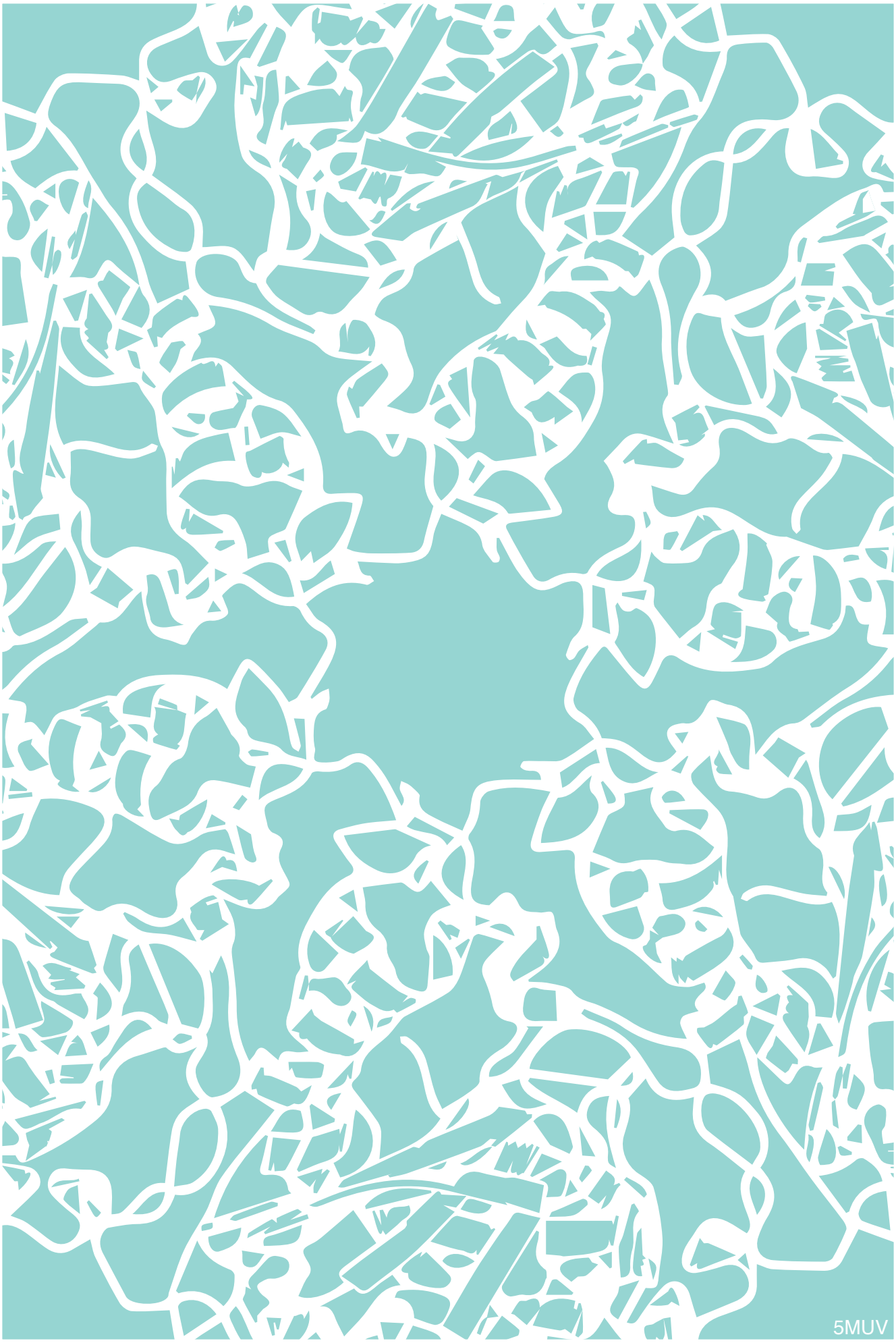
2019 INSTRUCT-ERIC ANNUAL REPORT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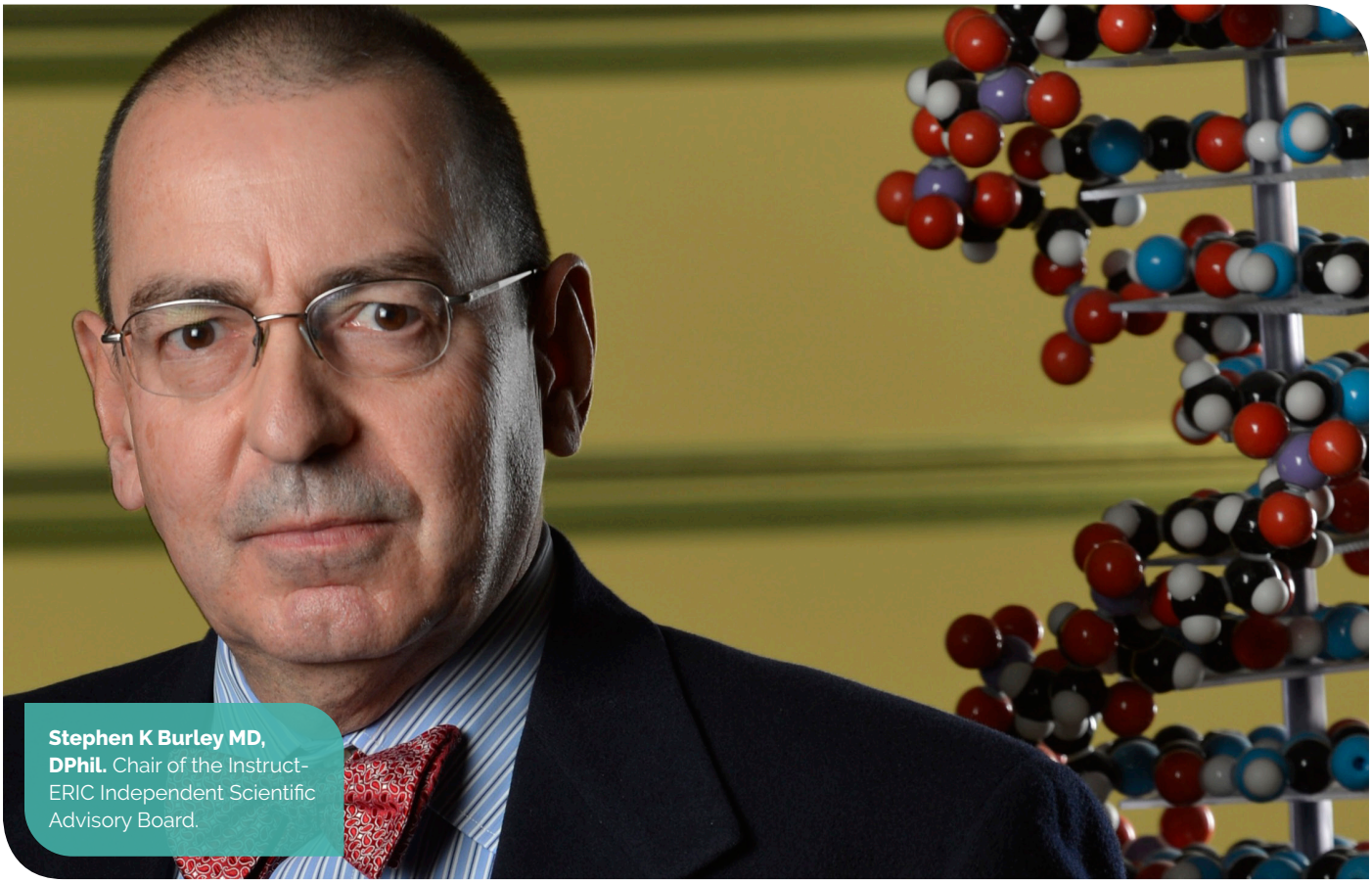
FOREWORD BY CHAIR OF THE INDEPENDENT SCIENTIFIC ADVISORY BOARD



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

As Chair of the Instruct-ERIC Independent Scientific Advisory Board, it is an honour to contribute this foreword to the 2019 Instruct-ERIC Annual Report.

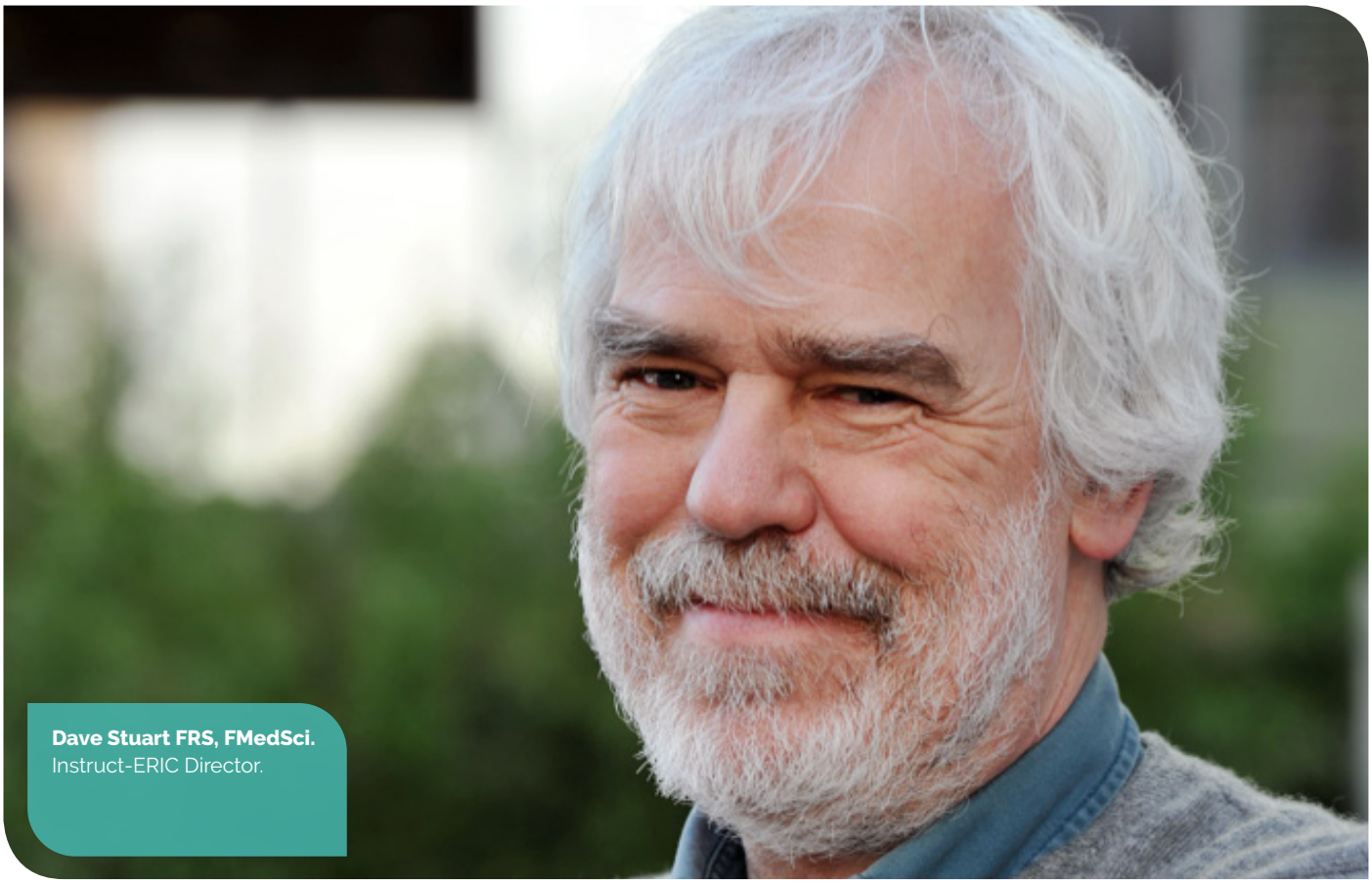
Instruct remains faithful to its overarching objective of fostering technical innovation and increasing the impact of structural biology on scientific achievements. The Instruct-ERIC Hub coordinates the work of nine science and technology centres providing user training and access to cutting-edge measurement techniques. Provision of high-cost instrumentation by many of the thirteen Member countries, individual Member resource and training commitments, and shared research infrastructure represent core elements of the Instruct mission.

European researchers play leading roles in structural and functional studies of biological macromolecules using synchrotron crystallography, nuclear magnetic resonance spectroscopy (NMR), and single-particle cryo-electron microscopy and cryo-electron tomography. During 2019, Instruct-ERIC helped structural biologists, their trainees, and collaborators contribute thousands of exciting new atomic-level, three-dimensional structures of macromolecules to the open access Protein Data Bank, informing every area of biology and medicine. Of particular importance are structures coming from studies of biological and biochemical systems in situ. State-of-the-art NMR instruments are being used to characterise proteins as they function inside living cells. Experimental measurements with X-ray free electron lasers are revealing the dynamics and structural nuances of enzyme catalysed chemical reactions as they occur in real time. New instruments coming from materials science are enabling structural studies of macromolecular machines inside cryogenically preserved cells at near atomic resolution. Looking ahead, Instruct researchers are working together with colleagues around the world to establish a new discipline known as integrative structural biology. This pioneering approach combines experimental measurements from multiple techniques with quantitative analysis tools to tackle ever larger biomolecular systems and study ever more complex biological phenomena that no one method alone can address.

Instruct-ERIC and its constituent Centres comprise a consortium wherein scientific excellence is the qualifying prerequisite. The organisation is similarly committed to high quality training for users of Instruct facilities. Of paramount importance are efforts aimed at ensuring the broadest possible access to infrastructure for robust sample preparation and experimental characterisation. Instruct researchers play critical roles in driving forward both development and implementation of methods that enable interdisciplinary research in fundamental biology, biomedicine, biotechnology and bioenergy for the benefit of all humanity.

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

FOREWORD BY INSTRUCT-ERIC DIRECTOR



Dave Stuart FRS, FMedSci.
Instruct-ERIC Director.

It is sobering to write the forward to the 2019 Instruct-ERIC Annual Report, looking back from a world shaken in 2020 by a viral pandemic. It is important to remember the longer trajectory and the period this report covers shows the steadily increasing power of integrated structural biology. During this period, political uncertainties around Brexit did not slow the growth of Instruct-ERIC – two new members, Latvia and Finland, joined, with Lithuania and EMBL almost there! It is a particular delight to welcome Finland, a country associated with Instruct from the very early days, that played a significant role in formulating our underlying precepts.

The underpinning methods of structural biology, NMR, X-ray crystallography and cryo-electron microscopy (cryo-EM) all continue to develop, although the greatest advances continue to come in electron imaging, with the arrival of the next generation of direct electron detectors substantially increasing throughput. Instruct continues to strive to democratise access to the leading equipment simply on the basis of scientific excellence, and to maintain Europe at the forefront technically. 2019 saw significant steps forward, as highlighted in the reports from the Centres. Especially notable on the cryo-EM front was the installation of the first high end 300 kV microscope in Spain, complemented on the sample side by the publication from the Belgium Centre published, with the Aricescu group, of a beautiful paper on the first successful exploitation of Megabodies: engineered larger versions of nanobodies designed to act as structure determination aids for cryo-EM. Alongside access and technical developments, Instruct carried on with very successful additional programmes, including training, internships and R&D awards. The 2019 Biennial held in Alcalá de Henares, Spain, was a huge success, with the prestigious Bertini award given to Wolfgang Baumeister, and an inspiring Women in Science workshop. Of course, the overall driver of Instruct is enabling science, and in 2019 the effectiveness is seen in the 193 papers published citing Instruct. Some highlights of the science are given in this report.

Finally, Instruct is critically dependent on the people who work together to build this success, ranging from our Council and Executive Committee, to the most important people on the ground in the Centres and the Hub. As a distributed infrastructure, the Centres are critical to Instruct, and the Hub, although quite small, not only pulls together all the activities I have mentioned and keeps them running smoothly, but also develops the ARIA software that underpins much Instruct activity and is central to a variety of EU-funded projects, for instance Instruct-ULTRA, which has helped build the sustainability of Instruct. In addition, the Hub engages with users, stakeholders and policy makers to keep Instruct a successful part of the mosaic of European research infrastructures. So especial thanks to everyone at the Hub and Centres for their commitment to the project through difficult times. At a time when there is a lot resting on science globally, it is crucial to maintain our vision of open international collaborative research, driven by scientific excellence.

LOOKING FORWARD

2019 saw strong progress in Instruct-ERIC operations, underpinning the key objectives to deliver excellent science for our user community and to elevate skills in structural biology through our training programme. Two new members, Latvia and Finland, joined Instruct-ERIC, with Lithuania and EMBL in the final stages of achieving membership as the year closed.

Initiatives to prepare for the next 5-year funding cycle of Instruct-ERIC (due to begin in July 2022) were started, with the formation of the Landscape Group who will review the state of structural biology facilities in Europe and Instruct's role within this.

This year has already seen significant expansion of Instruct facilities with the addition of the Astbury Centre in Leeds to Instruct Centre UK, and several new facilities made available through the three sites of Instruct Centre Finland. In addition, a test period for a revised access funding model was concluded in December and the results provided a strong case to retain the revised model for general implementation.

Finally, Instruct-ERIC has benefitted from the excellent work undertaken through Instruct-ULTRA,[1] and the legacy of this work will carry forward into more efficient planning processes with the implementation of our updated business and sustainability plans, and an active outreach programme to potential members and collaborators.

Thanks go to everyone who enables Instruct to provide excellent services through its Centres and the Hub. We look forward to 2020 with confidence and enthusiasm to continue to expand and improve the experience of those using and working with Instruct-ERIC.

Susan Daenke
Instruct-ERIC Hub Coordinator

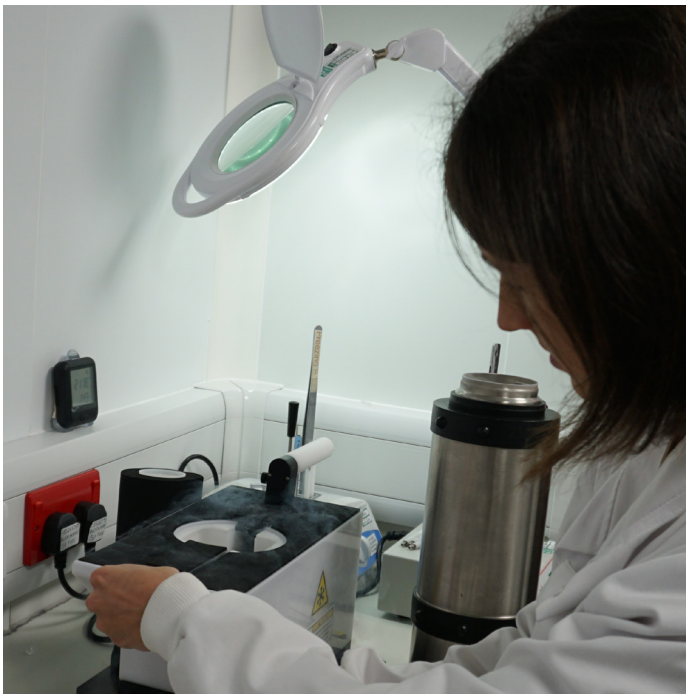
1. Instruct-ULTRA is a Horizon 2020 implementation project for Instruct-ERIC. The project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 731005.

EXECUTIVE SUMMARY

Proposals received	Publications	Average journal impact factor	Additional external funding	Students and researchers trained	Days of access provided
122	193	8.2	€775 257	123	782

DELIVERY OF EXCELLENT SCIENCE

2019 has been characterised by a significant expansion of the Instruct infrastructure made available to researchers. In the X-ray technology area, this includes the availability of Megabodies to potentiate structural characterisation of membrane proteins; an increase in automation and optimisation of conditions for crystal growth and sample handling. In the NMR field, the installation and commissioning of a 1.2 GHz instrument has been planned and is expected to be completed in 2020. This will enhance capabilities to provide NMR spectra of exquisite resolution, including detection methods for intrinsically disordered proteins. In addition, the development of a modular bioreactor for live cells allows NMR measurements to monitor biological events such as protein folding, ligand binding and protein-protein interactions in live cells. In the microscopy and imaging area, improved detector technology and fast data pre-processing continues to deliver 3D structural information for many sample types. The introduction of cellular imaging which allows ultra-thin sections to be stained by immunolabeling, and cryo-correlative light EM (cryo-CLEM) is also new in the Instruct infrastructure catalogue of technologies.



In 2019, proposal submissions were similar to 2018 with 122 full proposals received, delivering 782 units of access. 65.5% of these were transnational, demonstrating the strong delivery of infrastructure to researchers across Europe. Publications arising from Instruct activities continued to be strong with 191 publications acknowledging Instruct services, of which 25.4% were in high impact (>10 journal impact factor) journals.

External income from EU funded grants and ARIA revenue increased to €775,257, again showing the competitiveness of Instruct in attracting external funding and the potential for a modest but helpful revenue stream from ARIA.

TRAINING

An important component of Instruct's work is to support students and early researchers using structural biology approaches in their research. We launch a call for training courses each year and aim to cover the major fields in which there is demand in the community, as well as some complementary courses to help career development.

In 2019, a total of nine courses/workshops were offered and each of these were in high demand and very well attended. Feedback gathered from participants showed a high satisfaction rating.

Our first 'Women in Science' workshop was held in Alcalá de Henares in Spain and was especially well received. Funded through Instruct-ULTRA and attracting excellent speakers from academia and industry, it championed women who have had successful careers in science and highlighted some of their experiences. A second workshop in this series is planned for 2021.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INTRODUCTION

David S. Goodsell, RCSB Protein Data Bank. doi: 10.2210/rcsb_pdb/goodsell-gallery-020

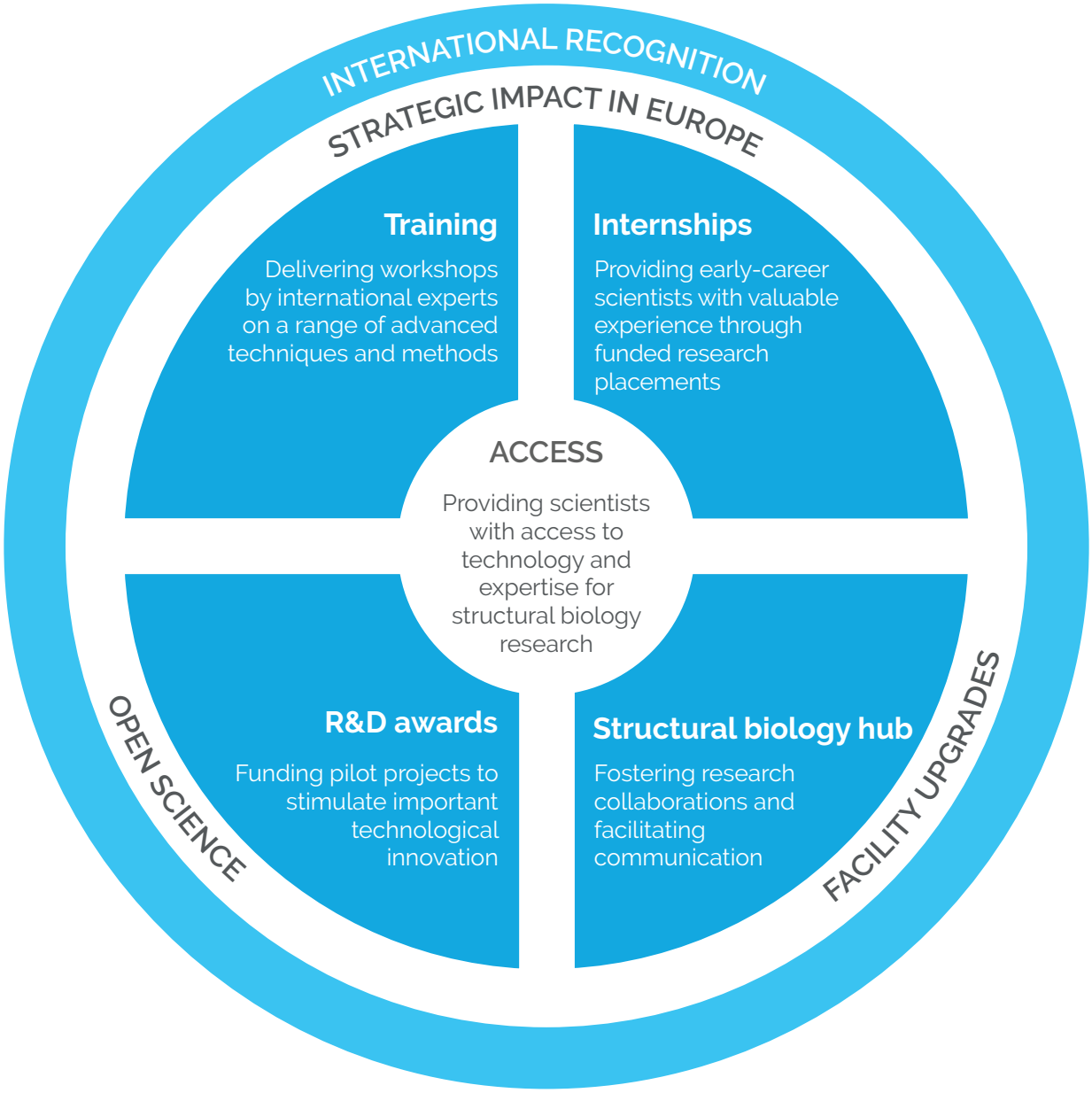
A GROWING COMMUNITY

Structural biology is developing rapidly, from characterising the structures of single molecules, to understanding and being able to predict the behaviour of biological systems in four dimensions. Structural biology uses a powerful set of tools to reveal fundamental biological structure-function relationships and this knowledge can be used to propose new intervention or prevention measures in models of health and disease.

Instruct-ERIC is a distributed research infrastructure making high-end technologies and methods in structural biology available to users. Instruct promotes innovation in biomedical science and operates on a non-economic basis according to the ERIC Regulation.

Instruct-ERIC brings together a remarkable organisation of high-quality instrumentation and expertise, and provides open access to cutting-edge structural biology technologies and methods - particularly championing the value of integrated approaches and technologies. Its mission to promote innovation in biomedical sciences and facilitate high impact science is underpinned by the following principles:

- Scientific excellence is a priority in offering Instruct services in support of structural biology research
- Transparency, equality and legality are the cornerstone of Instruct's operational model.



As of 31 December 2019, Instruct-ERIC has 13 Member Countries: Belgium, Czech Republic, Denmark, Finland, France, Israel, Italy, Latvia, Netherlands, Portugal, Slovakia, Spain and United Kingdom, and two Observers: Greece and EMBL.

MEMBERSHIP

Instruct-ERIC membership continues to grow, with the addition of Latvia and Finland in 2019, bringing membership to 13 countries (Fig 1). The membership fee is unchanged from the founding tiered model of cash contribution with in-kind contributions sustaining much of the infrastructure hardware and staff support at Instruct Centres.

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

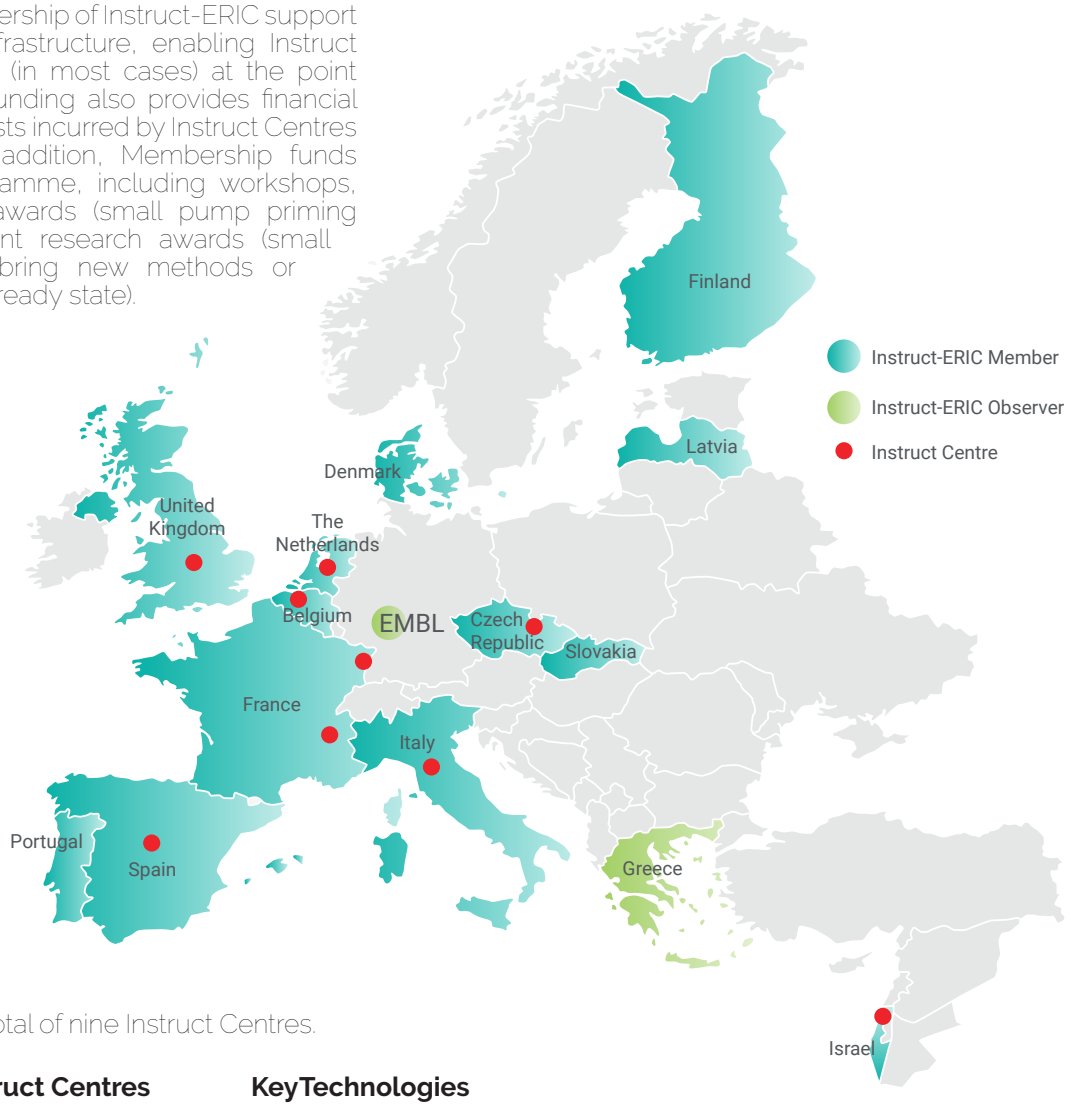


FIG 1. Map showing Instruct-ERIC Member Countries (Greece and EMBL are retained as Observers) and Instruct Centres.

Member Countries host a total of nine Instruct Centres.

Instruct Members	Instruct Centres	KeyTechnologies
Belgium	Instruct Centre BE	Nanobody production and testing
Czech Republic	Instruct Centre CZ	Cryo-EM, cryo-ET, high-field NMR, X-ray, structural MS
Denmark	-	-
Finland	-	-
France	Instruct Centre FR1	Specialist sample preparation, cryo-EM
	Instruct Centre FR2	Sample preparation, ESPRIT, NMR, MS
Israel	Instruct Centre IL	Sample preparation, bioinformatics
Italy	Instruct Centre IT	Advanced high field NMR and relaxometry, in-cell NMR
Latvia	-	-
Netherlands	Instruct Centre NL	High field NMR, specialist MS, cryo-EM, biophysical methods
Portugal	-	-
Slovakia	-	-
Spain	Instruct Centre ES	Advanced cryo-EM software for imaging
UK	Instruct Centre UK	Cryo-EM, X-ray methods, sample preparation, MS, computational analysis

Centralised coordination of Instruct service delivery is undertaken by the Instruct Hub located in the UK, currently comprising a small, variable core staff of up to six members led by the Instruct Director and supported by membership funds for a total of up to 3.55 FTEs. An additional 13 staff members make up the Hub team and work on EU-funded projects in which Instruct-ERIC is the Coordinator (Instruct-ULTRA, RI-VIS) or a beneficiary participant. Three members of the core team and those working on the Instruct-ULTRA grant are seconded to Instruct-ERIC from the University of Oxford. Instruct-ERIC directly employs 12 staff members.

MEMBERSHIP RATES

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

Member Country	Group	YR 1	YR 2	YR 3	YR 4	YR 5	SUM YR 1-5
UK	A	100.00	102.00	104.04	106.12	108.24	520.40
FR	A	100.00	102.00	104.04	106.12	108.24	520.40
ES	B	75.00	76.50	78.03	79.59	81.18	390.30
IT	B	75.00	76.50	78.03	79.59	81.18	390.30
BE	B	75.00	76.50	78.03	79.59	81.18	390.30
NL	B	75.00	76.50	78.03	79.59	81.18	390.30
IL	B	75.00	76.50	78.03	79.59	81.18	390.30
CZ	C	50.00	51.00	52.02	53.06	54.12	260.20
PT	C	50.00	51.00	52.02	53.06	54.12	260.20
DK	C	50.00	51.00	52.02	53.06	54.12	260.20
LV	C		29.75	52.02	53.06	54.12	188.95
SK	C	50.00	51.00	52.02	53.06	54.12	260.20
FI	C		4.33	52.02	53.06	54.12	163.53
Total		775.00	824.58	910.35	928.55	947.10	4,385.58

VALUE ADDED TO INSTRUCT-ERIC

In 2019, Instruct continued to provide access to its infrastructure, supported by the in-kind contributions of members that host an Instruct Centre. Their commitment to Instruct is to provide, maintain and update technologies and methods for the user community, and this is supplemented with Instruct funds that support consumable costs up to a maximum of €1500 per visit to the Centre. In most cases, this means that the infrastructure continues to be free to users at the point of access.

ADOPTION OF A NEW ACCESS FUNDING MODEL

2019 saw a review of the access funding model in response to the escalation of the costs associated with higher value instruments and methods being added to the Instruct catalogue.

Notably, Instruct's cryo-EM capacity increased in 2019, and complex sample labelling and deuteration was increasingly used for NMR methods. Using the capped limit of Instruct funding to support the consumable costs of these methods made it unsustainable for Centres to meet the user demand while supplementing the running costs of access provision from institutional budgets.

The Instruct Access Committee undertook a detailed review of access funding and implemented a pilot study to compare costs using two parallel contribution models over a period of six months. A revised access contribution model, more accurately representing the real cost of infrastructure provision, was compared with the previously approved model. The outcome of the pilot indicated that the new model would increase the cost of access provision to Instruct two- to three-fold, based on the existing demand. However, the impact of adopting the new model would be to enable the continued provision of cutting-edge technologies that are increasing in demand. The revised access contribution model was adopted in principle at the end of 2019, subject to approval by the Instruct-ERIC Council in 2020, allowing us to secure additional capacity in the Instruct catalogue (the addition of two cryo-EM instruments to the Instruct Centre UK, FR2 and CZ, and significant enhancements to existing cryo-EM detector configurations and automation).

TIMELINE
selected events from 2019

JAN

- Final review of West-Life in Brussels.
- Welcome to the new Instruct-ERIC website!

New Instruct Member Country

LITHUANIA

FEB

- **As project coordinators, members of the Instruct Hub and Centres organised and participated in the kick-off meeting for the Horizon 2020 project, RI-VIS.**

RI-VIS

MAR

- As part of our continued collaboration with structural biologists in Latin America, the Instruct Hub participated in the kick-off meeting for the project *Microbes*, organised by ITQB in Lisbon.

APR

400th PROPOSAL ACCEPTED

MAY

- **The fourth Instruct-ERIC Biennial Structural Biology Conference took place in Alcalá de Henares, Spain.**
- Prof Wolfgang Baumeister was awarded the 2019 Ivano Bertini Award for outstanding contributions to structural biology.
- The Instruct-ERIC Council meeting was held in Florence, Italy,

JUN

- **The Instruct-ERIC Hub participated in the fourth and final annual meeting of the iNEXT project in Lund, Sweden.**

iNEXT

New Instruct Member Country

FINLAND

JUL

- **7th Instruct-ERIC Internship programme was launched.**
- The Astbury Centre joined Instruct Centre UK.
- The new Instruct-ERIC access funding pilot was launched.
- Instruct-ULTRA coordinated a structural biology symposium at the EBSA conference in Madrid, Spain.

- **ARIA updated to Version 2.2.**

aria

AUG

400th VISIT COMPLETED

SEP

- Instruct-ERIC Coordinator, Susan Daenke, presented RI-VIS at the Global BioImaging Exchange of Experience IV Conference in Singapore.

- **Instruct-ULTRA hosted a Sustainability Workshop in Brussels, Belgium.**
- The Instruct-ERIC Council meeting was held in London, UK.

OCT

- **Instruct-ERIC inaugurates the Astbury Centre in Leeds to Instruct Centre UK.**

NOV

- Instruct-ERIC Coordinator, Susan Daenke, accepted the Chair of the Life Sciences Research Infrastructure (LS RI) Strategy Group from EATRIS at a meeting in Brussels, Belgium.
- **EMBL membership given preliminary approval by Instruct-ERIC Council.**
- 2019 Instruct-ERIC Internships announced.

EMBL

DEC

INSTRUCT BIENNIAL STRUCTURAL BIOLOGY CONFERENCE 2019

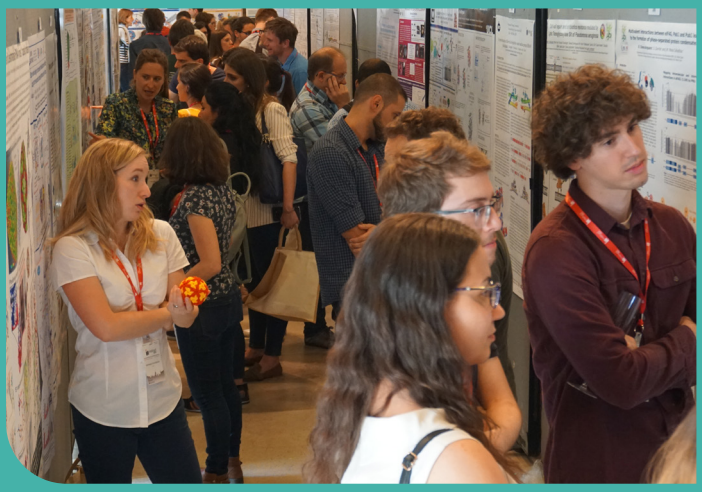
Following the success of previous Instruct Biennial Structural Biology Conferences, the fourth conference took place from 22 - 24 May 2019 in Alcalá de Henares, Spain.

The conference was officially opened by Prof. Rafael Rodrigo Montero, Ministry of Science, Innovation and Universities in Spain.

The programme showcased cutting-edge research, with international scientists demonstrating the important role of structural biology in addressing key challenges in health and life science, and looking towards emerging technologies and trends that are making an impact in biological research and biomedicine.

Twenty speakers presented the scientific programme split into four sessions covering:

- Structural biology towards cellular biology – integrating biology
- Structural biology and health – current challenges
- Emerging technologies in integrative structural biology
- New trends



Notable were presentations showing new approaches to identify fine molecular structure, revealing cellular function in situ; challenging research areas being addressed using new methods and imaginative ideas; and some important successes in X-ray and cryo-EM methods to improve resolution and increase sample throughput using automation.

The full programme is available on the Instruct website at instruct-eric.eu/biennial2019

BERTINI AWARD

The Conference is the major Instruct event at which the Bertini Award is made, recognising a significant achievement in frontier research that utilises an integrative structural biology approach. It is awarded in memory of Ivano Bertini, who built a world class NMR facility at the University of Florence and championed NMR as a key component of the integrated structural biology approach. Former laureates were Stephen Cusack (2015) and Lucia Banci (2017). The award is endowed by Bruker BioSpin.

The 2019 award was presented to Wolfgang Baumeister by the previous recipient Lucia Banci. Prof Baumeister was recognised for his pioneering and outstanding achievements in the field of cryo-electron tomography and its use to characterise molecular and supramolecular structures in intact cells at high spatial resolutions. He has embraced other structural methods in combination with cryo-EM in his research and his award lecture was entitled 'The molecular machinery of protein degradation: structural studies ex situ and in situ'.

FIG 1. Left to right: David Stuart, Lucia Banci and Wolfgang Baumeister.

WOMEN IN SCIENCE

The Conference hosted the first Women in Science Workshop, celebrating senior women scientists from industry and academia and offering advice on supporting women to progress their careers in science.

Chaired by Dr Evangelia Chrysina from NHRF, Greece, presentations from speakers were followed by a panel discussion on shared experiences and career advancement. This workshop received broad support from the structural biology community at large and an enthusiasm to repeat this at the next Instruct Biennial Conference scheduled for May 2021 in The Netherlands.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INSTRUCT CENTRES

INSTRUCT CENTRE BE - NANOBODIES4INSTRUCT

The Nanobodies4Instruct Centre generates Nanobodies and Megabodies to facilitate the structural analysis of proteins that are notoriously difficult to purify, crystallise, or study by other methods. The collective efforts of several laboratories have demonstrated that Nanobodies (Nbs) are exquisite chaperones for crystallising complex biological systems, such as membrane proteins, multiprotein assemblies, transient conformational states and intrinsically disordered proteins. Recently, Nanobodies have found their way into cryo-EM.

Nanobodies4Instruct: providing Nanobodies and Megabodies for Structural Biology

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

Nanobodies to lock functional conformations of dynamic proteins

The active-state conformations of GPCRs are unstable in the absence of specific cytosolic signalling partners representing key challenges for structural biology. In collaboration with Brian Kobilka, Nanobodies4Instruct generated Nanobodies against the ß2 adrenergic receptor (ß2AR), the muscarinic acetylcholine receptor (M2R) and the μ-opioid receptor (MOR) that exhibit G protein-like behaviour, and obtained agonist-bound, active-state crystal structures of receptor•Nb complexes of ß2AR, M2R and MOR.[3] Nanobodies from the facility were also instrumental to lock and solve the structures of single functional conformations of transporters.[4,5]

FIG 1. Llamas for Instruct.

Nanobodies to stabilise protein complexes

Other Nanbodies were developed that stabilise the ß2AR-Gs complex. One of the Nanobodies that inhibits the GTP driven dissociation of ß2AR-Gs was instrumental in obtaining the high-resolution crystal structures of several transmembrane signalling complexes, providing the first views of transmembrane signalling by GPCRs.[6] In two more recent collaborations, the facility developed Nanobodies that stabilise the PINK1-Ubiquitin enzyme-substrate complex[7] and the Vps34 complex II[8] of yeast, which is composed of four proteins.

NEW TECHNOLOGIES

Megabodies as innovative tools for cryo-EM

Nanobodies are highly popular and versatile tools for X-ray crystallography but they also hold promise for cryo-EM because they can lock well-defined conformations and stabilise multi-protein complexes. Recently, we reformatted our Nanobodies into Megabodies by rigidly grafting Nbs to selected protein scaffolds to increase their molecular weight, while retaining full antigen binding specificity.

Megabodies can be 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.[9] The Megabody design principles are applicable to different scaffold proteins and recognition domains of compatible geometries, and are amenable for efficient selection from yeast display libraries.

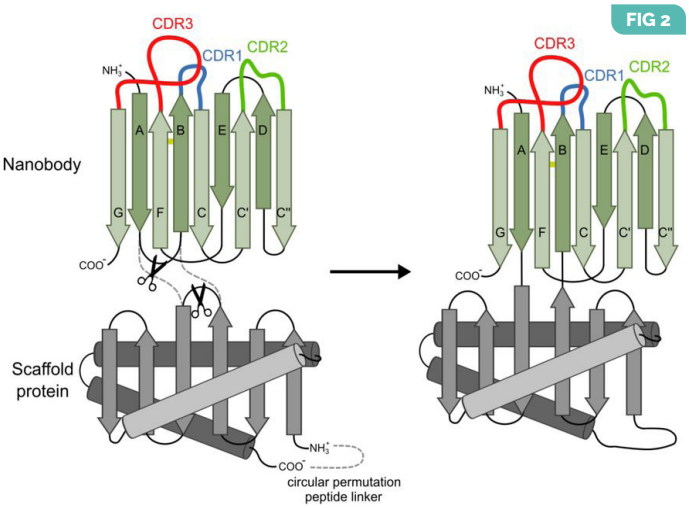

FIG 2. Megabodies are assembled from a nanobody (or a similar single-domain antigen-binding protein) and a scaffold protein. The scaffold protein is placed between β-strand A and β-strand B of a nanobody. The megabody is encoded by a single gene, comprising a nanobody that is grafted into the scaffold protein via two peptide bonds.[9]

In a recent Instruct-ERIC collaboration with the Aricescu lab, Nanobodies4Instruct successfully used such Megabodies to solve the first cryo-EM structures of the heteropentameric $GABA_A$ receptor[10,11] in complex with common drugs, including Xanax and Valium, amongst other pharmacological compounds.

The facility also developed Megabodies that bind to the membrane scaffold protein (MSP) commonly used to prepare nanodiscs. These MSP-binding Mbs help randomise the orientation of particles to facilitate cryo-EM studies of any membrane protein that is reconstituted in nanodiscs of different size and lipid composition.

1. Muyldermans S. *Ann. Rev. Biochem.* 2013;82:775-797.
2. Pardon E, Laeremans T, Triest S, Rasmussen SG, Wohlkönig A, Ruf A, Muyldermans S, Hol WG, Kobilka BK, Steyaert J. *Nat. Protoc.* 2014;9:674-693
3. Manglik A, Kobilka BK, Steyaert J. *Annu. Rev. Pharmacol. Toxicol.*. 2017;57:19-37.

SCIENCE HIGHLIGHTS

The morphogen Sonic hedgehog inhibits its receptor patched by a pincer grasp mechanism[12]

The modest resolution of the PTCH1 cryo-EM structures (at best 3.5 Å) did not allow for the conclusive identification of the ligands, nor for visualisation of the precise pose in which they interact with PTCH1. To address these limitations, the high-resolution crystal structures of the two soluble extracellular domains of PTCH1 were needed. The Nanobodies4Instruct facility raised Nbs against PTCH1ECD1-ECD2 (Nb64 and Nb75) that bound to PTCH1ECD1 and PTCH1ECD2, respectively, with nanomolar affinity. Rudolf et al. determined the crystal structures and used them to reassess the previously solved cryo-EM structures.[12]

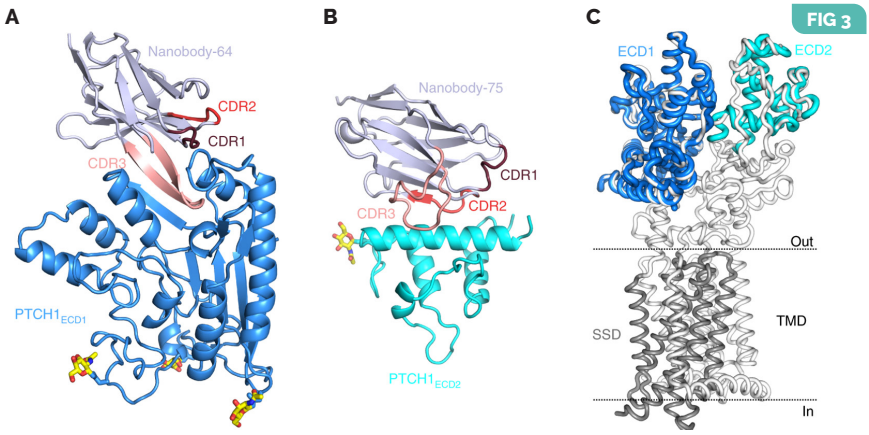

FIG 3. Cartoon representations of the high-resolution crystal structures of the PTCH1ECD1–Nb64 **(A)** and PTCH1ECD2–Nb75 **(B)** complexes. **(C)** Superposition of the PTCH1ECD1 and PTCH1ECD2 crystal structures on the previously determined cryo-EM PTCH1TM structure (PDB accession 6E1H).

The molecular mechanism of transport by the mitochondrial ADP/ATP carrier[4]

By using a specific nanobody, Ruprecht et al. were able to determine the structure of the ADP/ATP carrier (AaC) inhibited by BKA, which represents the first structure of a carrier in an m-state conformation and reveals the molecular mechanism of transport utilised by mitochondrial carrier proteins.and an mitochondrial ADP-ATP carrier (Fig 4A).[4]

L amino acid transporter structure and molecular bases for the asymmetry of substrate interaction[5]

The crystal structures of the bacterial alanine-serine-cysteine exchanger (BasC), a prokaryotic LAT transporter, which has 26–28% sequence identity with human LATs, were determined by Errasti-Murugarren et al. (Fig 4B). BasC crystallises in complex with a BasC nanobody (Nb74), in a non-occluded inward-facing conformation in the apo state and in complex with the substrate analog 2-aminoisobutyrate (2-AIB).[5]

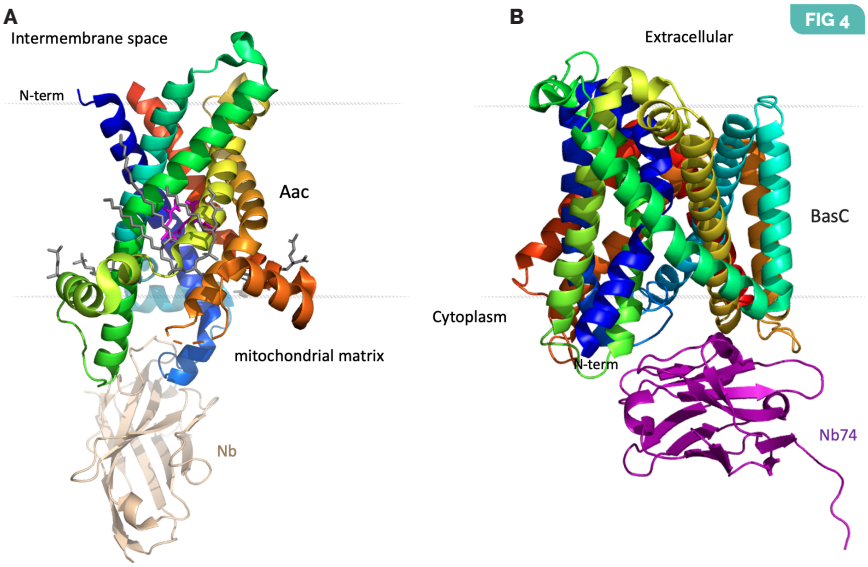

FIG 4. (A) The structure of TtAac (rainbow cartoon) bound to bongkrekic acid (magenta) and a nanobody (Nb, wheat). Cardiolipin molecules associated with the carrier are shown as sticks (grey). **(B)** Crystal structure of BasC apo in complex with Nb74 at 2.9 Å (PDB ID 6F2G). Helices are colored blue to red from the N-termini and Nb74 is magenta.

$GABA_A$ receptor signalling mechanisms revealed by structural pharmacology[10]

In collaboration with the Aricescu lab, Megabodies were successfully used to solve the first cryo-EM structures of the heteropentameric $GABA_A$ receptor[10,11] in complex with common drugs including Xanax and Valium.

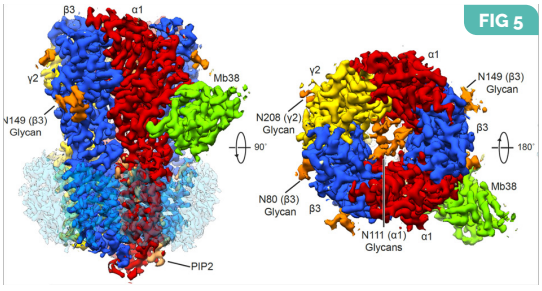

FIG 5. Side view of the sharpend cryo-EM map of 132L GABAA receptor-Mb38 complex (Nb domain from Mb in green).

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Instruct Centre Lead Scientists

Els Pardon

Jan Steyaert

INSTRUCT CENTRE CZ

The Czech Infrastructure for Integrative Structural Biology (CIISB) is a distributed infrastructure comprised of the central laboratories of CEITEC (Central European Institute of Technology), located in Brno, and BIOCEV (Biotechnology and Biomedicine Centre), located in Vestec near Prague. CIISB offers open access to ten high-end core facilities, and assisted expertise in NMR, X-ray crystallography and crystallisation, cryo-electron microscopy and tomography, biophysical characterisation of biomolecular interaction, nanobiotechnology, proteomics and structural mass spectrometry. CIISB constitutes Instruct Centre CZ.

NEW TECHNOLOGIES

Combined AFM-Raman microscope

A combined AFM-Raman microscope, consisting of Bruker Dimension Icon and Renishaw Raman microscope, was installed in the Nanobio CF lab at CEITEC MU during August 2019 (Fig 1). The unique combination extends the high resolution of AFM microscopy with the localisation specificity of Raman spectroscopy. Complex characterisation of amyloid fibres can be seen as an interesting application of this technique.

FIG 1

Q Exactive HF-X Mass Spectrometer

Q Exactive HF-X Hybrid Quadrupole-Orbitrap MS System (Thermo Fisher Scientific) was installed at the Proteomics Core Facility, CEITEC MU, in May 2019 (Fig 2). The new mass spectrometer brings higher sensitivity, higher MS/MS acquisition speed (up to 40Hz), flexibility in data dependent and data independent acquisition modes and improves capabilities in label free quantification of low-abundant ions in the most complex matrices.

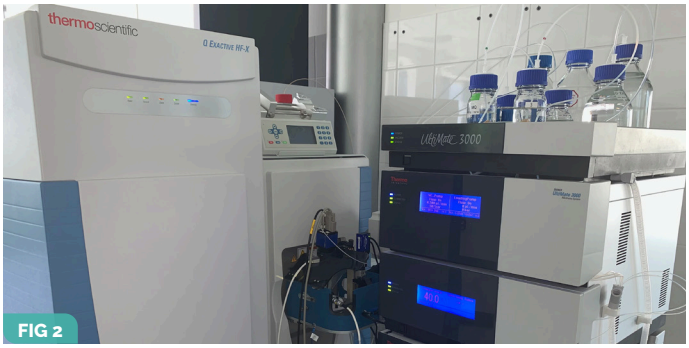


FIG 2

Cutting edge crystallisation robotics

The crystallisation facility at IBT-BIOCEV has been equipped with the crystallisation robot NT8 (Formulatrix) with several unique features: Crystallisation drops are set in an area with a controlled level of humidity, the minimal transferred volume can be 50 nl, the robot can be used for seeding and enables crystallisation in the lipidic cubic phase (Fig 3).

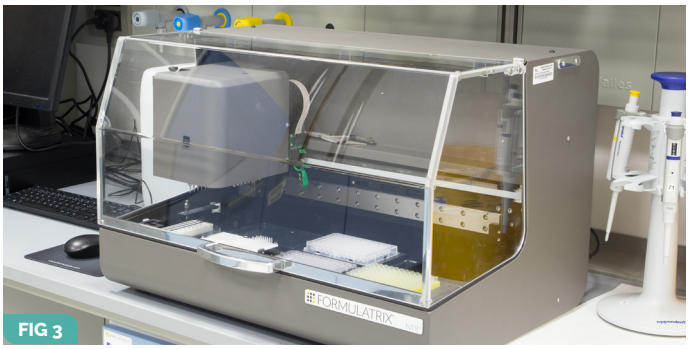


FIG 3

New experimental possibilities with dehumidifier on in-house diffractometer D8 Venture

The single crystal X-ray diffractometer in the diffraction lab at IBT-BIOCEV was recently equipped for controlling humidity levels of the crystal environment (HC-Lab, Arinax). The crystals can be treated at room temperature, while constantly monitoring their diffraction quality. Dramatic improvement of diffraction quality by dehumidification (Fig 4) is now available as a service, together with the possibility of room temperature data collection and sample modification during experiment.

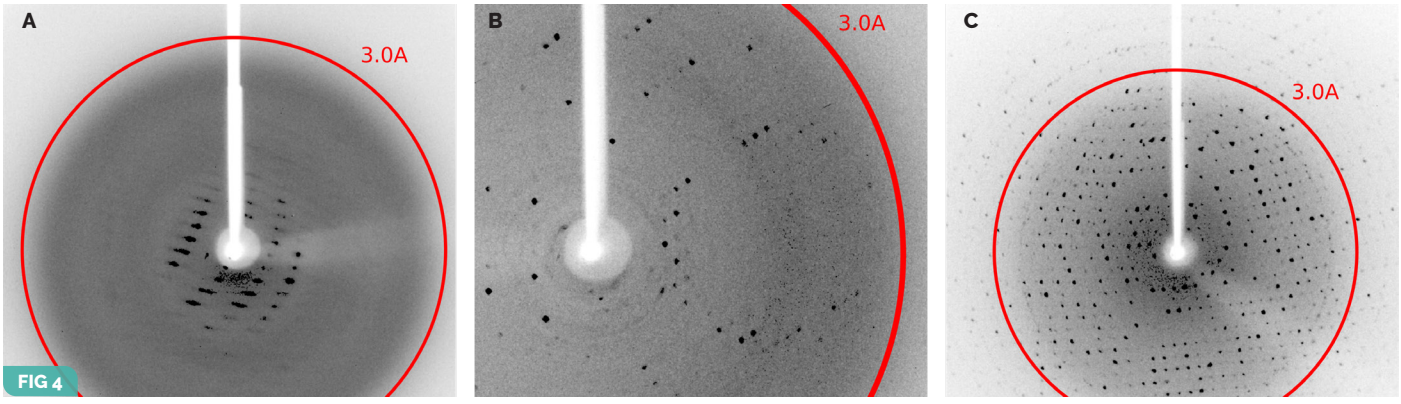


FIG 4

FIG 4. (A) Untreated cryo-cooled crystal, **(B)** Untreated crystal at room temperature, in controlled humidity using HC-Lab, **(C)** Crystal with removed solvent in HC-Lab, and subsequently cryo-cooled

SCIENCE HIGHLIGHTS

Structure and genome ejection mechanism of Staphylococcus aureus phage P68 - Pavel Plevka Research Group, CEITEC[1]

Phages infecting Staphylococcus aureus can be used as therapeutics against antibiotic-resistant bacterial infections. However, there is limited information about the mechanism of genome delivery of phages that infect Gram-positive bacteria. The publication presents the structures of native S. aureus phage P68, genome ejection intermediate, and empty particle. The P68 head contains 72 subunits of inner core protein, 15 of which bind to and alter the structure of adjacent major capsid proteins and thus specify attachment sites for head fibres. Unlike in the previously studied phages, the head fibres of P68 enable its virion to position itself at the cell surface for genome delivery. The unique interaction of one end of P68 DNA with one of the 12 portal protein subunits is disrupted before the genome ejection. The inner core proteins are released together with the DNA and enable the translocation of phage genome across the bacterial membrane into the cytoplasm.

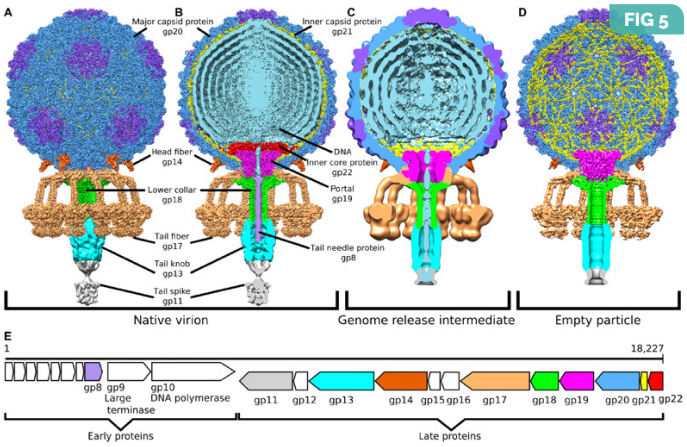


FIG 5. Virion and genome organisation of phage P68.[1]

Binding of eIF3 in complex with eIF5 and eIF1 to the 40S ribosomal subunit is accompanied by dramatic structural changes –Leoš S. Valášek Research Group, Institute of Microbiology CAS[2]

eIF3 is a large multiprotein complex serving as an essential scaffold promoting binding of other eIFs to the 40S subunit, where it coordinates their actions during translation initiation. Perhaps due to a high degree of flexibility of multiple eIF3 subunits, a high-resolution structure of free eIF3 from any organism has never been solved. Employing genetics and biochemistry, a 2D interaction map of all five yeast eIF3 subunits was previously built. In the publication, the authors further improved the previously reported in vitro reconstitution protocol of yeast eIF3, which they cross-linked and trypsin-digested to determine its overall shape in 3D by advanced mass-spectrometry. The obtained cross-links support the 2D subunit interaction map and reveal that eIF3 is tightly packed with its WD40 and RRM domains exposed. This contrasts with reported cryo-EM structures depicting eIF3 as a molecular embracer of the 40S subunit. Since the binding of eIF1 and eIF5 further fortified the compact architecture of eIF3, the authors suggest that its initial contact with the 40S solvent-exposed side makes eIF3 to open up and wrap around the 40S head with its extended arms.

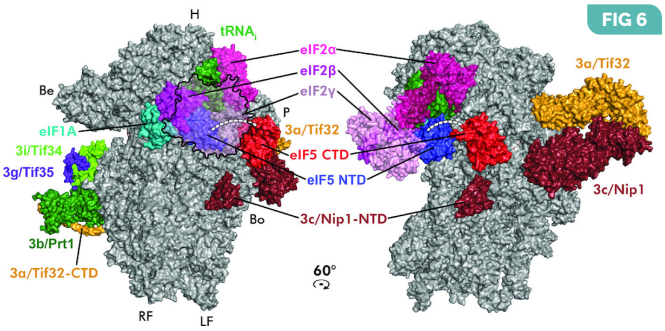


FIG 6. Model of the eIF5-containing PIC at the late initiation stage.[2]

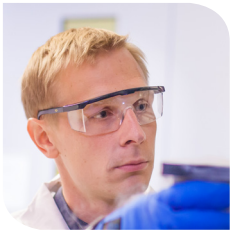
TRAINING

Instruct-ERIC workshop on Computational Approaches in Integration of Structural Biology Techniques

On 8-10 October, the Institute of Biotechnology of the Czech Academy of Sciences organised an Instruct-ERIC workshop on Computational Approaches in Integration of Structural Biology Techniques, with a total of 20 registered participants and 18 speakers.

The workshop provided a unique opportunity to communicate some of the latest developments in structural biology techniques, to enable participants to see instruments at work, and to discuss practical issues around sample properties and data acquisition. The workshop featured excellent speakers and developers of structural biology techniques, as well as scientists performing integrative structural biology studies at a high level. The participants gained practical experience of advanced mass spectrometry, biophysical methods, crystallisation and X-ray diffraction, and SAXS during four tutorials. Seven computer-based tutorials helped to develop computational skills and practical applications of algorithms devoted to either individual methods or integration of structural results.

Instruct Centre Lead Scientists



Jiří Nováček

Petr Pompach

1. Hrebík D, Štveráková D, Škubník K, Füzik T, Pantůček R, Plevka P. *Sci. Adv.* 2019;5:eaaw7414.
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INSTRUCT CENTRE ES

The Instruct Image Processing Center (I2PC) in Madrid has traditionally focused on image processing for structural biology, especially oriented towards electron microscopy (single particle analysis and electron tomography).

The facility develops new image processing algorithms covering the whole processing pipeline, from the raw data acquisition to the final structure with the finest structural details. These algorithms are publicly available through the software package Xmipp. I2PC also integrates other software packages into a workflow platform called Scipion, which is also publicly available and serves thousands of structural projects around the world. During 2019, the scope of I2PC has significantly broadened with the acquisition of a series of new instruments that will be integrated into Instruct-ERIC. In this way, our technological offer not only includes the traditional image processing, but also access to high end cryo-EM equipment, including CLEM.

FIG 1. Left: Talos Arctica cryo-electron microscope. Right: JEOL CryoARM300 microscope.

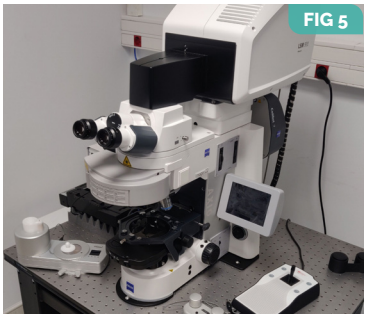
NEW TECHNOLOGIES

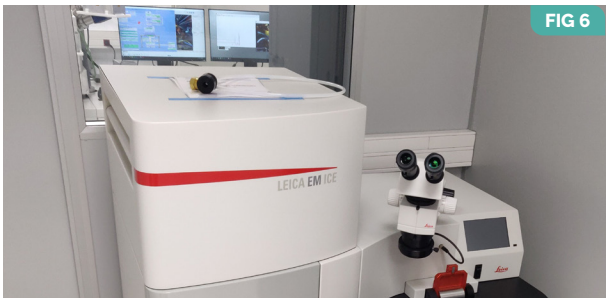
The cryo-electron microscopy facility (cryo-EM) of the Centro Nacional de Biotecnología (CNB-CSIC), (already hosting a 200 kV FEI Talos Arctica equipped with a Falcon III electron direct detector) has increased its capabilities for single-particle analysis and cryo-electron tomography with the installation of a 300 kV cryo-electron microscope; a JEOL CryoARM300 equipped with a K3 electron direct detector and an energy filter.

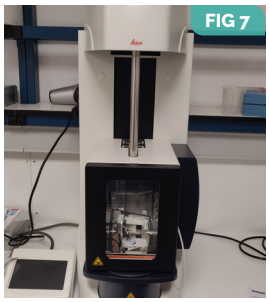
The cryo-electron microscopy facility is also implementing a new cryo-correlative light electron microscopy (cryo-CLEM) platform, which will include:

- Sample preparation capabilities, plunge and high pressure-freezing
- Cryo-fluorescence microscopy, cryo-epifluorescence microscopy and cryo-confocal microscopy
- Cryo-FIB-SEM microscopy, including lamella preparation and cryo-FIB-SEM tomography.

The cryo-CLEM platform aims to provide images to resolve the structure of protein complexes in this native environment by means of lamella preparation and subsequent tomography, and to provide three-dimensional ultrastructural information of whole cells and parts of tissue by means of cryo-FIB-SEM tomography.







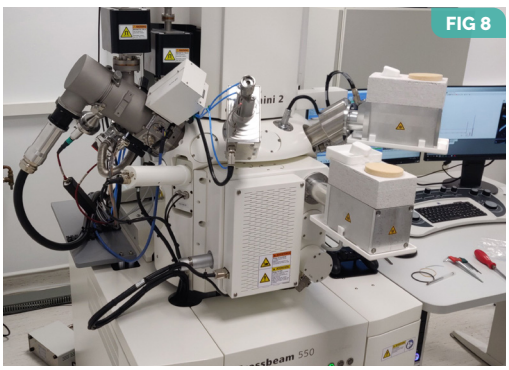


FIG 2. Talos Arctica cryo-electron microscope. **FIG 3.** JEOL CryoARM300 microscope. **FIG 4.** Thermo Fisher plunge-freezing machine. **FIG 5.** Zeiss cryo confocal microscope. **FIG 6.** Leica EM ICE 600 machine for high pressure sample preparation. **FIG 7.** Leica GP2 plunge sample preparation machine. **FIG 8.** Zeiss Crossbeam550 Microscope.

SCIENCE HIGHLIGHTS

Structural characterisation of cooperative couples of mAbs recognising a vaccine component[1]

The objective of this project was to use 3D electron microscopy to determine the structures of the lipoprotein factor H-binding protein (fHbp) present on the meningococcal surface in complex with the two mAbs and also in complex with the two Fabs derived from the mAbs. fHbp is known to elicit strong antibody responses in infected individuals and is a key vaccine target.

The reconstructions were used to i) determine the angle between the Fab arms simultaneously bound to the antigen and ii) to build models to assess if the synergy in complement activation by the cooperative mAbs could be explained by a facilitated assembly of the hexameric mAb complex, previously shown to be required for complement activation. The aim was to broaden understanding of the molecular details of vaccine-induced protection against Neisseria meningitidis.

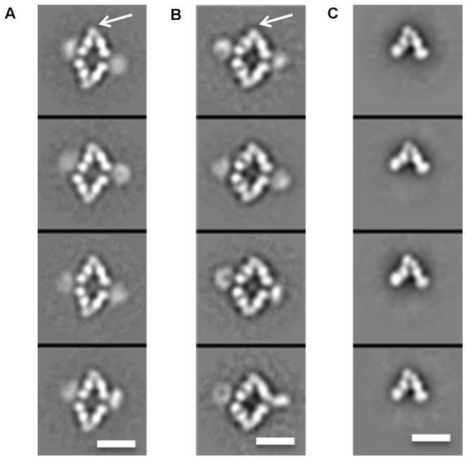


FIG 9

FIG 9. Comparison of representative reference-free 2D class averages of two mAb-based complexes and one fAb-based complex.

Subtomogram averaging of cryo-electron tomograms of chromatin plates from metaphase chromosomes[2]

In a previous Instruct project 'High-resolution structure of metaphase chromatin plates', good cryo-electron tomography results were obtained that allowed the reconstruction of metaphase chromatin plates. However, the arrangement of nucleosomes within this layered structure was not clear given the dimensional analysis of the multi-layered plates.

The objective of this project was to perform an extensive subtomogram averaging study to identify nucleosomes within the chromatin plates. The results reveal that long chromatin filaments in human metaphase chromosomes are folded to form multilaminar structures that are probably stacked along the chromosome axis. The compact organisation of chromatin may have a role in protecting genomic DNA during cell division.

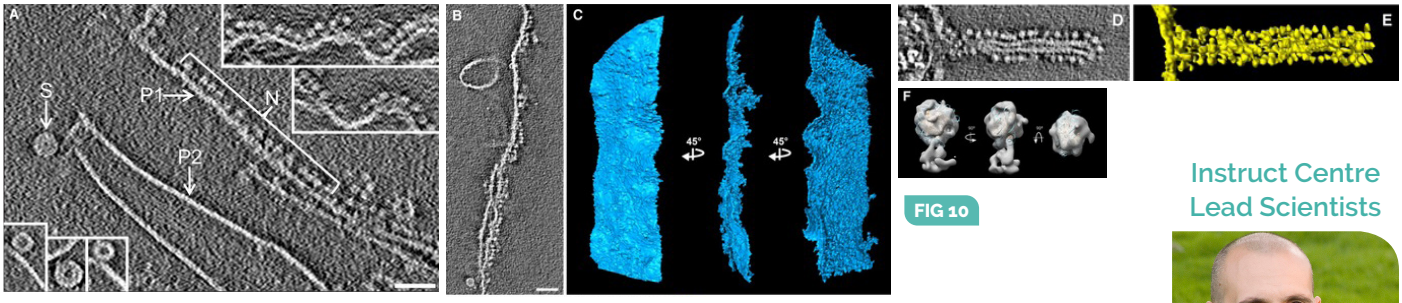


FIG 10

FIG 10. Nucleosomes (N) decorating a relaxed plate (P1); insets in the upper right show additional examples. Nucleosomes are not visible as individual units in typical compact plates (P2). Short compact interdigitated solenoids are shown in the main image (S) and in the bottomleft insets. B–E Slice (B) and segmentations in three different orientations (C) of a large relaxed plate decorated with many nucleosomes on its right side. Slice (D) and segmentation (E) of a relaxed plate forming a tube decorated with nucleosomes. F Structure of the decorative particles (like those shown in N, panel A) after subtomogram averaging.

TRAINING

Instruct course on image processing for electron microscopy and hybrid modelling

During the course, participants learned the basics of image processing in cryo-electron microscopy. Participants also learned how to fully perform an image processing project in Scipion, integrating several software packages at all the processing steps along the workflow - from the raw acquisition data, to the cryo-EM map and subsequently to the atomic model. Participants learned the theoretical foundations of the data analysis algorithms behind the programs, as well as how to design their own strategies to develop an image processing workflow that extracts the most information from the electron micrographs. Attendees also learned how to evaluate the quality and correctness of their map. In the modelling stage, participants learned how to perform a modelling by homology, globally align the atomic model to the cryo-EM map, refine it and evaluate its quality. All this knowledge was acquired through theoretical sessions interleaved with practical work so that participants could reproduce this kind of analysis at their home institutions.

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2. Chicano A, Crosas E, Otón J, Melero R, Engel BD, Daban JR. Frozenhydrated chromatin from metaphase chromosomes has an interdigitated multilayer structure. *EMBO J.* 2019:e99769.

Instruct Centre Lead Scientists

Francisco Javier Chichón

Jose Javier Conesa

Roberto Melero del Rio

INSTRUCT CENTRE FR1

Instruct Centre France 1 is hosted at 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). Instruct Centre FR1 provides an integrative environment for structural studies of protein and macromolecular complexes.

The integrated structural biology at Instruct Centre FR1 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 (the flagship for Instruct-ERIC), X-ray crystallography, small angle X-ray scattering, NMR of proteins and macromolecular complexes, including nucleoprotein complexes. Together, this allows integration of 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.

FIG 1. The Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC).

NEW TECHNOLOGIES

Gatan K3 direct electron detector and on-the-fly processing

The Centre has expertise in all aspects of cryo-EM, either by single particle analysis or cryo-electron tomography, and ranging from sample preparation and screening, to automated data acquisition and processing of large, high-resolution datasets. The facility hosts a high-end electron microscope, the Titan Krios, equipped with two direct electron detectors, allowing structure determination of macromolecular biological object at near atomic resolution.

To face the growing demand of data acquisition time of the community, and the need for higher quality data, the Gatan K2 camera has been upgraded to the latest generation of direct electron detector, the Gatan K3 camera. Compared to its predecessor, the K3 has an improved DQE, improving the quality of images at high resolution. The larger detector also displays an increased number of pixels and covers a wider field of view, improving the acquisition speed and efficiency. Combined with a higher frame rate, this allows the acquisition of larger datasets with improved image quality.

The main bottleneck in cryo-EM analysis is often the sample itself. Sample quality, and its potential to attain high resolution during analysis, cannot be estimated directly from the raw images, and requires processing of the data to be assessed. Thus, acquisition time on the Titan Krios can be wasted on samples that are unsuitable for high-resolution analysis. To optimise Titan Krios running time, the Centre has set up a complete, on-the-fly processing workflow, allowing analysis of image quality, as well as the direct structure determination at high resolution in single particle analysis, in parallel to data acquisition. The results obtained at this stage provides important feedback to tune the parameters of the running session.

SCIENCE HIGHLIGHTS

Access to the protein production platform

Onchocerca volvulus is a tissue dwelling nematode responsible for onchocerciasis or river blindness, the world's second leading cause of infectious blindness. Ferdinand Ngale Njume visited the baculovirus expression platform from the Instruct Centre France 1 to express, purify and characterise the Onchocerca volvulus Excretory Secretory product Ov28CRP, a putative GM2 activator protein as well as its C. elegans homologue. Ov28CRP was shown to bind phospholipids and to discriminate serum samples of infected and uninfected individuals.[1]

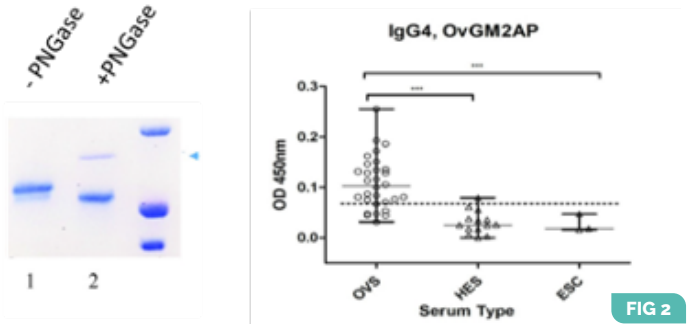


FIG 2. Production in insect cells and characterisation of the Onchocerca volvulus Excretory Secretory product Ov28CRP, a putative GM2 activator protein. The recombinant glycoprotein the recombinant protein discriminates serum samples of infected and uninfected individuals OVS = O.volvulus Serum (n = 30), HES = Hypo-endemic Serum (n = 14), ESC = European serum control (n = 03).

TRAINING

Third Instruct-ERIC Best Practices in Cryo-EM Workshop, 19 - 20 November 2019

On 19 November 2019, scientists from facilities across the world gathered at Instruct Centre France 1 for the annual Instruct Workshop for Best Practice in Cryo-EM. The two-day workshop, which received funding from the Instruct-ULTRA project, was in its third year, following the success and popularity of previous workshops in Harwell (Oxfordshire, UK) in May 2017 and NeCEN (Leiden, Netherlands) in October 2018.

The Instruct-ERIC Workshop for Best Practice in Cryo-EM was established as a forum for the exchange of knowledge and experience between EM facility scientists, managers and computational experts in academia and industry. The workshops enable sharing of best practice in the delivery of cryo-EM services and help to identify common issues and solutions in service provision. The workshops also encourage interaction between scientists and instrument manufacturers, in order to direct developments in training and technology for cryo-EM.

FIG 2. Group photo from the 3rd Instruct Workshop for Best Practice in Cryo-EM, hosted at Instruct Centre FR1.

At the third Best Practice Workshop, experts from leading cryo-EM facilities gave presentations under the themes of sample preparation, on the fly processing/data collection strategies, and cryo-electron tomography/FIB. The workshop also included a breakout session, which facilitated productive discussions on the topic of software workflows and data management and storage. The participants expressed their enjoyment of the workshop, in particular the 'nice talks and exchanges'.

It was also announced that the 4th Instruct Workshop for Best Practice in Cryo-EM will be hosted at the Astbury Biostructure Laboratory at the University of Leeds (Instruct Centre UK) in 2020.

Instruct Centre Lead Scientist

Patrick Schultz

1. Njume FN, Ghogomu SM, Shey RA, Gainkam LO, Poelvoorde P, Humblet P, Kamgno J, Robert A, Mutesa L, Lelubre C, Edelweiss E, Poterszman A, Anheuser S, Vanhamme L, Souopgui J. *PLoS Negl. Trop. Dis.* 2019;13:e0007591.

INSTRUCT CENTRE FR2

Instruct Centre France 2 in Grenoble provides technician-supported user access to some of the highest-level structural biology instrumentation in France. Its services/technologies address areas of sample preparation, characterisation and structural determination with NMR and cryo-EM as our flagships. All platforms have ISO 9001 quality certification.

User access to the platforms of the Institut de Biologie Structurale (IBS) is managed by the Integrated Structural Biology Grenoble (ISBG) service unit. The IBS is on the EPN Science Campus, an international science hub that also hosts three major European institutes – EMBL, ESRF and ILL, each with major research and user access programs in structural biology. Consequently, there is a long tradition of hosting users during their experimental visits during which they can experience French and Alpine culture.

FIG 1. The Glacios electron microscope at Instruct Centre FR2.

NEW TECHNOLOGIES

Complementing ongoing developments in single particle cryo-EM at the centre's EM platform (installation of a Glacios microscope (Fig 1), France 2 has been strengthening its activity and user access in cellular imaging by EM.

Samples are prepared using fixation techniques (chemical, high pressure-freezing) and are embedded in a resin prior to generation of ultra-thin sections at room temperature (ultrastructure) or embedded in gelatin for cryo-ultra-thin sections (protein localisation). Prior to imaging by TEM, sections can be stained or processed for immunolabeling.

Microscopy is performed on the FEI T12 or F20 instruments of the platform. These approaches allow users to understand their systems at the cellular dimension.

FIG 2. Cutting thin resin sections using an ultramicrotome at the Instruct Centre France 2 electron microscopy platform.

SCIENCE HIGHLIGHTS

New electron microscope used to visualise beta-galactosidase

A Glacios electron microscope has replaced the Polara electron microscope at Instruct Centre France 2, following a joint purchase by the CEA, ESRF and CNRS (FRISBI).

The microscope is equipped with a Falcon 2 and a K2 summit direct electron detector, and EPU and SerialEM automation packages are installed. The latter, working with the K2, facilitates data collection at about 60 images per hour, allowing researchers to obtain a 2.6 Å resolution 3D reconstruction of beta-galactosidase. The microscope is now available to Instruct users.

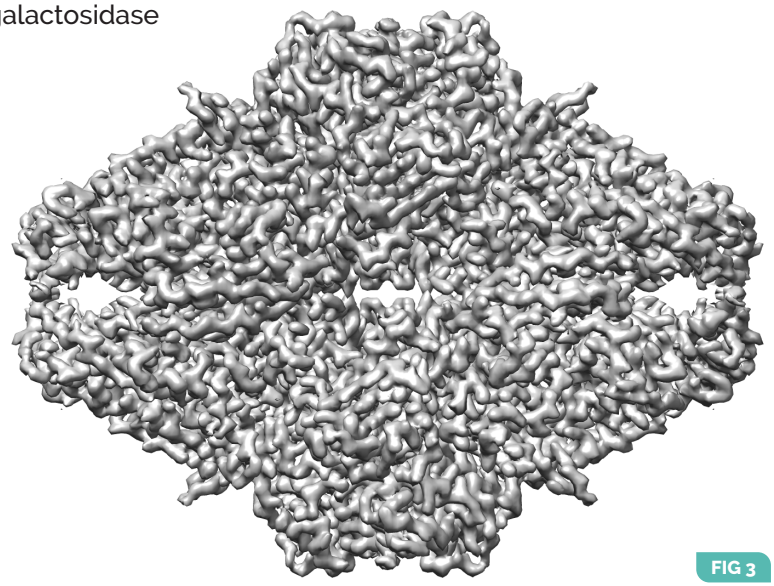


FIG 3

FIG 3. 3D reconstruction of the Beta Galactosidase at 2.6 Å resolution obtained using the Glacios/K2 setup. Estrozi L, Schoehn G, Arragain B.

Investigating the biogenesis of iron sulfur clusters and identifying reaction intermediates using native mass spectrometry[1]

Iron is a vital element for prokaryotic and eukaryotic life. For instance, iron metabolism is a key step of bacterial infection. Iron–sulfur (Fe–S) clusters (Isc) are critical protein cofactors required for many important biological events, including photosynthesis and oxidative respiration fixation.

The biogenesis of Isc is controlled by macromolecular machines that are highly conserved throughout species. Despite their relevance, the function of cluster biosynthesis remained elusive because their investigation requires very specific and demanding experimental conditions.

Using native mass spectrometry, scientists studied prokaryotic iron–sulfur cluster biogenesis machines, dynamic molecular complexes based on multiple weak interactions.

FIG 4

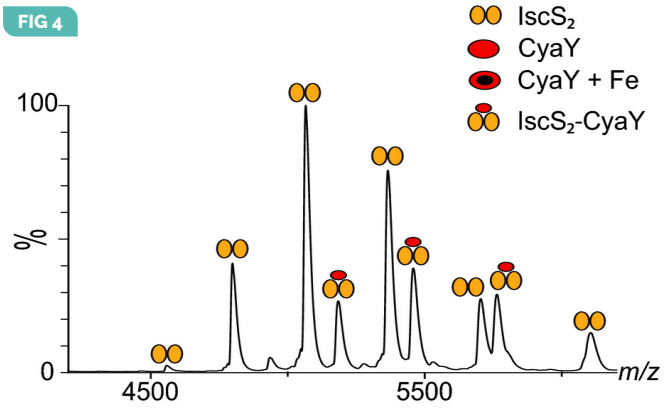


FIG 4. User Rita Puglisi from King's College London used native MS to study an iron-sulfur cluster-containing protein during a visit to Instruct Centre France 2. She observed a new species (CyaY-IscS2 complex) after mixing CyaY (an iron binding protein) and IscS in the presence of NH4Fe(SO4)2.

FIG 5

FIG 5. Analysing samples at the mass spectrometry platform of Instruct Centre France 2.

1. Puglisi R, Boeri Erba E, Pastore A. *FEBS J.* 2020. doi: 10.1111/febs.15281

Instruct Centre Lead Scientists

Darren Hart

Guy Schoehn

INSTRUCT CENTRE IL

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

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

FIG 1. Staff Scientists at the Instruct Centre IL. Left to right: Dr. Tamar Unger, Head of the Protein Expression Units; Dr. Shira Albeck, Head of the Protein Purification Unit; Dr. Yoav Peleg, Head of the DNA Manipulation Unit; Dr. Orly Dym, Head of the Crystallization & Structure Determination Unit.

NEW TECHNOLOGIES

Line-FRAP, a versatile method to measure diffusion rates in vitro and in vivo

Line-FRAP is a technique to measure the diffusion rate measurements of fast-diffusing molecules based on the recovery of photobleaching under a confocal microscope. Line-FRAP is a versatile method that can be applied to a range of fast-diffusing molecules of varying sizes, in a range of environments, and can be performed using standard equipment.

In summary:

- Protein diffusion rates in crowded environments dictates their action.
- Line-FRAP is a method to measure diffusion rates of biological molecules in prokaryotic and eukaryotic cells using physiological protein concentrations.
- Line-FRAP is superior than conventional FRAP due to the much-improved time resolution.
- Line-FRAP has been used to measure diffusion rates of various proteins within crowded environments and within prokaryotic and eukaryotic cells.
- The simplicity of using Line-FRAP opens it to a wide range of biologists for better understanding the effect of molecular motion on biological activity.

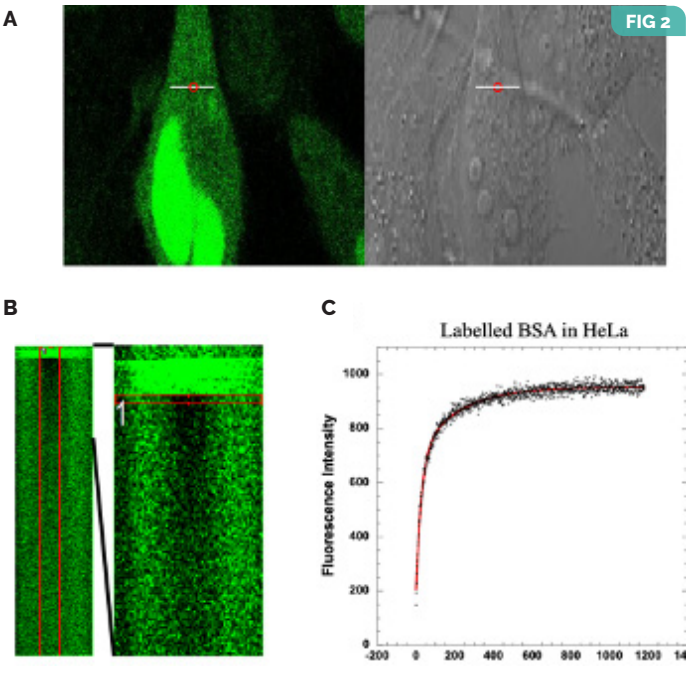


FIG 2. (A) Line-FRAP performed in a CFP transfected HeLa cell. White line represents the scanning line, Red circular area denotes the bleaching area. **(B)** A single scanning line as a function of time (in vertical direction) including the bleach. The red rectangular marks the region of interest (ROI), which was used for analysis. A zoomed view of Line-FRAP profile, is also shown where horizontal rectangular area (red colored marks ROI) denotes the fluorescence intensity as a function of length across the scanning line. **(C)** Average of 30 FRAP recovery curves as a function of time. (N=30; Correlation coefficient R=0.98).

SCIENCE HIGHLIGHTS

Flexible regions govern promiscuous binding of IL24 to receptors IL20R1 and IL22R1[3]

In close collaboration with the Instruct Centre CZ at BIOCEV, scientists from Instruct Centre IL studied the flexible regions that govern promiscuous binding of IL-24 to its receptors IL-20R1 and IL-22R1.

Interleukin 24 (IL-24) is a cytokine with the potential to be an effective treatment for autoimmune diseases and cancer. However, its instability and difficulties in its production have hampered detailed biological and biophysical studies. We approached the challenges of IL-24 production by using the PROSS algorithm to design more stable variants of IL-24. We used homology models built from the sequences and known structures of IL-20 and IL-19 and predicted and produced several extensively mutated IL-24 variants that were highly stable and produced in large yields; one of them was crystallised (IL-24B, PDB ID 6GG1; 3D Interactive at proteopedia.org/w/Journal:FEBS_Journal:1). The mutated variants, however, lost most of their binding capacity to the extracellular parts of cognate receptors. While the affinity to the receptor 2 (IL-20R2) was preserved, the variants lost affinity to IL-20R1 and IL-22R1 (shared receptors 1). Back engineering of the variants revealed that reintroduction of a single IL-24 wild-type residue (T198) to the patch interacting with receptors 1 restored 80% of the binding affinity and signalling capacity, accompanied by an acceptable drop in the protein stability by 9 °C. Multiple sequence alignment explains the stabilising effect of the mutated residues in the IL-24 variants by their presence in the related and more stable cytokines IL-20 and IL-19. The homology-based approach can enhance existing methods for protein engineering and represents a viable alternative to study and produce difficult proteins for which only in silico structural information is available, estimated as >40% of all important drug targets.

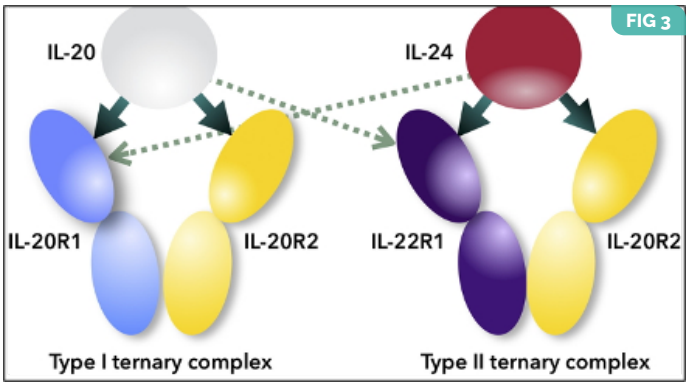


FIG 3. Schema of promiscuous binding of IL-20 and IL-24 to signalling receptors IL-20R1 and IL-22R1. Arrows indicate binding between cytokines and their receptors; thick arrows show preferential binding, dashed arrows show alternative binding to the promiscuous receptors.

TRAINING

Proteopedia workshops at European Universities

To achieve even greater impact, the 3D coordinate information available in the Protein Data Bank (PDB) has to be supplemented by information providing biological and chemical context and enriched by value-added annotations. It is not straightforward to derive this information from the limited annotations available in the PDB archive, and indeed this biological and chemical context may change as scientific knowledge progresses. To address this issue, an effective training strategy that provides infrastructure for understanding structural data, its quality, and to add information on biological context needs to be developed. Implementation of such a training strategy alongside multiple mechanisms for delivery of these data will ensure greater impact of the macromolecular structure information available in the PDB.

To begin to approach this, Instruct Centre IL collaborated with the PDBe as part of an ELIXIR / Instruct-ERIC project to investigate expanding and using Proteopedia throughout Europe for both academia and industry, as well as to be a resource for effective training and for collection of annotation information on biological and chemical context.

Between January – February, a series of four joint PDBe/Proteopedia outreach workshops were held in Europe at the University of Utrecht, the University of Strasbourg (Fig. 3), Charles University (Fig. 4) and the University of Oulu.

These workshops demonstrated the close synergy between PDBe and Proteopedia and demonstrated some of the newest features, including Proteopedia's Interactive 3D Complements (I3DCs) for publications in journals.

The participants were extremely impressed with Proteopedia, in fact one participant at the University of Strasbourg, Prof. Philippe Dumas suggested that '… maybe Proteopedia's I3DC will actually become a journal or just replace the journals'. Jaime Prilusky (Proteopedia) explained that for all accepted manuscripts to be published in any of the journals of the International Union of Crystallography (IUCr) (eg Acta Crystallographica, The IUCr Journal etc), the editors contact the authors of the manuscript and let them know that the IUCr is working with Proteopedia to create an interactive 3D complement (I3DC) to the paper which will be embedded in the online version of the paper.

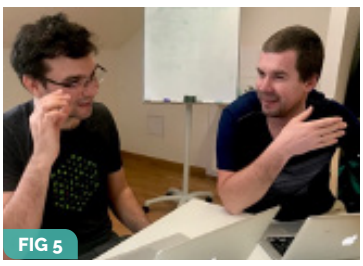


FIG 4 & 5. PDBe/Proteopedia outreach workshops.

Instruct Centre Lead Scientists

Gideon Schreiber

Joel Sussman

1. Dey D, Marciano S, Schreiber G. Line-FRAP, a versatile method based on fluorescence recovery after photobleaching to measure diffusion rates in vitro and in vivo. *bioRxiv*. 2020.
2. Zahradník J, Kolářová L, Peleg Y, Kolenko P, Svidenská S, Charnavets T, Unger T, Sussman JL, Schneider B. *FEBS J.* 2019;286:3858-73.

INSTRUCT CENTRE IT

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

The Instruct Centre IT 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 structural and dynamical characterisation of biological macromolecules and their complexes. The NMR platform comprises eleven high-resolution NMR spectrometers ranging from 400 MHz to 950 MHz and and the installation of the world's first commercially available 1.2 GHz NMR spectrometer will begin in January 2020. 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. Instruct Centre IT completed the magnetic resonance platform with a newly installed last-generation Q-Band CW/FT-EPR (with CW-X-band capability) EPR spectrometer.

Besides using the standard approaches for spectroscopic, structural and dynamical characterisation of proteins in solution and in other aggregation states, CERM/CIRMMP researchers have developed ^{13}C direct detection protocols for the characterisation of intrinsically disordered proteins,[1] experimental schemes for in-cell NMR spectroscopy,[2] and tailored pulse sequences for structural determination of paramagnetic systems.[3] Over the years, Instruct Centre IT has also expanded the panel of systems accessible through solid-state NMR with the introduction of NMR of sedimented solutes[4] and the first high-resolution characterisation of enzymes immobilised in solid supports.[5]

Advanced molecular biology laboratories are available, providing expertise for stable isotope labelling. Eukaryotic cell biology labs are also available, which include CO_2 incubators for growth and transfection of mammalian cells, and equipment for immunohistochemistry and Western Blotting. Instruct Centre IT also features a biophysical laboratory with dynamic light scattering, CD, stopped-flow, fluorimetry, UV-visible spectrophotometers, isothermal micro-calorimeter and differential scanning calorimeter, and atomic absorption. Laboratories equipped for mass spectrometry and X-ray diffraction flank the NMR platform.

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

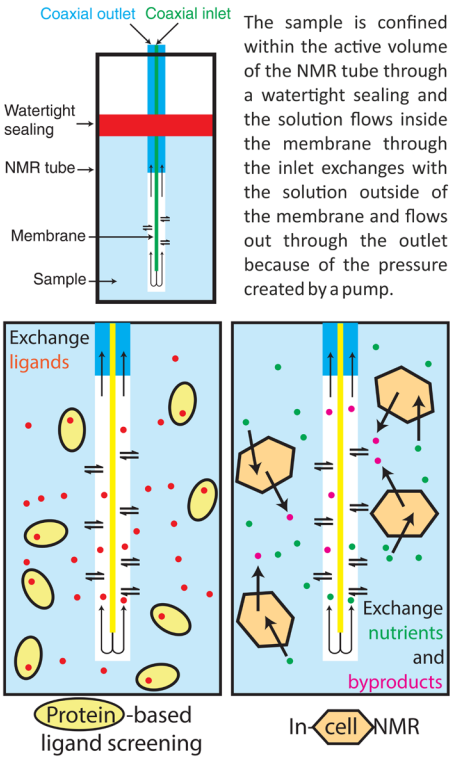
NEW TECHNOLOGIES

Real-time cellular structural biology

At Instruct Centre IT, the existing in-cell NMR workflow has been further developed to extend its range of applications. In order to overcome the short lifetime of the human cell sample, a modular NMR bioreactor has been developed and implemented. The bioreactor ensures cell viability and metabolic stability through a continuous flow of fresh nutrients and removal of toxic by-products. The setup builds upon a commercially available NMR flow unit, which has been modified to allow exchange of nutrients between the growth medium and the cells, and the removal of metabolic by-products through a microdialysis membrane.[6] This setup allows in-cell NMR experiments to be performed on human cells for prolonged periods of time, and can be applied, in principle, to monitor biological events involving protein structural change (such as folding, maturation, cofactor binding, protein-ligand and protein-protein interactions) in real time. The modularity allows alternative setups to be implemented, such as those relying on hydrogel-encapsulated cells for nutrient exchange without the use of the membrane.

Another exciting application of the mammalian expression approach for in-cell NMR developed at Instruct Centre IT is intracellular protein-observed ligand screening. It was demonstrated that protein-ligand interactions can be monitored directly in the cell cytoplasm, allowing the structural characterisation of the binding mode and early insights on the potency of potential drugs. The approach was applied to sulfonamide-class inhibitors of human carbonic anhydrase II.[7] Dose- and time-dependent intracellular binding curves revealed strikingly different diffusion behaviours, independent on the binding affinity in vitro, that correlated with drug potency.

The NMR bioreactor experimental setup

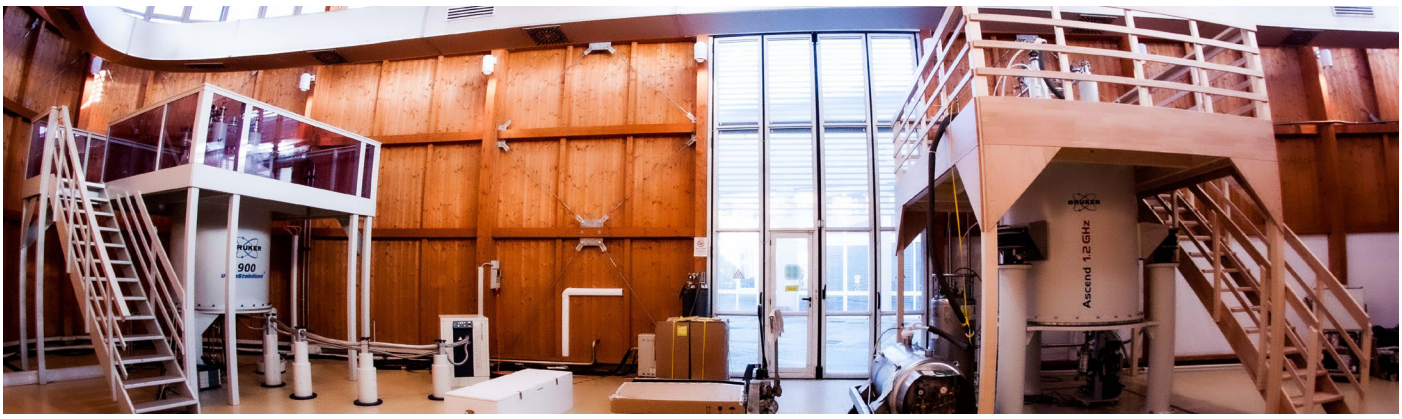


Service available at Instruct-IT

SCIENCE HIGHLIGHTS

Cutting edge NMR methodologies and advanced sample preparation strategies for life sciences

Proteins are not only a part of natural biology, physiology and pathology but also find 'artificial' uses, with applications in industrial catalysis, drugs and other healthcare increasing. The growth in the applications of proteins is paralleled by a need to understand and characterise samples at an unprecedented level of detail. To this end, NMR provides great opportunities. An example of this is the characterisation of PEGylated proteins, which are used in the therapy of leukaemias and other diseases. PEGylated proteins reorient too slowly to be profitably studied by solution NMR and escape crystallisation, but they give rise to extremely well-resolved solid-state NMR spectra in the sedimented state. The collection of structural restraints is possible, even if challenging because of the low protein content in these samples, and can be used in integrated approaches to ascertain the preservation of the native fold.[8] Solid-state NMR is also the only way to access atomic-level structural features of a protein adsorbed on aluminium hydroxide matrix for vaccine formulations, providing the way to identify the regions of the protein that are mainly affected by the presence of the inorganic matrix.[9]



A success story illustrating how Instruct-ERIC services can be fruitfully combined with that of other Life Science Research Infrastructures (funded by CORBEL)

With the goal of identifying a targeted therapy against triple-negative breast cancer (TNBC), a particularly aggressive form of breast cancer, a user first accessed the selection of a focused array of compounds against specific components of the Wnt pathway through EU-OPENSCREEN-ERIC.

Subsequent access to the NMR services of Instruct Centre IT for fragment screening allowed the identification of possible interacting ligands, as well as the validation and characterisation of the protein regions involved in the interaction and analysis of the binding affinity screening against pharmaceutical targets.

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3. Bertini I, Luchinat C, Parigi G, Ravera E. NMR of paramagnetic molecules: applications to metallobiomolecules and models. Elsevier; 2016.
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5. Fragai M, Luchinat C, Martelli T, Ravera E, Sagi I, Solomonov I, Udi Y. *Chem. Commun.* 2014;50:421-3.
6. Cerofolini L, Giuntini S, Barbieri L, Pennestri M, Codina A, Fragai M, Banci L, Luchinat E, Ravera E. *Biophys. J.* 2019;116:239-47.
7. Luchinat E, Barbieri L, Cremonini M, Nocentini A, Supuran CT, Banci L. *Angew. Chem. Int. Ed.* 2020;59:6535-9.
8. Cerofolini L, Giuntini S, Carlon A, Ravera E, Calderone V, Fragai M, Parigi G, Luchinat C. *Chem. A Eur. J.* 2019;25:1984-91.
9. Cerofolini L, Giuntini S, Ravera E, Luchinat C, Berti F, Fragai M. *npj Vaccines*. 2019;4:1-5.

Instruct Centre Lead Scientists

Lucia Banci

Simone Ciofi-Baffoni

Roberta Pierattelli

Antonio Rosato

INSTRUCT CENTRE NL

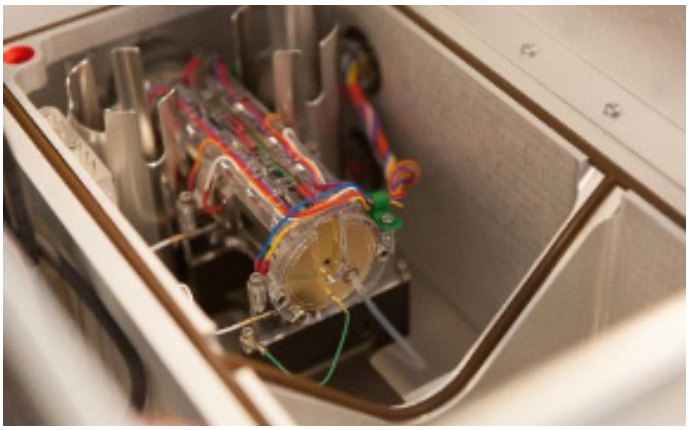
Instruct Centre NL is a coalition of three internationally operating and well-known research facilities that cover a wide range of structural biology technology: the biomolecular NMR and Mass Spectrometry facilities at the Bijvoet Centre for Biomolecular Research at Utrecht University; NeCEN, the national facility for high-end electron nanoscopy at Leiden University; and the NKI Protein Facility at the Division of Biochemistry of the Netherlands Cancer Institute in Amsterdam. Several well-recognised research groups are operating attached to these facilities, creating a vibrant academic environment.

The Bijvoet Centre incorporates computational structural biology (Alexandre Bonvin), structure determination by X-ray and cryo-EM (Piet Gros, Bert Janssen, Friedrich Förster), high-throughput Mass Spectrometry (Albert Heck, Maarten Atelaar), and high-field solid state NMR (Marc Baldus, Markus Weingarth) and solution NMR (Hugo van Ingen). As part of Instruct-ERIC, the Bijvoet node offers access to solid state NMR, solution state NMR, and Mass Spectrometry facilities.

The NeCEN cryo-Electron Microscopy core is aligned with the groups of Meindert Lamers, focusing on single particle cryo-EM, and of Ariane Briegel and Bram Koster, mainly using cryo-EM cellular tomography. NeCEN houses two Krios cryo-electron microscopes, equipped with a K3, and a K2 and Falcon 3 camera, respectively, managed by Ludovic Renault.

The NKI department of Biochemistry is close to the research groups of Anastassis Perrakis and Titia Sixma that focus on function-structure studies of proteins related to cancer. The NKI Protein Facility offers access to protein production, characterisation and crystallisation services (Patrick Celie) as well as to a collection of biophysical instruments and methods for data analysis for quantifying macromolecular interactions (Alex Fish).

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



NEW TECHNOLOGIES

New cryo-electron microscope installed at NKI

In 2019, NKI acquired new cryo-EM equipment, namely a JEM-F200 "F2" from JEOL, equipped with a K2 detector (Fig 1). This microscope is being commissioned as an Oncode Institute facility, focusing on single particle cryo-EM. The commissioning process is led by a new NKI EM-facility manager, Xiaohu Guo, and NKI aspires to offer access to this new instrument to Instruct-NL in the future.

FIG 1

FIG 1. New cryo-EM equipment at NKI.

New sample preparation methods for structural characterisation of IDPs

The importance of intrinsically disordered proteins (IDPs) in regulating cell biology has become abundantly clear in recent years. Yet, the structural characterisation of IDPs and their complexes with other proteins remains challenging. This is particularly true for large, multi-subunit complexes, and for characterisation of such complexes in the cellular environment.

As part of the Instruct-ULTRA project, scientists at Utrecht University developed sample preparation methods using differential deuteration to allow parallel quantitative investigations by NMR and SAXS/SANS of large IDP complexes (Fig 2). In addition, a protocol for in-cell NMR investigation of chromatin binding IDPs was developed. Support for both approaches is offered to Instruct users.

FIG 2

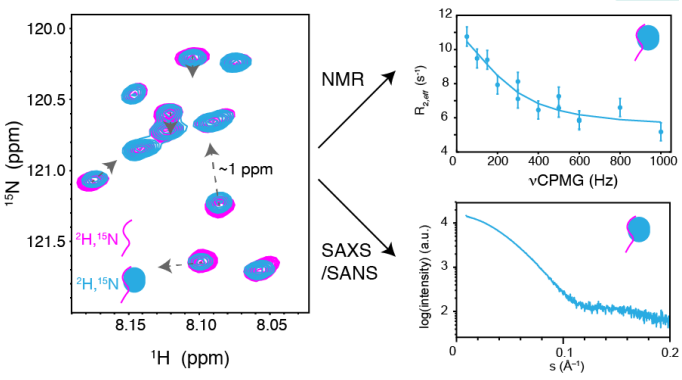


FIG 2. Parallel quantitative investigations of large IDP complexes by NMR and SAXS/SANS.

SCIENCE HIGHLIGHTS

Reconstituting immunological signalling pathways in vitro – exploring the role of 2D confinement for receptor specificity

The Ganzinger Lab used the NKI insect cell protein expression and purification facility and experience to purify four interleukin proteins that are involved in cytokine receptor signalling: IL-2Rα, IL-2Rβ, IL-2Rγ and IL-15Rα. Three of the proteins were produced in 2019, and the last one early in 2020. The purified proteins are now being studied at the user's laboratory to unravel the biophysical principles that enable immune cells to distinguish between different signals beyond the lock-and-key principle of molecular recognition of classical receptors.

FIG 3

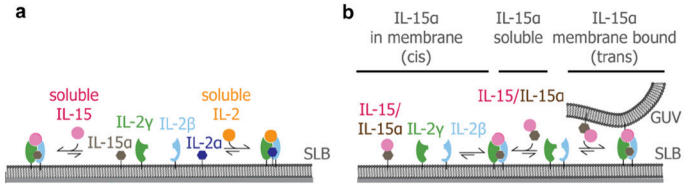


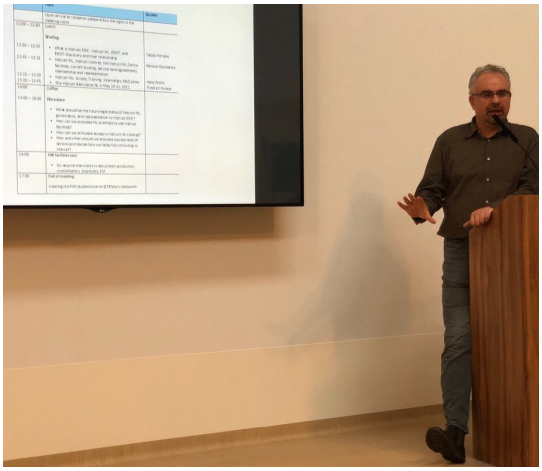
FIG 3. In vitro reconstitution of cytokine receptor signaling: schematic overview of extracellular and membrane-bound interleukin functioning.

NETWORKING HIGHLIGHTS

National meeting of Instruct-NL

A national structural biology community highlight was a focused meeting to consolidate the Instruct-NL core community and expand Instruct-NL to create an active and recognisable body for the Dutch structural biology and wider life sciences communities.

The Instruct-NL meeting was organised in October at the NKI in Amsterdam. Over 30 national structural biology group leaders and senior researchers attended, as well as representatives from the Dutch Science Funding Agency NWO and the Instruct-ERIC Council. After introducing the audience to Instruct, lively discussions took place on the future organisational and legal status of Instruct-NL, future representation in the ERIC, and how to stimulate Dutch scientists to make use of Instruct access and training opportunities. There was particular emphasis on stimulating access for international researchers, evaluating success levels, and lobbying for future funding of Instruct-NL.



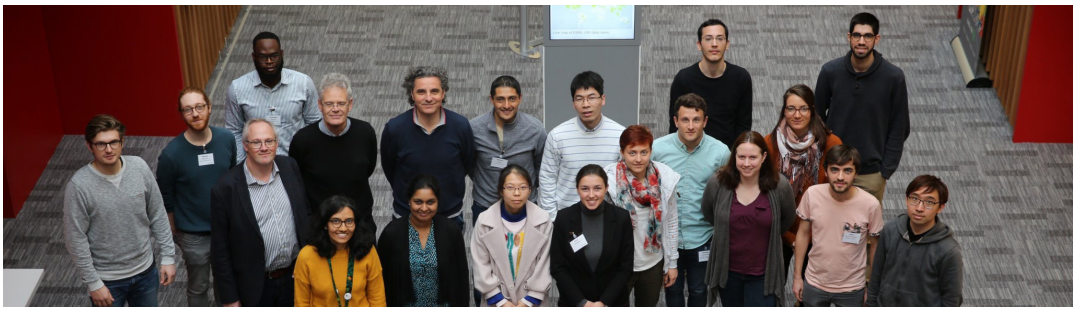
iNEXT-Discovery

In 2019, the Instruct-NL team from NKI, in close collaboration with the Instruct-ERIC Hub, prepared and submitted the iNEXT-successor Horizon 2020 project, iNEXT-Discovery (inext-discovery.eu). As a result, Instruct-ERIC is firmly represented in the consortium, together with about 20 European structural biology facilities and four other health and food ESFRI landmarks and projects (EATRIS, Euro-BioImaging, EU-OPENSCREEN, and METROFOOD-RI).

TRAINING

Workshop on Integrated Modelling of Protein-Protein Interactions

A joint Instruct-CAPRI Workshop on Integrated Modelling of Protein-Protein Interactions was organised on 1-2 April 2019 by the Computational Structural Biology group of the Bijvoet Centre and the EMBL European Bioinformatics Institute. The workshop took place at the Wellcome Genome Campus in Hinxton (UK). The aim of the course was to train young researchers in the computational modelling of protein-protein interactions using a variety of data sources, such as X-ray crystallography, NMR, SAXS, cross-linking mass spectrometry and cryo-EM. The course introduced and made extensive use of common software and e-science portals to facilitate the adoption of the methods and techniques by the students in their everyday work (www.capri-docking.org/events/#instruct-eric-workshop).



Instruct Centre Lead Scientists

Alexandre Bonvin

Hugo van Ingen

Anastassis Perrakis

Ludo Renault

Hans Wienk

INSTRUCT CENTRE UK

Instruct Centre UK includes facilities located within the University of Oxford in the Structural Biology Division (including the Oxford Particle Imaging Centre, OPIC) and the Department of Chemistry, which hosts the Biomolecular Mass Measurement Centre.

Additional infrastructure is provided by Diamond Light Source (the UK's national 3rd generation synchrotron facility) where a suite of 32 beamlines spans the physical and life sciences. Diamond also hosts a range of integrated facilities, including the UK's national cryo-EM centre (eBIC), the UK XFEL-Hub, an XChem fragment screening facility, and the Membrane Protein Laboratory (MPL). As an Instruct Centre, Diamond provides access to its suite of MX beamlines (I03, I04, I04-1, I23, I24), the bioSAXS beamline B21, the cryo-X-ray tomography beamline B24, and single particle cryo-EM and cryo-electron tomography at eBIC.

The Astbury Centre at the University of Leeds joined Instruct Centre UK in 2019 and brings together researchers from physics, the biological sciences and chemistry to enable interdisciplinary approaches to be harnessed to understand the molecular basis of life. The Astbury Centre has outstanding expertise and research infrastructure in chemical biology, biophysics and major techniques in structural molecular biology. As of Autumn 2019, the site was able to offer electron microscopy, NMR and mass spectrometry services to Instruct.

NEW TECHNOLOGIES

Astbury Centre

In 2019, two Titan Krios microscopes equipped with direct electron detectors for high resolution imaging were made available through Instruct, supporting both single particle and tilt series data acquisition. The facility offers enhanced support, where users can access expertise from the facility team to work up their sample from negative stain, cryo-EM grid preparation and screening, through to data collection and on-the-fly data processing.

The Astbury Biostructure Laboratory NMR facility provides state-of-the-art Bruker Avance III HD spectrometers operating at 600, 750 and 950 MHz, the last specialising in direct heteronuclear detection (^{15}N and ^{13}C). The combination of high field and direct nitrogen or carbon detection has advantages for complex or large intrinsically disordered proteins. New techniques using ^{15}N direct detection are under development and are of interest in chemically exchanging systems, as an alternative to C detection and when deuteration is not feasible.

The Biomolecular Mass Spectrometry facility provides peptide-level hydrogen/deuterium exchange (HDX)-MS capabilities through Instruct. Based around LEAP sample handling automation, pepsin proteolysis and Waters Synapt G2-Si MS, the system can be used to probe changes in protein dynamics, conformation and hydrogen-bonding across different treatment conditions. Binding epitopes, protein-protein interaction sites and conformational changes upon inhibitor binding are just some examples of the type of system that can be characterised.

FIG 1

FIG 1. The cryo-EM facility at the Astbury Centre.

Diamond Light Source

- Time resolved work at I24: Initial light-activated experiments were successfully carried on the microfocus and serial MX beamline, I24 using PORTO, the PORtable femTOsecond pump laser for serial synchrotron crystallography (SSX). This, when coupled with other techniques, enables dynamic crystallography.
- At VMXi (the versatile MX in situ beamline), custom goniometry was installed and commissioning is now being finalised. Once commissioning is completed, the beamline will also be available to Instruct users.
- The first Eiger 2 XE 16M detectors were installed in December 2018 and April 2019 on beamlines I04 and I03 respectively, providing an improvement in data rates, quality, and spot separation.
- BioSAXS beamline B21 optimised its in-vacuum Eiger 4M detector, improving the instrument's signal-to-noise while allowing sample changes to occur in minutes.
- B24, the cryoTXM beamline, saw the installation of phase contrast optics on the microscope that will allow data acquisition at higher energies and exploit phase contrast imaging.
- eBIC currently offers a user service programme on cryoFIB/SEM and provides access to four Titan Krios microscopes. Two cryoFIBs, the Scios and Aquilos, were added in May 2019, along with a cryo-capable Helios Hydra.
- The XChem facility is integrated with the I04-1 beamline and offers a highly streamlined process for full X-ray screening experiments. In 2019 data collection methodologies for fragment screening were improved to achieve a 30% faster crystal-to-crystal turnaround.

FIG 2

FIG 2. The mass spectrometry facility at the Astbury Centre.

FIG 3

FIG 3. The NMR facility at the Astbury Centre.

SCIENCE HIGHLIGHTS

Structures of influenza A virus RNA polymerase offer insight into viral genome replication

This research exemplifies integrative structural biology, using complementary techniques (including nanobody technology from Instruct Centre BE) to gain new insights into the replication mechanisms of influenza A RNA polymerase (FluPolA). The polymerase plays a crucial role in replication of the virus and is a key target for antivirals.

Cryo-EM imaging at eBIC and macromolecular crystallography at Diamond Light Source were used to analyse the influenza virus polymerase. The first high-resolution crystal structures of FluPolA of human and avian influenza viruses revealed crucial details of how the structure is vital in initiating RNA synthesis. Cryo-EM imaging revealed the mechanism of the protein binding to an RNA template. The results suggest a target by which virus reproduction might be inhibited and this could lead to the development of antiviral drugs against the flu.

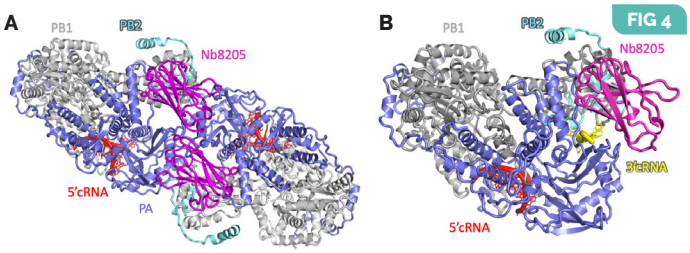


FIG 4

FIG 4. Structures of **(A)** dimeric and **(B)** monomeric cRNA-bound human H3N2 FluPolA heterotrimer in complex with Nb8205 solved by single-particle cryo-EM analysis. Structures are in the same orientation but not to scale.

TRAINING

EMBO Practical Course: High-throughput protein production and crystallisation

In June 2019, this course introduced well-established high-throughput approaches for expression screening and purifying proteins for crystallisation. Through hands-on training, participants were able to reconstitute integral membrane proteins into detergent-free systems.

Electron diffraction method for cryo-electron microscopes (MicroED Workshop)

This workshop aimed to teach users the electron diffraction method in cryo-EM microscopes, from setting up the microscope for diffraction data collection to image processing. The workshop, held in November 2019, was principally hands-on, with students working on the Talos Arctica and Titan Krios microscopes, as well as attending image processing tutorials.

eBIC Cryo-EM Sample Preparation Workshop

This workshop, held in November 2019, included practical work, with students preparing vitrified samples using the latest generation of plunge-freezing devices. Students were subsequently able to screen these samples on Titan Krios, Talos Arctica, and Glacios electron microscopes.

The OPEN-Sesame and Instruct Macromolecular Crystallography Thematic School

This course was run jointly with the H2020 funded project OPEN-Sesame, which is helping to develop skills and methodologies in support of the new Sesame synchrotron in Jordan. The objective of this school was to train young researchers from the Middle East in the techniques of macromolecular crystallography (MX) and BioSAXS. In December, three students attended a 2-day dedicated course in BioSAXS at Diamond Light Source, consisting of lectures, tutorials and hands-on data collection, processing, and data analysis at the B21 beamline.

1. Fan H, Walker AP, Carrique L, Keown JR, Martin IS, Karia D, Sharps J, Hengrung N, Pardon E, Steyaert J, Grimes JM, Fodor, E. Structures of influenza A virus RNA polymerase offer insight into viral genome replication. Nature. 2019;573:287-290.

Instruct Centre Lead Scientists

Jonathan Grimes

Nicola Harrington

Yvonne Jones

Rebecca Thompson

Martin Walsh

Yuguang Zhao

SPOTLIGHT ON MEMBER ACTIVITIES

Portugal and Slovakia were founding members of Instruct-ERIC and have played a central part in forming and helping to develop the Instruct that we see today. Although some Members do not currently host an Instruct Centre, all Members support Instruct activities and are involved in structural biology research that helps to consolidate and improve their national facilities and expertise.

PORTUGAL

In Portugal, integrated structural biology is well developed in several Universities:

University	Technology
ITQB NOVA, FCT NOVA, i3S/IBMC	Molecular biology, biophysical methods and X-ray methods
Minho University	Biochemistry, biology and related methods
Aveiro University	Biology and materials sciences
FCT NOVA, ITQB NOVA, Coimbra University	NMR
FCUL	Native mass spectrometry

Flagship Technology

At IBET/ITQB a link with the pharmaceutical industry has been developed with Merck KGaA at the gene-to-structure level by a combination of structural biology and molecular biophysics. A protein-based approach, involving state-of-the-art recombinant protein production and characterisation (such as protein construct design, thermal stability and dynamic light scattering studies, mass spectrometry, assay development and surface plasmon resonance) offers a valuable platform for drug screening, discovery and development, both focusing on small compounds interacting with target-proteins, or on the development of antibody-based therapies / biologics.

Collaborations

ITQB Nova also brought together funding from Instruct-ULTRA and the CYTED network, coordinated by Margarida Archer, to further develop collaborative work between Latin-American countries and Instruct Centres (Instruct Centres ES, FR1).

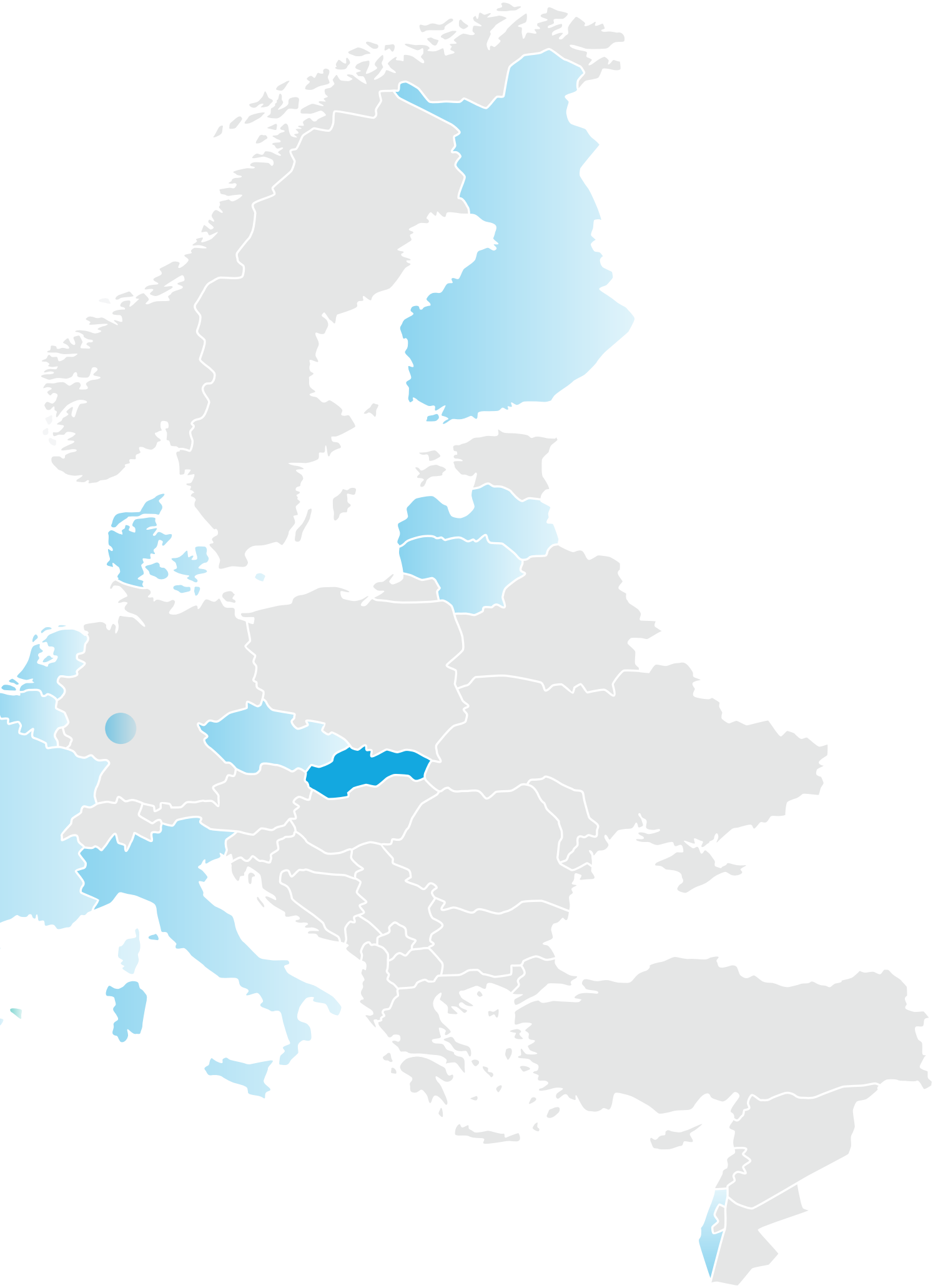
An EU Twinning project, IMpaCT is also running, coordinated by Pedro Matias and Célia Romão from ITQB with Instruct Centre ES, University of Helsinki and the Weizmann Institute (Instruct Centre IL) with the goal of training ITQB NOVA researchers in cryo-EM methods.

SLOVAKIA

Slovakia's main scientific site for Instruct activities is the Centre for Glycomics within the Institute of Chemistry at the Slovak Academy of Sciences in Bratislava.

Given the Centre's expertise in analysis of the structure, conformation, and dynamics of mono-, oligo- and poly-saccharides, their derivatives and glycoconjugates, and measuring weak intermolecular interactions between proteins and carbohydrate molecules, plans were initiated in 2019 to make these facilities open to scientists from Instruct Member Countries, as part of a pilot program to launch in 2020, for the structural analysis of glycan samples.

In November 2019, the Centre hosted the 3rd Structural Biology meeting, funded by Instruct-ULTRA, as a two-day scientific meeting showcasing the structural biology research from scientists in Slovakia and other European members. The quality of the presentations was appreciated by the 40 attendees.



INSTRUCT-ERIC HUB

The core staff of Instruct, both at the Hub and at the Centres, remained largely intact in 2019. Instruct has been built up through long relationships, and while new members have been added to our complement and have contributed new ideas and energy, the core has provided stability.

The Hub staff increased from 12 in 2018 to a total of 19 in 2019, with the extra staff being employed on EU-funded projects. Three staff members left Instruct and were replaced, and seven new members joined. Seven of the staff are seconded from the University of Oxford.

The Hub team has seen significant expansion in the IT and software development team. After a break of two years, spent working on reconfiguring big data computing infrastructure at the University of Oxford, Callum Smith re-joined Instruct to become a Senior Developer and to lead the IT team.

SPOTLIGHT ON STAFF

Susan Daenke

With a research background in virology and molecular immunology, Susan has always appreciated the ability of molecular structure to reveal functional architecture and interactions. Since she became involved in structural biology projects, the power of structural methods has advanced significantly and rapidly. This has made the first two and a half years of Instruct-ERIC both fascinating and challenging, since we now have capabilities to observe and measure structural dynamics of proteins and their complexes and follow interactions and structural changes in the native cell.

Susan's challenge lies in bringing these capabilities to researchers who need them and a large part of this has been to improve the awareness and understanding of Instruct's objectives and encourage researchers to become Instruct users. Her first priority is to find a way to support researchers, through Instruct, whether it is to access the infrastructure, training courses or the other services Instruct offers. She is also conscious of the value of involvement in the Horizon 2020 programme and interactions with the other research infrastructures, like Instruct-ERIC, on the ESFRI Roadmap and works to strengthen these connections.

As Hub Coordinator, Susan's role is to take an overview of Instruct operational delivery and, working with the Director and the governance bodies, implement a programme that is progressive and agile. To date, Instruct has successfully delivered an impressive catalogue of infrastructure facilities, excellent training courses and is coordinator in two and partner in several other H2020 funded projects. 2019 has been a successful year and Susan looks forward to building on this in 2020.

Callum Smith

Callum leads the IT team at the Instruct Hub, which is chiefly responsible for developing, maintaining and providing technical support for ARIA access management software and website functionality. Callum was the originator of ARIA and leads on strategies to develop its functionality with the changing needs of communities linked with Instruct.

Callum's role requires him to understand how the system is currently used and uses this as a foundation on which to build. The ARIA User Group is one advisory group that is useful here, as is also the Data Management and Computational Committee. He is responsible for oversight on all the different aspects of development, from React to PHP dev-ops through to our leading identity management solution. He ensures that software not only meets the needs of our growing user community but is also part of work to increase the stability and security of the platform.

Callum has responsibility for project work in EOSC-Life, iNEXT and RI-VIS, and works with members of these consortia to deliver for the projects.

CAPACITY BUILDING AND STAFF DEVELOPMENT

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

The Managers Group is one way to ensure that staff at Instruct Centres are in good communication with the Hub and can pass on information relating to new capabilities, difficulties or suggestions to improve Instruct. In 2019, the Manager Meeting was held in Alcalá de Henares in Spain and this meeting covered topics of quality assurance and management, shared experiences including best practices for common service provision amongst facility managers and the implementation of new ARIA features.

Work is ongoing to develop templates and standard operating procedures that all staff can use. In addition, the Instruct-ULTRA team are developing a communication strategy that will help all Centres to improve outreach to their local, regional and national communities while Instruct Hub will provide strong messages to promote transnational Instruct activities across Europe.

The next Manager Meeting is planned for February 2020, where there will be an emphasis on communications and working with industry.

NEW TEAM MEMBERS

Marysa Chapman
Project Assistant

Stephanie Chapman
Communications & Outreach Project Associate

Denis Nemytov
Front End Developer

Marcus Povey
Senior Software Developer

Swapna Rekala
Quality Assurance Engineer

Mark Reynolds
UX Designer

EXISTING TEAM MEMBERS

Claudia Alén Amaro
Senior Programme Manager

Twba Al-Shaghdari
Trainee Software Developer

Lorraine Donaldson
Financial Administrator

Madalena Gallagher
Administrative Officer

Naomi Gray
Project Manager

Natalie Haley
Project Manager

Estina Ketsetzopoulou
Finance Assistant

Ray Owens
Instruct-ULTRA Project Manager

David Rodríguez Aguiar
Software Developer

Fiona Sanderson
Software Developer

David Stuart
Director


6QN8

SERVICES

ACCESS

Providing access to Instruct infrastructure is the primary activity of Instruct-ERIC. Calls are advertised regularly on the website for proposals in any field of research utilising structural biology methods. Special calls with a particular scientific focus, or for a specific section of the community (for example from new user communities, industry users or pilot calls for international users) are launched periodically.

The application process continues to follow our on-line submission process via ARIA (Fig 1).

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

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

Instruct funding for Support Costs

The pilot study for a revised access contribution model tested the impact of raising the Instruct contribution to access costs for each service/technology. The new contribution at the academic rate was capped at €5000 per visit, which could be split between the following costs:

1. Consumable costs. Up to €5000 available per access visit or project to support experimental and instrument costs.
2. Travel and accommodation. If no other support is available, a contribution towards travel and accommodation paid to the researcher of up to €400 within mainland Europe or €600 from Israel. Within this limit, eligible accommodation costs are capped at €80pppn.

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

The pilot study concluded in December 2019 and was adopted in principle at that time, subject to approval by the Council in 2020. The benefits of the new model were found to be:

1. Fairer Instruct contribution to support highly specialist and expensive infrastructure, making it easier for Instruct Centres to pledge time for Instruct users.
2. An increase in infrastructure capacity delivered during the pilot period.

FIG 1

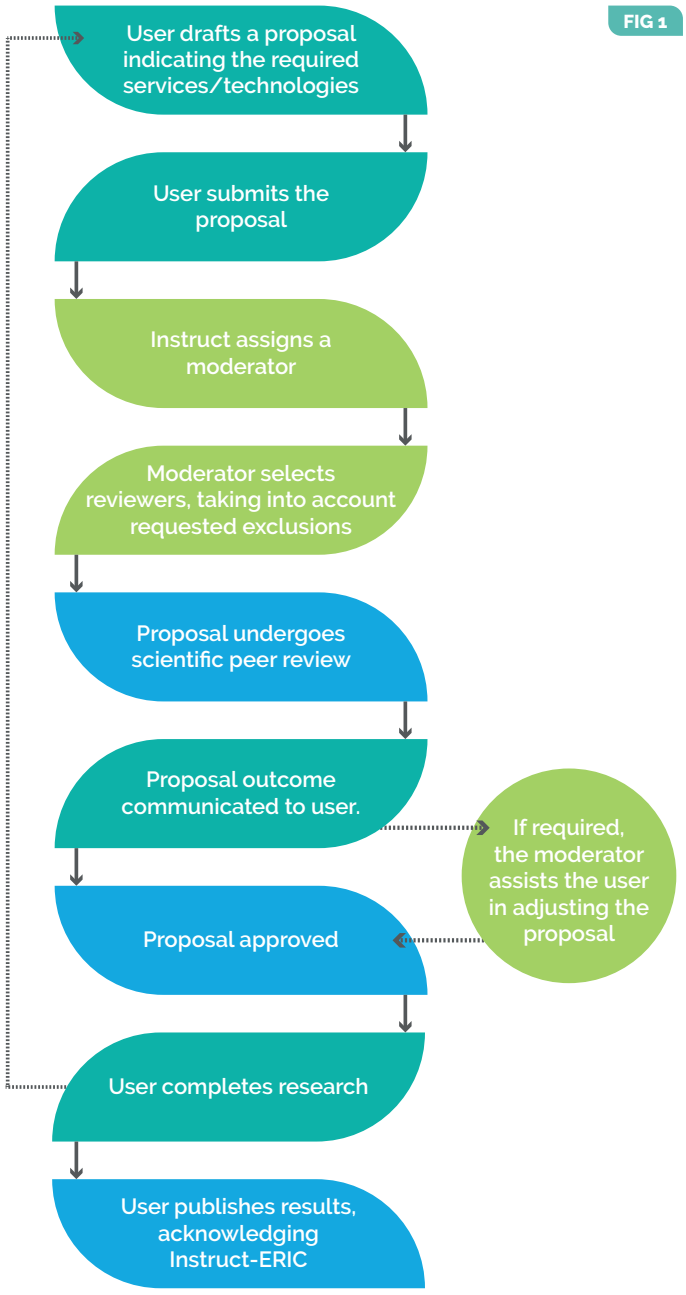


FIG 1. The pathway for access to Instruct-ERIC services.

ACCESS TO INFRASTRUCTURE 2019

In the period from 1 January to 31 December 2019, 122 proposals were received and 91 were approved following peer review, equating to a 75% approval rate.

Reasons for rejection include:

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

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

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

Overall, the feedback experiences from users of the Instruct infrastructure are positive. In 2019, 41% of applicants were return users. Instruct continues to engage new researchers in the structural biology community through website and social media, and Instruct-ULTRA has also worked to engage new users of Instruct access services through conferences and other networking events.

Access by Country

Applicants come from across Europe. Fig 2 shows the number of completed access visits per country in 2019. A total of 76 visits were undertaken by researchers from EU members, of which the highest numbers came from France, Spain and the United Kingdom.

A special call for pilot access to researchers in Latin America as part of an outreach programme funded through Instruct-ULTRA resulted in eight visits for researchers from Argentina and Brazil. In total, the access delivered to researchers as a result of these proposals equates to 782 days of infrastructure time.

The majority (65.5%) of access was transnational, with 34.5% was researchers accessing Instruct 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. In all cases where there are concerns, advice is sought from the receiving facility to determine if the request comes from outside, and a decision to proceed is sought from the proposal moderator.

FIG 2

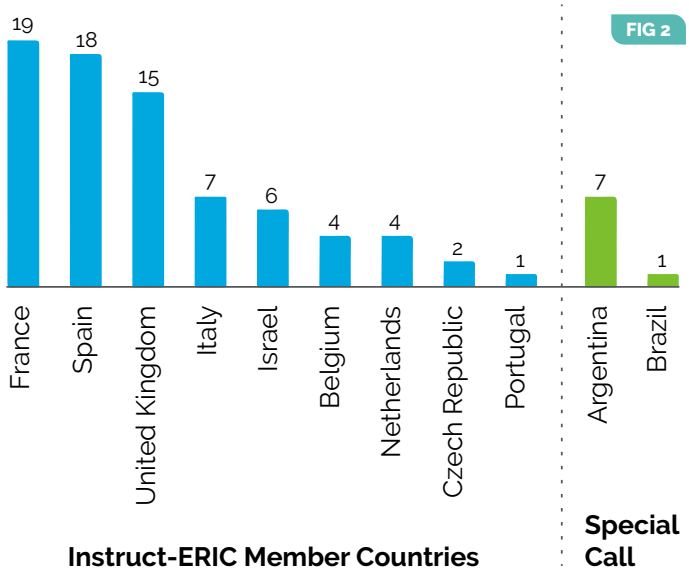


FIG 2. The number of completed access visits per country of applicant.

Access by Service Type

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

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

FIG 3

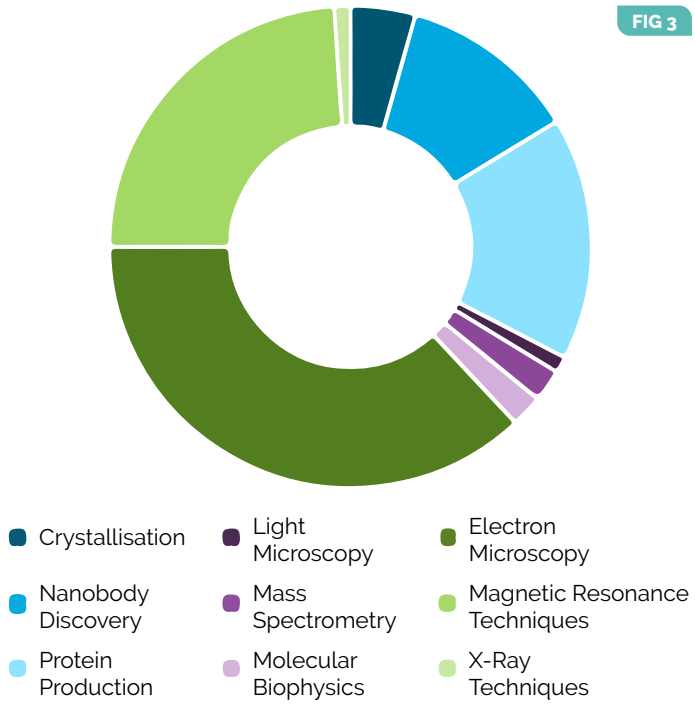


FIG 3. The number of completed access visits per country of applicant.

ACCESS HIGHLIGHTS

Cutting the message: structural insights into pre-mRNA recognition and cleavage by CPF endonuclease (PID2859)

Hill CH, Boreikaitė V, Kumar A, Casañal A, Kubík P, Degliesposti G, Maslen S, Mariani A, von Loeffelholz O, Girbig M, Skehel M, Passmore L. Activation of the endonuclease that defines mRNA 3 ends requires incorporation into an 8-subunit core cleavage and polyadenylation factor complex. *Mol. Cell.* 2019;73:1217-31.

Messenger RNAs (or mRNAs) carry the information required to make proteins in cells. The end of an mRNA is generated by a multi-protein complex called cleavage and polyadenylation factor (or CPF). Pre-RNA cleavage is essential in preparing the mRNA 3' end for transcription termination and addition of a string of adenosines – a modification that is important for translation of mRNA into protein and for mRNA stability.

A team in Lori Passmore's group at MRC Laboratory of Molecular Biology in Cambridge reconstituted the activities of CPF using recombinant proteins. Their work suggests redundancy in the composition of the dynamic mRNA 3' end processing complex.

The work, published in Molecular Cell, includes a cryo-EM reconstruction of the nuclease that cuts mRNAs, bound to its partner Mpe1. This was particularly challenging because the only part of the proteins that aligned well was 57 kDa in size – relatively small for cryo-EM. These data were collected on a Titan Krios with Volta phase plate at IGBMC Strasbourg through Instruct proposal PID2859. Further work on this proposal continued into 2019.

This research exemplifies an integrated structural biology method, including cryo-EM, protein crystallisation and X-ray structure determination, mass spectrometry and a range of software analysis tools.

FIG 1

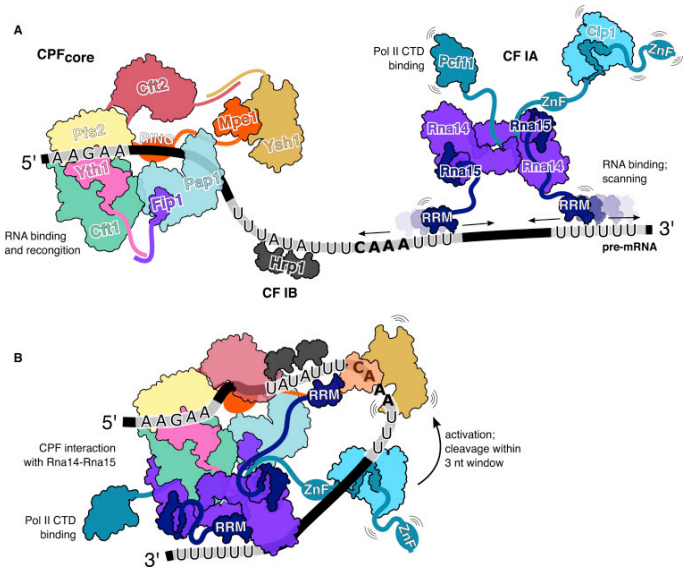


FIG 1. Model for 3' End Formation on the Minimal CYC1 Pre-mRNA Substrate

Nanobody selection against mitochondrial carriers (PID 1270)

Ruprecht JJ, King MS, Zögg T, Aleksandrova AA, Pardon E, Crichton PG, Steyaert J, Kunji ER. The molecular mechanism of transport by the mitochondrial ADP/ATP carrier. *Cell*. 2019 24;176:435-47.

Mitochondria generate energy for the body by making ATP from ADP, which needs to shuttle from the cell cytoplasm across the mitochondrial membranes. Once made, ATP then needs to be transported to other parts of the cell for use in chemical reactions. However, ATP and ADP need specific carriers to transport both molecules across the membrane. Jonathan Ruprecht and colleagues investigated the precise molecular mechanisms of the carrier.

Carrier molecules, like the mitochondrial ADP/ATP carrier, change shape in order to transport their cargo from one side of the membrane to the other. The small ADP/ATP carrier has to change shape significantly to allow passage from one side to another. The carrier transitions from the cytoplasmic-open state (for which the structure was already known) to the matrix-open state. To understand how the carrier changes between states, researchers first solved the 3D structure of the matrix-open state.

For 3D structure determination of the carrier, molecules were stably arranged into a 3D crystal. The carrier was trapped in the matrix-open state by using a compound called bongkrekic acid, a lethal toxin that binds to the protein and inactivates it. Crystals of the matrix-open state were grown bound to a nanobody prepared at Instruct Centre BE. X-ray diffraction data were then collected to reveal the 3D structure of the carrier molecule, using microfocus X-ray beamlines at Diamond Light Source (an Instruct facility) in the UK and the European Synchrotron Radiation Facility in France. The nanobodies and X-ray beamlines were essential to the success of this work.

FIG 2

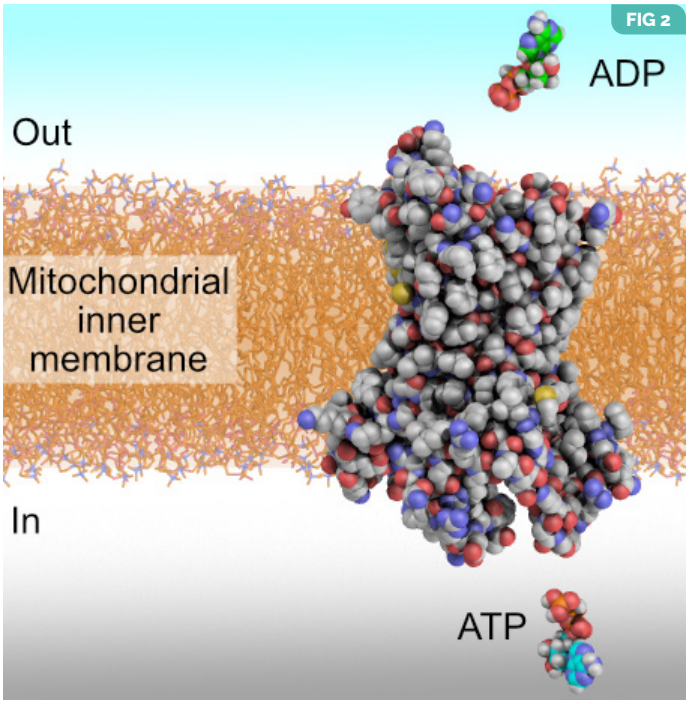


FIG 2. The mitochondrial ADP/ATP carrier protein, shown in the mitochondrial inner membrane, carries out the vital task of transporting ADP into mitochondria and ATP out.

JOINT RESEARCH AWARDS

2019 saw a new call for Joint Research Awards to support projects that develop new or improve existing access services delivered by Instruct to the user community. As new methods emerge, the Joint Research Awards aim to develop and progress methods and technologies to a stage where they can be made available through the Instruct access programme. In other words, to make them access-ready.

The awards were up to a value of €20,000 per project to be carried out at one or more Instruct Centres. Matched funding was encouraged. The call closed in August 2019 and three awards were made.

Experimental work for the Joint Research Awards runs for one year and a report on the outcome is due by the end of 2020. A key outcome is to provide a proof of principle workflow for providing a service to Instruct users.

Project 1 will explore the use of nanobodies coupled to a rigid scaffold for subsequent use in cryo-EM crystallography. Methods to achieve this using existing high throughput cloning techniques will underpin the work in this project.

Project 2 will develop new solid-state NMR methods to study the native modes of action of membrane-active drugs directly on cellular surfaces. Key to this work will be to develop new cellular sample preparation methods to preserve cellular integrity.

Project 3 will further develop and adapt a prototype set-up for in-cell NMR sample preparation which maintains cell viability and to make it available to non-expert users.

TRAINING

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

Each year Instruct opens a call for training course proposals, from which we select a broad range to offer the following year. In 2019, Instruct also co-organised four courses/ workshops to bring new communities and technological approaches together with structural biology methods.

The training programme in 2019 included nine courses or workshops, each of which saw an oversubscription. An analysis of the participants (Fig 1) shows that participants came from a total of 20 countries globally.

Of the total number of 123 participants, 78 were from Instruct Member Countries and a further 28 came from EU countries that are not yet Instruct members. Largely due to some training courses being jointly organised with other organisations, participants from 14 non-EU countries also attended some of the courses.

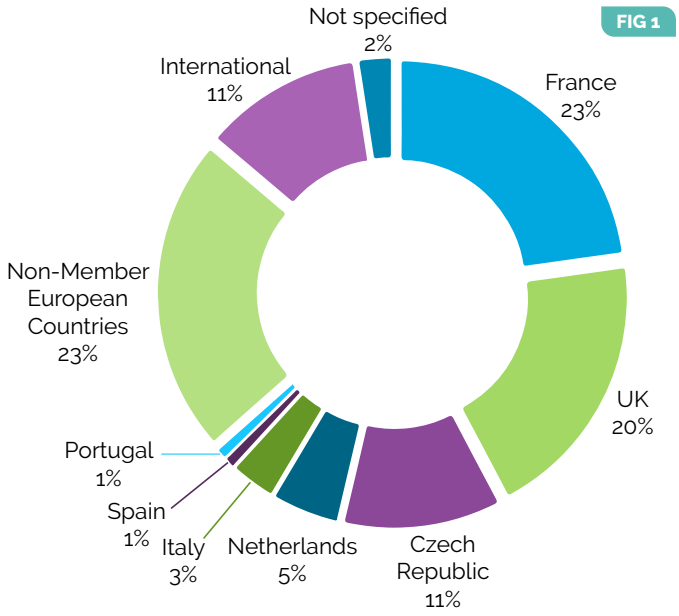


FIG 1. Training course participants by country of their home institution.

Joint Instruct-CAPRI workshop on interactive modelling of protein-protein interactions, 1 - 2 April 2019

This practical course trained young researchers in the computational modelling of protein-protein interactions using a variety of data sources, including X-ray crystallography, NMR, SAXS, cross-linking mass spectrometry and cryo-EM. The course introduced and made extensive use of common software and e-science portals to facilitate the adoption of the methods and techniques by the students in their everyday work.

Instruct-ERIC Centre Managers Workshop, 22 May 2019

This workshop introduced concepts of Quality Assurance in RIs and enabled shared experiences and best practices in the delivery of access to research infrastructure. A session on GDPR & future developments in ARIA was tailored for facility managers at Instruct Centres.

Women in Science Workshop, 22 May 2019

This workshop, supported through Instruct-ULTRA project, was aimed at women from all stages of their career, in academia, industry or management and men who wish to support women in their workplace. The program included presentations and discussion on why it is important to support women in science; women in companies; mentoring young women to achieve their career objectives.

Instruct-ERIC: Biology at different scales, 27 May - 7 June 2019

This course was aimed at PhD students and early-stage researchers. Bringing physics applications to modelling biological processes, topics covered included cell and tissue morphogenesis, role of membranes at mesoscopic and molecular scales, remodelling and transport processes.

Instruct course on image processing for electron microscopy and hybrid modelling, 8 - 11 July 2019

The course provided practical experience of using Scipion software with various integrated tools and services for image processing of single particles and atomic modelling.

Instruct workshop on integration of computational approaches in structural biology, 8 - 9 October 2019

This course introduced methods the combine computational tools for structural biology research to optimise the integration of data from various structural approaches, for example, mass spectrometry, NMR, SAXS and cryo-EM.

Joint Instruct-ARBRE MOBIEU workshop: The quality control training school, 14 - 17 October 2019

The workshop was aimed at structural biologists who wanted to improve their skills in sample analysis and optimisation for structural techniques, in particular cryo-EM. Trainees brought their own samples to work on during the practical sessions and were advised on translating their knowledge back to their home laboratories.

3rd Instruct workshop for best practices in cryo-EM, 19 - 20 November 2019

The third in this series of workshops focused on data collection and processing strategies, cryo-EM tomography/FIB, and shared best practices for solution of common issues encountered by researchers using these methods.

Open-Sesame and Instruct Macromolecular crystallography thematic school, 1 - 11 December 2019

This school formed part of Instruct's involvement in the training of Middle Eastern researchers in the techniques of macromolecular crystallography and small angle scattering (BioSAXS) to help develop these capabilities at the newly commissioned Sesame synchrotron in Jordan. Participants received practical training on the Dimaond Light Source beamlines and data processing methods.

INTERNSHIPS

Internships provide opportunities for students and young researchers to visit Instruct Centres for short periods of time (3-6 months) to allow them to become proficient in a new technology. The exchange programme provides in-depth skills training, along with valuable experience of a different scientific environment, including the implementation of standards, processes and culture. Internships are hosted at a Centre that specialises in a method or technology from which the intern's own research can benefit. There is an annual call for internship applications that is assessed by the Training Committee.

In 2019, all internships that were awarded in 2018 were completed. The three examples below show the breadth of the technologies covered during the internships and the dedication of Instruct Centres to the training of a new generation of structural biologists.

FERDINAND NGALE NJUME, a student from Belgium, visited Instruct Centre France 1 (Strasbourg) to carry out work on the 'Characterisation of a putative GM2 activator protein of onchocerca volvulus'. During the six-month internship, Ferdinand successfully expressed and purified both OvGM2AP (from Onchocerca volvulus) and the Caenorhabditis elegans orthologue CPP-1 following expression in baculovirus, and performed biophysical characterisation and crystallisation, including large scale deglycosylation, of both proteins. Demonstrating the integrative nature of the internship, the project continued with mass spectrometric analysis to identify the positions of the disulphide bridges in both proteins. When challenges were faced around the planned experimental design, Ferdinand carried out CRISPR-mediated knock-out of the C. elegans orthologous CPP-1 strains with altered cuticle permeability. To understand the mechanism of action of CPP-1 and OvGM2AP, a pull-down assay using anti-GFP nanobodies was attempted in order to purify the GFP fused CPP-1 from C. elegans.

JESÚS BALTANÁS COPADO from Spain visited Instruct Centre UK from June to December 2019. He was supervised by a multidisciplinary team including Juha Huiskonen, Thomas Walter, Bilal Qureshi, Karl Harlos and David Stuart. Jesús' visit included work on molecular biophysics, electron microscopy and macromolecular crystallisation.

IRREM LAAREB MOHAMMAD JABEEN from the University of Barcelona visited Instruct Centre CZ from May to July 2019. The aim of the project was the characterisation of the oncoprotein tyrosine kinase c-Src N-Terminal regulatory region. This region has been reported to form a fuzzy complex, in which the Intrinsically Disordered Region (IDR) (myristoylated SH4 and unique domains) interact with the adjacent SH3 domain. The intern carried out chemical shift perturbation experiments and NMR analysis including Saturation Transfer Difference Spectroscopy and Transferred NOESY experiments to further define the interactive interfaces.

In 2019, five internships were awarded to take place in 2020. The awards not only demonstrate a geographical distribution in the awardees but also in the Centres hosting the internships.

Name	Country of Home Institution	Internship Host
Ivan Polsinelli	Italy	Instruct Centre CZ
Yixin Lium	Finland	Instruct Centre UK
Andreia Fernandes	Portugal	Instruct Centre FI
Albert Galera Prat	Finland	Instruct Centre CZ
Gala Ramon	UK	Instruct Centre FR1

STAFF EXCHANGES

Through its participation in the CORBEL project, Instruct-ERIC has benefitted from staff exchange opportunities through the CORBEL Staff Programme. This initiative enables operational staff to enhance their expertise in data management, service provision, innovation and ethics by making short knowledge-exchange visits to other RIs that are noted for their excellence in this specific area.

Staff exchange to CERIC-ERIC

Naomi Gray from the Instruct ERIC Hub participated in a staff visit to the offices of CERIC-ERIC (Trieste, Italy) between 14 - 17 April 2019, to exchange experiences focussed on communication and outreach.

"Meeting the CERIC team, who were so enthusiastic and committed to their research infrastructure and users, was such an inspiration and there was so much to learn from their experience of running a thriving research infrastructure. Thank you to Nicoletta Carboni, Jana Kolar, Ornelia De Giacomo and Angela Zennaro and the entire CERIC team for your warm welcome."

Naomi Gray, Instruct-ERIC.

FIG 1. The CERIC-ERIC team and Naomi Gray on the final day of the visit.

COLLABORATIONS TO IMPROVE COMMON ADMINISTRATIVE METHODS AND PROCESSES

Crucial to the training of staff at the Instruct Hub is collaboration with other research infrastructures and Horizon 2020 funded projects, which incentivise the exchange of experience and knowledge between different European RIs.

In this context, Hub staff participated in workshops and meetings with the projects RISCAPE, RI-PATHS, MERIL, CatRIS, and OpenAIRE. The lessons learned at these meetings have helped the Instruct Hub to achieve and adopt standards and working practises in common with other RIs. In particular, experience in the area of RI landscape analysis will help the Hub in its work as part of the EU-LAC ResInfra project, whilst understanding the pathways to measure impact and how to mine information about publications will inform work on defining Key Performance Indicators (KPIs). Finally, interactions with MERIL and CatRIS will help Instruct to improve its visibility among the European user community at large.

In addition, the RI-VIS project, which is coordinated by Instruct, also promoted exchange of information and best practices between RIs, in particular in the area of communications. During 2019, EMBRC organised two communication workshops, at which Instruct Hub staff were active participants. These workshops we useful in the development of Instruct's communication strategy, which was undertaken as part of the work programme of the Instruct-ERIC implementation project, Instruct-ULTRA.

FIG 2. A team of RI-VIS communicators, including colleagues from Instruct, EMBRC, ESS, ERINHA, ECRIN, EU-OPENSCREEN and EATRIS, meet to exchange knowledge and best practices.

University of Oxford MicroInternships

In 2019, Instruct was invited to participate in the University of Oxford's 'Microinternship Programme'. Within this programme, the Instruct Hub received three interns from the University who were either postdoctoral scientists planning to move into management or current facility managers within University departments. The internships were a valuable opportunity for both the interns and the Hub to exchange ideas and perspectives. In 2019, the Hub also participated in the University of Oxford's network for communication officers and research facilitators (RISN). These activities promote Instruct-ERIC's visibility within the academic environment and more broadly with agencies linked to European funding programmes.

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

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

Access Management

ARIA provides a large suite of tools to form a service ecosystem, spanning proposal submission through to machine and facility management, and a fully email-integrated messaging system. ARIA is customisable to projects' needs and also facilitates the management of websites providing a presence for communities and infrastructures with integrated with a content management system.

Facility Management

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

Project Management

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

Data Access APIs

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

The focus in 2019 has been on improving the messaging system which has been re-engineered to support full communications via email. ARIA messaging is a unique tool that allows discussions relating to applications and proposals to be connected directly to the data for ease of management and simple archival procedures. It also allows for anonymous discussions between relevant parties, protecting the confidentiality of the reviewers. Enabling this to work via email whilst retaining key features that people require for service delivery is now fully implemented and has been positively received by the user community. ARIA has further extended its APIs and piloted a new data integration mechanism, initially with Diamond Light Source (Instruct-UK). We are now identifying opportunities to streamline usability throughout ARIA and the Instruct-ERIC website.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COLLABORATIVE WORK

EUROPEAN PROJECTS

instruct ULTRA

Instruct-ULTRA was launched in 2017 to expand and enhance Instruct-ERIC. The project is coordinated by Instruct and comprises 16 partner institutions, mainly from Instruct member states, with a budget of 3.9M Euros.

Expanding access through outreach activities

2019 was a busy and productive year for Instruct-ULTRA. To widen access to Instruct-ERIC, ULTRA took the lead in outreach to two new European countries, initiating dialogue with senior scientists and ministry representatives, and attending in-country meetings with key stakeholders to explain the mission of Instruct. As well as initiatives to expand European membership of Instruct, ULTRA has helped to promote Instruct globally by fostering links with structural biologists in South Africa, India and Latin America.

In another approach to expand Instruct's user base, ULTRA undertook a campaign to promote the Instruct technology catalogue to industry. A pilot SME campaign was launched in March 2019, culminating in a successful Open Event at Instruct Centre UK, with all materials made available for other Centres to replicate.


FIG 1

FIG 1. Attendees network at the Instruct for Industry event.

Supporting inclusivity in STEM

Responding to the challenge of Women in Science (WIS), Instruct-ULTRA hosted a successful WIS Workshop in May 2019 as a satellite event to the Instruct-ERIC Biennial Conference in Madrid. 75 participants, including 10 WIS fellowships, gathered to discuss the representation of females in faculty positions and practical ways to address the challenges facing women working in academia.

FIG 2

FIG 2. Speakers and organisers at the Instruct-ULTRA Women in Science workshop at the Instruct Biennial Structural Biology Conference.

Developing new technology and methods

Instruct-ULTRA has supported the development and refinement of structural biology technology through a series of pilot projects. Innovative approaches to sample preparation have included optimisation of an NMR bioreactor for in-cell applications, new techniques for streamlined production of membrane proteins, glycan analysis, and extending the use of nanobodies. Developments in cryo-EM have centred on the workflow for Focused Ion Beam (FIB) milling, remote access to data, and the design of a sterilisation device for cryo-EM samples. Structural biology data management challenges were addressed by new solutions in metadata processing, sharing and archiving, as well as improving connectivity between different software packages.

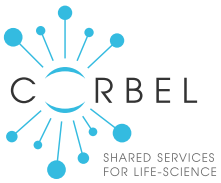
Planning for the future

Business and sustainability planning for Instruct-ERIC are two key areas that have been addressed in the ULTRA project, together with developing new communication strategies. These plans include a conference outreach programme that has already increased the visibility of Instruct-ERIC across Europe, generating new users of Instruct's technology and training services.

EUROPEAN PROJECTS

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

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



CORBEL: Coordinated research infrastructures building enduring life-science services

Within CORBEL, Instruct has contributed to a common access framework for all life sciences RIs to provide a seamless access pathway to services and resources across multiple RIs. This underpins some of the work planned for EOSC-Life.

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 one of the work packages.

iNEXT: Infrastructure for NMR, EM and X-rays for translational research

A transnational access network providing structural biology facilities to user communities for translational science projects. iNEXT ended in August 2019.

AARC2: Authentication and authorisation for research and collaboration

Working on the development and deployment of a broadly adopted AAI (Authentication and Authorisation Interface) that will serve EU research facilities, including ESFRIs.

Open-SESAME: Opening synchrotron light for experimental science and applications in the Middle East

This project is supporting the development and full exploitation of SESAME Light Source for the Middle-Eastern research community. Instruct provides specialist training for SESAME staff and users.

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.

ERIC Forum: The ERIC Forum implementation project

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

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

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

1. RI-VIS is coordinated by Instruct-ERIC.

INTERNATIONAL COLLABORATION

Instruct-ERIC's internationalisation efforts have proved particularly fruitful during 2019, developing initiatives in Latin America, India, South Africa, Middle East and India. Establishing joint activities with countries outside of Europe has increased international outreach, bringing benefits in terms of scientific exchange and new collaborations.

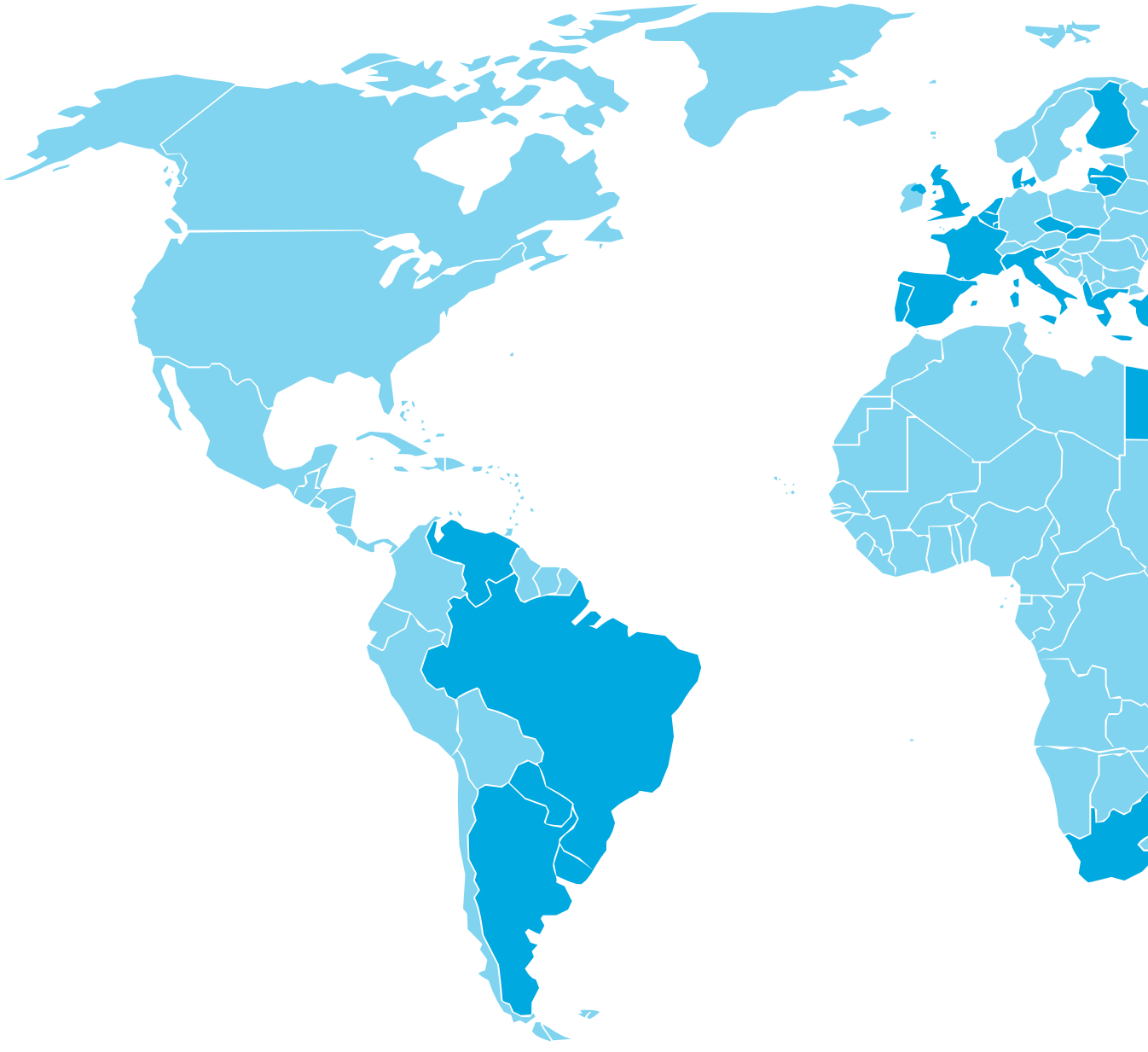
Latin America

During 2019, Instruct's relationship with countries in Latin America has been strengthened through Memoranda of Understanding (MoUs) with a further seven institutions, with CeBEM[1] playing a key role in validating their scientific standing in a regional context before an MoU was established.

In April, Instruct launched an Open Call welcoming the Institutes in CeBEM to apply for access to Instruct Centres. Eleven projects were accepted from five institutes. Successful applicants in the Open Call obtained financial support from either their home institute or through the MICROBES project (funded by Cyted[2]) recently awarded to ITQB (Instruct-PT). The first scientific visits took place during the second half of 2019 and others are planned for 2020.

Instruct-ERIC's track record in building collaborations with the structural biology community in Latin America has led to its participation in the project EU-LAC ResInfra (H2020-INFRASUPP-2019-1 no. 871140), which is funded for 30 months from 1 December 2019. The project aims to show the feasibility of collaboration between European Research Infrastructures and Latin America, in the context of the Community of Latin American and Caribbean States (CELAC). Instruct was invited to be one of four pilot projects that had already successfully engaged with the scientific community in Latin America. A landscape analysis of structural biology in the region will be carried as part of the project.

The activities planned within EU-LAC will complement work planned for the project RI-VIS (H2020 No 824063) that Instruct-ERIC coordinates. A joint event with EU-LAC ResInfra on international collaboration between Research Infrastructures is planned for 2020-2021 in Brazil at the CNPEM Campinas, the location of the new Sirius Synchrotron.



1. CeBEM is the structural biology network of the MERCOSUR countries: Argentina, Brazil, Paraguay, Uruguay and Venezuela.

2. Cyted is the Ibero-American Programme on Science and Technology for Development, created by the governments of Ibero-American countries in order to promote cooperation in science, technology and innovation for the harmonious development of Ibero-American countries.

India

India is now a major presence in the field of structural biology and has many collaborative links in Europe, including shared beamlines at Elettra (Trieste) and ESRF (Grenoble).

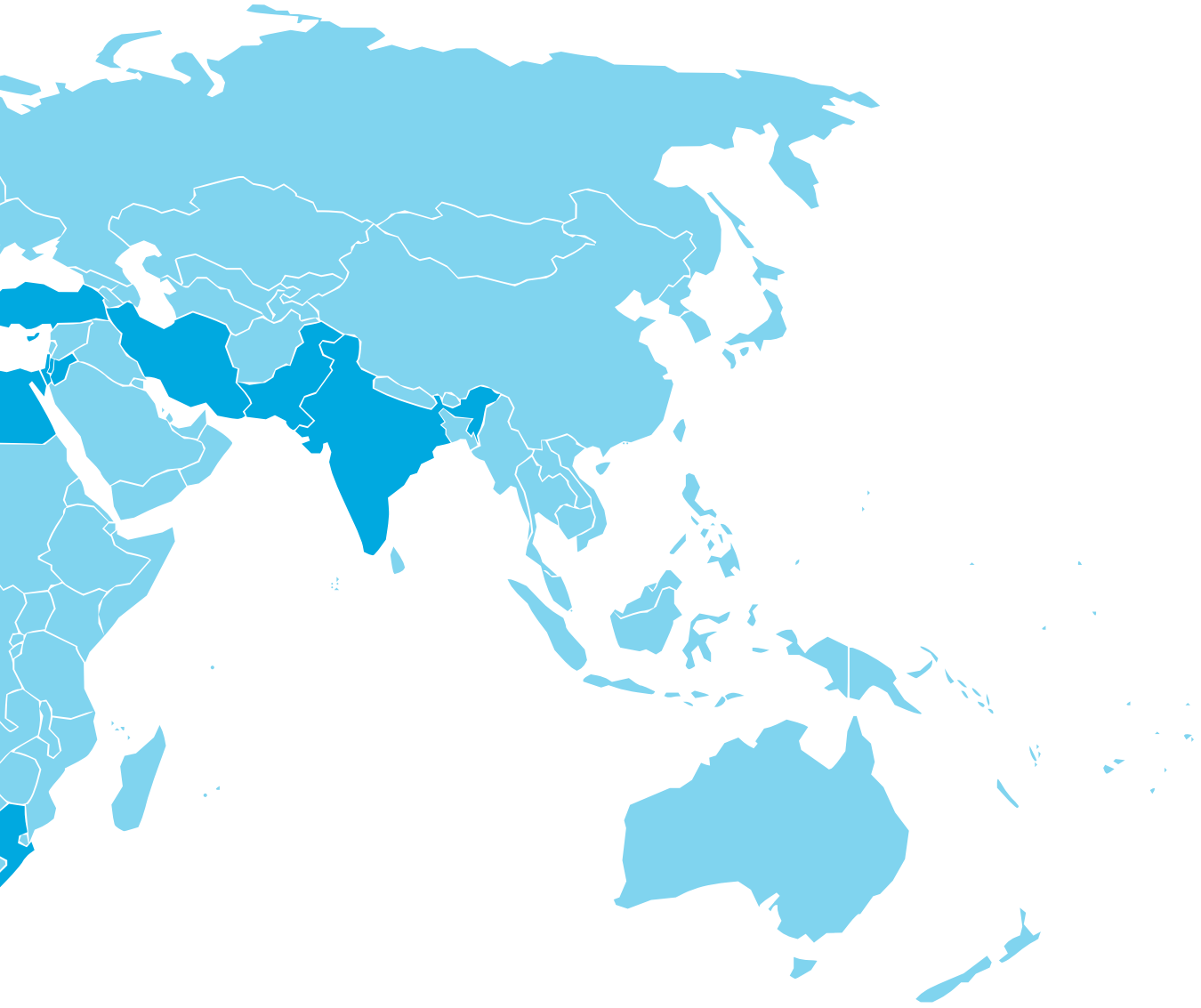
A significant meeting took place in September 2019, with senior Indian scientists invited by the CNRS to visit the University of Strasbourg (Instruct Centre FR1) for a joint meeting. The structural biology presentations illustrated the maturity and depth of science taking place in India and Instruct chaired a meeting looking at possibilities for collaboration with Indian scientists and institutes. Strong interest has emerged from a number of major institutional organisations in India. Follow-up meetings are scheduled in Delhi and Bangalore for January 2020, with the aim to explore potential collaborations and joint scientific meetings.

South Africa

Instruct has continued to explore opportunities to engage with structural biologists in South Africa. During 2019, Instruct established links with a UK-funded programme, Synchrotron Techniques for African Research and Technology (START), led by the Diamond Light Source (Instruct-UK). In January, Instruct participated in a START training workshop on 'Biophysics and Structural Biology at Synchrotrons' at the University of Cape Town and further collaborative events are planned for 2020.

Middle East

As a partner in the Open-SESAME project (H2020 project 730943), Instruct co-organised a training course on Macromolecular Crystallography for researchers from SESAME member countries (Cyprus, Egypt, Iran (Islamic Republic of), Israel, Jordan, Pakistan, the Palestinian Authority, and Turkey). The workshop was held at the Diamond Light Source (Instruct-UK), 1-11 December 2019.

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SUPPORTING ACTIVITIES AND OUTPUTS

COMMUNICATIONS

Since achieving the ERIC status in 2017, the Instruct community has continued to grow, with new European Members, facilities and users, and international collaborations. As the network expands, Instruct is developing its communications in order to maintain an effective and impactful dialogue with stakeholders and increase its visibility across Europe.

With support from Instruct-ULTRA, a Communication Strategy and Policy have been developed for Instruct-ERIC. As well as detailing the aims and objectives of Instruct communications, the Strategy document describes Instruct's key audiences, messages and communication channels. In turn, the accompanying Policy documents sets out principles and guidance for communications to ensure that all outputs consistent, effective and appropriate.

Preparations are underway to develop an Instruct-ERIC Communication Plan that builds on the Strategy and Policy documents by outlining key processes to increase awareness and recognition of Instruct and Research Infrastructures, and to enable dialogue with stakeholders.

Conference programme

To expand Instruct's reach to new academic communities, a conference outreach programme was developed for 2019 with support from Instruct-ULTRA. Through this programme, Instruct-ERIC participated at seven major European conferences, including FEBS 2019, EBSA 2019, and BASEL LIFE. Representation included talks to introduce Instruct-ERIC and booths in conference exhibition halls, often in collaboration with other RIs.

Additionally, a short 'Overview of Instruct' PowerPoint slide deck was made available to Instruct staff and partners to support them in their individual efforts to promote Instruct-ERIC at conferences and events.

Promotional material

A range of new print material has been produced for dissemination and promotion of Instruct-ERIC at conferences, training and events. These new resources include an eye-catching postcard, a travel coffee cup, pop-up banners for each Centre and Member Country, and personalised plaques for each Instruct facility. Targeted promotional material for industry, including a brochure with details of Instruct's services and a banner promoting Instruct's services for industry.

FIG 1. Staff from Instruct-ERIC, EU-OPENSCREEN, Euro-BioImaging and CORBEL host a joint European Research Infrastructure booth at the FEBS Congress 2019.

FIG 2. A selection of Instruct-ERIC promotional material.

Scientific Highlights

In 2019, a new section was introduced to the Instruct-ERIC website for scientific highlights that showcase high-impact research undertaken at Instruct Centres. The scientific highlights, (which are available at instruct-eric.eu/scientifichighlights) have received a very positive reception on social media.

Social media

Instruct-ERIC's social media presence has continued to grow, with the @instructhub Twitter account now at over 3,500 followers and gaining, on average, 90 new Twitter followers per month. A particular focus on quality scientific posts, conference and event promotion, and the production of bespoke graphics and audio-visuals has boosted engagement online.

Audio-visuals

This year Instruct has invested in photography and video equipment, along with editing software, so that a range of audio-visual resources can be produced in-house.

In 2019, a series of video podcasts were produced from scientific talks given at the Instruct-ERIC Biennial Structural Biology Conference. These podcasts allowed those who were unable to attend the event to view the material presented at the conference. During the satellite Women in Science event, female scientists were interviewed and the clips from these interviews were compiled into a short video on the topic 'Why is it important to support women in science?'. The final video was released on Twitter, gaining over 2000 impressions.

Videos were also filmed and produced to introduce Instruct facilities in Madrid, Strasbourg, and Leeds. These videos have been added to the Centre pages on the Instruct website to communicate the services available from these site. There are plans to continue with this initiative and film at other facilities in 2020.

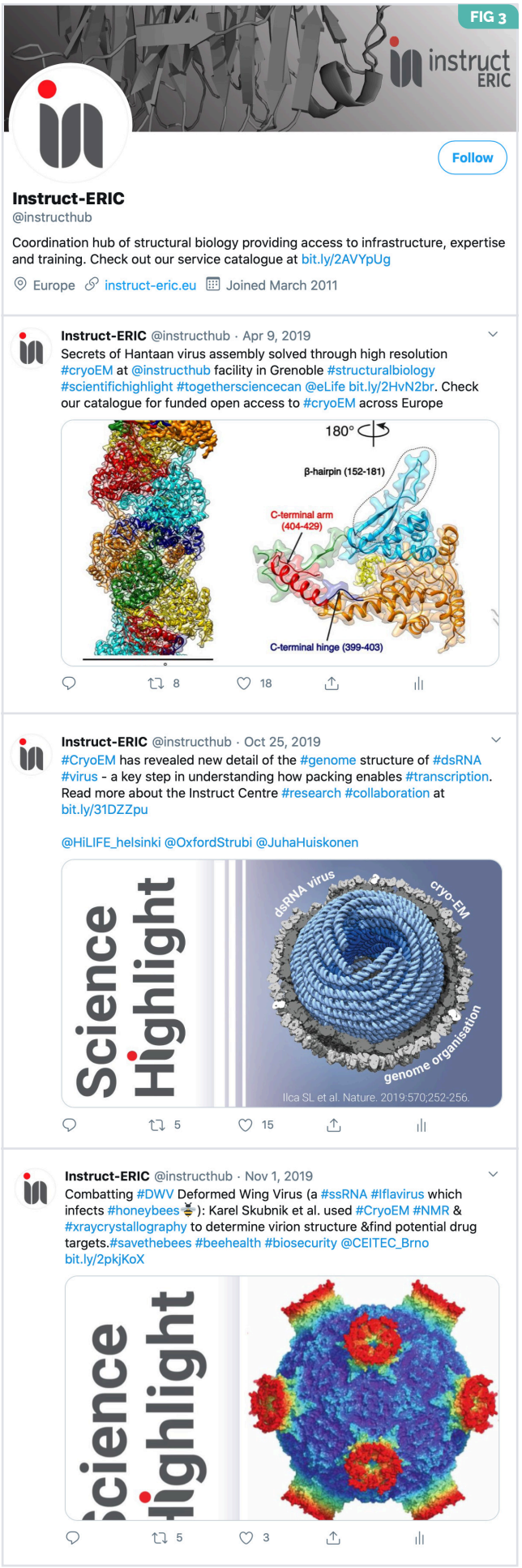

FIG 3. Instruct-ERIC Scientific Highlights are popular on Twitter.

SCIENTIFIC PUBLICATIONS

Instruct continues to consolidate and expand the ERIC organisation based on the scientific excellence of Instruct scientists that provide the drive, energy and vision that underpins our operational objectives. Instruct aims to deliver access to high quality infrastructure, supported by the expertise that makes it possible for researchers unfamiliar with the techniques to achieve good data and be able to understand and analyse it correctly.

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

For 2019, the list of peer-reviewed publications numbers 193. Of these, 49 publications appeared in journals of impact factor 10 or more, which is a slight increase compared with 45 published in 2018. The average journal impact factor for all publications acknowledging Instruct in 2019 was 8.2, again slightly higher than the 7.3 achieved in 2018.

The scientific impact of Instruct in the structural biology community in Europe is still relatively small, but these indicators suggest that Instruct is enabling good quality science, and aims to further increase Instruct's impact in Europe. As a research infrastructure, building our reputation for scientific excellence provides the credibility that is needed to engage with potential users, most of whom are from the academic community, where publication success is still the key criterion used to promote career progression.

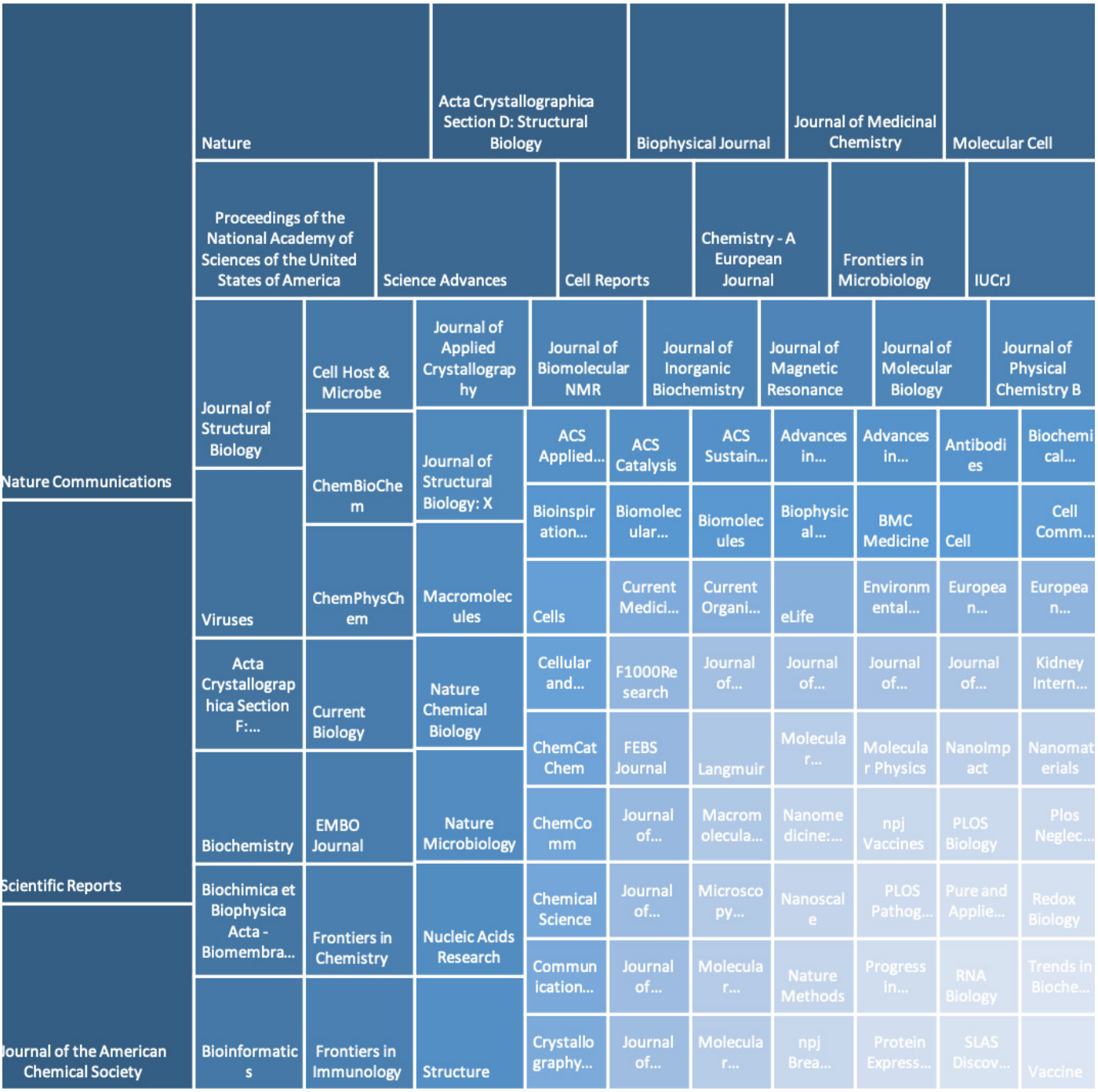

FIG 1. Relative number of publications acknowledging Instruct per peer-reviewed journal for 2019.

2019 PUBLICATIONS WITH JOURNAL IMPACT FACTOR >10

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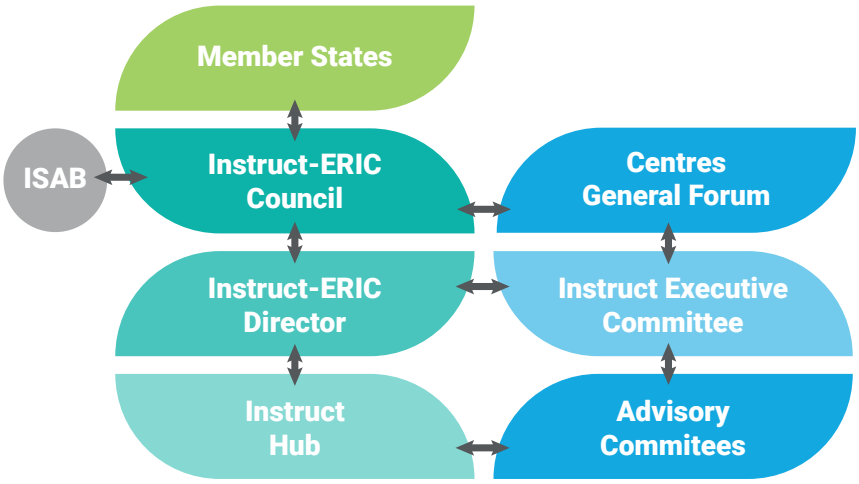
GOVERNANCE

Representation on the Instruct-ERIC Council saw a change of leadership with the Chairmanship passing from the UK to France with Eric Guittet taking the role of Chairman, and Inmaculada Figueroa from Spain supporting him as Vice-Chair. The Council was further supported by the addition of Ondrej Hradil (CZ) as a special advisor. The Council continued to be supported by the Independent Scientific Advisory Board, chaired by Prof Stephen Burley, with its membership unchanged and this ensured continuity in strategic oversight and advice to the Council.

Within 2019, the Director, David Stuart, indicated his intention to step down from his role due to his commitments to his other roles of Life Sciences Director of Diamond Light Source Limited, and his Professorship and co-Directorship of the Structural Biology Division at the University of Oxford. The Council were therefore tasked with establishing a process for recruitment of a new Director, aiming to achieve this by the end of 2020.



Scientific direction and implementation of the Instruct objectives continued to be managed by the Executive Committee, Chaired by the Director. Representation on the Executive Committee was bolstered by the addition of members from the new Instruct Centre in Finland, which comprises three sites and is coordinated centrally from the University of Helsinki by Prof Sarah Butcher. Both the Executive Committee and Council warmly welcomed Finland to Instruct-ERIC.

The Executive Committee continued to be advised by smaller subcommittees managing specific activity areas of the Instruct workplan. These included the Access Committee, Training Committee, Data Management and Computational Committee, and the Landscape Analysis Committee, which was formed late in 2019 to review the status of structural biology technology capabilities and capacity in Europe with reference to the global context. The Landscape Analysis Committee will report ahead of the internal 5-year review of Instruct-ERIC (in mid-2022) to help identify priority areas to be considered for future strategic planning.

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

Group/Panel/Project	Role	Representatives
ERIC Forum	Member	Susan Daenke
Life Sciences Research Infrastructure Strategy Board	Vice-Chair	Susan Daenke
ESFRI Health and Food Strategic Working Group	Member	Lucia Banci
Transvac2 Steering Group	Member	Susan Daenke, Francesca Morelli
CORBEL Executive Board	Member	David Stuart, deputised by Susan Daenke
Instruct-ULTRA Executive Board	Chair	Ray Owens
iNEXT Management Committee	Member	David Stuart

INDEPENDENT SCIENTIFIC ADVISORY BOARD

Chair: Prof Stephen Burley, Rutgers University, USA

Members

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

INSTRUCT-ERIC GOVERNING BODIES AND COMMITTEES

COUNCIL

Chair: Dr Eric Guittet, FR
Vice-Chair: Dr Inmaculada Figueroa, ES

Country	Scientific Delegate	Administrative delegate
Belgium	Michele Oleo	Laurence Lenoir
Czech Republic	Vladimir Sklenar	Jan Burianek
Denmark	Thomas Vosegaard	Troels Rasmussen
Finland	Sarah Butcher	Marko Uutela
France	Winfried Weissenhorn	Eric Guittet
Israel	Joel Sussman	Ilana Lowie
Italy	Lucia Banci	Grazia Pavoncelloa
Latvia	Kaspars Tars	Janis Paidus
Netherlands	Reinout Raijmakers	Marijn Goes
Portugal	Margarida Archer	Maria Armenia Carrondo
Spain	Jose Maria Carazo	Inmaculada Figueroa
Slovakia	Milos Hricovini	Lukas Zendulka
United Kingdom	Anne McGavigan	Megan Dowie
Observers		
Greece	Evangelia Chrysina	Maria Koutrokoi
EMBL	-	Silke Schumacher

EXECUTIVE COMMITTEE

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

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

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

Training Committee	**Chair:** Lucia Banci
Access Committee	**Chair:** Darren Hart
Data Management Committee	**Chair:** Jose Maria Carazo
Landscape Analysis Committee	**Chair:** Lucia Banci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FINANCIAL DATA

FINANCIAL DATA

This report presents the financial statements for the period 1 January 2019 to 31 December 2019.

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 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 financial report takes account of the residual financial impact of a previous operational legal form of Instruct, Instruct Academic Services Limited (IASL), which was a subsidiary company of the University of Oxford and was managed by a Board of Directors using financial administration through University of Oxford services. IASL financial management accounts were audited by KPMG LLP, Statutory Auditor, Reading UK and the appropriate aspects of the transition from IASL financial management to Instruct-ERIC financial management were included in the Instruct-ERIC Annual Report 2018.

As of December 2018, all financial transfers between the two legal entities were completed and no operations of any sort have gone through IASL.

This report covers the period 1 January 2019 – 31 December 2019 and was compiled in the period starting February 2020, at which time the emerging SARS-CoV2 pandemic imposed a significant impact on Instruct activities and those of essential advisors and contractors involved in the making of this report. The publication of the report was therefore delayed.

BALANCE SHEET FOR INSTRUCT-ERIC

As at 31 December 2019

Assets	GBP	EUR	Notes
Euro bank	1,081,484	1,277,795	
Sterling bank	50,370	59,513	
Total Bank	1,131,854	1,337,308	
Current Assets			
Accounts Receivable	110,007	129,975	1
Prepayments	2,094	2,474	
Accrued income	28,007	33,090	2
Amounts due from IASL	296	349	
Rental deposits	5,339	6,308	
Total Current Assets	145,743	172,196	
Fixed Assets			
Computer Equipment	12,474	14,738	
Depreciation on Computer Equipment	(2,975)	(3,514)	
Office Equipment	3,796	4,485	
Depreciation on Office Equipment	(1,209)	(1,547)	
Total Fixed Assets	11,986	14,162	
Total Assets	**1,289,583**	**1,523,666**	

Liabilities	GBP	EUR	Notes
Current Liabilities			
Accruals	47,097	55,646	
Accruals for Internship	2,616	3,091	3
Accruals for R&D awards	14,388	17,000	4
Accruals for Access awards	4,030	4,762	5
Amounts to be paid and Unclaimed Access Awards	222,000	262,298	6
Income in Advance - Members subscription	44,917	53,070	7
Income in Advance - Other inc deferred grants	692,726	818,469	8
Income in Advance - ARIA Support	917	1,083	
Other creditors	6,779	8,010	
Payroll taxes due	11,820	13,963	
Pensions due	-	-	
Total Current Liabilities	1,047,290	1,237,392	
Total Liabilities	**1,047,290**	**1,237,392**	
Net Assets	**242,293**	**286,274**	
Surplus Brought Forward	5,093	6,017	
Surplus for the Year	237,200	280,257	
Surplus Carried Forward	242,293	286,274	

Exchange rate for reporting period: 0.84636739

1. Membership income receivable
2. Final West-Life payment owing to pay the grant in full (accepted by EC)
3. Brought forward from IASL 2017
4. Brought forward from IASL 2017
5. Brought forward from IASL 2017
6. Access and other service accruals
7. Invoiced deferred membership subscriptions
8. Deferred project income

PROFIT AND LOSS FOR INSTRUCT-ERIC

For Year Ended 31 December 2019

Income	GBP	EUR	Notes
External grant income	516,056	609,730	9
External grant overhead contribution income	100,348	118,563	
Member state contributions	760,875	898,989	10
Other miscellaneous income	39,748	46,964	11
Total Income	1,417,027	1,674,246	
Less Cost of Service Provision			
Instruct staff salaries	269,618	318,559	
R&D Pilot awards	(650)	(769)	
JRA awards	53,299	62,974	
Access Cost	145,432	171,831	12
Meetings	96,816	114,390	
Project activities	274,892	324,791	13
Total Cost of Service Provision	983,639	991,776	
Gross Surplus	577,620	682,470	
Less Operating Expenses			
Commissioned services (Insurance, financial, HR, legal)	64,933	76,719	
	76,559	90,456	
Conference costs	25,446	30,065	
Consultants	3,331	3,936	14
Depreciation charge	85,202	100,668	
Foreign Currency Gains and Losses	6,726	7,964	
General admin (postage, copying, bank charges)	-	-	
Irrecoverable VAT	8,905	10,521	
Licenses & software	168	200	
Miscellaneous	1,465	1,731	
Office Stationery	64,436	76,133	
Premises and support	24,507	28,956	
Project overhead expenses	5,199	6,143	
Publicity	3,133	3,701	
Telephone	(29,590)	(34,962)	
Write off IASL balance owed	340,420	402,213	
Total Operating Expenses			
Net Surplus	**237,200**	**280,257**	

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

10. Membership income receivable

11. Sponsorship

12. 2018 - Access released – not claimed

13. WIP on research grants. Project activities delivered through IASL and carried forward to ERIC

14. IT, website and API work by IT consultant

SUPPORTING INFORMATION FOR THE FINANCIAL STATEMENTS

Accounting Policies

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

Reporting and Disclosure Exemptions

Going concern

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

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 recognise the ERIC as an international body or organisation 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 01/01/19 - 31/12/19	Payment received
UK	103,683	103,683
FR	103,683	103,683
ES	76,500	76,500
IT	77,763	77,763
BE	77,763	77,763
NL	23,763	23,763
IL	77,763	77,763
CZ	51,842	51,842
PT	51,842	51,842
DK	51,842	51,842
SK	51,425	51,425
LV	51,425	51,425
FI	26,010	26,010
Total	825,304	825,304

Grant Recipts

EU Grants	Income Jan - Dec 2019	Other income from Projects
iNEXT	72,666	2,084
CORBEL	160,692	40,174
WestLife	68,807	468
Instruct Ultra	104,282	26,072
Open-SESAME	29,968	10,843
Transvac2	1,715	429
AARC2	1,350	480
EOSC-Life	27,713	6,928
ERIC Forum	7,769	1,942
RI-VIS	42,846	10,712
EU-CELAC	868	217
Total	518,677	100,348

Overhead contribution recognised: 25%

C. Deficit/surplus on activities €5092.70

D. Employees

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

E. Debtors

Invoices outstanding from Members (present total figure outstanding against 2019 invoices) €694699.23

Accrued income (€429.34)

Grant accrued income (WestLife €21,076.57)

TOTAL €716205.14

F. Creditors

Accruals for services and awards (Access, Int, R&D, Training, unclaimed access) €279,302.60

Amounts due to IASL €32,042.88

Members subscriptions in advance €710,300.22

Advances on Research Grants €437,055.74

Payroll taxes and pensions €1,005.48

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 an 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 is agreed in principle and has been implemented to comply with the UK terms of mandatory pension enrolment of all eligible employees within 1 month of employment. Council will consider ratification of the Pension Plan at this meeting.

J. Grant Agreements

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

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

RI-VIS: €1,500,000 (total value) – projected start date February 2019

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

iNEXT: €128,500

West-Life: €152,750

CORBEL: €563,000

AARC2: €8710

Open-SESAME: €61,563

Transvac2: €29,260

EOSC-Life: €358,051

ERIC Forum: €43,300

EU-LAC ResInfra: €106,875

GLOSSARY

Term	Definition
AARC2	The second phase of the Authentication and Authorisation for Research and Collaboration initiative, which is continuing to develop and pilot an integrated cross-discipline authentication and authorisation framework.
Access	The unit of use of Instruct Research Infrastructure being in person (visit) or remotely (by sending samples)
Access Committee	A body established to manage the review of prospective users' proposals and applications for access to the tools and services provided by the Instruct-ERIC.
ARBRE MOBIEU	A network for the development of innovative integrative biophysical approaches.
ARIA	Access to Research Infrastructure Administration: Instruct-ERIC's access management system.
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
CERIC-ERIC	Central European Research Infrastructure Consortium provides open access to facilities in Central and Eastern EU for research in all fields of materials, biomaterials and nanotechnology.
CORBEL	An initiative of thirteen biological and medical Research Infrastructures to create a platform for harmonised user access to biological and medical technologies, biological samples and data services required by cutting-edge biomedical research.
CYTED	The Ibero-American Program of Science and Technology for Development, created by the governments of Latin American countries to promote cooperation in science, technology and innovation for the development of Latin America.
EATRIS	A European infrastructure for translational medicine that provides access to translational Research Infrastructure and expertise.
eBIC	The Electron Bio-Imaging Centre at the Diamond Light Source: provides equipment and expertise in cryo-electron microscopy, for both single particle analysis and cryo-tomography.
ECRIN	The European Clinical Research Infrastructure Network: a non-profit, intergovernmental organisation that supports the conduct of multinational clinical trials in Europe.
EMBL	The European Molecular Biology Laboratory: an intergovernmental organisation specialising in research in the life sciences, funded by its 20 member states.
EMBO	The European Molecular Biology Organization: a professional organization of life scientists promoting research in life science and enabling international exchange between scientists.
EMBRC	The European Marine Biological Resource Centre: a pan-European Research Infrastructure for marine biology and ecology research.
EOSC-Life	The European Open Science Cloud: bringing together biological and medical Research Infrastructures to create an open, collaborative space for digital biology.
ERIC	European Research Infrastructure Consortium: a specific legal form that facilitates the establishment and operation of Research Infrastructures with European interest.
ERIC Forum	A Horizon2020 project bringing together European Research Infrastructure Consortia to strengthen their coordination and enhance their collaborations.
ERINHA	European Research Infrastructure on Highly Pathogenic Agents is a pan-European distributed life sciences research Infrastructure dedicated to the study of highly infectious emerging and re-emerging high-consequence pathogens

GLOSSARY CONTINUED

Term	Definition
ESFRI	European Strategy Forum on Research Infrastructures: an organisation with members nominated by European member states ministries to support a coherent and strategy-led approach to policy-making on Research Infrastructures in Europe.
EU-LAC ResInfra	The European Union – Latin America and Caribbean partnership in Research Infrastructures pursues the construction of a bi-regional collaboration between European Union and the LAC countries.
EU-OPENSCREEN	A European Research Infrastructure Consortium providing open access to a range of technologies and tools for the systematic screening of chemical substances for their biological effects.
Euro-BioImaging	A European Research Infrastructure providing open access to a broad range of technologies in biological and biomedical imaging for life scientists.
FEBS	The Federation of European Biochemical Societies: a charitable organisation supporting research and education in molecular life sciences.
FRISBI	The French Infrastructure for Integrated Structural Biology: an infrastructure for integrative structural biology approaches.
H2020	Horizon 2020 is the biggest EU Research and Innovation programme, making €80 billion of funding available over 7 years.
iNEXT	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.
MERCOSUR	Southern Common Market: a South American trade bloc.
MERIL	Mapping of the European Research Infrastructure Landscape: a portal providing access to a database that stores information about openly accessible Research Infrastructures in Europe, across all scientific domains, including the social sciences and humanities.
METROFOOD-RI	METROFOOD-RI is a distributed-Research Infrastructure for promoting Metrology in Food and Nutrition, which will provide high level metrology services for enhancing food quality and safety.

GLOSSARY CONTINUED

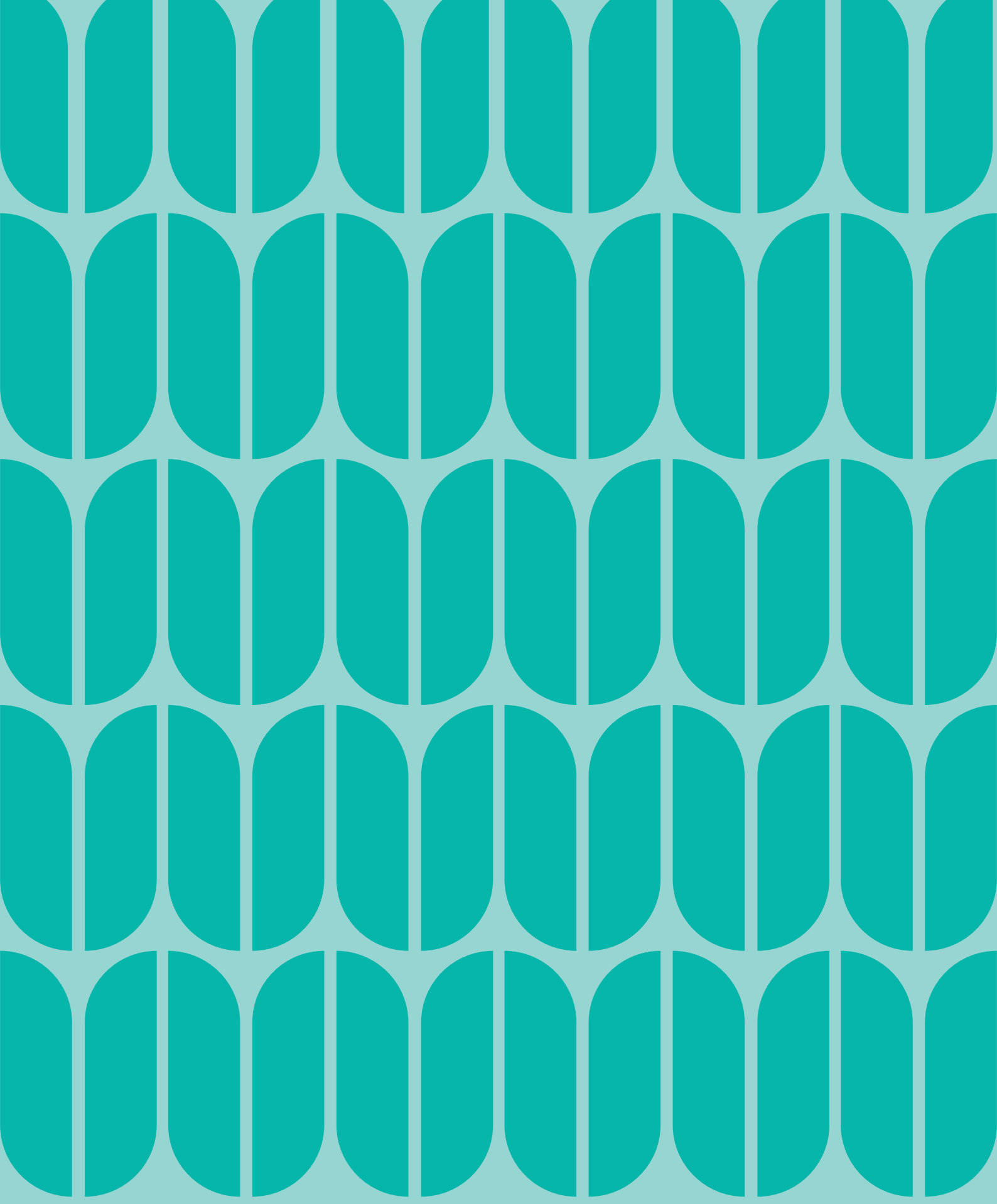
Term	Definition
Moderator	A person assigned to an Instruct proposal by the Secretary of Moderators in order to select reviewers and decide the outcome of user proposals.
Open-SESAME	A H2020 project to support the optimal use of the SESAME synchrotron light source for experimental science and applications in the Middle East.
OpenAIRE	The Open Access Infrastructure for Research in Europe is a network of dedicated Open Science experts promoting and providing training on Open Science as well as a technical infrastructure harvesting research output from connected data providers.
Proposal	A user's request for access to technology or other services.
Reviewer	Assigned by the moderator, a reviewer assesses the science of an Instruct proposal. Three reviewers are assigned to each proposal: all are external to the Instruct Centre that has been requested for access, and at least one is external to Instruct-ERIC.
RI-VIS	A H2020 funded project to increase the visibility of European Research Infrastructures (RIs) to new communities in Europe and beyond.
RISCAPE	RISCAPE is a European H2020 project to provide a consistent report of the international research infrastructure landscape.
Stakeholder	A person, or group of people with an interest or concern in Instruct-ERIC.
Transvac2	The TRANSVAC2 consortium comprises a comprehensive collection of leading European institutions that propose to further advance with the previous initiative towards the establishment of a fully operational and sustainable European vaccine R&D infrastructure.
West-Life	West-Life provides services for computation and data management to researchers in structural biology.

ABBREVIATIONS

API	Application Programming Interface
BIOCEV	Biotechnology and Biomedicine Centre (Czech Republic)
CAPRI	Critical Assessment of Predicted Interactions
CBI	Center of Integrative Biology (France)
CeBEM	Bolivian Center for Multidisciplinary Studies
CEA	French Alternative Energies and Atomic Energy Commission
CEITEC	Central European Institute of Technology (Czech Republic)
CELAC	Community of Latin American and Caribbean States
CERM	Magnetic Resonance Center of the University of Florence (Italy)
CIISB	The Czech Infrastructure for Integrative Structural Biology
CIRMMP	The Interuniversity Consortium for Magnetic Resonance of Metallo Proteins (Italy)
CNB	Spanish National Centre for Biotechnology
CNPEM	National Center for Research in Energy and Materials (Brazil)
CNRS	French National Centre for Scientific Research
CLEM	Correlative Light Electron Microscopy
CSIC	Spanish National Research Council
EBSA	European Biophysical Societies' Association
EC	The European Commission

ABBREVIATIONS CONTINUED

EM	Electron Microscopy
ESRF	The European Synchrotron Radiation Facility (France)
ET	Electron Tomography
FTE	Full-time equivalent
FRAP	Fluorescence recovery after photobleaching
GDPR	General Data Protection Regulation
I2PC	Instruct Image Processing Center (Spain)
IBET	Institute of Experimental Biology and Technology (Portugal)
IBS	Institute of Structural Biology (France)
IGBMC	The Institute of Genetics and Molecular and Cellular Biology (France)
ILL	Institut Laue–Langevin
ISAB	Independent Scientific Advisory Board
ISAL	Instruct Academic Services Limited
ISBG	Integrated Structural Biology Grenoble (France)
ISO	International Organisation for Standardisation
ISPC	The Israel Structural Proteomics Center
ITQB	Institute of Chemical and Biological Technology (Portugal)
JRA	Joint Research Award
KPIs	Key Performance Indicators
LS RI	Life Science Research Infrastructures
MoU	Memoranda of Understanding
MPL	Membrane Protein Laboratory
MRC	Medical Research Council (UK)
MS	Mass Spectrometry
Nbs	Nanobodies
NeCEN	Netherlands Centre for Electron Nanoscopy
NKI	Netherlands Cancer Institute
NMR	Nuclear Magnetic Resonance
OPIC	Oxford Particle Imaging Centre (UK)
PDB	Protein Data Bank
PID	Proposal Identification number
R&D	Research and development
RCSB	Research Collaboratory for Structural Bioinformatics
RI	Research Infrastructure
RISN	Research & Innovation Support Network (Oxford University)
SME	Small and medium-sized enterprises
START	Synchrotron Techniques for African Research and Technology
STEM	Science, Technology, Engineering and Mathematics
WIS	Women in Science



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Thank you to all authors and colleagues who have contributed to this publication.

Published 2020