Rapid Reviews COVID-19

Review 2: "Single-Molecule Dynamics of SARS-CoV-2 5' Cap Recognition by Human eIF4F"

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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:

This manuscript is potentially informative, but several controls need to be done and there are other possible interpretations of some of the data. The authors claim that eIF4A enhances cap accessibility based on the data that eIF4E binding is increased in the presence of additional eIF4A. This is an interesting result, but it is not evident that it is due to increased cap accessibility. Several of the studies with eIF4A and ATP should be repeated with a non-hydrolyzable ATP analog to determine the effects—if any—of helicase reactions. All of these studies would be more believable if a non-functional cap were used—such as ApppA—to determine if the reactions are truly with the mRNA cap or possibly with other structures in the 5' UTR. EIF 4G, for example, is known to interact with RNA secondary structures. It seems likely that SARS-CoV translation is more eIF4A-dependent than some mRNA and this is an interesting avenue to pursue. It is also well known that cap-binding depends on the nearby RNA structures, so this finding is not surprising. Two additional controls would provide more confidence in the conclusions. One is to measure ensemble binding to the mRNA to eIF4E when the mRNA is not immobilized. While the ensemble binding would not provide the same information as the single-molecule measurements, this data should be similar in overall affinity if the binding is not perturbed by the immobilization. Along these same lines, the authors do not state how they refolded the RNA oligonucleotides, which is likely important in binding and translation. Lastly, the authors labeled the eIF4E and added a small peptide extension. It is not shown whether or not this modification alters cap-binding or translation. This should be compared with wild-type eIF4E. Overall, there are some potentially intriguing results that should be further developed.