CERM
Centro Risonanze Magnetiche
FIRENZE

CIRMMP
FIRENZE

SCIENTIFIC ANNUAL REPORT

2023

UNIVERSITÀ DEGLI STUDI FIRENZE

Contents

Foreword

2023 was a crucial year for our Infrastructure. The University of Florence has recognized CERM as a Service Centre, thus making CERM independent from the Department of Chemistry. Following this institutional change, a new President and a new Board of Directors have been appointed among tenured professors and researchers of the University of Florence. This new status will give CERM greater independence in the acquisition and management of scientific equipment, as well as in taking part in national and international research and development projects, cooperation, and technology transfer, together with the Interuniversity Consortium CIRMMP, which supports CERM in its activities.

In 2023, the funds obtained through the National Recovery and Resilience Plan (NRRP) provided a significant boost to research initiatives in Italy. The NRRP investment injected momentum into Italy's research landscape, fostering innovation, driving collaboration among academia and industry, and ultimately contributing to the nation's socio-economic development. CERM/CIRMMP also received help from the NRRP funds: through the project **ITACA.SB** we secured funds for potentiating the Italian center of Instruct-ERIC and for implementing new facilities in Italy. This project will provide the Italian Structural Biology community with methodologies to meet their needs for performing top-level research with the overall goal of strengthening the Italian role in Instruct-ERIC by ensuring the European user community high-end services at the Italian center, and boosting the exploitation of Instruct-ERIC resources in general.

The attractiveness of CERM for national and international users relies on the quality of the research performed by its researchers. Again, in 2023 the level of scientific research developed at CERM/CIRMMP is confirmed to be of high quality as shown by looking at several parameters such as the large number of peer-reviewed publications (68) with an average publication impact factor of about 6.3. This average impact factor is remarkable not only because of the presence of works published in excellent journals (*Cell, Nature Methods, Angew. Chemie, J. Am. Chem. Soc…*) but also because of an overall general quality increase; indeed, about one third of the publications were in journals with an impact factor higher than 5. Structural and cellular biology dominate the landscape of topics but material science, new NMR methods and metabolomics are also well represented. The various research areas feature in more detail in the Research Activities section of this report.

Scientific excellence has always represented our strength and constantly attracts new users, who often establish collaborations with us. Indeed, users of our infrastructure find not only an excellent NMR service but also the expertise to properly analyze the data and translate them into scientific results. The role of CERM/CIRMMP in the European Research Infrastructure landscape was further reinforced. CERM/CIRMMP is the Italian center (Instruct-IT) of Instruct-ERIC, an ESFRI Landmark. The key role of the Italian center within Instruct-ERIC was strongly reaffirmed thanks to our involvement in most Instruct-ERIC activities, with a leading role in the Council and in the Executive Committee, as well as in the

support to training, internationalization, access, and data management. The latter is provided within the EOSC-Life project, which enables the management, storage, and reuse of data in the European Open Science Cloud (EOSC).

The activities of CERM/CIRMMP related to Instruct-ERIC were framed also within the canSERV and the ISIDORe projects, which coordinate the Biological and Medical European Research infrastructures (BMS RIs) to create platforms for access provision tackling cancer research and infectious disease outbreak, respectively.

At the national level, the activities of **Instruct-ITALIA**, the national consortium of infrastructures providing access to national users in structural biology, started in 2020, have rapidly increased, as detailed within this report. Instruct-ITALIA is a powerful tool for the Italian researchers who now have access to complementary techniques on different research fields: from NMR to Cryo-EM, to optical microscopy and X-ray techniques.

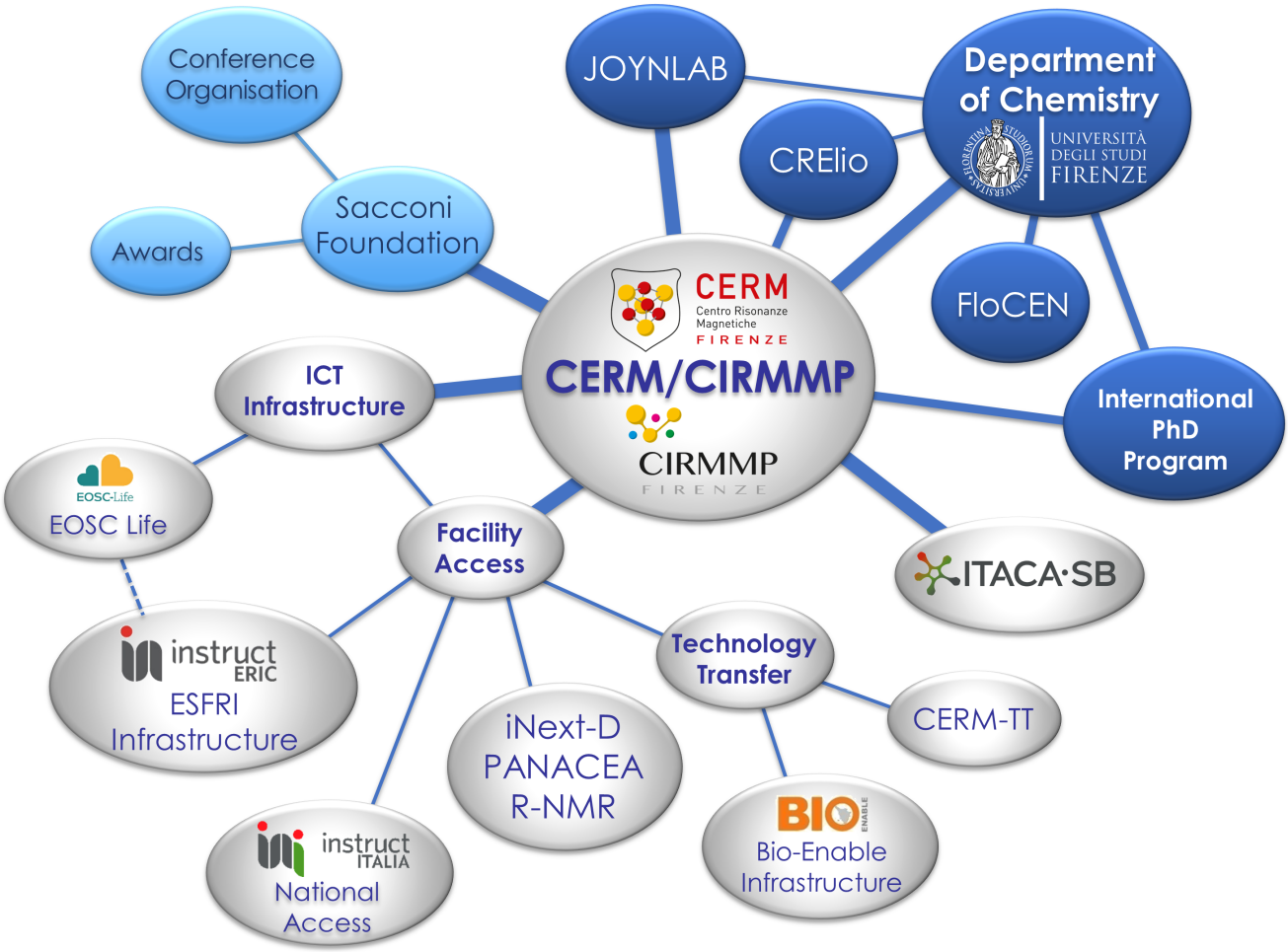
Figures

Also for 2023, the Italian Ministry of Education, University, and Research (MIUR) confirmed its support to the Italian Center of Instruct-ERIC within the International Action of the FOE funding. CERM/CIRMMP Investments and costs in 2023 amounted to € 2.970.000,00: 260.000,00 towards training and education, € 1.830.000,00 for new equipment, € 675.000,00 towards research activities and an additional € 205.000,00 covering operational costs. Furthermore, during 2023 the majority of the NRRP funding to the ITACA.SB project (€ 9,388,567,00) have been invested mainly in the acquisition and/or upgrade of the instrumentation in all the areas of activitiy of CERM/CIRMMP.

In 2023, besides the faculty staff, the body of researchers included 25 PhD students, 6 postdoctoral scientists, 2 graduate students, and 18 undergraduate students, as well as 14 between non-permanent and permanent technical staff.

We want to thank all the people that contributed to make CERM/CIRMMP what it is today and who continue to drive it forward, and all the Institutions that provided their support.

Prof. Lucia Banci Prof. Claudio Luchinat Prof. Paola Turano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Facility Access
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ESFRI Infrastructure
Technology Transfer
CERM-TT
iNext-D
PANACEA
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instruct ITALIA
National Access
BIO ENABLE
Bio-Enable Infrastructure

Who we are

Introduction

CERM is the Centre for Magnetic Resonance of the University of Florence. Since 2023 CERM has been recognized as a University's Service Centre, thus becoming independent from the Chemistry Department. It operates in synergy and collaboration with the Inter-University Consortium for Magnetic Resonance of MetalloProteins (CIRMMP) which includes three Italian Universities: Florence, Siena, and Bologna. CERM/CIRMMP is an infrastructure for Life Sciences with a particular focus on structural biology and specialization in NMR spectroscopy and relaxometry, bioinformatics, molecular and cellular biology, novel drug and vaccine design, and metabolomics. Nevertheless, it is open towards interfaces with other research fields, for example new materials and biomaterial developments, contrast agents and MRI techniques, and ICT technology.

Being a leading laboratory at both national and international level, CERM/CIRMMP receives funding from competitive project calls from the Tuscan Regional Government, the Italian Ministry of Higher Education and Research (MUR), the Italian Ministry of Health, and the European Commission (EC), as well as from private institutions.

The core technology at CERM/CIRMMP is NMR spectroscopy, and the onsite instrumentation is among the most advanced in the world. Since 1994 a European transnational access service, funded by EC, flanked the service provision at national level, that was already active since 1990. This long-term expertise places CERM/CIRMMP at the top of the list among the European NMR Research Infrastructures in Life Sciences. CERM/CIRMMP

actively stimulates interactions between private industry and public research institutions such as Universities, National Research Council (CNR) Institutes, and European counterparts, promoting synergistic activities such as collaborations and services to SMEs.

CERM/CIRMMP is the Italian Centre of Instruct-ERIC, which is the European research infrastructure in integrated structural biology defined in the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. CERM/CIRMMP is also included in the "Roadmap Italiana delle Infrastrutture di Ricerca di interesse Pan-Europeo" since 2010. In parallel, CERM/CIRMMP is also the core centre of the Instruct-ITALIA network, an infrastructure to promote and foster an integrated approach at the national level providing access to X-ray crystallography, NMR, Cryo-EM, as well as protein expression and crystallization. Instruct-ITALIA has started its activity in early 2020, promoting a more effective interaction within Italian structural biologists, as well as supporting access to the facilities of its national network.

Under the Next Generation EU scheme, Italy is receiving resources (NRRP Program) and, through this scheme, CERM/CIRMMP scientists participated in several projects. Of high relevance and impact is the awarding to CERM/CIRMMP, together with a few CNR laboratories in Italy, of the project, named ITACA.SB, which allocated significant resources to reinforce the Italian Center of Instruct-ERIC and Integrated Structural Biology in Italy.

CERM/CIRMMP is also an e-infrastructure, participating in a European GRID-based platform, providing access to user-friendly platforms and CPU resources for a broad range of services for structural biology. These services leverage technologies created in the context of EOSC (European Open Science Cloud) development initiatives and are made available through the EOSC Hub (for example, https://www.wenmr.eu/services/).

CERM/CIRMMP has also developed a centre for research and technology transfer: CERM-TT, funded by the Tuscany Region. Finally, CERM/CIRMMP is coordinating the activities of Bio-Enable, a distributed Infrastructure promoting technology transfer to industry and funded by the Regional Government of Tuscany in the frame of POR FESR 2014-2020.

CERM/CIRMMP is in the Scientific Campus ("Polo Scientifico") of the University of Florence in Sesto Fiorentino, an area just west of the city of Florence. The campus borders Florence International Airport and yet is a mere 30 minutes from the centre of Florence, world-renowned cradle of renaissance art and culture.

The Infrastructure

CERM/CIRMMP labs

The CERM/CIRMMP building covers an area of 3000 square meters hosting several laboratories, offices, and common rooms. The hallmark of the Center is the impressive collection of NMR spectrometers which feature the largest magnetic field range in the world (from 1.2 GHz - installed in early 2020, the first instrument in the world at this field - to the earth magnetic field of FFC relaxometers) and ranks it among the best equipped laboratories in the world. The NMR labs are flanked by molecular and cellular biology laboratories that are optimized for NMR sample production. A complete list of the instruments available at CERM/CIRMMP is reported at pag. 37. In addition to the main building, further 500 square meters in adjacent buildings are available to CERM scientists and researchers scientifically associated to CERM/CIRMMP: laboratories at the Department of Chemistry Ugo Schiff and at Genexpress; Da Vinci European Biobank; X-rays facilities; Helium liquefier. www.cerm.unifi.it

Instruct-ERIC

CERM/CIRMMP is an Instruct-ERIC Centre. Instruct-ERIC is the European research infrastructure in integrated structural biology, making cutting-edge technologies and high-end methods in a palette of tools for structural characterization available to users.

Structural biology is one of the key approaches that contribute to the understanding of the molecular and cellular functions. The main experimental technologies are complementary, and increasingly link detailed atomic structure with cellular context. Structural biology is currently in the middle of a revolution enabled by significant advances in various technologies (direct electron detectors in EM, advances in synchrotron sources and detectors, XFELs, ultra-high field NMR, super-resolution cryo-light microscopy, AI-driven prediction of protein structures).

Instruct-ERIC buildups on a number of Centres featuring the most advanced structural biology instrumentation and top-level expertise in the various methods. Instruct-ERIC offers a **single point of access** to both multiple techniques integrated at one Center or over various Centres, or to some Centres specialized in specific techniques. www.instruct-eric.eu

Instruct-ITALIA is the Italian Infrastructure for Integrated Structural Biology. It consists of a core of excellent research institutions and large centres that have a proven track record in structural biology and in service and expertise provision to users. Instruct-ITALIA aims to serve as a national consortium covering all main areas of structural biology research within Italy. https://www.cerm.unifi.it/instruct-it/

CERM TT

The CERM TT Competence Centre *dedicated to Ivano Bertini*, one of the founders of CERM, was established in response to the request of the Tuscany Region to make available to the industries and production companies in Tuscany centres of technology transfer, innovation clusters with advanced equipment and skills to boost the economic growth of the region.

CERM TT strengthens and optimizes the services offered by CERM/CIRMMP to the industry of the area: NMR instrumentation and advanced computing, a molecular biology laboratory for the production of proteins, scientific expertise and excellence, together with the maximum protection of industrial IP.

CERM TT provides analytical services and research and development (R&D) for companies. Specifically, it offers the following services:

- screening of drug candidates and provision of drug-target interaction studies.
- smart design of drugs.
- analysis of pharmaceutical formulations.

Bio-Enable

BIO-ENABLE is a "distributed research infrastructure" led by CERM/CIRMMP and which includes a few other Centres in Tuscany. Bio-Enable provides access to equipment and expertise to support industrial research and innovation. Tuscan companies operating in fields ranging from pharmaceuticals to biotechnology, from vaccines to biomaterials, from food to nanotechnology, can exploit the services of Bio-Enable in the development of their activities to be competitive at the international level.

CERM leads the BIO-ENABLE consortium composed by:

- Magnetic Resonance Center (CERM/CIRMMP, coordinator)
- Institute of Neurosciences of the CNR – Pisa;
- BioRobotics Institute of Sant'Anna School of Advanced Studies - Pisa;
- Department of Medical Biotechnologies – University of Siena.

BIO-ENABLE can provide support at various levels and through different types of contracts: from simple access to instrumentation to specific types of advice, help and assistance to industrial research. BIO-ENABLE guarantees total confidentiality of the data collected at the various platforms, both during the analysis and in the management and archiving of the data. www.bio-enable.it

Funded projects

CERM/CIRMMP cooperates at the international level with several universities, research institutions, and private industries with which it is involved in numerous research projects funded by the European Commission. Projects ongoing during 2023 are:

Remote NMR (R-NMR): Moving NMR infrastructures to remote access capabilities (HORIZON-CSA grant agreement n. 101058595, 01/07/2022-30/06/2025)

ISIDORe Integrated Services for Infectious Disease Outbreak Research. Grant agreement ID: 101046133 (01/02/2022-31/01/2025)

BeYond-COVID (BY-COVID) Grant agreement ID: 101046203 (1/10/2021-30/09/2024)

PANACEA "A Pan-European Solid-State NMR Infrastructure for Chemistry-Enabling Access", (H2020, contract n. 101008500, 01/09/2021-31/08/2025)

ITN "GLYTUNES – A multidisciplinary training network for the bioinspired development of glycomimetics tuning the Siglec-Sialoglycan axis" n. 956758 (01/03/2021-28/02/2025)

H2020 -INFRAIA iNEXT-Discovery - Structural Biology Research Infrastructures for Translational Research and Discovery (#871037) https://inext-discovery.eu (01/02/2020-31/07/2024)

ITFoC Information Technology: The Future of Cancer Treatment https://itfoc.eu/

THE INFRASTRUCTURE

EOSC-Life "Providing an open collaborative space for digital biology in Europe" (H2020, contract n. 824087, 01/03/2019-28/02/2023)

HIRES-MULTIDYN "Multiscale Dynamics with Ultrafast High-Resolution Re (H2020, contract n. 899683, 1/10/2020-30/09/2024)

EGI-ACE: Advanced Computing for EOSC (Horizon 2020 grant agreement n. 101017567, 01/01/2021-30/06/2023)

ITACA.SB: Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC (Call MUR 3264 - M4/C2/L3.1.1 - ID Proposal IR0000009)

Fragment-Screen: From fragments to high affinity binders interfacing integrated structural biology, medicinal chemistry and artificial intelligence (HORIZON-INFRA-2022-TECH-01 grant agreement n. 101094131 - 01/01/2023-31/12/2025

FC-RELAX: NMR relaxometry for biomedicine and advanced materials. A multidisciplinary doctoral network for field-cycling NMR relaxometry. (HORIZON-MSCA-DN-2021 grant agreement: 101072758 - 01/03/2023-28/02/2027)

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Funded by the European Union
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NRRP and CERM/CIRMMP

The CERM/CIRMMP Infrastructure is also strongly involved in the National Recovery and Resilience Plan (NRRP), participating in several projects directly as infrastructure or with the direct involvement of its researchers. Specifically, ITACA.SB is an infrastructure project empowering the Structural Biology services offered by Instruct-IT (https://www.itaca-sb.it/).

ITACA.SB: Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC

The activities of the ITACA.SB project at the Italian center of Instruct-ERIC aimed at maintaining the excellence of NMR services of the Italian Centre of Instruct-ERIC, empowering and integrating the service capacity for protein production and biophysical characterization, potentiating data management, and computational tools available for widening the exploitation of structural biology technologies. Furthermore, it promotes a reduction of the environmental impact of NMR structural biology activities at Instruct-IT. Finally, ITACA.SB promotes outreach and networking to build a strong Italian SB community.

National Recovery and Resilience Plan

Call MUR 3264/2021 – M4/C2/L3.1.1

Applicant: Consiglio Nazionale delle Ricerche (CNR)

Co-Applicant: Università degli Studi di Firenze

Starting date: 01.11.2022

Length: 30 months

Total amount: 17.977.617,89€ (40% of funds to South Italy infrastructures)

CERM@UniFi: 9.388.657,28€
CNR: 8.588.960,61€

Research Facilities involved in ITACA.SB: CERM/CIRMMP (Florence), IC: Institute of Crystallography (Bari, Caserta, Catania), IBPM: Institute of Molecular Biology and Pathology (Rome), ICB: Institute of Biomolecular Chemistry (Catania), IPCB: Institute for Polymers and Composite (Catania).

Staff: The ITACA.SB project has enlarged the CERM staff with the recruitment of 5 technologists, 3 Type A fixed-term Researchers and 6 PhD students.

Instrumentation upgrade: During 2023, CERM has received a major upgrade of its instrumentation, especially regarding NMR equipment: two Bruker NEO Consoles for the 700 MHz WB and the 950 MHz spectrometers, a QCI-P Cryoprobe (for the excitation of 1H, 13C, 15N and 31P), and an ENDOR Module for EPR experiments were purchased. Further instrumentation will be installed in 2024.

THE INFRASTRUCTURE

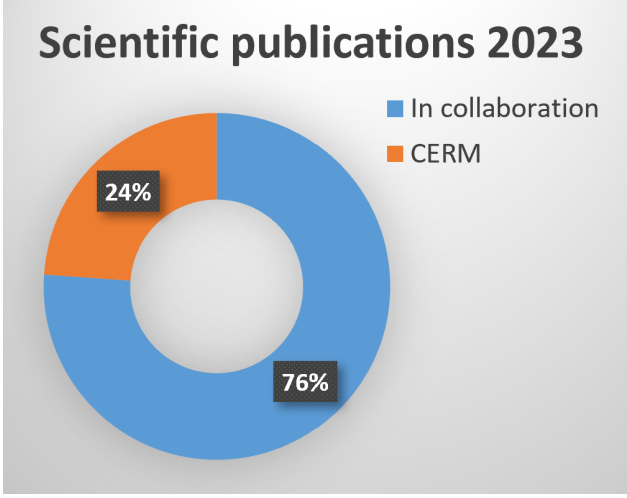
The laboratories were upgraded with the acquisition of additional instrumentation, such as a Nikon Eclipse Ts2R-FL Optical Microscope, a Varian Eclipse Fluorometer, a Stopped Flow SFM-4000, an Isothermal Titration Calorimeter MicroCal PEAQ-ITC, a Dawn-18 system (a SEC-MALS complete with HPLC, DLS, and FFF) and a Guava easyCyteTM Flow Cytometer. The laboratories of cellular and molecular biology have also been upgraded through the acquisition of ÄKTA Pure and Akta Go machines to scale up protein purification.
Publications: The project was acknowledged in 18 publications during 2023; the full list of publications is available at https://www.itaca-sb.it

SUMMARY OF NRRP PROJECTS WITH CERM/CIRMMP STAFF INVOLVED			
PROJECT			**Research Activities**
RI – ITACA.SB	Banci, Pierattelli, Turano, Felli, Fragai, Ravera, Rosato, Camponeschi, Cerofolini, Schiavina, Allegrozzi, Del Conte, Gonnelli	Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC	pg. 16-28
CN3 - SPOKE 5	Pierattelli, Fragai	Inflammatory and infectious diseases	pg. 20, 22
THE - SPOKE 4	Banci, Cantini, Del Conte, Gonnelli	Nanotechnologies for diagnosis and therapy	pg. 16
THE - SPOKE 6	Rosato	Precision Medicine & Personalized Healthcare	pg. 17
THE - SPOKE 7	Ciofi Baffoni, Piccioli	Innovating Translational Medicine	pg. 24
THE - SPOKE 8	Pierattelli, Felli, Parigi, Allegrozzi	Biotechnologies and imaging in neuroscience	pg. 22
PE8 - SPOKE 2	Tenori, Vignoli	Improving the understanding of the biology of ageing	pg. 27
PE12 - SPOKE 6	Felli	Mechanisms of neuronal cell degeneration and drug dependent reversal	pg. 20, 22

Research Activities

Introduction

During 2023, several research projects have been carried out, either as an extension of the activities of previous years or as new projects. Most of these projects receive specific funding from national and/or European organizations.
NMR is the core technology of CERM, but year by year CERM research has been oriented more and more toward new applications and toward the integration with other techniques. This is one of the principles of the Integrated structural biology that underlies the Instruct-ERIC consortium, where CERM/CIRMMP is the Italian node. In the following pages it can be appreciated how much the present research in CERM/CIRMMP is spanning a wide range of applications, from the structural biology to the bioinformatics methods and Information Technology, from paramagnetic NMR methods to the development of new contrast agents for MRI, from the metabolomics and biomedicine to the development of new solid-state NMR methods for the characterization of material surfaces and biomaterials.
In line with our mission to develop NMR as a technique and to integrate NMR with other techniques, most of our publications were done in collaboration with other research groups (76% of the overall number of publications). During 2023 we published 68 papers in international peer-reviewed journals, with several publications on very high impact factors journals (*Cell, Nature Methods, Angew. Chemie, J. Am. Chem. Soc.*). Our publications have an average publication impact factor of about 6.3, with 32% of the publications on journals with impact factors higher than 5. A complete list of publications is available at page 48.



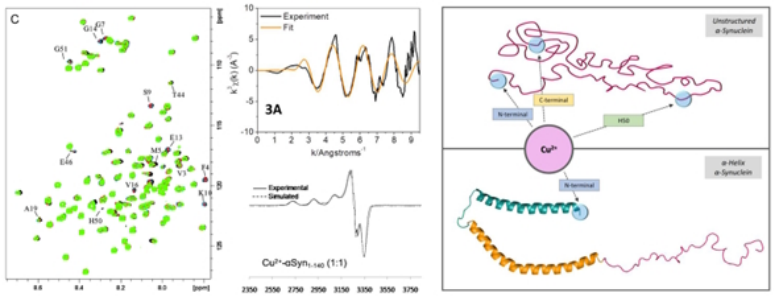
The interdisciplinary character of CERM/CIRMMP research projects, combined with the excellence of its instrumentation, constitutes a point of reference for the scientific community and for the cultural growth in the country, as demonstrated by the significant usage of the infrastructure by national scientists.

The Role of Solution NMR in Integrated Structural Biology

Understanding the function of biological molecules at the atomic level and their interplay in complex cellular pathways is the main goal of Structural Biology. The elucidation of complex, dynamic processes at the atomic level and possibly in the cellular environment requires interdisciplinary approaches combining techniques spanning different scales and resolutions.

Modern research in structural biology increasingly relies on multiple experimental and computational techniques to describe structure and function of challenging biomolecular complexes. High-resolution NMR, with its unique capability to elucidate at the atomic level macromolecular structure, dynamics, and weak or transient interactions in solution and in cells, plays a unique role in hybrid approaches. Combined with other techniques, NMR provides a complete picture of the dynamics and architecture of large biomolecular complexes. At CERM, NMR, combined with EPR spectroscopy and other biochemical techniques, was applied to rationalize the severe phenotype associated with a rare pathogenic mutation of BOLA3, a protein involved in the mitochondrial Fe-S cluster biogenesis.[1] NMR is also instrumental to shed light on how intrinsically disordered proteins (IDPs) bind metals: applications include the Cu binding to α-Synuclein in a membrane-like environment and how Zn binding affects the dynamics of transcriptional regulators YY1 and CzrA.[2-4]

In addition to traditional NMR-active nuclei, ^{1}H, ^{13}C, and ^{15}N, the highly sensitive ^{19}F offers an attractive alternative to study challenging macromolecules. A novel approach has been developed at CERM for labelling proteins at specific positions through the use of fluorine labels chemically linked to aromatic residues.[5] Current research also focuses on the development of fast 2D NMR methods to investigate protein dynamics in real time.[6]



Integration of residue-level data by NMR (left) with EXAFS and EPR (middle) allows characterizing metal binding to dynamic systems such as α-synuclein (right).

References:

1) B. Bargagna, L. Banci, F. Camponeschi, ***Int. J. Mol. Sci.*** 2023, 24, 11734.

2) C. Bacchella, F. Camponeschi, P. Kolkowska, et al. ***Biomolecules*** 2023, 13, 287.

3) M. Figiel, F. Szubert, E. Luchinat, et al. BBA - ***Gene Regul. Mech.*** 2023, 1866.

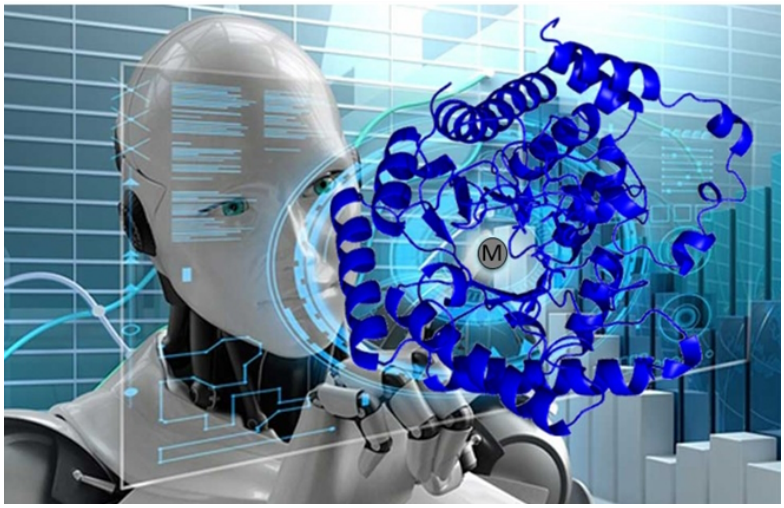
4) M. Villarruel Dujovne, M. Bringas, I. C. Felli, et al., ***J. Magn. Reson. Open*** 2023, 16–17.

5) V. Vitali, F. Torricella, L. Massai, et al. ***Sci. Rep.*** 2023, 13, 22017.

6) F. Bruno, E. Luchinat, K, Kazimierczuk, E. Ravera, Fast 2D NMR to Investigate Dynamic Events in Biomolecules. In ***Fast 2D Solution-state NMR: Concepts and Applications*** 2023.

Computing for Integrative Structural Biology

Innovative computational techniques in structural biology leverage the availability of extensive data repositories that integrate structural information in the PDB providing biological context for proteins, to which we contribute our expertise on metal sites[1,2].
Our expertise in metal-sites contributed to the rationale design of Ru(II)-based photo-responsive antibacterial agents[3]. We also furthered our interest in the development of force fields for MD simulations of metalloproteins, showing that such simulations can reproduce experimental NMR data very satisfactorily[4].
Additionally, we recently opened a new line of research with the aim of applying machine learning to the identification of zinc-sites in 3D structures[5].



Integrative structural biology combines data from multiple techniques to obtain a deeper understanding of complex biological systems. To progress towards this goal, our work focused on providing thorough information on metal-binding systems through European databases and on the automation of tools for data analysis.

References:

1) Bazayeva, M.; Laveglia, V.; Andreini, C.; Rosato, A. ***Journal of Inorganic Biochemistry*** 2023, 238

2) EOSC consortium. ***EMBO Journal*** 2023, 42 (23).

3) Pagliai, M.; Andreini, C.; Guerri, A.; Valtancoli, B.; Giorgi, C. et al. ***Inorganic Chemistry*** 2023, 62 (20), 7716–7727

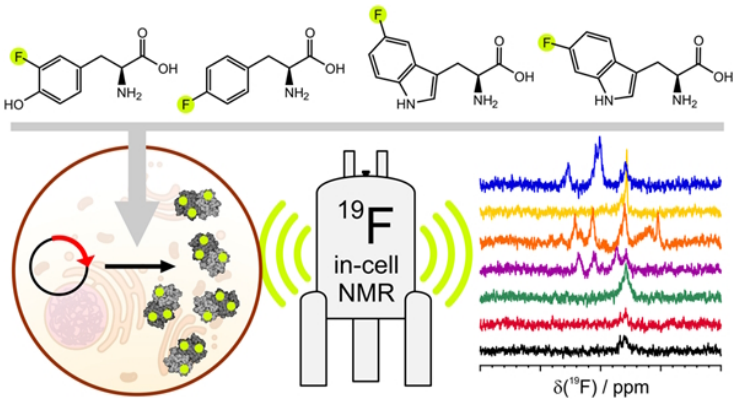
4) Bazayeva, M.; Giachetti, A.; Pagliai, M.; Rosato, A. I***nternational Journal of Molecular Sciences*** 2023, 24 (6)

5) Laveglia, V.; Bazayeva, M.; Andreini, C.; Rosato, A. ***Bioinformatics*** 2023, 39 (11).

In-cell NMR and EPR in Human Cells

CERM continues to pioneer the development of NMR methods to investigate proteins and small molecules in living human cells at atomic resolution. A novel approach has been developed to incorporate fluorinated amino acids in proteins expressed in human cells. ^{19}F in-cell NMR analysis results in simple, virtually background-free spectra, and can be applied to investigate protein structure, dynamics and interactions with the cellular environment, protein partners or external molecules.

In-cell spectroscopic approaches provide unique details on the structure, dynamics and function of macromolecules inside living cells. Such approaches provide highly physiologically relevant data, which integrates and complement the array of structural techniques available in vitro. Complex cellular processes, such as those regulating cellular metal homeostasis, can be elucidated.[1] Among the structural techniques suitable for in situ studies, NMR stands out as an ideal non-destructive atomic-resolution approach to investigate proteins and nucleic acids in the cellular milieu. Recent technological advancements have greatly extended its applicability, and will continue to do so in the near future.[2] At CERM, a novel approach has been reported, that allows introducing fluorine atoms in proteins expressed in human cells, enabling a new kind of ^{19}F in-cell NMR applications. Specifically, it is shown that fluorinated aromatic amino acids, 3-fluorotyrosine, 4-fluorophenylalanine, 5- and 6-fluorotryptophan, can be incorporated in intracellular proteins by switching to a custom-made growth medium during protein expression. Fluorinated proteins are easily detected and investigated in virtually background-free 1D spectra which exploits the ^{19}F nucleus, facilitating the readout of structural features and interactions with ligands or other proteins.[3]



Fluorinated aromatic amino acids are introduced in proteins transiently overexpressed in human cells, allowing ^{19}F in-cell NMR analysis of protein conformational changes and interactions with ligands..

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1) F. Camponeschi, L. Banci, ***FEBS Letters*** 2023, 597, 122–133.

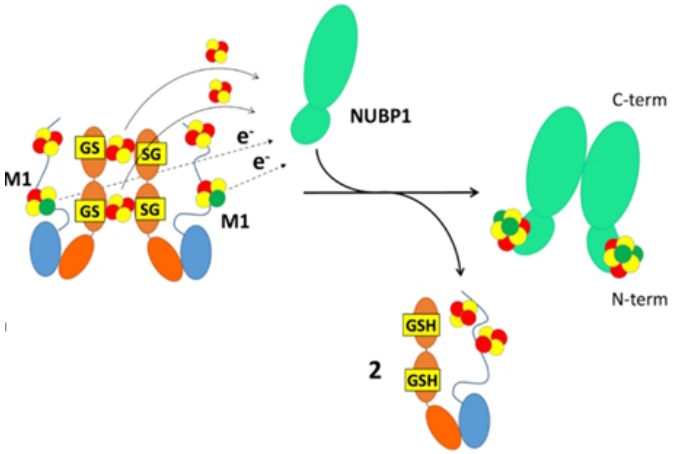
2) E. Luchinat, L. Banci, ***Rendiconti Lincei*** 2023, 34, 653–661.

3) L. B. T. Pham, A. Costantino, L. Barbieri, V. Calderone, E. Luchinat, L. Banci, ***J. Am. Chem. Soc.*** 2023, 145, 1389–1399.

Molecular Mechanisms of Iron-Sulfur Protein Biogenesis in Humans

In humans, the maturation of Fe-S proteins is performed by two distinct protein machineries, responsible for the biogenesis of mitochondrial, and cytosolic and nuclear Fe-S proteins. In 2023, we focused our attention on the investigation of how the [4Fe-4S] clusters are assembled and distributed in the mitochondria and in the cytosol of human cells. Using a combination of biochemical and spectroscopic techniques, we demonstrated that in the human cytosol, the GLRX3 protein and the anamorsin protein form a stable, hetero-tetrameric complex, that is able to synergically provide two $[2Fe\text{-}2S]^{2+}$ clusters from GLRX3 and two electrons from anamorsin for the assembly of a $[4Fe\text{-}4S]^{2+}$ cluster on the NUBP1 protein.[1]
SAXS coupled with solution NMR information reveals that the structural plasticity of NFU1, an accessory protein of the mitochondrial [4Fe-4S] machinery, is crucial to drive protein partners recognition and modulate $[4Fe\text{-}4S]^{2+}$ cluster transfer from the ISCA1-ISCA2 complex to the ISCA1-NFU1 complex, as well as to provide a first rational for the molecular function of the N-domain of NFU1 as a modulator in the $[4Fe\text{-}4S]^{2+}$ cluster transfer.[2]

Iron-sulfur proteins are The biogenesis of Fe-S proteins is a highly complex process in all living cells, and several human diseases are related to its misfunctioning. The comprehension of the mechanisms of Fe-S protein biogenesis is fundamental to boost the development of disease treatments.



Proposed model of the [4Fe-4S] cluster assembly process on NUBP1.

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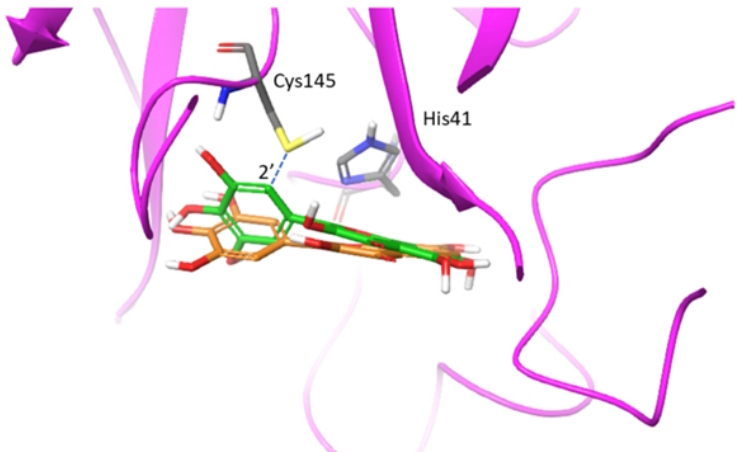
Proteins as Drugs and Drug Targets

Proteins are important pharmaceutical targets, and an increasing number of drugs are proteins. Studying the structure of the protein targets is crucial for finding new drugs and developing potential treatments. Using NMR to assess the higher-order structure of biological drugs is a powerful method for understanding their features and investigating the stability.

Non-structural protein 5 is a cysteine protease that plays a key role in SARS-CoV-2 replication, suppressing host protein synthesis and promoting immune evasion. The research activity of CERM/CIRMMP led to the identification of natural products as lead compounds for the development of antiviral agents.[1] The scientists of CERM/CIRMMP have also described a novel interaction between lipoproteins and α-syn aggregates that inhibits the formation of α-syn fibrils, which have relevant implications for seed amplification assays.[2] Further, the effect of low-molecular weight inhibitors on Aβ42 aggregation kinetics has been characterised through a specific computation model and the analysis by NMR of the fibrillation kinetics.[3]

In another line of research, new drugs targeting PD-1/PD-L1 immune checkpoint have been studied.[4] Moreover, a high-affinity mutant of PD-1, namely, HACTR-PD-1 with nanomolar affinity vs PD-L1, as an alternative to antibodies, has been designed and conjugated with rhamnosyl derivatives to improve immunological activity.[5] At CERM/CIRMMP, NMR has been also integrated in an analytical workflow to evaluate the effects of oxidative stress on monoclonal antibodies.[6] NMR has finally provided information about the dynamics of a mutant of STIM1,[7] the interaction of Hur protein with new ligands,[8] and the structure of a newly designed antimicrobial peptide.[9]



Overlap of the experimental and predicted binding mode of myricetin on the non-structural protein 5.

References:

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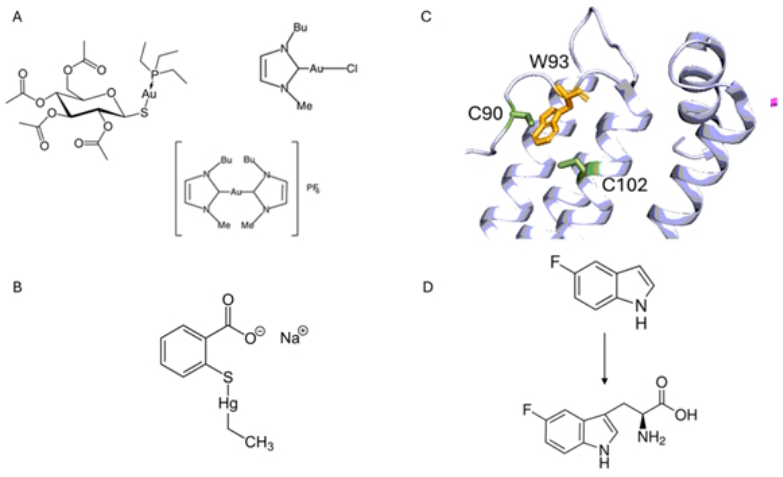
Metal-based drugs: metabolomic alterations and ferritin-mediated delivery

The recombinant homopolymeric human ferritin (HuHf) was exploited as a nanocarrier of metal-based drugs for the targeted delivery of cancer cells expressing high levels of the receptor TfR1. Two solvent-exposed cysteine residues, Cys90 and Cys102, provide an efficient binding site to coordinate soft metals like the gold(I) of auranofin (AF) and the mercury(II) of Thimerosal. The binding to these cysteines is proved by studying via ESI MS and ICP OES the interaction between the metallodrug and HuHf variants where one or both Cys residues are replaced by Ala[1].

^{19}F NMR on 5-F-Trp93 was efficiently used to further monitor the metal binding to the nearby Cys90 and Cys102 as well as the uptake of free and bound HuHf by cells[2].

The effect of free gold(I) compounds (AF and two cytotoxic carbene compounds) in A2780 ovarian cancer cells on the cell metabolome was monitored through 1H NMR[3] and compared to that of the HuHf@AF bioconjugate.

HuHf is exploited as a nanocarrier of metallodrugs containing soft metal ions via the binding to solvent exposed cysteines. ^{1}H and ^{19}F NMR are used to evaluate the effect of free and HuHf-bound metal compounds on the metabolome of A2780 cells and the metal uptake from the growth media, respectively.



Investigated metal compounds: A) Gold(I) compounds, i.e. Auranofin, Monocarbene, Dicarbene; B) Thimerosal, C) Close up of the area of the ferritin subunit containing the metal binding Cysteines and the only Tryptophan. D) Fluorination of Trp93 using 5-Fluorindole as the precursor.

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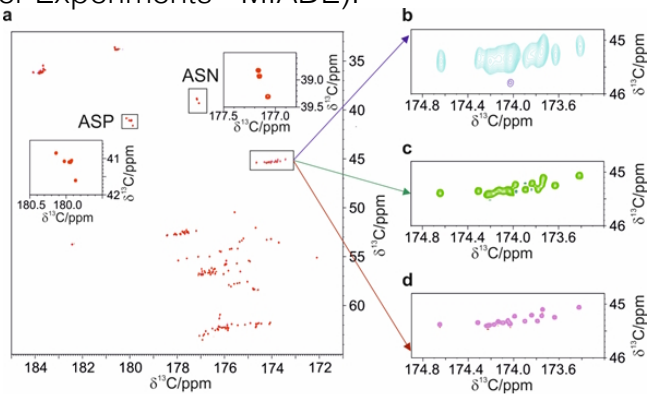
Intrinsically Disordered Proteins by NMR

NMR spectroscopy is pivotal for understanding atom-level details of flexible intrinsically disordered proteins (IDPs/IDRs). A refined 1.2 GHz protocol was developed describing ^{13}C direct detection NMR: the excellent resolution that can be achieved enables the study of complex systems like cancer-related IDPs. Two cancer-associated IDPs were studied via NMR. Novel experiments, exploiting cross-correlation in proline residues were developed. Guidelines (MIADE) were proposed to improve the presentation of experimental findings on IDPs.

IDPs are under the spotlight of current structural biology. NMR is one of the most relevant technologies for studying IDPs and the suite of ^{13}C detected NMR experiments developed at CERM has been recognized as an indispensable investigation tool. A protocol describing tips and tricks for optimal experimental performance exploiting ^{13}C-direct detection at 1.2 GHz to study IDPs was established. This protocol also illustrated some of the advantages of studying IDPs at ultra-high magnetic fields, like the increased resolution that can be obtained if compared to lower fields.[1]

Research at CERM/CIRMMP deals also with the functional studies of IDPs and of intrinsically disordered regions (IDRs) of heterogeneous proteins. Among them some related with the insurgence of cancer and its progression provided the ground to start investigation of liquid-liquid phase separation[2] and to design novel experiments to investigate the dynamic properties of proline-rich polypeptides, often involved in relevant aspects of IDPs function and misfunction.[3]

The long-lasting expertise at CERM in studying IDPs was key for defining guidelines to report findings of experimental studies of IDPs and of intrinsically disordered regions (IDRs) (Minimum Information About Disorder Experiments - MIADE).[4]



The figure shows the 2D CACO spectrum acquired using the 1.2 GHz NMR instrument on ^{13}C,^{15}N-labeled α-synuclein (a) together with a zoom of the region where the C′-Cα correlation peaks of glycine residues fall (d). It can be noted how the resolution increases moving from acquired at 500 MHz (b) to 700 MHz (c) up to 1.2 GHz (d) fields.

References:

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Solid-state NMR in Structural Biology

Antibiotics acting through new biological mechanisms are important to combat antimicrobial resistance. We introduced Clovibactin, a new antibiotic isolated form uncultured soil bacteria, that efficiently kills Gram-positive bacteria without any detectable drug resistance. Solid-state NMR and in particular ^{1}H-^{31}P ssNMR 2D correlation spectra, in combination with biochemical investigation and atomic force microscopy allowed us to disclose the mechanism of action of Clovibactin who is targeting the pyrophosphate of essential peptidoglycan precursors (C55PP, lipid II, and lipid IIIWTA). In addition, Clovibactin forms fibrils with an hydrophobic interface that wrap around the targeted pyrophosphate bypassing the variable structural element of the precursor, and for that, it lacks any antimicrobial resistance.[1]

We also developed new methodologies to characterise protein-drug conjugates, an innovative class of biological drugs for the treatment of cancer diseases. Through the integration of solid-state and solution NMR with crystallographic studies, a new cytotoxic molecule derived from paclitaxel, and able to bind with high affinity to transthyretin protein, has been developed. By binding to transthyretin, paclitaxel overcomes the very low solubility problems and can exploit the receptors for physiological transthyretin present on the surface of target cells in order to be internalized.[2,3]

We used spin-label-based EPR spectroscopy to investigate whether lysozyme shows an orientational preference with respect to the silica surface within the bioinspired composite.[4]

Solid state NMR (ssNMR) is one of the most powerful technique to characterise not soluble biomolecules and membrane proteins. In the example here reported we shows how through ssNMR it is possible to understand the action mode of clovibactin, a new powerful antibacterial molecule, and to characterise protein drug coniugate formulations.

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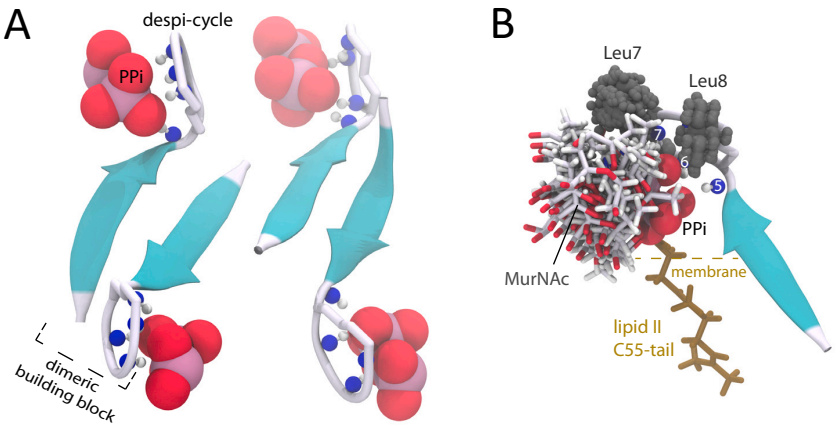
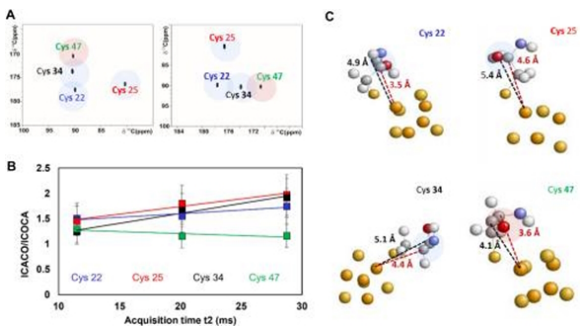


Action mode of Clovibactin: A) Solid-state derived structural model of the interaction with lipid-II pyrophosphate.
B) the hydrophobic depsi-cycle side chains of Clovibaction wrap like a glove around lipid II PPi group, interacting with the hydrophobic side of MurNAc.

NMR of Paramagnetic Systems

Tailored experiments can revive signals in the surrounding of a paramagnetic center; however, signals identification without specific residue assignment remains useless. Optimized ^{13}C direct detected experiments extend the available assignments, improving the overall knowledge of these systems.

Paramagnetic relaxation can drive the signal assignment of residues in the proximity of the paramagnetic center(s). This has allowed us to identify the potential key players of the biological function of the protein CISD3, a [2Fe-2S] containing protein[1,2]. The different relaxation properties of C^{α} and C' nuclei are exploited in CACO vs COCA experiments and the complementarity of the two experiments is used to obtain structural information[3]. In the case of the [4Fe-4S] HiPIP protein PioC, ^{13}C R1 values can be measured also at very short distances from the paramagnetic center and added to other classical and paramagnetism based NMR restraints to improve quality and quantity of the NMR information[4]. Paramagnetic NMR approaches have been essential to characterize the maturation pathway for the nickel-dependent enzyme urease, which utilizes UreE as a metallochaperone to supply Ni(II) ions[5] and, similarly, to study the protein-protein interactions in Rhodopseudomonas palustris TIE-1, responsbile for Photoferrotrophic Iron Oxidation in Rhodopseudomonas palustris TIE-1[6].
We have used paramagnetic NMR and EPR to study the coordination of cobalt(II) in the active site of carbonic anhydrase in the presence of thiocyanate, which is both a ligand for the metal center and a chaotropic agent. The different coordination models that explain the NMR and EPR data at high concentrations of thiocyanate have been discussed and interpreted[7]. Finally, we reviewed the use of paramagnetic NMR to probe protein structural rearrangements[8].



A) COCA-AP (left-half) and CACO-AP (right-half) spectra. B) ICACO/ICOCA peak intensity ratios related to increasing acquisition time in the direct dimension of the spectrum (t2). C) Molecular view of PioC's cluster binding residues.

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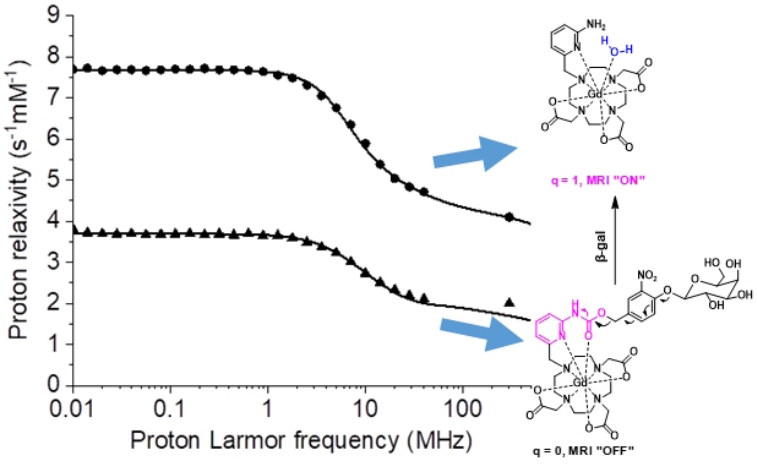
Field Cycling Relaxometry

Paramagnetic complexes are largely employed in magnetic resonance imaging (MRI) for their ability to increase the image contrast. Bio-responsive Gd(III) MRI probes are widely used for visualizing biological targets such as enzyme activities. We have described a new pyridyl-carbamate-based self-immolative Gd(III) bioresponsive MRI complex, acting as a probe for the β-galactosidase enzyme.[1] The enzymatic activation of the complex creates an open coordination site for water molecules to bind Gd(III), resulting in a significant increase of relaxation.

The amount, and thus the toxicity, of contrast agents to be injected can be lowered b y replacing the Gd(III) ion with Mn(II) ions[2], by increasing its relaxivity[3] or with iron oxides.[4] Gd-labeled protein cages can determine a huge signal amplification through both a high payload of Gd(III) in each protein cage and increased relaxivity due to a slow tumbling.[3] Nanogels based on Mn(II) bisamide derivatives of CDTA incorporated into chitosan also show improved relaxation performances.[2]

Field cycling relaxometry data suggests that superparamagnetic iron-oxide particles may also prove useful as T1 contrast agents for low-field MRI.[4]



[1]H relaxivity profiles of a non-activated (q=0) and activated (q=1) Gd-complex, acting as a β-galactosidase-responsive probe (25 °C).[1]

Field cycling relaxometry is an extremely informative tool for the characterization and optimization of contrast agents for MRI. This technique provides the field dependence of the water proton nuclear relaxation rates, thus revealing mechanistic information about the paramagnetic complexes, their hydration and dynamics.

References:

1) J.-H. Tang, H. Li, C. Yuan, G. Parigi, C. Luchinat, T.J. Meade, ***J. Am. Chem. Soc. 2023,*** 145, 10045–10050.

2) F. Carniato, M. Ricci, L. Tei, F. Garello, C. Furlan, E. Terreno, E. Ravera, G. Parigi, C. Luchinat, M. Botta, ***Small*** 2023, 19, 2302868.

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Solid-state Methods and DNP for Materials

Solid-state NMR (ssNMR) suffers for sensitivity problems, here we present two different approaches to increase the signal to noise in ssNMR, the first based on a new hyperpolarization method, and the second exploiting signal analysis denoising algorithms. These methods can be applied to the investigation of material sample.

Sensitivity is one of the major problems in solid-state NMR, and for this reason there is a growing interest in DNP and in any hyperpolarisation technique. Here we introduced first a new hyperpolarisation technique for solid-state NMR, that is based on a specific photo-CIDNAP effect in the solid phase. The innovative aspect of this technique is that, upon light illumination of the sample, we can arrive to hyperpolarize the bulk proton lattice of the solid-state sample, with the possibility to hyperpolarize the whole sample by ^{1}H-^{1}H spin diffusion.[1] This technique, demonstrated here at low field, can be in principle scale up to high magnetic field and room temperature, with an enormous potential in materials and biomolecular NMR investigation.

We also intensified our studies on liquid crystals extending the used of deuterium NMR in the investigation of the phase transitions in various nematic phases.[4]

Another approach to increase the signal to nose ratio, is to perform denoising processing on the acquired spectra. Signal analysis methods based on dimensionality reduction of the dataset have been evaluated for denoising series of solid-state NMR data. This is relevant in the case of signals with low signal-to-noise-ratio (SNR). We have identified the minimum SNR that allows for successful extraction of (e.g.) CP buildup curves for a preset measurement time.[3]

This approach has been successfully applied for understanding the poisoning of graphene oxide-based catalysts.[5]

References:

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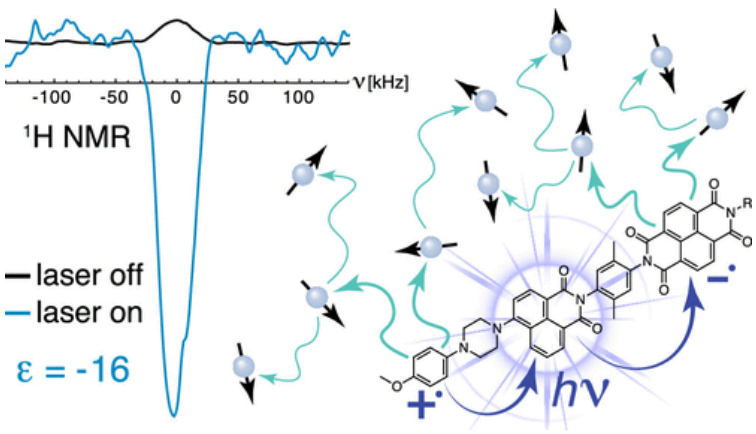


Schematic description of the optically enhanced solid-state NMR. The nature of the polarising agent (reported in the figure), is crucial for the successful polarisation enhancement.

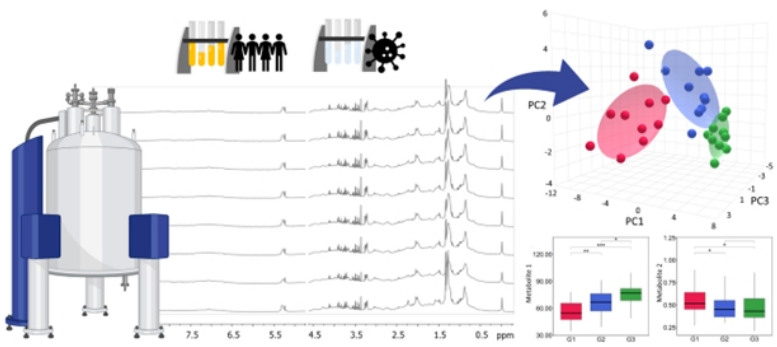
Metabolomics in Biomedicine

NMR-based metabolomics at CERM spans a broad range of activities. During 2023, our attention has been largely focused on the application of metabolomics in cancer research. In particular, we investigated the prognostic role of serum metabolomics for breast cancer and sarcoma patients[1,2], and the metabolic alterations occurring in the evolution from adenoma to colorectal cancer[3,4]. In addition, the research line on aging-related diseases has been further exploited, with investigation into cerebrovascular and neurodegenerative pathologies[5,6].

From the beginning of the pandemic, we used NMR-based profiling to define the metabolic alterations induced by COVID-19. The plasma of patients exhibits a robust metabolomic and lipoproteomic signature, associated with the severity of the disease, as evidenced by the analysis of a large cohort of patients sampled during various waves of infection[7].

Another thread of interest concerns the use of metabolomics to study the impact of therapeutical interventions on individual metabolism[8]. In particular, we used NMR-based metabolomics to explore the effects of probiotic administration on urinary and serum metabolic profiles in healthy individuals[9].

Attention has been also devoted to methodological improvements, to make NMR metabolomics more versatile and practical[10]. We discussed a combination of instrumental and data analysis strategies to speed NMR acquisition and to obtain a robust and efficient data matrix for classification[11,12].



A schematic representation of the workflow of metabolomics by NMR.

Metabolomics offers a comprehensive, dynamic, and accurate picture of a cellular model, a biofluid, an organ, or an organism at a molecular level. Thus, it is an invaluable instrument to obtain information on diseases' underlying biochemistry, to diagnose and to prognosticate pathological conditions.

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Other Applications of Metabolomics

Metabolomics encompasses various areas of basic and applied research.
It is utilized in the agricultural field for quality assessment and to understand the impact of processing on foodstuff. Exploring microorganisms and plants can help in the identification of novel metabolites with relevant ecological applications.

Even though conventional quality, safety, and authenticity control in food is based on targeted strategies, high-resolution 1H-NMR, performed under well-defined instrumental specifications, offers several advantages, including producing an extremely reproducible food fingerprint and fully quantitative data by means of a single experiment.

In this framework, NMR spectroscopy was employed to study the effects of biofortification of tomatoes with selenium on the physiological, metabolic, and molecular processes during the last stages of fruit development, particularly through ripening[1]. Se enrichment resulted in improved nutraceutical properties of tomatoes both directly, by increasing the Se content, and indirectly, by improving the levels of polyphenols, amino acids, terpenoids, and carotenoids. In the dairy sector is important to characterize the metabolites in milk for safety or traceability purposes. This was performed using NMR and NIR spectroscopy[2].

New applications are emerging in the field of ecology: metabolomics is a valuable tool for describing interactions between microorganisms and external environment, which could aid in the identification of molecular adaptations to environmental fluctuations. In particular, the mechanisms through which metabolic homeostasis is maintained in a cold-adapted marine bacterium at different temperatures was investigated[3].

References:

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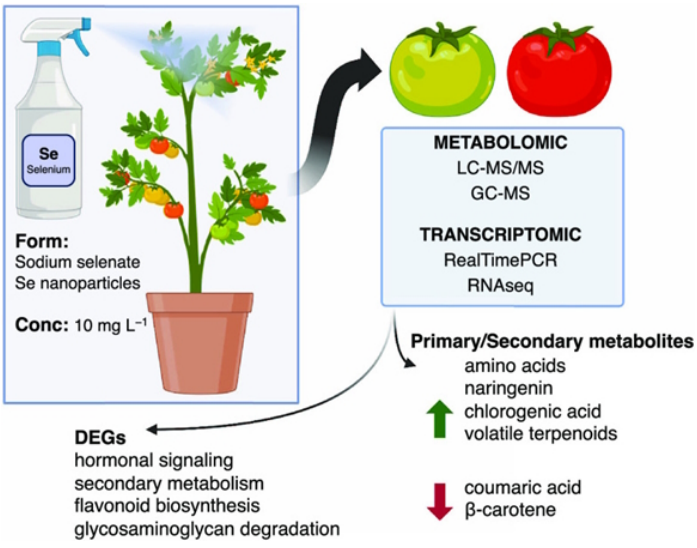


Effects of Se enrichment in tomatoes during ripening, metabolomic and transcriptomic results are summarized in figure.

National and Transnational access

Instruct-ERIC ESFRI Infrastructure – European and National NMR Research Infrastructure

CERM/CIRMMP is the key centre for application and development of NMR spectroscopy within Instruct-ERIC, an ESFRI infrastructure operative since 2012.

Instruct-ERIC provides access to unique instrumentation in a variety of different structural techniques (see page 9). This innovative approach allows for a description of biological cells at the molecular level, in order to understand how living organisms function in normal and pathological conditions and to design drugs and vaccines. Since 2022, dedicated access routes are available for research tackling cancer and infectious diseases through the canSERV and ISIDORe projects, respectively. The possibility of access to Instruct-ERIC represents a unique opportunity for researchers, both at the national and European level, as well as at the international one, to strengthen the innovation capacity of the research performed.

The project PANACEA (https://panacea-nmr.eu/), started in 2021, is funded by the HORIZON2020 program to offer European researchers access to advanced Solid-State NMR instruments for the investigation of chemical and pharmaceutical solid compounds, as well as organic and inorganic materials. The platform is open to scientists and industrial partners with or without previous experience in solid-state NMR.

In addition, CERM/CIRMMP continues to provide access to its instrumentation to all national users whose research is outside the Instruct-ERIC scope, provided their research project matches quality criteria in terms of scientific interest, excellence and feasibility. CERM/CIRMMP is promoting the development of a national platform Instruct-ITALIA to favour the development of a consortium of infrastructures in structural and cellular biology for national access service.

In all cases, access is granted on the basis of peer-review of the received proposals, and after a feasibility check by the staff scientists of CERM/CIRMMP. Technical assistance is provided for the acquisition of the data. Scientific collaborations are welcome but not required. The uniqueness of access provision at CERM/CIRMMP infrastructure lies in the wide number of available NMR instruments, the variety of the NMR equipment (probes, automatic sample changers,…) and the exceptional expertise of the scientific and technical stuff, which represents an ideal environment for NMR research, especially in the field of structural and functional characterization of biological systems. The description of the NMR instrumentation made available at CERM/CIRMMP is reported in the dedicated paragraph at page 37. Notably, in 2020 we have installed the first world 1.2 GHz instrument operative since April 2020, and its contribution to research is already visible in the research session.

NATIONAL AND TRANSNATIONAL ACCESS

Molecular biology and cellular biology labs are also strategic for the users' needs to prepare and/or optimize a large variety of samples for structural characterization, together with other biophysical equipments for EPR, CD, UV-vis, stopped-flow measurements, manual and automated crystallization facilities and X-ray diffractometry. Users can also access other university facilities available in the campus, such as those for cryo-electron microscopy (FloCEN), mass spectrometry, Raman resonance, and non-linear spectroscopies.

CERM/CIRMMP also provides access to its computational e-infrastructure, which includes a cluster for the more intensive calculations, with 16 blades harboring a total of 80 CPU cores. Ten servers are used to host services from web pages to databases and to enable access to the European Grid. A number of graphic stations are available for interactive NMR data analysis.

During 2023 we recorded 449 days of external access to the NMR spectrometers. A more detailed analysis shows that 354 days NMR access were provided to academic users via Instruct-ERIC, Instruct-ITALIA, iNEXT-Discovery and PANACEA, while 95 days were provided to industry users, either as services or through formal collaborations.

Beside NMR access provision, the infrastructure provided 40 days of access to protein production services via Instruct-ERIC and to other structural biology techniques via Instruct-ITALIA.

Worth to mention the implementation of a platform for the management of NMR access (https://amp.cerm.unifi.it/) improving data findability and experiment reproducibility and, thanks to new in-house LIMS, track of all the experiments performed and allows long-term data storage.

Collaborations with Industries

CERM/CIRMMP has a long tradition in collaborations with industries: from simply providing access and service to its instrumentation, to establishing a more collaborative activity in research projects or to the participation as partners in international project calls. This number does not include the access provided to industrial partners through collaborative projects.

We warmly thank the following companies for stimulating interactions:

Bracco SpA

Bruker BioSpin

Dompé Pharmaceutical

Italmatch Chemicals

Glaxo Smith Kline

Giotto Biotech Srl

Merck

COLLABORATION WITH INDUSTRIES

Menarini Srl

Valagro S.p.a.

Abiogen S.p.a.

Infineum

Danger and Safety

Buona Steve Jones

INOTREM, control innate immunity

Probiotical S.p.a.

COLLABORATION WITH INDUSTRIES

Indena S.p.a.

Stelar S.r.l.

Extra Byte S.r.l

Latus Pet

Maven Health GmbH

A special acknowledgment to Gruppo SAPIO Srl,
official supplier of all the cryogenic gases of CERM/CIRMMP

Other Institutions

Florence Center for Electron Nanoscopy (FloCEN)

FloCEN is a laboratory located at the Department of Chemistry of the University of Florence, which houses state-of-the-art equipment for Cryo-Electron Microscopy (Cryo-EM), which includes a ThermoFisher Transmission Electron cryo-Microscope Glacios at 200-kV (also equipped with a Falcon III direction electron detector), a ThermoFisher Vitrobot Mark IV for specimen preparation, and a PELCO easiGlow™ Discharge Cleaning System (optimized for cleaning TEM grids). Furthermore, FloCEN has a low humidity room and a shielding system to keep the microscope free from electromagnetic interference, thus guaranteeing a very stable environment. FloCEN was established thanks to the funding provided by MUR (grant Dipartimento di Eccellenza 2018-2022), with an important co-financing with the MUR International Action of FOE dedicated to the Italian centre of Instruct-ERIC. Cryo-EM and NMR are complementary techniques, providing insights into biomolecular structures at different resolutions and under diverse conditions.

https://www.flocen.unifi.it/index.

Recombinant Proteins JOYNLAB

The “Recombinant Proteins JOYNLAB” is a joint laboratory established between CERM, the Department of Chemistry (DICUS) “Ugo Schiff”, and Giotto Biotech S.r.l. (https://www.giotto-biotech.com/). JOYNLAB, through various activities including the execution of shared research and development projects, aims to achieve scientific and applied objectives in the development and study of:

- Recombinant proteins in both natural and isotopically enriched forms;
- Methodologies for the metabolomic analysis of biofluids;
- Reference standards for NMR in solution and solid state;
- Organic compounds of pharmaceutical and industrial interest.

In 2023, JOYNLAB was involved in a European MSCA project called “GLYTUNES - A multidisciplinary training network for the bio-inspired development of glycomimetics tuning the Siglec-Sialoglycan axis” (No. 956758 – Call H2020-MSCA-ITN-2019) funded and financed by the European Community; within this project a PhD student has been hired starting from November 2021 and she is involved in structural biology research. Additionally, in the same year, the funding for another Marie-Curie project named ENSCC (No. 101119492 - Call HORIZON-MSCA-2022-DN-01) was announced; within this project a new PhD student will be

hired. As a result of this collaboration, in 2023, the staff of JOYNLAB co-authored 10 scientific publications, among which 8 co-authored with CERM staff (page 48, publ. 3-4-9-11-19-36-43-44).

CRElio

CRElio is the Service Center of the University of Florence dedicated to the recovery and liquefaction of helium gas. Helium is a non-renewable resource. The extraction of helium is energy-intensive and has a non-negligible environmental impact. Therefore, the recovery and liquefaction of helium are important to ensure a stable and sustainable supply of this resource to support the needs of scientific applications. Liquid helium plays a crucial role in NMR spectroscopy by providing the necessary cooling for superconducting magnets. CERM joins CRElio together with a series of University Departments and other structures. For its activities, CERM is the main supplier of He gas to CRElio and the main user of liquefied He. This partnership with CRElio allows CERM to obtain a good share of the helium needed for refilling its NMR instruments in a sustainable manner.

Fondazione Sacconi

The Luigi Sacconi Foundation (https://www.cerm.unifi.it/fondazione-luigi-sacconi) was established in 1996 to honour the memory of Prof. Luigi Sacconi, who was a prominent figure in Chemistry and founder of the General and Inorganic Chemistry School in Florence where many international scientists have been educated. The Luigi Sacconi Foundation has its register office at CERM and members of CERM/CIRMMP are involved in the Foundation's Administrative Council. The aim of the Foundation is to promote scientific research in the molecular sciences at the local, national, and international levels. Particular attention is devoted to chemistry, in its implications and applications concerning health, quality of life, environment, energy, and technological and industrial development. For this purpose, the Luigi Sacconi Foundation collects documents and publications, promotes seminars, courses and meetings and other activities supporting the exchange of scientific knowledge, subsidizes the activity of Italian and foreign researchers, and establishes awards.

On April 14th, 2023, Professor Claudio Pettinari, Rector of the University of Camerino, delivered the "Luigi Sacconi Memorial Lecture in Chemistry" (2023 edition) titled "Materials of Future for Energy and the Environment". The Sacconi Medal Lecturer 2023 has been awarded to Prof. Silvio Aime. The award ceremony took place on 15 September 2023 during the XLIX National Congress of Inorganic Chemistry held in Perugia. The Foundation also collaborated in organizing AMYC-BIOMED 2023, the "Autumn Meeting for Young Chemists in Biomedical Sciences," held in Florence on October 16-18, 2023.

Instrumentation

Solution and Solid-State NMR Spectrometers

In 2020, the first 1.2 GHz NMR instrument operating at 28.2 T was installed at CERM. This instrument is currently operating with solution TCI and TXO cryoprobes. All NMR instruments are state-of-the-art, digital spectrometers equipped with a variety of cryo-probes, as well as with specific probes covering a broad range of frequencies and of observable nuclei. In addition, all the standard pulse sequences for spectroscopic, structural, dynamical, and functional characterization, tailored pulse sequences for structural determination of high molecular weight proteins and paramagnetic systems are implemented. ^{13}C direct-detection solution protocols for “protonless” NMR experiments and structural characterization of biomolecules, including unfolded or partially unfolded ones, are developed and updated. Pulse sequences and experiment setup have been implemented for the detection and characterization of paramagnetic systems, and in this field CERM has been pioneer since decades. For this reason, the 400 MHz instrument is equipped with a special 3 mm High Power probe designed for the investigation of paramagnetic systems. Solid-state MAS probes cover almost all the presently achievable MAS frequencies, from a few hundred of Hz to ultra-fast MAS regime, and since 2017 CERM/CIRMMP features a new 0.7 mm CP MAS probe spinning up to 111 kHz. Special protocols and devices are available for solid state experiments both for biological and inorganic material characterization. Set-up and pulse sequences for in-cell NMR experiments are also implemented. In 2023, a prototype shuttle system for high-resolution relaxometry measurements has been installed at 700 MHz as part of the HIRES-MULTIDYN research activities. By moving the sample inside the stray field of the magnet, this device allows for nuclear spin relaxation measurements at fields as low as 47 mT (~2 MHz ^{1}H Larmor frequency), while providing high resolution readout through high field detection.

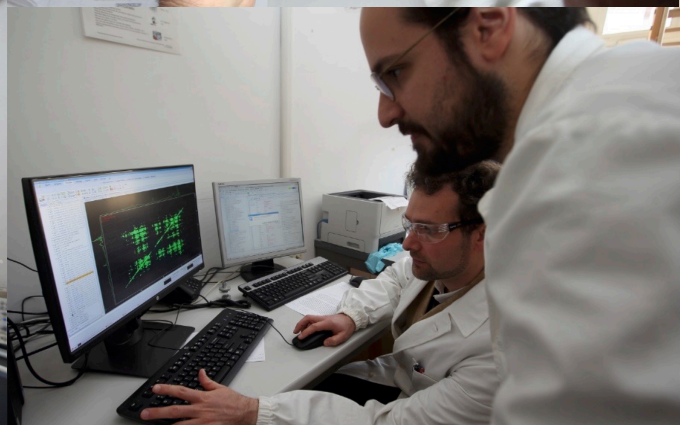
INSTRUMENTATION

B_0 Field (T)	^{1}H Larmor Frequency (Bore)	Probe heads
28.2	1200 MHz (NB 54 mm)	TCI Cryo 3 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) TXO Cryo 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) PI HR RT 3 mm solution $^{1}H/^{13}C/^{15}N$/ with ^{2}H decoupling)
22.3	950 MHz (NB 54 mm)	TCI Cryo 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
21.1	900 MHz (NB 54 mm)	2x TCI Cryo 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) TXI RT 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
20.0	850 MHz (WB 89 mm)	3.2 mm CP MAS DVT $^{15}N/^{13}C/^{1}H$ 1.3 mm CP MAS ^{1}H-^{19}F/BB/^{15}N 0.7 mm CP MAS $^{1}H/^{13}C/^{15}N$
18.8	800 MHz (NB 54 mm)	TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) QXI RT 5 mm solution($^{1}H/^{13}C/^{15}N/^{31}P$ with ^{2}H decoupling) ^{1}H-Selective High Power RT (prototype) 3.2 mm CP MAS DVT Low-E $^{15}N/^{13}C/^{1}H$ 1.3 mm CP MAS ^{1}H-^{19}F/BB-X/BB-Y 1.3 mm CP MAS $^{1}H/^{13}C/^{15}N$
16.4	700* MHz (NB 54 mm)	TCI Cryo 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
16.4	700 MHz (NB 54 mm)	TXO Cryo 5 mm solution($^{13}C/^{15}N/^{1}H$ with ^{2}H decoupling) TXO RT 5 mm solution($^{13}C/^{15}N/^{1}H$ with ^{2}H decoupling) TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
16.4	700 MHz (WB 89 mm)	3.2 mm CP MAS $^{15}N/^{13}C/^{1}H$ 4.0 mm CP MAS $^{15}N/^{13}C/^{1}H$
14.1	600 MHz (NB 54 mm)	2 x TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) HR-MAS 4.0mm ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) ^{1}H - Selective High Power RT, 5 mm solution ^{1}H - Selective RT, 5 mm solution BBI RT 5 mm solution BBO RT 5 mm solution BBO RT 10 mm solution / BB RT -Low-γ -10 mm solution

INSTRUMENTATION

14.1	600** MHz (NB 54 mm)	TXI RT 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
11.7	500 MHz (NB 54 mm)	QCI-P Cryo 5 mm solution($^{1}H/^{13}C/^{31}P/^{15}N$) TCI Cryo 5 mm solution($^{1}H/^{13}C/^{15}N$) TXI RT 5 mm solution ($^{1}H/^{13}C/^{15}N$) TBO RT 5 mm solution ($^{1}H/^{31}P$/BB) BBI RT 5 mm solution
9.4	400* MHz (NB 54 mm)	BBO RT 5 mm solution BBI RT 5 mm solution (^{1}H/BB) BBI RT 3 mm solution (^{1}H/BB) ^{1}H-Selective High Power 5 mm solution
0.33-1.25	EPR	X and Q Band cavities, X (9.43 GHz), Q-Band (35 GHz), ENDOR Module
0.00024-1	Fast Field Cycling Relaxometer	0.01-45 MHz 10 mm solution tubes

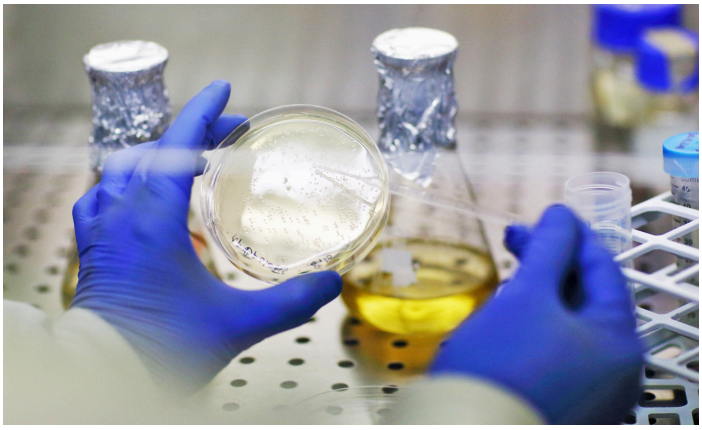
* With sample changer. ** Standardized for metabolomics: equipped with the SampleJet robotic and refrigerated charger, along with dedicated routines for the analysis of biofluids through the Bruker IVDr platform.



Biological and Biophysical Facilities and Services

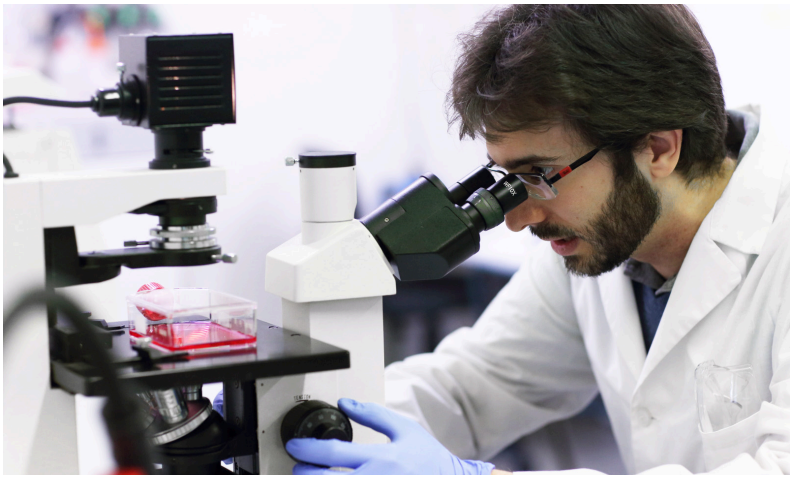
X-ray Crystallography

CERM/CIRMMP is equipped with standard crystallization facilities and with an automated nano-dispensing device (Mosquito, TTP Labtech). Furthermore, it has full access to the Interdepartmental Crystallography Centre of the University of Florence (CRIST, https://www.crist.unifi.it), equipped, among other instruments, with two sealed-tube diffractometers. The most recent one is a Bruker D8 Venture with double microsource (Cu and Mo) bearing a Photon III Pixel Array detector and the older one is an Xcalibur PX Ultra (Oxford Diffraction) equipped with a 165 mm CCD detector for routine in-house data collections. Both diffractometers are equipped with a liquid nitrogen cryosystem. Regular access to synchrotron beam time slots in European facilities is also available.



Molecular and Cellular Biology

CERM/CIRMMP is equipped with state-of-the-art facilities for gene cloning and protein expression and purification. Large scale protein expression in prokaryotes and yeast is available through the use of fermenters. Different isotope labelling schemes, including specific labelling schemes oriented to NMR characterization, can be achieved using auxotrophic strains. Fully equipped facilities for protein purification are available, including last-generation instruments for streamlined purification (ÄKTA chromatography system, including two newly installed ÄKTA Pure and ÄKTA Go machines) and equipment for protein purification.

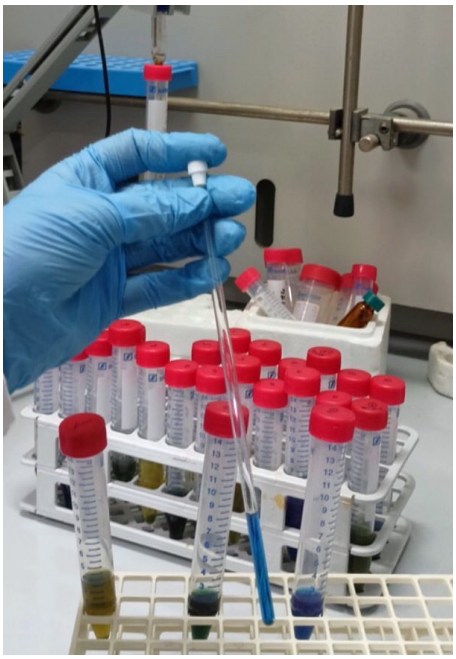


A dedicated modern glove box, equipped for protein purification and reconstitution in anaerobic environment is also available as support for the biomolecular Lab.

A mammalian expression lab for in-cell NMR is also equipped with modern instrumentation, including a newly installed Nikon Eclipse Ts2R-FL Optical Microscope, a Guava Easycyte 5 HT flow cytometer and a second laminar flow hood.

EPR

A Bruker ELEXYS E580 spectrometer allows operating in continuous wave at X-band (9.8 GHz) and in pulsed mode at Q-band (34 GHz). The spectrometer is equipped with a newly installed a DICE-II Pulse ENDOR System E560D-P-RF for spectroscopic characterization of the molecular and electronic structure of paramagnetic species.



Multi Angle/Dynamic Light Scattering

A new Dawn-18 system (SEC-MALS complete with HPLC DLS and FFF) instrument for measurements on batch samples or on in-flow samples (FPLC coupling) has been installed, which allows for high-sensitivity measurements of proteins, polymers and nanoparticles to determine sample polydispersity, molar mass, size, conformation, and interactions.

Isothermal Calorimetry (ITC)

A new Isothermal Titration Calorimeter (MicroCal PEAQ-ITC) to measure thermodynamical parameters in micro-samples has been installed. The instrument is fully equipped for studying protein-ligand and protein-protein thermodynamical parameters.

Optical Spectroscopy

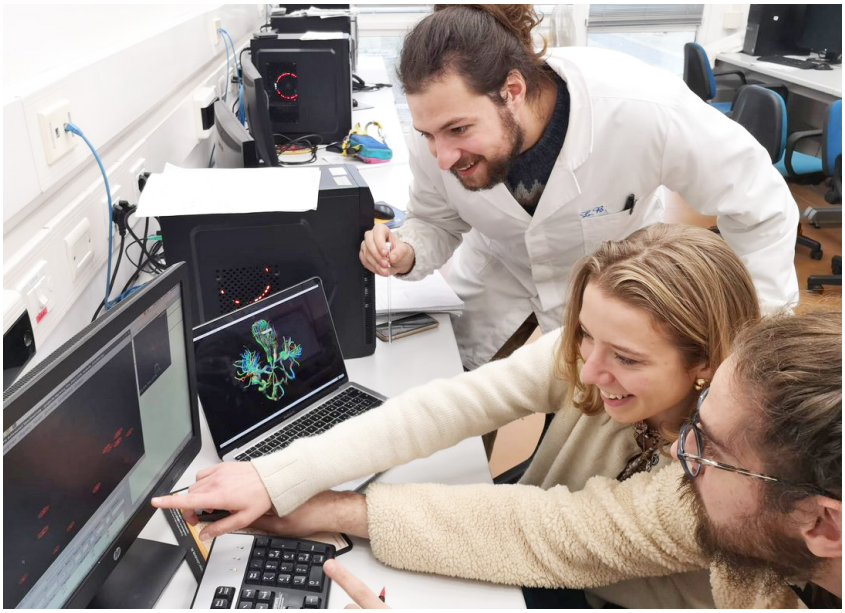
Absorption/Fluorescence Spectrophotometer (newly installed Varian Cary Eclipse Spectrophotometer) operating from 1000 to 200 nm, *Circular Dichroism* (CD) spectrometer operating form 1200 to 200 nm (Near-IR, Visible, UV) to derive information on the proteins secondary structure or protein-metal interaction, and newly installed SFM-4000 stopped-flow spectrophotometer are available in the infrastructure.

Computational Structural Biology Tools

CERM/CIRMMP provides integrated databases and software for genome browsing, metal binding analysis, structure calculation with/without paramagnetic restraints, sequence exploration, domain organization, protein complex analysis. Access to programs for NMR data processing and structural calculations is also provided via web.

Electronic infrastructure (e-infrastructure)

The grid and cloud-based services of CERM/CIRMMP are part of the WeNMR thematic services (https://www.egi.eu/case-study/wenmr/), which have been developed throughout a variety of collaborative European projects, the most recent ones being the EOSC-Hub and EGI-

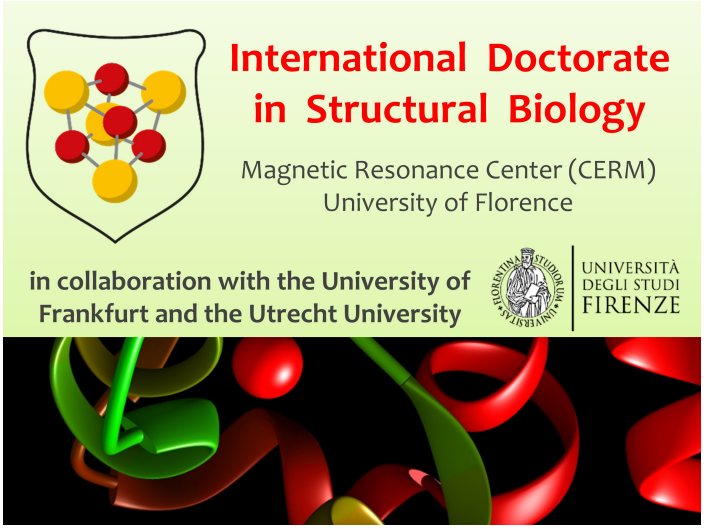


ACE initiative. Services for structural biology are also a crucial component of technological development ongoing in the context of the European Open Science Cloud (EOSC). In particular, the EOSC-Life project has provided a framework to create curated software pipelines spanning all aspects from data processing to the deposition of the final results (a.k.a. scientific workflows), using standardized approaches and management systems. This enables our structural biology workflows to be deposited in public repositories and be reused also by other NMR centres on their own computing infrastructure. The WeNMR thematic services provide application-level services specific to different cases in Structural Biology, with special focus on NMR-based tools. Those services are supported thanks to the strong commitment of providers giving access to grid, cloud and data storage computing resources, through a Service Level Agreement signed with the EGI Federation. The user community served by the WeNMR services encompasses over 12000 registered users over the years from more than 95 different countries. Among recently added services, there are pipelines for data analysis in fragment screening campaigns, which will be exploited in conjunction with EOSC services and other European projects. CERM/CIRMMP maintains a node of the European Grid Initiative. The available hardware comprises two clusters with 80 and 1024 CPU-cores respectively, and four TB of shared storage. A cluster with six Nvidia Tesla K20 GPU cards is also available.

Training & Education

International Doctorate in Structural Biology

The **International PhD course in Structural Biology** is a research doctorate of the *University of Florence*, administered by the Department of Chemistry and hosted at CERM, that runs in collaboration with the *Frankfurt and Utrecht Universities*. The scientific fields cover most of the molecular aspects of life sciences.



The main objective of the International PhD course in Structural Biology is the training of research doctors at the forefront of the knowledge in modern methodologies in molecular and structural biology, biotechnology and systems biology. It provides both theoretical and hands-on training in structural techniques applied to biological macromolecules in solution and in the crystalline state, as well as in non-crystalline materials such as fibrils or amyloid, and to biological macromolecules in their cellular environment. It also provides state-of-the-art training in molecular biology for the expression of isotope-enriched recombinant proteins and specifically those for NMR studies. Finally, it offers top level ICT training thanks to the well-established expertise and the exploitation of the e-infrastructure. Bioinformatics, biostatistics and NMR-metabolomics training is offered as well.

The scientific themes covered by the PhD course are:

1. **NMR spectroscopy** (in solution and in the solid state) and X-ray crystallography aimed at studying structure, function and dynamics in biological macromolecules and protein-protein adducts;

2. **Molecular and cellular biology techniques** for the production of proteins, DNA and bacterial and prokaryotic cell growth;

3. **Drug and vaccine development**, through rational design techniques

and structural characterization of biological drugs;

4. **Bioinformatics** to understand the structure-function relationship in biomolecules and in particular in metalloproteins through the large scale analysis of databases;
5. **In-cell NMR** studies, by which molecular pathways and cell import-export mechanisms are investigated;
6. **Metabolomics** studies, in which the individual metabolic fingerprints are related to disease states and fingerprints are utilized to provide early diagnosis or even identification of pre-disease states.

The added value of this PhD course is in the development of a *transnational educational project,* able to form PhDs at the forefront regarding the scientific formation, knowledge and development of research and technology, capable to consider multi-disciplinary, transnational cooperation and mobility as primary needs, and to evaluate collaborative projects as a requirement for high quality research. The doctoral program also relies on Faculty members who, in addition to scientists from CERM, include professors from other departments of the University of Florence and from the Universities of Frankfurt, Utrecht, Oxford and Lyon, all top places for structural biology.

Full-time attendance is mandatory, as is commitment to research activities. In addition to seminars and courses, students are asked to provide research seminars as a basic tool for their own training. Every PhD student is encouraged to liaise with foreign universities and take part in teaching and research training as well as in internships abroad.

Post-Doctorate

CERM/CIRMMP hosts several post-doctoral researchers. Some of them are former PhD students who remain at CERM after the end of the PhD, others come from all over the world for performing research projects and being trained in the methodologies in which CERM/CIRMMP excels. There are also several short- or long-term visitors coming from Italian and foreign universities.

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Prof. Alejandro Vila (Instituto de Biologia Molecular y Celular de Rosario, Argentina)

List of publications

SCIENTIFIC ARTICLES

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BOOK CHAPTERS

67) Bruno, F.; Luchinat, E.; Kazimierczuk, K.; Ravera, E. Fast 2D NMR to Investigate Dynamic Events in Biomolecules. In Fast 2D Solution-state NMR: Concepts and Applications; Dumez, J.-N., Giraudeau, P., Eds.; New Developments in NMR; 2023. https://doi.org/10.1039/BK9781839168062-00284.

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Meetings and Events Organized by CERM

Seminars Held at CERM

Dr. Sebastiano Di Pietro

Pharmacy Department, University of Pisa, Italy "Every time NMR solved my Organic Chemist's problems" November 15, 2023 at 13.00 - CERM Conference room

Prof. R.G. Griffin

Massachusetts Institute of Technology, USA "Atomic Resolution Structures of Amyloid Fibrils Time Domain DNP, Diamond Rotors, 1H Detected 17O NMR" November 17, 2023 at 18.00 - CERM Conference room

Philip Grandinetti

The Ohio State University, USA "Statistical Learning of Structure in Inorganic Oxide Glasses from 2D NMR Spectroscopy" November 16, 2023 at 18:00 - CERM Conference Room

Prof. Angela M. Gronenborn

Department of Structural Biology, University of Pittsburgh Medical School, Pittsburgh, USA

Present: "Integrating Science and Adventure in Structural Biology and Magnetic Resonance:

The Magic of Linking Rings: Discovery of a Unique Photoinduced Fluorescence Protein Crosslink;

November 13, 2023 at 17:30 - CERM Conference Room

Prof. Tatyana Polenova

Department of Chemistry and Biochemistry, University of Delaware, Newark, USA

Present: "Integrating Science and Adventure in Structural Biology and Magnetic Resonance:

Structural Basis for HIV Maturation Inhibitors Binding and Resistance: An Atomic Level View

November 13, 2023 at 17:30 - CERM Conference Room

Dr. Niklas Blomberg

Director of ELIXIR, Hinxton, UK "Ten years of ELIXIR - reflections on use, impact and sustainability of a research infrastructure for data" September 4, 2023 at 17:00 - CERM Conference Room

Dr. Lucia B. Chemes

Universidad Nacional de San Martin, Buenos Aires, Argentina "Evolution of SLiM-mediated interactions and their hijack by viral pathogens" July 27, 2023 at 14:30 - CERM Conference Room

Prof. Luis Rubio

Universidad Politécnica de Madrid, Spain “Can we engineer plants to metabolize atmospheric nitrogen?” July 12, 2023 at 12:30 - CERM Conference Room

Prof. Kenneth Merz

Department of Chemistry, College of Natural Science, Michigan State University, USA "Studying Metalloproteins Using Free Energy Methods" June 6, 2023 at 18:00 - CERM Conference room

Dr. Lorenzo Baronti

Bavarian NMR Center, TUM School of Natural Sciences and Helmholtz Munich - Institute of Structural Biology, Munich, Germany “Structural takes on non-coding RNA biology” April 12, 2023 at 12:30 - CERM Conference room

The Luigi Sacconi Memorial Lecture in Chemistry 2023

Prof. Claudio Pettinari

Rector of University of Camerino "Materials of future for energy and the environment" April 14, 2023 at 17:00 - Aula Magna, Polo Scientifico Università di Firenze, Sesto Fiorentino

Prof. Paul Vasos

University of Bucharest and Institute for Nuclear Physics, Extreme Light Infrastructure (ELI-NP) "Extended timescales for NMR via symmetry in biomolecular structures" March 1, 2023 at 12:00 - CERM Conference room

Meetings and Conferences

Annual consortium meeting HIRES-MULTIDYN

30 November - 1 December 2023, Florence. H2020 FET-Open project HIRES-MULTIDYN "Multiscale Dynamics with Ultrafast High-Resolution Relaxometry"

PANACEA 2nd annual user meeting and industrial day

15 - 16 November 2023, Florence

AMYC-BIOMED 2023

16 - 18 October 2023, Florence, Autumn Meeting for Young Chemists in Biomedical Sciences. 4th Edition

iNEXT-Discovery In-cell NMR Training Course

25 - 29 September 2023, CERM, Sesto Fiorentino. Course organized within the activities of iNEXT-Discovery

COST Training School: Application of NMR and other bio-tools to study FeS proteins

5 - 7 September 2023, CERM, Sesto Fiorentino

ICGEB course “NMR for combatting diseases: from cancer to SARS-CoV-2”

27 – 31 March 2023, CERM, Sesto Fiorentino

ITACA.SB Kick-off Meeting

31 January 2023, CERM, Sesto Fiorentino

Research Seminars

Friday, October 20th, 2023 at 1:00 pm **Prof. Paola Turano and Lucrezia Cosottini** "Ferritin-Au(I) bioconjugates as anticancer agents" CERM Conference room

Friday, October 13th, 2023 at 1:00 pm **Prof. Isabella Felli and Angela S. Tino** "NMR-based investigation of intrinsically disordered regions of modular proteins for tailored design of interacting peptides" CERM Conference room

Friday, September 22nd, 2023 at 1:00 pm **Prof. Lucia Banci and Valentina Vitali** "Spectroscopically orthogonal labelling to disentangle site-specific nitroxide label distributions" CERM Conference room

Friday, September 15th, 2023 at 1:00 pm **Prof. Roberta Pierattelli and Lorenzo Bracaglia** "Characterization of structurally heterogeneous proteins with NMR: the case of CBP-TAZ4" CERM Conference room

Friday, July 14th, 2023 at 1:00 pm **Prof. Lucia Banci and Bach Tung Lan Pham** "Method Development for 19F in-cell NMR. Assessments of Fluorinated Proteins Expressed in Human Cells by Intact Direct Mass Spectrometry on Cell Lysates" CERM Conference room

Friday, June 30th, 2023 at 1:00 pm **Prof. Enrico Ravera and Letizia Fiorucci** "A report from a on-field research" CERM Conference room

Friday, June 23rd, 2023 at 1:00 pm **Prof. Enrico Ravera and Francesco Bruno** "Clock is ticking!...during NMR experiments" CERM Conference room

Friday, June 16th, 2023 at 1:00 pm **Prof. Moreno Lelli and Naomi Anna Consoli** "A journey inside materials -From Solid State NMR spectroscopy to relaxometry-" CERM Conference room

Friday, June 9th, 2023 at 1:00 pm **Prof. Marco Fragai and Luis Padilla** "Enzymatic synthesis of Siglec-7 ligands and sialoglycan recognition" CERM Conference room

Friday, May 26th, 2023 at 1:00 pm **Prof. Marco Fragai and Giulia Roxana Gheorghita** "Expression and biophysical characterization of Siglecs" Online

MEETINGS & EVENTS

Friday, May 19th, 2023 at 1:00 pm **Prof. Marco Fragai and Francesca Sacco** "Integration of NMR spectroscopy and mass spectrometry for a new analytical workflow to characterize the oxidative stress in mAbs - Beyond the fingerprint -" CERM Conference room

Friday, May 12th, 2023 at 1:00 pm **Prof. Moreno Lelli and Lorenzo Niccoli** "Novel Polarizing Agents for High-Field and Fast MAS DNP Solid-State NMR" CERM Conference room

Friday, May 5th, 2023 at 1:00 pm **Prof. Francesca Cantini and Valentina Monaci** "Optimizing vaccine design for prevention of neonatal sepsis" CERM Conference room

Friday, April 28th, 2023 at 1:00 pm **Prof. Leonardo Tenori and Massimiliano Vinci** "NMR-based metabolomics in biomedical research: application to ageing and ageing-related diseases" CERM Conference room

Friday, April 21st, 2023 at 1:00 pm **Prof. Lucia Banci and Beatrice Bargagna** "Multiple Mitochondrial Dysfunctions Syndrome 2: Structural Consequences of the H96R BOLA3 Mutation" CERM Conference room

Friday, March 24th, 2023 at 1:00 pm **Tessa Bolognesi** "Expression of Nucleocapsid protein (N) from SARS-CoV 2 and its characterization through high-field NMR" CERM Conference room

Friday, March 17th, 2023 at 1:00 pm **Martina Rosati** "Incorporation of labeled amino acids in human cells towards cost-effective isotope-labeling schemes for in-cell NMR" CERM Conference room

Friday, March 10th, 2023 at 1:30 pm **Maria Anna Rodella** "Optimal NMR investigation of Intrinsically Disordered Proteins at ultra-high field exploiting 13C detected experiments" CERM Conference room

Friday, March 3rd, 2023 at 1:00 pm **Alessia De Santis** "Viral main proteases as potential pharmacological targets in developing broad-spectrum antiviral drugs" CERM Conference room

Friday, February 24th, 2023 at 1:00 pm **Francesco Currò** "Advanced structural and morphological characterization by NMR technologies of different biomolecules and biotechnological systems" CERM Conference room

MEETINGS & EVENTS

Friday, February 17th, 2023 at 1:00 pm **Azzurra Costantino** "19F In-cell NMR to investigate protein-ligand interactions in living human cells" CERM Conference room

Friday, February 10th, 2023 at 1:30 pm **Giulia Licciardi** "Ultrafast High Resolution Relaxometry: an overview of the technique and possible applications" CERM Conference room

Friday, February 3rd, 2023 at 1:00 pm **Maria Salobehaj** "Expression of biopharmaceuticals for the development of analytic and drug delivery strategies" CERM Conference room

Friday, January 27th, 2023 at 1:00 pm **Milana Bazayeva** "Towards a prediction tool for metal-binding sites" CERM Conference room

FUNDING INSTITUTIONS

Acknowledgements

University of Florence

Italian Ministry of University and Research

European Commission

Tuscany Regional Government

Fondazione Cassa di Risparmio di Firenze

Italian National Research Council

Italian National Institute of Health

FUNDING INSTITUTIONS

Cariplo Foundation

National Institutes of Health

Italian Association for Cancer Research

National Recovery and Resilience Plan (NRRP)

Italian Ministry of Agricolture

Italian Ministry of Health

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