ACE2 structural residues are highly conserved in a variety of mammals

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Outline

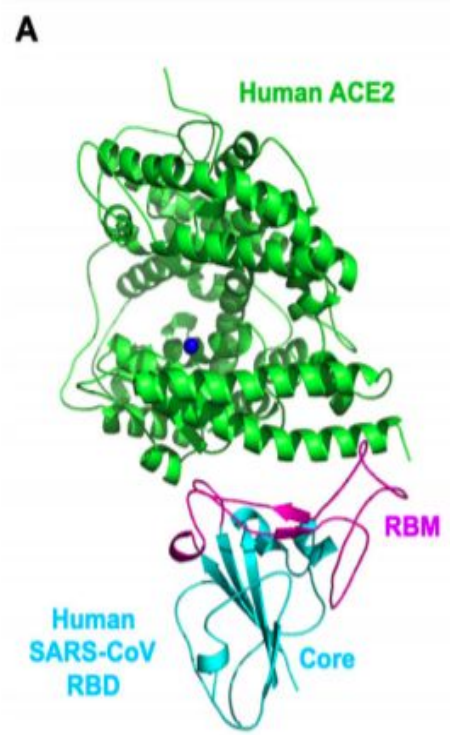
- **The ACE2 receptor found in mammals acts as the receptor for SARS-CoV-2, as well as other coronaviruses.**

- **Eight key ACE2 residues show high conservation in several mammals, indicating their importance to function & structure.**

- **The identified ACE2 structural & functional residues and ACE2 virus-binding hotspots are distinct from one another.**

ACE2 acts as the receptor for the SARS-CoV-2 spike RBD

- Mutations in spike protein key residues cause SARS-CoV-2 to recognize ACE2 at varying affinities (Wan et. al., 2020).
- SARS-CoV-2 recognizes ACE2 residues Lys 31, Glu 35, Asp 38, Met 82, and Lys 353 (Wan et. al., 2020).
- SARS-CoV-2 originated in bats, and likely used an intermediate host before mutating to infect humans (Wan et. al., 2020).



(Wan et al., 2020)

The ACE2 receptor is found in mammals, and is responsible for modulating Angiotensin

- Mammalian ACE2 proteins are expressed in vascular endothelium, myocardium, lungs, kidneys, and intestines (Fam et al., 2020).
- The primary function of ACE2 is to cleave Angiotensin (Ang) II into a heptapeptide, Ang 1-7 (Samavati & Uhal, 2020).
- Increased Ang II is associated with hypertension and accelerated blood clotting in arterioles (Samavati & Uhal, 2020).
- Therefore, ACE2 activation is necessary to prevent the harmful effects of Ang II on organisms.

How well conserved are ACE2 amino acid sequences in different mammals, and how does the level of conservation indicate sequence importance in function and structure?

ACE2 receptor sequences were obtained from UniProt and NCBI GenBank databases

- 10 species were selected based on 2 criteria:
  - Share a close common ancestor with humans, e.g. chimpanzees.
  - ACE2 sequence indicates binding affinity with the 2019-nCoV spike RBD, e.g. pigs, ferrets, and cats (Wan et al., 2020).

- Sequences were obtained from the UniProt and NCBI GenBank databases.
  - Three unreviewed UniProt sequences were used, due to a lack of reviewed sequences.

A variety of mammals were selected for ACE2 sequence analysis

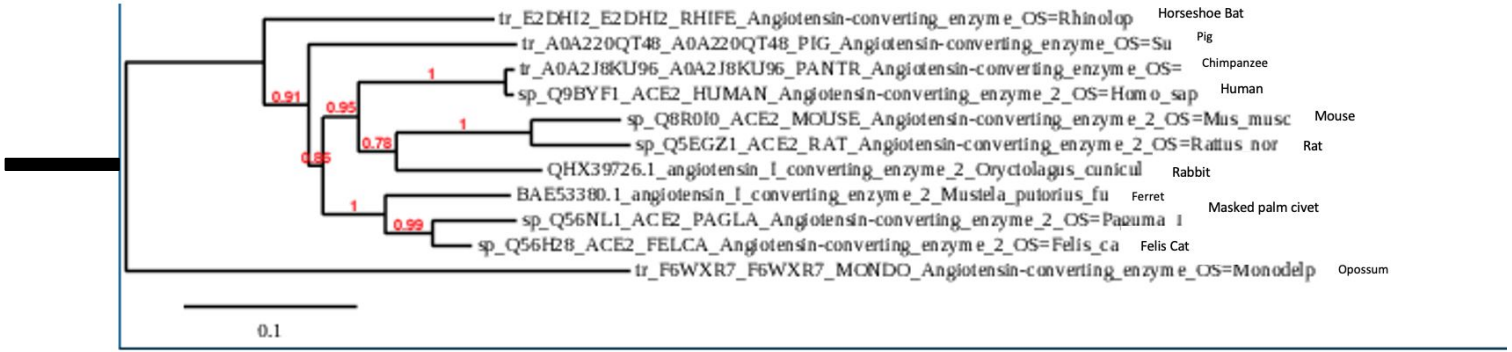
ACE2 sequences collected from UniProt	ACE2 sequences collected from NCBI GenBank
- Human - Chimpanzee* - Pig* - Rat - Mouse - Masked palm civet - Horseshoe bat* - Opossum** - Domestic Cat	- Rabbit - Ferret

*Unreviewed UniProt sequences

**Outgroup sequence

SARS-CoV-2 host species are not more closely related than the less favorable hosts

- Phylogeny.fr was used to generate a phylogenetic tree of the sequences.



- Opossum ACE2 sequence is the outgroup.
- Humans are most closely related to chimpanzees, in terms of the ACE2 protein.
- More favorable hosts for SARS-CoV-2 were not more closely related to one another than the non-favorable hosts.

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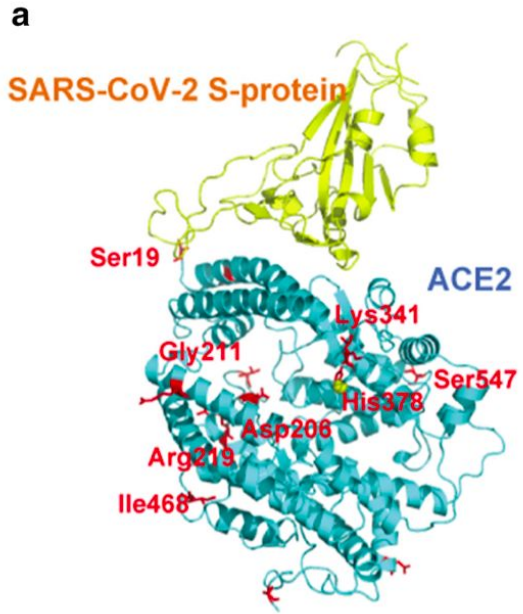
Human ACE2 has 8 key amino acids for structure and function

- A previous study identified 8 residues that are key to ACE2 structure and function in humans (Guo et al., 2020).

➔ His 378	➔ Arg 219
➔ Ser 19	➔ Lys 341
➔ Gly 211	➔ Ile 468
➔ Asp 206	➔ Ser 547



(Guo et al., 2020)

A multiple sequence alignment revealed that ACE2 structural residues are highly conserved in mammals

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ACE2 key structural residues are highly conserved in a variety of mammals

	378	19	211	206	219	341	468	547
Opossum	His	Asn	Glu	Asp	Arg	Lys	Ile	Ser
Bat	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Mouse	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Rat	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Pig	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Rabbit	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Chimpanzee	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Human	His	Ser	Gly	Asp	Arg	Lys	Ile	Ser
Ferret	His	Ser	Trp	Asp	Arg	Lys	Ile	Ser
Palm civet	His	Ser	Trp	Asp	Arg	Lys	Ile	Ser
Cat	His	Ser	Trp	Asp	Arg	Lys	Ile	Ser

- Six of the key structural residues are invariantly conserved.
- One key residue shows weak conservation (19).
- One key residue shows no conservation (211).

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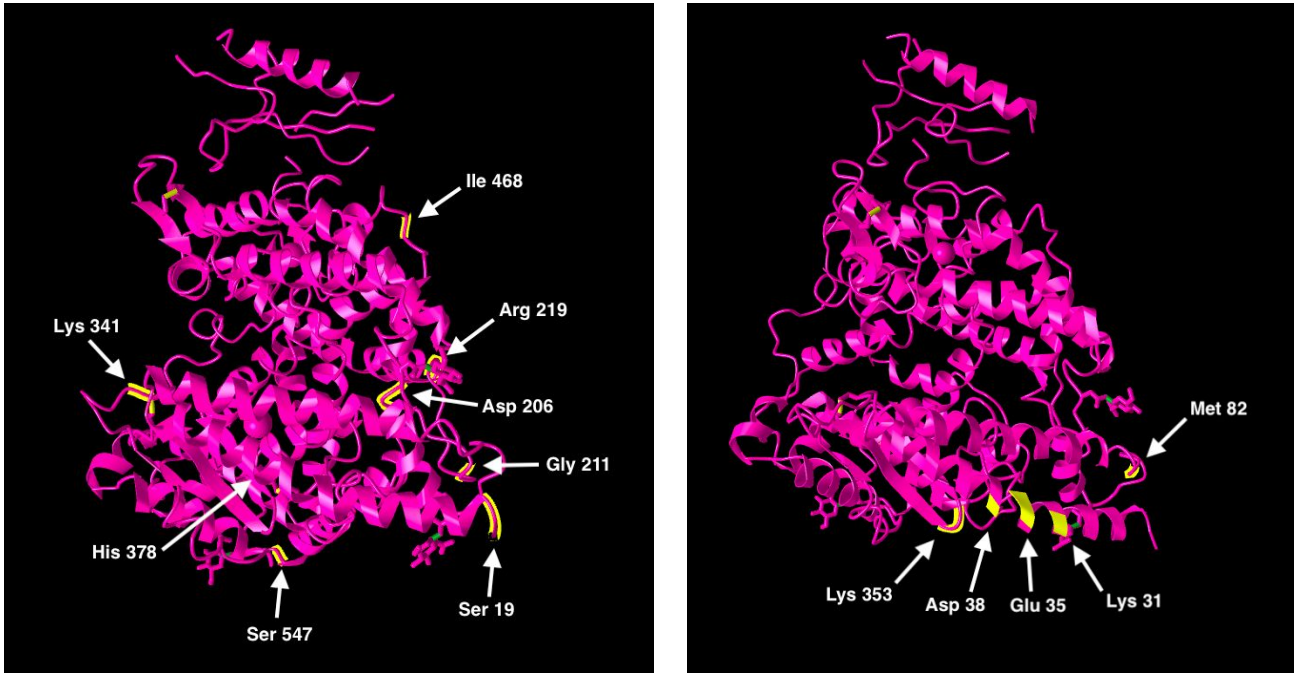
Structural models of ACE2 show the locations of viral-binding hotspots and structural residues



- The highly conserved structural & functional residues that were analyzed are distinct from the virus-binding hotspots on ACE2.

Identification of key structural residues on ACE2 may contribute to treatment for SARS-CoV-2

- Identifying which residues are critical for ACE2 structure and function may contribute to the development of treatments for SARS-CoV-2.

- Would it be possible to target the ACE2 receptor in a way that disrupts viral-binding hotspots without impacting structure & function?

Summary

- Phylogeny of ACE2 sequences did not play a role in its binding compatibility with SARS-CoV-2.
- Of the 8 key amino acids in human ACE2, 6 were invariantly conserved, 1 showed weak conservation, and 1 had no conservation.
- None of the invariant key amino acids were the same as the amino acid residues that bound to the novel SARS-CoV-2.

Acknowledgments

- Dr. Dahlquist for helping us with formulating the research question and locating sequences.

- The Wan et. al. 2020 paper for showing the role that the ACE2 receptor has in binding to the 2019-nCoV.

- NCBI and UniProt protein databases for giving access to the ACE2 sequences.

- iCn3D structure tool for allowing us to study the ACE2 structure.

References

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