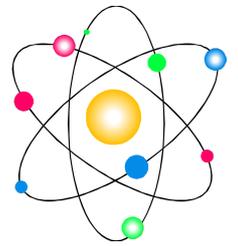**CERM**

Centro di Risonanze Magnetiche

Università di Firenze

CIRMMP

Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine

SCIENTIFIC ANNUAL REPORT

2019

UNIVERSITÀ DEGLI STUDI FIRENZE

Contents

Foreword

The world of NMR is entering a new era: after about fifteen years of research and development, **the new 1.2 GHz NMR magnet (28.2 T) is ready**. During 2019 Bruker announced the completion of the development of this new high field magnet, a sizeable field increase, ten years after the previous highest commercial magnetic field achievement (1.0 GHz (23.4 T). The world first 1.2 GHz NMR magnet will be installed in CERM beginning 2020, and we have already recorded the first, exciting spectra on the new machine already in September 2019 (arXiv:1910.07462v1 [physics.chem-ph]). A significant step forward in resolution and sensitivity is achieved by this new instrument for solution samples, and we expect similar improvements on solid state samples.

Having the first 1.2 GHz NMR spectrometer installed in Florence will make our center one major-player in this new course of NMR. This ultra high-field offers new opportunities to NMR, making it possible to obtain detailed characterisations of biomolecules, complex chemical mixtures, living cells, and new materials, unravelling processes that were inaccessible before. Especially in biomolecular research, NMR confirms to be an inescapable technique to thoroughly characterise biological systems at the molecular level. The research activities that we outline below in this report clearly evidence the uniqueness and the complementarity of NMR with respect to other techniques, including the emerging fields of molecular microscopies and other structural-oriented techniques. For example, NMR is the only technique providing both structural and dynamic information in vitro and in living systems. NMR has also a primary role in material/biomaterial characterisation, as well as in metabolomics. NMR fingerprinting analysis ranges from metabolomics to the characterisation of biological drugs, which is increasingly attracting the interest of pharmaceutical companies.

The research developed at CERM in 2019 follows this spirit of interdisciplinary and integrated approaches: by browsing through the research section of this report, the variety of applications of NMR that move toward the interfaces with other disciplines is clearly apparent: from structural biology to medicine, from material science to information technology. The dialog with other disciplines also stimulates our research to improve its theoretical and methodological bases, in order to be more and more effective in the applications. For this reason we dedicate a special section of the Research Areas of this report to emerging methods.

In parallel, the role in the European Research Infrastructure scenario of CERM/CIRMMP was further reinforced. CERM/CIRMMP is the Italian centre of Instruct-ERIC, an ESFRI landmark Research Infrastructure. During 2019, the key role of the Italian centre (Instruct IT) within Instruct-ERIC was strongly reaffirmed: as an outcome of Brexit, should Instruct-ERIC not being able to maintain its statutory seat in Oxford, United Kingdom, the statutory seat of Instruct-ERIC shall be located in Florence, Italy.

This was possible thanks to our strong involvement in most Instruct-ERIC activities, with a leading role in the Council and in the Executive Committee, as well as in the support to training, internationalization, access and industry engagement, the latter within the Instruct-ULTRA project which aims at releasing the full potential of Instruct to expand and consolidate infrastructure services for integrated structural life science research. The activity of CERM/CIRMMP related to Instruct-ERIC were framed also within the CORBEL initiative that coordinates 13 Biological and Medical European Research infrastructures (BMS RIs) to create a platform for harmonised user access to biological and medical technologies, biological samples, and data services required by cutting-edge biomedical research. From 2019, these BMS RIs are also committed in the EOSC-Life project for creating an open, digital and collaborative space for biological and medical research and to drive the evolution of the RI repository infrastructure for the European Open Science Cloud (EOSC).

Beside structural biology services provided as Instruct-ERIC centre, the access provision for translational research continued thanks to the iNEXT project and secured for next 4 years by the awardee of iNEXT-Discovery.

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

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

Figures

Also for 2019, the Italian Ministry of Education, University, and Research (MIUR) confirmed its support to the Italian centre of Instruct-ERIC within the International Action of the FOE funding. CERM/CIRMMP investments and costs in 2019 amounted to € 3.530.000,00: € 395.000,00 towards training and education, € 1.840.000,00 for new equipment and € 1.050.000,00 towards research activities. An additional € 245.000,00 covered operational costs. The actual replacement value of the instrumentation at CERM is close to € 53.000.000,00.

In 2019, in addition to the faculty staff, the body of researchers included 22 PhD students, 20 postdoctoral scientists, and 28 undergraduate students.

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

Prof. Claudio Luchinat   Prof. Lucia Banci
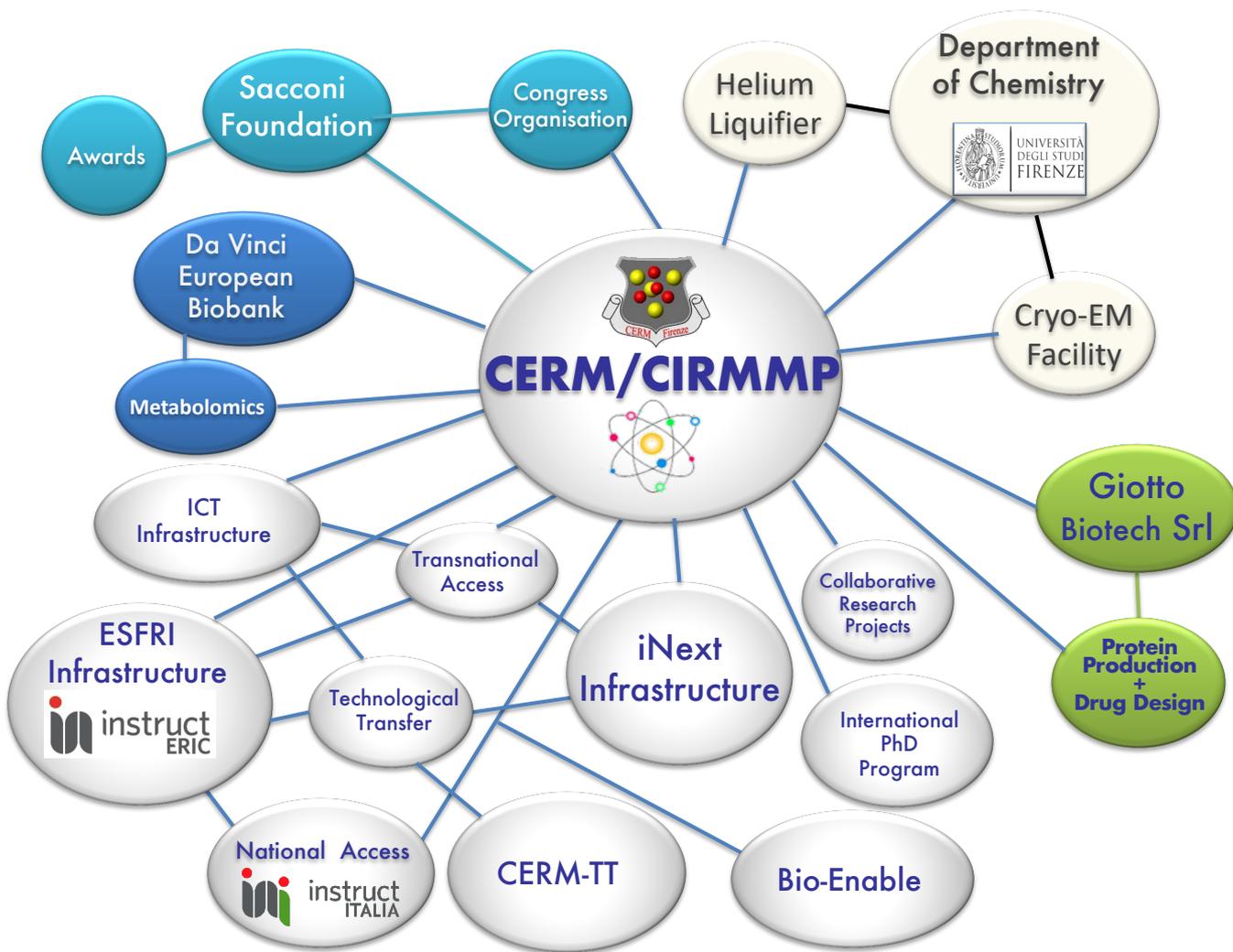
Who we are

Introduction

CERM, Centre for Magnetic Resonance, is a *scientific institution for research*, technology transfer and higher education of the University of Florence. It operates in synergy and collaboration with the Inter-University Consortium for Magnetic Resonance of MetalloProteins (CIRMMP) which includes three Italian Universities: Florence, Siena, and Bologna. CERM/CIRMMP is an *infrastructure for Life Sciences* with a particular focus on structural biology and specialisations in NMR spectroscopy, bioinformatics, molecular and cellular biology, novel drug and vaccine design, and metabolomics. Nevertheless it is open towards interfaces with other research fields, for example new material and biomaterial development, contrast agent and MRI techniques, and ICT technology.

Being a leading laboratory at both national and international level, CERM/CIRMMP receives funding from competitive project calls from the Tuscan Regional Government, the Italian Ministry of Higher Education and Research (MIUR), and the European Commission (EC), as well as from private institutions. Since 1994, CERM/CIRMMP is providing a transnational access to its instrumentation for its expertise and state-of-the-art instrumentation for NMR in Life Sciences.

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

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

CERM/CIRMMP is also an e-infrastructure, managing an European GRID-based platform, providing access to user-friendly platforms and CPU resources for a broad range of 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 co-ordinating the activities of BIO-ENABLE, a new distributed Infrastructure promoting technology transfer to industry and funded by the Regional Government of Tuscany in the frame of POR FESR 2014-2020.

CERM/CIRMMP is located in the Scientific Campus ("Polo Scientifico") of the University of Florence in Sesto Fiorentino, an area just west of the city of Florence. The campus borders Florence International Airport and yet is a mere 15 minutes from the centre of Florence, world-renowned cradle of renaissance art and culture.
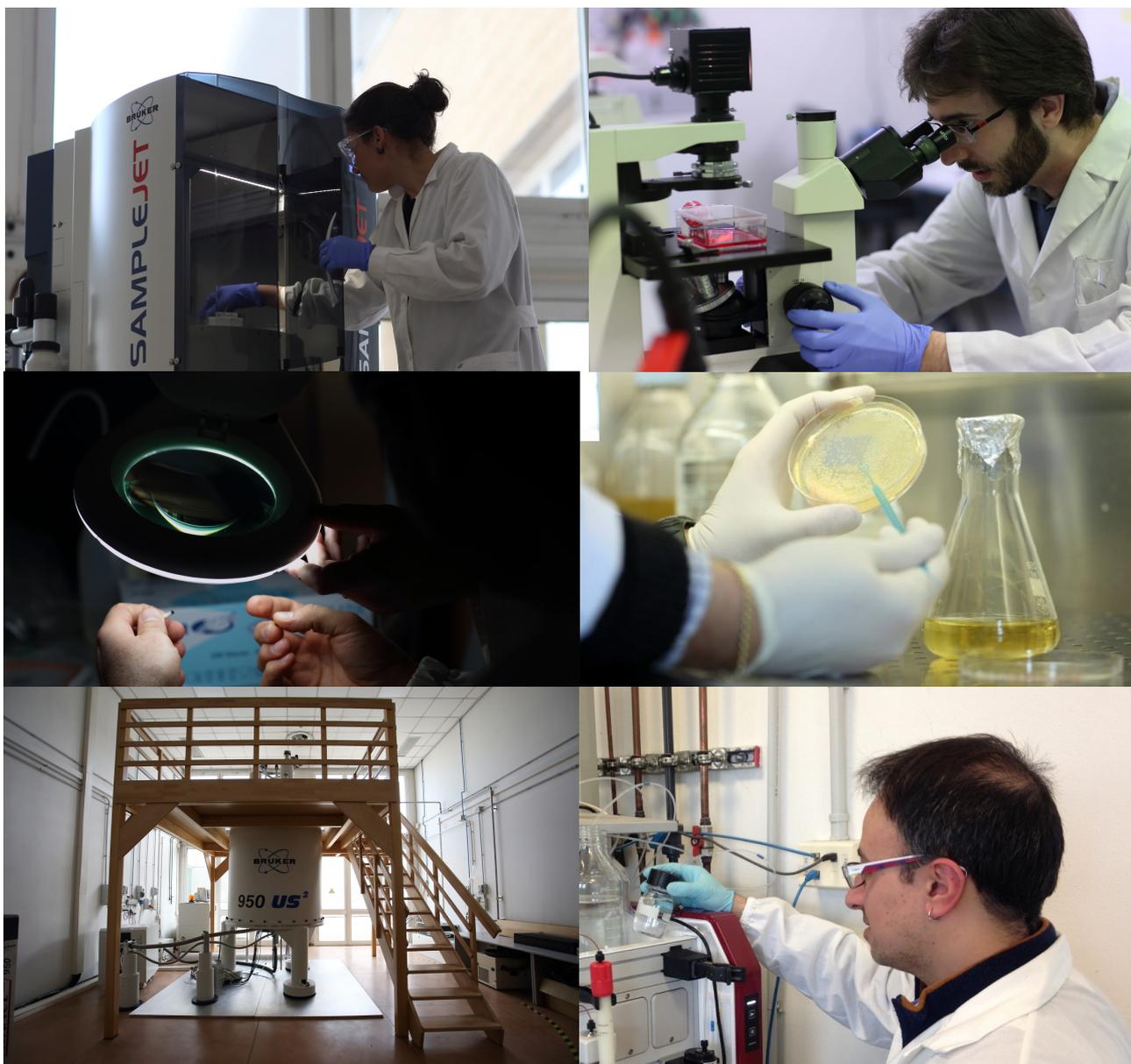
The Infrastructure

CERM/CIRMMP labs

The CERM/CIRMMP building covers an area of 3000 square meters hosting a number of laboratories, offices, and common rooms. The flagship of the Center is the impressive collection of NMR spectrometers which feature the largest magnetic field range in the world (from 950 MHz to the earth field, 1.2 GHz installed in early 2020) and ranks it among the best equipped laboratories in the world. The NMR labs are flanked by molecular and cellular biology laboratories where samples for the NMR are produced. A complete list of the instruments available at CERM/CIRMMP is reported at pag. 38. In addition to the main building, further 500 square meters in adjacent buildings are available to CERM scientists and researchers scientifically associated to CERM/CIRMMP: laboratories at the Department of Chemistry Ugo Schiff and at GENEXPRESS; DA VINCI European Biobank; X-rays facilities; Helium liquefier. **www.cerm.unifi.it**

Instruct-ERIC

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

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

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

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

- 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 2019 are:

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

H2020-INFRAIA iNEXT - Infrastructure for NMR, EM and X-ray crystallography for translational research (#653706)
http://www.inext-eu.org/

H2020-INFRADEV CORBEL - Coordinated research infrastructures building enduring life-science services (#654248)
http://www.corbel-project.eu/home.html

TRANSVAC2 - Improving and accellerating vaccine development in Europe

H2020-PHC Propag-ageing - The continuum between healthy ageing and idiopathic Parkinson disease within a propagation perspective of inflammation and damage: the search for new diagnostic, prognostic and therapeutic targets (#634821) https://www.propag-ageing.eu/

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

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

TIMB3 "Twin to Illuminate Metals in Biology and Bio-catalysis through Biospectroscopy" (H2020, #810856, 01/09/2018- 31/08/2021)

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

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

EOSC-Life brings together the 13 Life Science 'ESFRI' research infrastructures (LS RIs) to create an open, digital and collaborative space for biological and medical research. https://www.eosc-life.eu/


National Highly Relevance Projects - "Italia - Argentina"

Research Activities

Introduction

During 2019 a number of projects have been carried out, either as an extension of the activities of previous years or as new projects. Most of these projects receive specific funding from national and/or European organisations.

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

Scientific publications 2019

22%

In collaboration

CERM

78%
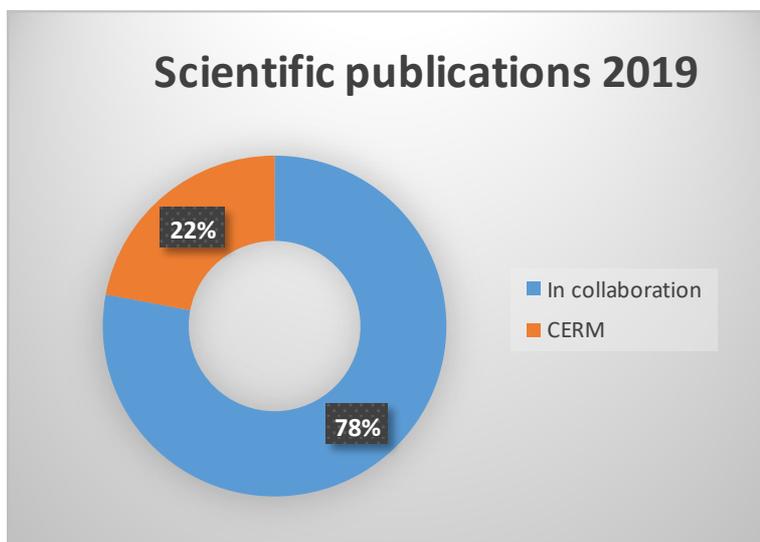
In line with our mission to develop NMR as a technique and to integrate NMR with other techniques, most of our publications were done in collaboration with other research groups (78% of the overall number of publications). During 2019 we published 60 papers in international peer-reviewed journals, with an average impact factor of 5.2. A complete list of publications is available at page 47.

CIRMMP has been ranked first among the Italian Inter-University Consortia in Chemical Science in the last evaluation of the quality of research (VQR 2011-14) by the National Agency for the Evaluation of the University and Research Systems. This excellent level of research of CERM/CIRMMP also contributed to having the Chemistry Department of the University Florence, to which most CERM scientists belong, ranked in the first place in the last Research Evaluation in the Chemical Science Area of the Italian Universities (VQR 2011-14). The Chemistry Department of the University of Florence was also winner of the national Project for Departments of Excellence. With the funds arrived with this Excellence National Project and in collaboration with CERM, the Department

of Chemistry will be endowed with a Cryo-EM microscope for high-resolution investigation of biomolecules and materials, and it will be accessible also to CERM researchers. This witnesses the impact of our research not only in the NMR field, but also in the larger chemical community and in the whole Italian research community.

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

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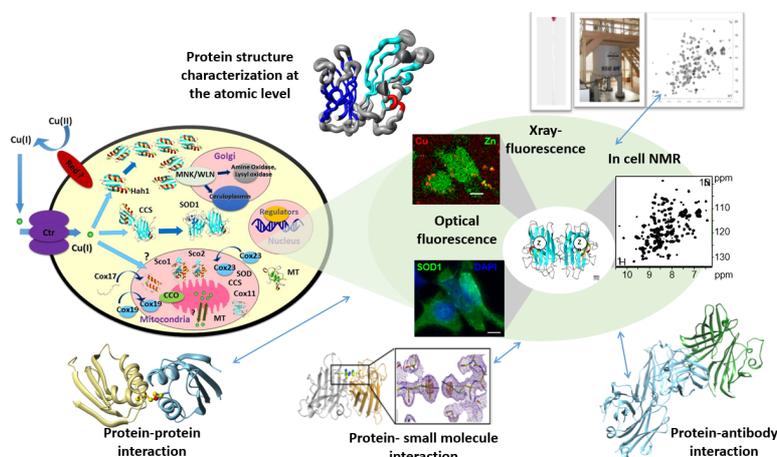
The Role of Solution NMR in Integrated Structural Biology

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

CERM applies solution NMR in an integrated systems biology approach for addressing more and more challenging questions. Such approach is routinely used to understand the role played by a protein in the frame of cellular metabolism, or to rationally engineer an enzyme for a specific industrial process, or to determine how to design novel drugs that target a particular protein, or to understand what changes might improve them.[1-3]

The major challenge of structural biology is understanding how proteins function at the cellular level, within macromolecular complexes, or in a cellular pathway.

Understanding dynamic processes that are co-ordinated at a cellular level is not possible using a single technology, but it becomes potentially accessible through the integration of a number of approaches, spanning different resolution scales.

References:

(1) Gourdoupis, S.; Nasta, V.; Ciofi-Baffoni, S.; Banci, L.; Calderone, V. *Acta Crystallogr. D Struct. Biol.* **2019**, 75, 317-324.

(2) Polykretis, P.; Luchinat, E.; Bonucci, A.; Giachetti, A.; Graewert, M.A.; Svergun, D.I.; Banci, L., *IUCrJ* **2019**, 6, 948-957.

(3) Cerofolini, L; Fragai, M; Ravera, E; Diebolder, CA; Renault, L; Calderone, V. *Biomolecules* **2019**, 9, 370.

The potential of integrated structural biology in unravelling biological processes.

Integrated Structural Techniques

While the number of high resolution structures and data obtained through different techniques increases steadily, it emerges the need of computational approaches to extract the most of the information, without over-interpretation.

NMR can be used to refine protein X-ray structures, drive EM reconstruction and monitor the presence of multiple conformational states.

Data integration in structural biology has become a paradigm for the characterisation of biomolecular systems, and it is now accepted that combining different techniques can fill the gaps in each other's blind spots.[1] We discuss the effect of imposing restraints based on the properties of the system to reduce the number of experimental data needed to obtain a more complete picture. We have introduced a set of new features of REFMAC-NMR that allow for including *a-priori* knowledge in the interpretation of NMR data for multi-domain and multi-subunit systems.[2] We have also developed methods to obtain information on the conformational variability of proteins with intrinsically disordered regions by using the MaxOcc approach. It aimed at identifying individual conformations, or groups thereof, that can exist for a large share of time in agreement with the average experimental data.[3,4] This conformational variability is also witnessed in the crystal state where two identical molecules, in the same asymmetric unit, can show different conformation geometries giving rise to a well known (yet largely unexplained) phenomenon like partial symmetry.
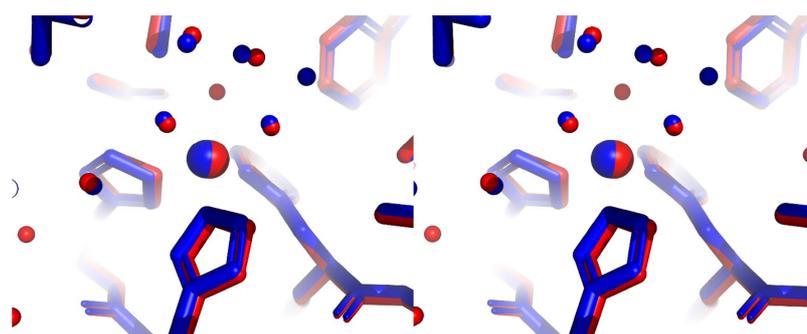
Stereo representation of the superposition of the active site region between molecule A and B in the asymmetric unit of Ni-hCAII. Penta-coordinated in blue and hexa in red.

References:

(1) Cerofolini, L.; Fragai, M.; Ravera, E.; Diebolder, C.A.; Renault, L.; Calderone, V. *Biomolecules* **2019**, 9, 370.

(2) Carlon, A.; Ravera, E.; Parigi, G.; Murshudov, G.N.; Luchinat, C. *J. Biomol. NMR* **2019**, 73, 265-278

(3) Carlon, A.; Gigli, L.; Ravera, E.; Parigi, G.; Gronenborn, A.M.; Luchinat, C. *Biophys. J.* **2019**, 117, 1948-1953

(4) Silva, J.M.; Giuntini, S.; Geraldes, C.F.G.C.; Macedo, A.L.; Ravera, E.; Fragai, M.; Luchinat, C.; Calderone, V. *J. Biol. Inorg. Chim. Acta* **2019**, 24, 91-101.

(5) Amato, J. Et al. *Nucleic Acid Res.* **2019**, 47, 9950-9966

In-cell NMR in Human Cells

Understanding of cellular processes requires all the involved partners to be characterised at the structural level.[1] To this aim, the *in-cell* NMR approach developed at CERM is being further extended and applied to human cells. The direct protein expression approach allows monitoring functional processes such as protein folding and maturation, cofactor binding and redox changes in response to external stimuli.[2,3]

The approach was applied to investigate the effect of cadmium, a highly toxic metal, on the maturation of intracellular superoxide dismutase 1 (SOD1).[4] We found that cadmium does not bind directly to SOD1 in place of zinc or copper. Instead, it induces the over-expression of metallothioneins (MTs) at NMR-detectable levels, in a zinc-dependent manner. With low-zinc, MT induction is inhibited and the consequent Cd-induced redox stress causes the premature formation of SOD1 disulfide bond. Conversely, high-zinc restores MT induction and prevents SOD1 oxidation.

To further expand the applicability of the methodology, a modular flow-NMR system was developed and optimized for multiple applications, both *in-cell* and *in vitro*.[5] Specifically, the NMR bioreactor improves cell viability and metabolic stability within the NMR spectrometer and allows time-resolved NMR experiments. *In vitro*, it allows efficient protein-based ligand screening under sample-limiting conditions. The design is now being applied to study protein-ligand interactions in human cells by time-resolved *in-cell* NMR.

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

References:

(1) Camponeschi, F., Banci, L.; *Pure Appl. Chem.* **2019**, 91, 231-245.

(2) Luchinat, E., Banci, L.; in *In-Cell NMR Spectroscopy: From Molecular Sciences to Cell Biology* (Eds.: Ito, Y., Dötsch, V., Shirakawa, M.), The Royal Society of Chemistry, **2019**, 45-61.

(3) Luchinat, E., Banci, L.; in *In-Cell NMR Spectroscopy: From Molecular Sciences to Cell Biology* (Eds.: Ito, Y., Dötsch, V., Shirakawa, M.), The Royal Society of Chemistry, **2019**, 207-227.

(4) Polykretis, P., Cencetti, F., Donati, C., Luchinat, E., Banci, L.; *Redox Biol.* **2019**, 21, 101102.

(5) Cerofolini, L., Giuntini, S., Barbieri, L., Pennestri, M., Codina, A., Fragai, M., Banci, L., Luchinat, E., Ravera, E.; *Biophys.l J.* **2019,** *116*, 239-247.

The NMR bioreactor design (left) exploits a microdialysis membrane to exchange fresh nutrients and remove metabolic by-products. Cell metabolic activity is measured by ^{31}P-NMR ATP measurement (right).

Structure-Based Vaccine Design

Vaccination is one of the major contributors to the global control of infections in human population. Solid-state NMR and isotope labelling methods can be used to characterise the structural features of the protein antigen components of vaccines and to investigate the preservation of the folding state of proteins adsorbed on Aluminium–OH matrix.

References:

(1) Cerofolini, L.; Giuntini, S.; Ravera, E.; Luchinat, C.; Berti, F.; Fragai, M. *NPJ Vaccines*. **2019**, 4, 20.

(2) Scarselli, M.; Aricò, B.;, Brunelli, B.;, Savino, S.;, Di Marcello, F.; Palumbo, E.;, Veggi, D.; Ciucchi, L.; Cartocci, E.;, Bottomley, M.J.; Malito, E.; Lo Surdo, P.; Comanducci, M.; Giuliani, M.M.; Cantini, F.; Dragonetti, S.; Colaprico, A.; Doro, F.; Giannetti, P.; Pallaoro, M.; Brogioni, B.; Tontini, M.; Hilleringmann, M.; Nardi-Dei, V.; Banci, L.; Pizza, M. Rappuoli; R. *Sci. Transl Med*. **2011**, 3, 91ra62.

The aluminum salts are the most commonly used adjuvants for commercial vaccines and, also due to their long-term success, they still remain the "gold standard" against a new adjuvant. In the final formulation the antigen is adsorbed on the Aluminium-based adjuvant and administered as precipitate. However, the mechanism of action of aluminum adjuvants for enhancing the immune response remains not fully understood, although they have been used over many years in vaccines for human use. The interaction with the adjuvant may alter folding, conformation and stability of the antigen. Alteration of folding and native conformational state of the epitopes may affect the immune response by influencing the antigen processing and presentation, the amount of protein bound to the adjuvant and its binding affinity. At CERM, the use of structural biology to develop new vaccines has already proved its effectiveness.[1] Now, we have shown that atomic structural details on protein antigens adsorbed on Aluminum gel adjuvant can be achieved, by solid-state NMR, from vaccine formulations obtained starting from isotopically enriched antigens and stored for several months.[2] The information provided by solid-state NMR offer a completely new approach, providing semiquantitative information on the adsorbed protein antigen and on its folding state. This is suitable for driving structure-based optimisation of vaccine formulation and manufacturing process.
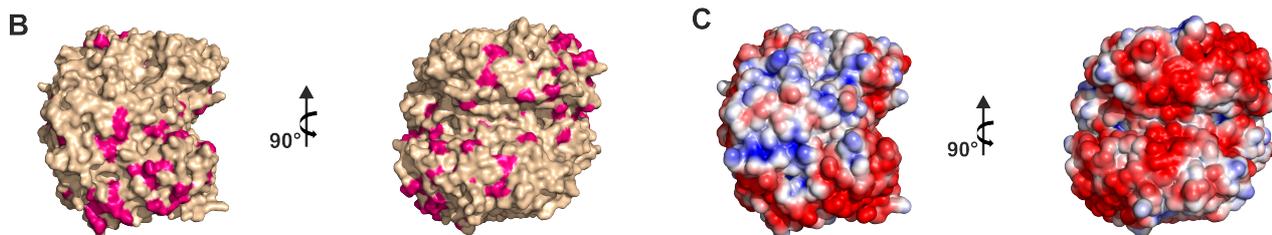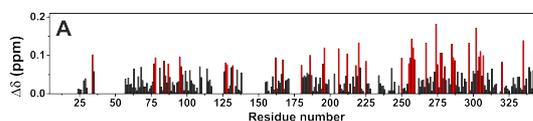

Chemical shift perturbations (CSP) of ANSII-AlumOH with respect to rehydrated freeze-dried ANSII and electrostatic potential on ANSII crystal structure.

Bioinformatics Tools for Metallo-Biology

In 2019 our work mainly focused on the application of our more recently developed software tools as well as on the exploitation of the contents of the newest release of METALPDB. In particular, we extended the METALPREDATOR tool to include additional metal ions with respect to its first release, and applied it to determine the zinc-proteome of *Acinetobacter baumannii*. The prediction was used to support the interpretation of experimental data on the expression of zinc proteins under different environmental conditions (zinc depletion/repletion). Further, we developed and validated a novel force-field in the context of the AMBER parameterisation for the simulation of zinc(II)-binding proteins. The proposed force-field assumes nonbonded spherical interactions between the central zinc(II) and the coordinating residues. A crucial innovative aspect of our approach is to account for the polarisation effects of the cation by redefining the atomic charges of the coordinating residues and an adjustment of Lennard-Jones parameters of Zn-interacting atoms to reproduce mean distance distributions.[1,2]

Despite metallo-proteins are present in all living organisms, playing a huge variety of fundamental biochemical processes, there is a paucity of computational resources focusing on these systems. We have developed several online resources and algorithms to investigate the role of metal ions in biological systems.
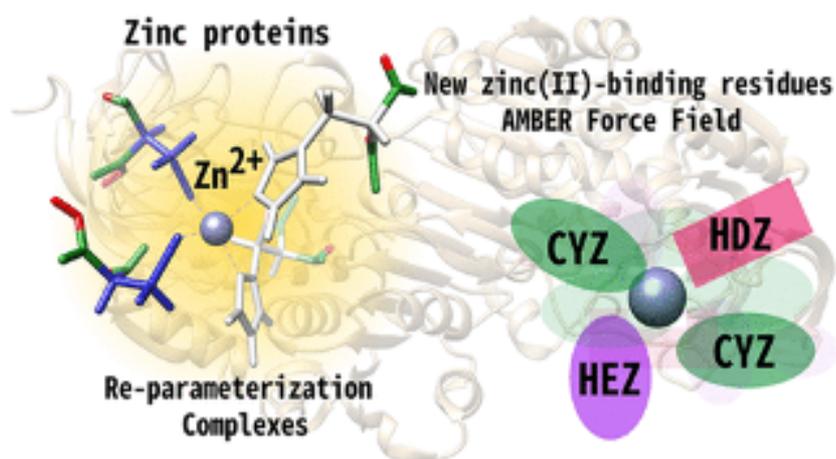
References:

(1) Macchiagodena M.; Pagliai M.; Andreini C.; Rosato A.; Procacci P.; *J. Chem. Inf. Model.* **2019**, 59 (9), 3803-3816

(2) Wang J.; Lonergan Z.R.; Gonzalez-Gutierrez G.; Nairn G.L.; Maxwell C.L.; Zhang Y.; Andreini C.; Karty J.A.; Chazin W.J.; Trinidad J.C.; Skaar E.P.; Giedroc D.P. *Cell Chem. Biol.* **2019**, 26 (5), 745-755. e7

Graphical representation of the reparametrisation procedure applied to the development of force-field parameters specific for zinc-bound Cys and His residues based on a non-bonded model.

Molecular Grounds of Iron-Sulfur Protein Biogenesis in Humans

Iron-sulfur (Fe-S) clusters are ancient protein cofactors involved in fundamental cellular processes. Despite the chemical simplicity of Fe-S cluster, their synthesis and assembly into apoproteins is a highly complex process in living cells. An increasing number of human diseases related to the misfunction of Fe-S protein biogenesis documents the importance of investigating such process in humans.

References:

(1) Nasta, V.; Suraci, D.; Gourdoupis, S.; Ciofi-Baffoni, S.; Banci, *FEBS J.* **2019** doi: 10.1111/febs.15140.

(2) Nasta, V.; Da Vela, S.; Gourdoupis, S.; Ciofi-Baffoni, S.; Svergun, D.; Banci, L.; *Sci. Rep.* **2019**, 18986.

CERM/CIRMMP focused one of its research activities to the investigation of the molecular mechanisms responsible of iron-sulfur protein biogenesis in humans. During 2019, structural information on the last steps of a mitochondrial machinery responsible of assembling [4Fe-4S] clusters and involving several proteins, namely ISCA1, ISCA2, IBA57, BOLA3 and NFU1, have been obtained. The use of an integrative approach, utilising information from small-angle X-ray scattering (SAXS) and bioinformatics-driven docking prediction, allow to determine a low-resolution structural model of the human mitochondrial [2Fe-2S]$^{2+}$ ISCA2-IBA57 complex. The latter model allowed us to define the molecular grounds of the pathogenic Arg146Trp mutation of IBA57. We also defined NFU1 protein as an 'assembler' of [4Fe-4S] clusters, that is, a protein able of converting two [2Fe-2S]$^{2+}$ clusters into a [4Fe-4S]$^{2+}$ cluster. We showed indeed that a [2Fe-2S]$^{2+}$ GLRX5-BOLA3 complex transfers its cluster to monomeric apo NFU1 to form, in the presence of a reductant, a [4Fe-4S]$^{2+}$ cluster bound to dimeric NFU1.[1,2]
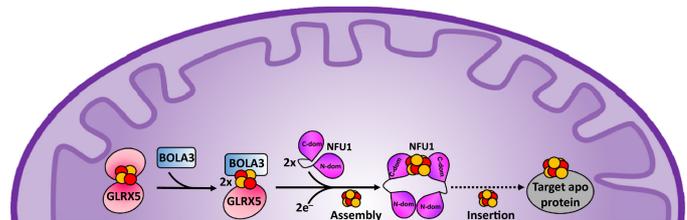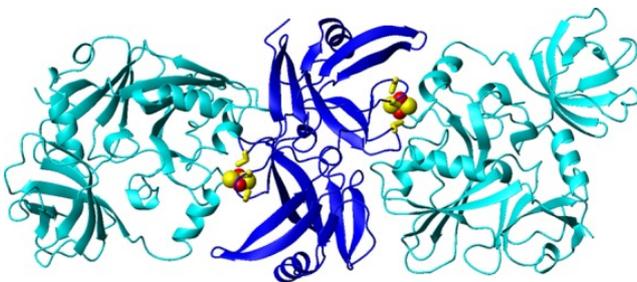
Left: Structure of the dimer of dimers of [2Fe-2S] ISCA2-IBA57. Right: Model of [4Fe-4S] cluster assembly pathway on NFU1.

Driving Iron Through the Ferritin Cage

Ferritin, in animals, is a polymer of 24 subunits that auto-assemble forming a spherical nanocage pierced by channels that keep in contact the external environment with the internal cavity. The biological function of ferritin is to sequester and concentrate iron inside its cavity in a hydrated ferric oxide mineral form, in order to prevent Fenton chemistry reactions within cells.

Ferritin's molecular weight is around 480–500 kDa. It represented a very challenging system for NMR study on the iron paths into the cage that required an integrated approach of solid-state MAS NMR and ^{13}C-^{13}C NOESY in solution.[1,2]

Time-lapse anomalous X-ray diffraction methods have been developed, allowing to follow the progressive population of transient iron binding sites along the path towards a well-defined di-iron ferroxidase site where they get oxidised by oxygen. Coupling structure determination of iron-loaded ferritin with solution stopped-flow kinetic measurements permits to validate the role played by His54, a key accessory iron binding residue affecting the catalytic iron oxidation.[3] The functional relevance of this site has been validated by measuring the reaction efficiency in solution in the H54N ferritin variant produced upon mutation of H54 observed to act as iron ligand.

Ferritin is a protein nanocage involved in cellular iron homeostasis. The iron pathways across different ferritins are continuously studied. A combination of time-lapse X-ray crystallography and kinetic data on wild type and selected ferritin variants, revealed new understandings about iron handling in ferritin from bullfrog.

References:

(1) Ciambellotti, S.; Turano, P. Eur. *J. Inorg. Chem.* **2019**, *5*, 569-576.

(2) Ghini, V.; Chevance, S.; Turano, P. *J. Inorg. Biochem.* **2019,** *192,* 25-32.

(3) Pozzi, C.; Di Pisa, F.; Lalli, D.; Rosa, C.; Turano, P.; Mangani, S. *J. Inorg. Biochem.* **2019,** *197,*110697.
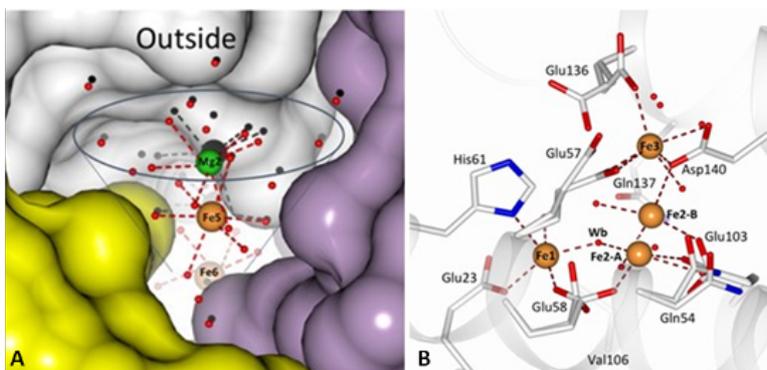
Iron pathway across ferritin: A) iron uptake at the entry-channel; B) population of iron binding sites that guide iron towards the catalytic center of ferritin and C) iron oxidation kinetics of ferritin.
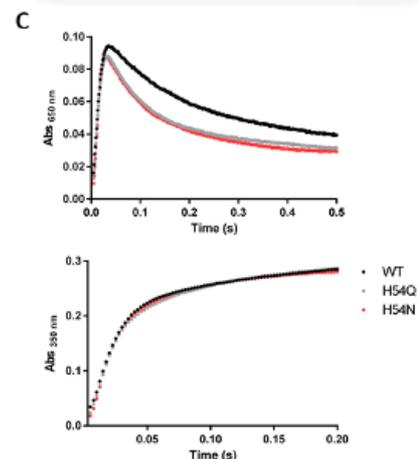
Intrinsically Disordered Proteins by NMR

Intrinsically Disordered Proteins (IDPs) represent an emerging field of research in modern protein chemistry. Present in any living organism they play key roles in a variety of different cellular pathways. NMR represents a unique tool for their investigation. Novel methods are under continuous development to identify functional modules enabled by disorder.

Novel methods based on NMR ^{13}C-direct detection were developed to determine simultaneous snapshots of IDPs in solution exploiting multiple receivers and to quantify paramagnetic relaxation enhancements originating from C', HN and Hα. In parallel, several IDPs of high biomedical interest were investigated. These include α-synuclein, E7 from papillomavirus, the N-terminal tail of the androgen receptor (AR) as well as osteopontin (OPN). Highlights from these studies include the elucidation of the role of aromatic-proline motives in the stabilisation the compact state of OPN and the identification of the mechanisms through which poly-Q tracts in the N-tail of AR adopt an α-helical conformation that protects them from aggregation.[1-5]

References:

(1) Schiavina, M.; Murrali, M.G.; Pontoriero, L.; Sainati, V.; Kümmerle, R., Bermel, W.; Pierattelli, R.; Felli, I.C. *Biophys. J.* **2019**, 117, 46-55.

(2) Mateos, B.; Konrat, R.; Pierattelli, R.; Felli, I.C. *CheBioChem*. **2019**, 20, 335-339.

(3) Mateos, B.; Conrad-Billroth, C.; Schiavina, M.; Beier, A.; Kontaxis, G.; Konrat, R.; Felli, IC.; Pierattelli, R. *J Mol. Biol.* **2019**, doi.org/10.1016/j.jmb.2019.11.015

(4) Kukic,P.; Lo Piccolo, M.; Nogueira, M.; Svergun, D.; Vendruscolo, M.; Felli, I.C.; and Pierattelli R. *Sci. rep.* **2019**, 9, 5822

(5) Escobedo, A. et al. *Nat. Commun.* **2019**, 10, 2034.
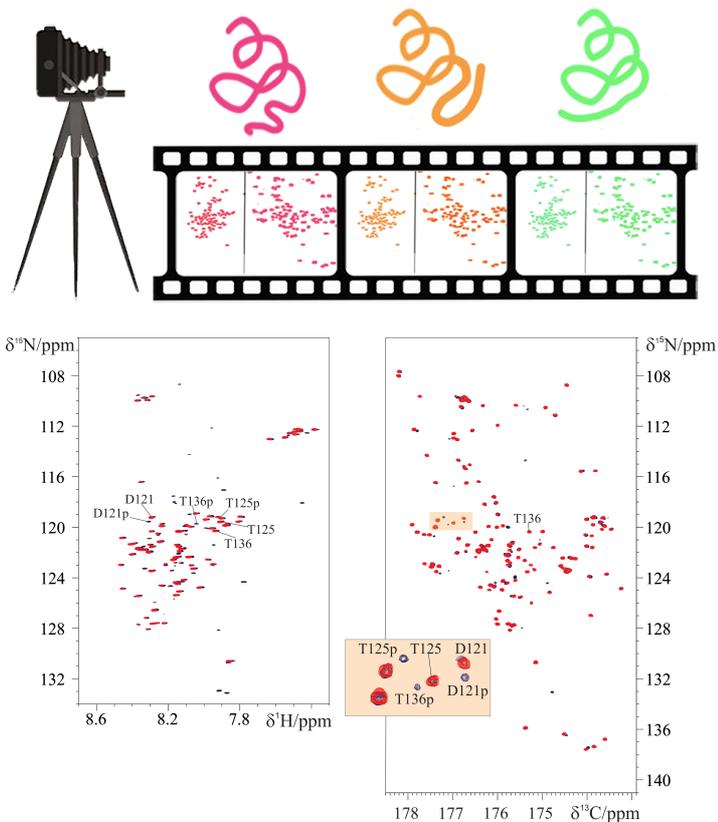
Acquiring simultaneous snapshots of IDPs in solution.

Distributed Computing in Structural Biology

Molecular Dynamics (MD), a computer simulation of the physical movements of atoms as a function of time, is a key computational technique in Structural Biology.
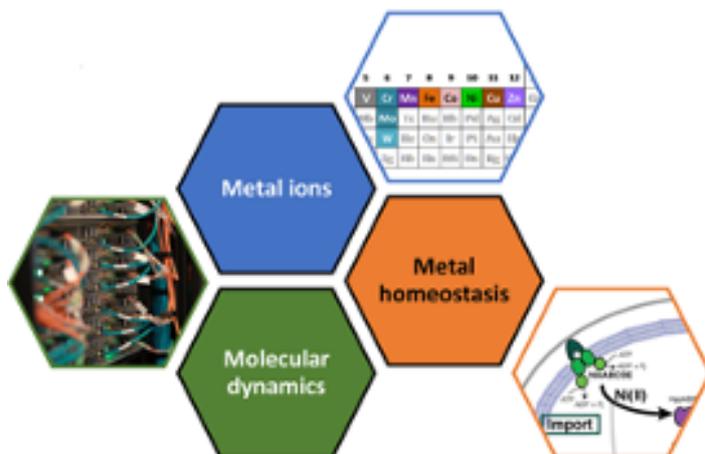
MD simulations capture the behaviour of biological macromolecules in full atomic detail. Such simulations provide information into biomolecular mechanisms at spatial and temporal scales that are difficult to observe experimentally. MD simulations are used also to improve the energetics and geometry of newly determined 3D structures of biological macromolecules.

Over the years, we developed methods for free and restrained MD simulations using a grid computational infrastructure, exploiting both traditional CPUs and GPUs. We applied these methods to obtain insight into the mechanisms of metal transport within cells.

To secure the computational power needed to make advanced MD techniques available to a large user basis, we are collaborating with EGI to have access to cloud-based computational resources.[1-4]

The processing and analysis of data describing the 3D structure and dynamics of biological macromolecules require the combined use of various software tools. To facilitate this task we have developed and applied standardised workflows using web interfaces.

The same concept will be extended to other workflows in the whole field of biomedicine.

The central role of MD simulations to understand the role of metal ions in cells, with a specific focus on metal homeostasis.

References:

(1) Morris, C.; [...] Rosato, A. et al. *J. Struct. Biol.*: X, **2019**, 100006.

(2) Peters, K.; [...] Rosato, A. et al. *GigaScience*, **2019**, 8, giy149.

(3) Sala, D.; Giachetti, A.; Rosato, A. *Biochim. Biophys. Acta (BBA) -Gen. Subj.* **2019**, 1863, 1560-1567.

(4) Macchiagodena M.; Pagliai M.; Andreini C.; Rosato A.; Procacci P. *J. Chem. Inf. Model.* **2019**, 59 (9), 3803-3816.

NMR of Paramagnetic Systems

The experimental and the-oretical developments that are achieved to study paramagnetic systems by NMR are continuously in-creasing. This offers the researchers a unique view on the molecular process-es underlying, for example, the catalytic activity of iron-sulfur enzymes or the be-haviour of small complexes in solution.
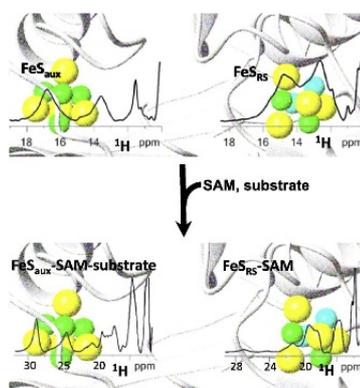
In paramagnetic compounds, from small complexes to complicated metal sites in protein and protein-protein complexes, NMR spectra are broad and/or distributed over wide frequency ranges. Tailored experiments can be performed to detect these resonances, and en-hance them over the other resonances. This spectral information is key to understanding the electronic structure of the paramagnetic center. Iron-Sulfur clus-ters in proteins (see page 20) give rise to very peculiar NMR spectra, which can be used as a diagnostic tool for the identification the electron distribution across FeS cluster. As an example, the events leading to catalysis in a S-Adenosylmethionine enzyme could be probed through the spin distribution across two [4Fe-4S] clusters via paramagnetic ^{1}H NMR spec-troscopy.[1]

The paramagnetic properties are faithful reporters of the electronic structure of metal centres: a straightfor-ward biunivocal relation among the electronic structure and the NMR observables would greatly improve the insight on the coordination geometry of metal centres in proteins.[2-4] We have used copper(II)-substituted human carbonic anhydrase to demonstrate the relation between EPR and NMR observables.[5]

Another example is the case of [Yb((S)-THP)]$^{3+}$ a chiral complex widely used for its magnetic and optical properties. Through the NMR investigation we were able not only to determine the detailed solution struc-ture, but we monitored also the dimerisation process occurring in solution, and the structural rearrangement induced by the intermole-cular interaction.[6]

References:

(1) Camponeschi, F.; Muzzioli, R.; Ciofi-Baffoni,S.; Piccioli, M.; Banci, L. *J. Mol. Biol.* **2019**, 431, 4514-4522;

(2) Parigi, G.; Benda, L.; Ravera, E.; Romanelli, M.; Luchinat, C. **2018**, *ArXiV*:1804.09055

(3) Parigi, G.; Ravera, E.; Luchinat, C. *Prog. NMR Spectrosc.* **2019**, 114-115, 211-236

(4) Parigi, G.; Ravera, E.; Luchinat, C. *J. Magn. Reson.* **2019**, 306, 173-179

(5) Cerofolini, L.; Silva, J.M.; Ravera, E.; Romanelli, M.; Geraldes, C.F.G.C.; Macedo, A.L.; Fragai, M.; Parigi, G.; Luchinat, C. *J. Phys. Chem. Lett.* **2019**, 10, 3610-3614.

(6) Lelli, M.; Di Bari, L.; *Dalton Trans.* **2019**, 48, 882-890.

The events of binding of a substrate to an iron-sulfur enzyme, and the catalytic mechanism can be investigated by the large shifts that the cluster imposes on the neighbouring residues.

Proteins and Drugs as Drug Targets

Structural characterisation of pharmacologically relevant proteins improves understanding and opens new ways for a drug rational design. However, the PEG coating that is used to evade the immune system also causes these molecules to "evade" the standard structural biology methodologies. For this reason, we have integrated complementary techniques, such as solution NMR, solid-state NMR and X-ray crystallography, to investigate the protein prior to and after PEGylation and to assess the structure preservation of large protein therapeutics upon bioconjugation.[1]

The pathological relevance of some proteins, such as matrix metalloproteinases, has prompted for many years the research and development of new drugs against these targets. We have summarised the recent advancements in the understanding of the mechanism of collagenolysis and elastolysis, and discussed the perspectives of new therapeutic strategies for targeting MMPs.[2]

Pharmaceutical research demands, with always greater extent, the characterisation at atomic detail of protein targets and biological drugs to improve both drug activity and safety. The advancements of the last years in the field of nuclear magnetic resonance can help to answer these growing needs.

References:

(1) Cerofolini, L.; Giuntini, S.; Carlon, A.; Ravera, E.; Calderone, V.; Fragai, M.; Parigi, G.; Luchinat, C. *Chem. Eur. J.* **2019**, 25, 1984-1991.

(2) Cerofolini, L.; Fragai, M.; Luchinat, C. *Curr. Med. Chem.* **2019**, 26, 2609-2633.
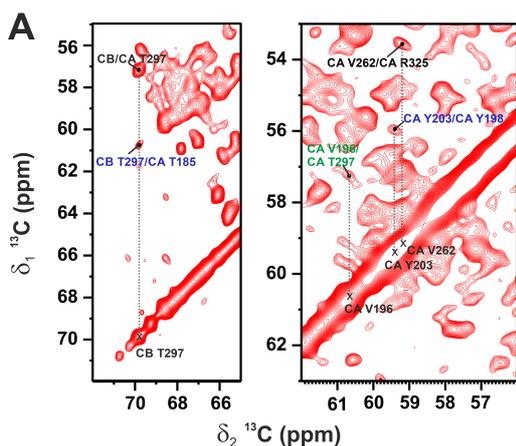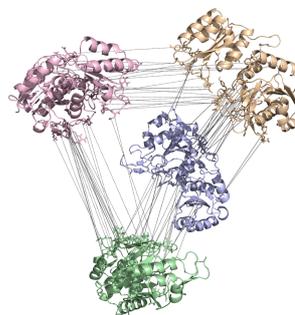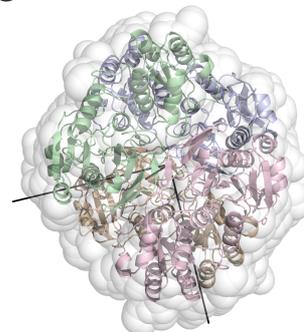
Assessment of the quaternary structure of asparaginase II after PEGylation, by collecting restraints from the analysis of solid-state NMR spectra and calculation of a model using computational programs.

Protein Aggregation Studied with Solution and Solid-State NMR

α-Synuclein (α-syn) is found to be naturally present in biofluids such as cerebrospinal fluid (CSF) and serum. Human serum albumin (HSA) is the most abundant protein found in these biofluids. HSA, at the concentration found in human serum interacts with α-syn and slows its aggregation.

References:

(1) Bellomo, G.; Bologna, S.; Cerofolini, L.; Paciotti, S.; Gatticchi, L.; Ravera, E.; Parnetti, L.; Fragai, M.; Luchinat, C. *J. Phys. Chem. B.* **2019**, 123, 4380-4386.

Misfolding and uncontrolled aggregation of α-synuclein (α-syn) is linked to the onset and progression of a branch of neurological disorders named synucleinopathies, which include Parkinson's disease (PD). Nowadays, the diagnosis of PD and other synucleinopathies still relies mainly on clinical symptoms. In this respect, the recent developments of real-time quaking-induced conversion (RT-QuIC) and α-syn protein misfolding cyclic amplification (PMCA) have provided new perspectives for the early-stage diagnosis of synucleinopathies.
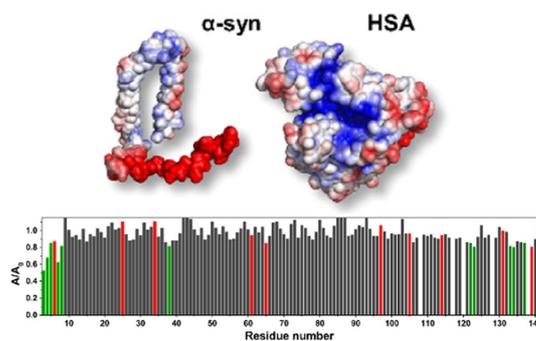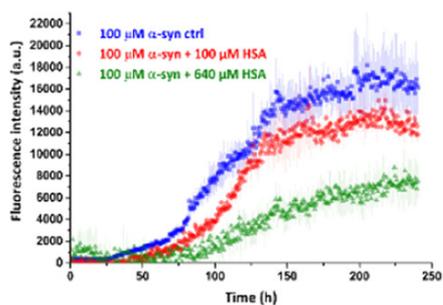
Human serum albumin (HSA) is the most abundant protein found in serum and recently, it has been reported to inhibit α-syn aggregation and membrane damage, produced by α-syn aggregates. However, these results appear in contrast to two previously published studies. To shed light on these contradictions and to clarify the partnership between α-syn and the most common protein in serum and CSF, we characterised the binding region of HSA on α-syn by high-field solution NMR and the inhibitory effect of HSA on α-syn aggregation kinetics by ThT fluorescence assays both in a low ionic strength environment and under physiological conditions. At CERM we found that HSA, at the concentration found in human serum, significantly slows the aggregation of α-syn. Furthermore, the interaction among these proteins is sensitive to ionic strength- and pH-dependent manner.[1]

Monomeric α-syn was left to aggregate in the presence of ThT and different HSA concentrations. Surface charge representation of α-syn and HSA, with blue and red representing regions of positive and negative electrostatic potentials, respectively. Intensity decreases of the signals of two-dimensional (2D) $^{15}N–^{1}H$ HSQC experiments acquired at 950 MHz on α-syn, after the addition of HAS.

FFC Relaxometry

FFC relaxometry is used for characterising the efficacy of paramagnetic complexes as contrast agents for MRI. Caspase probe 1 (CP1) is a bimodal fluorescence-magnetic resonance probe that exhibits response to caspase-3/7, which are enzymes activated during apoptosis. CP1 has three distinct components: a DOTA-Gd(III) chelate that provides the MR signal enhancement, tetraphenylethylene as the aggregation induced emission luminogen, and DEVD peptide which is a substrate for caspase-3/7. In response to caspase-3/7, the water-soluble peptide DEVD is cleaved and the remaining Gd(III)-conjugate aggregates leading to increased relaxation rates, thus providing an effective biomarker for apoptosis. Monitoring apoptosis in real-time provides invaluable information for evaluating cancer therapy response and screening preclinical anticancer drugs. In fact, effective cancer therapy largely depends on inducing apoptosis in cancer cells via chemotherapy and/or radiation.[1-4]

Fast field cycling (FFC) relaxometry can provide access to the structural and dynamic parameters on which nuclear relaxation depends, and it represents a precious tool for the optimisation of contrast agents for MRI, for the characterisation of dynamics in IDPs, and for understanding the mechanisms responsible for Overhauser DNP.
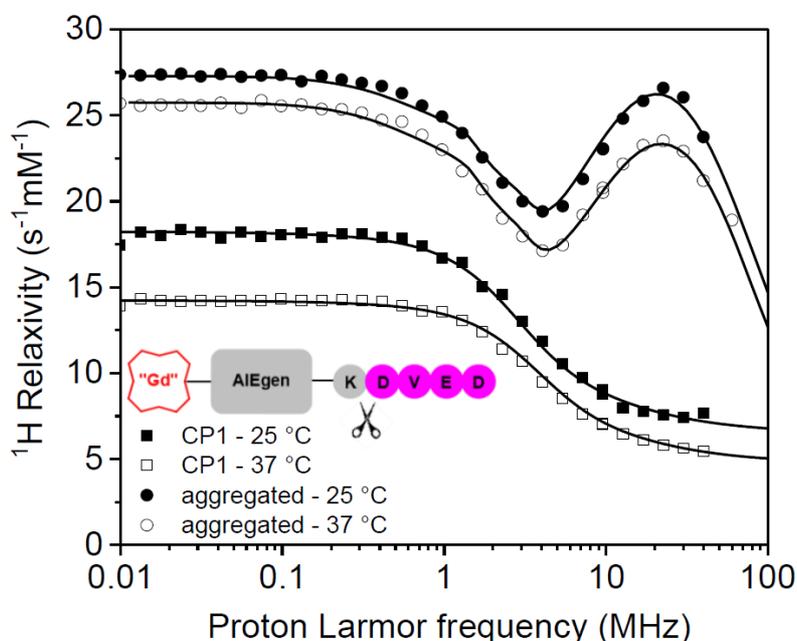
References:

(1) Li, H.; Parigi, G.; Luchinat, C.; Meade, T.J. *J. Am. Chem. Soc.* **2019,** *141*, 6224-6233.

(2) Rezaei-Ghaleh, N.; Parigi, G.; Zweckstetter, M. *J. Phys. Chem. Lett.* **2019,** *10,* 3369-3375.

(3) Parigi, G.; Ravera, E.; Bennati, M.; Luchinat, C. *Mol. Phys.* **2019,** *117,* 888-897.

(4) Fragai, M.; Ravera, E.; Tedoldi, F.; Luchinat, C.; Parigi, G. *Chemphyschem* **2019,** *20,* 2204-2209.

^{1}H NMRD profiles of CP1 and of its cleaved and aggregated form

Materials, Solid-state NMR Methods and DNP

Dynamic Nuclear Polarisation (DNP), makes it possible to increase sensitivity in solid-state NMR (ssNMR) by more than two orders of magnitude. This revolutionises the application of ssNMR in the characterisation of materials and biomolecule allowing, for example, the investigation of pharmaceutical directly in their commercial formulation.

References:

(1) Odermatt, N.T.; Lelli, M.; Herrmann, T; Abriata, L.A.; Japaridze, A.; Voilquin, H.; Singh, R.; Piton, J.; Emsley, L.; Dietler, G.; Cole, S.T. *J. Struct. Biol.* **2019**, *doi: 10.1016/j.jsb.2019.107434.*

(2) Viger-Gravel, J.; Avalos, C.E.; Kubicki, D.J.; Gajan, D.; Lelli, M.; Ouari, O.; Lesage, A.; Emsley, L. *Angew. Chem. Int. Ed. Engl.* **2019**, *58,* 7249-7253.

Solid state NMR (ssNMR) is the method of choice for the characterisation of many liquid crystals, solid chemicals and materials. In analogy with solution NMR, which is a well-established technique for structural and dynamic investigation in small molecules and even in complex biomolecules,[1] This technique is particularly useful for characterising commercial pharmaceutical formulations, which are often compound mixtures that include several solid phases. The main limitation to ssNMR is sensitivity, which is why we devote our efforts to extend DNP methods to characterise active pharmaceutical ingredients (API) and formulations. Here, we present a new method based on the generation of enhanced polarisation directly on ^{19}F nuclei and its propagation in the pharmaceutical formulation using an optimised fluorinated solvent. ^{19}F spectra with sensitivity improvements of the order of 70-80 can be obtained by exploiting the fluorine-fluorine spin diffusion. This approach allows us to characterise formulations with API in sub-milligram amounts and in natural isotopic abundance. The enhanced polarisation can then be transferred to spin ^{13}C or even used to acquire multidimensional correlation spectra. The impact of this technique on the pharmaceutical research and in the pharmaceutical industry can be enormous.

^{19}F Hahn echo spectra of 5-fluorouracil (12 kHz MAS, 9.4 T, 110 K), acquired with or without μwave irradiation (black). Sample (a) corresponds to 30 mg of API, containing 230 mmol of ^{19}F. Sample (b) is 0.293 mg of API mixed with 30.8 mg of cellulose, (2 mmol of F), and 12 mM AMUPol in TFE-d$_3$. The red trace is the simulated spectra.
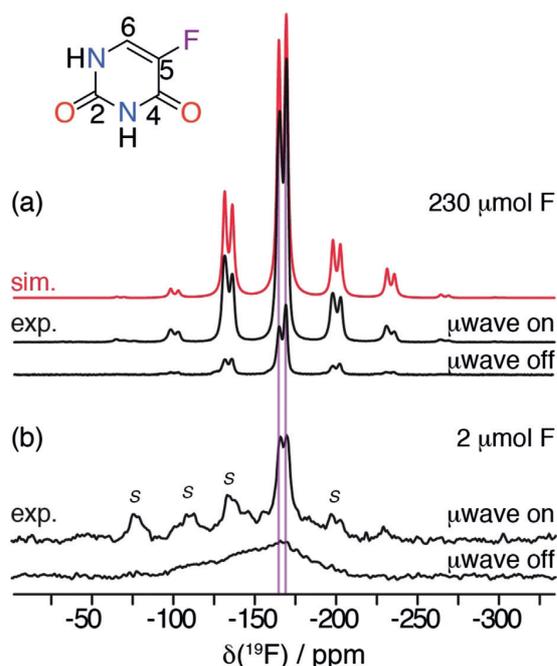
NMR in Metabolomic Fingerprinting

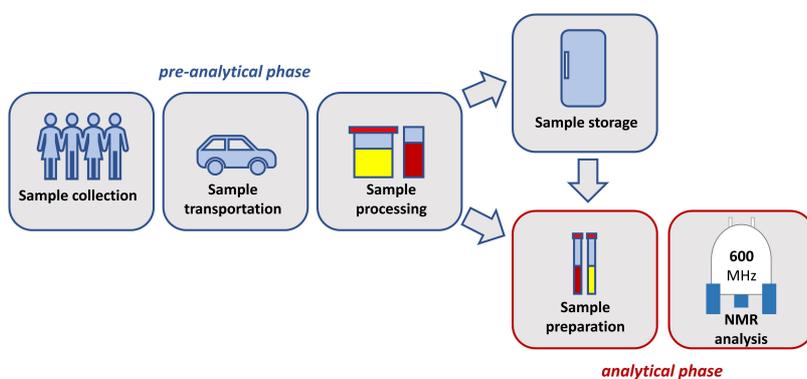Metabolomics deals with the -omic analysis of low molecular weight metabolites present in biological samples. NMR is one of principal analytical techniques used in metabolomics. NMR is intrinsically quantitative and highly reproducible and it allows for fast fingerprinting and profiling of a variety of samples.[1-3] The reliability, accuracy and reproducibility of NMR-based metabolomic analysis depends on the preservation of the chemical composition (in terms both of nature and concentration of metabolites) of the 'original' metabolome of a sample during the entire metabolomic workflow.

The pre-analytical phase includes different steps (sample collection, processing, transport and storage) that may influence the composition of the sample. Experimental validated procedures are developed to obtain high quality samples and to reduce errors in the analysis of biofluids like urine, plasma and serum.[4,5] Processing procedures affecting the metabolomic composition of food matrices, like olive oil, were also investigated.[6]

Also the analytical procedures, including sample preparation and NMR measurements, may influence the reliability of the results and detailed procedures are required to reduce errors and increase accuracy as far as possible. In this context, the addition of a small amount of paramagnetic gadolinium chelate, during the NMR sample preparation, enables faster and more efficient quantitative NMR experiments.[7]

A typical metabolomic workflow is made up of the pre-analytical phase, from sample collection to storage, and the analytical phase, including sample preparation and NMR measurements. Both phases comprise several steps that influence the final results. Validated and detailed procedures are required to improve the accuracy and the reproducibility of the analyses.

References:

(1) Vignoli, A.; *et al.* Angew *Chem. Int. Ed. Engl.* **2019**, 58, 968-994.

(2) Panteleimon, G.; *et al. TrAC* **2019**, 120, 115300.

(3) Saccenti, E.; *et al. Metabolites* **2019**, 9,E123.

(4) Ghini, V.; Quaglio, D.; Luchinat, C.; Turano, P. N *Biotechnol*. **2019**, 52, 25-34.

(5) Dagher, G.; *et al.* N *Biotechnol*. **2019**, 52,121-125.

(6) Dourou, A.M.; Brizzolara, S.; Meoni, G.; Tenori, L.; Famiani, F.; Luchinat C.; Tonutti, P. *Food Res. Int.* **2019**, 129,108861.

(7) Mulder, F.A.A.; Tenori, L.; Luchinat, C. A*ngew. Chem. Int .Ed. Engl.* **2019** 58, 15283-15286.

pre-analytical phase

Sample collection · Sample transportation · Sample processing · Sample storage · Sample preparation · 600 MHz NMR analysis

analytical phase

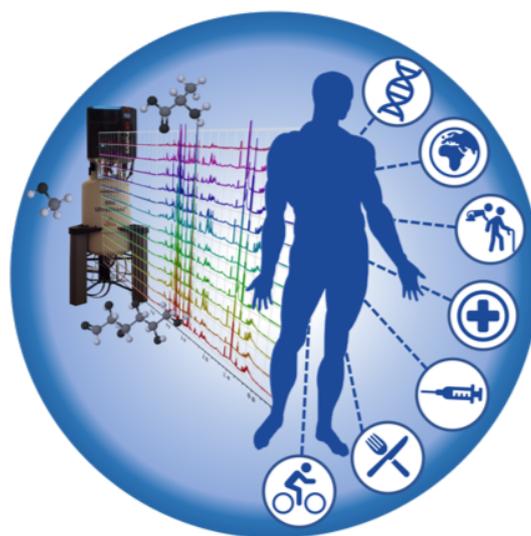Typical metabolomic workflow scheme.[4]

Metabolomics in Biomedicine

^{1}H NMR based metabolomics provides a dynamic portrait of the metabolic status of an individual, permitting the simultaneous identification and quantification of metabolites and small intermediate molecules. Thus, NMR metabolomics offers a molecular description of the health status of an individual for the characterisation of the metabolic signature of diseases for diagnostic and prognostic purposes and for the definition of individual susceptibility to treatments and environmental factors.

We have successfully exploited the application of NMR-based metabolomics in different pathological contexts, providing significant information on a wide range of pathologies. For example, we were able to characterise patients affected by hepatitis C infection with respect healthy controls and hepatitis B patients[1] and patients affected by primary cholangitis in comparison with patients affected by coeliac disease.[2] Metabolomics is particularly appropriate to develop prognostic models: we were able to derive a prognostic score based on NMR spectra able to identify the patients at risk of death within 2 years after the acute phase of a myocardial infarction.[3] Similarly, we developed a score to identify breast cancer patients with high risk to develop a relapse in the following 10 years.[4] Large scale population screening to assess the nutritional behaviour of individuals are also possible.[5] Urine and serum are the most employed human biofluids for metabolomic investigations, because they are easy to be collected and rich of systemic information. However, other more localized biofluids can be analysed, such as exhaled breath condensate (to study pulmonary diseases)[6] and saliva (to investigate oral pathologies).[7] Beside human samples, metabolomics can be used to study cellular models to investigate in vitro the effects of dietary[8] or pharmaceutical[9] interventions.

References:

(1) Meoni, G.; et al. *Sci. rep.* **2019**, 4128.

(2) Vignoli, A.; et al. *J. Proteom. Res.* **2019**

(3) Vignoli, A.; et al. *BMC Medicine.* **2019**, 17, 3.

(4) McCartney, A.; et al. *NPJ Breast Cancer.* **2019** 5, 26.

(5) Trimigno, A.; et al. *Mol. Nutr. Food Res.* **2019**, 63, 1.

(6) Vignoli, A.; et al. *J. Proteom. Res.* **2019** 1228.

(7) Romano, F.; at al. *Arch. Oral Biol.* **2019**, 97, 208.

(8) Ghini, V.; et al. *Nutrients.* **2019**.

(9) D'Alessandro, G.; et al. *Cell Commun. Signal.* **2019**, 108.

NMR-based metabolomics can investigate different aspects of human health.

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.

Since 2016, the access to European users is also provided through the newly funded iNEXT project (http://www.inext-eu.org). iNEXT is a consortium funded by the HORIZON2020 program to offer European researchers access to a wide range of advanced structural biology technologies (including X-ray technologies, NMR spectroscopy, Electron Microscopy and biophysics), to study the structure and function of biological macromolecules and their assemblies, and aspires to promote biomedicine, biotechnology, and biomaterials, involving scientists with or without previous experience in structural biology.

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

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

In all cases access is granted on the basis of peer-review of the received proposals, and after a feasibility check by the staff scientists of the receiving infrastructure. Technical assistance is

provided for the acquisition of the data. Scientific collaborations are welcome but not required. The uniqueness of access provision at CERM/CIRMMP infrastructure lies in the wide number of available NMR instruments, the variety of the NMR equipment (probes, automatic sample changers,…) and the exceptional expertise of the scientific and technical stuff, which represents an ideal environment for NMR research, especially in the field of structural and functional characterisation of biological systems. This allows the optimal use of the instrumentation also in a combined way, when needed. 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 39.
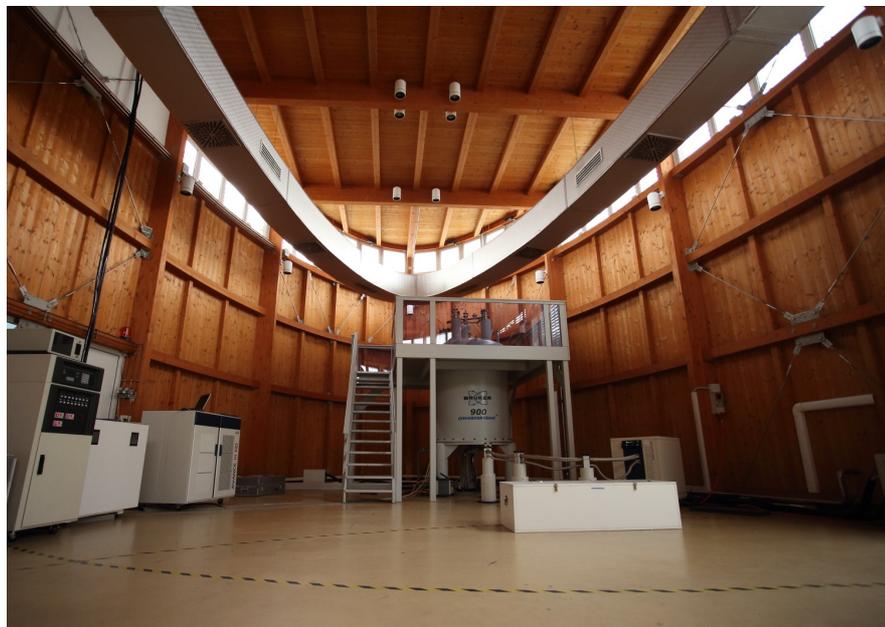
Molecular biology and cellular biology labs are also strategic for the users needs to prepare and/or optimise the large variety of samples for structural characterisation, together with other biophysical equipment for EPR, CD, UV-vis, stopped-flow measurements, manual and automated crystallisation facilities and X-ray diffractometry. Users can also access other university infrastructures available in the campus, such as those of mass spectrometry, Raman resonance, and non-linear spectroscopies.

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

During 2019 CERM/CIRMMP provided overall 539 days of NMR access to external users. A more detailed analysis shows that the access in the frame of INSTRUCT-ERIC, INSTRUCT-ITALIA, CORBEL and iNEXT, were overall 368 days in 2019.

Collaborations with Industries

CERM/CIRMMP has a long tradition in collaborations with industries: from simply providing access and service to its instrumentation, to establishing a more collaborative activity in research projects or to the participation as partners in international project calls. In 2019, thanks to the freshly inaugurated centre CERM TT and the Bio-Enable project, the pay-for-services access to industries was overall 171 days. This number does not include the access provided industrial partners through collaborative projects.

We warmly thank the following companies for stimulating interactions:

Bracco SpA

Bruker BioSpin

Dompé Pharmaceutical

Italmatch Chemicals

Glaxo Smith Kline

Giotto Biotech Srl

Merck

COLLABORATION WITH INDUSTRIES

Menarini Srl

Valagro S.p.a.

Abiogen S.p.a.

Infineum

Danger and Safety

Buona Steve Jones

A special acknowledgment to Gruppo SAPIO Srl,

official supplier of all the cryogenic gases of CERM/CIRMMP

Flanking Institutions

Da Vinci European BioBank

The Da Vinci European Biobank (daVEB) is handled by CsaVRI (Centro Servizi Di Ateneo Per la Valorizzazione della Ricerca e la Gestione dell'Incubatore) and it is certified ISO9001:2015. It is a research biobank that stores human biospecimens (plasma, serum, urine, tissues, cells), and bacterial expression vectors at cryogenic temperatures (Mechanical freezers for storage at –80 °C, equipped with auxiliary LN2 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 and its spin-off FiorGen foundation.

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: 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 in-

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

In 2019 Giotto Biotech has been involved in several research projects funded at the European or National level (FLAG-ERA-ITFoC, Information Technology: Future of Cancer Treatment; ITN EC RNAct, Enabling proteins with RNA recognition motifs for synthetic biology and bio-analytics.; SENSOGM, Development of biophotonic sensors for environmental determination of GMOs, funded by the Tuscany Region; SATURNO, Scarti organici e Anidride carbonica Trasformati in carbURanti, fertilizzanti e prodotti chimici; applicazione concreta dell'ecoNOmia circolare, funded by Piemonte Region).

Giotto Biotech research activity is carried out in synergy with CERM scientists. As an outcome of this collaboration, in 2019 Giotto Biotech and CERM researchers co-authored twelve scientific publications. Among industrial collaborations, Giotto Biotech is partner with the NMR manufacturer, Bruker Biospin, in the development of expert systems to assign metabolite signals in biofluids to perform quantitation without human intervention.

http://www.giottobiotech.com/

Fondazione Luigi Sacconi

The Luigi Sacconi Foundation was established in 1996 to honour the memory of *Prof. Luigi Sacconi* who was a prominent figure in Chemistry and founder of the General and Inorganic Chemistry School in Florence where many international scientists have been educated.

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

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

The Sacconi Memorial Lecture 2019 has been awarded to Prof. Prof. Gaetano T. Montelione director of the Northeast Structural Genomics Consortium (Department of Molecular Biology and Biochemistry, Rutgers University The State University of New Jersey, Piscataway, NJ, USA).

The Sacconi Medal Lecturer 2019 has been awarded to Dr. Liberato Manna, director of the Nanotechnology Department of the Italian institute of Technology (IIT, Genoa)

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

Instrumentation

Solution and Solid-State NMR Spectrometers

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.

B_0 Field (T)	^{1}H Larmor Frequency (Bore)	Probe heads
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-band (9.43 GHz), Q-Band (35 GHz)	X and Q Band cavities
0.00024-1	Fast Field Cycling Relaxometer	0.01-45 MHz 10 mm solution tubes

*With sample changer

X-ray Crystallography

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

Biological and Biophysical Facilities and Services

Molecular and Cellular Biology

CERM/CIRMMP is equipped with state-of-the-art facilities for gene cloning and protein expression and purification. Large scale protein expression in prokaryotes and yeast is available through the use of fermenters. Different isotope labelling schemes, including specific labelling schemes oriented to NMR characterisation, can be achieved through the use of auxotrophic strains. Fully equipped facilities for protein purification are available, including last-generation instruments for streamlined purification (ÄKTA pure chromatography system) and equipment for protein purification and reconstitution in anaerobic environment (glove box). A mammalian expression lab for in-cell NMR is also available.

EPR

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

Multi Angle/Dynamic Light Scattering

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

Isothermal Calorimetry (ITC)

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

Optical Spectroscopy

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

Computational Structural Biology Tools

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

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

Electronic infrastructure (e-infrastructure)

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

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

Training & Education

International Doctorate in Structural Biology

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

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

The scientific themes covered by the PhD course are:

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

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

3. **Drug and vaccine development**, through rational design techniques and structural characterisation of biological drugs;

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

5. *In cell* **NMR** studies, by which molecular pathways and cell import-export mechanisms are investigated;

6. **Metabolomics** studies, in which the individual metabolic fingerprints are related to disease states and fingerprints are utilised to provide early diagnosis or even identification of pre-disease states.

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

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

Post-Doctorate

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

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PUBLICATIONS

List of publications

1. Li H, Parigi G, Luchinat C, Meade TJ. Bimodal Fluorescence-Magnetic Resonance Contrast Agent for Apoptosis Imaging, **J. Am. Chem. Soc.** (2019) 141, 6224-6233 (IF 14.695)

2. Mulder FAA, Tenori L, Luchinat C. Fast and Quantitative NMR Metabolite Analysis Afforded by a Paramagnetic Co-Solute. **Angew. Chem. Int. Ed. Engl.** (2019), 58, 15283-15286. doi: 10.1002/anie.201908006. (IF 12.257)

3. Vignoli A, Ghini V, Meoni G, Licari C, Takis PG, Tenori L, Turano P, Luchinat C. High- Throughput Metabolomics by 1D NMR. **Angew. Chem. Int. Ed. Engl.** (2019), 58, 968-994. doi: 10.1002/anie.201804736. (IF 12.257).

4. Viger-Gravel J, Avalos CE, Kubicki DJ, Gajan D, Lelli M, Ouari O, Lesage A, Emsley L. 19F Magic Angle Spinning Dynamic Nuclear Polarization Enhanced NMR Spectroscopy. **Angew. Chem. Int. Ed. Engl.** (2019), 7249-7253, (IF 12.257)

5. Escobedo A, Topal B, Kunze MBA, Aranda J, Chiesa G, Mungianu D, Bernardo-Seisdedos G, Eftekharzadeh B, Gairí M, Pierattelli R, Felli IC, Diercks T, Millet O, García J, Orozco M, Crehuet R, Lindorff-Larsen K, Salvatella X. Side chain to main chain hydrogen bonds stabilize a polyglutamine helix in a transcription factor. **Nat. Commun.** (2019) 2; 102034. doi: 10.1038/s41467-019-09923-2. (IF 11.878)

6. Eftekharzadeh B, Banduseela VC, Chiesa G, Martínez-Cristóbal P, Rauch JN, Nath SR, Schwarz DMC, Shao H, Marin-Argany M, Di Sanza C, Giorgetti E, Yu Z, Pierattelli R, Felli IC, Brun-Heath I, García J, Nebreda ÁR, Gestwicki JE, Lieberman AP, Salvatella X. Hsp70 and Hsp40 inhibit an inter-domain interaction necessary for transcriptional activity in the androgen receptor. **Nat. Commun.** (2019),10, 3562. (IF 11.878)

7. Amato J, Cerofolini L, Brancaccio D, Giuntini S, Iaccarino N, Zizza P, Iachettini S, Biroccio A, Novellino E, Rosato A, Fragai M, Luchinat C, Randazzo A, Pagano B. Insights into telomeric G-quadruplex DNA recognition by HMGB1 protein. **Nucleic Acids Res.** (2019), 47, 9950-9966 (IF 11.147)

8. Parigi G, Ravera E, Luchinat C. Magnetic susceptibility and paramagnetism-based NMR. **Prog. NMR Spectrosc.** (2019), 114-115, 211-236. (IF 8.848)

9. 10.Takis PG, Ghini V, Tenori L, Turano P, Luchinat C. Uniqueness of the NMR approach to metabolomics. **TrAC.** (2019), 120, 115300, (IF 8.428)

10. Vignoli A, Tenori L, Giusti B, Takis PG, Valente S, Carrabba N, Balzi D, Barchielli A, Marchionni N, Gensini GF, Marcucci R, Luchinat C, Gori AM. NMR-based Metabolomics identifies patients at high risk of death patients within two years after acute myocardial infarction in the AMI-Florence II cohort. **BMC Med.** (2019), 17, 3, (IF 8.285)

PUBLICATIONS

11. Polykretis P, Cencetti F, Donati C, Luchinat E, Banci L. Cadmium effects on superoxide dismutase 1 in human cells revealed by NMR. **Redox Biol.** (2019); 21:101102. (IF 7.793)

12. Cerofolini L, Silva JM, Ravera E, Romanelli M, Geraldes CFGC, Macedo AL, Fragai M, Parigi G, Luchinat C. How Do Nuclei Couple to the Magnetic Moment of a Paramagnetic Center? A New Theory at the Gauntlet of the Experiments. **J. Phys. Chem. Lett.** (2019), 10, 3610-3614. (IF 7.329)

13. Rezaei-Ghaleh N, Parigi G, Zweckstetter M. Reorientational Dynamics of Amyloid-β from NMR Spin Relaxation and Molecular Simulation, **J. Phys. Chem. Lett.** (2019), 10, 3369-3375. (IF 7.329)

14. Cerofolini L, Giuntini S, Carlon A, Ravera E, Calderone V, Fragai M, Parigi G, Luchinat C. "Characterization of PEGylated asparaginase: new opportunities from NMR analysis of large pegylated therapeutics", **Chem. Eur. J.** (2019), 25, 1984-1991. (IF 5.160)

15. D'Alessandro G, Quaglio D, Monaco L, Lauro C, Ghirga F, Ingallina C, De Martino M, Fucile S, Porzia A, Di Castro MA, Bellato F, Mastrotto F, Mori M, Infante P, Turano P, Salmaso S, Caliceti P, Di Marcotullio L, Botta B, Ghini V, Limatola C. 1H-NMR metabolomics reveals the Glabrescione B exacerbation of glycolytic metabolism beside the cell growth inhibitory effect in glioma. **Cell Commun. Signal.** (2019), 108. doi: 10.1186/s12964-019-0421-8. (IF 5.111)

16. Varone A, Mariggiò S, Patheja M, Maione V, Varriale A, Vessichelli M, Spano D, Formiggini F, Lo Monte M, Brancati N, Frucci M, Del Vecchio P, D'Auria S, Flagiello A, Iannuzzi C, Luini A, Pucci P, Banci L, Valente C, Corda D. A signalling cascade involving receptor-activated phospholipase A2, glycerophosphoinositol 4-phosphate, Shp1 and Src in the activation of cell motility. **Cell Commun .Signal.** (2019), 17(1):20. doi: 10.1186/s12964-019-0329-3. (IF 5.111)

17. Camponeschi F, Muzzioli R, Ciofi-Baffoni S, Piccioli M, Banci L. Paramagnetic 1H NMR Spectroscopy to Investigate the Catalytic Mechanism of Radical S-Adenosylmethionine. **J. Mol. Biol.** (2019), S0022-2836(19)30542- (IF 5.067)

18. Cerofolini L, Giuntini S, Ravera E, Luchinat C, Berti F, Fragai M. Structural characterization of a protein adsorbed on aluminum hydroxide adjuvant in vaccine formulation. **NPJ Vaccines.** (2019). doi: 10.1038/s41541-019-0115-7. eCollection 2019. (IF 5.020)

19. Polykretis P, Luchinat E, Bonucci A, Giachetti A, Graewert MA, Svergun DI, Banci L. Conformational characterization of full-length X-chromosome-linked inhibitor of apoptosis protein (XIAP) through an integrated approach. **IUCrJ** (2019), 26(5) (IF 4.756)

20. Nasta V, Suraci D, Gourdoupis S, Ciofi-Baffoni S, Banci L. A pathway for assembling [4Fe-4S]2+ clusters in mitochondrial iron-sulfur protein biogenesis. **FEBS J.** (2019). doi: 10.1111/febs.15140 (IF 4.739)

21. Cerofolini L, Fragai M, Ravera E, Diebolder CA, Renault L, Calderone V. Integrative Approaches in Structural Biology: A More Complete Picture from the Combination of Individual Techniques. **Biomolecules** (2019), 9(8), 370. (IF 4.694)

22. Trimigno A, Khakimov B, Savorani F, Tenori L, Hendrixson V, Čivilis A, Glibetic M, Gurinovic M, Pentikäinen S, Sallinen J, Garduno Diaz S, Pasqui F, Khokhar S, Luchinat C, Bordoni A, Capozzi F, Balling Engelsen S. Investigation of Variations in the Human Urine Metabolome amongst European Populations: An Exploratory Search for Biomarkers of People at Risk-of- Poverty. **Mol. Nutr. Food Res.** (2019) 63(1):e1800216. doi: 10.1002/mnfr.201800216.(IF 4.653)

23. Lelli M, Di Bari L. Solution structure and structural rearrangement in chiral dimeric ytterbium(iii) complexes determined by paramagnetic NMR and NIR-CD. **Dalton Trans.** (2019); 48, 882-890. doi: 10.1039/c8dt03090a. (IF 4.052)

24. Meoni G, Lorini S, Monti M, Madia F, Corti G, Luchinat C, Zignego AL, Tenori L, Gragnani L. The metabolic fingerprints of HCV and HBV infections studied by Nuclear Magnetic Resonance Spectroscopy. **Sci. Rep.** (2019), 4128. doi: 10.1038/s41598-019-40028-4 (IF 4.011)

25. Kukic P, Lo Piccolo GM, Nogueira M, Svergun D, Vendruscolo M, Felli I, Pierattelli R. The free energy landscape of the oncogene protein E7 of human papilloma virus type 16 reveals a complex interplay between ordered and disordered regions. **Sci. Rep.** (2019), 9, 5822 (IF 4.011)

26. Daniels MJ, Nourse JB, Kim JrH, Sainati V, Schiavina M, Murrali MG, Pan B, Ferrie J, Haney CM, Moons R, Gould NS, Natalello A, Grandori R, Sobott F, Petersson EJ, Rhoades E, Pierattelli R, Felli IC, Uversky VN, Caldwell KA, Caldwell GA, Krol ES, Ischiropoulos H. Cyclized NDGA modifies dynamic -synuclein monomers preventing aggregation and toxicity. **Sci. Rep.** (2019), 9, 2937 (IF 4.011)

27. Nasta V, Da Vela S, Gourdoupis S, Ciofi-Baffoni S, Svergun D, Banci L. Structural properties of [2Fe-2S] ISCA2-IBA57: a complex of the mitochondrial iron-sulfur cluster assembly machinery". **Sci. Rep.** (2019), 9, 18986 (IF 4.011)

28. Becatti M, Bencini A, Nistri S, Conti L, Fabbrini MG, Lucarini L, Ghini V, Severi M, Fiorillo C, Giorgi C, Sorace L, Valtancoli B, Bani D. Different Antioxidant Efficacy of Two MnII- Containing Superoxide Anion Scavengers on Hypoxia/Reoxygenation-Exposed Cardiac Muscle Cells. **Sci Rep.** (2019); 9, 10320. (IF 4.011)

29. Macchiagodena M, Pagliai M, Andreini C, Rosato A, Procacci P. Upgrading and Validation of the AMBER Force Field for Histidine and Cysteine Zinc(II)-Binding Residues in Sites with Four Protein Ligands. **J. Chem. Inf. Model.** (2019), 59, 3803-3816. doi: 10.1021/acs.jcim.9b00407. (IF 3.966)

30. Cerofolini L, Fragai M, Luchinat C. Mechanism and Inhibition of Matrix Metalloproteinases. **Curr. Med. Chem.** (2019); 26, 2609-2633. (IF 3.894).

31. Vignoli A, Orlandini B, Tenori L, Biagini MR, Milani S, Renzi D, Luchinat C, Calabrò AS. Metabolic Signature of Primary Biliary Cholangitis and Its Comparison with Celiac Disease. **J. Proteome Res.** (2019) 1228-1236. doi: 10.1021/acs.jproteome.8b00849 (IF 3.780)

32. J. Malanho Silva, L. Cerofolini, S. Giuntini, V. Calderone, C. Geraldes, A. Macedo, G. Parigi, M. Fragai, E. Ravera, C. Luchinat, Metal centers in biomolecular solid-state NMR. **J. Struct. Biol.** (2019), 206, 99-109 (IF 3.754)

33. Odermatt NT, Lelli M, Herrmann T, Abriata LA, Japaridze A, Voilquin H, Singh R, Piton J, Emsley L, Dietler G, Cole ST. Structural and DNA binding properties of mycobacterial integration host factor mIHF. **J. Struct. Biol.** (2019), 107434. doi: 10.1016/j.jsb.2019.107434 (IF. 3.754)

34. Dagher G, Becker KF, Bonin S, Foy C, Gelmini S, Kubista M, Kungl P, Oelmueller U, Parkes H, Pinzani P, Riegman P, Schröder U, Stumptner C, Turano P, Sjöback R, Wutte A, Zatloukal K. Pre-analytical processes in medical diagnostics: New regulatory requirements and standards. **N. Biotechnol.** (2019), 52:121-125. doi: 10.1016/j.nbt.2019.05.002 (IF 3.739)

35. Ghini V, Quaglio D, Luchinat C, Turano P. NMR for sample quality assessment in metabolomics. **N. Biotechnol.** (2019), 52, 25-34 (IF 3.739)

36. Sala S, Giachetti A, Rosato A. An atomistic view of the YiiP structural changes upon zinc (II) binding. **BBA Gen. Subj.** (2019), 1863,1560-1567 (IF 3.681)

37. Carlon A, Gigli L, Ravera E, Parigi G, Gronenborn AM, Luchinat C. Assessing Structural Preferences of Unstructured Protein Regions by NMR. **Biophys. J.** (2019), 117, 1948-1953. (IF 3.665)

38. Cerofolini L, Giuntini S, Barbieri L, Pennestri M, Codina A, Fragai M, Banci L, Luchinat E, Ravera E. Real-Time Insights into Biological Events: In-Cell Processes and Protein-Ligand Interactions. **Biophys J.** (2019), 116, 239-247. (IF 3.665)

39. Schiavina M, Murrali MG, Pontoriero L, Sainati V, Kümmerle R, Bermel W, Pierattelli R, Felli IC. Taking Simultaneous Snapshots of Intrinsically Disordered Proteins in Action. **Biophys. J.** (2019),117, 46-55 (IF 3.665)

40. Silva JM, Giuntini S, Cerofolini L, Geraldes CFGC, Macedo AL, Ravera E, Fragai M, Luchinat C, Calderone V. Non-crystallographic symmetry in proteins: Jahn-Teller-like and Butterfly-like effects? **J. Biol. Inorg. Chem.** (2019), 24, 91-101. doi: 10.1007/s00775-018-1630-0. (IF 3.632)

41. Dourou AM, Brizzolara S, Meoni G, Tenori L, Famiani F, Luchinat C, Tonutti P. The inner temperature of the olives before processing affects the volatile profile and the composition of the oil. **Food Res. Int.** (2019), 129, 108861. doi: 10.1016/j.foodres.2019.108861 (IF 3.579)

42. Emwas AH, Roy R, McKay RT, Tenori L, Saccenti E, Gowda GAN, Raftery D, Alahmari F, Jaremko L, Jaremko M, Wishart DS. NMR Spectroscopy for Metabolomics Research. **Metabolites** (2019), pii: E123. doi: 10.3390/metabo9070123. (IF 3.303)

43. Gourdoupis S, Nasta V, Ciofi-Baffoni S, Banci L, Calderone V. In-house high-energy-remote SAD phasing using the magic triangle: how to tackle the P1 low symmetry using multiple orientations of the same crystal of human IBA57 to increase the multiplicity. **Acta Crystallogr. D Struct. Biol.** (2019), 75(Pt 3):317-324 doi: 10.1107/S2059798319000214 (IF 3.227)

44. Ghini V, Chevance S, Turano P. About the use of 13C-13C NOESY in bioinorganic chemistry. **J. Inorg. Biochem.** (2019), 192:25-32. doi: 10.1016/j.jinorgbio.(IF 3.224)

45. Pozzi C, Di Pisa F, Lalli D, Rosa C, Turano P, Mangani S. Effect of the point mutation H54N on the ferroxidase process of Rana catesbeiana H' ferritin. **J. Inorg. Biochem.** (2019), 197:110697. doi: 10.1016/j.jinorgbio.2019.110697.(IF 3.224)

46. Fragai M, Ravera E, Tedoldi F, Luchinat C, Parigi G. Relaxivity of Gd-based MRI contrast agents in crosslinked hyaluronic acid as a model for tissues. **ChemPhysChem.** (2019), 20, 2204- 2209 (IF 3.077)

47. Parigi G, Benda L, Ravera E, Romanelli M, Luchinat C. Pseudocontact shifts and paramagnetic susceptibility in semiempirical and quantum chemistry theories, **J. Chem. Phys.** (2019), 150, 144101 (1-11). (IF 2.997)

48. Bellomo G, Bologna S, Cerofolini L, Paciotti S, Gatticchi L, Ravera E, Parnetti L, Fragai M, Luchinat C. Dissecting the Interactions between Human Serum Albumin and α-Synuclein: New Insights on the Factors Influencing α-Synuclein Aggregation in Biological Fluids. **J. Phys. Chem. B** (2019), 123, 4380-4386. (IF 2.923)

49. Tsoukalidou S, Kakou M, Mavridis I, Koumantou D, Calderone V, Fragai M, Stratikos E, Papakyri-akou A, Vourloumis D. Exploration of zinc-binding groups for the design of inhibitors for the oxy-tocinase subfamily of M1 aminopeptidases. **Bioorg. Med. Chem.** (2019), 27(24):115177. doi: 10.1016/j.bmc.2019.115177. (IF 2.802)

50. Ravera E, Parigi G, Luchinat C, What are the methodological and theoretical prospects for para-magnetic NMR in structural biology? A glimpse into the crystal ball. **J. Magn. Reson.** (2019), 306, 173-179. (IF 2.689)

51. Davey NE, Babu MM, Blackledge M, Bridge A, Capella-Gutierrez S, Dosztanyi Z, Drysdale R, Ed-wards RJ, Elofsson A, Felli IC, Gibson TJ, Gutmanas A, Hancock JM, Harrow J, Higgins D, Jeffries CM, Le Mercier P, Mészáros B, Necci M, Notredame C, Orchard S, Ouzounis CA, Pancsa R, Papa-leo E, Pierattelli R, Piovesan D, Promponas VJ, Ruch P, Rustici G, Romero P, Sarntivijai S, Saun-ders G, Schuler B, Sharan M, Shields DC, Sussman JL, Tedds JA, Tompa P, Turewicz M, Von-drasek J, Vranken WF, Wallace BA, Wichapong K, Tosatto SCE. An intrinsically disordered proteins community for **ELIXIR**. F1000Res. (2019). doi: 10.12688/f1000research.20136.1.(IF 2.640)

52. Mateos B, Konrat R, Pierattelli R, Felli IC. NMR characterization of long-range contacts in intrinsi-cally disordered proteins from paramagnetic relaxation enhancement in 13C direct-detected ex-periments. **CheBioChem.** (2019), 20, 335-339 (IF 2.593)

53. Ciambellotti S, Turano P. Structural Biology of Iron-Binding Proteins by NMR Spectroscopy. **Eur. J. Inorg. Chem.** (2019), 5, pp. 569-576 (IF 2.578)

54. Camponeschi F, Banci L. Metal cofactors trafficking and assembly in the cell: a molecular view. **Pure Appl. Chem.** (2019), Volume 91, 231-245(IF. 2.350)

55. Carlon A, Ravera E, Parigi G, Murshudov GN, Luchinat C. Joint X-ray/NMR structure refinement of multidomain/multisubunit systems. **J. Biomol. NMR.** (2019), 73, 265-278 (IF 2.319)

56. Romano F, Meoni G, Manavella V, Baima G, Mariani GM, Cacciatore S, Tenori L, Aimetti M. Effect of non-surgical periodontal therapy on salivary metabolic fingerprint of generalized chronic periodontitis using nuclear magnetic resonance spectroscopy. **Arch. Oral Biol.** (2019), 97:208-214. (IF 1.663)

57. Parigi G, Ravera E, Bennati M, Luchinat C. Understanding Overhauser Dynamic Nuclear Polarisation through NMR relaxometry. **Mol. Phys.** (2019), 117, 888-897.(IF 1.571)

58. Barbieri L, Luchinat E. Backbone resonance assignment of human DJ-1 in the reduced state and in the cysteine sulfinic acid state. **Biomol. NMR Assign.** (2019). doi: 10.1007/s12104-019-09908-8 (IF 0.576)

59. McCartney A, Vignoli A, Tenori L, Fornier M, Rossi L, Risi E, Luchinat C, Biganzoli L, Di Leo A. Metabolomic analysis of serum may refine 21-gene expression assay risk recurrence stratification. **NPJ Breast Cancer.** (2019), 5:26. doi: 10.1038/s41523-019-0123-9. eCollection 2019.

60. Morris C, Andreetto P, Banci L, Bonvin A, Chojnowski G, Del Cano L, Carazo JM, Conesa P, Daenke Damaskos S, Giachetti A, Haleyf N, Hekkelmang ML, Heuser P, Joosteng RP, Kouřilh D, Křenek A, Kulhanek T, Lamzin V,Nadzirinj N, Perrakisg A, Rosato A, Sanderson F, Segura J, Schaarschmidt J, Sobolev J et al. West-Life: A Virtual Research Environment for structural biology, **J. Struc. Biol. X** (2019), 1, 100006.

BOOKS

1. Calderone V, Marco Fragai M and Luchinat C. *Reviewing the crystal structure of S100Z and other members of the S100 family: implications in calcium-regulated quaternary structure.* In C.W. Heizmann (Ed.) Calcium-Binding Proteins of the EF-hand Superfamily: from Basics to Medical Applications. **Methods Mol Biol.** (2019), 1929:487-499, Springer Protocols, Humana Press

Meetings and Events Organised by CERM

Seminars Held at CERM

Wednesday, January 16th, 6:00 pm, Dr. **Christine Cavazza**, CEA Grenoble, France, *"On the way to understanding carbon monoxide dehydrogenase maturation"*

Wednesday, February 20th, 6:00 pm, Dr. **Ricardo O. Louro**, Center for Magnetic Resonance António Xavier, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal, "*Using NMR to explore the molecular mechanisms of electricity production by bacteria*"

Thursday, March 7th, 12:00 pm, Dr. **Jiafei Mao**, Goethe-Universität Frankfurt am Main, Germany, "*Exploring Protein Structures and Functions via DNP-Enhanced Methyl SSNMR Spectroscopy*"

Thursday, March 21th, 5:30 pm, Dr. **Andreas G. Tzakos**, Department of Chemistry, University of Ioannina, Greece, "*Targeting the tumor microenvironment with prodrugs*"

Monday, April 8th, 12:00 pm, Dr. **Pablo Conesa**, Universidad Católica San Antonio de Murcia, Spain, "*Discovery of biomarkers and molecular targets in serrated colorectal carcinoma: from histology to molecular profiles*"

Wednesday, May 8th, 12:00 pm, Dr. **Andrzej Gorecki**, Jagiellonian University, Krakow, Poland, "*Strategy of transcription regulation by proteins YY1 and YY2*"

Thursday, May 30th, 12:00 pm, **Filip Szubert**, Jagellonian University, Krakow, Poland, "*Human transcription factor Yin Yang 1 - preparation of protein specimens for NMR studies*"

Monday, May 27th, 12:00 pm, Prof. **Robert Konrat**, Max F. Perutz Laboratories, University of Vienna, Austria, "*High-Content NMR for Molecular Biology and Drug Development*"

Tuesday, June 18th, 12:00 pm, Prof. **Frédéric Barras**, Département Microbiologie, Institut Pasteur, France, "*Fe-S protein biogenesis : evolution, stress adaptation and role in antibiotic resistance*"

Friday, July 12th, 5:30 pm, Dr. **Michaela Krafcikova**, Institute of Biophysics, Brno, Czech Republic, "*Study of protein-DNA interactions by using in-cell NMR*"

Thursday, September 19th, 5:30 pm, Prof. **Songi Han**, Department of Chemistry and Biochemistry, UCSB, USA, "*Study of protein hydration, assembly and aggregation by Overhauser DNP relaxometry and EPR spectroscopy*"

Friday, November 22th, 5:30 pm, Prof. Dr. **Silvio Tosatto**, Department of Biomedical Sciences, University of Padova, CNR Institute of Neuroscience, Padova, Italy, "*Computational resources for intrinsically disordered proteins*"

Group Meetings

10/01 **Maria Grazia Murrali** "Functional interaction studies of intrinsically disordered proteins"

01/02 **Valeria Putignano** "hMeProt web resource"

08/02 **Davide Sala** "Matching evolutionary couplings and ambiguous NMR contacts to derive homo-oligomers structure"

15/02 **Veronica Ghini** "Metabolomics to assess response to immune checkpoint inhibitors in patients with nonsmall-cell LUng cancer"

22/02 **Michele Invernici** "Paramagnetic NMR spectroscopy of FeS proteins"

01/03 **Sara Bologna** "Interaction of alpha-synuclein with human biofluids components: a step further"

08/03 **Sara Matteucci** "Iron trafficking in cytosolic iron-sulfur cluster assembly"

22/03 **Domenico Rizzo** "Expression and characterization by NMR of pharmaceutically relevant recombinant biomolecules"

05/04 **Giovanni Saudino** "Production of ISCA1, a protein involved in the biogenesis of mitochondrial iron sulfur proteins"

12/04 **Marco Schiavina** "Taking simultaneous snapshots of Intrinsically Disordered Proteins in action"

17/05 **Matteo Cremonini** "Segmental labelling approaches applied to NMR studies of multidomain proteins: a step further"

31/05 **Cristina Licari** "Fingerprinting by ^{1}H-NMR-based metabolomics"

07/06 **Nihar Ranjan Prusty** "Iron-sulphur (Fe-S) cluster binding proteins in human cytosol: a precise view on "scaffold" and "CIA targeting complex" proteins"

14/06 **Dafne Suraci** "NFU1 and ISCA2: new insights into mitochondrial Fe/S protein network"

21/06 **Lucia Gigli** "Looking at the coordination in paramagnetic metalloproteins from the distance"

28/06 **Letizia Barbieri** "Protein-ligand interactions by in-cell NMR"

02/09 **Dr. José Pedro Silva** "Human Carbonic Anhydrase 2: a paramagnetic tale"

20/09 **Dr. Gaia Meoni** "Metabolomics by NMR: applications in food research"

04/10 **Dr. Stefano Giuntini** "Characterization of protein-based bioconjugates and biomaterials by SSNMR"

11/10 **Dr. Silvia Ciambellotti** "Ferritin-based nanocarriers for cancer photodynamic therapy"

25/10 **Dr. Alessia Vignoli** "NMR metabolomic fingerprint of heart failures"

15/11 **Dr. Panagis Polykretis** "A harmful endogenous enemy"

06/12 **Dr. Enrico Luchinat** "Intracellular drug screening by protein-observed NMR"

19/12 **Dr. Alexander Röntgen** "Analysis of hydrogel scaffolds for bioreactor in-cell NMR"

Journal Clubs

15/03 - M. Cremonini, N. Prusty

29/03 - C. Licari, D. Suraci

10/05 - D. Sala, V. Putignano

24/05 - S. Bologna, M.G. Murrali

27/09 - L. Gigli

08/11 - S. Matteucci, D. Rizzo

13/12 - G. Saudino, M. Schiavina

Acknowledgements

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