

Rapid Reviews COVID-19

Review 2: "Phase transitions may explain why SARS-CoV-2 spreads so fast and why new variants are spreading faster"

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RR:C19 Evidence Scale rating by reviewer:

- **Potentially informative.** The main claims made are not strongly justified by the methods and data, but may yield some insight. The results and conclusions of the study may resemble those from the hypothetical ideal study, but there is substantial room for doubt. Decision-makers should consider this evidence only with a thorough understanding of its weaknesses, alongside other evidence and theory. Decision-makers should not consider this actionable, unless the weaknesses are clearly understood and there is other theory and evidence to further support it.

Review:

I have been requested to comment on “whether the above-quoted manuscript's main claims are reliable and trustworthy” and further to advise whether “the main claims are not informative or even misleading.”

Firstly, I would like to gist my overall impression that the manuscript certainly presents a potentially good piece of work (and no way misleading!) but would need **Major revisions** before acceptance can be recommended. I find it interesting from several angles. Having said that, there are some discrepancies that caught my eyes which I would like to bring to the concern of (first and foremost) the authors and also the journal Editorial Office.

The strong points:

The narrative presented in the manuscript is a physicist's view of molecular evolution. Starting from the first principles, the authors gradually add on complexities and logically derive well-argued conclusions, often backed up by supporting literature. The logical flow is exquisite at patches (especially in the Methods section), written with intellect and brevity.

The conformational transition of the SARS CoV-2 Spike glycoprotein between its metastable resting (prefusion) state and membrane-bound active (post-fusion) state has recently been well surveyed from various angles and has been revealed to be kinetically driven (‘surprisingly low kinetic barrier’ [1, 2]) and triggered by the accompanying conformational change of the S RBD from its ‘down’ to ‘up’ states. The conformational switch is a precondition to the host cell attachment of the virus leading

to its virulence and spread. The proposed approach treats the structural dynamics for the S glycoprotein functionality as a thermodynamic object immersed in water. The model is then used to probe thermodynamic criticality and phase transitions based on evolutionary changes in the sequence space. Importantly, ASA profiles for individual amino acid types embedded at the center of a protein fragment scales as a power-law of the fragment-length with well-ordered negative exponents for all 20 amino acids. The existence of such characteristic (universal) amino acid-water interaction parameters is strongly supportive of the proposed thermodynamic phase transition model. Key mutations that enhance viral attachment and infectiousness have been identified and linked with vaccine efficacy.

For analytical purposes, the authors formulate *"a measure of the average hydrophathy"* for each amino acid residue type placed *"at the center of an arbitrary background neighborhood"*. The 'hydrophathy' statistic is said to be modeled from the exponents of the characteristic power-law distributions attributed to individual amino acid residues from their respective ASA profiles.

The authors then talk of an abstract network view of the Spike glycoprotein based on evolutionary changes in the edges connecting hydrophilic regions. These edges are derived based on the comparative hydrophathy profiles ($\Psi(R, W=35)$) of the Spike proteins plotted for different coronavirus strains.

The network treatment of protein folds is nothing new but the differential order of (thermodynamic) phase transitions revealed by fractals is (for example) a key unique highlight.

"To our knowledge, proteins are the only large-scale networks that exhibit both first-order unfolding phase transitions and second-order conformational phase transitions described by fractals."

One of the key observations as well as one of the final concussive remarks of the paper is that the proposed evolutionary increase in hydrophobicity from the earlier to the later strains of the coronavirus could actually *"stabilize the virus in aerosols"*. This is indeed simple and rational, and, coupled with the proposed thermodynamic criticality and phase transitions makes the proposition interestingly unique.

Weak Points / Discrepancies:

- The paper suffers from a lack of care and clarity in describing the results with appropriate display items.

1. For example, I find the following sentence extremely difficult to imagine and conceptualize given the absence of an appropriate pictorial (network) representation.

"The minima represent edges of hydrophilic regions. Edge 1 (454) is located in the Receptor Binding Domain (RBD), 2 (569) in C-Terminal Domain 1 (CTD1), 3 in the Linker between S1 and S2 (694), 4 in the Fusion peptide in S2 (803), 5 (952) in heptad repeat 1 (HR1), and 6 (1156) in the Linker to the stem of S2"

2. *"These mutations do not affect the hydrophilic edges compared to CoV-2, but they do make the hydrophobic peak located at residue 227 larger, which is in the N-terminal domain adjacent to the receptor-binding domain. ... The near leveling of the hydrophobic peak near 227 with the receptor binding peak near 380 suggests that the two peaks could bind together to aerosol surfaces."* → where should one try to find traces of these so-called hydrophobic peaks? What about the quoted residue sequence numbers? Should one refer to the relative hydropathy profiles overlaid for the different coronavirus strains (Spike proteins) in Figure 2? No citing figures called, so, remains elusive. Overall, fuzzy and unclear without an appropriate pictorial description mapped onto the Spike protein structure.

3. **Table 2** has been wrongly cited as **Table 1** at least twice.

*"Edges 1, 2, 4, and 6 are the most hydrophilic. They have similar magnitudes and are much more similar than the corresponding edges of CoV-1 (see **Table 1**)." → should actually be referring to **Table 2**.*

*"The differences between $\Psi(R,35)$ for CoV-2 and B.1.1.7 are small, but close inspection of the hydrophilic edges shows that edges 1, 2, 4 and 6, which were nearly equal already in CoV-2, have become even more equal in B.1.17 (see **Table 1**)." → should also be referring to **Table 2**.*

Table 1. The shifted and rescaled hydropathic values Ψ for the Moret Zebende (MZ) and Kyte- Doolittle (KD) scales.

Table 2. Scores for main hydrophilic edges (based on CoV-2 sites).

4. *"**Table 3.** Scores for a selection of hydrophobic edges (based on CoV-2 sites). While most of the hydrophobic peaks are similar between CoV-2 and the new variants, there was a significant increase in hydrophobicity for peak B in B.1.351."*

If peak B (net increase: 3.1, percentage increase: 1.9%) in B.1.351 has a significant increase in hydrophobicity compared to that of CoV-2, then, why is it not applicable for

peak D (2.9, 1.7%) in B.1.351 & also peak D (3.1, 1.8%) in B.1.1.7 ?

- Some sentences suffer due to unfamiliarity with appropriate terminologies and/or poor grammar and may be better rephrased. For example:

1. "*near the outside of the protein*" may be rewritten as "near the protein periphery"

2. "*They (referring to 'long-range or allosteric interactions in motor proteins') are known to occur in principle but when small but are detectable using the hydrophobic scale with 20 exponents.*" → does not convey any rational meaning. Most probably they meant to say, '*... and are still detectable even when small using the hydrophobic scale with 20 exponents.*'

Since this is not a traditional peer review, I would restrict myself at this point. The paper, however, can certainly be improved to a great extent by the incorporation of appropriate additional analysis and better display, and a rigorous round of regular peer-review.

I repeat my gist that the preprint certainly presents a potentially good piece of work (and no way misleading!) but would need **Major revisions** (as prescribed in the report) before acceptance can be recommended.

References

1. Cai Y, Zhang J, Xiao T, et al (2020) Distinct conformational states of SARS-CoV-2 spike protein. Science. <https://doi.org/10.1126/science.abd4251>
2. Basu S, Saha P, Bhattacharyya D, Patra HK (2020) Plausible Blockers of Spike RBD in SARS-CoV-2 - Molecular Design and Underlying Interaction Dynamics from High-Level Structural Descriptors. <https://doi.org/10.20944/preprints202009.0006.v1>