OAK RIDGE National Laboratory

Neutron crystallography to inform drug design targeting SARS-CoV-2 main protease

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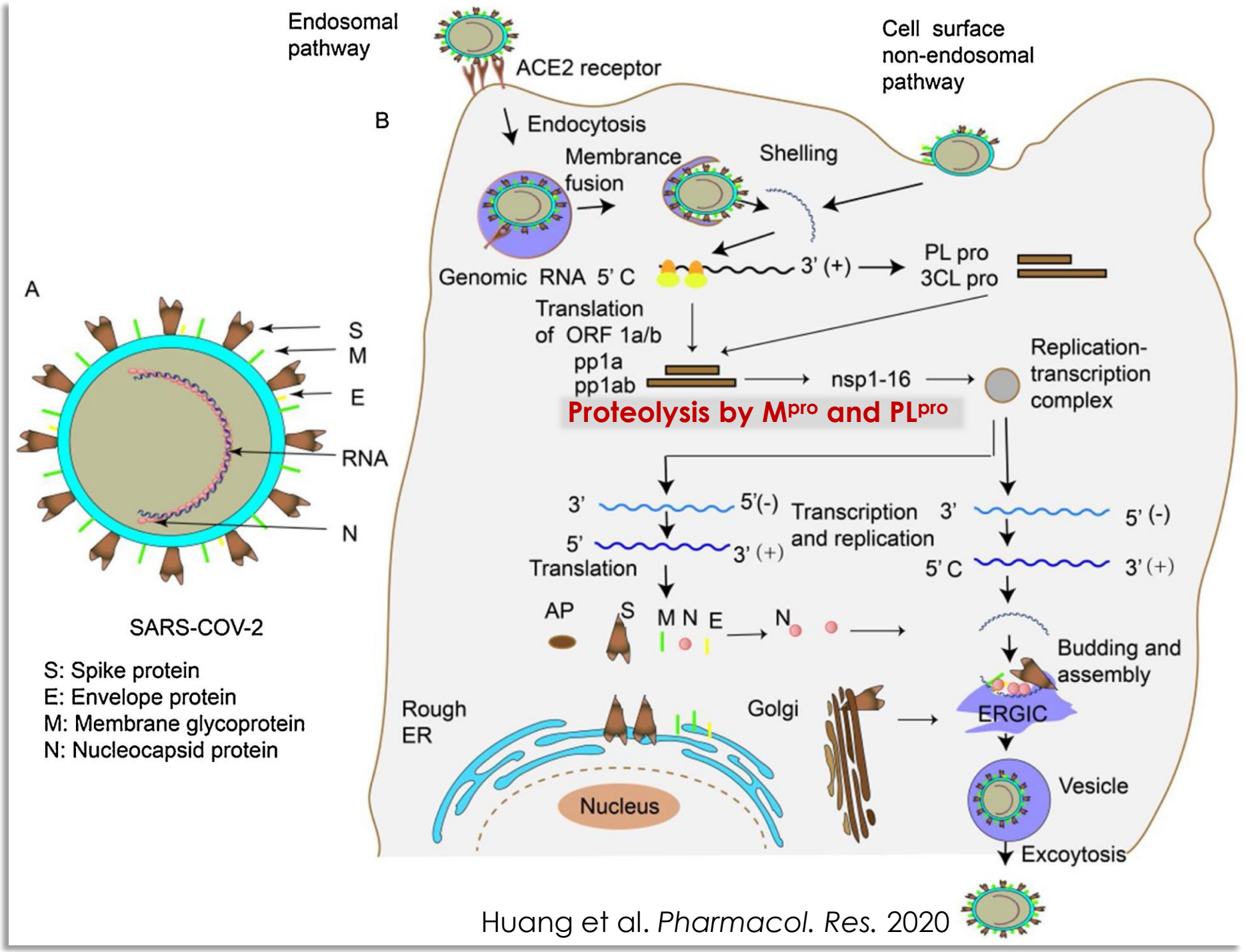
U.S. DEPARTMENT OF ENERGY

SARS-CoV-2 life cycle

- Proteolysis of pp1a and pp1ab by 3CL M^{pro} and PL^{pro} produces NSPs
- Action by the two enzymes is vital for the viral replication cycle
- Inhibition of the proteases can stop the viral replication



Huang et al. *Pharmacol. Res.* 2020

Main protease is the heart of SARS-CoV-2 replication

Stopping the heart of SARS-CoV-2

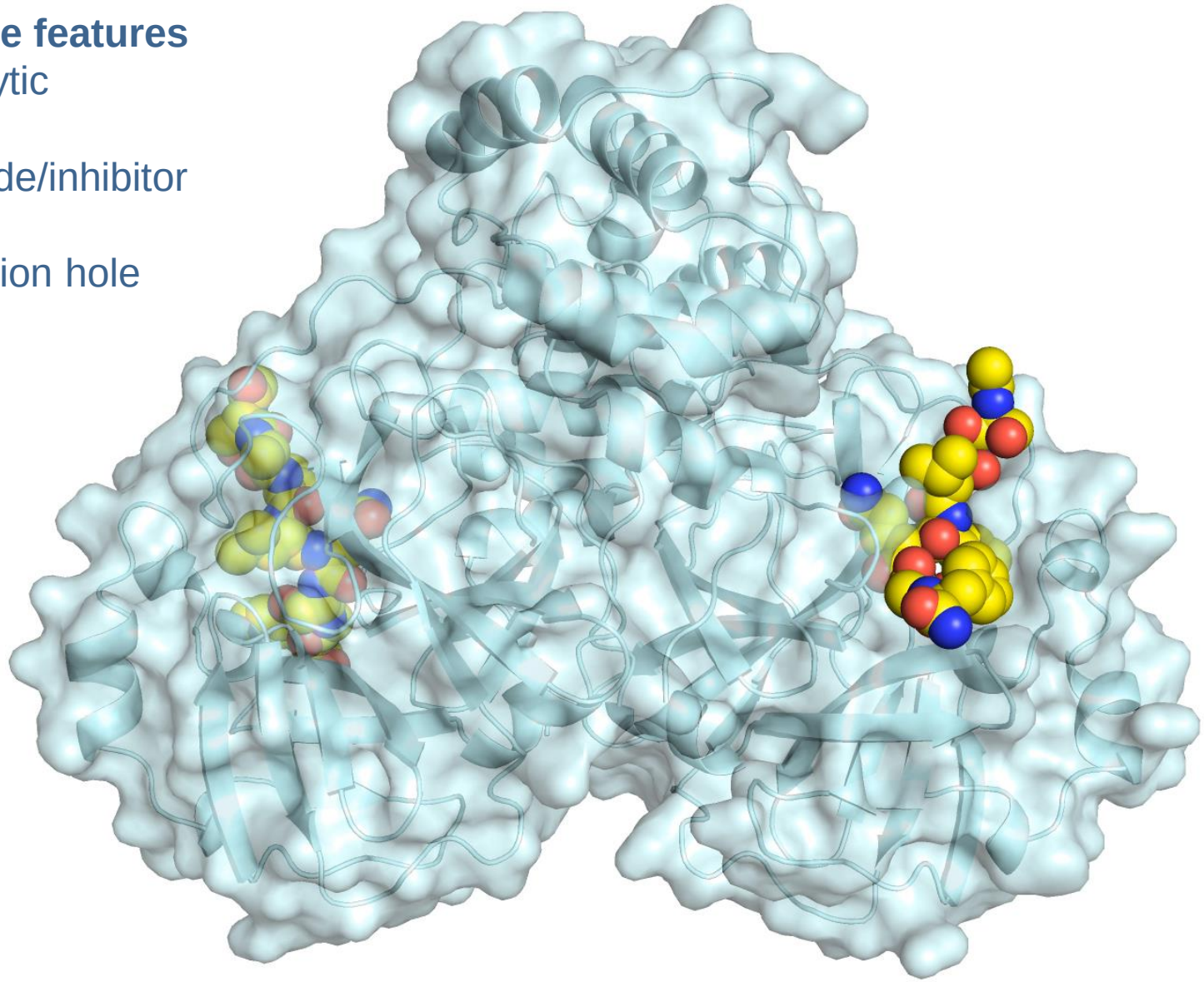
1) Polyproteins are cleaved into components of the replication machinery
2) Protease inhibitors bind active site, blocking substrate processing
3) Viral replication is prevented

Active site of SARS-CoV-2 M^{pro}

Substrate binding to M^{pro}

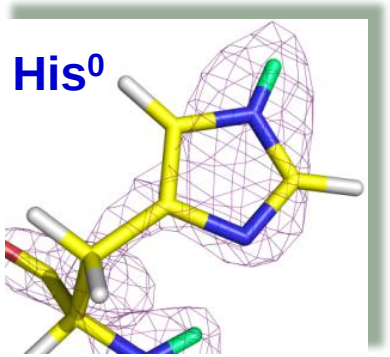
Targetable active site features

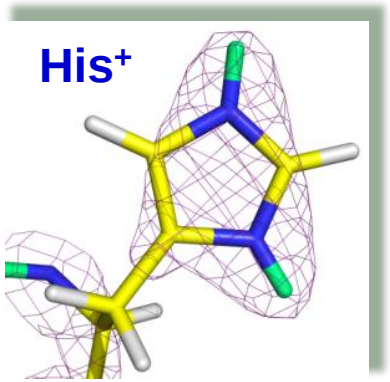
- Non-canonical catalytic Cys145-His41 dyad
- Room for ~6-7 peptide/inhibitor groups (P2'-P5)
- Characteristic oxyanion hole

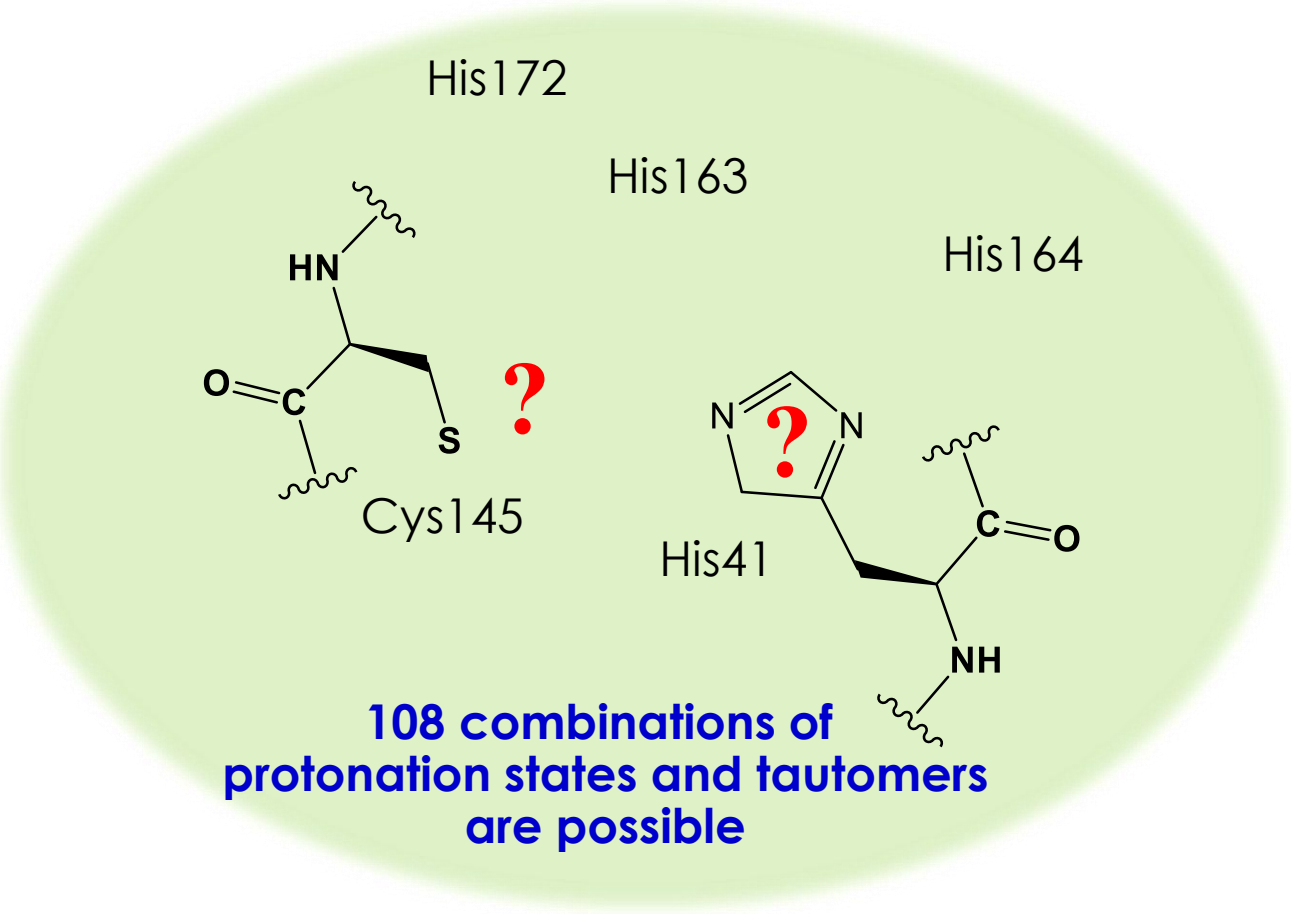


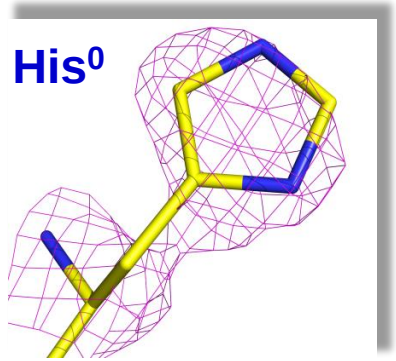
Kneller *et al.* 2020 *IUCR J.* 7, 1028-1035

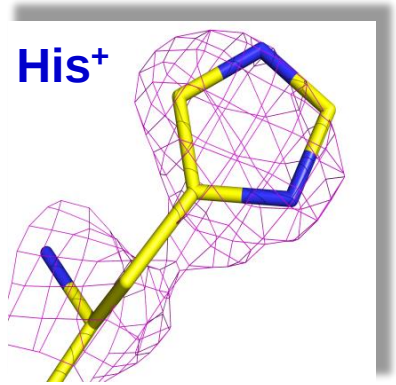
M^{pro} active site: Where are the hydrogens?





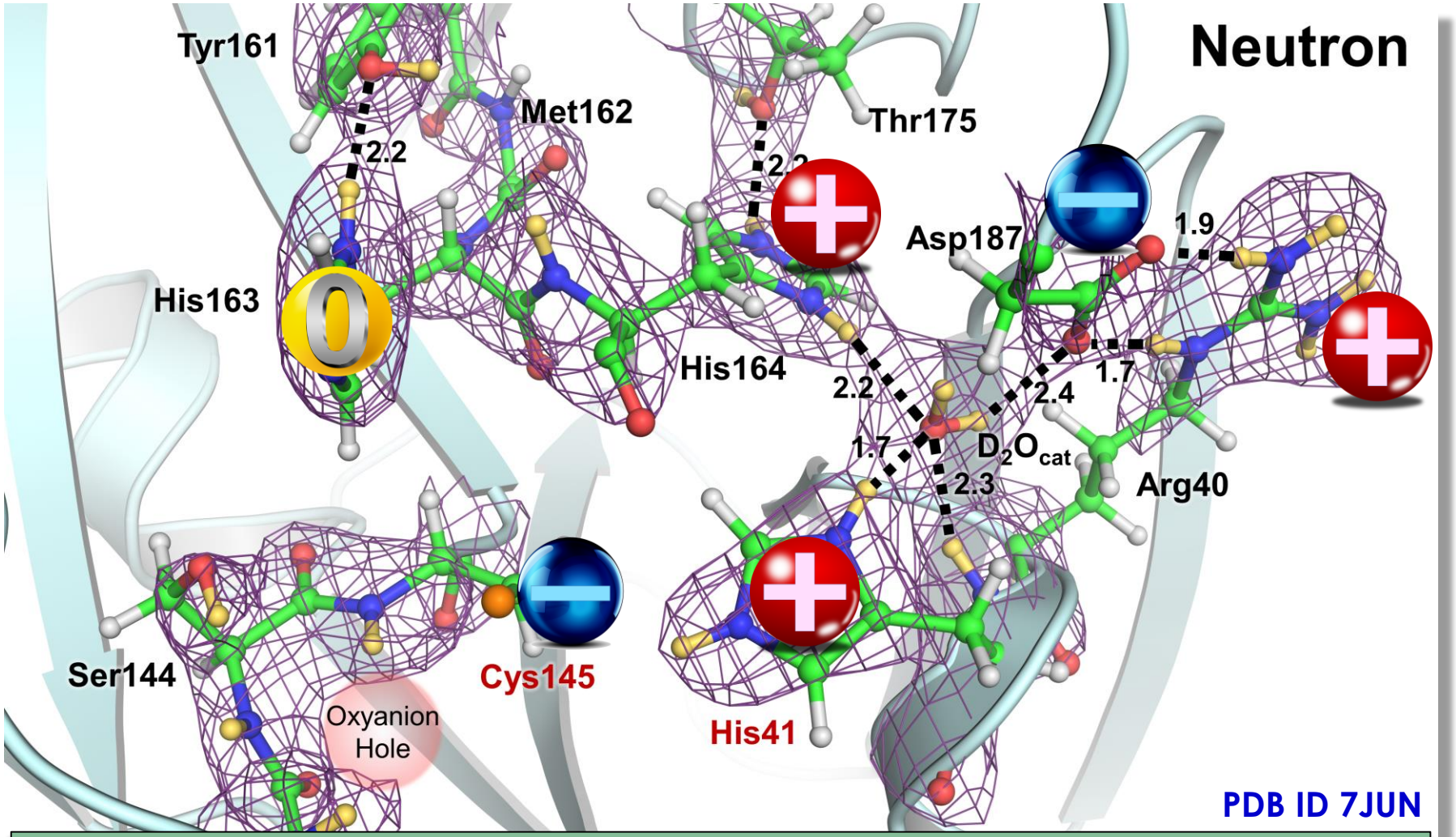






Kneller *et al.* 2020 *Acta Cryst.* F18, 688-699

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



First cysteine (coronavirus) protease neutron structure:
non-canonical catalytic dyad is zwitterionic

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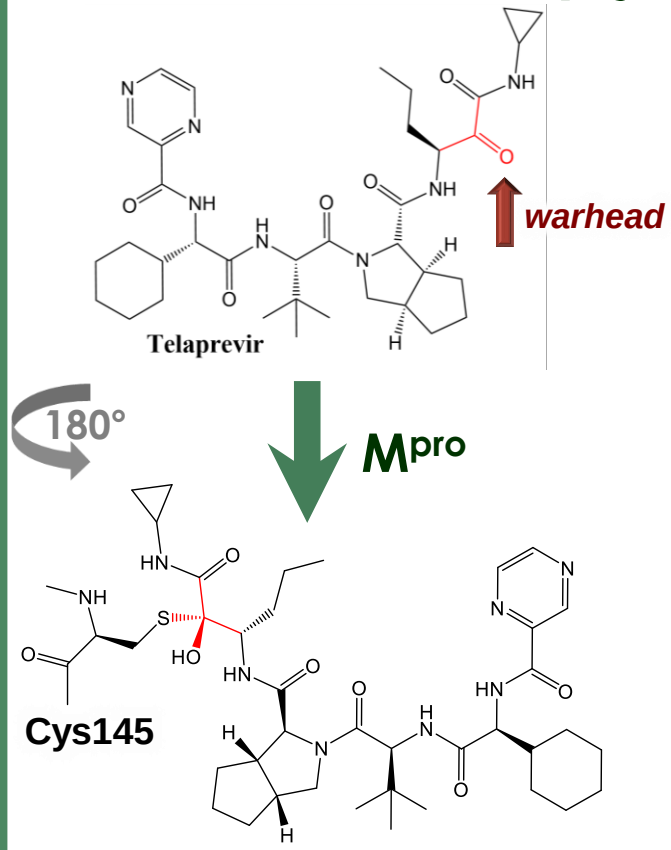
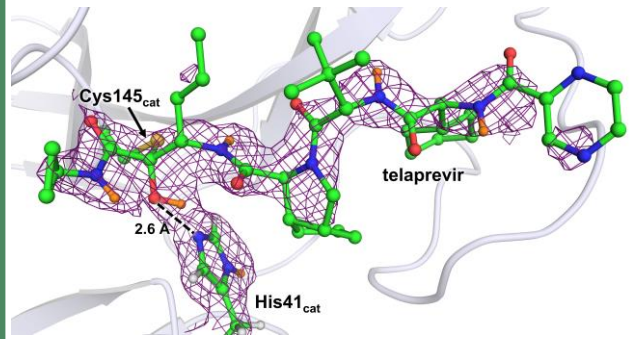
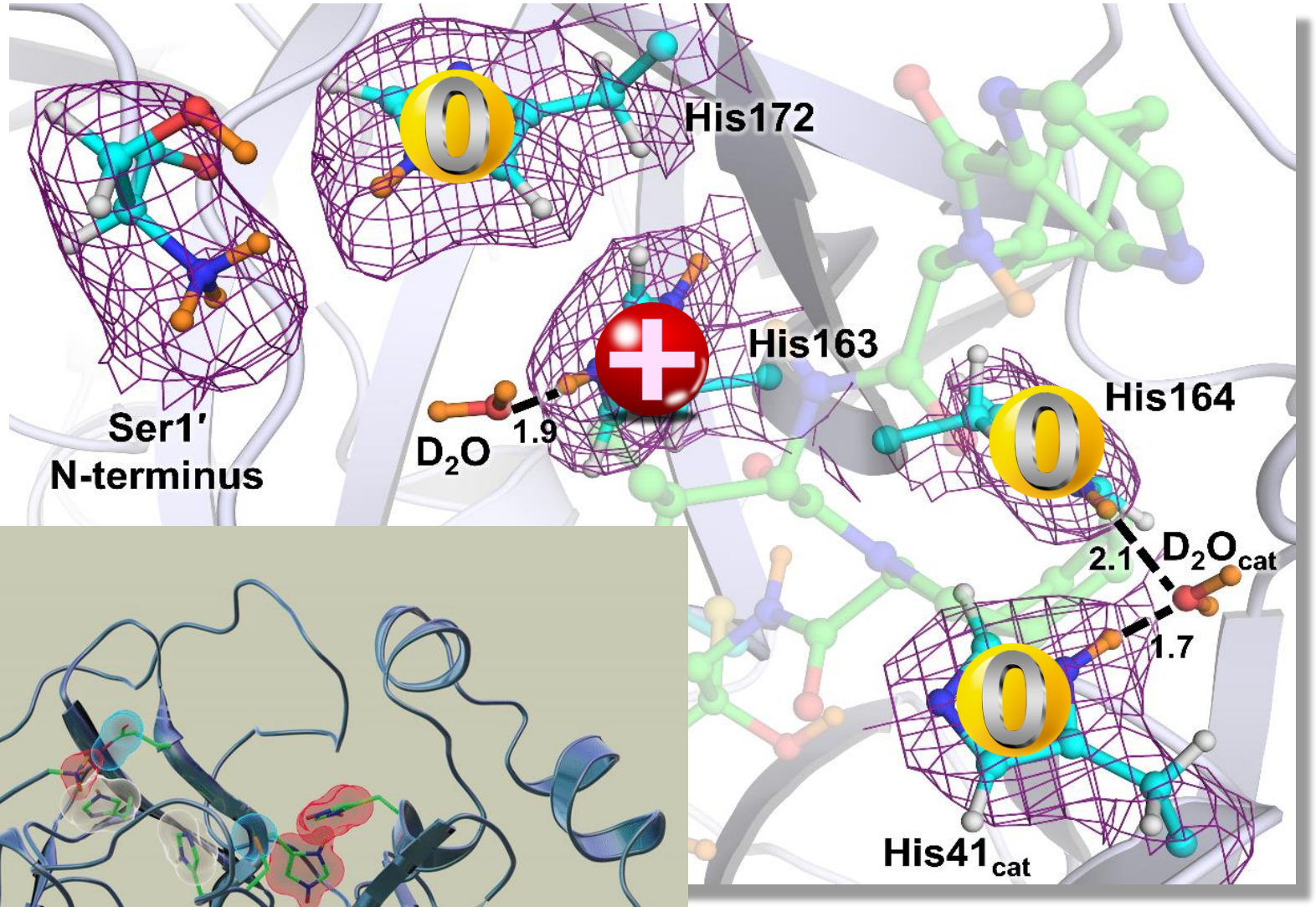
Kneller *et al.* 2020 *Nat. Commun.* 18, 688-699
Kneller et al. 2020 *J. Biol. Chem.* 295, 17365-17373

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







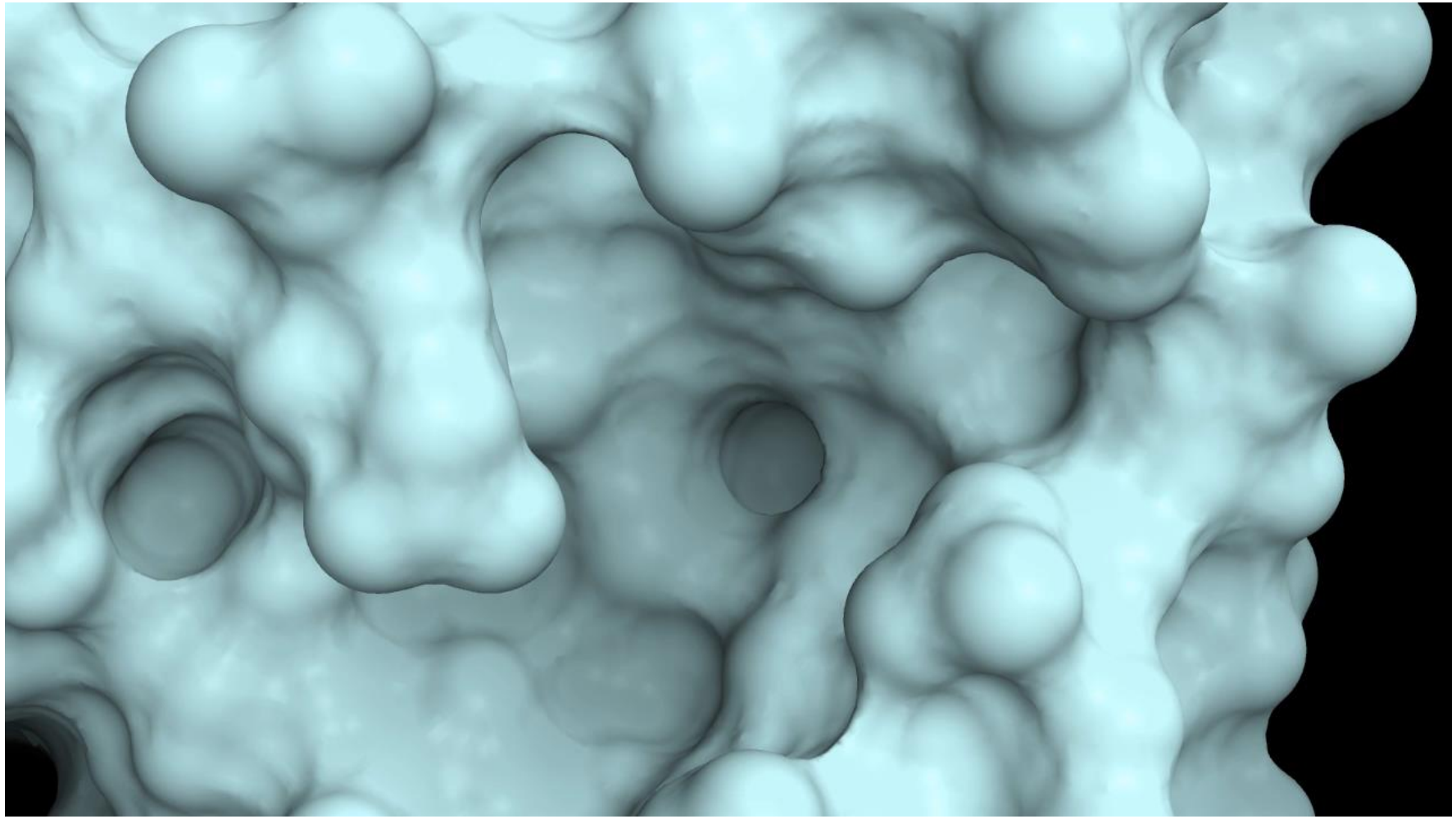
PDB ID 7LB7

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

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

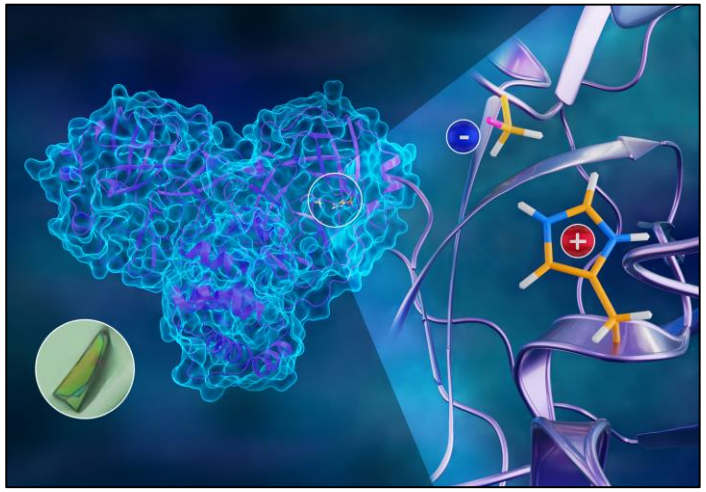
M^{pro} active site is malleable adapting to ligand size

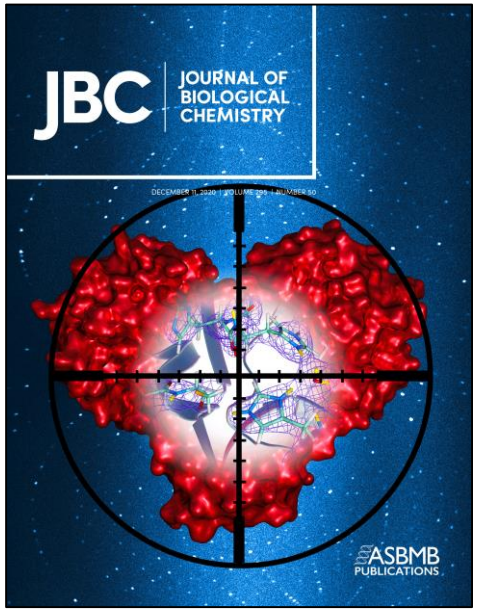
Subsites S2 and S4 are *cryptic*



Tunability and malleability of M^{pro}

- Active site protonation states are tunable, but overall electrostatic charge is maintained at +1
- Active site conformation dynamically adapts to inhibitor properties
- Cryptic binding subsites and plasticity presents challenges for inhibitor design and *in silico* modeling





Structure-activity relationship (SAR) study for noncovalent inhibitors:

Exploring structural, electrostatic and electronic determinants for binding to subsites S1 and S2

Supercomputer screen identifies noncovalent inhibitor

6.5 Million ligand library

↓

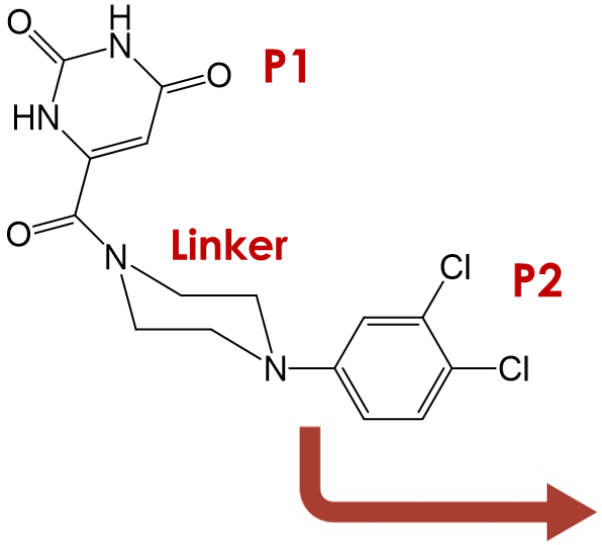
336 Billion conformer dockings

↓

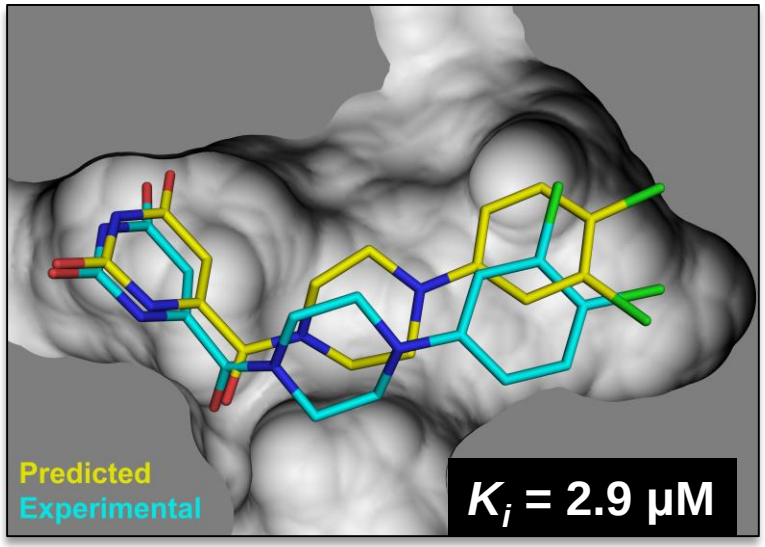
72 tested by *in vitro* assay

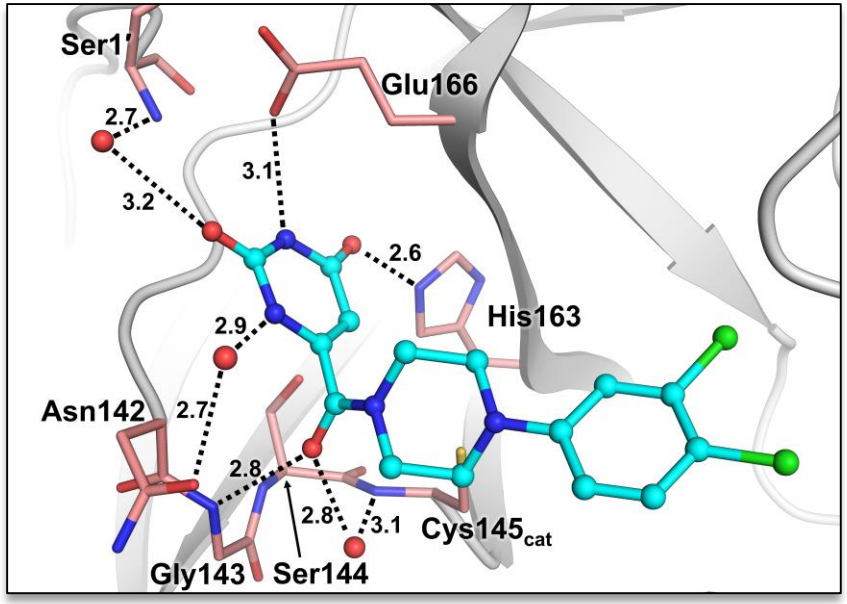
↓

1 active compound



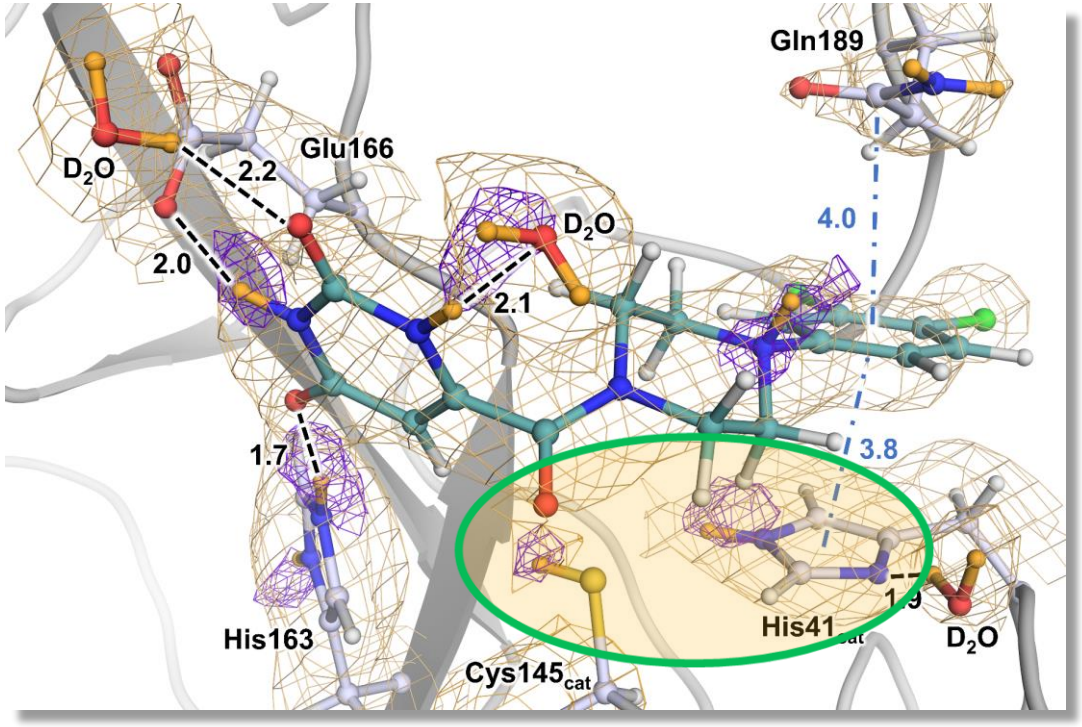
1 room temperature X-ray structure



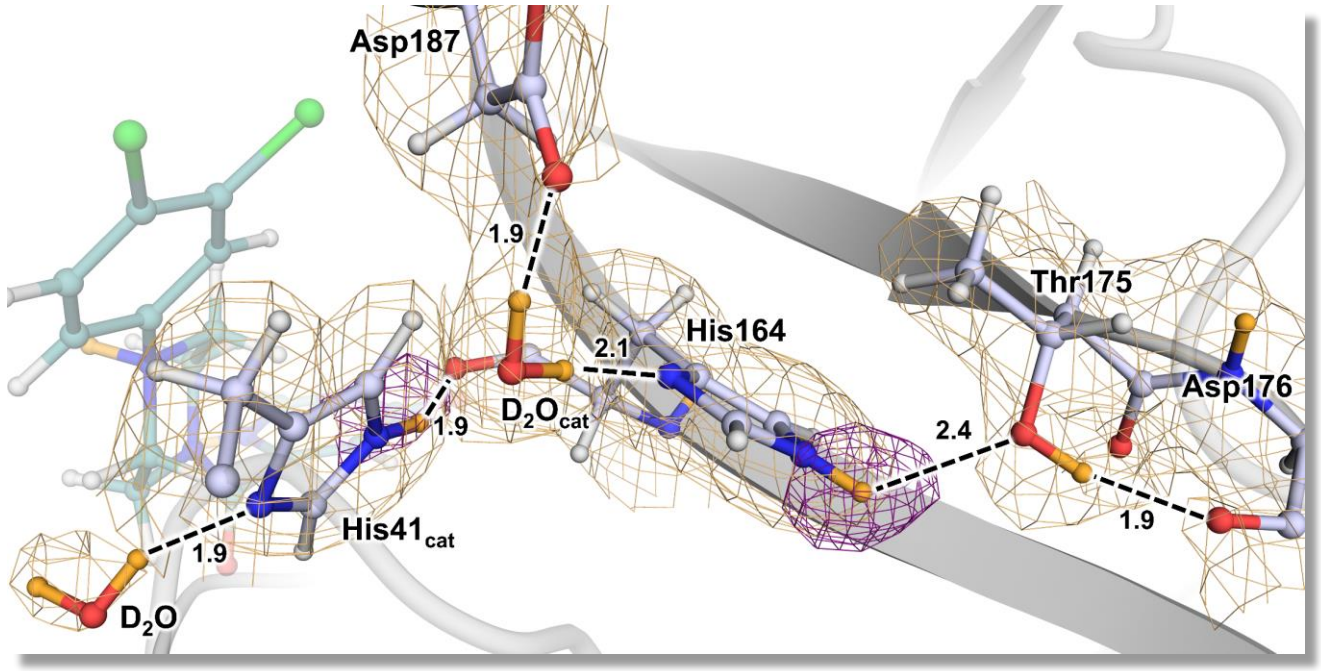


Clyde *et al.* 2022 *J. Chem. Inf. Model.* 62, 116-128

Room-temp joint XN structure of M^{pro}-1 complex @ 2.5Å resolution



Direct characterization of protein-ligand hydrogen bond network

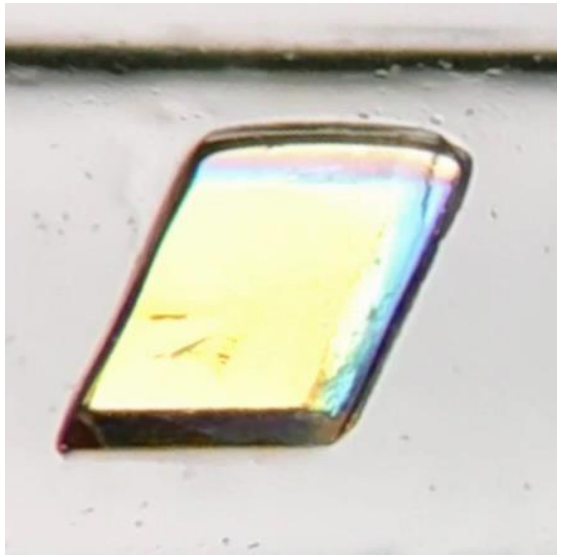


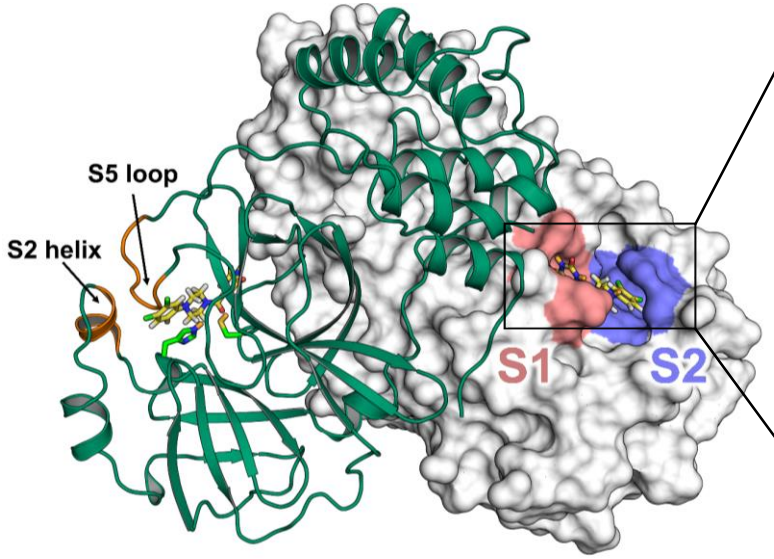
Neutrons enable observation of new catalytic water molecule orientation

First neutron structure of M^{pro}-Non-covalent inhibitor complex

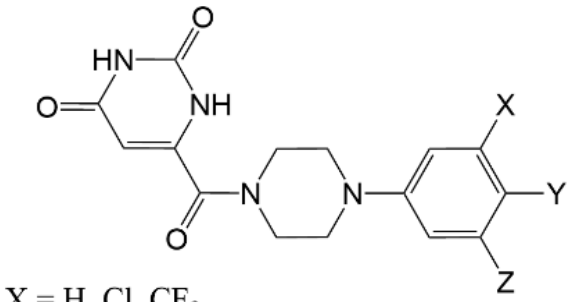
Kneller *et al.* 2021 *J. Med. Chem.* 64, 17366-17383

Neutron structure-led VR-assisted SAR

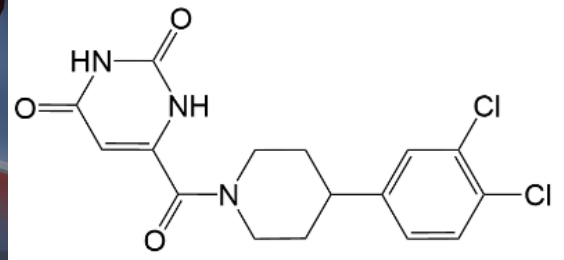




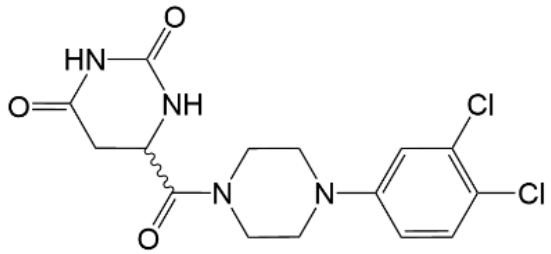
HL-3 series:



X = H, Cl, CF_3
Y = H, Cl, F, Br, I, CN, CF_3, CH_3, CHO, CH_2OH
Z = H, Cl

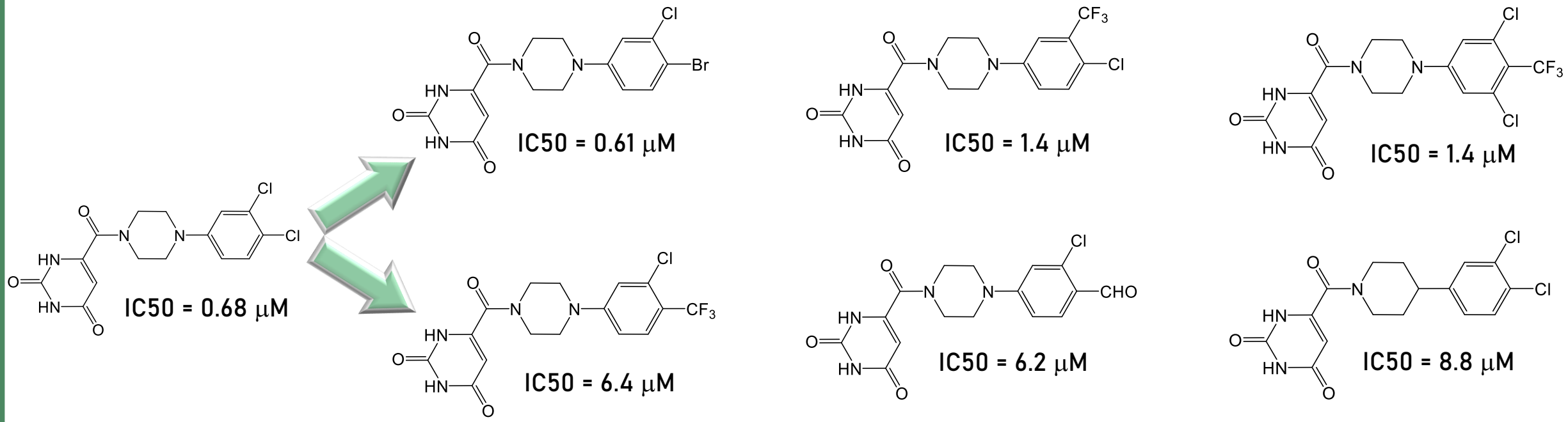


HL-3-69



HL-3-51 (*S*), HL-3-53 (*R*)

SAR study guided by the neutron structure M^{pro}-1 complex (HL-3 series)



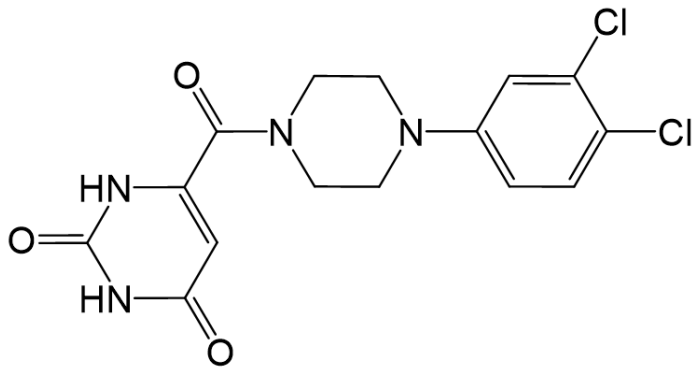
- P1 must have H bond acceptor capability and correct geometry to make H bond with protonated His163.
- Substituents on the aromatic P2 group should have both moderate steric size and electronegativity.
- P2 group with one substituent on the aromatic ring is disadvantageous.
- Highly electronegative substituents, such as -F or -CF_3 are disadvantageous, as are less electronegative but sterically larger ones.
- A third substituent such as -Cl at P2 group is favorable.

U.S. Provisional Patent Application serial No. 63/232,331

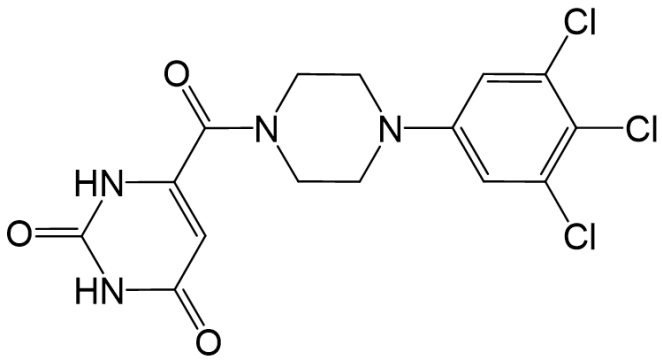
In vitro assessment of chemical modifications

Compound	Inhibition K_i (μM)	Affinity K_d (μM)	ΔH (kcal/mol)	ΔS (cal/mol·K)	ΔG (kcal/mol)
MCULE-5948770040	2.9	1.30	-8.32	-0.7	-8.11
HL-3-68	0.89	0.69	-7.75	2.4	-8.5
Mcule-CSR-494190-S1	1.4	1.32	-9.1	-3.16	-8.15

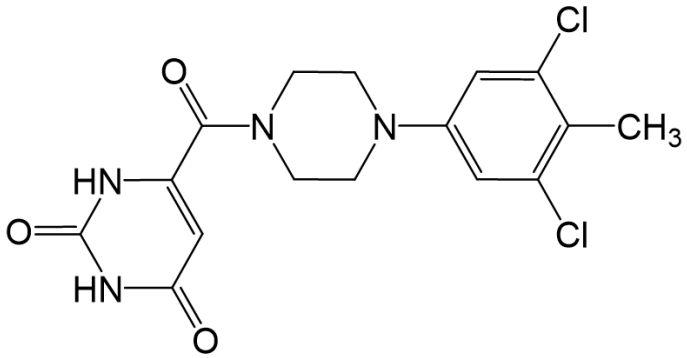
MCULE-5948770040



HL-3-68

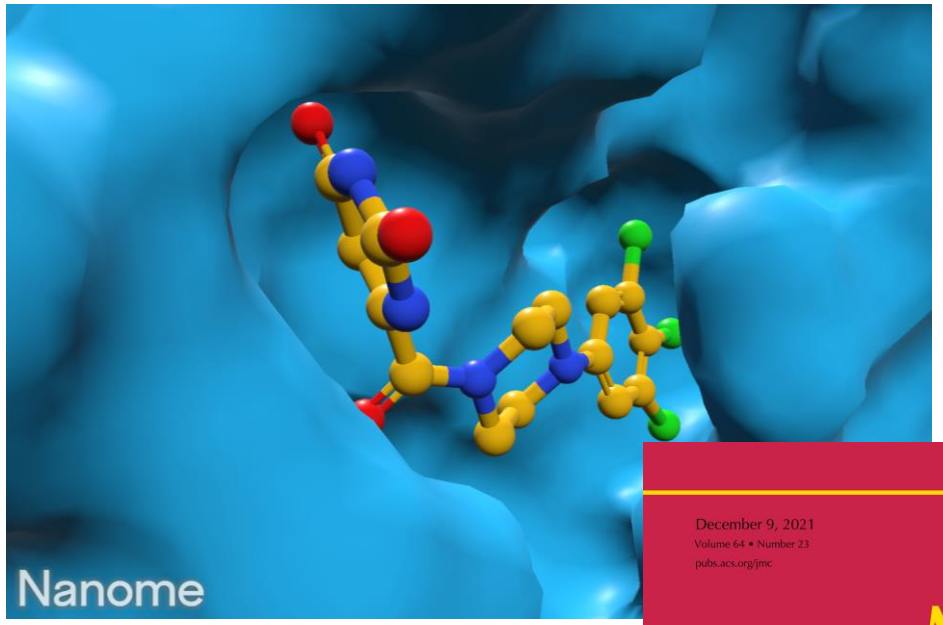


Mcule-CSR-494190-S1



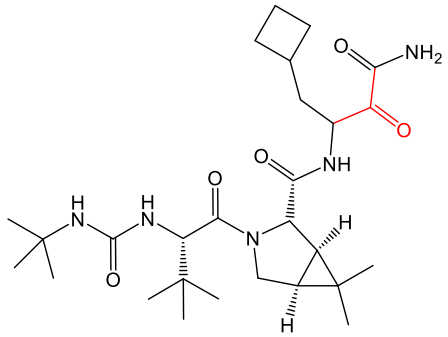
M^{pro} is structurally and electronically malleable

- One atom difference can significantly alter inhibitor binding potency
- VR allowed true 3D structural analysis and inhibitor building
- VR allowed inhibitor structures to be tailored to the binding site

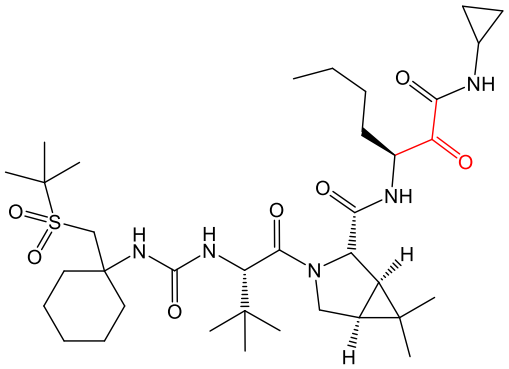


Design of covalent inhibitors based on hepatitis C virus protease inhibitors

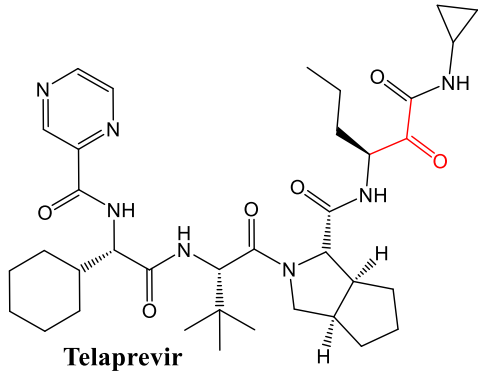
Hepatitis C virus protease inhibitors bind and inhibit M^{pro}



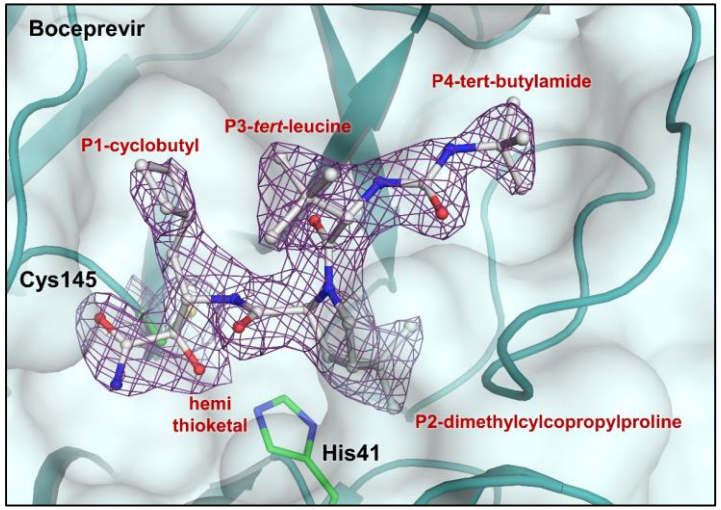
Boceprevir



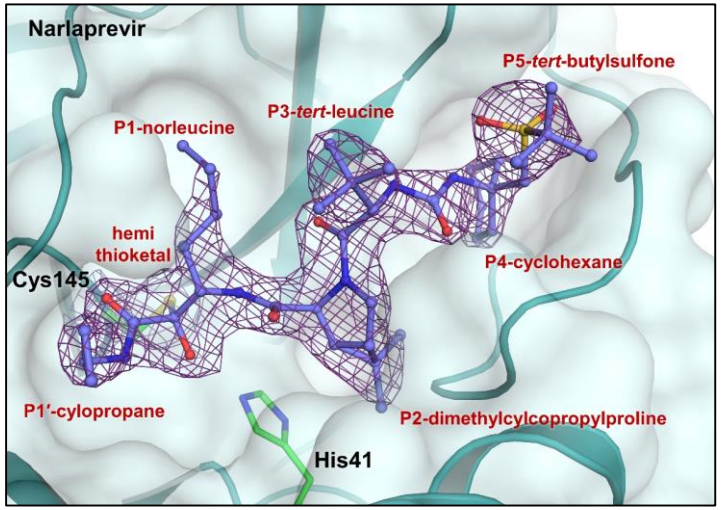
Narlaprevir



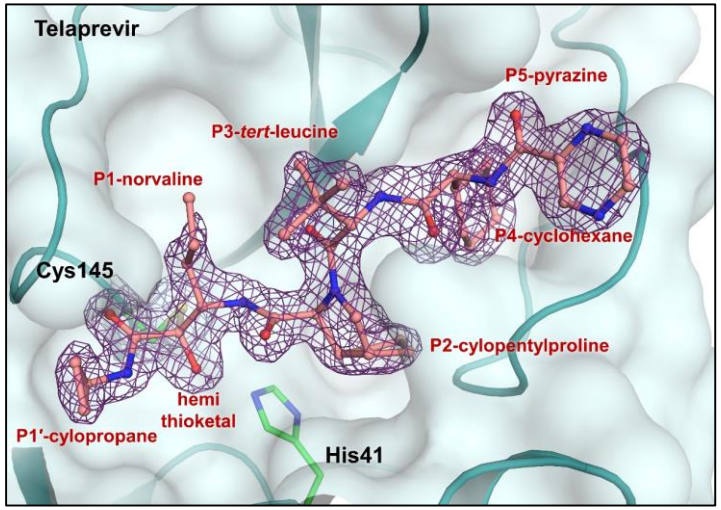
Telaprevir



PDB 6XQU IC50 = 3 μM



PDB 6XQT IC50 = 5 μM

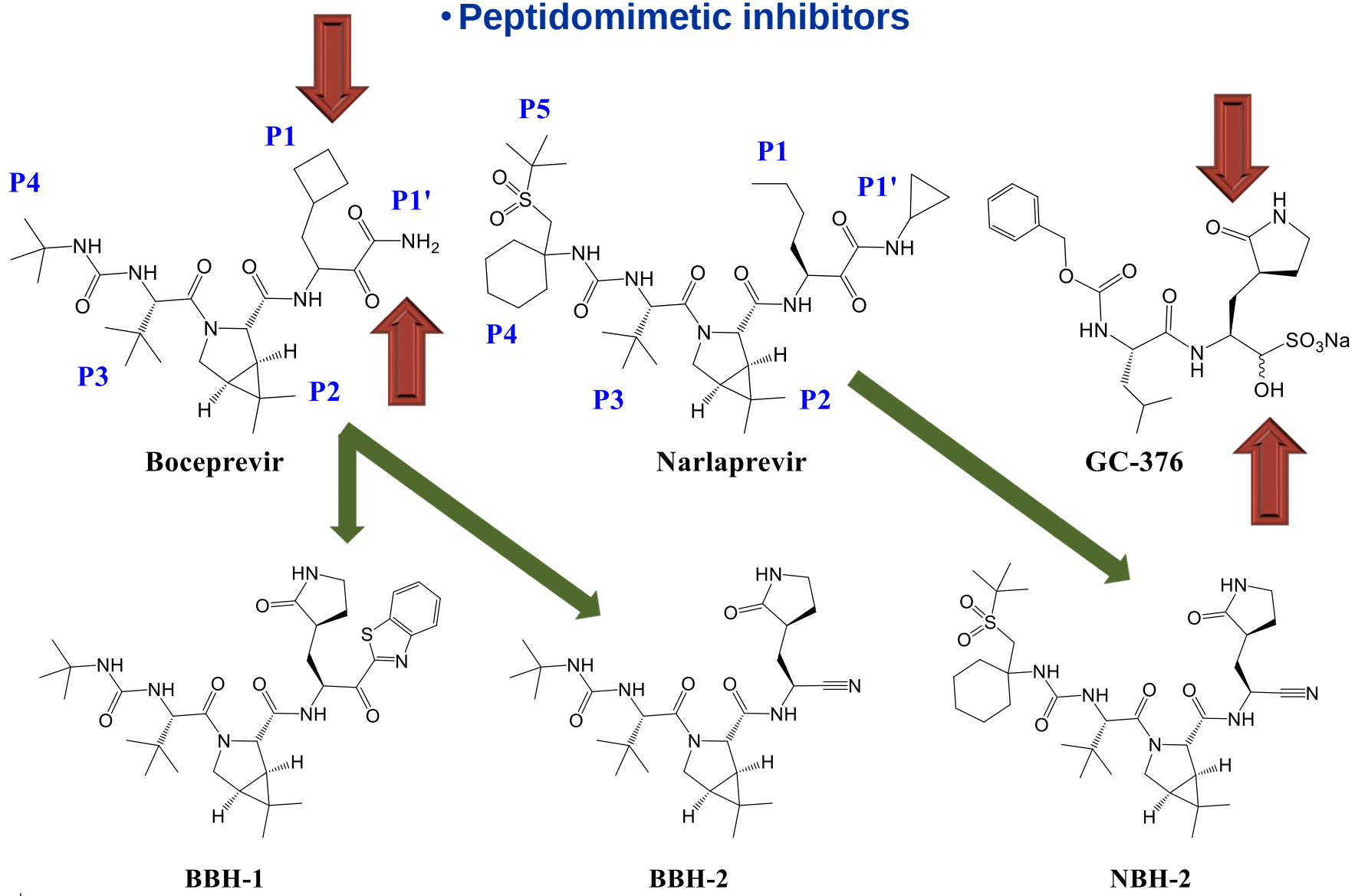


PDB 6XQS IC50 = 18 μM

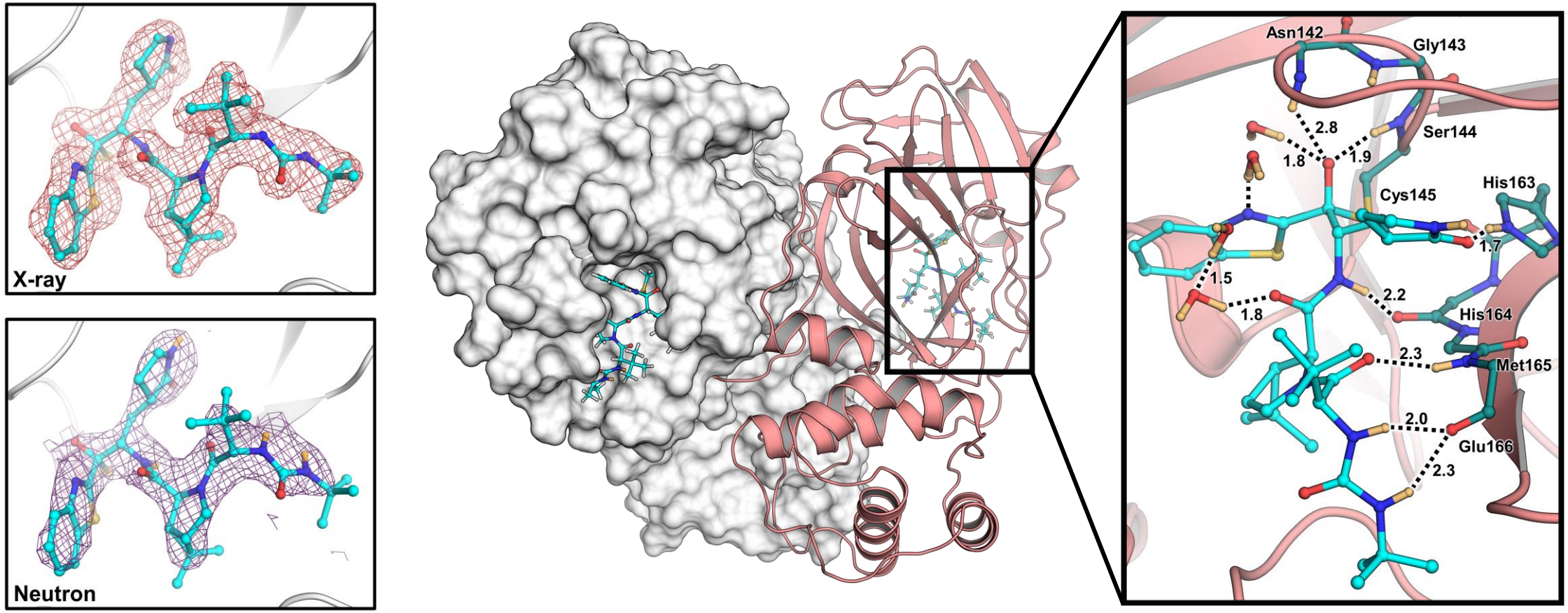
Kneller *et al.* 2020 *Structure 28*, 1313-1320

Design of covalent hybrid inhibitors of M^{pro}

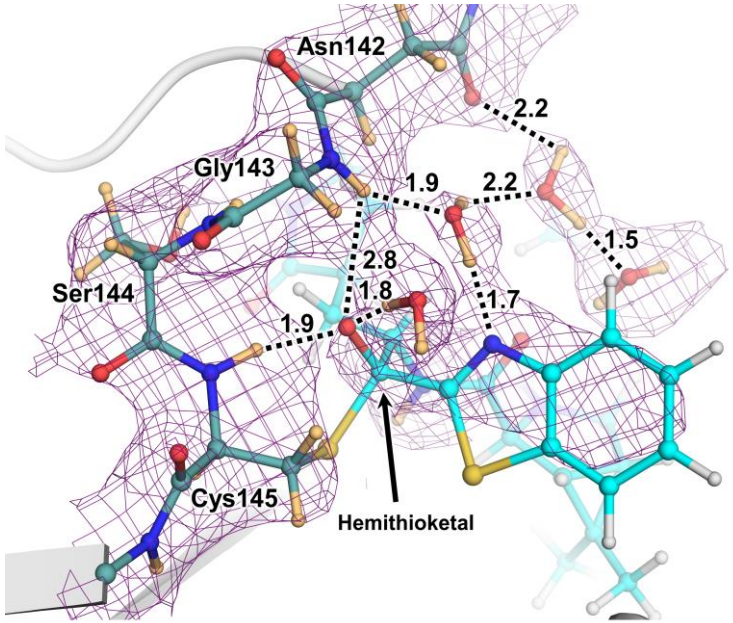
- Peptidomimetic inhibitors

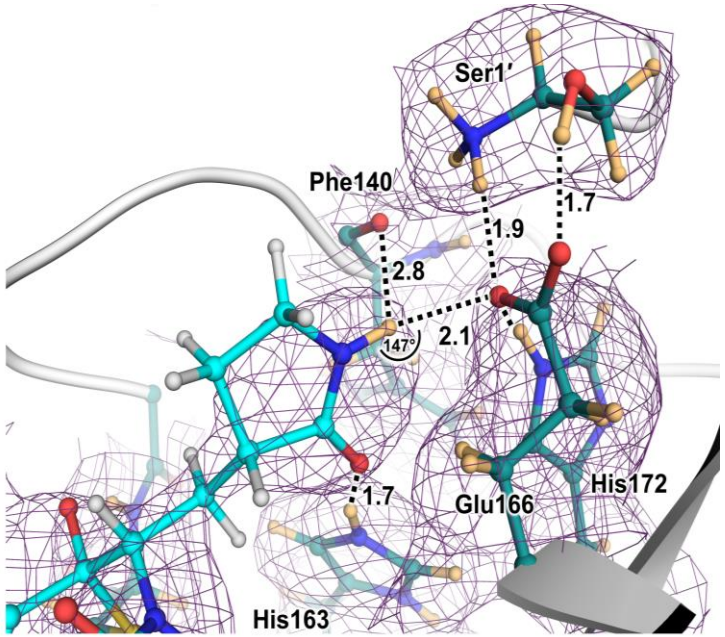


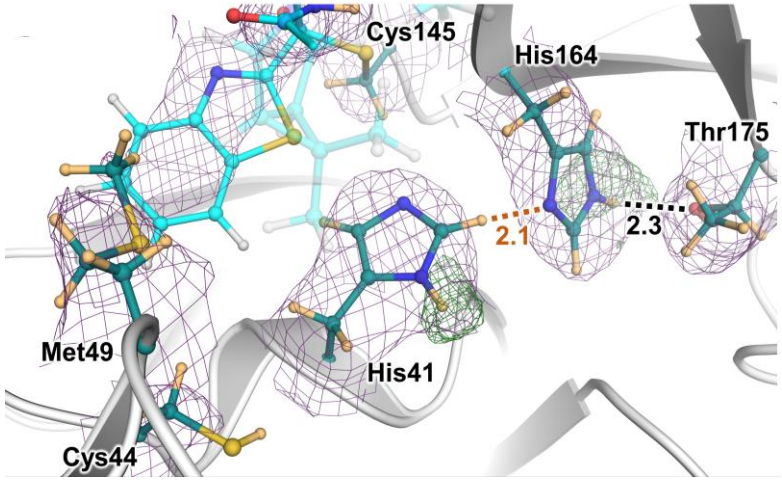
Unique binding of BBH-1 to M^{pro}: *A neutron structure perspective*



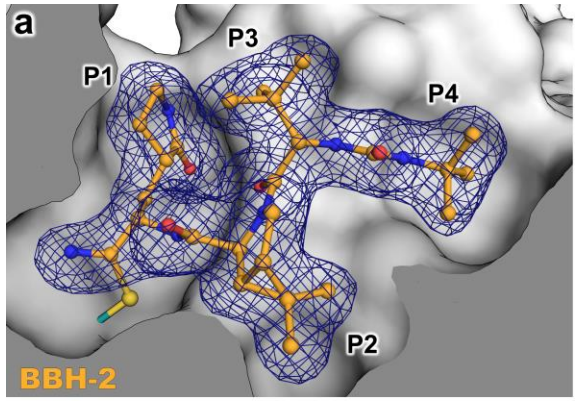
Unique binding of BBH-1 to M^{pro}: *A neutron structure perspective*

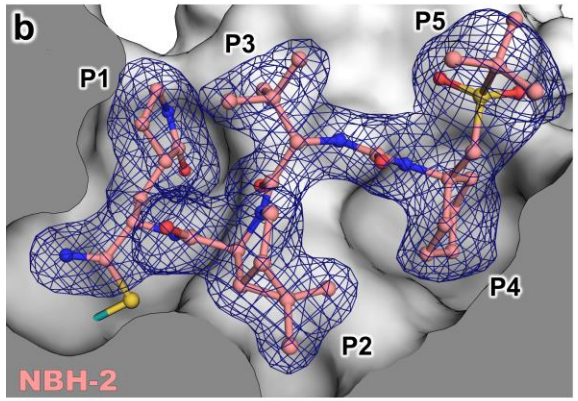


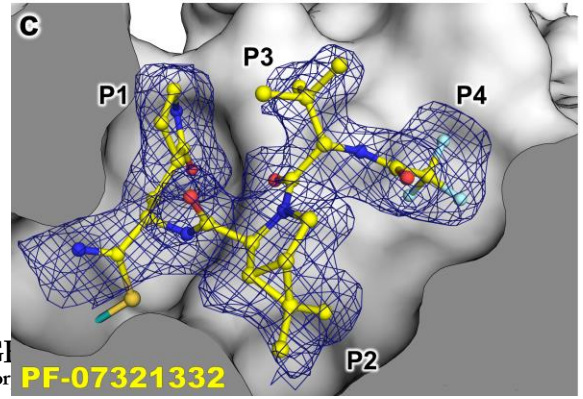


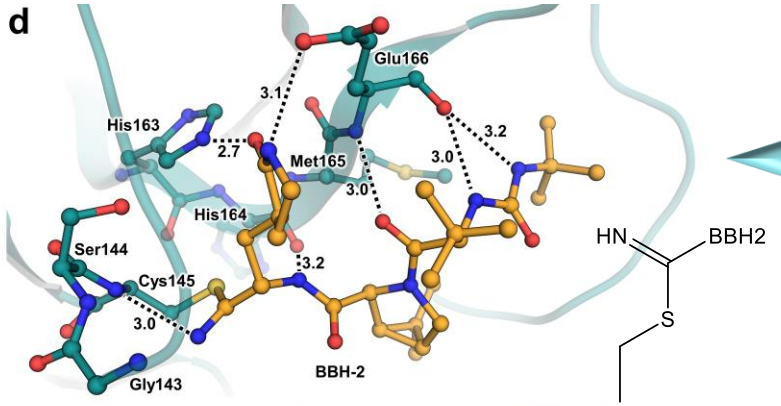


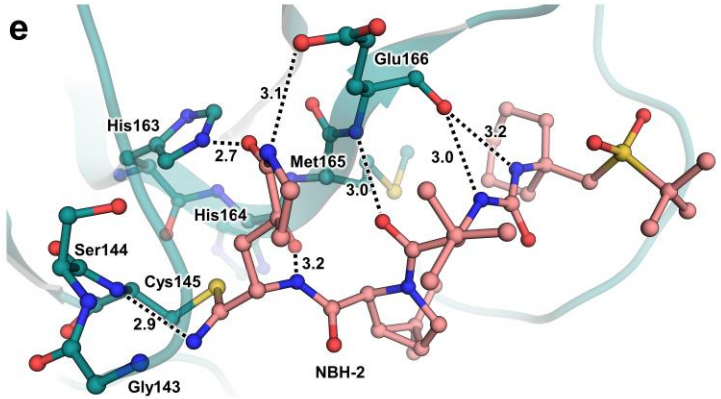
Covalent inhibitors with nitrile warhead

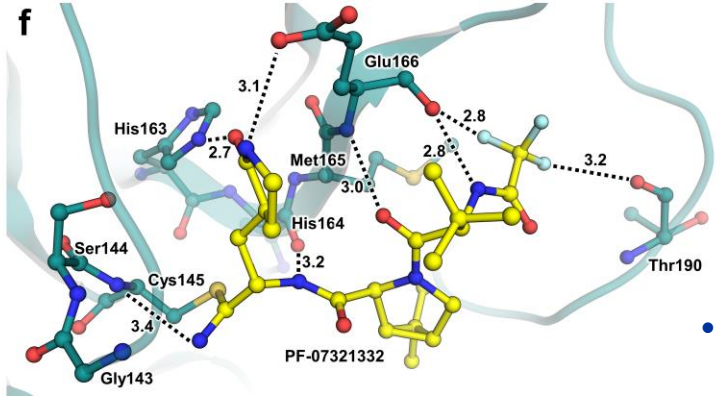


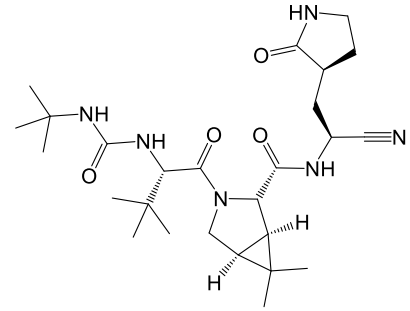


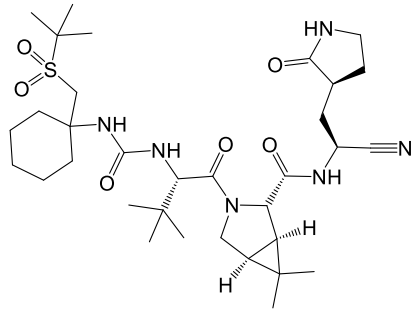


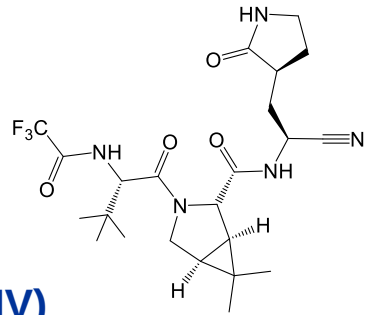








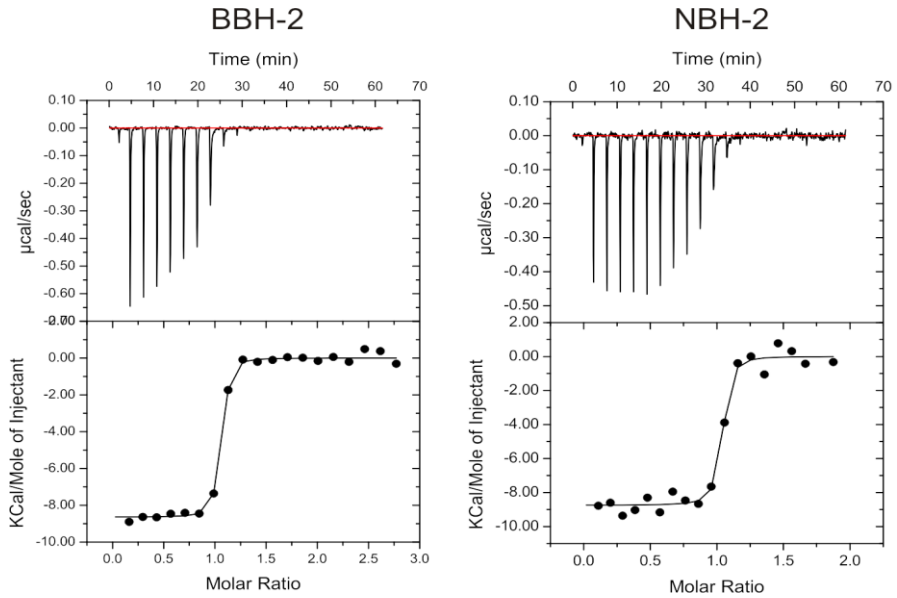


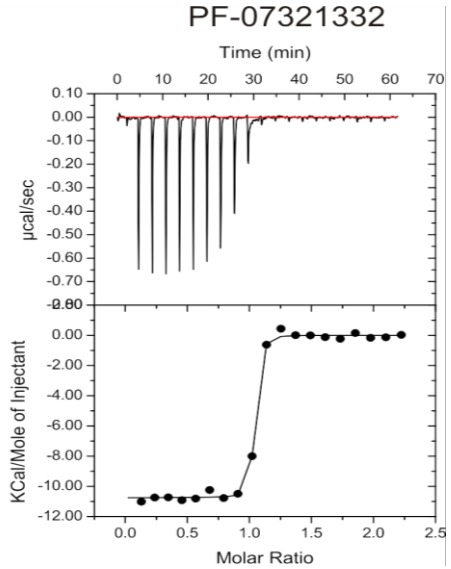


- Nirmatrelvir (NMV)

Isothermal titration calorimetry

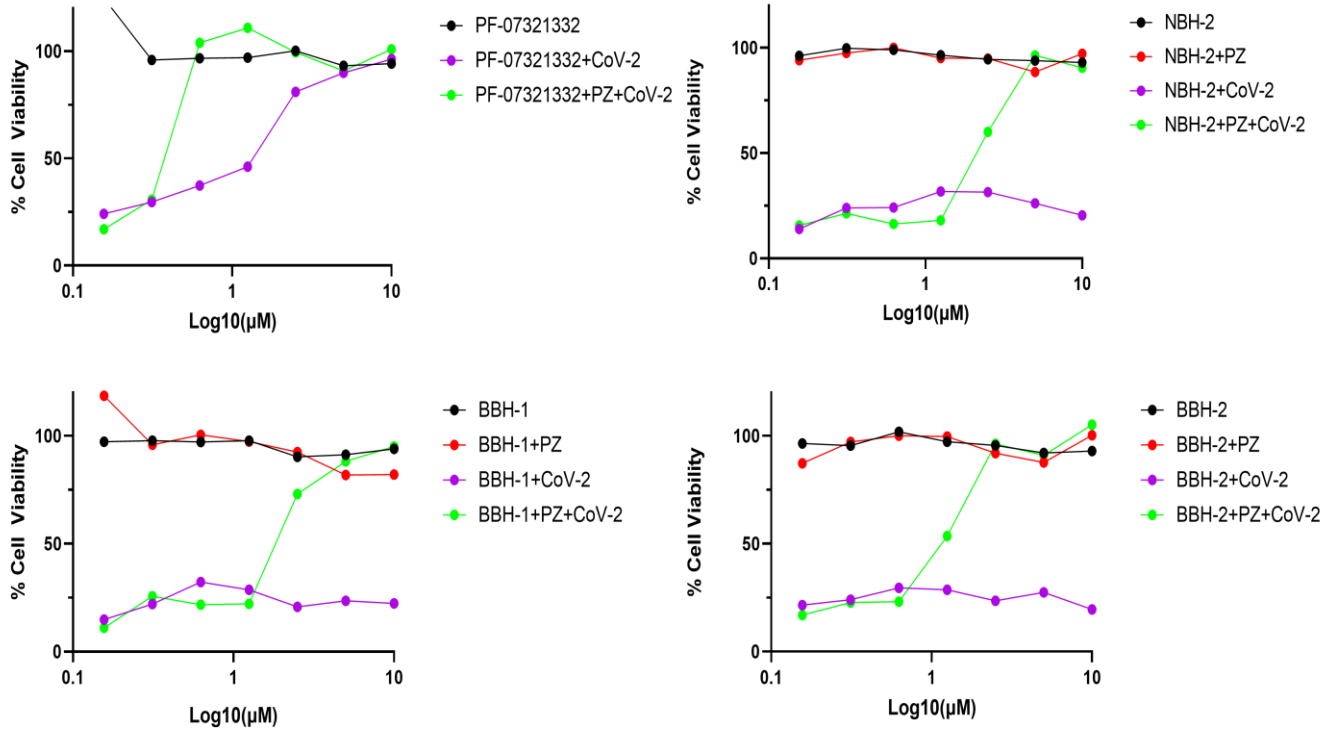
Compound	K_d, μM	Stoichiometry, N	ΔH, kcal·mol^{-1}	ΔS, cal·mol^{-1}·K^{-1}	ΔG, kcal·mol^{-1}
BBH-2	0.030 ± 0.007	1.000 ± 0.005	–8.74 ± 0.08	5.40	–10.4
NBH-2	0.026 ± 0.016	0.990 ± 0.009	–8.76 ± 0.17	5.63	–10.5
PF-07321332	0.007 ± 0.003	0.990 ± 0.003	–10.75 ± 0.70	1.57	–11.2
GC-376	0.15 ± 0.03[a]	0.99 ± 0.01	–6.7 ± 0.1	9.1	–9.4





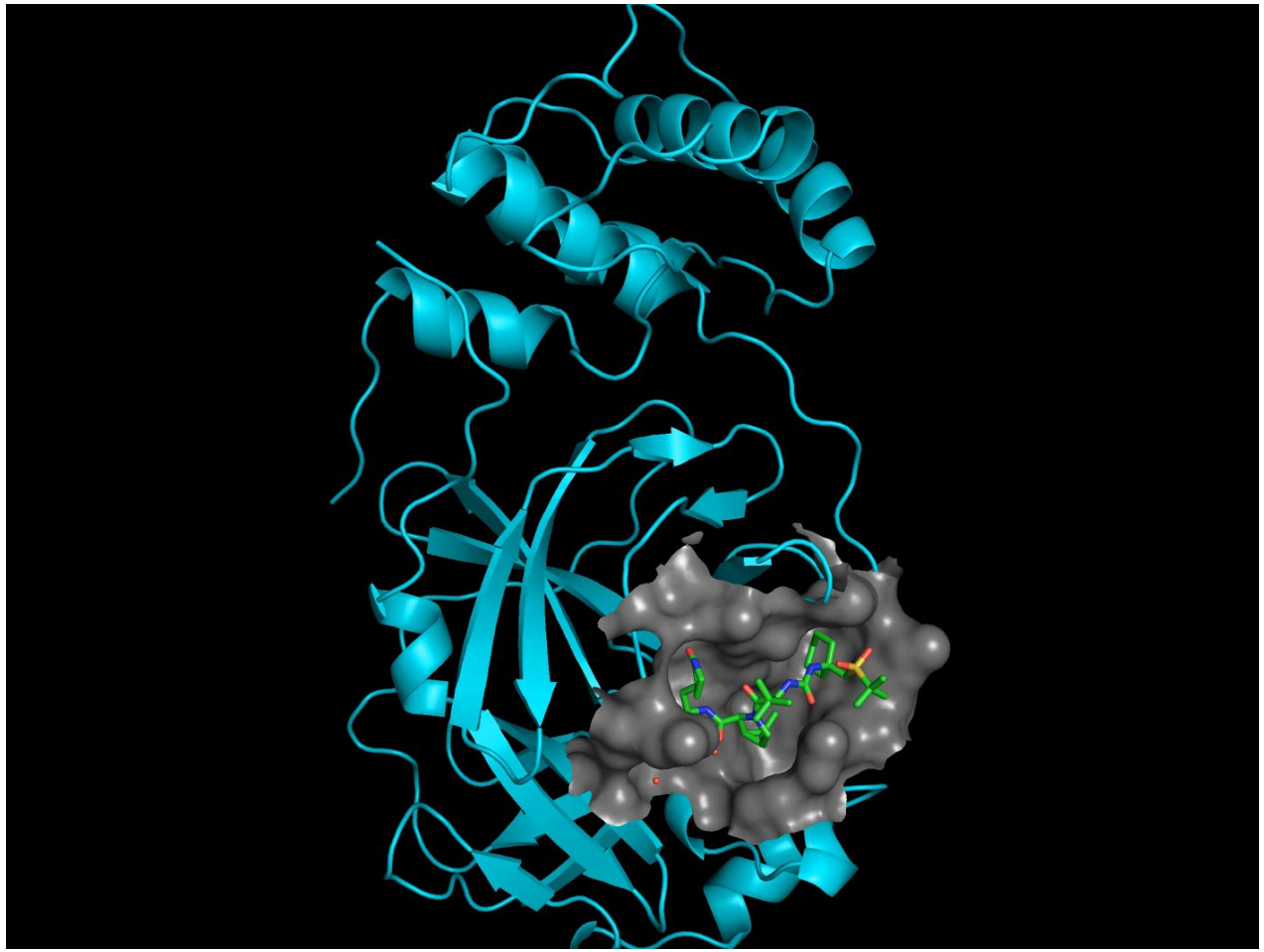
Antiviral data

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



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



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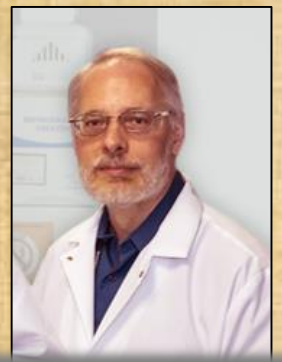

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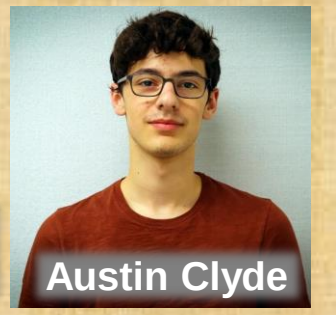

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