Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors provide a description of how they have applied various tools from statistical physics to networks composed of human interacomes integrated with a number of viral host-pathogen protein-protein interactomes, including the recently published interactome from SARS-CoV2. The technical elements of the manuscript are correct, however, I was left wanting more in terms of biological interpretation/ conclusions. The authors should do more systematic validation of their findings.

Major points:
1) The abstract did not sufficiently motivate why the idea of finding similarities/differences between viruses is interesting/useful.

2) I found the section of results describing the percolation analysis to be overly long, not very interesting, and could perhaps be cut altogether, or at least be much shorter. Since the fraction of total edges in the interactomes contributed by the viruses are very small, I am very unsurprised that this method shows that the various interactomes are highly similar.

3) None of the figure panels are labeled, making it hard to follow and interpret the authors' meaning.

4) I remain unconvinced of the utility of using these statistical physics methods to cluster the viral-human interactomes. For example, how do these results improve upon a much simpler metric of # shared human interactors? I'd expect that SARS-CoV2 and SARS-CoV at the least share many of their interactors, and so these complex methods are not needed. If the authors can show better clustering of the other respiratory illnesses with their methods than with a simple # shared interactors approach, that could be more interesting.

5) Regarding the 'large scales' clustering results, it is strange to me that now SARS-CoV and SARS-COV2 do not appear in the same cluster. Why are they similar at small scales, and dissimilar at large scales?

6) Further in the large scales results, the authors mention anecdotally that a treatment for (lopinavir-ritonavir) HIV has been proposed to treat SARS-COV2. However, this treatment has not shown promising results in Covid19 patients, and trials have been suspended. The authors could undertake a more thorough examination of drugs proposed for repurposing, and demonstrate that they find more from drugs within the SARS-COV2 cluster than from drugs outside the SARS-COV2 cluster (e.g. remdesivir, dexamethasone, drugs tested in the Krogan papers, etc). In general, the reader needs more to convince them to believe these results. Can the authors add something about what is driving the observed similarity? (e.g. shared genes, biological pathways, etc?).

Minor points:
1) Sentences like 'the results are presented in Fig. 4' should be changed to refer to figures parenthetically (e.g lines 129, 135)

2) Fig 6 lower left panel, dots should not be connected- no intrinsic order in the points.

3) BetaU should be included in the definitions in Figure 6.

Reviewer #2 (Remarks to the Author):

The study is interesting and original, but in multiple parts the analysis lacks depth and the conclusions are fairly vague. Several of the presented results seem to be caused by modeling choices instead of actual patterns in data, or, at best, they are not developed enough to allow me to distinguish between the two possibilities. Overall, I think there are several critical issues that need to be addressed to make the analysis convincing:

- The authors show that percolation analysis is not able to discriminate between the different virus interactomes, concluding that more convoluted techniques are needed to tackle the problem. However, the reasoning seems backwards here. The authors first show the percolation results where the curves collapse onto each other for all the networks, and then observe that the network are nearly identical both in terms of nodes and edges (up to 0.9996). Isn't the percolation result quite expected given the extreme similarity of the networks? The observed similarity makes the whole application of percolation analysis seem unnecessary and redundant. Also, isn't this similarity expected given how the networks have been built? If I understood it well, each network is essentially a very large network (~20k nodes) where few nodes are added for each virus (around 10-20), so the resulting networks are bound to be essentially the same. Barring some extremely unlikely cases, these few nodes are not going to produce an appreciable difference in such a global metric. This result does not suggest, in my opinion, that a purely topological analysis is not able to differentiate between viruses, but only that percolation on the full network is not the right tool to differentiate between these networks. A more targeted analysis on the neighborhoods of the virus-specific proteins would be more convincing here.

- The modeling of the bio-chemical dynamics should be explained more clearly. Specifically, is the last term on the right side of the equation (sum of A_ij x_i x_j) modeling the formation of complexes through pairwise binding? Since these complexes are different molecules (resulting in a net reduction of the unbound protein i), shouldn't the term be negative instead of positive? In this case, what is the evolution equation of this newly created hetero-dimers and how are they connected to the rest of the network? Also, since A_ij is a binary element, isn't the coupled term missing a binding rate constant, to make it comparable to the F_i and B_i terms? As further confirmation of this, the equation for the bio-chemical dynamics is similar to the one defined in SI of Ref 31 (which is referenced later in the manuscript), but in Ref. 31 the coupled term is negative and there is a binding rate constant R. The authors should explain their modeling choices more clearly or at least provide references that directly expose the equations they state (I couldn't find them in Refs 27-29, which seem quite generic).

- The regulatory dynamics equation seems to be missing some rate constants and seems to be modeling only activation of target gene (no inhibition). Is there a reason why the modeling is different from e.g. eq. 136 in SI of Ref 31? In general, the authors should describe and justify more clearly all these modeling choices and the values assigned to parameters such as F_i and B_i. In the same way, what are the starting concentrations x_i of each protein? Are they all set to 1? The difficulty in setting this values is one of the major challenges in modeling dynamical behavior in real biological systems, so it is surprising to me that these details are left out.

- The authors state "In our case, φ = 0 for both processes, therefore the perturbation from i has the same impact on neighbors, regardless of its degree". I didn't understand this sentence. Is the independence from the degree imposed or measured? In the former case, where is it imposed? In the latter case instead, how can phi be exactly zero?

- Eq. (3) is not clear. The term dx_k is a differential (at least if we are adhering to the same notation of Eq.(2), which describes a total derivative), and thus it is an infinitesimal quantity. How is G^v calculated? Is it approximated as deviation of x_i after perturbation?

- I am puzzled by the top plot of Fig. 4. The whole measure |G^v| seems to be a very convoluted

way to obtain some quantity that is essentially a linear rescaling of the number of affected nodes (up to some fluctuations that are ignored in the analysis). In the following I am assuming that G^v_k is proportional to the total variation of concentration of protein k when all the affected nodes are perturbed (which I am not sure of, see my previous point). Since the matrix elements are all of the same sign, G^v_k seems to be a non-decreasing function of the number of affected nodes, because each affected node adds a positive contribution to the total variation of concentration of every node in the network. The authors conclude "the overall impact of a perturbation generated by a virus is proportional to the amount of human proteins it interacts with". This seems like circular reasoning, since we are introducing an extensive quantity, which is by design proportional to the number of affected nodes, and at the same time presenting this behavior as a result. Since I have no data at hand, the authors are free to prove me wrong here: would this pattern (i.e. |G^v| being a linear rescaling of the number of affected nodes) appear also when evaluating G^v on networks where the links of the initial perturbed nodes are rewired randomly? If this is the case, how does this result help in the analysis of the virus-specific interactomes?

- The Sars-Cov-2 data seems to be very different from the data of other viruses (number of affected nodes), which may be likely caused by the fact that different sources are used for Sars-Cov-2 as compared to the other virus interactomes. While these batch effects are unavoidable because of the novelty of the Sars-cov-2 data (as aptly stated by the authors), I think that the optimal course of action would be to work on quantities that are not too affected by these biases. As per my previous point, the quantity |G^v| seems to be trivially dependent on the number of affected nodes, which in turn is the most evident bias in the Sars-cov-2 data against the other datasets (unless there is a plausible biological justification for why there is such a striking difference in the number of affected proteins).

- The clustering results in Fig. 5 are quite superficial and do not provide insights on what is really going on. The authors compute a hierarchical clustering of the networks based on their G^v vectors, but limit their analysis to essentially saying that having similar sets of affected proteins will result in having similar vectors G^v and being clustered together. Was the whole perturbation response analysis really necessary to come to this very basic conclusion? I think that the analysis would had been more convincing if the perturbation response highlighted some non-trivial similarities between the networks that weren't evident by simply counting the number of shared affected proteins.

- The last part focused on the study of the density matrix, and several quantities are defined to study the difference between the networks. However, it seems to me that this statistical framework is plugged in without particular care on why this would be relevant for the current application. What is the biological hypothesis here? Are the different virus networks supposed to have different information content? What kind of biological process is the density matrix modeling? Or is it simply proposed as a generic embedding technique?

- The part on the k-means clustering is quite devoid of details (as a side note, I find it questionable that some clarifications are only in the caption of Fig. 6, instead of the main text). What is the space of features for the clustering? Is it the 2D space of (delta phi, delta S)? What is the metric used and why? How has the number of clusters k=4 been chosen? Also, in the text it is said that the colors of the three plots on the right hand side of Fig. 6 have been selected according to the clustering results for beta=3. However, if the three beta plots in Fig. 6 are to be taken as a reference, it seems to me that beta=1 is the actual space used for the clustering.

- The authors introduce a number of (properly referenced) concepts to arrive to what seems to be a 2D vector embedding of perturbed graphs. However, it should be shown that there is some actual advantage in going through all these hoops instead of sticking with baseline approaches. It would be beneficial to show, for example, what we would obtain if we performed clustering on the matrix of jaccard similarities between the sets of affected proteins of each virus, and demonstrate

that the results obtained through the proposed methodology are more insightful.

- The main conclusions of the paper depend on the final clustering results (i.e. coronavirus PPI being clustered together with Sars-cov at small scales and HIV at large scales), yet the clustering results are just provided "as is" with only two sentences in the main text. No further assessment of the quality of the clustering are provided. Also, it is well known that clustering is crucially dependent on the parameters/methods/metrics used for clustering and it is often a very hard task to establish a "final" clustering result. How stable are these results with respect to changes of the parameters (such as k) or clustering methodologies?

- Most result figures are barely addressed, they seem completely replaceable and do not really seem to bring more insights to the understanding of the results. For example, what does the bottom plot of Fig. 4 tell us, specifically?
Also, was it necessary to show all the 8 plots of delta phi, delta S and delta U in various configurations (Fig. 6), when the only conclusion we have in the text is a description of how the interactomes are clustered (which is already summarized in the tables)?

In conclusion, this work proposes a number of different approaches aimed at finding distinguishing features of the virus interactomes (percolation, dynamics, spectral information), but several of these analyses feel rushed and superficial. I think that a more focused analysis with just one of such approaches, and more detailed descriptions of the reasonings, technical details and caveats would make the contribution more appealing for the scientific community.

Reviewer #3 (Remarks to the Author):

Review comments on "Multiscale statistical physics of the Human-Sars-CoV-2 interactome"

Summary:
In this paper the authors provided an interesting perspective of viewing the virus-human protein interactions as perturbation to the human PPI, and used classic statistical physic measures to evaluate them, clustering the viruses in the end. The paper is well written and easy to follow. However, the authors failed to provide any biological evidence to support their methodology, neither from mechanistic side nor from phenotypic side, thus the manuscript cannot convince the reader that the proposed measures are meaningful. In addition, the paper spends too much discussions on methodologies that do not work for obvious reasons.

Major comments
1. The authors need to support their method/model by evidence, both from the biological mechanism side (e.g. why is this clustering a reasonable way to classify viruses?); and from the phenotypic side (e.g. what biological features does the method capture? What biological observations support the clustering result?). Despite their well-establishment in statistical physics, these measures may lose their meaning in a protein-protein interactome context. For example, a scale-free network such as the protein interactome, are often known to shown heterogeneity, i.e. 'nodes cannot be treated as the same', as contradictory to a convention mean field assumption where 'every node is the same'. Without support, it is difficult to claim the proposed measures meaningful.

2. The authors might need to make sure that these measures (e.g. von Neumann entropy) are not biased by less relevant measures, such as number of viral proteins, number of viral-human protein interactions, etc. The reason is that, as biological datasets are often incomplete, we often observe a biased subset of the complete data, thus it is important that the proposed methodology should be independent of this incompleteness. For example, if a measure correlates with the number of virus-host protein interactions, then a well-studied virus will appear different from a not-well-

studied one, even if they are biologically similar, because the not-well-studied virus will have much less known interactions due to data incompleteness. It seems to me the measure(s) the author proposed might not work well under incompleteness of data. This problem might be addressed better if the authors can show biological insight of their measures.

3. As the authors pointed out in the introduction, percolation analysis is not expected to show difference between viruses as the viral proteins are too few and thus have too little impact on the network. However, the authors dedicated quite some detailed discussion to it. Would it be better to shorten this section, so that the readers can focus more on the rest?

4. Similarly to above, as the Cumulative Correlation is proportional to the number of proteins each virus binds to, it is also a trivial measure in the sense that it does not show more information than "the number of proteins each virus binds to". The similarity analysis (Figure 5), if I understood correctly, is dictated by the overlap of human genes different viruses bind to, and thus is another trivial measure. The authors might want to consider if these could be shortened, too.

5. The fact that the SARS-COV-2 protein has 332 number of targets in PPI, dramatically different from the rest of the viruses, may suggest the author's choice of representation for SARS-COV-2 is questionable. Can the authors validate this choice? I understand there may not exist alternatives in this matter, nevertheless this may reduce the reliability in the authors conclusions on SARS-COV-2

Minor comments:
1. Figure 2-left panel is not in vector format, i.e. one cannot zoom-in to see the small protein labels
2. Figure 1: Would it be more informative to also show additional numbers, e.g. the total number of human proteins each virus binds to?
3. Figure 2 caption has a grammatic problem: "BIOSTR Human PPI used in this study, this obtained from data fusion of two comprehensive public repositories"
4. Figure 2 caption: "On the other hand, distinct colored markers of constant size encode distinct viruses (45 in total, including SARS-CoV-2)": I don't get what 'constant size' here means
5. Line 98, Formula "Law of Mass Action" has typo in 'Sigma_j=1j'
6. Page 10 , line 122 'asses' is not correctly spelled
7. Page 13, "Dii = k_i delta_ij "should be "Dij = k_i delta_ij "
8. Figure 6 caption did not explain the upper half of the bottom right sub-figure very clearly. I suppose each dot is a virus, right? And why is beta=1 shown in bottom left, but beta=3 shown in bottom right?
9. Page 20, Paragraph 3. The statement 'viruses tend to preferentially target similar regions related to specific functions of the human interactome' needs more evidence to support. Based on the evidence presented in the manuscript, one could only conclude "the top virus-targeted proteins have interesting functions". In addition, defining 'similar regions' in human PPI is a tricky and difficult problem. If this is indeed the authors meant, the authors will have to explain how they define these regions.

Trento, December 2020

We thank the reviewers for their insightful comments which undoubtedly helped us to enhance the computational analysis and, consequently, strengthen the message of our manuscript.

The resubmitted manuscript is extensively changed according to the comments we received from the reviewers. To improve the manuscript with a valid biological perspective, we have had the pleasure to extend the research team to a computational biologist, who played a fundamental role to including the analysis of *first and second interactors*, *Gene Ontology and Pathways Enrichment* which provide more biological insights and evidence to support some of our results. While a full biological verification of the presented results is beyond the scope of this work, the new manuscript changes perspective and combines statistical physics and computational biology to gain new insights about SARS-CoV-2.

We doubled our statistics by adding another 48 virus-human interactomes, leading to 93 viruses overall, and updated our previous data to include all the interactions between viruses and human proteins, instead of limiting to the top interactions. In order to better explain the goal of our paper, we added a first figure emblematically showing why we include various analysis, from micro up to the macroscopic. Remarkably, we have added a whole new section about the mesoscale analysis, which further enhanced our message. Finally, the percolation and propagation of perturbations analyses have been reviewed and shortened.
In short, the goal of this paper is to shed light on the multiscale physics of interactomes, in the hope that it can trigger future fruitful cross-pollination between statistical physics and computational biology for application to systems biology and medicine.

In the following, we go through the referees' comments one by one, providing our answers. All the changes made are highlighted in the revised manuscript.

Sincerely,

The authors

Referee #1

Firstly, we thank the reviewer for the encouragement we received from the given summary:

The authors provide a description of how they have applied various tools from statistical physics to networks composed of human interacomes integrated with a number of viral host-pathogen protein-protein interactomes, including the recently published interactome from SARS-CoV2. The technical elements of the manuscript are correct, however, I was left wanting more in terms of biological interpretation/ conclusions. The authors should do more systematic validation of their findings.

According to this enlightening summary, we added a full comprehensive analysis of Pathways enrichment and Gene Ontology. In the following, we go through the major and minor points, one by one, and describe the changes we made to cope with the raised issue or provide our answers.

Major points:

1) The abstract did not sufficiently motivate why the idea of finding similarities/differences between viruses is interesting/useful.

We thank the reviewer for this insightful comment. Indeed, we agree that the abstract could be enhanced to explain better the importance of such analysis. Therefore, we changed the abstract, especially by adding the following sentence:

"Characterizing (dis)similarities between SARS-CoV-2 and other viral agents at a molecular level allows one to exploit existing information about the alteration of key biological processes due to known viruses in order to predict the potential impact of this new virus."

Also, we concluded the abstract in a different way to highlight the importance of the obtained results:

"Overall, our results indicate that SARS-CoV-2 exhibits similarities to expected viruses such as Influenza A and Human Respiratory Syncytial virus, and unexpected ones with different infection types and from distant families, like HIV1 and Human Herpes virus. Our results indicates that in the latter case COVID--19 is a systemic disease with potential effects on multiple organs and human body sub-systems"

Although there is existing evidence for the above claim, as far as we know, this is the first study to obtain such information using network science techniques, and more specifically, the statistical physics of networks.

2) I found the section of results describing the percolation analysis to be overly long, not very interesting, and could perhaps be cut altogether, or at least be much shorter. Since the fraction of total edges in the interactomes contributed by the viruses are very small, I am very unsurprised that this method shows that the various interactomes are highly similar.

We thank the reviewer for this comment. We totally agree that the results of the percolation section are not surprising. We followed the reviewer's comment and shrunk the section into almost a half, and also mentioned in the text that the result could be expected given the small fraction of viral interactions.

3) None of the figure panels are labeled, making it hard to follow and interpret the authors' meaning.

We thank the reviewer for this comment. We fully agree that lacking labels can affect the readability of the figures. Therefore, to the large figures with multiple panels, we added the required labels.

4) I remain unconvinced of the utility of using these statistical physics methods to cluster the viral-human interactomes. For example, how do these results improve upon a much simpler metric of # shared human interactors? I'd expect that SARS-CoV2 and SARS-CoV at the least share many of their interactors, and so these complex methods are not needed. If the authors can show better clustering of the other respiratory illnesses with their methods than with a simple # shared interactors approach, that could be more interesting.

We thank the referee for this important comment. We added the full analysis of shared interactors at first and second range. In fact, the results do not confirm the above expectations. Hierarchical clustering presented in section "Gene ontology and pathways enrichment analysis" shows that at the level of first interactors, SARS-CoV-2 is not similar to any other viruses. Also, we discuss this result in the same section as:

"To validate that these findings were biologically relevant, we have further performed a clustering analysis based on shared human proteins targeted by the viruses, enriched Reactome pathways within these targeted proteins or shared biological processes as defined by Gene Ontology. Figure.7 shows that SARS-CoV-2 does not cluster with any other virus on the basis of shared protein interactors, probably due to its relatively higher number of viral proteins and human interactors compared to other viruses. However, out of the 332 human proteins targeted by SARS-CoV-2, 18 of them were found to be targeted as well by other viruses, including Herpes viruses, HPV type 16, Reovirus or Encephalomyocarditis virus."

And further in the discussion comment :

"Concerning the direct interactors, although the approach solely based on protein similarity did not allow to highlight any relevant cluster, 18 human proteins were found to be targeted both by SARS-CoV-2 and by other viruses. Surprisingly, no other members from the coronavirus family were found to share human targets with SARS-CoV-2."

In fact, the biological analysis and the statistical physics method used in this article both indicate the emergence of non-trivial virus clusters at higher order interactions, finding SARS-CoV-2 similar to viruses such as HIV1 and Herpes virus.

5) Regarding the 'large scales' clustering results, it is strange to me that now SARS-CoV and SARS-COV2 do not appear in the same cluster. Why are they similar at small scales, and dissimilar at large scales?

We thank the referee once again. As we mentioned in the article, it is crucial to highlight that small \beta encodes short range interactions, mostly limited to the neighbors. At this scale, we might expect to see trivial (dis)similarities between viruses, like the similarity between SARS-CoV and other respiratory

diseases such as SARS-CoV-2. While according to our updated and more comprehensive dataset SARS-CoV-2 appears in the same cluster with HRSV, another respiratory disease, it is worth mentioning that this cluster is very close to SARS-CoV-1, confirming the similarity. However, we would like to mention that, despite the high genetic similarity, the two mentioned viruses show significant differences in their function, in terms of lethality, infectiousness, etc.

At larger \beta, where the higher order interactions between the nodes are considered, the complexity plays a more important role. This is the reason we observe unexpected similarities, for example betweens SARS-CoV-2, HIV1 and Herpes. We think that this result, coupled with our biological validation presented in the revised manuscript, works as strong evidence for the claim that SARS-CoV-2's functionality is still unknown to a large extent and suggest that this novel virus might have systemic effects.

6) Further in the large scales results, the authors mention anecdotally that a treatment for (lopinavir-ritonavir) HIV has been proposed to treat SARS-COV2. However, this treatment has not shown promising results in Covid19 patients, and trials have been suspended. The authors could undertake a more thorough examination of drugs proposed for repurposing, and demonstrate that they find more from drugs within the SARS-COV2 cluster than from drugs outside the SARS-COV2 cluster (e.g. remdesivir, dexamethasone, drugs tested in the Krogan papers, etc). In general, the reader needs more to convince them to believe these results. Can the authors add something about what is driving the observed similarity? (e.g. shared genes, biological pathways, etc?).

We thank the reviewer for this comment. Indeed, the medical treatment for SAR-CoV-2 is still eluding us, considering the variety of drugs being used. Of course, the full medical analysis is beyond the scope of this work and expertise of the authors and we have removed the anecdotal evidence in the revised manuscript, rephrasing its content.

Concerning the similarity, note that it is derived from the multiscale analysis of protein-protein interactions. Interestingly, viruses of different families appear to affect the higher order interactions in human cells, in similar ways. With respect to the original manuscript, we have extended the research team with a computational biologist to include the analysis of Gene Ontology and Reactome biological pathways to gain biological insights about the observed (dis)similarities. For the specific case of HIV, it is worth noting that both viruses are of RNA type and, according to increasing literature (now cited in the manuscript), SARS-CoV-2 might trigger autoimmune response with potentially overlapping pathways with the autoimmune disease caused by HIV.

Minor points:

1) Sentences like 'the results are presented in Fig. 4' should be changed to refer to figures parenthetically (e.g lines 129, 135)

We thank the reviewer for this comment. The paper is changed, accordingly.

2) Fig 6 lower left panel, dots should not be connected- no intrinsic order in the points.

We thank the reviewer for this comment. The paper is changed, accordingly.

3) BetaU should be included in the definitions in Figure 6.

We thank the reviewer for this comment. In fact, the only parameters used in this analysis are spectral entropy and Massiue function. Since internal energy (U) was not used as a clustering feature, we excluded it from the paper, completely.

We thank the reviewer once again for his/her time and effort. We think that the changes added value to our paper and enhanced the discussion about the biological relevance.

Referee #2

Firstly, we would like to thank the reviewer for his/her time and effort for considering our manuscript. In the following, we answer the comments one by one.

The study is interesting and original, but in multiple parts the analysis lacks depth and the conclusions are fairly vague. Several of the presented results seem to be caused by modeling choices instead of actual patterns in data, or, at best, they are not developed enough to allow me to distinguish between the two possibilities.

Before answering all of the raised issues, we would like to provide some explanations on our reasons for choosing multiple analyses. In fact, complex systems often show interesting properties when studied at different scales. Their microscopic, mesoscopic and macroscopic behavior might show similar or different patterns that are worth investigating and comparing to reach a comprehensive understanding. We tried to better explain this goal by adding an emblematic first plot describing all these approaches in one frame.

Furthermore and from another point of view, we must admit that there is no ground truth for the problem we are aiming to tackle. In fact, SARS-CoV-2 is too novel and our knowledge about it is very limited, still. Additionally, our understanding of how topology of the human interactome might influence the effects of viral infections is incomplete as well. Therefore, a comprehensive analysis seems more reasonable, where one can use multiple approaches to get a more comprehensive insight about the unknown process under investigation. Indeed, in the new version, we show how biological analysis confirms parts of the results obtained from the statistical physics, indicating similarities between the novel coronavirus and viruses like HIV1 and Herpes.

Overall, I think there are several critical issues that need to be addressed to make the analysis convincing:

- The authors show that percolation analysis is not able to discriminate between the different virus interactomes, concluding that more convoluted techniques are needed to tackle the problem. However, the reasoning seems backwards here. The authors first show the percolation results where the curves collapse onto each other for all the networks, and then observe that the network are nearly identical both in terms of nodes and edges (up to 0.9996). Isn't the percolation result quite expected given the extreme similarity of the networks? The observed similarity makes the whole application of percolation analysis seem unnecessary and redundant. Also, isn't this similarity expected given how the networks have been built? If I understood it well, each network is essentially a very large network (~20k nodes) where few nodes are added for each virus (around 10-20), so the resulting networks are bound to be essentially the same. Barring some extremely unlikely cases, these few nodes are not going to produce an appreciable difference in such a global metric. This result does not suggest, in my opinion, that a purely topological analysis is not able to

differentiate between viruses, but only that percolation on the full network is not the right tool to differentiate between these networks. A more targeted analysis on the neighborhoods of the virus-specific proteins would be more convincing here.

We thank the reviewer for this comment. We totally agree that the results of the percolation section are not surprising. We followed the reviewer's comment and shrunk the section into almost a half, and also mentioned in the text that the result could be expected given the small fraction of viral interactions.

We would like to mention that the data we currently have for SARS-CoV-2 has a lot more interactions with human proteins than other viruses, as explained in the paper. It poses limitations on the base-line and simpler approaches such as analysis of the shared interactors. Yet, in the light of this comment, we complemented the percolation section with another topological analysis, showing that the effect of viral components on the mesoscale organization of human PPI can be used to characterize the similarities between viruses.

- The modeling of the bio-chemical dynamics should be explained more clearly. Specifically, is the last term on the right side of the equation (sum of A_ij x_i x_j) modeling the formation of complexes through pairwise binding? Since these complexes are different molecules (resulting in a net reduction of the unbound protein i), shouldn't the term be negative instead of positive? In this case, what is the evolution equation of this newly created hetero-dimers and how are they connected to the rest of the network? Also, since A_ij is a binary element, isn't the coupled term missing a binding rate constant, to make it comparable to the F_i and B_i terms? As further confirmation of this, the equation for the bio-chemical dynamics is similar to the one defined in SI of Ref 31 (which is referenced later in the manuscript), but in Ref. 31 the coupled term is negative and there is a binding rate constant R. The authors should explain their modeling choices more clearly or at least provide references that directly expose the equations they state (I couldn't find them in Refs 27-29, which seem quite generic).

We thank the reviewer for this comment. We used the bio-chemical equation defined in SI of Ref 31 (now Ref 24 in the reviewed version) (https://doi.org/10.1038/nphys2741), in the numerical calculations performed the term is indeed negative (while the text had a typo, for which we apologize) and the dynamics model indeed requires a binding rate constant R. In this analysis we chose to work with constants F, B and R all set equal to 1. Since we do not have the values of the rate constants for each pair of PPIs in the human proteome we used the choice of constants that made use of the lower number of priors. We limited ourselves to studying the propagation of information in the network for the given dynamics and given the set of targeted proteins, not with the specific aim of recovering the full biology, but rather on characterizing the interactome on the perspective of the propagation of biochemical and regulatory signals from targeted proteins. We reviewed and shortened this section by limiting the analysis only to a regulatory dynamics process, as it shares the same propagation properties as the bio-chemical equation.

- The regulatory dynamics equation seems to be missing some rate constants and seems to be modeling only activation of target gene (no inhibition). Is there a reason why the modeling is different from e.g. eq. 136 in SI of Ref 31? In general, the authors should describe and justify more clearly all these modeling choices and the values assigned to parameters such as F_i and B_i. In the same way, what are the starting concentrations x_i of each protein? Are they all set to 1? The difficulty in setting this values is one of the

major challenges in modeling dynamical behavior in real biological systems, so it is surprising to me that these details are left out.

We thank the reviewer for this comment. Similarly to bio-chemical dynamics, the constants chosen for regulatory are equal to 1. The concentrations in which the perturbation is generated are obtained by calculating the steady state given the set of equations. The steady state is obtained starting from a homogeneous distribution of concentrations (all equal to 0.1) and solving the set of equations until a steady state is reached. In light of these comments we reviewed this analysis. For the regulatory process we used B = 10^-2 and R = 10^-1, as arbitrary interaction constants to simulate a degradation rate one order of magnitude slower than the interaction rate. These details have been added in a dedicated section in the supplementary material.

- The authors state "In our case, φ = 0 for both processes, therefore the perturbation from i has the same impact on neighbors, regardless of its degree". I didn't understand this sentence. Is the independence from the degree imposed or measured? In the former case, where is it imposed? In the latter case instead, how can phi be exactly zero?

We thank the reviewer for this comment. The independence is obtained analytically from the set of ordinary differential equations that describes the dynamics itself. It is a scaling property that can be derived analytically and therefore is equal to zero exactly (Ref 31 (now Ref 24 in the reviewed version) https://doi.org/10.1038/nphys2741)

- Eq. (3) is not clear. The term dx_k is a differential (at least if we are adhering to the same notation of Eq.(2), which describes a total derivative), and thus it is an infinitesimal quantity. How is G^v calculated? Is it approximated as deviation of x_i after perturbation?

We thank the reviewer for this comment. It is treated analytically as a total derivative, but numerically is approximated as the deviation of x_i after the perturbation. The constant perturbation from the targeted protein forces the system to a new steady state, hence all other proteins go from x_i to x_i + dx_i, which can be measured numerically and used as an approximation to determine G^v elements.

- I am puzzled by the top plot of Fig. 4. The whole measure |G^v| seems to be a very convoluted way to obtain some quantity that is essentially a linear rescaling of the number of affected nodes (up to some fluctuations that are ignored in the analysis). In the following I am assuming that G^v_k is proportional to the total variation of concentration of protein k when all the affected nodes are perturbed (which I am not sure of, see my previous point). Since the matrix elements are all of the same sign, G^v_k seems to be a non-decreasing function of the number of affected nodes, because each affected node adds a positive contribution to the total variation of concentration of every node in the network. The authors conclude "the overall impact of a perturbation generated by a virus is proportional to the amount of human proteins it interacts with". This seems like circular reasoning, since we are introducing an extensive quantity, which is by design proportional to the number of affected nodes, and at the same time presenting this behavior as a result. Since I have no data at hand, the authors are free to prove me wrong here: would this pattern (i.e. |G^v| being a linear rescaling of the number of affected nodes) appear also when evaluating G^v on networks where the links of the initial perturbed nodes are rewired randomly? If this is the case, how does this result help in the analysis of the virus-specific interactomes?

We thank the reviewer for this important comment. Calculation of the propagation pattern G^v from a given source can be informative as it depends on the local topology of the impacted nodes, characterizing how the interconnected system responds from a perturbation in that specific node. Which means that in the general case also |G^v| is dependent on the local topology of the interactome, e.g. targeted nodes with high degree and high assortativity (neighbors having too a high degree) can introduce higher |G^v| in some dynamics processes, in which the exponent Phi is greater than 0. In this specific case with regulatory and biochemical dynamics ODEs characterized by a Phi exponent equal to 0, there is no dependence on the local topology of targeted proteins. In light of this comment we decided to review the analysis on perturbation propagation and provide more understanding of this result, specifically by calculating cumulative correlation from single sources. We find that the cumulative correlation is independent of the PPI connectivity of the targeted protein/source, which explains why the cumulative response of all targeted proteins by the virus (G^v) is then proportional to the number of targeted proteins.

- The Sars-Cov-2 data seems to be very different from the data of other viruses (number of affected nodes), which may be likely caused by the fact that different sources are used for Sars-Cov-2 as compared to the other virus interactomes. While these batch effects are unavoidable because of the novelty of the Sars-cov-2 data (as aptly stated by the authors), I think that the optimal course of action would be to work on quantities that are not too affected by these biases. As per my previous point, the quantity |G^v| seems to be trivially dependent on the number of affected nodes, which in turn is the most evident bias in the Sars-cov-2 data against the other datasets (unless there is a plausible biological justification for why there is such a striking difference in the number of affected proteins).

We thank the reviewer for this comment. To improve this section and avoid the bias introduced by the number of sources, we reviewed the analysis of the propagation of signals, analyzing perturbations from single targeted proteins and showing their independence from the degree of source nodes. We then show how the cumulative quantity |G^v| is a consequence of the previous result.

- The clustering results in Fig. 5 are quite superficial and do not provide insights on what is really going on. The authors compute a hierarchical clustering of the networks based on their G^v vectors, but limit their analysis to essentially saying that having similar sets of affected proteins will result in having similar vectors G^v and being clustered together. Was the whole perturbation response analysis really necessary to come to this very basic conclusion? I think that the analysis would had been more convincing if the perturbation response highlighted some non-trivial similarities between the networks that weren't evident by simply counting the number of shared affected proteins.

We thank the reviewer for this comment. In light of a more extensive analysis and clustering based on integrated information from the other analyses (UMAP section on mesoscale structure, macroscopic functions and biological information) we decided to remove clustering results based on perturbation vectors. We notice its bias on the set of shared source nodes: this is ultimately a consequence of the dynamic processes we have modeled and the exponential decay of perturbations. The perturbation response in other scenarios (different dynamics processes for which propagation properties have a strong scaling dependence on the degree of nodes - https://doi.org/10.1038/nphys2741) can show topological differences that go beyond the set of targeted proteins, despite our analysis not falling in this case.

- The last part focused on the study of the density matrix, and several quantities are defined to study the difference between the networks. However, it seems to me that this statistical framework is plugged in without particular care on why this would be relevant for the current application. What is the biological hypothesis here? Are the different virus networks supposed to have different information content? What kind of biological process is the density matrix modeling? Or is it simply proposed as a generic embedding technique?

We thank the reviewer for this important comment. Gibbsian-like density matrices encode the interactions between the nodes of complex networks (please see our reference Ghavasieh, Nicolini, De Domenico 2020), from lower interactions to higher ones. In fact, \beta acts as a resolution parameter, where its small values encode the local interactions mostly limited to the neighbors and large values correspond to the non-trivial higher order interactions among the units. Therefore, it is beyond being only an embedding technique, and is based on physical approaches to quantify the non-trivial interactions from a multiscale view.

To better highlight the meaning of Gibbsian-like density matrices, we extended the introduction of the corresponding section by:

"In fact, it has been shown that such density matrices describe the short to long range interactions between the nodes, and their Von Neumann entropy encodes the diversity of information dynamics within the structures [Ghavasieh, Nicolini, De Domenico 2020]. The goal of this section is to study and compare the effect of viral components on the state of information dynamics in the human protein-protein network."

- The part on the k-means clustering is quite devoid of details (as a side note, I find it questionable that some clarifications are only in the caption of Fig. 6, instead of the main text). What is the space of features for the clustering? Is it the 2D space of (delta phi, delta S)? What is the metric used and why? How has the number of clusters k=4 been chosen? Also, in the text it is said that the colors of the three plots on the right hand side of Fig. 6 have been selected according to the clustering results for beta=3. However, if the three beta plots in Fig. 6 are to be taken as a reference, it seems to me that beta=1 is the actual space used for the clustering.

We thank the reviewer for this comment. We certainly agree that the manuscript missed a detailed description of the clustering technique. We confirm that our 2 dimensional space is delta phi and delta S. Also, the k-means clustering algorithm has been used coupled with the elbow method to determine the number of clusters at each value of beta. To make the necessary clarification, we changed the relevant part of the figure caption as follows:

"Taking the von Neumann entropy and Massieu function perturbations to be features defining a two dimensional space, k-means algorithm is used to cluster the viruses at different scales corresponding to distinct choices of beta (the three plots on the top of panel c). It is worth mentioning that the Elbow method has been used to find the optimal number of clusters. The trajectories at the bottom of the panel indicate how the perturbation changes with beta. The color of each trajectory is set by the clustering plot given at beta = 5 ."

Additionally, we added the required sentences to the main text:

"... the perturbations in Von Neumann entropy and Massiue function shape our two dimensional clustering space and the number of clusters has been calculated using the Elbow method for each choice of beta=1,2,3."

We believe that the part about the colors is related to the two plots at the bottom of the panel (c) where the trajectories have been depicted. Their colors are indeed selected according to the beta=3 clustering. It is worth mentioning that in the new revision, we decided to adopt a unified clustering method and apply it to all the analyses done in the paper, in a brand new section. More specifically, we used Umap dimensionality reduction and HDBSCAN method for the final clustering presented in the paper. Therefore, in the current version, the k-means clustering presented in the section is only to visualize the formation of virus clusters and their stability over propagation time scales.

- The authors introduce a number of (properly referenced) concepts to arrive to what seems to be a 2D vector embedding of perturbed graphs. However, it should be shown that there is some actual advantage in going through all these hoops instead of sticking with baseline approaches. It would be beneficial to show, for example, what we would obtain if we performed clustering on the matrix of jaccard similarities between the sets of affected proteins of each virus, and demonstrate that the results obtained through the proposed methodology are more insightful.

We thank the reviewer for this comment. In the light of this comment, we decided to add a section devoted to a comparative analysis of first and second interactors as well as Gene Ontology and pathways enrichment. At the level of first interactors, the binary hierarchical clustering results shows that SARS-CoV-2 does not appear similar to any other, while sharing 18 targeted proteins with the 92 viruses. We would like to mention that the data we currently have for SARS-CoV-2 has a lot more interactions with human proteins than other viruses. Therefore, the baseline and simpler approaches such as analysis of the shared interactors or the number of components fail to capture the similarities and differences between the novel coronavirus and others. For the same reason, our analysis of *Propagation of a perturbation from virus interactions* has not been successful in case of SARS-CoV-2.

We discussed it in the mentioned section as:

"To validate that these findings were biologically relevant, we have further performed a clustering analysis based on shared human proteins targeted by the viruses, enriched Reactome pathways within these targeted proteins or shared biological processes as defined by Gene Ontology. Figure.7 shows that SARS-CoV-2 does not cluster with any other virus on the basis of shared protein interactors, probably due to its relatively higher number of viral proteins and human interactors compared to other viruses. However, out of the 332 human proteins targeted by SARS-CoV-2, 18 of them were found to be targeted as well by other viruses, including Herpes viruses, HPV type 16, Reovirus or Encephalomyocarditis virus."

Interestingly, when 2nd range interactors are considered for the analysis the result was different, as we point out in the discussion:

"When the second-range targets were included in the clustering analysis, SARS-CoV-2 was observed to share secondary targets, and thus clustered with a wider range of viruses, including viruses responsible for skin and eye infections (Varicella, Cytomegalovirus), or attacking the hepatic (Hepatitis B/C), immune (HIV-1/2), respiratory (Influenza, Epstein-Barr), neurological system (Bunyavirus) or more

systemic-infectious viruses (Herpes). This apparent similarity with such diverse viruses may help explain the wide variety of symptoms and organs involved with SARS-CoV-2 infection and COVID-19."

This result is partially validating the statistical physics of information dynamics clustering for higher order interactions, indicating similarity between the novel coronavirus, HIV1 and Herpes. Please, note that HIV1 and Herpes are both implicated in several autoimmune diseases, such as AIDS, multiple sclerosis and systemic lupus erythematosus. Very recently, a new experimental study is providing evidence that inflammation caused by SARS-CoV-2 is able to trigger autoimmune responses, providing a compelling plausible explanation for the similarities we have found. We refer to Refs. 78-79 for details of the recent findings.

- The main conclusions of the paper depend on the final clustering results (i.e. coronavirus PPI being clustered together with Sars-cov at small scales and HIV at large scales), yet the clustering results are just provided "as is" with only two sentences in the main text. No further assessment of the quality of the clustering are provided. Also, it is well known that clustering is crucially dependent on the parameters/methods/metrics used for clustering and it is often a very hard task to establish a "final" clustering result. How stable are these results with respect to changes of the parameters (such as k) or clustering methodologies?

We thank the reviewer for this comment. We acknowledge that the previous version lacked a detailed description of the clustering algorithm. We resolved the issue as explained above, in the light of this comment and a previous one we received from the reviewer. Indeed, we agree that there is no universal clustering method and the results might depend on different parameters. However, we think that a k-means algorithm coupled with the elbow method is a standard approach to the problem.

Nevertheless, to strengthen our message, we decided to use a unified clustering method and apply it to all the analyses presented in the paper. More specifically, we used UMAP dimensionality reduction and HDBSCAN for clustering, both considered to be the state of the art for this purpose. Therefore, in the current version, the k-means clustering presented in the section is only to visualize the formation of virus clusters and their stability over propagation time scales. Notably, HDBSCAN provides the reliability of cluster assignments and we now include this information directly in the corresponding plots.

- Most result figures are barely addressed, they seem completely replaceable and do not really seem to bring more insights to the understanding of the results. For example, what does the bottom plot of Fig. 4 tell us, specifically?

We thank the reviewer for this note. We reviewed and shortened the propagation of perturbations analysis and updated the figures, removing redundant plots.

Also, was it necessary to show all the 8 plots of delta phi, delta S and delta U in various configurations (Fig. 6), when the only conclusion we have in the text is a description of how the interactomes are clustered (which is already summarized in the tables)?

We thank the reviewer for this comment. According to this suggestion, we removed the mentioned redundant subplots and kept only the three most important panels.

In conclusion, this work proposes a number of different approaches aimed at finding distinguishing features of the virus interactomes (percolation, dynamics, spectral information), but several of these analyses feel rushed and superficial. I think that a more focused analysis with just one of such approaches, and more detailed descriptions of the reasonings, technical details and caveats would make the contribution more appealing for the scientific community.

We thank the reviewer for his/her effort in correcting these issues. We believe that, in the new revision, the raised issues are fully addressed and in the light of the comments, the missing parts are added and unexplained parts are better explained. We also hope that the reviewer will be satisfied with our explanation of why a multi-method analysis is more beneficial than a single one which would not be able to provide insights across multiple scales and from multiple perspectives.

Referee #3

Firstly, we thank the reviewer for the encouragement we received:

In this paper the authors provided an interesting perspective of viewing the virus-human protein interactions as perturbation to the human PPI, and used classic statistical physic measures to evaluate them, clustering the viruses in the end. The paper is well written and easy to follow.

We agree that lack of biological evidence has been an important weakness of our manuscript, as the reviewer rightfully mentions:

However, the authors failed to provide any biological evidence to support their methodology, neither from mechanistic side nor from phenotypic side, thus the manuscript cannot convince the reader that the proposed measures are meaningful.

As we explain in the following, we have added a section to provide a comparative analysis of first and second range interactors, Gene Ontology and Pathways enrichment. Interestingly, we show that the results of these biology-driven analyses work as further evidence for our central claim of SARS-CoV-2 being a systemic disease, as well as they complement the results obtained from statistical physics.

We also agree that some of the methodologies had been discussed more than required:

In addition, the paper spends too much discussions on methodologies that do not work for obvious reasons.

In the following, we go through the major and minor comments, one by one, and describe the changes we made to resolve the raised issue or provide our answers.

Major comments

1. The authors need to support their method/model by evidence, both from the biological mechanism side (e.g. why is this clustering a reasonable way to classify viruses?); and from the phenotypic side (e.g. what biological features does the method capture? What biological observations support the clustering result?). Despite their well-establishment in statistical physics, these measures may lose their meaning in a protein-protein interactome context. For example, a scale-free network such as the protein interactome, are often known to shown heterogeneity, i.e. ‘nodes cannot be treated as the same’, as contradictory to a convention mean field assumption where ‘every node is the same’. Without support, it is difficult to claim the proposed measures meaningful.

We thank the reviewer for this important comment. To better highlight the biological mechanism we have in mind, the Introduction, as well as the Results and the Discussion sections have been extended, and a dedicated figure was added to better highlight the hypothesis behind each analytical method.
Also, we are pleased to inform the reviewer that the Von Neumann entropy is extremely sensitive to all types of inhomogeneities in the system that she/he is mentioning. Remarkably, it has been shown that it can be used to differentiate the nodes even in networks with homogeneous degree distribution, distinguishing the modular from hierarchical modular networks. We confidently ensure that the adopted framework is not a mean-field one which takes nodes to be the same, but in contrast, it has been shown that the Von Neumann entropy measures all types of local and global diversity of node, across multiple scales (please see our recent publication on this matter: Ghavasieh, Nicolini, De Domenico 2020).
In fact, Gibbsian-like density matrices encode the interactions between the nodes of complex networks (please see our reference Ghavasieh, Nicolini, De Domenico 2020), at the level of first interactors and beyond. In this framework, \beta plays the role of a resolution parameter, where its small values encode the local interactions mostly limited to the neighbors and large values correspond to the non-trivial higher order interactions among the units.

The reference we mentioned in the above paragraphs has been published after the submission of the current manuscript and could not be used to support our claim at that time. Therefore, we have added the required citation and wrote in the relevant section:

"In fact, it has been shown that such density matrices describe the short to long range interactions between the nodes, and their Von Neumann entropy encodes the diversity of information dynamics within the structures [Ghavasieh, Nicolini, De Domenico 2020]. The goal of this section is to study and compare the effect of viral components on the state of information dynamics in the human protein-protein network."

to ensure the framework of spectral information is fully explained.

2. The authors might need to make sure that these measures (e.g. von Neumann entropy) are not biased by less relevant measures, such as number of viral proteins, number of viral-human protein interactions, etc. The reason is that, as biological datasets are often incomplete, we often observe a biased subset of the complete data, thus it is important that the proposed methodology should be independent of this incompleteness. For example, if a measure correlates with the number of virus-host protein interactions, then a well-studied virus will appear different from a not-well-studied one, even if they are biologically similar, because the not-well-studied virus will have much less known interactions due to data incompleteness. It seems to me the measure(s) the author proposed might not work well under incompleteness of data. This problem might be addressed better if the authors can show biological insight of their measures.

We totally understand the second concern indicated by the reviewer. We certainly acknowledge that "if a measure correlates with the number of virus-host protein interactions, then a well-studied virus will appear different from a not-well-studied one". However, we approached the problem in a way to minimize such errors. In fact, the statistical physics of complex networks is used to capture the effect of viral components on the information dynamics within the whole human interactome. It is evident from our results that interactomes with high numbers of viral-human edges are being clustered with interactomes of lower

numbers of viral-human edges: e.g., SARS-CoV-2 has an exceedingly large number of interactions compared to Human Respiratory Syncytial virus, but they exhibit similarities well captured by our analyses. This fact indicates that, despite the unproportional number of interactions, they both have similar impact on the human protein-protein interactome.

Additionally, in the light of the comments, we added the analysis of first and second range interactors, as well as Gene Ontology and Pathways enrichment, a brand new approach independent from physics which is purely based on computational biology. Interestingly, when 2nd range interactors are considered, the results of these analyses are in agreement with the central claim of our paper:

"When the second-range targets were included in the clustering analysis, SARS-CoV-2 was observed to share secondary targets, and thus clustered with a wider range of viruses, including viruses responsible for skin and eye infections (Varicella, Cytomegalovirus), or attacking the hepatic (Hepatitis B/C), immune (HIV-1/2), respiratory (Influenza, Epstein-Barr), neurological system (Bunyavirus) or more systemic-infectious viruses (Herpes). This apparent similarity with such diverse viruses may help explain the wide variety of symptoms and organs involved with SARS-CoV-2 infection and COVID-19."

This is partially validating the statistical physics of information dynamics clustering for higher order interactions, indicating similarity between the novel coronavirus and viruses such as HIV1 and Herpes. Please, note that HIV1 and Herpes are both implicated in several autoimmune diseases, such as AIDS, multiple sclerosis and systemic lupus erythematosus. Very recently, a new experimental study is providing evidence that inflammation caused by SARS-CoV-2 is able to trigger autoimmune responses, providing a compelling plausible explanation for the similarities we have found. We refer to Refs. 78-79 for details of the recent findings.

3. As the authors pointed out in the introduction, percolation analysis is not expected to show difference between viruses as the viral proteins are too few and thus have too little impact on the network. However, the authors dedicated quite some detailed discussion to it. Would it be better to shorten this section, so that the readers can focus more on the rest?

We thank the reviewer for this comment. We totally agree that the results of the percolation section are not surprising. We followed the reviewer's comment and shrunk the section into almost a half, and also mentioned in the text that the result could be expected given the small fraction of viral interactions.

4. Similarly to above, as the Cumulative Correlation is proportional to the number of proteins each virus binds to, it is also a trivial measure in the sense that it does not show more information than "the number of proteins each virus binds to". The similarity analysis (Figure 5), if I understood correctly, is dictated by the overlap of human genes different viruses bind to, and thus is another trivial measure. The authors might want to consider if these could be shortened, too.

We thank the reviewer for this comment. We extended the analysis concerning the propagation of perturbations, providing more insight on the reason behind the Cumulative Correlation being proportional to the number of sources. We also reviewed and shortened other parts of the perturbation analysis.

5. The fact that the SARS-COV-2 protein has 332 number of targets in PPI, dramatically different from the rest of the viruses, may suggest the author's choice of representation for SARS-COV-2 is questionable. Can the authors validate this choice? I understand there may not exist alternatives in this matter, nevertheless this may reduce the reliability in the authors conclusions on SARS-COV-2

We thank the reviewer for this comment and acknowledge the mentioned possibility that our results might be affected by the limitations of the currently available data. Nevertheless, the data is gathered from the widely accepted available repositories and studies, and the whole scientific community is relying on exactly the same data sets, including the one for SARS-CoV-2 released by Gordon et al.
We will be looking forward to any updates in the available data sets and ready to complement this work with follow-ups. In addition to the Methods section, where we have discussed this particular vulnerability, we added the following paragraph to the discussion:

"It is worth mentioning that the SARS-CoV-2 outbreak is very recent and its PPI is not yet available on the STRING repository. Therefore, for this particular virus, we relied on a study published in Nature [Gordon2020], in April. We acknowledge the possibility that our results might be affected by the limitations of the currently available data sets."

to transparently inform the reader about the potential bias due to data limitations.

Minor comments:
1. Figure 2-left panel is not in vector format, i.e. one cannot zoom-in to see the small protein labels

We thank the reviewers for this comment. We updated the figure with a one with higher quality and resolution. We will be sharing the full data, in case the readers want to explore the targeted proteins.

2. Figure 1: Would it be more informative to also show additional numbers, e.g. the total number of human proteins each virus binds to?

We thank the reviewer for this comment. We added the suggested plot showing the number of viral components vs the number of human proteins targeted by them. Please see our second figure, supplementary materials, where we moved also the mentioned figure to obtain space for the novel analyses presented in the revised manuscript.

3. Figure 2 caption has a grammatic problem: "BIOSTR Human PPI used in this study, this obtained from data fusion of two comprehensive public repositories"

We thank the reviewer for this comment. We resolved the issue and we have also added a citation to the paper where BIOSTR has been presented in detail.

4. Figure 2 caption: “On the other hand, distinct colored markers of constant size encode distinct viruses (45 in total, including SARS-CoV-2)”: I don’t get what ‘constant size’ here means

We thank the reviewer for this comment. We had been referring to the number of human proteins, that is the same for all interactomes. To avoid confusion, we removed it from the caption.

5. Line 98, Formula “Law of Mass Action” has typo in ‘Sigma_j=1j’

We thank the reviewer for this comment. We solved the issue in the reviewed and shortened version of the section.

6. Page 10 , line 122 ‘asses’ is not correctly spelled

We thank the reviewer for this comment. We resolved the issue.

7. Page 13, “Dii = k_i delta_ij “should be “Dij = k_i delta_ij “

We thank the reviewer for this comment. In the revised manuscript, we adopted the suggested notation which is surely more rigorous.

8. Figure 6 caption did not explain the upper half of the bottom right sub-figure very clearly. I suppose each dot is a virus, right? And why is beta=1 shown in bottom left, but beta=3 shown in bottom right?

We thank the reviewer for this comment. In the revised manuscript, we removed some of the subplots which were not informative. We believe that the new version is not confusing. We thank the reviewer for this comment once again.

9. Page 20, Paragraph 3. The statement ‘viruses tend to preferentially target similar regions related to specific functions of the human interactome’ needs more evidence to support. Based on the evidence presented in the manuscript, one could only conclude “the top virus-targeted proteins have interesting functions”. In addition, defining ‘similar regions’ in human PPI is a tricky and difficult problem. If this is indeed the authors meant, the authors will have to explain how they define these regions.

We thank the reviewer for pointing this out. We have rephrased the statement as:

“Remarkably, viruses target a certain number of proteins which have interesting functions in the interactome.”

We thank the reviewer again for his/her time and effort and the invaluable comments.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors addressed all of my comments. Paper is much better now.

Reviewer #2 (Remarks to the Author):

The authors provided clear and thoughtful answers to my concerns, adding several interesting analyses for clarifying the biological implications of their findings. While I still have some reservations in part of the responses, I think that these points are debatable and out of the scope of this review. Overall, I can say that the manuscript has significantly improved. I have some final minor points about the new version of the manuscript.

Minor points:

- I am not fully convinced about the authors' response on my point about |G^v| being a convoluted quantity only telling us the number of affected nodes. The authors openly expand on its proportionality with the number of affected nodes, but in this case the measure isn't really helping in understanding some specific properties of the virus interactions, besides what we know already (i.e. the number of affected proteins). To reiterate my point: if we randomized the set of affected proteins, and repeated the same analysis, would we get a substantially different behavior in the plots of Fig. 4 and would the conclusions in the main text section change? If possible, I would appreciate to see the result here, even if only on a restricted set of virus interactomes or with few samples. If the results were the same or very similar, I think that the whole section shouldn't occupy such a sizable portion of the manuscript, since it would only dilute the message of the work without adding any specific information about the connectivity patterns of the virus interactomes.

- I couldn't find information on the sets of parameters used for producing the umap embedding and the HDBSCAN clusterings. These parameters affect the outputs of these procedures quite heavily (such as the number of neighbors in UMAP), so I think that their values should be disclosed even if they were left as default.

Reviewer #3 (Remarks to the Author):

I thank the authors for the point-to-point response to my previous comments.
The revisions have improved the manuscript significantly.

While I think the authors did very good in studying the virus-interactome perturbation problem in a classical physics context/setup, I'm not convinced that the authors methodologies are valid in real biological context. Specifically, I worry the authors' hypotheses/models made by the authors are under-validated in the biological context; some of authors approach, e.g. percolation, does not connect with the starting hypotheses well; and some results appears trivial, or inconsistent with each other.

Comments:
1. I like the type I&II hypothesis of how viruses affect the PPI: they seem perfect hypothesis from the physics perspective (and I also agree that these simpler hypotheses are better than more sophisticated ones). However, I'm a little worried if biological experts would agree. To be honest, I

don't really follow why the hypotheses can differentiate the authors' example of "DNA and RNA viruses behave differently".
Note I have no biology expertise, so sorry I cannot provide more constructive comment, but can only express a potential concern.

2. On "Percolation of the interactomes." Section:
If I understood it correctly, the hypothesis here is that the virus 'kill' all human proteins they interact with, and the authors wan t to evaluate the 'damage' by the virus. However the percolation analysis is done on the whole virus-PPI network, which is not making sense to me. In other words, I think a more specific percolation approach focusing on the virus-target-specific node removals, instead of node removal by centrality measures, seems to be the more reasonable approach.

While the introduction of percolation is comprehensive, I wonder if the authors could improve by adding one summary sentence at the beginning, highlighting what has been done in this section.

Figure 3: Notation "S" on y-axis refers to 'largest connected component size' in the caption, but this is not explicitly mentioned.

3. On the "Analysis of perturbation propagation":

The authors concluded the overall impact of a perturbation generated by a virus is proportional to the amount of human proteins it interacts with, which seems trivial. Is it indeed trivial?
In addition, is there an interpretation of this from the biological side? An interpretation from the biological side could help distinguish a signal from a study bias.

Is the Michaelis-Menten model the right assumption to a PPI? I'm very concerned if this hypothesis is the right one to capture the protein-interaction biology: In my impression (but I may be wrong), only regulations, i.e. a small proportion of all PPIs works with this models; the majority of the PPIs do not. The authors might need to show that this method is reasonable on the whole PPI level (maybe more specific references demonstrating its successful usage is helpful?).

4. On "Functional organization in modules and hierarchy: mesoscale analysis":
I like the evaluation of modularity under virus's effect. However, I'm confused by the interpretation from the two modularity models (Fig. 5): they seem contradictory to me. With absence of biological interpretation (e.g. what are the modules/clusters in biology?), the result might not be convincing.

5. On "Gene ontology and pathways enrichment analysis":
Some details in the enrichment study are missing, e.g. how did the authors define 'shared targets' of viruses? This definition could affect the result in 7A. For example, a direct count of 'shared targets' will not make any sense here, since the SARS-COV-2 has way too many targets. One might have to convert it to, e.g., a statistical significance. If direct overlap is indeed what the authors used, then I worry the result in Fig 7A is not very meaningful.

I'm under the impression that this affects the rest of the clustering analysis: the clustering results all seem to be dependent on the overlap. I.e. the SARS-COV-2 virus appears to be clustered with other viruses that share targets. If this is true, then the clustering is meaningless, because it becomes a trivial repetition of target overlap.
In other words, the authors might want to show that their methodology is not biased towards overlap of shared genes.

6. Notations and features on some figures (e.g. Fig 7 & 8) might be too small to read.

7. The description of the clustering study, especially how the authors combined physics and

biological aspects using machine learning to get the clustering results, appears to be missing in the main text. I found it difficult to follow, and wonder if it would be more helpful, to include one or two summary sentences describing the problem set-up and what features are being used in the ML approach (if possible).

Also, I'm skeptical if the overlap of targets between viruses is the main (trivial) reason that causes the clustering. Is there a (simple) way to check this?

8. Some items in the supplementary file are not displayed correctly. E.g. Supp. Fig.6 caption has a question mark "R package clusterProfiler ?".

Trento, 3 Feb, 2021

We thank the referees for their encouraging reviews. The resubmitted manuscript is changed to resolve the minor issues mentioned by the second reviewer and the comments we received from the third reviewer.
In the following, we go through the referees' comments one by one, providing our answers. All the changes made are highlighted in the revised manuscript.

Sincerely,

The authors

Referee #1

The authors addressed all of my comments. Paper is much better now.

We thank the reviewer for his/her time and effort reviewing the manuscript. We are pleased to hear that our work is improved thanks to his/her suggestions.

Referee #2

The authors provided clear and thoughtful answers to my concerns, adding several interesting analyses for clarifying the biological implications of their findings. While I still have some reservations in part of the responses, I think that these points are debatable and out of the scope of this review. Overall, I can say that the manuscript has significantly improved. I have some final minor points about the new version of the manuscript.

We thank the reviewers for his/her time and effort considering our manuscript. We are pleased to know that the manuscript is improved. In the following, we go through the last minor points raised by the reviewer, providing our answers in the hope that his/her concerns will be addressed.

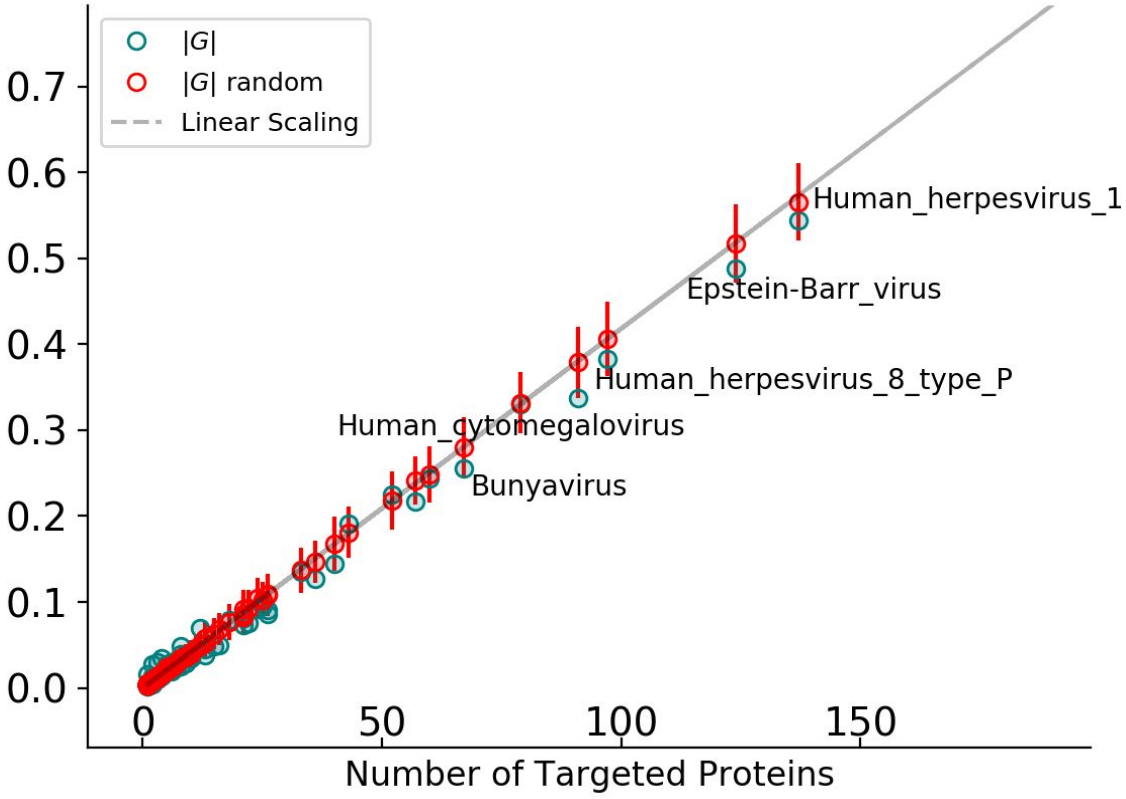
Minor points:

- I am not fully convinced about the authors' response on my point about |G^v| being a convoluted quantity only telling us the number of affected nodes. The authors openly expand on its proportionality with the number of affected nodes, but in this case the measure isn't really helping in understanding some specific properties of the virus interactions, besides what we know already (i.e. the number of affected proteins). To reiterate my point: if we randomized the set of affected proteins, and repeated the same analysis, would we get a substantially different behavior in the plots of Fig. 4 and would the conclusions in the main text section change? If possible, I would appreciate to see the result here, even if only on a restricted set of virus interactomes or with few samples. If the results were the same or very similar, I think that the whole section shouldn't occupy such a sizable portion of the manuscript, since it would only dilute the message of the work without adding any specific information about the connectivity patterns of the virus interactomes.

We thank the referee for this comment and we agree with his/her concern. To this aim, we performed additional analyses on a randomized set of affected proteins, as suggested. The following figure shows viruses impact against average cumulative |G^v| for randomized sets (the randomization process was repeated 100 times, bars showing standard deviation).

While there are virus-specific differences when it comes to single targeted proteins (|G^t|), the process of summing contributions is less sensitive to protein-specific information and the conclusion regarding the cumulative virus impact |G^v| would not change. Nonetheless, this result is meaningful as it is a

consequence of the Regulatory dynamics under study, which is relevant for studying protein networks. The study of a dynamical system on top of the real network of PPIs represents an interesting new methodology (it returns interesting insight regarding the amount of perturbation distributed by single targeted proteins, and also allows the study of relative differences in the effects of external agents in a theoretical framework of a dynamical system).



However, we agree with the referee that methods such as spectral entropy are more sensitive to the topological differences. We therefore decided to reduce the text of this analysis in the main manuscript and we moved the full discussion, figure and results regarding it in the Supplementary Material, to avoid diluting the message of the main manuscript. Thanks once again for further stressing this point.

- I couldn't find information on the sets of parameters used for producing the umap embedding and the HDBSCAN clusterings. These parameters affect the outputs of these procedures quite heavily (such as the number of neighbors in UMAP), so I think that their values should be disclosed even if they were left as default.

We thank the referee for the comment and we apologize for not being more specific. We added, in a dedicated section in the Supplementary Material, the parameters used for UMAP and HDBSCAN, and a link to the corresponding R documentation for full reproducibility of our results.

Referee #3

I thank the authors for the point-to-point response to my previous comments.

The revisions have improved the manuscript significantly.

We thank the referee for his/her time and effort considering our manuscript. We are pleased to know that the manuscript is improved during the last revision.

While I think the authors did very good in studying the virus-interactome perturbation problem in a classical physics context/setup, I'm not convinced that the authors methodologies are valid in real biological context. Specifically, I worry the authors' hypotheses/models made by the authors are under-validated in the biological context; some of authors approach, e.g. percolation, does not connect with the starting hypotheses well; and some results appears trivial, or inconsistent with each other.

We thank the reviewer for his/her encouraging view of the physical framework we adopt to study the virus-human interactions, as interdependent systems. We certainly acknowledge that our approach to the problem differs from the traditional biological approaches. However, we are rather optimistic and confident that novel methods grounded in physics can help to shed light on problems emerging in other disciplines, including biology. We believe the reviewer agrees with us that a more in-depth biological validation of our physical methods is out of the scope of a single paper like ours, where our goal is to provide another opportunity to establish a bridge between statistical physics and biology, which for sure should be followed by more communication and collaborations between the involved scientific communities.

In the following, we go through all the comments of the reviewer and provide our answers in detail, in the hope that his/her concerns will be addressed.

Comments:

1. I like the type I&II hypothesis of how viruses affect the PPI: they seem perfect hypothesis from the physics perspective (and I also agree that these simpler hypotheses are better than more sophisticated ones). However, I'm a little worried if biological experts would agree. To be honest, I don't really follow why the hypotheses can differentiate the authors' example of "DNA and RNA viruses behave differently".

Note I have no biology expertise, so sorry I cannot provide more constructive comment, but can only express a potential concern.

We thank the Referee for this comment and its potential concern. In fact, it highlights on our side a lack for an adequate explanation in the previous version of our manuscript.
On the one hand, the original text provided only a quick explanation of how RNA and DNA viruses are different, based on their interactome. We have updated the sentence in the text and added a reference. On

the other hand, the type I/II classification is not related to these DNA/RNA viruses differences and aims more at testing different perturbations from the physics perspective. Therefore, the difficulty found by the referee is perfectly justified and we have edited the text accordingly to avoid confusion in the reader.
The Type-I hypothesis represents a situation where a viral protein binds strongly to a human protein, preventing it to interact with its physiological protein partners and ***inhibiting it completely*** (node removal). The Type-II hypothesis represents a slightly different situation where the viral protein is able to ***modify the activity*** of the human protein it binds to (e.g. hyperactivation through allosteric binding, or conformational changes leading to loss or gain of functions/interactions). Therefore, as the referee correctly points out, the Types I and II are not at all expected to differentiate DNA and RNA viruses, but their role is to provide the reader with an operational framework explaining the hypothesis behind the choice of our analytical techniques.

We have updated the text to better clarify that these hypotheses are not meant to differentiate DNA and RNA viruses, as follows:

"It is worth remarking that our hypotheses in this work do not correspond to a difference between DNA and RNA viruses but, instead, they are intended to provide an operational framework to support the choice of the analytical techniques used in this study. Here, we will consider the following mapping, regardless of the type of virus, whether RNA or DNA, to allow for a consistent comparison of results across all families of viruses considered in this work"

We thank the referee once again for this comment.

2. On "Percolation of the interactomes." Section:

If I understood it correctly, the hypothesis here is that the virus 'kill' all human proteins they interact with, and the authors want to evaluate the 'damage' by the virus. However the percolation analysis is done on the whole virus-PPI network, which is not making sense to me. In other words, I think a more specific percolation approach focusing on the virus-target-specific node removals, instead of node removal by centrality measures, seems to be the more reasonable approach.

We thank the reviewer for this comment. In fact, in the percolation section we are not assuming that a viral protein kills the human proteins that interact with it. Actually, in this section we are not making any biological assumption but that connectivity is a first step to better understand functionality. We certainly acknowledge that in the previous version, the nature of the node removal was left undescribed.

In the revised text, we better explain the fact that this process can have different possible origins. Our goal is to compare the response of human-virus interdependent systems to removal strategies, pinpointing structural responses that could be later mapped to functional differences. Of course, since no virus stands out from the rest, there would be no need to proceed with more convoluted analyses. In the light of this comment we received from the referee, we added the following sentence to better clarify the point of percolation analysis:

"[The] absence [of proteins] can be justified from several viewpoints: viral proteins inhibiting human proteins or strongly perturbing their function, a drug targeting viral proteins and/or human ones, etc."

While the introduction of percolation is comprehensive, I wonder if the authors could improve by adding one summary sentence at the beginning, highlighting what has been done in this section.

We thank the referee for this comment. An introductory sentence in the section has been added and we have structured the first paragraph in order to make it more linear with the entire section.

Figure 3: Notation "S" on y-axis refers to 'largest connected component size' in the caption, but this is not explicitly mentioned.

We thank the referee for spotting this missing point. In the revised version, we have added in the main text that "S" is the normalized size of the largest connected component.

3. On the "Analysis of perturbation propagation":

The authors concluded the overall impact of a perturbation generated by a virus is proportional to the amount of human proteins it interacts with, which seems trivial. Is it indeed trivial?

We thank the reviewer for this comment. We fully agree with the reviewer that this proportionality seems trivial. However, we would like to point out that it is meaningful, as a direct consequence of the Regulatory dynamics, relevant for studying protein networks. In the references we mentioned here, it has been analytically (Ref. 24 - Universality in network dynamics) shown that in Regulatory networks, the impact of a node (the source of perturbation) on its neighborhood is independent of its degree. We exploit this measure for quantifying instead the cumulative impact on the entire interactome, providing insights about the effect of external agents. However, we agree with the referee that methods such as spectral entropy are more sensitive to the topological differences. Therefore, to give more space to other analyses more focused in identifying the distinguishing features between interactomes, we decided to reduce the size of the perturbation propagation section in the main manuscript and we moved the corresponding discussion and results to the Supplementary Material. We thank the reviewer once again for this comment.

In addition, is there an interpretation of this from the biological side? An interpretation from the biological side could help distinguish a signal from a study bias.

We thank the reviewer for this comment. From a biological perspective, the analysis of perturbation propagation has been studied in the literature and provides an interesting tool to understand signal propagation in PPI networks (Perturbation waves in proteins and protein networks: applications of percolation and game theories in signaling and drug design). Here, instead of retrieving the exact biology, the focus of this analysis is to construct a dynamical system which is eventually perturbed by the viral agent. This approach of quantifying the propagation of perturbations on processes on top of networks has been the focus of recent studies (Ref 24 and Ref 49:Universality in network dynamics, Quantifying the connectivity of a network: The network correlation function method and related works such as: Dynamic

patterns of information flow in complex networks.); which can yield understanding of complex interacting systems such as protein-protein networks.

Is the Michaelis-Menten model the right assumption to a PPI? I'm very concerned if this hypothesis is the right one to capture the protein-interaction biology: In my impression (but I may be wrong), only regulations, i.e. a small proportion of all PPIs works with this models; the majority of the PPIs do not. The authors might need to show that this method is reasonable on the whole PPI level (maybe more specific references demonstrating its successful usage is helpful?).

We thank the reviewer for this valuable point. We verified the information encoded in PPIs, and out of 719,552 PPI interactions, 668,372 of them belong either to category "experiments" (corresponding to biochemical or genetic data) or "database" (corresponding to imported interactions from databases of physical interaction or databases of curated biological pathway knowledge). We would like to mention that the functional links, such as the interaction of a transcription factor (a protein) with the promoter (DNA) of its target gene/protein and the downstream regulatory feedback loops, can be also represented by the M-M model (Supplementary information of Ref 24 Universality in network dynamics and Supplementary information of Dynamic patterns of information flow in complex networks). Additionally, physical links such as those involving enzymatic post-translational modifications (e.g. phosphorylation, ubiquitination, acetylation, methylation, etc.) can also be modelled using the Michaelis Menten equation (Dynamics of Posttranslational Modification Systems: Recent Progress and Future Directions).

We acknowledge that these choices represent an approximation over the real system of interactions. The rationale behind this choice is to approximate the complex biology of a PPI network to a simpler, dynamical system based on regulatory interactions that mimics the original one, and we focus on the relative differences between viruses in impacting this system.

4. On "Functional organization in modules and hierarchy: mesoscale analysis":

I like the evaluation of modularity under virus's effect. However, I'm confused by the interpretation from the two modularity models (Fig. 5): they seem contradictory to me. With absence of biological interpretation (e.g. what are the modules/clusters in biology?), the result might not be convincing.

We thank the reviewer for this valuable comment. We acknowledge that in the previous version of the manuscript, the differences between the two methods were not clearly explained. However, before getting to that point, we would like to better clarify the biological interpretation of modules in our study. As explained in the caption of Fig.1:

"The underlying hypothesis is that viral proteins alter the function of the human interactome at the mesoscopic level, i.e., interfering with the functional organization in modules typical of biomolecular systems"

Therefore, what we are doing in that section is measuring those interferences using network science tools. Moreover, functional modules are indeed meaningful in biological networks. For instance, we cite here two

works (ref. 26 and ref. 27) that study the functional modular structure of complex networks in biology. Specifically, using the original words from those studies:

1) in Functional cartography of complex metabolic networks is indeed discussed the identification of functional modules in metabolic networks: "*The information about modules and roles enables us to build a cartographic representation of the metabolic network [...]. This representation enables us to recover relevant biological information*"
2) in Yeast Protein Interactome topology provides framework for coordinated-functionality the link between modular structure and function is studied in the Yeast protein interactome: "*The second of these is the concept of biological functional modularity. In the context of proteins, at the extreme, this takes the form of protein machines performing specific functions in a cell. More generally, it consists of an expectation that the density of protein–protein interactions will rise as we zoom into an increasingly functionally related set of proteins*. We believe these works give the necessary context under which the study of modular structure of a protein-protein network becomes relevant.

Regarding the application of two different methods to assess the modularity structure, it should be noted that the two algorithms employed are based on two strongly different methodologies, since in network science there is no consensus on which method is the "right one". In fact, the whole community detection approach is a rather debated field of network science where there are essentially two approaches: the ones based on heuristic detection and the ones based on Bayesian inference. To the best of our knowledge, both methods have advantages and disadvantages, and an accurate analysis of a complex system should be based on results based on both of them, not just one.

Specifically, the Louvain algorithm searches for the partition that maximizes the modularity, while DC-SBM is based on Bayesian inference and finds the partition that maximizes the likelihood of observing that specific network. In fact, we expected different results regarding the number of modules and the modularity evaluation, since the two algorithms are based on fundamentally different methodologies, while complementing each other. However, the partitions generated by these two apparently different methods are consistent and show that while these two methods complement each other, they are not contradictory: this is a strong indicator of robustness for our results. To this end we would like to point the attention of the referee to this part discussed in the manuscript:

"Interestingly, some of the largest variations are detected for human coronaviruses in the Coronaviridae family. SARS-CoV and Coronavirus-229E, despite not having shared targeted human proteins with SARS-CoV-2, and impacting a sensibly lower number of proteins, show a variation in the new modular structure comparable to SARS-CoV-2. A result confirmed by both Louvain and DCSBM community detection methods.''

This indicates that despite being different, the methods capture the impact of viruses, consistently and with high sensitivity. Similarly, and to better clarify, we conclude the section "**Functional organization in modules and hierarchy**" with:

"SARS-CoV and Coronavirus-229E, despite not having shared targeted human proteins with SARS-CoV-2, and impacting a sensibly lower number of proteins, show a variation in the new modular structure comparable to SARS-CoV-2."

We thank the reviewer once again for this comment.

5. On "Gene ontology and pathways enrichment analysis":

Some details in the enrichment study are missing, e.g. how did the authors define 'shared targets' of viruses? This definition could affect the result in 7A. For example, a direct count of 'shared targets' will not make any sense here, since the SARS-COV-2 has way too many targets. One might have to convert it to, e.g., a statistical significance. If direct overlap is indeed what the authors used, then I worry the result in Fig 7A is not very meaningful.

I'm under the impression that this affects the rest of the clustering analysis: the clustering results all seem to be dependent on the overlap. I.e. the SARS-COV-2 virus appears to be clustered with other viruses that share targets. If this is true, then the clustering is meaningless, because it becomes a trivial repetition of target overlap.

In other words, the authors might want to show that their methodology is not biased towards overlap of shared genes.

We thank the reviewer for this very important comment. We acknowledge that our similarity assessment was not clearly explained in the previous version of the manuscript, although it was briefly mentioned in the supplementary materials. We assure the reviewer that the clustering obtained for the shared targets provides information fundamentally different from the one obtained using the propagation of perturbations. In fact, in the latter, only the overall impact of viruses is considered and shown to be proportional to the number of targeted human proteins. Please note that this number might be equal for two viruses while their target proteins are utterly different, having no overlap. In contrast, here, we use statistical measures, namely binary (Jaccard) distance, to compare the overlap in shared targets.

The "shared targets", as listed in the Supplementary Table 1, correspond to the human proteins reported to be both targeted by SARS-CoV-2 and by other viruses. To resolve the ambiguity of the previous version of the manuscript, we explain this in more details and clarity in the revised text:

"We consider a human protein as a "shared target" if it was reported to bind both to a SARS-CoV-2 protein and another virus' protein, according to the PPI data retrieved from http://viruses.string-db.org (Suppl. Table 1)."

We fully agree with the reviewer that the overlap result should have a direct impact on the further clustering results and that this type of analysis is quite standard, when comparing binary vectors. In fact, it was firstly suggested by the second referee, in our previous communication, as he/she explained:

"It would be beneficial to show, for example, what we would obtain if we performed clustering on the matrix of jaccard similarities between the sets of affected proteins of each virus, and demonstrate that the results obtained through the proposed methodology are more insightful."

In fact, Fig.7A shows the matrix of Jaccard similarities between the sets of affected proteins of each virus and illustrates that SARS-CoV-2 does not cluster with any other virus, as it does not share many of its targets with other viruses (only 18, see Suppl. Table 1). This low overlap in direct interactors is part of the reason why we further compare shared biological processes (GO-BP) and pathways (Reactome) rather than proteins themselves. As pathways and processes can involve hundreds of different proteins, viruses can

ultimately impact the same biological pathways without sharing any common targets. We used binary distance to perform all the hierarchical clusterings in Fig. 7A.

Furthermore, we show that viruses which cluster with SARS-CoV-2 based on the biological pathways and processes they impact, did not primarily share protein targets, e.g. "although Bunyavirus does not share any human protein target with SARS-CoV-2 (Suppl. Table 1), it is still found to be the most similar to SARS-CoV-2 based on their shared targeted pathways (which are mostly related to mitotic checkpoint controls, see Suppl. Table 2)."

Regarding the second order interactors overlap and whether these are the main drivers in the biological pathways/processes' overlap, similar results are observed: for example, Human SARS coronavirus does not cluster with SARS-CoV-2 based on their shared targets (2nd range interactors, Fig. 7C) but they do based on shared biological processes (Fig.7D)

6. Notations and features on some figures (e.g. Fig 7 & 8) might be too small to read.

We thank the reviewer for this relevant note. Unfortunately, given the size of the data set of 93 viruses, it is impossible to use larger fonts for this specific figure. For the sake of clarity, we have removed the line feature annotations corresponding to genes and pathways and we share the full data as supplementary material (Suppl. Tables A-F), where one can access all the information about the figure.

7. The description of the clustering study, especially how the authors combined physics and biological aspects using machine learning to get the clustering results, appears to be missing in the main text. I found it difficult to follow, and wonder if it would be more helpful, to include one or two summary sentences describing the problem set-up and what features are being used in the ML approach (if possible).

We thank the reviewer for this comment. We have extended the introduction to this section by adding a specific reference to the features used. These features are presented in detail in the dedicated section in the Supplementary Material:

"We combine the result of different analyses as features to perform the UMAP embedding, to provide an integrated view of virus clusters, identified via HDBSCAN algorithm (for more information and a detailed list of features used, see Suppl. Mat.)"

Also, I'm skeptical if the overlap of targets between viruses is the main (trivial) reason that causes the clustering. Is there a (simple) way to check this?

We thank the reviewer for the valuable comment. As we discuss in the main text, section "Gene ontology and pathways enrichment analysis", shared interactors (the overlap of targets) can not be used to find the similarities between SARS-CoV-2 and other viruses:

"Figure 6A shows that SARS-CoV-2 does not indeed cluster with any other virus on the basis of shared protein interactors"

In contrast, in the UMAP analysis and using combinations of micro/meso/macroscopic measures from other analyses, we obtain different clustering results--- very different from the analysis of shared targets. We would also like to discuss two examples, as these two points will clarify the fact that the overlap of targets between some viruses is not the main reason behind the clustering results in UMAP + HDBSCAN analysis.

From a theoretical standpoint, some of the features and metrics used for node embedding via UMAP come from measurements that take into account the macroscopic state of the network (for example the perturbations of the Von Neumann entropy), where removal, addition or rewiring of different nodes or connections in the network (with no overlap) can generate the same perturbed state, and therefore a feature for clustering. For instance, we provide a specific example in which viruses do not share targets, but have similar meso/macroscopic properties of the targeted interactome structure. The variation of information (VI - which is used as a feature in UMAP) between the mesoscale structures is similar for SARS-CoV-2 and the other human coronaviruses (therefore if only that feature would be considered for clustering they would be clustered together) but they (SARS-CoV-2 versus other human coronaviruses) do not have shared human interactors (targets, see Supplementary Table 1).

We thank the reviewer once again for this important point. We assure the reviewer that the clustering result can not be obtained using more basic approaches, due to the complexity of the problem.

8. Some items in the supplementary file are not displayed correctly. E.g. Supp. Fig.6 caption has a question mark “R package clusterProfiler ?”.

We thank the reviewer for pointing this error out. We resolved the issue

REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

The authors addressed my remaining concerns. To be honest, I remain skeptical of their considerations: “Nonetheless, this result is meaningful as it is a consequence of the Regulatory dynamics under study, which is relevant for studying protein networks.” The “burden of proof” should be on the authors to demonstrate that MM-like modeling is relevant for studying this *particular* system, beside any previous successful application on other systems in literature. The fact that we obtain exactly the same result in a random network is a failure of the model (or at best of the chosen measures, such as the global correlation function) in discriminating between a completely random pattern and a real one. As I said, this, coupled with the fact that the number of targeted proteins is the most evident batch effect of the Sars-cov-2 network against the other networks (because of different data sources), would warrant more caution in proposing this model for this situation. All the conclusions drawn in Sec. “Analysis of perturbation propagation”, (including supp. fig. 11a and b) seem to be a direct consequence of the modeling assumptions and are completely detached from the data, in that they would have been obtained in any other network or biological problem we were working with.

While this section is now less central in the whole manuscript, adding this up with the section on the percolation analysis, which also showed behaviors that were fairly expected (regardless of the data), we get a considerable portion of the manuscript discussing about something that is not really related to the virus networks, and is not expanding our knowledge on the models as well.

I already expressed this concern in my previous reviews, but I wanted to point out that the authors interventions in the sections did not really solve the problem. However, I do concede that the clustering and GO analyses the authors performed in the successive reviews added more interesting and context-specific content to the manuscript.

Overall, I have no further issues to highlight.

Reviewer #3 (Remarks to the Author):

I thank the authors for the comprehensive and detailed reply to my questions! I'm very satisfied with the authors' answers, and agree that the authors have done their best in the parts that I had doubts about. I don't have further questions.

Trento, 20 Feb, 2021

We thank the referees for their reviews and comments that surely enhanced our manuscript up to the current version. In the following, we go through the revision suggested by the second referee, indicating how we moved the less crucial sections to the supplementary materials and gave more space to the more important analyses.

Sincerely,

The authors

Referee #2

The authors addressed my remaining concerns. To be honest, I remain skeptical of their considerations: "Nonetheless, this result is meaningful as it is a consequence of the Regulatory dynamics under study, which is relevant for studying protein networks." The "burden of proof" should be on the authors to demonstrate that MM-like modeling is relevant for studying this *particular* system, beside any previous successful application on other systems in literature. The fact that we obtain exactly the same result in a random network is a failure of the model (or at best of the chosen measures, such as the global correlation function) in discriminating between a completely random pattern and a real one. As I said, this, coupled with the fact that the number of targeted proteins is the most evident batch effect of the Sars-cov-2 network against the other networks (because of different data sources), would warrant more caution in proposing this model for this situation. All the conclusions drawn in Sec. "Analysis of perturbation propagation", (including supp. fig. 11a and b) seem to be a direct consequence of the modeling assumptions and are completely detached from the data, in that they would have been obtained in any other network or biological problem we were working with. While this section is now less central in the whole manuscript, adding this up with the section on the percolation analysis, which also showed behaviors that were fairly expected (regardless of the data), we get a considerable portion of the manuscript discussing about something that is not really related to the virus networks, and is not expanding our knowledge on the models as well.

I already expressed this concern in my previous reviews, but I wanted to point out that the authors interventions in the sections did not really solve the problem. However, I do concede that the clustering and GO analyses the authors performed in the successive reviews added more interesting and context-specific content to the manuscript.

Overall, I have no further issues to highlight.

We thank the reviewer for this important comment. In light of this, with the aim of providing more space to analyses more effective in differentiating viruses, we have decided to move both Percolation and Perturbation propagation sections in the Supplementary Material. In place of them we have added a brief section that cites the analyses and results, adding the references to their specific sections and figures in SM.

"Percolation of the interactomes and Perturbation propagation: microscopic analyses. In this section we introduce two analyses performed on virus-host interactomes at the microscopic scale to detect virus (dis)similarities. A complete discussion of methods and results is present in the Supp. Sections 5 and 6. On the one hand, we investigate percolation processes, that, in the past, have been proved useful to shed light on several aspects of protein-related networks, such as in the identification of functional clusters and protein complexes, the verification of the quality of functional annotations or identification of critical properties. These successful applications motivate us to investigate percolation properties of virus-host interactomes. However, it turns out that percolation does not offer valuable insights when it comes at differentiating the topological response of our set of viruses under protein removals (see Supp. Figure 11), because the interactomes are too similar between each other. On the other hand, we take a dynamical approach and consider a regulatory dynamic process evolving on top of the reconstructed interactome with the aim of assessing differences between viral agents in the way they impact this system, by means of a dynamic perturbation in its steady state. We employ recent definitions of correlation functions to quantify the system response. We find that while this approach returns interesting insight regarding the amount of perturbation distributed by single targeted proteins (see Supp. Figure 12), there is need for more analyses to bring a comprehensive picture. Therefore, these types of microscopic analyses do not allow us to achieve our goal and we devote the rest of the article to investigate alternative approaches to differentiate between these so topologically similar interactomes."

We thank again the referee for this comment, we believe that this new revised version allows now a reading more focused on the insightful results about viruses similarities

Referee #3

I thank the authors for the comprehensive and detailed reply to my questions! I'm very satisfied with the authors' answers, and agree that the authors have done their best in the parts that I had doubts about. I don't have further questions.

We thank the reviewer for his/her time and effort reviewing the manuscript. We are pleased to know that our work is improved and that the reviewer is satisfied with our answers