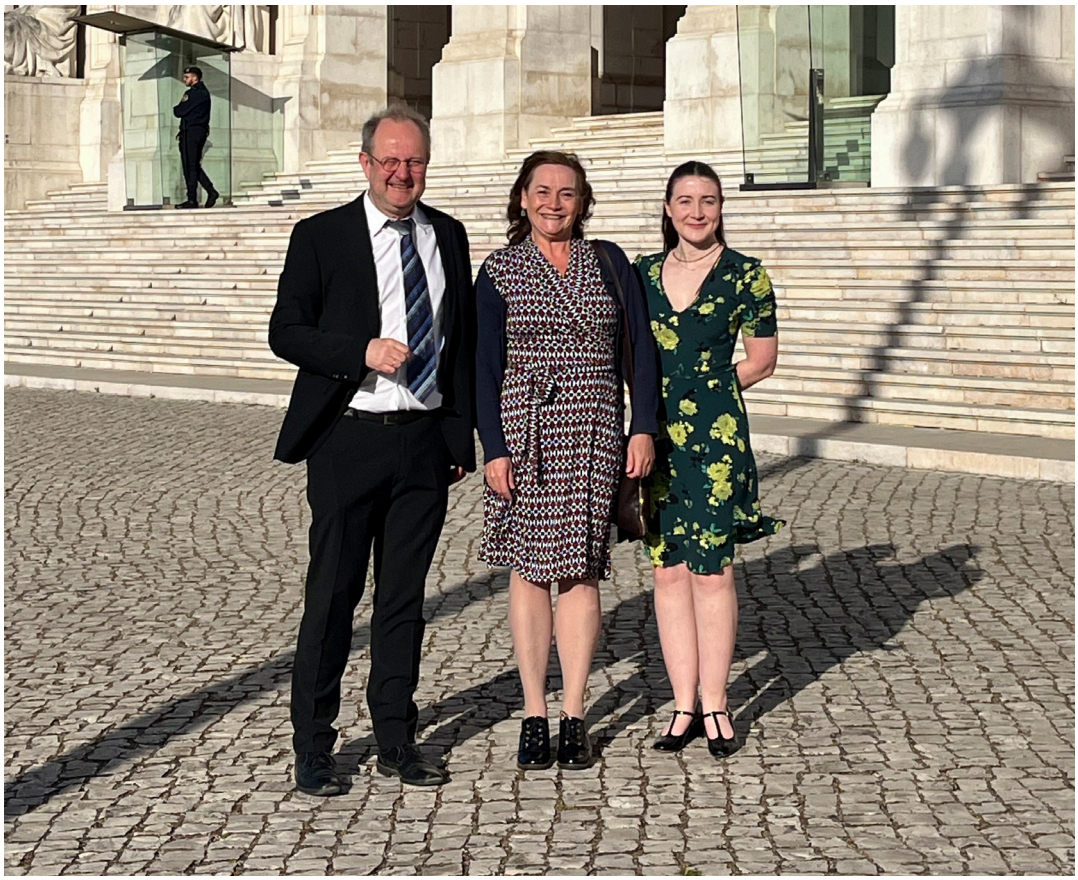
2022
INSTRUCT-ERIC ANNUAL REPORT

8AUA

CONTENTS

FOREWORD BY THE INSTRUCT-ERIC DIRECTOR & COORDINATION TEAM



Instruct-ERIC is the European Infrastructure for integrated Structural Biology. Its strength is the diversity of approaches thanks to its distributed organisation. Its *raison d'etre* is to provide democratised access to cutting-edge structural biology methodologies: crystallography, nuclear magnetic resonance spectroscopy, cryo-electron microscopy and supportive technologies in sample preparation and biomolecular analysis, delivered by expert scientists in each technology.

Providing access to structural biology has been the mission of Instruct since its foundation – what has been new in 2022?

2022 was a year of great change in Instruct-ERIC. Some of the changes are at scientific level, some at institutional and some at the personal level. On the personal level, Instruct-ERIC also underwent a change of leadership in 2022 with us starting our roles as Director and Hub Coordination team respectively, building on the fantastic legacy of David Stuart, Susan Daenke and Silke Schumacher. We hope to bring fresh perspectives for the future growth of Instruct and we are delighted to take on this new challenge within this organisation we have seen grow since becoming an ERIC in 2017.

2022 saw a return to normal operation across our Centres following the disruption caused by the COVID-19 pandemic, but what will be a lasting effect is the increased uptake of remote service provision. The disruption made clear the need for more widespread remote access to structural biology infrastructure. In the EU-funded projects eRImote and R-NMR, Instruct together with other ERICs is working to optimise the procedures for remote access.

The thematic access projects, CanSERV and ISIDORe, began their work to provide cross-domain access to research infrastructure services to tackle the global challenges of infectious disease and cancer. These projects should increase preparedness for future epidemics and further the EU cancer mission objectives. Instruct has a critical role in both projects, delivering cutting-edge structural biology services but also software support to these gargantuan initiatives with its ARIA access management software.

In 2022, we could meet again face-to-face at the Biennial Instruct conference in Utrecht. During this conference, the prestigious Bertini award was given to Sjors Scheres, honouring his ground-breaking discovery that the exact fibrillary structures of Alzheimer's amyloids is different for brain material isolated from different sources. At this meeting, Instruct scientists exchanged their newest findings on a plethora of different systems, but also highlighted the very rapid response of the global scientific community to solve all structures of the viral proteome and the viral genome of SARS-CoV-2, in order to target these structures by small molecules to help develop antiviral drugs and to monitor the effects of mutations observed in variants of concern on-the-fly. The combat of the pandemic has united scientists to form complementary teams in unprecedented manner – and lessons learnt start to emerge for the way how we conduct science and how we share scientific resources globally. Besides conducting science, making scientific findings openly available, in a findable and highly curated manner remains a key objective of Instruct. This task is far from trivial, as legitimate questions are often not easy to answer: Who owns data? How can the experimentalists, who worked hard, often over many years, into producing high quality samples, be acknowledged for their efforts? Often, a single new structure opens new avenues to what cannot yet be predicted. Instruct generates large quantities of high-quality experimental data and structural models. It are these experimental data, in particular the deposition of the structural models in the protein data bank (PDB), that made AI-based protein structure prediction possible by AlphaFold.

With AlphaFold, is integrated structural biology out of business? No, not at all. In fact, such meaningful predictions of protein structure space will sharpen Instruct's portfolio. Science will become faster; data will be harvested more rapidly. And we can then focus on the next level of structural questions: what are the structures of intermediates, transiently populated? What are the structures that are responsible for catalytic effects? What are the structures of high energy intermediates? How can we assemble increasingly complex structures as found to determine the structural architecture of our cells?

Even in 2021, many of the technologies now already offered for access to Instruct users were only emerging: access to rapid freeze quenching in cryo-EM, new sample specimen preparations for cryo-ET, new generations of 1.2 GHz NMR spectrometers changing liquid- and solid-state NMR, especially also for in-cell NMR applications. From the viewpoint of progress in fundamental science, these technologies pave the way for 4D cellular structural biology: the mapping of changes of native structure at utmost time and space resolution. Interfacing with scientists in the biomedical research area, the speed of drug screening by X-ray, NMR and, at dawn, by cryo-EM will impact pharmaceutical drug discovery. In this direction, Instruct-ERIC was successful in its Horizon Europe application for a new €10M technology development project Fragment-Screen as coordinator. Supported by the infrastructures EU-OPENSCREEN for medicinal chemistry and ELIXIR for data management, alongside several industrial partners, this project aims to improve the quality and throughput of Fragment-Screening technologies through hardware, software and method development. Fragment-Screen will harness the generative power of AI to inform fragment growth and merger strategies and chemical synthesis. This project is due to start in early 2023 and technological outputs developed will be made available to the research community through Instruct-ERIC.

Given these developments, 2022 was a timely moment for the formulation of Instruct's strategic implementation plan. The plan covers the planned activities and priorities for the coming 5-year funding cycle 2023-2027 as we look back on a fruitful first five years and forward to a promising future for Instruct-ERIC.

Stay tuned for more to come in 2023.

Prof Harald Schwalbe
Instruct-ERIC Director

Dr Claudia Alén Amaro
Head of Operations

Dr Natalie Haley
Head of Strategy

EXECUTIVE SUMMARY

INSTRUCT-ERIC MEMBERSHIP

In 2022 Instruct-ERIC had 14 Member Countries and Organisations and one Observer.
The members hosted 11 Instruct Centres with 27 facilities providing access to structural biology technology and expertise.

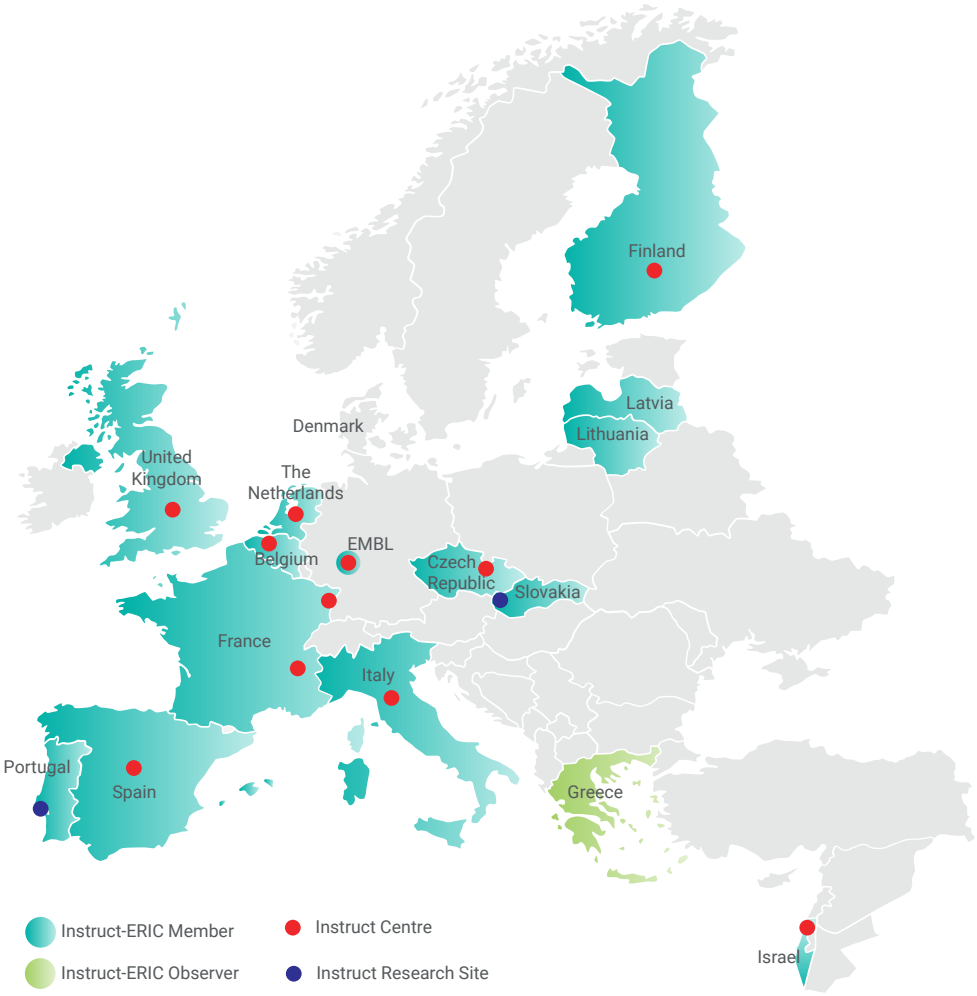
14 Members

1 Observer

11 Centres

27 Facilities

2 Research Sites



INSTRUCT-ERIC SERVICE CATALOGUE

Instruct-ERIC provides access to high-end structural biology services and techniques. In 2022, 80 services were offered accross nine service types.

Sample Preparation

- Crystallisation
- Nanobody Discovery
- Protein Production

Biomolecular analysis

- Imaging
- Mass Spectrometry
- Molecular Biophysics

3D Structural Analysis

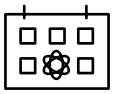
- Electron Microscopy
- Magnetic Resonance Techniques
- X-Ray Techniques

New services added to the Instruct-ERIC cataloge in 2022 were:

- Gene tagging, a CRISPR-Cas9 enhanced protein production service in Instruct Centre FR1,
- Cryo-correlative light and electron microscopy (Cryo-CLEM) in Instruct Centre EMBL,
- Kinetic ITC (kinITC) for determination of kinetic and thermodynamic parameters at Instruct Centre FR1

INSTRUCT-ERIC SERVICE PROVISION

153
Access proposals received



1056.3
Days of access provided

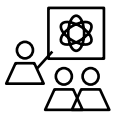
329
Scientific Publications

In 2022 Instruct-ERIC received 153 proposals for access from researchers in 20 countries of which 82% were approved.

Instruct-ERIC supported 134 research visits providing 1056.3 days of access to Instruct Facilities covering both national and transnational access.

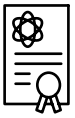
Scientific output resulted in 329 publications in peer-reviewed journals.

INSTRUCT-ERIC TRAINING AND CAREER DEVELOPEMNT



6
Training Courses

14
Internships



6
R&D Awards

In 2022, Instruct-ERIC provided funding to support six training courses.

Additionally, Instruct-ERIC supported 14 internships of three to six months for early career researchers.

Six Research & Development Pilot Awards were awarded for small scale pilot research projects in integrated structural biology.

INSTRUCT-ERIC HORIZON PROJECT PARTICIPATION

14
Projects

In 2022, Instruct-ERIC coordinated the RI-VIS project and participated in 13 Horizon projects, AI4Life, BY-COVID, canSERV, EOSC Future, EOSC-Life, EOSC4Cancer, ERIC Forum, eRImote, EU-LAC ResInfra, iNEXT-Discovery, ISIDORe, TRANSVAC-DS and TRANSVAC2.

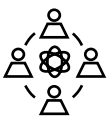
Two new projects, Fragment-Screen and IMAGINE, were approved to start in 2023.

ARIA - ACCESS MANAGEMENT SYSTEM

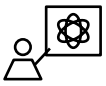
ARIA is a cloud software platform, developed and maintained by Instruct-ERIC Hub, which provides an integrated suite of tools for research infrastructure management.

In 2022, ARIA supported 15351 registered user. A record number of 756 proposals were submitted in ARIA resulting in 1129 peer reviews performed in 2022. Additionally, 2573 messages were sent in ARIA to connect the users, access managers, reviewers and facility teams.

INSTRUCT BIENNIAL STRUCTURAL BIOLOGY CONFERENCE 2022



252
Attendees



20
Speakers

Changes in structural biology: challenges in studying dynamics
19 - 20 May 2022, Utrecht, The Netherlands

This popular conference organised by Instruct-ERIC attracted 252 attendees that heard from 20 speakers on the topics of recent structural biology highlights, emerging methods and technologies and results of biomedical importance.

A highlight of the conference was the award of the 2022 Ivano Bertini Prize to Dr Sjors Scheres (UK) presented by previous winner Wolfgang Baumeister.

TIMELINE
selected events from 2022

JAN

Professor Harald Schwalbe becomes new Instruct-ERIC Director.

FEB

Second International Call Awarded

Start of the new Horizon Europe project **ISIDORe**

MAR

50th internship awarded

APR

800th access proposal approved

MAY

Instruct Biennial Structural Biology Conference in Utrecht, NL

Bertini Award Winner 2022:
Sjors Scheres

- Instruct-ERIC Council meeting in Madrid, ES
- Training Course *4th Instruct hands-on workshop on sample preparation for Single Particle cryo-EM*

JUN

Start of the new Horizon Europe project **eRImote**

5th Anniversary of Establishment of Instruct-ERIC

JUL

- Approval of the Horizon Europe projects Fragment-Screen and IMAGINE
- Training Course *Instruct-ing structural biologists towards at integration*

AUG

7th round of Instruct-ERIC R&D Pilot Awards awarded

SEP

Start of the new Horizon Europe projects **canSERV, EOSC4 Cancer** and **AI4Life**

eosc | cancer

AI4Life

Training Course *Joint Instruct-iNEXT Course: Integrating X-ray Instruct Practical School on Advanced Isotopic Labelling Methods for Integrated Structural Biology*

OCT

Instruct-ERIC is repersented at multiple events at ICRI2022

ICRI 2022

Instruct-ERIC signs trilateral MoU with Euro-BioImaging and EU-OPENSCREEN

Instruct-ERIC Council meeting in Brno, CZ

Instruct-ERIC signs MoU with the Brazilian Centre for Research in Energy and Materials (CNPEM)

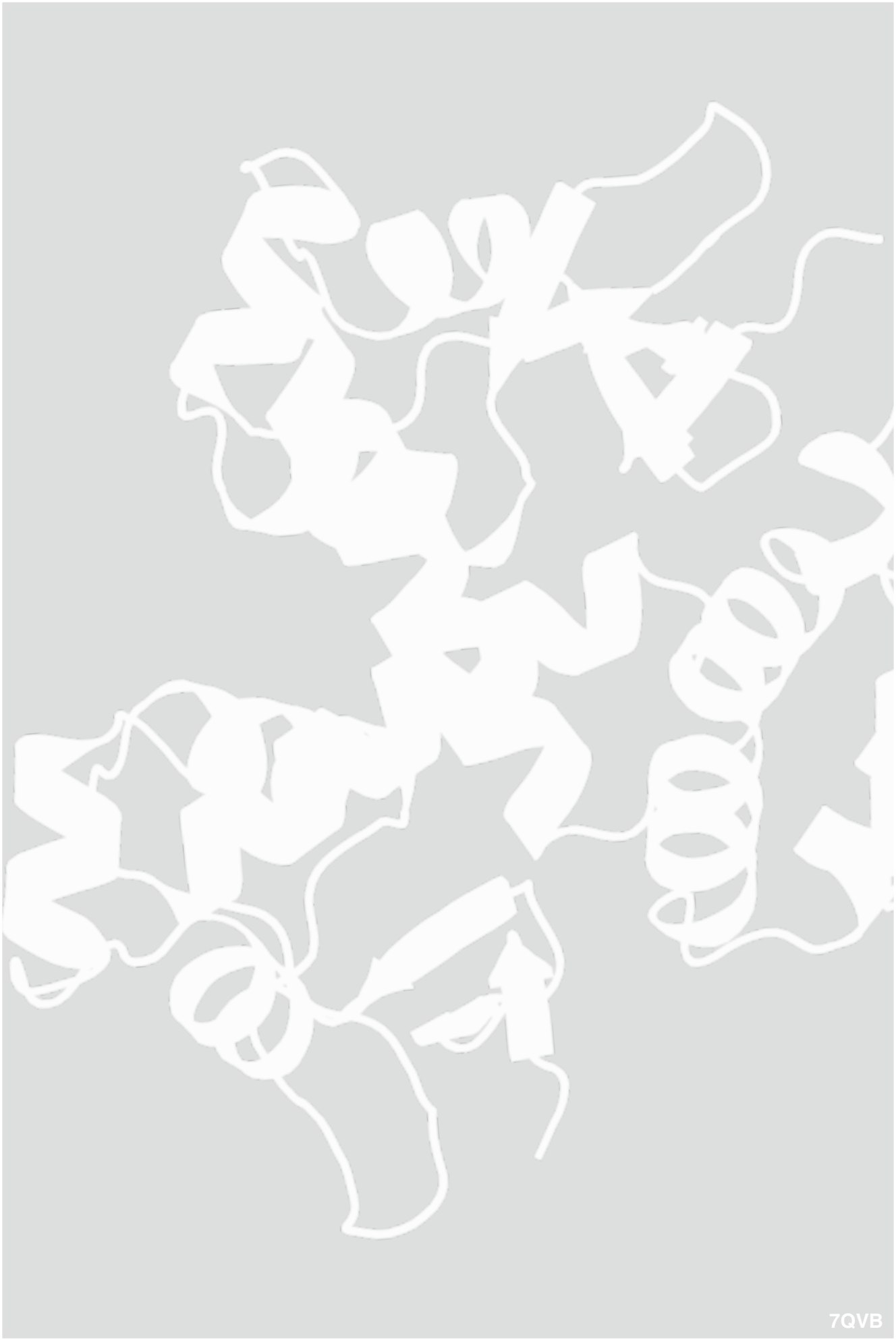
CNPEM

NOV

Instruct Hub retreat in Lille, FR

DEC

Training Course *Instruct hybrid course on Electron Tomography by CryoEM*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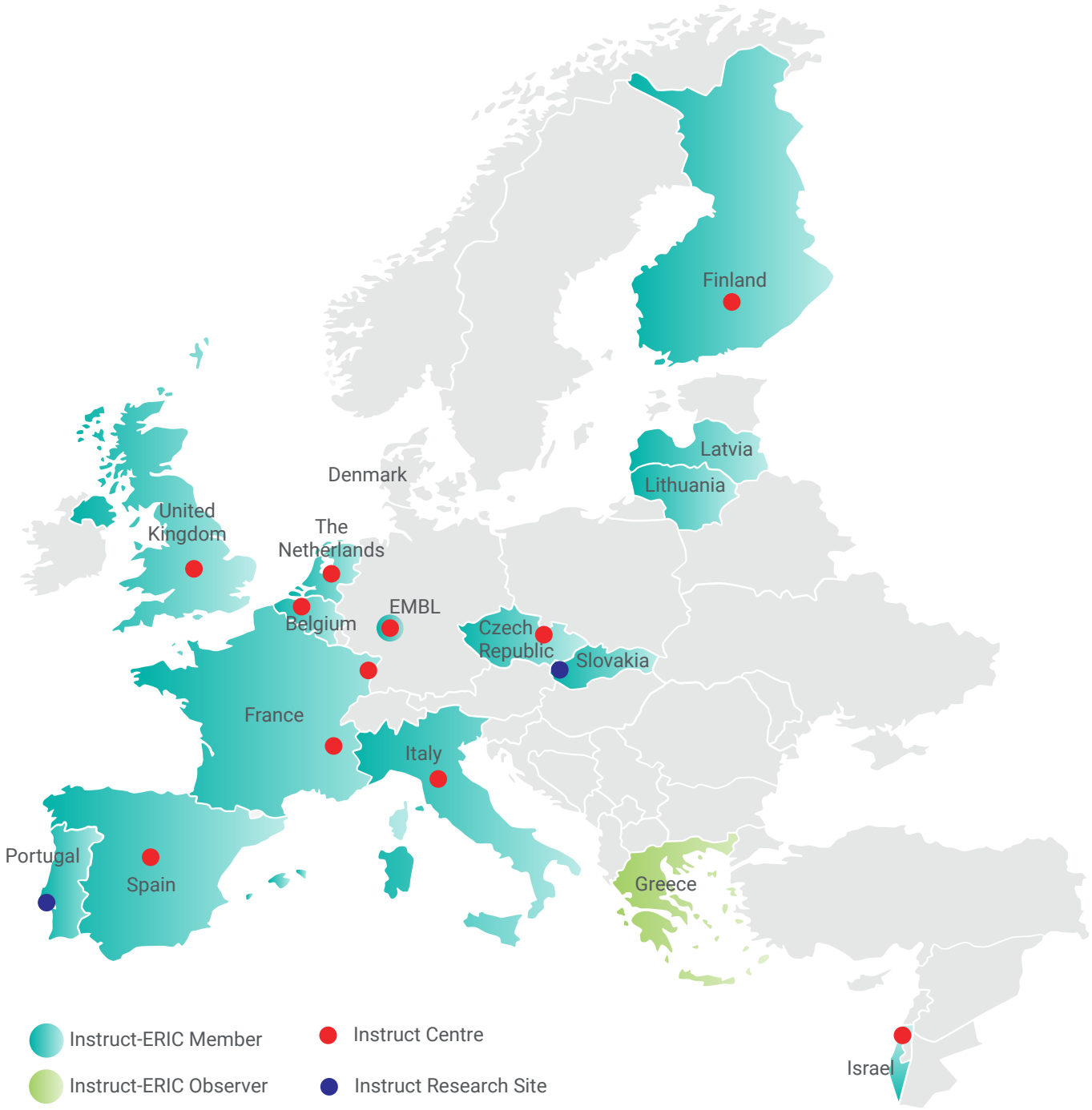
INSTRUCT MEMBERS

INSTRUCT-ERIC MEMBERSHIP

2022 marked the end of the initial 5-year funding cycle of Instruct-ERIC which began in July 2017. To reflect on the achievements, evaluate the progress over the first five years and renew the membership commitments for the next period 2023-2027, Instruct published its Quinquennial review analysis and report to its members in November 2021. The report was reviewed by the ISAB (Independent Scientific Advisory Board) and by Instruct-ERIC Council in May 2022 and sets the re-commitment of the current membership to the next 5 years of the infrastructure, including an increase of 10% in the membership contribution for all members.



In 2022 Instruct-ERIC continued discussions with prospective new members, in particular Slovenia and Germany, where progress towards a membership application advanced well. Sadly 2022 was also the year Instruct-ERIC said goodbye to colleagues in Denmark which withdrew its membership effective January 2022. As the financial pressures stimulated by global events in recent years continue to put pressure on national budgets, it is vital to reinforce the strength, value and impact of of contributing to the research infrastructure and ensure that research communities in Instruct-ERIC members get the maximum benefit from their membership. To this end, Instruct-ERIC launched a call for Research Sites, hubs of Instruct-ERIC activity, networking and training in member countries without Instruct Centres in 2021. Two new research sites in Slovakia and Portugal awarded from that call began their activities in 2022.

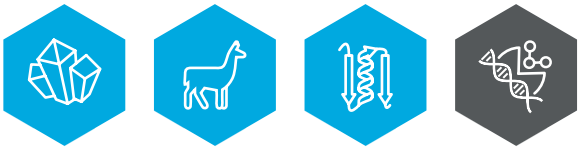
INSTRUCT-BE

Instruct Centre BE

https://instruct-eric.org/centres/instruct-be/

Nanobodies4Instruct, Brussels
Robotein for Instruct, Liège and Brussels

Services



Highlights in 2022

New Instruments, Upgrades and Tools

- New Ultraflextreme Enhanced MALDI-TOF/TOF mass spectrometer at Nanobodies4Instruct.
- Robotein® developed a high throughput assay to measure the avidity of polyclonal antibodies for the receptor binding domain of the SARS-CoV-2 spike protein.
- A new tool for the detection and inhibition of proteins involved in antimicrobial resistance was developed at Robotein.
- New biosensor tool to control the quality of therapeutic antibodies using ATR-FTIR spectroscopy.

Meetings and Outreach

- VUB in Brussels hosted the BECM/Belgian Cryo-EM user meeting in September 2022. The aim was to highlight exciting research involving cryo-EM within BECM and the greater Belgian EM community.
- Nineteenth Meeting of the Belgian Biophysical Society on “Protein Folding and Assembly”, held at University of Liège, in September 2022.

Find the full highlights here https://instruct-eric.org/content/instructbe-annual-report-2022

INSTRUCT-CZ

Instruct Centre CZ

https://instruct-eric.org/centres/instruct-cz/

BIOCEV - Biotechnology and Biomedicine Centre, Vestec, Prague-West
CEITEC - Central European Institute of Technology, Brno

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- A high-resolution plasma-FIB/SEM dual beam microscope with an integrated fluorescence microscope was installed at CEITEC.
- Also at CEITEC, proteomic analysis has been enhanced with new mass spectrometer timsTOF Pro 2 and liquid chromatograph Evosep One.
- The screening equipment for protein crystallisation at BIOCEV was upgraded by installing the SONICC instrument (Formulatrix).
- Also at BIOCEV, the facility acquired a Refeyn Two mass photometer, which allows the measurement of the mass, oligomerization and interaction of biomolecules in solution based on light scattering.

Meetings and Outreach

- The conference Discussions in Structural Molecular Biology and the User Meeting of CIISB was held in Nove Hrady, South Bohemia, March 2022.

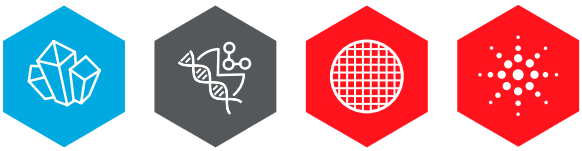
Find the full highlights here https://instruct-eric.org/content/instructcz-annual-report-2022

INSTRUCT-EMBL

Instruct Centre EMBL
https://instruct-eric.org/centres/instruct-embl/

EMBL Grenoble
EMBL Hamburg
EMBL Heidelberg

Services



Highlights in 2022

New Instruments, Upgrades and Tools

- EMBL Grenoble and ESRF have integrated a CrystalDirect™ harvester into the MASSIF-1 beamline, allowing simultaneous crystal harvesting and data collection.
- EMBL Hamburg's Sample Preparation and Characterisation Facility has expanded its eSPC bioinformatics services, now including mass photometry, and dynamic light scattering apps on the eSPC platform.
- Cryo-CLEM is now available at EMBL Heidelberg, allowing users to navigate large cellular volumes, and then acquire high-resolution snapshots of cellular events.

EMBL Initiatives

- The new EMBL Imaging Centre was inaugurated in 2022

Find the full highlights here https://instruct-eric.org/content/instructembl-annual-report-2022

INSTRUCT-ES

Instruct Centre ES
https://instruct-eric.org/centres/instruct-es/

CryoEM CNB-CSIC, Madrid
I2PC - Instruct Image Processing Center, Madrid

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- The Electron Microscopy Flexibility Hub Service was launched in June 2022. This is an innovative approach for image processing of challenging samples presenting a large degree of flexibility, especially if this flexibility is continuous, making it difficult to assign images to discrete classes.
- The SCIPION framework continues to develop which includes movie alignment, particle picking, classification 2D-3D, volume reconstruction and atomic structure determination.
- At the CNB cryo-EM facility, the recently acquired infrastructure for cryoelectron diffraction and cryocorrelative techniques has been initiated. The techniques are ready and have been first opened for the national community, and then Instruct in 2023.

Meetings and Outreach

- Online meeting: Working Day on Research Infrastructures (RI) in Biomedical Sciences, March 2022.

Find the full highlights here https://instruct-eric.org/content/instructes-annual-report-2022

INSTRUCT-FI

Instruct Centre FI

https://instruct-eric.org/centres/instruct-fi/

University of Eastern Finland, Joensuu
University of Helsinki, Helsinki
University of Oulu, Oulu

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- Newly developed asymmetrical flow field flow fractionation as a new way to purify and analyse RNA at University of Helsinki. Applicability of asymmetrical flow field flow fractionation (AF4) combined to multidetector platform was addressed to study single-stranded and double-stranded RNA molecules.
- Characterization of macromolecular complexes using light scattering, University of Oulu. In 2022 the multi-angle light scattering instrument coupled to a size exclusion chromatography (SEC-MALS) was updated at the University of Oulu. A new refractive index detector was installed and the system was enhanced by an additional online dynamic light scattering detector (QELS).

Meetings and Outreach

- Structural Biology Platform FINStruct and Instruct-ERIC Centre Finland 2022 Annual Meeting was held at the University of Eastern Finland, Joensuu, in November 2022. The programme included presentations of infrastructure facilities and scientific presentations.

Find the full highlights here https://instruct-eric.org/content/instructfi-annual-report-2022

INSTRUCT-FR

Instruct Centres FR1 & FR2

https://instruct-eric.org/centres/instruct-fr1/
https://instruct-eric.org/centres/instruct-fr2/

IGBMC, Strasbourg
IBS-ISBG, Grenoble

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- Gene tagging with the CRISPR-Cas9 system for introduction of affinity tags has been developed at Instruct-FR1, facilitating the purification of endogenous complexes or for tagging proteins with fluorescent reporters.
- Flow-Induced Dispersion Analysis (FIDA) is now availible at Instruct-FR1 for use in plasma, serum, cell lysate and fermentation media.
- The flagship high field 950 MHz instrument has been upgraded with a new Bruker NEO console at Instruct-FR2
- A new SAFe360 microscope from Abbelight and Olympus (SR-M4D) at Instruct-FR2 implemented to reinforce the super-resolution capabilities of the platform.

National Initiatives

- The project EquipEx+ France-Cryo-EM received funding to purchase three cryo electron microscopes of 300kV, one at the centre Instruct-FR1, one at Instruct-FR2 and one at SOLEIL, Paris-Saclay.

Meetings and outreach

- Organisation of 6th FRISBI users meeting held the 6th December 2022 at CNRS.

Find the full highlights here https://instruct-eric.org/content/instruct-member-france-annual-report-2022

INSTRUCT-IL

Instruct Centre IL

https://instruct-eric.org/centres/instruct-il/

ISPC - Weizmann Institute of Scince, Rehovot
Centre for Bioinformatics, Tel Aviv University, Tel Aviv

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- A Rigaku Liquid-metal-jet (LMJ) X-ray diffraction system was installed at the Structural Proteomics Unit (SPU). This system has had an enormous impact on structural biochemistry/biophysics at the WIS and Instruct-ERIC users. The Excillum LMJ source has high power load with a very small size focused beam which results in unprecedented specific high intensity.
- Another unique feature of the Rigaku LMJ system is its capacity to collect X-ray data directly from crystallisation trays at room temperature (RT). Data collection at RT can provide information on the conformational heterogeneity of a protein, and its conformation when bound to its protein partner or ligand.

Meetings and Outreach

- Members of the Instruct Centre IL participated in the FISEB conference in February 2023 of the Societies of Experimental Biology in Eilat, Israel. Multiple sessions in this exciting meeting were dedicated to structural biology and the many disciplines it relates to.

Find the full highlights here https://instruct-eric.org/content/instructil-annual-report-2022

INSTRUCT-IT

Instruct Centre IT

https://instruct-eric.org/centres/instruct-it/

CERM, Florence

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- The flagship 1.2 GHz NMR spectrometer has been equipped with a novel cryoprobe for ^{13}C direct detection experiments, and well-tailored for novel application using ^{15}N direct detection.
- The focus this year was on the expression of fluorinated proteins in human cells for ^{19}F NMR spectroscopy in-cell. Soluble targets may interact with large cellular components, preventing the observation of ^{1}H-^{15}N NMR signals – ^{19}F NMR can overcome this issue, as it allows detection of broad lines thanks to the absence of cellular background.
- Instruct-IT have developed a methodology to incorporate different fluorinated amino acids in proteins expressed in human cells.

National Initiatives

- A gap analysis of the services offered to the Italian scientific community in various areas of Life Science, including Structural Biology was promoted by Italian Ministries.
- Instruct-IT secured the ITACA.SB project for potentiating the Italian centre of Instruct-ERIC and for implementing new facilities in the country thanks to the Next Generation EU recovery funds.

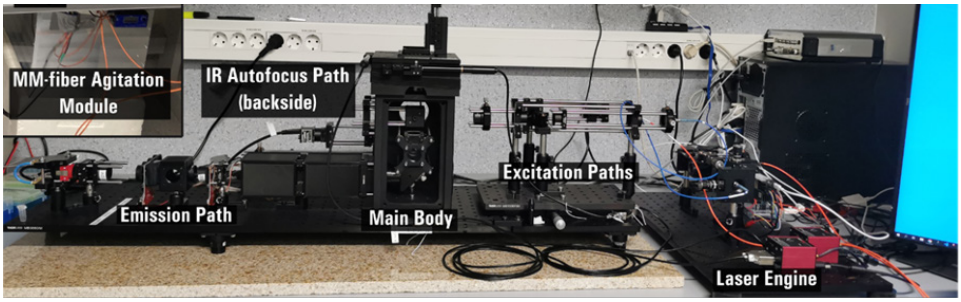
Find the full highlights here https://instruct-eric.org/content/instruct-it-annual-report-2022

INSTRUCT-LT

Highlights in 2022

New Instruments, Upgrades and Tools

- Vilnius University Life Sciences Center (VU LSC) is developing of a highly capable, yet inexpensive super-resolution (SR) imaging system “miEye”.
- Newly developed Electrochemical Impedance Spectroscopy (EIS) – a method of characterising the electrical properties of interfaces.



National Initiatives

- Over 10 scientists, PhD students, and Masters students were trained in cryo-EM sample preparation and data acquisition through courses offered by ThermoFisher Scientific. This should greatly enhance the utilisation of cryo-EM techniques, both at a local level using the Glacios microscope at VU LSC, and on a broader scale, utilising European infrastructure.
- Two PhD students improved their knowledge and expertise in intensive high-speed AFM summer courses in France. The acquired knowledge will be applied in the infrastructure available at VU LSC and abroad.

Find the full highlights here https://instruct-eric.org/content/instructlt-annual-report-2022

INSTRUCT-LV

Highlights in 2022

New Instruments, Upgrades and Tools

- Construction and opening of Biotechnology facility at Latvian Institute of Organic Synthesis.On March 29, 2023, the Latvian Institute of Organic Synthesis (LIOS) opened its new Biotechnology facility, located in a two-story building next to its main site. The laboratory spaces are equipped with the latest research equipment for molecular and structural biology research, which will enhance LIOS’s capabilities and potential for discovering new drugs. The new facility will be used for recombinant protein production as well as structural and interaction studies in the contexts of rational drug design, biomaterials, biocatalytic processes and bio-pharmaceutical development. Until now, performing these studies at LIOS was challenging due to various technological constraints.

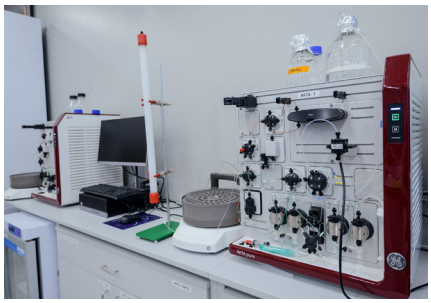


Photo credit: Latvian Institute of Organic Synthesis

Meetings and outreach

- Several structural biology related talks were presented during locally organized annual meeting of Latvian Biochemical Society in February 2022.
- THe FEBS3+ conference in June 2022 in Tallinn, organized in part by structural biology communities in Latvia, Estonia and Lithuania.

Find the full highlights here https://instruct-eric.org/content/instructlv-annual-report-2022

INSTRUCT-NL

Instruct Centre NL

https://instruct-eric.org/centres/instruct-nl/

Bijvoet Centre - Utrecht University, Utrecht
NKI Protein Facility, Amsterdam
NeCEN - Leiden University, Leiden

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- The NKI Protein Facility invested in a new High Five insect cell line to expand their portfolio of protein production services.

Meetings and Outreach

- Instruct-NL organised the 2022 Instruct-ERIC Biennial Structural Biology Conference. The event took place on May 19 and 20 in the monumental Muntgebouw building in Utrecht, and attracted over 250 structural biologists globally.
- The NeCEN facility restarted their monthly cryo-EM seminar series that was interrupted during the COVID-19 pandemic. To kick off this series, they organised a symposium with prominent speakers from the cryo-EM field.
- The exhibition *Onvoorstelbaar* showcased the advances in microscopy since the time of Antoni van Leeuwenhoek 350 years ago. The nearby NeCEN facility was present via display images, an interview with staff scientist Willem Noteborn and with co-director Ariane Briegel about cryo-EM at NeCEN, and a movie based on research done at the facility called *Treponema through the ages*.

Find the full highlights here https://instruct-eric.org/content/instructnl-annual-report-2022

INSTRUCT-PT

Instruct Research Site PT

https://instruct-eric.org/content/instruct-research-sites-portugal

FC-ULisboa, Lisboa
ITQB NOVA, Oeiras

Highlights in 2022

National Initiatives

- The Instruct-PT research site was created, providing a platform for training, outreach, or networking activities of interest to Instruct users and members.

New Instruments, Upgrades and Tools

- New a chip based nanoESI infusion system coupled to a magnetic resonance mass spectrometer (Solarix XR MRMS) with the added capability of sample preparation.

Meetings and Outreach

- ITCB-NOVA organised the 2022 Portuguese Centre for Integrated Structural Biology (PCISBIO) Meeting. The meeting provides an opportunity for the structural biology community in Portugal to come together and get a first-hand view of how this research infrastructure is available to Portuguese researchers.
- The 14th European Fourier Transform Mass Spectrometry Workshop & 3rd EFTMS School were held hosted by FC-ULisboa. The workshop promoted interaction and exchange between academy and industry researchers working in the field of Fourier transform mass spectrometry.

Find the full highlights here
https://instruct-eric.org/content/instructpt-annual-report-2022

INSTRUCT-SK

Instruct Research Site SK

https://instruct-eric.org/content/instruct-research-sites-slovakia

IC SAS - Slovak Academy of Sciences, Bratislava

Highlights in 2022

National Initiatives

- The Instruct-SK research site was created, providing a platform for training, outreach, or networking activities of interest to Instruct users and members.

Meetings and outreach

- The *Chemistry towards Biology 10 - Instruct* is the 10th continuation of the series of successful meetings aimed at the exchange of scientific results and ideas in the fields of chemistry and biology. The theme of the conference held in Bratislava, in September 2022 was *Biomolecular structure* and covered primarily topics - structure and dynamics of biomolecules, intermolecular interactions, experimental and theoretical methods in biomolecular research.

Find the full highlights here https://instruct-eric.org/content/instructsk-annual-report-2022

INSTRUCT-UK

Instruct Centre UK

https://instruct-eric.org/centres/instruct-uk/

Astbury Biostructure Laboratory, Leeds
Diamond Light Source, Didcot
Oxford Particle Imaging Centre, Oxford
Oxford Mass Spectrometry Centre, Oxford
Research Complex Harwell, Didcot
Molecular Biophysics Suite, Oxford
STRUBI, Oxford

Services

Highlights in 2022

New Instruments, Upgrades and Tools

- BIC, part of Diamond Light Source, completed the upgrade to Aquilos 2 with autoTEM to allow semiautomated lamellae milling with overnight hold time under cryo conditions.
- At Diamond Light Source, new undulator sources for beamline I04 and I04-1 have increased photon flux to in excess of 10^{12} photons/s and increased the scope of samples that can be studied.
- Developments in the preparation of small membrane protein (100 kDa) samples for cryo-EM have been applied to the Membrane Protein Laboratory pipeline in collaboration with eBIC.
- The Oxford Mass Spectrometry facility has a new building with proteomics, lipidomics, and glycomics equipment.
- OPIC acquired the PRIMO (alveole) Micropatterning device for improving the positioning of cells on grids.

Meetings and outreachw

- Frontiers in Native Mass Spectrometry and Single-Molecule Imaging Conference in Oxford in August 2022.
- Astbury Conversation, University of Leeds. Took place online April 2022.

Find the full highlights here https://instruct-eric.org/content/instructuk-annual-report-2022


8DKT

SCIENTIFIC HIGHLIGHTS

Teixobactin kills bacteria by a two-pronged attack on the cell envelope[1]

 # New Antibiotic # MRSA

The continuous rise in drug-resistant bacteria is a significant concern for global health, made even more critical as few new antibiotics have been added to the market in recent years. That is, until teixobactin was discovered as a potential new antibiotic, which displayed broad activity against several drug-resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA).

This study, from Shukla et al. (2022), aimed to understand the mechanism by which teixobactin attacked bacterial cells, in order to get a clearer picture of the potential role it could play as a clinical antibiotic, with the key technology being solid-state NMR (ssNMR).

Markus Weingarth, Associate Professor at Utrecht University, said, "In 2019, my colleague Prof. Moreno Lelli from CERM/ The University of Florence, and I obtained an Instruct- ERIC Research award (JRA award) that was endowed with €20,000. Our project was entitled 'Solid-State NMR to monitor native drug - receptor interactions in cellular surfaces'. This was a project that I incited because we had a major bottleneck in our understanding of the mode of action of teixobactin."

It was through the use of ssNMR and several other techniques, that Markus, Moreno, and the team investigated the binding mechanism of teixobactin to the bacterial membrane and discovered how it displays such potent antibiotic efficiency. These techniques included HS-AFM, confocal microscopy and molecular dynamic simulations.

Moreno Lelli, CERM/CIRMMP, said, "Thanks to the Instruct-ERIC JRA, the collaboration with Markus team was the perfect frame to exploit at its best our expertise and instrumentation at CERM/CIRMMP for the development of experiments to obtain the phosphorous-proton distance information".

Markus: "Teixobactin, using backbone amino-protons, targets the pyrophosphate group of the cell-wall precursor molecules Lipid II. But it was not known how exactly this worked. To understand this precisely, it was necessary to determine proton - phosphorous distances with solid-state NMR."

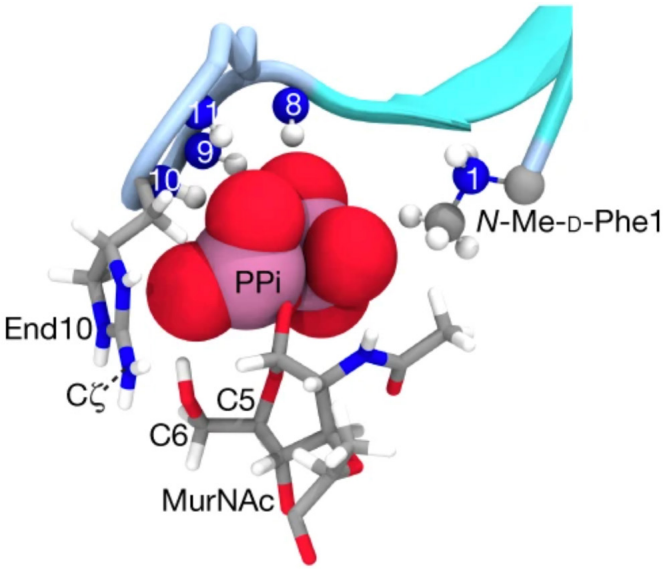


FIG 1. The backbone amino-protons of teixobactin, the End10 sidechain and the N terminus of an adjacent teixobactin coordinate the lipid II PPi group. In addition, End10 interacts with the MurNAc sugar via hydrogen bonds. Blue spheres represent backbone nitrogens; numbers indicate the residue numbers[1].

The C-terminus of teixobactin contains the amino acid, enduracididine (End10). The team found that this bound to lipid II in the sugar moiety (specifically the MurNAc- PPi site). This is also then anchored by the N-terminus of teixobactin.

Lipid II is a peptidoglycan precursor, and is essential for bacterial cell wall and membrane function and synthesis. It is not found in eukaryotic cells. The use of ssNMR at CERM, Florence, through Instruct funding, helped the team to make the discovery. The binding of End10 to MurNAc-PPi of lipid II is shown in Figure 1.

Markus: "Measuring H-P distances in solids required a special piece of hardware (a 31P-capable ssNMR probehead that enables fast sample spinning up to 60 kHz MAS) that we did not have in Utrecht, but that was available in Florence. With the JRA award, we could kick off our collaboration, and these H-P distances were a key contribution to precisely resolve the teixobactin - Lipid II interface."

The result was that as teixobactin bound to lipid II, it formed β-sheets. This created an irreversible supramolecular complex as more teixobactin molecules bound, forming more β-sheets. This complex inhibited the function of peptidoglycan (limiting cell membrane synthesis), but more importantly thinned the membrane itself through hydrophobic interactions within the complex, allowing ion leaking and a drop in membrane potential.

This explains the two-pronged method by which teixobactin attacks the cell envelope, but it has several other properties that make it such an exciting antibiotic prospect.

Lipid II is made of two components: the sugar moiety (bound to by enduracididine in teixobactin), and a pentapeptide (bound to by other antibiotics such as vancomycin, and ignored by teixobactin). The pentapeptide is liable to variation and, therefore, drug-resistance. By contrast, the sugar component (specifically MurNAc-PPi) is invariable, and present in several cell wall precursors (lipid I, lipid II, and lipid III).

This ground-breaking study should provide the foundation for further teixobactin development, and hopefully will lead to the generation of a novel, non-resistant antibiotic – something which is sorely needed in the medicinal world. The access to ssNMR at CERM through Instruct allowed the team to carry out their research into this potentially crucial antibiotic.

[1]Shukla *et al.,* (2022) Teixobactin kills bacteria by a two-pronged attack on the cell envelope, *Nature*. https://doi.org/10.1038/s41586-022-05019-y

RecA-LexA complex[2]

 # Nanobodies # *E. coli* SOS complex

In collaboration with the Cendron lab, the Nanobodies4Instruct facility discovered nanobodies via llama immunization and phage display, that bind LexA with sub-micromolar affinity and block autoproteolysis, repressing SOS response in *Escherichia coli*. Biophysical characterization of the nanobody-LexA complexes revealed that they act by trapping LexA in an inactive conformation and interfering with RecA engagement. These studies pave the way to the development of new-generation antibiotic adjuvants for the treatment of bacterial infections.

FIG 2

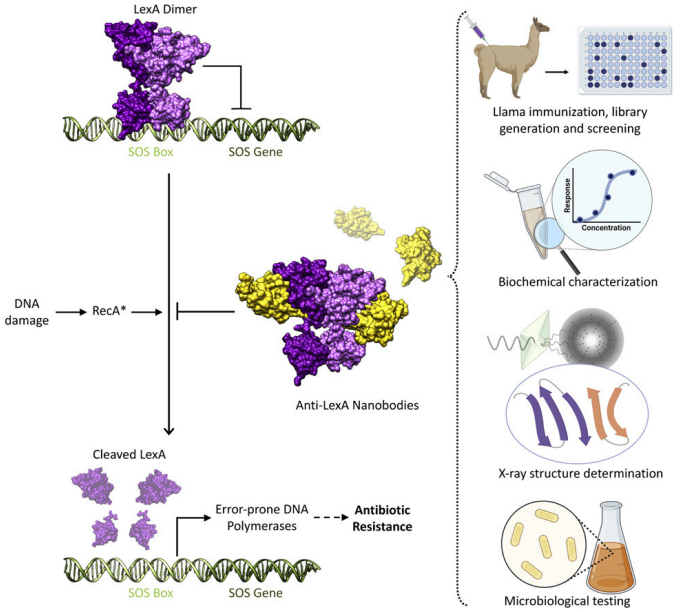


FIG 2. Graphical summary of the work performed by the Cendron lab in collaboration with Nanobodies4Instruct facility to block the *E. coli* SOS response using nanobodies.[2]

[2]Maso, L. *et al.* (2022) Nanobodies targeting LexA autocleavage disclose a novel suppression strategy of SOS-response pathway. *Structure* 30, 1479-1493.e9.

Structural basis of rapid actin dynamics in the evolutionarily divergent Leishmania parasite[3]

 # F-action # Depolymerizing Factor # *Leishmania major*

FIG 1

Actin is highly conserved in eukaryotes and plays a pivotal role in cell migration, endocytosis, and muscle contraction that is achieved via coordinated polymerisation and disassembly of actin filaments. Different organisms have quite different methods and machineries for facilitating actin filament dynamics. Kotila et al.[3] studied actin filaments of *Leishmania major*, a trypanosomatid parasite responsible for severe disease in humans, and compared it to mammalian actin. The aim of the study was to understand the differences in actin depolymerisation and the enzymes and cofactors involved. Formation of long *L. major* actin (LmActin) filaments from subunits in solution was assessed through microfluidics. They formed long filaments when polymerising at the barbed end. LmActin formed long filaments also when combined with rabbit skeletal muscle α-actin (RbActin), albeit at a much slower rate. Cryo-EM at the University of Helsinki was used to obtain advanced structural data. The structure of parasitical and mammalian actin revealed deletions and alterations that resulted in weaker connections between actin subunits that may explain the five-times more rapid depolymerisation rate of LmActin than mammalian actin. The role of actin-regulatory machinery on the actin filament dynamics was examined by saturating filaments of LmActin and RbActin with cofilin (Fig. 3), a key actin regulator promoting actin filament disassembly. Cofilin is inefficient at disassembly in yeast and mammals, and requires a cofactor (Aip1 the most common). However, Aip1 is not found in *L. major*. Kotila *et al.*, 2022 showed that mammalian cofilin bound to RbActin, but did not rapidly depolymerise in absence of cofactors such as Aip1. LmCofilin disassembled LmActin hundred-times more efficiently. Cryo-EM examination found that key differences in the N- and C-termini of cofilins and actin filament structures were responsible for this difference. The study provides a foundation for future systematic mutagenesis and molecular dynamic simulation studies, as well as open new avenues for developing specific inhibitors against parasitic actin, particularly in devastating trypanosomes such as *L. major*.

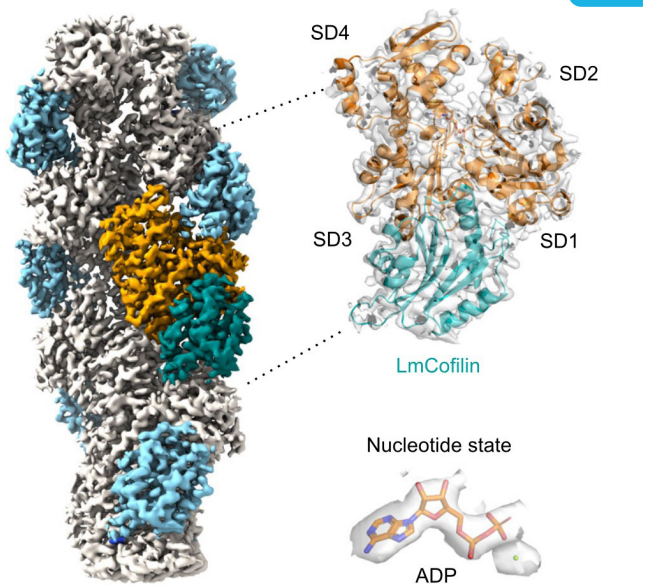


FIG 3. The cryo-EM map of LmActin filament decorated with LmCofilin (orange / blue section). Density for the model, around ADP and associated Mg2+ ion is also shown. Figure modified from Kotila et al, 2022, under Creative Common license.

[3]Kotila T, *et al* (2022) Structural basis of rapid actin dynamics in the evolutionarily divergent Leishmania parasite. *Nat Commun.* 13(1):3442. DOI10.1038/s41467-022-31068-y.

Posttranslational modification of microtubules by the MATCAP detyrosinase[1]

 # cryo-EM # Microtubule # Detyrosination

FIG 1

The tubulin detyrosination-tyrosination cycle involves removal and re-attachment of the C-terminal tyrosine of α-tubulin and is implicated in cognitive, cardiac, and mitotic defects. The effort of the vasohibin–small vasohibin-binding protein (SVBP) complex accounts for much but not all detyrosination activity. The research team from Instruct-NL used haploid genetic screens to identify a previously unannotated protein, 'microtubule associated tyrosine carboxypeptidase' (MATCAP), as a remaining detyrosinating enzyme. Cryo-EM structures from NeCEN and X-ray crystallography established MATCAP's cleaving mechanism, substrate specificity, and microtubule recognition. Paradoxically, whereas abrogation of tyrosine re-ligation is lethal in mice, the co-deletion of MATCAP and SVBP is not. Although viable, defective detyrosination caused microcephaly, which can be associated with proliferative defects during neurogenesis, and abnormal behaviour. Thus, MATCAP is a missing component of the detyrosination-tyrosination cycle, revealing the importance of this modification in brain development.

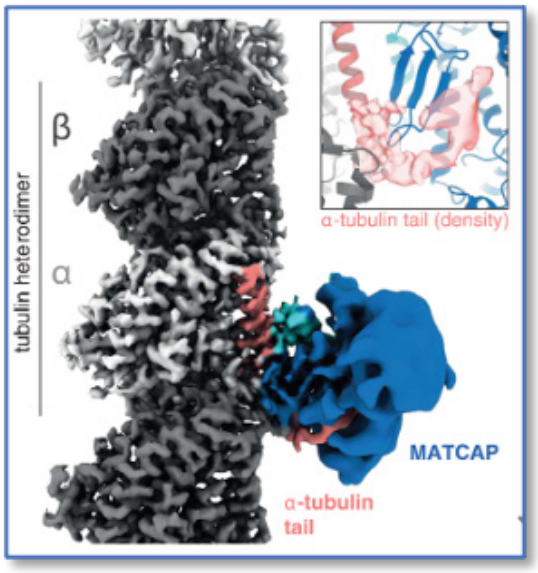


FIG 1. Cryo-EM map of MATCAP (blue) bound to the α-β-tubulin heterodimer (gray). Density corresponding to the C-terminal end of α-tubulin is in salmon pink; MATCAP density interacting with tubulin is in cyan. The displayed map is shown at isocontour 0.5 and 0.05 for the density belonging to tubulin and MATCAP, respectively, to better visualize the map features at each region. (Inset) Highlight of density that is attributed to the α-tubulin tail.

[1]Landskorn, L. *et al.* (2022) Posttranslational modification of microtubules by the MATCAP detyrosinase. *Science.* 22, 8875–88828876. DOI 10.1126/science.abn6020

Tail proteins of phage SU10 reorganize into the nozzle for genome delivery[2]

 # cryo-EM # Bacteriophage #Evolution

FIG 2

Escherichia coli phage SU10 belongs to the genus *Kuravirus* from the class *Caudoviricetes* of phages with short non-contractile tails. In contrast to other short-tailed phages, the tails of *Kuraviruses* elongate upon cell attachment. The research provided evidence that the virion of SU10 has a prolate head, containing genome and ejection proteins, and a tail, which is formed of portal, adaptor, nozzle, and tail needle proteins and decorated with long and short fibres. The binding of the long tail fibres to the receptors in the outer bacterial membrane induces the straightening of nozzle proteins and rotation of short tail fibres. After the re-arrangement, the nozzle proteins and short tail fibres alternate to form a nozzle that extends the tail by 28 nm. Subsequently, the tail needle detaches from the nozzle proteins and five types of ejection proteins are released from the SU10 head. The nozzle with the putative extension formed by the ejection proteins enables the delivery of the SU10 genome into the bacterial cytoplasm. It is likely that this mechanism of genome delivery, involving the formation of the tail nozzle, is employed by all *Kuraviruses*.

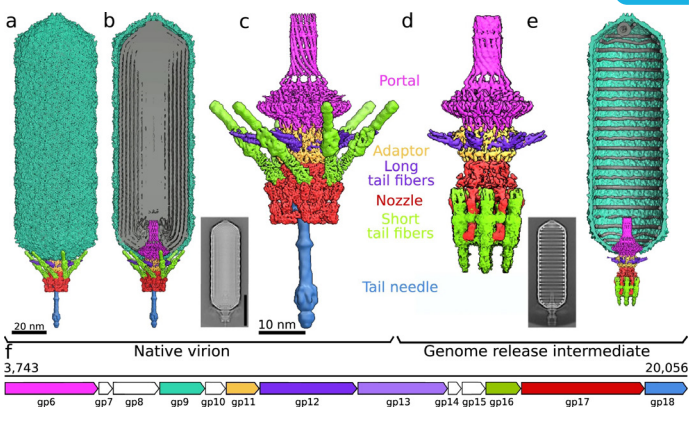


FIG 2. (a) Surface representation of composite cryo-EM map of virion of phage SU10. (b) The same as a, sliced lengthways to show the structure of the genome in grey. The inset shows a 2D class average of the SU10 virion. (c) Composite cryo-EM map of portal and tail complexes of SU10 virion. (d) Cryo-EM reconstruction of portal and tail complexes from an SU10 genome release intermediate. (e) Composite cryo-EM map of genome-release intermediate of SU10 sliced lengthways to show the structure of the genome remaining in the capsid. The inset shows a 2D class average of the SU10 genome release intermediate. (f) Schematic representation of segment of SU10 genome encoding structural proteins color-coded the same as the proteins in panels a to e[4].

[2]Šiborová, M. *et al.* (2022) Tail proteins of phage SU10 reorganize into the nozzle for genome delivery. *Nat Commun* 13, 5622. DOI 10.1038/s41467-022-33305-w

Deamidation of SARS CoV 2 spike protein and its role in immune evasion

 # Staff Exchanges # PTM

FIG 3

Leonardo Alonso (University of Buenos Aires) visited Instruct Centre EMBL Hamburg as part of a ResInfra Staff Exchanges Call, within EU-LAC ResInfra. During the exchnage he researched the role of asparagine deamidation in coronavirus proteins. Deamidation is a post-translational modification (PTM) that occurs spontaneously in proteins containing the dipeptide sequence NG. Despite its significance, the quantitative assessment of time-dependent accumulation of deamidated species at specific positions in large and heavily post-translationally modified proteins poses significant experimental challenges.

As a result, the potential functional role of deamidation has often been overlooked. It is commonly perceived as a degradative reaction that is detrimental to protein function and stability. The Receptor Binding Motif (RBM) of the SARS-CoV-2 spike protein plays a crucial role in viral infectivity. To better understand the impact of deamidation on the spike protein, we have developed a quantitative model. This model combines experimental data on the deamidation rates of hotspot residues and the number of spike trimers present on the surface of an average virion. Expanding our analysis to other members of the Sarbecovirus family, including human, bat, and pangolin spike proteins, we have observed that the presence of deamidation hotspots is a common feature within the RBM. Interestingly, some of these hotspots exhibit conservation patterns, suggesting a potential functional role that has yet to be identified. Understanding the impact of deamidation on viral infectivity and its correlation with viral evolution can contribute to the development of more effective therapeutic strategies and vaccine design. Asked about his motivation for visiting EMBL Hamburg, Leonardo stated, “EMBL Hamburg is renowned as one of the leading institutes worldwide for conducting structural analysis of proteins. The institute houses a diverse team of scientists who are at the forefront of protein structural analysis”.

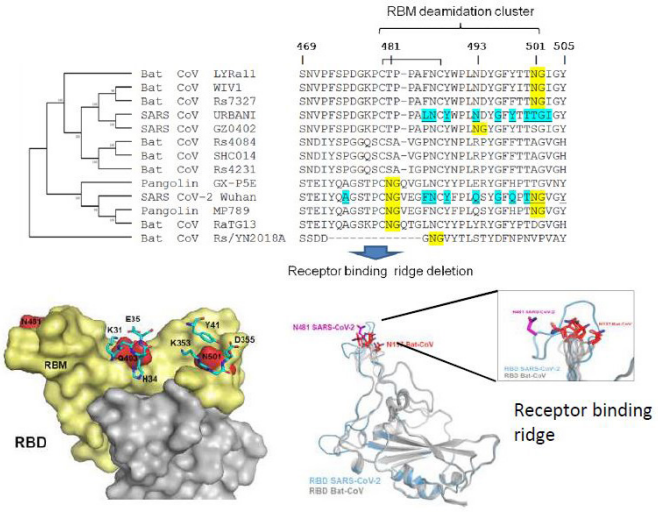


FIG 3. Conservation pattern of deamidation hotspots at the RBM of Sarbecoviruses.

This project is a continuation of work, that resulted in the following publication: Lorenzo R (2021) Deamidation drives molecular aging of the SARS-CoV-2 spike protein receptor-binding motif. J Biol Chem.297(4):101175. doi: 10.1016/j.jbc.2021.101175.

xia2.multiplex:a multi-crystal data-analysis pipeline to facilitate collection of data from challenging samples at cryo-temperatures and enable routine room-temperature data collection[3]

 # Neurodegenerative diseases # Nanoparticles # Drug Discovery # IDP

FIG 4

Radiation damage remains an issue for collection of complete diffraction data sets for a range of samples at cryo-temperatures and more so at ambient temperatures. This makes it necessary to merge data from multiple crystals to obtain complete data which is not always straightforward and has required significant manual intervention. To facilitate multicrystal data collection and enable automated data analysis a new program has been developed, xia2.multiplex. The program has been integrated into the auto processing pipelines at Diamond Light Source (part of Instruct Centre UK) and provides near real time results facilitating this mode of data collection and increasing throughput accordingly. The program has also been used to aid in collection of in situ room-temperature fragment/ligand screening. The improved data processing pipeline is now available to users of the MX beamlines at Diamond Light Source.

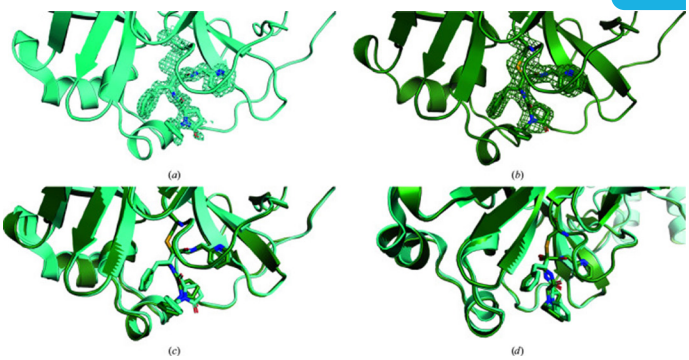


FIG 4. Views of the active site of SARS-CoV-2 Mpro in complex with ABT-957 (Redhead et al (2021) Sci. Rep. 11, 13208 (a) under cryogenic conditions and (b) at room temperature. Contours for the ligand density are drawn at 3σ. (c, d) Two slightly displaced views of the active site of SARS-CoV-2 Mproin complex with ABT-957 to show the conformational differences observed[1]

[3]Gildea, R.J., *et al.* (2022) xia2.multiplex: a multi-crystal data-analysis pipeline. *Acta Crystallogr D Struct Biol.* 78(Pt 6):752-769. DOI 10.1107/S2059798322004399.

Production of the full-length nucleolin for structural studies[1]

 # M. Tuberculosis # Regulation # Transcription Initiation

FIG 1

In an Instruct user project at the Cell-Free Expression service of the instruct Centre FR2 in Grenoble (assisted by J. Boisbouvier and L. Imbert), Carla Cruz and colleagues produced a domain of the protein nucleolin (NCL) with double labelling 15N 13C. At the Health Sciences Research Centre-University of Beira Interior (CICS-UBI), in collaboration with E. Cabrita, they then determined the 3D structure by NMR that illuminated the interaction of NCL with ligands and G-quadruplex (G4) motif found in precursor miRNA 149. The material produced in FR2 has subsequently enabled the team to develop several biophysical techniques for further structural and functional analysis of protein/RNA complexes. Two papers were published in 2022 on these results.

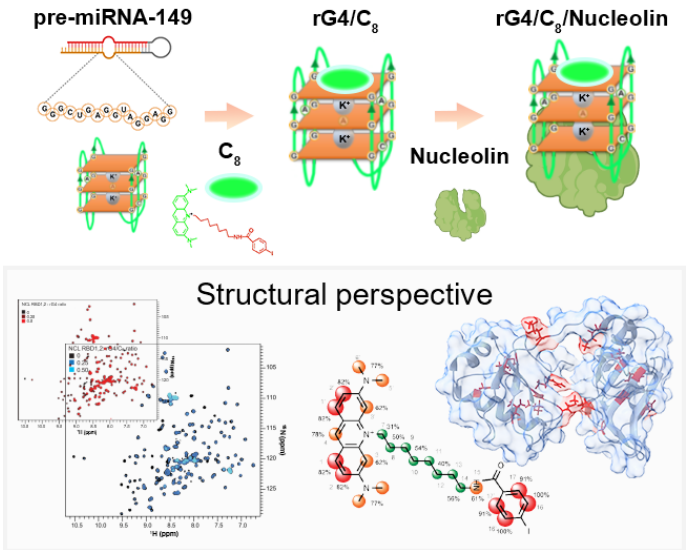


FIG 1. Graphical summary of the structural study of the complex between nucleolin and the RNA G4 found in pre-miRNA 149 stabilized by the compound C8.

[1]Santos, T. *et al.* (2022) Structural Perspective into the Interaction of an Oncogenesis-Relevant pre-miRNA G-Quadruplex Ligand Carrier with the Protein Nucleolin.*J., Chem. Eur. J.* 29, e202301181. DOI 0.1002/chem.202301181

Collaborations with industrial researches to expand solution and solid-state NMR strategies for the assessment of HOS

 # Biologics # Monoclonal antibodies # Industry collaboration

FIG 2

The assessment of the Higher Order Structure (HOS) by NMR is a powerful methodology to characterise the structural features of biologics. The researchers at the Instruct Centre IT have established an excellent collaboration with national and international pharmaceutical companies for the development of analytical methodologies for the characterisation of monoclonal antibodies and more broadly biological therapeutics. Development of innovative analytical procedures for the characterisation of antibodies against COVID-19 and anti-tumor have recently emerged from this research activity. These protocols, however, are more applicable and relevant in a broader sense and serve as a benchmark for characterising biological therapeutics. Recently, results of two projects carried out in collaboration with GSK Vaccines and Merck Serono respectively were published in specialised reference journals. In the first article published in Pharmaceuticals[2], the researcher of CERM/CIRMMP compared 2D ^{1}H-^{13}C ALSOFAST-HMQC NMR spectra with immunochemical ELISA-based analysis to evaluate their sensitivity in assessing the HOS of a potent human monoclonal antibody (mAb) for the treatment of coronavirus disease 2019 (COVID-19). The study confirmed that the methyl region of the 2D ^{1}H-^{13}C NMR spectrum is sensitive to changes in the secondary and tertiary structure of the mAb, more than ELISA immunoassay. Because of its highly detailed level of characterisation (i.e., many ^{1}H-^{13}C cross-peaks are used for statistical comparability), it was proved that the NMR technique provides a more informative outcome for the product characterisation of biopharmaceuticals. In the second article solid-state NMR has been used to identify the pattern of the residues of the programmed cell death ligand 1 (PD-L1) ectodomain that are involved in the interaction with a new multispecific biological drug[3]. This is possible because the large size and the intrinsic flexibility of the complexes are not limiting factors for solid-state NMR. These findings show the potential of solid-state NMR and provide new perspectives for the characterisation of biological therapeutics

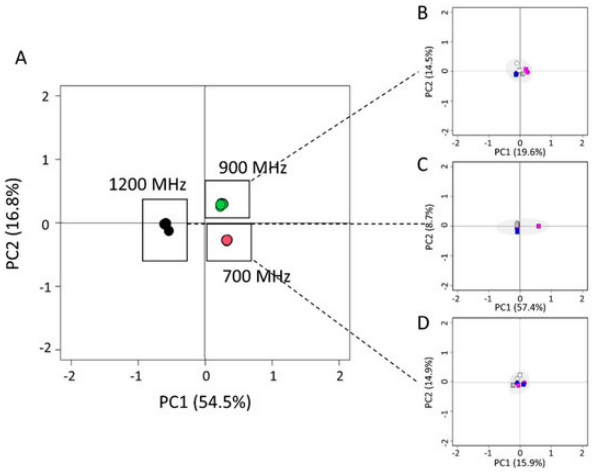


FIG 2. PCA score plot of 1H-13C methyl correlation spectra acquired on batches 19G and 20G at 700 (red dots), 900 (green dots), and 1200 MHz (black dots) over time[1].

[2]Cantini, F. *et al.* (2022) 2D NMR Analysis as a Sensitive Tool for Evaluating the Higher-Order Structural Integrity of Monoclonal Antibody against COVID-19. *Pharmaceutics.*14(10):1981. DOI 10.3390/pharmaceutics14101981

[3]Rizzo, D. *et al.* (2022) Epitope Mapping and Binding Assessment by Solid-State NMR Provide a Way for the Development of Biologics under the Quality by Design Paradigm. *J Am Chem Soc.* 144(22):10006-10016. DOI 10.1021/jacs.2c03232

RAssembly principles of the leptin receptor signalling complex via AUC[4]

 # Obesity # Immunity # Fertility # Cancer

The adipokine Leptin activates its receptor LEP-R in the hypothalamus to regulate body weight and exerts additional pleiotropic functions in immunity, fertility and cancer. Despite decades of research on leptin and its receptor as key regulators of body weight and homeostasis, the mechanism for the assembly of the leptin signaling complex is poorly understood at the molecular level. The overarching goal of this research project was to dissect the assembly principles of the leptin signalling complex via a multidisciplinary approach that combines biophysical studies (SEC-MALLS, AUC, BLI, ITC) and high-resolution structural studies (crystallography and cryoEM) with single-molecule fluorescence microscopy studies on living cells. The user access to the biophysical platform to study the assembly of higher-order leptin-receptor complex via analytical ultracentrifugation (AUC). In the paper[4], the authors show by X-ray crystallography and cryo-EM that,in contrast to long-standing paradigms, Leptin induces type I cytokine receptor assemblies featuring 3:3 stoichiometry and demonstrate such Leptin-induced trimerization of LEP-R on living cells via single-molecule microscopy.

FIG 3

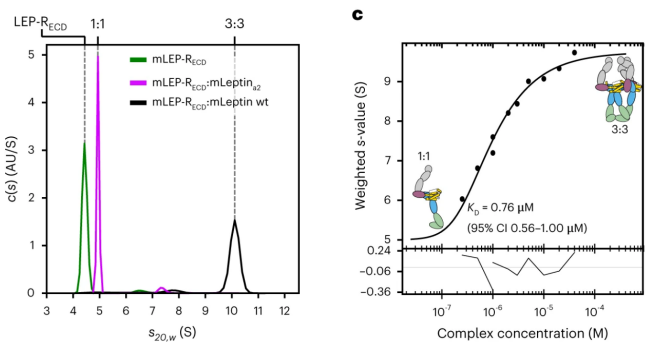


FIG 3. AUC-SV to determine the KD of the 1:1 to 3: 3 self-association.[1]

[4]Tsirigotaki, A., *et al.* (2023) Mechanism of receptor assembly via the pleiotropic adipokine Leptin. *Nat Struct Mol Biol* 30, 551–563. DOI /10.1038/s41594-023-00941-9 .

Structure and Function of a Dodecameric Machine: The Human RuvBL1/2 and its Role in the PAQosome

 # Internship # cryo-EM

The Instruct-ERIC Internship at the University of Helsinki aimed at provision of structural insights on the macromolecular Particle for Arrangement of Quaternary structure (PAQosome), which is a multi-unit chaperone complex essential in cellular processes. PAQosome consist of proteins RuvBL1, RuvBL2, UXT and URI1. During the 3-months internship, PhD student Catarina Paiva from ITQB-NOVA, Portugal prepared homogeneous samples of two sub-PAQosome sub-complexes – RuvBL1/RuvBL2/UXT and RuvBL1/RuvBL2/URI1 – at the Biomolecular Complex Purification facility utilising AF4 and the samples were structurally characterised by single-particle cryo-EM and image processing at the cryoEM facility (Fig. 4). The internship included training in AF4 and cryo-EM sample preparation and data analysis for both techniques.

FIG 4

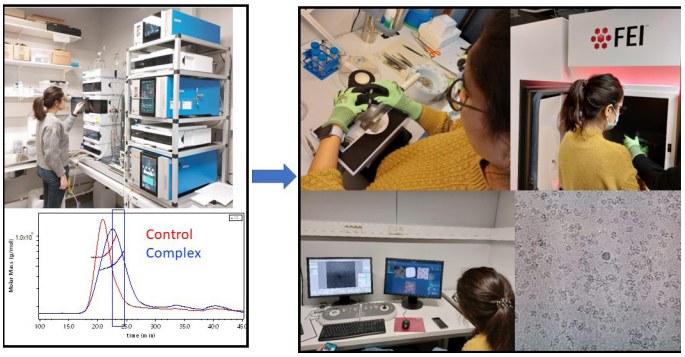


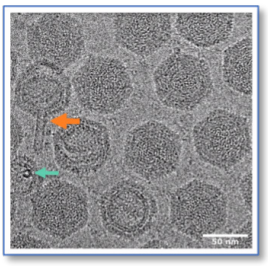
FIG 4. 4 AF4 fractionation of PAQosome sub-complexes (left, blue line) allowed collection of high MW sample components followed by grid preparation, sample screening, cryo-EM data collection (right) and data processing.

Cryo-EM structure of a proteo-lipidic viral tail-tube for viral genome

 # cryo-EM # Virology

Instruct-ERIC users Ane Martinez-Castillo and Nicola Abrescia from CIC BioGUNE in Spain received cryo-EM access at NeCEN to pursue their research on bacteriophages infecting bacteria. Even though the data is still being combined with other results and analysis is still ongoing, the work has been presented by the users to an audience of 60-70 people during the Instruct-ERIC *Structure Meets Function* webinar on 12 April 2022 that was composed and moderated by Instruct-NL staff.

FIG 5



BE

Molecular mechanisms of Sodium-Taurocholate Cotransporting Polypeptide[1]

 # Nanobodies # Antiviral-Drug Target #Liver Disease Therapies

The liver takes up bile salts from blood to generate bile, enabling absorption of lipophilic nutrients, and excretion of metabolites and drugs. Human Na+-taurocholate co-transporting polypeptide (NTCP) is the main hepatic bile salt uptake system, and the cellular entry receptor of human hepatitis B and D viruses (HBV/HDV), constituting an important antiviral-drug target. Here, we present human NTCP cryo-electron microscopy structures in complexes with nanobodies, revealing important architectural and mechanistic aspects of its function. The structures show an unexpected conformational transition for an active transporter, whereby NTCP opens a wide transmembrane pore that serves as the transport pathway for bile salts, and exposes key determinants of HBV/HDV binding on its surface. A nanobody that precludes pore opening impairs HBV/HDV recognition, demonstrating strong selectivity of the viruses for NTCP conformational state.

FIG 1

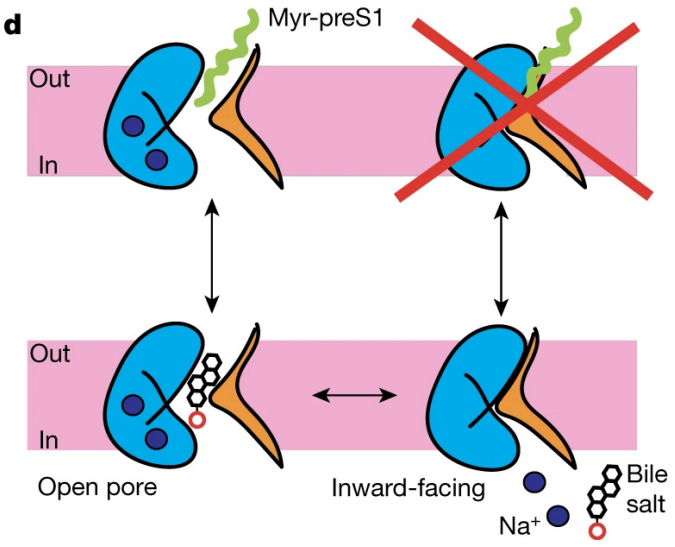


FIG 1. Cartoon representation of the NTCP gated-pore transport mechanism and the relative movements of the core (blue) and panel (orange) domains. The myr-preS1 domain of HBV/HDV (green) preferentially binds to open-to-outside states of the NTCP transport cycle[2].

[1]Goutam, K., *et al.* (2022) Structural basis of sodium-dependent bile salt uptake into the liver. *Nature* 1–6. DOI 10.1038/s41586-022-04723

IL

The importance of X-ray data collection at room temperature (RT) & cryogenic temperatures for studying the conformational changes induced upon ligand binding to 2-Enoyl-CoA Hydratase

 # Mitochondria # Regulation # Transcription Initiation

This project aims to tailor the substrate specificity properties of rat, mitochondrial 2E-enoyl-CoA hydratase (ECH), such that the biocatalytic properties of this enzyme can be exploited for making high-value compounds that can be used as intermediates in the synthesis of new therapeutics.

ECH is a CoA dependent enzyme of the β-oxidation fatty acid degradation pathway. The enzyme catalyzes the hydration of the 2E-enoyl-CoA substrate (Fig. 2) to the 3S-hydroxyacyl-CoA product.

FIG 2

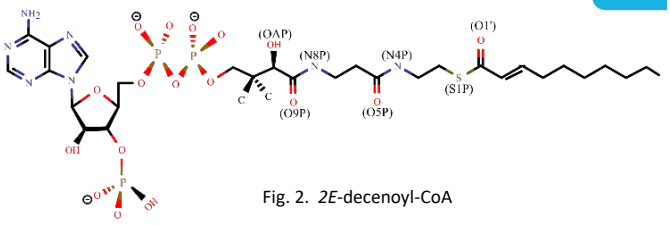


Fig. 2. *2E*-decenoyl-CoA

The enzyme belongs to the crotonase fold enzymes. The acyl tail of the physiological substrate is the linear 2E-enoyl moiety having chain lengths between 4 and 16 carbon atoms. The 3'-phosphate-5'-phosphate ADP moiety of CoA functions as an anchor when these substrates bind to the enzyme allowing the thioester-2E-enoyl tail to bind at the catalytic site.

The results of the crystallographic binding studies that have been done until now, suggest that the crystal treatment required for cryo cooling of the ECH crystals can influence the mode of binding of the thioester-2E-enoyl tail of 2E-enoyl-CoA substrates and substrate analogous. Therefore, collection of the X-ray data required RT to isolate the binding mode of this ligand. For this study, Dr. Subhadra Dalwani from the University of Oulu, Finland visited the SPU at the Weizmann Institute of Science (WIS), part of Instruct Centre IL, in November 2022 and used the Rigaku liquid metal jet (LMJ) X-ray diffraction system at RT which can provide information on the conformational heterogeneity of the protein, and its conformation when bound to its protein partner or ligand, and help to relate such information to function.

The experiments performed at WIS were aimed at characterising the diffraction properties of the ECH crystals at RT, using the plate scanning in situ data collection possibilities of the Rigaku LMJ X-ray system. The in situ data collection protocol is in several important ways different from the standard data collection from cryoprotected crystals. (i) It allows characterising the crystals, without removal from the original mother liquor and (ii) there is no need for cryoprotection in a stream of liquid nitrogen. Therefore, the major questions related to the project have been (i) is it possible to collect high-resolution data from the ECH crystals using the RT/cryo LMJ setup at Weizmann and (ii) to what extent does the mode of binding of the acyl tail differ when comparing RT data collection and cryo data collection. Comparing data collection at RT (at Weizmann) from 7 crystals to that obtained on a single crystal at Cryogenic temperature in the University of Oulu revealed a large variation in diffraction properties, which previously was not observed when doing the routine cryo-data collection. There is also a large variation in cell dimensions upon freezing, which could influence the mode of binding of the ligands.

Single particle cryo-EM of functional RNA polymerase complexes coupled to DNA topoisomerase

 # Cryo-EM # Regulation of Gene Expression

The project aimed to combine biochemistry and structural biology to understand how transcription is regulated and coupled to other important cellular processes. RNA polymerase (RNAP), which transcribes DNA to RNA, is a processive enzyme. It moves unidirectionally along DNA. As a consequence, topological stress builds up in DNA as a function of transcript length.

DNA supercoiling affects transcription because it produces hindering forces and may stall RNAP. Thus, DNA topology affects gene expression directly or indirectly. Biochemical data suggests a direct interaction of bacterial RNAP with DNA topoisomerase I (TopoI), which releases negative supercoils forming upstream of a transcribing RNAP. Supercoiling affects transcription initiation and pausing, which is a common and temporary interruption of transcription and plays a key role in transcription regulation. Cryo-EM reconstructions of *Escherichia coli* RNAP bound to transcription factors NusA and NusG showed that RNAP conformational changes correlate with transcriptional pausing.

FIG 3

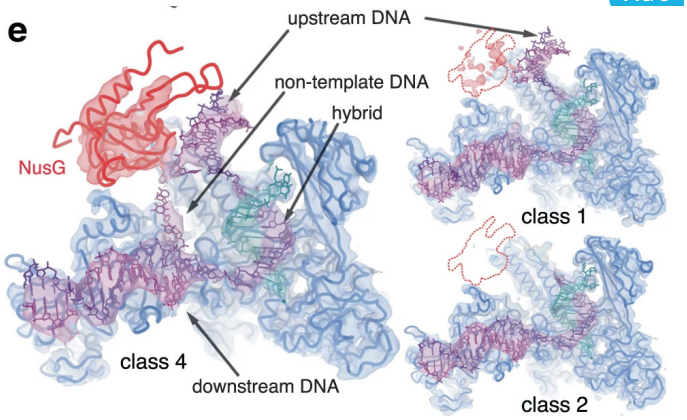


FIG 1. NusG (red) stabilises the upstream DNA duplex and the non-template DNA in the transcription bubble. Electron density of non-template DNA for NusG-containing class 4 (left) and classes lacking NusG (right).

[3]Zhu, C. *et al.* (2022) Transcription factors modulate RNA polymerase conformational equilibrium. *Nat Commun* 13, 1546 (2022). DOI 10.1038/s41467-022-29148-0

Cryo-EM structures of the smallest programmable nuclease TnpB[4]

 # Neurodegenerative diseases # Nanoparticles # Drug Discovery # IDP

CRISPR-Cas nucleases, such as Cas9 or Cas12, also known as gene scissors, have revolutionized the field of genome editing by enabling precise editing of genomes and correction of disease-causing mutations. However, the large size of Cas9 or Cas12 limits their delivery to target cells using Adeno-associated viruses (AAV), which are already utilized in gene therapy.

In their previous publication[4], scientists from VU LSC within Instruct-LT reported the discovery of a new class of programmable nucleases called TnpBs. These nucleases are associated with mobile genetic elements known as transposons. The researchers demonstrated that TnpB is the smallest programmable nuclease capable of efficient gene editing. However, the structural organisation and mechanism of TnpB remained unknown.

FIG 4

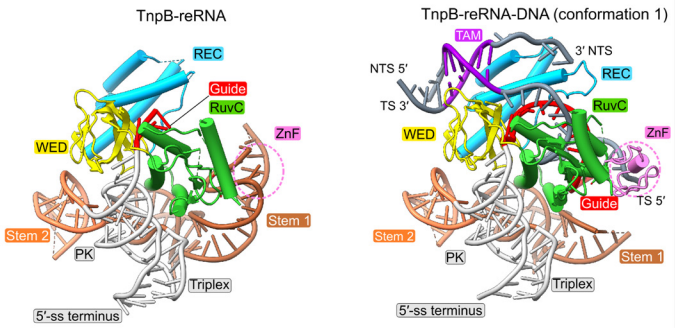


FIG 4. Cryo-EM structures of the D. radiodurans ISDra2 TnpB binary and ternary complexes[2].

In 2022, they made significant progress by solving three different structures of TnpB using cryo-EM. These structures included the TnpB-reRNA binary complex and TnpB-reRNA-DNA ternary complex in two different conformations. The cryo-EM studies unveiled the fundamental architecture of TnpB nuclease and provided insights into its molecular mechanism for DNA target recognition and cleavage.

Moreover, this study confirmed that TnpB represents the minimal structural and functional core of the Cas12 protein family, laying the foundation for the development of TnpB-based genome editing tools. The results were published in Nature in 2023[5]. This first cryo-EM study conducted by VU LSC scientists marks the dawning of a new era for the structural studies in Lithuania.

[4]Karvelis, T., *et al.* (2021) Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease. *Nature* 599, 692–696. DOI 10.1038/s41586-021-04058-1

[5]Sasnauskas, G., *et al.* (2023) TnpB structure reveals minimal functional core of Cas12 nuclease family. *Nature* 616, 384–389. DOI 10.1038/s41586-023-05826-x

Structural studies of spider silk N-terminal domain

 # Spider Silk # NMR # X-ray Crystallography

Spider silk is one of nature's strongest materials, which is formed through a precisely controlled structural conversion of the silk proteins. A complete understanding of the spider silk formation mechanism could allow the research team from Instruct-LV to reproduce it and make artificial spider silk with equivalent properties. The team performed structural studies of the spider silk N-terminal domain under variable environmental conditions using solution and solid-state NMR as well as X-ray crystallography. The studies revealed that pH-dependent dimerisation of the N-terminal domain is conserved between different silk types, although the residues that mediate this process are different. This highlights the importance of the N-terminal for fiber assembly. Furthermore, at elevated temperatures the N-terminal domain was found to form self-supporting and transparent hydrogels useful for immobilisation of active proteins, which is caused by an α-helix to β-sheet conversion and formation of amyloid-like fibrils. The work was performed as part of a collaboration with Profs. Jan Johansson and Anna Rising from Karolinska Institute in Stockholm, Sweden. The study has resulted in three research papers in 2022.

Arndt T., *et al.* (2022) N-terminal domain forms amyloid-like fibril based hydrogels and provides a protein immobilization platform. *Nat. Commun.* 13: 4695.

Šede M., *et al.* (2022) Solution Structure of Tubuliform Spidroin N-Terminal Domain and Implications for pH Dependent Dimerization. *Front. Mol. Biosci.* 9: 936887.

Sarr M., *et al.* (2022) The dimerization mechanism of the N-terminal domain of spider silk proteins is conserved despite extensive sequence divergence. *J. Biol. Chem.* 298: 101913.

Aromatic amino acid rotations: resolving a long-standing paradox in protein dynamics [1]

 # NMR # Protein Dynamics

FIG 1

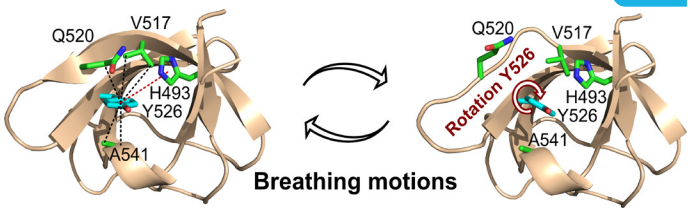


FIG 1. Structural insights into the protein breathing motions that are associated with ring flipping.

Nuclear magnetic resonance (NMR) studies conducted in the 1970s surprisingly demonstrated that aromatic amino acids in proteins can undergo rotations. Paradoxically, these aromatic amino acids are, in many cases, located in the hydrophobic core, where they engage in multiple interactions to maintain the protein's folding and thus its function. At the time, it was proposed that large-scale 'respiratory motions' of the protein were required to facilitate these rotations, but until now the structural details of these motions have remained enigmatic. By combining NMR spectroscopy and X-ray crystallography, researchers from IBS (NMR Flexibility and Protein Dynamics Group) in collaboration with researchers from IAB (Palencia Group) using the solution NMR service at Instruct Centre FR2 in Grenoble have been able to reveal for the first time the structural changes associated with aromatic rotations in the core of a protein. The study shows how a void volume is generated around the aromatic to allow rotation of its side chain. This discovery has implications for both protein design and structure prediction, highlighting how even small alterations in the delicate balance of interactions that stabilise the hydrophobic core can lead to major changes in protein structure.

[1]Mariño Pérez, L. *et al.* (2022) Visualizing protein breathing motions associated with aromatic ring flipping. *Nature* 602, 695–700. DOI 10.1038/s41586-022-04417-6

SAFE Coating - anti viral coating for wide spread use

 # Mass Spectrometry # COVID-19 # Industry Collaboration

SARS-CoV-2 viable viruses were detected for more than 72 hours on several surfaces. A surface able to neutralise the virus quickly will eliminate the possibility of virus transfer to humans body through surface contact. Biomimetx, a biotech start-up, developed an additive able to degrade biological macromolecules, once incorporated in a coating or paint, hence neutralising viruses coming into contact with additive coated surfaces.

Research was focused on structural studies of the spike protein, monitoring its stability loss, dissociation and degradation, mainly through native mass spectrometry. Our results shown that the active principles that may be incorporated into paints and other surface coating materials is capable to cause spike trimer dissociation through proteolysis. All spike variants tested were shown to be susceptible, paving the way for the creation of SarsCOV2 inactivating surfaces that may be useful in places with high viral loadings such as health care units and test centers. This project was made possible through Instruct-ERIC and the availability of its advanced structural facilities, in this case, native MS at Instruct-PT.

FR1

Structural transitions in the Mycobacterium tuberculosis transcription complexes: a basis for deciphering mechanisms of drug resistance[2]

 # M. Tuberculosis # Regulation # Transcription Initiation

FIG 2

Mycobacterium tuberculosis, causing agent of tuberculosis, is one of the most widespread human pathogens that has infected one third of the world population and continues to kill ~1.5 millions people each year (WHO report 2013). Development of a new therapeutic strategies and identification of the new drug targets necessitates deep understanding of the molecular mechanisms employed by bacteria to invade host and to survive antibiotic treatment. In the paper, results reveal that a non-conserved region of the stress factor σ is an allosteric controller of transcription initiation and demonstrate how basal transcription factors can regulate gene expression by modulating the RNAP holoenzyme assembly and hibernation.

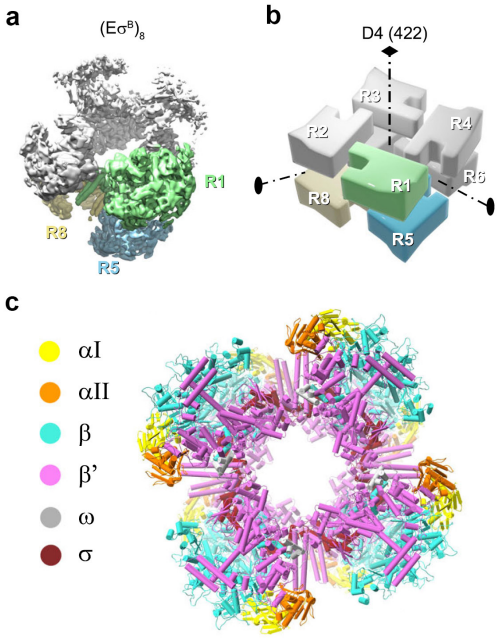


FIG 1. Cryo-EM structure of the M. tuberculosis EσB octamer.

a. Cryo-EM map of the octamer (EσB)8. RNAP protomers with well-defined density are in light green (protomer R1), sky blue (protomer R5), and khaki (protomer R8). b. 3D-model of the D4 symmetric octamer with the protomers numbered R1 to R8 and the symmetry axes indicated. Color codes are as in a. c. Molecular model of the octamer (EσB)8. Views from the top (protomers R1, R2, R3, R4) with the RNAP subunits color-coded as indicated on the left[2]

[2]Morichaud, Z. *et al.* (2023) Structural basis of the mycobacterial stress response RNA polymerase auto-inhibition via oligomerization. *Nature Comm.* DOI 10.1038/s41467-023-36113-y

CZ

Structure, assembly and substrate channeling of membrane associated E. coli anaerobic trifunctional enzyme (anEcTFE)[3]

 # Fatty Acid β-Oxidation # Trifunctional Enzyme #Crotonase Fold

FIG 3

Facultative anaerobic bacteria such as *Escherichia coli* have two $\alpha_2\beta_2$ heterotetrameric trifunctional enzymes (TFE), catalysing the last three steps of the β-oxidation cycle: soluble aerobic TFE (EcTFE) and membrane-associated anaerobic TFE (anEcTFE), closely related to the human mitochondrial TFE (HsTFE). The cryo-EM structure of anEcTFE and crystal structures of anEcTFE-α show that the overall assembly of anEcTFE and HsTFE is similar. However, their membrane-binding properties differ considerably. The shorter A5-H7 and H8 regions of anEcTFE-α result in weaker α-β as well as α-membrane interactions, respectively. The protruding H-H region of anEcTFE-β is therefore more critical for membrane-association. Mutational studies also show that this region is important for the stability of the anEcTFE-β dimer and anEcTFE heterotetramer. The fatty acyl tail binding tunnel of the anEcTFE-α hydratase domain, as in HsTFE-α, is wider than in EcTFE-α, accommodating longer fatty acyl tails, in good agreement with their respective substrate specificities.

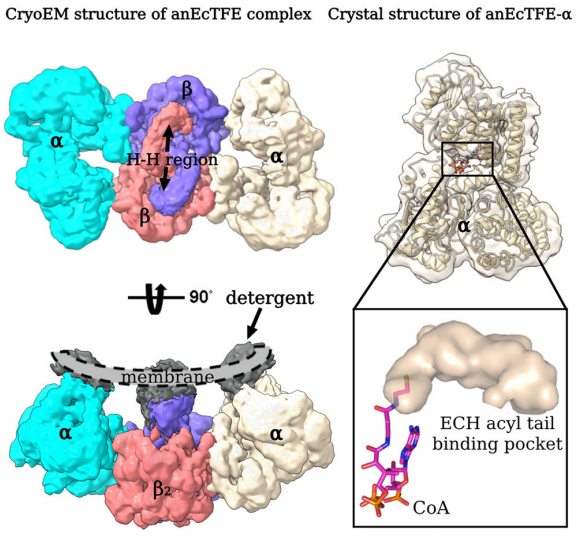


FIG 3. Graphical summary of the worked performed at CEITEC on the structure, assembly and substrate channeling of membrane associated *E. coli* anaerobic trifunctional enzym[3].

[3]Sah-Teli *et al.*, (2023) Structural basis for different membrane-binding properties of E. coli anaerobic and human mitochondrial b-oxidation trifunctional enzymes. *Structure* 31, 812–825. DOI 10.1016/j.str.2023.04.011

Nanoparticle-induced conformational transitions of the amyloidogenic tau protein[1]

 # Neurodegenerative diseases # Nanoparticles # Drug Discovery # IDP

FIG 1

Understanding the interactions between nanoparticles (NPs) and proteins is crucial for the successful application of NPs in biological contexts. Protein adsorption is dependent on particle size, and protein binding to ultrasmall (1–3 nm) NPs is considered to be generally weak. Most studies have involved structured biomacromolecules, while the interactions of ultrasmall NPs with intrinsically disordered proteins (IDPs) have remained elusive. IDPs are abundant in eukaryotes and found to associate with NPs intracellularly. As a model system, this project at Instruct-IT focused on ultrasmall gold nanoparticles (usGNPs) and the tau protein, a cytosolic IDP associated with Alzheimer's disease. Using site-resolved NMR, steady-state fluorescence, calorimetry, and circular dichroism, it was discovered that tau and usGNPs form persistent multimolecular complexes, suggesting a new type of nano-bio interaction. Specifically, the observed interaction hot spots explain the influence of usGNPs on tau conformational transitions, with implications for the intracellular targeting of aberrant IDP aggregation.

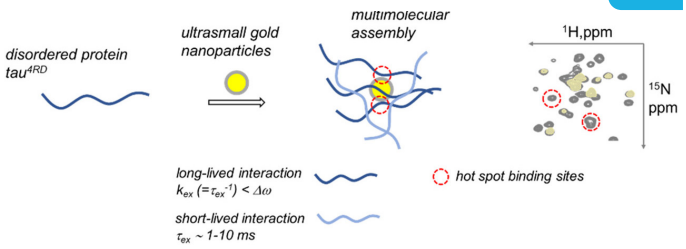


FIG 1. Graphical summary of nanoparticle-induced conformational transitions of the amyloidogenic Ttu protein.

[1]Viola, G. *et al.* (2022) New Paradigm for Nano–Bio Interactions: Multimolecular Assembly of a Prototypical Disordered Protein with Ultrasmall Nanoparticles. *NanoLett.* ,22, 8875–88828876. DOI 10.1021/acs.nanolett.2c02902

IS21 family transposase cleaved donor complex traps two right-handed superhelical crossings[2]

 # Transposase # cryo-EM

FIG 2

Spinola-Amilibia *et al.* have used a structure-function approach to define the molecular determinants by which IStA, a transposase present in the widespread IS21 family of mobile elements, catalyzes efficient DNA transposition. They determined the structure of IStA using cryo-EM to a resolution of 3.4 A and showed that it self-assembles into a highly intertwined tetramer that synapses two supercoiled terminal inverted repeats (Fig 1). The work confirms earlier biochemical data and shows the transposase engages the transposon ends using a mechanism similar to that of retroviral integrages.

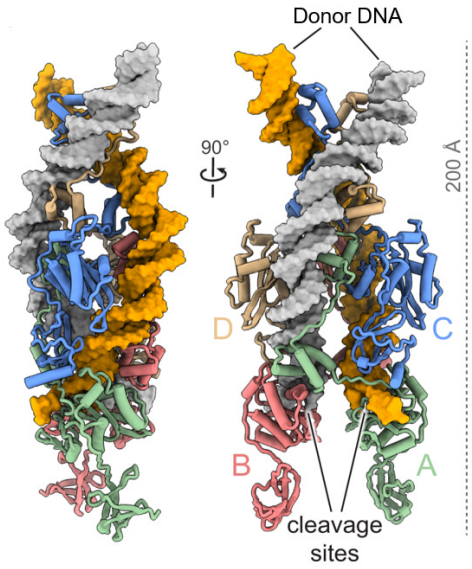


FIG 2. Two orthogonal views of the IstA tetramer bound to two pre-cleaved TIR DNAs

[2]Spínola-Amilibia, *et al.* (2023) IS21 family transposase cleaved donor complex traps two right-handed superhelical crossings. *Nat Commun* 14, 2335. DOI 10.1038/s41467-023-38071-x

Structure of the substrate-bound cytidine deaminase[3]

 # X-ray Crystallography # Metabolism

Cytidine deaminases (CDAs) catalyse hydrolytic deamination of cytidine and deoxycytidine to uridine and deoxyuridine. Researchers from VU LSC within Instruct-LT have found that prokaryotic homo-tetrameric CDAs catalyse the nucleophilic substitution at the fourth position of N4-acyl-cytidines, N4-alkyl-cytidines, and N4-alkyloxycarbonyl-cytidines, and S4-alkylthio-uridines and O4-alkyl-uridines, converting them to uridine and corresponding amide, amine, carbamate, thiol, or alcohol as leaving groups[3]. A 1.2Å X-ray structure of a metagenomic CDA_F14 showcased a relationship between the bulkiness of the leaving group and the volume of the binding pocket. Authors of this work proposed that CDAs that are active toward a wide range of substrates participate in salvage and/or catabolism of modified pyrimidine nucleosides, and play a key role in the cellular turnover of cytidine derivatives, including the pyrimidine-based prodrugs.

[3]Urbeliene, N. *et al.* (2022) Cytidine deaminases catalyze the conversion of N(S,O)4-substituted pyrimidine nucleosides. *Sci Adv.* 9(5):eade4361. DOI 10.1126/sciadv.ade4361

Project Title: Mechanism of action of Ecumicin on the drug target MtbClpC1

 # Antibiotics # cryo-EM # Unfordase # Drug

The discovery of four potent and chemically diverse natural product antibiotics (NPAs) targeting the ClpC1 unfoldase has proven its potential as a target against *Mycobacterium tuberculosis* (Mtb). These NPAs, including Ecumicin, Cyclomarin, Rufomycin, and Lassomycin, are among the most powerful anti-TB molecules discovered recently. However, their complex multi-ring structures pose challenges for medicinal chemistry, and the precise mode of action and follow-on processes leading to Mtb cell death are still largely unknown. ClpC1 consists of a N-terminal domain (NTD) and two ATP-binding modules, D1 and D2. While the full-length structure of ClpC1 was not available until recently, significant structural work has been done on the NTD, which is easier to handle. Interestingly, despite representing only a small portion of the protein, all NPAs have been found to bind to the NTD, and high-resolution X-ray structures of the binding sites have been obtained for Cyclomarin, Ecumicin, and Rufomycin. Although this allows for proper mapping of the binding pockets, it remains unclear how binding to the NTD translates into functional impairment of the rest of the protein. The project from scientists at University of Porto, PT, aims to solve the ClpC1 in its apo, cyclomarin-, and ecumicin-bound states via cryo-EM.

The Electron Microscopy facility we have prepared samples and collected datasets for ClpC1-Ecumycin complex.

In the Instruct Image Processing Center, I2PC has been working on the image processing helping the users to maximise the extraction of biological knowledge from the electron microscopy images using SCIPION package, including movie alignment, particle picking, classification, volume reconstruction.

Thanks to this Instruct project, the first structures of *Mycobacterium tuberculosis* ClpC1 in its apo, cyclomarin and ecumycin states have been determined by cryo-EM, providing a map for further drug development

[4]Weinhäupl, K., *et al.* (2022) Structure of the drug target ClpC1 unfoldase in action provides insights on antibiotic mechanism of action. *J. Biol. Chem.* 298(11) 102553. DOI 10.1016/j.jbc.2022.102553

FIG 3

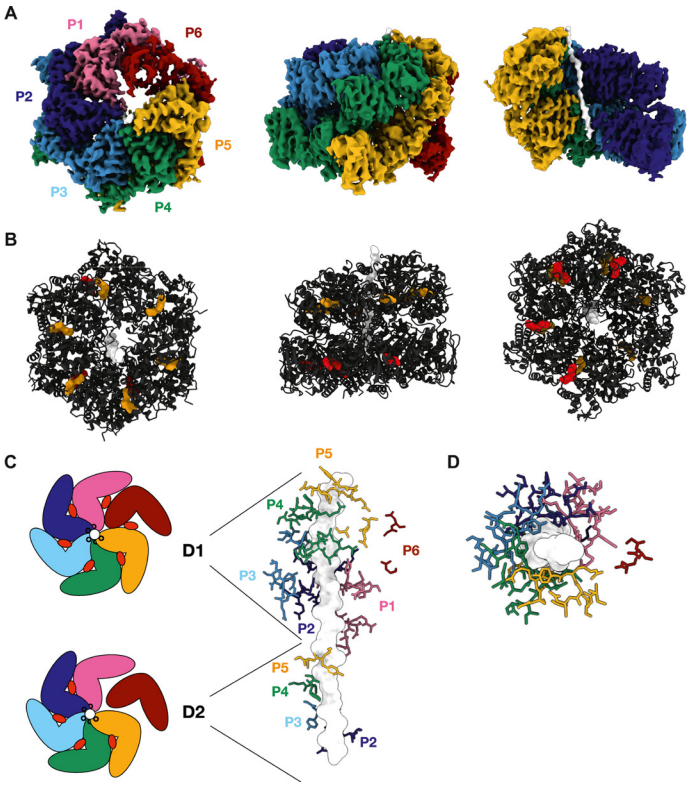


FIG 3. The MtbClpC1 active hexameric structure. A, cryo-EM map of the apo MtbClpC1 hexamer bound to a substrate peptide in top, side view and with the bound substrate visible (without the protomers P1 and P6). Individual protomers are colored independently and labeled P1 to P6. B, occupation of the nucleotide-binding pockets in the MtbClpC1 hexamer with ADP (in the D1 domain orange, in the D2 domain red). From left to right hexamer in top view (substrate entry pore), side view, and bottom view (interface with ClpP1P2). C, cartoon image of the nucleotide occupation in the D1 and D2 domains and the attachment of the pore loops in D1 and D2 to the substrate. P6 is detached from the substrate in both D1 and D2. D, top view of the ClpC1-bound substrate with pore loops attached in the typical spiral arrangement and the pore loop of P6 detached. Mtb, *Mycobacterium tuberculosis*.[4].


7QYD

CORE ACTIVITIES

ACCESS

What is Access?

As a research infrastructure, one of Instruct's core functions is to enable researchers to make use of scientific resources such as high-end technologies, equipment and expertise to facilitate innovation. This process by which researchers can use these scientific services is termed "access". Providing access to integrated structural biology infrastructure is a fundamental task of Instruct-ERIC. Any researcher worldwide can make use of Instruct-ERIC services. They must first submit a short research proposal detailing the research they would like to undertake, and the services from Instruct-ERIC's technology catalogue (instruct-eric.org/platform-catalogue) they wish to access. Proposals then undergo review by experts in structural biology to check that the proposal is of sufficient quality to warrant Instruct-ERIC access. Selection is on the basis of scientific excellence. Following successful scientific review, a proposal is checked for technical feasibility by the Instruct-ERIC centres offering the requested services. If the proposal is deemed technically feasible access can proceed.

Funding for Access

A particular benefit for researchers working in Instruct-ERIC member countries and international organisations is that they are eligible for access funding. This means that access is provided free to the user in most instances, due to a service-dependent contribution to the cost of providing access paid to the Instruct-ERIC centres from the Instruct-ERIC budget. Additionally, a contribution of €400 is granted towards the travel and accommodation (or in the case of remote access, sample shipping costs) of the researcher. Through funding our access programme in this way, Instruct-ERIC helps to democratise access such that the best scientific projects can benefit from the world-leading integrated structural biology technologies and expertise of Instruct-ERIC, regardless of the availability of technologies at their home institution or their ability to pay for access.

New technologies available in 2022

In 2022, Instruct-ERIC expanded its catalogue with three new technologies: Gene tagging, a CRISPR-Cas9 enhanced protein production service in Instruct Centre FR1, Cryo-correlative light and electron microscopy (Cryo-CLEM) in Instruct Centre EMBL, and Kinetic ITC (kinITC) for determination of kinetic and thermodynamic parameters at Instruct Centre FR1, together expanding the breadth of integrated structural biology techniques available for access.

Access through Horizon Europe projects

Through new Horizon Europe projects ISIDORe and canSERV, Instruct-ERIC services are also offered specifically for researchers in thematic areas of infectious disease research and cancer research. Under Horizon Europe, full costs of access provision are covered by the respective grants meaning access is free to European researchers, and to non-European researchers up to a threshold.

ISIDORe opened its first access call in June 2022 for SARS-CoV-2 and COVID-19. A call dedicated to Monkeypox followed in September and Instruct-ERIC services have been popular in these calls with 18 applications received. The canSERV project launched in September 2022 and will be opening its access calls in 2023.

Though all these initiatives benefit the community through the quantity of access to Instruct-ERIC that can be provided and funded and visibility of Instruct-ERIC services in new thematic areas, the different pathways to access Instruct-ERIC services can cause confusion to users of how to make the best use of what is available to them. To make the process as clear as possible, a dedicated webpage was published directing Instruct-ERIC users to the most appropriate access route for their needs. Find it at https://instruct-eric.org/content/funding-through-european-projects

Infectious Disease

ISIDORe

Cancer and Oncology

canSERV
providing cutting edge cancer research services across europe

Translational Research

iNEXT DISCOVERY

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ARIA: Access management software

Access on such a large scale requires intensive process support, enabled by a dedicated team in the Instruct-ERIC Hub and our pioneering access management software ARIA. ARIA is constantly evolving to encompass new features for access management, reporting and application handling. In 2022 developments included the new ability for moderators assessing research proposals to request resubmission of proposals instead of rejecting them, application weblinks to send applicants directly to the application system with a desired service already selected (rather than having to browse the catalogue for it) and improvements to the interface and speed of the ARIA messaging panel used for access and application-related communication.

ACCESS TO INFRASTRUCTURE 2022

Service demand

In the period from 1 January to 31 December 2022, demand for access remained high with 153 proposals were received of which 135 were approved, equating to a 88% approval rate. This high approval rate shows the excellent quality of the research project proposed.

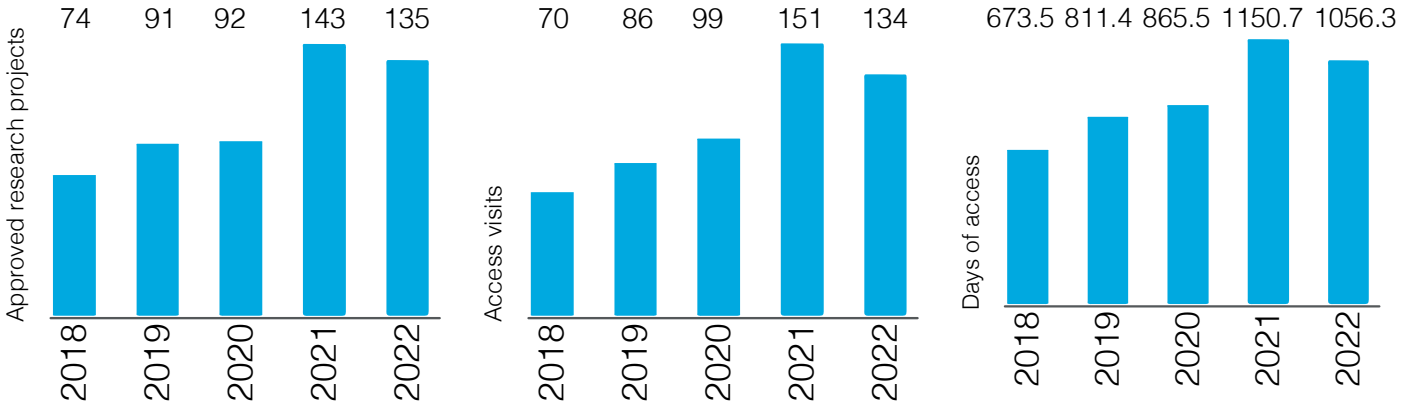


FIG 1. Instruct-ERIC access statistics [status 04.08.2023]

Service provision

In 2022, 134 research visits were completed. 51 of those (38%) were national visits and the majority of 83 (62%) visits was transnational access to a research facility in a different county.

The visits combined to a total of 1056.3 days of access of which 312 days were used nationally (30%) and 744.3 transational (70%).

Services were provided to researchers from 15 different countries (AR, BE, DK, EMBL, ES, FI, FR, IL, IT, NL, PT, SI, SK, UK, UY). Those were majoritively from Instruct-ERIC Members but special calls allowed for access from outside Europe as well and honoured commitments made in 2022 and in the years before.

3D structural analysis technologies (EM, NMR and x-ray) were the most accessed services in 2022. This was followed by sample preparation services (protein production, crystallisation and nanobody discovery) which are essential for for subsequent 3D analysis (Fig.2).

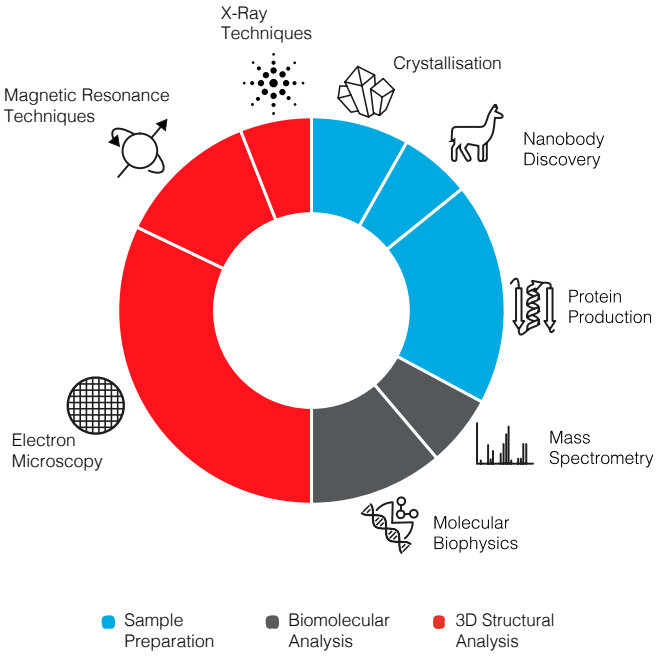


FIG 2. Instruct-ERIC access visits per service technology in 2022 [status 04.08.2023]

Peer-reviewed publications

A key performance indicator of success in this endeavour is the number of publications arising from use of Instruct-ERIC infrastructure, whether this is from the direct access to infrastructure through our Access programme, or through the various training and career development opportunities that Instruct supports (for example, internships, R&D pilot awards and joint research activities).

The list of peer-reviewed papers published in 2022 acknowledged Instruct-ERIC was 329 – even higher than the number achieved in 2020 and 2021.

These publications featured work of researchers from more than 47 different countries. This is indicative of Instruct-ERIC's presence within the global structural biology community – and is further emphasised by Instruct's growing international reputation.

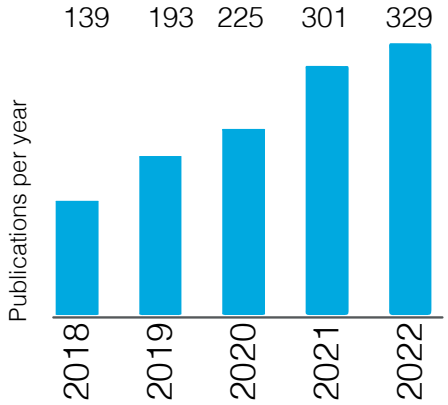


FIG 3. Instruct-ERIC publications statistics [staus 04.08.2023]

TRAINING AND CAREER DEVELOPMENT

The Instruct-ERIC training programme helps researchers to develop new structural biology methods and skills. It includes structured courses, workshops, and online webinars, along with access to tools, resources and expertise. In addition to the dedicated training schedule, Instruct-ERIC also develops structural biology careers through visits to Instruct Centres via the access programme, internships, and R&D projects. The expertise and best practice training available at Instruct Centres can be invaluable.

TRAINING COURSES 2022

Since 2012, 73 training courses have been approved for Instruct funding or co-funding. In 2022, the dedicated training programme included courses on cryo-EM, NMR, X-ray crystallography, cryo-ET, as well as workshops on combining several methods together for true integrated structural biology.

In 2022, Instruct also organised a workshop on the ARIA proposal submission system, allowing users of all research infrastructures using ARIA to ask their questions and see new features. In addition to sponsored courses, Instruct promotes training courses and conferences from Centres and the wider structural biology community on the Instruct-ERIC events page.

4th Instruct hands-on workshop on sample preparation for Single Particle cryo-EM

23-25 May 2022 **Instruct Centre FR2**

This biannual practical workshop has taken place since 2018 at the European Photon and Neutron Campus (EPN) in Grenoble, France. Four local institutes, the European Synchrotron (ESRF), the French Institute de Biologie Structurale (IBS), the European Molecular Biology Laboratory (EMBL Grenoble) and the Institut Laue Langevin (ILL) as well as an instructor from the microscope manufacturer ThermoFisher, provide a theoretical background and practical hand-on instructions of sample preparation for single particle cryo-EM. The course gives scientists at all stages of their career the opportunity to get an intense hands-on experience on the optimal preparation of sample grids. The course has an ever-increasing number of applicants; the practical workshop had 12 participants selected from over 70 applications and half of them opted to bring their own protein samples.

Joint Instruct-iNEXT Course: Integrating X-ray Instruct Practical School on Advanced Isotopic Labelling Methods for Integrated Structural Biology

16-23 September 2022 **Instruct Centre FR2**

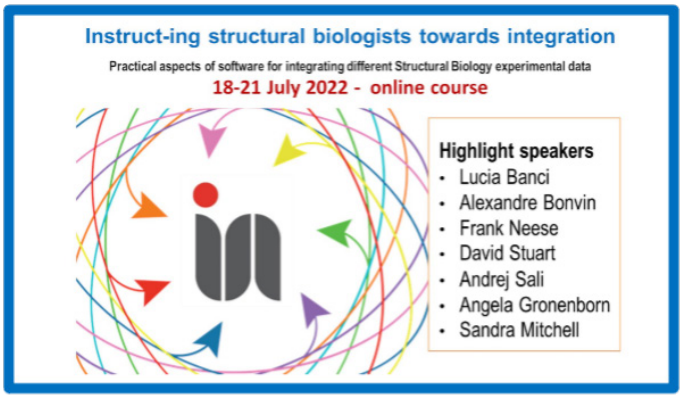
Biomolecular NMR spectroscopy provides invaluable insights into macromolecular structures, dynamics and interactions. Such studies require the production of macromolecules (proteins or nucleic acids) that have been enriched with NMR-visible stable isotopes. Recent advances in isotopic labelling techniques have allowed increasingly complicated biomolecular systems to be investigated by NMR spectroscopy. This Instruct Practical School has provided both practical and theoretical training in state-of-the-art isotopic-labelling approaches for NMR studies. The participants gain hands-on experience in a broad range of labelling methods, including innovative protocols for specific labelling, segmental labelling and in vitro expression of RNA and proteins. The 16 participants were encouraged to bring their own protein and/or RNA constructs for use in practical demonstrations. The School provided an environment in which attendants were able to exchange practical experience on protein labelling with instructors and other participants.

FIGs. All figures in this Instruct-ERIC training programme section correspond to the respective training course to their left.

Instruct-ing structural biologists towards integration

18-21 July 2022 **Instruct Centre IT [Virtual]**

Instruct-IT, together with Institut Pasteur and the Hebrew University of Jerusalem, organised the online course Instruct-ing Structural Biologists Towards Integration, that covered several aspects of integration in structural Biology. The aim of the course was to provide the participants with the possibility of experiencing different software for integrating different Structural Biology experimental data (NMR, Cryo-EM, X-ray, SAXS). Renowned structural biologists presented their research for which integration of different techniques proved to be the only way to achieve the result. The aspects of science communication and philosophical implication of integrative approaches, were also approached.



Crystallography, Nuclear Magnetic Resonance, Magnetic Resonance Mass Spectrometry and Electron Microscopy

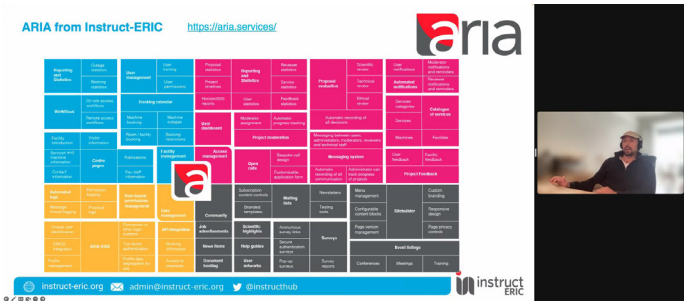
26-30 September 2022 **Instruct PT**

The main objective of this course was to provide the fundamental concepts and integrative hands-on applications of Macromolecular Crystallography (MX), Nuclear Magnetic Resonance (NMR), Magnetic Resonance Mass Spectrometry (MRMS) and single-particle analysis cryo-Electron Microscopy (EM) in structural biology. The major strengths and limitations associated with each technique were also highlighted and discussed along with their complementarity to achieve truly integrative structural biology approaches to tackle challenging scientific projects.

ARIA Training Workshop

3 November 2022 **Instruct Hub [virtual]**

ARIA is a collection of cloud services provided by Instruct to research infrastructures, facilities and user communities, to help drive RI application and management. The ARIA Training Workshop was an opportunity for attendees to learn how to make the best use of ARIA, hear about recent and upcoming new features with opportunities to pose their questions directly to the ARIA development team. The virtual session covered a wide range of ARIA topics, including what it is, how it works, and answering any specific questions proposed by the group; the workshop was aimed primarily at facility managers, researchers, administrators, technical managers, and support staff.



Instruct hybrid course on Electron Tomography by CryoEM

13-16 December 2022 **Instruct Centre ES**

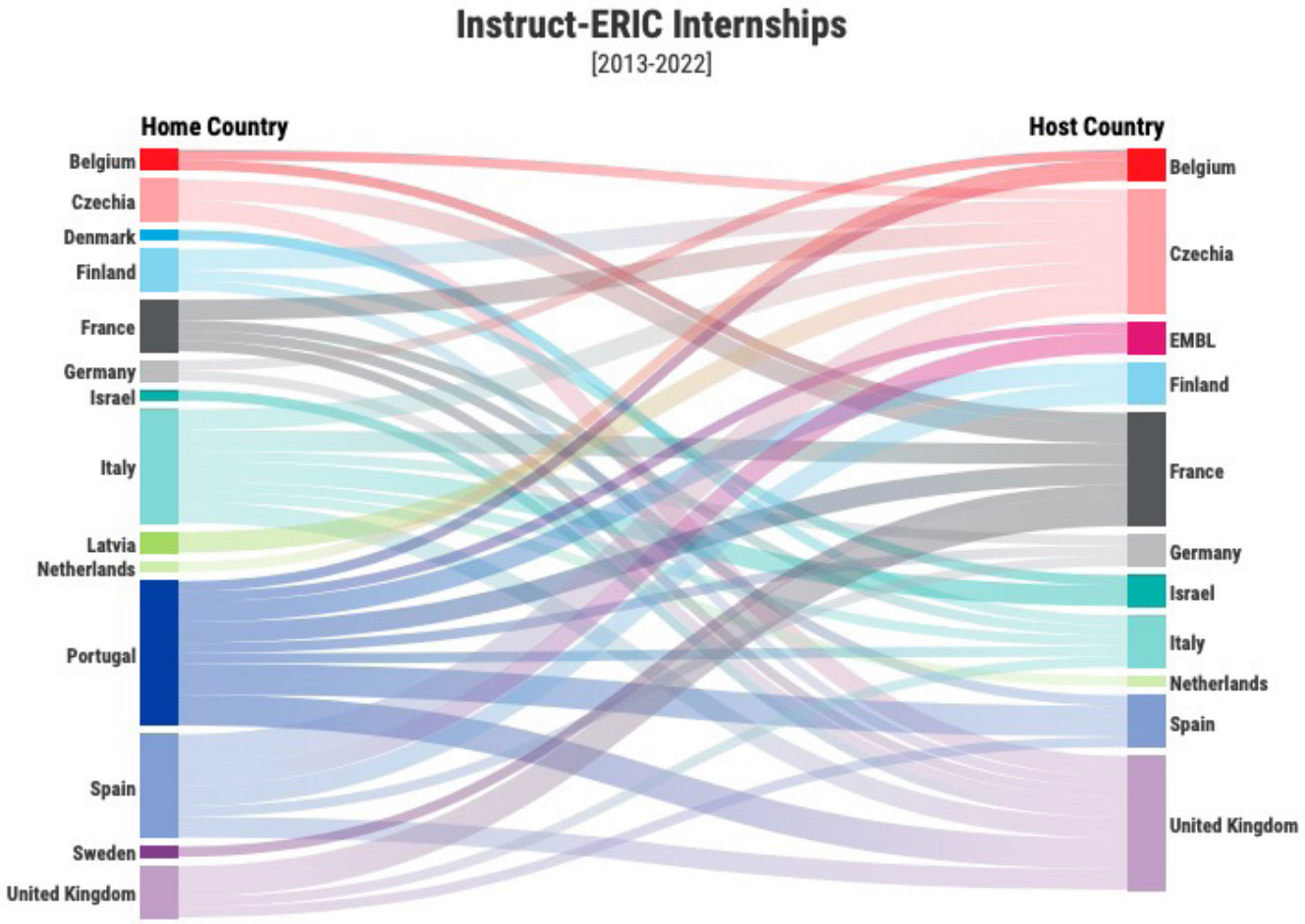
Cryo-electron Tomography is a rapidly growing technique that can visualise cell organelles and biological macromolecules in their cellular context. Image processing algorithms allow the identification of the macromolecules of interest in the electron tomograms. Then, by extracting small subtomograms around those locations and identifying those similar particles, we may average them and recognize their different conformations. This latter process is called subtomogram averaging and has allowed solving a growing number of macromolecules with a resolution between 20-3A.

Guided by the I2PC team, the course aims were to provide a comprehensive overview of the whole image processing pipeline in Electron Tomography (ET) from the movie alignment of the tilt series, 3D reconstruction of the tomogram, particle identification, and subtomogram averaging, all inside SCIPION integration framework.

INTERNSHIPS

The Instruct-ERIC Internship programme aims to train structural and cell biologists in a wide range of technologies with 3 to 6 months visits to Instruct Facilities. The exchange programme provides in-depth skills training for pre-doctoral, and early-stage postdoctoral fellows, along with valuable experience of a different scientific environment, including the implementation of standards, processes and culture. Internships are hosted at a Instruct Facility that specialises in a method or technology from which the intern's own research can benefit. All internships require the intern to exchange with a facility in a different country or member organisation to their own.

Since 2013 Intruct has awarded 63 internship that have facilitated research exchanges across Europe:



In **2022**, 14 internships applciations were approved in three calls accross the year with a 74% approval rate. The awards not only demonstrate a geographical distribution in the awardees but also in the Centres hosting the internships.

Name	Country of Home Organisation	Internship Host
Juan Luis Pacheco Garcia	Spain	Instruct Centre CZ
Lavinia Carlini	Italy	Instruct Centre UK
Janis Rumnieks	Latvia	Instruct Centre CZ
Eros Antonio Lampitella	Italy	Instruct Centre IL
Diogo Athayde	Portugal	Instruct Centre ES
Ginsts Kalnins	Latvia	Instruct Centre CZ
Giedre Ratkeviciute	UK	Instruct Centre ES
Alba Morán Vaquero	Spain	Instruct Centre CZ
Priscillia Lagoutte	France	Instruct Centre UK
Anna Catarina Paiva	Portugal	Instruct Centre FI
Izabella Tambones	France	Instruct Centre CZ
Adria Alcaide Jimenez	Spain	Instruct Centre EMBL
Pieter-Jan Vermeire	Belgium	Instruct Centre CZ
Ines Trindade	Portugal	Instruct Centre IT

R&D AWARDS

From time to time, Instruct-ERIC publishes calls for small scale pilot research projects in integrated structural biology. Resources are allocated to support a limited number of pilot studies proposed by researchers from Instruct member countries. Pilot projects may be funded up to a maximum of €15,000. The funds are expected to cover research expenses but not normally salaries or overheads. The intent of this support is to help researchers develop external funding for projects, i.e. the expectation is that a pilot study will lead to a grant submission to national or international funding bodies.

Instruct has run several calls for R&D projects since 2013, with funding awarded to more than 50 projects in that time. The seventh call was opened in February 2022 and received 58 applications of which six applications were awarded:

Name	Country of Home Organisation
Olivier Duss	EMBL
Angelita Simonetti	France
Michael Assfalg	Italy
Bram Koster	Netherlands
Carla Cruz	Portugal
Peter Harrison	UK

R&D Award Highlights

Angelita Simonetti: R&D Award APPID 2434

Structural and mechanistic studies of the hypoxia-induced translation regulation of HIF-1α mRNA in pediatric high-grade gliomas

Pediatric high-grade gliomas (PHGGs) are common tumors of the central nervous system in children under 15 years. Their molecular profiles are different from adult ones, opening the prospect of developing specific therapies for pediatric gliomas. Hypoxia-Inducible Factor 1α protein (HIF-1α) is a key player in PHGGs enabling cell adaptation to hypoxic stress. Recently, we demonstrated by in vitro translation assay that the RNA G4 (rG4) present within the 5'UTR of HIF1a drives its alternative translation initiation playing a key role on its hypoxia-induced translation regulation. Our main aim is to gain mechanistic insight into the Hypoxia-induced translation initiation mechanism of HIF-1α which expression during O2 stress is related to angiogenesis, cancer progression and metastasis. To achieve our goal, we will obtain the Cryo-EM structures of the human 48S Initiation Complex (48S IC) assembled on HIF1α and its factors composition by mass spectrometry (LS-MS/MS assay). Importantly, we will isolate 48S IC directly from the cell extracts of patient's derived PHGGs cell lines (PDCL-PHGGs) cultured under normoxic and/or hypoxic conditions, using Grad-Cryo-EM method. Studying its hypoxia-induced alternative translation initiation mechanism should provide new opportunities to target specific RNA/protein interactions allowing development of a new class of anticancer drugs targeting PHGGs.

Carla Cruz: R&D Award APPID 2473

Novel approach to control let-7e miRNA biogenesis: Unveiling the relationship between structure and function of let-7e precursor miRNA/nucleolin complex

Our project aims to unveil the relationship between nucleolin, a protein deeply associated with miRNA biogenesis and the let-7e precursor miRNA, that has the potential to adopt a G-quadruplex (G4) structure and is dysregulated in lung cancer. In this project we will label let-7e miRNA precursor for the NMR studies. Biophysical and biochemical techniques will be also considered to fully characterize nucleolin/let 7e precursor miRNA complex and their implication in miRNA biogenesis pathway and lung cancer.

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

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

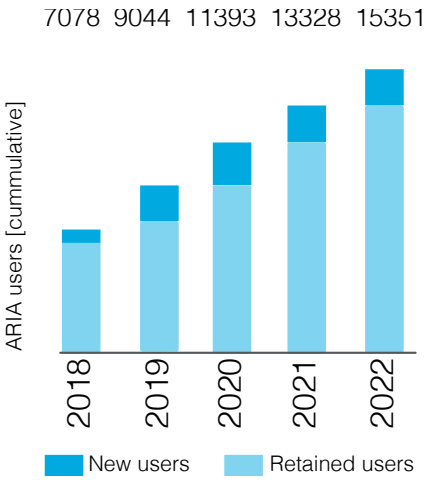
ARIA was initially developed to service Instruct’s own needs due to a lack of suitable commercial software, however it has since grown to support projects and infrastructures throughout the life sciences, and beyond. As other research infrastructures reach maturity and begin offering access to their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

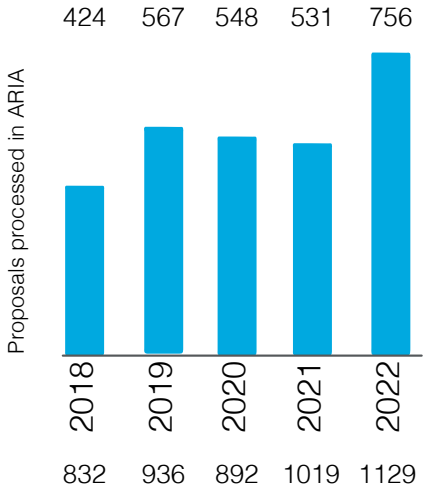
2022 saw the introduction of some powerful new functionality, further expanding the capability of the ARIA platform. This included the introduction of “Access Calls”, allowing access administrators the ability to offer time limited thematic access to all or part of their service catalogue, as well as a new “white label” customisable theme to streamline onboarding of new projects.

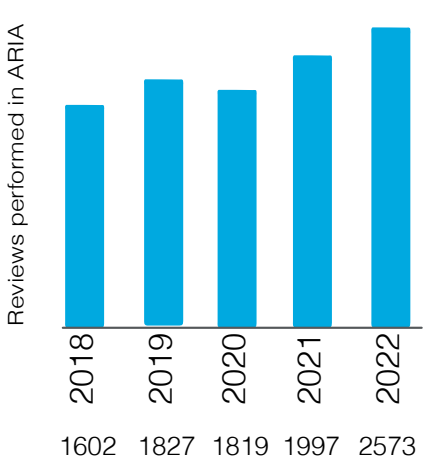
2022 also saw an increased focus on interoperability, and an “API first” development approach, which will allow third party applications the ability to integrate with the platform.

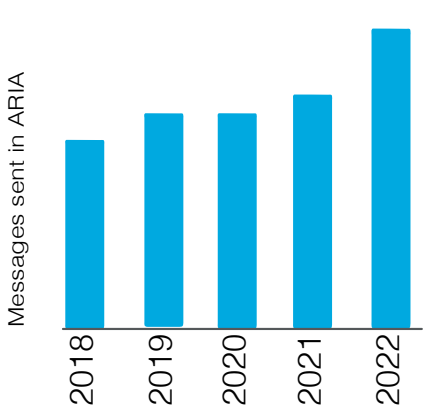
ARIA in numbers

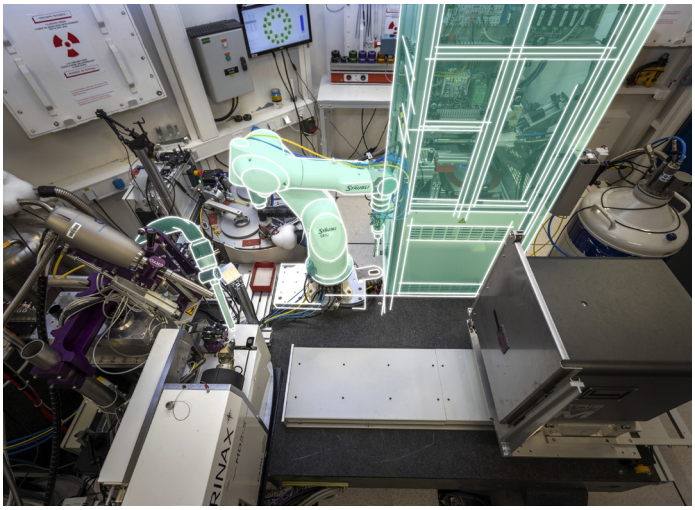
Overall, ARIA welcomed 2023 new users in 2022, bringing the total number of registered users to 15351. A new yearly high of 756 proposals were submitted in ARIA though the different access routes resulting in 1129 performed reviews in 2022. Additionally, the access management was supported by the upgraded messaging system that processed 2573 messages in 2022 connecting the users, access managers, reviews and facility teams.

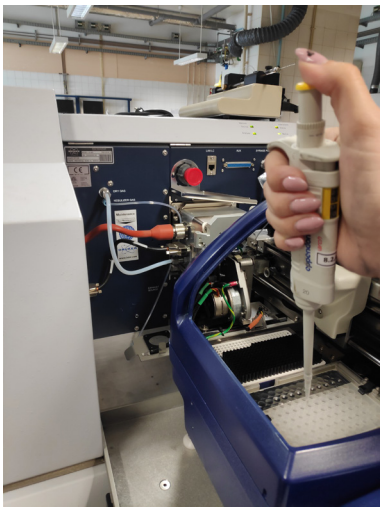


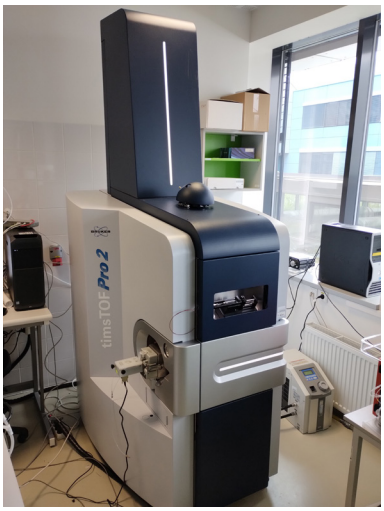








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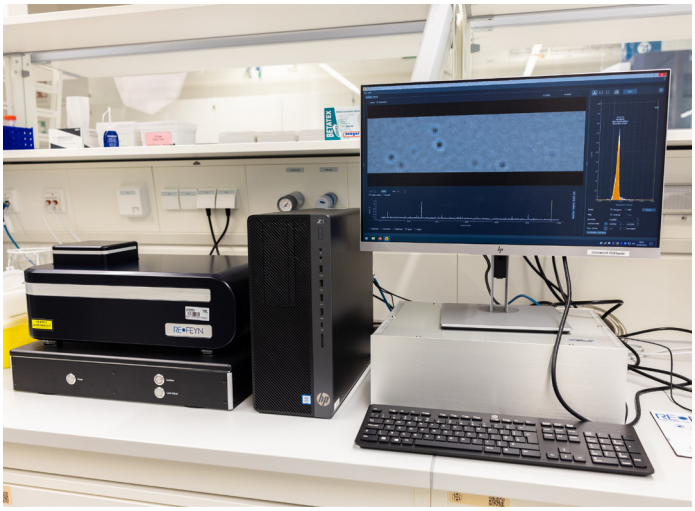
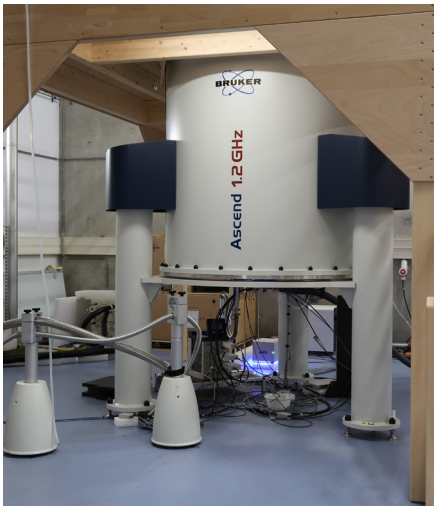



cytiva
Biacore S200


TITAN KRIOS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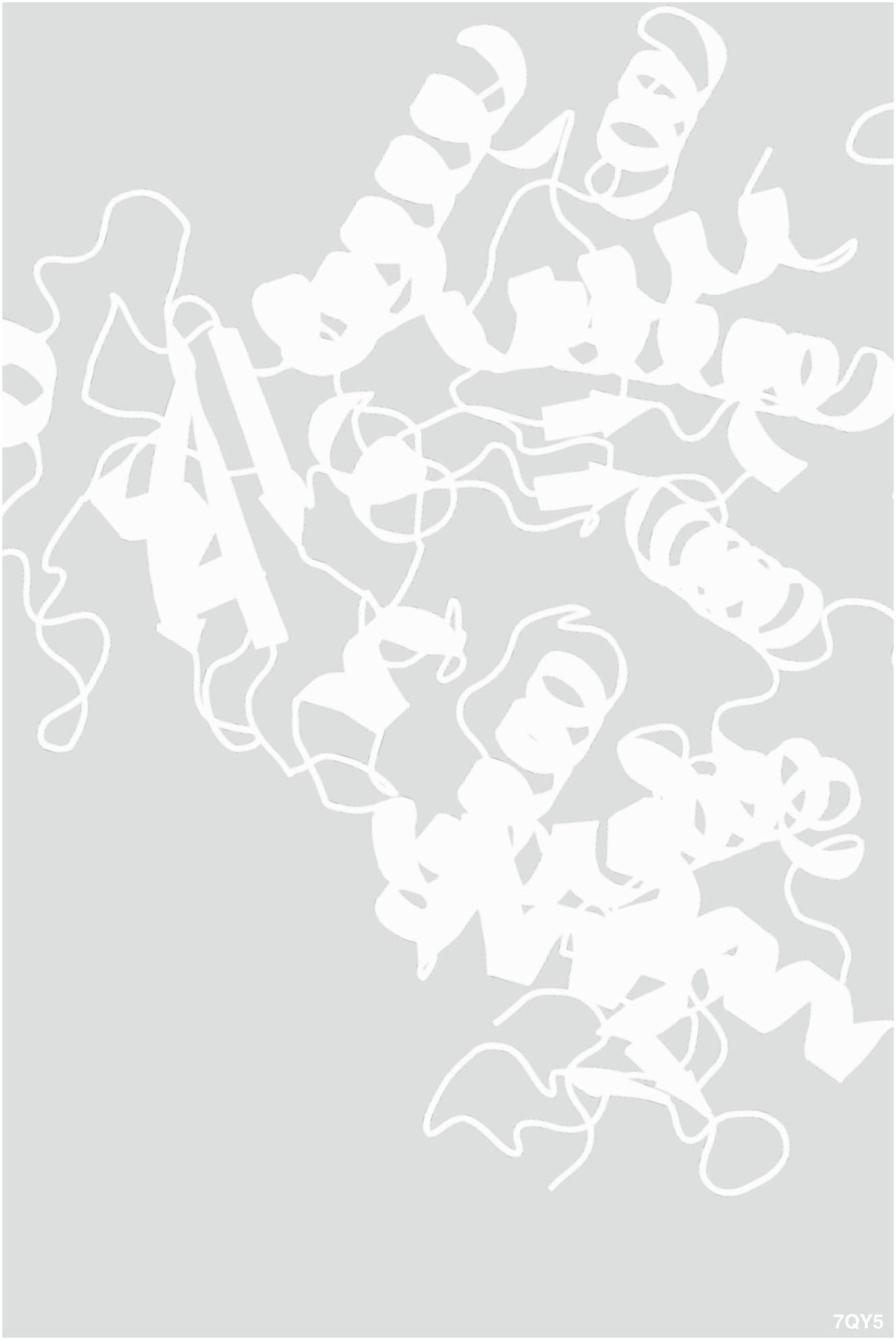
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COLLABORATIVE WORK

EUROPEAN PROJECTS

COORDINATION:

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

This project established working methods and tools tohelp any research infrastructure (across the domains of life sciences, physics, humanities, social sciences etc.) to improve their visibility and impact. RI-VIS helped European research infrastructures to target new communities and to promote international partnerships.

Instruct coordinated the RI-VIS project and co-led the work on international cooperation, analysing the international partnerships and priorities of European research infrastructures and creating tools and resources to aid international partnerships such as a template MOU.

Timeframe:	February 2019 to January 2022
Total budget:	€ 1 500 000
Instruct participation:	Instruct Hub [Coordination]
Instruct budget:	€ 290 266

PARTICIPATION:

AI4Life: Artificial Intelligence for Image Data Analysis in the Life Sciences

AI4LIFE aims to build bridges between the life science community and the machine learning/artificial intelligence community. AI has enormous potential for advancing life sciences however access to the necessary equipment, software and training in AI and machine learning for life science research infrastructures is scarce. AI4LIFE will build an open, accessible, community-driven repository of FAIR pre-trained AI models and develop services to deliver these models to life scientists. Instruct-ERIC is disseminationg the ouputs o this project to the structural biology community.

Timeframe:	September 2022 to August 2025
Total budget:	€ 4 141 166
Instruct participation:	Instruct Hub
Instruct budget:	€ 34 500

BY-COVID: BeYond-COVID

BY-COVID builds and expands upon the successful COVID-19 Data Platform. Rather than focus purely on providing technical solutions to the biological data, BY-COVID will work with partners from a broad range of disciplines, including public health and social sciences, to incorporate a wider range of data. Instruct-ERIC plays a key role in the project, with a number of Instruct partners involved in the FAIRification and interoperability of COVID-related data.

Timeframe:	October 2021 to September 2024
Total budget:	€ 12 000 000
Instruct participation:	Instruct Hub, Instruct-CZ, Instruct-ES, Instruct-IT
Instruct budget:	€ 166 316

canSERV: Providing cutting edge cancer research services across Europe

canSERV's mission is to make cutting-edge and customised research services available to the cancer research community EU wide, enable innovative R&D projects and foster precision medicine for patients benefit across Europe. Structural biology is a key field in the pipeline of oncology, so Instruct will be offering its services to the canSERV community. Additionally, the ARIA submission system will be utilised as the central access management software for access provision in canSERV.

Timeframe:	September 2022 to August 2025
Total budget:	€ 14 866 441
Instruct participation:	Instruct Hub and all Instruct Centres as service providers
Instruct budget:	€ 1 376 753

EOSC-Future: Advancing European research through Open Science

EOSC Future aims to integrate, consolidate, and connect e-infrastructures, research communities, and initiatives in Open Science to further develop the EOSC Portal, EOSC-Core and EOSC-Exchange of the European Open Science Cloud (EOSC). Instruct-ERIC is involved in two interdisciplinary test science projects within EOSC-Future: Working to enhance access to secure social science data sets using our ARIA software in collaboration with social science research infrastructure CESSDA, and working on improving FAIRness of COVID-19 imaging data i n collaboration with life science research infrastructures EU-OPENSCREEN and Euro-BioImaging.

Timeframe:	April 2021 to September 2023
Total budget:	€ 40 877 089
Instruct participation:	Instruct Hub, Instruct-CZ, Instruct-ES
Instruct budget:	€ 217 421

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

EOSC-Life launched in April 2019 as the life-science domain specific area of the European Open Science Cloud (EOSC). The project aims to further cloud adoption through life sciences, increase data standards and interoperability and improve access to tools and data. Instruct-ERIC is utilising its cross-research infrastructure support tool, ARIA, to support the project activities and enhance integration with other research infrastructures in the life-sciences domain. As co-leads of WP5 Instruct-ERIC will also be supporting the delivery of Life Science Login, a single sign on technology that will allow for seamless data sharing across multiple life-science domains.

Timeframe:	March 2019 to August 2023
Total budget:	€ 26 145 996
Instruct participation:	Instruct Hub, Instruct-ES, Instruct-FI, Instruct-IL, Instruct-IT, Instruct-NL, Instruct-UK
Instruct budget:	€ 1 731 012

EOSC4Cancer: A European-wide foundation to accelerate Data-driven Cancer Research

EOSC4Cancer will make cancer genomics, imaging, medical, clinical, environmental and socio-economics data accessible, using and enhancing existing federated and interoperable systems for securely identifying, sharing, processing and reusing FAIR cancer data. Instruct represents the structural biology community in this project and provides a connection between EOSC4Cancer and sister project canSERV which is a generator of cancer research data.

Timeframe:	September 2022 to February 2025
Total budget:	€ 7 814 549
Instruct participation:	Instruct Hub
Instruct budget:	€ 82 500

eRImote: Pathway to Improved Resilience and Digital and Remote Access

eRImote considers solutions for digital and remote service provision across RI domains, as well as transferable practices and new developments that will improve accessibility and resilience of RI infrastructures. The aim of eRImote is to create an online information platform with a publicly available data store on best practices and tools for remote provision of RI services. Instruct is heavlily involved in all aspects of this project.

Timeframe:	June 2022 to November 2024
Total budget:	€ 1 429 877
Instruct participation:	Instruct Hub
Instruct budget:	€ 272 375

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.

Timeframe:	January 2019 to December 2022
Total budget:	€ 1 495 281
Instruct participation:	Instruct Hub
Instruct budget:	€ 43 300

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

EU-LAC ResInfra will develop a bi-regional collaboration of RIs between EU and LAC countries to build on common interests. Within this project Instruct-ERIC is responsible for the Pilot on Infrastructure cooperation and coordination on Structural Biological for Health.

Timeframe:	December 2019 to February 2023
Total budget:	€ 1 499 719
Instruct participation:	Instruct Hub
Instruct budget:	€ 106 875

iNEXT Discovery: Structural biology for translational research and discovery

iNEXT-Discovery aims to enable access to structural biology research infrastructures for all European researchers, and especially non-experts in structural biology. For that reason it brings together a diversity of large research facilities and other groups in a single consortium. Instruct provides the proposal system for the project and the many Instruct-ERIC facilities also offer access to their services through iNEXT-Discovery and contribute to networking and communication activities. Instruct is responsible of the sustainability planning for the activities of the project.

Timeframe:	February 2020 to July 2024
Total budget:	€ 9 987 744
Instruct participation:	Instruct Hub, Instruct-CZ, Instruct-EMBL, Instruct-ES, Instruct-FR, Instruct-IT, Instruct-LT, Instruct-NL, Instruct-PT, Instruct-UK
Instruct budget:	€ 6 689 519

ISIDORe: Integrated Services for Infectious Disease Outbreak Research

ISIDORe provides research services from structural biology through to clinical trials to support infectious disease epidemic research including SARS-CoV-2. With a special emphasis on addressing the emergent SARS-CoV-2 variants, ISIDORe accelerates the generation of knowledge and tools to tackle epidemic prone pathogens, while avoiding fragmentation and duplication among EU initiatives. Structural biology is a key field in tinfectiouse disease research, so Instruct will be offering its services to the canSERV communit

Timeframe:	February 2022 to January 2025
Total budget:	€ 20 998 624
Instruct participation:	Instruct Hub, Instruct-BE, Instruct-CZ, Instruct-EMBL, Instruct-ES, Instruct-FI, Instruct-FR, Instruct-IL, Instruct-IT, Instruct-NL, Instruct-SK, Instruct-UK
Instruct budget:	€ 676 053

TRANSVAC-DS: Towards a sustainable European vaccine infrastructure

TRANSVAC-DS aims to consolidate the conceptual and technical design and ultimate implementation of a European vaccine R&D infrastructure. • Contribute to gaps and needs analysis (WP2). Instruct is contributing to the feasibility study and business plan as well as participating in the socioeconomic impact analysis.

Timeframe:	May 2017 to April 2023
Total budget:	€ 1 879 216
Instruct participation:	Instruct Hub, Instruct-IT
Instruct budget:	€ 35 000

TRANSVAC2: European vaccine research and development infrastructure

TRANSVAC2 focuses on making new vaccines quicker. It bridges the translational gap in biomedical research, and supports cooperation between public vaccine R&D institutions of excellence, related initiatives and networks in Europe, and industrial partners. Instruct makes its infrastructure available to TRANSVAC2 researchers on request.

Timeframe:	May 2017 to April 2023
Total budget:	€ 14 556 732
Instruct participation:	Instruct Hub
Instruct budget:	€ 29 260

EUROPEAN PROJECTS

Instruct-ERIC has enjoyed a long-standing connection with Latin America, which provided a solid foundation for the bi-regional partnerships of the EU-LAC ResInfra Project. As a central pilot representing life sciences, Instruct carried out several tasks and collaborative projects within EU-LAC ResInfra to boost cooperation between Latin America and Europe.

International Calls

In the context of EU-LAC ResInfra, Instruct-ERIC organised an International Call. This led to eight visits being awarded to researchers from Latin American institutions that hold a Memorandum of Understanding (MOU) with Instruct-ERIC, utilising advanced structural biology technologies available in Europe. The research projects helped foster collaborations between Latin American and European facilities, which will continue on into the future.

FIG 1. EU-LAC ResInfra consortium meeting in Montevideo, Uruguay, in July 2022.

Staff Exchange Visits

In addition to the International Call, Instruct-ERIC opened a staff exchange call to allow researchers from Latin American institutions to visit European facilities, also open to researchers from Latin American institutions that hold an MOU with Instruct-ERIC.

Unlike the International Call, and unlike regular access visits, the staff exchanges took place over many months, with one being carried out throughout the entirety of 2022. The main aim was for the researchers to gain experience using new techniques, which they could then transfer to their team in their home laboratory, whilst also imparting their considerable expertise to the host institution team. Additionally, the long-term exchanges further solidify relationships between the home and host facilities. The participating researchers are shown below:

Roberto Salinas of University of Sao Paulo (USP) visited the Bijvoet Centre at Instruct-NL Utrecht University (UU). See video.

Leonardo Alonso of University of Buenos Aires (UBA) visited EMBL Hamburg, to access the mass spectrometry facilities at the Instruct Centre.

Alejandro Buschiazzo of Institut Pasteur Montevideo (IPMontevideo) visited the Instruct Image Processing Centre (I2PC) In Madrid, Spain.

FIG2. Report on the landscape analysis of structural biology research infrastructure in Latin America.

Landscape Analysis of Structural Biology Research Infrastructure

The Landscape Analysis of Structural Biology Research Infrastructure in Latin America reviewed survey and direct interview responses from structural biologists across Latin America, and developed several conclusions and recommendations for both researchers and policy makers. The analysis involved survey responses from more than 150 researchers in Latin America, in addition to in-depth interviews with 29 respondents. The report was published in 2022, and is available in English, Spanish, and Portuguese.

Memoranda of Understanding Signed in Context of EU-LAC ResInfra

Through collaboration with the EU-LAC ResInfra project and the RI-VIS project, Instruct-ERIC has advanced its cooperation with institutions in Latin America, signing MoUs with the University of San Martin, and the University of Buenos Aires in 2021, as well as the Brazilian Centre for Research in Energy and Materials (CNPEM) in Brazil in 2022. This takes the total number of international institutions that hold an MoU with Instruct-ERIC to 11.

FIG 1. Instruct Director Harald Schwalbe (right) alongside Rodrigo Portugal (CNPEM) signing the MoU with the Brazilian Centre for Research in Energy and Materials (CNPEM) at ICRI2022 in Brno, Czechia.

EUROPEAN COLLABORATION

Trilateral MoU between Instruct-ERIC, Euro-BioImaging and EU-OPENSCREEN

2022 saw the formalisation of a longstanding collaboration between Instruct-ERIC and two other ERICs also operating in broad area of analytical molecular biology: Euro-BioImaging and EU-OPENSCREEN. Complementing Instruct's infrastructure in integrated structural biology, Euro-BioImaging provides advanced imaging technologies and EU-OPENSCREEN offers screening and chemistry facilities for chemical biology and early drug discovery.

The collaboration between the three ERICs has grown over the last decade, as all three worked together to provide joint access to their infrastructures and harmonised supporting services in projects such as BioMedBridges, CORBEL and EOSC-Life. Over time, more joint projects, either involving all three of the ERICs, or projects involving two of the three, have been developed and are being delivered.

Instruct-ERIC, Euro-BioImaging and EU-OPENSCREEN share a common mission to provide open democratised access to high-end technological infrastructure and, by doing so, advance excellent science. The three ERICs offer highly complementary techniques crucial to address cross-disciplinary user needs as exemplified by joint access provision in CORBEL and now ISIDORe. There is great potential for overlaps and synergies within this large pool of research collectively covered by the three ERICs. Complementarity between the three ERICs is not only on a scientific level, but also on an organisational level. Each has the same organisational structure as a distributed ERICs composed of national scientific Centres orchestrated from an operational Hub, and consequently faces similar challenges and opportunities which can be addressed synergistically.

The foundations of this shared experience, long-term collaboration, and shared vision, also formed the basis for the trilateral Memorandum of Understanding between EU-OPENSCREEN, Instruct-ERIC, and Euro-BioImaging, which was signed in a ceremony at the International Conference on Research Infrastructures ICRI in Brno, in October 2022. This MoU solidifies the alliance and lays the groundwork for continued collaboration and interactions.

The three ERICs look to build on this MOU with joint initiatives in areas including, access, data management, infrastructure operation, internationalisation, training and communication in the coming years.

FIG 1

FIG 2

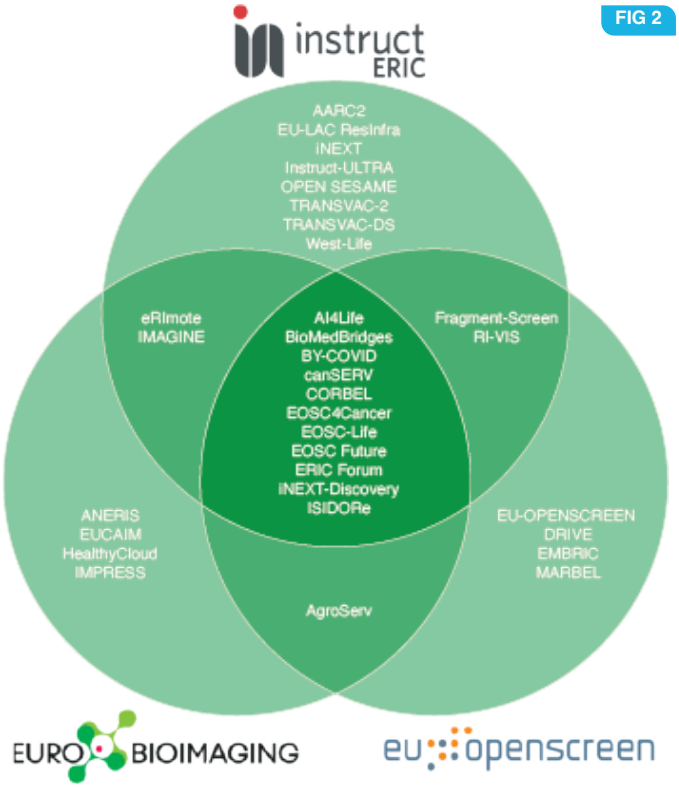


FIG 3

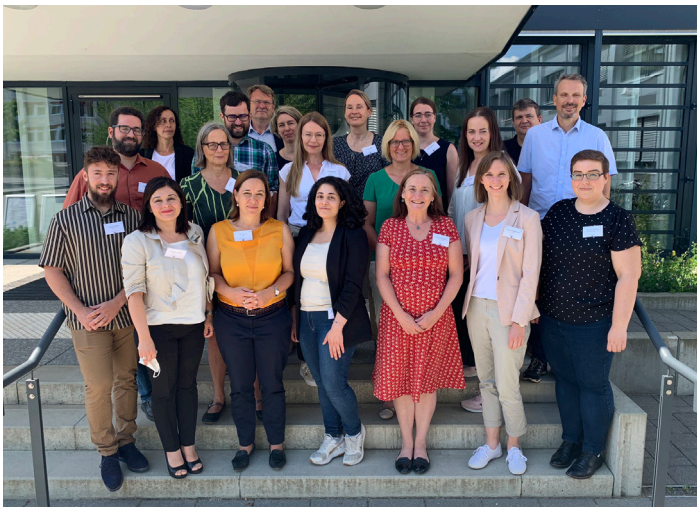
FIG 1. Harald Schwalbe (middle), Antje Keppler (left) and Wolfgang Fecke (right) with the signed trilateral MoU between Instruct-ERIC, Euro-BioImaging and EU-OPENSCREEN.

FIG 2. The three research infrastructures have a long history of working together on European projects.

FIG 3. Representatives of Instruct-ERIC, Euro-BioImaging and EU-OPENSCREEN at a joint outreach booth during the CORBEL project.


RI-VIS
BRNO


ISIDORe

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SUPPORTING ACTIVITIES AND OUTPUTS

COMMUNICATIONS

Instruct-ERIC celebrated its tenth year of operation, and also its fifth year with ERIC status, both in 2022. Additionally, being a Biennial year, much of the communication in 2022 was centred around the conference in May. However, the breadth of Instruct's activities extend far beyond the Biennial Conference, including international events, developing new materials and toolkits, and enhancing the website to become a central hub for structural biology activities in Europe and beyond.

Conferences and Exhibitions

IBSBC2022: The Biennial Conference was a significant communication exercise, acting as a draw for researchers, academics and students from the global structural biology community. Instruct-ERIC had an engaging presence on social media before, during, and after the event, and attracted hundreds to the website, who could then find out more about our catalogue of services and apply for access.

ICRI2022: ICRI, the International Conference on Research Infrastructures, is always an important conference in the research infrastructure calendar, but as Instruct-ERIC was part of the main plenary session, and was involved in a plethora of satellite and side events, there was much to disseminate from the structural biology community. From international access news in the EU-LAC ResInfra and RI-VIS satellite events, to a talk in the main conference on the merits of transnational access, this was a huge event in the Instruct conference programme for 2022.

Member conferences: Instruct-ERIC Members and Centres also held their own conferences in 2022, highlighting and disseminating the importance of a distributed research infrastructure to the decision makers in their own countries. Slovakia, Finland, and Portugal all held widely attended conferences.

New communication materials

In partnership with the EU-LAC ResInfra project, three researchers in Latin America conducted long-term exchanges with labs in Europe, utilising technology and sharing best practices and experiences with the teams there. Instruct interviewed two of these researchers, generating **staff exchange videos** on their thoughts and experiences, to inspire others to take up similar opportunities should they be presented.

The portfolio of **Instruct Centre videos** was expanded, with facilities in Instruct-ES and Instruct-NL represented with interview videos detailing the services, technologies and expertise available. Researchers and administrators from I2PC, NKI, NeCEN, and the Bijvoet Centre gave interviews inviting researchers across the world to utilise the technologies at their facilities.

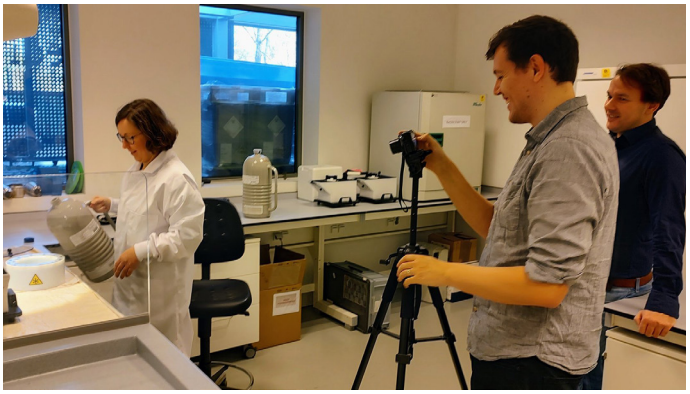


FIG 1. Instruct-ERIC Communications Offcier John Dolan filming the new facility communications videos (bottom right). With an example Instruct Centre screenshot for NKI (bottom left) and staff exchange screenshot (top right).

Newsletter

The Instruct-ERIC Newsletter continued its distribution in 2022, to almost 3,500 accounts in the ARIA network. The themes for the Summer (Biennial Conference) and Winter (International Collaboration) were apt, as the major advances Instruct had made throughout the year. In addition, the newsletters kept readers informed of project information, science highlights and testimonials, as well as new technologies and equipment available at Instruct centres.

Social media

Instruct-ERIC social media developed further in 2022, reaching more than 5,400 followers on Twitter, and more than 600 on LinkedIn. The power of social media as a tool for engagement and promotion, as well as a mechanism to reach new users to further develop structural biology on a global scale, cannot be understated.

Scientific Highlights

Instruct-ERIC published eight science highlights in 2022, covering a wide variety of topics from SARS-CoV-2 and vaccine studies, to protein folding research and AI developments. A key highlight was the study from Marcus Weingarth from Instruct-NL on the Teixobactin, a potential new antibiotic with strong activity against many antibiotic-resistant pathogens, which utilised instrumentation from several Instruct facilities.

Webinars

The *Structure Meets Function* webinar series continued to provide information and background to Instruct services and centres. This year, the shift focused to users who had accessed Instruct facilities, to give a testimonials and a detailed account of how their access visit worked, and a description of the science they could achieve with access to services through Instruct.

ARIA Communication

In addition to core Instruct-ERIC communication, great attention was paid towards disseminating the power and importance of ARIA to the Instruct and research infrastructure community. The ARIA Twitter account was filled with the latest releases and new features added to the platform, as well as detailing the conferences and events that ARIA was presented at. Additionally, the ARIA Training Workshop in November 2022 provided a widespread opportunity for users of ARIA to see talks on new features, and discuss new features that they would like added to the system.

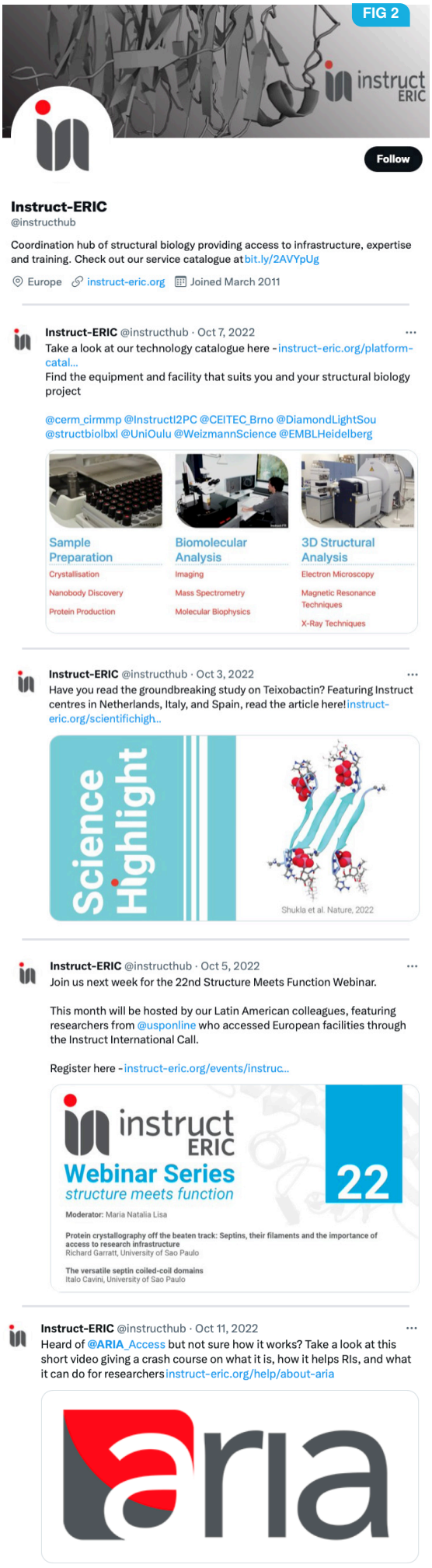


FIG 2. Instruct-ERIC presence on Twitter.

INSTRUCT BIENNIAL STRUCTURAL BIOLOGY CONFERENCE 2022

The 5th Instruct Biennial Structural Biology Conference titled *Challenges in Structural Dynamics* took place in the beautiful city of Utrecht, Netherlands from 19 to 20 May 2022 hosted by Instruct-NL Bijvoet Centre, NeCEN and NKI.

250 scientists from across the global structural biology community participated at the conference including researchers who presented their results obtained through access to major cutting-edge technologies available at Instruct Centres. This was the fifth edition of the Biennial conference, the previous ones being hosted in Madrid, Brno, Heidelberg and Florence and the programme included sessions representing recent structural biology highlights, emerging methods and technologies and results of biomedical importance.

The conference covered a huge range of topics and technologies within structural biology, from structural studies into RNA and understanding protein dynamics, to utilising cryo-EM for advanced molecular structures and studying SARS-CoV-2 with NMR. The full complement of techniques and methodologies were on display, once again showcasing structural biology as an integral cornerstone of the life sciences and beyond.

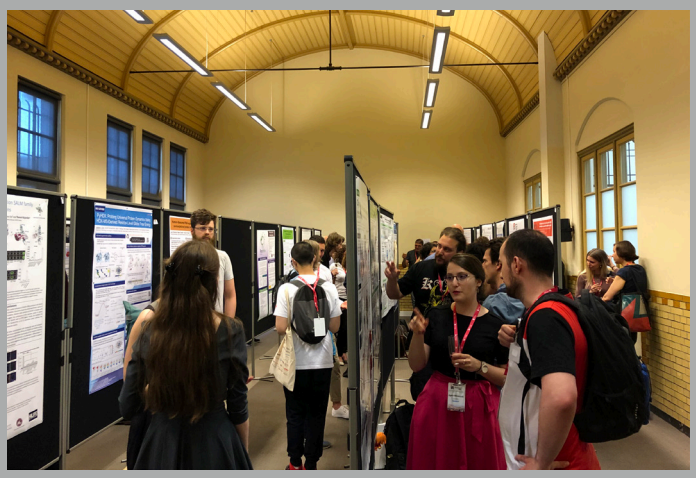
Several new techniques were also explored, including the AlphaFold system, as well as some other AI technologies and how they integrate with existing structural biology machinery. The final session of the conference on *Future Challenges and Perspectives* analysed the direction that structural biology is going in, and how novel innovations can help the field grow and develop further.

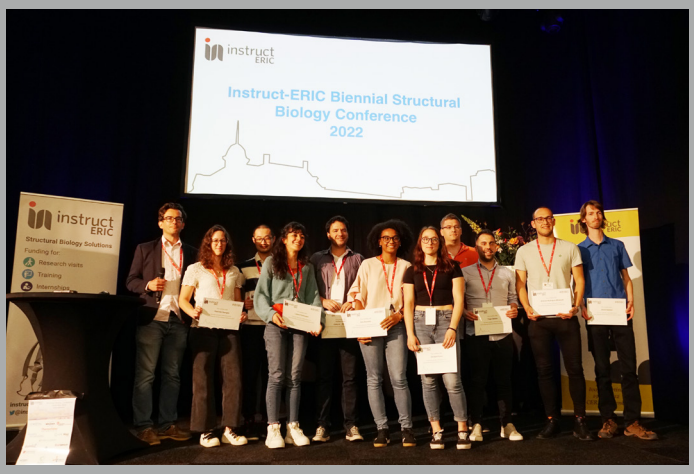
In addition to the main keynote speakers, several abstracts were chosen from attendees to give plenary talks, as well as the selected students who had been given fellowships to attend the conference. These talks were a great opportunity to see how the next generation of structural biologists were exploring and undertaking their research, providing a fresh perspective on the latest technologies and methodologies in structural biology.

BERTINI AWARD

As always, the Ivano Bertini Award was presented at the conference. The Ivano Bertini Award is offered by Instruct to recognise a significant achievement in frontier research that utilises an integrative structural biology approach. The 2022 award was given to Dr Sjors Scheres, Joint Head of The Division of Structural Studies at MRC Molecular Biology Lab, in Cambridge, UK by the previous awardee Wolfgang Baumeister (Max Planck Institute of Biochemistry). Dr Scheres was recognised for his work in the field of Cryo-EM: he developed the world-renowned RELION software package, which is based on Bayesian methods, to solve the structures of biological macromolecular assemblies by Cryo-EM.

FIG 1. Left to right: Wolfgang Baumeister, Ruediger Weisemann (Bruker), Sjors Scheres, Harald Schwalbe, Jose-Maria Carazo and Rolf Boelens.





The high profile list of speakers included:

Montserrat Bárcena	Leiden University Medical Center, Netherlands
Juri Rappsilber	Institute of Biotechnology, Technische Universität Berlin, Germany
Wolfgang Baumeister	Max Planck Institute, Germany
Ora Schueler-Furman	Hebrew University, Israel
Peijun Zhang	Division of Structural Biology, Oxford University, UK
Ben Schuler	Department of Biochemistry, University of Zurich, Switzerland
Cristina Paulino	University of Groningen, Netherlands
Wei Yang	NIDDK, Maryland, USA
Katja Petzold	Karolinska Institute, Sweden
Giulia Zanetti	University of London, UK
Philipp Selenko	Weizmann Institute of Science, Israel
Tassos Perrakis	Netherlands Cancer Institute
Hugo Van Ingen	Utrecht University, Netherlands
Liang Xue	EMBL Heidelberg, Germany
David Zapletal	CEITEC, Czech Republic
Giorgia Fiorini	University of Oxford, UK
Agata Misiaszek	EMBL Heidelberg, Germany
Ida de Vries	Netherlands Cancer Institute
Ioannis Skalidis	Martin Luther Universität, Germany
Harald Schwalbe	Instruct-ERIC Director

INSTRUCT-ERIC HUB

2022 saw changes in leadership at the Instruct-ERIC Hub. Prof Harald Schwalbe succeeded Prof David Stuart as Director at the start of the year and Hub Coordinator Dr Susan Daenke retired from Instruct in early 2022. Dave and Susan had been instrumental in the establishment and successful maturation of the infrastructure and long may their legacy continue.

Dr Silke Schumacher took on the role of Instruct Hub Coordinator until October 2022 looking to strengthen the relationships between Instruct and both new and existing member countries leveraging her expertise in international relations. From November 2022 onwards Dr Claudia Alén Amaro and Dr Natalie Haley were jointly promoted to the role of Hub Coordination as Head of Operations and Head of Strategy respectively. Claudia and Natalie have been integral part of Instruct-ERIC operations from the start of the ERIC.

In the IT team, Instruct-ERIC welcomed Junior Developer Alec Matthews and Developer Joshua Ruff in 2022 expanding the ARIA team to five members. This was made possible due to ARIA development work planned in the EOSC Future and canSERV projects. To support further support the newly active canSERV project, Trainee Project Manager Corinna Brockhaus also joined the Hub. Corinna will be primarily responsible project managing both the access and development aspects of this project designated to Instruct-ERIC, and its sister project EOSC4Cancer. Project managers Dr Regina Günster and Dr Pauline Audergon progressed to Project Managers in 2022 from trainee positions in recognition of their experience and began managing new Horizon Europe projects eRImote and ISIDORe on behalf of Instruct-ERIC. Carla Marques Wood provided additional administrative support towards the end of 2022. Other existing project management, IT and administrative staff present in 2021 remained in post in 2022 providing continuity and retaining skills in the Hub.

HUB RETREAT

To cement the new team relationships and give time and space to explore ideas and future visions for Instruct, the first Hub retreat was held in November 2022 in the city of Lille. The team was supported by an external moderator who guided staff through a MBTI assessment and structured activities to improve team working when tackling workplace challenges. Feedback on the event was overwhelmingly positive and a second retreat will be scheduled in 2023.

FIG 1. Instruct Hub members at the Hub retreat in Lille, France.

SPOTLIGHT ON STAFF

Professor Harald Schwalbe

Prof. Schwalbe holds the position of full professor at the Goethe-University Frankfurt, Germany. His major training and interest are in biomolecular NMR spectroscopy on RNAs, DNAs, proteins and their complexes. From his background in NMR spectroscopy, he has collaborated with X-ray crystallographers (Prof. Klebe, Marburg, Dr. Weiss, Berlin) to understand protein-inhibitor complexes and conducted medicinal chemistry work, recently towards EphA2 inhibitors (Prof. Tosato, NIH) and FXR nuclear hormone receptors (Prof. Merk, LMU Munich). He has further characterised the codon-dependence of nascent polypeptide chains within the ribosomal exit tunnel (with Profs. Frangakis and Glaubitz, Frankfurt, Prof. Blackledge, Grenoble) using liquid- and solid-state NMR, single particle cryo-EM and MD simulations.

Since 2020, Prof. Schwalbe coordinates the NMR-based covid19-nmr consortium. Covid19-nmr has characterised the 2nd structure of the RNA genome of the SARS-CoV-2, made 85% of the viral proteome amenable for NMR studies, and screened fragment libraries against 20 viral RNA and 25 viral protein targets. These endeavours form the basis form the basis for antiviral inhibitors, especially those that target viral RNAs.

Prof. Schwalbe was appointed Director of Instruct-ERIC in January 2022. For the Instruct community, he is coordinating several recently awarded EU grants.

HUB TEAM MEMBERS

Claudia Alén Amaro
Head of Operations

Omran Alhaddad
IT Junior Developer

Pauline Audergon
Project Manager

Corinna Brockhaus
Trainee Project Manager

Susan Daenke
Hub Coordinator

John Dolan
Communications Officer

Lorraine Donaldson
Financial Administrator

Madalena Gallagher
Administrative Officer

Regina Guenster
Project Manager

Francisco Guimaraes
Finance and Admin Officer

Natalie Haley
Head of Strategy

Marcus Lowndes
IT Junior Developer

Carla Marques-Wood
Administrative Support

Alec Matthews
IT Junior Developer

Marcus Povey
Senior Software Developer

Joshua Ruff
Software Developer

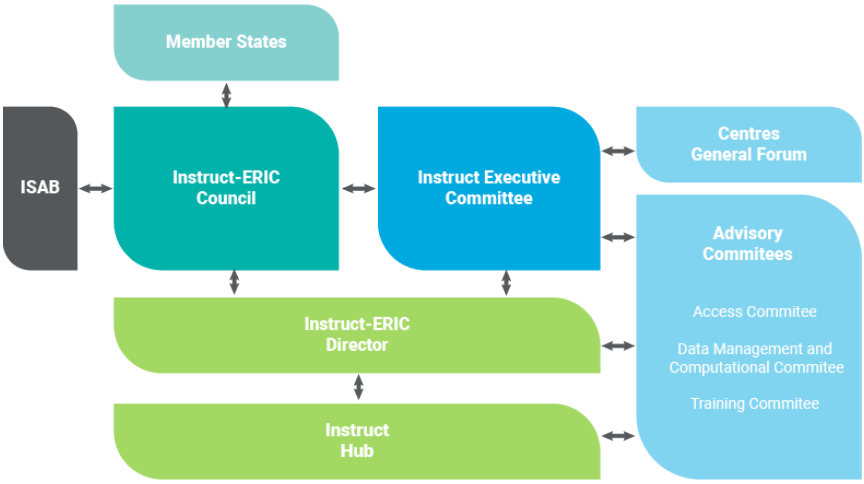
Silke Schumacher
Hub Coordinator

Harald Schwalbe
Director

FIG 2. Instruct Hub members at the summer picnic, retreat and christmas party (left to right).

GOVERNANCE

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



The most significant change in the governance of Instruct-ERIC in 2022 was the appointment of Prof Harald Schwalbe as Director at the start of the year. Elsewhere the leadership of Instruct-ERIC Council continued unchanged in 2022 with Dr Eric Guittet (FR) and Prof Sarah Butcher (FI) in the roles of Chair and Vice-Chair respectively. May 2022 saw the first in-person Council meeting since the COVID-19 pandemic hosted by Instruct-ES in Madrid. The Independent Scientific Advisory Board (ISAB), chaired by Prof Stephen Burley remained in place to advise the Council and the Director.

2022 marked a significant year for Instruct-ERIC as the end of the first 5-year cycle. Council approved a step increase of 10% to the membership contribution to begin from January 2023, in addition to the annual 2% per annum increase to strengthen the financial sustainability of the infrastructure. Building on the results of the Quinquennial review undertaken in 2021, and looking to the future, the Director developed a strategy implementation plan (SIP) for the next five years 2023-2027. The SIP was approved by Council as a living document to shape the strategic direction of the infrastructure. It sets forth ambitious plans for expanding the infrastructure and further initiatives to enable excellent structural biology research. The SIP primarily focusses on operational and strategic direction of the infrastructure will be proceeded in 2023 by a Scientific Plan positioning Instruct within the rapidly evolving field of Integrated Structural Biology and setting the scientific vision for the coming years.

Instruct's Executive Committee made the case for increased support for Structural Biology access provision. It is foreseen that following the end of the Horizon 2020 project iNEXT-Discovery, without further mechanisms set in place the capacity for structural biology access in Europe will be reduced and this could have a catastrophic effect on the field. Opportunities such as the possibility of co-funding access programmes with the EC are being pursued.

Much work was undertaken in the sub-committees reporting to Executive Committee: Access Committee, Training Committee, Data Management and Computational Committee (DMCC) and R&D committee. Of special note, the DMCC recommendations for the future of data management for data generated at Instruct-ERIC facilities were presented to Executive Committee. The recommendation is that Instruct collects sufficient minimal metadata to enable reuse of data generated from user access at its facilities in such a way that the facility can automatically publish this data after embargo. This would further increase the availability of high-quality structural data for reuse in accordance with the FAIR principles. DMCC is currently working to revise Instruct's policies to address these recommendations and technical work to implement the proposals is planned in the Fragment-Screen project starting in 2023.

INDEPENDENT SCIENTIFIC ADVISORY BOARD

Chair: Stephen Burley, Rutgers University, USA

Members

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

COUNCIL

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

Chair: Eric Guittet, FR

Vice-Chair: Sarah Butcher, FI

Country	Delegates
Belgium	Michele Oleo & Virginie Storms
Czech Republic	Vladimir Sklenar/ Pavel Plevka & Jan Burianek
EMBL	Christoph Mueller & Plamena Markova
Finland	Sarah Butcher & Anni Kleino
France	Winfried Weissenhorn & Eric Guittet
Israel	Joel Sussman & Iris Eisenberg
Italy	Lucia Banci & Grazia Pavoncello
Latvia	Kaspars Tars & Uldis Berkis
Lithuania	Gintaras Valincius
Netherlands	Reinout Raijmakers & Nienke Klomp
Portugal	Maria Armenia Carrondo & Marta Abrantes
Spain	Jose Maria Carazo & Inmaculada Figueroa/ Ignacio Baanante Balastegui
Slovakia	Milos Hricovini & Barbora Liptakova/ Simona Tanhäuserová
United Kingdom	Megan Dowie & Anne McGavigan/ Robert Deller

Observers

Greece	Evangelia Chrysina

EXECUTIVE COMMITTEE

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

Chair: Harald Schwalbe (Instruct Director)

Instruct Centre	Head of Centre	Deputy
Instruct BE	Jan Steyaert	Han Remaut
Instruct CZ	Vladimir Sklenar/ Pavel Plevka	Ondrej Hradil
Instruct EMBL	Stephen Cusack/ Kristina Djinovic Carugo	Matthias Willmans
Instruct ES	Jose Maria Carazo	Carlos Oscar Sanchez Sorzano
Instruct FI	Sarah Butcher	Markku Varjosalo
Instruct FR1	Patrick Schultz	Jean Cavarelli
Instruct FR2	Darren Hart	Martin Blackledge
Instruct IL	Michal Sharon	Joel Sussman
Instruct IT	Lucia Banci	Roberta Pierattelli
Instruct NL	Ariane Briegel	Rolf Boelens
Instruct UK	David Stuart	Andrew Quigley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FINANCIAL DATA

FINANCIAL DATA

This report presents the financial statements for the period 1 January 2021 to 31 December 2022.

Appointment of Members to Council

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

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

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

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

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

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

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

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

This report covers the period 1 January 2022 – 31 December 2022.

BALANCE SHEET FOR INSTRUCT-ERIC

As at 31 December 2022

Assets	GBP	EUR	Notes
Euro bank	1,217,277	1,375,159	
Sterling bank	47,119	53,230	
Total Bank	1,264,396	1,428,389	
Current Assets			
Accounts Receivable	-	-	1
Prepayments	10,875	12,286	
Accrued income	77,923	88,030	2
Rental deposits	3,973	4,488	
Total Current Assets	92,771	104,804	
Fixed Assets			
Computer Equipment	22,780	25,735	
Depreciation on Computer Equipment	(16,997)	(19,201)	
Office Equipment	4,997	5,645	
Depreciation on Office Equipment	(4,455)	(5,033)	
Total Fixed Assets	6,325	7,146	
Total Assets	**1,363,492**	**1,540,339**	

Liabilities	GBP	EUR	Notes
Current Liabilities			
Accruals	12,298	13,893	
Amounts to be paid and Unclaimed Access Awards	542,279	612,613	3
Income in Advance - Members subscription	-	-	4
Income in Advance - Other inc deferred grants	684,722	773,531	5
Income in Advance - ARIA Support	(5,278)	(5,962)	
Other creditors	5,044	5,698	
Payroll taxes due	12,530	14,155	
Pensions due	-	-	
Total Current Liabilities	1,251,595	1,413,928	
Total Liabilities	**1,251,595**	**1,413,928**	
Net Assets	**111,897**	**126,411**	
Surplus Brought Forward	77,473	92,295	
Exchange rate movement - revalue opening reserves	4,224	-	6
Surplus for the Year	30,200	34,116	
Surplus Carried Forward	111,897	126,411	

Exchange rate for reporting period: 0.88519

1. Membership income receivable

2. Grant income recoverable at year end

3. Access and other service accruals

4. Invoiced deferred membership subscriptions

5. Deferred project income

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

PROFIT AND LOSS FOR INSTRUCT-ERIC

For Year Ended 31 December 2022

Income	GBP	EUR	Notes
External grant income	346,048	390,928	7
External grant overhead contribution income	86,405	97,612	
Member state contributions	886,416	1,001,385	8
Other miscellaneous income	67,797	76,590	9
Total Income	1,386,666	1,566,515	
Less Cost of Service Provision			
Instruct core staff salaries	313,895	354,607	
R&D Pilot awards	48,296	54,560	
Training Courses	38,889	43,933	
Internships	29,393	33,205	
Access Cost	250,655	283,165	
Meetings	48,361	54,633	
Project activities	309,745	349,919	10
Total Cost of Service Provision	1,039,234	1,174,022	
Gross Surplus	347,432	392,493	
Less Operating Expenses			
Commissioned services (Insurance, financial, HR, legal)	44,341	50,092	
Conference costs	89,551	101,166	
Consultants	86,992	98,275	11
Recruitment costs	-	-	
Depreciation charge	6,149	6,947	
Foreign Currency (Gains)/Losses	1,547	1,748	
General admin (postage, copying, bank charges)	2,668	3,014	
Licenses & software	20,989	23,711	
Miscellaneous	356	402	
Office Stationery	443	500	
Premises and support	56,378	63,690	
Project overhead expenses	11,400	12,879	
Publicity	171	193	
Telephone	1,201	1,357	
Exceptional costs	-	-	
Total Operating Expenses	322,186	363,974	
Other Operating Gains/(Losses)			
Profit on disposal of fixed assets	4,954	5,597	
Total Other Operating Gains/(Losses)	4,954	5,597	
Net Surplus	**30,200**	**34,116**	

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

8. Membership income receivable

9. ARIA support and conference sponsorship

10. WIP on research grants. Project activities delivered.

11. Staff costs recharged from the University of Oxford and external consultans

SUPPORTING INFORMATION FOR THE FINANCIAL STATEMENTS

Accounting Policies

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

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

Reporting and Disclosure Exemptions

Going concern

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

Expenditure

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

Foreign Exchange

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

Corporation Tax

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

Basis of preparation

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

Income

1. The amounts derived from membership subscriptions. This income is recognised evenly over the subscription period.
2. EC Grants and projects income is 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 01/01/22 - 31/12/22	Payment received
UK	108,243	108,243
FR	108,243	108,243
ES	81,180	81,180
IT	81,180	81,180
BE	81,180	81,180
NL	81,180	81,180
IL	80,253	80,253
CZ	54,120	54,120
PT	54,120	54,120
DK	54,120	54,120
SK	54,120	54,120
LV	54,120	54,120
FI	54,120	54,120
LT	54,120	54,120
EMBL	54,120	54,120
Total	1,001,226	1,001,226

Grant Recipts

EU Grants	Income Jan - Dec 2022	Other income from Projects
AIALife	569.04	142.26
BY-COVID	7,618.69	1,904.66
canSERV	41,314.80	10,328.70
EOSC Future	16,502.75	4,125.69
EOSC-Life	38,261.30	9,565.31
EOSC4Cancer	2,982.36	745.59
ERIC Forum	6,930.39	806.46
eRImote	36,558.15	9,139.54
EU-LAC ResInfra	58,654.10	15,468.21
iNEXT-Discovery	46,432.27	11,608.06
ISIDORe	73,503.50	18,375.87
RI-VIS	54,801.70	13,701.44
TRANSVAC-DS	1,392.38	348.09
TRANSVAC2	5,406.62	1,351.66
Total	390,928.05	97,611.54

Overhead contribution recognised: 25%

C. Deficit/surplus on activities: 34,116

D. Employees

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

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

E. Debtors

Invoices outstanding from Members (present total figure outstanding against 2022 invoices): €0
Other accrued income: €88,030
Grant accrued income: €12,286

F. Creditors

Accruals for services and awards (Access, Internships, R&D, Training, unclaimed access) €626,506
Advances on Research Grants: €773,531
Other creditors: €13,891

G. Related Parties

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

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

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

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

H. Commitments

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

I. Pensions

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

J. Grant Agreements

Instruct ERIC acts as host (Coordinator) in respect of the following grants:
RI-VIS: €1,500,000 (total value) – start date 01 February 2019, end date 31 Jan 2022.

Instruct-ERIC is a beneficiary partner in the following grants with a project lifetime award to Instruct-ERIC shown below:
AI4Life: €34,500
BY-COVID: €45,874.50
canSERV: €1,376,752.63
EOSC Future: € 92,421.05
EOSC-Life: €681,442.26
EOSC4Cancer: €82,500
ERIC Forum: €43,300
eRImote: €272,375
EU-LAC ResInfra: €106,875
iNEXT-Discovery: €147,500
ISIDORe: €606,487.50
TRANSVAC-DS: €14,375
TRANSVAC2: €29,260

ABBREVIATIONS AND GLOSSARY

Term	Definition
Access	The unit of use of Instruct Research Infrastructure being in person (visit) or remotely (by sending samples)
Access Committee	A body established to manage the review of prospective users' proposals and applications for access to the tools and services provided by the Instruct-ERIC.
AF4	Asymmetrical flow field-flow fractionation
AI	Artificial intelligence
AI4Life	AI4LIFE aims to build bridges between the life science community and the machine learning/ artificial intelligence community
AlphaFold	AlphaFold is an artificial intelligence program developed by DeepMind which performs predictions of protein structure.
API	Application Programming Interface
ARIA	Access to Research Infrastructure Administration: Instruct-ERIC's access management system
ATR-FTIR	Attenuated total reflectance sampling methodology for Fourier transform infrared spectroscopy
AUC	Analytical Ultracentrifugation
BIOCEV	Biotechnology and Biomedicine Centre (Czech Republic)
BLI	Biolayer Interferometry
BY-COVID	The BeYond-COVID project aims to make COVID-19 data accessible to scientists in laboratories but also to anyone who can use it, such as medical staff in hospitals or government officials.
canSERV	canSERV's mission is to make cutting-edge and customised research services available to the cancer research community EU wide, enable innovative R&D projects and foster precision medicine for patients benefit across Europe.
CEITEC	Central European Institute of Technology (Czech Republic)
CERM	Magnetic Resonance Center of the University of Florence (Italy)
CIISB	The Czech Infrastructure for Integrative Structural Biology
CIRMMP	The Interuniversity Consortium for Magnetic Resonance of Metallo Proteins (Italy)
CLEM	Correlative light and electron microscopy
CNB	Spanish National Centre for Biotechnology
COVID-19	Coronavirus disease caused by the SARS-CoV-2 virus
CRISPR-Cas9	Natuarally occuring gene editing system in bacteria now used by geneticists to alter genome sequences in other cell types organisms.
CSIC	Spanish National Research Council
DLS	Dimond Light Source (UK)
DMCC	Data Managementand Computational Committee
eBIC	Electron Bio-Imaging Centre (UK)
EC	The European Commission
EIS	Electrochemical Impedance Spectroscopy
EM	Electron Microscopy
EMBL	The European Molecular Biology Laboratory: an intergovernmental organisation specialising in

ABBREVIATIONS AND GLOSSARY CONTINUED

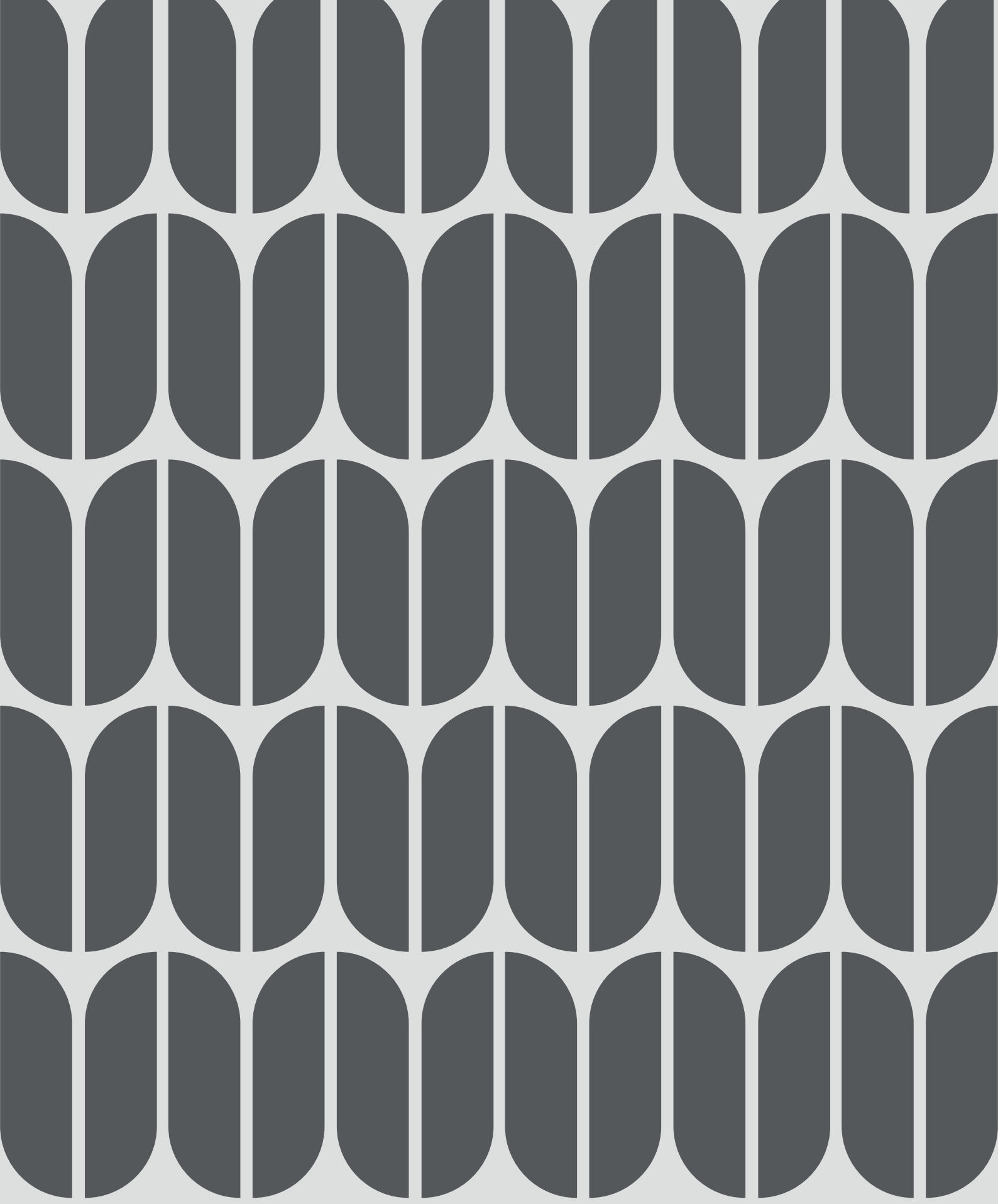
Term	Definition
	research in the life sciences, funded by its 28 member states.
EOSC Future	EU-funded EOSC Future project to integrate, consolidate and connect e-infrastructures, research communities and initiatives in Open Science to advance the EOSC platform of services.
EOSC-Life	The European Open Science Cloud: bringing together biological and medical Research Infrastructures to create an open, collaborative space for digital biology.
EOSC4Cancer	EOSC4Cancer will make cancer data accessible, using and enhancing existing federated and interoperable systems for securely identifying, sharing, processing and reusing FAIR cancer data
ERIC	European Research Infrastructure Consortium: a specific legal form that facilitates the establishment and operation of Research Infrastructures with European interest.
ERIC Forum	A Horizon2020 project bringing together European Research Infrastructure Consortia to strengthen their coordination and enhance their collaborations.
eRImote	eRImote considers solutions for digital and remote service provision across RI domains.
ESFRI	European Strategy Forum on Research Infrastructures: an organisation with members nominated by European member states ministries to support a coherent and strategy-led approach to policy-making on Research Infrastructures in Europe.
ESRF	The European Synchrotron Radiation Facility (France)
ET	Electron Tomography
EU-LAC ResInfra	The European Union – Latin America and Caribbean partnership in Research Infrastructures pursues the construction of a bi-regional collaboration between European Union and the LAC countries.
Euro-BioImaging	A European Research Infrastructure providing open access to a broad range of technologies in biological and biomedical imaging for life scientists.
EU-OPENSCREEN	A European Research Infrastructure providing access to all stages of a chemical tool development projects.
FEBS	The Federation of European Biochemical Societies: a charitable organisation supporting research and education in molecular life sciences.
FIB-SEM	Focused Ion Beam Scanning Electron Microscopy
Fragment-Screen	Horizon Europe project: From fragments to high affinity binders interfacing integrated structural biology, medicinal chemistry and artificial intelligence.
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.
I2PC	Instruct Image Processing Center (Spain)
IBS	Institute of Structural Biology (France)
IGBMC	The Institute of Genetics and Molecular and Cellular Biology (France)
IMAGINE	Horizon Europe project: Next generation imaging technologies to probe structure and function of biological specimen across scales in their natural context
iNEXT-Discovery	A consortium funded by the Horizon2020 program, offering European researchers access to a range of structural biology technologies.
Instruct Centre	An organisation that delivers access through the Instruct funding route.

ABBREVIATIONS AND GLOSSARY CONTINUED

Term	Definition
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 Research Site	An Instruct facility or organisation, or a consortium of organisations within a country that can offer a centralised national hub to provide training, outreach or networking activities of interest to Instruct users and members.
Instruct User	A person that has applied, or is in the process of applying to access services through Instruct.
ISAB	Independent Scientific Advisory Board
ISBG	Integrated Structural Biology Grenoble (France)
ISIDORE	The ISIDORe project provides research services from structural biology through to clinical trials to support infectious disease epidemic research including SARS-CoV-2.
ITC	Isothermal titration calorimetry
ITQB	Institute of Chemical and Biological Technology (Portugal)
JRA	Joint Research Award
LIOS	Latvian Institute of Organic Synthesis
LMJ	Liquid-metal-jet
MALDI-TOF	Matrix Assisted Laser Desorption/Ionisation coupled to time-of-flight mass spectrometry
Moderator	A person assigned to an Instruct proposal by the Secretary of Moderators in order to select reviewers and decide the outcome of user proposals.
MoU	Memoranda of Understanding
MS	Mass Spectrometry
MX	Macromolecular Crystallography
NeCEN	Netherlands Centre for Electron Nanoscopy
NKI	Netherlands Cancer Institute
NMR	Nuclear Magnetic Resonance
OPIC	Oxford Particle Imaging Centre (UK)
PCISBIO	Portuguese Centre for Integrated Structural Biology
PID	Proposal Identification number
Proposal	A user's request for access to technology or other services.
QELS	Quasi-elastic light scattering

ABBREVIATIONS AND GLOSSARY CONTINUED

Term	Definition
R&D	Research and development
Reviewer	Assigned by the moderator, a reviewer assesses the science of an Instruct proposal. Three reviewers are assigned to each proposal: all are external to the Instruct Centre that has been requested for access, and at least one is external to Instruct-ERIC.
RI	Research Infrastructure
RI-VIS	A H2020 funded project to increase the visibility of European Research Infrastructures (RIs) to new communities in Europe and beyond.
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2 causing the COVID-19 pandemic
Scipion	Integrative image processing workflow engine
SEC-MALLS	Size Exclusion Chomatography - Multi-Angle Laser Light Scattering
SPC	The Israel Structural Proteomics Center
SPU	Structural Proteomics Unit
SR	Super Resolution
ssNMR	solid-state NMR
Stakeholder	A person, or group of people with an interest or concern in Instruct-ERIC.
TRANSVAC-DS	The TRANSVAC-DS project aims to consolidate the conceptual and technical design and ultimate implementation of a European vaccine R&D infrastructure.
TRANSVAC2	The TRANSVAC2 consortium comprises a comprehensive collection of leading European institutions that propose to further advance with the previous initiative towards the establishment of a fully operational and sustainable European vaccine R&D infrastructure.
WIS	Weizmann Institute of Science (Israel)
VU LSC	Vilnius University Life Science Center



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

Published 2023