Leritrelvir (RAY1216):
A SARS-CoV-2 Main Protease inhibitor for the treatment of COVID-19

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BioKin Ltd.

Brandeis University :: 5 Apr 2024

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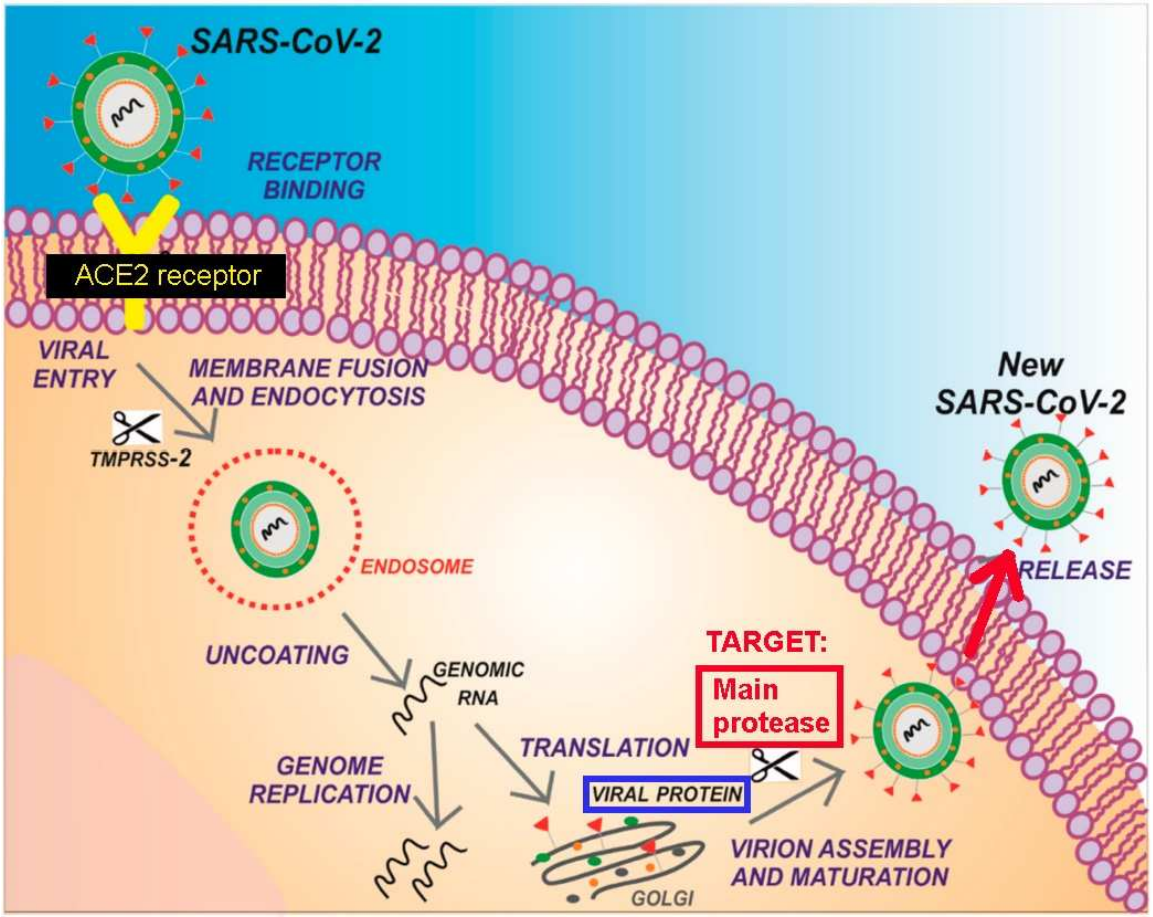
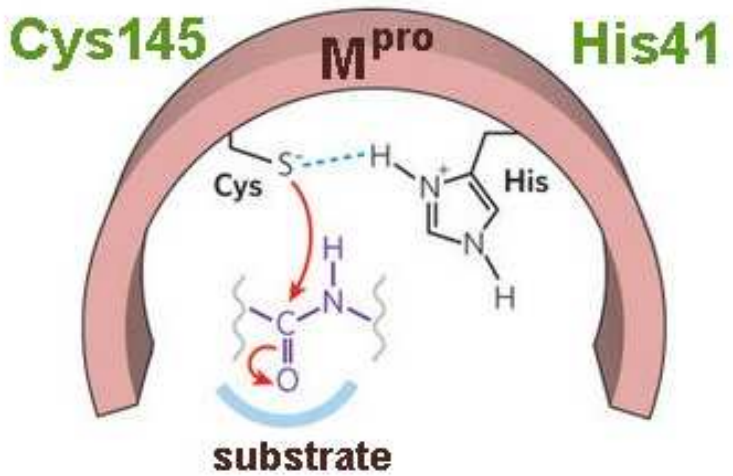
SARS-CoV-2 viral life cycle



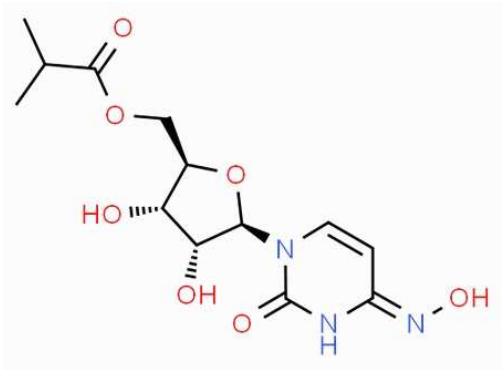
Adapted from Citarella *et al.* (2021)

- M^{pro} is relatively conserved across SARS-CoV-2 variants.

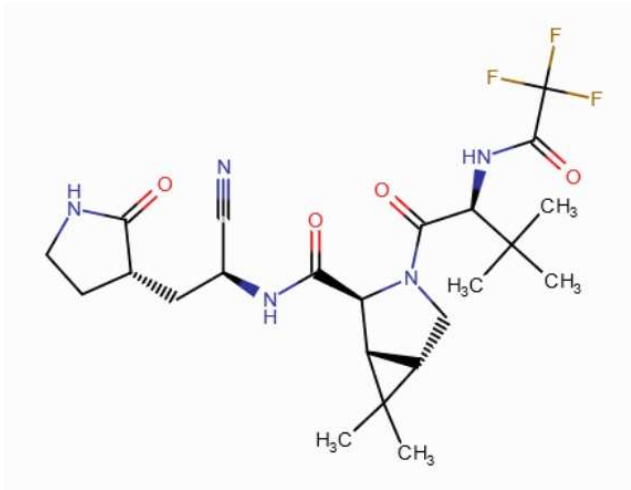
M^{pro} is a cysteine protease



The only two anti-COVID-19 drugs currently approved in the United States



molnupiravir (Merck)



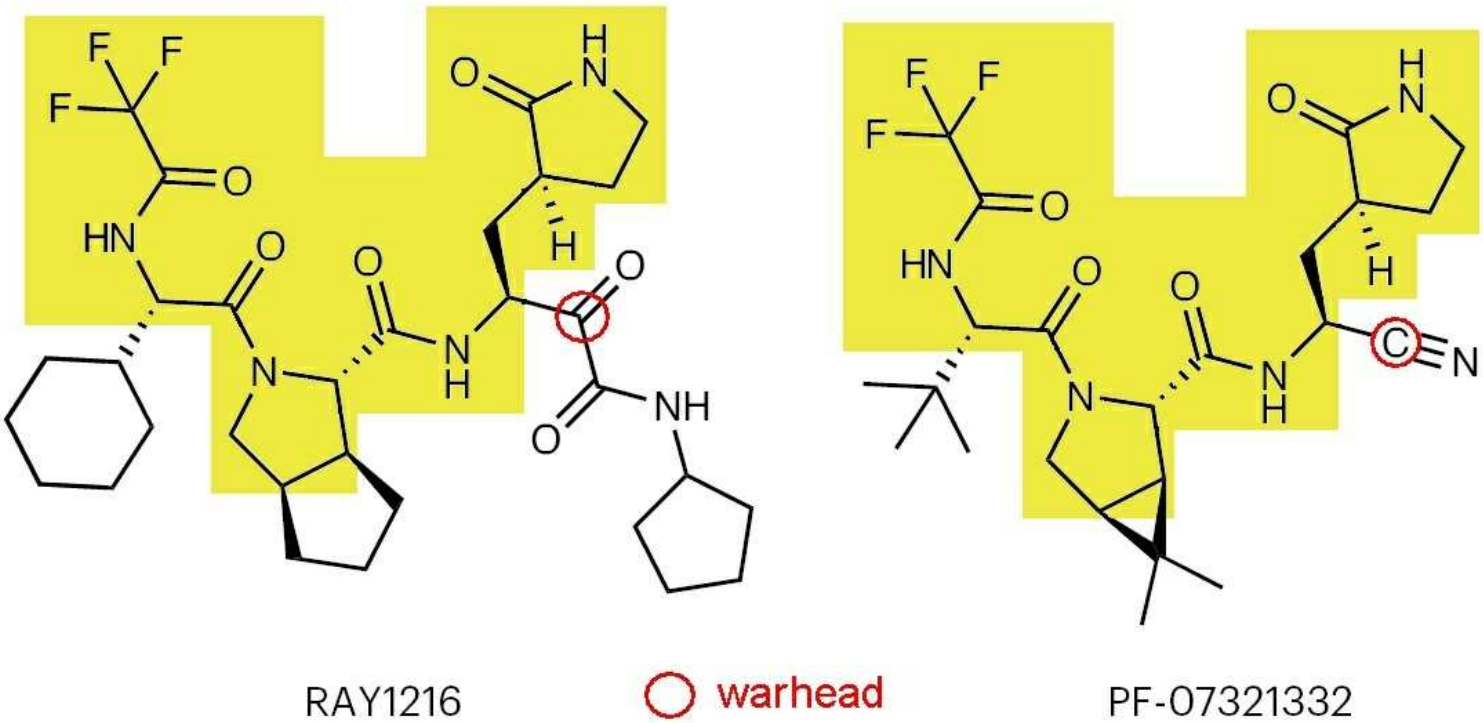
nirmatrelvir (Pfizer) = **PF-0731332**
Selective inhibitor of M^{pro}

Problems:

- **molnupiravir** is mutagenic; it randomly scrambles viral RNA.
- **nirmatrelvir** requires ritonavir, which inhibits liver enzymes to slow down drug metabolism.

Structure of the novel M^{pro} inhibitor drug: RAY1216 = "leritrelvir"

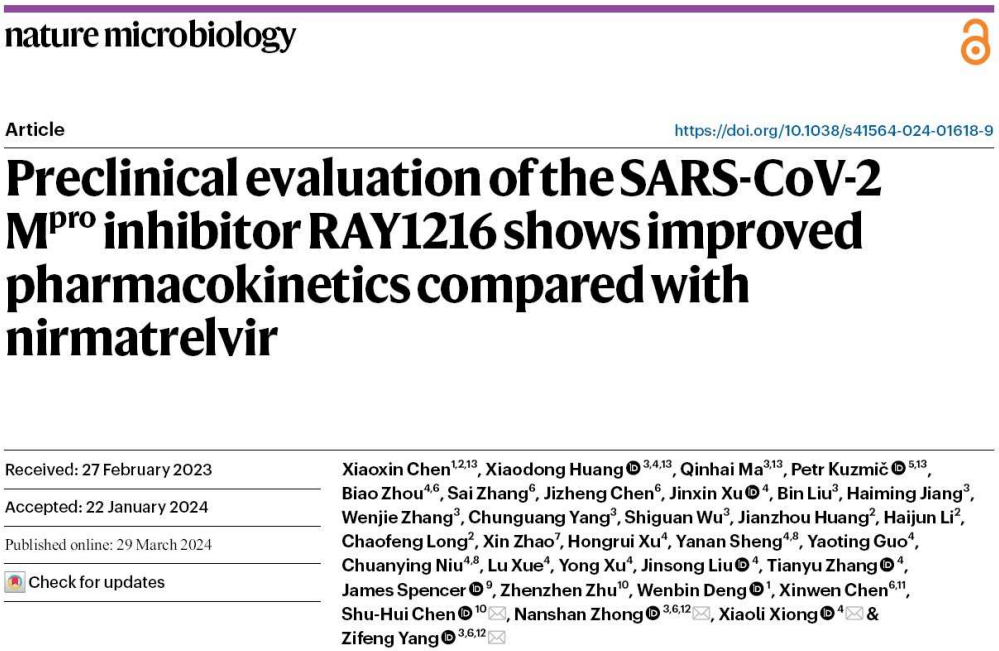
- Yellow-shaded areas are identical in both drugs.



- **RAY1216** (*leritrelvir*): Raynovent Biotechnology Co., Guangzhou, China
- **PF-0731332** (*nirmatrelvir*): Pfizer Inc., New York, USA

RAY1216 vs. PF-0731332: head-to-head comparison

- Chen, X.; Huang, X.; Ma, Q.; Kuzmič, P.; *et al.* (2024) *Nat. Microbiol.* **9**, 1075 – 1088
- Published in print: **April 5, 2024**.



nature microbiology

Article

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Preclinical evaluation of the SARS-CoV-2 M^{pro} inhibitor RAY1216 shows improved pharmacokinetics compared with nirmatrelvir

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Check for updates

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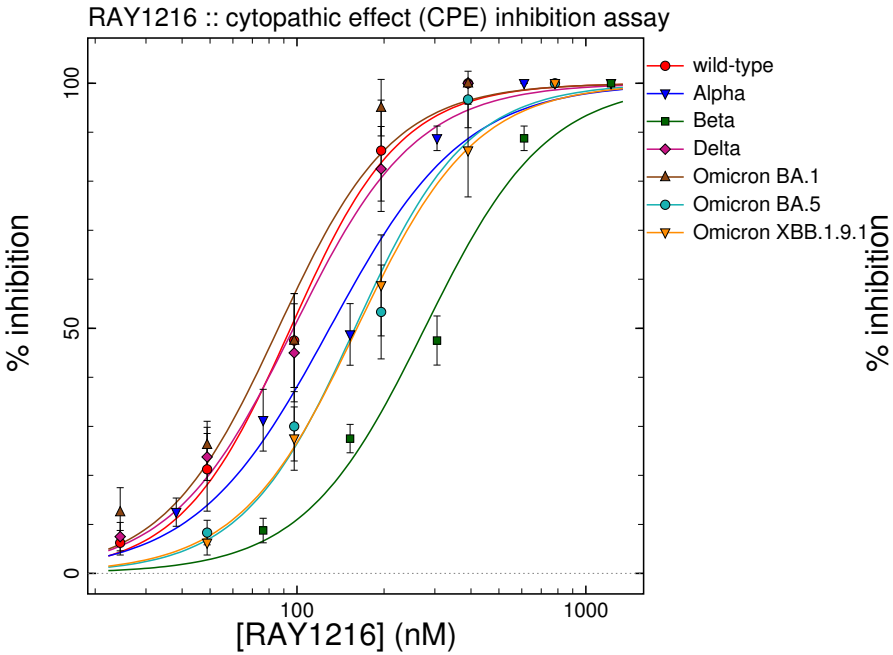
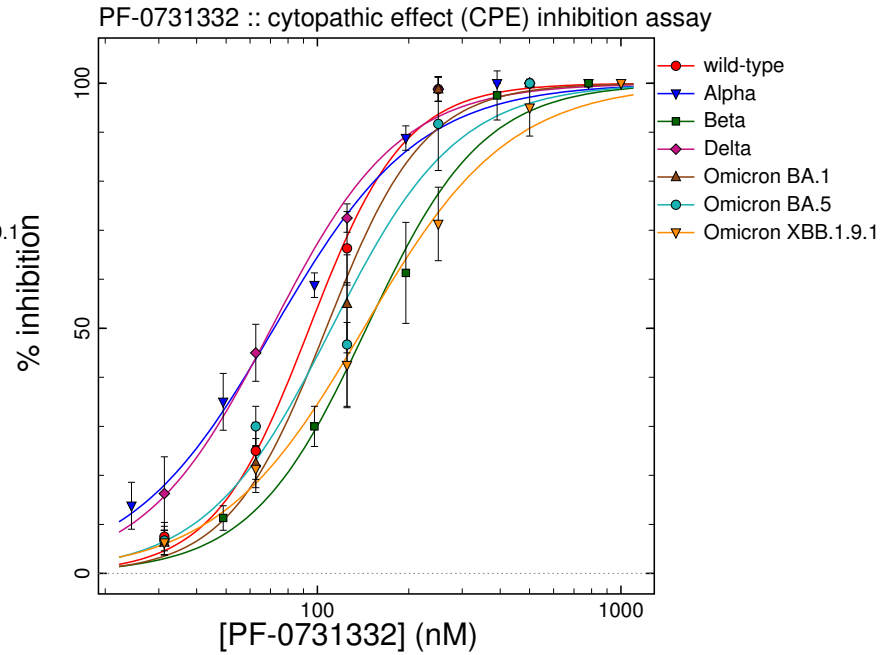
- Almost all pre-clinical results are very similar for both drugs.
- The only – crucially important – difference is in pharmacokinetics (plasma stability).

Example of nearly identical results: cytopathic effect (CPE) inhibition assay



RAY1216

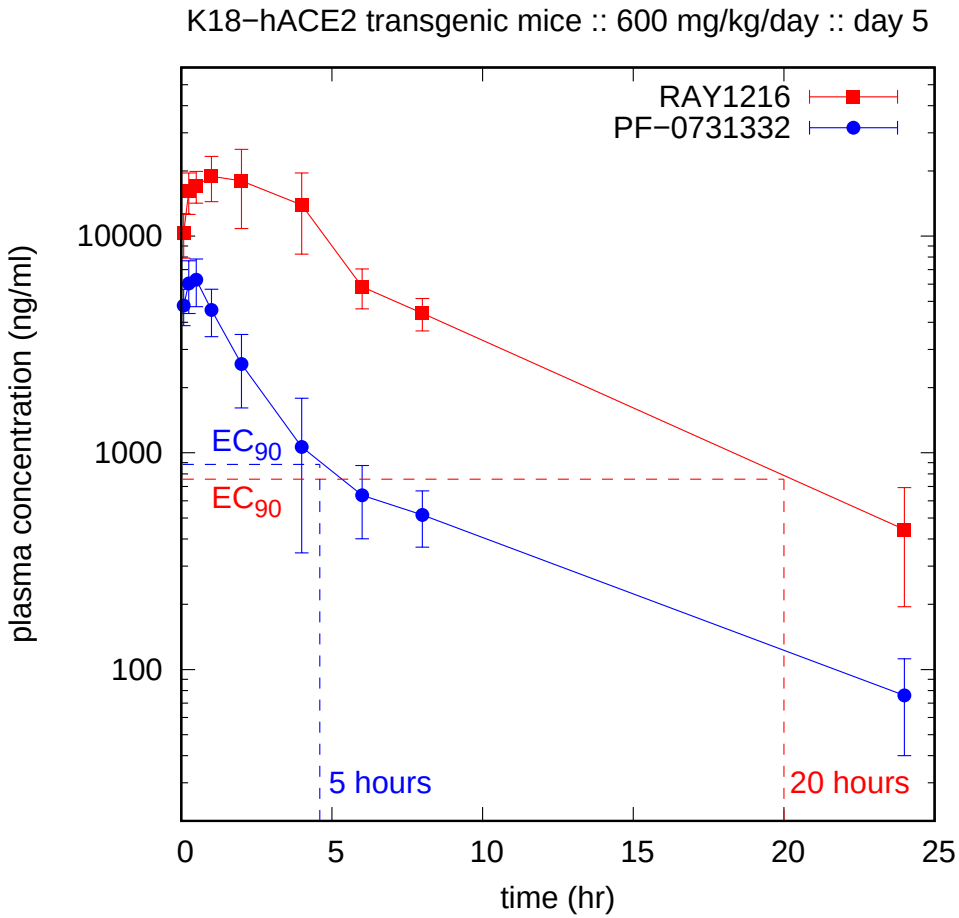


PF-0731332

Delta variant CPE EC_{90} corrected for plasma binding:

760 ng/ml (RAY1216)
880 ng/ml (PF-0731332)

Major difference in pharmacokinetics



Phase 3 human clinical trial results

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December 2023

Articles

Leritrelvir for the treatment of mild or moderate COVID-19 without co-administered ritonavir: a multicentre randomised, double-blind, placebo-controlled phase 3 trial

Yangqing Zhan,[a,b,aa] Zhengshi Lin,[a,b,aa] Jingyi Liang,[a,b,aa] Ruilin Sun,[c,aa] Yueping Li,[d,aa] Bingliang Lin,[e,aa] Fangqi Ge,[f,aa] Ling Lin,[g,aa] Hongzhou Lu,[h,aa] Liang Su,[i] Tianxin Xiang,[j] Hongqiu Pan,[k] Chaolin Huang,[l] Ying Deng,[m] Furong Wang,[n] Ruhong Xu,[o] Dexiong Chen,[p] Ping Zhang,[q] Jianlin Tong,[r] Xifu Wang,[s] Qingwei Meng,[s] Zhigang Zheng,[t] Shuqiang Ou,[u] Xiaoyun Guo,[u] Herui Yao,[v] Tao Yu,[v] Weiyang Li,[w] Yu Zhang,[x] Mei Jiang,[a] Zhonghao Fang,[a,b] Yudi Song,[y] Ruifeng Chen,[a,b] Jincan Luo,[a,b] Changyuan Kang,[a,b] Shiwei Liang,[a,b] Haijun Li,[z] and other Collaborative Institutes, Jingping Zheng,[a,b,***] Nanshan Zhong,[a,b,**] and Zifeng Yang[a,b,*]

RESULTS: *Time to sustained clinical recovery*

- Placebo group: **11 days**
- Treatment group: **10 days**
- Dose: 400 mg three times a day for 5 days

Single-component drug vs. combination therapy

- **RAY1216 (leritrelvir)**: single component
- **PF-0731332 (nirmatrelvir)**: co-administered with **ritonavir** (severe drug-drug interactions)

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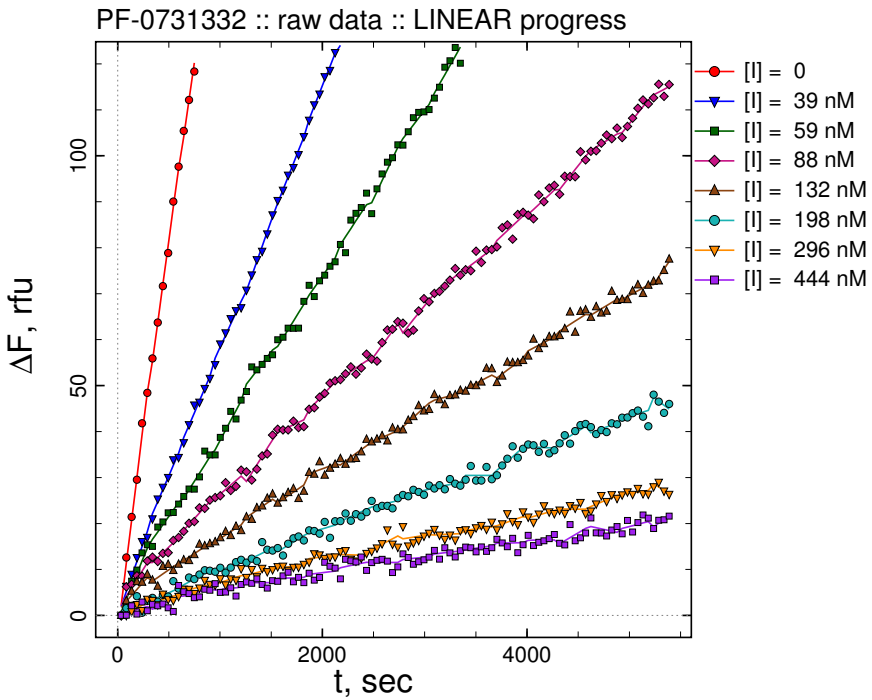
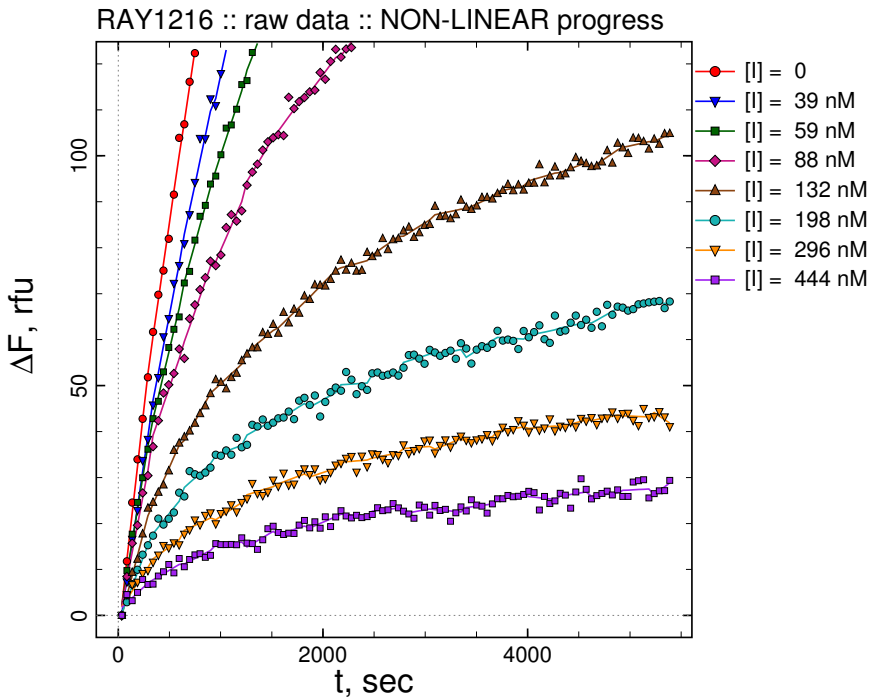
"Fast" vs. "slow" binding kinetics: Raw experimental data

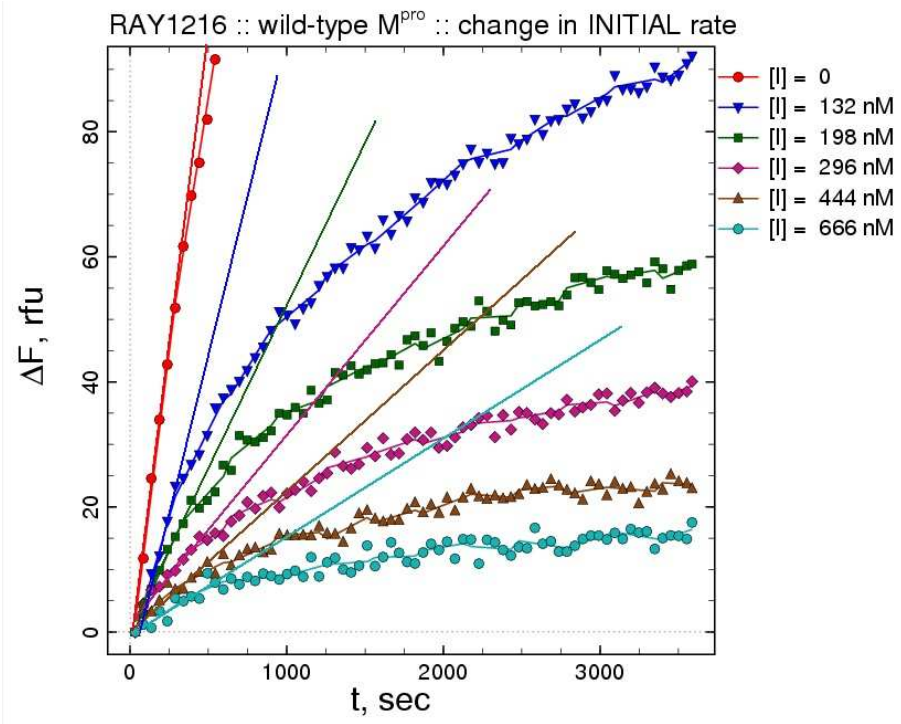




- **PF-0731332**: instantaneous binding
- **RAY1216**: "slow-binding"[1]

1. Morrison, J. F., and Walsh, C. T. (1988) "The behavior and significance of slow-binding enzyme inhibitors". *Adv. Enzymol.* **61**, 201-301.

Stepwise binding of RAY1216: "slow" rearrangement of the E.I complex



raw experimental data

$$E + I \underset{}{\overset{K_d}{\rightleftharpoons}} E{\bullet}I \underset{k_r}{\overset{k_f}{\rightleftharpoons}} EI$$

rapid equilibrium

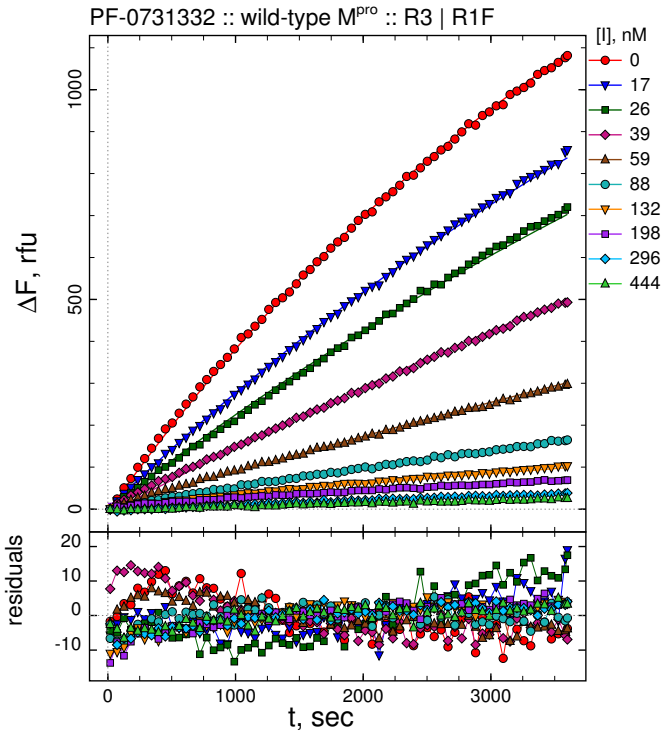
implied mechanism

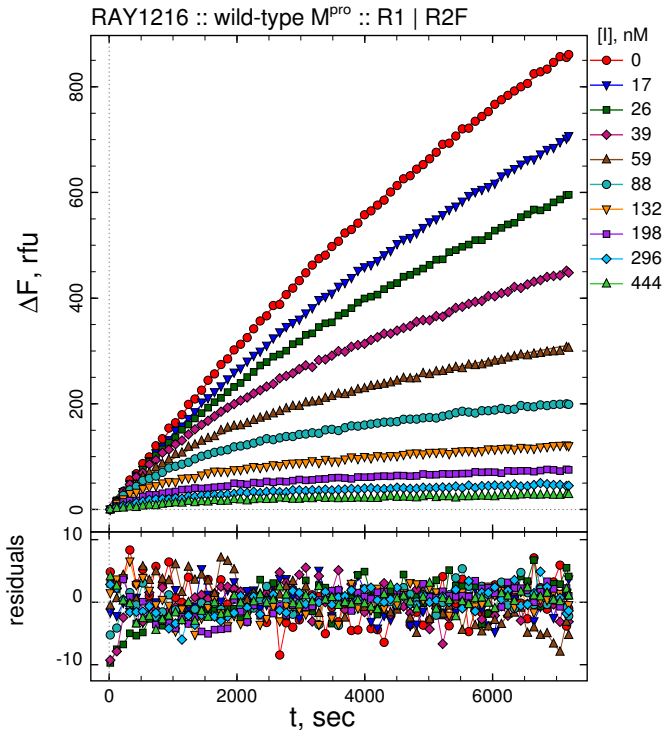
- two-step binding
- first step (binding proper) is "fast"
- second step (rearrangement) is "slow"

"Fast" vs. "slow" binding kinetics: Data analysis method

- **Experimental design:**
  Three independent replicates of each dose-response series.
- **Mathematical model:**
  Systems of first-order ordinary differential equations.
- **Regression method:**
  Global fit of combined progress curves.
- **Competing mechanisms:**
  (1) Two-step "fast/slow"
  (2) One-step "slow"
  (3) One-step "fast"
- **Software implementation:**
  Program *DynaFit* (*Methods in Enzymology* **467**, 247-280, 2009).

“Fast” vs. “slow” binding kinetics: Model discrimination results





- **PF-0731332**: one-step “fast” mechanism

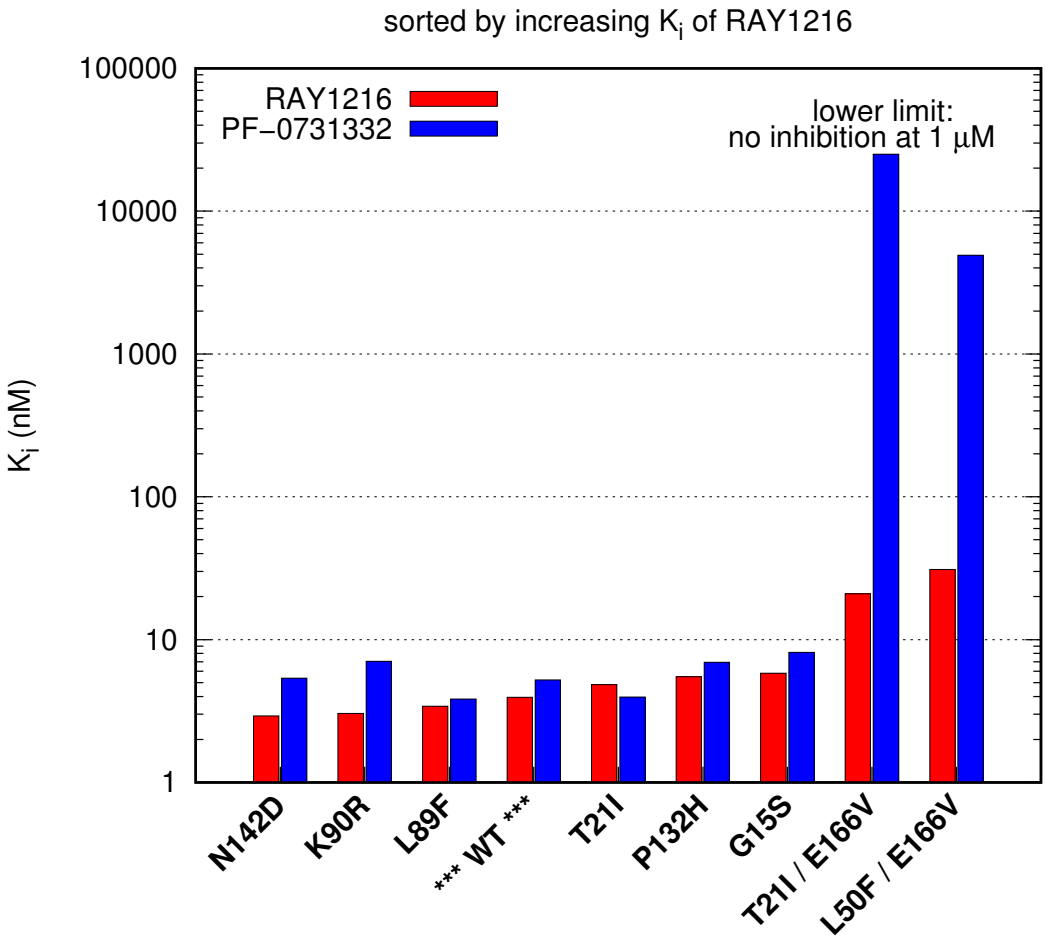
$K_\mathrm{i} = K_\mathrm{d} = 5.2$ nM
$t_\mathrm{resid} < 1$ min

- **RAY1216**: two-step “fast / slow” mechanism

$K_\mathrm{d} = 154$ nM
$k_\mathrm{f} = 0.0030\ \mathrm{s}^{-1}$
$k_\mathrm{r} = 0.000078\ \mathrm{s}^{-1}$

$K_\mathrm{i} = K_\mathrm{d}/(1 + k_\mathrm{f}/k_\mathrm{r}) = 4.0$ nM
$t_\mathrm{resid} = 1/k_\mathrm{r} = 210$ min

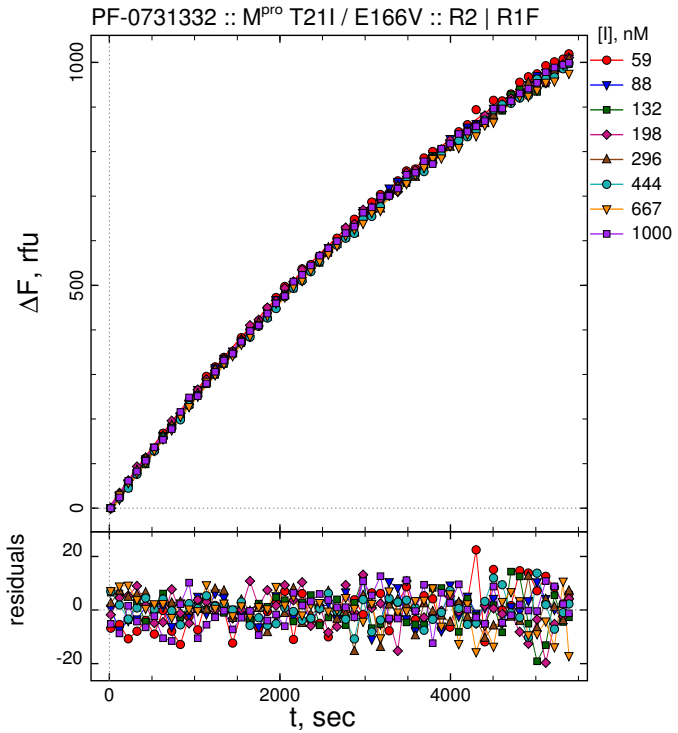
Overall K_i results for selected M^{pro} mutants

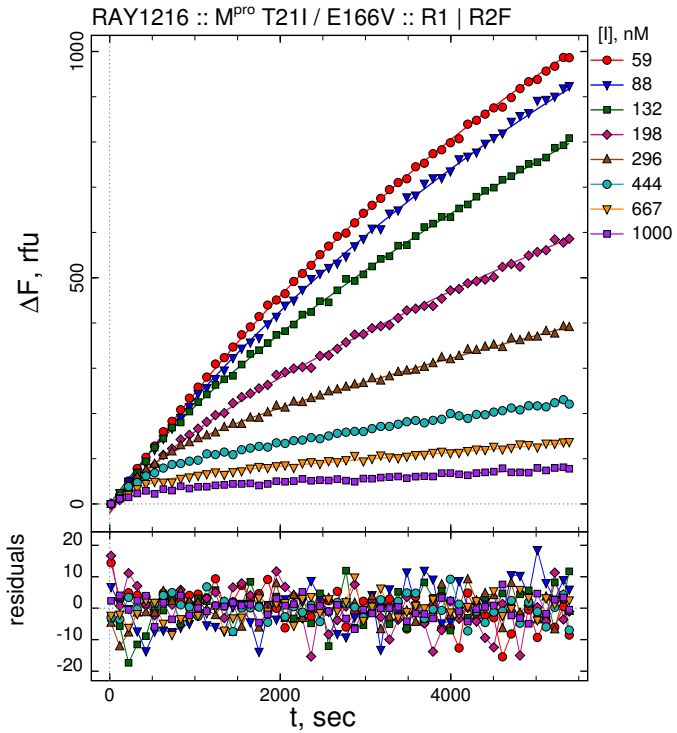


See also:

Duan *et al.* (2023) *Nature* **622**, 376-382
"Molecular mechanisms of SARS-CoV-2 resistance to nirmatrelvir"

One of several regognized *nirmatelvir-resistant* mutants: T21I / E166V





- **PF-0731332**: no inhibition

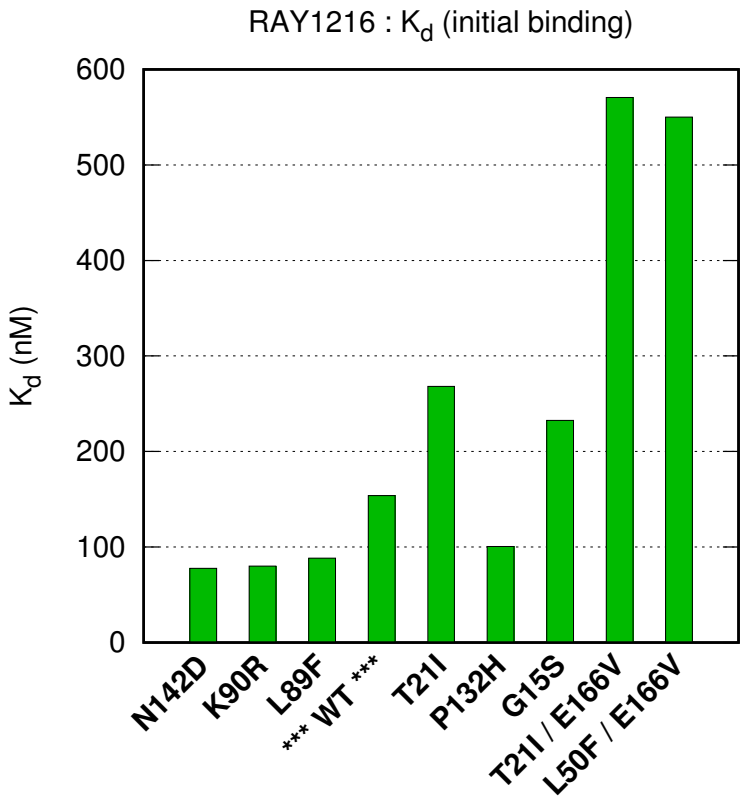
  $K_\mathrm{i} > 25{,}000$ nM

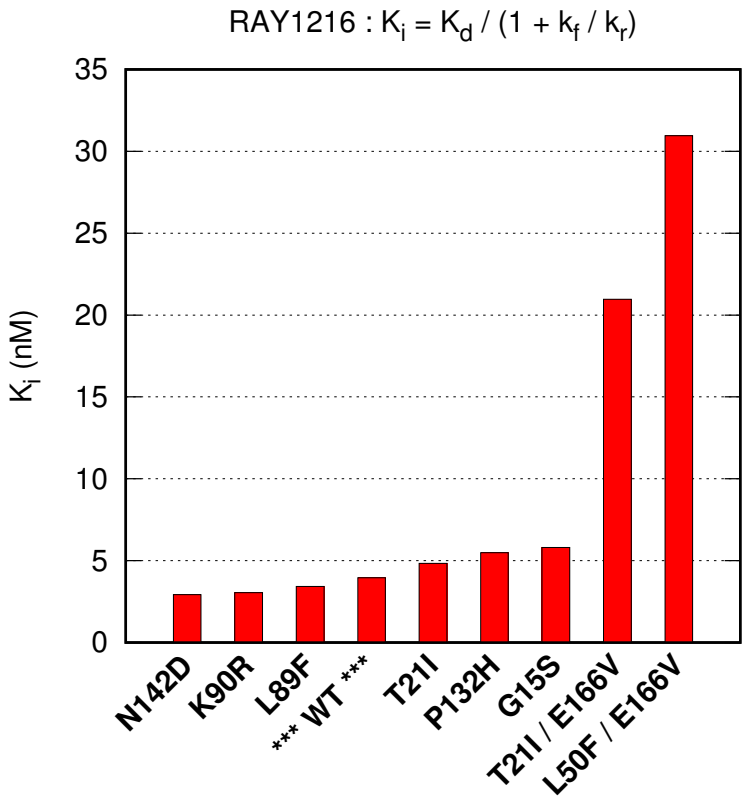
- **RAY1216**: two-step "fast / slow" mechanism

  $K_\mathrm{d} = 470$ nM
  $k_\mathrm{f} = 0.0048\ \mathrm{s}^{-1}$
  $k_\mathrm{r} = 0.00024\ \mathrm{s}^{-1}$

  $K_\mathrm{i} = K_\mathrm{d}/(1 + k_\mathrm{f}/k_\mathrm{r}) = 22$ nM
  $t_\mathrm{resid} = 1/k_\mathrm{r} = 70$ min

RAY1216: Overall K_i and initial K_d results for selected M^{pro} mutants





- **RAY1216**: Initial binding affinity

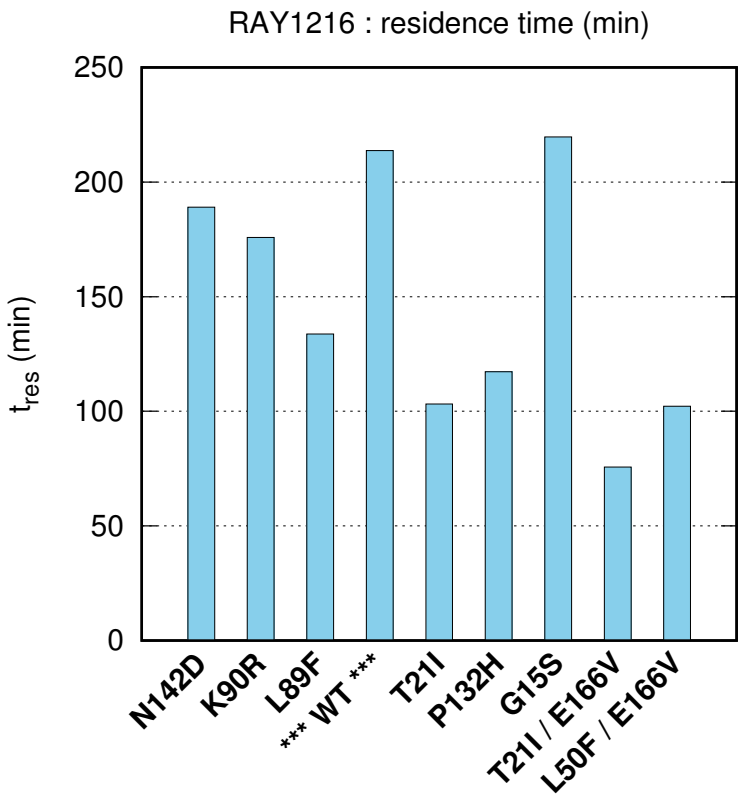
$$K_d = k_{off}/k_{on}$$

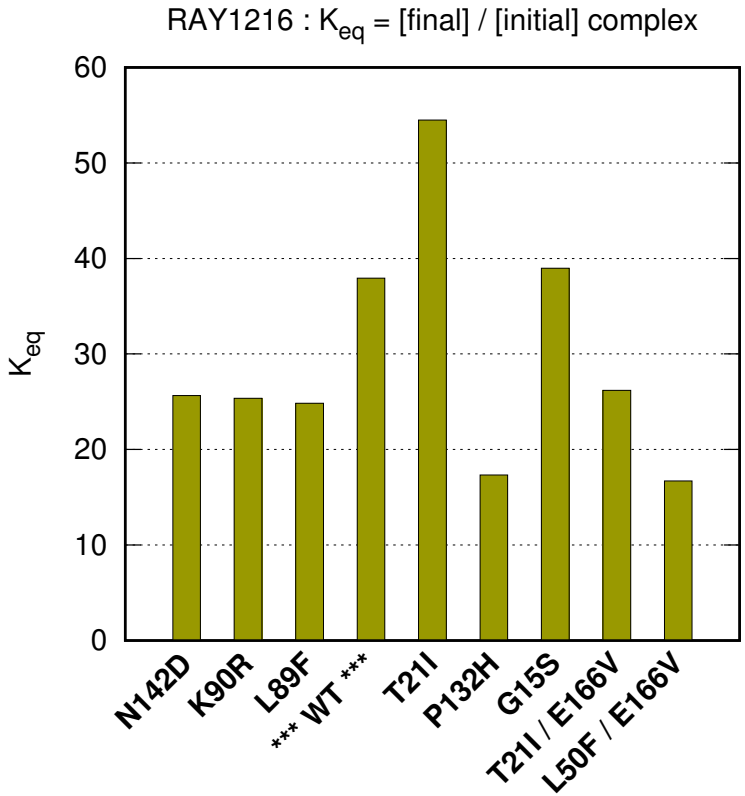
- **RAY1216**: Overall binding affinity

$$K_i = K_d/(1 + k_f/k_r)$$

- Binding to most M^{pro} mutants involves two distinct enzyme–inhibitor complexes.

RAY1216: Drug-receptor residence time for selected M^{pro} mutants





- **RAY1216**: Drug-receptor residence time

$$t_{res} = 1/k_r$$

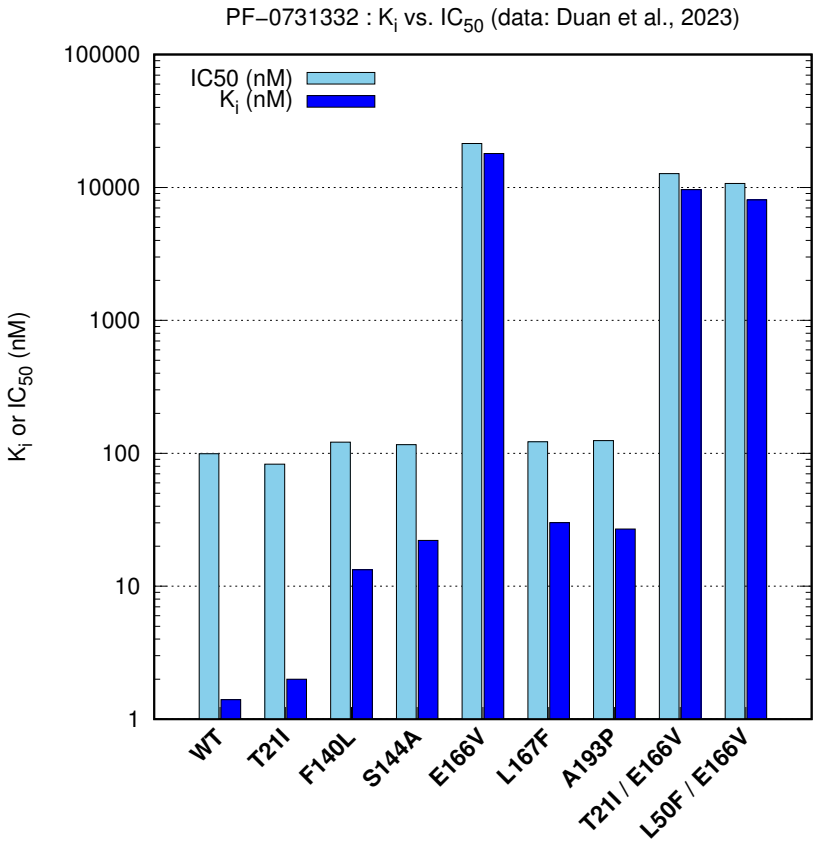
- **RAY1216**: Equilibrium between complexes

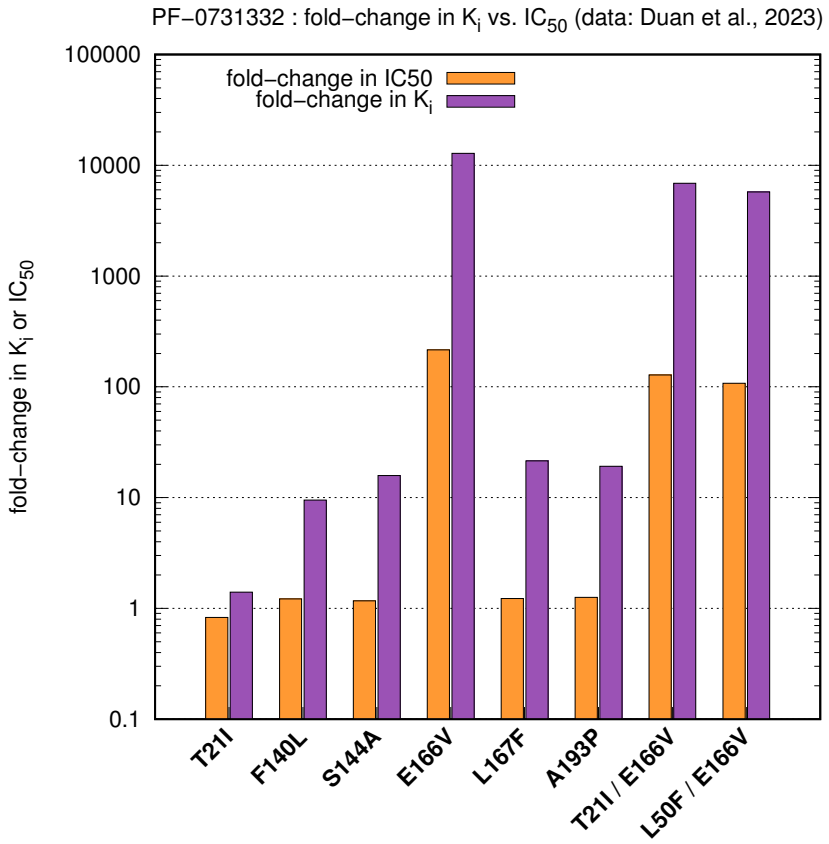
$$K_{eq} = k_f/k_r$$

- Drug-resistance of M^{pro} mutants is determined by more than just overall binding affinity.

PF-0731332: A cautionary tale of IC_{50} vs. the inhibition constant, K_i

- IC_{50} is not a suitable measure of potency for "tight-binding"[1] inhibition.





Experimental data: Duan *et al.* (2023) *Nature* **622**, 376-382

- $IC_{50} = [E]/2 + K_i^{app}$

1. Williams, J. W., and Morrison, J. F. (1979) "The kinetics of reversible tight-binding inhibition". *Meth. Enzymol.* **63**, 437-467.

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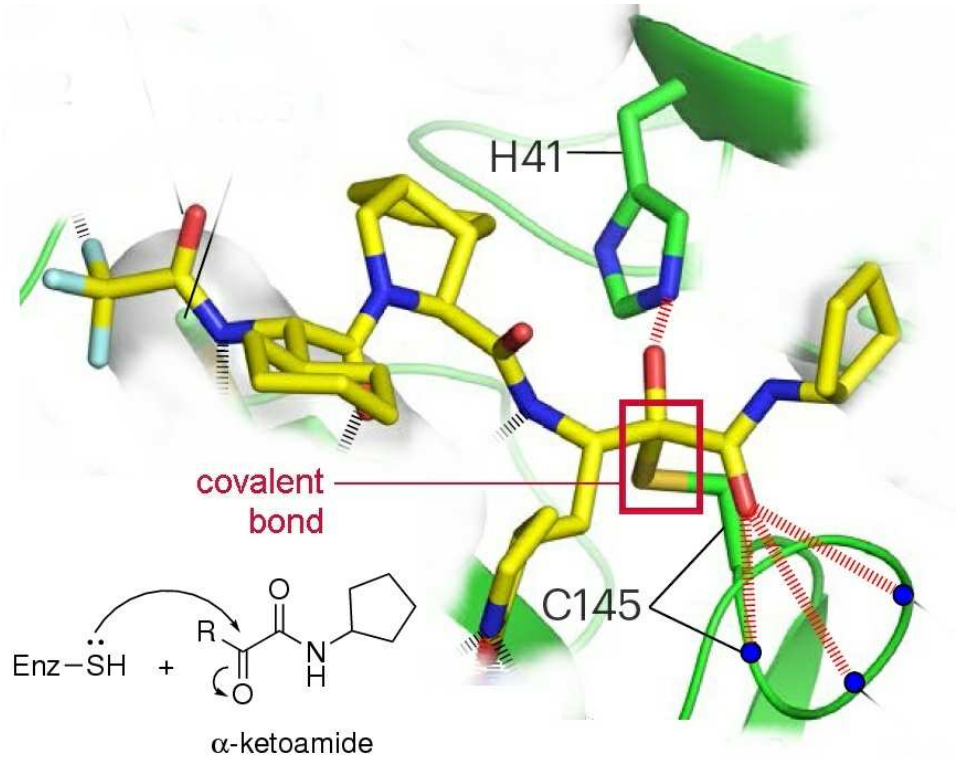
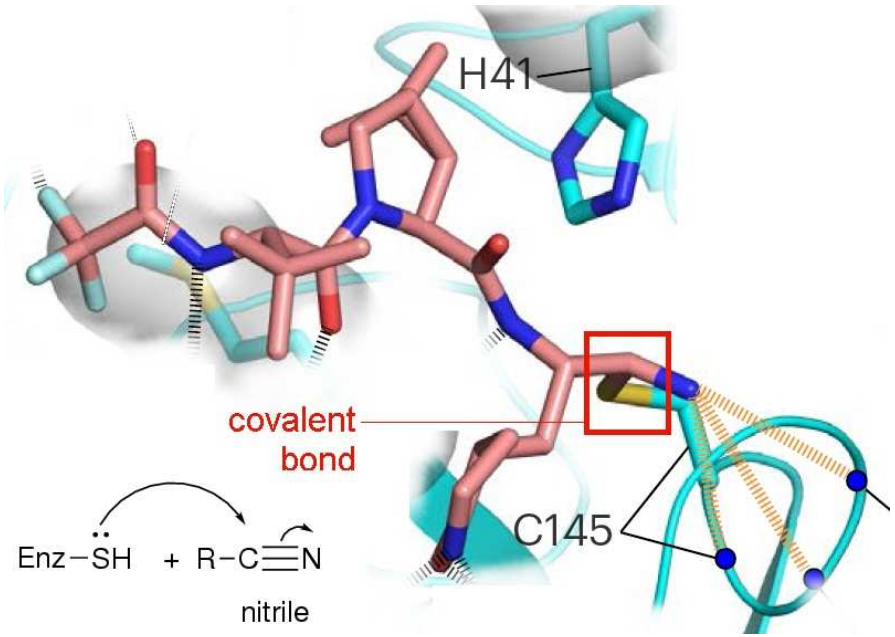
Covalent enzyme-inhibitor complexes seen in X-ray structures



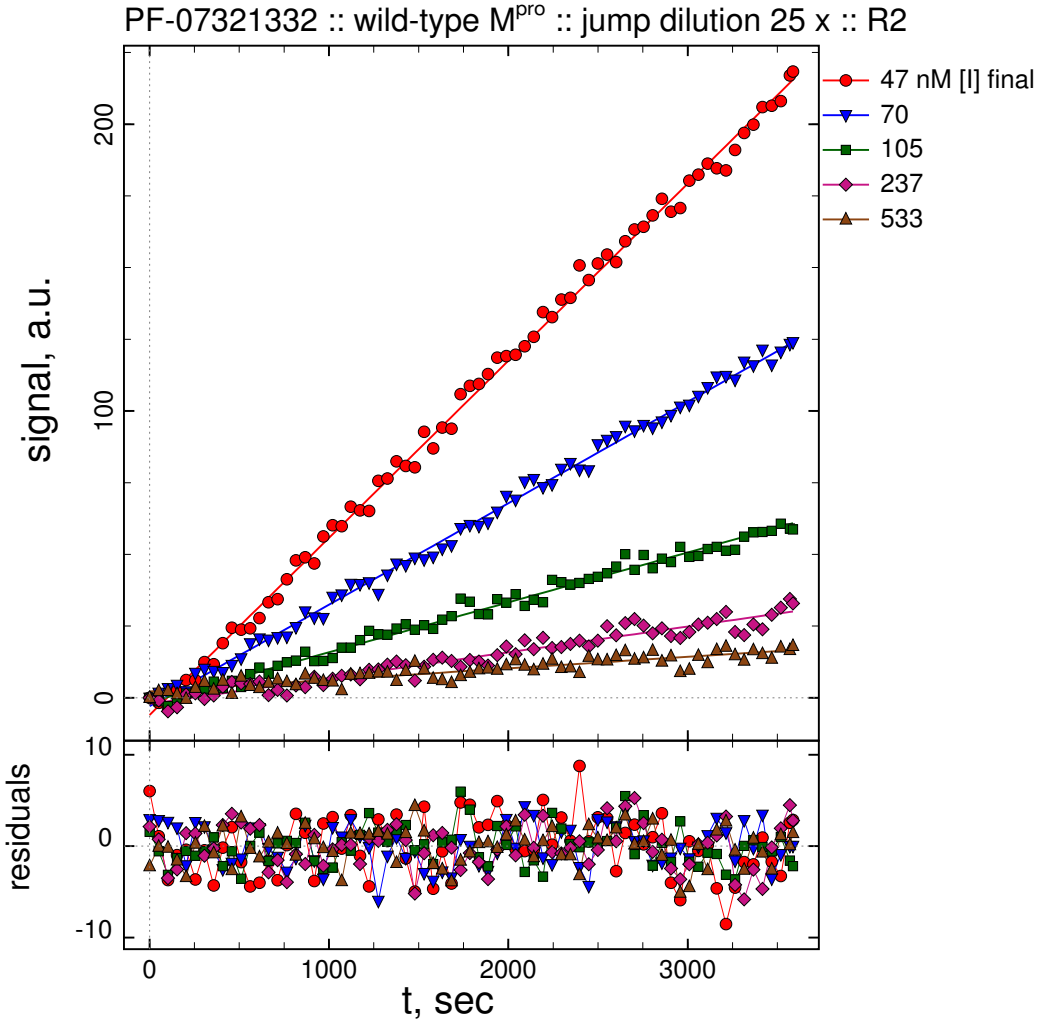
RAY1216



PF-0731332

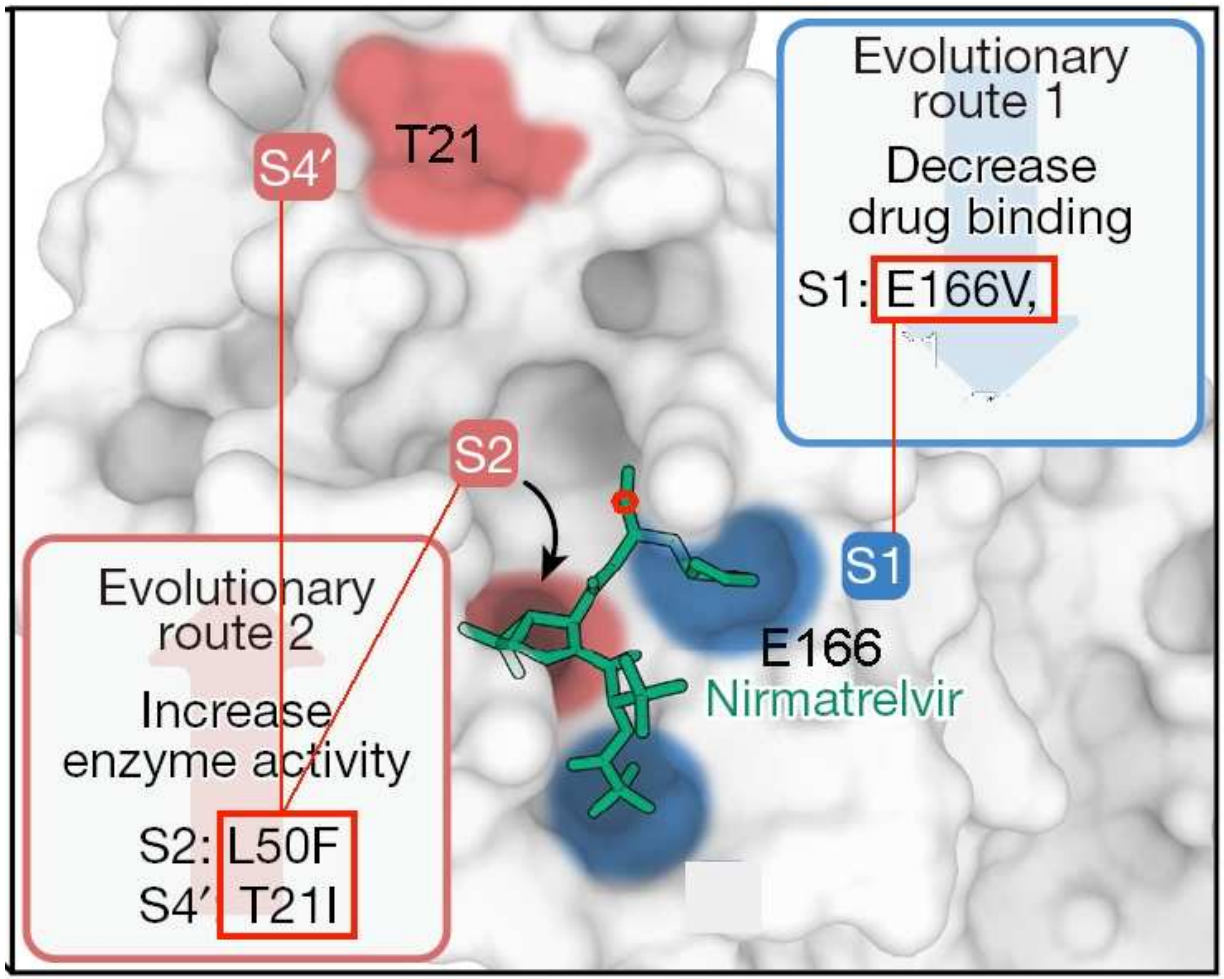
- Both inhibitors form a covalent ("irreversible") complex in the crystalline phase.
- In contrast, solution-phase kinetics shows perfect reversibility.

The "jump-dilution" experiment: A standard test of reversibility



- The recovery of enzymatic activity is full and instantaneous.
- There is no solution-kinetic evidence for a covalent (irreversible) complex.

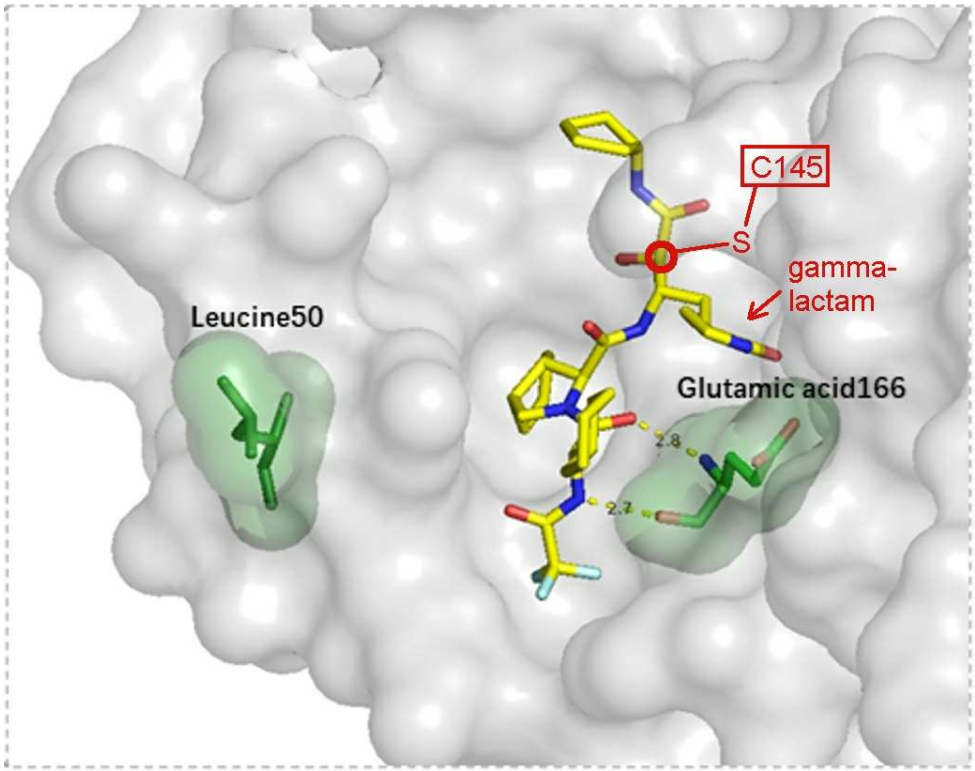
Proposed evolutionary routes for the development of nirmatrelvir resistance



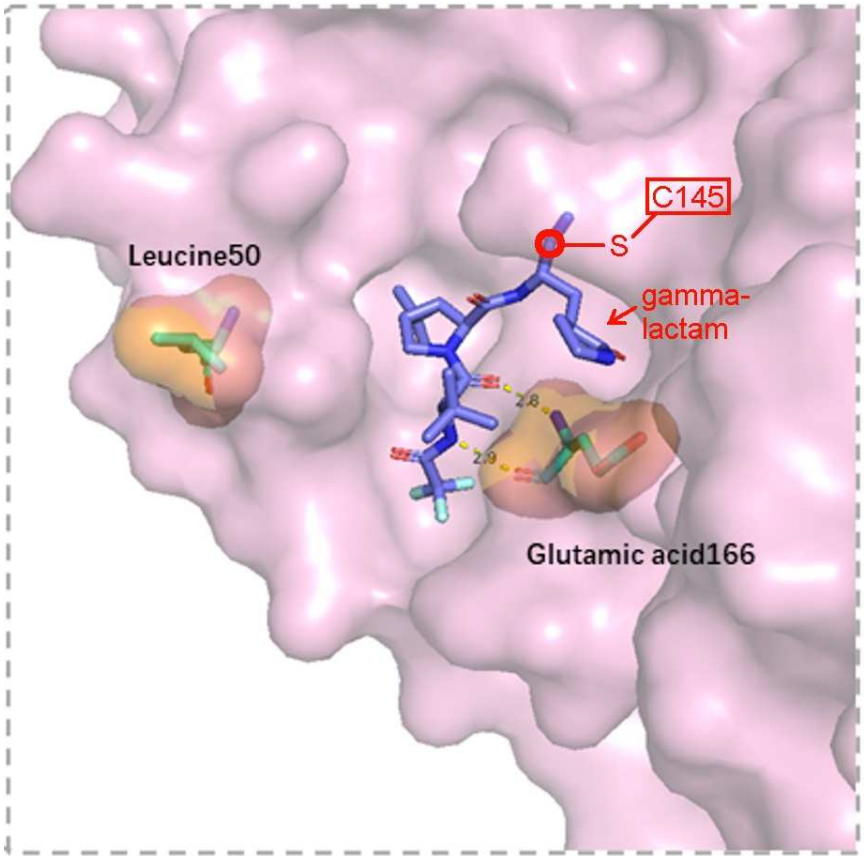
Adapted from Duan *et al.* (2023)

- Why the huge difference between **RAY1216** and **PF-0731332** in binding to **E166V mutants**?

Detailed view of E166 / inhibitor contacts in *wild-type* M^{pro}



RAY1216

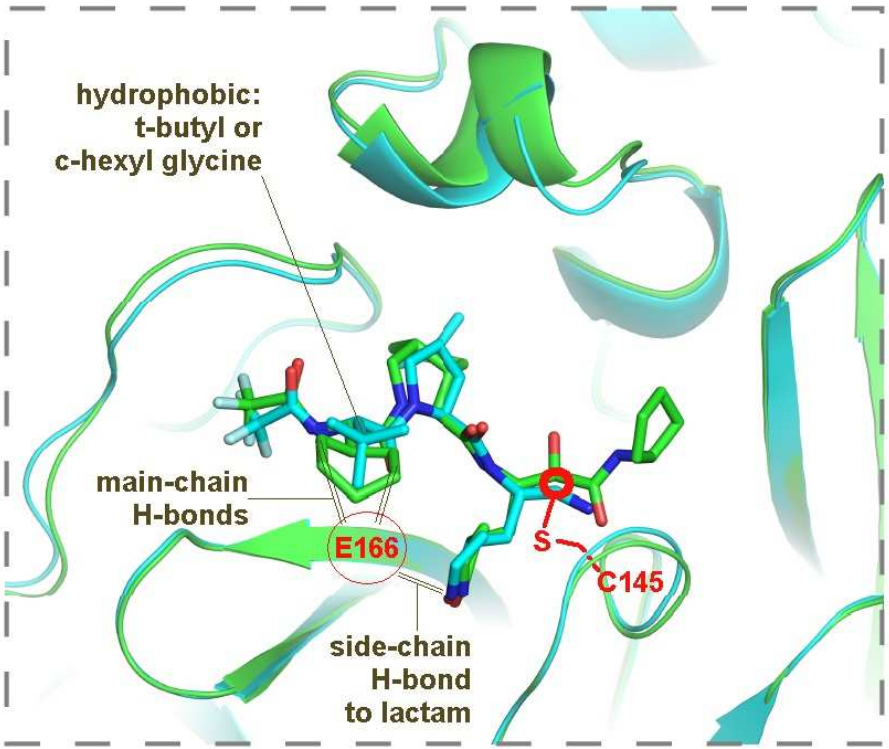


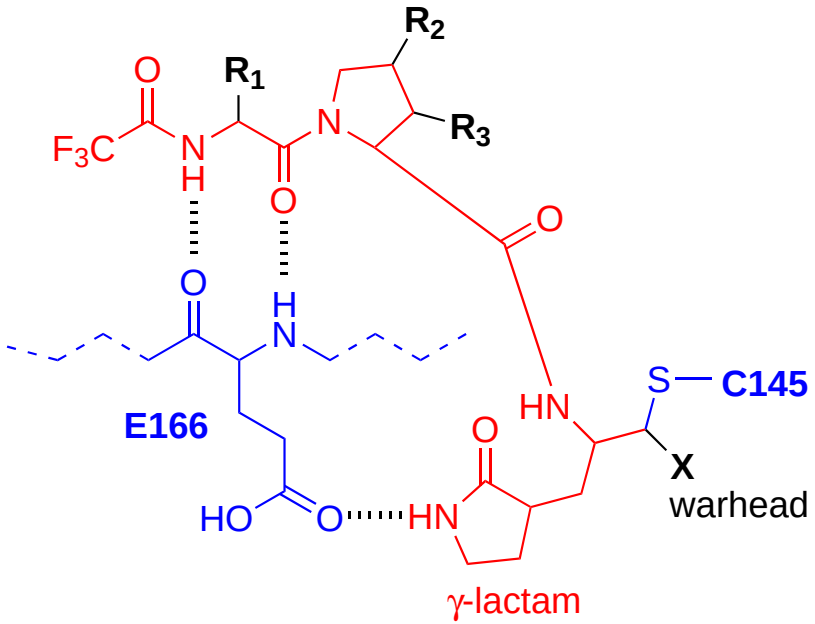
PF-0731332

Images courtesy of Xiaodong Huang (GIBH, Guangzhou)

- To this untrained eye, these two structures look very similar.

Another detailed view of E166 / inhibitor contacts in *wild-type* M^{pro}





H-bond between and E166 side-chain and lactam NH pointed by Duan *et al.* (2023).

- Why the huge difference between **RAY1216** and **PF-0731332** in binding to **E166V mutants**?

Summary and conclusions

Current results:

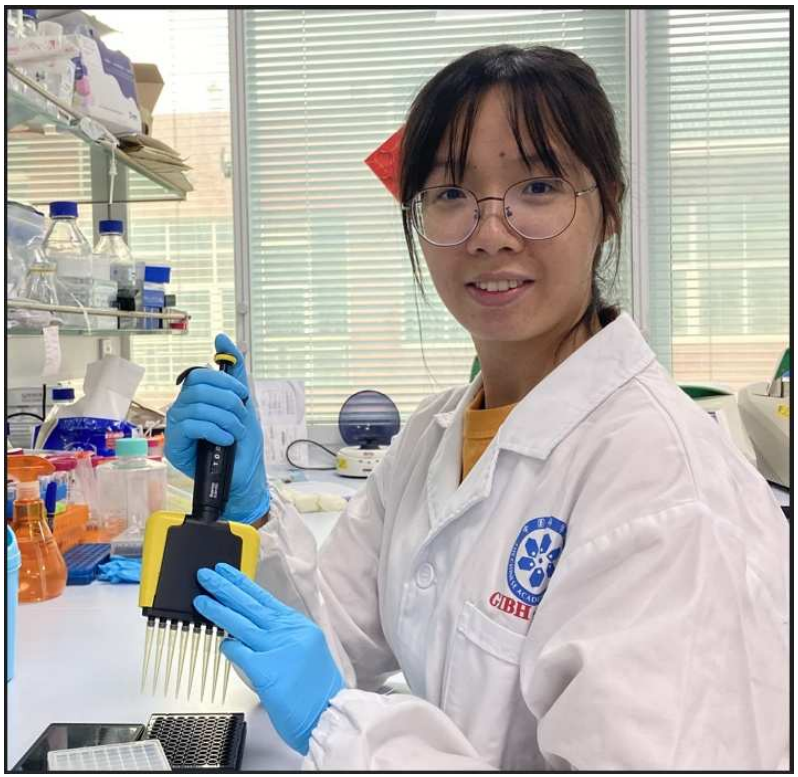
- **Leritrelvir (RAY1216)** is the only highly selective COVID-19 drug used as mono-therapy.
- It is unusually "slow-binding", with very slow association and long residence time.
- The main advantage of **RAY1216** over **PF-0731332** is in increased plasma stability.

Still to be figured out:

- Which structural features of **RAY1216** are responsible for its unusually slow binding?
- Does long residence time correlate with pharmacological efficacy in this particular case?
- Why do E166 mutations strongly reduce the potency of **PF-0731332**, but not of **RAY1216**?
- Understand better the very intriguing monomer–dimer properties of M^{pro}.

Acknowledgments

- *Nature Microbiology* manuscript: **33** co-authors from **12** institutions.
- Closest collaborators from the **Guangzhou Institute of Biomedicine and Health (GIBH)**:

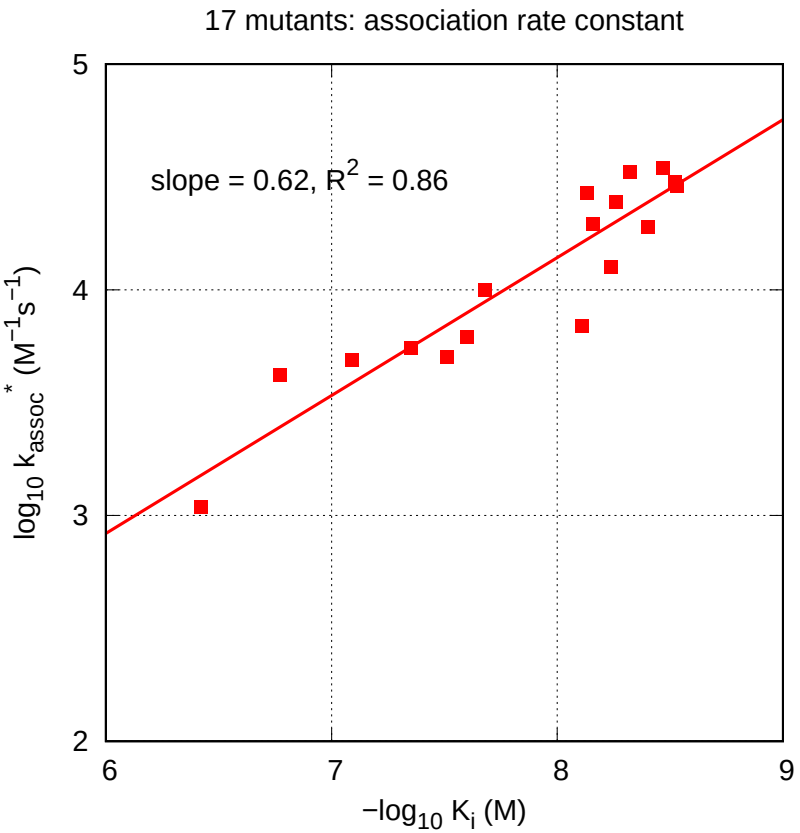


Xiaodong Huang*
PhD Student

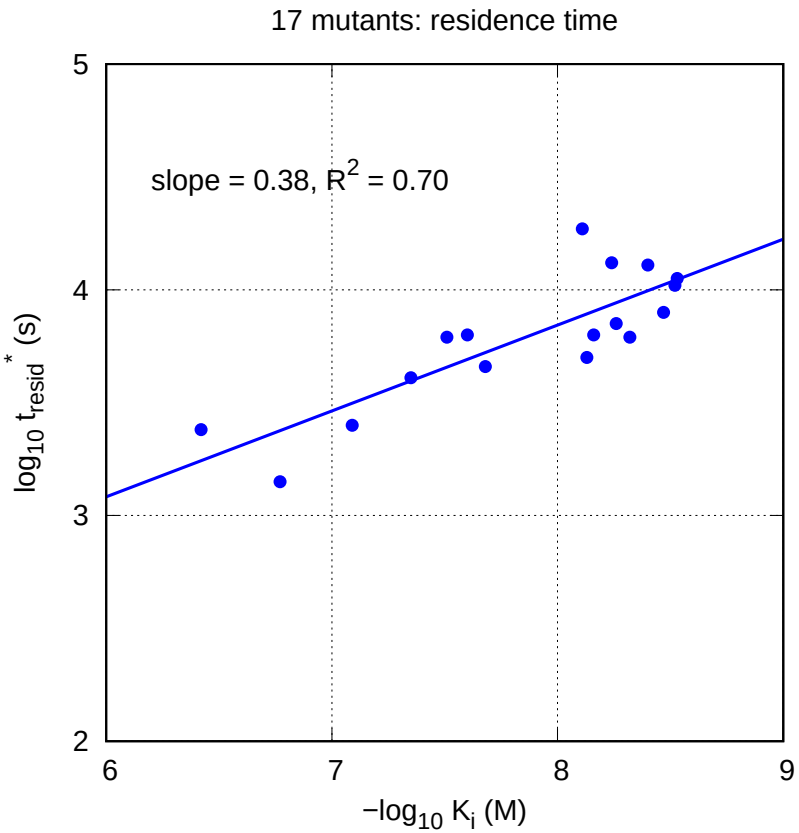
Xiaoli (Alex) Xiong
Principal Investigator
Group Leader – Structural Biology

* Protein expression and purification; single-crystal analysis (absolute stereochemistry); protein crystallography (grow co-crystals with drugs; collect and interpret synchrotron data); **enzyme assays**; detailed manuscript management; *and more*.

All available mutants: RAY1216 "Kinetic Equilibrium Correlation" plot



enzyme-inhibitor association
slope: **0.62**: slightly dominant



dissociation of the EI complex
slope: **0.38**