THE EMBO JOURNAL

Protein translocation by the SecA ATPase occurs by a power-stroke mechanism

Marco A. Catipovic, Benedikt W. Bauer, Joseph J. Loparo and Tom A. Rapoport.

Review timeline:

Submission date:	9th November 2018
Editorial Decision:	3rd December 2018
Revision received:	13th December 2018
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Editor: Elisabetta Argenzio

Transaction Report:

(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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 3rd December 2018

Thank you for submitting your manuscript on a molecular mechanism for SecA ATPase activation to The EMBO Journal. Your study has been sent to three referees for evaluation, and we have now received reports from them, which are enclosed below for your information.

As you can see, the referees concur with us on the potential interest of your findings. However, they also raise critical points that need to be addressed before they can support publication in The EMBO Journal. In particular, referee #1 and referee #2 request you to further investigate the movement of the clamp of SecA and test the existence of intermediate FRET states. In addition, referee #2 asks you to discuss the position of the clamp in light of SecA's crystal structure. Similarly, referee #3 suggests you to comment on: i) possible artefacts arising from non-physiological jammed intermediates; ii) and how translocation occurs when the two-helix finger of SecA is cross-linked to SecY.

Addressing these issues as suggested by the referees is required to warrant publication in The EMBO Journal. Given the overall interest of your study, I would like to invite you to revise the manuscript in response to the referee reports.

REFEREE REPORTS

Referee #1:

In this paper, authors study the molecular mechanism of SecA ATPases through single-molecule FRET. They find that upon ATP binding, the two-helix finger of SecA undergoes a conformational change for its function. Based on their data, authors hypothesize a power-stroke mechanism. More analysis or experiments should be conducted before publication.

Major issues:
1. As shown in Figures 1E & S7D, there is a long low FRET state followed by a short period with fast FRET changes corresponding to several rounds of actions. It's more like the spring-loaded model.
2. For clamp, much more frequent FRET changes are shown in Figures 3D & S9D. However, authors only compared dwell times on the low or high FRET states. It seems that the clamp moves more frequently. This could significantly influence their model shown in Figure 5.
3. For clamp, only two major FRET states are shown. As shown in Figure 5, the clamp rotates. Therefore, one would expect more intermediate states since it may stop at any angle.

Minor issue:
1. Based on example traces (such as Figure 2A), only two FRET states are clear. Authors should show more traces with other FRET states.

Referee #2:

This is an interesting MS that supplies supporting evidence, via single molecule FRET studies, for a model of SecA action that was articulated some years ago by this group in a paper presenting the structure of SecA in complex with SecY. There are two major "moving parts" of SecA that are examined here, the two helix finger, which binds substrate protein and inserts it into the SecY channel, and a putative clamp structure involving the PPXD (polypeptide crosslinking domain), which putatively closes on the polypeptide either just prior to or during ATP hydrolysis, preventing backsliding of the chain as the two helix finger retracts. In general, the design of the first FRET pair and data concerning the two helix finger are entirely consistent with the idea of insertion into SecY. (Can the investigators suggest how far the finger inserts?) Less clear, however, are the studies of the clamp. There is surely a groove present in the proposed position for a clamp in the crystal structure from Zimmer 2008, and, as shown in Fig.2 of that work, there is potential for major movement of the PPXD toward the nucleotide binding domain. Such preceding information really needs to be briefly described and illustrated (showing the positions of the probes used here, not in a space filler of AY as in Fig.3A but in e.g. a ribbons image that better shows PPXD, like Fig.S1b, also including Y), offering an easier understanding of why and where the probes have been placed to make the second set of FRET observations. This should include comment about the referred-to earlier functional data involving e.g. cys Xlinking/sliding experiments that would support the nature of the PPXD movement to prevent sliding, as well as any temporal observations that would lead to the notion that this closure event is occurring at the particular proposed point in the cycle. In this latter vein, it would be great if the two FRET sets could be directly connected to each other (beyond the differing behavior in nucleotide and extrapolation to the cycle). One wonders whether such connection would be possible either by supplying a third probe (3-color FRET) or perhaps by supplying a quencher.

Referee #3:

The study by Catipovic et. al. uses single-molecule FRET to study the mechanism of Sec-mediated post-translational translocation in gram-negative bacteria. Overall, the results of this study validate and expand upon findings presented in a previous high-quality paper from the same group (Bauer et. al., 2014), which postulated that the SecA ATPase catalyzes protein translocation via a "push-and-slide" mechanism. According to this model, ATP binding to SecA leads to a "power stroke" which pushes the translocating substrate forward into the SecY channel. After ATP hydrolysis, conformational changes within SecA allow the substrate to passively diffuse either forward or backwards within the SecY channel, until a subsequent "power-stroke" event.

The current study refines this model by elucidating fine details of the conformational changes within SecA after the power-stroke occurs. Specifically, their findings reveal how SecA minimizes non-productive "back-sliding" of the preprotein substrate during the translocation reaction. They state that their results have implications for the mechanisms of other related motor ATPases.

The paper is well-written, and the experimental workflow is logical and well-presented. I have several minor comments that should be addressed to improve the manuscript before publication:

1. Discussion: are the authors concerned that their observations may be an artefact caused by the fact that they are studying conformational changes of SecA in the presence of a non-physiological jammed translocation intermediate? It would be nice to see a brief comment on this in the discussion.

2. Discussion: how do the authors reconcile their findings with the fact that translocation is still able to occur even when the SecA two helix finger is immobilized via cross-linking to SecY? It would be nice to see an explanation in the discussion beyond the current text: " Surprisingly, the two-helix finger of SecA 17 can be crosslinked to a cytosolic loop in SecY without abolishing translocation activity 18 (Whitehouse et al, 2012), perhaps because the finger remains flexible".

3. Figure 2B: please indicate on the graph which bar corresponds to which FRET state (either low, intermediate or high) - this will improve clarity for the reader. This also applies to the relevant panels in Figure S7 and S9.

1st Revision - authors' response 13th December 2018

Referee #1:

1. As shown in Figures 1E & S7D, there is a long low FRET state followed by a short period with fast FRET changes corresponding to several rounds of actions. It's more like the spring-loaded model.

There seems to be a misunderstanding about the “rounds of action”. The periods of fast FRET changes occur when labeled SecA binds (shown in the bottom trace). The long periods of “silence” are periods between SecA binding events. We have now clarified this in the figure by grey boxes and also show other sets of traces in a new Fig. S6, in which SecA binds for a longer duration, as in the trace for the clamp (Fig. 3D; see point #2).

2. For clamp, much more frequent FRET changes are shown in Figures 3D & S9D. However, authors only compared dwell times on the low or high FRET states. It seems that the clamp moves more frequently. This could significantly influence their model shown in Figure 5.

The longer periods of rapid FRET changes with the clamp are due to longer SecA binding in this particular trace. We are now providing additional sets of traces in Fig. S7, in which SecA happened to be bound for short periods. We hope that these additional examples in the supplemental figures, and the indication of SecA bound periods in the main figures (grey boxes), will clear any misunderstanding.

3. For clamp, only two major FRET states are shown. As shown in Figure 5, the clamp rotates. Therefore, one would expect more intermediate states since it may stop at any angle.

Our results show only two FRET states. Obviously, it is possible that the THF or clamp occupy intermediate states, but these are too short-lived to be observed. Whether the clamp “stops” at intermediate positions is uncertain, but likely not of biological importance.

Minor issue:
1. Based on example traces (such as Figure 2A), only two FRET states are clear. Authors should show more traces with other FRET states.

There are only two FRET states in our experiments. Perhaps, the reviewer asks for more traces, which we now provide in Figs. S6 and S7.

Referee #2:

This is an interesting MS that supplies supporting evidence, via single molecule FRET studies, for a model of SecA action that was articulated some years ago by this group in a paper presenting the structure of SecA in complex with SecY. There are two major "moving parts" of SecA that are examined here, the two helix finger, which binds substrate protein and inserts it into the SecY channel, and a putative clamp structure involving the PPXD (polypeptide crosslinking domain), which putatively closes on the polypeptide either just prior to or during ATP hydrolysis, preventing backsliding of the chain as the two helix finger retracts. In general, the design of the first FRET pair and data concerning the two helix finger are entirely consistent with the idea of insertion into SecY.

(Can the investigators suggest how far the finger inserts?)

Distance estimates are inaccurate at the extremes of the FRET range (i.e. close to zero and 1). Although our FRET data are in good agreement with distance estimates from X-ray structures in the sensitive range (~0.4-0.6), we feel that it would not be appropriate to give distances for the extreme positions of the THF and speculate how far the THF reaches into the SecY channel.

Less clear, however, are the studies of the clamp. There is surely a groove present in the proposed position for a clamp in the crystal structure from Zimmer 2008, and, as shown in Fig.2 of that work, there is potential for major movement of the PPXD toward the nucleotide binding domain. Such preceding information really needs to be briefly described and illustrated (showing the positions of the probes used here, not in a space filler of AY as in Fig.3A but in e.g. a ribbons image that better shows PPXD, like Fig.S1b, also including Y), offering an easier understanding of why and where the probes have been placed to make the second set of FRET observations.

As requested by the reviewer, we have clarified Fig. 3A. We now show a ribbon diagram with views from the side and the top (Fig. 3A and 3B). These figures should make it clearer where the probes were placed. To show the movement of the PPXD (rotation towards NBD2), we also added a panel to Fig. EV1.

This should include comment about the referred-to earlier functional data involving e.g. cys Xlinking/sliding experiments that would support the nature of the PPXD movement to prevent sliding, as well as any temporal observations that would lead to the notion that this closure event is occurring at the particular proposed point in the cycle.

We now cite previous experiments that show that the polypeptide chain moves through the clamp, as well as structural data that show the rotation of the PPXD (pg. 11, lines 6-11). The role of the clamp in holding the polypeptide chain during a stage of the ATP hydrolysis cycle has not previously been described and is a novel finding in this paper.

In this latter vein, it would be great if the two FRET sets could be directly connected to each other (beyond the differing behavior in nucleotide and extrapolation to the cycle). One wonders whether such connection would be possible either by supplying a third probe (3-color FRET) or perhaps by supplying a quencher.

As mentioned above, we strongly feel that these experiments are beyond the scope of the present paper.

Referee #3:

The study by Catipovic et. al. uses single-molecule FRET to study the mechanism of Sec-mediated post-translational translocation in gram-negative bacteria. Overall, the results of this study validate and expand upon findings presented in a previous high-quality paper from the same group (Bauer et. al., 2014), which postulated that the SecA ATPase catalyzes protein translocation via a "push-and-slide" mechanism. According to this model, ATP binding to SecA leads to a "power stroke" which pushes the translocating substrate forward into the SecY channel. After ATP hydrolysis, conformational changes within SecA allow the substrate to passively diffuse either forward or backwards within the SecY channel, until a subsequent "power-stroke" event.

The current study refines this model by elucidating fine details of the conformational changes within SecA after the power-stroke occurs. Specifically, their findings reveal how SecA minimizes non-productive "back-sliding" of the preprotein substrate during the translocation reaction. They state that their results have implications for the mechanisms of other related motor ATPases.

The paper is well-written, and the experimental workflow is logical and well-presented. I have several minor comments that should be addressed to improve the manuscript before publication:

1. Discussion: are the authors concerned that their observations may be an artefact caused by the fact that they are studying conformational changes of SecA in the presence of a non-physiological jammed translocation intermediate? It would be nice to see a brief comment on this in the discussion.

We added a sentence to the discussion to mention that the polypeptide chain is continuously sliding away from the channel and being re-inserted by SecA (pg. 14, lines 2-5). The re-insertion corresponds to real translocation, even if the C-terminus of the polypeptide does not enter the channel.

2. Discussion: how do the authors reconcile their findings with the fact that translocation is still able to occur even when the SecA two helix finger is immobilized via cross-linking to SecY? It would be nice to see an explanation in the discussion beyond the current text: " Surprisingly, the two-helix finger of SecA 17 can be crosslinked to a cytosolic loop in SecY without abolishing translocation activity 18 (Whitehouse et al, 2012), perhaps because the finger remains flexible".

We added a sentence to the discussion (pg. 14 lines 22-26). Whitehouse et al. actually tested crosslinks of SecA's THF to two positions in SecY. With one position, translocation was abolished, which would indicate that THF mobility is in fact required. The other position crosslinked the THF to the 6/7 loop of SecY and allowed some translocation to occur. This loop is long and loosely structured and might not have prevented the movements of the THF.

3. Figure 2B: please indicate on the graph which bar corresponds to which FRET state (either low, intermediate or high) - this will improve clarity for the reader. This also applies to the relevant panels in Figure S7 and S9.

The bars in Figs. 2B, EV2, and EV4 do not represent particular FRET states, but give the number of conformations observed with the indicated nucleotides.

With the revised manuscript, we also provide a two-sentence summary, bullet points on our major findings, and a graphical abstract. We also formatted the paper to meet the requirements of EMBO J.

2nd Editorial Decision 15th January 2019

Thank you for submitting a revised version of your manuscript. Please accept my apologies for the extended duration of the review process due to the recent holidays. Your study has now been seen by the original referees whose comments are shown below.

As you can see, while both referee #1 and referee #3 find that criticisms have been sufficiently addressed and recommend the manuscript for publication, referee #2 still stresses that the finger insertion/retraction and clamp closure/opening FRET analyses have not been coupled experimentally and requests you to employ either a mutant or a crosslinked/trapped state(s) to resolve this issue.

I also consulted with the other referees regarding this point and referee #3 responded and agreed that testing a mutant would be important to find the missing link between two FRET sets.

I would hence invite you to address the remaining issue from referee #2. Please do not hesitate to contact me for any questions you might have.

REFEREE REPORTS

Referee #1:

The authors have satisfactorily revised the manuscript.

Referee #2:

I've studied this revised MS and believe the authors have improved it concerning the specific text/fig items asked for. This said, however, it remains that the two FRET studies, of finger insertion/retraction and clamp closure/opening have not really been coupled experimentally. Yes, we know the respective dwell times, but we do not really know the points of overlap as diagrammed in the final figure. Perhaps a mutant or a crosslinked/trapped state(s) could be explored to resolve this if an additional probe is not feasible? More immediately, do the data collected in ATPgammasS support their model?

Referee #3:

The authors have responded with my comments/remarks sufficiently/appropriately. thank you.

2nd Revision - authors' response 25th January 2019

We were disappointed that the paper was still not accepted for publication. In the original report, reviewer #2 asked for further confirmation of the coupling between the movements of the two-helix finger and clamp. We do think that we provided evidence for the coupling, based not only on the dwell times, but also on the generation of trapped ATP hydrolysis intermediates. We used ADP plus BeFx to generate a state corresponding to the transition state of ATP hydrolysis (Fig. 1C,D and Fig. 3B,C), and ADP plus Pi or vanadate (Vi) to generate a state after ATP hydrolysis, but before Pi release (Fig. EV3 and Fig. 4E). In both cases, we found the clamp to be closed, while the two-helix finger moved from an intermediate state in ADP•BeFx to a de-inserted state in ADP•Pi or Vi. It is unclear to us which other trapped states the reviewer suggests we study, or which mutant or crosslinked product would generate a meaningful trapped state. It is possible that the reviewer was thinking of using a Walker B mutant (D209N), in which SecA would be locked into the ATP-bound state, but such a mutant would not allow the generation of a translocation intermediate in the first place, so no FRET experiments could be performed with it.

The trapped states we have studied led us to the diagrams in the last figure. We have changed the diagrams (Figs. 5A and B) and the text (legend, p. 12 lines 24-26, and p. 13 lines 17-19) to admit that we do not know the exact point at which the clamp closes during the ATP hydrolysis cycle; it could close either upon ATP binding or during ATP hydrolysis.

We added several sentences to the Discussion to better explain the basis for the coupling of the FRET experiments carried out for the two-helix finger and clamp (p.13 line 24 – p. 14 line 1). We also now explicitly mention that three-color FRET will be required to follow the movements of the two-helix finger and clamp at the same time (p. 14 lines 1-2) As we said before in response to reviewer #2's suggestion, such experiments are not only difficult with respect to data analysis, but also currently beyond reach for us because of equipment limitations.

We now explain better that the ATPgS experiments are fully consistent with the proposed model (p. 11 lines 22-25) The analog extends the high FRET state of the clamp, indicating that the clamp closes either during ATP binding or hydrolysis.

We hope that the reviewers will accept this new version of the manuscript or suggest specific experiments (specific mutants or crosslinking experiments) that would further test our model.

Editor Correspondance 25th January 2018

Thank you for submitting a revised version of your manuscript. As already anticipated, referee #2 finds that the remaining criticism has been sufficiently addressed and recommends the manuscript for publication.

However, before we can formally accept the manuscript there are a few editorial issues concerning text and figures that I need you to address.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Tom A. Rapoport
Journal Submitted to: Embo Journal
Manuscript Number: EMBOJ-2018-101140R

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 justified
- 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?	Sample sizes were not determined beforehand, but rather they were established so that the deprivation of any single FRET trace of data from the dataset did not significantly change the observed results or the conclusions drawn therefrom. The sample sizes for each reported dataset can be found in the corresponding figure legends.
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?	Colocalized fluorescent spots were excluded from the analysis if the fluorophores were not in 1:1 stoichiometry or if there was no measurable anti-correlation in the fluorophores' intensities.
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.	Randomized group allocation was not applicable to this study. Datasets were characterized simply by the identity of the cysteine mutations in SecA and SecY, as well the nucleotides used during imaging.
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.	The investigators were not blinded to data identity when assessing results.
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.	The only formal statistical test reported is the two sample Kolmogorov-Smirnov test, which makes no assumptions as to distributions underlying the data.
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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).	NA
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

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
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

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	See Materials and Methods
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).	Source data for figures included with submission.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
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.	NA

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.	This study does not fall under dual use research restrictions.