CHARACTERIZING THE ROLES OF CHEMOMECHANICAL COUPLINGS IN THE DIFFERENTIAL BEHAVIOR OF SARS-CoV-1 AND SARS-CoV-2 SPIKE GLYCOPROTEINS

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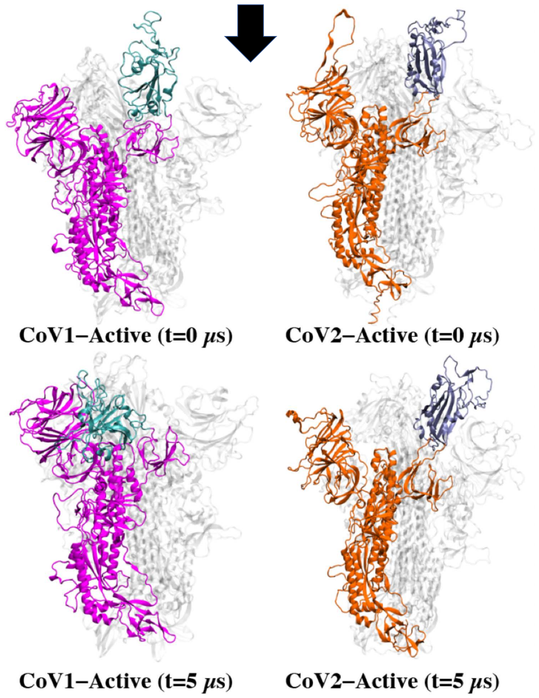
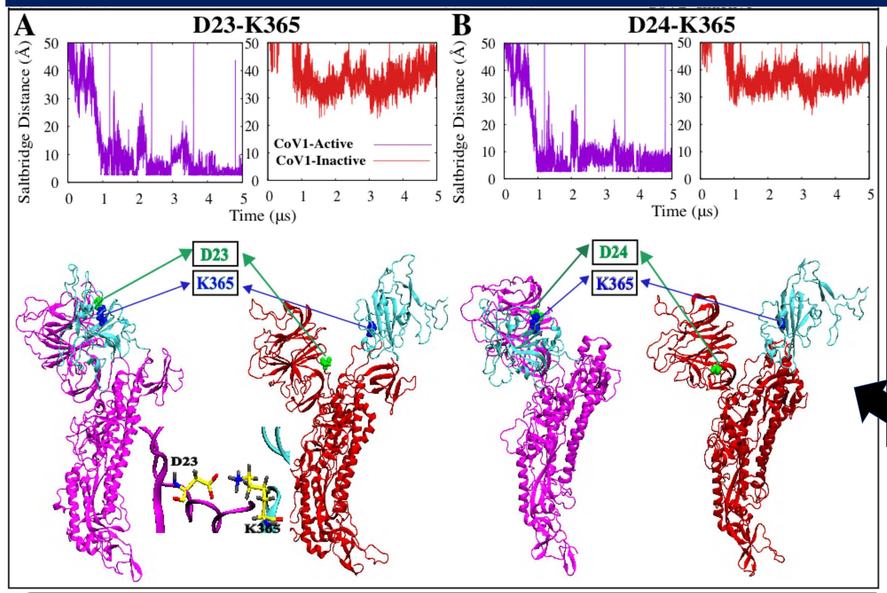
INTRODUCTION

The highly contagious severe acute respiratory syndrome (SARS) coronavirus-2 (CoV-2) is closely related to CoV-1 which broke out as an epidemic in 2003. CoV-1 and CoV-2 spike proteins share similarities in sequence identity and binding patterns to human angiotensin converting enzyme 2 (ACE2) receptor. Despite these similarities, CoV-2 spike protein has a higher infectivity and transmissibility, with emergence of new mutated variants raising concerns about the efficacies of the vaccines. As such, in addition to studies discussing the binding mechanism of receptor-binding domain (RBD) to ACE2, it is expedient to explore the events prior to the binding of RBD-ACE2 in comparison to CoV-1. We propose that this can lead to design and possible modifications of therapeutic agents that can inhibit the binding and prevent the spread of COVID-19 irrespective of mutating variations. For our study, we have used cryogenic electron microscopy (Cryo-EM) structures of active and inactive models of CoV-1 and 2. Our research discusses the conformational changes and differences seen through electrostatic interactions in CoV-1 and CoV-2 prior to ACE2 binding, and considers hot spot regions outside the RBD that could be contributing to the differences in transmissibility. Our extensive electrostatic interaction analysis reveals that the driving force behind a unique conformational transition observed in the initially active CoV-1 spike protein simulation (see below) is at least partly a set of salt bridge interactions that are unique to CoV-1. Residues D23 and D24 (not conserved) in the N-terminal domain (NTD) interacts with K365 in the RBD, forming stable salt bridges in the active CoV-1 spike protein but not in the inactive state. We also found that conserved residues within the RBD and NTD form strong salt bridge interactions in the CoV-2 spike protein but not in the CoV-1 spike protein

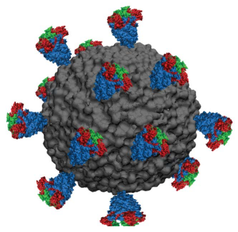
Active form of CoV-1 spike protein undergoes a spontaneous large-scale conformational transition and essentially becomes inactivated.



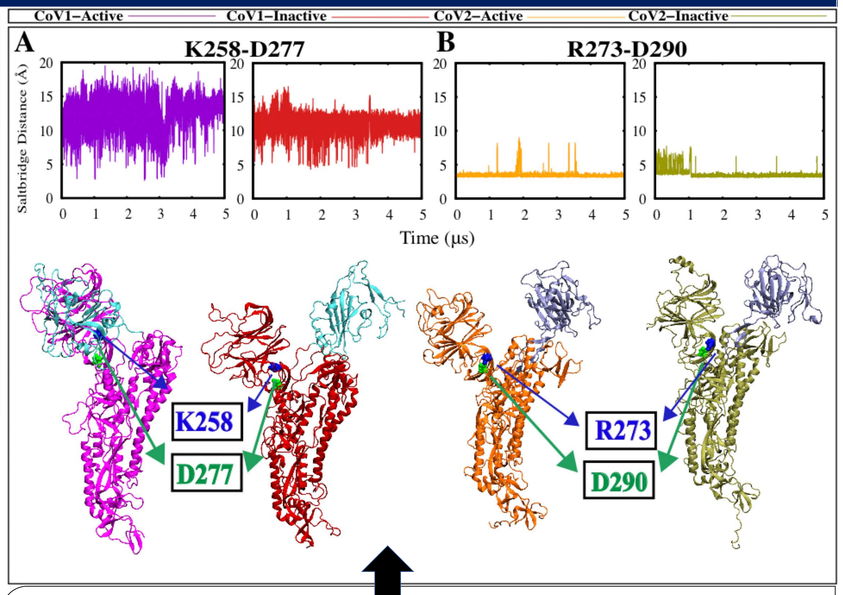
RESULTS



Unique salt bridge interactions between the RBD and NTD of the active CoV-1 spike protomer facilitate the transition to a pseudo-inactive conformation. D23 and 24 are not present in the CoV-2 spike protein.

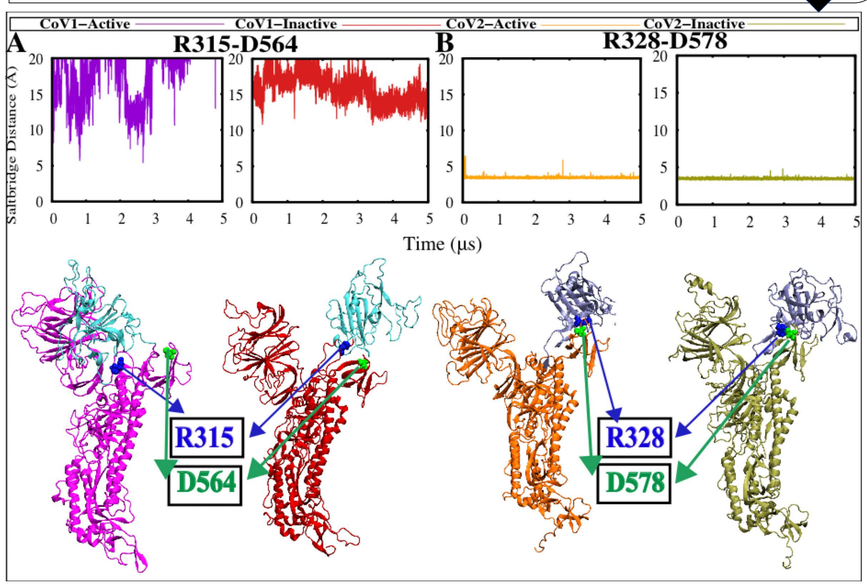


SCAN ME

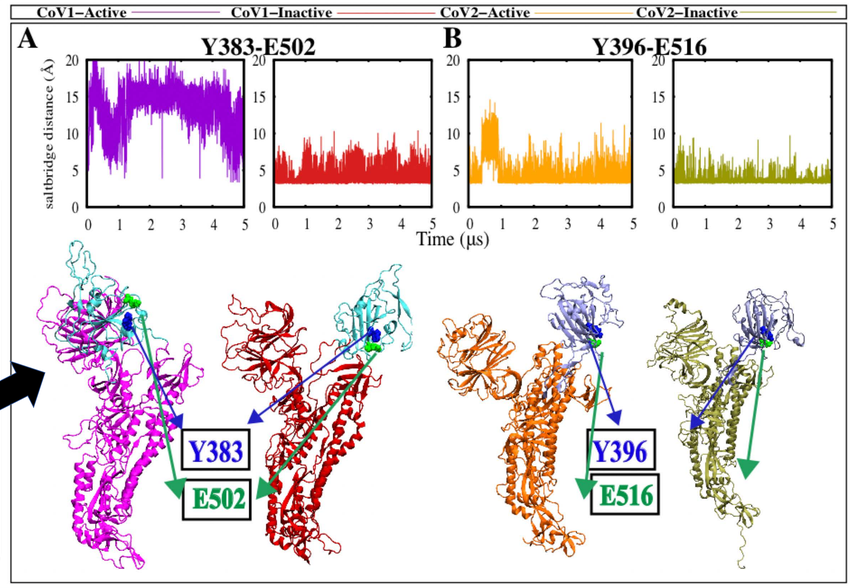


Similarly, R273 and D290 form a stable salt bridge in both active and inactive CoV-2 spike proteins while K258 and D277 do not form a salt bridge in the CoV-1 spike proteins. These electrostatic interactions thus potentially contribute to the relative stability of the active SARS-CoV-2 spike protein.

R328 and D578 form a strong salt bridge in both active and inactive CoV-2 spike proteins while the corresponding conserved residues (R315 and D564) do not form a salt bridge in the CoV-1 spike proteins.



Additionally, a conserved pair of residues form an intra-RBD hydrogen bond in the active/inactive CoV-2 spike protein (Y396-E516) and the inactive CoV-1 spike protein (Y383-E502), but not in the active CoV-1 spike protein (Y383-E502).



METHODS

MD Simulations were run for 5μs for both inactive and active CoV-1 and CoV-2 spike proteins. The active CoV-1 and CoV-2 simulations were repeated additionally twice for another 5 microseconds each. All simulations have been performed in an explicit water environment. Cryo-EM structures of active and inactive forms of SAR-CoV-1 (5X5B and 5X58) and active and inactive forms of SAR-CoV-2 (PDB ID – 6VYB and 6VXX) respectively were used as initial models. Engineered residues (P986, P987) in the CoV-2 spike protein were mutated back to the wildtype residues (K986, V987). System sizes were 680615 and 454608 atoms for CoV-1, 730937 and 577927 atoms for CoV-2, active and inactive forms respectively.

CONCLUSION

We show that the active form of the CoV-2 spike protein is more stable than that of CoV-1. The RBD of the active CoV-1 spike protein moves toward the NTD to form a pseudo-inactive state, while the CoV-2 stays open. Electrostatic interaction analysis shows a unique salt bridge interaction between the NTD and RBD of the active CoV-1 spike protein, which contributes to the conformational change seen in the active CoV-1 spike protein. Aside from the receptor binding domain (RBD), the N-terminal domain (NTD) plays a significant role in the differential behavior of the CoV-1 and 2 spike proteins . Strong salt bridge and hydrogen bond interactions observed in the CoV-2 spike protein but not the CoV-1 spike protein, potentially contribute to the relative stability of the active SARS-CoV-2 spike protein and might prevent such a conformational transition from occurring.

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