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

# *Materiale didattico di supporto*

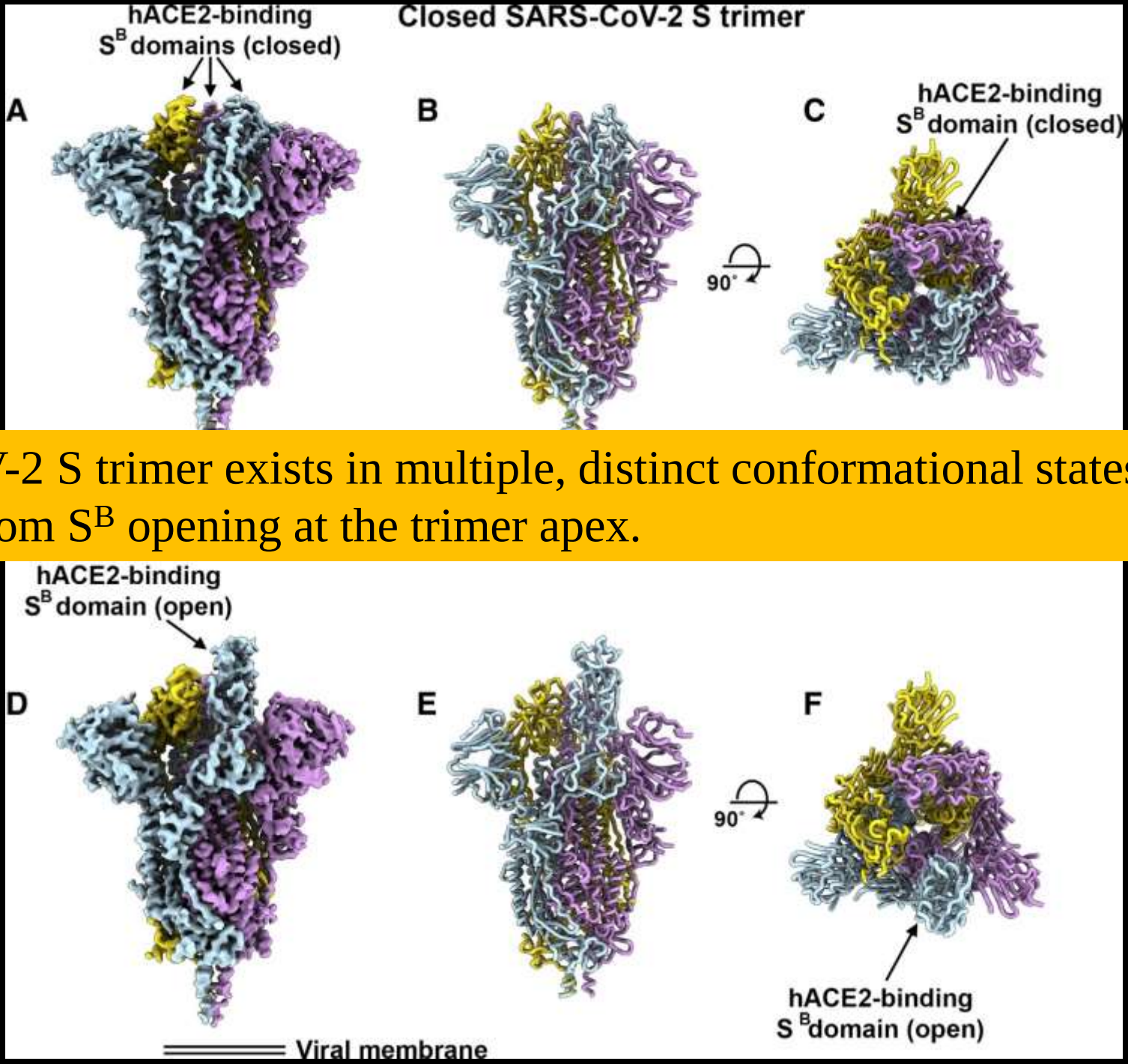
Tutto il materiale fornito a supporto delle lezioni e reperibile nel minisito dell'insegnamento o sulla piattaforma online UniFE deve essere inteso come traccia degli argomenti svolti e non sostituisce il libro di testo.

# Sommario (in pillole) 4

- La struttura, conformazione e sequenze dello spike sono state comparate tra coronavirus in relazione alla loro antigenicità.

Queste informazioni aiutano lo sviluppo di di vaccini,

di anticorpi inibitori, e di saggi immunologici

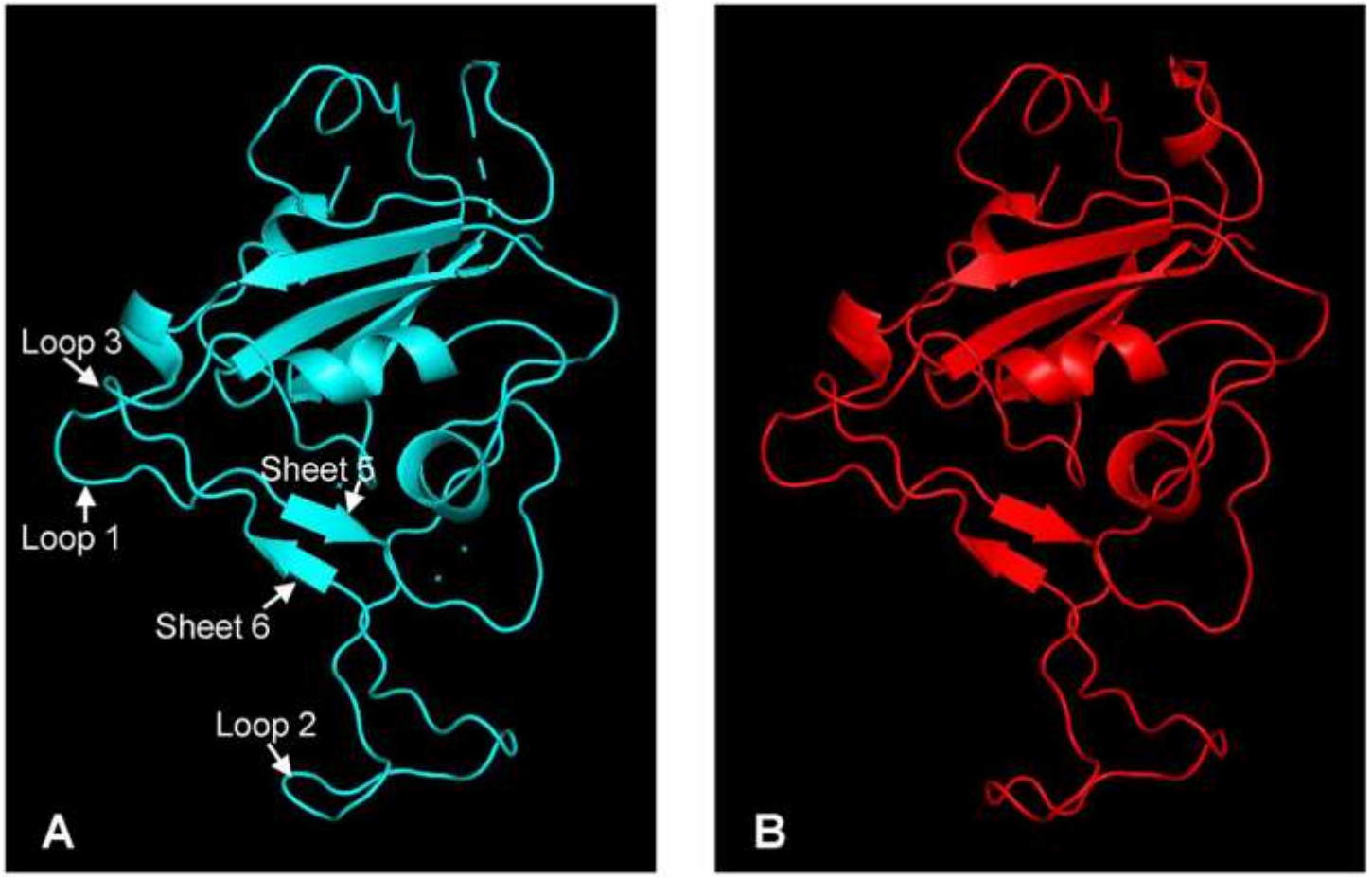



SARS-CoV-2 S trimer exists in multiple, distinct conformational states resulting from $S^B$ opening at the trimer apex.

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

Due filmini interessanti al seguente sito soprattutto Movie S2

https://science.sciencemag.org/content/suppl/2020/02/18/science.abb2507.DC1




# Comparazione del RBD tra coronavirus

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

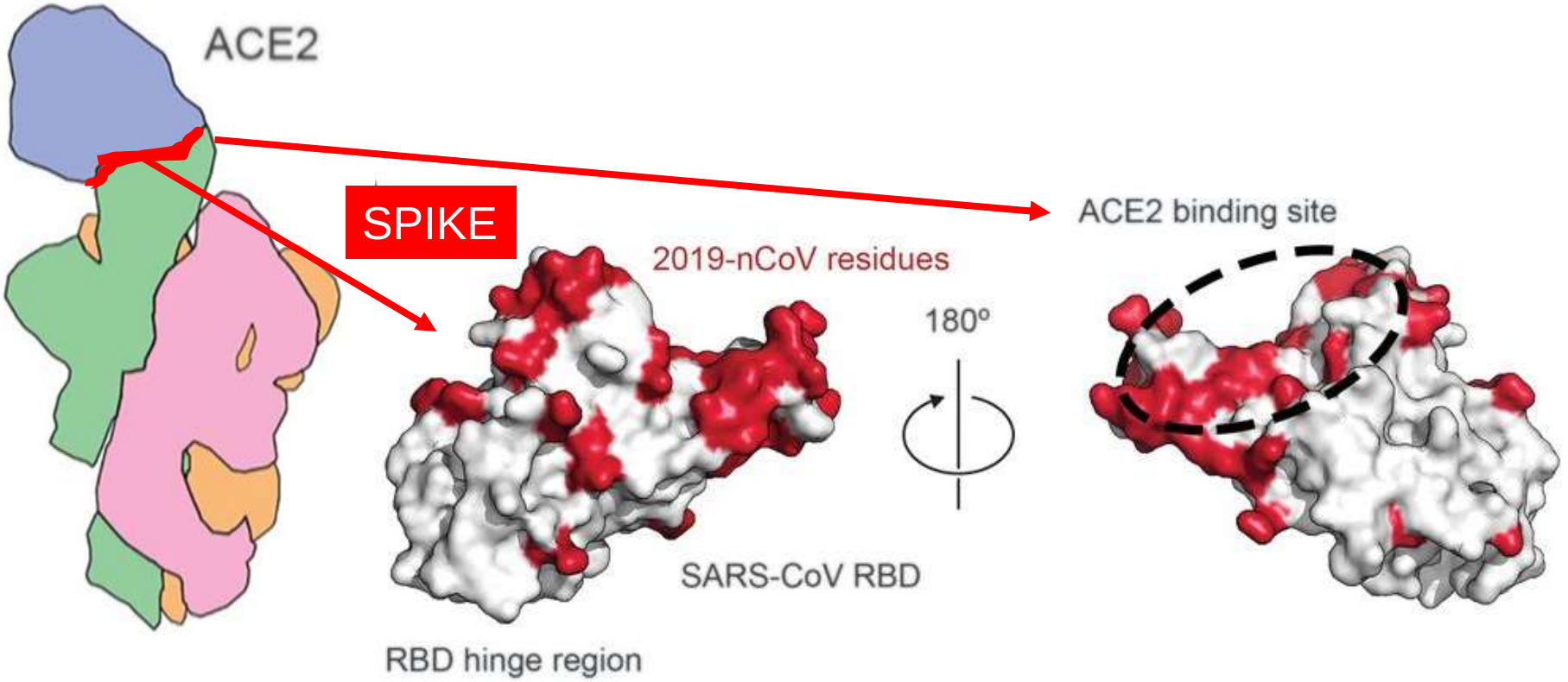
# Comparazione del RBD tra coronavirus


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 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**



Science
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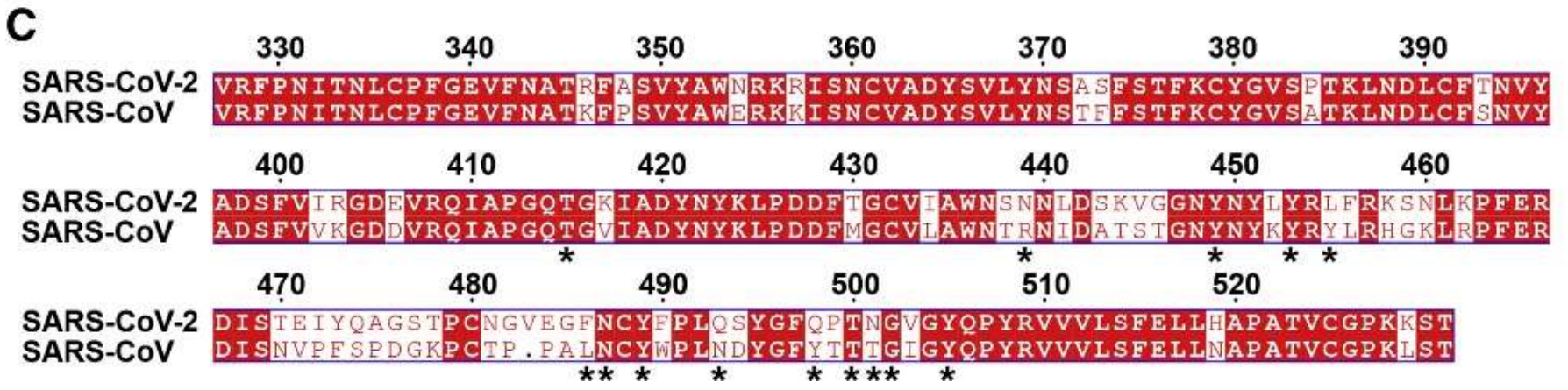
# SARS-CoV-2 S / SARS-CoV S AA sequence comparison




WHITE conserved AA

RED differing AA

key AA (n=14) for binding of SARS-CoV $S^B$ to hACE2 are labeled with a star

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

Variazioni di sequenza tra  Spike (S)
del  CoV-19 (SARS-CoV-2 S)
e
Virus SARS precedente (SARS-CoV S)

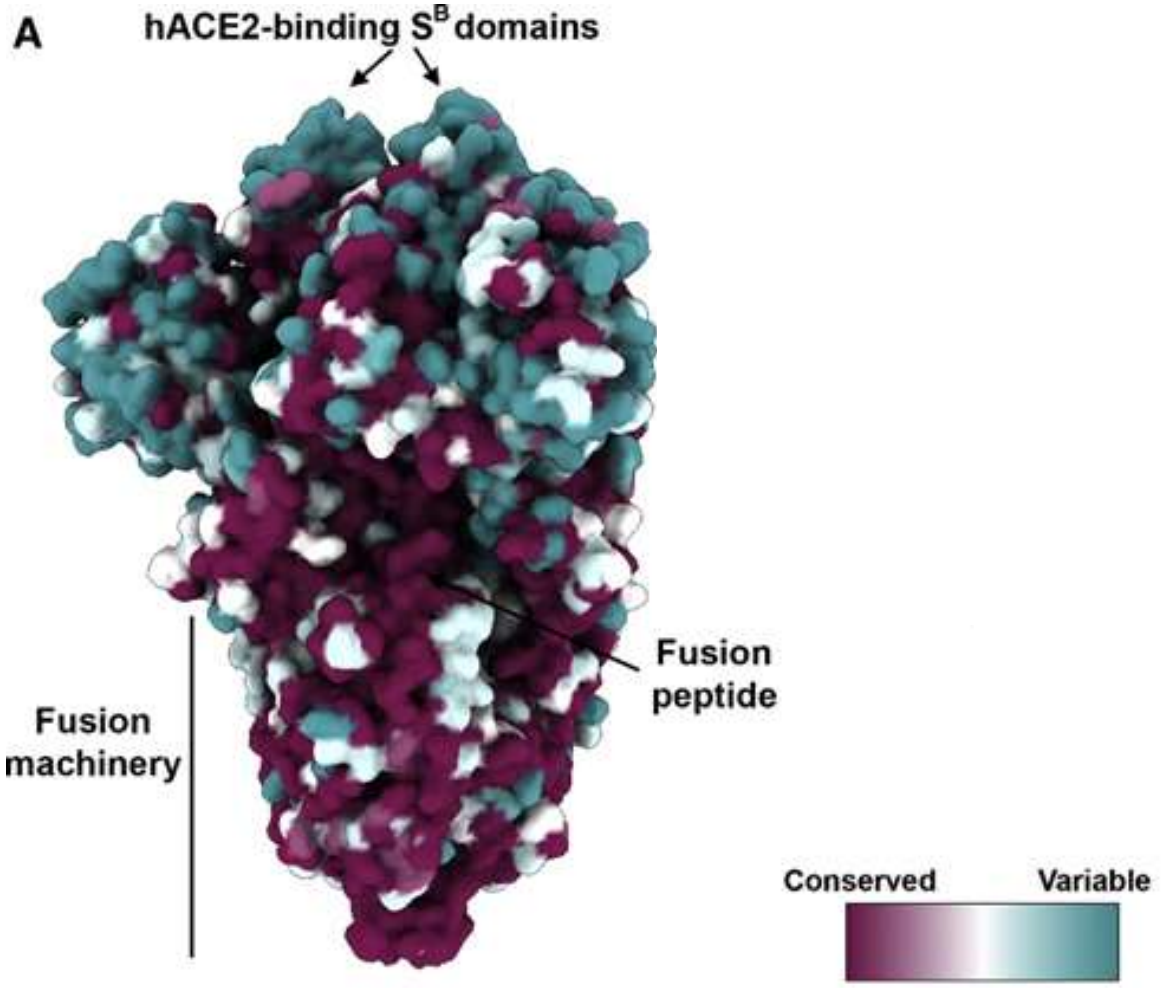
# Valutazione complessiva della superficie dello Spike

# SARS-CoV-2 S / SARS-CoV S AA sequence comparison

S2 fusion machinery is more conserved than the S1 subunit

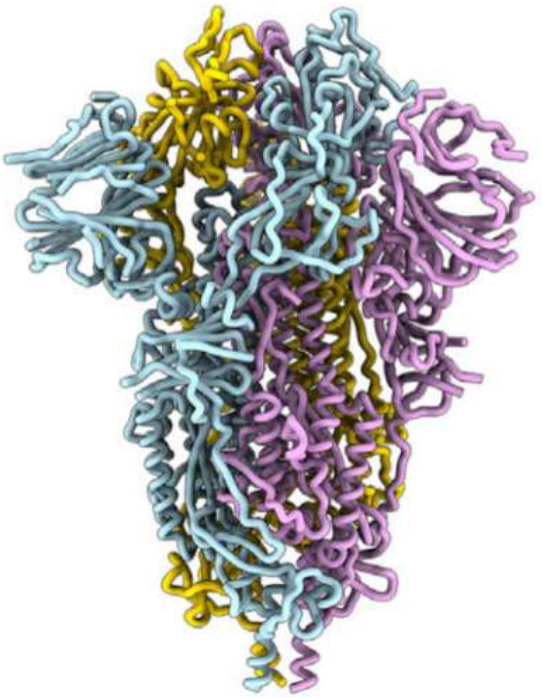
S1

S2

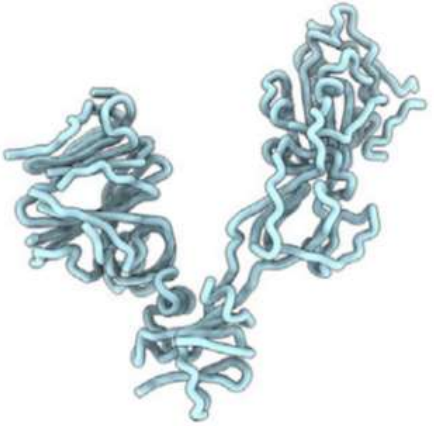


SARS-CoV-2 S

A

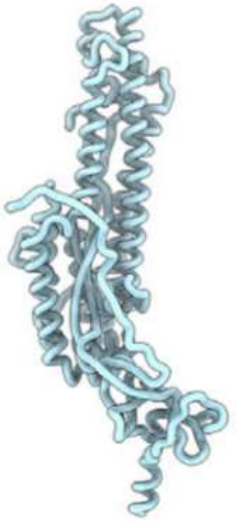


B



$S_1$ subunit

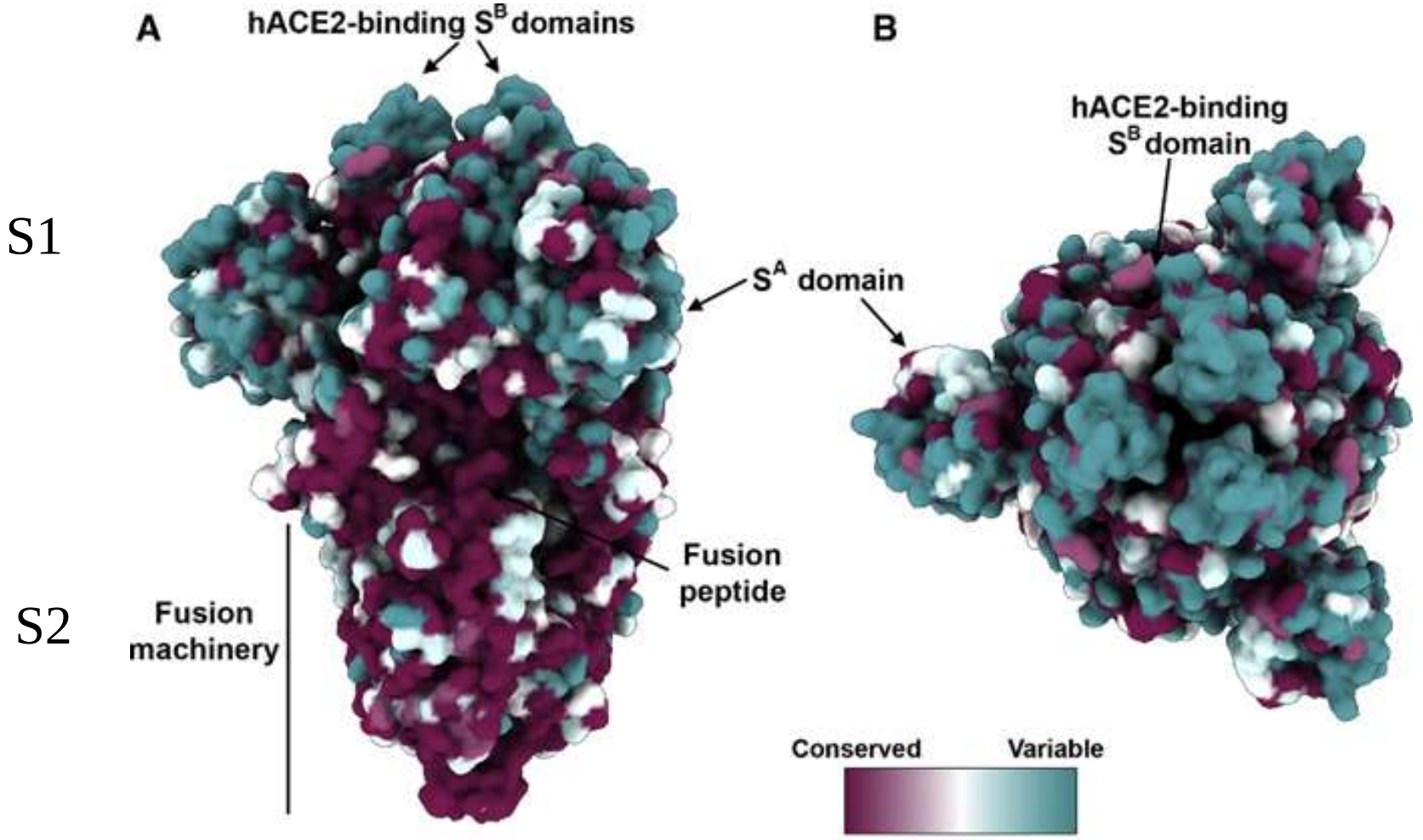
C



$S_2$ subunit

# SARS-CoV-2 S / SARS-CoV S  A A sequence comparison

the highest divergence found within SA and SB domains




S1

S2

# Conservazione differenziata in diversi domini dello Spike

The SARS-CoV-2 and SARS-CoV S2 subunits share **88%** sequence identity.
They are structurally conserved and can be superimposed with 1.2 A˚ root-mean-square deviation (rmsd) over 417 aligned Calpha positions).

The SARS-CoV-2 and SARS-CoV S Binding domains share **75%** amino acid sequence identity, and **50%** identity within their receptor-binding motifs (RBMs).

20 out of 22 SARS-CoV-2 S N-linked glycosylation sites are conserved in SARS-CoV S (9 out of 13 glycans in the S1 subunit and **all 9** glycans in the S2 subunit )

# Antigenicità

Accessibility to Abs of the  S2 subunit  (fusion machinery-88% glycan 9/9 glycan conserved-) will be comparable among these viruses.

Most SARS-CoV neutralizing Abs isolated to date target the SB domain and that several of them recognize the receptor-binding motifs (RBMs)

The SARS-CoV-2 and SARS-CoV SB domains share only 75% amino acid sequence identity, future work will be necessary to evaluate whether any of these Abs neutralize the newly emerged coronavirus

# Anticorpi contro lo SPIKE

# Uomini e Topi

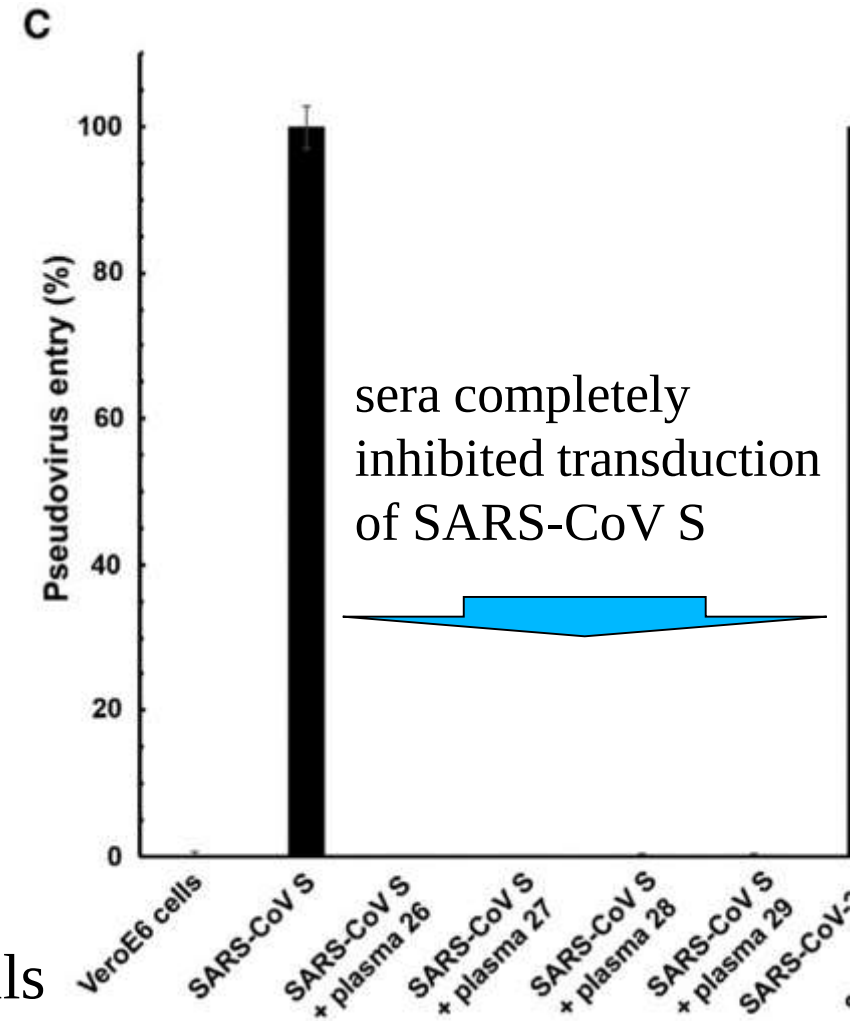
# Ipotesi- cross-reattività

Based on these observations, we hypothesized that exposure to one of the two viruses could elicit cross-reactive and potentially neutralizing Abs against the other virus.

# Esperimento (anticorpi da modelli murini)

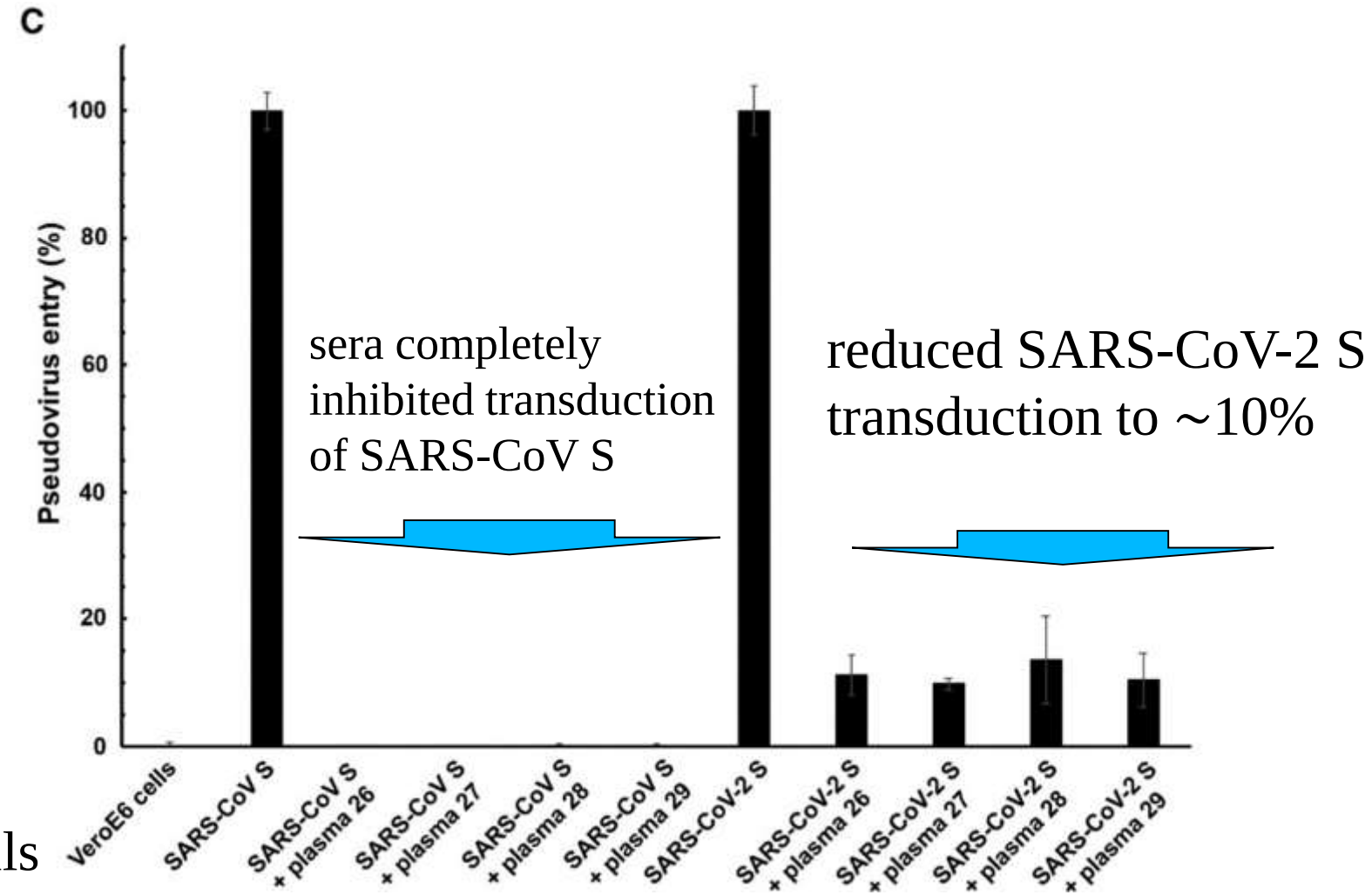
We therefore investigated the ability of plasma from four mice immunized with SARS-CoV S ectodomain trimer to inhibit

i) SARS-CoV S-mediated and ii) SARS-CoV-2 S- mediated entry into target cells.




control cells

Walls et al., Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein, Cell (2020)




control cells

Vero E6 monkey kidney epithelial **cells**

Walls et al., Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein, Cell (2020)

L’immunizzazione contro lo spike della SARS potrebbe rendere immuni anche alla SARS CoV2? Forse si

The “heterotypic” response blocking SARS-CoV-2 S-mediated entry into host cells suggests that immunity against one virus can potentially provide protection against related viruses.

CONSIDERAZIONI per il Futuro

These findings also indicate that it might be difficult to distinguish exposure to SARS-CoV-2 from other SARSr-CoVs in serological studies using S ectodomain trimers and that specific assays will need to be designed.

Our results provide a structural framework to identify conserved and accessible epitopes across S glycoproteins that will support ongoing vaccine design efforts.

# Anticorpi contro lo SPIKE

# Umani
# (Pazienti SARS/Bcell librerie)

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

# Anticorpi dei pazienti contro SARS- CoV

SARS-CoV infection leads to generation of potent neutralizing Abs (nAbs).

Antibodies that neutralize the virus in vitro were detected in SARS-CoV-infected patients.

Several groups have developed human monoclonal Abs (hmAbs) to the SARS-CoV spike (S) glycoprotein that neutralize the virus and have potential for therapy and prophylaxis of SARS

# La storia «molecolare» dell'anticorpo s320

Epstein-Barr virus transformation of human B cells

We used this method to analyze the memory repertoire of a patient who recovered from severe acute respiratory syndrome coronavirus (SARS-CoV) infection and to isolate **monoclonal antibodies specific for different viral proteins**

One such antibody confers protection in vivo in a mouse model of SARS-CoV infection.

*Nat Med. 2004 Aug;10(8):871-5. An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus. Traggiai E et al*

# La storia “molecolare” dell’anticorpo m396

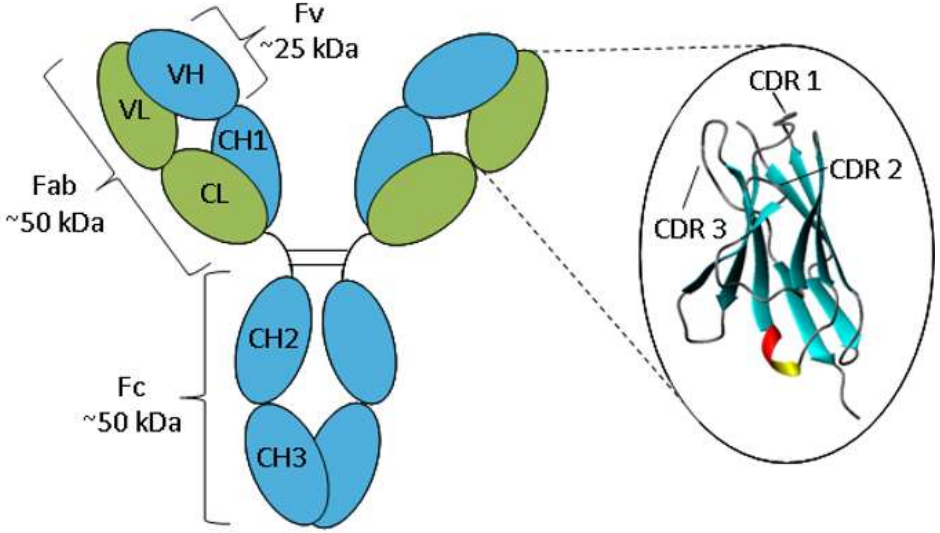
Residues critical for the binding of SARS-CoV to its receptor ACE2 (RBD residues 317–518) were cloned into a baculovirus vector, expressed in insect cells, and purified.

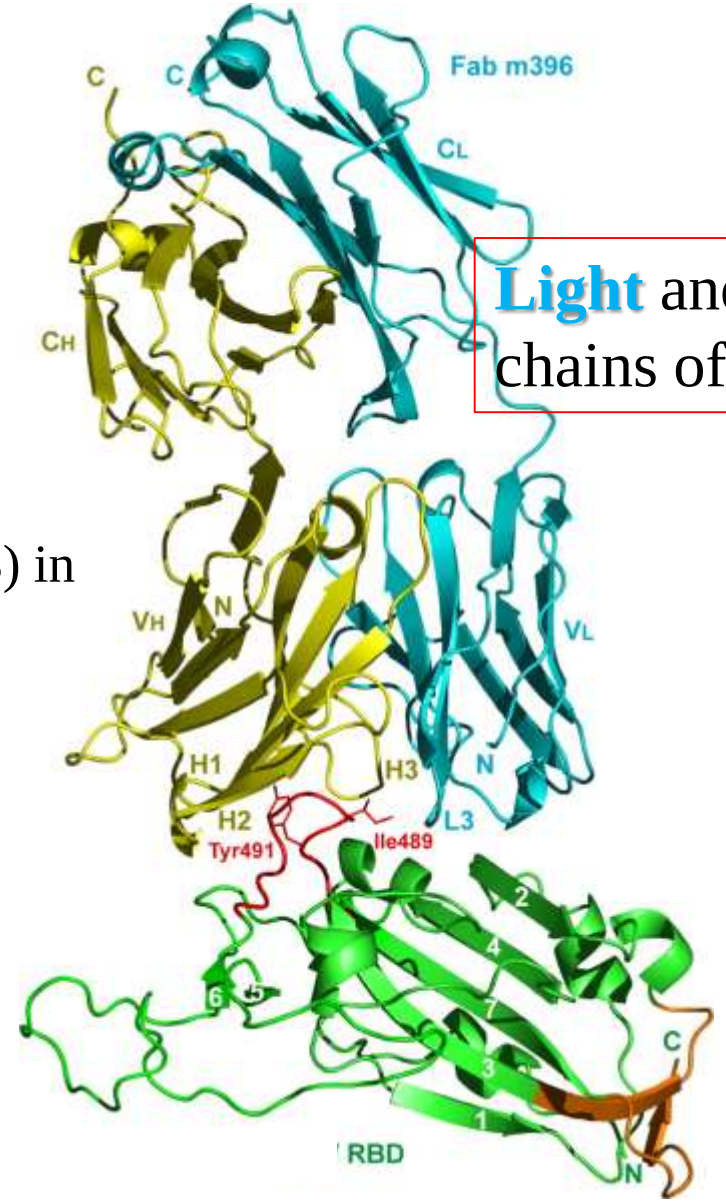
This fragment was used as a selecting antigen for a large (~$10^{10}$ different antibodies) **human antibody Fab library**, constructed from the B lymphocytes of **10 healthy volunteers**

The Fab with the strongest binding to the RBD, m396, was converted to full antibody (IgG1), expressed, and purified.

# The neutralizing antibody Fab m396.

antigen binding fragment (Fab),




**Light** and **heavy** chains of the Fab

Hypervariable Loops (H1, H2, H3, and L3) in the complementarity determining regions (CDRs) make contacts with the **RBD**

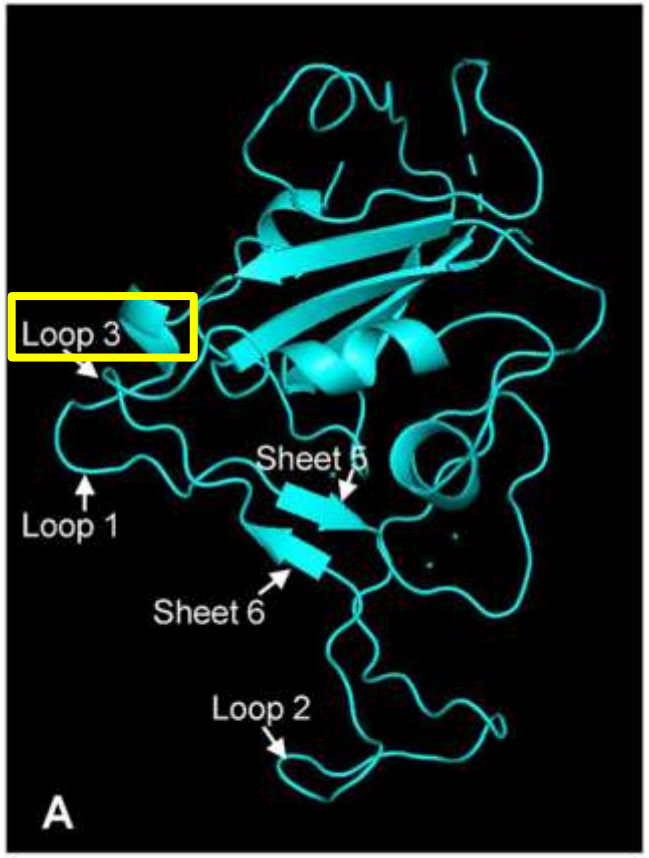
the neutralizing site -residues 482 through 491 (β6–β7 loop)- **is in red**

**Ponraj Prabakaran et al. J. Biol. Chem. 2006;281:15829-15836**

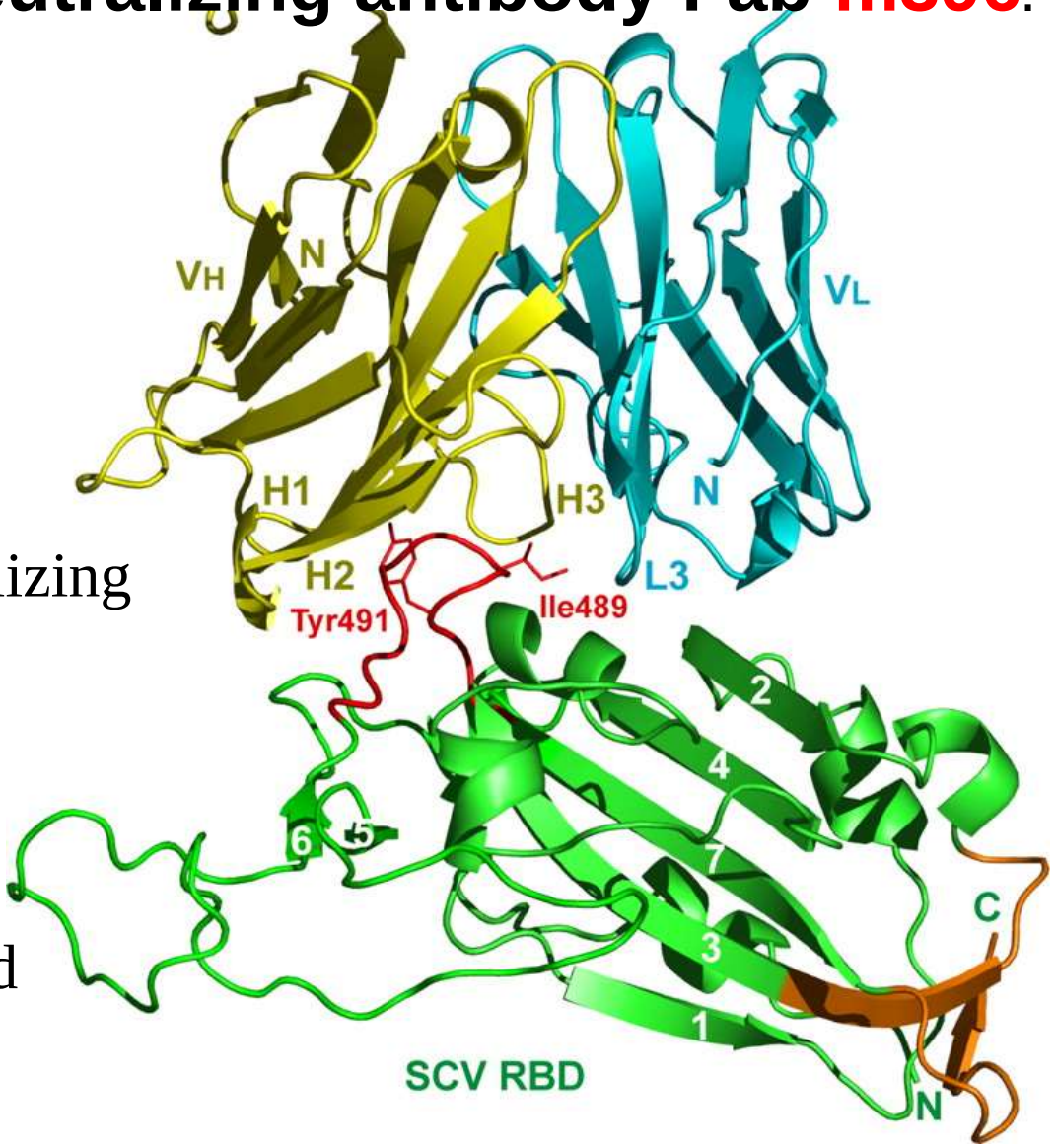
The American Society for Biochemistry and Molecular Biology, Inc.

JBC | JOURNAL OF BIOLOGICAL CHEMISTRY

# The neutralizing antibody Fab m396.




SARS-CoV RBD



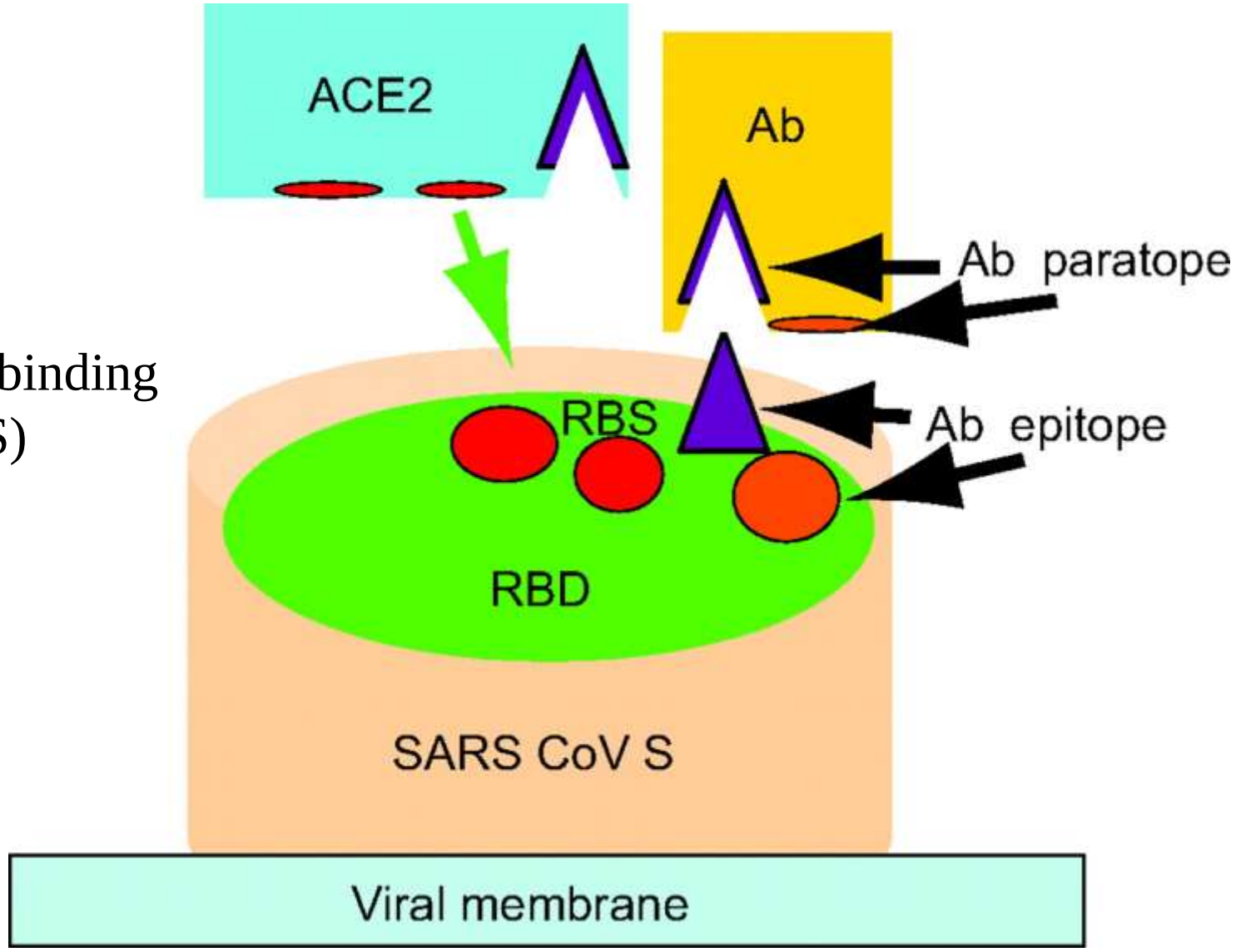

the neutralizing site **is in red**

Hypervariable Loops (H1, H2, H3, and L3) in the complementarity determining regions (CDRs)

**Ponraj Prabakaran et al. J. Biol. Chem. 2006;281:15829-15836**

**Schematic representation of the SARS-CoV neutralization mechanism.**

receptor-binding site (RBS)




**Zhongyu Zhu et al. PNAS 2007;104:29:12123-12128**

PNAS

# La storia dell'anticorpo 80R

The severe acute respiratory syndrome coronavirus (SARS-CoV) caused a worldwide epidemic in late 2002/early 2003 and a second outbreak in the winter of 2003/2004 by an independent animal-to-human transmission.

…the human monoclonal antibody 80R potently neutralizes the virus from the first outbreak but not the second …

*Proc Natl Acad Sci U S A. 2007 Jul 17;104(29):12123-8. Epub 2007 Jul 9. Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. Zhu Z et al*

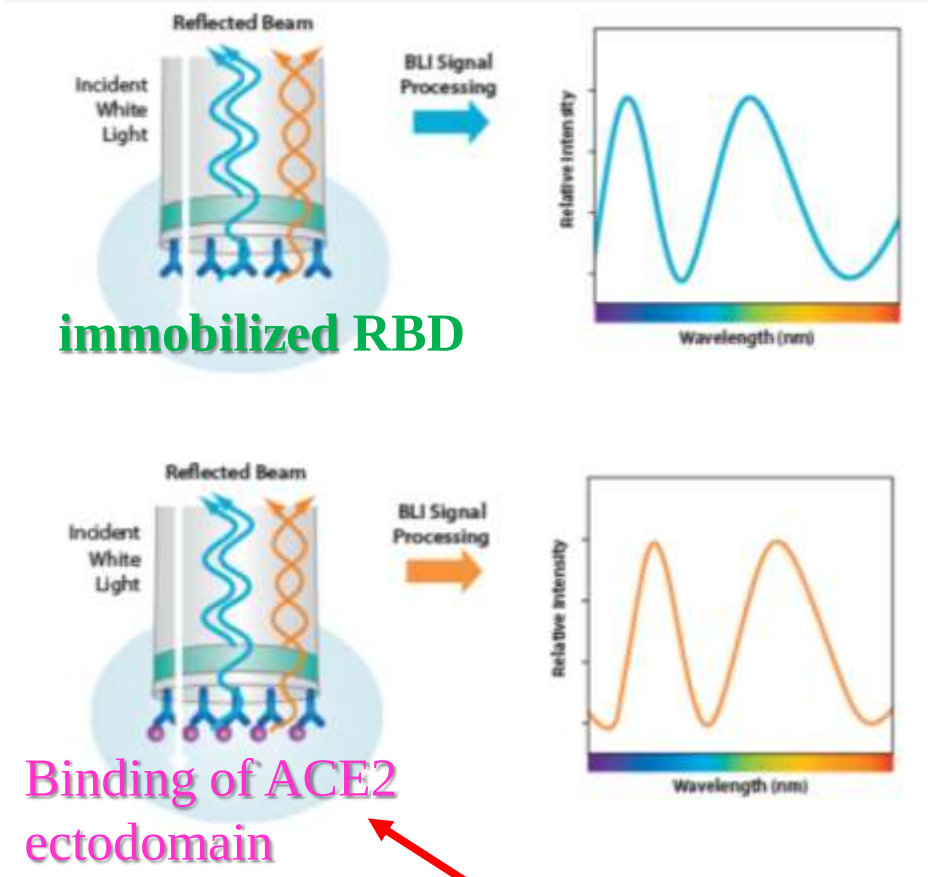
# *Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein*

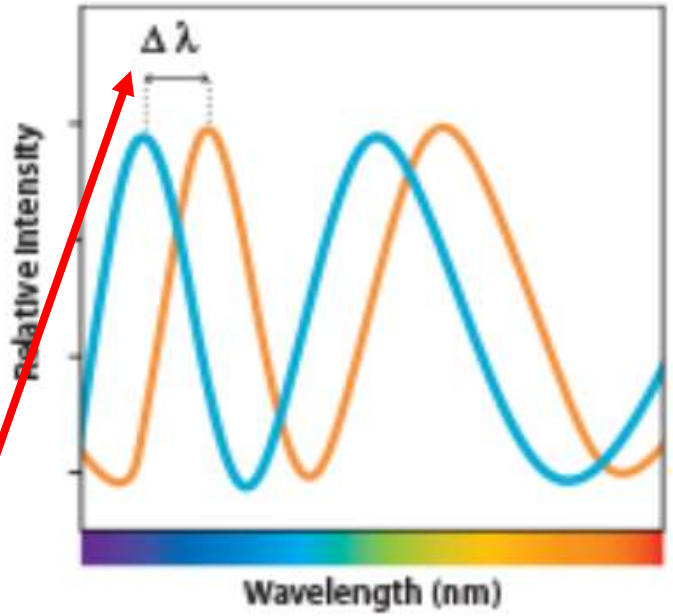
*Alexandra C. Walls, Young-Jun Park, M. Alejandra Tortorici, Abigail Wall, Andrew T. McGuire, David Veesler*

*Cell*

DOI: 10.1016/j.cell.2020.02.058

# Bio-Layer Interferometry (BLI) measures biomolecular interactions

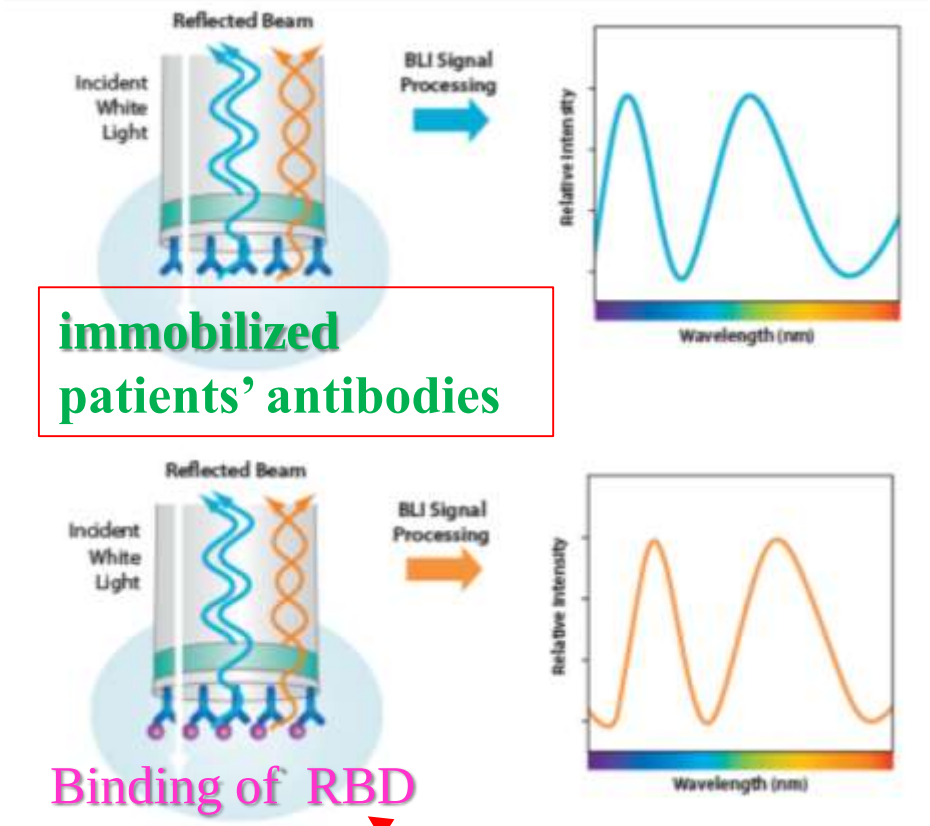


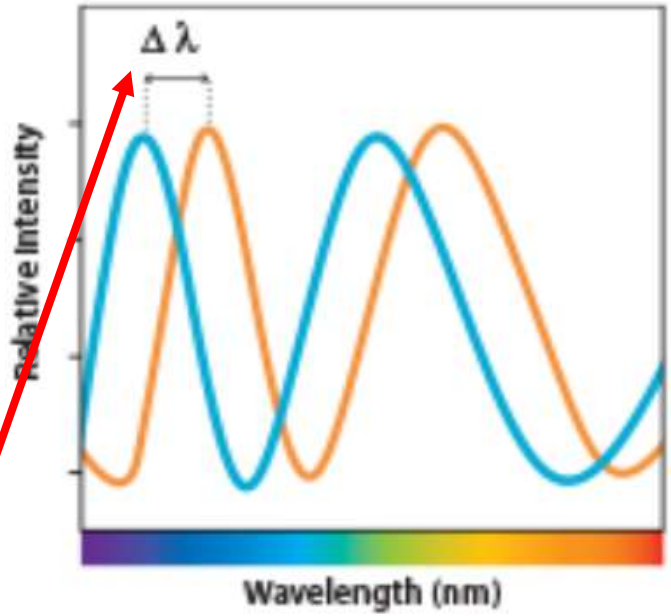




Change in the number of molecules bound to the biosensor tip causes a shift (Delta Lambda) in the interference pattern

# Bio-Layer Interferometry (BLI) measures biomolecular interactions

antibodies from patients




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

# Affinità Spike (RBD) con anticorpi dei pazienti

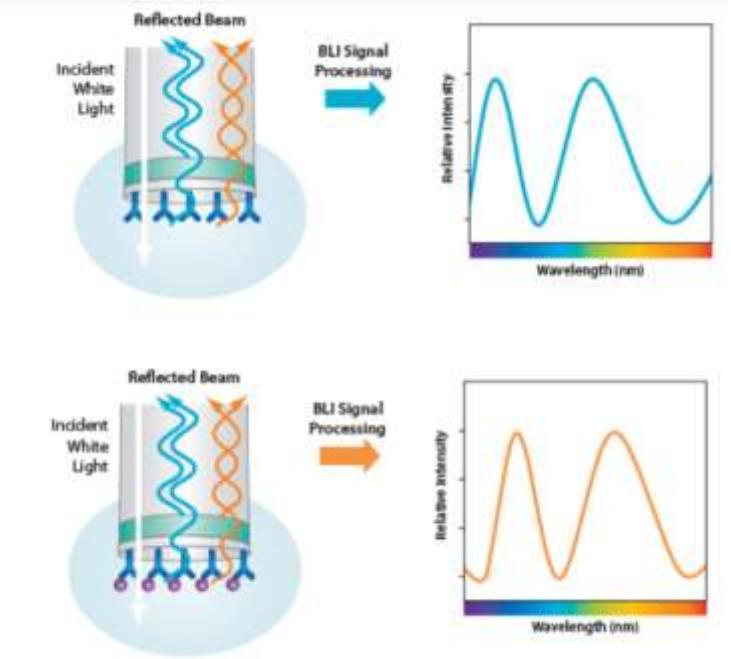
**Biolayer interferometry**

S230, 80R and m396 IgGs were immobilized to anti-human capture sensortips

and dipped into wells containing

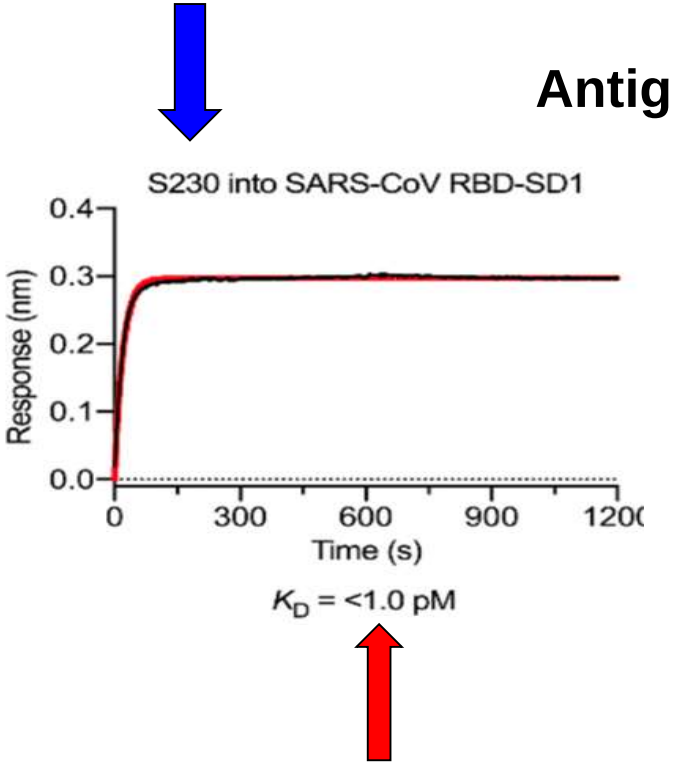
1) 1 microM 2019-nCoV RBD to measure association

2) containing only buffer to measure dissociation




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

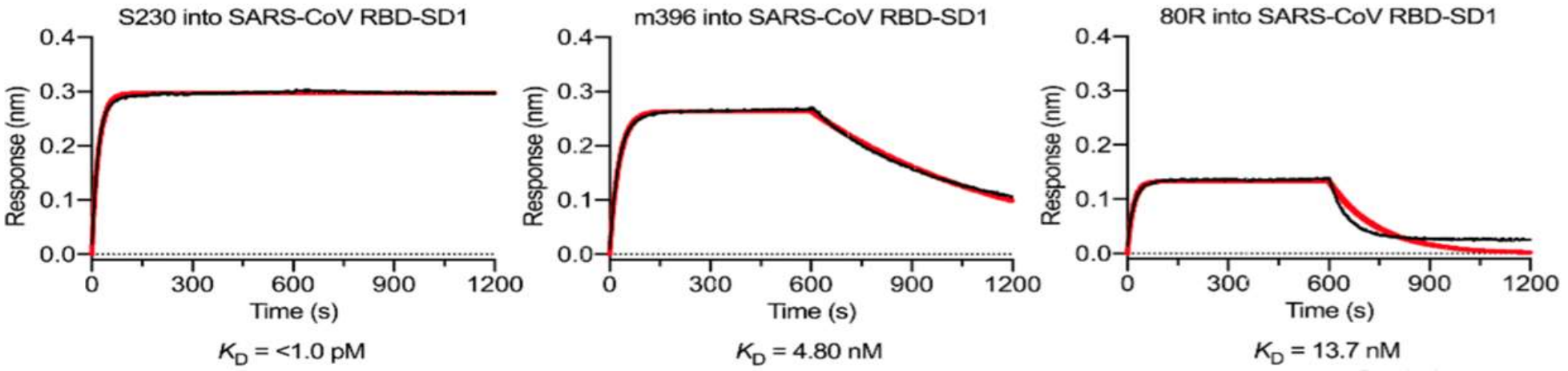
# Antigenicity of the SARS-CoV RBD






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

## Strong Binding to the SARS-CoV RBD



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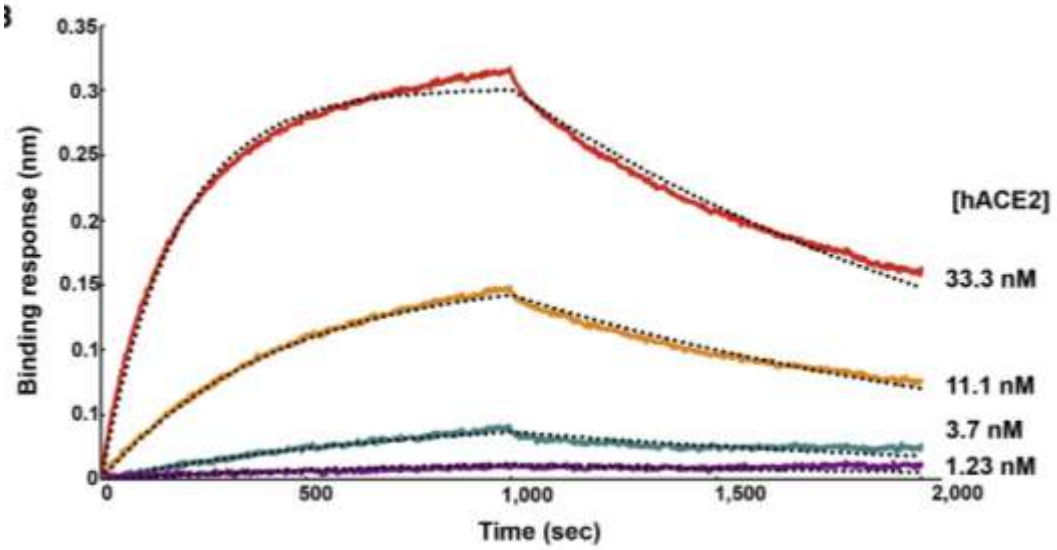
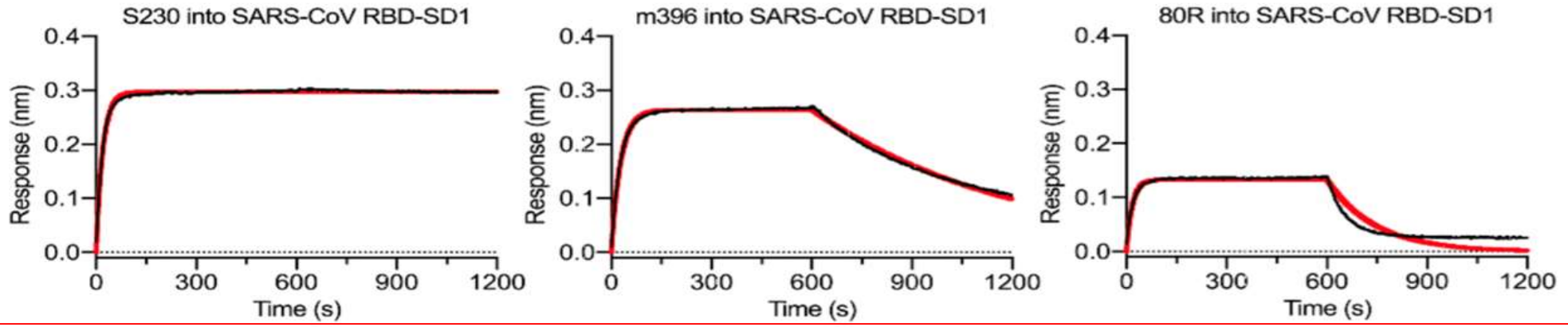
# Comparazione della costante di dissociazione all'equilibrio $K_D$

**SARS-CoV RBD/ ACE2**

$K_D = 5.0$ nM




## Strong Binding to the SARS-CoV RBD




$K_D$ = <1.0 pM | $K_D$ = 4.80 nM | $K_D$ = 13.7 nM



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

# Sommario (in pillole)

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

L’affinità degli anticorpi umani per lo spike del SARS  è paragonabile o maggiore di quella tra Spike SARS e ACE2, il recettore.

# Antigenicity of the 2019-nCoV RBD ?



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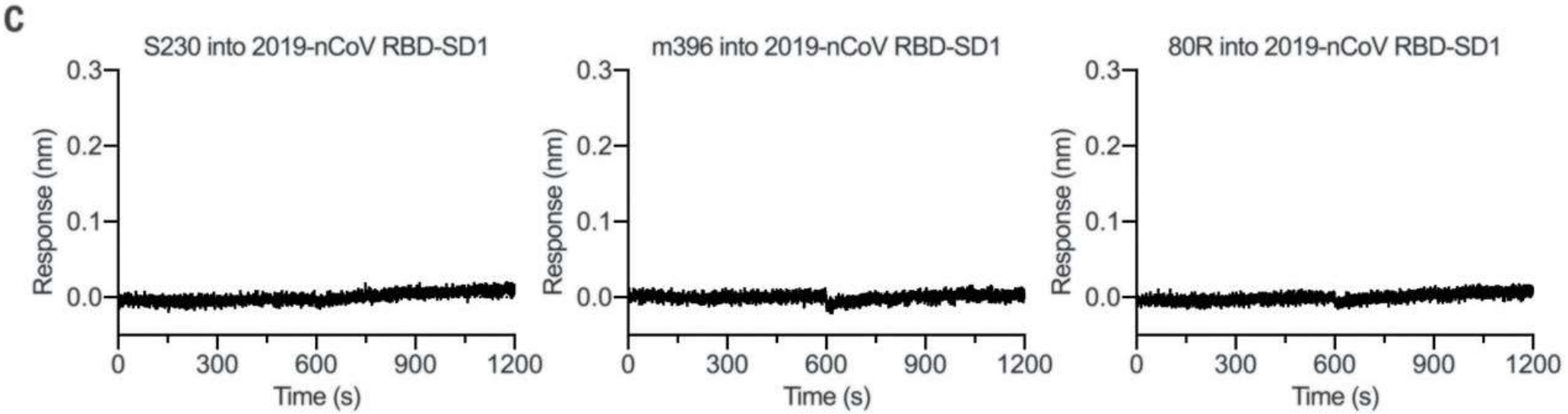
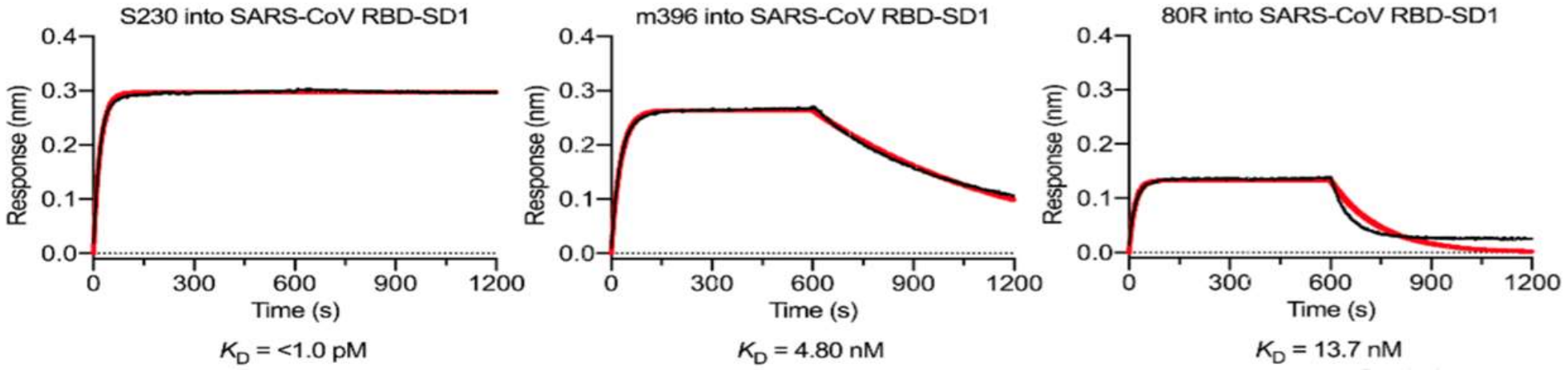
Daniel Wrapp et al. Science 2020;367:1260-1263

# NO Binding to the 2019-nCoV RBD




# Strong Binding to the SARS-CoV RBD



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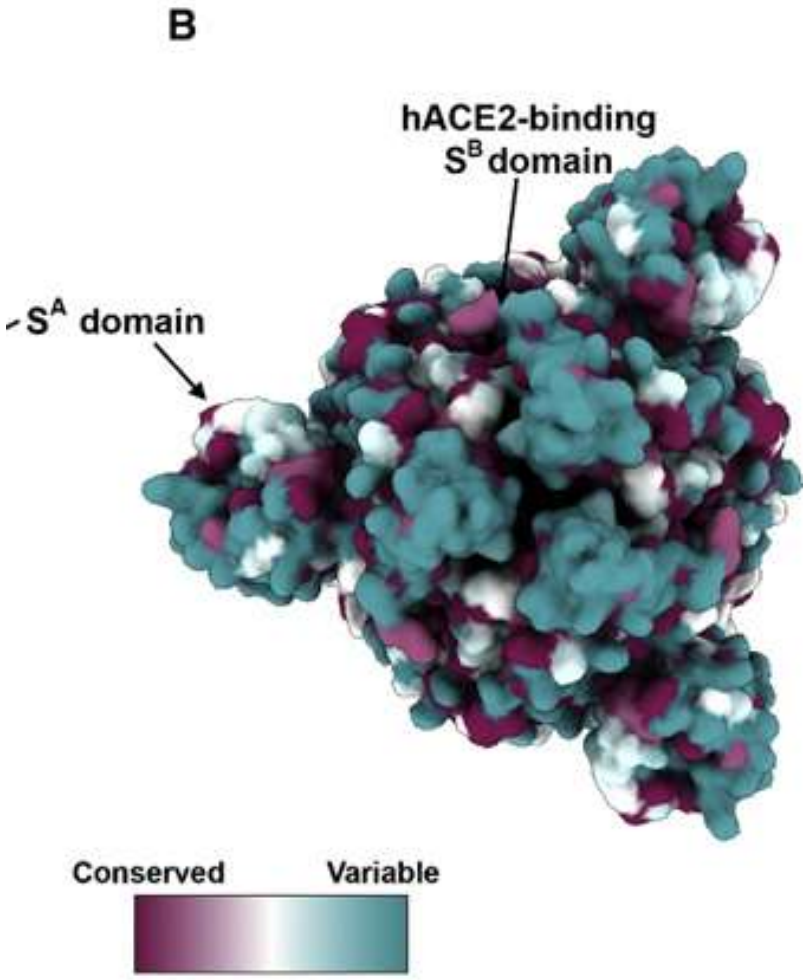
# SARS-CoV-2 S / SARS-CoV S  A A sequence comparison

the highest divergence found within SA and SB domains



# Affinità Spike (RBD) con anticorpi umani

Premessa Despite the relatively high degree of structural homology between the 2019-nCoV RBD and the SARS-CoV RBD,

Osservazione NO binding to the 2019-nCoV RBD could be detected for any of the three mAbs

Osservazione In contrast strong binding was observed to the SARS-CoV RBD.

Ipotesi the lack of observed binding suggests that SARS-directed mAbs will not necessarily be cross-reactive

.

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

# Sviluppi

Future antibody isolation and therapeutic design will benefit from using 2019-nCoV S proteins as probes. “Anticorpi contro 2019-nCoV S solo usando 2019-nCoV S”

Knowing the atomic-level structure of the 2019-nCoV spike will allow protein-engineering efforts that could improve antigenicity and protein expression for vaccine development.

The structural data will facilitate the evaluation of 2019-nCoV spike mutations that will occur as the virus undergoes genetic drift

Help to define whether those residues have surface exposure and map to sites of known antibody epitopes

# Sviluppi 2

The protein produced by this construct is in the prefusion conformation, which should maintain the most neutralization-sensitive epitopes when used as candidate vaccine antigens or B cell probes for isolating neutralizing human mAbs (vaccine design).

The atomic-level detail will enable the design and screening of small molecules with fusion-inhibiting potential (antiviral therapeutics).

# Sommario (in pillole) 4

- La struttura, conformazione e sequenze dello spike sono state comparate tra coronavirus in relazione alla loro antigenicità.

Queste informazioni aiutano lo sviluppo di vaccini,

di anticorpi per inibire il virus e per saggi immunologici