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Perspective

Accelerating COVID-19 Research Using Molecular Dynamics Simulation

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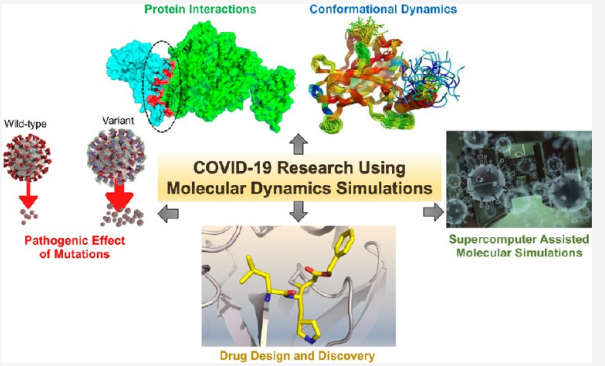
ABSTRACT: The COVID-19 pandemic has emerged as a global medico-socio-economic disaster. Given the lack of effective therapeutics against SARS-CoV-2, scientists are racing to disseminate suggestions for rapidly deployable therapeutic options, including drug repurposing and repositioning strategies. Molecular dynamics (MD) simulations have provided the opportunity to make rational scientific breakthroughs in a time of crisis. Advancements in these technologies in recent years have become an indispensable tool for scientists studying protein structure, function, dynamics, interactions, and drug discovery. Integrating the structural data obtained from high-resolution methods with MD simulations has helped in comprehending the process of infection and pathogenesis, as well as the SARS-CoV-2 maturation in host cells, in a short duration of time. It has also guided us to identify and prioritize drug targets and new chemical entities, and to repurpose drugs. Here, we discuss how MD simulation has been explored by the scientific community to accelerate and guide translational research on SARS-CoV-2 in the past year. We have also considered future research directions for researchers, where MD simulations can help fill the existing gaps in COVID-19 research.



1. INTRODUCTION

Molecular dynamics (MD) simulation is a numerical method to study many-particle systems, such as molecules, clusters, and even macroscopic systems like gases, liquids, and solids. Broadly, it is a form of computer simulation in which atoms and molecules are allowed to interact for a fixed time period, which typically solves the classical equations of motion for atoms and molecules and obtains the time evolution information on a system. The initial grand success of MD simulation in material science and chemical physics paved the way for a broad yet unexplored field of biological sciences.[1] It represents an interface between wet- and dry-lab and, therefore, is often described as a "virtual microscope" with high temporal and spatial resolution. MD simulation provides complete knowledge of a studied system, where if all trajectories are known, the thermodynamic, dynamic, and physicochemical properties of the molecules can be extracted and analyzed. As biological macromolecules exert their functions due to their dynamic rather than static nature, MD simulation serves as an ideal approach to investigate the range of accessible configurations and conformations of biomolecules as a function of time by the simultaneous integration of Newton's equations of motion.[2] Over the past decades, MD simulations have been utilized in numerous studies, starting from understanding biomolecular structure−dynamics−function relationships, conformational dynamics, allostery, drug design, and structure prediction refinement, to understanding disease pathophysiologies by mimicking physiological conditions and generating experimentally testable hypotheses and predictions (Figure 1).[3−6] Inevitably, performing biochemical experiments on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is time-consuming and requires sophisticated safety protocols. In comparison, the computational studies are quick and easily performed and provide information that is sometimes challenging to obtain from the wet-lab experiments.[7] Thus, MD simulation has emerged as the most common yet obvious method to investigate biomolecular interactions and conformational dynamics. Multiscale coarse-grained models have been used to understand the behavior of the complete SARS-CoV-2 virion.[8] The experimentally determined high-resolution 3-D structures of SARS-CoV-2 proteins have been used for simulation studies to determine their detailed mechanistic attributes and dynamics and identify conformational changes. The combination of docking and MD simulation-based binding free energy calculations has proven valuable in understanding protein−protein interaction and

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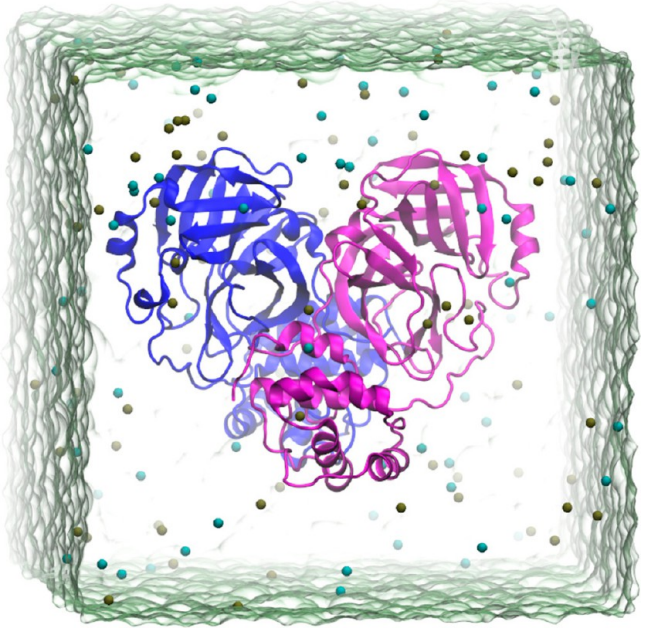

Figure 1. MD simulation system for the SARS-CoV-2 M^{pro}. A simulation box with two monomers of the M^{pro} dimer (PDB ID: 6LU7) is shown in blue and purple cartoons. Water is shown as transparent, and ions K^+ and Cl^- are shown in tan and cyan van der Waals spheres, respectively. Reproduced with permission from ref 71. Copyright 2020 American Chemical Society.

identifying potential inhibitors. In addition, the MD studies have revealed crucial information on virus−host interactions. All of this information has helped accelerate COVID-19 research and has improved our knowledge of SARS-CoV-2 biology. In this Perspective, we discuss how MD simulation has been utilized to address various aspects of SARS-CoV-2-induced pathogenesis, with the specific intent being to help fill the gaps in our understanding of the new disease.

2. PROTEIN INTERACTIONS AND CONFORMATIONAL DYNAMICS

Perhaps the most crucial application of MD simulations in COVID-19 research has been its ability to reveal the structural dynamics and conformational arrangements of the viral proteins and associated protein−protein interactions. MD simulations have been instrumental in studying the structure, flexibility, packing, and interactions of SARS-CoV-2 proteins. In this section, we discuss the application of MD simulations for obtaining information on the structure and dynamics of viral proteins.

2.1. Spike Glycoprotein. Spike glycoprotein (S-protein), one of the most prominent structures of SARS-CoV-2, is present on the surface of the virus envelope and helps in attaching to the target cell receptor, specifically the angiotensin-converting enzyme 2 (ACE2) receptor. The S-protein is club-shaped and exists as a trimer, with each monomer consisting of two domains (S1 and S2). It is also heavily glycosylated with the N-linked and O-linked glycans. While the S2-domain is embedded in the viral membrane, the

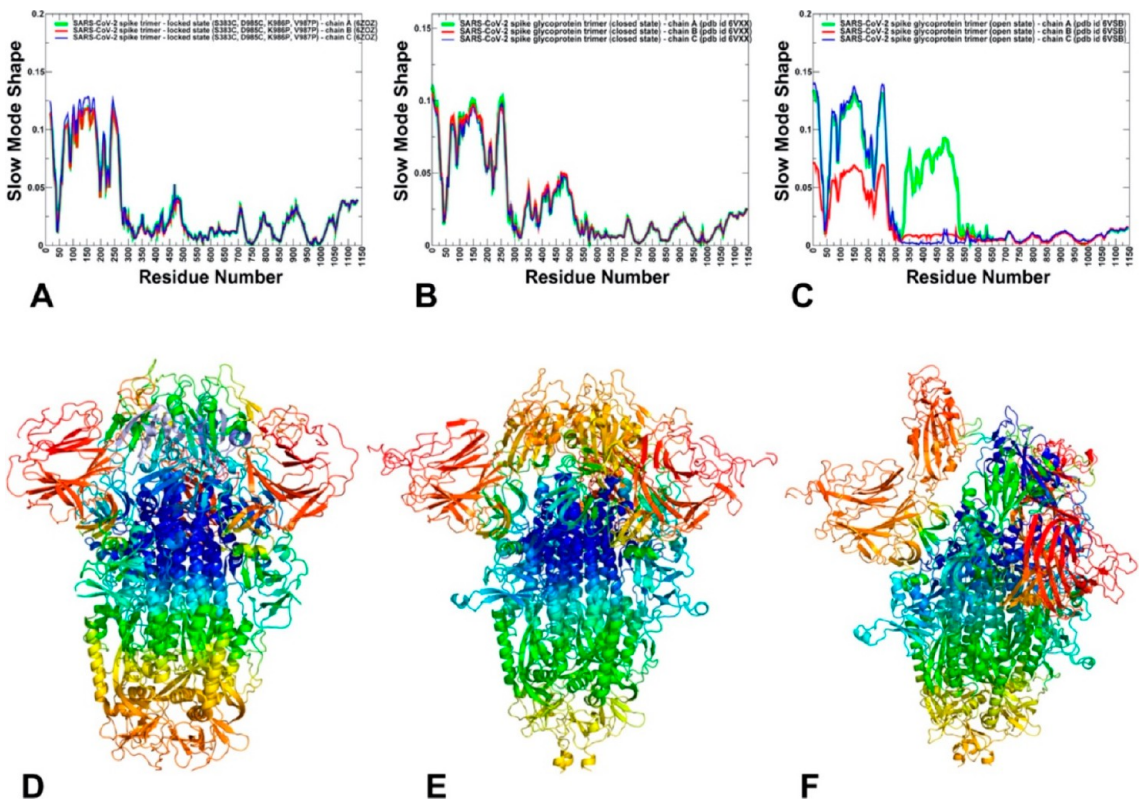

Figure 2. Functional dynamics and analysis of collective motions in the locked, closed, and open states of the SARS-CoV-2 S-trimer prefusion form. (A) Mean-square fluctuations of the locked state averaged over the three lowest-frequency modes for the cryo-EM structure of the disulfide-stabilized SARS-CoV-2 S-trimer. (B) Essential mobility profiles averaged over the three lowest-frequency modes for the cryo-EM structure of the S-trimer in the closed state and (C) the open state are shown. Structural maps of the essential mobility profiles for the locked state of the S-prefusion trimer (D), closed state (E), and open state (F) are shown. Reproduced with permission from ref 17. Copyright 2020 American Chemical Society.

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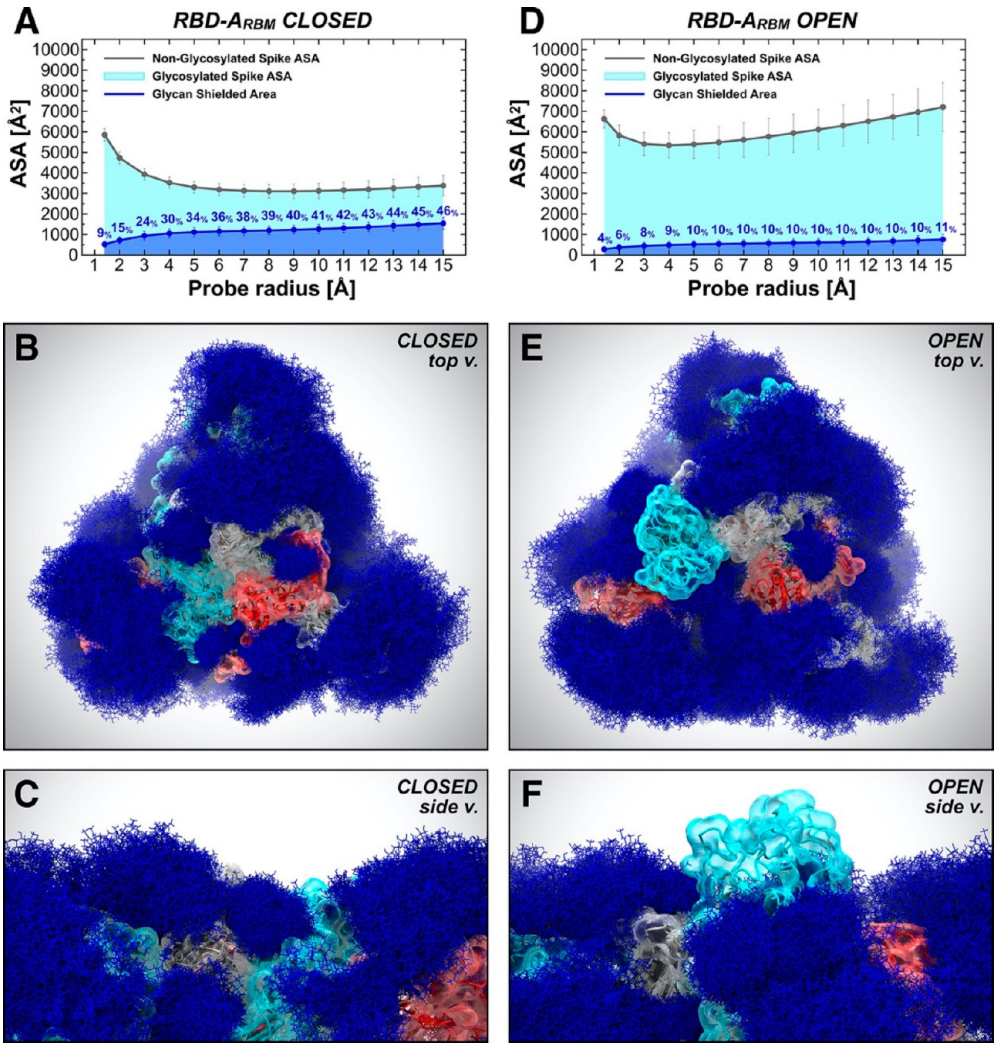

Figure 3. Glycan shield of the RBD-ACE2 interacting region. The accessible surface area of the receptor-binding motif (RBM-A) and the area shielded by neighboring glycans in the closed (A) and open (D) states. The values have been averaged across replicas and are reported with standard deviation. Highlighted in cyan is the RBM-A area that remains accessible in the presence of glycans, which is also graphically depicted on the structure in the panels located below the plots. Molecular representation of closed and open systems from the top (B and E, respectively) and side (C and F, respectively) views. In panels E and F, RBD within chain A (cyan) is in the "up" conformation and emerges from the glycan shield. Reproduced with permission from ref 19. Copyright 2020 American Chemical Society.

S1-domain is exposed to the surface. Since the S-protein is involved in the viral attachment and entry, numerous studies have been carried out to understand the structural dynamics associated with the interactions of S-protein with the ACE2 receptor. The X-ray crystal structures of S-protein revealed interesting and unique dynamics.[9−11] The S-protein can exist in three conformational states: open (or up), semiopen, and closed (or down). These conformations refer to the structure of the receptor-binding domain (RBD, residues 319−541) found on the S1 region of the protein. The transition of the S-protein from open to closed conformation occurs via a semiopen conformation. In the closed form, large numbers of intermolecular salt bridges and hydrogen-bonded interactions were present between the monomers, which reduced the overall dynamics of the RBD. However, interactions were gradually lost when it traversed to the open conformation, as revealed by a steered MD study.[12] Interestingly, residues that bind to ACE2 were solvent accessible in the closed conformation, but they could not bind to ACE2 due to steric hindrance. The closed and open conformations occupied two different energy wells in the free energy landscape, whereas the semiopen conformation had the intermediate energy well. The comparison of 83 different β-coronavirus S-proteins revealed that the incidence of an open and closed conformation was dependent on the interdomain contacts, which also determines the surface antigenicity.

SARS-CoV-2 is highly adaptable to external environmental conditions; the S-protein acquires the closed conformation at high temperatures and a more open conformation between temperatures 20 and 40 °C, indicating temperature-sensitivity.[13] The influence of the environment can also be ascertained from the well-known D614G mutation, which changes the conformation of RBD, allowing it to form better interactions with ACE2. Introducing mutations that alter interdomain interactions altered the RBD-domain, at times exposing its immunogenic receptor-binding regions.[14] Understanding such dynamics is crucial for the rational design of S-protein and ACE2 decoys for therapeutic applications.[15] The S1- and S2-domains have a tiny contact area, which allows considerable conformational flexibility in S1. The RBD, N-terminal domain

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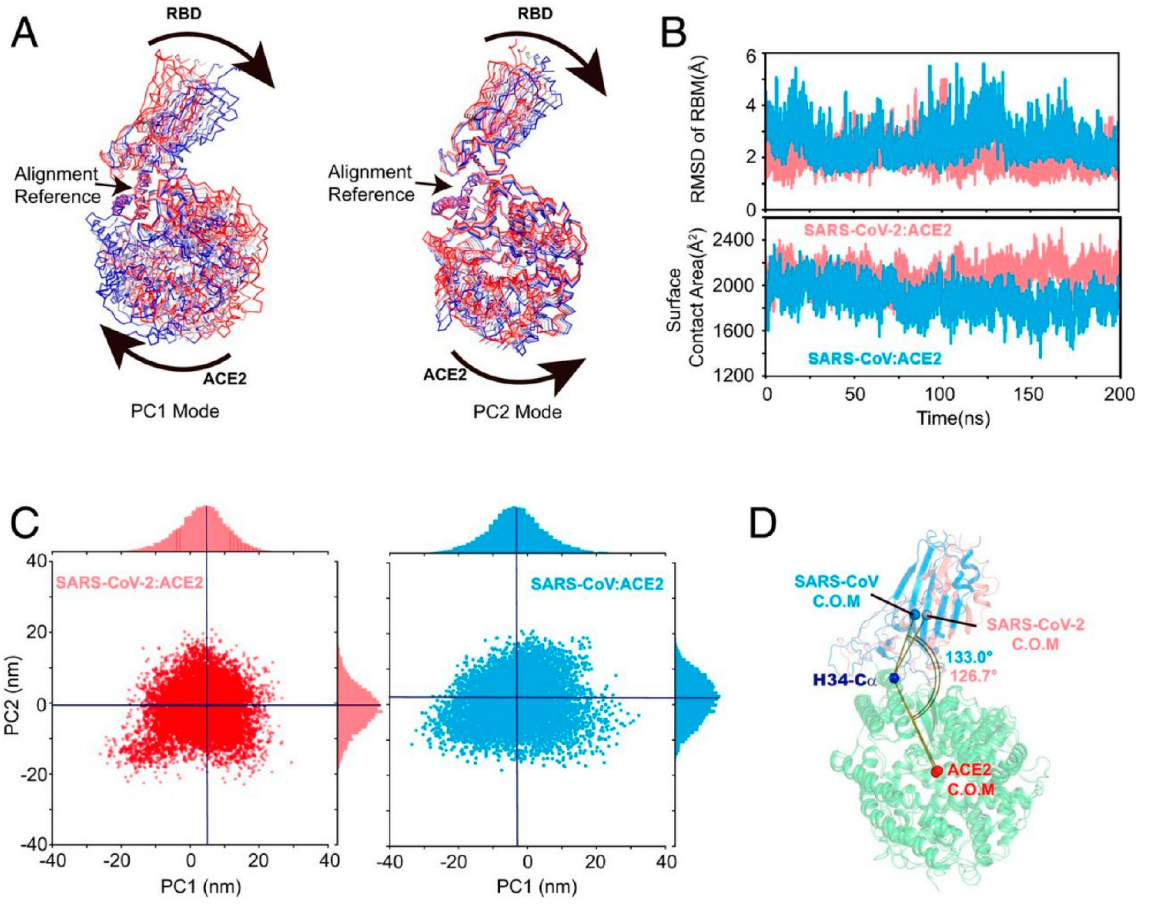

Figure 4. Characteristic dynamic fluctuations of the RBD-ACE2 complexes of SARS-CoV-2 and SARS-CoV. Fluctuations depicted by the two lowest-frequency principal components, PC1 and PC2 (A), and dynamic conformations projected onto the two PC vectors (C) are shown. In panel B, the RMSD and surface contact areas of the RBM in both complexes during the 200 ns MD simulations are shown. In panel D, the tilt angles of the two RBD-ACE2 complexes near the center of the N-terminal helix to the centers of mass (COM) for RBD and ACE2 are shown, respectively. Reproduced from ref 27, open-access article distributed under CC-BY. Copyright 2020 National Academy of Sciences, USA.

(NTD, residues 14−305), and subdomains of S1 move along with the connector domain as a single subunit. The connector domain comprises three "hinges" called the "hip, knee, and ankle" regions. The hinges allow the large RBD and the NTD of S1 to adjust their height and proximity to ACE2 receptors for stronger binding. A 2.5-μs-long MD simulation of the S-protein revealed that the head region of the S-protein remained largely stable, but the connector region with the three hinges was flexible.[16] Although the spike's head is heavily tilted, the movement of the hinge region allows the heads to connect accurately to the membranes. Notably, the hinges are heavily N-glycosylated (90% glycosylation) that protects them from antibody recognition and binding. Molecular simulations and network modeling studies characterized the dynamics and mobility features of the distinct functional states and revealed that the bending dynamics of the stalk region helped the virus to scan the cell surface more efficiently[17] (Figure 2). Another all-atom MD analysis further identified the mechanisms of SARS-CoV-2 membrane fusion and showed that a trimeric unit of SARS-CoV-2 S-protein was efficient in triggering initial stages of membrane fusion, where the residues 816−855 of S-protein represent the fusion peptide.[18]

The SARS-CoV-2 S-protein is heavily glycosylated, with 22 N-linked and 4 O-linked glycosylation sites. The heavy glycosylation helps the virus to evade the humoral immune response of the host. The MD simulation studies helped significantly with understanding the distribution and role of glycans in the S-protein.[19,20] While the stalk region is heavily glycosylated, shielding it from possible antibodies, the RBD has glycan holes providing an opportunity for the antibodies to bind. Only 62% of the RBD region is shielded from antibodies compared to 90% of the stalk region. Amaro et al. performed a microsecond MD simulation of the fully glycosylated S-protein on a realistic membrane system and identified that the S1-domain has relatively lesser glycans; however, they play a significant role in the S-protein binding and dynamics.[19] The NTD also has fewer glycans providing greater access to its epitopes. The glycan mutants N165A and N243A shifted the conformation of the S-protein from an open to a closed state, thereby affecting the protein function. These residues are crucial for stabilizing the open conformation of the RBD-domain.[19] The N234 stabilizes the open conformation, whereas the N165 glycan resists the open to close transition. Network analysis also revealed that they have a high betweenness centrality, which aids in the "scissoring" motion between the NTD of the two monomers of the S-protein trimer (Figure 3).[19] Additionally, all-atom MD simulations of the up and down forms of a fully glycosylated S-protein of SARS-CoV-2 revealed key interdomain interactions responsible for stabilizing each form and inducing large-scale conformational transitions.[21] Moreover, the huge size of the S-protein creates challenges in understanding its key conforma-

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tional transitions. To address this, Li and co-workers developed a reliable simulation protocol that considers many short simulations, which resulted in obtaining insights into the opening of the fusion peptide on a submicrosecond time-scale following cleavage at the S2′ site.[22]

A comparison of the interface of the S-protein and ACE2 receptor of SARS-CoV and SARS-CoV-2 using MD simulations revealed that the interfacial residues of SARS-CoV S-protein were mutated when compared to that of SARS-CoV-2. Interestingly, an approximately 1.5-fold increase was observed in the interaction energy between the proteins after mutation. The contact between the S-protein and ACE2 receptor was studied from a detailed contact map constructed from data obtained from all-atom MD simulations. It was observed that the total interaction energies between SARS-CoV-2 S-protein and ACE2 receptor primarily increased due to the structural rearrangements, which increased the electrostatic and van der Waals contacts. The surface electrostatics of SARS-CoV-2 showed an increase in the negative charge on the S1-domain. Although the overall dynamics of S-protein in SARS-CoV-2/ACE2 and SARS-CoV/ACE2 receptors were largely similar, the loop dynamics in SARS-CoV-2 showed a higher correlation toward ACE2. Simulations also revealed that ACE2 rearranges its structure to fit well with the S-protein.[23] Millisecond MD simulation studies showed that mutations in the SARS-CoV-2 S-protein present an evolutionary advantage for the virus. The new conformation permits the S-protein to readily bind to the ACE2 at the cost of very little entropy, leading to an increased rate of infectivity of the SARS-CoV-2 virus. Several crucial hydrogen bonds and salt bridges have been observed in the RBD-ACE2 interface of the SARS-CoV-2, which result in higher free energy binding; however, they were absent in SARS-CoV.[24−26]

Detailed MD and free energy simulations of the binary complexes of the RBD-domains of SARS-CoV and SARS-CoV-2 with ACE2 revealed that enhanced binding of SARS-CoV-2 RBD to ACE2 occurs through complex networks of hydrogen-bonding and hydrophobic interactions. The results elegantly showed the dynamic fluctuations and interplay of differential hydrophobic contacts on one side of the RBD and hydrogen-bonding network extended to the opposite end of RBD in SARS-CoV-2 (Figure 4).[27] Additionally, a key mutation at the 417 position resulted in greater electrostatic complementarity.[27] Collectively, these MD-derived data suggested that the interactions of the specific residues of SARS-CoV-2 S-protein and corresponding human ACE2 residues were due to an extensive network of hydrogen bonds and a large surface of noncovalent interactions.[27,28] In another study, researchers used an integrated approach combining coarse-grained MD simulations with protein stability- and perturbation-based network analyses to examine the role of mutations in the SARS-CoV-2 S-protein trimer to explain the dynamics and allosteric regulation in great detail.[29]

The analysis of atomistic MD simulations of glycosylated S-protein helped identify the protein energetics and immunoreactive potential of different subdomains in the S-protein.[30] Using energy decomposition and the matrix of low coupling energies, different immunoreactive subdomains were identified, including the RBD, regions corresponding to NTD, the central part of the S1A-domain, and a highly reactive carbohydrate cluster in the S2-domain. Few other potential epitopes for antibody design were also identified by analyzing the top 5% weakly coupled residue pairs on the S-protein.[30]

One of the prominent genetic variations that emerged in SARS-CoV-2 is the insertion of a novel furin cleavage site, which is absent in its predecessors. The dynamics of furin protease binding to the furin cleavage site of S-protein were studied using protein docking and MD simulations. The studies showed that the furin molecules bind at the middle of the S-trimer at the adjacent sides. Subsequently, the furin protease binds tightly to the S-protein/furin complex by burying a vast surface area. The protease residues that interact with furin molecules were well-placed in the complex. A large number of van der Waals and hydrogen-bonding interactions enabled the binding of protease to the S-protein. When potential inhibitors were added along with furin, the resultant protease/S-protein complex was found to have stronger binding affinities mediated by hydrogen-bond and polar interactions and thus could act as a possible drug target.[31]

2.2. Main Protease. After the initiation and assembly of the SARS-CoV-2, proteolytic processing generates functional subunits of the virus. The central proteolytic enzyme is the main protease (M^{pro}), also called the 3C-like protease ($3CL^{pro}$). The M^{pro} is a homodimer comprising three structural domains (domain I, II, and III). Domains I and II are made up of β-sheets and form the main catalytic subunit, whereas domain III is a compact α-helical domain connected to domain II by a long linker loop. The M^{pro}'s of SARS-CoV and SARS-CoV-2 share 96% sequence identity. Although domains I and II are crucial for the catalytic activity, deletion of domain III has been found to prevent the dimerization of the enzyme and significantly reduce the enzyme's activity. Like the other proteases, the catalytic activity of M^{pro} depends on the generation of oxyanion holes, mediated by residues Cys145, His41, and other catalytic residues having specificity for a Gln present in the peptide substrate. To understand the molecular mechanism of M^{pro} action, a 2-μs-long MD simulation of the SARS-CoV-2 M^{pro} in various conformations (monomer, dimer, with a short model peptide substrate, and without peptide substrate) was performed.[32] The data revealed that mutations in the protease induced rearrangements of domain II and domain III. The salt bridge interactions observed in wild-type protease significantly changed in the mutants. The rearrangement of the domains unfavorably impacts the binding of a model peptide substrate (mimicking the polyprotein sequence recognized at the active site). The monomer form bound to the substrate rigidifies the structure and prevents rotation of domain III, facilitating enzyme dimerization required for the catalytic activity. A comparison of the dimer in peptide bound and unbound states revealed a hydrophobic cluster that allosterically controls the domain III rotation. Further analysis of the simulations indicated a 3_{10} helical twist in the monomer near the enzyme active site. This reversible twist structure blocks the peptide's accessibility to active site residues in the monomeric form. However, the active site residues were observed to be completely solvent-exposed in the dimeric state.[32]

Comparison of the MD simulation-based analyses of protein residue interaction networks between SARS-CoV and SARS-CoV-2 M^{pro} revealed that the overall topological properties of both the structures differ only by 5%.[33] The average SARS CoV-2 M^{pro} is 1900% more sensitive than SARS CoV M^{pro} in transmitting small structural changes across the complete protein through long-range interactions. Thus, minor structural changes in SARS-COV-2 M^{pro} lead to an enormous increase in enzyme efficiency by transmitting small changes between long-

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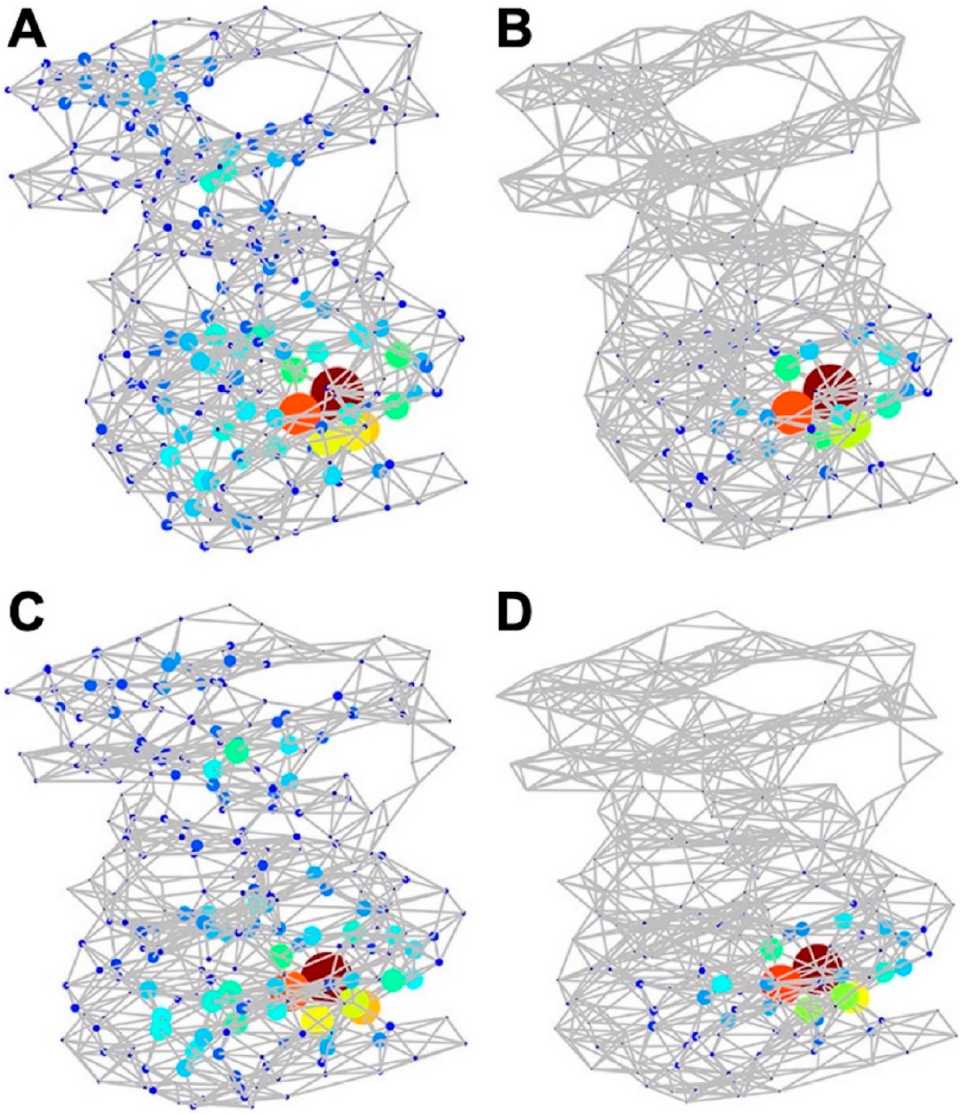

Figure 5. Illustration of the M^{pro} topology. Illustration of subgraph (A, C) and LR subgraph (B, D) centralities of the amino acid residues of the chain A of SARS-CoV M^{pro} of (top) and of SARS-CoV-2 (bottom) is shown. Reproduced with permission from ref 33. Copyright 2020 AIP Publishing.

range amino acid residues network in the structure (Figure 5).[33] The network of proteases in the presence of inhibitors constructed from crystal structures revealed that although long-range transmission occurred in the protease, most of the effect was concentrated around the inhibitor binding site, specifically the catalytic Cys145 residue.[33] Although structural changes were not evident, hidden complexity was deciphered using protein residue networks. The elastic network models of the SARS-CoV-2 M^{pro} supported the long-range interactions. Moreover, the protease has a highly dynamic and flexible structure. The analysis also hinted that although the wild-type protease does not show cooperativity, it could be introduced by mutations. The study helped identify novel residues in the protein, which dynamically control the active site microenvironment and can be explored to design noncompetitive inhibitors.[34] Additionally, eight residues around the dimer interface of M^{pro} were found to be involved in regulating the enzyme activity. Multiple pH-dependent MD simulations revealed the role of solvent pH on the M^{pro} activity. The maximum number of native contacts and the optimal concentration of the catalytic triad formed at pH 7. At pH above or below 7, the integrity of the M^{pro} structure was seriously compromised.[35]

2.3. Nucleocapsid. The SARS-CoV-2 nucleocapsid (N-protein) is one of the crucial proteins involved in the packaging of the viral genome. It can homo-oligomerize and associate with other viral proteins or with the viral genome. The N-protein phase separates with the viral RNA, indicating a strong correlation. Structurally, the N-protein has five domains: N- and C-terminal domains (NTD and CTD), RNA binding domain, a central linker domain, and a dimerization domain. The NTD and central domain are intrinsically disordered in structure. Apart from the dimerization domain, the RNA binding domain can also form higher oligomers. The structure and dynamics of the N protein were determined using single-molecule spectroscopy in combination with atomistic MD simulations. The NTD and the RNA binding domain are closely associated with each other. The central domain showed rapid rearrangements, resulting in no links between the dimerization and the RNA binding domains; however, the

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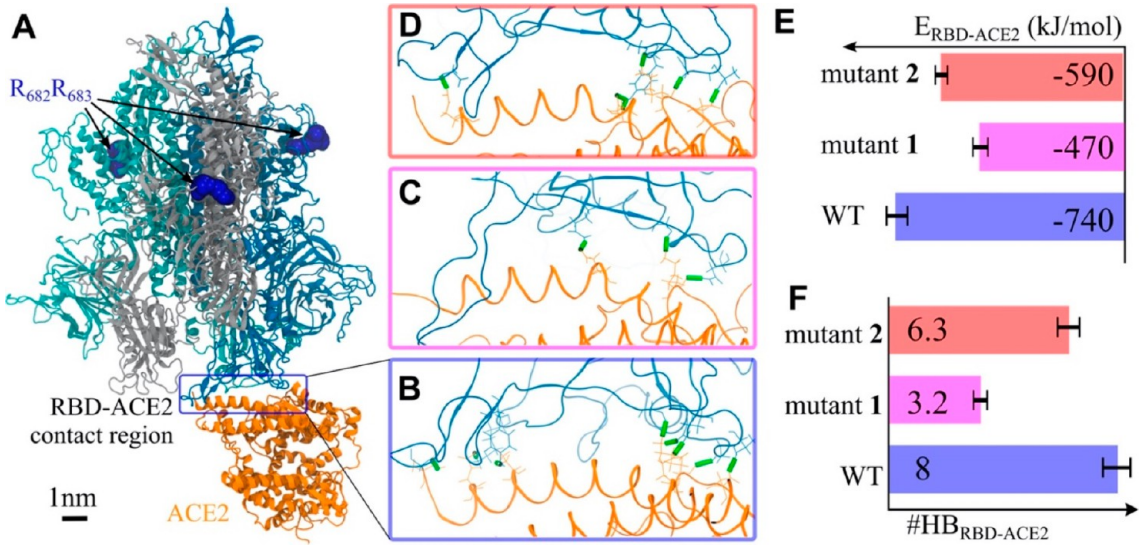

Figure 6. Distal mutants 1 and 2 both weaken the binding affinity between the RBD and ACE2. (A) Final simulation snapshot of the wild-type S-protein trimer. (B) Contact region between the wild-type S-protein and ACE2, where the eight RBD-ACE2 intermolecular hydrogen bonds are highlighted. (C, D) Similar to panel B, except for the mutants 1 and 2, respectively. (E) Potential energy between the SARS-CoV-2 RBD and ACE2. (F) The number of intermolecular hydrogen bonds (HB) between the RBD and ACE2. In panels E and F, the averages and standard deviations are calculated from five parallel runs. In mutant 1, the $N_{679}SPRRA_{684}$ residues were removed, and mutant 2 is denoted by $E_{682}E_{683}$. Reproduced with permission from ref 48. Copyright 2020 American Chemical Society.

CTD and dimerization domain interacted with each other.[36] Computational data further revealed the formation of multiple transient helices in the protein. A transient helix H3 from the central linker region was involved in the RNA binding, while the adjacent transient helix H4 was involved in protein−protein interactions. Similarly, in the CTD, the helices supported the binding of the N-protein to RNA, membranes, or other proteins.[36] A study using NMR-restraint-driven docking simulations combined with mutational analysis of N-protein NTD revealed that the region is positively charged and can bind to both single-stranded and double-stranded RNA molecules, thereby providing detailed insights into RNA recognition. However, the binding surface was U-shaped indicating that single-stranded RNA would adopt a half-turn conformation for optimal binding to the protein.[37]

2.4. Nonstructural Proteins. Apart from structural proteins, SARS-CoV-2 encodes several nonstructural proteins (NSP1 to NSP16) that are also known to play various important functions.[38] Several studies have utilized MD simulations to investigate the effect and binding modes of approved and repurposed drugs, natural bioactive and metabolite molecules, and inhibitors against NSPs.[39−45] However, as they are at the early stages of possible therapeutics development, it is outside the scope of the present perspective to discuss them in detail.

3. EFFECT OF MUTATIONS

Within one year of their discovery, the SARS-CoV-2 proteins have accumulated a vast number of mutations, leading to the emergence of various strains. Understanding the roles of mutations is critical in establishing infectivity, pathogenicity, and subsequent drug and vaccine development. Toward this, several MD simulation-based studies revealed crucial information. The MD simulations of 50 M^{pro} mutations of SARS-CoV-2 at the early pandemic stage showed that several important residues, such as Gly15, Val157, and Pro184, mutated more than once in SARS CoV-2. The data also suggested that the introduction of Glu48 mutation instead of Asp48 resulted in a novel "TSEEMLN" loop at the binding pocket, and the residue Phe140 widens the substrate-binding surface.[46] Another exciting study demonstrated that a key mutation at position 417 of the S-protein to Lys417 establishes a salt bridge in the hydrophobic interface of the RBD-ACE2, resulting in a higher electrostatic complementarity as well as enhanced hydrophobic packing as a result of the elimination of four proline residues in the interacting loop of the SARS-CoV-2 complex.[27]

The effect of Asp to Glu mutation at residue 614 (D614G) on the structure and thermodynamic stability of the S-protein was analyzed by MD simulations, which revealed that the mutation introduced structural mobility, decreased thermal stability, and further established a strong binding affinity with furin as compared to the wild-type.[47] Using large-scale atomistic MD simulations, it was shown that mutations distal from the RBD of the S-protein affect the transmissibility of SARS-CoV-2. For instance, certain missense mutations, although located 10 nm away from the RBD, enhanced the electrostatic and hydration interactions between RBD and ACE2 (Figure 6).[48] Another study employed MD simulations and network modeling and identified the regulatory centers of allosteric interactions for distinct functional states of the wild-type and missense variants of the prefusion S-protein trimer of SARS-CoV-2.[17] The mechanism of the enhanced binding affinity of SARS-CoV-2 N501Y S-protein mutation for the ACE2 receptor was investigated using extensive MD simulations, where the hydrophobic interactions were found to be crucial for the increased binding energy.[49]

In a related study conducted to better understand the stability of the RBD/ACE2 complex, extensive MD simulations revealed the effect of mutations identified in the RBD of SARS-CoV-2 strains isolated from humans. The study demonstrated that most of the naturally occurring mutations in the RBD have better binding affinity to ACE2 when compared to the wild-type Wuhan reference genome (NC_045512.2) (http://cov-glue.cvr.gla.ac.uk/#/home), thereby highlighting the crucial

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role of certain hotspot residues at the interface of RBD and ACE2.[50] On the other hand, it is important to understand the effect of naturally occurring missense mutations in human ACE2 on the RBD-ACE2 interaction. To address this, multiple computational studies, including MD simulations, were used that showed the specific mutations affecting the affinity between ACE2 and S1-domain through long-range interactions and various structural dynamics and conformational events arising due to the closed and open states of the S-protein of SARS-CoV-2.[51] An artificial intelligence-based model showed the incidence of mutations based on duration, dispersal, and frequency of occurrences.[52]

4. DRUG DESIGN AND DISCOVERY

An all-atom MD simulation has been at the forefront of *in silico* techniques used in drug design and discovery against COVID-19. The MD simulations have been widely employed in understanding the viral protein−drug and protein−inhibitor interactions, evaluating their binding affinities, energetics, and stability. Since the beginning of the COVID-19 pandemic, researchers across the globe have extensively worked on exploring and prioritizing hundreds of thousands of inhibitors and potential drugs (using drug repurposing) using MD simulations. Several repurposed and investigational drugs have been proposed to target the key SARS-CoV-2 proteins, including (i) S-protein, (ii) RNA-dependent RNA polymerase (RdRp), and (iii) M^{pro}/$3CL^{pro}$.

Remdesivir, which targets the viral RNA-dependent RNA polymerase (RdRp) and induces the evasion of proofreading and subsequent inhibition of viral RNA synthesis, has been studied in detail using MD simulations. It is an adenine-based nucleotide analogue that was developed against the Ebola virus. During the early days of the pandemic, the binding of remdesivir to the SARS-CoV-2 RdRp was investigated using MD simulations, and free energy perturbation methods were used to understand its inhibition mechanism.[53] The binding of remdesivir to SARS-CoV-2 RdRp was significantly stronger compared to its natural substrate, ATP. MD simulations coupled with ensemble docking of the apo and RdRp-bound remdesivir revealed the blocking of template entry sites in the presence of remdesivir. Analysis of the principal components suggested significant conformational changes leading to the establishment of strong contacts between several catalytic residues, including Ser759, Asp760, and Asp761.[54] Later, using molecular docking, steered MD, and umbrella sampling, it was shown that remdesivir could strongly bind to both SARS-CoV-2 RdRp (via electrostatic interactions) and M^{pro} (via van der Waals interactions).[55]

MD simulations combined with free energy and fragment molecular orbital calculations showed that the anti-HIV drugs, lopinavir and ritonavir, bind to the active site residues of SARS-CoV-2 M^{pro}.[56] Ritonavir formed a higher number of contacts and bound efficiently using several specific electrostatic, dispersion, and charge transfer contacts than lopinavir. Extensive MD simulations of the dimeric M^{pro} revealed the dynamics of seven HIV inhibitors (darunavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, and tipranavir) and binding mechanisms to the active site of M^{pro}, at least twice in 28 simulations of 200 ns each. The microsecond-long timescale simulations further revealed a wide variation in the geometry of the binding sites in M^{pro} and binding poses of the HIV-1 protease inhibitors. The M^{pro} has a relatively flexible pocket when bound to inhibitors. Three different binding sites were identified, suggesting that the inhibitor binding should not be restricted only to the enzyme's active site. The drugs were seen flipping and changing their binding poses during MD simulations. The binding of the drugs also induced conformational changes in the C-terminal of M^{pro}, which participates in stabilizing the substrate or the ligand binding. Such data provide an opportunity to improve and optimize the drugs for stronger binding and specificity for inhibiting the SARS-CoV-2 M^{pro}.[57]

An extensive MD simulation study on the anti-influenza drug umifenovir (Arbidol) demonstrated that Arbidol binds at the RBD/ACE2 interface with a high affinity by forming stronger intermolecular interactions with key residues of RBD compared to that with ACE2. It was proposed that the binding of Arbidol induces structural rigidity in the virus glycoprotein, resulting in restriction of the conformational rearrangements associated with membrane attachment and virus entry.[58] In a related study, MD simulations and structural analyses were carried out to highlight that Arbidol blocks the S-protein trimerization, which is crucial for host cell adhesion and hijacking.[59] A supervised MD study further explored the druggability of the SARS-CoV-2 S-protein, which showed that six FDA-approved drugs were capable of significantly blocking the RBD/ACE2 interaction.[60] A recent report employing all-atom MD simulations and binding enthalpy calculations showed that an active site inhibitor (MLN-4760) could reduce the adherence of S-protein with ACE2 by weakening and destabilizing the interactions at the ACE2-RBD interface of SARS-CoV-2.[61] A similar work studied the effect of small molecules SSAA09E2 and Nilotinib on the ACE2-RBD interface through MD simulations and found that they intervene with hydrogen bonds at the interface and hence the flexibility of the proteins.[62] Interestingly, stapled peptides have also been designed that target the RBD of the SARS-CoV-2, which upon analysis through MD simulations and binding free energy calculations has been shown to bind with a potency that is similar to that of experimentally proven peptide inhibitors.[63]

Several drug repurposing studies have identified a plethora of potential drugs against M^{pro}. The FDA-approved antivirals, lopinavir, ritonavir, tipranavir, and raltegravir, and several HIV protease inhibitors (HPIs) were identified as the potential M^{pro} inhibitors.[64,65] An e-pharmacophore approach combined with MD simulations revealed that drugs like binifibrate and bamifylline bind to the active site of M^{pro}.[66] A further study screened 1615 FDA-approved drugs and 4266 other approved drugs, using an array of computational methods in combination with MD simulations, and found that simeprevir (anti-HCV drug) and pyronaridine (antimalarial drug) bind M^{pro}.[67] Several other drugs, including the anti-HIV drugs indinavir and darunavir, also interact with M^{pro}.[68,69] Another study explored the mechanism of covalent inhibition of M^{pro} by an α-ketoamide inhibitor.[70] Multiple MD simulations and theoretical approaches were used to investigate the binding mechanisms of 19 marketed drugs to the ligand-binding pocket of M^{pro}. The data demonstrated that ligand binding to the M^{pro} pocket could be improved if a part of the ligand occupies a specific anchor site. This finding provides an opportunity to explore new binding mechanisms and designs and optimize the related M^{pro} inhibitors with higher binding affinities.[71] In addition, using MD simulations and free energy calculations, several studies showed that natural compounds, including polyphenols, flavones, coumarin, and green tea extract, could

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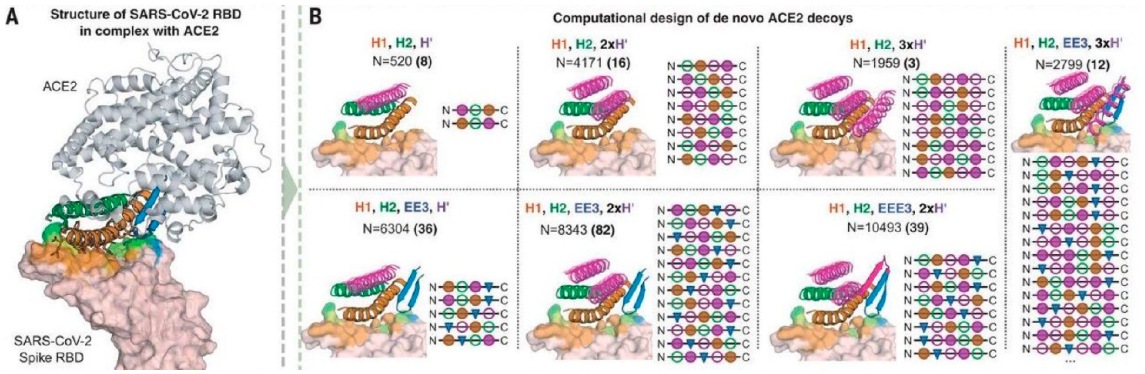

Figure 7. Design and characterization of *de novo* ACE2 decoys. (A) ACE2 and its binding motifs in complex with SARS-CoV-2 RBD are shown. (B) *De novo* secondary structure elements were computationally generated. Seven combinations of the secondary structure elements were considered. Reproduced with permission from ref 78. Copyright 2020 The American Association for the Advancement of Science.

bind to the M^{pro}.[53,55,72] Pyranonigrin A, a secondary fungal metabolite, was also shown to bind to the M^{pro} with a high affinity.[73] The MD simulations also showed that ligands, such as vitamins, retinoids, and steroids, could bind to the free fatty acid pocket of the S-protein.[74] A free tool, interactive MD in virtual reality (iMD-VR), has also been developed to create M^{pro} complexes. The iMD-VR allows the users to perform flexible docking of the M^{pro} inhibitors and oligopeptide substrates, interact with MD simulations, and build protein complexes in a physically rigorous and flexible manner.[75]

The MD simulation-based lead identification studies on other SARS-CoV-2 proteins are also available (reviewed in ref 76). Additionally, multitarget studies have also been performed, where MD simulations were used to understand the binding dynamics and affinity of the inhibitors (reviewed in refs 76 and 77). However, a critical limitation of most of the studies is that they were not confirmed using wet-lab approaches. Nonetheless, they provided significant information about the active site interaction and dynamics of the enzymes and guided the improvement and optimization in the design of the existing drugs for improved binding and inhibition.

5. DESIGN OF PROTEIN-BASED THERAPEUTICS

MD simulations have also been used to validate novel computationally designed therapeutics, such as the design of ACE2 decoys.[78] A high-throughput study used a *de novo* protein design approach to construct 35 000 ACE2 decoys computationally; the binding of the top-ranked 196 designs to the SARS-CoV-2 RBD was tested experimentally (Figure 7).[78] The data revealed that certain decoys were able to strongly neutralize a SARS-CoV-2 infection *in vitro*. Notably, a single intranasal dose of decoy protected Syrian hamsters from a subsequent lethal SARS-CoV-2 challenge.[78] In another study, RBD-based peptides were computationally designed, and their ACE2 binding and inhibiting potential were analyzed.[79]

6. APPLICATIONS OF SUPERCOMPUTER-ASSISTED MD SIMULATIONS

Not only high-throughput computational methodologies but also massive large-scale hardware has been effectively utilized to understand the molecular mechanisms of SARS-CoV-2-triggered infection. Massive-scale MD simulations using state-of-the-art supercomputer machines have been used to gain insights into the biology of SARS-CoV-2. The Amaro lab at the University of California, San Diego used ~250 000 processing cores and ~4000 processor nodes to run MD simulations on one of the world's top supercomputers named "Frontera" to complete an all-atom simulation of the influenza virus envelope.[80] Similarly, researchers at the RIKEN Center for Biosystems Dynamics Research, Japan, used a drug discovery supercomputer MDGRAPE-4A to analyze the structure-dynamics relationship of the M^{pro} of SARS-CoV-2 (https://www.riken.jp/en/news_pubs/news/2020/20200323_1/). Their MD model comprised 98 694 atoms, including 29 712 water molecules and a total of 10-μs-long simulations. Researchers conducted the simulations and made the raw simulation data publicly available on the Mendeley Data repository for researchers across the globe (https://data.mendeley.com/datasets/vpps4vhryg/2). Furthermore, researchers at the Department of Energy's Oak Ridge National Laboratory used a supercomputer called "Summit" to perform MD simulations on 8000 compounds to screen for potent binders to S-protein and identified 77 potential small-molecule drug compounds.[81] Interestingly, two research groups, one at the University of Arkansas, used the Anton 2 supercomputer to understand the activation process of SARS-CoV-2 S-protein (https://www.psc.edu/tag/covid/, https://www.psc.edu/psc-covid-19-update-june-15-2020/), while the other research group at the University of California, Riverside, employed the Anton 2 supercomputer to evaluate how the CRISPR-Cas gene-editing system recognizes the genetic material of SARS-CoV-2 with microsecond-long MD simulations (https://insideucr.ucr.edu/stories/2020/06/17/uc-riverside-engineers-are-using-supercomputers-investigate-rapid-crispr-based). Remarkably, over a million citizen scientists performed an unprecedented 0.1 s of MD simulations through the Folding@home computing project to create the world's first Exascale computer and simulate protein dynamics of SARS-CoV-2.[82] This, in turn, revealed how the S-protein of SARS-CoV-2 uses conformational masking to evade host immunity and subsequently identified the hidden cryptic pockets that were not captured or were extremely difficult to capture in experiments. A consortium of high-performance computing (HPC) for research on COVID-19 was constituted in early 2020. The consortium houses some of the largest supercomputers from academia, federal agencies, industries, and national laboratories from around the world. It actively supports researchers by providing computational grants for accelerating their research on COVID-19. Currently, more than 100 different research projects on COVID-19 are part of the consortium (https://covid19-hpc-consortium.org/).

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7. CONCLUSION AND FUTURE PERSPECTIVES

Science has never been more dynamic than it is in the current pandemic. Thousands of researchers in academia and the pharmaceutical industry throughout the world paused their research curiosity and began working on COVID-19, rendering science CORONA-ized. The way the pandemic resulted in a shift in scientific priorities is unprecedented. Many bold and creative approaches helped develop several vaccines against COVID-19, and immunization started globally at a record-breaking speed. However, the deployment of COVID-19 vaccines should not deflect us from learning the biology of SARS-CoV-2, and the search for an effective long-term therapeutic strategy should be our top priority.

The COVID-19 pandemic offered researchers the opportunity to explore high-performance computational methods, including MD simulation, as a technological prospect for biological discoveries of SARS-CoV-2. With the increasing availability of technical infrastructures and computational power, MD simulations can be performed and managed even from off-site. Thus, understanding the biology of the virus has become an integral part of COVID-19 research. Today, a large number of scientific publications are available that systematically used MD simulations to provide a comprehensive understanding of the molecular mechanism of SARS-CoV-2 infection. However, SARS-CoV-2 research will continue for many more years to come, and as expected, with time, the computing power, resources, and mathematical basis of simulations will keep increasing. These methods will be exploited by computational biologists and are likely to emerge as a strong and supplementary pillar for the mechanistic understanding of COVID-19. The future of COVID-19 research promises many exciting opportunities for unsolved problems where MD simulation can prove its worth magnanimously. We underline a few suggestions and directions where MD simulations may be helpful for COVID-19 research.

1. More than 1400 structures (crystals, cryo-EM, and others) of SARS-CoV-2 proteins have been deposited in the Protein Data Bank in the past 18 months. However, these structures have been solved with great speed and under immense pressure at the time of crisis. Thus, it is highly possible that the structures can contain errors. Even minor errors in structure calculation may severely compromise the process of structure-based drug design as the potential inhibitor can be misinterpreted as biologically and pharmaceutically relevant. The Coronavirus Structural Task Force is continuously working to evaluate, improve, and remodel the structures (https://insidecorona.net/). Additional analyses of these structures through computer simulations may provide significant biological insights into their conformational dynamics and functional interactions.
2. Few simulation studies have identified allosteric sites and cryptic pockets in certain SARS-CoV-2 proteins. However, all the allosteric inhibitor binding sites and cryptic pockets in all the SARS-CoV-2 proteins remain explored. More efforts are thus required for the purpose of drug design.
3. In the future, many antivirals will be developed. It is imperative to exhaustively study their inhibitory action using MD simulations. Pharmacophore modeling in combination with MD simulations can help develop better and potent antivirals.
4. As new variants of SARS-CoV-2 are emerging and will continue to emerge, an understanding of the structure−function−disease relationship of new variants will be required for the management of COVID-19 and future disease outbreaks. In such studies, MD simulations can provide crucial information that will complement the results of biochemical experiments. The variation in functions of the potential mutants can also be studied further using MD simulations by characterizing their structure and dynamics. The MD simulations can also be used to analyze possible residues that, upon mutation, can render the protein structurally compatible for target-based drug design.
5. With the advancement of the pandemic, the SARS-CoV-2 may develop resistance mutations in various proteins as a response to drug or immune pressure by undergoing positive selection. Using high-throughput protein design approaches, few studies have identified potential residues that could mutate in a short time to develop drug resistance in the virus.[83,84] Validation of such data using MD simulations will be helpful to predict mutations that may emerge in the future. Resistance against various antibiotics is being reported due to their excessive use to prevent secondary infection in COVID-19 patients. Such aspects should be investigated by multiple computational methods, including MD simulations.
6. Molecular dynamics simulations in combination with other assistive technologies might be helpful to assess the contributions of the genetic profile of a patient and viral genome variability to the differential clinical outcomes of COVID-19 patients.
7. An initial coarse-grained molecular model of the SARS-CoV-2 virion has been developed. However, these were early results. Additional whole-cell simulations of the complete virion and the various virion components should be performed. Information from holistic models of the virion and its components can help understand new routes to tackle the virus by exploiting viral mechanisms involving large-scale features. Additionally, the interplay between proteins and genetic components will provide information for the development of defense strategies, which, in turn, will help in preventing damage from similar pandemics in the future.

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