



Research at LEAPS facilities fighting COVID-19

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LEAPS

League of European
Accelerator-based
Photon Sources



Europe's Accelerator-Based Photon Sources join forces in fighting COVID-19

LEAPS facilities are joining forces in front of the coronavirus pandemic, offering their capacities to the whole scientific community. Several facilities have opened calls for rapid access to dedicated beamtime for prioritizing the research on the SARS-CoV-2 virus, its therapy and vaccine, above the rest, aiming at minimizing the time from proposal to paper submissions. Sections A to G summarize the LEAPS instrumentations and methods that are at the service of the world scientific community. Examples of the first results, which are being obtained and publicly available, are also shown. The Annex lists the particular information available at several of LEAPS facilities related to COVID-19 including the calls for rapid access.

Boosting the existing capacities with adequate national and European funding programs, will place the LEAPS consortium in a privileged position to be one of the mayor players for addressing the present and future viral threats the society is facing.




A. High-Precision Structure Determination of Relevant Biological Systems

The determination of the three-dimensional structure of proteins at atomic level can be achieved by macromolecular X-ray crystallography and to a somewhat lesser resolution by cryo-electron microscopy. These structures are important to understand the biological and physiological processes of SARS-CoV-2, such as replication and adhesion to the human target cells at a molecular level. Structural information on complexes of drug-targets together with inhibitors relevant to COVID-19 would speed up the process of drug and vaccine development.

Investigations on the binding of small molecule effectors (e.g. inhibitors or drug precursors) to their macromolecular target molecule need to be performed, and modern high-throughput crystallography beamlines are ideally suited for this purpose. A full three-dimensional structure determination will not only provide a yes/no answer with respect to the binding event itself, it will also deliver the binding location and the precise binding mode including the detailed interactions between the effector and the target.

Due to the high degree of automation, which has been developed, in particular at the participating macromolecular X-ray crystallography beam lines, they may even be used as a screening technique. Nowadays it is possible to collect diffraction data sets of several hundred samples within a 24-hour period. It is hoped that such experiments will reveal new chemical scaffolds binding to macromolecular targets, which may in the future be developed into promising drug candidates.



While XFEL facilities are presently not as automated or high-throughput as synchrotrons, and hence cannot be considered screening facilities, they have the unique capability to also explore the dynamics of the systems at room temperature using the femtosecond X-ray pulses that enable getting the diffraction patterns before radiation damage occurs.

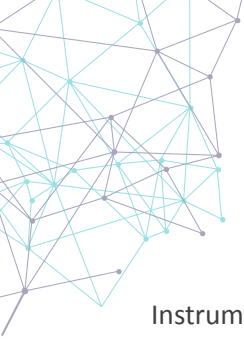
The SPB/SFX instrument of the European XFEL and the SwissFEL Aramis beamline for SFX and fixed target studies, dedicated to structural biology applications, enable the researchers to perform Serial Femtosecond Crystallography experiments at temperatures close to physiological conditions. In addition, researchers may be able observe the dynamics of biological reactions and processes such as the interaction between viral and host proteins.

Nearly all facilities participating in LEAPS (ALBA, DESY, DIAMOND, ELETTRA, FERMI, ESRF, EuXFEL, MAX IV, HZB, PSI, SOLARIS and SOLEIL) offer access to their equipment for corona-related research.

B. X-ray Imaging

Understanding the viral life cycle of SARS-CoV-2 including steps such as adhesion, endocytosis, replication, assembly and release of new virus particles is important for identifying possible targets for therapy. Imaging techniques can give information at a sub-cellular level which is ideally suited to study processes such as adhesion, endocytosis etc.

Within LEAPS a large variety of such X-ray imaging techniques are available at several institutes. In addition, in many of these instruments, imaging is combined with other techniques such as fluorescence which provides information on the distribution and content of specific elements.



Instruments offered throughout LEAPS in the soft X-ray range and including 3D cryotomography in the water window are operating at ALBA, BESSY-II (HZB) and DIAMOND. They can give information with ca. 30 nm spatial resolution and are specialized in imaging internal organelles of cells. The ELETTRA TwinMic microscope combines full field and scanning modes, allows simultaneous detection of X-ray absorption signal and X-ray fluorescence spectra, and has ptychography option as well. At SLS (PSI) a scanning microscope with resolution in the range 20-50 nm for microspectroscopy applications in carbon based and other materials is available. At PETRA III (DESY) and MAX IV, the scanning micro and nanoprobe instruments provide 2D chemical maps by combining imaging and fluorescence at micro and nanometer scales. Similarly, at SOLEIL and ESRF, microspectroscopy beamlines allow 2D X-ray absorption spectroscopy at submicrometer resolutions. Higher lateral resolution is available at ESRF that is operating a scanning microscope with ca. 10 nm resolution.

Hard X-rays are also used at SLS and ESRF for 3D tomographic imaging with spatial resolutions in the range 1-10 μm typically, complemented by high-resolution imaging with 10-100 nm resolution of samples under cryo conditions at the SLS. This large variety of imaging and microspectroscopy instruments can be combined to develop correlative microscopy that allows merging morphological and chemical images of the same samples using different instruments.

As sub-cellular imaging is an emerging field, LEAPS will play an instrumental role stimulating this development that could have a wide impact for the study of infectious diseases, should timely and adequate funding be made available.

C. X-ray Scattering

Small Angle X-ray Scattering (SAXS) is a structural method, that provides direct information on the overall shape of macromolecules in solution, from individual proteins to virus particles or even entire cells. The dynamics of complex formation can also be followed, thus potentially informing on e.g. viral adhesion and docking onto the human target cells or virus capsid assembly processes in the millisecond time scale by the use of rapid mixing devices. In particular, recent studies have demonstrated the power of time-resolved SAXS method combined with advanced computational modeling to elucidate the complete assembly pathway (and associated free-energy landscape) from capsid proteins to matured viral particles. The assembly process is highly cooperative and the inverse process of disintegration under hostile condition follows a different pathway. Similar studies on nonpathogenic variants of SARS-Cov-2 would yield a deeper understanding of the replication process within host. Furthermore identifying and disrupting the critical step of the assembly process is key to halting the infection. Using a nonpathogenic model system, the destruction process can be followed by SAXS, which is relevant for probing the efficacy of different detergent formulations or alcohols, and determining the time scale involved in the process. This information is particularly relevant for cleaning and disinfecting hands, surfaces, etc.

SAXS can also provide precise confirmation in solution of macromolecular structures derived by X-ray protein crystallography or by Cryo-Electron Microscopy involving surface deposition. This technique is available at ESRF, SOLEIL, PETRA III, Diamond, ALBA and ELETTRA and will become available at MAX IV.

D. Circular Dichroism

Synchrotron radiation circular dichroism (SRCD) is a fast spectroscopic technique which can be used in inhibitor and diagnostic testing tools development. It can be used to screen SARS-CoV-2 proteins and nucleotides, such as RNA, individually as well as their complexes with inhibitors. Rapid time resolved circular dichroism allows that structural changes caused by e.g. inhibitor binding to a target protein can be followed in real time.

SRCD measurements can be performed at the deep ultraviolet (DUV) at ASTRID2 (ISA), while high-throughput capacity on microplates is offered at DIAMOND and at SOLEIL using an automatic sample changer. ASTRID2 and SOLEIL offer rapid time resolved circular dichroism capabilities.

E. IR Spectroscopy

Vibrational spectroscopy methods in the fingerprint spectral region using highly brilliant infrared synchrotron radiation are used to study functional processes in biological systems from the molecule to the single cell and tissue level, therefore many steps in the life-cycle of SARS-CoV-2 can be studied by this technique. For instance, changes in lipid membranes as happening in the endocytosis step, adhesion caused protein conformations, and changes in cell metabolism upon virus infection and drug reception at the single cell level can be studied with subcellular resolution by Fourier transform microspectroscopy (ALBA, ELETTRA, SOLEIL, BESSY II). Chemical imaging with resolution down to single virus particle size (SOLEIL, ELETTRA) is also possible.

Furthermore, conformational changes of protein-ligand interaction can be studied and more specifically the docking proteins of virus particles following their non-repetitive kinetics triggered by the

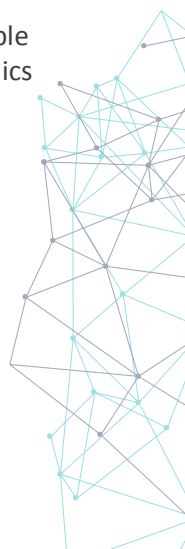
photolysis of cage compounds by means of single-shot spectroscopy (BESSY II). In addition, infrared ion spectroscopy with a free electron laser (FELIX) gives access to the molecular structure in mass-spectrometry based metabolomics, e.g. in small-molecule biomarker discovery which is important for the development of diagnostics.

F. Soft X-ray Transient Absorption

Conformational changes of bio-molecules are responsible for many biological functions and observing these changes increases the knowledge on e.g. the adhesion of SARS-CoV-2 to the target molecule on human cells. The element- and enantio-selective soft X-ray produced by FELs coupled to table-top UV laser in pump-probe configuration give a unique approach to study these changes in intermolecular interactions, as well as interactions with drug molecules as a function of time. This approach can be applied to the case of drug-target interactions, as in the case of antiviral compounds on the phospholipidic membranes of vesicles.

Studying these conformational changes will help to understand the details of the dynamical deformations in the drug-membrane interaction. In this context the FERMI free electron laser based at ELETTRA and FLASH (DESY), thanks to their unique features, are able to investigate with atomic and enantiomeric selectivity the dynamics in pharmaceutical molecules.

A campaign of measures on antiviral drugs such as chloroquine, whose efficacy against COVID19 is much discussed, is currently being carried out on FERMI.



G. UV Fluorescence Imaging

Proteins (and most biomolecules or organic molecules) present specific fluorescence when excited in the deep ultraviolet (DUV, below 350 nm) wavelength range with synchrotron light, opening the way to fluorescence imaging without any probe at sub 100 nm resolution making it a very versatile method to study different physiological processes caused by SARS-CoV-2. For viruses, SOLEIL developed DUV fluorescence imaging of infected cells to follow the aggregation of viral peptides inducing high virulence.

First scientific results from LEAPS facilities on COVID-19 related research

The activity within LEAPS has been altered by the pandemic itself, in all cases reduced and in some cases even stopped for weeks. But maintaining the facilities ready to serve the research community investigating on COVID-19 related issues is a priority, with the activation of rapid access, and the intensification of remote access or sample mail-in service.

Many experiments, including those performed by pharmaceutical companies, are being performed and analyzed; several initiatives of participation in collaborations with research institutions are being developed, in some cases with projects already funded (as for example at HZB and SOLEIL); proteins have been resolved and are being deposited in the Protein Data Base (PDB) (<http://biosync.sbkb.org/index.jsp>); and first publications on SARS-CoV-2 with the contribution of LEAPS members start to appear, as shown hereafter with some examples.



A team from the University of Lübeck and from Helmholtz Centre for Infection Research (HZI) is working on a promising approach to prevent the viruses from multiplying. At BESSY II, they have decoded the three-dimensional architecture of the main protease of SARS-CoV-2, involved in the reproduction of the virus. Analyzing its 3D architecture will help in development of drugs for inhibiting the virus reproduction (see Fig.1 and <https://science.sciencemag.org/content/368/6489/409> doi: 10.1126/science.abb3405).

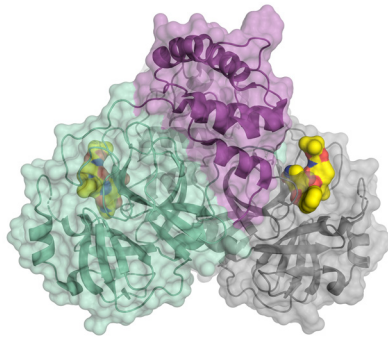


Figure 1. Three-dimensional structure of SARS-CoV-2 M^{pro}.



Scientists from ELETTRA participated in a study on how the COVID-19 virus is evolving revealing that European, North American and Asian strains might coexist, each of them characterized by a different mutation pattern (see Fig. 2 and Pachetti et al, J Trans Med (2020) <https://doi.org/10.1186/s12967-020-02344-6>). A further study (paper under preparation) is focused on possible correlation of the mutations with SARS-CoV-2 mortality rates and X-ray transient absorption experiments are on-going in collaboration with EPFL, UCI and ICGEB testing possible antiviral drugs.

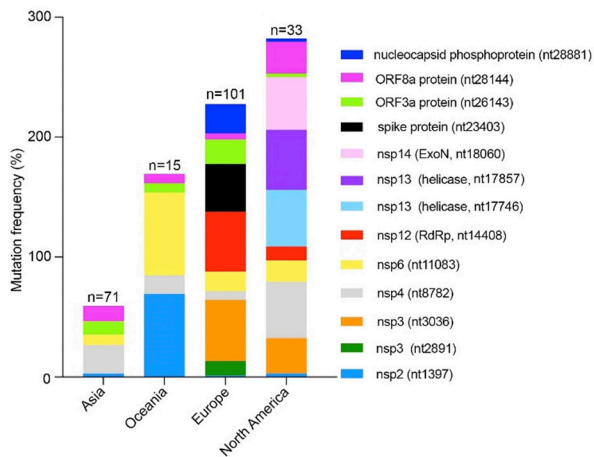


Figure 2. SARS-CoV-2 mutation frequency in different geographic areas. The figure shows that genomes from European and North American patients present an increase in mutation frequency compared to Asia. It is also possible to observe that Europe and North America show a differential pattern of mutations: mutation 14408 (red), 23404 (black), 28881 (electric blue) and 26143 (light green) are present mostly in Europe, whereas 18060 (pink), 17875 (purple) and 17746 (light blue) are present mostly in North America.

At SLS, PSI, a research group from the Institute of Biochemistry II, Frankfurt, Germany is studying the inhibition of papain-like protease PLpro blocks SARS-CoV-2 spread and the promotion of anti-viral immunity has obtained interesting results using the MX-Beamline X06SA-PXI (see Fig. 3 from the paper under review in Nature, Donghyuk Shin, et al. I. Dikik DOI:10.21203/rs.3.rs-27134/v1)

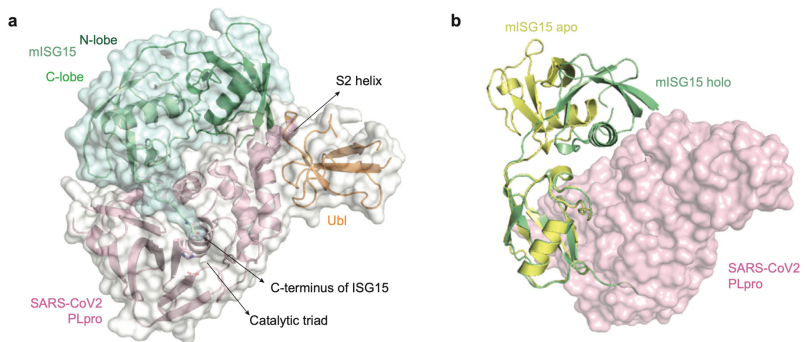


Figure 3. Crystal structure of a PLproCoV2 - ubiquitin-like protein ISG15 complex.

The Strategic Consortium

LEAPS - the League of European Accelerator-based Photon Sources - is a strategic consortium initiated by the Directors of the Synchrotron Radiation and Free Electron Laser (FEL) user facilities in Europe. LEAPS' primary goal is to actively ensure and promote the quality and impact of the fundamental, applied and industrial research carried out at their respective facilities to the greater benefit of European science and society.

The consortium is composed of sixteen members hosting facilities in different European countries, representing a multidisciplinary research community of more than 24000 scientists, encompassing physicists, chemists, biologists, materials scientists, as well as experts from medicine, geology and cultural heritage.

Links to COVID-19 related webpages of LEAPS facilities

ALBA – <https://www.albasynchrotron.es/en/covid-19-information/>

DESY (PETRA III) – https://photon-science.desy.de/users_area/fast_track_access_for_covid_19/index_eng.html

DIAMOND – <https://www.diamond.ac.uk/Users.html>

ELETTRA – <https://www.elettra.trieste.it/userarea/covid-19-virus-elettra-rapid-access-proposals.html>

ESRF – <http://www.esrf.eu/home.html>

EuropeanXFEL – https://www.xfel.eu/news_and_events/news/index_eng.html?openDirectAnchor=1777&two_columns=0

FELIX – <https://www.ru.nl/felix/vm/rapid-access-corona-related-research-felix/>

HZB (BESSY II) – https://www.helmholtz-berlin.de/forschung/oe/np/gmx/index_en.html

MAX IV – <https://www.maxiv.lu.se/covid-19/>

PSI (SLS) – <https://www.psi.ch/en/psd/covid-19>

SOLARIS – <https://www.ceric-eric.eu/2020/03/10/covid-19-fast-track-access/>

SOLEIL – <https://www.synchrotron-soleil.fr/en/news/covid-19-research-synchrotron-soleil-and-rapid-access>

Mission

LEAPS will use the power of its combined voice to ensure that member light source facilities continue to be world-leading, to act as a powerful tool for the development and integration of skills with a view to address 21st century global challenges, and to consolidate Europe's leadership in the field.

Vision

A world where European science is a catalyst for solving global challenges, a key driver for competitiveness and a compelling force for closer integration and peace through scientific collaboration.



Working together in LEAPS