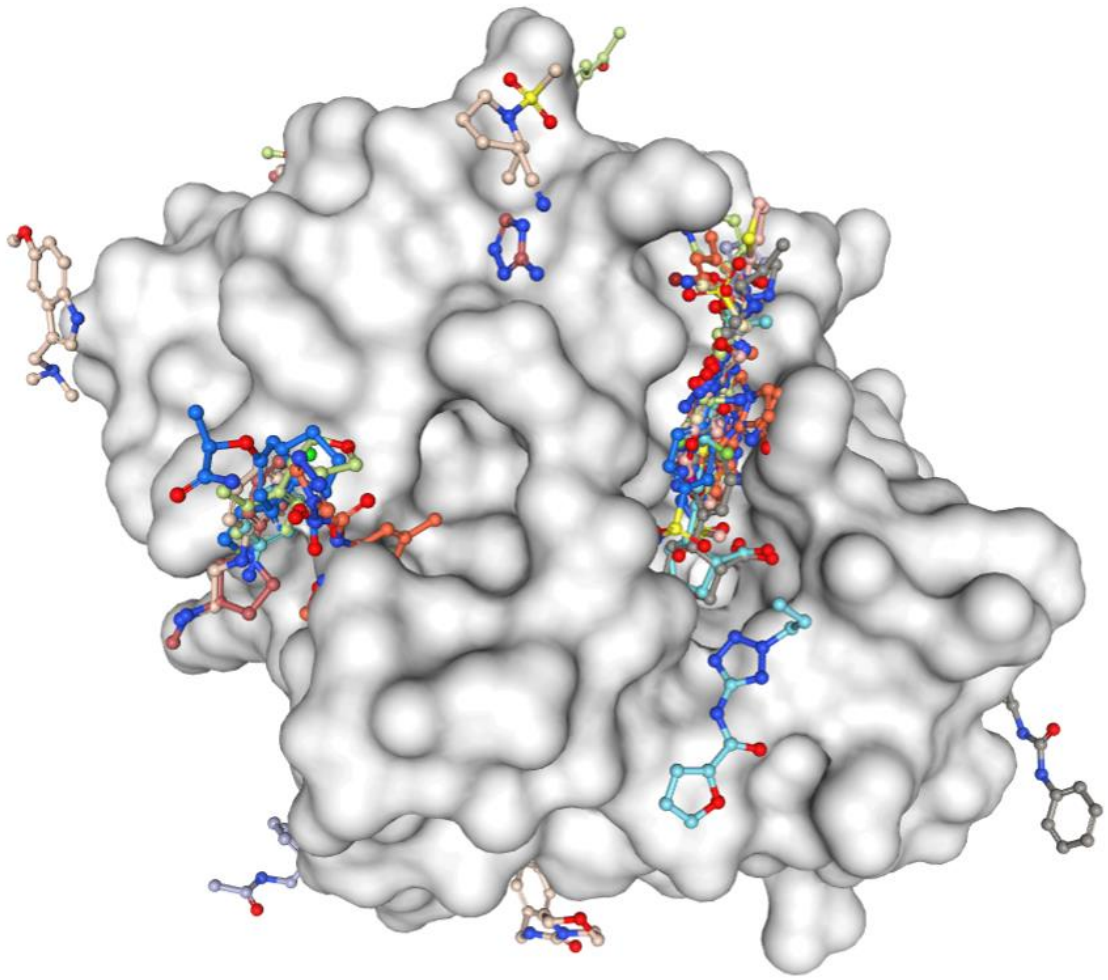
Nsp3 macrodomain ADP-ribosyl hydrolase and XChem fragment screen

Summary



In light of the ongoing coronavirus (COVID-19) pandemic, the Ivan Ahel Laboratory at the University of Oxford joined together with the XChem team at Diamond Light Source to contribute to the current global efforts discovering and developing much-needed novel antiviral therapeutic possibilities. For this, the Ivan Ahel team has been able to rapidly solve the SARS-CoV-2 macrodomain structure to near atomic resolution, and then, together with Frank von Delft's XChem team, completed a large crystallographic fragment screen against this target.

The macrodomain (also called Macro X domain) is a 150 amino acid protein module with (ADP-ribosyl)-hydrolase activity that is a part of the SARS-CoV-2 multidomain protein nsp3. ADP-ribosylation is a reversible post-translational modification of proteins synthesised by the PARP family of enzymes, and regulates many pathways in human cells, including the DNA damage response and antiviral defences. Several of the human PARPs such as PARP10, PARP13 and PARP14 act specifically as antiviral proteins to generate an antiviral environment and prevent virus replication. In contrast, the viral macrodomain removes the ADP-ribosylation modifications, thereby enabling the virus to counteract the PARP-induced innate immunity (Fehr et al, Trends Microbiol, 2018; Figure 1).

The macrodomain enzyme represents a promising drug target for the treatment of coronavirus infections, since macrodomain-deficient viruses (including all studied coronaviruses, alphaviruses and hepatitis E virus) are unable to replicate in human cells (eg. Fehr et al, mBio, 2016). Nevertheless, no inhibitors for this enzyme have been developed to date.

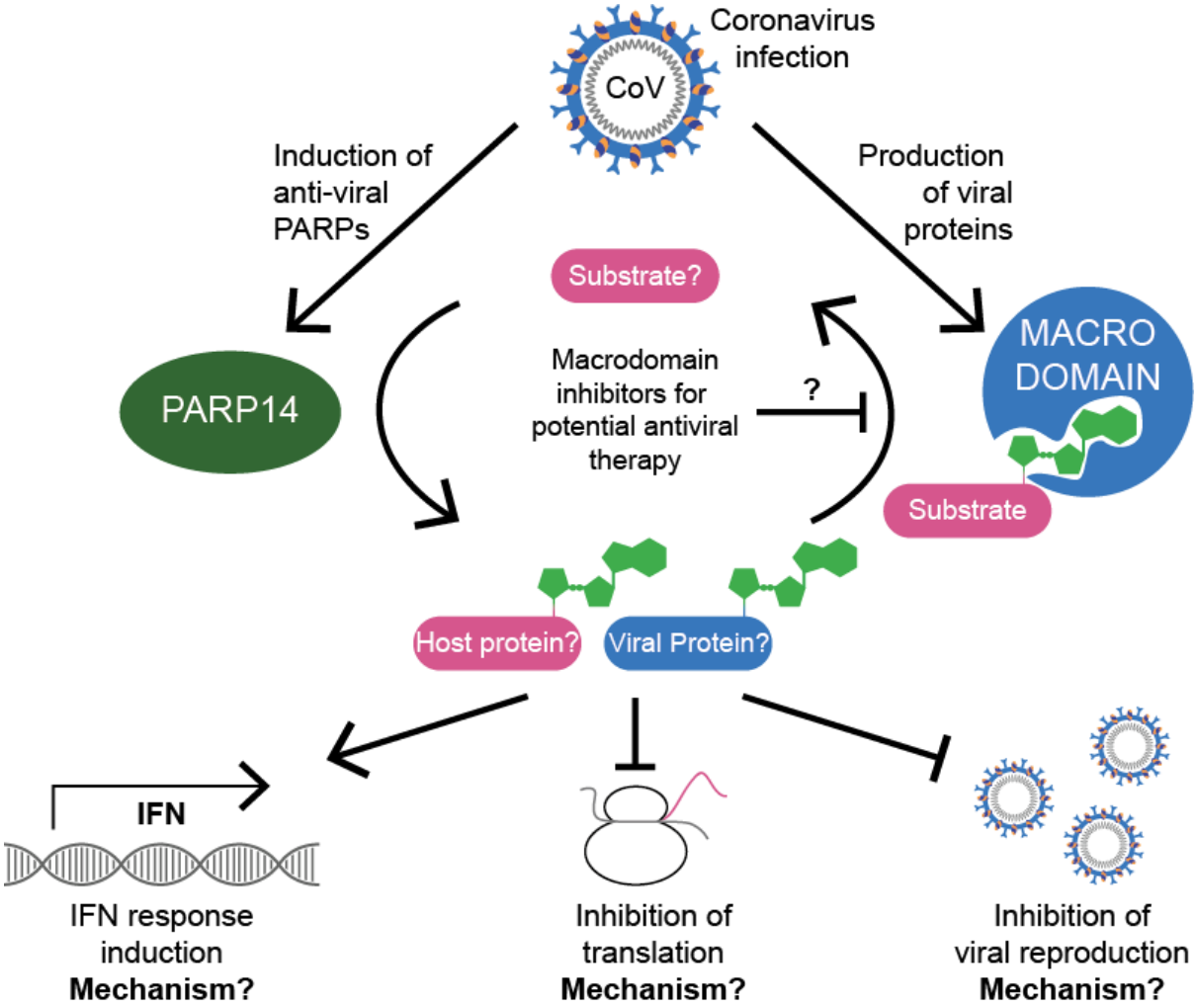
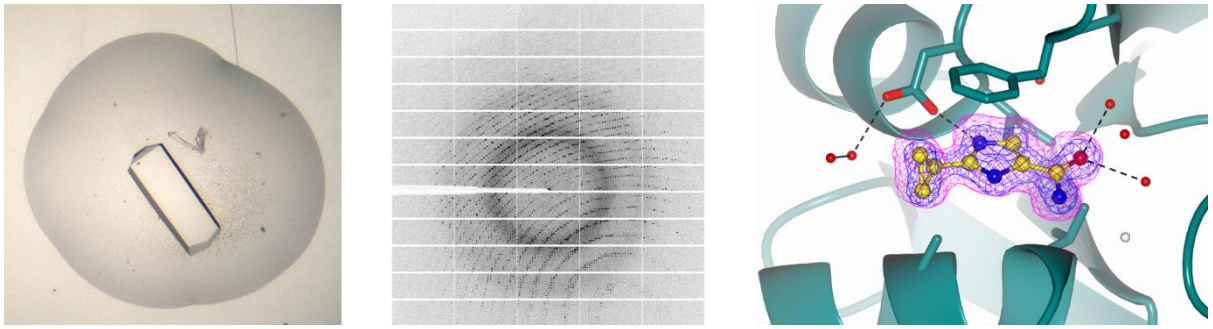


Figure 1. Model for the role of ADP-ribosylation following coronavirus infection. Infection of cells with coronavirus leads to the accumulation of human antiviral proteins as well as viral proteins, such as human PARP14 and viral macrodomain, respectively. The ADP-ribosylation activity of PARP14 leads to the induction of the interferon (IFN) response, down-regulation of translation and prevents viral replication. The viral macrodomain exhibits an (ADP-ribosyl)-hydrolase activity which is required for evasion of immune responses and efficient viral replication through antagonizing PARP14 activity.

Highlights on progress



In March this year, postdocs in Ivan Ahel's group (Marion Schuller, Johannes Rack and Kang Zhu) cloned, purified and tested a range of SARS-CoV-2 macrodomain protein constructs. The best protein construct yielded, after optimisation of crystallisation conditions, crystals of the un-liganded enzyme that diffracted to very high resolution (1.0 Å) on beamline I03, allowing the structure to be rapidly determined and refined.

Crucially, these tests showed both that the crystals were tolerant to high DMSO concentrations, and that the active site (ADP-ribose binding) was empty and solvent accessible. Thus, an ideal crystallisation system for macrodomain fragment screening has been established in just a few weeks.

The XChem campaign and analysis were performed by Marion Schuller (Ahel group) and Daren Fearon, Alice Douangamath, Anthony Aimon and Victor Rangel (Frank von Delft's XChem team).

After growing a large number of crystals at Diamond and further optimisation of soaking conditions for adaptation to the screening process, the 1200-crystal experiment was completed within 72 hours, including all steps from fragment soaking, crystal harvesting and data collection on beamline I04-1 by the 22nd of May.
Analysis of the screening data using PanDDA (Nick Pearce and Conor Wild) for hit identification in the XChemExplorer interface (Tobias Krojer) revealed over 100 potential binding events which yielded a final total number of 80 hits (hit rate: 7%), which were refined and released on the 9th of July.

Following screening of the EU-OPENSCREEN fragment library (968 fragments at 100 mM designed by the EU-OPENSCREEN partner sites in collaboration with iNEXT-Discovery/Instruct-ERIC partners), a further 24 hits were identified and released on Fragalysis on the 16th of September, taking the total number to 124 (5.7%).

XChem Fragment Screen

The crystallographic fragment screen against the SARS-CoV-2 Nsp3 macrodomain encompassed multiple fragment libraries: the DSI-poised library, MiniFrags (Astex), FragLites &Peplites (CRUK Newcastle Drug Discovery Unit (Newcastle University)), York3D (University of York), SpotFinder (Hungarian Academy of Sciences) the Edelris Keymical library and the EU OPENSCREEN fragment library.
The screen identified 88 binding events in the four sites of known biological interest (along with the usual artefactual hits that bind to the surface and crystal contacts):

i. 54 fragments (58 binding events) bind to the adenine subsite of the active site, indicating how to compete with the natural ligand, i.e. ADP-ribose
ii. 9 fragments (10 events) bind to the proximal ribose subsite of the active site, again indicating how to compete with the natural ligand, i.e. ADP-ribose
iii. 7 fragments (8 events) bind near the catalytic loop indicating how to compete with the macrodomain interaction with ADP-ribosylated host proteins
iv. 8 fragments bind near a loop previously-observed to be involved in pyrophosphate binding

International collaboration

During the process of finalising our data, we were contacted by the James Fraser laboratory and QBI Coronovirus Research Group collaborators at the University of California, San Francisco who had made a similar, independent effort also running a fragment screen on the SARS-CoV-2 macrodomain, however, with a slightly different crystal form of the macrodomain and fragment libraries.
The Fraser team identified additional 13 hits, 3 of which were in the active site. Together with the XChem data, the two fragment screens give unique insights for developing novel chemical matter targeting the active site of the macrodomain, and show the way to developing effective, first-in-class inhibitors of the SARS-CoV-2 Nsp3 macrodomain .
The Ahel lab will now join forces with the von Delft and Fraser groups, as well as recruiting further collaborators, in an international collaboration sharing expertise and resources in computational and medicinal chemistry as well as crystallographic, biochemical and up to *in vivo* inhibitor characterisation, in the hope to contribute a step forward to combat the COVID-19 pandemic.
Both our and the Fraser lab data can be viewed on Fragalysis.

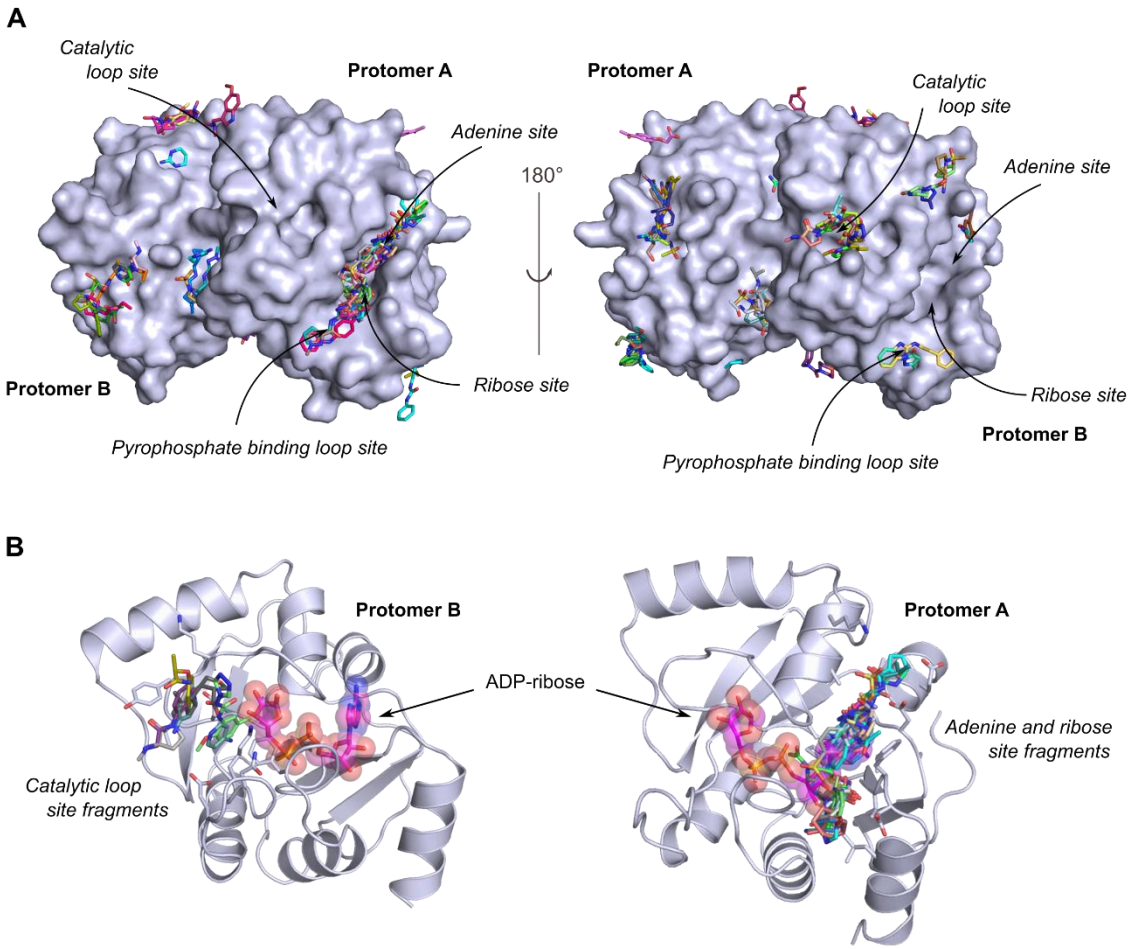
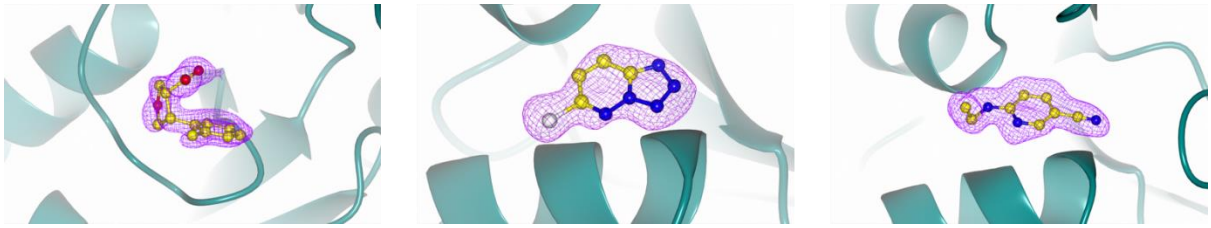


Figure 2. SARS-CoV-2 Nsp3 macrodomain fragment hits. (A) Fragment hits were identified at different sites around the macrodomain crystallographic dimer*. Of particular interest are fragments binding to site 1 as being the adenine binding site of the natural ligand ADP-ribose and fragments binding to site 4 as being the substrate binding site which enables macrodomain interactions with ADP-ribosylated host proteins. The macrodomain is shown in surface representation and the fragments as atom-coloured stick model.* ***(B) Overlay of fragments targeting the adenine (right) and substrate (left) binding site of the Nsp3 macrodomain.*** *The natural ligand ADP-ribose in stick/sphere representation was superimposed to the selected structures.*

Electron density evidence



XChem fragment hits are detected and modelled using the PanDDA paradigm (Pearce *et al*, 2016), which explicitly treats the low occupancy binding of fragments as a superposition of bound and unbound states. This requires on the one hand a 3D density background correction to detect and model fragments; and on the other to model both bound and unbound states for refinement (Pearce *et al*, 2017).

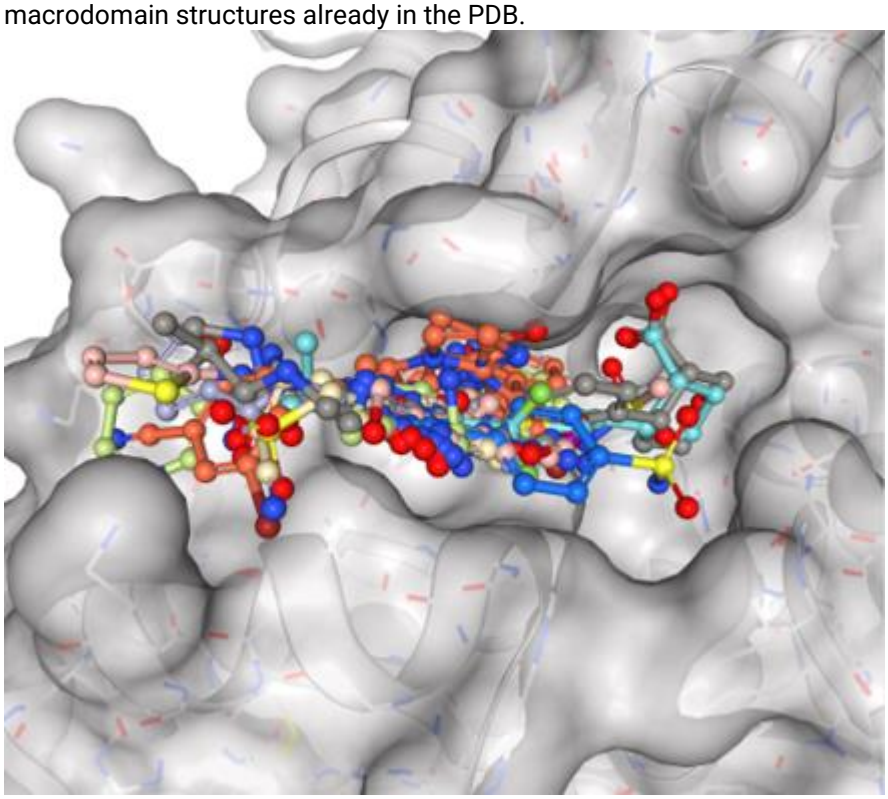
These corrected density maps are named "Event maps", and are the primary evidence for the fragment models. They are deposited in the PDB along with the refined models and diffraction data;

however, the existing validation processes of the PDB do not yet correctly account for these data, and consequently the validation reports flag up several of the structures as extreme outliers.

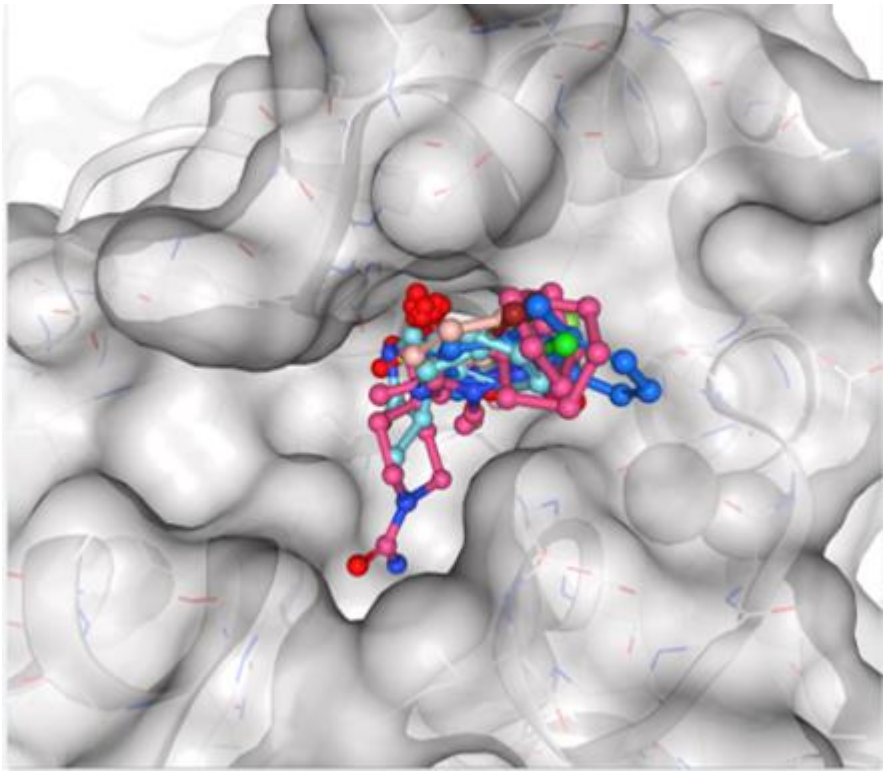
Instead, to check the electron density evidence for the fragment hits, consult the density and model quality evidence presented here, where the data that explain the model are collated in easily reviewable form. *This is a stop-gap solution while we work with the PDB to simplify the process of accessing this evidence directly on their pages*.

Interactive views

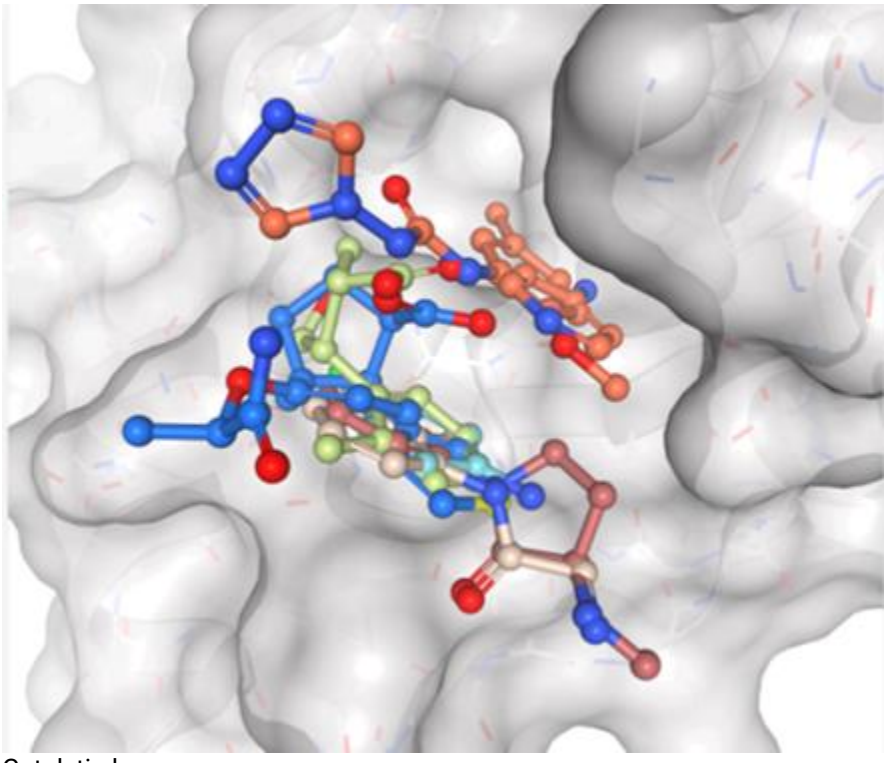
All hit structures (XChem and UCSF) can be viewed on Fragalysis, alongside ligand-bound macrodomain structures already in the PDB.



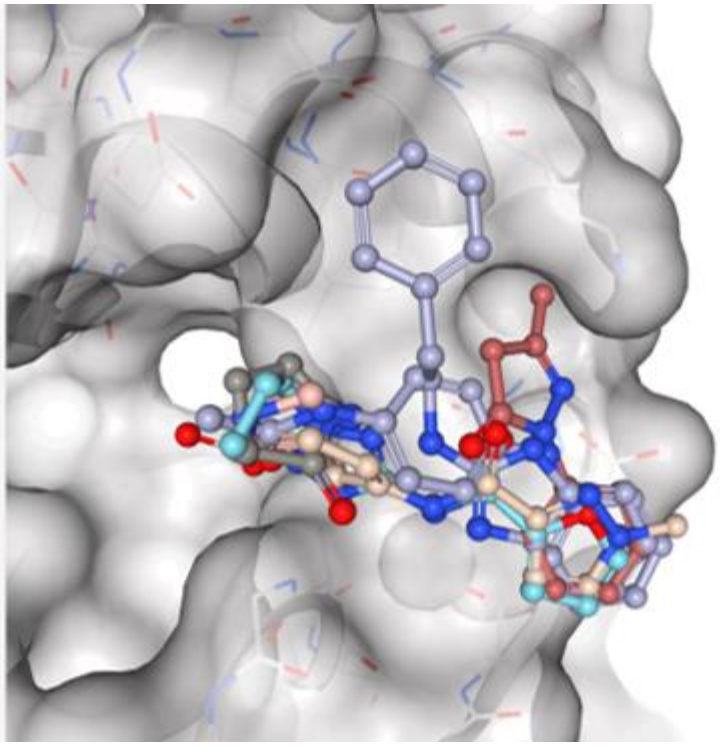
Adenine site.



Proximal ribose site.



Catalytic loop.



Glycine rich loop.

Credits

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- Yuliana Yosaatmadja

XChem Team (Frank von Delft)

- Alice Douangamath
- Daren Fearon
- Tyler Gorrie-Stone
- Louise Dunnett
- Jose Brandao-Neto
- Anthony Aimon
- Rachael Skyner
- Warren Thompson
- Ailsa Powell
- Alex Dias
- Felicity Bertram