Corso di laurea in Scienze Biologiche
Corso di laurea magistrale in Scienze Biomolecolari e dell'Evoluzione

Materiale didattico di supporto

Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.

Sommario (in pillole) 3

- La struttura, conformazione e sequenze dello spike sono state comparate tra coronavirus in relazione alla loro affinità per il recettore.

- L'affinità dello spike per ACE2 è stata determinata e comparata con quelle dei Coronavirus precedenti

Interazione tra Spike e recettore ACE2

Strutture ed affinità 1

Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2

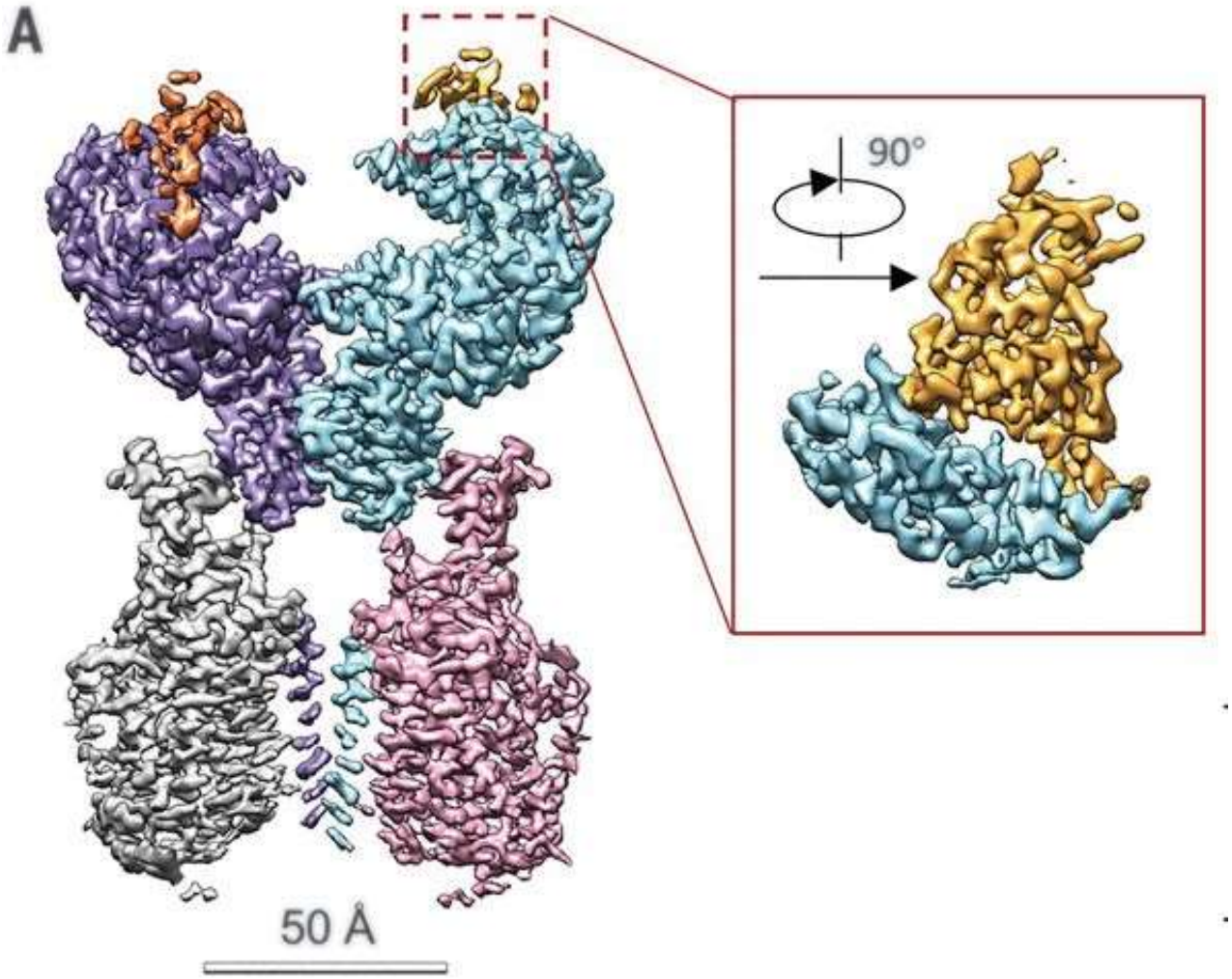
- EM reconstruction of the ternary complex
- Sructure determined to an overall resolution of 2.9 Å from 527,017 particles
- Resolution of 3.5 Å for the RBD

Reliable modeling and analysis of the interface

by Renhong Yan et Al Science Volume 367(6485):1444-1448 March 27, 2020

Science AAAS

Cryo–electron microscopy structures of full-length human ACE2 in the presence of the neutral amino acid transporter B^0AT1 with the receptor binding domain (RBD) of the surface spike glycoprotein (S protein) of SARS-CoV-2



The RBD (Spike) is recognized by the extracellular peptidase domain PD
of ACE2 mainly through polar residues.

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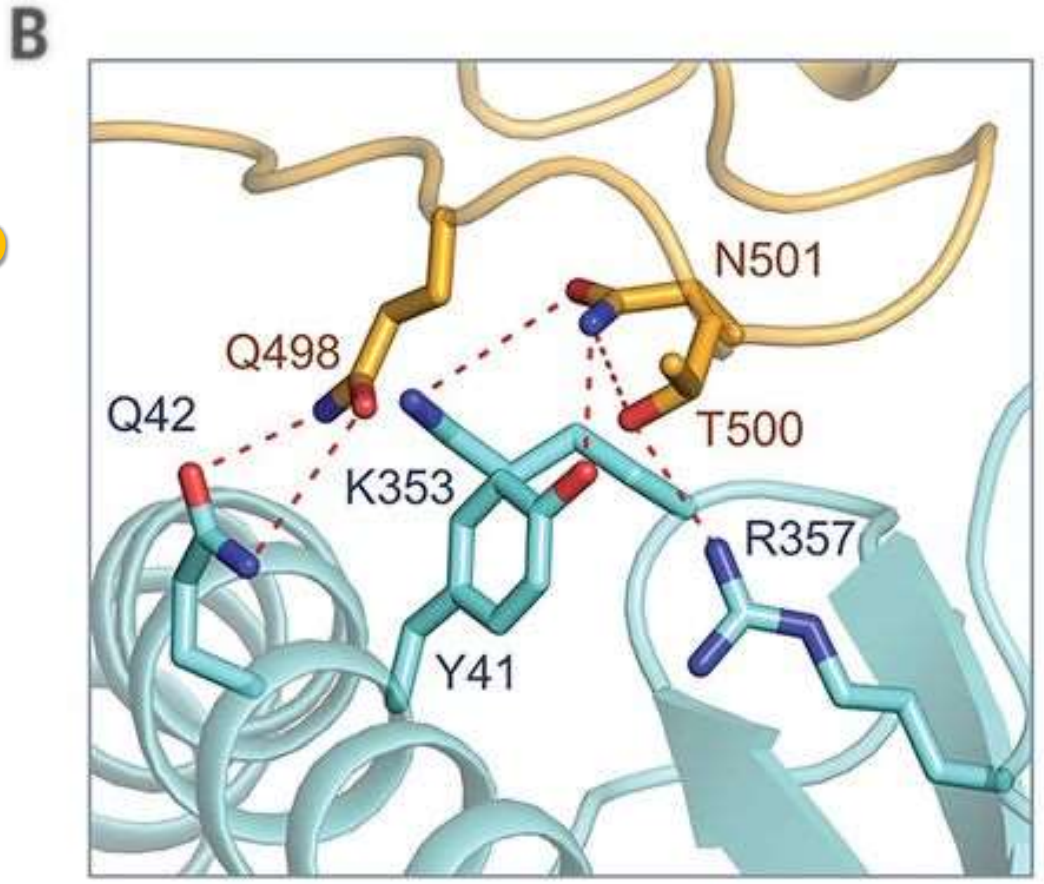
Renhong Yan et al. Science 2020;367:1444-1448

Fig. 4 Interactions between SARS-CoV-2-RBD and ACE2.

SARS-CoV-2-RBD

ACE2



Polar interactions
red dashed lines

"The overall interface is similar to that between SARS-CoV and ACE2 mediated mainly through polar interactions"

Renhong Yan et al. Science 2020;367:1444-1448

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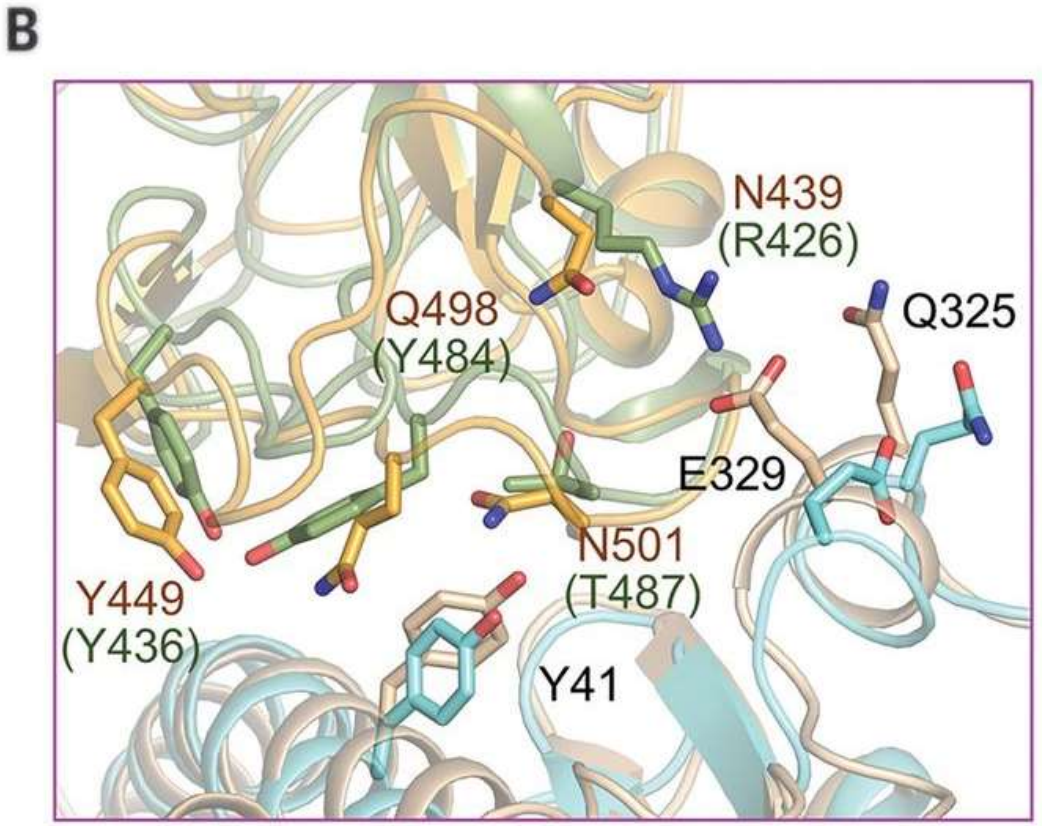
Interface comparison between SARS-CoV-2-RBD and SARS-CoV-RBD with ACE2

.



Change of residues
CoV-2-RBD (**brown**)
SARS-CoV-RBD (**green**).

Interazioni meno forti

Replacement of Arg426 (R426) with Asn439 (N439) appears to weaken the interaction by eliminating one important salt bridge with Asp329 (E329)

Renhong Yan et al. Science 2020;367:1444-1448

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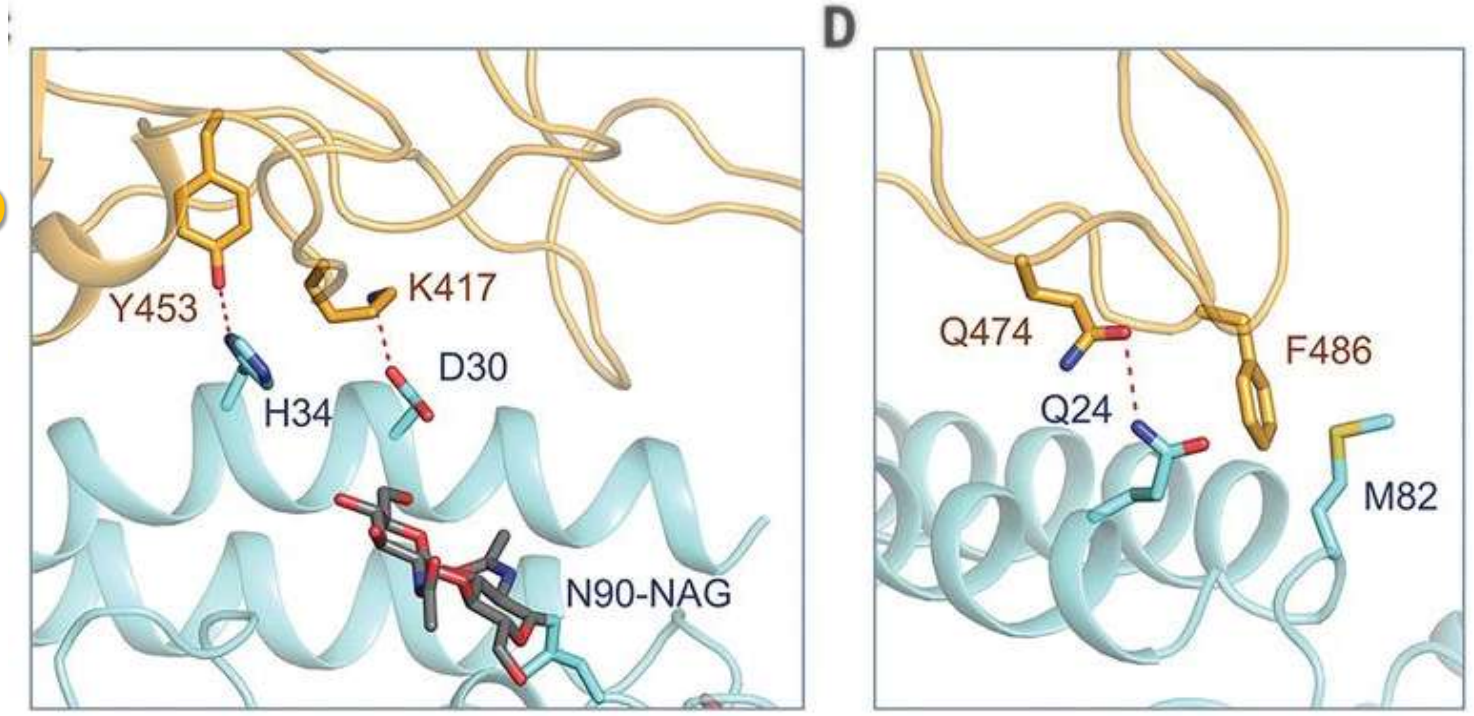
New Interactions between SARS-CoV-2-RBD and ACE2.

SARS-CoV-2-RBD

ACE2



The change fromVal404 to Lys417 (K417) may result in a tighter association because of the salt bridge formation between Lys417 and Asp30 (D30) of ACE2

Interazioni piu forti

The change from Leu472 to Phe486 (F486) may result in a stronger van der Waals contact with Met82 (M82)

Renhong Yan et al. Science 2020;367:1444-1448

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Comparazione del RBD tra coronavirus

RED conserved amino acid interacting with ACE2 in the SARS-CoV Receptor Binding Domain and in other viruses

GREEN Major altered amino acids in 2019-nCoV

Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140

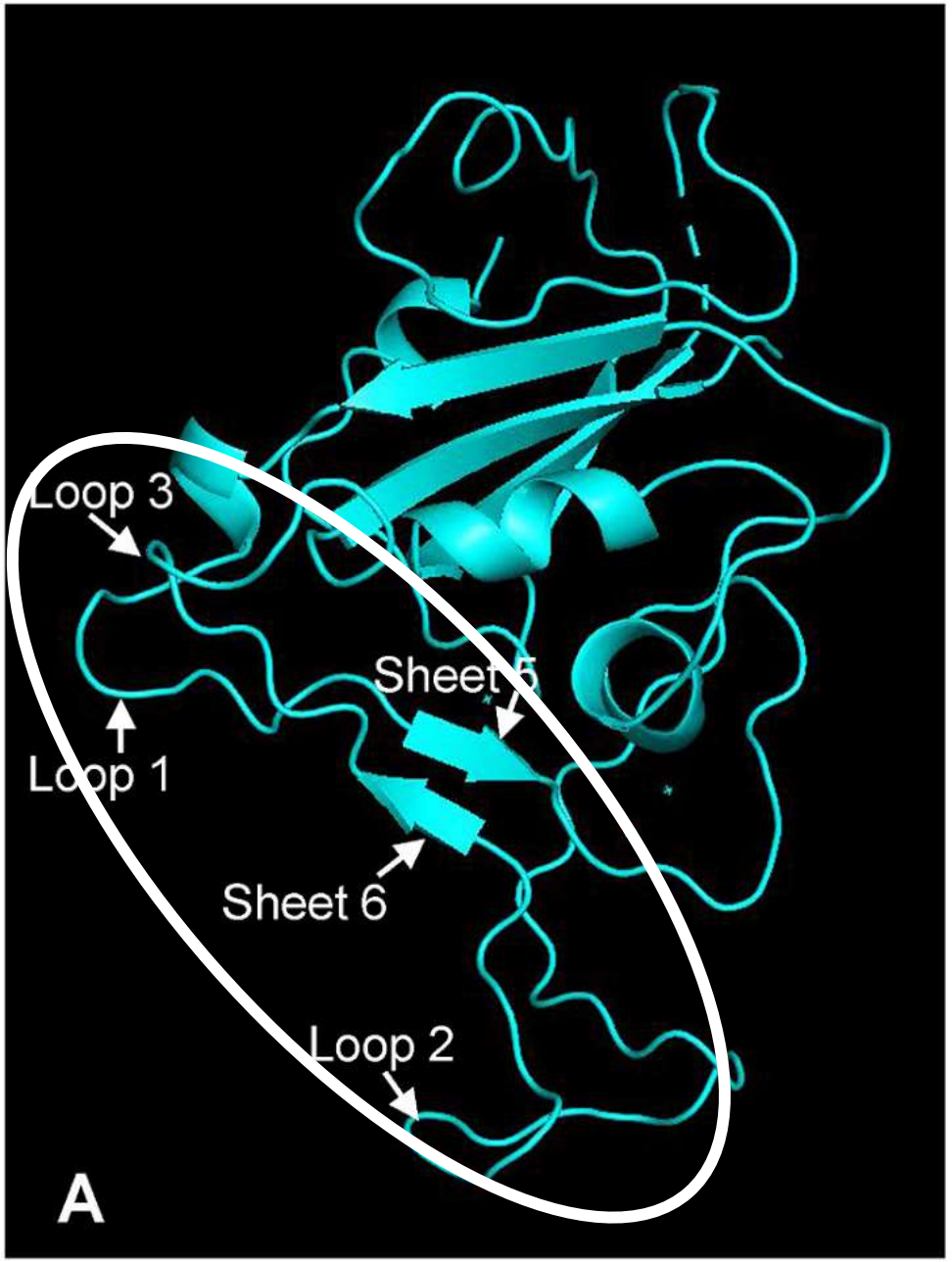
Comparazione del RBD tra coronavirus

RED conserved amino acid interacting with ACE2 in the SARS-CoV Receptor Binding Domain and in other viruses

GREEN Major altered amino acids in 2019-nCoV

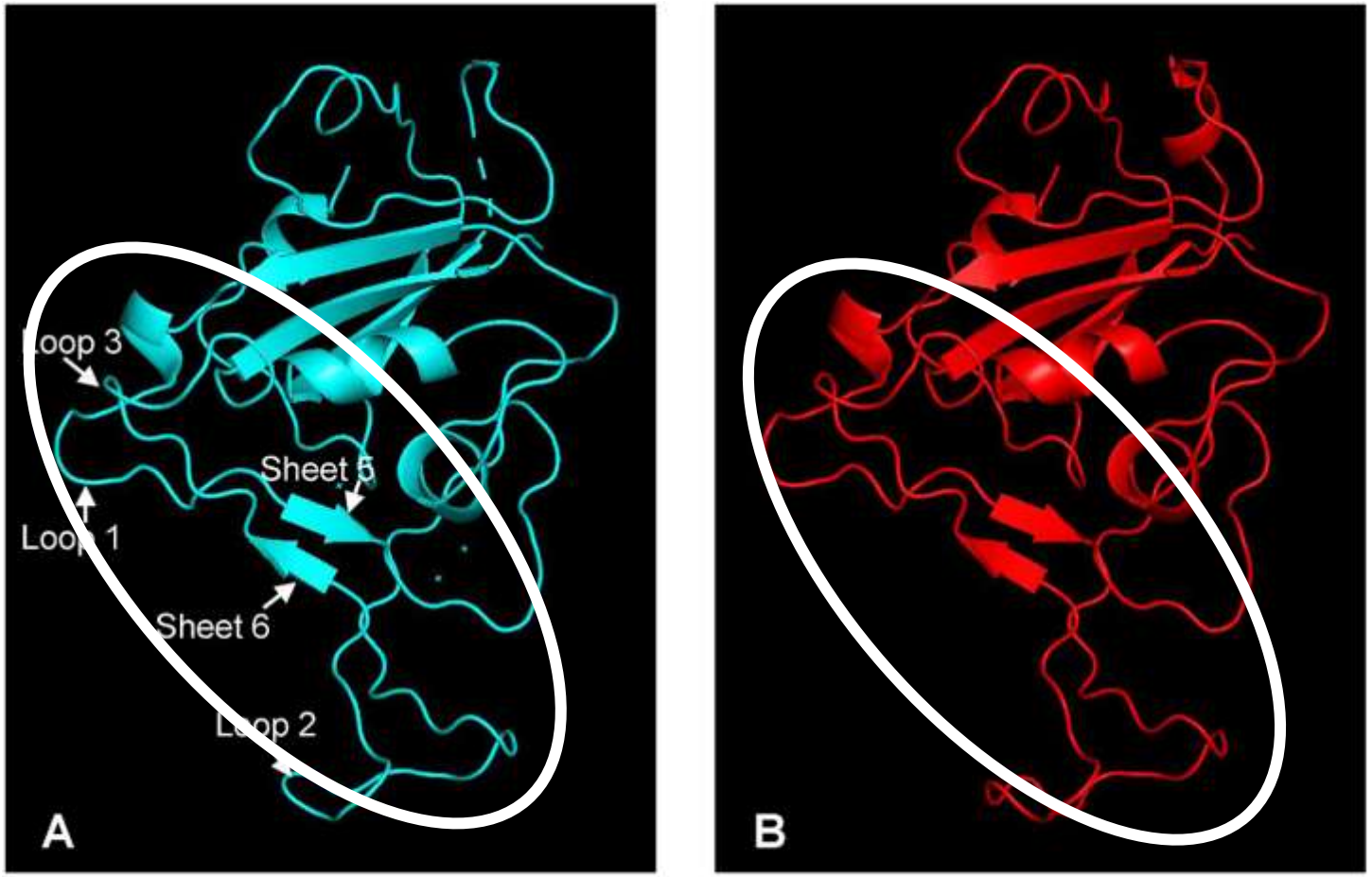
The structure of spike glycoprotein RBD of 2019-nCoV has unique features that potentially allow a high affinity binding to ACE2 in human cells.

Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140



SARS-CoV RBD

Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140

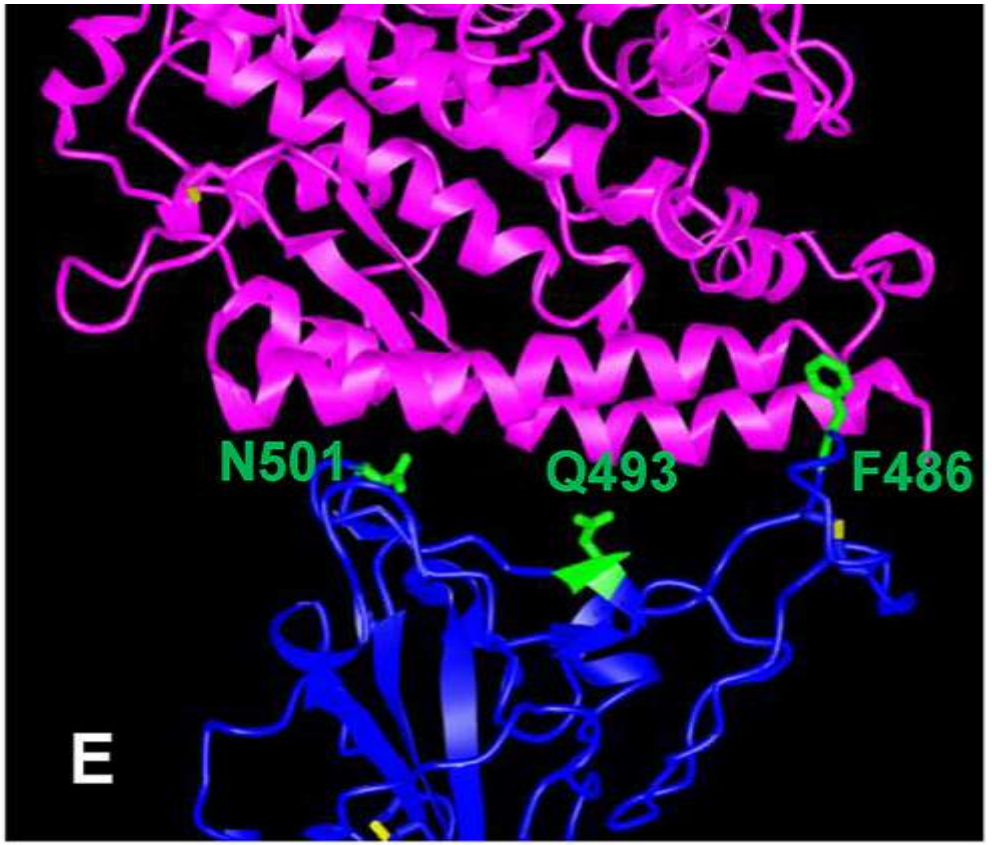


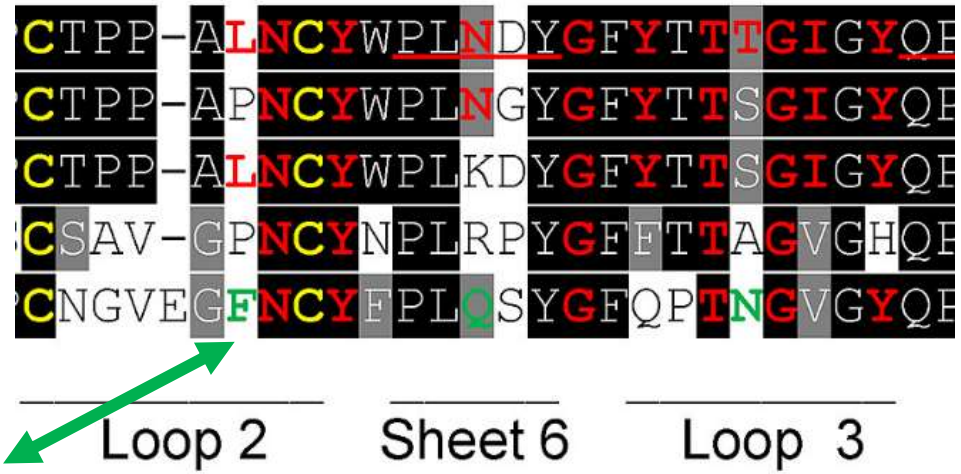
Comparazione del RBD tra coronavirus

Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140

Comparazione del RBD tra coronavirus

ACE2





GREEN Major altered amino acids in 2019-nCoV

RBD
2019-nCov

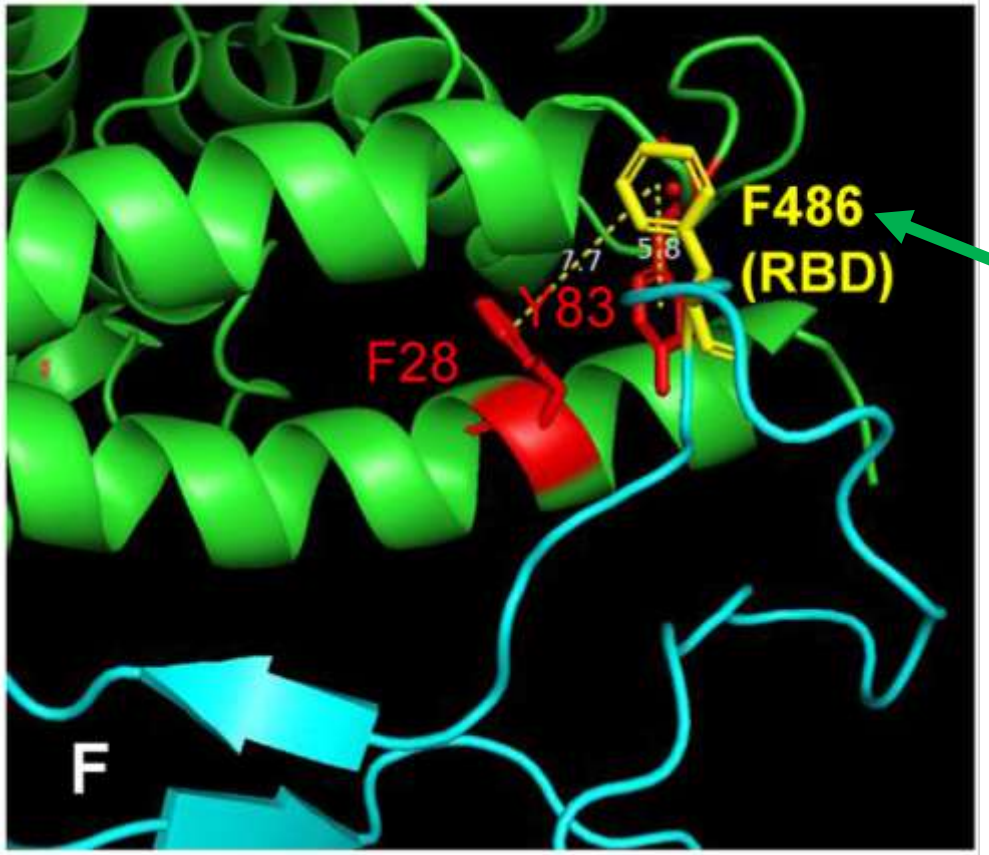
2019-nCov RBD/ACE2

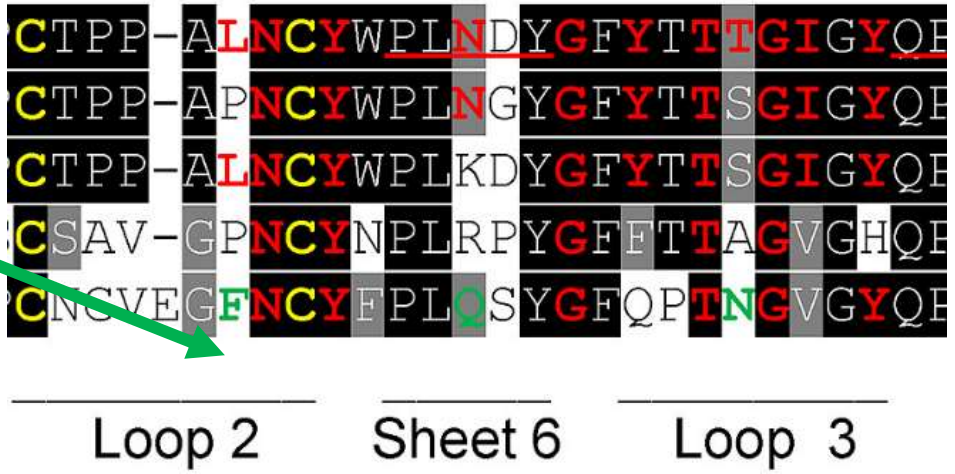
phenylalanine F486 in the flexible loop can penetrate deep into a hydrophobic pocket in ACE2

Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140

Comparazione del RBD tra coronavirus

ACE2





GREEN Major altered amino acids in 2019-nCoV

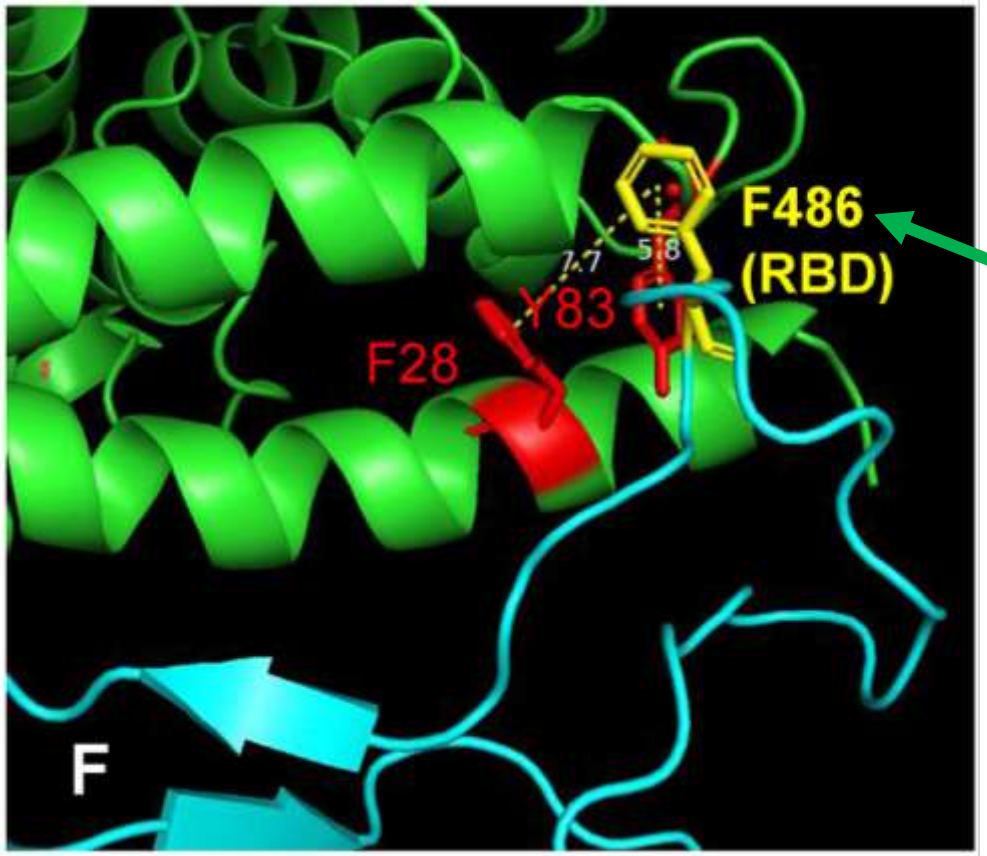
RBD
2019-nCov

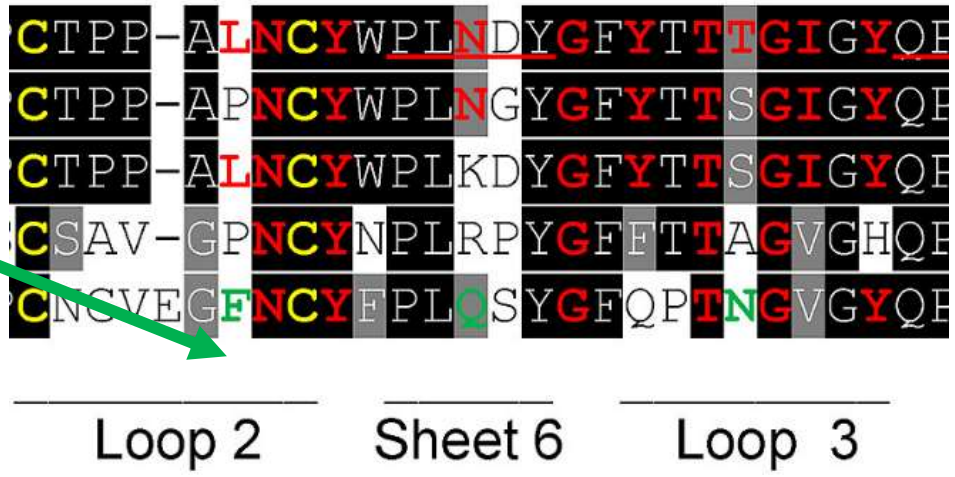
phenylalanine F486 in the flexible loop can penetrate deep into a hydrophobic pocket in ACE2 formed by **F28, L79, Y83, and L97**

The presence of two aromatic amino acids in the pocket may provide additional binding force via π-stacking interactions.

Comparazione del RBD tra coronavirus Y. Chen et al

ACE2





GREEN Major altered amino acids in 2019-nCoV

RBD
2019-nCov

Structural features of the spike glycoprotein RBD of 2019-nCoV confer potentially higher affinity binding for its receptor than found with SARS-CoV

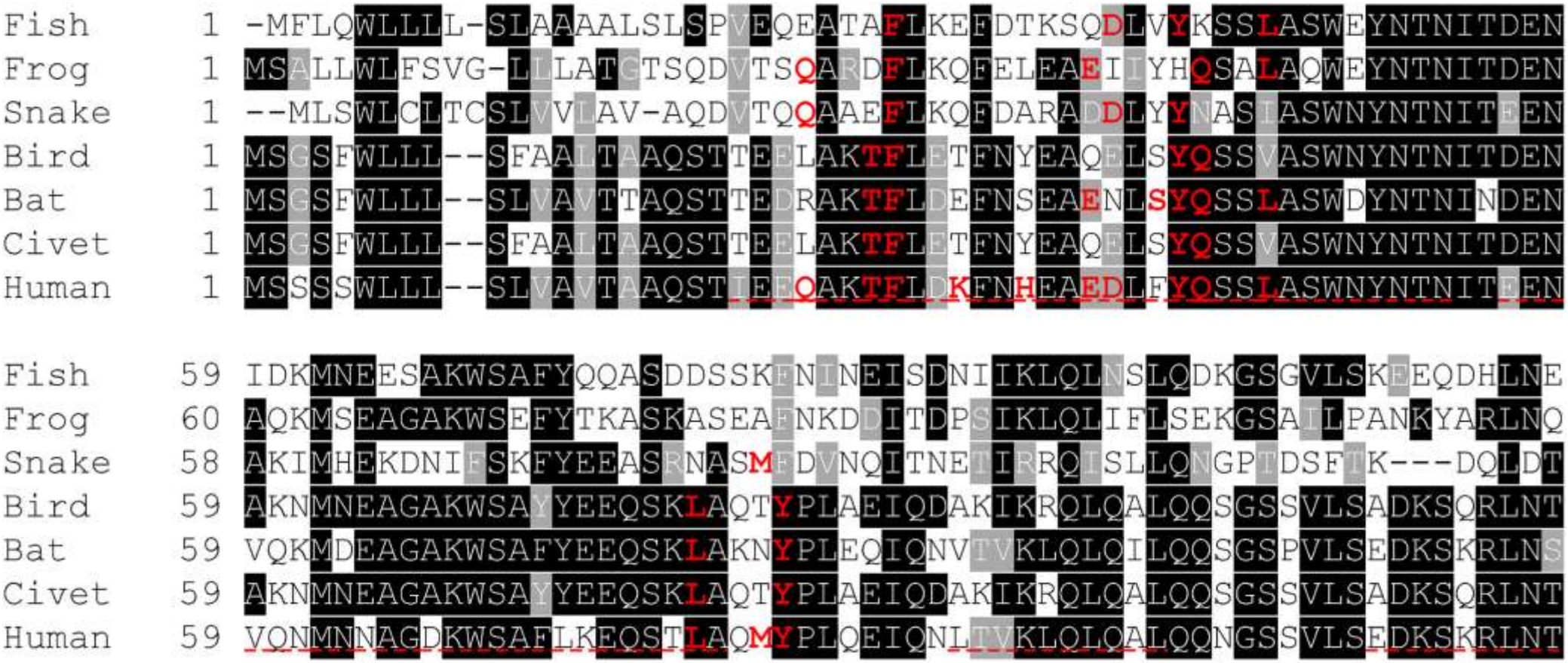
ACE2 nel Regno Animale

Amino acid sequence alignment of ACE2 molecules from 7 animals

amino acid residues in human ACE2 that directly interact with RBD of SARS-CoV **highlighted in red**.

dash-lines for α-helix

Amino acid sequence alignment of ACE2 molecules from 7 animals



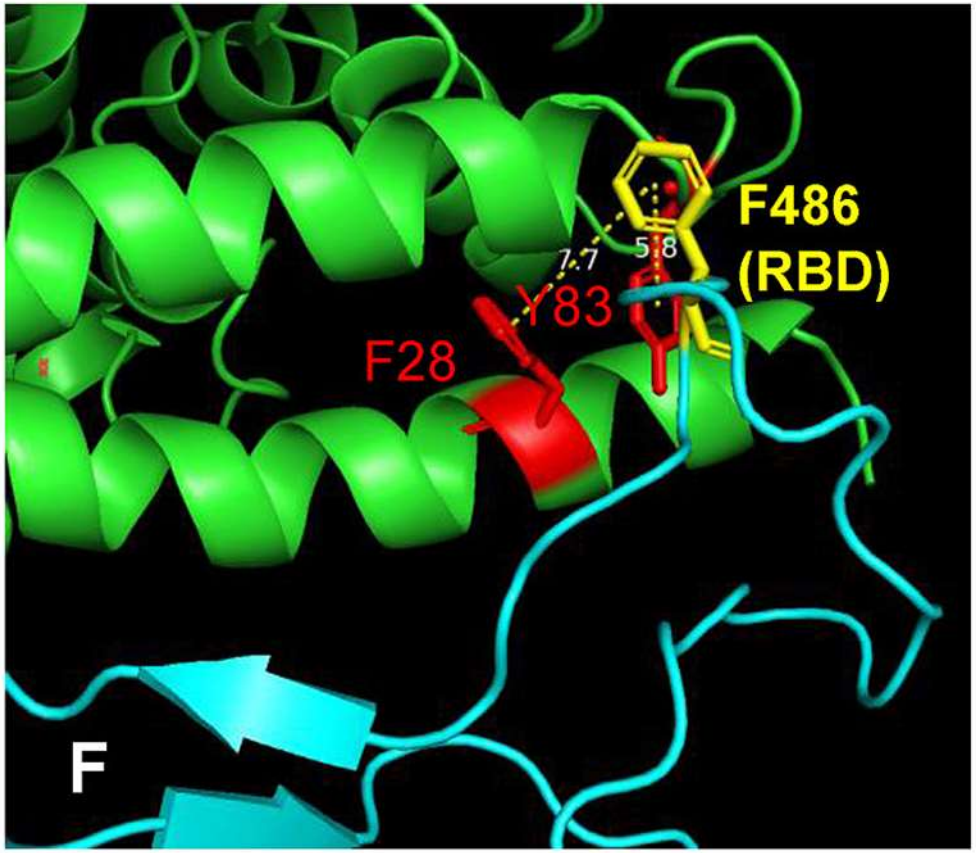
amino acid residues in human ACE2 that directly interact with RBD of SARS-CoV **highlighted in red**.

Correspondent amino acids for other animals are also highlighted in red if they are shared by human ACE2

dash-lines for α-helix

Out of ACE2 20 amino acid residues involved in the direct interaction, **4 of them are shared by all seven species of animals analyzed**

F28 that interacts with F486 of spike glycoprotein from 2019-nCoV is **shared by all seven species**



Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140

IPOTESI

ACE2 molecules from any of these animals has the potential to interact with RBD of 2019-nCoV with high affinity.

SARS-CoV-like coronaviruses have been found in many bats that are considered as natural reservoirs for the viruses.

It would not be a surprise if any of these wild animals is found to be a primary or secondary host of 2019-nCoV.

Y. Chen et al. / Biochemical and Biophysical Research Communications 525 (2020) 135e140

Interazione tra Spike e recettore ACE2

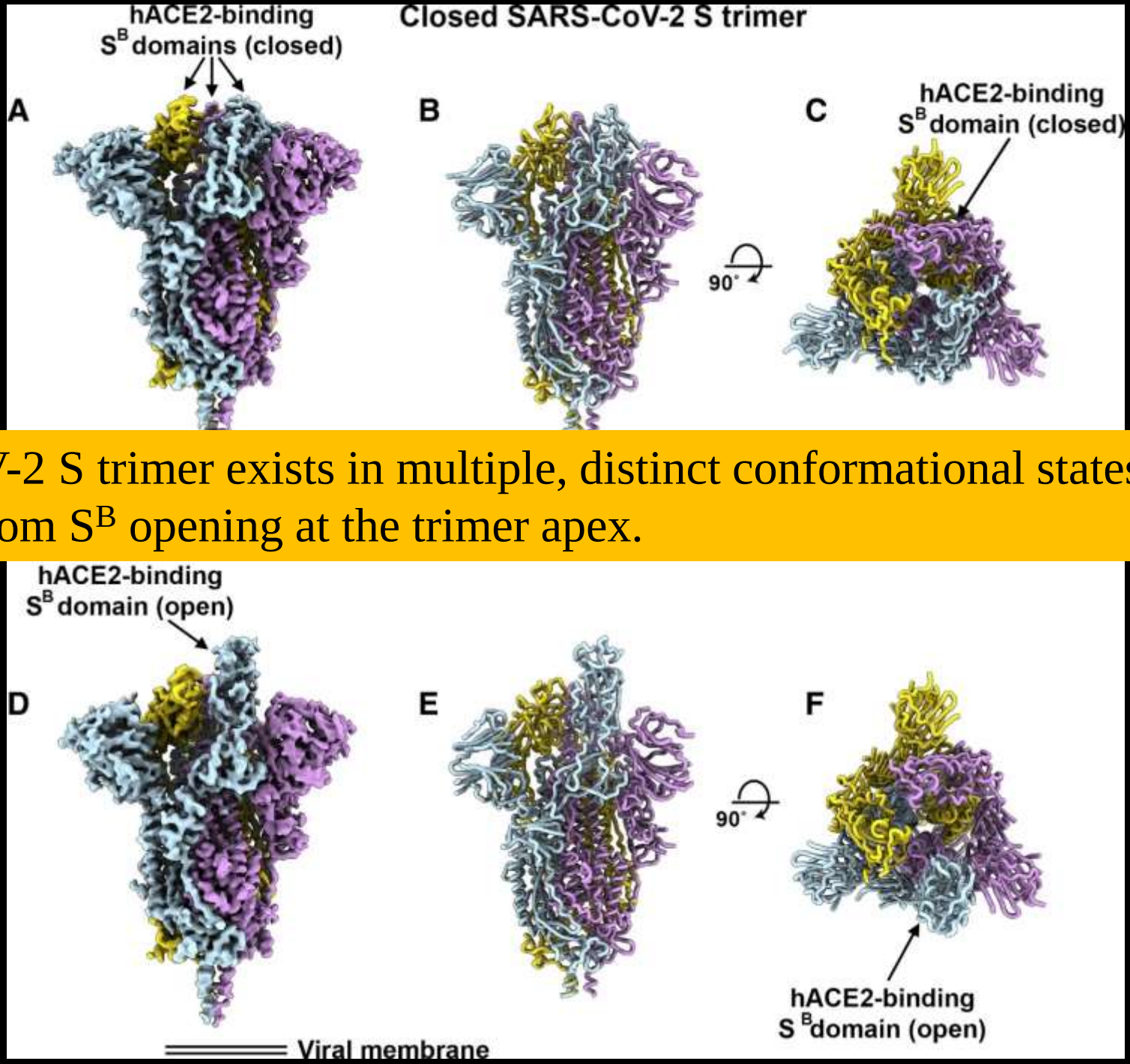
Strutture ed affinità 2

Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein

Alexandra C. Walls, Young-Jun Park, M. Alejandra Tortorici, Abigail Wall, Andrew T. McGuire, David Veesler

Cell

DOI: 10.1016/j.cell.2020.02.058



SARS-CoV-2 S trimer exists in multiple, distinct conformational states resulting from S^B opening at the trimer apex.

Cell DOI: (10.1016/j.cell.2020.02.058)

I cambi strutturali dello spike sono essenziali per la fusione delle membrane

Structural changes of spike S are necessary for receptor engagement of virus and lead to initiation of membrane fusion

In SARS-CoV-2 coronavirus, S glycoprotein trimers appear to exist in partially opened states, while they remain largely closed in human coronaviruses associated with common colds.

L’affinità di legame tra spike ed ACE2 e patogenicità del virus

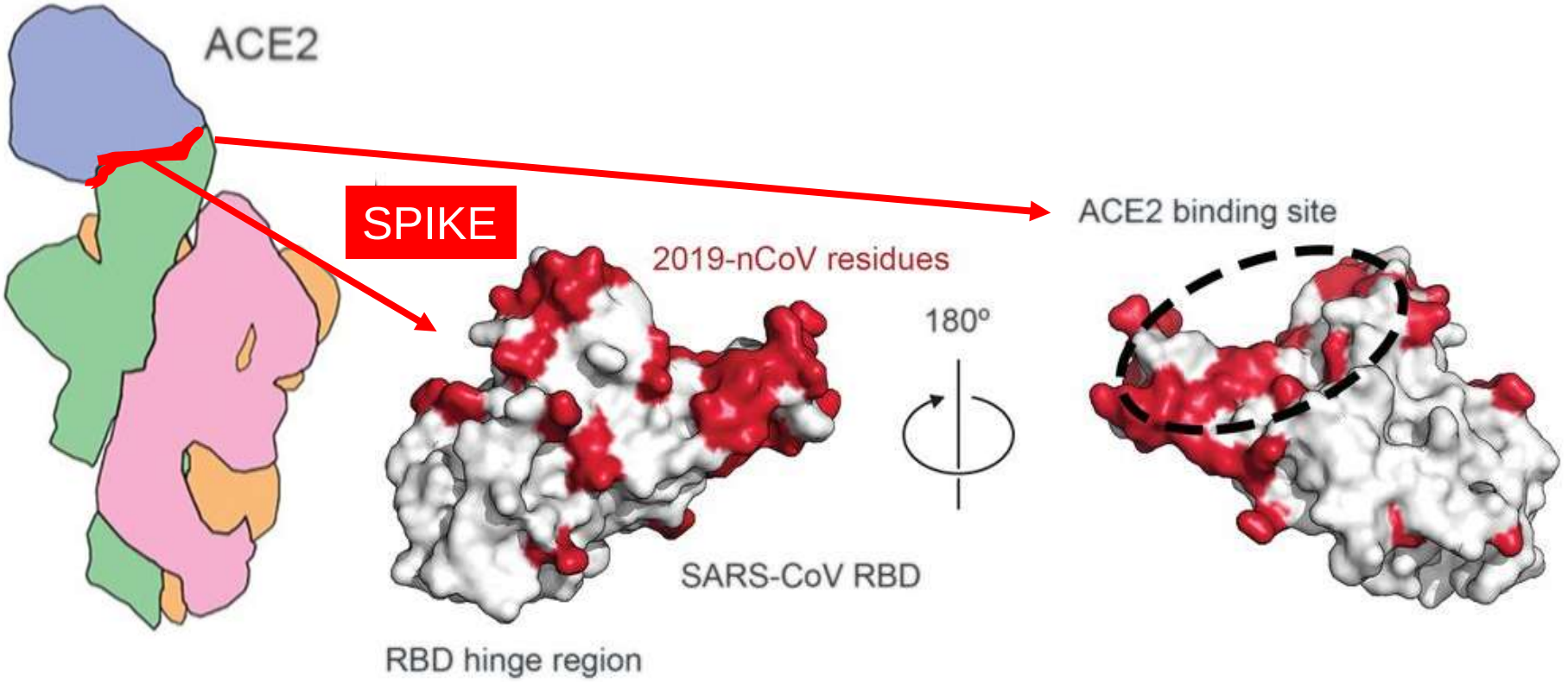
The binding affinity of SARS-CoV for hACE2 could be correlated with with the rate of transmissibility, viral replication in distinct species, and disease severity.

The most pathogenic coronaviruses will exhibit S glycoprotein trimers spontaneously sampling closed and open conformations, as is the case for SARS-CoV-2, SARS-CoV and MERS-CoV.

Lo Spike ed il recettore cellulare interagiscono con grande affinità

- The SARS-CoV2 S B domain engages human ACE2 (hACE2) with tight binding which could partially explain the efficient transmission of SARS-CoV-2 in humans.

Daniel Wrapp et al. Science 2020;367:1260-1263



SARS-CoV Receptor Binding Domain shown as a white molecular surface (PDB ID: 2AJF), with residues that **vary** in the 2019-nCoV RBD **colored red**. The ACE2-binding site = black dashed line.

Daniel Wrapp et al. Science 2020;367:1260-1263

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Misura dell'affinità con biosensori

Surface plasmon resonance

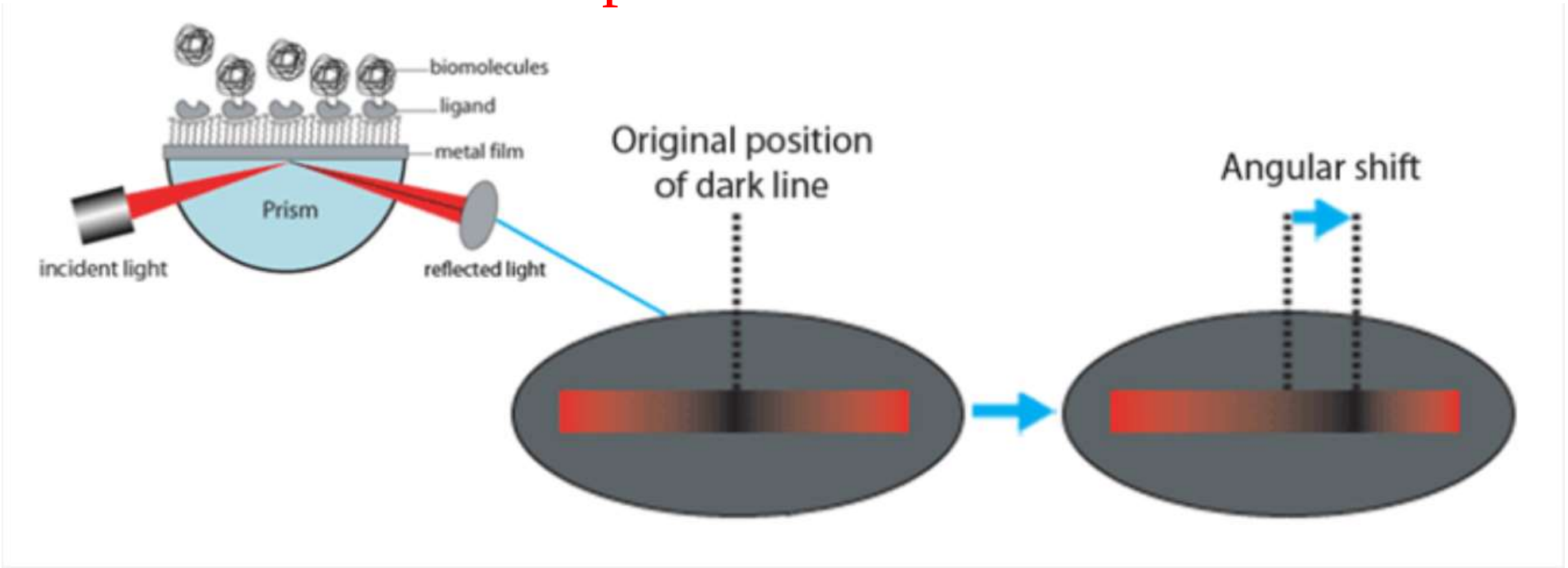
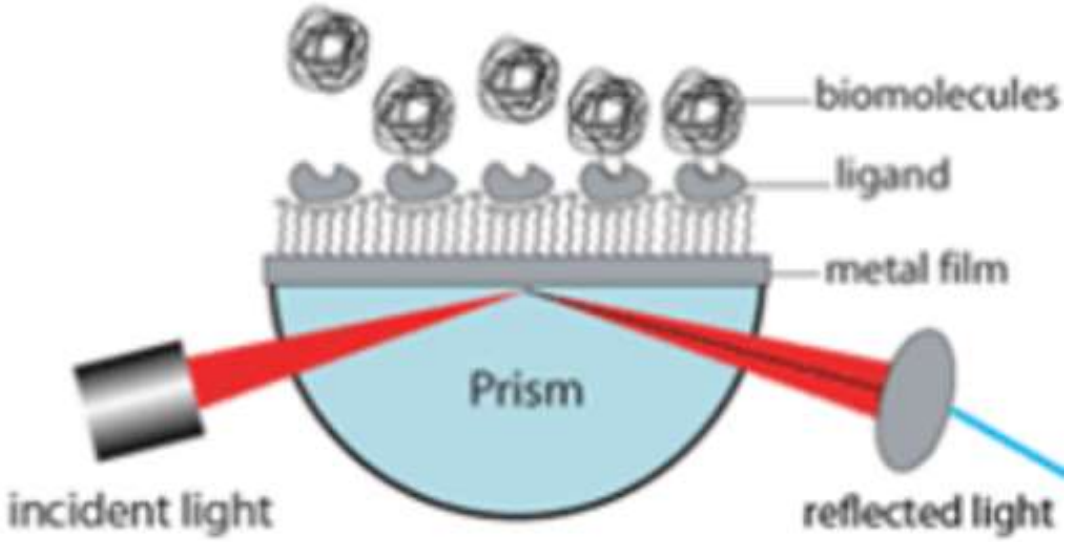


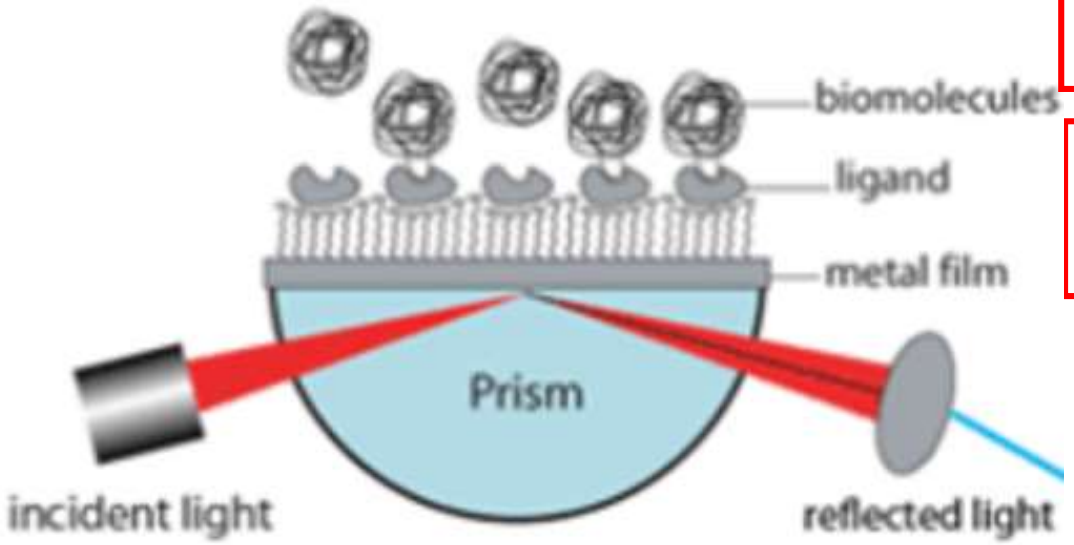
FIG. 2 The excitation of surface plasmons results in a dark line in the reflected beam, and the angular position of the dark line shifts as a molecule binding event takes place.

Misura dell'affinità con biosensori



His-tagged 2019-nCoV S RBD was immobilized to a sensorchip

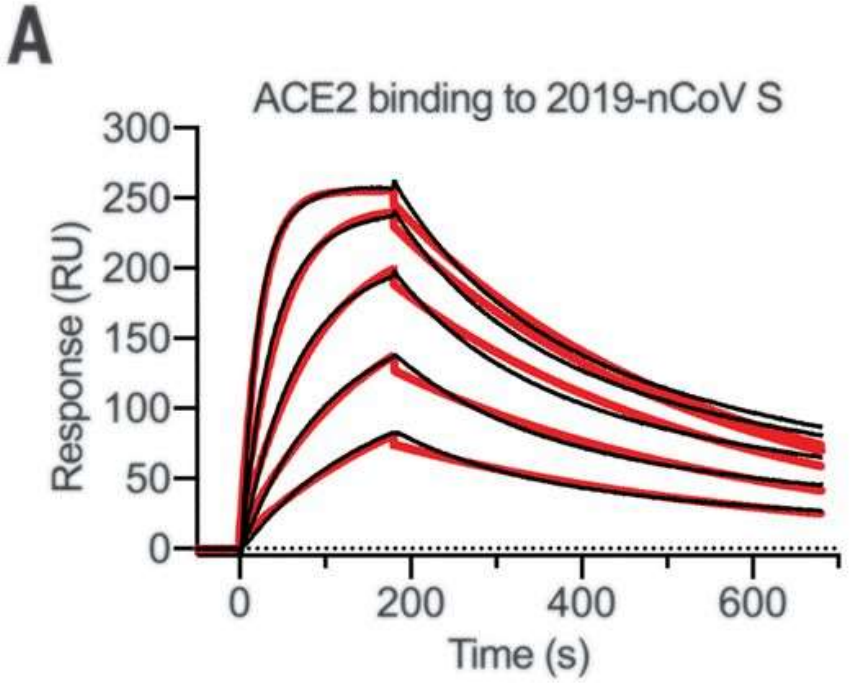
Misura dell'affinità con biosensori



Serial dilutions (250 to 15.6 nM) of purified ACE2 were injected

His-tagged 2019-nCoV S RBD was immobilized to a sensorchip

2019-nCoV S binds human ACE2 with high affinity.



K_D = 14.7 nM equilibrium dissociation constant

Surface plasmon resonance sensorgram showing the binding kinetics for human ACE2 and immobilized 2019-nCoV S.

Data are shown as black lines (best fit of the data in red)

Daniel Wrapp et al. Science 2020;367:1260-1263

Science AAAS

Misura dell'affinità con biosensori Comparazione tra RBD

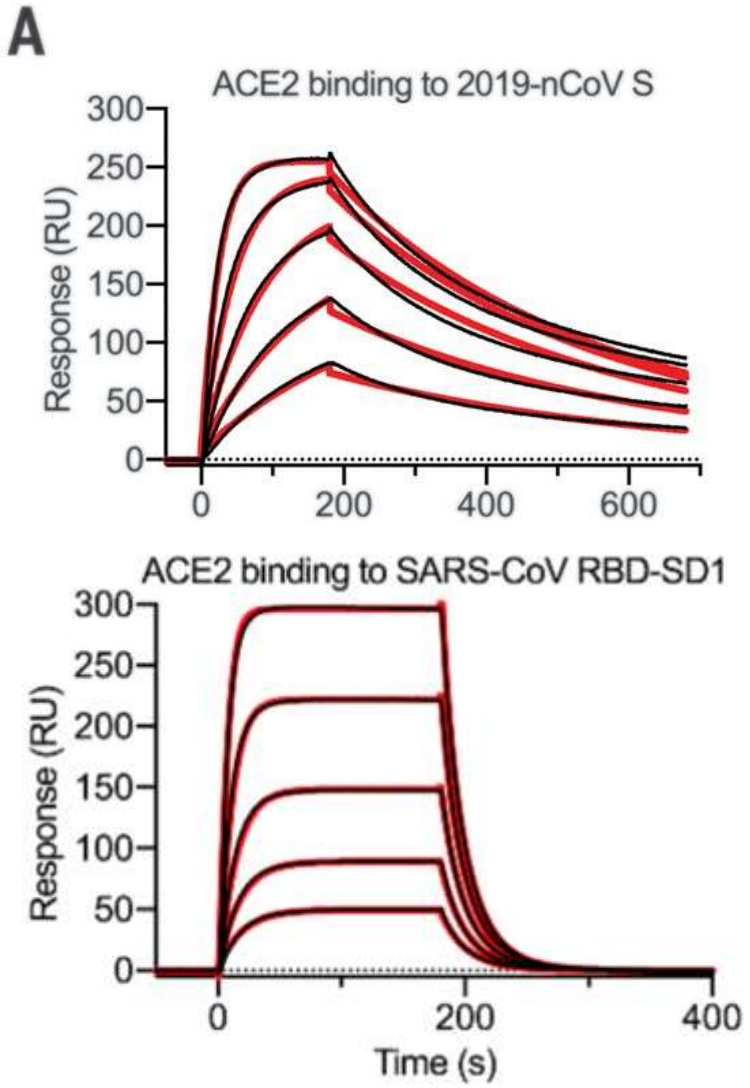
1 His-tagged 2019-nCoV S RBD was immobilized to a sensorchip

Serial dilutions (250 to 15.6 nM) of purified ACE2 were injected

2 His-tagged SARS-CoV RBD was immobilized to a sensorchip

Serial dilutions of purified and untagged ACE2 were injected ranging in concentration from 500 to 31.3 nM.

2019-nCoV S binds human ACE2 with high affinity.



K_D = 14.7 nM
k_a = 1.88 x10^5 $M^{-1}s^{-1}$
k_d = 2.76 x 10^{-3} s^{-1}

Equilibrium dissociation constant

Rate constant

K_D = 325.8 nM
k_a = 3.62 x10^5 $M^{-1}s^{-1}$
k_d = 0.112 s^{-1}

Equilibrium dissociation constant

Rate constant

Comparazione affinità
Spike-ACE2
tra coronavirus

Daniel Wrapp et al. Science 2020;367:1260-1263

Science
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CONCLUSIONI

ACE2 bound to the 2019-nCoV S ectodomain with ~15 nM affinity, which is ~10- to 20-fold higher than ACE2 binding to SARS-CoV

The 2019-nCoV S protein binds angiotensin-converting enzyme 2 (ACE2) with higher affinity than does severe acute respiratory syndrome (SARS)-CoV S.

Ipotesi

The high affinity of 2019-nCoV S for human ACE2 may contribute to the apparent ease with which 2019-nCoV can spread from human to human; however, additional studies are needed

Misura dell'affinità

Risultati e interpretazione parzialmente diversi in un'altra pubblicazione

Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein

Alexandra C. Walls, Young-Jun Park, M. Alejandra Tortorici, Abigail Wall, Andrew T. McGuire, David Veesler

Cell

DOI: 10.1016/j.cell.2020.02.058

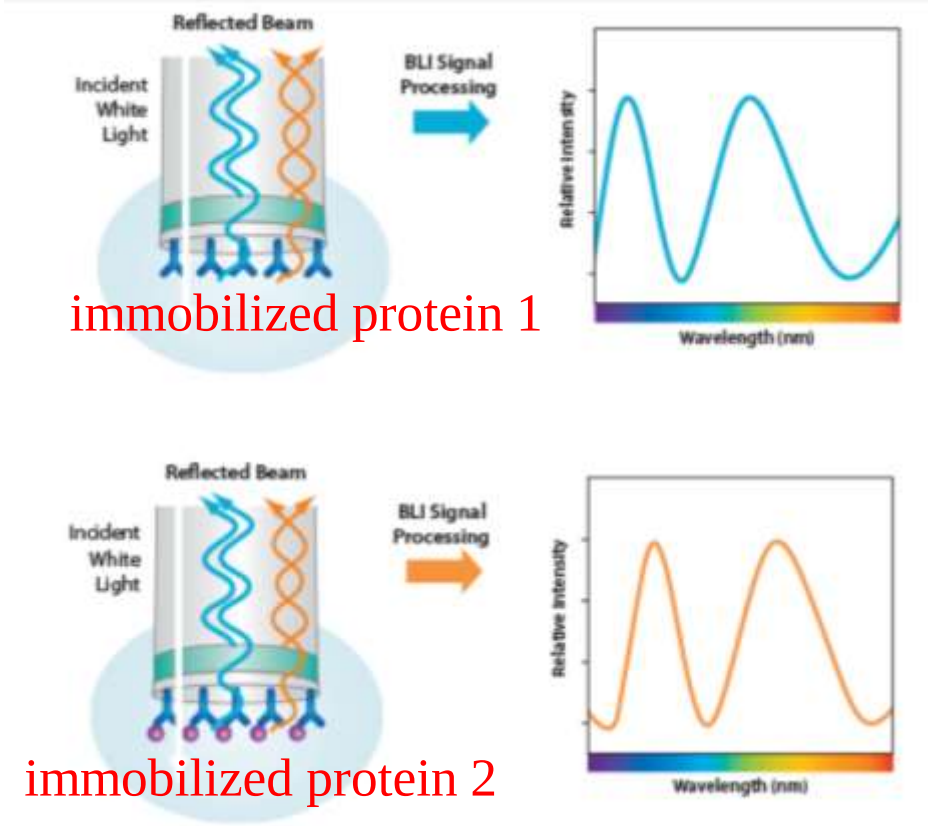
PREMESSA

The binding affinity of SARS-CoV for hACE2 correlates with the overall rate of viral replication in distinct species as well as with transmissibility and disease severity

PROPOSTA SPERIMENTALE
To understand the contribution of receptor interaction to the infectivity of SARS-CoV-2, we characterized engagement of hACE2 by SARS-CoV-2 S^B and SARS-CoV S^B side-by-side.

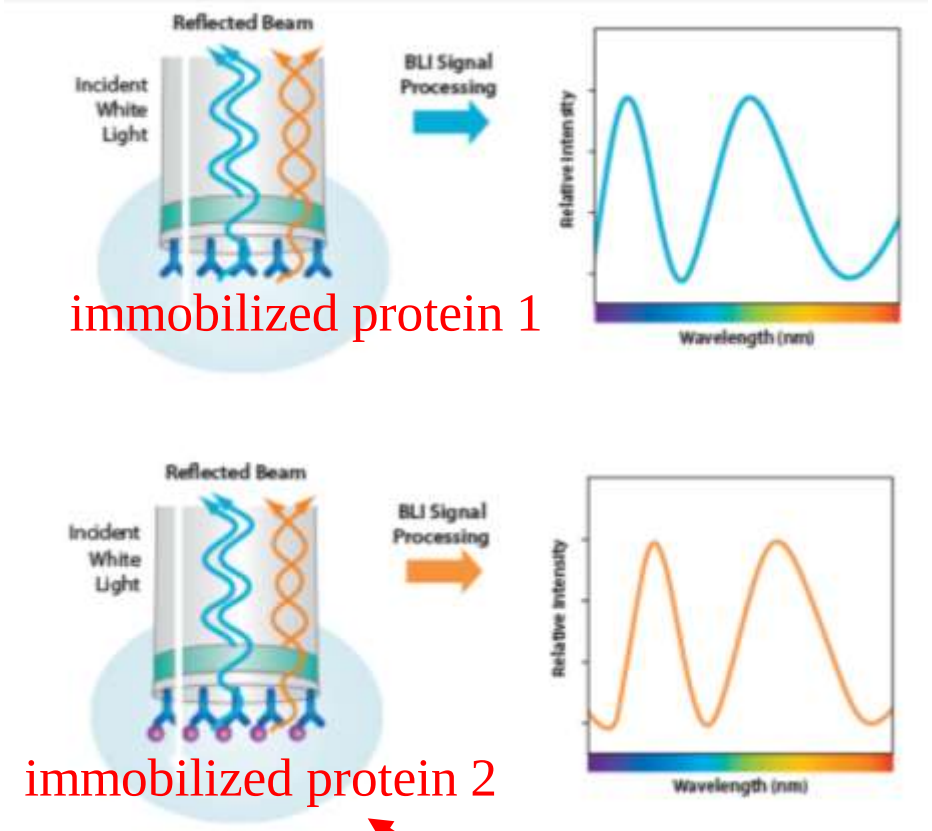
We used **biolayer interferometry to study binding kinetics and affinity** of the purified hACE2 ectodomain to SARS-CoV-2 S^B and SARS-CoV S^B immobilized at the surface of biosensors.

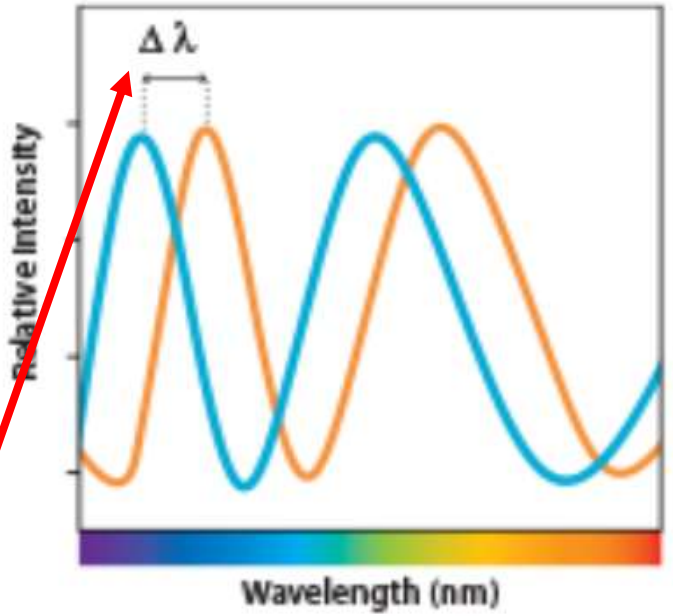
Bio-Layer Interferometry (BLI) measures biomolecular interactions



Optical analytical technique that analyzes the interference pattern of white light reflected from a layer of immobilized protein on the biosensor tip

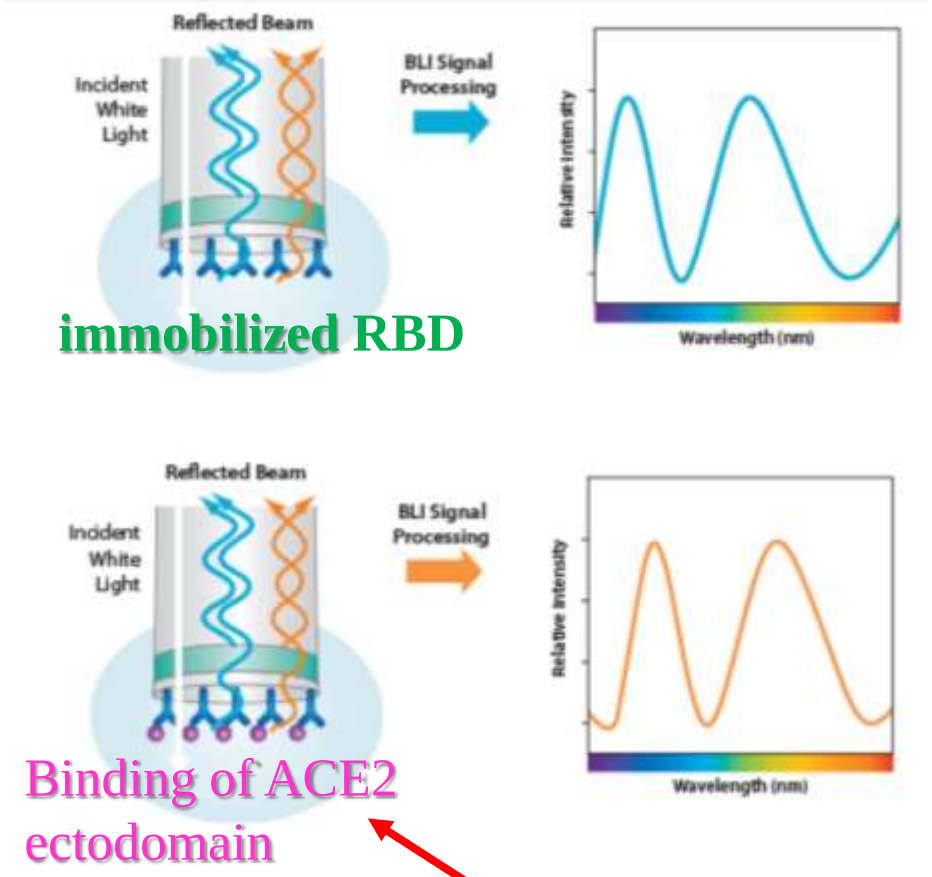
Bio-Layer Interferometry (BLI) measures biomolecular interactions

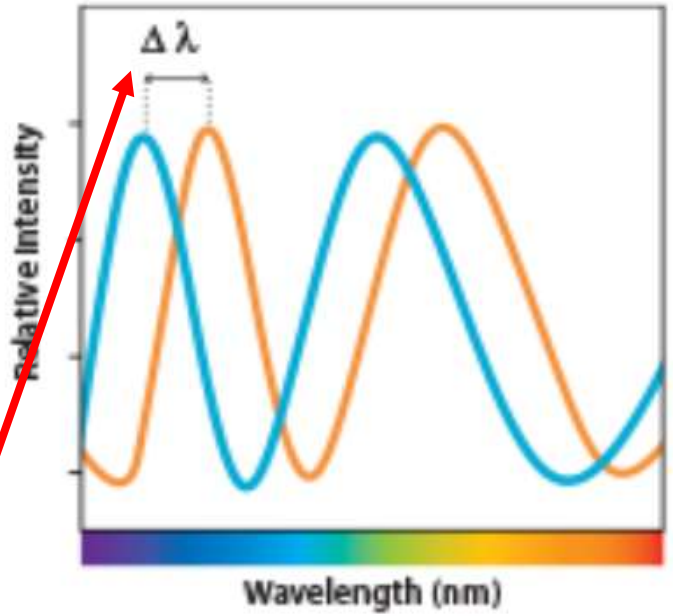




Change in the number of molecules bound to the biosensor tip causes a shift (Delta Lambda) in the interference pattern

Bio-Layer Interferometry (BLI) measures biomolecular interactions





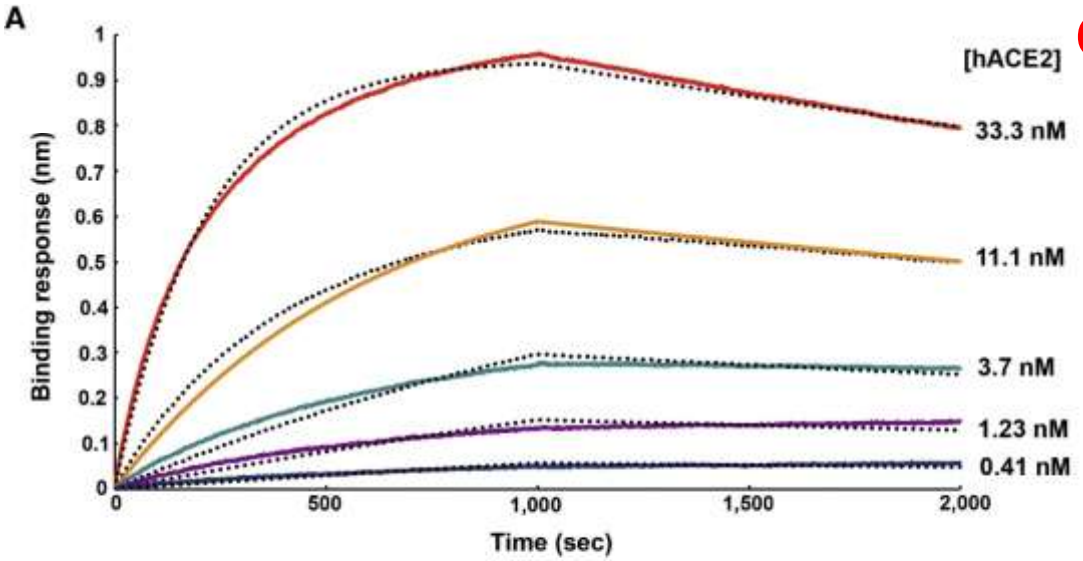
Change in the number of molecules bound to the biosensor tip causes a shift (Delta Lambda) in the interference pattern

SARS-CoV-2 S Recognizes hACE2 with Comparable Affinity to SARS-CoV S

equilibrium dissociation Constant (KD)

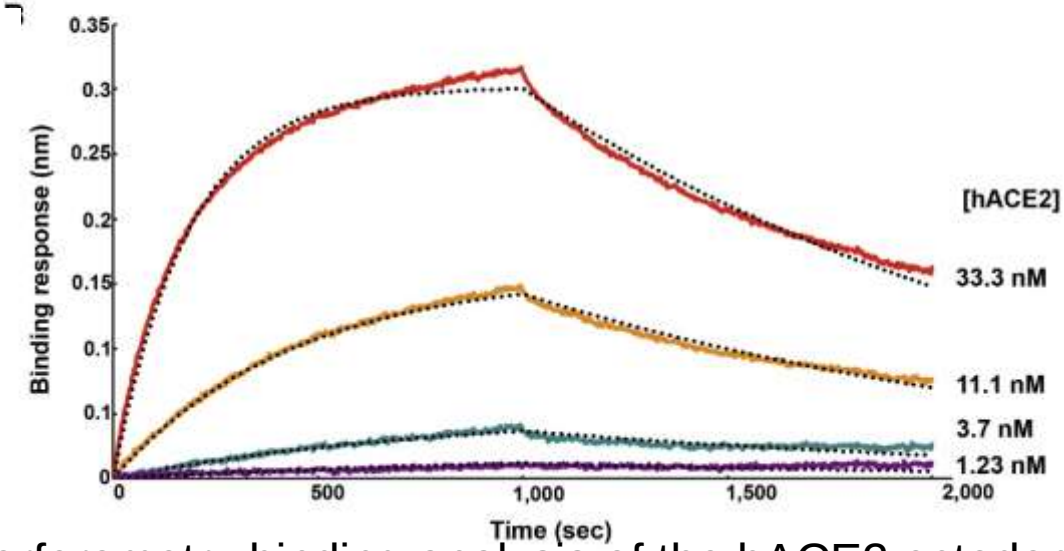
SARS-CoV-2 S^B

Comparazione affinità Spike-ACE2 tra coronavirus



1.2 nM

SARS-CoV S^B



5.0 nM

Biolayer interferometry binding analysis of the hACE2 ectodomain to immobilized SARS-CoV-2 S^B (A) or SARS-CoV S^B (B).

Cell DOI: (10.1016/j.cell.2020.02.058)

Comparazione affinità Spike-ACE2 tra coronavirus

Table 1. Kinetic Analysis of hACE2 Binding to SARS-CoV-2 S^B and SARS-CoV S^B by Biolayer Interferometry

	SARS-CoV-2 S^B	SARS-CoV S^B
K_D (nM)	1.2 ± 0.1	5.0 ± 0.1
k_{on} ($M^{-1}.s^{-1}$)	1.4×10^5 ($2.3 \pm 1.4 \times 10^5$)	1.4×10^5 ($1.7 \pm 0.7 \times 10^5$)
k_{off} (s^{-1})	1.6×10^{-4} ($1.7 \pm 0.8 \times 10^{-4}$)	7.1×10^{-4} ($8.7 \pm 5.1 \times 10^{-4}$)

K_D Costante dissociazione all'equilibrio

k_{on}
k_{off} Costanti di velocita

"We found that hACE2 bound to SARS-CoV-2 S^B and SARS-CoV S^B with respective equilibrium dissociation constants of 1.2 nM and 5.0 nM, and comparable kinetic rate constants"

Cell DOI: (10.1016/j.cell.2020.02.058)

Sommario (in pillole) 3

- La struttura, conformazione e sequenze dello spike, e dell'interfaccia con il recettore ACE2, sono state comparate tra coronavirus in relazione alla loro affinità per il recettore ACE2.

- L'affinità dello spike per ACE2 è stata determinata con diversi metodi (biosensori) e comparata con quelle dei Coronavirus precedenti

Il coronavirus comparso nel 2019 sembra essere più affine al recettore, e quindi più infettivo

Sommario (in pillole) 3

- Gli spike dei coronavirus sono stati studiati in relazione alla loro antigenicità.

Queste informazioni aiutano lo sviluppo di di vaccini,
di anticorpi per inibire il virus e per saggi immunologici

La prossima
Anticorpi contro lo SPIKE

Uomini e Topi