LEAGUE OF ADVANCED EUROPEAN NEUTRON SOURCES

LENS and the fight against Covid-19: How neutrons will contribute

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As with HIV before it, Europe's advanced neutron sources will make an essential contribution to the fight against SARS-CoV-2

On 26 October 1895 Louis Pasteur announced to the National Academy of Science in Paris the first successful vaccination of a 9-year-old boy infected with rabies, a viral disease that up to then was considered unavoidably fatal. Viral diseases continue to haunt humanity, as demonstrated by the current pandemic of Covid-19 that, as we write, has killed tens of thousands, is straining our health systems dangerously beyond their capacity and is a severe threat to the global economy. However, the means we have to confront our foe have improved tremendously since the times of Pasteur, and this is one of the merits of modern science.

While Pasteur did not even know what enemy he was dealing with, SARS-CoV-2, the virus at the origin of the coronavirus disease, Covid-19, was deciphered immediately after its discovery. Its full genome sequence is available on the internet. This sequence and the processes developed in the context of genetic research are at the origin of the kits employed to test for Covid-19. The sequencing equally allowed expression of many of the proteins that make up the virus, and today—only three months after the discovery of the virus—the three-dimensional structures of these proteins are available. This structural information is already in use by pharmaceutical researchers hunting for the inhibitors that will impede the virus's reproduction.

But the global race for both a vaccine and therapeutics for Covid-19 requires more than genetic information. Modern analytical tools such as synchrotron X-ray radiation, cryo-electron microscopy and neutron scattering are indispensable for important insights into the morphology and functionality of viruses. Neutron scattering's particular role here is to provide unique information on the chemistry of enzymatic reactions that often involve proton transfer. Recent studies on HIV-1 protease, an enzyme essential for the life-cycle of the HIV virus, perfectly illustrate the case.

Treating the disease and stopping the virus

Proteases are like biological scissors that cleave polypeptide chains at precise locations. If the cleavage is inhibited, for example, by appropriate anti-retroviral drugs, then so-called poly-proteins remain in their original state and the machinery of virus replication is blocked. For the treatment to be efficient this inhibition has to be robust—that is, the drug occupying the active site should be strongly bound to the backbone of the protease. In this way the likelihood that treatments can be effective in the long run, despite mutations of the enzyme, is increased. **Neutron crystallography** data adds supplementary structural information to X-ray data by providing key details regarding hydrogen atoms, critical players in the binding of such drugs to their target enzyme through hydrogen bonding, and revealing important details of protonation and hydration. In this way neutron crystallography data can help towards the design of more effective medications.

High-resolution X-ray data on the protease of SARS-CoV-2 are already available and efforts are being deployed to obtain crystals for neutron crystallography studies. Proteases are, however, not the only proteins where neutron crystallography can provide essential information. For example, virus spike proteins responsible for mediating the attachment and entry into human cells are of great relevance for developing therapeutic defense strategies against the virus. Here neutron crystallography can provide unique information about the precise coupling mechanism of the virus and the receptor proteins of the cell membrane.

The big picture and the moving picture

When it comes to studying the function of larger biological complexes such as assembled viruses, **small angle neutron scattering** becomes an important analytical tool. The technique's capacity to distinguish specific regions (RNA, proteins and lipids) of the virus—thanks to advanced deuteration methods—enables researchers to map out the arrangement of the various components, contributing invaluable information to structural studies of SARS-CoV-2.

While NMR and cryo-electron microscopy provide the detailed atomic-resolution structure of small biological assemblies, neutron scattering allows researchers to pan back to see the larger picture of full molecular complexes at lower resolution. Neutron scattering is also uniquely suited to determining the structure of functional membrane proteins in physiological conditions. Neutron scattering will therefore make it possible to map out the structure of the complex formed by the SARS-CoV-2 spike protein—the protein surrounding the virus—and its human receptor.

A full understanding of the virus's life cycle requires the study of the interaction of the virus with the cell membrane and the mechanism it uses to penetrate the host cell. Covid-19 is one of those viruses, like HIV, possessing a viral envelope composed of lipids, proteins and sugars. By providing information on its molecular structure and composition, the technique of neutron reflectometry helps to elucidate the precise mechanism the virus uses to penetrate the cell. This may be by direct fusion of the virus membrane with the external cell membrane, or with the plasma membrane, or, in the case that the virus is internalized, by endocytosis. **Neutron reflectometry** can in fact provide detailed structural information on the interaction of small protein fragments, so-called peptides, that mimic the spike protein and that are believed to be responsible for binding with the receptor of the host cell.

Finally, we should not forget that viruses in their physiological environments are highly dynamic systems. Knowing how they move, deform and cluster is essential to the optimisation of diagnostic and therapeutic processes. **Neutron spectroscopy**, which is ideally suited to follow the motion of matter from the small chemical group to large macromolecular assemblies, is the tool of choice to provide this information.

Assembling the data from all of these neutron-based analyses of the coronavirus will be essential to control its spread and limit its societal impact over the long term.

LENS facilities recognize and have quickly embraced their responsibility to contribute to the fight against Covid-19, and are fully mobilized to rapidly conduct all relevant experiments. These will take priority over all other scientific activities at the facilities. Special access channels to beam time have been put in place to allow the scientific community to respond without delay to the challenge posed by Covid-19. Details can be found on the respective sites of the LENS partners.

LENS members tackling COVID-19

Budapest Neutron Centre (BNC)

https://www.bnc.hu/?q=covid-19-update2

The beamline proposal deadline for the 2020 autumn cycles has been postponed until 15 May. However, rapid access proposals are still being accepted, where priority access will be given to projects related to the fight against the CoVid-19 pandemic. Email communication of the users with the BNC user office and with the instrument staff is encouraged via addresses available on the facility web at https://www.bnc.hu. The reactor is presently scheduled to restart on 11 May. Priority measurements will be performed as the actual state regulations allow the staff, users and reactor to be back in operation.

European Spallation Source (ESS)

https://europeanspallationsource.se/article/2020/03/27/ess-demax-lab-prioritise-proposals-covid-19-related-research

The European Spallation Source Deuteration and Macromolecular Crystallisation Support Lab, DEMAX, is already functioning on a small scale and we can provide expertise, advice, and limited materials to support research to address the critical need to understand and shut down the coronavirus disease, COVID-19. ESS will prioritise requests for the production of deuterated proteins, DNA, and some types of small molecules such as detergents and lipids that are all potentially useful for neutron scattering studies of viral components. As an enveloped virus, the membrane of COVID-19 is composed of lipid membranes derived from the host cell. DEMAX can make deuterated eukaryotic lipids derived from yeast that could be used to study viral-host cell interactions and maybe even viral assembly interactions with proteins. ESS will also actively engage with external subject matter experts to support their efforts in collaborative projects. In addition, as experienced scientists in small angle scattering, chemistry, structural biology, reflectometry, and crystallography we can also assist with structural modelling and data analysis.

Strategies on how to contribute to COVID-19 research fit well into the already defined LENS WG3 PA 'role of cell membranes in health and disease'. This PA involves partners from ESS (coordinator), ILL, FZJ, STFC, MLZ and PSI, and liaises with relevant external subject matter experts in the field.

Forschungszentrum Jülich/Jülich Centre for Neutron Science (FZJ/JCNS)

https://www.fz-juelich.de/SharedDocs/Pressemitteilungen/UK/EN/2020/2020-03-23-corona-fzj-en.html

In order to contribute to the research on the corona virus SARS-CoV-2, JCNS has decided to offer its instruments via a special proposal round with privileged access, giving them priority over all other applications for beam time. To this respect, regular proposal rounds (next Deadline March 27, 2020) would imply too long waiting time to access the JCNS instruments, and therefore all interested scientists are kindly invited to submit their experiment proposal to the JCNS User Office per email (useroffice@mlz-garching.de) without any pre-defined template and without any deadline. Submitted proposals will be assessed as soon as possible to ensure access to the instrument during the next reactor cycle planned between May 5 and June 3, 2020.

Institut Laue-Langevin (ILL)

https://www.ill.eu/news-press-events/news/general-news/coronavirus-covid-19/

At the ILL, we plan to run 2 more cycles in 2020 which will constitute a significant opportunity to perform early research related to Covid-19 on many of our neutron scattering instruments. In addition to the instruments, we have deuteration facilities and share biology and soft matter facilities with our partner institutes on the EPN campus - the ESRF, EMBL and IBS. For rapid access to beam time, Director's Discretionary Time (for full experiments) and Easy Access (for short measurements) should be used.
We also expect the research community to work on Covid-19 on a longer timescale. We encourage you to think about the role of neutrons and discuss longer-term projects with scientists at ILL. These projects may require PhD students which the ILL can support through its PhD programme (next call autumn 2020) and the InnovaXN programme, running with the ESRF, for which the second call for projects involving industry partners will be opened shortly, with a view to selecting 20 more projects by July 2020.

ISIS Neutron & Muon Source (ISIS)

https://www.isis.stfc.ac.uk/Pages/Coronavirus-(Covid-19)-and-the-ISIS-Neutron-&-Muon-Source.aspx

Corona virus functions through a viral to membrane receptor route in which the protein “spikes” on the surface of the virus interact with a specific protein receptor (angiotensin converting enzyme 2, ACE2) on the mammalian cell membrane surface. This interaction promotes the fusing of the virus with the plasma membrane and the subsequent transfer of the viral genetic material into the host cell where it hijacks the cellular machinery ultimately producing more viral particles.
At the ISIS pulsed neutron and muon source we have a series of techniques and model biological membrane systems which enable both structural and dynamical studies on complex biological architectures. Macromolecular interactions as well as the relative distribution and changes in components during a biochemical event can be examined in realistic environments such as mammalian cellular membrane surfaces. Unique insight into the virus (sars-cov-2) and the resultant disease (covid-19) can be provided by neutron scattering techniques and would include measurements such as:

- Through neutron reflectometry and small angle neutron scattering, the overall details of viral–membrane binding could be resolved and the effect of pharmaceutical compounds on disrupting this interaction examined.
- Using Small angle neutron scattering, the structure of the virus could be examined and the relative distribution of macromolecular components within that structure, both before and after the interaction of antiviral drugs, resolved. This could be complemented by dynamical

studies on the effects of the drugs on the virus components using quasi-elastic neutron scattering.

- Verifying the absolute molecular level details of precision assay and sensor systems for viral detection could be examined using neutron reflectometry to aid in the development of these field deployable diagnostics.

Building on detailed atomic structural information from the research community, and working across the Harwell campus, the application of neutron techniques is capable of providing unique insights, particularly in relation to the fundamental understanding and benchmarking of the diagnosis and therapeutics development stages. The ISIS source has rapid and flexible access mechanisms in operation routinely which will enable to these research tools and capabilities to be available for the international research effort upon the earliest resumption of operations.

Laboratoire Léon Brillouin (LLB)

http://www.cea.fr/Pages/actualites/sante-sciences-du-vivant/covid19-CEA.aspx

We have mobilised our laboratory equipment and are participating in the CEA and CNRS efforts to fight against the COVID-19. Concerning our CRG instrument IN6 at ILL, we will join the ILL efforts and share its policy on this subject.

Heinz Maier-Leibnitz Zentrum (MLZ)

https://www.mlz-garching.de/englisch/news-und-press/news-articles/rapid-access-for-research-on-coronavirus-sars-cov-2.html?back=yes

Researchers worldwide are trying to develop drugs or a vaccine against the coronavirus SARS-CoV-2. In view of the corona pandemic, the Heinz Maier-Leibnitz Zentrum (MLZ) also offers special access to measurements with neutrons, which can provide important insights into the behaviour of the virus. Scientists who want to use neutrons to study the corona virus SARS-CoV-2 or the disease COVID-19 will have special and privileged access. They do not have to apply for measurement time through the usual channels. “Research on the coronavirus SARS-CoV-2 and on the disease COVID-19 currently has priority over all other applications for measurement time at the MLZ,” said Prof. Dr. Peter Müller-Buschbaum, Scientific Director of the FRMII. That is why the MLZ has set up a rapid access for research on the coronavirus SARS-CoV-2 with immediate effect. Guest scientists should contact the user office directly with their applications for measurement time. A scientific evaluation of the application will then take place as soon as possible.

Paul Scherrer Institute (PSI)

https://www.psi.ch/de/psd/covid-19

A better understanding of COVID-19 and its spread is essential in order to detect the disease, treat and protect patients and ultimately control the epidemic. This is why the Paul Scherrer Institut is open for collaboration with other research groups and industry to develop treatments and diagnostics to better tackle the COVID-19 outbreak and to increase preparedness for future possible outbreaks. PSI’s facilities provide unique characterization techniques to efficiently and promptly support these needs. Further, our interdisciplinary teams comprising physicists, molecular and structural biologists, chemists and beamline scientists are ready to assist with their expertise.