



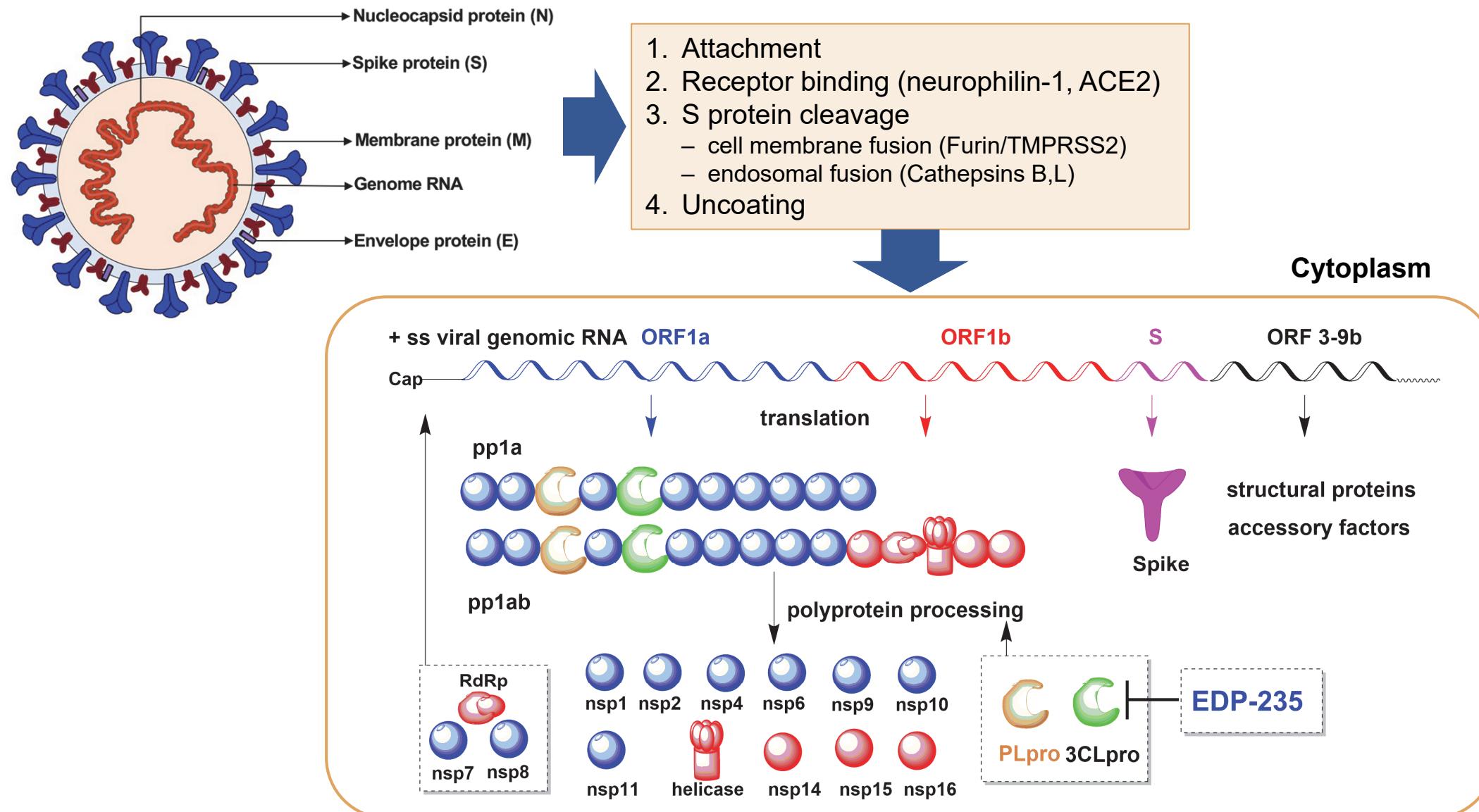
Molecular Basis for the Antiviral Action of EDP-235: A Potent and Selective SARS-CoV-2 3CLpro Inhibitor

Anand Balakrishnan

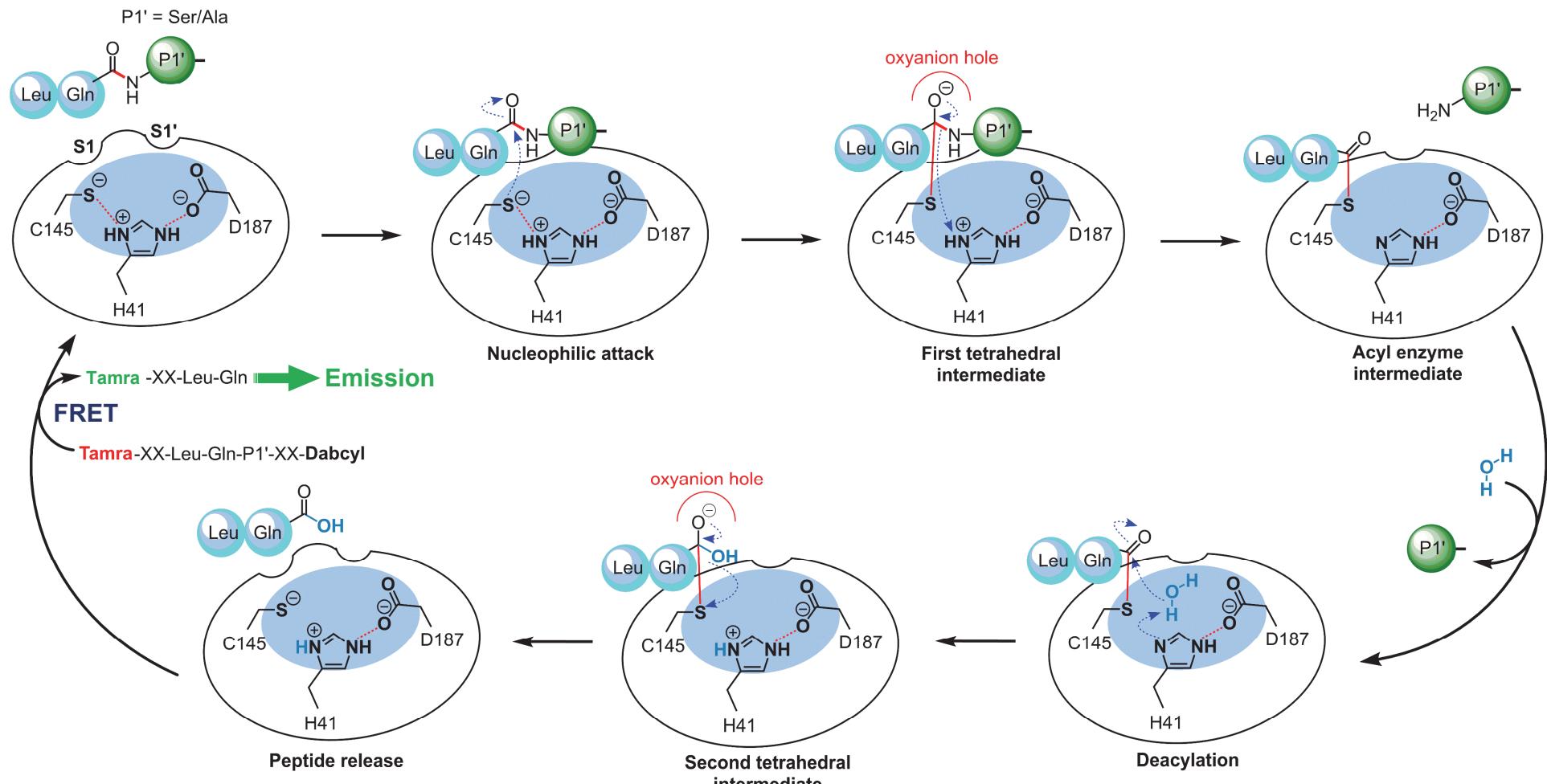
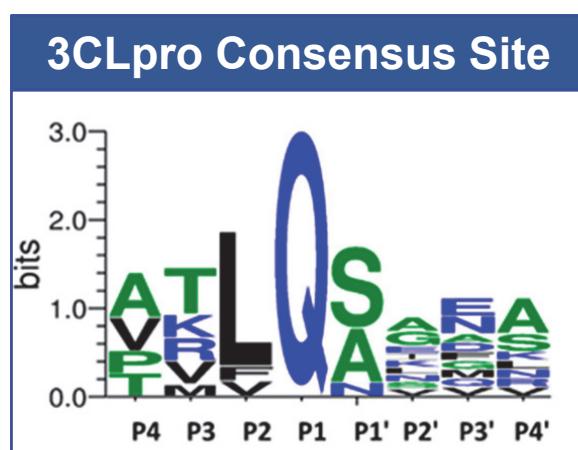
2022 Annual Meeting of ASBMB
April 4th, 2022



SARS-CoV-2 life cycle and enzyme targets for antiviral development



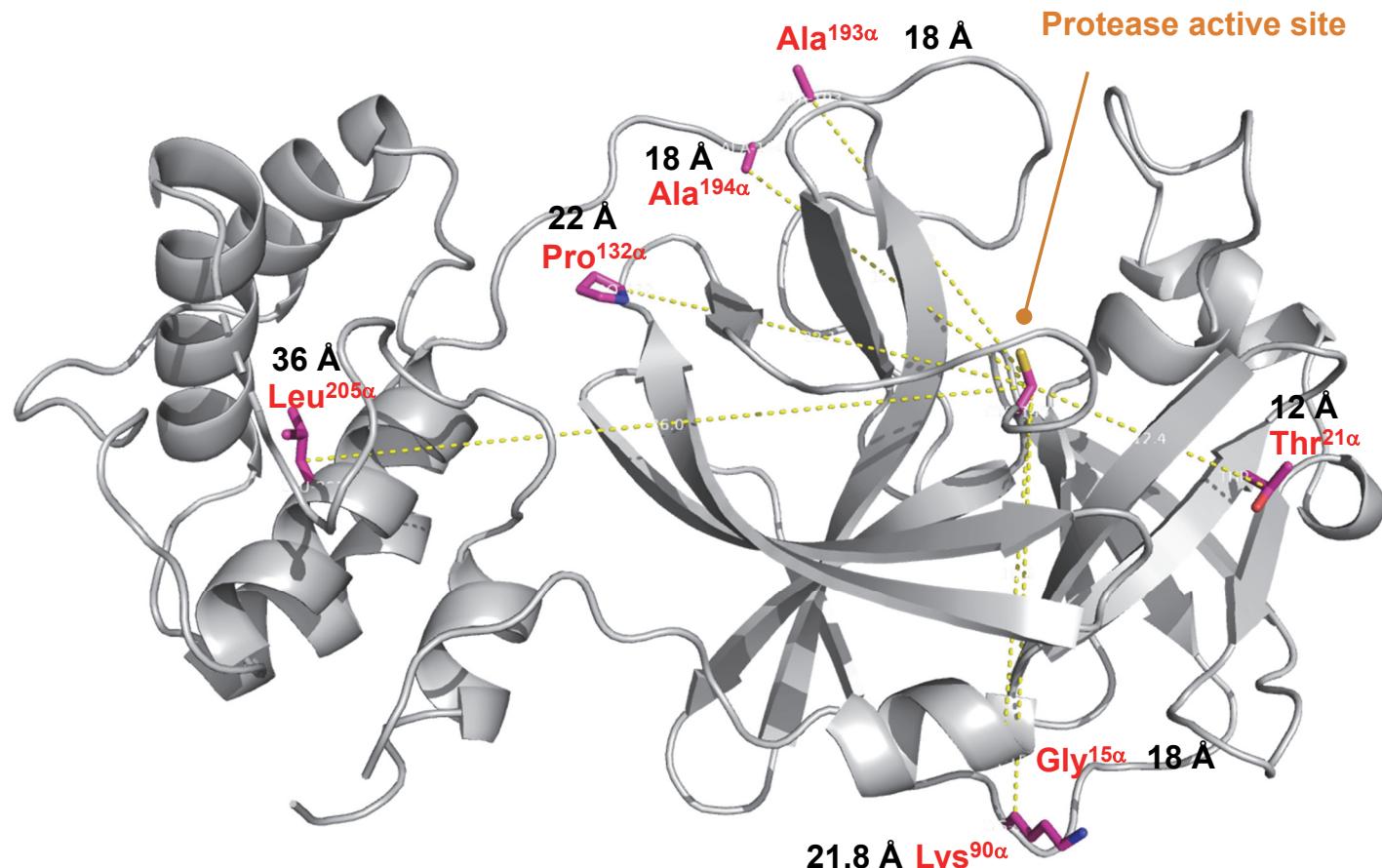
Catalytic cycle of 3C-like protease from SARS-CoV-2



Catalytic activity monitored with FRET [fluorescence resonance energy transfer] assay

EDP-235 is a highly potent 3CLpro inhibitor and retains activity against SARS-CoV-2 variants

- 3CLpro is highly conserved across SARS-CoV-2 variants
- All variant enzymes were active in protease biochemical assays

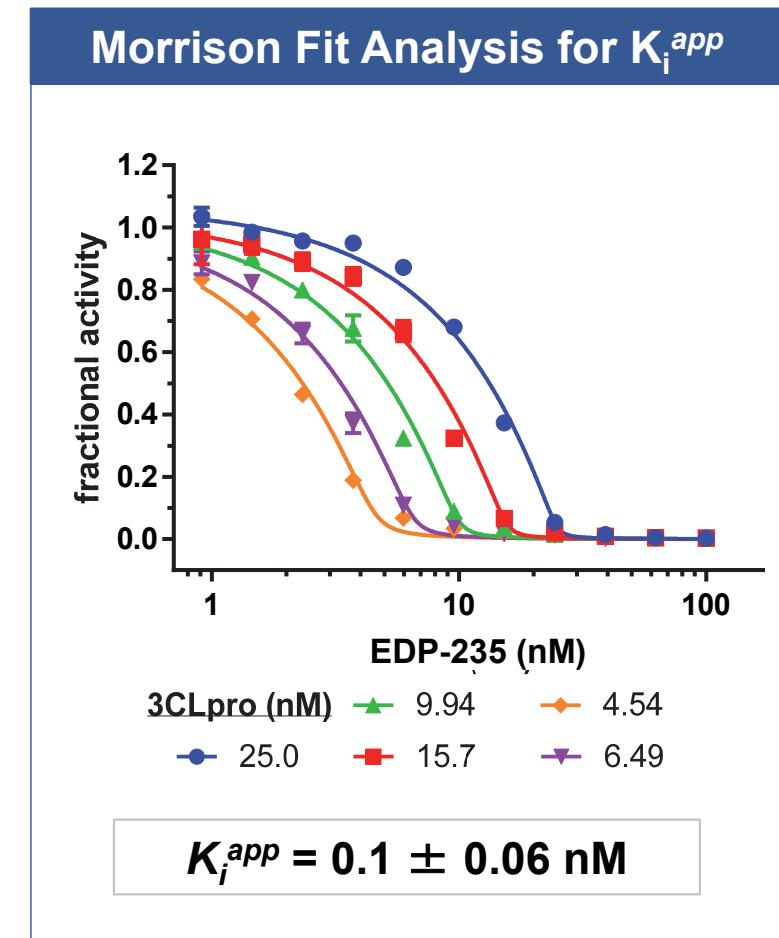
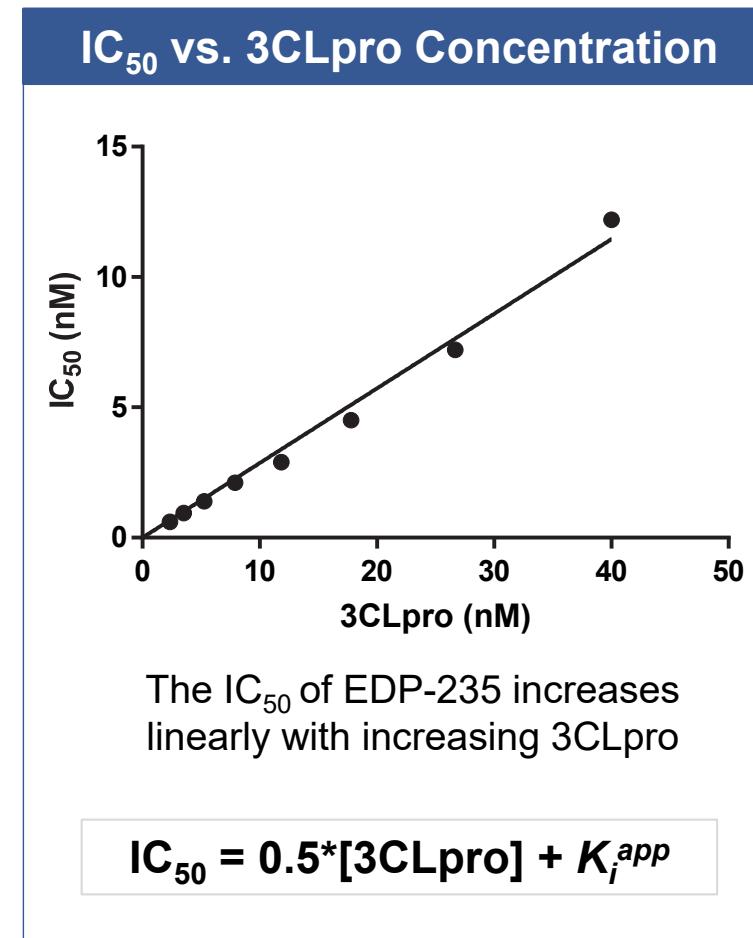
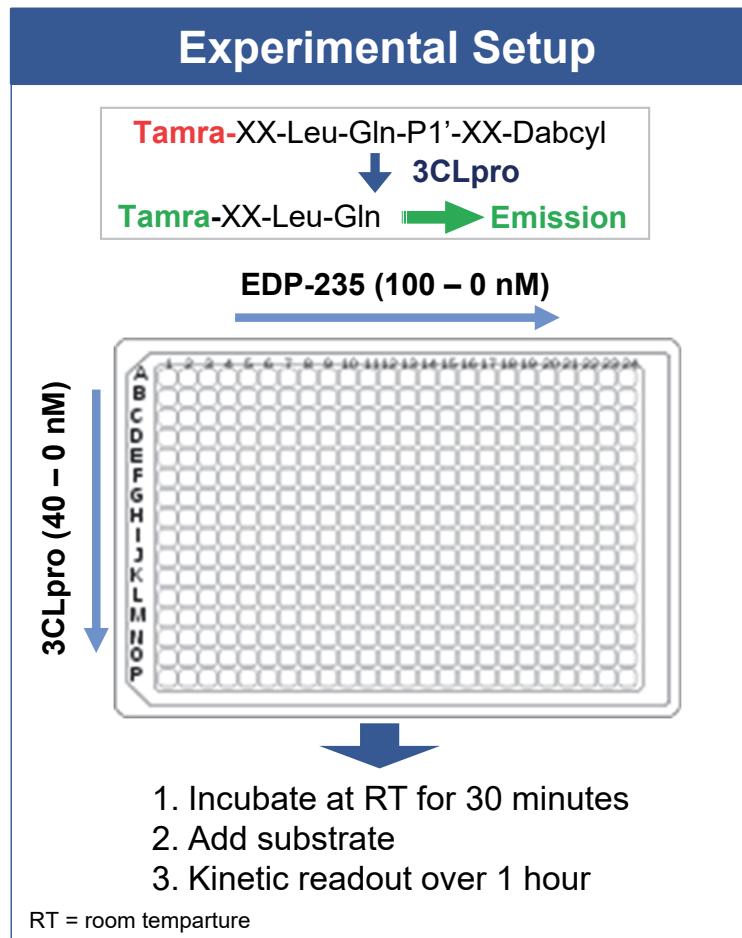


3CLpro Enzyme Assay			
SARS-CoV-2 Lineage	WHO Classification	3CLpro Mutation	EDP-235 IC ₅₀ (nM)
A	n/a	-	5.8 ± 3.7
B.1.351	Beta	K90R	2.8 ± 0.9
B.1.351.2	Beta	K90R/A193V	5.4 ± 1.0
P.2	Zeta	L205V	3.4 ± 1.0
B.1.617.3	n/a	A194S	5.7 ± 0.5
B.1.1.318	n/a	T21I	2.0 ± 0.1
C.36.3, C.37	n/a	G15S	4.7 ± 2.5
B.1.1.529	Omicron	P132H	4.1 ± 0.8

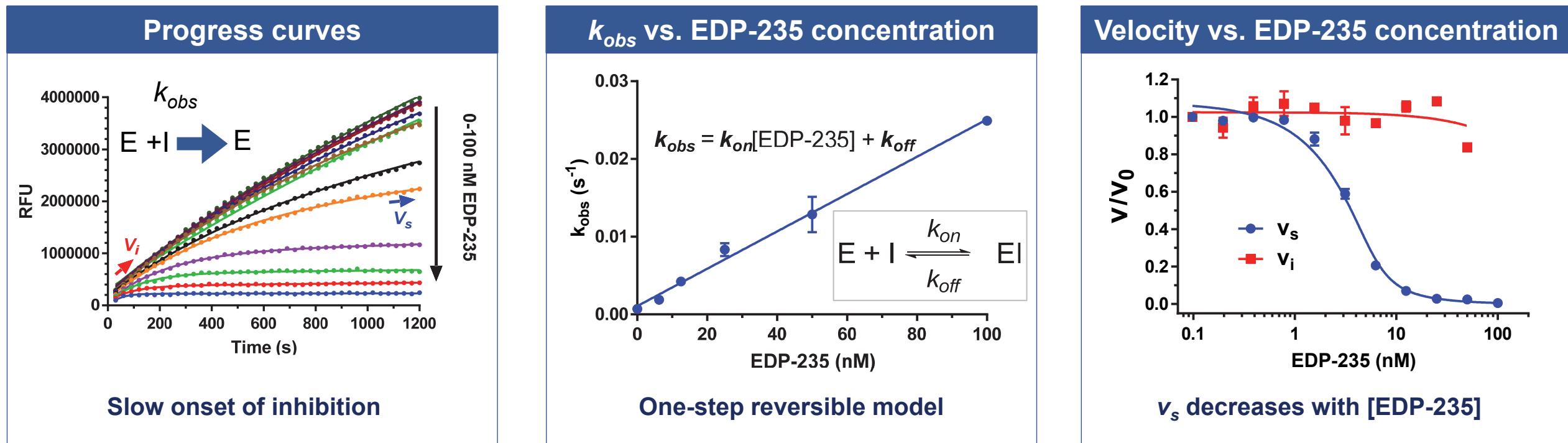
Live Virus			
SARS-CoV-2 Lineage	WHO Classification	3CLpro Mutation	EDP-235 EC ₅₀ (nM)
A	n/a	-	5.1
B.1.617.2	Delta	-	4.3
B.1.1.529	Omicron	P132H	7.3

IC₅₀ = half-maximal inhibitory concentration. *The 3CLpro sequences for the ancestral A lineage and B.1.617.2 (Delta) variant are identical. Antiviral activity determined in the presence of a p-glycoprotein inhibitor.

EDP-235 is a tight-binding inhibitor of SARS-CoV-2 3CLpro



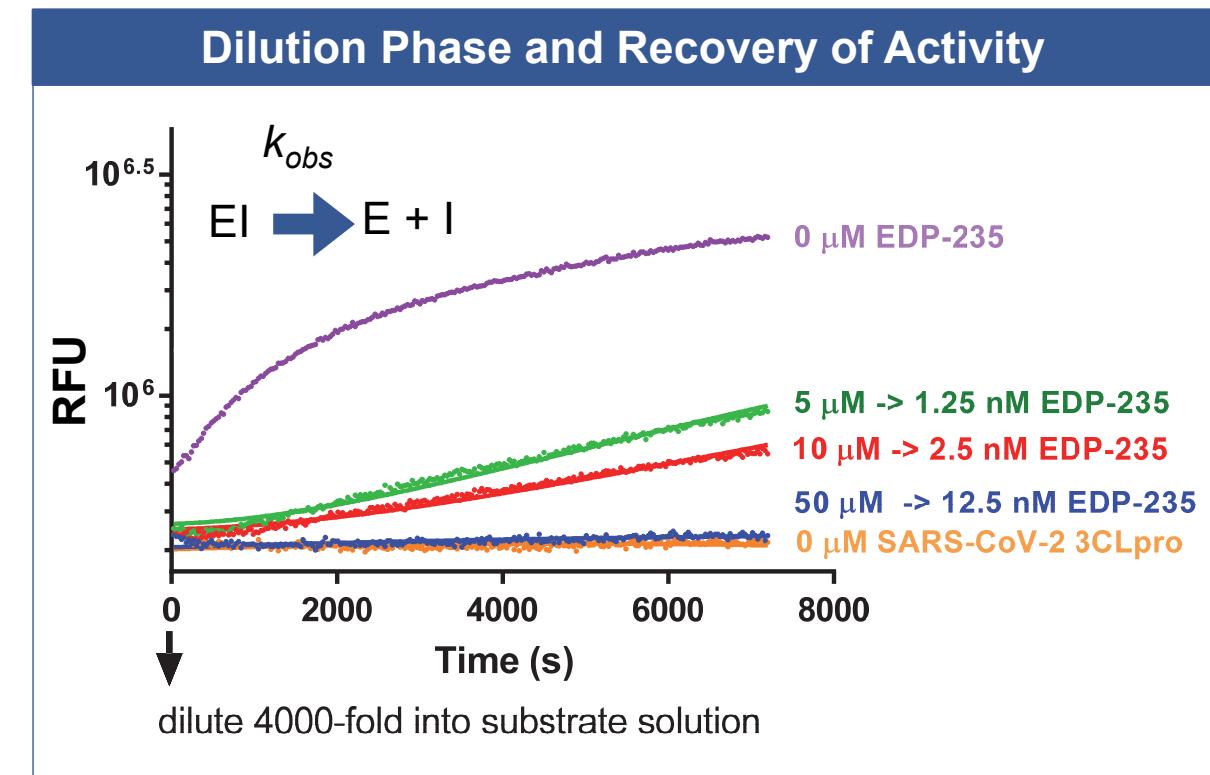
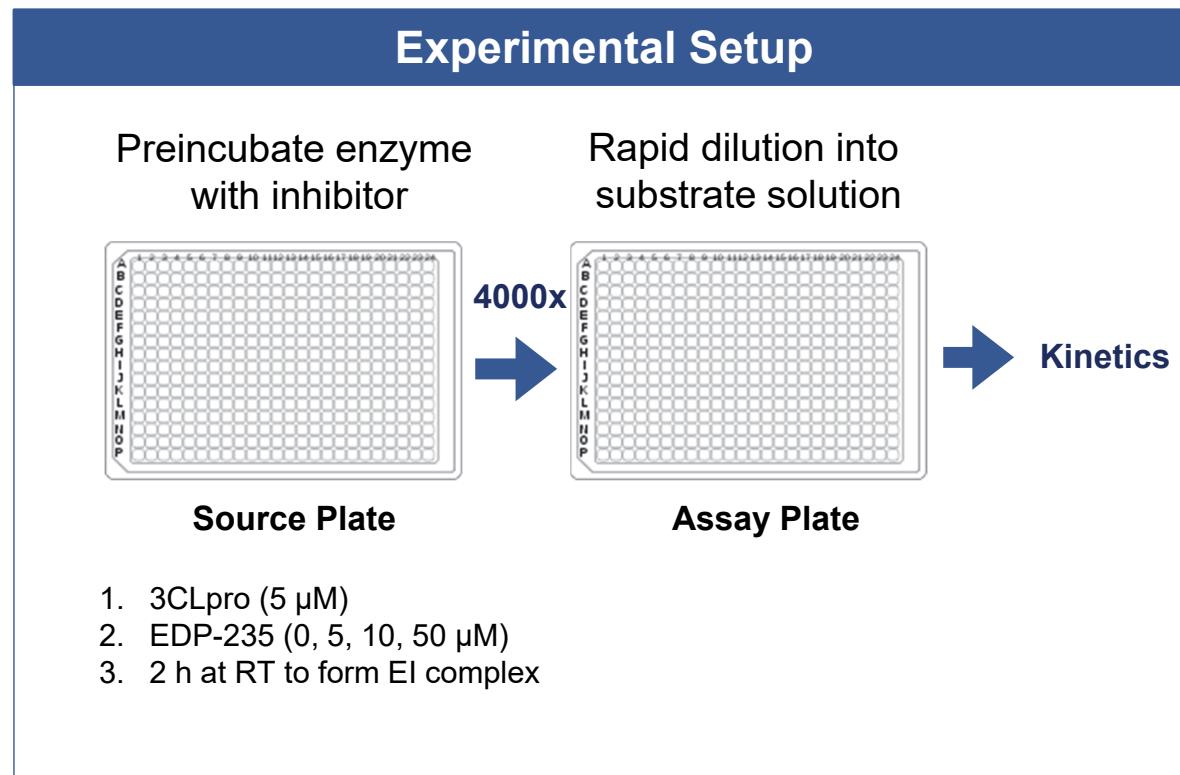
EDP-235 is a time-dependent inhibitor of SARS-CoV-2 3CLpro



Parameter	Value Mean \pm SD, n=3
k_{on}	$(2.6 \pm 1.6) \times 10^5 \text{ M}^{-1}\text{s}^{-1}$
k_{off}	$(9 \pm 1) \times 10^{-4} \text{ s}^{-1}$
K_i^{app}	$4.7 \pm 2.4 \text{ nM}$

$$\sim k_{cat}/K_m = 2.1 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$$

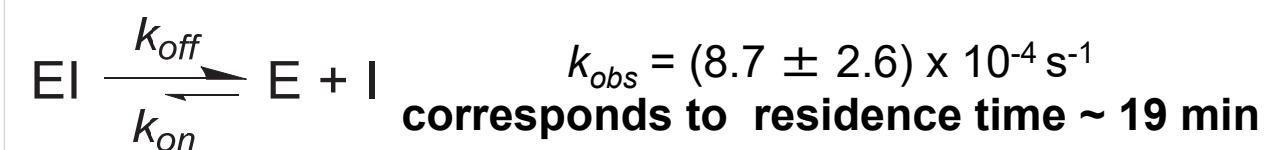
EDP-235 is a reversible inhibitor of SARS-CoV-2 3CLpro



RT = room temperature

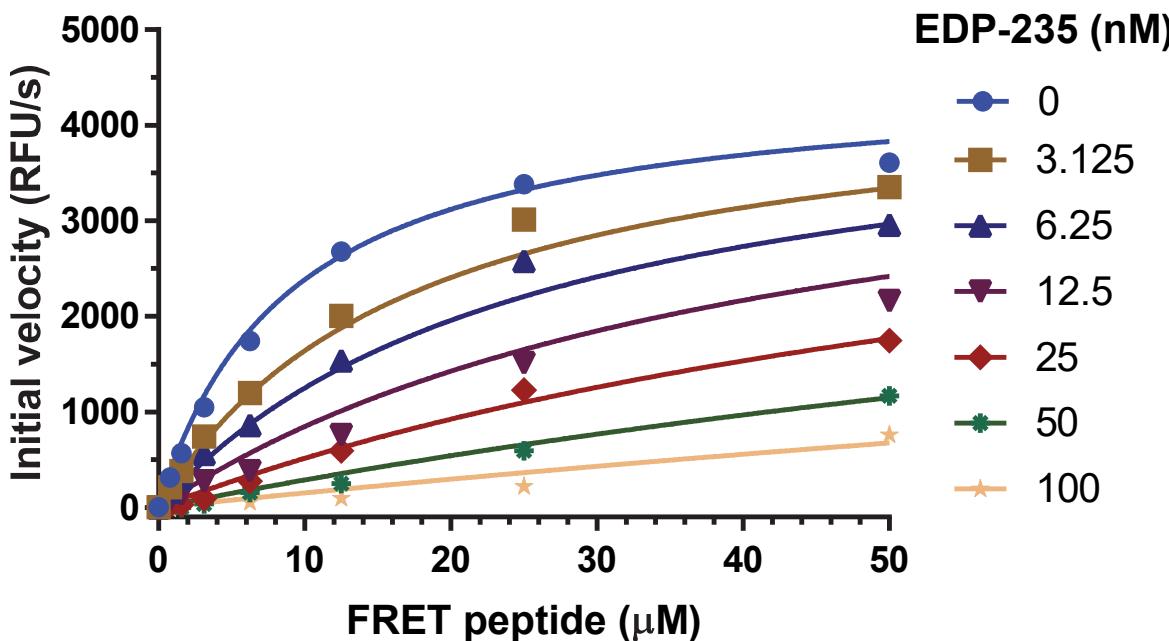
E = Enzyme

I = Inhibitor



EDP-235 is a substrate competitive inhibitor of SARS-CoV-2 3CLpro

Kinetic Analysis with Respect to Substrate



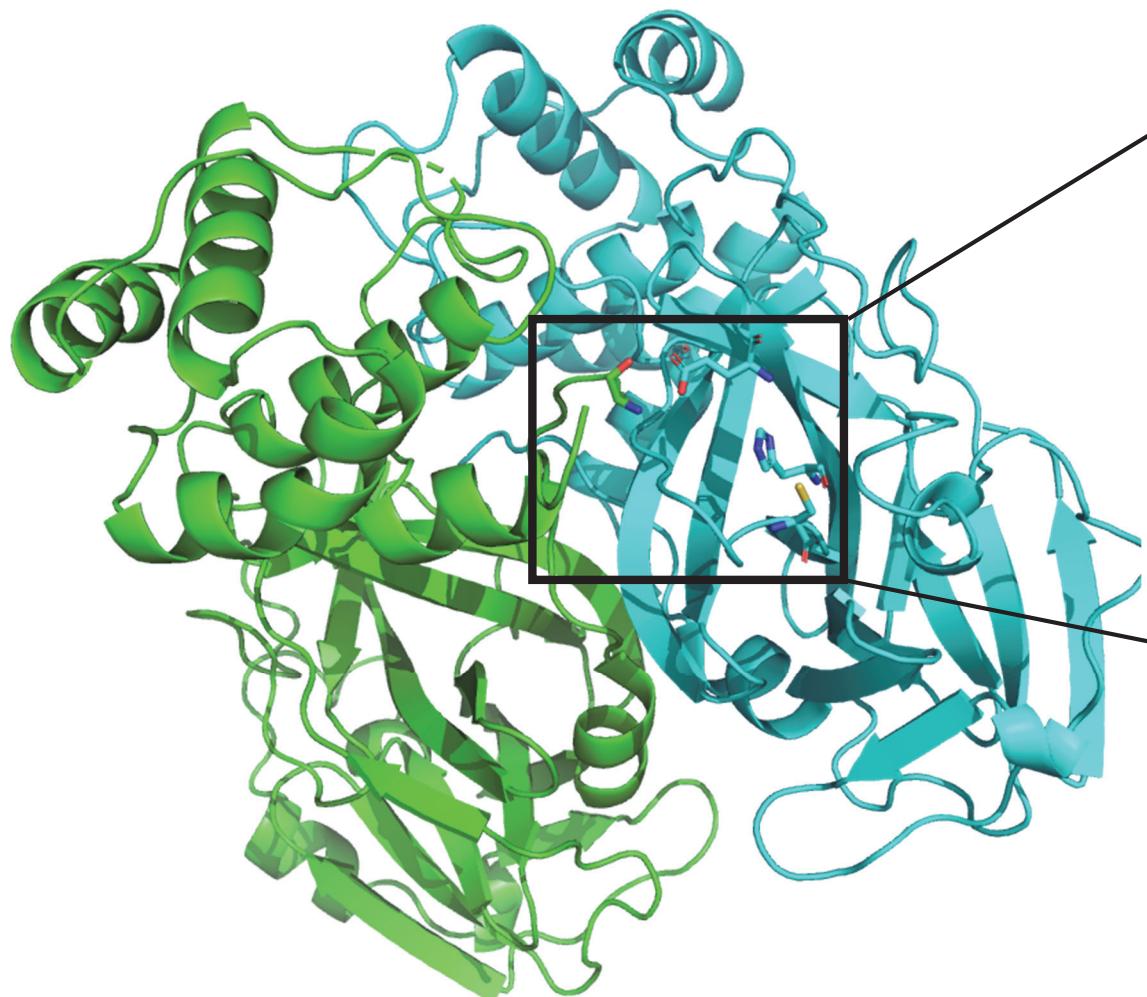
EDP-235 is a competitive inhibitor with respect to the FRET peptide substrate

Summary of Kinetic Mechanism Characterization

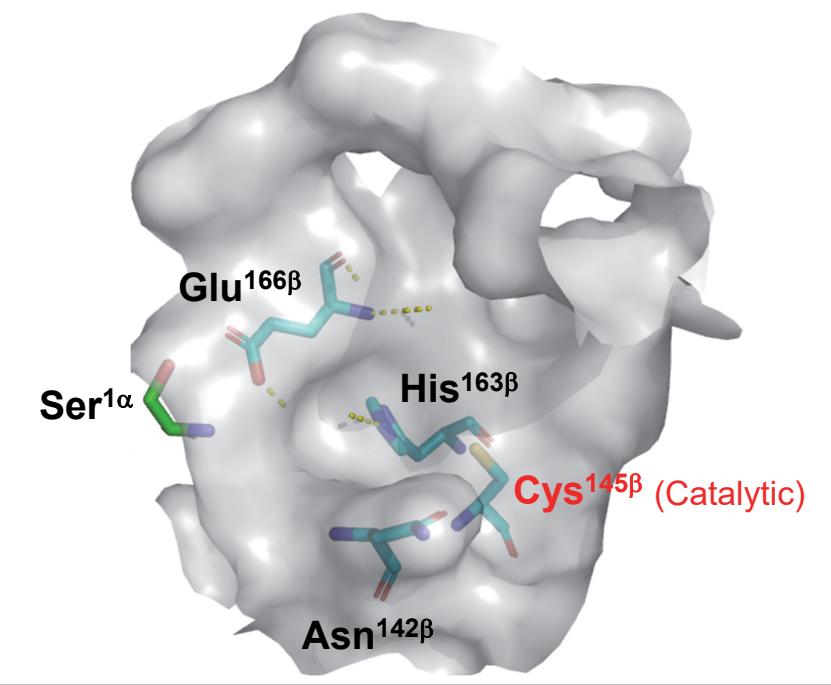
Parameter	Value
IC ₅₀	5.8 nM
K_i	3.0 ± 1.6 nM
k_{on}	(2.6 ± 1.6) $\times 10^5 \text{ M}^{-1}\text{s}^{-1}$
k_{off}	(8.7 ± 2.6) $\times 10^{-4} \text{ s}^{-1}$
Residence time	~ 19 min
Kinetic Mechanism	Time-dependent, reversible, Substrate competitive inhibition

EDP-235 analog binds at the active site of SARS-CoV-2 3CLpro

Quaternary structure of SARS-CoV-2 3CLpro dimer



Compound binding site with key residues



- Crystal structures of apo 3CLpro and co-structures of key compounds were obtained at 2.5-2.8 Å.
- 3CLpro crystallized as a dimer and active site residues make essential polar contacts with compounds.
- Structures provide support for mechanism of inhibition of EDP-235.

EDP-235 shows antiviral activity against all human coronaviruses



Virus	3CLpro Enzyme Assay IC_{50} (nM)	Live Virus Assay					SI
		Cell Type	Endpoint	EC_{50} (nM)	CC_{50} (nM)		
SARS-CoV-2	5.8	Vero E6*	CPE	5.1	>10,000	>1,960	
HCoV-229E	5.4	MRC-5	CPE	3.6	>50,000	>13,889	
HCoV-HKU1	3.8	-	-	-	-	-	
HCoV-NL63	1.8	LLC-MK2	RT-qPCR	6.1	-	-	
HCoV-OC43	3.4	HCT-8*	RT-qPCR	56	-	-	
SARS-CoV	1.9	Vero E6*	CPE	24	>3,000	>125	
MERS-CoV	70	Vero 76	CPE	150	>26,000	>173	
			Viral Yield	130 [EC₉₀]	>26,000	>200	

*Assay performed in the presence of a P-glycoprotein inhibitor (CP-100356, 2 μ M) to prevent transporter-mediated efflux. CPE = cytopathic effect; P-gpi = P-glycoprotein inhibitor CP-100356 (2 μ M); qPCR = quantitative polymerase chain reaction. HCoV-229E, HCoV-HKU1, HCoV-OC43, HCoV-NL63 = human coronavirus 229E, HKU1, OC43, and NL63, respectively; SARS-CoV = severe acute respiratory syndrome; MERS-CoV = Middle East respiratory syndrome. Vero 76 and Vero E6 cells are derived from African green monkey kidney epithelia, MRC-5 are human lung fibroblasts, HCT-8 are derived from a human ileocecal adenocarcinoma, and LLC-MK2 are Rhesus monkey epithelial cells. pHAEc = primary human airway epithelial cells.

EDP-235 shows highly selective inhibition of 3CLpro compared to human proteases

Cysteine Proteases

- SARS CoV 2 – PLpro, 3CLpro
- Caspases 1 – 11, 14
- Cathepsins B, C, D, E, G, H, K, L, S, V
- Papain, Calpain 1

Serine Proteases

- Trypsin
- TMPRSS 2
- Furin

Aspartyl protease

- BACE1

Zn metalloprotease

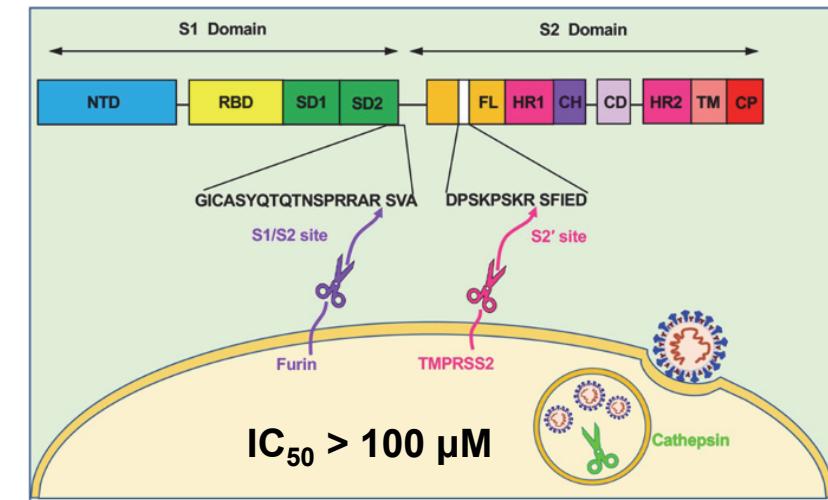
- ACE 1, 2

- Tested against 31 host proteases of diverse classes
- $IC_{50} > 100 \mu M$ against 23 out of 31 including host proteases relevant to viral infection

Target	EDP-235 IC_{50} (μM)
Caspase 2	4.6
Caspase 3	4.2
Caspase 6	2
Caspase 7	4.7
Caspase 8	22.5
Caspase 9	4.7
Caspase 14	9
Cathepsin K	18.5
SARS-CoV-2 3CLpro	0.0058
Selectivity Index	> 340

Cysteine Proteases

Host proteases in viral entry/fusion



- ACE 2
- Cathepsin B
- Cathepsin L
- Trypsin
- TMPRSS 2
- Furin

1. *Signal Transduction and Targeted Therapy* (2021). 6:233
2. *Int. J. Mol. Sci.* (2020), 21(24), 9523

Molecular mechanism of EDP-235 action and its pharmacological effect

Molecular Observations	In vitro Pharmacology	Preclinical Properties	EDP-235 ¹
Time-dependence/on-target residence time	High potency and antiviral efficacy	Mechanism	Protease Inhibitor
Substrate competitive /active site binder	Broad spectrum anti-coronaviral	Potency	Enzyme IC₅₀ (nM) 5.8
Strong interactions with highly conserved active-site residues	High barrier to resistance		Vero Cell EC₅₀ (nM) 5.1
High degree of selectivity over other mammalian proteases	On target pharmacology and low off-target effects	Oral Bioavailability ²	95%
		Lung/Plasma ratio ³	4.1
		Projected Efficacious Dose	100 – 500mg QD

1. Jiang *et al.*, ISIRV Poster #120, Oct 19, 2021

2. Oral bioavailability in rats for EDP-235

3. AUC lung to plasma ratio in rats for EDP-235

Acknowledgements

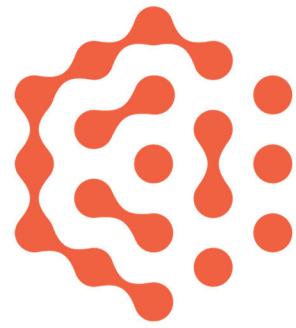
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