CERM
Centro Risonanze Magnetiche
FIRENZE

CIRMMP
FIRENZE

SCIENTIFIC ANNUAL REPORT

2024

UNIVERSITÀ DEGLI STUDI FIRENZE

Contents

Foreword

The year 2024 has been a landmark year for our centre, as we celebrated the 25th anniversary of the foundation of CERM and the 30th anniversary of the foundation of the Interuniversity Consortium for Magnetic Resonance of Metalloproteins (CIRMMP). Additionally, this year marked the 40th anniversary of the Chianti Workshop, a conference traditionally organized by Ivano Bertini and several generations of his collaborators to highlight recent advancements and future perspectives in Magnetic Resonance, spanning from Chemistry to Life and Material Sciences with a special focus of electron and nuclear relaxation. To commemorate these milestones, CERM organized a special edition of the Chianti Workshop with a two-day event featuring presentations on various Magnetic Resonance topics by leading international experts.

The attractiveness of CERM/CIRMMP for national and international users relies on the quality of the research performed by its researchers. In 2024, the high quality of scientific research conducted at CERM/CIRMMP is once again confirmed. This is evidenced by the substantial number of peer-reviewed publications (52) and their high scientific impact not only because of the presence of works published in excellent journals (*Nat. Microbiol., Angew. Chem., J. Am. Chem. Soc., Nat. Commun. …*) but also because of an overall quality increase. Indeed, 63% of papers have been published in journals ranked in the first JIF quartile of their subject areas (more than one half 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 analyse 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 centre (Instruct-IT) of Instruct-ERIC, an ESFRI Landmark. The key role of the Italian centre 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 activities of CERM/CIRMMP related to Instruct-ERIC were framed also within the canSERV, ISIDORe and FHERITALE projects, which coordinate the Biological and Medical European Research infrastructures (BMS RIs) to create platforms for access provision tackling cancer research, infectious disease outbreak, and effects of exposure to artificial materials, 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.

In 2024, the funds obtained through the National Recovery and Resilience Plan (NRRP) continued to provide a significant boost to research initiatives in Italy. CERM received help from the NRRP funds through the project ITACA.SB, which secured funds for potentiating the Italian centre of Instruct-ERIC and for implementing new facilities in Italy. This project is providing 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 centre and boosting the exploitation of Instruct-ERIC resources in general.

Figures

Also for 2024, the Italian Ministry of University and Research (MUR) confirmed its support to the Italian Centre of Instruct-ERIC within the International Action of the FOE funding.

In 2024, besides the faculty staff, the body of researchers included 30 PhD students, 8 postdoctoral scientists, and 6 graduate students, as well as 14 between non-permanent and permanent technical and research 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.

Introduction

CERM is the Centre for Magnetic Resonance of the University of Florence. 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 material 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.

WHO WE ARE

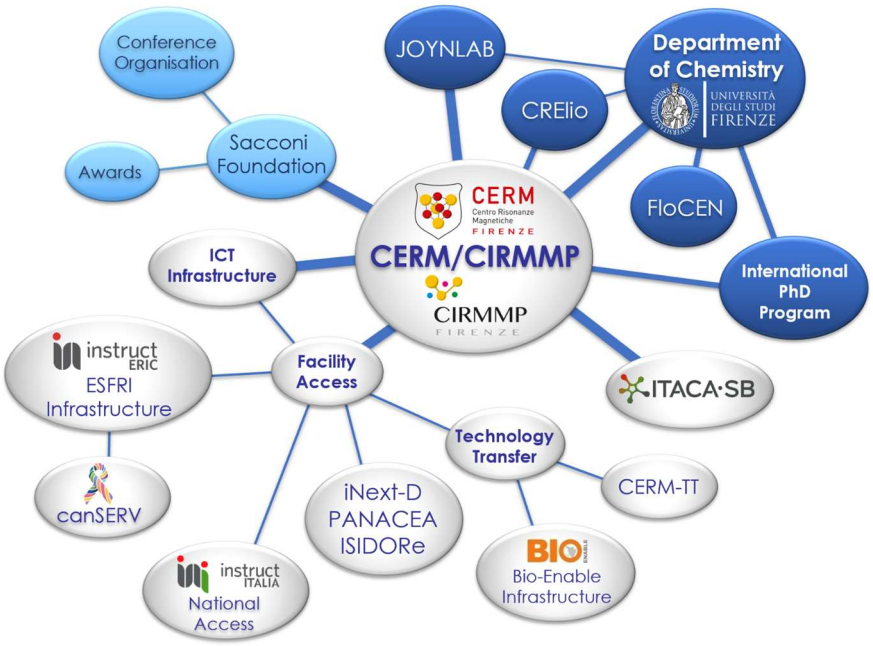
CERM/CIRMMP is the Italian Centre of Instruct-ERIC, which is the European research infrastructure in integrated structural biology defined in the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. Since 2010, CERM/CIRMMP is also included in the "Roadmap Italiana delle Infrastrutture di Ricerca di interesse Pan-Europeo". 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. Since 2023, CERM has been operating as an independent University Service Centre.

Under the Next Generation EU scheme, Italy is receiving resources (NRRP Program) and, through this scheme, CERM/CIRMMP scientists participated in several projects. Remarkably, CERM/CIRMMP is leading a consortium with a few CNR laboratories in Italy within the project ITACA.SB, which allocated significant resources (9.4 M€) to reinforce the Italian node of Instruct-ERIC, the European Infrastructure of Integrated Structural Biology in Italy.

CERM/CIRMMP is an e-infrastructure, participating in a European GRID-based platform, providing access to user-friendly platforms and CPU/GPU 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 inside the "Campus Sesto" site (formerly known as "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 Centre is the impressive collection of NMR spectrometers which feature the largest magnetic field range in the world (up to 1.2 GHz - installed in early 2020, the first commercial instrument in the world at this field) 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. 11. In addition to the main building, a further 500 square meters in adjacent buildings are available to CERM scientists: laboratories at the Department of Chemistry Ugo Schiff and at Genexpress; 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 Centre 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/

The Infrastructure

CERM TT

The CERM TT Competence Centre dedicated to Ivano Bertini, founder 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.

https://www.unifi.it/it/ricerca-e-innovazione/innovazione/collaborazioni-strategiche/centri-di-competenza-e-associazioni-lo

Bio-Enable

BIO-ENABLE is a "distributed research infrastructure" led by CERM/CIRMMP and 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 Centre (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

In addition to the individual research projects of CERM/CIRMMP members, 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 2024 are:

W-BioCat - Heavy metal enzymes for sustainable industrial biocatalysis. (HORIZON-EIC-2023-PATHFINDEROPEN-01 grant agreement n. 101129798 - 1/02/2024 - 31/01/2028)

MR LATVIA - Development of Magnetic Resonance in Latvia (HORIZON-WIDERA-2023-ACCESS-02 Grant agreement n. 101160091 - 01/09/2024 - 31/08/2027)

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)

FHERITALE Food, Health and Environment Research Infrastructures to Tackle Emerging Priorities (HORIZON-INFRA-2023-DEV-01 Grant agreement ID: 101131588 01/01/2024 - 31/12/2026)

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)

HIRES-MULTIDYN "Multiscale Dynamics with Ultrafast High-Resolution Relaxometry" (H2020-FETOPEN-2018-2020 grant agreement n. 8996830 - 01/10/2020-30/09/2025)

PANACEA “A Pan-European Solid-State NMR Infrastructure for Chemistry-Enabling Access” (H2020-INFRAIA-2018-2020 grant agreement n. 101008500 - 01/09/2021-31/08/2025)

ISIDORe Integrated Services for Infectious Disease Outbreak Research (HORIZON-RIA grant agreement n. 101046133 - 01/02/2022 - 31/07/2025)

The Infrastructure

Remote NMR (R-NMR): Moving NMR infrastructures to remote access capabilities (HORIZON-CSA grant agreement n. 101058595 - 01/07/202 2- 30/06/2025)

ITACA.SB: Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC National Recovery and Resilience Plan Strengthening and creation of Research Infrastructures – Mission 4 – Investment line 3.1 (CUP: B53C22001790006 - 01/11/2022 - 30/04/2025)

ITN "GLYTUNES – A multidisciplinary training network for the bioinspired development of glycomimetics tuning the Siglec-Sialoglycan axis" (H2020-MSCA-ITN-2020 grant agreement n. 956758 - 01/03/2021 - 28/02/2025)

BeYond-COVID (BY-COVID) (HORIZON-INFRA-2021-EMERGENCY-01 grant agreement n. 101046203 - 1/10/2021-30/09/2024)

iNEXT-Discovery - Structural Biology Research Infrastructures for Translational Research and Discovery (H2020-INFRAIA-2018-2020 grant agreement n. 871037 - 01/02/2020-31/07/2024)

NRRP and CERM/CIRMMP

The CERM/CIRMMP Infrastructure is also strongly involved in the National Recovery and Resilience Plan (NRRP), funded by NextGeneration EU, and participates in several projects either directly as infrastructure or through the 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 aim 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, ITACA.SB 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

Project Duration: 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 expanded the CERM staff by recruiting 6 technologists, 3 fixed-term Type A Researchers, and 7 PhD students.

Instrumentation upgrade: Since the start of the ITACA.SB project, CERM has received a major upgrade of its instrumentation: two Bruker NEO Consoles for the 700 MHz WB and the 950 MHz spectrometers, a QCI-P Cryoprobe (for the excitation of ^{1}H, ^{13}C, ^{15}N and ^{31}P), and an ENDOR Module for EPR experiments were purchased. The biotechnological laboratories were upgraded

with the acquisition of 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) a Guava easyCyteTM Flow Cytometer, and two ÄKTA Pure and Akta Go machines for protein purification.

In 2024, an additional 600 MHz Bruker spectrometer has been installed, equipped with a NEO Console and a QCI-F Cryoprobe (for excitation of ^{1}H, ^{19}F, ^{13}C and ^{15}N). A 0.7 mm 0.7 mm CP MAS HCN solid-state NMR probe for 1.2 GHz and a BBO probe with a gradient amplifier for 700 MHz were also installed. Finally, five NMR instruments have been upgraded with BSNL (Bruker Smart Nitrogen Liquefier) units, which use the extra cooling capacity of the CryoPlatforms to drastically reduce the need of liquid N_2 refills of the magnets.

Publications: By the end of 2024, ITACA.SB has been acknowledged in 46 peer-reviewed publications. An up-to-date 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. 19-32
CN3 - SPOKE 5	Pierattelli, Fragai	Inflammatory and infectious diseases	pg. 25, 26
THE - SPOKE 4	Banci, Cantini, Del Conte, Gonnelli	Nanotechnologies for diagnosis and therapy	pg. 27
THE - SPOKE 6	Rosato	Precision Medicine & Personalized Healthcare	pg. 21
THE - SPOKE 7	Ciofi Baffoni, Piccioli	Innovating Translational Medicine	pg. 23
THE - SPOKE 8	Pierattelli, Felli, Parigi, Allegrozzi	Biotechnologies and imaging in neuroscience	pg. 25
PE8 - SPOKE 2	Tenori, Vignoli	Improving the understanding of the biology of ageing	pg. 31
PE12 - SPOKE 6	Felli	Mechanisms of neuronal cell degeneration and drug dependent reversal	pg. 25, 26

Solution and Solid-State NMR Spectrometers

All NMR instruments at CERM 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 to the standard pulse sequences for spectroscopic, structural, dynamical, and functional characterization, tailored pulse sequences for structural determination of high molecular weight proteins, intrinsically disordered proteins and paramagnetic systems are implemented. Furthermore, protocols and pulse sequences tailored for in-cell NMR experiments are also implemented, including two flow NMR bioreactor units that can fit any 5-mm probe and preserve cell viability for prolonged NMR acquisitions, up to ~3 days. Pulse sequences and experiment setup for the detection and characterization of paramagnetic systems have been pioneered at CERM for decades. Solid-state MAS probes cover almost all the presently achievable MAS frequencies, for both biological and inorganic material characterization. A prototype shuttle system for high-resolution relaxometry measurements at 700 MHz, part of the HIRES-MULTIDYN research activities, allows for nuclear spin relaxation measurements at fields as low as 47 mT (~2 MHz ^{1}H Larmor frequency) by moving the sample inside the stray field of the magnet, while providing high resolution readout through high field detection.

In 2024, an additional 600 MHz spectrometer has been installed at CERM, equipped with a NEO console and a QCI-F Cryoprobe (^{1}H/^{19}F/^{13}C/^{15}N). In addition to the triple resonance channels for biomolecular applications, this probe features a fourth channel for ^{19}F acquisition and decoupling, allowing a vast range of applications towards fluorinated macromolecules and drugs both in vitro and in cells. A double-resonance broadband diffusion probe has also been installed at 700 MHz, which is designed for diffusion applications and optimized for very strong gradient pulses and fast switching times. With the aim of reducing the cost and environmental impact of the NMR facility, two nitrogen generators have been installed, which produce gaseous N_2 for the NMR instruments and the biotechnology labs.

Instrumentation

Furthermore, two 700 MHz, the 900 MHz, 950 MHz and the newly installed 600 MHz magnets have been upgraded with Bruker Smart Nitrogen Liquefier (BSNL) units, which use the extra cooling capacity of the CryoPlatforms to drastically reduce the need of liquid N_2 refills of these magnets. Combined with the existing BSNL unit of the 1.2 GHz, the newly installed instrumentation reduces the consumption of nitrogen even further.

B_0 Field (T)	^{1}H Larmor Frequency (Bore)	Probe heads
28.2	1.2 GHz (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

Instrumentation

16.4	700* MHz (NB 54 mm)	TXO Cryo 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 (NB 54 mm)	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 $^{15}N/^{13}C/^{1}H$ DIFF-DR-BB/^{1}H&^{19}F-D-Z-5mm
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
14.1	600** MHz (NB 54 mm)	TXI RT 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
14.1	600 MHz (NB 54 mm)	QCI-F Cryo 5mm solution ($^{1}H/^{19}F/^{13}C/^{15}N$)
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.

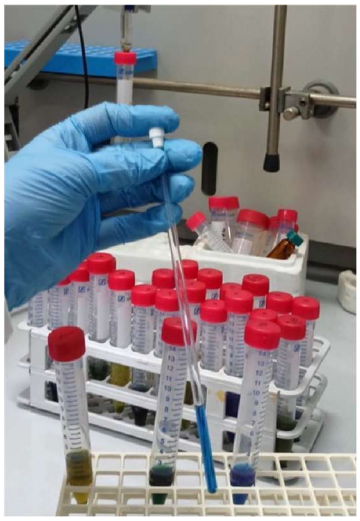
Molecular and Cellular Biology

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 equipped with a CO_2 incubator, two laminar flow hoods, a brightfield microscope, a Nikon Eclipse Ts2RFL Optical Microscope, and a Guava Easycyte 5 HT flow cytometer.

Instrumentation

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 from 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, the analysis and prediction of metal bindings, NMR-based macromolecular structure calculations with/without paramagnetic restraints, investigation of protein complexes. 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 involving the European Grid Infrastructure and other partners. At present this collaboration is funded via the EOSC Data Commons initiative (https://www.eosc-data-commons.eu/).

Services for structural biology are indeed 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 framework is still being used as it has enabled 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. The user community served by the WeNMR services encompasses over 14000 registered users over the years from nearly 100 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.

The available hardware at CERM/CIRMMP comprises two clusters with 80 and 1024 CPU-cores respectively, a cluster with 16 Nvidia L40 GPU cards and 256 CPU Cores, and two NAS storage units with 120 TB each.

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 5). 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.

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 equipment for EPR, CD, UV-vis, stopped-flow measurements, manual and automated crystallization facilities and X-ray diffractometry. Users can also access other university facilities

National and Transnational Access

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 and participate to the WeNMR thematic services. Furthermore, a number of graphic stations are available for interactive NMR data analysis.

During 2024 we recorded 472 days of external access to the NMR spectrometers. A more detailed analysis shows that 146 days of NMR access were provided to academic users via Instruct-ERIC, Instruct-ITALIA, iNEXT-Discovery and PANACEA, 269 days through formal collaborations, while 57 days were provided to industry users as services.

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

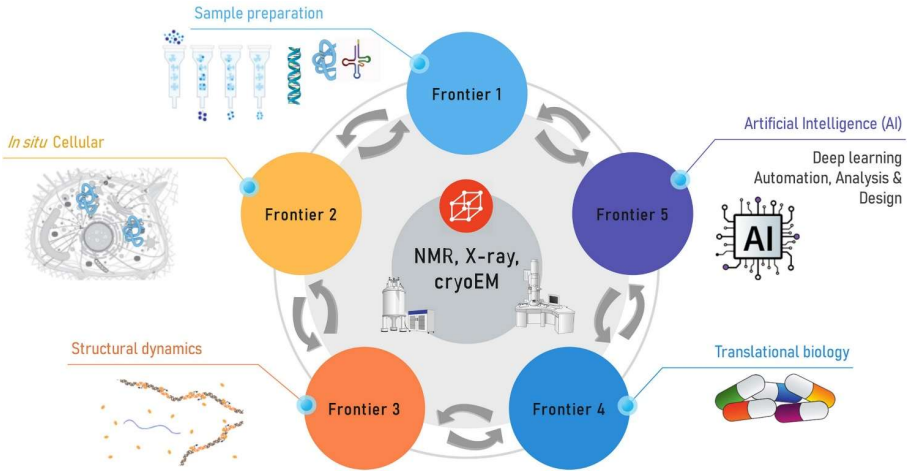
Worth to mention the implementation of a platform for the management of NMR access (https://amp.cerm.unifi.it/) improving data findability and experiment reproducibility and, thanks to new in-house LIMS, track of all the experiments performed and allows long-term data storage.

Introduction

During 2024, several research projects have been carried out at CERM, 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.

While NMR remains the core technology at CERM/CIRMMP, research over the years has progressively expanded to new applications, integrating NMR with a range of complementary techniques. This approach reflects the principles of integrated structural biology, which underlie the Instruct-ERIC consortium, where CERM/CIRMMP serves as the Italian node. Indeed, by providing access to high-end technologies and fostering collaboration, Instruct-ERIC plays a crucial role in driving progress in this field of research.

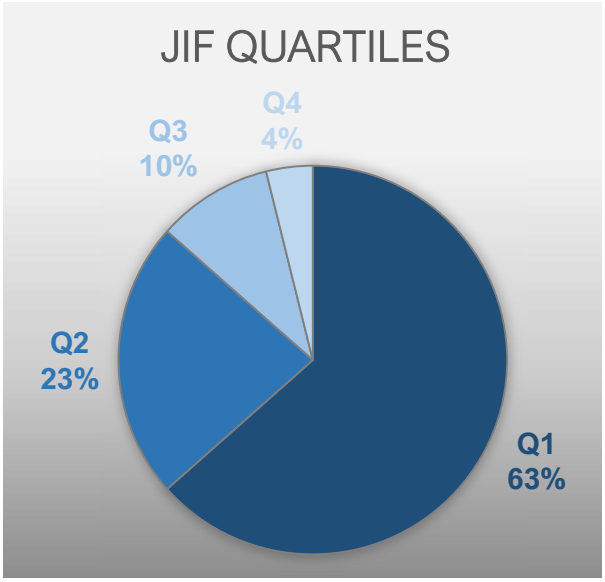
Current research at CERM/CIRMMP spans a broad spectrum of applications, from NMR studies of proteins in vitro and in intact cells, to bioinformatics and IT-based methods; from paramagnetic NMR and EPR spectroscopy to the development of novel MRI contrast agents; from investigating proteins and peptides as drugs or therapeutic targets to the development of metal-based drugs; and from metabolomics and biomedical applications to advanced solid-state NMR techniques for material characterization.



Future directions of integrated structural biology.
(from: Schwalbe, et al. Structure 2024)

The central goal of structural biology is to understand the function of biological molecules at atomic resolution and their roles in complex cellular pathways. Modern structural biology increasingly depends on a hybrid approach that combines experimental and computational techniques to investigate the structure and dynamics of complex biomolecular systems (*Schwalbe, et al. Structure 2024, 32 (10), 1563–1580*). High-resolution NMR spectroscopy plays a pivotal role in this framework, providing unique insights into macromolecular structure, conformational dynamics, and weak or transient interactions—both in solution and in living cells. When integrated with other methods, NMR significantly enhances our understanding of the architecture and behavior of large biomolecular assemblies. CERM/CIRMMP stands out as a leading European center in this field, contributing extensively to integrated structural biology through its expertise in magnetic resonance. This is clearly reflected in the breadth of research activities described in the following pages.

Research Activities



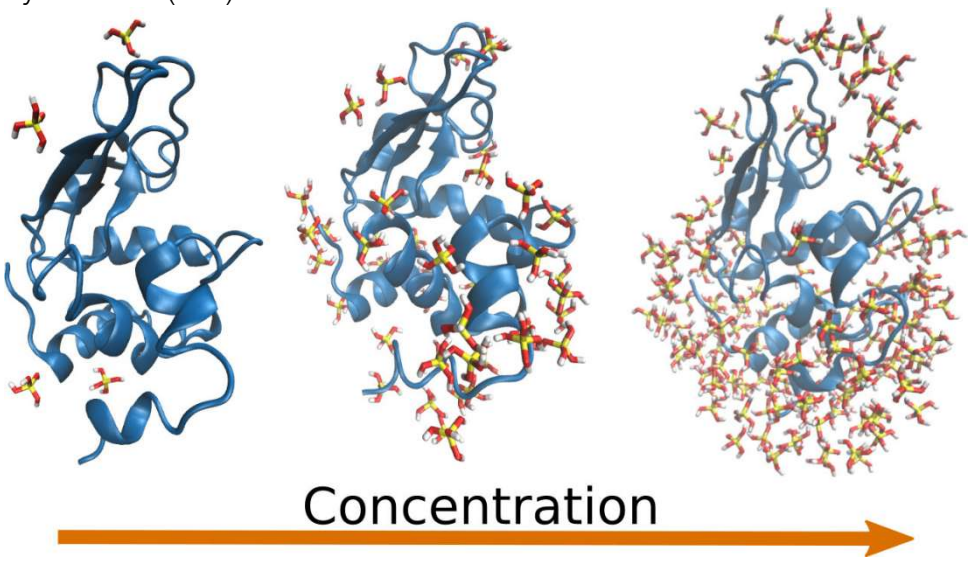
During 2024 we published 52 papers in international peer-reviewed journals, with several publications on high-impact journals. Our publications have an average journal impact factor (JIF) of about 6, with 35% of the publications on journals with JIF higher than 5. Notably, 63% of papers have been published in journals ranked in the first JIF quartile of their subject areas (Q1). A complete list of publications is available at page 44.

As a member of Instruct-ERIC and a partner in numerous interdisciplinary projects, at national and international levels, CERM/CIRMMP serves as a hub for integrative structural biology, providing access to state-of-the-art facilities, fostering cross-disciplinary collaboration, and acting as a reference point for both the scientific community and broader cultural growth in the country.

Computing for Integrative Structural Biology

CERM is committed to the development and application of innovative computational methods for the prediction and analysis of metal-binding sites in proteins. These methods entail mainly machine learning techniques, quantum mechanics and molecular dynamics simulations. The metal systems addressed can be diamagnetic as well as paramagnetic. Our results encompass the investigation of the interaction between proteins and metal-containing compounds as well as of the structural properties of metal-binding sites.

Computational methods are crucial in studying protein structure as they enable accurate modelling and prediction of inter-molecular interactions. They help in understanding protein folding, stability, and function, which is essential for drug design and disease research. In metal-binding systems, a specific challenge is posed by the presence of metal ions, which require specific computational strategies. Machine learning (ML) methods leverage big data to predict selected properties of the systems of interest. Such data can be extracted from databases, such as MetalPDB.[1] ML approaches were used for speeding up *ab initio* quantum chemical calculations of the electronic structure of a paramagnetic metal centre in response to structural perturbations.[2] Another line of activity has focused on structural modelling and molecular dynamics (MD) simulations to understand the interactions between metal-containing compounds/particles and proteins. In the case of the reaction of ferritin with aurothiomalate, this approach allowed us to rationalize the biological activity of the system (see page 24). Molecular simulations were also used to study the interaction of lysozyme with soluble inorganic species in the initial phases of silica polycondensation.[3]



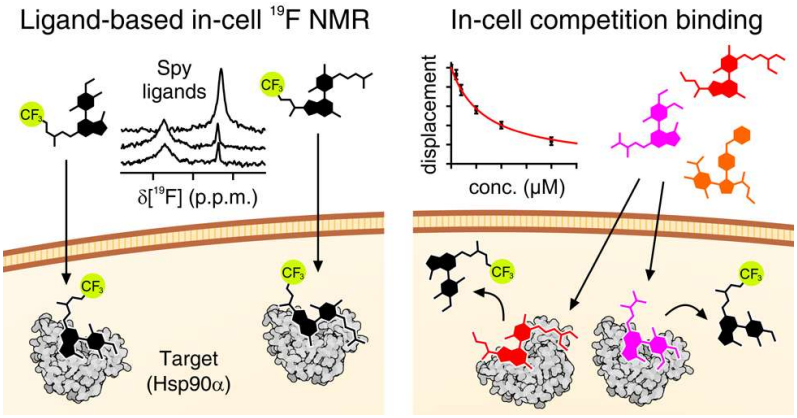
The mechanism of formation of silica templated by lysozyme was studied by molecular dynamics simulations. It appeared that the charged protein electrostatically attracted the precursor. The increase in local concentration of the latter thus accelerated silica polycondensation.

1) M. Bazayeva, C. Andreini, A. Rosato, ***Acta Cryst D*** 2024, 80, 362–376. 2) L. Gigli, J.M. Silva et al., ***Inorg. Chem.*** 2024, 63, 10713–10725. 3) M. Macchiagodena, M. Fragai et al., ***Chemistry Eur. J.*** 30, e20241249.

In-cell NMR and EPR in Human Cells

At CERM, novel methods are being developed to investigate proteins and small molecules in living human cells by NMR and EPR. ^{19}F in-cell NMR has been applied to monitor drug binding to otherwise 'invisible' intracellular target proteins. Novel cost-effective strategies have been developed to achieve selective ^{13}C or ^{19}F,^{13}C side-chain labelling of proteins expressed in human cells. The relaxation properties of spin labels for in-cell EPR have been further investigated.

In-cell NMR and EPR provide unique details on the structure, dynamics and function of macromolecules inside living cells. The highly physiologically relevant data obtained can complement the structural characterization usually carried out in vitro, and allow elucidating important phenomena such as protein-drug interactions. We have previously reported a novel approach for introducing fluorine atoms in proteins expressed in human cells for ^{19}F in-cell NMR applications. We have further characterized the incorporation efficiency of fluorinated aromatic amino acids (3-fluorotyrosine, 4-fluorophenylalanine, 5- and 6-fluorotryptophan) in different protein expression conditions, and defined the optimal conditions for maximizing either sensitivity or sample homogeneity.[1] ^{19}F in-cell NMR was further applied to monitor drug binding to an 'NMR-invisible' intracellular target. The approach relied on competition experiments, in which a fluorinated spy ligand is observed as it is displaced from the target upon treatment with a second unknown ligand, providing quantitative information on its binding affinity.[2] In parallel, novel methods have been developed to enable selective side-chain labelling of proteins with either ^{13}C or ^{19}F,^{13}C in human cells.[3,4] ^{13}C-labelled side-chains can be introduced in a cost-effective manner by employing labelled α-ketoacid precursors. Finally, the relaxation properties of spin labels for in-cell EPR have been further investigated in protonated and deuterated cellular environments.[5]



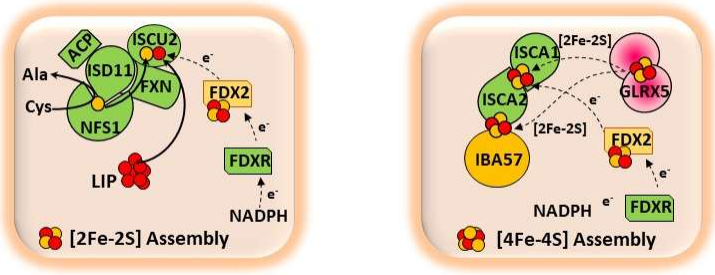
The binding of fluorinated ligands to an intracellular target can be monitored by in-cell ^{19}F NMR. A fluorinated spy ligand allows measuring the relative affinity of other ligands by competition binding experiments.

1) A. Costantino, L. B. T. Pham, L. Barbieri, V. Calderone, G. Ben-Nissan, M. Sharon, L. Banci, E. Luchinat, ***Prot. Sci.*** 2024, 33, e4910. 2) E. Luchinat, L. Barbieri, B. Davis, P. A. Brough, M. Pennestri, L. Banci, ***J. Med. Chem.*** 2024, 67, 1115–1126. 3) M. Rosati, L. Barbieri, M. Hlavac, S. Kratzwald, R. J. Lichtenecker, R. Konrat, E. Luchinat, L. Banci, ***J. Biomol. NMR*** 2024, 78, 237–247. 4) G. Toscano, M. Rosati, L. Barbieri, K. Maier, L. Banci, E. Luchinat, R. Konrat, R. J. Lichtenecker, ***Chem. Commun.*** 2024, 60, 14188–14191. 5) F. Torricella, V. Vitali, L. Banci, ***Phys. Chem. Chem. Phys.*** 2024, 26, 20246–20250.

Molecular Mechanisms of Iron-Sulfur Protein Biogenesis in Humans

CERM continues to pioneer the investigation of the molecular mechanisms of iron-sulfur protein biogenesis by applying a solution NMR-based approach. Novel molecular models have been established that rationalize the pathogenicity of single-point mutations in FDX2 and IBA57, as well as investigate the molecular role of nitric oxide in CISD3, a protein involved in cancer, diabetes, and neurodegenerative disorders.

Over the last decade, the structural aspects involving iron-sulfur (Fe/S) protein biogenesis have played an increasingly important role in understanding the high mechanistic complexity of the mitochondrial and cytosolic machineries maturing Fe/S proteins. In this regard, solution NMR has had a significant impact, particularly due to its ability to monitor transient protein-protein interactions, which are prevalent in the networks of pathways involved in Fe/S cluster biosynthesis and transfer.[1] These studies also provide solid basis to unravel the molecular determinants of rare human disorders. In this respect, recent results from CERM showed that: i) The pathogenic P144L mutation of FDX2 negatively affects the electron transfer pathway from NADPH-FDXR to FDX2, thereby reducing its capacity to assemble both [2Fe-2S] and [4Fe-4S] clusters; ii) The pathogenic G104C mutation significantly affects the stability of IBA57, both in its isolated form and in complex with its protein partner ISCA2, thus providing a rationale for the severe phenotype associated with this variant; iii) The molecular bases of cluster release and the destabilizing effects of nitric oxide in CISD3, a protein involved in various human diseases, are provided.[4] Overall, we demonstrated that solution NMR spectroscopy plays a crucial role in understanding how the molecular aspects of Fe/S protein biogenesis are linked to human diseases, thereby providing fundamental information necessary for designing specific potential therapeutic approaches.



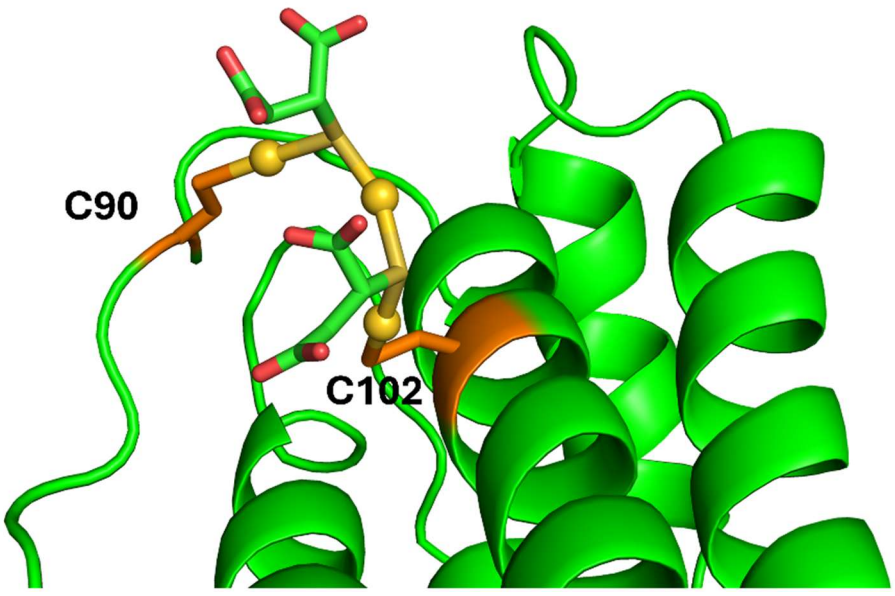
Human [2Fe-2S] and [4Fe-4S] cluster assembly pathway. FDX2 is part of an electron transfer chain consisting of NADPH and FDXR driving the biosynthesis of [2Fe-2S] on ISCU2 protein and [4Fe-4S] clusters on ISCA1-ISCA2-IBA57 complex.

1) L. Querci, M. Piccioli, S. Ciofi-Baffoni, L. Banci, ***Biochim Biophys Acta Mol Cell Res.*** 2024, 1871, 119786. 2) D. Grifagni, D. Doni, B. Susini, B.M. Fonseca, R.O. Louro, P. Costantini, S. Ciofi-Baffoni, ***Protein Science*** 2024, 33, e5197. 3) B. Bargagna, T. Staderini, S.H. Lang, L. Banci, F. Camponeschi, ***Int J Mol Sci.*** 2024, 25, 10466. 4) D. Grifagni, J.M. Silva, L. Querci, M. Lepoivre, C. Vallières, R.O. Louro, L. Banci, M. Piccioli, M.-P. Golinelli-Cohen, F. Cantini, ***J Biol Chem.*** 2024, 300, 105745.

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

We continued investigating the alterations in the metabolome of cancers cells induced by metallodrugs by focusing on the cellular effects induced by the gold(I) aurothiomalate (AuTM) in A2780 ovarian cancer cells. AuTM was shown to give rise to a well-defined adduct with human H ferritin (HuHf@AuTM). The association with ferritin strongly enhances the cytotoxicity of the drug, which can be interpreted in terms of a far more efficient drug uptake when mediated by the TfR1 receptor.

Several metal-based drugs are currently in clinical use for the treatment of various diseases and metabolomics can help defining the main effects induced by these multitarget molecules.[1] Here, we have concentrated our efforts in analyzing the metabolome alterations induced by AuTM, an FDA-approved antiarthritic gold(I) drug now under repurposing as an anticancer. To enhance its antitumoral activity, we prepared a bioconjugate with human apoferritin; the adduct contains 3 gold atoms per ferritin subunit under the form of a Au_3TM_2 moiety (as it results from ESI MS) bound to the solvent-exposed Cys90 and Cys102.[2] These two residues do not play any significant structural nor functional role in HuHf, but are correctly positioned to cooperatively bind soft metal ions.[3] HuHf@AuTM was found to be more cytotoxic than free AuTM against A2780 cancer cells, mainly due to higher gold uptake driven by TfR1-mediated HuHf internalization. NMR-metabolomics showed that free and bound AuTM qualitatively induce similar changes in treated A2780 cells.[2] ^{1}H NMR metabolomics was also used to establish differences between the widespread commercial A2780 cell line and patient-derived primary cells.[4]



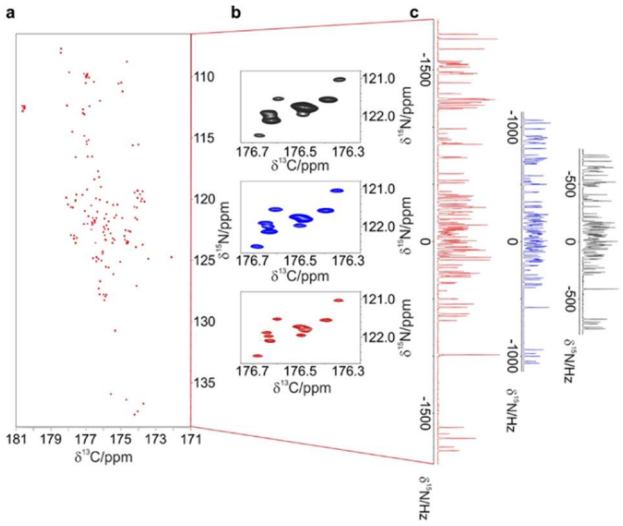
The Au_3TM_2 cluster bound to Cys90 and Cys102 in a HuHf subunit, from computational modeling and MD simulations.

1) V. Ghini, ***Inorganics*** 2024 12, 168. 2) L. Cosottini et al., ***Angew. Chem.*** 2024, 63, e202410791. 3) L. Cosottini, J. Buzzigoli, P. Turano, ***Eur. J. Inorg. Chem.*** 2024, 27, e202400486. 4) V. Ghini et al., ***Cells*** 2024, 13, 661.

Intrinsically Disordered Proteins

A protocol for investigating intrinsically disordered proteins at ultra-high field using ^{13}C detection was developed as part of CERM's methodological advancements in this research area, which are widely recognized by the scientific community. Ongoing contributions in this field include the design of novel experiments and the development of methods for describing IDPs structural ensembles.

Nowadays intrinsically disordered proteins (IDPs) are recognized as a central area of research in the structural biology community. Their conformational malleability, typically represented by conformational ensembles, whose characterization is crucial for studying IDP behavior, is key to their function, making it essential to understand their flexibility. Researchers at CERM are actively engaged in IDPs' characterization, from NMR experimental design to ensembles collection and description. To optimize ultra-high field NMR experiments, a protocol was developed illustrating 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 (Fig. 1).[1,2] Hardware innovations in spectrometers continuously provide new experimental tools. Among them, "multiple receivers" represent a particularly exciting advancement. Combining multiple receivers with complementary labeling schemes enabled the study of interactions between a cancer-related IDP and a large heterodimeric complex.[3] The contribution of CERM researchers to the Protein Ensemble Database (PED) project was useful to stimulate progress in the collection and distribution of ensembles of IDPs among the scientific community.[4] A geometrical approach providing a framework for understanding the agreement of a conformational ensemble to the experimental observations was also developed.[5]



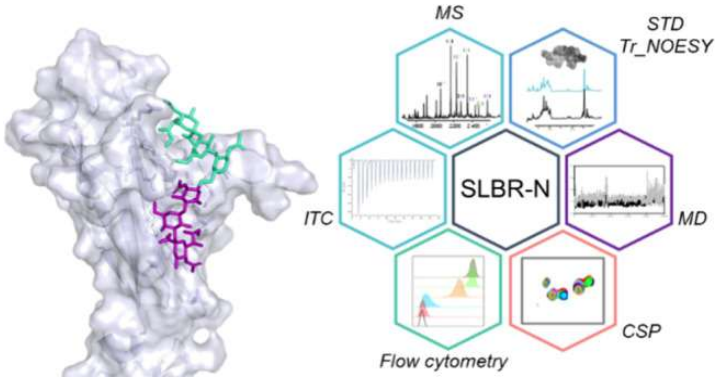
The 2D CON (Fig. a) experiment relies on $^{13}C'$ direct detection while ^{15}N chemical shift is encoded in the indirect dimension. The higher the external magnetic field, the higher the resolution that can be achieved (Fig. b), as observed going from 500 (red) to 700 MHz (blue) to 1.2 GHz (black). Notably, if the same spectral window in ppm is used, a larger bandwidth in Hz is observed when a higher B_0 is used (Fig. c).

1) M. Schiavina, L. Bracaglia, M. A. Rodella, R. Kümmerle, R. Konrat, I. C. Felli, R. Pierattelli, ***Nat. Protocols.*** 2024, 19, 406–440. 2) M. Schiavina, L. Bracaglia, T. Bolognesi, M. A. Rodella, G. Tagliaferro, A. S. Tino, R. Pierattelli, I. C. Felli, **J. *Magn. Reson. Open*** 2024, 18, 100143. 3) S. Knödlstorfer, M. Schiavina, M. A. Rodella, K. Ledolter, R. Konrat, R. Pierattelli, I. C. Felli, ***J.Am.Chem.Soc.*** 2024, 146, 27983–27987. 4) H. Ghafouri, T. Lazar, et al. ***Nucleic Acids Res.***, 2024, 52 (D1), D536–D544. 5) L. Fiorucci, M. Schiavina, I. C. Felli, R. Pierattelli, E. Ravera, ***J. Chem. Inf. Model.,*** 2024, 64, 5392–5401.

Proteins as drugs and drug targets

Proteins are important pharmaceutical targets, and an increasing number of drugs are proteins. Studying protein structure of the protein targets and their interactions with partners is crucial for finding new drugs and developing potential treatments. Ligand-based NMR assays are also routinely applied to identify and validate the binding of drug candidates to their target.

The development of new drugs is a fundamental research topic at CERM/CIRMMP. Understanding the mechanism of protein targets, such as those involved in cancer or in multidrug-resistant infections, is crucial for designing new therapeutics. A multidisciplinary approach was applied to elucidate ligand specificity of the serine-rich repeat glycoprotein exposed on the bacterial surface of *Streptococcus gordonii*, paving the way for the design and development of potential therapeutics against streptococcal infections.[1] Also, the development of a safe and effective vaccine against *Neisseria gonorrhoeae* is urgently needed. We have identified a cyclic peptide (CP2) that mimics the sugar epitope of *N. gonorrhoeae* and performed a structural analysis of the complex between CP2 and a monoclonal antibody.[2] The recognition of endotoxic lipopolysaccharides of Gram-negative *Akkermansia muciniphila* by the innate immune system via Toll-Like Receptors was investigated, revealing unprecedented structural and signalling properties.[3] A first-in-class pyrazole-isoxazole molecule showing remarkable growth inhibition of *Candida albicans* in combination with voriconazole has been also discovered and analysed.[4] The binding of monastrol, a known anticancer drug, to the actin-binding protein fascin has been characterized, confirming the potential of this compound as an antimetastatic agent.[5] The direct binding of novel compounds to the mitochondrial membrane protein VDAC1, a therapeutic target of inflammation-related diseases, was validated in vitro by ^{19}F NMR. NMR, coupled with X-ray crystallography, revealed the largest known protein–calixarene interface, offering a platform to probe multivalency in biomolecular systems.[6] Finally, the assignment of the 20 kDa MrkA subunit from *Klebsiella pneumoniae*, a pathogen of increasing concern, was reported, which will allow mapping the antibody interaction sites, enabling the development of novel therapeutic strategies.[7]



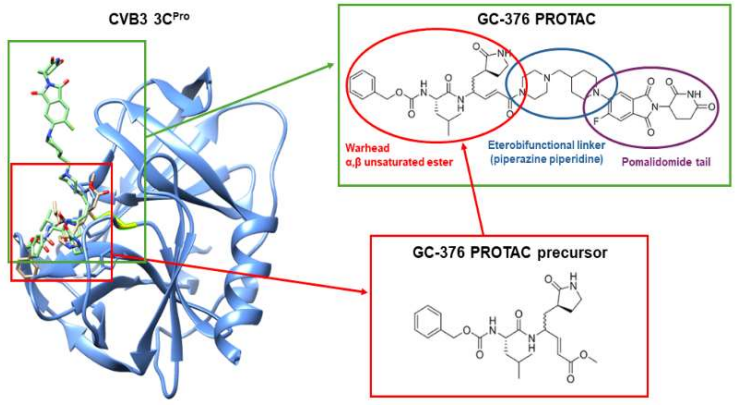
A multidisciplinary approach has been applied to investigate the mechanism of infection by Streptococcus gordonii.

1) C. Di Carluccio, *et al.* ***ACS Cent. Sci.*** 2024, 10, 447–459. 2) P. T. Beernink, *et al.* ***JACS Au*** 2024, 4, 2617–2629. 3) P. Garcia-Vello, *et al.* ***Nat. Commun.*** 2024, 15, 8411. 4) S. Pelliccia, *et al.* ***J. Med. Chem.*** 2024, 67, 14256–14276. 5) B. Alburquerque-González, *et al.* ***Biomed. Pharmacother.*** 2024, 175, 116785. 6) S. Conti Nibali, *et al.* ***iScience*** 2024, 27, 109853. 6) R. J., Flood, *et al.* ***Biomacromolecules*** 2024, *25* (2), 1303–1309. 7) V. Monaci, *et al.* **Biomol. NMR Assign.** 18 (2), 171–179.

Development of a Peptidomimetic PROTAC molecule as broad-spectrum antiviral agent

The conservation of the main protease in viral genomes, combined with the absence of a homologous protease in humans, makes this enzyme family an ideal target for developing broad-spectrum antiviral drugs with minimized host toxicity. We develop a peptidomimetic molecule able to trigger the degradation of the viral proteases of SARS-CoV-2 and of Coxsackievirus B3.

Proteolysis targeting chimera (PROTAC) technology provides an attractive approach to modulate protein levels of therapeutic target by hijacking the cellular machinery responsible for the physiological elimination of endogenous proteins. This strategy has been widely established to be successful in targeting cancer-related proteins, with more than a dozen drug candidates entering clinical investigation. On the contrary, the applicability of PROTAC technology in the field of antivirals remains marginal. Only a few studies of PROTAC molecules targeting viral proteins have been reported in the case of hepatitis and influenza viruses, and just a preliminary attempt investigating the applicability of PROTAC technology against coronaviruses (CoV) has been documented. We have here applied the PROTAC technology to obtain a peptidomimetic molecule able to trigger the degradation of SARS-CoV-2 3-chymotrypsin-like protease ($3CL^{Pro}$) and of the main protease of Coxsackievirus CVB3 ($3C^{Pro}$). The PROTAC molecule has been synthesized in the laboratory of Prof. Trabocchi (DICUS). The interaction of PROTAC with CVB3 $3C^{Pro}$ and SARS-CoV-2 $3CL^{Pro}$ has been characterized at CERM through X-ray crystallography, NMR and biochemical techniques. The degradation efficiency in the cellular context driven by our PROTAC molecule was analysed in collaboration with University of Verona. These studies show that our PROTAC molecule is a potential broad-spectrum antiviral agent.



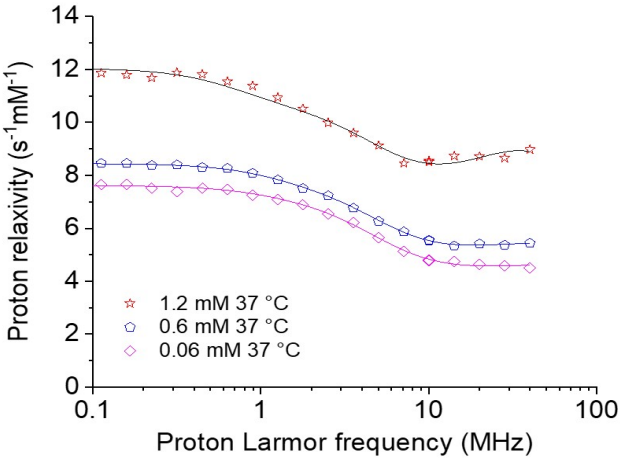
Schematic representation of synthetized GC-376 based PROTAC and structure of CVB3 $3C^{Pro}$ complexed with GC-376 based PROTAC and its precursor (PDB ID 8S6F).

1) A. De Santis, D. Grifagni, A. Orsetti, E. Lenci, A. Rosato, M. D'Onofrio, A. Trabocchi, S. Ciofi-Baffoni, F. Cantini, V. Calderone, ***Biomolecules*** 2024, 14 (10). 2) D. Grifagni, E. Lenci, A. De Santis, A. Orsetti, C. G. Barracchia, F. Tedesco, R. Bellini Puglielli, F. Lucarelli, A. Lauriola, M. Assfalg, F. Cantini, V. Calderone, D. Guardavaccaro, A. Trabocchi, M. D'Onofrio, S. Ciofi-Baffoni, ***ACS Medicinal Chemistry Letters*** 2024, 15 (2), 250–257.

NMR and EPR of Paramagnetic Systems

Paramagnetic metals largely affect NMR spectra and nuclear relaxation rates of the molecules in which they are hosted. Paramagnetic complexes are largely employed in magnetic resonance imaging (MRI) for their ability to increase the image contrast. At CERM, paramagnetic NMR effects are investigated for getting information on molecular structure and dynamics, and field cycling relaxometry measurement are performed for the characterization and optimization of

Magnetic resonance (MR) imaging is a non-invasive clinical modality with unlimited depth penetration and high spatial and temporal resolution. The intensity of the MR signal depends on the proton relaxation rates of water molecules in tissues. Paramagnetic metals increase the relaxation rates in such a way to generate brighter signals and thus to enhance the sensitivity of MR imaging. Gadolinium(III) complexes are the most frequently used agents developed to increase the contrast in clinical applications. Field cycling relaxometry, providing the field dependence of the water proton nuclear relaxation rates, is an extremely informative tool for revealing mechanistic information about the paramagnetic complexes, their hydration and dynamics. This technique has been employed to characterize a multimeric galactose functionalized gadolinium(III) chelate with the feature of spontaneous dynamic aggregation in aqueous condition.[1] Indeed, relaxation measurements showed a remarkable increase in the reorientation time of the complex by increasing its concentration, reporting on the occurrence of aggregation.



^{1}H relaxivity profiles of galactose functionalized gadolinium(III) complex at different concentrations (0.06, 0.6 and 1.2 mM).

Paramagnetic NMR is receiving increasing attention, and the key to make it successful is to link the optimization of the experimental conditions to the knowledge of the electronic structure of the metal centre under investigation. Over the years, we derived rules for different metals in different coordination environments. More recently, we have started collecting these rules in an accessible manner, making it easier for the users - even unexperienced - to get into this fascinating problem.[2,3,4]

1) J.-H. Tang, M. Luo, W. Tsao, E.A. Waters, G. Parigi, C. Luchinat, T.J. Meade, ***Inorg. Chem.*** 2024, 63, 24662–24671. 2) L. Querci, L. Fiorucci, E. Ravera, M. Piccioli, ***Inorganics*** 2024, 12. 3) V. Vitali, K. Ackermann, G. Hagelueken, B.E. Bode, ***Applied Magn. Reson.*** 2024, 55, 187–205. 4) A. Coelho, J.M. Silva, F. Cantini, M. Piccioli, R.O. Louro, C.M. Paquete, ***Biomol. NMR Assignments*** 2024, 18, 139–146.

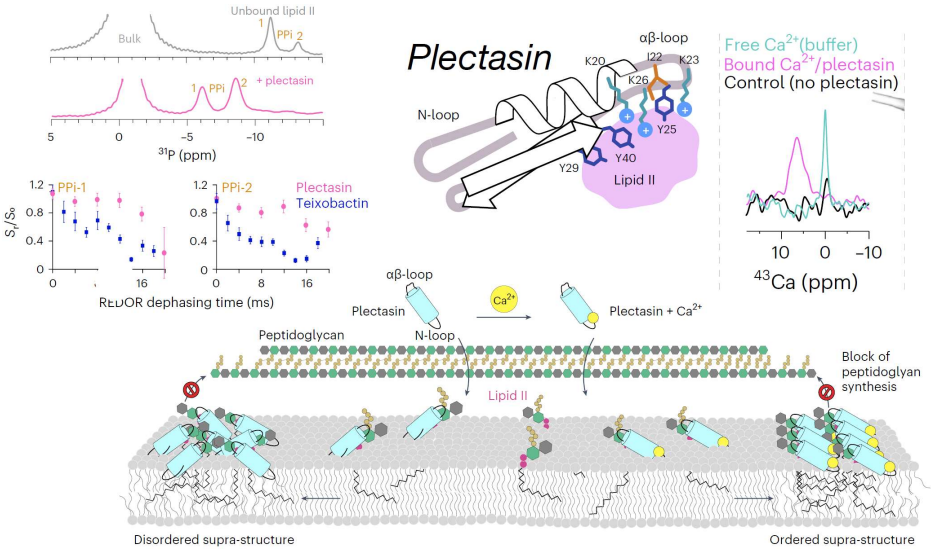
Solid-state NMR in Structural Biology

Solid state NMR (ssNMR) is one of the most powerful techniques to characterise not soluble biomolecules and membrane proteins. In the examples here reported, we show how through ssNMR it is possible to understand the mechanism of action of plectasin, a new antibiotic, but also a complex study to understand the evolution of the different colour in marine bacteria proteorhodopsin. We also report an example about the application to the investigation of a new pharmaceuticals.

Antimicrobial resistance is a leading cause of mortality, calling for new antibiotics. By using a combination of ssNMR, AF microscopy and activity assays, we have investigated the mechanism of action of *plectasin*, a fungal antibiotic peptide that blocks the cell wall synthesis in a variety of Gram-positive bacteria. In particular, ^{31}P NMR ssNMR spectra and ^{13}C–^{31}P REDOR evidence that *plectasin* binds the highly conserved pyrophosphate of Lipid-II, a peptidoglycan precursor of the bacteria cell wall, sequestering the precursor and blocking the cell wall synthesis. This mechanism is even more effective in the presence of calcium: ^{43}Ca ssNMR spectra showed that Plectsin/Lipid-II oligomers bind Ca^{2+} ions forming densely packed plaques on the cell membrane that also alters the membrane structure.[1]

Proteorhodopsins are widely distributed photoreceptors from marine bacteria with evolutionary adapted blue- and green-absorbing variants that correlate with a conserved glutamine/ leucine at position 105. Integrating DNP ssNMR and computational (QM/MM) methods, we show that this single residue is responsible for a variety of coupled structural and electrostatic changes along the ionone ring, the retinal polyene chain, and within the binding pocket, that collectively explain the observed colour shift.[2]

We also report an example about the application of ssNMR to the investigation of a new pharmaceuticals.[3]



Mechanism of action of Plectasin interacting with Lipid-II, as shown in ^{31}P and REDOR ssNMR spectra. In presence of Ca^{2+} is forming ordered aggregates weakening the bacteria membrane.

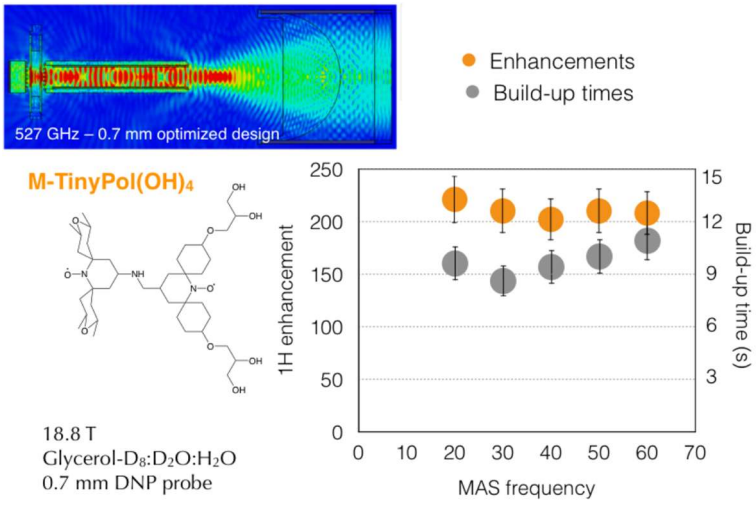
1) S. Jekhmane, *et al.*, ***Nature Microbiol.*** 2024, 9, 1778–1791. 2) J. Mao *et al.*, ***Science Adv.*** 2024, 10, eadj0384. 3) Z. Wan, *et al.* ***Molecules*** 2024, 2, 29.

Spectroscopic methods for Materials

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 techniques. We introduced a new family of Polarizing Agents, dubbed TinyPols that are expressly designed to be efficient at high field and fast MAS. Indeed, they provides enhancements of the order of 200, at 18.8 T (800 MHz of ^{1}H Larmor frequency) and at 65 kHz MAS. The key element in these molecules is the introduction of "antenna" aliphatic sidechains, in proximity of the nitroxide moieties. These "antennas" act promoting the ^{1}H-^{1}H spin-diffusion accelerating the propagation of the hyperpolarization in the bulk sample, increasing enhancement and reducing build-up time, with a strong impact in the sensitivity gain.[1]

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 Polarizing Agents for DNP ssNMR, and the second on a new strategy for increasing DNP transfer by changing the MAS frequency during the experiment. These methods are useful for the characterisation of polymers or materials in general.

The second method also act increasing the spin-diffusion of the polarization to better increase the hyperpolarization inside a target material. This is done with a new sequence where the recovery time is performed at low MAS frequency, with a much faster nuclear spin-diffusion, and detection is performed at fast-MAS by taking advantage of the higher resolution. This makes it possible to have significant gain in signal compared to "only fast MAS" methods.[2]



M-TinyPOL(OH)$_4$ shows the highest DNP efficiency performance for polarizing agents at fields ⩾18 T and water/glycerole environment. This is extremely relevant for samples compatible with aqueous media.

A mechanochemical process is proposed as an innovative approach to the synthesis of polypropylene-based macromolecular stabilizers with outstanding thermal behaviour. The study reports a quantitative evaluation of the degree of functionalization based on ATR-FTIR quantification technique and the solid-state NMR to characterise the ensuing materials. This strategy is a significant step towards safe and sustainable chemical synthesis in the field of macromolecular materials.[3]

1) L. Niccoli, *et al.* ***Chemical Science*** 2024, *15*, 16582–16593. 2) S. Badoni, *et al.* ***Journal of Physical Chemistry A*** 2024, *128*, 7005–7012. 3) Di Maro, M. *et al.* ***Reactive and Functional Polymers*** 2024, 197, 105858.

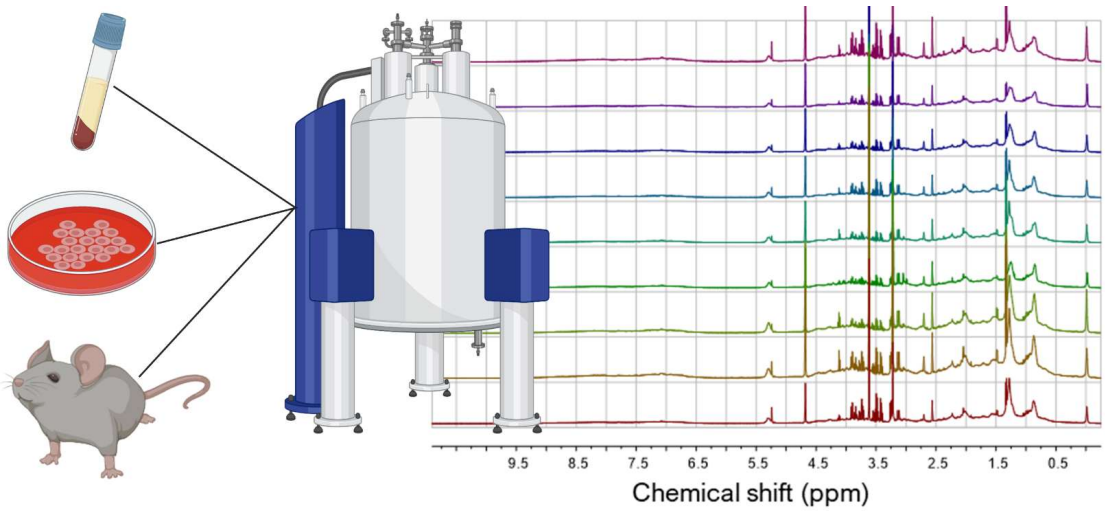
Metabolomics in Biomedicine

NMR-based metabolomics at CERM spans a broad range of activities. In 2024, the research focus was primarily oriented towards two specific areas: the investigation of the effects of anticoagulation drugs in elderly patients with atrial fibrillation[1], and the impact of sperm membrane composition and serum lipoprotein profiles on male reproductive potential[2]. In addition, NMR-based metabolomics coupled with proteomics was used to characterize the molecular signature of colitis in symptomatic and presymptomatic stages of the inflammatory process at the tissue and fecal level in mouse model. The results obtained highlight the diagnostic potential of metabolomics for inflammatory diseases[3].

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

Another intriguing research trajectory pertains to the utilization of metabolomics to study the impact of therapeutic interventions. In particular, we investigated the effects of direct and alternating current on the metabolome of a 3D bioconstruct composed of fibroblasts and keratinocytes within a collagen matrix to explore the anti-inflammatory effects of electrostimulation[4].

NMR metabolomics has also a role in the study of animal diseases. We described, by analyzing serum extracts, the positive effects of boron supplementation in dairy calves after birth[5].



A schematic summary of the NMR-based metabolomics activities performed on different biosamples.

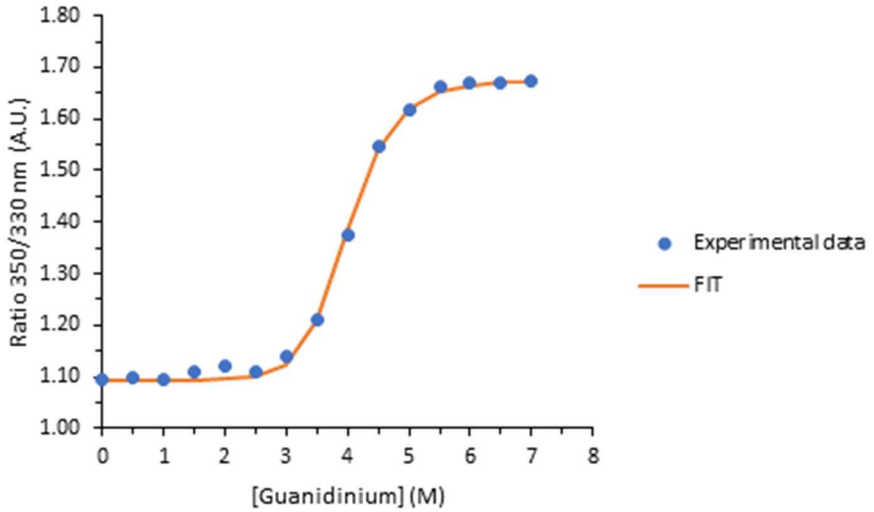
1) A. Vignoli, *et al*. ***Life Sci.*** 2024, 351. 2) A. Di Nisio, *et al*. ***Int. J. Mol. Sci.*** 2024, *25*, 1. 3) E.Shimshoni, I. Solomonov, I. Sagi, V. Ghini, ***J. Proteome Res.*** 2024, *23*, 4, 1420–1432. 4) B. Di Pietro, *et al*. ***Int. J. Mol. Sci.*** 2024, *25*, 18. 5) A. A.-A., Adam, *et al*. ***Assiut Vet. Med. J.*** 2024, *70*, 180, 10–25.

Biophysical Characterization of Proteins

Biophysical characterization of proteins is an integrated approach that combines several advanced techniques to elucidate protein structure, dynamics, kinetics, and interactions. Traditional methods -such as fluorescence spectroscopy, circular dichroism, isothermal titration calorimetry, SEC/FFF-MALS, stopped-flow are also available in CERM and are complementary to nuclear magnetic resonance. These combined approaches allow for a detailed analysis of protein folding, conformational transitions, and transient interaction events that govern regulatory mechanisms.

The integration of multiple biophysical techniques with NMR and computational methods significantly enhances protein studies in complex environments. This approach improves the accuracy of kinetic data and provides deeper insights into allosteric modulation and transient interactions. By combining complementary methods, researchers can achieve a more comprehensive understanding of protein dynamics, structural transitions, and regulatory mechanisms, ultimately advancing the study of biological systems.

The synergy between all those techniques, among NMR and computational methodologies, has significantly expanded our capacity to study proteins in complex environments, enhancing the reliability of kinetic data and deepens our understanding of allosteric modulation and transient binding events, ultimately contributing to a more robust framework for decoding the intricate dynamics of biological systems. Examples of these integrations can be founded in recent published papers from our scientists[1,2].



Stability studies of Lysozyme with increasing concentrations of guanidinium hydrochloride which allows to determine the free energy of denaturation.

1) G. Pérez-Ropero, A. Pérez-Ràfols, T. Martelli, U. H. Danielson, J. Buijs, ***Biochemistry*** 2024, 63 (21), 2816–2829. 2) R. Dolcemascolo, M. Heras-Hernández, L. Goiriz, R. Montagud-Martínez, A. Requena-Menéndez, R. Ruiz, A. Pérez-Ràfols, R. A. Higuera-Rodríguez, G. Pérez-Ropero, W. F. Vranken, T. Martelli, W. Kaiser, J. Buijs, Rodrigo, G. ***eLife*** 2024, 12.

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

Glaxo Smith Kline

Giotto Biotech Srl

Merck

Menarini Srl

Infineum

Stelar S.r.l.

Extra Byte S.r.l

Latus Pet

Collaborations

Maven Health GmbH

Evonik

Achilles Vaccines

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

Florence Center for Electron Nanoscopy (FloCEN)

FloCEN (https://www.flocen.unifi.it/index.) 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.

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.giottobiotech.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 2024 the agreement between DICUS and Giotto Biotech S.r.l. has been renewed. JOYNLAB participated in the European MSCA project “GLYTUNES - A multidisciplinary training network for the bio-inspired development of glycomimetics tuning the Siglec-Sialoglycan axis”, funded by the European Community. Within this project, a PhD student was hired in November 2021 and successfully defended her thesis in November 2024. As part of another Marie-Curie project, ENSCC, in November 2024 a PhD student was enrolled in the PhD program in Structural Biology. Additionally, in 2024, JOYNLAB was involved in two further projects: the Tuscany regional project EMILE; the Marie-Curie Staff Exchange Project McGEA ("Metallo-enzymes and Cells for Green Environmental Alternatives," Call HORIZON-MSCA-2023-SE-01 - Action HORIZON-TMA-MSCA-SE, No. 101183014).

Other Institutions

CRElio

CRElio is the Service Centre 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 awards, seminars, courses and meetings and other activities supporting the exchange of scientific knowledge, subsidizes the activity of Italian and foreign researchers.

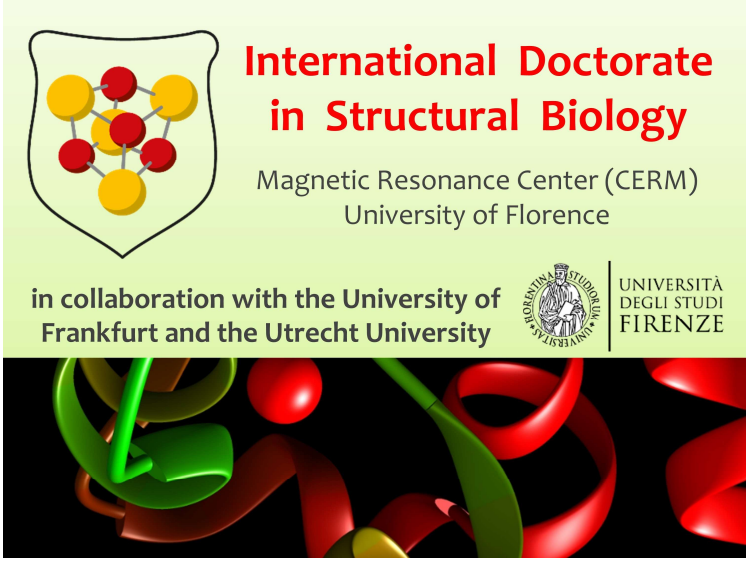
On May 3rd, 2024, at Palazzo Vecchio in Florence, the XVII edition of the "Città di Firenze" Prize for Molecular Sciences (2024 Edition) took place. The event was organized by the Luigi Sacconi Foundation, with the support of the Fondazione Cassa di Risparmio di Firenze. The awardee, Prof. Daniel G. Nocera, Patterson Rockwood Professor of Energy at the Department of Chemistry and Chemical Biology at Harvard University, delivered a lecture titled *"Addressing the Global Energy Challenge: Creating Food and Fuels from Sun, Water, and Air"*. During the XXVIII National Congress of the Italian Chemical Society (23-30 August, 2024), which will take place in Milan, the 2024 Sacconi Medal will be awarded. The recipient, Prof. Anne-Marie Caminade, was selected jointly by the Board of Directors of the Foundation and the Executive Council of the Inorganic Chemistry Division of the Italian Chemical Society. The "Luigi Sacconi Memorial Lecture in Chemistry" in 2024 was delivered by Prof. Philip Grandinetti. The lecture was titled "*Real-Time Monitoring of Battery Chemistry with Nuclear Magnetic Resonance Spectroscopy*". The

Other Institutions

Foundation also collaborated in organizing the 2024 edition of the Chianti Workshop (May 16-17, 2024) - *"A Journey Through 40 Years of Magnetic Resonance Discoveries: From Pioneers to Gen"* and the 13th International Copper Meeting (September 15-20, 2024).

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;

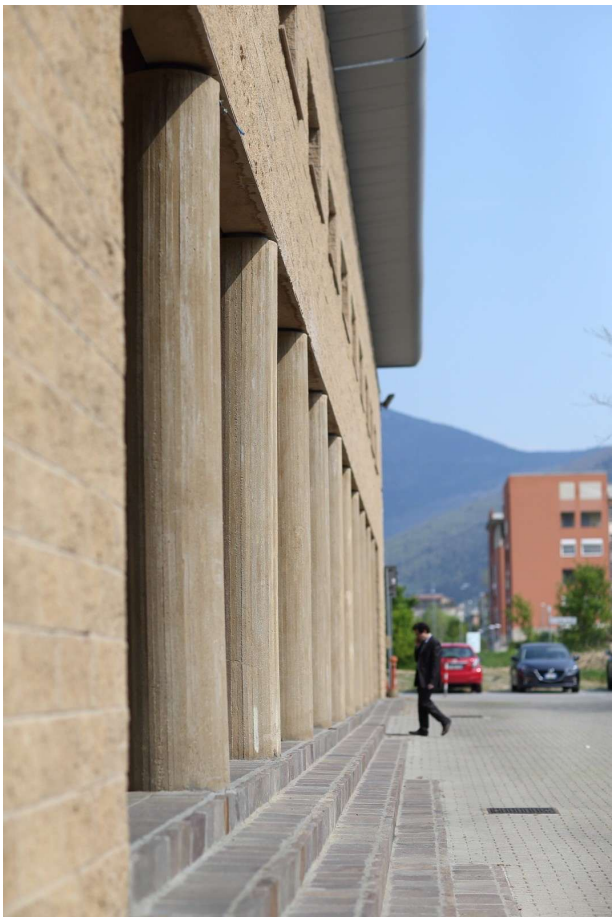
4. Bioinformatics to understand the structure-function relationship in biomolecules and in particular in metalloproteins through the large-scale analysis of databases;

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 of considering 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 the Universities of Frankfurt, Utrecht, Madrid 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 internships abroad. Post-Doctorate



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 J. Vila (Instituto de Biologia Molecular y Celular de Rosario, CONICET - Universidad Nacional de Rosario, Argentina)

Prof. Anthony Watts (Department of Biochemistry, University of Oxford,UK)

List of Publications

SCIENTIFIC ARTICLES

1. Jekhmane, S.; Derks, M. G. N.; Maity, S.; Slingerland, C. J.; Tehrani, K. H. M. E.; Medeiros-Silva, J.; Charitou, V.; Ammerlaan, D.; Fetz, C.; Consoli, N. A.; Cochrane, R. V. K.; Matheson, E. J.; van der Weijde, M.; Elenbaas, B. O. W.; Lavore, F.; Cox, R.; Lorent, J. H.; Baldus, M.; Künzler, M.; Lelli, M.; Cochrane, S. A.; Martin, N. I.; Roos, W. H.; Breukink, E.; Weingarth, M. Host Defence Peptide Plectasin Targets Bacterial Cell Wall Precursor Lipid II by a Calcium-Sensitive Supramolecular Mechanism. *Nature Microbiology* **2024**, *9* (7), 1778–1791. https://doi.org/10.1038/s41564-024-01696-9. (IF 20.5)

2. Ghafouri, H.; Lazar, T.; Del Conte, A.; Tenorio Ku, L. G.; Tompa, P.; Tosatto, S. C. E.; Monzon, A. M.; Aspromonte, M. C.; Bernadó, P.; Chaves-Arquero, B.; Chemes, L. B.; Clementel, D.; Cordeiro, T. N.; Elena-Real, C. A.; Feig, M.; Felli, I. C.; Ferrari, C.; Forman-Kay, J. D.; Gomes, T.; Gondelaud, F.; Gradinaru, C. C.; Ha-Duong, T.; Head-Gordon, T.; Heidarsson, P. O.; Janson, G.; Jeschke, G.; Leonardi, E.; Liu, Z. H.; Longhi, S.; Lund, X. L.; Macias, M. J.; Martin-Malpartida, P.; Mercadante, D.; Mouhand, A.; Nagy, G.; Nugnes, M. V.; Pérez-Cañadillas, J. M.; Pesce, G.; Pierattelli, R.; Piovesan, D.; Quaglia, F.; Ricard-Blum, S.; Robustelli, P.; Sagar, A.; Salladini, E.; Sénicourt, L.; Sibille, N.; Teixeira, J. M. C.; Tsangaris, T. E.; Varadi, M. PED in 2024: Improving the Community Deposition of Structural Ensembles for Intrinsically Disordered Proteins. *Nucleic Acids Research* **2024**, *52* (D1), D536–D544. https://doi.org/10.1093/nar/gkad947. (IF 16.7)

3. Cosottini, L.; Geri, A.; Ghini, V.; Mannelli, M.; Zineddu, S.; Di Paco, G.; Giachetti, A.; Massai, L.; Severi, M.; Gamberi, T.; Rosato, A.; Turano, P.; Messori, L. Unlocking the Power of Human Ferritin: Enhanced Drug Delivery of Aurothiomalate in A2780 Ovarian Cancer Cells. *Angewandte Chemie - International Edition* **2024**, *63* (40). https://doi.org/10.1002/anie.202410791. (IF 16.1)

4. Garcia-Vello, P.; Tytgat, H. L. P.; Elzinga, J.; Van Hul, M.; Plovier, H.; Tiemblo-Martin, M.; Cani, P. D.; Nicolardi, S.; Fragai, M.; De Castro, C.; Di Lorenzo, F.; Silipo, A.; Molinaro, A.; de Vos, W. M. The Lipooligosaccharide of the Gut Symbiont Akkermansia Muciniphila Exhibits a Remarkable Structure and TLR Signaling Capacity. *Nature Communications* **2024**, *15* (1). https://doi.org/10.1038/s41467-024-52683-x. (IF 14.7)

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BOOK CHAPTER

Luchinat, C.; Parigi, G. Metalloproteins, Structural Determination Of. In *Encyclopedia of Condensed Matter Physics*; 2024; p V4:718-V4:730. https://doi.org/10.1016/B978-0-323-90800-9.00313-9.

Seminars Held at CERM

ITACA.SB Webinar Series (https://edu.meet.garr.it/itaca_webinar)

October 16th, 2024, 15.00-16.00

Glycoproteins and in-cell NMR

Chair: Marco Fragai (University of Florence, Italy)

Speaker 1: Alba Silipo (University of Naples, Italy)

Title: Carbohydrates as keywords in the molecular dialogue

Speaker 2: Enrico Luchinat (University of Florence, Italy)

Title: “Detecting protein-drug interactions in human cells by real-time ^{19}F NMR”

November 13th, 2024, 15.00-16.00

Integrated approaches to study intrinsically disordered proteins

Chair: Isabella Felli (University of Florence, Italy)

Speaker 1: Michael Assfalg (University of Verona, Italy)

Title: "The impact of ubiquitination on the conformational properties of tau protein in solution"

Speaker 2: Marco Schiavina (University of Florence, Italy)

Title: “Exploring the structural and dynamic landscape of IDPs and multidomain proteins using direct ^{13}C NMR”

December 9th, 2024, 16.30-17.30

SAXS & other low-resolution techniques

Chair: Enrico Ravera (University of Florence, Italy)

Speaker 1: Dina Schneidman (The Hebrew University of Jerusalem, Israel)

Title: "Modeling large macromolecular assemblies in the age of deep learning"

Speaker2: Fabio Baroni (Merck Group, Guidonia, Italy)

Title: "Development of high-resolution biophysical methods for the structural characterization of biologics under the Quality by Design paradigm"

Dr. Riccardo Muzzioli

Radiopharmaceutical Chemist, Solve Therapeutics, Durham, North Carolina “From CERM to the US, protein chemistry in a radiochemistry world”, Wednesday, 18 December 2024 at 12.00, CERM Conference room.

Prof. Anthony Watts

Department of Biochemistry, University of Oxford, UK, Friday, 18 October 2024 at 14:00, "The importance of waters in membrane receptor sensitization - implications for optogenetics”, CERM Conference room.

Meetings and Events Organized by CERM

Dr. Luca Unione
CIC bioGUNE, Basque Research & Technology Alliance, Spain, Madrid, "Sugars, Carbohydrates, Saccharides, or simply Glycans", 25 September 2024 at 17:00, CERM Conference room.

Prof. Luis Rubio
Spanish National Research Council – CSIC, Madrid, "Cross-kingdom assembly of metallocofactors by integration of prokaryotic nitrogenase into eukaryotic hosts", 8 July 2024 at 12:30, CERM Conference room.

Prof. Alejandro J. Vila
Instituto de Biologia Molecular y Celular de Rosario, CONICET - Universidad Nacional de Rosario, Argentina, "Periplasmic Protein Quality Control in Live Cells by NMR", 26 June 2024 at 12:30, CERM Conference room.

Prof. Philip Grandinetti
The Ohio State University, USA, *The Luigi Sacconi Memorial Lecture in Chemistry 2024,* "Real-Time Monitoring of Battery Chemistry with Nuclear Magnetic Resonance Spectroscopy", Thursday, May 30, 2024, 5 p.m., Room 35, Blocco Aule, Polo Scientifico, Sesto Fiorentino.

Premio Città di Firenze sulle Scienze Molecolari 2024
Prof. Daniel G. Nocera
Patterson Rockwood Professor of Energy, Harvard University, USA, “Affrontare la sfida energetica globale: creare cibo e combustibile da sole, acqua e aria”, May 3rd, 2024 at 17.00, Salone dei Cinquecento, Palazzo Vecchio, Florence.

Prof. David Huffman
Western Michigan University, Kalamazoo, USA, "The State of Copper in the Cell", Thursday, February 29, 2024, CERM Conference room at 18.00.

Prof. Lapo Bogani
Dipartimento di Chimica "Ugo Schiff", Università di Firenze, "A new wave of molecular Carbon: from synthesis to quantum electronics and topological states", Friday, January 26, 2024, CERM Conference room at 17.00.

Meetings and Conferences

Exchanging news and views on NMR research activities at MPI Goettingen and CERM

18-19 July 2024, Sesto Fiorentino (FI) - CERM conference room

Chianti Workshop 2024

“A journey through 40-years of magnetic resonance discoveries: From pioneers to Gen-Z”

16-17 May, 2024, Blocco Aule, Polo Scientifico, Sesto Fiorentino

Fheritale Kick-off meeting

HORIZON-INFRA-2023-DEV-01 " Food, Health and Environment Research Infrastructures to Tackle Emerging Priorities".

Florence, 16 - 17 January 2024

Group Meetings

Friday, October 11th, 2024 at 1:00 pm
Prof. Roberta Pierattelli - Tessa Bolognesi
"From Expression to Interaction: NMR Insights into the Structural Features of the SARS-CoV-2 Nucleocapsid Protein and its Binding to Enoxaparin"

Friday, October 4th, 2024 at 1:00 pm
Prof. Roberta Pierattelli - Maria Anna Rodella
"15N-detected TROSY to study intrinsically disordered proteins: strategies to increase spectral quality"

Friday, September 27th, 2024 at 1:00 pm
Prof. Lucia Banci - Azzurra Costantino
"19F In-cell NMR to investigate protein-ligand interactions in living human cells"

Friday, September 20th, 2024 at 1:00 pm
Prof. Francesca Cantini - Alessia De Santis
"Investigation of CRBN E3 ubiquitin ligase within the development of a broad-spectrum antiviral drug"

Friday, September 13th, 2024 at 1:00 pm
Prof. Marco Fragai and Francesco Curro'
"Advanced structural and morphological characterization by NMR technologies of different biomolecules and biotechnological systems"

Friday, July 12th, 2024 at 1:00 pm
Prof. Lucia Banci and Martina Rosati
"Selective isotope labelling schemes to uncover the interactions of BRCA1 in human cells"

Friday, July 5th, 2024 at 1:00 pm
Prof. Marco Fragai and Bianca Susini
"Expression and characterization of protein involved in neurodegenerative disease"

Friday, June 28th, 2024 at 1:00 pm
Prof. Antonio Rosato and Cosimo Ciofalo
"A benchmark for the assessment of tools for the prediction of metalloproteins"

Friday, June 21st, 2024 at 1:00 pm
Prof. Marco Fragai and Siyu Lin
"Expression And NMR Characterization Of Labelled P-domains Of Emerging Norovirus"

Meetings and Events Organized by CERM

Friday, June 14th, 2024 at 1:00 pm
Prof. Lucia Banci and Martina Masini
"Exploring the role of mitoNEET in the reactivation of the iron master regulator IRP1"

Friday, May 31st, 2024 at 1:00 pm
Prof. Marco Fragai and Jlenia Bindi
"Relaxometric studies for the development of protein-based contrast agent for MR"

Friday, May 24th, 2024 at 1:00 pm
Prof. Antonio Rosato and Giulio Tassini
"Antibody-antigen binding characterization for the rational development an in vitro potency assay for a strep A vaccine"

Friday, May 10th, 2024 at 1:00 pm
Prof. Lucia Banci and Rosanna Cuccaro
"The role of human GLRX3 and NDOR1 in the maturation of cytosolic Fe-S proteins"

Friday, April 19th, 2024 at 1:00 pm
Prof. Cristina Nativi and Andrea Baldi
"Rational Design, Synthesis and Screening of Inhibitors in Enzyme-mediated Diseases: Novel Macrocyclic Pyrimidines as MerTK-Specific Inhibitors"

Friday, April 12th, 2024 at 1:00 pm
Prof. Enrico Ravera and Francesco Bruno
"The Mixture Demixer and Other Stories"

Friday, April 5th, 2024 at 1:00 pm
Prof. Moreno Lelli and Naomi Consoli
"Characterization of innovative materials for hydrogen storage"

Friday, March 29th, 2024 at 1:00 pm
Prof. Lucia Banci and Beatrice Bargagna
"MMDS type 3 caused by IBA57 mutations: the challenging expression and characterization of G104C IBA57 mutant"

Friday, March 22nd, 2024 at 1:00 pm
Prof. Giacomo Parigi and Adam Kubrak
"FC NMR Relaxometry on complex biological mixtures - Urine Relaxation in High- and Low-Fields"

Friday, March 15th, 2024 at 2:00 pm
Prof. Marco Fragai and Francesca Sacco

Meetings and Events Organized by CERM

"Integration of NMR spectroscopy and mass spectrometry for a new analytical workflow for the higher order structure characterization of biotherapeutics. Beyond the epitope mapping."

Friday, March 8th, 2024 at 1:00 pm
Prof. Marco Fragai and Luis Padilla
"Sialoglycan Binding Proteins – in Eukaryotic and Prokaryotic systems"
CERM Conference room

Friday, March 1st, 2024 at 1:00 pm
Prof. Francesca Cantini and Valentina Monaci
"Optimizing vaccine design for prevention of neonatal sepsis"

Friday, February 23rd, 2024 at 1:00 pm
Prof. Moreno Lelli and Lorenzo Niccoli
"Efficient Dinitroxides for DNP at High Fields and Fast MAS: from Proton Density to Solvation effects"

Friday, February 16th, 2024 at 1:00 pm
Prof. Marco Fragai and Giulia RoxanaGheorghiță
"Siglec-Sialic acid interaction axis: different binding contexts"

Friday, February 9th, 2024 at 1:00 pm
Prof. Enrico Ravera and Letizia Fiorucci
"Field dependent effects in paramagnetic NMR: What you never knew you didn't know"

Friday, February 2nd, 2024 at 1:00 pm
Prof. Lucia Banci and Beatrice Bargagna
"MMDS type 3 caused by IBA57 mutations: the challenging expression and characterization of G104C IBA57 mutant"

Friday, January 19th, 2024 at 1:00 pm
Dr. Debora Grifagni
"Looking for the Electron Transfer Partner (ETP) of human SAND: a possible candidate"

Friday, January 12th, 2024 at 1:00 pm
Dr. Veronica Ghini
"NMR detection of the cellular effects of gold(I)-based anticancer compounds and of their bioconjugates with ferritin"

Funding Institutions

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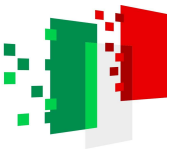

National Recovery and Resilience Plan (NRRP)

Italian Ministry of Agriculture

Ministero dell'agricoltura, della sovranità alimentare e delle foreste

Italian Ministry of Health

Ministero della Salute

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