely to be cellulose (indicating cell debris) or hemicellulose.

• When the IAGP sequence is presented in Figure 3 it is shown with a GPI anchor signal. Also in the scheme Figure 6 the GPI anchor structure, its attachment and release are shown in detail. However, the prediction tool that was used does not predict IAGP as GPI anchored (score = -76.05) and even the 'most likely' omega-site is different from the one shown in Figure 3. In my opinion the question whether or not IAGP is GPI anchored is irrelevant to the story and related suggestions may be removed without reducing the informative value.

• Figure 3C is mentioned in the context of the results section which is misleading. However it only shows a generic structure of a hypothetical AGP. In fact this is not even a classic (sic) AGP as claimed in the text. The literature uses the term "classical AGP" for extremely reduced proteins that contain only a backbone for O-glycosylation (typically XP repeats) and not other potentially functional protein domains (see Ref Ellis et al 2010).

• The sequence of IAGP does not resemble any known AGP. As such this is not remarkable because the important domains that define AGPs are so called AG-modules, stretches of XP, which are lacking from IAGP altogether. However, it bears remarkable sequence similarity to cytochrome oxidase subunit 5b-2. Are the authors sure that they have cloned the right cDNA?

• It would be important to estimate the degree of glycosylation of IAGP, especially when there is access to relatively large amounts of deglycosylated IAGP, MS/MS analysis could reveal hydroxyprolines in isolated positions which is an indirect indicator of AG-glycosylation. It would also confirm the identity of the suggested protein sequence which seems doubtful to me. Another possibility to confirm the nature of the cloned cDNA would be to express the sequence in a heterologous host (e.g. tobacco or Arabidopsis) and test its post-translational modifications.

References:

• The original papers where monoclonal antibodies were introduced and where they were characterized should be referenced.

• The SDS-PAGE procedure is not referenced.

Comments on presentation style:

• The English requires professional editing as the work contains numerous grammatical errors.

• Figure 3C is mentioned in the text before Figures 3A and B.

• Figure 5: the bottom part of the figure should be deleted and the glycan groups should be indicated just below the antibody names or even better above the bars.

• Figure 6 is far too elaborate and is not only confusing but also gives some quite false impressions (e.g. the existence of GPI-PLC in plants or the assumption that AGPs are glycosylated in the Golgi neither of which is proven). Only the relevant parts of the figure should be retained. Delete GPI-anchor, biosynthetic pathway (ribosomes, ER, Golgi), PLD/PLC.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes
Supplemental Material Warranted?: Yes

Comments:
The submission [redacted]. describes in some detail a study to determine the mechanism of adhesion in English Ivy.

Approaches are varied and include (nano-)mechanical studies, proteomics, genomics and biochemistry. The conclusions are interesting and sound. They build upon previous work by this group, but offer significant new insight and 'proof of concept'. The methods and results are described well and concisely. The study of adhesion of plants lags behind that of animals and, for this reason, it is particularly interesting to see such a comprehensive report presented.

I have no major comments on the body of the text, except that I found the discussion to be rather short and lacking in depth. Similarly, I believe that the significance statement could be strengthened. What are the implications of the findings? The possible applications and routes to exploitation? Page 13, line 11, should this measurement of lap shear not be expressed as a stress in Pa?

My other comments are minor and include:

Page 2, line 14 - I don't think "conversely" is the right word.

Line 18 - Here and throughout the MS, 'by' is usually more appropriate than 'via'.

Line 18 - "the characteristic physicochemical..."

Line 20 - Remove "revealed".

Page 5, line 4 - "developed previously"

Page 6, line 5 - "This result was..."

Line 9 - "verified". Here and throughout tenses are mixed, often in the same sentence. e.g. "verifies" and "displayed".

Page 9, line 8 - "those of pectin and sodium..."

Line 12 - "In the current work..."

Line 13 - "objective was to reveal..."

Line 15 - ": surface wetting"

Line 20 - "beneficial for surface wetting by the ivy..."

Page 12, line 14 - "The manner in which nanoparticles..."

Line 16 - "product prepared via emulsion polymerisation. It is composed of nano-sized polymer-based particles dispersed in an aqueous..."

Line 17 - "Upon application to a ..."

Page 13, line 6 - "developed by integrating..."

Line 10 - "variations in adhesion force".

Line 10 - "enhanced with increasing hardening time".

Page 14, line 3 - "interfere with the..."

Page 15, line 1 - this sentence does not seem to make sense.

Page 15, line 20 - replace "has been established early"

Page 16, line 1 - remove "for"

Page 21, line 22 - remove "for"

Page 23, line 7 - "Once removed..."

Page 24, line 19 - "described earlier"

Page 25, line 5 - replace "via" with "by"

Line 10 - same as above

Line 13 - "were employed as adherends"

Line 15 - remove "sample"

Reviewer #3:

Suitable Quality?: No
Sufficient General Interest?: Yes
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
This paper reports on the characterization of Ivy AGP and the adhesion strength it creates between glass slides.

There are a significant number of technical problems with the paper which make it unacceptable for publication in the current form. The comments are listed below.

1. The evidence to support that fact that the AGP is exuded and indeed the critical molecule used by the Ivy to adhere is not presented. The statement is made that it is "presumed" that the AGP is involved in the adhesion.

2. While the characterization of the AGP seems sound, it is not clear whether the nanoparticles are single molecules or aggregates. The aggregation number should be determined or at least estimated.

3. The rheological measurements are deficient. The method used is determining a component of shear yield stress? There is no stress strain data reported to show the rheological behavior. This is critical to the paper. I suggest that "pull off" measurements are performed where the stress is normal to the surface are made. Furthermore, stress strain curves should be reported. These will give an indication of the creep and yield type behavior. The current measurements are made for silica surfaces. This is a good model for the wall however the Ivy is a biological system. The true measurement if possible should be between the Ivy and a silica slide. Furthermore the methods description does not contain enough detail to permit replication.

4. There is no mechanism or model for the action of the AGP in the adhesion presented. The data is therefore not properly analyzed.

5. The grammar is poor. Generally the paper is not well written.

Given the above technical problems with the paper it is not acceptable for publication in PNAS as the research is not well designed and executed.

****

Decision letter for 2016-00406:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406 Decision Notification
Message: February 9, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your resubmitted manuscript [MS# 2016-00406] has obtained 3 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due April 9, 2016. If you require additional time, notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the reviewers' comments. If you submitted a single PDF file at initial submission, you will be required to submit individual publication-ready files (e.g., Word file for manuscript text; EPS, TIFF, or high-resolution PDF for figures; Word file for tables; etc.)

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors are encouraged to use Supporting Information to show all necessary data, or to deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

Authors are responsible for obtaining waivers of any institutional open access mandates before publication with PNAS. Many institutions require that their authors transfer a nonexclusive author license to the institution and deposit the final author manuscript, with edits from peer review incorporated, into institutional repositories. These mandates conflict with PNAS policy because authors must provide the National Academy of Sciences with an exclusive license to publish their work. Authors employed by an institution with such a mandate should obtain a waiver for the nonexclusive license and upload the file during resubmission. A list of OA mandates can be found online (http://roarmap.eprints.org/).

When you are ready to submit your revised manuscript, go to the site and begin your submission:
<http://www.pnascentral.org/cgi-bin/main.plex?el=A5B3CgWM5A6GBcF6I3A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>.

Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Heather Snijdewind
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) HSnijdewind@nas.edu

****
Editor's Remarks to Author:

The authors have substantially revised the manuscript, performed additional experiments, and clarified all issues of the first three reviewers.

In summary, the authors: (1) included a phylogenetic tree corroborating the identity of the IAGP (2) performed Mass. spec analyses to confirm the IAGP protein sequence, (3) added a tensile test to confirm the molecular nature of the ivy-derived adhesive, (4) expanded AFM analyses to show that ivy nanoparticles observed in the AFM images are individual molecules rather than aggregates, (5) addressed the issue on hemicellulose impurities, (6) added stress strain data to show the rheological behavior, (7) included more details on methods, (8) removed GPI anchor discussion, (9) improved the model of ivy adhesion, (10), performed English and grammar proofreading.

After re-evaluation, several issues remain to be addressed. The authors are asked to improve style, add sub-headings, and re-write the discussion. Furthermore, the importance of pectin should be emphasized. The significance of the current work should be expanded, especially in the discussion section. Questions and suggestions regarding the figures need to be addressed. Further grammatical editing is requested.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: No
Procedures Described?: No

Comments:
The submission [redacted]. details the identification and characterisation of arabinogalactan-like proteins in the adhesive substance of English ivy. Further, the authors develop a mimic with which to test the principles that they argue govern adhesion by the species.

As I stated in my first review, I find the science to be sound and of sufficiently broad interest to be published in PNAS. The findings are significant and also represent an advance in the specific field of research.

My comments and requests for improvement of the original manuscript mainly revolved around style and presentation. Unfortunately this has not improved and is, if anything, worse in this more recent version. I think that this is a consequence of adding the extra material requested by review and the re-drafting/re-organising of the text that this required. Therefore, while I find the research interesting, sound and would like to see it published in PNAS, I am unable to recommend publication of the manuscript in its current form.

First and foremost the text needs to be thoroughly edited by a native English speaker. Before this is done, however, there is significant restructuring that must be undertaken. Currently the style of the paper is that of one long chronological narrative that includes methods, results and discussion. The discussion section simply re-states the general concepts as described throughout. In this format I find the paper very difficult to follow and it certainly does not help the reader to identify points of interest. It is my opinion that the authors should sub-divide the paper much more, halving the length of the results section, make more use of sub-headings and move all of the methods out to the methods section. This would improve the clarity, readability and impact of the findings which, in the current format, are somewhat lost.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
This paper reports on the active component of English Ivy as the active adhesive component used by these plants. The paper is of general interest to the scientific community and the readership of PNAS. The revised version has been significantly improved in accord with the comments of all reviewers. I support publication in PNAS in the current form.

Reviewer #3:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes

Clearly Written?: Yes
Procedures Described?: Yes

Comments:
The authors isolated and characterized nanoparticles found in ivy-derived adhesive. They identified a component of the nanoparticles as being an arabinogalactan protein. They demonstrated that the nanoparticles were found in close proximity to pectin and interacted in a calcium-dependent manner. Bulk adhesion testing was done to demonstrate that all three components - nanoparticles, pectin, and calcium - resulted in a stronger adhesive bond than individual components. Overall, the experiments are interesting and performed well, but there are items that should be addressed before publication:

1. Overall, the abstract and introduction (and the results discussing Figures 1-3) seem to emphasize the importance of the arabinogalactan protein (AGP) nanoparticles in ivy adhesive. The importance of pectin is not emphasized strongly in the beginning part of the manuscript. However, it seems that pectin is at least equally (if not more) important to the adhesive as the nanoparticles are - in Figure 6, pectin by itself has higher bulk adhesion strength than nanoparticles by themselves. Thus, it is unclear why there is such an emphasis on AGP and not on pectin. Is it because that pectin was already known to be an important component and that this paper is characterizing the second component? Or is it because the AGPs are less viscous and thus can penetrate the substrates more and provide mechanical interlocking (although if this is the case, more experiments need to be performed to show this phenomenon). More context would be helpful.

2. There are some allusions to other botanic adhesives that have been studied (page 6, lines 778-785). The text states that "arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and Ficus pumila." Given that arabinogalactans and pectin are found to be the major components of the English ivy adhesive studied in this paper, it would be helpful to expand on the significance of the current work in the abstract, intro, and, especially, the discussion section. How are the findings significant and different from papers published about other plant adhesives? Are the results in this paper already known for other botanic adhesives or is there some additional insight provided here or something that is unique and compelling about the English ivy adhesive?

3. Figure 2: Panel D has a lane labeled as being from Fraction 1, but the legend only acknowledges Fractions 2-5. The text is also unclear - it states that "apart from the solvent peak designated as fraction 1," which could imply that Fraction 1 was not run on the gel. Also, one gel lane is labeled "Marker", but the legend refers to it as "Lane M."

4. Figure 3: For panel C, the figure legend states "Amino acids that are proposed to play adhesive function, comprising Ile, Leu, and Val, are indicated by black triangles." This statement is confusing as this reviewer interpreted the resulting experiments as showing that calcium mediated adhesive interactions between negatively charged residues on AGPs and pectin. Thus, it is not clear how Ile, Leu, and Val are involved in adhesion. Perhaps they are involved in adhesion to the substrates, but no data are shown to support the role of these amino acids in adhesion.

5. Figure 4B: This reviewer could not find the text description in the results section for the EDX data.

6. Figure 5: The second schematic (after evaporation) is confusing. Is pectin supposed to be the gray area that surrounds the yellow spheres? If so, the schematic only appears to be showing that calcium interacts directly with pectin (the gray portion) and not the AGP particles.

7. Figure 6D: The text (page 8, lines 984-993) would be stronger if it explained why the change in pH would affect adhesion. The text states "given that the cross-linking extent...is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here." However, rather than saying it should vary, it would be useful to explain why it is expected to vary at pH 4 and 9. Are we near the pKa values? Are residues no longer negatively charged at one or both of those values?

8. Page 7, lines 905-910: The text hypotheses that the nanoparticles allow for mechanical interlocking. However, are there no chemical adhesive forces (e.g., covalent bonds, van der Waals forces, etc) that are expected to occur?

9. Page 8, line 1035: It is unclear what is meant by "partially reflects the physiological implications of the associated low intrinsic viscosity."

10. Figure 6: For panel G, the meaning of the asterisk is not clear. The legend says the asterisk is compared to "EGTA-free adhesive composites containing 2 mM Ca2+"; however, the asterisk is placed above the composite group with Ca2+ and no EGTA. Also, it seems like it would make more sense if an ANOVA were performed and Tukey groups were shown so that one could determine which groups were statistically similar or different.

11. Materials and Methods (SI page 2, line 21): It would be helpful to report centrifugation in terms of g and not just rpm.

12. Figure S5: In the legend, please explain the green dotted line in panels C-F.

13. Figure S7: Given that there is an arrow with the word "agglomeration" connecting the two panels, it is not clear whether the left and right panels are from the same sample that have agglomerated over time. Or, are they from different samples or different areas of the same sample?

14. Figure S8: Given that two different secondary antibodies were used in the ELISA, is it valid to show results from all of the ELISAs on the same graph? In other words, are the absorbance values from ELISA wells using different secondary antibodies comparable? Were standard curves performed to show that the absorbance values would equate to the same amount?

15. The still image derived from movie S1 and the latter portion of movie S1 (that shows that ivy is stuck to a surface) do not appear to be in focus.

16. In the response to reviewers' comments, point 1.1 says that the nanostructures are "individual molecules consisting of covalently bonded AGP and pectin domain." What is meant by pectic domain? Is this a domain in the AGP that binds pectin? Or do you mean that the molecules contain AGP and short fragments of pectin (in which case they would not be individual molecules)?

17. Further grammatical editing of the paper would be helpful. For example, on page 4, there are multiple instances of "faction 4" instead of "fraction 4." There are other examples of minor grammatical or typographical errors in the manuscript.

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PUBLICATION INFORMATION AND CHARGES

High resolution files are required for figures that will appear in the main text of the article and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. For more information on preparing digital art, please review the PNAS digital art guidelines.

Authors are invited to submit scientifically interesting and visually arresting cover illustrations. To view accepted cover art, please visit the
PNAS cover archive. Please note that images must be original and that exclusive rights to publish will convey to PNAS. If selected for the cover, the image also may be used further in promotional materials, including but not limited to brochures, advertisements, and posters. All submissions should be

accompanied by a brief lay-language caption (50-60 words) and appropriate credit information (e.g., photograph courtesy of...). Images should be 21.5 cm wide by 22.5 cm high. Files should be .eps or .tif and should be in RGB (red, green, blue) color mode. Please submit electronic files with your revised manuscript in the PNAS online submission system or via e-mail to PNASCovers@nas.edu as soon as possible. If files are too large to e-mail, contact the PNAS office for ftp instructions or send the files on CD-ROM by courier to the PNAS Editorial Office (2101 Constitution Ave NW, PNAS 340, Washington DC 20418, phone: 202-334-2679). If you cannot submit electronic files, please contact the PNAS office for assistance. All submissions should include the manuscript number, author name, phone, fax, and email. See the Information for Authors for more details. Please note covers are selected from papers that have been assigned to an issue, after authors have returned proofs.

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Decision letter for 2016-00406R

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406R Decision Notification
Message: April 26, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406R
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your manuscript [MS# 2016-00406R] has obtained 2 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due June 25, 2016. If you require additional time, please notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the editor's and reviewer's comments.

We also require that you amend your title. We are seeking a descriptive title without the use of an em/en dash or colon (i.e. a single declarative title). This is non-negotiable and an exception will not be made.

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors must deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

When you are ready to submit your revised manuscript, go to the site and begin your submission:
<http://www.pnascentral.org/cgi-bin/main.plex?el=A2B3CgWM7B5GBcF2I1A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>

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Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Tom Myers
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) pnas@nas.edu

****

Editor Comments:
We thank the authors for their efforts to improve the mansucript. The manuscript mertis publication in PNAS. We kindly ask to provide a high resolution image of Fig. 4.

Reviewer Comments:
Reviewer #1:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
I find this revised manuscript to be a significant improvement on the former and in my opinion can be published in its current form. All amendments requested by previous reviews appear to have been included.

Reviewer #3:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
The authors have substantially revised and edited their paper in response to the reviewers' comments. The writing more clearly conveys the context of their work and is suitable for the general audience that reads PNAS. I support acceptance by PNAS for publication. I have only one minor comment that may be addressed in typesetting - Figure 4 has many important panels, and at its current size, it is difficult to see and interpret all of the data. I was able to adequately see the data when enlarging the figure to >300% of its size and want to ensure that the resolution is maintained in the final published form so that others can enlarge the figure without it becoming pixelated. Alternatively, a larger version of the figure could be placed in the supplementary material.

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PUBLICATION INFORMATION AND CHARGES

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Decision letter for 2016-00406RR

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406RR Decision Notification
Message: April 29, 2016

Title: "Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406RR
Authors: [redacted]

Dear Dr. Zhang,

We are pleased to inform you that the PNAS Editorial Board has given final approval of your article for publication. Peter Ladurner, the Editor who conducted the initial review of your manuscript [MS# 2016-00406RR], will also be informed of the decision.

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Sincerely yours,
Inder M. Verma
Editor-in-Chief

****

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Thursday, July 21, 2016 3:53 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavangh,
Thank you for your quick response and willingness to provide those communications. I hope you enjoy your conference and we will look for those items when you are back in the office.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Thursday, July 21, 2016 3:17 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: Re: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I'm out of the office at a conference this week, but I will send our correspondence with Dr. Zhang when I'm back in the office next week.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: "Yucel, Jennifer" <yucel.4@osu.edu>
Date: Thursday, July 21, 2016 at 11:28 AM
To: Kavanagh Etta <ekavanagh@nas.edu>
Cc: "hart.322@osu.edu" <hart.322@osu.edu>, "Moses, Randolph" <moses.2@osu.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

Dr. Hart forwarded your email to me. I am the University's research integrity officer and I am confirming that the university did receive these concerns and we are looking into this matter.

Would it be possible for you to share with me any communications that the journal had with Dr. Zhang during the publication process? Those communications would greatly assist us in the review of this matter and would be greatly appreciated. Once we have completed our review we will inform you of our determination.
Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: "Kavanagh, Etta" <EKavanagh@nas.edu>
Date: July 15, 2016 at 5:21:57 PM EDT
To: "'hart.322@osu.edu'" <hart.322@osu.edu>
Subject: FW: confidential complaint about scientific misconduct
Dear Dr. Hart,

I am contacting you regarding the complaint PNAS received regarding the paper "Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy" [redacted]. We shared the complaint with the editor, Peter Ladurner, and he provided the following comments:

"I want to ensure my support for PNAS regarding manuscript 2016-00406RR.

The question if the gene identified is indeed the ivy AGP cought my attention after the initial submition. Please note that I questioned this finding myself. For their first revision I demanded that the authors have to add a phylogenetic tree showing the true AGP relationship of their protein. In their revision the authors provided the respective tree (and mass spec data) corroborating their finding.

I want to state that such data - under normal circumstances and if the data were generated according to best scientific practice - are sufficient to support the authors statement that their protein is an AGP.

However, if the genes for generating the phylogenetic tree were highly hand picked (and not selected according to their statement in the paper: "Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 were the closest relatives, as shown in Fig. S4A.") any tree can be fabricated.
I hope this is not true.

The next steps require detailed sequence analyses using BLAST, reciprocal BLAST, thourough protein alignments and phylogenetic analyses of the submitted sequence.

Depending on the result the authors need to provide raw data and lab book level information on gene isolation, details on clones with gene inserts from PCR and RACE experiments, sequencing raw files, details of their BLAST search settings and databases, information on the selection and generation of alignments and the phylogenetic tree, Mass Spectrometry raw data, details on GPI anchor bioinformatics.

Please let me know if I can help with the sequence analyses."

Is Ohio State University investigating these concerns? Should I contact the research integrity office? Thank you very much for your help.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Response to the Editor's and the Reviewers' Comments for the Manuscript:

"*Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy***"**

Reference: 2015-01638

Authors: [redacted], Yongzhong Wang, Li Tan, [redacted], Mei-Zhen Cui, Feng Hao, and Mingjun Zhang

Dear Editor and the Reviewers,

Thank you very much for your effort in handling and reviewing this paper. We greatly appreciate the comments, which have significantly helped us to improve the manuscript. To address the editor's and all the reviewers' comments, several additional experiments were performed and included in this revision to support the conclusions. We have answered all the questions as enclosed below. Corresponding changes made in response to the reviewers' comments are marked in blue in the revised manuscript.

We would like to thank you for the opportunity to improve our manuscript. Should you need any further information, please let us know.

Thank you very much!

Sincerely yours,

Mingjun Zhang, PhD & D.Sc.
Professor and Investigator
Department of Biomedical Engineering
Davis Heart and Lung Research Institute
The Ohio State University
340C/D Biomedical Research Tower
460 W 12th Ave.,
Columbus, OH 43210
Email: zhang.4882@osu.edu
Tel.: 001-614-292-1591

Response to the Editor's Comments:

Editor's Remarks to Author:

The manuscript cannot be published in PNAS in the current form. The reviewers raise several concerns regarding the isolated AGP, the glycosyl composition on silicon wafers, the nature of nanoparticle shown in the AFM images, and other technical problems. If the authors can address ALL of the reviewers' concerns, a resubmission could be possible. The manuscript requires a substantial major revision accompanied by additional experiments to confirm the results presented. All comments of the reviewers need to be carefully considered. Specifically, all of the following matters should be expanded:

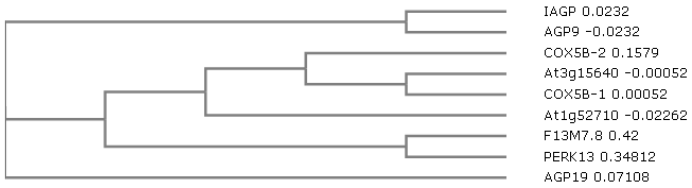
1. In particular, it is questioned if the correct gene was isolated since important domains are lacking. There is convincing doubt that the isolated IAGP represents an AGP at all. Therefore, I request a molecular phylogenetic tree including AGPs and Cytochrome Oxidase subunits to demonstrate the correct placement of IAGP within the AGP clade. In addition, as suggested by a reviewer, the degree of AG-glycosylation should be estimated as indicator that the cloned sequence is the correct AGP. In this respect, Mass Spectrometry also needs to be performed to corroborate that IAGP peptides are indeed present in abundance in purified particles.

Response: Thanks for the comments. We have established and included a phylogenetic tree in this revised manuscript (**Fig. S4*A***), also shown below (**Fig. R1*A***). Since the genome of *Hederal helix* is still unknown, analogous proteins chosen for phylogenetic analysis were selected according to a BLASTp search in the genome of *Arabidopsis thaliana.* In particular, four hydroxyproline-rich glycoproteins (HRGPs) and four cytochrome c oxidase subunits which are estimated to possess the most similar amino acid sequences to the IAGP by BLASTp, were introduced into the phylogenetic comparison to determine the relative placement of the IAGP. Among all the comparative proteins, the AGP9 exhibited the closest relationship to the IAGP, implying the apparent homology between the IAGP and other typical AGPs in *A. thaliana*. To further validate the identity of the IAGP, MALDI-TOF MS was carried out. As shown in **Fig. S4*B*** and **R1*B***, several molecular masses detected in the MS analysis well matched the expected masses of the putative tryptic peptides of the deglycosylated IAGP. Additionally, the numbers and distributions of the hydroxyprolines (Hyps) within each identified peptide were deduced from the information gained from the MS analysis. Greater than 90% of the prolines within the IAGP appear in the form of Hyp, which commonly defines the positions and extent of *O*-glycosylation in typical AGPs (1). Thus, the IAGP is estimated to possess a high degree of AG-glycosylation (*O*-glycosylation). As such, since typical AGPs are characterized by their high ratio of Hyps within the protein backbones, the sequence cloned by RACE should be regarded as a correct gene encoding AGP (1). The lacking of several common motifs may be attributed to the diversity of the AGP molecules among distinct species.

Given that the AGPs are a superfamily of glycoproteins present in the plant cell wall, as the first AGP molecular that has been identified in *H. helix*, it is reasonable to estimate that the IAGP contains certain level of variations in amino acid sequences.

A Phylogram

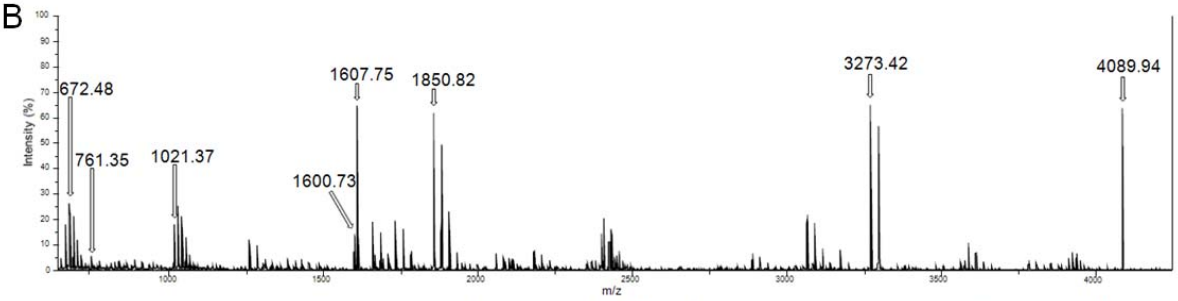
Branch length: Cladogram Real



Abbreviation	Full name of comparative proteins[a]	NCBI reference No.	Species
COX5B-2	cytochrome c oxidase subunit 5b-2	NP_178140.1	*A. thaliana*
At1g52710	putative cytochrome c oxidase subunit 5b-like	NP_175680	*A. thaliana*
At3g15640	cytochrome c oxidase subunit 5b-1	NP_001078161.1	*A. thaliana*
COX5B-1	cytochrome c oxidase subunit 5b-1	NP_188185.1	*A. thaliana*
AGP19	arabinogalactan protein 19	NP_177041.3	*A. thaliana*
AGP9	arabinogalactan protein 9	NP_973463.1	*A. thaliana*
PERK13	proline-rich extensin-like receptor kinase 13	NP_177203.1	*A. thaliana*
F13M7.8	hydroxyproline-rich glycoprotein family protein	NP_563722.1	*A. thaliana*
IAGP	*Hedera helix* arabinogalactan protein	KM820289	*H. Helix*

[a] Various proteins with amino acid sequences homologous to the IAGP, including four cytochrome c oxidase subunits and four HRGPs, were selected for phylogenetic comparison according to a BLASTp search in the genome of *A. thaliana*.

B



Tryptic peptides[a]	Predicted mass[b]	Mass detected[c]	Corresponding sequence with modifications[d]
(-)APPPTDAEQATGLER(L)	1552.7601	1600.7326	AOOOTDAEQATGLER
(R)LEILGK(M)	672.4290	672.4759	LEILGK
(K)MQGVDIFDMRPLDASR(L)	1850.8887	1850.8197	MQGVDIFDMRPLDASR
(R)LGTPEDPIVVNSAGNEQYVGCTGFPVDSHGVLWI TLTR(E)	4042.0014	4089.9372	LGTOEDOIVVNSAGNEQYVGCTGFOVDSHGVLWI TLTR
(R)EEPQSR(C)	745.3475	761.3524	EEOQSR
(R)CMECGSTYK(M)	1021.3787	1021.3695	CMECGSTYK
(K)MHYVGPADDPHAHDHGHGHDHHGPPPKPK(D)	3177.4356	3273.4176	MHYVGOADDOHAHDHGHGHDHHGOOOKOK
(K)DMADFLKPEYLHI(-)	1591.7824	1607.7533	DMADFLKOEYLHI

[a] Theoretical amino acid sequences of the peptides obtained after the digestion with trypsin. [b] Predicted molecular masses of respective peptides. [c] Molecular masses of corresponding peptides determined by MALDI-TOF MS. [d] Amino acid sequences of corresponding tryptic peptides deduced from the MALDI-TOF MS results, including the information regarding the number and distribution of the Hyps. O represents the Hyp.

Fig. R1. Validation of the identity of the IAGP by establishing a phylogenetic tree and MALDI-TOF MS analysis. (***A***) A phylogenetic tree, comprising the IAGP, four cytochrome c oxidase subunits, and four HRGPs, was constructed by Clustal Phylogeny. Apart from the IAGP, all other proteins were selected from the *A. thaliana* according to a BLASTp search. In addition to typical HRGPs, given that the IAGP demonstrates moderate extent of similarity to several cytochrome c oxidase subunits, the most analogous ones estimated by the BLASTp were chosen for the phylogenetic analysis to determine the identity of the IAGP. (***B***) MALDI-TOF MS spectrum of the tryptic peptides harvested from the deglycosylated fraction 4 derived from

RP-HPLC. In comparison to the expected masses of respective tryptic peptides, the number and positions of the Hyps (O) within each peptide were identified by the masses determined by MALDI-TOF MS.

2. Further evidence that IAGP is in fact the Ivy adhesion molecule is required.

Response: Thanks for the comment. In this revision, the tensile test proposed to substantiate the molecular basis for the ivy-derived adhesive was intensified. In particular, the specific roles of each component in the constructed adhesive composites, including the AGP-rich ivy nanoparticles, pectic polysaccharides, and calcium ions, were quantitatively evaluated in the lap joint shear strength test and the tensile strength test. In contrast to the adhesive composites comprising pectin and Ca^{2+}, markedly stronger shear strength and tensile strength were reached by the ivy-mimetic adhesive constructs consisting of equivalent total amount of the AGP-rich ivy nanoparticles, pectin, and calcium ions 7 days after the preparation, as shown in **Fig. 6 *B* and *G***, respectively, indicating that the IAGP-based ivy nanoparticles indeed possess the capacity to favor the adhesive events upon the integration with pectin.

3. The issue whether nanoparticles in AFM images are individual molecules or aggregates should be clarified.

Response: Thanks for the comment. Yes, the ivy nanoparticles observed in the AFM images (**Fig. 1*C* and Fig. 2 *E* and F**) are individual molecules rather than aggregates. We included additional experimental evidence and explanations to support the conclusion. The ivy nanoparticles, either isolated and purified *in vitro* (**Fig. 1*C***) or observed in the mucilage exuded by the adventitious roots in situ (2, 3), always demonstrate a uniform appearance in terms of their size, shape, and morphology, while being characterized by AFM. In addition, relevant morphological information is also validated by scanning electron microscopic (SEM) examination of both *in vitro* purified nanoparticles (4) and those captured in the sticky exudates (**Fig. 4*B***). In contrast, the aggregates of the ivy nanoparticles exhibit a dramatic difference in morphology, as presented in **Fig. S6**. More importantly, the ivy nanoparticles purified via SEC were further fractionated by RP-HPLC and each fraction harvested was characterized by AFM, as shown in **Fig. 2 *C*-*F***. Notably, the fraction 4 was composed of spherical nanostructures analogous to the SEC-purified nanoparticles in form and size. Given that any structural domains with loosely noncovalent binding should be separated during the gradient elution in RP-HPLC, this result suggests that all the constituents essential for the construction of the overall architecture of the ivy nanoparticles are covalently connected. In this respect, the purified ivy nanoparticles displayed in **Fig. 1*C*** should be regarded as individual molecules, instead of clusters of multiple molecules. Accordingly, a brief description to clarify the statement was included in the revised manuscript on Page 7, Lines 14-17, shown as below:

"Given that structural domains with loosely noncovalent binding should be separated during the gradient elution in RP-HPLC, these data suggest that the purified ivy nanoparticles observed in **Fig. 1*C*** are individual molecules, rather than clusters of multiple molecules."

4. The nature of adhering residues on silicon wafers (Table 2) needs to be illuminated - can these represent cellulose or hemicellulose impurities?

Response: Thanks for the suggestion. Due to the limited amount of mucilage secreted by the adventitious roots of English ivy, an in situ collection strategy was developed in this study to harvest sufficient adhesive substances by recovering the dried exudates remnant on the silicon wafers. We agree that this method also has its own shortcoming, even though it seems to be the most effective approach. Partial components of the plant cell wall, including cellulose and hemicellulose, may be encapsulated into the solid mucilage upon the detachment of the clinging adventitious roots and thus resuspended with other adhesive substances in the sample prepared for monosaccharide composition analysis (**Table 2**) and ELISA screening test (**Fig. S8**). As described in the previous studies (2, 5), for the vast majority of the climbing plants which secrete mucilage from respective climbing organs, an adhesive pad is formed at the junction of the climbing organs and corresponding substrates, comprising adhesive substances and partial components derived from the plant cell wall. However, the existence of cellulose or hemicellulose in the mucilage recovered from the imprints remnant on the silicon wafers does not conflict with the conclusion that the AGPs and the pectic polysaccharides are the predominant components in the sticky exudates of English ivy. In fact, earlier studies with respect to the botanic bioadhesives have reached an agreement that the pectins and AGPs are indeed present in abundance in these adhesive secretions, by both biochemical assays and immunohistochemical analyses (5-11). Consistently, our results here indicate that these two types of acidic polysaccharides/glycoproteins are also abundantly distributed in the ivy-derived adhesive, similar to other bioadhesives observed in Virginia creeper, Boston ivy, *Ficus pumila*, and many others (5-11).

Kevin C. Vaughn *et al.* have described the pectins and the AGPs in the botanic adhesive as "mucilaginous molecules that are spread across the surface of the structure to be attached, filling in the gaps" (12). In particular, "arabinans and AGPs appear to be an even more mobile component of the adhesive, filling in spaces between the papillate epidermal cells and even moving into small cracks in the structure that is attached" (12).

Accordingly, the presence of penitential cellulose and/or hemicellulose in the adhesive substances recovered from the imprints remnant on the silicon wafers is interpreted in the revised manuscript, shown as below:

"Meanwhile, from the monosaccharide composition analyses, it is also noteworthy that the proportion of Glc, Man and Xyl as a whole is greater than 65% (mol%) of the total monosaccharides, suggesting the presence of cellulose and/or hemicellulose in the imprints remnant on the silicon wafers. These substances presumably

arise from the encapsulation of partial components of the plant cell wall within the cured adhesive, a phenomenon that has been detailed in the previous studies (2, 5).” (Page 14, Lines 6-12)

5. Stress strain data to show the rheological behavior need to be included.

Response: We agree. In this revision, all the data obtained from the lap joint shear strength test and the tensile strength test 7 days after the preparation of the adhesive composites have been converted to stress-strain curves, as shown in **Fig. 6 *C* and *F***. Accordingly, brief descriptions regarding the rheological behavior of respective adhesive constructs are included in this revision, shown as following:

“The stress-strain curves of respective adhesive composites, as measured and plotted by the lap shear test on day 7, are shown in **Fig. 6*C***. It could be observed that for the adhesive composites comprising both the ivy nanoparticles and pectic polysaccharides, the strains at failure were substantially lower than those of the constructs containing either ivy nanoparticles or pectic substances alone. In particular, the minimum strain at failure was reached by the ivy-mimetic EGTA-free adhesive composite in the presence of Ca^{2+}.” (Page 20, Lines 4-10)

“Similar to the information gained from the lap shear test, the stress-strain curves show that the strain at failure of the reconstructed ivy-mimetic adhesive composite in the presence of Ca^{2+} and in the absence of EGTA is approximately 57% and 37% lower than those of the cases containing 2 mM Ca^{2+} integrated with either ivy nanoparticles or pectic polysaccharides, respectively, on day 7 (**Fig. 6*F***).” (Page 21, Lines 3-7)

6. Methods descriptions need to be more detailed.

Response: Thanks for the suggestion. This manuscript has been thoroughly revised and polished by a native English speaker and co-authors. Part of the Methods section has been rewritten to describe the experimental procedures in detail. Imprecise descriptions have been eliminated. Due to the length limitation, the majority of the Materials and Methods have been removed to the supporting information, designated as ***SI Materials and Methods***. Thanks again for the comments.

Responses to the Reviewers' Comments:

Reviewer #1:

The paper investigates the molecular and bio-physical basis of the adhesive secreted by Ivy. A fraction of apparently spherical particles is isolated from exudate and is analysed biochemically and biophysically. It is suggested that the particles mainly consist of an arabinogalactan protein (AGP) that is termed IAGP. Based on a N-terminal peptide sequence of the deglycosylated protein, a putative cDNA for IAGP is cloned. The purified particles display low inherent viscosity and can form adhesive glues when combined with pectin and calcium. From a biophysical perspective the case looks more convincing than from a molecular biological one.

1. Comments on scientific content:

1.1 AFM: It is not clear what is actually shown with the AFM images. Do the authors suggest that we are looking at individual AGP molecules or aggregates of many AGP and e.g. pectin molecules? Even though the texture of the mages suggests spherical particles the individual particles i.e. connected shapes are rarely ever spherical but are clusters. I am not an AFM expert but it seems helpful to image the particles at increasingly lower density so that individual particles might become the predominant structures. Otherwise it might mean that the spheres are not spheres after all.

Response: Thanks for the comments. We apologize for the confusion. For the nanostructures presented in the AFM images (**Fig. 1*C* and Fig. 2 *E* and F**), they are individual molecules consisting of covalently bonded AGP and pectic domain (please refer to the response to the editor's comment 3). These nanostructures were discovered in 2008 (2), and their spherical shape has been examined and determined by both AFM and SEM analyses in the earlier studies (2-4). The ivy nanoparticles, either purified *in vitro* (4) or in situ detected in the mucilage exuded by the adventitious roots (2), demonstrate a spheroidal architecture consistently, while the measurement in the z-direction using AFM often tortures the exact number.

Per the reviewer's suggestion, we have examined the purified ivy nanoparticles at a lower density using AFM. As shown in **Fig. 1*C* (inset),** individual spherical nanoparticles could be clearly distinguished from clusters. Notably, clusters formed by the aggregation of the purified ivy nanoparticles do exist in the AFM images. It is difficult to avoid them since the trend in aggregation in a dry state is a characteristic feature of typical AGPs (13). In addition, the spherical shape of the ivy nanoparticles was further validated by the SEM imaging of the nanostructures within the sticky exudates remnant on the silicon wafers, as shown in **Fig. 4*B***. More importantly, as described above, the fraction 4 obtained from RP-HPLC was detected to be composed of spherical nanostructures analogous to the SEC-purified nanoparticles in terms of form and size, as shown in

Fig. 2 *C-F*. Given that any structural domains with loosely noncovalent binding should be separated during the gradient elution in RP-HPLC, this result here suggests that the constituents essential for the construction of the overall architecture of the ivy nanoparticles are covalently connected. In this respect, the ivy nanoparticles shown in **Fig. 1*C*** should be considered as individual spherical molecules comprising AGP and pectic domains, with a strong capability of agglomeration. To clarify this issue, a brief description was included in the revised manuscript, shown as below:

"Given that structural domains with loosely noncovalent binding should be separated during the gradient elution in RP-HPLC, these data suggest that the purified ivy nanoparticles observed in **Fig. 1*C*** are individual molecules, rather than clusters of multiple molecules." (Page 7, Lines 14-17)

1.2 Fig. 1D and 1E. I don't know whether the methods applied for these figures are commonly known among physicists. As a molecular biologist I would find it helpful to get a better explanation of the method and why it should be applied.

Response: Thanks for the reminder. Yes, they are common tools used by physicists. We agree that it is a good idea to include detailed explanations.

Dynamic light scattering (DLS), also called photo correlation spectroscopy or quasi-elastic light scattering, is an analytical tool capable of noninvasively determining the hydrodynamic size and size distribution of particles, including polymers, proteins, and colloids, ranging from 0.5 nm to 6 μm, in a liquid environment (14-16). This technique is even sensitive enough to dynamically monitor small changes in size of the tested particles. The principle of DLS is based on the Brownian motion of small particles, resulting in a Doppler shift of incident laser light (17, 18). The diffusion constant of particles measured can be interpreted using the Stokes-Einstein equation to obtain the average hydrodynamic diameter of particles (17-19).

Similarly, electrophoretic light scattering (ELS) is a technique also based on DLS (17). This powerful tool is designed for characterizing the surface charges of colloidal particles under liquid condition, according to the same Doppler effect arising from the oscillating electric field (17, 20). The zeta potential is determined by measuring the electrophoretic mobility (20, 21).

In this study, these common techniques were employed to characterize the hydrodynamic size, size distribution, and surface charges of the purified ivy nanoparticles. The physicochemical information provided regarding the purified ivy nanoparticles allows us to better explore the functions of these nanostructures. A brief introduction of these two techniques was included in the ***SI Materials and Methods*** section in this revision, shown as following:

"The size distribution, mean size and the zeta potential of the purified ivy nanoparticles were characterized by DLS and ELS at 25 ℃, using a Malvern Zetasizer, Nano ZS (Malvern Instruments Ltd, Worcestershire, UK), with a He-Ne laser (wavelength of 633 nm) and a detector angle of 173°. DLS is an analytical tool capable of noninvasively determining the hydrodynamic size and size distribution of particles, including polymers, proteins, and colloids, ranging from 0.5 nm to 6 μm, in a liquid environment (14-16), while ELS is a technique designed for characterizing the surface charges of colloidal particles under liquid condition (17, 20)." (***SI Materials and Methods*** section: Page 4, Lines 1-7)

1.3 Tables 1 and 2: While the meaning of table 1 is relatively well explained and is interpreted that the linkages probably represent both AGII and pectic structures, the presence Glc, Xyl and Man (together >12 Mol%) is not explained. The sugars are reminiscent of hemicellulose but the terminal Glc is not. Is this an impurity or a component of the AGP/pectin particles? Table 2 describes the sugars found in adhering residues and the text states that "pectin is one of the main components ...". However the predominant monosaccharide in the remnant is glucose. Together with xylose and mannose it accounts for >65% meaning the main constituent is likely to be cellulose (indicating cell debris) or hemicellulose.

Response: Thanks for the comments. We agree with the reviewer's comment that several types of sugars identified in the purified ivy nanoparticles, i.e. Glc, Xyl and Man, are not commonly regarded as characteristic monosaccharides in AGPs. However (also interestingly as reported in the ample literature), these three types of monosaccharide are indeed detected in multiple AGPs extracted from a variety of species, including those isolated from *Carica papaya* (22), *Gossypium hirsutum* (23), *A. thaliana* (24), *Spondias dulcis* (25), *Aegle marmelos* (26), *Prosopis chilensis* (27), *Prosopis glandulosa* (27), *Chlorisia speciose* (28), and many others. In particular, for the AGPs isolated and purified from *Daucus carota*, the proportion of the Glc identified is up to 25.1% (Mol%) of the total monosaccharides (13). We believe that the difference in the monosaccharide compositions among various species is attributed to the diversity of the AGP molecules. For the ivy nanoparticles isolated from the adventitious roots of *H. helix*, the monosaccharide composition was determined from the Complex Carbohydrates Research Center (CCRC) at the University of Georgia, using the purified fraction 4 harvested from RP-HPLC. The presence of Glc, Xyl and Man should not be derived from any impurity, but be associated with the chemical constituents of the ivy nanoparticle itself. To validate the results of the monosaccharide composition analysis, all the tests were repeated to ensure the accuracy and reliability of the data obtained. As suggested by the reviewer, a brief description of the existence of these three types of monosaccharides was included in the revised manuscript on Page 8, Lines 17-20, shown as below:

"Notably, even though Glc, Man and Xyl are not commonly regarded as characteristic monosaccharides in AGPs and pectin, they accounted for a proportion of greater than 12% (mol%) of the total monosaccharides as a whole in the fraction 4."

For the reviewer's concern with regard to the monosaccharide composition analysis of the adhesive substances recovered from the imprints remnant on the silicon wafers, please refer to the response to the editor's comment 4. As aforementioned, given the limited amount of mucilage exuded by the adventitious roots of English ivy, the difficulty in gathering sufficient amount of adhesive substances in liquid form has restrained our capability of exploring the chemical constituents within the ivy-derived adhesive. As a result, alternative strategy to harvest massive adhesive substances by recovering the dried exudates remnant on the silicon wafers seems to be the most effective approach for such a purpose, even though this method also has its own shortcoming. Partial components within the plant cell wall, including cellulose and hemicellulose, may be encapsulated into the solid mucilage upon the detachment of the clinging adventitious roots and thus resuspended with other adhesive substances in the sample prepared for monosaccharide composition analysis (**Table 2**) and ELISA screening test (**Fig. S8**). As described in the previous studies (2, 5), for the vast majority of the climbing plants which secrete mucilage from respective climbing organs, an adhesive pad is formed at the junction of the climbing organs and corresponding substrates, as shown below (**Fig. R2**), comprising adhesive substances and partial components derived from the plant cell wall. However, the existence of cellulose or hemicellulose in the mucilage recovered from the imprints remnant on the silicon wafers does not conflict with the conclusion that the AGPs and the pectic polysaccharides are the predominant components in the sticky exudates of English ivy. Actually, earlier studies with respect to the botanic bioadhesives have reached an agreement that the pectins and AGPs are indeed present in abundance in these adhesive secretions, by both biochemical assays and immunohistochemical analyses (5-11). Consistently, our results here indicate that these two types of acidic polysaccharides/glycoproteins are also abundantly distributed in the ivy-derived adhesive, similar to other bioadhesives observed in Virginia creeper, Boston ivy, *Ficus pumila*, and many others (5-11).

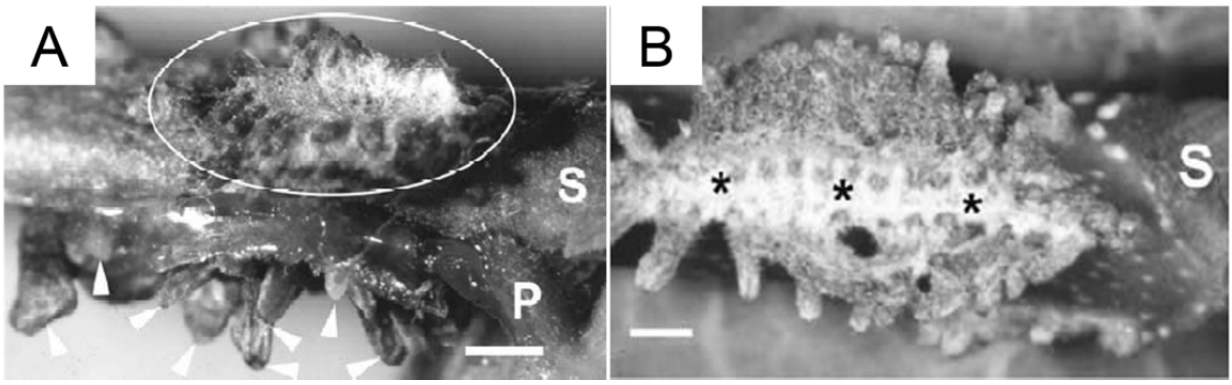


Fig. R2. Adhesive pad formed by climbing fig (*Ficus pumila*). (***A***) Stem two weeks after treatment with auxin paste. Auxin induced roots (arrowheads) are present on the treated side of the stem. They lack root hairs and many appear dried and shrunken. The untreated side of the stem has a normally developed pad (oval) with root hairs. The petiole (P) and the stipule (S) are visible. Scale bar 1 mm. (***B***) A adhesive pad adhered to a glass substrate. Root hairs are obvious as a white or tan pubescence. Scale bar 1 mm. The images are cited from (5).

Kevin C. Vaughn *et al.* have appropriately described the pectins and the AGPs in the botanic adhesive as "mucilaginous molecules that are spread across the surface of the structure to be attached, filling in the gaps" (12). In particular, "arabinans and AGPs appear to be an even more mobile component of the adhesive, filling in spaces between the papillate epidermal cells and even moving into small cracks in the structure that is attached" (12).

Accordingly, a brief interpretation of the possible presence of cellulose and/or hemicellulose in the adhesive substances recovered from the imprints remnant on the silicon wafers was included in the revised manuscript, shown as below:

"To determine the chemical constituents of this matrix, the remnant substances exuded from the adventitious roots on the silicon wafers were re-suspended in PBS and examined using ELISA screening test, with 38 mAbs raised against the vast majority of the polysaccharides present in the plant cell wall listed in **Table S1**. As shown in **Fig. S8**, accompanied by arabinogalactans, diverse pectic epitopes are also richly distributed in the adhesive secretions. Moreover, subsequent glycosyl composition assay identified that 4.09% (mol%) GalA*p* residues were contained in the adhesive substances recovered from the remnant on the silicon wafers, as listed in **Table 2**, further suggesting the existence of pectic polysaccharides in the mucilage derived from the adventitious roots of English ivy. Meanwhile, from the monosaccharide composition analyses, it is also noteworthy that the proportion of Glc, Man and Xyl as a whole is greater than 65% (mol%) of the total monosaccharides, suggesting the presence of cellulose and/or hemicellulose in the imprints remnant on the silicon wafers. These substances presumably arise from the encapsulation of partial components of the plant cell wall within the cured adhesive, a phenomenon that has been detailed in the previous studies (2, 5). Given that the arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and *Ficus pumila*, as shown in the previous cytochemical analyses (5-11), it is logical to expect that these two acidic polysaccharides possess exceptional capacity to effectively support the adhesive function of the sticky exudates at the interface." (Page 13, Line 21 to Page 14, Line 17)

1.4 When the IAGP sequence is presented in Figure 3 it is shown with a GPI anchor signal. Also in the scheme Figure 6 the GPI anchor structure, its attachment and release are shown in detail. However, the prediction tool that was used does not predict IAGP as GPI anchored (score = -76.05) and even the 'most likely' omega-site is different from the one shown in Figure 3. In my opinion the question whether or not IAGP is GPI anchored is irrelevant to the story and related suggestions may be removed without reducing the informative value.

Response: We agree that this is a good way to avoid confusion. All the discussion regarding the GPI anchor structure has been removed from this revision. **Fig. 3** and **Fig. 5** (previous **Fig. 6**) have been corrected

accordingly. The tool used for the GPI prediction described in the previous Methods section has been removed as well.

1.5 Figure 3C is mentioned in the context of the results section which is misleading. However it only shows a generic structure of a hypothetical AGP. In fact this is not even a classic (sic) AGP as claimed in the text. The literature uses the term "classical AGP" for extremely reduced proteins that contain only a backbone for O-glycosylation (typically XP repeats) and not other potentially functional protein domains (see Ref Ellis et al 2010).

Response: We apologize for this confusion. In the **Fig. 3*A*** (original **Fig. 3*C***) and also in the main text, we do not intend to classify the IAGP identified from *H. helix* into any specific subfamily of AGPs. We mistakenly used the word "classical" in the previous manuscript. All the information we would like to provide in the **Fig. 3*A*** is just the generic structure of AGPs. This generic schematic drawing may allow researchers from different fields to understand relevant discussion. To avoid this confusion, in this revision, the term "classic AGP" has been removed and replaced with "typical AGP".

1.6 The sequence of IAGP does not resemble any known AGP. As such this is not remarkable because the important domains that define AGPs are so called AG-modules, stretches of XP, which are lacking from IAGP altogether. However, it bears remarkable sequence similarity to cytochrome oxidase subunit 5b-2. Are the authors sure that they have cloned the right cDNA?

Response: Thanks for the comments. Yes, we are confident about the cDNA sequence cloned previously. To address the reviewer's concern, several additional analyses were performed to substantiate the identity of the IAGP. In light of the editor's suggestion, since the IAGP identified indeed demonstrates moderate similarity to some cytochrome oxidase subunits via a BLASTp search in the genome of *A. thaliana*, a phylogenetic tree was built comprising four HRGPs and four cytochrome c oxidase subunits which exhibited the highest similarities to the IAGP in the BLASTp search, in the revised manuscript (**Fig. S4*A***), shown as above (**Fig. R1*A***). The aim is to estimate the correct placement of the IAGP within the AGP clade. Among all the comparative proteins, the closest relatives are the AGP9 and the IAGP, implying the apparent homology between the IAGP and other typical AGPs in *A. thaliana*. To further validate the identity of the IAGP, MALDI-TOF MS was carried out as described below. The molecular masses obtained from the MS analysis well matched the expected masses of several theoretically predicted tryptic peptides of the IAGP. The lacking of several common motifs relevant to AGPs may be attributed to the diversity of the AGP molecules among distinct species. Given that the AGPs are a superfamily of glycoproteins present in the plant cell wall, as the first AGP molecular that has been identified in *H. helix*, it is reasonable to estimate that the IAGP contains certain level of variations in amino acid sequences.

1.7 It would be important to estimate the degree of glycosylation of IAGP, especially when there is access to relatively large amounts of deglycosylated IAGP, MS/MS analysis could reveal hydroxyprolines in isolated positions which is an indirect indicator of AG-glycosylation. It would also confirm the identity of the suggested protein sequence which seems doubtful to me. Another possibility to confirm the nature of the cloned cDNA would be to express the sequence in a heterologous host (e.g. tobacco or Arabidopsis) and test its post-translational modifications.

Response: Thanks for the comments. MALDI-TOF MS was performed to determine the distribution of the Hyps and evidence the identity of the IAGP in this revision. As shown in **Fig. S4*B*** and **R1*B***, several molecular masses observed in MS spectrum of the tryptic peptides harvested from the deglycosylated fraction 4 obtained from RP-HPLC well matched the expected masses of the putative tryptic peptides of the IAGP. Additionally, the numbers and distributions of the Hyps within each identified peptide were deduced from the information gained from the MS analysis. Greater than 90% of the prolines within the IAGP appear in the form of Hyp, which commonly defines the positions and extent of *O*-glycosylation in typical AGPs (1). As such, the IAGP is estimated to possess a high degree of *O*-glycosylation.

Accordingly, the phylogenetic comparison and the MALDI-TOF MS analysis were described in the revised manuscript, shown as following:

"Notably, in a BLASTp search in the genome of the *A. thaliana*, the IAGP identified from the *H. helix* also exhibited a moderate extent of similarity to several cytochrome c oxidase subunits, implying potential homology between these two types of proteins. Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 demonstrated to be the closest relatives, as shown in **Fig. S4*A***. The identity of the IAGP obtained from the RACE cloning was further validated by MALDI-TOF MS analysis by determining the masses of the tryptic peptides of the deglycosylated fraction 4 obtained from RP-HPLC. As shown in **Fig. S4*B***, three peptides with respective masses detected by the MS matched the predicted molecular masses of expected tryptic peptides. Additionally, the hydroxylation degree and the distribution of the Hyps in other estimated tryptic peptides were also deduced from relevant molecular masses gained from the MS test. Given that greater than 90% of the prolines within the IAGP appear in the form of Hyp, which commonly bears type II AGs and short oligoarabinosides in typical AGPs (1), the IAGP should be highly *O*-glycosylated in the ivy nanoparticles, similar to other AGPs identified from *A. thaliana*." (Page 9, Line 16 to Page 10, Line 8)

2. References:

2.1 The original papers where monoclonal antibodies were introduced and where they were characterized should be referenced.

Response: Thanks for the reviewer's suggestion. All mAbs used in this study have been appropriately referenced in the revised manuscript, as listed in **Table S1**.

2.2 The SDS-PAGE procedure is not referenced.

Response: Thanks for the suggestion. The SDS-PAGE procedure has been referenced in the revised manuscript, as shown in ***SI Materials and Methods*** on Page 4, Lines 12-13.

3. Comments on presentation style:

3.1 The English requires professional editing as the work contains numerous grammatical errors.

Response: We appreciate the suggestion. This manuscript has been thoroughly revised and polished to eliminate grammatical and syntactic errors as well as typos by a native English speaker. The majority of the paragraphs have been rewritten to avoid imprecise interpretation of results and inaccurate discussion.

3.2 Figure 3C is mentioned in the text before Figures 3A and B.

Response: Thanks for the reminder. The **Fig. 3** has been reorganized and sequentially presented in the main text of the revised manuscript. In addition, the **Fig. 1**, **Fig. 4** and **Fig. 6** (previous **Fig. 7**) have been adjusted accordingly.

3.3 Figure 5: the bottom part of the figure should be deleted and the glycan groups should be indicated just below the antibody names or even better above the bars.

Response: Thanks for the suggestion. Due to the length limitation, the previous **Fig. 5** has been removed to the supporting information, designated as **Fig. S8**, as suggested during the quality check by the journal. In the revised manuscript, the bottom part of the **Fig. S8** has been removed and the glycans have been grouped above the bars instead.

3.4 Figure 6 is far too elaborate and is not only confusing but also gives some quite false impressions (e.g. the existence of GPI-PLC in plants or the assumption that AGPs are glycosylated in the Golgi neither of which is proven). Only the relevant parts of the figure should be retained. Delete GPI-anchor, biosynthetic pathway (ribosomes, ER, Golgi), PLD/PLC.

Response: Thanks for the suggestion. The **Fig. 5** (previous **Fig. 6**) has been redrawn. Biosynthetic pathway, including PLD/PLC and GPI anchor, has all been removed. Information relevant to the proposed molecular basis for the ivy-derived adhesive is retained. Thanks again for the reviewer's comments.

Reviewer #2:

Comments:

The submission [redacted] describes in some detail a study to determine the mechanism of adhesion in English Ivy. Approaches are varied and include (nano-)mechanical studies, proteomics, genomics and biochemistry. The conclusions are interesting and sound. They build upon previous work by this group, but offer significant new insight and 'proof of concept'. The methods and results are described well and concisely. The study of adhesion of plants lags behind that of animals and, for this reason, it is particularly interesting to see such a comprehensive report presented.

1. I have no major comments on the body of the text, except that I found the discussion to be rather short and lacking in depth. Similarly, I believe that the significance statement could be strengthened. What are the implications of the findings? The possible applications and routes to exploitation?

Response: Thanks for the suggestion. The discussion section has been intensified in this revision. In particular, the similarities in molecular bases for the ivy-derived adhesive and the mussel-derived adhesive have been analyzed and the implied correlations between these two types of bioadhesives have been discussed, shown as below:

"More importantly, it has been proposed that the genes encoding the HRGPs may originate from a "superfamily" of ancient genes relevant to diverse adhesive events in both animals and plants (29). In comparison the cell walls of plants to the corresponding extracellular matrices of animals, with respective frameworks built with HRGP extensin family and collagen, obviously, considerable commonalities are shared by these two types of polymeric networks (29). In particular, repeat motifs dominated by Hyp are observed in both matrices, defining and determining the helical conformation during the complicated assembly and cross-linking process (30). Meanwhile, the helical conformation is thought to be stabilized by the arabinosyl/galactosyl-modication of the Hyps within both networks (29, 30). Notably, similar Hyp-rich motif is also identified in the adhesive proteins isolated from mussel (31, 32), suggesting the potential evolutionary homology between the adhesive proteins derived from animals and plants (29). As another typical subfamily of HRGPs, the AGPs identified in the ivy nanoparticles are also rich in Hyps. In this respect, there may be still some ubiquitous principles between these two types of bioadhesives waiting for us to explore." (Page 22, Lines 6-19)

In the meanwhile, as suggested by the reviewer, the significance statement has been rewritten to emphasize the significance and implications of this study, as well as the potential areas of application. However, due to the length limitation, these aspects are just concisely described, shown as following:

"Despite significant progress has been made in exploring molecular bases for multiple adhesive events in animal kingdom, the exceptional adhesion behavior of climbing plants, such as English ivy, is still poorly understood. In this study, the spheroidal nanoparticles observed in the mucilage exuded by the English ivy are identified to be predominantly composed of arabinogalactan proteins (AGPs) and the roles of these AGP-rich nanoparticles in favoring the generation of strong adhesion strength are elucidated. The Ca^{2+}-driven electrostatic interactions among uronic acids within different biopolymers upon curing could be exploited as guidelines in the design and fabrication of novel synthetic adhesives, and the ivy-derived adhesive composite is capable of serving as a template for inspiring the development of diverse adhesive biomaterials." (Page 3, Lines 2-11)

2. Page 13, line 11, should this measurement of lap shear not be expressed as a stress in Pa?

Response: Thanks for the comments. As also suggested by the reviewer 3, all the values obtained from the lap shear test and tensile strength test were converted to stress-strain curves, as shown in **Fig. 6**. Shear strength (Pa) and tensile strength (Pa) of different adhesive composites were evaluated in this revision, instead of the adhesion force (N) described in the previous manuscript.

3. My other comments are minor and include:

Page 2, line 14 - I don't think "conversely" is the right word.

Line 18 - Here and throughout the MS, 'by' is usually more appropriate than 'via'.

Line 18 - "the characteristic physicochemical..."

Line 20 - Remove "revealed".

Page 5, line 4 - "developed previously"

Page 6, line 5 - "This result was..."

Line 9 - "verified". Here and throughout tenses are mixed, often in the same sentence. e.g. "verifies" and "displayed".

Page 9, line 8 - "those of pectin and sodium..."

Line 12 - "In the current work..."

Line 13 - "objective was to reveal..."

Line 15 - ": surface wetting"

Line 20 - "beneficial for surface wetting by the ivy..."

Page 12, line 14 - "The manner in which nanoparticles..."

Line 16 - "product prepared via emulsion polymerisation. It is composed of nano-sized polymer-based particles dispersed in an aqueous..."

Line 17 - "Upon application to a ..."

Page 13, line 6 - "developed by integrating..."

Line 10 - "variations in adhesion force".

Line 10 - "enhanced with increasing hardening time".

Page 14, line 3 - "interfere with the..."

Page 15, line 1 - this sentence does not seem to make sense.

Page 15, line 20 - replace "has been established early"

Page 16, line 1 - remove "for"

Page 21, line 22 - remove "for"

Page 23, line 7 - "Once removed..."

Page 24, line 19 - "described earlier"

Page 25, line 5 - replace "via" with "by"

Line 10 - same as above

Line 13 - "were employed as adherends"

Line 15 - remove "sample"

Response: We appreciate the reviewer's help on improving our English writing. All the errors mentioned above have been corrected in the revised manuscript. In addition, this manuscript has been thoroughly revised and polished. Considerable paragraphs have been reorganized or rewritten to ensure the precise and concise interpretation of data. As a result, multiple grammatical or syntactic suggestions indicated above are not present in this revision due to the changes in the overall paragraphs. Thanks again for the reviewer's comments.

Reviewer #3:

Comments:

This paper reports on the characterization of Ivy AGP and the adhesion strength it creates between glass slides. There are a significant number of technical problems with the paper which make it unacceptable for publication in the current form. The comments are listed below.

1. The evidence to support that fact that the AGP is exuded and indeed the critical molecule used by the Ivy to adhere is not presented. The statement is made that it is "presumed" that the AGP is involved in the adhesion.

Response: Thanks for the comments. We apologize for this confusion. In this study, the ivy nanoparticles are identified to be predominantly composed of AGPs. The secretory procedures of these AGP-rich nanoparticles from the adventitious roots toward the substrates have been monitored in real time and described in detail in our previous study (2, 3). With respect to the specific roles of these AGP-rich ivy nanoparticles within the ivy-derived adhesive during the generation of strong adhesion strengthen, multiple tests from physical, biochemical, immunohistochemical and mechanical perspectives have been carried out to comprehensively explore the molecular events in which these AGP-rich ivy nanoparticles are involved. However, the experimental data supporting our hypothesis were not well explained and interpreted in the previous manuscript. In this revision, we rephrased most of the experimental descriptions to ensure that the informative value of our experimental results can be effectively and precisely delivered. Together with the newly included tensile tests as suggested by the reviewer (**Fig. 6**), we believe that the experimental data presented in this revision are sufficient to reflect the essential roles of these AGP-rich nanoparticles in the ivy-derived adhesive. In particular, the low intrinsic viscosity of these AGP-rich molecules is capable of improving the surface wetting of the ivy-derived adhesive, as discussed from Page 11, Line 6 to Page 12, Line 11. The calcium-dependent electrostatic interactions among carboxyl groups of uronic acids within the AGPs and the pectic polysaccharides are determined to be the driving force for the cross-linking of the ivy-derived adhesive, via dot blotting test and fluorescent combination assay as described from Page 15, Line 7 to Page 17, Line 9. More importantly, the tensile test was intensified and improved as suggested by the reviewer. In particular, in order to validate the specific function of the AGPs in the ivy-derived adhesive, the adhesive behavior of the reconstructed ivy-mimetic composite consisting of AGP-rich ivy nanoparticles, pectic polysaccharides and calcium ions was compared to that of the composite comprising equivalent total amount of pectin incorporated with calcium ions by quantitatively assessing the shear strengths and tensile strengths of respective adhesive constructs, as shown in **Fig. 6**. In contrast to the adhesive composite comprising pectin and Ca^{2+}, markedly stronger shear strength and tensile strength were reached by the reconstructed ivy-mimetic composite 7 days after the preparation, as shown in **Fig. 6 *B* and *G***. This result here strongly supports the fact that the AGP-rich ivy nanoparticles indeed possess the capacity to favor the adhesive events upon the integration with pectin.

2. While the characterization of the AGP seems sound, it is not clear whether the nanoparticles are single molecules or aggregates. The aggregation number should be determined or at least estimated.
Response: Thanks for the comments. Please refer to the response to the reviewer 1's comment 1.1. As described above, more evidence is included in this revision to show that the ivy nanoparticles observed in the AFM images (**Fig. 1*C* and Fig. 2 *E* and F**) should be regarded as individual molecules instead of clusters of multiple molecules. As shown in **Fig. 2 *C*-*F***, the ivy nanoparticles purified via SEC-HPLC were further fractionated by RP-HPLC and each fraction harvested was characterized by AFM. It was observed that the fraction 4 was composed of spherical nanostructures analogous to the SEC-purified nanoparticles in terms of form and size. Given any structural domains with loosely noncovalent binding should be separated during the gradient elution in RP-HPLC, this result here suggests that all the constituents essential for the construction of the overall architecture of the ivy nanoparticles are covalently connected. In this respect, in light of the information gained from the RP-HPLC analysis, the purified ivy nanoparticles observed in **Fig. 1*C*** should be considered as individual molecules, rather than clusters of multiple molecules. However, in the meanwhile, these individual molecule-assembled nanostructures indeed demonstrate a strong tendency to aggregate, a characteristic trait of typical AGPs as interpreted on Page 13, Lines 6-16. To avoid potential confusion, a brief description of the unimolecular nature of the ivy nanoparticles was included in the revised manuscript on Page 7, Lines 14-17, shown as following:

"Given that structural domains with loosely noncovalent binding should be separated during the gradient elution in RP-HPLC, these data suggest that the purified ivy nanoparticles observed in **Fig. 1*C*** are individual molecules, rather than clusters of multiple molecules."

3. The rheological measurements are deficient. The method used is determining a component of shear yield stress? There is no stress strain data reported to show the rheological behavior. This is critical to the paper. I suggest that "pull off" measurements are performed where the stress is normal to the surface are made. Furthermore, stress strain curves should be reported. These will give an indication of the creep and yield type behavior. The current measurements are made for silica surfaces. This is a good model for the wall however the Ivy is a biological system. The true measurement if possible should be between the Ivy and a silica slide. Furthermore the methods description does not contain enough detail to permit replication.
Response: Thanks for the suggestion. The rheological measurements have been substantially improved by re-conducting the tensile tests as suggested. In this study, we intend to compare the adhesion strengths of different adhesive composites prepared by mimicking the ivy-derived adhesive substances. For such a purpose, the shear strengths and tensile strengths at failure of respective composites were quantitatively evaluated by the lap joint shear strength test and the tensile strength test. The method we used in the adhesive lap joint shear

strength test is according to the ASTM D1002 which has been extensively applied to examine the adhesion strength of numerous bioadhesives in the previous studies (33-39). Per suggestion of the reviewer, all the data obtained from the tensile test were converted to stress-strain curves shown in **Fig. 6 *C* and *F***, and adhesion strength (Pa), instead of adhesion force (N), was used to evaluate respective adhesive composites. In addition, a new tensile test where the stress in normal to the surface was designed and performed as suggested by the reviewer, illustrated in **Fig. 6*E*** and shown as below (**Fig. R3**). For the tensile test proposed by the reviewer between the adventitious roots of English ivy and the silica slides, we also made a try but failed to get repeatable results and thus did not include the data here. The adventitious roots are difficult to be fixed in a quantitative fashion in this mechanical test and most of the time the mechanical strength of the root tissue is markedly weaker than the adhesion strength of the applied adhesive composites, resulting in the break of root tissue during the test. Relevant description of the methods used for the tensile test has been rephrased to show more detail. In addition, due to the length limitation, the majority of the Materials and Methods have been removed to the supporting information, as suggested during the quality check by the journal, designated as ***SI Materials and Methods***. Corresponding changes made in the revised manuscript are shown as following:

"To evaluate the behavior of the prepared adhesive composites, the adhesion strength of this reconstructed adhesive was examined by both lap joint shear strength test and tensile strength test, two strategies that have been extensively applied to quantitatively assess bioadhesives (**Fig. 6*A***) (36, 40-43). The variation in shear strength of the prepared adhesive constructs was monitored over time to reflect the curing progress. As shown in **Fig. 6*B***, in general, the shear strength of the ivy-mimetic composites at failure was elevated with increase in time and reached a plateau of maximum value in approximately three days. To explore the specific roles of each component within the developed material, the shear strengths of the Ca^{2+}-free and EGTA-containing adhesive constructs, as well as those of the sole ivy nanoparticles/pectin incorporated with calcium ions, were also traced throughout the test under the same condition. Similarly, the maximal shear strengths of respective control groups could not be approached until approximately three days. However, significant difference in the shear strength values that could be reached after three days was observed among distinct adhesive composites. On day 7, a shear strength up to 0.53 ± 0.033 MPa was achieved by the ivy-mimetic adhesive construct prepared in the presence of Ca^{2+} and in the absence of EGTA, substantially greater than those of the Ca^{2+}-free or EGTA-containing counterparts, with respective shear strengths of ~ 0.31 ± 0.013 and 0.40 ± 0.010 MPa, indicating the significance of the calcium ions in developing an ivy-mimetic adhesive composite with expected level of performance. Meanwhile, the shear strengths of the composites consisting of 2 mM Ca^{2+} integrated with either ivy nanoparticles or pectin alone were approximately 0.12 ± 0.036 and 0.40 ± 0.017 MPa, respectively, still markedly weaker than that of the ivy-mimetic adhesive constructs 7 days after preparation. The stress-strain curves of respective adhesive composites, as measured and plotted by the lap shear test on day 7, are shown in **Fig. 6*C***. It could be observed that for the adhesive composites comprising both the ivy nanoparticles and pectic polysaccharides, the strains at failure were substantially lower than those of the

constructs containing either ivy nanoparticles or pectic substances alone. In particular, the minimum strain at failure was reached by the ivy-mimetic EGTA-free adhesive composite in the presence of Ca^{2+}.

In addition to the apparent effects of the ionic strength, the influence of pH value on the shear strength of the reconstructed ivy-mimetic adhesive was also evaluated by preparing the adhesive composites at distinct pH. As shown in **Fig. 6*D***, in comparison to that of the adhesive composite prepared under neutral condition, slightly but significantly weaker shear strengths obtained on day 7 were observed in response to the pH variations. In particular, in contrast to the composite developed in TBS at pH 7.6, approximately 1.4- to 1.5-fold lower shear strengths were reached by the cases prepared at pH 4 or 9 after 7 days. Given that the cross-linking extent of the ivy-mimetic adhesive constructs is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here.

To ideally reflect the interfacial behavior of the reconstructed ivy-mimetic adhesive composites, an alternative tensile strength test was performed by applying the prepared adhesive materials to the joint of two clevis pins, as illustrated in **Fig. 6*E***. Similar to the information gained from the lap shear test, the stress-strain curves show that the strain at failure of the reconstructed ivy-mimetic adhesive composite in the presence of Ca^{2+} and in the absence of EGTA is approximately 57% and 37% lower than those of the cases containing 2 mM Ca^{2+} integrated with either ivy nanoparticles or pectic polysaccharides, respectively, on day 7 (**Fig. 6*F***). In addition, the tensile strength of this reconstructed ivy-mimetic adhesive composite was ~ 3.9-, 1.6-, 3.8-, and 1.7-fold stronger than respective adhesive composites consisting of ivy nanoparticles with Ca^{2+}, pectin with Ca^{2+}, ivy nanoparticles with pectin, or ivy nanoparticles with pectin in the presence of both Ca^{2+} and EGTA, 7 days after preparation, as shown in **Fig. 6*G***." (Page 19, Line 6 to Page 21, Line 11)

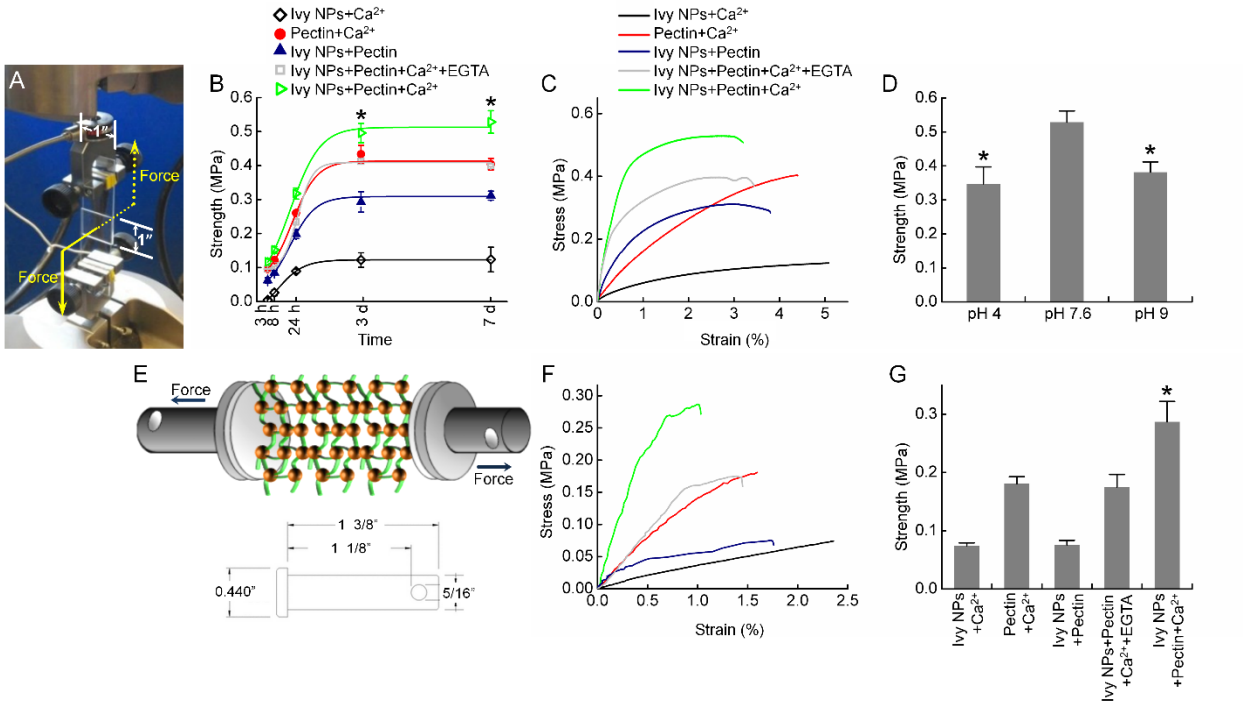


Fig. R3. Tensile test of the reconstructed ivy-mimetic adhesive composites. (***A***) For the adhesive lap joint shear strength test, prepared adhesive composites were applied to a 1" × 1" overlapping area of two glass slides. (***B***) Shear strengths of the ivy-mimetic adhesive composites 3 h, 8 h, 24 h, 3 days, and 7 days after preparation. The shear strengths of the calcium-free and EGTA-containing adhesive constructs, as well as the composites consisting of individual pectin/ivy nanoparticles and Ca^{2+}, were also measured at the same time intervals, respectively. The strength curves were fitted by OriginPro 8.0 using a growth/sigmoidal function ($y = \frac{A2 - A1}{1 + 10^{(LOGx0 - x)\,p}} + A1$). Error bars indicate SD; *$P < 0.05$, versus the shear strengths of the EGTA-free adhesive composites in the presence of 2 mM Ca^{2+}. (***C***) Stress-strain curves of respective composites measured by lap shear test 7 days after preparation. (***D***) Influence of pH value on the shear strengths of the ivy-mimetic adhesive constructs, as measured by lap shear test on day 7. Error bars represent SD; *$P < 0.05$, versus the shear strength of the composites prepared at pH 7.6. (***E***) A schematic drawing of the adhesive tensile strength test. Adhesive composites were applied to the interface of two clevis pins and the tensile strength was examined on an MTS system as shown in ***A***. Bottom, the dimensions of the clevis pin employed in this test. (***F***) Stress-strain curves of respective composites measured by adhesive tensile strength test 7 days after preparation. (***G***) Tensile strengths of respective adhesive composites on day 7. Error bars indicate SD; *$P < 0.05$, versus the tensile strength of the EGTA-free adhesive composites containing 2 mM Ca^{2+}.

"To quantitatively evaluate the performance of the developed adhesive materials at the interface, a lap joint shear strength test and a tensile strength test were employed to examine the adhesion strength of the constructed adhesive composites, according to the standard procedure (ASTM D1002) described in the previous studies with slight modification (36, 40-43). All measurements were conducted on an MTS 831 elastomer testing system (MTS, Eden Prairie, MN) mounted with 45 N, 107 N, or 2 kN load cells. Uniform load was imposed across the bond area at a rate of 1 mm/min and the force needed to pull the two adherends apart was monitored. Values were converted to stress-strain curves. For the lap shear test, microscope glass slides (3" × 1" × 1 mm, Thermo Fisher Scientific, Rockford, IL) were selected as the adherends and cleaned by

pretreating with H_2SO_4/H_2O_2 (7:3, v/v). After rinsing and air-drying, 10 μl of the prepared adhesive composites was applied to a 1" × 1" overlapping area, as illustrated in **Fig. 6*A***. Glass slides were then clamped together by two binder clips with a force of ~ 25 N until the tensile test. The shear strengths of each adhesive composite were determined at a series of time intervals after the preparation, i.e. 3 h, 8 h, 24 h, 3 days, and 7 days. Similarly, a tensile strength test was carried out by applying 5 μl of the adhesive composites to the joint of two clevis pins (McMaster-Carr, Elmhurst, IL), as illustrated in **Fig. 6*E***. The junction was sealed with parafilm for 2 days and the tensile strength test was performed 7 days after the preparation. A minimum of ten samples per group were tested.

Reconstructed ivy-mimetic adhesive composite was prepared by integrating 5 mg/ml ivy nanoparticles, 10 mg/ml pectin extracted from citrus fruit, and 2 mM $CaCl_2$ in TBS, pH 7.6. To evaluate the role of the calcium ions in the generation of strong adhesion strength, the adhesive composite consisting of 10 mg/ml pectin and 5 mg/ml ivy nanoparticles as well as that comprising 10 mg/ml pectin, 5 mg/ml ivy nanoparticles, 2 mM $CaCl_2$, and 1 mM EGTA were also prepared and investigated. In addition, the adhesion strengths of the composites containing 2 mM $CaCl_2$ and either 15 mg/ml ivy nanoparticles or 15 mg/ml pectic polysaccharides were also examined under the same experimental condition for comparison. The influence of pH value on the performance of the ivy-mimetic adhesive constructs was also assessed by determining the shear strengths of respective adhesive composites prepared in (*i*) 0.01 M sodium acetate/acetic acid buffer, pH 4.0, (*ii*) TBS, pH 7.6, and (*iii*) 0.01 M Tris-HCl buffer, pH 9.0." (***SI Materials and Methods*** section: Page 12, Line 20 to Page 14, Line 3)

4. There is no mechanism or model for the action of the AGP in the adhesion presented. The data is therefore not properly analyzed.

Response: Thanks for the reminder. In fact, the calcium-driven electrostatic interactions among uronic acid residues of AGPs and pectic acids are evidenced to be the predominant driving force for the cross-linking of the ivy-derived adhesive during the curing progress. This calcium-dependent event has been validated by several biochemical assays. However, relevant results were poorly interpreted and discussed in the previous manuscript. We are sorry for this confusion. To avoid such confusion and precisely present the molecular mechanisms, the data interpretation as well as relevant discussion have all be rewritten in this revision, shown as below:

"In this respect, the effort in the exploration of the potential interactions among these acidic polysaccharides/glycoproteins may substantially improve our understanding of the molecular events controlling the generation of strong adhesion force within the ivy-derived bioadhesive. For such a purpose, a dot blotting test and a fluorescent combination assay were carried out to evaluate the hypothetical binding between the AGP-rich ivy nanoparticles and the pectic polysaccharides, apart from the aforementioned

covalent conjugation of the AGPs and the pectic fraction within the ivy nanoparticles. To assess the potential interactions using dot blotting, 20-34% esterified commercial pectic acid extracted from citrus fruit was dried down on PVDF membrane and subsequently incubated, after blocking, with the suspension of the purified ivy nanoparticles in the presence of 2 mM Ca^{2+}. The level of the AGP-rich nanoparticles to which pectin bound was assessed by sequential incubation with JIM13 primary antibody and HRP-conjugated secondary antibody. As shown in **Fig. 4*F***, while the AGP-rich ivy nanoparticles indeed demonstrated concentration-dependent interactions with the adsorbed pectic polysaccharides, this binding was susceptible to the addition of external Ca^{2+}-chelating agent, EGTA, which apparently suppressed the affinity of these two components. Additionally, this electrostatic interaction was further quantitatively evaluated using a fluorescent combination assay. Similar to the dot blotting examination, the pectic polysaccharides with a methyl esterification degree of 20-30% were coated onto 96-well plates prior to the blocking, and the Ca^{2+}-containing suspension of the FITC-labeled ivy nanoparticles was subsequently applied to each well. After 1 h incubation, the reaction buffer was removed and replaced with TBS, and the amount of the AGP-rich nanoparticles arrested by the adsorbed pectin was quantitatively determined by capturing the fluorescent intensity using plate reader. As expected, the concentration-dependent interactions between the ivy nanoparticles and the pectic polysaccharides were consistently observed in this assay, as shown in **Fig. 4*G***. In the meanwhile, for the ivy nanoparticles tested at an initial concentration of 5 mg/ml, the amounts of the immobilized ivy nanoparticles detected in the incubation buffers containing 2 mM Ca^{2+} were approximately 2.1- to 2.5-fold and 1.5- to 1.8-fold greater than those of the nanoparticles remnant in Ca^{2+}-free buffers and EGTA-containing buffers, respectively, at pectin concentrations ranging from 1 to 10 mg/ml, suggesting that the calcium ions are capable of promoting the electrostatic binding of the AGP-rich ivy nanoparticles and the pectin. To further validate this Ca^{2+}-dependent interaction, 1 mg/ml ivy nanoparticles were applied to the combination assay under the same condition. As shown in **Fig. 4*G***, in comparison to respective amounts of the ivy nanoparticles attached to the adsorbed pectic polysaccharides either in the absence of calcium ions or in the presence of excess EGTA, at least 2.0- and 1.2-fold greater amounts of the FITC-conjugated nanoparticles were detected to be bound to the pectin in the EGTA-free reaction buffer containing 2 mM Ca^{2+}. In addition, this Ca^{2+}-modulated electrostatic interaction between the AGP-rich ivy nanoparticles and the pectic polysaccharides upon binding was further evidenced by testing the affinity of the AGP-rich ivy nanoparticles toward other electroneutral molecules, including BSA and fully esterified pectin, in the same assay. As shown in **Fig. 4*G***, in contrast to the case containing 2 mM Ca^{2+}, significant difference in the amount of the FITC-conjugated nanoparticles attached after 1 h incubation was not observed in response to either the absence of calcium ions or the presence of external EGTA, indicating that the calcium ion-driven interaction between the AGP-rich ivy nanoparticles and the acidic pectic polysaccharides is the predominant force aiding in their binding. This electrostatic interaction is displayed by calcium ions in facilitating the cross-linking among carboxyl groups of the uronic acid residues within the AGPs and the pectic acids (**Fig. 4*H***). It is noteworthy that this Ca^{2+}-driven event has been frequently discussed in the earlier reports (13, 44, 45), with experimental results consistent with that of the current study.

Meanwhile, given that the Ca^{2+} is one of the richest and physiologically vital ions present in the extracellular space of plant cell (46-48), it is reasonable to conclude here that the Ca^{2+}-regulated cross-linking among these acidic polysaccharides/glycoproteins undoubtedly renders potent driving force, effectively promoting the curing (hardening) progress of the sticky exudate derived from the adventitious roots of English ivy." (Page 15, Line 4 to Page 17, Line 9)

5. The grammar is poor. Generally the paper is not well written. Given the above technical problems with the paper it is not acceptable for publication in PNAS as the research is not well designed and executed.

Response: Thanks for the suggestion. This manuscript has been thoroughly revised and polished by a native English speaker. Several grammatical and syntactic errors, as well as typos, have been corrected. In this revision, the majority of the descriptions regarding the analysis, interpretation and discussion of the experimental data have been reorganized or rewritten to ensure the precise delivery of information. Inaccurate statement and overstated conclusion have all been rephrased. We believe that this manuscript has been substantially improved and we expect the reconsideration of the reviewer. Thanks again for the reviewer's comments.

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Response to the Editor's and the Reviewers' Comments for the Manuscript:

"*Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy***"**

Reference: 2016-00406

Authors: [redacted], Yongzhong Wang, Li Tan, [redacted], Mei-Zhen Cui, Feng Hao, and Mingjun Zhang

Dear Editor and the Reviewers,

We would like to thank you for all your insightful comments while reviewing this manuscript. We greatly appreciate the suggested revisions, which have significantly helped us to improve this manuscript. Please find our point-by-point revisions and responses to each reviewer's comments below. In addition, as you have suggested, the new manuscript has an improved style with sectional sub-headings, has been professionally edited, and has a re-written discussion section that better highlights the significance of our work. Questions and suggestions regarding the figures have also been addressed and can be found below. You will find that all corresponding changes made in response to the comments are marked in blue in the revised manuscript.

Thanks for this opportunity to have our manuscript considered for publication in your journal.

Should you need any additional information, please let us know.

Sincerely yours,

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Response to the Editor's Comments:

Editor's Remarks to Author:

The authors have substantially revised the manuscript, performed additional experiments, and clarified all issues of the first three reviewers.

In summary, the authors: (1) included a phylogenetic tree corroborating the identity of the IAGP (2) performed Mass. spec analyses to confirm the IAGP protein sequence, (3) added a tensile test to confirm the molecular nature of the ivy-derived adhesive, (4) expanded AFM analyses to show that ivy nanoparticles observed in the AFM images are individual molecules rather than aggregates, (5) addressed the issue on hemicellulose impurities, (6) added stress strain data to show the rheological behavior, (7) included more details on methods, (8) removed GPI anchor discussion, (9) improved the model of ivy adhesion, (10), performed English and grammar proofreading.

After re-evaluation, several issues remain to be addressed. The authors are asked to improve style, add sub-headings, and re-write the discussion. Furthermore, the importance of pectin should be emphasized. The significance of the current work should be expanded, especially in the discussion section. Questions and suggestions regarding the figures need to be addressed. Further grammatical editing is requested.

Response: Thank you very much for your comments, suggestions, and opportunity to improve our manuscript by revision. To further improve our work, we have addressed all concerns raised. In particular, the overall manuscript style has been revised. Additional sub-headings were included to improve the flow and the delivery of concepts, results, and conclusions. The discussion section has also been re-written, as shown on Page 20, Line 13 to Page 25, Line 20, and expands topics involving the unique features of the ivy adhesive, the physiological functions of the AGPs and the pectin, and the importance/impact of our data scientifically supporting the "wattle blossom" model. The discussion section also highlights similarities and differences between animal and plant adhesives and the future bioengineering applications for the ivy adhesive. The importance of pectin has been further emphasized, in the abstract (Page 2, Lines 18 to 21), introduction (Page 4, Lines 9 to 13), and discussion section (Page 22, Lines 15 to 23 and Page 20, Line 14 to Page 21, Line 23). All the concerns raised about figures and figure legends have been addressed as detailed below. As suggested, this manuscript has been polished by a native English speaker and edited by Oxford Editing that is recommended by PNAS. Thanks again for the comments.

Responses to the Reviewers' Comments:

Reviewer #1:

The submission [redacted] details the identification and characterisation of arabinogalactan-like proteins in the adhesive substance of English ivy. Further, the authors develop a mimic with which to test the principles that they argue govern adhesion by the species.

As I stated in my first review, I find the science to be sound and of sufficiently broad interest to be published in PNAS. The findings are significant and also represent an advance in the specific field of research.

1. My comments and requests for improvement of the original manuscript mainly revolved around style and presentation. Unfortunately this has not improved and is, if anything, worse in this more recent version. I think that this is a consequence of adding the extra material requested by review and the re-drafting/re-organising of the text that this required. Therefore, while I find the research interesting, sound and would like to see it published in PNAS, I am unable to recommend publication of the manuscript in its current form. First and foremost the text needs to be thoroughly edited by a native English speaker.

Response: Thank you very much for the helpful comments. As suggested, we have revised the manuscript thoroughly and had the manuscript polished by a native English speaker and edited by a professional writing service (Oxford Editing) recommended by PNAS. In particular, the overall presentation style of this manuscript has been improved by separating the methodological descriptions from the results section, and by placing partial content to the discussion section. Additional sub-headings have been included to divide the results section into more portions, in order to improve the readability of this manuscript.

2. Before this is done, however, there is significant restructuring that must be undertaken. Currently the style of the paper is that of one long chronological narrative that includes methods, results and discussion. The discussion section simply re-states the general concepts as described throughout. In this format I find the paper very difficult to follow and it certainly does not help the reader to identify points of interest. It is my opinion that the authors should sub-divide the paper much more, halving the length of the results section, make more use of sub-headings and move all of the methods out to the methods section. This would improve the clarity, readability and impact of the findings which, in the current format, are somewhat lost.

Response: We fully agree with the comments. To improve the readability of this manuscript, we have re-organized the overall structures. In particular, additional sub-headings have been included in the results section, and the length of the results section has been reduced by moving all of the methodological descriptions into the ***SI Materials and Methods*** section. Considerable content has been removed from the original results section and placed into the discussion section. The discussion section has been rewritten to better communicate the impact of our findings, i.e., (*i*) the uniqueness of the ivy adhesive in contrast to other botanic adhesives (Page 20, Line 14 to Page 21, Line 23), (*ii*) the new insight into the physiological functions of AGPs and pectin (Page 22, Lines 2 to 23), (*iii*) the new evidence provided by this study to support the "wattle blossom" model (Page 23, Lines 2 to 16), (*iv*) the similarities between the ivy adhesive and the animal adhesives (Page 23, Line 18 to Page 24, Line 9), and (*v*) the bioinspired engineering applications of the findings (Page 24, Line 11 to Page 25, Line 12). Thanks again for the reviewer's comments.

Reviewer #2:

This paper reports on the active component of English Ivy as the active adhesive component used by these plants. The paper is of general interest to the scientific community and the readership of PNAS. The revised version has been significantly improved in accord with the comments of all reviewers. I support publication in PNAS in the current form.

Response: Thank you very much for your comments and support for the publication of our manuscript.

Reviewer #3:

The authors isolated and characterized nanoparticles found in ivy-derived adhesive. They identified a component of the nanoparticles as being an arabinogalactan protein. They demonstrated that the nanoparticles were found in close proximity to pectin and interacted in a calcium-dependent manner. Bulk adhesion testing was done to demonstrate that all three components - nanoparticles, pectin, and calcium - resulted in a stronger adhesive bond than individual components. Overall, the experiments are interesting and performed well, but there are items that should be addressed before publication:

1. Overall, the abstract and introduction (and the results discussing Figures 1-3) seem to emphasize the importance of the arabinogalactan protein (AGP) nanoparticles in ivy adhesive. The importance of pectin is not emphasized strongly in the beginning part of the manuscript. However, it seems that pectin is at least equally (if not more) important to the adhesive as the nanoparticles are - in Figure 6, pectin by itself has higher bulk adhesion strength than nanoparticles by themselves. Thus, it is unclear why there is such an emphasis on AGP and not on pectin. Is it because that pectin was already known to be an important component and that this paper is characterizing the second component? Or is it because the AGPs are less viscous and thus can penetrate the substrates more and provide mechanical interlocking (although if this is the case, more experiments need to be performed to show this phenomenon). More context would be helpful.

Response: Thank you very much for the helpful suggestion. We recognize that pectic polysaccharides have a significant role in the generation of strong adhesion strength within the ivy-derived bioadhesive. Both pectin and AGPs are molecules that are classically known to be adhesive substances exuded by climbing plants (1-4). However, since the presence of bulk spherical nanoparticles is the most unique feature that distinguishes the ivy adhesive from other botanic adhesives and they are identified to be predominantly composed of AGPs in the current study, we feel that the nanomorphological effect arising from these spherical architectures needs to be comprehensively elaborated. We would like to define that as the uniqueness and novelty of this paper. It was never our intention to disregard the essential role of the pectin within the ivy adhesive. To avoid understating the contribution of pectin, the revised manuscript now highlights the synergetic effect between pectin and AGP-rich nanoparticles. In particular, the importance of the pectic polysaccharides in the plant-derived bioadhesives has been described in the abstract and introduction section, shown as following:

"Given that AGPs and pectic polysaccharides are also observed in bioadhesives exuded by other climbing plants, the adhesion mechanisms revealed by English ivy may forward the progress toward understanding the general principles underlying diverse botanic adhesives." (Abstract: Page 2, Lines 18 to 21)

"Diverse polysaccharides and glycoproteins, comprising mucilaginous pectins, arabinogalactans, arabinogalactan proteins (AGPs), and many others, have been identified to be the predominant components in these adhesive substances (1-4); however, the molecular mechanisms underlying the high-strength adhesion remain elusive." (Introduction section: Page 4, Lines 9 to 13)

In the meantime, we emphasize the functions of the AGP-rich nanostructures since they drive a favorable surface wetting of the ivy adhesive, aiding in the generation of strong adhesion strength eventually. The viscosities of the AGP-rich nanoparticles and the individual pectic polysaccharides have been examined and compared as shown in **Fig. 4*A***. As expected, apparently lower intrinsic viscosity was observed in the AGP-rich nanoparticles as compared to that of the pectin and other typical polysaccharides (**Fig. 4*A***). According to the basic principles of adhesion, lower viscosity promotes a preferable wetting behavior (5-9). In this respect, the AGP-rich ivy nanoparticles are prone to penetrating the irregularities on the substrates and spreading over the surface, acting as mobile phase in the ivy-derived adhesive (**Fig. R1**).

The conclusion is consistent with the previous study regarding the bioadhesive derived from the Virginia creeper, claiming that "AGPs may be produced de novo and serve as a highly mobile phase of the adhesive, penetrating areas of the substrate too small for the pad to penetrate" (1). Meanwhile, Kevin C. Vaughn *et al.* have described that in contrast to pectin, "arabinans and AGPs appear to be an even more mobile component of the adhesive, filling in spaces between the papillate epidermal cells and even moving into small cracks in the structure that is attached" (10). Additionally, it has reached an agreement that the substances are capable of promoting mechanical interlocking in an adhesive if they exhibit sufficient capacity to penetrate substrates upon the curing given that considerable intra- and inter-molecular actions, including but not limited to van der Waals forces and frictions, arise during this process (6, 11-13). Accordingly, our argumentation about the mechanical interlocking favored by the penetration of AGP-rich ivy nanoparticles is logical and evidenced.

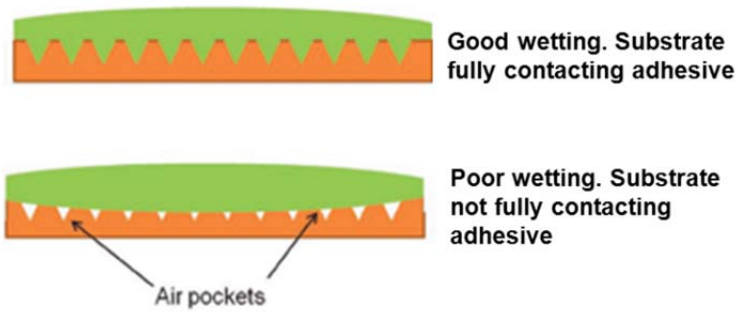

Fig. R1. A schematic drawing of the difference in surface wetting realized by adhesives with distinct viscosities. Better wetting allows the adhesive substances to penetrate the substrate and spread over the surface. Image is cited from (14), with slight modification.

2. There are some allusions to other botanic adhesives that have been studied (page 6, lines 778-785). The text states that "arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and Ficus pumila." Given that arabinogalactans and pectin are found to be the major components of the English ivy adhesive studied in this paper, it would be helpful to expand on the significance of the current work in the abstract, intro, and, especially, the discussion section. How are the findings significant and different from papers published about other plant adhesives? Are the results in this paper already known for other botanic adhesives or is there some additional insight provided here or something that is unique and compelling about the English ivy adhesive?

Response: Thank you for the suggestion. The significance of the current work, especially the contributions and breakthroughs in contrast to the previous studies regarding botanic adhesives, as well as the uniqueness of the ivy-derived bioadhesive, has been expanded on in the abstract, introduction, and discussion sections shown as below:

"Given that AGPs and pectic polysaccharides are also observed in bioadhesives exuded by other climbing plants, the adhesion mechanisms revealed by English ivy may forward the progress toward understanding the general principles underlying diverse botanic adhesives." (Abstract: Page 2, Lines 18 to 21)

"Diverse polysaccharides and glycoproteins, comprising mucilaginous pectins, arabinogalactans, arabinogalactan proteins (AGPs), and many others, have been identified to be the predominant components in these adhesive substances (1-4); however, the molecular mechanisms underlying the high-strength adhesion remain elusive." (Introduction section: Page 4, Lines 9 to 13)

"*The uniqueness of the ivy adhesive in contrast to other botanic adhesives.*
One of the early efforts in exploring the behaviors of bioadhesives derived from climbing plants was made by Charles Darwin who described and documented the process of the "viscid fluid" exuded by the adventitious roots of *Ficus repens* (15). Since then, unfortunately, few studies have dealt with the molecular events within the botanic adhesives, and accordingly, little is known about the adhesion mechanisms underlying these mucilages. In recent years, the chemical components of several types of botanic adhesives produced by Virginia creeper (Parthenocissus quinquefolia), Boston ivy, and *Ficus pumila* have been examined by immunocytochemical identification and cytochemical stains (1-4, 16). Consistently, acidic polysaccharides and glycoproteins, involving pectic acids, arabinogalactans, and AGPs, are recognized as the predominant constituents of these adhesive substances. As a result, production of a pectic mucilage that sets and adheres the vines to the support surfaces is thought be involved in the clinging of most climbing plants (1). Additionally,

arabinogalactans and AGPs are hypothesized to be the highly mobile phase within the botanic adhesives, capable of penetrating the interstices of the substrates and facilitating the sealing of the adhesives into numerous tiny holes on the surface of the support substrates (1, 10). Kevin C. Vaughn *et al.* have appropriately described the pectins and the AGPs in the botanic adhesive as "mucilaginous molecules that are spread across the surface of the structure to be attached, filling in the gaps" (10). In particular, "arabinans and AGPs appear to be an even more mobile component of the adhesive, filling in spaces between the papillate epidermal cells and even moving into small cracks in the structure that is attached" (10). In this study, pectic polysaccharides and AGPs are also identified to be the predominant components of the mucilage exuded by the adventitious roots of English ivy and the AGPs are substantiated to be present in a nano-sized spherical appearance within the sticky liquid. These spherical architectures are the most unique feature that distinguishes the ivy adhesive from other botanic mucilages. The spherical shape results in the low intrinsic viscosity of the AGP molecules and thus allows a favorable surface wetting of bioadhesive over the support surfaces. In this respect, this study provides the first experimental evidence to support the hypothetical theory that the AGPs serve as a mobile phase in the adhesive exudates. More importantly, the molecular interactions within the ivy-derived adhesive were investigated in detail and calcium-dependent electrostatic binding between uronic acid residues on the pectic substances and the AGPs is evidenced to be the driving force for the effective cross-linking (curing) of the mucilage. Analogous or identical molecular events might be present in other botanic adhesives owing to their similar functions and chemical constituents." (Discussion section: Page 20, Line 14 to Page 21, Line 23)

3. Figure 2: Panel D has a lane labeled as being from Fraction 1, but the legend only acknowledges Fractions 2-5. The text is also unclear - it states that "apart from the solvent peak designated as fraction 1," which could imply that Fraction 1 was not run on the gel. Also, one gel lane is labeled "Marker", but the legend refers to it as "Lane M."

Response: Thank you for catching this error. Yes, in the **Fig. 2*D***, the second lane of the gel is an empty lane and mistakenly marked as "Fraction 1". We have deleted this confusing label and replaced it with "Empty". The peak 1 shown in the RP-HPLC profile (**Fig. 2*C***) was a solvent signal and no component was obtained after freeze-drying. However, in order to ensure that the gel information is consistent with that of the chromatography profile, an empty lane was included prior to the fraction 2, corresponding to the solvent peak designated as fraction 1. In the meantime, the first lane of the gel has been re-labeled as "M" instead of "Marker" in this revision (**Fig. 2*D***). The **Fig. 2*A*** was also corrected accordingly.

4. Figure 3: For panel C, the figure legend states "Amino acids that are proposed to play adhesive function, comprising Ile, Leu, and Val, are indicated by black triangles." This statement is confusing as this reviewer interpreted the resulting experiments as showing that calcium mediated adhesive interactions between negatively charged residues on AGPs and pectin. Thus, it is not clear how Ile, Leu,

and Val are involved in adhesion. Perhaps they are involved in adhesion to the substrates, but no data are shown to support the role of these amino acids in adhesion.

Response: We agree. Experimental evidence supports the conclusion that the curing of the ivy-derived adhesive is driven by the calcium-dependent interactions between the uronic acid residues on AGPs and pectic polysaccharides. We claimed the adhesive function of several amino acids since they are commonly regarded as conserved residues involved in multiple cell adhesion events (17, 18). However, as indicated by the reviewer, given that there is no direct evidence to validate their contribution to the adhesive activities of English ivy, we decided to remove relevant claim and discussion to avoid potential confusions. Accordingly, the black triangles indicating the positions of these amino acids in **Fig. 3*C*** were all deleted and corresponding description in figure legends was also removed.

5. Figure 4B: This reviewer could not find the text description in the results section for the EDX data.

Response: Thank you for finding this error. A brief description of the EDX analysis was included in the results section, shown as below:

"Meanwhile, energy-dispersive X-ray (EDX) spectra showed that apart from a silicon signal derived from the substrate, the vast majority of the surface elements were carbon and oxygen, suggesting the organic nature of these nanostructures (**Fig. 4*B*, bottom right panel**)." (Page 13, Lines 6 to 9)

6. Figure 5: The second schematic (after evaporation) is confusing. Is pectin supposed to be the gray area that surrounds the yellow spheres? If so, the schematic only appears to be showing that calcium interacts directly with pectin (the gray portion) and not the AGP particles.

Response: We apologize for the confusion. Yes, pectin should be the gray area surrounding the yellow spheres that represent the exuded nanoparticles and the calcium-driven interactions should be present between pectin and pectin, as well as pectin and AGP-rich nanoparticles, given that uronic acids are present in both pectin and AGPs. We have modified the schematic to eliminate the potential confusion, as shown in **Fig. 5** and **Fig. R2**.

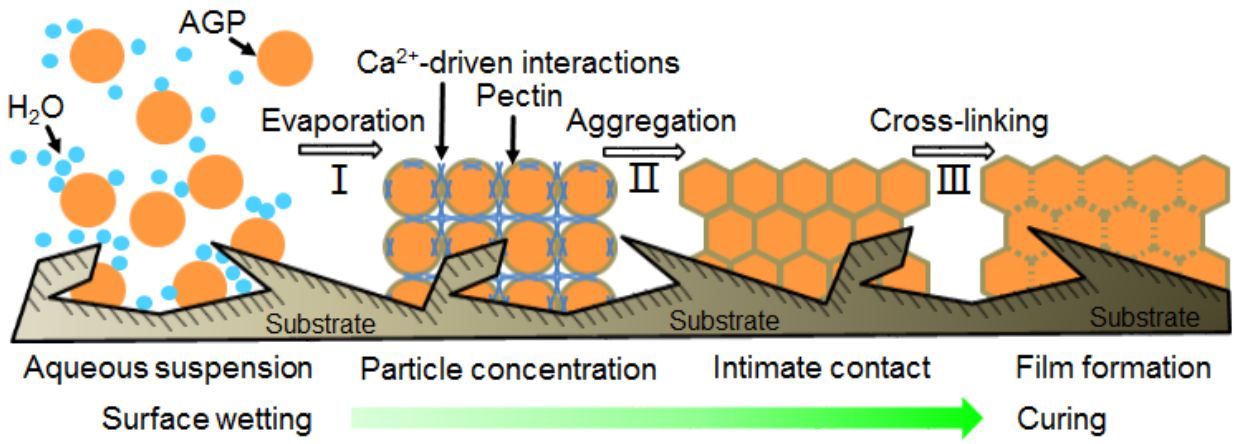

Fig. R2. A schematic drawing of the molecular basis for the ivy-derived adhesive. Adhesive substances are exuded toward the extracellular spaces of root cells, containing AGP-rich nanoparticles, in a manner that has been documented earlier (19). The spheroidal shape of these nanoparticles allows them liable to spread at the interface and permeate the substrates, as a result of their low intrinsic viscosity. Upon evaporation, nanoparticles are concentrated and packed to reach tight contact, giving rise to the formation of a film. Further calcium-dependent cross-linking among carboxyl groups of the AGPs and the pectic polysaccharides elevates the cohesive strength of this film, and the intimate contact of the nanoparticles with the corresponding substrates causes an effective mechanical interlocking at the interface.

7. Figure 6D: The text (page 8, lines 984-993) would be stronger if it explained why the change in pH would affect adhesion. The text states "given that the cross-linking extent...is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here." However, rather than saying it should vary, it would be useful to explain why it is expected to vary at pH 4 and 9. Are we near the pKa values? Are residues no longer negatively charged at one or both of those values?

Response: Thank you for the suggestion. A detailed interpretation regarding the influence of pH values on the adhesion strength during the tensile test has been included in this revision, shown as following:

"Since the p*K*a of pectin is approximately 3.5 (20, 21), and the isoelectric point of the AGP-rich ivy nanoparticles should be located in the acidic range given their negative charge displayed under neutral condition (**Fig. 1*E***), the deprotonation of the carboxyl groups of the uronic acid residues within both components is suppressed at pH 4 in contrast to that in a neutral environment, resulting in less surface charge and thus weaker electrostatic interactions with calcium ions. On the contrary, Ca^{2+} is prone to form slightly soluble $Ca(OH)_2$ under an alkaline condition (pH = 9), also undermining the electrostatic binding. In both cases, the cross-linking degree of the adhesive composites is affected upon the curing process, giving rise to lower adhesion strengths than that of the adhesive material prepared at pH 7.6." (Page 19, Lines 14 to 22)

8. Page 7, lines 905-910: The text hypotheses that the nanoparticles allow for mechanical interlocking. However, are there no chemical adhesive forces (e.g., covalent bonds, van der Waals forces, etc) that are expected to occur?

Response: Thank you for the comments. Yes, adhesion at the interface is a comprehensive process. Several chemical and mechanical forces may be involved in such process, aiding in the generation of strong adhesion strength, such as chemical bonding, electrostatic interactions, adsorption, mechanical interlocking, molecular interactions, van der Waals interactions, self/inter-diffusion, and many others (13, 22, 23). In this study, we explored the adhesion mechanisms underlying the mucilage exuded by English ivy, especially the unique roles of the AGP-rich spherical nanoparticles within the adhesive exudate. We have evidenced that calcium-dependent electrostatic interaction among uronic acids within the AGPs and the pectic polysaccharides is the predominant driving force for the curing of the ivy-derived bioadhesive. This electrostatic interaction drives the cross-linking of the adhesive substances, enhances the cohesive strength, and thus favors the generation of high-strength surface adhesion. The strategy of mechanical interlocking is also employed by the ivy-derived nanoparticles during the curing process, given that they can penetrate the rough surfaces readily, a behavior similar to that of the engineered synthetic nanoparticles contained in the poly(vinyl acetate) adhesive (5, 6, 24-26). In addition to the electrostatic interactions and mechanical interlocking, van der Waals force is also substantiated to be involved in the adhesion process, favoring the formation of strong adhesion strength, in our previous study (27). In the meanwhile, even though direct evidence to support the covalent cross-linking between the adjacent AGP-rich ivy nanoparticles was not observed through our experiments, the AGPs indeed possess the capacity to bind one another via oxidative cross-linking in some cases, rigidifying the cell wall (28-30). In this respect, it seems logical to hypothesize that an analogous cross-linking pattern might be present between the AGP-rich nanostructures. In fact, we have tried H_2O_2 and peroxidase to trigger and catalyze this potential reaction, according to the procedures described in the previous study (30), whereas similar result was not observed in the AGP-rich nanoparticles. However, the possibility of oxidative cross-linking might still exist during the curing of the ivy-derived adhesive, given that it is still unclear if other oxidants instead of H_2O_2 are present in such mucilage. This may be worth further exploration in the future studies. Accordingly, relevant discussion regarding the contribution of van der Waals interactions to the high-strength adhesion was included, shown as following:

"In addition to the calcium-dependent electrostatic interactions and mechanical interlocking, the van der Waals force is also evidenced to be involved in the curing process, as interpreted in our previous study (27)." (Page 17, Lines 14 to 16)

9. Page 8, line 1035: It is unclear what is meant by "partially reflects the physiological implications of the associated low intrinsic viscosity."

Response: We apologize for the confusion. This sentence has been revised as "elucidates the physiological meaning of the low intrinsic viscosity." (Page 23, Lines 15 to 16)

10. Figure 6: For panel G, the meaning of the asterisk is not clear. The legend says the asterisk is compared to "EGTA-free adhesive composites containing 2 mM Ca2+"; however, the asterisk is placed above the composite group with Ca2+ and no EGTA. Also, it seems like it would make more sense if an ANOVA were performed and Tukey groups were shown so that one could determine which groups were statistically similar or different.

Response: We agree with the comments. A one-way ANOVA was performed and Tukey's post hoc test was applied for multiple comparisons. The Tukey groups are shown in the revised **Fig. 6*G***. Meanwhile, the confusing figure legend has been removed. In addition, a description of the statistical approach applied has been included in the ***SI Materials and Methods*** section accordingly, shown as following:

"Data are expressed as mean ± SD. For two groups' comparison, statistical significance was determined using a two-tailed Student's *t*-test. For more than two group comparisons, one-way ANOVA was applied followed by Tukey's post hoc multiple comparison test. $P < 0.05$ was considered to be statistically significant." (***SI Materials and Methods*** section: Page 14, Lines 14 to 17)

11. Materials and Methods (SI page 2, line 21): It would be helpful to report centrifugation in terms of g and not just rpm.

Response: Thank you for the suggestion. The centrifugal speed has been reported in the form of relative centrifugal force (RCF, g) in this revision, shown as below:

"The extract was centrifuged at 12000 rpm (12396 g, Eppendorf 5418 microcentrifuge, Hauppauge, NY) for 1 min to remove any debris." (***SI Materials and Methods*** section: Page 2, Lines 21 to 23)

12. Figure S5: In the legend, please explain the green dotted line in panels C-F.

Response: Thank you for the suggestion. A detailed explanation of the green dotted lines has been included in the figure legend, shown as following:

"The green dotted lines in ***C-F*** reflect the trend of the adventitious roots in bending parallel to the substrates in order to create more contact area, a phenomenon that has been described previously (19)." (Supporting Information: Page 19, Lines 11 to 13)

13. Figure S7: Given that there is an arrow with the word "agglomeration" connecting the two panels, it is not clear whether the left and right panels are from the same sample that have agglomerated over time. Or, are they from different samples or different areas of the same sample?

Response: Thank you for pointing this out. These two images are from different samples. We included these two images here to show the difference in appearance of the nanostructures within the exudates at distinct stages during curing. The ideal way to monitor this process is to trace the change of the same sample over time; however, the technical difficulty in real-time tracking by SEM compelled us to use an alternative approach. As shown in **Fig. S7, left panel**, individual nanoparticles and clusters generated by the aggregation of nanoparticles are both present, implying the tendency to form bulky pads and further adhesive film at the interface (**Fig. S7, right panel**). A brief description of the samples used in the SEM observation has been included in the figure legend to avoid potential confusion, shown as below:

"Two images were captured from different samples, demonstrating the morphological differences of the nanostructures within the exudates at distinct stages during curing." (Supporting Information: Page 21, Lines 4 to 6)

14. Figure S8: Given that two different secondary antibodies were used in the ELISA, is it valid to show results from all of the ELISAs on the same graph? In other words, are the absorbance values from ELISA wells using different secondary antibodies comparable? Were standard curves performed to show that the absorbance values would equate to the same amount?

Response: Thank you for the comments. Here the ELISA screening test was carried out to qualitatively identify the presence of potential glycans within an unknown substance, instead of precisely determining the amounts of respective candidates. This method has been well established and extensively applied for screening glycans in the previous studies (31-37). Pilot experiments have ensured that the absorbance values of the ELISA panel screening are comparable (37, 38). During the ELISA screening test, even though two different secondary antibodies were applied (either goat anti-mouse IgG [Sigma A4416] or goat anti-rat IgG [Sigma A9037]), depending on the primary antibodies used (mouse for CCRC series and rat for JIM series), horseradish peroxidase is conjugated with both secondary antibodies. The same TMB peroxidase substrate kit SK-4400 (Vector Laboratories, Inc.) and the identical dilutions 1:5000 (for secondary antibodies) allow this

methodology to be reliable. A brief description of the experimental detail has been included to eliminate potential concern regarding this methodology, shown as following:

"The ELISA screening analysis was performed according to the method detailed in the earlier study (37). Specifically, 10 ng/μl residual substance recovered from the ivy imprints on the silicon wafers was applied to a 96-well plate at 50 μl per well, which was then dried overnight at 37 °C. 38 types of mAbs, which recognize the glycan epitopes of non-fucosylated xyloglucan, xylan, HG backbone, RG-I backbone, RG-I, RG-I/AG, and AG, respectively, as listed in **Table S1**, were used as primary antibodies. Horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (for CCRC mAbs series) or horseradish peroxidase-conjugated goat anti-rat IgG antibodies (for JIM mAbs series) (Sigma-Aldrich, St. Louis, MO), depending on the primary mAbs used, was diluted 1:5000 in TBS, and 50 μl was applied to each well and incubated for 1 h. Wells were then rinsed five times with 300 μl of TBS. 50 μl of freshly prepared 3, 3', 5, 5'-tetramethylbenzidine substrate solution (Vector Laboratories, Inc., Burlingame, CA) was added to each well according to the manufacturer's instructions. The reaction was stopped after 20 min, via the addition of 50 μl of 0.5 N sulfuric acid to each well. The absorbance of each well was read and recorded at a wavelength of either 450 nm or 655 nm using a model 680 microplate reader (Bio-Rad, Hercules, CA)." (***SI Materials and Methods*** section: Page 12, Line 10 to Page 13, Line 2)

15. The still image derived from movie S1 and the latter portion of movie S1 (that shows that ivy is stuck to a surface) do not appear to be in focus.

Response: Thank you for pointing this out. In this revision, the still image and the **Movie S1** have been replaced with clear ones that are in focus, to better display the interfacial peeling and the adhesive substances remaining on the substrate.

16. In the response to reviewers' comments, point 1.1 says that the nanostructures are "individual molecules consisting of covalently bonded AGP and pectin domain." What is meant by pectic domain? Is this a domain in the AGP that binds pectin? Or do you mean that the molecules contain AGP and short fragments of pectin (in which case they would not be individual molecules)?

Response: We apologize for the confusion. It should be defined as fragments of pectin rather than pectic domain. As interpreted in the previous response letter, the individual nanoparticles should possess two portions, i.e., AGPs and covalently bonded pectic glycans (short fragments), as evidenced by monosaccharide composition analyses (**Table 1**) and NMR spectra (**Fig. S3**). Even though these two molecules are commonly regarded as distinct cell wall components, they have been identified to be covalently linked in the arabinoxylan pectin arabinogalactan protein 1 (APAP1) and the RG-I-enriched fraction named Ara101P, which are both

reported as individual molecules in the recent studies (39). In this respect, the covalently conjugated proteoglycans-assembled individual nanoparticles should be considered as individual molecules as well. The chemical structure of the individual nanoparticles is homogeneous. To avoid potential confusion, we used the term “short fragments of pectin” instead of “pectic domain” in this revision.

17. Further grammatical editing of the paper would be helpful. For example, on page 4, there are multiple instances of "faction 4" instead of "fraction 4." There are other examples of minor grammatical or typographical errors in the manuscript.

Response: Thank you for the suggestion. This revision has been thoroughly polished by a professional writing service (Oxford Editing) recommended by PNAS. The typo “faction 4” mentioned above has been corrected and we have made extra effort to clean up the rest of the manuscript. Thanks again for the reviewer’s comments.

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Response to the Editor's and the Reviewers' Comments for the Manuscript:

"*Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy***"**

Reference: 2016-00406R

Authors: [redacted], Yongzhong Wang, Li Tan, [redacted], Mei-Zhen Cui, Feng Hao, and Mingjun Zhang

Dear Editors and the Reviewers,

We would like to thank you all very much for your insightful comments. We greatly appreciate the suggested revisions, which have significantly helped us to improve this manuscript. Please find our point-by-point revisions and responses to your comments below.

In particular, the title has been amended to be descriptive without a colon, i.e., "***Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy.***" An amplified **Fig. 4** with a high resolution has been included in the manuscript to ensure that relevant information can easily be extracted and captured by the readers.

Thank you for this opportunity to have our manuscript considered for publication in your journal.

Should you need any additional information, please let us know.

Sincerely yours,

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Response to the Editorial Office's Comments:

We also require that you amend your title. We are seeking a descriptive title without the use of an em/en dash or colon (i.e. a single declarative title). This is non-negotiable and an exception will not be made.

Response: Thank you very much for the suggestion. The title has been amended as "***Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy.***"

Response to the Editor's Comments:

Editor Comments:

We thank the authors for their efforts to improve the manuscript. The manuscript mertis publication in PNAS. We kindly ask to provide a high resolution image of Fig. 4.

Response: Thank you very much for your comment, suggestion, and the opportunity to publish our manuscript. A high-resolution image of **Fig. 4** has been uploaded with this revision. The size of **Fig. 4** has also been enlarged to ensure that the contained information can be clearly conveyed. Due to the length limitation of articles for PNAS Plus, we have moved the original **Table 1** and **Table 2** to the Supporting Information, designated as **Table S1** and **Table S3**, respectively. The original **Table S1** has been renamed as **Table S2**, accordingly. The above format changes will not cause any changes in scientific contents, but will significantly increase the readability of the manuscript.

Responses to the Reviewers' Comments:

Reviewer #1:

I find this revised manuscript to be a significant improvement on the former and in my opinion can be published in its current form. All amendments requested by previous reviews appear to have been included.

Response: Thank you very much for your comment and support for the publication of our manuscript.

Reviewer #3:

The authors have substantially revised and edited their paper in response to the reviewers' comments. The writing more clearly conveys the context of their work and is suitable for the general audience that reads PNAS. I support acceptance by PNAS for publication. I have only one minor comment that may be addressed in typesetting - Figure 4 has many important panels, and at its current size, it is difficult to see and interpret all of the data. I

was able to adequately see the data when enlarging the figure to >300% of its size and want to ensure that the resolution is maintained in the final published form so that others can enlarge the figure without it becoming pixelated. Alternatively, a larger version of the figure could be placed in the supplementary material.

Response: Thank you very much for your comment and support for the publication of our manuscript. Per your advice, we have enlarged the size of **Fig. 4** during typesetting and uploaded a high-resolution image for this revision.

From:	Yucel, Jennifer
To:	Yucel, Jennifer
Subject:	CONFIDENTIAL -- FW: confidential complaint about scientific misconduct
Date:	Thursday, August 25, 2016 7:49:43 AM
Attachments:	Misconduct complaint June 2016.pdf ATT00001.htm

From: "Kavanagh, Etta" <EKavanagh@nas.edu>
Date: July 15, 2016 at 5:21:57 PM EDT
To: "'hart.322@osu.edu'" <hart.322@osu.edu>
Subject: FW: confidential complaint about scientific misconduct

Dear Dr. Hart,

I am contacting you regarding the complaint PNAS received regarding the paper "Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy" [redacted]. We shared the complaint with the editor, Peter Ladurner, and he provided the following comments:

"I want to ensure my support for PNAS regarding manuscript 2016-00406RR.

The question if the gene identified is indeed the ivy AGP cought my attention after the initial submition. Please note that I questioned this finding myself. For their first revision I demanded that the authors have to add a phylogenetic tree showing the true AGP relationship of their protein. In their revision the authors provided the respective tree (and mass spec data) corroborating their finding.

I want to state that such data - under normal circumstances and if the data were generated according to best scientific practice - are sufficient to support the authors statement that their protein is an AGP.

However, if the genes for generating the phylogenetic tree were highly hand picked (and not selected according to their statement in the paper: "Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 were the closest relatives, as shown in Fig. S4A.") any tree can be fabricated.
I hope this is not true.

The next steps require detailed sequence analyses using BLAST, reciprocal BLAST, thourough protein alignments and phylogenetic analyses of the submitted sequence.

Depending on the result the authors need to provide raw data and lab book level information on gene isolation, details on clones with gene inserts from PCR and RACE experiments, sequencing raw files, details of their BLAST search settings and databases, information on the selection and generation of alignments and the phylogenetic tree, Mass Spectrometry raw data, details on GPI anchor bioinformatics.

Please let me know if I can help with the sequence analyses."

Is Ohio State University investigating these concerns? Should I contact the research integrity office? Thank you very much for your help.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Dear Sir/Madam,

I am writing to request retraction of the paper “Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy” [redacted]. published in PNAS in May 2016.

www.pnas.org/cgi/doi/10.1073/pnas.1600406113

The corresponding author is Dr Mingjun Zhang, Department of Biomedical Engineering, College of Engineering, The Ohio State University, Columbus, OH 43210 USA.

The allegations specifically are:

The authors have knowlingly, intentionally, repeatedly, and substantially misrepresented data in order to publish the manuscript.

This serious misrepresentation has misled the scientific community. Such inappropriate behaviour has serious negative implications for the integrity of the scientific community, the careers of honest researchers, and the use of public funds.

I believe the seriousness of this misrepresentation calls into question the integrity and validity of the entire work, and thus retraction rather than correction is appropriate.

I further request an investigation into the scientific conduct of the authors, to be carried out perhaps by Ohio State University.

Evidence:

1. Misrepresentation: The data in PNAS paper figure 3 claims to show the sequence of an Ivy protein, that is glycosylated to be an AGP. There is an alignment with some Arabidopsis proteins, claiming to show moderate similarity. PNAS paper Figure S4 shows a cladogram on the basis of alignment to Arabidopsis proteins. However, the protein is in fact a fungal cytochrome oxidase subunit. The sequence when aligned to all proteins, not just Arabidopsis, shows up to 90% identity to fungal mitochondrial cytochrome oxidase subunits. Please see figure 1 below. By showing in this manuscript selective alignment only against Arabidopsis plant proteins, the authors have intentionally concealed this identity. This high sequency identity is so clear, that any responsible biologist will agree that there is no doubt about this misrepresentation. It is inconceivable that the authors responsible for the figure preparation were unaware of the issue. The mispresentation is serious, in that the data for one of the main figures in the paper is not what it claims to be. The authors have not identified an Ivy arabinogalactan protein, which is the topic of the paper.

2. The authors have knowingly, intentionally and repeatedly, misrepresented the data in their manuscript submissions. I reviewed the manuscript in July 2015, when it was apparently re-submitted to Nature Communications (manuscript NCOMMS-14-00999A-Z). I found serious errors in the manuscript at that time. It appeared to me that the authors made falsified claims in that submission, but at that stage I could not rule out incompetence as an alternative explanation. Please see my brief review of the figure 3 in that submission: Appendix 1 below. I pointed out to them at that time that the protein is a fungal cytochome oxidase subunit. The authors received the review: I have now contacted Nature communications, and the editor confirmed the authors received the review. Second, they have altered the text of the article by discussing the cytochrome oxidase similarity, but attempting to conceal the truth by alignments only to plant proteins. There were additional problems with that submission, which appear also to be falsified claims. Specifically, they claimed that Big-PI predicts a GPI anchor site for this protein. I used the same predictor with their sequence, and found no such prediction. It is not possible to make an honest mistake about such a claim. They dropped this claim from the PNAS submission after my review. Appendix 2 shows that the point about low likelihood this is an AGP was raised by an earlier referee, so the authors have been fully aware of the issue for many months.

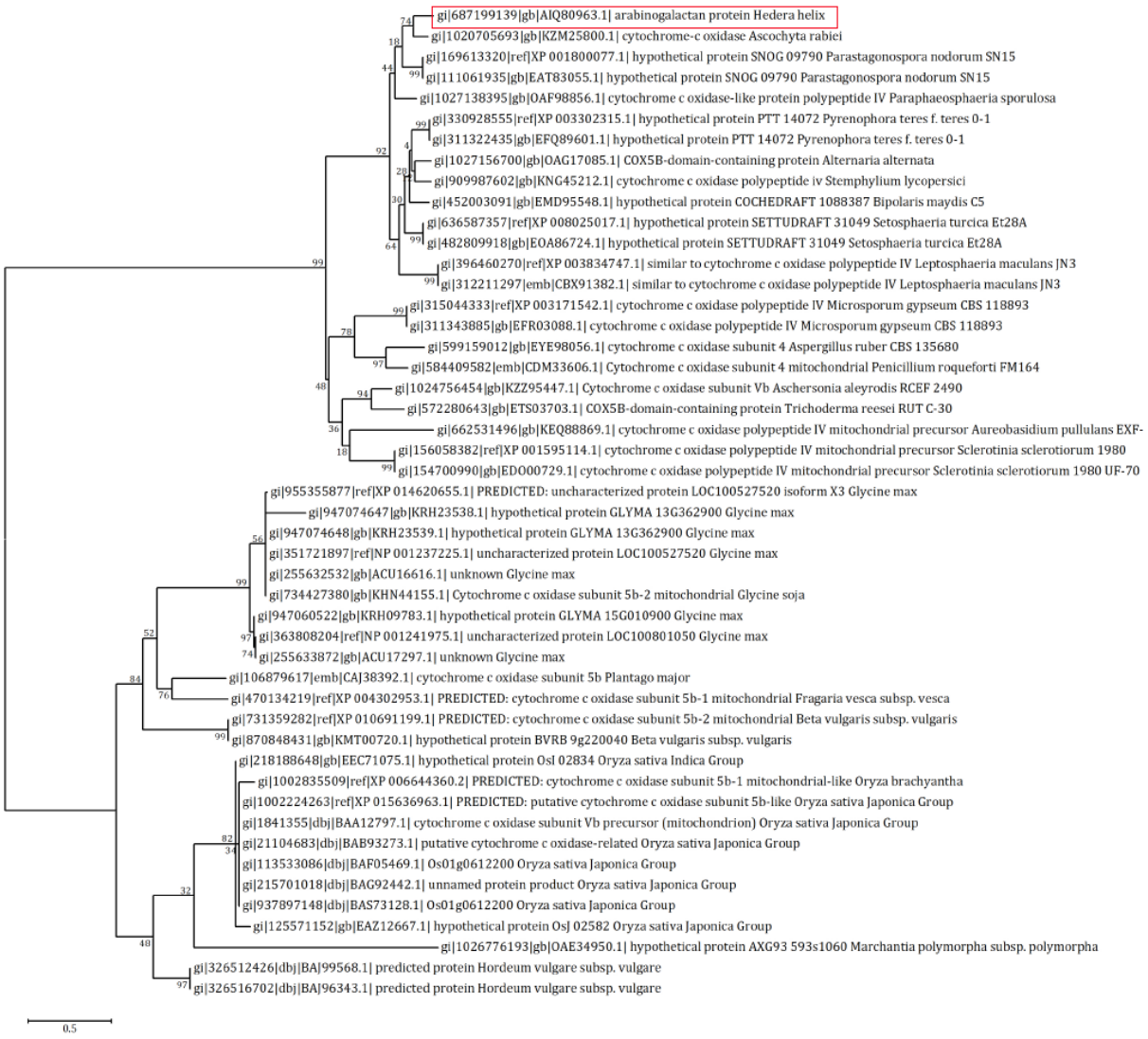

Figure 1. A phylogenetic tree using alignments against all proteins in the databases, rather than selectively Arabidopsis plants. The ‘ivy AGP’ is clearly a fungal protein.

Appendix 1:

My Nature communications review July 2015

This manuscript describes an intriguing hypothesis that a calcium dependent interaction between arabinogalactan and pectin provides a mechanism for adhesion of ivy.

If true, this would be of considerable interest, given the poor understanding of arabinogalactan mechanism and function, and also given interest in plant adhesives and cell adhesion.

Unfortunately, as I studied the experiments in this paper to understand fully the evidence, it became clear to me that many of them are very poorly conducted, and very much over interpreted. The experiments are sometimes not as they are described, they are redundant, or they are uninformative. I have therefore not reviewed all aspects of the manuscript.

A few examples will suffice.

Figure 3.

1.

The title: "**Figure 3: Chemical analyses of the ivy nanoparticles."** Does not describe the content, which is nucleotide and protein sequence.

2.
P7, line 12: "a full-length cDNA was amplified and cloned (**Fig. 3a**)."
However, the sequence in Fig3a is genomic sequence, as it contains an intron, as described in the legend. Why show the genomic sequence?

3.
P7, line 13 "The protein sequence was designated as IVY ARABINOGALACTAN PROTEIN (IAGP) and was also aligned to other HRGPs in *Arabidopsis*, demonstrating a moderate sequence similarity (**Fig. 3b**).

There are no motifs in this sequence that suggest arabinogalactan addition- such as APAP- non-contiguous proline. Contiguous hydroxyproline such as OOO at the N-terminus is suggestive of extensin arabinosylation only at the N terminus.

4.
The model in Fig. 3c is entirely fanciful and does not reflect any evidence presented for glycosylation in the manner shown. There is no evidence for some of the prolines becoming hydroxylated, or the position of any of the TypeII AG additions.

5.
A GPI anchor signal is suggested and the omega (w) site shown. GPI-anchor addition requires a short hydrophobic sequence. The C-terminus of this protein has many charged residues and cannot be such a signal.

6.

In the Methods it is stated that GPI-prediction was done by big-PI plant predictor. I put this sequence into the predictor. Outcome: No GPI anchor predicted. How is it then that this is presented in the figure?

7.
The alignment in Fig3b is shown to four Arabidopsis AGPs. The names of these should be shown in conventional format, such as AtAGP19. F13M7.8 is the name of a BAC ORF and precedes completion of the genome sequence in the year 2000.

8.
The authors wish to claim the sequence is related to AGP sequences. Fig 3 legend says “Identical residues are shaded in black,”. Amino acid 10 is shaded in black to show identity of a conserved proline in the other proteins. Unfortunately, the amino acid in the ivy protein is alanine, not proline.

9.
There being almost nothing correct in this figure, I searched the amino acid sequence against NCBI. It is identical to a fungal cytochrome C oxidase subunit. The authors have not identified an ivy AGP. They have cloned a fungal protein.

On the basis of this figure alone, the manuscript shows that the authors have not taken due care and attention to their experimentation, or to manuscript preparation.

Figure 3 of the Nature communications resubmission July 2015:

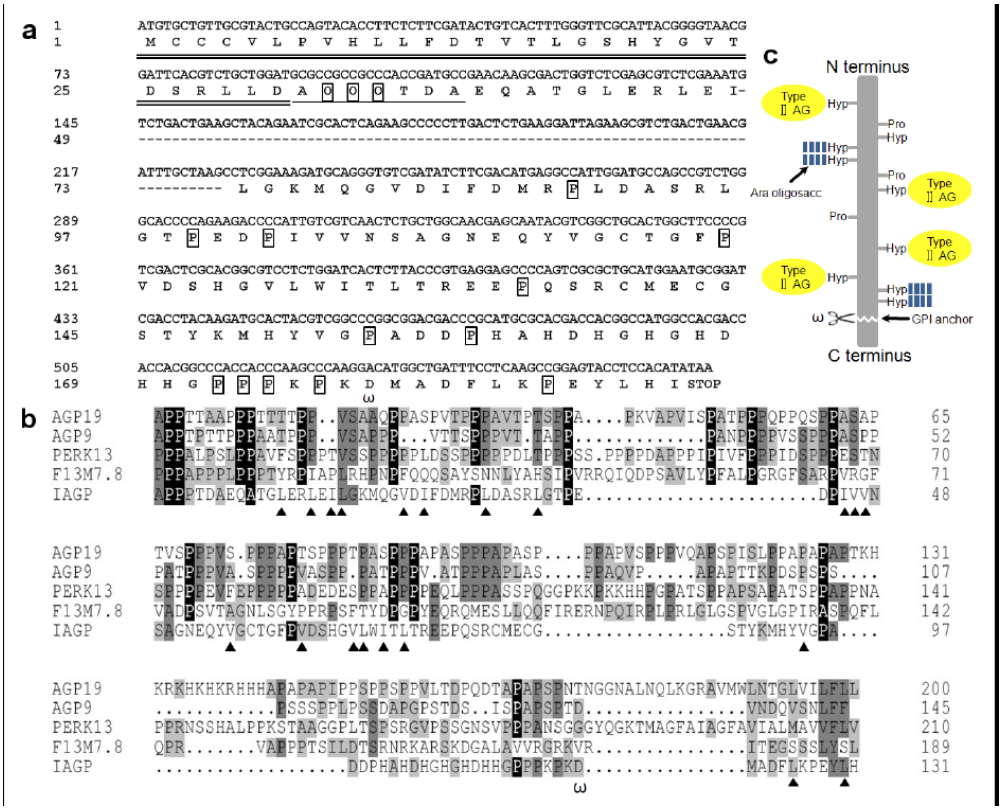

Figure 3: Chemical analyses of the ivy nanoparticles.

(**a**) Sequence of nucleotides and corresponding sequence of the protein backbone in the ivy nanoparticles. The amino acids obtained from *N*-terminal sequencing are single underlined. O represents hydroxyproline. The signal sequence at the *N*-terminus is double underlined. Genomic *iagp* contains an 83-bp intron which is indicated with a dashed line. 15 proline (or hydroxyproline) residues (boxed) out of 132 amino acids are obtained in the protein backbone. The predicted C-terminal site that is modified with a GPI anchor is indicated by the ω. (**b**) Multiple sequence alignment of IAGP with four other HRGPs in *Arabidopsis*. These four glycoproteins were chosen for multiple sequence alignment since they are the most analogous HRGPs to IAGP, according to a blast search in *A. thaliana*. The alignment was generated by ClustalX and edited manually using DNAman. Identical residues are shaded in black, while conserved and similar residues are indicated in dark gray and light gray, respectively. Amino acids that are proposed to play adherence function, including Ile, Leu, and Val, are indicated by black triangles [49]. The C-terminal site that is predicted to be modified with a GPI anchor is indicated by the ω. (**c**) A schematic drawing of the glycosylated protein backbone. Some of the proline residues are hydroxylated and glycosylated with short oligoarabinosides or type Ⅱ AGs [25].

Appendix 2

Response to earlier submission referee comment, provided to me in review July 2015.

a) Homology argument to other AGPs is not convincing.

Response: As commented by the second reviewer, English ivy (*Hedera helix*) is not a genetic model plant. The genome of *H. helix* has not been sequenced, and the *iagp* reported in this manuscript is the first gene sequence that has been deposited in the GenBank through this study, regarding the AGP backbones within *H. helix*. For the multiple sequence alignment, we chose other four analogous hydroxyproline-rich glycoproteins (HRGPs) from *Arabidopsis thaliana* according to a blast search. Even though the interspecific difference may influence the alignment, the result indeed demonstrates moderate similarity among five sequences. Moreover, even in the same species, such as *A. thaliana*, distinct AGPs exhibit noticeable diversity in the backbone sequences [3]. Therefore the result of the multiple sequence alignment is reasonable. The description about the sequence alignment was revised, shown as below:

"The protein sequence was designated as IVY ARABINOGALACTAN PROTEIN (IAGP) and was also aligned to other HRGPs in *Arabidopsis*, demonstrating a moderate sequence similarity (**Fig. 3b**)." (Page 7, Lines 13-15)

From: Kavanagh, Etta
To: Yucel, Jennifer
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct
Date: Thursday, December 20, 2018 5:30:30 PM
Attachments: image001.png

Hi Jen,

Thanks very much.

Etta

Etta Kavanagh
Editorial Manager
PNAS
phone: 202-334-1386
fax: 202-334-2739
email: ekavanagh@nas.edu

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Thursday, December 20, 2018 7:35 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,
Please call me Jen. I am working on this but we have a number of institutional officials out for the holidays. I will get you a response as quickly as I can. I can say as indicated in my original email that our recommendation is that the paper needs to be retracted. We will provide more information to support our request shortly.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 18, 2018 1:36 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I am checking in to see if you have any updates on this matter. Thank you very much.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 10, 2018 9:57 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you, your assistance with this matter is much appreciated.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, December 6, 2018 10:17 AM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

We will hold off on making a decision on Dr. Zhang's request until we hear back from you.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Wednesday, December 5, 2018 8:31 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. I will need to speak to institutional leadership about this situation. That may take me a few days. Can I ask that you please hold on processing Dr. Zhang's request until I can get back to you? I will do my best to have that be in the next week or 2.
Thank you for any assistance on this you can provide.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 4, 2018 5:21 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

Dr. Zhang contacted our office to request that we publish a correction. He said that he did not think that a retraction was warranted and provided the attached documents.

Are you able to provide us with a copy of the report, or an official letter detailing the concerns with the paper? Thank you very much for your help.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 3, 2018 12:53 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

The Ohio State University has completed its investigation of this matter, relating to the [redacted] 2016 PNAS 113 publication (attached). The university has determined that serious erroneous research was reported in the paper and we are requiring the authors to retract the paper.

I am writing to confirm that Dr. Mingjun Zhang [redacted] have contacted the journal to request the retraction. Can you please confirm that the authors have submitted this request for retraction? If not, please let me know.
Your assistance is much appreciated.

Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, July 26, 2016 5:11 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've attached the three letters that Dr. Zhang provided in response to the reviewer comments. Author letter 1 is the response provided with the first resubmission (2016-00406).

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Tuesday, July 26, 2016 12:00 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. Would it be possible to also get copies of what Dr. Zhang submitted with his revised manuscripts? Of particular importance to our review would be his response to reviewers comments and to the editor accompanying the first resubmission [manuscript # 2016-00406] in which he addressed the identity of IAGP with phylogenic analysis and mass spec.
Would it be possible to get this from you? Again, we greatly appreciate your assistance with this matter.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Monday, July 25, 2016 5:20 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've pasted below the four decision letters that our office sent [redacted]. The manuscript was originally submitted in early 2015. It was rejected, but the authors were invited to resubmit it. As far as we can tell, these are the only correspondence that our office had with Dr. Zhang.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Decision letter for 2015-01638:

From: pnas@nas.edu

To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2015-01638 Decision Notification
Message: March 18, 2015

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2015-01638
Authors: [redacted]

Dear Dr. Zhang,

I apologize for the delay and regret to inform you that the PNAS Editorial Board has rejected your manuscript [MS# 2015-01638]. The expert who served as editor obtained 3 reviews, which are included below. After careful consideration, the editor decided that we cannot accept your manuscript.

Note that the PNAS License to Publish conveyed at initial submission is terminated.

However, because the reviewers think the work is of interest and the editor concurs, we are willing to consider one resubmission that constructively addresses all of the concerns raised in the critiques. The paper would have to satisfy both the reviewers and the editor, and new criticisms could arise upon re-evaluation. We cannot guarantee success and will be unable to consider further resubmissions.

Thank you for submitting your work to PNAS.

Sincerely yours,
Inder M. Verma
Editor-in-Chief

****
Editor's Remarks to Author:

The manuscript cannot be published in PNAS in the current form. The reviewers raise several concerns regarding the isolated AGP, the glycosyl composition on silicon wafers, the nature of nanoparticle shown in the AFM images, and other technical problems. If the authors can address ALL of the reviewers' concerns, a resubmission could be possible.

The manuscript requires a substantial major revision accompanied by additional experiments to confirm the results presented. All comments of the reviewers need to be carefully considered. Specifically, all of the following matters should be expanded:

(1) In particular, it is questioned if the correct gene was isolated since important domains are lacking. There is convincing doubt that the isolated IAGP represents an AGP at all.

Therefore, I request a molecular phylogenetic tree including AGPs and Cytochrome Oxidase subunits to demonstrate the correct placement of IAGP within the AGP clade. In addition, as suggested by a reviewer, the degree of AG-glycosylation should be estimated as indicator that the cloned sequence is the correct AGP. In this respect, Mass Spectrometry also needs to be performed to corroborate that IAGP peptides are indeed present in abundance in purified particles.

(2) Further evidence that IAGP is in fact the Ivy adhesion molecule is required.

(3) The issue whether nanoparticles in AFM images are individual molecules or aggregates should be clarified.

(4) The nature of adhering residues on silicon wafers (Table 2) needs to be illuminated - can these represent cellulose or hemicellulose impurities?

(5) Stress strain data to show the rheological behavior need to be included.

(6) Methods descriptions need to be more detailed.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: No
Sufficient General Interest?: No
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
The paper investigates the molecular and bio-physical basis of the adhesive secreted by Ivy. A fraction of apparently spherical particles is isolated from exudate and is analysed biochemically and biophysically. It is suggested that the particles mainly consist of an arabinogalactan protein (AGP) that is termed IAGP. Based on a N-terminal peptide sequence of the deglycosylated protein, a putative cDNA for IAGP is cloned. The purified particles display low inherent viscosity and can form adhesive glues when combined with pectin and calcium. From a biophysical perspective the case looks more convincing than from a molecular biological one.

Comments on scientific content:

• AFM: It is not clear what is actually shown with the AFM images. Do the authors suggest that we are looking at individual AGP molecules or aggregates of many AGP and e.g. pectin molecules? Even though the texture of the mages suggests spherical particles the individual particles i.e. connected shapes are rarely ever spherical but are clusters. I am not an AFM expert but it seems helpful to image the particles at increasingly lower density so that individual particles might become the predominant structures. Otherwise it might mean that the spheres are not spheres after all.

• Fig. 1D and 1E. I don't know whether the methods applied for these figures are commonly known among physicists. As a molecular biologist I would find it helpful to get a better explanation of the method and why it should be applied.

• Tables 1 and 2: While the meaning of table 1 is relatively well explained and is interpreted that the linkages probably represent both AGII and pectic structures, the presence Glc, Xyl and Man (together >12 Mol%) is not explained. The sugars are reminiscent of hemicellulose but the terminal Glc is not. Is this an impurity or a component of the AGP/pectin particles? Table 2 describes the sugars found in adhering residues and the text states that "pectin is one of the main components ...". However the predominant monosaccharide in the remnant is glucose. Together with xylose and mannose it accounts for >65% meaning the main constituent is likely to be cellulose (indicating cell debris) or hemicellulose.

• When the IAGP sequence is presented in Figure 3 it is shown with a GPI anchor signal. Also in the scheme Figure 6 the GPI anchor structure, its attachment and release are shown in detail. However, the prediction tool that was used does not predict IAGP as GPI anchored (score = -76.05) and even the 'most likely' omega-site is different from the one shown in Figure 3. In my opinion the question whether or not IAGP is GPI anchored is irrelevant to the story and related suggestions may be removed without reducing the informative value.

• Figure 3C is mentioned in the context of the results section which is misleading. However it only shows a generic structure of a hypothetical AGP. In fact this is not even a classic (sic) AGP as claimed in the text. The literature uses the term "classical AGP" for extremely reduced proteins that contain only a backbone for O-glycosylation (typically XP repeats) and not other potentially functional protein domains (see Ref Ellis et al 2010).

• The sequence of IAGP does not resemble any known AGP. As such this is not remarkable because the important domains that define AGPs are so called AG-modules, stretches of XP, which are lacking from IAGP altogether. However, it bears remarkable sequence similarity to cytochrome oxidase subunit 5b-2. Are the authors sure that they have cloned the right cDNA?

• It would be important to estimate the degree of glycosylation of IAGP, especially when there is access to relatively large amounts of deglycosylated IAGP, MS/MS analysis could reveal hydroxyprolines in isolated positions which is an indirect indicator of AG-glycosylation. It would also confirm the identity of the suggested protein sequence which seems doubtful to me. Another possibility to confirm the nature of the cloned cDNA would be to express the sequence in a heterologous host (e.g. tobacco or Arabidopsis) and test its post-translational modifications.

References:

• The original papers where monoclonal antibodies were introduced and where they were characterized should be referenced.

• The SDS-PAGE procedure is not referenced.

Comments on presentation style:

• The English requires professional editing as the work contains numerous grammatical errors.

• Figure 3C is mentioned in the text before Figures 3A and B.

• Figure 5: the bottom part of the figure should be deleted and the glycan groups should be indicated just below the antibody names or even better above the bars.

• Figure 6 is far too elaborate and is not only confusing but also gives some quite false impressions (e.g. the existence of GPI-PLC in plants or the assumption that AGPs are glycosylated in the Golgi neither of which is proven). Only the relevant parts of the figure should be retained. Delete GPI-anchor, biosynthetic pathway (ribosomes, ER, Golgi), PLD/PLC.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes
Supplemental Material Warranted?: Yes

Comments:
The submission [redacted] describes in some detail a study to determine the mechanism of adhesion in English Ivy.

Approaches are varied and include (nano-)mechanical studies, proteomics, genomics and biochemistry. The conclusions are interesting and sound. They build upon previous work by this group, but offer significant new insight and 'proof of concept'. The methods and results are described well and concisely. The study of adhesion of plants lags behind that of animals and, for this reason, it is particularly interesting to see such a comprehensive report presented.

I have no major comments on the body of the text, except that I found the discussion to be rather short and lacking in depth. Similarly, I believe that the significance statement could be strengthened. What are the implications of the findings? The possible applications and routes to exploitation? Page 13, line 11, should this measurement of lap shear not be expressed as a stress in Pa?

My other comments are minor and include:

Page 2, line 14 - I don't think "conversely" is the right word.

Line 18 - Here and throughout the MS, 'by' is usually more appropriate than 'via'.

Line 18 - "the characteristic physicochemical..."

Line 20 - Remove "revealed".

Page 5, line 4 - "developed previously"

Page 6, line 5 - "This result was..."

Line 9 - "verified". Here and throughout tenses are mixed, often in the same sentence. e.g. "verifies" and "displayed".

Page 9, line 8 - "those of pectin and sodium..."

Line 12 - "In the current work..."

Line 13 - "objective was to reveal..."

Line 15 - ": surface wetting"

Line 20 - "beneficial for surface wetting by the ivy..."

Page 12, line 14 - "The manner in which nanoparticles..."

Line 16 - "product prepared via emulsion polymerisation. It is composed of nano-sized polymer-based particles dispersed in an aqueous..."

Line 17 - "Upon application to a ..."

Page 13, line 6 - "developed by integrating..."

Line 10 - "variations in adhesion force".

Line 10 - "enhanced with increasing hardening time".

Page 14, line 3 - "interfere with the..."

Page 15, line 1 - this sentence does not seem to make sense.

Page 15, line 20 - replace "has been established early"

Page 16, line 1 - remove "for"

Page 21, line 22 - remove "for"

Page 23, line 7 - "Once removed..."

Page 24, line 19 - "described earlier"

Page 25, line 5 - replace "via" with "by"

Line 10 - same as above

Line 13 - "were employed as adherends"

Line 15 - remove "sample"

Reviewer #3:

Suitable Quality?: No
Sufficient General Interest?: Yes
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
This paper reports on the characterization of Ivy AGP and the adhesion strength it creates between glass slides.

There are a significant number of technical problems with the paper which make it unacceptable for publication in the current form. The comments are listed below.

1. The evidence to support that fact that the AGP is exuded and indeed the critical molecule used by the Ivy to adhere is not presented. The statement is made that it is "presumed" that the AGP is involved in the adhesion.

2. While the characterization of the AGP seems sound, it is not clear whether the nanoparticles are single molecules or aggregates. The aggregation number should be determined or at least estimated.

3. The rheological measurements are deficient. The method used is determining a component of shear yield stress? There is no stress strain data reported to show the rheological behavior. This is critical to the paper. I suggest that "pull off" measurements are performed where the stress is normal to the surface are made. Furthermore, stress strain curves should be reported. These will give an indication of the creep and yield type behavior. The current measurements are made for silica surfaces. This is a good model for the wall however the Ivy is a biological system. The true measurement if possible should be between the Ivy and a silica slide. Furthermore the methods description does not contain enough detail to permit replication.

4. There is no mechanism or model for the action of the AGP in the adhesion presented. The data is therefore not properly analyzed.

5. The grammar is poor. Generally the paper is not well written.

Given the above technical problems with the paper it is not acceptable for publication in PNAS as the research is not well designed and executed.

****

Decision letter for 2016-00406:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406 Decision Notification

Message: February 9, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your resubmitted manuscript [MS# 2016-00406] has obtained 3 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due April 9, 2016. If you require additional time, notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the reviewers' comments. If you submitted a single PDF file at initial submission, you will be required to submit individual publication-ready files (e.g., Word file for manuscript text; EPS, TIFF, or high-resolution PDF for figures; Word file for tables; etc.)

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors are encouraged to use Supporting Information to show all necessary data, or to deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

Authors are responsible for obtaining waivers of any institutional open access mandates before publication with PNAS. Many institutions require that their authors transfer a nonexclusive author license to the institution and deposit the final author manuscript, with edits from peer review incorporated, into institutional repositories. These mandates conflict with PNAS policy because authors must provide the National Academy of Sciences with an exclusive license to publish their work. Authors employed by an institution with such a mandate should obtain a waiver for the nonexclusive license and upload the file during resubmission. A list of OA mandates can be found online (http://roarmap.eprints.org/).

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A5B3CgWM5A6GBcF6I3A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>.

Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Heather Snijdewind
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) HSnijdewind@nas.edu

****
Editor's Remarks to Author:

The authors have substantially revised the manuscript, performed additional experiments, and clarified all issues of the first three reviewers.

In summary, the authors: (1) included a phylogenetic tree corroborating the identity of the IAGP (2) performed Mass. spec analyses to confirm the IAGP protein sequence, (3) added a tensile test to confirm the molecular nature of the ivy-derived adhesive, (4) expanded AFM analyses to show that ivy nanoparticles observed in the AFM images are individual molecules rather than aggregates, (5) addressed the issue on hemicellulose impurities, (6) added stress strain data to show the rheological behavior, (7) included more details on methods, (8) removed GPI anchor discussion, (9) improved the model of ivy adhesion, (10), performed English and grammar proofreading.

After re-evaluation, several issues remain to be addressed. The authors are asked to improve style, add sub-headings, and re-write the discussion. Furthermore, the importance of pectin should be emphasized. The significance of the current work should be expanded, especially in the discussion section. Questions and suggestions regarding the figures need to be addressed. Further grammatical editing is requested.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: No
Procedures Described?: No

Comments:
The submission [redacted] details the identification and characterisation of arabinogalactan-like proteins in the adhesive substance of English ivy. Further, the authors develop a mimic with which to test the principles that they argue govern adhesion by the species.

As I stated in my first review, I find the science to be sound and of sufficiently broad interest to be published in PNAS. The findings are significant and also represent an advance in the specific field of research.

My comments and requests for improvement of the original manuscript mainly revolved around style and presentation. Unfortunately this has not improved and is, if anything, worse in this more recent version. I think that this is a consequence of adding the extra material requested by review and the re-drafting/re-organising of the text that this required. Therefore, while I find the research interesting, sound and would like to see it published in PNAS, I am unable to recommend publication of the manuscript in its current form.

First and foremost the text needs to be thoroughly edited by a native English speaker. Before this is done, however, there is significant restructuring that must be undertaken. Currently the style of the paper is that of one long chronological narrative that includes methods, results and discussion. The discussion section simply re-states the general concepts as described throughout. In this format I find the paper very difficult to follow and it certainly does not help the reader to identify points of interest. It is my opinion that the authors should sub-divide the paper much more, halving the length of the results section, make more use of sub-headings and move all of the methods out to the methods section. This would improve the clarity, readability and impact of the findings which, in the current format, are somewhat lost.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
This paper reports on the active component of English Ivy as the active adhesive component used by these plants. The paper is of general interest to the scientific community and the readership of PNAS. The revised version has been significantly improved in accord with the comments of all reviewers. I support publication in PNAS in the current form.

Reviewer #3:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
The authors isolated and characterized nanoparticles found in ivy-derived adhesive. They identified a component of the nanoparticles as being an arabinogalactan protein. They demonstrated that the nanoparticles were found in close proximity to pectin and interacted in a calcium-dependent manner. Bulk adhesion testing was done to demonstrate that all three components - nanoparticles, pectin, and calcium - resulted in a stronger adhesive bond than individual components. Overall, the experiments are interesting and performed well, but there are items that should be addressed before publication:

1. Overall, the abstract and introduction (and the results discussing Figures 1-3) seem to emphasize the importance of the arabinogalactan protein (AGP) nanoparticles in ivy adhesive. The importance of pectin is not emphasized strongly in the beginning part of the manuscript. However, it seems that pectin is at least equally (if not more) important to the adhesive as the nanoparticles are - in Figure 6, pectin by itself has higher bulk adhesion strength than nanoparticles by themselves. Thus, it is unclear why there is such an emphasis on AGP and not on pectin. Is it because that pectin was already known to be an important component and that this paper is characterizing the second component? Or is it because the AGPs are less viscous and thus can penetrate the substrates more and provide mechanical interlocking (although if this is the case,

more experiments need to be performed to show this phenomenon). More context would be helpful.

2. There are some allusions to other botanic adhesives that have been studied (page 6, lines 778-785). The text states that "arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and Ficus pumila." Given that arabinogalactans and pectin are found to be the major components of the English ivy adhesive studied in this paper, it would be helpful to expand on the significance of the current work in the abstract, intro, and, especially, the discussion section. How are the findings significant and different from papers published about other plant adhesives? Are the results in this paper already known for other botanic adhesives or is there some additional insight provided here or something that is unique and compelling about the English ivy adhesive?

3. Figure 2: Panel D has a lane labeled as being from Fraction 1, but the legend only acknowledges Fractions 2-5. The text is also unclear - it states that "apart from the solvent peak designated as fraction 1," which could imply that Fraction 1 was not run on the gel. Also, one gel lane is labeled "Marker", but the legend refers to it as "Lane M."

4. Figure 3: For panel C, the figure legend states "Amino acids that are proposed to play adhesive function, comprising Ile, Leu, and Val, are indicated by black triangles." This statement is confusing as this reviewer interpreted the resulting experiments as showing that calcium mediated adhesive interactions between negatively charged residues on AGPs and pectin. Thus, it is not clear how Ile, Leu, and Val are involved in adhesion. Perhaps they are involved in adhesion to the substrates, but no data are shown to support the role of these amino acids in adhesion.

5. Figure 4B: This reviewer could not find the text description in the results section for the EDX data.

6. Figure 5: The second schematic (after evaporation) is confusing. Is pectin supposed to be the gray area that surrounds the yellow spheres? If so, the schematic only appears to be showing that calcium interacts directly with pectin (the gray portion) and not the AGP particles.

7. Figure 6D: The text (page 8, lines 984-993) would be stronger if it explained why the change in pH would affect adhesion. The text states "given that the cross-linking extent...is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here." However, rather than saying it should vary, it would be useful to explain why it is expected to vary at pH 4 and 9. Are we near the pKa values? Are residues no longer negatively charged at one or both of those values?

8. Page 7, lines 905-910: The text hypotheses that the nanoparticles allow for mechanical interlocking. However, are there no chemical adhesive forces (e.g., covalent bonds, van der Waals forces, etc) that are expected to occur?

9. Page 8, line 1035: It is unclear what is meant by "partially reflects the physiological implications of the associated low intrinsic viscosity."

10. Figure 6: For panel G, the meaning of the asterisk is not clear. The legend says the asterisk is compared to "EGTA-free adhesive composites containing 2 mM Ca2+"; however, the asterisk is placed above the composite group with Ca2+ and no EGTA. Also, it seems like it would make more sense if an ANOVA were performed and Tukey groups were shown so that one could determine which groups were statistically similar or different.

11. Materials and Methods (SI page 2, line 21): It would be helpful to report centrifugation in terms of g and not just rpm.

12. Figure S5: In the legend, please explain the green dotted line in panels C-F.

13. Figure S7: Given that there is an arrow with the word "agglomeration" connecting the two panels, it is not clear whether the left and right panels are from the same sample that have agglomerated over time. Or, are they from different samples or different areas of the same sample?

14. Figure S8: Given that two different secondary antibodies were used in the ELISA, is it valid to show results from all of the ELISAs on the same graph? In other words, are the absorbance values from ELISA wells using different secondary antibodies comparable? Were standard curves performed to show that the absorbance values would equate to the same amount?

15. The still image derived from movie S1 and the latter portion of movie S1 (that shows that ivy is stuck to a surface) do not appear to be in focus.

16. In the response to reviewers' comments, point 1.1 says that the nanostructures are "individual molecules consisting of covalently bonded AGP and pectin domain." What is meant by pectic domain? Is this a domain in the AGP that binds pectin? Or do you mean that the molecules contain AGP and short fragments of pectin (in which case they would not be individual molecules)?

17. Further grammatical editing of the paper would be helpful. For example, on page 4, there are multiple instances of "faction 4" instead of "fraction 4." There are other examples of minor grammatical or typographical errors in the manuscript.

****

PUBLICATION INFORMATION AND CHARGES

High resolution files are required for figures that will appear in the main text of the article and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. For more information on preparing digital art, please review the PNAS digital art guidelines.

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Decision letter for 2016-00406R

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406R Decision Notification
Message: April 26, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406R
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your manuscript [MS# 2016-00406R] has obtained 2 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due June 25, 2016. If you require additional time, please notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the editor's and reviewer's comments.

We also require that you amend your title. We are seeking a descriptive title without the use of an em/en dash or colon (i.e. a single declarative title). This is non-negotiable and an exception will not be made.

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors must deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A2B3CgWM7B5GBcF2I1A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>

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Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Tom Myers
PNAS Editorial Office

(p) 202.334.2679
(f) 202.334.2739
(e) pnas@nas.edu

****

Editor Comments:
We thank the authors for their efforts to improve the mansucript. The manuscript mertis publication in PNAS. We kindly ask to provide a high resolution image of Fig. 4.

Reviewer Comments:
Reviewer #1:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
I find this revised manuscript to be a significant improvement on the former and in my opinion can be published in its current form. All amendments requested by previous reviews appear to have been included.

Reviewer #3:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
The authors have substantially revised and edited their paper in response to the reviewers' comments. The writing more clearly conveys the context of their work and is suitable for the general audience that reads PNAS. I support acceptance by PNAS for publication. I have only one minor comment that may be addressed in typesetting - Figure 4 has many important panels, and at its current size, it is difficult to see and interpret all of the data. I was able to adequately see the data when enlarging the figure to >300% of its size and want to ensure that the resolution is maintained in the final published form so that others can enlarge the figure without it becoming pixelated. Alternatively, a larger version of the figure could be placed in the supplementary material.

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PUBLICATION INFORMATION AND CHARGES

High resolution files are required for figures that will appear in the print journal and must be uploaded with the final version of your manuscript.

Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. PNAS Plus articles will cost $2,150 per research article, with no additional charges for color figures or SI. For more information on preparing digital art, please review the PNAS digital art guidelines.

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Decision letter for 2016-00406RR

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406RR Decision Notification
Message: April 29, 2016

Title: "Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406RR
Authors: [redacted]

Dear Dr. Zhang,

We are pleased to inform you that the PNAS Editorial Board has given final approval of your article for publication. Peter Ladurner, the Editor who conducted the initial review of your manuscript [MS# 2016-00406RR], will also be informed of the decision.

Please note PNAS Plus articles are held to a strict 10-page maximum length. As your work is prepared for publication, you may be contacted by our printer to reduce the length of your article during the proof stage.

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may talk with the press about their work prior to the embargo but should coordinate this with the PNAS News Office or their institution's press office so that reporters are aware of PNAS policy and understand that papers are embargoed until the week of publication. If you plan to present your embargoed paper at a conference prior to publication, please contact the PNAS News Office immediately at 202-334-1310, or PNASnews@nas.edu.

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<http://www.pnascentral.org/cgi-bin/main.plex?el=A2B4CgWM1C6GBcF3F3A9ftdbJEbeP06uSdnKALMAw9MwZ>

Sincerely yours,
Inder M. Verma
Editor-in-Chief

****

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Thursday, July 21, 2016 3:53 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavangh,
Thank you for your quick response and willingness to provide those communications. I hope you enjoy your conference and we will look for those items when you are back in the office.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Thursday, July 21, 2016 3:17 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: Re: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I'm out of the office at a conference this week, but I will send our correspondence with Dr. Zhang when I'm back in the office next week.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: "Yucel, Jennifer" <yucel.4@osu.edu>
Date: Thursday, July 21, 2016 at 11:28 AM
To: Kavanagh Etta <ekavanagh@nas.edu>
Cc: "hart.322@osu.edu" <hart.322@osu.edu>, "Moses, Randolph" <moses.2@osu.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

Dr. Hart forwarded your email to me. I am the University's research integrity officer and I am confirming that the university did receive these concerns and we are looking into this matter.

Would it be possible for you to share with me any communications that the journal had with Dr. Zhang during the publication process? Those communications would greatly assist us in the review of this matter and would be greatly appreciated. Once we have completed our review we will inform you of our determination.
Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: "Kavanagh, Etta" <EKavanagh@nas.edu>
Date: July 15, 2016 at 5:21:57 PM EDT
To: "'hart.322@osu.edu'" <hart.322@osu.edu>
Subject: FW: confidential complaint about scientific misconduct
Dear Dr. Hart,

I am contacting you regarding the complaint PNAS received regarding the paper "Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy' [redacted] We shared the complaint with the editor, Peter Ladurner, and he provided the following comments:

"I want to ensure my support for PNAS regarding manuscript 2016-00406RR.

The question if the gene identified is indeed the ivy AGP cought my attention after the initial submition. Please note that I questioned this finding myself. For their first revision I demanded that the authors have to add a phylogenetic tree showing the true AGP relationship of their protein. In their revision the authors provided the respective tree (and mass spec data) corroborating their finding.

I want to state that such data - under normal circumstances and if the data were generated according to best scientific practice - are sufficient to support the authors statement that their protein is an AGP.

However, if the genes for generating the phylogenetic tree were highly hand picked (and not selected according to their statement in the paper: "Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 were the closest relatives, as shown in Fig. S4A.") any tree can be fabricated.
I hope this is not true.

The next steps require detailed sequence analyses using BLAST, reciprocal BLAST, thourough protein alignments and phylogenetic analyses of the submitted sequence.

Depending on the result the authors need to provide raw data and lab book level information on gene isolation, details on clones with gene inserts from PCR and RACE experiments, sequencing raw files, details of their BLAST search settings and databases, information on the selection and generation of alignments and the phylogenetic tree, Mass Spectrometry raw data, details on GPI anchor bioinformatics.

Please let me know if I can help with the sequence analyses."

Is Ohio State University investigating these concerns? Should I contact the research integrity office? Thank you very much for your help.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer
To: Kavanagh, Etta
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct
Date: Friday, January 18, 2019 10:46:25 AM
Attachments: image001.png

Hi Etta,
I apologize for the delay in responding. This is a sensitive and complicated situation that we are working on with the author and his legal counsel. We will provide more information as soon as we are able. We appreciate your patience.
Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Wednesday, January 16, 2019 4:32 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Jen,

I am checking in to see if you have any updates. Thanks very much.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Thursday, December 20, 2018 7:35 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,
Please call me Jen. I am working on this but we have a number of institutional officials out for the holidays. I will get you a response as quickly as I can. I can say as indicated in my original email that our recommendation is that the paper needs to be retracted. We will provide more information to support our request shortly.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 18, 2018 1:36 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I am checking in to see if you have any updates on this matter. Thank you very much.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 10, 2018 9:57 AM

To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you, your assistance with this matter is much appreciated.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, December 6, 2018 10:17 AM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

We will hold off on making a decision on Dr. Zhang's request until we hear back from you.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Wednesday, December 5, 2018 8:31 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. I will need to speak to institutional leadership about this situation. That may take me a few days. Can I ask that you please hold on processing Dr. Zhang's request until I can get back to you? I will do my best to have that be in the next week or 2.
Thank you for any assistance on this you can provide.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 4, 2018 5:21 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

Dr. Zhang contacted our office to request that we publish a correction. He said that he did not think that a retraction was warranted and provided the attached documents.

Are you able to provide us with a copy of the report, or an official letter detailing the concerns with the paper? Thank you very much for your help.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 3, 2018 12:53 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

The Ohio State University has completed its investigation of this matter, relating to the [redacted] 2016 PNAS 113 publication (attached). The university has determined that serious erroneous research was reported in the paper and we are requiring the authors to retract the paper.

I am writing to confirm that Dr. Mingjun Zhang [redacted] have contacted the journal to request the retraction. Can you please confirm that the authors have submitted this request for retraction? If not, please let me know.
Your assistance is much appreciated.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, July 26, 2016 5:11 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've attached the three letters that Dr. Zhang provided in response to the reviewer comments. Author letter 1 is the response provided with the first resubmission (2016-00406).

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Tuesday, July 26, 2016 12:00 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. Would it be possible to also get copies of what Dr. Zhang submitted with his revised manuscripts? Of particular importance to our review would be his response to reviewers comments and to the editor accompanying the first resubmission [manuscript # 2016-00406] in which he addressed the identity of IAGP with phylogenic analysis and mass spec.
Would it be possible to get this from you? Again, we greatly appreciate your assistance with this matter.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Monday, July 25, 2016 5:20 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've pasted below the four decision letters that our office sent to Dr. Yang. The manuscript was originally submitted in early 2015. It was rejected, but the authors were invited to resubmit it. As far as we can tell, these are the only correspondence that our office had with Dr. Zhang.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Decision letter for 2015-01638:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2015-01638 Decision Notification
Message: March 18, 2015

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2015-01638
Authors: [redacted]

Dear Dr. Zhang,

I apologize for the delay and regret to inform you that the PNAS Editorial Board has rejected your manuscript [MS# 2015-01638]. The expert who served as editor obtained 3 reviews, which are included below. After careful consideration, the editor decided that we cannot accept your manuscript.

Note that the PNAS License to Publish conveyed at initial submission is terminated.

However, because the reviewers think the work is of interest and the editor concurs, we are willing to consider one resubmission that constructively addresses all of the concerns raised in the critiques. The paper would have to satisfy both the reviewers and the editor, and new criticisms could arise upon re-evaluation. We cannot guarantee success and will be unable to consider further resubmissions.

Thank you for submitting your work to PNAS.

Sincerely yours,
Inder M. Verma
Editor-in-Chief

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Editor's Remarks to Author:

The manuscript cannot be published in PNAS in the current form. The reviewers raise several concerns regarding the isolated AGP, the glycosyl composition on silicon wafers, the nature of nanoparticle shown in the AFM images, and other technical problems. If the authors can address ALL of the reviewers' concerns, a resubmission could be possible.

The manuscript requires a substantial major revision accompanied by additional experiments to confirm the results presented. All comments of the reviewers need to be carefully considered. Specifically, all of the following matters should be expanded:

(1) In particular, it is questioned if the correct gene was isolated since important domains are lacking. There is convincing doubt that the isolated IAGP represents an AGP at all.

Therefore, I request a molecular phylogenetic tree including AGPs and Cytochrome Oxidase subunits to demonstrate the correct placement of IAGP within the AGP clade. In addition, as suggested by a reviewer, the degree of AG-glycosylation should be estimated as indicator that the cloned sequence is the correct AGP. In this respect, Mass Spectrometry also needs to be performed to corroborate that IAGP peptides are indeed present in abundance in purified particles.

(2) Further evidence that IAGP is in fact the Ivy adhesion molecule is required.

(3) The issue whether nanoparticles in AFM images are individual molecules or aggregates should be clarified.

(4) The nature of adhering residues on silicon wafers (Table 2) needs to be illuminated - can these represent cellulose or hemicellulose impurities?

(5) Stress strain data to show the rheological behavior need to be included.

(6) Methods descriptions need to be more detailed.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: No
Sufficient General Interest?: No
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
The paper investigates the molecular and bio-physical basis of the adhesive secreted by Ivy. A fraction of apparently spherical particles is isolated from exudate and is analysed biochemically and biophysically. It is suggested that the particles mainly consist of an arabinogalactan protein (AGP) that is termed IAGP. Based on a N-terminal peptide sequence of the deglycosylated protein, a putative cDNA for IAGP is cloned. The purified particles display low inherent viscosity and can form adhesive glues when combined with pectin and calcium. From a biophysical perspective the case looks more convincing than from a molecular biological one.

Comments on scientific content:

• AFM: It is not clear what is actually shown with the AFM images. Do the authors suggest that we are looking at individual AGP molecules or aggregates of many AGP and e.g. pectin molecules? Even though the texture of the mages suggests spherical particles the individual particles i.e. connected shapes are rarely ever spherical but are clusters. I am not an AFM expert but it seems helpful to image the particles at increasingly lower density so that individual particles might become the predominant structures. Otherwise it might mean that the spheres are not spheres after all.

• Fig. 1D and 1E. I don't know whether the methods applied for these figures are commonly known among physicists. As a molecular biologist I would find it helpful to get a better explanation of the method and why it should be applied.

• Tables 1 and 2: While the meaning of table 1 is relatively well explained and is interpreted that the linkages probably represent both AGII and pectic structures, the presence Glc, Xyl and Man (together >12 Mol%) is not explained. The sugars are reminiscent of hemicellulose but the terminal Glc is not. Is this an impurity or a component of the AGP/pectin particles? Table 2 describes the sugars found in adhering residues and the text states that "pectin is one of the main components ...". However the predominant monosaccharide in the remnant is glucose. Together with xylose and mannose it accounts for >65% meaning the main constituent is likely to be cellulose (indicating cell debris) or hemicellulose.

• When the IAGP sequence is presented in Figure 3 it is shown with a GPI anchor signal. Also in the scheme Figure 6 the GPI anchor structure, its attachment and release are shown in detail. However, the prediction tool that was used does not predict IAGP as GPI anchored (score = -76.05) and even the 'most likely' omega-site is different from the one shown in Figure 3. In my opinion the question whether or not IAGP is GPI anchored is irrelevant to the story and related suggestions may be removed without reducing the informative value.

• Figure 3C is mentioned in the context of the results section which is misleading. However it only shows a generic structure of a hypothetical AGP. In fact this is not even a classic (sic) AGP as claimed in the text. The literature uses the term "classical AGP" for extremely reduced proteins that contain only a backbone for O-glycosylation (typically XP repeats) and not other potentially functional protein domains (see Ref Ellis et al 2010).

• The sequence of IAGP does not resemble any known AGP. As such this is not remarkable because the important domains that define AGPs are so called AG-modules, stretches of XP, which are lacking from IAGP altogether. However, it bears remarkable sequence similarity to cytochrome oxidase subunit 5b-2. Are the authors sure that they have cloned the right cDNA?

• It would be important to estimate the degree of glycosylation of IAGP, especially when there is access to relatively large amounts of deglycosylated IAGP, MS/MS analysis could reveal hydroxyprolines in isolated positions which is an indirect indicator of AG-glycosylation. It would also confirm the identity of the suggested protein sequence which seems doubtful to me. Another possibility to confirm the nature of the cloned cDNA would be to express the sequence in a heterologous host (e.g. tobacco or Arabidopsis) and test its post-translational modifications.

References:

• The original papers where monoclonal antibodies were introduced and where they were characterized should be referenced.

• The SDS-PAGE procedure is not referenced.

Comments on presentation style:

• The English requires professional editing as the work contains numerous grammatical errors.

• Figure 3C is mentioned in the text before Figures 3A and B.

• Figure 5: the bottom part of the figure should be deleted and the glycan groups should be indicated just below the antibody names or even better above the bars.

• Figure 6 is far too elaborate and is not only confusing but also gives some quite false impressions (e.g. the existence of GPI-PLC in plants or the assumption that AGPs are glycosylated in the Golgi neither of which is proven). Only the relevant parts of the figure should be retained. Delete GPI-anchor, biosynthetic pathway (ribosomes, ER, Golgi), PLD/PLC.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes
Supplemental Material Warranted?: Yes

Comments:
The submission [redacted] describes in some detail a study to determine the mechanism of adhesion in English Ivy.

Approaches are varied and include (nano-)mechanical studies, proteomics, genomics and biochemistry. The conclusions are interesting and sound. They build upon previous work by this group, but offer significant new insight and 'proof of concept'. The methods and results are described well and concisely. The study of adhesion of plants lags behind that of animals and, for this reason, it is particularly interesting to see such a comprehensive report presented.

I have no major comments on the body of the text, except that I found the discussion to be rather short and lacking in depth. Similarly, I believe that the significance statement could be strengthened. What are the implications of the findings? The possible applications and routes to exploitation? Page 13, line 11, should this measurement of lap shear not be expressed as a stress in Pa?

My other comments are minor and include:

Page 2, line 14 - I don't think "conversely" is the right word.

Line 18 - Here and throughout the MS, 'by' is usually more appropriate than 'via'.

Line 18 - "the characteristic physicochemical..."

Line 20 - Remove "revealed".

Page 5, line 4 - "developed previously"

Page 6, line 5 - "This result was..."

Line 9 - "verified". Here and throughout tenses are mixed, often in the same sentence. e.g. "verifies" and "displayed".

Page 9, line 8 - "those of pectin and sodium..."

Line 12 - "In the current work..."

Line 13 - "objective was to reveal..."

Line 15 - ": surface wetting"

Line 20 - "beneficial for surface wetting by the ivy..."

Page 12, line 14 - "The manner in which nanoparticles..."

Line 16 - "product prepared via emulsion polymerisation. It is composed of nano-sized polymer-based particles dispersed in an aqueous..."

Line 17 - "Upon application to a ..."

Page 13, line 6 - "developed by integrating..."

Line 10 - "variations in adhesion force".

Line 10 - "enhanced with increasing hardening time".

Page 14, line 3 - "interfere with the..."

Page 15, line 1 - this sentence does not seem to make sense.

Page 15, line 20 - replace "has been established early"

Page 16, line 1 - remove "for"

Page 21, line 22 - remove "for"

Page 23, line 7 - "Once removed..."

Page 24, line 19 - "described earlier"

Page 25, line 5 - replace "via" with "by"

Line 10 - same as above

Line 13 - "were employed as adherends"

Line 15 - remove "sample"

Reviewer #3:

Suitable Quality?: No
Sufficient General Interest?: Yes
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
This paper reports on the characterization of Ivy AGP and the adhesion strength it creates between glass slides.

There are a significant number of technical problems with the paper which make it unacceptable for publication in the current form. The comments are listed below.

1. The evidence to support that fact that the AGP is exuded and indeed the critical molecule used by the Ivy to adhere is not presented. The statement is made that it is "presumed" that the AGP is involved in the adhesion.

2. While the characterization of the AGP seems sound, it is not clear whether the nanoparticles are single molecules or aggregates. The aggregation number should be determined or at least estimated.

3. The rheological measurements are deficient. The method used is determining a component of shear yield stress? There is no stress strain data reported to show the rheological behavior. This is critical to the paper. I suggest that "pull off" measurements are performed where the stress is normal to the surface are made. Furthermore, stress strain curves should be reported. These will give an indication of the creep and yield type behavior. The current measurements are made for silica surfaces. This is a good model for the wall however the Ivy is a biological system. The true measurement if possible should be between the Ivy and a silica slide. Furthermore the methods description does not contain enough detail to permit replication.

4. There is no mechanism or model for the action of the AGP in the adhesion presented. The data is therefore not properly analyzed.

5. The grammar is poor. Generally the paper is not well written.

Given the above technical problems with the paper it is not acceptable for publication in PNAS as the research is not well designed and executed.

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Decision letter for 2016-00406:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406 Decision Notification
Message: February 9, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your resubmitted manuscript [MS# 2016-00406] has obtained 3 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due April 9, 2016. If you require additional time, notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the reviewers' comments. If you submitted a single PDF file at initial submission, you will be required to submit individual publication-ready files (e.g., Word file for manuscript text; EPS, TIFF, or high-resolution PDF for figures; Word file for tables; etc.)

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors are encouraged to use Supporting Information to show all necessary data, or to deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

Authors are responsible for obtaining waivers of any institutional open access mandates before publication with PNAS. Many institutions require that their authors transfer a nonexclusive author license to the institution and deposit the final author manuscript, with edits from peer review incorporated, into institutional repositories. These mandates conflict with PNAS policy because authors must provide the National Academy of Sciences with an exclusive license to publish their work. Authors employed by an institution with such a mandate should obtain a waiver for the nonexclusive license and upload the file during resubmission. A list of OA mandates can be found online (http://roarmap.eprints.org/).

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A5B3CgWM5A6GBcF6I3A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>.

Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Heather Snijdewind
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) HSnijdewind@nas.edu

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Editor's Remarks to Author:

The authors have substantially revised the manuscript, performed additional experiments, and clarified all issues of the first three reviewers.

In summary, the authors: (1) included a phylogenetic tree corroborating the identity of the IAGP (2) performed Mass. spec analyses to confirm the IAGP protein sequence, (3) added a tensile test to confirm the molecular nature of the ivy-derived adhesive, (4) expanded AFM analyses to

show that ivy nanoparticles observed in the AFM images are individual molecules rather than aggregates, (5) addressed the issue on hemicellulose impurities, (6) added stress strain data to show the rheological behavior, (7) included more details on methods, (8) removed GPI anchor discussion, (9) improved the model of ivy adhesion, (10), performed English and grammar proofreading.

After re-evaluation, several issues remain to be addressed. The authors are asked to improve style, add sub-headings, and re-write the discussion. Furthermore, the importance of pectin should be emphasized. The significance of the current work should be expanded, especially in the discussion section. Questions and suggestions regarding the figures need to be addressed. Further grammatical editing is requested.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: No
Procedures Described?: No

Comments:
The submission [redacted]. details the identification and characterisation of arabinogalactan-like proteins in the adhesive substance of English ivy. Further, the authors develop a mimic with which to test the principles that they argue govern adhesion by the species.

As I stated in my first review, I find the science to be sound and of sufficiently broad interest to be published in PNAS. The findings are significant and also represent an advance in the specific field of research.

My comments and requests for improvement of the original manuscript mainly revolved around style and presentation. Unfortunately this has not improved and is, if anything, worse in this more recent version. I think that this is a consequence of adding the extra material requested by review and the re-drafting/re-organising of the text that this required. Therefore, while I find the research interesting, sound and would like to see it published in PNAS, I am unable to recommend publication of the manuscript in its current form.

First and foremost the text needs to be thoroughly edited by a native English speaker. Before this is done, however, there is significant restructuring that must be undertaken. Currently the style of the paper is that of one long chronological narrative that includes methods, results and discussion. The discussion section simply re-states the general concepts as described throughout. In this format I find the paper very difficult to follow and it certainly does not help the reader to identify points of interest. It is my opinion that the authors should sub-divide the paper much more, halving the length of the results section, make more use of sub-headings and move all of the methods out to the methods section. This would improve the clarity, readability and impact of the findings which, in the current format, are somewhat lost.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
This paper reports on the active component of English Ivy as the active adhesive component used by these plants. The paper is of general interest to the scientific community and the readership of PNAS. The revised version has been significantly improved in accord with the comments of all reviewers. I support publication in PNAS in the current form.

Reviewer #3:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
The authors isolated and characterized nanoparticles found in ivy-derived adhesive. They identified a component of the nanoparticles as being an arabinogalactan protein. They demonstrated that the nanoparticles were found in close proximity to pectin and interacted in a calcium-dependent manner. Bulk adhesion testing was done to demonstrate that all three components - nanoparticles, pectin, and calcium - resulted in a stronger adhesive bond than individual components. Overall, the experiments are interesting and performed well, but there are items that should be addressed before publication:

1. Overall, the abstract and introduction (and the results discussing Figures 1-3) seem to emphasize the importance of the arabinogalactan protein (AGP) nanoparticles in ivy adhesive. The importance of pectin is not emphasized strongly in the beginning part of the manuscript. However, it seems that pectin is at least equally (if not more) important to the adhesive as the nanoparticles are - in Figure 6, pectin by itself has higher bulk adhesion strength than nanoparticles by themselves. Thus, it is unclear why there is such an emphasis on AGP and not on pectin. Is it because that pectin was already known to be an important component and that this paper is characterizing the second component? Or is it because the AGPs are less viscous and thus can penetrate the substrates more and provide mechanical interlocking (although if this is the case, more experiments need to be performed to show this phenomenon). More context would be helpful.

2. There are some allusions to other botanic adhesives that have been studied (page 6, lines 778-785). The text states that "arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and Ficus pumila." Given that arabinogalactans and pectin are found to be the major components of the English ivy adhesive studied in this paper, it would be helpful to expand on the significance of the current work in the abstract, intro, and, especially, the discussion section. How are the findings significant and different from papers published about other plant adhesives? Are the results in this paper already known for other botanic adhesives or is there some additional insight provided here or something that is unique and compelling about the English ivy adhesive?

3. Figure 2: Panel D has a lane labeled as being from Fraction 1, but the legend only acknowledges Fractions 2-5. The text is also unclear - it states that "apart from the solvent peak designated as fraction 1," which could imply that Fraction 1 was not run on the gel. Also, one gel lane is labeled "Marker", but the legend refers to it as "Lane M."

4. Figure 3: For panel C, the figure legend states "Amino acids that are proposed to play adhesive function, comprising Ile, Leu, and Val, are indicated by black triangles." This statement is confusing as this reviewer interpreted the resulting experiments as showing that calcium mediated adhesive interactions between negatively charged residues on AGPs and pectin. Thus, it is not clear how Ile, Leu, and Val are involved in adhesion. Perhaps they are involved in adhesion to the substrates, but no data are shown to support the role of these amino acids in adhesion.

5. Figure 4B: This reviewer could not find the text description in the results section for the EDX data.

6. Figure 5: The second schematic (after evaporation) is confusing. Is pectin supposed to be the gray area that surrounds the yellow spheres? If so, the schematic only appears to be showing that calcium interacts directly with pectin (the gray portion) and not the AGP particles.

7. Figure 6D: The text (page 8, lines 984-993) would be stronger if it explained why the change in pH would affect adhesion. The text states "given that the cross-linking extent...is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here." However, rather than saying it should vary, it would be useful to explain why it is expected to vary at pH 4 and 9. Are we near the pKa values? Are residues no longer negatively charged at one or both of those values?

8. Page 7, lines 905-910: The text hypotheses that the nanoparticles allow for mechanical interlocking. However, are there no chemical adhesive forces (e.g., covalent bonds, van der Waals forces, etc) that are expected to occur?

9. Page 8, line 1035: It is unclear what is meant by "partially reflects the physiological implications of the associated low intrinsic viscosity."

10. Figure 6: For panel G, the meaning of the asterisk is not clear. The legend says the asterisk is compared to "EGTA-free adhesive composites containing 2 mM Ca2+"; however, the asterisk is placed above the composite group with Ca2+ and no EGTA. Also, it seems like it would make more sense if an ANOVA were performed and Tukey groups were shown so that one could determine which groups were statistically similar or different.

11. Materials and Methods (SI page 2, line 21): It would be helpful to report centrifugation in terms of g and not just rpm.

12. Figure S5: In the legend, please explain the green dotted line in panels C-F.

13. Figure S7: Given that there is an arrow with the word "agglomeration" connecting the two panels, it is not clear whether the left and right panels are from the same sample that have agglomerated over time. Or, are they from different samples or different areas of the same sample?

14. Figure S8: Given that two different secondary antibodies were used in the ELISA, is it valid to show results from all of the ELISAs on the same graph? In other words, are the absorbance values from ELISA wells using different secondary antibodies comparable? Were standard curves performed to show that the absorbance values would equate to the same amount?

15. The still image derived from movie S1 and the latter portion of movie S1 (that shows that ivy is stuck to a surface) do not appear to be in

focus.

16. In the response to reviewers' comments, point 1.1 says that the nanostructures are "individual molecules consisting of covalently bonded AGP and pectin domain." What is meant by pectic domain? Is this a domain in the AGP that binds pectin? Or do you mean that the molecules contain AGP and short fragments of pectin (in which case they would not be individual molecules)?

17. Further grammatical editing of the paper would be helpful. For example, on page 4, there are multiple instances of "faction 4" instead of "fraction 4." There are other examples of minor grammatical or typographical errors in the manuscript.

****

PUBLICATION INFORMATION AND CHARGES

High resolution files are required for figures that will appear in the main text of the article and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. For more information on preparing digital art, please review the PNAS digital art guidelines.

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Decision letter for 2016-00406R

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406R Decision Notification
Message: April 26, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406R
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your manuscript [MS# 2016-00406R] has obtained 2 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due June 25, 2016. If you require additional time, please notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the editor's and reviewer's comments.

We also require that you amend your title. We are seeking a descriptive title without the use of an em/en dash or colon (i.e. a single declarative title). This is non-negotiable and an exception will not be made.

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors must deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-

bin/main.plex?el=A2B3CgWM7B5GBcF2I1A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>

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Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Tom Myers
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) pnas@nas.edu

****

Editor Comments:
We thank the authors for their efforts to improve the mansucript. The manuscript mertis publication in PNAS. We kindly ask to provide a high resolution image of Fig. 4.

Reviewer Comments:
Reviewer #1:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
I find this revised manuscript to be a significant improvement on the former and in my opinion can be published in its current form. All amendments requested by previous reviews appear to have been included.

Reviewer #3:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
The authors have substantially revised and edited their paper in response to the reviewers' comments. The writing more clearly conveys the context of their work and is suitable for the general audience that reads PNAS. I support acceptance by PNAS for publication. I have only one minor comment that may be addressed in typesetting - Figure 4 has many important panels, and at its current size, it is difficult to see and interpret all of the data. I was able to adequately see the data when enlarging the figure to >300% of its size and want to ensure that the resolution is maintained in the final published form so that others can enlarge the figure without it becoming pixelated. Alternatively, a larger version of the figure could be placed in the supplementary material.

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High resolution files are required for figures that will appear in the print journal and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. PNAS Plus articles will cost $2,150 per research article, with no additional charges for color figures or SI. For more information on preparing digital art, please review the PNAS digital art guidelines.

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Decision letter for 2016-00406RR

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406RR Decision Notification
Message: April 29, 2016

Title: "Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406RR
Authors: [redacted]

Dear Dr. Zhang,

We are pleased to inform you that the PNAS Editorial Board has given final approval of your article for publication. Peter Ladurner, the Editor who conducted the initial review of your manuscript [MS# 2016-00406RR], will also be informed of the decision.

Please note PNAS Plus articles are held to a strict 10-page maximum length. As your work is prepared for publication, you may be contacted by our printer to reduce the length of your article during the proof stage.

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<http://www.pnascentral.org/cgi-bin/main.plex?el=A2B4CgWM1C6GBcF3F3A9ftdbJEbeP06uSdnKALMAw9MwZ>

Sincerely yours,
Inder M. Verma
Editor-in-Chief

****

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Thursday, July 21, 2016 3:53 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavangh,
Thank you for your quick response and willingness to provide those communications. I hope you enjoy your conference and we will look for those items when you are back in the office.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Thursday, July 21, 2016 3:17 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: Re: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I'm out of the office at a conference this week, but I will send our correspondence with Dr. Zhang when I'm back in the office next week.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: "Yucel, Jennifer" <yucel.4@osu.edu>
Date: Thursday, July 21, 2016 at 11:28 AM
To: Kavanagh Etta <ekavanagh@nas.edu>
Cc: "hart.322@osu.edu" <hart.322@osu.edu>, "Moses, Randolph" <moses.2@osu.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

Dr. Hart forwarded your email to me. I am the University's research integrity officer and I am confirming that the university did receive these concerns and we are looking into this matter.

Would it be possible for you to share with me any communications that the journal had with Dr. Zhang during the publication process? Those communications would greatly assist us in the review of this matter and would be greatly appreciated. Once we have completed our review we will inform you of our determination.
Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: "Kavanagh, Etta" <EKavanagh@nas.edu>
Date: July 15, 2016 at 5:21:57 PM EDT
To: "'hart.322@osu.edu'" <hart.322@osu.edu>
Subject: FW: confidential complaint about scientific misconduct
Dear Dr. Hart,

I am contacting you regarding the complaint PNAS received regarding the paper "Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy" [redacted] We shared the complaint with the editor, Peter Ladurner, and he provided the following comments:

"I want to ensure my support for PNAS regarding manuscript 2016-00406RR.

The question if the gene identified is indeed the ivy AGP cought my attention after the initial submition. Please note that I questioned this finding myself. For their first revision I demanded that the authors have to add a phylogenetic tree showing the true AGP relationship of their protein. In their revision the authors provided the respective tree (and mass spec data) corroborating their finding.

I want to state that such data - under normal circumstances and if the data were generated according to best scientific practice - are sufficient to support the authors statement that their protein is an AGP.

However, if the genes for generating the phylogenetic tree were highly hand picked (and not selected according to their statement in the paper: "Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 were the closest relatives, as shown in Fig. S4A.") any tree can be fabricated.
I hope this is not true.

The next steps require detailed sequence analyses using BLAST, reciprocal BLAST, thourough protein alignments and phylogenetic analyses of the submitted sequence.

Depending on the result the authors need to provide raw data and lab book level information on gene isolation, details on clones with gene inserts from PCR and RACE experiments, sequencing raw files, details of their BLAST search settings and databases, information on the selection and generation of alignments and the phylogenetic tree, Mass Spectrometry raw data, details on GPI anchor bioinformatics.

Please let me know if I can help with the sequence analyses."

Is Ohio State University investigating these concerns? Should I contact the research integrity office? Thank you very much for your help.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer
To: Kavanagh, Etta
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct
Date: Friday, March 22, 2019 7:57:47 AM
Attachments: image001.png

Thanks Etta, very much appreciate you working with us to correct the scientific record.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, March 21, 2019 7:11 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Jen,

If the authors refuse to retract the paper, an Expression of Concern is a possibility, but we're hoping that won't be necessary. I'm in contact with Dr. Zhang, and I hope to have more information next week.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS
phone: 202-334-1386
fax: 202-334-2739
email: ekavanagh@nas.edu

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Thursday, March 21, 2019 3:40 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,
Thank you for the update. Our experience in this matter is that you will most likely not get a statement and they will not agree to a retraction. We definitely want to ensure that the bad data is removed from the scientific literature and will help however we can. Can PNAS put an expression of concern on the paper while waiting for the authors to comply? We just want to make sure that we are preventing people from using bad data.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, March 21, 2019 3:34 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Jen,

We will share the retraction statement with you once the authors have prepared it. Although we've asked them to prepare a statement, they are still pushing for a correction. I let Dr. Zhang know that if they did not prepare a statement, we would draft one for him. I'll be in touch once I have more information.

Sincerely,
Etta Kavanagh

Editorial Manager
PNAS
phone: 202-334-1386
fax: 202-334-2739
email: ekavanagh@nas.edu

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Tuesday, March 19, 2019 6:59 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Etta,
Thanks for the quick response and for the information. Would it be possible for the institution to review the proposed retraction statement? We have done this with other journals in the past, especially when the authors disagree with the need for a retraction. We want to make sure that the retraction is factually correct and doesn't attempt to concentrate blame inappropriately.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Monday, March 18, 2019 5:24 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Jen,

Our Editorial Board has determined that a retraction is the most appropriate course of action, and we have asked the authors to prepare a retraction statement. I'll let you know when we have a publication date scheduled. Thank you very much for your help with this matter.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, March 18, 2019 2:39 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,
I was wondering if you might be able to provide an update on the status of the editorial board review. Any information you can provide would be appreciated.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Friday, February 8, 2019 5:26 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,
Thank you for letting us know. I look forward to hearing from you at some point in the near future.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, February 7, 2019 2:43 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Schriver, Emily M. <schriver.21@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Jen,

I wanted to acknowledge receipt of your message. We are discussing the matter with our Editorial Board, and I will be in touch when I have more information.

Best regards,
Etta

Etta Kavanagh
Editorial Manager
PNAS
phone: 202-334-1386
fax: 202-334-2739
email: ekavanagh@nas.edu

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Tuesday, January 29, 2019 3:36 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Cc: Schriver, Emily M. <schriver.21@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Etta,
Attached please find a letter from me regarding this matter. Thank you for your patience with us while we worked through this difficult situation.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Wednesday, January 16, 2019 4:32 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Jen,

I am checking in to see if you have any updates. Thanks very much.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Thursday, December 20, 2018 7:35 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,

Please call me Jen. I am working on this but we have a number of institutional officials out for the holidays. I will get you a response as quickly as I can. I can say as indicated in my original email that our recommendation is that the paper needs to be retracted. We will provide more information to support our request shortly.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 18, 2018 1:36 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I am checking in to see if you have any updates on this matter. Thank you very much.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 10, 2018 9:57 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you, your assistance with this matter is much appreciated.
Best,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, December 6, 2018 10:17 AM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

We will hold off on making a decision on Dr. Zhang's request until we hear back from you.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Wednesday, December 5, 2018 8:31 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. I will need to speak to institutional leadership about this situation. That may take me a few days. Can I ask that you please hold on processing Dr. Zhang's request until I can get back to you? I will do my best to have that be in the next week or 2.
Thank you for any assistance on this you can provide.

Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 4, 2018 5:21 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

Dr. Zhang contacted our office to request that we publish a correction. He said that he did not think that a retraction was warranted and provided the attached documents.

Are you able to provide us with a copy of the report, or an official letter detailing the concerns with the paper? Thank you very much for your help.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 3, 2018 12:53 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

The Ohio State University has completed its investigation of this matter, relating to the [redacted]., 2016 PNAS 113 publication (attached). The university has determined that serious erroneous research was reported in the paper and we are requiring the authors to retract the paper.

I am writing to confirm that Dr. Mingjun Zhang [redacted] have contacted the journal to request the retraction. Can you please confirm that the authors have submitted this request for retraction? If not, please let me know.
Your assistance is much appreciated.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, July 26, 2016 5:11 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've attached the three letters that Dr. Zhang provided in response to the reviewer comments. Author letter 1 is the response provided with the first resubmission (2016-00406).

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Tuesday, July 26, 2016 12:00 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. Would it be possible to also get copies of what Dr. Zhang submitted with his revised manuscripts? Of particular importance to our review would be his response to reviewers comments and to the editor accompanying the first resubmission [manuscript # 2016-00406] in which he addressed the identity of IAGP with phylogenic analysis and mass spec.
Would it be possible to get this from you? Again, we greatly appreciate your assistance with this matter.
Sincerely yours,
Jen

THE OHIO STATE UNIVERSITY

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Monday, July 25, 2016 5:20 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've pasted below the four decision letters that our office sent to Dr. Yang. The manuscript was originally submitted in early 2015. It was rejected, but the authors were invited to resubmit it. As far as we can tell, these are the only correspondence that our office had with Dr. Zhang.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Decision letter for 2015-01638:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2015-01638 Decision Notification
Message: March 18, 2015

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2015-01638
Authors: [redacted]

Dear Dr. Zhang,

I apologize for the delay and regret to inform you that the PNAS Editorial Board has rejected your manuscript [MS# 2015-01638]. The expert who served as editor obtained 3 reviews, which are included below. After careful consideration, the editor decided that we cannot accept your manuscript.

Note that the PNAS License to Publish conveyed at initial submission is terminated.

However, because the reviewers think the work is of interest and the editor concurs, we are willing to consider one resubmission that constructively addresses all of the concerns raised in the critiques. The paper would have to satisfy both the reviewers and the editor, and new criticisms could arise upon re-evaluation. We cannot guarantee success and will be unable to consider further resubmissions.

Thank you for submitting your work to PNAS.

Sincerely yours,
Inder M. Verma

Editor-in-Chief

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Editor's Remarks to Author:

The manuscript cannot be published in PNAS in the current form. The reviewers raise several concerns regarding the isolated AGP, the glycosyl composition on silicon wafers, the nature of nanoparticle shown in the AFM images, and other technical problems. If the authors can address ALL of the reviewers' concerns, a resubmission could be possible.

The manuscript requires a substantial major revision accompanied by additional experiments to confirm the results presented. All comments of the reviewers need to be carefully considered. Specifically, all of the following matters should be expanded:

(1) In particular, it is questioned if the correct gene was isolated since important domains are lacking. There is convincing doubt that the isolated IAGP represents an AGP at all.

Therefore, I request a molecular phylogenetic tree including AGPs and Cytochrome Oxidase subunits to demonstrate the correct placement of IAGP within the AGP clade. In addition, as suggested by a reviewer, the degree of AG-glycosylation should be estimated as indicator that the cloned sequence is the correct AGP. In this respect, Mass Spectrometry also needs to be performed to corroborate that IAGP peptides are indeed present in abundance in purified particles.

(2) Further evidence that IAGP is in fact the Ivy adhesion molecule is required.

(3) The issue whether nanoparticles in AFM images are individual molecules or aggregates should be clarified.

(4) The nature of adhering residues on silicon wafers (Table 2) needs to be illuminated - can these represent cellulose or hemicellulose impurities?

(5) Stress strain data to show the rheological behavior need to be included.

(6) Methods descriptions need to be more detailed.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: No
Sufficient General Interest?: No
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
The paper investigates the molecular and bio-physical basis of the adhesive secreted by Ivy. A fraction of apparently spherical particles is isolated from exudate and is analysed biochemically and biophysically. It is suggested that the particles mainly consist of an arabinogalactan protein (AGP) that is termed IAGP. Based on a N-terminal peptide sequence of the deglycosylated protein, a putative cDNA for IAGP is cloned. The purified particles display low inherent viscosity and can form adhesive glues when combined with pectin and calcium. From a biophysical perspective the case looks more convincing than from a molecular biological one.

Comments on scientific content:

• AFM: It is not clear what is actually shown with the AFM images. Do the authors suggest that we are looking at individual AGP molecules or aggregates of many AGP and e.g. pectin molecules? Even though the texture of the mages suggests spherical particles the individual particles i.e. connected shapes are rarely ever spherical but are clusters. I am not an AFM expert but it seems helpful to image the particles at increasingly lower density so that individual particles might become the predominant structures. Otherwise it might mean that the spheres are not spheres after all.

• Fig. 1D and 1E. I don't know whether the methods applied for these figures are commonly known among physicists. As a molecular biologist I would find it helpful to get a better explanation of the method and why it should be applied.

• Tables 1 and 2: While the meaning of table 1 is relatively well explained and is interpreted that the linkages probably represent both AGII and pectic structures, the presence Glc, Xyl and Man (together >12 Mol%) is not explained. The sugars are reminiscent of hemicellulose but the terminal Glc is not. Is this an impurity or a component of the AGP/pectin particles? Table 2 describes the sugars found in adhering residues and the text states that "pectin is one of the main components ...". However the predominant monosaccharide in the remnant is glucose. Together with xylose and mannose it accounts for >65% meaning the main constituent is likely to be cellulose (indicating cell debris) or hemicellulose.

• When the IAGP sequence is presented in Figure 3 it is shown with a GPI anchor signal. Also in the scheme Figure 6 the GPI anchor structure, its attachment and release are shown in detail. However, the prediction tool that was used does not predict IAGP as GPI anchored (score = -76.05) and even the 'most likely' omega-site is different from the one shown in Figure 3. In my opinion the question whether or not IAGP is GPI anchored is irrelevant to the story and related suggestions may be removed without reducing the informative value.

• Figure 3C is mentioned in the context of the results section which is misleading. However it only shows a generic structure of a hypothetical AGP. In fact this is not even a classic (sic) AGP as claimed in the text. The literature uses the term "classical AGP" for extremely reduced proteins that contain only a backbone for O-glycosylation (typically XP repeats) and not other potentially functional protein domains (see Ref Ellis et al 2010).

• The sequence of IAGP does not resemble any known AGP. As such this is not remarkable because the important domains that define AGPs are so called AG-modules, stretches of XP, which are lacking from IAGP altogether. However, it bears remarkable sequence similarity to cytochrome oxidase subunit 5b-2. Are the authors sure that they have cloned the right cDNA?

• It would be important to estimate the degree of glycosylation of IAGP, especially when there is access to relatively large amounts of deglycosylated IAGP, MS/MS analysis could reveal hydroxyprolines in isolated positions which is an indirect indicator of AG-glycosylation. It would also confirm the identity of the suggested protein sequence which seems doubtful to me. Another possibility to confirm the nature of the cloned cDNA would be to express the sequence in a heterologous host (e.g. tobacco or Arabidopsis) and test its post-translational modifications.

References:

• The original papers where monoclonal antibodies were introduced and where they were characterized should be referenced.

• The SDS-PAGE procedure is not referenced.

Comments on presentation style:

• The English requires professional editing as the work contains numerous grammatical errors.

• Figure 3C is mentioned in the text before Figures 3A and B.

• Figure 5: the bottom part of the figure should be deleted and the glycan groups should be indicated just below the antibody names or even better above the bars.

• Figure 6 is far too elaborate and is not only confusing but also gives some quite false impressions (e.g. the existence of GPI-PLC in plants or the assumption that AGPs are glycosylated in the Golgi neither of which is proven). Only the relevant parts of the figure should be retained. Delete GPI-anchor, biosynthetic pathway (ribosomes, ER, Golgi), PLD/PLC.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes
Supplemental Material Warranted?: Yes

Comments:
The submission [redacted] describes in some detail a study to determine the mechanism of adhesion in English Ivy.

Approaches are varied and include (nano-)mechanical studies, proteomics, genomics and biochemistry. The conclusions are interesting and sound. They build upon previous work by this group, but offer significant new insight and 'proof of concept'. The methods and results are described well and concisely. The study of adhesion of plants lags behind that of animals and, for this reason, it is particularly interesting to see such a comprehensive report presented.

I have no major comments on the body of the text, except that I found the discussion to be rather short and lacking in depth. Similarly, I believe

that the significance statement could be strengthened. What are the implications of the findings? The possible applications and routes to exploitation? Page 13, line 11, should this measurement of lap shear not be expressed as a stress in Pa?

My other comments are minor and include:

Page 2, line 14 - I don't think "conversely" is the right word.

Line 18 - Here and throughout the MS, 'by' is usually more appropriate than 'via'.

Line 18 - "the characteristic physicochemical..."

Line 20 - Remove "revealed".

Page 5, line 4 - "developed previously"

Page 6, line 5 - "This result was..."

Line 9 - "verified". Here and throughout tenses are mixed, often in the same sentence. e.g. "verifies" and "displayed".

Page 9, line 8 - "those of pectin and sodium..."

Line 12 - "In the current work..."

Line 13 - "objective was to reveal..."

Line 15 - ": surface wetting"

Line 20 - "beneficial for surface wetting by the ivy..."

Page 12, line 14 - "The manner in which nanoparticles..."

Line 16 - "product prepared via emulsion polymerisation. It is composed of nano-sized polymer-based particles dispersed in an aqueous..."

Line 17 - "Upon application to a ..."

Page 13, line 6 - "developed by integrating..."

Line 10 - "variations in adhesion force".

Line 10 - "enhanced with increasing hardening time".

Page 14, line 3 - "interfere with the..."

Page 15, line 1 - this sentence does not seem to make sense.

Page 15, line 20 - replace "has been established early"

Page 16, line 1 - remove "for"

Page 21, line 22 - remove "for"

Page 23, line 7 - "Once removed..."

Page 24, line 19 - "described earlier"

Page 25, line 5 - replace "via" with "by"

Line 10 - same as above

Line 13 - "were employed as adherends"

Line 15 - remove "sample"

Reviewer #3:

Suitable Quality?: No
Sufficient General Interest?: Yes
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
This paper reports on the characterization of Ivy AGP and the adhesion strength it creates between glass slides.

There are a significant number of technical problems with the paper which make it unacceptable for publication in the current form. The comments are listed below.

1. The evidence to support that fact that the AGP is exuded and indeed the critical molecule used by the Ivy to adhere is not presented. The statement is made that it is "presumed" that the AGP is involved in the adhesion.

2. While the characterization of the AGP seems sound, it is not clear whether the nanoparticles are single molecules or aggregates. The aggregation number should be determined or at least estimated.

3. The rheological measurements are deficient. The method used is determining a component of shear yield stress? There is no stress strain data reported to show the rheological behavior. This is critical to the paper. I suggest that "pull off" measurements are performed where the stress is normal to the surface are made. Furthermore, stress strain curves should be reported. These will give an indication of the creep and yield type behavior. The current measurements are made for silica surfaces. This is a good model for the wall however the Ivy is a biological system. The true measurement if possible should be between the Ivy and a silica slide. Furthermore the methods description does not contain enough detail to permit replication.

4. There is no mechanism or model for the action of the AGP in the adhesion presented. The data is therefore not properly analyzed.

5. The grammar is poor. Generally the paper is not well written.

Given the above technical problems with the paper it is not acceptable for publication in PNAS as the research is not well designed and executed.

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Decision letter for 2016-00406:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406 Decision Notification
Message: February 9, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your resubmitted manuscript [MS# 2016-00406] has obtained 3 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due April 9, 2016. If you require additional time, notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the reviewers' comments. If you submitted a single PDF file at initial submission, you will be required to submit individual publication-ready files (e.g., Word file for manuscript text; EPS, TIFF, or high-resolution PDF for figures; Word file for tables; etc.)

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors are encouraged to use Supporting Information to show all necessary data, or to deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

Authors are responsible for obtaining waivers of any institutional open access mandates before publication with PNAS. Many institutions require that their authors transfer a nonexclusive author license to the institution and deposit the final author manuscript, with edits from peer review

incorporated, into institutional repositories. These mandates conflict with PNAS policy because authors must provide the National Academy of Sciences with an exclusive license to publish their work. Authors employed by an institution with such a mandate should obtain a waiver for the nonexclusive license and upload the file during resubmission. A list of OA mandates can be found online (http://roarmap.eprints.org/).

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A5B3CgWM5A6GBcF6I3A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>.

Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Heather Snijdewind
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) HSnijdewind@nas.edu

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Editor's Remarks to Author:

The authors have substantially revised the manuscript, performed additional experiments, and clarified all issues of the first three reviewers.

In summary, the authors: (1) included a phylogenetic tree corroborating the identity of the IAGP (2) performed Mass. spec analyses to confirm the IAGP protein sequence, (3) added a tensile test to confirm the molecular nature of the ivy-derived adhesive, (4) expanded AFM analyses to show that ivy nanoparticles observed in the AFM images are individual molecules rather than aggregates, (5) addressed the issue on hemicellulose impurities, (6) added stress strain data to show the rheological behavior, (7) included more details on methods, (8) removed GPI anchor discussion, (9) improved the model of ivy adhesion, (10), performed English and grammar proofreading.

After re-evaluation, several issues remain to be addressed. The authors are asked to improve style, add sub-headings, and re-write the discussion. Furthermore, the importance of pectin should be emphasized. The significance of the current work should be expanded, especially in the discussion section. Questions and suggestions regarding the figures need to be addressed. Further grammatical editing is requested.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: No
Procedures Described?: No

Comments:
The submission [redacted]. details the identification and characterisation of arabinogalactan-like proteins in the adhesive substance of English ivy. Further, the authors develop a mimic with which to test the principles that they argue govern adhesion by the species.

As I stated in my first review, I find the science to be sound and of sufficiently broad interest to be published in PNAS. The findings are significant and also represent an advance in the specific field of research.

My comments and requests for improvement of the original manuscript mainly revolved around style and presentation. Unfortunately this has not improved and is, if anything, worse in this more recent version. I think that this is a consequence of adding the extra material requested by review and the re-drafting/re-organising of the text that this required. Therefore, while I find the research interesting, sound and would like to see it published in PNAS, I am unable to recommend publication of the manuscript in its current form.

First and foremost the text needs to be thoroughly edited by a native English speaker. Before this is done, however, there is significant restructuring that must be undertaken. Currently the style of the paper is that of one long chronological narrative that includes methods, results and discussion. The discussion section simply re-states the general concepts as described throughout. In this format I find the paper very difficult to follow and it certainly does not help the reader to identify points of interest. It is my opinion that the authors should sub-divide the paper much more, halving the length of the results section, make more use of sub-headings and move all of the methods out to the methods section.

This would improve the clarity, readability and impact of the findings which, in the current format, are somewhat lost.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
This paper reports on the active component of English Ivy as the active adhesive component used by these plants. The paper is of general interest to the scientific community and the readership of PNAS. The revised version has been significantly improved in accord with the comments of all reviewers. I support publication in PNAS in the current form.

Reviewer #3:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
The authors isolated and characterized nanoparticles found in ivy-derived adhesive. They identified a component of the nanoparticles as being an arabinogalactan protein. They demonstrated that the nanoparticles were found in close proximity to pectin and interacted in a calcium-dependent manner. Bulk adhesion testing was done to demonstrate that all three components - nanoparticles, pectin, and calcium - resulted in a stronger adhesive bond than individual components. Overall, the experiments are interesting and performed well, but there are items that should be addressed before publication:

1. Overall, the abstract and introduction (and the results discussing Figures 1-3) seem to emphasize the importance of the arabinogalactan protein (AGP) nanoparticles in ivy adhesive. The importance of pectin is not emphasized strongly in the beginning part of the manuscript. However, it seems that pectin is at least equally (if not more) important to the adhesive as the nanoparticles are - in Figure 6, pectin by itself has higher bulk adhesion strength than nanoparticles by themselves. Thus, it is unclear why there is such an emphasis on AGP and not on pectin. Is it because that pectin was already known to be an important component and that this paper is characterizing the second component? Or is it because the AGPs are less viscous and thus can penetrate the substrates more and provide mechanical interlocking (although if this is the case, more experiments need to be performed to show this phenomenon). More context would be helpful.

2. There are some allusions to other botanic adhesives that have been studied (page 6, lines 778-785). The text states that "arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and Ficus pumila." Given that arabinogalactans and pectin are found to be the major components of the English ivy adhesive studied in this paper, it would be helpful to expand on the significance of the current work in the abstract, intro, and, especially, the discussion section. How are the findings significant and different from papers published about other plant adhesives? Are the results in this paper already known for other botanic adhesives or is there some additional insight provided here or something that is unique and compelling about the English ivy adhesive?

3. Figure 2: Panel D has a lane labeled as being from Fraction 1, but the legend only acknowledges Fractions 2-5. The text is also unclear - it states that "apart from the solvent peak designated as fraction 1," which could imply that Fraction 1 was not run on the gel. Also, one gel lane is labeled "Marker", but the legend refers to it as "Lane M."

4. Figure 3: For panel C, the figure legend states "Amino acids that are proposed to play adhesive function, comprising Ile, Leu, and Val, are indicated by black triangles." This statement is confusing as this reviewer interpreted the resulting experiments as showing that calcium mediated adhesive interactions between negatively charged residues on AGPs and pectin. Thus, it is not clear how Ile, Leu, and Val are involved in adhesion. Perhaps they are involved in adhesion to the substrates, but no data are shown to support the role of these amino acids in adhesion.

5. Figure 4B: This reviewer could not find the text description in the results section for the EDX data.

6. Figure 5: The second schematic (after evaporation) is confusing. Is pectin supposed to be the gray area that surrounds the yellow spheres? If so, the schematic only appears to be showing that calcium interacts directly with pectin (the gray portion) and not the AGP particles.

7. Figure 6D: The text (page 8, lines 984-993) would be stronger if it explained why the change in pH would affect adhesion. The text states "given that the cross-linking extent...is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here." However, rather than saying it should vary, it would be useful to explain why it is expected to vary at pH 4 and 9. Are we near the pKa values? Are residues no longer negatively charged at one or both of those values?

8. Page 7, lines 905-910: The text hypotheses that the nanoparticles allow for mechanical interlocking. However, are there no chemical adhesive forces (e.g., covalent bonds, van der Waals forces, etc) that are expected to occur?

9. Page 8, line 1035: It is unclear what is meant by "partially reflects the physiological implications of the associated low intrinsic viscosity."

10. Figure 6: For panel G, the meaning of the asterisk is not clear. The legend says the asterisk is compared to "EGTA-free adhesive composites containing 2 mM Ca2+"; however, the asterisk is placed above the composite group with Ca2+ and no EGTA. Also, it seems like it would make more sense if an ANOVA were performed and Tukey groups were shown so that one could determine which groups were statistically similar or different.

11. Materials and Methods (SI page 2, line 21): It would be helpful to report centrifugation in terms of g and not just rpm.

12. Figure S5: In the legend, please explain the green dotted line in panels C-F.

13. Figure S7: Given that there is an arrow with the word "agglomeration" connecting the two panels, it is not clear whether the left and right panels are from the same sample that have agglomerated over time. Or, are they from different samples or different areas of the same sample?

14. Figure S8: Given that two different secondary antibodies were used in the ELISA, is it valid to show results from all of the ELISAs on the same graph? In other words, are the absorbance values from ELISA wells using different secondary antibodies comparable? Were standard curves performed to show that the absorbance values would equate to the same amount?

15. The still image derived from movie S1 and the latter portion of movie S1 (that shows that ivy is stuck to a surface) do not appear to be in focus.

16. In the response to reviewers' comments, point 1.1 says that the nanostructures are "individual molecules consisting of covalently bonded AGP and pectin domain." What is meant by pectic domain? Is this a domain in the AGP that binds pectin? Or do you mean that the molecules contain AGP and short fragments of pectin (in which case they would not be individual molecules)?

17. Further grammatical editing of the paper would be helpful. For example, on page 4, there are multiple instances of "faction 4" instead of "fraction 4." There are other examples of minor grammatical or typographical errors in the manuscript.

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PUBLICATION INFORMATION AND CHARGES

High resolution files are required for figures that will appear in the main text of the article and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. For more information on preparing digital art, please review the PNAS digital art guidelines.

Authors are invited to submit scientifically interesting and visually arresting cover illustrations. To view accepted cover art, please visit the PNAS cover archive. Please note that images must be original and that exclusive rights to publish will convey to PNAS. If selected for the cover, the image also may be used further in promotional materials, including but not limited to brochures, advertisements, and posters. All submissions should be accompanied by a brief lay-language caption (50-60 words) and appropriate credit information (e.g., photograph courtesy of...). Images should be 21.5 cm wide by 22.5 cm high. Files should be .eps or .tif and should be in RGB (red, green, blue) color mode. Please submit electronic files with your revised manuscript in the PNAS online submission system or via e-mail to PNASCovers@nas.edu as soon as possible. If files are too large to e-mail, contact the PNAS office for ftp instructions or send the files on CD-ROM by courier to the PNAS Editorial Office (2101 Constitution Ave NW, PNAS 340, Washington DC 20418, phone: 202-334-2679). If you cannot submit electronic files, please contact the PNAS office for assistance. All submissions should include the manuscript number, author name, phone, fax, and email. See the Information for Authors for more details. Please note covers are selected from papers that have been assigned to an issue, after authors have returned proofs.

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Decision letter for 2016-00406R

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]

Subject: PNAS MS# 2016-00406R Decision Notification
Message: April 26, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406R
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your manuscript [MS# 2016-00406R] has obtained 2 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due June 25, 2016. If you require additional time, please notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the editor's and reviewer's comments.

We also require that you amend your title. We are seeking a descriptive title without the use of an em/en dash or colon (i.e. a single declarative title). This is non-negotiable and an exception will not be made.

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors must deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A2B3CgWM7B5GBcF2I1A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>

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Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Tom Myers
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) pnas@nas.edu

****

Editor Comments:
We thank the authors for their efforts to improve the mansucript. The manuscript mertis publication in PNAS. We kindly ask to provide a high resolution image of Fig. 4.

Reviewer Comments:
Reviewer #1:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
I find this revised manuscript to be a significant improvement on the former and in my opinion can be published in its current form. All amendments requested by previous reviews appear to have been included.

Reviewer #3:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
The authors have substantially revised and edited their paper in response to the reviewers' comments. The writing more clearly conveys the context of their work and is suitable for the general audience that reads PNAS. I support acceptance by PNAS for publication. I have only one minor comment that may be addressed in typesetting - Figure 4 has many important panels, and at its current size, it is difficult to see and interpret all of the data. I was able to adequately see the data when enlarging the figure to >300% of its size and want to ensure that the resolution is maintained in the final published form so that others can enlarge the figure without it becoming pixelated. Alternatively, a larger version of the figure could be placed in the supplementary material.

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PUBLICATION INFORMATION AND CHARGES

High resolution files are required for figures that will appear in the print journal and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. PNAS Plus articles will cost $2,150 per research article, with no additional charges for color figures or SI. For more information on preparing digital art, please review the PNAS digital art guidelines.

Authors are invited to submit scientifically interesting and visually arresting cover illustrations. To view accepted cover art, please visit the PNAS cover archive. Please note that images must be original and that exclusive rights to publish will convey to PNAS. If selected for the cover, the image also may be used further in promotional materials, including but not limited to brochures, advertisements, and posters. All submissions should be accompanied by a brief lay-language caption (50-60 words) and appropriate credit information (e.g., photograph courtesy of...). Images should be 21 cm wide by 22.5 cm high. Files should be .eps or .tif and should be in RGB (red, green, blue) color mode. Please submit electronic files with your revised manuscript in the PNAS online submission system or via e-mail to PNASCovers@nas.edu as soon as possible. All submissions should include the manuscript number, author name, phone, fax, and email. See the Information for Authors for more details. Please note covers are selected from papers that have been assigned to an issue, after authors have returned proofs.

****

Decision letter for 2016-00406RR

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406RR Decision Notification
Message: April 29, 2016

Title: "Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406RR
Authors: [redacted]

Dear Dr. Zhang,

We are pleased to inform you that the PNAS Editorial Board has given final approval of your article for publication. Peter Ladurner, the Editor who conducted the initial review of your manuscript [MS# 2016-00406RR], will also be informed of the decision.

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Sincerely yours,
Inder M. Verma
Editor-in-Chief

****

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Thursday, July 21, 2016 3:53 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavangh,
Thank you for your quick response and willingness to provide those communications. I hope you enjoy your conference and we will look for those items when you are back in the office.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Thursday, July 21, 2016 3:17 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: Re: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I'm out of the office at a conference this week, but I will send our correspondence with Dr. Zhang when I'm back in the office next week.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: "Yucel, Jennifer" <yucel.4@osu.edu>
Date: Thursday, July 21, 2016 at 11:28 AM
To: Kavanagh Etta <ekavanagh@nas.edu>
Cc: "hart.322@osu.edu" <hart.322@osu.edu>, "Moses, Randolph" <moses.2@osu.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

Dr. Hart forwarded your email to me. I am the University's research integrity officer and I am confirming that the university did receive these concerns and we are looking into this matter.

Would it be possible for you to share with me any communications that the journal had with Dr. Zhang during the publication process? Those communications would greatly assist us in the review of this matter and would be greatly appreciated. Once we have completed our review we will inform you of our determination.
Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: "Kavanagh, Etta" <EKavanagh@nas.edu>
Date: July 15, 2016 at 5:21:57 PM EDT
To: "'hart.322@osu.edu'" <hart.322@osu.edu>
Subject: FW: confidential complaint about scientific misconduct
Dear Dr. Hart,

I am contacting you regarding the complaint PNAS received regarding the paper "Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy" [redacted]. We shared the complaint with the editor, Peter Ladurner, and he

provided the following comments:

"I want to ensure my support for PNAS regarding manuscript 2016-00406RR.

The question if the gene identified is indeed the ivy AGP cought my attention after the initial submition. Please note that I questioned this finding myself. For their first revision I demanded that the authors have to add a phylogenetic tree showing the true AGP relationship of their protein. In their revision the authors provided the respective tree (and mass spec data) corroborating their finding.

I want to state that such data - under normal circumstances and if the data were generated according to best scientific practice - are sufficient to support the authors statement that their protein is an AGP.

However, if the genes for generating the phylogenetic tree were highly hand picked (and not selected according to their statement in the paper: "Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 were the closest relatives, as shown in Fig. S4A.") any tree can be fabricated.
I hope this is not true.

The next steps require detailed sequence analyses using BLAST, reciprocal BLAST, thourough protein alignments and phylogenetic analyses of the submitted sequence.

Depending on the result the authors need to provide raw data and lab book level information on gene isolation, details on clones with gene inserts from PCR and RACE experiments, sequencing raw files, details of their BLAST search settings and databases, information on the selection and generation of alignments and the phylogenetic tree, Mass Spectrometry raw data, details on GPI anchor bioinformatics.

Please let me know if I can help with the sequence analyses."

Is Ohio State University investigating these concerns? Should I contact the research integrity office? Thank you very much for your help.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer
To: Salsbury, Daniel
Cc: Schriver, Emily M.; Lester, Brandon
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct (16-00406)
Date: Friday, March 29, 2019 1:44:28 PM
Attachments: image002.png
image003.png
NSF RM Regulations - 45-CFR-689.pdf

Dear Dr. Salsbury,

The primary funding agency for the work in question was NSF and they are currently reviewing the matter as required under 45 CFR Part 689 (attached). OSU has not yet received NSF's final review and determination but it is important to point out that OSU's findings are final and will not change regardless of NSF's findings. The NSF investigator who is handling this case is Dr. Erik Runko erunko@nsf.gov and he may be able to provide you with more information regarding this matter.
Best,
Jen

The Ohio State University
Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Salsbury, Daniel <DSalsbur@nas.edu>
Sent: Friday, March 29, 2019 11:54 AM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: FW: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct (16-00406)

Dear Dr. Yucel,
We have been informed by a representative of Mingjun Zhang that he has appealed to the National Science Foundation for review of OSU's findings. Can you confirm and provide any additional information regarding the status of this matter?

Sincerely,
Daniel Salsbury

Daniel Salsbury
Deputy Executive Editor
PNAS
2101 Constitution Ave, NW
Washington, DC 20418
Ph: 202-334-2682
dsalsbur@nas.edu

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Tuesday, January 29, 2019 3:36 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Cc: Schriver, Emily M. <schriver.21@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Etta,
Attached please find a letter from me regarding this matter. Thank you for your patience with us while we worked through this difficult situation.
Best,
Jen

The Ohio State University
Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Wednesday, January 16, 2019 4:32 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Jen,

I am checking in to see if you have any updates. Thanks very much.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Thursday, December 20, 2018 7:35 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Hi Etta,
Please call me Jen. I am working on this but we have a number of institutional officials out for the holidays. I will get you a response as quickly as I can. I can say as indicated in my original email that our recommendation is that the paper needs to be retracted. We will provide more information to support our request shortly.
Best,
Jen

The Ohio State University

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 18, 2018 1:36 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I am checking in to see if you have any updates on this matter. Thank you very much.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 10, 2018 9:57 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you, your assistance with this matter is much appreciated.
Best,
Jen

The Ohio State University

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Thursday, December 6, 2018 10:17 AM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

We will hold off on making a decision on Dr. Zhang's request until we hear back from you.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Wednesday, December 5, 2018 8:31 AM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. I will need to speak to institutional leadership about this situation. That may take me a few days. Can I ask that you please hold on processing Dr. Zhang's request until I can get back to you? I will do my best to have that be in the next week or 2.
Thank you for any assistance on this you can provide.
Sincerely yours,
Jen

The Ohio State University

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, December 4, 2018 5:21 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

Dr. Zhang contacted our office to request that we publish a correction. He said that he did not think that a retraction was warranted and provided the attached documents.

Are you able to provide us with a copy of the report, or an official letter detailing the concerns with the paper? Thank you very much for your help.

Sincerely,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer <yucel.4@osu.edu>
Sent: Monday, December 3, 2018 12:53 PM
To: Kavanagh, Etta <EKavanagh@nas.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

The Ohio State University has completed its investigation of this matter, relating to the [redacted] 2016 PNAS 113 publication (attached). The university has determined that serious erroneous research was reported in the paper and we are requiring the authors to retract the paper.

I am writing to confirm that Dr. Mingjun Zhang [redacted] have contacted the journal to request the retraction. Can you please confirm that the authors have submitted this request for retraction? If not, please let me know.
Your assistance is much appreciated.
Sincerely yours,
Jen

The Ohio State University

Jennifer K. Yucel, Ph.D. | Associate Vice President & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 304, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta <EKavanagh@nas.edu>
Sent: Tuesday, July 26, 2016 5:11 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've attached the three letters that Dr. Zhang provided in response to the reviewer comments. Author letter 1 is the response provided with the first resubmission (2016-00406).

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Tuesday, July 26, 2016 12:00 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,
Thank you for providing this information, it is very helpful. Would it be possible to also get copies of what Dr. Zhang submitted with his revised manuscripts? Of particular importance to our review would be his response to reviewers comments and to the editor accompanying the first resubmission [manuscript # 2016-00406] in which he addressed the identity of IAGP with phylogenic analysis and mass spec.
Would it be possible to get this from you? Again, we greatly appreciate your assistance with this matter.
Sincerely yours,
Jen

The Ohio State University
Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

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From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Monday, July 25, 2016 5:20 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I've pasted below the four decision letters that our office sent to Dr. Yang. The manuscript was originally submitted in early 2015. It was rejected, but the authors were invited to resubmit it. As far as we can tell, these are the only correspondence that our office had with Dr. Zhang.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

Decision letter for 2015-01638:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2015-01638 Decision Notification
Message: March 18, 2015

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2015-01638
Authors: [redacted]

Dear Dr. Zhang,

I apologize for the delay and regret to inform you that the PNAS Editorial Board has rejected your manuscript [MS# 2015-01638]. The expert who served as editor obtained 3 reviews, which are included below. After careful consideration, the editor decided that we cannot accept your manuscript.

Note that the PNAS License to Publish conveyed at initial submission is terminated.

However, because the reviewers think the work is of interest and the editor concurs, we are willing to consider one resubmission that constructively addresses all of the concerns raised in the critiques. The paper would have to satisfy both the reviewers and the editor, and new criticisms could arise upon re-evaluation. We cannot guarantee success and will be unable to consider further resubmissions.

Thank you for submitting your work to PNAS.

Sincerely yours,
Inder M. Verma
Editor-in-Chief

****
Editor's Remarks to Author:

The manuscript cannot be published in PNAS in the current form. The reviewers raise several concerns regarding the isolated AGP, the glycosyl composition on silicon wafers, the nature of nanoparticle shown in the AFM images, and other technical problems. If the authors can address ALL of the reviewers' concerns, a resubmission could be possible.

The manuscript requires a substantial major revision accompanied by additional experiments to confirm the results presented. All comments of the reviewers need to be carefully considered. Specifically, all of the following matters should be expanded:

(1) In particular, it is questioned if the correct gene was isolated since important domains are lacking. There is convincing doubt that the isolated IAGP represents an AGP at all.

Therefore, I request a molecular phylogenetic tree including AGPs and Cytochrome Oxidase subunits to demonstrate the correct placement of IAGP within the AGP clade. In addition, as suggested by a reviewer, the degree of AG-glycosylation should be estimated as indicator that the cloned sequence is the correct AGP. In this respect, Mass Spectrometry also needs to be performed to corroborate that IAGP peptides are indeed present in abundance in purified particles.

(2) Further evidence that IAGP is in fact the Ivy adhesion molecule is required.

(3) The issue whether nanoparticles in AFM images are individual molecules or aggregates should be clarified.

(4) The nature of adhering residues on silicon wafers (Table 2) needs to be illuminated - can these represent cellulose or hemicellulose impurities?

(5) Stress strain data to show the rheological behavior need to be included.

(6) Methods descriptions need to be more detailed.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: No
Sufficient General Interest?: No
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:

The paper investigates the molecular and bio-physical basis of the adhesive secreted by Ivy. A fraction of apparently spherical particles is isolated from exudate and is analysed biochemically and biophysically. It is suggested that the particles mainly consist of an arabinogalactan protein (AGP) that is termed IAGP. Based on a N-terminal peptide sequence of the deglycosylated protein, a putative cDNA for IAGP is cloned. The purified particles display low inherent viscosity and can form adhesive glues when combined with pectin and calcium. From a biophysical perspective the case looks more convincing than from a molecular biological one.

Comments on scientific content:

• AFM: It is not clear what is actually shown with the AFM images. Do the authors suggest that we are looking at individual AGP molecules or aggregates of many AGP and e.g. pectin molecules? Even though the texture of the mages suggests spherical particles the individual particles i.e. connected shapes are rarely ever spherical but are clusters. I am not an AFM expert but it seems helpful to image the particles at increasingly lower density so that individual particles might become the predominant structures. Otherwise it might mean that the spheres are not spheres after all.

• Fig. 1D and 1E. I don't know whether the methods applied for these figures are commonly known among physicists. As a molecular biologist I would find it helpful to get a better explanation of the method and why it should be applied.

• Tables 1 and 2: While the meaning of table 1 is relatively well explained and is interpreted that the linkages probably represent both AGII and pectic structures, the presence Glc, Xyl and Man (together >12 Mol%) is not explained. The sugars are reminiscent of hemicellulose but the terminal Glc is not. Is this an impurity or a component of the AGP/pectin particles? Table 2 describes the sugars found in adhering residues and the text states that "pectin is one of the main components ...". However the predominant monosaccharide in the remnant is glucose. Together with xylose and mannose it accounts for >65% meaning the main constituent is likely to be cellulose (indicating cell debris) or hemicellulose.

• When the IAGP sequence is presented in Figure 3 it is shown with a GPI anchor signal. Also in the scheme Figure 6 the GPI anchor structure, its attachment and release are shown in detail. However, the prediction tool that was used does not predict IAGP as GPI anchored (score = -76.05) and even the 'most likely' omega-site is different from the one shown in Figure 3. In my opinion the question whether or not IAGP is GPI anchored is irrelevant to the story and related suggestions may be removed without reducing the informative value.

• Figure 3C is mentioned in the context of the results section which is misleading. However it only shows a generic structure of a hypothetical AGP. In fact this is not even a classic (sic) AGP as claimed in the text. The literature uses the term "classical AGP" for extremely reduced proteins that contain only a backbone for O-glycosylation (typically XP repeats) and not other potentially functional protein domains (see Ref Ellis et al 2010).

• The sequence of IAGP does not resemble any known AGP. As such this is not remarkable because the important domains that define AGPs are so called AG-modules, stretches of XP, which are lacking from IAGP altogether. However, it bears remarkable sequence similarity to cytochrome oxidase subunit 5b-2. Are the authors sure that they have cloned the right cDNA?

• It would be important to estimate the degree of glycosylation of IAGP, especially when there is access to relatively large amounts of deglycosylated IAGP, MS/MS analysis could reveal hydroxyprolines in isolated positions which is an indirect indicator of AG-glycosylation. It would also confirm the identity of the suggested protein sequence which seems doubtful to me. Another possibility to confirm the nature of the cloned cDNA would be to express the sequence in a heterologous host (e.g. tobacco or Arabidopsis) and test its post-translational modifications.

References:

• The original papers where monoclonal antibodies were introduced and where they were characterized should be referenced.

• The SDS-PAGE procedure is not referenced.

Comments on presentation style:

• The English requires professional editing as the work contains numerous grammatical errors.

• Figure 3C is mentioned in the text before Figures 3A and B.

• Figure 5: the bottom part of the figure should be deleted and the glycan groups should be indicated just below the antibody names or even better above the bars.

• Figure 6 is far too elaborate and is not only confusing but also gives some quite false impressions (e.g. the existence of GPI-PLC in plants or the assumption that AGPs are glycosylated in the Golgi neither of which is proven). Only the relevant parts of the figure should be retained. Delete GPI-anchor, biosynthetic pathway (ribosomes, ER, Golgi), PLD/PLC.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes
Supplemental Material Warranted?: Yes

Comments:
The submission [redacted] describes in some detail a study to determine the mechanism of adhesion in English Ivy.

Approaches are varied and include (nano-)mechanical studies, proteomics, genomics and biochemistry. The conclusions are interesting and sound. They build upon previous work by this group, but offer significant new insight and 'proof of concept'. The methods and results are described well and concisely. The study of adhesion of plants lags behind that of animals and, for this reason, it is particularly interesting to see such a comprehensive report presented.

I have no major comments on the body of the text, except that I found the discussion to be rather short and lacking in depth. Similarly, I believe that the significance statement could be strengthened. What are the implications of the findings? The possible applications and routes to exploitation? Page 13, line 11, should this measurement of lap shear not be expressed as a stress in Pa?

My other comments are minor and include:

Page 2, line 14 - I don't think "conversely" is the right word.

Line 18 - Here and throughout the MS, 'by' is usually more appropriate than 'via'.

Line 18 - "the characteristic physicochemical..."

Line 20 - Remove "revealed".

Page 5, line 4 - "developed previously"

Page 6, line 5 - "This result was..."

Line 9 - "verified". Here and throughout tenses are mixed, often in the same sentence. e.g. "verifies" and "displayed".

Page 9, line 8 - "those of pectin and sodium..."

Line 12 - "In the current work..."

Line 13 - "objective was to reveal..."

Line 15 - ": surface wetting"

Line 20 - "beneficial for surface wetting by the ivy..."

Page 12, line 14 - "The manner in which nanoparticles..."

Line 16 - "product prepared via emulsion polymerisation. It is composed of nano-sized polymer-based particles dispersed in an aqueous..."

Line 17 - "Upon application to a ..."

Page 13, line 6 - "developed by integrating..."

Line 10 - "variations in adhesion force".

Line 10 - "enhanced with increasing hardening time".

Page 14, line 3 - "interfere with the..."

Page 15, line 1 - this sentence does not seem to make sense.

Page 15, line 20 - replace "has been established early"

Page 16, line 1 - remove "for"

Page 21, line 22 - remove "for"

Page 23, line 7 - "Once removed..."

Page 24, line 19 - "described earlier"

Page 25, line 5 - replace "via" with "by"

Line 10 - same as above

Line 13 - "were employed as adherends"

Line 15 - remove "sample"

Reviewer #3:

Suitable Quality?: No
Sufficient General Interest?: Yes
Conclusions Justified?: No
Clearly Written?: No
Procedures Described?: No
Supplemental Material Warranted?: Yes

Comments:
This paper reports on the characterization of Ivy AGP and the adhesion strength it creates between glass slides.

There are a significant number of technical problems with the paper which make it unacceptable for publication in the current form. The comments are listed below.

1. The evidence to support that fact that the AGP is exuded and indeed the critical molecule used by the Ivy to adhere is not presented. The statement is made that it is "presumed" that the AGP is involved in the adhesion.

2. While the characterization of the AGP seems sound, it is not clear whether the nanoparticles are single molecules or aggregates. The aggregation number should be determined or at least estimated.

3. The rheological measurements are deficient. The method used is determining a component of shear yield stress? There is no stress strain data reported to show the rheological behavior. This is critical to the paper. I suggest that "pull off" measurements are performed where the stress is normal to the surface are made. Furthermore, stress strain curves should be reported. These will give an indication of the creep and yield type behavior. The current measurements are made for silica surfaces. This is a good model for the wall however the Ivy is a biological system. The true measurement if possible should be between the Ivy and a silica slide. Furthermore the methods description does not contain enough detail to permit replication.

4. There is no mechanism or model for the action of the AGP in the adhesion presented. The data is therefore not properly analyzed.

5. The grammar is poor. Generally the paper is not well written.

Given the above technical problems with the paper it is not acceptable for publication in PNAS as the research is not well designed and executed.

****
Decision letter for 2016-00406:

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406 Decision Notification
Message: February 9, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your resubmitted manuscript [MS# 2016-00406] has obtained 3 reviews, which are included below. The

editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due April 9, 2016. If you require additional time, notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the reviewers' comments. If you submitted a single PDF file at initial submission, you will be required to submit individual publication-ready files (e.g., Word file for manuscript text; EPS, TIFF, or high-resolution PDF for figures; Word file for tables; etc.)

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors are encouraged to use Supporting Information to show all necessary data, or to deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

Authors are responsible for obtaining waivers of any institutional open access mandates before publication with PNAS. Many institutions require that their authors transfer a nonexclusive author license to the institution and deposit the final author manuscript, with edits from peer review incorporated, into institutional repositories. These mandates conflict with PNAS policy because authors must provide the National Academy of Sciences with an exclusive license to publish their work. Authors employed by an institution with such a mandate should obtain a waiver for the nonexclusive license and upload the file during resubmission. A list of OA mandates can be found online (http://roarmap.eprints.org/).

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A5B3CgWM5A6GBcF6I3A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>.

Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Heather Snijdewind
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) HSnijdewind@nas.edu

****
Editor's Remarks to Author:

The authors have substantially revised the manuscript, performed additional experiments, and clarified all issues of the first three reviewers.

In summary, the authors: (1) included a phylogenetic tree corroborating the identity of the IAGP (2) performed Mass. spec analyses to confirm the IAGP protein sequence, (3) added a tensile test to confirm the molecular nature of the ivy-derived adhesive, (4) expanded AFM analyses to show that ivy nanoparticles observed in the AFM images are individual molecules rather than aggregates, (5) addressed the issue on hemicellulose impurities, (6) added stress strain data to show the rheological behavior, (7) included more details on methods, (8) removed GPI anchor discussion, (9) improved the model of ivy adhesion, (10), performed English and grammar proofreading.

After re-evaluation, several issues remain to be addressed. The authors are asked to improve style, add sub-headings, and re-write the discussion. Furthermore, the importance of pectin should be emphasized. The significance of the current work should be expanded, especially in the discussion section. Questions and suggestions regarding the figures need to be addressed. Further grammatical editing is requested.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: No
Procedures Described?: No

Comments:

The submission [redacted]. details the identification and characterisation of arabinogalactan-like proteins in the adhesive substance of English ivy. Further, the authors develop a mimic with which to test the principles that they argue govern adhesion by the species.

As I stated in my first review, I find the science to be sound and of sufficiently broad interest to be published in PNAS. The findings are significant and also represent an advance in the specific field of research.

My comments and requests for improvement of the original manuscript mainly revolved around style and presentation. Unfortunately this has not improved and is, if anything, worse in this more recent version. I think that this is a consequence of adding the extra material requested by review and the re-drafting/re-organising of the text that this required. Therefore, while I find the research interesting, sound and would like to see it published in PNAS, I am unable to recommend publication of the manuscript in its current form.

First and foremost the text needs to be thoroughly edited by a native English speaker. Before this is done, however, there is significant restructuring that must be undertaken. Currently the style of the paper is that of one long chronological narrative that includes methods, results and discussion. The discussion section simply re-states the general concepts as described throughout. In this format I find the paper very difficult to follow and it certainly does not help the reader to identify points of interest. It is my opinion that the authors should sub-divide the paper much more, halving the length of the results section, make more use of sub-headings and move all of the methods out to the methods section. This would improve the clarity, readability and impact of the findings which, in the current format, are somewhat lost.

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
This paper reports on the active component of English Ivy as the active adhesive component used by these plants. The paper is of general interest to the scientific community and the readership of PNAS. The revised version has been significantly improved in accord with the comments of all reviewers. I support publication in PNAS in the current form.

Reviewer #3:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes

Comments:
The authors isolated and characterized nanoparticles found in ivy-derived adhesive. They identified a component of the nanoparticles as being an arabinogalactan protein. They demonstrated that the nanoparticles were found in close proximity to pectin and interacted in a calcium-dependent manner. Bulk adhesion testing was done to demonstrate that all three components - nanoparticles, pectin, and calcium - resulted in a stronger adhesive bond than individual components. Overall, the experiments are interesting and performed well, but there are items that should be addressed before publication:

1. Overall, the abstract and introduction (and the results discussing Figures 1-3) seem to emphasize the importance of the arabinogalactan protein (AGP) nanoparticles in ivy adhesive. The importance of pectin is not emphasized strongly in the beginning part of the manuscript. However, it seems that pectin is at least equally (if not more) important to the adhesive as the nanoparticles are - in Figure 6, pectin by itself has higher bulk adhesion strength than nanoparticles by themselves. Thus, it is unclear why there is such an emphasis on AGP and not on pectin. Is it because that pectin was already known to be an important component and that this paper is characterizing the second component? Or is it because the AGPs are less viscous and thus can penetrate the substrates more and provide mechanical interlocking (although if this is the case, more experiments need to be performed to show this phenomenon). More context would be helpful.

2. There are some allusions to other botanic adhesives that have been studied (page 6, lines 778-785). The text states that "arabinogalactans and pectic substances have been identified to be two of the predominant components in the majority of botanic adhesives, including those obtained from the climbing organs of Virginia creeper, Boston ivy, and Ficus pumila." Given that arabinogalactans and pectin are found to be the major components of the English ivy adhesive studied in this paper, it would be helpful to expand on the significance of the current work in the abstract, intro, and, especially, the discussion section. How are the findings significant and different from papers published about other plant adhesives? Are the results in this paper already known for other botanic adhesives or is there some additional insight provided here or something that is unique and compelling about the English ivy adhesive?

3. Figure 2: Panel D has a lane labeled as being from Fraction 1, but the legend only acknowledges Fractions 2-5. The text is also unclear - it states that "apart from the solvent peak designated as fraction 1," which could imply that Fraction 1 was not run on the gel. Also, one gel lane is labeled "Marker", but the legend refers to it as "Lane M."

4. Figure 3: For panel C, the figure legend states "Amino acids that are proposed to play adhesive function, comprising Ile, Leu, and Val, are indicated by black triangles." This statement is confusing as this reviewer interpreted the resulting experiments as showing that calcium mediated adhesive interactions between negatively charged residues on AGPs and pectin. Thus, it is not clear how Ile, Leu, and Val are involved in adhesion. Perhaps they are involved in adhesion to the substrates, but no data are shown to support the role of these amino acids in adhesion.

5. Figure 4B: This reviewer could not find the text description in the results section for the EDX data.

6. Figure 5: The second schematic (after evaporation) is confusing. Is pectin supposed to be the gray area that surrounds the yellow spheres? If so, the schematic only appears to be showing that calcium interacts directly with pectin (the gray portion) and not the AGP particles.

7. Figure 6D: The text (page 8, lines 984-993) would be stronger if it explained why the change in pH would affect adhesion. The text states "given that the cross-linking extent...is determined by the calcium-modulated electrostatic interaction which is susceptible to the pH variations, it is reasonable to expect this pH-responsive event here." However, rather than saying it should vary, it would be useful to explain why it is expected to vary at pH 4 and 9. Are we near the pKa values? Are residues no longer negatively charged at one or both of those values?

8. Page 7, lines 905-910: The text hypotheses that the nanoparticles allow for mechanical interlocking. However, are there no chemical adhesive forces (e.g., covalent bonds, van der Waals forces, etc) that are expected to occur?

9. Page 8, line 1035: It is unclear what is meant by "partially reflects the physiological implications of the associated low intrinsic viscosity."

10. Figure 6: For panel G, the meaning of the asterisk is not clear. The legend says the asterisk is compared to "EGTA-free adhesive composites containing 2 mM Ca2+"; however, the asterisk is placed above the composite group with Ca2+ and no EGTA. Also, it seems like it would make more sense if an ANOVA were performed and Tukey groups were shown so that one could determine which groups were statistically similar or different.

11. Materials and Methods (SI page 2, line 21): It would be helpful to report centrifugation in terms of g and not just rpm.

12. Figure S5: In the legend, please explain the green dotted line in panels C-F.

13. Figure S7: Given that there is an arrow with the word "agglomeration" connecting the two panels, it is not clear whether the left and right panels are from the same sample that have agglomerated over time. Or, are they from different samples or different areas of the same sample?

14. Figure S8: Given that two different secondary antibodies were used in the ELISA, is it valid to show results from all of the ELISAs on the same graph? In other words, are the absorbance values from ELISA wells using different secondary antibodies comparable? Were standard curves performed to show that the absorbance values would equate to the same amount?

15. The still image derived from movie S1 and the latter portion of movie S1 (that shows that ivy is stuck to a surface) do not appear to be in focus.

16. In the response to reviewers' comments, point 1.1 says that the nanostructures are "individual molecules consisting of covalently bonded AGP and pectin domain." What is meant by pectic domain? Is this a domain in the AGP that binds pectin? Or do you mean that the molecules contain AGP and short fragments of pectin (in which case they would not be individual molecules)?

17. Further grammatical editing of the paper would be helpful. For example, on page 4, there are multiple instances of "faction 4" instead of "fraction 4." There are other examples of minor grammatical or typographical errors in the manuscript.

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High resolution files are required for figures that will appear in the main text of the article and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. For more information on preparing digital art, please review the PNAS digital art guidelines.

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Decision letter for 2016-00406R

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406R Decision Notification
Message: April 26, 2016

Title: "Nano-spherical Arabinogalactan Protein: A Key Component in the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406R
Authors: [redacted]

Dear Dr. Zhang,

The expert who is serving as editor for your manuscript [MS# 2016-00406R] has obtained 2 reviews, which are included below. The editor requests that you constructively address the concerns of the reviewers in a revised manuscript. Please note that multiple revisions are rarely permitted and there is no guarantee that the paper will be accepted.

PNAS allows 60 days to submit a revision. Your revision is due June 25, 2016. If you require additional time, please notify the editorial office and we will extend the due date.

When submitting revised materials, we require that you include a revision cover letter with a detailed point-by-point response to the editor's and reviewer's comments.

We also require that you amend your title. We are seeking a descriptive title without the use of an em/en dash or colon (i.e. a single declarative title). This is non-negotiable and an exception will not be made.

Please note that statements such as "data not shown" and "personal communication" cannot be used to support claims in the work. References to "data not shown" are not allowed and should be removed prior to submission. Authors must deposit their data in a publicly accessible database if posting as Supporting Information is not possible.

When you are ready to submit your revised manuscript, go to the site and begin your submission: <http://www.pnascentral.org/cgi-bin/main.plex?el=A2B3CgWM7B5GBcF2I1A9ftdRsbOzWK8lCQkf8qGnDnfLQZ>

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Adding, removing, or reordering your author line will require approval from all coauthors before the editorial office can proceed with your manuscript. Also, if you would like to add an additional corresponding author on your manuscript, please note this in the "Comments for Editorial Staff" text box when completing your revision.

Thank you for submitting to PNAS. We look forward to receiving the revision.

Sincerely yours,
Tom Myers
PNAS Editorial Office
(p) 202.334.2679
(f) 202.334.2739
(e) pnas@nas.edu

****

Editor Comments:
We thank the authors for their efforts to improve the mansucript. The manuscript mertis publication in PNAS. We kindly ask to provide a high

resolution image of Fig. 4.

Reviewer Comments:
Reviewer #1:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
I find this revised manuscript to be a significant improvement on the former and in my opinion can be published in its current form. All amendments requested by previous reviews appear to have been included.

Reviewer #3:

Suitable Quality?:
Yes

Sufficient General Interest?:
Yes

Conclusions Justified?:
Yes

Clearly Written?:
Yes

Procedures Described?:
Yes

Comments (Required):
The authors have substantially revised and edited their paper in response to the reviewers' comments. The writing more clearly conveys the context of their work and is suitable for the general audience that reads PNAS. I support acceptance by PNAS for publication. I have only one minor comment that may be addressed in typesetting - Figure 4 has many important panels, and at its current size, it is difficult to see and interpret all of the data. I was able to adequately see the data when enlarging the figure to >300% of its size and want to ensure that the resolution is maintained in the final published form so that others can enlarge the figure without it becoming pixelated. Alternatively, a larger version of the figure could be placed in the supplementary material.

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High resolution files are required for figures that will appear in the print journal and must be uploaded with the final version of your manuscript. Resolution of at least 1200 dpi is needed for all line art, 600 dpi for images that combine line art with photographs/halftones, and 300 dpi for color or grayscale photographic images. PNAS Plus articles will cost $2,150 per research article, with no additional charges for color figures or SI. For more information on preparing digital art, please review the PNAS digital art guidelines.

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credit information (e.g., photograph courtesy of...). Images should be 21 cm wide by 22.5 cm high. Files should be .eps or .tif and should be in RGB (red, green, blue) color mode. Please submit electronic files with your revised manuscript in the PNAS online submission system or via e-mail to PNASCovers@nas.edu as soon as possible. All submissions should include the manuscript number, author name, phone, fax, and email. See the Information for Authors for more details. Please note covers are selected from papers that have been assigned to an issue, after authors have returned proofs.

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Decision letter for 2016-00406RR

From: pnas@nas.edu
To: zhang.4882@osu.edu
CC: [redacted]
Subject: PNAS MS# 2016-00406RR Decision Notification
Message: April 29, 2016

Title: "Nano-spherical Arabinogalactan Proteins are a Key Component of the High-strength Adhesive Secreted by English Ivy"
Tracking #: 2016-00406RR
Authors: [redacted]

Dear Dr. Zhang,

We are pleased to inform you that the PNAS Editorial Board has given final approval of your article for publication. Peter Ladurner, the Editor who conducted the initial review of your manuscript [MS# 2016-00406RR], will also be informed of the decision.

Please note PNAS Plus articles are held to a strict 10-page maximum length. As your work is prepared for publication, you may be contacted by our printer to reduce the length of your article during the proof stage.

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<http://www.pnascentral.org/cgi-bin/main.plex?el=A2B4CgWM1C6GBcF3F3A9ftdbJEbeP06uSdnKALMAw9MwZ>

Sincerely yours,
Inder M. Verma
Editor-in-Chief

****

From: Yucel, Jennifer [mailto:yucel.4@osu.edu]
Sent: Thursday, July 21, 2016 3:53 PM
To: Kavanagh, Etta
Cc: Hart, Rich; Moses, Randolph
Subject: RE: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavangh,
Thank you for your quick response and willingness to provide those communications. I hope you enjoy your conference and we will look for those items when you are back in the office.
Best,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 | yucel.4@osu.edu

From: Kavanagh, Etta [mailto:EKavanagh@nas.edu]
Sent: Thursday, July 21, 2016 3:17 PM
To: Yucel, Jennifer <yucel.4@osu.edu>
Cc: Hart, Rich <hart.322@osu.edu>; Moses, Randolph <moses.2@osu.edu>
Subject: Re: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Yucel,

I'm out of the office at a conference this week, but I will send our correspondence with Dr. Zhang when I'm back in the office next week.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

From: "Yucel, Jennifer" <yucel.4@osu.edu>
Date: Thursday, July 21, 2016 at 11:28 AM
To: Kavanagh Etta <ekavanagh@nas.edu>
Cc: "hart.322@osu.edu" <hart.322@osu.edu>, "Moses, Randolph" <moses.2@osu.edu>
Subject: CONFIDENTIAL -- FW: confidential complaint about scientific misconduct

Dear Dr. Kavanagh,

Dr. Hart forwarded your email to me. I am the University's research integrity officer and I am confirming that the university did receive these concerns and we are looking into this matter.

Would it be possible for you to share with me any communications that the journal had with Dr. Zhang during the publication process? Those communications would greatly assist us in the review of this matter and would be greatly appreciated. Once we have completed our review we will inform you of our determination.

Sincerely yours,
Jen

Jennifer K. Yucel, Ph.D. | Director & Research Integrity Officer
The Ohio State University |Office of Research Compliance| 1960 Kenny Road, Room 208, Columbus, OH 43210 | (614) 247-8831 |
yucel.4@osu.edu

From: "Kavanagh, Etta" <EKavanagh@nas.edu>
Date: July 15, 2016 at 5:21:57 PM EDT
To: "'hart.322@osu.edu'" <hart.322@osu.edu>
Subject: FW: confidential complaint about scientific misconduct
Dear Dr. Hart,

I am contacting you regarding the complaint PNAS received regarding the paper “Nanospherical arabinogalactan proteins are a key component of the high-strength adhesive secreted by English ivy” [redacted] We shared the complaint with the editor, Peter Ladurner, and he provided the following comments:

"I want to ensure my support for PNAS regarding manuscript 2016-00406RR.

The question if the gene identified is indeed the ivy AGP cought my attention after the initial submition. Please note that I questioned this finding myself. For their first revision I demanded that the authors have to add a phylogenetic tree showing the true AGP relationship of their protein. In their revision the authors provided the respective tree (and mass spec data) corroborating their finding.

I want to state that such data - under normal circumstances and if the data were generated according to best scientific practice - are sufficient to support the authors statement that their protein is an AGP.

However, if the genes for generating the phylogenetic tree were highly hand picked (and not selected according to their statement in the paper: "Accordingly, a phylogenetic analysis was carried out to determine the relationship of the IAGP with these analogous proteins obtained from BLASTp. Among them, the IAGP and the AGP9 were the closest relatives, as shown in Fig. S4A.") any tree can be fabricated.
I hope this is not true.

The next steps require detailed sequence analyses using BLAST, reciprocal BLAST, thourough protein alignments and phylogenetic analyses of the submitted sequence.

Depending on the result the authors need to provide raw data and lab book level information on gene isolation, details on clones with gene inserts from PCR and RACE experiments, sequencing raw files, details of their BLAST search settings and databases, information on the selection and generation of alignments and the phylogenetic tree, Mass Spectrometry raw data, details on GPI anchor bioinformatics.

Please let me know if I can help with the sequence analyses."

Is Ohio State University investigating these concerns? Should I contact the research integrity office? Thank you very much for your help.

Best regards,
Etta Kavanagh
Editorial Manager
PNAS

THE OHIO STATE UNIVERSITY

Office of Research

Office of Research Compliance
Research Administration Building
Room 208
1960 Kenny Road
Columbus, OH 43210

Jennifer K. Yucel, PhD
Associate Vice President &
Research Integrity Officer
614-247-8831 Phone
614-292-0225 Fax
yucel.4@osu.edu

CONFIDENTIAL/SENSITIVE

January 29, 2019

Ms. Etta Kavanagh
Editorial Manager
PNAS
EKavanagh@nas.edu

RE: RETRACTION REQUEST [redacted] 2016 PNAS 113:E3139-E3202

Dear Ms. Kavanagh:

As noted in my email dated December 3, 2018, the Ohio State University has completed its investigation of allegations of possible Research Misconduct relating to [redacted]., 2016, PNAS 113:E3139-E3202. The University determined that data and text for Figures 3 and S4A regarding the acquisition, identification, characterization, and reporting of Ivy arabinogalactan protein (*IAGP*) were intentionally falsified [redacted] and then intentionally and recklessly misreported in the manuscript [redacted] M. Zhang). As these actions constitute Research Misconduct, the University is requesting that the manuscript be retracted and that the sequences deposited in GenBank for *IAGP* (KM820289, KF752597, AKN58855) be withdrawn. The University does not believe that correction of the article would be appropriate or scientifically valid.

To date, Dr. Zhang has not provided to the University any additional data or any records supporting the information that was provided to you for the proposed correction, and we cannot validate, nor verify the accuracy or authenticity of any of those data. Further, the new sequence information provided to PNAS for the correction does not identify a new replacement sequence for the previously published *IAGP*, but rather provides six (6) new candidate proteins based on tryptic peptide hits. This does not correct the scientific record and the relevancy of these new candidates is questionable.

We understand that the final decision on whether or not to retract or correct the manuscript ultimately resides with the journal, but we strongly suggest that retraction is the appropriate course of action. If the journal does decide to allow for a correction, please also note that the University did identify a number of other errors in the text, including misrepresentation of the materials and methods described for the mass spectrometry data presented in Figure S4B.

Due to confidentiality requirements, we are limited in what information we can provide regarding this matter, but we will work with you if there are specific questions or information that would be helpful. Please contact me at 614-247-8831 or yucel.4@osu.edu, if you require additional information or materials concerning this matter.

Sincerely,

Jennifer K. Yucel, Ph.D.
Associate Vice President, Office of Research Compliance
Research Integrity Officer

CC: Office of Legal Affairs