molecular systems biology

SARS-CoV-2 infection remodels the host protein thermal stability landscape

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

The reviewers' comments and authors' responses are not available with this article, as the initial review process took place with another journal.

1st Editorial Decision 22nd Dec 2020

Thank you again for sending us your manuscript "SARS-CoV-2 infection remodels the host protein thermal stability landscape". The reviews from the other journal are quite constructive and we can definitely use them rather than reviewing the study from scratch. I would therefore invite you to submit your manuscript once you have performed the revisions addressing the issues raised. Please include a detailed point by point response, so that we can easily evaluate the changes.

In summary, the two main issues raised were the lack of a decisive methodological advance and the relatively limited biological insights into SARS-CoV-2 infection. The methodological advance is not an issue for Molecular Systems Biology, as we do not see this as a Method paper. In my view, and along the lines of the reviewers' comments, the value of the study is mostly at the resource level. Regarding the level of biological insight, we do not think that extensive functional follow up analyses or deep novel mechanistic insights are essential for acceptance in Molecular Systems Biology. That said, I think that it would be important to provide some further support for the value of the presented resource (and its combination with the PPI and phosphorylation data from previous work) for understanding SARS-CoV-2 infection and identifying drug targets.

As you have not yet provided your revision plan, I am not sure what additional data you have at hand, but looking at the reviewers' comments I would propose the following:

- While a fully fletched functional follow up on CYP1A1 does not seem necessary, in case you have some further data it would be nice to strengthen this part of the study and increase the overall level of biological insight, which was a general concern raised by the reviewers.

- Additional assessment of viral replication by qPCR of the supernatant as independent validation of the compound effects would be a nice addition.

- In order to increase the confidence in the value of the resource, some evidence that the prioritized drugs could not have been identified by other approaches would need to be provided.

- Comparing protein destabilization and stabilization seems interesting and relatively easily addressable.

We thank you for considering our manuscript and appreciate the opportunity to resubmit our revised version according to the reviewers' comments from the other journal. Please find our detailed point by point response to the reviewers' comments below as well as our response to the points you raised in our previous correspondence on December 22nd 2020.

Due to the current Germany-wide lockdown regulations which limit experimental work, our revisions focus primarily on addressing the comments raised by rewriting sections of the text and expanding on some of the issues raised to more clearly illustrate the biological insight provided by our findings.

1. While a fully fletched functional follow up on CYP1A1 does not seem necessary, in case you have some further data it would be nice to strengthen this part of the study and increase the overall level of biological insight, which was a general concern raised by the reviewers.

We have not acquired additional data on CYP1A1 however, we have revised the discussion to further highlight the biological significance of CYP1A1 in SARS-CoV-2 infection, particularly with respect to upstream regulators i.e. Ahr and HSP90. We explain how HSP90 inhibition – which suppresses CYP1A1 expression in Caco-2 cells – serves as indirect evidence of the biological relevance of CYP1A1 for SARS-CoV-2 infection.

L306-320: "One of the many pathways regulated by HSP90 chaperones includes the aryl hydrocarbon receptor (AhR) signaling pathway (Pongratz, 1992). During SARS-CoV-2 infection, AhR promotes interferon-induced mucus production in a rodent model (Liu et al., Cell Research). This buildup of alveolar mucus is thought to underly the obstructed blood-gas exchange seen in SARS-CoV-2 patients, thus leading to hypoxia and reduced lung capacity. In line with a role for AhR activation during SARS-CoV-2 infection, we detected a pronounced increase in the abundance of the well-known target gene product, CYP1A1 (4-12 hours post infection). Interestingly, pharmacological inhibition of HSP90 with 17-AAG is known to potently suppresses CYP1A1 abundance by promoting proteasome-mediated degradation of the upstream transcriptional regulator, AhR (Kasai et al, 2010; Hughes et al., 2008). It is therefore conceivable that at least part of the mechanism by which 17-AAG suppresses SARS-CoV-2 replication is through reducing CYP1A1 abundance, whose activity we show is required for SARS-Cov-2 replication. Although it remains unclear how CYP1A1 activity is required for SARS-CoV-2 replication in this context, further exploration of HSP90 dependent pathways required for SARS-CoV-2 replication could lead to the identification of additional therapeutic host targets."

2. Additional assessment of viral replication by qPCR of the supernatant as independent validation of the compound effects would be a nice addition.

We agree that independent validation by qPCR of culture supernatants would provide additional confirmation of the effects of these compounds on SARS-CoV-2 replication. However, these were not collected at the time of the previous experiments and these experiments would need to be repeated. Given the current lockdown, and the uncertainty of its duration, such experiments are unfeasible for us at this point in time.

3. In order to increase the confidence in the value of the resource, some evidence that the prioritized drugs could not have been identified by other approaches would need to be provided.

We prioritized a unique panel of ten drugs solely based on their selectivity for seven distinct host targets that displayed thermal stability (6/7) or abundance (1/7) changes upon SARS-CoV-2 infection entirely based on our data. We note that four of the seven prioritized drugs have been previously tested by others for SARS-CoV-2 inhibition based on indirect evidence (e.g. overall pathway enrichments) and not due to direct detection of changes in the protein state of the drug target itself. We have elaborated on this in our response to reviewer # 2 point #3 below and have amended the main text to clarify how we prioritized drugs as well as the discussion to highlight the above point.

From: “We manually shortlisted seven druggable host target proteins which we found to exhibit altered abundance (CYP1A1: inhibited by rhapontigenin) or thermostability (CAPN1: PD-150606, IMPDH1/2: ribavirin and mycophenolic acid, HSP90: tanespimycin, PTRG1: acetylsalicylic acid and diclofenac, CTSV: E64d and CA-074-Me) upon SARS-CoV-2 infection.”

To L244-245 “*Based solely on our ability to find compounds that selectively modulate the activity of host proteins which were significantly altered in our data*, we manually shortlisted seven druggable host target proteins which we found to exhibit altered abundance (CYP1A1: inhibited by rhapontigenin) or thermostability (CAPN1: PD-150606, IMPDH1/2: ribavirin and mycophenolic acid, HSP90: tanespimycin, PTRG1: acetylsalicylic acid and diclofenac, CTSV: E64d and CA-074-Me) upon SARS-CoV-2 infection.”

4. Comparing protein destabilization and stabilization seems interesting and relatively easily addressable."

We agree that this is indeed an interesting analysis to perform. We have now separated GO term enrichments based on the directionally of stability changes. This analysis is now included as supplementary Figure S3, S4 and referred to in the main text as follows;

L138-139: “Hits related to protein folding were mostly the result of thermal destabilization and cell-cell adhesion proteins were mostly stabilized (Figure S3, S4 and Table 2).”

Please also see our detailed response below.

The remainder of the authors' point-by-point response to the reviewers’ comments has been removed, as it contains the reviewers' comments from the initial review process, which took place with another journal.

2nd Editorial Decision 14th Jan 2021

Thank you again for submitting your work to Molecular Systems Biology. As we discussed previously, the reviews from the other journal were quite constructive so we decided to use these reports rather than reviewing the study from scratch. Thank you for the detailed point by point response describing how the issues raised have been addressed. I feel that the additional analyses, discussions, edits and responses to the reviewers' comments seem to satisfactorily address the points raised. As most of the reviewers' concerns referred to the need to better support the main conclusions, but there were no concerns related to the technical aspects of the core experiments reported in the study, we have decided to proceed with making a decision based on our evaluation of the study and your responses, without involving an Editorial Advisory Board member or external expert.

We understand that given the current lockdown situation it is difficult to perform experiments. Therefore, we think that further experimental data on CYP1A1 are not required at this stage. Given that as the reviewers acknowledged, the main value of the study is at the resource level and considering that the study deals with a very timely and intensely researched topic, we have decided to proceed with publishing the study in its current form.

Before we can formally accept your study for publication in Molecular Systems Biology, I would ask you to perform some minor revisions listed below.

2nd Authors' Response to Reviewers 15th Jan 2021

The authors have made all requested editorial changes.

Accepted 18th Jan 2021

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

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Manuscript Number: MSB-2020-10188

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	We performed all experiments in triplicate and no sample size pre-determination was performed.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded from the analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes.
Is there an estimate of variation within each group of data?	Yes.

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Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	No antibodies were used
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Caco-2 cells were acquired from APC Cork (Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland), HeLa cells (CCL-2), VeroE6 and Calu-3 cells from ATCC. Caco-2 cells were recently authenticated. All cells were routinely checked for Mycoplasma contamination.

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD022115.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
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21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	All code to reproduce this analysis has been deposited at https://github.com/andrenmateus/TPP-SARSCoV2/.

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No.