2021
INSTRUCT-ERIC ANNUAL REPORT


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CONTENTS

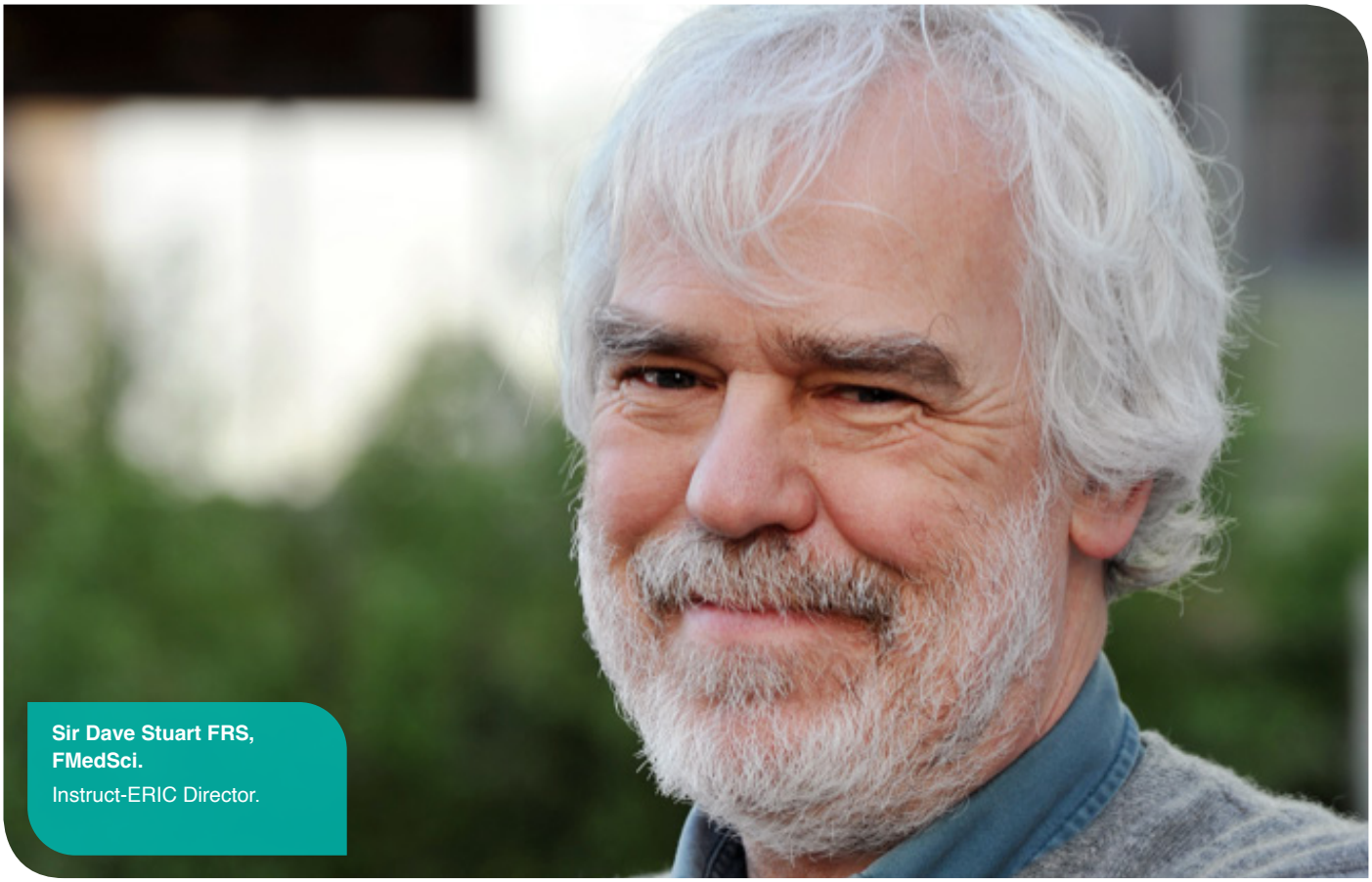
FOREWORD BY CHAIR OF THE INDEPENDENT SCIENTIFIC ADVISORY BOARD

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

International Scientific Advisory Board (ISAB) members strongly support Instruct-ERIC's commitment to providing the broadest possible access to structural biology infrastructure and expertise aimed at maximising the impact of three-dimensional (3D) biostructure data on research and education across the life sciences. The organisation is rightly focused on using new capabilities for molecular resolution studies and understanding structural dynamics to characterise biological systems at atomic and near-atomic resolution in the cellular context. Instruct infrastructure continues to evolve and expand to keep pace with new technical developments. In electron microscopy and tomography, for example, Instruct-ERIC now offers access to a comprehensive choice of state-of-the-art cryo-electron microscopes and ancillary instruments housed by seven Instruct Centres. Scientific excellence remains the top priority of projects supported by the organisation, which is clearly exemplified by the 301 peer-reviewed scientific publications appearing in 2021 that benefitted from Instruct services.

Instruct-ERIC will be developing the five-year strategic programme for 2023-2027. Access to infrastructure for macromolecular crystallography, nuclear magnetic resonance spectroscopy, and electron microscopy will continue to among be the most important services provided to Instruct users. As Instruct membership expands and its internationalisation programmes develop, the burgeoning user community will present new challenges and create new opportunities for the organisation. The ISAB is confident that Instruct-ERIC has a strong foundation from which to plan, consolidate, and strengthen its position in Europe and globally as a research infrastructure provider of choice for structural, cellular, and organismal biologists.

FOREWORD BY INSTRUCT-ERIC DIRECTOR



Sir Dave Stuart FRS, FMedSci.
Instruct-ERIC Director.

2021 continued to be a challenging year for Instruct as uncertainties around the global management of the COVID-19 pandemic continued to impact on Instruct activities. While some programmes were inevitably delayed again, remote access to infrastructure services became even more important to support research through this critical time. New services were added to the Instruct catalogue including X-ray fragment screening, CRISPR-Cas9 tagging and mass photometry and the addition of more instruments and significant upgrades to existing technologies and methods expanded the choice and value of infrastructures for the user community.

Data management also became a more obvious and urgent priority as the global COVID-19 response brought into sharp focus the problems in integrating data from different technological platforms and across national boundaries. The development of the European Open Science Cloud (EOSC) is driving many of the data management processes and Instruct-ERIC has been involved in this process through membership of a number of consortia funded through Horizon 2020 and Horizon Europe framework funding. The Instruct access management system ARIA has continued to develop to enable efficient integration with the pan-European EOSC systems and this has strengthened ARIA which has had several software upgrades to enable data exchange and to improve the user interface.

To the tremendous credit to the staff at Instruct Centre facilities and in the Hub service provision and the day-to-day project and administrative processes continued apace throughout the year. Access projects supported by Instruct increased from 2020 with the highest financial commitment for access provision recorded in 2021 since Instruct-ERIC was founded. Furthermore, most training events were completed, albeit some in virtual mode instead of in person.

This Annual Report provides a summary of the 2021 Instruct year in which the scale of our infrastructure increased along with the access provision and the scientific publications arising from research in which Instruct played a part. 2022 will see some significant changes in leadership and a new strategic plan for the next five years and this report records the status of Instruct from which the future will be built.

At a personal level it has been a tremendous privilege to have been associated with Instruct since its foundation and to have worked with people from across Europe to establish the principles and practice of making high quality structural biology infrastructure and expert support available to all researchers in member countries on the basis of scientific excellence. It is clear that there will major global challenges in the next few years, and we will need to defend those principles in order to allow Instruct to fully develop.

LOOKING FORWARD

2021 proved to be challenging and rewarding in almost equal measure:

- challenging because the early optimism in 2021 that the SARS CoV2 pandemic would be more manageable was dashed when renewed restrictions were imposed and the Omicron variant became the dominant strain;
- challenging because the accelerated calls for proposals in the first work programme of Horizon Europe meant that a great deal of work had to be done in a very short time to contribute to several applications in parallel, stretching Instruct's resources;
- rewarding because all of the applications to Horizon Europe calls that Instruct participated in were subsequently funded;
- rewarding because the negotiations for the appointment of a new Director for Instruct came to a close with an excellent candidate ready to take over from early 2022.

Instruct-ERIC also began preparations for its second five-year funding cycle, starting mid 2022, by preparing a summary report of Instruct activities in the first four years of ERIC operations. This report, termed the Quinquennial Report (QQR) was framed to provide the Instruct Members with sufficient information for a decision on future scientific and financial commitment to Instruct to be made for the period 2022-2027. The QQR provided a vision for future priorities for Instruct, leaving room for the incoming Director to add to this to form the final strategic programme. Instruct has much to look forward to in 2022 with a strong portfolio of existing and newly approved EU-funded projects, a new structure at the helm of Instruct and the ambition to expand and build on Instruct's strengths, within Europe and internationally.

Susan Daenke
Instruct-ERIC Hub Coordinator

EXECUTIVE SUMMARY

15
Members

11
Centres

27
Facilities

In 2021 Instruct-ERIC had 15 member countries and organisations.

These members hosted 11 Instruct Centres with 27 facilities providing research access. A new addition in 2021 included the Instruct Centre EMBL located in EMBL Hamburg, Heidelberg and Grenoble as well as CryoEM CNB-CSIC facility in Spain.

165
Access proposals received

1069.2
Days of access provided

301
Scientific Publications

In 2021 Instruct recieved 165 proposals for access from researchers in 19 countries of which 85% were approved.

Instruct supported 131 research visits providing 1069.2 days of access to Instruct Facilities covering both national and transnational access.

Scientific output resulted in 301 publications in peer-reviewed journals.

80
Services

80 infrastructure services offered in nine categories.

A number of significant additions and upgrades to the infrastructure at Instruct Centres took place. This includes the Instruct Centre EMBL offering sample preparation, characterisation and 3D structural analysis services. The CryoEM facility at Centro Nacional de Biotecnologia, CSIC to the Instruct Centre-Spain, closely aligned with the Instruct Image Processing Centre to form a seamless workflow from sample preparation, image collection and image data analysis. Hydrogen-deuterium exchange mass spectrometry and the mass photometry facilities at the Kavli Institute for Nanoscience Discovery were added to the Instruct Centre-UK. The gene-tagging with CRISPR-Cas6 platform for adding affinity tags to expression constructs through a partnership with Instruct-FR1 and the Museum d'Histoire Naturelle Paris, FR.

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. Additionally, several of the courses focused on remote access to research infrastructure services both for Research Infrastructure managers and users. Remote access has become more prevalent during the COVID-19 pandemic and is rapidly growing throughout the community.

8
Projects

Instruct-ERIC participated in 7 Horizon projects, EOSC-Life, ERIC Forum, iNEXT-Discovery, RI-VIS, EU-LAC ResInfra, TRANSVAC2 and TRANSVAC-DS.
BY-COVID funded by the new Horizon Europe work programme started in October 2021.
Five further projects Instruct-ERIC applied to in 2021, ISIDORe, canSERV, EOSC4Cancer, AI4Life, and eRImote, were approved and are expected to start in 2022.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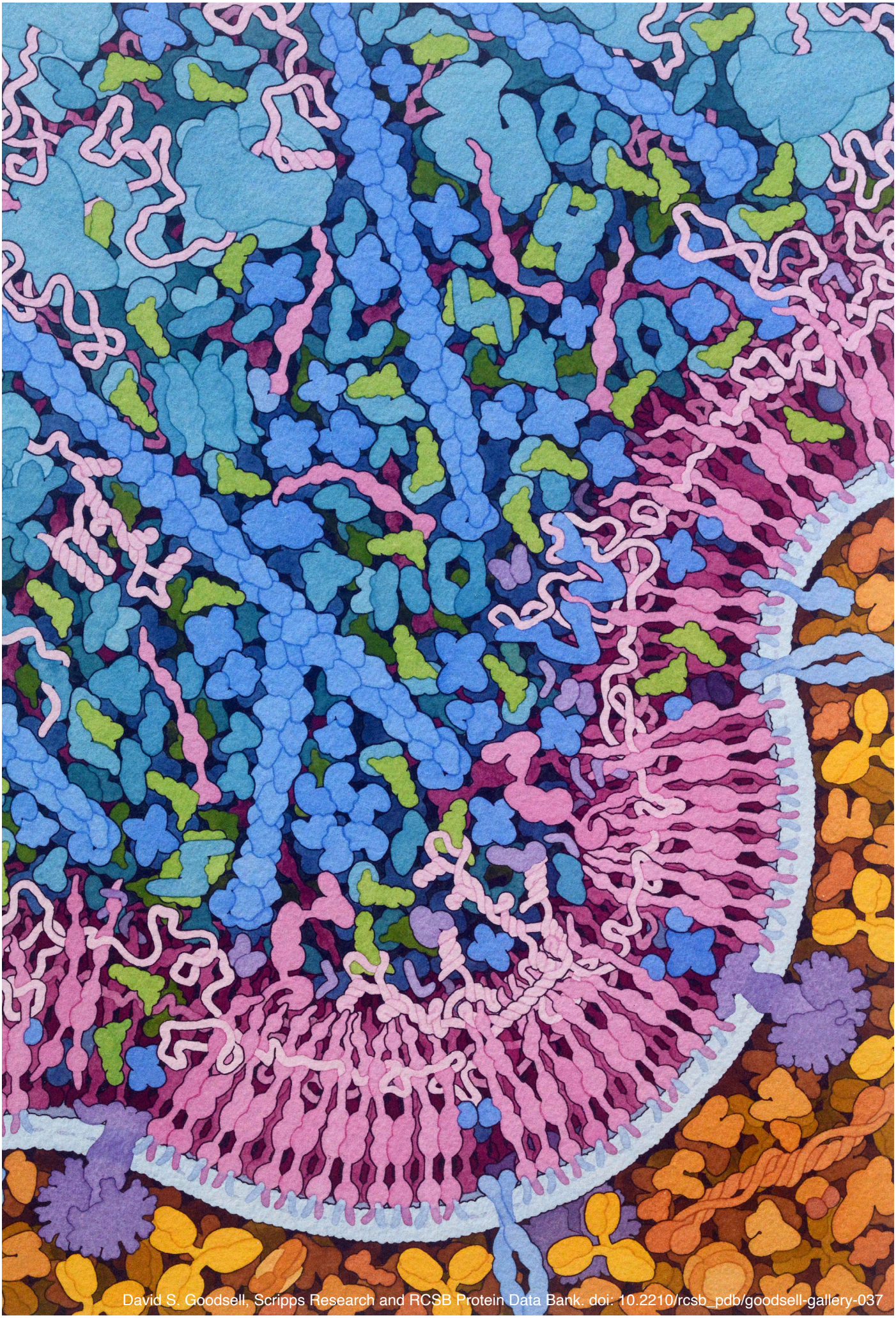
INTRODUCTION



David S. Goodsell, Scripps Research and RCSB Protein Data Bank. doi: 10.2210/rcsb_pdb/goodsell-gallery-037

INSTRUCT-ERIC ADVANCES IN 2021

Instruct-ERIC is built on its infrastructure access operations, providing access to an increasingly sophisticated and expansive network of instruments, with the expertise to enable our user community to achieve impressive results for their research. The success of the Instruct access programme depends on constant review, revision, addition and evolution of the infrastructure, and a number of Instruct Centres enhanced their infrastructure capabilities in 2021 to meet new demands from users. Notable were the addition of:

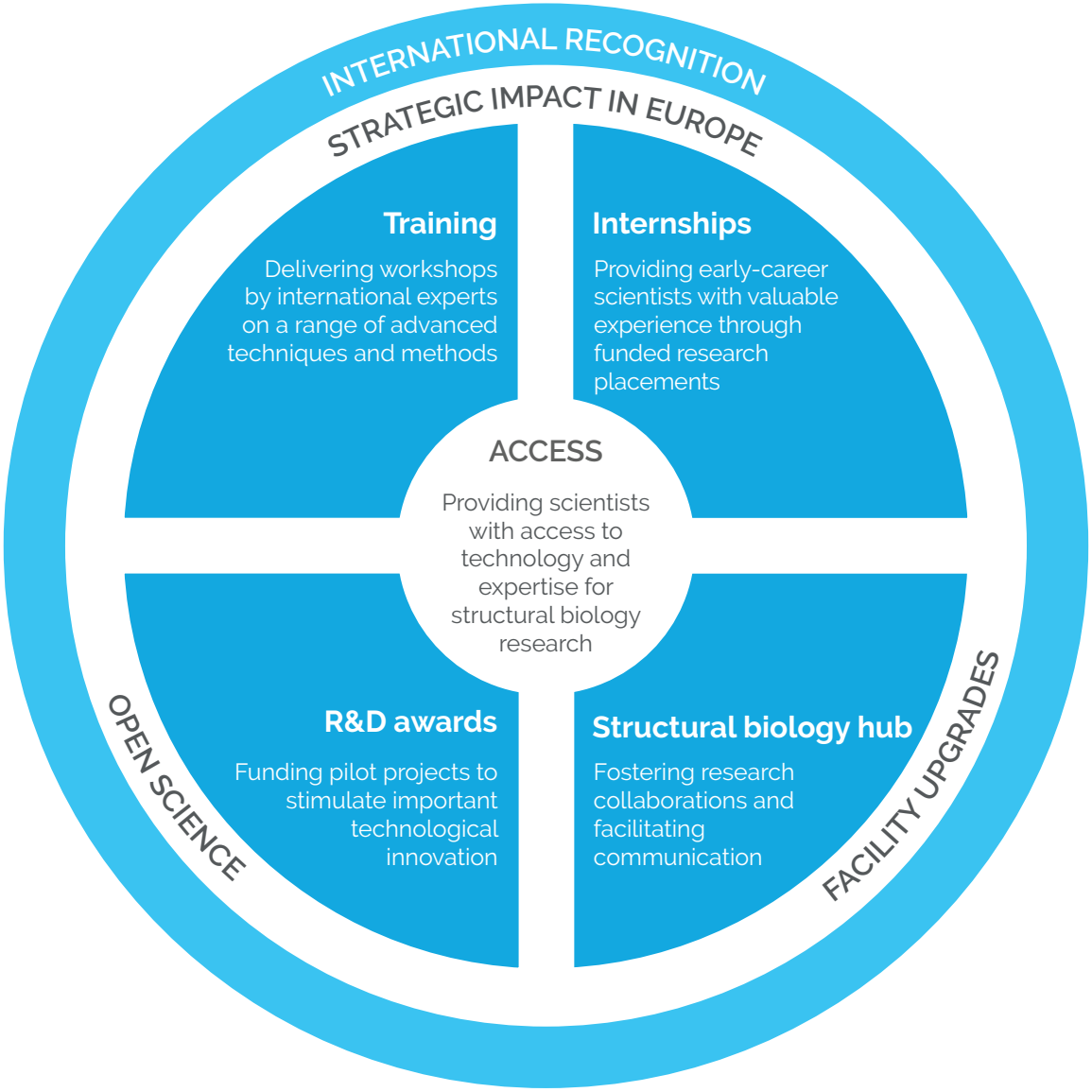
Instruct Centre EMBL with facilities in Grenoble, Hamburg and Heidelberg. Collectively the new Centre offers expertise and services in crystallisation, mass spectrometry, molecular biophysics, electron microscopy and X-ray techniques.

The **cryo-EM facility** at Centro Nacional de Biotecnologia, CSIC to the Instruct Centre Spain, closely aligned with the Instruct Image Processing Centre to form a seamless workflow from sample preparation, image collection and image data analysis.

The Instruct Landscape Working Group formed in 2021 to review the European and international landscapes in structural biology technologies and will provide information to help shape the future priorities for Instruct infrastructure.

Underpinning the access delivery, ARIA is constantly evolving and many major and minor improvements were made throughout 2021. An overhaul of the internal ARIA messaging system made this much more user-friendly and enabled direct operation through email. Changes to the ARIA core also improved operational speed and added functionalities for data reporting, managing new calls, joint calls and awards.

The Instruct access contribution model, which defines the funding contribution that Instruct makes to both user and facility in support of approved projects, was revised to better reflect current costs.



INSTRUCT-ERIC MEMBERSHIP

The Quinquennial Report set out targets for expanding EU membership of Instruct-ERIC in addition to providing its core operational programme. Even in a challenging financial environment, Instruct is confident that membership will expand to include more European member states and to engage internationally to best serve its community. The budget proposed to service these ambitions through to the next funding cycle forecasts the need for an uplift in the membership contribution model, for one year only, to reset the budget. This decision will be taken by Council in 2022 to become operational for 2023, and if approved will require a Statute amendment.

Membership of Instruct-ERIC stood at 15 members and one observer in 2021.

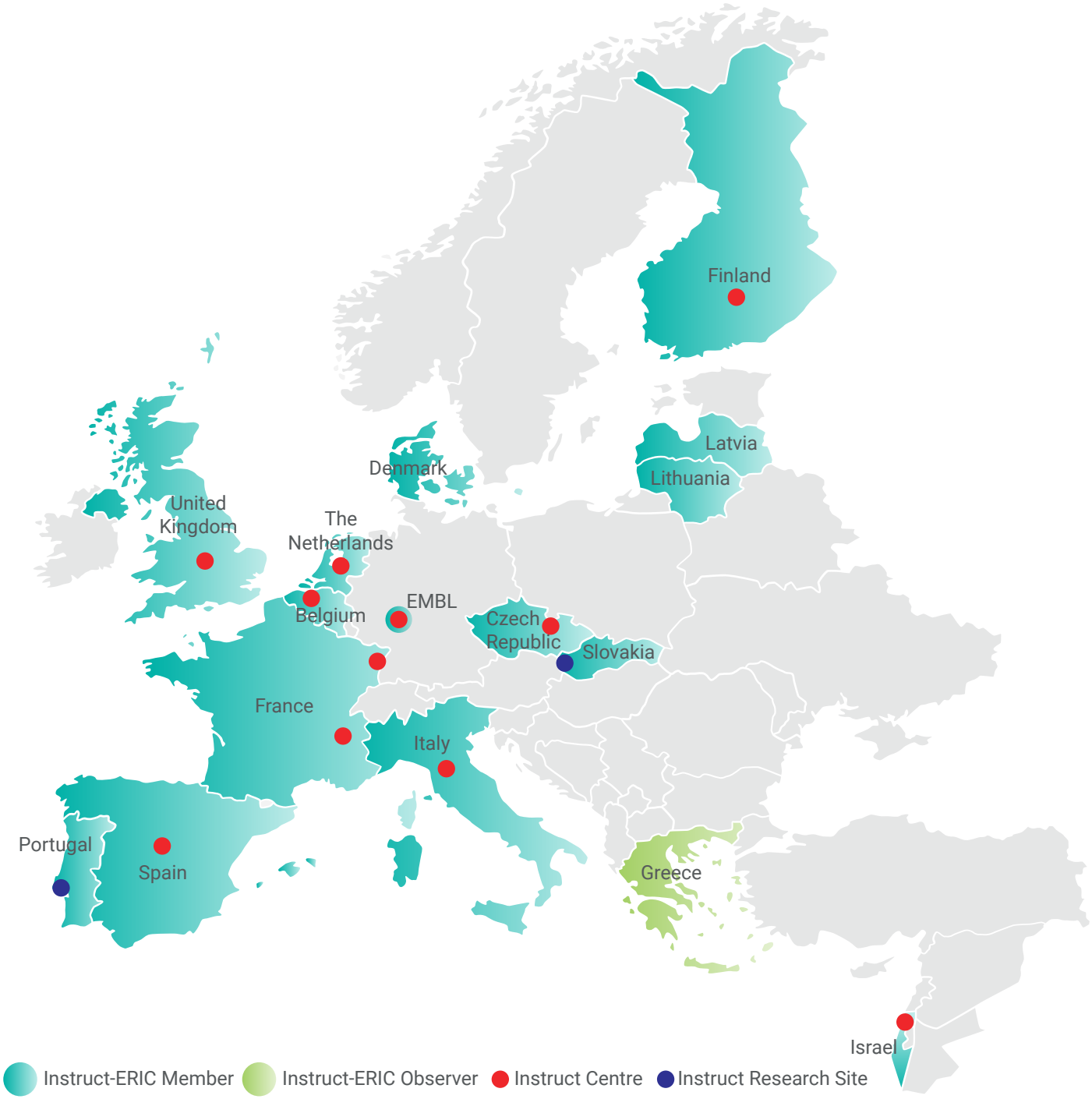


FIG 1. Map showing Instruct-ERIC Member Countries and Organisations (Greece was retained as an Observer) and Instruct Centres.

The Instruct-ERIC statutory seat remained in the UK in 2021 with the Coordination Hub located in Oxford, UK. Increasing pressure and concern was apparent regarding association of the UK to Horizon Europe, to settle UK eligibility for funding through the Horizon Europe Framework and to avoid destabilisation of Instruct-ERIC through a change to its structure. Any change to the fundamental governance structure would exert a significant impact on Instruct operations and efforts will continue to clarify this in 2022 with the utmost urgency. The goodwill and support of all members to define Instruct-ERIC as a true European Research Infrastructure has been essential to sustaining Instruct through the past 5 years of uncertainty.

INSTRUCT CENTRES

Members' in-kind contributions sustain the composition of the infrastructure that is made available for access and Instruct now hosts 11 Centres with 27 facilities.

	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-EMBL	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	153.34	565.50
FR	A	100.00	102.00	104.04	106.12	153.34	565.50
ES	B	75.00	76.50	78.03	79.59	115.01	424.13
IT	B	75.00	76.50	78.03	79.59	115.01	424.13
BE	B	75.00	76.50	78.03	79.59	115.01	424.13
NL	B	75.00	76.50	78.03	79.59	115.01	424.13
IL	B	75.00	76.50	78.03	79.59	115.01	424.13
CZ	C	50.00	51.00	52.02	53.06	76.67	282.75
PT	C	50.00	51.00	52.02	53.06	76.67	282.75
DK	C	50.00	51.00	52.02	53.06	22.55	228.63
LV	C		29.75	52.02	53.06	76.67	211.50
SK	C	50.00	51.00	52.02	53.06	76.67	282.75
FI	C		4.33	52.02	53.06	76.67	186.08
LT	C			8.67	53.06	76.67	138.40
Total		775.00	824.58	919.02	981.61	1,364.28	4,864.49

*The Year 5 membership contributions include the annual fee plus the remaining five months August-December 2022 to bring contributions in line with the calendar year which is also the reporting year.

TIMELINE
selected events from 2021

JAN

Instruct-ERIC Director David Stuart awarded knighthood for his research on virus protein strucures

New Instruct Centre EMBL

FEB

Africa - Europe Symposium on Research Infrastructures organised by the RI-VIS project coorindated by Instruct-ERIC

MAR

ARIA updated towards cloud migration through full containerisation of the software.

APR

- Joint Call for Research - Instruct-ERIC and iNEXT-Discovery
- Instruct-ERIC launched **Software Developer Exchange of Experience** webinar series

MAY

- Instruct-ERIC Council meeting
- Applications for the Horizon Europe projects ISIDORe and BY-COVID submitted

JUN

40th internship awarded

Latin America - Europe Symposium on Research Infrastructures organised by the RI-VIS project coordinated by Instruct-ERIC

Instruct Hub Coordinator Susan Daenke invited panelist at ICRI 2021

50th R&D project awarded

JUL

- Instruct-ERIC is now a partner in two newly approved Horizon Europe projects ISIDORe and BY-COVID
- Instruct-ERIC opens its first remote internship call

AUG

EU-LAC ResInfra Staff Exchange call: Bi-Regional Collaboration with Instruct-ERIC

SEP

- 2nd International Call Opens in a joint acitivity with EU-LAC ResInfra and iNext-Discovery
- Applications for the Horizon Europe projects canSERV, EOSC4Cancer, AI4Life and eRImote submitted

Australia - Europe Symposium on Research Infrastructures
organised by the RI-VIS project coorindated by Instruct-ERIC

700th access proposal approved

Instruct-ERIC at the UN General Assembly Science Summit

Instruct joins the Horizon Europe project

BY-COVID

OCT

Instruct-ERIC Council meeting

NOV

New Instruct Facility

CryoEM CNB-CSIC

New Instruct Research Sites

Instruct-PT

Instruct-SK

DEC

Instruct-ERIC Centre Finland Opening and Structural Biology Finland Conference 2021


5WYG

INSTRUCT CENTRES

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.

Instruct Centre Lead Scientists

Els Pardon, Jan Steyaert, André Matagne, Alain Brans, Ernesto Ambroggio, Erik Goormaghtigh, Joëlle De Meutter

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 to stabilise protein-protein interactions and other applications in (structural) biology. Their use extends to structural biology as once they are structurally characterised, the same Nanobody can be functionally expressed in eukaryotic cells to track their targets inside a living cell.

FIG 1

FIG 1. Llamas for Instruct.

Robotein® is a technology platform, which offers automated screening for optimal recombinant protein expression, purification, and formulation, together with biochemical/biophysical protein analysis. High throughput (HT) experiments are carried out with the help of two automated liquid handling workstations, in combination with capillary electrophoresis (LabChip GXII) for fast and easy nucleic acids and proteins separation, and biolayer interferometry (Octet HTX platform) for the analysis of biomolecular interactions and protein quantification. Thus, for example, this technique is used to measure the avidity and affinity of polyclonal and monoclonal antibodies, respectively, with specificity for any purified antigen. In addition, the combination of a protein microarrayer with FTIR imaging provides information on protein identity, post-translational modifications, and conformation.

NEW TECHNOLOGIES

A new tool for HT hydrogen/deuterium exchange (HDX) monitored by FTIR imaging.

We combined the building of protein microarrays with FTIR imaging to create HT HDX FTIR measurements. Slides bearing the protein microarrays are submitted to a flow of N_2 gas saturated with ^2H_2O. Exchange occurs simultaneously for all proteins and single images covering ca 96 spots of proteins can be recorded on-line at selected time points. Each protein spot contains ca 5 ng protein and the 96-protein array covers 2.5x2.5 mm^2. HDX can be monitored in real time, eliminating back-exchange problems encountered when the exchange reaction is stopped by decreasing the pH before subsequent processing. Analysis of the HDX curves by inverse Laplace transform and by fitting exponential curves provide quantitative information on different classes of amide protons. The whole process of analysis has been automated to yield fast analyses. This tool responds to an urgent need of scientists, both in the academia and industry, to assess any change in the state of folding of proteins.

Megabodies as innovative tools for cryo-EM

Nanobodies are highly popular and versatile tools for X-ray crystallography but they are also promising tools for cryo-EM because they can lock well-defined conformations and stabilise multi-protein complexes. We reformatted our Nanobodies into Megabodies, whereby Nbs are rigidly grafted into selected protein scaffolds to increase their molecular weight while retaining the full antigen binding specificity[3]. Megabodies have been used to obtain 3D reconstructions for membrane proteins that suffer from severe preferential orientation and can be applied as fiducial markers on small proteins that are otherwise too small to allow accurate particle alignment.

Measurement of polyclonal antibody avidity

A high throughput assay has been developed for the measurement of the avidity of polyclonal antibodies with

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. Uchański, T. *et al* (2021). Megabodies expand the nanobody toolkit for protein structure determination by single-particle cryo-EM." *Nat Methods* 18(1): 60-68.

specificity for the receptor binding domain (RND) of the spike protein, using biolayer interferometry.

This allows the assessment the quality of the immune response against SARS-CoV-2. Thus, this parameter, together with the known levels of Ab binding to various spike domains (RBD, S1 and S2) and the neutralising AB capacity led to the observation[4] of poor Ab responses to mRNA vaccination in infection-naive nursing home (NH) residents and in some naive staff members. These data suggested suboptimal protection against breakthrough infection, especially with variants of concern, and were used to support the administration of a third dose of mRNA vaccine to further improve protection of NH residents against COVID-19 (Fig. 2).

FIG 2

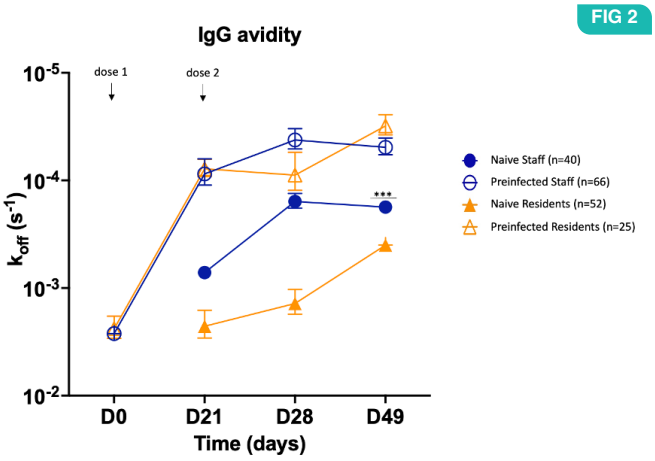


FIG 2. Avidity maturation after two doses of Pfizer/BioNTech mRNA vaccination. Medical staff individuals are represented in blue, whereas nursing home residents are represented in orange. Slow and weak avidity maturation is observed in naïve residents compared to naïve staff. Rapid and intense avidity maturation is observed in preinfected residents as for preinfected staff.

SCIENCE HIGHLIGHTS

Structure, mechanism, and inhibition of Hedgehog acyltransferase[5]

In collaboration with the Siebold lab, we generated Nanobodies to structurally and functionally characterise full-length human HHAT. Two Nbs bound to purified HHAT with low nanomolar affinity and also bound the HHAT expressed in cells. To overcome the technical hurdles of size and/or preferential orientation problems for cryo-EM structure analysis, we created Mbs with the YgjK scaffold resulting in 100 kDa megabodies[5]. These Megabodies were successfully used to solve the high-resolution cryo-EM structure of HHAT bound to substrate analog palmityl-coenzyme A and a SHH-mimetic megabody, revealing a heme group bound to HHAT that is essential for HHAT function (Fig. 3).

FIG 3

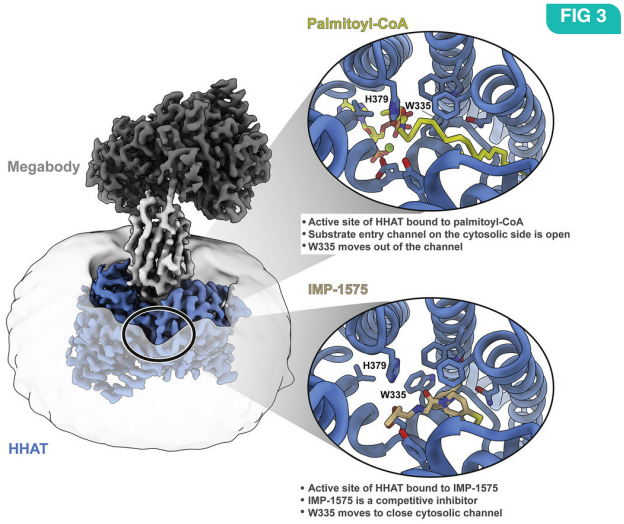


FIG 3. Crystal structure of nanobody-bound ECF-PanT. High resolution cryo-EM structure of HHAT-Megabody complex bound to ligand or inhibitor[5]

Protein Structural Denaturation Evaluated by MCR-ALS of Protein Microarray FTIR Spectra[6]

The loss of native structure is common in proteins. Among others, aggregation is one modification of particular importance as it is a major concern for the efficiency and safety of biotherapeutic proteins. We combined the use of protein microarrays spotted at a density of ca 2,500 samples per cm^2 and Fourier transform infrared (FTIR) imaging to analyse structural modifications (Fig. 4). Multivariate curve resolution alternating least square (MCR-ALS) was used to model a new spectral component appearing in the protein set subject to denaturing conditions. In the native protein set, 6 components were found to be sufficient to obtain a good modelling of the spectra. Their shape allowed them to be assigned to alpha-helix, beta-sheet and other structures. In the denatured proteins, a new component assigned to intermolecular beta-sheet was necessary and modelled by MCR-ALS. MCR-ALS therefore unveils a spectroscopic marker of protein aggregation and allows a semi-quantitative evaluation of its content. The high density of the arrays is associated with a fast and automatic processing of hundreds of proteins/environmental conditions.

FIG 4

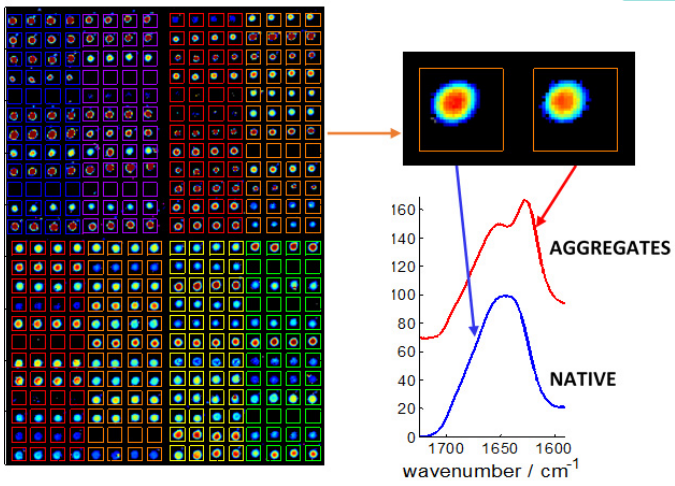


FIG 4. Detailed nanobody interaction sites in ELIC and channel pore analysis.[6]

4. Pannus, P. (2021) Poor antibody response to BioNTech/Pfizer COVID-19 vaccination in SARS-CoV-2 naïve residents of nursing homes, *Clin. Infect. Dis.* doi: 10.1093/cid/ciab998.
5. Coupland, C.E. *et al.* (2021) Structure, mechanism, and inhibition of Hedgehog acyltransferase. *Mol Cell* 81, 5025-5038.e10
6. De Meutter and Goormaghtigh E. (2021) Protein Structural Denaturation Evaluated by MCR-ALS of Protein Microarray FTIR Spectra. *Anal. Chem.* doi: 10.1021/acs.analchem.1c01416.

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 at CEITEC – Central European Institute of Technology, Brno and BIOCEV - Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University, Vestec, Prague-West. CIISB offers open-access and assisted expertise to 10 high-end core facilities for advanced 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

Thanks to the national funding provided by the OP VVV project UP CIISB through the Ministry of Education, Youth, and Sports of the Czech Republic, the CIISB core facilities (CF) have been continually upgraded since 2020. In 2021, the Josef Dadok National NMR Centre (CEITEC) obtained new cryo-probeheads of its 600, 700, 850, and 950 MHz systems with extended capabilities for 1H, 13C, 15N, and 19F detection and multiple resonance options, as well as a new diffusion with strong magnetic field gradients probe for the 700 MHz multi-purpose NMR spectrometer.

Diffraction Techniques CF (BIOCEV) upgraded its D8 Venture diffractometer and Biophysical Techniques CF (BIOCEV) purchased a new system Octet R8 System (Sartorius) for high-quality kinetic screening and affinity characterisation of biomolecular interactions (Fig. 1).

Also, the X-ray diffractometers at the X-ray Diffraction and Bio-SAXS CF (CEITEC) have been upgraded to stay at the state-of-the-art level.

Cryo-electron microscopy and tomography CF (CEITEC) (Fig. 2) acquired a new cryo-fluorescence microscope for imaging of fluorescently labeled samples at cryo-conditions. The setup is composed of a Leica DM6 FS microscope equipped with the cryo-stage and dedicated 50x cryo-objective with NA of 0.9. The system is further equipped with THUNDER technology for real-time deconvolution of the data during acquisition. The microscope will become an indispensable part of the correlative light and electron microscopy imaging (CLEM) of biological specimens at the near-native conditions.

In addition, a new Vitrification robot - Leica EM GP2 was installed. The instrument for the semi-automated vitrification of the specimen by plunge freezing enables sensor-controlled blotting of the sample. The automated blotting can be carried out from one side only (single-sided blotting) or from multiple sides.

CF Nanobio (CEITEC) got a new flagship technology, the AFM microscope JPK NanoWizard 4XP installed on a Leica DMi8 optical microscope with a fluorescence module (Fig. 3). The new AFM microscope is an imaging tool, which allows mapping of elastic properties of various samples with nanometer resolution. The new instrumental additions extend the portfolio of techniques applicable for biomolecular and chemical investigations.

FIG 1

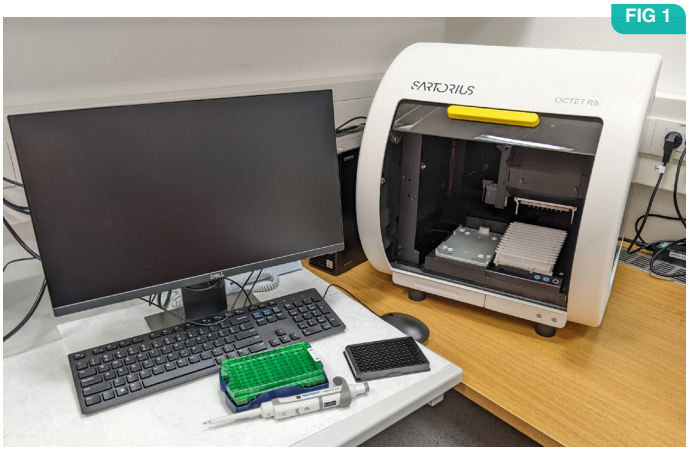


FIG 1 Octet R8 System (Sartorius) at BIOCEV.

FIG 2

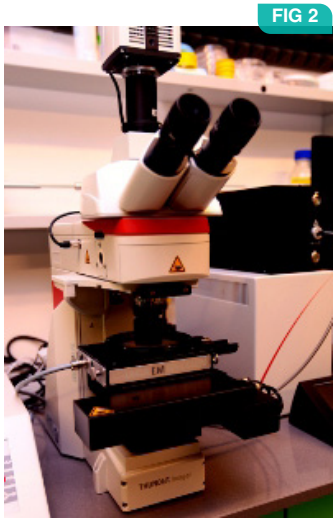


FIG 2 New cryo-fluorescence microscope for imaging of fluorescently labeled samples at cryo-conditions installed in 2021 at CEITEC. .

FIG 3

FIG 3 FM microscope JPK NanoWizard 4XP installed on a Leica DMi8 optical microscope with a fluorescence module at CF Nanobio (CEITEC).

SCIENCE HIGHLIGHTS

Anillin propels myosin-independent constriction of actin rings[1]

Constriction of the cytokinetic ring, a circular structure of actin filaments, is an essential step during cell division. Mechanical forces driving the constriction are attributed to myosin motor proteins, which slide actin filaments along each other. However, in multiple organisms, ring constriction has been reported to be myosin independent. How actin rings constrict in the absence of motor activity remains unclear. Here, we demonstrate that anillin, a nonmotor actin crosslinker, indispensable during cytokinesis, autonomously propels the contractility of actin bundles. Anillin generates contractile forces of tens of pico-Newtons to maximise the lengths of overlaps between bundled actin filaments. The contractility is enhanced by actin disassembly. When multiple actin filaments are arranged into a ring, this contractility leads to ring constriction. Our results indicate that passive actin crosslinkers can substitute for the activity of molecular motors to generate contractile forces in a variety of actin networks, including the cytokinetic ring (Fig. 4)[1].

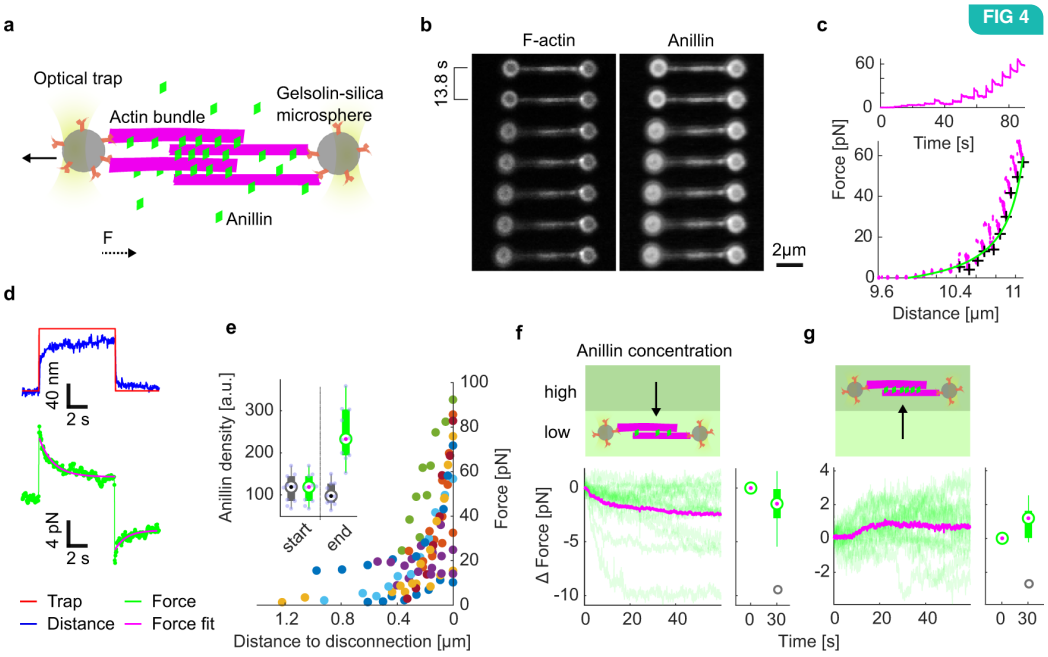


FIG 4. Anillin generates tens of pico-Newton forces to slide actin filaments. **(a)** Schematic representation of the experimental setup. **(b)** Time-lapse fluorescence micrographs showing an actin bundle attached between two silica microspheres. **(c)** Typical force time-trace (top) and the force-distance curve (bottom) corresponding to stretching of an anillin-actin filament bundle. **(d)** Temporal response of the construct to stretching and relaxation **(e)** The detected force increased with decreasing overlap length before the filaments slid apart completely **(f, g)** Force response of a pre-stretched actin-anillin bundle to a decrease (f) or increase (g) of anillin-GFP concentration[1].

Context-specific action of macrolide antibiotics on the eukaryotic ribosome[2]

Macrolide antibiotics bind in the nascent peptide exit tunnel of the bacterial ribosome and prevent polymerisation of specific amino acid sequences, selectively inhibiting translation of a subset of proteins. Because preventing translation of individual proteins could be beneficial for the treatment of human diseases, we asked whether macrolides, if bound to the eukar- yotic ribosome, would retain their context- and protein-specific action. By introducing a single mutation in rRNA, we rendered yeast Saccharomyces cerevisiae cells sensitive to macrolides. Cryo-EM structural analysis showed that the macrolide telithromycin binds in the tunnel of the engineered eukaryotic ribosome (Fig. 5). Genome-wide analysis of cellular translation and bio- chemical studies demonstrated that the drug inhibits eukaryotic translation by preferentially stalling ribosomes at distinct sequence motifs. Context-specific action markedly depends on the macrolide structure. Eliminating macrolide-arrest motifs from a protein renders its translation macrolide-tolerant. Our data illuminate the prospects of adapting macrolides for protein-selective translation inhibition in eukaryotic cells.

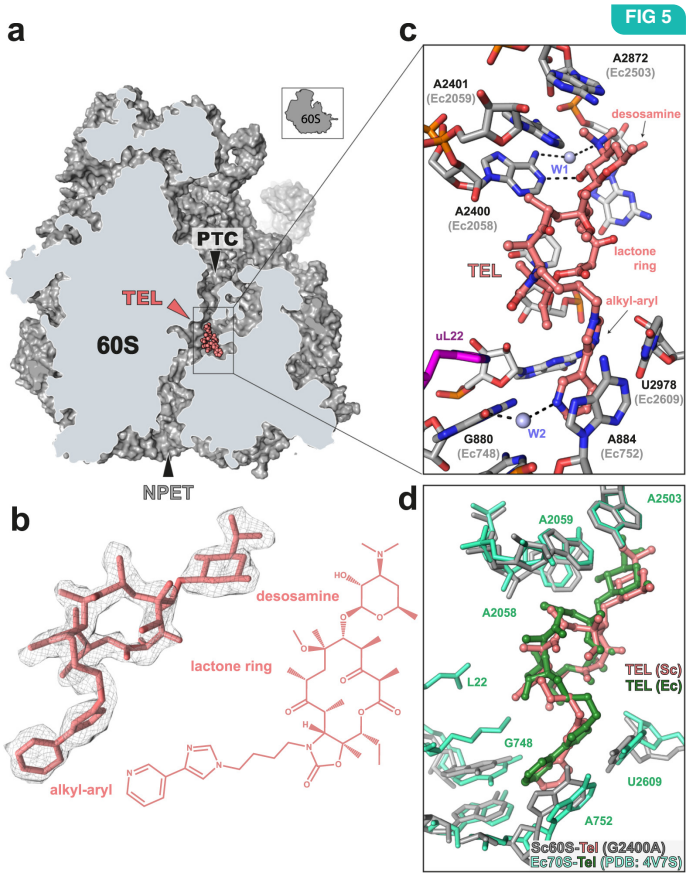


FIG 5 Cryo-EM structure of TEL bound to the yeast ribosome[2].

1. Kučera O., *et al,* (2021) Anillin propels myosin-independent constriction of actin rings, *Nature Comm.* doi: 10.1038/s41467-021-24474-1
2. Svetlov, M.S. *et al,* (2021) Context-specific action of macrolide antibiotics on the eukaryotic ribosome, *Nature Comm.* doi: 10.1038/s41467-021-23068-1

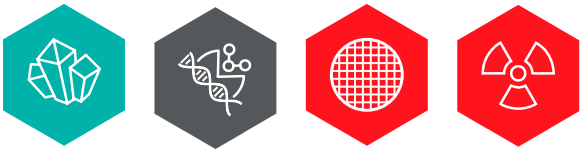
INSTRUCT CENTRE EMBL

The European Molecular Biology Laboratory (EMBL) is Europe's flagship laboratory for the life sciences. Instruct Centre EMBL is made up of three sites. EMBL Heidelberg, EMBL Hamburg, and EMBL Grenoble.

EMBL Heidelberg specialises in high-resolution cryogenic electron microscopy (cryo-EM) of biological samples, supporting users for single-particle and tomography projects.

EMBL Hamburg focusses on state-of-the-art structural biology methods using synchrotron radiation. EMBL Hamburg operates three beamlines on the PETRA III high-brilliance synchrotron: one dedicated to biological small-angle X-ray scattering and two for macromolecular crystallography.

EMBL Grenoble is one of the largest platforms for high-throughput crystallisation screening in Europe. The HTX lab offers automated protein-to-structure and fragment screening crystallography pipelines, including screening and structural characterisation of ligand-target complexes.



Instruct Centre Lead Scientists

Christoph Müller	Simone Mattei
Matthias Wilmanns	Thomas Schneider
Stephen Cusack	Jose A. Marquez

NEW TECHNOLOGIES

EMBL Heidelberg – the new EMBL Imaging Centre opens for users

In December 2021 the new EMBL Imaging Centre opened its doors to visits from external users. The centre will be the home of the EMBL Heidelberg Instruct Centre (IC).

Today, outstanding advances in imaging technologies enable us to directly visualise the molecular machinery of life at an unprecedented level of detail. Cutting-edge microscopy is becoming a central technology platform for the life sciences. However, only a minority of scientists have access to the latest imaging technologies as the devices are expensive and complex in their set-up, maintenance and usage.

Motivated by this, the IC will provide users access to state-of-the-art cryo-EM equipment for structure determination projects using the latest technology and methods in single-particle analysis and cryo-electron tomography (cryo-ET), including academically developed methods not yet commercially available.

The EMBL Imaging Centre hosts a Titan Krios G4 and Glacios microscope. The Titan Krios is a 300 kV transmission electron microscope (TEM) with a three-condenser lens system. It allows for high-end single-particle and tomography data acquisition using a cold field emission gun (C-FEG), SelectrisX energy filter, Falcon 4 electron counting camera and volta phase plate. It's configured for high-throughput automated single particle and tomography data acquisition.

The Glacios from Thermo Fisher Scientific is a 200 kV transmission electron microscope with a two-condenser lens system. It's equipped with an autoloader system which allows for quick screening of up to 12 cryo-EM samples. The system also features the latest SelectrisX energy filter and Falcon 4 electron counting camera which enables high-quality data collection for suitable single particle samples.

The facilitie's EM engineers and application specialists will help and support with cryo-EM data collection to tackle a broad range of biological questions and projects based on single-particle analysis and cryo-electron tomography for high-resolution structural determination of macromolecular complexes. The Imaging Centre is accessible for in person visits, or can be accessed fully remotely.

FIG 1. EMBL Imaging Centre. Credit: EMBL Photolab Kinga Lubowiecka.

EMBL Grenoble - new pipeline for the structural analysis of membrane proteins.

Membrane proteins are central to many physiological processes, and represent attractive targets for drug discovery programmes. Yet they remain very difficult to analyse structurally. Moreover, high-throughput structure-based drug discovery has not yet been fully exploited for membrane proteins because of lack of automation. The Marquez Team in collaboration with Sebastien Granier (IGF, Montpellier) have sought to remedy this by developing a versatile and easy to use platform for in meso membrane protein crystallisation, enabling rapid atomic structure determination at both cryogenic and room temperatures. Using the CrystalDirect method as a support for LCP crystallisation, the pipeline combines high-throughput microcrystal soaking, automated laser-based harvesting, and serial crystallography, enabling screening of small-molecule libraries with membrane protein crystals grown in meso (Fig. 2).

This new pipeline is integrated into the web-based Crystallographic Information Management System (CRIMS). This system allows automated tracking of crystallographic experiments and web-based access to experimental design and evaluation tools as well as shipment to and recovery of processed data from synchrotrons. This approach brings needed automation to this important class of drug targets and enables high-throughput structure-based ligand discovery with membrane proteins. This resource is open to scientists through Instruct-ERIC and other access programs, and could be replicated elsewhere.

FIG 2

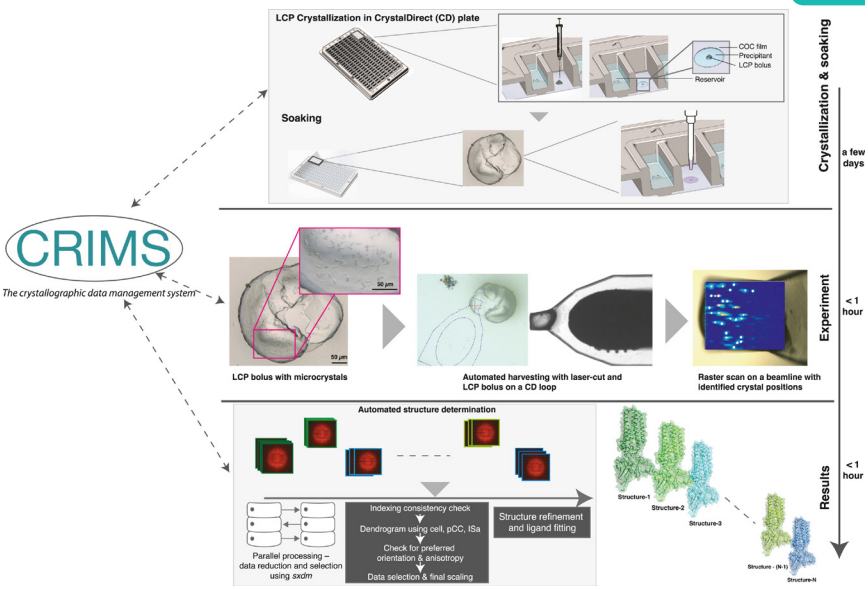


FIG 2. A pipeline for high-throughput ligand discovery with membrane protein crystals. After LCP crystallisation in CrystalDirect plates, crystals can be directly accessed by removal of the top plastic seal on the plate (top row). Soaking solutions are introduced into the experiment by a pipetting robot, after which the plate is resealed and incubated for the necessary time (top row). After soaking, LCP crystals are auto-harvested with the CrystalDirect robot into pins and stored in UniPucks for shipment to the synchrotron (middle row, "Experiment"). Serial diffraction data are collected for each uniquely soaked bolus and merged after established protocols before structure refinement and ligand fitting (bottom row, "Results"). The automated data flow in the pipeline, shown by double-arrowed dashed lines, is maintained through the CRIMS, through which users can access, monitor, and analyse data via web interfaces.

The joint EMBL Grenoble - ESRF structural Biology group (JSBG) is currently involved in the installation of a CrystalDirect harvester on the ID30A-1/MASSIF-1 beam line to enable plate-to-beam data collection modes, and in the upgrade of ID29 to a dedicated high brilliance serial synchrotron crystallography beam line for challenging dynamic structural biology projects. User access to CrystalDirect at MASSIF1 and to the ID29 beam line is expected to start in summer 2022.

SCIENCE HIGHLIGHTS

Very high-resolution structures by cryo-EM of Mycobacterium tuberculosis DNA Gyrase in complex with DNA and inhibitors.

DNA gyrase is an essential enzyme in bacteria but despite extensive structural studies the detailed architecture of the Mycobacterium tuberculosis full-length enzyme was still lacking when we started this work, and thus the molecular specificities and conformational intermediates of this highly flexible macromolecule. Cryo-EM allowed us to finally obtain snap-shot structures of various conformational states of this nanomachine and also high-resolution maps of the cleavage core in complex with DNA and inhibitors (Fig. 3). Such maps allow a detailed understanding of small-molecule coordination. Taken together, the data open exciting new avenues for structure-based drug design.

FIG 3

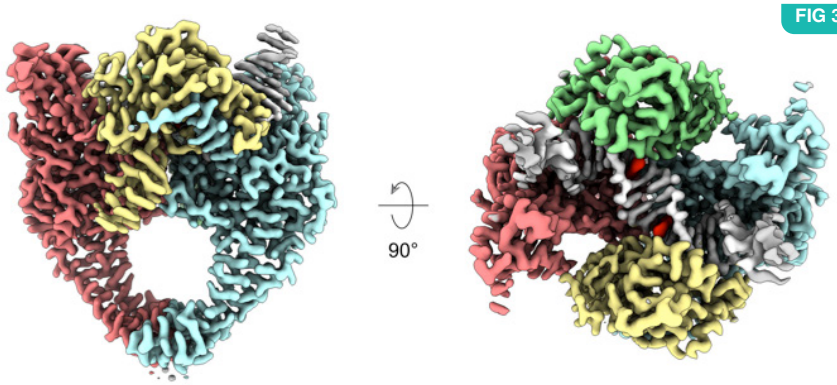


FIG 3. 2.65-Å resolution structure of the cleavage core in complex with DNA (in grey) and 2 inhibitor-molecules (in red).

1. Healey R.D. *et al.* (2021). An automated platform for structural analysis of membrane proteins through serial crystallography. *Cell Rep Methods.* doi: 10.1016/j.crmeth.2021.100102
2. Cornaciu I. *et al.* (2021). The Automated Crystallography Pipelines at the EMBL HTX Facility in Grenoble. *J Vis Exp.* doi: 10.3791/62491.

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

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Francisco Javier Chichón
Javier Collado Ávila

The Instruct Image Processing Center supports Instruct users whose projects are approved by Instruct Access committee. An image processing specialist is assigned to each project and the reconstructed map along with the whole image processing workflow followed to reach it and reproduce it is returned to the user. Along the process, multiple interactions with the user help to focus the analysis and obtain optimal results. The lab is also open to internships through Instruct so that the incoming visitor learns the image processing techniques and brings them back to thier home laboratory. The I2PC also develops infrastructure software to automate and facilitate the image processing data analysis in the Structural Biology community. Our software is open-source and serves thousands of structural projects worldwide.

The CryoEM CNB–CSIC facility provides expertise to researchers with samples in the first stages of characterisation. Samples are first analysed by negative staining in search of the best conditions (pH, ionic strength, etc). Once found, samples are subjected to different vitrification conditions in search of the best parameters, which are then tested in a cryomicroscope FEI 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.

With the recent installation of a JEOL CryoARM300 300 kV equipped with a Gatan K3 electron direct detector and an Omega energy filter, the facility will be in the position of acquiring large data sets and to perform cryoelectron tomography.

FIG 1

FIG 1. Instruct Centre-ES

NEW TECHNOLOGIES

During 2021 the Instruct Image Processing Center has been working on the development of automatic workflows that allows on-the-fly processing of the acquired data and give immediate feedback to the facility operator in order to maximise the microscope throughput.

The CryoEM CNB– CSIC facility has established a solid remote access system and most of the jobs are carried out this way. Besides, a YouTube channel has been created to cover the highlights of the facility and facilitate the visualisation of the facility outcomes. This comes together with a streaming data transfer (secure-rsync direct connection with the Center) and Globus transfer system.

Instruct Centre ES 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

SCIENCE HIGHLIGHT - INTERNSHIP

Structural intermediates of the CHIKV nsP1 capping pathway

This scientific highlight corresponds to an **Instruct-ERIC Internship** at the CryoEM CNB-CSIC facility. In this context Dr. Rhian Jones, visited us from October 18, 2021 to January 31, 2022. In this project the applicant aimed to structurally characterise key intermediates in the viral system she was working with by cryo-EM.

Chikungunya virus is an emerging RNA virus of importance for global health. Viral replication, transcription, shut down of the cell translation and evasion from the innate immune system are essential processes for infection that are mediated mainly by four viral non-structural proteins (nsP 1 to 4) encoded by the virus.

NsP1 is responsible for the capping of the viral RNA and the applicants had recently reported the structure of the nsP1 capping rings by cryo-EM at 2.6 Å resolution describing the structural basis of membrane binding, oligomerisation and the allosteric activation of the capping reaction4. NsP1 caps in a three-step reaction, first methylating the GTP with a SAM methyl donor cofactor to form SAH and m7GTP, and then covalently linking m7GMP to the histidine residue H34 for subsequent transfer to a 5' diphosphate viral RNA.

At the CNB, the two Instruct facilities have been working:

- The Electron Microscopy Sample Characterisation facility has prepared samples and collected datasets for the SAH and m7GTP substrate bound, m7GMP covalently linked and RNA bound complexes to capture these intermediates of reaction.
- The Instruct Image Processing Center, I2PC has been working on the image processing helping them to maximise the extraction of biological knowledge from the electron microscopy images using the SCIPION package, including movie alignment, particle picking, classification, volume reconstruction, … (Fig 2).

Thanks to this Instruct project, the applicants have begun to understand the role of different residues in the active site on the configuration of the active site, and the dynamics required for the reaction to occur. These represent a very important asset for antiviral design targeting the viral capping reaction.

FIG 2

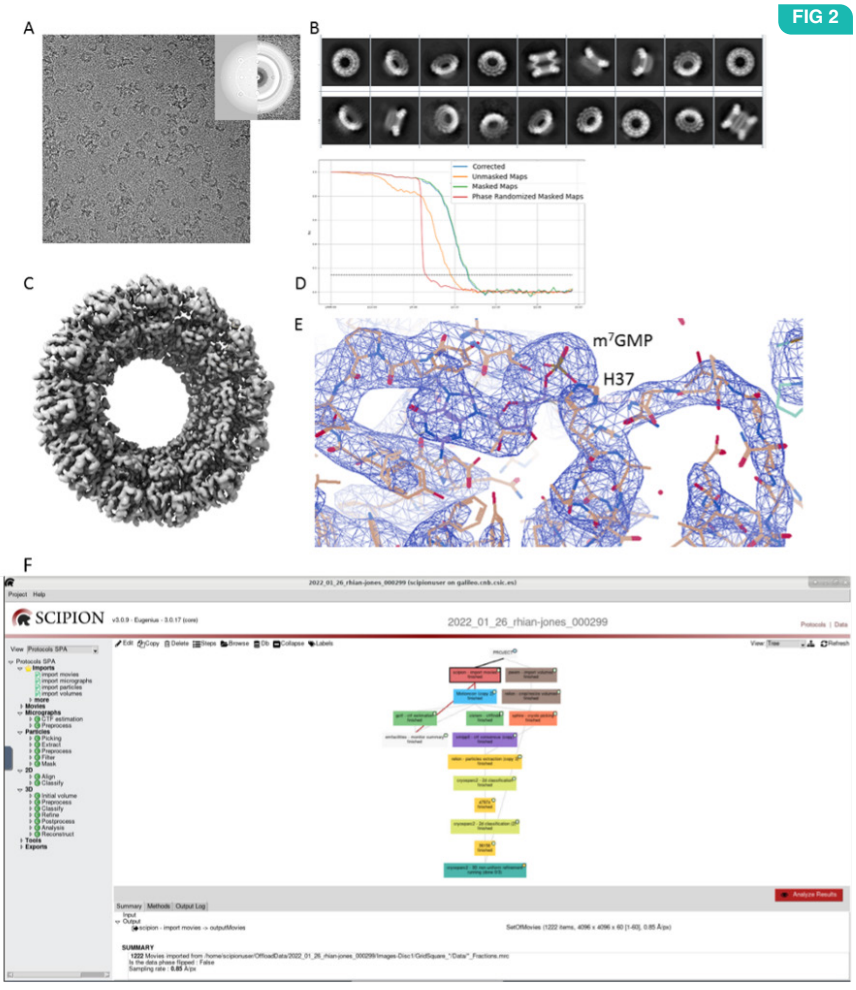

FIG 2. Representative data for the nsP1-m7G covalent complex. **(a)** Representative micrograph recorded at 1.8um defocus, with calculated CTF inset. **(b)** Class averages of the data taken forward for further processing. **(c)** 3D reconstruction of the complex with **(d)** FSC demonstrating an overall map resolution of 2.9Å at the 0.143 threshold. **(e)** Maps with detail of one of the active site pocket showing an m7GMP ligand (purple) covalently linked to the catalytic histidine of the nsP1 main chain (orange). Maps were rendered in Coot and ChimeraX. **(f)** Image processing workflow using Scipion (http://scipion.i2pc.es/). In coloured boxes each of the steps used in the processing.

INSTRUCT CENTRE FI

Instruct Centre FI is formed by three Centers of Excellence at the Universities of Helsinki, Oulu, and Eastern Finland. The Centre provides expert-supported user access in state-of-the-art sample preparation, characterisation, and structural biology techniques.

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Minna Poranen
Hideo Iwai
Markku Varjosalo
Lari Lehtiö
Lloyd Ruddock
Juha Rouvinen
Janne Jänis

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.

NEW TECHNOLOGIES

New blot-free vitrification device a Cryosol “Vitrojet”

Irreproducibility in cryo-EM grid preparation can be a source of inefficiency in material usage, sample denaturation at the air-water interface and extended imaging time on the microscope. Blot-free vitrification methods have been recently developed, that can potentially overcome some of these issues. We have recently installed a Cryosol Vitrojet (University of Helsinki, Fig. 1), a new blot-free vitrification instrument as the first within the Instruct-ERIC centres and the second in Europe. With this cutting-edge development, sample preparation allowing the production of homogeneous grids from small sample volumes should be greatly improved as only 1 µL of sample is required to make up to 12 grids. The service will be offered as part of the sample screening service in early 2022.

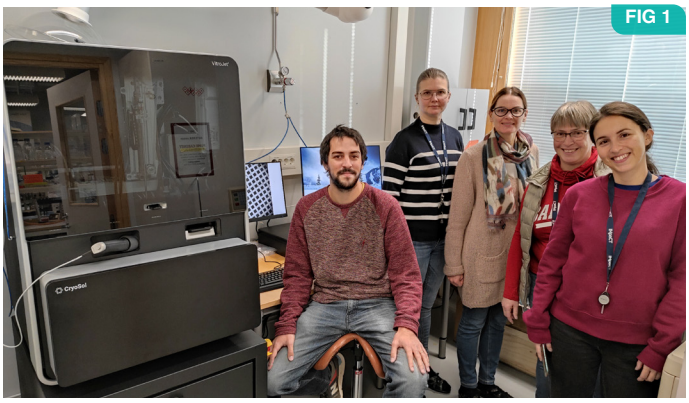


FIG 1

New design dispersion inlet channel for asymmetric flow field-flow fractionation (AF4)

The new AF4 multidetection platform was installed at the Biocomplex, University of Helsinki at the end of 2020 and the service launched in 2021. Various sample types e.g., nucleic acids, proteins and their complexes, viruses and virus-like particles as well as extracellular vesicles have been analysed so far. The latest development in the AF4 service is a new design dispersion inlet channel (Fig. 2) for sample separation that allows the analysis of sticky samples as the focusing step is omitted.

FIG 2

High-resolution fractionation and profiling option for ultracentrifugation gradients

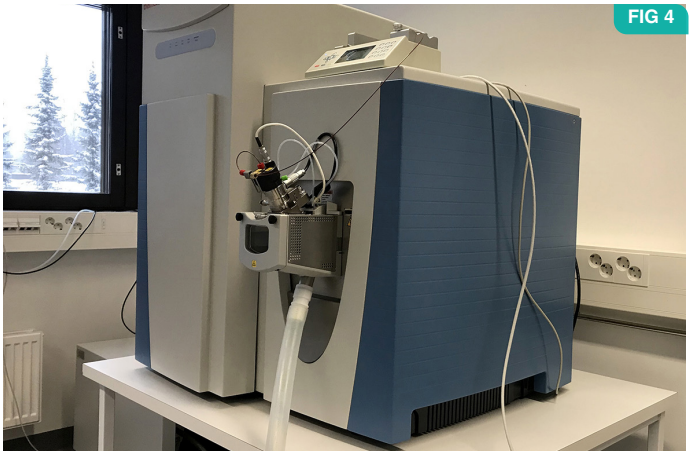
Biocomplex preparative ultracentrifugation facility obtained a BioComp Piston Gradient Fractionator with Triax three wavelength flow cell for UV (A260 and A280) and fluorescence (eGFP or Cy5) profiling (Fig. 3). The new instrument enables automated fraction collection coupled to UV and fluorescence data collection for sample components separated by ultracentrifugation.

FIG 3

Ultra-high mass range mass spectrometer for native mass spectrometry

The new Thermo Scientifc Q Exactive UHMR Orbitrap mass spectrometer (Fig. 4) was installed at native mass spectrometry facility at the University of Eastern Finland. The instrument has very high sensitivity and high mass resolution for larger proteins and large protein assemblies such as viruses (up to 20 MDa). It is especially suitable for studying large protein complexes, protein-protein, and protein-ligand interactions.

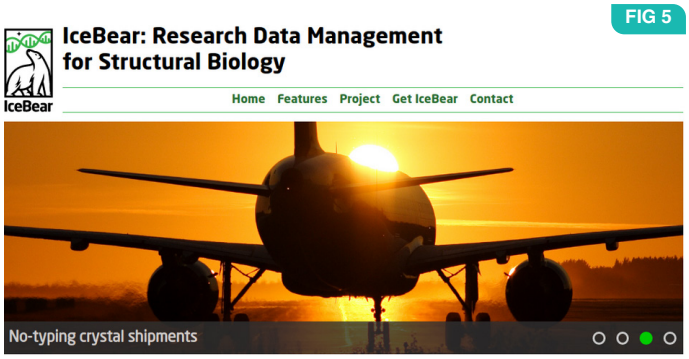
FIG 4



Cloud version of the data management software IceBear

The software package IceBear (Integrated Crystal data-tracking Enhancing Biochemistry Education And Research, Fig. 5) includes now sample meta data transfer with the ISPyB database used at the DLS, MAXIV and ESRF synchrotrons. New features, including links back to IceBear from ISPyB EXI and transfer of data collection statistics to IceBear, have been implemented through collaboration of the MAXIV team. A new cloud version of IceBear is now in use at the University of Oulu and the cPouta ELIXIR cloud environment of CSC - IT Center for Science. As cPouta environment is within the high-performance computing facility, it will allow development of new features such as an automated image scoring to help the users in the future. IceBear system has also been described in a publication[1]. This work was supported by the EOSC-Life project.

FIG 5



SCIENCE HIGHLIGHT - R&D AWARD

Raman/Fluorescence Tweezers Microspectroscopy (RFTM) for single bio-nanoparticle quality control

The fastest growing sector in nanomedicine is drug delivery based on bio-nanoparticles. However, the development of bio-nanoparticles is challenging and delayed by the lack of fast and rigorous in-house quality control protocol at single-particle level. The R&D pilot project on Single-Particle Raman/Fluorescence Tweezers Microspectroscopy technology addresses this issue by providing a quality control of individual bio-nanoparticles via their biomolecular composition. To set-up and validate the sensitivity and performance of the method, highly purified membrane-containing dsDNA bacteriophage PRD1 and genome-less PRD1 Sus1 mutant particles were purified at the Biomolecular Complex Purification facility at the University of Helsinki / Instruct FI. The optical trapping of single particles with subsequent recording of Raman spectra from the trapped bioparticles, was developed and optimised. The presence of nucleic acid inside a single bioparticle was assessed from the recorded Raman spectra using Biomolecular Component Analysis, which was based on measured spectra from protein shell, internal membrane and DNA constituting PRD1 bacteriophage. The DNA concentration inside bioparticle can be directly estimated from the fitting coefficient of corresponding spectral component, which is in turn proportional to the DNA spectrum area. The limits of RFTM method sensitivity with respect to DNA concentration, number of trapped bioparticles, and experiment duration were evaluated. The project is a joint effort of Sergei Kruglik, Sorbonne University, France; Juan Manuel Falcon and Nicola Abrescia, CIC bioGUNE, Spain with Instruct FI.

FIG 6

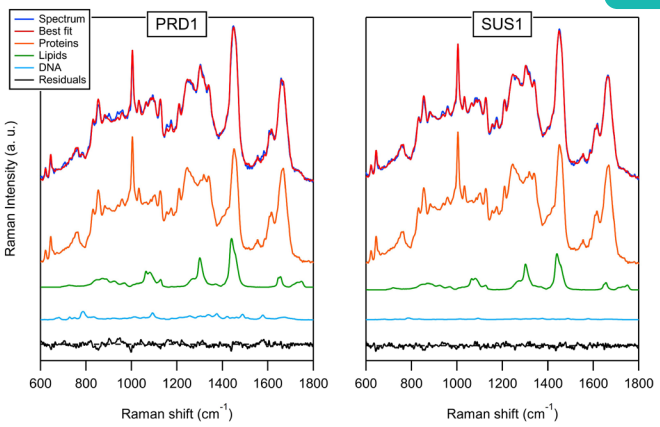


Fig. 6 Raman spectra of a single PRD1 (dark blue curve, left panel) and genome-less Sus1 particle (right panel), with the non-linear least-square best fit (red) based on quantitative spectral decomposition into proteins (orange), lipids (green) and DNA (blue) contributions, using Biomolecular Component Analysis. Spectra were recorded using Raman Tweezers Microspectrometer built at Laboratory Jean Perrin, Sorbonne University.

FIG 7

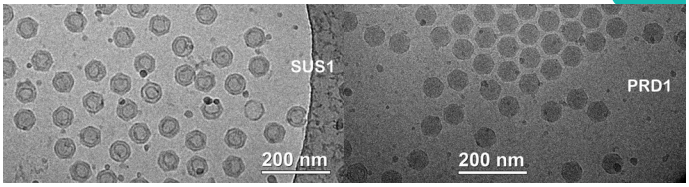


Fig. 7 Cryogenic transmission electron microscopy images of PRD1 and Sus1 samples. Images were recorded using JEOL2100 microscope and Gatan 626 liquid-nitrogen cooled cryo-holder, at IMPMC Institute, Sorbonne University.

1. Daniel, E., *et al.* (2021) IceBear: an intuitive and versatile web application for research-data tracking from crystallization experiment to PDB deposition. *Acta Cryst. D.* doi: 10.1107/S2059798320015223

INSTRUCT CENTRE FR1

The Instruct-Centre FR1 at Strasbourg hosted in the Center of Integrative Biology (CBI), located on the IGBMC site at Illkirch/ Strasbourg, which is also the coordinating Centre for FRISBI (French Infrastructure for Integrated Structural Biology), 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

New cryogenic transmission electron microscope

Cryo-EM is constantly pushing the frontier of imaging in life science and structural biology and opens new opportunities to study challenging biological structures, or tackle difficult target for drug discovery. To answer the growing demand of the scientific community for access to state of the art equipment, a cryogenic Glacios electron microscope has been installed to reinforce and complement the Titan Krios. This new generation of instruments features a highly coherent XFEG operated at 200 kV and is equipped with a Gatan K2 Summit direct electron detector. The Autoloader ensure an easy, contamination free sample transfer from the Glacios to the Titan Krios, allowing robust Single Particles analysis (SPA) or tomography workflows from grid quality screening to high resolution, high throughput data collection on both microscopes. The Glacios also features a CETA-D camera, optimised for micro-electron diffraction data collection.

FIG 1

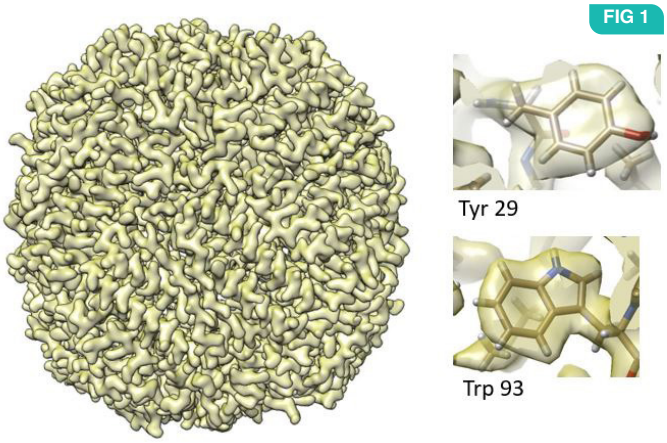


FIG 1. Cryo-EM map of apoferritin at 2.21 Å resolution recorded on the new Glacios microscope. Inserts show the well resolved tyrosine and tryptophan side chains.

Affinity grids for single particle cryo EM

The streptavidin Affinity Grid (SAG) is a unique tool for cryo-EM sample preparation that takes advantage of controlled sample immobilisation to image challenging biological macromolecules. SAGs are composed of a holey carbon support on which a 2D streptavidin crystal was grown, which is still able to bind biotin, as well as a variety of peptide tags, with high affinity. The SAG captures biotinylated/tagged targets and concentrates dilute samples on an EM support for imaging, sample distribution and orientation are controlled and only tagged species are specifically retained. Most importantly, the macromolecules are protected from the adverse effects of the air-buffer interface such as surface denaturation.

FIG 2

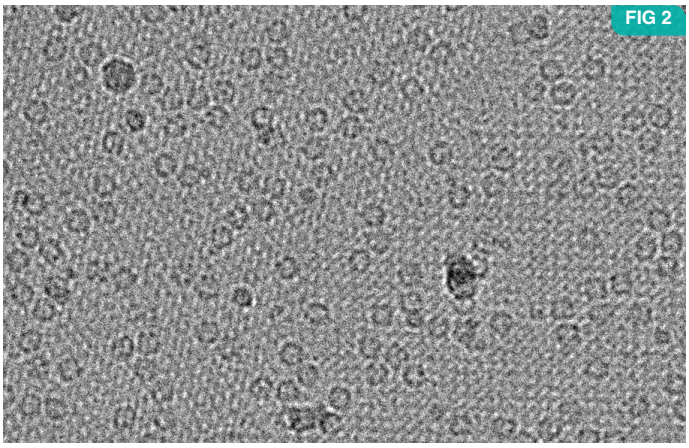


FIG 2. Two-dimensional streptavidin crystal coated with biotinylated apoferritin.

HR-Bac, a toolbox based on homologous recombination for expression, screening and production of multiprotein complexes using the baculovirus expression system[1]

The Baculovirus/insect cell expression system is a powerful technology for reconstitution of eukaryotic macromolecular assemblies. Most multigene expression platforms rely on Tn7-mediated transposition for transferring the expression cassette into the baculoviral genome. This allows a rigorous characterisation of recombinant bacmids but involves multiple steps, a limitation when many constructs are to be tested. For parallel expression screening and potential high throughput applications, we have established an open source multigene-expression toolbox exploiting homologous recombination, thus reducing the recombinant baculovirus generation to a single-step procedure and shortening the time from cloning to protein production to 2 weeks.

The HR-bac toolbox is composed of a set of engineered bacmids expressing a fluorescent marker to monitor virus propagation and a library of transfer vectors. They contain single or dual expression cassettes bearing different affinity tags and their design facilitates the mix and match utilisation of expression units from Multibac constructs. The overall cost of virus generation with HR-bac toolbox is relatively low as the preparation of linearised baculoviral DNA only requires standard reagents.

FIG 3

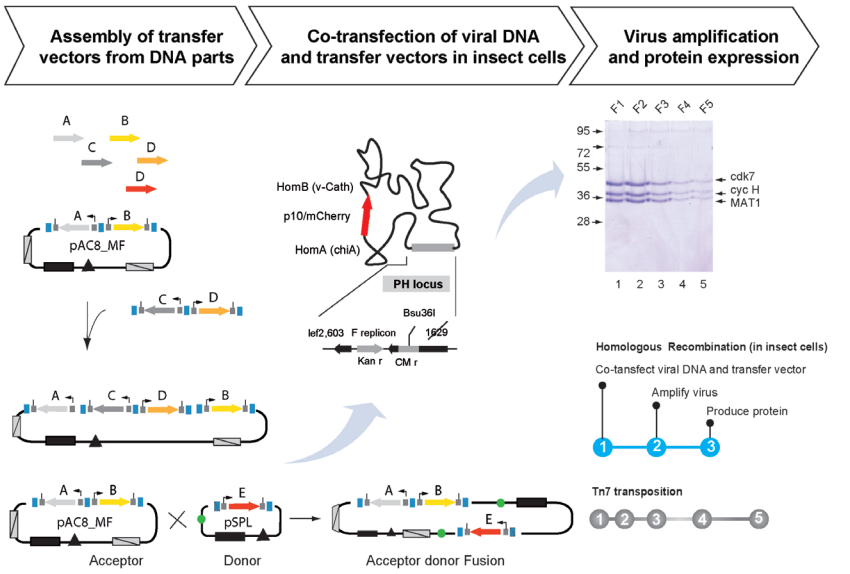


FIG 3. The HR-Bac toolbox

SCIENCE HIGHLIGHTS

NLRP3 decamer bound to the cytokine release inhibitor CRID3[2]

FIG 4

NLRP3 is an intracellular sensor protein whose activation by a broad spectrum of exogenous and endogenous stimuli leads to inflammasome formation and pyroptosis. The conformational states of NLRP3 and the way antagonistic small molecules act at the molecular level remain poorly understood. Here we report the cryo-electron microscopy structures of full-length human NLRP3 in its native form and complexed with the inhibitor CRID3 (also named MCC950). Inactive, ADP-bound NLRP3 is a decamer composed of homodimers of intertwined LRR domains that assemble back-to-back as pentamers. The NACHT domain is located at the apical axis of this spherical structure. One PYD dimer is additionally formed inside the LRR cage. Molecular contacts between the concave sites of two opposing LRRs are mediated by an acidic loop extending from an LRR transition segment. Binding of CRID3 significantly stabilises the NACHT and LRR domains relative to each other, allowing structural resolution of 3.8-4.2 Å. CRID3 binds into a cleft, connecting four subdomains of the NACHT with the transition LRR. Its central sulfonylurea group interacts with the Walker A motif of the NLRP3 nucleotide-binding domain and is sandwiched between two arginines, explaining the specificity of NLRP3 for this chemical entity. With the determination of the binding site of this lead therapeutic, specific targeting of NLRP3 for the treatment of autoinflammatory and autoimmune diseases and rational drug optimisation are within reach[2].

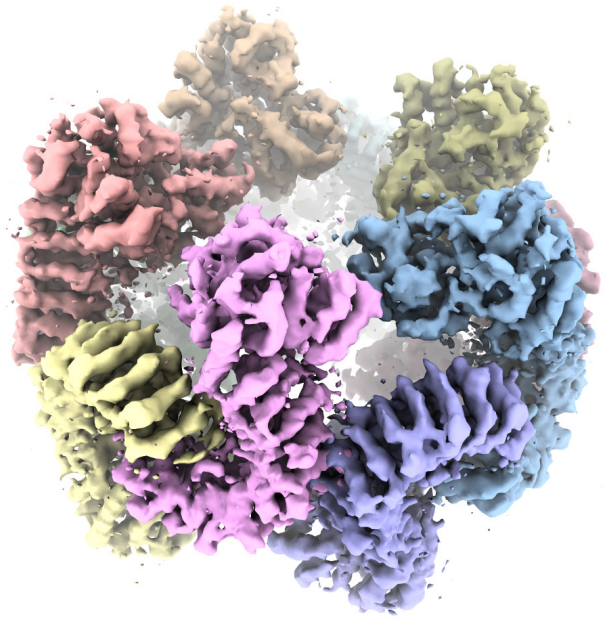


FIG 4. Cryo-EM map of the NLRP3 decamer

1. Kolesnikova O., *et al.* (2022) HR-Bac, a toolbox based on homologous recombination for expression, screening and production of multiprotein complexes using the baculovirus expression system. *Sci Rep.* doi: 10.1038/s41598-021-04715-5
2. Hochheiser, I.V., *et al.* (2022) Structure of the NLRP3 decamer bound to the cytokine release inhibitor CRID3. *Nature.* doi: 10.1038/s41586-022-04467-w

INSTRUCT CENTRE FR2

Instruct Centre FR2 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 provides 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 our platforms follow a Quality Assurance programme, managed by a full-time quality engineer, and are certified ISO 9001 NFX 50-900.

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

NEW TECHNOLOGIES

Super-resolution microscopy for structural biology

In the last year, we have purchased and installed a commercial super-resolution system at the Instruct FR2 imaging platform in the IBS. Following the recruitment of Dr. O. Glushonkov with funding from the French Infrastructure for Structural Biology (FRISBI), the system is now open for user access. The Abbelight microscope offers exclusive multi-colour 3D super-resolution imaging in STORM, PALM and PAINT modalities, with single-particle tracking capabilities. The attainable resolution of Single Molecule Localisation Microscopy (SMLM) is in the range of tens of nanometres which bridges conventional fluorescence microscopy techniques with structural approaches. The data we obtain is fitted for image reconstruction and can be further used to retrieve additional information during post-processing steps such as segmentation, clustering, and co-localisation analysis. Typically, users are structural biologists who wish to understand how their systems function at the sub-cellular level, by analysing the locations, dynamics and potential interactions of their proteins.

FIG 1

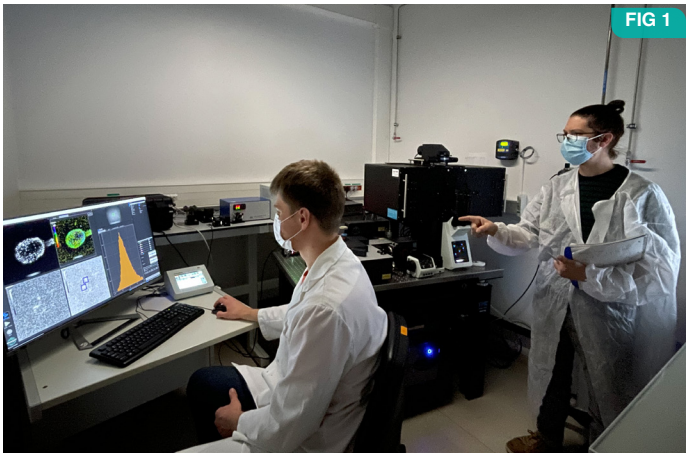


FIG 1. Super-resolution microscopy at the Instruct FR2 imaging platform on the new Abbelight microscope with O. Glushonkov and a user.

Anaerobic freezing of oxygen sensitive proteins for cryo-electron microscopy.

Some proteins, such as those containing iron-sulfur centres, are sensitive to oxygen and their activity or structure can be damaged when exposed to air. Studying them by cryo-EM can therefore be problematic as the freezing of the grids takes place in the laboratory atmosphere. In collaboration with the IBS metalloproteins group of Dr. Y. Nicolet, we have developed a protocol to freeze cryo-EM grids under anaerobic conditions using a Vitrobot installed in an oxygen-free glove box. Proof-of-principle results were obtained on two complexes: one insensitive to oxygen: apoferritin, and another that is sensitive: pyruvate ferredoxin oxidoreductase (PFOR). For apoferritin, the quality of the freezing and the absence of contamination resulted in a high-resolution 3D structure (2.4 Å) on the Glacios of the FR2 centre. For the second

FIG 2

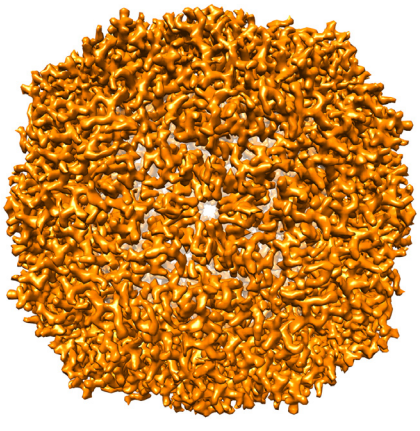


FIG 2. T3D structure of the Apoferritin obtained under anaerobic conditions

complex, which is dimeric, we obtained a structure at 2.9 Å resolution that is very similar to one obtained 20 years ago by X-ray crystallography showing the well-preserved iron-sulfur centres. A paper summarising these results has been published and the experimental setup will be accessible via the platform.

SCIENCE HIGHLIGHTS

Pre-miRNA-149 G-quadruplex as a molecular agent to capture nucleolin[2]

One of the most significant challenges in capturing and detecting biomarkers is the choice of an appropriate biomolecular receptor. Recently, RNA G-quadruplexes emerged as plausible receptors due to their ability to recognise with high-affinity their target proteins. One of the main obstacles in these studies has been to produce the proteins necessary to complete structural and biological studies. To that end, the group of Carla Cruz at CICS-UBI submitted an application to the Instruct FR2 cell-free expression platform (PID: 10168 "Production of the full-length nucleolin for structural studies"), where the expression of nucleolin was performed by L. Imbert in remote access mode due to the restrictions on travel. The availability of this challenging purified protein permitted unveiling the interaction between nucleolin and the RNA G-quadruplex structure of pre-miRNA-149 in their recent publication. Furthermore, this proof-of-concept study successfully used microfluidics for the capture of nucleolin and could open new opportunities to detect nucleolin in complex samples.

FIG 3

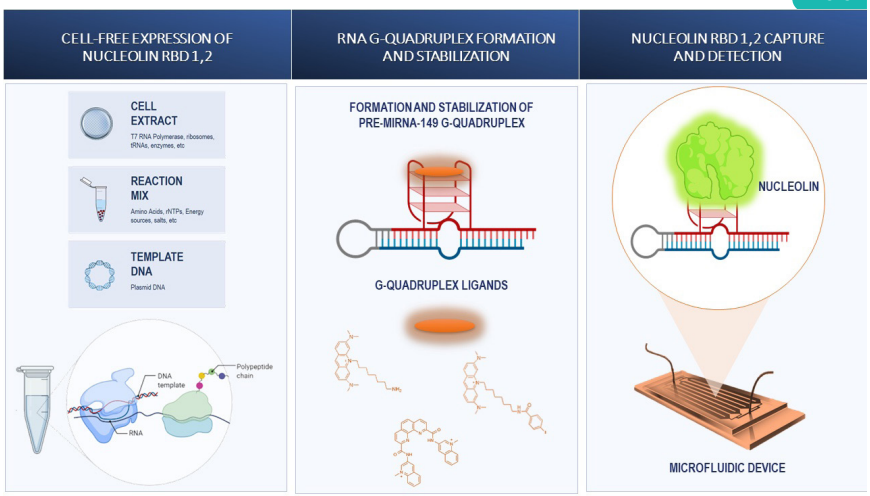
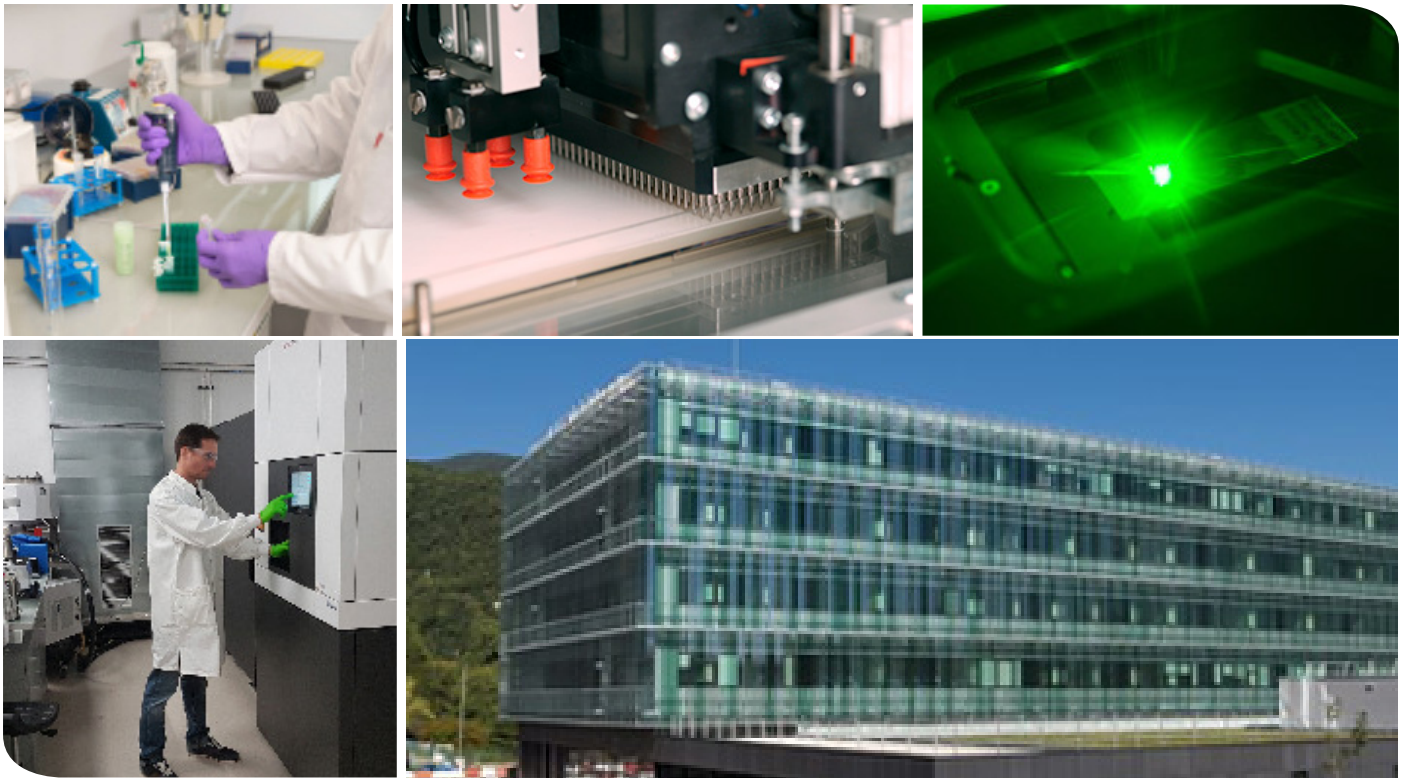


FIG 3. Studies using nucleolin expressed at the Instruct FR2 cell-free platform by Portuguese user, Carla Cruz. The nucleolin was then used to study the interaction with RNA G-quadruplexes.



1. Cherrier M.V., *et al.* (2022) Oxygen-Sensitive Metalloprotein Structure Determination by Cryo-Electron Microscopy. *Biomolecules.* doi: 10.3390/biom12030441

2 Santos T.,*et al.* (2022) C. Pre-miRNA-149 G-quadruplex as a molecular agent to capture nucleolin. *Eur J Pharm Sci.* doi: 10.1016/j.ejps.2021.106093

INSTRUCT CENTRE IL

The Israel Structure Proteomics Center (ISPC) at the Weizmann Institute of Science was established 18 years ago through a large financial contribution of the Israel Ministry of Science and Technology.

Instruct Centre Lead Scientists

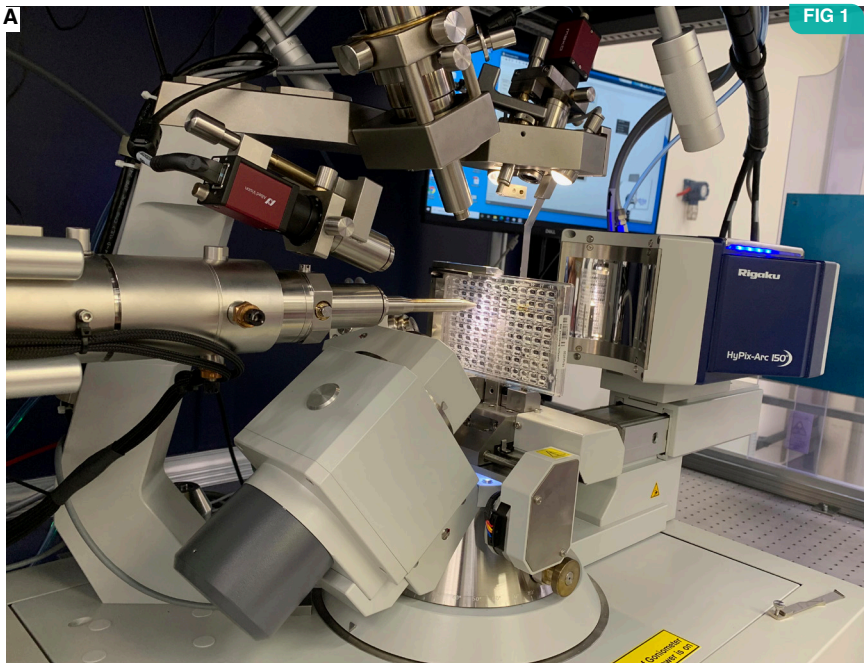
Shira Albeck
Yoav Peleg
Tamar Unger
Orly Dym

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. Michal Sharon 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 Center for implementing all steps of the pipeline from gene to the 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 and biotech/pharma companies in Israel and its Instruct-ERIC partners; it also offers training and consultation for students and staff.

NEW TECHNOLOGIES

Liquid-metal-jet (LMJ) X-ray diffraction system in action

Last year the liquid-metal-jet (LMJ) X-ray diffraction system was installed at the Structural Proteomics Unit (SPU) (Fig. 1A). This system produces unprecedented brightness and is considered 'state-of-the-art' in-house X-ray system. This has 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 high intensity and therefore greatly reduces a whole data set collection time. In the last year the lab collected high resolution (1.5-2.5 Å) data sets of few protein crystals some in the presence of ligands which might contribute to the development of drugs (Fig. 1B).



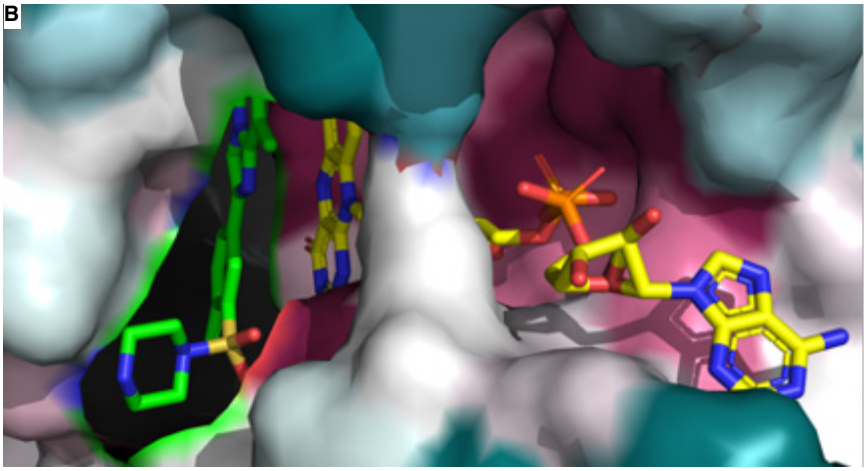


FIG 1. (A) Rigaku Liquid Metal Jet (LMJ) Synergy System with an Excillum LMJ source and Rigaku HyPix Arc 150°-pixel detector operating at the Weizmann Institute Instruct-ERIC Center. **(B)** Ligand binding to Quinone Reductase 2 (hQR2) (paper in preparation).

SCIENCE HIGHLIGHTS

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

The PROSS algorithm for improving protein stability and heterologous expression levels has been applied to a range of challenging enzymes, receptor binding proteins and vaccine immunogens. The PROSS algorithm is as a stand-alone tool for protein scientists with no or limited experience in modelling[1]. To probe PROSS's generality, ISPC launched a community effort by 12 labs that are part of the Protein Production and Purification Partnership in Europe (P4EU) (https://p4eu.org). Each lab selected 1-4 protein target(s) for protein expression for a total of 14 different targets across the benchmark, without support from the PROSS developers. Up to six automatically generated designs were evaluated in each case for expression, stability, and, in some cases, protein function. In eleven cases, the designs exhibited increased heterologous expression levels and/or thermal stability relative to the parental protein. In addition, in six out of seven proteins in which activity was tested, a conservation of activity was observed. In two prime examples, the human Stem Cell Factor (hSCF) and human Cadherin-Like Domain (CLD12) from the RET receptor, the wild type proteins were not expressible as soluble proteins in E. coli, yet the PROSS designs exhibited high expression levels in E. coli and HEK293 cells, respectively, and showed improved thermal stability. This study demonstrates the strengths of community-wide efforts to probe the generality of new methods and recommends areas for future research to advance practically useful algorithms for protein science. See Fig. 2 for schematic illustration of the PROSS algorithm.

The benchmark study was coordinated from the Weizmann Institute (by Dr. Yoav Peleg). The PROSS developers (Dr. Adi Goldenzweig and Prof. Sarel Fleishman) were involved only in the analysis of the results. The results of the benchmark were recently published in the Journal of Molecular Biology[2].

FIG 2

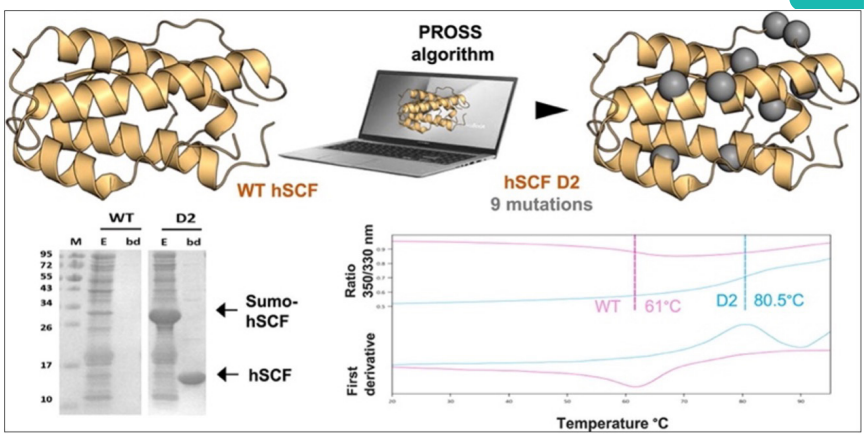


FIG 2. Use of PROSS to obtain high expression and stabilisation of proteins: (A) PROSS algorithm predicts several designs containing mutations which will stabilise the protein without altering activity and structure. (B) The expression levels of the designs (D1-D3) are tested by SDS PAGE. (C) DSF analysis of the D2 exhibits enhanced stability (T_m = 80.5°C) compared to the WT (T_m = 61°C).[1]

Instruct-IL uncovers role of metal ions in protein folding, structure and function[3]

Metal ions are fundamental to the folding and structure of proteins such as enzymes. Research from Silman et al (2021) has described a novel 4D binding motif for divalent metal ions in acetylcholinesterase (AChE), an enzyme that hydrolyses acetylcholine (ACh). The motif was first identified in TcAChE, purified from the electric organ of Torpedo californica. The study subsequently identified the motif in 31 AChE sequences, as well as in 28 butyrylcholinesterase (BChE) sequences.

The study, conducted at the Weizmann Institute, found that irreversible thermal inactivation of the enzyme was reduced by the presence of divalent metal ions, namely Mn^{+2}, Mg^{+2} and Ca^{+2} (Fig. 3). The three cations both strongly increase the thermostability of the TcAChE, and also the activation energy for denaturation. Mg^{+2} and Mn^{+2} also protect zebrafish AChE, which contains the 4D motif. However, the two divalent metal ions do not protect either human or electric eel AChE, both of which lack the motif.

X-ray crystallography was then used to visualise the exceptionally versatile motif, which is capable of binding 1-3 cations. In addition to the aforementioned 31 AChE sequences that exhibit the 4D motif, around 200 proteins were identified using the ASSAM programme, which contain the same 4D motif, many of which contain a divalent metal ion.

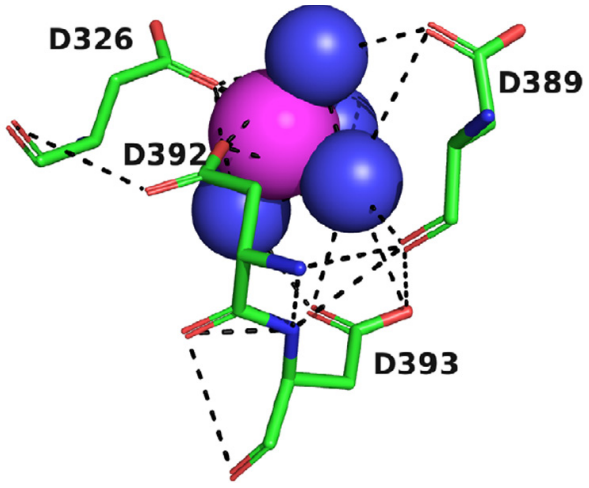


FIG 3. Crystal structure of the Mg^{+2} complex with TcAChE. The four Asp residues, D326, D389, D392, and D393, are shown as sticks, with carbons in green, oxygens in red, and nitrogens in blue. Waters are shown as blue spheres, and the magnesium ion as a magenta sphere. Hydrogen bonds and ionic bonds are shown as dashed black lines.

1. Goldenzweig A., *et al.* (2016) Automated Structure-and Sequence-Based Design of Proteins for High Bacterial Expression and Stability. *Mol Cell.* doi: 10.1016/j.molcel.2016.06.012
2. Peleg., *et al.* (2021) Community-Wide Experimental Evaluation of the PROSS Stability-Design Method. *J. of Mol. Biol.* doi: 10.1016/j.jmb.2021.166964
3. Silman I., *et al.* (2021) Torpedo californica acetylcholinesterase is stabilized by binding of a divalent metal ion to a novel and versatile 4D motif. *Protein Science.* doi: 10.1002/pro.4061

INSTRUCT CENTRE IT

The Magnetic Resonance Centre (CERM) of the University of Florence together with the Interuniversity Consortium CIRMMP constitute an infrastructure for Life Sciences, which 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 — Isabella Caterina Felli

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 structural 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 probes 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 to advancing methodologies and protocols for both solution and solid state NMR. Examples of these are the development of the 13C direct detection protocols for characterisation of challenging protein,[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 the 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

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 2020 and in 2021 has been fully operational for users (Figure 1). The 1.2 GHz NMR system is unique in its capabilities and is equipped with a triple-resonance inverse detection cryogenically cooled probehead with outstanding performances in terms of resolution.

As a response to travel restrictions imposed by the Covid-19 pandemic, CERM/CIRMMP engaged to optimise and widen NMR remote access modality. This was also the occasion to implement an Access Management Platform to facilitate all the steps necessary to access the infrastructure, starting from sample delivery to collection and storage of the data following the FAIR principles. The Platform is now fully operative.

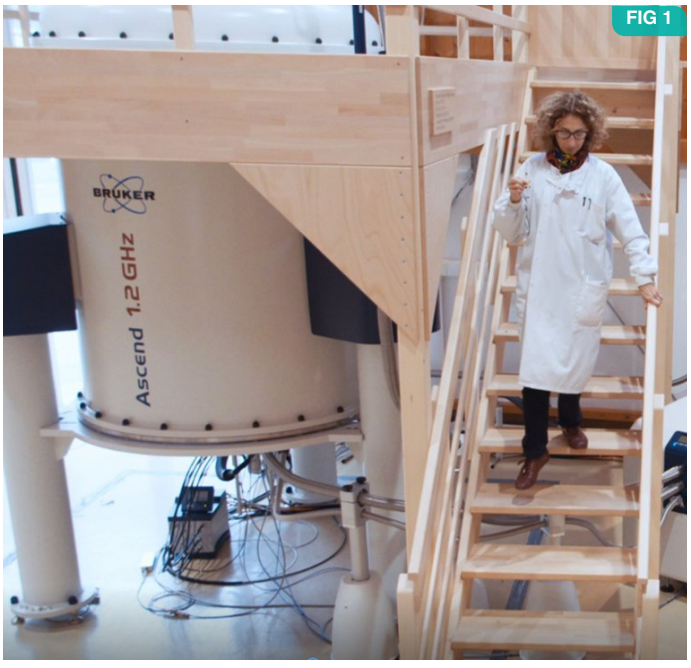


FIG 1. The new 1.2 GHz NMR spectrometer at Instruct-IT.

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

High Order structure (HOS) of "biologics"

CERM/CIRMMP scientists have developed new strategies for the structural characterisation and for the evaluation of the High Order structure (HOS) of "biologics". Together with the conventional approaches based on recording 1D 1H or 2D 1H-13C heteronuclear correlation for the HOS evaluation and comparability studies, already available at CERM/CIRMMP, now the integrative use of solution and solid-state NMR allows us to obtain a characterisation at atomic level of biomolecules, large PEGylated proteins and antigens in vaccine formulations and hydrogels. This methodology relies on the resonance assignment of the proteins achieved by recording 13C- and 1H-detected solid-state spectra, and by performing a comparative analysis of the spectra to obtain a map of the chemical shift variations. This approach has been successfully used to prove that the L-Asparaginase preserves its native folding after PEGylation and to obtain a semi-quantitative evaluation of the conjugation degree and pattern in glycoconjugate vaccine model (Figure 2).[4]

FIG 2

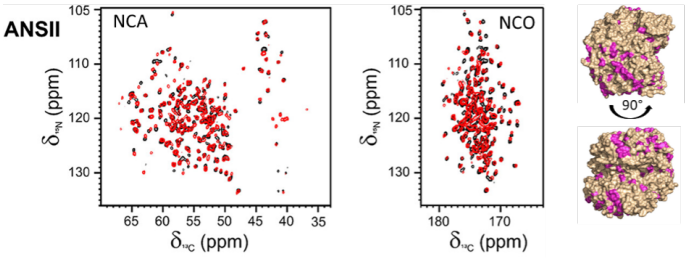


FIG 2. 2D 15N 13C NCA and NCO spectra of ANSII encapsulated in the hyaluronic acid hydrogel superimposed with NCA and NCO of the rehydrated freeze-dried reference protein. The residues experiencing the largest variations, highlighted in magenta, are mapped on the protein surface (PDB 3ECA) and involve hydrophobic and slightly polar surface patches.[4]

Structure and function of NDRG1, a Ni(II)-induced target for lung cancer therapy[5]

Accumulation of the protein Tau in neurofibrillary tangles in the brain is a major histopathological hallmark of Alzheimer's disease (AD) and several other conditions, collectively referred to as tauopathies. There is general agreement that Tau-directed therapeutic strategies would significantly impact the treatment of AD and other tauopathies. However, the intrinsic conformational dynamics and the multiple existing proteoforms make the targeting of Tau extremely challenging, and common drug molecules often fail to redirect aberrant conformational transitions. Nanoparticles offer an alternative to traditional drugs and show promise as potential artificial chaperones capable to mitigate abnormal protein aggregation. The success of the latter strategy requires a detailed description of nanoparticle-induced structural changes and high resolution NMR techniques are best suited to address this issue. A number of Tau domains and their interactions were investigated exploiting complementary 13C- and 1H-detected NMR experiments. The spectra allowed complete backbone resonances assignment and observation of the perturbations induced by the interacting E3 ligase CHIP (Figure 3). This study demonstrated the possibility to investigate by NMR the dynamic interaction of Tau with large objects such as enzymes and nanoparticles.[5]

FIG 3

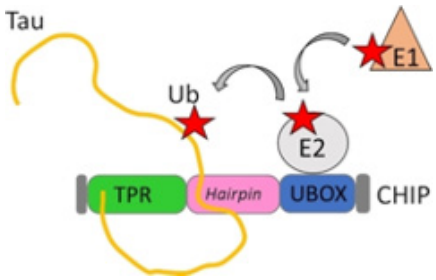


FIG 3. An NMR-based structural investigation of the Tau–CHIP complex revealed a multi-domain, dynamic interaction that explains the origin of the multiple-site ubiquitination of Tau operated by CHIP, as well as the ability of this ligase to modify Tau in the absence of chaperones.[5]

A Real-Time Approach to Study Cellular Metabolism in Mammalian Cells

In the frame of a 3-months internship at CERM/CIRMMP, Simone Fjordside, a Ph.D. student at Aarhus University, employed the in-cell NMR bioreactor system to perform real-time metabolomic studies directly in living cells. The aim is to establish a robust setup to record in real time the metabolic fingerprint of mammalian cells, which are encapsulated in a hydrogel and continuously supplied with fresh medium (Figure. 4). With use of the bioreactor, time series of NMR spectra were successfully recorded on mammalian cells, and cellular viability and metabolic activity were evaluated. Besides the scientific work carried out, the internship was instrumental to train the student in the operation of the bioreactor and will allow her to implement and further optimise a similar bioreactor at Aarhus University.

FIG 4

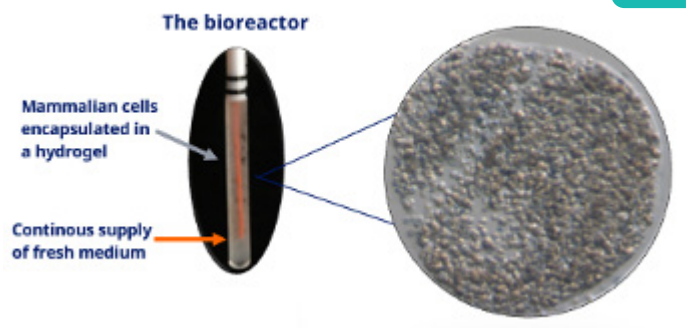


FIG 4 The assembled in-cell NMR bioreactor containing encapsulated mammalian cells and the inlet tube for fresh medium supply (left) and a slice of the hydrogel thread showing the encapsulated cells (right).

4. LRizzo D., *et al.*. (2021) Evaluation of the Higher Order Structure of Biotherapeutics Embedded in Hydrogels for Bioprinting and Drug Release. *Anal. Chem.* doi: 10.1021/acs.analchem.1c01850
5. Munari F., *et al.*. (2022) Structural Basis for Chaperone-Independent Ubiquitination of Tau Protein by Its E3 Ligase CHIP. *Angew Chem Int Ed Engl.* doi: 10.1002/anie.202112374

INSTRUCT CENTRE NL

Instruct Centre NL makes available some high-end research facilities from three Dutch institutes to cover a broad range of structural biology technology. These are the NMR and mass spectrometry facilities at the Bijvoet Centre for Biomolecular Research at Utrecht University, the national facility for high-end electron nanoscopy at Leiden University - NeCEN, and the NKI Protein Facility at the Division of Biochemistry of the Netherlands Cancer Institute in Amsterdam. All of these facilities are hosted by well-recognised research groups, contributing to an excellent academic environment.

Instruct Centre Lead Scientists
Meindert Lamers
Ariane Briegel
Anastassis Perrakis
Marc Baldus
Albert Heck
Alexandre Bonvin

Utrecht University offers access to their NMR and mass spectrometry facilities. The groups of Marc Baldus and Markus Weingarth offer high-field solid state NMR resources, whereas Hugo van Ingen is taking care of liquid state NMR services. In addition, Albert Heck and Maarten Altelaar take care of UU's mass spectrometry facility. The Bijvoet Centre includes also the relevant research groups of Alexandre Bonvin for computational structural biology, and the group for structure determination by X-ray crystallography and cryo-electron microscopy with Piet Gros, Bert Janssen, Friedrich Förster.

The NeCEN cryo-Electron Microscopy facility in Leiden houses two Krios microscopes equipped with K3, K2 and Falcon 3 cameras, and is headed by Ludovic Renault. Meindert Lamers and Ariane Briegel act as NeCEN co-directors. Their research groups as well as the Bram Koster group are located in the nearby university and medical center focusing on single particle cryo-EM and cryo-EM cellular tomography.

The NKI Protein Facility offers access to their protein production, characterisation and crystallisation services (Patrick Celie), as well as to a vast collection of complementary biophysical technologies for quantifying macromolecular interactions (Alexander Fish). The Division of Biochemistry that hosts the facility also houses the closely associated research groups of Anastassis Perrakis and Titia Sixma, which are well-known for their structural biology research related to cancer.

All the facilities and research groups that are affiliated to Instruct Centre NL provide access to national and foreign users from all Instruct members, and efficiently assist their visitors with experimental planning, data collection, hands-on training, data analyses and discussions. Access is open for researchers from academia and industry.

NEW TECHNOLOGIES

NMR at 1.2 GHz

In 2021, one of the first ultra-high field 1.2 GHz Avance nuclear magnetic resonance (NMR) spectrometers worldwide was installed at the NMR group at Utrecht University that coordinates the national Dutch ultra-high field magnetic resonance consortium (uNMR-NL, http://unmr-nl.science.uu.nl/). With the new 1.2 GHz system, uNMR-NL researchers as well as (inter)national users will be able to study complex biomolecular systems ranging from dynamic molecular networks to membrane and cell-embedded protein machines and the processes operating on nucleosomes within cells with unprecedented resolution. In addition, the new instrumentation that can be accessed remotely will support diverse scientific areas including research on (bio)materials, plants, metabolomic studies. Lasty, the NMR system will enable micro-imaging studies using specialised probe technology to obtain both the molecular structural information and 3D magnetic resonance images of samples.

FIG 1. The 1.2 GHz NMR set-up installed at Utrecht University

PDB-REDO-Cloud and EOSC-Life

Led by main scientist Robbie Joosten, the NKI team started two EOSC-Life in 2021 projects for cloudifying their X-ray structure refinement software PDB-REDO, both mediated via Instruct-ERIC. The first of these projects opens access to a 'FAIRified' databank of PDB-REDO refined structures, by (i) including complete version history as richer content for all kinds of structural bioinformatics and computational

approaches, and by (ii) adopting the annotation of the PDBe Knowledge Base for the PDB-REDO data and metadata. The second project cloudifies the PDB-REDO software itself, to make available a flexible and scalable engine through the EOSC platform that is also capable to efficiently populate the cloudified PDB-REDO databank. Ready-to-go workflows for dataset generation are being developed and implemented, together with a front-end for experienced users to design and run computational experiments with all available PDB-REDO features, and a back-end for external parties for using "PDB-REDO-Cloud" for their workflows. Already, these developments helped us to intensify our PDB-REDO access offer, for instance also through the ISIDORe project that was awarded later in 2021.

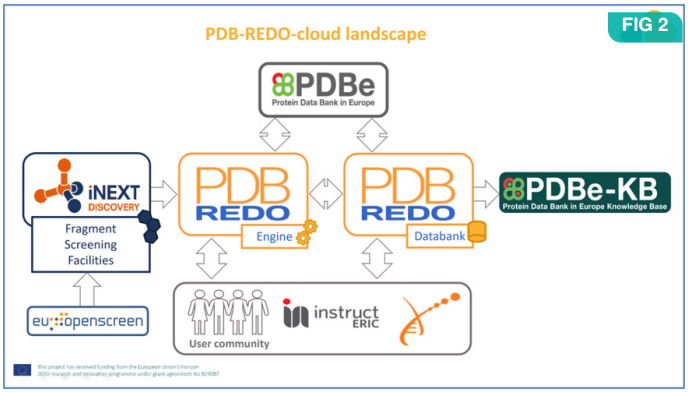


FIG 2. Different remote access approaches that have been developed at NeCEN. Top: Option 1 – Direct access via TeamViewer or other remote desktop technology; Bottom: Option 2 – Using VPN access and remote pads.

SCIENCE HIGHLIGHTS

Determination of Notch1 – Jagged1 complex affinity in solution

The Notch signaling system connects cells for driving proliferation, apoptosis, and differentiation in metazoans, and its distortion leads to debilitating developmental disorders and cancers. Other than forming a five-by-five domain complex, it is unclear how 40 extracellular domains of the Notch1 receptor engage the 19 domains of its canonical ligand, Jagged1. Using cross-linking mass spectrometry, biophysical, and structural techniques five regions were identified, two on Notch1 and three on Jagged1, that form distinct interaction networks. These, together with Notch1 and Jagged1 dimensions and flexibility as determined by SAXS, support the formation of different nonlinear architectures. The MST-services provided by NKI turned out absolutely crucial for getting unambiguous information regarding the interactions.

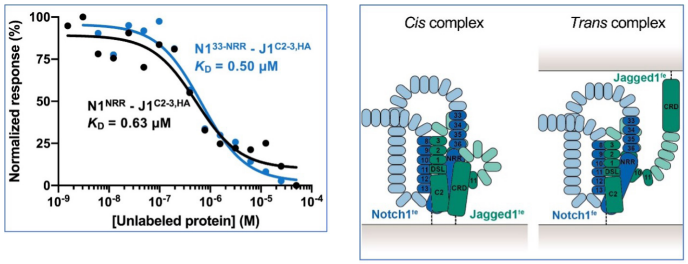


FIG 3. Left: MST-binding curves of Notch1NRR (black) and Notch1EGF33–NRR (blue) to Jagged1C2–EGF3,HA. Right: Models of the complete Notch1–Jagged1 ectodomain complex based on interaction data, either assuming a 'cis' or 'trans' arrangement (not all interactions that are shown might occur simultaneously).

Understanding Glucose Activation by UGPases

Activation of monosaccharides is necessary in many glucosyl transfer reactions and critical for synthesising various glycans. These reactions are catalysed by NDP-sugar pyrophosphorylases that use nucleotide tri-phosphate to tag sugars with a nucleotidyl-group. UDP-glucose pyrophosphorylase (UGPase), synthesising the activated nucleotide sugar UDP-glucose, is fundamental for viability of Aspergillus species. This may be related to UDP-glucose being the primary metabolite synthesising the disaccharide trehalose and glycogen, two major carbohydrate storage molecules, as well as cell wall 1,3-β-D-Glucan in fungi. Revealing such mechanisms may lay the basis for developing novel anti-fungal drugs by targeting fungal UGPases.

An early, preliminary cryo-EM single-particle reconstruction of apo-UGPase showed a low-resolution octameric configuration with D4 symmetry. For refinement, the project collected additional high-resolution data at NeCEN. The resulting new reconstruction of the octamer reveals a well-defined central core constituted by the C-terminal UGPase Left-Handed Beta-helix domains. However, the definition of the particle decreases towards the N-terminus, indicating movement in the catalytic domains. To better define also the catalytic domain, the symmetry-expanded reconstruction of the UGPase protomer was performed, achieving ~3.5 Å resolution. This allowed to model critical residues implicated in catalysis, and to define protomer-protomer interactions that participate in the cooperativity of this enzyme.

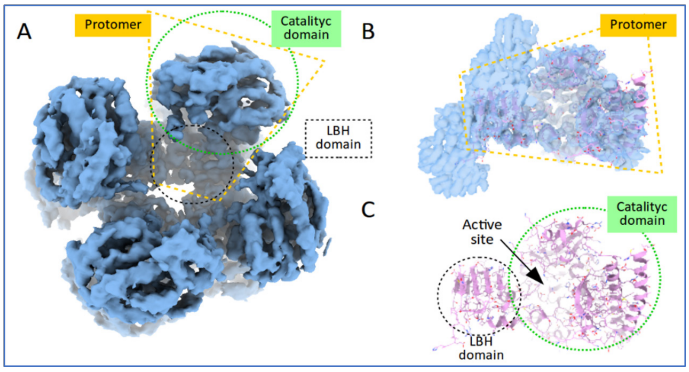


FIG 4. Cryo-EM single particle analysis of Aspergillus UGPase. A) The D4 symmetry reconstruction of the enzyme. The orange box shows a region corresponding to protomer density; N-terminal catalytic domain and the C-terminal LBH regions are shown in green and black circles. B) Density for a symmetry-enhanced reconstruction in which the protomer is fitted; the refined model for the LBH and catalytic domains is displayed in panel C.

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), the Molecular Biophysics Suite and the Oxford Mass Spectrometry Centre.

Instruct Centre
Lead Scientists
Rebecca Thompson
Martin Walsh
Jonathan Grimes
Justin Benesch

NEW TECHNOLOGIES

Astbury Centre

Astbury Biostructure Laboratory cryo-EM received funding from Wellcome multi-user equipment award to upgrade our two Titan Krios microscopes with latest Selectris/Falcon 4 direct electron detectors, along with the implementation of fringe free imaging. These new detectors and other upgrades have increased the quantity and quality of cryo-EM data acquisition, reducing the time needed to collect data. The implementation of multi-grid EPU allows us to more efficiently collect data across multiple grids, which is ideal for projects where small datasets are needed. This might mean collecting multiple small datasets to assess which grid will likely yield highest resolution, or has a labile complex intact. We are able to offer remote control of our Krios' to Instruct users.

The Electron Bioimaging Centre (eBIC)

During 2021, eBIC completed the fringe-free imaging (FFI) upgrades on Krios I, II and IV, and secured funds for the installation of Selectris X/Falcon 4i on Krios III, in addition to Windows 10 upgrades on Krios I, II, and IV. Starting in April 2022, all four cryo-electron microscopes will be able to perform continuous EPU data collections across multiple grids allowing faster data throughput. Along with medium magnification montaging in Thermo Scientific's Tomography 5 software, this will assist in identifying positions from which to collect data. All data collection at eBIC is remote, and we have brand new pictorial user guides to assist researchers in the collection of their own data for both EPU and tomography, as well as data transfer, shipping and processing.

Diamond - B21 beamline

B21 shifted to 100% mail-in services during the pandemic, and 6000 sample shipping holders were injection-moulded to support the mail-in SAXS community. These containers were specifically designed to physically protect PCR strip samples at dry ice temperatures. All holders are 2D barcoded to allow for sample tracking during the lifetime of the sample from user to Diamond. To support the 100% mail-in operations, data acquisition software updates for unattended data collection (UDC) were deployed to allow for switching between SEC-SAXS and batch mode measurements. In addition, B21 acquired the next generation X-ray exposure unit called "coflow" that will minimise radiation damage during data collection in Diamond-II. The coflow will be tested and commissioned on I22 in 2022.

The Membrane Protein Laboratory (MPL)

The Membrane Protein Laboratory (MPL) was awarded funding from Wellcome to enhance and continue operations for the next five years. The focus of this next phase is to augment access routes for integrative approaches to membrane protein structural biology. The Harwell Crystallisation Facility also secured funds for upgrades, including the introduction of a new multi-fluorescence imaging option as well as an integrated platform for screen design and dispensing. These will be available to the user community through Instruct-ERIC in the near future.

Macromolecular Crystallography (MX) beamlines

MX at Diamond continued to operate its beamlines through responsive mode access, which responds to user needs aligned effectively with their lab workflows. This is reflected across the suite of capabilities, with data collected automatically (typically between 1-4 days of samples being delivered to Diamond) and remote access shifts scheduled (typically within the following week) around more co-ordinated access for experiments. These included: serial synchrotron crystallography and room temperature studies, long wavelength anomalous phasing or metal identification, and fragment screening. With the advent of AI-driven model generation, we have added this, via AlphaFold2, into our data analysis pipelines alongside improving multi-crystal dataset processing.

OPIC

This past year, 2021, has been busy for the facility, with the installation of our long-awaited Aquilos-2 FIB-SEM, Windows-10 upgrades of our Glacios and Krios microscopes, FFI and AFIS, in addition to the installation of a Falcon-4

detector with SelectrisX energy filter, to replace our long-serving K2/Gif. With the significant increases in data collection rate and contrast boasted by Falcon-4/SelectrisX technologies, we anticipate a huge boost in tomographic and single particle data quality and quantity. Both machines are now operating with the latest version of EPU and Tomo5 and fringe free illumination allowing for more rapid data collection set up and acquisition, in addition to the option of setting up multiple data collections to run overnight.

In readiness for category-3 level virus samples, such as SARS-CoV2, much effort has been put in to provide robust testing and operation procedures and training for our containment suite, which we have started to execute.

We have also recently gained approval for the purchase and installation of an integrated fluorescent microscope for correlative imaging as well as an Alvéole Automated Bioengineering Platform for the micropatterning of EM grids to optimise cell positioning.

Oxford Mass Spectrometry Centre

In 2021 The Oxford Mass Spectrometry Centre opened two new services for Instruct-ERIC access. Dedicated instrumentation for hydrogen-deuterium exchange mass spectrometry customised for the study of protein assemblies; and mass photometry, a new way of measuring the mass of molecules in solution, are now available through the Instruct-ERIC catalogue.

SCIENCE HIGHLIGHTS

Discriminative SKP2 interactions with CDK-cyclin complexes support a cyclin A-specific role in p27KIP1 degradation[1]

The first publication resulting from HDX-MS access at the Astbury Centre through Instruct-ERIC was published in 2021 through a collaboration with Jane Endicott's group at Newcastle University which aims to unravel the complex interplay found in cell cycle regulation.

The work identified the SKP2 binding site on cyclin A, it showed that this site was not present in cyclin B or E and that the binding site overlapped with p27KIP1 leasing to the proposal that the SKP2 interaction with CDK2-cyclin A by more than one mechanism allows fine tuning of the degradation of p27KIP1 during cell cycle progression. 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 (Fig. 1). Work is underway to further understand the mechanistic implications of the new structural information and design experiments to test the resulting hypotheses rigorously.

FIG 1

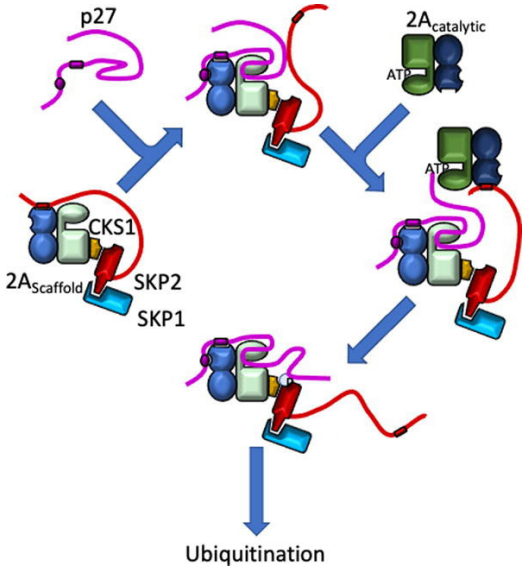


FIG 1. Proposed roles for the cyclin A-SKP2 interaction in regulating the cell cycle.[1]

SARS-CoV-2 research at OPIC

To aid the effort to tackle the pandemic, OPIC facilities were used extensively to obtain structures of SARS-CoV-2 spike protein with a host of antibodies derived from convalescent patients that may have therapeutic or diagnostic applications[2,3,4]. In addition, data collected on the OPIC Krios provided insight into an exciting new vaccine candidate[5]. Another OPIC-Krios derived structure has also been recently published as part of a collaboration with bioGUNE, Spain[6].

FIG 2

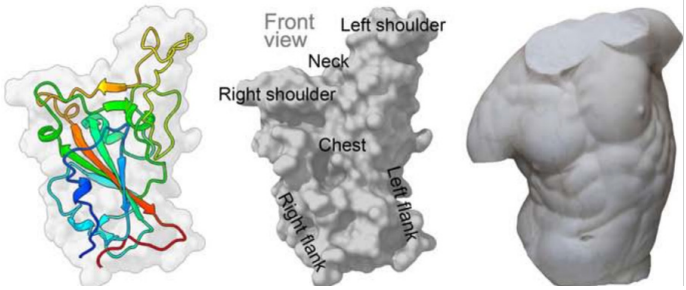
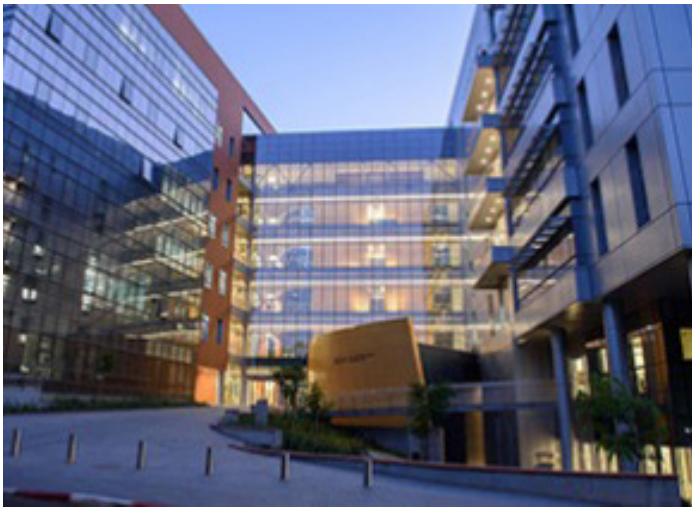


FIG 2. The antigenic anatomy of SARS-CoV-2 receptor binding domain.[2]

1. Salamina M. *et al.* (2021). Discriminative SKP2 interactions with CDK-cyclin complexes support a cyclin A-specific role in p27KIP1 degradation. *J of Mol Biol,* doi:10.1016/J.JMB.2020.166795
2. Dejnirattisai, W. *et al.* (2021) The antigenic anatomy of SARS-CoV-2 receptor binding domain." *Cell.* doi: 10.1016/j.cell.2021.02.032
3. Liu, C., *et al.* (2022) The antibody response to SARS-CoV-2 Beta underscores the antigenic distance to other variants. *Cell Host & Microbe.* doi: 10.1016/j.chom.2021.11.013
4. Huang, K-Y A., *et al.* (2021) Structures and therapeutic potential of anti-RBD human monoclonal antibodies against SARS-CoV-2. *Theranostics.* doi: 10.7150/thno.65563
5. Tan, T., *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. *Nature Com.* doi: 10.1038/s41467-020-20654-7.
6. Duyvesteyn, H., *et al.* (2021) Bacteriophage PRD1 as a nanoscaffold for drug loading. *Nanoscale* doi: 10.1039/d1nr04153c


CEITEC


50
50 YEARS WITH OUR EYE ON THE FU



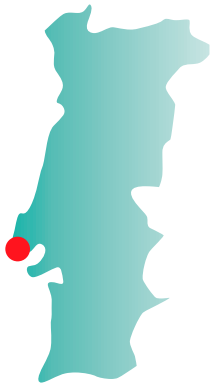

EMBL

NEW INSTRUCT-ERIC RESEARCH SITES

An Instruct-ERIC Research Site is an individual facility or organisation, or a consortium of organisations within a country that can offer a centralised national hub to provide training, outreach or networking activities of interest to Instruct users and members.

Separate to Instruct centres/facilities, research sites do not offer access to research equipment/technologies, instead delivering supplementary services to benefit the wider Instruct structural biology community.

PORTUGAL



Instruct Research Site-PT
FC-ULisboa, Lisboa and
ITQB NOVA, Oeiras.

The Portuguese Instruct Research Site (Instruct-PT) is formed by two centres located at FC-ULisboa, Lisboa and ITQB NOVA, Oeiras. Together, they provide expertise and advanced integrative training in native and structural mass spectrometry, macromolecular crystallography, NMR spectroscopy, SAXS and biophysical characterisation. Instruct-PT hosts state of the art equipment in MRMS and NMR, and provides pilot access to MRMS.

The Portugal research site will host annual meetings of the Portuguese structural biology community, fostering networking, cohesion and esprit de corps. These activities will benefit young researchers by providing contact with the latest technologies and, most importantly, world leading researchers in the field that will provide inspiring role-models and outreach opportunities.

FIG 1. FC-ULisboa, Lisboa, PT

FIG 2

FIG 2. ITQB NOVA, Oeiras, PT

SLOVAKIA

Instruct Research Site-SK
Institute of Chemistry, Center for Glycomics, Bratislava, Slovakia

Slovak Instruct Research Site is located at the Slovak Academy of Sciences (SAS) in Bratislava. Institute of Chemistry (IC), one of the largest academic SAS institutions, provides access to services in the field of glycan and glycoform structure analysis by applying spectroscopic methods. IC SAS analytical services (high-resolution NMR, MALDI, ESI MS and theoretical analysis) will be provided for other research groups through either short-term internships or remote access via the Instruct website.

Apart from analytical services, the Slovak research site organised Instruct scientific meetings in 2017, 2018 and 2019. The next meeting will be organised in September 2022 as a combination of the two conferences (Chemistry towards Biology 10 and Instruct) and will focus on the biomolecular structure.

FIG 3

FIG 3. IInstitute of Chemistry, Center for Glycomics, Bratislava, SK

INSTRUCT-ERIC HUB

The project management and administration teams were unchanged, providing continuity and retaining skills in the Hub.

2021 saw the loss of the IT lead senior developer who had established ARIA as the main access management system for Instruct. This role was immediately filled by Marcus Povey with no major impact on service delivery or ambition for further development goals. Two trainee developers were recruited to strengthen the IT capacity. However in December 2021, the second Senior Developer left Instruct and recruitment to fill this role will be undertaken in 2022.

The recruitment process for the Instruct-ERIC Director continued with an outcome expected early 2022.

CAPACITY BUILDING AND STAFF DEVELOPMENT

Our success is built on the professionalism and commitment of our staff and we take staff development very seriously. All staff undergo an annual staff assessment to monitor performance and to address any issues. In 2021 our two trainee project managers were mentored through the requirements of project delivery, proposal preparation and submission for Horizon Europe grant funding, and administrative duties in support of the various committees and governance bodies of Instruct-ERIC.

In addition Instruct hosted a number of events to increase knowhow and skills that help deliver Instruct services to the user community. This included two Software Developer Exchange of Experience events (May and September 2021), Best Practices in cryo-EM (June 2021) and the Centres General Forum (June 2021) where all parties exchange opinions and experiences to improve the functionality of Instruct. A Project managers meeting, dealing with issues encountered in the preparation of proposals to calls in the Horizon Europe work programme, was held in July 2021.

SPOTLIGHT ON STAFF

Sir David Stuart

Professor Stuart concuded his time as Scientific Director of Instruct-ERIC at the end of 2021. As a world class structural biologist, specialising in X-ray crystallography for structural virology he has determined the structures of major pathogens including foot-and-mouth-disease virus, Bluetongue virus, hand-foot-and-mouth-disease virus and hepatitis A virus and has worked on key drug discovery targets such as HIV-1 reverse transcriptase, Ebola virus glycoprotein and most recently SARS-CoV2 spike protein. An early advocate of electron microscopy for 3D structural analysis at atomic resolution, Professor Stuart has been instrumental in establishing integrated workflows bringing different technologies (X-ray crystallography, cryo-EM and cryo-ET, SAXS) together. With a long track record in setting up infrastructure facilities in the UK (Division of Structural Biology at the University of Oxford, STRUBI; Oxford Centre for Particle Imaging, OPIC; Oxford Protein Production Facility, OPPF) he also supported European science initiatives and was a founding member, along with Dino Moras, Stephen Cusack, Lucia Banci, Joel Sussman and Wolfgang Baumeister, of Instruct, which became Instruct-ERIC in 2017. Professor Stuart is currently Life Sciences Director at Diamond Light Source Ltd, Joint Head of The Division of Structural Biology at Oxford University and MRC Research Professor. He is the recipient of many prizes and medals for his scientific work and in 2021 received a Knighthood for his services to medical research and the scientific community. He will retain his role as lead of the Instruct Centre UK but will concentrate on his other duties. Professor Stuart has provided the vision and ambition for Instruct to become a successful research infrastructure.

FIG 1. Instruct Hub members enjoying the summer picnic and Christmas party.

HUB TEAM MEMBERS

Claudia Alén Amaro
Senior Programme Manager

Omran Alhaddad
IT Junior Developer

Pauline Audergon
Trainee Project Manager

Stephanie Chapman
Communications Associate

Susan Daenke
Hub Coordinator

John Dolan
Communications Officer

Lorraine Donaldson
Financial Administrator

Madalena Gallagher
Administrative Officer

Regina Guenster
Trainee Project Manager

Francisco Guimaraes
Finance and Admin Officer

Natalie Haley
Project Manager

Marcus Lowndes
IT Junior Developer

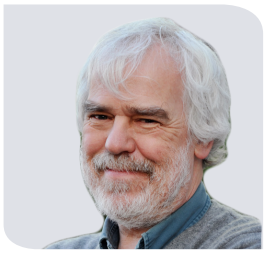
Denis Nemytov
Front End Developer

Ray Owens
Instruct-ULTRA Project Manager

Marcus Povey
Senior Software Developer

Fiona Sanderson
Software Developer

Callum Smith
IT Team Lead



David Stuart
Director

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SERVICES

ACCESS

In 2021, as the world began to adapt to the continuing COVID-19 pandemic, Instruct-ERIC access demand continued to increase. Structural biology access has been demonstrably crucial in the fight against COVID-19 and as restrictions on mobility reduce across Europe, access for other types of project can also resume. Taken together with our growing user base and community, Instruct-ERIC services in 2021 were more highly demanded than ever before.

To meet this growing demand and ensure delivery of access to as many researchers as possible, whilst maintaining a fair financial contribution. The Instruct-ERIC Access committee recommended further refinements to the 2020 access funding model in a review after two years of its operation.

These included differential maximum financial contributions per service type based on actual costs of providing these services and a unification of the contribution towards user travel costs across the whole infrastructure. Also explicitly clarified was the decision that what was formerly user travel costs can be used towards either travel and accommodation or sample shipping, addressing the growing provision of remote access.

To ensure that the access budget could benefit a larger number of projects and researchers, individual projects requesting multiple visits to use the same service were discouraged to promote integrated projects where multiple visits are to a range of services.

FIG 1

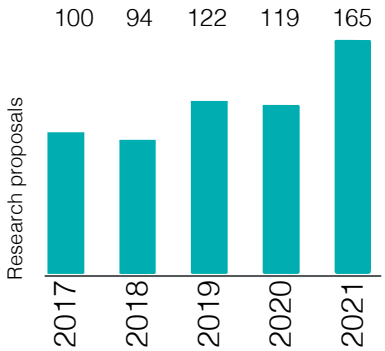


FIG 1. Instruct-ERIC proposal statistics.

Access requests were accepted on a rolling basis throughout 2021. The process for requesting access to Instruct-ERIC services continues to make use of our access management software ARIA. The established process remains unchanged from previous reports where users submit proposals, together with their research team, for one or more services from the Instruct-ERIC catalogue. The secretary of moderators assigns a suitable moderator who sends the proposal for scientific peer review by three reviewers including at least one reviewer external to Instruct-ERIC. Projects with sufficient scientific excellence then undergo a technical evaluation to ensure that the services requested are feasible and can be provided. Access provision is recorded and tracked within the ARIA system by the Instruct Centre staff before feedback is collected from user and facility once the access is completed. These data are aggregated to provide KPIs and access statistics.

The ARIA development team are constantly responding to feedback to enhance the functionality of ARIA to streamline the work done in the Instruct Centres, committees and the Hub. In 2021 a feature to record against a visit when it had been claimed for from the Hub and when the claim had been paid was implemented. Information on the specialisations, workload and response rate of reviewers and moderators were added to make it easier to select appropriate moderators and reviewers for each proposal.

Enhancements in ARIA were also made to better monitor and export access KPI and statistics, significant improvements were made to the reporting outputs and statistics in conjunction with the KPI working group led by Ludo Renault (NL) and Regina Günster in the Instruct Hub.

FIG 2

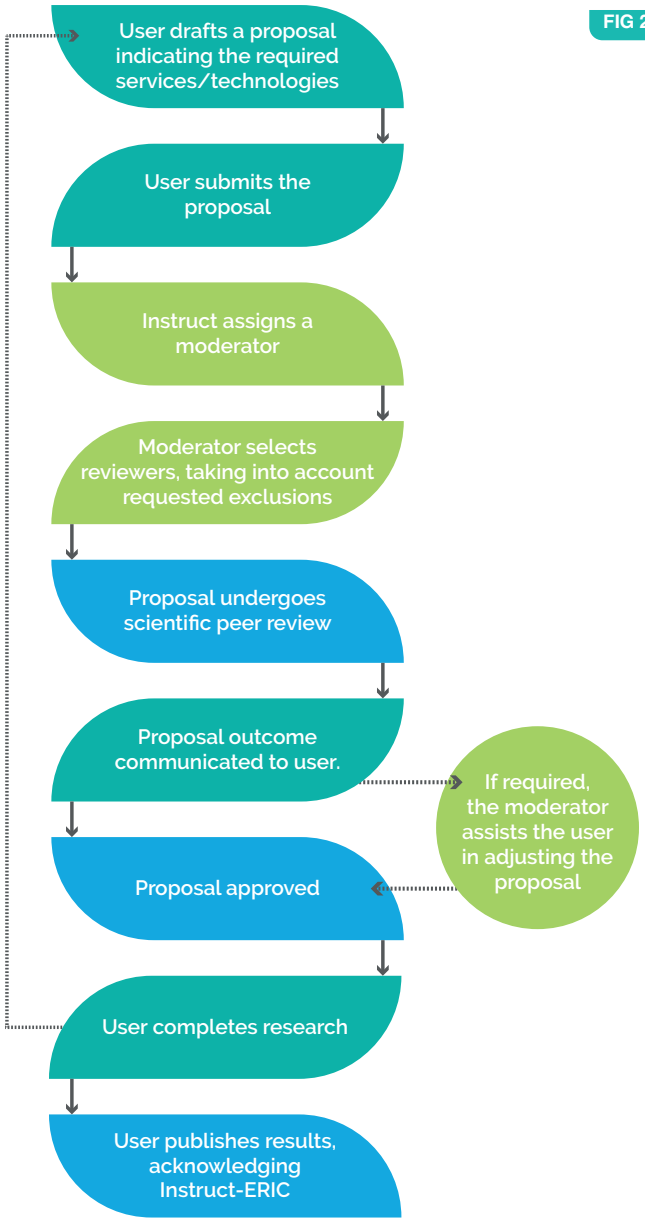


FIG 2. The pathway for access to Instruct-ERIC services.

ACCESS TO INFRASTRUCTURE 2021

In the period from 1 January to 31 December 2021, 165 proposals were received of which 141 were approved, equating to a 85% approval rate.

This high approval rate shows the excellent quality of the research project proposed and reasons for rejection included:
The applicant is ineligible (applicant id located outside an Instruct-ERIC member country organisation)
The scientific content does not achieve the required standard (as judged by peer review).

In 2021 Instruct-ERIC supported 131 research visits providing 1069.2 days of access to Instruct Facilities covering both national and transnational access.

Access by Country

Projects come from across the world with Figure 3 showing the number of completed access visits per country in 2021. A total of 131 visits were undertaken by researchers from Instruct-ERIC members, of which the highest numbers came from Spain, France, and the United Kingdom and three visits were undertaken by researchers from non-member countries via collaboration. The majority (57.2%) of access was transnational, with 42.7% was researchers accessing Instruct-ERIC infrastructure in their own country. In managing access submissions, care is taken to ensure that researchers do not request Instruct-funded access from their home laboratory.

FIG 3

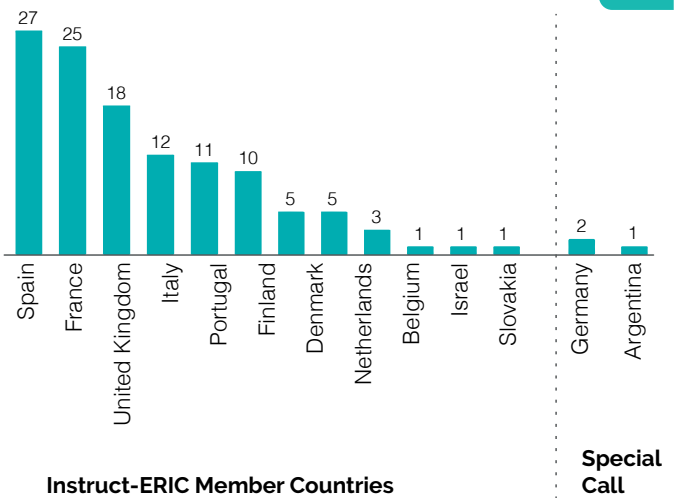


FIG 3. The number of completed access visits per country of applicant.

Access by Service Type

Figure 4 shows the infrastructure service types selected and subsequently accessed by researchers. Most users accessed electron microscopy and image processing facilities, 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-ERIC services to ensure the quality of the preparation before structural data is gathered.

FIG 4

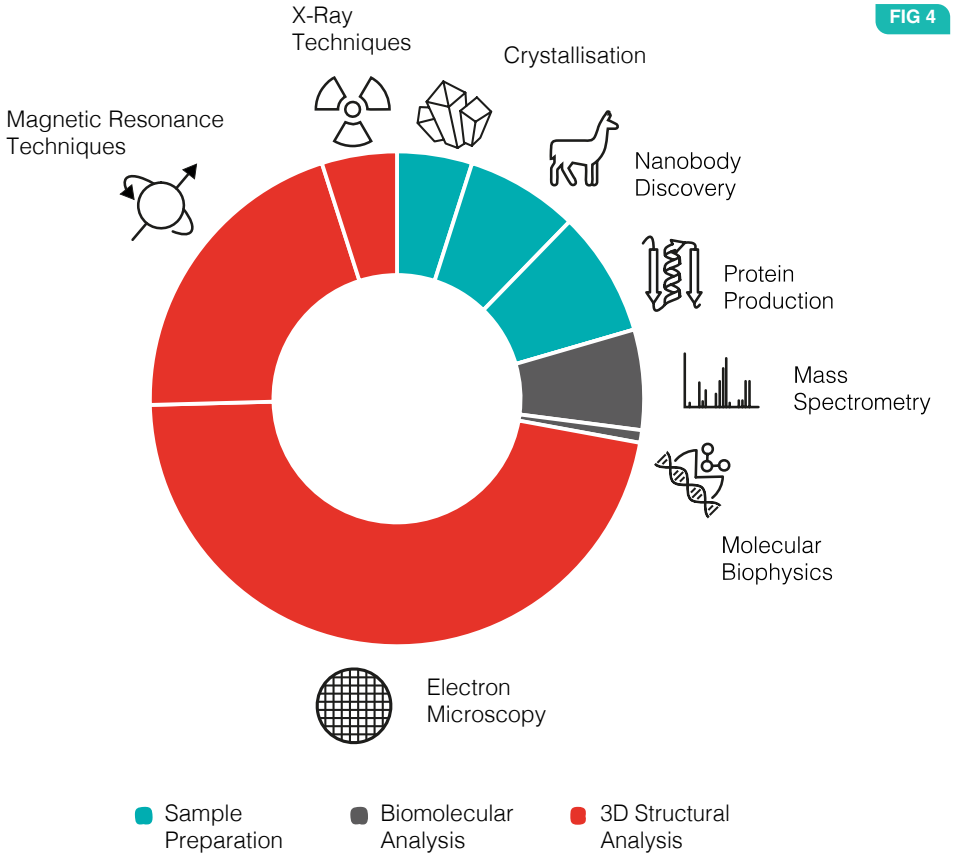


FIG 4. Representation of the service demand in 2021.

TRAINING

For the last 10 years, Instruct training programme has increased the skills of researchers in structural biology through the organisation and support of training courses and internships.

Since the COVID-19 pandemic started, we have increased our remote training and continued some in-person training when possible. Virtual courses have been recorded with content available in our YouTube channel, increasing the reach of our training. To add to our traditional training routes it is worth mentioning the strong training element in the access visits where early career researcher learns from the expertise of the facility scientists.

Our Centres have organised courses, workshops and webinars using our centralised Zoom licence in addition to the technical support from the Hub. All events were advertised and outcomes communicated through our mailing lists, website and social media channels. In the case of internships, we changed our traditional individual deadline to an open call with submissions being reviewed as soon as they were submitted. In this way we were able to fund internships when travel conditions permitted.

This year we launched a new scheme: the Instruct Remote Internships. These pilot internships were dedicated to early career researchers (PhD students or Postdocs in their first 5 years after graduation) for providing the opportunity to discuss their own structural biology project(s) with one of the Instruct Centres' leaders. This gives the intern the opportunity to train through tutoring.

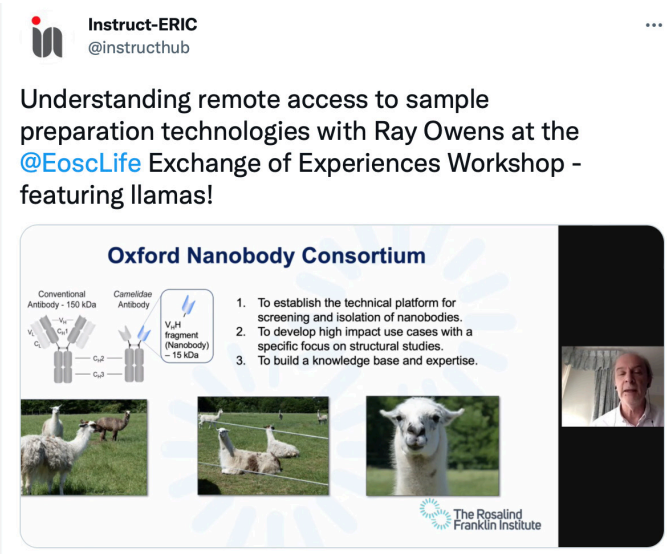
Instruct-ERIC allocated funds to support two remote internships. In contrast to the from standard Instruct-ERIC Internship, succesful applicants are supervised remotely by a Scientific Tutor from one of the Instruct Centres, with whom they discuss and improve their projects. Projects proposed basic or applied research of a preliminary nature, with an expectation of receiving hints and collecting data in support of a project plan wider in scope, potentially object of an application for more substantial funding through conventional routes. Projects that include technology and/or software development were encouraged. All proposals included the use of technologies available at Instruct Centres or developed technologies that would benefit the aims of Instruct. Following the remote internship, access to the technologies available in the Instruct-ERIC catalogue is achieved by submitting a regular request for access.

INSTRUCT-ERIC TRAINING COURSES 2021

Exchange of Experience Workshop: Remote Access and User Training

14 April 2021 Instruct-ERIC, Euro-BioImaging, EOSC Life [Virtual]

This one-day, hands-on workshop was aimed at life science researchers, to address the challenges of moving face-to-face trainings online, and to ensure maximum effectiveness of online trainings. We worked in close collaboration with colleagues from Euro-BioImaging with strong participation of research and management staff from our centres in the UK, France, the Netherlands, EMBL and Czech Republic. Topics addressed included: Online facilitation, keeping attention of participants and community building. Parallel breakout sessions allowed for more in-depth discussion of teaching computer-based hands-on tutorial courses and challenges faced by RIs requiring physical infrastructures.



Instruct training course on fully remote data collection on eBIC Cryo-EMs

10-21 May 2021 Instruct Centre-UK [Virtual]

This course was designed to provide training in data acquisition for single particle analysis (SPA), paying particular attention to how to set up and run an SPA workflow entirely remotely. The course delivered training to 4 cohorts of 2 people per cohort. Each cohort was scheduled two 8 hr Talos sessions followed by one 8 hr Krios session.

Participants were invited to ship pre-screened single particle grids in each cohort for use during the course. In the case that evidence could be found of a high-quality single particle sample and preliminary high- resolution analysis, the access to the Krios session was extended to 48 hrs for a full data collection.

Instruct-ERIC course in biophysics, structural mass spectrometry, and X-ray techniques

24-27 May 2021 Instruct Centre-CZ [Virtual]

This online course organised by the Centre of Molecular Structure, IBT, BIOCEV, welcomed 48 registered participants and provided 4 afternoons of intensive teaching and training from 10 speakers and tutors. Each day was dedicated to one area of integrative structural biology. The lectures were focused on young scientists and novices in the field. The training sessions provided basic insights into the instrumentation used and specific applications. The Course participants could discuss directly with speakers and tutors. A 30-minute block of informal discussions with breakout rooms enabled contacts among the participants, speakers, and organisers. Teaching material was provided via a cloud link and both the talks and training sessions were recorded and the recordings provided to the Course participants. The online platform Zoom was utilised; some training sessions were pre-recorded and the remaining ones were broadcast online, both with assistance from a professional AV company.

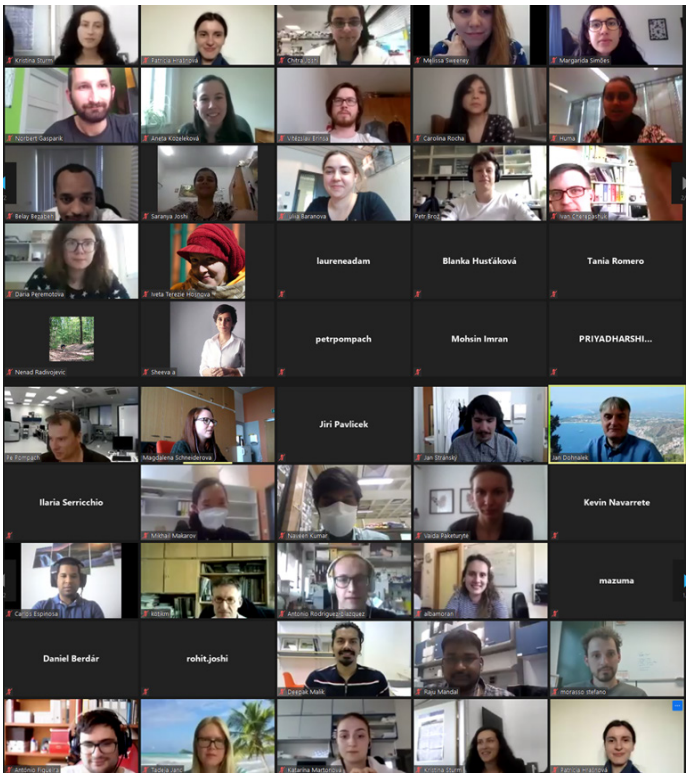


FIG 1. Participants of the Instruct-ERIC course in biophysics, structural mass spectrometry, and X-ray techniques

Instruct workshop on hydrogen/deuterium exchange studied by mass spectrometry and nuclear magnetic resonance spectroscopy

21 June – 02 July 2021 Instruct Centre-UK [Virtual]

This course aimed at early career researchers (postgraduate students, postdoctoral research fellows) with either no or limited experience with MS, NMR or other structural biology techniques, and an interest in applying hydrogen-deuterium exchange methods.

Instruct course on X-ray, electron and neutron crystallography

29 June – 06 July 2021 Instruct Centre-FI [Virtual]

This course was aimed at PhD students and postdocs whose research projects focus on solving structures using crystallography. Students from 14 countries participated in the training event. Course covered state of the art techniques in crystallography, but also provided training in basic principles on the diffraction experiment, data collection strategies, structure solution as well as refinement and validation. Use of neutron and electron crystallography was covered by experts focusing on specific approaches in sample preparation and data handling. The course was organised virtually including a range of hands-on practical sessions for example a remote data collection session on four beamlines in Diamond Light source, UK with a possibility for course participants to send crystals for data collection.

Instruct virtual course on Single Particle Analysis by Cryo-EM

28 June 28 – 02 July 2021 Instruct Centre-ES [Virtual]

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. This edition of the course has included the sample preparation and image acquisition part. The CNB CryoEM facility was in the process of being incorporated into the offer of the Instruct catalogue. For this reason, the course was extended from the traditional 3 days course into a 5 days course that included sample preparation and image acquisition.

The course was a success with regard to the number of attendees, and the final survey showed an excellent evaluation of the quality of the course and content learned. There were some comments indicating that they would have preferred a course in person, but the current travel restrictions have prevented this. The attendees were able to process tutorial data in cloud machines and were acquainted with most of the theoretical and practical ideas of image processing and atomic modelling in cryo-EM.

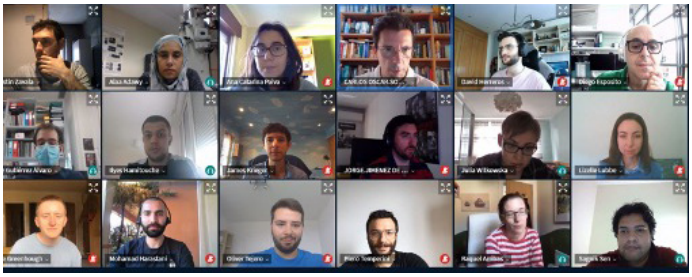


FIG 2. Instruct virtual course on Single Particle Analysis by Cryo-EM.

Instruct-ERIC Best Practices in Cryo-EM Workshop 2021

18-19 October 2021 Instruct Centre-EMBL [Virtual]

Now in its fifth year, this virtual workshop brought together those involved in the running of high-end cryo-EM facilities to discuss and share best practices. It was open to EM facility scientists, managers, and computing specialists both from academia and industry.

This two-day workshop welcomed speakers from a wide variety of institutions. Their presentations covered a range of topics on operating a Cryo-EM facility, including best practice in sample preparation, imaging and data handling/processing, operational models, remote operations, and practical challenges. During the presentations and round table sessions the future perspectives, opportunities, and challenges in Cryo-EM facility management were discussed.

Instruct virtual course on Electron Tomography by Cryo-EM

13-16 December 2021 Instruct Centre-ES [Virtual]

The aim of the course was to provide a comprehensive overview of the whole image processing pipeline in Electron Tomography (ET) from the movie alignment of the tilt series, to 3D reconstruction of the tomogram, particle identification, and subtomogram averaging.

The course was a success with regard to the number of attendees, and the final survey showed an excellent evaluation of the quality of the course and its practical usefulness. There were some comments indicating that they would have preferred a course in person, but the current travel restrictions have prevented this. The attendees were able to process tutorial data in cloud machines and were acquainted with most of the theoretical and practical concepts and methodologies of image processing in Electron Tomography (ET).

FIG 3. Instruct virtual course on Electron Tomography by Cryo-EM

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 the July 2020 receiving 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 were announced early in 2021.

Elias Adriaenssens, Belgium
Leonardo Almeida-Souza, Finland
Mark Tully, France
Moreno Lelli, Italy
Hugo van Ingen, Netherlands
Hugo Oliveira, Portugal
Nicola G. A. Abrescia, Spain
Javier Garcia Nafria, Spain
Steve Prince, UK
Theo Karamanos, UK

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 any time after discussions with the host Instruct Centre. Until further notice there is no fixed deadline with applications being reviewed as received. In 2021 there were 16 applications submitted to the 8th call and out of those 11 were approved:

Subhadra Dalwani, Finland - Internship at Instruct Centre ISPC (WIS) Israel
Matteo Ardini, Italy - Internship at Instruct Centre-FR2
Ramita Sulu, Oulu - Internship at Instruct Centre-CZ (CEITEC)
Elda Bauda, France - Internship at Instruct Centre-CZ (CEITEC)
Luca Mauro Invernizzi, Italy - Internship at Instruct Centre-CZ (BIOCEV)
Rhian Jones, France - Internship at Instruct Centre-ES (I2PC)
Miguel Cantero Reviejo, Spain - Internship at Instruct Centre Instruct-FI (Helsinki)
Jana Nedvedova, Czech Republic - Internship at Instruct Centre Instruct-FR2
Simone Fjordside, Denmark - Internship at Instruct Centre Instruct-IT
Giuditta Dal Cortivo, Italy - Internship at Instruct Centre Instruct-IT
Priscillia Lagoutte, France -Internship at Instruct Centre Instruct-UK (OPIC)

STAFF EXCHANGE

The Hub organised virtual meetings with Instruct managers to continue the improvement of our services and the development of the use of ARIA as a tool for access management.

During 2021 Instruct continued 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. In this context, Instruct has been selected as one of the recipients of ENRIITC support to organise an industry outreach event which will take place next year.

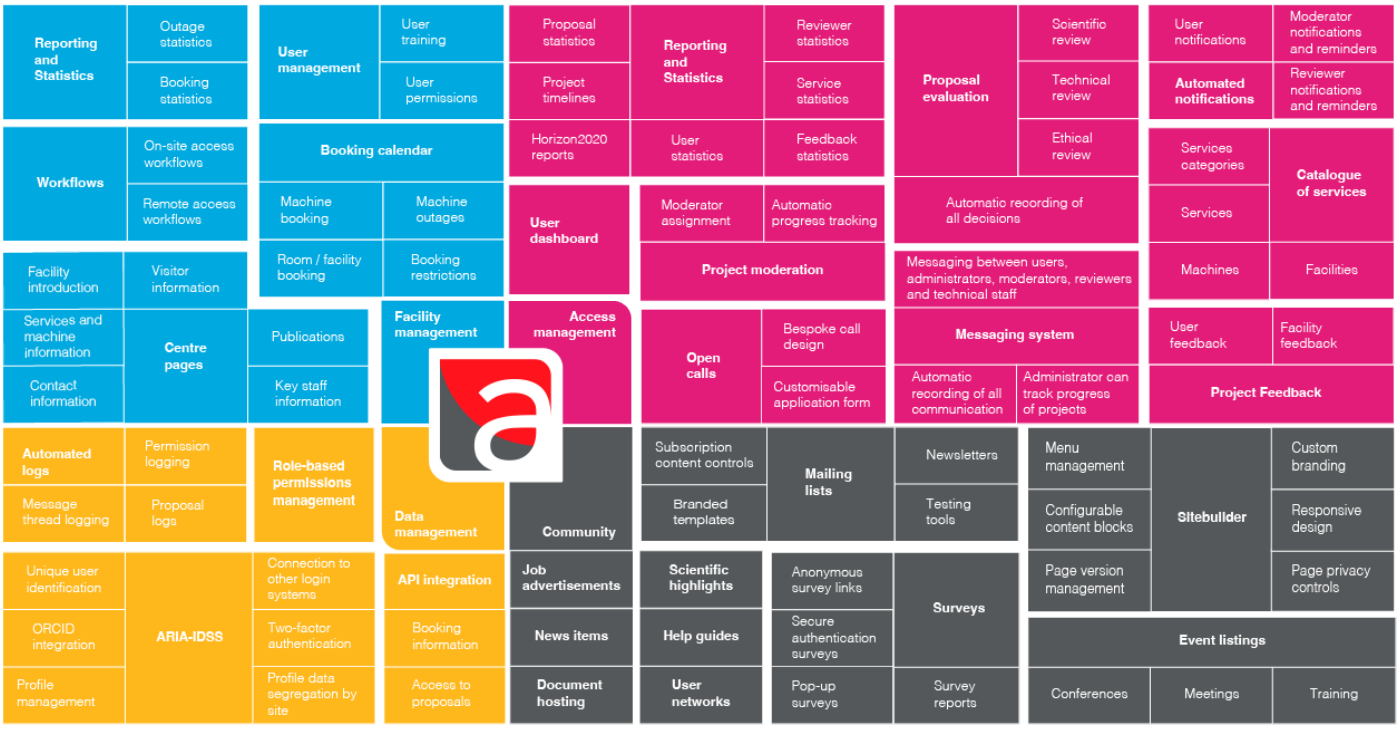
In collaboration with EOSC-Life colleagues, Instruct organised a staff exchange workshop in the topic of remote access and remote training. To advance on the issue of training for research infrastructure users and personnel, members of the Instruct Hub participated in a 4 day staff exchange event with members of the EATRIS and ELIXIR Hubs. Collaboration with other research infrastructures has been instrumental in the training of members of the Instruct Hub.

During 2021 a new scheme for international staff exchanges was set up in the frame of the project EU-LAC ResInfra. A call was open and 3 applications were selected. The staff exchanges will take place in 2022.

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

ARIA is a cloud software platform, developed and maintained by Instruct-ERIC Hub, which provides an integrated suite of tools for research infrastructure management.

The acronym “ARIA” is an abbreviation of “Access to Research Infrastructure Administration” and the functionality covers a range of access management functions: access catalogue, proposal submission, scientific peer review, technical evaluation, access delivery, feedback collection and access reporting. In addition, ARIA provides software tools for facility management such as instrument booking; website management, document management, events/ news/job postings, survey tool, CRM; and APIs for data integration. These tools are supported by context-dependent integrated internal messaging and automated notifications and reminders.



ARIA was initially developed to service Instruct’s own needs due to a lack of suitable commercial software, however it has since grown to support projects and infrastructures throughout the life sciences, and beyond. As other research infrastructures reach maturity and begin offering access to their scientific communities the secure, a scalable solution for access management offered by ARIA is required.

The focus of 2021 has been the rollout of a new login system, making ARIA ready for future support for EOSC-Life's LifeScience Login. It also saw the launch of the new “Sites” model which will make it much easier for ARIA customers to administer their projects, as well as increasing ARIA’s already robust GDPR position.

In 2021, ARIA is the go-to solution for access management within the European science community.

First established in 2020, the ARIA user group chaired by Rebecca Thompson of Instruct Centre UK continued to work through 2021 providing insights and feedback towards current and future ARIA developments. As a legacy of the former H2020 funded project CORBEL, which saw the use of ARIA expanded beyond the Instruct community to other research infrastructures, a mechanism to allow feedback from super-users of the ARIA system from a range of different organisations was required to ensure ARIA’s utility as a pan-infrastructure management platform.

Suggestions for improvements to existing features or new functionality are added by members of the user group to an online ideas board using the software Trello. These proto-ideas are then expanded upon by online discussion on the items and prioritised for implementation by the ARIA team. In 2021 the user group contributed to the specification for several new features now available in ARIA including ORCID IDs in user profiles, text formatting and hyperlinks in proposal form fields, and configurable guidelines for technical evaluation.


7AEG

COLLABORATIVE WORK

EUROPEAN PROJECTS

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

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.

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.

BY-COVID: BeYond-COVID

The BeYond-COVID project aims to make COVID-19 data accessible to scientists in laboratories but also to anyone who can use it, such as medical staff in hospitals or government officials.

EUROPEAN PROJECTS

The RI-VIS Project, a consortium of 12 research infrastructures coordinated by Instruct-ERIC, was designed to increase international visibility of research infrastructures and to promote international cooperation – whilst also tackling visibility of European research infrastructures beyond the existing RI community.

International Outreach

The RI-VIS project organised three International Symposia on Research Infrastructures, each between Europe and three different global regions: Africa, Latin America, and Australia. These symposia were initially supposed to take place as in-person events, but the COVID-19 outbreak meant these needed to be postponed, and then were decided to take place as virtual events.

The symposia were backed up by white papers, offering recommendations towards cooperation between Europe and each region. These were shared widely throughout the project and were a central part of each symposium.

The symposia brought together delegates from across multiple regions and disciplines; researchers, policy makers, and research infrastructure managers all discussed how best to forge ongoing partnerships and collaborations in the regions. The symposia, supplemented by satellite events for specific discussions to take place, were a success, with almost 600 attendees across the three events. Such was the success, that the CREMLINplus project requested to organise the Europe-Russian Federation Symposium, a similar one-day virtual event inspired by the earlier symposia.

Increasing Research Infrastructure Visibility

The RI-VIS Project developed a series of tools, workshops, and documents designed to directly enhance research infrastructure visibility, and provide a platform for sustainable development into the future.

RI-VIS provided an online communications workshop, aimed at research infrastructure managers and communications officers. This workshop, run by ClearEurope, provided an in-depth course on how to maximise visibility for research infrastructures in the digital space, and reach wider audiences in research and beyond.

RI-VIS also developed communications guidelines for European research infrastructures looking to engage with collaborators in Africa and in Latin America. The documents are structured as step by step guides to produce regional communication strategies, and are informed from feedback received via survey responses from regional experts.

Other tools provided by RI-VIS include the Communications Toolkit – an expansive toolkit made available online using ARIA Sitebuilder as a resource for research infrastructures to understand how best to develop a communications strategy. RI-VIS also developed an MOU template, which can be used as a basis to formalise relationships between RIs and regional institutions. In addition, the RI-VIS Slack channel continues to be used by over 400 users from across global research infrastructures, with more than 30,000 messages shared – demonstrating the value of the tool as a knowledge sharing platform.

RI-VIS launched in Febuary 2019 and came to an end in January 2022, and had a total budget of 1.5M Euros.

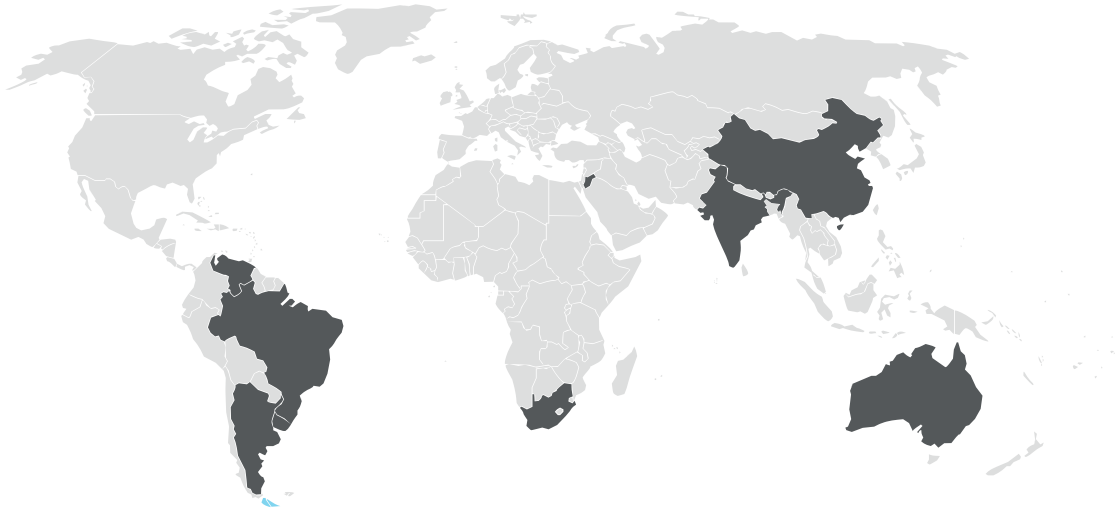
FIG 1. RI-VIS kick-off meeting

FIG 2. International RI-VIS Symposia held in 2021.

FIG 3. RI-VIS Communicaiton Toolkit.

INTERNATIONAL COLLABORATION AND OUTREACH

As Instruct-ERIC has developed and grown over the years, its reach extends far beyond Europe, collaborating with structural biologists worldwide. In conjunction with several Horizon Europe projects (notably RI-VIS and EU-LAC ResInfra) Instruct-ERIC has had the opportunity to engage, collaborate, and most significantly, provide access for researchers in regions at a global scale. This has built on many years of ever-increasing collaboration with regions such as Latin America, Africa and Australia. The first MoU between Instruct and another facility was signed in 2016, and Instruct signed two in 2021 – with many more in between. The RI-VIS Symposia provided a platform for engagement with researchers around the world, whilst calls for access in conjunction with the EU-LAC ResInfra project allowed Instruct to invite global researchers to visit Instruct's European facilities.



RI-VIS Symposia

The RI-VIS Symposia on Research Infrastructures were an excellent opportunity for Instruct to engage directly with researchers in different global regions. Postponed one year due to the COVID-19 outbreak, the symposia brought together delegates from across many scientific fields, from research infrastructures, policy makers and funders – creating a platform for discussion on the topics that most affect researchers and access to research infrastructure.

The three symposia took place between Europe and Africa, Latin America and Australia, in February, June and October, respectively. Instruct-ERIC, as coordinator of the RI-VIS project, was given the opportunity to present Instruct and what it can offer for researchers in structural biology at each event. In addition to the main symposia, a series of thematic satellite events were organised, allowing researchers and RIs in individual scientific fields to discuss their specific needs with policy makers in the region.

Building on the success of a similar satellite event to the Africa-Europe symposium organised in 2020, Instruct arranged a satellite event to the Latin America – Europe Symposium, titled "Strengthening the Structural Biology Community in Latin America". This two-hour meeting brought together structural biology researchers from across Latin America, as well as researchers in Europe and policy makers from both regions. The discussion was largely focused on how both regions can help each other, and how best to implement a research infrastructure-type hub in Latin America. More than 120 people attended the meeting, with more than 70% of participants joining from Latin America. Another satellite event to the Latin America Symposium was the SIRIUS Synchrotron Tour. Hosted by Ana Zeri of SIRIUS, the tour took participants virtually through the synchrotron, showcasing the beamlines, labs, and research teams.

Instruct Staff Exchange Call

As part of its ongoing collaboration with Latin America in the context of the EU-LAC ResInfra project, Instruct-ERIC opened a staff exchange call to allow researchers from Latin American institutions to visit European facilities. The call invited researchers from any Latin American institution which has an MoU with Instruct-ERIC to submit an application to utilise the equipment and machineries available at Instruct's European centres. Three researchers will be visiting Instruct-ERIC facilities throughout 2022, accessing the equipment and sharing best practises and guidance with the host institutions to further advance research and understanding not only of the structural biology techniques but also of the procedures to allow open access to the infrastructure.

Instruct 2nd International Call

The second Instruct-ERIC International Call was completed in 2021 – researchers from institutions that held an MoU with Instruct were eligible to apply. Applications were received both from researchers in Latin America and from South Africa. With COVID-19 restrictions potentially lifting over the course of 2022, the aim is that these international visits will be able to take place physically over the next year. Some visits from the 1st International Call are still due to take place, having been postponed as a result of the COVID-19 outbreak.

Memoranda of Understanding Signed

With collaboration with the EU-LAC ResInfra project, Instruct-ERIC has advanced its cooperation with institutions in Latin America. In 2021, Instruct signed 2 more MoU with institutions in Latin America, taking the current total to 10 institutions globally.

University of Buenos Aires (UBA) and University of San Martin (UNSAM), both in Argentina, were the institutions that signed with Instruct.

In conjunction with the RI-VIS Africa-Europe Symposium on Research Infrastructures, an MoU was also signed with the University of Cape Town (UCT) in South Africa which was also the local co-organiser of the symposium.

These MoU open the possibility for access provision between Instruct and the international facilities, but also for networking and training events.

In 2021 Instruct-ERIC held MoUs with the following international organisations

Instituto de Investigaciones Bioquímicas de La Plata INIBIOLP (CONICET-UNLP), Argentina

Instituto de Químicay Fisicoquímica Biológicas (Universidad de Buenos Aires), Argentina

Instituto de Biología Molecular y Celular de Rosario (IBR), Argentina

Fundacion Instituto Leloir (FIL, Buenos Aires), Argentina

University of Buenos Aires (UBA), Argentina

University of San Martin (UNSAM), Argentina

University of Sao Paulo (USP), Brazil

Institute Pasteur (Montevideo), Uruguay

University de la Republica (Montevideo), Uruguay

University of Cape Town (UCT), South Africa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SUPPORTING ACTIVITIES AND OUTPUTS

COMMUNICATIONS

Instruct-ERIC has developed its communications strategy over many years, identifying new tactics and platforms to ensure the brand is effectively publicised to as wide an audience as possible. As Instruct's reach grows beyond Europe, increasing its collaborative efforts in other regions of the world, such as Latin America and Africa, the communications have also evolved to incorporate new audiences.

Strategic communications

The impact of restricted movement and a reliance on virtual platforms continued throughout 2021, reinforcing the need for creative and effective digital communication strategies. Instruct continued to produce online content for the website, social media, through ARIA, and for other projects. Almost all events, workshops and meetings were held over online platforms, which required dissemination strategies aimed towards boosting virtual registrations, rather than in-person.

Communications activities

RI-VIS Social Media and Marketing Training Series

The RI-VIS project organised the Social Media and Marketing Training series, a weekly online seminar open to project managers and communication officers from research infrastructures across Europe to enhance their communication and social media expertise. The 12-week course, run by Clear Europe, took participants through the most effective way to use various techniques on several social media platforms to promote their own RI and to engage with others.

Instruct Conferences and Exhibitions

Instruct-ERIC was represented at a number of international conferences and exhibitions in 2021, despite these all taking place in a virtual format. This ensured that Instruct remained on the world stage of structural biology and research infrastructures, and was able to promote its services to a wider audience.

ICRI 2021: The 2021 ICRI (International Conference on Research Infrastructures) edition took place virtually, having initially been scheduled to take place in Canada. Instruct had a virtual booth at the conferenc and was invited to participate in the panel discussion "International Research Infrastructures, the way forward", which positioned Instruct as a knowledge leader on global research infrastructure.

FEBS 2021: Instruct participated at FEBS (Federation of European Biochemical Societies) 2021 alongside three other European Research Infrastructures to promote Instruct-ERIC individually and the collective of life science research infrastructures in Europe, manning the virtual booth and giving a presentation on each infrastructure.

CellBio 2021: Instruct gave a presentation at the virtual CellBio 2021, highlighting the training opportunities available at Instruct-ERIC, as part of the training programme of workshops and courses, as well as internships available to students and early career researchers.

EFMC-ISMC 2021: Instruct had a virtual booth at EFMC-ISMC (European Federation for Medicinal Chemistry - International Symposium on Medicinal Chemistry), providing visitors with information on how Instruct services can enhance their medical research.

Newsletter

The newsletter continued to provide a snapshot of Instruct news, events and partner project information. Both newsletters were well-received, providing users with an overview of what Instruct-ERIC had achieved in the previous 6 months. The theme of the summer newsletter was “Data Resources and Open Science”, regarding the importance of data accessibility when physical research access was made more difficult by the pandemic. The winter newsletter focused on international collaboration, which had been a major theme for Instruct in 2021.

Social media

The Instruct Twitter account has continued to grow in stature, both in terms of followers and engagement reaching more than 5,000 followers in 2021. The account has benefitted from coordinated posting efforts during events, webinars and meetings, as well as the opportunity to engage with Twitter accounts set up specifically for Horizon Europe projects. Increased social media activity from these projects, as well as from other research infrastructures as virtual communication increased throughout the year, led to a boost in followership and engagement for Instruct.

Scientific Highlights

Scientific highlights are an evergreen source of high-engagement communication material. The combination of fresh, high-quality science mixed with the social media benefit of engaging with the facilities/centres in question makes science highlights an important part of Instruct’s communication strategy. In 2021, the vast majority of highlights revolved around COVID-19, and the part Instruct facilities played in combatting it. As COVID is still such a key topic around the world, being able to showcase Instruct’s role in studying the virus is a crucial communication tool.

Webinars

The Instruct-ERIC Structure Meets Function Webinar series has gone from strength to strength in 2021. 10 webinars were organised: 6 representing Instruct centres 4 from Instruct’s non-centre members. Across the 10 webinars, more than 1,200 attendees joined, providing an engaged audience, bountiful Q&A sessions and a boost in social media activity. The webinars are recorded and displayed on the Instruct-ERIC website, which draw hundreds of views organically and on social media.

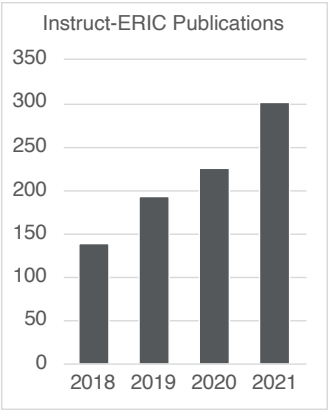
FIG 2. Instruct-ERIC Scientific Highlights are popular on Twitter.

SCIENTIFIC PUBLICATIONS

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

A key performance indicator of success in this endeavour is the number of 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 2021, the list of peer-reviewed publications totalled 301 – even higher than the number achieved in 2019 and 2020. This emphasises both the impact of Instruct-ERIC on the structural biology community, offering access for researchers to European infrastructure, technology and equipment. This is then made even more significant as physical access was still heavily impacted by travel restrictions due to the pandemic.



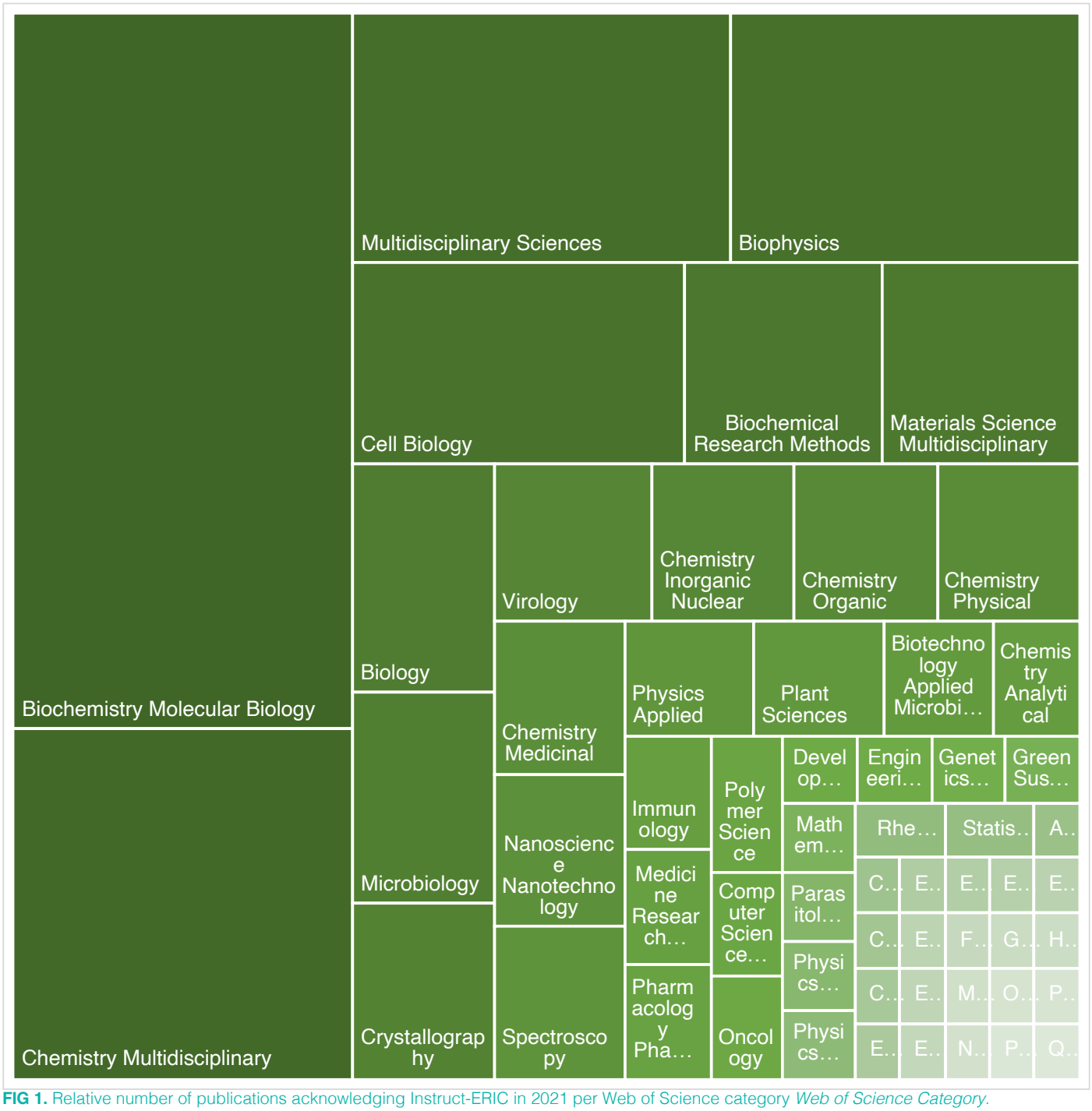


FIG 1. Relative number of publications acknowledging Instruct-ERIC in 2021 per Web of Science category *Web of Science Category*.

These publications came in 153 different journals, featuring researchers from more than 57 different countries. This is indicative of Instruct's presence within the global structural biology community – and is further emphasised by Instruct's growing international reputation. Instruct's breadth of service and technology offering is further underlined by the journals covering 58 different categories within structural biology – researchers can access the infrastructure for their precise research needs through Instruct.

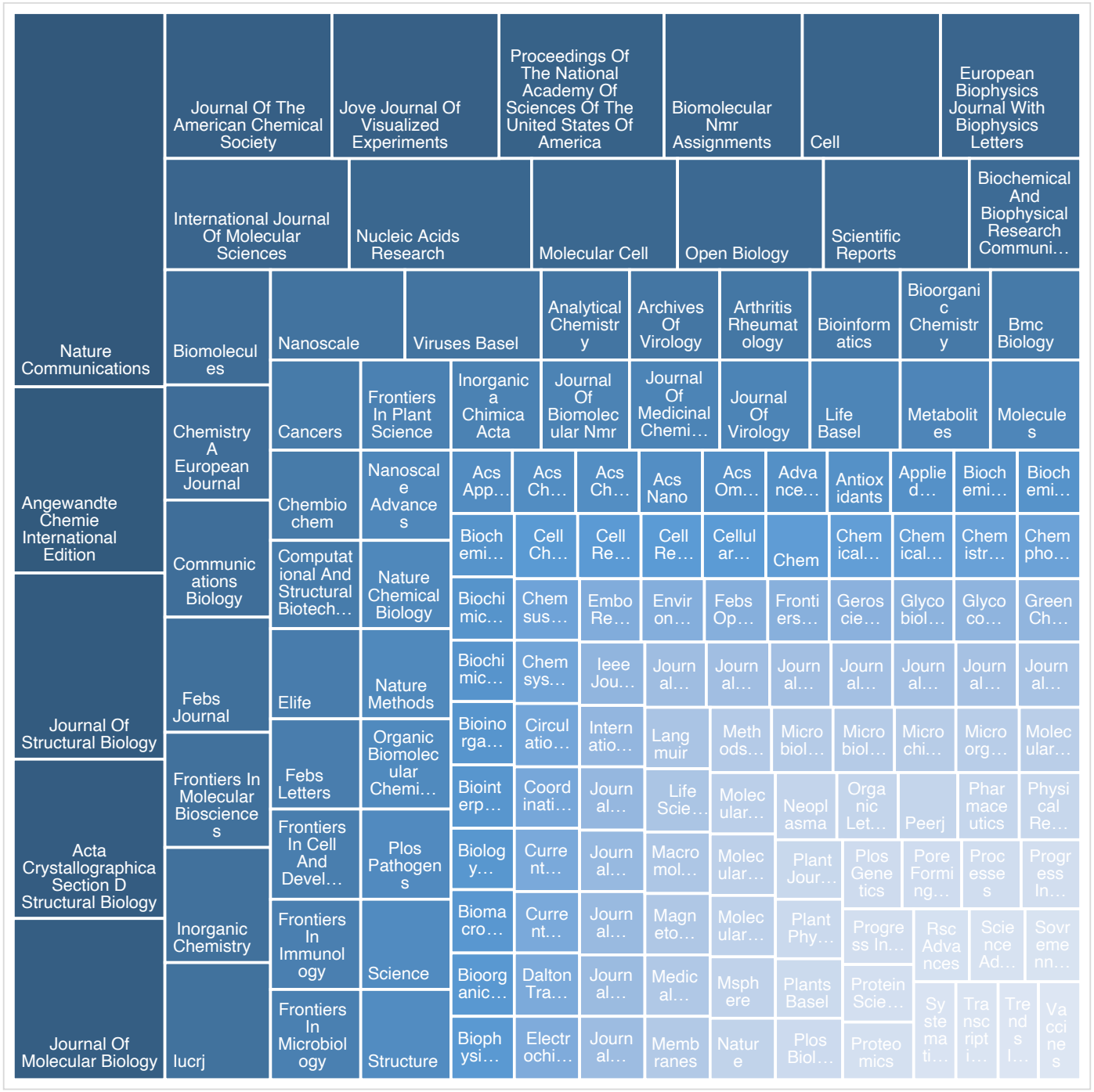


FIG 1. Relative number of publications acknowledging Instruct-ERIC in 2021 per Web of Science category *Publication Totle*

Instruct-ERIC science highlights are periodically added to the website. These offer a snapshot of the hundreds of publications acknowledging Instruct every year, showcasing the latest research from Instruct centres.

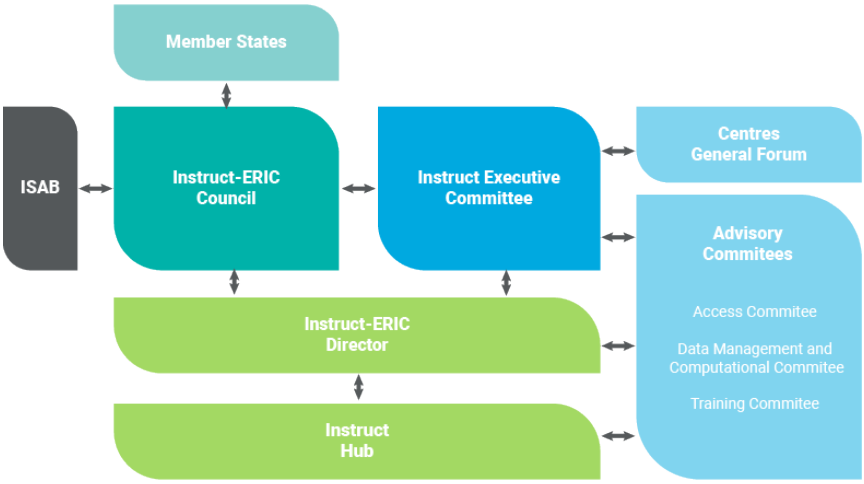
In 2021, most highlights revolved around COVID-19, and the part Instruct facilities played in combatting it. The role of research infrastructures and scientific networks could not be made clearer than in the COVID-19 outbreak; the science highlights demonstrate how collaborations between researchers were able to provide swift and effective action in understanding and controlling the virus.

GOVERNANCE

The Instruct-ERIC Council continued to be chaired by Eric Guittet (FR), with Sarah Butcher (FI) replacing Inmaculada Figueroa (ES) as Vice-Chair. The Independent Scientific Advisory Board (ISAB), chaired by Stephen Burley remained in place to advise the Council.

The Instruct-ERIC Council concluded their search for the next Instruct Director with the expectation to have the appointee in place early 2022. The Hub Coordinator gave notice to leave Instruct and a search for candidates was initiated late 2021 by the Director with the expectation that the appointment would be made by the incoming Director in 2022.

An important task for Council was to prepare for the second Instruct (5-year) funding cycle, to start from 1st August 2022. A number of policy issues concerning the future financial commitment from members, the management of the access budget, future priorities for ARIA development and internationalisation all need decisions by Council as part of the preparatory work.



The status of the UK as a participant in H2020 and Horizon Europe framework projects was unchanged from 2020, and the Instruct-ERIC statutory seat and Coordination Hub remained in the UK with all the appropriate taxation benefits implemented.

Scientific direction and implementation of the Instruct objectives continued to be managed by the Executive Committee, Chaired by the Director. The Committee worked on the preparation of the Quinquennial Report (QQR) with a delivery timeline of November 2021 and agreed the launch of a call for Instruct Research Sites, a new initiative to include all members in training, networking and event organisation for Instruct to increase visibility for all members in the RI Landscape.

The impact of Instruct on SARS CoV-2 research continued with 21 SARS CoV-2 related Instruct publications (out of a total of 301) in press in 2021.

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 formed a number of task forces and working groups to review data handling by users and facilities, and web tools with a plan to report to the Executive Committee in Q1 2022.

Working Group/Panel/Project	Role	Representatives
ERIC Forum	Member	Dave Stuart /deputised by Susan Daenke
LS RI Strategy Board	Member/Chair [until July 2021]	Susan Daenke
Transvac2 Steering Group	Member	Susan Daenke, Francesca Morelli
iNEXT-Discovery Management Committee	Member	David Stuart
iNEXT-Discovery Executive Board	Member	Susan Daenke
EOSC-Life Executive Board	Member	Dave Stuart /deputised by Susan Daenke
RI-VIS General Assembley	Chair	Susan Daenke
RI-VIS Steering Group	Member	Susan Daenke and Natalie Haley

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 (Q1/2 2021) / Sarah Butcher, FI (Q3/4 2021)

Hub support: Susan Daenke, Instruct Hub

Country	Scientific Delegate	Administrative delegate
Belgium	Michele Oleo	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 Pavoncello
Latvia	Kaspars Tars	Uldis Berkis
Lithuania	Gintaras Valincius	
Netherlands	Reinout Raijmakers	Nienke Klomp
Portugal	Maria Armenia Carrondo	Marta Abrantes
Spain	Jose Maria Carazo	Inmaculada Figueroa
Slovakia	Milos Hricovini	Barbora Liptakova
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: 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, Max IV, 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 : David Stuart (Instruct Director)

Vice-Chair: 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 EMBL	Stephen Cusack	Matthias Willmans
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	Michal Sharon	Joel Sussman
Instruct IT	Lucia Banci	Roberta Pierattelli
Instruct NL	Rolf Boelens	Anastassis Perrakis/Ariane Briegel
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: Dino Moras

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: Pauline Audergon

Hub support: John Dolan

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: Natalie Haley & Pauline Audergon

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, provideing 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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FINANCIAL DATA

FINANCIAL DATA

This report presents the financial statements for the period 1 January 2021 to 31 December 2021.

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 2021 – 31 December 2021 which concided with the SARS-CoV-2 pandemic which imposed a significant impact on Instruct-ERIC activities.

BALANCE SHEET FOR INSTRUCT-ERIC

As at 31 December 2021

Assets	GBP	EUR	Notes
Euro bank	897,809	1,069,597	
Sterling bank	123,059	146,605	
Total Bank	1,020,868	1,216,202	
Current Assets			
Accounts Receivable	755,242	899,751	1
Prepayments	8,056	9,597	
Accrued income	38,454	45,812	2
Rental deposits	3,767	4,488	
Total Current Assets	805,519	959,648	
Fixed Assets			
Computer Equipment	20,384	24,284	
Depreciation on Computer Equipment	(15,556)	(18,533)	
Office Equipment	4,126	4,915	
Depreciation on Office Equipment	(3,249)	(3,871)	
Total Fixed Assets	5,705	6,795	
Total Assets	**1,832,092**	**2,182,645**	

Liabilities	GBP	EUR	Notes
Current Liabilities			
Accruals	11,083	13,204	
Amounts to be paid and Unclaimed Access Awards	650,761	775,279	3
Income in Advance - Members subscription	794,991	947,106	4
Income in Advance - Other inc deferred grants	271,779	323,781	5
Income in Advance - ARIA Support	3,848	4,584	
Other creditors	4,783	5,698	
Payroll taxes due	10,463	12,465	
Pensions due	6,911	8,233	
Total Current Liabilities	1,754,619	2,090,350	
Total Liabilities	**1,754,619**	**2,090,350**	
Net Assets	**77,473**	**92,295**	
Surplus Brought Forward	290,214	325,103	
Exchange rate movement - revalue opening reserves	(17,325)	-	6
Surplus for the Year	(195,416)	(232,808)	
Surplus Carried Forward	77,473	92,295	

Exchange rate for reporting period: 0.83939

1. Membership income receivable

2. Grant income recoverable at year end

3. Access and other service accruals

4. Invoiced deferred membership subscriptions

5. Deferred project income

6. Revalue opening reserves from prior year exchange rate to the exchange rate used for current reporting period.

PROFIT AND LOSS FOR INSTRUCT-ERIC

For Year Ended 31 December 2021

Income	GBP	EUR	Notes
External grant income	260,504	310,349	7
External grant overhead contribution income	65,160	77,628	
Member state contributions	881,827	1,050,557	8
Other miscellaneous income	16,788	20,000	9
Total Income	1,224,279	1,458,534	
Less Cost of Service Provision			
Instruct staff salaries	235,397	280,438	
R&D Pilot awards	116,402	138,675	
JRA awards	-	-	
Access Cost	513,534	611,794	
Instruct Centre Cost	-	-	
Meetings	3,034	3,615	
Project activities	248,367	295,890	10
Total Cost of Service Provision	1,116,734	1,330,412	
Gross Surplus	107,545	128,122	
Less Operating Expenses			
Commissioned services (Insurance, financial, HR, legal)	33,291	39,661	
Conference costs	-	-	
Consultants	129,248	153,979	11
Recruitment costs	30,088	35,845	
Depreciation charge	8,153	9,713	
Foreign Currency (Gains)/Losses	(3,210)	(3,824)	
General admin (postage, copying, bank charges)	1,407	1,676	
Licenses & software	17,478	20,822	
Miscellaneous	10,366	12,350	
Office Stationery	217	258	
Premises and support	56,667	67,510	
Project overhead expenses	17,387	20,714	
Publicity	587	699	
Telephone	1,282	1,527	
Write off IASL balance owed	-	-	
Total Operating Expenses	302,961	360,930	
Net Surplus	**(195,416)**	**(232,808)**	

7. Project income including 25% contribution to Instruct-ERIC overheads, against expenditure

8. Membership income receivable

9. ARIA support

10. WIP on research grants. Project activities delivered.

11. Staff costs recharged from the University of Oxford and EMBL

SUPPORTING INFORMATION FOR THE FINANCIAL STATEMENTS

Accounting Policies

The financial statements are prepared under the historical cost convention, and 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 01/01/21 - 31/12/21	Payment received
UK	107,005	107,005
FR	107,005	107,005
ES	80,253	80,253
IT	80,253	80,253
BE	80,253	80,253
NL	80,253	80,253
IL	80,253	80,253
CZ	53,502	53,502
PT	53,502	53,502
DK	53,502	53,502
SK	53,502	53,502
LV	53,502	53,502
FI	53,502	53,502
LT	53,502	53,502
Total	989,789	989,789

Grant Recipts

EU Grants	Income Jan - Dec 2020	Other income from Projects
Transvac2	2,797	699
EOSC-Life	133,932	33,483
ERIC Forum	11,952	2,988
RI-VIS	110,037	27,509
EU-LAC ResInfra	36,656	9,164
iNext Discovery	13,174	3,293
TRANSVAC-DS	634	158
Total	309,182	77,294

Overhead contribution recognised: 25%

C. Deficit/surplus on activities (€232,808)

D. Employees

EMBL in-kind contribution in lieu of membership fees supported 1 FTE Trainee Project Manager.

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 2021 invoices) €899,751
Other accrued income €9,597
Grant accrued income €45,812

F. Creditors

Accruals for services and awards (Access, Internships, R&D, Training, unclaimed access) €788,483
Advances on Research Grants €323,781
Advances on Member Subscription €947,106
Advances on Grant Income €323,781
Other creditors €10,282
Payroll taxes and pensions €20,698

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:
RI-VIS: €1,500,000 (total value) – start date 01 February 2019, end date 31 Jan 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: €464,862
ERIC Forum: €43,300
EU-LAC ResInfra: €106,875
iNext-Discovery: €147,500
TRANSVAC-DS: €14,375
BY COVID: €45,875

New awards
ISIDORe: €606,488 (start date: 01/02/2022)
eRImote: €272,375 (start date: 01/06/2022)
canSERV: €1,376,753 (start date: 01/09/2022)
EOSC4Cancer: €82,500 (start date: 01/09/2022)
AIALife: €34,500 (start date: 01/09/2022)

ABBREVIATIONS AND GLOSSARY

Term	Definition
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.
AF4	Asymmetrical flow field-flow fractionation
AI4Life	AI4LIFE aims to build bridges between the life science community and the machine learning/ artificial intelligence community
API	Application Programming Interface
ARIA	Access to Research Infrastructure Administration: Instruct-ERIC's access management system.
AUC	Analytical Ultracentrifugation
Bio-SAXS	Biological small angle X-ray scattering
BIOCEV	Biotechnology and Biomedicine Centre (Czech Republic)
BREXIT	Brexit was the withdrawal of the United Kingdom from the European Union and the European Atomic Energy Community on 31 January 2020.
BY-COVID	The BeYond-COVID project aims to make COVID-19 data accessible to scientists in laboratories but also to anyone who can use it, such as medical staff in hospitals or government officials.
canSERV	canSERV's mission is to make cutting-edge and customised research services available to the cancer research community EU wide, enable innovative R&D projects and foster precision medicine for patients benefit across Europe.
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
CBI	Center of Integrative Biology (France)
CD	Circular dichroism
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
COVID-19	Coronavirus disease caused by the SARS-CoV-2 virus
CPU	Central processing unit
CRISPR-Cas9	Natuarally occuring gene editing system in bacteria now used by geneticists to alter genome sequences in other cell types organisms.
CSIC	Spanish National Research Council
DLS	Dimond Light Source (UK)
DMCC	Data Managementand Computational Committee
eBIC	Electron Bio-Imaging Centre (UK)
EC	The European Commission

ABBREVIATIONS AND GLOSSARY CONTINUED

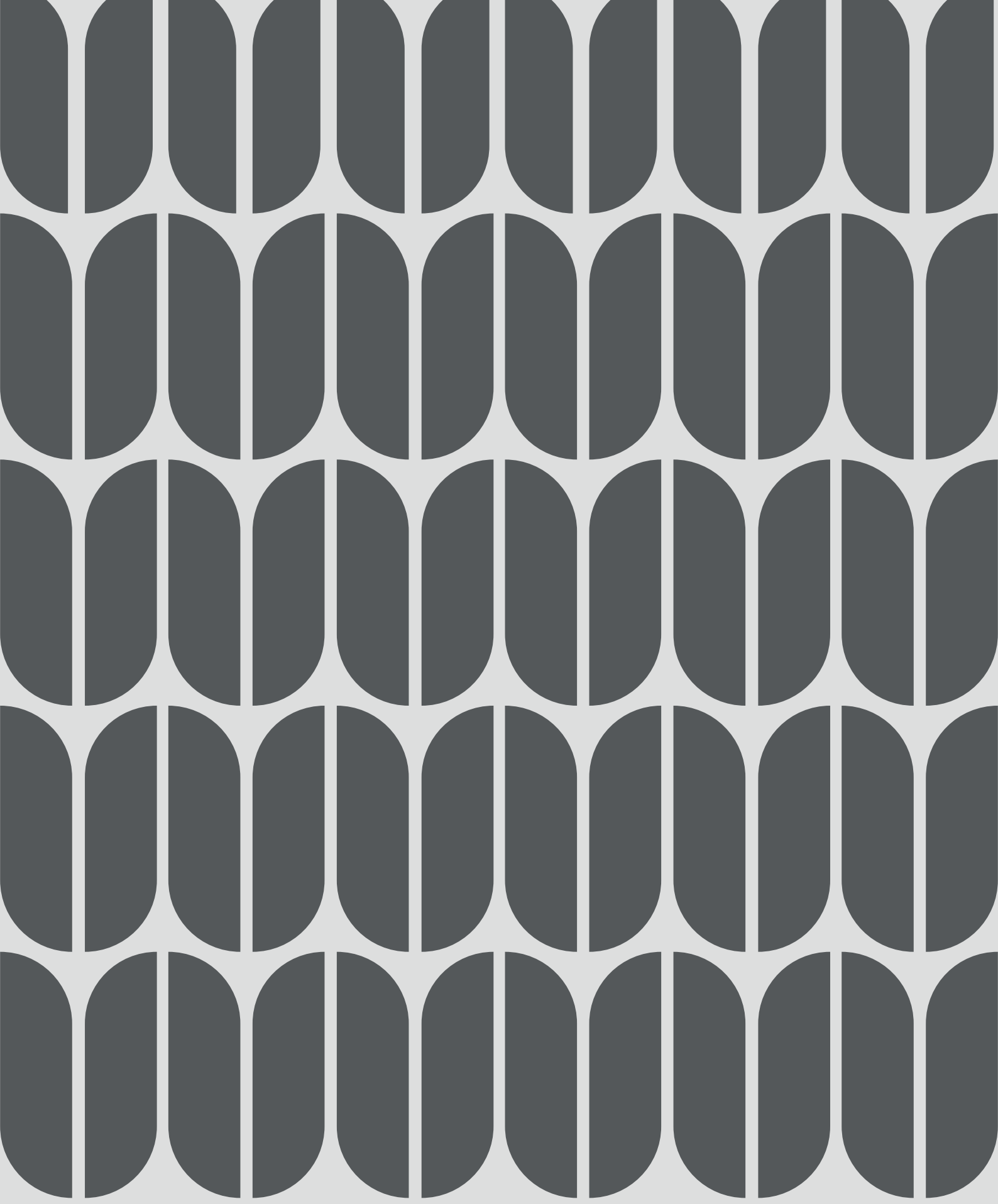
Term	Definition
EM	Electron Microscopy
EMBL	The European Molecular Biology Laboratory: an intergovernmental organisation specialising in research in the life sciences, funded by its 20 member states.
ENRIITC	The ENRIITC project aims to build a permanent pan-European network of Industrial Liaison and 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.
EOSC4Cancer	EOSC4Cancer will make cancer data accessible, using and enhancing existing federated and interoperable systems for securely identifying, sharing, processing and reusing FAIR cancer data
EPR	Electron Paramagnetic Resonance
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.
eRImote	eRImote considers solutions for digital and remote service provision across RI domains.
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.
ESPRIT	Soluble Proteins by Random Incremental Truncation
ESRF	The European Synchrotron Radiation Facility (France)
ET	Electron Tomography
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.
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.
FTIR	Fourier transform infrared
GDPR	General Data Protection Regulation
H2020	Horizon 2020 is the biggest EU Research and Innovation programme, making €80 billion of funding available over 7 years.
HDX	Hydrogen Deuerium exchange
HTX	High-throughput crystallisation
I2PC	Instruct Image Processing Center (Spain)
IBS	Institute of Structural Biology (France)
IceBear	Integrated Crystal-data-tracking Enhancing Biochemistry Education And Research Software
I GBMC	The Institute of Genetics and Molecular and Cellular Biology (France)

ABBREVIATIONS AND GLOSSARY CONTINUED

Term	Definition
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.
ISAB	Independent Scientific Advisory Board
ISAL	Instruct Academic Services Limited
ISBG	Integrated Structural Biology Grenoble (France)
ISIDORE	The ISIDORe project provides research services from structural biology through to clinical trials to support infectious disease epidemic research including SARS-CoV-2.
ISO	International Organisation for Standardisation
ISPyB	Laboratory Information Management System used for sample tracking and experiment reporting at synchrotron beamlines
ITC	Isothermal titration calorimetry
ITQB	Institute of Chemical and Biological Technology (Portugal)
JRA	Joint Research Award
KPIs	Key Performance Indicators
LMJ	Liquid-metal-jet
LS RI	Life Science Research Infrastructures
MALDI	Matrix Assisted Laser Desorption/Ionisation
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.
MoU	Memoranda of Understanding
MS	Mass Spectrometry
MST	Microscale Thermophoresis
MX	Macromolecular Crystallography

ABBREVIATIONS AND GLOSSARY CONTINUED

Term	Definition
NeCEN	Netherlands Centre for Electron Nanoscopy
NKI	Netherlands Cancer Institute
NMR	Nuclear Magnetic Resonance
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.
OPIC	Oxford Particle Imaging Centre (UK)
PAINT	Points accumulation for imaging in nanoscale topography
PALM	Photo-activated localization microscopy
PCR	Polymerase chain reaction
PDB	Protein Data Bank
PID	Proposal Identification number
Proposal	A user's request for access to technology or other services.
R&D	Research and development
RBD	Receptor-binding domain
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	Research Infrastructure
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
SEC-MALLS	Size Exclusion Chomatography - Multi-Angle Laser Light Scattering
SEM	Scanning electron microscope
SPC	The Israel Structural Proteomics Center
SPR	Surface Plasmon Resonance
SPU	Structural Proteomics Unit
Stakeholder	A person, or group of people with an interest or concern in Instruct-ERIC.
STORM	Stochastic optical reconstruction microscopy
TEM	Tansmission electron microscopy
TRANSVAC-DS	The TRANSVAC-DS project aims to consolidate the conceptual and technical design and ultimate implementation of a European vaccine R&D infrastructure.
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.
WIS	Weizmann Institute of Science (Israel)



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