# Molecular Aspects of Spike–ACE2 Interaction

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A new betacoronavirus (CoV-2) is responsible for the pandemic of severe acute respiratory syndrome (SARS) that began in China at the end of 2019, today known as COronaVIrus Disease 2019 (COVID-19). Subsequent studies confirmed the human angiotensin-converting enzyme 2 (hACE2) as the main cell receptor of spike trimeric glycoprotein, located on the viral envelope, mediating the CoV-2 invasion into the host cells through the receptor-binding domain (RBD) of the spike. Computational analysis of the known experimental 3D structures of spike–ACE2 complexes evidenced distinguishing features in the molecular interactions at the RBD-cell receptor binding interface between CoV-2 and previous CoV-1. The spike represents a key target for drug design as well as an optimal antigen for RNA/viral vector vaccines and monoclonal antibodies in order to maximize prevention and therapy of COVID-19.

COVID-19 SARS coronavirus CoV-1 CoV-2 viral spike protein receptor-binding domain (RBD) human ACE2 binding interface

A pneumonia outbreak of unknown origin first emerged in Wuhan, Hubei province of China, during the last months of 2019 [1][2][3]. The etiologic agent was identified by the Coronaviridae Study Group of the International Committee on Taxonomy of Viruses (CSG-ICTV) as an unknown positive-strand RNA betacoronavirus (CoV-2) [4], the seventh coronavirus known to infect humans, designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It emerged as highly transmissible from human to human, and was related to SARS-CoV-1 of 2002 with 82% genome identity. Based on the established practices and studies of phylogeny, it was defined as being of probable zoonotic origin [5][6][7][8]. The new disease, named the CoronaVIrus Disease 2019 (COVID-19), rapidly spread worldwide due to human-to-human contacts, and the World Health Organization (WHO) declared global pandemic status in March 2020. There is high variability in disease severity, with asymptomatic and paucisymptomatic cases of COVID-19, while severe cases can evolve towards a life-threatening SARS. CoV-2 showed a death rate lower than that of CoV-1, although with pronounced geographical variability [1][9][10]. This suggests that the molecular interactions between the host receptor and the coronavirus variants play an important role in successful infection. However, different host proteins play the roles of receptors/interactors in coronavirus infection, with consequences in comorbidities [11].

Recent researches on infectious capacity of this novel coronavirus established that human angiotensin-converting enzyme 2 (hACE2), a homodimer protein attached to cell membrane and exposed at the external environment, is a host receptor for CoV-2 as for CoV-1 in 2002 [12][13][14][15][16][17][18][19][20][21][22][23]. Also known as ACEH (ACE homologue), hACE2 belongs to the angiotensin-converting enzyme family of peptidyl dipeptidases (zinc-dependent metalloprotease) and it is homologous to human angiotensin-converting enzyme 1 (hACE1), but with a broader

substrate specificity. While hACE1 generates the vasoconstrictor peptide angiotensin 2 (AT2), hACE2 can lower blood pressure by catalyzing the cleavage of angiotensin 1 (AT1) (inactive decapeptide precursor of AT2) into AT1–9 (vasodilator), and AT2 (vasoconstrictor octapeptide) into the vasodilator AT1–7 [21]. Both hACE1 and hACE2 are AT1-converting enzymes and regulators of blood pressure that have counterbalance roles by acting on vasoactive peptides from the renin-angiotensin-aldosterone system (RAAS). In the bloodstream, the renin protease secreted from the kidneys cleaves the angiotensinogen (AGT) secreted by the liver to form AT1, which in turn generates AT2 or AT1–9 from the actions of hACE1 and hACE2, respectively. AT2 is cleaved by multiple enzymes, most importantly hACE2, to form AT1–7. AT2 also forms AT4 via the action of aminopeptidases (APs), both AT2 and AT4 act via AT1 receptors. As hACE2 opposes the actions of AT2, it has a key role in the RAAS. Therefore, there is a beneficial effect in hypertension and cardiovascular diseases when AT2 concentration decreases.

Although hACE2 is hijacked by some coronaviruses, its primary physiological role is in the hydrolysis of AT1 and AT2, peptide hormones that control vasoconstriction and blood pressure [21]. It is a type I membrane protein primarily expressed in the lungs, heart, kidneys, liver, and small intestine, while its decreased expression is associated with cardiovascular diseases. The monomer of hACE2 consists of an N-terminal peptidase domain (PD) and a C-terminal collectrin-like domain that ends with a transmembrane alpha helix and an approximately 40-residue intracellular segment. Collectrin is a renal protein without catalytic domain and has no similarity with hACE1. Thus, hACE2 may have evolved as a chimera between the ACE-like domain and the collectrin domain. The PD of hACE2 is responsible for processing AT1 to produce AT1–9, which is then processed by other enzymes to become AT1–7, or directly to cleave AT2 to give AT1–7. In contrast with hACE1, hACE2 does not hydrolyze bradykinin and is not inhibited by hACE1 inhibitors (ACE-I). At the onset of the pandemic, the use over time of ACE-I has been postulated to increase susceptibility to COVID-19 in patients with hypertension [24]. RAAS-inhibition can upregulate the expression of the hACE2 receptor, so the therapeutic treatment with ACE-I might promote the infection. However, to date, no evidence exists that RAAS inhibitors can increase the host's susceptibility to COVID-19.

The trimeric glycoprotein spike of coronaviruses is anchored in the envelope of the characteristic virion, and many copies of these macromolecules give the likeness of a crown, "corona" in Latin, the word used for the name of these viruses. The spike mediates the recognition of the host hACE2 receptor throughout its receptor-binding domain (RBD), corresponding to the 318–510 sequence region of CoV-2. One of the three spike chains exposes RBD in a structural conformation easy to reach by the hACE2 receptor. So, the spike directly binds with its RBD to the host receptor hACE2-PD, exploiting hACE2 to carry on the infection of the human host (Figure 1).

The specific amino acid sequence of this spike portion plays a key role in conferring to CoV-2 the ability to infect humans, being known as responsible for the species specificity [6][9][25]. Following infection, the ligand-receptor interaction occurs, the complex spike–ACE2 is formed, and spike is cleaved into two fragments by serine proteases, such as transmembrane protease serine 2 (TMPRSS2): the N-terminal S1 fragment containing the RBD bound to ACE2–PD, and the C-terminal S2 fragment responsible for the membrane fusion of the virus with the host cell [13]. Therefore, the molecular interaction and the stable binding between spike RBD and ACE2-PD are propaedeutic to the invasion of host cells by CoV-2. The S1 fragment of CoV-2 has a sequence portion with around

70% shared amino acid identity with the corresponding sequence of CoV-1, while the S2 fragment shares 99% identity with the corresponding sequence of CoV-1 [5]. More particularly, inside the S1 fragment, RBD shows high sequence identity between the two coronaviruses with the exception of its C-terminal region, which is involved in the direct binding with the hACE2 receptor [5]. Therefore, spike RDB is under selective pressure to evade host immune response. This aspect would explain the high mutation frequency observed in spike RBD. Thus, anticoronavirus antibodies (Ab) designed against the core domain of spike RBD and the S2 fragment should be potentially effective with a broad spectrum versus genetic variants of spike [26]. Novel spikes may likely increase the virulence by evading the host immunity within species and enabling host-switches by altering cross-species receptor recognition. Additionally, the spike has been identified as a critical recombination hotspot [5][27]. Therefore, it is very likely that the sequence variability of the spike, as well as of the hACE2 host receptor, may modulate virion intake and consequent disease severity. Consequently, the spike represents an optimal target for the development of RNA/viral vector vaccines, monoclonal Ab, diagnostics, and therapies [9][26]. Moreover, spike RBD can be extremely useful for in silico function predictions based on structure, also as a result of mutations, in the interactions with neutralizing Ab and hACE2 [9][15][26].

CoV-2 is being spread much more rapidly than CoV-1. Although many studies have been carried out, it has not yet been definitely established whether the interaction of spike–hACE2 is stronger with CoV-1 or CoV-2, and how this aspect can be put in relation with their different infectivity levels. The computational studies in the literature seem to report conflicting results. Preliminary in silico analysis predicted the free energy values of binding between the spike RBD (CoV-1 and CoV-2) and hACE2. First molecular docking simulations between the spike RBD of CoV-1 and hACE2 showed that their interaction is energetically favored with respect to CoV-2, which nevertheless showed a significant binding affinity to hACE2 [7]. Further in silico researches by molecular modeling and docking showed low binding energy in the CoV-2 spike–ACE2 complex as compared to CoV-1 [6]. Based on the structure of the CoV-2 spike experimentally solved in the prefusion conformation and on the kinetics of this interaction quantified by surface plasmon resonance, another study evidenced that the CoV-2 spike binds to hACE2 with higher affinity (10- to 20-fold) than the spike of CoV-1 [20]. In the same study, many differences at molecular level were evidenced when the spikes of CoV-1 and CoV-2 interacted with hACE2. In this regard, the spike of CoV-2 uses the hACE2 receptor less efficiently than the spike of the CoV-1 strain of 2002, but more efficiently than the CoV-1 strain of 2003 [18][25]. The CoV-2 mutations located in the spike-RBD region likely cause higher infectivity and lower pathogenicity than CoV-1 of 2002 with around 10% mortality rate [15][18][27]. Although the genome of CoV-2 has 82% nucleotide identity with CoV-1, and the spike of CoV-2 shares about 76% sequence identity with that of CoV-1 [5], the free energy of binding between the spike and hACE2 is comparable for CoV-2 and CoV-1 [17]. These results are compatible with the fact that the CoV-2 spike-RBD residues at the spike–ACE2 interface may have evolved in extremely complex ways from different common ancestors. Overall, the results of these studies differ on how the structural peculiarities between the two coronaviruses in the binding of spike to hACE2 can differently stabilize the ligand–receptor interactions of the complex spike–ACE2.

Several other proteins have been investigated for the potential activity as receptor or interactor of the spikes of coronaviruses, including CoV-2. TMPRSS2 is a serine protease upregulated by androgen hormones; it is involved in the infection process of many viruses, including coronaviruses, acting on the spike and hACE2, and facilitating

virus–cell membrane fusion [13][28]. Another receptor/interactor of the spike is neuropilin-1 (NRP1), a cell-surface receptor for vascular endothelial growth factor 165 (VEGF-165) and semaphorins. NRP1 binds furin-cleaved substrates, and is implicated in CoV-2 infectivity [29][30][31]. Other receptors or interactors of coronaviruses, and potentially of CoV-2, are also known. Dipeptidyl peptidase 4 (DDP4) is a glycoprotein membrane receptor involved in T-cell activation, with peptidase activity, and it is known as the MERS receptor [32]. Another serine protease, TMPRSS11D, cleaves and activates the spike of human coronavirus 229E (HCoV-229E), facilitating its cell entrance [33]. The C-type lectin domain family 4 member M (CLEC4M) is a membrane protein, known as an attachment receptor for hepatitis C virus (HCV), Ebola virus, human coronavirus 229E, and CoV-1 [34]. Many of these receptors are involved in several pathologies, and more in particular in the main COVID-19 comorbidities, thus suggesting that tissue expression of these proteins may be related to the epidemiological features of COVID-19 patients [11]. Moreover, several studies have proposed sialic acids on the host cell surface as possible co-receptors, acting as a further attachment mechanism that facilitates CoV-2 to enter the cell [35].

To discover potential molecular targets, as there are only a few functional therapeutic agents and several vaccines are currently available, a more in-depth understanding of the molecular interaction mechanisms underlying the initial steps of infection is required. In fact, massive interventions were directed against the first CoV-2 that appeared in 2019, but the emergence of its genetic variants, above all related to spike mutations, presents new challenges based on their high transmissibility and putting in doubt the efficacy of the first vaccines. Therefore, more recent studies have set out to investigate the structural interactions at the chain–chain interface in the spike–ACE2 complexes of crystallographic structures of both CoV-1 and CoV-2 available in the Protein Data Bank (PDB), the single worldwide repository of information about the 3D structural data of biological macromolecules [36]. Structures of the claw-like ACE2-PD in complex with the RBD or spike have revealed the molecular details of their interaction. On the one hand, these studies showed what specific amino acid residues are involved in ligand–receptor binding and how they interact at the spike–ACE2 interface. On the other hand, they allowed to evidence interesting structural characteristics, and differences between CoV-1 and CoV-2. Therapy and prevention of COVID-19 could benefit from these findings, because the spike is a key target for designing therapeutic agents and a viral antigen for optimal vaccine and monoclonal Ab development.

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