

# ***In silico* study of the spike protein from SARS-CoV-2 interaction with ACE2: similarity with SARS-CoV, hot-spot analysis and effect of the receptor polymorphism**

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## **Abstract**

The spread of the COVID-19 caused by the SARS-CoV-2 outbreak has been growing since its first identification in December 2019. The publishing of the first SARS-CoV-2 genome made a valuable source of data to study the details about its phylogeny, evolution, and interaction with the host. Protein-protein binding assays have confirmed that Angiotensin-converting enzyme 2 (ACE2) is more likely to be the cell receptor via which the virus invades the host cell. In the present work, we provide an insight into the interaction of the viral spike Receptor Binding Domain (RBD) from different coronavirus isolates with host ACE2 protein. We used homology-based protein-protein

docking, binding energy estimation, and decomposition, aiming to predict both qualitative and quantitative aspects of the interaction. Using *in silico* structural modelling, we brought additional evidence that the interface segment of the spike protein RBD might be acquired by SARS-CoV-2 via a complex evolutionary process rather than mutation accumulation. We also highlighted the relevance of Q493 and P499 amino acid residues of SARS-CoV-2 RBD for binding to hACE2 and maintaining the stability of the interface. Finally, we studied the impact of eight different variants located at the interaction surface of ACE2, on the complex formation with SARS-CoV-2 RBD. We found that none of them is likely to disrupt the interaction with the viral RBD of SARS-CoV-2.

key words: COVID-19, ACE2, viral spike Receptor Binding Domain, 1  
homology-based protein-protein docking, variants. 2

## Introduction 3

The coronavirus SARS-CoV-2 (previously known as nCoV-19) has been 4  
associated with the recent epidemic of acute respiratory distress syndrome 5  
[9], [2]. Recent studies have suggested that the virus binds to the ACE2 6  
receptor on the surface of the host cell using the spike protein and explored 7  
the binary interaction of these two partners [4, 12, 28]. In this work, we 8  
focused our analysis on the interface residues to get insight into four main 9  
subjects: (1) The architecture of the interface of the spike protein and 10  
whether its evolution in many isolates supports an increase in affinity 11  
toward the ACE2 receptor; (2) How the affinity of SARS-COV-2-RBD and 12  
SARS-CoV-RBD toward different ACE2 homologous proteins from different 13  
species is dictated by a divergent interface sequences (3); A comparison of 14  
the interaction hotspots between SARS-CoV and SARS-CoV-2; and finally, 15  
(4) whether any of the studied ACE2 variants may show a different binding 16  
property compared to the reference allele. To tackle these questions we 17  
used multiscale modelling approaches combined to sequence and phylogeny 18  
analysis. 19

## Materials and Methods

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### Sequences and data retrieval

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Full genome sequences of 10 Coronaviruses isolates were retrieved from NCBI GeneBank corresponding to the following accessions: AY485277.1 (SARS coronavirus Sino1-11), FJ882957.1 (SARS coronavirus MA15), MG772933.1 (Bat SARS-like coronavirus isolate bat-SL-CoVZC45), MG772934.1 (Bat SARS-like coronavirus isolate bat-SL-CoVZXC21), DQ412043.1 (Bat SARS coronavirus Rm1), AY304488.1 (SARS coronavirus SZ16), AY395003.1 (SARS coronavirus ZS-C), KT444582.1 (SARS-like coronavirus WIV16), MN996532.1 (Bat coronavirus RaTG13) in addition to Wuhan seafood market pneumonia virus commonly known as SARS-CoV-2 (accession MN908947.3).

The sequences of the surface glycoprotein were extracted from the Coding Segment (CDS) translation feature from each genome annotation or by locally aligning the protein from SARS-CoV-2 with all possible ORFs from the translated genomes. ACE2 orthologous sequences from Human (Uniprot sequence Q9BYF1), Masked palm civet (NCBI protein AAX63775.1 from *Paguma larvata*), Chinese rufous horseshoe bat (NCBI protein AGZ48803.1 from *Rhinolophus sinicus*), King cobra snake (NCBI protein ETE61880.1 from *Ophiophagus hannah*), chicken (NCBI protein XP\_416822.2, *Gallus gallus*), domestic dog (NCBI protein XP\_005641049.1, *Canis lupus familiaris*), Wild boar (NCBI protein XP\_020935033.1, *Sus scrofa*) and Brown rat (NCBI protein NP\_001012006.1 *Rattus norvegicus*) were also computed and retrieved.

Human variants of the ACE2 gene were collected from the gnomAD database [7]. Only variants that map to the protein coding region and belonging to the interface of interaction with the RBD of the spike protein were retained for further analyses.

### Sequence analysis and phylogeny tree calculation

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MAFFT was used to align the whole genome sequences and the protein sequences of viral RBDs [8]. For the genome comparison, we selected the best site model based on lowest Bayesian Information Criterion calculated

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using model selection tool implemented in MEGA 6 software [21]. The General Time Reversible (GTR) model was chosen as the best fitting model for nucleotide substitution with discrete Gamma distribution (+G) with 5 rate categories. For the RBD sequences, the best substitution model for ML calculation was selected using a model selection tool implemented on MEGA 6 software based on the lowest Bayesian Information criterion (BIC) score. Therefore, the WAG model [24] using a discrete Gamma distribution (+G) with 5 rate categories has been selected.

Phylogenetic trees were generated using a maximum likelihood (ML) method in MEGA 6. The consistency of the topology, for the RBD sequences, was assessed using a bootstrap method with 1000 replicates. The resulting phylogenetic tree was edited with iTOL [13].

## Homology based protein-protein docking and binding energy estimation

The co-crystal structure of the spike protein of SARS coronavirus complexed to human human-civet chimeric receptor ACE2 was solved at 3 Å resolution (PDB code 3SCL). We used this structure as a template to build the complex of spike protein from different virus isolates with the human ACE2 protein (Uniprot sequence Q9BYF1). The template sequences of the ligand (spike protein) and the receptor (ACE2) were aligned locally with the target sequences using the program Water from the EMBOSS package [16]. Modeller version 9.22 [18] was then used to predict the complex model of each spike protein with the ACE2 using a slow refining protocol. For each model, we generated ten conformers from which we selected the model with the best DOPE score [19].

To calculate the binding energy we used the PRODIGY server [27]. The Calculation of contribution of each amino acid in a protein partner was computed with MM-GBSA method implemented in the HawkDock server [23]. Different 3D structures of hACE2, each comprising one of identified variants, were modeled using the BuildModel module of FoldX5 [3]. Because it is more adapted to predict the effect of punctual variations of amino acids, we used DynaMut at this stage of analysis [17].

## Flexibility analysis 84

We ran a protocol to simulate the spike RBD fluctuation of SARS-CoV-2 and SARS-Cov using the standalone program CABS-flex (version 0.9.14) [11]. Three replicates of the simulation with different seeds were conducted using a temperature value of 1.4 (dimensionless value related to the physical temperature). The protein backbone was kept fully flexible and the number of the Monte carlo cycles was set to 100.

## Results 91

### Sequence and phylogeny analysis 92

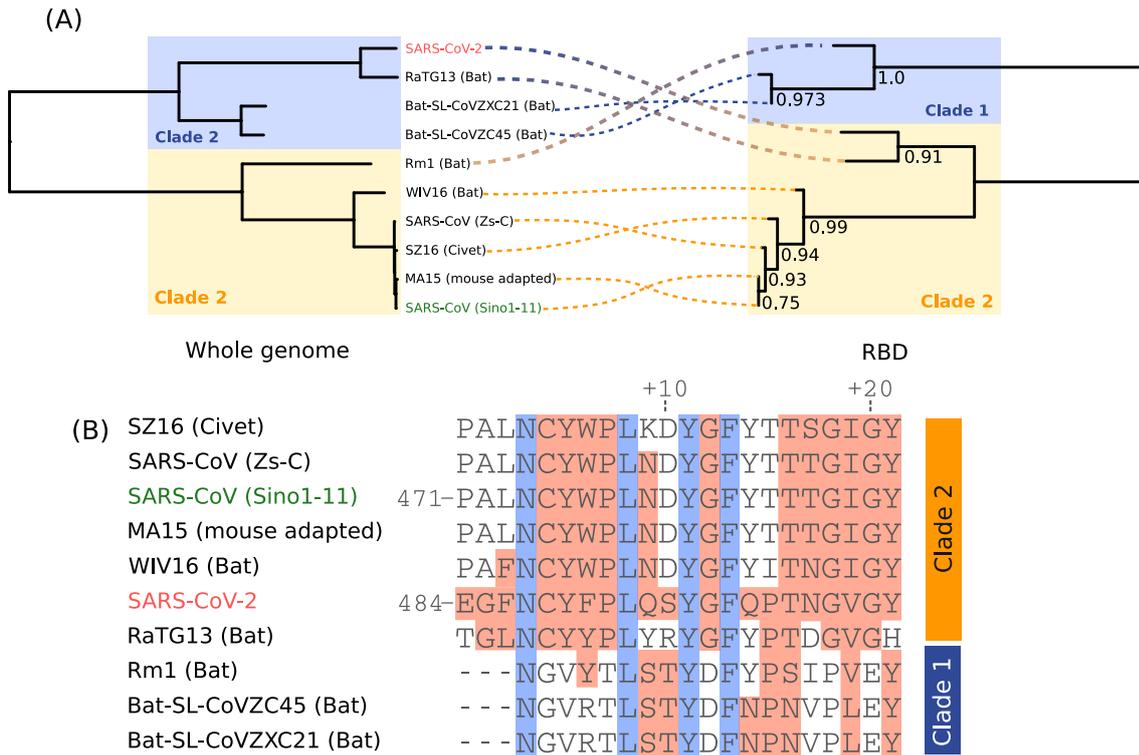
Phylogenetic analysis of the different RBD sequences revealed two well supported clades. Clade 2 includes SARS-CoV-2, RatG13, SZ16, ZS-C, WIV16, MA15, and SARS-CoV-Sino1-11 isolates (Figure 1A). SARS-CoV-2 and RatG13 sequences are the closest to the common ancestor of this clade. Clade 1 includes Rm1 isolate, Bat-SL-CoVZC45 and Bat-SL-CoVZXC21. These three isolates are closely related to SARS-CoV-2 as revealed by the phylogenetic tree constructed from the entire genome (Figure 1A). The exact tree topology is reproduced when we used only the RBD segment corresponding to the interface residues with hACE2. This is a linear sequence spanning from residue N481 to N501 in SARS-CoV-2.

Multiple sequence alignment showed that the interface segment of SARS-CoV-2 shares higher similarity to sequences from clade 2 (Figure 1B). However, we noticed that S494, Q498 and P499 are exclusively similar to their equivalent amino acids in sequences from clade 1. SARS-CoV-2 interface sequence is closely related to Bat-CovRaTG13 sequence, isolated from a *Rhinolophus affinis* bat.

### Prediction of the RBD/hACE2 complex structure 109

To investigate whether the interface of the spike protein isolates evolves by increasing the affinity toward the ACE2 receptor in the final host, we predicted the interaction models of the envelope anchored spike protein (SP) from several clinically relevant Coronavirus isolates with the human receptor

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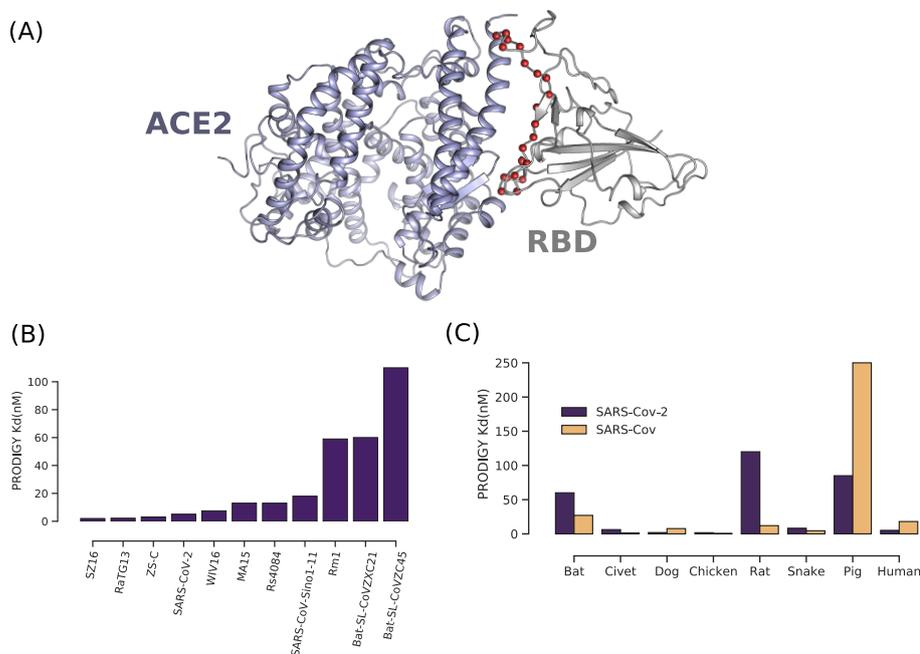


**Figure 1.** Phylogeny and sequence analysis based on full genome and RBDs from the different isolates included in this study. (A) Phylogeny trees are opposed to each other to show the clade discrepancies and discontinuous lines shows the equivalent taxon between each tree. (B) Multiple sequence alignment of the interface residues of RBD. Blocks in red indicate the residues with similar biochemical properties to the positions in SARS-CoV-2. Conserved residues are colored in blue.

ACE2 (hACE2). The construction of the complex applies a comparative- 114  
 based approach that uses a template structure in which both partners 115  
 (ligand and receptor) are closely related to those in the target system 116  
 respectively. In our study, we only modeled the interaction of the RBD 117  
 which was shown to be implicated in the physical interaction with ACE2 118  
 receptor (Figure 2A). The lowest sequence identity of the modeled spike 119  
 proteins as well as those of any of the orthologous ACE2 sequences (Human, 120  
 civet, bat, pig, rat, chicken and snake) do not fall below 63% compared to 121  
 their respective templates. At such values of sequence identities between 122  
 the equivalent partners of the receptor or the ligand, it is expected that the 123  
 template and the target complexes share the same binding mode [1, 10]. 124

## Analysis of energy Interaction of hACE2 with other virus

We calculated the binding energy of RBD from different virus isolates interacting with hACE2 using the PRODIGY method (Figure 2b). The binding energies are converted to dissociation constant estimations (Kd). SP proteins from bat-SL-CoVZC45, bat-SL-CoVZXC21 and Rm1-Cov show the highest values (least favorable) which are all above 50 nM. All the other estimations fall below 18 nM. The interaction between hACE2 and the RBDs of SARS-CoV-2 isolate (Whuhan-Hu-1) and the SARS-CoV-Sino1-11 show Kd values of 5.1 and 18 nM, respectively.



**Figure 2.** Homology based protein-protein docking of RBD/ACE2 and binding energy analysis of spike RBD with ACE2 receptor. (A) Homology based protein-protein docking complex of SARS-CoV-2 RBD with hACE2. The red spheres are the interface residues of the RBD. (B) Binding energies were converted to Kd values calculated for spike RBD from different coronavirus isolates interacting with hACE2. (C) Dissociation constant estimation calculated between SARS-CoV-2 RBD and homologous ACE2 from different model animals.

## 0.1 Energy analyses of human SARS-CoV-2 and SARS-CoV with different animal ACE2 receptors 136

We made this analysis to investigate the tendency of SARS-CoV-2 and SARS-CoV to interact with different orthologous forms of ACE2 which is dictated by the divergence in their interacting surfaces. Homology based protein-protein docking was conducted to generate the interaction model of SARS-CoV-2 RBD with ACE2 receptor from different animal species (Figure 2C). We noticed that for the civet, dog, chicken and snake forms, the interaction energy is very low and very similar either for SARS-CoV-2 or SARS-CoV. Although the Kd are relatively low for the rat and bat forms interacting with SARS-CoV RBD, those of SARS-CoV-2 are high and go beyond 50 nM. On the other hand, it seems that the interaction with pig ACE2 is more favorable for SARS-CoV-2 isolate since the estimated Kd is 3 folds lesser compared to SARS-CoV-2. 137  
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### Decomposition of the interaction energy 149

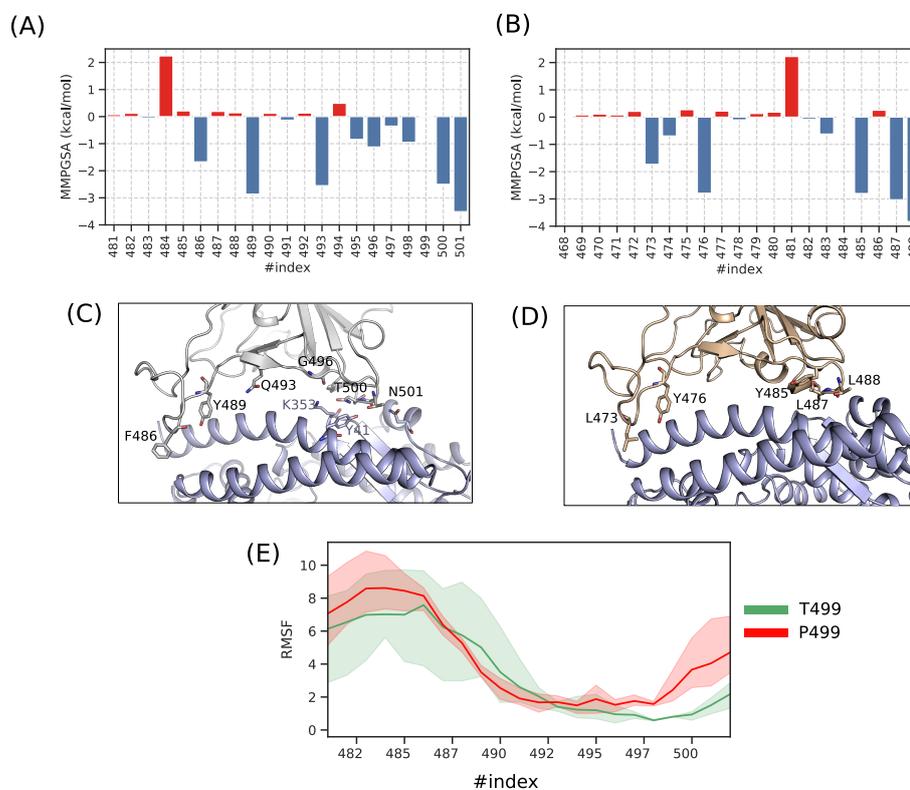
The MM-GBSA calculation allowed us to assign the contribution in the binding energy of each amino acid in the interface with hACE2. We carried this analysis using both sequences of the SARS-CoV-2 Wuhan-Hu-1 (Figure 3A) and the Sino1-11 SARS-CoV (Figure 3B) isolates. Residues F486, Y489, Q493, G496, T500 and N501 of SARS-CoV-2 RBD form the hotspots of the interface with hACE2 protein were investigated (we only consider values  $> 1$  or  $< 1$  kcal/mol to ignore the effect due to the thermal fluctuation). All these amino acids form three patches of interaction spread along the linear interface segment (Figure 3C): two from the N and C termini and one central. T500 establishes two hydrogen bonds using its side and main chains with Y41 and N330 of hACE2. N501 forms another hydrogen bond with ACE2 residue K353 buried within the interface. On the other hand, SARS-CoV RBD interface contains five residues (Figure 3D), L473, Y476, Y485, T487 and T488 corresponding to the equivalent hotspot residues of RBD from SARS-CoV-2 F487, Y490, G497, T501 and N502. Therefore, Q493 as a hotspot amino acid is specific to SARS-CoV-2 interface. The equivalent residue N480 in SARS-CoV only shows a non-significant contribution of 0.18 kcal/mol. 150  
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## Flexibility analysis

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Sequence analysis and the visual inspection of RBD/hACE2 complex might reflect the substitution of P499 in SARS-CoV-2 RBD as a form of adaptation toward a better affinity with the receptor. In order to further investigate its role, we performed a flexibility analysis using a reference structure (SARS-CoV-2 RBD containing P499) and an *in silico* mutated form P499T, a residue found in SARS-CoV and most of the clade 2. Our results show that the mutation caused a significant decrease in stability for nine residues of the interface corresponding to segment 482-491 (Figure 3E). Indeed, the RMSF variability per amino acid for this sequence increases compared to the reference structure.

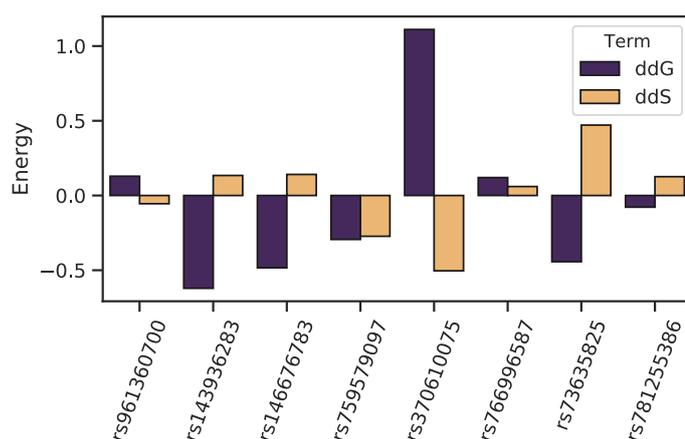
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**Figure 3.** Analysis of the interaction between RBD and hACE2. Decomposition of the MMGBSA energy for each amino acid of the binding surface from SARS-CoV-2 (A) and SARS-CoV Sino1-11 isolate (B). Position of the hotspot residues of the complexes RBD-SARS-CoV-2/hACE2 (C) and RBD-SARS-CoV/hACE2 (D). (E) Flexibility of RBD interface residue expressed as Root Mean Square Fluctuation (RMSF) for two forms of RBD-SARS-CoV-2, T499 and P499.

## Analysis of ACE2 variability and affinity with the virus 179

A total of eight variants of hACE2 that map to the interaction surface 180  
are described in the gnomAD database. All these variants are rare (Ta- 181  
ble 1) and mostly found in European non-Finnish and African populations. 182  
Considering both the enthalpy (ddG) and the vibrational entropy in our 183  
calculation (ddS), we found no significant change in the interaction energy 184  
( $> 1$  or  $< 1$  kcal/mol) (Figure 4). 185



**Figure 4.** Estimation of the energy changing upon mutation for hACE2 variants. Both Enthalpy and entropy terms are computed.

## Discussion 186

Since the Covid-2019 outbreak, several milestone papers have been published 187  
to examine the particularity of SARS-CoV-2 spike protein and its putative 188  
interaction with ACE2 as a receptor [25, 26]. In the current study, we 189  
focused our analysis on the interface segments of SARS-CoV-2 spike RBD 190  
interacting with ACE2 from different species by estimating interaction 191  
energy profiles. 192

We have studied the effect of eight variants of ACE2 in order to detect 193  
polymorphisms that may increase or decrease the virulence in the host. We 194  
concluded that if ACE2 is the only route for the infection in humans, variants 195  
interacting physically with RBD are not likely to disrupt the formation of 196  
the complex and would have a marginal effect on the affinity. Therefore, it 197

is unlikely that any form of resistance to the virus, related to the ACE2 198  
gene, exists. However, this analysis merits to be investigated in depth in 199  
different ethnic groups for a better assessment of the contribution of genetic 200  
variability in host-pathogen interaction. The binding of SARS-CoV-2 RBD 201  
to different forms of ACE2 shows similar values compared to SARS-CoV 202  
which is in agreement with the ability of the virus to cross the species barrier. 203  
However, although we estimated low Kd value between SARS-CoV RBD 204  
and Gallus gallus ACE2, there have been no reported cases of SARS-CoV 205  
isolated from chicken. On the other hand, while Kd is high for SARS-CoV 206  
RBD interacting with porcine ACE2, there are only few reported cases 207  
of such type [20]. Indeed, host-pathogen interaction is a complex process 208  
unlikely to be controlled only by the binding of the spike protein with 209  
ACE2 [5]. For model animals where Kd values are very low, ACE2 analysis 210  
may play a key role in targeting the main animal reservoir of SARS-CoV-2. 211  
For high Kd values, other factors might regulate the infection including the 212  
implication of different receptors or the response of the immune system but 213  
that does not mean that the infection is unlikely to occur. On the other 214  
hand, whole-genome phylogenetic analysis of the different isolates included 215  
in this study is consistent with previous works that place the Wuhan-Hu-1 216  
isolate close to Bat-SL-CoVZC45 and Bat-SL-CoVZXC21 isolates [14,15,22] 217  
within the Betacoronavirus genus. The use of RBD sequences, however, 218  
places the virus in a clade that comprises SARS-CoV related homologs 219  
including isolates from Bat and Civet. The clade swapping as seen in 220  
figure 1A, seems also to occur for RaTG13 and Rm1 isolated from bat. This 221  
is expected as the use of different phylogenetic markers may considerably 222  
affect the topology of the tree. However, given the functional implication 223  
of the spike RBD in host-pathogen infection, we have raised the question 224  
about where the virus obtained its RBD binding interface. The binding of 225  
the spike glycoprotein to ACE2 receptor requires a certain level of affinity. 226  
In the case where the RBD evolves from an ancestral form closer to that of 227  
Bat-SL-CoVZC45 and Bat-SL-CoVZXC21, we expected a decrease of the 228  
binding energy through the evolution process following incremental changes 229  
in the RBD. In such a scenario, we presume that there are other intermediary 230  
forms of coronavirus that describe such variation of the binding energy to 231  
reach a level where the pathogen can cross the species barriers and infect 232

humans with high affinity toward hACE2. On the other hand, our results 233  
show that the binding energy and the interface sequence of SARS-CoV-2 234  
RBD are closer to SARS-CoV related isolates (either from Human or other 235  
species). Therefore a recombination event involving the spike protein that 236  
might have occurred between SARS-CoV and an ancestral form of the 237  
current SARS-CoV-2 virus might be also possible. This will allow for the 238  
virus to acquire a minimum set of residues for the interaction with hACE2. 239  
The recombination in the spike protein gene has been previously suggested 240  
by Wei et al in their phylogenetic analysis [6]. Thereafter, incremental 241  
changes in the binding interface segment will occur in order to reach a 242  
better affinity toward the receptor. One of these changes may involve P499 243  
residue which substitution to threonine seems to drastically destabilize the 244  
interface segment and has a distant effect. Moreover, the decomposition 245  
of the interaction energy showed that 5 out of 6 hotspot amino acids in 246  
SARS-CoV-2 have their equivalent in SARS-CoV including N501. Contrary 247  
to what Wan et al [22] have stated, the single mutation N501T does not 248  
seem to enhance the affinity. Rather, the residue Q493 might be responsible 249  
for such higher affinity due to a better satisfaction of the Van der Waals 250  
by the longer polar side chain of asparagine. Indeed, when we made the 251  
same analysis while mutating Q493 to N493, the favorable contribution 252  
decreases from -2.55 kcal/mol to a non significant value of -0.01 kcal/mol, 253  
thus supporting our claim. 254

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**Table 1.** Population frequencies of hACE2 missense variants located on the interaction surface with SARS-CoV-2 RBD ( $\times 10^{-5}$ )

rs ID	European (non-Finnish)	African	Latino	Ashkenazi Jewish	East Asian	South Asian	Finnish	Other	Global
rs961360700	2.59	0	0	0	0	0	0	0	1.17
rs143936283	6.51	0	0	0	0	0	0	19.05	3.443
rs146676783	0	0.105	0	0	0	0	32.22	0	3.897
rs759579097	0	0.1056	0	0	0	0	0	0	0.9842
rs370610075	1.274	0	0	0	0	0	0	0	0.5756
rs766996587	0	26.23	0	0	0	0	0	0	2.442
rs73635825	0	332.3	0	0	0	0	0	18.82	31.29
rs781255386	0	0	7.303	0	0	0	0	0	1.091