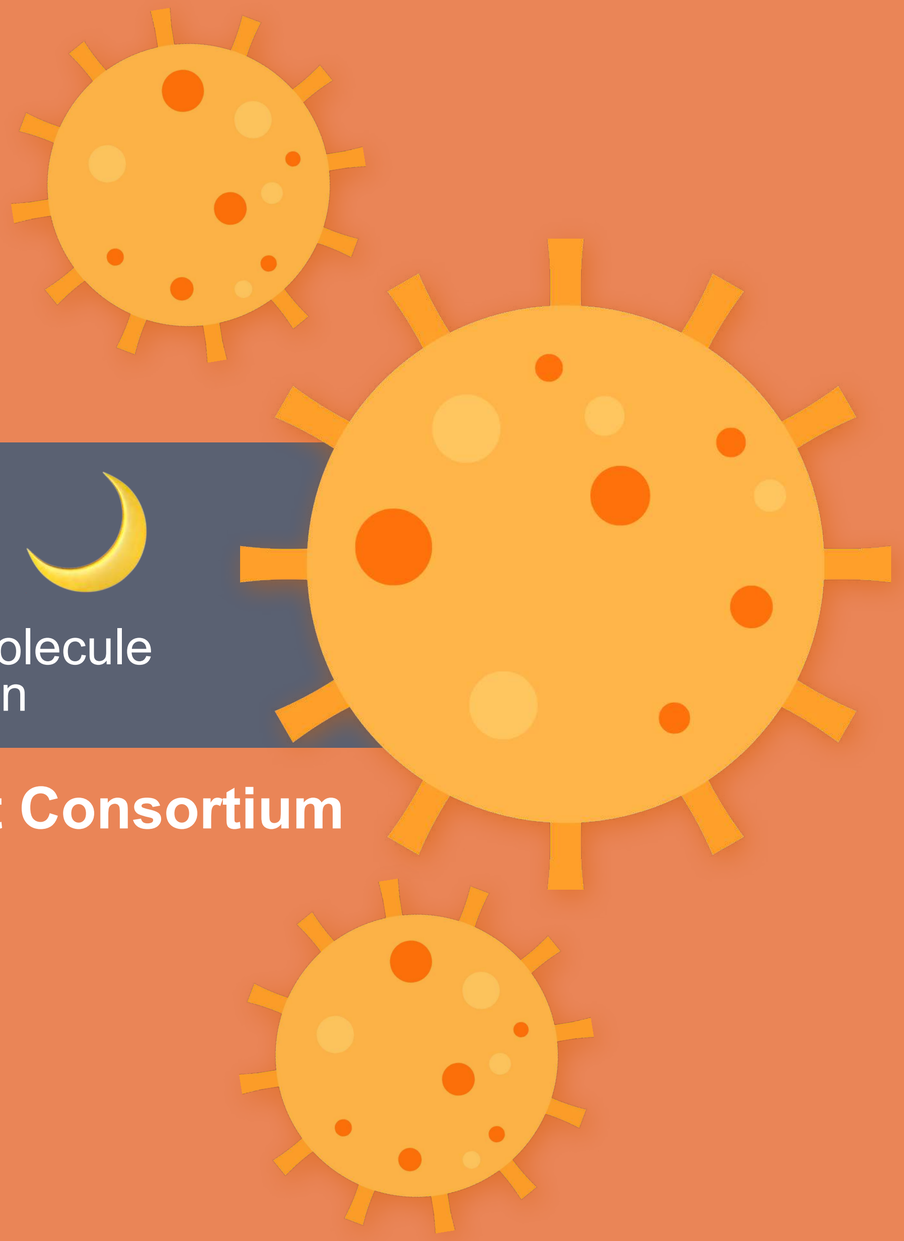
OK to tweet/share!



# THE COVID MOONSHOT 🌙

Closing in on an orally-bioavailable non-peptidomimetic small molecule inhibitor of SARS-CoV-2 Mpro with an open science collaboration

**John D. Chodera (MSKCC)** for the **COVID Moonshot Consortium**

data: http://postera.ai/covid

slides: http://choderalab.org/news

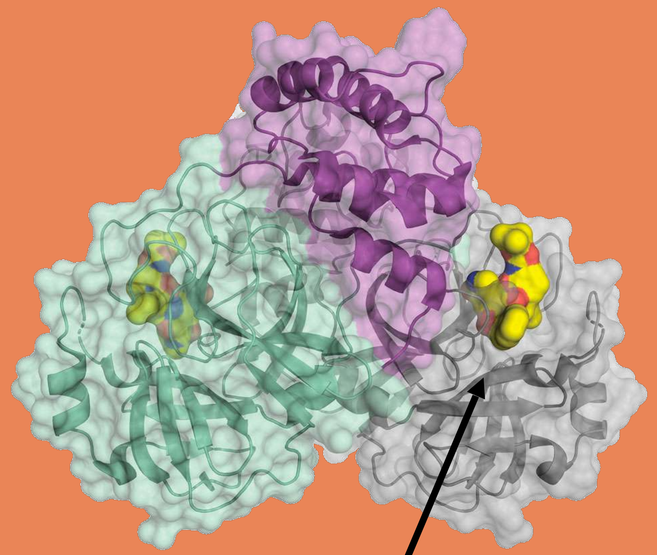
**DISCLOSURES:**

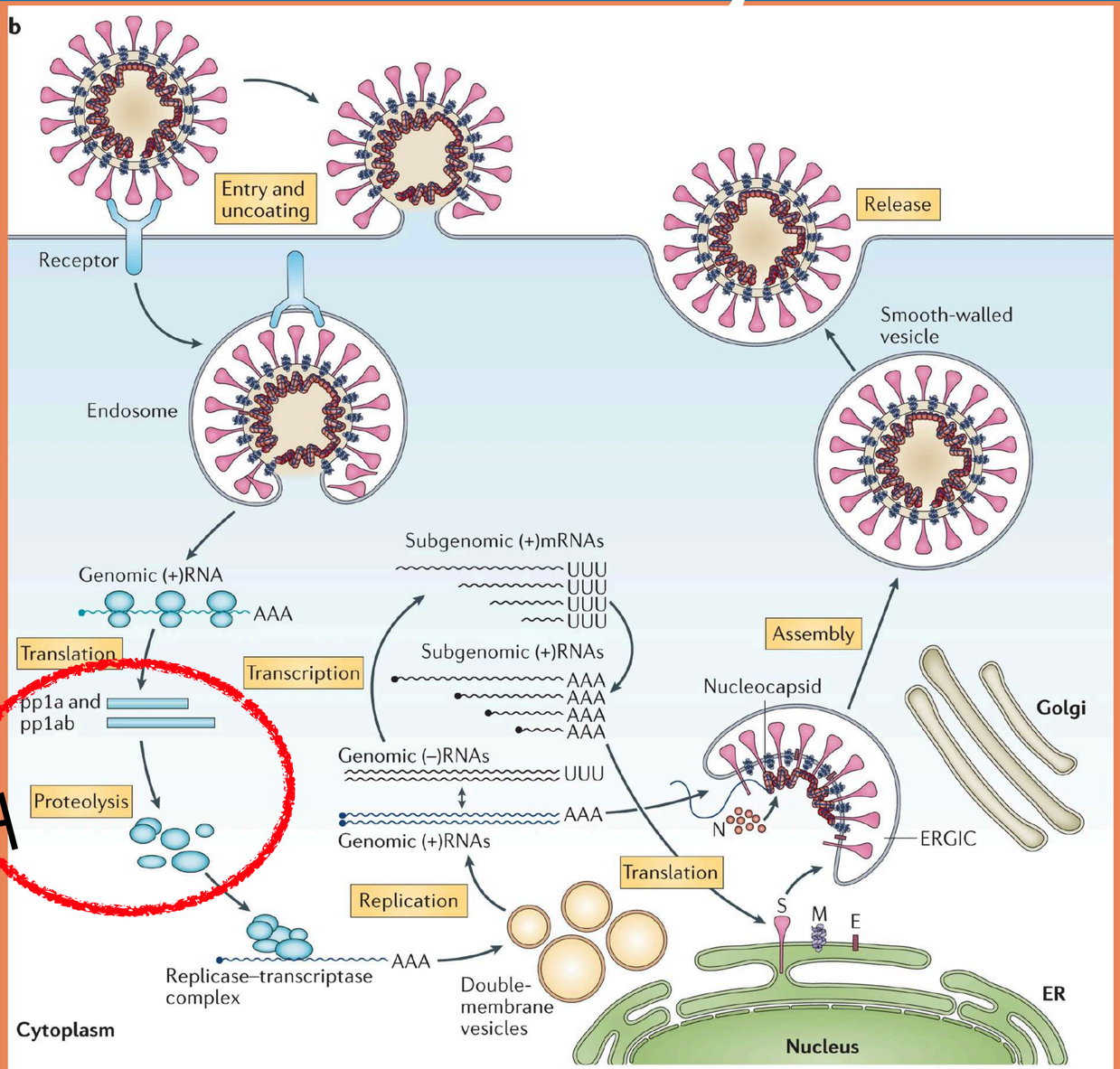
- Scientific Advisory Board: OpenEye Scientific, Redesign Science
  Scientific Consultant: Interline
  All funding: http://choderalab.org/funding

# The SARS-CoV-2 main viral protease (Mpro) is essential for a key stage in the viral life cycle

**M$^{pro}$**

also: nsp5, 3CL$^{Pro}$

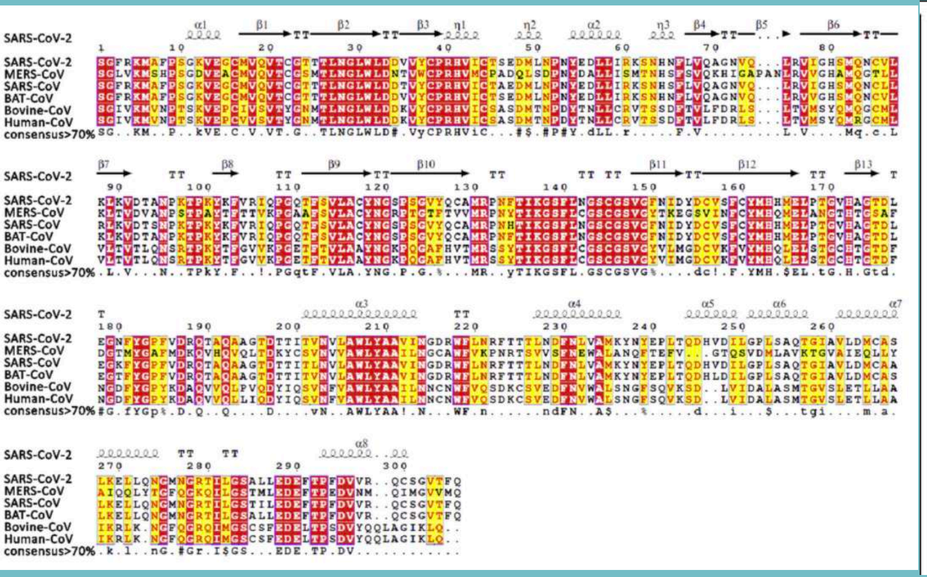





de Wit et al. Nat. Rev. Microbiology (2016)

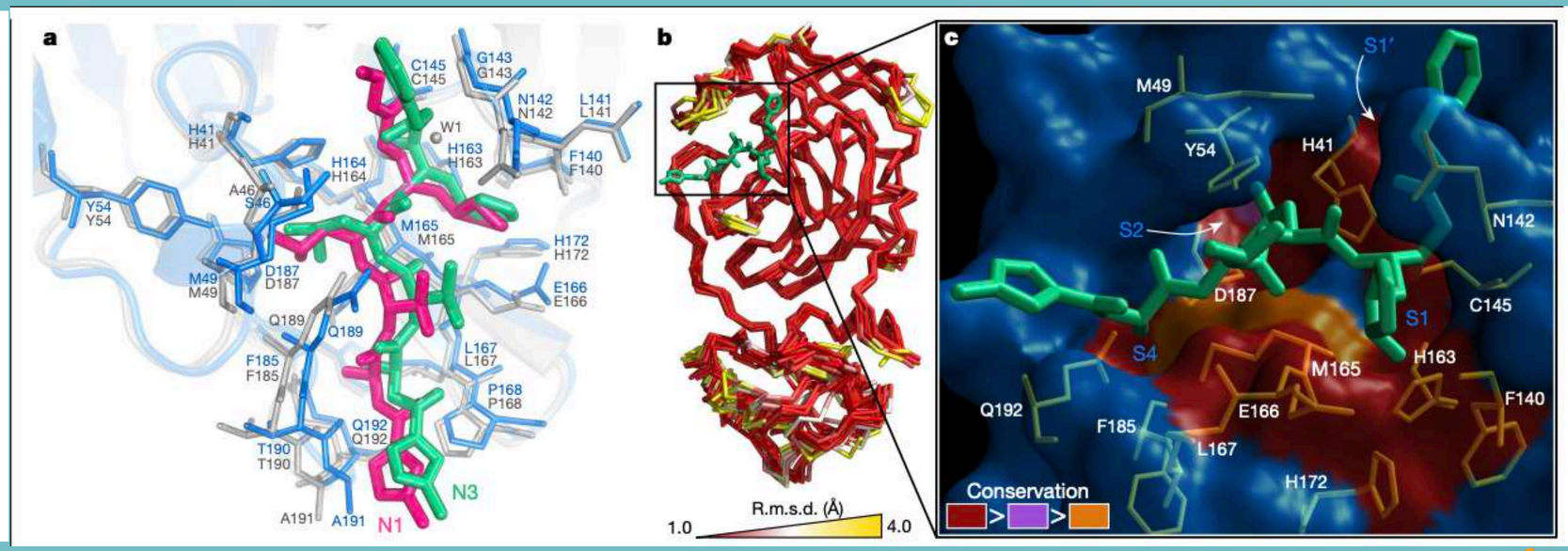
# Mpro is highly conserved among viruses that cause SARS, MERS, and COVID

**sequence** (24 Jan 2020)




Tahir ul Qamal et al. J Pharm Anal, in press
doi:10.1016/j.jpha.2020.03.009

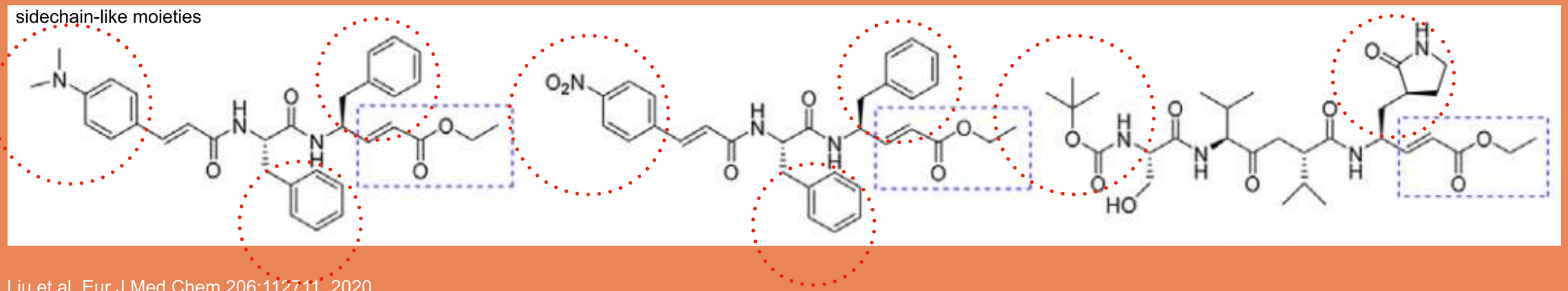
**structure** (PDB structure released 5 Feb 2020)




Jin et al. Nature 582:289, 2020
doi:10.1038/s41586-020-2223-y

**Mpro appears to be a viable target for antiviral therapy and potentially pan-coronavirus therapy**

# Previously known Mpro inhibitors mimic peptides, which are difficult to develop into useful oral drugs




Liu et al. Eur J Med Chem 206:112711, 2020

**We needed a new potent small molecule drug.**
**How do we get there *quickly*?**

# Why do we need oral drugs if we have vaccines?

If vaccinating ~100% public, need complete safety

Drug doesn’t require 100% compliance by public

Oral drugs could be deployed early, unlike IV drugs

Could remain effective against mutations that vaccine may provide incomplete protection against

Oral inhibitor without cold chain storage requirements would be practical and inexpensive enough to deploy globally

Could provide prophylaxis following exposure or treat acute illness at onset of symptoms

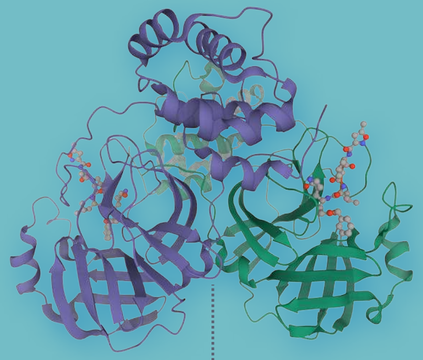
# Diamond Light Source prosecuted a high-throughput X-ray fragment screen in a matter of weeks

**Frank von Delft**
**Diamond Light Source / XChem / SGC**

**February 14**

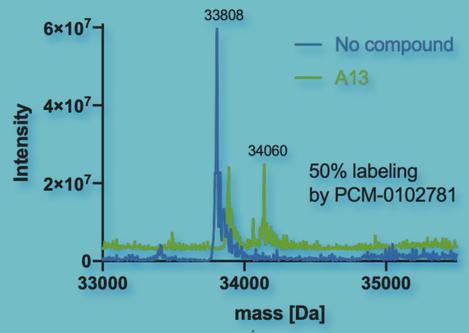
Main protease cloned and produced

***Martin Walsh***



**February 20**

Atomic resolution structure of the protease determined

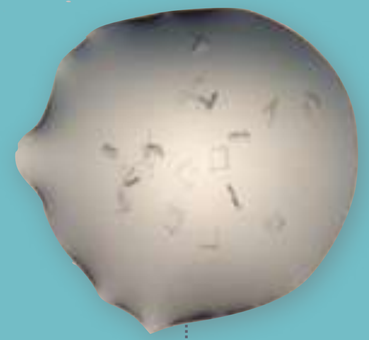



**February 25**

Covalent screen finds 150 active site hits

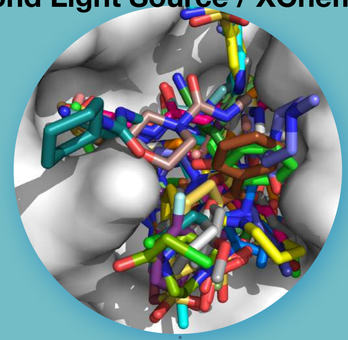
>40 hits validated

***Nir London***



**March 5**

1,500 crystals collected in one day (!)

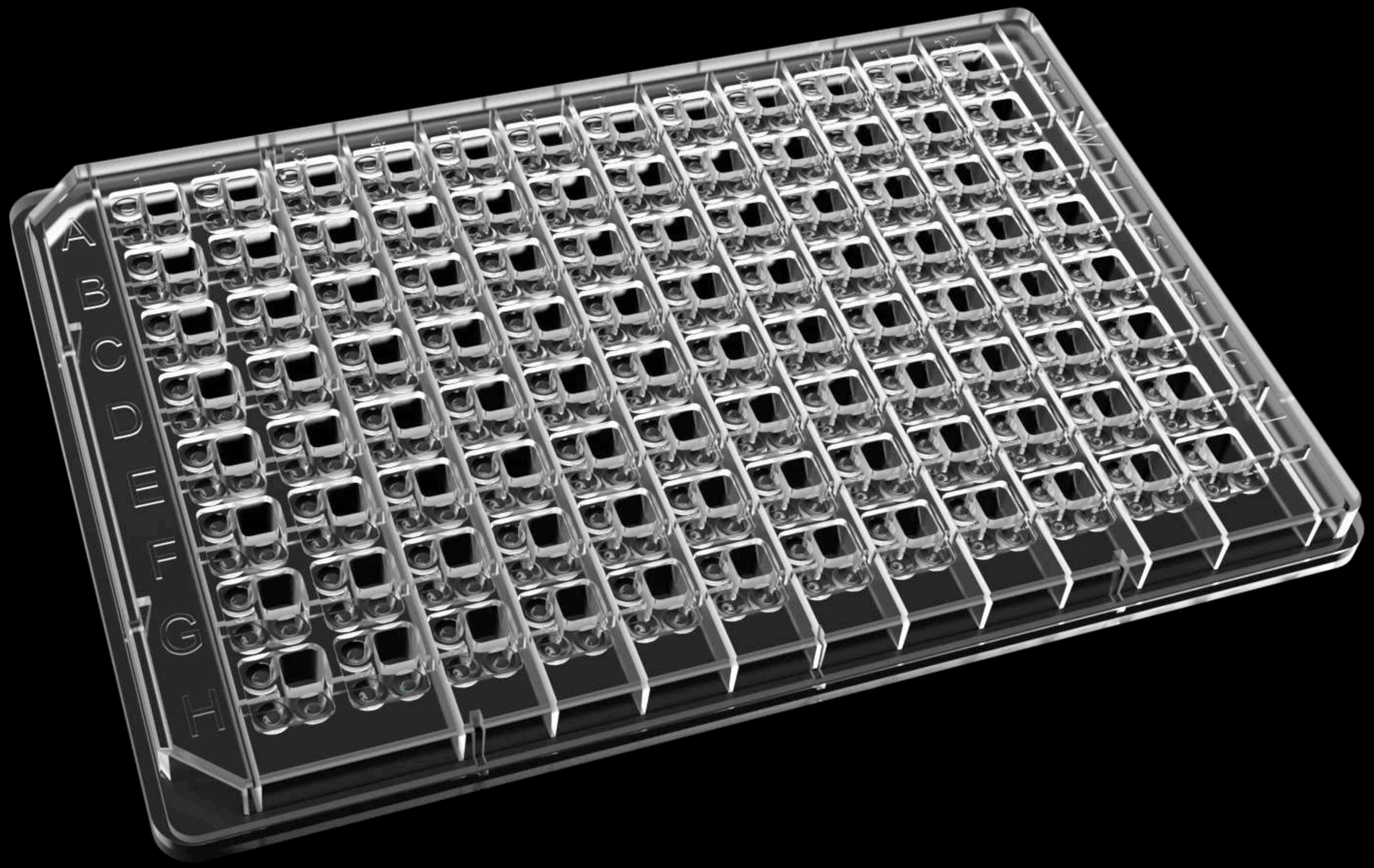


**March 18**

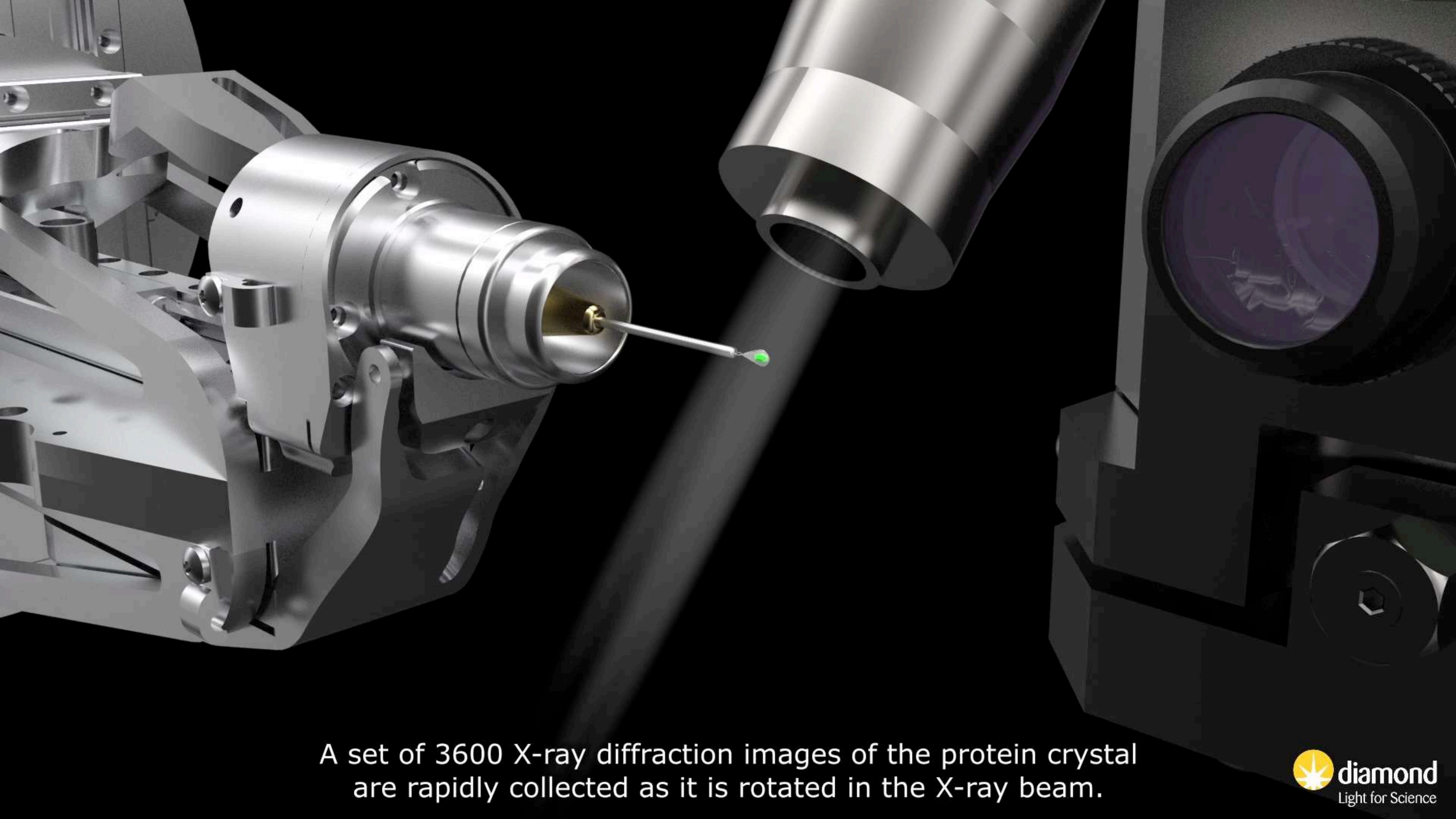
78 fragment-bound structures solved and released to the web

48 covalent fragments
71 active site fragments

https://www.diamond.ac.uk/covid-19/for-scientists/Main-protease-structure-and-XChem.html

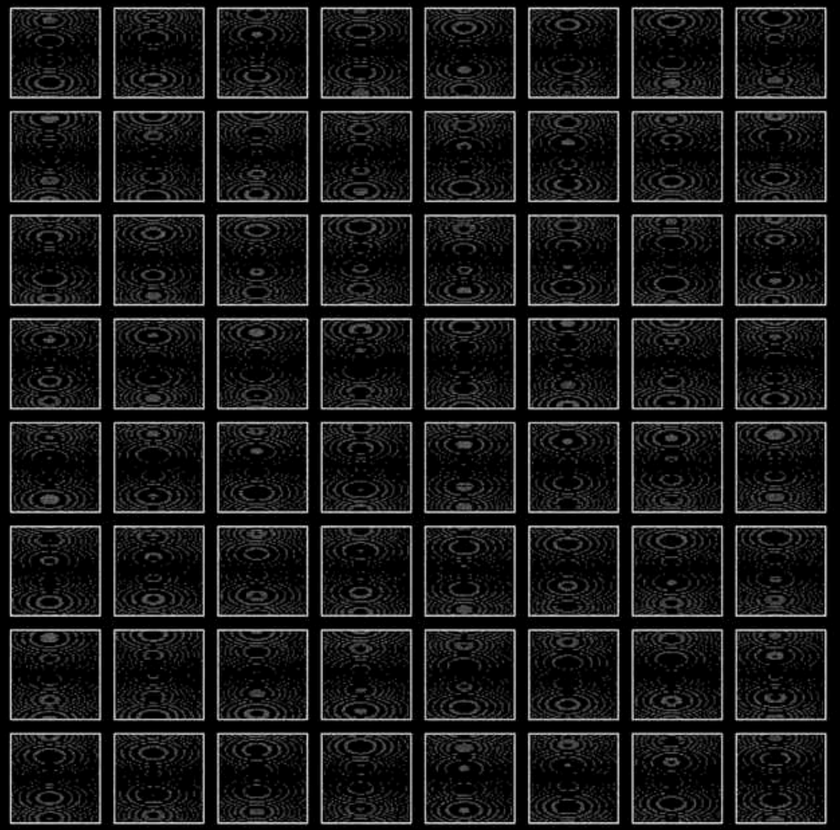



diamond
Light for Science



A set of 3600 X-ray diffraction images of the protein crystal are rapidly collected as it is rotated in the X-ray beam.

diamond
Light for Science

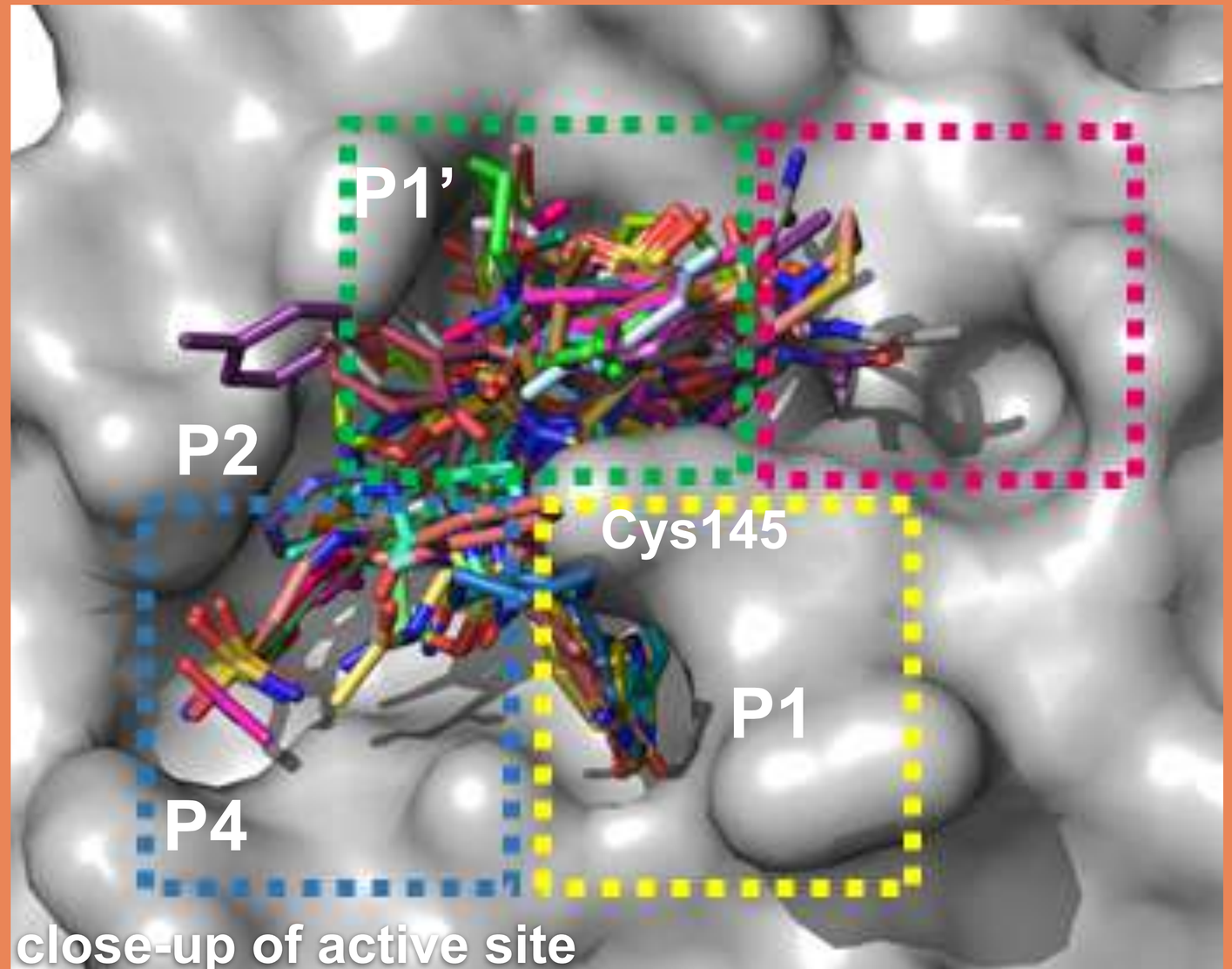


From the diffraction patterns, the three dimensional structure of the SARS-CoV-2 Mpro protein can be determined.

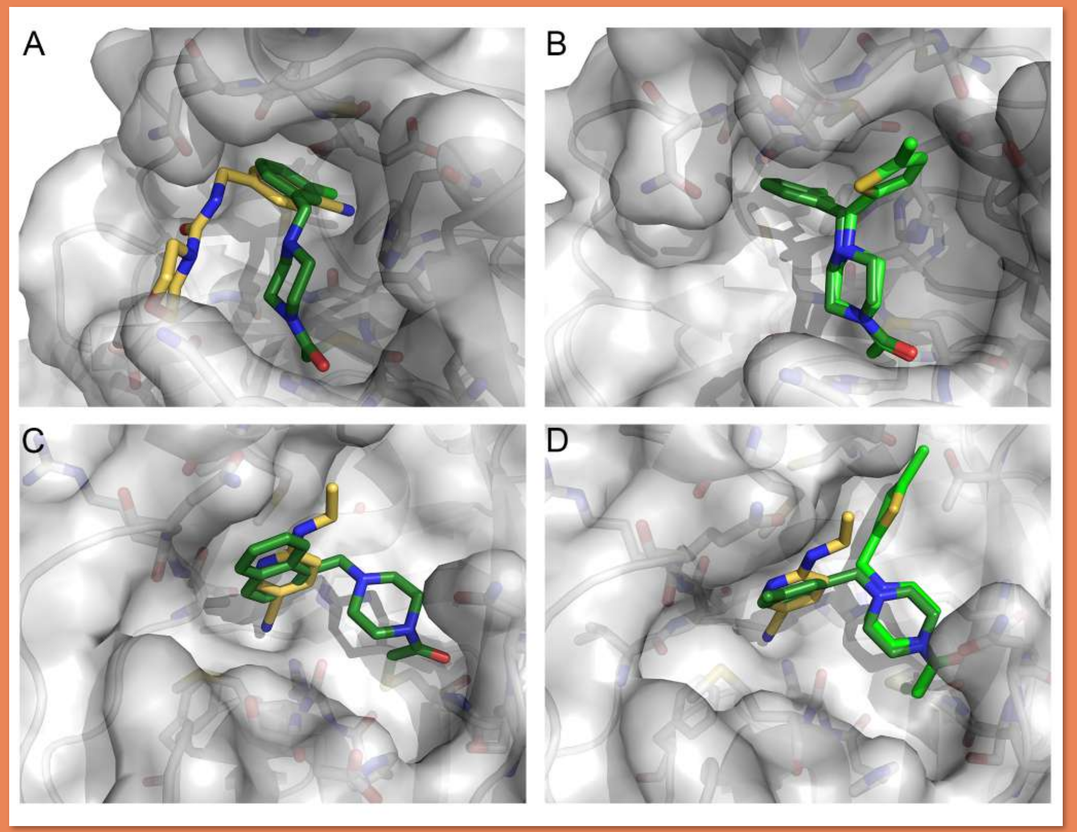
diamond
Light for Science

# The Diamond fragments completely cover the active site

interactive view: https://fragalysis.diamond.ac.uk/viewer/react/preview/target/Mpro




close-up of active site




**Could fragment merges reveal a path to potent inhibition?**

Douangamath et al., Nature Communications 11:5047, 2020
https://www.nature.com/articles/s41467-020-18709-w

# All data was immediately released online (pre-preprinted!)

diamond | Coronavirus Science

Home | For Scientists | For Journalists | For the Public | For Staff | Diamond Website

In This Section

- Main protease structure and XChem fragment screen
  - COVID MoonShot - Taking fragments to impact
  - Electron density evidence
  - Downloads
  - Highlights on progress
  - Credits
  - FAQ
- Nsp3 macrodomain ADP-ribosyl hydrolase and XChem fragment screen
- New scientific animations
- Rapid Access
- Research Areas
- Our collaborators

## Main protease structure and XChem fragment screen

### Summary

To contribute to the global effort to combat COVID-19, Diamond has been able to solve a new structure of the SARS-CoV-2 main protease ($M^{Pro}$) at high resolution (PDB ID: 6YB7), and complete a large XChem crystallographic fragment screen against it (detailed below). Data have been deposited with the PDB, but we are making the results available immediately to the world on this page; additional work is ongoing, and updates will be continually posted here in coming days and weeks.

This work builds on the sensationally fast crystal structure of $M^{Pro}$ at 2.16 Å in complex with a covalent inhibitor, released in January this year by Prof Zihe Rao (6LU7, published here, described here). We thus ordered the synthetic gene and cloned the full length protein as previously described for the SARS main protease (Xue et al 2007). This yielded crystals of the unliganded enzyme that diffracted to high resolution (1.25 Å) on beamline I04-1, in a different space group to the inhibitor complex, and the structure was determined and refined rapidly. ***Critically, this showed it had the active site empty and solvent accessible - perfect for fragment screening.***

So it proved: the first 600-crystal experiment could be completed in 72 hours, through growing large numbers of crystals, optimising the soaking conditions, soaking and harvesting all 600 crystals and completing the data collection run on beamline I04-1. The hits from this initial run and other details were pre-released on March 6th.

By the 24th of March, the initial 1500-crystal experiment was complete, and the results made publicly available. Screening additional libraries throughout April brought the total number of active site fragments to 71, with 48 fragments binding covalently (full timeline here and download page here). This was an exceptionally large screen which yielded a remarkably rich readout, with vast opportunities for fragment growing and merging.

We have already triggered computationally-driven follow-up work internally, and externally joined forces to launch a fully-open crowdsourcing and crowdfunding initiative – the COVID Moonshot - to establish urgently the shortest route possible to clinical impact by maximally exploiting the readout - you can help, read more here.

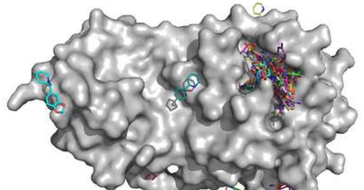
On the 11th of May, the first biochemical and structural data from Moonshot compounds was released and by the 12th of June over 500 compounds had been tested, demonstrating that the design-make-test process is fully in place.

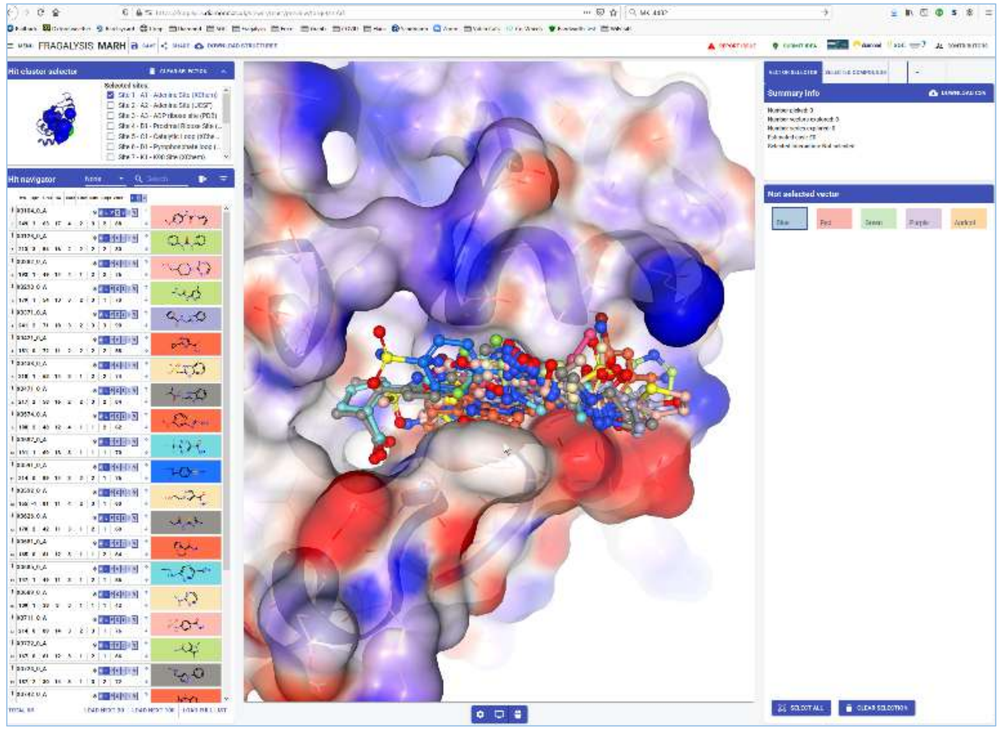
### XChem fragment screen

The initial screen encompassed multiple fragment libraries: the DSI-poised library, MiniFrags (Astex) FragLites & Peplites (CRUK Newcastle Drug Discovery Unit (Newcastle University)), York3D (University of York), SpotFinder and heterocyclic electrophilic fragment library (Hungarian Academy of Sciences) and an electrophilic fragment library designed and pre-screened by mass spec at the Weizmann Institute (see below).

There were 74 hits of high interest - data and extensive details are here, and some interactive views here:

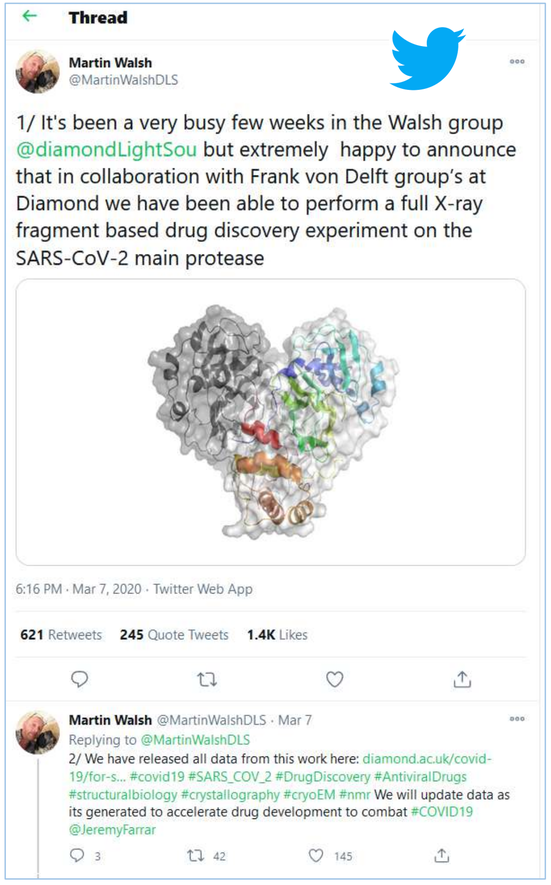
- 23 non-covalent hits in the active site
- 48 covalent hits in the active site
- 3 hits in the dimer interface, one in a calculated hotspot






https://fragalysis.diamond.ac.uk

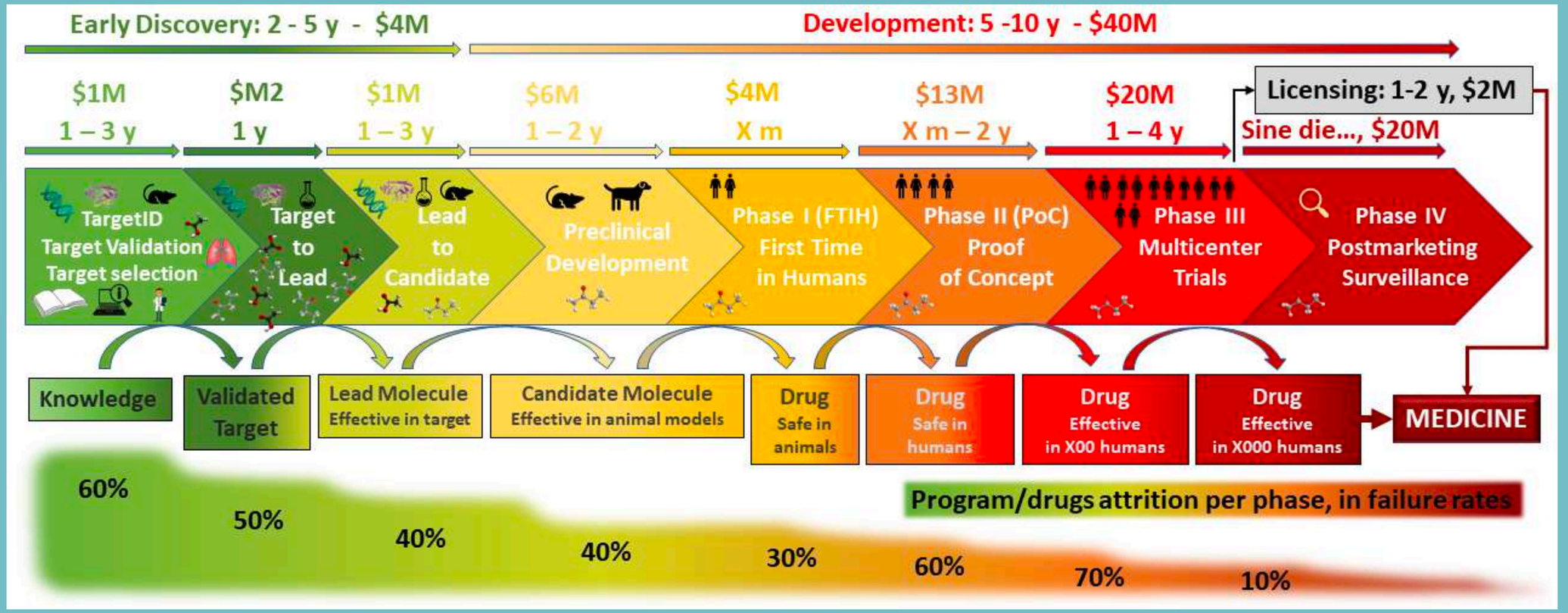
https://www.diamond.ac.uk/covid-19/for-scientists/Main-protease-structure-and-XChem.html




COVID Moonshot

Centre for Medicines Discovery
University of Oxford
XCHEM Diamond Fragment Screening
diamond

# Drug discovery is usually a long and expensive process



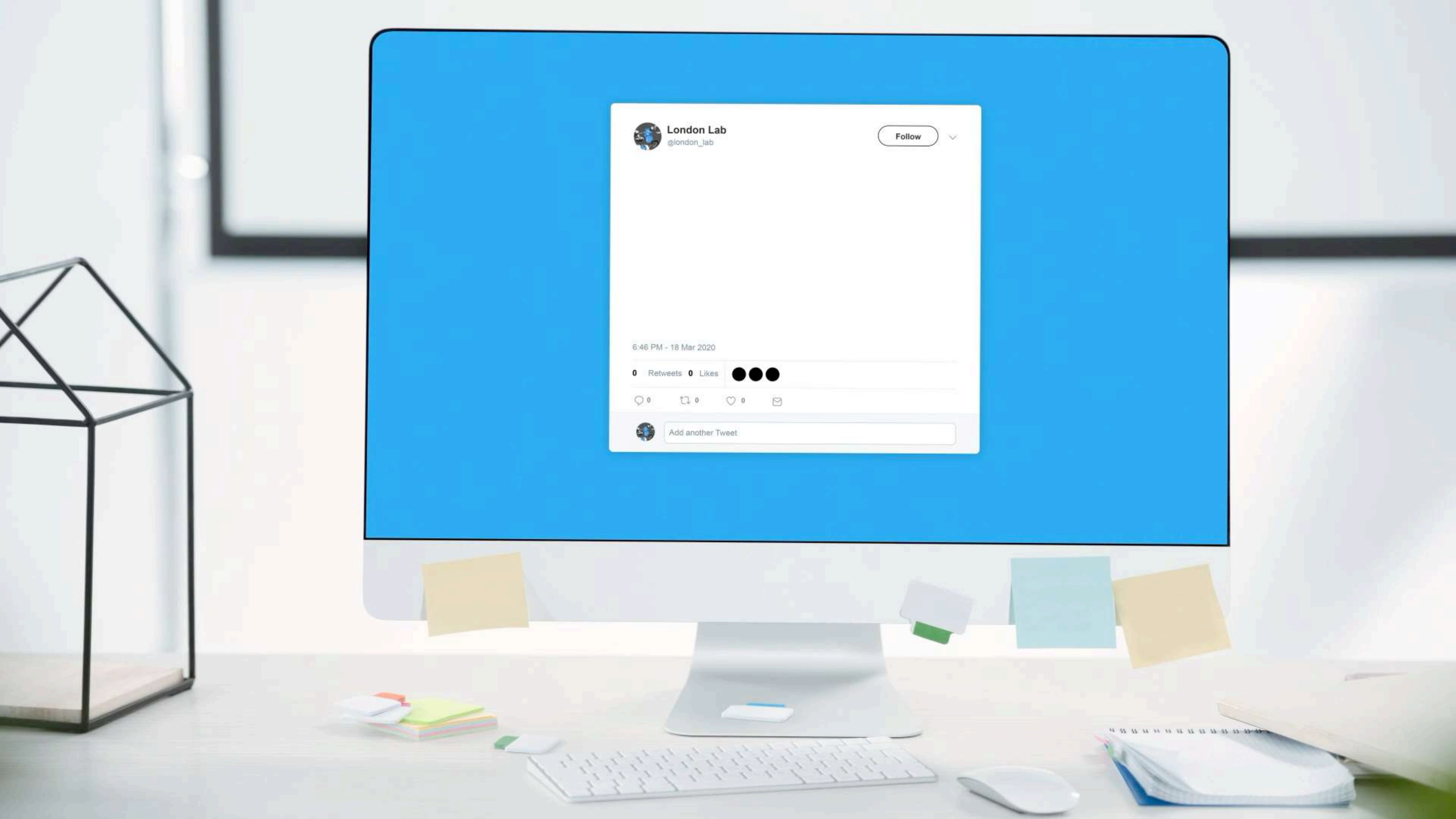

https://doctortarget.com/machine-learning-applied-drug-discovery/

## How can we cut down this timeline?

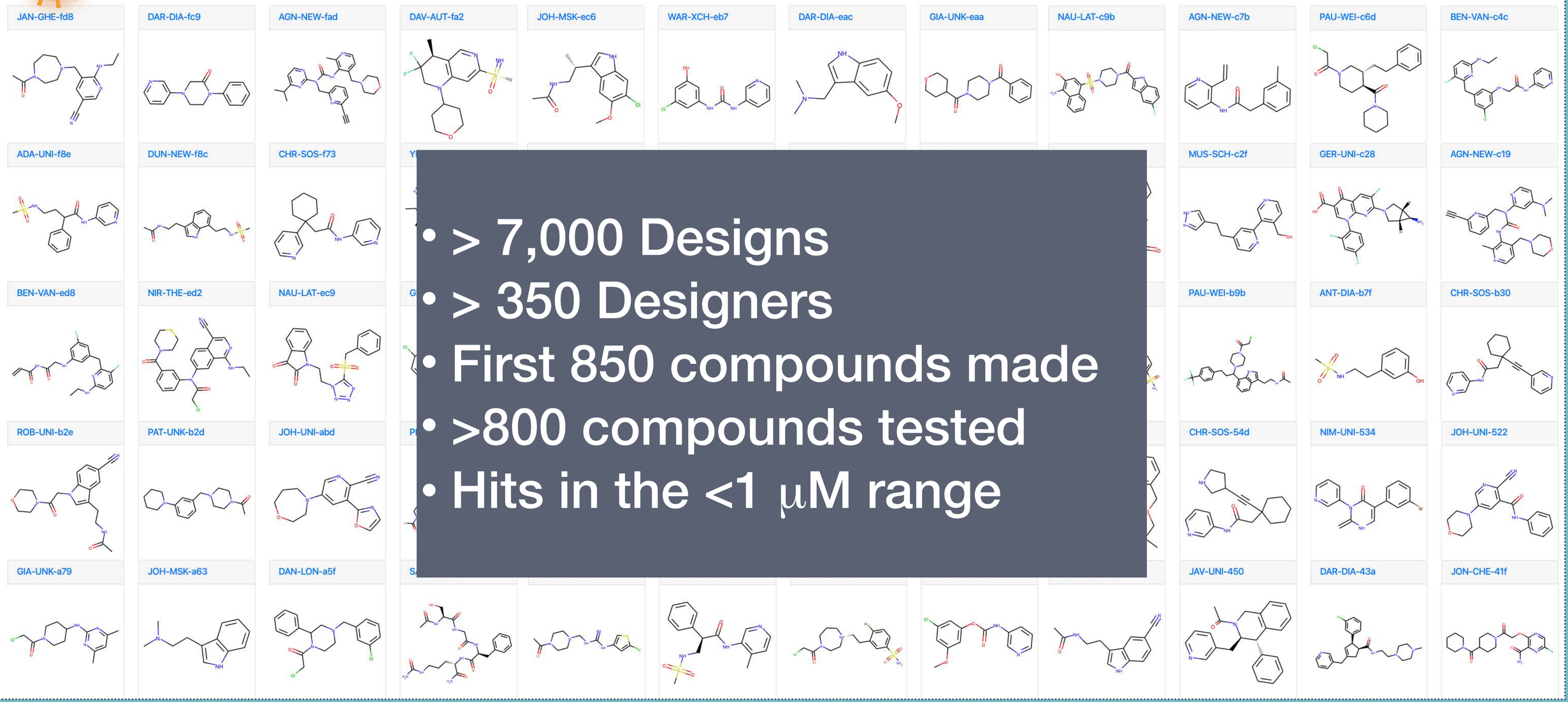
# Which strategies would most quickly get us from fragment structures all the way to a useful drug?

**Nir London**
Weizmann Institute

## What if we tried ALL OF THEM?

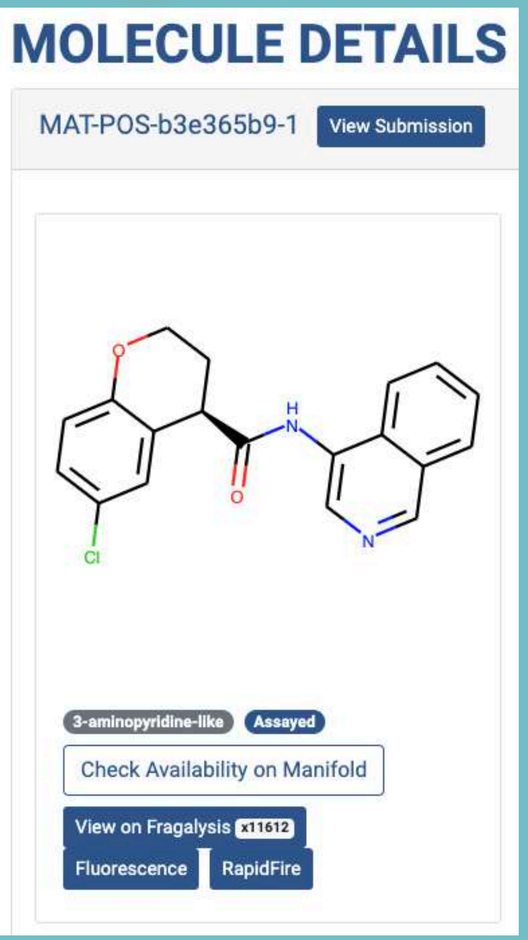

London Lab
@london_lab
Follow
6:46 PM - 18 Mar 2020
0 Retweets 0 Likes
Add another Tweet

# …and there was overwhelming response

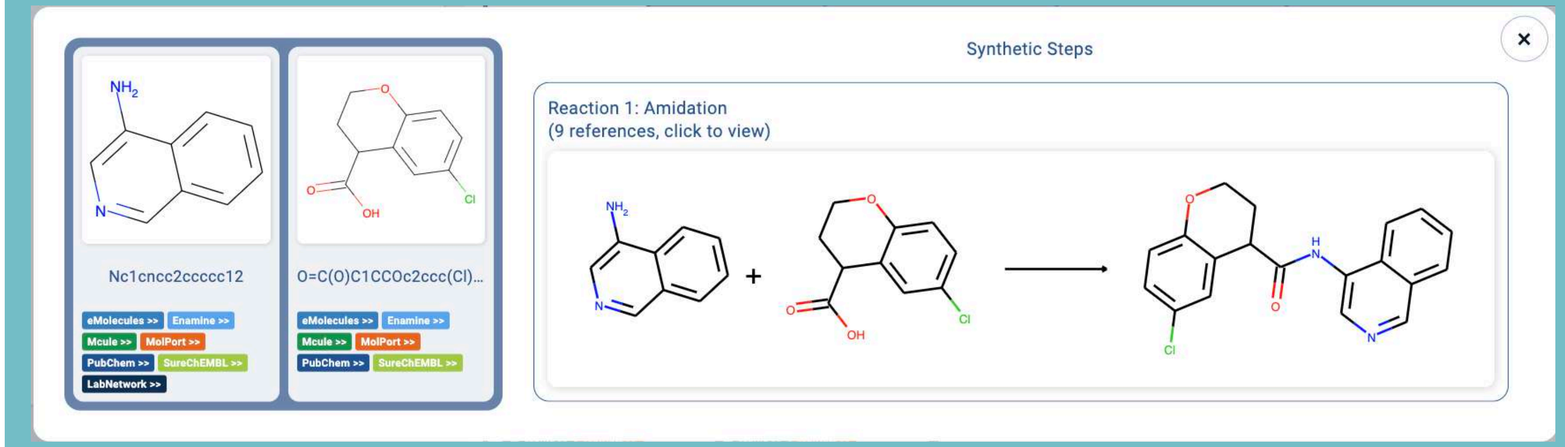



- > 7,000 Designs
- > 350 Designers
- First 850 compounds made
- >800 compounds tested
- Hits in the <1 μM range

# PostEra used synthetic route prediction AI to quickly identify with designs could be rapidly synthesized




## CRO catalogue-aware optimal synthetic route



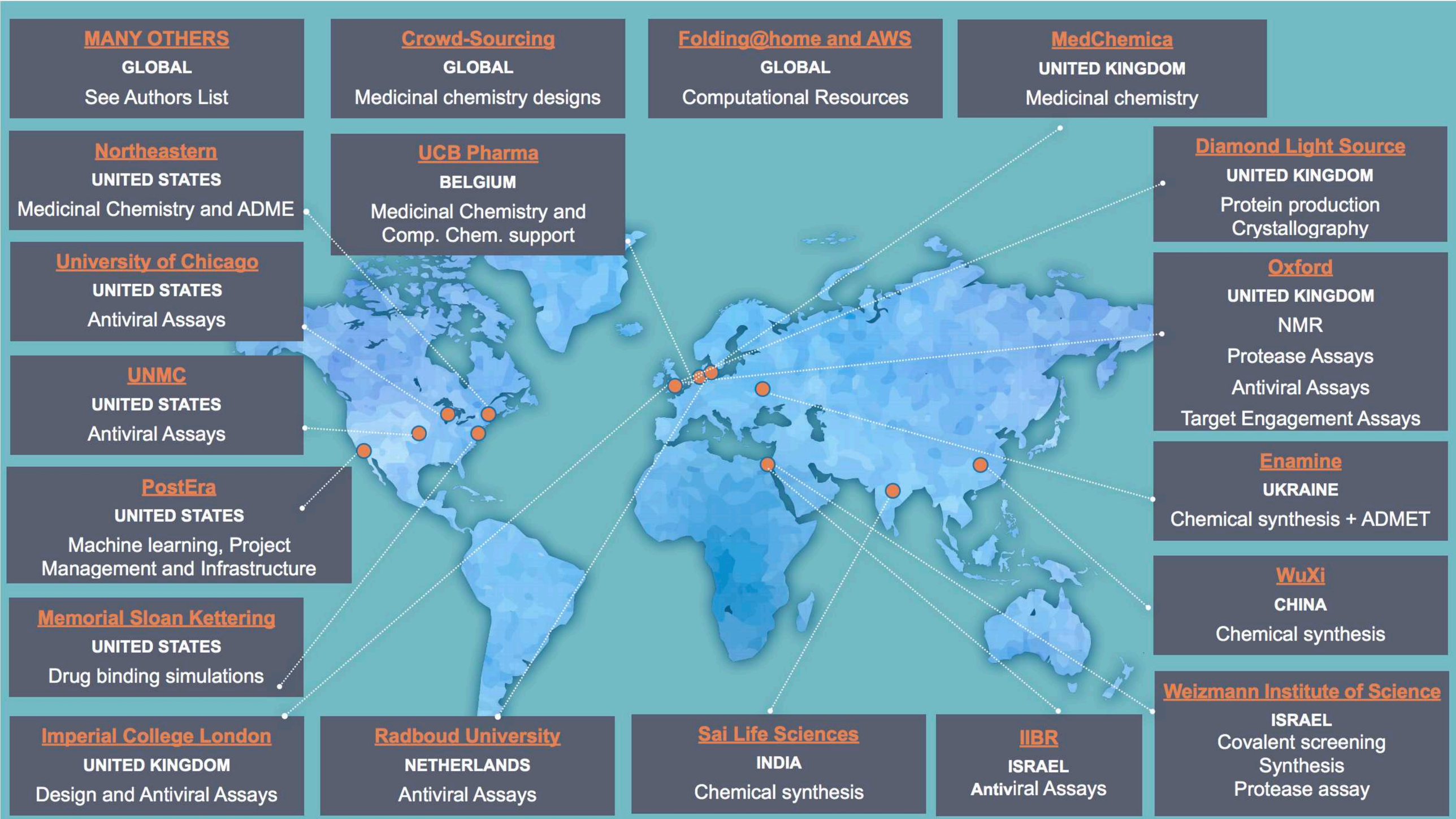

http://postera.ai/manifold

## CROs donating effort

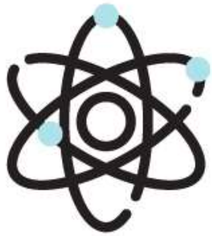
- Enamine
- WuXi
- Sai

http://postera.ai/covid

Schwaller et al. ACS Central Science 5:9, 2019
https://pubs.acs.org/doi/10.1021/acscentsci.9b00576

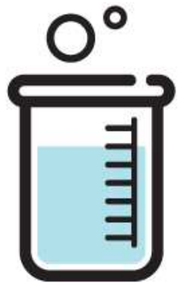

MANY OTHERS
GLOBAL
See Authors List
Crowd-Sourcing
GLOBAL
Medicinal chemistry designs
Folding@home and AWS
GLOBAL
Computational Resources
MedChemica
UNITED KINGDOM
Medicinal chemistry
Northeastern
UNITED STATES
Medicinal Chemistry and ADME
UCB Pharma
BELGIUM
Medicinal Chemistry and Comp. Chem. support
Diamond Light Source
UNITED KINGDOM
Protein production
Crystallography
University of Chicago
UNITED STATES
Antiviral Assays
Oxford
UNITED KINGDOM
NMR
Protease Assays
Antiviral Assays
Target Engagement Assays
UNMC
UNITED STATES
Antiviral Assays
Enamine
UKRAINE
Chemical synthesis + ADMET
PostEra
UNITED STATES
Machine learning, Project Management and Infrastructure
WuXi
CHINA
Chemical synthesis
Memorial Sloan Kettering
UNITED STATES
Drug binding simulations
Weizmann Institute of Science
ISRAEL
Covalent screening
Synthesis
Protease assay
Imperial College London
UNITED KINGDOM
Design and Antiviral Assays
Radboud University
NETHERLANDS
Antiviral Assays
Sai Life Sciences
INDIA
Chemical synthesis
IIBR
ISRAEL
Antiviral Assays

# The COVID Moonshot is an open science, patent-free drug discovery project



## Open science

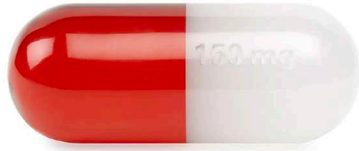
COVID Moonshot



## Open data

http://postera.ai/covid

## Patent-free

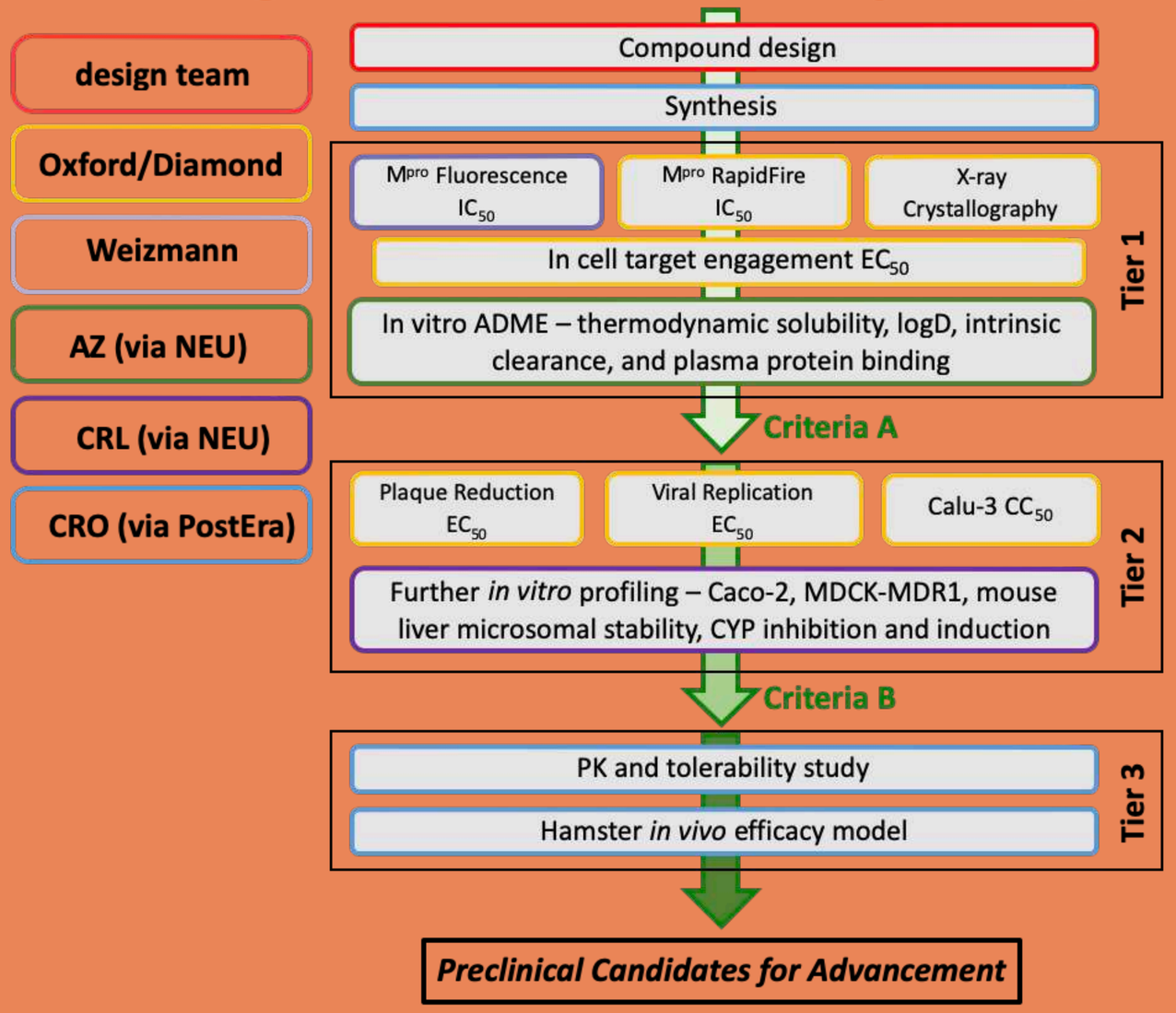


# Defined a target product profile (TPP) for oral Mpro inhibitor for use in early disease or prophylactic use following exposure

| Property | Target range | Rationale |
|---|---|---|
| **protease assay** | $IC_{50}$ < 10 nM | Extrapolation from other anti-viral programs |
| **viral replication assay** | $EC_{50}$ < 5 µM | Suppression of virus at achievable blood levels |
| **plaque reduction assay** | $EC_{50}$ < 5 µM | Suppression of virus at achievable blood levels |
| **route of administration** | oral | bid/tid - compromise PK for potency if pharmacodynamic effect achieved |
| **solubility** | > 5 mg/mL | Aim for biopharmaceutical class 1 assuming <= 750 mg dose |
| **half-life** | > 8 h (human) est from rat and dog | Assume PK/PD requires continuous cover over plaque inhibition for 24 h max bid dosing |
| **safety** | Only reversible and monitorable toxicities<br>No significant DDI - clean in 5 CYP450 isoforms<br>hERG and NaV1.5 $IC_{50}$ > 50 µM<br>No significant change in QTc<br>Ames negative<br>No mutagenicity or teratogenicity risk | No significant toxicological delays to development<br>DDI aims to deal with co-morbidities / therapies,<br>cardiac safety for COVID-19 risk profile<br>cardiac safety for COVID-19 risk profile<br>Low carcinogenicity risk reduces delays in manufacturing<br>Patient group will include significant proportion of women of childbearing age |

# Assay cascade constructed to help us reach TPP goals as rapidly as possible

**Ed Griffen**
Medchemica




**Does it inhibit Mpro? How does it bind?**

**Does it enter cells and inhibit Mpro?**

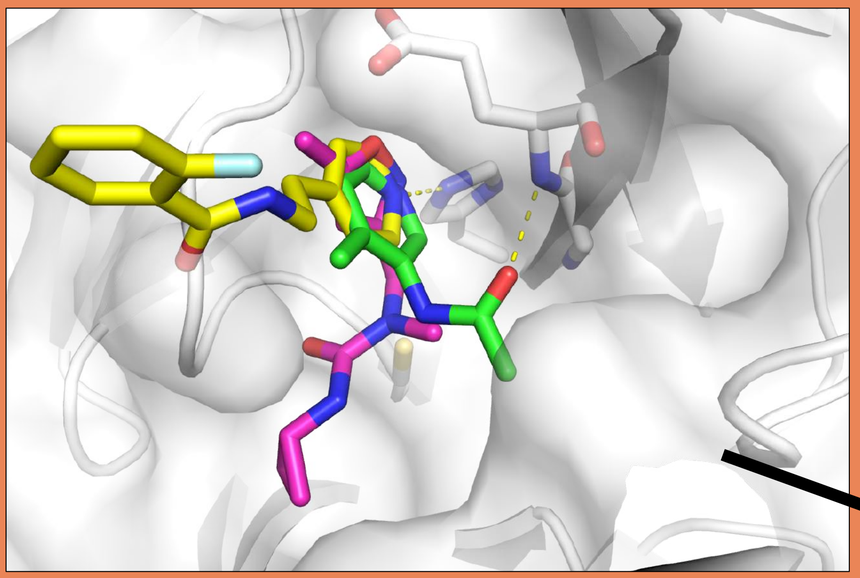
**Does it have a chance of working in humans?**

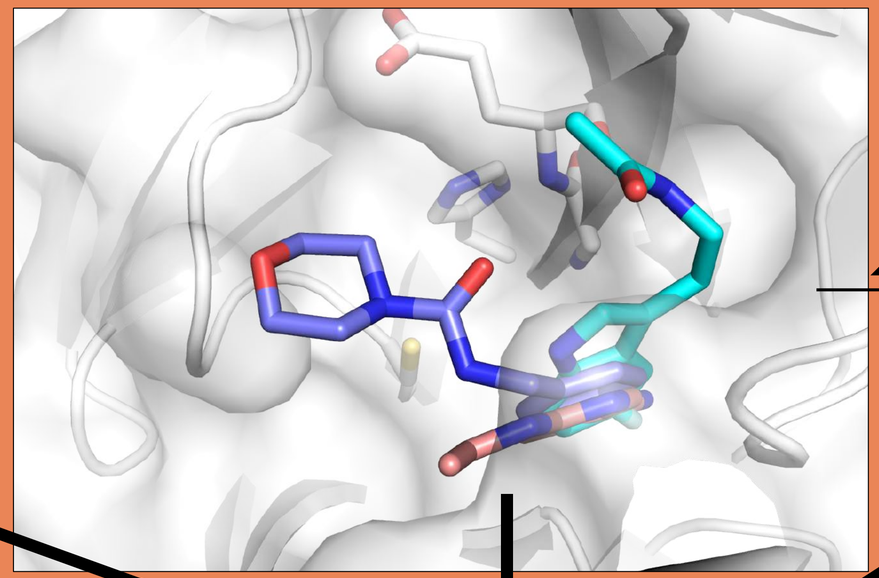
**Does it kill virus in infected cells, sparing healthy cells?**

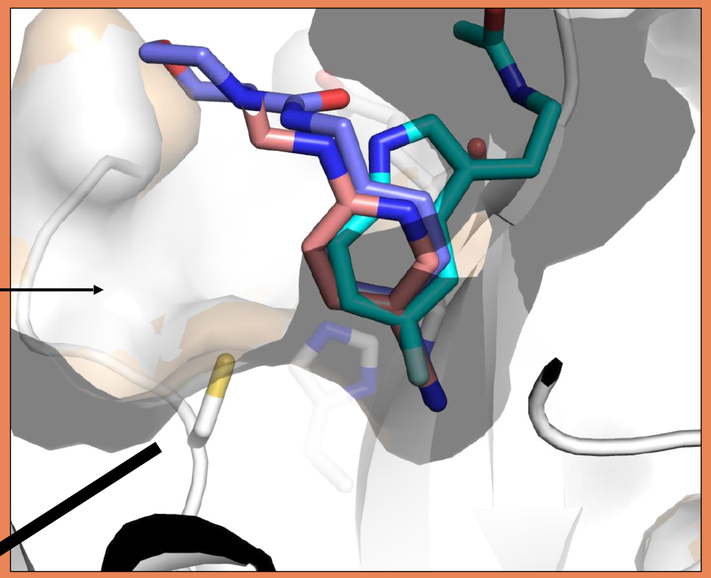
**Does it have a favorable safety profile?**

**Is it orally bioavailable at required concentrations?**

# Crowdsourcing generated a number of novel chemical series by fragment merging





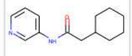


**Contributor:** Tryfon Zarganis - Tzitzikas, University of Oxford, TDI MedChem

**Design Rationale:**

The design of the molecules was done by superimposing the different fragments from the crystal structures (by eye). The reactions should be fairly easy urea formation or amide coupling all from readily available starting materials. Fragments used for the conception of the ideas are the following x0107, x0434, x0678, x0748, x0995, x1382

**Inspired By:**



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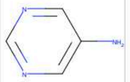

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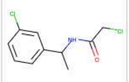

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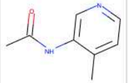

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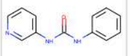

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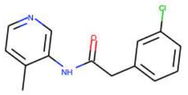


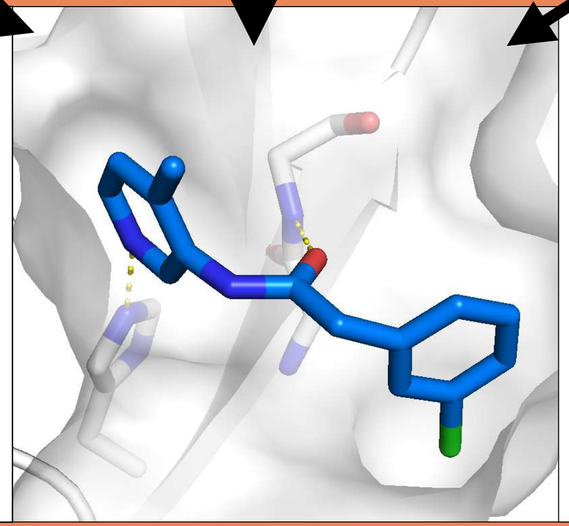
TRY-UNI-714a760b-6

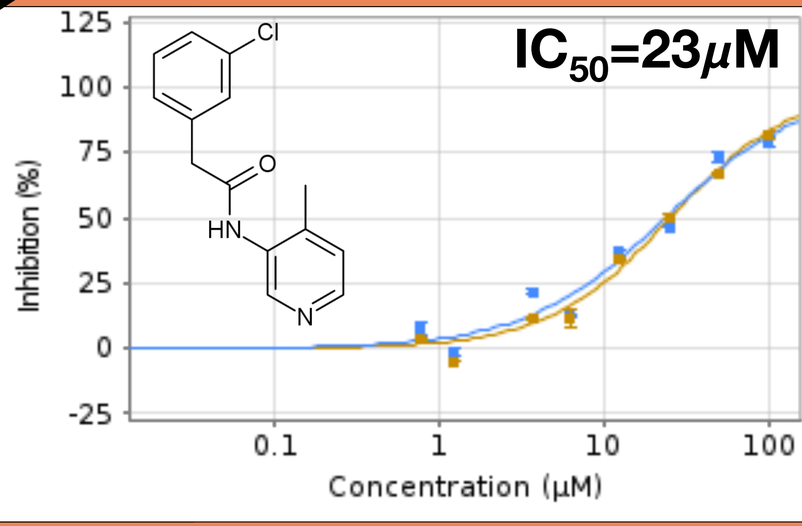
Cc1ccncc1NC(=O)Cc1cccc(Cl)c1

3-aminopyridine-like

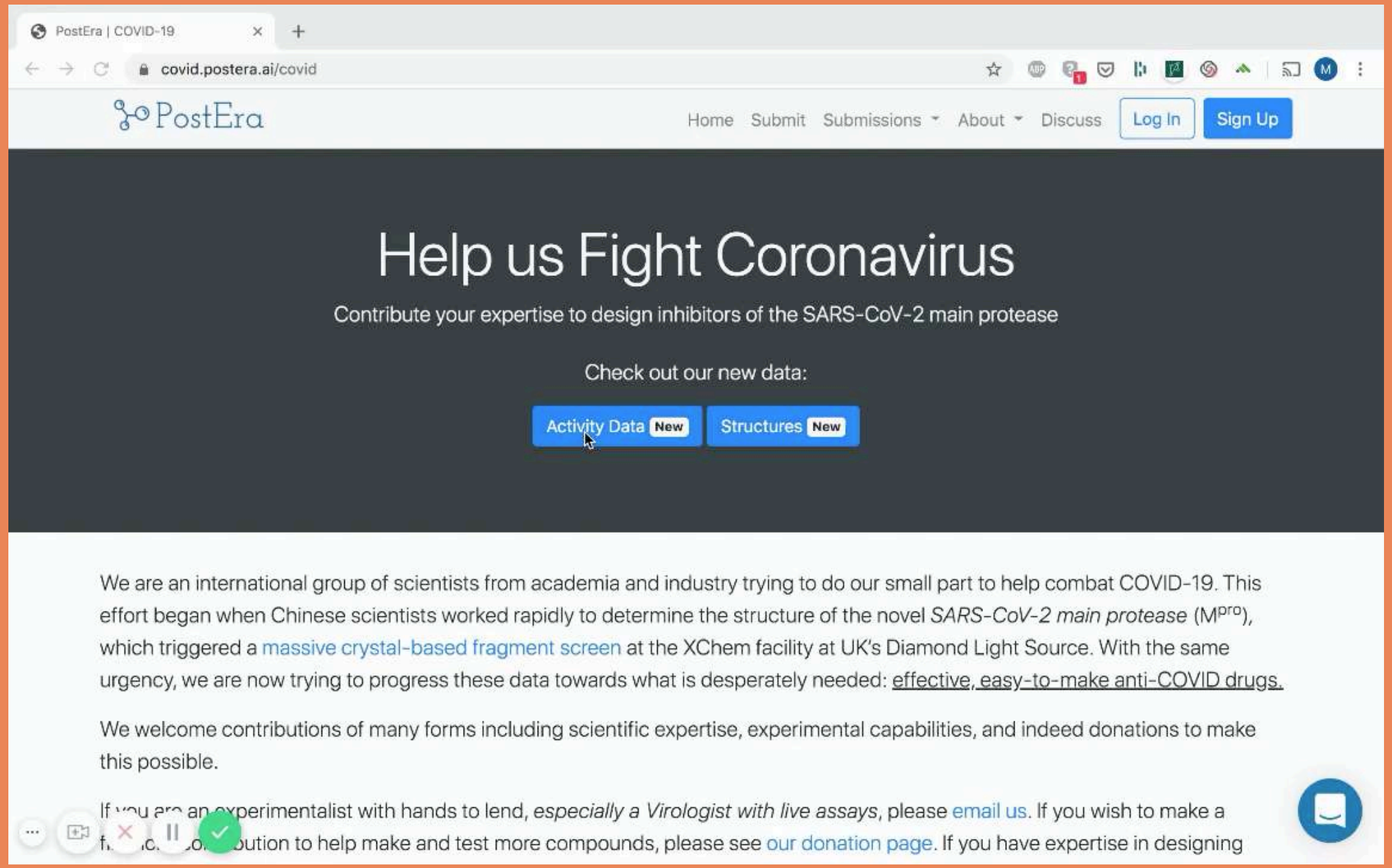
Enamine Mcule MolPort Assayed

View



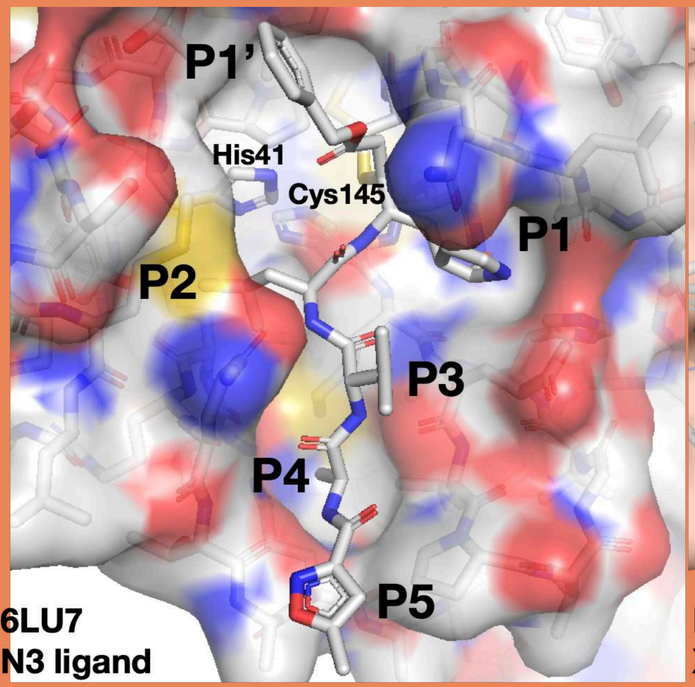


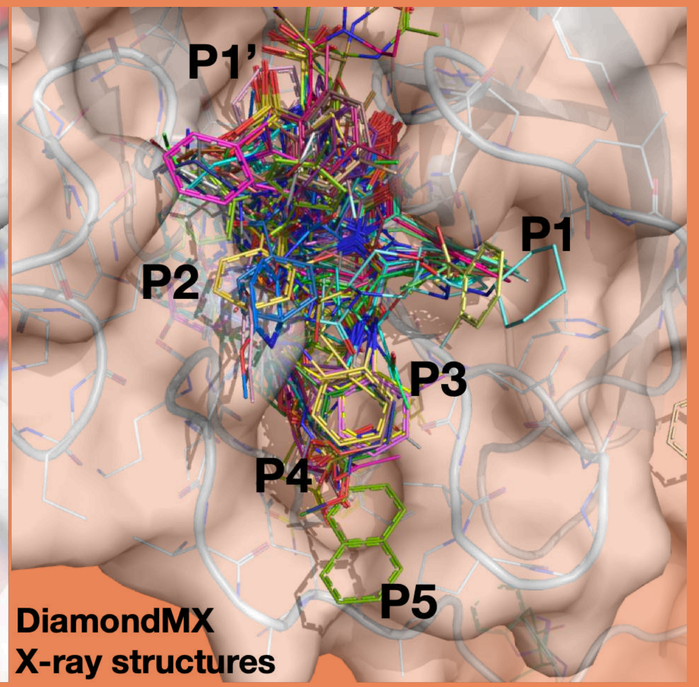
# Data reported back to community

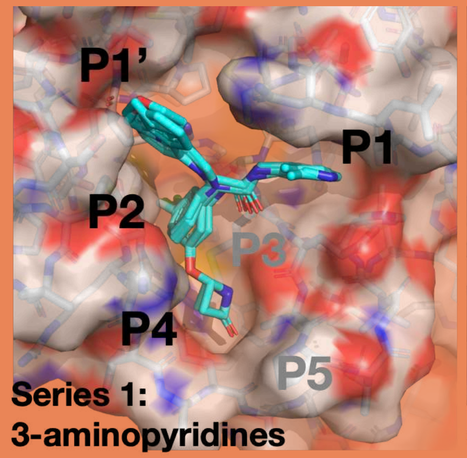


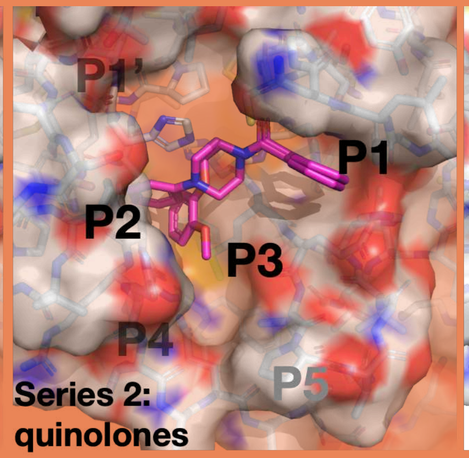

http://postera.ai/covid

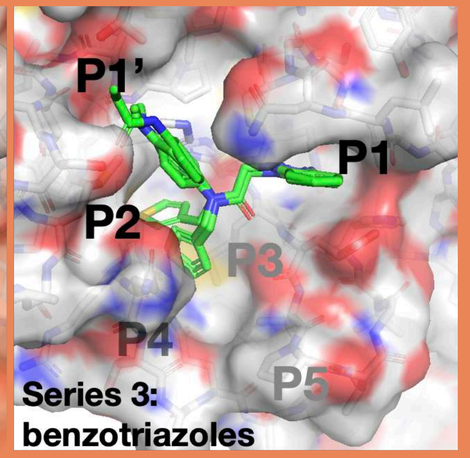
# We focused on three primary noncovalent series



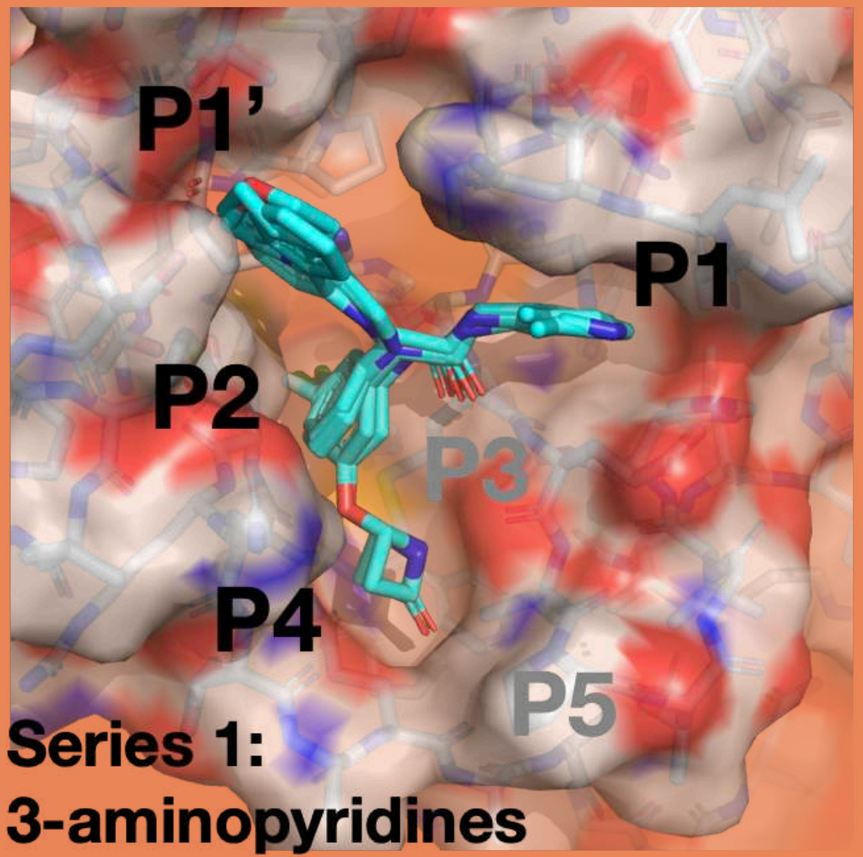



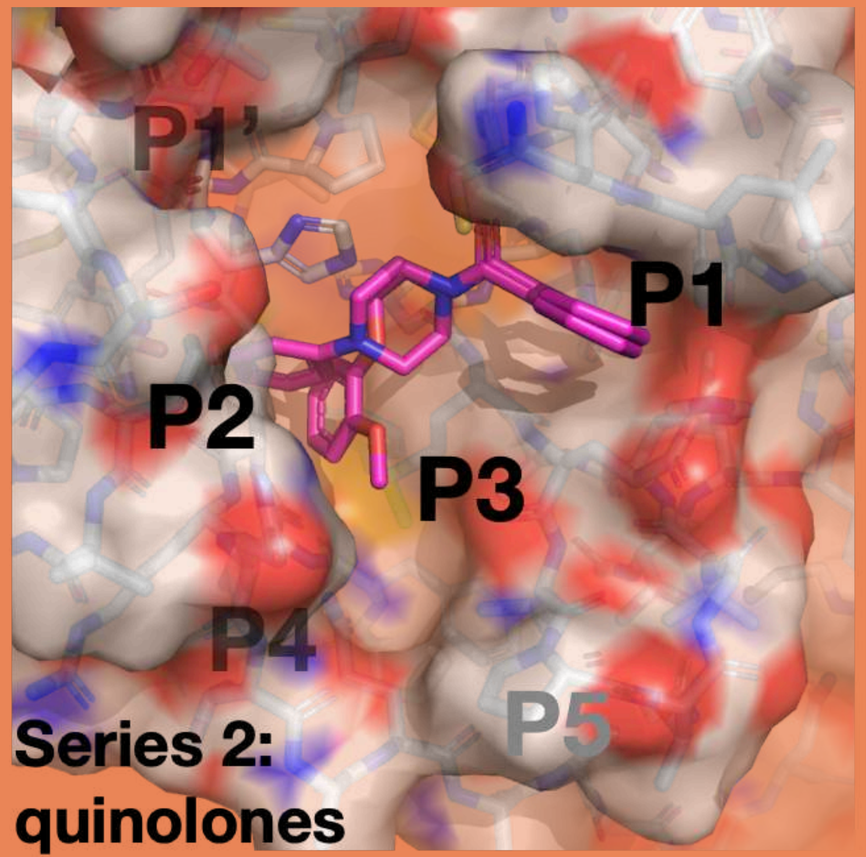



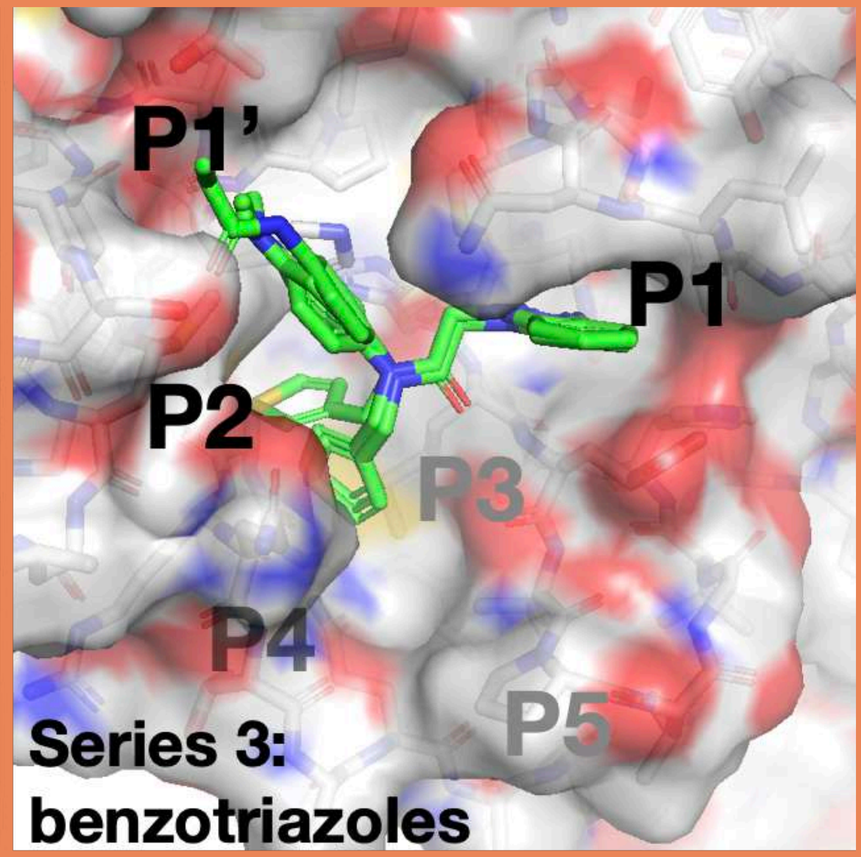




http://postera.ai/covid

# We focused on three primary noncovalent series



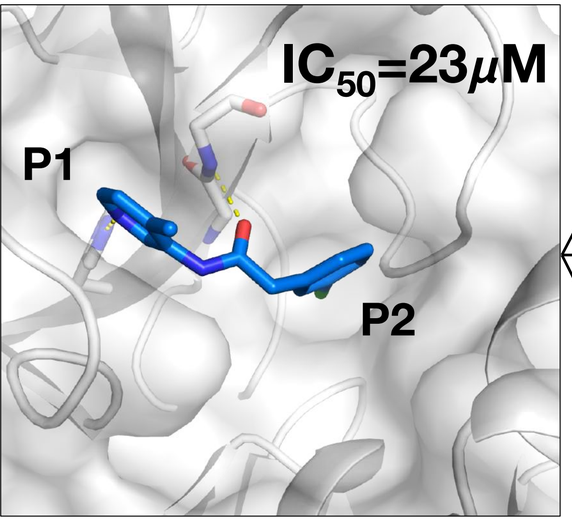



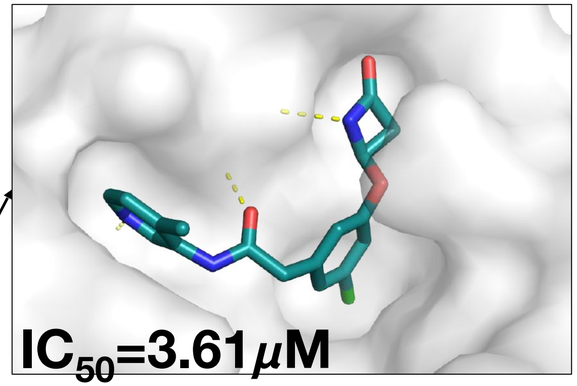

http://postera.ai/covid

# 3-aminopyridines provide a potent P1-P2 scaffold capable of accessing P4 and P1’ pockets

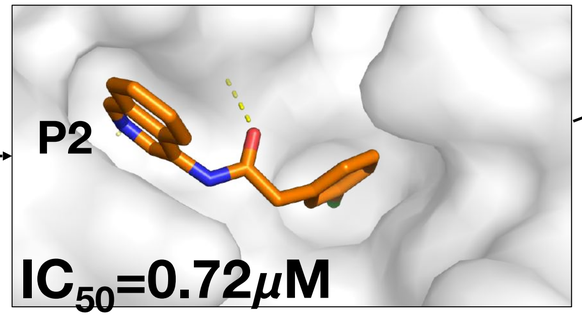
**>300 aminopyridine compounds synthesized**



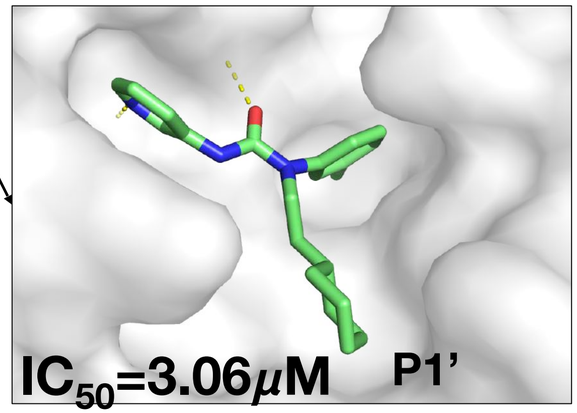

6.4x



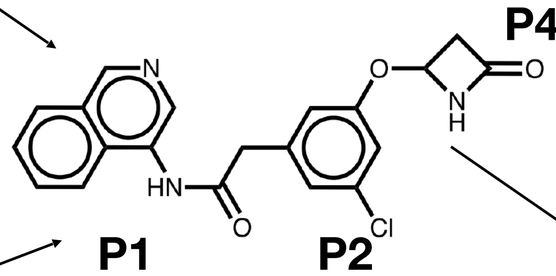

32x

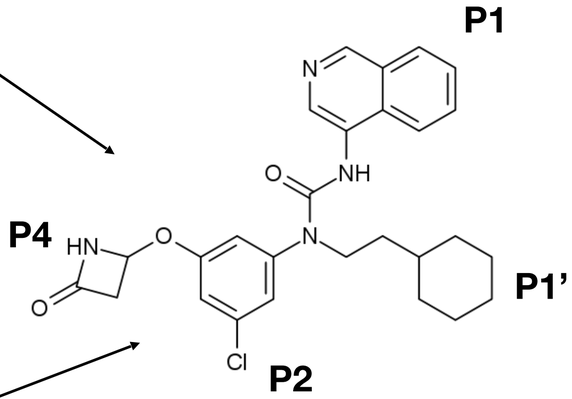



7.5x

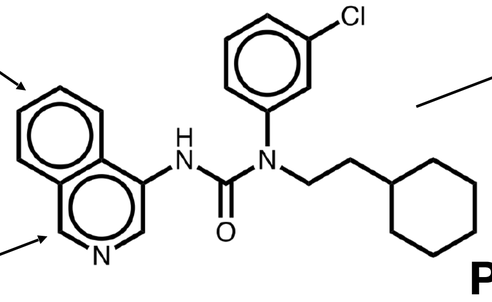



$IC_{50}$=260 nM




$IC_{50}$= 105 nM




$IC_{50}$=270 nM

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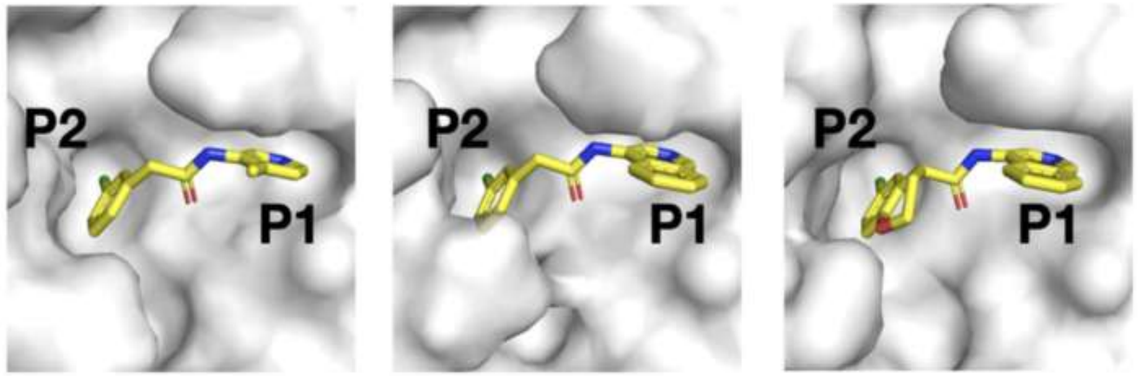
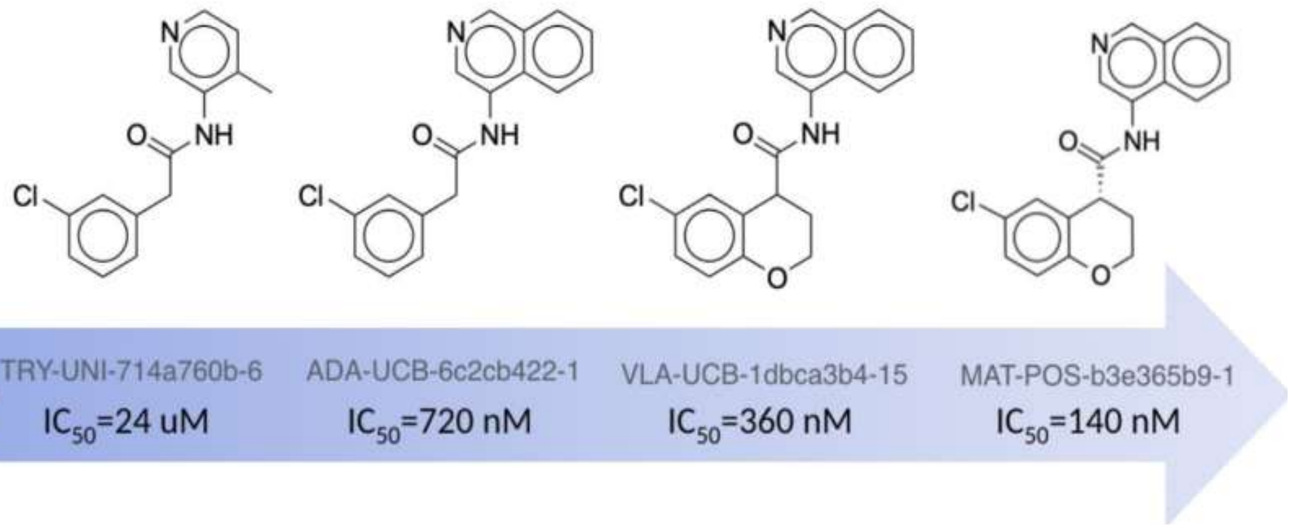
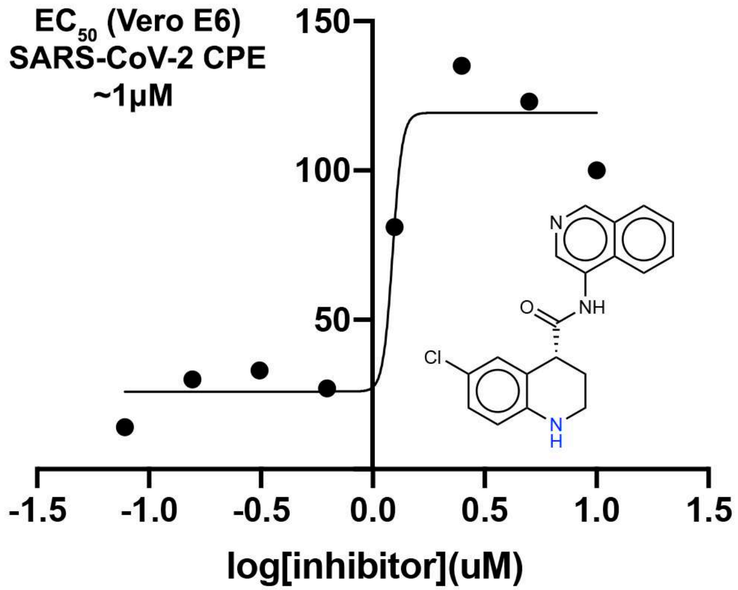
# Aminopyridine series has evolved into a potent P1-P2 scaffold with ~1 µM antiviral activity




DiamondMX/XChem x2646 | DiamondMX/XChem x10959 | DiamondMX/XChem x11498




## Viral activity assay




http://postera.ai/covid

With the Israel Institute of Biological Research

# Meanwhile, our lab had started to use Folding@home to study COVID-19 targets…

FOLDING @HOME


## Client statistics by OS

| OS Type | Native TFLOPS* | x86 TFLOPS* | Active CPUs | Active Cores | Total CPUs |
|---|---|---|---|---|---|
| Windows | 857 | 857 | 67,467 | 187,104 | 5,857,235 |
| Mac OS X | 91 | 91 | 8,083 | 85,382 | 217,033 |
| Linux | 87 | 87 | 6,383 | 26,457 | 882,200 |
| NVIDIA GPU | 1 | 2 | 4 | 4 | 348,371 |
| ATI GPU | 10,243 | 21,613 | 7,178 | 7,178 | 426,335 |
| NVIDAI Fermi GPU | 36,065 | 76,097 | 21,570 | 21,587 | 624,822 |
| Total | 47,344 | 98,747 | 110,685 | 327,712 | 8,355,996 |

1924085 people have non-anonymously contributed to Folding@home.

**Table last updated at Sat, 19 Oct 2019 18:23:11**


**~100 pflop/s!**

# …building the first exaFLOP/s computing platform as the public joined in our effort

## FOLDING@HOME TAKES UP THE FIGHT AGAINST COVID-19 / 2019-NCOV

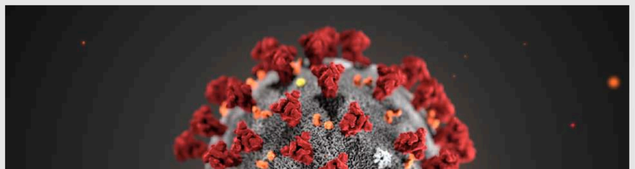
February 27, 2020
by Greg Bowman

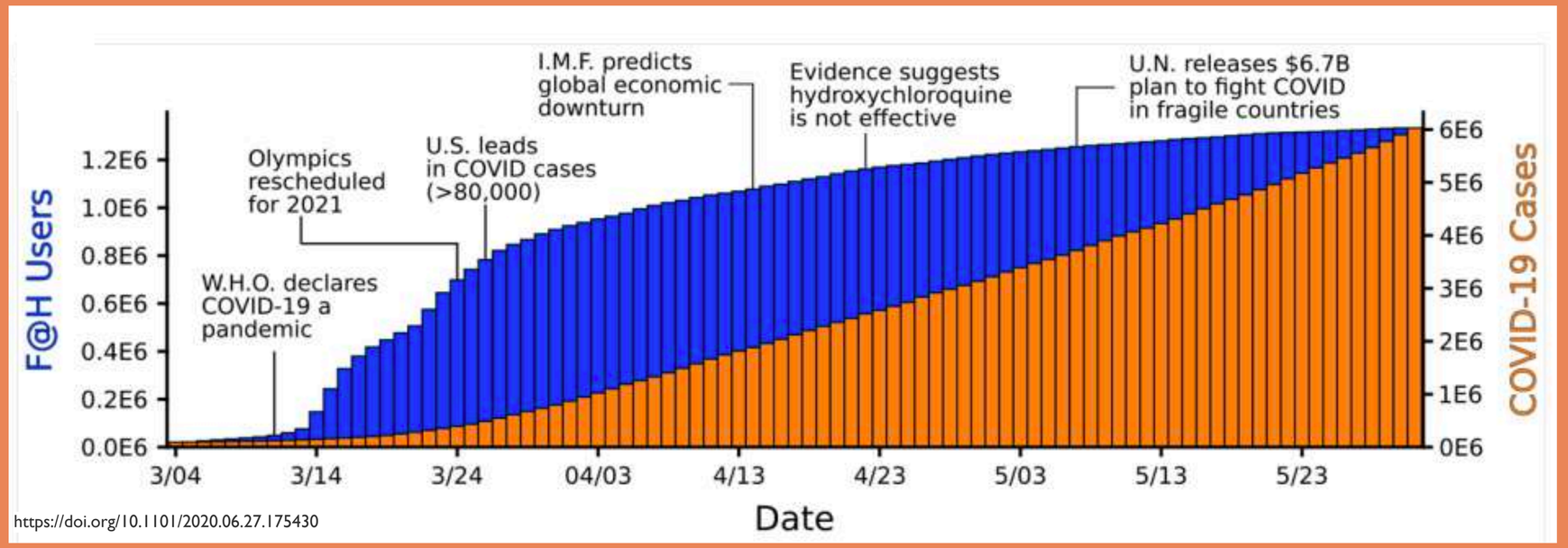
We need your help! Folding@home is joining researchers around the world working to better understand the 2019 Coronavirus (2019-nCoV) to accelerate the open science effort to develop new life-saving therapies. By downloading Folding@Home, you can donate your unused computational resources to the Folding@home Consortium, where researchers working to advance our understanding of the structures of potential drug targets for 2019-nCoV that could aid in the design of new therapies. The data you help us generate will be quickly and openly disseminated as part of an open science collaboration of multiple laboratories around the world, giving researchers new tools that may unlock new opportunities for developing lifesaving drugs.

2019-nCoV is a close cousin to SARS coronavirus (SARS-CoV), and acts in a similar way. For both coronaviruses, the first step of infection occurs in the lungs, when a protein on the surface of the virus binds to a receptor protein on a lung cell. This viral protein is called the spike protein, depicted in red in the image below, and the receptor is known as ACE2. A therapeutic antibody is a type of protein that can block the viral protein from binding to its receptor, therefore preventing the virus from infecting the lung cell. A therapeutic antibody has already been developed for SARS-CoV, but to develop therapeutic antibodies or small molecules for 2019-nCoV, scientists need to better understand the structure of the viral spike protein and how it binds to the human ACE2 receptor required for viral entry into human cells.

Proteins are not stagnant—they wiggle and fold and unfold to take on numerous shapes. We need to study not only one shape of the viral spike protein, but all the ways the protein wiggles and folds into alternative shapes in order to best understand how it interacts with the ACE2 receptor, so that an antibody can be designed. Low-resolution structures of the SARS-CoV spike protein exist and we know the mutations that differ between SARS-CoV and 2019-nCoV. Given this information, we are uniquely positioned to help model the structure of the 2019-nCoV spike protein and identify sites that can be targeted by a therapeutic antibody. We can build computational models that accomplish this goal, but it takes a lot of computing power.

This is where you come in! With many computers working towards the same goal, we aim to help develop a therapeutic remedy as quickly as possible. By downloading Folding@home here [LINK] and selecting to contribute to "Any Disease", you can help provide us with the computational power required to tackle this problem. One protein from 2019-nCoV, a protease encoded by the viral RNA, has already been crystallized. Although the 2019-nCoV spike protein of interest has not yet been resolved bound to ACE2, our objective is to use the homologous structure of the SARS-CoV spike protein to identify therapeutic antibody targets.






https://doi.org/10.1101/2020.06.27.175430

**Ariana Brenner (CBM)**

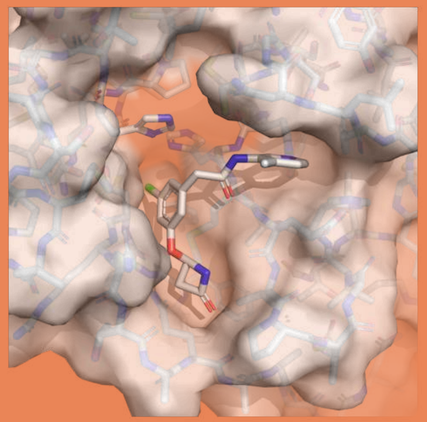
**Rafal Wiewiora (TPCB)**

**Ivy Zhang (CBM)**

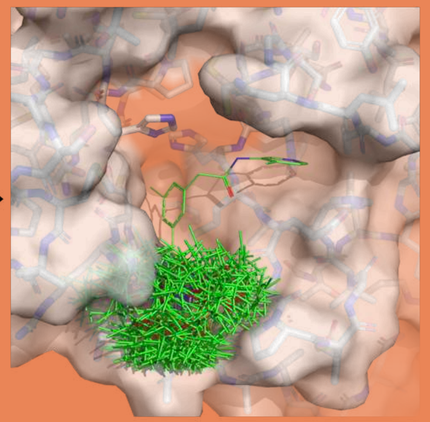
# Folding@home is running free energy calculations at planetary scale in 1-2 week sprints

**Dominic Rufa**
**Tri-I TPCB PhD student**

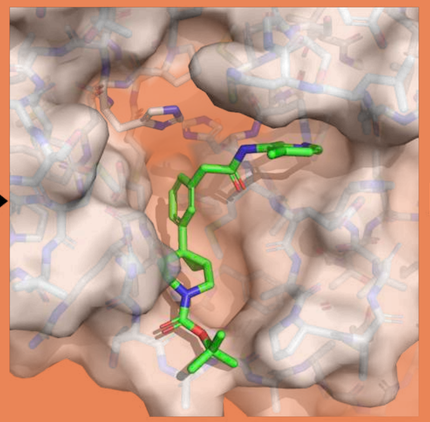
**X-ray structure as reference**



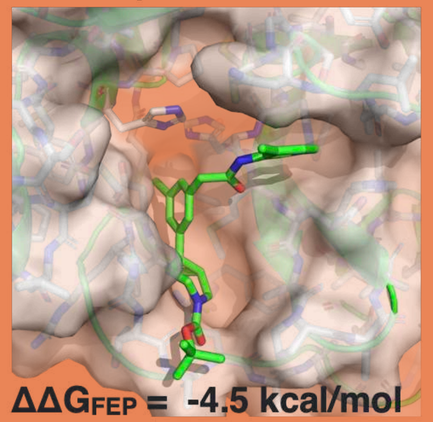
**constrained enumeration of poses for proposed molecule**



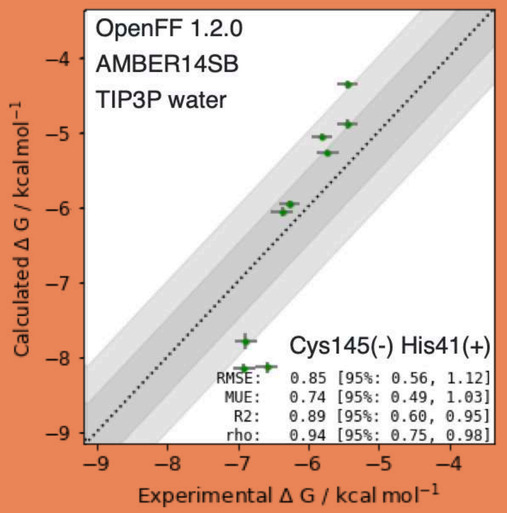
**selection of pose with best docking score**



**nonequilibrium alchemical free energy calculation final posed structure**




**retrospective performance on 3-aminopyridine lead series**

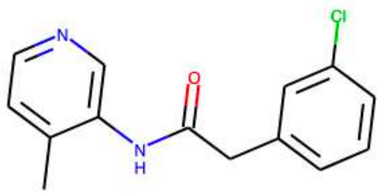



**perses:** open source relative alchemical free energy calculations
http://github.com/choderalab/perses
**Open Force Field Initiative** OpenFF 1.2.0 (“Parsley”) force field
http://openforcefield.org

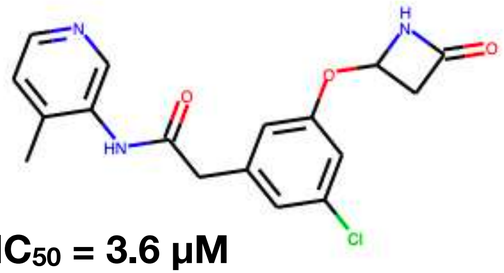
# Our Folding@home free energy calculations aim to identify optimal P1’ and P4 substituents

**Hannah Bruce Macdonald**

**MolSSI Investment Postroctoral Fellow, MSKCC**



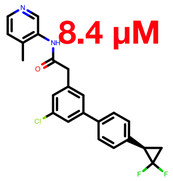
**7x better**



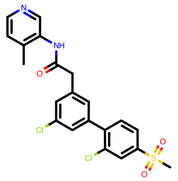
**$IC_{50}$ = 25 µM**
**TRY-UNI-714a760b-6**

**$IC_{50}$ = 3.6 µM**
**TRY-UNI-2eddb1ff-7**

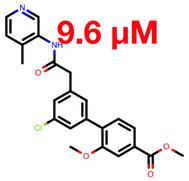
EN300-26624333 **8.4 µM**



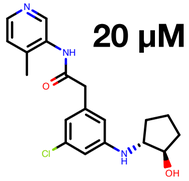
EN300-365771



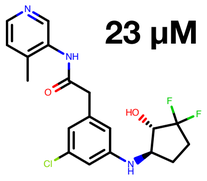
EN300-316592 **9.6 µM**



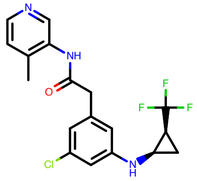
EN300-60314 **20 µM**



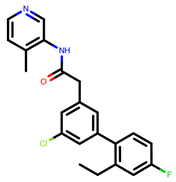
EN300-6734624 **23 µM**



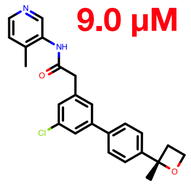
EN300-20814457



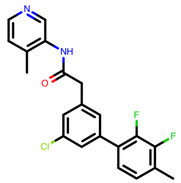
EN300-298506



EN300-1086686 **9.0 µM**



EN300-106778



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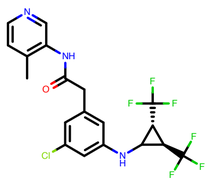
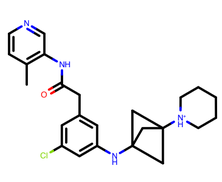
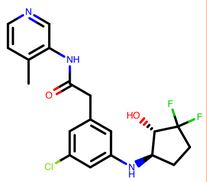


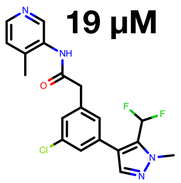
EN300-2515954



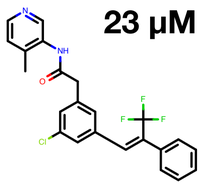
EN300-6734624



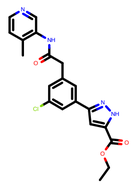
EN300-1425849 **19 µM**



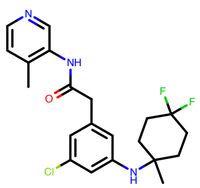
EN300-1704613 **23 µM**



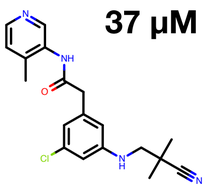
EN300-299518

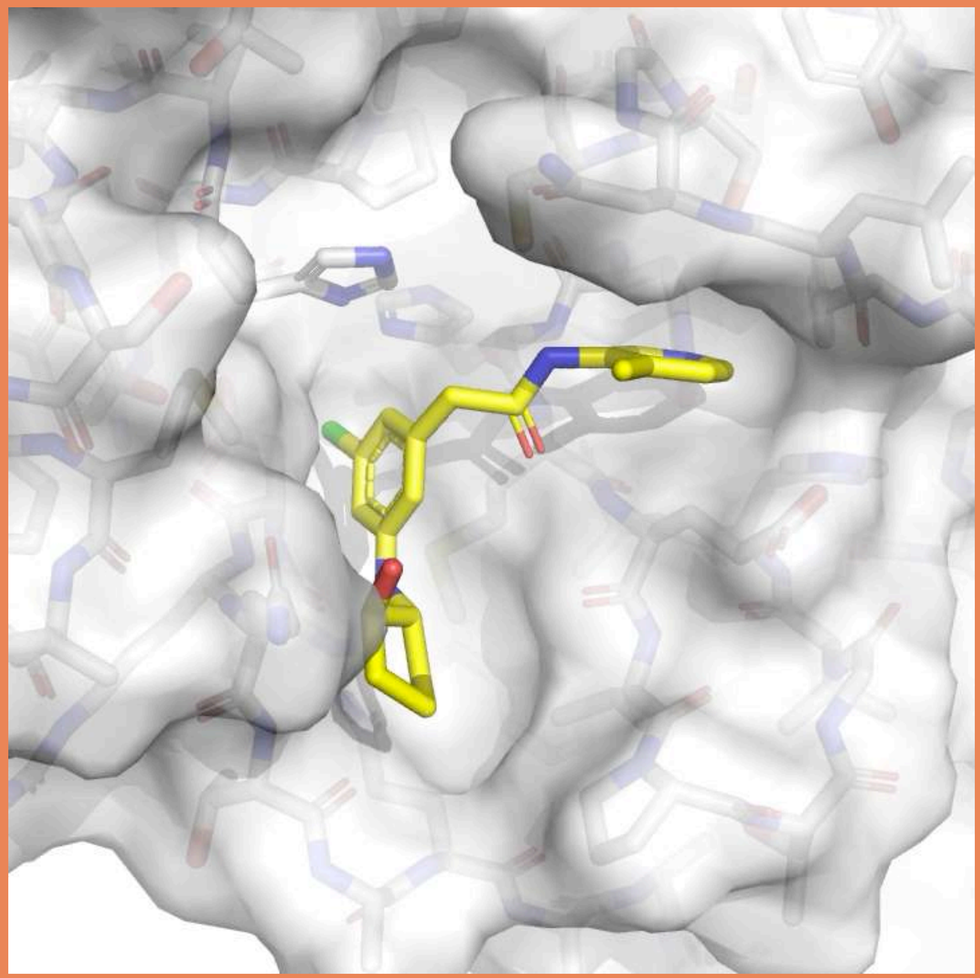


EN300-124496



EN300-212829 **37 µM**

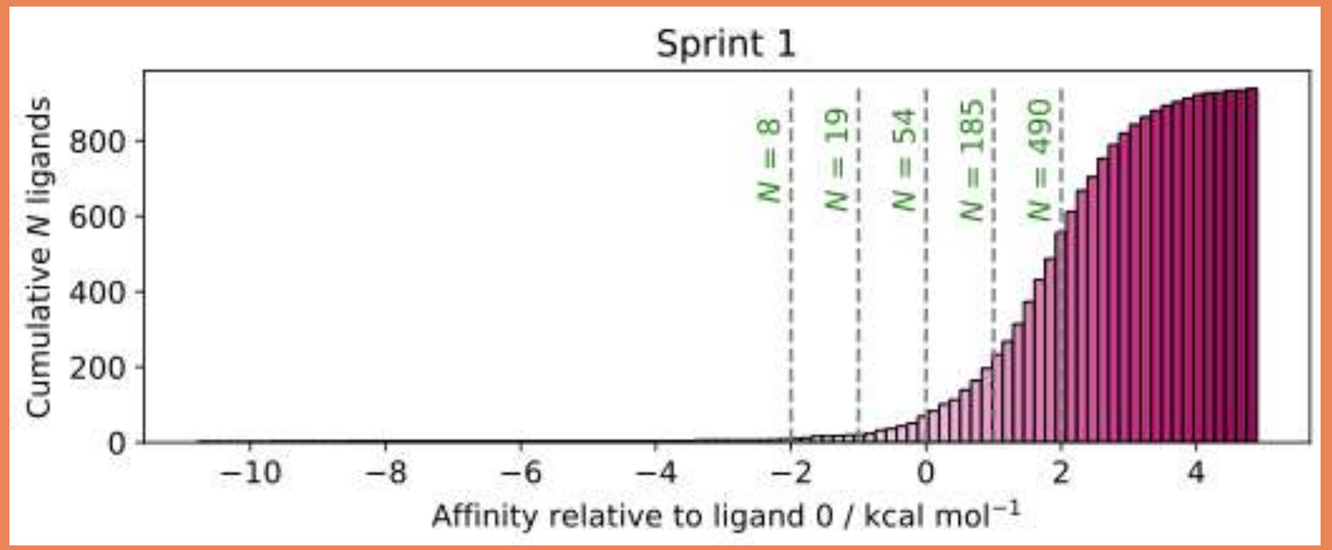




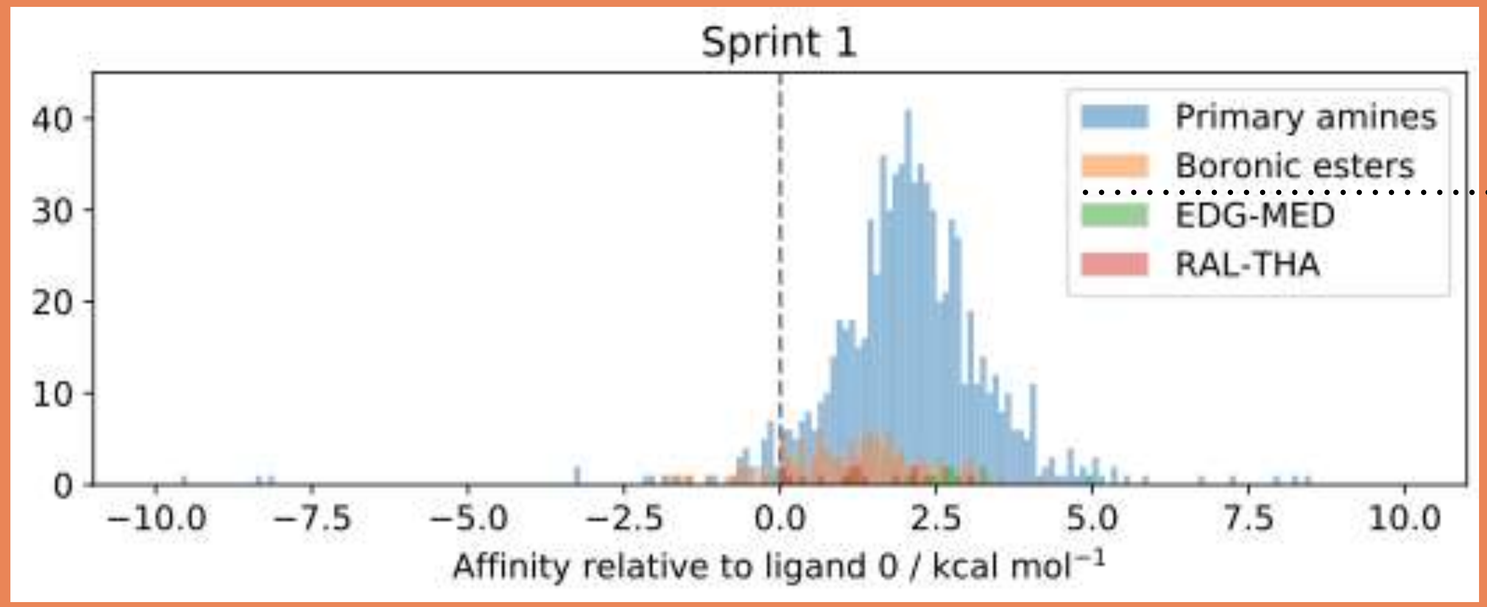
**DATA**: https://covid.postera.ai/covid/submissions/f42f3716-f86f-41d8-9906-c4fb7b6f5773

# Most ideas were bad ideas

**better** **worse**



# Human chemists seem better than random, but it's hard to get them to generate enough ideas



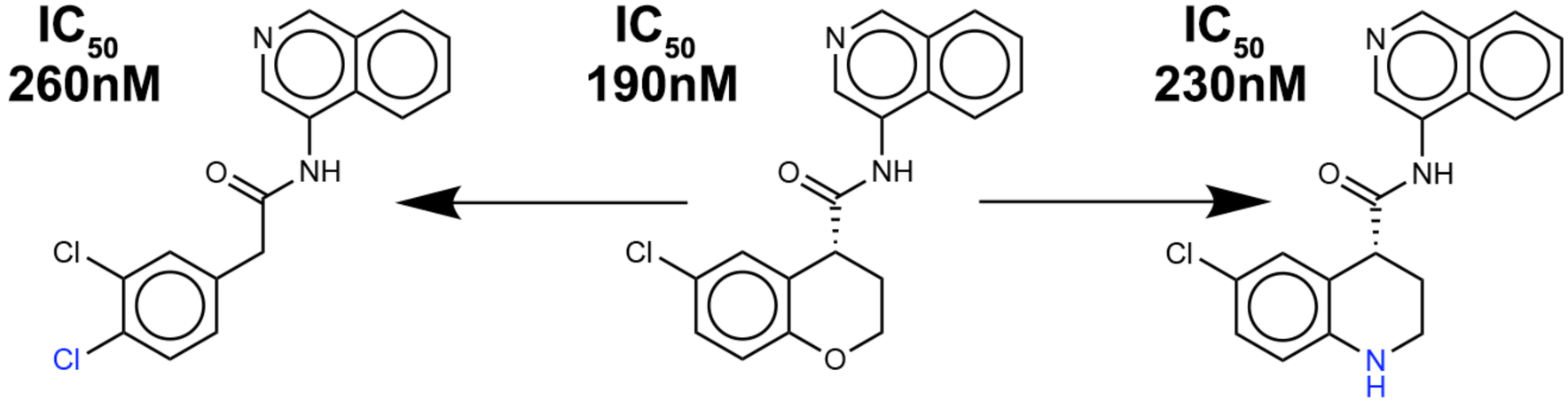

computer

humans

# Good SAR during lead optimization points the way toward meeting our goals for selecting a clinical candidate

**Solubility**

**189 µM** | **33 µM** | **94 µM (racemate)**

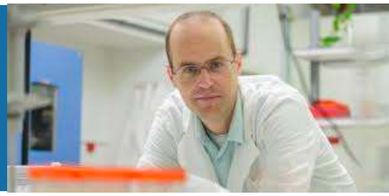



**103.3 min** | **14.1 min.** | **94.8 min**

**Human Liver Microsomes ($t_{1/2}$)**

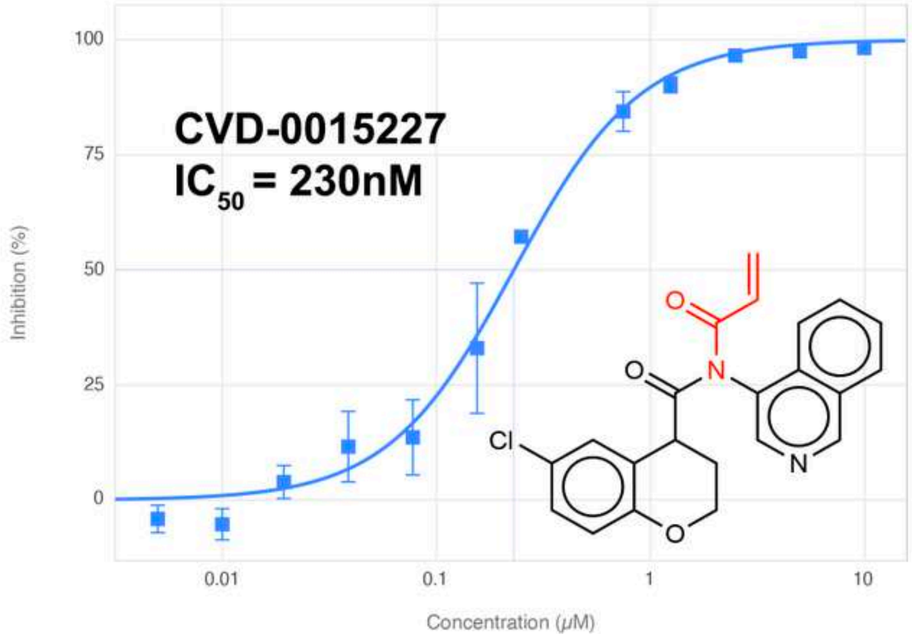
http://postera.ai/covid

# Lead series is well-poised for covalentization

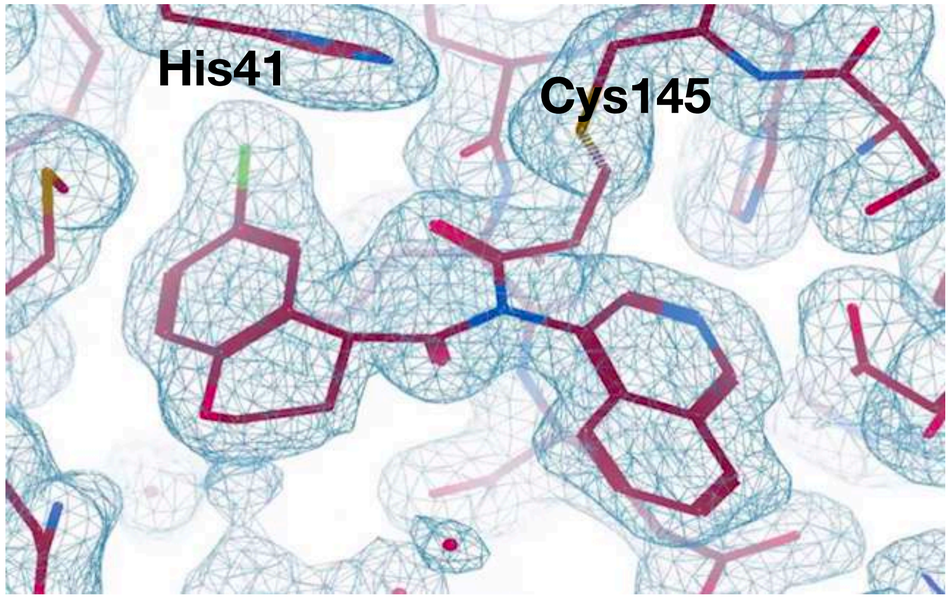


**Nir London**
**Weizmann Institute**

MAT-POS-090737b9-1




**Vladas Oleinkovas, UCB**
**Matt Robinson, PostEra**




**Diamond Light Source / XChem**
**Daeron Fearon**

http://postera.ai/covid

# We aim to nominate a clinical candidate in Mar 2021

**Goal**: new potent antiviral: therapeutic & prophylactic

- *simple synthesis*
- *orally available*
- *pharmacologically behaved*
- *pre-clinically safe*

**Strategy**: work fully open to ensure rapid global availability

- *no IP encumbrance*
- *generic drug*
- *assays/structures/discussions: http://postera.ai/covid*
- *protocols: https://doi.org/10.1101/2020.10.29.339317*

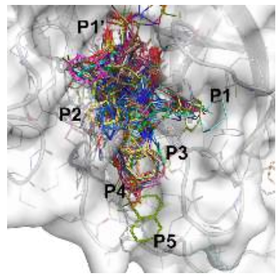
*April* — *Sept* — *Dec* — *Mar '21*

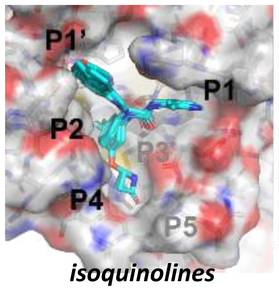
***XChem & data release***

**fragment-to-lead**
*$350k, 30 groups*

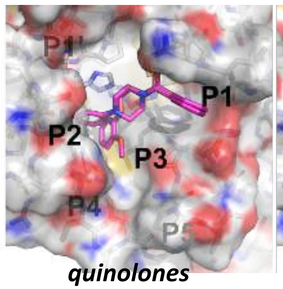
**lead optimization**
*$400k, 40 groups*

**toxicity, pre-clinical**
*$1.5-2m, 50 groups*

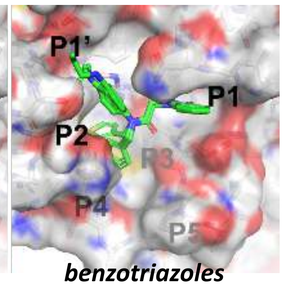


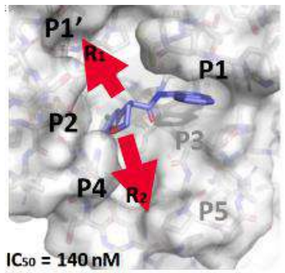

***isoquinolines***




***quinolones***




***benzotriazoles***




6 months:
*3 lead series*
*100nM enzyme inhibition*
*cellular antiviral activity*
*(some philanthropic funding)*

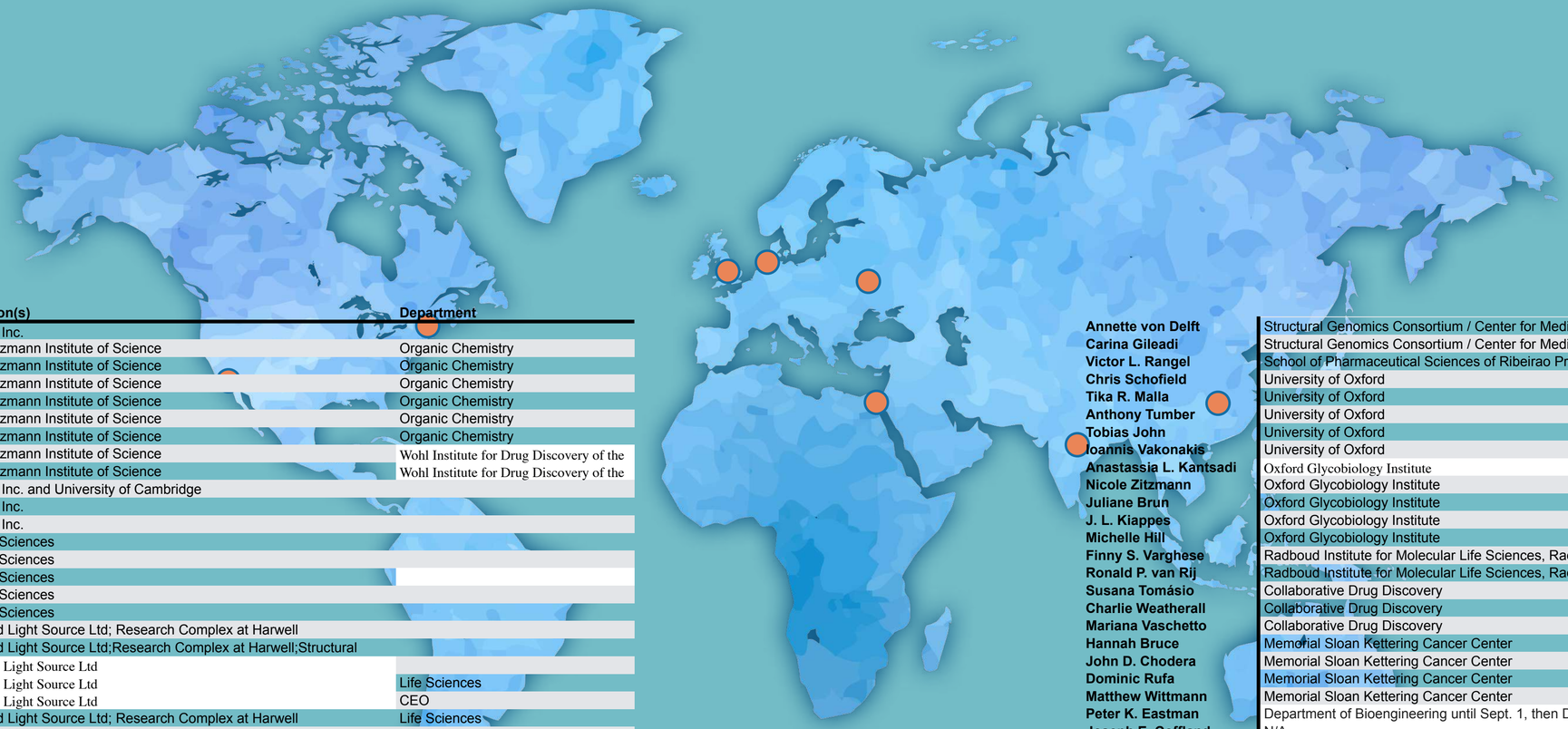
achieved:
*oral availability*
*antiviral $IC_{50}$ <1μM*
*protease selectivity*

improving:
*potency*
*solubility*
*metabolic stability*

seeking:
***critical mass funding***
*partners (curr: charity, gov)*
*formulation & manufacturing*
*clinical trials*

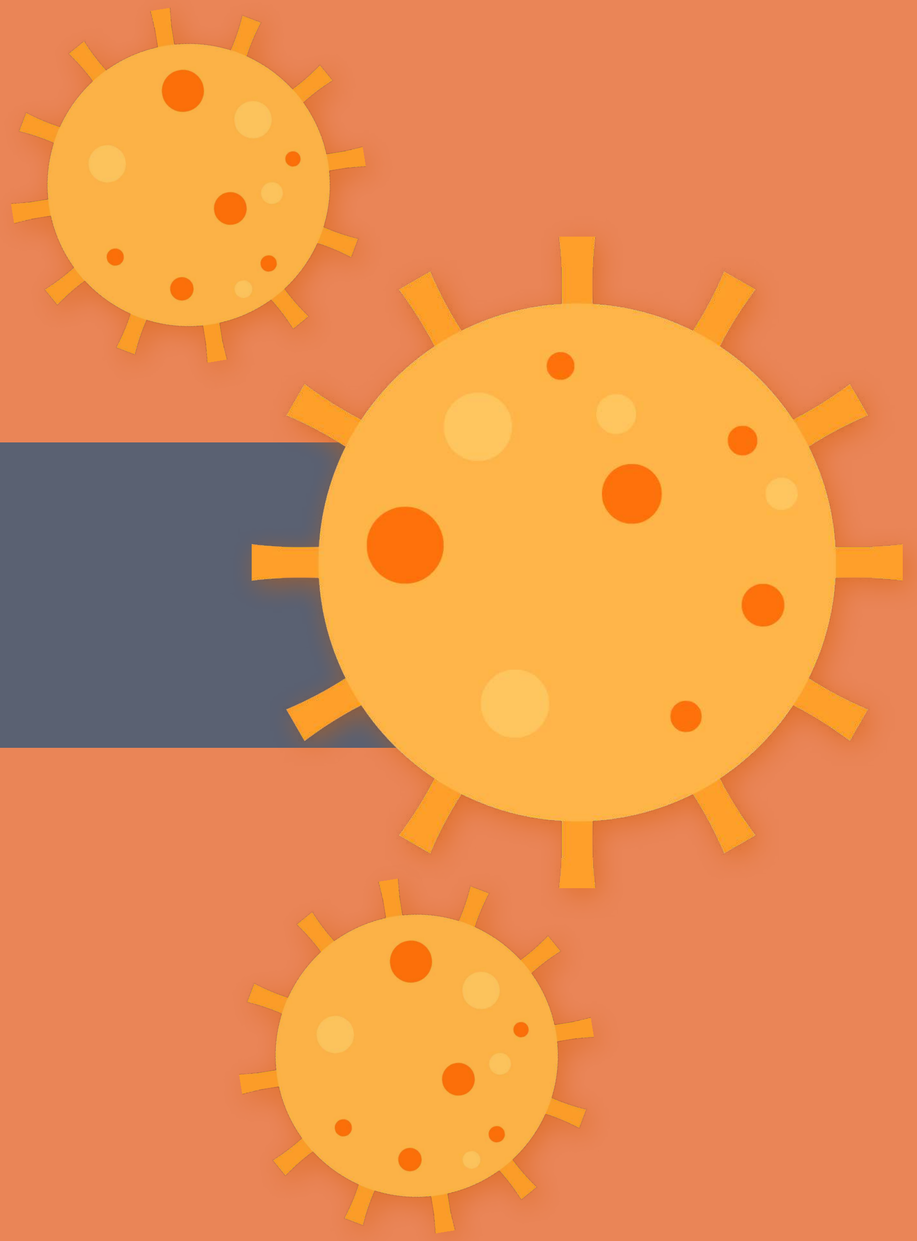
**http://postera.ai/covid**

# The COVID Moonshot collaboration is worldwide

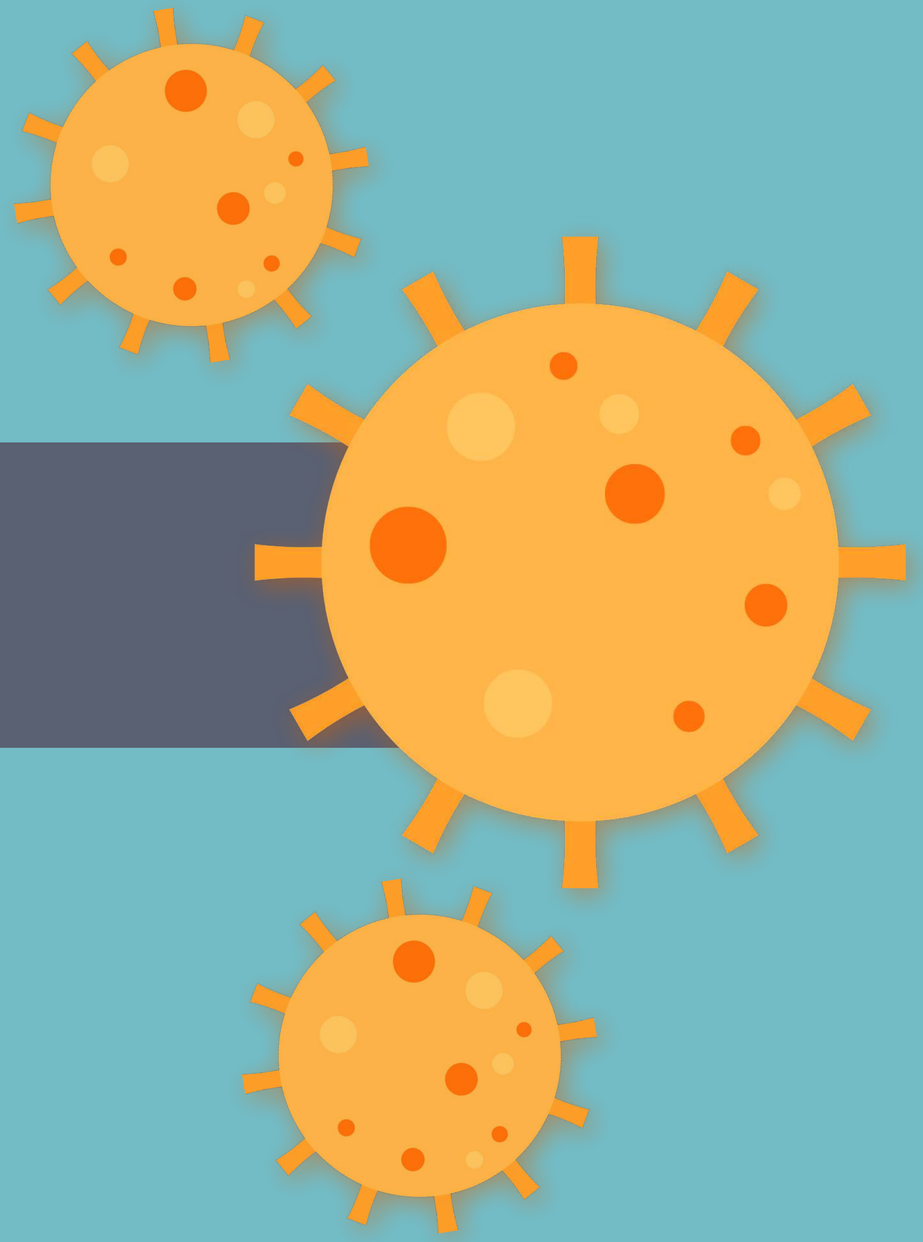


| Name | Institution(s) | Department |
|---|---|---|
| Matthew C. Robinson | PostEra Inc. | |
| Nir London | The Weizmann Institute of Science | Organic Chemistry |
| Efrat Resnick | The Weizmann Institute of Science | Organic Chemistry |
| Daniel Zaidmann | The Weizmann Institute of Science | Organic Chemistry |
| Paul Gehrtz | The Weizmann Institute of Science | Organic Chemistry |
| Rambabu N. Reddi | The Weizmann Institute of Science | Organic Chemistry |
| Ronen Gabizon | The Weizmann Institute of Science | Organic Chemistry |
| Haim Barr | The Weizmann Institute of Science | Wohl Institute for Drug Discovery of the |
| Shirly Valter | The Weizmann Institute of Science | Wohl Institute for Drug Discovery of the |
| Alpha Lee | PostEra Inc. and University of Cambridge | |
| Andrew Jajack | PostEra Inc. | |
| Milan Cvitkovic | PostEra Inc. | |
| Aarif Shaikh | Sai Life Sciences | |
| Iakir Piniari | Sai Life Sciences | |
| Vishwanath Swamy | Sai Life Sciences | |
| Maneesh Pingle | Sai Life Sciences | |
| Sarma BVNBS | Sai Life Sciences | |
| Anthony Aimon | Diamond Light Source Ltd; Research Complex at Harwell | |
| Frank von Delft | Diamond Light Source Ltd;Research Complex at Harwell;Structural | |
| Daren Fearon | Diamond Light Source Ltd | |
| Louise Dunnett | Diamond Light Source Ltd | Life Sciences |
| Ailsa Powell | Diamond Light Source Ltd | CEO |
| Jose Brandao Neto | Diamond Light Source Ltd; Research Complex at Harwell | Life Sciences |
| Rachael Skyner | Diamond Light Source Ltd; Research Complex at Harwell | Life Sciences |
| Warren Thompson | Diamond Light Source Ltd | |
| Tyler Gorrie-Stone | Diamond Light Source Ltd; Research Complex at Harwell | Life Sciences |
| Lizbé Koekemoer | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Tobias Krojer | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Mike Fairhead | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Beth MacLean | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Andrew Thompson | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Conor Francis Wild | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Mihaela D. Smilova | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Nathan Wright | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Annette von Delft | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Carina Gileadi | Structural Genomics Consortium / Center for Medicines Discovery | Nuffield Department of Medicine |
| Victor L. Rangel | School of Pharmaceutical Sciences of Ribeirao Preto | Pharmaceutical Sciences |
| Chris Schofield | University of Oxford | Department of Chemistry |
| Tika R. Malla | University of Oxford | Department of Chemistry |
| Anthony Tumber | University of Oxford | Department of Chemistry |
| Tobias John | University of Oxford | Department of Chemistry |
| Ioannis Vakonakis | University of Oxford | Department of Biochemistry |
| Anastassia L. Kantsadi | Oxford Glycobiology Institute | Department of Biochemistry, |
| Nicole Zitzmann | Oxford Glycobiology Institute | Department of Biochemistry, |
| Juliane Brun | Oxford Glycobiology Institute | Department of Biochemistry, |
| J. L. Kiappes | Oxford Glycobiology Institute | Department of Biochemistry, |
| Michelle Hill | Oxford Glycobiology Institute | department of Biochemistry, |
| Finny S. Varghese | Radboud Institute for Molecular Life Sciences, Radboud University | Department of Medical Microbiology |
| Ronald P. van Rij | Radboud Institute for Molecular Life Sciences, Radboud University | Department of Medical Microbiology |
| Susana Tomásio | Collaborative Drug Discovery | |
| Charlie Weatherall | Collaborative Drug Discovery | |
| Mariana Vaschetto | Collaborative Drug Discovery | |
| Hannah Bruce | Memorial Sloan Kettering Cancer Center | Computational and Systems Biology |
| John D. Chodera | Memorial Sloan Kettering Cancer Center | Computational and Systems Biology |
| Dominic Rufa | Memorial Sloan Kettering Cancer Center | Computational and Systems Biology |
| Matthew Wittmann | Memorial Sloan Kettering Cancer Center | Computational and Systems Biology |
| Peter K. Eastman | Department of Bioengineering until Sept. 1, then Department of | Stanford University |
| Joseph E. Coffland | N/A | Cauldron Development LLC |
| Ed J. Griffen | MedChemica Ltd | Research and Development |
| Willam McCorkindale | University of Cambridge | |
| Aaron Morris | PostEra Inc | CEO |
| Robert Glen | University of Cambridge | Department of Chemistry |
| Jason Cole | Cambridge Crystallographic Datacentre | |
| Richard Foster | University of Leeds | School of Chemistry |
| Holly Foster | University of Leeds | School of Chemistry |
| Mark Calmiano | UCB | |
| Jag Heer | UCB | |
| Jiye Shi | UCB | |
| Eric Jnoff | UCB | |
| Matthew F.D. Hurley | Temple University | Department of Chemistry |

# THANK YOU!



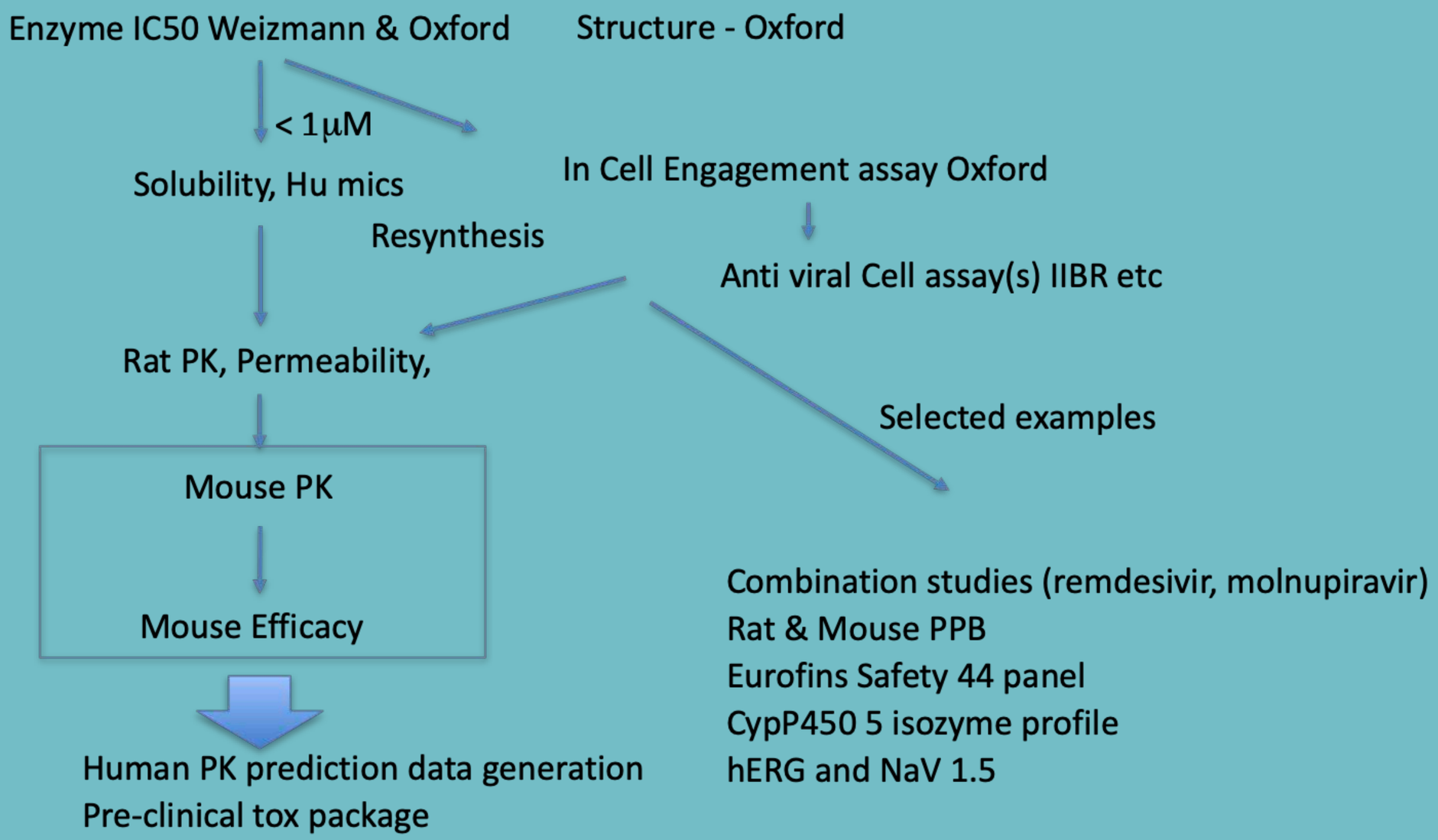
# BACKUP SLIDES



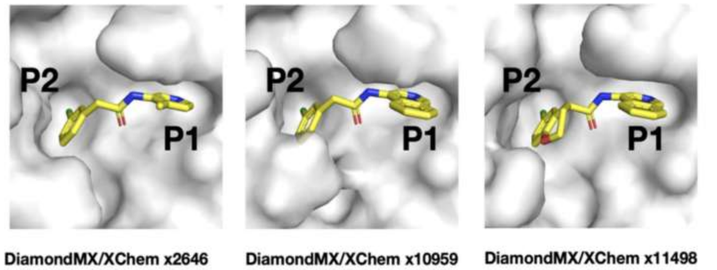
# Current TPP for oral Mpro inhibitor

| Property | Target range | Rationale | |
|---|---|---|---|
| Protease assay | $IC_{50}$ < 50 nM (compromise if clean and anti viral activity sufficient) | Extrapolation from other anti-viral programs | 105 nM |
| Viral replication | $EC_{50}$ < 0.2µM (Vero-E6, and Calu-3) | Suppression of virus at achievable blood levels | 0.4-1 µM |
| Plaque reduction | $EC_{50}$ < 0.2µM (Vero-E6, and Calu-3) | Suppression of virus at achievable blood levels | in progress |
| PK-PD | Cmin > EC90(plaque reduction) for 24h | Assume constant suppression of viral replication | |
| Coronavirus spectrum | SARS-CoV2 B1.1.7 , B.1.1.248 variants essential, SARS-CoV1 & MERS desirable | Treat vaccine resistant variants and future pandemic preparation. | oral exposure observed |
| Route of administration | oral | bid/tid(qid)- compromise PK for potency if pharmacodynamic effect achieved | |
| Solubility | > 5 mg/mL | Aim for biopharmaceutical class 1 assuming <= 750 mg dose | < 1 mg/mL |
| Half-life | Ideally>= 8 h (human) estimated from rat and dog PK | Assume PK/PD requires continuous cover over viral replication for 24 h | rat 2h |
| Safety | No significant protease activity > 50% at 10µM (Nanosyn 61 protease panel)<br>Only reversible and monitorable toxicities (NOAEL > 30x Cmax)<br>No significant DDI - clean in 5 CYP450 isoforms<br>hERG and NaV1.5 $IC_{50}$ > 50 µM<br>No significant change in QTc<br>Ames negative<br>No mutagenicity or teratogenicity risk | No significant toxicological delays to development<br>Avoid DDI to support co-morbidities & combination therapy,<br>Critical cardiac safety for COVID-19 risk profile<br>Low carcinogenicity risk reduces delays in manufacturing<br>Patient group will include significant proportion of women of childbearing age | clean protease panel<br>live phase planned<br>CYP450s in progress<br>cardiotoxicity in vivo testing planned<br>Ames planned |

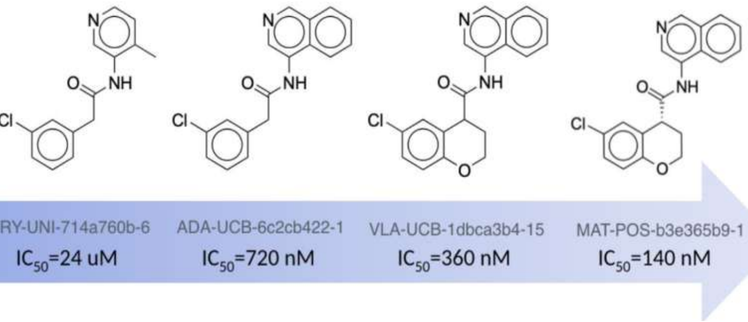
# Critical path for assay cascade



# Primary series: Aminopyridines




DiamondMX/XChem x2646 DiamondMX/XChem x10959 DiamondMX/XChem x11498




| Assay | Type | August | December | December | TPP goal |
|---|---|---|---|---|---|
| **Tier 1** | | **JOR-UNI-2fc98d0b-12** | **MAT-POS-b3e365b9-1** | **MAT-POS-53907a1c-3** | |
| Mpro inhibition (Fluorescence) | IC50 | 3.1 µM | 141 nM | 58 nM | <50 nM |
| Mpro inhibition (RapidFire) | IC50 | 3.3 µM | 257 nM | | <50 nM |
| thermodynamic solubility | solubility | | 34 µM | | >10 µM |
| plasma protein binding | fraction unbound | | 12±2% unbound | | >1% unbound |
| **Tier 2** | | | | | |
| VeroE6 antiviral activity (CPE) | IC50 | | 1.57 µM | | <5 µM |
| VeroE6 antiviral activity (qPCR) | IC50 | 7.31 µM | 2.63 µM | | <5 µM |
| VeroE6 cytotoxicity | CC50 | 25.5 µM | >500 µM | | >100 µM |
| A549 cytotoxicity | CC50 | 14.1 µM | >100 µM | | >100 µM |
| Calu-3 cytotoxicity | CC50 | 18.2 µM | >100 µM | | >100 µM |
| protease selectivity at 100 µM | 40 human protease panel | | <12% | | <40% |
| MDCK-MDR1 | Papp | | 41±1 x10^-6 cm/s | | >10 x10^-6 cm/s |
| human liver microsomal stability | CLint | | 98.3 µg/min/mg protein | | <10 µg/min/mg protein |
| | t 1/2 | | 14.1 min | | >120 min |
| **Tier 3** | | | | | |
| rat oral bioavailability | t 1/2 | | 1 h | | >8 h |

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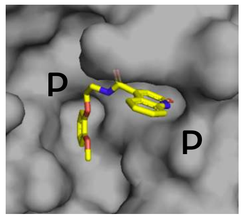
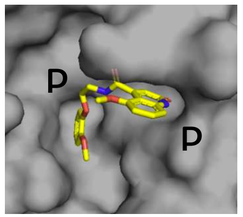
# Backup series 1: Quinolones

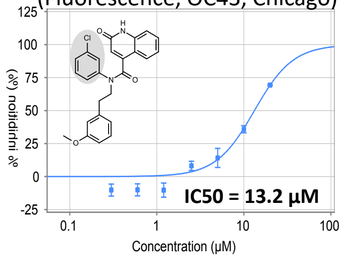


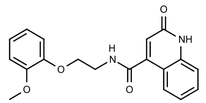

DiamondMX/XChem x2910



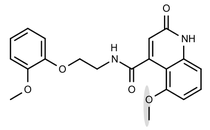

DiamondMX/XChem x11294

ALP-POS-ddb41b15-4
(Fluorescence, OC43, Chicago)

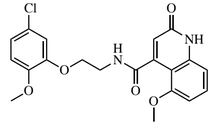


MAT-POS-916a2c5a-2
$IC_{50}$ = 7 μM



EDJ-MED-6af13d92-3
$IC_{50}$ = 2 μM



MAT-POS-3b536971-1
$IC_{50}$ = 870 nM

| Assay | Type | August | December | December | TPP goal |
|---|---|---|---|---|---|
| **Tier 1** | | **MAT-POS-916a2c5a-2** | **EDJ-MED-6af13d92-3** | **MAT-POS-3b536971-1** | |
| Mpro inhibition (Fluorescence) | IC50 | 7.5 μM | 2.03 μM | 870 nM | <50 nM |
| Mpro inhibition (RapidFire) | IC50 | 3.5 μM | 2.08 μM | | <50 nM |
| thermodynamic solubility | solubility | | 84 μM | | >10 μM |
| plasma protein binding | fraction unbound | | 29.5±0.7% unbound | | >1% unbound |
| **Tier 2** | | | | | |
| VeroE6 antiviral activity (fluorescence, OC43) | IC50 | | >20 μM | | <5 μM |
| VeroE6 antiviral activity (CPE) | IC50 | | not active | | <5 μM |
| VeroE6 cytotoxicity | CC50 | | >20 μM | | >100 μM |
| A549 cytotoxicity | CC50 | | >10 μM | | >100 μM |
| Calu-3 cytotoxicity | CC50 | | >100 μM | | >100 μM |
| protease selectivity at 100 μM | 40 human protease panel | | <10% | | <40% |
| MDCK-MDR1 | Papp | | 2.0±0.1 x 10^-6 cm/s | | >10 x 10^-6 cm/s |
| human liver | CLint | | 19.3 μg/min/mg protein | | <10 μg/min/mg protein |
| microsomal stability | t 1/2 | | 71.9 min | | >120 min |
| **Tier 3** | | | | | |
| rat oral bioavailability | t 1/2 | | 43 min | | >8 h |

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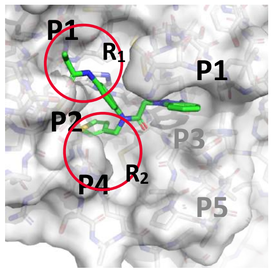
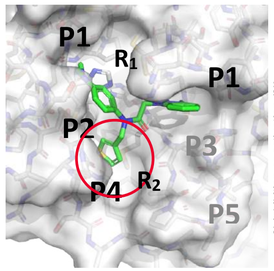
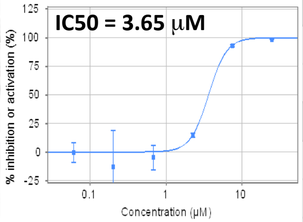
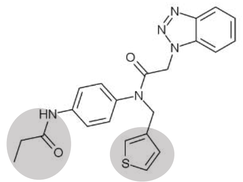
# Backup series 2: Benzopyrans

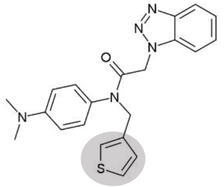


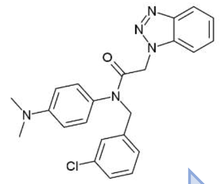




**ALP-POS-c59291d4-2**
(qPCR, SARS-CoV-2, Radboud)







**ALP-POS-c59291d4-2**
IC50 12.56 µM

**ALP-POS-c59291d4-2**
IC50 5.369 µM

**ALP-POS-6d04362c-2**
IC50 0.391 µM

| Assay | Type | August | December | TPP goal |
|---|---|---|---|---|
| **Tier 1** | | **ALP-POS-c59291d4-2** | **ALP-POS-6d04362c-2** | |
| Mpro inhibition (Fluorescence) | IC50 | 1.63 µM | 497 nM | <50 nM |
| Mpro inhibition (RapidFire) | IC50 | 12.6 µM | 391 nM | <50 nM |
| **Tier 2** | | | | |
| VeroE6 antiviral activity (Fluorescence, OC43) | IC50 | >20 µM | | <5 µM |
| VeroE6 antiviral activity (CPE) | IC50 | not active | | <5 µM |
| VeroE6 antiviral activity (CPE) | IC50 | 3.65 µM | | <5 µM |
| VeroE6 cytotoxicity | CC50 | >100 µM | | >100 µM |
| A549 cytotoxicity | CC50 | >20 µM | | >100 µM |
| Calu-3 cytotoxicity | CC50 | >100 µM | | >100 µM |
| protease selectivity at 100 µM | | <35% | | <40% |
| MDCK-MDR1 | Papp | | | >10 x10^-6 cm/s |
| human liver | CLint | 641 µg/min/mg protein | | <10 µg/min/mg protein |
| microsomal stability | t 1/2 | 2.16 min | | >120 min |

http://postera.ai/covid