How is the international scientific community identifying new antivirals to tackle Covid-19?

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The Covid-19 pandemic has inspired international collaborative efforts to find effective antiviral agents. Fragment-based drug discovery is a well-established tool for early drug discovery. Advances in synchrotron capabilities and the introduction of streamlined crystal soaking facilities, such as the world first XChem platform at Diamond Light Source, have provided substantial improvements in throughput and integration between sample preparation, data collection and hit identification. Other new technologies such as computational docking also contribute to the intense international efforts to tackle the pandemic.

Why will antiviral drugs be needed in the fight against coronavirus?

Coronaviruses have dramatically entered the public consciousness during the Covid-19 pandemic. As the pandemic took hold, research groups swiftly collaborated on a range of studies to better understand the SARS-CoV-2 virus and to develop potential antiviral drugs.

Although the SARS-CoV-2 virus is now well known, other coronaviruses have been responsible for a number of outbreaks over the past two decades, including the SARS (severe acute respiratory syndrome) epidemic and Middle East respiratory syndrome (MERS).

Intense international partnership has developed effective vaccines remarkably fast and worldwide vaccination is now well underway but some countries in Africa, Asia and South America will have slow vaccine coverage. Vaccines are clearly not 100% effective in preventing infection and vaccine-resistant strains are already spreading. Without a therapeutic treatment, Covid-19 is likely to persist like seasonal flu. Antiviral drugs will, therefore, play an important role in the long-term fight against this virus. The fact that there are still no approved treatments for MERS is a timely reminder of the urgent action required.

Fortunately, there has also been a remarkable level of international collaboration on the screening of antiviral agents, and new technologies such as high-throughput fragment screening using X-ray crystallography and computational docking techniques have allowed this work to be carried out at a rapid pace, and for the data to be disseminated openly to the wider scientific community. Much of these data have been released before publication to allow the wider scientific community to build on the achievements more rapidly.

This article highlights recent collaborative work between the UK's national synchrotron, Diamond and many other research groups around the world that has added to the global knowledge base on Covid-19 and in the development of potential antiviral agents.

Figure 1. Diamond Light Source (DLS) aerial view (Credit: Diamond Light Source 2020)

Did you know the XChem Fragment Screening platform can screen 1000 compounds in less than a week?

Fragment-based screening is now well-established as a powerful approach to early drug discovery. The XChem facility at Diamond Light Source (*Figure 1*) was established in 2015 as a world first to provide routine medium/high-throughput fragment screening by X-ray crystallography. Similar platforms are now being established at other synchrotrons around the world. The facility gives users access to dedicated laboratory facilities and synchrotron beamtime on the beamline I04-1, as well as local support and expertise, allowing them to implement a highly streamlined process, which can screen up to 1000 compounds individually in less than a week. (Diamond website; v Delft F, Translational Scientist 2016) [1].

Rather than starting from a larger, substrate-based molecule or screening hundreds of thousands of drug-sized molecules, fragment-based drug discovery starts with more limited libraries of smaller molecules, or fragments. Finding chemical fragments that may bind only weakly, but efficiently, to the target, and combining them to produce a compound with useful affinity, requires a much smaller library than traditional high-throughput screening, and can generate more hits (*Figure 2*).

	NMR	SPR	X-ray crystallography
Sensitivity	High (< 5 mM)	Medium (< 500 µM)	**Very high (≥10 mM)**
Throughput	Medium	High	Medium
Protein requirements	High	Low	Medium
Other requirements	Labelled protein for protein-observed NMR	Protein must be immobilised on chip	**Robust, soakable crystal system**
Information on binding site?	Possible through competition assays	Possible through competition assays	Yes
Pros	In solution method, can provide estimates on K_D	Fast, affinity and kinetics of binding can be determined	**Direct structural information immediately available**
Cons	Slow, typically screen cocktails	Compound interference with chip possible	Static system

Figure 2. Comparison of the most commonly used biophysical techniques used to identify fragment hits.

This approach can facilitate lead identification as small fragments tend to bind to more sites on proteins. In addition, fragment screening directly in crystals using the automation provided by XChem enables throughput orders of magnitude faster than previous techniques.

A range of new technologies, techniques and algorithms have allowed the development of a fast, streamlined process covering crystal soaking, harvesting, automatic data collection, and data analysis with lower overheads. These include:

- Acoustic liquid dispensing – powerful in dispensing compounds into specific drops.
- Image identification – to spot and select crystals.
- Robot-assisted manual harvesting – allowing fast selection of crystals.
- Algorithms to analyse diffraction data – to automatically identify outliers and extract the

signal optimally - the platform has developed tools to streamline density interpretation and refinement (PanDDA and XChemExplorer).

- Web-applications – allowing annotation and public dissemination of protein-ligand complex data(cite Fragalysis)
- Development of new fragment libraries (cite Keseru, Nat Comms, SpotXplorer paper)

The wealth of experience from more than 100 academic projects, 30 projects from industry and over 40 collaborative projects meant that the XChem platform was well placed to carry out fragment screens early on in the pandemic. The XChem facilities have hosted a number of international groups of researchers including the colleagues from University of Oxford, University of Cambridge, University of California San Francisco and the University of Sao Paulo.

Can we identify fragments that bind to SARS-CoV-2 proteins?

Collaborative Covid-19 research projects

A number of potential drug targets against the SARS-CoV-2 virus (*Figure 3*) have been identified and numerous international studies and collaborative Covid-19 research projects are taking place at pace.

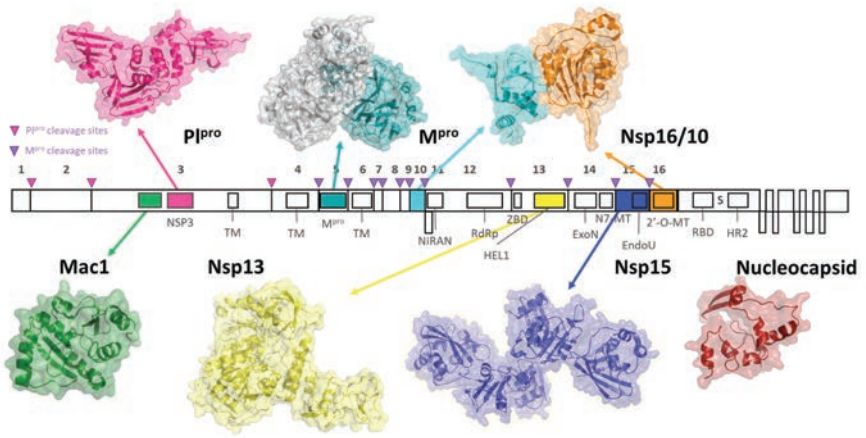


Figure 3. Potential drug targets from SARS-Cov-2 that have been screened at XChem

An international team led by Martin Walsh's group and the XChem team at Diamond (*Figure 4*), plus Nir London's group at the Weizmann Institute of Science, Israel used fragment-based screening to test a library of nearly 1250 fragments against the SARS-CoV-2 Main protease (M^{pro}) (*Figure 5*) (Douangamath, A. et al. 2020) [4]. The screen identified 71 hits that spanned the entire active site, as well as 3 hits at the dimer interface. These structures reveal routes to rapidly develop more potent inhibitors through merging of covalent and non-covalent fragment hits and offer valuable structural and reactivity information for on-going structure-based drug design against SARS-CoV-2 main protease.

Figure 4. Team(s) at Diamond responsible for Mpro fragment screen (left to right: Martin Walsh, Claire Strain-Damerell, Daren Fearon, David Owen, Ailsa Powell, Frank von Delft, Rachael Skyner, Petra Lukacik, Louise Dunnett, Alice Douangamath, Jose Brandao-Neto, Conor Wild).

In total 68 covalent fragments and 1176 non-covalent fragments were screened by either co-crystallisation or soaking of the compounds into the crystals. 1877 crystals were mounted at Diamond and 1638 datasets with a resolution better than 2.8 Å were collected and yielded structures of 96 fragments bound to Mpro. The study was achieved at remarkably high speed with protein crystals first obtained on 13 February 2020, and all experimental data were collected by 7th March. The first crystal structures were made public only three days later, and by the beginning of April 2020 all final structures were released in the midst of the evolving pandemic.

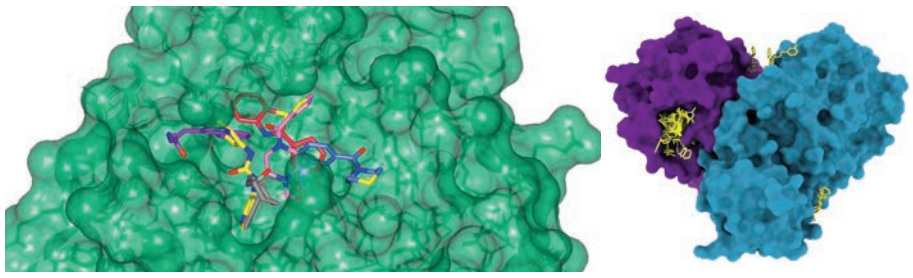


Figure 5. Surface representation of SARS CoV Mpro dimer with fragment hits from XChem screen shown as yellow sticks.

How can fragment hits be developed into potent inhibitors?

Another collaboration between an international group of scientists called the COVID Moonshot aims to rapidly develop a clinically effective antiviral.

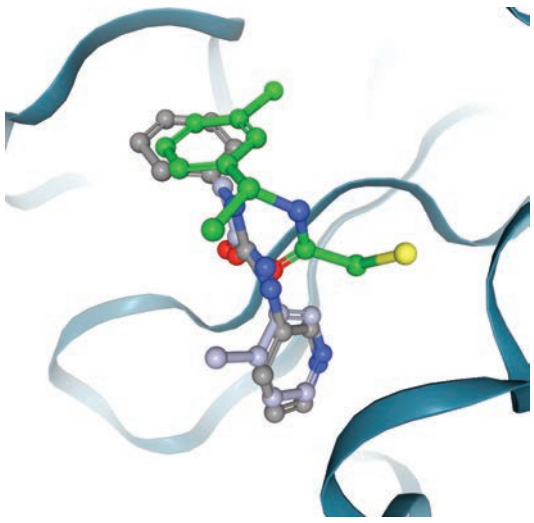
The COVID Moonshot(II) started as a spontaneous virtual collaboration in March 2020. As countries locked down, a group of scientists, academics, pharmaceutical research teams and students began a worldwide, twitter-fuelled race against the clock to identify new molecules that could block SARS-CoV-2 infection and develop pills that would be readily available to the most vulnerable communities.

Ultimately more than 150 scientists - including dozens of students who put their own projects on hold – joined Moonshot to crowdsource ideas for molecular compounds, model them and evaluate them in-vitro against the virus. Their goal: a safe, globally affordable, not-for-profit oral treatment for COVID-19 and related viral pandemics.

One of the founders of the Moonshot initiative; Frank von Delft, Professor of Structural Chemical Biology at the University of Oxford and Principal Beamline Scientist at Diamond Light Source commented in an article about the project in Nature in June 21; 'Open drug discovery efforts are invariably super slow – ours has been an express train on tracks we have laid down as we go, it is a way of working none of us realised was possible.'

Partners of the Moonshot project include academic and industrial groups such as Diamond Light Source, UK's national synchrotron; the Weizmann Institute of Science (Israel); the Nuffield Department of Medicine at the University of Oxford (UK); PostEra (US/UK); the Memorial Sloan Kettering Cancer Center (US); drug discovery consultants including MedChemica Ltd (UK) and the Drugs for Neglected Diseases initiative (Switzerland), which is now taking the lead in coordinating the drive towards the clinic which was recently awarded £8 million in funding from Wellcome.

The project combines crowdsourcing designs of new inhibitors from chemists around the world with machine learning and robotic experiments. All data are being released in real time to enable worldwide collaboration and rapid progress (Covid Moonshot Consortium; PostEra website) [3].



The Moonshot is targeting the SARS-CoV- 2 main protease Mpro which is highly conserved in coronaviruses and essential for viral replication, making it a key drug target. To date, the project has involved more than 7000 designs and 350 designers around the world with over 800 compounds tested, many based on hits from the original fragment screen carried out at Diamond (*Figure 6*). It is hoped that the open science approach will enable the development of many new tools, approaches and ultimately viable treatment candidates.

Figure 6. Overlay of fragment hits used to design more potent inhibitors by the Covid Moonshot collaboration

Thanks to unprecedented collaboration, rapid progress has been made and the team now aims to identify pre-clinical candidate molecules by end of 2021 – compounds that will be simple to manufacture in the form of pills and which will exert an anti-viral effect via potent inhibition of the main protease (MPro) of the SARS-CoV-2 virus. The project has now entered the more capital-intensive phase: tweaking, optimising and testing these molecules to develop them into a safe treatment.

Moonshot compounds have been screened in a number of centres including the Diamond XChem facility, the London group of the Weizmann Institute (Rehovot, Israel), as well as the Schofield group (Chemistry Research Laboratory, University of Oxford).

Why are scientists targeting the Nsp3 macrodomain?

Another potential antiviral drug target is the macrodomain (Mac1) portion of the non-structural protein 3 (Nsp3) from the SARS-2-CoV-2 virus. The Nsp3 macrodomain allows the virus to evade the immune response and is essential for viral replication. Previous studies have shown that viruses that lack Mac1 cannot replicate in human cells, suggesting that blocking it with a drug would have the same effect.

A recent international study involving more than 50 multi-national collaborators used the beamline and laboratory facilities at XChem (*Figure 6*) and the Advanced Light Source in California to undertake a massive computational docking and crystallographic screening effort to identify compounds primarily targeting the active site of this domain. The study included researchers from the University of Oxford, the QCRG Structural Biology Consortium, at the University of California, San Francisco and scientists on the XChem platform and may represent the largest collection of high-resolution crystallographic fragment hits against any target that has been made publicly available.

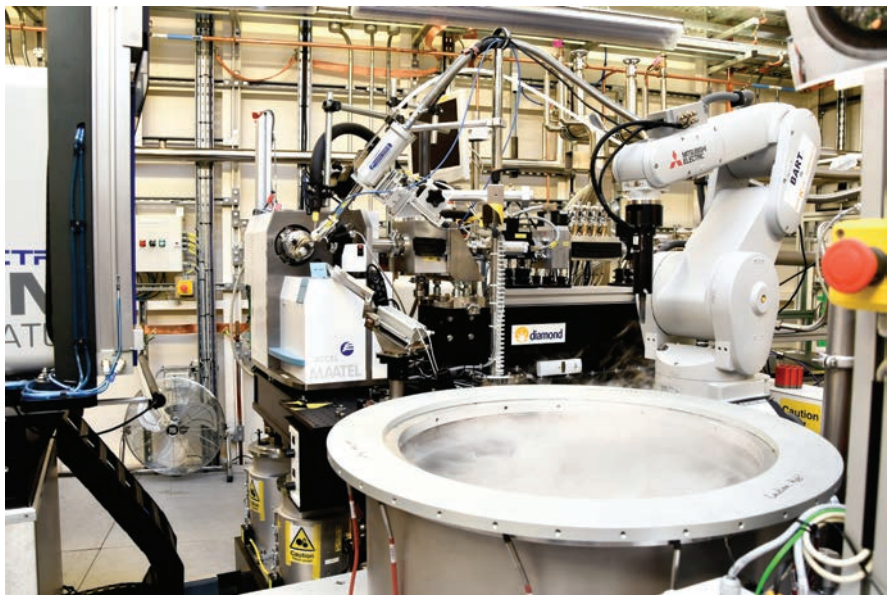


Figure 7. The Diamond Light Source beamline 104-1 experimental hutch showing sample changer and sample environment. Copyright Diamond Light Source Ltd 2020.

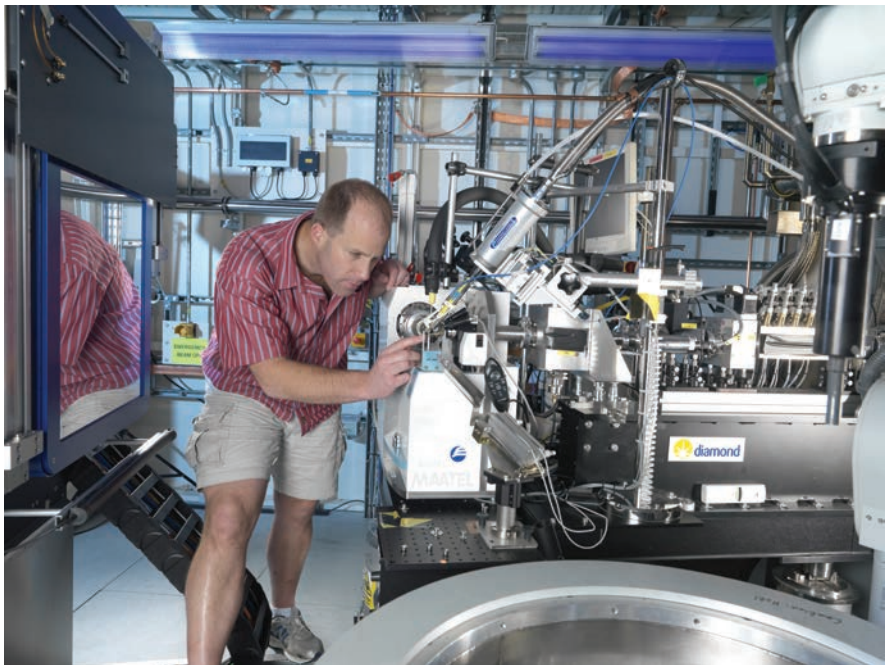
Crystallographic screening of over 2500 fragments resulted in the discovery of 234 fragment compounds that directly bind to sites of interest on the surface of the protein. All of these crystal structures have been released in the public domain.

Independently, the researchers screened more than 20 million fragments (mostly from ZINC15) against the protein using computational docking, another innovative drug discovery technique employing computer models and simulations to assess the likely interactions of virtual molecules for favourable interactions with Mac1 and their promise as starting points for drug discovery. Of 60 top hits chosen for crystallographic soaking, 20 yielded structures, all at high resolution (0.94-1.01 Å). Most of the 20 experimentally determined structures confirmed the docking predictions.

The study reaffirms the growing importance of crystallographic and computational screening, both in providing numerous drug leads for SARS-CoV-2 antivirals and in vital training for new computational methods.

Conclusion

These, and many other ongoing studies, have demonstrated the power of international cooperation in tackling a major global issue. Open sharing of data in real time has allowed research groups to swiftly build on findings, and the use of pioneering new technologies such as fragment-based screening and computational docking methods have revolutionised our approach to new drug discovery.



Frank von Delph

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BCA Spring Meeting

The British Crystallographic Association (https://crystallography.org.uk/) meeting will be held in Leeds from 11th-14th April, 2022. This exciting meeting provides an opportunity for scientists working in the area of structural physics, chemistry and biology to meet and discuss their work. In this years' BCA we have a wide range of sessions specific to Structural Biology led by Professor Randy Read who is providing the Plenary talk on Alphafold2 and the Hodgkin Prize lecture is being given by Professor Elspeth Garman on the life of Dorothy Hodgkin. The individual sessions include a highly relevant session on Covid Drug Discovery which will focus on structural methods used to identify ligands which bind to coronavirus proteins and how these methods have been applied to further develop these ligands into promising leads and drugs. The keynote lecture for this session will be given by Dr Ivan Ahel from the University of Oxford.