OAK RIDGE National Laboratory

Neutron crystallography to uncover SARS-CoV-2 main protease function and design potent inhibitors

Andrey Kovalevsky
Neutron Scattering Division
Oak Ridge National Laboratory

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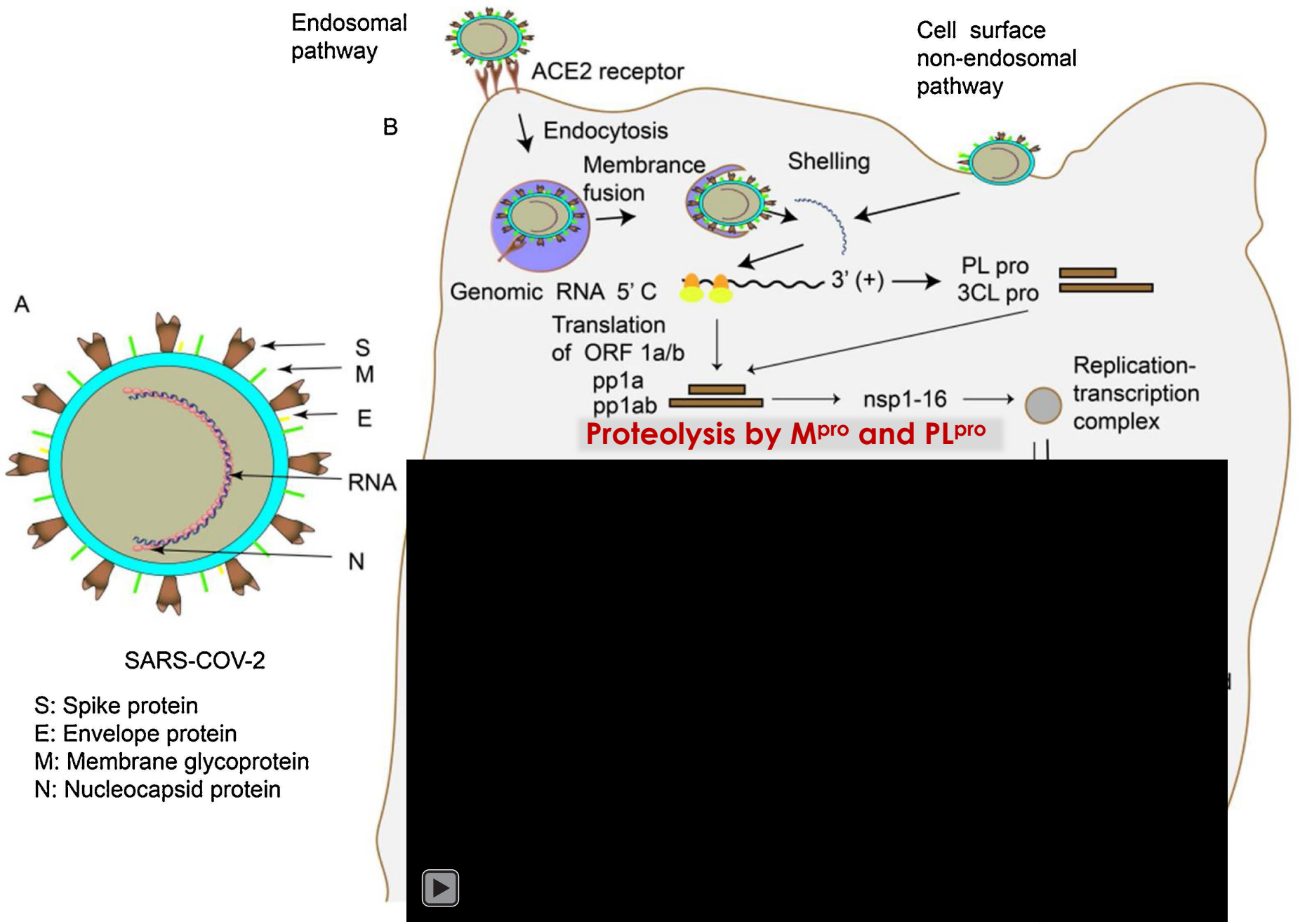
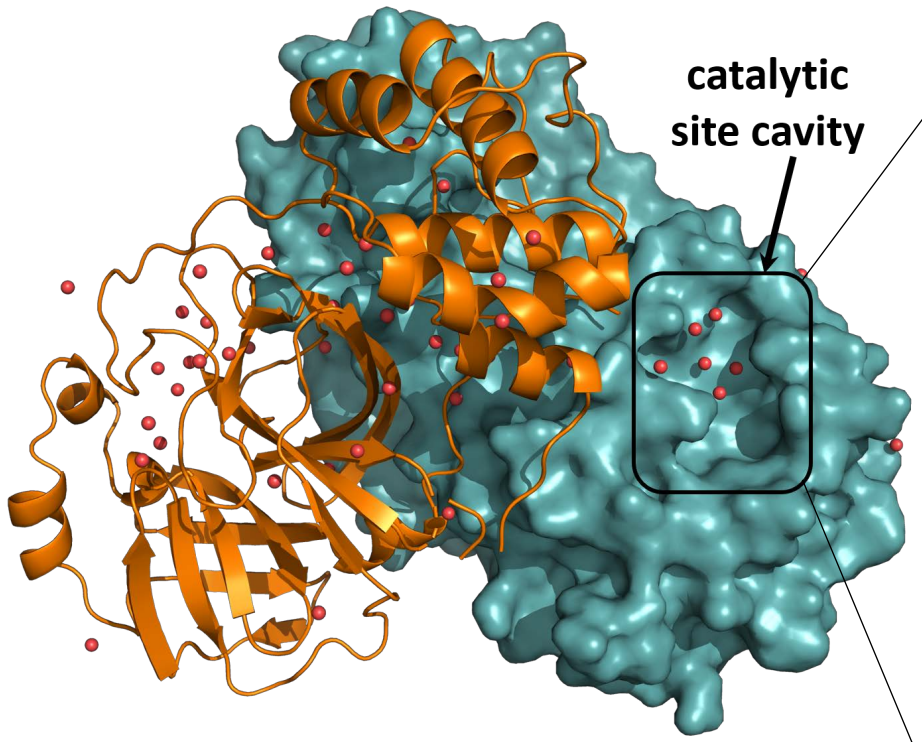
SARS-CoV-2 replication cycle

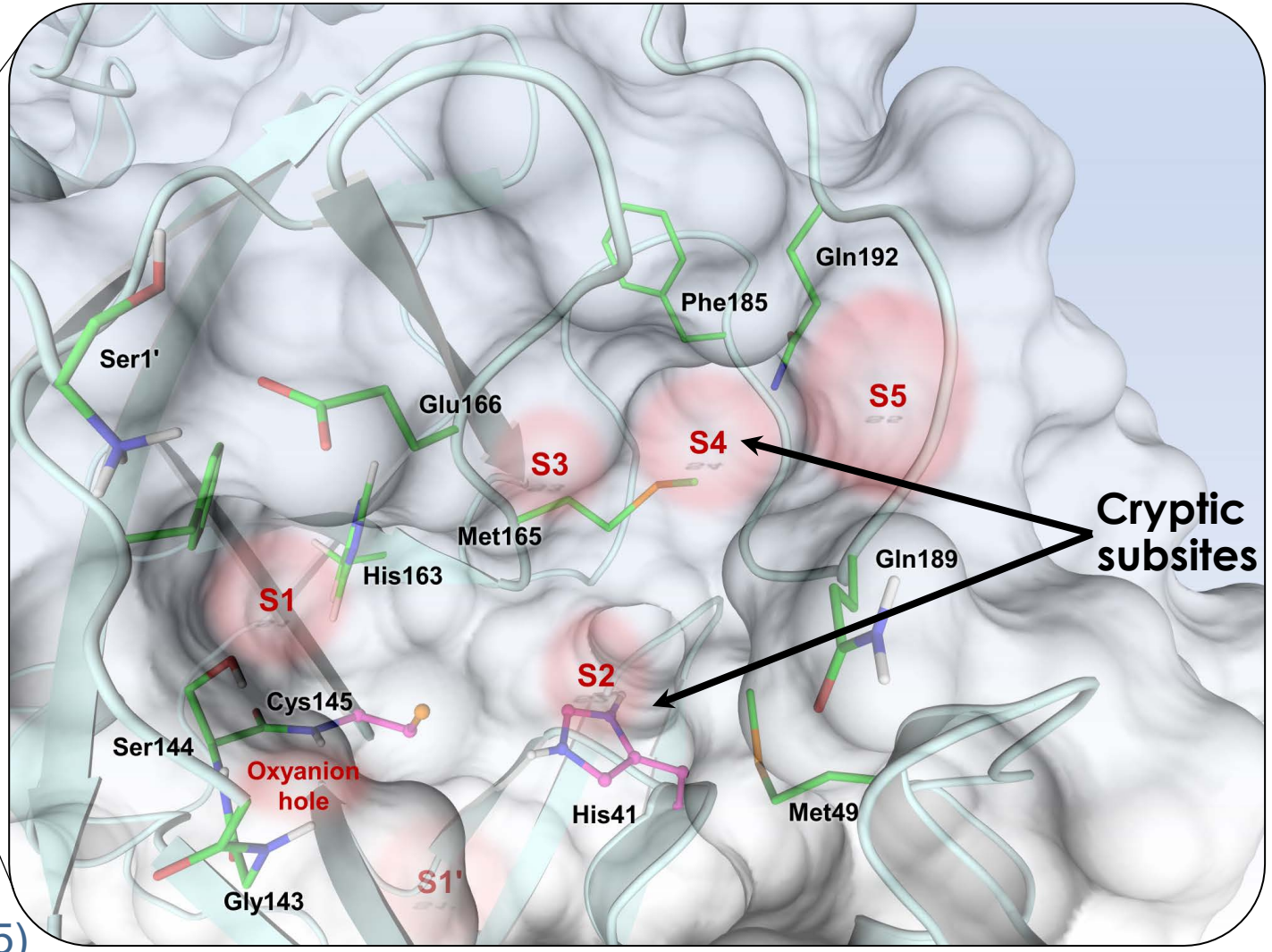
- Proteolysis of pp1a and pp1ab by M^{pro} and PL^{pro} produces NSPs
- Action by the two enzymes is central to virus replication



Huang et al. *Pharmacol. Res.* 2020

Homodimeric SARS-CoV-2 M^{pro} and its active site

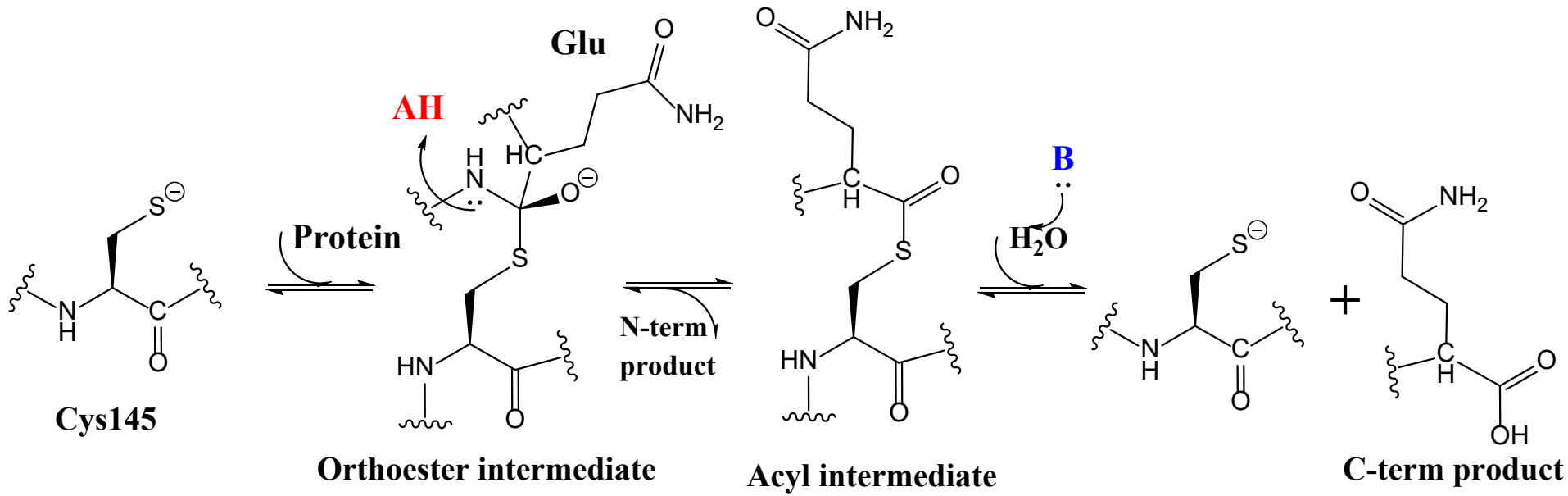




Targetable active site features

- Non-canonical catalytic Cys145-His41 dyad
- Room for ~6-7 peptide/inhibitor groups (P2'-P5)
- Characteristic oxyanion hole

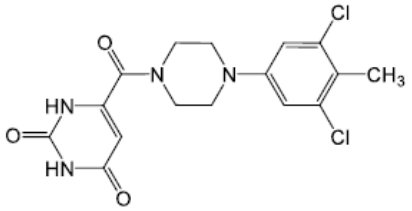
M^{pro} catalytic mechanism



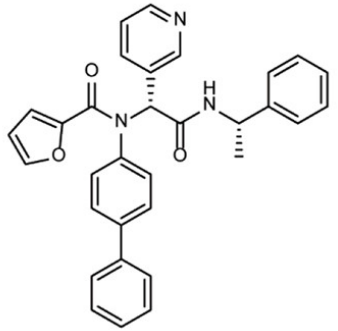
- Catalytic His41 is the best candidate to act as general acid (**AH**) in the first step and as general base (B) in the second step of the reaction.

M^{pro} inhibitors

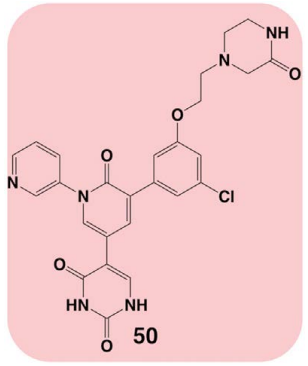
❖ Noncovalent inhibitors



Kneller *et al.* 2021 *J. Med. Chem.*



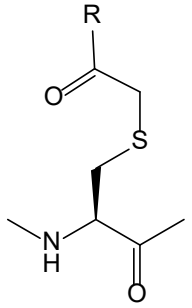
Kitamura *et al.* 2021 *J. Med. Chem.*

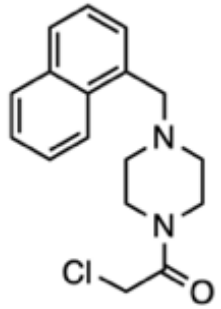


Deshmukh *et al.* 2021 *Structure*

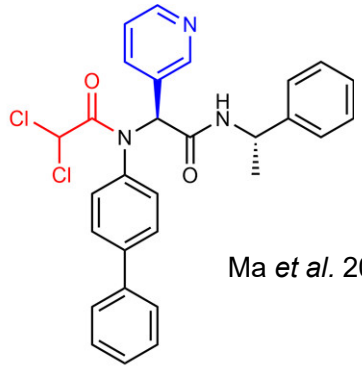
Drayman *et al.* 2021 *Science*

❖ Irreversible covalent inhibitors

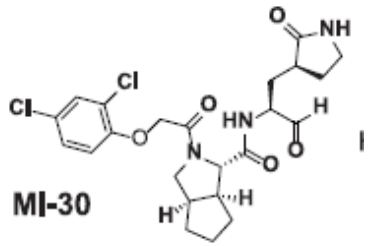




Joshi *et al.* 2021 *Commun. Biol.*
Under review

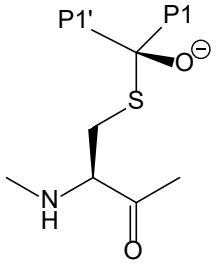


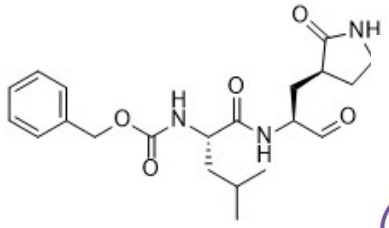
Ma *et al.* 2021 *JACS*



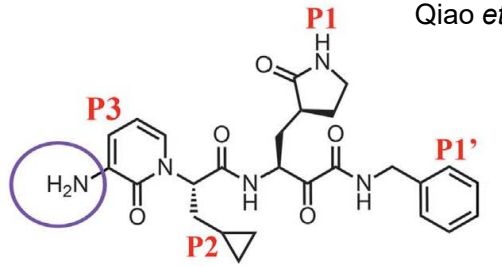
Qiao *et al.* 2021 *Science*

❖ Mechanism-based reversible covalent inhibitors





Fu *et al.* 2020 *Nat. Commun.*



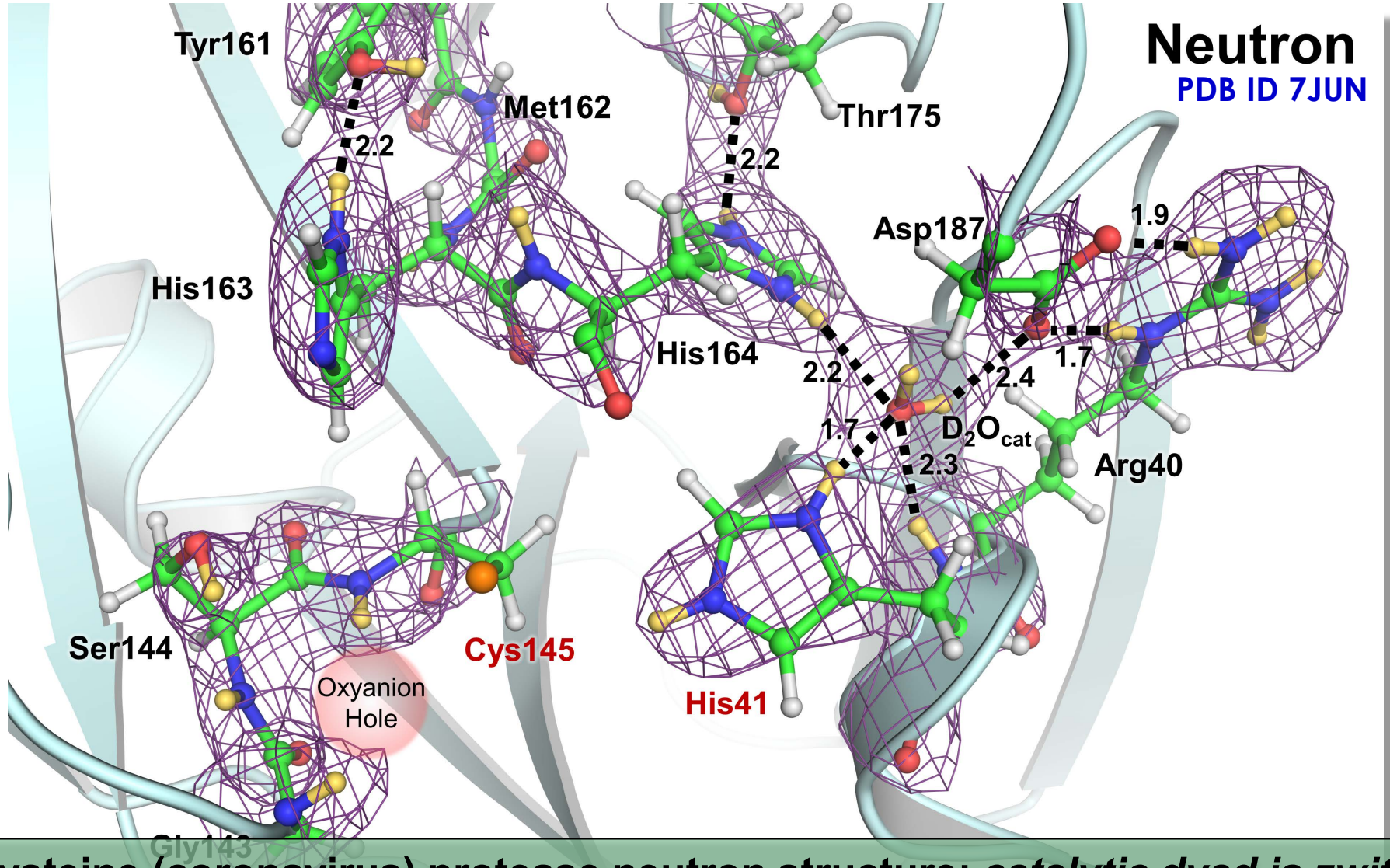
Zhang *et al.* 2020 *Science*

Hydrogen: The most important but most neglected atom

Today's practitioners of drug design often have a bias toward nonhydrogen atoms, forgetting the central role that hydrogens play in molecular recognition.

Darryl B. McConnell
J. Med. Chem. 2021, **64**, 16319.

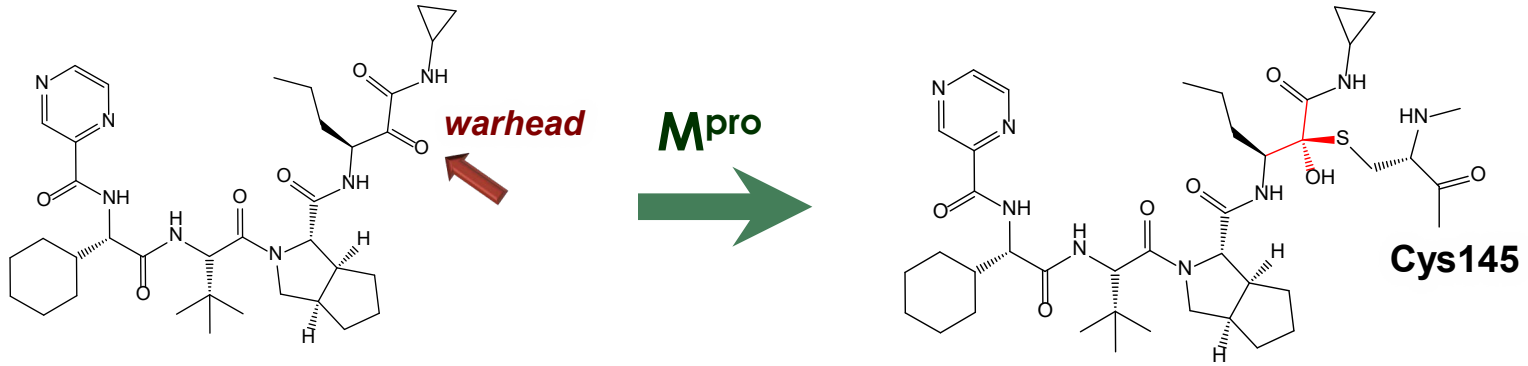
Room-temp joint XN structure of M^{pro} in the native form @ 2.5Å resolution

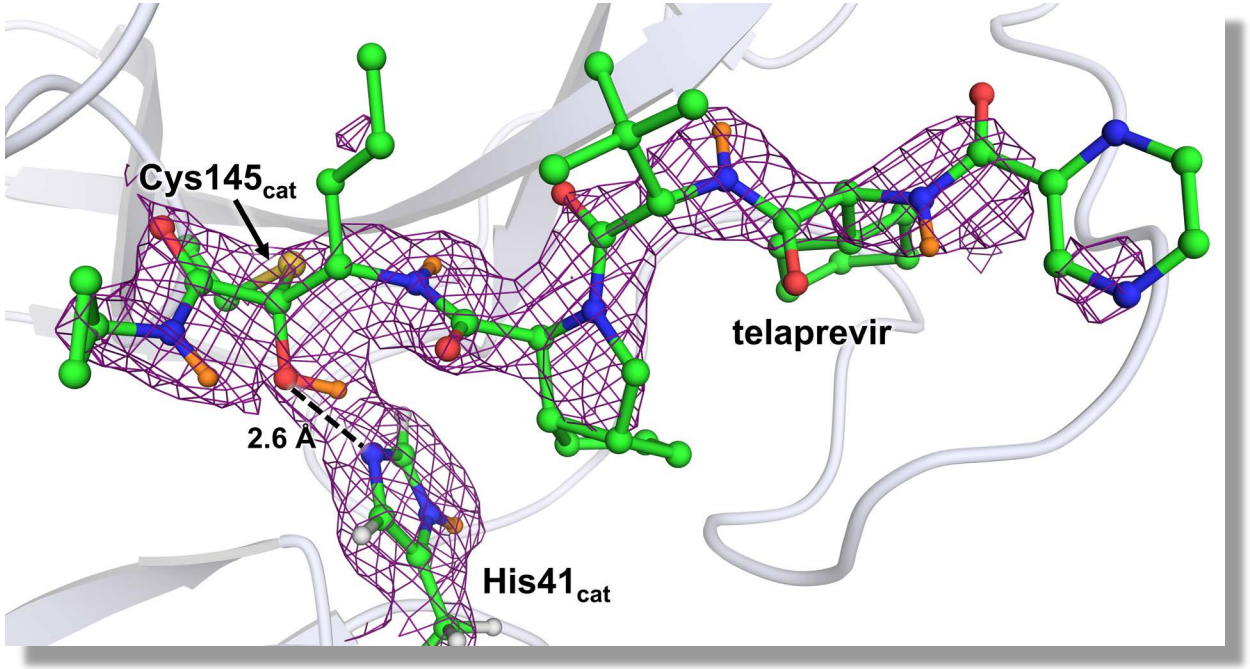


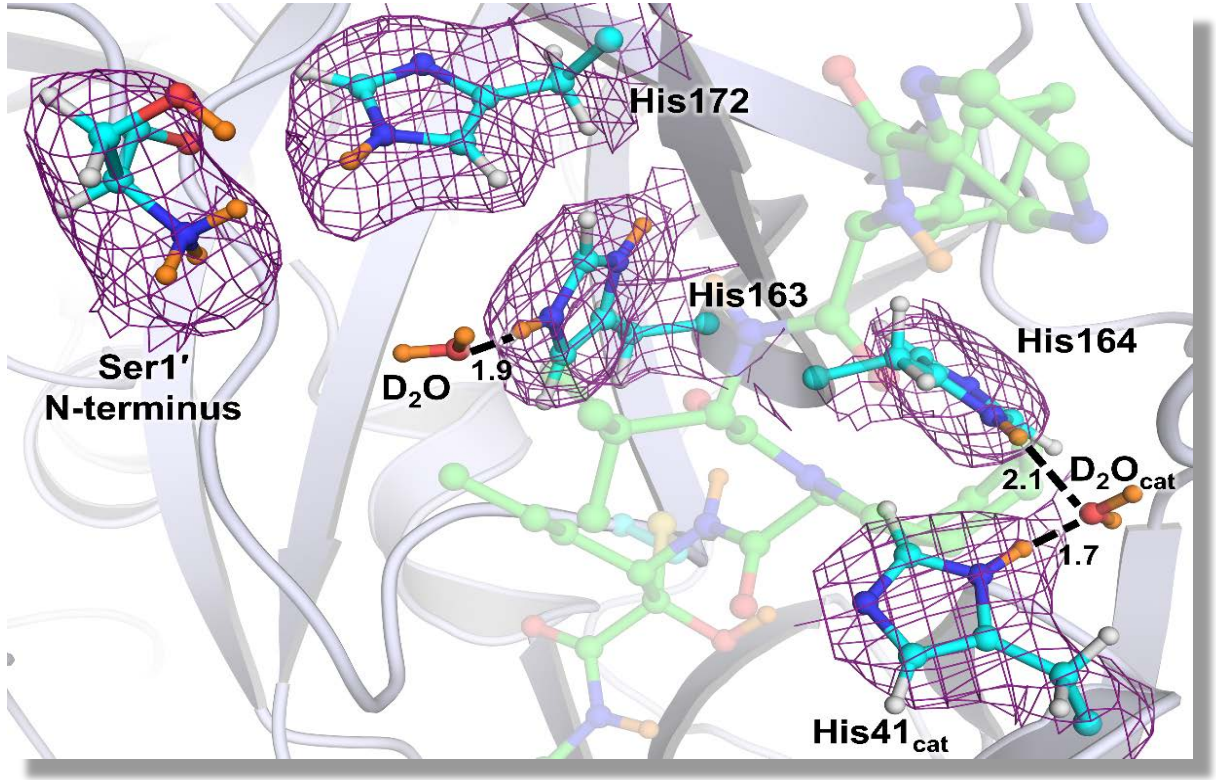
First cysteine (coronavirus) protease neutron structure: *catalytic dyad is zwitterionic*

Kneller *et al.* 2020 *Acta Cryst.* F18, 688-699
Kneller *et al.* 2020 *Nat. Commun.* 18, 688-699
Kneller et al. 2020 *J. Biol. Chem.* 295, 17365-17373

Room-temp joint XN structure of M^{pro}-Telaprevir @ 2.4Å resolution







Hemithioketal is protonated with short H-bond to His41, but unfavorable geometry

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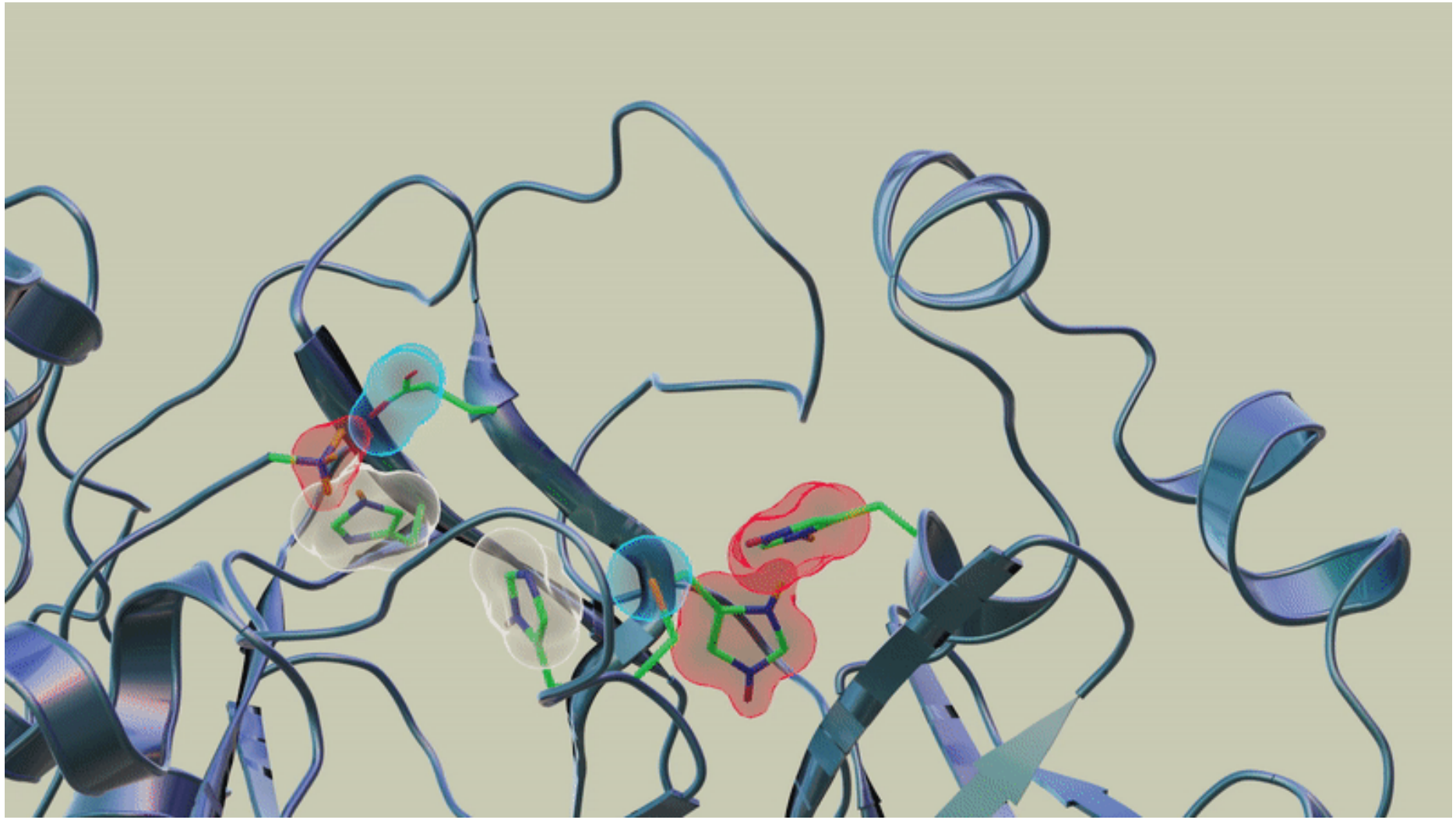
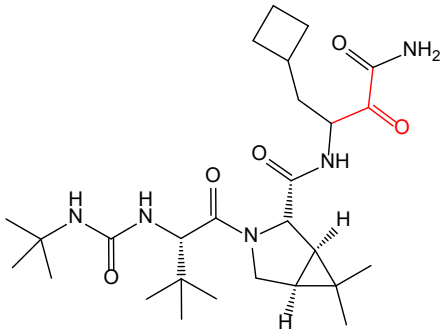
Kneller *et al.* 2021 *J. Med. Chem.* 64, 4991-5000

M^{pro} active site is tunable and malleable

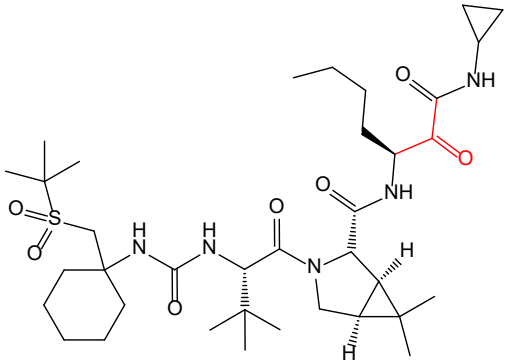


Design of mechanism-based covalent inhibitors by splicing technique

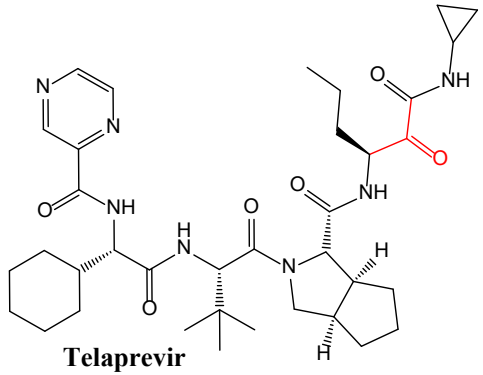
Hepatitis C virus protease inhibitors bind and inhibit M^{pro}



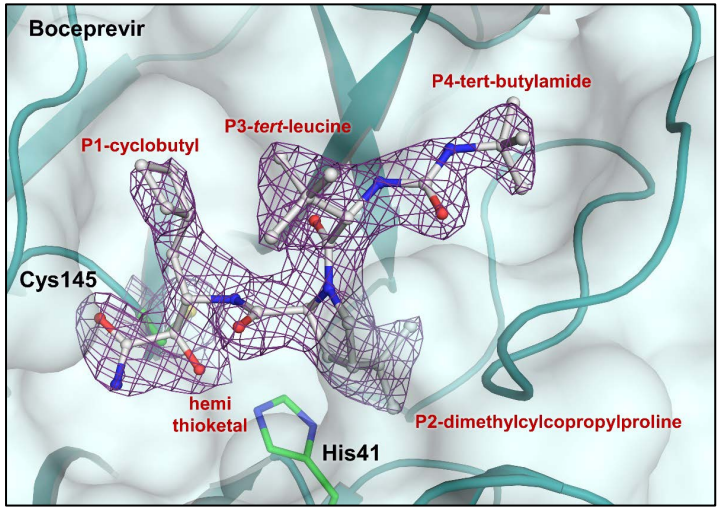
Boceprevir



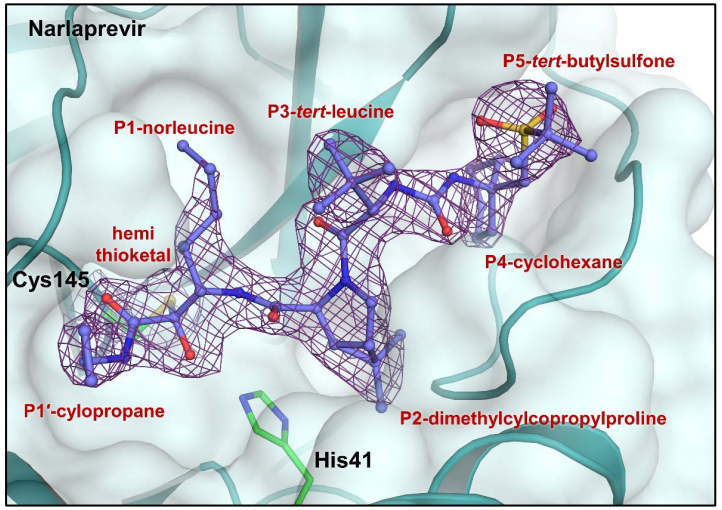
Narlaprevir



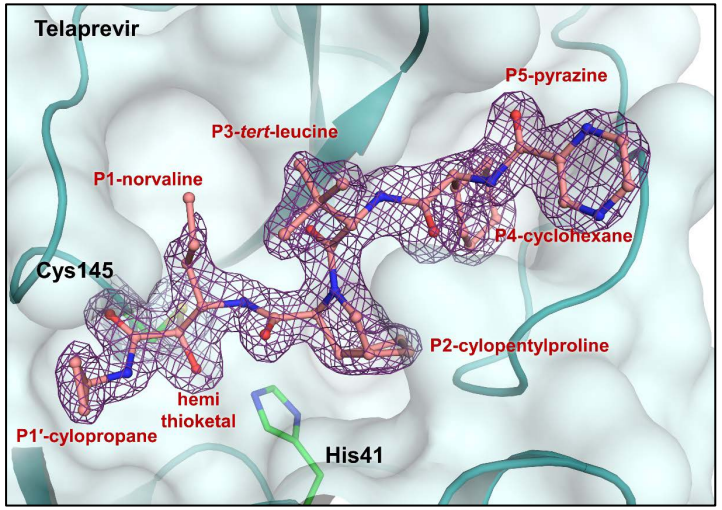
Telaprevir



PDB 6XQU IC50 = 3 μM



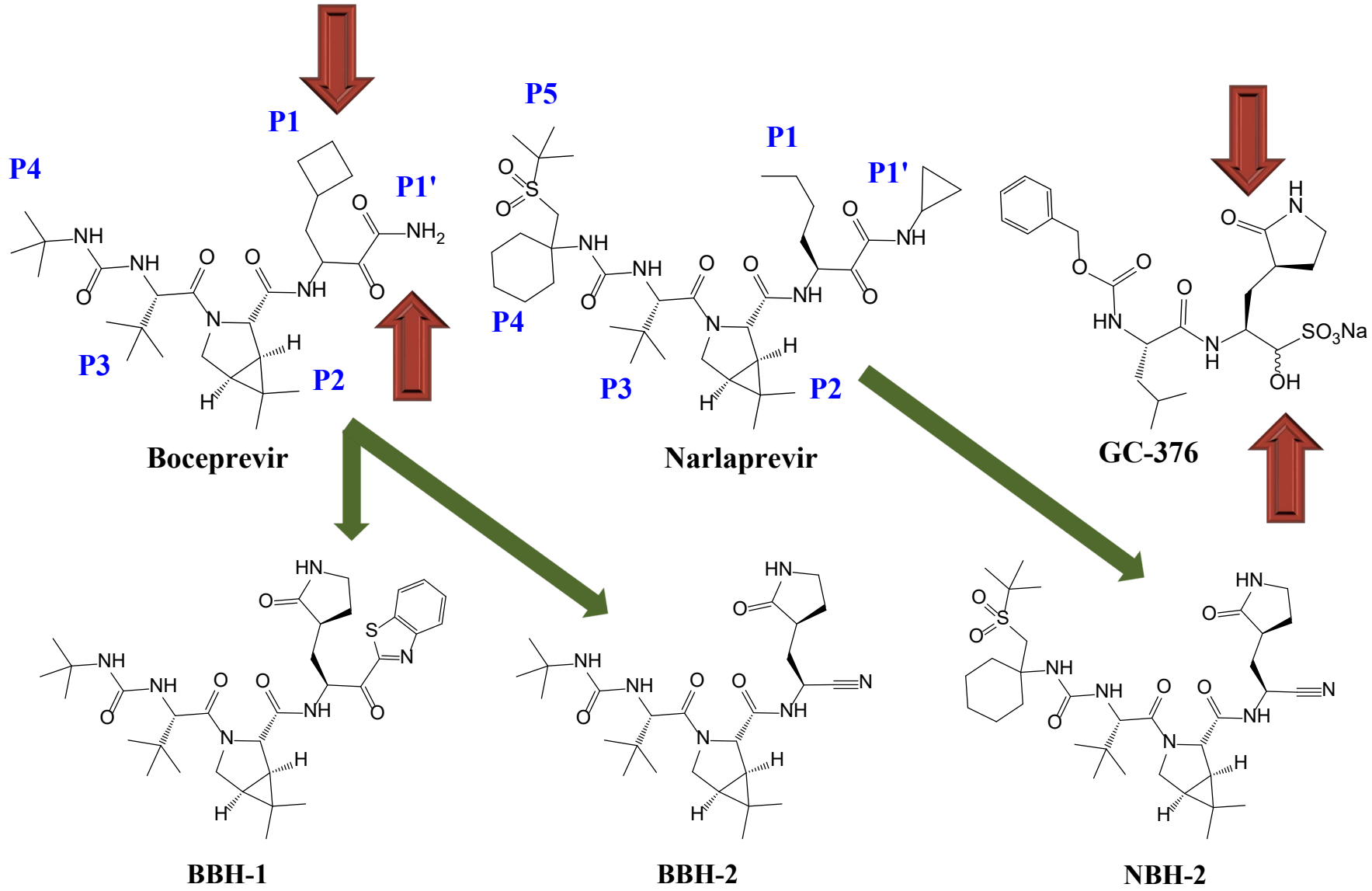
PDB 6XQT IC50 = 5 μM



PDB 6XQS IC50 = 18 μM

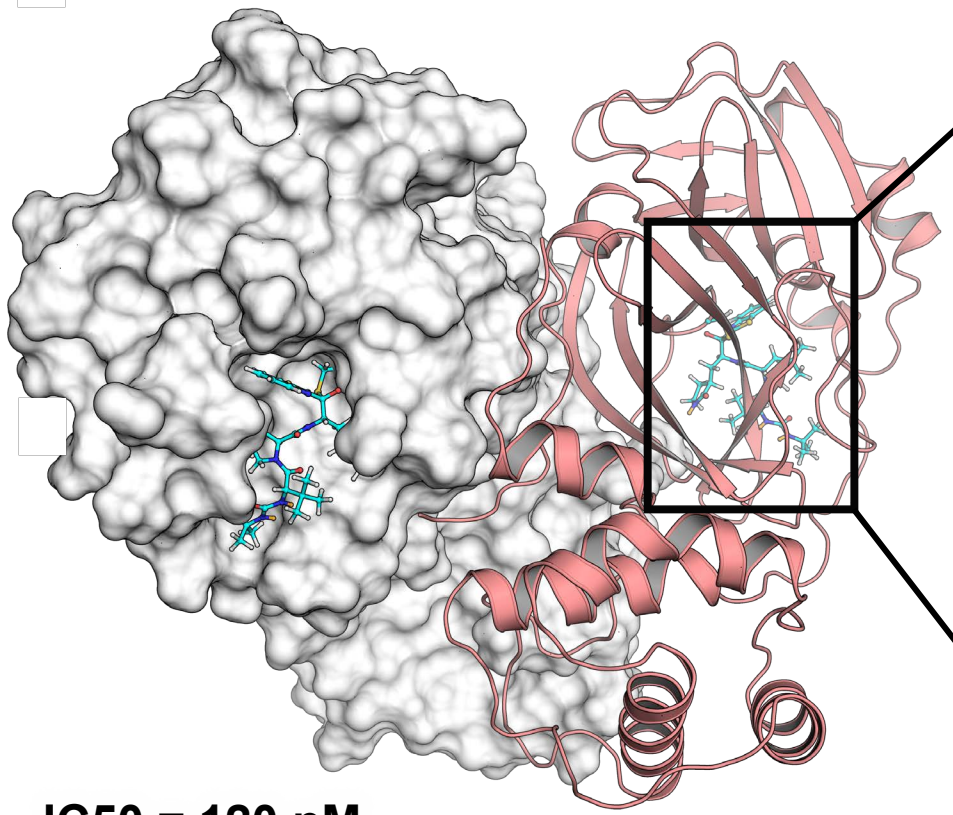
Kneller *et al.* 2020 *Structure* 28, 1313-1320

Design of covalent hybrid inhibitors of M^{pro}

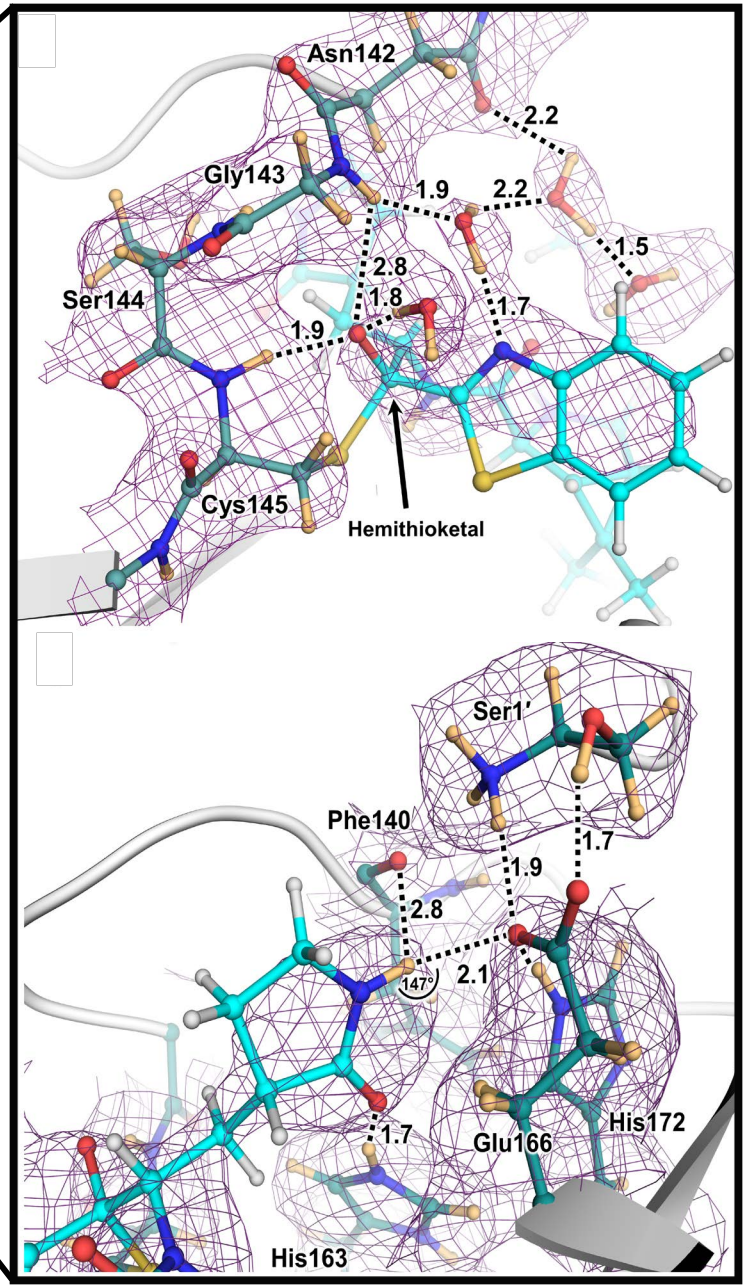


Kneller *et al.* 2022 *Nat. Commun.* 13, 2268

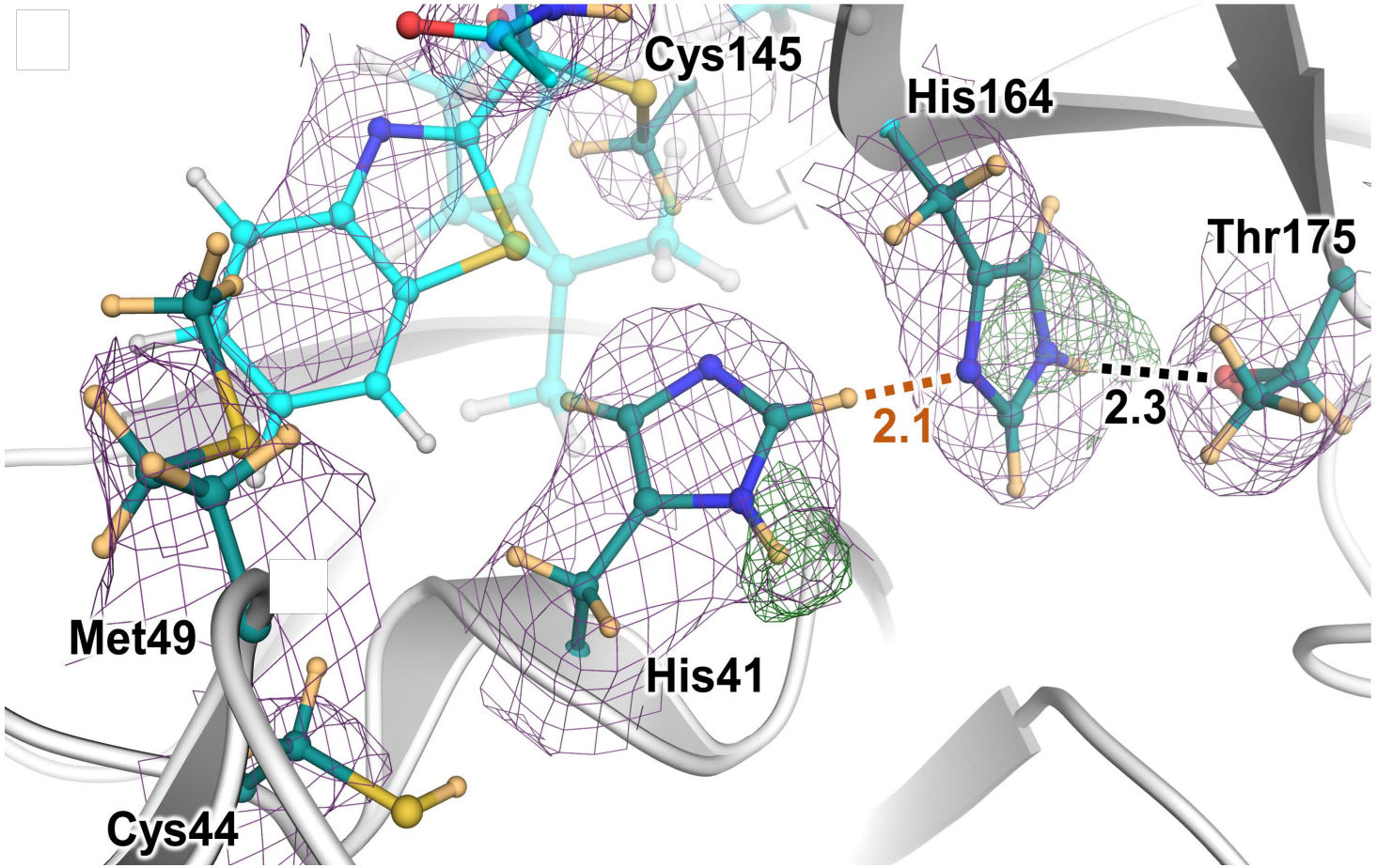
Unique binding of BBH-1 to M^{pro}: *A neutron structure perspective*



IC50 = 120 nM



Unique binding of BBH-1 to M^{pro}: *A neutron structure perspective*



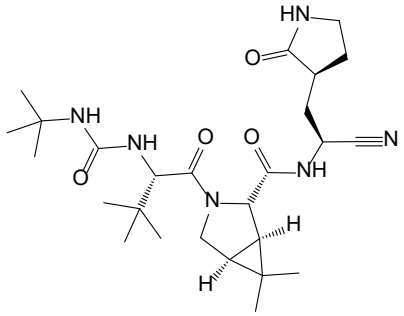
Unconventional C-H…N hydrogen bond is visualized in the neutron structure, mistakenly identified as conventional N-H…N hydrogen bond in the X-ray structure

Kneller *et al.* 2022 *Nat. Commun.* 13, 2268

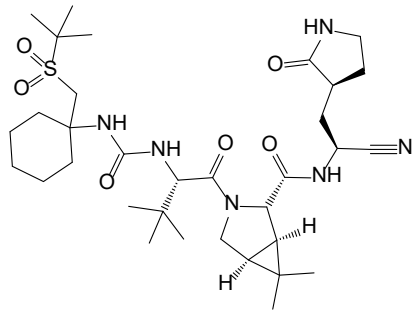
Binding affinity and antiviral activity of hybrid inhibitors

Compound	K_d, μM	Stoichiometry, N	ΔH, kcal mol^{-1}	ΔS, cal mol^{-1} K^{-1}	ΔG, kcal mol^{-1}
BBH-2	0.030 ± 0.007	1.000 ± 0.005	–8.74 ± 0.08	5.40	–10.4
NBH-2	0.026 ± 0.016	0.990 ± 0.009	–8.76 ± 0.17	5.63	–10.5
NMV	0.007 ± 0.003	0.990 ± 0.003	–10.75 ± 0.70	1.57	–11.2
GC-376	0.15 ± 0.03	0.99 ± 0.01	–6.7 ± 0.1	9.1	–9.4

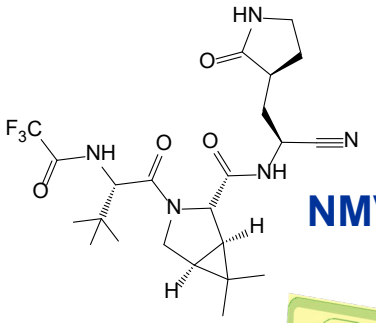
Compound	EC_{50}, μM (without CP-100356)	EC_{50}, μM (with CP-100356)	CC_{50}, μM
BBH-1	16.1	1.5	> 10
BBH-2	15.4	0.88	> 10
NBH-2	13.9	1.82	> 10
PF-07321332	0.88	0.25	> 10



BBH-2

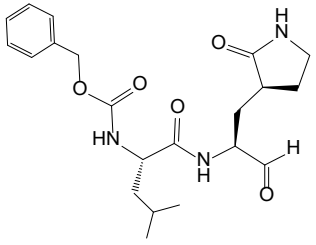


NBH-2



PF-07321332

NMV

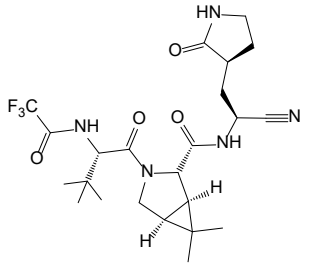


GC-376

Kneller *et al.* 2022 *Nat. Commun.* 13, 2268

Hybrid inhibitors – fruitful path to clinical drugs

- Protonation states adapt to a specific inhibitor
- Active site geometry adapts to inhibitor steric and electronic properties
- Hybrid inhibitors are conceptually superior to previous designs



PF-07321332

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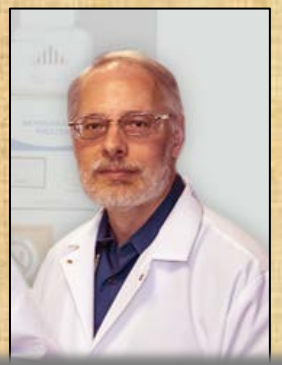

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Swati Pant

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ITC Data

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THE UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER

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Colleen Jonsson

Surekha Surendranathan

Jyothi Parvathareddy

ILL, Neutrons

Matthew Blakeley

NVBL:
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