2017-18
INSTRUCT-ERIC ANNUAL REPORT


DISPO-SAFE

CONTENTS

FOREWORD BY CHAIR OF THE INDEPENDENT SCIENTIFIC ADVISORY BOARD

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

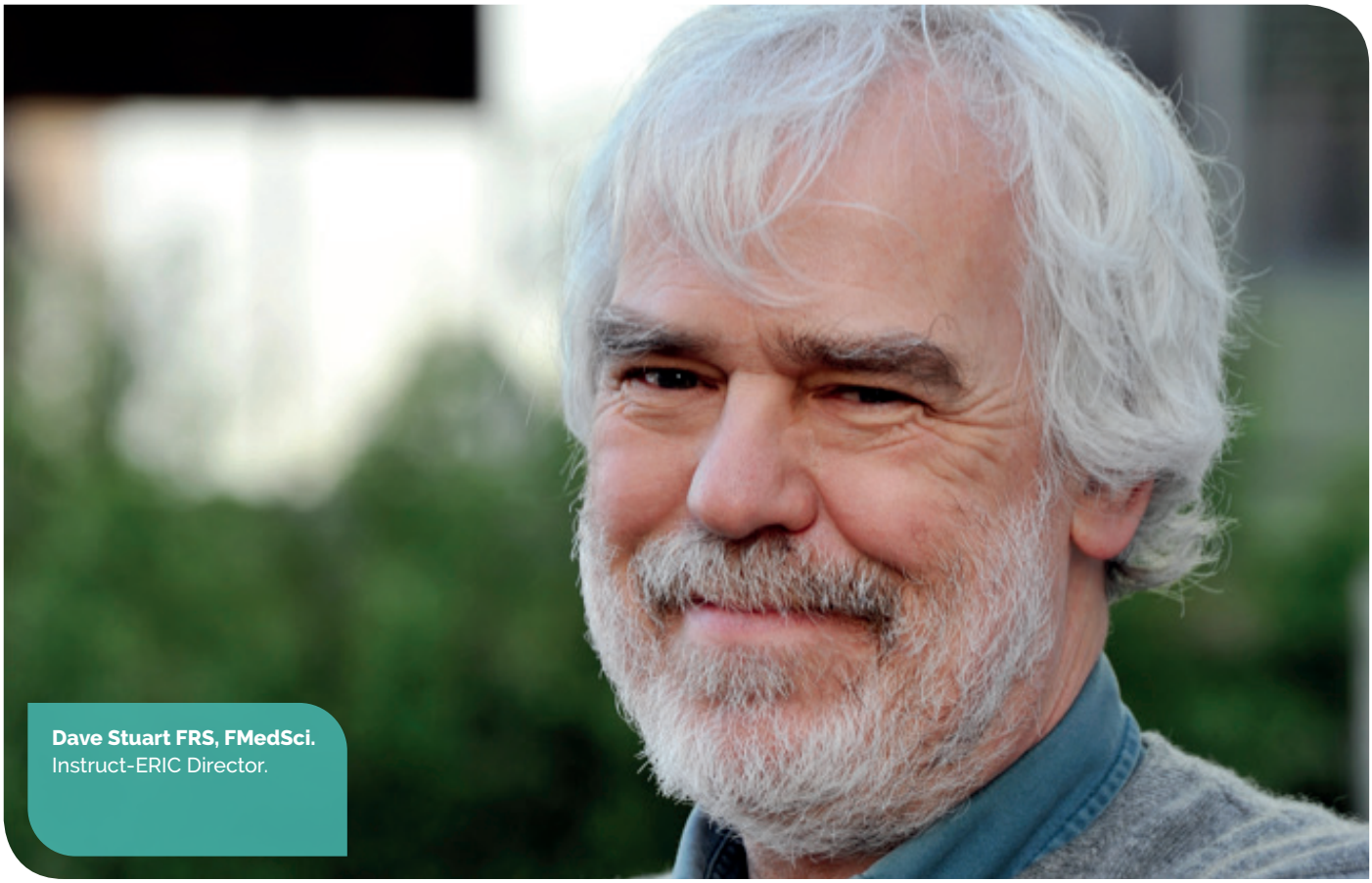
The Instruct-ERIC Independent Scientific Advisory Board (ISAB) is charged with providing advice to Council, the Executive Committee, and the Director. Current Members include Stephen K. Burley (Chair; RCSB Protein Data Bank, Rutgers University and University of California San Diego, United States), Ilaria Ferlenghi (GSK Vaccines, Italy), Angela M. Gronenborn (University of Pittsburgh, United States), Jürgen M. Plitzko (Max-Planck-Institut für Biochemie, Martinsried, Germany), and Marjolein Thunnissen (MAX IV and University of Lund, Sweden).

As ISAB Chair, it is an honour to contribute this foreword to the first Instruct-ERIC Annual Report published following adoption of the European Research Infrastructure Consortium legal framework in mid 2017. Founded in 2002, Instruct (now Instruct-ERIC) has remained faithful to its overarching objective of fostering technical innovation and automation, to thereby increase the impact of structural biology. Today, the Instruct-ERIC Hub coordinates the work of nine science and technology Centres in user training and providing access to cutting-edge measurement techniques. Given the limited availability of high-cost instrumentation in many of the twelve Member countries, individual commitments to the Instruct-ERIC vision and sharing of research infrastructure show how much can be accomplished through cooperation and collective action.

Europe continues to play an international leadership role 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 with sub-tomogram averaging. Atomic-level visualisation of biomolecules transforms every area of biology that is fortunate enough to succumb to one or more of these techniques. There is, simply put, no substitute for a "direct look" at the molecules of life. Supported by Instruct-ERIC, scientists and their trainees and collaborators are producing some of the most exciting new structures entering the open access Protein Data Bank archive on a daily basis. Of particular significance are NMR structures obtained by studying proteins at work inside living cells, and structures of enormous macromolecular machines revealed using state-of-the-art electron imaging methods. On the immediate horizon, new opportunities for studying biology and biochemistry in the 4th dimension (time) will come from serial-femtosecond X-ray crystallography using the European X-ray Free-Electron Laser and pink beam serial crystallography at both national and regional synchrotron radiation sources. Going beyond the limitations of single measurement techniques, the next decade will see ever larger molecular systems and ever more complex biological phenomena yielding their secrets to integrative structural biology approaches that combine complementary experimental and computational tools.

These advances will depend critically on delivering quality training to users and providing the broadest possible access to state-of-the-art sample preparation and characterisation using quantitative methods of measurement and analysis. Instruct-ERIC and its constituent Centres have equally important roles to play in shaping the evolution of structural biology and the future of interdisciplinary research in fundamental biology, biomedicine, and bioenergy for the good of all humanity. My ISAB colleagues and I look forward to working across the organisation to help ensure that Instruct-ERIC is well prepared to surmount the myriad scientific, technical, financial, and political challenges ahead, in order to continue delivering value to researchers, educators, and early-career trainees, patients and their families, national and supra-national funders, and Member-nation taxpayers.

FOREWORD BY INSTRUCT-ERIC DIRECTOR



Dave Stuart FRS, FMedSci.
Instruct-ERIC Director.

Instruct-ERIC is a multinational, distributed research infrastructure funded by contributions from its Members and is a landmark in the European Research Infrastructure Landscape. Instruct has delivered excellent science through the provision of services, expertise and training in cutting-edge technology and has achieved a tangible influence on technical development in structural biology. Instruct aims to remain at the forefront of structural biology methods and services, enabling high-impact research through to the end of the current funding cycle in 2022. David Stuart has been the Director of Instruct-ERIC since it was established in 2017.

This report provides data from the period of Instruct-ERIC launch (July 2017) through to 31 December 2018.

It is a delight to write a foreword to the first Instruct-ERIC Annual Report, describing the situation in the first 18 months of operation following our adoption of the ERIC legal framework. Instruct, now a distributed infrastructure coordinated from the Oxford Hub with Centres in eight countries, had its origins in earlier EU Framework Programme grants, which starting in 2002,[1] represented a European response to the structural genomics activities active in the USA and Japan. The European response was, from the outset, distinctive, in that it aimed to mobilise technological innovation and automation to increase the impact of structural biology. It has become increasingly clear that physical science, technology and engineering advances are indeed major drivers of discovery, as NMR, synchrotron macromolecular crystallography and cryo-electron microscopy (cryo-EM) have continued to advance. For cryo-EM the advances have revolutionised the field, so that the analysis in atomic detail of hugely complex biological assemblies is becoming almost routine. These advances have increased the sophistication of the methods, and also the cost of cutting edge infrastructure. Instruct has championed these developments and proposes a model for the democratisation of access to high-end infrastructure.[2] With the transition to Instruct-ERIC, what started as a bottom-up development has been anchored firmly at the national level, with strong support at ministerial level. Currently we have twelve Member countries, each paying a subscription, to allow access to the infrastructure and training provision of Instruct-ERIC. Whilst Instruct-ERIC is now strong, the storms of external politics has introduced uncertainty and this remains as I write this. In times such as these, pan-European research infrastructures are powerful examples of what can be achieved by working together, fostering cooperation and the exchange of people and ideas, to accelerate discovery for the benefit of all. Instruct aims to facilitate the delivery of excellent science through the provision of services, expertise and training in cutting-edge technology and, although there is much still to do, has already achieved a tangible influence on technical development in structural biology. Our aim is to continue to shape the way access and training is delivered, so that Europe remains at the forefront of structural biology methods and services to enable high impact research, initially through the current funding cycle which completes in 2022. Finally, I would like to thank everyone who has made this possible, from funders who have had patience with academic scientists, through to the dedicated staff in the Centres, and especially the Hub, where commitment, determination and good humour have prevailed. I hope this report will give a glimpse of the current excitement in the field; certainly there is much more to come!

1 Stuart DI, Jones EY, Wilson KS, Daenke S. SPINE: Structural Proteomics IN Europe - the best of both worlds. Acta Crystallogr. D. 2006;62:ii-i.
2 Stuart DI, Subramaniam S, Abrescia NG. The democratization of cryo-EM. Nat. Methods. 2016;13:607.

LOOKING FORWARD

The period of this report has seen significant changes to Instruct since its launch in 2017. This includes the addition of new Members, the addition of new technologies to the catalogue of services, considerable expansion of its user community, and a transition procedure taking the Instruct Hub out from the umbrella of the University of Oxford to become independent.

One third into our first, five-year funding cycle, we are now in a position to review our services based on the data collected within this first reporting period, to identify strategies for improvement and new opportunities. These include a review of the funding support model for access, and a new funding route for targeted development projects. Instruct has been successful in developing a strategic partnership with a consortium of countries in Latin America and the first pilot access call is planned for early 2019, paving the way for a more permanent relationship. These, and many other objectives for the remaining funding term, will be challenging and will require the help and resolve of all Instruct Members, Centres, advisors and staff to deliver for our user community.

I would like to thank everyone involved in the development and delivery of Instruct services, and those supporting the vision and execution of its objectives. In particular, I thank the Hub staff for their forbearance through recent times and their commitment to Instruct's future.

Susan Daenke (Hub Coordinator)

EXECUTIVE SUMMARY

Activity indicators	First reporting period (2017-18)
Proposals received	139
Publications	204
Average journal impact factor	7.3
Additional external funding	€469 134
Students and researchers trained	155
Presentations at events	42

DELIVERY OF EXCELLENT SCIENCE

Europe is historically strong in structural biology and has pioneered a number of excellent technologies for structure determination. Involving access users in Instruct exposes the Centers to an increased diversity of research fields and stimulates advances in technology to accommodate emerging needs. Users, therefore, are an integral part of Instruct's strategy for developing highly specialist technologies that are accessible to all. Instruct-ERIC has continued to provide access to structural biology technologies using the same model that was established prior to ERIC status being achieved. Between 2017-18 Instruct received 139 proposals through its ARIA access management portal, which facilitated:

- processing of proposals through peer review
- contact between all parties
- scheduling of technical instrument time
- reporting from user and facility

In the same period, ARIA managed 415 proposals for iNEXT,[1] and 23 for CORBEL,[2] demonstrating the value and impact of ARIA as a core tool for pan-European access. Demand for access was received from a total of 15 countries, including three from outside of Europe.

The quality of scientific output was demonstrated by the >200 publications that were published in the 18 month reporting period, including 45 publications in high-impact journals (JIF >10). The average journal impact factor of all Instruct publications in this time was 7.3, with 593 non-self citations.

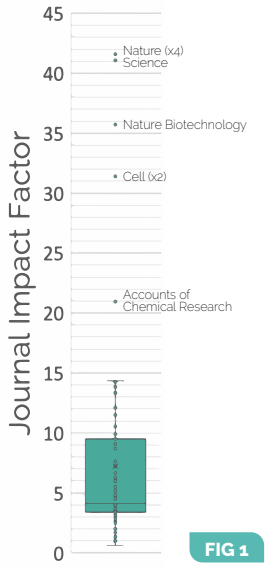
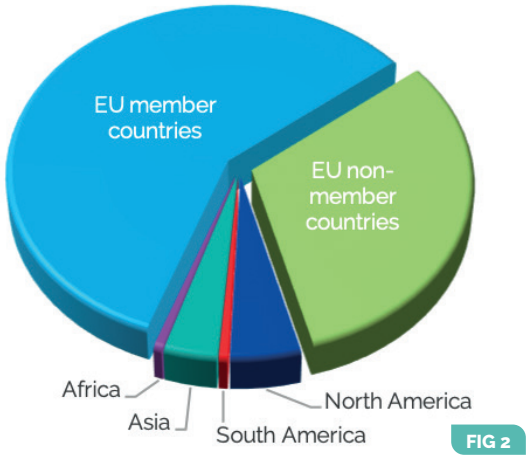


FIG 1. The distribution of journal impact factors for publications during the reporting period.

TRAINING

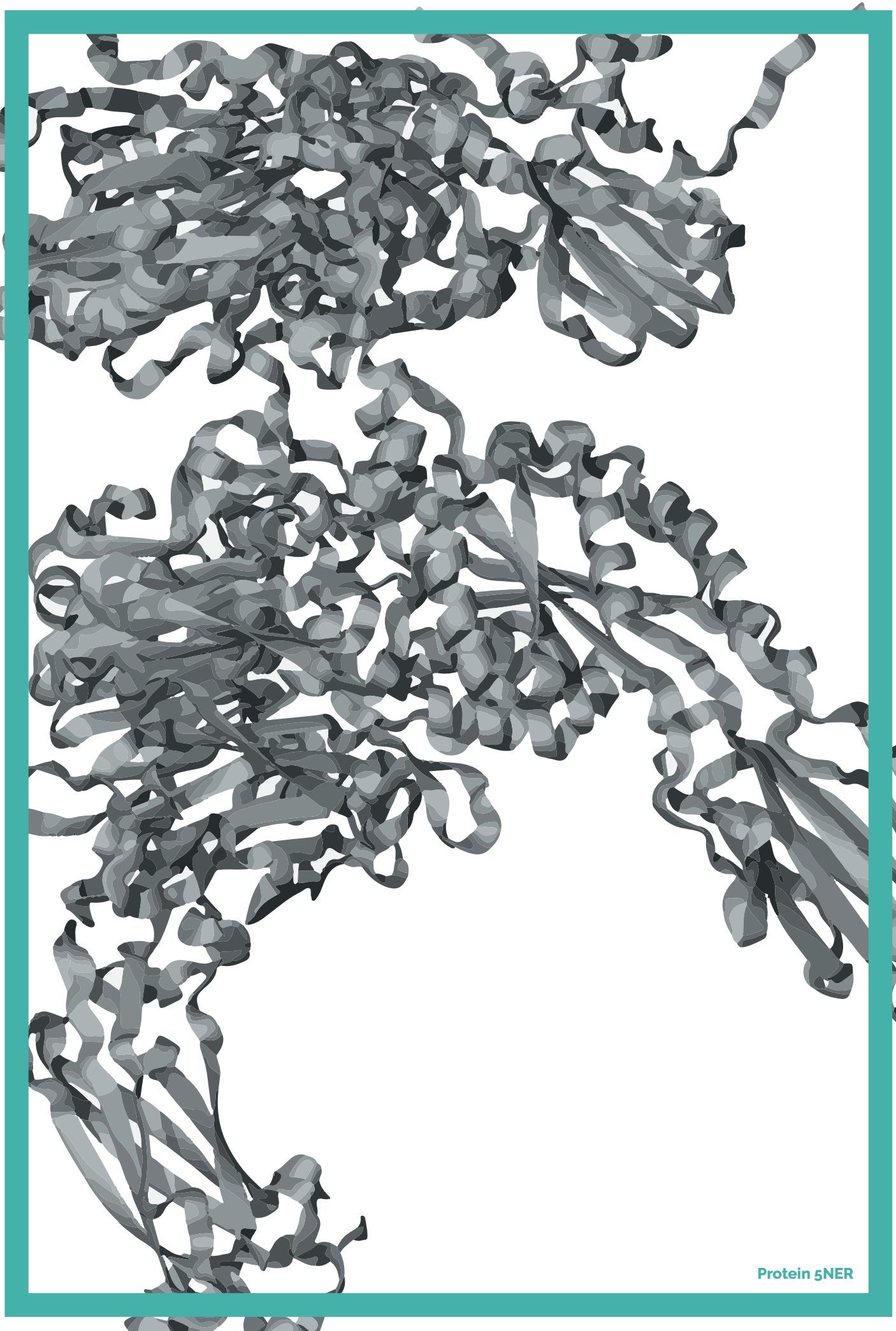
Training is an important priority for Instruct. Our training aims to be of value to scientists at different stages in their career, but is mainly taken up by early-career researchers who need to master an increasing array of techniques in structural biology. The need for training can be seen from the extreme demand for some of the courses, for instance 12 times over-subscription for the Instruct Cryo-EM Sample Preparation Workshop at Diamond Light Source in the UK. Nine courses have been funded in topics related to the use of methods and technologies in structural biology, ranging from sample preparation to advanced and integrated methods. The training programme has had strong support, with all courses and workshops over-subscribed. Training courses that were delivered in the 2017 - 18 reporting period include:



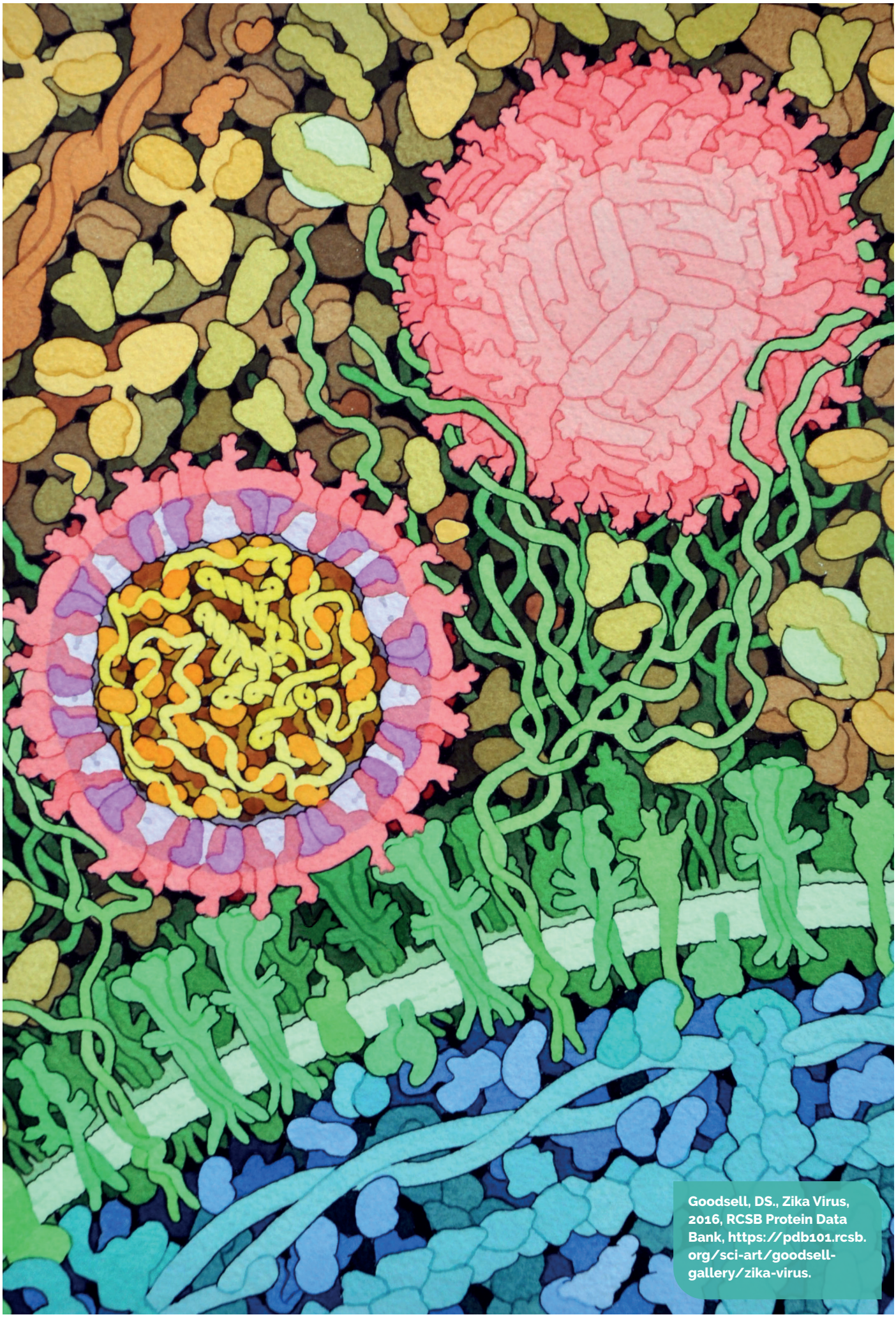
- Hydrodynamic and thermodynamic analysis of biological macromolecules and their interactions. Hosted by BIOCEV Centre of Molecular Science, Instruct Centre CZ (Prague), 23-28 September 2018.
- Instruct course on model building and refinement for high resolution EM maps. Hosted by CCISB, Harwell Campus, Instruct Centre UK, 1-4 May 2018.
- Instruct course in image processing for electron microscopy in the cloud. Hosted by National Center for Biotechnology Madrid, Instruct Centre ES, 17-29 January 2018.
- Open-SESAME and Instruct-ERIC workshop on remote X-ray data collection from European synchrotrons. Hosted by the Weizmann Institute Israel, Instruct Centre IL, 14-18 May 2018..

FIG 2. Representation of participants at training courses in the reporting period.

1 iNEXT: consortium offering European researchers access to Structural Biology technologies.
2 CORBEL: platform for multidisciplinary access to multiple RIs in the Life Sciences domain.


Protein 5NER

INTRODUCTION



Goodsell, DS., Zika Virus, 2016, RCSB Protein Data Bank, https://pdb101.rcsb.org/sci-art/goodsell-gallery/zika-virus.

AIMS OF INSTRUCT

Instruct is a European Research Infrastructure in structural biology, launched operationally in 2012 and recognised as a Landmark in the European Strategic Forum for Research Infrastructures (ESFRI) group in 2016. Instruct was seed-funded by the European Commission (Framework 7) and in July 2017, Instruct was granted European Research Infrastructure Consortium (ERIC) status in accord with European Regulation (EC) No 723/2009 modified by Council Regulation (EU) No 1261/2013. The new legal entity, Instruct-ERIC, is a consortium comprising twelve European Member countries. Instruct operates on a tiered national subscription model, is hosted by the UK, and the Instruct Hub is located in Oxford, UK.

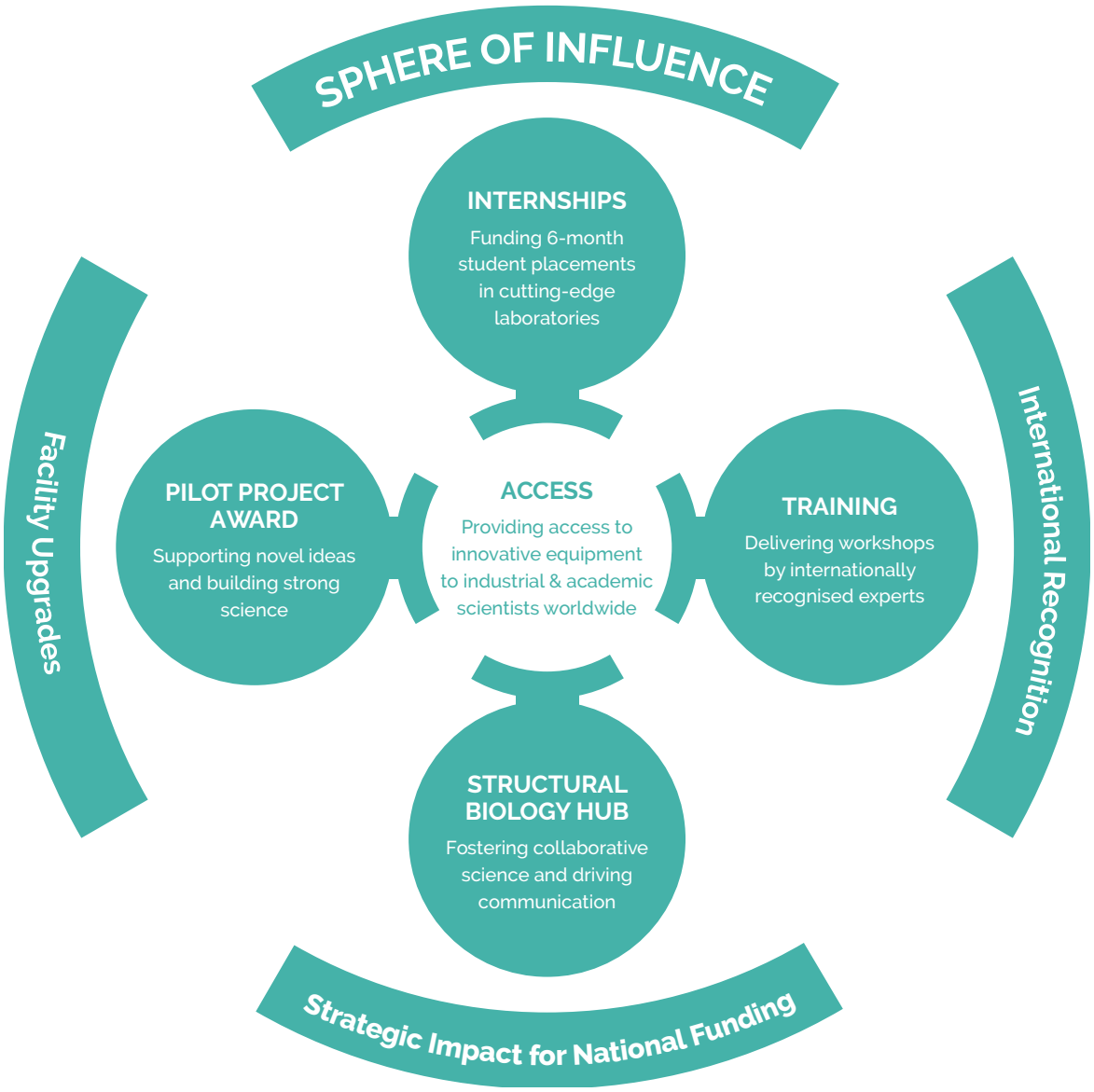
Instruct's mission: **To provide peer-reviewed, open access to a broad, integrated palette of state-of-the-art structural biology infrastructure, promoting innovation in biomedical science and facilitating high impact research.**

Instruct provides researchers in Europe with access to specialist, advanced, structural biology infrastructure through its Centres. It also hosts training courses and workshops in new or emerging technologies, and funds internships and small research awards to stimulate career progression and methods developments. Instruct-funded access is provided for researchers from Instruct Member countries, otherwise researchers can access services on a fee-for-service basis.

Instruct has a prominent profile in the European life sciences community and is an influential participant in policy determination through its membership of the ERIC Forum and membership on EC panels and committees. Instruct is currently in receipt of eleven H2020 grants, two of which Instruct is leading.

Instruct has a role in bringing together and strengthening the scientific communities in its Member countries. This is exemplified by FRISBI, PCISBIO and CIISB. Instruct developed its own access management system (ARIA), which is used by several independent organisations.

This report spans the 17 month period following the Instruct-ERIC launch, from 1 August 2017 through to 31 December 2018. Hereafter, subsequent annual reports will span each calendar year to coincide with the dates of our financial year. Instruct activities are presented to illustrate performance against key criteria but where metrics in this first report are unavailable, achievements are structured in a narrative fashion and, where appropriate, specific points are illustrated by exemplars.



MEMBERSHIP

Instruct is built upon a model that includes a modest cash contribution and a more substantial in-kind contribution for the provision of infrastructure access and supporting activities at the Centres. Members pay a subscription fee that varies according to the size of their science community, enabling them to apply for access to scientific infrastructure, training, internships and pump priming R&D funds. The model is explained on page 13. Countries with an intention to join can apply for observer status on our Council. The majority of Members host a Centre that provides access to cutting-edge structural biology infrastructure and it is expected that new Members will work towards establishing a Centre. Instruct-ERIC launched with ten founding Members in 2017. Spain joined as a Member at the start of the 2018 calendar year, and negotiations were completed during 2018 for Latvia to join as a Member from January 2019. Discussions continued with Finland towards prospective membership. Greece and EMBL are retained as observers.

Member countries host a total of nine Instruct Centres.

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

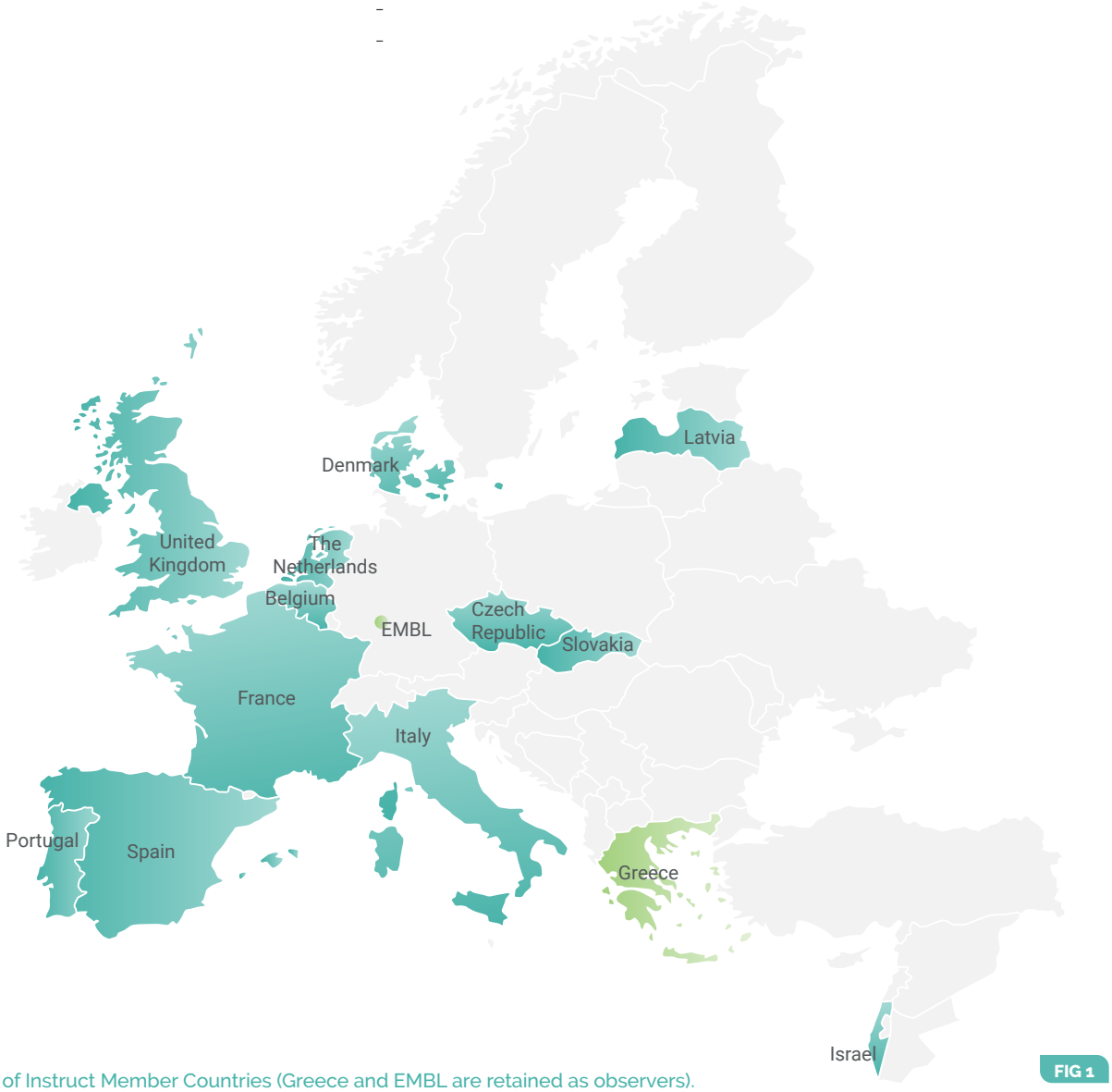


FIG 1

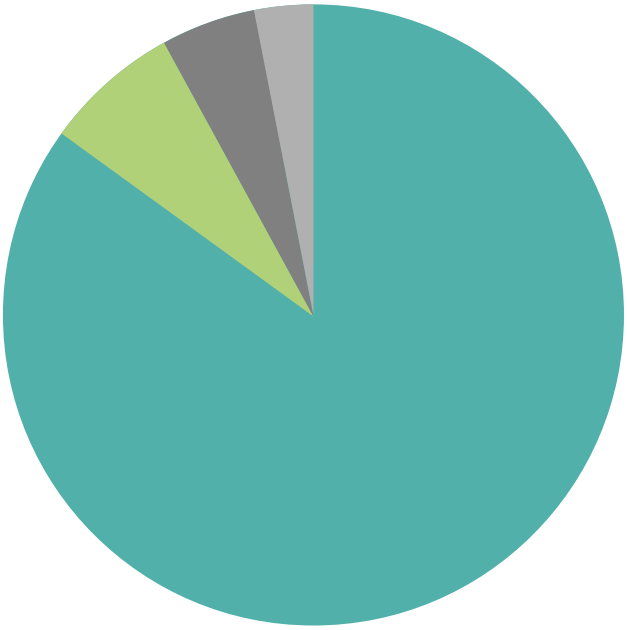
FIG 1. Map of Instruct Member Countries (Greece and EMBL are retained as observers).

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			52.02	53.06	54.12	260.20
SK	C	50.00	50.00	52.02	53.06	54.12	260.20
Total		775.00	790.50	858.33	875.50	893.01	4293.33

VALUE ADDED TO INSTRUCT-ERIC



In-kind contributions | Group A membership | Group B membership | Group C membership

Instruct was founded on the principle that researchers needing access to high-end infrastructure for their research, should get access that is free at the point of service. This is the only truly democratic model, but it requires a significant commitment from Member states whose infrastructure facilities are made available for Instruct use.

Since the annual cash contribution from Members is low, the largest contribution is made in-kind by those Members hosting an Instruct Centre. In all cases, the infrastructure provided through Instruct Centres has been established by existing academic institutions at significant cost, with the costs of staffing and maintenance an ongoing commitment. In some cases (e.g. in the Czech Republic), part of the investment to establish advanced capabilities in structural methods was leveraged from European Structural Funds with Instruct's help. The Instruct membership model is therefore based on the goodwill and ongoing commitment of each Member, which also ensures the quality of Instruct services for its community.

Advances that might be added to the Instruct infrastructure are constantly monitored internally and more formally by the ISAB, such that the infrastructure as a whole evolves to meet user demand.

TIMELINE
selected events from 2017-18

2017

JUL

- **Instruct-ERIC launch ceremony, London**
- ARBRE-MOBIEU conference, Edinburgh. Instruct-ULTRA broker new staff exchange programme to start November 2017 between CNR Institute of Biophysics, Photon and Nanotechnologies.

AUG

- Instruct Academic Services Limited Board meeting. Full operational and administrative transfer to Instruct-ERIC.
- ARIA v2.0 launched.

SEP

FEBS Congress 2017, Israel. Joint Instruct-ERIC/Euro-BioImaging/EU-OPENSCREEN session on access to RIs. Instruct Director presents a plenary address.

- 2nd Exchange of Experience workshop organised by Euro-BioImaging, India. Representation from Instruct-ULTRA.
- Visit from Alejandro Buschiazzo of Institute Pasteur Montevideo, Uruguay. Consolidate engagment with Brasil, Argentina and Uruguay via CeBEM, brokered by Instruct-ULTRA.
- Staff exchange from Instruct Hub to CCMAR (EMBRC), Faro, on best practices for RI management.

OCT

- **Sustainability workshop for West-Life and iNEXT hosted at Instruct offices to discuss strategy for legacy hosting of services.**
- Scientific Organising Committee convened for the 4th Instruct Biennial Scientific Conference to be held in Madrid 2019.

NOV

- **ARIA v2.1 launch, providing role-based help guides, new forums, admin search facility and upgraded dashboard.**
- 2nd General meeting of AARC2. Instruct activities are to help provide a federated authentication service for Life Sciences RIs.
- EOSC Stakeholders Forum, Brussels.

DEC

- **American Society for Cell Biology/ EMBO meeting, Philadelphia. Instruct present on Access Management for RIs.**

2018

JAN

- EMPHASIS meeting, London, UK. Instruct present on RI Operational Processes and Networking.
- **ARIA webinar for access management.**

FEB

- Instruct Managers Meeting, Amsterdam. Training and networking event for Instruct Centre facility managers and colleagues from iNEXT.

MAR

- ARBRE-MOBIEU symposium, Warsaw. Instruct-ULTRA participate in 'Talking molecules: the networks that shape the living world'
- EMBRIC meeting: 'A successful road from organisms to molecular tools', Berlin. Instruct-ULTRA participation.
- Instruct-ULTRA General Assembly, Oxford
- CYTED Grant awarded.
- **Bulgarian Presidency Flagship Conference, Sofia**

30th internship awarded

30

2018 CONTINUED

APR

- Instruct-ERIC move to new premises at Oxford House.
- Staff exchange from Instruct Hub to University of Bonn through ERASMUS.
- Instruct host 2nd ERIC Forum meeting in Oxford.

MAY

- Instruct-ERIC Strategic Meeting, Viana do Castelo
- West-Life All Partners Meeting, Madrid.
- Open-SESAME/Instruct-ULTRA joint workshop at the Diamond Light Source, UK.
- Staff exchange funded through Instruct-ULTRA NKI for the purpose of developing ARIADNE software.

40th training course completed

JUN

- CORBEL Quality Management Expert meeting in Munich, hosted by Infrafrontier.
- Instruct ES ceremony organised by the Ministry of Economy and Industry in Madrid, registering intent to join Instruct-ERIC.
- MERIL Interoperability workshop, Athens.

JUL

- UKRO Annual Meeting, Southampton UK.
- CORBEL 'Train the Trainer' workshop, Amsterdam.
- PCISBIO conference, Oeiras. Instruct Hub Coordinator presents the current activities and achievements of Instruct-ERIC.
- Forum of Neuroscience Conference, Berlin. Instruct-ULTRA host an Instruct-ERIC focus-session.
- FEBS Congress 2018, Prague. Instruct-ULTRA host an Instruct-ERIC focus-session.

AUG

- Staff exchange from EU-OPENSCREEN to Instruct Hub.
- Anton Ussi (EATRIS) visits the Instruct Hub to discuss collaboration opportunities.

SEP

- EC workshop on H2020 audit practices, London, organised by the UK Ministry for Business, Energy and Industrial Strategy.
- Hackathon to implement the transfer of tools from West-Life to Instruct is hosted by the Instruct Hub.
- 4th ICRI Conference, Vienna.
- UK Ministry for Business, Energy and Industrial Strategy Conference in London.
- iNEXT face-to-face meeting, Amsterdam.

OCT

- InRoad Validation workshop , Brussels.
- Instruct-ULTRA mid-term review, Brussels
- Digital Infrastructure for Research (DI4R), Lisbon.

1500th person trained

1500

NOV

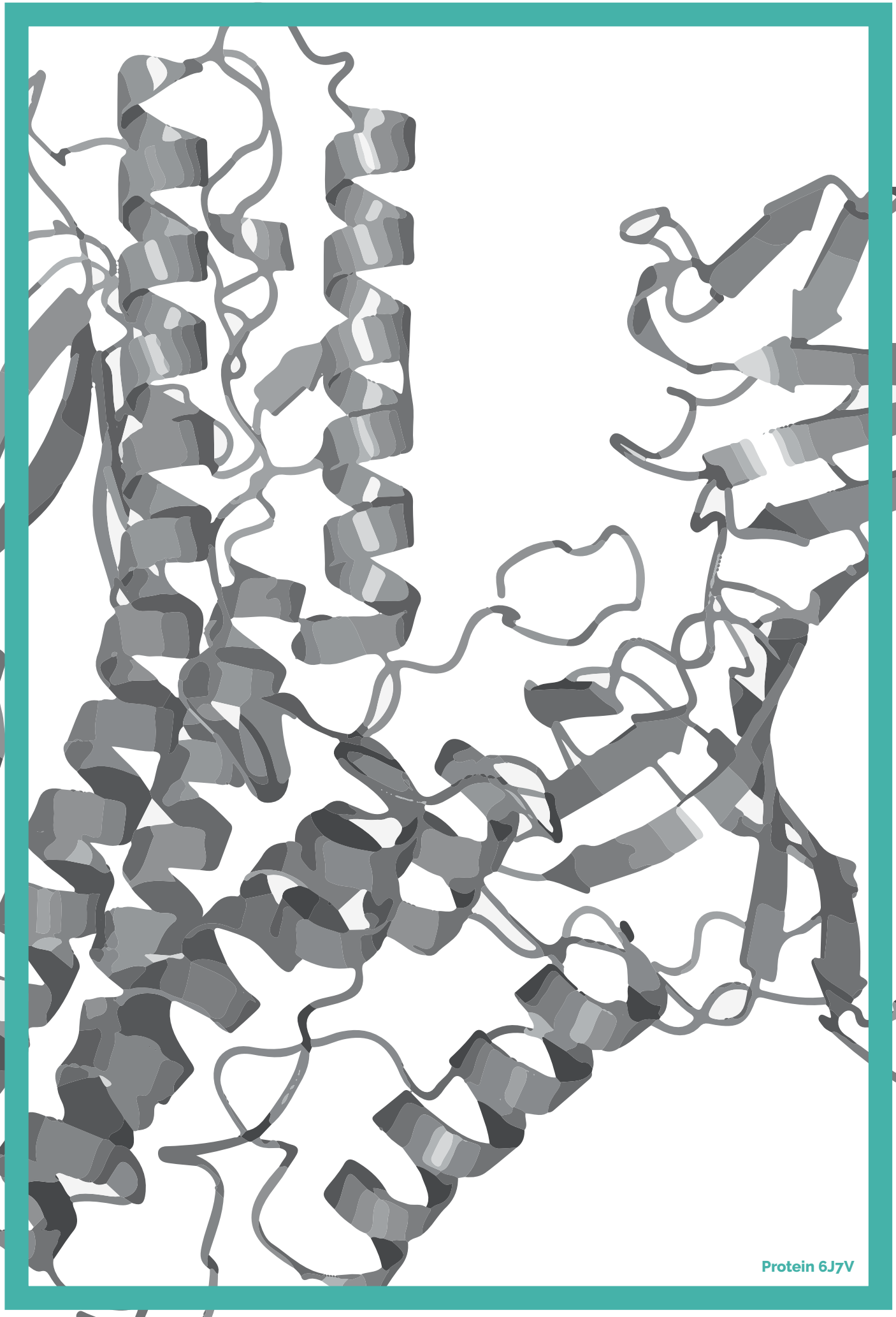
7000th registered user

7000

- Staff exchange from Instruct Hub to ECRIN, Paris.
- RI-Path workshop on infrastructures for basic research, CERN.
- Instruct Computational Structural Biology Group meeting to finalise legacy software and tools migration to Instruct.
- 3rd ERIC Forum meeting, Seville. Instruct Hub Coordinator presents to the Forum.

DEC

- Instruct's Service Catalogue upgrade completed.
- **Re-launch of the Instruct-ERIC website.**
- InRoad Conference to present the Final Report.


Protein 6J7V


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. Collective efforts of several laboratories have demonstrated that Nanobodies are exquisite chaperones for crystallising complex biological systems such as membrane proteins, multiprotein assemblies, transient conformational states and intrinsically disordered proteins.

Nanobodies4Instruct: providing Nanobodies and Megabodies for Structural Biology:

Nanobodies are 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. Recently, Nanobodies have also found their way to cryo-EM.

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,[3] M2R,[4] and MOR.[5]

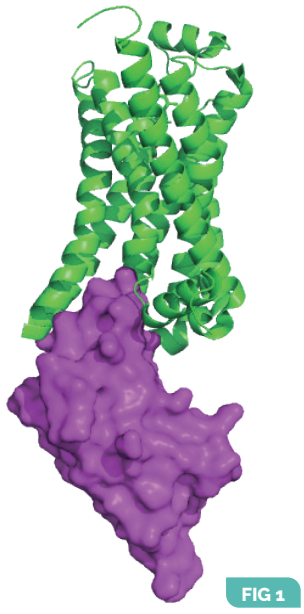


FIG 1

FIG 1. Crystal structure of ß2 adrenergic receptor (ß2AR, green) in a complex with Nb80 (pink).

Nanobodies to stabilize protein complexes:

Other Nanobodies were developed that stabilise the ß2AR-Gs complex. One of these 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 signaling by GPCRs.[6] In two more recent collaborations, Nanobodies were developed that stabilise the PINK1-Ubiquitin enzyme-substrate complex[7] and the Vps34 complex II[8] of yeast that is composed of four proteins.

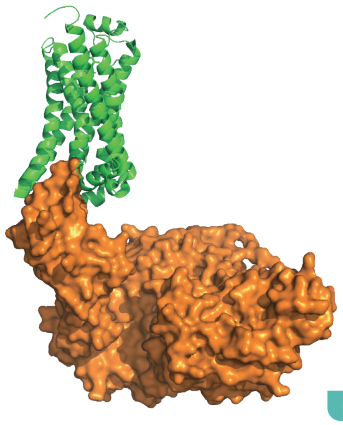


FIG 2

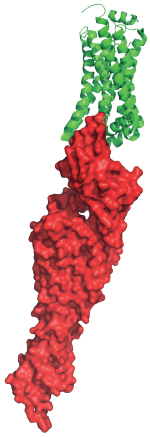


FIG 3

FIG 2 & 3. Model of Nb80-derived chimeric proteins, called megabodies (orange & red).

FIG 4

FIG 4. Llamas for Instruct.

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, whereby Nanobodies are rigidly grafted into selected protein scaffolds to increase their molecular weight, while retaining the full antigen binding specificity. With this innovation, we expand cryo-EM analysis to proteins that are small and/or display preferential orientation in ice, two major factors that limit the resolution of reconstructed density maps. In a recent Instruct collaboration with the Ariescu lab, we successfully used such Megabodies to solve the first cryo-EM structures of the heteropentameric GABAA receptor[9, 10] in complex with common drugs including Xanax and Valium, amongst other pharmacological compounds.

Training the next generation of structural biologists on site:

From 9 to 19 September 2018, Instruct Centre BE organised the first Nanobody4Instruct Workshop in Brussels. The training course was devoted to the hands on discovery of nanobodies and focused on its applications in structural biology. 12 researchers/students from Instruct Member countries contributed their own pet protein and discovered their own Nanobodies to be used in their future research. In parallel, the researchers were trained in applying such antibodies in X-ray crystallography and cryo-EM and introduced to other developments in the field.

FIG 5

FIG 6

FIG 5 & 6. Nanobody4Instruct Workshop 2018 in Brussels, Belgium.

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Instruct Centre Lead Scientists

Els Pardon

Jan Steyaert

INSTRUCT CENTRE CZ

Instruct Centre CZ is coordinated within the Czech Infrastructure for Integrative Structural Biology (CIISB) and formed by two Centres of Excellence for structural biology at CEITEC (Central European Institute of Technology), Brno and BIOCEV (Biotechnology and Biomedicine Centre), Vestec, Prague-West. CIISB offers open-access and assisted expertise to ten, core facilities for high-end cryo-electron microscopy and tomography, high-field NMR, X-ray crystallography and crystallisation, biophysical characterisation of biomolecular interaction, nanobiotechnology, proteomics, and structural mass spectrometry.

FLAGSHIP TECHNOLOGIES

Cryo-electron Microscopy and Tomography - CEITEC

Provides access for the acquisition of cryo-electron microscopy images for both single particle and cryo-electron tomography, and assisted expertise for sample preparation and data analysis.

Key equipment: Titan Krios (80 - 300 kV, energy filter, Volta phase-plate, Gatan K2, Falcon F3); Talos Arctica (40 - 200 kV, Falcon F3); Tecnai F20 (200 kV); Dual beam Versa3D FIB/SEM; vitrification robot Vitrobot Mark IV.

FIG 1

FIG 1. Titan Krios cryo -electron microscope.

FIG 2

Josef Dadok National NMR Centre - CEITEC

Provides access to high-field NMR spectrometers for study of the structure, dynamics, and molecular interactions of biomacromolecules (proteins, nucleic acids, carbohydrates) and their complexes.

Key equipment: High-field NMR spectrometers for high-resolution spectroscopy in liquids: 600 MHz, 700 MHz, 850 MHz, and 950 MHz equipped with cryogenic probes; 500 MHz and 700 MHz NMR spectrometers for high-resolution spectroscopy in liquids and solids.

FIG 2: 950 MHz high-field NMR spectrometer.

Structural Mass Spectrometry - BIOCEV

Provides access for the acquisition of structural proteomics data to determine the composition of metabolites, nucleic acid, proteins, and carbohydrates, and to analyse post-translational modifications and structural states of proteins and complexes in solution.

Key equipment: 15T-Solarix XR FT-ICR mass spectrometer with atmospheric pressure ionisation technique including electrospray/native electrospray and vacuum ionisation technique; Autoflex Speed MALDI-TOF mass spectrometer (Bruker Daltonics); Excimer laser (coherent)


FIG 3

FIG 3. 15T-Solarix XR FT-ICR mass spectrometer.


FIG 4

Diffraction Techniques - BIOCEV

Provides assisted use of the equipment for X-ray diffraction and SAXS measurements, in-house experimental phasing, "service" data collection at synchrotrons, and long-term documented cryo-storage.

Key equipment: D8 Venture diffractometer (Gallium X-ray Metal Jet C2 source, Photon II detector, and Kappa goniometer, ISX stage for D8 Venture – motorised stage for in situ X-ray diffraction experiments; SAXSpoint 2.0 instrument (Gallium X-ray Liquid Metal Jet C2+ source, Eiger 1M detector, and in situ UV/Vis spectrophotometric probe).

FIG 4: D8 Venture X-ray diffractometer.

RESEARCH HIGHLIGHTS

Automated NMR resonance assignments and structure determination using a minimal set of 4D spectra - Konstantinos Tripsianes Research Group CEITEC (Nature Communications 2018).

The automation of NMR structure determination remains a significant bottleneck towards increasing the throughput and accessibility of NMR as a structural biology tool to study proteins. Newly developed 4D-CHAINS/autoNOE-Rosetta algorithm represents a complete, highly time efficient pipeline for NOE-driven structure determination of medium- to larger-sized proteins using just two 4D spectra obtained using non-uniform sampling. Results on four protein targets ranging in size from 15.5 to 27.3 kDa illustrate that the NMR structures of proteins can be determined accurately and in an unsupervised manner in a matter of days.

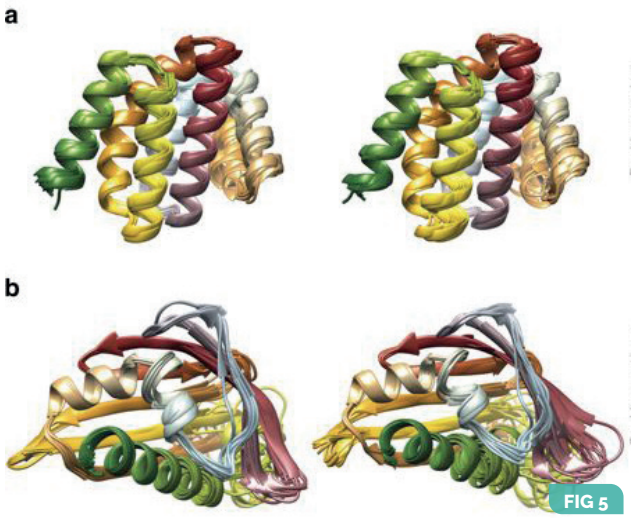


FIG 5

FIG 5. Comparison of structural ensembles calculated from supervised versus fully automated assignments. (a) alpha-lytic protease (aLP, 198 aa) and (b) Enzyme I (nEIt, 248 aa).[1]

Enterovirus particles expel capsid pentamers to enable genome release - Pavel Plevka Research Group CEITEC (Nature Communications 2019).

Viruses from the genus Enterovirus are important human pathogens. Receptor binding or exposure to acidic pH in endosomes converts enterovirus particles to an activated state that is required for genome release. Cryo-electron microscopy was used to visualise virions of human echovirus 18 in the process of genome release and discovered that the exit of the RNA from the particle of echovirus 18 results in a loss of one, two, or three adjacent capsid-protein pentamers. The opening in the capsid, which is more than 120 Å in diameter, enables the release of the genome without the need to unwind. Capsids lacking pentamers during genome release from echovirus were also observed. These findings uncover a mechanism of enterovirus genome release that could become a target for antiviral drugs.

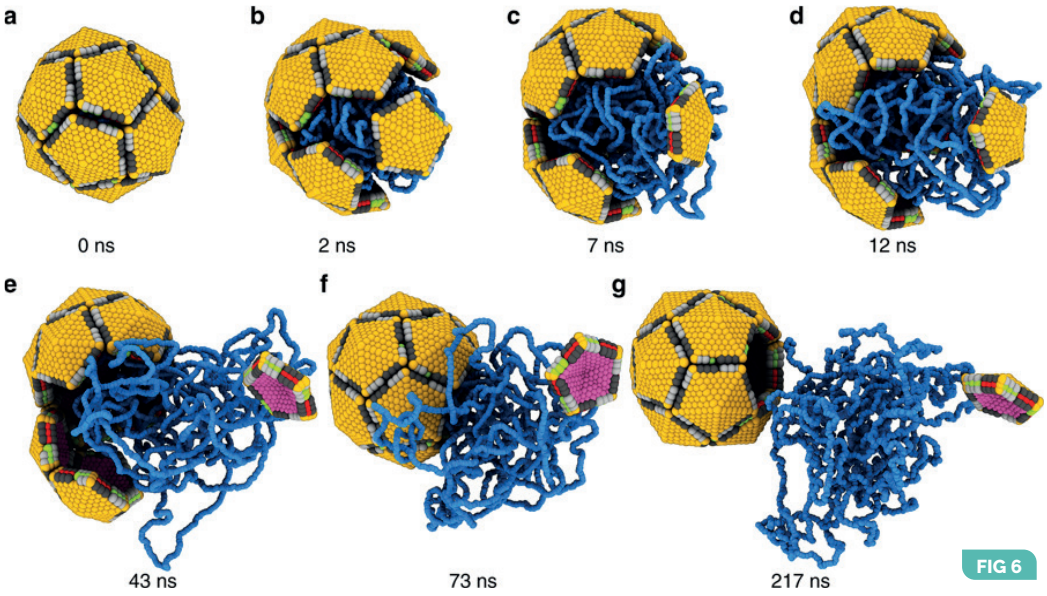


FIG 6

FIG 6. Seven snapshots from the molecular dynamics simulation of echovirus 18 genome release.[2]

The new methodology developed to obtain the above highlighted results is available to all users through Instruct access.

Instruct Centre Lead Scientists

CEITEC – Vladimír Sklenář

BIOCEV – Jan Dohnálek

1. Evangelidis T, Nerli S, Nováček J, Brereton AE, Karplus PA, Dotas RR, Venditti V, Sgourakis NG, Tripsianes K. Automated NMR resonance assignments and structure determination using a minimal set of 4D spectra. *Nature Commun.* 2018;9:384.

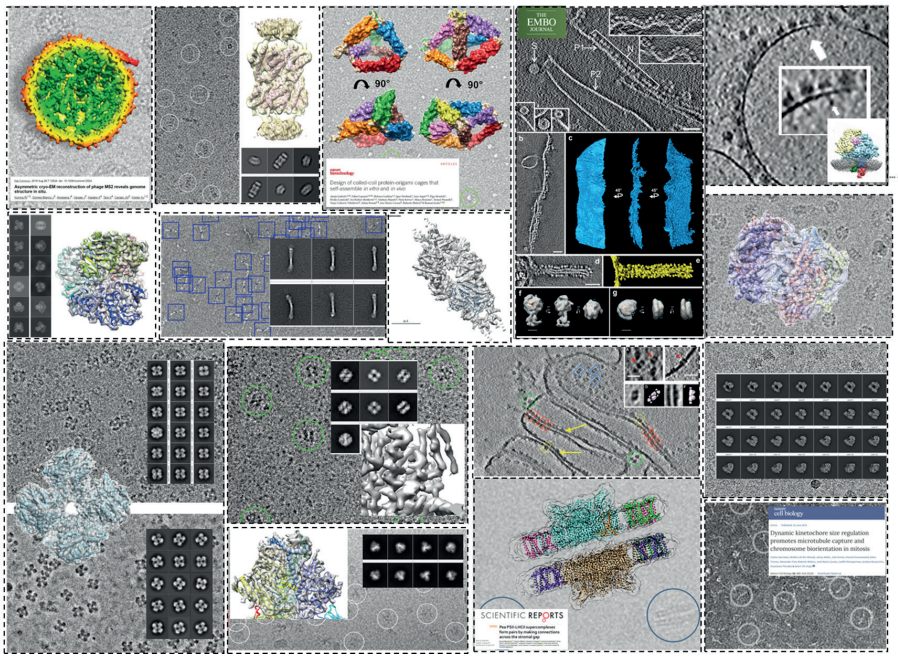
2. Buchta D, Füzik T, Hrebík D, Levdansky Y, Sukeník L, Mukhamedova L, Moravcová J, Vácha R, Plevka P. Enterovirus particles expel capsid pentamers to enable genome release. *Nature Commun.* 2019;10:1138.

INSTRUCT CENTRE ES - IMAGE PROCESSING CENTER (I²PC)

The I²PC is a world-leading centre for image processing for structural biology, especially focused on electron microscopy (single particle analysis and electron tomography). Instruct Centre ES has developed 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 has integrated other software packages into a workflow platform called Scipion, which is also publicly available and serves thousands of structural projects around the world. Scipion allows very flexible data analysis and visualisation with access to over 20+ image processing software suites. Scipion is specially designed to offer full control of the underlying algorithms with a user-friendly interface. Image processing workflows can be smoothly executed in local workstations or clusters, large high performance computers, or even the computing cloud. It has been adopted for stream processing in many EM facilities. In this way, the quality of the data being acquired can be evaluated early and key decisions can be undertaken saving time and money.

Through Instruct, I²PC help European researchers to analyse their images and to succeed in elucidating the three-dimensional structure of the macromolecule under study. Researchers may send their images for analysis (the access pack provides personnel, computing time and storage), or physically visit the Centre for a limited period. Instruct also grants mid-term internships that have been used by some researchers to dive deeply into sample preparation, image acquisition and data analysis. I²PC provide training courses on Image Processing for Electron Microscopy worldwide, and are particularly active in Europe and Latin America, supporting Instruct's efforts towards internationalisation.



RESEARCH HIGHLIGHTS

3D map of the plant Photosystem II supercomplex: towards atomic resolution by single-particle cryo electron microscopy.

Researchers visiting I²PC performed the biochemical, structural and functional characterisation of pairs of PSII-LHCII supercomplexes, which were isolated under physiologically-relevant cation concentrations. Using single-particle cryo-electron microscopy, the three-dimensional structure of paired C2S2M PSII-LHCII was determined.[1]

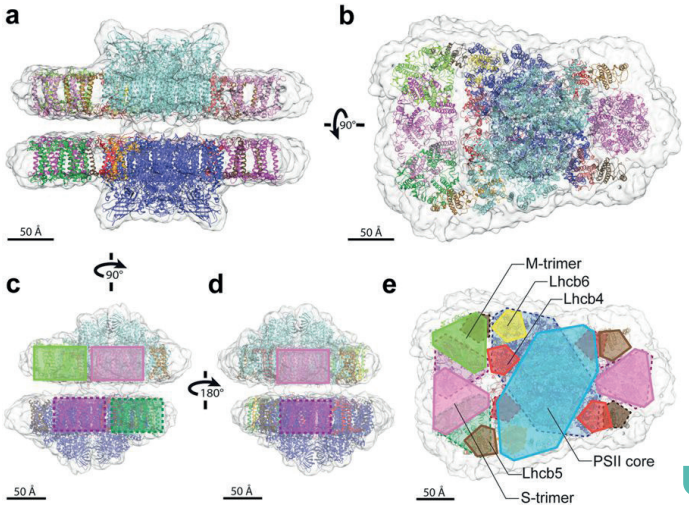


FIG 1

FIG 1. Fitting the cryo-EM density map of paired C2S2M supercomplexes with high-resolution structures.

Subtomogram averaging of cryo-electron tomograms of chromatin plates from metaphase chromosomes.

Researchers have used cryo-electron tomography and small-angle X-ray scattering to investigate the chromatin folding in metaphase chromosomes. The tomographic 3D reconstructions show that frozen-hydrated chromatin from chromosomes is planar and forms multilayered plates. The layer thickness was measured accounting for the contrast transfer function fringes at the plate edges, yielding a width of ~ 7.5 nm, which is compatible with the dimensions of a monolayer of nucleosomes slightly tilted with respect to the layer surface. Individual nucleosomes are visible decorating distorted plates.[2]

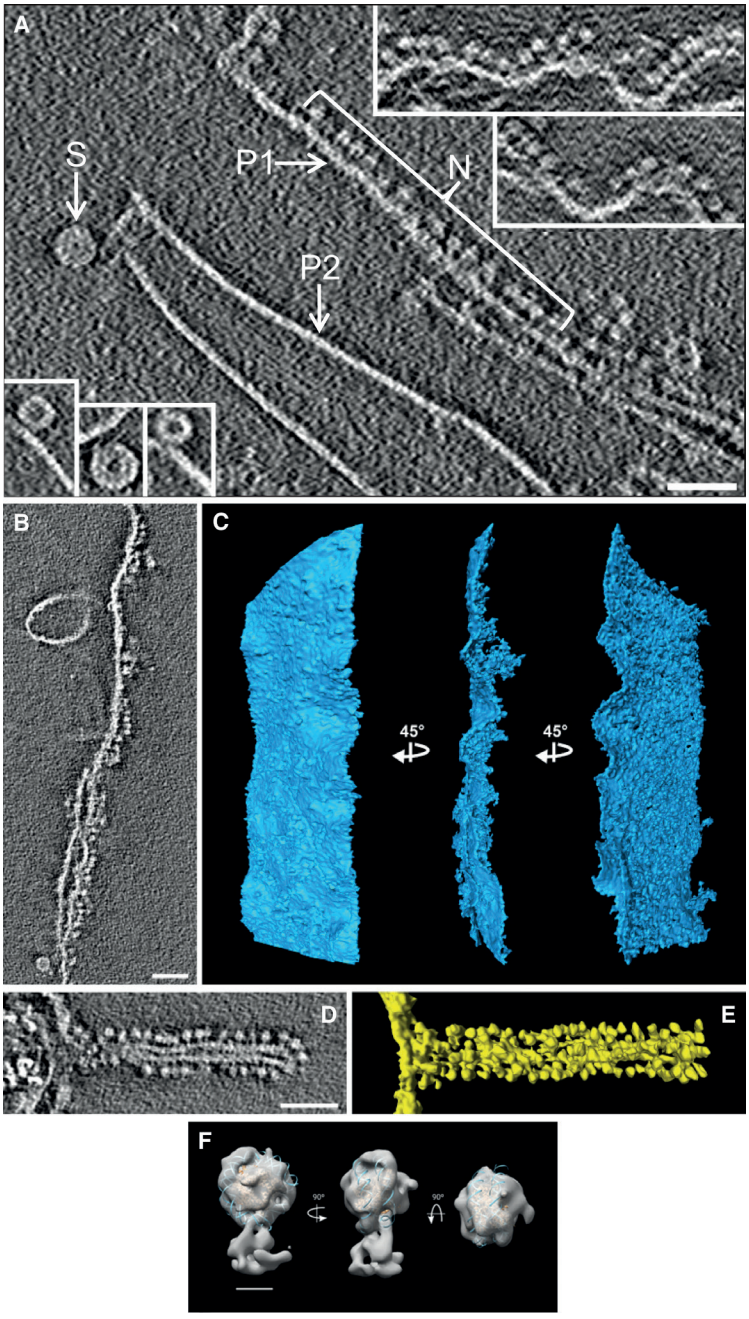


FIG 2. Cryotomograms of compact plates and distorted plates decorated with nucleosomes.

FIG 2

At the I²PC we develop the image processing algorithms and technology to extract the most from electron microscopy and help researchers to successfully use it.

Instruct Centre Lead Scientists

José María Carazo García

Carlos Oscar Sorzano Sanchez

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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 FR1 – CENTER OF INTEGRATIVE BIOLOGY, IGBMC

The Instruct Centre FR1 in Strasbourg is hosted in the Center of Integrative Biology (CBI), located on the IGBMC site at Illkirch/Strasbourg, which is also the coordinating Centre for FRISBI (French Infrastructure for Integrated Structural Biology). The Centre provides an integrated environment for structural studies of protein and macromolecular complexes.

Instruct Centre FR1 provides a cutting-edge technological environment for integrative structural biology approaches, from the molecular to the cellular level, with an emphasis on protein production linked with preparation of samples for electron microscopy and crystallography.

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

FLAGSHIP TECHNOLOGIES

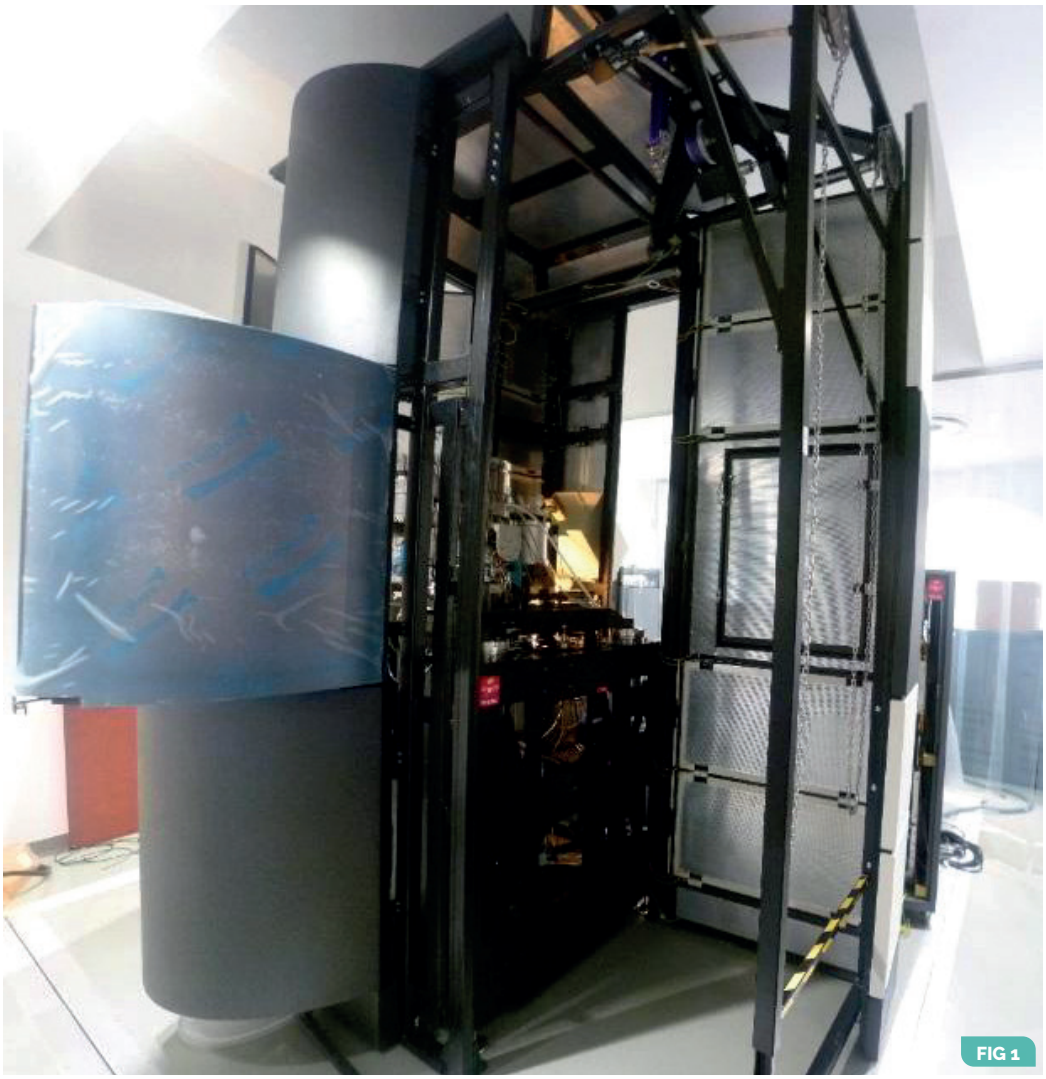


FIG 1. The cryo-electron microscope at Instruct Centre FR1.

The Instruct-Centre France-1 flagship platform: Electron microscopy

The Centre has expertise in all aspects related to single particle cryo-EM analysis and cryo-ET, from sample optimization and grid preparation to automated acquisition of large data sets and image analysis including on-the-fly data processing. The EM facilities at the CBI include well-equipped laboratories dedicated to sample preparation for electron microscopy (negative staining and vitrification) and EM instrumentation covering the full range from initial characterization to final high-resolution cryo-EM and cryo-ET data collection. This includes microscopes for sample screening and preliminary structure determination (Polara, Tecnai F20 and CM120) and a high-end microscope (Titan Krios, equipped with Cs corrector, Gatan Energy Filter, Volta Phase-Plate and two direct electron detectors (Gatan K2 Summit (coming soon K3) and Thermo Scientific Falcon III). There is also dedicated IT support for data storage and on-the-fly image data pre-processing.

RESEARCH HIGHLIGHTS

Access to the electron microscopy platform (PID 2859), Hill CH, *et al.* Activation of the endonuclease that defines mRNA 3' ends requires incorporation into an 8-subunit core cleavage and polyadenylation factor complex. *Mol. Cell.* 2019;73:1217-1231.e11

This highlights a user access in 2018 for specific technologies in cryo-EM including Volta phase plate routinely operated at the CBI, to analyse the multi-protein cleavage and polyadenylation factor (CPF/CPSF), which is essential for the formation of eukaryotic mRNA 3' ends through pre-mRNAs cleavage and poly(A) tail addition. User access enabled the analyisis of the cryo-EM structure of the Ysh1–Mpe1–Yjr141w sub-complex, which has a relatively small molecular weight (177 kDa). In a combined approach using X-ray crystallography, electron microscopy and mass spectrometry, the structure of Ysh1 bound to Mpe1 was determined, which revealed the subunit arrangement within the CPF core.

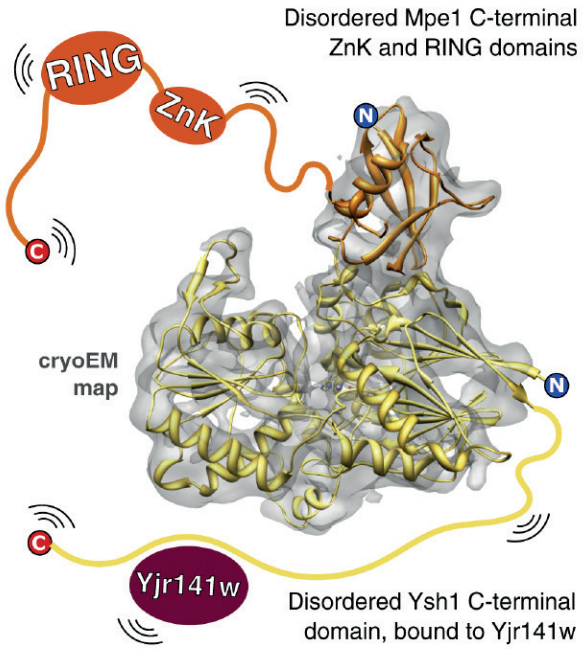
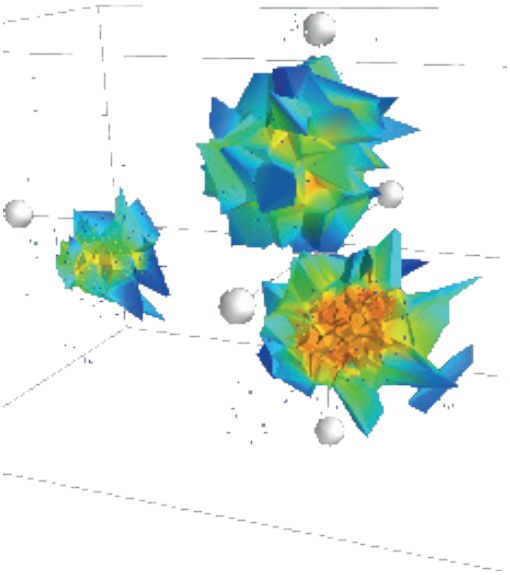


FIG 2

FIG 2. Mass spectrometry and cryo-EM define the interactions among full length Ysh1, Mpe1, and Yjr141w. The crystal structure was docked into the EM map filtered to 6 Å resolution.

RESEARCH & DEVELOPMENT



Developments for the segmentation of super-resolution microscopy data (dSTORM, PALM etc.) based on Voronoi diagrams (Andronov et al., Bioinformatics, 2018), including a software 3DClusterViSu: http://cbi-dev.igbmc.fr/cbi/voronoi3D have been performed at the CBI (Instruct Centre FR1).

Instruct/FRISBI course on Preparation & Characterisation of Macromolecular Complexes, November 2018;

16 students and postdocs selected, originated from 5 different European countries. Participants had the opportunity to gain hands-on experience of genome editing technologies in order to produce recombinant complexes in insect cells using the baculovirus expression system, and to characterise protein samples using complementary biophysical methods including nanoDSF, AUC-SV, SEC-MALS and EM analysis of negatively stained particles.

Instruct Centre Lead Scientist

Alberto Podjarny

INSTRUCT CENTRE FR2 - INSTITUT DE BIOLOGIE STRUCTURALE (IBS)

The Instruct Centre FR2 in Grenoble provides supported user access to some of the highest level structural biology instrumentation in France. Platforms are located at the Institut de Biologie Structurale (IBS) and the EMBL with user access managed by the Integrated Structural Biology Grenoble (ISBG) service unit.

Sample preparation includes mass spectrometry, cell-free expression, ESPRIT construct library screening, isotopic labelling, N-ter sequencing and Robiomol for automated molecular biology. The Molecular Biophysics platforms provides AUC, SEC-MALLS, MST, BLI, ITC, CD, DLS and SPR. Cellular imaging is available using cellular EM, confocal, video, PALM and STORM microscopy. Membrane protein crystallisation is available, as is structural analysis (beyond the X-ray and neutron facilities of the Grenoble site) are provided by cryo-EM and NMR platforms. All our platforms follow a Quality Assurance programme, managed by a full-time quality engineer, and are certified ISO 9001 NFX 50-900.

Upgrades in 2018 include a Glacios electron microscope with Falcon II direct electron detector (soon with a K2 summit), ESI-TOF and MALDI TOF/TOF mass spectrometers and the addition of a biolayer interferometer to our park of biophysics instruments.

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

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

FIG 2. The Institut de Biologie Structurale in Grenoble.

RESEARCH HIGHLIGHTS

Several snapshots of the 5-HT3 serotonin receptors in action

An international collaboration of scientists from France, Denmark and the USA, headed by Hugues Nury of the IBS in Grenoble, used the Instruct FR2 EM platform, together with the ESRF Titan Krios, to understand how the 5-HT3 serotonin receptors operate at the molecular level.

Drugs that alleviate nausea and vomiting for cancer patients undergoing radiotherapy or chemotherapy are 5-HT3 receptor inhibitors. Sitting in the membrane of an excitable cell (typically a neuron), they provide cation-selective pores through the membrane that transiently opens when serotonin is bound to the receptors. This results in the modification of the electrical potential of the cell. 5-HT3 receptors are thus ligand-activated ion channels and participate in fast neurotransmission, both in the central nervous system and in peripheral systems such as the enteric nervous system.

Four conformations of the 5-HT3 pentameric receptor were imaged using cryo-EM, representing snapshots of the receptor along its functional cycle. For instance, the inhibited conformation reveals how the binding site looks like when an antagonist (the anti-emetic drug tropisetron) is present. An active conformation shows how the binding site is re-arranged when serotonin is bound and also features an open pore permeant to cations. In the structure with the best resolution (3.2 Å), the disposition of bound serotonin and its interactions can be seen in detail and without ambiguity.

Put together, the data deepens our understanding of the molecular mechanism of operation for the 5-HT3 receptors.

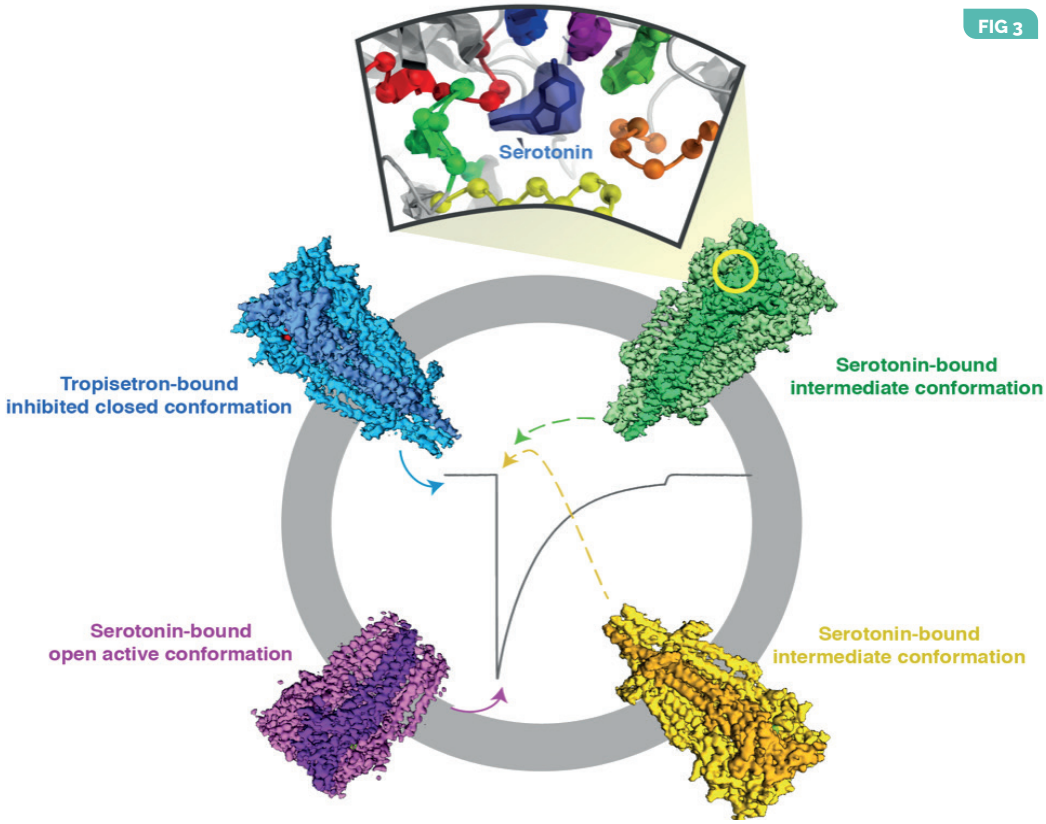


FIG 3. The four conformations obtained by cryo-electron microscopy are shown. The green conformation was obtained at the ESRF CM-01 microscope at 3.2 Å resolution. An inset represents the binding site of serotonin with the experimental information shown as a blue surface The grey line illustrates the electrical response to serotonin recorded in a cell expressing 5-HT3 receptors.[1]

Instruct Centre Lead Scientist

Darren Hart

1. Polovinkin L, Hassaine G, Perot J, Neumann E, Jensen AA, Lefebvre SN, Corringer PJ, Neyton J, Chipot C, Dehez F, Schoehn G, Nury H. Conformational transitions of the serotonin 5-HT3 receptor. *Nature*. 2018;563:275-279.

INSTRUCT CENTRE IL - THE DANA AND YOSSIE HOLLANDER CENTER FOR STRUCTURAL PROTEOMICS (ISPC) IN THE WEIZMANN INSTITUTE

The ISPC is an Israeli Instruct Center at the Weizmann Institute of Science that implements all steps in the pipeline, from the gene to the 3D protein structure. The Center collaborates with, and provides services for the scientific community and for clinicians by making available its expertise in DNA manipulation, protein expression, protein purification and protein structure determination and analysis.

Description of Technology

The 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 Facilities of the Weizmann Institute, 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 Center for protein production, biochemical/biophysical studies and structure determination using state-of-the-art, high-throughput infrastructures. The ISPC's principal mission is to provide a service for 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 at the Weizmann Institute and at other academic institutions, to biotech/pharma companies in Israel, and to its Instruct-ERIC partners. It also offers training and consultation for students and staff.

Key technologies include state-of-the-art protein production and purification equipment. In addition, the ISPC just purchased an Ultra-Bright, liquid-metal jet (LMJ) X-ray Diffraction System with in situ screening capabilities for crystallisation plates. The ISPC also employ three crystallisation robots, including an LCP-equipped Mosquito for crystallisation of membrane proteins and two 1,000 plate Formulatrix visualisation robots (at 4˚ and 19˚C) using the CRIMS visualisation system.

Proteopedia, developed at the ISPC, is being used for outreach in 60 countries with over 4,100 contributors.

SUCCESS STORIES

PROSS "Protein Repair One Stop Shop" to produce much more stable proteins

Low stability and low (or no) heterologous expression yields are major practical bottlenecks in research, let alone in applying proteins as therapeutics. The solutions to this challenge have so far been protein-specific, laborious, and time consuming (e.g. expression in eukaryotic systems such as HEK293 and insect cells). To address this challenge, Dr. Sarel Fleishman and his student Adi Goldenzweig (in the Dept. Biomolecular Science at the Weizmann Institute) developed a novel and general algorithm that combines phylogenetic analysis with energy design to identify dozens of mutations that improve stability and enable high-yield E. coli expression without affecting function. It is called Protein Repair One Stop Shop (PROSS) [http://pross.weizmann.ac.il].

In an initial test of this method, the Israel Structural Proteomics Center collaborated with the Fleishman group to try to express human AChE in E. coli. AChE is large (60kDa), membrane-associated, disulfide-linked, and glycosylated. These four hallmarks are often found in proteins that are challenging for bacterial over-expression. Indeed, AChE had not been expressed in prokaryotic systems, despite 20 years of intensive attempts due to its fundamental interest and its therapeutic importance as the target of organophosphate (OP) poisoning. The designs generated automatically by PROSS, in contrast, yielded 2 mg active enzyme per liter E. coli culture, and are ~20 ˚C more resistant to thermal denaturation, while maintaining the catalytic efficiency of the WT enzyme and is virtually identical in 3D structure (Fig 1). In the designed human AChE variant displayed in the figure, 51 mutations are introduced throughout the structure, both internal and on the surface. These designs will now serve as robust OP-detoxification reagents, and as potential countermeasures against poisoning by pesticides and nerve agents, such as VX, soman, and sarin.

In order to aid in seeing the overall structure in 3D as well as the position of the mutated individual amino acids, a Proteopedia 'Interactive 3D Complement' of the Molecular Cell paper was created to complement the published paper (Fig 2).

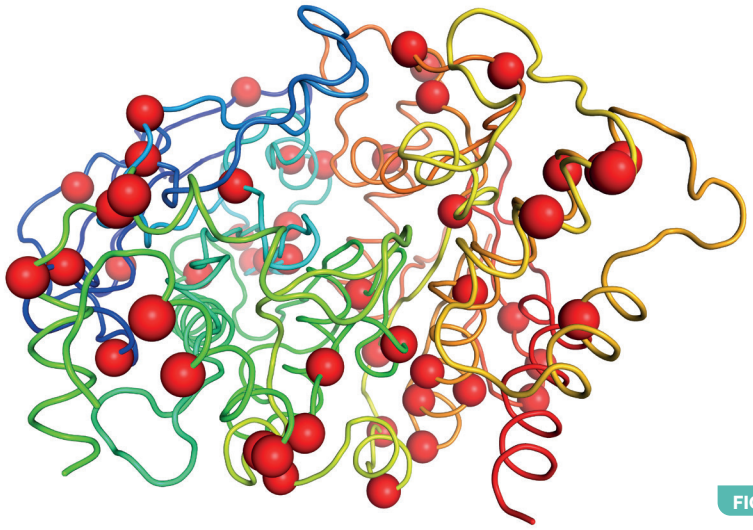


FIG 1

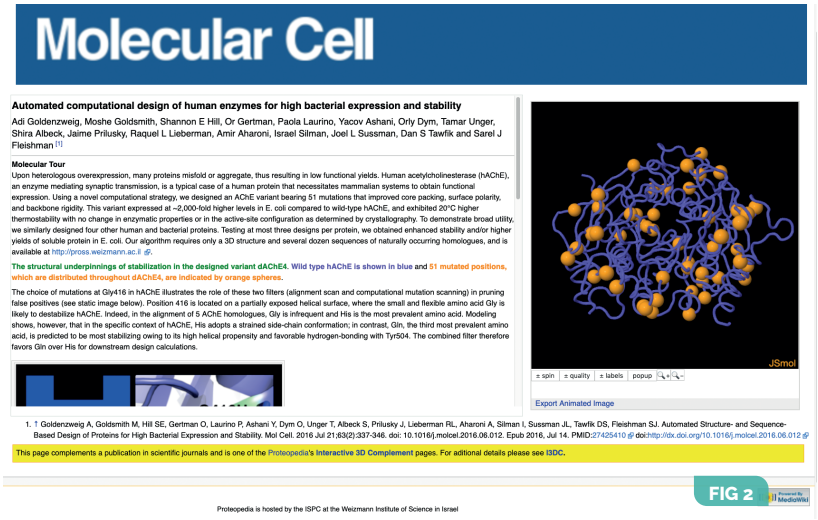


FIG 2

The ISPC has used the PROSS algorithm for improved expression of multiple protein targets, including:

- Bacterial Rhodopsin
- MMP14
- Glucocerebrosidase (GCase)
- Human Stem Cell Factor (hSCF)
- IL24

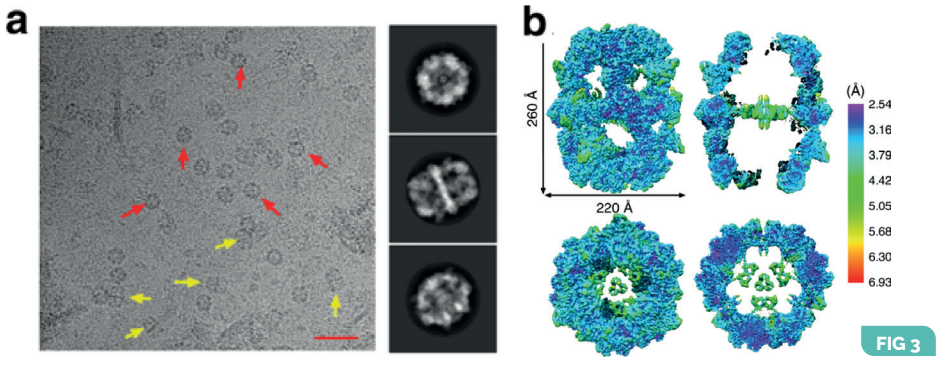


FIG 3

Cryo-EM structure of Type-I Mycobacterium tuberculosis fatty acid synthase (FAS1) at 3.3Å resolution

Mtb fatty acid synthase type-I (FAS-I) is an essential ~2 MDa enzymatic complex that contributes to the virulence of Mtb, and thus a prime target for anti-TB drugs. The enzyme was cloned and co-expressed in E. coli with its activator AcpS and isolated using an engineered strep tag producing various oriented single particles in cryo-EM (Fig. 3).[2]

1. Goldenzweig A, Goldsmith M, Hill SE, Gertman O, Laurino P, Ashani Y, Dym O, Unger T, Albeck S, Prilusky J, Lieberman RL, Aharoni A, Silman I, Sussman JL, Tawfik DS, Fleishman SJ. Automated structure-and sequence-based design of proteins for high bacterial expression and stability. *Mol. Cell.* 2016;6:337-346.

2. Elad N, Baron S, Peleg Y, Albeck S, Grunwald J, Raviv G, Shakked Z, Zimhony O, Diskin R. Structure of Type-I Mycobacterium tuberculosis fatty acid synthase at 3.3 Å resolution. *Nature Commun.* 2018;9:3886.

Fig 1. Designed human AChE showing 51 mutations, which are distributed throughout the structure. The enzyme is ~20 °C more thermally stable than the WT enzyme, but maintains its catalytic activity. Its structure is virtually identical to that of the WT mammalian expressed enzyme.

Fig 2. An Interactive 3D Complement (I3DC) in Proteopedia for the recent paper in Molecular Cell,[1] showing a 3D interactive view (right side of the page) of the 51 amino changes (shown as orange spheres) in the structure of the human AChE automatically designed by PROSS. It is clear from the image that the mutations are spread all over the structure of AChE, and not grouped in one particular region. This I3DC can be viewed at: https://proteopedia.org/w/Journal:Molecular_Cell:1.

Fig 3. FAS1 3D structure (a) A 3.3Å resolution structure (PDB; 6GJC) was determined. (b) One of the distinct features that differentiate this structure from previously determined fungal FAS-1 systems is its larger catalytic cleft which provide its unique ability to produce C26 fatty acids.

Instruct Centre Lead Scientists

Gideon Schreiber

Joel Sussman

INSTRUCT CENTRE IT - MAGNETIC RESONANCE CENTER (CERM)

The Magnetic Resonance Center (CERM) of the University of Florence, together with the Interuniversity Consortium CIRMMP, constitute an infrastructure for life sciences, which provides a unique environment for research in the field of structural biology. The infrastructure is specialised in structural biology, molecular biology, protein/complex structure determination, functional characterisation, drug-discovery, structure-based vaccine design, bioinformatics, NMR methodology, relaxometry and metabolomics.

FLAGSHIP TECHNOLOGIES

The CERM/CIRMMP NMR platform offers unique research capabilities in the field of high-resolution NMR, providing users with state-of-the -art instrumentation and expertise to perform the most comprehensive array of NMR experiments needed for the structure and dynamic characterisation of biological macromolecules and their complexes. In the near future, it will host the world's first 1.2 GHz NMR spectrometer. The NMR platform comprises eleven high-resolution NMR spectrometers ranging from 400 MHz to 950 MHz. Each instrument is equipped with state-of-the art consoles and several probes to meet all conceivable experimental conditions. Five spectrometers are equipped with CryoProbes™, one of them specifically designed for ^{13}C-direct detection NMR experiments. The 800 MHz is shared between solid-state NMR with fast-MAS probe and solution NMR with high-power probe for the study of fast-relaxing systems. Additional probes to cover a wide range of frequencies, including very low-frequency nuclei and several metal nuclei, are also available. A 700 MHz and the 850 MHz are wide-bore instruments equipped with ultra-fast MAS accessories for solid-state samples with double and triple resonance probes while one of the 600 MHz is equipped with HR-MAS accessories. 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.

Beside using the standard pulse-sequences 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]

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

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

Cellular structural biology

CERM scientists have developed a unique strategy for in-cell NMR, based on protein expression and labelling in human cells.[4] Cutting-edge NMR technologies are required as well as high-level protein expression and labelling efficiency.

Challenging questions in cellular biology can be addressed: protein folding and maturation; post-translational modifications; chaperone interactions; redox-dependent folding, etc.. Thus, the number of researchers who want to exploit the great potential of this technique is continuously increasing. NMR applications to living cells require that the cells remain viable and metabolically active during the duration of the NMR measurement to preserve the biological significance of the experiment. To guarantee these conditions also in challenging samples with high density of cells, either in suspension or immobilised in a gel matrix, CERM/CIRMMP scientists, in collaboration with Bruker UK Ltd, have developed and are currently improving a modular NMR bioreactor. The innovative design of the flow system provides fresh nutrients and removes metabolic byproducts, allowing at the same time multiple flow unit configurations, making the technique widely applicable for Instruct-ERIC users[5]

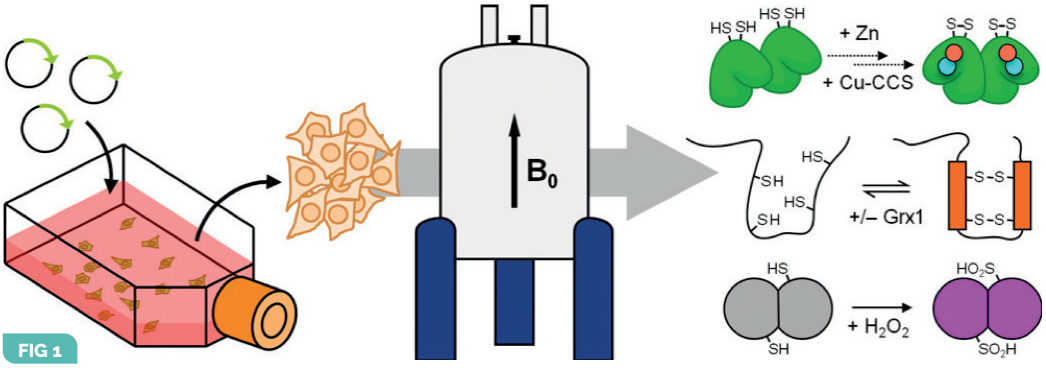


FIG 1

Fig 1. In-cell NMR in human cells: direct protein expression allows structural studies of protein folding and maturation.

Fe-S cluster interactomics CERM/CIRMMP scientists are recognised world-wide for their expertise in the characterisation of metalloproteins and the cellular pathways in which these are involved, including biogenesis. Several users working in this area accessed the CERM/CIRMMP NMR platform to boost their research goals, e.g. to study iron-sulfur (Fe-S) proteins, on which CERM researchers have a long standing tradition. Despite the chemical simplicity of Fe-S cluster, their synthesis and assembly into apoproteins is a highly complex and coordinated process in living cells. The interaction networks responsible for maturing human mitochondrial and cytosolic Fe-S proteins were largely characterised at CERM by combining solution NMR standard experiments with those tailored to paramagnetic systems. These studies showed the tremendous contribution of NMR in providing a molecular view of Fe-S protein biogenesis, since protein-protein interactions occurring in this process are often weak and transient, and thus difficult to be characterised at high resolution with other methodologies.[6,7] The obtained picture of the molecular mechanisms that are at the basis of Fe-S protein biogenesis is fundamental to boost the development of treatments of human diseases strictly related to the misfunction of this process.

Instruct Centre Lead Scientists

Lucia Banci

Simone Ciofi-Baffoni

Roberta Pierattelli

Antonio Rosato

1. Felli IC, Pierattelli R, editors. Intrinsically disordered proteins studied by NMR spectroscopy. Springer; 2015.
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INSTRUCT CENTRE NL - BIJVOET CENTRE, NECEN, NKI

Instruct NL consists of three internationally renowned sites with facilities covering a wide range of techniques. The Bijvoet Center of Utrecht University offers biomolecular NMR spectroscopy, computational structural biology and mass spectrometry. The NKI Protein Facility provides access to biophysical characterisation and crystallisation, and NeCEN offers access to two cyro-electron microscopes.

Bijvoet Center of Utrecht University (UU)

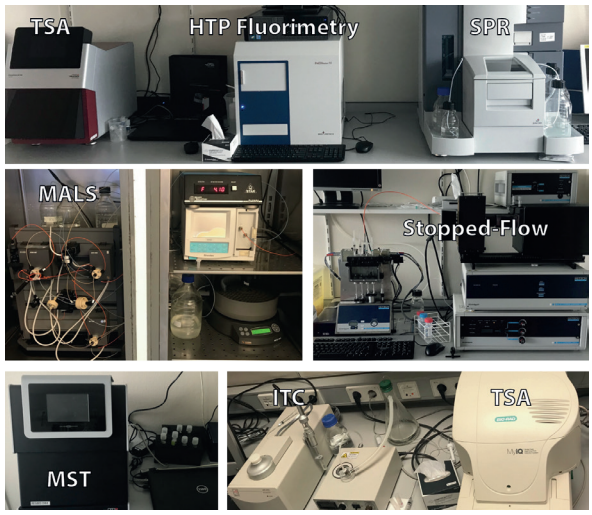
The Bijvoet Center has facilities for high-throughput mass spectrometry on proteins and peptides, X-ray crystal structure determination of biomolecules and small molecules, and high-field solid and liquid-state NMR.

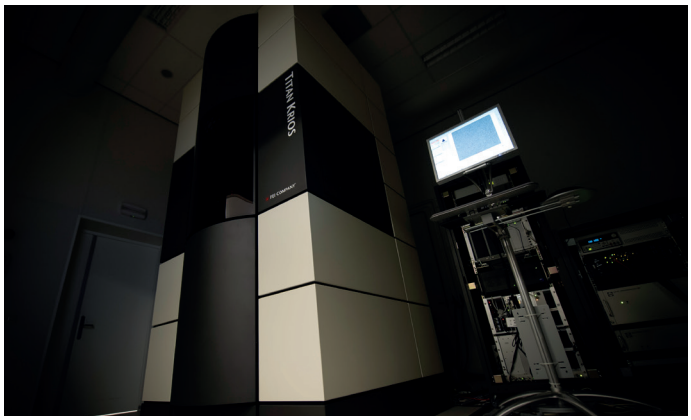
UU offers native protein mass spectrometry, which allows determination of protein mass, complex stoichiometry, and - in combination with ion mobility separation - characterisation of the overall structure of protein complexes. The NMR technology offered enables structure determination of biomolecules and/or the characterisation of macromolecular complexes.

Protein Facility of the Netherlands Cancer Institute (NKI)

The Protein Facility at the Division of Biochemistry of The Netherlands Cancer Institute (NKI) provides support for production, purification, biophysical characterisation and crystallisation of proteins. Researchers without experience or local facilities to produce and analyse proteins can request assistance with the design and performance of experiments. A particular strength of the facility is the characterisation of macromolecular interactions using a multitude of biophysical methods.

The facility designs protein fragments with structural- and bio-informatics tools to increase the chance for success. Platforms for protein expression in E. coli, insect cells and mammalian systems are offered and chromatography purification can be performed on different systems. Various biophysics equipment for characterisation of macromolecular properties and interactions is available. Finally, the facility offers automated high-throughput crystallisation screening, where conditions can be screened for in 96-well format in 100-200 nanoliter droplets.





Netherlands Centre for Electron Nanoscopy at Leiden University (NeCEN)

NeCEN provides access to cryo-transmission electron microscopy (FEI Titan Krios cryo-TEM). NeCEN offers two major aspects of cryo-TEM. One microscope is for visualising cellular structures in 3D with nanometer precision (tomography), the other is equipped for analysing single particles with highest resolution possible. In addition to data collection, NeCEN offers a wide range or cryo-EM related services, including sample preparation, sample screening, image processing and customer tailored trainings and courses.

2018 COMPLETED PROJECTS

PID 6749 (NeCEN): Structural study of H2A ubiquitination regulation via USP48, Xiaohu Guo and Titia Sixma (NL)

PID 6401 (NeCEN): Structural characterization of the bovine mitoribosome, Xabier Agirrezabala (ES)

PID 3765 (NeCEN): Cellular ultrastructure of viable but nonculturable Vibrio cholerae from environmental water cultures, Ariane Briegel (DE)

PID 1579 (UU): Labelling Mass Spectrometry, Roberta Montanari (IT)

2018 PUBLICATIONS

PID 1498 (NeCEN): Bilokapic A, Strauss M, Halic M. Histone octamer rearranges to adapt to DNA unwrapping. *Nat. Struct. Mol. Biol.* 2018;25:101.

PID 3765 (NeCEN): Brenzinger S, van der Aart LT, Van Wezel GP, Lacroix JM, Glatter T, Briegel A. Structural and proteomic changes in viable but non-culturable Vibrio cholerae. *Front. Microbiol.* 2019;10:793.

Van Beusekom B, Heidebrecht T, Adamopoulos A, Fish A, Pardon E, Steyaert J, Joosten RP, Perrakis A. Characterization and structure determination of a llama-derived nanobody targeting the J-base binding protein 1. *Acta Crystallogr. F.* 2018;74:690-695.

Koning RI, Koster AJ, Sharp TH. Advances in cryo-electron tomography for biology and medicine. *Ann. Anat.* 2018;217:82-96.

2018 SCIENTIFIC HIGHLIGHT

PID 368: 4-month internship (UU): Pascal Albanese (IT)

Photosynthesis drives life on earth by exploiting solar energy to split water molecules through the Photosystem (PS) II enzyme. In plant thylakoid membranes, PSII binds a set of light harvesting complexes (LHCII) to form different types of supercomplexes.[1] Plant PSII-LHCIIsc are localised in the stacked region of thylakoid grana,[2] in which they form paired conformations of type (C2S2M2)x2[3] and (C2S2M)x2,[4] with the two supercomplexes facing each other at the stromal surface. Although the atomic structure of PSII-LHCIIsc has recently been solved,[3] there is a lack of knowledge on their mutual interactions when facing each other, and their structural dynamics in response to light variation. Major challenges in the structure determination of the stromal interactions are not only the dynamic nature of these megadalton assemblies, but also the heterogeneity of the LHCII subunits and high flexibility of their stromally exposed N-terminal loops. At the Heck-Lab (UU) we combined top-down mass spectrometry (TD-MS) and crosslinking mass spectrometry (XL-MS) to reveal so far hidden structural details.

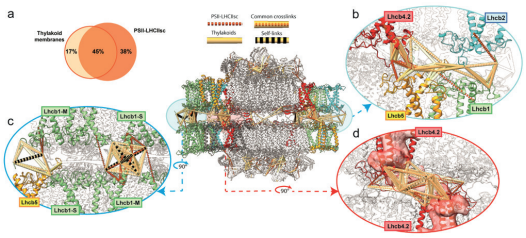


FIG 1. Detection of paired PSII-LHCIIsc in chloroplast thylakoids by in situ XL-MS.

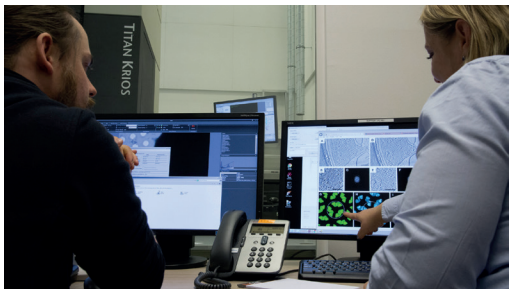
We identified and quatified 35 proteins, with 71 different proteoforms. N-termini of all PSII-LHCIIsc sequences were identified, which are largely missing in the high-resolution structures available. Structures of all proteins with unknown N-termini were predicted using the sequence of the most abundant proteoform, then fitted in the intermediate-resolution cryo-EM structure of the paired C2S2M. We treated PSII-LHCIIsc from three light conditions with two complementary chemical cross-linkers (DSSO and EDC), targeting different residues and producing partially overlapping distance restraints. We detected 104 crosslinks for DSSO and 54 for EDC that are uniquely ascribable to subunits interacting across the stromal gap, including three self-links (i.e. crosslinked peptide pairs involving the same residues). The positioning of either Lhcb2 or Lhcb1 near the PSII reaction center suggests functional hubs, and interplay of N-terminal acetylation and known phosphosites might control PSII-LHCIIsc macro-organisation. Most interactions detected in vitro on isolated paired supercomplexes were supported by XL-MS results obtained in situ with DSSO on thylakoid membranes, thereby validating our structural model in a close-to-native state (Fig. 1).

INSTRUCT NL 2018 - OTHER HIGHLIGHTS

In October 2018, an Instruct workshop for EM facilities was organised by and hosted at NeCEN (https://www.necen.nl/oct-2018-2nd-instruct-workshop-on-cryo-em-best-practices-91).

To promote collaboration between Instruct service providers, NKI - via the H2020 project Instruct-ULTRA - has been developing a virtual laboratory environment and common gateway for using protocols and equipment, aiming to be spread to all protein production and biophysical facilities in Europe that want to work with Instruct.

With respect to technical developments of the Instruct NL center, UU is preparing to house a 1.2 GHz NMR system, allowing access to the highest magnetic field worldwide for increased sensitivity and spectral resolution. In addition, whereas UU already includes EM-tomography facilities, also NKI is setting



up lower-end equipment for EM screening and lower-resolution structural biology approaches.

At the moment, an Instruct NL meeting is being planned to expand and streamline the Dutch national Instruct NL community. Instruct NL has been selected to organise the 2021 Instruct Biennial Structural Biology Conference.

1. Dekker JP, Boekema EJ. Supramolecular organization of thylakoid membrane proteins in green plants. *Biochim. Biophys. Acta - Bioenerg.* 2005;1706:12-39.
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3. Su X, Ma J, Wei X, Cao P, Zhu D, Chang W, Liu Z, Zhang, Li M. Structure and assembly mechanism of plant C2S2M2-type PSII-LHCII supercomplex. *Science* 2017;357:815-820.
4. Albanese P, Melero R, Engel BD, Grinzato A, Berto P, Manfredi M, Chiodoni A, Vargas J, Sorzano CÓ, Marengo E, Saracco G, Zanotti G, Carazo JM, Pagliano C. Pea PSII-LHCII supercomplexes form pairs by making connections across the stromal gap. *Sci. Rep.* 2017;7:10067.

Instruct Centre Lead Scientists

Marc Baldus

Rolf Boelens

Alexandre Bonvin

Albert Heck

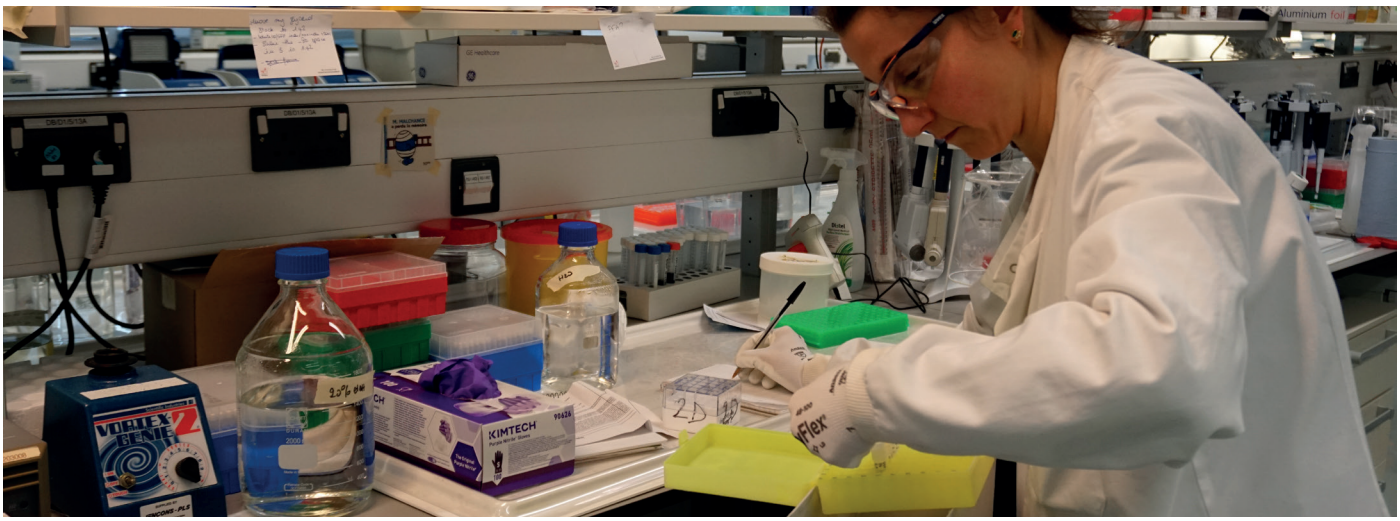
Anastassis Perrakis

Ludo Renault

INSTRUCT CENTRE UK

Instruct UK brings together six facilities on three sites in and around Oxford. Sample preparation, notably using mammalian expression systems (at STRUBI) and specialist (containment) cryo-EM facilities (OPIC) are co-located in the Structural Biology Division of the University of Oxford. The mass spectrometry and biophysics suites, located in the Biochemistry and Chemistry faculties of the University in Oxford city, are easily integrated with users accessing STRUBI facilities. More sample preparation, characterisation and crystallisation is available on the Harwell Campus, which houses both the Membrane Protein Laboratory at the Research Complex, and the Diamond Light Source, the UK's national synchrotron facility. The site, located to the south of Oxford, provides access to various beamlines for X-ray data collection, and also the new national cryo-EM facility (eBIC).

As far as possible, the UK Centre aims to provide a full process pipeline from protein expression and sample quality control, through to crystallisation and data collection at the X-ray facilities at Diamond. Alternatively, sample preparation and preliminary quality checks for cryo-EM are also available and if appropriate, good samples can be scheduled for cryo-EM analysis on one of several Krios Titan instruments. Specialist methods for virus structure and large macromolecular complexes are available.



FLAGSHIP TECHNOLOGIES - OXFORD MASS SPECTROMETRY CENTRE

MASS PHOTOMETRY: WEIGHING BIOMOLECULES WITH LIGHT

A recent development from the Oxford Mass Spectrometry Centre, mass photometry is an optical technique with a unique capability for imaging and characterising biomolecules. By exploiting light scattering phenomena, mass photometry can weigh biomolecules with high accuracy and single-molecule sensitivity, potentially transforming the way that we study protein structure and dynamics.

Single-molecule characterisation has revolutionised our ability to probe the nanoscale structure and interactions of biomolecules. Nevertheless, many technologies lack the spatiotemporal resolution to track and quantify the diverse and dynamic structures found in biological systems, and fewer still can image unlabelled, single molecules in a biologically-relevant environment.

Mass photometry is a new optical technique for quantitative analysis of unlabelled biomolecules in vitro.[1] In the mass photometer, coherent light is scattered from an aqueous solution of biomolecules, producing an interference pattern. Although the scattering effect is weak, the optimised optics of the mass photometer can detect and transform the interferometric signal into a high-precision, contrast image of the biomolecule and its surroundings. Significantly, the interference contrast signal is strongly correlated with the mass of the biomolecule, leading to applications in mass photometry. At its current sensitivity, mass photometry has been used to mass-image individual biomolecules of 50 to 800 kDa to within 2 % mass accuracy and 1 kDa precision.

In their formative publication, Young et al. have demonstrated the immense scope of mass photometry for biomolecular characterisation. Using the mass photometry technique, the authors were able to resolve oligomeric distributions, quantify rare complexes, and observe the growth of nanoscopic objects at low concentration. The authors also demonstrated the benefit of time-resolved mass-imaging in extracting kinetic and thermodynamic data for complex biological mechanisms.

Currently, there are few other techniques that offer the sensitivity, spatiotemporal resolution, and wide applicability of mass photometry for the study of native biomolecules in vitro. As commercial apparatus is made available,[2] mass photometry is anticipated to become a powerful and accessible characterisation tool in structural biology.

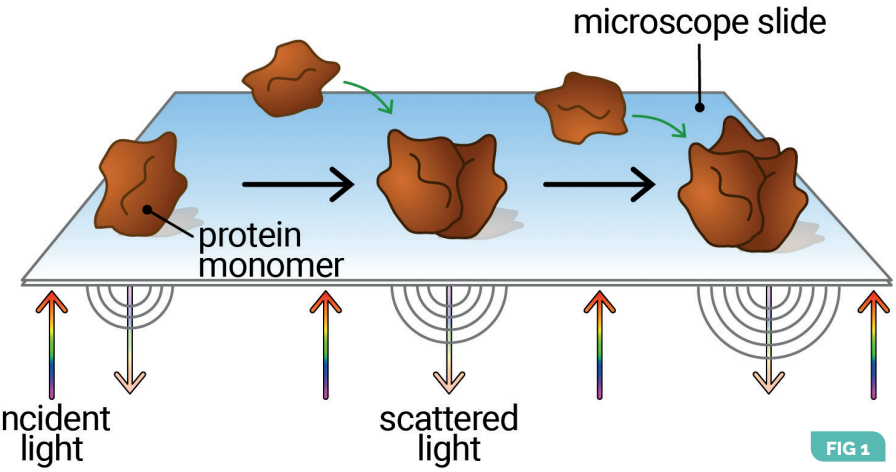


FIG 1

FIG 1. Mass photometry can be used to mass-image the complexation of protein in real-time.

Advanced mass spectrometry techniques, such as mass photometry, are available through Instruct-ERIC at the Oxford Mass Spectrometry Centre.

2018 SCIENTIFIC HIGHLIGHT

PID 1195, 1199, 1201, 1202: A collaborative project initiated in 2014 involving researchers from the University of Finland, pre-dating Instruct-ERIC but resulting in a publication "Assembly of complex viruses exemplified by a halophilic euryarchaeal virus" *Nat. Commun.* 2019;10:1456.

The PDR1-adeno viruses are characterised by a conserved pseudo-hexameric capsomere composed of three copies of a single major capsid protein. High resolution cryo-EM analysis showed that a class of archaeal viruses, exemplified by a double-stranded DNA virus SH1, possess hetero-hexameric capsid proteins that mimic the PDR1 pattern. The cryo-EM structure images obtained indicate that the archaeal viruses use common structural building-block components to assemble into PRD1-like capsids and may represent precursors of the PDR1-adeno lineage, with similar receptor engagement mechanisms.

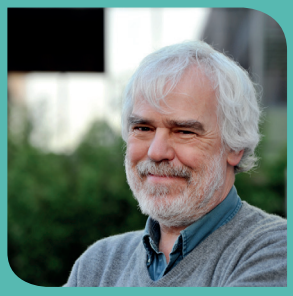
1. Young G, Hundt N, Cole D, Fineberg A, Andrecka J, Tyler A, Olerinyova A, Ansari A, Marklund EG, Collier MP, Chandler SA, Tkachenko O, Allen J, Crispin M, Billington N, Takagi Y, Sellers JR, Eichmann C, Selenko P, Frey L, Riek R, Galpin MR, Struwe WB, Benesch JLP, Kukura P. Quantitative mass imaging of single biological macromolecules. *Science.* 2018;360:423-427.

2. https://www.refeyn.com.

Instruct Centre Lead Scientists


Juha Huiskonen


Ray Owens


Dave Stuart


Martin Walsh


Yuguang Zhao

INSTRUCT-ERIC HUB

At the launch of Instruct-ERIC, the Hub had six members of staff seconded from the University of Oxford. The Director, the Hub Coordinator and the Instruct Project Manager have been involved with the initiation and setup of Instruct since 2006, and provide a degree of experience and continuity that is a strong anchor for Instruct.

Changes to the Hub have included the recruitment of Fiona Sanderson and Twba Al-Shaghdari to the IT Team as Software Developer and Trainee Software Developer, replacing Callum Smith and Narayanan Krishnan. Fiona has driven important quality control measures in the access management software ARIA and expanded its functionality. Natalie Haley joined in October 2017 as Project Officer for CORBEL activities and soon demonstrated her skills in software implementation, adding value to the IT team profile. The IT effort has been supplemented by the work of two external contractors who were brought in to develop the new Instruct-ERIC and Instruct-ULTRA websites, and an API for data exchange between Instruct facility sites and ARIA.

Naomi Gray was recruited as Project Manager for the Instruct-ULTRA Implementation Grant in February 2018, with Ray Owens leading the ULTRA Coordination team.

Financial and general administrative duties are carried out by Lorraine Donaldson and Madalena Gallagher. Estina Ketsetzopoulou joined as Finance and Administrative Assistant in September 2018, as Lorraine reduced her hours. As of the 31 December 2018, the Hub complement was eleven staff including the Director.

NEW TEAM MEMBERS

Fiona Sanderson

Twba Al-Shaghdari

Naomi Gray

Estina Ketsetzopoulou

EXISTING TEAM MEMBERS

Madalena Gallagher

Lorraine Donaldson

David Rodríguez Aguiar

Ray Owens

Susan Daenke

David Stuart

NEW PREMISES FOR INSTRUCT-ERIC

On 1 April 2018, the Hub moved from their previous office space within the Division of Structural Biology at the University of Oxford into commercial office space. The Hub occupies a suite of three offices and a meeting room which is set up for videoconferencing.

The new address is:

Instruct-ERIC, Oxford House, Parkway Court, John Smith Drive, Oxford, OX4 2JY, UK.

Our primary telephone contact is Madalena Gallagher on +44 (0)1865 988 639.

We are situated close to the centre of Oxford and maintain a strong link with the University of Oxford through the European Research Office and related interest groups. The Harwell Campus, where the Diamond Light Source, Research Complex, and eBIC are situated, and the new Rosalind Franklin Institute will be built in the next five years, is just 27km to the south.

SPOTLIGHT ON STAFF

Claudia Alén Amaro

Claudia is a Pharmaceutical Chemistry Masters graduate from the Universidad Mayor de la Republica Oriental del Uruguay and holds a DPhil in Biochemistry from the University of Oxford. Following postdoctoral laboratory work, Claudia joined as Project Manager to the Instruct Preparatory Phase project in 2008 and has since helped Instruct to grow and develop through to ERIC status.

Now the Senior Instruct Programme Manager, she manages the day-to-day operational activities of Instruct, specialising in the access, training and communications work. She has taken a special interest in developing Instruct's outreach to Latin American countries, firstly by securing funding from the British Council for a joint training workshop, and subsequently through a number of events which have expanded the consortium into Uruguay, Brazil and Argentina and established MOUs with several major scientific institutions. Claudia runs the Instruct Twitter account and has built Instruct visibility and the current following over several years.

Natalie Haley

Natalie holds a Master of Physics and a DPhil degree from the University of Oxford, graduating in 2017. She joined Instruct to work on the CORBEL Project and almost immediately expanded her portfolio to include the tail-end of the West-Life project. Natalie has grown to be a valued contributor to a number of projects, specialising in those that deliver services through the ARIA digital interface. Her supreme organisation skills will be well used when she takes the role of Project Manager to the new project RI-VIS, which is coordinated by the Hub, and to EOSC-Life, in which Instruct is a partner.

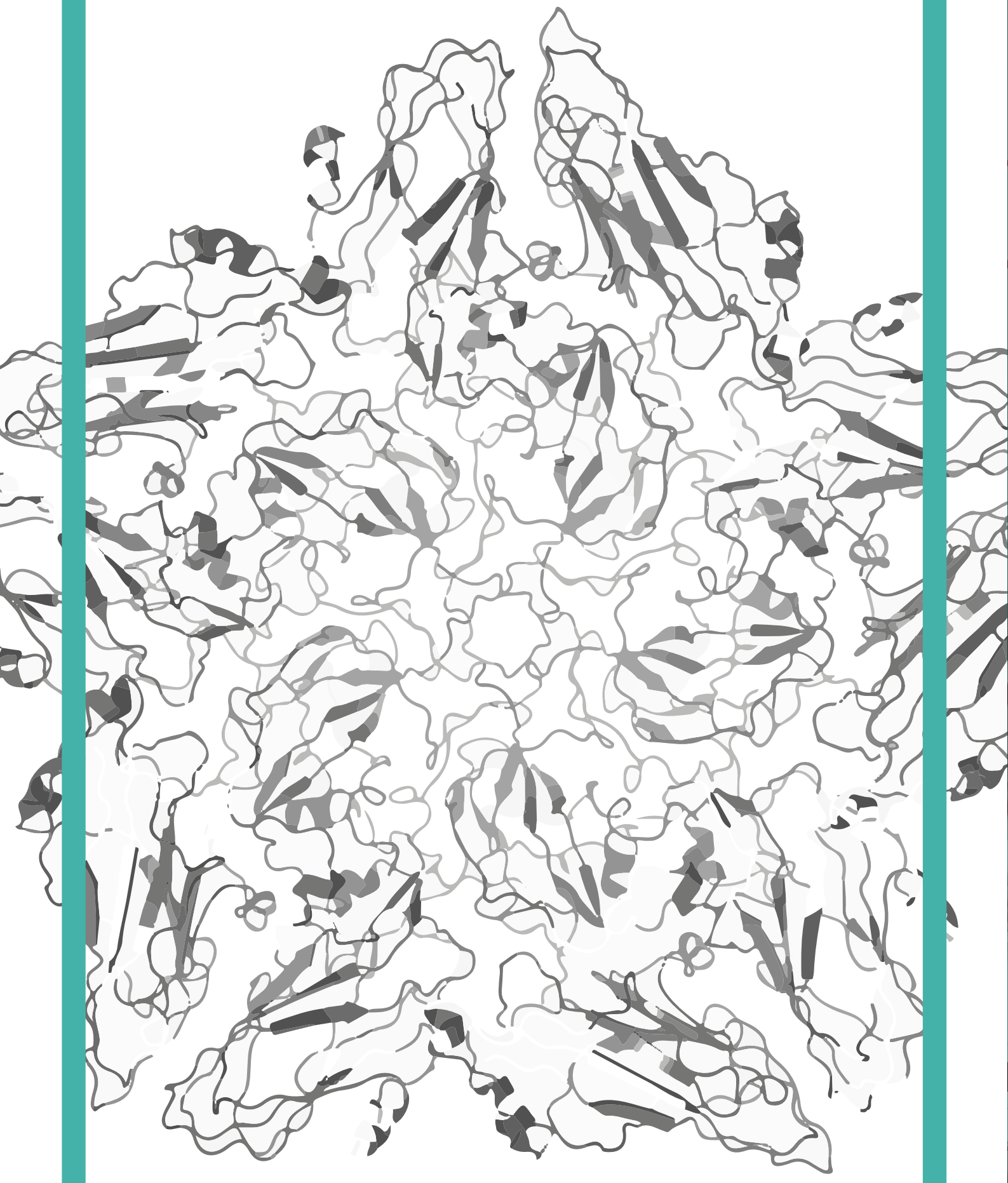
CAPACITY BUILDING AND STAFF DEVELOPMENT

Instruct Centres have developed and refined the processes for providing access to infrastructure through several years of operation. The Instruct-ERIC Managers Group has been formed which is a forum for exchanging information and building on experiences gained. The Group includes membership from all Centres, represented by facility managers and by supporting staff who contribute to access delivery.

The Managers Group convenes a workshop once per year to discuss issues such as the following:

- Quality Assurance in Research Infrastructures
- Best practices for quality management in the delivery of access
- Requirements for GDPR
- Future developments in ARIA
- Opportunities for training
- Identify future requirements for facility managers

The Group is working towards establishing some common standards for access delivery and organise staff exchanges, training courses, podcasts and webinars related to management and administrative procedures. ARIA use and training is also available for existing and new facility staff. The Group has strengthened links between Centres and is helping to build the Instruct mark of quality.



Protein 5OYI


SERVICES
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ACCESS

Access to infrastructure is invited via calls that are published on the website and circulated through other web channels and social media to reach a wide user community. General calls for access are frequent and, from time to time, a specific call is launched to describe a particular focus for research, or to target a specific user community, for example users from new communities, industry users, or pilot calls for non-European users.

Proposal applications are submitted via the ARIA web-portal on the Instruct site and outline a scientific case and the selection of infrastructure(s) that a user needs. Proposals are sent for peer review to a panel that includes two reviewers external to the Instruct membership and one reviewer from within our 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 Access to each service/ technology is capped at €1500 per visit. This can be split between the following costs:

1. Consumable costs: the Centre can claim up to €1100 per access visit or project.
2. Travel and accommodation: if there is no other support available, a contribution towards travel and accommodation can be paid to the researcher of up to €600, depending on region.

The progress of each proposal is actively tracked through an internal messaging system that links all correspondence between user, moderator and host Centres. All proposals are monitored daily to ensure that any issues that arise can be managed immediately.

Access is available to any researcher or user from industry and the amount of support funding available from Instruct is agreed before work commences. In cases where the access costs are in excess of the Instruct support available, or the user requires access for proprietary work, the user may be asked to cover the costs from grants or other means.

The Instruct model for funding access to infrastructure at Centres is being evaluated for revision. The European Commission has developed a funding model for EC access networks which is a proven benchmark for infrastructure provision. At the Strategic meeting in May 2018, Instruct resolved to evaluate a similar version of the EC model for implementation by Instruct. A six-month pilot is planned for the third and fourth quarter of 2019, and the outcome will be reported in the next Annual report.

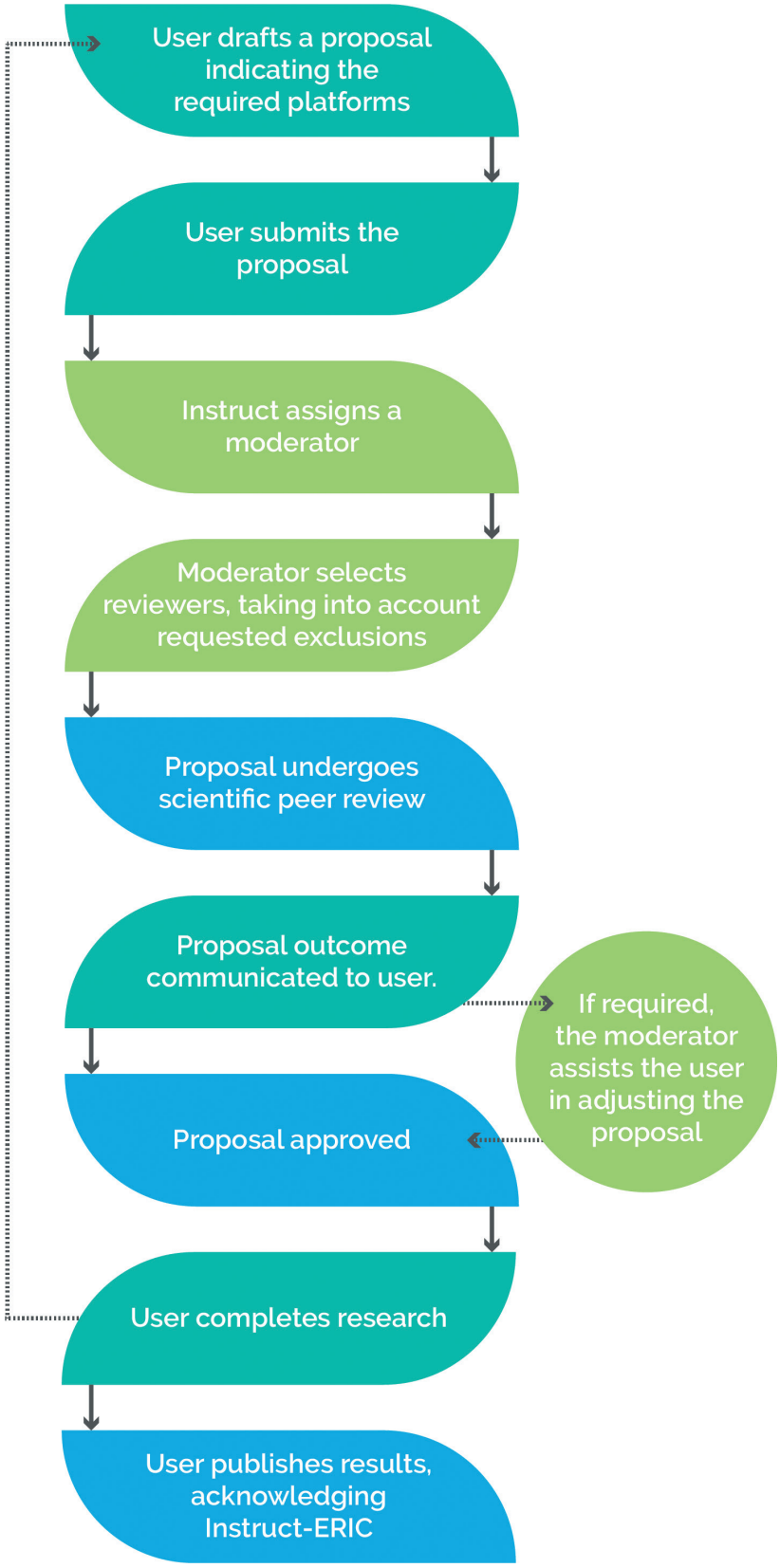


FIG 1. The pathway for access to Instruct-ERIC services.

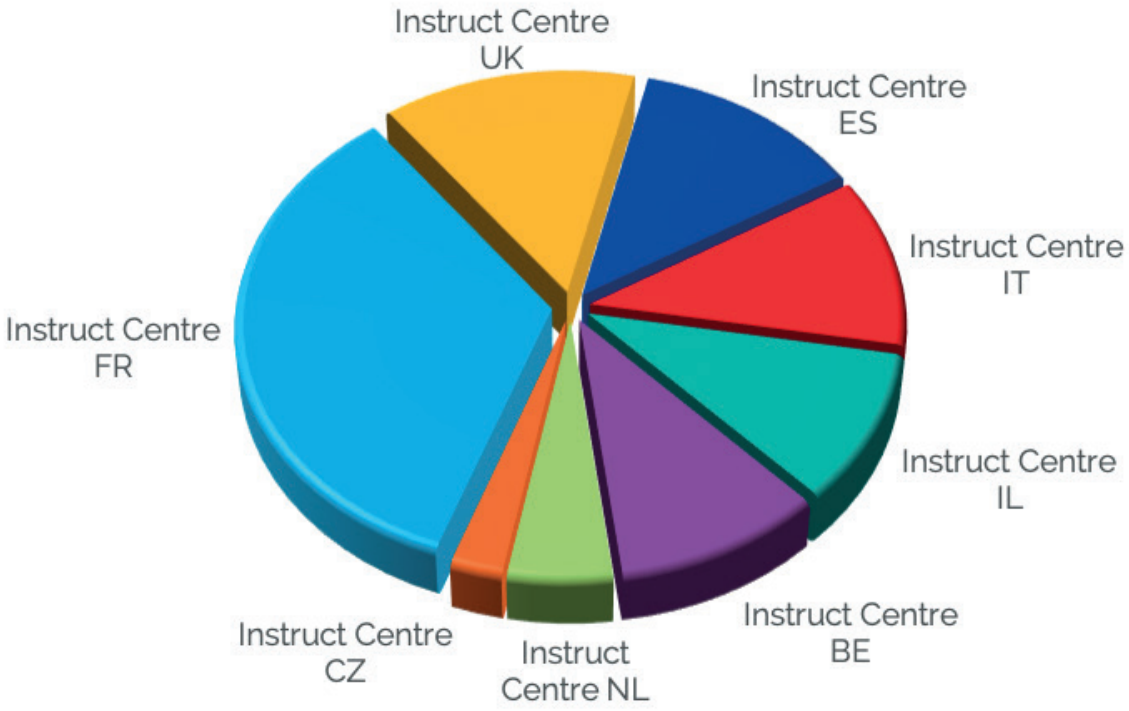


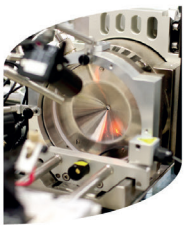
FIG 2. The share of access visits to each Instruct Centre during the reporting period.

Access is the cornerstone of Instruct activity. In the period from 1 August 2017 to 31 December 2018 a total of 139 proposals were received by Instruct. Of these, 15 were rejected.

Applicants requested access to platforms in France most frequently, followed by platforms on the UK and Spain. France provides infrastructure at two Instruct Centres: Strasbourg and Grenoble. Both offer highly specialist methods and these Centres also provide national access services via FRISBI (French Infrastructure for Integrated Structural Biology).

sample preparation

- crystallisation
  - macromolecular crystallisation
  - membrane protein crystallisation
- nanobody discovery
  - in vitro
  - E. coli (isotope labelling)
  - insect cells
  - mammalian cells
  - library-based screening
- protein production
  - membrane protein production
  - automated molecular biology



biomolecular analysis

- imaging
  - confocal microscopy
  - video microscopy
  - flow-cytometry
- mass spectrometry
  - native MS
  - proteomic MS
- molecular biophysics
  - differential scanning calorimetry
  - differential scanning fluorescence
  - microscale thermophoresis
  - thermal shift assay
  - analytical ultracentrifugation
  - surface plasmon resonance
  - isothermal calorimetry
  - fluorescence spectroscopy
  - circular dichroism
  - microscale thermophoresis
  - dynamic light scattering
  - SEC-MALLS

3D structural analysis

- electron microscopy
  - cryo-EM
  - SEM
  - TEM
  - image processing
- magnetic resonance
  - EPR
  - solid state NMR
  - solution NMR
  - relaxometry
- X-ray techniques
  - bio-SAXS
  - X-ray diffraction

FIG 3. The catalogue of technologies accessible through Instruct-ERIC.

ACCESS

The technology types that applicants have requested reflect recent advances in methods such as cryo-electron microscopy and imaging. Along with this, there is an increasing demand for some sample preparation methods such as protein production, biophysical methods for sample characterisation, and eukaryotic expression technologies.

FIG 1

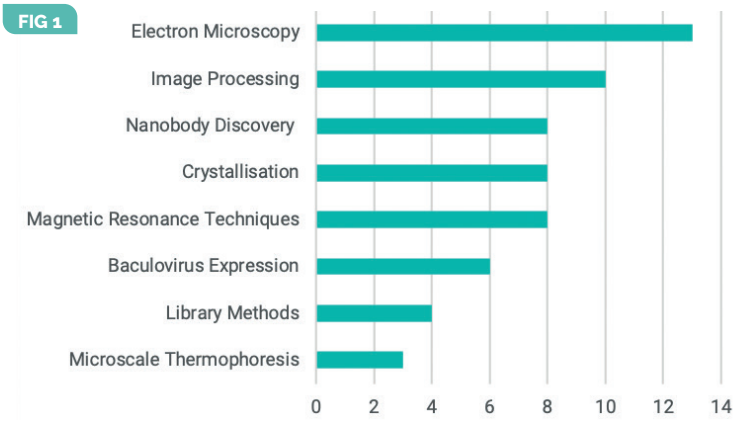


FIG 1. Top platforms requested for access visits..

FIG 2

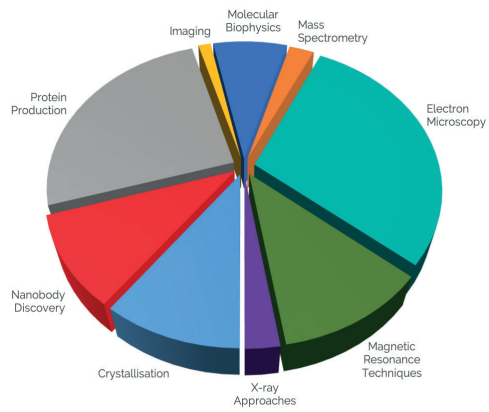


FIG 2. Share of user access by technology type.

Nanobody production, provided by the Instruct Centre Belgium, is a technology in high demand but of limited capacity. In this reporting period, 12 access projects for nanobodies were received, of which four were rejected. Each project takes up to three months to prepare the immunogen, immunise and test the animals and then undergo selection e-screening for antibodies. This access service is provided, passing only some consumable costs on to Instruct, with the remaining costs being covered by external grants held by the Instruct Centre BE host institution. The average cost to Instruct per nanobody project is €1100. Overall the impact of nanobody production has been significant, with 20 publications in the reporting period.

Applicants for access services are gathered from a very broad area: 15 countries of origin were represented in the data selection, of which four were non-Instruct Member EU countries (Germany, Poland), and three were non-EU countries (Switzerland, Turkey, Ghana). Applications from non-Member countries are identified pre-review as being ineligible for Instruct-funded access.

FIG 3

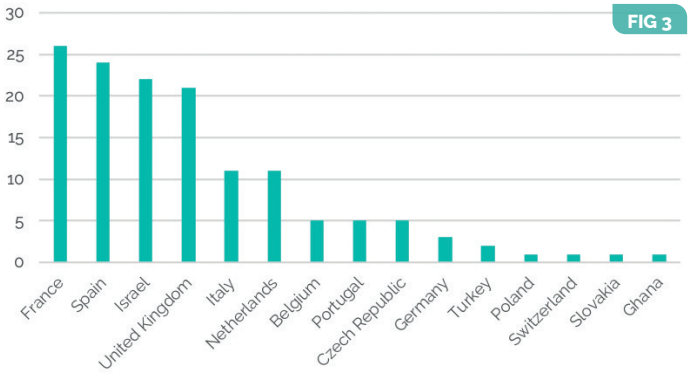
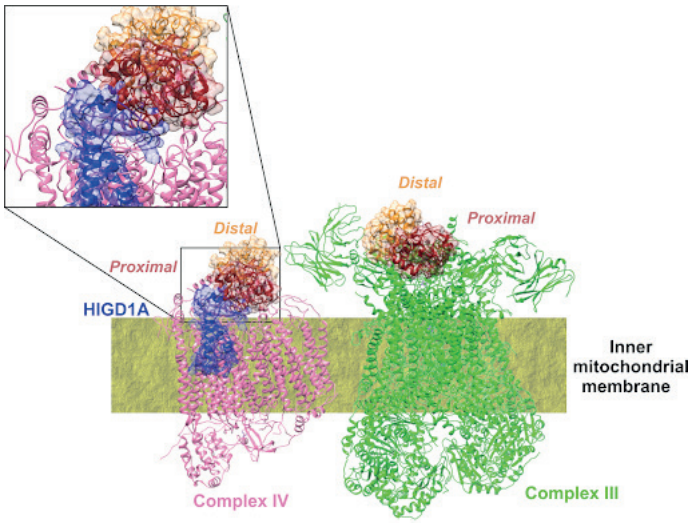


FIG 3. User access by the country of their host institution.

ACCESS HIGHLIGHTS

PID2921 - Respiratory supercomplexes: the interaction between hypoxia-inducible proteins and cytochrome c in humans

Proper working and regulation of the mitochondrial electron transport chain (ETC) are essential for cell energy production and detoxification. ETC malfunction is linked to a variety of human diseases, namely aging, cancer, etc.. The ETC consists of four membrane-embedded protein complexes, with cytochrome c as electron carrier between complex III and IV. With the help of the so-called respiratory factors, i.e. hypoxia-inducible domain family member 1A (HIGD1A), the four ETC complexes can increase their catalytic efficiency upon association in supercomplexes. Based on former findings from the research group (Guerra-Castellano et al., PNAS 2018), the Instruct access proposal PID2921 focussed on the interaction between cytochrome c and HIGD1A to better understand the assembly of mitochondrial supercomplexes at the biophysical and structural level.



Publication:

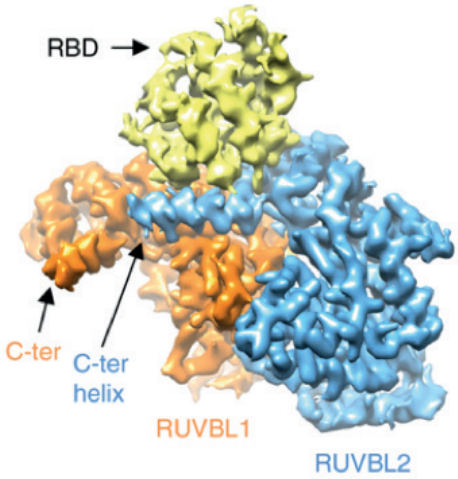
Guerra-Castellano A, Díaz-Quintana A, Pérez-Mejías G, Elena-Real CA, González-Arzola K, García-Mauriño SM, De la Rosa MA, Díaz-Moreno I. Oxidative stress is tightly regulated by cytochrome c phosphorylation and respirasome factors in mitochondria *Proc. Natl. Acad. Sci.*. 2018;115:7955-7960.

PID2463 - Cryo-EM of a yeast Hsp90 complex

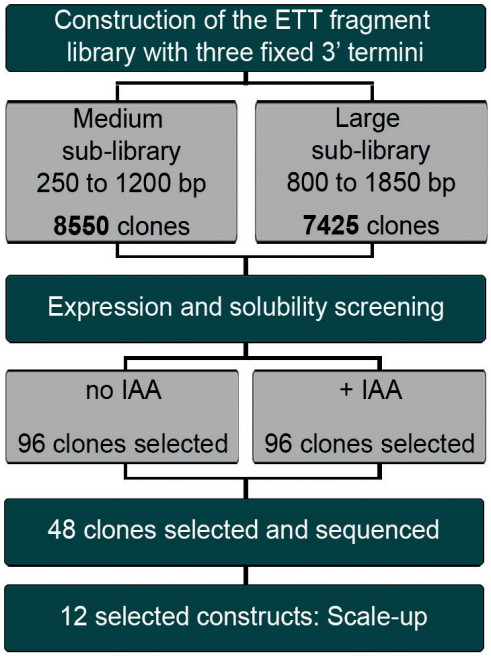
The R2TP complex is a specialised Hsp90 co-chaperone required for the assembly and maturation of multi-subunit complexes, including the small nucleolar ribonucleoproteins, RNA polymerase II and complexes containing phosphatidylinositol-3-kinase-like kinases. However, the mechanism by which the R2TP/Prefoldin-like co-chaperone and HSP90 facilitate assembly and cellular stability is poorly understood. In this investigation, Cryo-EM and biochemical studies were used to study the structure of the complex between yeast R2TP and Hsp90.

Publication:

Martino F, Pal M, Muñoz-Hernández H, Rodríguez CF, Núñez-Ramírez R, Gil-Carton D, Degliesposti G, Skehel JM, Roe SM, Prodromou C, Pearl LH. RPAP3 provides a flexible scaffold for coupling HSP90 to the human R2TP co-chaperone complex. *Nat. Commun.* 2018;9:1501.



View of RUVBL inner cavity



PID1526 - ETT protein production through the ESPRIT method

The plant hormone, auxin, plays a key role in plant development and growth. Auxin signalling is mediated by auxin response factors (ARFs) that dimerise with modulating Aux/IAA repressors. ARF3 (ETTIN or ETT) encodes an atypical ARF that is impaired in dimerisation with Aux/IAA repressors. Due to its inability to behave as a canonical ARF, ETT has evolved an alternative mechanism to respond to changes in auxin concentration without Aux/IAA regulation. A detailed analysis of the 3D protein structure of the ETT-IND complex would be a major step in understanding this mechanism. In the proposal, a soluble ETT protein was obtained using the innovative ESPRIT method to screen >16 thousand constructs for soluble expression of ETT in the absence and presence of IAA. The successful identification and purification of the domain and characterisation as an intrinsically disordered protein (IDP) containing newly identified interaction motifs will pave the way towards an improved understanding of auxin-mediated regulation in plants, which could improve the yield of a wide variety of economically important crops.

Publication:

Simonini S, Mas PJ, Mas CM, Østergaard L, Hart DJ. Auxin sensing is a property of an unstructured domain in the Auxin Response Factor ETTIN of Arabidopsis thaliana. *Sci. Rep.* 2018;8:13563.

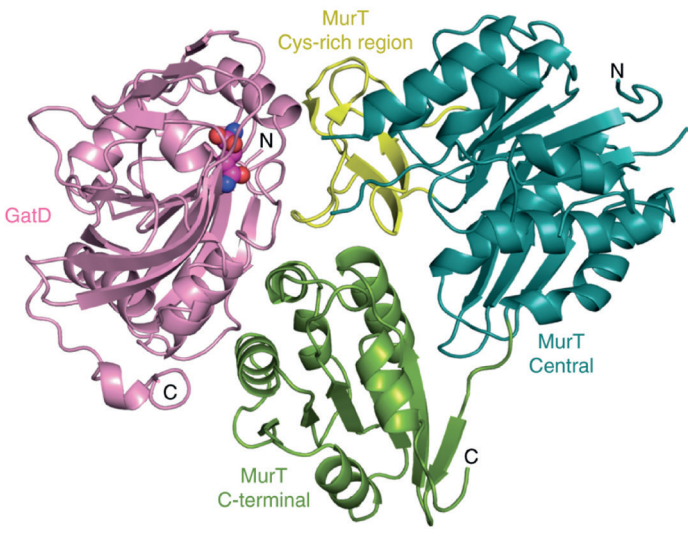
PID1876: MurT-GatD protein complex, a drug target in Staphylococcus aureus pathogenicity

Antibiotic resistant bacteria (ARB) pose a significant threat to global health, and considerable efforts are being made to develop new generations of antibiotics that combat ARB. Modifications of peptidoglycan (a mesh-polymer that surrounds some bacterial cells) offers a new antibacterial target against Gram-positive species such as Streptococcus pneumoniae, Staphylococcus aureus, or Mycobacterium tuberculosis. At the second residue of the precursor peptide, D-glutamate is amidated into D-iso-glutamine in many Gram-positive bacteria by the MurT-GatD amidotransferase complex. Genetic studies have shown that impairment of the MurT-GatD complex reduces the rate of cell growth and decreases resistance to -lactam antibiotics and lysozyme. In this proposal, the structure and functionality of the MurT-GatD complex was investigated in order to identify druggable binding sites to impair its function.

Publications:

Leisico F, Vieira D, Figueiredo TA, Silva M, Cabrita EJ, Sobral RG, Ludovice AM, Trincão J, Romão MJ, Lencastre H, Santos-Silva T. First insights of peptidoglycan amidation in Gram-positive bacteria-the high-resolution crystal structure of Staphylococcus aureus glutamine amidotransferase GatD. *Sci. Rep.* 2018;8:5313.

Morlot C, Straume D, Peters K, Hegnar OA, Simon N, Villard AM, Contreras-Martel C, Leisico F, Breukink E, Gravier-Pelletier C, Le Corre L. Structure of the essential peptidoglycan amidotransferase MurT/GatD complex from Streptococcus pneumoniae. *Nat. Commun.* 2018;9:3180.



RESEARCH AND DEVELOPMENT AWARDS

Research and Development (R&D) awards are small pump-priming grants evaluated by external peer review. The purpose is to obtain preliminary data that might lead to a conventional grant award from a major funder. Encouraged to be ambitious, they carry an award typically of €10000 for a project of 1 year duration, although the amount can be elevated at the discretion of the R&D Review Panel, if funds allow. Although the work can be undertaken in the applicant's own institution, awardees are encouraged to use Instruct technologies to take the work further than might otherwise be the case. This is managed through the access system, with proposals linked with an R&D award being fast-tracked. R&D awards are offered by a call once per year - these awards are very popular and heavily oversubscribed. Of 60 applications in the 5th call, seven awards were made and 13 proposals were recommended for access. In the previous call (4th call), nine of 51 applications were awarded and 18 were recommended for access. Most of these were carried out within the reporting period..

RESEARCH AND DEVELOPMENT HIGHLIGHTS

Hugo Fraga: R&D award APPID301

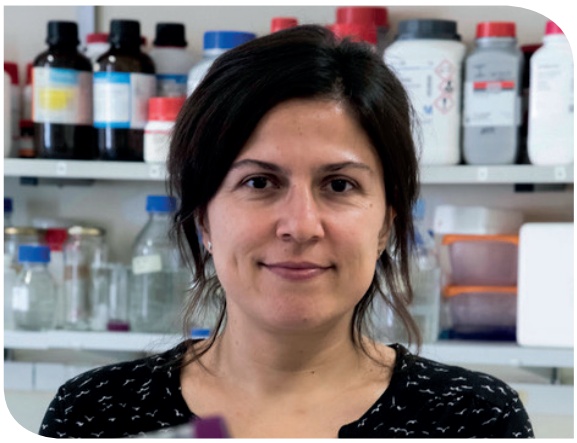
An Innovative EM/NMR approach for the characterisation of the drug target ClpP

As part of the R&D project, Hugo used Instruct Centre FR2 at Grenoble to study the proteolytic complex ClpP. Using an integrated approach that included X-ray crystallography, solid- and solution-state NMR, molecular dynamics simulations and isothermal titration calorimetry, Hugo and an IBS multidisciplinary team showed that the proteasome-inhibitor bortezomib binds to the ClpP active site serine mimicking a peptide substrate and induces a concerted allosteric activation of the complex. The bortezomib-activated conformation also exhibits a higher affinity for its cognate unfoldase ClpX. They propose an allosteric mechanism, whereby substrate binding to a single subunit locks ClpP into an active conformation optimized for chaperone association, as well as protein processive degradation.

Publications:

Fraga H, Arnaud CA, Gauto DF, Audin M, Kurauskas V, Macek P, Krichel C, Guan JY, Boisbouvier J, Sprangers R, Breyton C. & Schanda P. Solid-state NMR H–N–(C)–H and H–N–C–C 3D/4D C correlation experiments for resonance assignment of large proteins. ChemPhysChem. 2017;18:2697-2703.

Felix J, Weinhäupl K, Chipot C, Dehez F, Hessel A, Gauto DF, Morlot C, Abian O, Gutsche I, Velazquez-Campoy A, Schanda P & Fraga H. Mechanism of the allosteric activation of the ClpP protease machinery by substrates and active-site inhibitors. Sci Adv, 2019 in press.



Maria Jose Sanchez-Barrera: R&D award APPID99

The development of new drugs against autism: the Ca2+ sensor NCS-1 as a novel pharmacological target.

María José investigated the modulation of the complex between NCS-1 and Ric8a with small compounds to regulate synaptic function. A phenothiazine molecule, FD44, was identified to bind at the interaction surface of NCS-1 and Ric8a to inhibit the protein complex, reduce synapse number and increase associative learning in an autistic-like syndrome animal model.

Publications:

Mansilla A, Chaves-Sanjuán A, Campillo N, Semelidou O, Martínez-González L, Infantes L, González-Rubio JM, Gil C, Conde S, Skoulakis E, Ferrús A, Martínez A, Sánchez-Barrena MJ. Interference of the complex between NCS-1 and Ric8a with phenothiazines regulates synaptic function and is an approach for fragile X syndrome. Proc. Natl. Acad. Sci. USA 2017;114:E999-1008.

Roca C, Martínez-González L, Daniel-Mozo, M, Sastre, J, Infantes, L, Mansilla A, Chaves-Sanjuán A, González-Rubio JM, Gil C, Cañada J, Martínez A, Sánchez-Barrena MJ, Campillo NE. Deciphering the inhibition of the neuronal calcium sensor 1 and the guanine exchange factor Ric8a with a small phenothiazine molecule for the rational generation of therapeutic synapse function regulators. J. Med. Chem. 2018;6:5910-5921.

FD44 is the subject of a patent application (WO2017051046A1).

The review panel recommended 13 proposals that would benefit from the use of Instruct infrastructure through the access system. Applicants were offered access based on the review undertaken by the R&D panel.

The R&D calls are unrestricted in scientific focus, and in discussions held at the Strategic Meeting in Portugal in May 2018, Instruct agreed subsequent calls would be targeted to specific areas of scientific development need. From 2019, the R&D budget will be reassigned for Joint Research Awards (JRA) that develop new or improve existing access services delivered by Instruct to the user community. €80000 will be made available for JRA, with €10000 kept in reserve for an R&D call if the Executive Committee agrees.

The areas chosen for the first JRA call are:

1. Cryo-electron tomography: to move towards a robust and partially automated workflow for cellular cryotomography that can be made routinely available.
2. Automated screening for binding reagents (e.g. nanobodies): to determine the relative values of libraries obtained, for instance, from randomised synthesis, naïve animals and vaccination and to optimise production of reagents to extend the capability of cryo-EM.
3. Develop in-cell NMR to access readiness: to develop a standard workflow to provide routine user access to a flow bioreactor system that improves the stability of in-cell NMR samples and allows real-time measurements of cellular processes.

TRAINING

The Instruct training programme helps researchers to access new methods and improve their skills. It includes structured courses, internships and online webinars, along with access to tools, resources and expertise. Through visits to Instruct Centres via the access programme, users also receive on-site training and mentoring in new methods to immediately benefit their own research.

Instruct Training Courses

In total, seven courses and workshops were delivered during the reporting period. Participants cover a broad geographical area, with the UK showing the highest percentage of participation (16.7% of attendees), with Spain (14.1%) and France (8.3%) following in rank order. In total, 32 countries have been represented; 17 EU member states and 15 external (Australia, Belarus, Brazil, Canada, China, India, Israel, Japan, Russia, South Africa, South Korea, Switzerland, Tunisia, Ukraine, USA).

Instruct Centres that have hosted at least one training course in the period are Czech Republic (2), Israel (1), Netherlands (1), Spain (2), UK (1).

FIG 1

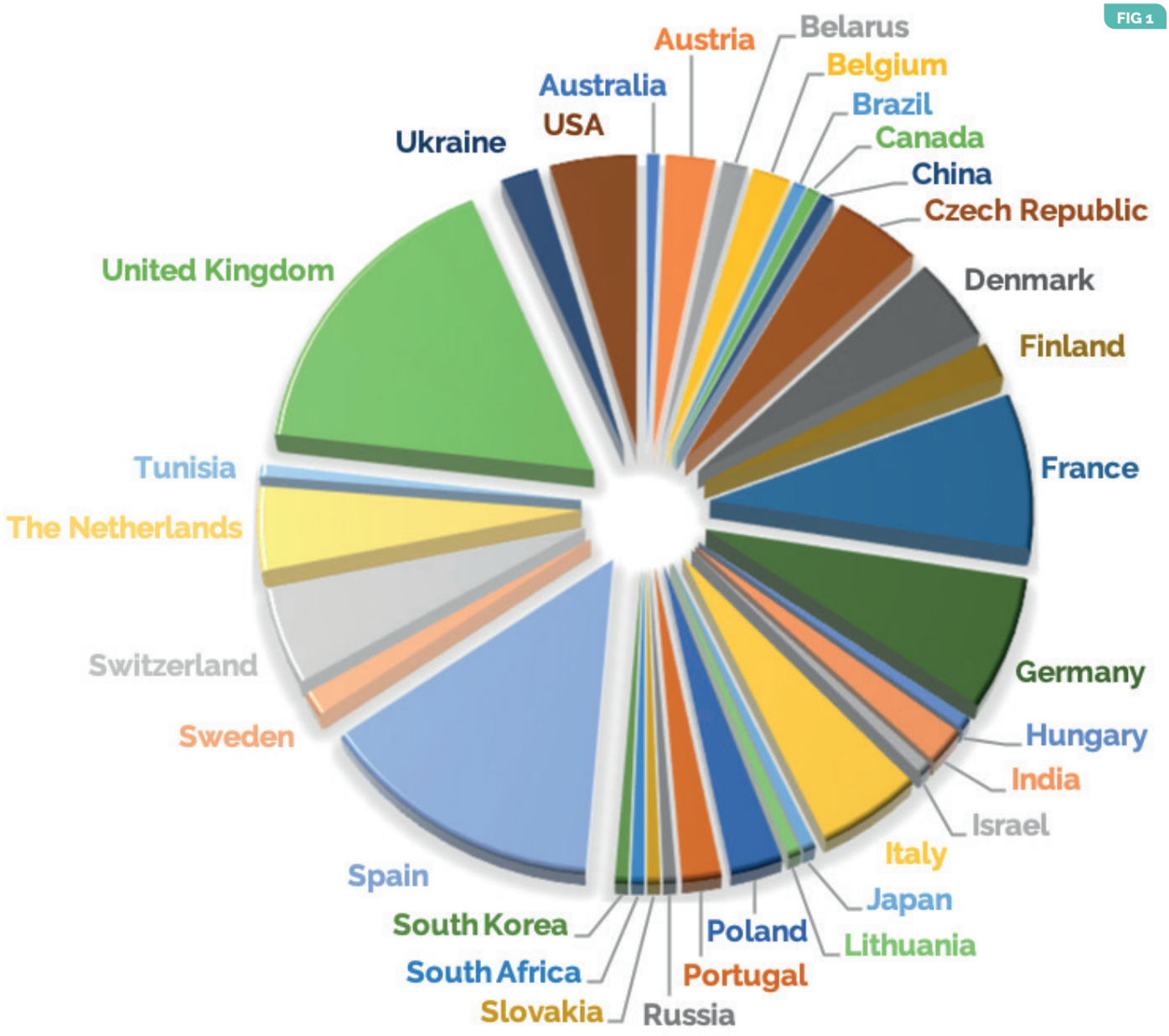


FIG 1. Training course participants by the country of their home institution.

INTERNSHIPS

Internships for students and young researchers support short-term (3-6 months) exchange programmes at an Instruct Centre. This programme provides in-depth skills training along with valuable experience of a different scientific environment including standards, processes and culture, and is hosted from a Centre that specialises in a method or technology from which the intern's own research can benefit.

Eleven internships were awarded, with a geographical distribution of Centres hosting the awardees. There is an annual call for internship applications that is assessed by the Training Committee.

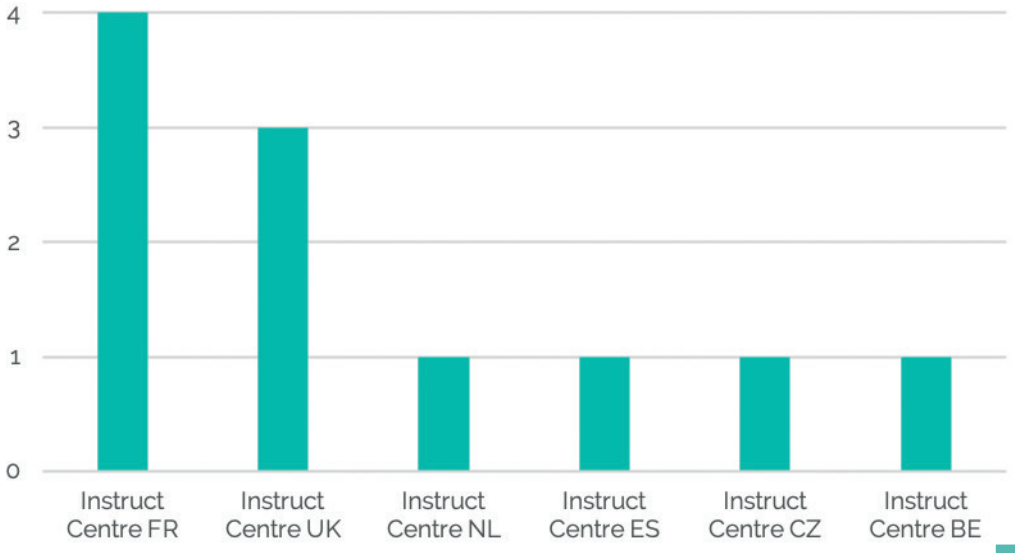


FIG 2

FIG 2. The number of interns hosted by each Instruct Centre.

INTERNSHIP HIGHLIGHT

Ritu Rathi: Internship APPID 577 and Access PID5260

More than 600 human E3-ligases specifically determine the ubiquitylation of about 50,000 human protein targets and consequently regulate most cellular pathways. But what regulates these E3s? Recently, a self-ubiquitylation dependent allosteric mechanism was found to regulate members of HECT E3 ligase family. The discovery was made by employing an E. coli-based expression/purification system to purify several ubiquitylated HECT E3s. During the internship, Ritu used a structural biology approach to elucidate the allosteric regulatory mechanism of these HECTs, and also contributed to a review on ubiquitin signaling and degradation of aggregate-prone proteins in normal and diseased states.

Galves M, Rathi R, Prag G, Ashkenazi A. Ubiquitin Signaling and Degradation of Aggregate-Prone Proteins. *Trends Biochem. Sci.* 2019.

WEBINARS AND PODCASTS

Training webinars are available on YouTube, via the Instruct website, for training in the use of ARIA. In addition, a number of webinars on the use and application of computational tools can be accessed through the Instruct website.

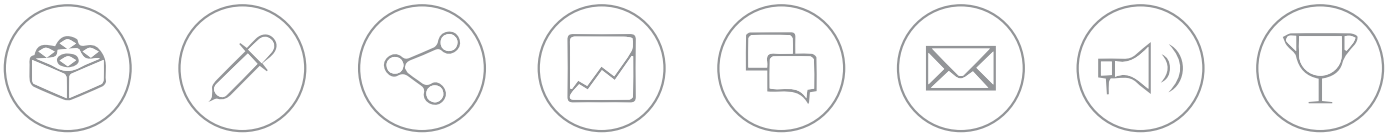
Podcasts on areas of relevance to Instruct or advances in structural methods are also accessible from the Instruct website.

View our webinars and podcasts.

ARIA is an integrated cloud service designed, built, and provided by Instruct to research infrastructures, facilities and user communities. As a cloud service, ARIA has the opportunity to centralise access offerings from multiple biomedical science research domains in order to provide cross-disciplinary scientific proposals and truly integrative science.

ARIA hosts a huge number of tools targeted at all stages of scientific workflows, with the original flagship product built to deliver access proposal management for research infrastructures. Since the initial launch it has been further expanded to support and integrate facility management with a powerful visit management workflow and machine booking calendar. Additionally there are a suite of tools for managing an online website and presence for communities and infrastructures with support for news, events, jobs, forums and custom page editors. All of these tools can be completely white-labelled to be included seamlessly into an existing website or brand presence.

Access Management



Over the past year we have seen a huge increase in ARIA adoption throughout the biomedical research community, with a range of new infrastructures and projects taking advantage of the high quality service and tools offered by ARIA. 6 new agreements with infrastructures to provide access management have been negotiated, bringing new revenue streams for the team to grow and further support the platform.

Facility Management

The ARIA team has grown significantly to deliver new functionality and features in context of the CORBEL and EOSC-Life projects. Internally there has been new management frameworks and structures put in place to further improve the dependability of the service and tighten release management to ensure that ARIA continues to provide an essential and resilient service to its consumers.

Community Hubs

In the coming year development will be focussed on extending existing functionality to enhance the usability of the system and ensure that it supports users in their native way of working. Additionally, there is planned support for quality management frameworks for facilities with checkpointing and ISO compliance. In the context of EOSC-Life, new central identity management will be integrated as the production service of LifeScience ID comes online along with further engagement with other research infrastructures to offer ARIA through other channels. Lastly, specifications for the next major version of ARIA, Version 3, are being drafted to take advantage of modern technologies (both browser and infrastructure) to allow continued growth of the platform.

Data Access APIs

TESTIMONIAL

ARIA has a number of users around the world who represent key organisations within structural biology.

Frauke Leitner - CORBEL Project Manager

We were charged with the exciting task to organise an Open Call for projects, spanning access to services and technologies offered by ten European research infrastructures. Without ARIA, the management of user applications and reviews across so many partners would have been close to impossible. Thanks to the great support by the ARIA team, we managed it successfully!

IMPACT

Key metrics demonstrate impact in our community and to wider stakeholder communities. Keyword data mined from publication titles and abstracts show the predominant targets where structural studies have contributed information (Fig 1). Data drawn from the participation at Instruct events show the complex interconnections established through Instruct activities (Fig 2). Likewise, similar data from Instruct-ULTRA activities highlight the impact in establishing new community involvement from Latin American institutions as part of the outreach programme (Fig 3).

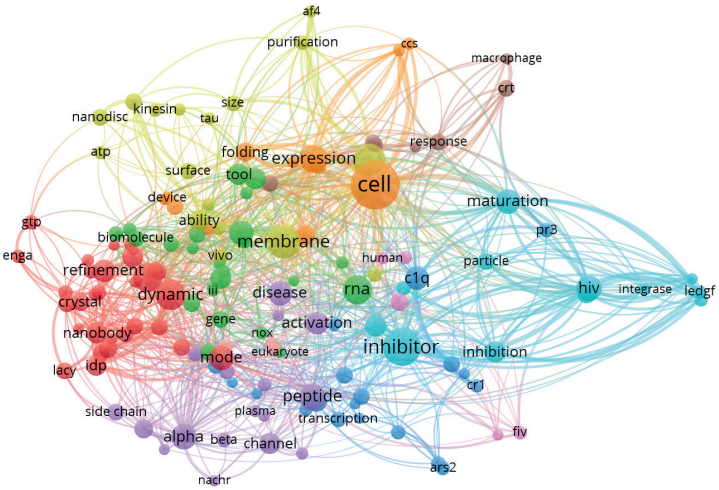


FIG 1

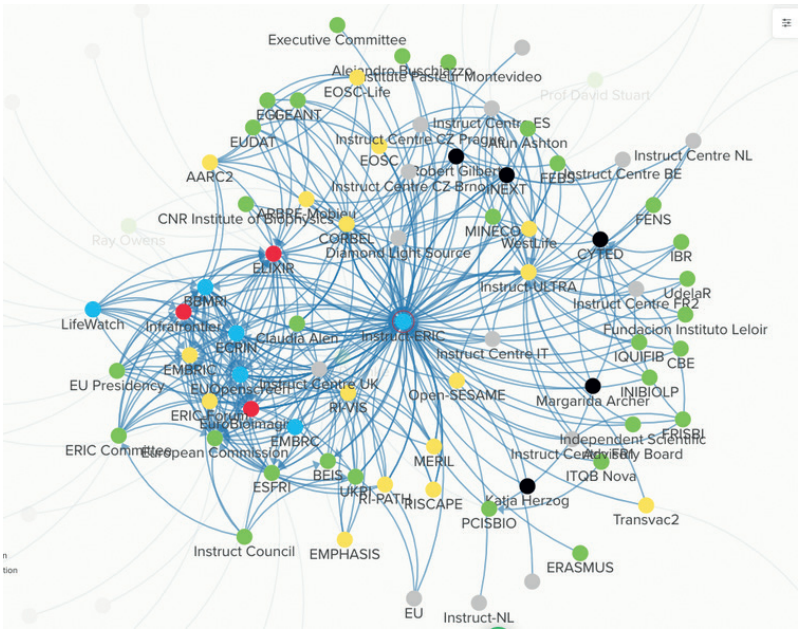


FIG 2

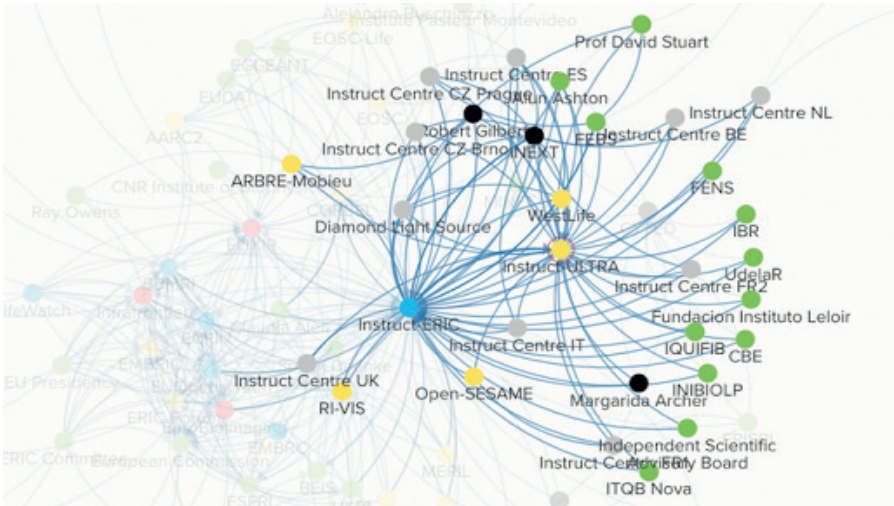


FIG 3

FIG 1. The occurence of keywords from the titles and abstracts of publications during the reporting period.

FIG 2. Network connections between participant organisations and key individuals that have been involved in providing Instruct-ERIC services and events.

FIG 3. Network connections centred around Instruct-ULTRA show specific involvement of Latin American institutions as part of the outreach programme.

INTERNATIONAL COLLABORATION

Instruct aims to increase its international outreach with the ultimate prospect of recruiting Member states as third-party countries from outside of Europe. Instruct-ULTRA has effort dedicated to this, and has helped to consolidate scientific links in Latin America, where interactions were initiated prior to the launch of Instruct-ERIC using funds secured from a British Council grant.

Instruct-ERIC and Instruct-ULTRA scientists launched discussions with CeBEM, the Structural Biology Centre of MERCOSUR (the South American common market). CeBEM integrates nine major centres of the region from Brazil, Argentina and Uruguay, that already have a strong profile in structural biology and protein science. Instruct aims to expand into the structural biology community in Latin American, with CeBEM acting as a point of contact and validator of potential partner institutions.

Preliminary discussions have been held with the following institutions in Argentina: Universidad Nacional de La Plata (Buenos Aires), Instituto Leloir (Buenos Aires), Universidad Nacional de La Plata (Buenos Aires) Instituto de Biología Molecular y Cellular de Rosario and Universidade Federal de Minas Gerais in Brazil. MOUs defining joint activities are in preparation with these groups. These were followed by a visit to Instruct UK by Alejandro Buschiazzo (University of Sao Paulo, Brazil) in September 2017.

A number of MOUs have been established and approved by the Instruct Council:

Country	Institution	Date Signed	Duration
Uruguay	Institute Pasteur (Montevideo)	17/02/16	5 years
Argentina	Leloir Institute, Buenos Aires	01/03/16	5 years
Argentina-Brazil-Uruguay	CeBEM	30/10/18	5 years
Brazil	University of São Paulo	21/09/18	5 years
Uruguay	Universidad de la Republica (Montevideo)	01/03/19	5 years

A foresight meeting to discuss strategies for internationalisation was held in March 2018

A call for a project entitled "Expanding cryo-electron microscopy to Latin America" was launched in March 2018 as part of Instruct-ULTRA Work Package 4. The project was supported by Jose M. Carazo (CNB-CSIC and Instruct-ES) and Margarida Archer (ITQB and Instruct PT), in close collaboration with Bruno Klaholz (IGBMC and Instruct FR1). The project work will begin in 2019.

Links for further information

University of Sao Paulo:

http://www5.usp.br/english/?lang-en

University of the Republic of Uruguay:

https://www.fcien.edu.uy

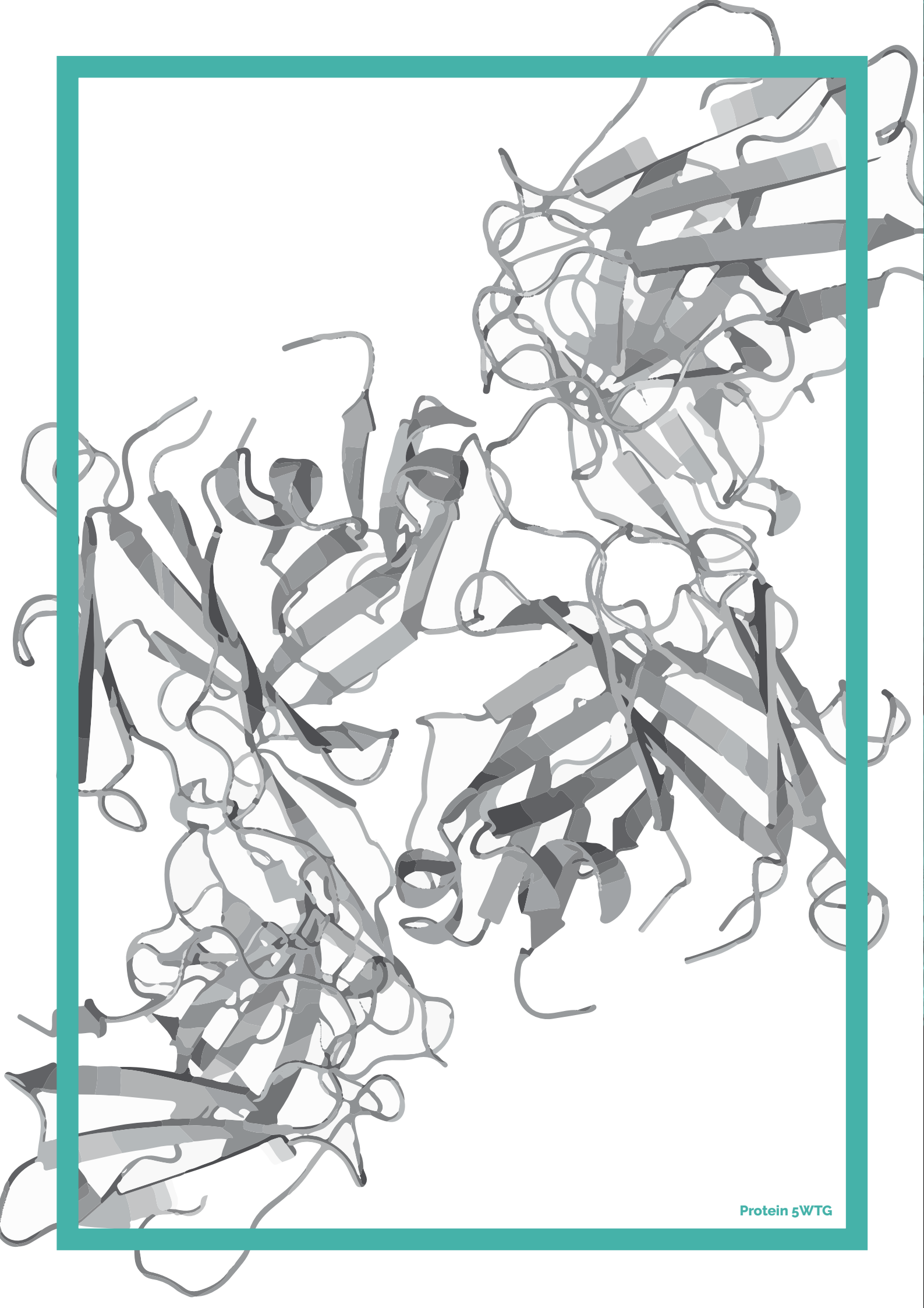
MERCOSUR Centre for Structural Biology (CeBEM):

http://www.cebem-lat.org/en/general/quienes/

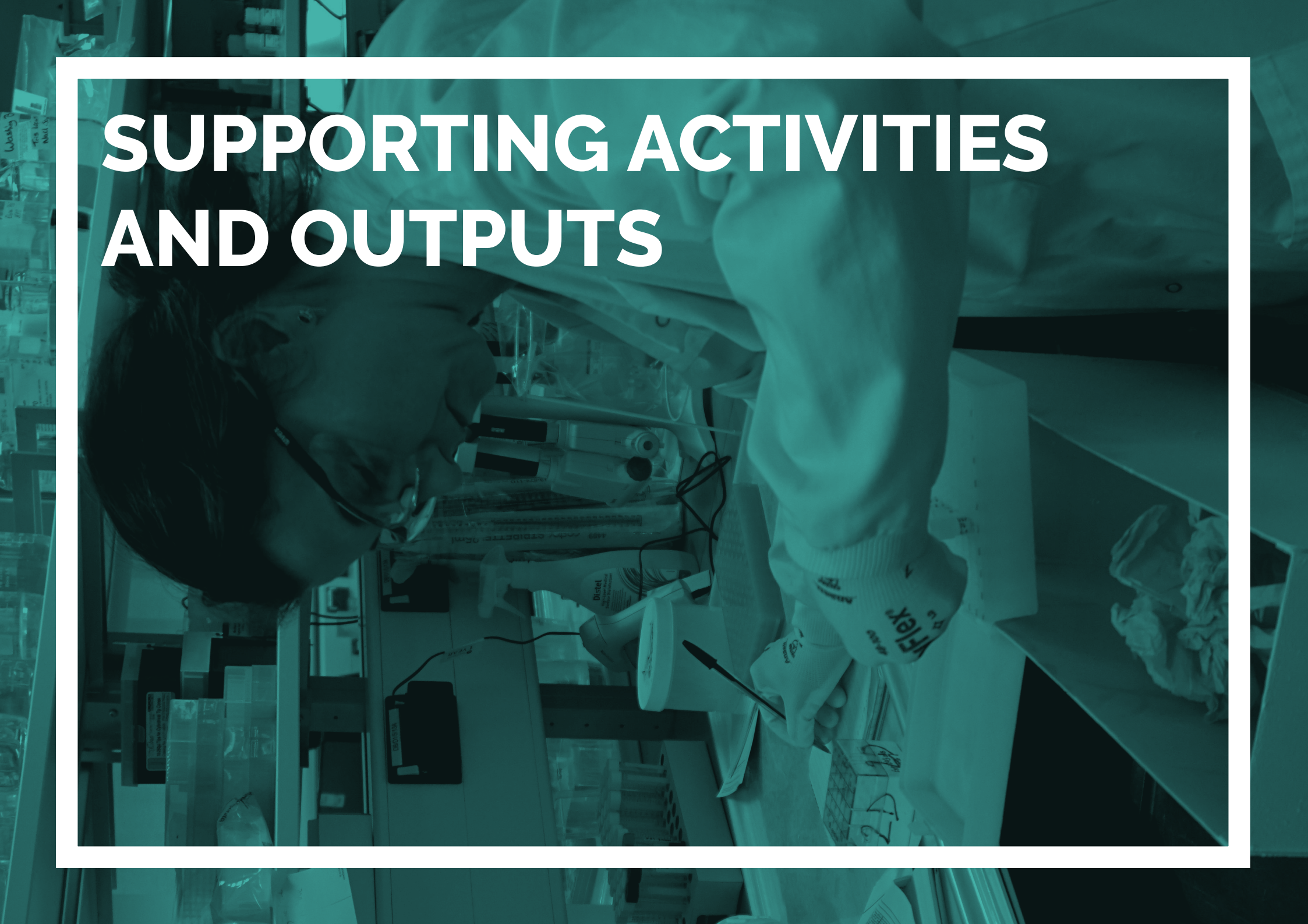
Engagement with other international regions is less well developed than with Latin America. South Africa has a well-developed but relatively limited structural biology community that has recently been boosted by investment for cryo-EM instruments. Some discussions have been initiated and supported by a workshop "Training the next generation of structural biologists in South Africa", held jointly with the Diamond Light Source and supported by Instruct-ULTRA in University of Cape Town in January 2019. Workshops such as these are particularly significant in building up a global structural biology community, and are a key step in bringing access to the Instruct infrastructure and expertise to new regions.

FIG 1

FIG 1. Map highlighting collaborations with Latin America.


Protein 5WTG

SUPPORTING ACTIVITIES AND OUTPUTS



COMMUNICATIONS

Website

The layout, design and content of the Instruct-ERIC website were revised and the new website launched in November 2018. Principal design changes have simplified the navigation menu tree, implemented the revised catalogue of access services, brought the news and Twitter feed to the front page to enable a live turnover of current news, and introduced a snapshot of Instruct activity in a banner at the bottom of the homepage.

The content was reviewed and updated throughout, and subtle but important changes were made to the page design. Feedback on the new design from users was positive.

Analysis of the Instruct-ERIC website show a slow but steady increase in visits to the site, with peaks of up to 840 unique visits per day.

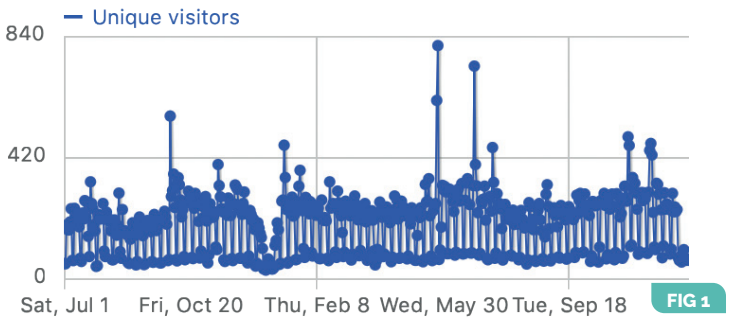


FIG 1

Webpage traffic monitoring shows that most visits come through other internal pages, or by direct entry URLs. The most popular single page views are for instrument booking and the Instruct access dashboard.

FIG 1. The number of unique visitors to the Instruct-ERIC website per day, from 1 July 2017 to 31 December 2018.

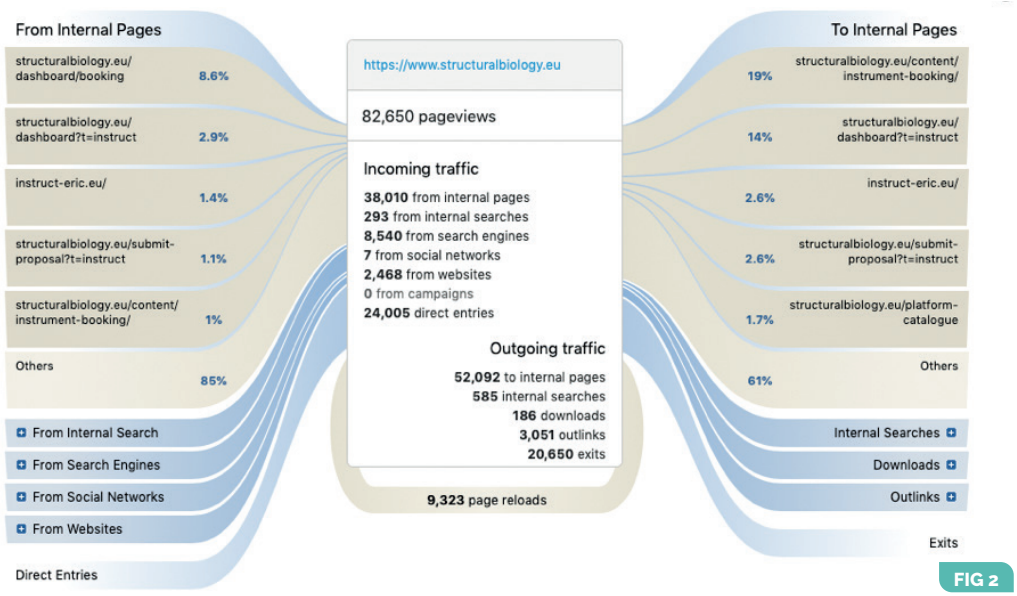


FIG 2

FIG 2. The traffic within the Instruct-ERIC website.

Conferences and Workshops

Instruct-ERIC was present at many conferences, workshops, panel discussions and events, where the Instruct banner, posters and literature were made available. The Instruct flyer was revised for distribution and the new brochure will follow in 2019.

Cross-disciplinary communication was strengthened by our participation in the Exchange of Experience workshop in India, 15-19 September 2017, and staff exchanges: Instruct manager Claudia Alen to EMBRC (CCMAR), 17-19 September 2017, and EU-Openscreen European Relations and Grant Officer Katja Herzog to Instruct, 14-17th August 2018. We were also represented at the CORBEL Quality Management Expert meeting hosted by Infrafrontier in Munich and the MERIL-Interoperability Workshop in Athens.

SOCIAL MEDIA

Instruct-ERIC continues to increase its presence on Twitter with more than 7500 tweets and 2500 followers. This medium is increasingly effective to provide instant information on breaking news from meetings and conferences, and Instruct-ERIC is widely re-tweeted.

Alice Clark @AtomsAlice • Sep 17

Many thanks to the wonderful Dimple Karia and to @instructhub for collecting me a lovely dataset on the Krios @ STRUBI over the weekend. This is a really amazing resourse for those of us from smaller Uni's withouth big microscopes.

Chris H Hill @chillzaa • 27m

Access to phase-plate data collection on the Titan Krios @IGBMC through @instructhub was a crucial part of our work. The application/ review process was very quick. Highly recommend!

EU-OPENSCREEM ERIC @EuOpenscreen • Oct 26

@CORBEL_eu enables staff exchanges between #EU_RIs to exchange knowledge and operations. @instructhub welcomed @ EuOpenscreen colleague Katja Herzog for a few days in Oxford in August. Picture: Chrust Church College, Oxford, Uk

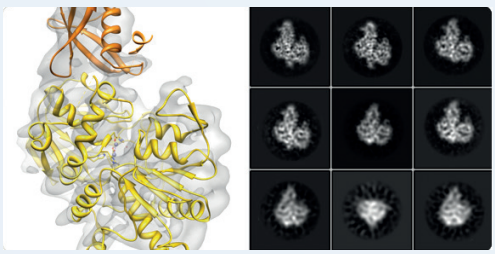
Ehmke Pohl @ehmke_pohl • 4h

Congrats - look at the diversity of the line-up - well done, what a fanstastic program !

Instruct-ERIC @instructhub
Submit your abstract to #IBSBC2019 bit.ly/2FERZhW be one of the 4 selected as speaker to join our great line up @SjorsScheres Sali @UCSF Yonath @WeizmannScience @AndyDor2 Pereiro ...

lori Passmore @lapassmore • 13h

Also determined a cyro-EM structure of this part of the complex - only 57 kDa @instructhub @IGBMC



CNB @CNB_CSIC • 17 OCT 2017

Replying to @Instruct2PC @instructhub @iNEXT_H2020

The story, now in English:

Building small self-assembling protein cages
A new method allows the design and construction of nanometric cages using proteins. The structures are able to self-assemble inside cells and living b...
cnb.csic.es

New Suite5 Analytics on MERIL portal's use

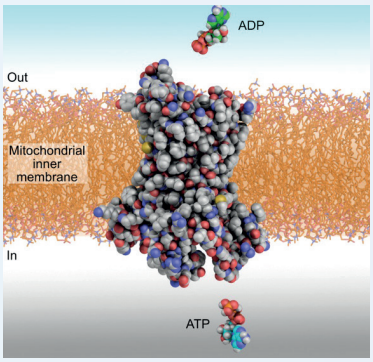
Meril is now receiving Suite5 anonymised analytics to learn more about MERIL users, their interests and search behaviour when browsing on the portal. From analysing the user behaviour, in particular we are hoping to gain more information about how to optimise the portal and make i more user-friendly.

Since September 2018, the MERIL portal has had over 16,000 clicks and over 4,000 search page views. Our MERIL users come from 81 different countries all over the world.

Below an example of the generated work cloud showcasing the most searched keyworks on the MERIL portal in the last three months:

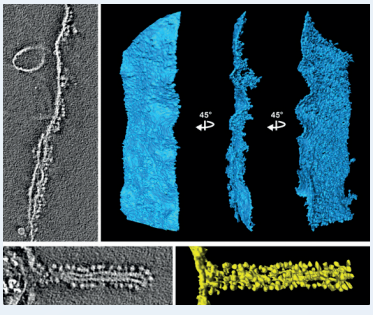
VIB @VIBLifeSciences • 16h

Important new insights in the molecular mechanism of transport by the mitochondrial ADP/ATP carrier published in @CellPressNews made possible thanks to different kinds of support from - amongst others - @instructhub and @FWOVlaanderen



Ben Engel @bengeliscious • Jan 4

After years in limbo, my @instructhub collaboration with Andrea Chicano, Eva Crosas, Joaquín Otón, @melero-roberto, and Joan-Ramon Daban is online! Im curious what people think about the controversial multilayered plate structure of metaphase chromatin



Chris Toseland @Chris Toseland

@AliaSantos and @RosieGough1 from the lab are over @NeCEN_2p0 optimising our CryoEM run. Thanks to @instructhub for funding!

SUSTAINABILITY

Instruct-ERIC has participated in a number of surveys from ESFRI, the European Commission, other research infrastructures and various evaluation projects in order to identify the metrics by which impact and sustainability can be measured. Instruct is also a member of the ERIC Forum, and hosted the 2nd ERIC Forum meeting in Oxford in April 2018.

FIG 1. Group photo from the 2nd ERIC Forum meeting in Oxford, April 2018.

Instruct attended the 3rd ERIC Forum in Seville in November 2018 and is a long term member of the CORBEL Biomedical Sciences Research Infrastructure Strategy Group and the Medical Infrastructure Users Forum. Instruct-ERIC is a signatory to a number of position papers that define the working practices of the research infrastructures in the biomedical domain, and views on evaluation processes applicable to these RIs.

EUROPEAN GRANTS

External grant income for Instruct came from EU H2020 grants, of which Instruct coordinates (leads) two and participates in a further nine. Coordination, project management and administration for these grants is all handled by Hub staff in the UK.

Project grants held during the 2017-18 reporting period include:

Project	Total Project Award	Instruct Award	End Date
West-Life	€3981125	€152750	31/10/18
iNEXT	€9999534	€128500	31/8/19
AARC2	€2999893	€8710	30/4/19
Open-SESAME	€1957324	€61563	31/12/19
CORBEL	€14837800	€563000	31/5/20
Instruct-ULTRA	€3950000	€419088	31/12/20
Transvac2	€10599993	€29260	30/4/22
Total	€48325669	€1362871	

New Grants Awarded	Total Project Award	Instruct Award	Start Date
RI-VIS	€1500000	€293781	Start 1/2/19 (30 months)
ERIC Forum	€1495281	€43300	Start 1/1/19 (48 months)
EOSC-Life	€23745996	€358051	Start 1/3/19 (48 months)
Total	€26741277	€695132	

Instruct-ULTRA: An implementation grant to help develop and consolidate Instruct-ERIC.[1]

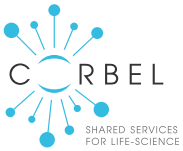
Instruct-ULTRA is developing relationships with new communities by targeted interactions with industry and non-EU countries and organisations. Development of potential new infrastructure services for the Instruct catalogue is a major aim of one of the work packages. Instruct-ERIC coordinates Instruct-ULTRA.

RI-VIS: Increasing the visibility of Research Infrastructures.[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. Instruct-ERIC coordinates RI-VIS.

West-Life: A Virtual Research Environment for Structural Biology (completed).

Now completed, West-Life delivered a comprehensive toolkit for data management and analysis by structural biology researchers through a single web-interface. access to West-Life tools and services is now available through Instruct.



CORBEL: Developing shared services for Life Science.

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: (New award) European Open Science Cloud for Life Sciences.

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 will finish 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.

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

SCIENTIFIC PUBLICATIONS

Peer reviewed publications are the currency of excellent science and a key metric of success for Instruct-ERIC. The number of publications acknowledging the use of Instruct infrastructure (via access projects, internships, R&D awards) is shown in Fig 1. Growing year on year, the number now exceeds 800 publications. In this reporting period, 45 were published in a journal of impact factor greater than 10 (see list below). All users of the Instruct infrastructure are required to cite Instruct's contribution in presentations and publications.

The relational diagram (Fig 2) shows the proportionate contribution to publications from Instruct Centres and their supporting academic institutions and funding bodies, based on acknowledgement.

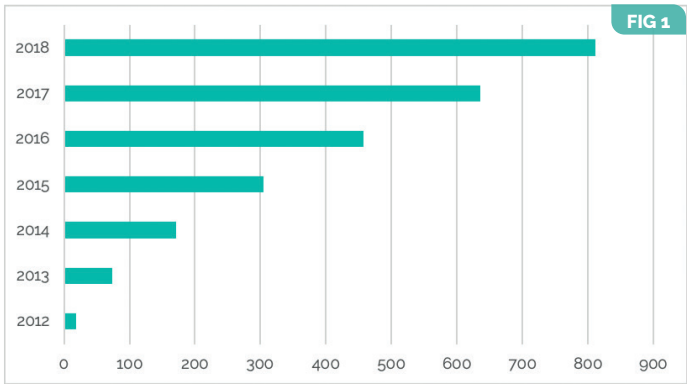


FIG 1. Cumulative publications between 2012 - 2018.

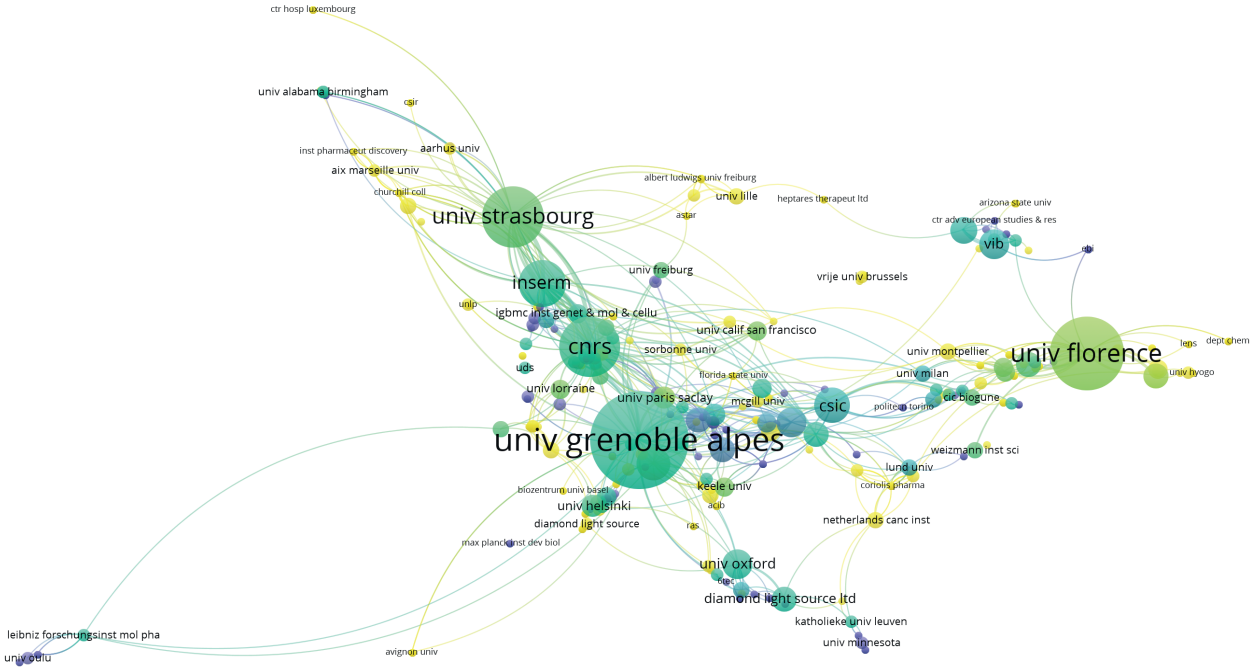


FIG 2. The contribution and relationships of Instruct Centres, supporting academic institutions and funding bodies to Instruct publications, based on acknowledgement.

PUBLICATIONS BY JOURNAL IMPACT FACTOR (JIF > 10)

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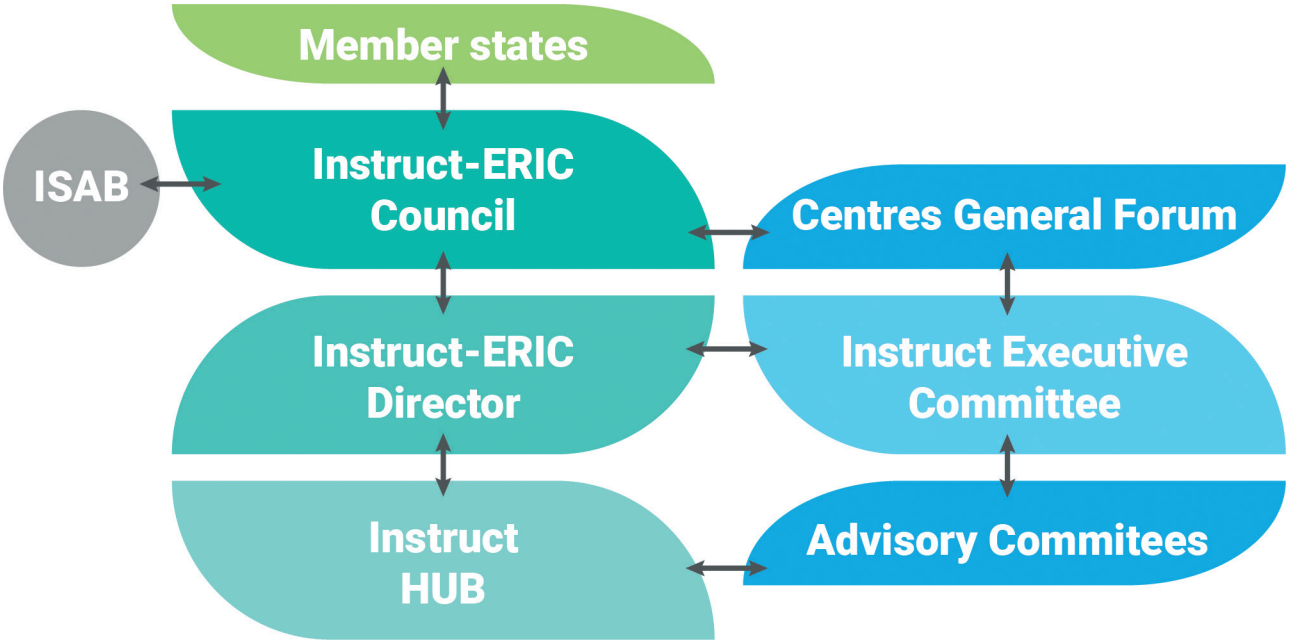
GOVERNANCE



Instruct was implemented as a European Research Infrastructure Consortium (ERIC) on 4 July 2017. Instruct-ERIC was set up in accordance with the Council Regulation (EC) No 732/2009 of 25 June 2009 on the Community legal framework for a European Research Infrastructure Consortium. Instruct-ERIC was implemented with 10 founding Member States and its operational oversight is governed by its statutes, published in the Official Journal of the European Union 2017/C 230/01.

The Instruct-ERIC Council comprises representatives of all the Instruct-ERIC Member States and monitors compliance with statutory elements and achievements against objectives. The Director, with advice from the Independent Scientific Advisory Board (ISAB), sets the strategic scientific priorities that are executed by the Instruct Centres and monitored by the Executive Committee. Coordination of these governing and monitoring bodies is achieved through the Hub. A number of sub-committees provide information and advice as required.

The Executive Committee manages the scientific evaluation of new Centres and the addition of new facilities to existing Instruct Centres, with the Council providing the final decisions based on a recommendation from the Executive Committee and their own judgement on the strategic benefit to Instruct. Scientific review of new Centres is undertaken by the ISAB which can be supplemented with additional specialists as required.

Both Council and the Executive Committee own and monitor risks associated with their respective responsibilities.

Instruct-ERIC has a voice on various panels to bring awareness of Instruct to policy makers and funders and to extend its membership and community impact. Instruct personnel are members of the following:

- ERIC Forum
- CORBEL Biomedical Sciences Research Infrastructure Strategy Group
- ESFRI Health and Food Strategic Working Group

Instruct-ERIC Lead scientists interface with their national policy makers, funders and infrastructure organisations to provide information on how Instruct serves the structural biology community and its ambitions for the future.

INSTRUCT-ERIC GOVERNING BODIES AND COMMITTEES

COUNCIL

CHAIR: Dr Nathan Richardson, UK
VICE-CHAIR: Dr Laurence Lenoir, BE

Country	Scientific Delegate	Administrative delegate
Belgium	Michele Oleo	Laurence Lenoir
Czech Republic	Vladimir Sklenar	Jan Burianek
Denmark	Thomas Vosegaard	Troels Rasmussen
France	Winfried Weissenhorn	Eric Guittet
Israel	Joel Sussman	Ilana Lowie
Italy	Lucia Banci	Grazia Pavoncello
Latvia	Kaspars Tars	Janis Paidus
Netherlands	Reinout Raijmakers	Marijn Goes
Portugal	Margarida Archer	Maria Armenia Carrondo
Spain	Jose Maria Carazo	Inmacculada Figueroa
Slovakia	Milos Hricovini	Lukas Zendulka
United Kingdom	Anne McGavigan	Nathan Richardson
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 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

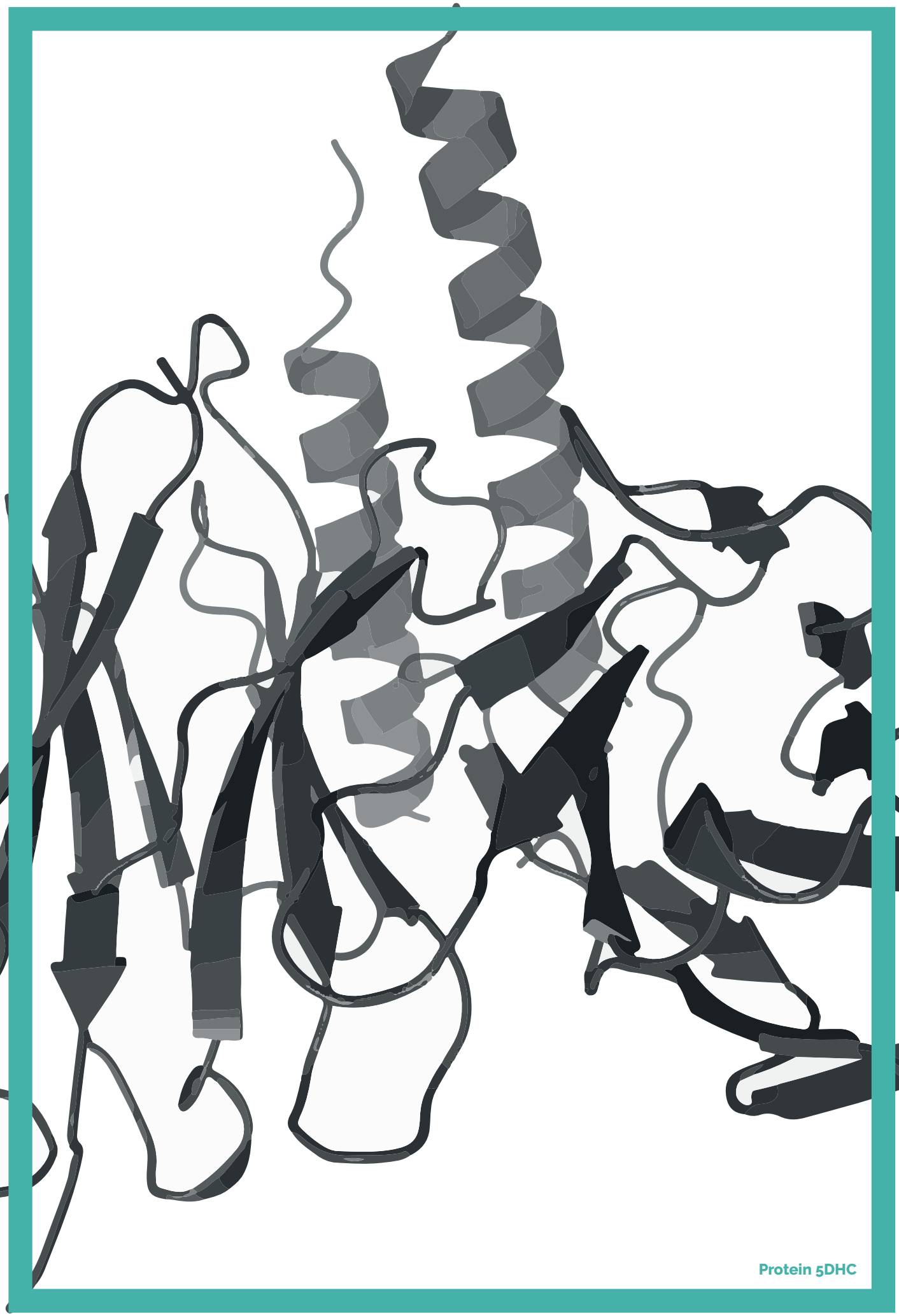
Independent Scientific Advisory Board

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

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

Training Committee: Chair - Lucia Banci
Access Committee: Chair – Darren Hart
Business Working Group: Chair – Ondrej Hradil
Data Management Committee: Chair – Jose Maria Carazo


Protein 5DHC

FINANCIAL DATA

FINANCIAL DATA

This report presents the financial statements for the period 1 August 2017 to 31 December 2018 and was approved by Council on 16 October 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 are required to select suitable accounting policies and then apply them consistently:

Select suitable accounting principles and then apply them consistently;

Make judgements and estimates that are reasonable and prudent;

State whether UK Accounting Standards have been followed, subject to any material departures and explained in the financial statements;

Assess Instruct-ERIC's ability to continue its activities, disclosing as applicable matters related to financial resilience;

Use 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 adequate accounting records that 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 are responsible for such internal control as they determine is necessary to enable the preparation of financial statements 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 transition from 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.

This report covers the period from the start of Instruct-ERIC as the legal entity, at which point there was a period of transition of operations from IASL to the ERIC which continued through to July 2018. IASL presented financial results to 31 July 2018 to the IASL Board on 20th November 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

BALANCE SHEET FOR INSTRUCT-ERIC

As at 31 December 2018

Assets	GBP	EUR	Notes
EURO BANK	593,284	668,598	
STERLING BANK	26,736	30,130	
Total Bank	620,020	698,728	
Current Assets			
Accounts Receivable	558,430	629,320	1
Accrued income	387	436	
Grant accrued income	18,998	21,410	2
Total Current Assets	645,573	727,525	
Fixed Assets			
Computer Equipment	3,731	4,204	
Depreciation on Computer Equipment	(358)	(404)	
Depreciation on Office Equipment	(594)	(670)	
Office Equipment	3,566	4,019	
Total Fixed Assets	6,344	7,149	
Total Assets	1,271,937	1,433,403	

Liabilities	GBP	EUR	Notes
Current Liabilities			
Accruals for Internship	10,756	12,121	3
Accruals for R&D awards	68,426	77,113	4
Accruals for Training	8,791	9,907	5
Accruals for recharges	19,622	22,113	
Amount due to IASL	28,433	32,043	
Accruals for Access awards	29,419	33,153	6
Amounts to be paid and Unclaimed Access Awards	82,393	92,852	7
Access Awards			
Income in Advance - Members subscription	71,858	80,980	8
Income in Advance - Other inc deferred grants	387,824	437,056	9
Payroll taxes due	749	844	
Pensions due	142	161	
Total Current Liabilities	1,266,845	1,427,664	

Liabilities	GBP	EUR
Total Liabilities	1,266,845	1,427,664
Net Assets	**5,093**	**5,739**
Equity		
Surplus for the year	5,093	5,739

Exchange rate for reporting period: 0.887354909

Approved by the Council Members representative and signed on behalf of the Council

Professor David Stuart 19 December 2019

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. Brought forward from IASL 2017
7. Access and other service accruals
8. Invoiced deferred membership subscriptions
9. Deferred project income

PROFIT AND LOSS FOR INSTRUCT-ERIC

For the period 1 July 2017 to 31 December 2018

Income	GBP	EUR	Notes
External grant income	110,205	124,196	10
Member state contributions	948,378	1,068,770	11
Other miscellaneous income	12,656	14,262	12
Total Income	1,071,240	1,207,228	
Less Cost of Service Provision			
Instruct staff salaries	202,177	227,842	
R&D Pilot awards	6,959	7,842	
Access Cost	(25,286)	(28,496)	13
Instruct Centre Costs	455,765	513,622	14
Meetings (organisation, venue, travel, catering, materials)	32,457	36,577	
Project activities	311,568	351,119	15
Total Cost of Service Provision	983,639	1,108,507	
Gross Surplus	87,601	98,721	
Less Operating Expenses			
Commissioned services (Insurance, financial, HR, legal)	36,624	41,273	
Consultants	12,403	13,978	16
Depreciation charge	953	1,073	
Foreign Currency Gains and Losses	(16,084)	(18,126)	
General admin (postage, copying, bank charges)	1,905	2,147	
Irrecoverable VAT	951	1,072	
Licenses & software	2,118	2,386	
Miscellaneous	751	847	
Office Stationery	668	753	
Premises and support	39,711	44,752	
Publicity (posters, flyers, banners etc.)	2,247	2,532	
Telephone	262	295	
Total Operating Expenses	82,508	92,982	
Net Surplus	5,093	5,739	

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

11. Membership income receivable

12. Sponsorship

13. Access released – not claimed (credit)

14. Recharges from IASL for services that were delivered to IASL and charged across to ERIC

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

16. IT, website and API work by IT consultant

Approved by the Council Members representative and signed on behalf of the Council

Professor David Stuart 19 December 2019

SUPPORTING INFORMATION FOR THE FINANCIAL STATEMENTS

Accounting Policies

The financial statements are prepared under the historical cost convention, and in accordance with the Statutes of Instruct.

The principal accounting policies set out below have, unless otherwise stated, been applied consistently to all periods presented in these financial statements.

Reporting and Disclosure Exemptions

Going concern

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

Expenditure

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

Foreign Exchange

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

Corporation Tax:

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

Basis of preparation

The financial statements have been prepared in accordance with applicable United Kingdom accounting standards, and under the historical cost accounting rules used and approved for IASL.

Income

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

Depreciation

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

Taxation

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

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

Cash and cash equivalents

Cash and cash equivalents comprise cash balances and call deposits.

ACCOUNTING JUDGEMENTS AND ESTIMATES

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

Judgements: Grant Income recognition

Estimations, uncertainties and assumptions: Going concern

B. Income

List of Members and their cash contribution (EUR)

Member Country	Invoiced 1/8/17 - 31/12/18	Payment received	Invoice adjustment
UK	142,500	141,667	833
FR	142,500	141,667	833
ES	75,000	75,000	0
IT	106,875	106,250	625
BE	106,875	106,250	625
NL	106,875	106,250	625
IL	106,875	106,250	625
CZ	71,250	70,833	417
PT	71,250	70,833	417
DK	71,250	70,833	417
SK*	50,417	51,000	(583)
LV**	0	0	0
Total	1,051,667	1,046,833	4,834

Grant Recipts

EU Grants	Income Aug '17 - Dec '18	Overhead Contribution Recognised
Instruct-ULTRA	148,427	37,107
iNEXT	45,510	11,378
West-Life	63,645	15,911
CORBEL	167,912	41,978
AARC2	6,171	1,543
Open-SESAME	29,071	7,268
Transvac2	8,398	2,100
Total	469,134	117,285

Overhead contribution recognized: 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. Instruct-ERIC supported three direct employees of the University of Oxford at 31 December 2018. 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) €629319.79

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 €80,980

Advances on Research Grants €437,055.74

Payroll taxes and pensions €1,005.48

G. Reserves

To be determined.

H. 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."

I. 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.

J. 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.

K. 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

New awards:

EOSC-Life: €358,051 – projected to start March 2019

ERIC Forum: €43,300 – projected to start February 2019

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.
ARIADNE	A virtual laboratory environment for protein production and characterisation.
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-ERIC	The European Marine Biological Resource Centre: a pan-European Research Infrastructure for marine biology and ecology research.
EMBRIC	The European Marine Biological Research Infrastructure Cluster: to accelerate scientific discovery and innovation from marine bio-resources, promoting new applications derived from marine organisms.
EMPHASIS	The European Infrastructure for Multi-Scale Plant Phenomics and Simulation: a distributed Research Infrastructure developing and providing access to facilities and services that address multi-scale plant phenotyping in different agro-climatic scenarios in Europe.
EOSC-Life	The European Open Science Cloud: bringing together biological and medical Research Infrastructures to create an open, collaborative space for digital biology.
ERASMUS	A European Union programme supporting education, training, youth and sport in Europe.
ERIC	European Research Infrastructure Consortium: a specific legal form that facilitates the establishment and operation of Research Infrastructures with European interest.
ERIC Forum	A Horizon2020 project bringing together European Research Infrastructure Consortia to strengthen their coordination and enhance their collaborations.
ESFRI	European Strategy Forum on Research Infrastructures: an organisation with members nominated by European member states ministries to support a coherent and strategy-led approach to policy-making on Research Infrastructures in Europe.
EU-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.
European Structural Funds	A European programme providing funds to help local areas grow by supporting investment in innovation, businesses, skills and employment and create jobs.
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.
Infrafrontier	A European Research Infrastructure for the generation, phenotyping, archiving and distribution of model mammalian genomes.
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.

Term	Definition
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.
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.
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.
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.
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.
UKRO	The UK Research Office: aims to maximise UK engagement in EU-funded research, innovation and higher education activities.
UKSB	The United Kingdom Society for Biomaterials: a non-profit organization working to develop novel biomaterials for clinical applications in medical devices, prosthetics and for regenerative medicine.
West-Life	West-Life provides services for computation and data management to researchers in structural biology.

ABBREVIATIONS

API	Application Programming Interface
APPID	Application Identification number.
BIOCEV	Biotechnology and Biomedicine Centre (Czech Republic)
CBI	Center of Integrative Biology (France)
CCMAR	Center of Marine Sciences (Portugal)
CeBEM	Bolivian Center for Multidisciplinary Studies
CEITEC	Central European Institute of Technology (Czech Republic)
CERM	Magnetic Resonance Center of the University of Florence (Italy)
CERN	The European Organization for Nuclear Research (Switzerland)
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
CNR	National Research Council (Italy)
CSIC	Spanish National Research Council
DI4R	Digital Infrastructure for Research conference
EC	The European Commission.
ESRF	The European Synchrotron Radiation Facility (France)
GDPR	General Data Protection Regulation
I2PC	Instruct Image Processing Center (Spain)
IBS	Institute of Structural Biology (France)
ICRI	International Conference for Research Infrastructures.
IGBMC	The Institute of Genetics and Molecular and Cellular Biology (France)
ISAB	Independent Scientific Advisory Board
ISAL	Instruct Academic Services Limited
ISBG	Integrated Structural Biology Grenoble (France)
ISO	International Organisation for Standardisation
ISPC	The Israel Structural Proteomics Center
JIF	Journal Impact Factor
JRA	Joint Research Award
NeCEN	Netherlands Centre for Electron Nanoscopy
NKI	Netherlands Cancer Institute.
OPIC	Oxford Particle Imaging Centre (UK)
PCISBIO	Portuguese Centre for Integrated Structural Biology
PID	Proposal Identification number
R&D	Research and development
RCSB	Research Collaboratory for Structural Bioinformatics
RI	Research Infrastructure
STRUBI	The Division of Structural Biology at the University of Oxford (UK)
UU	Utrecht University

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