Interactions of the SARS-CoV-2 variants with human ACE2 receptor

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Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, responsible for COVID-19) is enveloped, large, moderately pleomorphic, positive-stranded RNA (+ssRNA) virus belonging to the genus *Betacoronaviruses*. The genome of SARS-CoV-2 encodes the non-structural replicase polyprotein, four structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N), and several additional non-structural proteins called accessory proteins.

The spike protein (150 kDa) is a highly glycosylated homotrimer that is distributed on the surface of the virion particles and protrudes radially from the viral envelope, forming a "crown-like" structure. The S-glycoprotein is a class I fusion protein and is responsible for the binding of the virion to the host receptor, its fusion with it and entry into the virus. The SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2), an enzyme found on the outer surface of a variety of cells, as their cellular receptor.

Each coronavirus spike protein consists of three segments: an ectodomain, a transmembrane anchor and an intracellular tail. Two subunits can be distinguished in the ectodomain of the S protein. The amino-terminal subunit (S1) is responsible for the binding of the virus to the ACE2 receptor, while the carboxyl-terminal subunit (S2) is responsible for the fusion of the virion with the cell membrane, during which it undergoes a conformational change and rejects the S1 subunit. The S protein of SARS-CoV-2 has 1273 amino acid residues. The S1 subunit of coronaviruses includes the N-terminal domain (NTD), the receptor-binding domain (RBD) and two subdomains SD1 and SD2. A critical, initial step for SARS-CoV-2 to enter the host cells is specific interaction the SARS-CoV-2 virus spike protein receptor binding domain (RBD) with angiotensin-converting enzyme 2 (ACE2) on the cell surface. The RBD contains a core and an extended loop called the receptor-binding motif (RBM), which interacts directly with ACE2 [1-7].

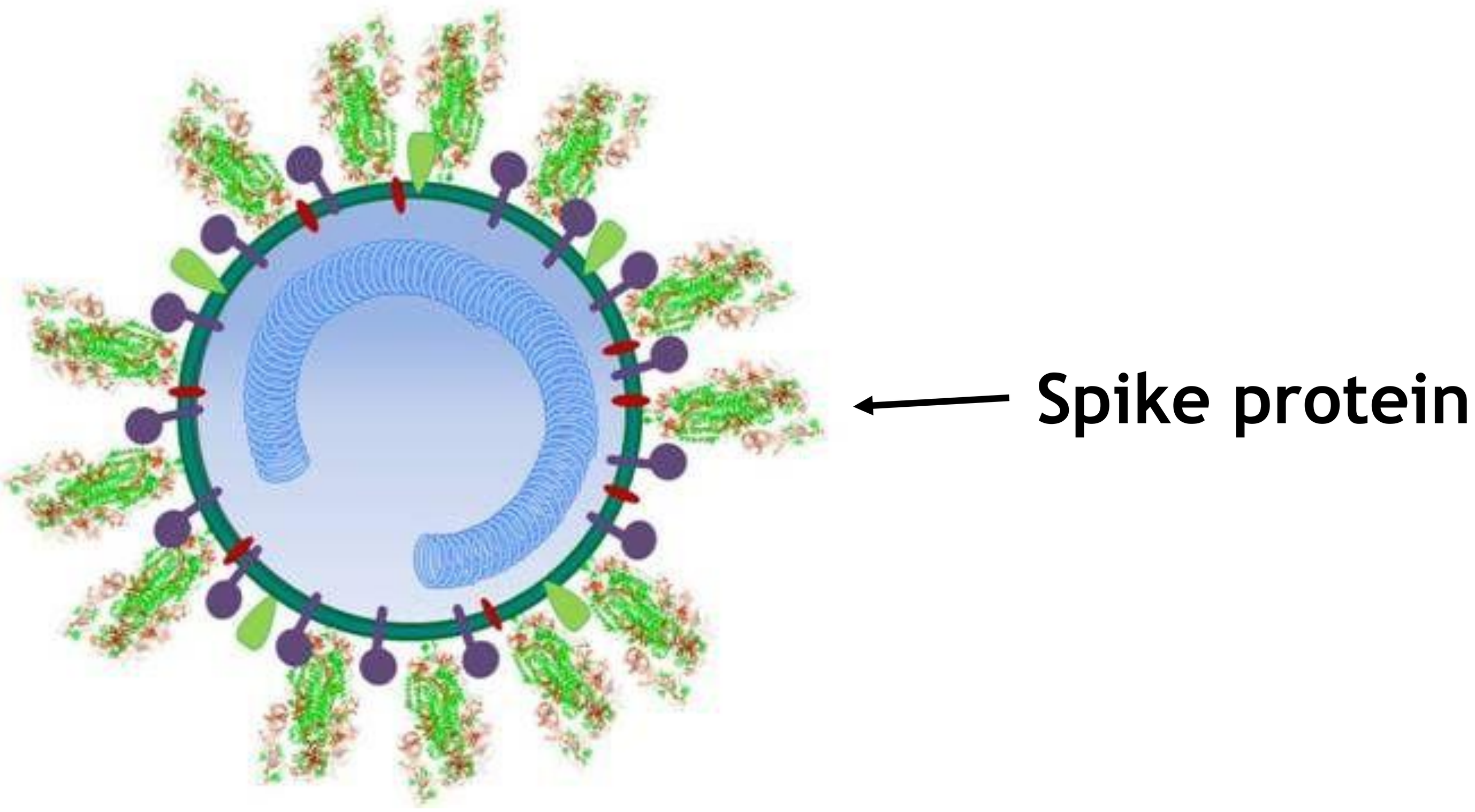

Fig. 1. The SARS-CoV-2 virion

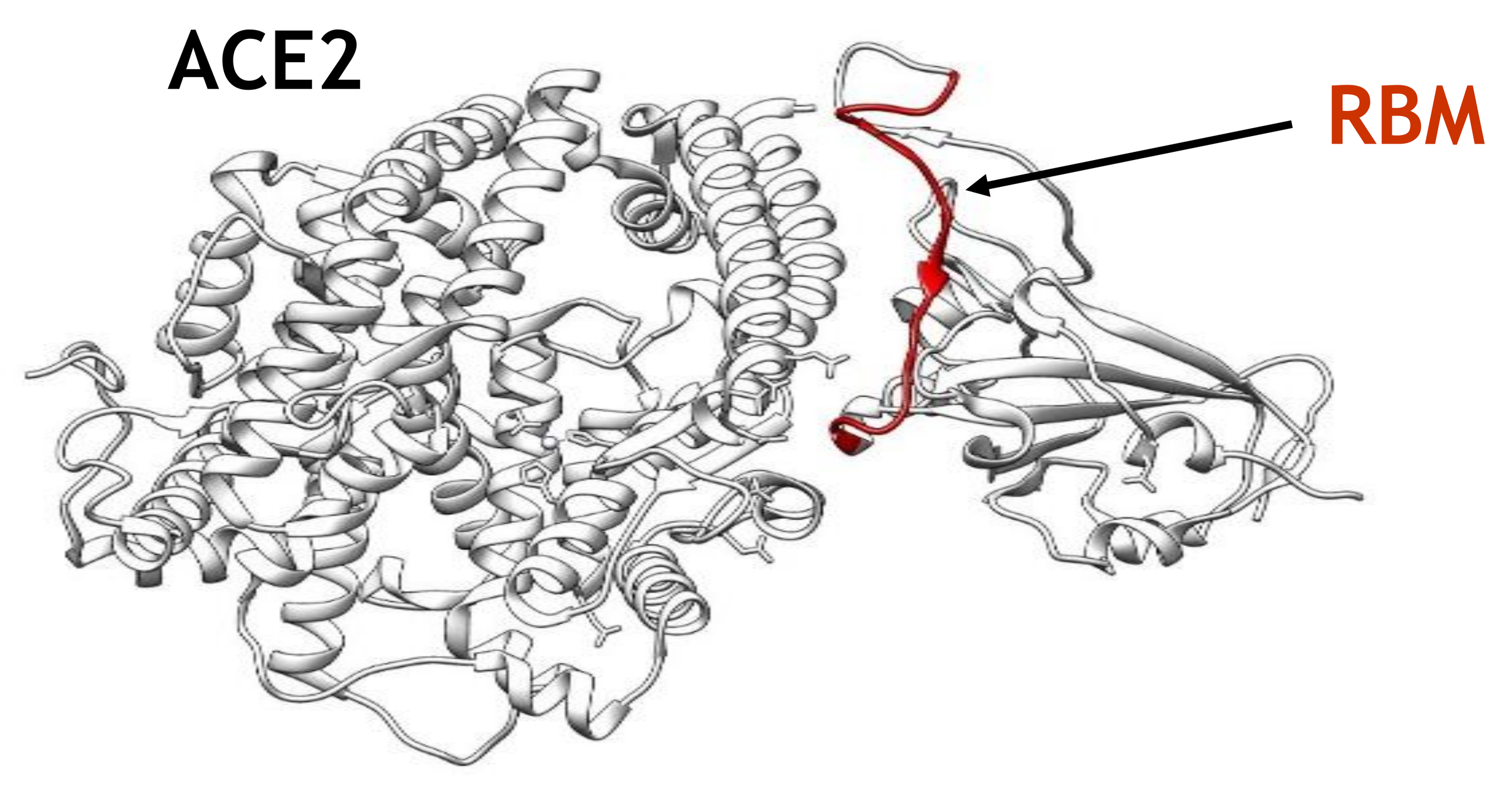

Fig. 2. The structure of the ACE2 and S1 of the SARS-CoV-2 complex

Mutations in RBD of SARS-CoV-2

Mutations in RBD present in SARS-CoV-2 variants of concern, classified by the WHO, have emerged independently around the world. The common mutations found in SARS-CoV-2 RBD, which have direct potential to affect key characteristics of the virus, are N501Y, E484K, K417T and K417N. The studies indicated, that the mutations N501Y, E484K, and S477N enhance the in++weeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeeek QQQQQQQQQQQQQQQQQQQQQQQQQQQQPE\AZ+W-teraction of SARS-CoV-2 S protein with receptor, while the mutations K417T and K417N decreased the affinity. Some studies show that the mutations in RBD that enhance binding to the ACE
47555;/.L.;2 receptor provide facilitate of SARS-CoV-2 immune escape. Moreover these findings suggest that these mutations may directly affect infectivity [5-7].

Table 1. Characteristic of mutations [3]

Mutation	Seen in variant	Effects on transmissibility
N501Y	B.1.1.7 P.1	Increased. Stronger binding affinity . Up to 7.1 fold affinity increase.
E484K	B.1.351 P.1	Increased. Stronger binding affinity with ACE2
K417N	B.1.351 B.1.617.2	Decreased. Lowers ACE2 affinity.

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SARS-CoV-2 Wuhan    331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV  395
SARS-CoV-2 Alfa     331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV  395
SARS-CoV-2 Beta     331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV  395
SARS-CoV-2 Gamma    331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV  395
SARS-CoV-2 Delta    331  NITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNV  395
SARS-CoV-2 Omicron  331  NITNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNLAPFSTFKCYGVSPTKLNDLCFTNV  395

SARS-CoV-2 Wuhan    396  YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN  460
SARS-CoV-2 Alfa     396  YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN  460
SARS-CoV-2 Beta     396  YADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN  460
SARS-CoV-2 Gamma    396  YADSFVIRGDEVRQIAPGQTGTIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRKSN  460
SARS-CoV-2 Delta    396  YADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYRYRLFRKSN  460
SARS-CoV-2 Omicron  396  YADSFVIRGDEVRQIAPGQTGNIADYNYKLPDDFTGCVIAWNSNKLDSKVSGNYNYRYRLFRKSN  460

SARS-CoV-2 Wuhan    461  LKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATV   524
SARS-CoV-2 Alfa     461  LKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATV   524
SARS-CoV-2 Beta     461  LKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATV   524
SARS-CoV-2 Gamma    461  LKPFERDISTEIYQAGSTPCNGVKGFNCYFPLQSYGFQPTYGVGYQPYRVVVLSFELLHAPATV   524
SARS-CoV-2 Delta    461  LKPFERDISTEIYQAGSKPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATV   524
SARS-CoV-2 Omicron  461  LKPFERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPTYGVGYQPYRVVVLSFELLHAPATV   524
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Fig. 3. Sequence alignment of SARS-CoV-2 S1 RBD.
Variable amino acids residues SARS-CoV-2 indentified in Wuhan and variants of concern are in red

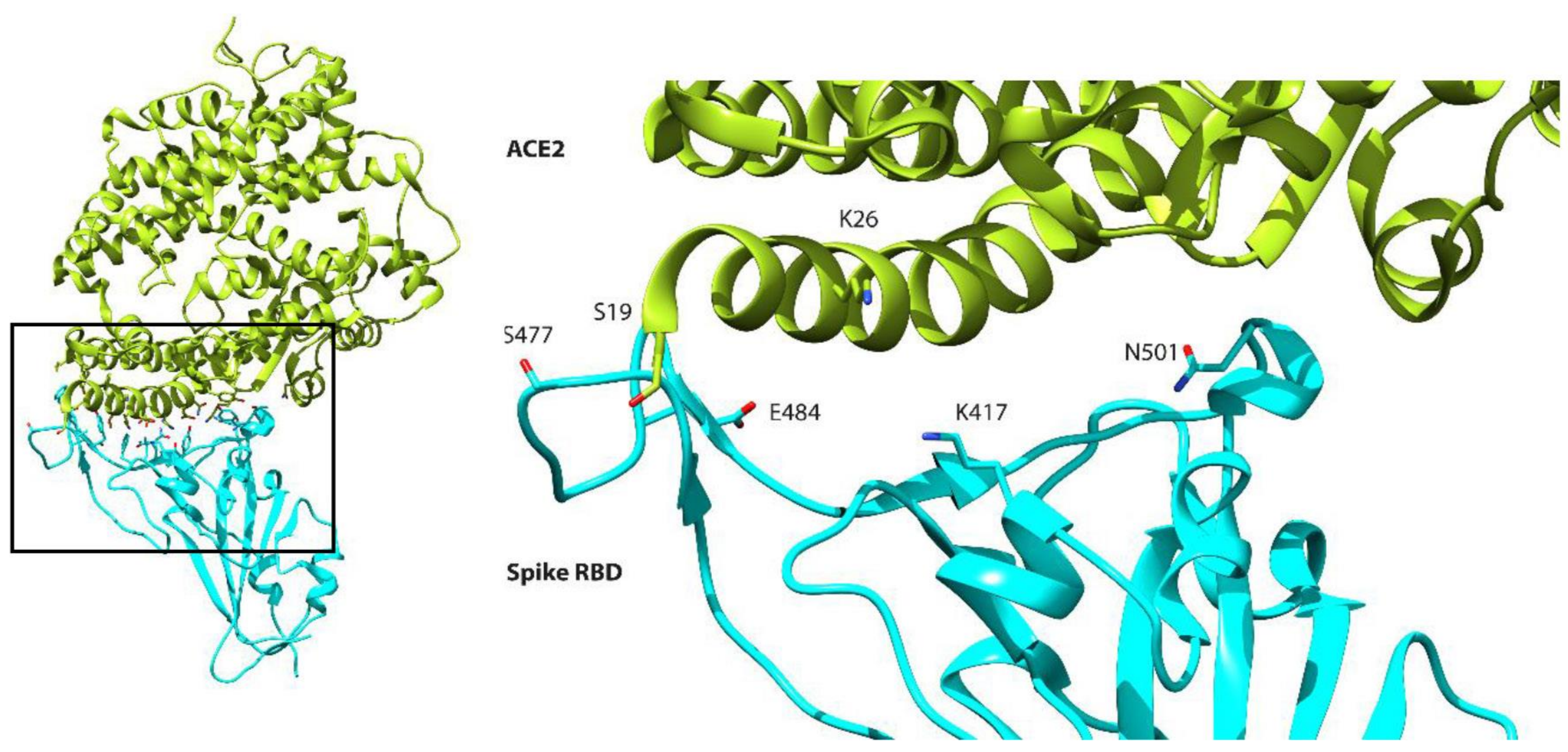

Fig. 4. The structure of human ACE2 (green) in complex with SARS-CoV-2 spike RBD (cyan). The residue mutated labelled [6]

<u>Variants of concers (by WHO):</u>

Alfa - B.1.1.7, the British Variant, identified in UK in September 2020
Beta - B.1.351, the South African Variant, identified in RPA in December 2020
Gamma - P.1, the Brasilian Variant, identified in Japan in January 2021
Delta - B.1.617.2, the Indian Variant, detected in India in October 2020
Omicron - B.1.1.529, identified in South Africa in November 2021

Perspectives

The receptor binding motif of the S1 protein forms an unstructured loop (Fig. 4), our future perspective is to design a spike protein fragment containing this loop of SARS-CoV-2 and its variants to identify the unique effects of S1-specific mutations on ACE2 binding. Full thermodynamic properties may provide an answer to the questions, which mutations are central to this interaction and why some of the new variants of SARS-CoV-2 spread more rapidly. We designed five peptides (20 amino acid residues, fragment of RBD) with common mutations (Fig. 5). We are going to use a more sophisticated method - isothermal titration calorimetry (ITC). ITC is indeed the most efficient quantitative method for determining the thermodynamic properties related to interactions between two molecules.

1. 482 Ac-GVEGFNCYFPLQSYGFQPTNG-NH_2 502
2. 482 Ac-GVEGFNCYFPLQSYGFQPTYG-NH_2 502
3. 482 Ac-GVKGFNCYFPLQSYGFQPTYG-NH_2 502
4. 482 Ac-GVKGFNCYFPLQSYGFQPTNG-NH_2 502
5. 482 Ac-TPPALNCYWPLNDYGFYTTTG-NH_2 502

Fig. 4. Peptide sequences with common mutations (in red)

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