

## COMPUTATIONAL STUDIES TO ANALYZE EFFECT OF CURCUMIN INHIBITION ON CORONAVIRUS D614G MUTATED SPIKE PROTEIN

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#### Abstract

COVID-19 disease created worldwide chaos with millions of casualties worldwide. The infection initiates when the viral spike protein interacts with the human ACE2 receptor. Developing an effective therapeutic drug or vaccine for the disease is a challenging task since the virus can mutate itself. For instance, recently viral spike protein D614G mutation has been identified which makes it more contagious. In this study, we have investigated the efficacy of curcumin, a natural bioactive compound in inhibiting the binding of spike protein to ACE2 protein in native and D614G mutated viruses using molecular docking tool. We have used an I-TASSER server to computationally model virus spike protein. We have obtained a reliable C-score value. The PyMol molecular visualization tool was employed to generate spike protein D614G mutation. The docking studies were performed on a Swiss Dock server and the complex was visualized using JMOL software. The free energy change for curcumin-spike protein complex for native and mutated forms were found to be substantially unaltered. Taken together, our studies indicate that among natural medicinal compounds studied so far curcumin is an ideal candidate to inhibit spike-ACE2 interaction irrespective of the viral mutation.

**Keywords:** SARS-CoV-2, Curcumin, Spike Protein, ACE2 Protein, D614G Mutation, Molecular Docking

#### INTRODUCTION

The extremely infectious novel coronavirus disorder 2019 (COVID 19) outbreak is a pandemic that has been marked by acute severe respiratory syndrome (SARS), which is induced by SARS coronavirus 2 (SARS CoV 2) infection. Therefore, developing immediate therapy against this

virus is the need of the hour. The SARS CoV 2 is a big, single-stranded RNA virus that has a genome of about 30 kilobases in size. It is wrapped by a capsid. The spike

(S) Protein is one of the four structural proteins found in the coronavirus. Viral entrance, replication, assembly, and spread in lung as well as other cells are all dependent on structural proteins. The entry of virus is mediated via angiotensin converting enzyme (ACE) 2 transmembrane receptor of the host cell (Chen et al., 2020). During infection, the S protein collaborates with the ACE2 protein in the host plasma membrane to pave the way for viral entrance (Y. Chen, Liu, & Guo, 2020). In this regard, pharmacological intervention aimed at the SARS-CoV-2 spikes glycoprotein could be useful. Unfortunately, traditional vaccine development is now a lengthy process that could be jumpstarted by computationally-assisted medication design.

The infection of the virus starts when S protein interacts with the transmembrane ACE2 protein expressed on the host cell plasma membrane. After receptor binding, the virus makes an entry to the host cell cytoplasm. In general, this is achieved by proteolytic cleavage of S protein, followed by fusion of the viral particle and plasma membrane. Subunit 1 (S1) of the viral S glycoprotein contains the receptor binding domain (RBD), which is responsible for determining the virus-host range or cellular tropism; subunit 2 (S2) mediates virus and cellular membranes fusion by means of two tandem domains. A replication-transcription complex (RTC) is formed within the double-membrane sac-like vesicles when viral RNA is liberated into the cytoplasm once membrane fusion is complete. Viral RNA production is followed by the formation of the antiviral replicase complex. Subgenomic RNAs are synthesised by the RTC complex and then translated into functional and structural proteins. By budding into to the Endoplasmic Reticulum-Golgi intermediate compartment, virions are generated when the progeny genomes are encapsidated by N protein, and then merge with the membrane-bound components. Finally, membrane-bound vesicles containing virion fuse with cell membrane, releasing the virus into infect further cells.

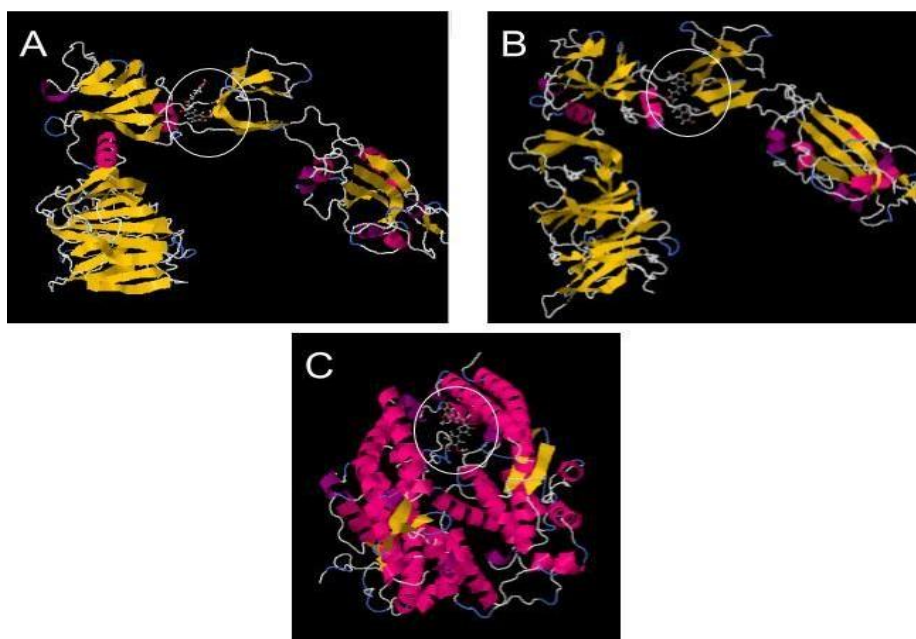
Confronting the novel viruses has always been a challenging task. Viral genome has a high rate of mutation because of the lack of RNA polymerase proofreading ability (Elena & Sanjuán, 2005). This promotes the evolution of RNA virus resistance to currently available antiviral drugs (Bolken & Hruby, 2008; Sahin et al., 2020). One of the recently known D614G mutation in coronavirus spike (S) protein is more contagious than the wild type protein. In the present study, we have investigated the efficacy of curcumin in inhibiting the binding of host ACE2 transmembrane protein to viral S protein in wild type and D614G mutated viruses.

The lack of therapeutic treatment in the form of medications or vaccines in this age of extremely infectious coronavirus has driven scientists to hunt for hints amongst safe herbal extract constituents. Developing new therapies sometimes requires the time-consuming process of creating new lead chemicals; however, medicinal plant products may provide an alternative. The current research focuses on the bioactive component curcumin, which is found in the Indian spice turmeric, a rhizomatous herbaceous plant belonging to the ginger family. Several different strains of viruses can be treated using turmeric's chemical components, which are widely utilised for treatment. Curcumin's therapeutic efficacy against SARS-CoV-2 was

investigated in this work using a computer-aided medication design approach.



**Fig 1: Computational model of Spike2 protein using I-TASSER database. The C-score is about 1.05. The C-score value suggests a reasonable probability of a predicted model**



**Fig 2: A docking comparison between curcumin binding to A) spike protein B) Mutated Spike Protein (D614G) and C) ACE2 protein (PDB ID: 1R42). The Swiss Dock server was used for molecular docking and analysed using JMOL tool. The circle depicts the binding site for protein-ligand interaction. The spike protein- curcumin binding in native and mutated form yields a  $\Delta G$  value of  $-7.78$  kcal/mol and  $-7.64$  kcal/mol respectively. The ACE2 binding to curcumin generates  $\Delta G$  value of  $-7.25$  kcal/mol.**

## DISCUSSION

Molecular docking is a computer-based method to identify potential drugs complementary to target proteins. Researchers rely heavily on it to make predictions about the binding and affinity of ligand for macromolecules like protein before undertaking the far more time- and cost-intensive process of conducting experimental experiments. Furthermore, the accuracy, speed, and reliability of molecular docking approach nowadays make it a suitable choice to predict the molecular structure of drugs. The ligand-receptor complex of the turmeric bioactive compound such as curcumin used in COVID19 therapies was studied in terms of docking scores, the binding free energy of ligand-receptor complex. In the present study, the orientation of the docked curcumin in the active site of wild type and mutant S protein was analyzed in detail.

SARS-CoV-2 is responsible for unprecedented pandemic with worldwide concern. To overcome SARS-CoV-2 infection, a large number of drug candidates that include antiviral, antibiotics and anti-malarial properties have been tested in treating COVID19 patients and they are useful in preventing viral infection to some extent. However, considering the fatal side effects of these compounds, the potential of curcumin and its derivatives to inhibit SARS-CoV-2 S protein becomes more important. Curcumin is a molecule of interest to us because it is a natural bioactive compound and importantly it is not toxic to humans even at dose of 5 g/day (Soleimani et al., 2018). Given curcumin interacts with the spike and plasma membrane receptor proteins at very high concentrations and hence toxicity won't be an issue. We hope that our molecular docking study along with future *in vivo* and *in vitro* experiments will establish curcumin as a potential drug candidate for COVID19 treatment.

There is insufficient information in structural databases about the protein complexes of SARS-CoV-2, a member of the genus Beta corona virus. Therefore, we have used an I-TASSER database to computationally model virus S protein. We have obtained a C-score of 1.05 which is an indicative of a reliable model on the basis of different interactions (Figure 1).

With molecular docking approach, the orientation of the docked curcumin in the potential drug binding site of wild type and mutant S protein was analyzed in detail. For molecular docking, we used SwissDock server and analyzed using JMOL program. The S protein- curcumin binding in native and mutated form yields a deltaG value of -7.78 kcal/mol and -7.64 kcal/mol respectively (Figure 2A and B). The circle in figure 2 represents the binding site for protein-ligand interaction. This data demonstrate that binding of curcumin to wild type S-protein is slightly stronger than the mutated S protein harboring D614G mutation. Intriguingly, the free energy change for curcumin-S protein complex for wild type and mutated forms of S protein were found to be almost unchanged inferring curcumin efficacy in inhibiting the wild and mutated version of viral S protein equally. By contrast, the ACE2 binding to curcumin generates deltaG value of -7.25 kcal/mol. This comparatively high deltaG value reflects the slightly weak binding of curcumin to ACE2 protein when compared with binding to S protein (Figure 2C). Most importantly, the high binding energy of curcumin to S or ACE2 protein suggests that curcumin might act as a bridge between S-ACE2 proteins interaction thereby inhibiting the COVID19 infection.

Curcumin does have a strong affinity energy of -7.9Kcal/mol for S protein & -7.8Kcal/mol for ACE2, according to a study by Jena et al. The curcumin-S protein binding is consistent with our result. However, the curcumin-ACE2 protein binding is comparatively higher than ours. This distinction might be the different programs that were used for molecular docking and analysis or different docking site was chosen in these studies. In another independent study, Suravajhala *et al.* obtained the binding energy of -4.9 kcal/mol corresponding to curcumin and S protein binding. In yet another study, Shanmugarajan *et al.* found the calculated binding free energy was  $-4.067 \text{ kcal mol}^{-1}$  for curcumin-S protein interaction. Moreover, Fazal *et al.* reported via animal studies that curcumin can inhibit the expression of ACE2 receptor. Based on these studies, we speculate that curcumin not only makes human less vulnerable to SARS-CoV-2 infection by inhibiting the ACE2 receptor expression but it can also inhibit the ACE2-S protein binding.

## CONCLUSION

We propose curcumin as a therapeutic target against SARS-CoV-2 infection. In this work, we have investigated the efficacy of curcumin, a natural bioactive compound in inhibiting the binding of S protein to ACE2 protein in wild and D614G mutated S protein of SARS-CoV-2 using molecular docking tool. The S protein D614G mutation is found to be more contagious. The free energy change for curcumin-S protein complex for native and mutated forms were found to be substantially unchanged meaning thereby curcumin is equally effective in inhibiting the wild and mutated version of viral S protein. Overall, it is concluded that among natural medicinal compounds reported in literature curcumin is an ideal candidate to inhibit S-ACE2 interaction regardless of the viral mutation.

Our data show that S protein is an important pharmacological target protein for COVID19, and it is likely that curcumin prevents viral infection by blocking key physiologically active target regions in S protein. Therefore, curcumin is a candidate for further study as a medicinal drug for the treatment of COVID19 based on the results of this molecular docking study. This was, however, only an exploratory study aimed at pinpointing curcumin's active pharmacophore and the complementary binding site on the target protein. Future plans include extensive *in vitro* and *in vivo* investigations to corroborate the effectiveness of curcumin on COVID19 and supplement the current findings. Our studies via *in silico* approach identifies curcumin as a potential therapeutic agent to prevent the viral infection.

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