2020
INSTRUCT-ERIC ANNUAL REPORT

CONTENTS

FOREWORD BY
CHAIR OF THE INDEPENDENT SCIENTIFIC ADVISORY BOARD

Stephen K Burley MD, DPhil. Chair of the Instruct-ERIC Independent Scientific Advisory Board.

As Chair of the Instruct-ERIC Independent Scientific Advisory Board, it is an honour to contribute this foreword to the 2020 Instruct-ERIC Annual Report.

The organization and its mission to foster technical innovation and increase the impact of structural biology on biological and biomedical research have never been more relevant. Despite a very challenging year, the Instruct-ERIC Hub has continued to coordinate the work of nine science and technology centres providing user training and access to cutting-edge measurement techniques. Lithuania and the European Molecular Biology Laboratory (EMBL) have come on board as members of Instruct-ERIC. Entry of EMBL has bolstered the quality of Instruct infrastructure, particularly with the cryo-electron microscopy facilities available at the new EMBL Imaging Centre, which are available for use by external scientists for both single particle and tomography imaging studies.

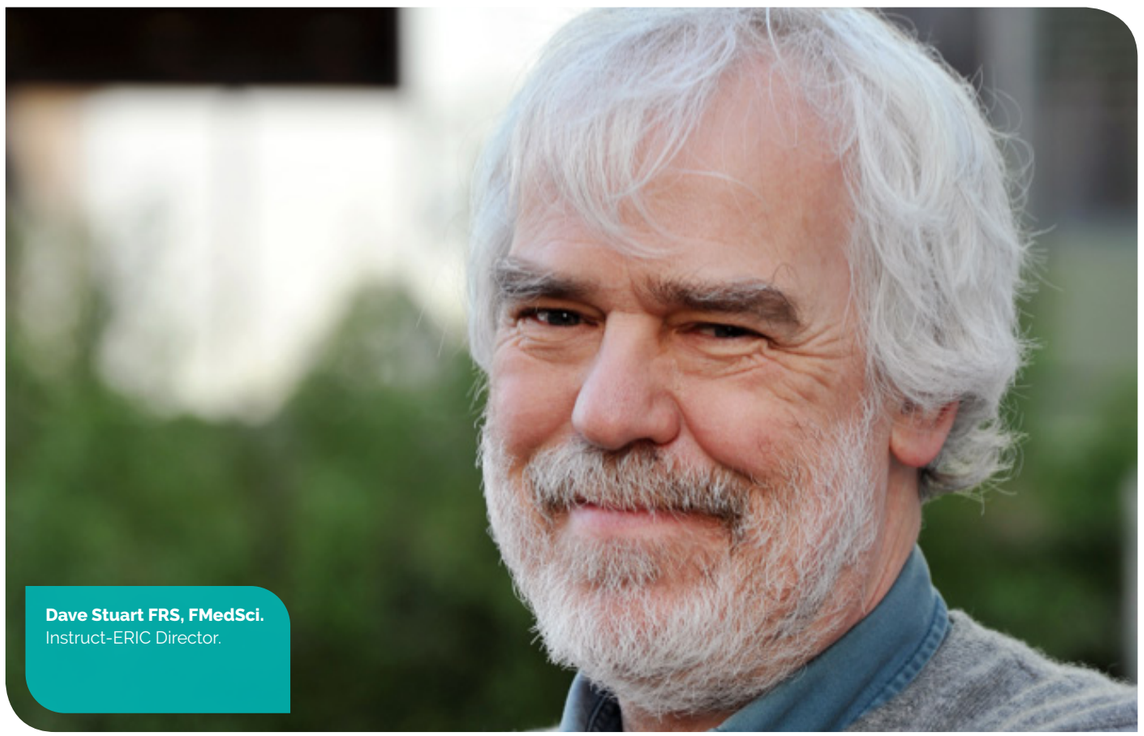
In the face of the COVID-19 pandemic, the Instruct-ERIC team rightly focused on bolstering remote access services, delivering online webinars and training tools, and making the ARIA access management software more resilient. The ARIA system is now in demand from a number of other research infrastructures. It is helping to bring these together to form a coherent 'super-infrastructure' across research fields in Europe.

Instruct-ERIC was also deeply involved in many SARS-CoV-2 related research initiatives, reflecting broader efforts made across the global structural biology community in fighting the pandemic. The organization helped build a COVID-19 protein reagent portal for researchers in the United Kingdom, which provided them with high quality reagents for, prevented duplication of effort, and ensured reagent integrity. During calendar 2020, nearly 1,000 experimentally-determined three-dimensional structures of coronavirus proteins were deposited to the Protein Data Bank archive. Much of this important work was carried out by structural biologists using research tools and instrumentation developed by Instruct member scientists.

The Instruct-ERIC consortium upholds scientific excellence as the qualifying prerequisite in the delivery of all its services including high quality training for users of Instruct facilities. From August 2020, Instruct-ERIC expanded its scientific communication with a new webinar series that presents Instruct scientific achievements and technology advancements. Instruct researchers themselves are the drivers of development and implementation of methods that enable interdisciplinary research in fundamental biology, biomedicine, biotechnology and bioenergy for the benefit of all humanity.

My ISAB colleagues and I are comm-itted to helping Instruct-ERIC prepare for the challenges ahead, and thereby ensure that the consortium continues to deliver value to researchers, educators, patient and social communities, funders of Instruct-ERIC, and member nation taxpayers.

FOREWORD BY
INSTRUCT-ERIC DIRECTOR



Dave Stuart FRS, FMedSci. Instruct-ERIC Director.

2020 was a year dominated by a pandemic which is still ongoing. In this context Instruct-ERIC has continued to develop its collection of technology platforms that support research needing structural biology methods and has driven the remote provision of these services. Following its mission to promote integrated technology approaches, Instruct has demonstrated the value of an infrastructure resource that is accessed via a single portal through which multiple facilities provide their instruments and expertise. The approach itself has gained traction to the extent that it is increasingly the practical realisation in research projects which use structural methods in combination and to complement other methodologies. The extensive catalogue of 75 services has delivered 95 access visits, contributing to an impressive number of publications acknowledging their contribution.

The Instruct membership has grown to fifteen with the addition of two new members in 2020, Lithuania and EMBL, and it is these members that provide the underpinning funding for Instruct-ERIC through a cash contribution and the significant in-kind contributions that sustain the excellent instrumentation and staff support at each Instruct Centre. Exciting new technology platforms have also been added to our Centre sites including the first 1.2GHz NMR spectrometer in Instruct-IT and an expansion of electron microscopy facilities in the UK, Netherlands, France, Czech Republic and Spain, where an €8 million investment has been established at Instruct Centre-ES located at the Spanish National Center for Biotechnology for national and Instruct users.

It is important to acknowledge the efforts that all Instruct members and support staff have made to continue their work for Instruct through the SARS-CoV-2 pandemic which dominated activities for 11 of the 12 months in 2020. In that time, transitions and new developments to enable remote access were accelerated so that by the end of the year almost 85% of service technologies could be accessed in this way. Not only did this keep the Instruct portal open for business to provide its infrastructure for projects but it has established, along with many other facilities providing structural biology methods, a new way of working that will outlive the pandemic to contribute to a 'new normal' workflow. Instruct was able to gear up by offering rapid access to its infrastructure for COVID-19 research projects, providing a COVID-19 resource centre and building a COVID-19 protein portal to help the effort. By the end of the year, a number of high impact publications were in the pipeline reporting work that had been supported at Instruct Centres. This report should give a sense of the 'business-as-usual' work which defied the pandemic restrictions while also making important advances and helping the global efforts to find solutions to the SARS-CoV-2 threat.

LOOKING FORWARD

2020 was a challenging year for Instruct-ERIC as almost from the start the potential impact of the SARS-CoV-2 epidemic as first described in China was clear. Almost all of the Instruct Centres were involved in COVID-19-related work from early in February 2020. It is a matter of great pride that the structural biologists around the world were quick to collaborate openly, sharing knowledge, reagents and data to solve important SARS-CoV-2 structures by early February which fed into many vaccine and therapeutics development efforts. Instruct provided infrastructure for COVID-19 research but also helped to develop a portal through which researchers could request reagents to help with their research. This reduced the need for each research group to make their own proteins, antibodies or other reagents at the appropriate purity, integrity, stability and quantity – something that takes time and experience to achieve. As the year progressed, the Instruct infrastructure access practices evolved to offer more access using remote platforms. While remote access processes were underway on some platforms anyway, the pandemic accelerated this to accommodate researchers who found themselves unable to travel to a facility to undertake work in person. Instead, while remote access enabled work to be done at the facility, in many cases the researcher could still follow the experiment in progress using a remote interface in real time. Reassuringly, the demand for Instruct infrastructure did not decrease over the 2020 year, showing that value was still there for Instruct users.

We continue to deliver Instruct activities to our user community in a situation that changes regularly. However we are confident that we have maintained our standards of service and we look forward to a 2021 that will ease the restrictions and allow us to engage with our community in ways other than online video-conferencing.

Susan Daenke
Instruct-ERIC Hub Coordinator

EXECUTIVE SUMMARY

15 Members

10 Centres

23 Facilities

In 2020 Instruct-ERIC welcomed EMBL and Lithuania as new members bringing the number of **member countries and organisations to 15**

These members hosted 10 Instruct Centres with **23 facilities** providing research access. A new addition in 2020 was the Instruct Centre-Finland.

119 Access proposals received

789.5 Days of access provided

225 Scientific Publications

In 2020 Instruct recieved **119 proposals for access** from researchers in 18 countries of which 77% were approved..

Instruct supported **95 research visits** providing **789.5 days of access** to Instruct Facilities covering both national and transnational access.

Scientific output resulted in **225 publications** in peer-reviewed journals.

75 Services

75 infrastructure services offered in nine categories.

A number of significant additions and upgrades to the infrastructure at Instruct Centres took place. These included implementation of the Robotein high throughput screening facility to test buffers for protein stability at Instruct-BE; the liquid metal Jet in-house X-ray diffraction system at Instruct-IL can achieve fast data collection from microcrystals using a pixel detector; a single-cell proteomic SCoPE mass spectrometer at Instruct Centre-FI allows single cell proteome profiles to be linked to functional phenotypes; the cryo-EM-CSIC facility acquired a 300kV JEOL cryoARM300 with a Gatan K3 direct electron detector to directly expand the capabilities of the Instruct Centre-ES, linking directly with the image processing infrastructure. Several significant upgrades were also made to existing infrastructure and smaller instrumentation.

Sample Preparation
- Crystallisation
- Nanobody Discovery
- Protein Production

Biomolecular analysis
- Imaging
- Mass Spectrometry
- Molecular Biophysics

3D Structural Analysis
- Electron Microscopy
- Magnetic Resonance Techniques
- X-Ray Techniques

8 Training Courses

Of the **eight training courses** offered, four of these addressed methods using cryo-EM. Courses are organised in response to demand by the structural biology community and the popularity of cryo-EM courses, all of which were significantly oversubscribed, indicates the expansion in this area. Two courses were organised to train facility managers, again to meet the demand from the community in understanding common processes to deliver infrastructure services from Instruct Centres.

H2020

9 Projects

Instruct-ERIC participated in **9 Horizon 2020 projects,** CORBEL, EOSC-Life, ERIC Forum, iNEXT-Discovery, Instruct-ULTRA, RI-VIS, EU-LAC ResInfra and TRANSVAC2,
New project in 2020 was TRANSVAC-DS.

INTRODUCTION

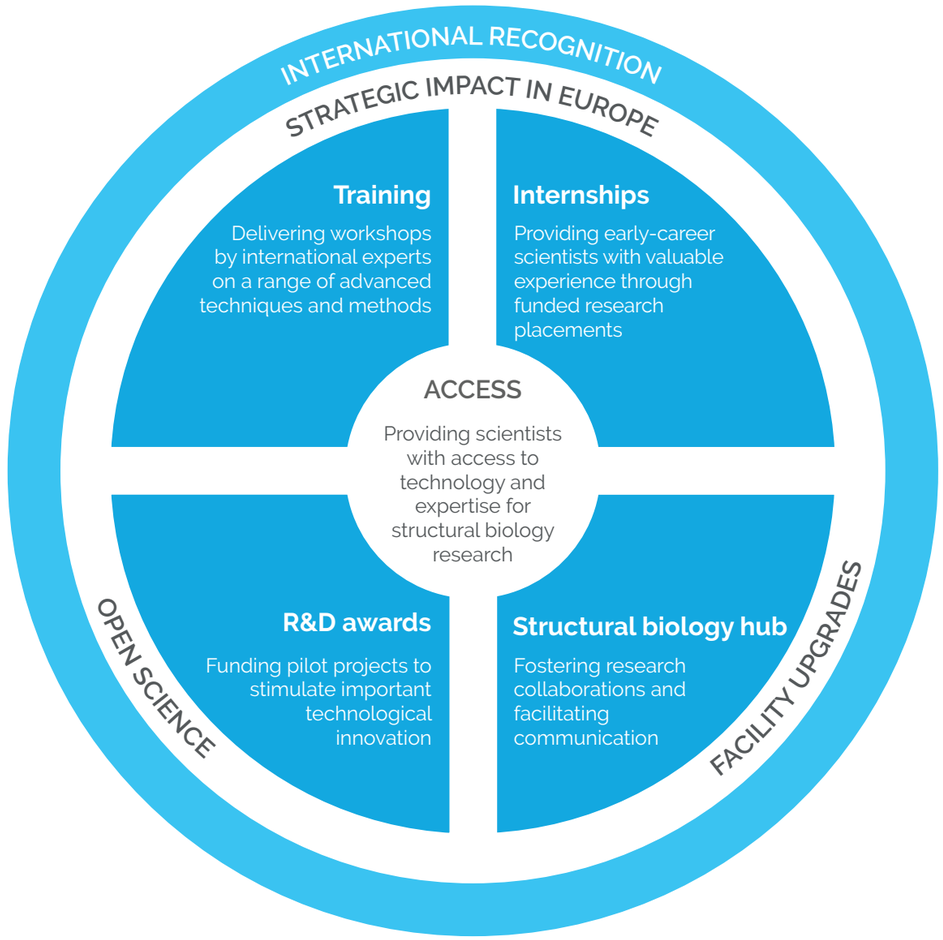
David S. Goodsell, RCSB Protein Data Bank and Springer Nature; doi: 10.2210/rcsb_pdb/goodsell-gallery-025

INSTRUCT ADVANCES IN 2020

While Instruct continues its core infrastructure access and training programmes, it also actively engages with new communities and looks to identify new technologies that will expand the value we offer to our users. The Instruct-ULTRA project helped to develop new contacts in European and international regions and to promote Instruct's mission. The addition of EMBL and Lithuania in 2020 extended Instruct-ERIC membership to 15, while Instruct's participation in the EU-LAC ResInfra project provided a valuable opportunity to establish new links with structural biology communities in Latin America. Likewise, the RI-VIS project targeted Africa, Latin America and Australia regions with plans to host three major international symposia with other European Research Infrastructures to establish new collaborative opportunities. While the timeframe for the symposia was delayed, they will now take place in 2021.

New and emerging technologies are a key component of Instruct's offer to the structural biology community, providing the means to tackle questions in a new way or with greater power. Many upgraded and several new technologies were added to the Instruct catalogue in 2020 and these can be found described in the Instruct Centres section of this report.

Finally, Instruct made every effort to keep the infrastructure open and accessible throughout the working restrictions of 2020. Using a rapid access scheme launched in March 2020, researchers were given priority access to infrastructure for COVID-19-related research and Centres reacted quickly to adjust their infrastructure to remote access where possible. Due to these and other strategies, Instruct maintained its service level in almost every area, with the exception of the internships which in most cases were postponed until travel is opened up again. This was only possible with the help and flexibility of the Centres and Instruct staff, for which we thank everyone for their dedication and hard work.



As of 31 December 2020, Instruct-ERIC has 15 Members: Belgium, Czech Republic, Denmark, EMBL, Finland, France, Israel, Italy, Latvia, Lithuania, Netherlands, Portugal, Slovakia, Spain and United Kingdom, and one Observer: Greece.

MEMBERSHIP

Instruct-ERIC membership continues to grow, with the addition of EMBL (formally approved as a member in late December 2019) and Lithuania (June 2020), bringing membership to 15 (Fig 1). The membership fee is unchanged from the founding tiered model of cash contribution with in-kind contributions sustaining much of the infrastructure hardware and staff support at Instruct Centres. EMBL membership fully funds a staff post in the Hub in lieu of a cash contribution.

The funds from core membership of Instruct-ERIC support the costs of access to infrastructure, enabling Instruct to deliver its services free (in most cases) at the point of access for users. The funding also provides financial support for consumable costs incurred by Instruct Centres in delivering services. Other Instruct activities, for example the training programme, including workshops, internships, pilot project awards (small pump priming research projects) and joint research awards (small development awards to bring new methods or technologies to an access-ready state) are also funded from the core membership contributions.

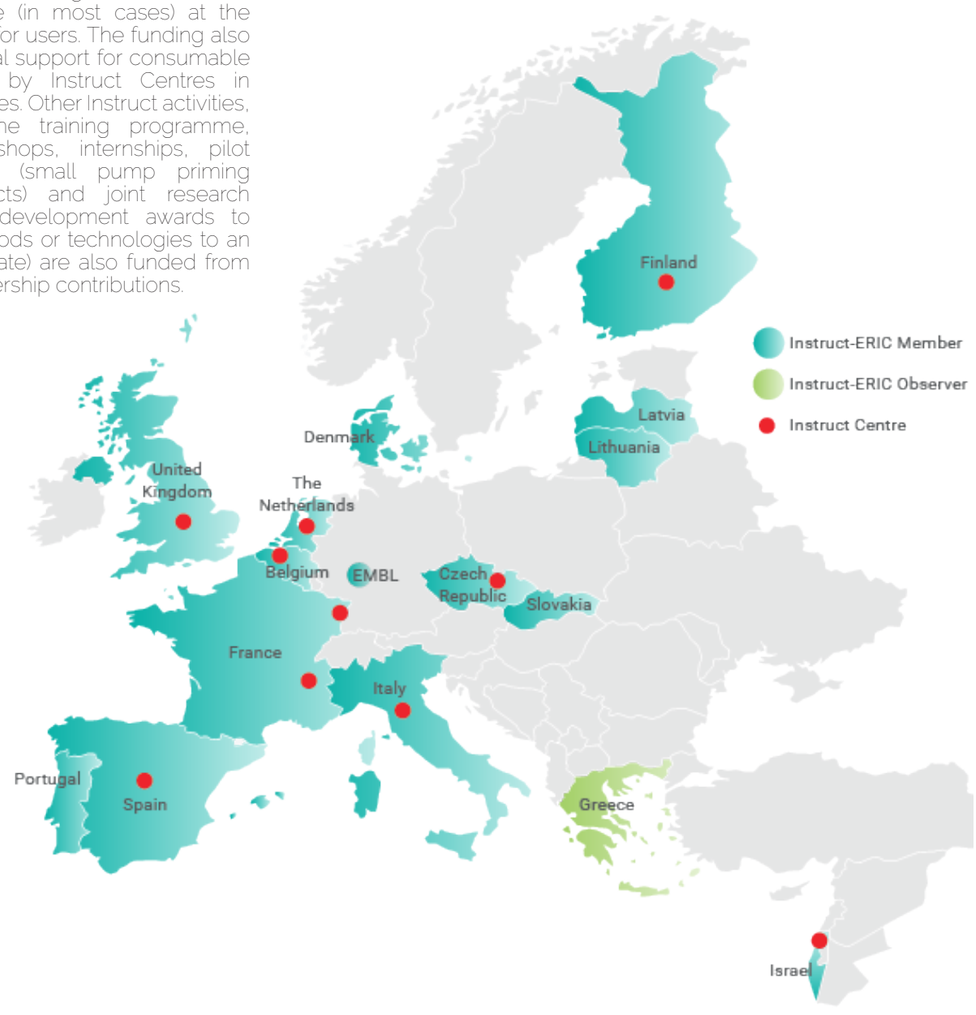


FIG 1. Map showing Instruct-ERIC Member Countries and Organisations (Greece was retained as an Observer) and Instruct Centres.

Centralised coordination of Instruct service delivery continues to be delivered from the Hub located in Oxford, UK and the UK retained the Instruct-ERIC statutory seat in 2020, pending the final implementation of the BREXIT withdrawal agreement in 2021. In each of the Instruct Centres providing infrastructure, the host institutions provide expert staff to support the experimental work and help with data management. This is a significant in-kind contribution by Instruct-ERIC members hosting Instruct Centres. An active and comprehensive dialog between the Hub and the Centres underpins the process of infrastructure access management through the ARIA system and this ensures that there is constant monitoring of the process from proposal submission through to completion of the work.

INSTRUCT CENTRES

In 2020 the member countries hosted 10 Instruct Centres that provided services in 23 facilities across Europe.

	Sample Preparation			Biomolecular Analysis			3D Structural Analysis		
	Crystallisation	Nanobody Discovery	Protein Production	Imaging	Mass Spectrometry	Molecular Biophysics	Electron Microscopy	Magnetic Resonance Techniques	X-Ray Techniques
Instruct Centre-BE		X	X			X			
Instruct Centre-CZ	X			X	X	X	X	X	X
Instruct Centre-ES							X		
Instruct Centre-FI	X		X		X	X	X	X	X
Instruct Centre-FR1	X		X	X		X	X	X	X
Instruct Centre-FR2	X		X	X	X	X	X	X	
Instruct Centre-IL	X		X				X		
Instruct Centre-IT			X			X		X	
Instruct Centre-NL	X		X		X	X	X	X	
Instruct Centre-UK	X		X		X	X	X	X	X

MEMBERSHIP RATES

Total cash contribution in kEUR per annum, with annual increase of 2%.

Member Country	Group	YR 1	YR 2	YR 3	YR 4	YR 5	SUM YR 1-5
UK	A	100.00	102.00	104.04	106.12	108.24	520.40
FR	A	100.00	102.00	104.04	106.12	108.24	520.40
ES	B	75.00	76.50	78.03	79.59	81.18	390.30
IT	B	75.00	76.50	78.03	79.59	81.18	390.30
BE	B	75.00	76.50	78.03	79.59	81.18	390.30
NL	B	75.00	76.50	78.03	79.59	81.18	390.30
IL	B	75.00	76.50	78.03	79.59	81.18	390.30
CZ	C	50.00	51.00	52.02	53.06	54.12	260.20
PT	C	50.00	51.00	52.02	53.06	54.12	260.20
DK	C	50.00	51.00	52.02	53.06	54.12	260.20
LV	C		29.75	52.02	53.06	54.12	188.95
SK	C	50.00	51.00	52.02	53.06	54.12	260.20
FI	C		4.33	52.02	53.06	54.12	163.53
LT	C			8.67	53.06	54.12	115.85
Total		775.00	824.58	919.02	981.61	1,001.22	4,501.43

INSTRUCT ADDED VALUE

The financial model for Instruct-ERIC was unchanged in 2020 comprising a combination of a cash contribution and in-kind contributions to support infrastructure provision at Instruct Centres and other activities (training, outreach) from all members. While the staff time committed to support Instruct activities is clear and can be measured, the scientific knowledge and experience that adds value for the user at each Centre largely goes unrecorded. While this may not be quantifiable, it is recognised and appreciated by the users and is frequently mentioned in feedback reports. Therefore the in-kind contribution is multidimensional, providing technological expertise with scientific advice and training, along with the physical provision of the infrastructure itself and is an important added value.

TIMELINE
selected events from 2020

JAN

The Instruct Hub participated in the kick-off meeting of the Horizon 2020 project EU-LAC ResInfra.

ResInfra
EU LAC

FEB

- Instruct Managers Meeting 2020 in Amsterdam
- ARIA Workshop in Amsterdam

New Instruct Centre
FINLAND

Instruct joined the Horizon 2020 project iNEXT-Discovery

iNEXT DISCOVERY

MAR

- The Instruct-ERIC Hub participated in the final annual meeting of the CORBEL project.
- **ARIA updated to Version 2.3.**

aria

Instruct offered priority access to COVID-19 research

APR

New Instruct Member Organisation

EMBL

MAY

- **UK consortium launches COVID-19 Protein Portal to provide essential reagents for national SARS-CoV-2 research**
- Instruct-ERIC Council meeting

JUN

Instruct joint the Horizon 2020 project TRANSVAC-DS

TRANSVAC DS

New Instruct Member Country
LITHUANIA

JUL

Open Call for Glycan Analysis funded by Instruct-ULTRA to send samples to the Institute of Chemistry at the Slovak Academy of Sciences

AUG

Instruct-ERIC inaugurates Robotein in Liège to Instruct Centre BE.

Robotein

- Royal Society Gabor Medal awarded to Instruct-ERIC Director Prof David Stuart
- Instruct-ERIC launched the webinar series

structure meets function

SEP

Instruct-ULTRA hosted the virtual event

Celebration of Instruct-ERIC

Instruct-ERIC wins FREYA Ambassador Competition.

FREYA

OCT

- Instruct-ERIC Council meeting
- Instruct presents its collabortation with the OpenAIRE project
- **Best practices in Cryo-EM Worshop** held at the Astbury Biostructure Laboratory, UK

NOV

- Instruct presentation at PaNOSC/ ExPaNDS Annual meeting
- First discussion with collaborators in Ireland towards a Science Foundation Ireland application to Instruct-ERIC

DEC

After four years the Instruct-ULTRA project concludes

instruct ULTRA

FinStruct & Instruct-ERIC Centre FI selected for inclusion on Finland's national research infrastructure roadmap

instruct ERIC

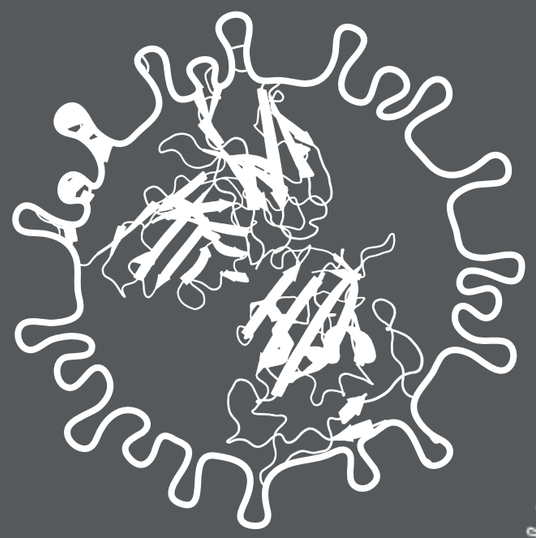
Supporting research relating to

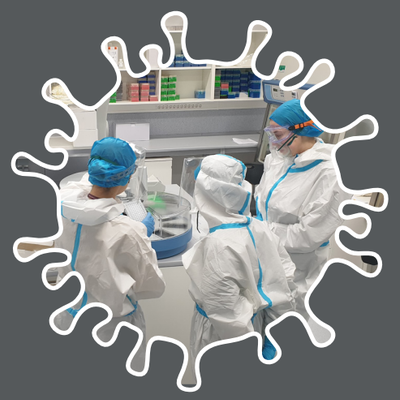
SARS-CoV-2 and COVID-19

Instruct-ERIC is committed to the use of our infrastructure in response to emerging societal needs. In the case of the COVID-19 virus, Instruct-ERIC made its resources available to support researchers in their efforts to study the virus, working towards the development of an effective vaccine or treatment.

Infrastructures like Instruct-ERIC are configured to respond to emerging needs in public health, from the development of antivirals to vaccines. Structural biology can be enormously effective as demonstrated in the SARS-CoV-2 pandemic emergency starting early 2020, with viral proteins structurally characterised weeks after the viral sequence was available. The continued open-access to Instruct-ERIC infrastructure throughout 2020 generated a strong demand for access to instrumentation for COVID-related research, aided by a dedicated rapid response call for projects launched by Instruct on 04 March 2020 which prioritised proposals in this topic. This was followed by the launch of the COVID-19 resource centre on 24 March and the 3D Bionotes online tool on 04 May 2020.

In this early stage of the pandemic, Instruct technologies quickly switched to remote access where possible, with many platforms developing new remote processes specifically to keep their instruments open for access. Thus cryo-EM facilities were made available at several Instruct Centres and the 1.2GHz NMR spectrometer at CERM/CIRMMP was offered to Instruct users in June 2020. Fragment screening on SARS-CoV-2 druggable targets was made available at Instruct Centres UK (X-Chem) and FR2 (CrystalDirect), providing remote crystallography pipelines for soaking and screening microcrystals with chemical fragment libraries.





PRIORITY ACCESS

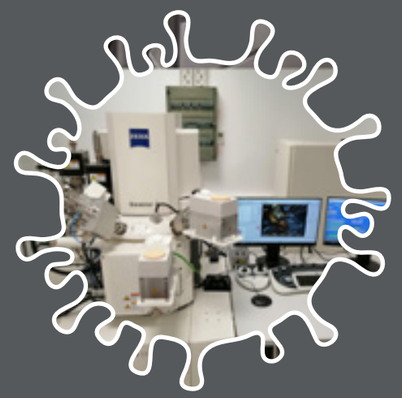
to structural biology services for research proposals relating to COVID-19 virus research

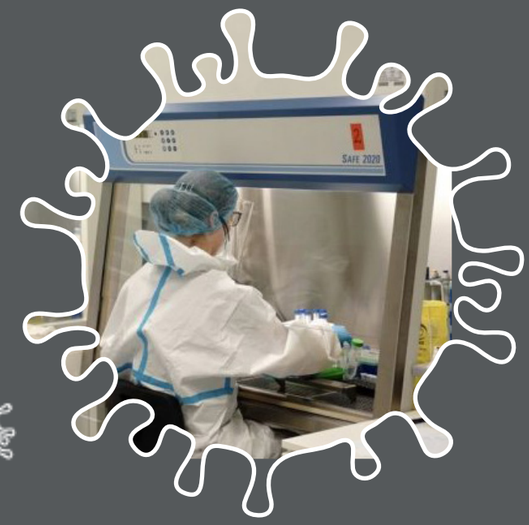
instruct ERIC

As a consequence, the projects supported by Instruct progressed quickly. In addition, it was quickly realised that the essential protein production expertise which produces proteins of exquisite quality for structural characterisation could be immediately engaged for many other purposes, for example as reagents for diagnostic testing, for research into immune responsiveness and drug screening. This led to the launch of the COVID-19 protein portal, which was a repurposed configuration of the ARIA access management system as an online platform that enabled scientists to search and request protein reagents for critical research in SARS-CoV-2. Initially launched in the UK supported by national funding, access for European users was also supported.

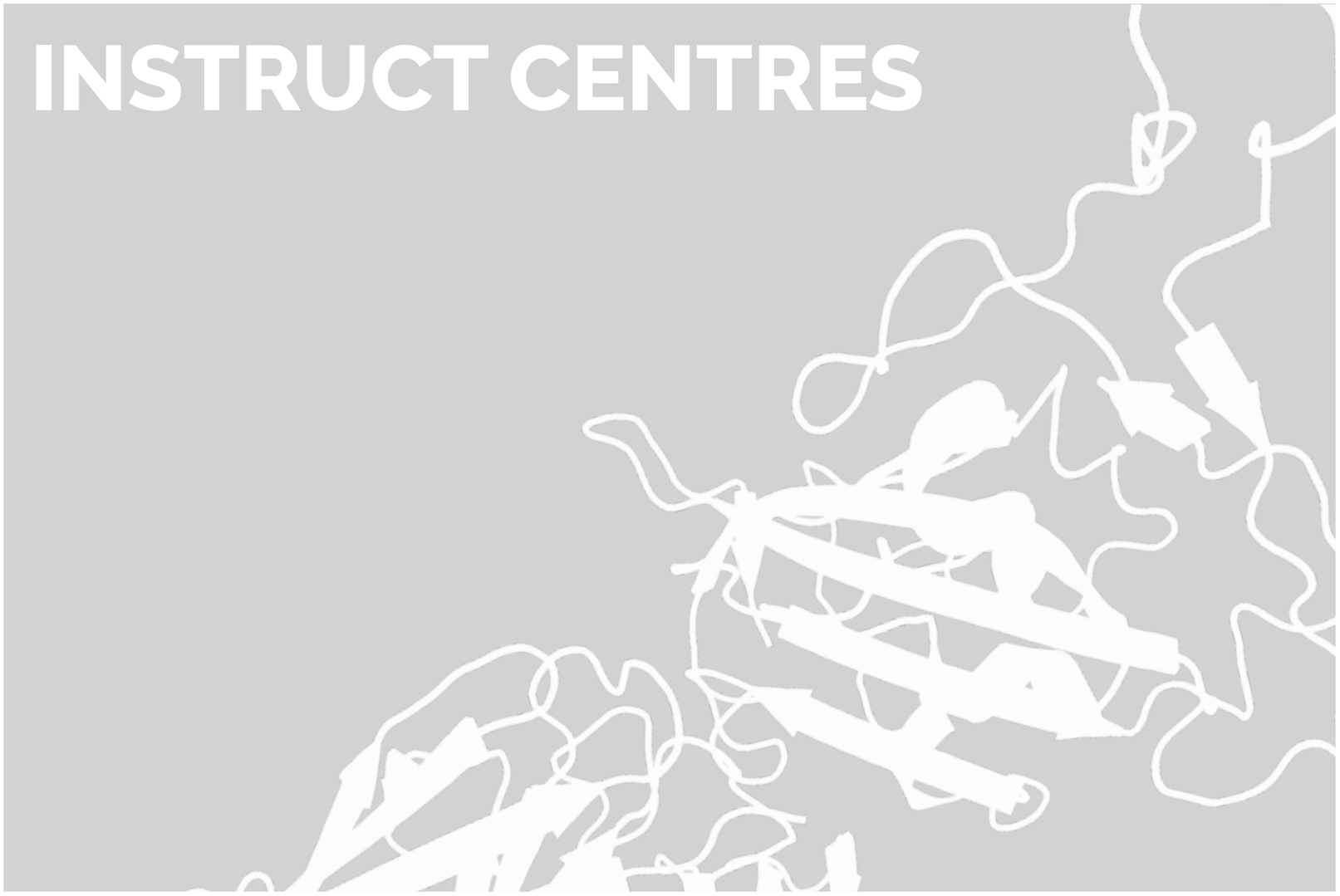
Instruct infrastructures remained functional during the restrictions on population movement and working conditions, facilitated by automation and remote access and by the end of 2020 several publications on SARS-CoV-2 research, facilitated by Instruct access to infrastructure, were public.

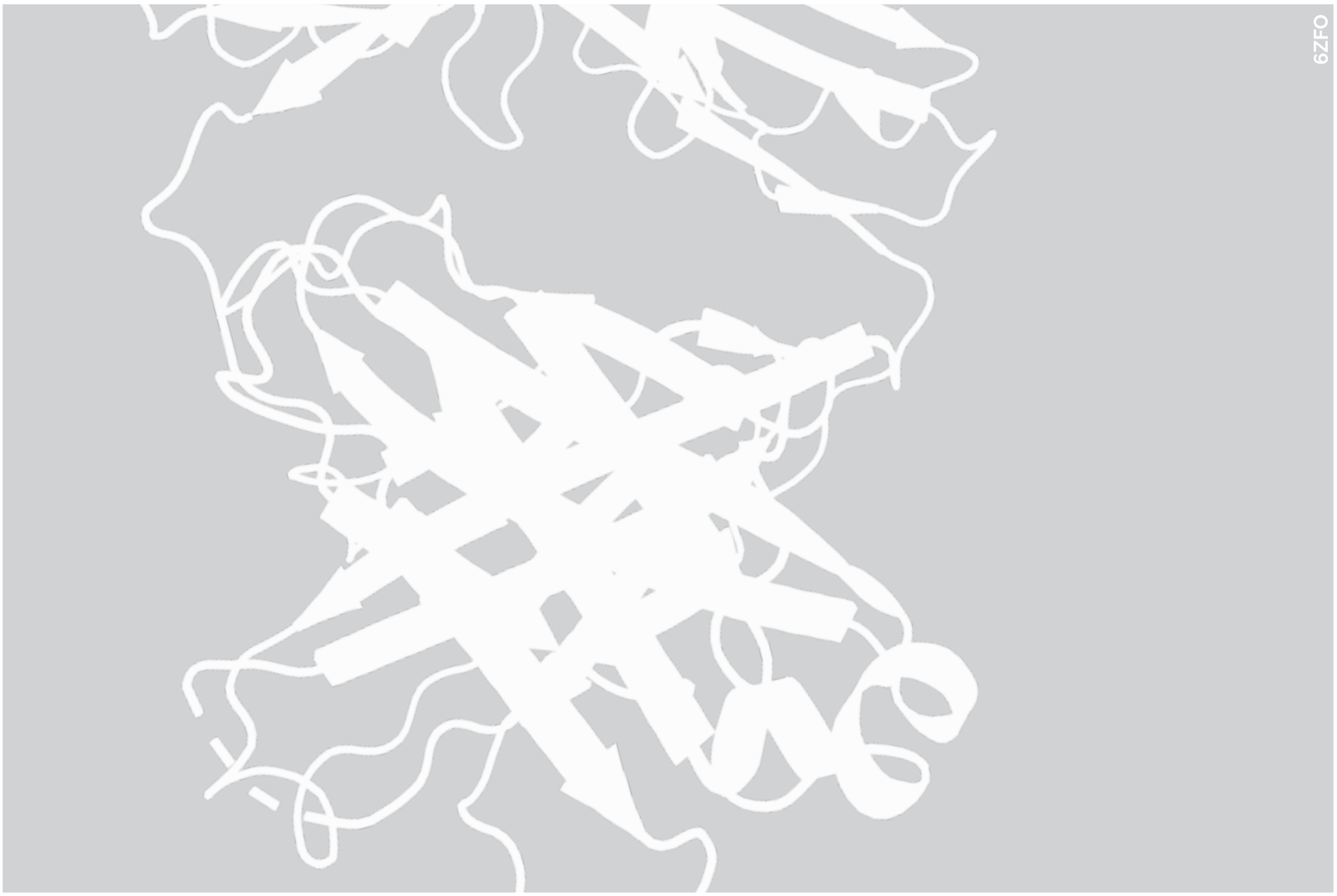
The experiences of 2020 demonstrated that research infrastructures like Instruct are fit for purpose and ready to respond quickly to emergency situations, including following the pattern of SARS-CoV-2 variant emergence and pathology and new pathogenic assaults.





INSTRUCT CENTRES





6ZFO

INSTRUCT CENTRE BE

The Nanobodies4Instruct facility generates Nanobodies and Megabodies to facilitate the structural analysis of proteins that are notoriously difficult to purify, to crystallise or to study by other methods. In 2019, the addition of a second independent facility was approved by the Instruct Executive Committee and then confirmed by the Council. Thus, the Robotein® facility was integrated within Instruct and this was formally celebrated by a launch event on 14 February 2020, during which the facility was formally inaugurated as a research site of Instruct Centre-BE. Finally, Robotein® was presented on 27 May 2020 at an Instruct access committee videoconference and access to users was subsequently announced on the Instruct website on 08 July 2020.

Instruct Centre Lead Scientists

Els Pardon, Jan Steyaert, André Matagne, Alain Brans, Marylène Vandevenne, Erik Goormaghtigh

Nanobodies4Instruct: providing Nanobodies and Megabodies for Structural Biology

Nanobodies are the small (15 kDa) and stable single domain fragments harbouring the full antigen-binding capacity of camelid heavy chain-only antibodies[1]. Nanobodies are exquisite chaperones[2] for crystallising membrane proteins, multiprotein assemblies, transient conformational states and intrinsically disordered proteins. Nanobodies can also be used for other applications in structural biology. Because Nanobodies can be functionally expressed as intrabodies in eukaryotic cells, these single-domain antibodies can also be used to track their targets inside a living cell.

FIG 1

FIG 1. Llamas for Instruct.

Robotein® offers automated screening for optimal recombinant protein expression, purification and formulation, together with biochemical/biophysical protein analysis

The high-throughput (HT) Robotein® facility is a technological platform built on competences and infrastructures available in the academic setting of two laboratories that offer a large portfolio for structural and functional analysis of proteins: the Centre for Protein Engineering at the University of Liège and the Structural Biology and Bioinformatics Centre at the University of Brussels. It offers HT screening for cloning, expression, purification, formulation and characterisation of proteins, including quantification, determination of protein secondary structure content, measurement of phosphorylation and glycosylation, enzymatic assays and interaction with ligands. instrumental to lock and solve the structures of single functional conformations of transporters.

NEW TECHNOLOGIES

Protein formulation through automated screening of pH and buffer conditions, using the Robotein® high throughput facility.

Buffer and pH are known to have a crucial effect on both the stability and activity of proteins. In particular, proper conditions can improve their stability and function significantly during purification, storage and handling. It can also promote data reproducibility, support the interpretation of experimental results and, finally, contribute to our general understanding of the biophysical properties of proteins.

Thus, we have developed[3] a HT screen of 158 different buffers/pH conditions, in which we evaluated both the stability and the function of various model proteins. The modular setup of the screen allows for easy implementation of other characterisation methods and parameters, as well as additional test conditions. Validation of the buffer/pH screen included a nanobody and an enzyme (i.e. CAS12). The latter is currently used for the development of a CRISPR/CAS assay, which, in combination with classical PCR technology, allows rapid detection of the SARS-CoV-2 virus.

1. Muyldermans S. (2031). Nanobodies: Natural Single-Domain Antibodies. *Annu. Rev. Biochem*. 82. 775–797
2. Pardon E. *et al.* (2014) A general protocol for the generation of Nanobodies for structural biology. *Nat. Protoc*. 9. 674–93
3. Kellner R.. *et al.* ((2021) Protein formulation through automated screening of pH and buffer conditions, using the Robotein® high throughput facility, *Eur. Biophys. J.* 50(3-4):473-490

FTIR Imaging of Protein Microarrays for High Throughput Secondary Structure Determination.

A new method was designed[4] for HT protein structure determination. It is based on spotting proteins as microarrays at a density of ca. 2000–4000 samples per cm^2 and recording Fourier transform infrared (FTIR) spectra by FTIR imaging. It also introduces a new protein library, called cSP92, which contains 92 well-characterised proteins. It has been designed to cover as well as possible the structural space, both in terms of secondary structures and higher-level structures. Ascending stepwise linear regression (ASLR), partial least square (PLS) regression, and support vector machine (SVM) have been used to correlate spectral characteristics to secondary structure features. ASLR generally provides better results than PLS and SVM. The observation that secondary structure prediction is as good for protein microarray spectra as for the reference attenuated total reflection spectra recorded with the same samples validates the HT microarray approach. Repeated double cross-validation shows that the approach is suitable for the high accuracy determination of the protein secondary structure with root mean square standard error in the cross-validation of 4.9 ± 1.1% for -helix, 4.6 ± 0.8% for -sheet, and 6.3 ± 2.2% for the "other" structures when using ASLR.

SCIENCE HIGHLIGHTS

In vitro reconstitution of dynamically interacting integral membrane subunits of energy-coupling factor transporters[5]

By using a specific nanobody, Setyawati *et al.* were able to determine the structure of pantothenate (ECF-PanT) a ECF transporter. Comparison of a crystal structure of ECF-PanT with previously determined structures of ECF-FolT2 revealed larger conformational changes upon binding of folate than pantothenate, which could explain the kinetic differences. The work shows that a minimal in vitro system with two reconstituted transporters recapitulates intricate kinetics behaviour observed in vivo.[5]

GABAA receptor signalling mechanisms revealed by structural pharmacology[6,7,8]

In collaboration with the Aricescu lab, Megabodies were successfully used to solve the first cryo-EM structures of the heteropentameric GABAA receptor[6,7] in complex with common drugs including Xanax and Valium. By using a new electron source, energy filter and camera, we also obtained a 1.7 Å resolution cryo-EM reconstruction for a the 3 GABAA homopentamer. Such maps allow a detailed understanding of small-molecule coordination, visualisation of solvent molecules and alternative conformations for multiple amino acids, and unambiguous building of ordered acidic side chains and glycans.[8]

Modulation of the Erwinia ligand-gated ion channel (ELIC) and the 5-HT3 receptor via a common vestibule site[9]

In the study of Brams *et al*, we developed nanobodies which are functionally active as allosteric modulators, the co-crystal structures of the pentameric prokaryote (Erwinia) ligand-gated ion channel ELIC was solved bound either to a positive or a negative allosteric modulator.[9]

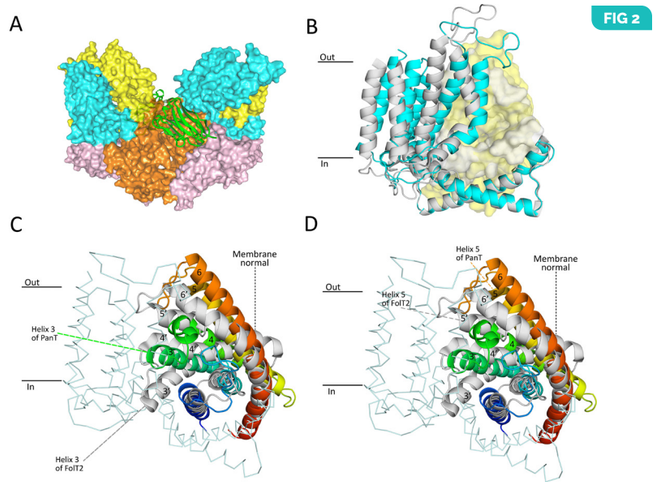


FIG 2

FIG 2. Crystal structure of nanobody-bound ECF-PanT.[5]

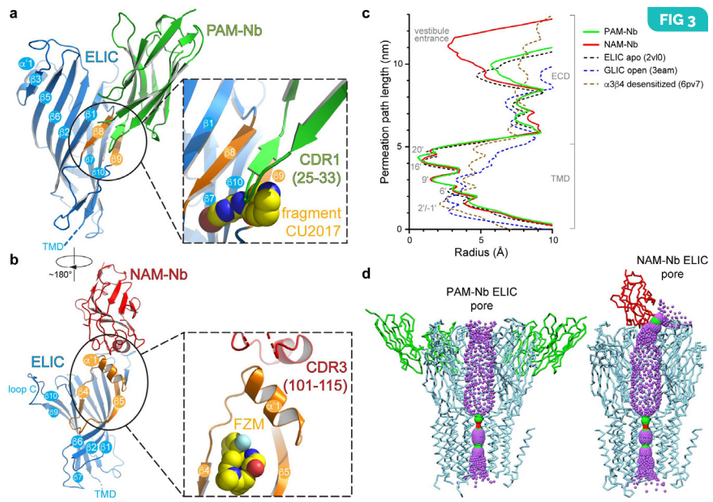


FIG 3

FIG 3. Detailed nanobody interaction sites in ELIC and channel pore analysis.[9]

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6. Masiulis S. *et al.* (2019) GABAA receptor signalling mechanisms revealed by structural pharmacology *Nature* 565, 454-459
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8. Nakane T. *et al.* (2020) Single-particle cryo-EM at atomic resolution *Nature* 587: 152-156.
9. Brams M. *et al.* (2020) Modulation of the Erwinia ligand-gated ion channel (ELIC) and the 5-HT3 receptor via a common vestibule site. *Elife* 9, e51511

INSTRUCT CENTRE CZ

Instruct Centre CZ is coordinated within the Czech Infrastructure for Integrative Structural Biology (CIISB) formed by two Centers of Excellence for Structural Biology: Central European Institute of Technology, Brno (CEITEC) and Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University, Vestec, Prague-West (BIOCEV). CIISB offers open-access and assisted expertise to 10 core facilities for high-end cryo-electron microscopy and tomography, high-field NMR, X-ray crystallography and crystallisation, biophysical characterisation of biomolecular interaction, nanobiotechnology, proteomics, and structural mass spectrometry. Flagship technologies include cryo-Electron Microscopy and Tomography (CEITEC), Josef Dadok National NMR Centre (CEITEC), Structural Mass Spectrometry (BIOCEV), and Diffraction Techniques (BIOCEV).

Instruct Centre Lead Scientists
Vladimír Sklenář
Jan Dohnálek

NEW TECHNOLOGIES in 2020

Since January 2020, a new online submission system has offered simplified protocol for submission of requests for open access to all ten CIISB Core Facilities. All received project proposals of potential users are immediately forwarded to the evaluation process. If the proposals are evaluated positively the applicants are instantly invited to conduct their experiments.

The Josef Dadok NMR National Centre (CEITEC) upgraded all high-field NMR spectrometers from 600 MHz to 950 MHz with new electronics Avance Neo. In addition, the liquification units called BSNL (Bruker Smart Nitrogen Liquefier) were installed on four systems equipped with the cryo-probes (600, 700, 850, and 950 MHz). The nitrogen refill interval is thus extended from 2-3 weeks to more than 6 months.

The CIISB cryo-electron microscopy core facility at CEITEC Masaryk University is expanding its services in the sample preparation for electron microscopy. The facility has recently acquired high-pressure freezer Leica EM ICE for vitrification of bulky biological specimens (up to 200 mm thickness). In addition, the freeze-substitution unit Leica EM AFS2 (for resin embedding of the high-pressure frozen samples) and the ultramicrotome Leica EM UC7 (with the adapter for cryo-ultramicrotomy) were purchased in order to provide the facility users with the complete workflow for preparation of thin section samples for both room-temperature electron microscopy and cryo-EM.

The X-ray Diffraction and Bio-SAXS core facility (CEITEC) upgraded the goniometer (from geometry partial chi to kappa-geometry) and the X-ray detector (from CCD to HPC, Hybrid Photon Counting detector) at the dual wavelength Rigaku single crystal diffractometer.

The Crystallisation of Proteins and Nucleic Acids core facility (BIOCEV) extended automated screening to low temperatures with a new crystallisation hotel RI182 (Formulatrix) to broaden the already available options of dedicated spaces for crystallisation at three different temperatures.

In the Proteomics (CEITEC) and Structural mass spectrometry (BIOCEV) core facilities new hybrid mass spectrometers with ion mobility module timsTOF Pro (Bruker) were installed for shotgun proteomics, hydrogen-deuterium exchange, covalent labelling experiments, and native mass spectrometry with ion mobility separation.

The Centre of Molecular Structure at BIOCEV established a new Protein Production facility. This initiative follows the survey mapping and preliminary interest from potential users and resulted in a platform for both routine protein production and demanding development of methods in semi high-throughput format. The facility has been in a pilot mode since July 2020 to evaluate the feasibility for the long run. Now the new Protein Production facility has been added to the CIISB application form and users can apply via the standard application process.

FIG 1

FIG 1. Structural Mass Spectrometry core facility at BIOCEV with a new timsTOF Pro spectrometer.

CIISB is committed to the use of its resources in response to the emergency situation of the COVID-19 virus pandemic. Successful proposals are free of charge, and no financial contribution is requested for the measurement/service. All Instruct-CZ - CIISB Core facilities have been fully functional. Visits of external foreign users are regulated by the Measures concerning foreigners and border crossing of the Czech Government. Typically, the samples are mailed in and handled as usual on the first-come-first-serve basis. Despite all COVID-19 regulations during 2020, the extent of open access to CIISB technologies was not negatively influenced by the adopted measures and remained at the level comparable to, or even higher than, previous years. The data obtained by CIISB contributed to 108 scientific papers published during 2020, compared to 95 in 2019 and 78 in 2018. Increase in the quantity goes along with the increase of the quality of published papers. 21 papers appeared in the Nature Index journals, including those in Science, Nature Communications, Nature Structural and Molecular Biology, Angewandte Chemie. Int. Ed., Journal of the American Chemical Society, Journal of Cell Biology, etc.

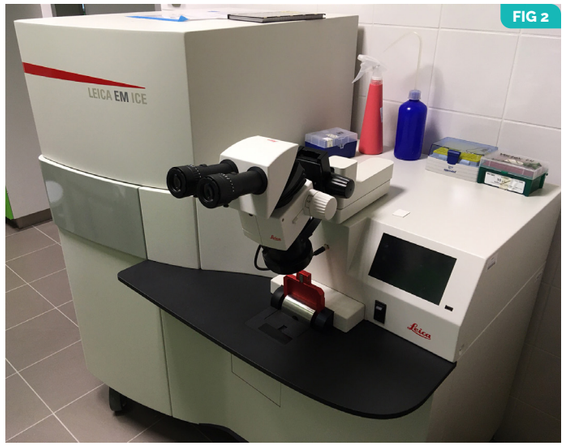

FIG 2

FIG 2. High-pressure freezer Leica EM ICE for vitrification of bulky biological specimen (up to 200 mm thickness) at CEITEC cryo-electron microscopy and tomography core facility.

SCIENCE HIGHLIGHTS

Capsid opening enables genome release of iflaviruses - Pavel Plevka Research Group, CEITEC[1]

The family Iflaviridae includes economically important viruses of the western honeybee such as deformed wing virus, slow bee paralysis virus, and sacbrood virus. Iflaviruses have nonenveloped virions and capsids organised with icosahedral symmetry. The genome release of iflaviruses can be induced in vitro by exposure to acidic pH, implying that they enter cells by endocytosis. Genome release intermediates of iflaviruses have not been structurally characterised. Here, P. Plevka *et.al.* show that conformational changes and expansion of iflavirus RNA genomes, which are induced by acidic pH, trigger the opening of iflavirus particles. Capsids of slow bee paralysis virus and sacbrood virus crack into pieces. In contrast, capsids of deformed wing virus are more flexible and open like flowers to release their genomes. The large openings in iflavirus particles enable the fast exit of genomes from capsids, which decreases the probability of genome degradation by the RNases present in endosomes.

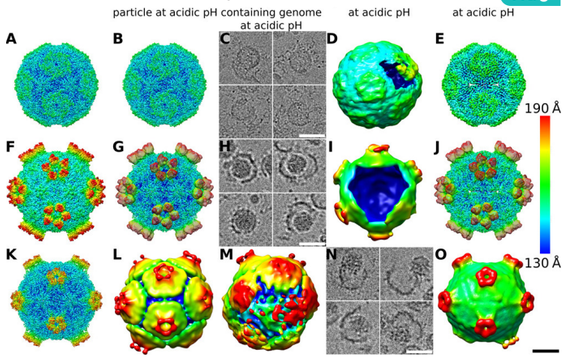

FIG 3

FIG 3. Structural changes in iflavirus particles that enable genome release of SBV, SBPV, and DWV. Structural changes in iflavirus particles that enable genome release of SBV, SBPV, and DWV.

Mycobacterial HelD is a nucleic acids-clearing factor for RNA polymerase – Libor Krásný Research Group, Institute of Microbiology CAS[2]

RNA synthesis is central to life, and RNA polymerase (RNAP) depends on accessory factors for recovery from stalled states and adaptation to environmental changes. Here, T. Kouba, J. Dohnálek, L. Krásný et. al. investigated the mechanism by which a helicase-like factor HelD recycles RNAP. They report a cryo-EM structure of a complex between the *Mycobacterium smegmatis* RNAP and HelD. The crescent-shaped HelD simultaneously penetrates deep into two RNAP channels that are responsible for nucleic acids binding and substrate delivery to the active site, thereby locking RNAP in an inactive state. They show that HelD prevents non-specific interactions between RNAP and DNA and dissociates stalled transcription elongation complexes. The liberated RNAP can either stay dormant, sequestered by HelD, or upon HelD release, restart transcription. Their results provide insight into the architecture and regulation of the highly medically-relevant mycobacterial transcription machinery and define HelD as a clearing factor that releases RNAP from nonfunctional complexes with nucleic acids.

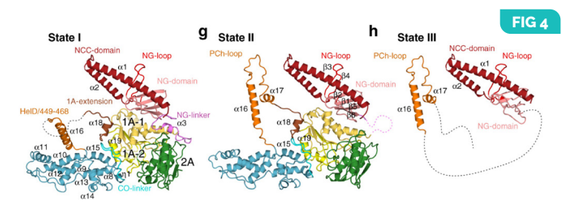

FIG 4

FIG 4 Three states of HelD colour-coded according to the domain structure .[2]

1. Škubnik K., *et al.* (2021) Capsid opening enables genome release of iflaviruses, *Sci. Adv.* 7(1):eabd7130
2. Kouba T. *et al.* (2020) Mycobacterial HelD is a nucleic acids-clearing factor for RNA polymerase, *Nature Comm.* 11:6419

INSTRUCT CENTRE ES

Instruct Centre ES hosts the Instruct Image Processing Center (I2PC) and the Instruct Cryo-EM – CSIC facility, working together to provide support to the Structural Biology Community.

Instruct Centre Lead Scientists

Rocio Arranz, Francisco Javier Chinchon, Teresa Bueno, Roberto Melero, Marcos Gragera

The Electron Microscopy Image Processing Service provides state-of-the-art software infrastructure, support and image processing services for elucidating the structure of biological macromolecules by Cryo-EM Single Particle Analysis and cellular subsections by cryo-Electron Tomography. The contribution to Instruct is two-fold. On one side, Instruct-ES produces an integrative image processing workflow engine (Scipion) that serves thousands of projects worldwide. This software allows the definition of image processing pipelines capable of analysing the raw data acquired by the microscope and turning it into insightful biological knowledge. On the other side, the scientific community is supported by creating and applying those image processing pipelines to specific projects approved by Instruct. The Centre is also open to medium-term internships through Instruct so that users can learn the image processing methodology and bring it back to their home laboratories.

The Cryo-EM – CSIC facility provides expertise to researchers with samples in the first stages of characterisation. Samples are analysed by negative staining in search of the best conditions. Once found, samples are subjected to different vitrification conditions in search of the best parameters, which are then tested in a cryomicroscope Talos Arctica 200 kV equipped with a Falcon III electron direct detector. The best grids are used to acquire data for image processing, and in this regard it is advisable to contact the EM Image Processing service for support in the data processing.

FIG 1

FIG 1. TITEL

NEW TECHNOLOGIES in 2020

In 2020 we have extensively improved our image processing workflow engine (Scipion) to facilitate its installation, update, and its adaptation to the currently supported Python standards. We have also made a strong effort in enlarging the number of plug-ins in the Single Particle Analysis and Atomic modelling pipelines. Similarly, we have also addressed the image processing pipeline required by cryo-EM facilities in order to obtain immediate feedback on the quality of the acquisition and a processing in streaming that the user may continue in his/her home laboratory. We have also substantially advanced in the design of plug-ins for electron tomography. These plug-ins are currently in a beta state so that it is actively used by early adopters of this new framework.

The Cryo-EM – CSIC facility has acquired state-of-the art infrastructure for different purposes. The main acquisition has been a 300 kV cryoelectron microscope JEOL CryoARM300 equipped with a Gatan K3 direct electron detector and an Omega filter, which will be used for single-particle analysis and for cryoelectron tomography.

A big effort has been expended in acquiring infrastructure for cryo correlative microscopy. The possibility of analysing biological events tracked by optical microscopy (using a cryo confocal microscope ZEISS LSM900 AiryScan2) and structurally characterised by cryo electron tomography, thanks to the recent acquisition of the cryo-FIBSEM microscope ZEISS CrossBeam 550, has a huge potential interest in the Spanish (and European) community of cell biologists. The infrastructure has recently been installed.

SCIENCE HIGHLIGHTS

Instruct Centre Spain's computational services do not require physical access and our instrumental ones were already used to conduct users projects remotely. For this reason, both the Instruct Image Processing Center and the Cryo EM-CSIC Facility have been accessible during most of the pandemic. Part of our work in COVID-19 image processing involved the analysis of the SARS-CoV-2 spike protein, which has been been published in:

Continuous flexibility analysis of SARS-CoV-2 spike prefusion structures[1] - PID 11775

Using a new consensus-based image-processing approach together with principal component analysis, the flexibility and conformational dynamics of the SARS-CoV-2 spike in the prefusion state have been analysed. These studies revealed concerted motions involving the receptor-binding domain (RBD), N-terminal domain, and subdomains 1 and 2 around the previously characterised 1-RBD-up state, which have been modeled as elastic deformations. It is shown that in this data set there are not well defined, stable spike conformations, but virtually a continuum of states. An ensemble map was obtained with minimum bias, from which the extremes of the change along the direction of maximal variance were modeled by flexible fitting. The results provide a warning of the potential image-processing classification instability of these complicated data sets, which has a direct impact on the interpretability of the results. The 3DBionotes web platform provides an interactive environment where structural and multi-omics data can be analysed and explored. The COVID-19 outbreak has heightened the need for a central, web-based resource to access the structure, related biochemical and biomedical annotations and new variants of all proteins associated with the SARS-CoV-2 virus.

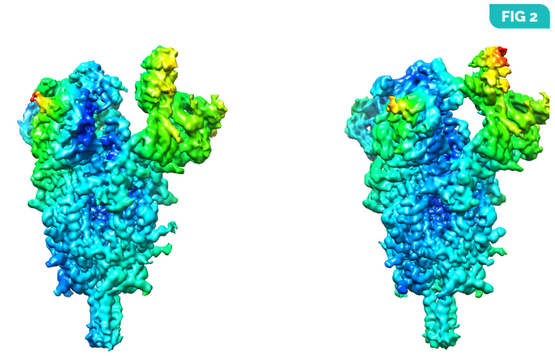

FIG 2

FIG 2. Different conformations adopted by the SARS-CoV-2 spike. The colors correspond to different levels of structural stress, from very stable areas (in blue) to very unstable (in red), passing through areas of intermediate stress (in green)

COVID-19: Structural resource with map annotations - PID: 11778

The COVID-19 3DBionotes platform has been created and hosted by the Instruct Image Processing Centre, in the Biocomputing Unit of the National Centre of Biotechnology in Spain (CNB-CSIC), in close collaboration with ELIXIR-ES. Data has been acquired from a wide range of sources, including cryo-EM, as well as X-Ray crystallography and NMR. A novel set of annotations for this COVID-19 edition includes functional mapping of the residues that likely constitute ligand and protein-protein interaction binding sites, drug screening, validation and quality measurements, as well as model refinements provided by the Coronavirus Structural Taskforce.

This has been a crucial resource for structural biology researchers throughout the COVID-19 pandemic, providing easy access to regularly updated data on the structure of the virus.

FIG 3. 3DBionotes-COVID-19 application screenshots. **(A)** Landing page, showing some of the main sections: representative examples, a simplified schema of the virus proteome that serves as index with links to the corresponding subsection for every protein, followed by various panels containing all the structures. **(B)** 3D viewer and annotations, showing the example EMDB-30915, corresponding to APO form spike glycoprotein of SARS-CoV-2. By clicking in any of the symbols representing an annotation, all the residues associated with it will be highlighted in the protein sequence alignment as well as in the atomic structure. At the same time, those residues will also be highlighted with vertical yellow bars so it is easier to locate in relation with other annotations types. Additionally, a panel will pop-up with more detailed information about the annotation, including links to the origin of the data source. In this case, values obtained with FSC-Q validation method are shown over the whole structure.

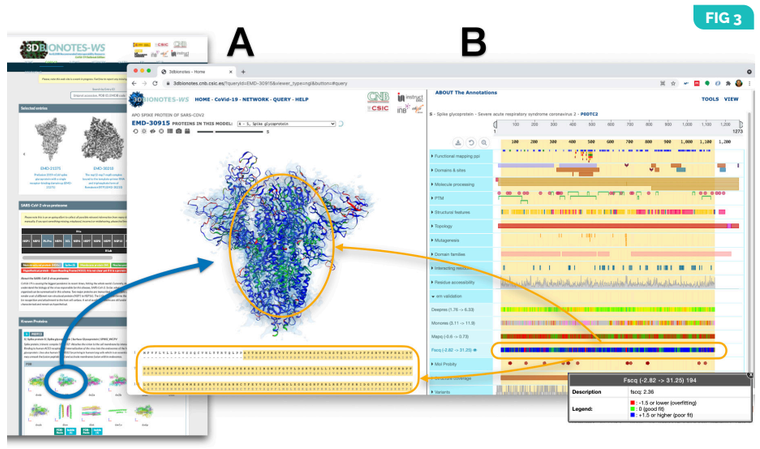

FIG 3

1. Melero R. *et al.* (2020). Continuous flexibility analysis of SARS-CoV-2 spike prefusion structures. *IUCrJ*. 7(6):1059-1069

INSTRUCT CENTRE FI

Instruct Centre FI is formed by three Centers of Excellence at the Universities of Helsinki, Oulu, and Eastern Finland. Since February 2020, the Centre has provided expert-supported user access in state-of-the-art sample preparation, characterisation, and structural biology techniques through the Instruct-ERIC service/technology catalogue.

University of Helsinki provides an integrative environment for purification, analysis and structural studies of biological complexes. The expertise includes biomolecular complex purification; cryo-electron microscopy; segmental isotope labelling for nuclear magnetic resonance and neutron scattering; and single cell proteomics. University of Oulu fosters the expertise and infrastructure for molecular biophysics, protein crystallography and in-house data collection and develops the IceBear crystal data management software. University of Eastern Finland offers high-resolution native mass spectrometry techniques for studies of protein folding and assembly of biological macromolecules as well as for quantitative biological interaction studies with large dynamic range.

Instruct Centre Lead Scientists
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Hideo Iwai
Markku Varjosalo
Lari Lehtiö
Lloyd Ruddock
Juha Rouvinen
Janne Jänis

NEW TECHNOLOGIES in 2020

New sample preparation strategies for biomolecular complexes

Asymmetrical flow field-flow fractionation (AF4) allows gentle size-based separation of fragile biological complexes in liquid phase for subsequent structural, biophysical or biochemical analyses. This technology is a new service in the Instruct-ERIC catalogue[1]. The instrument is the first new-generation Wyatt Eclipse Neon AF4 device installed in Europe. The advanced multi-detector system of the instrument includes multiangle and dynamic light scattering detectors as well as UV, refractive index and fluorescence detectors. This setup enables measurement of radius of gyration, absolute molar mass and hydrodynamic radius of the samples downstream of AF4 separation. AF4 can be used in analytical and semi-preparative mode for the characterisation and purification of samples over large dynamic size range. Samples produced at Biocomplex facility can be further studied at the cryo-EM facility located in the Instruct ERIC Centre-FI at the University of Helsinki.

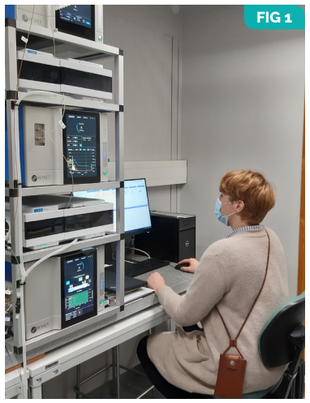


FIG 1. PhD student Johanna Puutio was the first user of the new AF4 Eclipse NEON instrument at the Instruct ERIC Centre FI, University of Helsinki. (Picture taken by Katri Eskelin)

Single-cell proteomic SCoPE-MS analyses

Recent technological developments in cell separation and mass spectrometry analyses enable single-cell proteome studies - deconstructing differential cell populations, and inference of protein abundance relationships. The proteome configuration of single cells can be linked to functional phenotypes. We have demonstrated the applicability of this technology also on human samples derived from COVID-19 patients. Single-cell level detailed protein analysis complements routine genomics and transcriptomics data.

Intein-based method for segmental isotopic labeling

Scarless intein-based protein ligation, which has been developed for segmental isotopic labeling of multi-domain proteins, is a powerful tool in structural investigation. We have recently developed a salt-inducible split intein where the ligated product has the native protein sequence without any modification at the ligation junction[2]. Protein ligation was used to produce antibody-like molecules against SARS-CoV-2 in vitro.

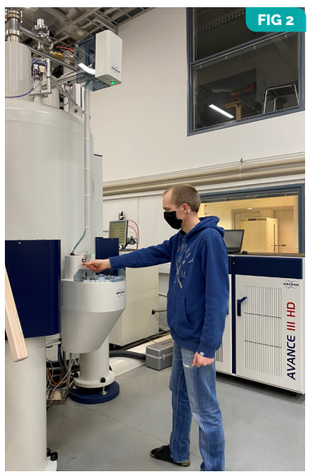


FIG 2. Laboratory Engineer Tuomas Niemi-Aro operating the 850 MHz magnet at the Instruct ERIC Centre FI, University of Helsinki.

Data management software IceBear for crystallisation

The software package IceBear (Integrated Crystal-data-tracking Enhancing Biochemistry Education And Research) has been developed at the University of Oulu in collaboration with the Weizmann Institute of Science and Diamond Light Source (www.icebear.fi). IceBear records individual crystallisation drop experiments at home laboratories through a series of time-dependent images generated by various drop imaging systems. It captures all the relevant crystallisation metadata. Modules have been added to exchange the metadata upon sample shipment with ISPyB, a Laboratory Information Management System used for sample tracking and experiment reporting at synchrotron beamlines. This functionality has been implemented at DLS and collaboration with MAXIV and ESRF has been initiated to extend the support to other European synchrotrons. The crystal-page in IceBear provides a link to the relevant ISPyB page and it can also store links to the published raw diffraction data, PDB, Proteopedia and publication(s).

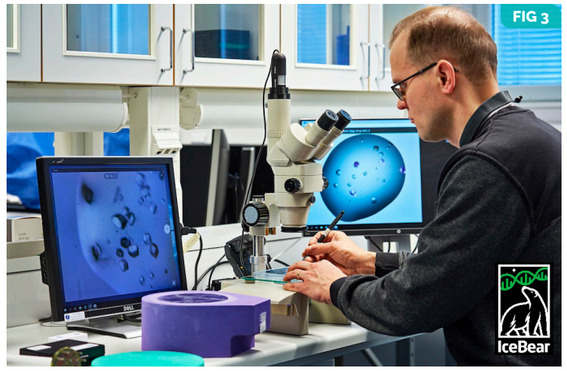


FIG 3. Instruct Centre FI Scientist Dr Kristian Koski fishing out crystals for data collection and tracking the samples simultaneously with the IceBear software at the Instruct ERIC Centre FI, University of Oulu.

Native mass spectrometry of proteins and their complexes

High-resolution mass spectrometry platform at University of Eastern Finland provides native mass spectrometry services for the life science community. The major instrument for these studies has been the ultra-high resolution 12T Bruker Solarix XR FTICR mass spectrometer which has been used for measuring protein-protein, protein-ligand, and protein-metal complexes. A recently installed Bruker timsTOF mass spectrometer offers new possibilities to analyse shape, size, and conformations of proteins in the native state. The analysis provides experimental values for the collision cross-section of proteins with high accuracy. This value is directly proportional to the protein structure. In 2020 we have tested and optimized timsTOF instrument in analysis of model proteins and achieved typical ion mobility resolution values of 20 (FWHM). The highest ion-mobilty resolution (130) was obtained for a zinc-finger peptide which showed the existence of two isomers.

SCIENCE HIGHLIGHTS

Structure determination of Deinococcus radiodurans DNA polymerase I (DrPolI) and DrPolI:DNA complex by cryo-EM - APPID926

Andreia Fernandes from Portugal visited the Instruct Centre FI for 3 months in 2020 and was supervised by Sarah Butcher and her cryo-EM team. Andreia said that "In my PhD project I have been developing experiments to determine the structure of Deinococcus radiodurans DNA polymerase I (DrPolI) and NAD+ dependent DNA ligase by X-ray crystallography, both have been challenging to crystallize. Cryo-EM is a promising structural biology methodology as an alternative to X-ray crystallography. Thus, for my Instruct internship, the specific research objectives were to determine the DrPolI (monomer 102 kDa) structure by cryo-EM and also the structure of DrPolI complexed with DNA. So far, I obtained a preliminary 14 Å model of DrPolI dimer, and a 9 Å model of DrPolI monomer using computer access to the Finnish CSC- IT Centre for Science. As part of the plan, in my host institution I gathered preliminary data and started data processing, but part of the structure determination is being finished in my home laboratory. The Instruct internship was important not only for my thesis work, but also for learning cryo-EM methodologies with support of experts in the field. I had the opportunity to learn how to prepare a sample for single particle imaging by cryo-EM, how to assess ice and sample quality, the steps of data collection in TEM, and data processing via Scipion and cryoSPARC. I used equipment such as glow discharger and different plungers. Moreover, I also performed grid clipping, transfer to the loading cassette, and to storage. I followed the atlas screening, setting up and selection of data for data collection in FEI TALOS Arctica microscope, an equipment that I do not have access in my home institution and country. I also took part in an international workshop on sample preparation for cryo-EM. Subsequently my prepared grids were shipped to and imaged at the ESRF on a Titan Krios and I am continuing with data collection."

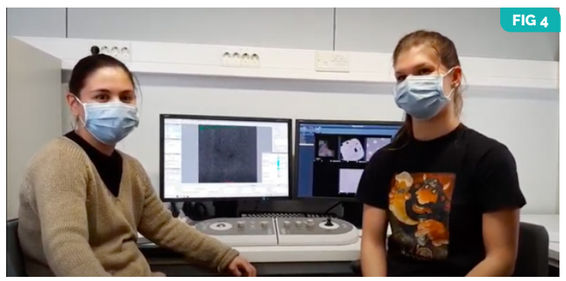


FIG 4. Andreia Fernandes (left) and Zlatka Plavek (right) collecting data at the Instruct Centre-FI cryo-EM facility in Helsinki during Andreia's Instruct Internship. Photo: IMpaCT project (Horizon 2020 Research and Innovation programme under GA 857203), picture from the IMpaCT Single Particle Workshop organised by the University of Helsinki partner.

1. De S. *et al.* (2020) Association of host protein VARICOSE with HCPro within a multiprotein complex is crucial for RNA silencing suppression, translation, encapsidation and systemic spread of potato virus A infection. *PLoS Pathog.* 12;16(10):e1008956

2. Ciragan A. *et al.* (2020) NMR Structure and Dynamics of TonB Investigated by Scar-Less Segmental Isotopic Labeling Using a Salt-Inducible Split Intein. *Front Chem.* 19;8:136

INSTRUCT CENTRE FR1

The Instruct-Centre France-1 at Strasbourg hosted in the Center of Integrative Biology (CBI), is located on the IGBMC site at Illkirch/ Strasbourg, which is also the coordinating Centre for FRISBI (French Infrastructure for Integrated Structural Biology). The centre provides an integrated environment for structural studies of protein and macromolecular complexes.

Instruct Centre Lead Scientists
Patrick Schultz
Jean Cavarelli
Arnaud Poterszman

Its integrated structural biology platform offers project-based access to a large panel of tools from sample preparation (bacterial, insect and mammalian cell expression systems), purification and biophysical characterisation to three-dimensional structure determination using cryo-EM (our flagship for Instruct-ERIC), X-ray crystallography, small angle X-ray scattering, NMR of proteins and macromolecular complexes including nucleoprotein complexes. Taken together, this allows integrating functional data and various multi-resolution structural data. These activities are supported by experienced engineers and technicians and by the strong scientific environment and know-how provided by the Department of Structural Biology at the CBI/IGBMC .

NEW TECHNOLOGIES in 2020

Genome editing to tag endogenous complexes for purification and imaging

Using genome editing to tag endogenous protein complexes, a pipeline for the purification of macromolecular complexes from endogenous sources was developed. In the frame of a collaborative effort with the Tacgene Platform (Museum National d'Histoire Naturelle, France), procedures for tagging proteins in mammalian cells using the CRISPR-Cas9 technology, clone validation and large scale cultures were established[1,2]. Within the frame of Instruct-ULTRA, a test access was provided to an external user that has prototyped the pipeline as a new technology service for Instruct.

Using genome engineering, standardised procedures for labelling complexes in cells for imaging (live cell confocal and super solution microscopy) was implemented. As a proof of concept, knocked in U2OS derived cell lines, where the XPB, XPD and MAT1 subunits of the transcription/ DNA repair complex have been labelled with the GFP or its mEOS2 derivative, were obtained. Tagging of the XPB subunit is detailed in Figure 1.

In addition, Instruct Centre-FR1 revised its procedures to assemble multigene construct for recombinant expression in insect cell using the baculovirus expression system.[3,4]

FIG 1

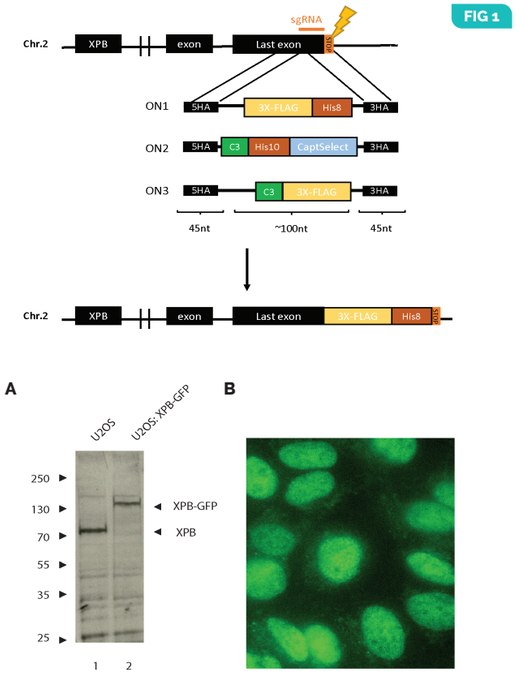


FIG 1. Protein labelling for imaging and functional proteomics. To introduce a GFP tag at the C-terminal end of the XPB gene, a sgRNA was selected to generate a CRISPR/Cas9-induced double strand breaks in vicinity of the stop codon of the XPB gene. The donor plasmid contains the eGFP-2A-Puro coding sequence flanked by respectively 300- and 800 bp homology arms (5' and 3' HA). The eGFP-2A-Puro coding sequence is inserted at the position of the XPB STOP codon and in frame with the last exon of the target protein. **(A)** Western blot analysis of engineered U2-OS cells in which wild-type XPB was replaced by C-terminal GFP-tagged version (U2-OS XPB::GFP, lane 2). Un-modified U2-OS cells were used as control (U2-OS, lane 1) (Adapted from Sandoz et al. 2019). **(B)** GFP fluorescence of living U2OS XPB::GFP cells observed using an inverted microscope equipped with a 63x immersion objective.

SCIENCE HIGHLIGHTS

Access to the Electron microscopy platform - PID 12263

V. cholerae is known to be a danger in Developing countries and the WHO has shortlisted *H. influenzae* as a pathogen, against which novel antibiotics are urgently needed. The sialic acid transporters HiSiaPQM and VcSiaPQM from Haemophilus influenza and Vibrio cholera are involved in a number of processes which protects these pathogens from the innate immune response of the host. The goal of this project is to determine for the first time the structure of a sialic acid TRAP transporter by cryo-EM. A high-resolution structure will certainly help to better understand its function and ultimately to use it as a target for novel antibiotics.

Gregor Hagelueken from the Institute of Structural Biology, University of Bonn, has used the electron microscopy facility to record a dataset of the transporters embedded in a lipid nanodisc. Nanobodies have been added to the sample to mark the location of the transporter inside the nanodiscs to facilitate its localisation. A full high-resolution dataset has been acquired. 2D classes obtained from this dataset clearly show the nanodisc and the presence of the nanobody helps to locate the transporter in order to overcome the variability in the protein positioning within the nanodisc. This tool will help to reconstruct the transporter from the dataset.

FIG 2

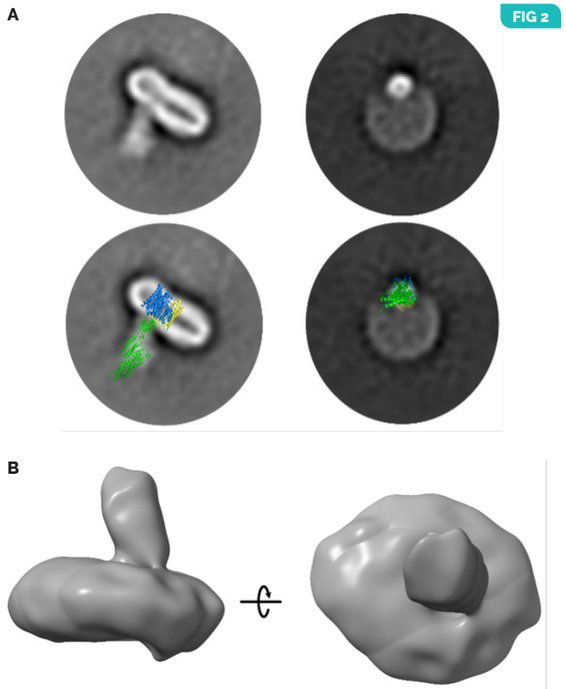


FIG 2. (A) Representative 2D class average (side and top view, top left and top right respectively). Structure of the nanobody (in green) is shown. Density in the nanodisc corresponding to the transporter is visible. **(B)** 3D reconstruction of the nanodisc with a nanobody bound.

Structural and functional aspects of the heme-binding protein p22HBP - PIDs 1046, 1797 and 3808

The SOUL, or heme-binding protein HBP/SOUL, family represents a group of evolutionary conserved putative heme-binding proteins that contains a number of members in animal, plant and bacterial species.

Brian Goodfellow from CICECO at the University of Aveiro, Portugal has used the protein expression platform to express the wild type murine and human HEBP1 proteins in bacteria. The expressed proteins were used to perform crystallography studies on the crystallography platform and functional assays.

This work discusses the structures of HEBP1 and HEBP2 in light of new X-ray data for heme bound murine HEBP1. The interaction between tetrapyrroles and HEBP1, initially proven to be hydrophobic in nature, was thought to also involve electrostatic interactions between heme propionate groups and positively charged amino acid side chains. However, the new X-ray structure, and results from murine HEBP1 variants and human HEBP1, confirm the hydrophobic nature of the heme-HEBP1 interaction, resulting in Kd values in the low nanomolar range, and rules out any electrostatic stabilisation. Results from NMR relaxation time measurements for human HEBP1 describe a rigid globular protein with no change in motional regime upon heme binding.

FIG 3

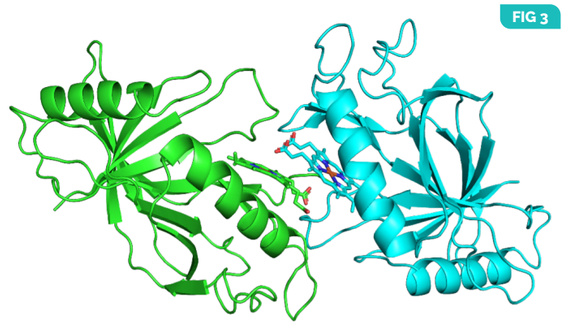


FIG 3. The crystal structure of heme-bound mHEBP1 at 2.45 Å resolution. Two molecules are seen in the asymmetric unit with the hemin molecules stacked against each other at a dimer interface.

1. Geny S, *et al.* (2021) Tagging Proteins with Fluorescent Reporters Using the CRISPR/Cas9 System and Double-Stranded DNA Donors. *Methods Mol Biol.* 2021;2247:39-57
2. Geny S, *et al.* (2021) Gene tagging with the CRISPR-Cas9 system to facilitate macromolecular complex purification. Methods Mol Biol. Structural Proteomics. *Methods Mol Biol.* 2305:153-17
3 Rossolilo *et al.* (2021) Production of multiprotein complexes using the Baculovirus expression system: Homology based and Restriction Free cloning strategies for construct design. *Methods Mol Biol.* 2247:17-3
4 Kolesnikova *et al.* (2021) HR-Bac, a toolbox based on homologous recombination for expression screening and production of multiprotein complexes using the baculovirus expression system. (submitted)

INSTRUCT CENTRE FR2

Instruct Centre France-2 in Grenoble provides supported user access to some of the highest-level structural biology instrumentation in France. Our platforms are located at the Institut de Biologie Structurale (IBS) with user access managed by the Integrated Structural Biology Grenoble (ISBG) service unit.

Instruct Centre Lead Scientists
Darren Hart
Winfried Weissenhorn
Guy Schoehn
Caroline Mas
Elisabetta Boeri
Bernhard Brutscher

Sample preparation includes mass spectrometry, cell-free expression, ESPRIT construct library screening and isotopic labelling. The Molecular Biophysics platform provides AUC, SEC-MALLS, MST, Mass Photometry, BLI, ITC, DLS and SPR. Cellular imaging is available using cellular EM, confocal, video, PALM and STORM microscopy. Membrane protein crystallisation is available in close collaboration with the EMBL HTX lab, and structural analysis capabilities are provided by cryo-EM and NMR platforms. All platforms follow a Quality Assurance programme, managed by a full-time quality engineer, and are certified ISO 9001 NFX 50-900.

The flagship technology is the Electron Microscopy platform with its T12, F20 and Glacios microscopes.

NEW TECHNOLOGIES

New Refeyn mass photometer (MP One) at the FR2 biophysics platform

Instruct Centre FR-2 has acquired a Refeyn mass photometer (MP One) at our biophysics platform, in collaboration with local partners including EMBL, that allows measurement of the mass of macromolecules in solution, in their native state and without labelling. One of the major advantages of this instrument is the small amount of sample required. Indeed, a few microlitres of protein solution are sufficient, so it is valuable for experiments where samples quantities are limiting. The relatively fast measurements allow determination of the mass distribution of a sample in order to evaluate complex formation, multimerisation states or stability. Our biophysics platform is proving popular with Instruct visitors and has been functioning in remote access mode during 2020 with users sending their samples.

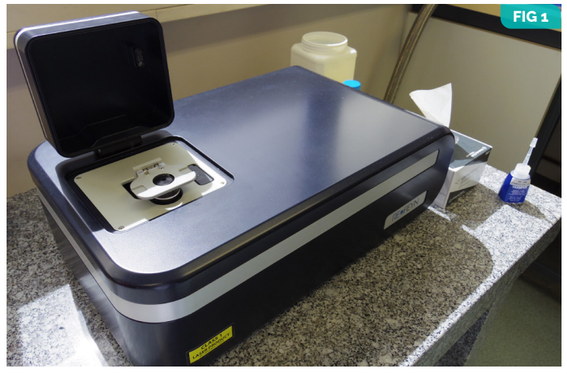


FIG 1

FIG 2. The new Mass Photometer at the France-2 biophysics platform permits rapid analysis of the mass distribution of a protein sample.

New Abbelight super resolution microscope

Complementing our current home-made PALM microscope that remains open to specific applications and future developments, the platform recently acquired, with the support of the GRAL Labex, a new user-friendly super-resolution microscope (Single Molecule Localisation Microscopy) to satisfy the high project demand. The new instrument, a Safe360 from Abbelight™ and Olympus, offers exclusive multicolour 3D super-resolution in STORM/ PALM and PAINT modalities, with single-particle tracking capabilities. The system is driven by a 6 high power lasers combiner and an EPI/HiLo/TIRF illumination module. Two high speed and high sensitivity sCMOS cameras can acquire more than 100 frames per second to drastically reduce the acquisition time. The majority of users of these microscopes are structural biologists seeking to complement their data with cellular observations and measurements.

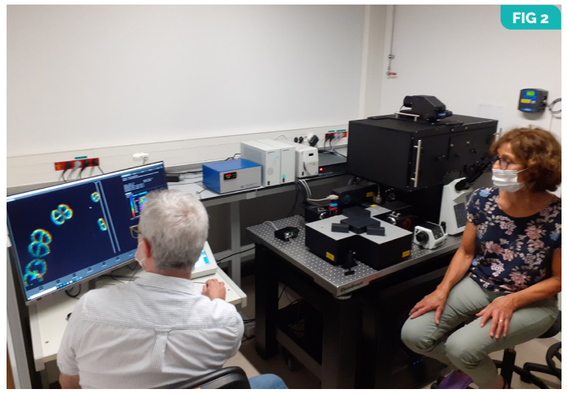


FIG 2

FIG 2. The new Abbelight super resolution microscope at France 2.

SCIENCE HIGHLIGHTS

Biophysical characterisation of the complex between the iron-responsive transcription factor Fep1 and DNA[1] - PID 6867

In order to get insights into the conformational modification of a metabolic yeast transcription factor upon DNA binding, the group led by Maria Carmela Bonaccorsi di Patti from Sapienza University of Rome (Italy) joined efforts with scientists from Instruct France-2 (IBS-IBSG Grenoble; Christine Ebel and Aline LeRoy), Université Claude Bernard Lyon 1 (Adriana E. Miele) and University of Molise (Giovanni Musci and Antimo Cutone).

Fep1 is a transcription factor that regulates the iron uptake in yeast by directly sensing the iron content thanks to the presence of [2Fe-2S] cluster in between two Cys2-Cys2 zinc fingers motifs.

By using a combination of analytical ultracentrifugation, small angle X-ray scattering, fluorescence spectroscopy and differential scanning calorimetry, the Authors have shown that this intrinsically disordered protein is able to gain structural features when wrapping around its cognate DNA sequence, the GATA responsive element. This binding event can also stabilise the complex and promote higher order assemblies, as evidenced by the presence of 1:1 and 2:1 (protein:DNA) stoichiometry. Recombinant purified Fep1 contains a sub-stoichiometric [2Fe-2S] cluster, nevertheless it is able to bind its cognate GATA-responsive DNA element. Once the protein is fully reconstituted with the iron-sulfur cluster, higher stoichiometry start to be present, therefore indicating a role of the cluster in recruiting accessory proteins. Given that the DNA binding constant is in the nM range independently of the content of [2Fe-2S] cluster, the authors hypothesised a role in enhancing/stabilising a conformation of Fep1 prone to recruit accessory proteins to better modulate gene transcription in high iron conditions.


FIG 3

FIG 3. Preparation of an AUC run at France2 (credit: © CEA/D. Morel)

This work exemplifies the potential of using complementary biophysics techniques for deciphering interactions within multicomponent systems. The Instruct access program supported a one-week visit by Maria Carmela to France-2 (IBS-ISBG) to perform analytical ultracentrifugation sedimentation velocity experiments and analyse the data.

Mass spectrometry studies on the murine S100A9 inflammatory mediator protein[2] - PID 6047

The protein S100A9 belongs to the S100 family of calcium-binding proteins found exclusively in vertebrates and it is a major constituent of neutrophils. In response to a pathological condition, S100A9 can be released extracellularly and induce both pro- and anti-inflammatory signals. It also acts as an antimicrobial agent, through metal sequestration. The mechanisms whereby divalent cations modulate the extracellular functions of S100A9 are still unclear. It has been proposed that these ions may affect both the ternary and quaternary structure of these proteins, thereby influencing their physiological properties. Laure Yatime solved the crystal structures of WT and C80A murine S100A9 (mS100A9) in the presence of calcium and zinc (at 1.45 and 2.35 Å resolution, respectively). These structures unravel an intramolecular disulfide bridge that stabilises the C-terminal tail in a rigid conformation, thus shaping a second Zn-binding site per S100A9 protomer. To date, no structure could be obtained in absence of calcium and zinc. Using different MS approaches at the IBS, we investigated the behaviour of S100A9 with and without divalent cations such as calcium and zinc. We demonstrate that mS100A9 can form both non-covalent and covalent homodimers with distinct disulfide bond patterns. The non-covalent homodimers contain an intramolecular disulfide bridge linking Cys91 to Cys111. The disulfide-crosslinked homodimers are characterised by distinct disulfide bond patterns depending on the metal present. MS data indicate that the relative proportion of these forms depends on the ions bound to the protein. Calcium appears to promote the non-covalent, canonical homodimer, whereas zinc enhances the formation of the S-S bridged homodimer(s). Overall, MS well complements X-ray crystallography through the analyses of samples that could not provide crystallographic data.


FIG 4

FIG 4. Analysing samples at the mass spectrometry platform of France2 (credit: © CEA/D. Morel)

1 Miele AE, *et al.* (2021) Biophysical characterization of the complex between the iron-responsive transcription factor Fep1 and DNA. *Eur Biophys J.* 12.
2 Signor L,*et al.* (2020) Divalent cations influence the dimerisation mode of murine S100A9 protein by modulating its disulfide bond pattern. *J Struct Biol.* 31;213(1):107689

INSTRUCT CENTRE IL

The Israel Structure Proteomics Center (ISPC) at the Weizmann Institute of Science was established 17 years ago through a large financial contribution of the Israel Ministry of Science and Technology.

Instruct Centre Lead Scientists

Shira Albeck — Tamar Unger
Yoav Peleg — Orly Dym

Since 2017, it has been part of the Life Science Core Facility of the Weizmann Institute of Science and under the scientific direction of Prof. Gideon Schreiber and Prof. Joel L. Sussman, with the operation managed by Dr. Tamar Unger, Dr. Yoav Peleg, Dr. Shira Albeck and Dr. Orly Dym. It serves as an Israeli Centre for implementing all steps of the pipeline from gene to 3D protein structure, using state-of-the-art technologies and infrastructures. The ISPC's principal mission is to provide a service for gene manipulation, producing proteins and/or determining 3D structures of protein targets selected by the investigators. The ISPC has developed high-throughput methodologies for cloning, expression, purification, crystallisation, structure determination and structure analysis. The ISPC provides its services to scientists both at the Weizmann Institute and at other academic institutions, biotech/pharma companies in Israel, and to its Instruct-ERIC partners. The ISPC also offers training and consultation for students and staff.

NEW TECHNOLOGIES

New liquid-metal-jet (LMJ) X-ray Diffraction System

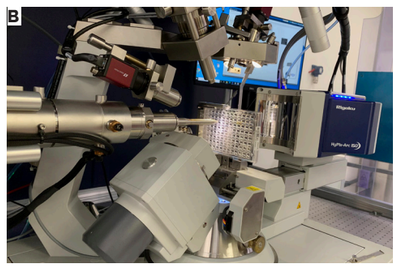
Over the last few years, the technology for in-house X-ray data collection has been revolutionised by the appearance of liquid-metal-jet (LMJ) technology for X-ray sources, combined with pixel detectors. This system produces unprecedented brightness and is considered 'state-of-the-art' in-house X-ray system. The Rigaku LMJ Synergy System with HyPix Arc 150° detector was recently installed at the Structural Proteomics Unit (SPU) (Fig. 1a). This will have an enormous impact on structural biochemistry/biophysics at the Weizmann Institute of Science (WIS) and Instruct-ERIC users.

The Excillum LMJ source has high power load and small electron focus which results in unprecedented specific intensity, yielding a ~3.5 times higher specific intensity than the current generation of rotating anodes.

It permits data collection on crystals down to 10 µm in size, true microcrystals which, until now, has only been possible at synchrotrons using synchrotron radiation. The Rigaku HyPix Arc 150°-pixel area detector is ~x10 more sensitive than current image plates, providing a ~x12,800 faster readout time. Therefore, it greatly reduces collection time per frame, which allows a whole data set to be collected within less than an hour, instead of a whole day on a rotating anode, image plate system.

One of the biggest advantages of the system is that it permits screening for crystal quality by X-ray data diffraction, in situ directly on 96-well crystallisation plates, at room temperature, thus eliminating the time-consuming and challenging task of mounting often small and fragile protein crystals and preventing the decrease in diffraction quality and resolution often observed in the cryo-cooling process (Fig. 1b). In fact, one can collect a full data set by merging partial data from few crystals at room temperature. Applying the in-situ setup we already collected an RT full data set which was merged from 4 crystals of hen egg-white lysozyme to 1.4 Å resolution yielding an excellent map. We also collected 1 Å resolution data on a single hen egg-white lysozyme at cryo-temperature (Fig. 1c).

FIG 1



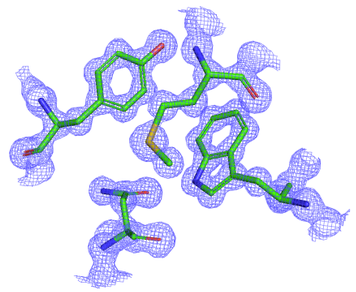


FIG 1. (A) Rigaku Liquid Metal Jet (LMJ) Synergy System with an Excillum LMJ source and Rigaku HyPix Arc 150°-pixel detector operating at the Weizmann Institute Instruct-ERIC Center **(B)** Screening diffraction directly from a 96-well crystallisation plate, at room temperature (RT), with the use of a Rigaku XtalCheck-S system. **(C)** Electron density maps at 1.0Å resolution based on X-ray data collected in about 20 min at 150° K. A model of hen egg-white lysozyme is seen fitted to a 2Fo-Fc electron density map after refinement (blue mesh at 2 rmsd).

SCIENCE HIGHLIGHTS

SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor[1]

SARS-CoV-2, which causes COVID-19, resulted in an epidemic of global reach. Structural and functional studies have shown that a single receptor-binding domain (RBD) of the SARS-CoV-2 homotrimer spike glycoprotein interacts with ACE2, which serves as its receptor. Blocking the ACE2 receptors by specific antibodies voids viral entry. Spike mutation that enhance ACE2 binding, such as N501Y, S477N, E484K and L452R also became major variants of concern which elevated the infectivity and severity of COVID-19.

Notably, the RBD domain itself can be used as a competitive inhibitor of the ACE2 receptor binding site. However, its affinity had to be significantly optimised, to reach pM affinity. We have recently developed an enhanced strategy for yeast display, based on C and N-terminal fusions of extremely bright fluorescence colors that can monitor expression at minute levels. Utilising this method, we obtained pM affinity between a mutant RBD and ACE2, based on multiple-steps of selection that combine enhanced binding with increase RBD protein thermostability. Intriguingly, the more contagious mutations, S477N, E484K, and N501Y, were prominently selected by yeast surface display due to their increased affinity. Further in vitro evolution resulted in a 600-fold improvement in binding affinity. A 2.9 Å EM structure of the ACE2-RBD-62 complex (Fig. 4A and B), with 9 mutations including all rapidly spreading mutations, provides structural insight into the pM affinity. Out of the nine mutations in the RBM four involve intramolecular interactions, stabilising the RBD-62 structure. The mutations S477N, Q498R, N501Y are forming new contacts with ACE2. The arginine at position 498 makes a salt bridge to Q42 and hydrogen contact to Y41 of ACE2 making together with mutation N501Y (Y has contact with K353) a strong network of new interactions supporting the impact of these residues. Calculating the electrostatic potential of the RBD-62 in comparison to RBD- WT shows a much more positive surface of the former, which is complementary to the negatively charged RBD binding surface on ACE2. The structure provides a way to design drugs and vaccines against virus strains that have not yet been seen, but may evolve. Finally, we want to point out the potential of RBD-62 as a drug, as it blocks very efficiently ACE2, without affecting its important enzymatic activity.

FIG 2

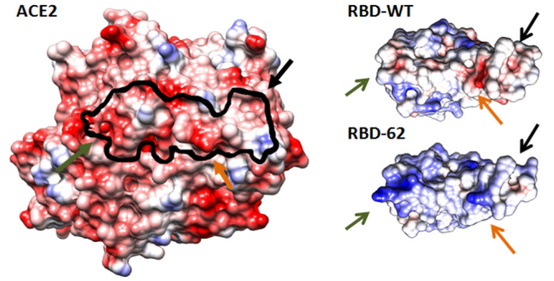


FIG 2. ACE2-RBD-62 complex Cryo-EM structure at 2.9 A resolutions. Electrostatic complementarity between RBD and ACE2 is strengthened in RBD-62 by positive charge N460K, E484K, and Q498R.

Use of Mass Photometry (Refeyn OneMP) to analyse protein-protein interaction & protein quality

The Refeyn OneMP system applies the principle of interference reflection microscopy and interferometric scattering microscopy to quantify light scattered by a single molecule on a glass surface. The amount of light scattered by each molecule is directly correlated to its molecular mass (Fig. 3)[2]

Based on the above principle, the Refeyn OneMP can monitor protein-protein interactions at a single-molecule level with high-sensitivity and simultaneously determine molecular weight of proteins and protein complexes with a high dynamic range and great accuracy.

The mass photometer is an ideal tool for quality control in the protein structural analysis workflow as it can assess the molecular mass and the oligomerisation status of a sample in one measurement.

The ISPC utilised this technology to test the quality and activity of various recombinant proteins expressed in the centre. In 2020 the ISPC expressed and purified several COVID-19 proteins such as the SARS-Cov-2 Spike protein and its Receptor Binding Domain (RBD) as well as the hACE2 receptor for various COVID-19 experiments. The proteins all expressed in ExpiF293F cells, were tested using the Refeyn OneMP instrument available to the centre. A typical experiment is shown demonstrating the binding of the spike and its RBD to ACE2 is summarised in Fig. 4.

FIG 3

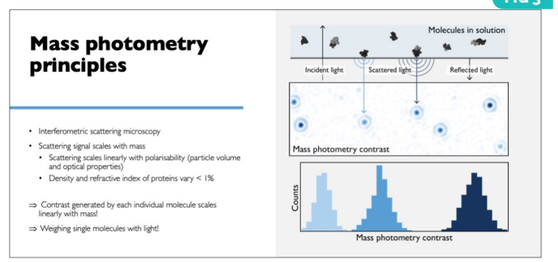


FIG 3. Mass Photometry principles

FIG 4

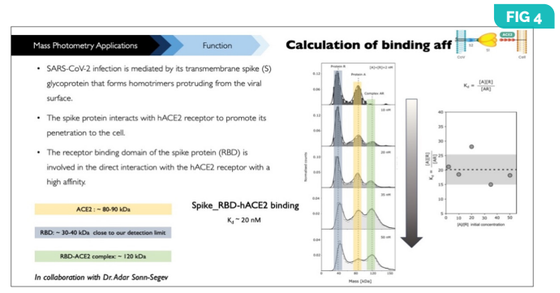


FIG 4. An example of an experiment using the mass photometry system

1. Zahradnik J, *et al.* (2021) SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor. *bioRxiv*. 2021.01.06.425392.
2. Young G, *et al.*. Quantitative mass imaging of single biological macromolecules. *Science*. 360(6387):423-427.

INSTRUCT CENTRE IT

Instruct Centre-IT is based at CERM/CIRMMP, an infrastructure for life sciences that provides a unique environment for research in the field of structural biology. The infrastructure is specialised in structural biology, molecular biology, protein/complex structure determination, functional characterisation, drug-discovery, structure-based vaccine design, bioinformatics, NMR methodology, relaxometry and metabolomics.

Instruct Centre Lead Scientists

Lucia Banci — Simone Ciofi-Baffoni
Roberta Pierattelli — Antonio Rosato

The CERM/CIRMMP NMR platform offers unique research capabilities in the field of high-resolution NMR, providing users with state of the art instrumentation and expertise to perform the most comprehensive array of NMR experiments needed for the structure and dynamic characterisation of biological macromolecules and their complexes. The NMR platform comprises twelve high-resolution NMR spectrometers ranging from 400 MHz to 1200 MHz. Each instrument is equipped with state-of-the art consoles and several probeheads to meet all conceivable experimental conditions. On the low field-end it offers unique instruments for measuring nuclear relaxation at various magnetic fields, including a Fast Field Cycling Relaxometer, operating in the 0.01- 40 MHz range.

Researchers at CERM/CIRMMP are strongly committed in advancing methodologies and protocols for both solution and solid-state NMR. For example, the development of the ^{13}C direct detection protocols for the characterisation of intrinsically disordered proteins[1] the experimental schemes for in-cell NMR spectroscopy[2], and tailored pulse sequences for structural determination of paramagnetic systems[3].

Advanced molecular biology laboratories are available, providing expertise for stable isotope labelling. Eukaryotic cell biology labs are also available, which include CO_2 incubators for growth and transfection of mammalian cells, and equipment for immunohistochemistry and Western Blotting.

Instruct-IT also features a biophysical laboratory with last-generation Q-Band CW/FT-EPR (with CW-X-band capability) EPR spectrometer, dynamic light scattering, CD, stopped-flow, fluorimetry, UV-visible spectrophotometers, isothermal micro-calorimeter and differential scanning calorimeter, atomic absorption. Laboratories equipped for mass spectrometry and X-ray diffraction and the newly installed cryo-EM are flanking the NMR platform.

CERM/CIRMMP is also an e-infrastructure, managing a GRID based platform for providing access to user friendly platforms and CPU resources for a broad range of computational programs and tools relevant for structural biology.

NEW TECHNOLOGIES in 2020

1.2 GHz NMR spectrometer

The world's first commercially available 1.2 GHz NMR spectrometer was delivered at Instruct-IT at the beginning of the year. The first spectra were recorded in May and since August the instrument has been available for external users.

This new 1.2 GHz NMR system is unique in its capabilities to study challenging protein systems and answer biological questions. These include the high-resolution structural and dynamic characterisation of proteins and their interactions in human living cells near physiological conditions. It will also enable improved research on intrinsically disordered proteins, that make up a large share of the human proteome.

As a response to travel restrictions imposed by the COVID-19 pandemic, CERM/CIRMMP engaged to optimise and widen NMR remote access modality. Such modality has been available to users for many years, but used by the more expert users only. Activities included the optimisation of internal user management and the development of specific guidelines aimed at encouraging non-expert users to exploit remote access, in an effort to maintain the training-through-access activity of the infrastructure.

FIG 1

FIG 1. The new 1.2 GHz NMR spectrometer at Instruct-IT.

1. Felli IC, Pierattelli R. (Eds) (2015) Intrinsically Disordered Proteins Studied by NMR Spectroscopy, *Springer*, ISBN 978-3-319-20164-1
2. Luchinat E, Banci L.. (2018) In-Cell NMR in Human Cells: Direct Protein Expression Allows Structural Studies of Protein Folding and Maturation. *Acc. Chem. Res.*, 51:1550-155
3. Bertini I. *et al.*. NMR of paramagnetic molecules, *Elsevier*, 2016

SCIENCE HIGHLIGHTS

In-cell drug-discovery

CERM/CIRMMP scientists have extended the previously developed in-cell NMR approach to perform intracellular protein-observe ligand screening in human cells[4]. The method allows direct observation of both the free and the ligand-bound target, enabling quantitative analysis of ligand binding in a dose- and time-dependent fashion. The methodology was applied to screen a set of newly-developed inhibitors of human carbonic anhydrase II (CA II), and to assess the permeability and the binding to CA II of previously approved drugs known for multi-target binding behaviour[5]. The approach provides precious information on both cell penetrance and binding selectivity towards the intracellular target, and could be used to improve the effectiveness of modern drug development pipelines.

FIG 2

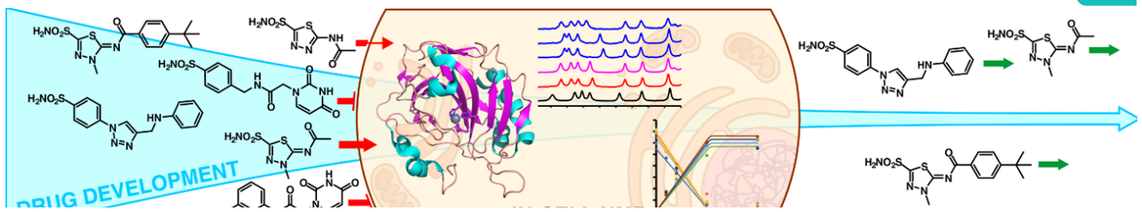


FIG 2. Protein-observed intracellular ligand screening by NMR in human cells provide pharmacologically relevant insights in the cellular context.

Structure of Toxic Amyloid-Oligomers by Electron Spin Resonance - PID 10299

CERM/CIRMMP offers the users with state-of-the-art equipment for EPR spectroscopy which can provide crucial information to obtain structural models of the toxic species involved in the development of Alzheimer's disease and other amyloid-linked disease. Among toxic species, soluble oligomers are particularly important because they are responsible for spreading cell damages thus rapidly impairing organs functions.

DEER experiments on Cu-Aß(1-42) samples in toxic oligomeric state, in protofibrillar state, and in low-toxicity amorphous state carried out within access proposal PID 10299 demonstrate the presence of assembly of Cu–crosslinked dimers into globular tetramers in the toxic oligomeric state. This technique also permitted the measure of a Cu-Cu distances within the oligomer, structural constraints suggesting that the Aß(1-42) dimer is the building block of soluble oligomers, providing a novel frame to interpret kinetic data of Aß(1-42) aggregation[6].

FIG 3

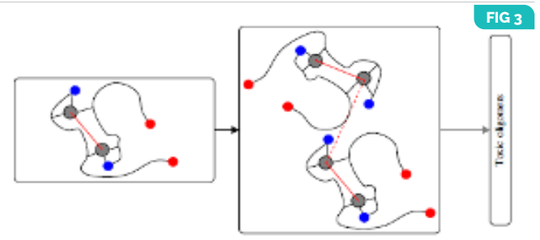


FIG 3. Schematic representations of computational models of tetramers formed by Cu-crosslinked dimers of Aß (1-42)

Structure and function of NDRG1, a Ni(II)-induced target for lung cancer therapy - PID 12704

Lung cancer is the first cause of tumor-related death worldwide. Inhalation of nickel compounds, contained in cigarette smoke and fine dusts, is a causative agent for this pathology. Understanding the mechanisms of nickel-induced carcinogenesis is of social importance, especially considering the ever-growing level of air pollution. Exposure to nickel causes higher expression of NDRG1, a Ni(II)-binding protein involved in various cellular pathways such as differentiation, proliferation, metastasis, and higher cancer aggressiveness and resistance to chemotherapy. Accordingly, NDRG1 has been proposed as a possible target for cancer treatment. In the absence of structural information, the NMR investigation at 1.2 GHz demonstrated that the C-terminal domain of NDRG1, known to be responsible for binding the toxic Ni(II) ions, exists in a fully disordered state. The completeness of the NMR data enabled also the detailed study of the effects of Ni(II) binding to NDRG1.[7]

FIG 4

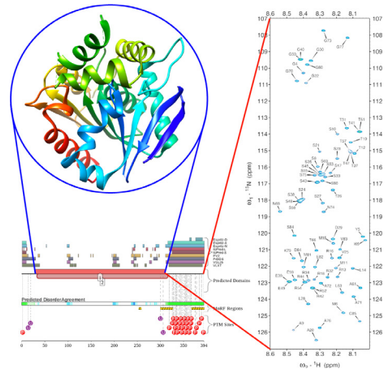


FIG 4. Structure of NDRG1: the globular domain by crystallography, and the Ni(II) binding intrinsically disordered region by NMR.[7]

4. Luchinat E., *et al.*. Drug screening in human cells by NMR spectroscopy allows the early assessment of drug potency. Angewandte Chemie International Edition, 59(16), 6535-6539, 2020.
5. Luchinat E., *et al.*. Intracellular binding/unbinding kinetics of approved drugs to carbonic anhydrase II observed by in-cell NMR. ACS chemical biology, 15(10), 2792-2800, 2020.
6. Banchelli M., *et al.*. Probing the Structure of Toxic Amyloid- Oligomers with Electron Spin Resonance and Molecular Modeling. ACS Chem Neurosci, on line ahead of print, 2021.
7. Beniamino Y. *et al.* Characterization of the intrinsically disordered region of the human N-myc downstream regulated gene-1 (hNDRG1) protein, a possible target for lung cancer therapy. (In preparation)

INSTRUCT CENTRE NL

Instruct Centre-NL is composed of research facilities from three Dutch institutes that together cover a wide range of structural biology technology: the NMR and mass spectrometry facilities at the Bijvoet Centre for Biomolecular Research at Utrecht University; NeCEN, the national facility for high-end electron nanoscopy at Leiden University; the NKI protein facility at the Division of Biochemistry of the Netherlands Cancer Institute in Amsterdam. These facilities are in close connection with well-recognised research groups, creating a lively academic environment.

Instruct Centre Lead Scientists

Meindert Lamers
Ariane Briegel
Anastassis Perrakis
Marc Baldus
Albert Heck
Alexandre Bonvin

The Bijvoet Centre offers access to their NMR and mass spectrometry facilities. The research groups of Marc Baldus and Markus Weingarth are associated with high-field solid state NMR and Hugo van Ingen with solution NMR. Furthermore, Albert Heck and Maarten Altelaar are focusing on the use of mass spectrometry for their research, and the Bijvoet Centre also hosts the closely related research groups of Alexandre Bonvin for computational structural biology, and Piet Gros, Bert Janssen and Friedrich Förster for structure determination by X-ray and cryo-Electron Microscopy.

The NeCEN cryo-Electron Microscopy core in Leiden offers two Krios microscopes, equipped with a K3, and a K2 and Falcon 3 camera, and is headed by Ludovic Renault. Meindert Lamers and Ariane Briegel act as NeCEN co-directors, and their research groups as well as that of Bram Koster are hosted in nearby university and medical departments for single particle cryo-EM and cryo-EM cellular tomography.

The NKI protein facility offers access to protein production, characterisation and crystallisation services (Patrick Celie), as well as to their large collection of biophysical technologies for quantifying macromolecular interactions (Alexander Fish). The Division of Biochemistry housing the facility also hosts the closely associated research groups of Anastassis Perrakis and Titia Sixma, which focus on structures of proteins related to cancer.

The facilities that are combined in Instruct Centre-NL provide access to national and foreign Instruct users, and efficiently assist their visitors with expert experimental planning, data collection, discussions, hands-on training and data analyses. Access is open for researchers from academia and industry.

NEW TECHNOLOGIES

All Instruct-NL facilities offer remote services, and these services have been rapidly improved to deal with the travel restrictions resulting from the COVID-19 pandemic. For example, the NeCEN node made, as part of the Instruct-ULTRA project, great progress to optimise their remote access services. Their developed architecture allows now for two different ways to connect remotely to the microscopes at the cryo-EM facility. The first is predominantly for internal remote-control access, and allows the user to fully operate the microscope by using control pads in a different room at the facility. This option allows control of the microscope like an operator sitting in front of it, and in some cases control pads are even placed at the remote site. This remote option is reserved for advanced users and EM managers of related facilities such as other Instruct-ERIC facilities. The second option for external remote access is a solution using a VPN server connection. Here, one connects via a direct dedicated connector onto the server using a certificate loaded onto the microscope. With this option, NeCEN controls what is available to the remote user (could be either view only, or control over an application). It is thus available for a wider audience of users of the facility.

FIG 1

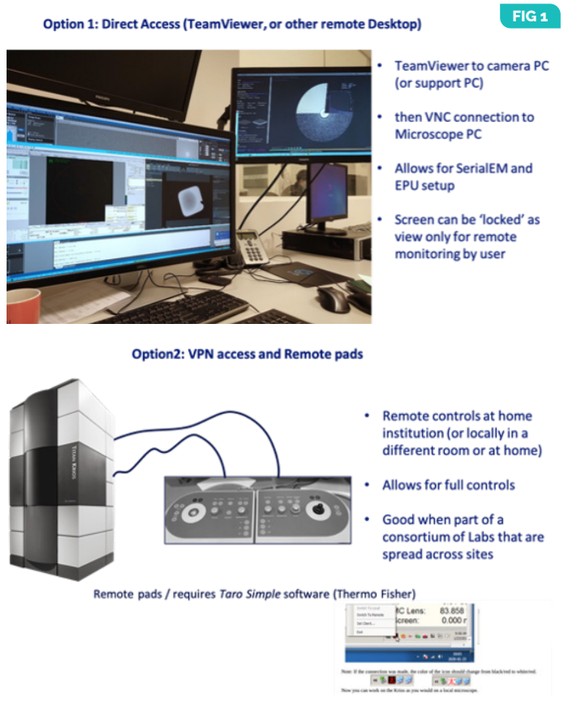
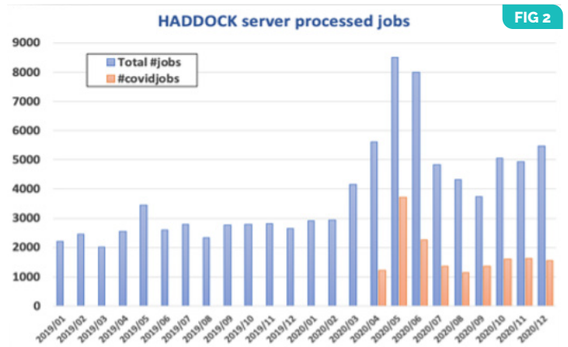


FIG 1. Different remote access approaches that have been developed at NeCEN. Top: Option 1 – Direct access via TeamViewer or other remote desktop technology; Bottom: Option 2 – Using VPN access and remote pads.

From a structural bioinformatics perspective, the Bijvoet Centre has more than doubled the processing capacity of their widely used HADDOCK web portal in response to the increased demand during the COVID-19 pandemic. This is reflected in an increase in both the number of users and of access submissions.

In 2020, the NMR facilities of Instruct-NL, together with other NMR partners from the Netherlands, were awarded almost 18 million Euro from the Dutch National Roadmap for Large-Scale Research Infrastructure to expand the range of applications and the overall capacity of the facilities. This consortium (uNMR-NL), led by the Instruct-NL NMR node in Utrecht, will use this funding to establish a nation-wide grid linking this core centre to other Dutch high-field NMR groups, thereby fostering access and exchange between local centres and user groups.

FIG 2



In 2020, the mass spectrometry facility in Utrecht started a new collaboration with the company Bruker to further develop crosslinking-mass spectrometry (XL-MS) into a mature tool for studying protein structures and interactions by mass spectrometry.

SCIENCE HIGHLIGHTS

Insights into PPAR gamma Phosphorylation and Its Inhibition Mechanism - PID 1579

PPAR gamma is a key target for the treatment of type 2 diabetes and metabolic syndrome. Synthetic antidiabetic drugs activating PPAR gamma are accompanied by serious undesirable side effects related to their agonism. In the search for new PPAR gamma regulators, inhibitors of PPAR gamma phosphorylation on S245 mediated by CDK5 represent an opportunity for the development of an improved generation of antidiabetic drugs acting through this nuclear receptor. In this study, the researchers employed a multidisciplinary approach including protein–protein docking, X-ray crystallography, NMR, HDX, MD simulations and site-directed mutagenesis to investigate conformational changes in PPAR gamma that impair the ability of CDK5 to interact with PPAR gamma and hence inhibit PPAR gamma phosphorylation. The Bijvoet Centre mass spectrometry facilities were used for the HDX experiments

FIG 3

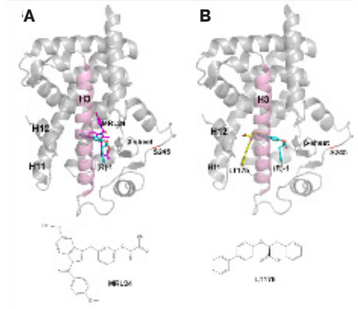


FIG 3. Comparison of different ligand binding modes in the PPAR gamma-ligand binding domain. **(A)** Superposition of (R)-1, (cyan) and MRL24 (purple); **(B)** Superposition of LT175 (yellow) and (R)-1. The phosphorylation target, S245, is shown in red, helix3 in pink and the beta-sheet in light blue. (PDB codes of LT175, (R)-1 and MRL24 structures are 3B3K, 3D6D and 2Q5P, respectively);

Structure of the Human Signal Peptidase Complex - PID 7385

Many proteins that function in the Endoplasmic Reticulum are produced on ribosomes as polypeptides with a short N-terminal extension, which acts as translocation signal and is cleaved off upon co-translational translocation. Manuel Liaci and co-workers revealed the structural features of the Signal Peptidase Complex from the human ER and reported on the mechanism of signal peptide cleavage upon translocation of these secretory proteins. The NKI protein facility was used for biophysical studies to support the suggested mechanism that could be revealed from the structure.

Determinants of this process were previously known, but the molecular details of signal peptide recognition and removal were still elusive. It was shown that the signal peptidase complex exists in two functional paralogs with distinct proteolytic subunits. Supported by the nanoDSF analyses at NKI, the atomic structures of both paralogs were determined using cryo-EM and structural proteomics. The active site is formed by a catalytic triad that abuts the ER membrane. Also, a membrane-spanning region collectively formed by all subunits in this hetero-tetrameric complex appears to locally thin the bilayer. This unique architecture could generate the specificity for the thousands of different signals peptides based on the length of their hydrophobic segments.

FIG 3

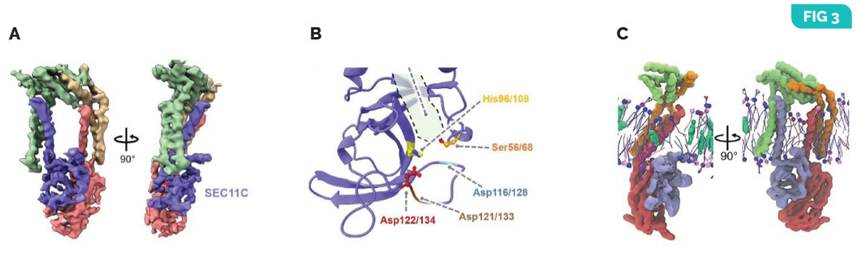


FIG 4. Overall architecture, structure and signal peptide binding pocket of the human Signal Peptidase Complex (SPC). **(A)** EM-map of SPC-C, the SEC11C protein containing the signal peptidase activity in purple; **(B)** Details of the conserved signal peptidase I fold of SEC11C. The c-region binding pocket and (candidate) catalytic residues are indicated; **(C)** Molecular dynamics simulation of human SPC-C in a complex with ER lipids highlight membrane insertion of the complex.

INSTRUCT CENTRE UK

Instruct Centre UK hosts a range of technologies across seven sites throughout the UK, including Astbury Biostructure Laboratory University of Leeds (cryo-EM, NMR and hydrogen deuterium exchange mass spectrometry), Diamond Light Source (cryo-EM, X-ray diffraction, Bio-SAXS and XChem fragment screening), Research Complex Harwell (Membrane protein production), and facilities located within the University of Oxford in the Structural Biology Division (including the Oxford Particle Imaging Centre, OPIC) and the Department of Chemistry, which hosts the Biomolecular Mass Measurement Centre.

Instruct Centre Lead Scientists
Rebecca Thompson
Martin Walsh
Jonathan Grimes

NEW TECHNOLOGIES in 2020

Astbury Centre

Like research facilities across the world, Instruct-UK was dominated by the COVID-19 crisis and our response to it. While the country was 'locked down', research facilities continued to host COVID-19 research, adapting their modes of access to allow work to continue safely. At the Astbury Biostructure Laboratory cryo-EM facility, Instruct funding enabled a team from the University of York to utilise cryo-EM to image a protein from SARS-CoV-2 which condenses and protects the viral genome. The team, led by Professor Fred Antson at the York Structural Biology Laboratory, were able to work remotely with the EM facility team to be involved with the sessions 'live' during sample preparation and grid screening. This remote access capability is now routinely offered for many research facilities.

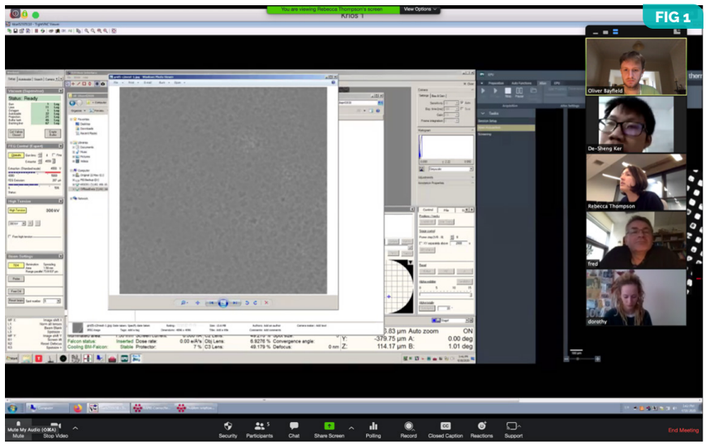


FIG 1. Science over Zoom.

Diamond Light Source

The Krios microscopes at electron Bio-Imaging Centre (eBIC), Diamond, have all recently been upgraded with Fringe-free illumination (FFI), which allows much smaller illuminated areas, up to 40% reduction than previously possible. With smaller illuminated areas, more movies per hole can be collected, and in combination with aberration free image shift (AFIS) this has dramatically increased the data collection throughput. With optimal grid types, collection setup and magnification we can now collect up to 1000 movies per hour.

A Bioquantum-K3 filter/detector has been installed on the eBIC Talos. This will facilitate screening, data collection and training in both single particle and tomography on this microscope.

The Aquilos cryoFIB/SEM has been upgraded to the Aquilos 2 platform providing the possibility to automate lamella preparation and giving increased hold times at cryogenic temperatures. Cryo-lift out (Cryo-LO) also allows regions of high pressure frozen cellular/tissue material to be extracted and subsequently thinned for cryo-electron tomography. This is still under commissioning.

Fully automated unattended data collection for MX was introduced as a new mode of operation in 2020, firstly on I03 and then rolled out to I04 and I04-1. This provides fast and efficient data collection with no user interaction with the beamlines.

In addition, the shift to remote operations helped drive the development of ISPyB to support bioSAXS experiments on B21. Users can now manage sample shipments and scheduling through a common web interface that has been the cornerstone of MX user experiences at Diamond. The bioSAXS ISPyB interface provides a common platform to our structural biology community allowing users to see and download their datasets.

The microfocus beamline I24 installed a cryogenic permanent magnet undulator providing higher fluxes, in particular at higher energies. A new CdTe Eiger2 9M detector optimised for use at these high energies has been installed and is available to users.

[An] OPIC Effort against SARS-CoV-2

The need to maintain safe, social distancing practices over the past year has been a struggle for all, including user facilities; demanding rapid and major changes in how we worked to keep science going in a safe environment. Thanks to the University green-lighting SARS-CoV-2 projects early in the outbreak, and an enormous effort from the administrative and support staff, OPIC has been able to almost continually operate both Glacios and Krios electron microscopes over this period, supporting numerous projects and initiatives in our efforts against SARS-CoV-2 virus.

Projects have been numerous and often part of collaborative efforts with groups as far reaching as Taiwan (Arthur Huang) and as close as the Rosalind Franklin Institute (Jim Naismith), Nuffield Department of Medicine (NDM, Gavin Screaton) and Jenner Institute (Sarah Gilbert). Stemming from this, OPIC has contributed to the structural aspect of a number of publications (with several still in the pipeline), including characterisation of convalescent-patient-derived antibody interactions with the ectodomain of SARS-CoV-2 Spike protein[1,2,3], potent nanobody-Spike binders[4], and a potential vaccine candidate (RBD-SpyCatcher with Alain Townsend)[5].

In addition, we have been performing a 'structural quality control' service, for the high throughput serology platform being developed in conjunction with ThermoFisher Scientific. More recently, the OPIC facility, in collaboration with Sarah Gilbert's group and eBIC, has been used extensively for in-cell characterisation of the Oxford/Astra-Zeneca vaccine.

Thanks to forward planning, we were able to welcome non-COVID related projects immediately after being allowed to do so, in addition to remote training and support sessions, resulting in both microscopes being in high demand ever since, already giving some structural data that is being prepared for publication.

We are looking forward to welcoming new Instruct proposals and hope to continue to support the pandemic effort. Our facility is unique in that our Krios microscope, fitted with double hepa-filters, is housed within a CL-3 laboratory and, in addition to CL2 experiments, we now have SOPs and risk assessments in place for imaging of some "live" CL-3 organisms.

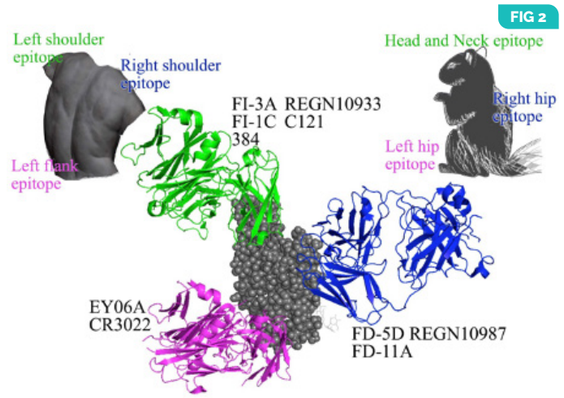


FIG 2. The diverse range of antibody epitopes identified by cryo-EM and X-ray crystallography mapped onto an anatomical representation of Spike Receptor Binding Domain in the context of potent fab binders. OPIC data have thus far contributed to the characterisation of FI-3A, 384, CR3022 and EY06A epitopes.

SCIENCE HIGHLIGHTS

First glimpses of the mechanism of electron-bifurcation catalysed by hydrogenases - PID 20432

Electron-bifurcating enzymes split electron pairs donated from a single reactant, passing one electron down-hill to 'high-potential' acceptors and using the released energy to push the other electron up-hill to 'low-potential' acceptors. These energetic changes have great biotechnological potential in synthetic-biological electrical circuits, acting a little like molecular transformers. However, how bifurcating hydrogenases 'sacrifice' the energy of one electron to boost the other is unknown and efforts to understand this transformation have been hindered by a lack of structural information.

Using the powerful Titan Krios electron microscopes at the Astbury Centre at the University of Leeds, researchers from the York Structural Biology Laboratory and the Max Planck Institute for Chemical Energy Conversion, led by Jamie Blaza and James Birrell respectively, solved the structure of one bifurcating system to a resolution of 2.3 Å. This resolution is very high for cryo-EM maps. This map has revealed how cofactors such as the FeS clusters are tightly coordinated by the protein lattice, shown in the figure. These cofactors are used to transport electrons efficiently between distant active sites, which is essential for catalysis. Work is underway to further understand the mechanistic implications of the new structural information and design experiments to test the resulting hypotheses rigorously.

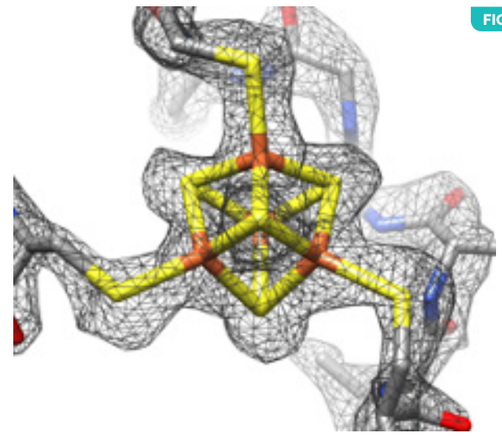


FIG 3. One of the 4Fe-4S clusters found within the bifurcating hydrogenase. The high-resolution allows the co-ordination of the FeS cluster to be unambiguously inferred.

1. Huo JD. *et al.*. (2020). Neutralization of SARS-CoV-2 by Destruction of the Prefusion Spike. *Cell Host & Microbe.* 28(3):445-454.e6
2. Zhou DRN. *et al.* (2020). Structural basis for the neutralization of SARS-CoV-2 by an antibody from a convalescent patient. *Nature Structural & Molecular Biology.* 27(10):950-958
3. Dejnirattisai, W. *et al.* (2021). The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell.* 184(8):2183
4. Huo J. *et al.* (2020). Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2. *Nature Structural & Molecular Biology.* 27(9):846-85
5. Tan TK. *et al.* (2021). A COVID-19 vaccine candidate using SpyCatcher multimerization of the SARS-CoV-2 spike protein receptor-binding domain induces potent neutralising antibody responses, *Nat Commun 12;542* .

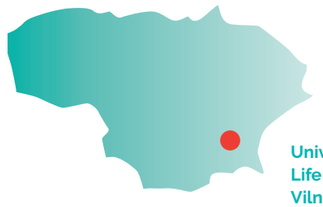
SPOTLIGHT ON MEMBER ACTIVITIES

While many of the key Instruct activities are managed at Instruct Centres, there is still very active engagement in members which do not currently host an Instruct Centre. Training, networking and outreach events and workshops can all be hosted by these members and Instruct supports the development of new methods as part of a progression to develop an Instruct Centre which could add services to the infrastructure catalogue.

Here some of these activities, from Slovakia, Lithuania and Portugal, are summarised.

LITHUANIA

In response to the COVID-19 challenges in Lithuania, VU Life Sciences Center (VU LSC) immediately provided its research infrastructure, personnel resources and knowledge to contribute to the national effort to get through the crisis.



University of Vilnius, Life Sciences Center, Vilnius, Lithuania

Temporary Molecular Diagnostics Laboratory for COVID-19 Testing

The VU Life Sciences Center joined the COVID-19 fighting forces aiming to increase the diagnostic throughput in the country. In March 2020, responding to the state-wide shortage of both technical and human resources, the LSC scientists established the Temporary Molecular Diagnostics Laboratory for diagnostic testing of COVID-19 samples.

A number of the LSC laboratories were transformed into controlled-access areas, complying to all the operational and safety requirements for the biological safety level-2 laboratories. Volunteers joined the COVID-19 diagnostics team and were assigned to the sample registry, sample preparation, viral RNA detection and result management groups.

In Autumn 2020, the diagnostic testing was resumed as Lithuania entered the second wave of the pandemic. By the end of November 2020, the number of volunteers reached 40 members, including both LSC personnel and students. Furthermore, the laboratory acquired new equipment (The KingFisher Flex Purification System) in order to increase the testing throughput.

The Nasopharyngeal Sample Pooling Methodology

With significant demand for virus testing, LSC scientists are developing the nasopharyngeal sample pooling methodology. Pooled-sample testing is a promising strategy to screen large populations rapidly with limited resources.

Environmental Surface Sample Testing for SARS-CoV-2 Traces

One of the reasons why this pandemic has been difficult to contain is the inability to identify presymptomatic and asymptomatic SARS-CoV-2 carriers as some of these individuals can be highly contagious when they have mild or no symptoms. Such individuals can shed a high viral load in their workplace and expose co-workers to constant fomite spread. We assayed over 200 samples of environmental surfaces in the Life Sciences Center of Vilnius University, which led to the identification of several pre-/ asymptomatic carriers among the community.

Serologic Testing of COVID-19

The scientists of Biotechnology of VU LSC contributed to the development and validation of COVID-19 serologic assays and employed them for testing of clinical samples. In May 2020, a group of volunteers validated commercial rapid serologic tests for detection of SARS-CoV-2-specific IgM and IgG antibodies. This study was performed in collaboration with the Biobank of Vilnius University Hospital Santaros Klinikos. The validated rapid serologic tests were employed for a sero-epidemiologic study performed by Vilnius University and the Lithuanian University of Health Sciences.

This study demonstrated that IgG antibodies are raised both after a symptomatic and asymptomatic SARS-CoV-2 infection and persist for at least 6 months.

Full Genome Analysis of SARS-CoV-2 Isolates

Sequencing of whole genome of SARS-CoV-2 is of great importance to understand virus evolution and identify mutations that have a potential impact on virus transmissibility and pathogenicity. The scientists of the Department of Eukaryote Gene Engineering of VU LSC and the Department of Bioinformatics of the Institute of Biotechnology of VU LSC, in collaboration with company Thermo Fisher Scientific Baltics, analysed full genomes of 15 SARS-CoV-2 isolates collected by the Biobank of Santaros Klinikos during the first outbreak and submitted these data to the GISAID database. Later VU Science Promotion Fund supported analysis of more SARS-COV-2 genomes isolated from Lithuanian patients collected by Biobank of Santaros Klinikos during the second outbreak.

Although this work was not undertaken within the Instruct service delivery system, it demonstrated the ability and flexibility to scale up and meet the urgent demand in the areas of SARS-CoV-2 research and provides important information on the capabilities of the infrastructure in Lithuania.

PORTUGAL

COVID-19 related research

The Macromolecular Crystallography (MX) unit at ITQB NOVA has participated in several COVID-19 related research initiatives at the institutional level. One of the initiatives was the development of a test based on LAMP (Loop-mediated isothermal amplification method) technology with colorimetric detection, yielding results within 30 to 60 minutes. The test is now being applied directly to saliva samples with very promising results. Its speed, sensitivity, easy collection, and reduced cost make it particularly suitable for rapid screening of SARS-CoV-2 infection. The MX-unit used their skills to produce recombinant Bst polymerase, an enzyme needed for the LAMP test, which was shown to be more efficient than the commercial alternative.

ITQB NOVA, Macromolecular Crystallography Unit, Lisbon, Portugal

Researchers of the MX-Unit are currently studying different proteins from SARS-CoV-2, aiming to unveil the mechanisms of molecular interactions between these proteins and the host innate immune system. A PhD fellowship was awarded in the scope of PhD4COVID program, FCT. Another project in collaboration with Molecular Modelling groups from ITQB involves small proteins designed to impair the interaction of SARS-CoV-2 with human ACE2.

Additional activities

ITQB NOVA was the first Instruct partner to be granted access to the Robotein® facility, CIP Liege; work is ongoing to produce a human target enzyme using a high-throughput approach coupling protein solubilisation with functional assays.

A PhD student from ITQB was the first student with an Instruct internship to visit the Lab of Professor Sarah Butcher, at the University of Helsinki, to learn single particle analysis by cryo electron microscopy (cryo-EM). During this period (September to November 2020) support was also provided from EU Twinning project IMpaCT.

SLOVAKIA

Glycan analysis funded by Instruct-ULTRA

Institute of Chemistry's analytical platform contributed service technologies in the field of glycan analysis through a pilot program initiated and funded by Instruct-ULTRA. The services were publicised on the Instruct website through the ARIA access management system in 2020. Four projects were submitted despite the fact that the COVID-19 pandemic considerably restricted research activities across Europe.

Institute of Chemistry, Center for Glycomics, Bratislava, Slovakia

The approved projects (delivered by remote access) were focused on the analysis of glycoforms:

1. Glycoproteomics of Sulfolobus SlaA proteins (University of Exeter)
2. Analysis of glycans on human primary amine oxidase to get insight into its complex with sialic acid-binding immunoglobulin-like lectin-9 (Åbo Akademi)
3. Identifying unknown glycans on pili from the bacterium Thermus thermophilus (University of Exeter)
4. Analysis of the glycan profile of recombinant human ceruloplasmin (Sapienza University of Rome)

The main aim of the project was to develop the analysis of structure, conformation, and dynamics of glycans as a service technology within Instruct. The analyses are performed by physico-analytical methods such as high-resolution NMR, Gas Chromatography Mass Spectrometry (GS MS), Matrix Assisted Laser Desorption/Ionisation (MALDI), Fourier-transform infrared spectroscopy (FTIR), methylation analysis, infrared (IR) and Raman spectroscopy, HPLC, surface plasmon resonance, atomic force microscope, DSC, RTG diffractometer. The analysis of the glycan profile of recombinant human ceruloplasmin (project 4) by MALDI TOF revealed the occupancy of ¾ theoretical N-glycosylation sites, each with high mannose glycans (up to 15-20 mannose residues) and piloted the workflows required to provide this service remotely

The second most important activity was focussed on organisation of the 'Chemistry towards Biology 10 / Instruct-ULTRA' conference Conference, planned for June. Due to the development of pandemic restrictions this was postponed first to late September and then to November 2020. However, the pandemic prevented us to from finalising this effort and the meeting is postponed to 2021.

INSTRUCT-ERIC HUB

The Hub experienced some changes in personnel in 2020 but was fortunate to avoid significant staff shortages due to the pandemic. A key change came at the end of the year with the completion of the Instruct-ULTRA project when the Hub lost the four staff members supported on that grant. Other changes were seen in the IT team and the finance team but importantly the core members of the Hub staff remained in place, providing continuity and retaining the knowledge and skills in-house for Instruct.

CAPACITY BUILDING AND STAFF DEVELOPMENT

The coordinating team in the Instruct Hub works closely with the Centres to ensure that Instruct meets the demands of the user community. The development of new technologies requires new working practices at the Centres delivering the infrastructure access and likewise, developments in ARIA affect almost all Instruct services and need to be communicated broadly through the Instruct-wide community.

The Managers Group is one way to ensure that staff at Instruct Centres are in good communication with the Hub and can pass on information relating to new capabilities, difficulties or suggestions to improve Instruct. In February 2020 the Managers meeting was hosted by Instruct-ULTRA in the Netherlands with an emphasis on the full operational capabilitied of the ARIA platform. This was held as a satellite meeting to an ARIA user workshop jointly by the CORBEL and iNEXT-Discovery projects.

SPOTLIGHT ON STAFF

Stephanie Chapmann

It was during her chemistry PhD that Stephanie discovered her affinity for communication and found great enjoyment in helping others to communicate their work too. So, on submitting her thesis, she knew that she wanted to move into a role that would allow her to pursue her enthusiasm for science and communication.

In April 2019, Stephanie joined the Instruct-ERIC Hub as Communication and Outreach Project Associate within the Instruct-ULTRA project. In her role, Stephanie is involved in a wide variety of dissemination and outreach activities, from creating content for the website, to representing Instruct at international conferences. With the wider Hub team, she has helped to develop a Communication Strategy for Instruct, which has since been developed into a targeted Communication Plan. She particularly enjoys writing Scientific Highlights for the Instruct website (which provide an excellent opportunity to keep up to date with the latest structural biology research), as well as creating eye-catching graphics and audio-visuals to accompany her communications. Although the pandemic may have changed the dynamics of Research Infrastructure operations in 2020, Stephanie and the Hub have found new opportunities to support and engage the research community, including the creation of an online COVID-19 Resource Centre and the launch of a popular webinar series: Structure Meets Function.

FIG 1

FIG 1. Instruct Hub members enjoying the socially distance summer picnic.

HUB TEAM MEMBERS

Claudia Alén Amaro
Senior Programme Manager

Pauline Audergon
Trainee Project Manager

Marysa Chapman
Project Assistant

Susan Daenke
Hub Coordinator

Lorraine Donaldson
Financial Administrator

Madalena Gallagher
Administrative Officer

Naomi Gray
Project Manager

Regina Guenster
Trainee Project Manager

Francisco Guimaraes
Finance and Admin Officer

Natalie Haley
Project Manager

Estina Ketsetzopoulou
Finance Assistant

Denis Nemytov
Front End Developer

Ray Owens
Instruct-ULTRA Project Manager

Marcus Povey
Senior Software Developer

Swapna Rekala
Quality Assurance Engineer

Mark Reynolds
UX Designer

Fiona Sanderson
Software Developer

Callum Smith
IT Team Lead

David Stuart
Director

SERVICES

ACCESS

A new access funding model was developed in 2019 and implemented fully from January 2020. The data provided in this report results from the first full year implementation of the new access model.

The new model increases the funding to Centres to support eligible Instruct access projects, with a cap of €5000 per access visit, which supports one defined experiment or data collection. On approval by the moderator of a proposal, more access visits may be authorised to complete the requested research if justification can be made.

In the 2020 COVID-19 crisis, most access was undertaken remotely and samples were mailed in or shipped to Instruct Centres. In these circumstances, where the researcher was unable to travel to an infrastructure facility to do the work due to national travel restrictions, Instruct agreed to use the travel fund allocation to cover the costs of sample shipment to the facility.

The process of application for access to infrastructure remains essential and managed by the online system ARIA. However, both significant and minor updates and improvements to ARIA were implemented in response to issues brought forward by users and managers. Some of the terminology has changed in the system to better segregate levels of service provision and the panel of moderators and reviewers was expandedas new scientists were identified who were willing to take on the role to review proposals. Calls for proposals spanned the year and in 2020 the rapid access call was opened for COVID-related projects. A table identifying those facility services that were available for remote access was posted on the Instruct website.

The application process continues to follow our online submission process via ARIA (Fig 1).

Applicants outline a scientific case and select the infrastructure(s) that they need to support their proposal. Proposals are sent for peer review to a panel that includes two reviewers external to the Instruct membership and one reviewer from within the Instruct membership – each selected for expertise in the technology being requested.

The aim is to get a decision on approval or rejection within two to three weeks from submission but for complex requests, or where more information is required, this may be slightly longer. Once approved, a fund is allocated to support the work, and a time frame is agreed with the host Instruct Centre for completing the access.

FIG 1

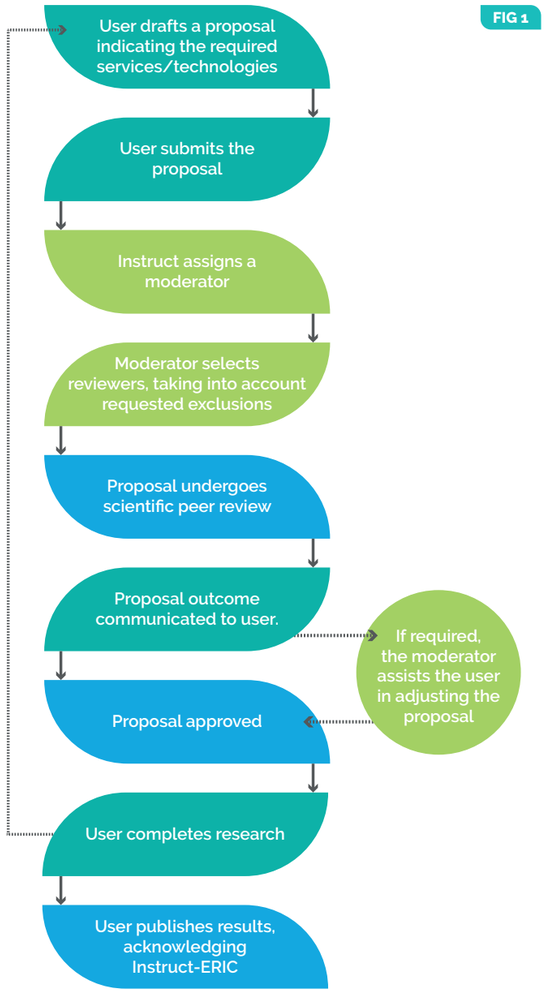


FIG 1. The pathway for access to Instruct-ERIC services.

Instruct funding for Support Costs

In 2019 the pilot study for a revised access contribution model tested the impact of raising the Instruct contribution to access costs for each service/technology. The new contribution towards access academic rate was capped at €5000 per visit, plus travel or shipping for the user as follows:

1. Contribution rates are set per technology type and are paid per day of access delivered up to €5000 to support experimental and instrument costs.
2. User travel and accommodation. If no other support is available, a contribution towards travel and accommodation paid to the researcher of up to €400 within mainland Europe or €600 from Israel. Within this limit, eligible accommodation costs are capped at €80pppn. In 2020, as infrastructure access switched to remote for more technologies, the funds allocated for travel and accommodation were repurposed to support mail-in costs for samples. In most cases, shipment was managed by the Instruct Centre receiving the sample.

In all cases, the amount of support funding available from Instruct is agreed before work commences. In rare cases where the access costs are in excess of the Instruct support available, the user may be asked to cover the extra costs from grants or other means.

ACCESS TO INFRASTRUCTURE 2020

In the period from 1 January to 31 December 2020, 119 proposals were received and 92 were approved following peer review, equating to a 77% approval rate.

Reasons for rejection include:

- The applicant is ineligible (fails to provide adequate evidence of research status)
- The scientific content does not achieve the required standard (as judged by peer review).

In all cases, the Hub operational team offers help to applicants to modify their proposals if the insufficiencies are a matter of providing basic profile information. In addition, advice is provided to help in the choices of infrastructures and facilities to best match their needs.

In the event that a selected facility is not available at the time required by the applicant, an alternative selection is offered, and support is provided in modifying the proposal to reflect the changes.

Overall, the feedback experiences from users of the Instruct infrastructure are positive. In 2020, 45% of applicants were return users. Instruct continues to engage new researchers in the structural biology community through website and social media, and Instruct-ULTRA has also worked to engage new users of Instruct access services through conferences and other networking events.

Access by Country

Applicants come from across Europe. Fig 2 shows the number of completed access visits per country in 2020. A total of 76 visits were undertaken by researchers from EU members, of which the highest numbers came from the United Kingdom, Spain and France.

A special call for pilot access to researchers in Latin America as part of an outreach programme funded through Instruct-ULTRA resulted in eight visits for researchers from Argentina and Brazil. In total, the access delivered to researchers as a result of these proposals equates to 789.5 days of infrastructure time.

The majority (65.5%) of access was transnational, with 34.5% was researchers accessing Instruct infrastructure in their own country. The implementation of remote access to more infrastructure facilities in 2020 may have skewed the figures for transnational access (reduced the proportion of transnational access) as it removes any barriers that may have been levied due to travel inconvenience (either distance, time or other issues), however this was not recorded and no assumptions can be made. In managing access submissions, care is taken to ensure that researchers do not request Instruct-funded access from their home laboratory. In all cases where there are concerns, advice is sought from the receiving facility to determine if the request comes from outside, and a decision to proceed is sought from the proposal moderator.

FIG 2

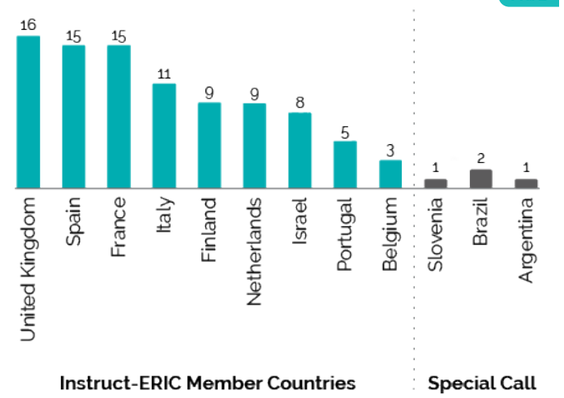


FIG 2. The number of completed access visits per country of applicant.

Access by Service Type

Fig 3 shows the infrastructure service types selected and subsequently accessed by researchers. Most users accessed electron microscopy and image processing facilities, and NMR services were also in very high demand.

Not surprisingly, protein production and sample preparation were the next highly requested, since these steps precede structural characterisation. While many researchers prepare samples in their home laboratory, some use Instruct services to ensure the quality of the preparation before structural data is gathered.

FIG 3

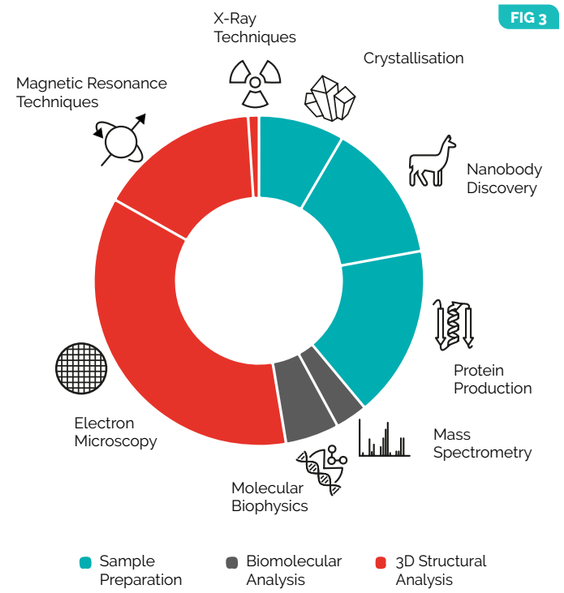


FIG 3. The number of completed access visits per country of applicant.

TRAINING

Instruct offers a range of workshops led by internationally recognised experts. Our aim is to train European researchers in a variety of structural biology methods, enabling them to expand their expertise and implement new techniques in their research.

Instruct training programmes aim to increase the skills level of researchers in structural biology through the organisation and support of training courses and internships. In addition to courses and internships there is a strong training element in the access visits where early career researchers learn from the expertise of the facility scientists. All the activities mentioned were based on in-person interactions and scientists' mobility. During 2020 in response to the COVID-19 crisis actions were taken to make sure that training activities were able to continue. Additionally, in response to the challenges posed by the pandemic, Instruct was able to identify new opportunities through remote and virtual training.

As the pandemic restricted travel and face-to-face activities such as in-person training, Instruct set out to re-structure training courses to virtual events where possible and re-scheduled events where it was not possible to hold the events online. Online platforms to use for virtual training were analysed and the Hub purchased a specialised Zoom license which is available to all Centres organising courses, workshops and webinars. The programme supported virtual events with the technical support of the Hub. Even when virtual, training courses continue to have a hands-on approach with a high ratio of trainers to trainees to ensure a high quality of training. In this context, we can highlight the event organised by Instruct Centre-FR1 which was to take place in Rosario Argentina. The event was converted to an online format which was a first for the local organisers. The virtual setting allowed a larger set of trainers from the Instruct Centre to be present and the Hub to be directly involved. The feedback received was very positive and events like this will continue even when the pandemic has ended.

While access moved from visits to remote access, increased communication through videoconferencing was set up to continue the training aspect of the access activities. Instruct has been working with colleagues in the project EOSC-Life to look into improving remote access and remote training activities. Diverse aspects from the equipment and software techniques used to keep the trainees attention during online courses and remote access were analysed. This work has helped to minimise the effects of the pandemic in the short term but will have long term effect on our capacity to train a higher number and more diverse set of students.

A

B

C

D

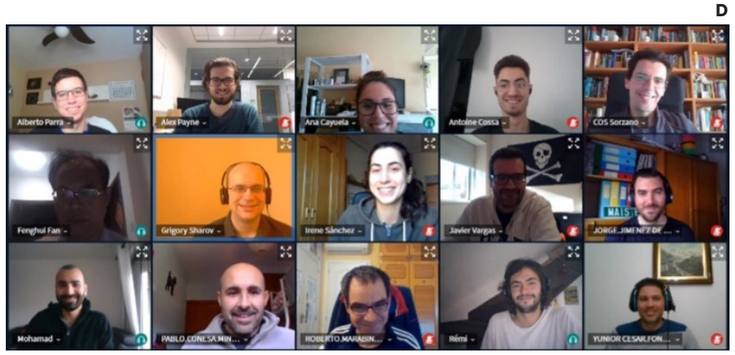


FIG 1. Instruct-ERIC training in 2020 (A) Instruct-ERIC Mangers Meeting (B) Instruct-ERIC and ARBRE-MOBIEU Workshop: Analysis and Optimization of Sample Quality for Cryo-electron Microscopy and Other Structural Techniques (C) Instruct theoretical and practical course: Integrative Structural Biology in Latin America (D) Instruct Course on the development of image processing workflows in streaming and structural data analysis components for Electron Microscopy

INSTRUCT-ERIC TRAINING COURSES 2020

Instruct Workshop for the hydrodynamic and thermodynamic analysis of biological macromolecules and their interactions: multi-method approaches and global data analyses

Grenoble, FR. January 26-31 2020.

This event was a fantastic opportunity to collaborate with colleagues from FEBS and ARBRE-MOBIOU in the important topic of hydrodynamic and thermodynamic analysis of macromolecules. Measuring and quantifying molecular interactions are in increasing demand from the biological community in response to the explosion of information coming from the application of high throughput methods. The opening of the field of biophysical measurements to new users creates the need for basic and advanced training courses covering fundamental knowledge of the instrumental methods, practice in setting-up and performing experiments, and data analysis and interpretation.

Instruct-ERIC and ARBRE-MOBIEU Workshop: Analysis and Optimisation of Sample Quality for Cryo-electron Microscopy and Other Structural Techniques

Brno, CZ. February 11-14, 2020.

This workshop was attended by 63 structural biologists from 8 European countries who wanted to improve their skills in sample analysis and optimisation for structural techniques, in particular cryo-EM. Training was focused on the importance of providing basic protein quality control as well as buffer and storage optimisation via using quick and relatively cheap techniques to save precious cryo-EM time. The course was divided into theoretical (1 day) and practical part (2 days).

Instruct-ERIC Managers meeting

Amsterdam, NL. February 24-25 2020

More than 50 project managers, research facilitators and communicators got together to discuss common issues to research infrastructure management. This included communication, outreach and, in particular, interaction with Industry. The agenda, with speakers from the Hub and Instruct Centres, centred on best practices in facility management, including receiving industry clients, quality management and communication.

ARIA-training for Facility Manager / Local Operators Workshop

Amsterdam, NL. February 24-25 2020

The NKI-node of Instruct Centre NL, together with Instruct-ERIC, organised an ARIA workshop supported by the CORBEL staff programme. This event attracted 50 participants from Instruct-Hub and Instruct Centres, but also from other stakeholder groups such as CORBEL, EU-OPENSCREEN, iNEXT-Discovery, and RI-VIS at the NKI in Amsterdam. Several information and Q&A sessions were spread over this event to cover the many aspects of ARIA.

Instruct Best Practices in Cryo-EM Workshop

Leeds, UK (Virtual), October 12-13 2020

Instruct has run a series of workshops on best practices in cryo-EM. The fourth edition of the series was organised by the Instruct Centre-UK team in Leeds. The workshops are aimed at managers involved in the running of high-end cryo-EM facilities to discuss and share best practices and is open to EM facility scientists, managers and computing specialists both from academia and industry. Sessions included presentation on best practice in sample preparation, imaging and data handling/processing, operational models in light of COVID-19, as well as round table discussions.

Instruct Course on the development of image processing workflows in streaming and structural data analysis components for Electron Microscopy

Madrid, ES (Virtual), October 26-30, 2020

The aim of the course was to show how to integrate any software tool related to electron microscopy data analysis or its integration with other biophysical or structural techniques into Scipion. The course also aimed to show the basic data management tools, the different interfaces, stream capabilities, and how to construct personalised workflows. This is particularly important for EM facilities as they can tailor the online processing to their computing capabilities and user needs. The course was aimed at programmers, engineers, computational scientists, physicists, mathematicians, and in general to information technology related scientists or staff with interest in Electron Microscopy as an application. Especially welcome were those developers of software packages or independent software tools for Electron Microscopy.

INSTRUCT theoretical and practical course: Integrative Structural Biology in Latin America

Rosario, ARG (Virtual) November 23-27 2020

This course was aimed at South American students and early career researchers presented a broad display of the fundamentals of X-ray crystallography, X-ray scattering, NMR and electron microscopy, with a focus on current strategies used to integrate the information gathered from different techniques. Due to the limitations imposed by the COVID-19 pandemic, the course was held remotely. Theoretical modules were presented by speakers with help of a moderator from the organising committee. Practical modules consisted of real dataset analyses. The course was organised by Instruct France-FR1 in collaboration with colleagues in CeBEM in the frame of our continued collaboration with Latin America.

Instruct virtual course on Single Particle Analysis by Cryo-EM

Madrid, ES (Virtual), December 14 – 18, 2020

The aim of the course was to give an overall overview of the whole process of single particle analysis (SPA) starting from sample preparation, image acquisition at the microscope, image processing (three-dimensional reconstruction) and atomic modelling. In this edition the course covered the sample preparation and image acquisition. The CNB cryo-EM facility is in the process of being incorporated into the offer of the Instruct catalogue. For this reason, the traditional 3 days course was extended into a 5 days course that included sample preparation and image acquisition.

TRAINING AND CAREER BUILDING

R&D AWARDS

Instruct opened a call for small scale pilot research projects in integrated structural biology. These pilot studies are expected to have well defined objectives and are funded up to a maximum of €15,000. The call was opened in July 2020 and received 84 applications from 10 member countries. During the last quarter of 2020, the proposals were moderated by the Hub and reviewed by a panel of more than a hundred internal and external reviewers. Successful applications will be announced early in 2021 and will be reported next year.

INTERNSHIPS

The Instruct Internship programme aims to train structural and cell biologists in a wide range of technologies with 3 to 6 months visits to Instruct Centres. The programme is aimed to pre-doctoral and early stage postdoctoral fellows and they specifically focus on the benefit to the applicant's research. From the last quarter 2020, due to the COVID-19 pandemic applications were invited for submission at anytime after discussions with the host Instruct Centre. Until further notice there is no fixed deadline with applications being reviewed as received.

Nanobody selection against mitochondrial carriers - PID 1270

How paired PSII–LHCII supercomplexes mediate the stacking of plant thylakoid membranes unveiled by structural mass-spectrometry

Grana are a characteristic feature of higher plant thylakoid membranes, consisting of stacks of appressed membranes enriched in Photosystem II (PSII) and associated light-harvesting complex II (LHCII) proteins, together forming the PSII-LHCII supercomplex. Grana stacks undergo light-dependent structural changes, mainly by reorganising the supramolecular structure of these PSII-LHCII supercomplexes. Also, LHCII is vital for grana formation, in which PSII-LHCII supercomplexes are also involved. By combining top-down and crosslinking mass spectrometry the spatial organisation of paired PSII-LHCII supercomplexes within thylakoid membranes were uncovered. The Bijvoet Centre mass spectrometry facilities were used for the mass spectrometry experiments through an Instruct Internship of first author Pascal Albanase at the Bijvoet Centre mass spectrometry group.

FIG 1

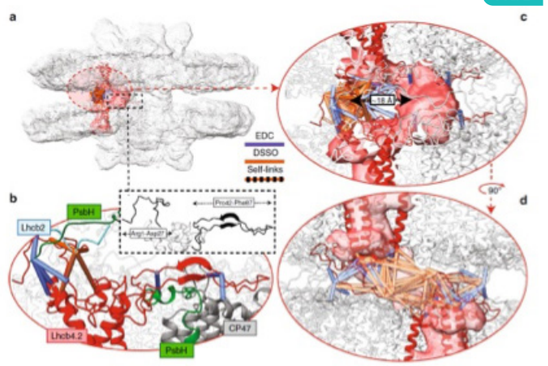


FIG 1. Mapping of Lhcb4.2 crosslinks putatively responsible for the structural anchor of paired PSII–LHCIIsc.

Albanese P. *et al.* How paired PSII–LHCII supercomplexes mediate the stacking of plant thylakoid membranes unveiled by structural mass-spectrometry. *Nature Communications* 11, 136

STAFF EXCHANGE

Communication and outreach was the focus of the Managers' Meeting which took place in Amsterdam in February 2020. In this event, Instruct-ERIC facility managers were able to exchange ideas on communication with different stakeholders with focus on industrial partners. These annual opportunities are vital for the staff of the distributed facilities to exchange ideas and to meet with staff from related research infrastructures. The managers meeting was followed by the annual ARIA workshop where staff using the software developed by Instruct learn about new features and discuss future requirements.

During 2020 Instruct began collaborating with the project ENRIITC in a series of events aiming to establish a new forum for best practice exchange in the area of industrial use of research infrastructures.

Instruct continued collaborating with CORBEL colleagues to set up the mechanisms to ensure the sustainability of the spaces of collaboration and exchange created during the project. Supported by the CORBEL staff programme, our communication officer participated in the Advanced Communication and PR management course in March 2020. In addition, were organised workshops covering diverse topics related to research infrastructure operation and they are still available at corbel-project.eu/webinars. Some of the themes of collaboration continue in the frame of the project EOSC-Life, in particular in matters related to online training and remote access. In this context, Instruct facilitated the session on challenges faced by RIs requiring physical infrastructure within the Informal Exchange of Experiences workshop organised online in July 2020.

Periodic meetings were organised between the Hub staff and staff from the Horizon 2020 projects CatRIS and OpenAIRE to advance in our common understanding in the crucial area of information flow for research infrastructures.

aria

ARIA is a collection of services designed for cloud deployment and built for Instruct, and it is available to other research infrastructures, facilities and user communities. ARIA provides a platform to centralise access to services from multiple research domains, thereby supporting cross-disciplinary scientific proposals and integrative science.

ARIA offers an access proposal management system for research infrastructures. This includes control over each step of submission, management, review, approval, post-approval and reporting. In addition, the integrated messaging system allows anonymised contact, administrative control and configurable email notifications.

Access Management

ARIA provides a large suite of tools to form a service ecosystem, spanning proposal submission through to ISO compliant machine and facility management, and a fully GDPR compatible messaging system. ARIA is customisable to project needs, and also facilitates the managing of online website functionality providing a presence for communities and infrastructures with an integrated content management system.

Simply configurable · Your branding matters · Manage workflows · Reporting & statistics · Full-fledged messaging · Instant notifications · Reach your users · Many more features

Facility Management

Managing access and scheduling within facilities can be done quickly and easily in ARIA with a specific submission system feeding directly into the single-point scheduling system. Individual configurable workflows for facility management integrate remote or access visits, booking calendars, user training and permissions.

Remote or visit access · Quick start-up · Calendar overview · User training & permissions · Simple notifications · Many more features

Project Management

Projects and communities can be hosted within ARIA, giving organisations a web presence and hub of activity, with news, events, jobs and forums to engage with their users.

Integrated forums · News and events · Ad-hoc call management · Integrated ARIA identity management

Data Access APIs

API integration is available to facilities and access providers to export data from ARIA into local management systems.

API Integration · Sample management · Virtual research environment

ARIA saw some big changes in 2020, with the introduction of unified visit and booking management "Workflows". Not only does this provide a common interface for internal bookings and external visits, it also allows facility managers to better manage their machines with per-machine customisable checkpoints, conversation threads and expenditure reporting. The ARIA team also began trialling ARIA Sitebuilder, a brand new, modern and customisable content management system used to build the COVID-19 data portal. ARIA Sitebuilder offers an easily customisable and responsive experience, with built-in access to ARIA functionality. Fundamentally, ARIA has seen significant architectural changes to better support GDPR requirements, as well as to make the platform ready for its move into the cloud. This includes a massive, and ongoing, expansion of APIs that underpin ARIA Sitebuilder, and new functionality going forward.

COLLABORATIVE WORK

EUROPEAN PROJECTS

CORBEL was a project bringing together life science research infrastructures to integrate and harmonise research services and enable interdisciplinary life science research. CORBEL launched in September 2015 and came to an end in May 2020 and had a total budget of 14.8M Euros.

Combining access to life science services

Building on the foundations set by a previous project BioMedBridges, CORBEL involved 13 research infrastructures in the life sciences specialising in a diverse range of life science research areas from biobanking, to plant phenotyping and imaging to clinical trials. Together these infrastructures developed harmonised access delivery, policies and procedures; common ethical legal and social implication (ELSI) support; shared innovation support and industrial partnerships; and joint data management exchange and integration practices.

Within CORBEL, Instruct led the activities towards building common solutions for user access, providing and expanding our ARIA research infrastructure management software to host inter-research-infrastructure access calls for cutting-edge interdisciplinary research projects; piloting a common authentication and authorisation platform, the Life Science AAI, in collaboration with AARC2 project; and promoting quality management through a community of practice.

Following the success of the open calls for inter-research-infrastructure access in CORBEL, many research infrastructures and transnational access projects decided to use ARIA access management software beyond CORBEL, including Euro-BioImaging, EU-OPENSCREEN, MIRRI and EMBRC.

From the CORBEL open calls it became clear that structural biology and advanced imaging services were commonly requested together. CORBEL also provided opportunities for Instruct and Euro-BioImaging to jointly showcase their infrastructures at research conferences. As a result of these synergies, a collaboration agreement was signed between Instruct-ERIC and Euro-BioImaging to continue to promote joint activities between the two sister infrastructures including integrated access.

Supporting SARS-CoV-2 research

CORBEL was also prioritised by the European Commission following the COVID-19 pandemic to refocus efforts from the life science infrastructures to tackle the global crisis. Instruct Centre Spain developed 3DBionotes COVID-19. This is an information integration resource where structural data and multiple other information sources on SARS-CoV-2 can be correlated and mined, with an interactive graphical representation. 3DBionotes-COVID-19 combines earlier developments in 3D-Bionotes, with Instruct-led work in fragment-based screening, among other large-scale efforts to elucidate future antiviral therapies.

Instruct Centre Spain was part of a collaboration to analyse the SARS-CoV-2 spike protein 3D structure without imposing the common cryo-EM approach that assumes the existence of defined, stable, conformational states. This alternative approach used a three-dimensional Principal Component Analysis computed directly from the raw initial images. The first results already show intricate patterns of spike flexibility, beyond the one already expected for the Receptor Binding Domain.

FIG 1. Building on the foundations set by a previous project BioMedBridges, CORBEL involved 13 research infrastructures in the life sciences (© Bildbad / BIOCOM within H2020 project CORBEL (Grant agreement No 654248)).

FIG 2. A collaboration agreement was signed between Instruct-ERIC and Euro-BioImaging to promote joint activities between the two sister infrastructures including integrated access.

FIG 3. CORBEL animation showcasing Instruct-ERICs response to COVID-19 (© Bildbad / BIOCOM within H2020 project CORBEL (Grant agreement No 654248)).

EUROPEAN PROJECTS

RI-VIS: Expanding Research Infrastructure visibility to strengthen strategic partnerships[1]

This project aims to establish working methods and tools that will aid any research infrastructure (across the domains of life sciences, physics, humanities, social sciences etc.) to improve their visibility and impact in order to target new communities and to promote international partnerships of European research infrastructures..

Instruct-ULTRA: Development and consolidation of Instruct-ERIC[1]

Instruct-ULTRA is developing relationships with new communities by targeted interactions with industry and non-EU countries and organisations. Development of potential new infrastructure services for the Instruct catalogue is a major aim of one of the work packages. Instruct-ERIC coordinates Instruct-ULTRA.

EOSC-Life: Providing an open collaborative space for digital biology in Europe

A four-year cluster project of 13 EU Life Sciences RIs, EOSC-Life is creating an open collaborative space for digital biology in Europe. Instruct is a beneficiary partner and co-leads three of the work packages.

iNEXT Discovery: Structural biology for translational research and discovery

iNEXT-Discovery aims to enable access to structural biology research infrastructures for all European researchers, and especially non-experts in structural biology.

TRANSVAC2: European vaccine research and development infrastructure

Provides scientific and technical services for 29 vaccine projects. Instruct makes its infrastructure available to TRANSVAC2 researchers on request.

TRANSVAC-DS: Towards a sustainable European vaccine infrastructure

TRANSVAC-DS aims to consolidate the conceptual and technical design and ultimate implementation of a European vaccine R&D infrastructure.

ERIC Forum: The ERIC Forum implementation project

Building on the voluntary work of the increasing number of ERICs to establish common practices through shared experiences, this implementation project is developing a more formal structure for collaborative activities between the ERICs.

EU-LAC ResInfra: Towards a new EU-LAC partnership in Research Infrastructures

EU-LAC ResInfra will develop a bi-regional collaboration of RIs between EU and LAC countries to build on common interests.

1. RI-VIS and Instruct-ULTRA are coordinated by Instruct-ERIC.

INTERNATIONAL COLLABORATION AND OUTREACH

Instruct-ERIC has developed partnerships with biological research organisations all over the world.

Instruct has signed Memoranda of Understanding (MoUs) with institutions to provide access to research equipment and technology. It provides training programmes and workshops rolled out to enhance understanding and expertise in other regions. Instruct also delivers events and symposia, providing networking and discussion opportunities across regions. In conjunction with several working groups, Instruct has been central to a wide range of international collaboration projects including Microbes, RI-VIS, EU-LAC ResInfra and Instruct-ULTRA, helping to enhance partnerships with research facilities in regions such as Africa, Latin America and the Caribbean, and Australia. An immediate indicator of impact has been the number of MoUs signed with LAC research facilities, as part of a wider landscape analysis to understand the influence that structural biology research infrastructures have in the region, and how collaboration with RIs such as Instruct-ERIC could reap significant rewards for everyone.

ResInfra EU-LAC

RI-VIS

Supporting Latin American Structural Biology

The Working Group for Landscape Analysis of Structural Biology Research Infrastructure in Latin America was established to advise on the tasks within work package 4 of the EU-LAC ResInfra project. This Horizon 2020 project aims to identify a number of Latin America and the Caribbean (LAC) Research Infrastructures that may be considered eligible for the construction of a bi-regional collaboration. The working group is chaired by Richard Garratt from the University of Sao Paulo, with representations from Instruct Centres, the Hub and LAC organisations with signed MOUs with Instruct.

The working group has been advising Instruct on how to identify tasks and stakeholders and to understand how Structural Biology is being prioritised in the different LAC countries. In this context, the working group has produced a list of over 250 structural biologists in LAC and one of their tasks will be to prepare a survey to identify the needs and challenges of the community.

The collaboration with the Latin American structural biology community in the area of training continued in 2020 with the workshop **"Instruct theoretical and practical course: Integrative Structural Biology in Latin America"** held virtually in Rosario funded and supported by Instruct-ULTRA. This workshop provided a broad display of the fundamentals of X-ray crystallography, X-ray scattering, NMR and electron microscopy, with a focus on current strategies used to integrate the information gathered from different techniques. Due to the limitations imposed by the COVID-19 pandemic, the course was held remotely. Theoretical modules were presented by speakers from Instruct Centres and our collaborators from CeBEM. The speakers were helped by moderators from the organising committee and technical support from the Hub. Practical modules consisted of real dataset analyses.

Training of early career researchers is at the forefront of Instruct international activities . In addition to the lectures and practical data analysis, the course included virtual poster presentations open for discussion, where participants showed their projects via slides.

Enhancing the global visibility of research infrastructures

Further international collaboration has been facilitated thanks to the RI-VIS project. Focused more broadly on research infrastructures as a whole, RI-VIS has been communicating the concept of RIs at the top level in international regions. In 2021 the RI-VIS project will be hosting three international symposia between Europe and Africa, Latin America, and Australia. Preparatory work for these symposia which have been postponed from 2020 as a result of the COVID-19 pandemic, has been underway this year.

In addition to the symposia, within the RI-VIS project Instruct-ERIC has been helping to prepare three white papers to provide recommendations on how to increase collaboration between European research infrastructures and counterparts from Africa, Latin America and Australia. The papers will be targeted at funders, policy makers and research infrastructure managers and collate the insights of experts from research infrastructures and policymakers from the respective regions, due to be published in 2021.

In the context of our work with RI-VIS, Instruct-ERIC held an online workshop in February 2020 "Furthering Structural Biology in Africa" in collaboration with the START project (Synchrotron Techniques for African Research and Technology). The event presented the Instruct-ERIC infrastructure to structural biologists in Africa, showcased some of the structural biology research taking place in South Africa, and initiated discussions on how Instruct-ERIC and the South-African structural biology community can assist each other in the future and establish a fruitful future relationship, exploring possible opportunities with the START project. Discussions had begun to establish a memorandum of understanding between the University of Cape Town and Instruct-ERIC towards joint activities.

This event was originally planned as an in-person satellite workshop to the RI-VIS Africa-Europe Symposium on Research infrastructures but after this symposium was postponed the Structural Biology workshop continued in an online format.

FIG 1. EU-LAC ResInfra Kick-off meeting in January 2020 held in Madrid, Spain.

FIG 2. Instruct-ERIC and collegues at the RI-VIS 2020 Annual General Meeting in Munich, Germany. (Febuary 2020)

SUPPORTING ACTIVITIES AND OUTPUTS

COMMUNICATIONS

Effective communications is vital for all research infrastructures, and Instruct is no different. Whether it is to enhance visibility and outreach with the wider scientific network, or establishing a dialogue with existing stakeholders and contacts, Instruct has developed its communications strategy over many years to maintain its position within the community.

Strategic communications

The Instruct-ERIC community has experienced rapid growth since it achieved ERIC status in 2017. As with all research infrastructures, it has benefitted from the increasing focus on visibility and communication in order to maintain an effective dialogue with key stakeholders and ensure that Instruct-ERIC remains sustainable and valuable to its users. This is highlighted by an ever-increasing network of European Members and facilities, as well as burgeoning international partnerships.

The Communication Strategy and Policy Plan created in conjunction with Instruct-ULTRA has evolved, with more emphasis placed on reaching new audiences through online media such as webinars and podcasts. This has been particularly successful in 2020 with restricted face-to-face meetings. The plan outlines the key audiences for Instruct-ERIC's communications and the primary channels to reach them. The plan identifies clear actions that can be taken to enhance the visibility of Instruct-ERIC.

Communications activities

In order to develop a deeper understanding of communications, and the benefits that it can have, Instruct-ULTRA delivered a Communications Workshop at the Instruct-ULTRA General Assembly in November 2020. This outlined to managers the importance of strong communications channels for RI's, and the substantial benefits that can be gleaned from a clear communications plan. The aim was to help each facility ascertain the most effective communications strategy, that would help both their individual outreach, but also benefit Instruct as a whole.

RI-VIS toolkit and communications strategy

A Communications toolkit for European Research infrastructures, developed through the RI-VIS project was launched in May 2020. 31 communication experts from 17 research infrastructures including Instruct-ERIC took part in three workshops to help build the workshop. The toolkit has proved a popular and helpful tool for all staff involved in communications activities within research infrastructures, providing recommendations and advice on use of language, social media strategy, targeting communications and more. It helps research infrastructures to improve their own specific communications, but also to better explain the concept of a research infrastructure more generally. The toolkit had been downloaded over 600 times from either Zenodo or the RI-VIS website in the three months following its launch. To improve the usability of this resource and enable future updates, work is underway to convert it into an online format using the new ARIA SiteBuilder platform.

To complement the toolkit RI-VIS also developed a guide for research infrastructures to create or update their own communication strategy. This work was co-led by Instruct-ERIC and EMPHASIS, and informed by previous work in the Instruct-ULTRA project and the Instruct Hub gained through developing the first Instruct-ERIC communication strategy.

RI-VIS slack channel

Through the development of the RI-VIS Communications toolkit, a community of communications experts was established, meeting at the workshops to develop the toolkit they also shared experiences, tips, and common challenges. As identified in an RI-VIS survey, many infrastructures have small communications teams, with one or fewer staff members fully dedicated to communications. A clear need emerged for peer-support within the research infrastructure communications community. A slack workspace was created to enable exchanges and discussions on topics primarily focussed on communications. The slack community has gained over 100 members and continues to grow.

FIG 1

FIG 1. Newsletter update

Newsletter

The popular Instruct newsletter was given a redesign in 2020, aimed at delivering a more user-friendly experience. As the newsletter highlights some of the key activities undertaken by Instruct and its partners throughout the six-month period, it's important that these messages are not lost. By implementing a clearer, streamlined visual design, engagement with the newsletter increased, leading to more clicks and more visits to the website.

Social media

Instruct-ERIC's social media presence continues to grow, gaining around 1,000 new followers on Twitter in the past 12 months, now at 4,500. The presence of specialist Twitter accounts for H2020 projects, such as RI-VIS, EU-LAC ResInfra and EOSC-Life, has led to a marked boost in engagement and followership, connecting the varied audiences that follow each account. Social media performance and outreach is often linked heavily to events – with many face-to-face meetings cancelled or postponed in 2020, this had a direct impact on social media impressions, engagement and new followers. However, increased social media management and activity for virtual events ensured a strong performance on social media in 2020.

Scientific Highlights

Scientific highlights continue to be a valuable form of communication and outreach, demonstrating to existing and potential users the work that can be done at Instruct-ERIC centres. The Scientific Highlight section of the website has gone from strength to strength in 2020, showcasing Instruct-ERIC's role in combatting the COVID-19 pandemic. This has become one of the most popular pages on the website and repeatedly deliver high levels of engagement on social media, as visitors and followers can see the tangible impacts of Instruct-ERIC.

Webinars

Since August, Instruct-ERIC has been running a monthly webinar series, entitled Structure Meets Function. Each webinar features expert speakers from one of Instruct's Centres, highlighting latest developments in structural biology and demonstrating how integrative methods are enabling scientists to decipher the mechanisms that underpin health and disease, made even more significant given the huge base of work carried out on COVID-19. The webinars have been a huge success, drawing hundreds of attendees from across the world. Through the work of Instruct-ULTRA, fully edited recordings of the webinars have been made available on the Instruct-ERIC website, which have drawn hundreds of views organically and on social media.

FIG 2

FIG 2. Instruct-ERIC Scientific Highlights are popular on Twitter.

SCIENTIFIC PUBLICATIONS

Instruct continues to offer access to high quality structural biology infrastructure, supported by scientists across Europe who operate at the highest levels of excellence for Instruct researchers – allowing those who are unfamiliar with the techniques to achieve good data and be able to understand and analyse it correctly.

Important indicators of success in this endeavour are the publications that arise from Instruct activities, whether this is from the direct access to infrastructure through our Access programme, or through the various training and career development opportunities that Instruct supports (for example, internships, R&D pilot awards and joint research activities).

In 2020, the list of peer-reviewed publications totalled 225 – even higher than the number achieved in 2019. This highlights the continued progression of Instruct-ERIC: not only increasing access for researchers to European infrastructure, but also providing the equipment and technology to uncover meaningful results – made even more significant in a year where physical access to research sites was made all the more difficult by the COVID-19 outbreak.

This is indicative of Instructs' presence within the European structural biology community – and is further emphasised by Instructs' growing reputation on a global scale. As a research infrastructure, building our reputation for scientific excellence provides the credibility that is needed to engage with potential users, most of whom are from the academic community, where publication success is still the key criterion used to promote career progression.

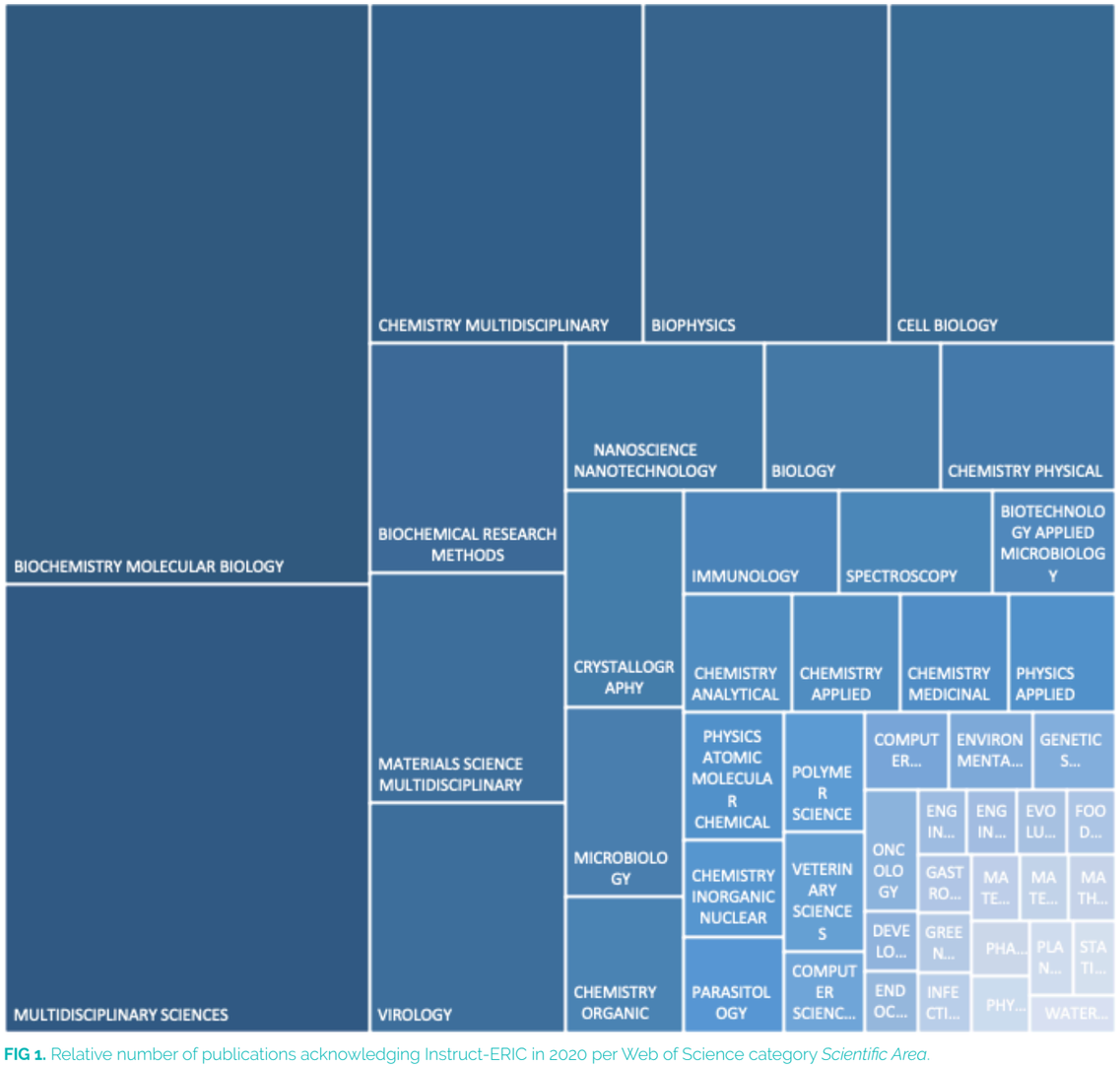


FIG 1. Relative number of publications acknowledging Instruct-ERIC in 2020 per Web of Science category *Scientific Area*.

2020 PUBLICATIONS

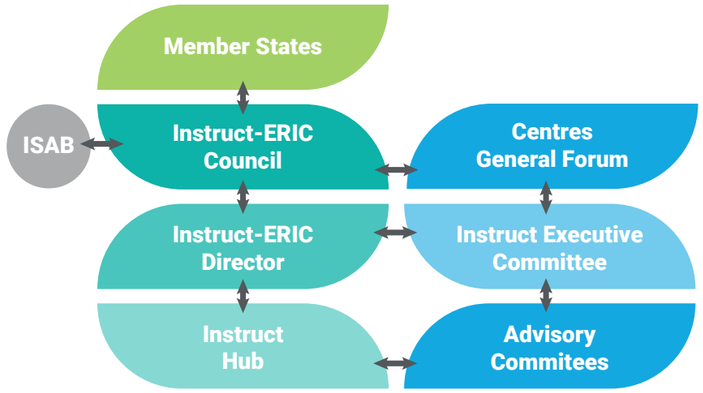
All Instruct Centres were invited to nominate two Instruct publicaitons from 2020 that they found of particular interest, developed novel techniques, were highly influential in their field or showcased Instruct-ERICs service offers:

- Albanese P, Tamara S, Saracco G, Scheltema RA, Pagliano C. How paired PSII-LHCII supercomplexes mediate the stacking of plant thylakoid membranes unveiled by structural mass-spectrometry. *Nature Communications*. 2020;11(1):1361. doi: 10.1038/s41467-020-15184-1
- Brams M, Govaerts C, Kambara K, Price KL, Spurny R, Gharpure A, Pardon E, Evans GL, Bertrand D, Lummis SCR, Hibbs RE, Steyaert J, Ulens C. Modulation of the Erwinia ligand-gated ion channel (ELIC) and the 5-HT3 receptor via a common vestibule site. *eLife*. 2020;9:e51511. doi: 10.7554/eLife.51511
- Camacho-Zarco AR, Kalayil S, Maurin D, Salvi N, Delaforge E, Milles S, Jensen MR, Hart DJ, Cusack S, Blackledge M. Molecular basis of host-adaptation interactions between influenza virus polymerase PB2 subunit and ANP32A. *Nature Communications*. 2020;11(1):3656. doi: 10.1038/s41467-020-17407-x
- Carter SD, Hampton CM, Langlois R, Melero R, Farino ZJ, Calderon MJ, Li W, Wallace CT, Tran NH, Grassucci RA, Siegmund SE, Pemberton J, Morgenstern TJ, Eisenman L, Aguilar JI, Greenberg NL, Levy ES, Yi E, Mitchell WG, Rice WJ, Wigge C, Pilli J, George EW, Aslanoglou D, Courel M, Freyberg RJ, Javitch JA, Wills ZP, Area-Gomez E, Shiva S, Bartolini F, Volchuk A, Murray SA, Aridor M, Fish KN, Walter P, Balla T, Fass D, Wolf SG, Watkins SC, Carazo JM, Jensen GJ, Frank J, Freyberg Z. Ribosome-associated vesicles: A dynamic subcompartment of the endoplasmic reticulum in secretory cells. *Science Advances*. 2020;6(14):eaay9572. doi: 10.1126/sciadv.aay9572
- De S, Pollari M, Varjosalo M, Makinen K. Association of host protein VARICOSE with HCPro within a multiprotein complex is crucial for RNA silencing suppression, translation, encapsidation and systemic spread of potato virus A infection. *PLoS Pathogens*. 2020;16(10). doi: 10.1371/journal.ppat.1008956
- Huo J, Le Bas A, Ruza RR, Duyvesteyn HME, Mikolajek H, Malinauskas T, Tan TK, Rijal P, Dumoux M, Ward PN, Ren J, Zhou D, Harrison PJ, Weckener M, Clare DK, Vogirala VK, Radecke J, Moynie L, Zhao Y, Gilbert-Jaramillo J, Knight ML, Tree JA, Buttigieg KR, Coombes N, Elmore MJ, Carroll MW, Carrique L, Shah PNM, James W, Townsend AR, Stuart DI, Owens RJ, Naismith JH. Neutralizing nanobodies bind SARS-CoV-2 spike RBD and block interaction with ACE2. *Nature Structural & Molecular Biology*. 2020;27(9):846-854. doi: 10.1038/s41594-020-0469-6
- Jung JH, Barbosa AD, Hutin S, Kumita JR, Gao M, Derwort D, Silva CS, Lai X, Pierre E, Geng F, Kim SB, Baek S, Zubieta C, Jaeger KE, Wigge PA. A prion-like domain in ELF3 functions as a thermosensor in Arabidopsis. *Nature*. 2020;585(7824):256-260. doi: 10.1038/s41586-020-2644-7
- Laurinmaki P, Shakeel S, Ekstrom JO, Mohammadi P, Hultmark D, Butcher SJ. Structure of Nora virus at 2.7 angstrom resolution and implications for receptor binding, capsid stability and taxonomy. *Scientific Reports*. 2020;10(1):19675. doi: 10.1038/s41598-020-76613-1
- Luchinat E, Barbieri L, Cremonini M, Nocentini A, Supuran CT, Banci L. Drug Screening in Human Cells by NMR Spectroscopy Allows the Early Assessment of Drug Potency. *Angewandte Chemie-International Edition*. 2020;59(16):6535-6539. doi: 10.1002/anie.201913436
- Melero R, Sorzano COS, Foster B, Vilas JL, Martinez M, Marabini R, Ramirez-Aportela E, Sanchez-Garcia R, Herreros D, del Cano L, Losana P, Fonseca-Reyna YC, Conesa P, Wrapp D, Chacon P, McLellan JS, Tagare HD, Carazo JM. Continuous flexibility analysis of SARS-CoV-2 spike prefusion structures. *IUCrJ*. 2020;7(6):1059-1069. doi: 10.1107/s2052252520012725
- Papai G, Frechard A, Kolesnikova O, Crucifix C, Schultz P, Ben-Shem A. Structure of SAGA and mechanism of TBP deposition on gene promoters. *Nature*. 2020;577(7792):711-716. doi: 10.1038/s41586-020-1944-2
- Pontoriero L, Schiavina M, Murrali MG, Pierattelli R, Felli IC. Monitoring the Interaction of alpha-Synuclein with Calcium Ions through Exclusively Heteronuclear Nuclear Magnetic Resonance Experiments. *ngewandte Chemie-International Edition*. 2020;59(42):18537-18545. doi: 10.1002/anie.202008079
- Setyawati I, Stanek WK, Majsnerowska M, Swier LJYM, Pardon E, Steyaert J, Guskov A, Slotboom DJ. In vitro reconstitution of dynamically interacting integral membrane subunits of energy-coupling factor transporters. *eLife*. 2020;9. doi: 10.7554/eLife.64389
- Webster MW, Takacs M, Zhu CJ, Vidmar V, Eduljee A, Abdelkareem M, Weixlbaumer A. Structural basis of transcription-translation coupling and collision in bacteria. *Science*. 2020;369(6509):1355-1359. doi: 10.1126/science.abb5036

GOVERNANCE

The Instruct-ERIC Council retained its Chairman as Eric Guittet (FR), with Inmaculada Figueroa (ES) supporting him as Vice-Chair. The Council continued to have the support of the Independent Scientific Advisory Board (ISAB), chaired by Prof Stephen Burley, with its membership unchanged. 2020 would normally have convened a meeting of the Executive Committee with the ISAB, but this was postponed to coincide with the next (also postponed) Instruct Biennial Scientific Conference which will be held in 2022. The Director and Hub Coordinator remained in contact with the ISAB Chair through the year however, to ensure that the Board was aware of the activities and particularly the COVID-19 initiatives being taken by Instruct.

The Instruct-ERIC Council initiated a search for the next Instruct Director. Progress was again compromised by the COVID pandemic and actions are now delayed. Prof David Stuart confirmed his intention to remain in post until a new Director was appointed. In addition, The Council worked with representatives from the European Commission to identify the steps remaining to finalise the implementations arising from the BREXIT withdrawal agreement. Instruct-ERIC was advised that the UK (as the current statutory host of the ERIC) was considered as an Associate Country until otherwise informed and the need to make changes to the ERIC structure or location was not immediate.



Scientific direction and implementation of the Instruct objectives continued to be managed by the Executive Committee, chaired by the Director. The Committee oversaw and implemented a rapid access response for COVID-related access proposals, accelerating infrastructure provision. At the same time, most Centre facilities improved their remote access provision such that this increased from approximately 20% in January 2020 to 80% by December 2020. Remote access availability was a crucial step in retaining infrastructure service provision during periods of restriction.

The Executive Committee subcommittees, including the Access Committee, Training Committee, Data Management and Computational Committee (DMCC) and the Landscape Analysis Committee continued their work. The DMCC expanded its remit to include more strategic planning for ARIA and for better integration with the various projects related to the development of the European Open Science Cloud. Led by Jose Maria Carazo, the DMCC formalised these discussions and was elected to represent Instruct in the EOSC Association, of which Instruct submitted a conditional application for Observer status in October 2020.

Instruct-ERIC expanded its involvement in various consultative panels and groups contributing to the continued integration of the research infrastructures in ESFRI, EC-funded projects and other stakeholder and interest groups.

Working Group/Panel/Project	Role	Representatives
ERIC Forum	Member	Susan Daenke
LS RI Strategy Board	Chair	Susan Daenke
ESFRI Health and Food Strategic Working Group	Member	Lucia Banci
Transvac2 Steering Group	Member	Susan Daenke, Francesca Morelli
CORBEL Executive Board	Member	David Stuart, deputised by Susan Daenke
CORBEL WP Leaders	Member	Natalie Haley
Instruct-ULTRA Executive Board	Chair	Ray Owens
iNEXT-Discovery Management Committee	Member	David Stuart
iNEXT-Discovery Executive Board	Member	Susan Daenke
EOSC-Life Executive Board	Member	Susan Daenke
EOSC-Life WP co-Leaders	Member	Susan Daenke & Natalie Haley & Callum Smith
RI-VIS Steering Group	Chair	Susan Daenke & Natalie Haley
RI-VIs General Assembley	Member	Susan Daenke

INSTRUCT-ERIC GOVERNING BODIES AND COMMITTEES

COUNCIL

The Instruct-ERIC Council is the ultimate decision-making body of the consortium. It consists of scientific and administrative representatives from each Instruct-ERIC Member.

Chair: Eric Guittet, FR

Vice-Chair: Inmaculada Figueroa, ES

Hub support: Susan Daenke, Instruct Hub

Country	Scientific Delegate	Administrative delegate
Belgium	Michele Oleo	Laurence Lenoir/Virginie Storms
Czech Republic	Vladimir Sklenar	Jan Burianek
Denmark	Thomas Vosegaard	Line Bekker Poulsen
EMBL	Christoph Mueller	Plamena Markova
Finland	Sarah Butcher	Marko Uutela
France	Winfried Weissenhorn	Eric Guittet
Israel	Joel Sussman	Iris Eisenberg
Italy	Lucia Banci	Grazia Pavoncelloa
Latvia	Kaspars Tars	Uldis Berkis
Lithuania	Gintaras Valincius	Tomas Simulevic
Netherlands	Reinout Raijmakers	Nienke Klomp
Portugal	Maria Armenia Carrondo	Margarida Archer/Marta Abrantes
Spain	Jose Maria Carazo	Inmaculada Figueroa
Slovakia	Milos Hricovini	Viera Petrasova
United Kingdom	Megan Dowie	Anne McGavigan

Observers

Greece	Evangelia Chrysina

The following working groups have responsibilities in defined areas of activity and report to the Council:

BREXIT Working Group

The BREXIT Working Group was formed to monitor and manage the processes required by the European Commission and the ERIC Committee to establish the status of Instruct-ERIC post BREXIT, in accordance with the Instruct-ERIC statutes and the ERIC Regulation.

Chair: Jan Burianek

Hub support: Susan Daenke

Internationalisation Working Group

The Internationalisation Working Group was established to consider a strategy for Instruct-ERIC to engage with organisations or countries outside of the EU member states and associated countries.

Chair: Reinout Raijmakers

Hub support: Susan Daenke

INDEPENDENT SCIENTIFIC ADVISORY BOARD

Chair: Prof Stephen Burley, Rutgers University, USA

Members

Angela Gronenborn, Pittsburgh University, USA
Juergen Plitzko, Max Plank Institute for Biochemistry, Germany
Ilaria Ferlenghi, GSK, Italy
Marjolein Thunnissen, MaxIV, Sweden

INSTRUCT-ERIC GOVERNING BODIES AND COMMITTEES

EXECUTIVE COMMITTEE

The Executive Committee is the principal executive management committee for Instruct-ERIC, comprising representatives drawn from Instruct Centres. It is the supervisory body for the execution of the project which reports to and is accountable to the instruct-ERIC Council.

Chair : Prof David Stuart (Instruct Director)

Vice-Chair: Prof Lucia Banci (Instruct Deputy Director)

Hub support: Claudia Alen Amaro

Instruct Centre	Head of Centre	Second
Instruct BE	Jan Steyaert	Han Remaut
Instruct CZ	Vladimir Sklenar	Ondrej Hradil
Instruct ES	Jose Maria Carazo	Carlos Oscar Sanchez Sorzano
Instruct FI	Sarah Butcher	Hanna Oskanen
Instruct FR1	Alberto Podjarny	Jean Cavarelli
Instruct FR2	Darren Hart	Martin Blackledge
Instruct IL	Gideon Schreiber	Joel Sussman
Instruct IT	Lucia Banci	Roberta Pierattelli
Instruct NL	Rolf Boelens	Anastassis Perrakis
Instruct UK	David Stuart	Ray Owens

The following sub-committees and working groups have responsibilities in defined areas of activity and report to the Executive Committee:

TRAINING COMMITTEE

The training committee manages the launch of training calls, selection, funding and delivery of courses. The Training Committee reports to the Executive Committee.

Chair: Lucia Banci

Hub support: Claudia Alen Amaro & Madalena Gallagher

R&D review panel

From time to time, Instruct publishes calls for small scale pilot research projects in integrated structural biology. This panel with membership approved by the Executive Committee awards the R&D pilot projects after proposal are sent to for external review.

Chair: Lucia Banci

Hub support: Claudia Alen Amaro & Madalena Gallagher

ACCESS COMMITTEE

The Access Committee monitors and manages the delivery of access and report to the Executive Committee with recommendations for operational amendments.

Chair: Darren Hart

Hub support: Claudia Alen Amaro & Madalena Gallagher

Centre pages Working Group

The Centre pages Working Group gives feedback to the Instruct Hub and guidelines to each Instruct Centre to ensure that each Instruct-ERIC Centre page provides clear and detailed information that users need to inform their access decisions. This WG includes members of different Instruct Centres, project managers from Instruct hub, a communication officer and a member of the ARIA team

Chair: Stephanie Chapman & Pauline Audergon

DATA MANAGEMENT AND COMPUTATIONAL COMMITTEE

To provide insight to computational and data needs of the structural biology community and help define Instruct-ERIC's strategy to support the scientific community in this capacity. This committee reports to the Executive Committee.

Chair: Jose Maria Carazo

Hub support: Marcus Povey

Web services Working Group

Instruct-ERIC was a partner of the West-Life project and offered a home for the West-Life tools in the catalogue. This working group is tasked to curate the content of these pages checking that tool information is up to date.

The group will also consider the mechanisms through which Instruct-ERIC can support structural biology software and software development.

Chair: Martyn Winn

Hub support: Natalie Haley

Instruct-ERIC User Data Working Group

Carry out an analysis across different disciplines and Instruct Facilities to explore how Instruct could support their users in implementing Open science and FAIR data polices.

Chair: Jose Marquez

Hub support: Fiona Sanderson & Claudia Alen Amaro

EOSC Task Force

The objectives of the EOSC Task Force are:

1) The coordination of activities within EOSC (in particular with EOSC-Life) where Instruct partners are involved

2) The identification of the needs of the whole Instruct computational community

3) The alignment of Instruct data management policies with the ones from EOSC-Life

4) Finding and advertising opportunities available to the Instruct-ERIC community through EOSC (e.g. EOSC-Life calls...)

Chair: Natalie Haley & Pauline Audergon

ARIA User Group

A group of super-users of the ARIA software was put together, prodeing a forum to generate ideas and requirements for new ARIA functionality, identify pain points with existing software for improvement, and to prioritise the proposed developments.

Chair: Rebecca Thompson

Hub support: Natalie Haley

LANDSCAPE ANALYSIS COMMITTEE

The working group was established by the Executive Committee to look into new directions of the provision of structural biology research infrastructure. The working group has launched a survey which will help to keep our catalogue updated according to the needs of the community.

Chair: Lucia Banci

Hub support: Susan Daenke

KPI Working Gourp

The KPI working group was formed to define which (ESFRI) Key Performance Indicators Instruct-ERIC will report, how the data will be gathered and to develop standard operting procedures for data collection and reporting of each relevant KPI.

Chair: Ludo Renault

Hub support: Regina Guenster

IBSBC Organisation Team

This Group organises the 5th Instruct Biennial Structural Biology Conference (IBSBC) held in Utrecht (Netherlands) from 18 - 20 May 2022.

Chair: Friedrich Förster

Hub support: Claudia Alen Amaro & Madalena Gallagher

FINANCIAL DATA

FINANCIAL DATA

This report presents the financial statements for the period 1 January 2020 to 31 December 2020.

Appointment of Members to Council

Council representation is by nomination of up to two delegates for each Instruct Member who are empowered with full authority to vote on all issues raised during meetings of the Council as laid out in Article 10 of the statutes. The rights, obligations and voting rules of the Council are set out in the Instruct-ERIC Statutes Article 13.

Statement of Council Members' responsibilities in respect of the Council's Report and the Financial Statements

The Council Members are responsible for preparing the Council's Report and the financial statements in accordance with applicable law and regulations.

The ERIC Regulation (EC) No 723/2009 Article 17 requires Instruct-ERIC to prepare an annual report which includes operational and financial aspects of its activities. The Report shall be approved by the Council and transmitted to the European Commission and the relevant public authorities within six months from the end of the corresponding financial year. The Report shall be made publicly available.

The financial statements are prepared in accordance with applicable law and the statutes of Instruct.

In preparing these financial statements, the Council Members accept the recommendations of the auditor and approve the application of the appropriate policies in the following decisions:

- Making judgements and estimates that are reasonable and prudent;
- Stating whether UK Accounting Standards have been followed, subject to any material departures and explained in the financial statements;
- Assessing Instruct-ERIC's ability to continue its activities, disclosing as applicable matters related to financial resilience;
- Using the 'going concern' basis of accounting unless they intend to cease operations or have no realistic alternative but to do so.

The Council is responsible for ensuring the Financial Statements are accurate and that the accounting records are sufficient to show and explain Instruct-ERIC's transactions and disclose with reasonable accuracy at any time the financial position of Instruct-ERIC and enable Council Members to ensure that the financial statements comply with the appropriate regulations and applicable law. Council Members aver that they are free from material misstatement, whether due to fraud or error, and have general responsibility for taking such steps as are reasonably open to them to safeguard the assets of Instruct-ERIC and to prevent and detect fraud and other irregularities.

This report covers the period 1 January 2020 – 31 December 2020 which concided almost exactly with the emerging SARS-CoV-2 pandemic which imposed a significant impact on Instruct activities and those of essential advisors and contractors involved in the making of this report. The publication of the financial report was therefore delayed.

BALANCE SHEET FOR INSTRUCT-ERIC

As at 31 December 2020

Assets	**GBP**	**EUR**	**Notes**
Euro bank	1,153,631	1,292,314	
Sterling bank	71,742	80,367	
Total Bank	1,225,373	1,372,681	
Current Assets			
Accounts Receivable	63,641	71,292	1
Prepayments	5,948	6,663	
Accrued income	219,786	246,208	2
Amounts due from IASL	-	-	
Rental deposits	4,006	4,488	
Total Current Assets	293,381	328,651	
Fixed Assets			
Computer Equipment	21,678	24,284	
Depreciation on Computer Equipment	(8,912)	(9,983)	
Office Equipment	4,388	4,915	
Depreciation on Office Equipment	(2,417)	(2,708)	
Total Fixed Assets	14,737	16,508	
Total Assets	**1,533,491**	**1,717,840**	

Liabilities	**GBP**	**EUR**	**Notes**
Current Liabilities			
Accruals	13,703	15,350	
Accruals for Internship	-	-	
Accruals for R&D awards	-	-	
Accruals for Access awards	-	-	
Amounts to be paid and Unclaimed Access Awards	605,377	678,152	3
Income in Advance - Members subscription	-	-	
Income in Advance - Other inc deferred grants	496,631	556,333	4
Income in Advance - ARIA Support	438	491	
Other creditors	113,493	127,137	
Payroll taxes due	6,446	7,221	
Pensions due	7,189	8,053	
Total Current Liabilities	1,243,277	1,392,737	
Total Liabilities	**1,243,277**	**1,392,737**	
Net Assets	**290,214**	**325,103**	
Surplus Brought Forward	242,293	286,274	
Exchange rate movement - revalue opening reserves	13,261	-	
Surplus for the Year	34,660	38,829	
Surplus Carried Forward	290,214	325,103	

Exchange rate for reporting period: 0.892686

1. Mebership income receivable

2. CORBEL funding recoverable at year-end

3. Access and other service accruals

4. Deferred project income

PROFIT AND LOSS FOR INSTRUCT-ERIC

For Year Ended 31 December 2020

Income	GBP	EUR	Notes
External grant income	475,029	532,135	5
External grant overhead contribution income	102,537	114,864	
Member state contributions	841,456	942,611	6
Other miscellaneous income	5,694	6,378	7
Total Income	1,424,716	1,595,988	
Less Cost of Service Provision			
Instruct staff salaries	282,510	316,472	
R&D Pilot awards	5,952	6,667	
JRA awards	(80)	(90)	
Access Cost	518,665	581,016	8
Instruct Centre Cost	-	-	
Meetings	23,583	26,418	
Project activities	375,685	420,848	9
Total Cost of Service Provision	1,206,315	1,351,331	
Gross Surplus	218,401	244,657	
Less Operating Expenses			
Commissioned services (Insurance, financial, HR, legal)	42,369	47,462	
Conference costs	-	-	
Consultants	-	-	
Depreciation charge	6,811	7,630	
Foreign Currency Gains and Losses	16,952	18,990	
General admin (postage, copying, bank charges)	2,882	3,228	
Licenses & software	23,104	25,881	
Miscellaneous	744	833	
Office Stationery	1,532	1,716	
Premises and support	72,827	81,582	
Project overhead expenses	10,059	11,268	
Publicity	4,376	4,902	
Telephone	2,085	2,336	
Write off IASL balance owed	-	-	
Total Operating Expenses	183,741	205,828	
Net Surplus	**34,660**	**38,829**	

5. Project income excluding 25% contribution to Instruct-ERIC overheads, against expenditure

6. Membership income receivable

7. ARIA support

8. 2018 - Access released – not claimed

9. WIP on research grants.

SUPPORTING INFORMATION FOR THE FINANCIAL STATEMENTS

Accounting Policies

The financial statements are prepared in accordance with the Statutes of Instruct. The principal accounting policies set out below have, unless otherwise stated, been applied consistently to all periods presented in these financial statements.

Reporting and Disclosure Exemptions

Going concern

The financial statements have been prepared on the assumption that Instruct-ERIC will continue as a going concern. Instruct-ERIC is expected to generate positive cash flows on its own account for the foreseeable future. The Council Members have a reasonable expectation that Instruct-ERIC has adequate resources to continue in operational existence for the foreseeable future. Thus the Council Members continue to adopt the going concern basis in preparing the financial statements.

Expenditure

Awards are recognised as expenditure when the relevant committee formally approves the award. Awards are given a 12 – 18 month window after which the beneficiary must reapply if unclaimed

Foreign Exchange

Currency transactions are recorded at the rate of exchange on the transaction date. Monetary assets and liabilities denominated in non-UK currencies are reported at the rates of exchange prevailing on the balance sheet. Non-monetary assets and liabilities measured at historical cost in a non-UK currency are translated using the exchange rate at the date of the transaction. Currency exchange differences are recognised in the Profit and Loss statement.

Corporation Tax:

In our opinion and under the terms of the Council Directive 2006/112/EC of 28 November 2006 on the common system of value added tax and Council Directive 92/12/EEC of 25 February 1992 on the general arrangements for products subject to excise duty and on the holding, movement and monitoring of such products, Instruct-ERIC has no liability to Corporation Tax.

Basis of preparation

The financial statements have been prepared in accordance with applicable United Kingdom accounting standards, and under the historical cost accounting rules used and approved for Instruct-ERIC in accordance with the requirements of the ERIC Regulation.

Income

1. The amounts derived from membership subscriptions. This income is recognised evenly over the subscription period.
2. EC Grants and projects income is recognised when the costs are incurred, attributing the contribution to overheads as per the Grant Agreement.

Depreciation

Tangible assets are calculated using an initial measurement at cost (including delivery and handling costs, installation costs) and the straight line method of depreciation to a zero salvage value at the end of the depreciation term. For computer equipment the depreciation term is 3 years. For furniture, fixtures and fittings, the depreciation term is 5 years. The following costs are not capitalised in this measurement: communication or training costs, repairs and maintenance. Software licenses are classified as intangible assets.

Taxation

The United Kingdom, as host Member State of Instruct-ERIC, has made a declaration to recognize the ERIC as an international body or organization for the purpose of the application of Council Directive 2006/112/EC of 28 November 2006 on the common system of value added tax and Council Directive 92/12/EEC of 25 February 1992 on the general arrangements for products subject to excise duty and on the holding, movement and monitoring of such products as of its setting up. Instruct-ERIC therefore benefits from certain exemptions as an international organisation for the purpose of applying Directive 2004/18/EC of the European Parliament and of the Council of 31 March 2004 on the coordination of procedures for the award of public works contracts, public supply contracts and public service contracts, in conformity with State aid rules.

Instruct-ERIC operates and reports on this basis of tax exemption except where irrecoverable tax is shown.

Cash and cash equivalents

Cash and cash equivalents comprise cash balances and call deposits.

ACCOUNTING JUDGEMENTS AND ESTIMATES

In its preparation of these financial statements, Instruct-ERIC has made material judgements, estimates and assumptions. Discussion of these judgements, estimates and assumptions and their impact is included in the relevant note disclosures; the main areas being:

Judgements: Grant Income recognition

Estimations, uncertainties and assumptions: Going concern

B. Income

List of Members and their cash contribution (EUR)

Member Country	Invoiced 01/01/20 - 31/12/20	Payment received
UK	104,040	104,040
FR	104,040	104,040
ES	78,030	78,030
IT	78,030	78,030
BE	78,030	78,030
NL	78,030	78,030
IL	78,030	78,030
CZ	52,453	52,453
PT	52,020	52,020
DK	52,020	52,020
SK	52,020	52,020
LV	52,020	52,020
FI	52,540	52,540
LT	30,778	30,778
Total	942,081	942,081

Grant Recipts

EU Grants	Income Jan - Dec 2020	Other income from Projects
CORBEL	224,174	33,619
Instruct-ULTRA	105,310	28,817
Transvac2	2,245	561
EOSC-Life	144,308	36,077
ERIC Forum	9,238	2,309
RI-VIS	34,185	8,546
EU-CELAC	4,246	1,061
iNext-Discovery	14,759	3,689
TRANSVAC-DS	727	181
i-NEXT/OPEN SESAME	(7057)	0
Total	532,135	114,860

Overhead contribution recognised: 25%

C. Deficit/surplus on activities €286,274

D. Employees

Some work is performed on behalf of Instruct-ERIC by employees of the University of Oxford. The cost of their services is charged to Instruct-ERIC by the University.

E. Debtors

Invoices outstanding from Members (present total figure outstanding against 2020 invoices) €71,291.67.

Other accrued income €11,151

Grant accrued income €246,208

F. Creditors

Accruals for services and awards (Access, Int, R&D, Training, unclaimed access) €678,151

Advances on Research Grants €556,333

Other creditors €127,628

Payroll taxes and pensions €15,271

G. Related Parties

Third parties are specified within each project Grant Agreement, particularly Articles 11-15 and in the Consortium Agreements (based on the DESCA H2020 Model Consortium Agreement, March 2016) between beneficiary partners.

The Consortium Agreement defines the responsibilities of beneficiary partners towards third parties that undertake project work, as follows:

"A Party (beneficiary partner) that enters into a subcontract or otherwise involves third parties (including but not limited to Affiliated Entities or Third parties linked to a Beneficiary identified under the Grant Agreement) in the Project remains responsible for carrying out its relevant part of the Project and for such third party's compliance with the provisions of the Consortium Agreement and of the Grant Agreement. The Party has to ensure that the involvement of third parties does not affect the rights and obligation of the other parties under the Consortium Agreement and the Grant Agreement.

Each Party shall be solely liable for any loss, damage or injury to third parties resulting from the performance of the said Party's obligations by it or on its behalf under the Consortium Agreement or from its use of Results or Background whether owned by that Party or obtained by it from another Party according the Grant Agreement or the Consortium Agreement."

H. Commitments

Instruct-ERIC has a lease agreement with PURE Offices Ltd, The Blade, Abbey Square, Reading, Berkshire RG1 3BE, UK to provide office space comprising Suites 8-11 including telephone, wireless and infrastructure services. The lease is on a rolling 1 month notice of termination.

I. Pensions

A Defined Contribution Pension Plan has been established through Aviva (www.aviva.co.uk/business/workplace-pensions/) with 8% employee contribution and 18% employer contribution. The Plan operates with an annual management charge of 0.3% which is levied annually on each Member portfolio investment. The Plan has been running successfully and has been implemented to comply with the UK terms of mandatory pension enrolment of all eligible employees within 1 month of employment.

J. Grant Agreements

Instruct ERIC acts as host (Coordinator) in respect of the following grants:

Instruct-ULTRA: €3,950,000 (total value - now completed)

RI-VIS: €1,500,000 (total value) – start date February 2019, end date 31 January 2022.

Instruct-ERIC is a beneficiary partner in the following grants with a project lifetime award to Instruct-ERIC shown below:

Transvac2: €29,260

EOSC-Life: €452,362

ERIC Forum:€43,300

EU-LAC ResInfra: €106,875

iNEXT-Discovery: €147,500

TRANSVAC-DS: €14,375

GLOSSARY

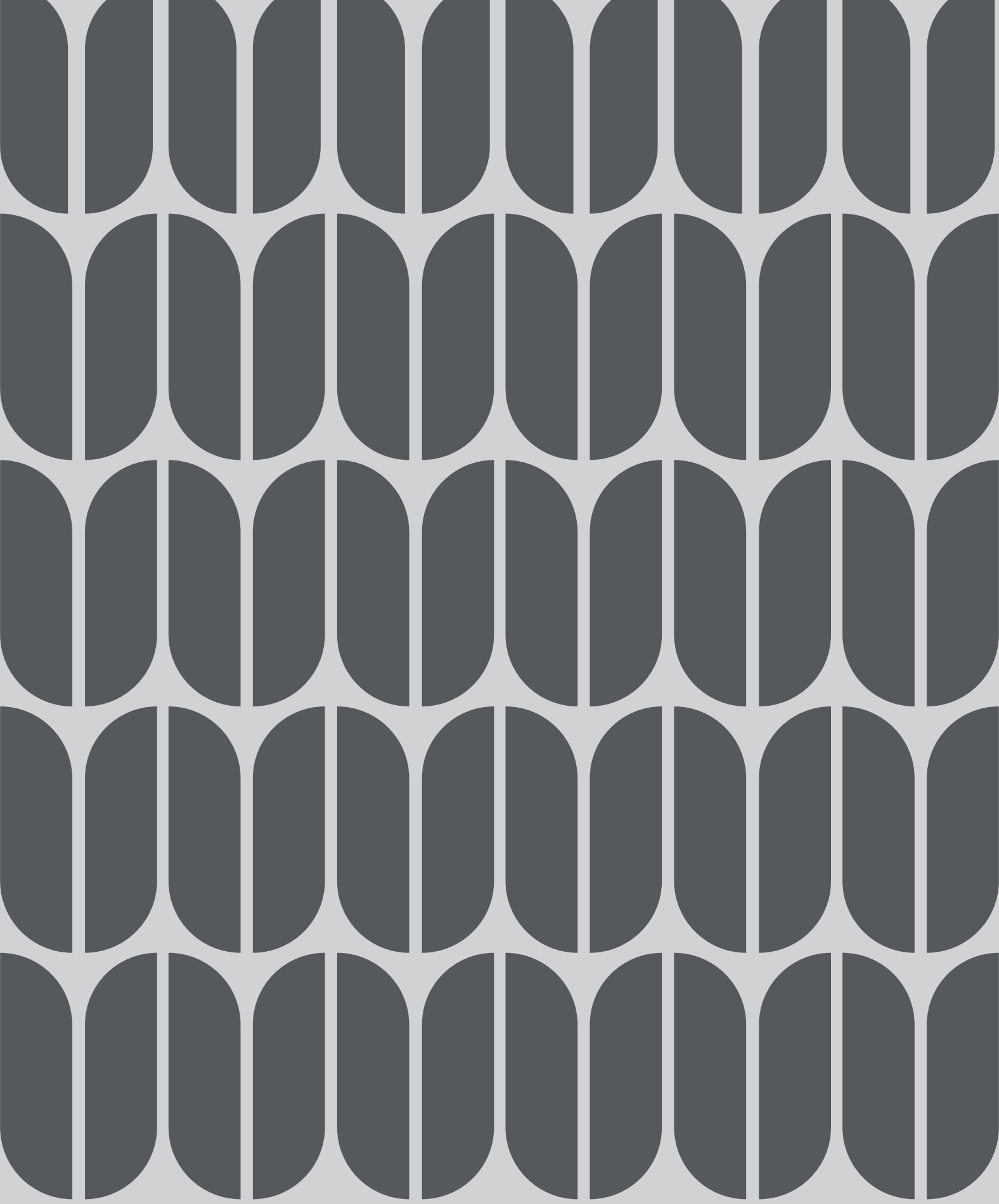
Term	Definition
AARC2	The second phase of the Authentication and Authorisation for Research and Collaboration initiative, which is continuing to develop and pilot an integrated cross-discipline authentication and authorisation framework.
Access	The unit of use of Instruct Research Infrastructure being in person (visit) or remotely (by sending samples)
Access Committee	A body established to manage the review of prospective users' proposals and applications for access to the tools and services provided by the Instruct-ERIC.
ARBRE MOBIEU	A network for the development of innovative integrative biophysical approaches.
ARIA	Access to Research Infrastructure Administration: Instruct-ERIC's access management system.
BREXIT	Brexit was the withdrawal of the United Kingdom from the European Union and the European Atomic Energy Community on 31 January 2020.
CatRIS	The Catalogue of Research Infrastructure Services is a harmonised and aggregated catalogue of services and resources provided by Research Infrastructures and Core Facilities across Europe
CORBEL	An initiative of thirteen biological and medical Research Infrastructures to create a platform for harmonised user access to biological and medical technologies, biological samples and data services required by cutting-edge biomedical research.
COVID-19	Coronavirus disease caused by the SARS-CoV-2 virus
CRISPR-Cas9	Natuarally occuring gene editing system in bacteria now used by geneticists to alter genome sequences in other cell types organisms.
EMBL	The European Molecular Biology Laboratory: an intergovernmental organisation specialising in research in the life sciences, funded by its 20 member states.
EMBRC	The European Marine Biological Resource Centre: a pan-European Research Infrastructure for marine biology and ecology research.
ENRIITC	The ENRIITC project aims to build a permanent pan-European network of Industrial Liaison and Contact Officers.
EOSC-Life	The European Open Science Cloud: bringing together biological and medical Research Infrastructures to create an open, collaborative space for digital biology.
ERIC	European Research Infrastructure Consortium: a specific legal form that facilitates the establishment and operation of Research Infrastructures with European interest.
ERIC Forum	A Horizon2020 project bringing together European Research Infrastructure Consortia to strengthen their coordination and enhance their collaborations.
ESFRI	European Strategy Forum on Research Infrastructures: an organisation with members nominated by European member states ministries to support a coherent and strategy-led approach to policy-making on Research Infrastructures in Europe.
EU-LAC ResInfra	The European Union – Latin America and Caribbean partnership in Research Infrastructures pursues the construction of a bi-regional collaboration between European Union and the LAC countries.
EU-OPENSCREEN	A European Research Infrastructure Consortium providing open access to a range of technologies and tools for the systematic screening of chemical substances for their biological effects.
Euro-BioImaging	A European Research Infrastructure providing open access to a broad range of technologies in biological and biomedical imaging for life scientists.
FEBS	The Federation of European Biochemical Societies: a charitable organisation supporting research and education in molecular life sciences.
FRISBI	The French Infrastructure for Integrated Structural Biology: an infrastructure for integrative structural biology approaches.
H2020	Horizon 2020 is the biggest EU Research and Innovation programme, making €80 billion of funding available over 7 years.
IceBear	Integrated Crystal-data-tracking Enhancing Biochemistry Education And Research Software
ISPyB	Laboratory Information Management System used for sample tracking and experiment reporting at synchrotron beamlines
iNEXT-Discovery	A consortium funded by the Horizon2020 program, offering European researchers access to a range of structural biology technologies.
Instruct Centre	An organisation that delivers access through the Instruct funding route.
Instruct Council	The governing body of Instruct-ERIC, deciding all issues of major importance including strategic objectives and targets and the deployment of finances and resources.
Instruct Executive Committee	The supervisory body for the execution of the project that reports to, and is accountable to the Instruct Council. Responsible for maintaining the progress and direction of the project.
Instruct Hub	The team responsible for coordinating Instruct-ERIC's operational activities.
Instruct Managers Group	A group of facility managers from across the Instruct RI, who discuss operational advances and support.
Instruct Member	A country paying a membership fee to allow its scientists to apply for funding to access Instruct-ERIC services.
Instruct Observer	Countries or international organisations that are considering Instruct membership can become an Observer for a period of 1 year.
Instruct User	A person that has applied, or is in the process of applying to access services through Instruct.
Instruct-ULTRA	A booster project to enhance and develop structural biology provision across Europe and beyond.
Moderator	A person assigned to an Instruct proposal by the Secretary of Moderators in order to select reviewers and decide the outcome of user proposals.
OpenAIRE	The Open Access Infrastructure for Research in Europe is a network of dedicated Open Science experts promoting and providing training on Open Science as well as a technical infrastructure harvesting research output from connected data providers.
Proposal	A user's request for access to technology or other services.
Reviewer	Assigned by the moderator, a reviewer assesses the science of an Instruct proposal. Three reviewers are assigned to each proposal: all are external to the Instruct Centre that has been requested for access, and at least one is external to Instruct-ERIC.
RI-VIS	A H2020 funded project to increase the visibility of European Research Infrastructures (RIs) to new communities in Europe and beyond.
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 causing the COVID-19 pandemic
Scipion	Integrative image processing workflow engine
Stakeholder	A person, or group of people with an interest or concern in Instruct-ERIC.
Transvac2	The TRANSVAC2 consortium comprises a comprehensive collection of leading European institutions that propose to further advance with the previous initiative towards the establishment of a fully operational and sustainable European vaccine R&D infrastructure.
TRANSVAC-DS	The TRANSVAC-DS project aims to consolidate the conceptual and technical design and ultimate implementation of a European vaccine R&D infrastructure.
West-Life	West-Life provides services for computation and data management to researchers in structural biology.

ABBREVIATIONS

AF4	Asymmetrical flow field-flow fractionation
API	Application Programming Interface
ASLR	Ascending stepwise linear regression
AUC	Analytical Ultracentrifugation
Bio-SAXS	Biological small angle X-ray scattering
BIOCEV	Biotechnology and Biomedicine Centre (Czech Republic)
BLI	Bio-layer interferometry
BSNL	Bruker Smart Nitrogen Liquefier
CBI	Center of Integrative Biology (France)
CD	Circular dichroism
CeBEM	Bolivian Center for Multidisciplinary Studies
CEA	French Alternative Energies and Atomic Energy Commission
CEITEC	Central European Institute of Technology (Czech Republic)
CELAC	Community of Latin American and Caribbean States
CERM	Magnetic Resonance Center of the University of Florence (Italy)
CIISB	The Czech Infrastructure for Integrative Structural Biology
CIRMMP	The Interuniversity Consortium for Magnetic Resonance of Metallo Proteins (Italy)
CNB	Spanish National Centre for Biotechnology
CPU	Central processing unit
CSIC	Spanish National Research Council
DLS	Dimond Light Source (UK)
DMCC	Data Managementand Computational Committee
DSC	Differential Scanning Calorimetry
eBIC	Electron Bio-Imaging Centre (UK)
EC	The European Commission
EM	Electron Microscopy
EPR	Electron Paramagnetic Resonance
ESPRIT	Soluble Proteins by Random Incremental Truncation
ESRF	The European Synchrotron Radiation Facility (France)
ET	Electron Tomography
FTIR	Fourier transform infrared
GDPR	General Data Protection Regulation
HDX	Hydrogen Deuerium exchange
HPLC	High-performance liquid chromatography
HT	High-troughput
HTX	High-throughput crystallisation
I2PC	Instruct Image Processing Center (Spain)
IBS	Institute of Structural Biology (France)
IBSBC	Instruct Biennial Structural Biology Conference
IGBMC	The Institute of Genetics and Molecular and Cellular Biology (France)
ISAB	Independent Scientific Advisory Board
ISAL	Instruct Academic Services Limited
ISBG	Integrated Structural Biology Grenoble (France)

ABBREVIATIONS CONTINUED

ISO	International Organisation for Standardisation
SPC	The Israel Structural Proteomics Center
ITC	Isothermal titration calorimetry
ITQB	Institute of Chemical and Biological Technology (Portugal)
JRA	Joint Research Award
KPIs	Key Performance Indicators
LMJ	LMJ
LS RI	Life Science Research Infrastructures
MALDI	Matrix Assisted Laser Desorption/Ionisation
MD	Molecular dynamics
MoU	Memoranda of Understanding
MS	Mass Spectrometry
MST	Microscale Thermophoresis
MX	Macromolecular Crystallography
NeCEN	Netherlands Centre for Electron Nanoscopy
NKI	Netherlands Cancer Institute
NMR	Nuclear Magnetic Resonance
OPIC	Oxford Particle Imaging Centre (UK)
PALM	Photo-activated localization microscopy
PAINT	Points accumulation for imaging in nanoscale topography
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PID	Proposal Identification number
PLS	Partial least square
R&D	Research and development
RBD	Receptor-binding domain
RI	Research Infrastructure
RTG	Radioisotope Thermoelectric Generator
SEC-MALLS	Size Exclusion Chomatography - Multi-Angle Laser Light Scattering
SEM	Scanning electron microscope
SOP	Standard operating procedure
SPR	Surface Plasmon Resonance
SPU	Structural Proteomics Unit
START	Synchrotron Techniques for African Research and Technology
STORM	Stochastic optical reconstruction microscopy
SVM	Support vector machine
TEM	Tansmission electron microscopy
VU LSC	Vilnius university Life Sciences Center
WHO	World Health Organization
WIS	Weizmann Institute of Science (Israel)



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