Controlling the dimerization of SARS-CoV-2 main protease to design broad spectrum antivirals

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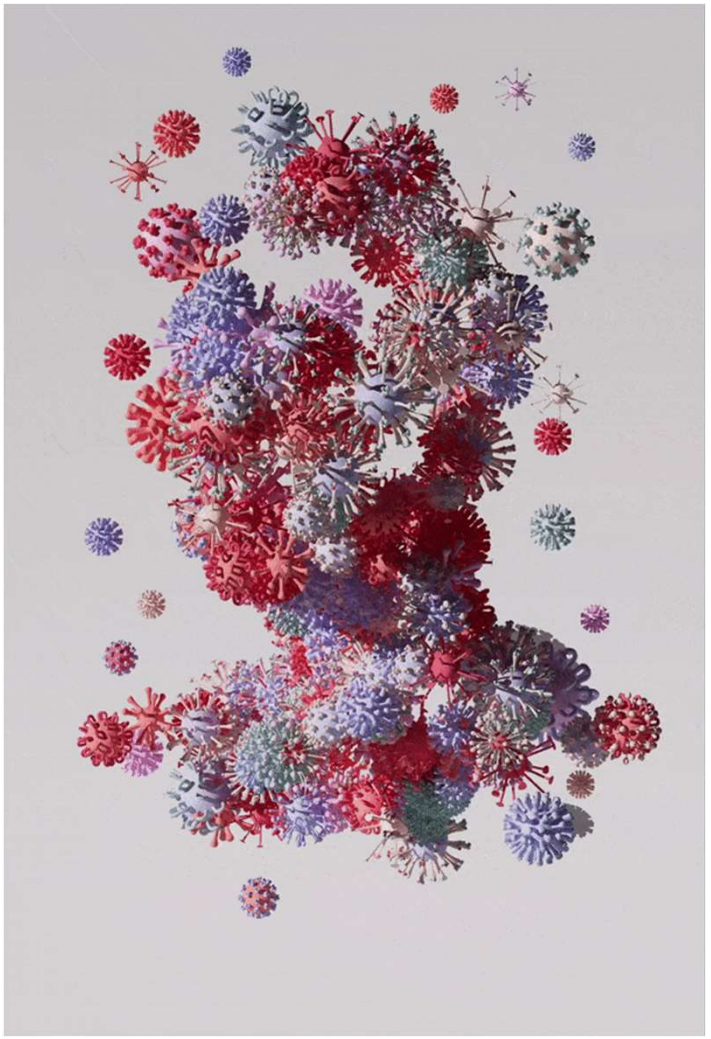
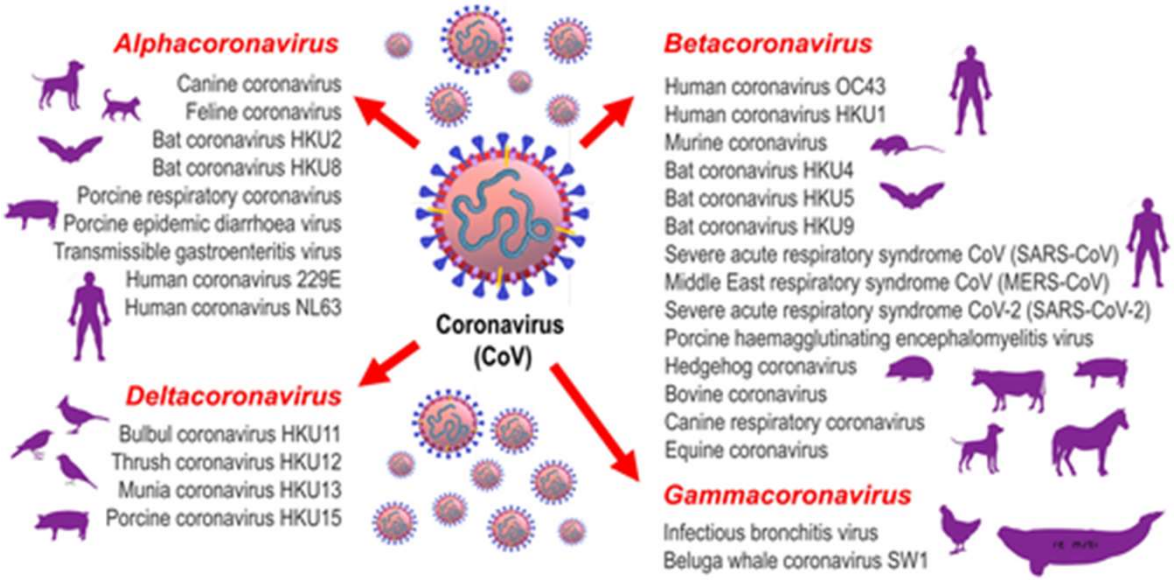
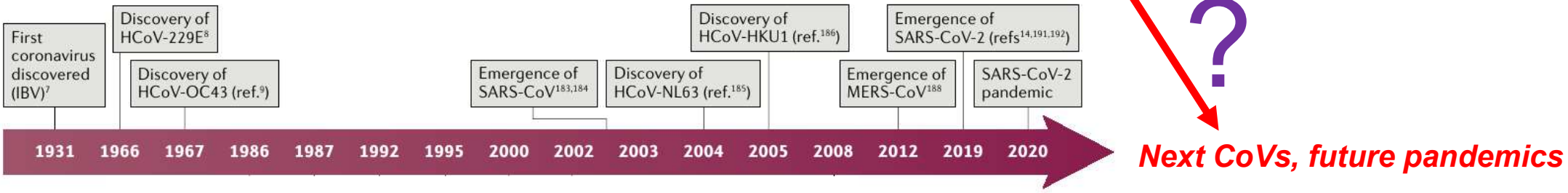
Comez L., Libera V., Macchiarulo A.,
Schiaroli E., Francisci D., Orecchini A., Sacchetti F., Petrillo C.



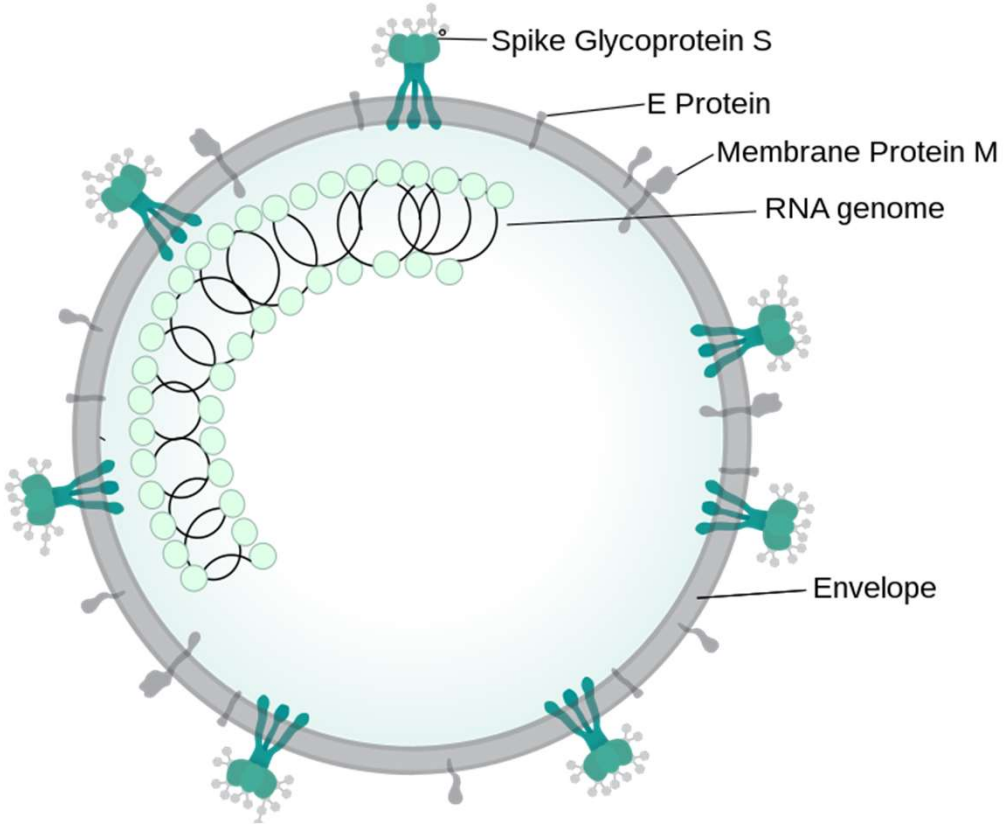
Ambiti PTSR: Fisica delle Biomolecole
e dei Materiali Avanzati per Terapia, Nanoscienze

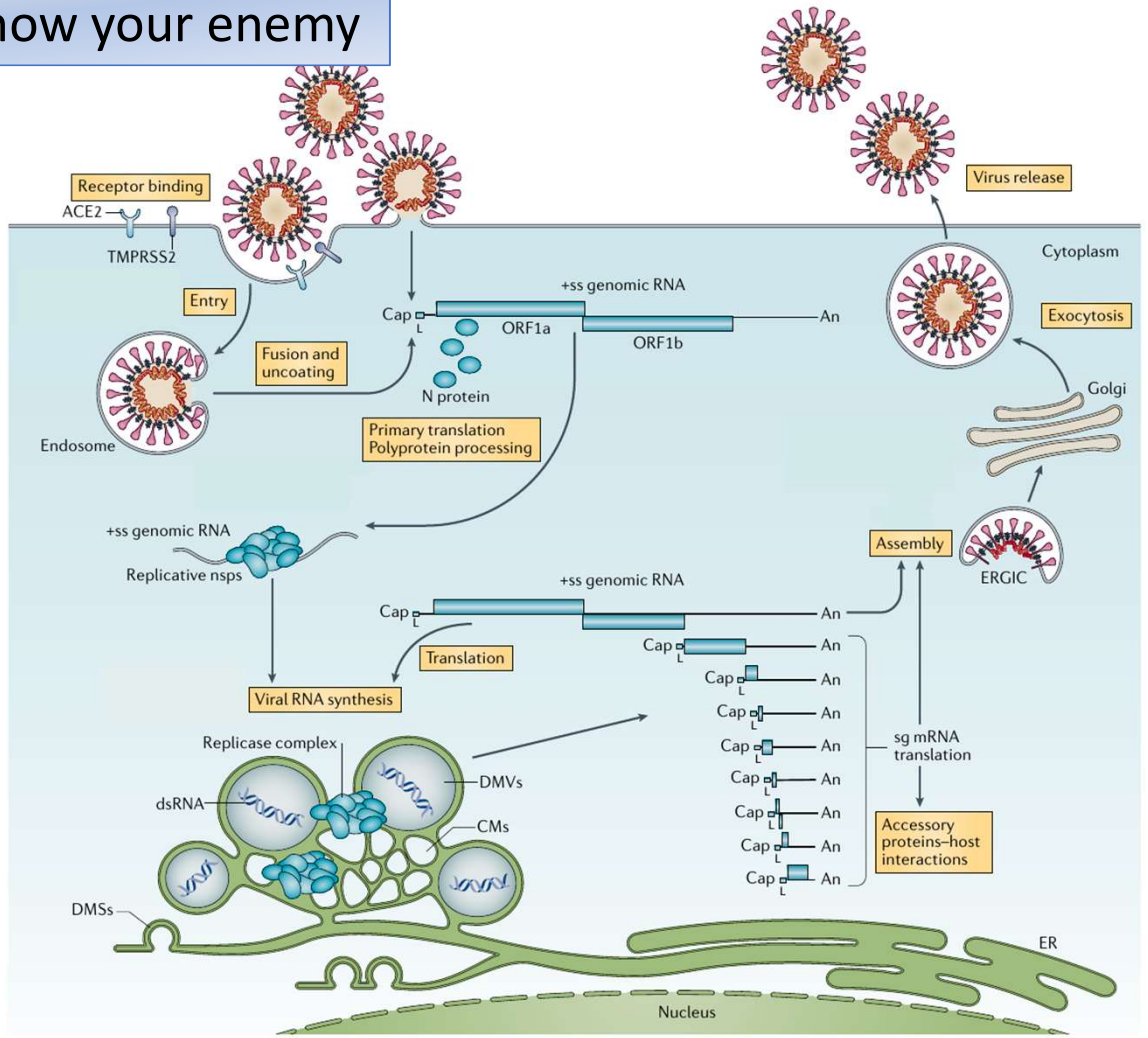
Toward broad spectrum antivirals





SARS-CoV-2: know your enemy





Disrupting SARS-CoV-2 life cycle

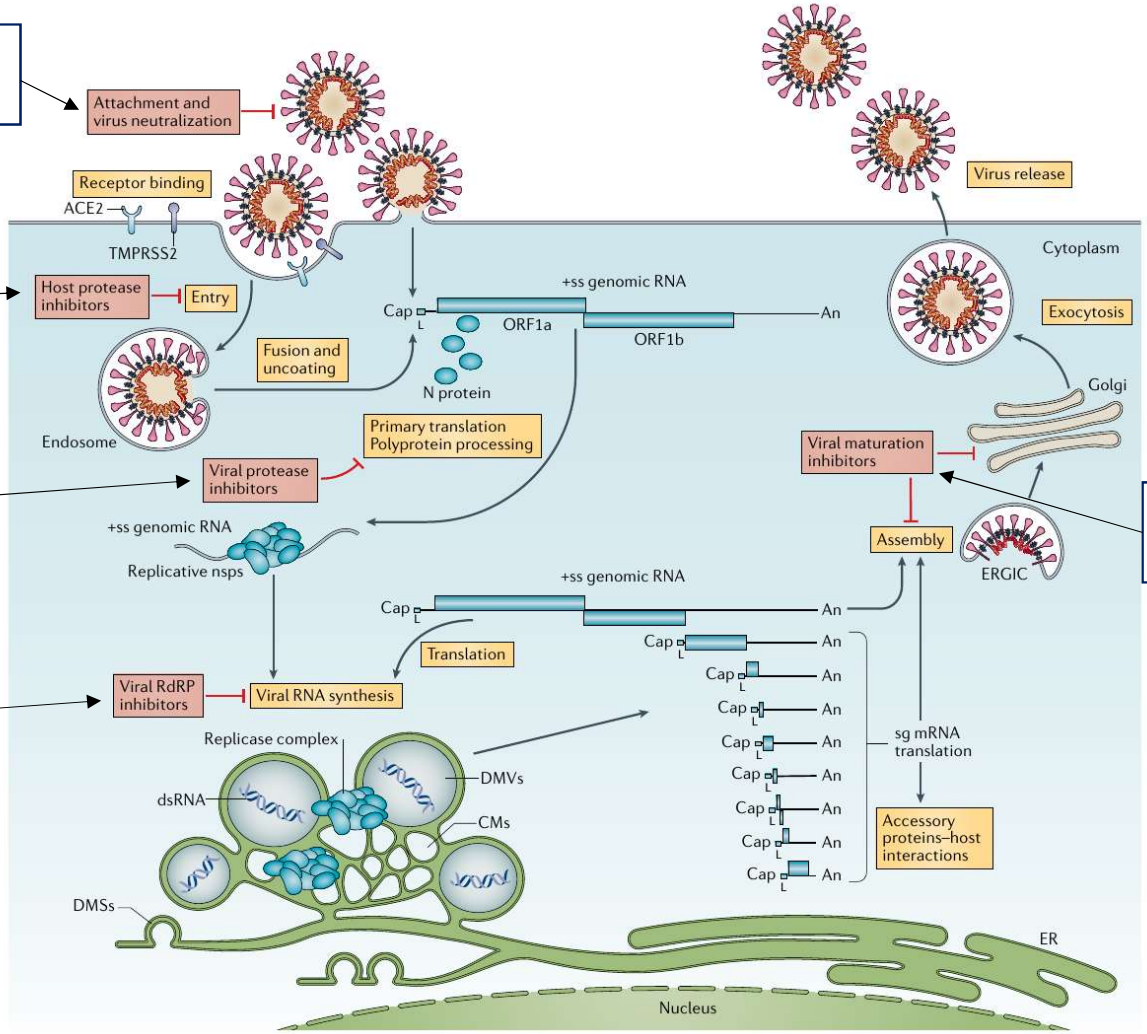
Vaccines, monoclonal antibodies

Antivirals (zapnometinib)

Antivirals (paxlovid)

Antivirals (remdesivir, molnupiravir)

Antivirals (oseltamivir)



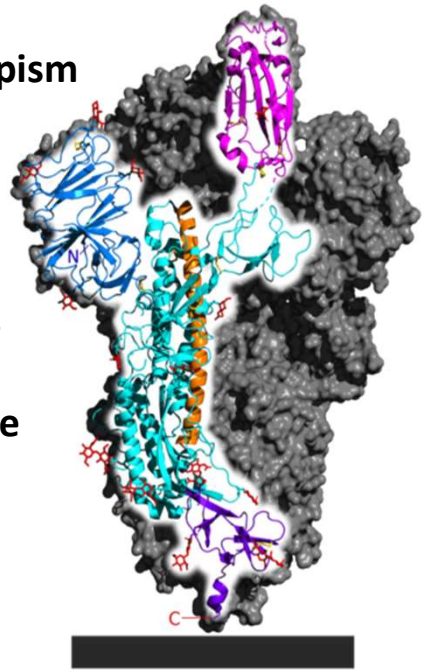
The major target (so far) Spike protein (S)

Host range and **cell tropism**

Highly **immunogenic**

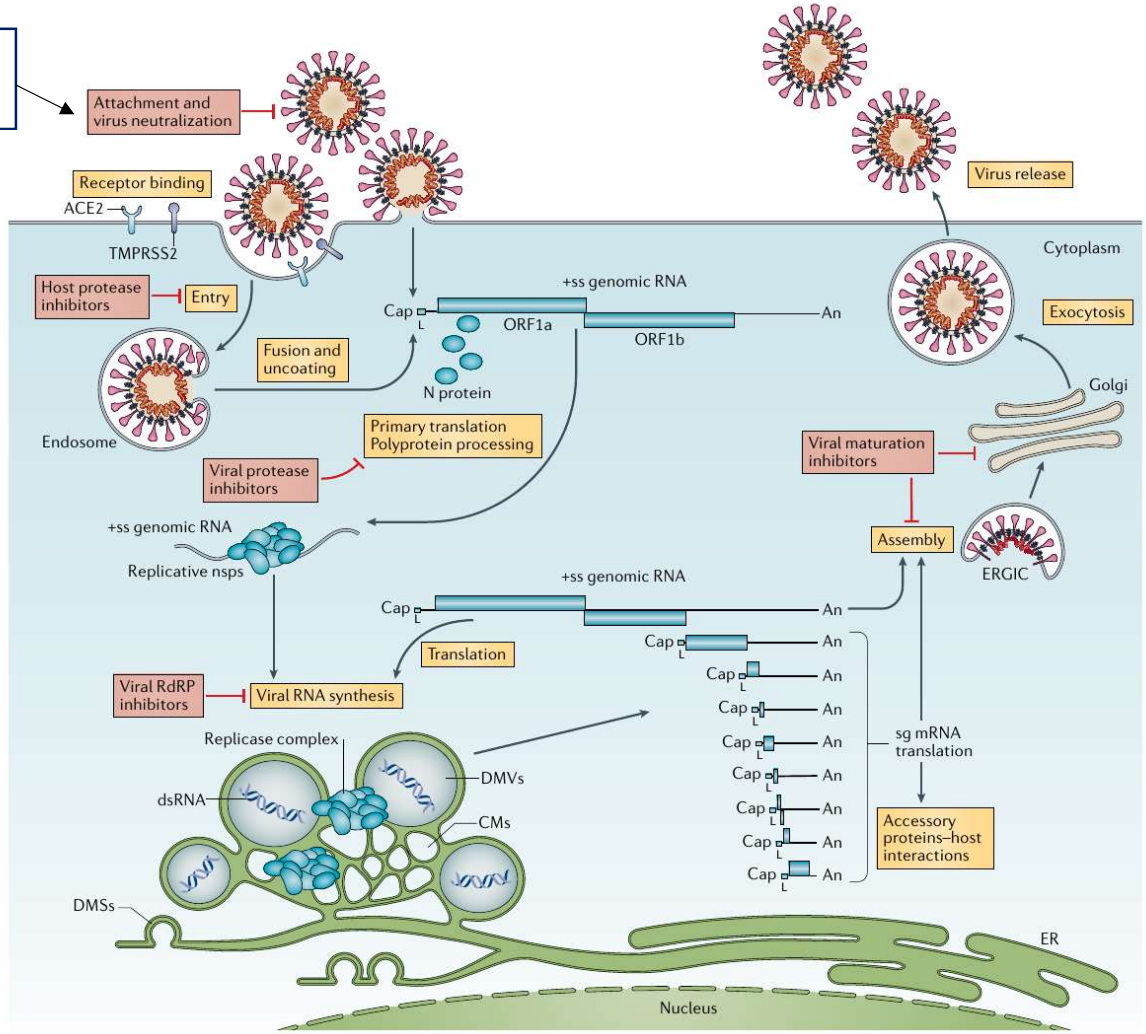
Target for **vaccines** and **monoclonal antibodies**

Very high evolution rate of the spike gene

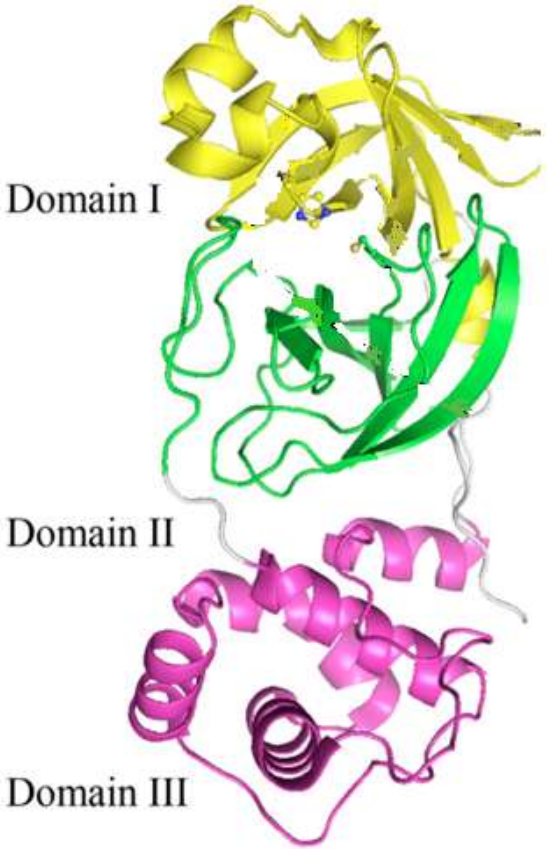


S1

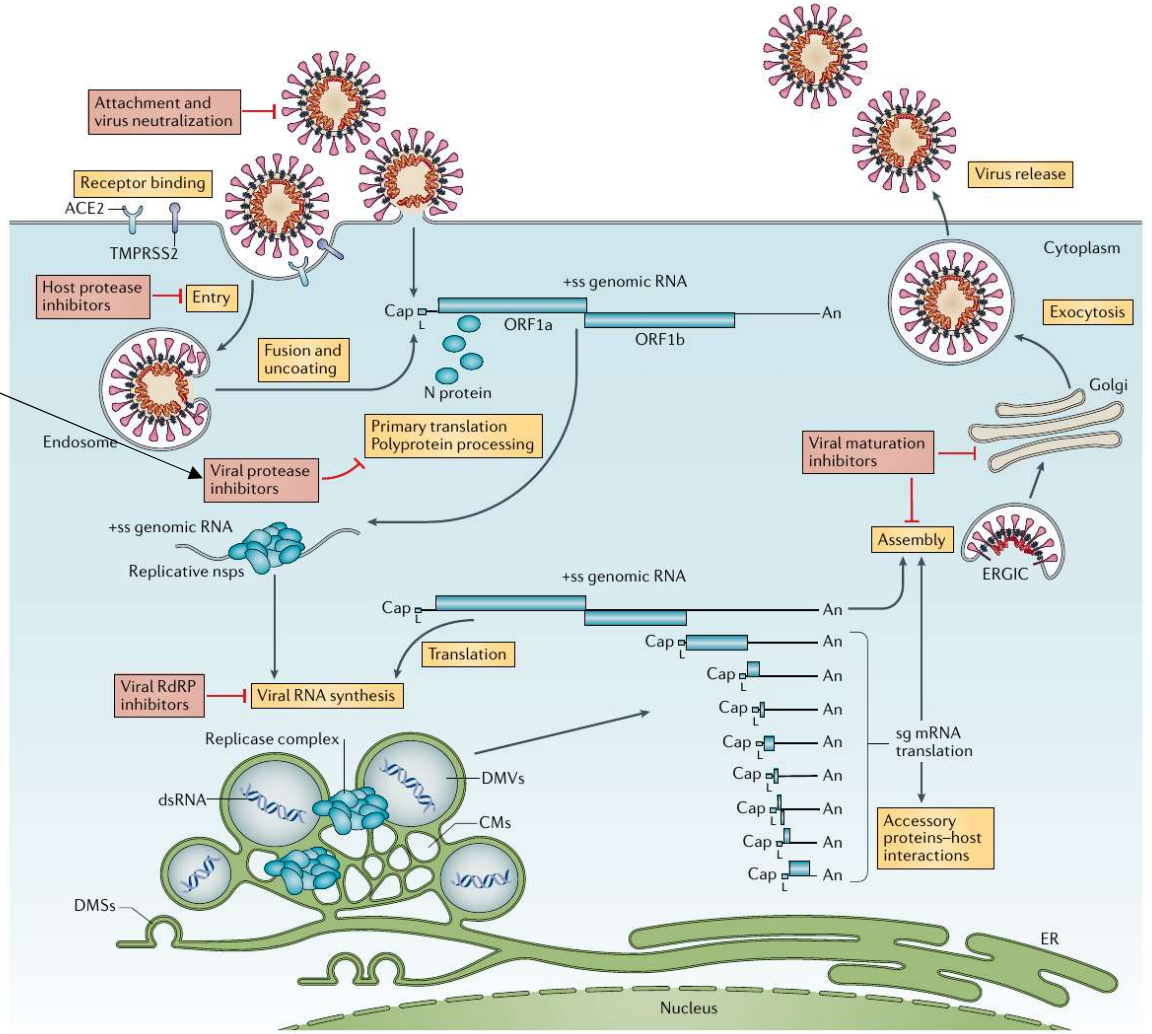
S2



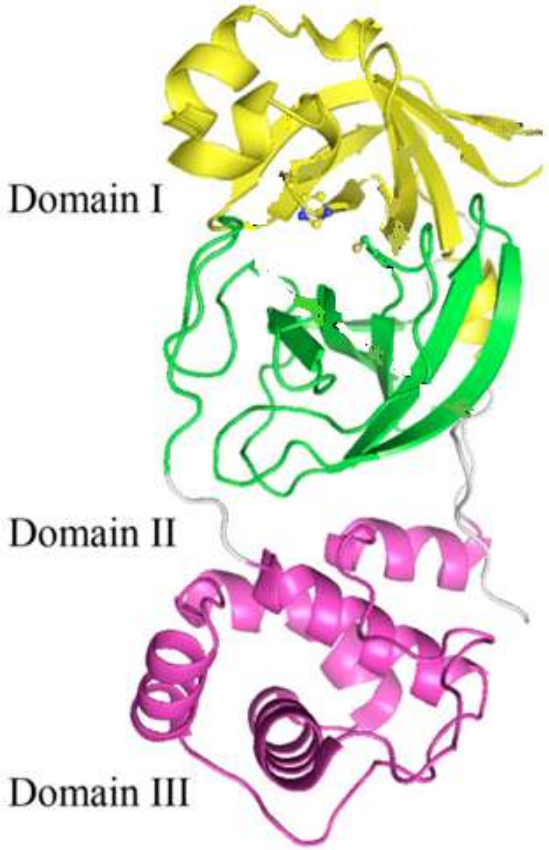
An actual and promising target
Main Protease (Mpro)

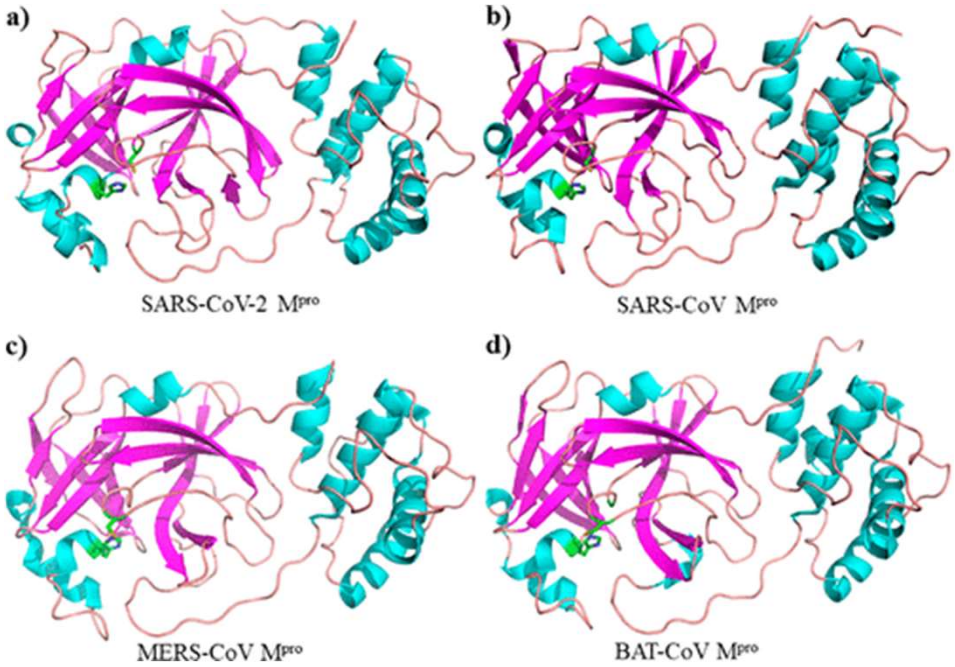


Antivirals (paxlovid)

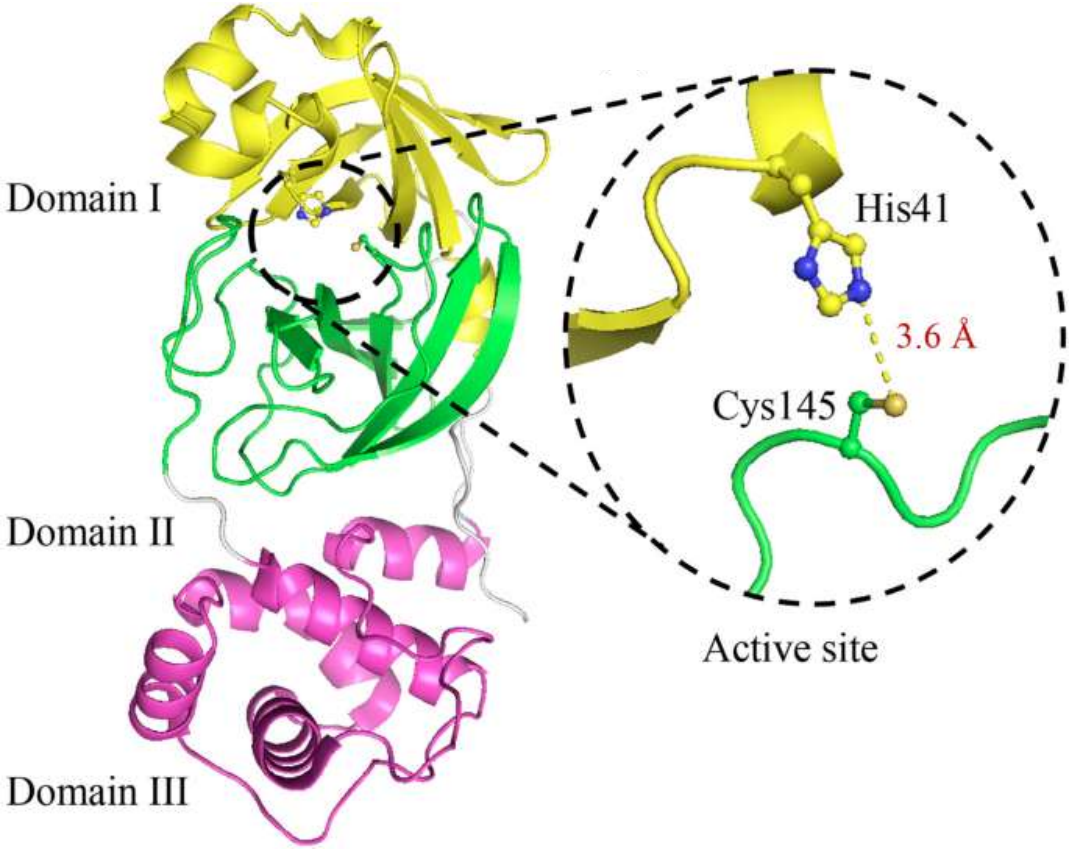


Mpro is highily conserved among CoVs

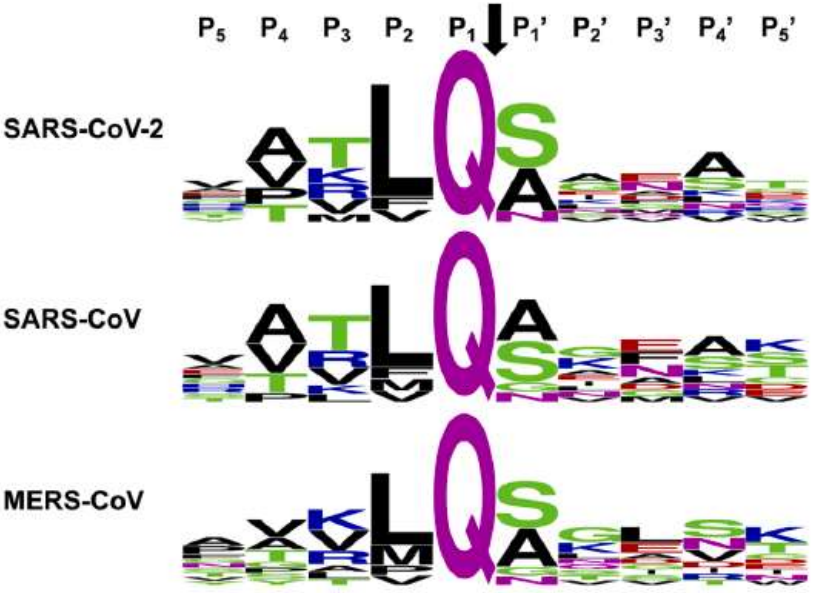




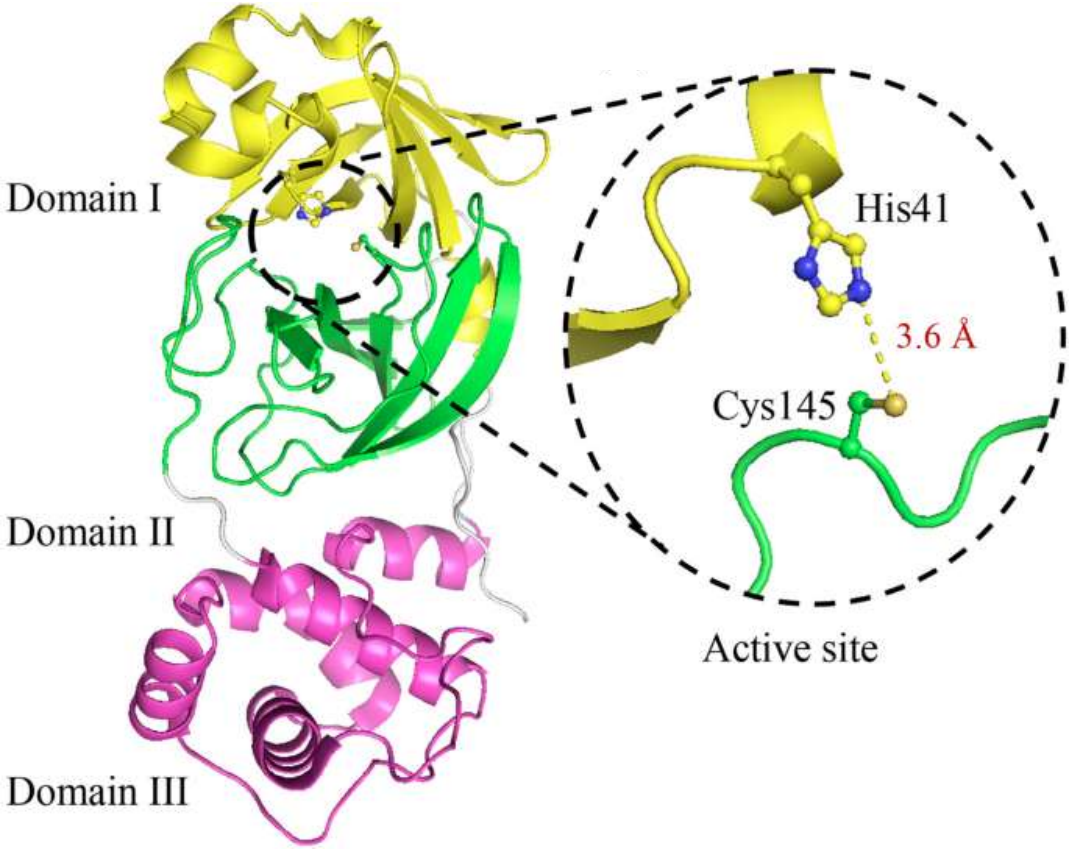
Mpro has a specific catalytic action



The **catalytic dyad** exclusively cleaves polypeptide sequences after a glutamine residue. **No human host-cell proteases** are known with this substrate specificity.



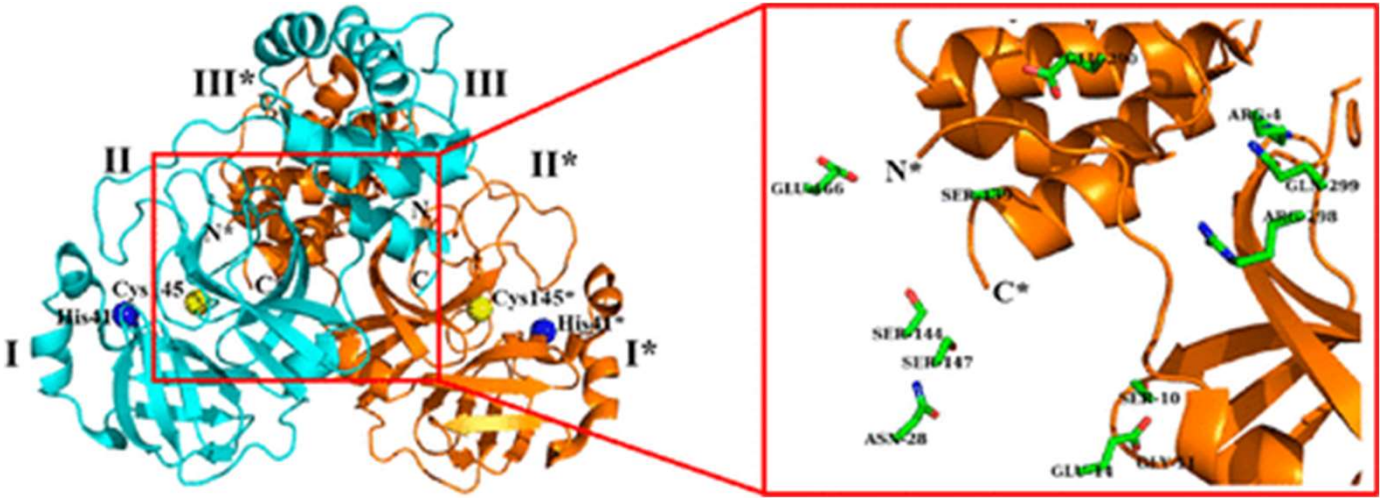
Mutations and Mpro



Mutation in Mpro is often **lethal to the virus** => SARS-CoV-2 variants tend to have a **very few mutations** in Mpro.

```
                                                                                *
SARS-CoV-2     1 SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIR
SARS-CoV       1 SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDTVYCPRHVICTAEDMLNPNYEDLLIR

SARS-CoV-2    61 KSNHNFLVQAG---NVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLAC
SARS-CoV      61 KSNHSFLVQAG---NVQLRVIGHSMQNCLLRLKVDTSNPKTPKYKFVRIQPGQTFSVLAC

                                                  *
SARS-CoV-2   118 YNGSPSGVYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDL
SARS-CoV     118 YNGSPSGVYQCAMRPNHTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDL

SARS-CoV-2   178 EGNFYGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKY
SARS-CoV     178 EGKFYGPFVDRQTAQAAGTDTTITLNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKY

SARS-CoV-2   238 NYEPL-TQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDV
SARS-CoV     238 NYEPL-TQDHVDILGPLSAQTGIAVLDMCAALKELLQNGMNGRTILGSTLLEDEFTPFDV

SARS-CoV-2   297 VRQCSGVTFQ
SARS-CoV     297 VRQCSGVTFQ
```

Mpro is a dimer



Mpro is a homodimer consisting of two monomers that are arranged almost perpendicular to one another.

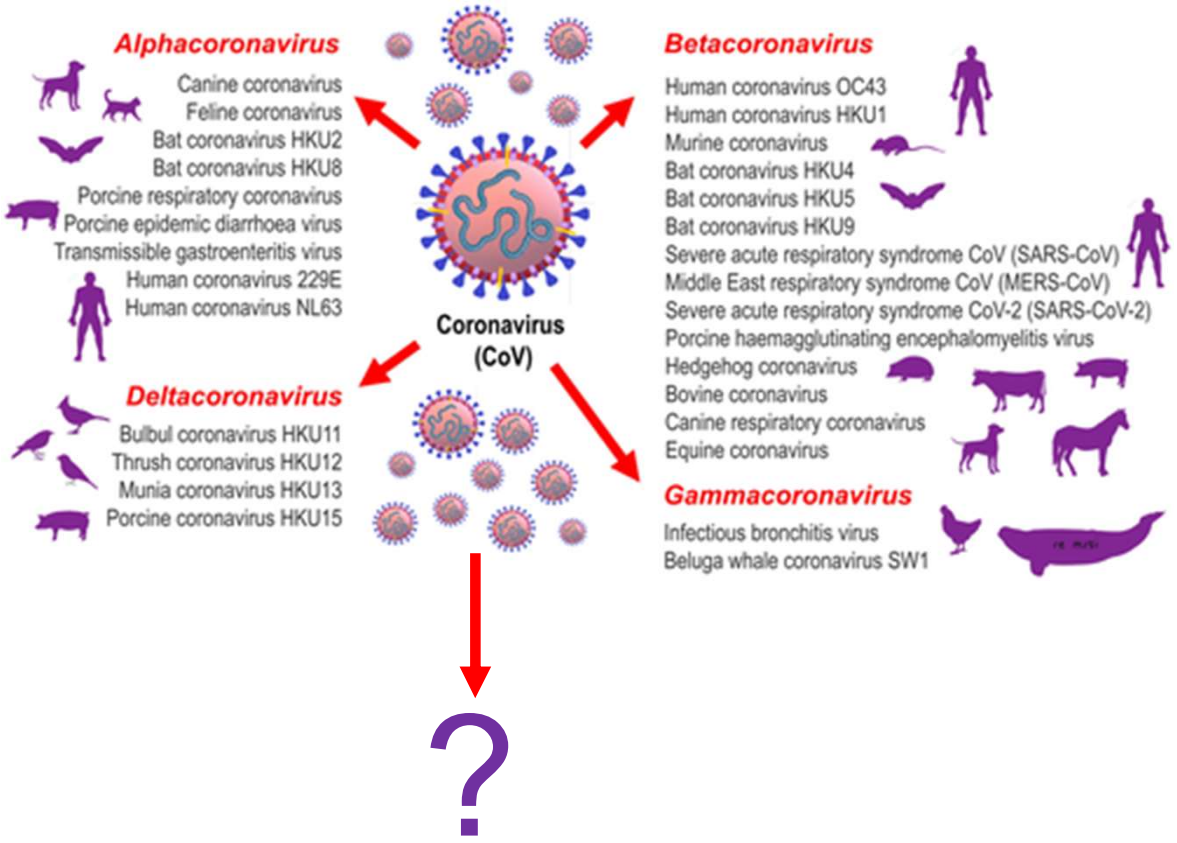
The **dimerization** is necessary for enzymatic activity.

Toward broad spectrum antivirals

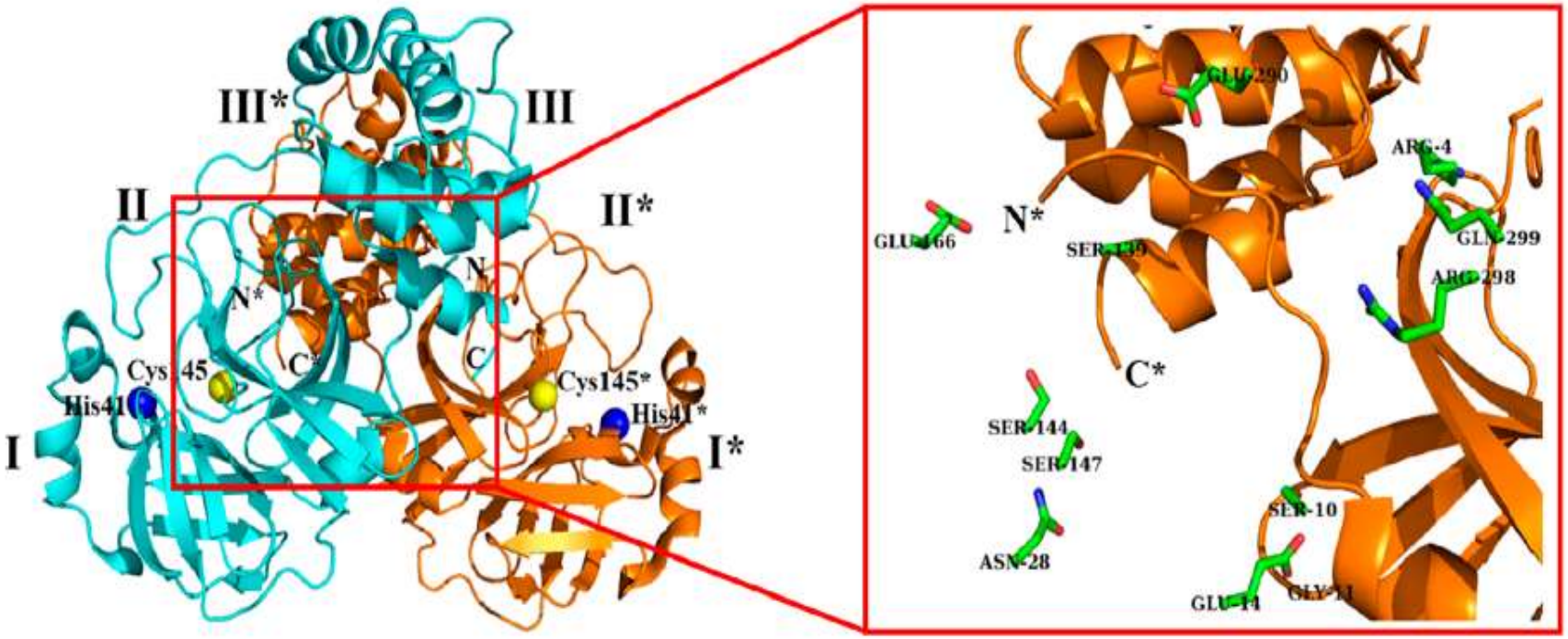
Targeting Mpro

1) **reduced risk** of **mutation-mediated** drug resistance

2) **antivirals** with **broad-spectrum activity**



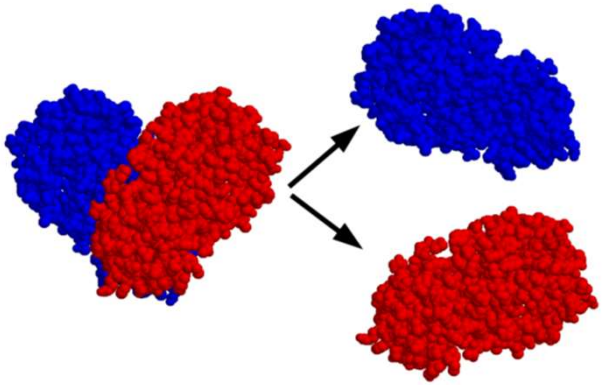
Inhibiting the Mpro: dimerization interface



Mpro equilibrium dimerization

$$M_2^{pro} \rightleftharpoons 2M_1^{pro}$$

$$K_D = \frac{[M_1^{pro}]^2}{[M_2^{pro}]} = \frac{2Cx_1^2}{1-x_1} = e^{-\Delta G_D/(RT)}$$



scientific reports

Check for updates

OPEN

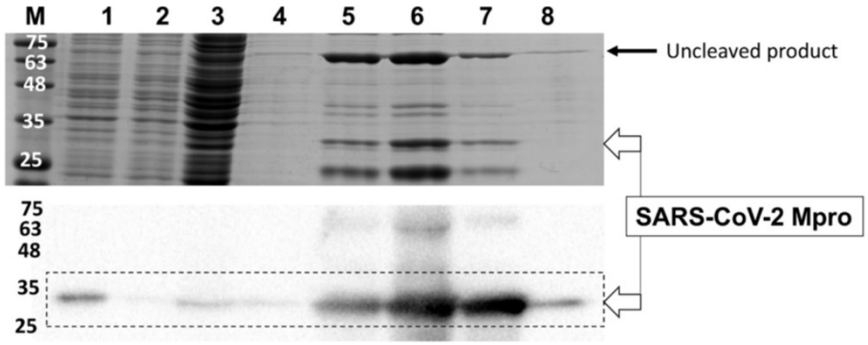
The dimer-monomer equilibrium of SARS-CoV-2 main protease is affected by small molecule inhibitors

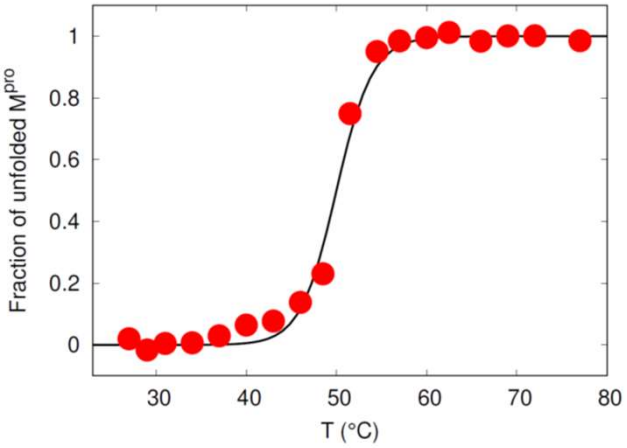
Lucia Silvestrini[1], Norhan Belhaj[2], Lucia Comez[3], Yuri Gerelli[2], Antonino Lauria[4], Valeria Libera[5], Paolo Mariani[2], Paola Marzullo[4], Maria Grazia Ortore[2], Antonio Palumbo Piccionello[4], Caterina Petrillo[5], Lucrezia Savini[1], Alessandro Paciaroni[5] & Francesco Spinozzi[2]✉

K_D=(2.5±0.2) μM Zhang, L. et al. Science 368, 409–412 (2020)

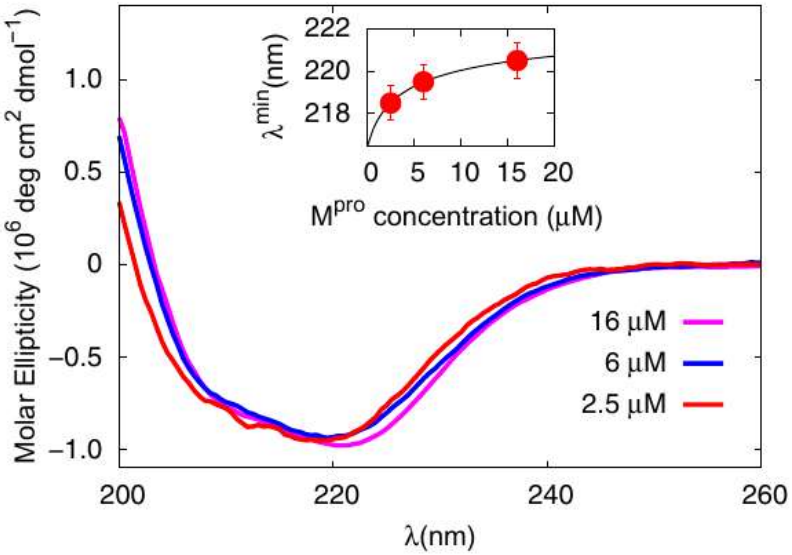
K_D=(0.14±0.03) μM El-Baba, T. J. et al. Angew. Chem. Int. Ed., 59, 23544 – 23548 (2020)

Producing (UNIVPM, MASBIC) Characterizing Mpro (UNIPG)





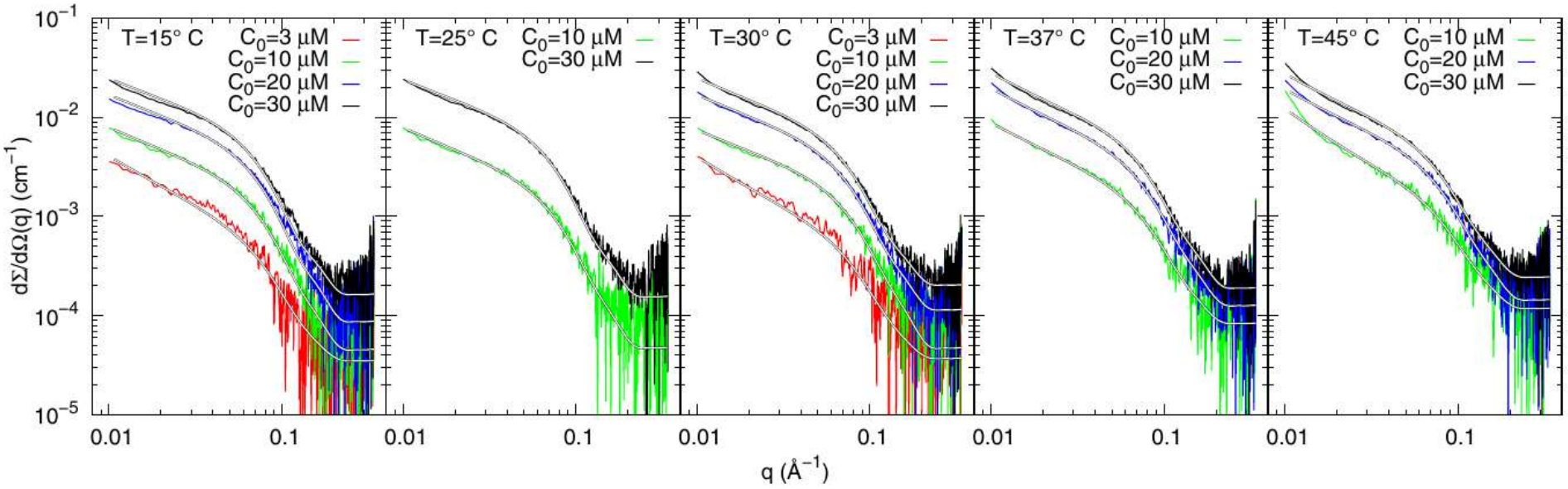
Mpro dimerization: far-UV CD (UNIPG)



$$K_D = 7 \pm 1\,\mu\mathrm{M}$$

Since April 2020

Mpro dimerization: SAXS (UNIVPM, UNIPG)

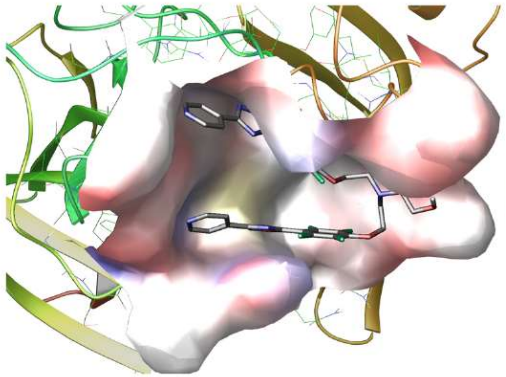


$$\frac{d\Sigma}{d\Omega}(q) = N_A \kappa C_N P(q) S_M(q)$$

$$P(q) = x_1 P_1(q) + \frac{1}{2}(1 - x_1) P_2(q)$$

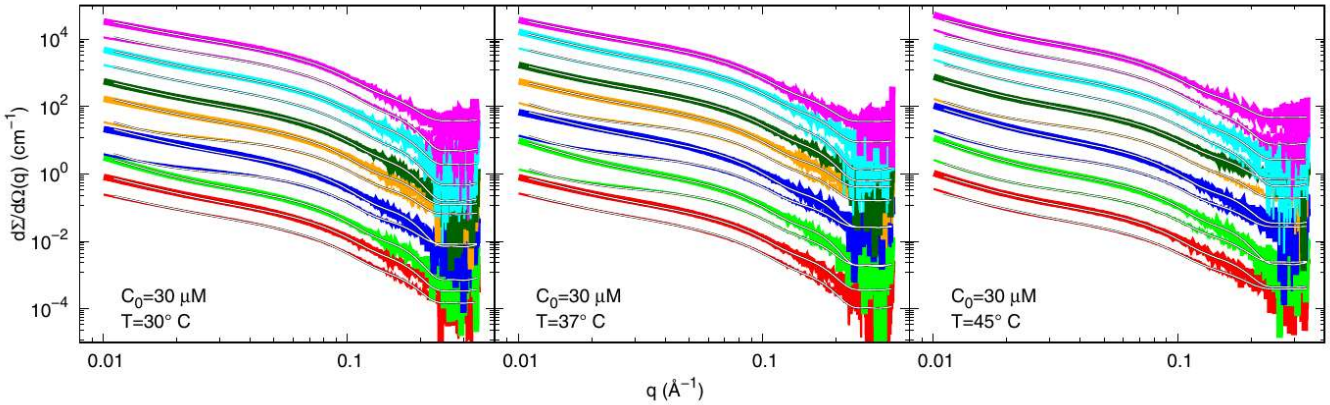
$$K_D = 7 \pm 1\,\mu\mathrm{M}$$

In-silico inhibitor selection (UNIPA)



Inhibitor	IFD_score
1	−584.958
2	−578.774
3	−591.618
4	−580.827
5	−584.772
6	−583.655
7	−588.468
13b	−594.758

Mpro dimerization with small molecules: SAXS (UNIVPM, UNIPG)



60 μM Inhibitor		1	2	3	4	5	6	7
K_D°	(μM)	26 ± 4	8 ± 6	7 ± 4	5 ± 4	19 ± 7	30 ± 10	30 ± 10

PROTECT project

Biophysics

Pharmaceutical, Biophysical features

Virology Cellular tests

Optimizing Mpro Inhibitors

Dip. Fis. e Geo. CNR-IOM

Dip. Sci. Farm.

Clin. Mal. Infett. Dip. Med. e Chir.

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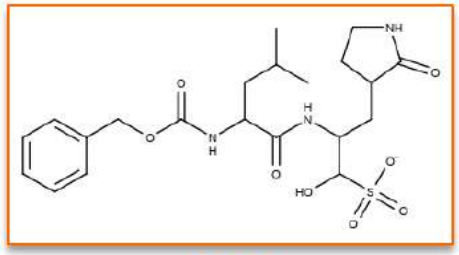
STUDIUM GENERALE CIVITATIS PERUSINI A.D. MCCCVIII

A.D. 1308 unipg UNIVERSITÀ DEGLI STUDI DI PERUGIA

PROTECT
Drug repurposing

MicroScale Thermophoresis Standard Assay (Lab. Prof. A. Macchiarulo, *Dip. Sci. Farm.*)

Compound	K_d (µM) 0 mM DTT
Lopinavir	n.d.
Ritonavir	n.d.
GC-376	0.17±0.04
Nelfinavir	> 1000



GC-376

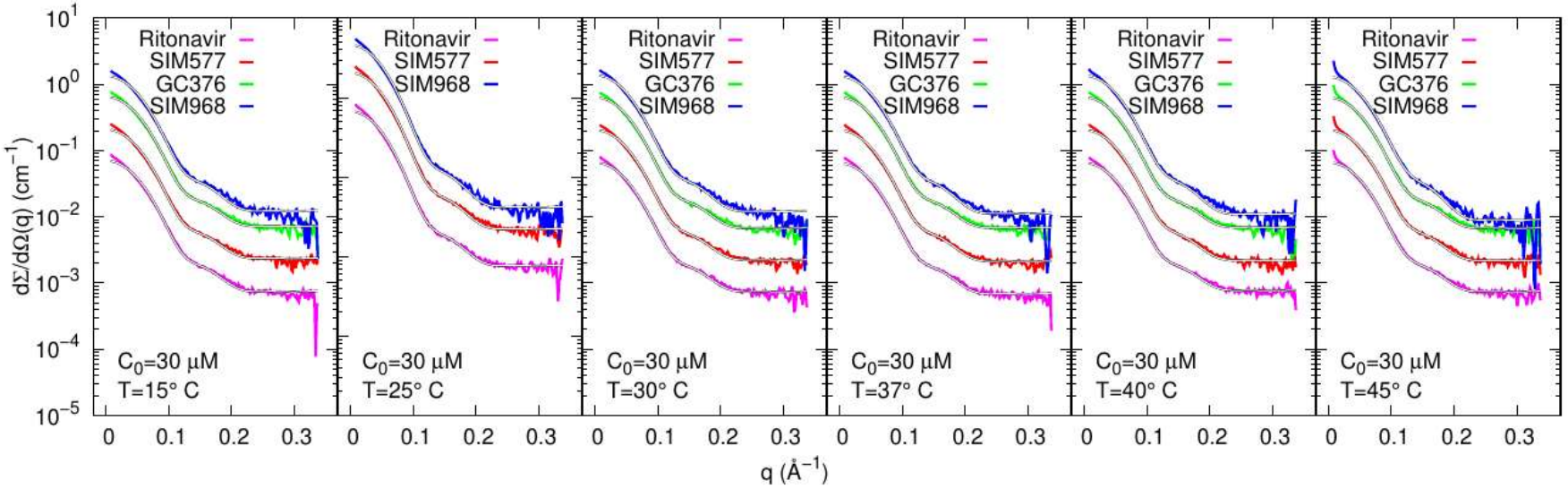
PROTECT
Drug repurposing

Titolazione con plaque assay dei surnatanti a 48 h (Clinica Malattie Infettive, Dip. Med. Chir. Prof. D. Francisci, Dr. E. Schiaroli)

GC376 → **EC50 0.74** µM

Remdesivir → **EC50 1.4** µM

PROTECT
Drug repurposing



30 μM Inibithor		Ritonavir	GC376
K_D°	(μM)	0.05±0.05	0.01±0.01

Fis.Geo.
L. Comez
C. Petrillo
A. Orecchini
V. Libera

Sci. Farm.
A. Macchiarulo Lab

Diamond SLS, Oxford
N. Belhaj

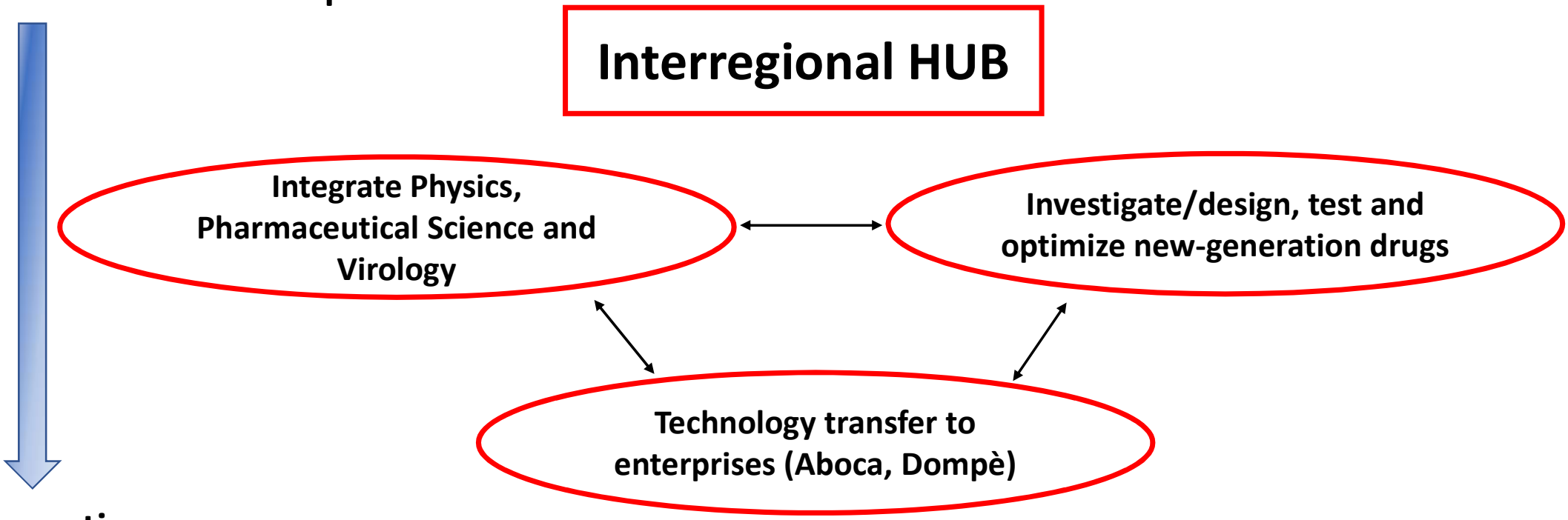
Mal. Inf., Med. Chir.
D. Francisci
E. Schiaroli

DISVA UNIVPM
F. Spinozzi
P. Mariani
M.G. Ortore

Tec. Bio. Chim. Farm. UNIPA
A. Palumbo Piccionello

Foresights

Research and Development



Innovation

Societal challenge: Health, demographic change and wellbeing, Emerging diseases

Actions and Strategy

Azioni collaborative di Ateneo (1.2 Sviluppo di prodotti e tecniche innovative diagnostiche e terapeutiche e 4.2 Nanoscienze e Nanotecnologie)

PNRR MUR (Ecosistema Innovazione, Centro Nazionale, Partenariato Esteso)

C-Labs di Ateneo (Imaging e Spettrometria)

Risorse Umane (Ateneo)

Progetto PROTECT (Fondazione CRPG)