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## Previews

# Hold your horses: The receptor-binding domains of SARS-CoV-2, SARS-CoV, and hCoV-NL63 bind equine ACE2

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In this issue of *Structure*, Lan and colleagues seek to identify regions on the ACE2 receptor and coronavirus spikes that are essential for the viral attachment. They achieve it through a detailed comparative analysis of the binding of coronaviruses NL63, SARS-CoV, and several SARS-CoV-2 variants with human and horse ACE2.

Coronaviruses infect a variety of hosts ranging from birds and whales to bats and humans. To do so, different coronaviruses utilize a range of proteins and glycans as receptors. However, even distant coronavirus families may sometimes use the same receptor, and this is the case with sarbecoviruses—the sub-lineage of betacoronaviruses, which include the causative agent of the current pandemic, SARS-CoV-2. In common with SARS-CoV and the more distantly related alpha-coronavirus NL63, sarbecoviruses use their spike proteins to gain entry to the host cell by engaging the cellular receptor ACE2 (Hofmann et al., 2005; Li et al., 2005; Shang et al., 2020).

SARS-CoV-2 and its close relatives have been shown to infect multiple animal species and are predicted to infect many more (Conceicao et al., 2020; Liu et al., 2021b; Starr et al., 2022; Wu et al., 2020). Yet, only a few reports have provided insights into the molecular details on how CoV spike proteins bind to animal ACE2 receptors (for example, Liu et al., 2021a; Wu et al., 2020), and therefore the essential conserved residues in both CoV spikes and animal ACE2s crucial for infecting multiple host species have not been well described. Such studies are urgently needed to aid understanding of the molecular basis of the large coronavirus host diversity and to predict the host range of emerging viruses.

Here, Lan et al. (2022) exploit the rare ability of the ACE2 of the horse *Equus caballus* to bind the three human coronaviruses that use ACE2 as their receptor: NL63, SARS-CoV, and SARS-CoV-2 (Liu

et al., 2021b). In the study in this issue of *Structure*, they present crystal structures of equine ACE2 (eACE2) bound to the receptor binding domains (RBDs) of the spikes of these three viruses. This is combined with surface plasmon resonance (SPR) measurements to compare binding of human ACE2 (hACE2) and eACE2 receptors with these RBDs, as well as with the RBDs of SARS-CoV-2 variants that have emerged within the human population during the current pandemic.

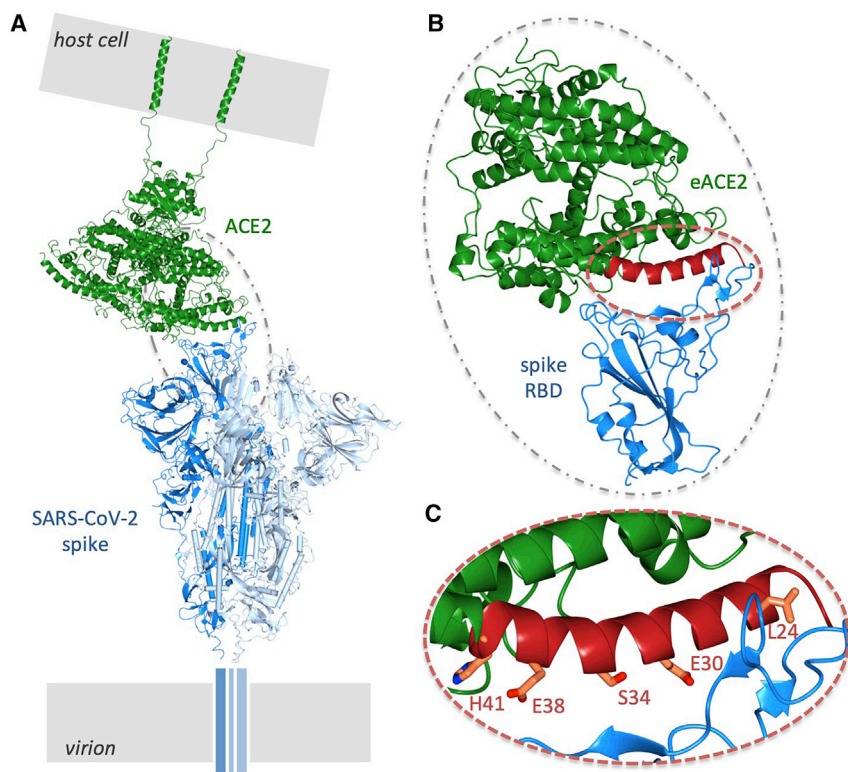
The structures of the three RBDs of NL63, SARS-CoV, and SARS-CoV-2 in complexes with eACE2 exploit the same membrane-distal binding interface observed previously in the structures of these RBDs with hACE2 (Li et al., 2005; Shang et al., 2020). The binding interface for all three involves a loop (residues 350–357) on ACE2, whereas the SARS-CoV-2 and SARS-CoV bind also to the long N-terminal helix (residues 19–50), and the smaller RBD of NL63 engages instead the two small neighboring loops 319–330 and 386–393. On all three CoV RBDs, the binding is localized mainly to their tip, known as the receptor binding motif(s) (RBMs), which in the native context of the whole spike trimer is furthest away from the viral membrane. Even though the structures of RBDs with hACE2 and eACE2 are very similar, the binding studies reveal that the affinity of all three spike proteins is lower for eACE2 than hACE2, and strikingly large differences have been observed for SARS-CoV and alpha SARS-CoV-2 (both > 50-fold weaker) and especially for the beta and gamma SARS-CoV-2 variants (both > 400-fold weaker). Inferring from the binding

data and structures of SARS-CoV and SARS-CoV-2 RBDs—those that showed the biggest variances in affinity between hACE2 and eACE2—Lan et al. attribute the main source of difference to residues in the initial (N-terminal) and middle parts of the N-terminal helix (residues 24, 30, 34, 38, and 41) of ACE2 (Figure 1). On the RBDs, residues N501 SARS-CoV-2 (Y501 in alpha, beta, and gamma variants) and possibly K417<sub>SARS-CoV-2</sub> (N417 in beta and gamma variants) are among the corresponding residues crucial for the interaction.

On ACE2, K417<sub>SARS-CoV-2</sub> and N501<sub>SARS-CoV-2</sub> are engaged by residues E30<sub>eACE2</sub>/D30<sub>hACE2</sub> and H41<sub>eACE2</sub>/Y41<sub>hACE2</sub>, respectively. Given the differences in eACE2 affinity between the alpha, beta, gamma variants, which carry Y501 substitution, and the N501 ancestral SARS-CoV-2, the authors hypothesize that the position 41 in ACE2, which directly engages N/Y501, is the crucial determinant of the RBD:ACE2 species preference. To confirm this, they introduce the H41Y substitution into eACE2, which rescues the strong binding of variant SARS-CoV-2 spikes to eACE2 and confirms the crucial importance of this residue for the interaction. These results are in line with another very recent study on eACE2 binding by sarbecovirus RBDs (Xu et al., 2022), which identified a similar set of residues crucial for the interaction while also highlighting the importance of S494<sub>SARS-CoV-2</sub>.

These studies have wider implications, as ACE2s of humans and many animals that can be infected with SARS-CoV-2, such as pangolins or cats, have Y41, while





**Figure 1. ACE2 region crucial for SARS-CoV-2 species specificity**

(A) A structural model of the SARS-CoV-2 spike trimer (colored in blue, with the monomer directly engaging the receptor in darker shade) binding to the dimeric hACE2 receptor (green) reconstructed from the cryogenic electron microscopy (cryo-EM) structures of the spike-receptor complex (PDB ID 7a54) and the dimeric ACE2 in complex with the spike RBD (PDB ID 6m17). The dotted line encircles the region shown in the next panel.

(B) The crystal structure solved by Lan et al. and reported in this issue of *Structure* (PDB ID 7fc5). SARS-CoV-2 spike RBD (blue) bound to eACE2 (green) with the ACE2 part of the binding interface variable among different animal species and thus likely essential for the zoonotic events shown in red. The dotted line encircles the region shown in the next panel.

(C) Residues in eACE2 that differ from those of hACE2 and are essential for binding to the SARS-CoV-2 RBD with side chains colored in orange.

several species, such as horses and donkeys, but also some bat species from the *Rhinolophus* genus, carry H41 instead. Remarkably, ACE2s of New World monkeys that also have H41—uniquely placed next to E42 rather than the more common Q42—were identified in the past as unable to support SARS-CoV-2 binding (Liu et al., 2021b), confirming the importance of residue 41 for potential species specificity. The Lan et al. study adds to the existing data and points to the initial part of the N-terminal ACE2 helix as the region that should be further investigated in structural and functional studies to gain a better understanding of the host specificity of coronaviruses. This seems especially important for investigating the origin of the SARS-CoV-2 pandemic, as many bat species from the family Rhinolophidae, regarded as hosts for sarbecoviruses, show

remarkable sequence diversity in this region. These recent studies also point to the crucial determinants of ACE2 binding in the SARS-CoV-2 spike, such as residues 417 and 501. It seems likely that, as the virus has evolved in the human population and optimized the interaction with hACE2 via substitutions such as N501Y, it is simultaneously becoming less capable of infecting animal hosts.

While Lan et al. rightly conclude that the spike-ACE2 affinity is only one of many factors determining whether a virus can infect a given host, their study undoubtedly adds to our understanding of coronavirus biology and our preparedness for future zoonotic events.

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#### DECLARATION OF INTERESTS

The authors declare no competing interests.

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