

Finding new ligands to the SARS CoV 2 Macrodomein

using Fragments, Neutrons, and Entropy

James Fraser
(he/him)
UCSF



**Dr. Galen
Correy**



**Dr. Stefan
Gahbauer**



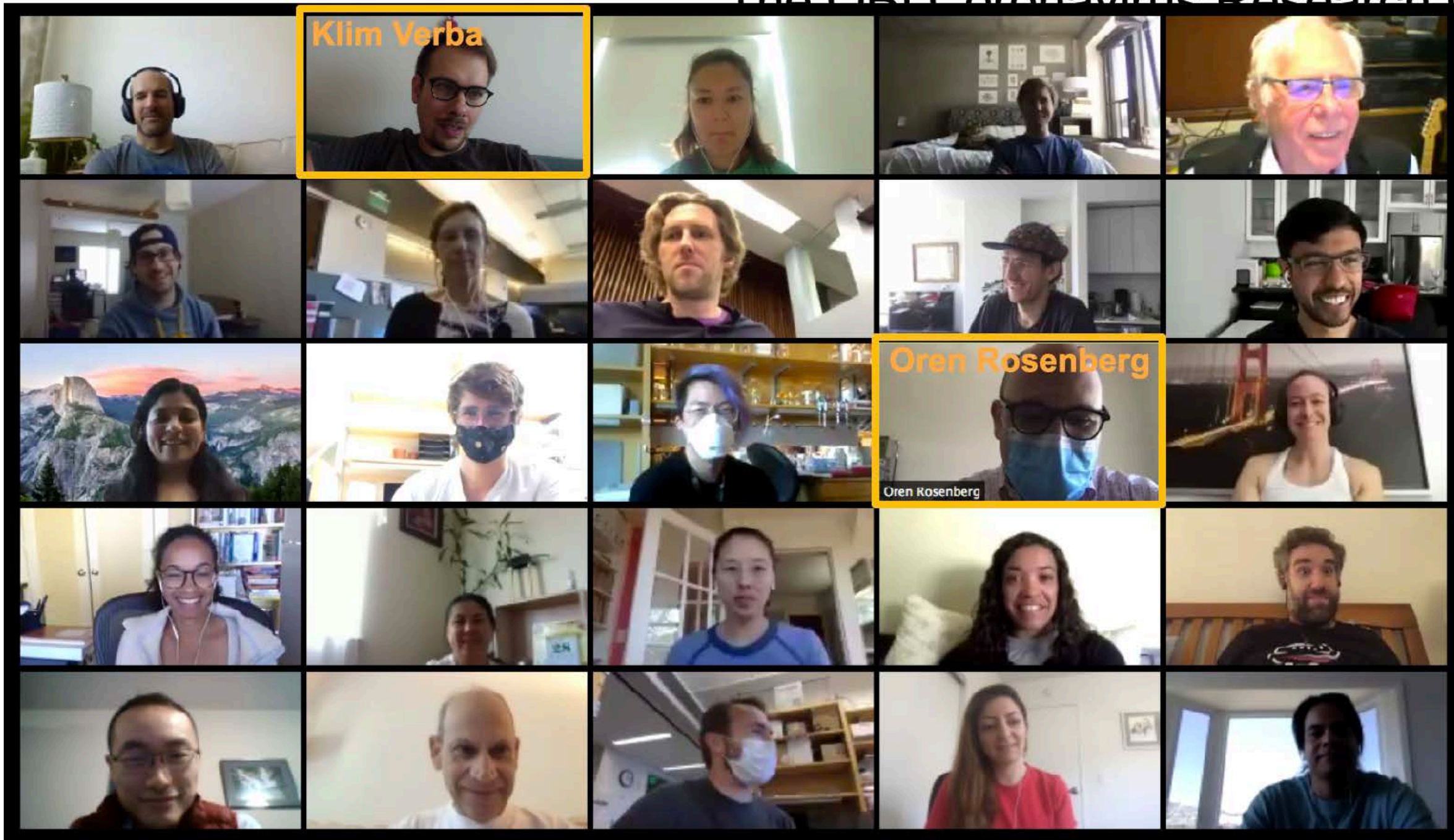
**Dr. Daren
Fearon**



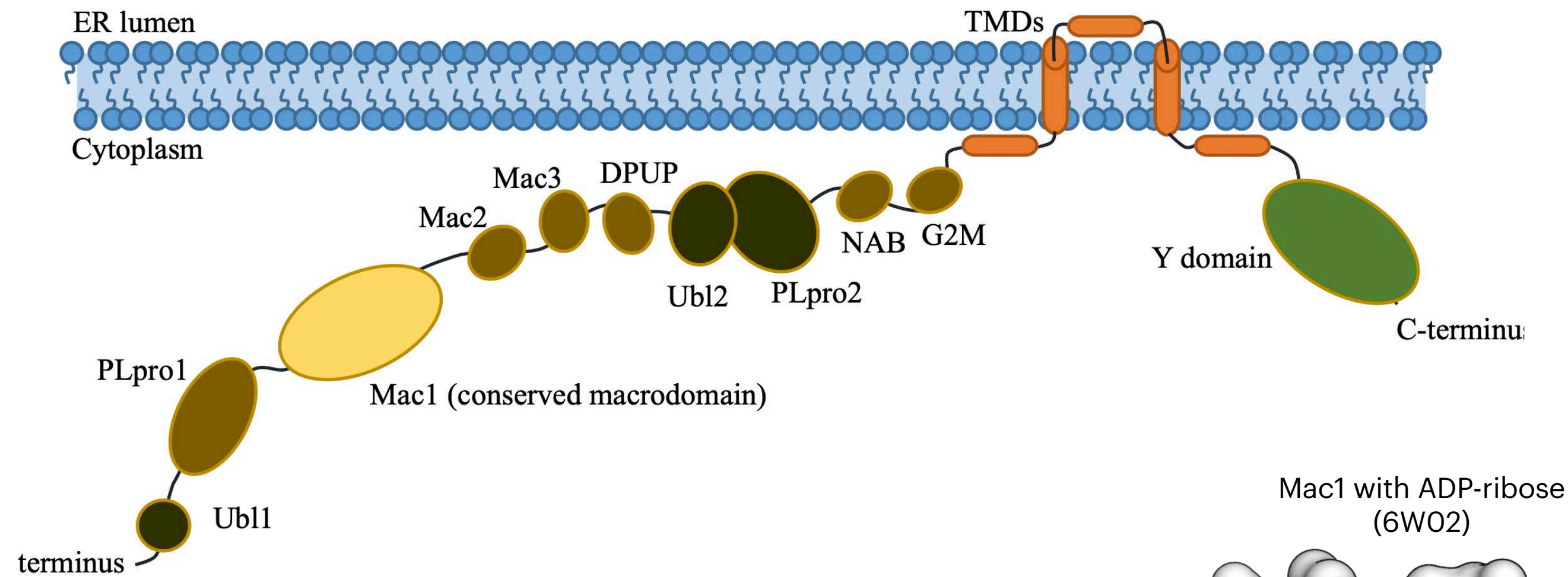
**Dr. Marion
Schuller**



@FRASER_LAB



The macrodomain of NSP3



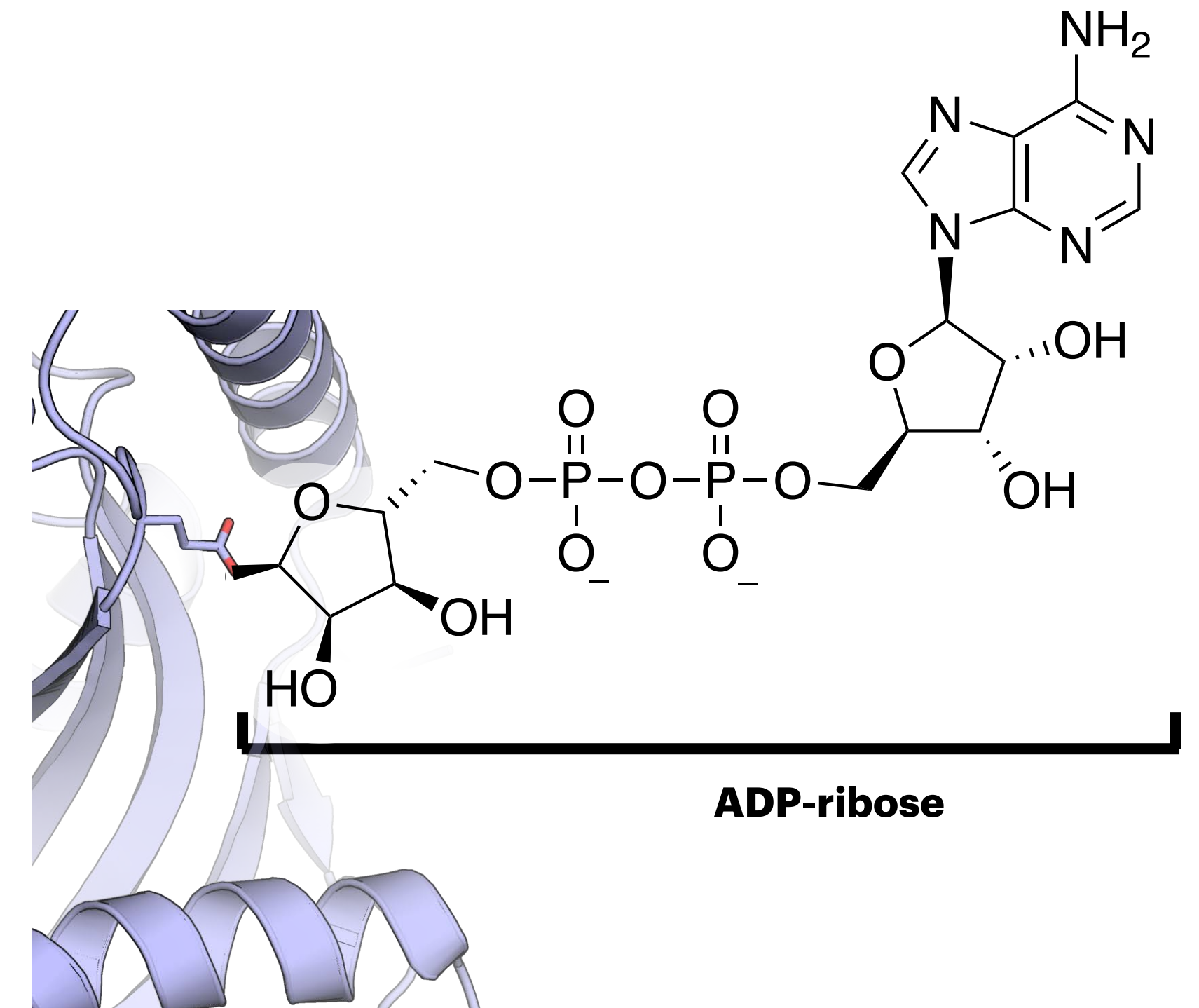
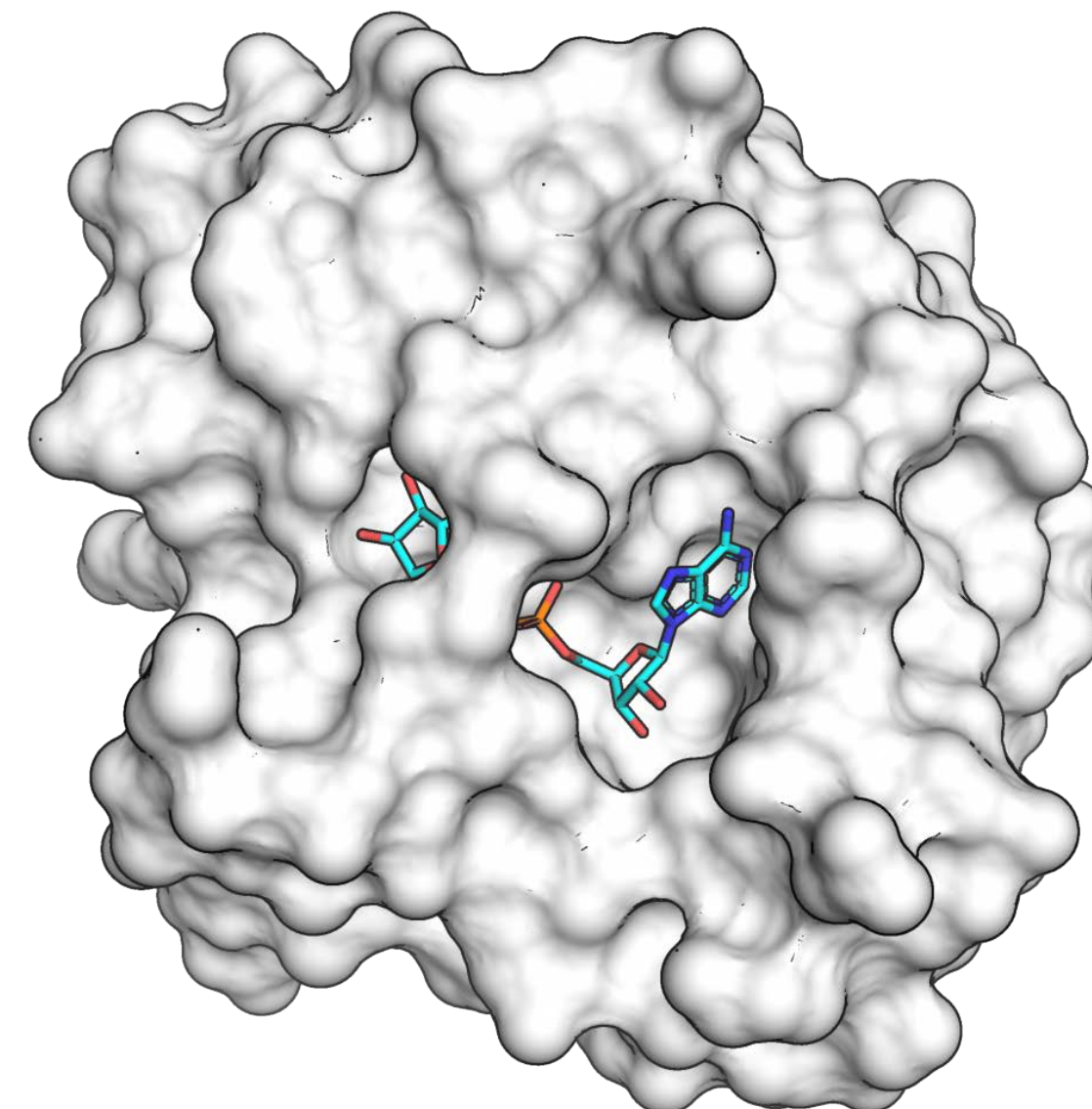
Alhammad... Fehr, J Virology, 2021



Prof. Brian
Shoichet

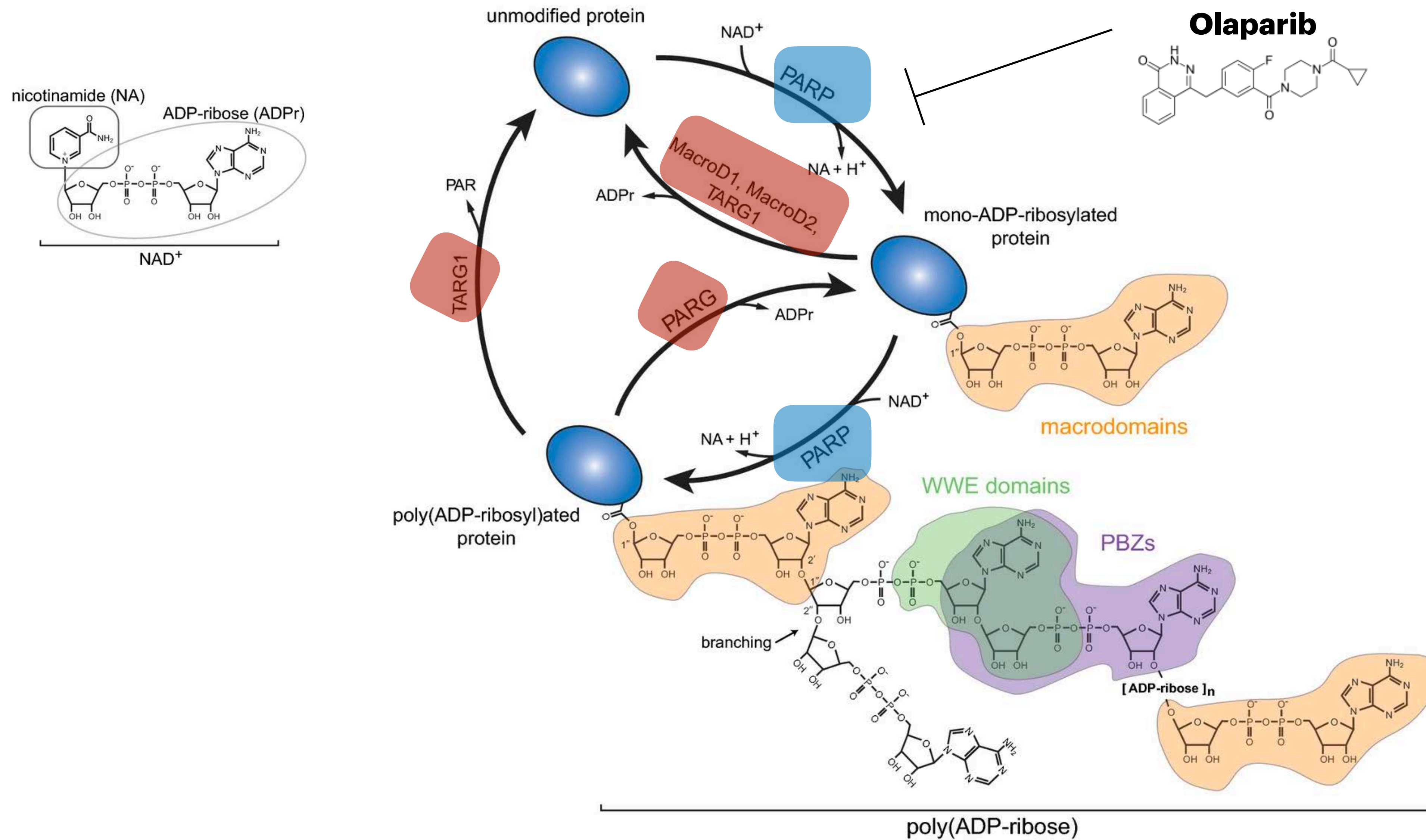


Dr. Stefan
Gahbauer



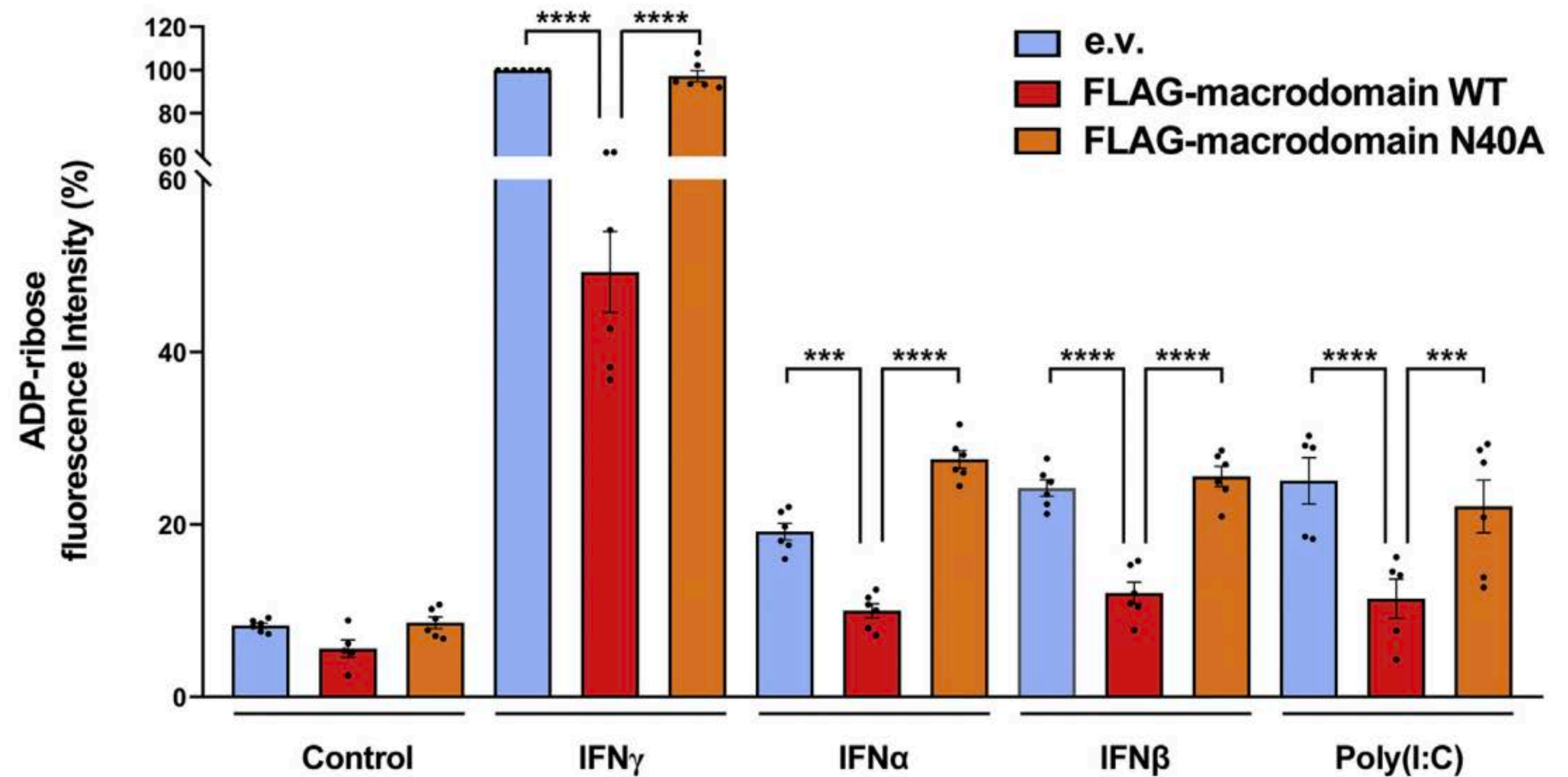
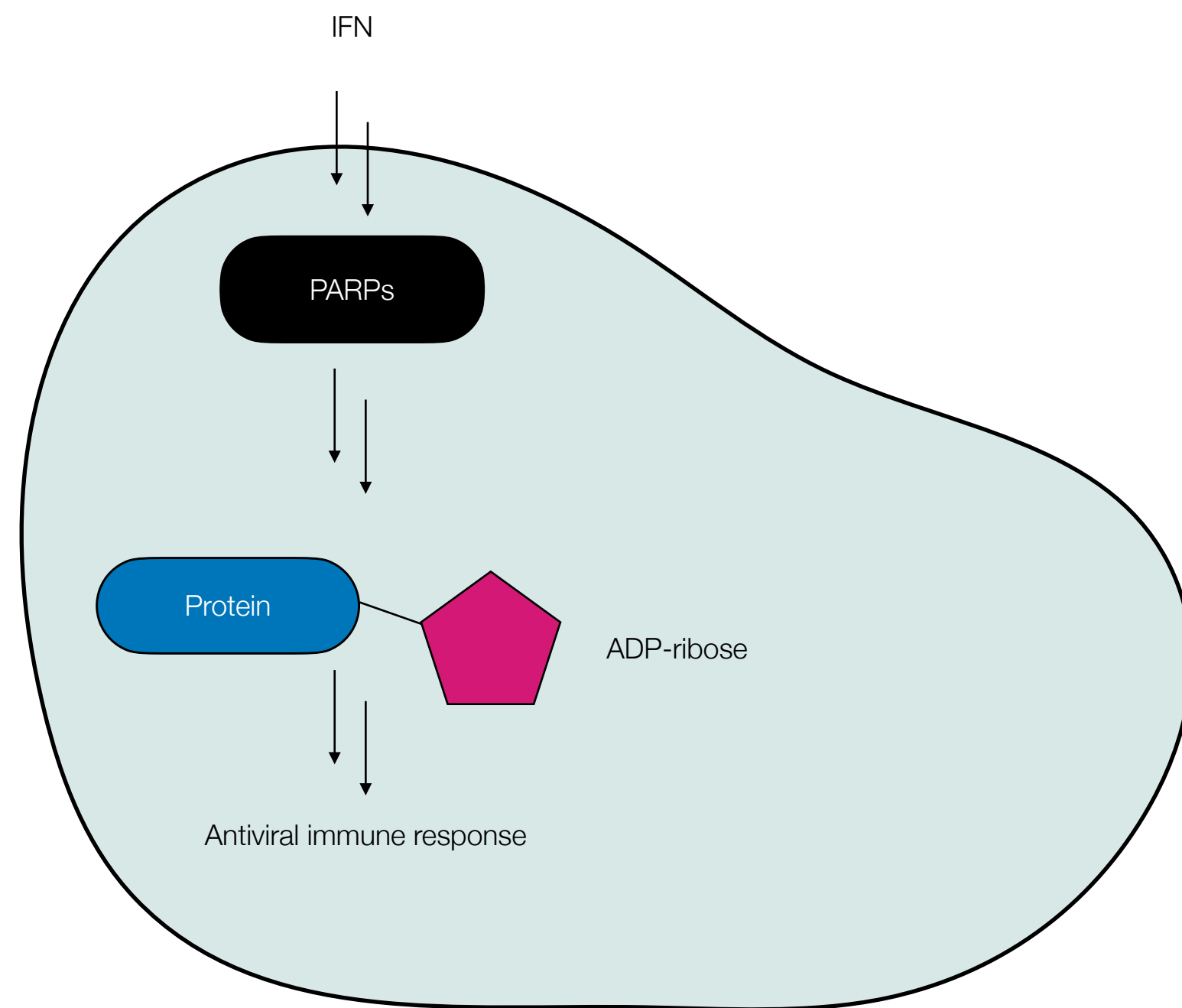
Prof. Alan
Ashworth

ADPr is a complex post-translational modification



Prof. Alan Ashworth

Macrodomain blocks the output of **interferon signaling**



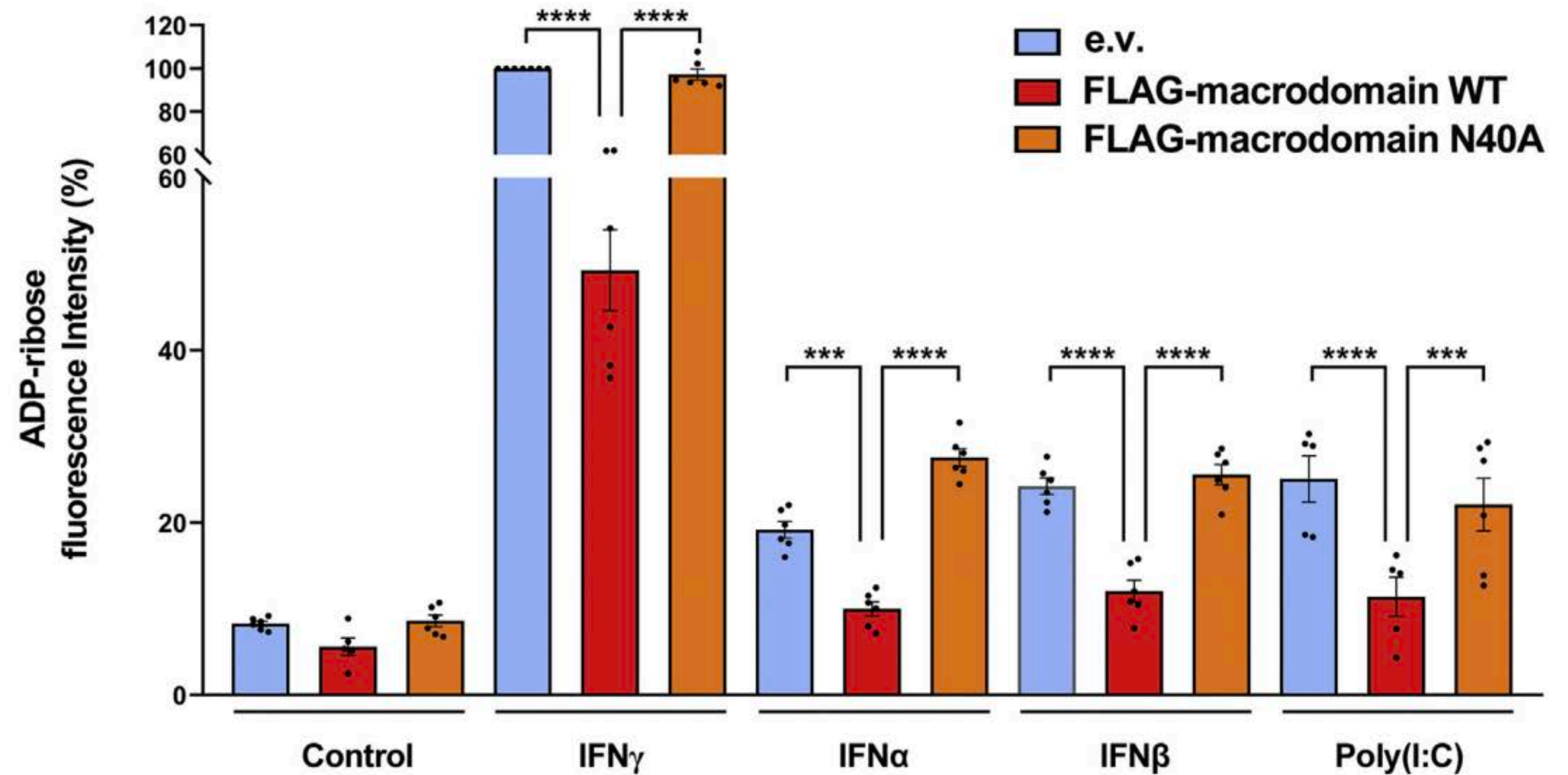
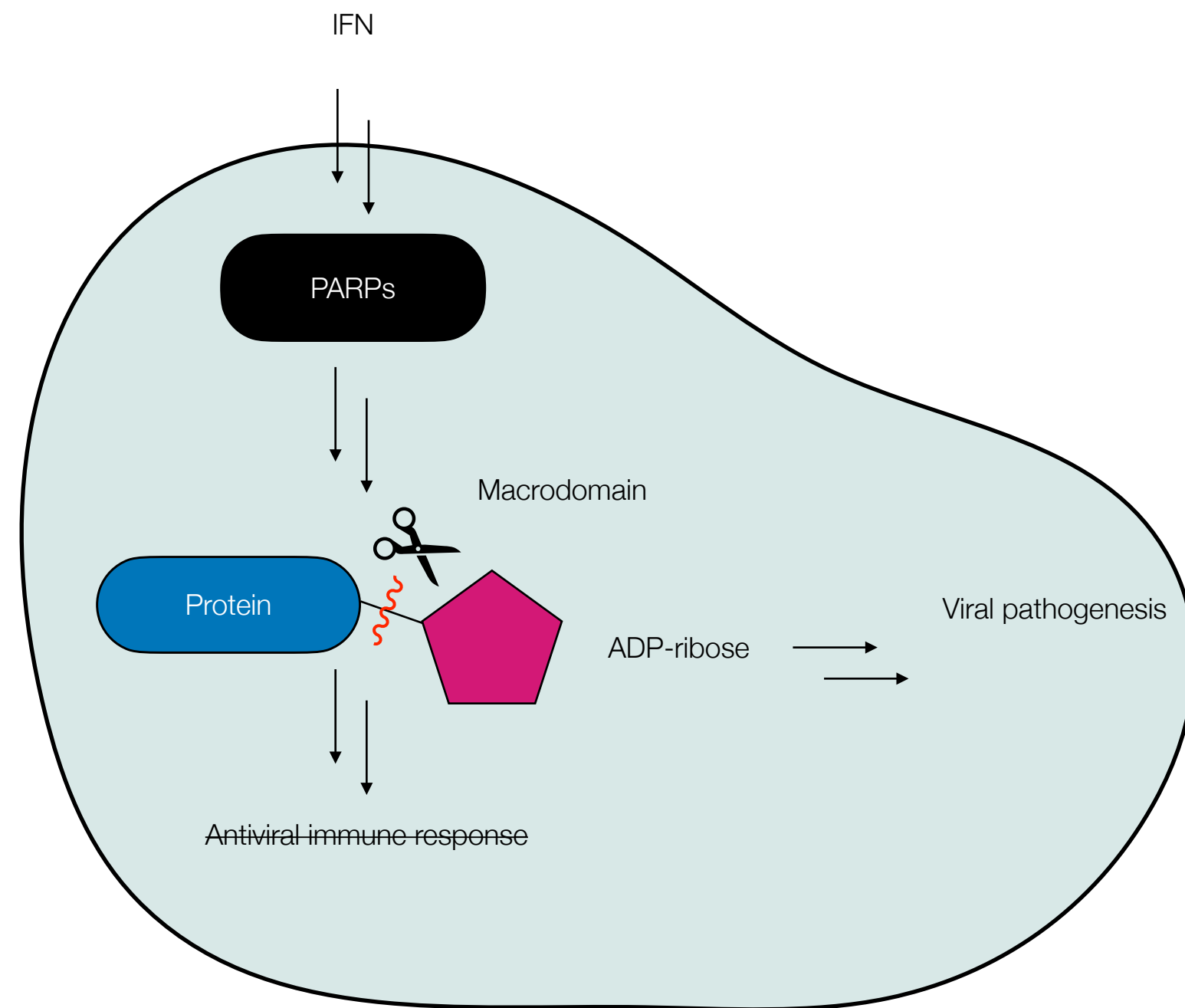
JBC RESEARCH ARTICLE

The SARS-CoV-2 Nsp3 macrodomain reverses PARP9/DTX3L-dependent ADP-ribosylation induced by interferon signaling

Received for publication, May 4, 2021, and in revised form, July 16, 2021. Published, Papers in Press, August 4, 2021, <https://doi.org/10.1016/j.jbc.2021.101041>

Lilian Cristina Russo¹, Rebeka Tomasin¹, Isaac Araújo Matos¹ , Antonio Carlos Manucci¹, Sven T. Sowa² ,
Katie Dale³, Keith W. Caldecott³, Lari Lehtiö², Deborah Schechtman¹, Flavia C. Meotti¹ ,
Alexandre Bruni-Cardoso¹, and Nicolas Carlos Hoch^{1,*} 

Macrodomain blocks the output of interferon signaling



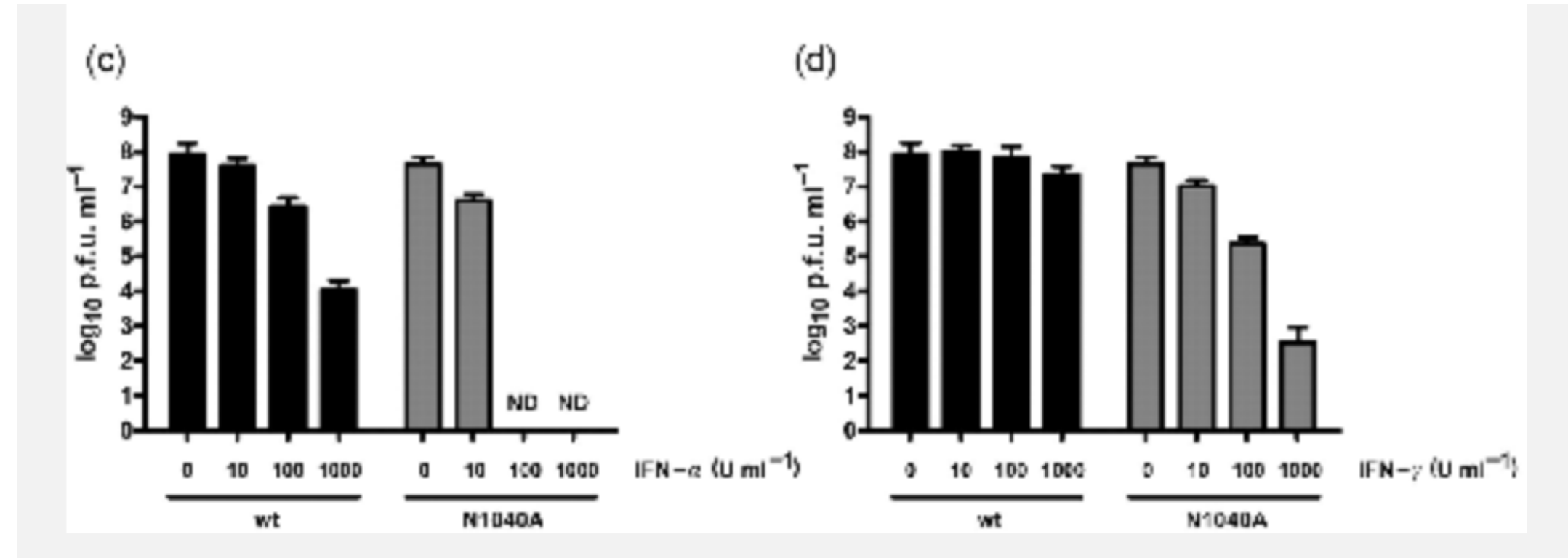
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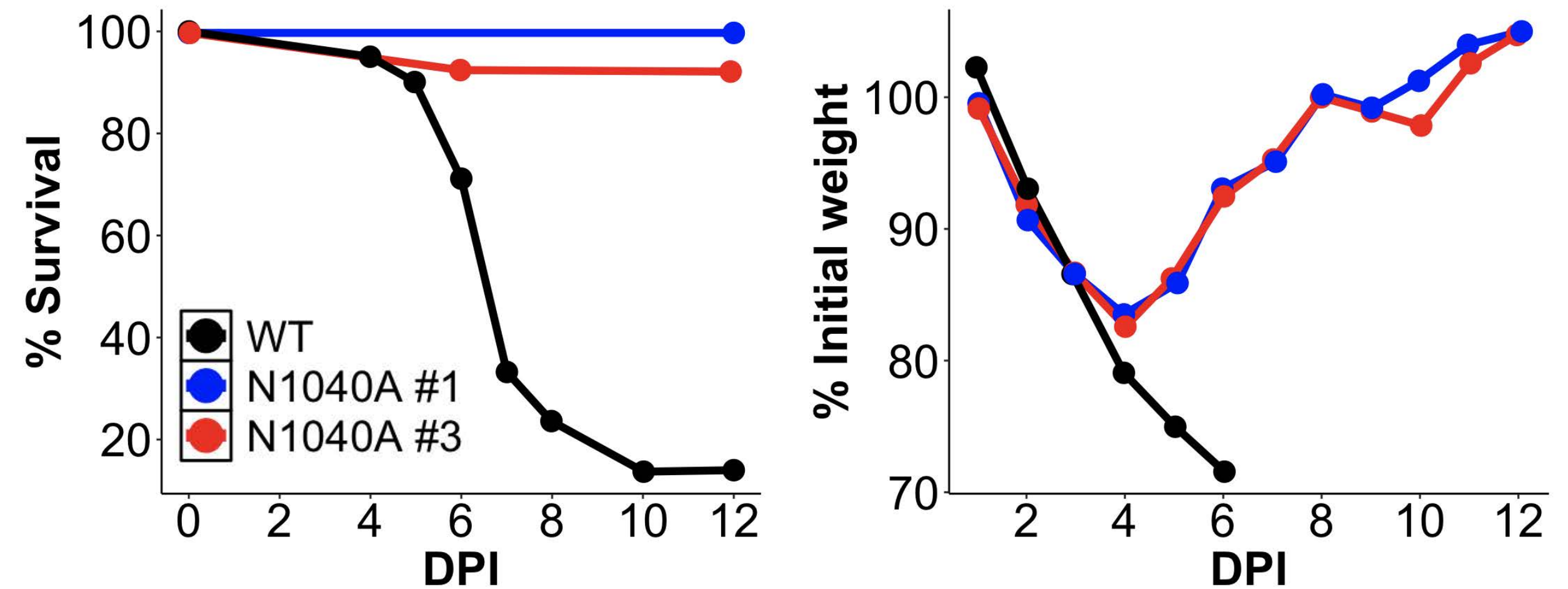
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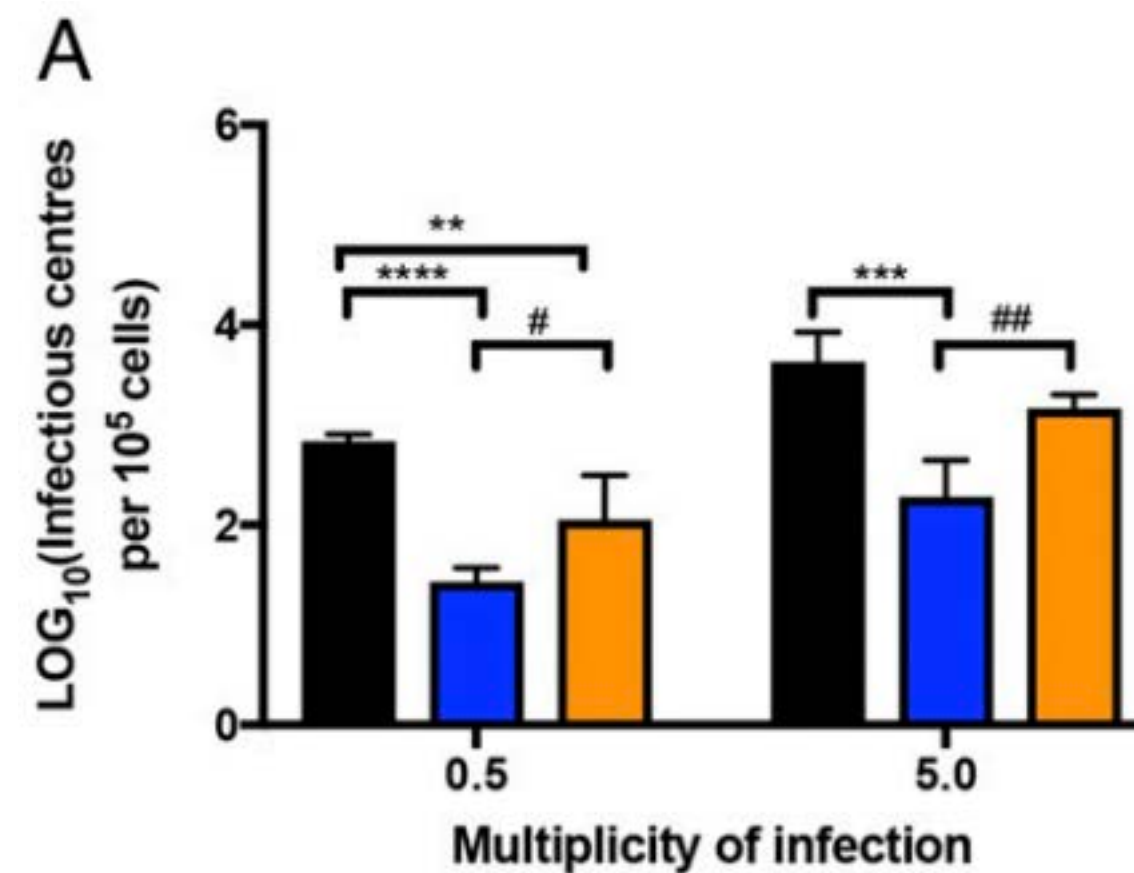
Macrodomain is a **validated target***



Kuri...Weber, J Gen Virology, 2011
SARS 1: Vero E6 +interferon



Fehr...Ahel, Perlman. mBio. 2016
SARS 1: animal model



Abraham...Griffin, PNAS, 2018
CHIKV: NSC34 cells

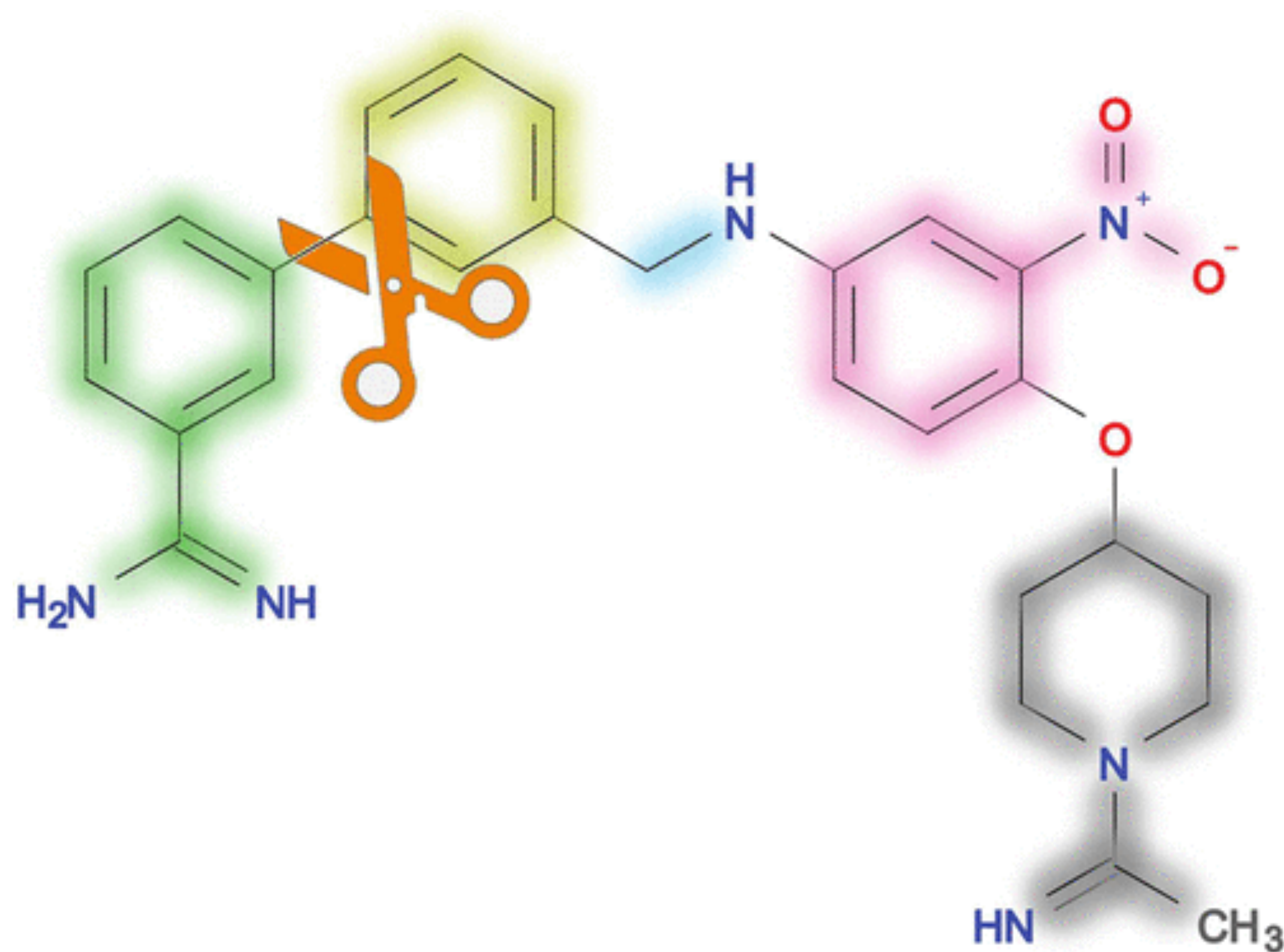
* for SARS-1, CHIKV

Want a chemical tool compound for:

RNAseq, proteomics, viral replication, animal studies, etc

No assays, no chemical matter, **no problem!**

What is a fragment?

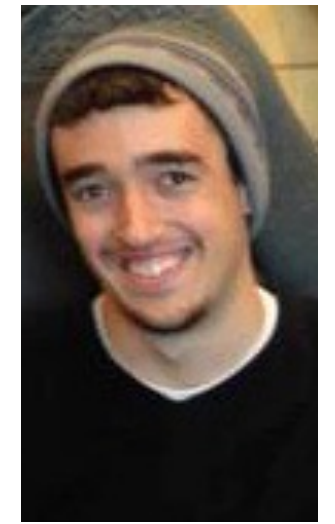


And why use
X-rays?



Prof. Frank
von Delft
(SGC, Diamond)

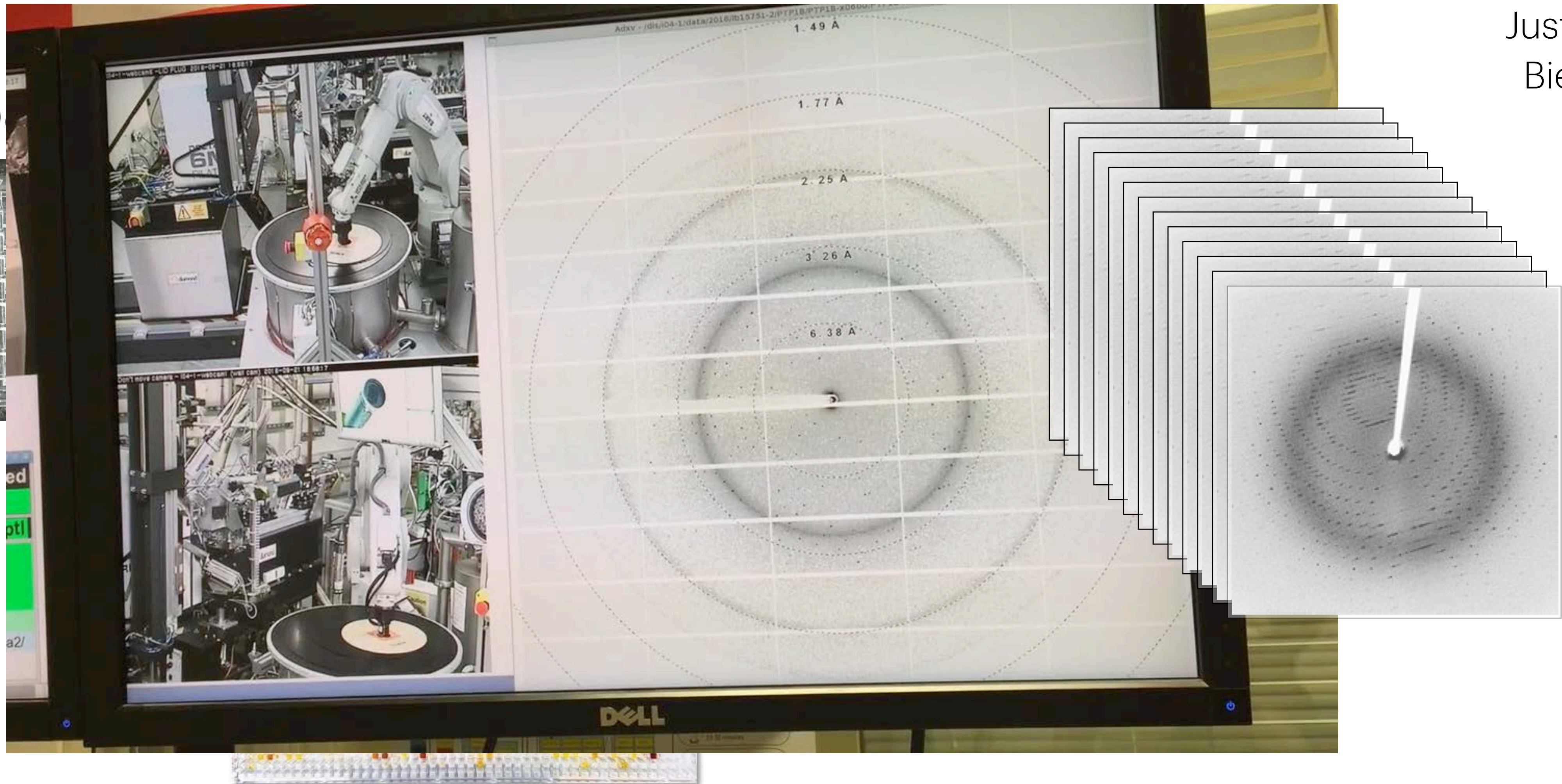
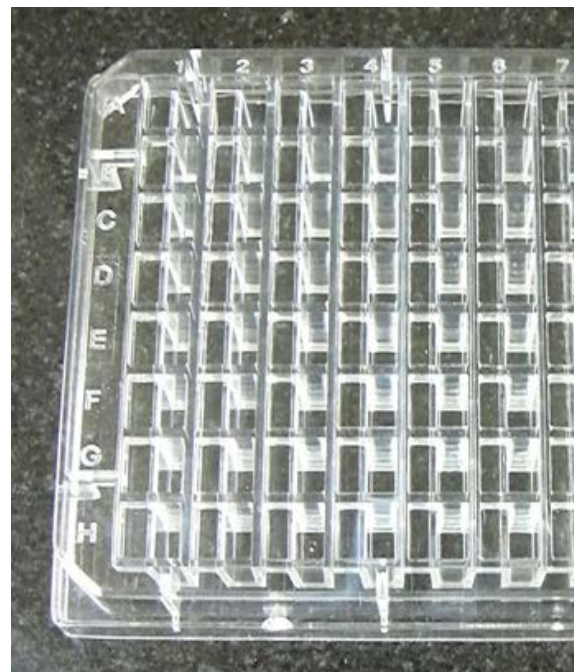
We have been building a **fragment-soaking** pipeline at UCSF modeled after X-Chem (UK)



Justin
Biel



Frank von D



Talking to Frank von Delft - they were going after macrodomain also - **we decided to team up!**



**Dr. Daren
Fearon
(Diamond)**



**Prof. Frank
von Delft
(SGC, Diamond, Oxford)**



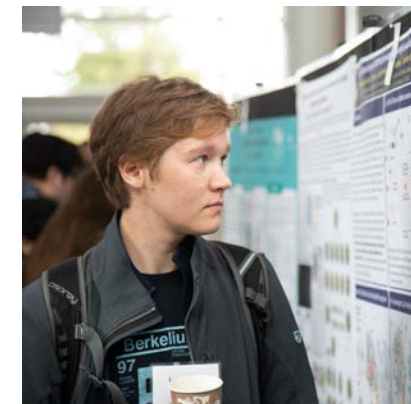
**Prof. Ivan
Ahel (Oxford)**



**Dr. Marion
Schuller (Oxford)**



**Prof. Michael
Thompson
(UC Merced)
(QCRG Structural Biology team!)**



**Dr. Iris
Young**

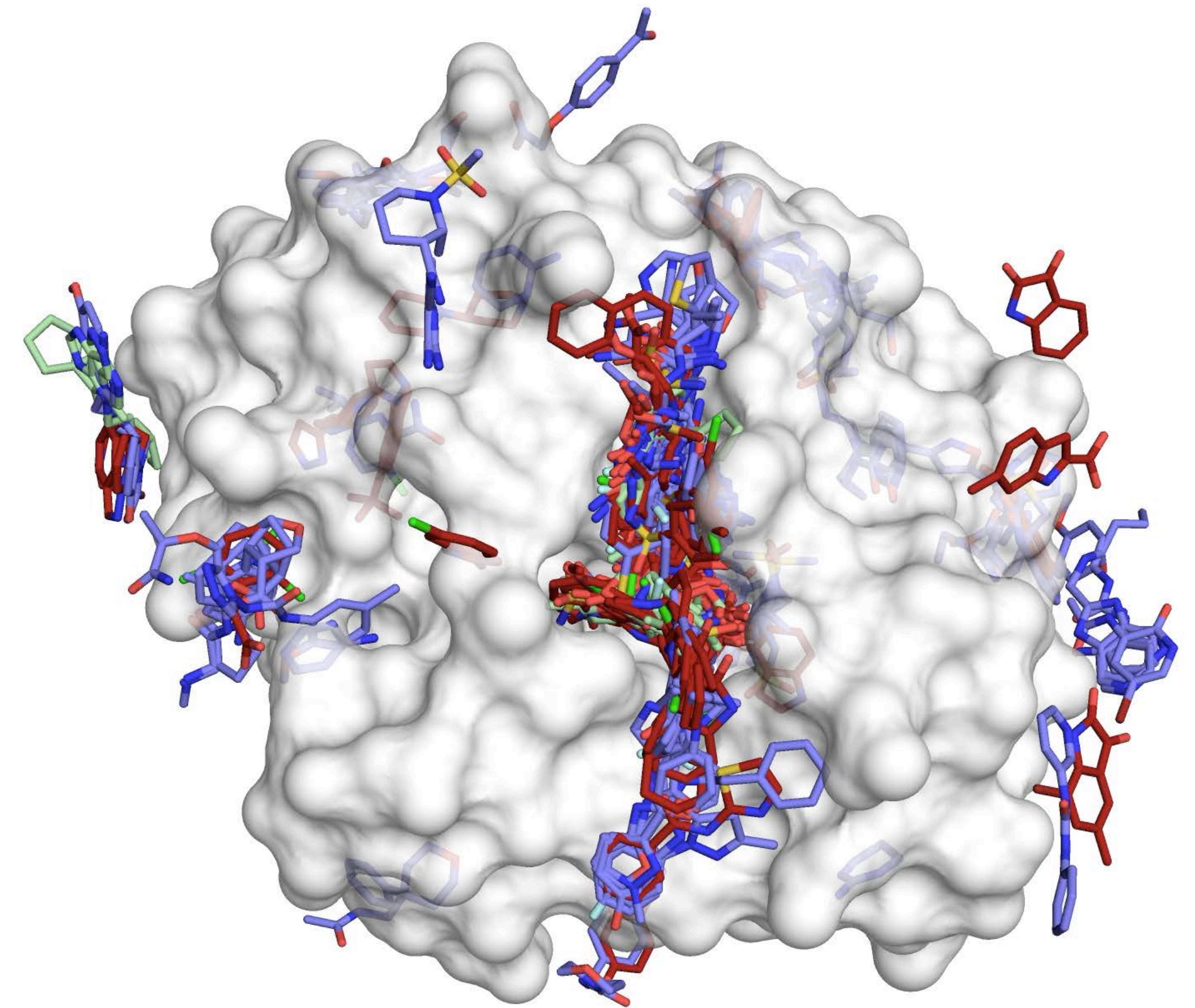
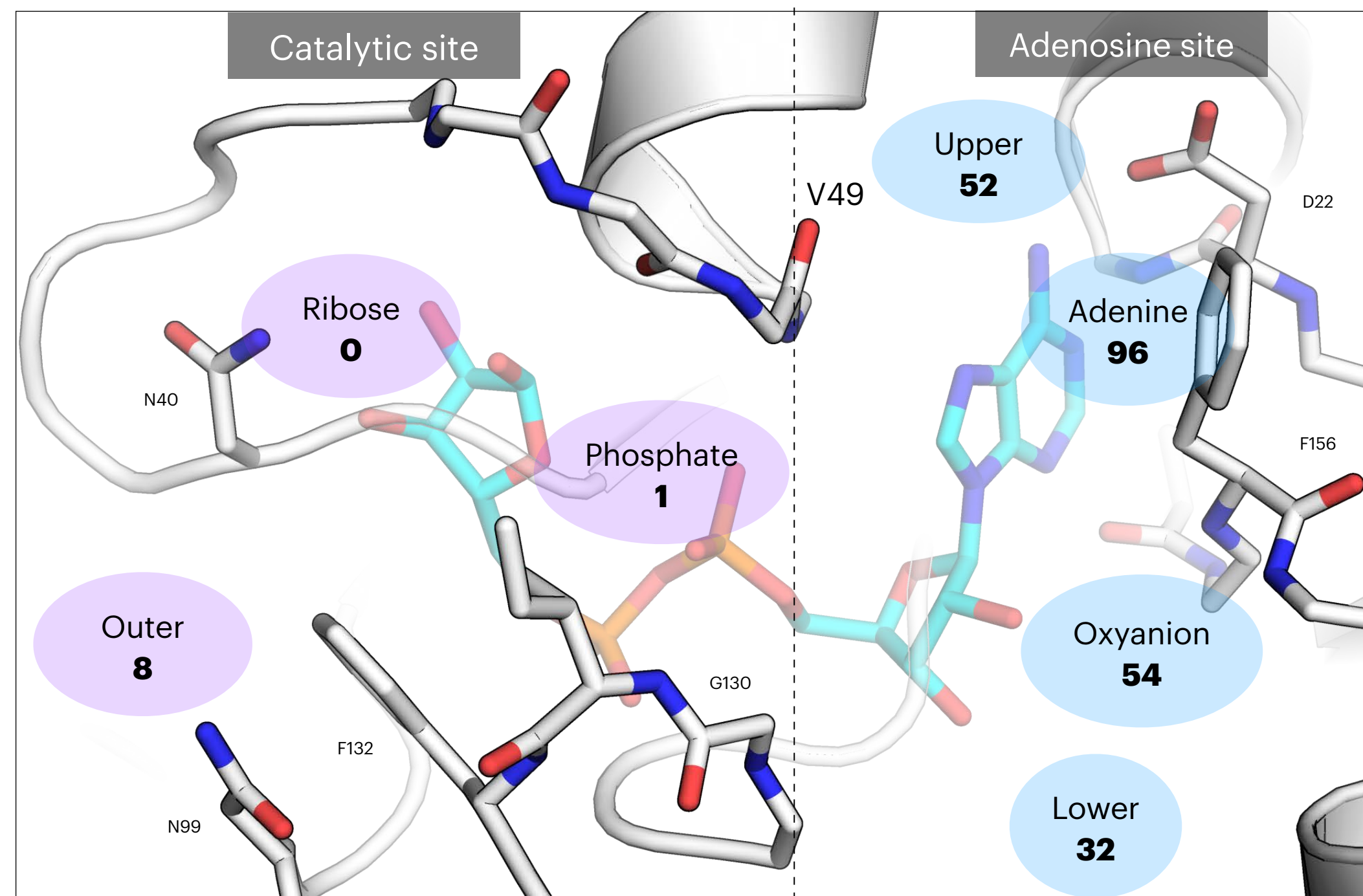


**Dr. Galen
Correy**

ALS: James Holton, George Meigs
SSRL: Aina Cohen, Silvia Russi,
Clyde Smith, Lisa Dunn,
Jeney Wierman
NSLS-II: Martin Fuchs, Alexei Soares

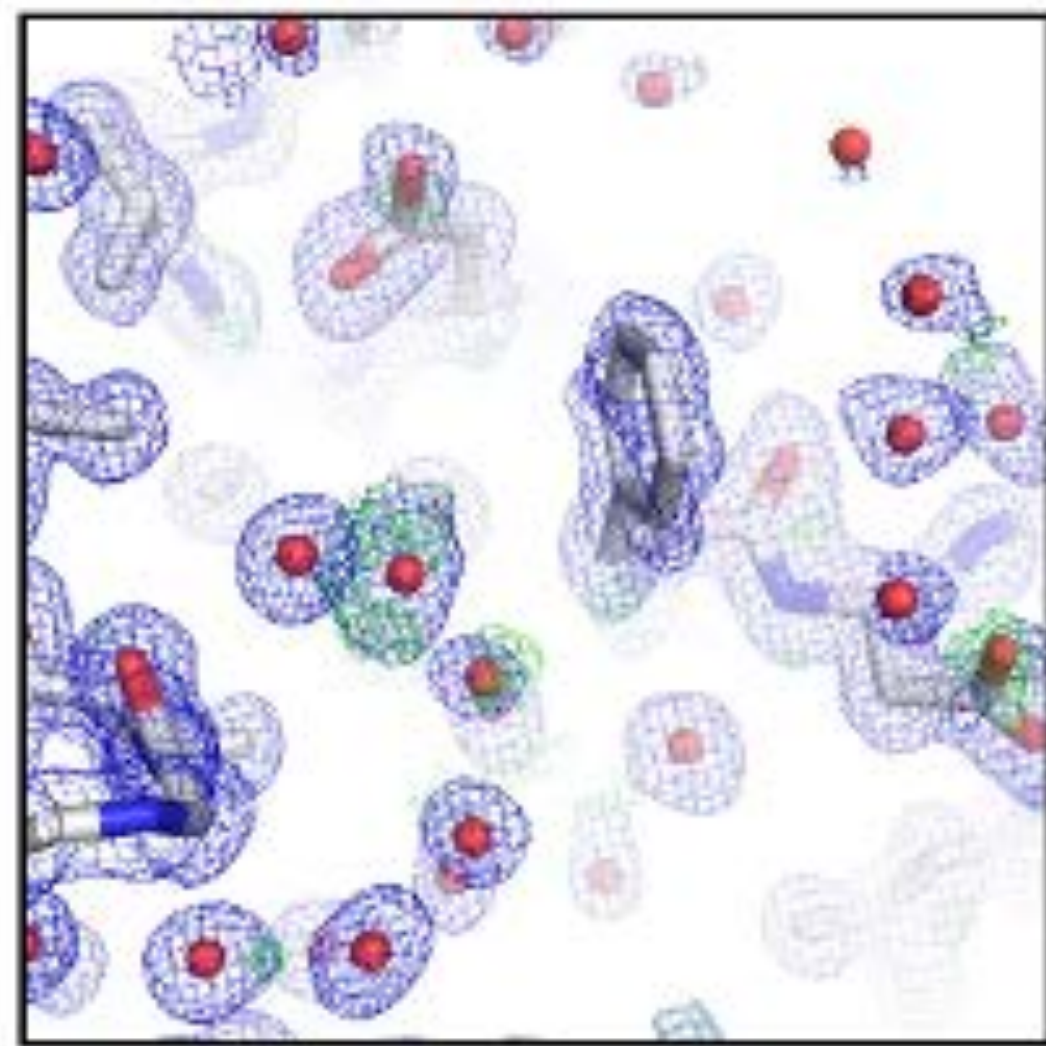


We were surprised by the location of hits
(**north-south** rather than **east-west**)



Schuller*, Correy*, Gahbauer*, Fearon*,
Science Advances, 2021

What makes a good structure?



"No useful conclusions can be derived by PDB users from this ligand ..."



“What concerned us most, however, were the discrepancies between the atomic coordinates and the electron-density maps calculated using the map coefficients in mtz format downloaded from the RCSB server of the PDB.”

PROTEIN|SCIENCE

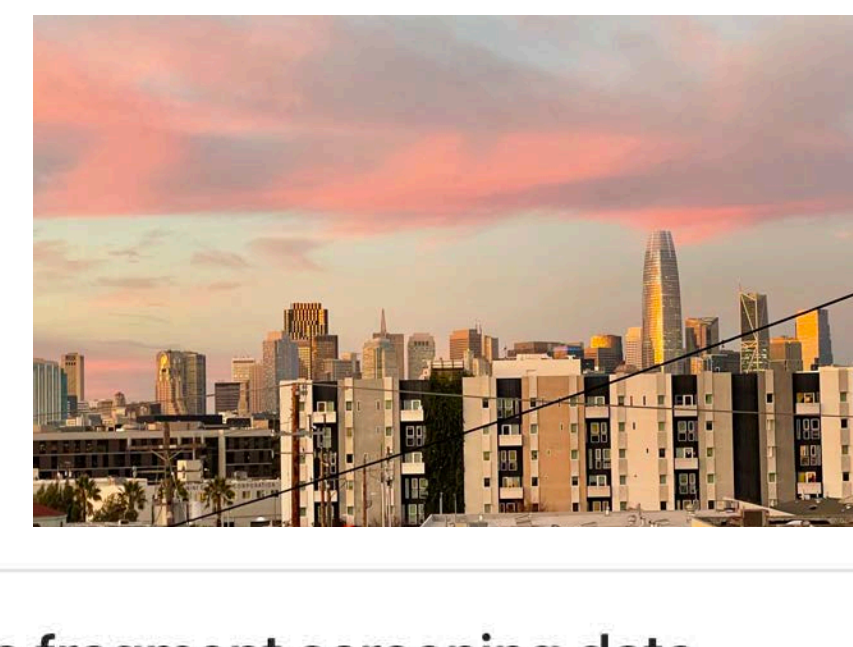
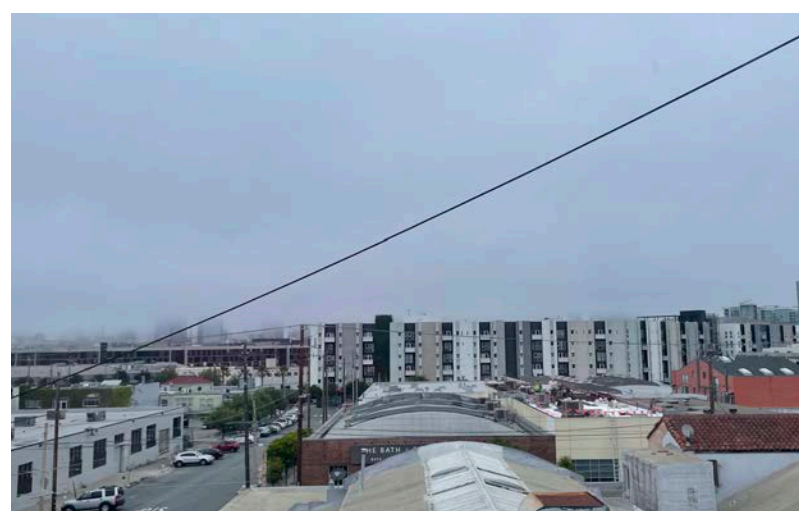
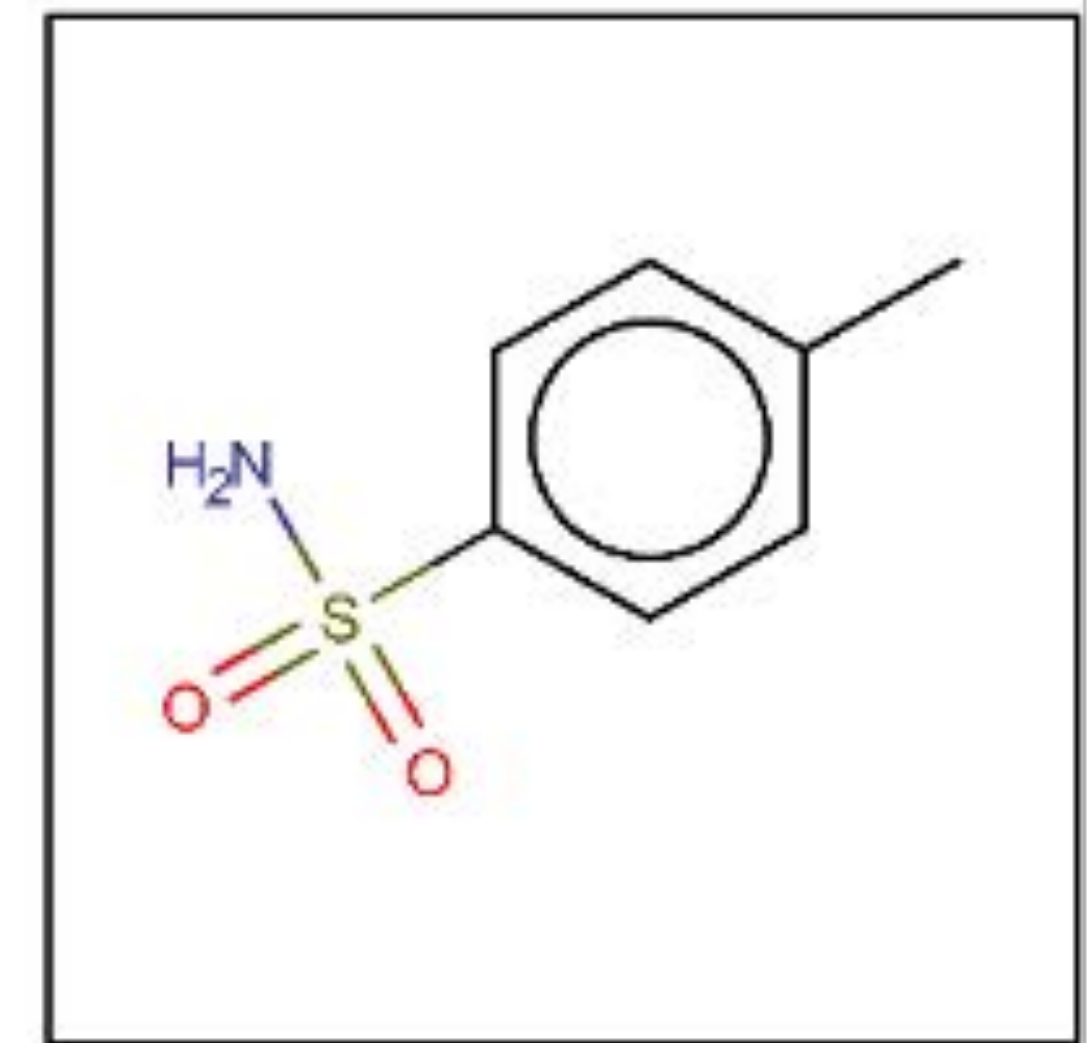
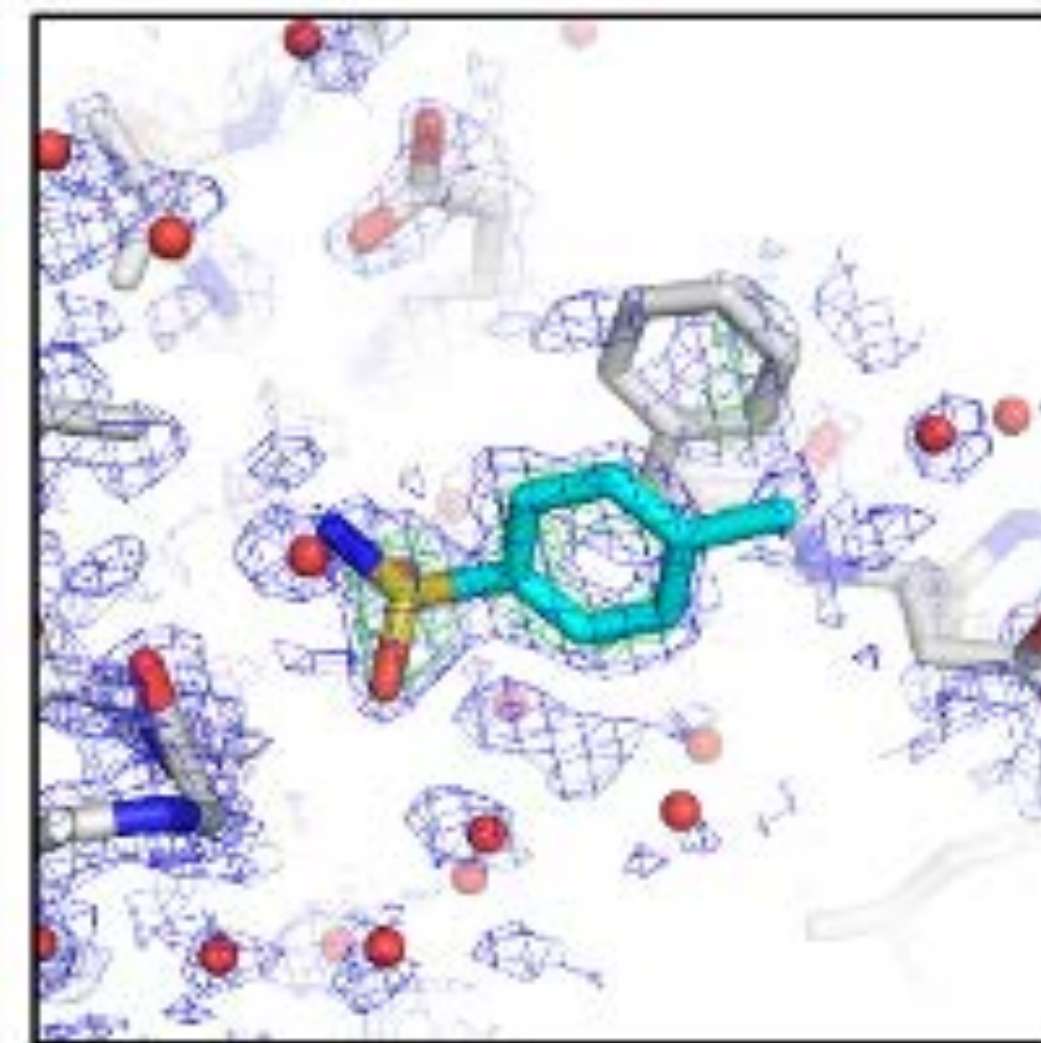
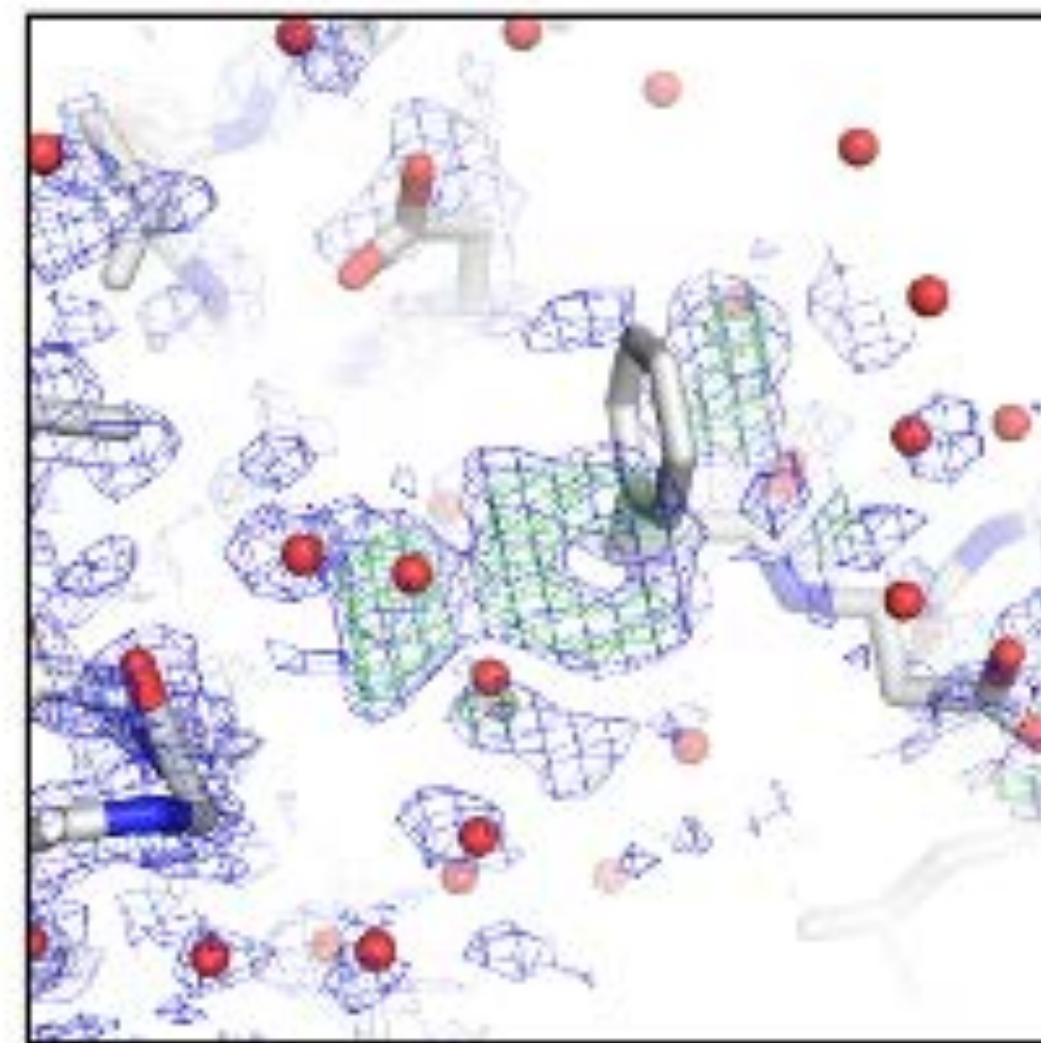
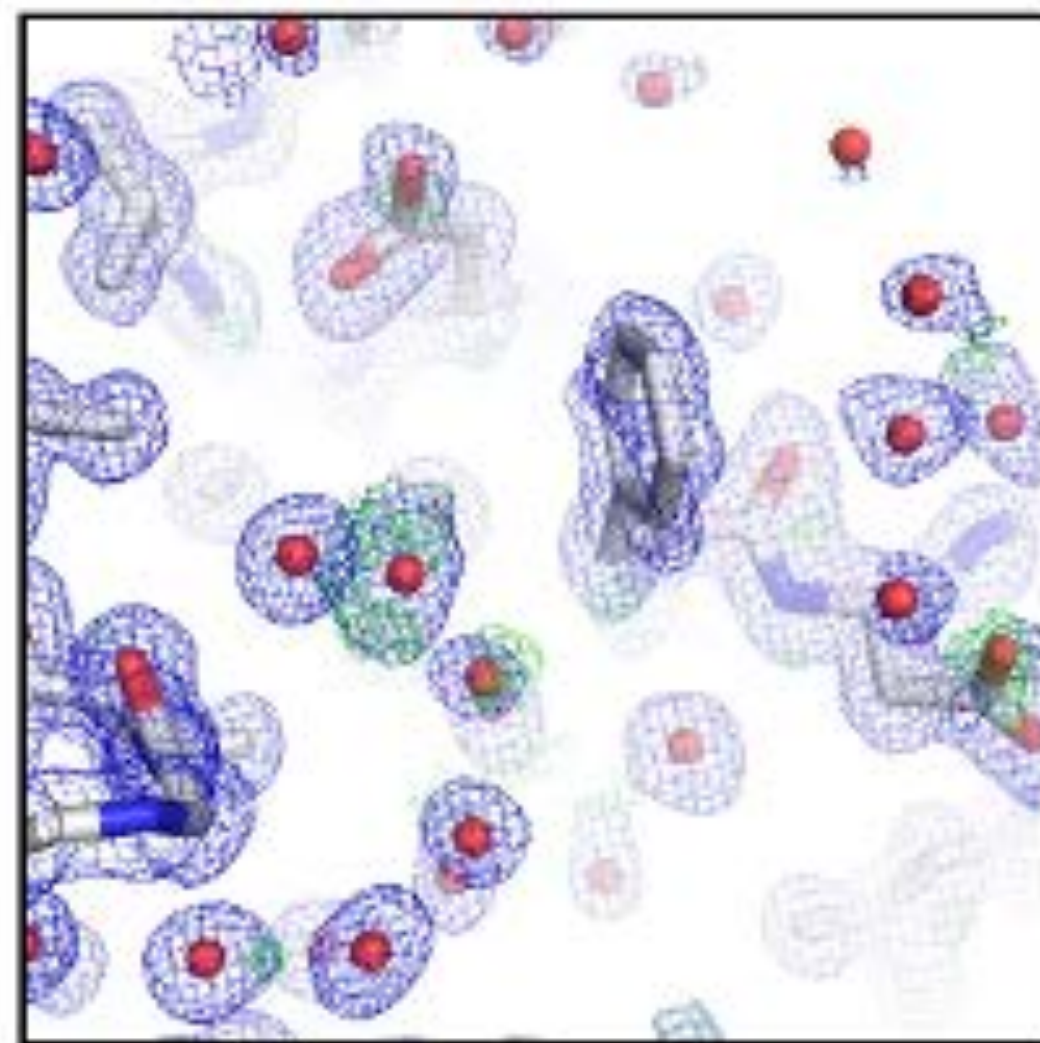
Commentary | [Free Access](#)

Group depositions to the Protein Data Bank need adequate presentation and different archiving protocol

Mariusz Jaskolski✉ Alexander Wlodawer, Zbigniew Dauter, Wlodek Minor, Bernhard Rupp✉

First published: 09 January 2022 | <https://doi.org/10.1002/pro.4271>

What makes a ~~good~~ useful structure?



Of problems and opportunities-How to treat and how to not treat crystallographic fragment screening data

Weiss MS, Wollenhaupt J, Correy GJ, Fraser JS, Heine A, Klebe G, Krojer T, Thunissen M, Pearce NM.

Protein Science, 2022

- **Public datasets**

- [Identifying new ligands for the SARS-CoV-2 Macrodomein by Fragment Screening and Multi-temperature Crystallography \[Interactively explore on Fragalys\]](#)

- **Preprints and papers**

- [Iterative computational design and crystallographic screening identifies potent inhibitors targeting the Nsp3 Macrodomein of SARS-CoV-2 \[DOI\]](#)
- [Fragment binding to the Nsp3 macrodomein of SARS-CoV-2 identified through crystallographic screening and computational docking \[DOI\]](#)
- [The mechanisms of catalysis and ligand binding for the SARS-CoV-2 NSP3 macrodomein from neutron and x-ray diffraction at room temperature \[DOI\]](#)
- [Of problems and opportunities—How to treat and how to not treat crystallographic fragment screening data \[DOI\]](#)

- **Tutorials**

- [Multi-state models from PanDDA](#)
- [Inspecting PanDDA event maps deposited in the Protein Data Bank](#)

NIH

National Institutes of Health

Office of the Director

Data Science at NIH

Integrated Resource for Reproducibility in Macromolecular Crystallography

This project is being funded by the [Targeted Software Development](#) award 1 U01 HG008424-01 as part of the [BD2K \(Big Data to Knowledge\)](#) program of the National Institute of Health. The project is developing tools for "wrangling" data from protein diffraction experiments. We are also creating a growing repository of diffraction experiments used to determine protein structures in the [PDB](#), contributed by the [CSGID](#), [SSGCID](#), [JCSG](#), [MCSG](#), [SGC](#), and other large-scale projects, as well as individual research laboratories.

Currently indexed projects: **6111**

Currently indexed datasets: **9541**

Gerard to Me & Clemens

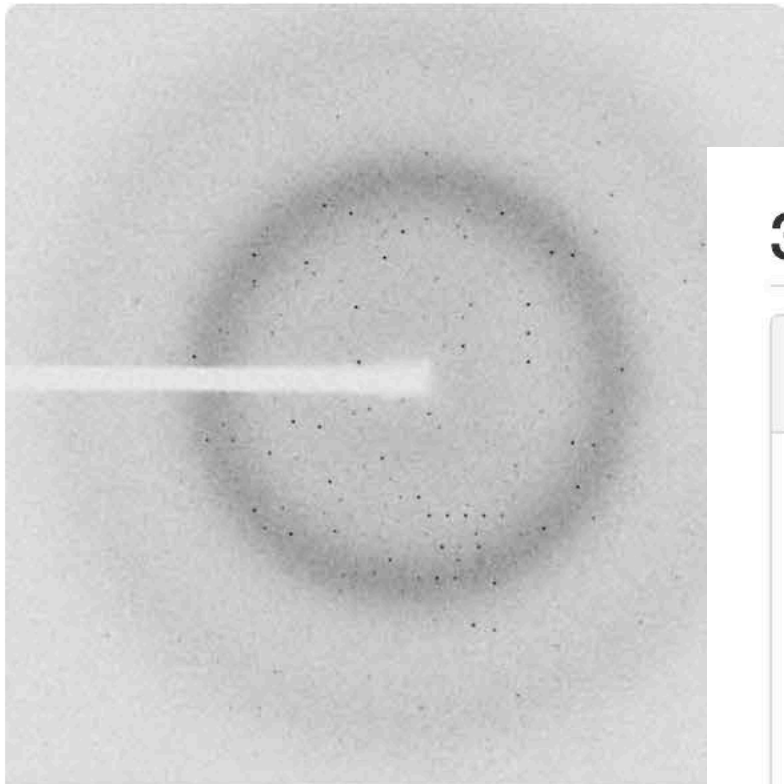
Dear Jamie,

We hope this message finds you well and deeply involved in truly "great stuff"!

We noticed the recent PDB entries 5spz to 5ssr (and perhaps a few more) that constitute a PanDDA deposition in conjunction with a bioRxiv preprint on the "Structure-based inhibitor optimization for the Nsp3 1 Macrodomein of SARS-CoV-2".

All the Supplementary Material is very nicely organised, but the Zenodo files only contain merged X-ray data and results derived from them.

Would it be possible to also have access to the raw diffraction images? These had been made available for an earlier version of this work, and we had found them very useful (as you may recall we

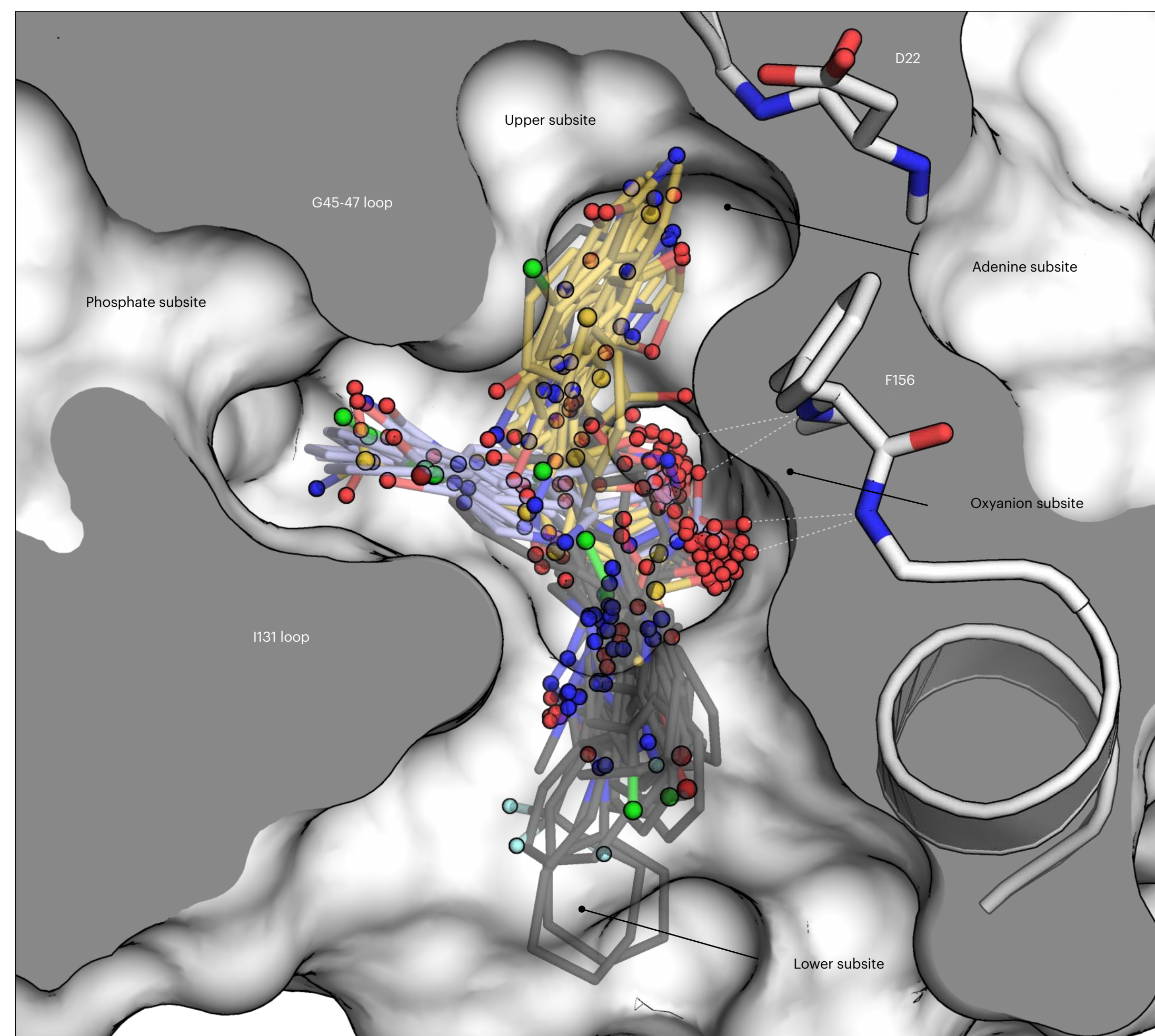


JUL 14

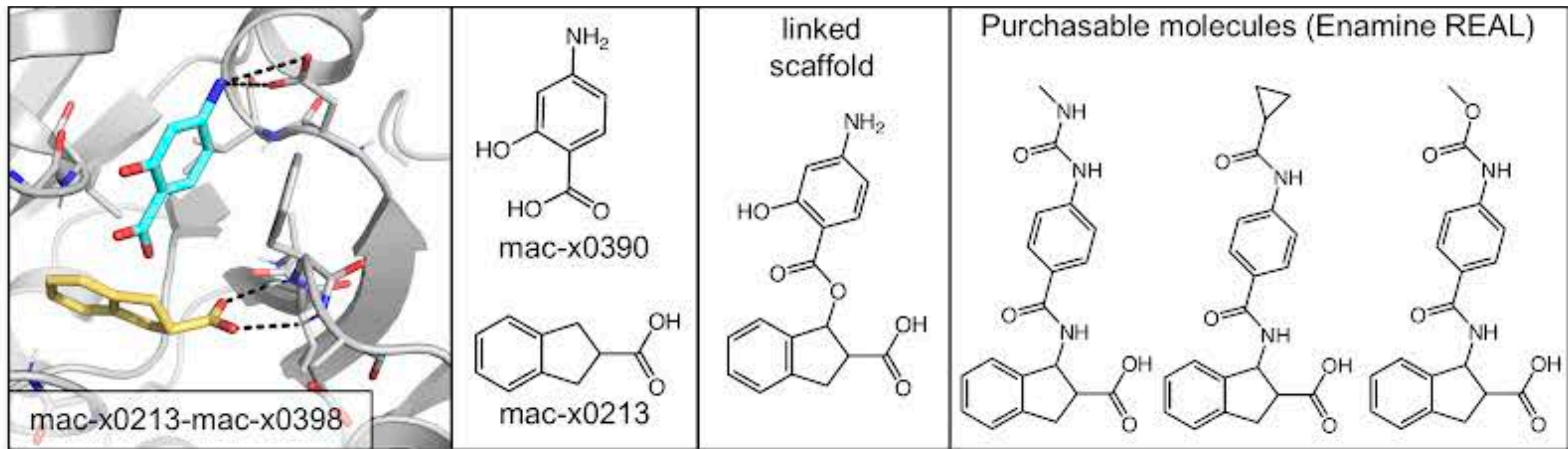
388 results

<

Fragment linking opportunities are revealed by the large set of fragment structures



Fragment **linking** proposes new molecules

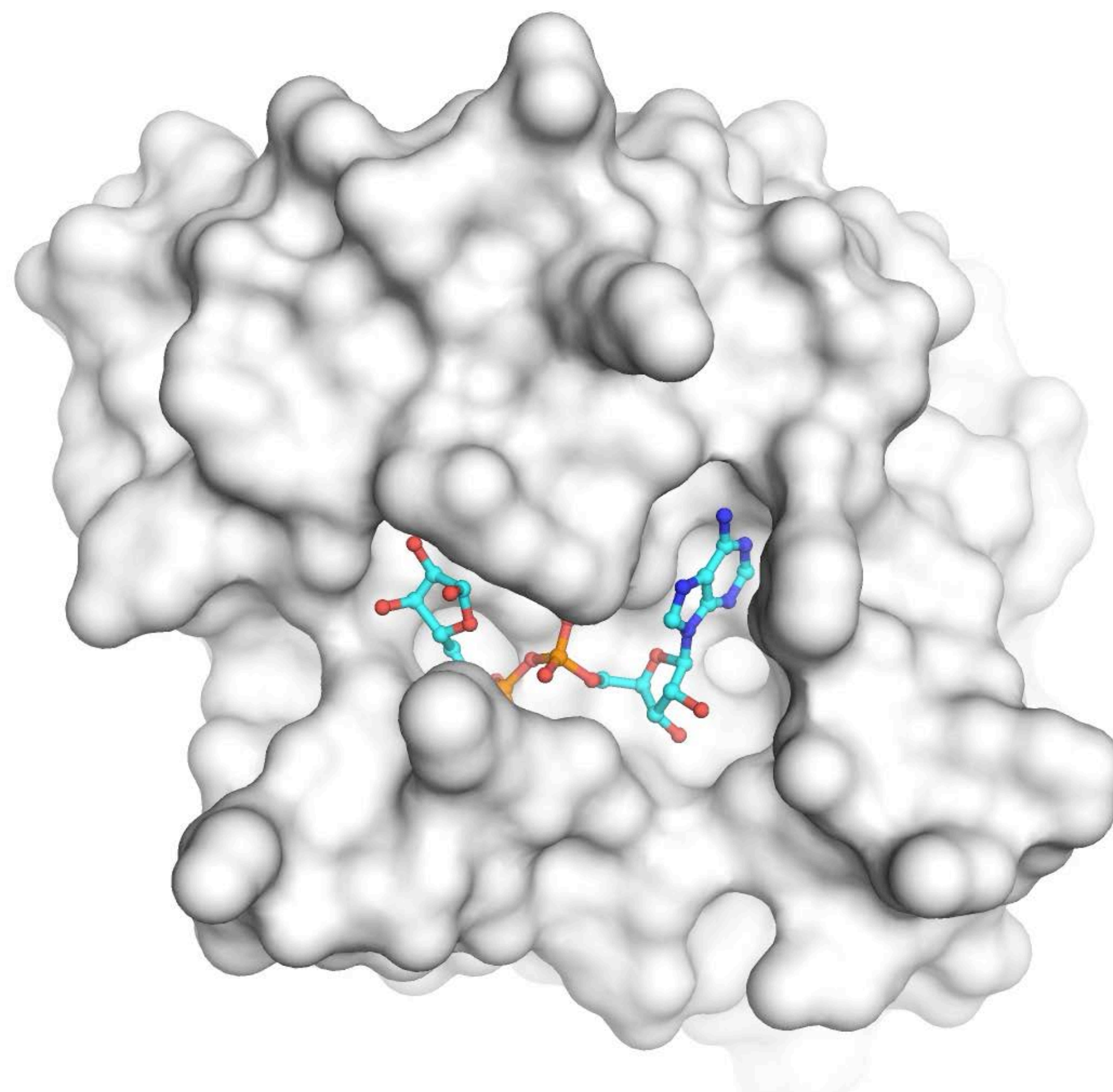


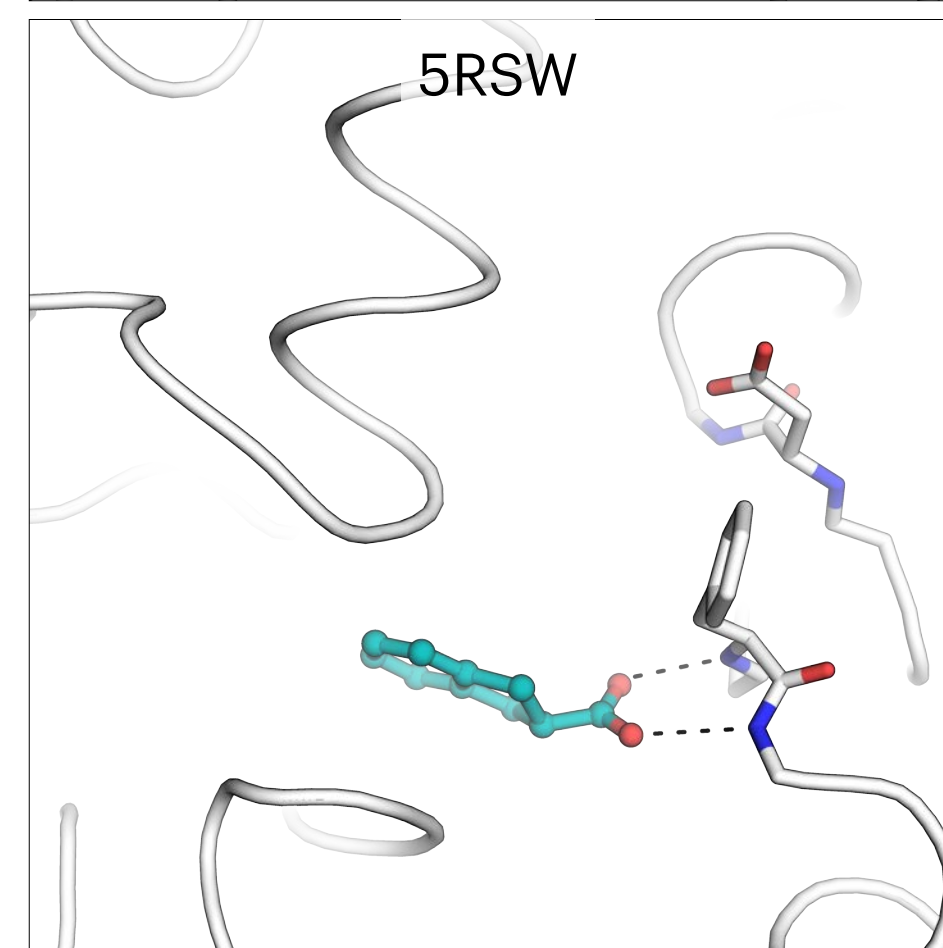
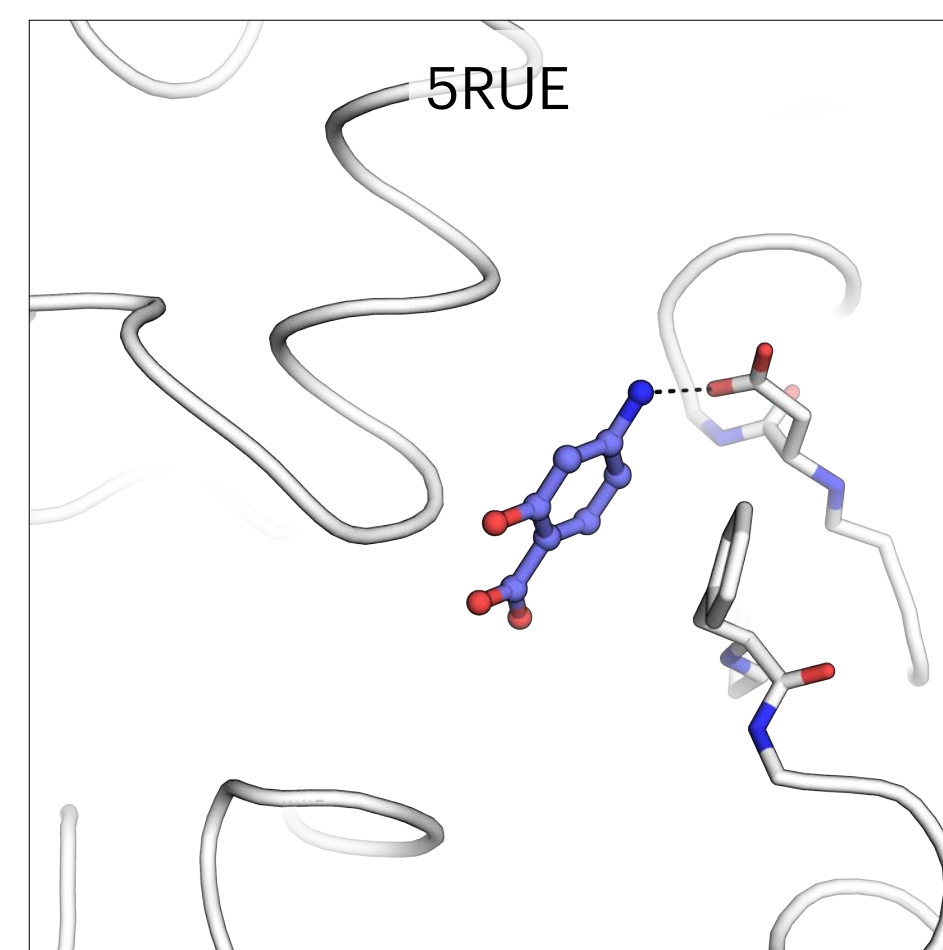
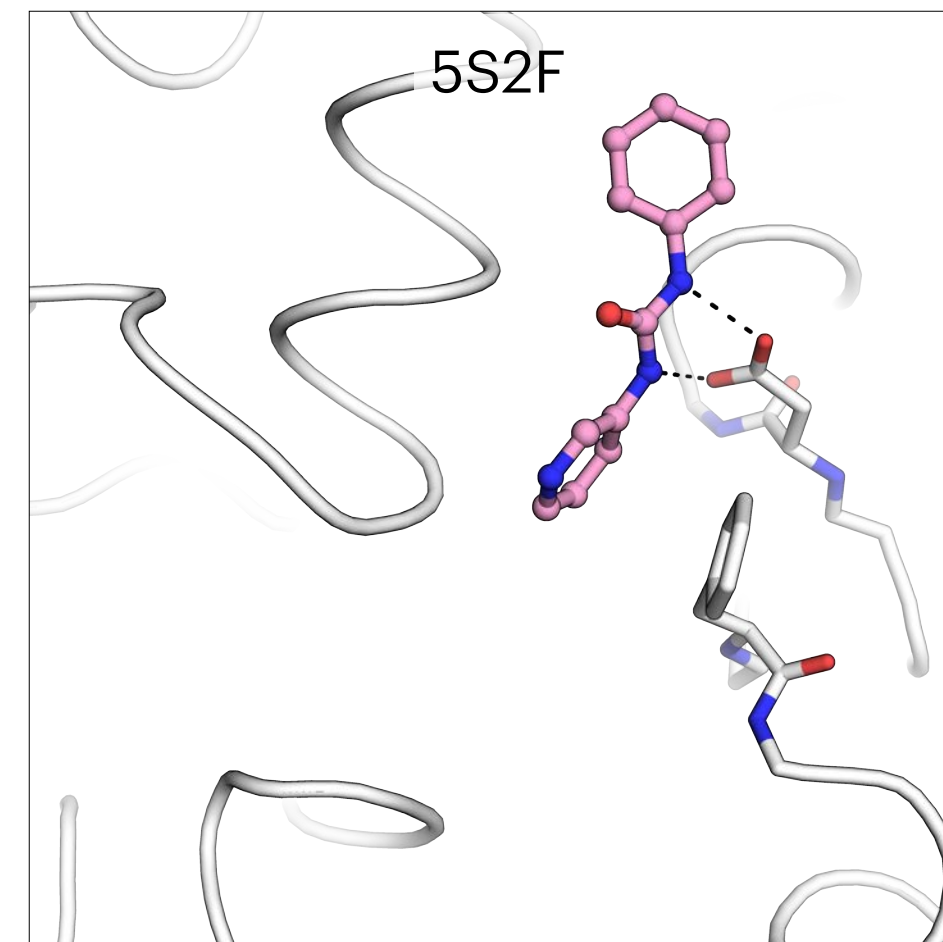
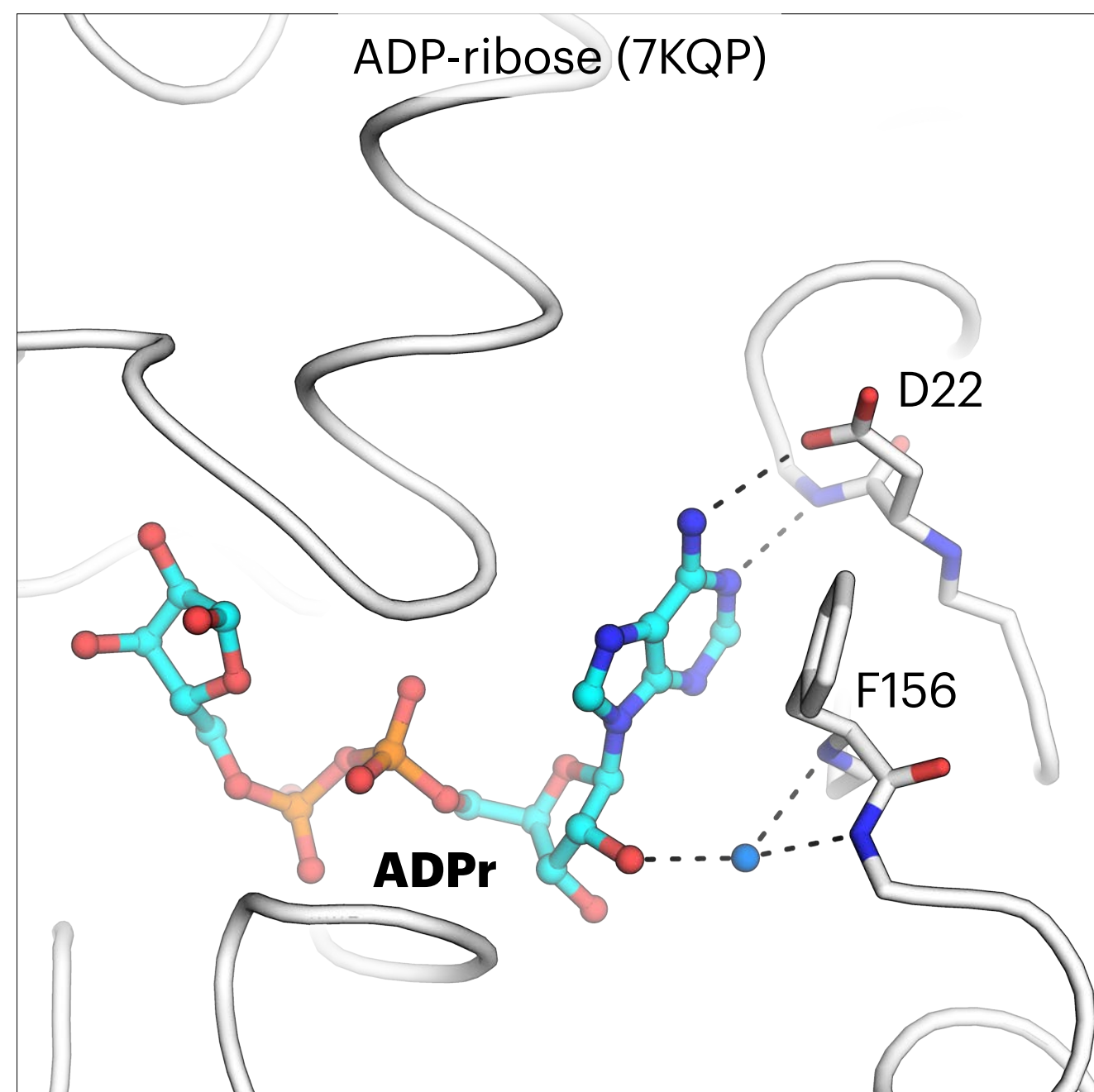
Dr. Matteo
Ferla

<https://github.com/matteoferla/Fragmenstein>

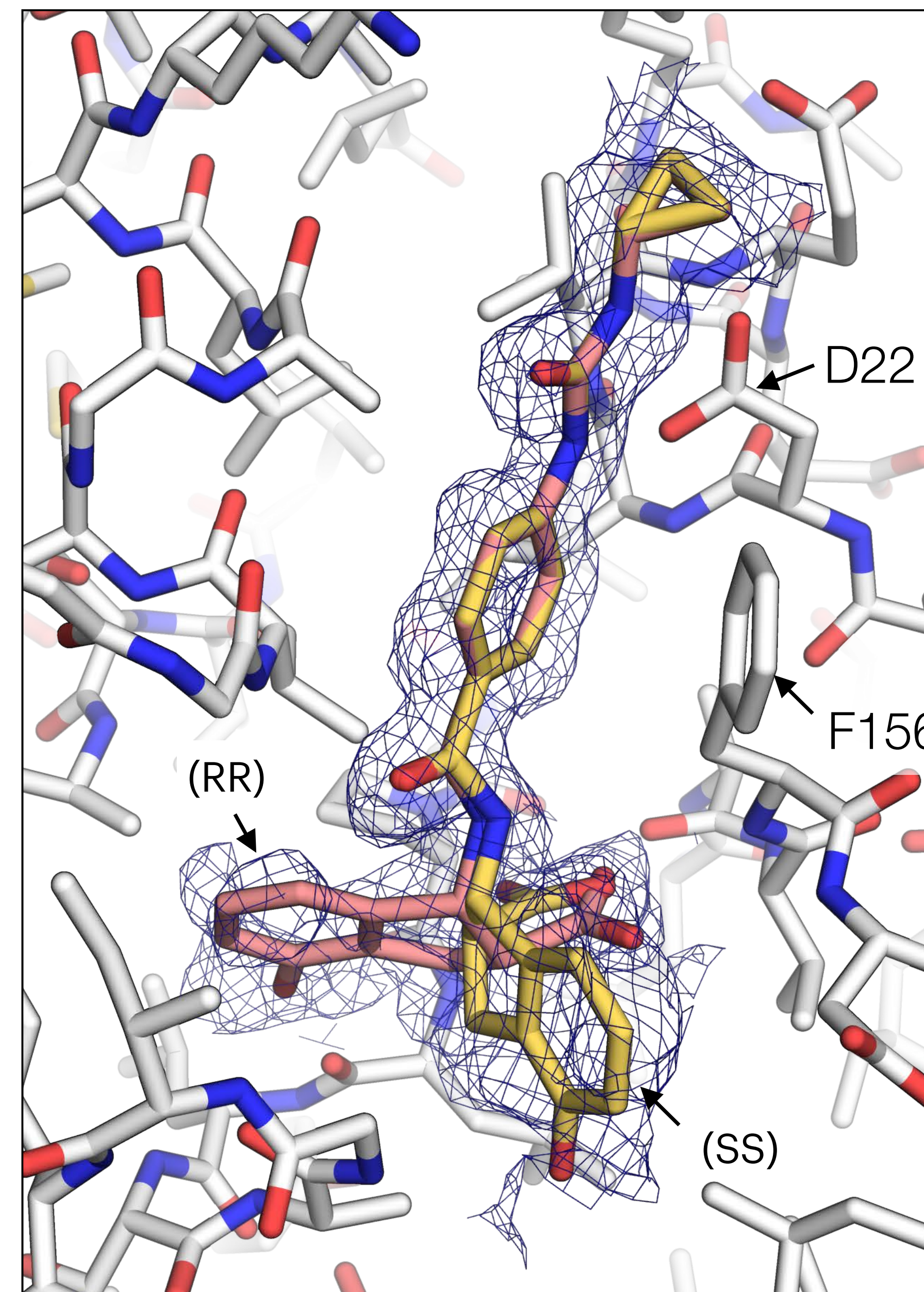


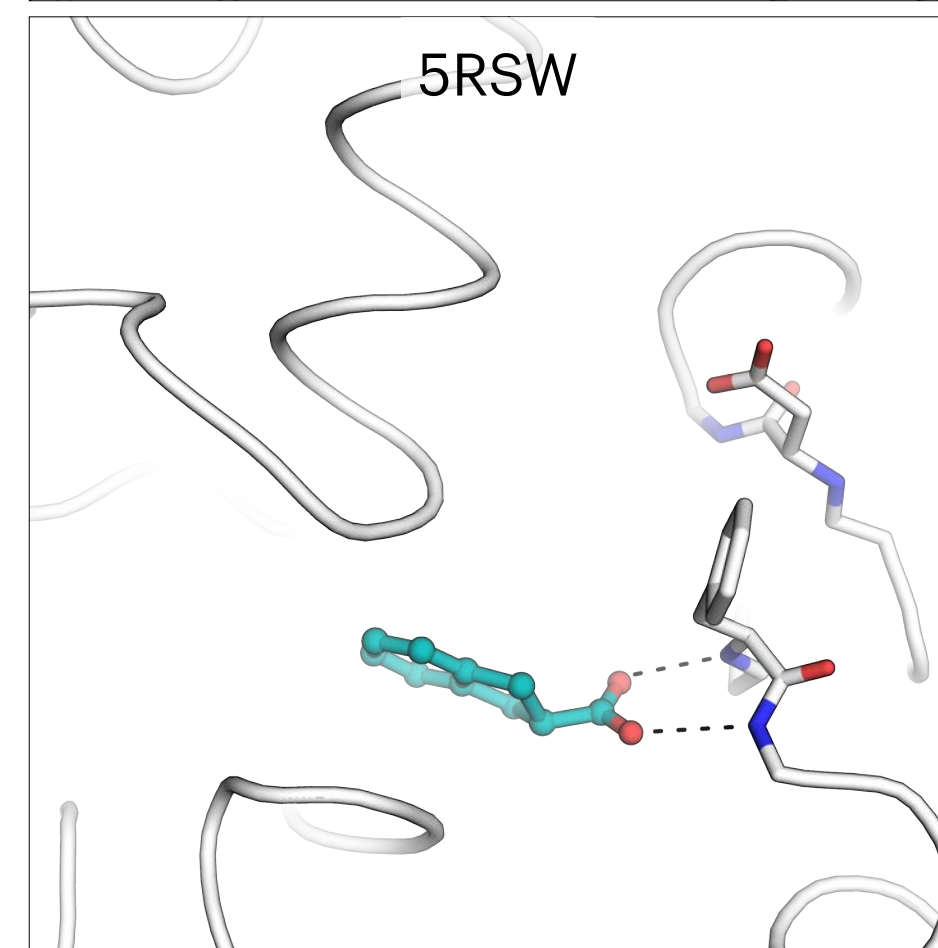
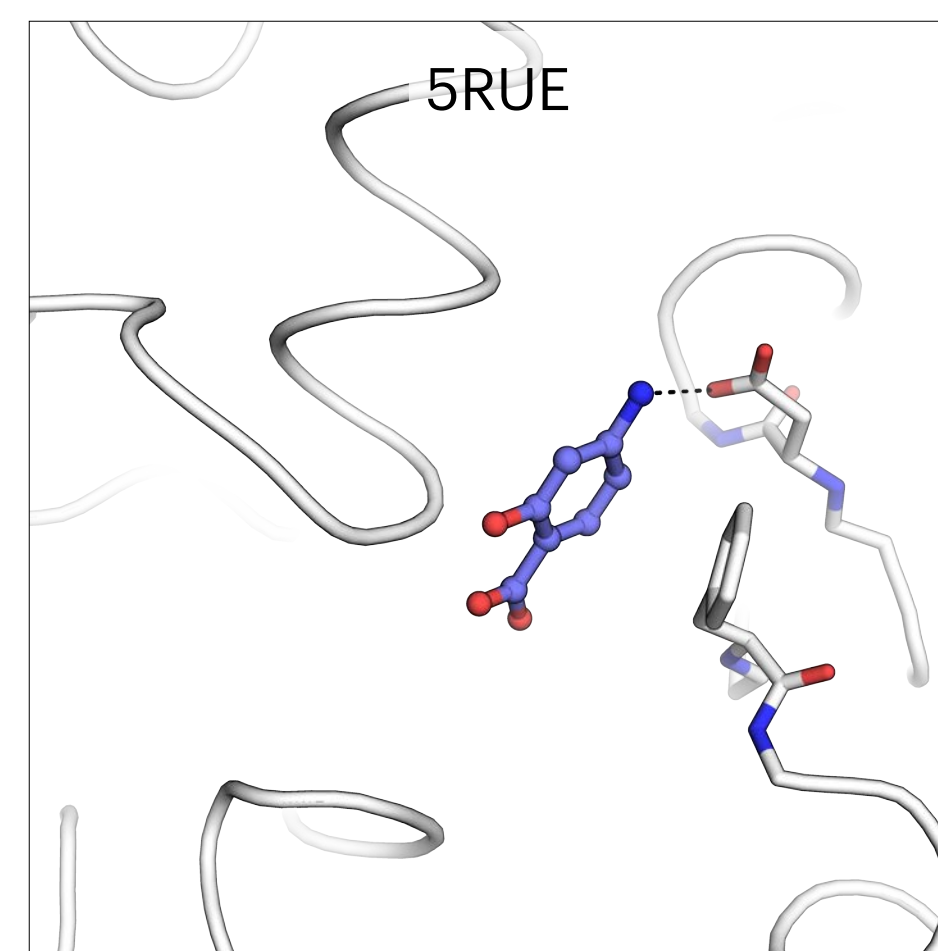
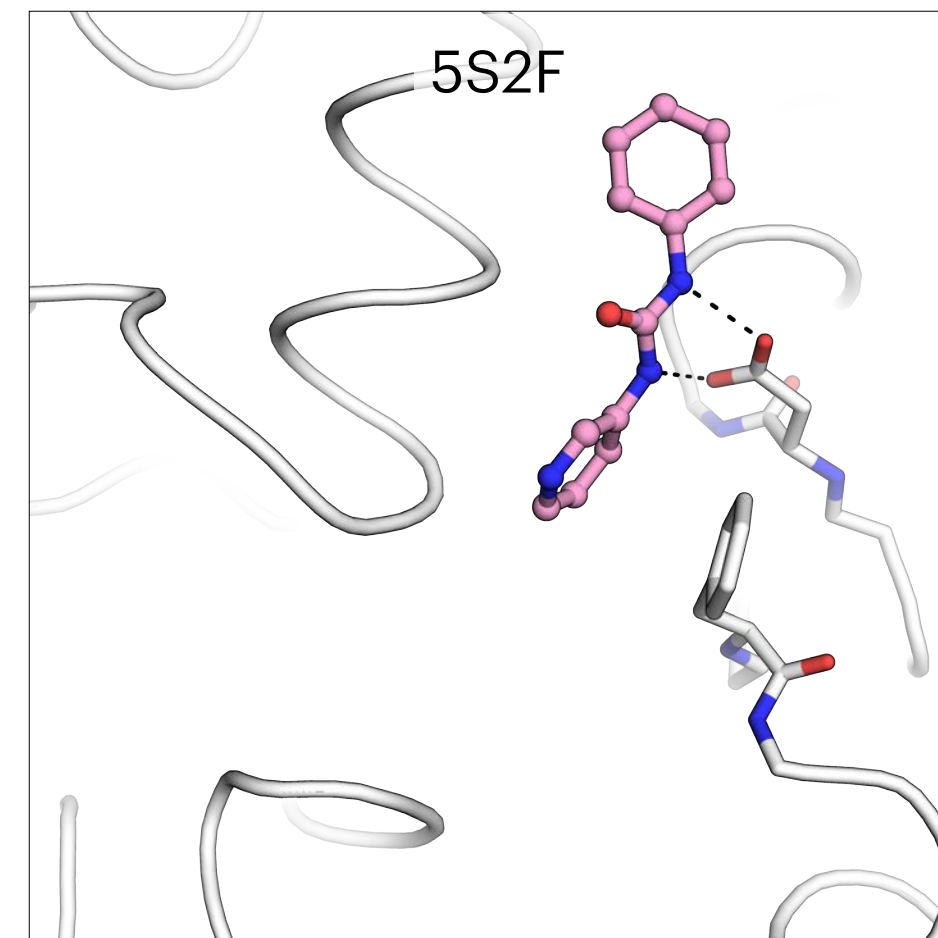
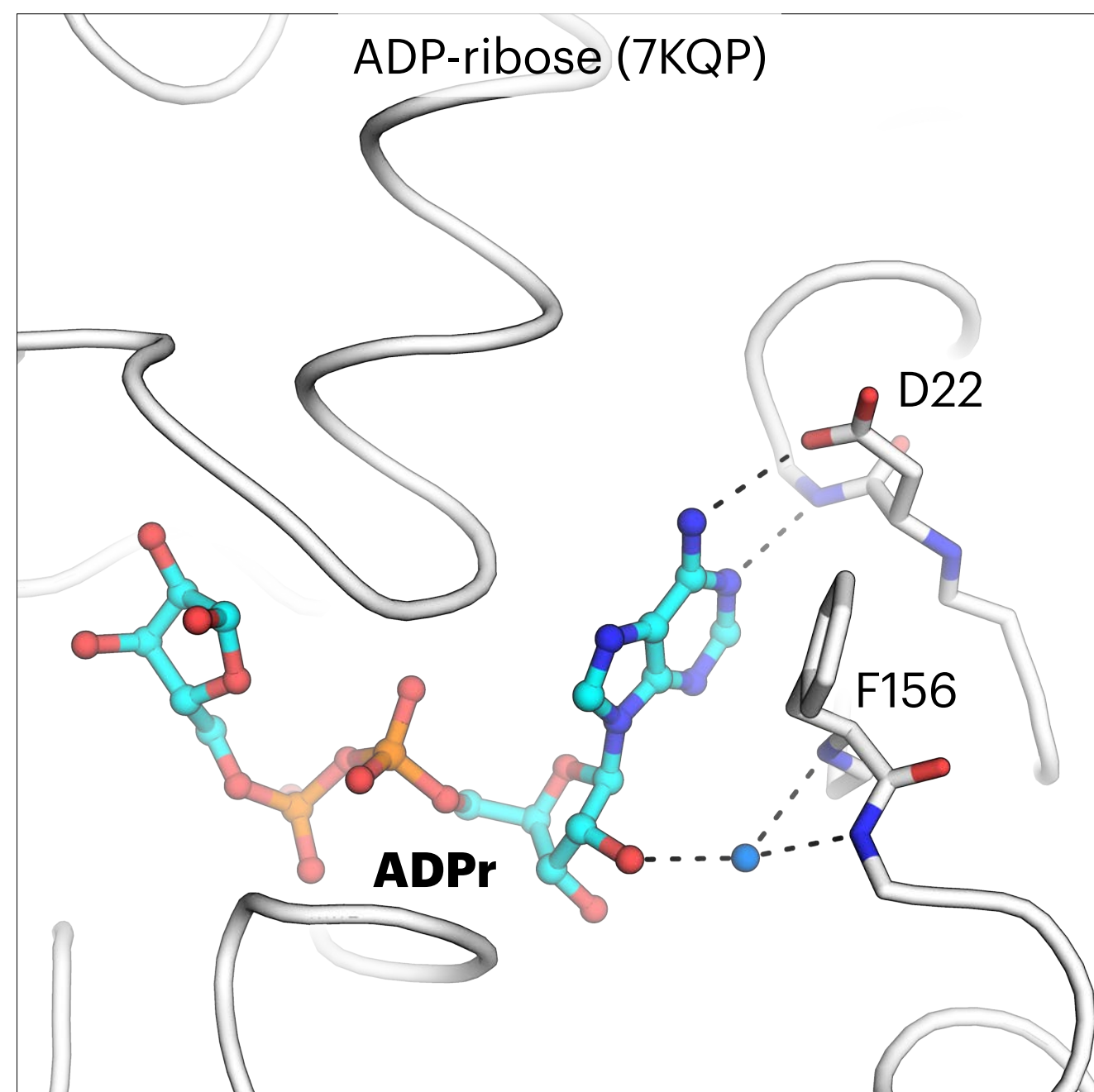
Linking molecules together creates reasonable, (cheap)
purchasable compounds



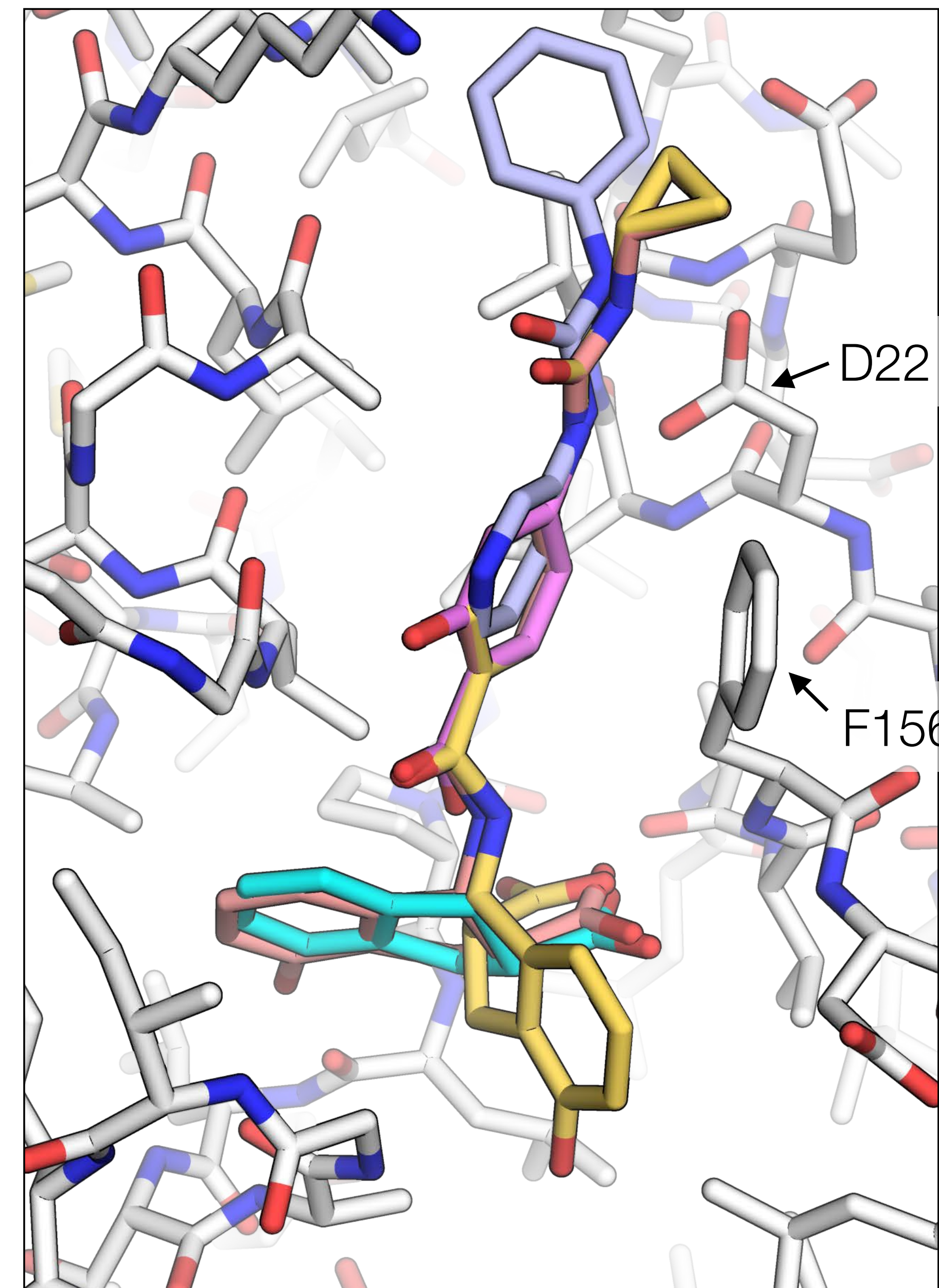


PanDDA event map

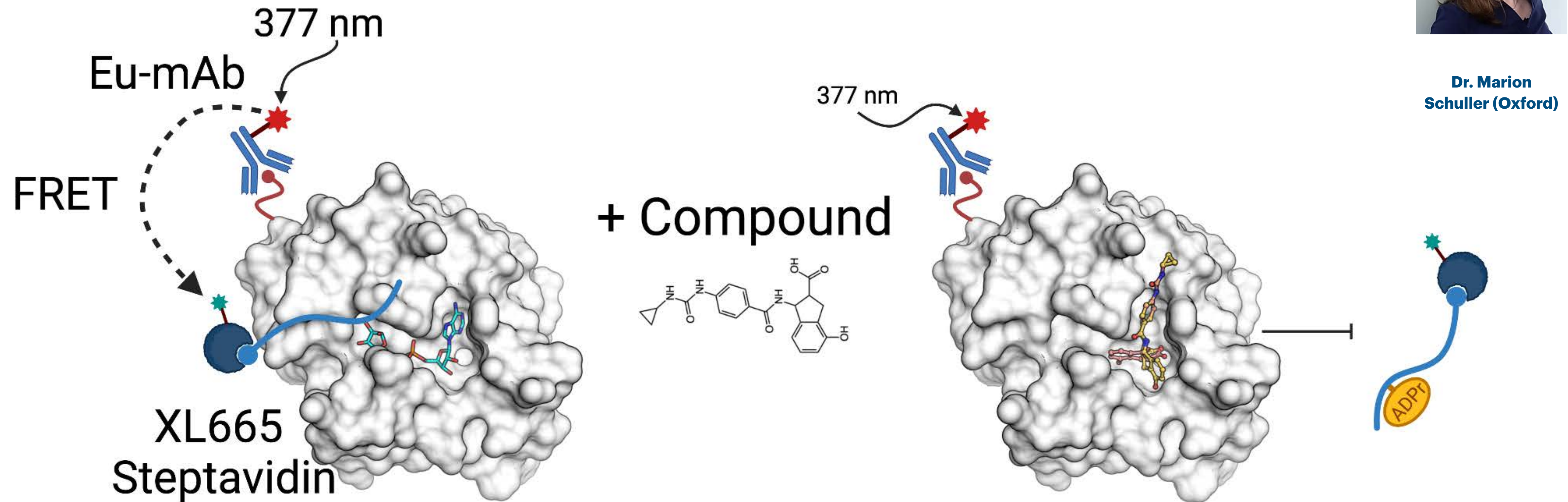




Aligned with fragments

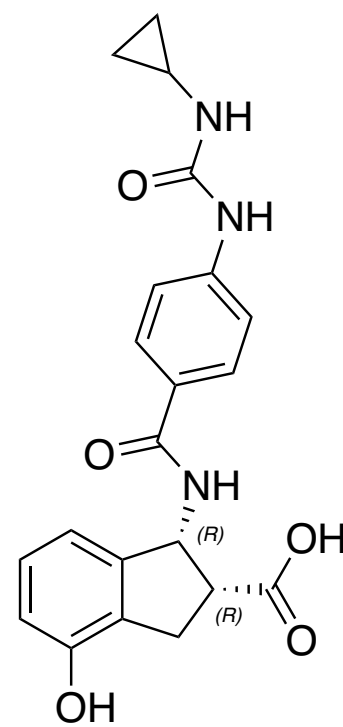


Homogenous Time Resolved Fluorescence (HTRF) displacement monitors **compound binding**



Dr. Marion
Schuller (Oxford)

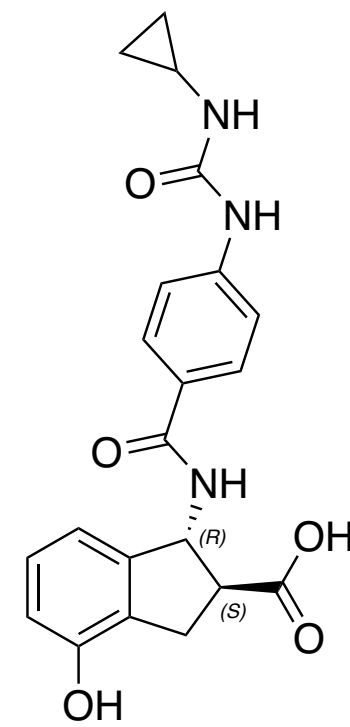
RR, which **overlaps** most with fragments, is most potent



Z8601 (5SQJ)

IC₅₀ = 0.5 μM

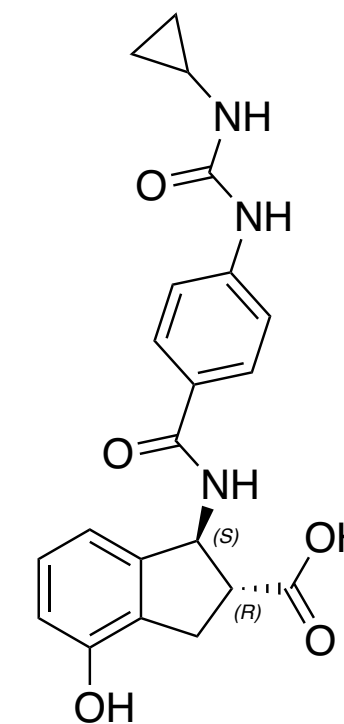
RR



E2160 (5SR2)

IC₅₀ = 43.4 μM

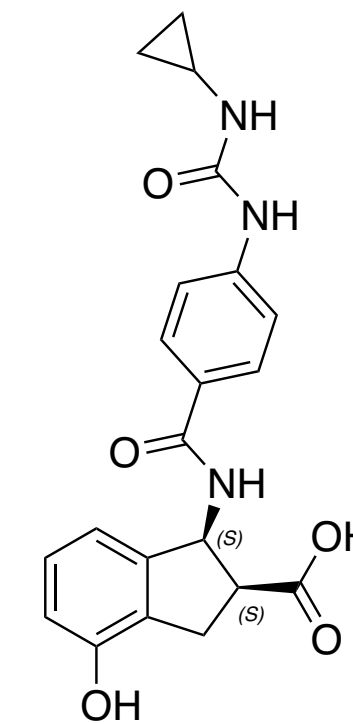
RS



E2158 (no structure)

IC₅₀ = 55.7 μM

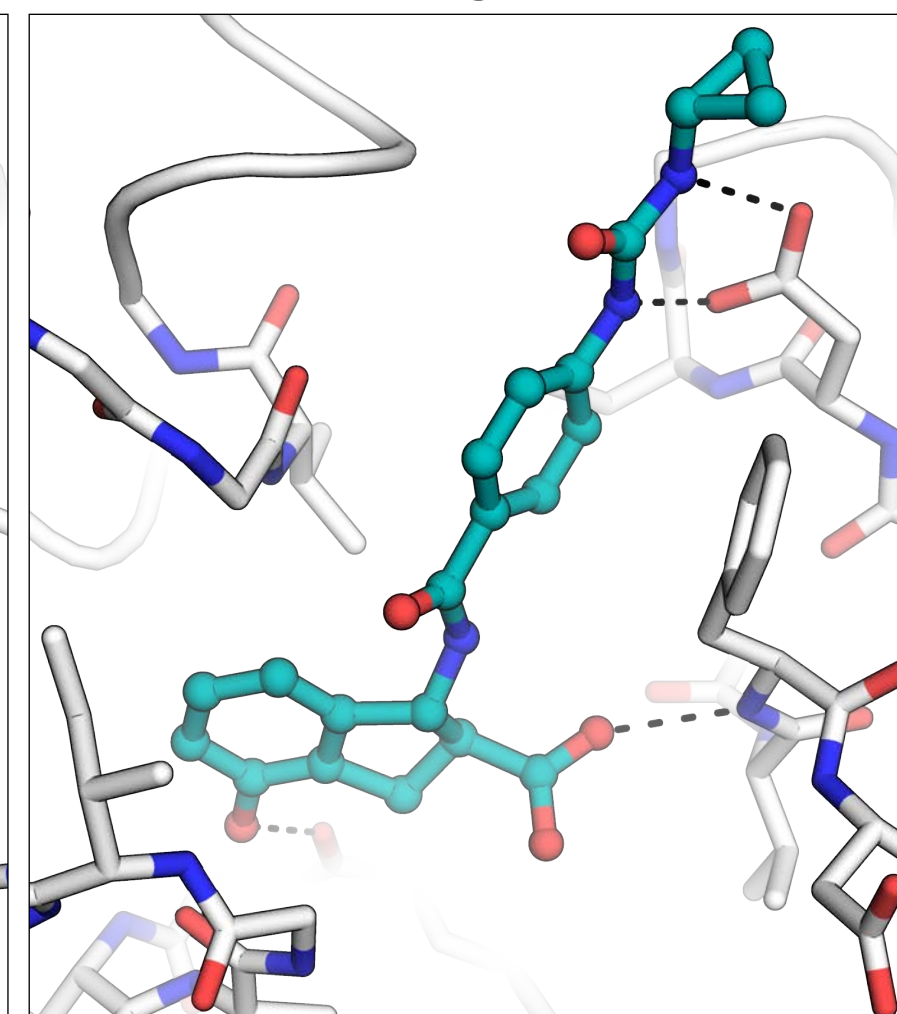
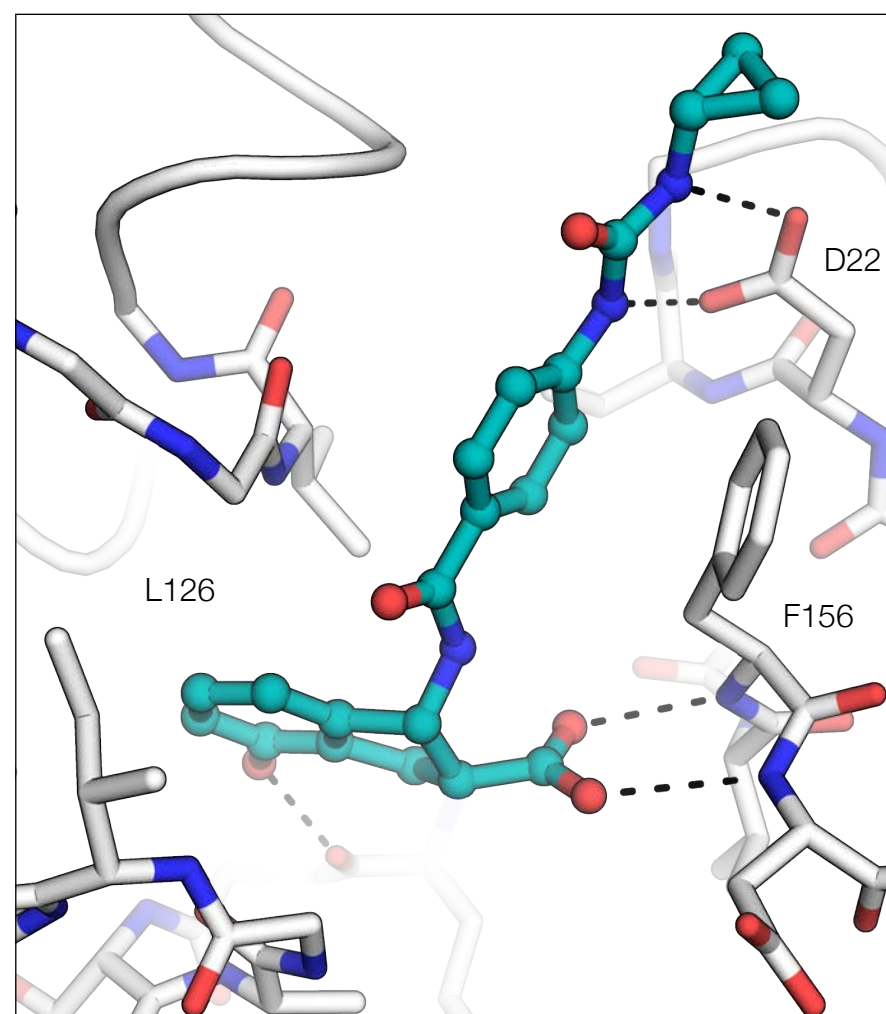
SR



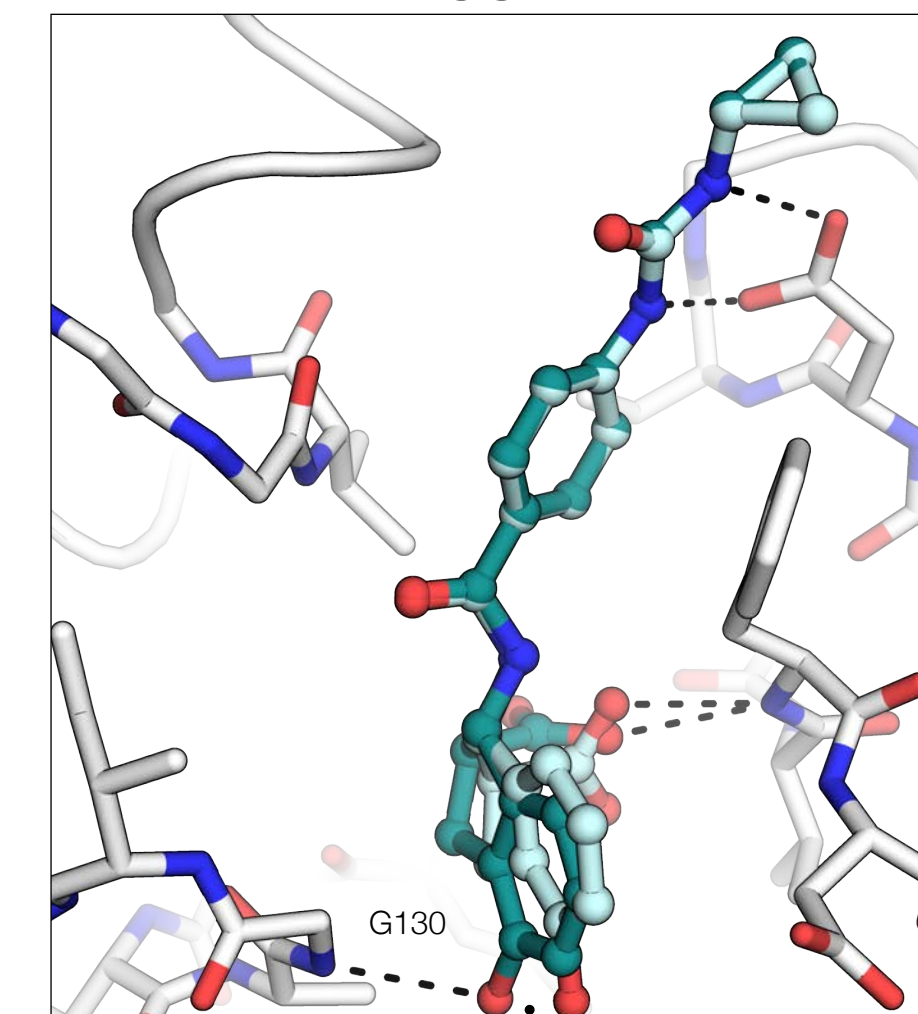
Z9050 (5SR3)

IC₅₀ = 3.2 μM

SS



No binding
detected
(5 crystals
screened)

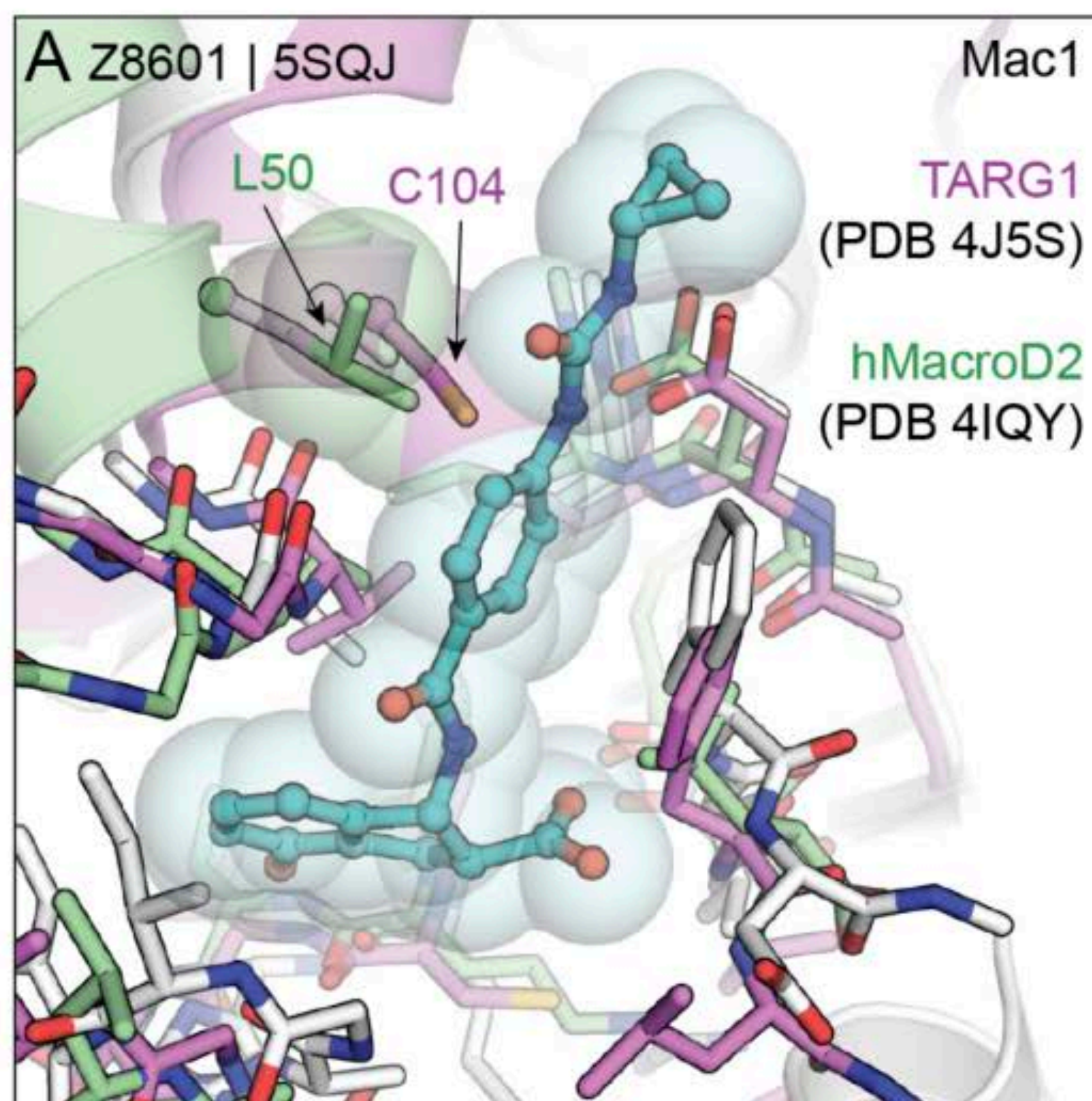


Two conformations

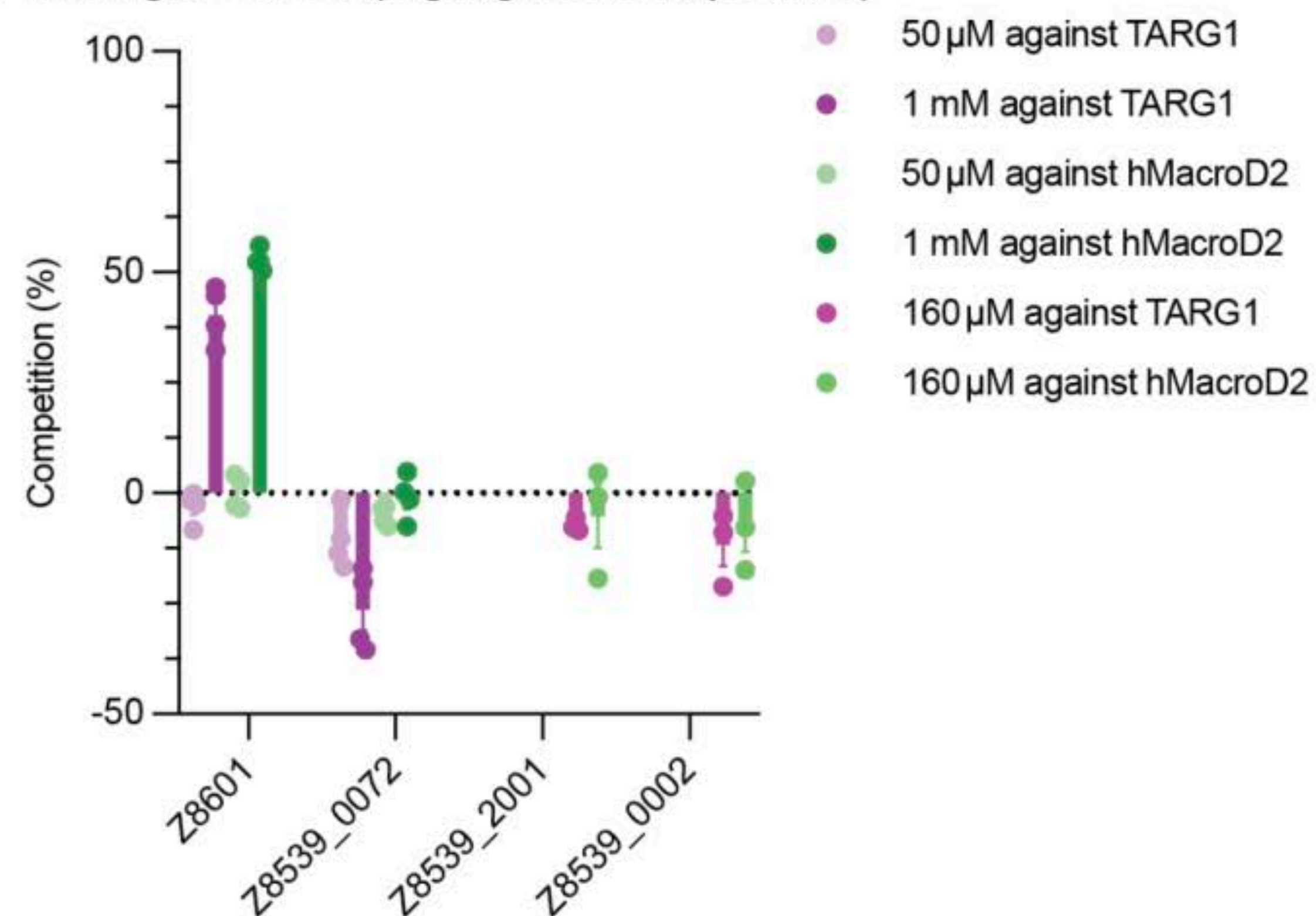


Yagmur Doruk

Selectivity data is promising so far



B Off-target screen (highlighted compounds)

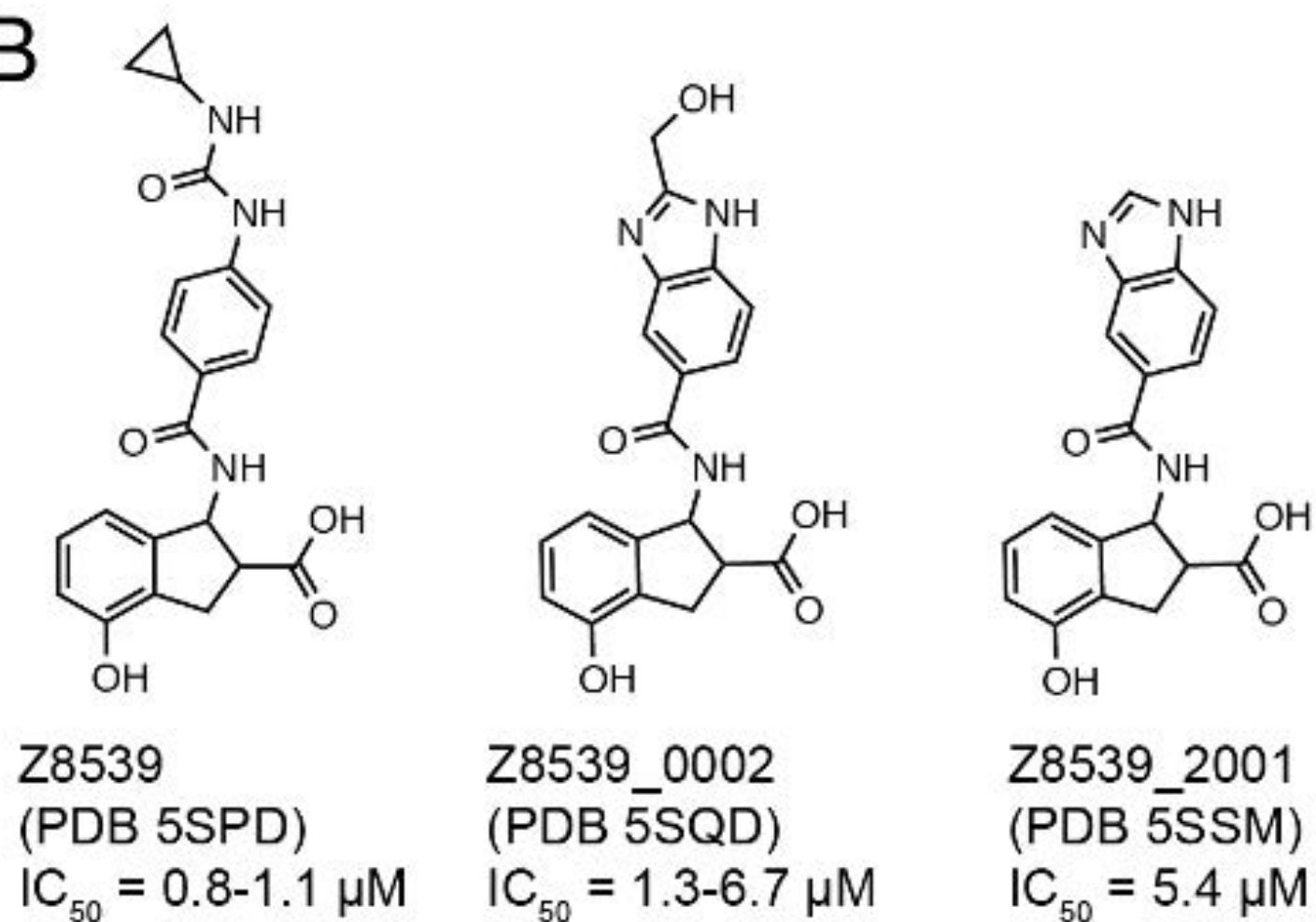


Permeability is a problem!

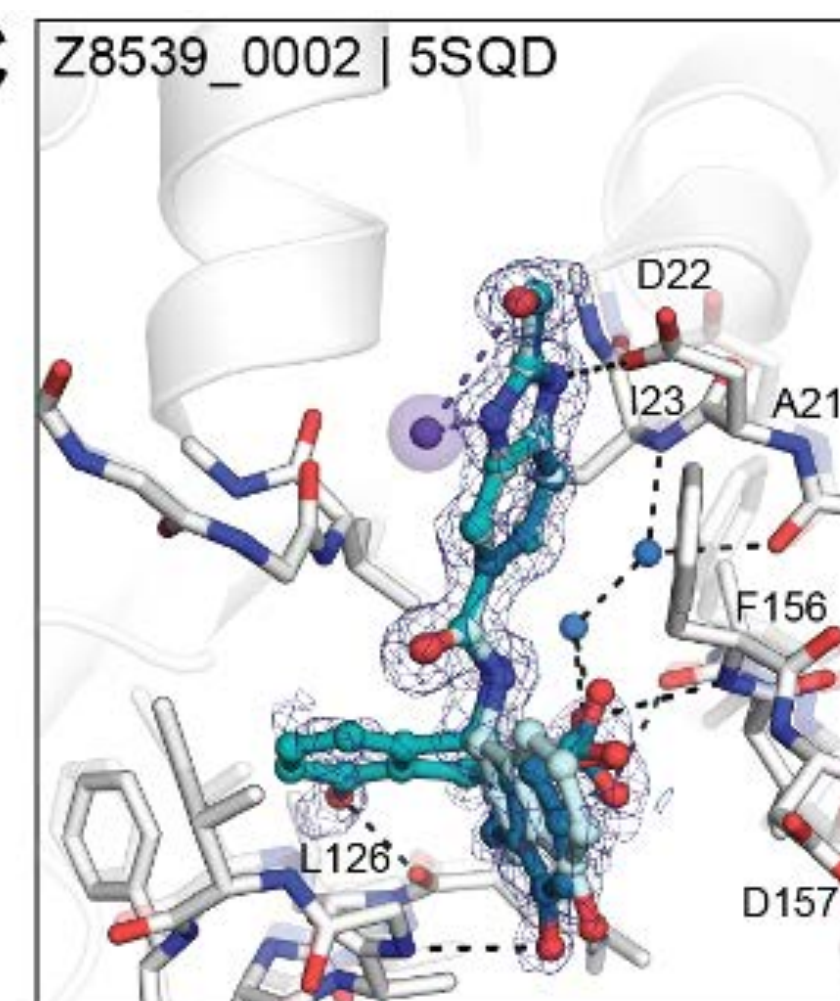
A

Compound	MDR1-MDCKII permeability [10 ⁻⁶ cm/s]	
	P _{app} (AB)	P _{app} (BA)
Z8539	1.1 ± 0.1	0.5 ± 0.0
Z8539_0002	0.8 ± 0.2	1.4 ± 1.0
Z8539_2001	1.1 ± 0.1	0.5 ± 0.0
Atenolol	0.9 ± 0.1	-
Ketoprofen	17.6 ± 2.5	16.1 ± 0.5

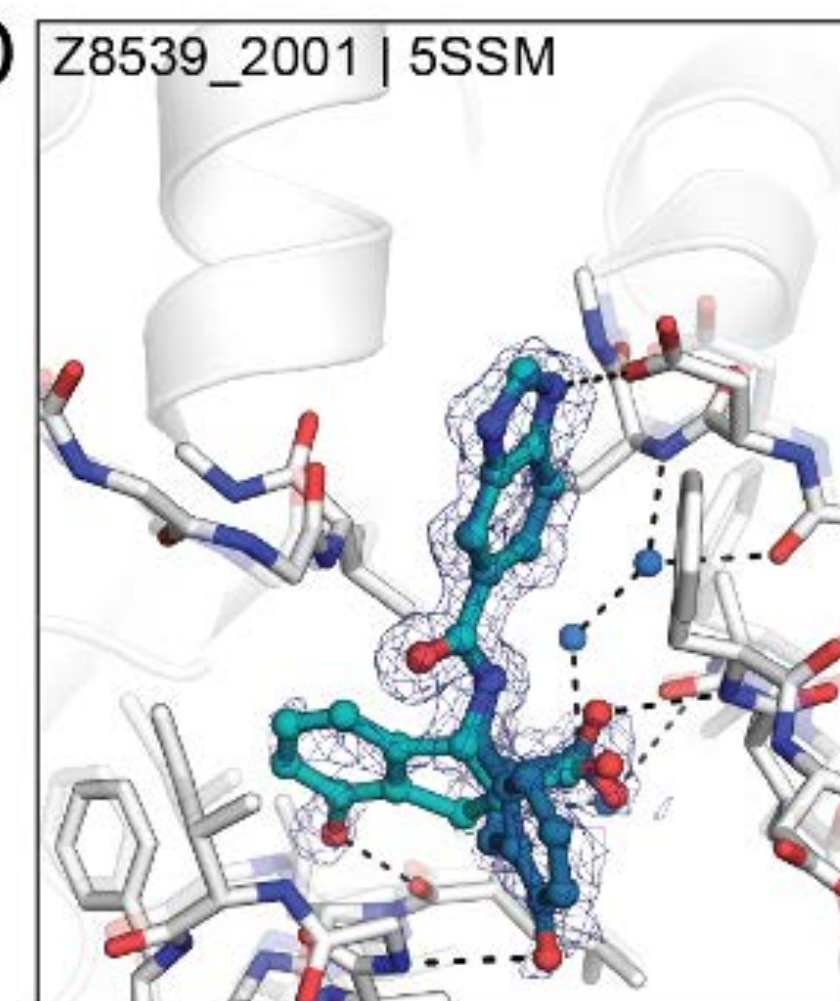
B



C

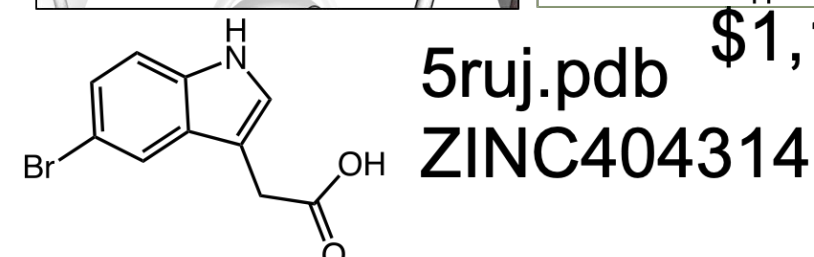
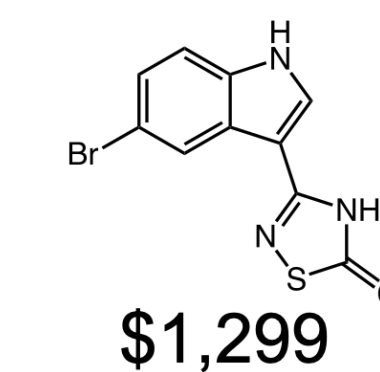
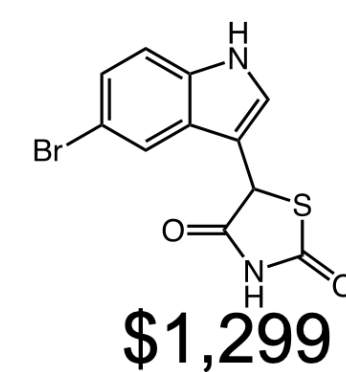
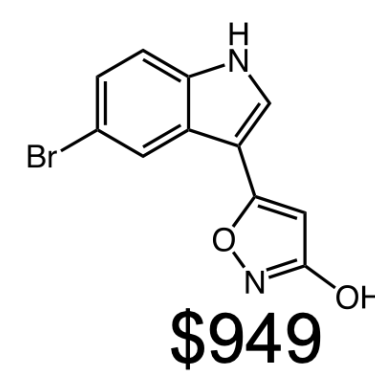
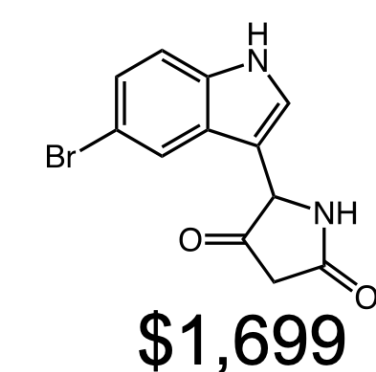
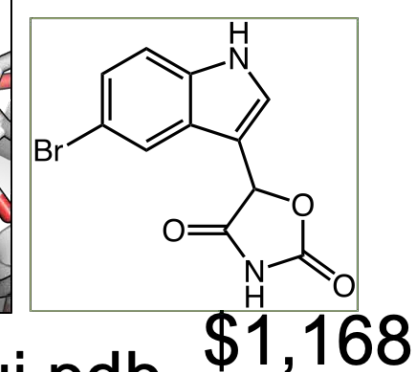
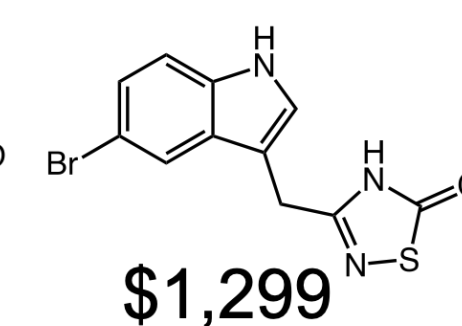
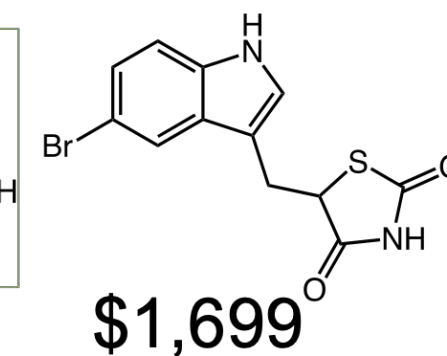
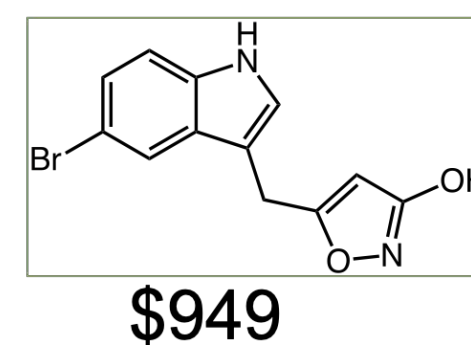
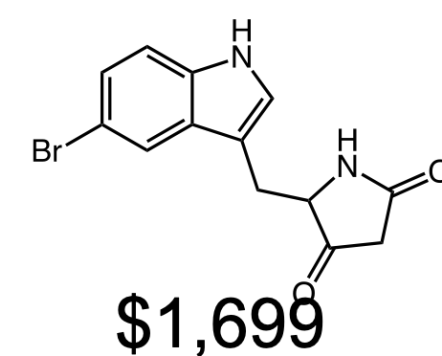
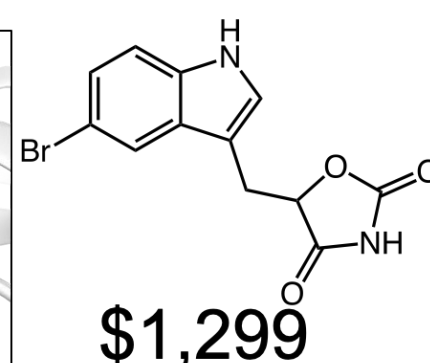
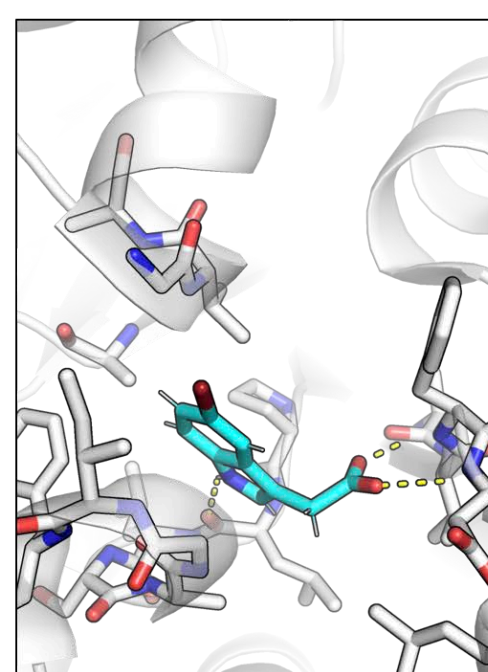
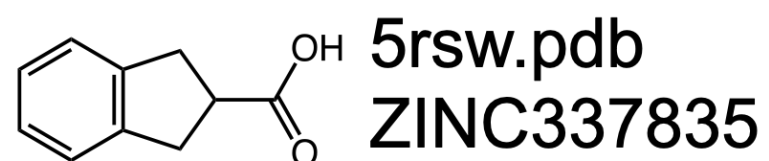
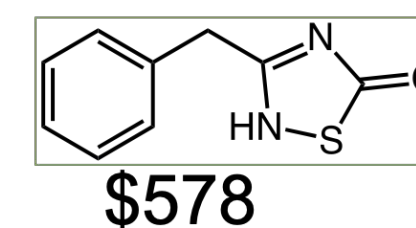
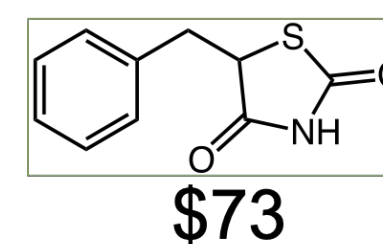
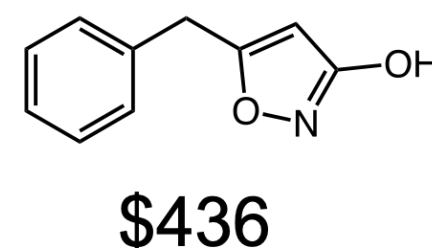
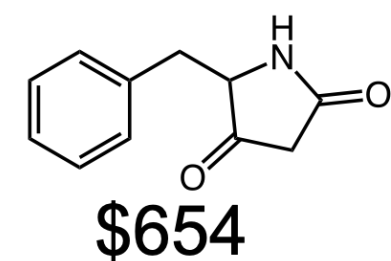
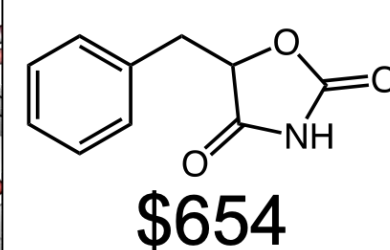
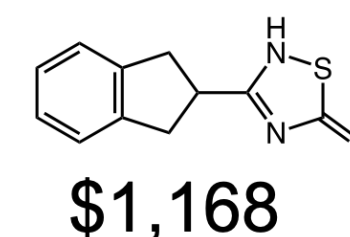
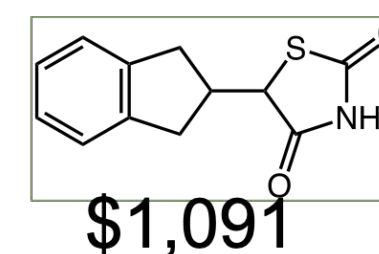
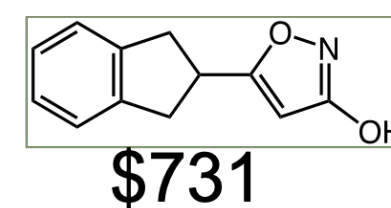
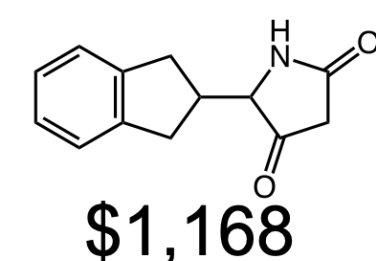
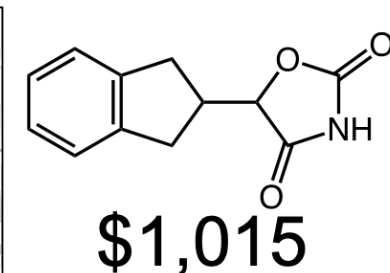
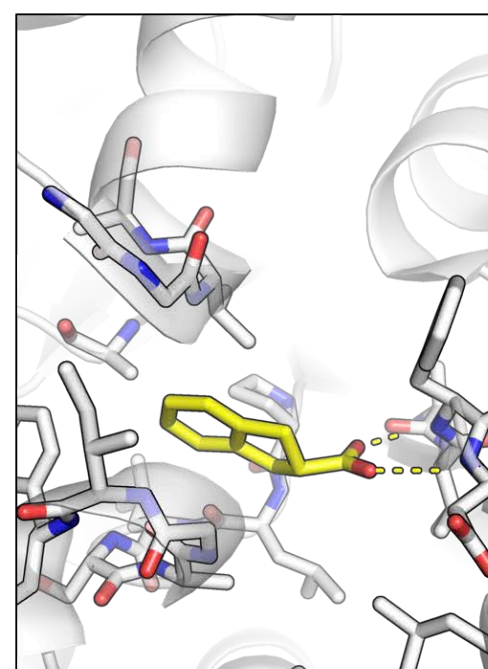
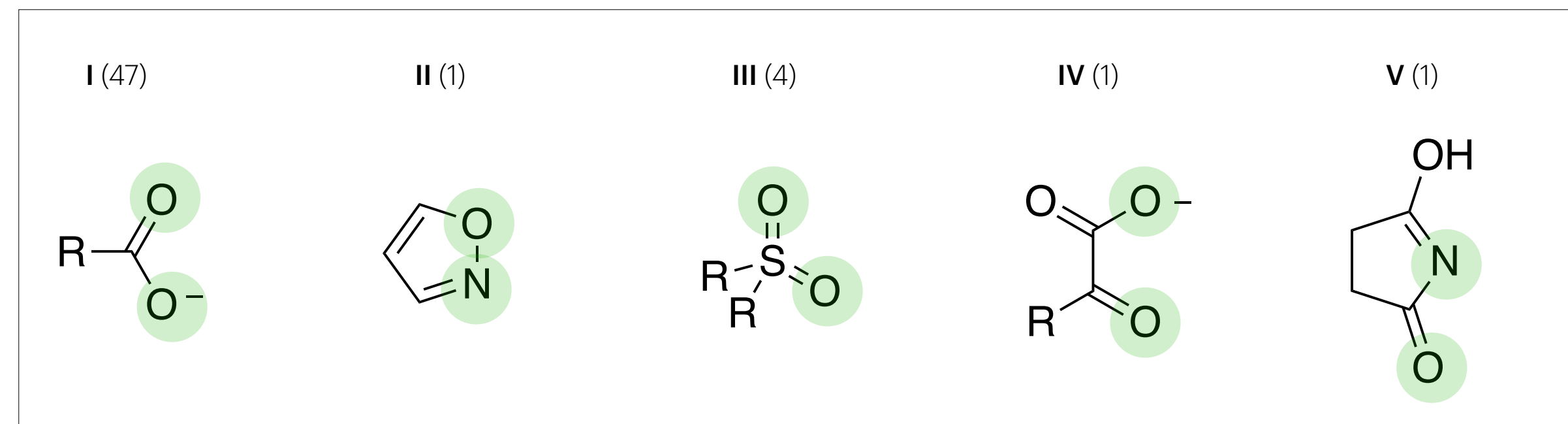


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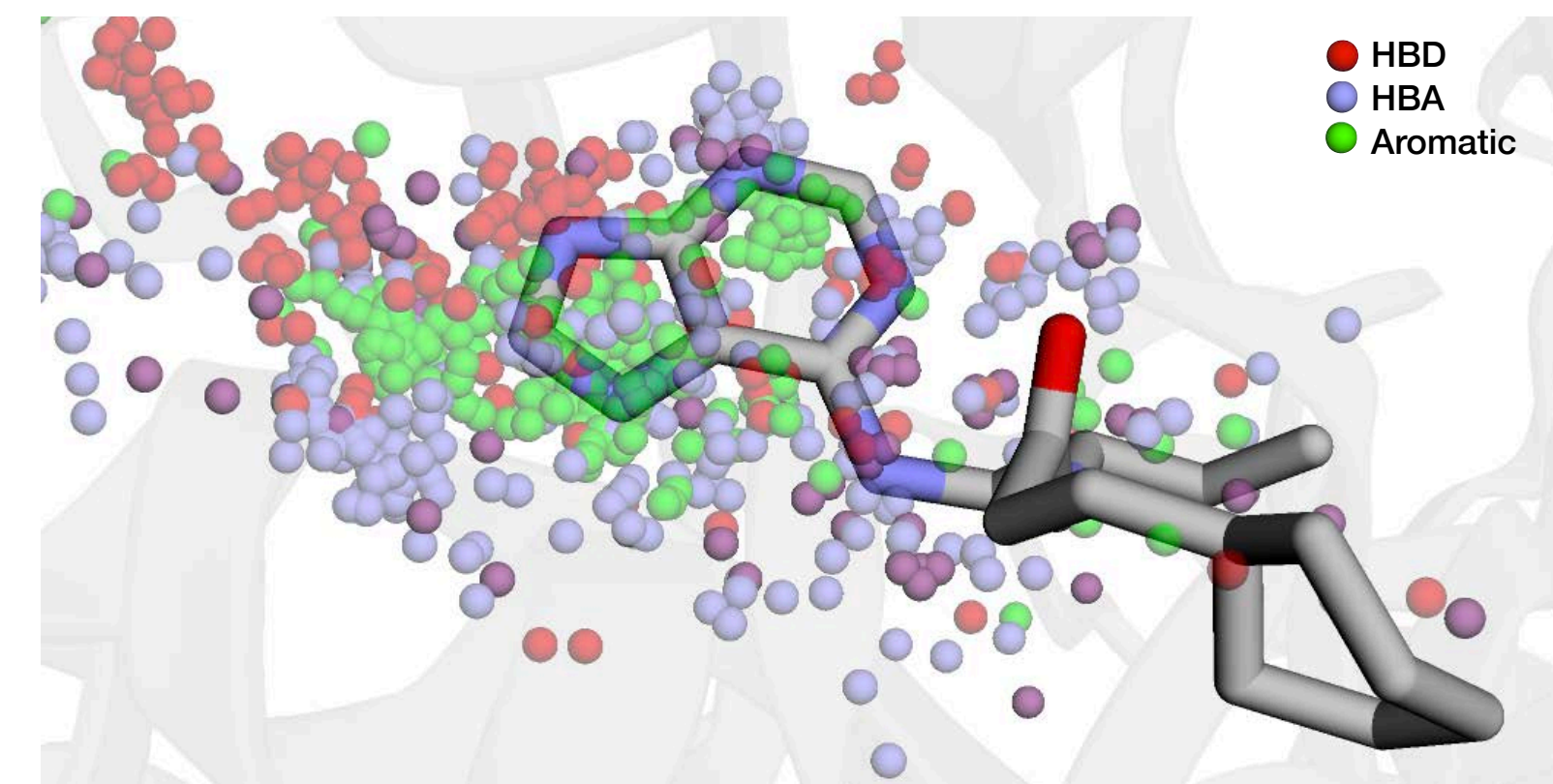
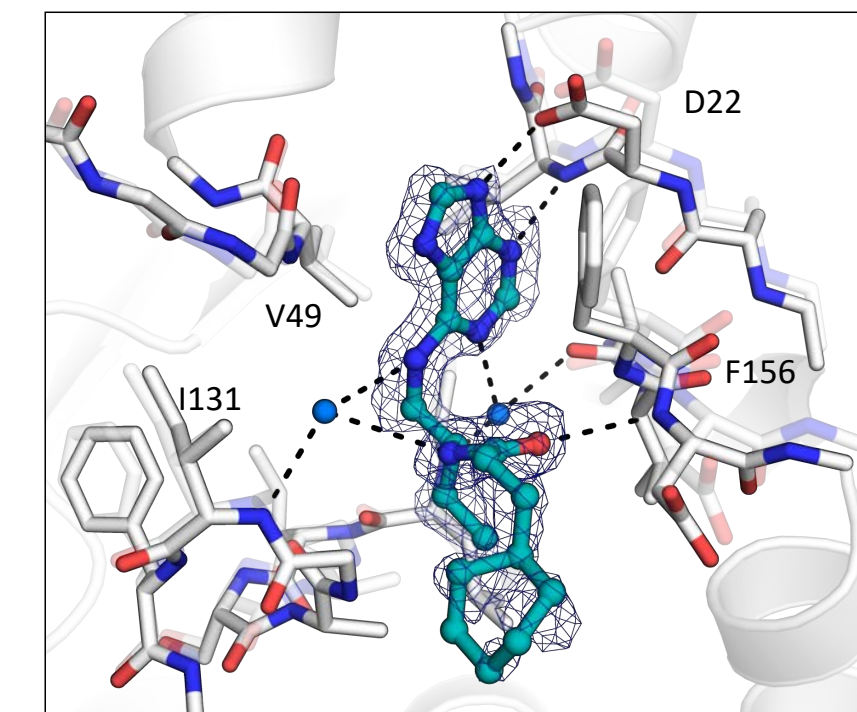
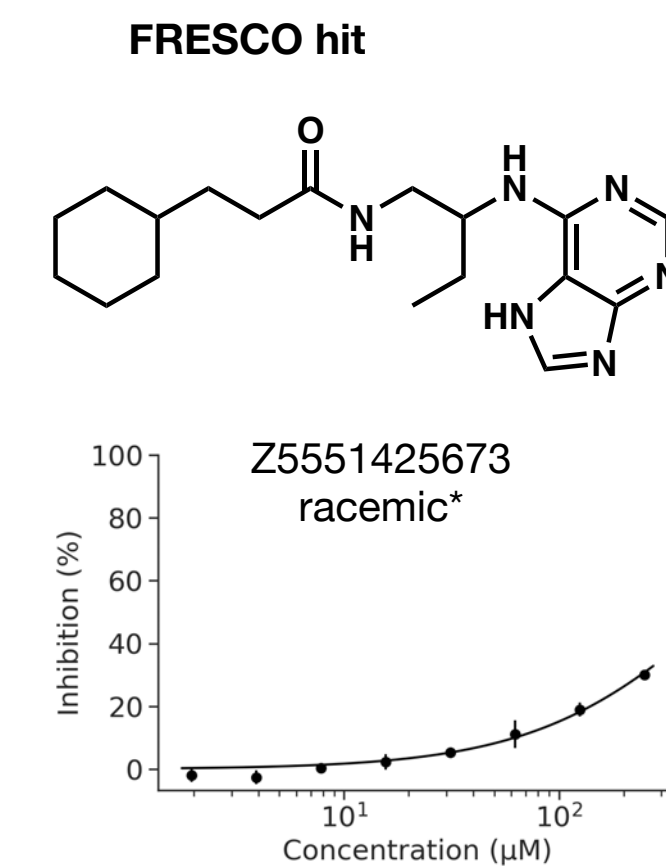
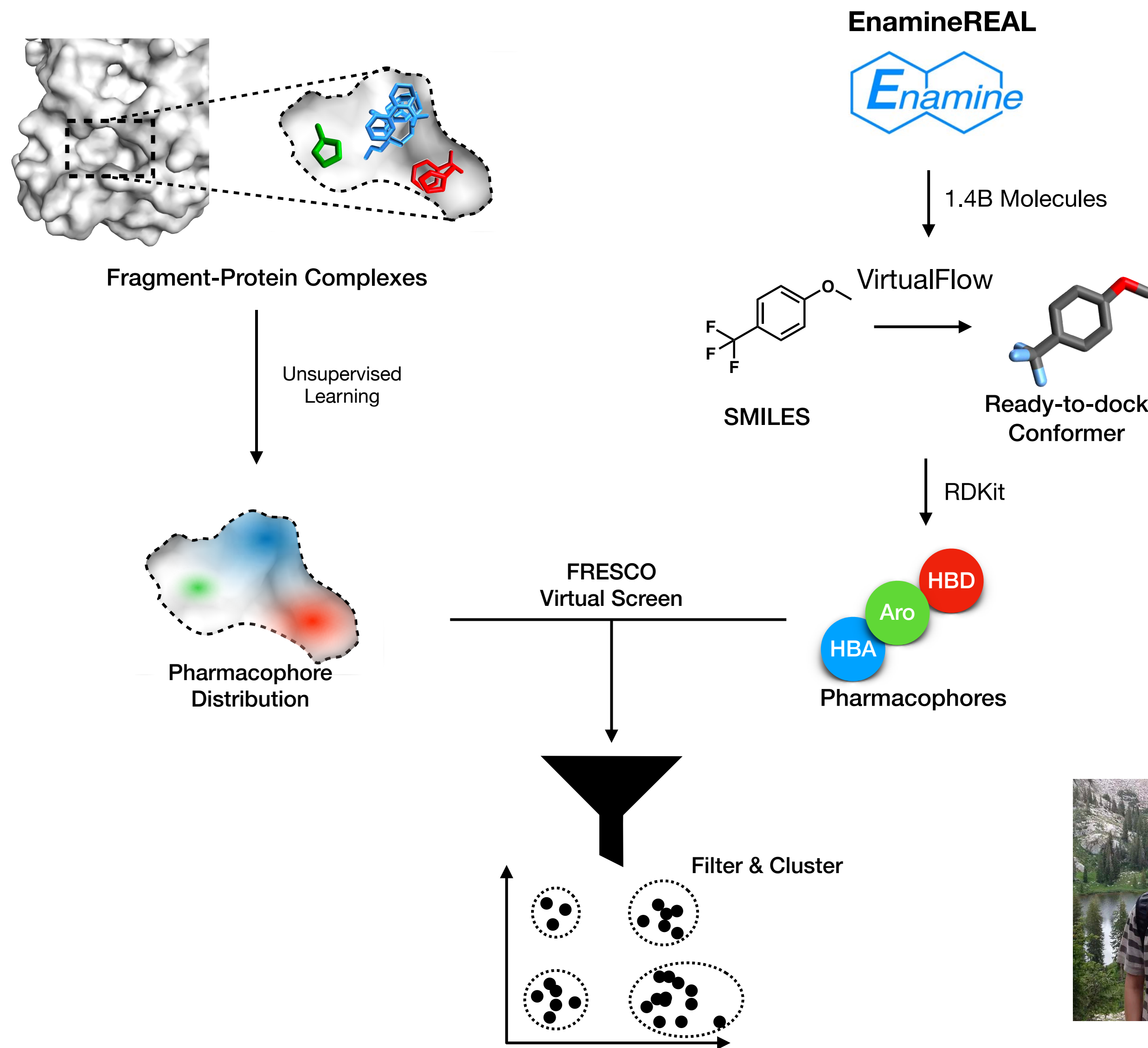


Attempting to overcome permeability liability with bioisostere analogs

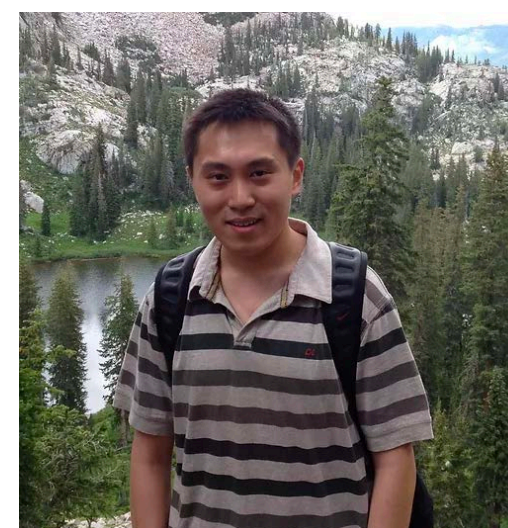
Five fragment classes binding in the **oxyanion site**



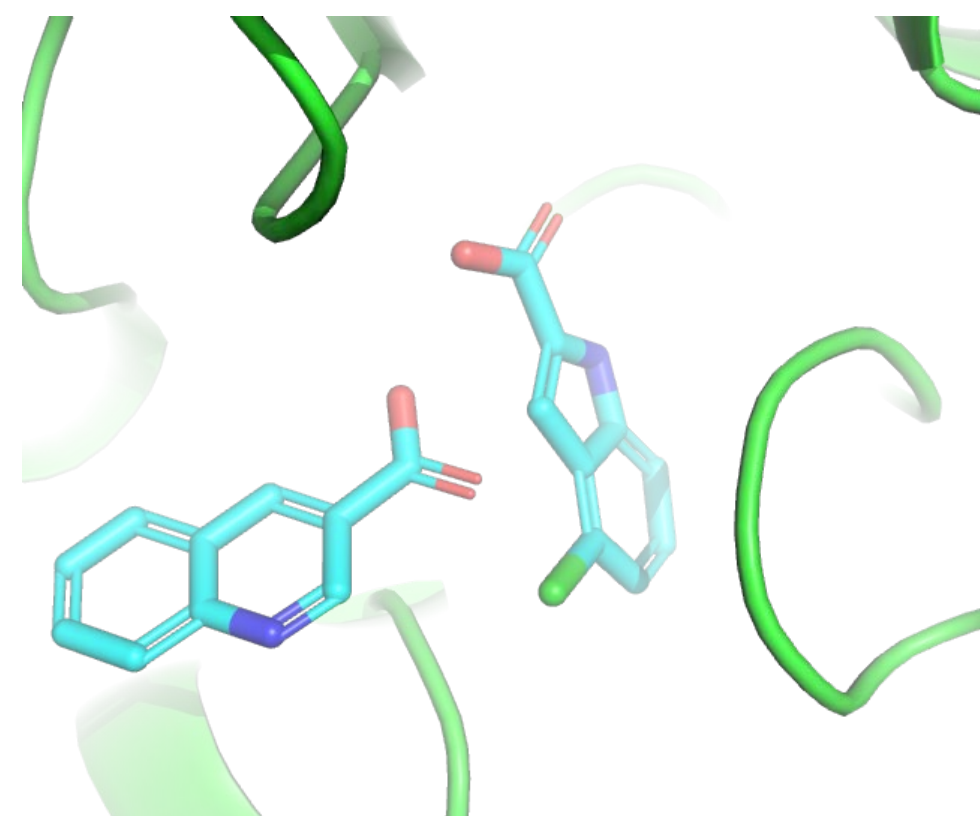
Pharmacophores learned from fragment data on Mpro, applied to Mac1



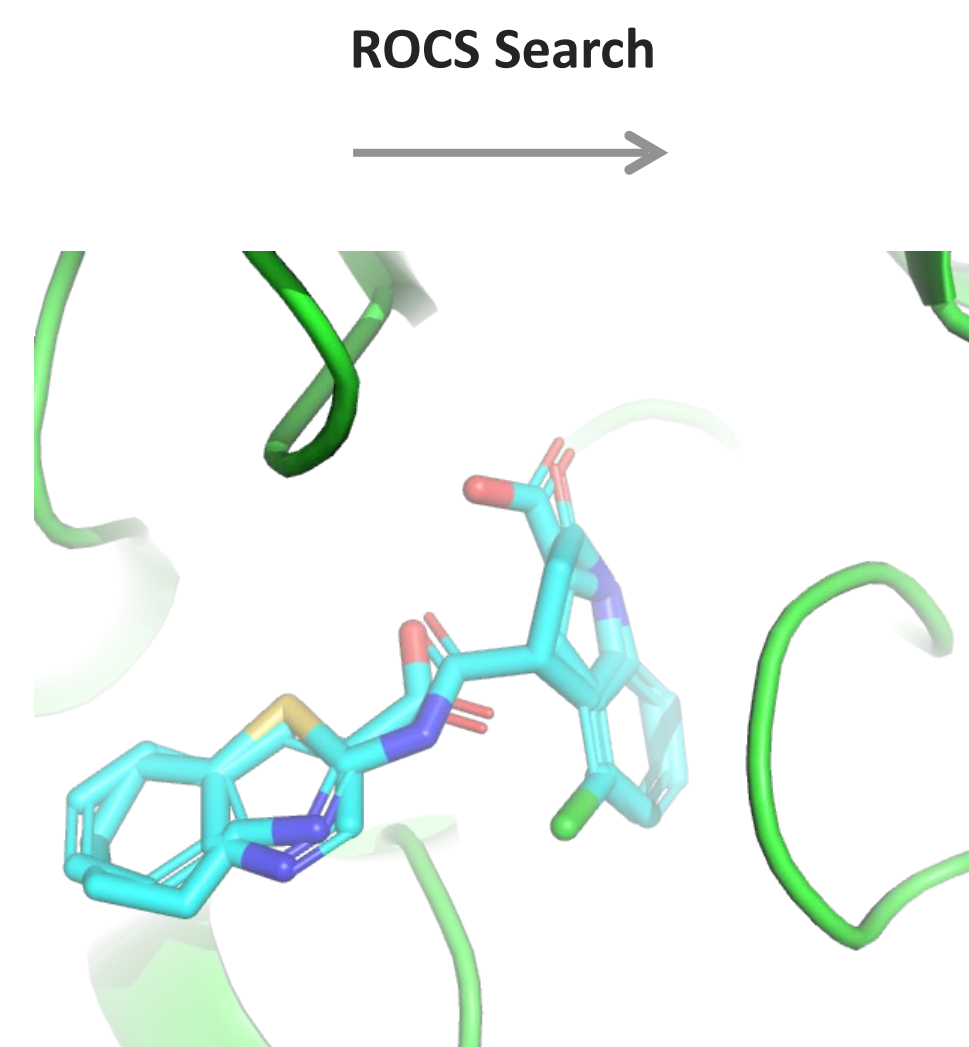
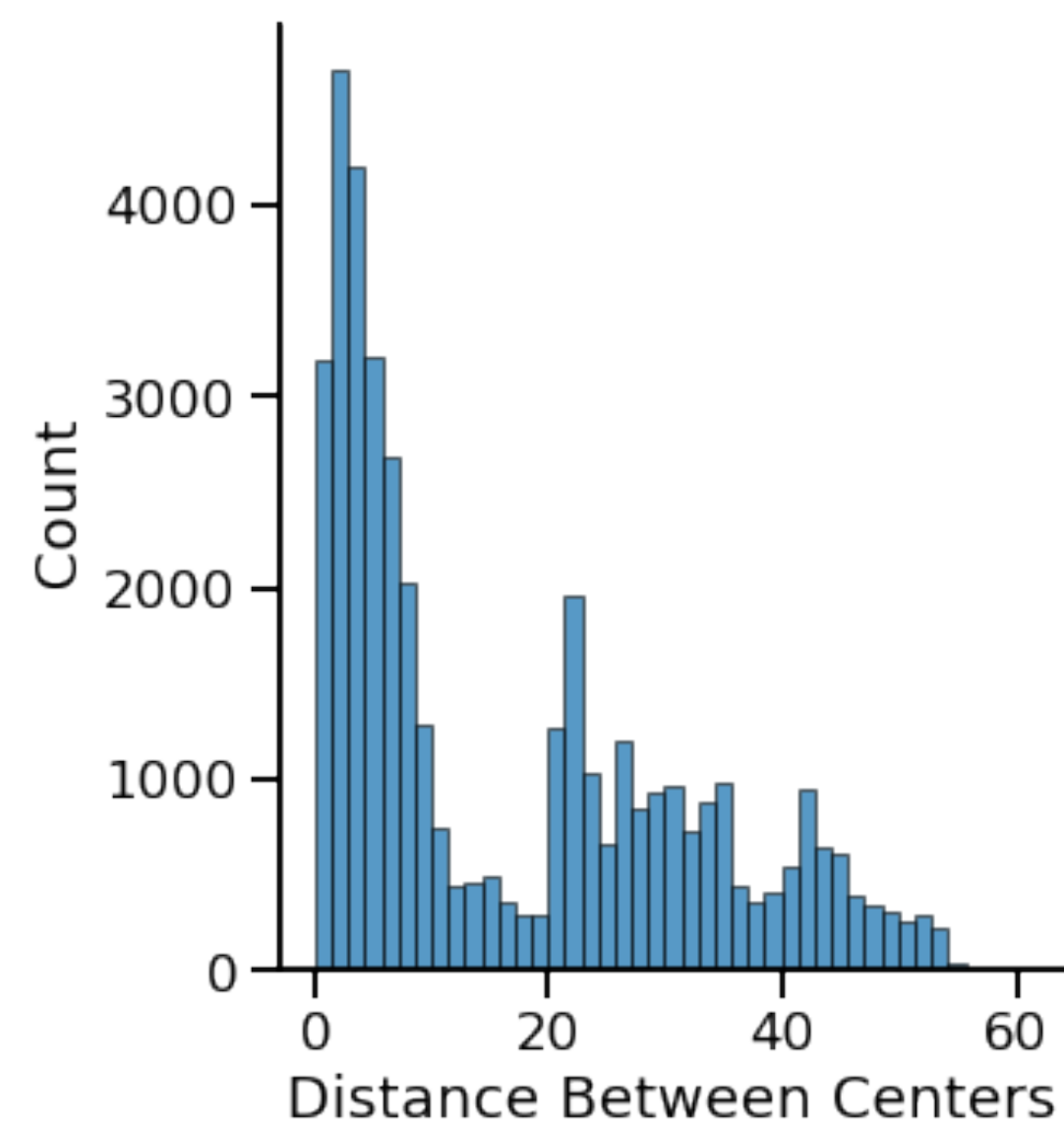
Alpha Lee (Cambridge/PostEra)
William McCorkindale



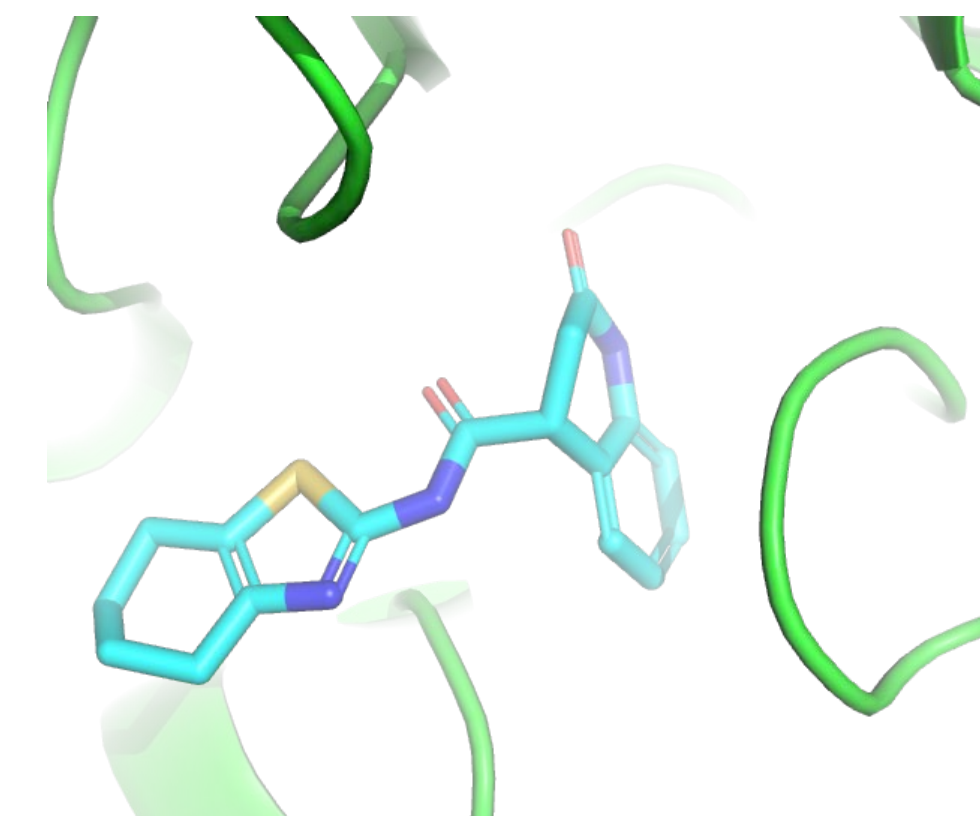
ROCS shape-based searching to identify mergers



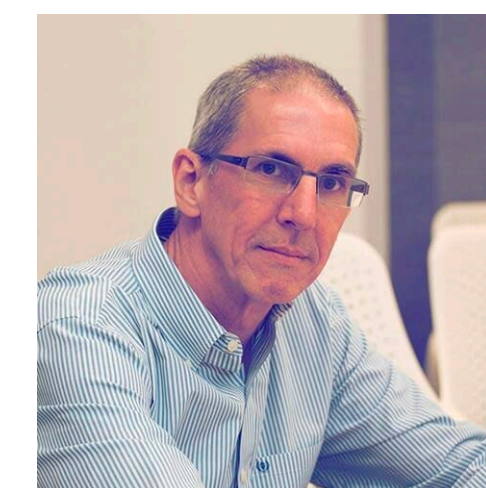
Identify fragment pairs



ROCS Search



Identify commercial analogs

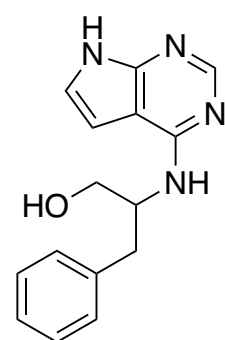


Pat Walters (RelayTx)



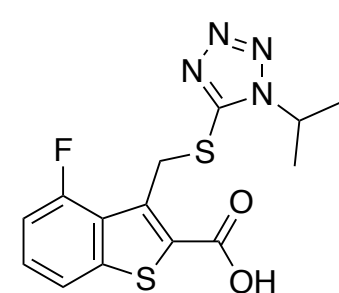
Shape-based approach gives distinct starting points

Z1129899617



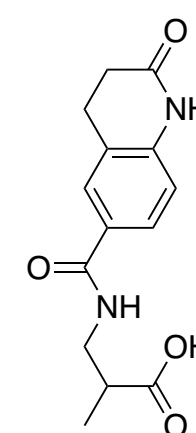
HTRF IC₅₀ = 110 μ M

Z226416444



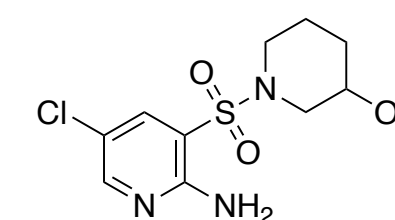
HTRF IC₅₀ = 640 μ M

Z1445218850



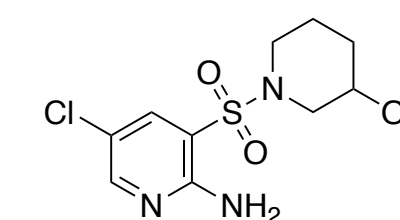
HTRF IC₅₀ = 130 μ M

Z1498462888

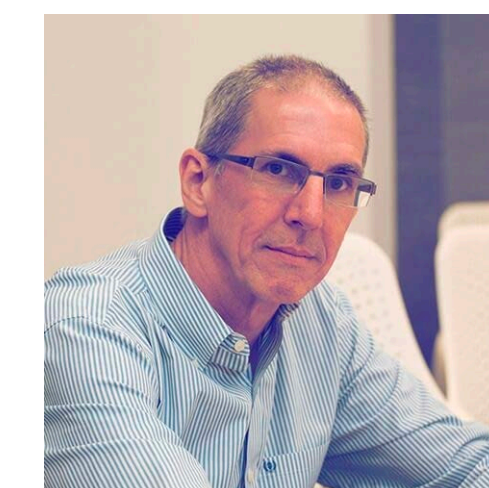
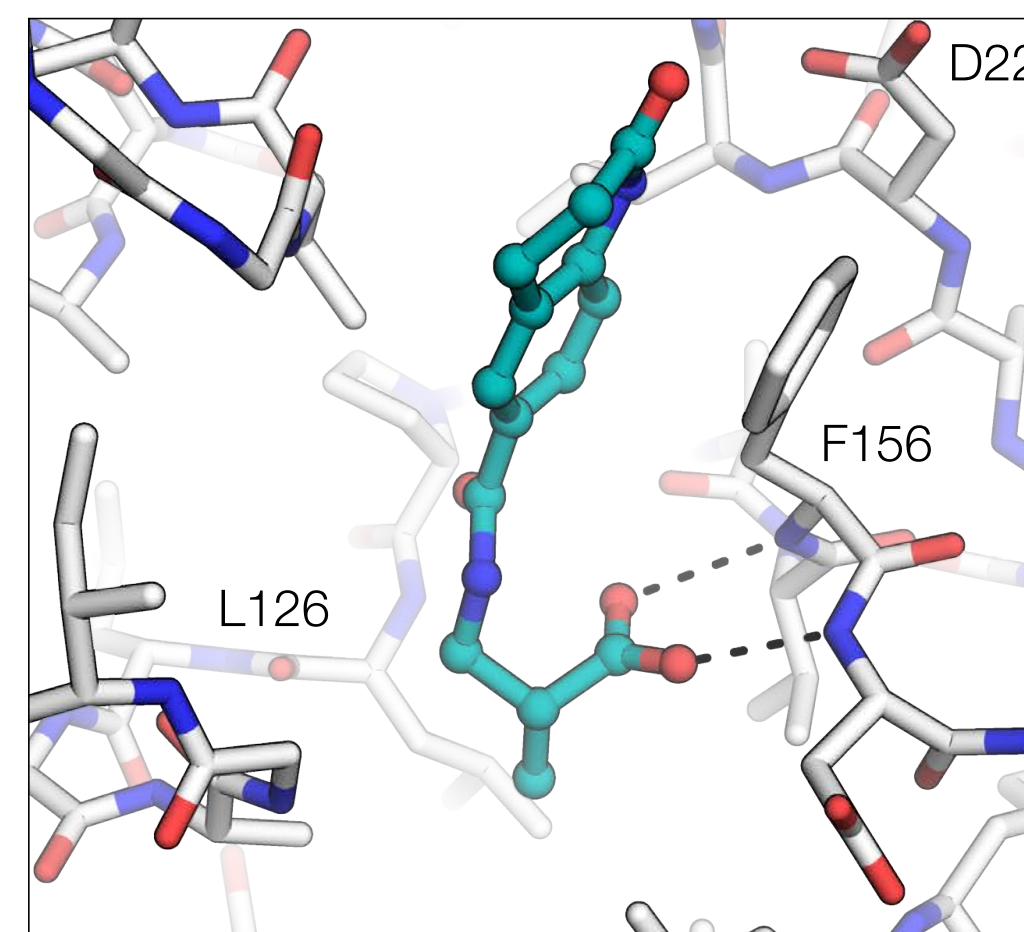
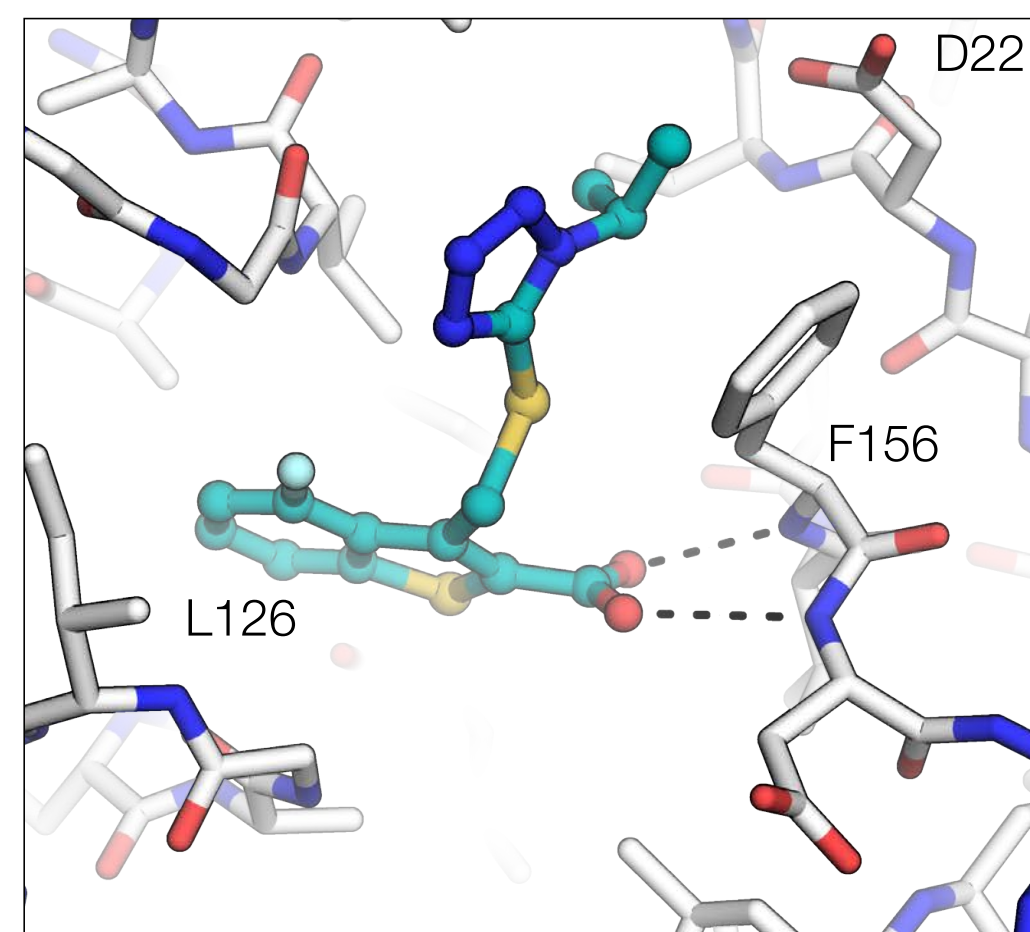
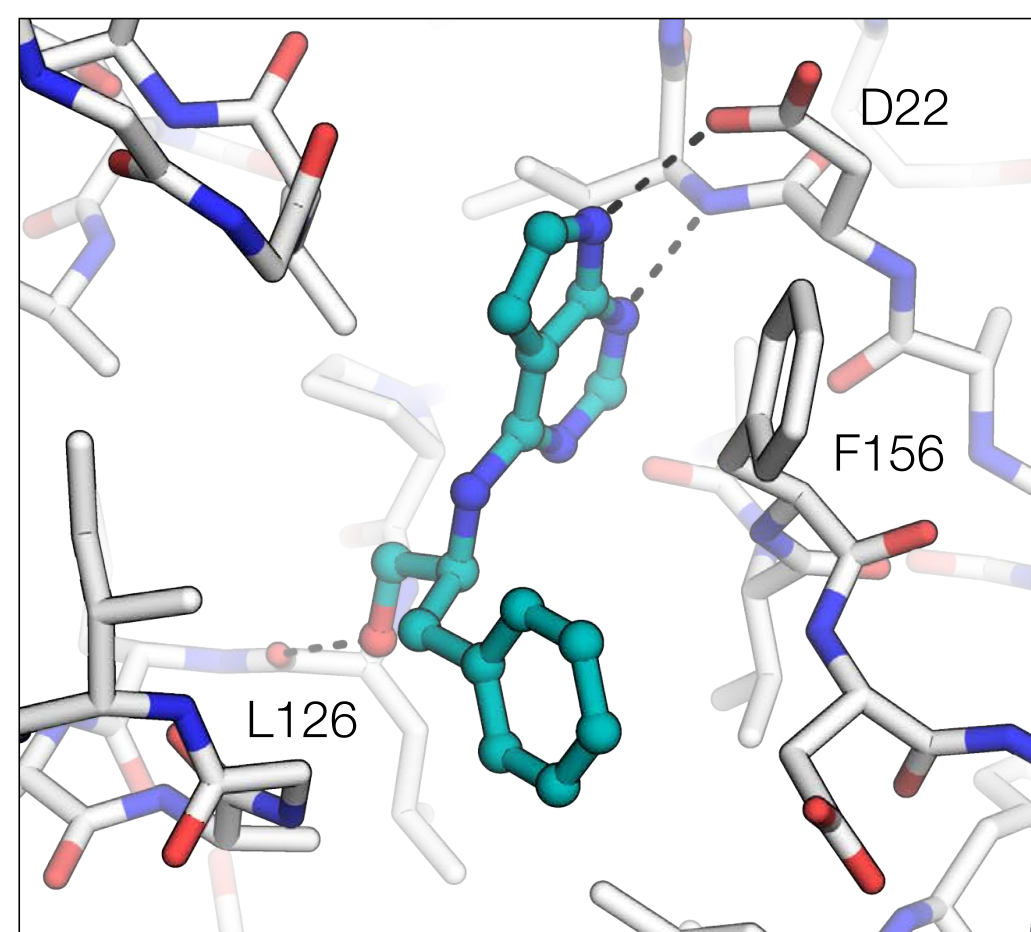


HTRF IC₅₀ = 56 μ M

Z1498497575



HTRF IC₅₀ = 54 μ M



Pat Walters (RelayTx)



10/17 mergers correspond to input fragments, 3 match partially, 4 find new pockets

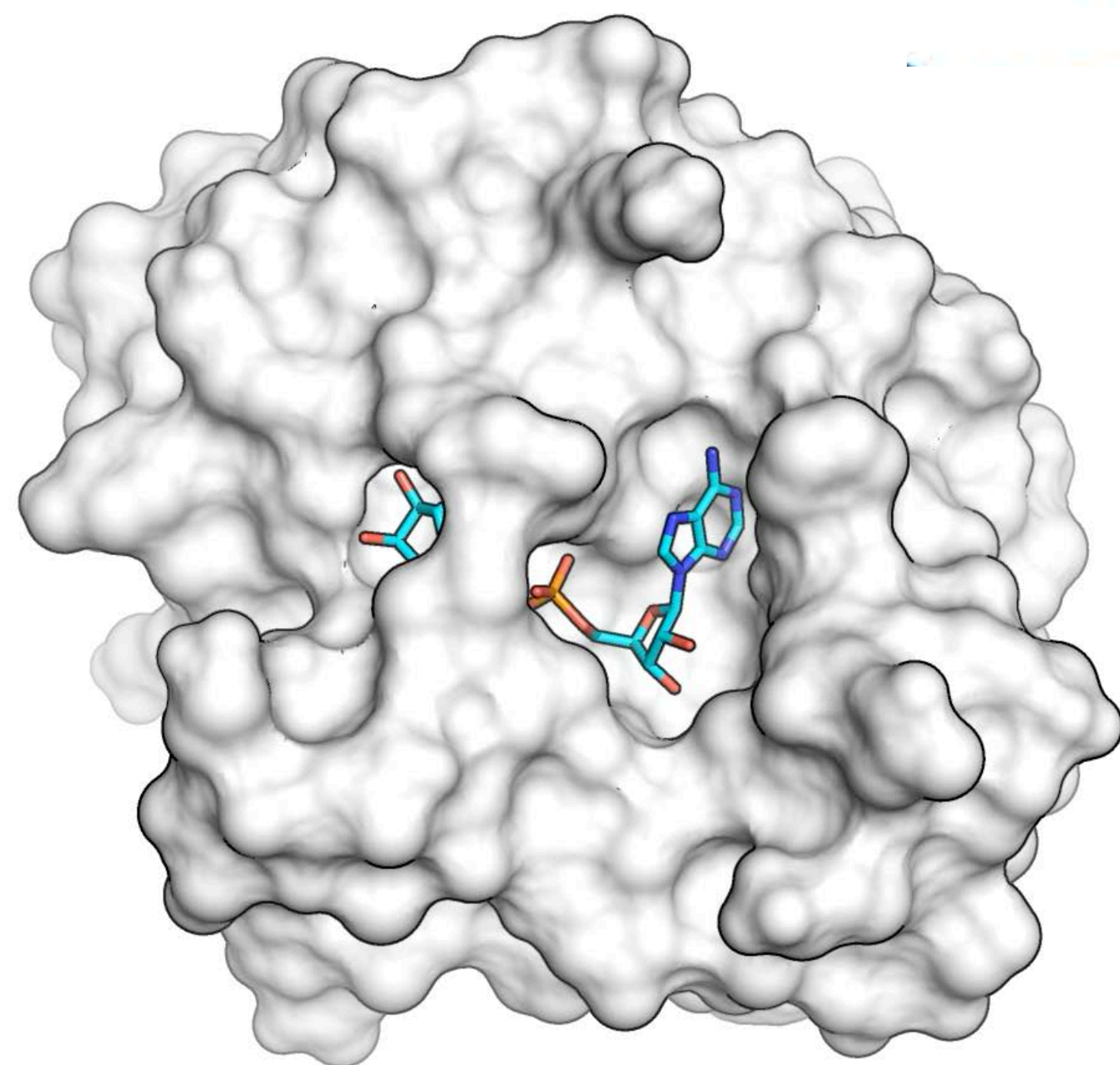
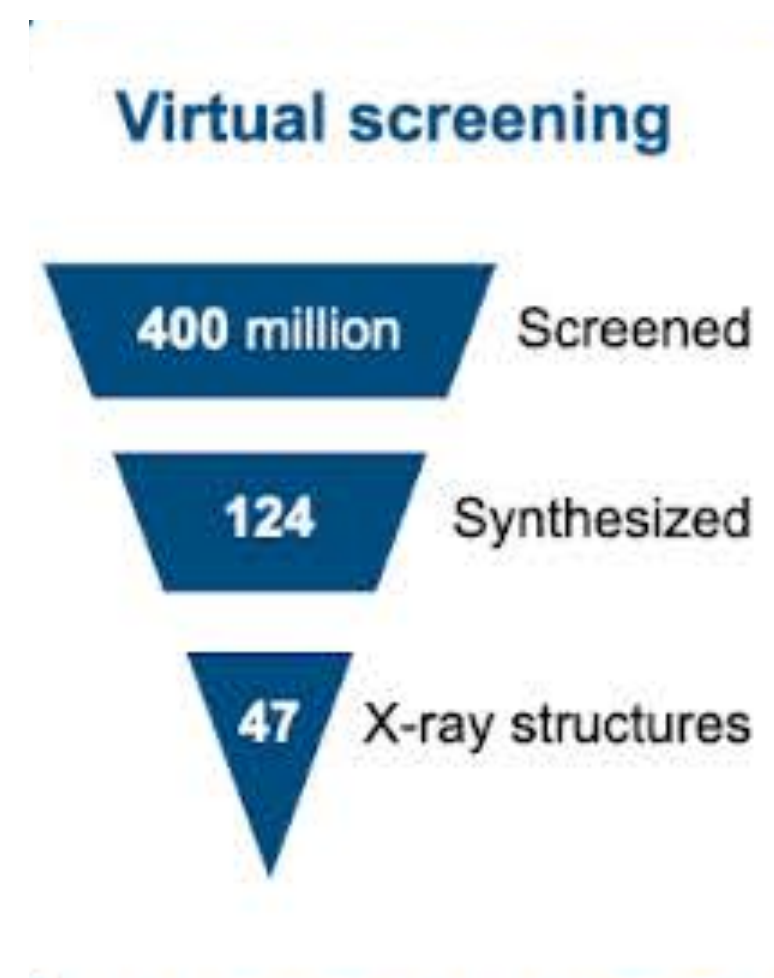
Screening and Structural Core have discovered new hits with docking



**Prof. Brian
Shoichet**



**Dr. Stefan
Gahbauer**



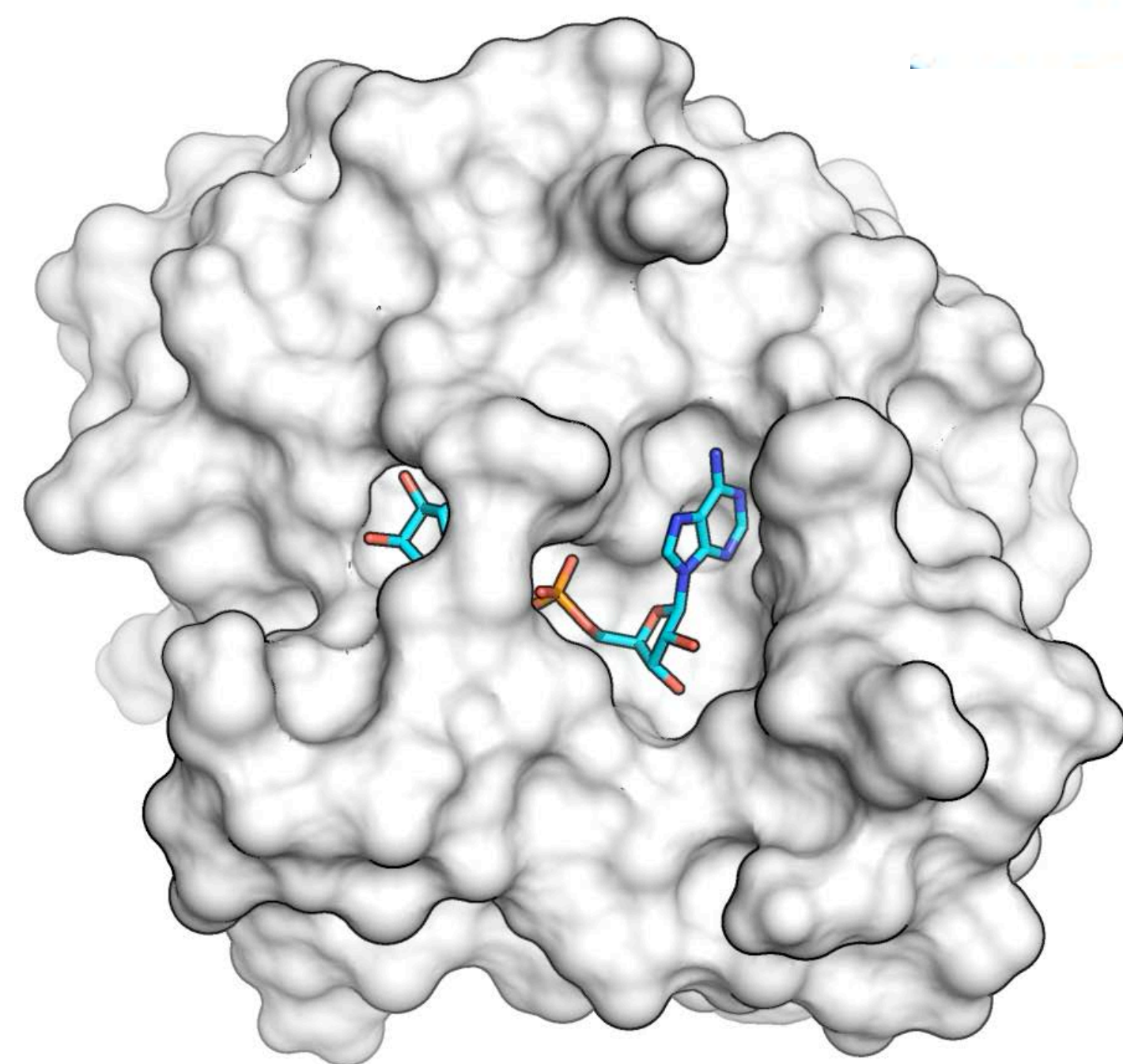
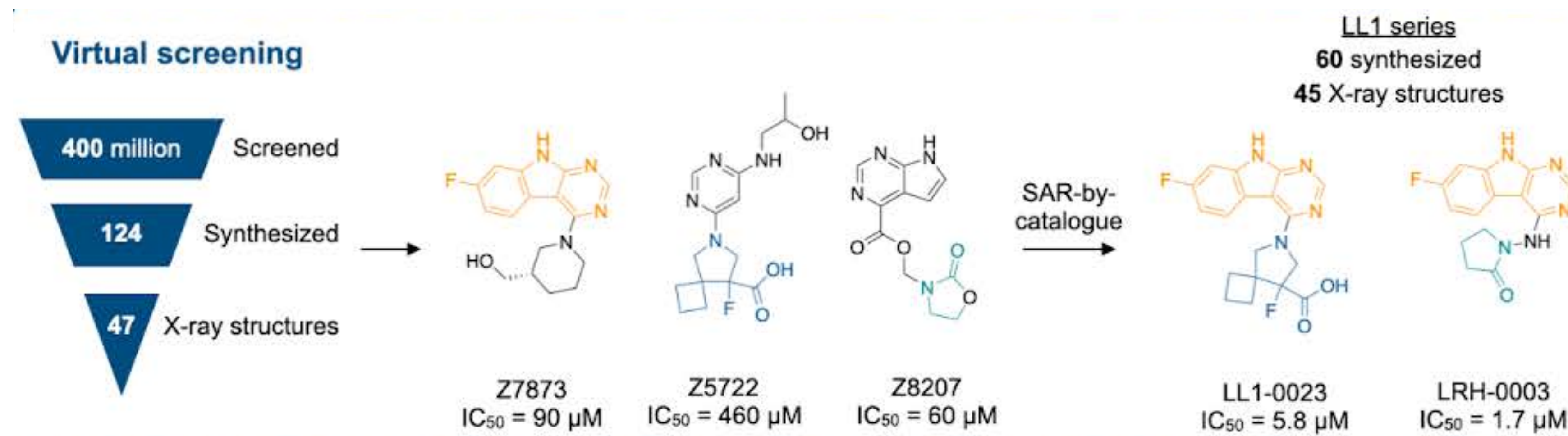
Docking has identified additional leads, with encouraging permeability



Prof. Brian
Shoichet



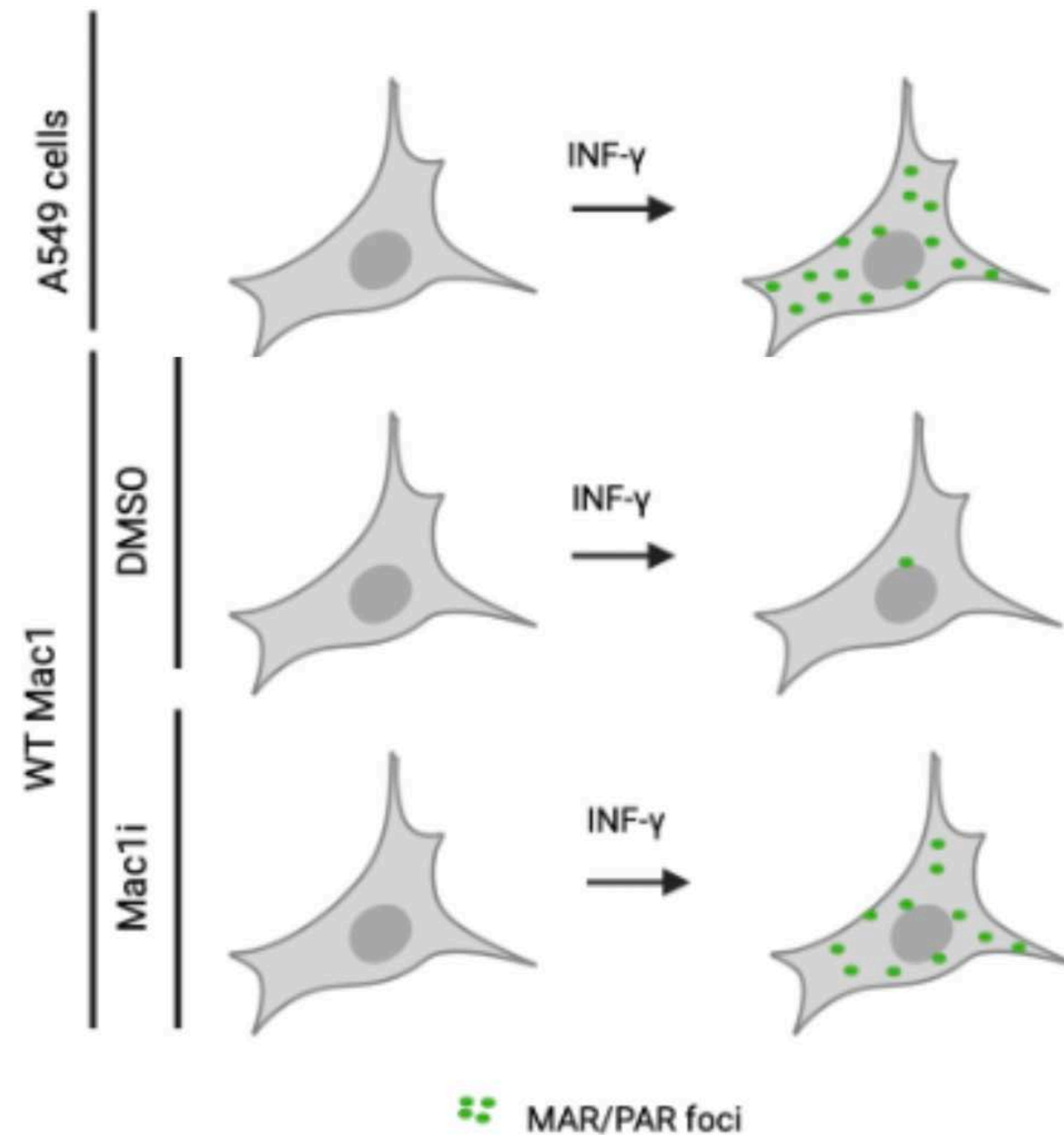
Dr. Stefan
Gahbauer



LRH-0003 and LRH-0021 obtained high permeability values in MDR1-MDCKII cell-based assays of 138 and 120 nm/s in apical to basal and 243 and 91 nm/s in basal to apical direction, respectively.

Gahbauer*, Correy* et al, BioRxiv, 2022

Interferon stimulation induces ADPr **puncta**



Dr. Morgan
Diolaiti

Dr. Manasi
Jogalekar

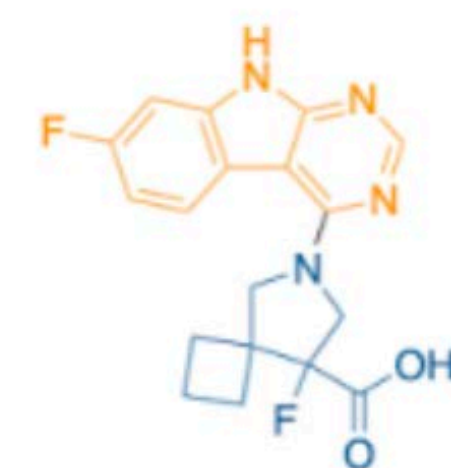
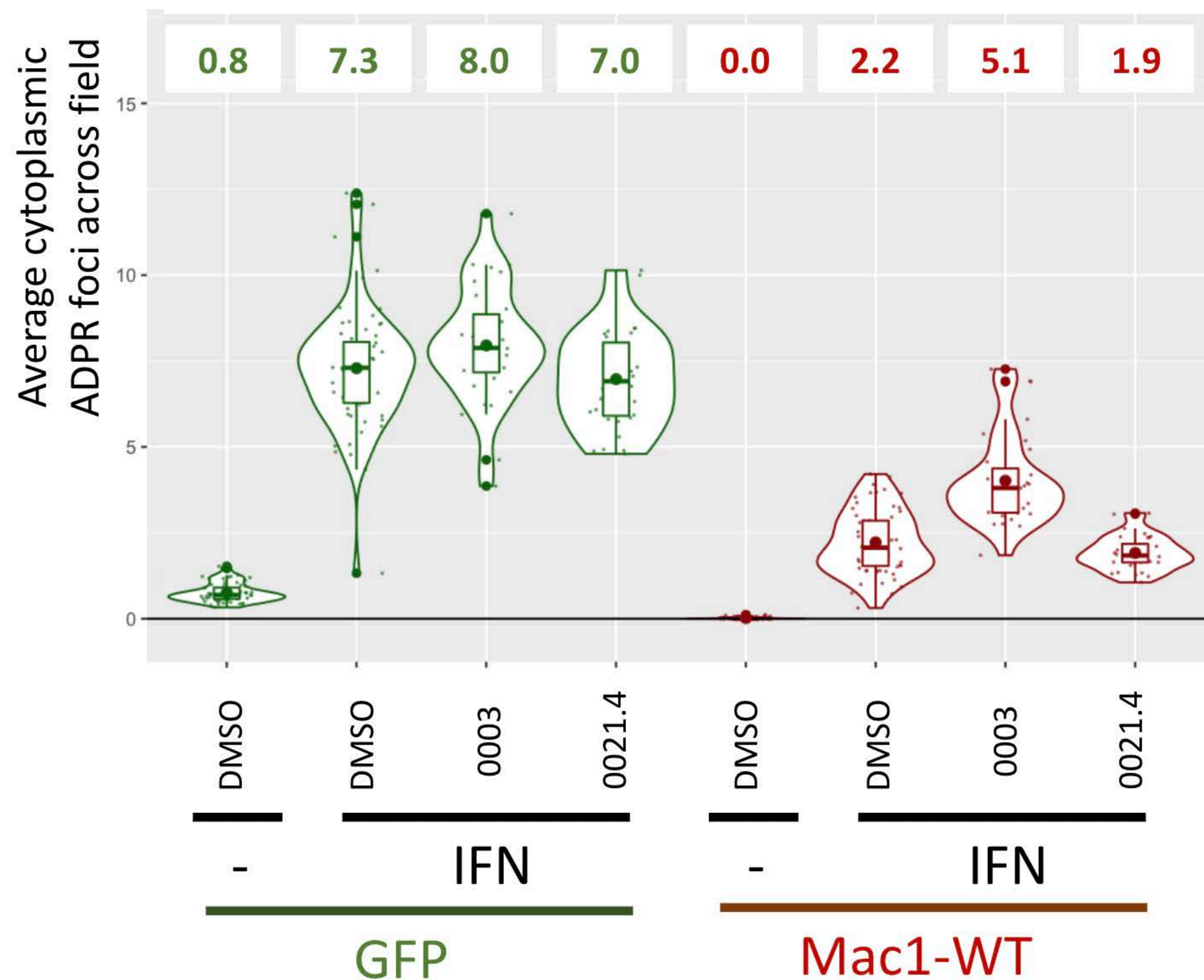


Prof. Alan
Ashworth

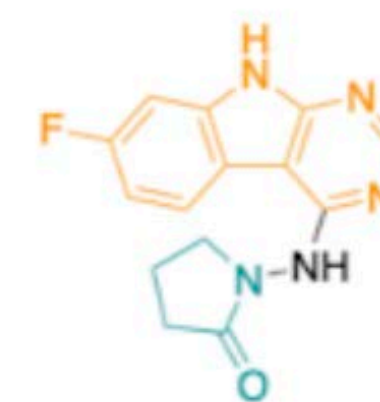


Dr. Paddy
O'Leary

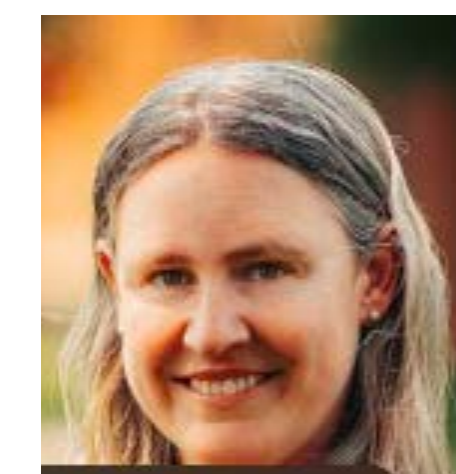
Cellular activity from docking ketone series



LL1-0023
 $IC_{50} = 5.8 \mu M$



LRH-0003
 $IC_{50} = 1.7 \mu M$



Dr. Morgan
Diolaiti



Dr. Manasi
Jogalekar

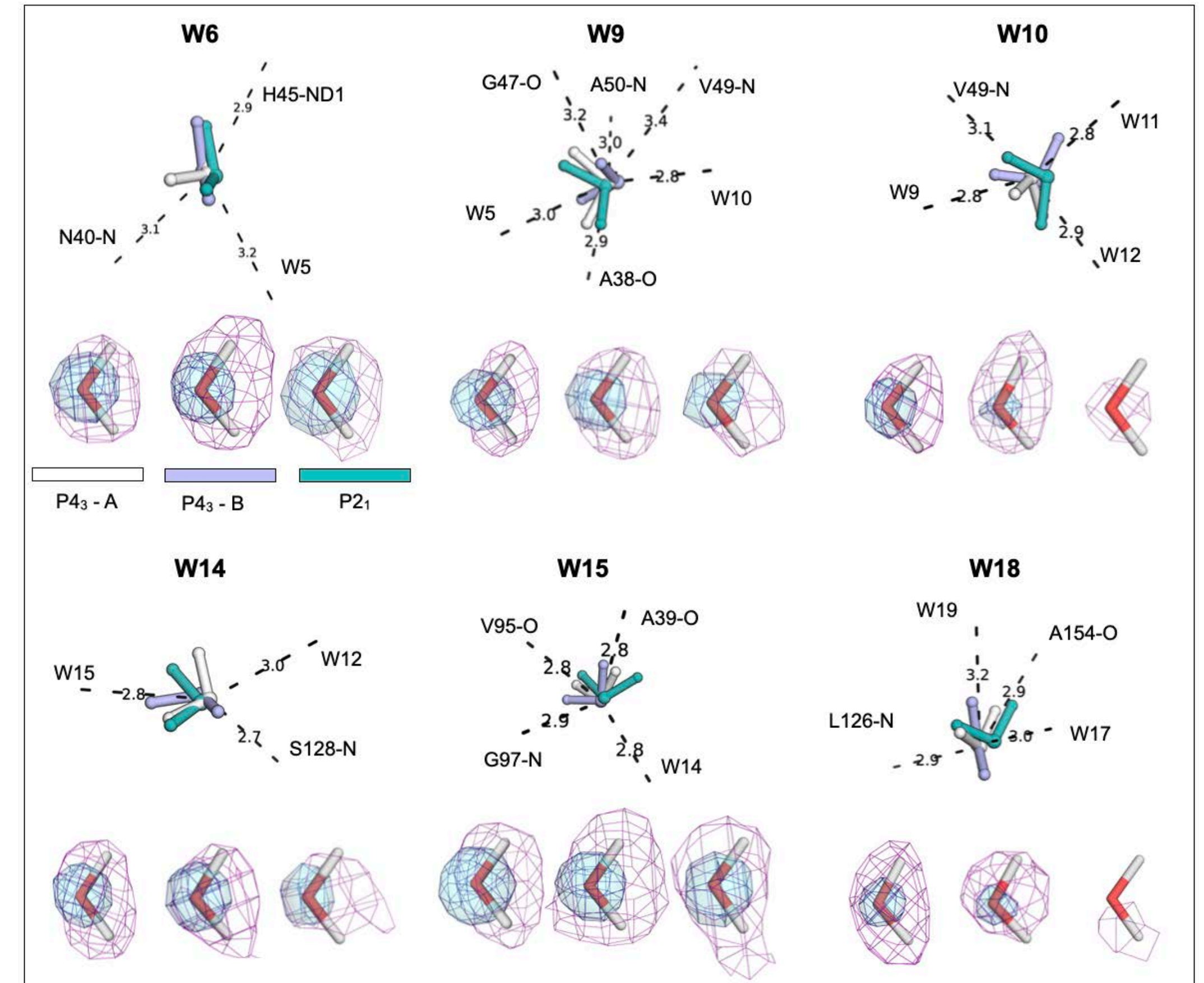
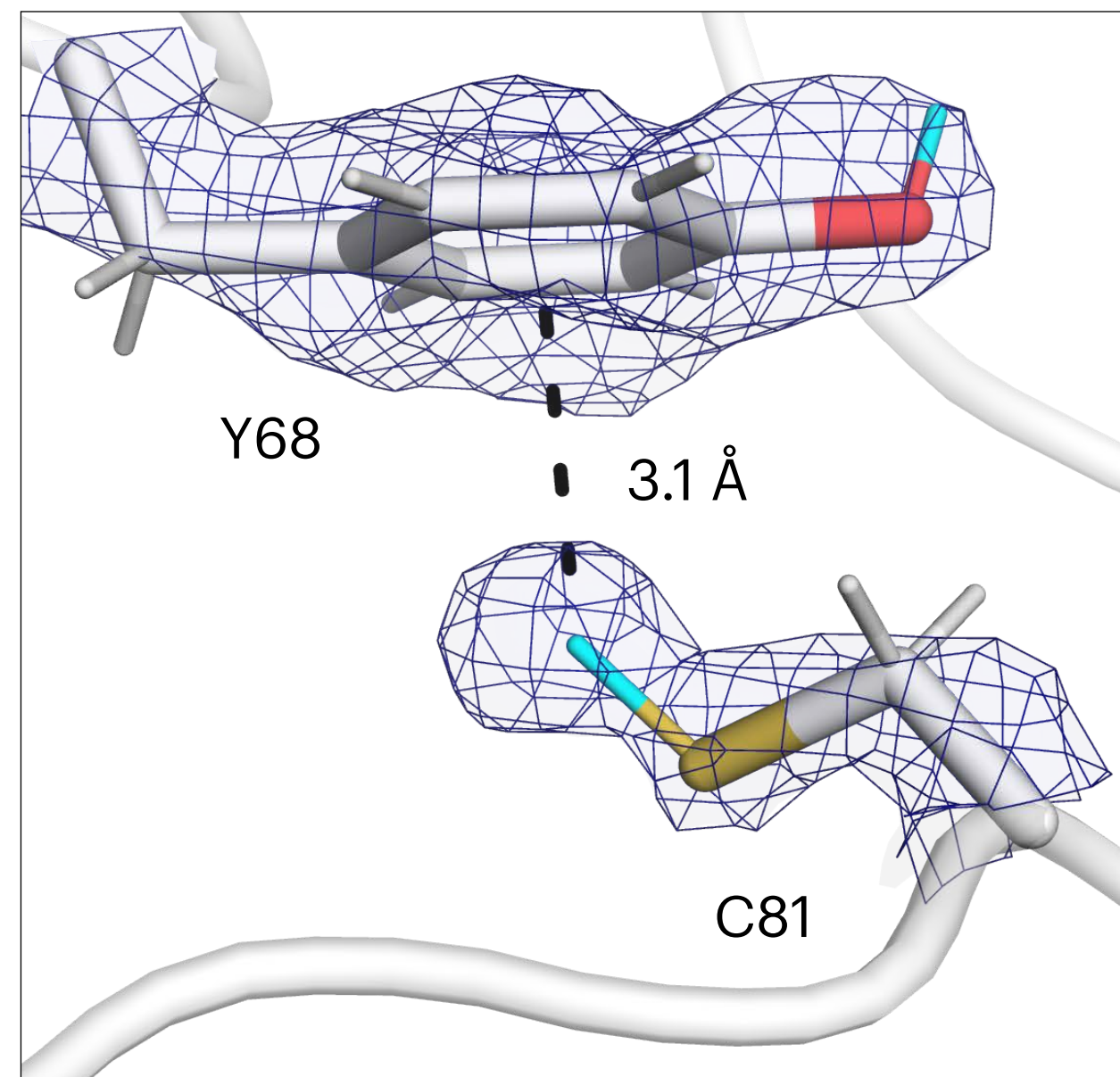


Prof. Alan
Ashworth

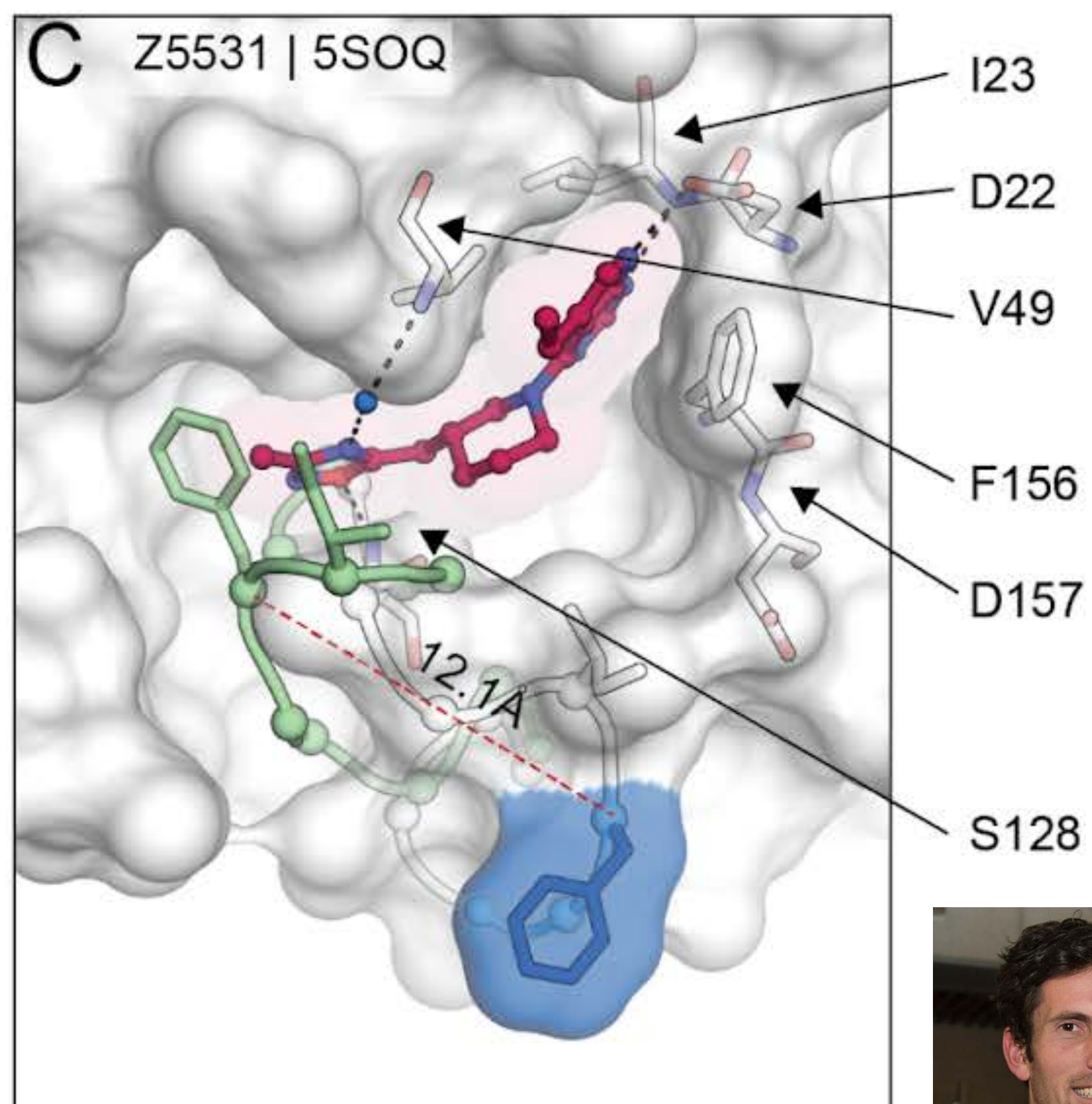


Dr. Paddy
O'Leary

Neutrons are cool!!



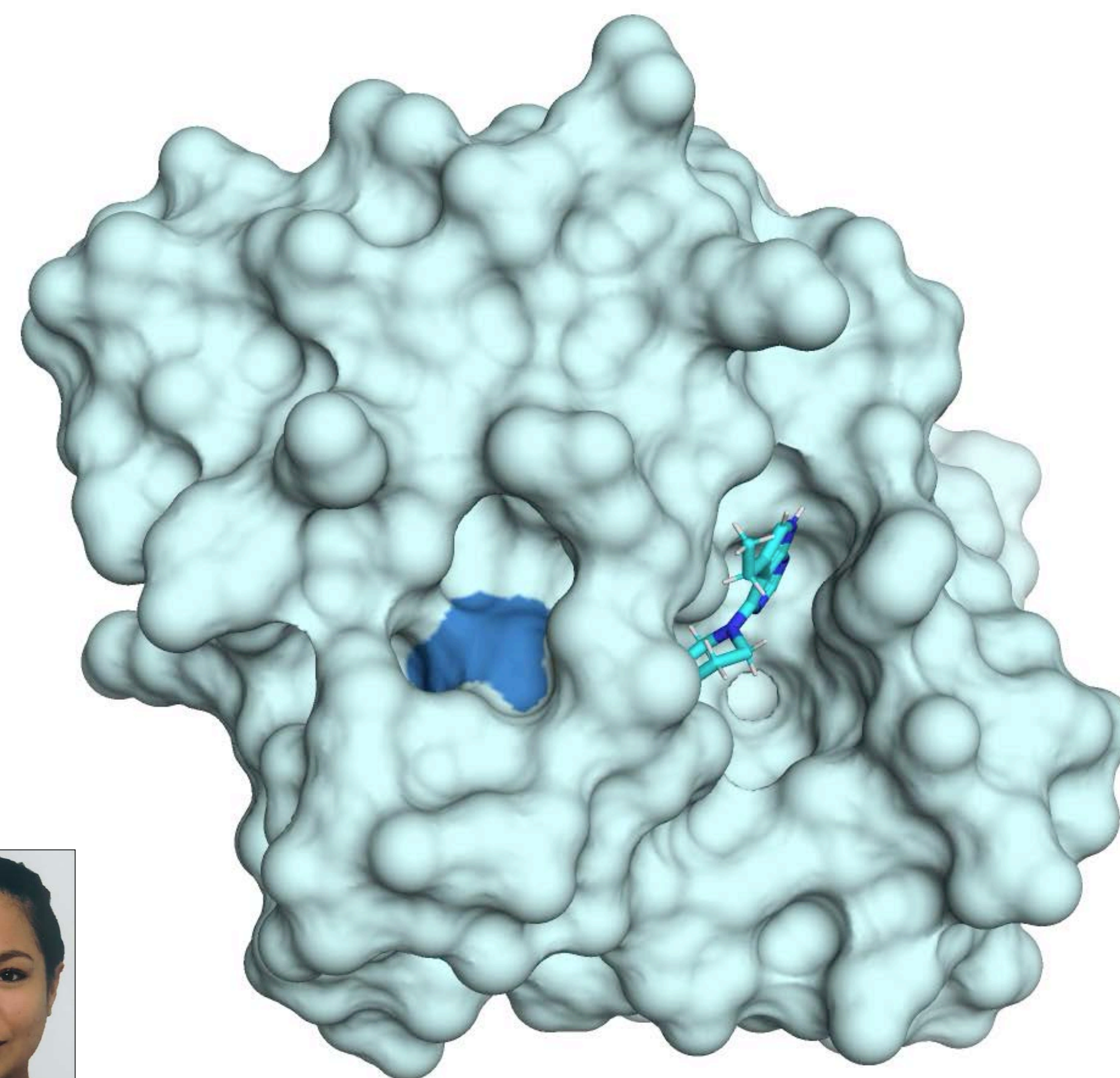
Some docked compounds stabilize a new “**open**” state



Dr. Galen
Correy



Dr. Moira
Rachman



QCRG: Team Macrodomain



Dr. Taha Y Taha



Dr. Galen
Correy



Yagmur Doruk



Dr. Moira
Rachman



Dr. Lena
Bergmann



Dr. Stefan
Gahbauer
(Now at
Deep Apple)



Dr. Morgan
Diolaiti



Dr. Manasi
Jogalekar



Dr. Jack Moen



Dr. Ryan
Gonciarz



Dr. Paddy
O'Leary



Krogan



Renslo



Shoichet



Ashworth



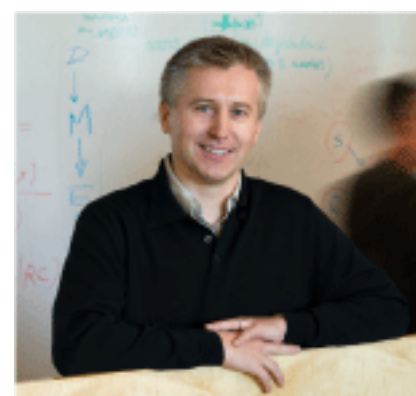
Zuliana-Alvarez



Swaney



Ott



Sali



THE FRASER LAB



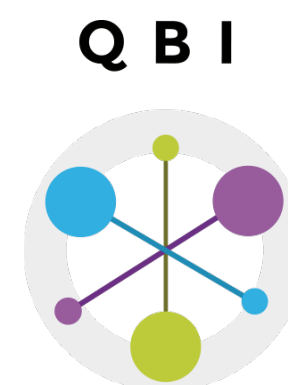

Marion Schuller, Daren Fearon
Frank von Delft, Ivan Ahel
(Oxford)

Galen
Correy



**Michael
Thompson**
(UC Merced)

ALS: James Holton, George Meigs
SSRL: Aina Cohen, Silvia Russi,
 Clyde Smith, Lisa Dunn, Jeney Wierman
NSLS-II: Martin Fuchs, Alexei Soares



Conflict of Interest:
Equity, Consulting,
Funding



Brian
Shoichet



**Stefan
Gahbauer**

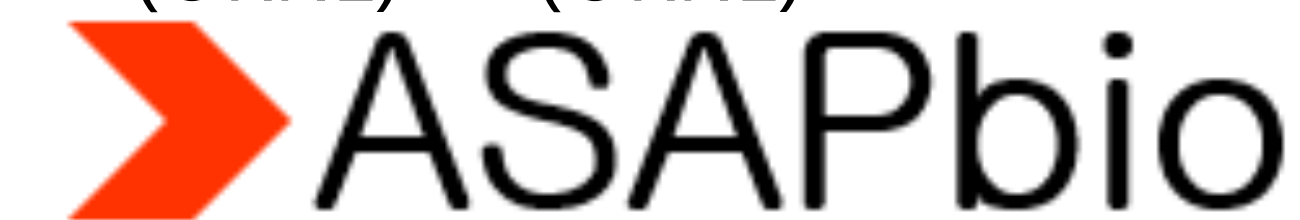


Alan
Ashworth

Lena
Bergmann



Andrii Kovalevsky (ORNL)
 Flora Meilleur (ORNL)



POST PREPRINTS!

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