Biophysical *Journal*
New and Notable

Biophysical Society

Bow to the enemy: How flexibility of host protein receptors can favor SARS-CoV-2

Stefano A. Serapian[1] and Giorgio Colombo[1,*]
[1]Department of Chemistry, University of Pavia, Pavia, Italy

Covid-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread all over the world in 2020, resulting in the worst global human health emergency in modern times. The pandemic has been causing significant numbers of deaths and dramatic consequences in terms of restrictions to the ways we live in our societies and in terms of economic disruptions.

Covid-19, on the other hand, sparked an unprecedented global mobilization in research, with the scientific community coming together in what is probably the greatest collective attempt to solve one single problem ever seen. In a matter of months, we have learned that SARS-CoV-2 high efficiency of spread relies on the virus's unprecedented capacity to effectively enter host cells and hijack the host's replicative machinery. Together with SARS-CoV-2's highly selective tropism, this determines the onset of a variety of diseases, including respiratory syndromes, heart diseases, hepatitis, and central nervous system complications.

The virus exploits specific and exquisitely evolved proteins and protein interactions to enter host cells, subvert the host cell physiology, and promote efficient replication and spreading. In this context, the homotrimeric viral spike protein is the key player in starting the process of cell entry docking onto the protein receptor angiotensin-converting enzyme 2 (ACE2) (1). The latter is highly expressed in various types of human cells. The publication of a number of high-resolution structures of SARS-CoV-2 proteins, in some cases in complex with human targets, represents a first fundamental step to understand the molecular determinants of the infection (2,3).

These types of interactions have been experimentally characterized at the level of structure, kinetics of binding, and effects of mutations/deletions on the disease phenotype. Despite this sophistication, there is still no experimental technique that can provide insight at an atomic level into the dynamics of the interacting partners and their impact on the recognition process itself. To understand the fundamental traits of protein dynamics linked to the initiation of the infection process at atomistic resolution, we have little choice but to turn to theoretical approaches.

In this framework, Barros et al. (4; in this issue of the *Biophysical Journal*) have used realistic, extensive all-atom molecular dynamics simulations to investigate the role of structural flexibility and the internal dynamics of the ACE2 receptor in a realistic membrane-mimicking environment (Fig. 1). They simulated the fully glycosylated system in its full-length form, both in its free state and bound to the spike receptor binding domain (RBD). The authors observe a high degree of flexibility in both ACE2 states, which reverberates in hinge-bending motions of the region connecting the head to the transmembrane helix. Such motions do not disrupt the ACE2 homodimer or ACE2-RBD interfaces but rather seem to favor the selection of an ensemble of ACE2 conformations that can accommodate binding of the spike trimer to more than one ACE2 homodimer. One of the crucial aspects of this work is the realization that the flexibility of the host receptor can be actively harnessed by the virus to favor effective attachment via multivalent interactions.

Interestingly, other recent investigations have begun to reveal flexibility themes in SARS-CoV-2 protein functions implicated in cell entry. The conformational heterogeneity of glycans in both the spike protein and ACE2 directly fine-tunes spike-ACE2 interactions (5,6), whereas molecular simulations of the open and closed conformations of the isolated spike protein reveal that the dynamic rearrangement of glycan chains can mask epitopes from being recognized by the human immune system and at the same time play a role in modulating the structural rearrangements of the RBDs (7). Global structural flexibility

Submitted November 29, 2020, and accepted for publication January 28, 2021.

*Correspondence: g.colombo@unipv.it

Editor: Susan Schroeder
https://doi.org/10.1016/j.bpj.2021.01.029

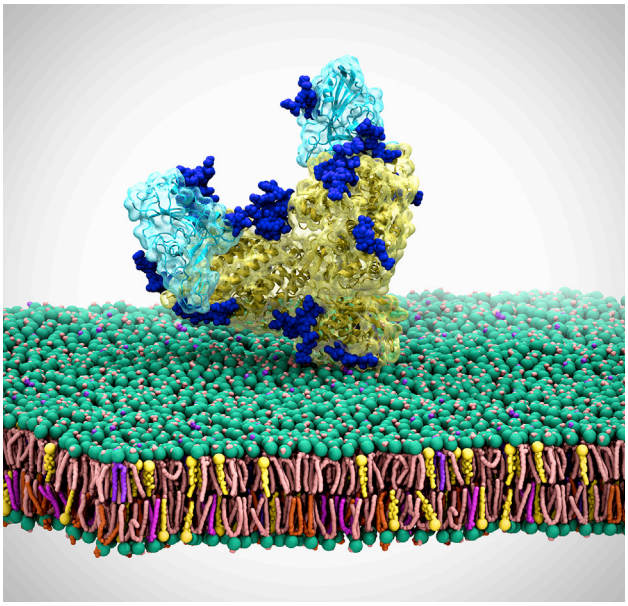

FIGURE 1 The structure of the full-length ACE2 on the model cell membrane simulated in Barros et al. (4). The image is by Lorenzo Casalino and based on Barros et al. (4). To see this figure in color, go online.

and large-scale rearrangements of the fully glycosylated membrane-bound spike protein have been directly observed in a recent impressive work by Turonova et al. (8). Combining cryo-electron tomography and molecular dynamics simulations, they observed that the viral spike protein populated mostly the closed prefusion conformation, whereas its stalk domain was shown to contain three hinges, giving the head large orientational freedom.

The importance of the Barros study (4), combined with the other aforementioned investigations, is that it clearly reveals how the peculiar dynamic traits of the host and virus proteins can help explain the remarkable efficiency of SARS-CoV-2 at infecting host cells. Hinge motions in the viral spike protein favor the efficacious scan of the host cell surface, whereas hinge motions in the ACE2 receptor permit it to sample conformations that can favorably adapt to the docking spike.

These mechanisms of concerted conformational adaptation represent a clear advantage for the virus.

Yet, to our advantage, the knowledge of their relevance at atomistic detail can generate novel opportunities for therapeutic development. Scanning different ACE2 and spike conformations for potential druggable pockets that are not immediately evident in the static structures of the proteins can be used to guide drug screening or de novo design efforts.

The fine characterization of the correlations between hinge motions and distal regions implicated in forming the protein-protein interface can reveal allosteric communication mechanisms. Allostery regulates function by selecting conformational states that meet functional requirements. The knowledge of such mechanisms can be used to design allosteric ligands that reshape protein-protein interaction surfaces by binding to sites far from the actual interface and can potentially influence the selection of one binding partner over another at a shared interface. This strategy might turn out to be particularly useful in targeting ACE2. Because the protein plays a role in a number of pathways relevant to normal cellular metabolism, targeting its receptor site would expectedly impact indiscriminately on its entire functional spectrum, leading to unwanted toxicity effects. By targeting conformational states with specific recognition profiles, allosteric drugs might provide better options for drugs that selectively reduce viral transmission, minimizing excess human toxicity.

In this context, the deep insights provided by large-scale simulations can be further expanded by distributed computing initiatives (9). Recently, the Folding@home distributed computing project simulated an unprecedented 0.1 s of the viral proteome, observing dramatic conformational changes across a wide variety of proteins and revealing more than 50 “cryptic” pockets for the design of new antiviral drugs (10).

Finally, high-resolution characterization of the conformational landscapes of viral proteins and their interactions with human partners can reveal surfaces potentially recognized by neutralizing antibodies (11). The knowledge of the (dynamic) architectures underlying these surfaces can be exploited in the biochemical design of novel antigens capable of eliciting a protective response, with optimized profiles of immunoreactivity and stability, making fundamental contributions to practical large-scale vaccination (12).

One important common trait shared by the studies from the Amaro lab and the Folding@home consortium (as well as other initiatives) is the fact that all data and models are made available to the community online, providing an unprecedented wealth of high-quality structural data.

In conclusion, at a time that the whole world is eagerly waiting for treatments that stop (or at least limit) this emergency condition and bring us back to our normal lives, the insights provided by computational detailed characterizations of protein properties, otherwise inaccessible to experiments, can open the way to the development of novel therapeutic agents, increasing the hope for a cure in the near future.

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