CERM
Centro Risonanze Magnetiche
FIRENZE

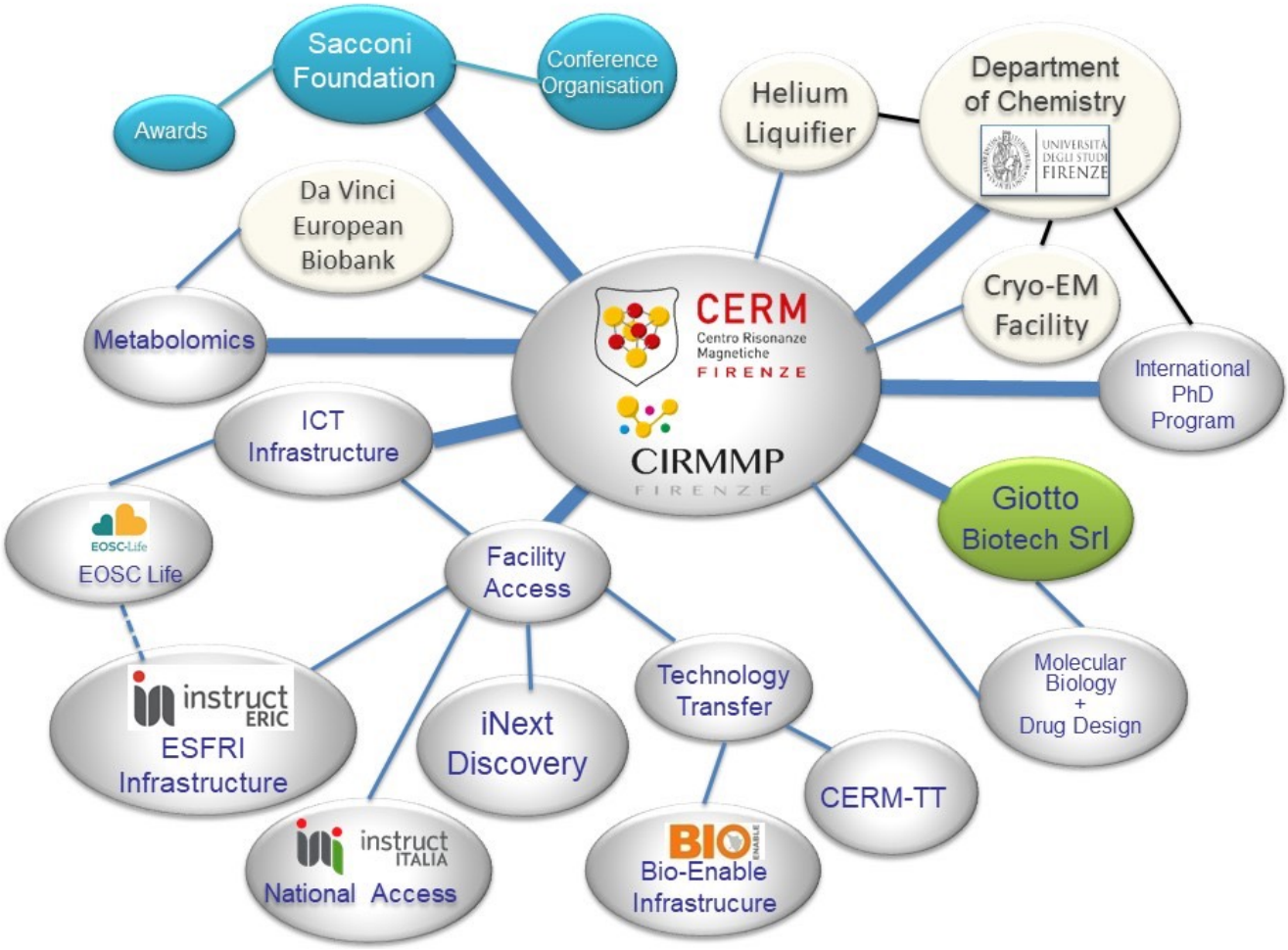
CIRMMP
FIRENZE

SCIENTIFIC ANNUAL REPORT

2021

UNIVERSITÀ DEGLI STUDI FIRENZE

Contents


Sacconi Foundation
Conference Organisation
Awards
Helium Liquifier
Department of Chemistry
UNIVERSITÀ DEGLI STUDI FIRENZE
Da Vinci European Biobank
CERM
Centro Risonanze Magnetiche
FIRENZE
CIRMMP
FIRENZE
Cryo-EM Facility
Metabolomics
International PhD Program
ICT Infrastructure
EOSC-Life
EOSC Life
Facility Access
Giotto Biotech Srl
instruct ERIC
ESFRI Infrastructure
iNext Discovery
Technology Transfer
Molecular Biology + Drug Design
CERM-TT
instruct ITALIA
National Access
BIO ENABLE
Bio-Enable Infrastrucure

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 (MUR), 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/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 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. 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 crystallisation. ***Instruct-ITALIA*** has started its activity in early 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 a European GRID-based platform, providing access to user-friendly platforms and CPU resources for a broad range of software 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 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 that are optimised for NMR sample production. A complete list of the instruments available at CERM/CIRMMP is reported at pag. 35. 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 2021 are:

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

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

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

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/ (01/01/2017-30/06/2021)

TRANSVAC2 - Improving and accellerating vaccine development in Europe

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

THE INFRASTRUCTURE

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

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

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

Research Activities

Introduction

During 2021, 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 node. In the following pages it can be appreciated how much the present research in CERM/CIRMMP is spanning a wide range of applications, from the structural biology to the bioinformatics methods and Information Technology, from paramagnetic NMR methods to the development of new contrast agents for MRI, from the metabolomics and biomedicine to the development of new solid-state NMR methods for the characterisation of material surfaces and biomaterials.

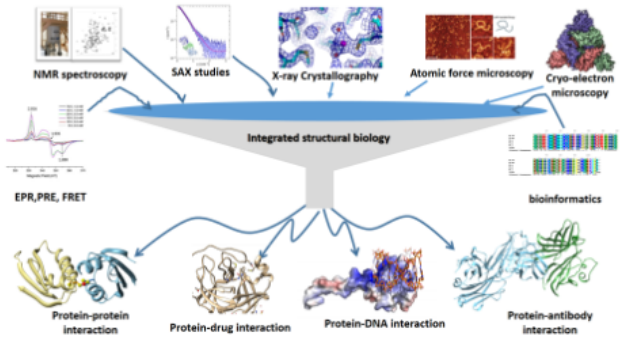
In line with our mission to develop NMR as a technique and to integrate NMR with other techniques, most of our publications were done in collaboration with other research groups (84% of the overall number of publications). During 2021 we published 80 papers in international peer-reviewed journals, more than 10% more publication with respect to last year. This is quite remarkable considering the limitations due to pandemic. The average impact factor slightly increased up to 5.68, which is relevant considering that also the number of publications also increased. A complete list of publications is available at page 43.

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.

RESEARCH ACTIVITIES

The Role of Solution NMR in Integrated Structural Biology

Nowadays solution NMR is an indispensable technology for determining structures of proteins and their interactions with other macromolecules, even when they are weak and transient. NMR is a unique technique for characterising small proteins and functional biological processes directly in solution and in living cells. In view of the complementarity and specificities of NMR, especially with respect to other techniques like Cryo-EM, the integration of solution NMR with other structural and experimental data, is profitable to understand how proteins, protein complexes or DNA-protein complexes dynamically interact with their functional environment. 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 structural 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, to rationally engineer an enzyme for a specific industrial process, to determine how to design novel drugs that target a particular protein, or to understand which molecular changes might improve them.[1-5]



Integrated structural biology to unravel biological processes.

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 coordinated 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.

References:

(1) Rathner. P.; Fahrner, M. *et al. Nat. Chem. Biol.,* **2021,** 17, 196.

(2) Romero, A.R.; Calderone, V. *et al. J. Comm. Biol.* **2021**, 4, 1111.

(3) Pirog, A.; Cantini, F. *et al. J Mol. Biol.* **2021,** 433, 167054.

(4) Wienk, H.; Banci, L. *et al. J. Vis. Exp.*, **2021**, 177, e63435.

(5) Duranti, C. *et al. Protein. Exp. Pur.,* **2021,** 184, 105879.

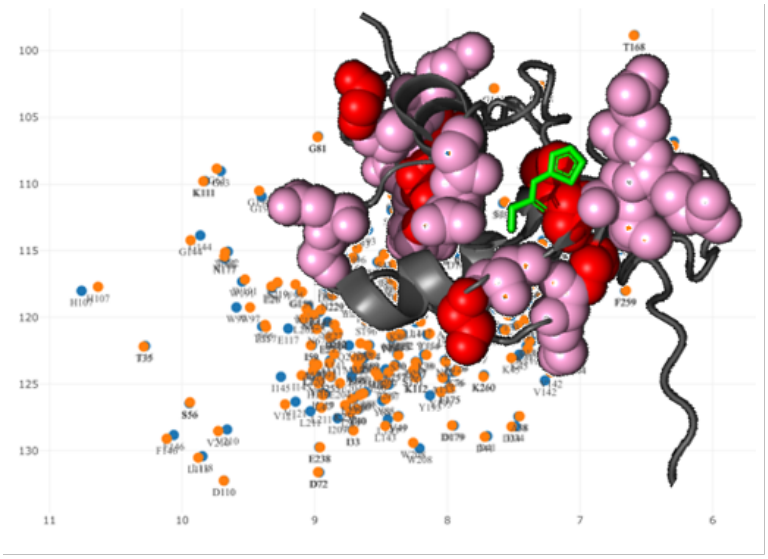
Computing for Integrative Structural Biology

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

A cornerstone for the development of innovative computational techniques in structural biology is the availability of extensive data repositories that integrate structural information in the PDB providing biological context for proteins, to which we contribute our expertise on metal sites.

In addition, we investigated the dynamic aspects of metal-protein interactions in transport systems, as well as in the interaction with cellular receptors.

Last but not least, our tools for automated data analysis and interpretation, from fragment screening to NMR-based structure refinement, have been available to the community via web servers also in the context of European collaborations.[1-5]



The PICASSO web server (https://picasso.cerm.unifi.it/) is a user-friendly tool that uses a pair of simple HSQC experiments to identify the ligand/fragment-binding region at the surface of a pharmacological protein target.

References:

(1) PDBe-KB consortium; Andreini, C.; Rosato, A.; Putignano, V. *et al. Nucleic Acids Res.* **2021,** 50, gkab988.

(2) Cafaro, A.; Andreini, C.; Banci, L. *et al. Int. J. Mol. Sci.* **2021,** 22, 317.

(3) Honorato, R.V.; Giachetti, A.; Rosato, A.; Bonvin, A.M.J.J. *Front. Mol. Biosci.* **2021,** 8, 729513.

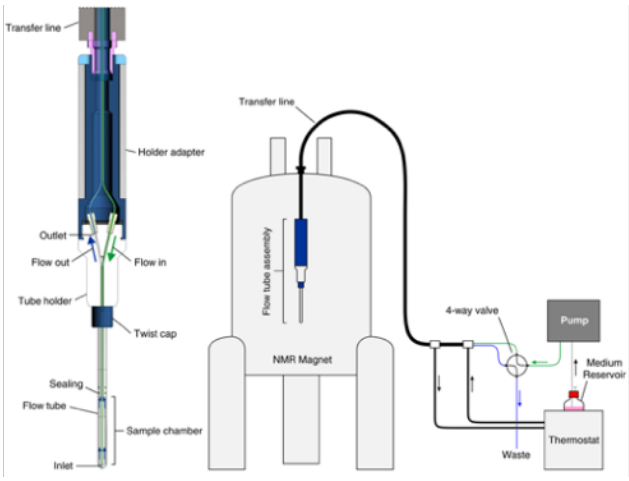
(4) La Veglia, V.; Giachetti, A.; Cerofolini, L.; Haubrich, K.; Fragai, M.; Ciulli, A.; Rosato A. *J. Chem. Inf. Model.* **2021,** 61, 5726-5733.

(5) Sala, D.; Giachetti, A.; Rosato, A. *J. Chem. Inf. Model.* **2021,** 61, 901-912.

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.
A detailed workflow to set up a modular flow-NMR bioreactor system previously developed was reported, together with a visualised protocol.[1] The bioreactor ensures high cell viability over prolonged periods of time by providing oxygen and fresh nutrients to hydrogel-embedded human cells in the NMR spectrometer.
The NMR bioreactor was applied to determine the intracellular binding affinity of inhibitors of carbonic anhydrase II. Competition binding with a reference compound allowed accurate measurement of dissociation constants in the nanomolar range.[2]
By using the protein expression approach in human cells previously developed at CERM, we assessed for the first time the performance of the 1.2 GHz NMR spectrometer when analysing ^{15}N-labeled proteins in living cells.[3]
When analysing unfolded proteins, such as α-synuclein, major improvements in terms of both resolution and sensitivity were observed, when compared to the same experiments recorded at 900 MHz and 950 MHz. TROSY-based experiments resulted in improved resolution also when analysing folded proteins, thereby overcoming the increased transverse spin relaxation that occurs at ultra-high fields.

CERM continues to pioneer the development of NMR methods to investigate proteins and small molecules in living human cells at atomic resolution.
Recent applications focused on the measurement of thermodynamic parameters of protein-ligand interaction in the cellular environment, and on the first example of protein in-cell NMR at the 1.2 GHz ultra-high field.



Scheme of the high-cell density bioreactor for in-cell NMR spectroscopy.

References:

(1) Barbieri, L.; Luchinat, E., *J. Vis. Exp.*, **2021**, 169, 62323.

(2) Luchinat, E.; Barbieri, L.; Cremonini, M.; Pennestri, M.; Nocentini, A.; Supuran, C.T.; Banci, L., *Acta Crystallogr. D Struct. Biol.*, **2021**, 77, 1270-1281.

(3) Luchinat, E.; Barbieri, L.; Cremonini, M.; Banci, L. *J. Biomol. NMR,* **2021**, 75, 97-107.

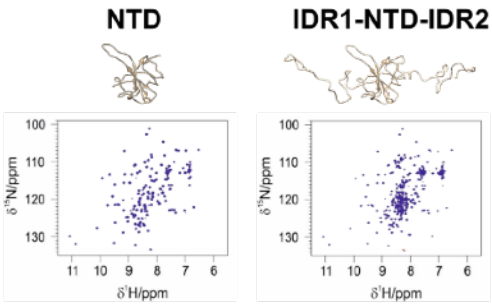
NMR Methods Fighting Covid-19 Pandemic

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

NMR has been successfully used with a double function: as a structural biology technique to characterise some SARS-CoV2 proteins and their druggability, as well as to define the metabolomic profile of coronavirus disease in human blood and the individual response to treatment. As far as the characterisation of viral proteins is concerned, we focused on two proteins: i) the nucleocapsid protein and ii) the main protease of the coronavirus (Mpro), which is one of the most interesting molecular targets for a pharmacological treatment of COVID-19.

In particular, we investigated by solution NMR the 1-248 construct of the nucleocapsid protein that comprises two disordered fragments (IDR1 and IDR2) in addition to the N-terminal globular domain (NTD). Concerning Mpro protein, by integrating X-ray crystallography and solution NMR, we discovered that zinc(II) inhibits the protease by binding at its active site. Our results may allow the design of potent inhibitors of SARS-CoV-2 Mpro.

NMR-based metabolomics and lipoproteomics, applied to the plasma of 30 patients hospitalized at the Florence University hospital during the first wave of COVID-19 in spring 2020, revealed an extremely strong signature of the disease. Upon treatment with Tocilizumab, a monoclonal antibody that interacts with the receptor of the cytokine interleukin-6, metabolites that are dysregulated in COVID-19 patients partially or completely revert towards the levels of control healthy subjects, whereas the lipoproteome components show a “slower recovery” towards healthy values.[1-4]



^{15}N HSQC spectra of NTD and IDR1-NTD-IDR2 constructs of nucleocaspid protein from SARS-CoV2.

References:

(1) Altincekic, N. *et al. Front. Mol. Biosci.* **2021,** 8, 653148.

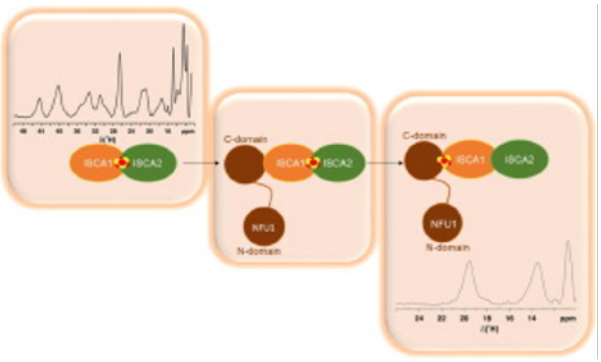
(2) Schiavina, M.; Pontoriero, L.; Uversky, V.N.; Felli, I.C.; Pierattelli, R. *Biomol. NMR Assign.,* **2021,** doi:10.1007/s12104-021-10009-8.

(3) Grifagni, D.; Calderone, V.; Giuntini, S.; Cantini, F.; Fragai. M.; Banci, L. *Chem. Commun.* **2021**, 7910-7913.

(4) Meoni, G.; Ghini, V.; Maggi, L.; Vignoli, A.; Mazzoni, A.; Salvati, L.; Capone, M.; Vanni, A.; Tenori, L.; Fontanari, P.; Lavorini, F.; Peris, A.; Bartoloni, A.; Liotta, F.; Cosmi, L.; Luchinat, C.; Annunziato, F.; Turano, P. *PLoS Pathog.* **2021,** 17, 1009243.

Molecular Mechanisms of Iron-Sulfur Protein Biogenesis in Humans

The mitochondrial Fe-S cluster assembly machinery is in charge of the maturation of mitochondrial [4Fe-4S] proteins. In humans, the last steps of the machinery, that involves the assembly and transfer of [4Fe-4S] cluster to mature mitochondrial [4Fe-4S] target proteins, is still not clearly identified. In 2021, we focused our attention on three proteins of the machinery, i.e. ISCA1, ISCA2 and NFU1. We present a NMR-based study showing a detailed molecular model of the succession of events performed in a coordinated manner by ISCA1, ISCA2 and NFU1 to make [4Fe-4S] clusters available to mitochondrial apoproteins. Our proposed mechanism guarantees that the [4Fe-4S] cluster can be safely moved from where it is assembled on the ISCA1-ISCA2 complex to NFU1, thereby resulting the [4Fe-4S] cluster available for the mitochondrial apoproteins specifically requiring NFU1 for their maturation. Within this frame, we have also investigated the molecular grounds of a rare pathogenic mutation of BOLA3 (Cys59Tyr), a protein also involved in the last step of the mitochondrial Fe-S cluster assembly machinery. These studies allowed us to rationalize the unique phenotype observed in the multiple mitochondrial dysfunctions syndrome-2 caused by Cys59Tyr mutation.[1-3]



[4Fe-4S] Cluster Transfer in the Maturation of Human Mitochondrial [4Fe-4S] proteins.

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:

1) Matteucci, S.; Camponeschi, F.; Clémancey, M.; Ciofi-Baffoni, S.; Blondin, G.; Banci, L. *Angew. Chem. Int. Ed. Engl.* **2021**, 60, 14841-14845.

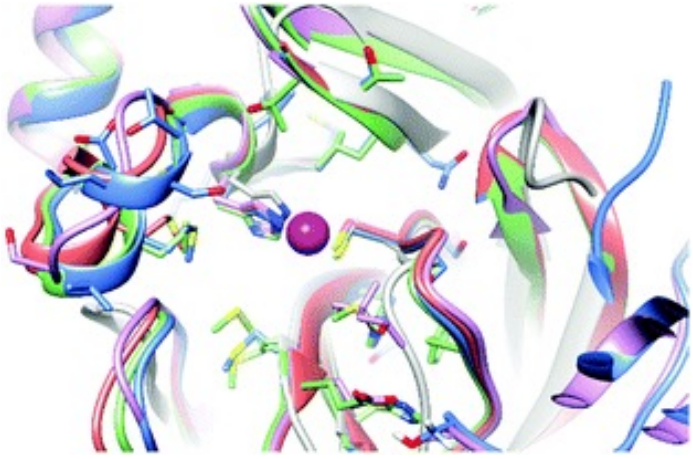
(2) Suraci, D.; Saudino, G.; Nasta, V.; Ciofi-Baffoni, S.; Banci, L. *J. Mol. Biol.* **2021**, 433, 166924.

(3) Saudino, G.; Suraci, D.; Nasta, V.; Ciofi-Baffoni, S.; Banci, L. *Int. J. Mol. Sci.* **2021**, 22, 4848.

Proteins as drugs and drug targets

SARS-CoV-2 main protease and PD-L1 are two important pharmaceutical targets. The elucidation of the structural basis of ionic zinc binding to the SARS-CoV-2 main protease and the NMR screening on PD-L1 protein have provided the way for the design of new potent and selective inhibitors of these two protein targets.

Structural data on the SARS-CoV-2 main protease in complex with a zinc-containing organic inhibitor are already present in the literature and gave hints on the presence of a zinc binding site involving the catalytically relevant cysteine and histidine residues. In this paper, the structural basis of ionic zinc binding to the SARS-CoV-2 main protease has been elucidated by X-ray crystallography. The zinc binding affinity and its ability to inhibit the SARS-CoV-2 main protease have been investigated. These findings provide solid ground for the design of potent and selective metal-conjugated inhibitors of the SARS-CoV-2 main protease. The inhibition of the PD-1/PD-L1 axis by small molecules is a promising strategy solid cancers. A series of 2,4,6-tri- and 2,4-disubstituted 1,3,5-triazines, have been designed, synthesised, and assayed for their PD-L1 binding by NMR and homogeneous time-resolved fluorescence. The most promising, compound demonstrated to strongly bind with the PD-L1 protein and challenged it in a co-culture of PD-L1 expressing cancer cells and peripheral blood mononuclear cells enhanced antitumor immune activity of the latter.[1-4]



Superposition of the zinc binding site in zinc bound SARS-CoV-2 Mpro (light blue), 2Z94 (red), 2ZU2 (pink), 7B83 (green), and 2ZTX (gray).

References:

(1) Grifagni, D.; Calderone, V.; Giuntini, S.; Cantini, F.; Fragai, M.; Banci, L. *Chem. Commun.,* **2021**, 57, 7910-7913.

2) Russomanno, P.; Assoni, G.; Amato, J.; D'Amore, V.M.; Scaglia, R.; Brancaccio, D. *et al. J. Med. Chem.*, **2021**, 64, 16020-16045.

3) Leone, G.; Pepi, S.; Consumi, M.; Lamponi S.; Fragai, M. *et al. Carbohydr. Polym.*, **2021**, 271, 118452.

(4) Daniele, S.; La Pietra, V.; Piccarducci, R.; Pietrobono, D.; Cavallini, C. *et al. Eur. J. Pharmacol.*, **2021**, 897, 173936.

Driving Iron Through the Ferritin Cage

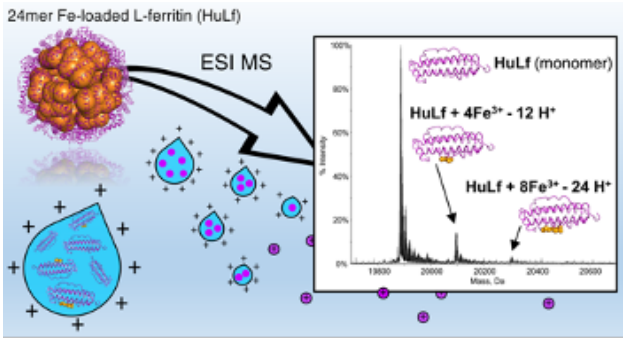
Since several years, and in collaboration with the group of Stefano Mangani in Siena, we have applied time-lapse X-ray crystallography to identify iron binding sites in eukaryotic ferritins. With the same approach we detailed the interaction between iron and human mitochondrial ferritin (hMFT).[1] hMFT is a natural homopolymer with ferroxidase activity. While providing the first structural evidence of iron ions binding to the ferroxidase site of hMTF, we identified transient iron binding sites occurring from the entry channel to the catalytic site. Differences in some accessory residues in the proximity of the catalytic site finely tune the iron path and modulate the reaction kinetics. A superoxide/peroxo-bound form at the di-iron center of the ferroxidase site might support the radical-scavenging role proposed for hMTF in mitochondria.
Additionally, human L-ferritin (HuLf) was loaded in solution with variable amounts of iron. ESI-MS was then successfully applied to detect tetra- and octa-iron clusters bound to L subunits, thus confirming the presence of such clusters - previously underscored by X-ray crystallography – also in solution samples.[2] This observation further supports the functional relevance of such species as precursors of the caged biomineral.

The characterisation of the iron binding sites in human ferritin has been further developed by applying time-lapse X-ray crystallography to mitochondrial ferritin and by developing an ESI-MS approach to observe inner-surface driven formation of iron biomineral precursors.

References:

(1) Massai, L.; Ciambellotti, S.; Cosottini, L.; Messori, L.; Turano, P.; Pratesi, A. *Dalton Trans.* **2021**, 50, 1646–16467.

(2) Ciambellotti, S.; Pratesi, A.; Tassone, G.; Turano, P.; Mangani, S.; Pozzi, C. *Chem. Eur. J.* **2021**, 27, 14690-14701.



Tetra- and octa-iron clusters bound to L-ferritin monomers are visible in the deconvoluted ESI mass spectrum of HuLf 10^{-6} M loaded with 350 Fe^{2+} ions.

Intrinsically Disordered Proteins

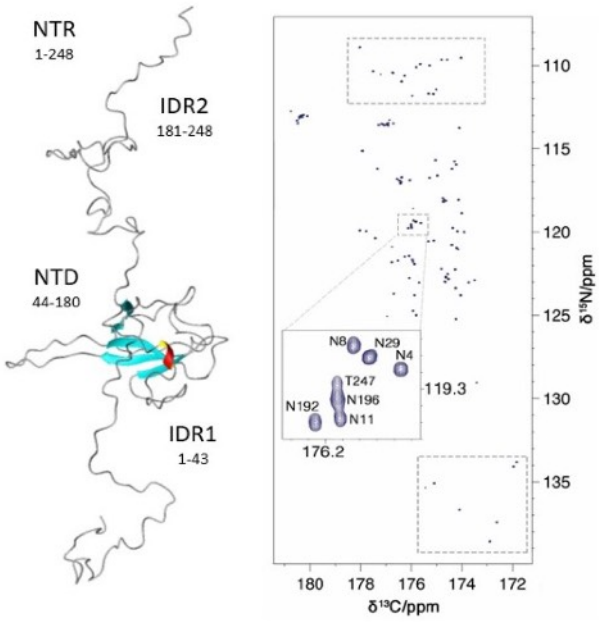
The intrinsically disordered regions of the nucleocapsid protein from SARS-CoV-2 were investigated within the N terminal construct (1-248). The suite of NMR experiments based on ^{13}C direct detection was crucial to achieve this objective. Novel experimental variants to focus on proline residues were developed.

The possibility to directly monitor correlations involving proline residues in clean regions of 2D CON spectra stimulated the design of a series of experiments to investigate the properties of these residues in intrinsically disordered proteins (IDPs) or protein regions (IDRs). A proline fingerprint can be easily obtained by exploiting their peculiar ^{15}N chemical shifts, providing the information that is missing in the widely used 2D HN spectra. This strategy was implemented in the triple resonance NMR experiments needed for sequence specific assignment (3D CBCACON, 3D CBCANCO, 3D COCON)[1], expanding the suite of NMR experiments based on carbonyl direct detection. The latter has become an established tool for the investigation of IDPs/IDRs and allowed us to perform the sequence specific assignment of the intrinsically disordered regions of the nucleocapsid protein (N) from SARS-CoV-2 within the N-terminal region (1-248)[2].

References:

(1) Felli, I.C.; Bermel, W.; Pierattelli, R. *Magn. Reson.* **2021**, 2, 511-522.

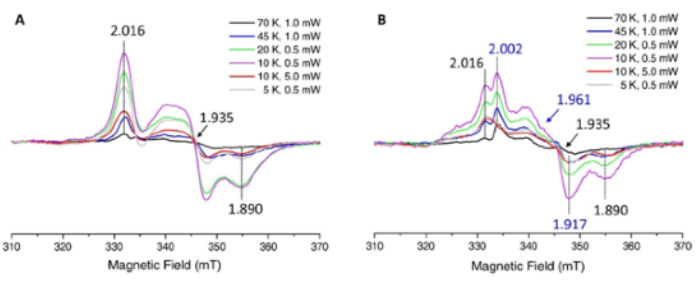
(2) Schiavina, M.; Pontoriero, L.; Uversky, V.N.; Felli, I.C.; Pierattelli, R.; *Biomol. NMR Assign.*, **2021**, 10.1007/s12104-012-10009-8.



The 2D CON spectrum of the N-terminal region (NTR, 1-248) of the nucleocapsid protein (N) from SARS-CoV-2 is shown next to a model of this construct (obtained combining the 3D structure of NTD with IDRs obtained through Flexible Meccano).

EPR and Pulsed EPR in Structural Biology

The powerful combination of EPR and Pulsed EPR spectroscopies gained a lot of interest due to its unique contributions to structural biology. 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). Indeed, the peculiar role of iron sulfur cluster proteins can be investigated using such method. During the last year, a work from Camponeschi *et al.*[1] investigated the properties of the human anamorsin, exploiting an interesting combination of EPR and Mössbauer spectroscopies to characterise the FeS cluster. Indeed, the molecular description achievable with pulsed EPR methods, such as DEER (Double Electron Electron Resonance), can be implemented to obtain information about highly disordered systems. Bonucci *et al.*[2] presented an efficient combination of various biophysical methods for the investigation of the intrinsically disordered nuclear chromatin protein NUPR1. In conclusion, the long distance distribution obtainable from DEER spectroscopy and the cw-EPR dynamical information give valuable information in the *in cell* context. In 2021, Torricella *et. al.*[3] introduced an additional method for protein delivery into living cells. In this paper, the authors show how a mild thermal treatment at 42 °C promotes the incorporation of exogenous protein inside the cytoplasmatic region of *E.coli* and *P.pastoris*. cw-EPR at room temperature was employed to retrieve dynamical information for the investigated proteins inside the intracellular environment.



cw-EPR characterization of the iron sulfur cluster present In A) M2-anamorsin B) WT-anamorsin. Adapted from ref. 1.

EPR and Pulsed EPR in combination either with site directed spin-labelling or native paramagnetic centres open the possibility to obtain information about paramagnetic systems. In addition to the structural biology tools, Pulse Dipolar Spectroscopy, such as Pulsed Electron Double Resonance (PELDOR), allows to measure long-range nanometric distance distributions (1.6-16 nm) between at least two paramagnetic centres.

References:

(1) Matteucci, S.; Camponeschi, F.; Clémancey, M.; Ciofi-Baffoni, S.; Blondin, G.; Banci, L. *Angew. Chem. Int. Ed.* **2021**, 60, 14841-14845.

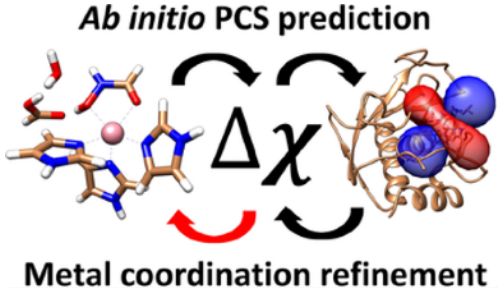
(2) Bonucci, A.; Palomino-Schätzlein, M.; Malo de Molina, P.; Arbe, A.; Pierattelli, R.; Rizzuti, B.; Iovanna, J.L.; Neira, J.L. *Front. Mol. Biosci.* **2021**, 8, 684622.

(3)Torricella, F.; Bonucci, A.; Polykretis, P.; Cencetti, F.; Banci, L. *Biochem. Biophys. Res. Commun.* **2021**, 570, 82-88.

NMR of Paramagnetic Systems

NMR of paramagnetic molecules is witnessing a renaissance, which is linked to the versatility of the paramagnetic effects for structural applications, and sustained by the improvements in instrumentation and in theoretical methods.

In the last few years, we have started exploring the possibility of calculating paramagnetic NMR observables from Quantum Chemical (QC) calculations. In collaboration with the group of Frank Neese (MPI Muelheim), we had demonstrated that the correct treatment of the complete Spin Hamiltonian is necessary to achieve a physical sense in the calculations. Now, still in collaboration with the Neese group, we have progressed to testing the software implementation of the complete theory starting from a relatively simple model. In this way, we were able to correct the assignments achieved in the late '60s, which would not have been possible without the use of QC calculations.[1] We then demonstrated that it is possible to refine the structure of the active site of a paramagnetic metalloprotein using long-range pseudocontact shifts and QC calculations of the magnetic susceptibility tensor.[2] This opens new possibilities in molecular magnetism,[3] but also to the characterization of metalloproteins, which bind naturally or not naturally paramagnetic metal ions (including lanthanoids).[4] Finally, with the availability of high fields, it is becoming increasingly complex to achieve spectra that can be phased over their whole spectral window, because of the finite bandwidth of the pulses and of the presence of a dead time over which magnetization evolves. We have proven that a statistical analysis of the data can be used to obtain phase-distortion-free spectra over spectral windows exceeding 2000 ppm, even in high field.[5]



Ab initio calculations can be used to link the geometry around the metal center to the long-range paramagnetic NMR observables, which can be recovered outside the "blind sphere".

References:

(1) Ravera, E.; Gigli, L.; Suturina, E.A.; Calderone, V.; Fragai, M.; Parigi, G.; Luchinat, C. *Angew. Chem. Int. Ed.* **2021**, 60, 14960-14966.

(2) Ravera, E.; Gigli, L.; Czarniecki, B.; Lang. L.; Kuemmerle, R.; Parigi, G.; Piccioli, M.; Neese, F.; Luchinat, C. *Inorg. Chem.* **2021**, 60, 2068–2075.

(3) Gigli, L.; Di Grande, S.; Ravera, E.; Parigi, G.; Luchinat, C. *Magnetochemistry* **2021**, *7*, 96.

(4) Ravera, E.; Cerofolini, L.; Fragai, M.; Parigi, G.; Luchinat, C. *Methods in Enzymol.* **2021**, 651, 103-137.

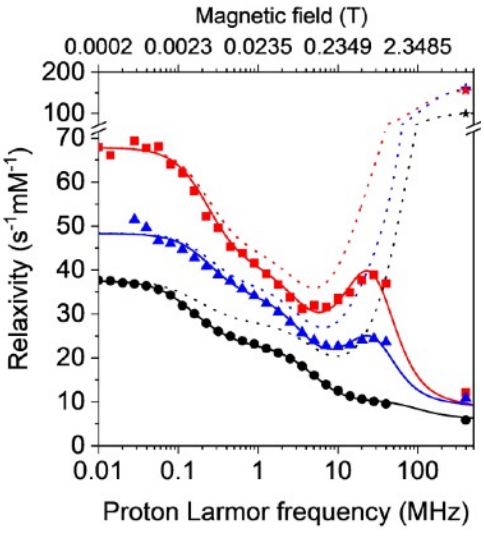
(5) Ravera, E. *J. Magn. Res. Open.* **2021**, 100022.

Fast-Field Cycling Relaxometry

High-resolution NMR relaxometry combines the analytical power of high-resolution NMR with relaxometry, exploiting the stray field of a commercial NMR magnet as variable field. From the field dependence of proton relaxation rates of metabolites in human blood serum, we demonstrated that weak interactions of metabolites with proteins can be identified.[1]

Fast field-cycling relaxometry can used for characterizing dynamics present in proteins in different conditions,[2] and the influence of electron relaxation on the nuclear relaxivity of paramagnetic nanoparticles.[3]

The relaxometry profiles of natural oral contrast agents like pineapple and blueberry juices were characterized in order to understand the origin of the increase in relaxation rates in reference to their content of manganese(II) ions.[4,5] The presence of alginate slows down reorientation, with a subsequent increase in the relaxation rates. These fruit juices have the advantages of better taste, tolerability, and lower price with respect to the artificial agents.



^{1}H longitudinal relaxivity profiles and transverse relaxivity at 400 MHz (stars) of Mn^{2+} ions in pineapple juice without (black) and with alginate (5%: blue; 15%: red) at 25 °C.

Field cycling relaxometry can provide access to the structural and dynamic parameters on which nuclear relaxation depends, and it represents a precious tool for the optimization of contrast agents for MRI and for investigating dynamic processing in nanoparticles and electron relaxation of paramagnetic systems.

References:

(1) Wang, Z.; Pisano, S.; Ghini, V.; Kaderávek, P.; Zachrdla, M.; Pelupessy, P.; Kazmierczak, M.; Marquardsen, T.; Tyburn, J.M.; Bouvignies, G.; Parigi, G.; Luchinat, C.; Ferrage, F. *JACS* **2021**, 143, 9393–9404.

(2) Parigi, G.; Ravera, E.; Fragai, M.; Luchinat, C. *Prog. NMR Spectrosc.* **2021**, 124, 85-98.

(3) Yon, M.; Gineste, S.; Parigi, G.; Lonetti, B.; Gibot, L.; Talham, D.; Marty, J.D.; Mingotaud, C. *ACS Appl. Nano Mater.* **2021**, 4, 4974-4982.

(4) Rizzo, D.; Ravera, E.; Fragai, M.; Parigi, G.; Luchinat, C. *Bioinorg. Chem. Appl.* **2021**, 6666018.

(5) Licciardi, G.; Rizzo, D.; Ravera, E.; Fragai, M.; Parigi, G.; Luchinat, C. *NMR Biomed.* **2021**, e4623.

Solid-state NMR methods and DNP for Materials and BioMaterials

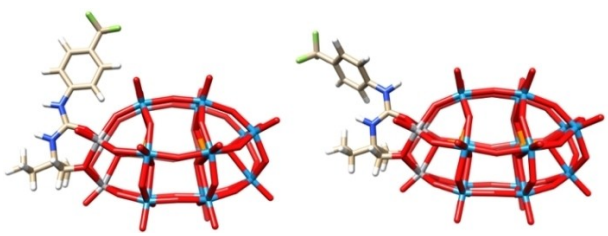
Solid state NMR (ssNMR) is the method of choice for the characterisation of many solid chemicals, materials and even in complex biomolecules in the solid phase. This technique is also combined with Dynamic Nuclear Polarisation (DNP) that provides sensitivity enhancements up to 2 orders of magnitude over a large variety of samples.

With ssNMR we have examined the conformational and dynamical changes that ubiquitin undergoes when it is physisorbed to hydrophilic (MCM41) or hydrophobic (SBA15) porous silica, as well as nascent bioinspired silica. In all three cases the stable globular fold of the protein is unaffected. Adsorption on the more hydrophilic MCM41 results in smaller perturbation of secondary structure with respect to adsorption on the more hydrophobic SBA15.[1]

Luckarift *et al.* brilliantly demonstrated that HEWL, which is a small polycationic protein, can promote the polycondensation of silica from solutions of tetraoxosilicic(IV) acid and titania. We have found that a hydrolysed species of the titanium precursor is located at a positive patch on the protein surface.[2]

We applied NMR and ssNMR also in the characterisation of organic and inorganic materials such as functionalised Polyoxometalates.[3,4]

The main limitation to ssNMR is sensitivity, which is why we devote our efforts to extend DNP methods to characterise materials and biomolecules. DNP is hampered by the need of working at cryogenic temperature (~100 K) to achieve high enhancements. We demonstrated in two publications that, if the sample is embedded into a sufficiently rigid matrix, it is possible to achieve high DNP enhancements also at temperatures above 230 K or close to room temperature, also high magnetic field.[5,6]



Structures for *trans–trans* (left) and *cis–trans* (right) conformations of urea@POM (ref. 4).

References:

(1) Adiram-Filiba, N. *et al. Biomacromolecules* **2021,** *doi.org/10.1021/acs.biomac.*

(2) Gigli, L. *et al. Biomolecules,* **2021**, 11, 43.

(3) Francesconi, O. *et al. Chem. Eur. J.* **2021**, 27, 10456-10460.

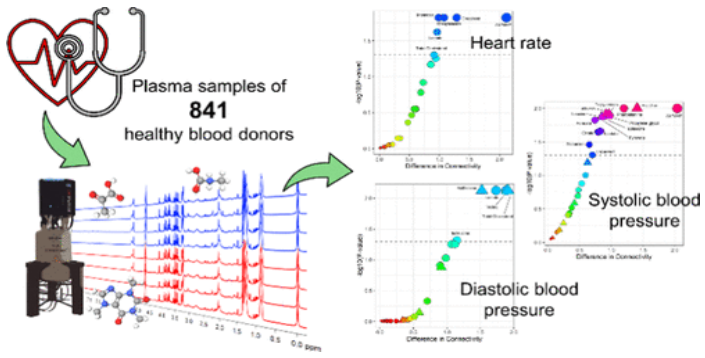
(4) Vilona, D.; Lelli, M.; Dumont, E.; Lacôte, E. *Chem. Eur. J.* **2021**, 27, 17761

(5) Hope, M.A. *et al. J. Phys. Chem. C* ***2021****,* 125, 18799−18809

(6) Menzildjian, G. *et al. J. Phys. Chem. B.* **2021**, 125, 13329-13338.

Metabolomics in Biomedicine

We explored the metabolic effects of age and sex to highlight metabolic patterns in a cohort of nonagenarian subjects[1] and to undeline the age-related changes of metabolites and lipoproteins in elderly.[2,3] In another study in elderly, NMR was employed to discriminate between early and metastatic colorectal cancer.[4] Because advanced age is the major risk factor for idiopathic Parkinson's disease (PD), a H2020 Europen project was set-up to characterize the contribution of the ageing process to PD development.[5] Breast cancer (BC) remains one of our main topics:[6,7] we designed a study to evaluate possible associations between the pre-diagnostic metabolomic profile and the risk of BC in high versus low mammographic density women.[8] Further, we explored the alterations in circulating lipoproteins in HER2-positive patients.[9] Colorectal cancer[10,11] and prostate cancer[12] were also investigated. Network analysis was applied to explore molecular pathways associated with blood pressure and heart rate in healthy subjects[13] and to study the molecular mechanisms associated with 3-month mortality in patients with acute ischemic stroke.[14]
We also addressed the extent to which samples of different cohorts were suitable to be used together for NMR metabolomics studies and whether data integration of studies performed on such samples was feasible and reliable.[15]



Subjects with subclinical high blood pressure and heart rate could present latent cardiometabolic dysregulations.[10]

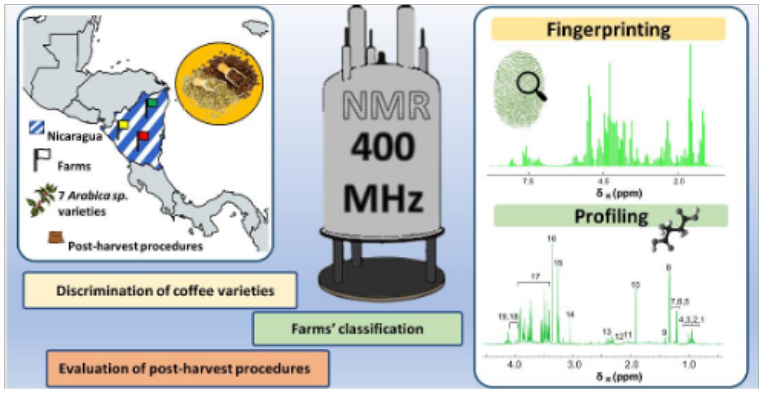
Metabolomics offers a molecular description of the health status of an individual and thus it has the potential to become the election technique to obtain new insights on the biochemistry of diseases and of the susceptibility to develop diseases.

References:
(1) Meoni, G. *et al. Geroscience* **2021**, 44, 1109.
(2) Di Cesare, F. *et al. J. Gerontol. A Biol. Sci. Med. Sci.* **2021**, 77, 918.
(3) Balder, Y. *et al. Metabolites* **2021**, 11, 326.
(4) Di Donato, S. *et al. Cancers*, **2021**, 13, 2762.
(5) Meoni, G. *et al. Mech. Ageing Dev.* **2021**, 194, 111426.
(6) Vignoli, A. *et al. IJMS* **2021**, 27, 6430.
(7) Tsopra, R. *et al. BMC Med. Inform. Decis. Mak.* **2021**, *274*.
(8) Bendinelli, B. *et al. Sci. Rep.* **2021**, 11, 13025.
(9) Corona, G. *et al. Cancers* **2021**, 13, 5845.
(10) Nannini, G. *et al. World J. Gastroenterol.* **2021**, 14, 27.
(11) Vignoli, A. *et al. Appl. Sci.* **2021**, 11, 11120.
(12) Cacciatore, S. *et al. Cancer Metab.* **2021**, 9, 29.
(13) Vignoli, A. *et al. J. Proteome Res.* **2021**, 20, 1040.
(14) Licari, C. *et al. J. Proteome Res.* **2021**, 20, 4758.
(15) Ghini, V. *et al. Clin. Chem.* **2021**, 67, 1163.

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.

Cell models are particularly useful in metabolomic studies, because they are tightly controlled systems with small metabolic noise. We have exploited NMR-based metabolomics to monitor the changes in the metabolic profile of A2780 ovarian cancer cells elicited by auranofin, a clinically approved gold drug now repurposed as an anticancer agent.[1] Skeletal muscle atrophy in cellular models was also investigated.[2] Metabolomics was also applied to design optimized protein production of the human cyclin-dependent kinase like 5, combining experimental data on protein production with metabolic modelling.[3] Bacteria play a pivotal role on modulating periodontal disease. Gingival crevicular fluid contains many bacteria-derived metabolites and can be used to find site-specific biomarkers of disease activity.[4] NMR metabolomics has also a role in the study of animal diseases: NMR spectroscopy was applied to investigate the association between milk metabolome and udder quarter health status in dairy cows.[5] Food processing has a great impact on the metabolic composition of foods. This aspect was investigated by phenotyping green and roasted beans of Nicaraguan coffee Arabica varieties processed with different post-harvest practices.[6]



We evaluated how different coffee varieties react to different post-harvest procedures such as fermentation time, type of drying and roasting.[5]

References:

(1) Ghini, V. et al. *Dalton Trans.* **2021**, 50, 6349.

(2) Bernacchioni, C. et al. *Int. J. Mol. Sci.* **2021**, 22, 1280.

(3) Fondi, M. et al. *Metabolites* **2021**, 11, 491.

(4) Baima, G. et al. *J. Periodontal Res.* **2021**, 56, 663.

(5) Bobbo, T. et al. *J. Dairy Sci.* **2021**, 105, 535.

(6) Meoni, G. et al. *App. Sci.* **2021**, 1, 24.

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 2021 the newly funded PANACEA project (https://panacea-nmr.eu/) has started. PANACEA is a consortium 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 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 33. 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 equipments 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.

Thanks to the implementation of the "mail in" access modality that foresees sample shipment, the access provision during 2021 has been in line with the pre-pandemic data, with 522 days of access to the NMR spectrometers. A more detailed analysis shows that 403 days NMR access were provided to academic users via Instruct-ERIC, Instruct-ITALIA and iNEXT-Discovery, and 119 days to industry users, either as services or through formal collaborations. Beside NMR access provision, the infrastructure provided access to protein production services via Instruct-ERIC and to other structural biology techniques via Instruct-ITALIA.

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

Collaborations with Industries

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

Menarini Srl

COLLABORATION WITH INDUSTRIES

Valagro S.p.a.

Abiogen S.p.a.

Infineum

Danger and Safety

Buona Steve Jones

INOTREM, control innate immunity

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 2021 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; ITN GLYTUNES, A multidisciplinary training network for the bioinspired development of glycomimetics tuning the Siglec-Sialoglycan axis; 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 2021 Giotto Biotech and CERM researchers co-authored five 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, 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 Interdepartmental 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.

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

A mammalian expression lab for in-cell NMR is also equipped with modern instrumentation.

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 infor-

mation 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)

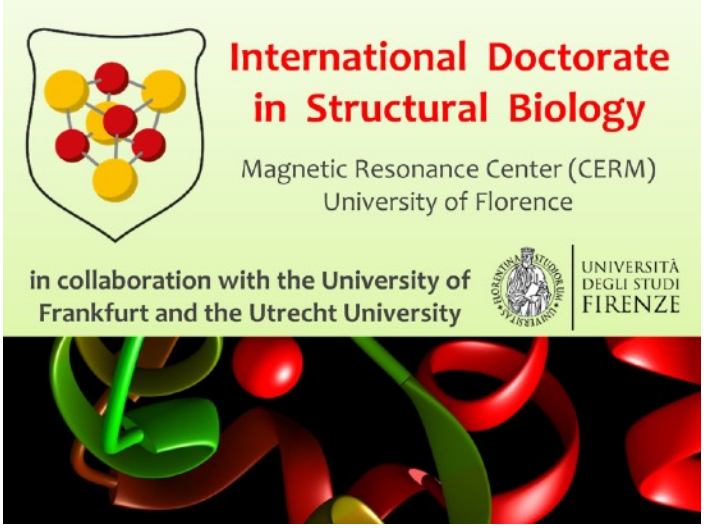
The grid and cloud-based services of CERM/CIRMMP are currently being provided via the WeNMR thematic services (https://www.eosc-hub.eu/services/WeNMR suite for Structural Biology) within the EOSC-Hub initiative. This leverages the success of the previously funded WeNMR e-Infrastructure and West-Life 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 characterisa-

tion 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, 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.

CERM/CIRMMP Organisation

SCIENTIFIC BOARD OF CERM

Prof. Lucia Banci (Director)
Prof. Claudia Andreini
Prof. Marco Fragai
Prof. Moreno Lelli
Prof. Paola Turano
Prof. Antonio Rosato

CIRMMP BOARD OF DIRECTORS

University of Florence
Prof. Claudio Luchinat (President)
Prof. Lucia Banci

University of Bologna
Prof. Stefano Ciurli
Prof. Francesco Capozzi

University of Siena
Prof. Daniela Valensin
Prof. Stefano Mangani

FACULTY MEMBERS

Prof. Claudia Andreini
Prof. Lucia Banci
Dr. Vito Calderone
Prof. Francesca Cantini
Prof. Simone Ciofi-Baffoni
Prof. Isabella Felli
Prof. Marco Fragai
Prof. Moreno Lelli
Prof. Claudio Luchinat
Prof. Giacomo Parigi
Prof. Mario Piccioli
Prof. Roberta Pierattelli
Dr. Enrico Ravera
Prof. Antonio Rosato
Dr. Leonardo Tenori
Prof. Paola Turano

CIRMMP SCIENTIFIC BOARD

Prof. Lucia Banci

University of Siena
Prof. Stefano Mangani

University of Bologna
Prof. Francesco Capozzi
Prof. Stefano Ciurli

ASSOCIATE FACULTY MEMBERS

University of Florence
Prof. Alessandro Mordini
Prof. Cristina Nativi
University of Siena
Prof. Stefano Mangani
University of Bologna
Dr. Elena Babini
Prof. Francesco Capozzi
Prof. Stefano Ciurli
Dr. Barbara Zambelli

CERM/CIRMMP ORGANISATION

Personnel

POST DOCTORAL FELLOWS

Argyriou Aikaterini

Letizia Barbieri

Francesca Camponeschi

Linda Cerofolini

Silvia Ciambellotti

Matteo Cremonini

Federico De Biasi

Veronica Ghini

Andrea Giachetti

Lucia Gigli

Tatiana Kozyreva

Vincenzo Laveglia

Enrico Luchinat

Tommaso Martelli

Gaia Meoni

Josè Pedro Malanho da Silva

Alessia Vignoli

PhD STUDENTS

Milana Bazayeva

Francesca Di Cesare

Deborah Grifagni

Angelica Ciarlone

Giulia Licciardi

Sara Matteucci

Francesco Milanesi

Anna Perez I Rafols

Bach Tung Lan Pham

Letizia Pontoriero

Domenico Rizzo

Maria Salobehaj

Giovanni Saudino

Marco Schiavina

Francesco Torricella

Valentina Vitali

UNDERGRADUATE STUDENTS

Sabrina Antonacci

Sofia Baldi

Tommaso Bayon

Rainer Bega

Chiara Biagioni

Niccolò Bianciardi

Lisa Boanini

Giulia Boso

Lorenzo Bracaglia

Francesco Bruno

Eleonora Ciuffi

CERM/CIRMMP ORGANISATION

Naomi Anna Consoli
Lucrezia Cosottini
Giuditta Dal Cortino
Michela Dal Pozzolo
Alessia De Santis
Maria Lidia Donnini
Lavinia Feliciangeli
Letizia Fiorucci
Leonardo Galeotti
Greta Giusti
Eleonora Guadagno
Dimitri Khodin
Claudio Mangano
Francesco Massaini
Beatrice Milazzo
Lorenzo Niccoli
Claudia Patiti
Valentina Pecchioli
Marta Ridolfi
Francesca Rossi
Enea Sacchi
Francesca Sacco
Aurora Silvestri
Davide Silvestri
Bianca Susini
Giuseppe Tagliaferro
Giulio Tassini

PROJECT MANAGER

Francesca Morelli

TECHNICIANS

Marco Allegrozzi
Fabio Calogiuri
Rebecca Del Conte
Leonardo Gonnelli
Massimo Lucci
Cristina Mescalchin
Enrico Morelli

ADMINISTRATIVE SUPPORT

Isabella Barbaro
Francesca Di Gloria
Milena Moazzi
Laura Norfini
Lisa Orlando

GIOTTO SCIENTISTS

Mercia Ferreira de Sousa
Tommaso Martelli

Visiting Scientists at CERM

Dr. Edoardo Saccenti - Assistant Professor

Wageningen University, Netherlands

Cristina Di Carluccio - PhD Student

University of Naples Federico II, Italy

Simone Fjordside - PhD Student

Aarhus University and Novo Nordisk, Denmark

Joel Roca - PhD Student

Universiteit Brussel, Belgium

Pasquale Russomanno - PhD Student

University of Naples Federico II, Italy

Luca Sperotto - PhD Student

Helmholtz Zentrum München, Germany

Juli Buhl - Erasmus Undergraduate

Aarhus University, Denmark

List of publications

1. PDBe-KB consortium: Rosato, A.; Putignano, V. *et al.* PDBe-KB: collaboratively defining the biological context of structural data. *Nucleic Acids Res.,* **2021***,* 2021,10gkab988. doi: 10.1093/nar/gkab988. Epub ahead of print (IF 16.971).

2. Wang, Z.; Pisano, S.; Ghini, V. Kadeřávek, P.; Zachrdla, M.; Pelupessy, P.; Kazmierczak, M.; Marquardsen, T.; Tyburn, J.M.; Bouvignies, G.; Parigi, G.; Luchinat, C.; Ferrage, F. Detection of metabolite-protein interactions in complex biological samples by high-resolution relaxometry: towards interactomics by NMR, *JACS*, **2021**, 143, 9393–9404, doi: 10.1021/ jacs.1c01388 (IF 15.419).

3. Ravera. E.; Gigli, L.; Suturina, E.A.; Calderone, V.; Fragai, M.; Parigi, G.; LuchinaT, C. A high-resolution view of the coordination environment in a paramagnetic metalloprotein from its magnetic properties, *Angew. Chem. Int. Ed.*, **2021**, 60, 14960-14966 doi: 10.1002/anie.202101149 (IF 15.336).

4. Francesconi, O.; Milanesi, F.; Nativi, C.; Roelens, S. A Simple Biomimetic Receptor Selectively Recognizing the GlcNAc2 Disaccharide in Water. *Angew. Chem. Int. Ed.*, **2021**, 10; 60, 11168-11172. doi: 10.1002/anie.202100560 (IF15.336).

5. Matteucci, S.; Camponeschi, F.; Clémancey, M.; Ciofi-Baffoni, S.; Blondin, G.; Banci, L. In-cellulo Mössbauer and EPR studies bring new evidences to the long-standing debate on the iron-sulfur cluster binding in human anamorsin. *Angew. Chem. Int. Ed.*, **2021**, doi: 10.1002/anie.202102910 (IF 15.336)

6. Rathner, P.; Fahrner, M.; Cerofolini, L.; Grabmayr, H.; Horvath, F.; Krobath, H.; Gupta, A.; Ravera, E.; Fragai, M.; Bechmann, M.; Renger, T.; Luchinat, C.; Romanin, C.; Müller, N. Interhelical interactions within the STIM1 CC1 domain modulate CRAC channel activation. *Nat. Chem. Biol.*, **2021**, 17, 196-204, doi: 10.1038/s41589-020-00672-8 (IF 15.040).

7. Parigi, G.; Ravera, E.; Fragai, M.; Luchinat C. Unveiling protein dynamics in solution with field-cycling NMR relaxometry, *Prog. NMR spec.*, **2021**, 124, 85-98, doi: 10.1016/j.pnmrs.2021.05.001 (IF 9.765).

8. Leone, G.; Pepi, S.; Consumi, M.; Lamponi, S.; Fragai, M.; Martinucci, M.; Baldoneschi, V.; Francesconi, O.; Nativi, C.; Magnani, A. Sodium hyaluronate-g-2- ((N-(6-aminohexyl)-4-methoxyphenyl)sulfonamido)-N-hydroxyacetamide with enhanced affinity towards MMP12 catalytic domain to be used as visco-supplement with increased degradation resistance. *Carbohydr Polym.,* **2021**, 271, 118452 (IF 9.381).

9. Ghini, V.; Abuja, P.M.; Polasek, O.; Kozera, L.; Laiho, P.; Anton, G.; Zins, M.; Klovins, J.; Metspalu, A.; Wichmann, H.E.; Gieger, C.; Luchinat, C.; Zatloukal, K.; Turano, P. Metabolomic Fingerprints in Large Population Cohorts: Impact of Preanalytical Heterogeneity. *Clin Chem.,* **2021** 67, 1153-1155. doi: 10.1093/clinchem/hvab092 (IF 8.327).

10. Rizzo, D.; Ravera, E.; Fragai, M.; Parigi, G.; Luchinat, C. Origin of the MRI Contrast in Natural and Hydrogel Formulation of Pineapple Juice, *Bioinorg. Chem. Appl.,* **2021**, doi: 10.1155/2021/666 6018 (IF 7.778).

11. Meoni, G.; Di Cesare, F.; Tenori, L.; Gori, A.M.; Marcucci, R.; Giusti, B.; Molino-Lova, R.; Macchi, C.; Pancani, S.; Luchinat, C.; Saccenti, E. Lipid and metabolite correlation networks specific to clinical and biochemical covariate show differences associated with sexual dimorphism in a cohort of nonagenarians. *Geroscience,* **2021** doi: 10.1007/s11357-021-00404-3 (IF 7.713).

12. Luchinat, E.; Barbieri, L.; Cremonini, M.; Pennestri, M.; Nocentini, A.; Supuran, C.T.; Banci, L. Determination of intracellular protein-ligand binding affinity by competition binding in-cell NMR. *Acta Crystallogr. D Struct. Biol.,* **2021**, 77, 1270-1281. doi: 10.1107/S2059798321009037 (IF 7.652).

13. Russomanno, P.; Assoni, G.; Amato, J.; D'Amore, V.M.; Scaglia, R.; Brancaccio, D.; Pedrini, M.; Polcaro, G.; La Pietra, V.; Orlando, P.; Falzoni, M.; Cerofolini, L.; Giuntini, S.; Fragai, M.; Pagano, B.; Donati, G.; Novellino, E.; Quintavalle, C.; Condorelli, G.; Sabbatino, F.; Seneci, P.; Arosio, D.; Pepe, S.; Marinelli, L. Interfering with the Tumor-Immune Interface: Making Way for Triazine-Based Small Molecules as Novel PD-L1 Inhibitors. *J. Med. Chem.*, **2021**, 64, 16020-16045 (IF 7.446).

14. Adiram-Filiba, N.; Ohaion, E.; Verner, G.; Schremer, A.; Nadav-Tsubery, M.; Lublin-Tennenbaum, T.; Keinan-Adamsky, K.; Lucci, M.; Luchinat, C.; Ravera, E.; Goobes, G. Structure and dynamics perturbations in ubiquitin adsorbed or entrapped in silica materials are related to disparate surface chemistries resolved by solid-state NMR spectroscopy, *Biomacromolecules*, **2021**, doi: 10.1021/acs.biomac.1c00495 (IF 6.988).

15. Rizzo, D.; Cerofolini, L.; Pérez-Ràfols, A.; Giuntini, S.; Baroni, F.; Ravera, E.; Luchinat, C.; Fragai M. Evaluation of the Higher Order Structure of Biotherapeutics Embedded in Hydrogels for Bioprinting and Drug Release, *Anal. Chem.*, **2021**, 93, 32, 11208–11214, doi: 10.1021/acs.analchem.1c01850 (IF 6.986).

16. Albuquerque-Gonzalez, B.; Bernabé-Garcia, M.; Bernabé-Garcia, A.; Ruiz-Sanz, J.; Lopez-Calderon, F.F.; Gonnelli, L.; Banci, L.; Peña-García, J,; Luque, I.; Nicolas, F.J.; Cayuela-Fuentes, M.L.; Luchinat, E.; Perez-Sanchez, H.; Monto-Garcia, S.; Conesa-Zamora, P. The FDA-Approved Antiviral Raltegravir Inhibits Fascin1-Dependent Invasion of Colorectal Tumor Cells In Vitro and In Vivo, *Cancers*, **2021**, 13, 861. doi: 10.3390/cancers13040861 (IF 6.860).

17. Corona, G.; Di Gregorio, E.; Vignoli, A.; Muraro, E.; Steffan, A.; Miolo, G. ^{1}H-NMR Plasma Lipoproteins Profile Analysis Reveals Lipid Metabolism Alterations in HER2-Positive Breast Cancer Patients. *Cancers*, **2021**, 13 (22), 5845. doi: 10.3390/cancers13225845 (IF 6.860).

18. Di Donato, S.; Vignoli, A.; Biagioni, C.; Malorni, L.; Mori, E.; Tenori, L.; Calamai, V.; Parnofiello, A.; Di Pierro, G.; Migliaccio, I.; Cantafio, S.; Baraghini, M.; Mottino, G.; Becheri, D.; Del Monte, F.; Miceli, E.; McCartney, A.; Di Leo, A.; Luchinat, C.; Biganzoli, L. A Serum Metabolomics Classifier Derived from Elderly Patients with Metastatic Colorectal Cancer Predicts Relapse in the Adjuvant Setting. *Cancers,* **2021**, 13 (11), 2762. doi: 10.3390/cancers1311276 (IF 6.860).

19. Meoni, G.; Ghini, V.; Maggi, L.; Vignoli, A.; Mazzoni, A.; Salvati, L.; Capone, M.; Vanni, A.; Tenori, L.; Fontanari, P.; Lavorini, F.; Peris, A.; Bartoloni, A.; Liotta, F., Cosmi, L.; Luchinat, C.; Annunziato, F.; Turano, P. Metabolomic/lipidomic profiling of COVID-19 and individual response to tocilizumab. *PLoS Pathog.*, **2021**, 17. doi: 10.1371/journal.ppat.1009243 e 1009243 (IF 6.823).

20. Romero, A.R.; Lunev, S.; Popowicz, G.M.; Calderone, V.; Gentili, M.; Sattler, M.; Plewka, J.; Taube, M.; Kozak, M.; Holak, T.A.; Dömling, A.S.S.; Goves, M.R. A fragment-based approach identifies an allosteric pocket that impacts malate dehydrogenase activity. *Commun. Biol.*, **2021**, 4, doi: 10.1038/s42003-021-02442-1 (IF 6.268).

21. Grifagni, D.; Calderone, V.; Giuntini, S.; Cantini, F.; Fragai, M.; Banci, L. SARS-CoV-2 Mpro inhibition by a zinc ion: structural features and hints for drug design. *Chem. Commun. (Camb).*, **2021** 10; 57(64), 7910-7913. doi: 10.1039/d1cc02956h (IF 6.222).

22. Di Cesare, F.; Luchinat, C.; Tenori, L.; Saccenti, E. Age and sex dependent changes of free circulating blood metabolite and lipid abundances, correlations and ratios. *J. Gerontol. A Biol. Sci. Med. Sci.*, **2021**, 335. doi: 10.1093/gerona/glab335 (IF 6.053).

23. Cafaro, A.; Barillari, G.; Moretti, S.; Palladino, C.; Tripiciano, A.; Falchi, M.; Picconi, O.; Cossut, M.R.P.; Campagna, M.; Arancio, A.; Sgadari, C.; Andreini, C.; Banci, L.; Monini, P.; Ensoli, B. HIV-1 Tat Protein Enters Dysfunctional Endothelial Cells via Integrins and Renders Them Permissive to Virus Replication, *Int. J. Mol. Sci.*, **2021**, 22, 317. doi:10.3390/ijms22010317 (IF 5.932).

24. Bernacchioni, C.; Ghini, V.; Squecco, R.; Idrizaj, E.; Garella, R.; Puliti, E.; Cencetti, F.; Bruni, P.; Donati, C. Role of Sphingosine 1-Phosphate Signalling Axis in Muscle Atrophy Induced by TNFα in C2C12 Myotubes. *Int. J. Mol. Sci.*, **2021**, 22, 1280. doi: 10.3390/ijms22031280 (IF 5.932).

25. Saudino, G.; Suraci, D.; Nasta, V.; Ciofi-Baffoni, S.; Banci, L. Molecular Basis of Multiple Mitochondrial Dysfunctions Syndrome 2 Caused by CYS59TYR BOLA3 Mutation. *Int. J. Mol. Sci.*, **2021**, 22(9), 4848. doi: 10.3390/ijms22094848 (IF 5.932).

26. Vignoli, A.; Risi, E.; McCartney, A.; Migliaccio, I.; Moretti, E.; Malorni, L.; Luchinat, C.; Biganzoli, L.; Tenori, L. Precision Oncology via NMR-Based Metabolomics: A Review on Breast Cancer. *IJMS*, **2021**, 22 (9), 4687. doi: 10.3390/ijms22094687 (IF 5.924).

27. Nannini, G.G.; Meoni, G.; Tenori, L.; Ringressi, M.N.; Taddei, A.; Niccolai, E.; Baldi, S.E.; Russo, C.; Luchinat, C.; Amedei, A. Fecal metabolomics' profile: A comparative study from colorectal cancer vs adenomatous polyps' patients. *World J. Gastroenterol.*, **2021**, 14, 27, 6430-6441. doi: 10.3748/wjg.v27.i38.6430 (IF 5.74).

28. Piróg, A.; Cantini, F.; Nierzwicki, Ł.; Obuchowski, I.; Tomiczek, B.; Czub, J.; Liberek, K. Two Bacterial Small Heat Shock Proteins, IbpA and IbpB, Form a Functional Heterodimer. *J. Mol. Biol.*, **2021**, 433(15):167054. doi: 10.1016/j.jmb.2021.167054 (IF 5.469).

29. Suraci, D.; Saudino, G.; Nasta, V.; Ciofi Baffoni, S.; Banci, L. ISCA1 orchestrates ISCA2 and NFU1 in the maturation of human mitochondrial [4Fe-4S] proteins, *J. Mol. Biol.*, **2021**, doi: 10.1016/j.jmb.2021.166924 (IF 5.469).

30. Cacciatore, S.; Wium, M.; Licari, C.; Ajayi-Smith, A.; Masieri, L.; Anderson, C.; Salukazana, A.S.; Kaestner, L.; Carini, M.; Carbone, G.M.; Catapano, C.V.; Loda, M.; Libermann, T.A.; Zerbini, L.F. Inflammatory metabolic profile of South African patients with prostate cancer. *Cancer Metab.*, **2021**, 9(1), 29. doi: 10.1186/s40170-021-00265-6 (IF 5.469).

31. Meoni, G.; Tenori, L; Luchinat, C.; Turano, P. *et al.* A geroscience approach for Parkinson's disease: Conceptual framework and design of PROPAG-AGEING project PROPAG-AGEING Consortium. *Mech. Ageing Dev.*, **2021**, 194:111426. doi: 10.1016/j.mad.2020.111426 (IF 5.432).

32. Felli, I.C.; Pierattelli, R.; Pontoriero, L.; Schiavina, M,. *et al*. Large-scale recombinant production of the SARS-CoV-2 proteome for high-throughput and structural biology applications, *Front. Mol. Biosci.*, **2021**, doi: 10.3389/fmolb.2021.653148 (IF 5.246).

33. Bonucci, A.; Palomino-Schätzlein, M.; Malo de Molina, P.; Arbe, A.; Pierattelli, R.; Rizzuti, B.; Iovanna, J.L.; Neira, J.L. Crowding Effects on the Structure and Dynamics of the Intrinsically Disordered Nuclear Chromatin Protein NUPR1. *Front. Mol. Biosci.*, **2021**, 8, 684622 (IF 5.246).

34. Honorato, R.V.; Koukos, P.I.; Jiménez-García, B.; Tsaregorodtsev, A.; Verlato, M.; Giachetti, A.; Rosato, A.; Bonvin, A.M.J.J. Structural Biology in the Clouds: The WeNMR-EOSC Ecosystem. *Front. Mol. Biosci.*, **2021**, 8:729513. doi: 10.3389/fmolb.2021.729513 (IF 5.246).

35. Ciambellotti, S.; Pratesi, A.; Tassone, G.; Turano, P.; Mangani, S.; Pozzi, C. Iron Binding in the Ferroxidase Site of Human Mitochondrial Ferritin. *Chem. Eur. J.*, **2021**, 27(59):14690-14701. doi: 10.1002/chem.202102270 (IF 5.236).

36. Francesconi, O.; Milanesi, F.; Nativi, C.; Roelens, S. Molecular Recognition of Disaccharides in Water: Preorganized Macrocyclic or Adaptive Acyclic? *Chem. Eur. J.*, **2021**, 27(40), 10456-10460. doi: 10.1002/chem.202101238 (IF 5.236).

37. Vilona, D.; Lelli, M.; Dumont, E. Lacôte E. Organo-Polyoxometalate-BasedHydrogen-BondCatalysis, *Chem. Eur. J.*, **2021**, 27,17761– 17764. doi: 10.1002/chem.202102807 (IF 5.236).

38. Ravera, E.; Gigli, L.; Czarniecki, B.; Lang, L.; Kuemmerle, R.; Parigi, G.; Piccioli, M.; Neese, F.; Luchinat, C. A quantum chemistry view on two archetypical paramagnetic pentacoordinate nickel(II) complexes offers a fresh look on their NMR spectra, *Inorg. Chem.*, **2021**, 60, 3, 2068–2075. doi: 10.1021/acs.inorgchem.0c03635 (IF 5.165).

39. Yon, M.; Gineste, S.; Parigi, G.; Lonetti, B.; Gibot, L.; Talham, D.; Marty, J.D.; Mingotaud, C. Hybrid Polymeric Nanostructures Stabilized by Zirconium and Gadolinium ions for Use as Magnetic Resonance Imaging Contrast Agents, ACS Appl. Nano Mater., **2021**, 4, 4974-4982. doi: 10.1021/acsanm.1c00495 (IF 5.097).

40. La Veglia, V.; Giachetti, A.; Cerofolini, L.; Fragai, M.; Ciulli, A.; Rosato, A. Automated Determination of Nuclear Magnetic Resonance Chemical Shift Perturbations in Ligand Screening Experiments: The PICASSO Web Server. *J. Chem. Inf. Model*, **2021**, doi: 10.1021/acs.jcim.1c00871 (IF 4.956).

41. Sala, D.; Giachetti, A.; Rosato, A. Insights into the Dynamics of the Human Zinc Transporter ZnT8 by MD Simulations. *J. Chem. Inf. Model.*, **2021** 22, 61 901-912. doi: 10.1021/acs.jcim.0c01139 (IF 4.956).

42. Fondi, M.; Gonzi, S.; Dziurzynski, M.; Turano, P.; Ghini, V.; Calvanese, M.; Colarusso, A.; Lauro, C.; Parrilli, E.; Tutino, M.L. Modelling hCDKL5 Heterologous Expression in Bacteria. *Metabolites*, **2021**, 11(8), 491. doi: 10.3390/metabo11080491 (IF 4.932).

43. Balder, Y.; Vignoli, A.; Tenori, L.; Luchinat, C.; Saccenti, E. Exploration of Blood Lipoprotein and Lipid Fraction Profiles in Healthy Subjects through Integrated Univariate, Multivariate, and Network Analysis Reveals Association of Lipase Activity and Cholesterol Esterification with Sex and Age. *Metabolites*, **2021**, 11 (5), 326. doi: 10.3390/metabo11050326 (IF 4.932).

44. Gigli, L.; Ravera, E.; Calderone, V.; Luchinat, C. On the mechanism of bioinspired formation of inorganic oxides: structural evidence of the electrostatic nature of the interaction between a mononuclear inorganic precursor and lysozyme. *Biomolecules*, **2021**, 11, 43, doi: 10.3390/biom11010043 (IF 4.879).

45. Salladini, E.; Gondelaud, F.; Nilsson, J.F.; Pesce, G.; Bignon, C.; Murrali, M.G.; Fabre, R.; Pierattelli, R.; Kajava, A.V.; Horvat, B.; Gerlier, D.; Mathieu, C.; Longhi, S. Identification of a Region in the Common Amino-terminal Domain of Hendra Virus P, V, and W Proteins Responsible for Phase Transition and Amyloid Formation. *Biomolecules*, **2021**, 11, 1324. doi: 10.3390/biom11091324 (IF 4.879).

46. Forgione, R.E.; Di Carluccio, C.; Milanesi, F.; Kubota, M.; Fabregat Nieto, F.; Molinaro, A,.; Hashiguchi, T.; Francesconi, O.; Marchetti, R.; Silipo, A. Characterization of Natural and Synthetic Sialoglycans Targeting the Hemagglutinin-Neuraminidase of Mumps Virus. *Front. Chem.*, **2021**, 9, 711346. doi: 10.3389/fchem.2021.711346. eCollection (IF 4.62).

47. Vignoli, A.; Tenori, L.; Luchinat, C.; Saccenti, E. Differential Network Analysis Reveals Molecular Determinants Associated with Blood Pressure and Heart Rate in Healthy Subjects. *J. Proteom. Res.*, **2021**, 20, 1040-1051. doi: 10.1021/acs.jproteome.0c00882 (IF 4.466).

48. Licari, C.; Tenori, L.; Giusti, B.; Sticchi, E.; Kura, A.; De Cario, R.; Inzitari, D.; Piccardi, B.; Nesi, M.; Sarti, C.; Arba, F.; Palumbo, V.; Nencini, P.; Marcucci, R.; Gori, A.M.; Luchinat, C.; Saccenti, E. Analysis of Metabolite and Lipid Association Networks Reveals Molecular Mechanisms Associated with 3-Month Mortality and Poor Functional Outcomes in Patients with Acute Ischemic Stroke after Thrombolytic Treatment with Recombinant Tissue Plasminogen Activator. *J. Proteom. Res.*, **2021**, 20, 4758-4770. doi: 10.1021/acs.jproteome.1c00406 (IF 4.466).

49. Daniele, S.; La Pietra, V.; Piccarducci, R.; Pietrobono, D.; CavallinI, C.; D'Amore, V.M.; Cerofolini, L.; Giuntini, S.; Russomanno, P.; Puxeddu, M.; Nalli, M.; Pedrini, M.; Fragai, M.; Luchinat, C. Novellino E, Taliani S, La Regina G, Silvestri R, Martini C, Marinelli L. CXCR4 antagonism sensitizes cancer cells to novel indole-based MDM2/4 inhibitors in glioblastoma multiforme. *Eur. J. Pharmacol.*, **2021**, 897, 173936. doi: 10.1016/j.ejphar.2021.173936 (IF 4.432).

50. Massai, L.; Ciambellotti, S.; Cosottini, L.; Messori, L.; Turano, P.; Pratesi, A. Direct detection of iron clusters in L ferritins through ESI-MS experiments. *Dalton Trans.*, **2021**, 50(45), 16464-16467. doi: 10.1039/d1dt03106f. (IF 4.39).

51. Ghini, V.; Senzacqua, T.; Massai, L.; Gamberi, T.; Messori, L.; Turano, P. NMR reveals the metabolic changes induced by auranofin in A2780 cancer cells: evidence for glutathione dysregulation. *Dalton Trans.*, **2021**, 50, 6349-6355. doi: 10.1039/d1dt00750e (IF 4.39).

52. Bendinelli, B.; Vignoli, A.; Palli, D.; Assedi, M.; Ambrogetti, D.; Luchinat, C.; Caini, S.; Saieva, C.; Turano, P.; Masala, G. Prediagnostic circulating metabolites in female breast cancer cases with low and high mammographic breast density. *Sci. Rep.*, **2021**, 11, 13025. doi: 10.1038/s41598-021-92508-1 (IF 4.379).

53. Bobbo, T.; Meoni, G.; Niero, G.; Tenori, L.; Luchinat, C.; Cassandro, M.; Penasa, M. Nuclear magnetic resonance spectroscopy to investigate the association between milk metabolites and udder quarter health status in dairy cows. *J. Dairy Sci.*, **2021**, S0022-0302(21)00964-4. doi: 10.3168/jds.2021-20906. (IF 4.354).

54. Baima, G.; Corana, M.; Iaderosa, G.; Romano, F.; Meoni, G.; Tenori, L.; Aimetti, M. Metabolomics of gingival crevicular fluid to identify biomarkers for periodontitis: a systematic review with meta-analysis. *J. Periodontal. Res.*, **2021**, 56, 633-645. doi: 10.1111/jre.12872 (IF 4.241).

55. Michael, A.; Hope, M.A.; Björgvinsdóttir. S.; Halat, D.M.; Menzildjian, G.; Wang, Z.; Zhang, B.; MacManus-Driscoll, J.; Lesage, A.; Lelli, M.; Emsley, L.; Grey, C.P. Endogenous ^{17}O Dynamic Nuclear Polarization of Gd-Doped CeO2 from 100 to 370 K, *J. Phys. Chem. C*, **2021**, 125, 18799−18809. doi: 10.1021/acs.jpcc.1c04479 (IF 4.126).

56. Licciardi, G.; Rizzo, D.; Ravera, E.; Fragai, M.; Parigi, G.; Luchinat, C. Not only manganese, but fruit component effects dictate the efficiency of fruit juice as an oral magnetic resonance imaging contrast agent. *NMR Biomed.*, **2021**, e4623. doi: 10.1002/nbm.4623 (IF 4.044).

57. Bellomo, G.; Paciotti, S.; Gatticchi, L.; Rizzo, D.; Paoletti, F.P.; Fragai, M.; Parnetti, L. Seed amplification assays for diagnosing synucleinopathies: the issue of influencing factors. *Front. Biosci.*, **2021**, 26, 1075-1088. doi: 10.52586/5010 (IF 4.009).

58. Torricella, F.; Bonucci, A.; Polykretis, P.; Cencetti, F.; Banci, L. Rapid protein delivery to living cells for biomolecular investigation. *Biochem. Biophys. Res. Commun.*, **2021**, 570, 82-88. doi: 10.1016/j.bbrc.2021.07.006 (IF 3.575).

59. Polykretis, P.; Luchinat, E. Biophysical characterization of the interaction between the full-length XIAP and Smac/DIABLO. *Biochem. Biophys. Res. Commun.*, **2021**, 568, 180-185. doi: 10.1016/j.bbrc.2021.06.077 (IF 3.575).

60. Trindade, I.B.; Hernandez, G.; Lebègue, E.; Barrière, F.; Cordeiro, T.; Piccioli, M.; Louro, R.O. Conjuring up a ghost: structural and functional characterization of FhuF, a ferric siderophore reductase from E. coli. *J. Biol. Inorg. Chem.*, **2021**, 26, 313-326. doi: 10.1007/s00775-021-01854-y (IF 3.358).

61. Selegato, D.M.; Bracco, C.; Giannelli, C.; Parigi, G.; Luchinat, C.; Sgheri, L.; Ravera, E. Comparison of Different Reweighting Approaches for the Calculation of Conformational Variability of Macromolecules from Molecular Simulations. *Chem. Phys. Chem.*, **2021**, *22*, 127-138, doi: 10.1002/cphc.202000714 (IF 3.102).

62. Menzildjian, G.; Lund, A.; Yulikov, M.; Gajan, D.; Niccoli, L.; Karthikeyan, G.; Casano, G.; JeschkE, G.; Ouari, O.; Lelli, M.; Lesage, A. Efficient Dynamic Nuclear Polarization up to 230 K with Hybrid BDPA-Nitroxide Radicals at a High Magnetic Field. *J. Phys. Chem. B.*, **2021**, 125, 13329-13338. doi: 10.1021/acs.jpcb.1c07307 (IF 2.991).

63. Luchinat, E.; Barbieri, L.; Cremonini, M.; Banci, L. Protein in-cell NMR spectroscopy at 1.2 GHz. *J. Biomol. NMR*, **2021**, 75(2-3), 97-107. doi: 10.1007/s10858-021-00358-w (IF 2.835).

64. Vignoli, A.; Mori, E.; Di Donato, S.; Malorni, L.; Biagioni, C.; Benelli, M.; Calamai, V.; Cantafio, S.; Parnofiello, A.; Baraghini, M.; Garzi, A.; Monte, F.D.; Romagnoli, D.; Migliaccio, I.; Luchinat, C.; Tenori, L.; Biganzoli, L. Exploring Serum NMR-Based Metabolomic Fingerprint of Colorectal Cancer Patients: Effects of Surgery and Possible Associations with Cancer Relapse. *Appl. Sci.*, **2021**, 11, 11120. doi: 10.3390/app112311120 (IF 2.679).

65. Meoni, G.; Luchinat, C.; Gotti, E.; Cadena, A.; Tenori, L. Phenotyping Green and Roasted Beans of Nicaraguan Coffea Arabica Varieties Processed with Different Post-Harvest Practices. *Appl. Sci.*, **2021**, 1, 24. doi10.3390/app112411779 (IF 2.679).

66. Prusty, N.R.; Camponeschi, F.; Ciofi Baffoni, S.; Banci, L. The human YAE1-ORAOV1 complex of the cytosolic iron-sulfur protein assembly machinery binds a [4Fe-4S] cluster, *Inorg. Chim. Acta*, **2021**, 518, 1, doi: 10.1016/j.ica.2021.120252 (IF 2.545).

67. Tsopra, R.; Fernandez, X.; Luchinat, C.; Alberghina, L.; Lehrach, H.; Vanoni, M.; Dreher, F.; Sezerman, O.U.; Cuggia, M.; de Tayrac, M.; Miklasevics, E.; Itu, L.M.; Geanta, M.; Ogilvie, L.; Godey, F.; Boldisor, C.N.; Campillo-Gimenez, B.; Cioroboiu, C.; Ciusdel, C.F.; Coman, S.; Hijano Cubelos, O.; Itu, A.; Lange, B.; Le Gallo, M.; Lespagnol, A.; Mauri, G.; Soykam, H.O.; Rance, B.; Turano, P.; Tenori, L.; Vignoli, A.; Wierling, C.; Benhabiles, N.; Burgun, A. A framework for validating AI in precision medicine: considerations from the European ITFoC consortium. *BMC Med. Inform. Decis. Mak.*, **2021**, 274. doi: 10.1186/s12911-021-01634-3 (IF 2.317).

68. Gigli, L.; Di Grande, S.; Ravera, E.; Parigi, G.; Luchinat, C. NMR for Single Ion Magnets *Magnetochemistry*, **2021**, 7, 96, doi: 10.3390/magnetochemistry7070096 (IF 2.193).

69. Cantini, F.; Giannì, P.; Savarin, P.; Bizzarri, A.R.; Sette, M. Solution structure of the anticancer p28 peptide in biomimetic medium. *J. Pept. Sci.*, **2021**, 27, e3357. doi: 10.1002/psc.3357 (IF 1.905).

70. Ravera, E.; Cerofolini, L.; Fragai, M.; Parigi, G.; Luchinat, C. Characterization of lanthanoid-binding proteins using NMR spectroscopy. *Meth. Enzymol.*, **2021**, 651, 103-137. doi: 10.1016/bs.mie.2021.01.039 (IF1.600).

71. Wienk, H.; Banci, L.; Daenke, S.; Pereiro, E.; Schwalbe, H.; Stuart, D.I.; Weiss, M.S.; Perrakis, A. iNEXT-Discovery and Instruct-ERIC: Integrating High-End Services for Translational Research in Structural Biology. *J. Vis. Exp.*, **2021**, 177, e63435 (IF 1.163).

72. Barbieri, L.; Luchinat, E. Monitoring Protein-Ligand Interactions in Human Cells by Real-Time Quantitative In-Cell NMR using a High Cell Density Bioreactor. *J. Vis. Exp.*, **2021**, 169. doi: 10.3791/62323. (IF 1.163).

73. Schiavina, M.; Pontoriero, L.; Uversky, V.N.; Felli, I.C.; Pierattelli, R. The highly flexible disordered regions of the SARS-CoV-2 nucleocapsid N protein within the 1–248 residue construct: sequence-specific resonance assignments through NMR. *Biomol. NMR Assign.*, **2021**, doi: 10.1007/s12104-021-10009-8 (IF 0.746).

74. Gallo, A.; Tsika, A.C.; Fourkiotis, N.K.; Cantini, F.; Banci, L.; Sreeramulu, S.; Schwalbe, H.; Spyroulias, G.A. ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the SUD domains of SARS-CoV-2 non-structural protein 3c: "The SUD-M and SUD-C domains", *Biomol. NMR Assign.*, **2021**, 1, 165-171. doi: 10.1007/s12104-020-10000-9 (IF 0.746).

75. Bellomo, G.; Ravera, E.; Calderone, V.; Botta, M.; Fragai, M.; Parigi, G.; Luchinat, C. Revisiting paramagnetic relaxation enhancements in slowly rotating systems: how long is the long range? *Magn. Reson.*, **2021**, 2, 25–31. doi: 10.5194/mr-2-25-2021 (IF 2.229).

76. Camponeschi, F.; Gallo, A.; Piccioli, M.; Banci, L. The long-standing relationship between Paramagnetic NMR and Iron-Sulfur proteins: the mitoNEET example. An old method for new stories or the other way around? *Magn. Reson.*, **2021**, 2, 203–221. doi: 10.5194/mr-2021-2 (IF 2.229).

77. Felli, I.C.; Bermel, W.; Pierattelli, R. Exclusively heteronuclear NMR experiments for the investigation of intrinsically disordered proteins: focusing on proline residues. *Magn. Reson.*, **2021**, 2, 511-522. doi: 10.5194/mr-2-511-2021 (IF 2.229).

78. Ravera, E. Phase distortion-free pNMR spectra, *JMRO*, **2021**, 100022. doi: 10.1016/j.jmro.2021.100022 (IF 4.668).

79. Di Carluccio, C.; Forgione, R.E.; Bosso, A.; Yokoyama, S.; Manabe, Y.; Pizzo, E.; Molinaro, A.; Fukase, K.; Marco, M.; Bensing, B.A.; Marchetti R.; Silipo, A. Molecular recognition of sialoglycans by streptococcal Siglec-like adhesins: toward the shape of specific inhibitors. *RCS Chem. Biol.*, **2021**, 2, 1618-1630. doi: 10.1039/D1CB00173F.

80. Duranti, C.; Lastraioli, E.; Iorio, J.; Capitani, C.; Carraresi, L.; Gonnelli, L.; Arcangeli, A. Expression and purification of a novel single-chain diabody (scDb-hERG1/β1) from Pichia pastoris transformants. *Protein Expr. Purif.*, **2021**, 184, 105879. doi: 10.1016/j.pep.2021.105879 (IF 1.65).

Meetings and Events Organised by CERM

Seminars Held at CERM

September 21st, **Prof. Michael J. Maroney,** University of Massachusetts Amherst, MA, USA, "Maturation of Ni superoxide dismutase: how does the enzyme get its nickel?" CERM Conference Room

Meetings and Conferences

October 22nd-29th, NMR Training School "Fundamentals of magnetic resonance spectroscopies and metal trafficking", virtual meeting

February 16th,"iNEXT-Discovery First Annual Scientific Meeting", virtual meeting

Group Meetings

15/01 **Moreno Lelli** "Structural Determination in the Solid-state of Very-Diluted Species Through Dynamic Nuclear Polarization NMR"

22/01 **Domenico Rizzo** "Expression and Characterization of human proteins for drug screening: STING and PD-L1"

29/01 **Giovanni Saudino** "Investigating the molecular role of NFU1 in the lipoyl synthase maturation"

05/02 **Sara Matteucci** "Production and Functional Characterization of NUBP1-NUBP2 Complex, Involved in Irun-Sulfur Cluster Biogenesis"

12/02 **Marco Schiavina** "SARS-CoV-2 Nucleoprotein: dealing with heterogeneity"

19/02 **Milana Bazayeva** "Metal-Binding Proteins"

05/03 **Giulia Licciardi** "Dealing with olive oil relaxation process"

12/03 **Bach Tung Lan Pham** "Characterization of SARS-CoV-2 Proteins by In-cell NMR Spectroscopy "

MEETINGS & EVENTS

19/03 **Milano Bazayeva** “Metal-Binding Proteins”

26/03 **Mathias Percipalle** “Structural, thermodynamic and kinetic comparison of camel- and llama-derived nanobodies”

09/04 **Maria Salobehaj** “Expression and characterization of Apolipoprotein A-I Milano variant”

23/04 **Valentina Vitali** “Towards tyrosine conjugation of proteins for NMR and EPR spectroscopy”

30/04 **Francesca Di Cesare** “^{1}H NMR-based metabolomics to investigate the effect of probiotics on human phenotype”

14/05 **Francesco Milanesi** “Design and Synthesis Of New Glycomimetics as Ligands of hCD22”

21/05 **Deborah Grifagni** “Zinc-mediate inhibition of SARS-CoV-2 M^{pro}: structural features and hints for drug design”

28/05 **Francesco Torricella** “Novel living systems protein delivery and progresses in ^{19}F NMR”

25/06 **Letizia Pontoriero** “Biochemical characterization of the N protein 1-248 construct from SARS-CoV2: dealing with a heterogeneous system”

17/09 **Francesca Camponeschi** “Assembling [4Fe-4S] clusters in the human cytosol”

01/10 **Veronica Ghini** “Metabolomic fingerprint of COVID-19”

08/10 **Vincenzo Laveglia** “Machine Learning Methods for Metal Binding Protein Analysis”

15/10 **Silvia Ciambellotti** “Ferritin nanosystems for targeted delivery towards cancer cells”

05/11 **Lucia Gigli** “What do we need for modelling the active site of a paramagnetic metalloprotein?”

12/11 **Letizia Barbieri** “Protein-Protein Interactions in Human Cells by NMR”

19/11 **Gaia Meoni** “Updates on COMETA project: metabolites and lipoproteins of COVID-19 patients and severity”

26/11 **Alessia Vignoli** “Serum or plasma, what is the difference?”

Journal Clubs

16/04 **Letizia Pontoriero** “High-resolution ex vivo NMR spectroscopy of human Z α1-antitrypsin”

Anna Pérez i Ràfols “Structure of SRSF1 RRM1 bound to RNA reveals an unexpected bimodal mode of interaction and explains its involvement in SMN1 exon7 splicing”

07/05 **Giovanni Saudino** “Cell-Specific Delivery Using an Engineered Protein Nanocage”

Domenico Rizzo “Hidden kinetic traps in multidomain folding highlight the presence of a misfolded but functionally competent intermediate”

04/06 **Francesca Di Cesare** “MCR-ALS analysis of 1H NMR spectra by segments to study the zebrafish exposure to acrylamide”

11/06 **Sara Matteucci** “Fe-S cofactors in the SARS-CoV-2 RNA-dependent RNA polymerase are potential antiviral targets”

Marco Schiavina “Conserved allosteric ensembles in disordered proteins using TROSY/anti-TROSY R2-filtered spectroscopy”

09/07 **Milana Bazayeva** “Assessment of protein-protein interfaces in cryo-EM derived assemblies”

Giulia Licciardi “Comment on the Optimal Parameters to Derive Intrinsically Disordered Protein Conformational Ensembles from Small-Angle X-ray Scattering Data Using the Ensemble Optimization Method”

10/09 **Bach Tung Lan Pham** “Real-Time Conformational Dynamics of SARS-CoV-2 Spikes on Virus Particles”

24/09 **Maria Salobejah** “The OP Protein Cage: A Versatile Molecular Delivery Platform”

Valentina Vitali “The cryo-EM structure of the bd oxidase from M. tuberculosis reveals a unique structural framework and enables rational drug design to combat TB”

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

Contact Information

CERM

Via Luigi Sacconi 6

50019 Sesto Fiorentino (FI), Italy

www.cerm.unifi.it

Phone: + 39 055 4574270

Fax: + 39 055 4574923

E-mail: cerm@cerm.unifi.it

@cerm_cirmmp

https://www.linkedin.com/company/cerm-cirmmp/