CERM
Centro Risonanze Magnetiche
FIRENZE

CIRMMP
FIRENZE

SCIENTIFIC ANNUAL REPORT

2020

UNIVERSITÀ DEGLI STUDI FIRENZE

Contents

Foreword

The 2020 is the year of Covid-19 pandemic. This major event dramatically affected the life of billions of people, and it is still profoundly affecting our present life. This event has also brought to the fore the urgent need to invest in an efficient healthcare system and to fund frontier scientific research. 2020 is also the year where Covid-19 vaccines has been developed, only just after about 11 months that this new virus has been officially discovered. This terrific result has been possible only because of the significant progress in chemistry and life sciences that we have observed in the last a few decades and that provided to scientists the tools to afford this unexpected pandemic crisis. If a few years ago we could have said the scientific research is the foundation for building the future, now we can say the scientific research is the foundation for our present.

For our Infrastructure, 2020 is also the year where we got installed **the new 1.2 GHz NMR magnet (28.2 T),** and our laboratory received first in the world this outstanding instrument. With 1.2 GHz, NMR is entering a new era: a sizeable field increase compared to previous high field magnets, that is achieved after about fifteen years of research and development, and after more than a decade that the highest NMR magnetic field was not progressing anymore. We are already observing a significant step forward in resolution and sensitivity, as it is detailed below in the research activities. The potential of this new instrument is also exploited in the SARS-Cov2 research: we are developing several Covid-19 projects and we are part of consortium “COVID-NMR” that aims at characterising all the SARS-Cov2 proteins, paving the way to understand and afford the mechanism of action of this virus. We are also part of the NMR COVID-19 Metabolic Network. The contribution of this new instrument in terms of quality of the spectra and achieved information is already consistent and we present some of these results in the research activities.

Having the first 1.2 GHz NMR spectrometer installed in Florence makes our centre one major-player in this new course of NMR. This ultra high-field offers new opportunities to NMR, making it possible to obtain detailed characterisations of biomolecules, complex chemical mixtures, living cells, and new materials, unravelling processes that were inaccessible before. Especially in biomolecular research, NMR confirms to be an inescapable technique to thoroughly characterise biological systems at the molecular level. The research activities that we outline below in this report clearly evidence the uniqueness and the complementarity of NMR with respect to other techniques, including the emerging fields of molecular microscopies and other structural-oriented techniques.

Despite the limitations due to Covid-19 pandemic we were able to keep our research always active arriving to publish about 20% more papers published this year compared to 2019. By browsing through the research section of this report, the variety of applications of NMR that move toward the interfaces with other disciplines is clearly apparent: from structural biology to medicine, from material science to information technology.

In parallel, the role in the European Research Infrastructure scenario of CERM/CIRMMP was further reinforced. CERM/CIRMMP is the Italian centre of Instruct-ERIC, an ESFRI landmark Research Infrastructure. During 2020, the key role of the Italian centre (Instruct IT) within Instruct-ERIC was strongly reaffirmed: as an outcome of Brexit, should Instruct-ERIC not being able to maintain its statutory seat in Oxford, United Kingdom, the statutory seat of Instruct-ERIC shall be located in Florence, Italy.

This was possible thanks to our strong 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, internationalisation, access and industry engagement, the latter within the Instruct-ULTRA project which aims at releasing the full potential of Instruct to expand and consolidate infrastructure services for integrated structural life science research. The activity of CERM/CIRMMP related to Instruct-ERIC were framed also within the CORBEL initiative that coordinates 13 Biological and Medical European Research infrastructures (BMS RIs) to create a platform for harmonised user access to biological and medical technologies, biological samples, and data services required by cutting-edge biomedical research.

Furthermore, we have proceeded with the **EuroBioNMR EEIG** consortium, which is being established to co-ordinate European NMR research in biology and to ensure user access to several NMR infrastructures for all excellent scientific projects.

At the national level, in February 2020 we started the **Instruct-ITALIA** national consortium of infrastructures providing access to national users in structural biology, offering access to complementary techniques and providing competences available in each facility on different research fields: from NMR, to Cryo-EM, to optical microscopy ad X-ray techniques.

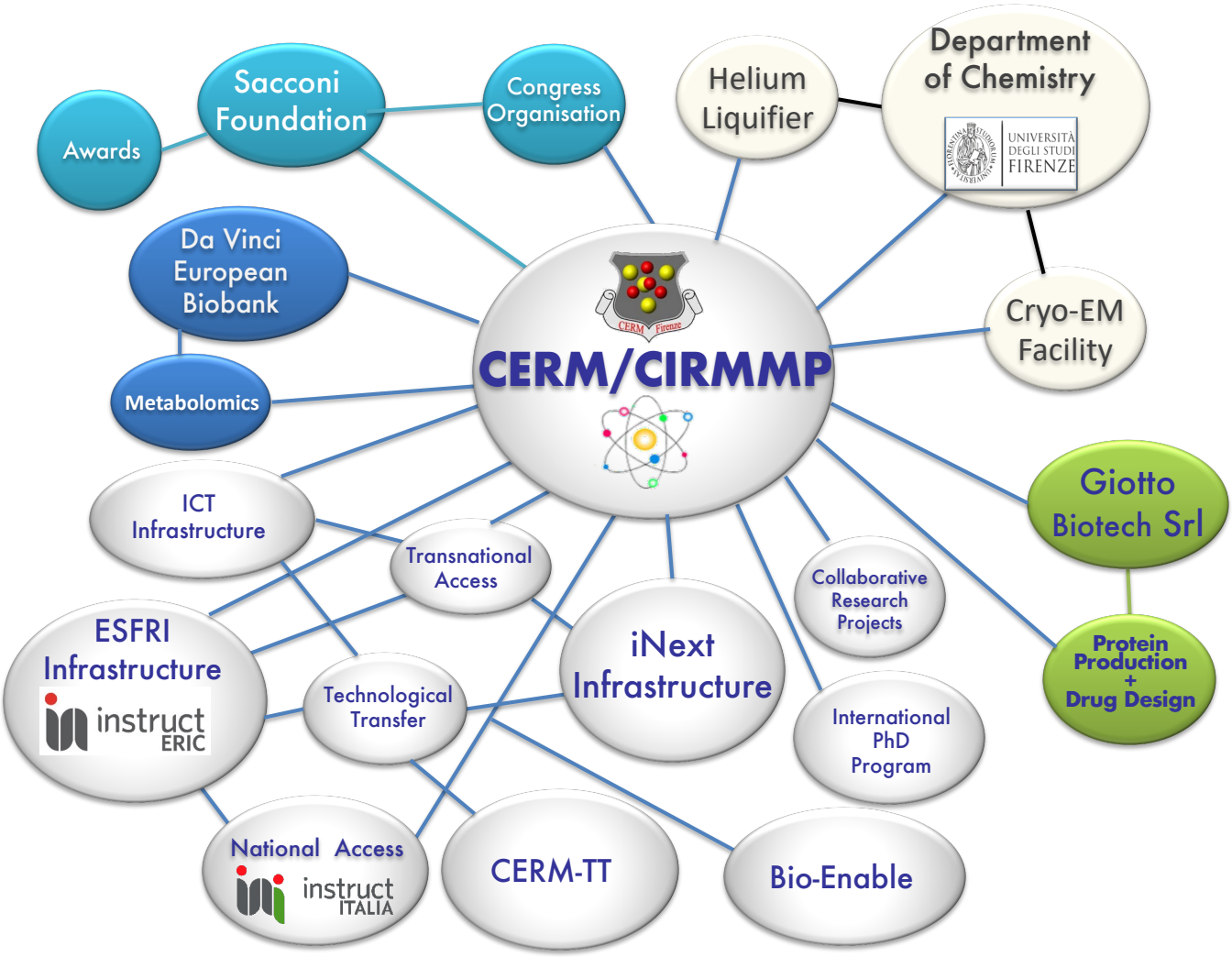
Figures

Also for 2020, the Italian Ministry of Education, University, and Research (MIUR) confirmed its support to the Italian centre of Instruct-ERIC within the International Action of the FOE funding. CERM/CIRMMP Investments and costs in 2020 amounted to € 3.380.000,00: € 320.000,00 towards training and education, € 1.920.000,00 for new equipment and € 910.000,00 towards research activities. An additional € 230.000,00 covered operational costs. The actual replacement value of the instrumentation at CERM is close to € 51.000.000,00.

In 2020, in addition to the faculty staff, the body of researchers included 17 PhD students 20 postdoctoral scientists and 15 undergraduate students.

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

Prof. Claudio Luchinat Prof. Lucia Banci


Awards
Sacconi Foundation
Congress Organisation
Helium Liquifier
Department of Chemistry
UNIVERSITÀ DEGLI STUDI FIRENZE
Da Vinci European Biobank
CERM Firenze
CERM/CIRMMP
Cryo-EM Facility
Metabolomics
ICT Infrastructure
Transnational Access
Giotto Biotech Srl
Collaborative Research Projects
ESFRI Infrastructure
instruct ERIC
iNext Infrastructure
Protein Production + Drug Design
Technological Transfer
International PhD Program
National Access
instruct ITALIA
CERM-TT
Bio-Enable

Who we are

Introduction

CERM, Centre for Magnetic Resonance, is a ***scientific institution for research***, technology transfer and higher education 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 specialisations in NMR spectroscopy, 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 development, contrast agent 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 (MIUR), and the European Commission (EC), as well as from private institutions. Since 1994, CERM/CIRMMP is providing a transnational access to its instrumentation for its expertise and state-of-the-art instrumentation for NMR in Life Sciences.

The core technology at CERM/CIRMMP is NMR spectroscopy, and the onsite instrumentation is among the most advanced in the world. A European transnational access service, funded by EC since 1994 in addition to service provision at national level operating since 1990, places CERM/CIRMMP at the top of the list for experience among the European NMR Research Infrastructures. CERM/CRIMMP actively stimulates interactions between private industry and public research institutions such as Universities, National Research Council (CNR) Institutes, and European counterparts, promoting synergistic activities such as collaborations and services to SMEs.

CERM/CIRMMP is a core 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. The Italian centre of INSTRUCT-ERIC, CERM/CIRMMP is also included in the "*Roadmap Italiana delle Infrastrutture di Ricerca di interesse Pan-Europeo*" since 2010. In parallel, ***CERM/CIRMMP*** is also the core center of the ***Instruct-ITALIA*** network, a new infrastructure to promote and to 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 at begin 2020 promoting a more effective interaction within Italian structural biologists as well as at supporting access to the facilities of its national network.

CERM/CIRMMP is also an e-infrastructure, managing an European GRID-based platform, providing access to user-friendly platforms and CPU resources for a broad range of soft-

ware tools for structural biology. CERM/CIRMMP also promoted the creation of the DA VINCI EUROPEAN BIOBANK, a "*biobank of biological samples and biomolecular resources*". CERM/CIRMMP has also developed a centre for research and technology transfer: CERM-TT, funded by the Tuscany Region and inaugurated in July 2015. Finally, CERM/CIRMMP is coordinating the activities of BIO-ENABLE, a new 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 located in the Scientific Campus ("Polo Scientifico") of the University of Florence in Sesto Fiorentino, an area just west of the city of Florence. The campus borders Florence International Airport and yet is a mere 15 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 a number of laboratories, offices, and common rooms. The flagship of the Center is the impressive collection of NMR spectrometers which feature the largest magnetic field range in the world (from 950 MHz to the earth field, 1.2 GHz installed in early 2020) and ranks it among the best equipped laboratories in the world. The NMR labs are flanked by molecular and cellular biology laboratories where samples for the NMR are produced. A complete list of the instruments available at CERM/CIRMMP is reported at pag. 38. In addition to the main building, further 500 square meters in adjacent buildings are available to CERM scientists and researchers scientifically associated to CERM/CIRMMP: laboratories at the Department of Chemistry Ugo Schiff and at GENEXPRESS; DA VINCI European Biobank; X-rays facilities; Helium liquefier. www.cerm.unifi.it

Instruct-ERIC

CERM/CIRMMP is an INSTRUCT-ERIC Centre. INSTRUCT-ERIC is the European research infrastructure in integrated structural biology, making cutting-edge technologies and high-end methods in a palette of tools for structural characterisation 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 miscroscopy).

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

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

CERM TT

The CERM TT Competence Centre *dedicated to Ivano Bertini*, 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 optimises the service 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 performs analytical services and research and development (R&D) for companies. In particular it offers the following services:

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

Bio-Enable

BIO-ENABLE is a "distributed research infrastructure" led by CERM/CIRMMP and includes a few of 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 international level.

CERM leads the BIO-ENABLE consortium composed by:

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

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

www.bio-enable.it

Funded projects

CERM/CIRMMP cooperates at the international level with several universities, research institutions and private industries with which is involved in numerous research projects funded by the European Commission. Projects ongoing during 2020 are:

H2020-INFRADEV **INSTRUCT ULTRA** - Releasing the full potential of Instruct to expand and consolidate infrastructure services for integrated structural life science research (#731005) https://www.instruct-ultra.eu/

H2020 -INFRAIA iNEXT-Discovery - Structural Biology Research Infrastructures for Translationa Research and Discovery (#871037) https://inext-discovery.eu

H2020-INFRADEV CORBEL - COordinated Research Infrastructures Building Enduring Life-science Services (H2020, contract n. 654248, 01/09/2015-31/05/2020)

TRANSVAC2 - Improving and accellerating vaccine development in Europe

"The Biogenesis of Iron-sulfur Proteins: from Cellular Biology to Molecular Aspects (FeSBioNet)" Cost Action CA15133 (H2020, 15/04/2016-14/10/2020)

EOSC-hub "Integrating and managing services for the European Open Science Cloud" (H2020, contract n. 777536, 01/01/2018- 31/12/2020)

TIMB3 "Twin to Illuminate Metals in Biology and Biocatalysis through Biospectroscopy" (H2020, contract n. 810856, 01/09/2018-31/08/2021)

THE INFRASTRUCTURE

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

SPIDIA4P - Standardisation and improvement of generic pre-analytical tools and procedures for in-vitro diagnostics. http://www.spidia.eu/

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

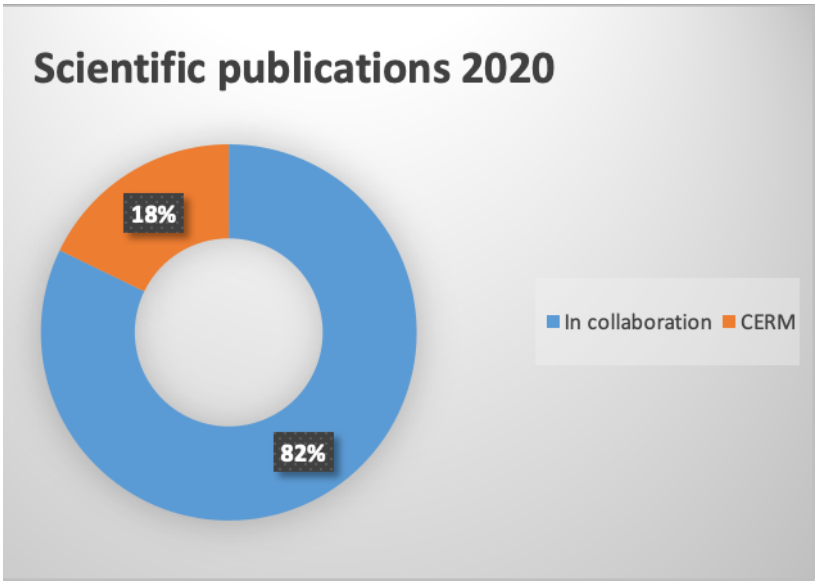
HIRES-MULTIDYN "Multiscale Dynamics with Ultrafast High-Resolution Re (H2020, contract n. 899683, 1/10/2020-30/09/2024)

Research Activities

Introduction

During 2020 a number of projects have been carried out, either as an extension of the activities of previous years or as new projects. Most of these projects receive specific funding from national and/or European organisations.

NMR is the core technology of CERM, but year by year CERM research has been oriented more and more toward new applications and toward the integration with other techniques. This is one of the principles of the Integrated Structural Biology that underlays the INSTRUCT-ERIC consortium, where CERM/CIRMMP is the Italian pole. In the following pages it can be appreciated how much the present research in CERM/CIRMMP is spanning a wide range of applications, from the structural biology to the bioinformatics methods and Information Technology, from paramagnetic NMR methods to the development of new contrast agents for MRI, from the metabolomics and biomedicine to the development of new solid-state NMR methods for the characterisation of material surfaces and biomaterials.



In line with our mission to develop NMR as a technique and to integrate NMR with other techniques, most of our publications were done in collaboration with other research groups (82% of the overall number of publications). During 2020 we published 73 papers in international peer-reviewed journals, more than 20% more publication with respect to last year. This is quite remarkable considering the limitations of the pandemic year. The average impact factor is of 5.34. A complete list of publications is available at page 47.

CIRMMP has been ranked first among the Italian Inter-University Consortia in Chemical Science in the last evaluation of the quality of research (VQR 2011-14) by the National Agency for the Evaluation of the University and Research Systems. The Chemistry Department of the University of Florence was also winner of the national Project for Departments of Excellence. With the funds arrived with this Excellence National Project and in collaboration with CERM, the Department of Chemistry will be endowed with a Cryo-EM microscope for high-resolution investigation of biomolecules and materials, and it will be accessible also to

CERM researchers. This witnesses the impact of our research not only in the NMR field, but also in the larger chemical community and in the whole Italian research community.

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

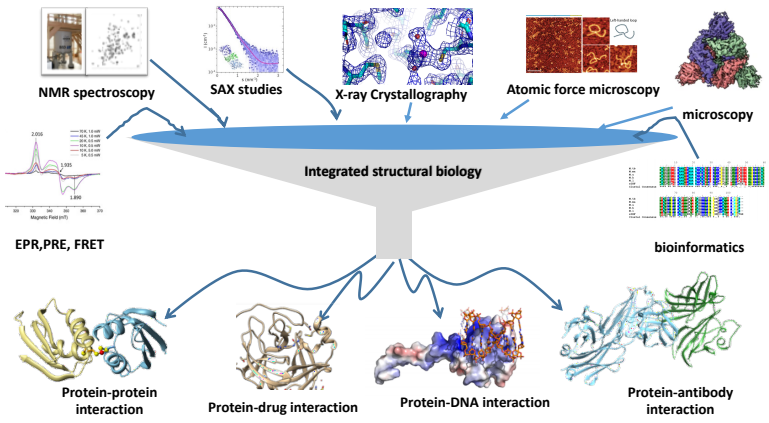
Finally, since 2016 CIRMMP has decided to implement a quality system of the NMR lab, and is presently undergoing an ISO9001 certification process. The long-term goal is the obtainment of ISO/IEC 17025 accreditation for a set of key validated NMR analyses.

The Role of Solution NMR in Integrated Structural Biology

Nowadays solution NMR is an indispensable technology for determining not only structures of proteins but also their weak and transient interactions with other macromolecules, as well as for characterising functional processes directly in living cells. Through the integration of solution NMR with other structural data on different length and time scales, we can understand how proteins, protein complexes or DNA-protein complexes dynamically interact with their functional environment.[1-2] This fundamental understanding will underpin our ability to provide new therapeutics to meet the grand challenges of an ageing society, public health and global pandemics.

CERM applies solution NMR in an integrated systems biology approach for addressing more and more challenging questions. Such approach is routinely used to understand the role played by a protein in the frame of cellular metabolism, or to rationally engineer an enzyme for a specific industrial process, or to determine how to design novel drugs that target a particular protein, or to understand what changes might improve them.[3-6]

The major challenge of structural biology is understanding how proteins function at the cellular level, within macromolecular complexes, or in a cellular pathway.
Understanding dynamic processes that are co-ordinated at a cellular level is not possible using a single technology, but it becomes potentially accessible through the integration of a number of approaches, spanning different resolution scales.



The potential of integrated structural biology in unravelling biological processes.

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(3) Schiavina M,. *et al. Sci Rep.* **2020,** *10,* 19574.

(4) Odermatt, N.T.; Lelli, M.; *et al.J. Struct. Biol.* **2020,** *209,107434.*

(5) Trindade I B; *et al. Inorg. Chim. Acta ,* ***2021,*** *514*, 119984.

(6) Chatzikonstantinou AV, *et al. Methods Enzymol.* ***2020,*** *633,* 71-101.

Computing for Integrative Structural Biology

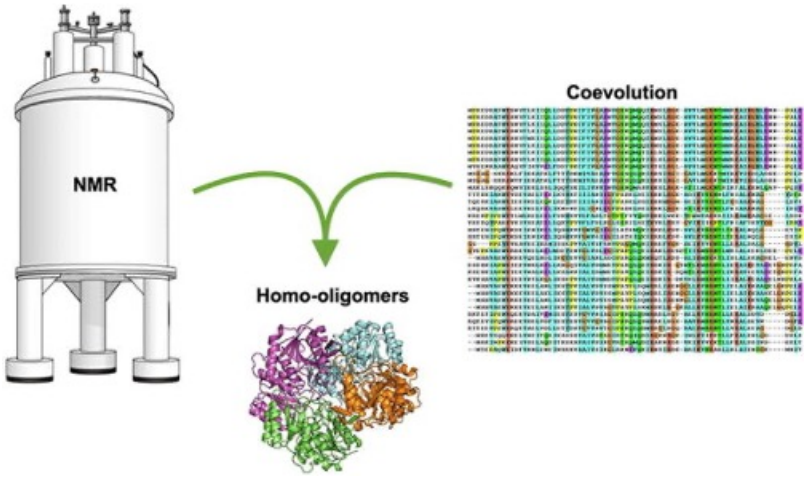
Integrative structural biology combines data from multiple techniques to generate complete structural models for complex biological systems. Our work explored two main lines: integration of NMR data with X-ray data or with bioinformatics data. In all cases, data integration improves the accuracy of the structural models.

The integration of data from two or more techniques, experimental or computational, significantly improves the accuracy of the structural models for large protein assemblies and flexible multi-domain proteins.

In this context we worked on the integration of solution or solid-state NMR data with evolutionary information to characterise homo-multimeric proteins [1].

The integration of NMR and computational data is useful also to define the conformational variability of proteins [2].

Finally, we showed how the integration of NMR and X-ray data enhances the structural determination of single-domain proteins [3].



The analysis of multiple sequence alignments provides information on co-evolution of residues within the protein sequence. Ambiguous NMR restraints involving co-evolving residues can be interpreted as inter-subunit contacts and used to drive protein-protein docking calculations [1].

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(3) Schirò, A.; Carlon, A.; Parigi, G.; Murshudov, G.; Calderone, V.; Ravera, E.; Luchinat, C. *J. Struct. Biol. X* **2020** 4, 100019

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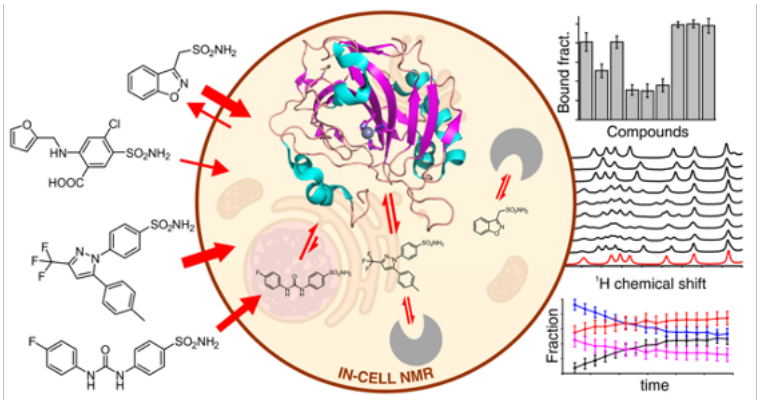
In-cell NMR in Human Cells

In-cell NMR spectroscopy allows structural and functional characterisation of macromolecules inside living cells, allowing the study of protein-protein and protein-ligand interactions in a highly physiological environment.
The protein expression approach in human cells previously developed at CERM was applied to perform a protein-observed intracellular ligand screening. The binding of sulfonamide-derived molecules to the human carbonic anhydrase II metalloenzyme was directly observed in human cells by in-cell NMR. Dose- and time-dependent binding curves were analysed with a diffusion-limited binding model and provided insights on cell penetrance, intracellular binding affinity and adduct stability for each molecule.[1,2]
A modular flow-NMR bioreactor system was developed, which maintains high cell viability over prolonged periods of time, up to 72 hours, by providing oxygen and fresh nutrients to human cells embedded in hydrogel within the NMR spectrometer. The NMR bioreactor allows time-resolved in-cell NMR experiments, which were applied to monitor protein-ligand interactions and chemical modifications by time-resolved in-cell NMR.[3]
Time-resolved NMR in-cell and in vitro can provide precious insights on physio-pathological processes at single residue level, as shown by monitoring the chemical modification of superoxide dismutase 1 by the toxic by-product methylglyoxal.[4]

In-cell NMR spectroscopy is a unique tool for characterizing biological macromolecules in their physiological environment at atomic resolution. At CERM, we have developed a protein expression approach in to observe proteins in human cells by NMR. Functional processes and changes in response to external stimuli can be monitored in their native environment.



Protein-observed intracellular ligand screening by NMR in human cells provide pharmacologically relevant insights in the cellular context.

References:

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NMR Methods Fighting Covid-19 Pandemic

The outbreak of the COVID-19 pandemic has prompted CERM to take part in series of activities (from international to local) concerning the use of NMR to learn about SARS-CoV2 and its mechanism of action. Main research areas concern structural biology and drugability of SARSCoV2 proteins and metabolomics fingerprinting COVID-19 patients of different disease severity and response to treatment.

References:

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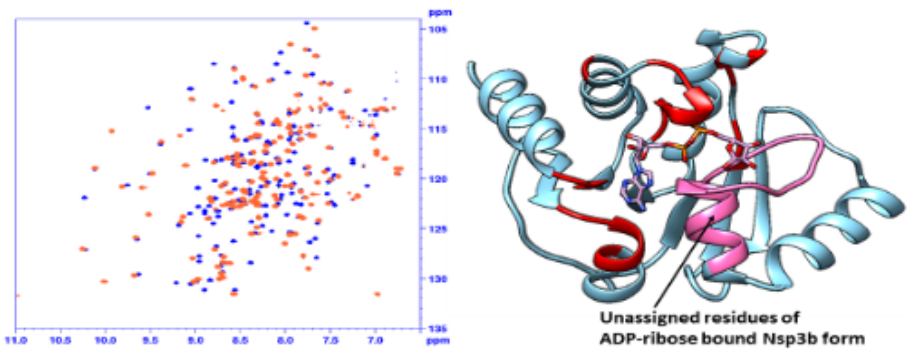
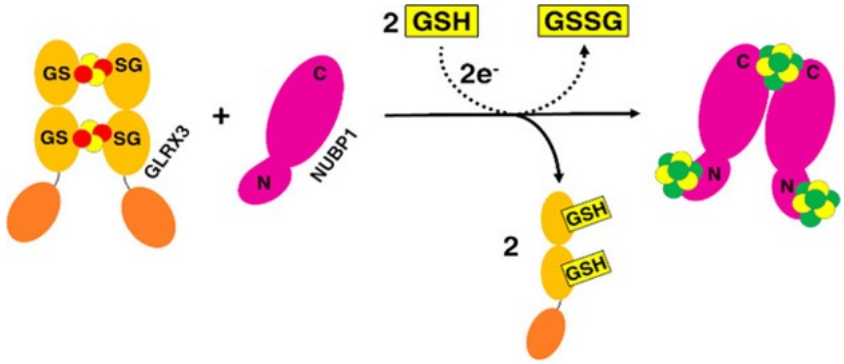
Within the international project Covid19-NMR (https://covid19-nmr.de/), aimed at determining RNA and protein structures of SARS-CoV-2 and to investigate their drugability by small molecules, CERM was involved in the NMR spectroscopic characterisation of the macrodomain (MD) Nsp3b, a subdomain of the 217 kDa Nsp3 from SARS-CoV-2. The MDs of SARS and MERS play a key role in viral replication and modulate host's immune response, making SARS-CoV-2 Nsp3b a highly relevant target in the viral replication process. The complete NMR backbone resonance assignment of the SARS-CoV-2 Nsp3b MD in its *apo* form and in complex with ADP-ribose has been obtained and will provide a basis for NMR investigations targeted towards small-molecule inhibitors of the catalytic activity of Nsp3b. CERM is also part of the COVID-19 Research Network coordinated by the Australian National Phenome Centre at Murdoch University, aimed at coordinating international efforts in metabolomics to better understand and mitigate the COVID-19 virus pandemic threat. At the local level CERM collaborates with AOU Careggi for the fingerprint of disease and individual response to various treatments; a joint research on the effect of Tocilizumab has appeared as MEDRXIV/2020/228361. CERM has received a two-year funding from for the biennial project COMETA (Study of the metabolomic fingerprint of patients affected by Covid 19 for an accurate diagnostic, prognostic and therapeutic classification - COVID-19 REGION OF TUSCANY RESEARCH NOTICE).



Overlay of HSQC spectra of apo (blue) and ADP-ribose (orange) Nsp3b. Residues showing large chemical shift perturbation effects for the titration of nsp3b with ADP-ribose are shown in red

Molecular Mechanisms of Iron-Sulfur Protein Biogenesis in Humans

The cytosolic Fe-S assembly (CIA) machinery, is in charge of the maturation of cytosolic and nuclear [4Fe-4S] proteins. In humans, the first steps of the CIA machinery involves a set of proteins, whose molecular role is still not clearly identified. In 2020, we focused our attention on how a [4Fe-4S] cluster is assembled on NUBP1 and received by CIAO3 protein within the CIA machinery. Specifically, we observed that i) $[2Fe\text{-}2S]^{2+}$ clusters are transferred from GLRX3 to monomeric apo NUBP1 and reductively coupled to form $[4Fe\text{-}4S]^{2+}$ clusters on both N-terminal and C-terminal cluster binding motifs of NUBP1 in the presence of glutathione that acts as a reductant, ii) cluster binding to the C-terminal motif of NUBP1 promotes protein dimerization, while cluster binding to the N-terminal motif does not affect the quaternary structure of NUBP1, and iii) the formation of a stable, [4Fe-4S]-bound, complex, composed by CIAO3 and the CIA2A-CIAO1 complex. Our findings provide the first evidence for GLRX3 acting as a [2Fe-2S] cluster chaperone in the early stage of the CIA machinery, and revealed for the first time that CIAO3 is able to establish a stable interaction only once the CIA2A-CIAO1 complex is formed. These studies provide solid bases on the molecular mechanisms of the CIA machinery.



[4Fe-4S] cluster assembly on NUBP1 shows that GLRX3 acts as [2Fe-2S] cluster chaperone in the CIA machinery.

Iron-sulfur (Fe-S) clusters are ancient protein cofactors involved in fundamental cellular processes. Fe-S protein biogenesis is a highly complex process in all living cells. Several human diseases are related to the misfunction of Fe-S protein biogenesis. A picture of the molecular mechanisms at the basis of Fe-S protein biogenesis is fundamental to boost the development of disease treatments.

References:

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(2) Maione, V.; Grifagni D.; Torricella F.; Cantini F.; Banci L. *J Biol Inorg Chem.* **2020**, 25, 501-508.

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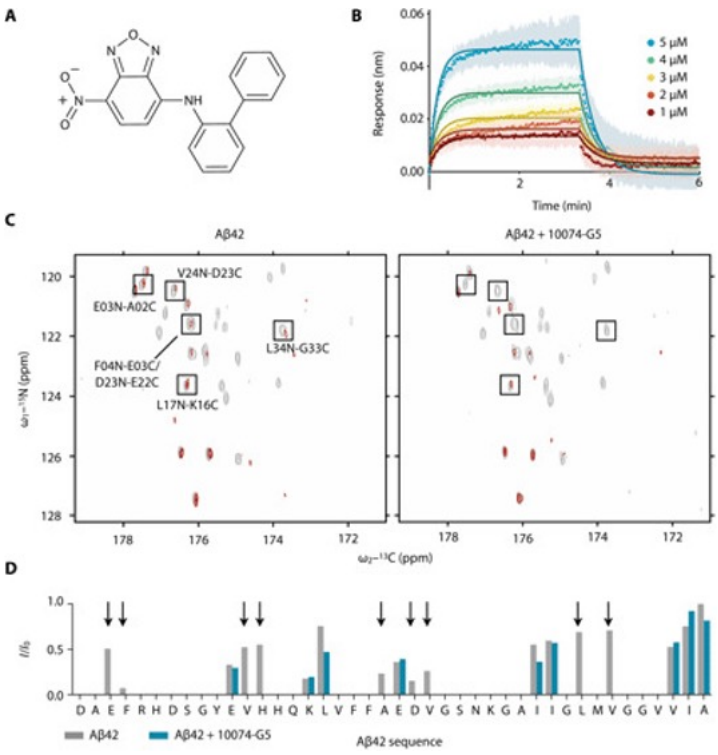
Proteins as drugs and drug targets

CERM has contributed to develop new methodologies for NMR screening and analytical procedure to assess the quality fragment libraries allowing the identification of a small molecule capable of binding and sequestering the intrinsically disordered amyloid-β (Aβ) peptide in its monomeric, soluble state.

Fragment screening has become a more structure-based approach to inhibitor development. However, it is often witnessed that the availability, immediate and long-term, of a high quality fragment-screening library is still beyond the reach of most academic laboratories. In 2020, CERM has contributed to develop analytical procedures to assess the quality of fragment libraries[1] by NMR spectroscopy and has described an efficient and simple method for the screening of PPI inhibitors (HOPPI-NMR), in which one of the two interacting proteins is replaced by a short peptide (hot-peptide).[2] Another relevant result of the research activity at CERM has been the identification of a small molecule capable of binding and sequestering the intrinsically disordered amyloid-β (Aβ) peptide in its monomeric, soluble state.[3]



Left: Structure of the dimer of dimers of [2Fe-2S] ISCA2-IBA57. Right: Model of [4Fe-4S] cluster assembly pathway on NFU1.

References:

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Driving Iron Through the Ferritin Cage

L-subunits in mammalian cytoplasmic ferritin assist the formation of encapsulated iron biomineral providing a nucleation site on the inner surface of the cage. The underlying mechanism is largely elusive, although literature data suggest that the growth starts from the inner cage surface. We have obtained a high-resolution evidence of this event.[1] The formation of the tri-nuclear seed is a surface-driven process. The subsequent development into an 8-iron cluster is only indirectly assisted by protein side chains. Analogous results have been obtained on natural horse spleen ferritin, a commercial heteropolymer with > 90 % of L- type sub units, sharing a sequence identity of 87.4 % to human L-subunits.[1] Thus, the 8-iron cluster formation appears as a common feature, of possible biological significance, in L-subunits of mammalian ferritins.

The properties of the inner cage surface also determine the encapsulation efficiency of exogenous species. We are now exploiting the use of human ferritin cages to encapsulate a variety of molecules, from small metal complexes to small proteins. In particular, the stability of mitochondrial cytochrome c under a variety of conditions has been investigated,[2] for the design of a high-pH disassembly and re-assembly procedure to create ferritin carriers with apoptotic activity.

X-ray structures of homopolymeric recombinant human L-ferritin and natural horse spleen ferritin revealed the growth of an octa-nuclear iron cluster on the inner surface of the protein cage. The growth of this cluster provides an atomic resolution view of how the caged iron-oxo biomineral develops starting from an initial nucleation seed.

References:

(1) Ciambellotti, S.; Pozzi, C.; Mangani, S.; Turano, P. *Chemistry* **2020**, 26, 5770-5773.

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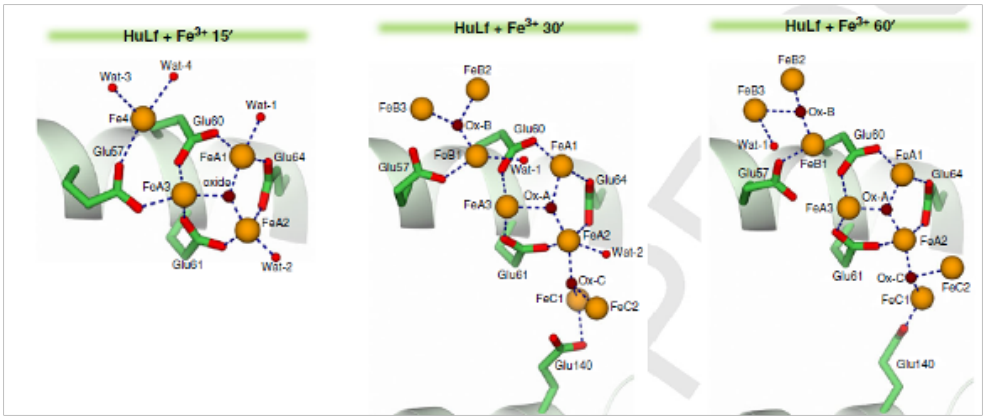

Snapshots of iron(III) binding events in human L ferritin as a function if the ion free diffusion time.

Intrinsically Disordered Proteins by NMR

NMR experiments, under continuous development at CERM, reveal interesting properties of intrinsically disordered proteins as well as of flexible linkers in complex protein machineries. Novel functional motifs within highly flexible, disordered protein regions are beginning to emerge.

Intrinsically disordered proteins continue to be in the spotlight of structural biology and NMR constitutes the key experimental technique to access atomic resolution information on their structural and dynamic properties. Novel NMR experiments based on ^{13}C direct detection were developed to monitor aminoacid side chains, solvent exchange processes (1) and to investigate proline residues in their cis and trans peptide bond isomers (2). These were used to study how α-synuclein senses calcium concentration jumps associated to the transmission of nervous signals revealing specific motifs (DPD and EPE) in aromatic rich regions involved in the interaction (1, Figure below). Proline-aromatic pairs were also identified as key residues promoting more compact, still highly flexible states in Osteopontin (2). Finally, the suite of ^{13}C detected experiments was used to investigate flexible linkers of CBP (ID4 and ID5) and their interactions (3,4). Elegant experiments were proposed to actively exploit exchange processes with the solvent (5).

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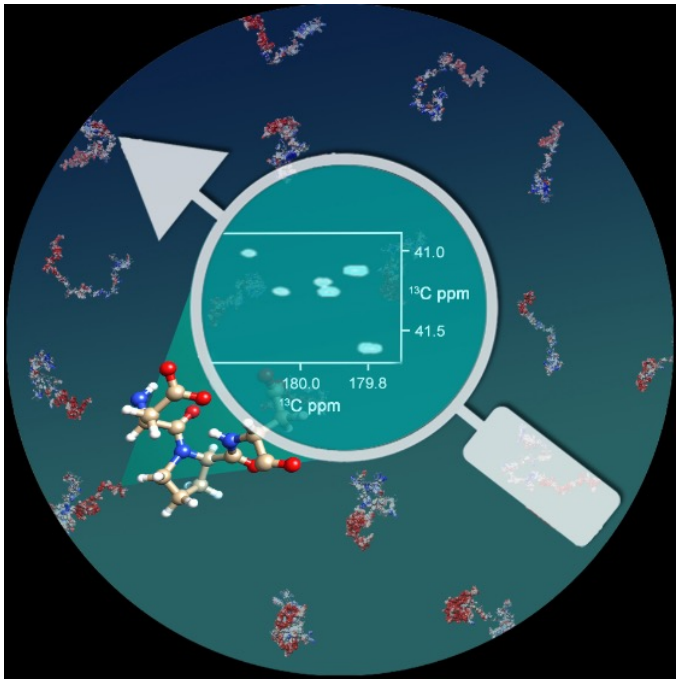


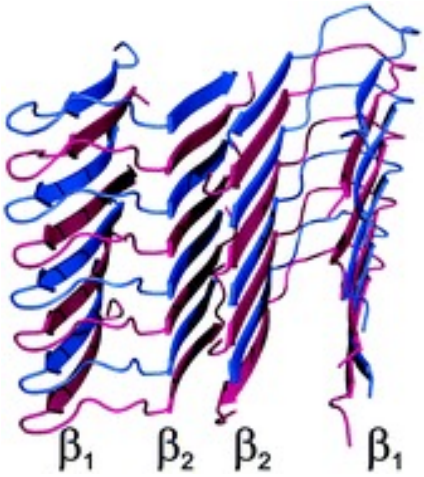
Zooming into aminoacid side chains through ^{13}C direct detection NMR experiments, allows us to identify peculiar motifs that are involved in sensing concentration jumps of calcium ions.

Solid-state NMR to study Biomolecules and Protein Aggregation

Solid-state NMR is once more the method of choice to structurally investigate solid proteins, protein aggregates. [1] Furthermore it provides unique dynamic information in proximities of the metal center also in systems that cannot be investigated otherwise[2,3].

We have applied this technique to investigate the Aβ fibrils, and the possible cofibrilization of mixed Aβ(1–40) and Aβ(1–42) fibrils. We have prepared and investigated Aβ fibrils in the same experimental conditions as those previously used to obtain well-shaped fibrils of pure Aβ(1–40), using a 1:1 ratio of the two isoforms Aβ(1–40) and Aβ(1–42). A new single species is spontaneously formed. The mixtures before fibrillization show a marked toxicity to cultured neurons. When a 3:7 Aβ(1–42):Aβ(1–40) ratio (previously found to be the most toxic mixture) is used, the same single species is observed, but with the excess Aβ(1–40) simultaneously forming the same pure fibrillar species previously characterised. Collectively, our solid-state NMR data demonstrate beyond any doubt that Aβ(1–40) and Aβ(1–42) can co-fibrillize in a 1:1 ratio to form an interlaced fibril.[1]

Solid-state NMR demonstrated also a powerful technique to investigate the binding mode of new antibiotics. We used ^{1}H-^{31}P correlation experiments at ultra fast MAS to find the binding mode of teixobactins with Lipid II cellular membrane. The atomistic details obtained shed new light in the binding mode of this newly discovered antibiotic teixobactins.[3]



Structural model of Aβ(1–40)/Aβ(1–42) interlaced mixed fibrils. The Aβ(1–42) polypeptide is coloured in magenta while the Aβ(1–40) polypeptide in blue.

CERM has identified and characterised by solid-state NMR a novel, structurally-uniform 1:1 mixed fibrillar species, which differs from both pure fibrils. It forms preferentially even when Aβ(1–42):Aβ(1–40) peptides are mixed in a non-stoichiometric ratio. Solid-state NMR is also applied to the investigation of antibiotic/cell membrane interaction, revealing the precise binding mode of the novel teixobactine antibiotic.

References:

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NMR of Paramagnetic Systems

Quantum chemical methods and computational approaches allowed us to calculate paramagnetic chemical shifts in solution and in solid state.
New experiments have been developed to measure relaxation rates and and to account them into structure calculations.
Novel paramagnetic tags were also developed.

Recently, it has been proposed to calculate PCS from first principle. In collaboration with Frank Neese (MPI Muelheim) we demonstrated that the difference between modern QC calculations and semiempirical treatment arises because of the neglect of the gauge correction term in the complete Hamiltonian.(1). In collaboration with Dr. Pintacuda and Prof. Emsley we have also introduced a new approach that enables the determination of a high-definition structure of the active site of a metalloprotein combining solid-state NMR and computational approaches (2).
The obtainment of structural restraints for the first coordination sphere of the metal ion(s) is crucial to understand their biological function. A novel HSQC scheme, termed R_2-weighted HSQC-AP, achieves this aim by detecting signals that escaped detection in a conventional HSQC experiment and provides fully reliable R_2 values in the range of ^1H R_2 rates ca. 50-400 s^{-1}. (3)
The High Potential Iron-Sulfur Protein (HiPIP) PioC, from *Rhodopseudomonas palustris* TIE-1 provides the first example of a protein structure determination by NMR where NOEs have been completely replaced by Paramagnetic Relaxation Enhancements (PRE) restraints. Comparison of the family of structures obtained by NOE and PER structural restraints reveals that the pairwise RMSD between them are comparable with the precision within each family (4). This approach can be extended also to proteins that are not naturally binding a paramagnetic metal. We have developed a new tag, which binds through a photocatalyzed thiol-ene reaction (5).

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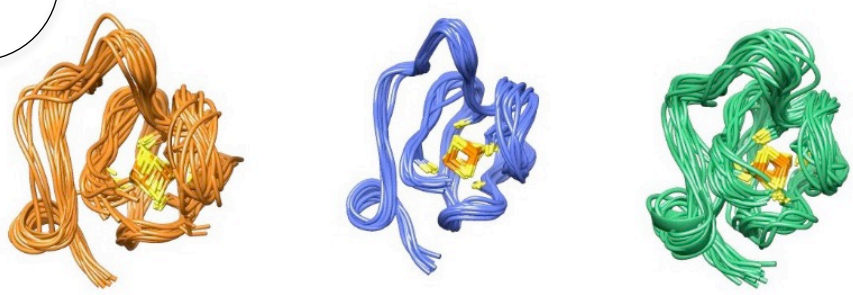
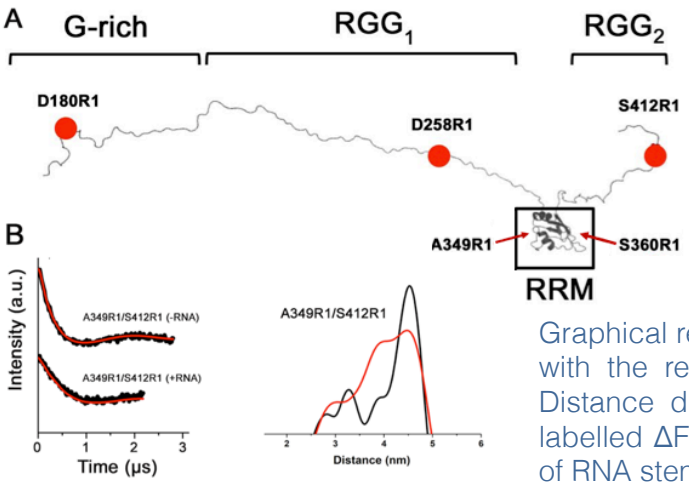


Solution structure of PioC obtained using NOEs only (orange), the full set of NMR restraints (blue), PREs only (green).

EPR and Pulsed EPR in Structural Biology

EPR and Pulsed EPR spectroscopies represent a group of powerful tools for structural biology investigations. Continuous wave (cw) EPR spectra at room or cryogenic temperature can be employed to gain detailed information about either protein native paramagnetic sites[1,2] (e.g: metal ion, FeS cluster) or site directed spin labelled biomolecules (e.g: nitroxide labelled).[2] On the other side pulsed EPR methods and especially pulse dipolar spectroscopy provides the unique contribute to structural biology to retrieve reliable distance distributions based on the dipolar coupling between two equal or orthogonal paramagnetic labels.[3,4] During the last year, the X/Q band EPR spectrometer at CERM has been employed to disentangle relevant biological answers. In particular the role of the CIAO3 protein has been investigated employing cryogenic temperature X-band cw-EPR spectra to retrieve for the first time the formation of a stable [4Fe-4S]-bound complex composed by CIAO3 and the hetero-CIA2A-CIAO1 complex[1] . Moreover an efficient NMR, EPR combined study has been optimised to characterise the structural properties of the ΔFUS construct from the Fused in Sarcoma tracriptional factor. Indeed the use of cw-EPR and PELDOR (Pulsed Electron Double Resonance) experiments established the interaction of the RGG regions with the RRM domain and the structural changes of the protein upon the RNA binding.

The use of continuous EPR and Pulsed EPR in combination either with Site directed spin labelling or native paramagnetic centres open the possibility to obtain information about the environment surrounding the paramagnetic probe. Moreover, in addition to the structural biology tools, PELDOR Spectroscopy allows to measure long range nanometric distances distribution (1.6 - 16 nm) between at least two paramagnetic centres.



Graphical representation of the ΔFUS modelled structure with the relative MTSSL(R1)-nitroxide labelling site (a). Distance distribution extracted from one of the doubly labelled ΔFUS protein mutant in presence and absence of RNA stem-loop (b). Adapted from Bonucci et. Al.[2]

References:

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Fast-Field Cycling Relaxometry

Fast field cycling (FFC) relaxometry can provide access to the structural and dynamic parameters on which nuclear relaxation depends, and it represents a precious tool for the optimisation of contrast agents for MRI and for investigating how particle size and pore size of paramagnetic nanoparticles influence the proton relaxation efficiency.

FFC relaxometry is used for characterising the efficacy of paramagnetic complexes as contrast agents for MRI.[1-3] The confinement of Gd(III) chelates within nanogels can be considered as a new route to hypersensitive MRI probes. In fact, besides the restriction of the local reorientation degrees of freedom related to the encapsulation of the Gd(III) chelates, the high water content entrapped within the matrix can favour a relaxivity enhancement. Hydrogel nanoparticles composed of chitosan and hyaluronate and incorporating Gd-based MRI contrast agents exhibit enhanced efficacy (relaxivity) as relaxation agents, over 6 times that of the free complexes. The analysis of the relaxometry profiles indicates that the confinement of the Gd complexes into the nanogel indeed increases their reorientation time to the nanoseconds time scale and that the lifetime of the water molecule(s) coordinated to the Gd ion remains short enough not to limit the relaxivity, as well as that between the water inside the nanogel and the bulk water.[1]

References:

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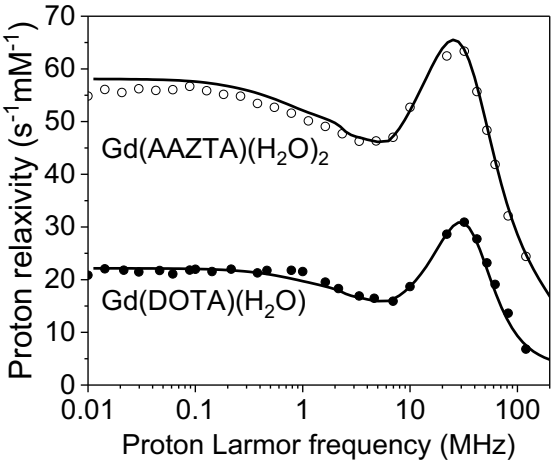

^1H relaxivity profiles for $[Gd(DOTA)(H_2O)]^-$ and $[Gd(AAZTA)(H_2O)_2]^-$ incorporated in chitosan/hyaluronate nanogels at 298 K.

Methods for Material and BioMaterials

The interest of composite materials having a matrix (organic or inorganic) and an enzyme component is considered a new paradigm in biotechnologies. The active part of these materials, at the protein-matrix interface intrinsically lacks a long-range order, and therefore is unaffordable by X-ray diffraction and, being solid, escapes solution NMR characterisation. These systems are ideally suited for a solid-state NMR-based characterisation, for which we have proposed a protocol.[1] An intriguing observation is related to the characterisation of a protein/inorganic oxide composite: it has long been demonstrated that polycationic molecules can promote the formation at physiological conditions of inorganic oxides, which would otherwise be formed under harsh conditions. The molecular processes underlying this behaviour is, however, still unclear. We have used X-day diffraction of a polycationic protein crystal soaked with the precursors of the inorganic oxides and obtained a conclusive structure-based evidence of the interaction of the protein with a mononuclear titanium(IV) species, in a region rich of positive charges, figure 1.[2]
New methodologies has been also introduced in the filed of liquid crystals investigation. A carefully performed NMR analysis on spectra acquired at ultra-high magnetic field clearly shows the by NMR is it possible to characterise the dynamics occurring in the phase transitions also monitoring the occurrence of magnetic field effects.[3]

With the complexity of new materials increasing steadily, the methods for their characterisation must be developed and improved. In this effort, new protocols are devised and unexpected observations may arise.

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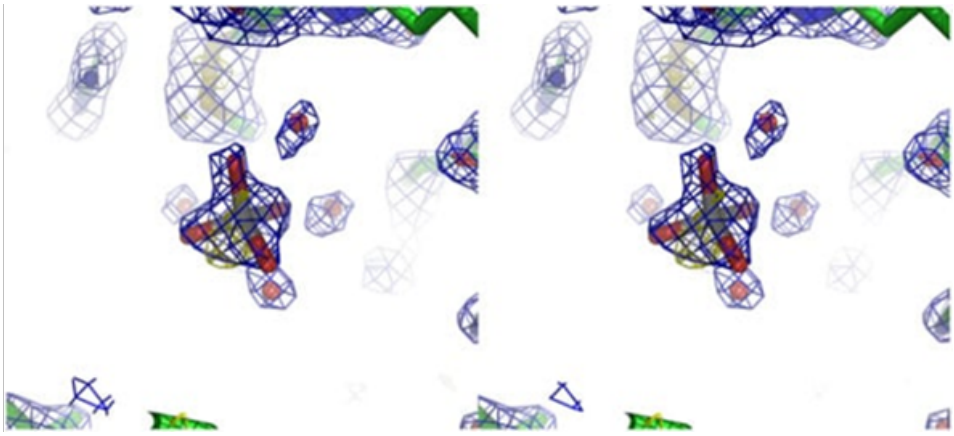

Cross-eye stereo view of a mononuclear titanium(VI) species interacting with lysozyme, a polycationic protein.

Materials, Solid-state NMR Methods and DNP

Dynamic Nuclear Polarisation (DNP), makes it possible to increase sensitivity in solid-state NMR by more than two orders of magnitude. We introduced new polarising agents with increased efficiency at high magnetic field. This technique, together with new processing strategies opens new ways in material characterisation.

Solid state NMR (ssNMR) is the method of choice for the characterisation of many solid chemicals , materials and even in complex biomolecules in the solid phase. The main limitation to ssNMR is sensitivity, which is why we devote our efforts to extend DNP methods to characterise material and biomolecules. This year, in collaboration with Prof.s L. Emsley, O. Ouari and Dr. A. Lesage, we actively developed new polarising agents, especially designed for bimolecular investigations. We introduced a strongly dipolarly coupled bi-nitroxides, dubbed TinyPols, that has excellent performances at high magnetic field (18.8 T) with enhancement close to 100.[1] If these biradicals are designed with conformationally open, solvent accessible bis nitroxides the enhancement improves further like in M-TinyPol and Open-HydroPol, that is actually the best performing biradical at 9.4 T ever before.[2] For non aqueous solvent HyTEK2 confirms to be the best polarising radical at high magnetic field, with enhancements up to 200 at 21.3 T (900 MHz) and 65 kHz MAS frequency.[3,4] In conventional solid-state NMR, the signal to noise ratio can be increased by alternative processing strategies. If the number of the spectral components is known *a priori*, we have demonstrated that multivariate curve resolution can be used to increase the sensitivity of 1H-^{29}Si heteronuclear correlation experiments.[5]

References:

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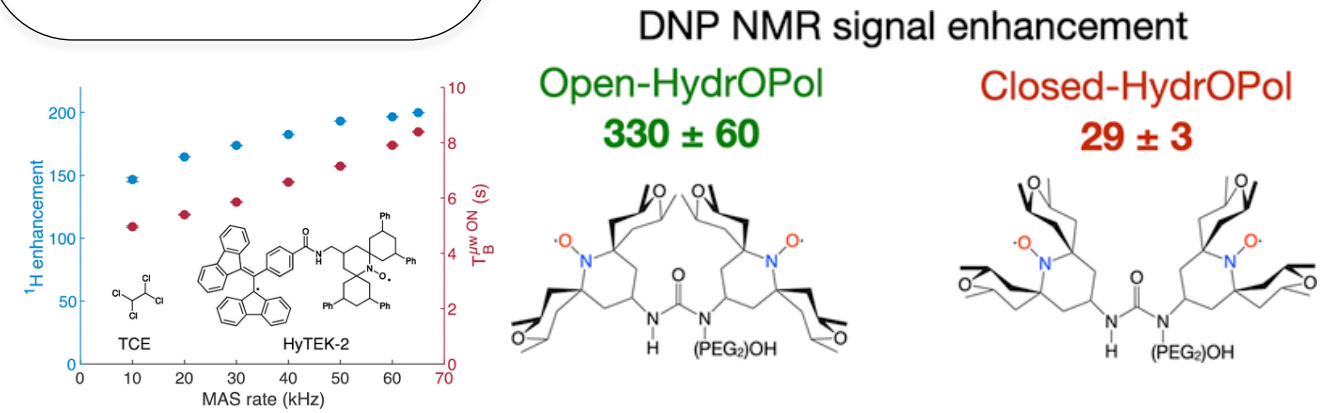
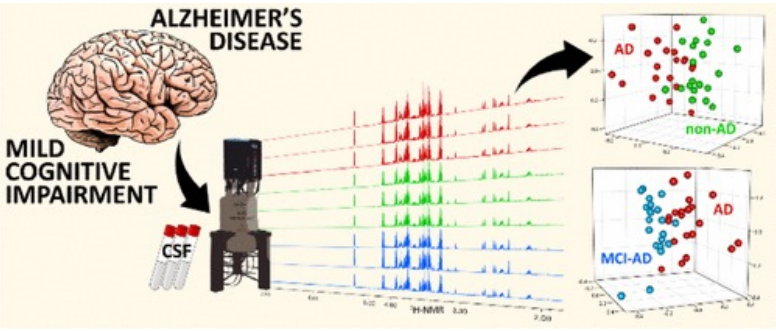


Left: DNP Enhancement as function of MAS frequency for HyTEK2 at 21.3 T up to 65 kHz. Right: stunning enhancement difference observed at 9.4 T for conformationally closed and open HydrOPol radicals. O-HydrOPol is actually the best performing polarising agent ever at 9.4 T.

Metabolomics in Biomedicine

Nuclear Magnetic Resonance (NMR) spectroscopy is a unique approach to provide a fast analysis of biological samples for metabolomic investigations [1]. We have successfully exploited the application of NMR-based metabolomics in different pathological contexts. For example, a clear signature of Down syndrome emerged from the comparison of sera and urine from diseased subjects and healthy controls [2]. Metabolomics is also particularly appropriate to develop prognostic models to characterise the risk of developing cardiovascular diseases [3,4]. Similarly, metabolomics find applications in the field of medical oncology [5]. We demonstrated that metabolomics can be applied to stratify breast cancer patients according to the risk of developing disease recurrence [6]. Urine and serum are the most employed human biofluids for metabolomic investigations because they are easy to be collected and rich of systemic information. However other more localized biofluids can be analysed, such as exhaled breath condensate (to study pulmonary diseases) [7], saliva (to investigate oral pathologies) [8] and cerebrospinal fluid (to study neurodegenerative diseases). We used the latter to extract a characteristic signature of Alzheimer's disease able to discriminate the pre-dementia from the dementia stage [9]. Pharmacometabolomics is also an exciting emerging field: using metabolomicst it is possible to select patients who are more likely responders to biological chemiotherapy before drug administration [10] or to predict adverse events in subjects treated with sildenafil [11]. In the same way we can assess the effects of dietary interventions with enriched foods [12] or with probiotics [13].



Cerebrospinal fluid metabolomics shows a signature characteristic of Alzheimer's disease patients (AD) with respect to pre-dementia (MCI-AD) and non-AD patients [9].

The contribution of NMR metabolomics to biomedicine includes the characterisation of the metabolic signature of diseases for diagnostic and prognostic purposes as well as for the definition of individual susceptibility to medical treatment, dietary intervention, and environmental factors.

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Other applications of metabolomics: from bacteria to animal models

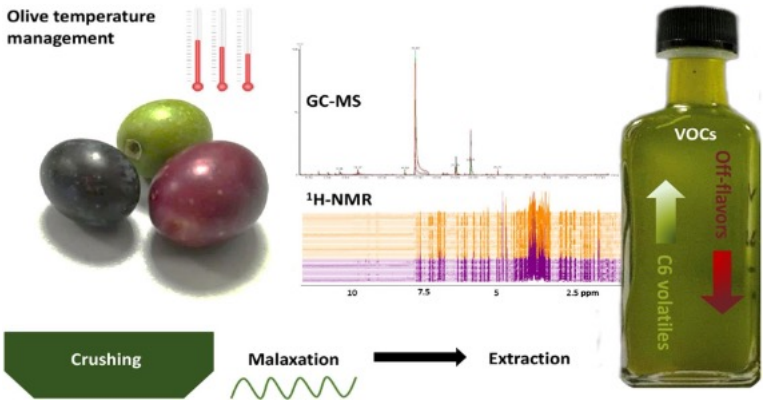
Metabolomics offers useful contributions to a comprehensive insight into the functional status of animals, plants, and cells. For this reason, metabolomics can be employed in several fields with applications spanning from the analysis of plants and foodstuff to veterinary sciences.

NMR-based metabolomics finds its application in various areas including, but not limited to bacterial and plant metabolism, food analysis, veterinary sciences.

The global regulation of the heterotrophic bacterium *Pseudoalteromonas haloplanktis*, when grown in a medium including multiple carbon sources, was studied by integrating a set of complementary -omics techniques with measured growth parameters. Time-resolved ^{1}H and ^{1}H-^{13}C NMR showed that the two main nutritional strategies (co-utilization and sequential uptake of multiple substrates) can coexist in the same growth experiment, leading to an efficient exploitation of the available carbon sources [1].

Agricultural practices and food processing have a great impact on the composition of resulting foods; thus, metabolomics has an emerging role in monitoring the influence of different manufacturing procedures on food quality [2,3].

NMR metabolomics has also a role in the study of animal diseases. We can report successful applications in the study of cow [4,5] and dog [6] heath. From the viewpoint of metabolomics, the use of animal models in interventional studies has the advantage of reducing the biological noise arising from inter-individual variations, and thus permitting the evaluation of, e.g., nutritional interventions using smaller groups of individuals with respect to large scale nutritional studies in humans [7].



Lowering the temperature of the olives before crushing modulates oil composition by reducing compounds associated with specific organoleptic defects of the oil [2].

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National and Transnational access

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

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

INSTRUCT-ERIC provides access to unique instrumentation in a variety of different structural techniques (see pages 9). This innovative approach allows for a description of biological cells at the molecular level, in order to understand how living organisms function in normal and pathological conditions and to design drugs and vaccines. The possibility of access to INSTRUCT-ERIC represents a unique opportunity for researchers, both at the national and European level, to strengthen the innovation capacity of the research performed. The request of access to Instruct-ERIC has exponentially increased since it became operational. The same trend is registered for the CERM/CIRMMP platform.

In 2020 has started the newly funded iNEXT-Discovery project (http://www.inext-eu.org). iNEXT-Discovery is a consortium funded by the HORIZON2020 program to offer European researchers access to a wide range of advanced structural biology technologies (including X-ray technologies, NMR spectroscopy, Electron Microscopy and biophysics), and aspires to promote biomedicine, biotechnology, and biomaterials, involving scientists with or without previous experience in structural biology.

Since 2016 has been operative the European access platform CORBEL. CORBEL is an initiative of eleven new biological and medical research infrastructures (BMS RIs), which includes INSTRUCT-ERIC, that together creates a platform for harmonised user access to biological and medical technologies, biological samples, and data services required by cutting-edge biomedical research.

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 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 the receiving infrastructure. 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 characterisation of biological systems. The description of the NMR instrumentation made available under the above mentioned access projects at CERM/CIRMMP is reported in the dedicate paragraph at page 38. Notably in 2020 we have installed the first world 1.2 GHz instrument operative since March, and its contribution to research is already visible in the research session.

Molecular biology and cellular biology labs are also strategic for the users needs to prepare and/or optimise the large variety of samples for structural characterisation, together with other biophysical equipment for EPR, CD, UV-vis, stopped-flow measurements, manual and automated crystallisation facilities and X-ray diffractometry. Users can also access other university infrastructures available in the campus, such as those of mass spectrometry, Raman resonance, and non-linear spectroscopies.

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

The Covid-19 pandemic restrictions strongly affected the access service provided in Europe. The CERM/CIRMMP infrastructure kept his service available as much as possible, also providing remote NMR services for users that could not visit the facility. Access provided during 2020 was reduced because the new project iNEXT-Discovery was ready for proposal submission only in late summer. As a consequence, the whole NMR access provision during 2020 was sensibly lower respect to previous years, with a total of 275 days. A more detailed analysis shows that the access related to Instruct-ERIC (Instruct-ERIC, Instruct-ITALIA and Corbel) was the great majority, with a total of 169 days, with only 17 days provided through iNEXT-Discovery.

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. In 2020, thanks to the freshly inaugurated centre CERM TT and the BIO-ENABLE project, the pay-for-services access to industries was overall 89 days. This number does not include the access provided industrial partners through collaborative projects.

We warmly thank the following companies for stimulating interactions:

Bracco SpA

Bruker BioSpin

Dompé Pharmaceutical

Italmatch Chemicals

Glaxo Smith Kline

Giotto Biotech Srl

Merck

COLLABORATION WITH INDUSTRIES

Menarini Srl

Valagro S.p.a.

Abiogen S.p.a.

Infineum

Danger and Safety

Buona Steve Jones

A special acknowledgment to Gruppo SAPIO Srl,

official supplier of all the cryogenic gases of CERM/CIRMMP

Flanking Institutions

Da Vinci European BioBank

The Da Vinci European Biobank (daVEB) is handled by CsaVRI (Centro Servizi Di Ateneo Per la Valorizzazione della Ricerca e la Gestione dell'Incubatore) and it is certified ISO9001:2015. It is a research biobank that stores human biospecimens (plasma, serum, urine, tissues, cells), and bacterial expression vectors at cryogenic temperatures (Mechanical freezers for storage at –80 °C, equipped with auxiliary LN_2 cooling system and tanks for cryopreservation in nitrogen vapour phase at –150 °C, with automatic nitrogen supply).

Thanks to the involvement of scientific and technical staff in the management of daVEB, CERM has established connections with the ESFRI European Biobank Infrastructure BBMRI, which are reinforced by the metabolomics research activities of CERM.

The interaction between daVEB and CERM is strategic and synergistic. Scientific collaborations in the metabolomic field contribute to the development of SOPs validated by NMR and to the enrichment of the biobank in terms of type and number of samples. daVEB currently houses a collection of unique samples (biofluids, tissues and DNA) of growing importance by number in the following areas: COVID-19, melanoma, rare skin diseases, diseases of the genital-urinary cancer, cardio-circulatory diseases, digestive diseases, breast cancer, non-Hodgkin's lymphoma, diseases associated with the ageing. On the other hand, the biobank acts as a support to the metabolomics research via NMR carried out at CERM by providing a storage service of samples and the associated data, following protocols in accordance with international standards.

The daVEB is a partner of the RISE project (Competence center-RISE Network infrastructure for industrial research and incubation for advanced services to innovative companies), coordinated by CSAVRI; PAR-FAS funding of Regione Toscana It operates as an infrastructure to support experimental development activities and provision of services, with open access to private companies.

https://www.unifi.it/vp-11370-da-vinci-european-biobank.html

Giotto Biotech Srl

Giotto Biotech S.r.l. is a SME founded in 2011 as a spin-off of CERM that aims at contributing to the biomedical sciences by providing enabling products and services, with a particular focus on complementary technologies in the field of NMR. Giotto Biotech provides a full range of compounds and custom manufacturing to supply research needs in the field of biomedical sciences, consulting and services. The company is active in various fields including protein pro-

duction and isotope labelling, organic synthesis, services for NMR, and information technology. The services include NMR metabolomics and statistical analysis.

In 2020 Giotto Biotech has been involved in several research projects funded at the European or National level (FLAG-ERA-ITFoC, Information Technology: Future of Cancer Treatment; ITN EC RNAct, Enabling proteins with RNA recognition motifs for synthetic biology and bio-analytics.; SENSOGM, Development of biophotonic sensors for environmental determination of GMOs, funded by the Tuscany Region; SATURNO, Scarti organici e Anidride carbonica Trasformati in carbURanti, fertilizzanti e prodotti chimici; applicazione concreta dell'ecoNOmia circolare, funded by Piemonte Region; RESPIRA - ROVER AND UAS FOR REMOTE AIR MONITORING, funded by ARTES 4.0 and MISE; NMR metabolomics: the Made in Italy revolution for food certification, Financed by Fondazione CR Firenze).

Giotto Biotech research activity is carried out in synergy with CERM scientists. As an outcome of this collaboration, in 2020 Giotto Biotech and CERM researchers co-authored seven scientific publications.

http://www.giottobiotech.com/

Fondazione Luigi Sacconi

The Luigi Sacconi Foundation 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.

Its aim is to promote scientific research in the molecular sciences at the local, national and international levels. Particular attention is addressed to chemistry, in its implications and applications concerning health, quality of life, the environment, energy, and technological and industrial development.

For this purpose the Luigi Sacconi Foundation collects documents and publications, promotes seminars, courses and meetings and other activities supporting the exchange of scientific knowledge, subsidises the activity of Italian and foreign researchers, and establishes awards.

The Sacconi Medal Lecturer 2020 has been awarded to Prof. Chi-Ming Che, Director of Laboratory for Synthetic Chemistry and Chemical Biology of the Hong Kong University. Because of the pandemic restriction the medal will be delivered on 2022.

http://www.cerm.unifi.it/fondazione

Instrumentation

Solution and Solid-State NMR Spectrometers

In 2020 the first 1.2 GHz NMR instrument, operating at 28.2 T has been installed at CERM. This instrument is operating with a solution TCI cryoprobe. All NMR instruments are state-of-the-art, digital spectrometers equipped with a variety of cryo-probes as well as of specific probes covering a broad range of frequencies and of observable nuclei. In addition to all the standard pulse sequences for spectroscopic, structural, dynamical, and functional characterisation, tailored pulse sequences for structural determination of high molecular weight proteins and paramagnetic systems are implemented, as well as ^{13}C direct-detection solution protocols for "protonless" NMR experiments and structural characterisation of biomolecules, including unfolded or partially unfolded ones. Pulse sequences and experiment setup have been implemented for the detection and characterisation of paramagnetic systems and in this field CERM has been pioneer since decades. For this reason we have now equipped a 400 MHz instrument with a special 3mm High Power probe designed for the investigation of paramagnetic systems. Solid-state MAS probes cover almost all the presently achievable MAS frequencies, from a few hundred of Hz to ultra-fast MAS regime, and since 2017 we have a new 0.7mm CP MAS probe spinning up to 111 kHz. Special protocols and devices are available for solid state experiments both for biological and inorganic material characterisation. Set-up and pulse sequences for ***in-cell*** NMR experiments are also implemented.

INSTRUMENTATION

B_0 Field (T)	^{1}H Larmor Frequency (Bore)	Probe heads
28.2	1200 MHz (NB 54 mm)	TCI Cryo 3 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) TXO Cryo 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) 0.7 mm CP MAS $^{1}H/^{13}C/^{15}N$ (soon available)
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)	TCI Cryo 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) TXI RT 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
20.0	850 MHz (WB 89 mm)	3.2 mm CP MAS DVT $^{15}N/^{13}C/^{1}H$ 1.3 mm CP MAS ^{1}H-^{19}F/BB/^{15}N 0.7 mm CP MAS $^{1}H/^{13}C/^{15}N$
18.8	800 MHz (NB 54 mm)	TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) QXI RT 5 mm solution($^{1}H/^{13}C/^{15}N/^{31}P$ with ^{2}H decoupling) ^{1}H-Selective High Power RT (prototype) 3.2 mm CP MAS DVT Low-E $^{15}N/^{13}C/^{1}H$ 1.3 mm CP MAS ^{1}H-^{19}F/BB-X/BB-Y 1.3 mm CP MAS $^{1}H/^{13}C/^{15}N$
16.4	700* MHz (NB 54 mm)	TCI Cryo 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
16.4	700 MHz (NB 54 mm)	TXO Cryo 5 mm solution($^{13}C/^{15}N/^{1}H$ with ^{2}H decoupling) TXO RT 5 mm solution($^{13}C/^{15}N/^{1}H$ with ^{2}H decoupling) TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
16.4	700 MHz (WB 89 mm)	3.2 mm CP MAS $^{15}N/^{13}C/^{1}H$ 4.0 mm CP MAS $^{15}N/^{13}C/^{1}H$
14.1	600 MHz (NB 54 mm)	2 x TXI RT 5 mm solution($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) HR-MAS 4.0mm ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling) ^{1}H - Selective High Power RT, 5 mm solution ^{1}H - Selective RT, 5 mm solution BBI RT 5 mm solution BBO RT 5 mm solution BBO RT 10 mm solution / BB RT -Low-γ -10 mm solution
14.1	600* MHz (NB 54 mm)	TXI RT 5 mm solution ($^{1}H/^{13}C/^{15}N$ with ^{2}H decoupling)
11.7	500 MHz (NB 54 mm)	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	X (9.43 GHz) Q-Band (35 GHz)	X and Q Band cavities
0.00024-1	Fast Field Cycling Relaxometer	0.01-45 MHz 10 mm solution tubes

*With sample changer

X-ray Crystallography

CERM/CIRMMP is equipped with standard crystallisation facilities and with an automated nano-dispensing device (Mosquito, TTP Labtech). Furthermore it has full access to the Inter-departmental Crystallography Centre of the University of Florence (CRIST) equipped, among other instruments, with a sealed-tube diffractometer bearing a CCD detector (AgilentTechnologies) for routine in-house data collections. Regular access to synchrotron beam time slots in Europe facilities is also possible.

Biological and Biophysical Facilities and Services

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 characterisation, can be achieved through the use of auxotrophic strains. Fully equipped facilities for protein purification are available, including last-generation instruments for streamlined purification (ÄKTA pure chromatography system) and equipment for protein purification and reconstitution in anaerobic environment (glove box). A mammalian expression lab for in-cell NMR is also available.

EPR

9.43 GHz (X-Band, continuous wave, Elexsys E 580E) and 35 GHz (Q-Band, pulsed, Elexsys E 580E) instrument.

Multi Angle/Dynamic Light Scattering

Instrument for measurements on batch samples or on in-flow samples (FPLC coupling).

Isothermal Calorimetry (ITC)

ITC device to measure thermodynamical parameters in micro-samples. The instrument is fully equipped for studying protein-ligand and protein-protein thermodynamical parameters.

Optical Spectroscopy

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

Computational Structural Biology Tools

CERM/CIRMMP provides integrated databases and software for genome browsing, metal binding analysis, structure calculation with/without paramagnetic restraints, sequence validation, domain organisation, evolution, protein complex analysis.

Access to programs for NMR data processing and structural calculations is also provided via web.

Electronic infrastructure (e-infrastructure)

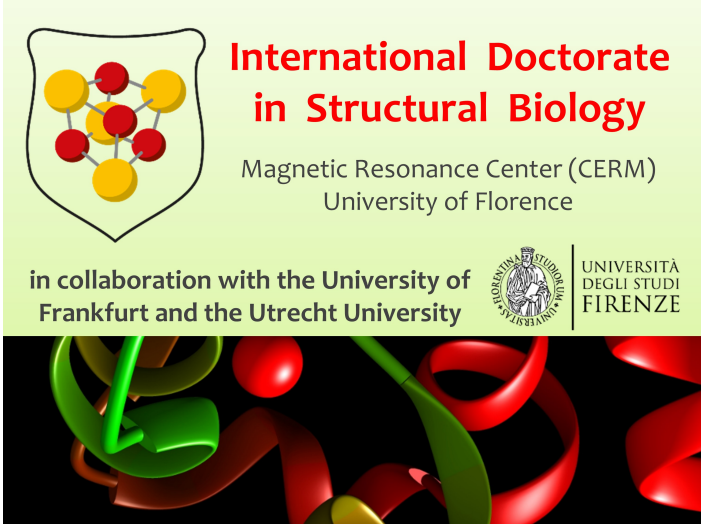
The grid and cloud-based services of CERM/CIRMMP are currently being provided via the WeNMR thematic services (https://www.eosc-hub.eu/services/WeNMR suite for Structural Biology) within the EOSC-Hub initiative. This leverages the success of the previously funded WeNMR e-Infrastructure and West-Life virtual research environment. The WeNMR thematic services provide application-level services specific to different cases in Structural Biology, with a main focus on NMR-based tools. Those services are supported thanks to the strong commitment of resource providers giving access to grid, cloud and data storage computing resources. This support has been formalised by a Service Level Agreement with the EGI Federation. The user community served by the WeNMR services encompasses over 12,000 registered users over the years from more than 95 different countries.

CERM/CIRMMP maintains a node of the European Grid Initiative. The available hardware comprises two clusters with 80 and 1024 CPU-cores respectively, and four TB of shared storage. A cluster with six Nvidia Tesla K20 GPGPU cards is also available.

Training & Education

International Doctorate in Structural Biology

The **International PhD course in Structural Biology** is a research doctorate of the *University of Florence*, 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 characterisation 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 utilised 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, the knowledge and development of research and technology, capable to consider multi-disciplinary, transnational cooperation and mobility as primary needs, and to evaluate collaborative projects as a requirement for high quality research. The doctoral program also relies on Faculty members who, in addition to scientists from CERM, include professors from other departments of the University of Florence and from the Universities of Frankfurt, Utrecht, Oxford and Lyon, all top places for Structural Biology.

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

Post-Doctorate

CERM/CIRMMP hosts a number of 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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1. Cerofolini L, Ravera E, Fragai M, Luchinat C, NMR of Immobilized Enzymes}, Immobilization of Enzymes and Cells, 363—383, 2020, Guisan, J.M.,Bolivar, J.M.,López-Gallego, F.,Rocha-Martín, J. Humana, New York, NY
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3. Banci L, Luchinat E, Protein folding, maturation and redox state, Serie: NEW DEVELOPMENTS IN NMR. In In-cell NMR Spectroscopy: From Molecular Sciences to Cell Biology, editors: Yutaka Ito, Volker Dötsch, Masahiro Shirakawa, RSC 2020
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Meetings and Events Organised by CERM

Seminars Held at CERM

Friday, January 24th at 5.00 pm **Prof. Alessandro Vannini** Human Technopole Foundation, Milano “Unveiling RNA Polymerase III (extra)transcriptional complexes: an integrated structural biology approach” CERM Conference Room

Tuesday, February 4th at 5.00 pm **Prof. Claudio Franceschi** Istituto delle Scienze Neurologiche di Bologna (AUSL-ISNB) “Inflammaging and age-related diseases” - CERM Conference Room

Wednesday, 14th October, 5:30 pm. **Prof. Daniela Rhodes** MRC Laboratory of Molecular Biology, Cambridge, UK NTU Institute of Structural Biology, Nanyang Technological University, Singapore "Telomere structure using cryo-EM and CLEM” Wednesday, 14th October, 5:30 CERM Conference Room (also online seminar)

Friday, 23rd October, 5:30 pm. **Prof. Rino Rappuoli** "Vaccines and monoclonals to regain our freedom” Conference Room at CERM, Sesto Fiorentino (also recorded online seminar)

Monday, 23rd November, 4.00 pm **Dr. Enrico Luchinat** iNext-Discovery webinar "Real-time ligand binding in human cells monitored by bioreactor-assisted in-cell NMR “ online seminar

Thursday, 26th November 2020, 4:00 p.m. **Prof. Roberta Pierattelli** Emerging Topics in Biomolecular Magnetic Resonance "Just flexible linkers? Un-structural biology by NMR“ online seminar

Group Meetings

17/01 **Denise Selegato** “Comparison of ensemble averaging methods in integrated structural biology”

27/01 **Matteo Cremonini** “Split inteins technology applied to NMR studies of multidomain proteins: new strategies”

07/02 **Cristina Licari** “META-MAGIC: ^{1}H-NMR-based metabolomics to predict poor outcomes in ischemic stroke treated with thrombolysis”

14/02 **Michele Invernici** “NOE-less protein structures determination and Glutathione Complexed Fe–S Centers”

MEETINGS & EVENTS

21/02 **Nihar Ranjan Prusty** “Proteins recruiting specific cytosolic Fe-S protein in human : Expression and Characterization”

15/05 **Letizia Pontoriero** "Zooming into the interaction of alpha synuclein with Calcium ions near physiological conditions: the elusive nature of the C-tail”

Anna Peréz i Ràfols "Expression on Multi-Domain RRMs for the characterization of protein dynamics by NMR”

12/06 **Sara Matteucci** "Production and Functional Characterization of CFD1 Involved in Iron-Sulfur Clusters Biogenesis”

Domenico Rizzo "Interaction of Alpha-Synuclein with Human Biofluids Components"

19/06 **Giovanni Saudino** " ISCA1: a late actor of 4Fe-4S cluster biosynthesis in the mitochondrial ISC assembly machinery"

26/06 **Marco Schiavina** "Driving Forces in IDPs: the Case of OPN's Compact State"

11/09 **Lucia Gigli** "Electrostatic nature of the interaction between lysozyme and inorganic oxides"

17/09 **Veronica Ghini** "Cell metabolomics- a tool to investigate the mechanism of action of Auranofin"

09/10 **Letizia Barbieri** "Drug screening in human cells by NMR"

30/10 **Gaia Meoni** "Metabolomics in the Time of Covid-19"

06/11 **Vincenzo Laveglia** "Automated Methods for the Detection and Analysis of Binding Sites in Proteins"

13/11 **Silvia Ciambellotti** "Ferritin-based nanocarriers for cancer photodynamic therapy"

27/11 **Alessia Vignoli** "COVID-19 Pandemic: what can metabolomics tell us?"

03/12 **Panagis Polykretis** "Tricky proteins, a revolutionary method & a subject for reflection"

11/12 **Valeria Putignano** "Development of bioinformatics resources for metalloprotein analysis”

Journal Clubs

20/11 Anna Perez I Raffle

22/10 Francesco Milanesi & Francesco Torricella

16/10 Francesca Di Cesare & Deborah Grifagni

02/10 Nivedita Nivedita

17/07 Dafne Suraci & Cristina Licari

10/07 Lucia Gigli & Letizia Pontoriero

29/05 Nihar Ranjan Prusty & Matteo Cremonini

22/05 Sara Matteucci & Domenico Rizzo

08/05 Marco Schiavina & Giovanni Saudino

FUNDING INSTITUTIONS

Acknowledgements

University of Florence

Tuscany Regional Government

European Commission

Italian Ministry of Education, University and Research

Ente Cassa di Risparmio Foundation

Italian National Research Council

Italian National Institute of Health

Fondazione Cariplo

American National Institutes of Health

Italian Association for Cancer Research

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