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.

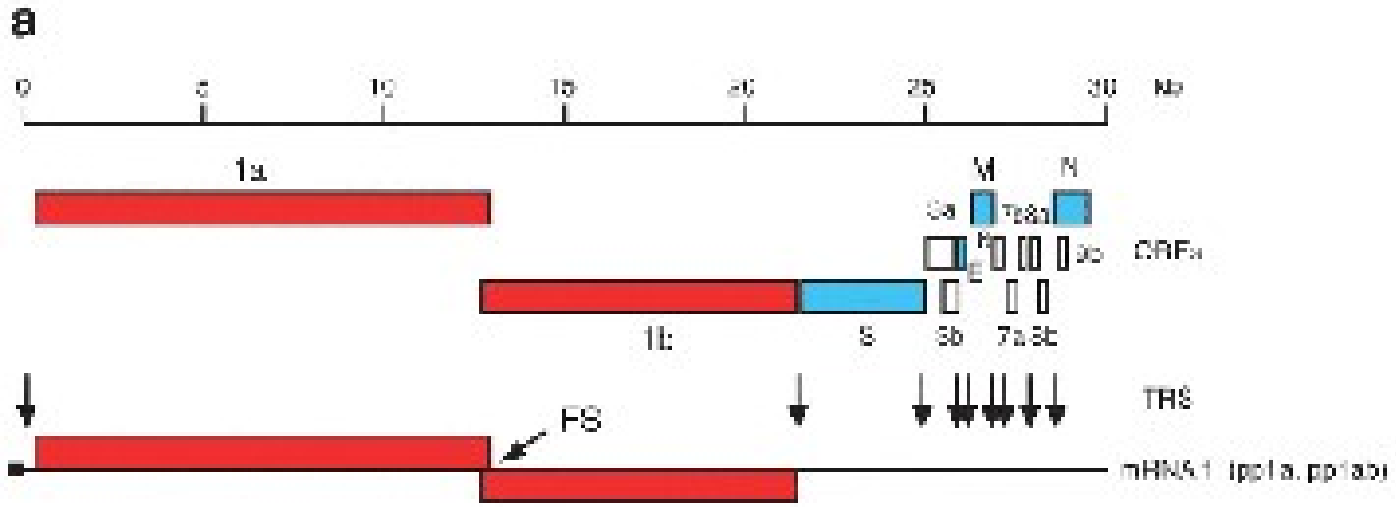
Premessa e Sommario (in pillole)

- Il virus “SARS-CoV-2” sta infettando gran parte delle popolazioni umane
- Le armi di attacco del virus sono le glicoproteine del suo “Spike”
- Spike lega con grande affinità l’enzima che converte l’angiotensina (ACE2) e lo usa come recettore per entrare ed invadere le nostre cellule.
- Recentemente la struttura dello spike e le sue conformazioni sono state identificate ad alta risoluzione
- La maturazione proteasica delle proteine virali è vitale per il suo ciclo vitale e per l’infezione

Queste informazioni favoriscono lo sviluppo di molecule capaci di inibire l’attacco, potenzialmente di grande significato terapeutico

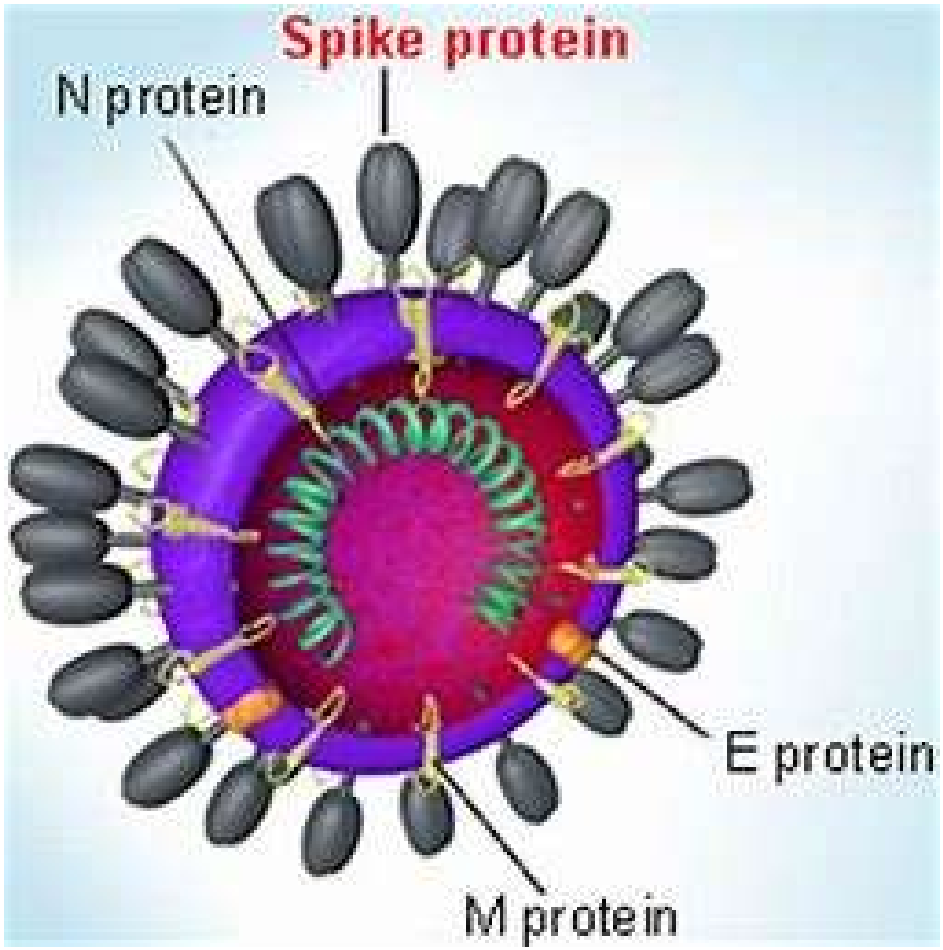
SARS-CoV genome organization and expression

Coronaviruses are positive-stranded RNA viruses with large viral RNA genomes (27 to 31 kb).



Two overlapping polyproteins [pp1a (replicase 1a, 450 kD) and pp1ab (replicase 1ab, 750 kD) mediate all the functions required for viral replication and transcription.

Lo «Spike»



Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation

by Daniel Wrapp, Nianshuang Wang, Kizzmekia S. Corbett, Jory A. Goldsmith, Ching-Lin Hsieh, Olubukola Abiona, Barney S. Graham, and Jason S. McLellan

Science
Volume 367(6483):1260-1263
March 13, 2020

Science
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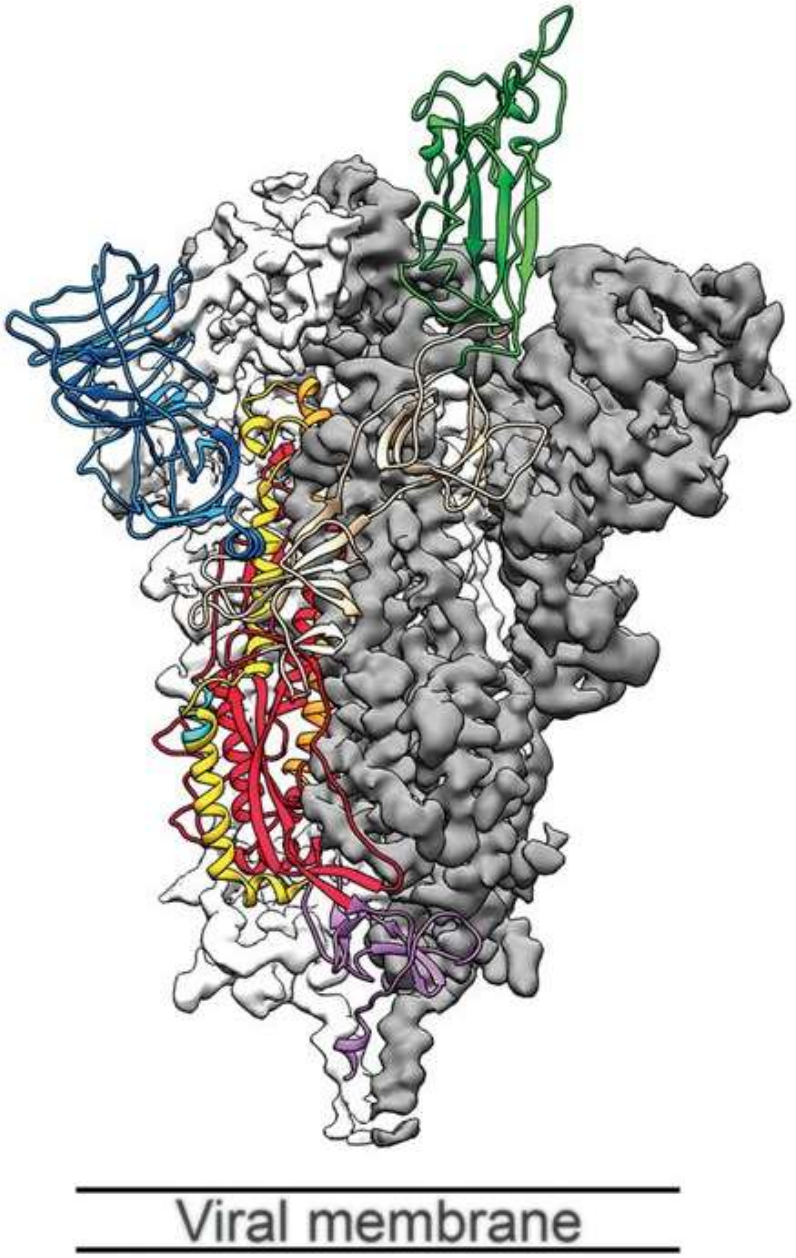
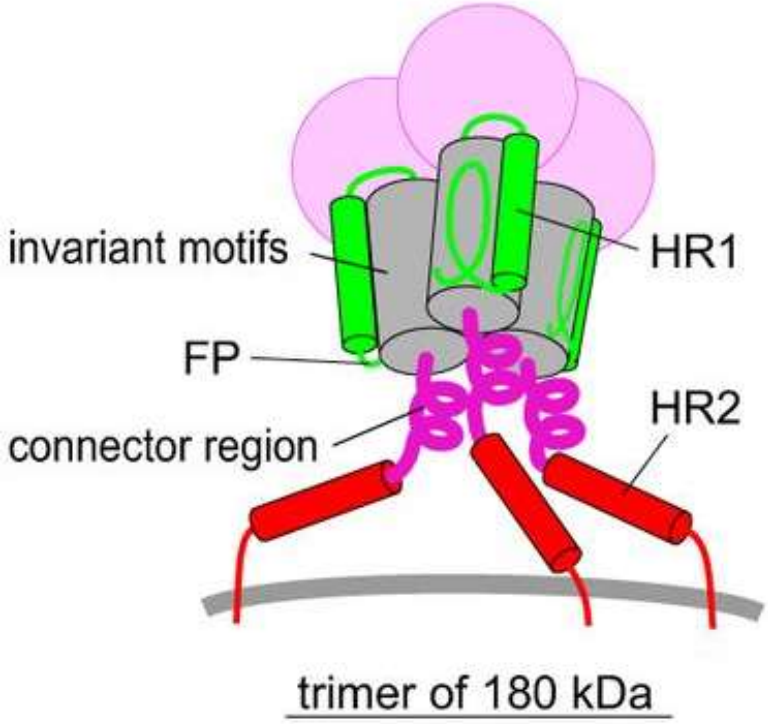
Lo «Spike»



Coronavirus entry into host cells is mediated by the transmembrane spike (S) glycoprotein that forms homotrimers protruding from the viral surface



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

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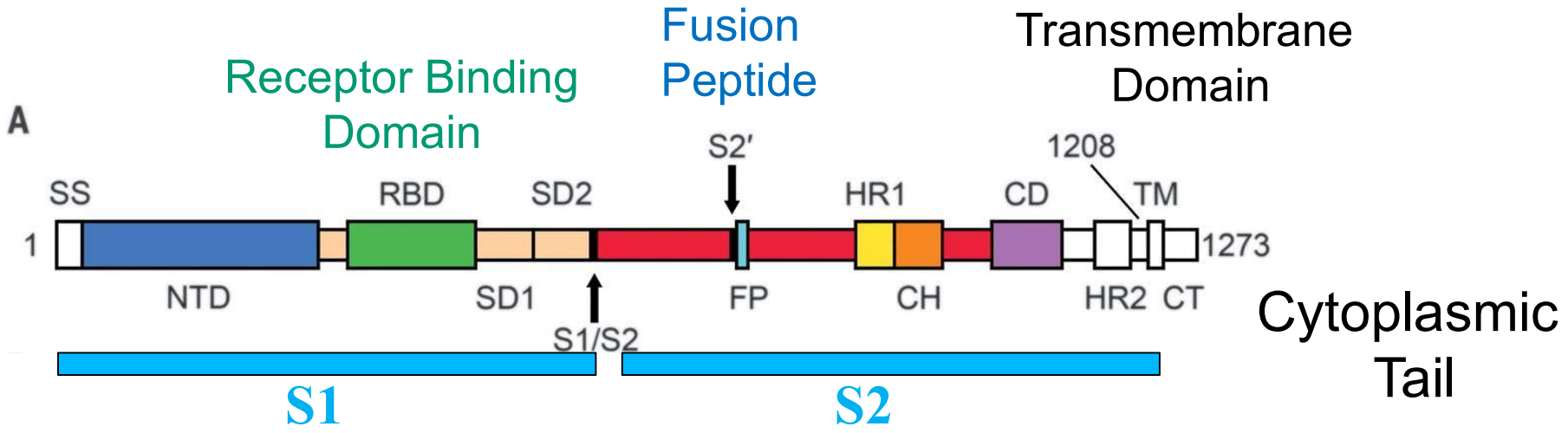
Lo «Spike»- struttura

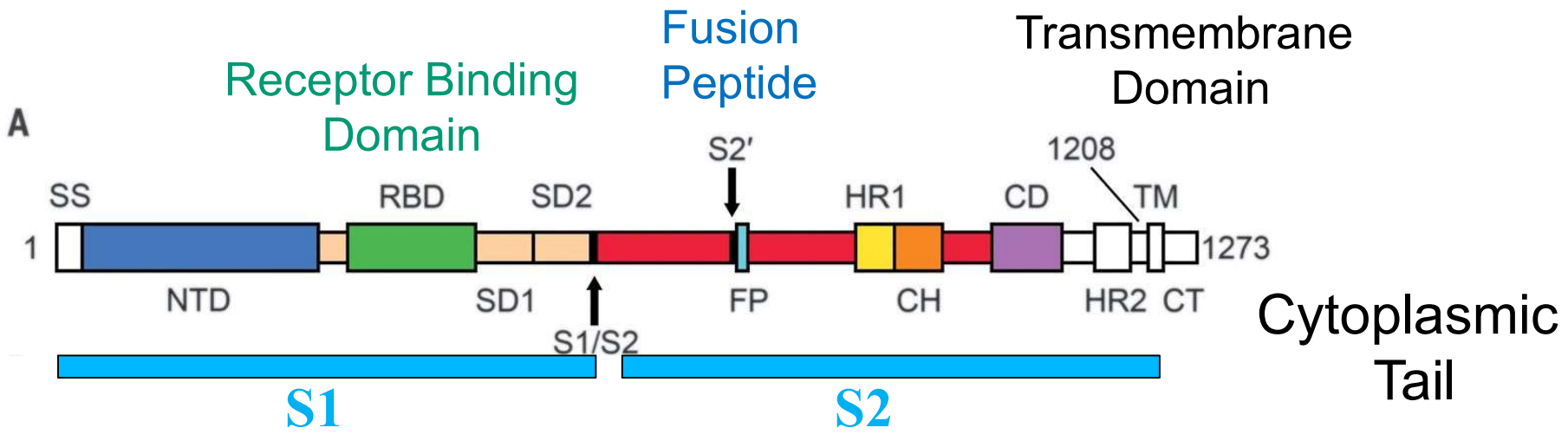
- S comprises two functional subunits responsible for binding to the host cell receptor (S1 subunit) and fusion of the viral and cellular membranes (S2 subunit).



The S1 subunit comprises the receptor-binding domain and contributes to stabilization of the membrane-anchored S2 subunit that contains the fusion peptide and machinery

Lo «Spike»- struttura e …maturazione proteolitica

- S is cleaved at the boundary between the S1 and S2 subunits, which remain non-covalently bound in the prefusion conformation



Arrows, protease cleavage sites

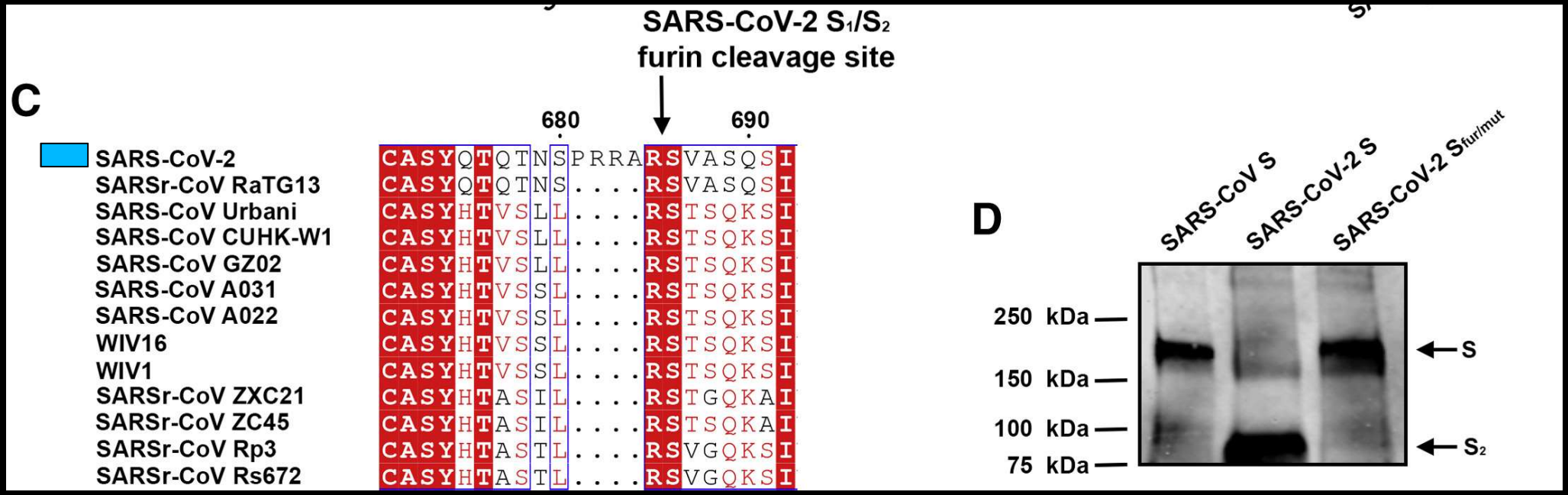
Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein

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

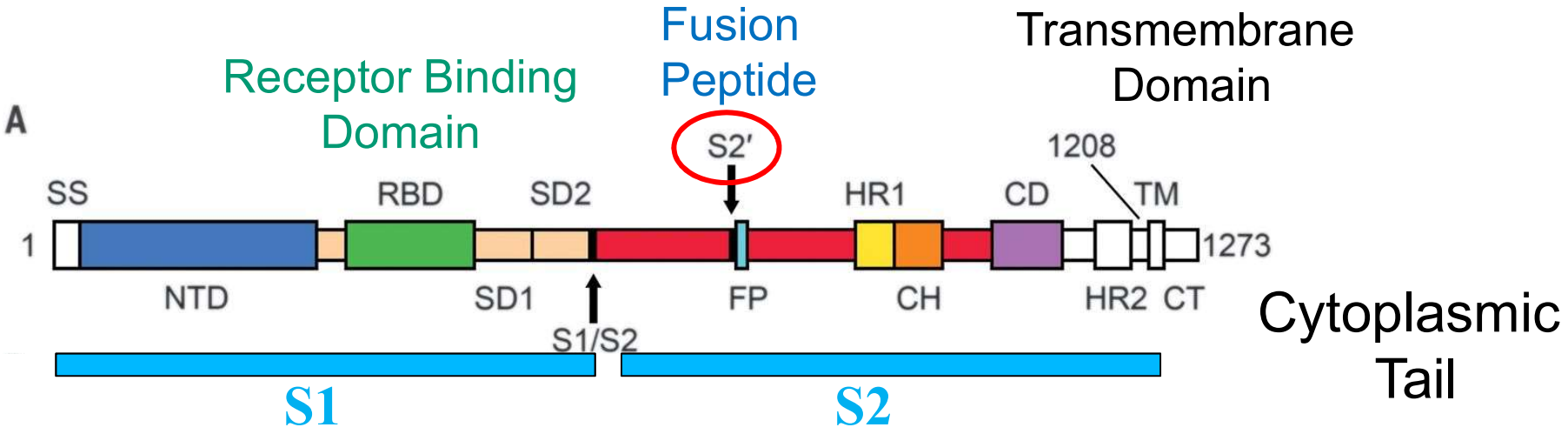
an S1/S2 furin cleavage site in this novel COV-2 coronavirus



SARS-CoV-2 S glycoprotein harbors a furin cleavage site at the boundary between the S1/S2 subunits, which is processed during biogenesis SARS-CoV-2 S differs from SARS-CoV and SARS-related CoVs

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

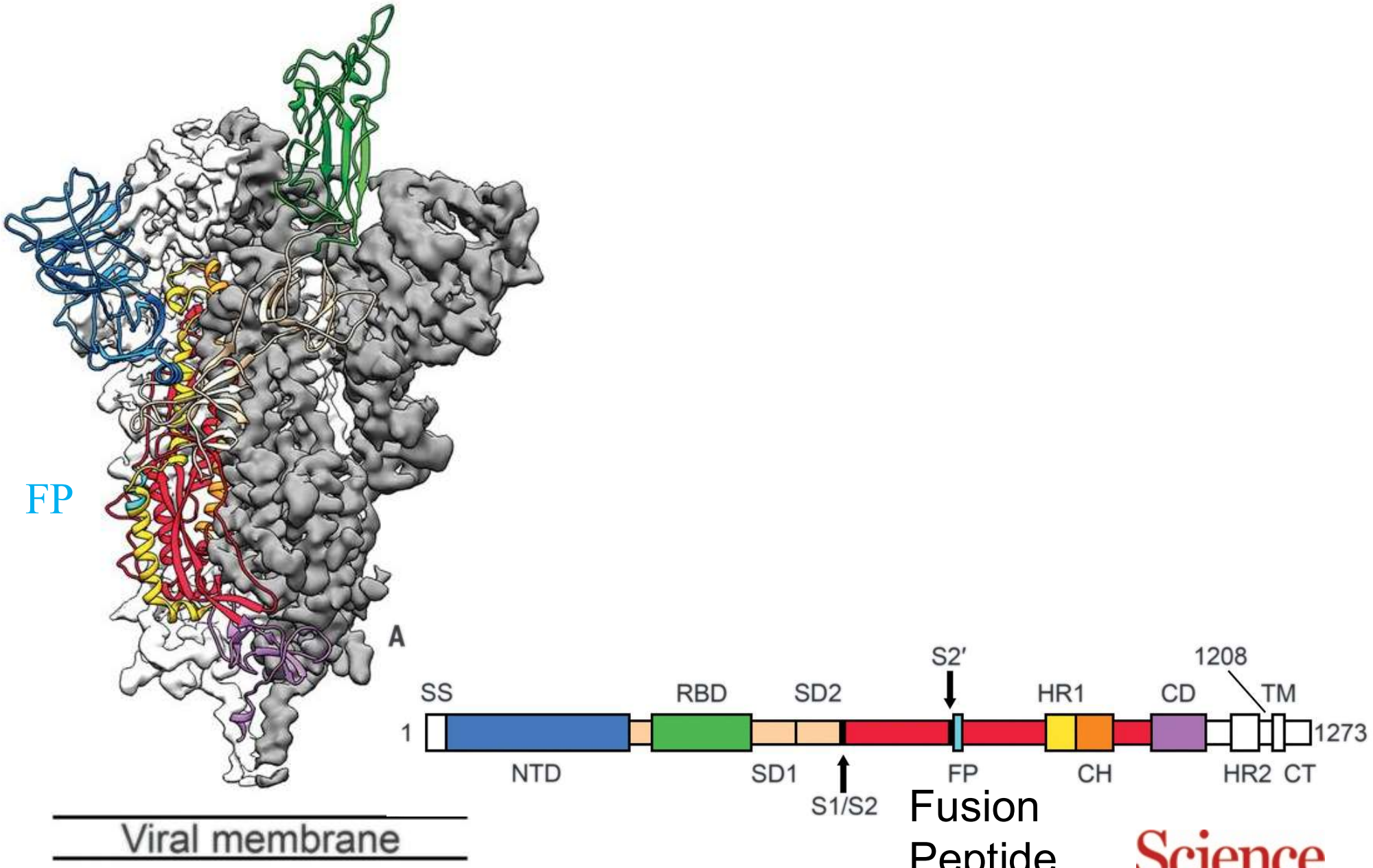
Lo «Spike»- struttura e …maturazione proteolitica



Arrows, protease cleavage sites

S is further cleaved by host proteases at the so-called S2 site located upstream of the fusion peptide. This cleavage activates the protein for membrane fusion via extensive irreversible conformational changes.

Fig. 1 Side view of the prefusion structure of the 2019-nCoV S protein with a single Receptor Binding Domain (RBD) in the up conformation.



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

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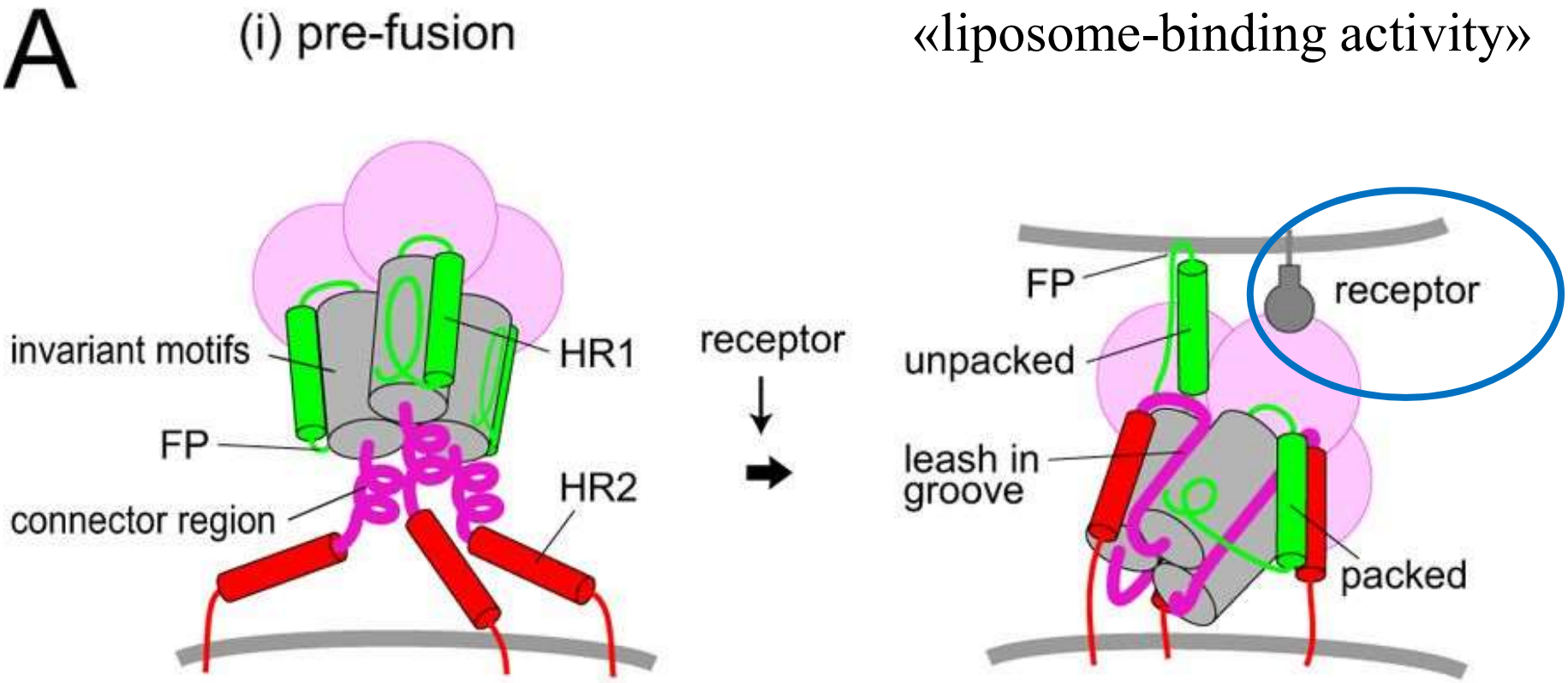
Schematic diagram of S protein activation.



Miyuki Kawase et al. J. Virol. 2019; doi:10.1128/JVI.00785-19

Il recettore cellulare

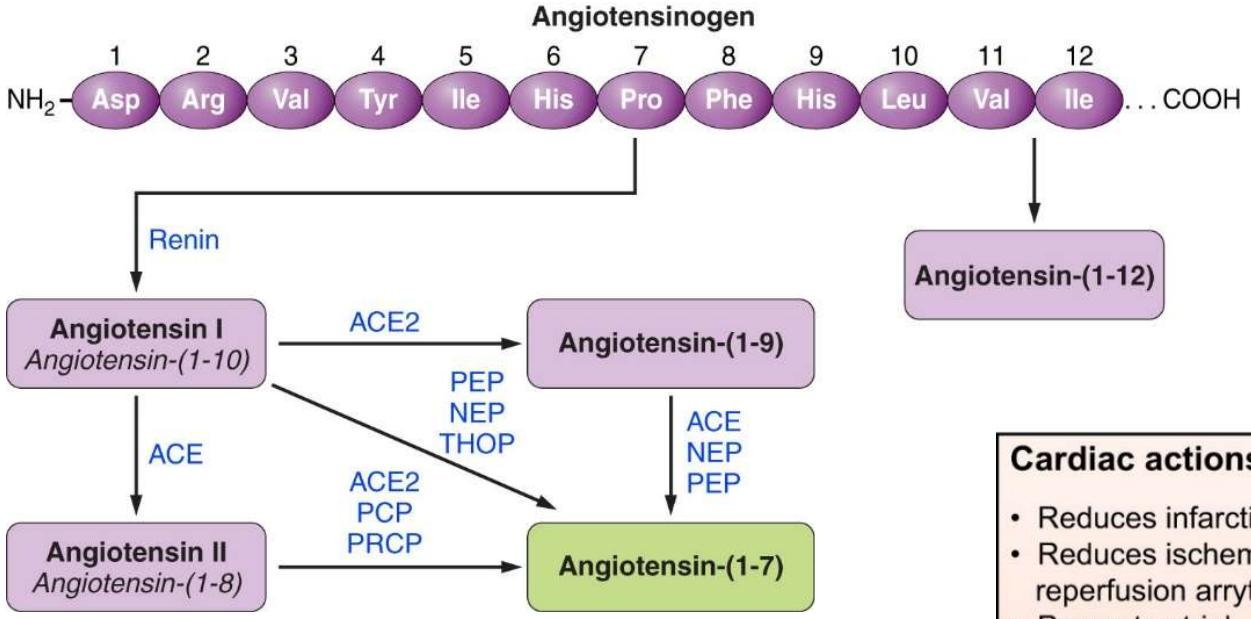
- Coronavirus entry into susceptible cells requires the concerted action of receptor-binding and proteolytic processing of the S protein to promote virus-cell fusion.
- Previous studies have identified

angiotensin converting enzyme 2 (ACE2)
as a functional receptor for SARS-CoV

- ACE2 physiological role is in the maturation of angiotensin, a peptide hormone that controls vasoconstriction and blood pressure

The renin-angiotensin system cascade

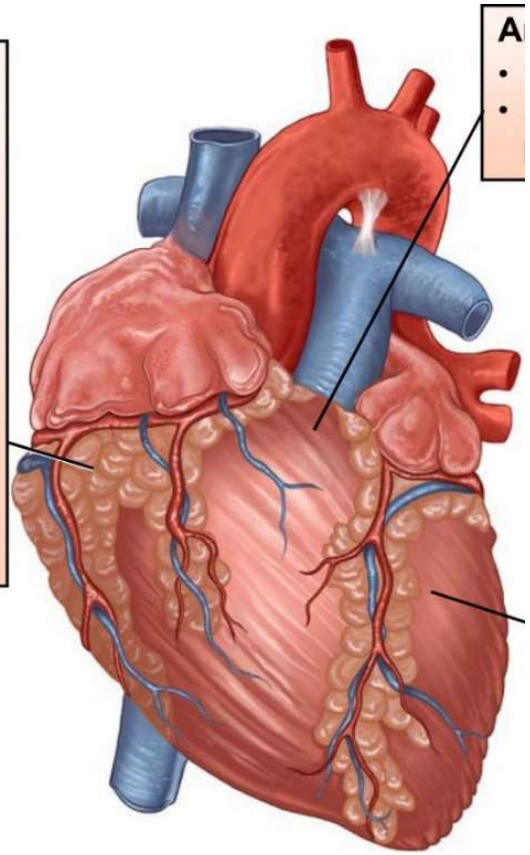
american physiological society | Physiological Reviews



ACE, angiotensin-converting enzyme;
ACE2, angiotensin-converting enzyme 2

Cardiac actions

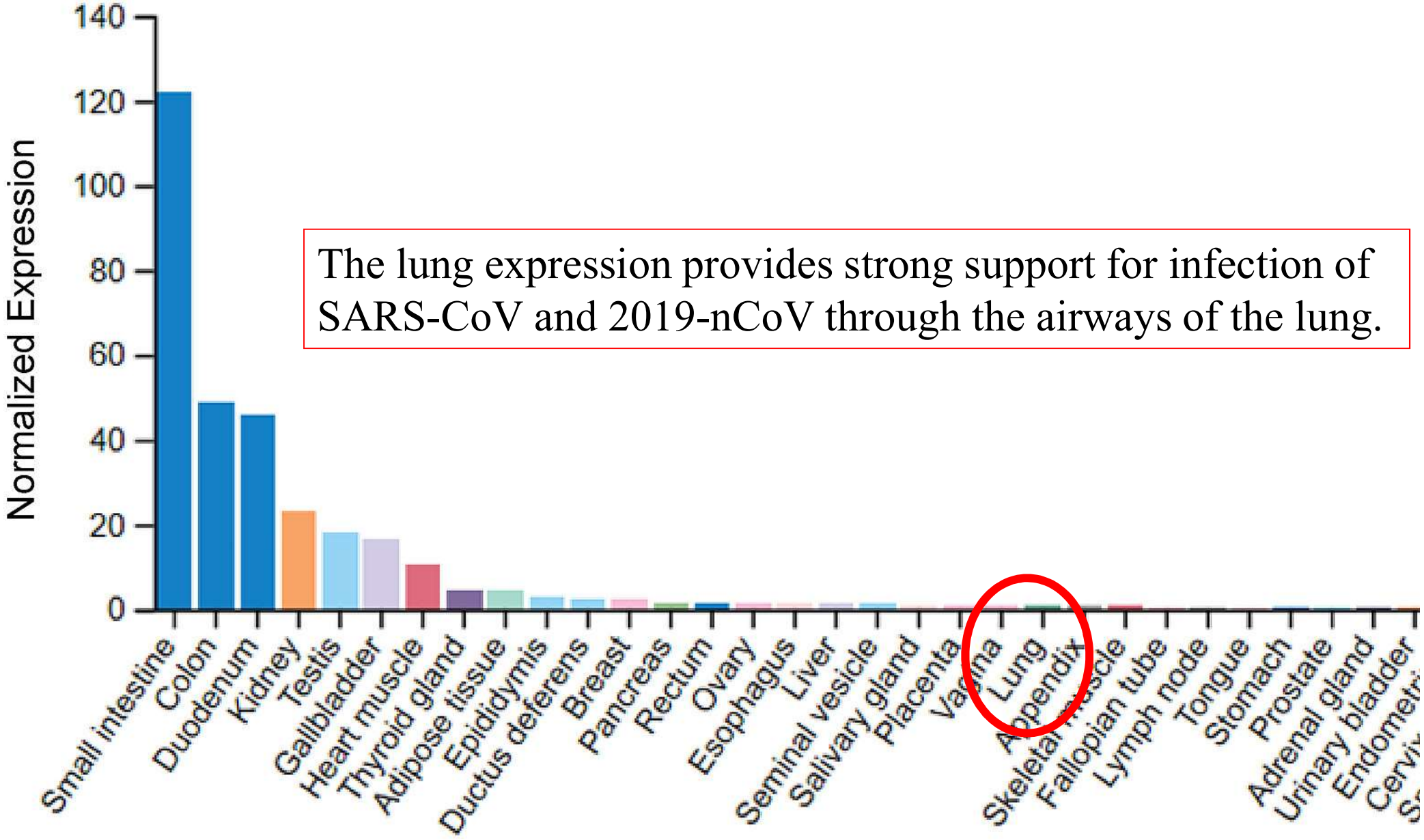
- Reduces infarction area
- Reduces ischemia-reperfusion arrythmias
- Prevents atrial tachycardia
- Improves autonomic function
- Prevents heart dysfunction
- Inhibits oxidative stress
- Upregulates nitric oxide synthase in SHR



DOI: (10.1152/physrev.00023.2016)

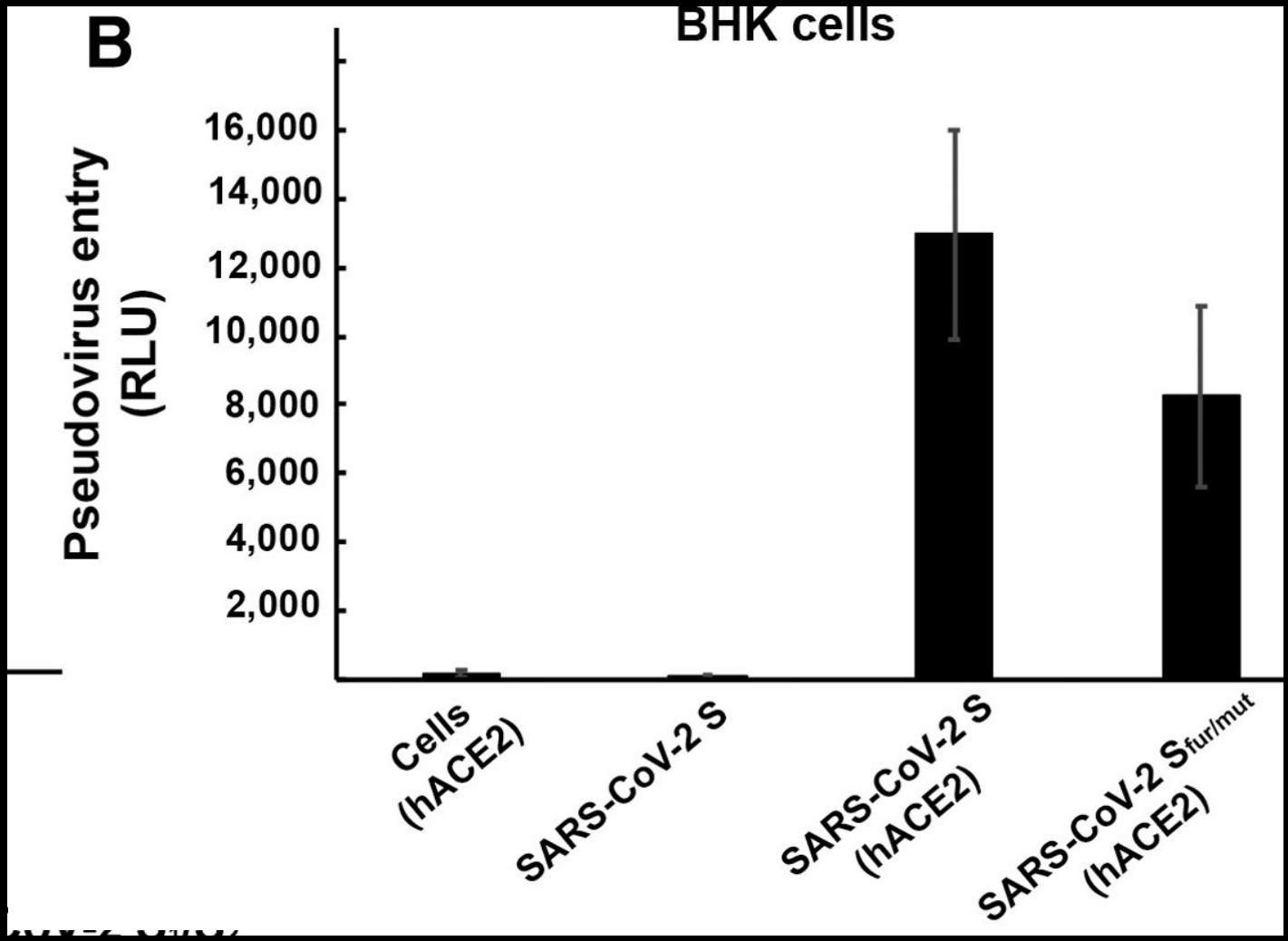
Espressione tissutale di ACE2

Expression of ACE2 in human tissues.
from The Human Protein Atlas (www.proteinatlas.org).



The lung expression provides strong support for infection of SARS-CoV and 2019-nCoV through the airways of the lung.

ACE2 Is a Functional Receptor for SARS-CoV-2 S

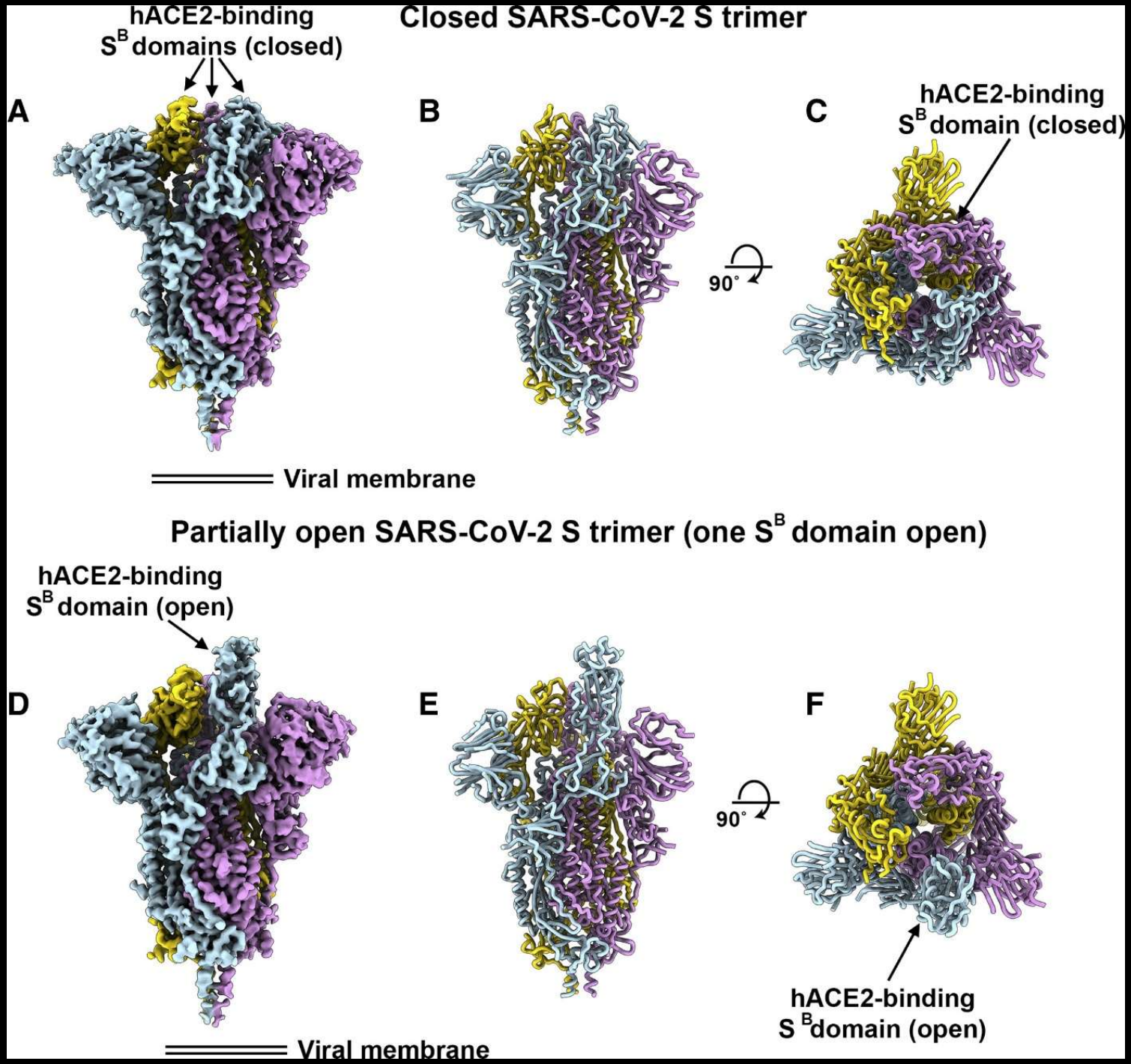


Entry of pseudotyped with SARS-CoV-2 S in BHK cells transiently transfected with hACE2.

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

Il recettore cellulare

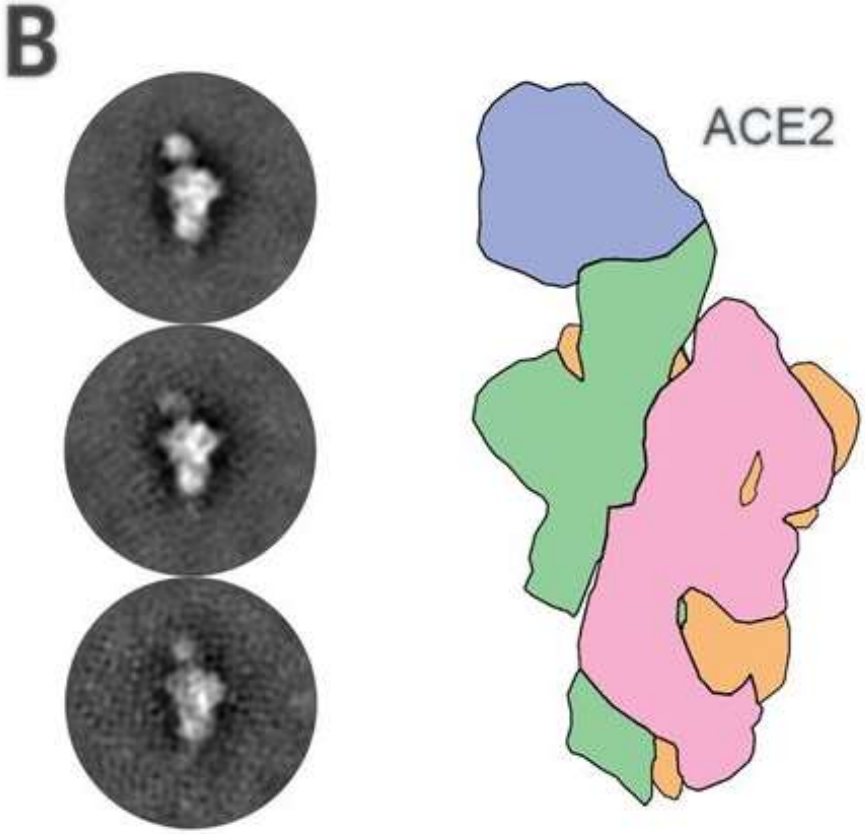
- SARS-CoV interact directly with angiotensin-converting enzyme 2 (ACE2) via the S B domain to enter target cells



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

2019-nCoV S binds human ACE2

Negative-stain EM and diagram of 2019-nCoV S bound by ACE2.



ACE2 (Blue) is positioned above
the 2019-nCoV S protein protomers tan, pink, and green

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

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

by Renhong Yan, Yuanyuan Zhang, Yaning Li, Lu Xia, Yingying Guo, and Qiang Zhou

Science
Volume 367(6485):1444-1448
March 27, 2020

Science
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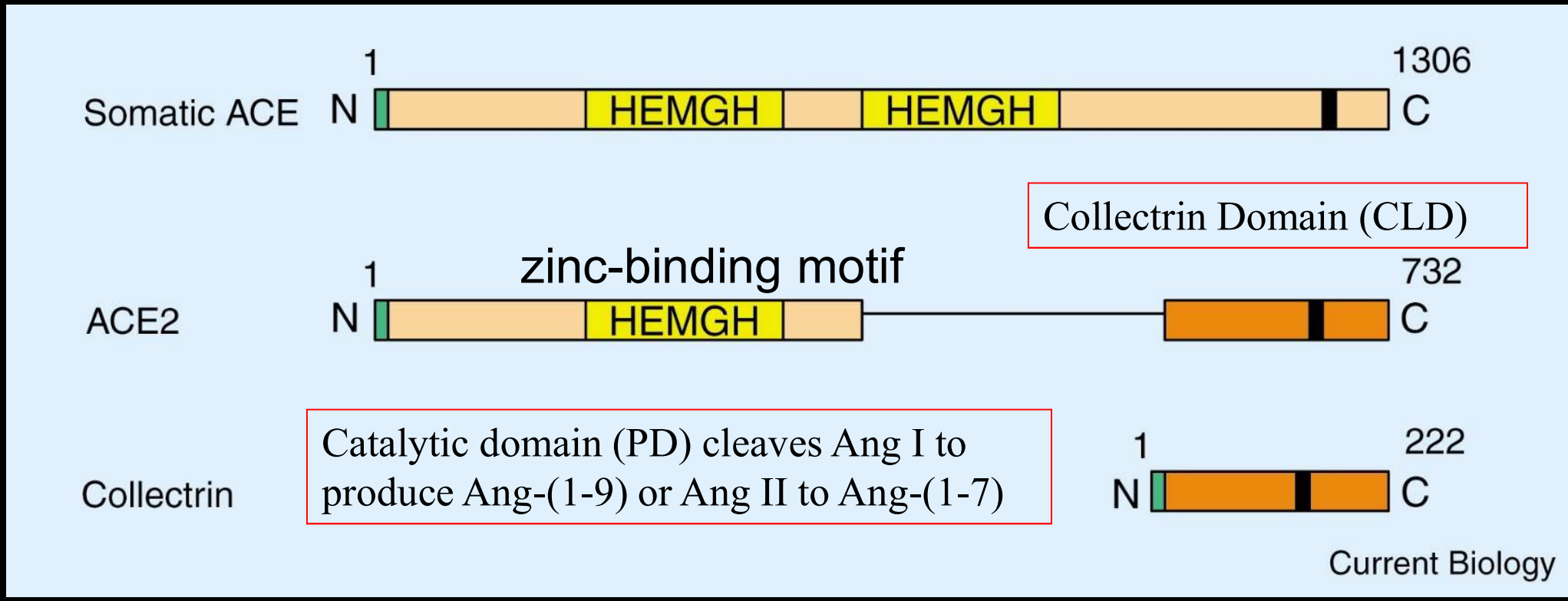
ACE e ACE2 proteasi del sistema Renina angiotensina



Collectrin is most abundantly expressed in the kidney where it has an important role in amino acid transport. Collectrin is also expressed in endothelial cells where it regulates L-arginine uptake.

Cell PRESS

Current Biology 2002 12, R745-R752DOI: (10.1016/S0960-9822(02)01255-1)

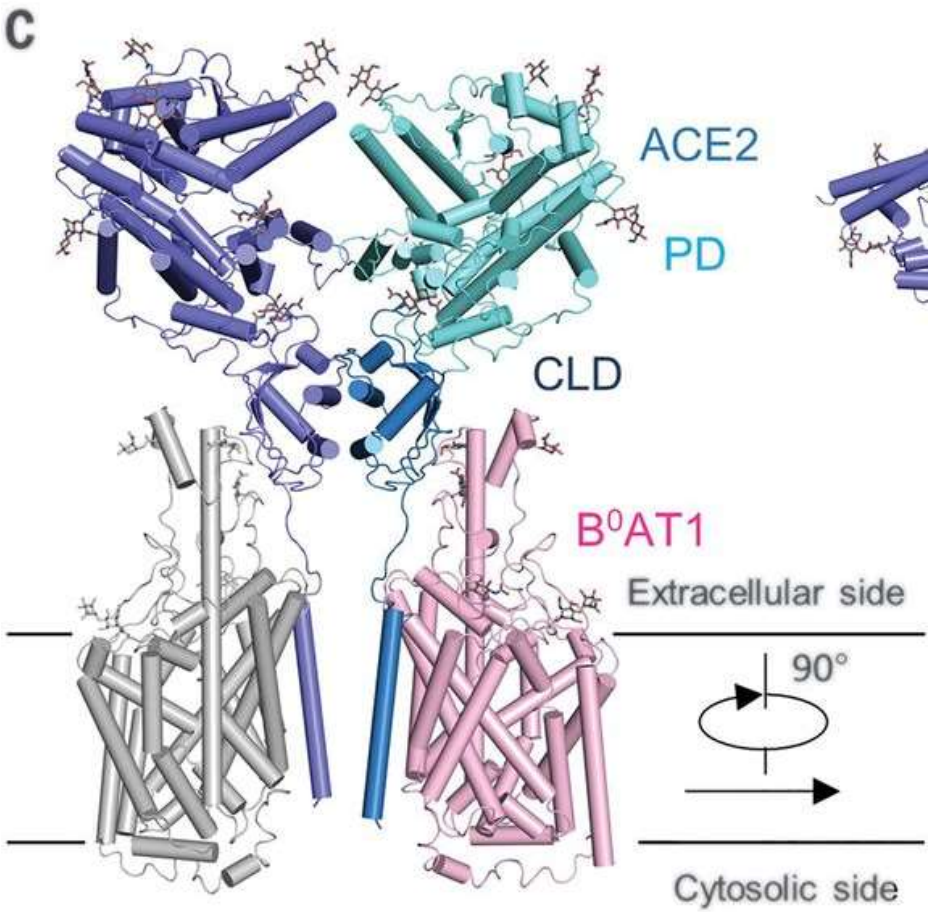
ACE2 ha altre funzioni ed interazioni

ACE2 also functions as the chaperone for membrane trafficking of the amino acid transporter B0AT1, also known as SLC6A19 (25)

B0AT1 mediates uptake of neutral amino acids into intestinal cells in a sodium-dependent manner.

Yan et al. present the structure of human ACE2 in complex with a membrane protein that it chaperones, B0AT1.

Cryo–electron microscopy structures of full-length human ACE2 in the presence of the neutral amino acid transporter B^0AT1



The ACE2-B^0AT1 complex is assembled as a dimer of heterodimers, with the collectrin-like domain CLD) of ACE2 mediating homodimerization.

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

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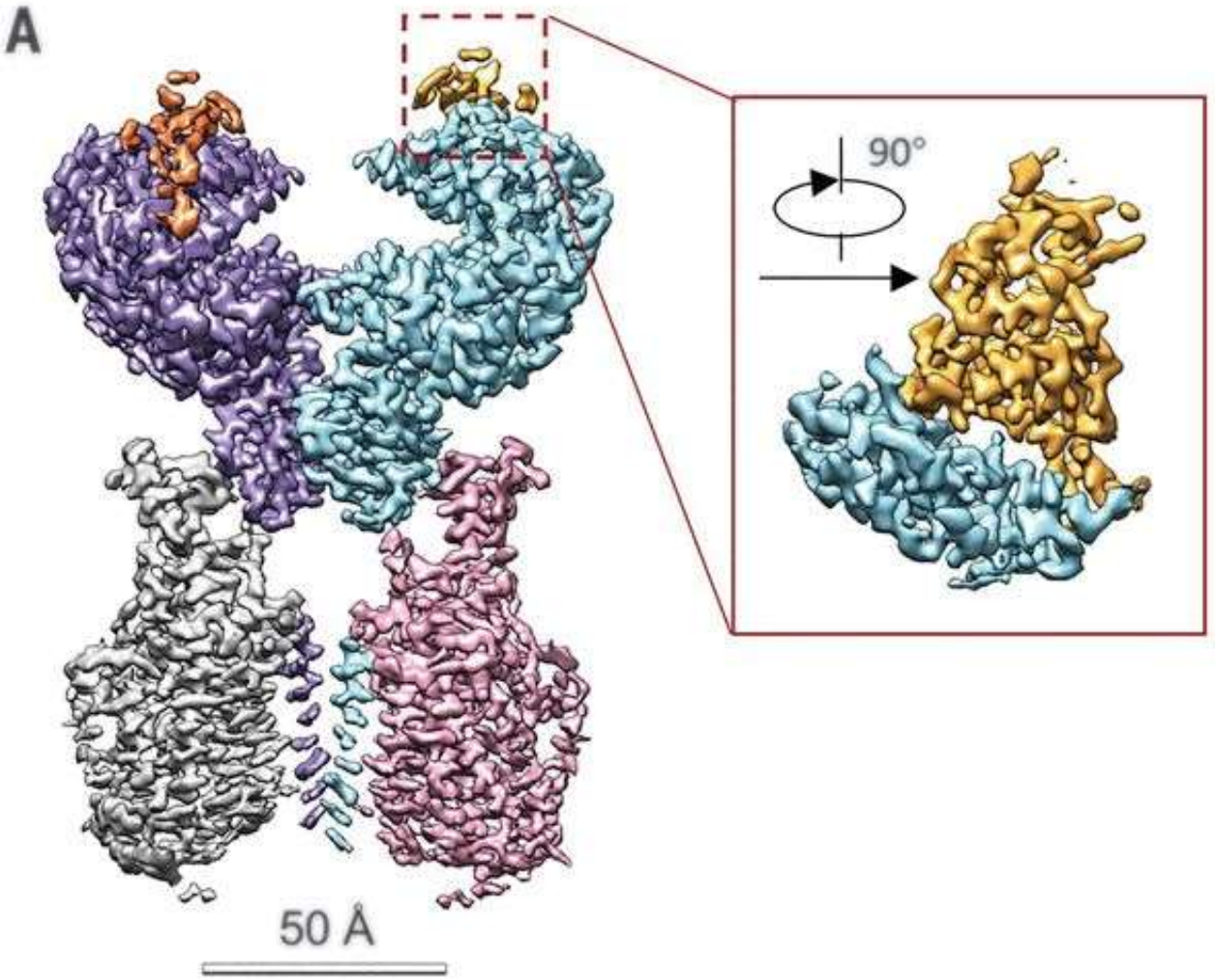
Cryo–electron microscopy structures of full-length human ACE2 in the presence of the neutral amino acid transporter B^0AT1 with the receptor binding domain (RBD) of the surface spike glycoprotein (S protein) of SARS-CoV-2



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

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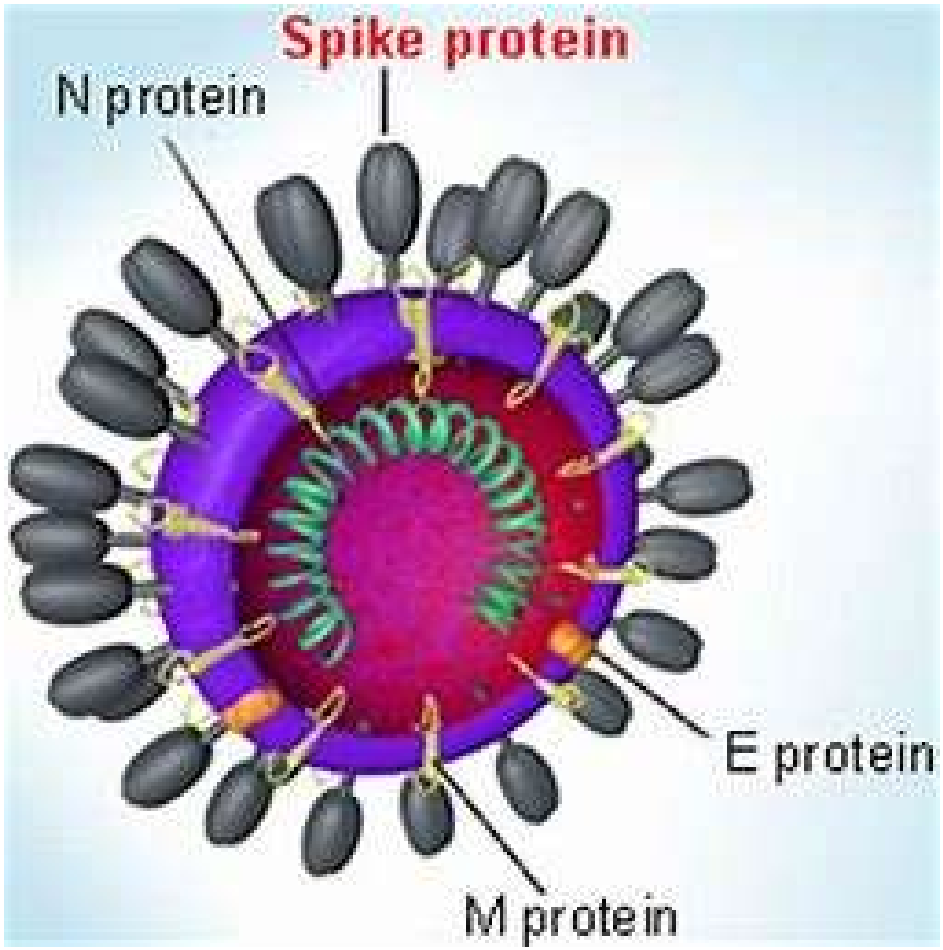
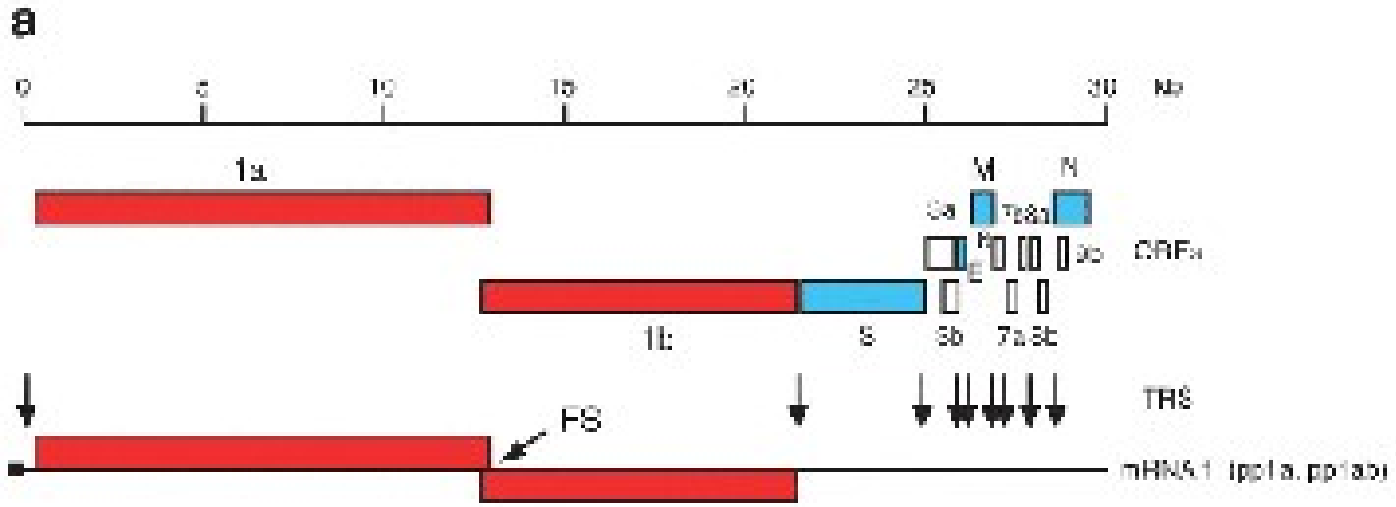
Renhong Yan et al. Science 2020;367:1444-1448

Lo «Spike»



SARS-CoV polyprotein expression and maturation



Two overlapping polyproteins [pp1a (replicase 1a, 450 kD) and pp1ab (replicase 1ab, 750 kD) mediate all the functions required for viral replication and transcription.

The functional polypeptides are released from the polyproteins **by extensive proteolytic processing**

SARS-CoV polyprotein proteolytic maturation

Polyprotein proteolytic maturation is primarily achieved by the 33.1-kD HCoV main proteinase (**Mpro**) (also called 3CLpro)

The Mpro (3CLpro) cleaves the polyprotein at no less than **11 conserved sites** involving Leu Gln (Ser,Ala,Gly) sequences

The process is initiated by the enzyme's own autolytic cleavage from pp1a and pp1ab.

SARS-CoV polyprotein proteolytic maturation

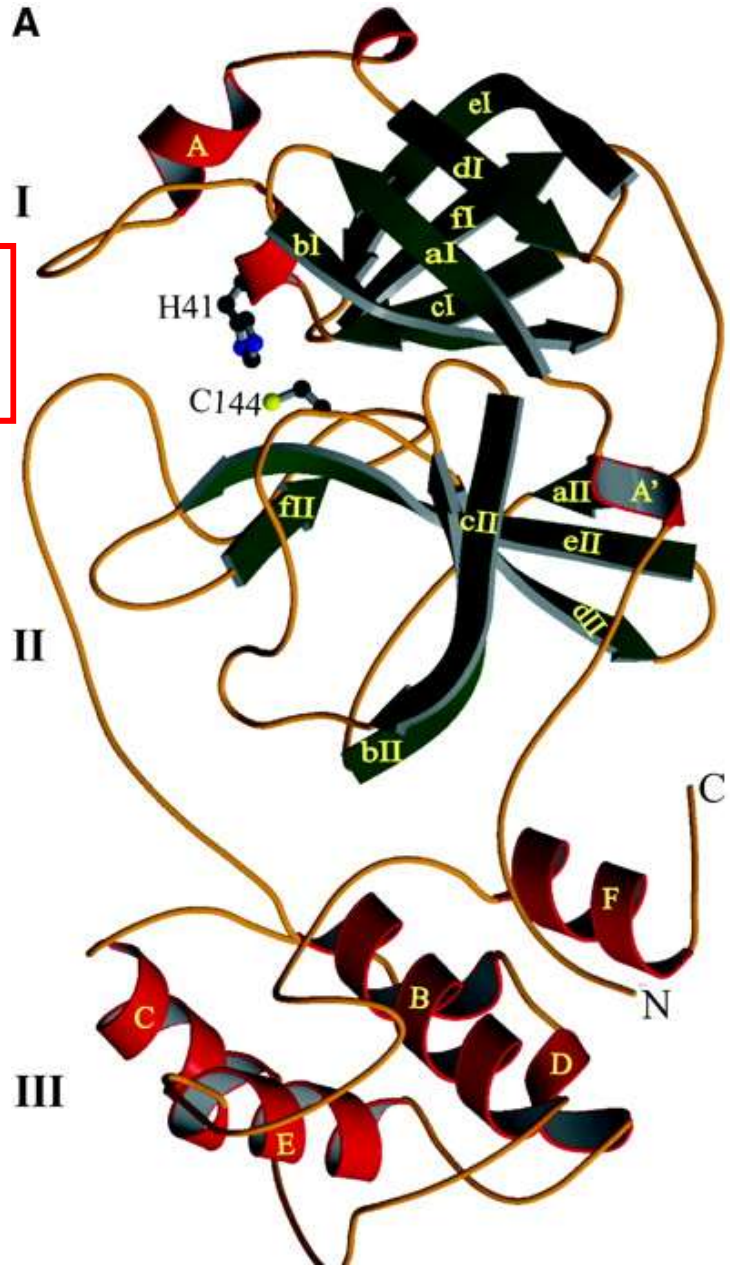
This cleavage pattern appears to be conserved in the Mpro from SARS coronavirus (SARS-CoV)

SARS-CoV polyproteins have three noncanonical Mpro cleavage sites with Phe, Met, or Val in the P2 position, but the same cleavage sites are unusual in other coronaviruses as well.

The functional importance of Mpro in the viral life cycle makes this proteinase an attractive target for the development of drugs directed against SARS and other coronavirus infections.

3D structure of coronavirus Mpro protease

catalytic dyad
Cys^{144} and His^{41}



Kanchan Anand et al. Science 2003;300:1763-1767

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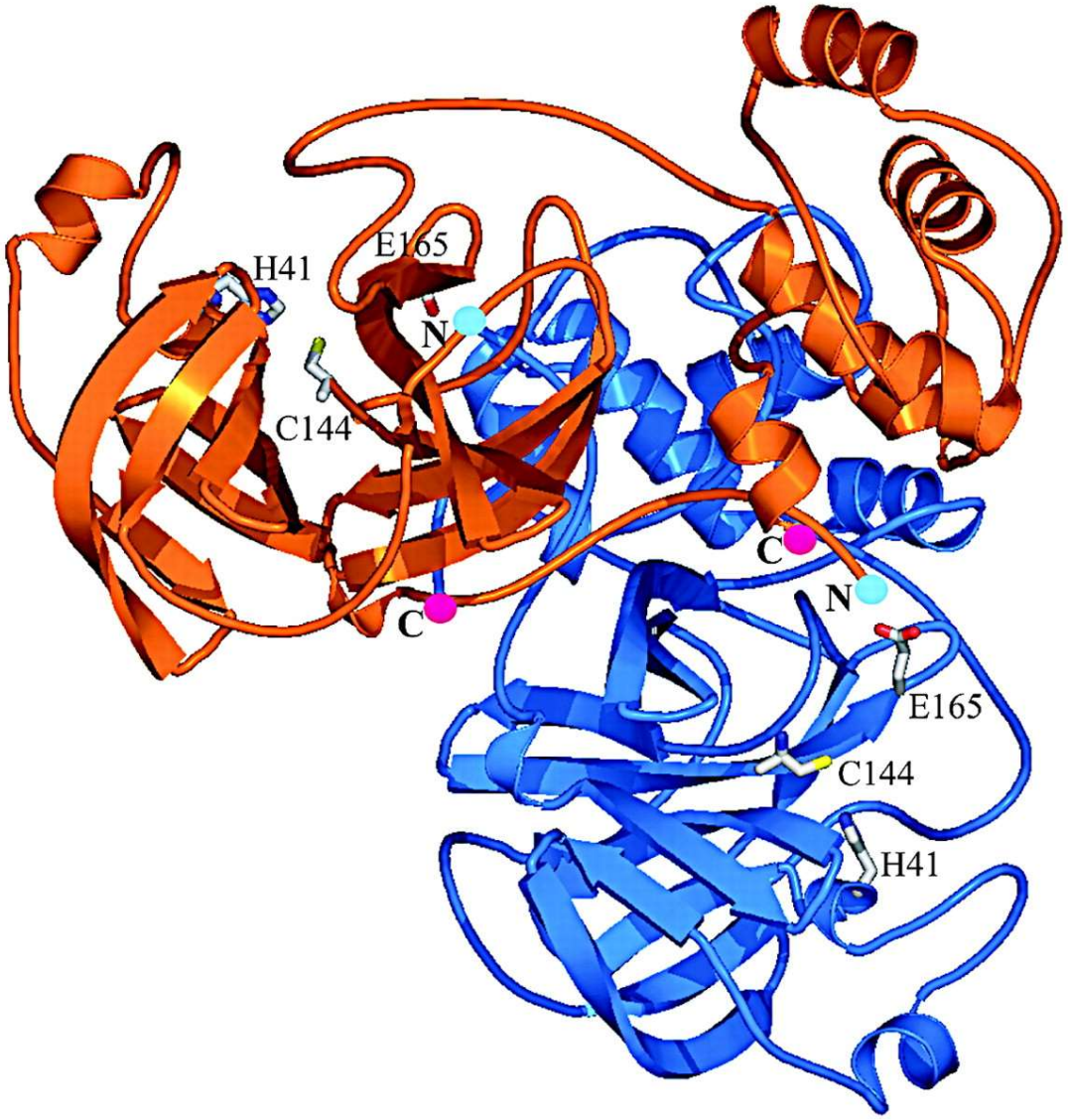
Fig. 2. Dimer of HCoV Mpro.



Kanchan Anand et al. Science 2003;300:1763-1767

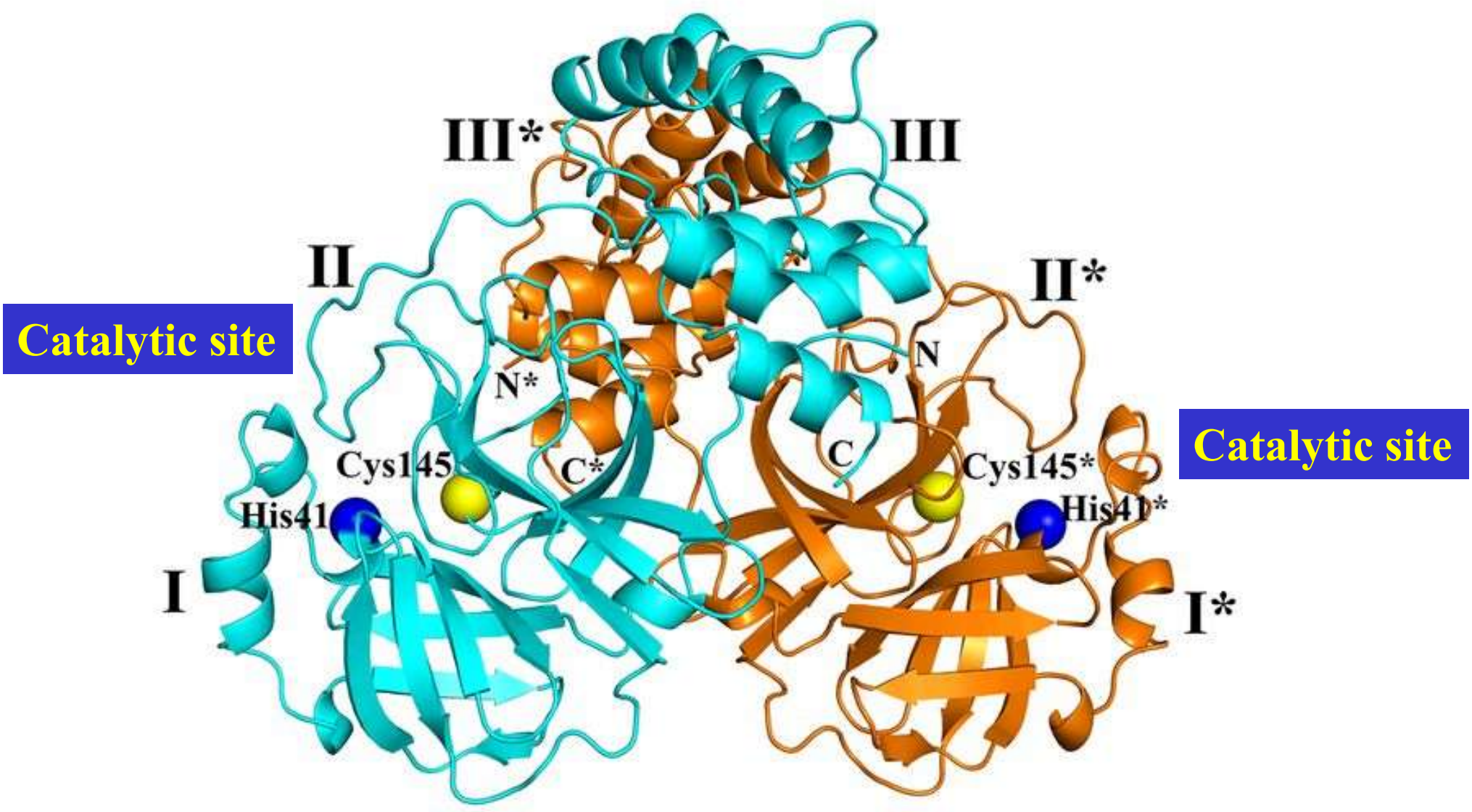
Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors

by Linlin Zhang, Daizong Lin, Xinyuanyuan Sun, Ute Curth, Christian Drosten, Lucie Sauerhering, Stephan Becker, Katharina Rox, and Rolf Hilgenfeld

Science
Volume ():eabb3405
March 23, 2020

Science
AAAS

Fig. 2 Three-dimensional structure of SARS-CoV-2 Mpro dimer



Linlin Zhang et al. Science 2020;science.abb3405

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COVID-19 Lezione 1

Scientists are racing to learn the secrets of severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2), which is the cause of the pandemic disease COVID-19.

The structures and functional studies provide a basis for the development of therapeutics targeting the crucial interaction between the Spike ACE2 and proteases needed for virus function and maturation.