CERM

Centro di Risonanze Magnetiche

Università di Firenze

CIRMMP

Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine

SCIENTIFIC ANNUAL REPORT

2018

UNIVERSITÀ DEGLI STUDI FIRENZE

Contents

Foreword

The world of NMR is undergoing rapid changes. On the one hand, the emerging new techniques for structural investigation (Cryo-EM, AFM, FEL,…) will resize the relative contribution of NMR to biomolecular structural determination, on the other hand they further highlight the uniqueness and the complementarity of the NMR technique with respect to new and old methods. 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 is going to attract a significant interest by pharmaceutical industries for the characterisation of biological drugs. As well DNP solid-state NMR is applied more and more in the investigation of solids and inorganic materials. These rapid scientific progresses offer us a powerful stimulus to continue to develop methodology, integrating the information coming from NMR with those from other techniques, making it possible to obtain a more and more detailed description of complex biological systems or advanced materials.

The research developed at CERM in 2018 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. With respect to 2017, we have a sizeable increase in the number of publications, and an average impact factor of our publications that is rated well above 5. The dialog with other disciplines forces our research to improve its theoretical and methodological bases, in order to be 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, we reinforced our role in the European Research Infrastructure scenario. CERM/CIRMMP is the Italian centre of the INSTRUCT European Research Infrastructure Consortium (ERIC), landmark of the 2018 ESFRI Roadmap. The **INSTRUCT-ULTRA** project aims to accelerate the expanded implementation of INSTRUCT-ERIC by opening up to new members in Europe and partnerships at the global level, with a sharp focus on increasing the effectiveness of user access to key technologies, also working together with manufacturers. Furthermore, we have been going on with the **EuroBioNMR EEIG** consortium, which is being established to co-ordinate European NMR research in biology and to ensure user access to them for all excellent scientific projects. The activity of INSTRUCT-ERIC and other 12 Biological and Medical European Research infrastructures (BMS RIs) is now coordinated by the **CORBEL** initiative, which creates a platform for harmonised user access to biological and medical technologies, biological samples and data services required by cutting-edge biomedical research. CORBEL will boost the efficiency, productivity and impact of European biomedical research.

FIGURES

Also for 2018, the Italian Ministry of Research confirmed its support to the Italian node of INSTRUCT-ERIC within the International Action of the FOE funding. CERM/CIRMMP investments and costs in 2018 amounted to € 3.380.000,00: € 320.000,00 towards training and education, € 1.920.000,00 for new equipment and € 910.000,00 towards research activities. An additional € 230.000,00 covered operational costs. The actual replacement value of the instrumentation at CERM is over € 50.000.000,00.

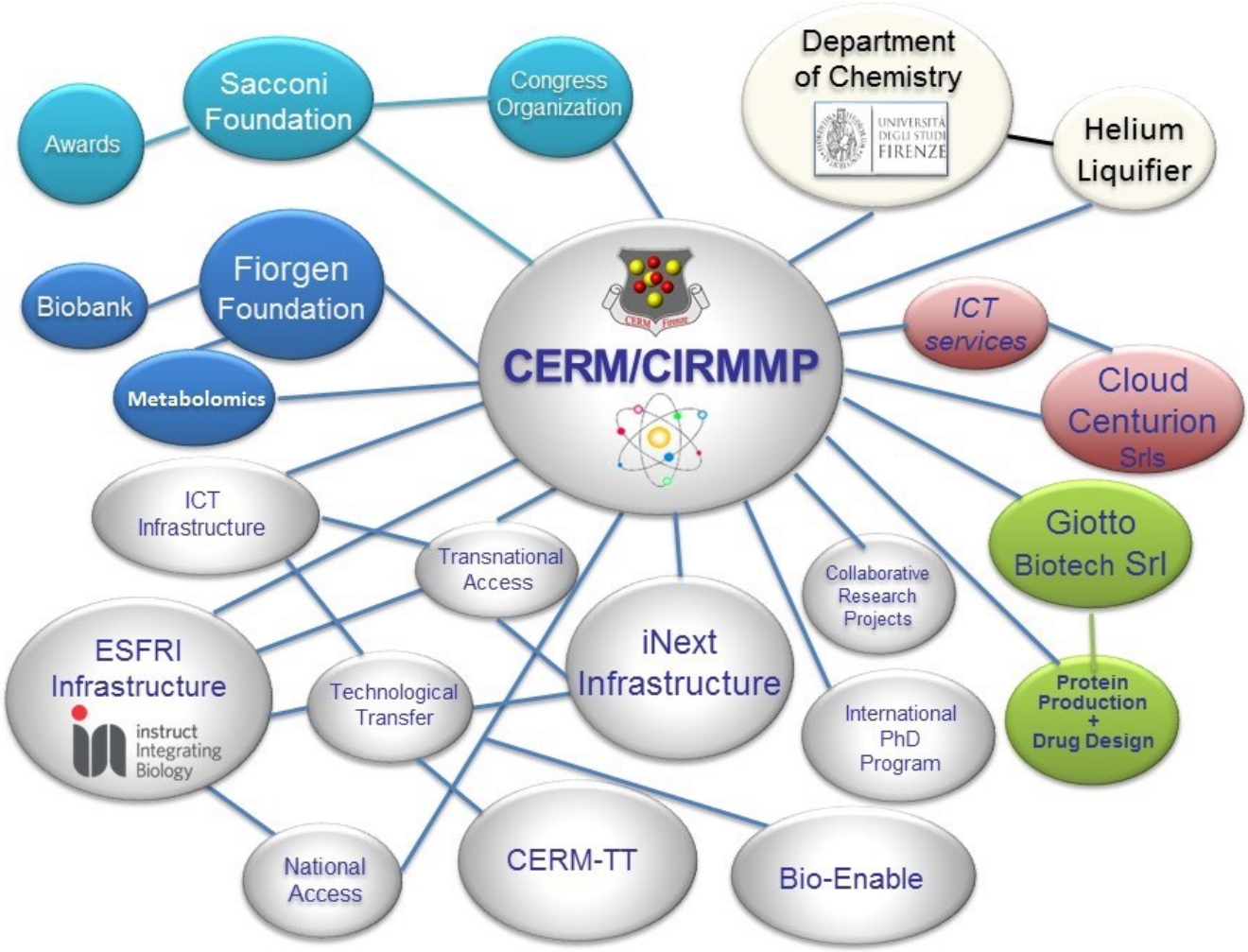
In 2018, in addition to the faculty staff, the body of researchers included 17 PhD students 12 postdoctoral scientists and 8 undergraduate students.

LOOKING AHEAD

The next big achievement for our lab will be the installation of the new 1.2 GHz Bruker NMR spectrometer which is expected by the end of 2019. An agreement with Bruker Biospin assures that CERM/CIRMMP will be the first in the world to have that instrument.

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


Department of Chemistry
UNIVERSITÀ DEGLI STUDI FIRENZE
Helium Liquifier
Sacconi Foundation
Congress Organization
Awards
Fiorgen Foundation
Biobank
Metabolomics
CERM/CIRMMP
ICT services
Cloud Centurion Srls
ICT Infrastructure
Transnational Access
Collaborative Research Projects
Giotto Biotech Srl
ESFRI Infrastructure
instruct Integrating Biology
Technological Transfer
iNext Infrastructure
International PhD Program
Protein Production + Drug Design
National Access
CERM-TT
Bio-Enable

Who we are

Introduction

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

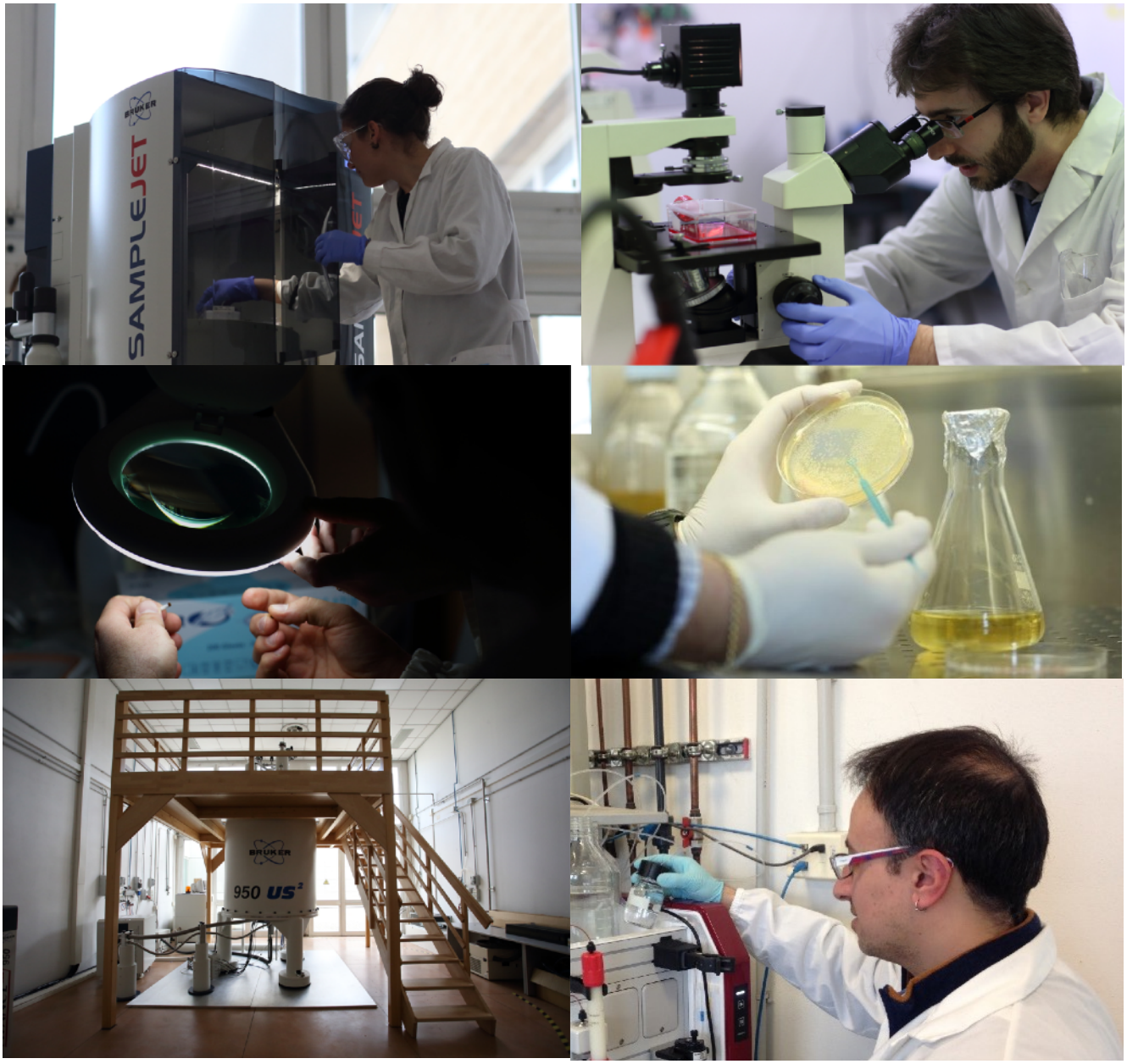
Being a leading laboratory at both national and international level, CERM/CIRMMP receives funding from competitive project calls from the Tuscan Regional Government, the Italian Ministry of Higher Education and Research (MIUR) and the European Commission (EC), as well as from private institutions. Since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

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

CERM/CIRMMP is a core Centre of Instruct-ERIC, which is the European research infrastructure in integrated structural biology defined in the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. The Italian centre of INSTRUCT-ERIC, CERM/CIRMMP is also included in the "*Roadmap Italiana delle Infrastrutture di Ricerca di interesse Pan-Europeo*" since 2010. ***CERM/CIRMMP is also an e-infrastructure***, managing a GRID-based platform, together with other laboratories in Europe, for providing access to user friendly platforms and CPU resources for a broad range of computational programs and tools relevant for structural biology. CERM/CIRMMP also promoted the creation of a "*biobank of biological samples and biomolecular resources*", the DA VINCI EUROPEAN BIOBANK. Finally, CERM/CIRMMP has promoted the creation of a centre for research and

technology transfer: CERM-TT, funded by the Tuscany Region, which was inaugurated in July 2015. Since November 2015, CERM/CIRMMP is coordinating the activities of BIO-ENABLE, a new distributed Infrastructure funded by the Regional Government of Tuscany in the frame of POR FESR 2014-2020, which is aiming at strengthening and widening the technology transfer to industry.

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 3 000 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) 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 buildup 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 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 and including 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 lead 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 2018 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.structuralbiology.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)

THE INFRASTRUCTURE

TIMB3 “Twin to Illuminate Metals in Biology and Biocatalysis 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/

H2020-EINFRA Phenomenal - A comprehensive and standardized e-infrastructure for analyzing medical metabolic phenotype data (#654241)http://phenomenal-h2020.eu/home/

H2020-EINFRA West-life - World-wide E-in-frastructure for structural biology (#675858) https://portal.west-life.eu/

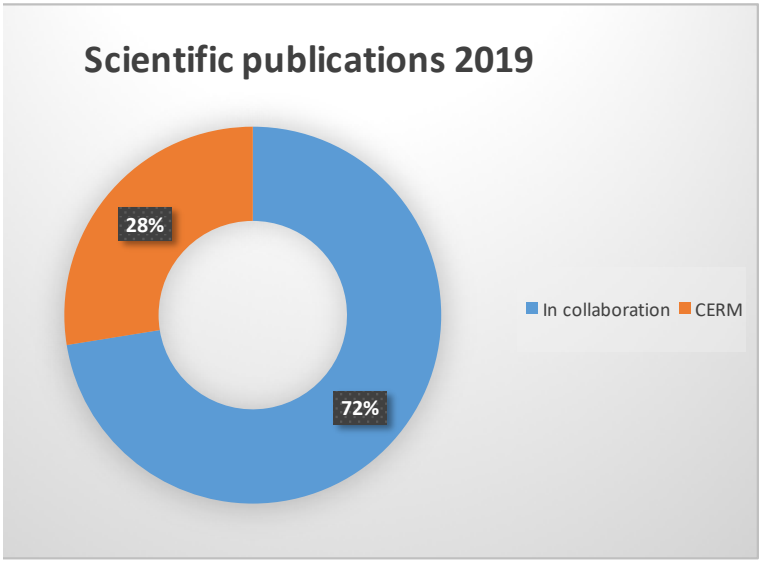
National Highly Relevance Projects - “Italia - Argentina”

Research Activities

Introduction

During 2018 a number of projects have been carried out, either as an extension of the activities of previous years or as new projects. Most of these projects receive specific funding from national and/or European organisations.

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



In line with our mission to develop NMR as a technique and to integrate NMR with other techniques, most of our publications were done in collaboration with other research groups (75% of the overall number of publications). With respect to 2017 we significantly increase the number of publications: 69 papers in international peer-reviewed journals in 2018 compared to 56 in 2017, with an average impact factor of 5.4. A complete list of publications is available at page 47.

CIRMMP has been ranked first among the Italian Interuniversity 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 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.

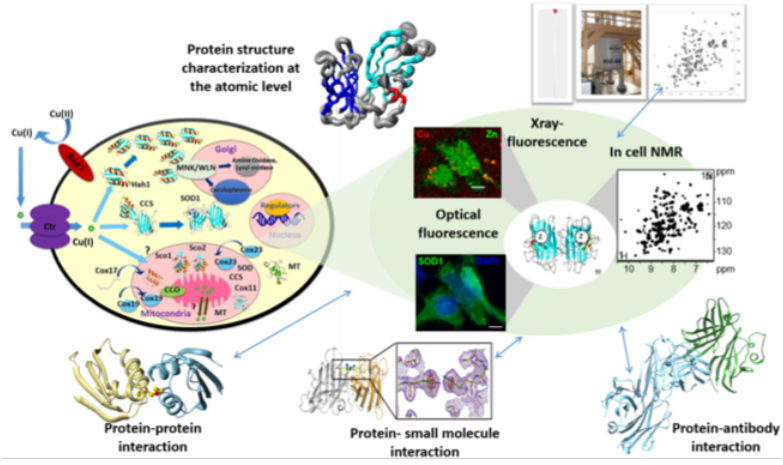
The interdisciplinary character of CERM/CIRMMP research projects, combined with the excellence of its instrumentation, constitutes a point of reference for the scientific community and for the cultural growth in the country, as demonstrated by the significant usage of the infrastructure by national scientists.

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 enabling technology for determining not only structures of biomolecules but also their interactions, even weak and transient, as well as for characterising functional processes in solution and also directly in living cells. Through the integration of solution NMR with other structural data derived using different technologies and on different length and time scales, we will be able to understand, on the basis of detailed atomic structure, how proteins, protein complexes or DNA-protein complexes interact dynamically 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.[1,2,3,4]



The potential of integrated structural biology in unravelling biological processes.

It is no longer sufficient to determine simply the structure and biochemical properties of a protein. In line with the trend towards systems biology, a major challenge now is understanding how that protein functions within a larger macromolecular assembly or in a cellular pathway or even at the organism level.

Understanding dynamic processes that are co-ordinated at a cellular level is not possible using a single technology, but becomes potentially accessible through the integration of a number of approaches. Complementary structural biology technologies are required to face complex systems in dynamic way.

References:

(1) Gourdoupis S, Nasta V, Calderone V, Ciofi-Baffoni S, Banci L. *J Am Chem Soc.* **2018***;140*, 14401-14412

(2)Saponaro A, Cantini F, Porro A, Bucchi A, DiFrancesco D, Maione V, Donadoni C, Introini B, Mesirca P, Mangoni ME, Thiel G, Banci L, Santoro B, Moroni A, *Elife.* **2018***: e35753*. doi: 10.7554/eLife.35753

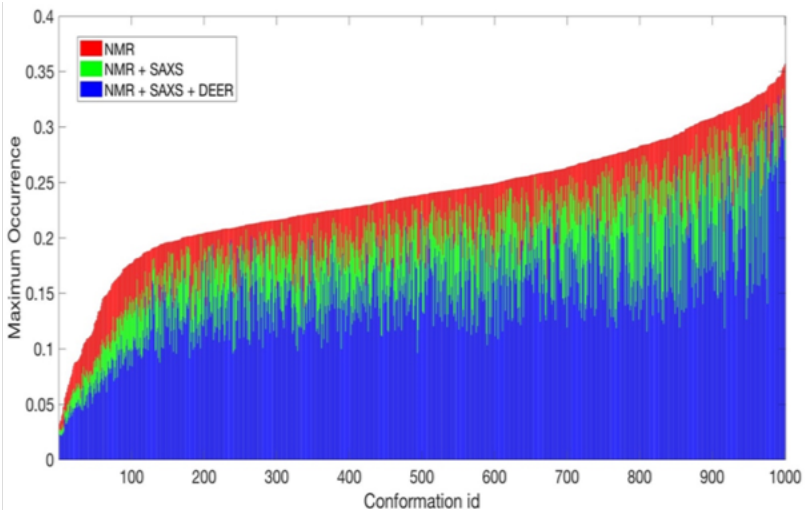
(3) Cantini F, Calderone V, Di Cesare Mannelli L, Korsak M, Gonnelli L, Francesconi O, Ghelardini C, Banci L, and Nativi C *ACS Med. Chem. Lett.* **2018** *9:* 1094−1098.

(4) Luchinat, E., Chiarella, S., Franceschini, M., Di Matteo, A., Brunori, M., Banci, L., & Federici, L. *The FEBS journal,* **2018** *285*, 832-847

Integrated Structural Techniques

There is an increasing need to develop approaches that permit to extract the most of the information from experimental data collected with different techniques, without over-interpretation.
NMR data can refine protein X-ray structures in solution and monitor the presence of multiple conformational states.

NMR data can monitor conformational rearrangements in systems composed of multiple domains. We have developed methods to obtain information on the conformational variability of multi-units systems using the MaxOcc approach, aimed at identifying individual conformations, or groups thereof, that can exist for a large share of time in agreement with the average experimental data. DEER data can now be analysed together with NMR and SAXS data to restrain the MaxOcc values.
NMR data have also be included as restraints in the widely-used X-ray refinement software REFMAC to assess whether they can be explained by a structural model derived from X-ray crystallography within the accuracy of its diffraction pattern. In this case, the NMR data can be used to provide a structure that complies with both solid state and solution data and is thus more reliable. The use of tensor constraints either in orientation or in magnitude can help the refinement of multiple domains systems.



MaxOcc values calculated for 1000 conformations of the two domain protein calmodulin using NMR, NMR and SAXS, or NMR, SAXS and DEER data. The use of the three types of data restricts the set of conformations with largest MaxOcc.

References:

(1) Gigli, L.; Andrałojć, W.; Dalaloyan, A.; Parigi, G.; Ravera, E.; Goldfarb, D.; Luchinat, C. *Phys. Chem. Chem. Phys.* **2018,** *43*, 27429-27438

(2) Carlon A.; Ravera, E.; Parigi, G.; Murshudov, G.N.; Luchinat, C. J. *Biomol. NMR* **2018,** *doi: 10.1007/s10858-018-0212-3*

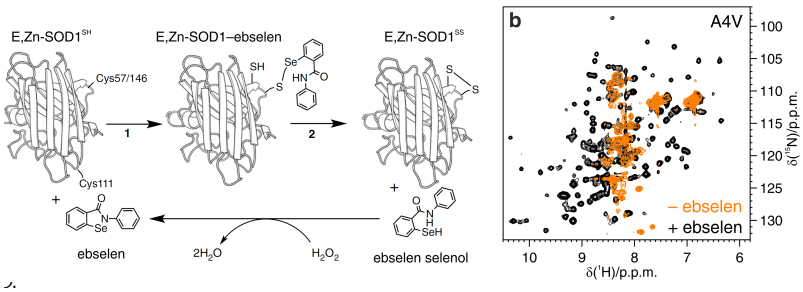
(3) Calderone, V.; Fragai, M.; Luchinat, C. *Inorg. Chim. Acta* ***2018,*** *470,* 402-406

(4) Ravera, E.; Carlon, A.; Fragai, M.; Parigi, G.; Luchinat, C. *Emerging Topics in Life Sciences* **2018,** *2,* 19-28.

(5) Ravera, E.; Takis, P.G.; Fragai, M.; Parigi, G.; Luchinat, C. *Eur. J. Inorg. Chem.* **2018,** *DOI 10.1002/ejic.201800875*

In-cell NMR in Human Cells

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, metal binding, redox regulation and other post-translational modifications at atomic resolution in human cells.[1,2] Conformational and redox changes in response to external stimuli can also be investigated. The approach was applied to investigate the effect of the small antioxidant molecule ebselen to intracellular SOD1. It was found that ebselen catalysed the formation of the intramolecular disulfide bond, and was able to rescue the correct folding of SOD1 mutants linked to familial variants of Amyotrophic Lateral Sclerosis (fALS).[3] In another work, the intracellular metal binding and redox behaviour of human DJ-1 was characterised in human cells, and the selective oxidation of a reactive cysteine in the active site of the protein was monitored in response to oxidative stress.[4] Furthermore, it was shown that single-cell synchrotron radiation infrared spectroscopy is a complementary approach to in-cell NMR to assess the overall chemical composition and isotopic labelling.[5] Further methodological developments are ongoing to implement in-flow bioreactor systems,[6] which will improve cell viability within the NMR spectrometer to allow time-resolved NMR experiments.



Ebselen stabilizes mutant SOD1 by catalyzing disulfide bond formation. (a) Proposed reaction mechanism between disulfide-reduced SOD1 and ebselen occurring in the cytosol of human cells. (b) ^{1}H-^{15}N in-cell NMR spectra of cells expressing the A4V SOD1 mutant in the absence (orange) and in the presence (black) of ebselen. The well-dispersed signals observed in the sample of cells treated with ebselen indicate the presence of correctly folded protein.

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 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) Luchinat, E.; Banci, L. *Accounts of Chemical Research,* **2018**, *51*, 1550.

(2) Luchinat, E.; Banci, L. *Emerging Topics in Life Sciences,* **2018,** *2,* 29.

(3) Capper, M. J.; Wright, G. S. A.; Barbieri, L.; Luchinat, E.; Mercatelli, E.; McAlary, L.; Yerbury, J. J.; O'Neill, P. M.; Antonyuk, S. V.; Banci, L.; Hasnain, S. S. *Nature Communications,* **2018,** *9*, 1693.

(4) Barbieri, L., Luchinat, E., Banci, L. *J. Biol. Inorg. Chem,* **2018,** *23*, 61-69

(5) Mitri, E., Barbieri, L., Vaccari, L.; Luchinat, E. *The Analyst,* **2018,** *143*, 1171.

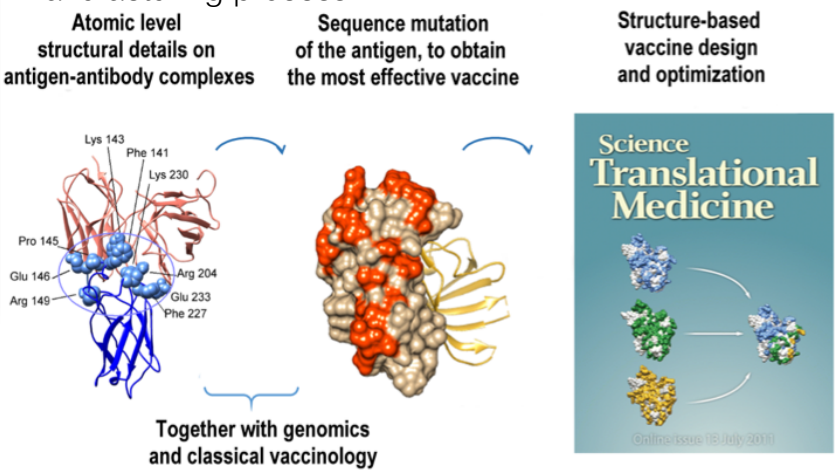
(6) Chatzikonstantinou, A, M. Chatziathanasiadou, E. Ravera, M. Fragai, G. Parigi, I. Gerothanassis, C. Luchinat, H. Stamatis, A. Tzakos, *BBA - General Subjects,* **2018**, *1862,* 1-8.

Structure-Based Vaccine Design

CERM/CIRMMP is a leading centre in the development of an innovative approach in vaccine design. The latter is based on the characterisation of the antigen structure and of its interacting regions with the antibody.
With this approach CERM/CIRMMP researchers and GSK Vaccines researchers, were able to create a broadly protective vaccine against more than 500 variants of the bacterial pathogen Neisseria meningitidis *serogroup B (MenB).*

Structurally vaccinology approach has been successfully applied with the final aim to engineer biomolecules able to trigger a neutralising effect in various strains of the *Neisseria meningitides* serogroup B pathogen (MenB). This approach allowed the design of multiple antigenic epitopes on the scaffold of factor H binding protein (fHBP, variant 1). The detailed knowledge of the residues recognised by protective antibodies is fundamental for modulating the immunogenicity of the antigen and to improve the bacterial potential vaccine candidate every time that the sequence variability of protective antigens is a major limit to the development of vaccines.[1-4]

Moreover the heterogeneous composition of vaccine formulations and the relatively low concentration make the characterisation of the protein antigens extremely challenging. Aluminum-containing adjuvants is used to enhance the immune response of several antigens. CERM researchers showed that solid-state NMR and isotope labelling methods can be used to characterise the structural features of the antigen in the presence of the adjuvants providing information about the regions of the protein that are affected by the presence of the inorganic matrix. This methodology can find application in several steps of the vaccine development pipeline, from the antigen optimisation, through the design of vaccine formulation, up to stability studies and manufacturing process[5].



Structural vaccinology: a genome-based approach combined with structural biology

References:

(1) Rubino, J.T.; Martinelli, M.; Cantini, F.; Banci, L.; Scarselli, M. et. al *J Biol Inorg Chem*. **2016**, *21*, 185.

(2) Liguori, A.; Malito, E.; Lo Surdo, P.; Fagnocchi, L.; Cantini, F.; Haag, A. F.; Brier, S.; Pizza, M.; Delany, I.; Bottomley, M. J. *PLoS Pathog*. **2016**, *12*, e1005557.

(3) Rippa, V.; Cantini, F.; Veggi, D.; Gentile, M.A.; Banci, L.; Pizza, M.; Scarselli, M. et. al *Clin Vaccine Immunol*. **2015**, *22*, 769.

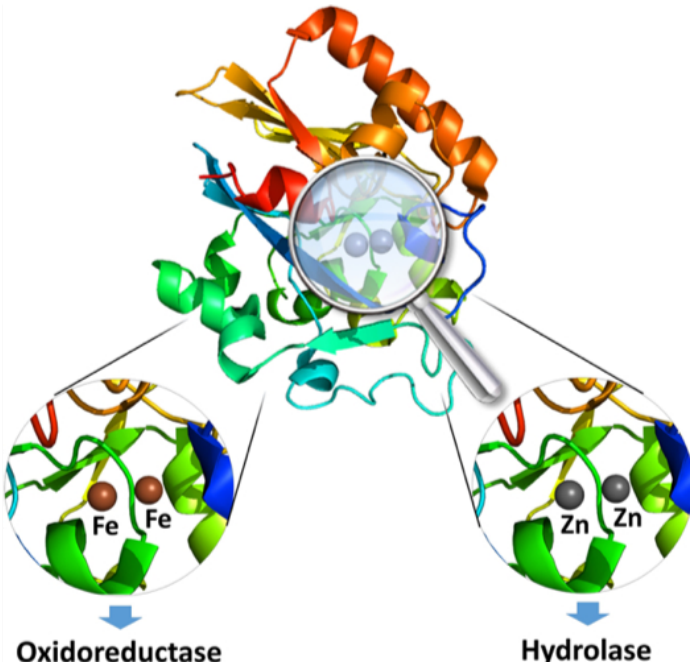
(4) Scarselli, M.; Cantini, F.; Dragonetti, S.; Banci, L.; Pizza, M.; Rappuoli, R. et al *Sci Transl Med*. **2011**, 3, 91ra62.

(5) Cerofolini, L.; Giuntini, S.; Ravera, E.; Luchinat, C.; Berti, F.; Fragai, M. *NPJ Vaccines*. **2019,** doi:0.1038/s41541-019-0115-7.

Bioinformatics Tools for Metallo-Biology

In 2018 our work mainly focused on the application of our more recently developed 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 human iron-proteome. A similar approach was followed to predict the zinc-proteome of yeast and used this prediction to support the interpretation of experimental data on the expression of zinc proteins under different environmental conditions (zinc depletion/repletion). Finally, we systematically mined MetalPDB together with enzyme databases to understand how functional diversity within superfamilies of metalloenzymes relates to structural changes at the catalytic site. We observed that evolution tends to conserve the metal site. When changes occur, they do not modify the catalytic role of non-redox metals whereas they affect the role of redox-active metals.

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.



Metalloenzymes belonging to a given structural superfamily can perform significantly different catalysis depending on whether the metal site contains redox-active metals.

References:

(1) Putignano, V.; Rosato, A.; Banci, L.; Andreini, C. *Nucleic Acids Res* **2018,** *46,* D459-D464.

(2) Andreini C.; Putignano V.; Rosato A.; Banci L. *Metallomics* **2018,** *10,* 1223-1231.

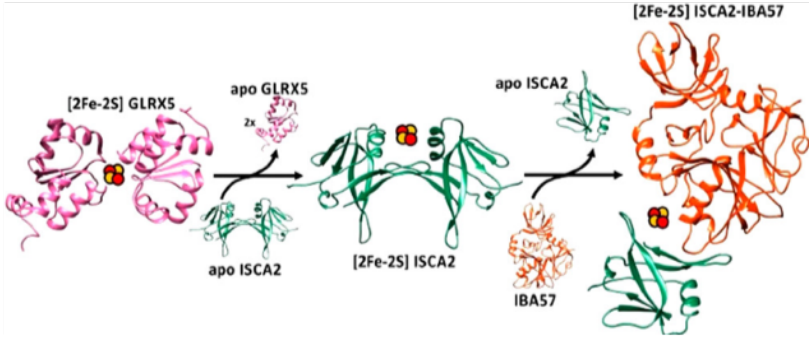
(3) Wang Y.; Weisenhorn E.; MacDiarmid CW.; Andreini C.; Bucci M.; Taggart J.; Banci L.; Russell J.; Coon JJ.; Eide D.J. *Metallomics* **2018,** *10,* 1755-1776.

(4) Valasatava Y.; Rosato A.; Furnham N.; Thornton JM.; Andreini C.; *J. Inorg. Biochem.* **2018,** *179,* 40-53.

Molecular Mechanisms 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 and coordinated process in living cells. Different biogenesis machineries in both bacteria and eukaryotes have been discovered that assist Fe-S protein maturation. An increasing number of human diseases related to misfunction of Fe-S protein biogenesis documents the importance of investigating such process in humans. A picture of the molecular mechanisms that are at the basis of Fe-S protein biogenesis is fundamental to boost the development of treatments on such human diseases.

In 2018, we investigated on the role of IBA57 protein family which acts in the late phase of the mitochondrial iron-sulfur cluster assembly machinery. IBA57 was proposed to mature [4Fe-4S] proteins along with GLRX5, ISCA1, and ISCA2, but a molecular picture on how these proteins cooperate is not defined yet. We solved the crystal structure of human IBA57 at 1.75 Å resolution, the first structure of IBA57 protein family. We also showed that i) IBA57 forms a heterodimeric complex with ISCA2 by bridging a [2Fe-2S] cluster, ii) [2Fe-2S] cluster binding is absolutely required to promote the complex formation, and iii) the cysteine of the conserved motif characterising IBA57 protein family and the three conserved cysteines of the ISCA protein family act as cluster ligands. We delineate a cluster transfer pathway involving GLRX5, ISCA2 and IBA57, which ends in the formation of a [2Fe-2S] cluster-mediated ISCA2-IBA57 complex, which is resistant to a highly oxidative environment and able to reactivate aconitase. The [2Fe-2S] ISCA2-IBA57 complex may be required as a specific system maturing Fe-S enzymes under aerobic cellular conditions, working similarly to the cytosolic human [2Fe-2S] mitoNEET protein that is capable of reactivating cytosolic aconitase in human cells.



A [2Fe-2S] cluster transfer pathway leading to the formation of the cluster-mediated [2Fe-2S] ISCA2-IBA57 complex.

References:

(1) Gourdoupis, S.; Nasta, V.; Calderone, V.; Ciofi-Baffoni, S.; Banci, L. *J Am Chem Soc.* **2018,** *201*, 140, 14401.

(2) Ciofi-Baffoni, S.; Nasta, V.; Banci, L. *Metallomics* **2018,** *10,* 49.

(3) Sturlese, M.; Manta, B.; Bertarello, A.; Bonilla, M.; Lelli, M.; Zambelli, B.; Grunberg, K.; Mammi, S.; Comini, M.A.; Bellanda, M. *Sci Rep.* **2018,** *8,* 13716.

Ferritin as a Carrier for Targeted Cellular Delivery

The demineralised (apo) form of commercial horse spleen ferritin is often used for drug cellular delivery upon encapsulation of different molecules.[1,2] On the other hand, human H-ferritin[3] has been proposed as a potentially superior carrier for drug delivery because, given its human origin, it should not activate inflammatory or immunological response. Tumour cells display selective ability of cells to internalise human ferritin cages rich in L- or H-subunits through specific receptors; the SCARA5 (Scavenger receptor class A type 5) for L-rich cages and TfR1 (Transferrin receptor 1) for H-rich cages. We have analysed the toxicity of iron-loaded H- and L-ferritin on HeLa cells taken as a tumour cell model that expresses both TfR1 and SCARA5 receptors.[1] This choice allowed us to compare the behaviour of the two proteins in the same cell line. Cellular uptake of human H-ferritin results in significant cytotoxicity on HeLa cells at submicromolar concentrations. Conversely, L-ferritin is toxic only at >1 order of magnitude higher protein concentrations. We proposed that the different cytotoxicity of the two ferritin cages originates from the presence in H-ferritin of a pool of non-biomineralised iron ions bound at the ferroxidase catalytic and accessory sites of H-ferritin subunits. This iron pool is readily released during the endosomal-mediated H-ferritin internalisation induced by Tfr1.

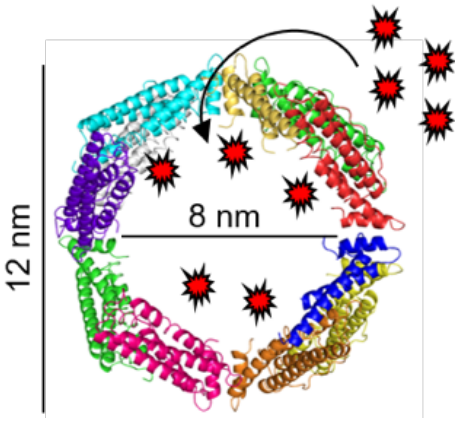
Iron-loaded recombinant human homopolymeric H-ferritin is taken-up by HeLa cells via the Tfr1 receptors and internalised via endocytosis. The process causes a release of iron that results cytotoxic due to the presence of an iron pool, which is labile at the low endosomal pH.

References:

(1) Cutrin, J.; Alberti, D.; Bernacchioni, C.; *et al. Oncotarget.* **2018**, 9, 27974-27984.

(2) Ciambellotti, S., Pratesi, A., Severi, M., *et al. Dalton Trans.* **2018**, 47, 11429-11437.

(3) Ciambellotti S, Turano P. *Eur. J. Inorg. Chem.* **2018,** doi: 10.1002/ejic.2018.01261

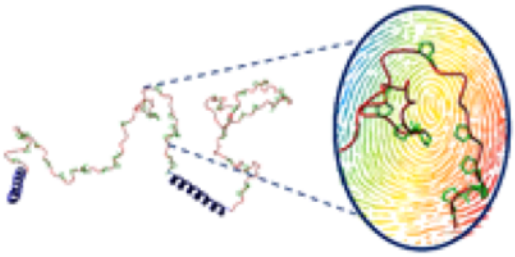


Representation of a loaded ferritin nanocage for targeted delivery.

Intrinsically Disordered Proteins

Intrinsically disordered proteins (IDPs) and disordered protein regions (IDRs) are now under the spotlights of structural biology. NMR represents a unique tool to access atomic resolution information.
Novel NMR methods for their investigation are presented.

Intrinsically disordered proteins (IDPs) carry out many biological functions. They lack a stable 3D structure and are able to adopt many different conformations in dynamic equilibrium. The interplay between local dynamics and global rearrangements is key for their function. Novel NMR methods based on ^{13}C detection were proposed to focus on their proline fingerprint as well as to determine observables providing long-range information that reveal compact states (paramagnetic relaxation enhancements). Relaxometry measurements performed on IDPs show that fast segmental motions are unable of averaging nuclear dipolar interactions completely and indicate the presence of motions with correlation times of 6-9 ns and squared order parameter of ca. 0.1. These motions are not related to specific long-range interactions but represent an intrinsic feature of IDPs dynamics. Finally, hyperpolarized water was used to highlight signals of amide protons in 2D spectra.



Prolines' signals are not directly visible in commonly-used NMR experiments and often escape investigation. We propose here an effective tool for capturing their features exploiting ^{13}C direct-detection NMR spectroscopy.

References:

(1) Rezaei-Ghaleh, N., Parigi, G., Soranno, A., Holla, A., Becker, S., Schuler, B., Luchinat, C., Zweckstetter, M.; *Angew. Chem. Int. Ed.* **2018,** *57,* 15262-15266.

(2) Murrali, M.G., Piai, A., Bermel, W., Felli, I.C., Pierattelli, R.; *ChemBioChem,* **2018,** *19,* 1625-1629.

(3) Mateos, B., Konrat, R., Pierattelli, R., Felli, I.C.; *ChemBioChem.* **2018** *doi: 10.1002/cbic.201800539*

(4) Murrali, M.G., Schiavina, M., Sainati, V., Bermel, W., Pierattelli, R., Felli, I.C.; *J. Biomol. NMR* **2018,** *70,* 167-175.

(5) Szekely, O., Olsen, G.L., Felli, I.C., Frydman, L.; *Anal Chem.* **2018,** *90,* 6169-6177.

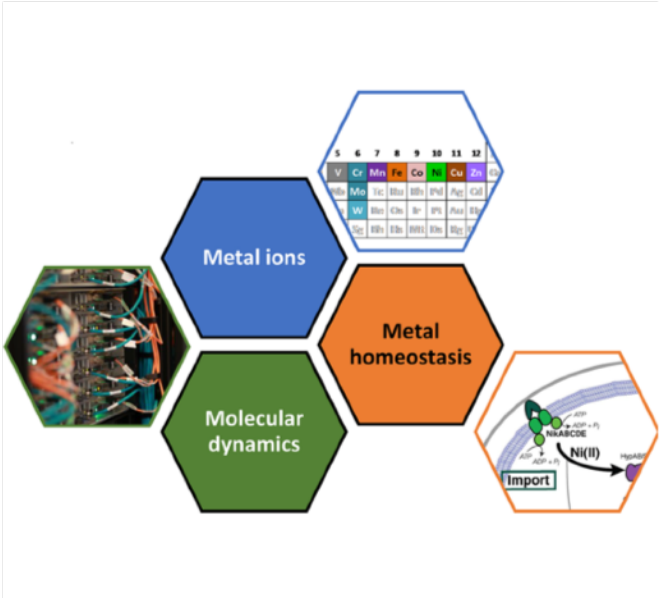
Distributed Computing in Structural Biology

Molecular Dynamics (MD), a computer simulation of the physical movements of atoms and molecules as a function of time, is 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. Over the years, we developed methods for free and restrained MD simulations using a grid computational infrastructure, exploiting both traditional CPUs and accelerated computing (GPGPUs). We applied these methods to understand the structural role of metal ions and mechanisms of metal transport.

The computational resources required to process and analyse this "big" data collected in life science studies are outgrowing the capabilities of individual research institutes. We are collaborating with other large research infrastructures to provide cloud-based compute resources and give open access to data to empower re-

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:

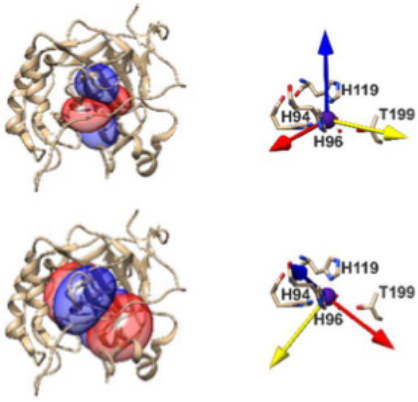
(1) Sala, D.; Musiani, F.; Rosato, A. *Eur. J. Inorg. Chem.* **2018,** *43*, 4661-4677.

(2) Sala, D.; Giachetti, A.; Rosato, A. *AIMS Biophysics* **2018**, *5,* 77-96.

NMR of Paramagnetic Systems

Paramagnetic systems offer a wide range of opportunities to the NMR spectroscopists, requiring continuous methodological advances in both experiments and theoretical treatments. The variety of systems that can be investigated, as well as the information that is possible to extract is therefore continuously increasing.

^{1}H-based NMR experiments tailored to observe spectra that are broad and/or distributed on a very large frequency range are still the method of choice for the characterisation of paramagnetic compounds, from small complexes to complicated metal sites in protein and protein-protein complexes. Iron-Sulfur clusters give rise to very peculiar NMR signatures, which can be used as a diagnostic tool for the identification the electron distribution across the irons of the FeS cluster. As an example, the spin distribution across a [Fe4S4]-[FeFe] cluster in a hydrogenase could be uniquely monitored via paramagnetic ^{1}H NMR spectroscopy.[1] A further example is the detailed atomic view of "Fe-S interactomics" and that it is particularly effective when protein-protein interactions are weak and transient.[2]
The paramagnetic properties are faithful reporters of the electronic structure of metal centres: a straightforward biunivocal relation among the electronic structure and the NMR observables would greatly improve the insight on the coordination geometry of metal centres in proteins.[3,4] An example of such an application is the characterisation of cobalt(II) and nickel(II)-substituted human carbonic anhydrase, bound to different ligands.[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 structure, but we monitored also the dimerisation process occurring in solution, and the structural rearrangement induced by the intermolecular interaction.[6]



Magnetic susceptibility tensors, represented as Iso-PCS surfaces (left) and orientation of the principal axes (right) obtained for free cobalt(II)-loaded human carbonic anhydrase(II), free (top) or inhibited with oxalate (bottom).

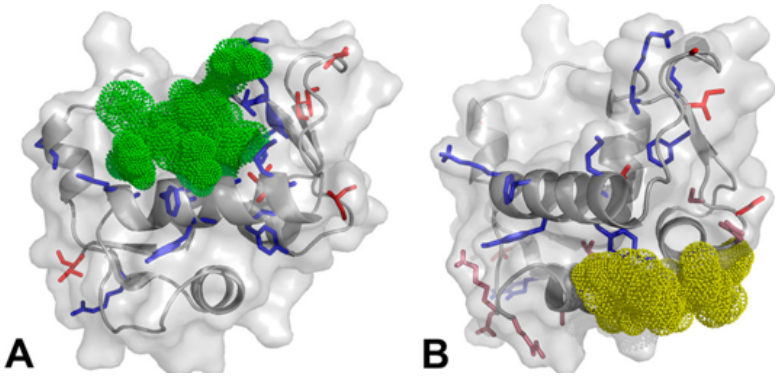
References:

(1) Rumpel, S.; Ravera, E.; Sommer, C.; Reijerse E.; Fasès, C.; Luchinat, C.; Lubitz, W. *J. Am. Chem. Soc* **2018**, 140, 131-134;
(2) Banci, L; Camponeschi, F.; Ciofi-Baffoni,S.; Piccioli, M.; *J. Biol. Inorg. Chem* **2018**, 23, 665-685;
(3) Ravera, E.; Takis, P.G.; Fragai, M.; Parigi, G.; Luchinat, C. *Eur. J. Inorg. Chem.* **2018**, 44, 4752-4770;
(4). Parigi, G.; Benda, L.; Ravera, E.; Romanelli, M.; Luchinat, C. **2018,** ArXiV:1804.09055
(5) Cerofolini, L.; Staderini, T.; Giuntini, S.; Ravera, E.; Fragai, M.; Parigi, G.; Pierattelli, R.; Luchinat, C. *J. Biol. Inorg. Chem.* **2018**, 23, 61-69;
(6). Lelli, M.; Di Bari, L.; *Dalton Trans.* **2018,** *doi: 10.1039/C8DT03090A.*

Proteins and mRNAs as Drug Targets

We have implemented a novel drug discovery approach based on biophysical screening of focused positional scanning libraries to identify candidate drugs for metalloproteins. Using this strategy we have discovered a novel and selective compound series targeting MMP-12.[1] In a different project we have discovered a compound, which inhibits both MDM2/4 and α5β1/αvβ3 integrins. A lead optimisation campaign was carried out on this first compound has provided a derivative turned out to be a potent MDM2/4 and α5β1/αvβ3 blocker. In p53-wild type glioma cells, 9 arrested cell cycle and proliferation and strongly reduced cell invasiveness, emerging as the first molecule of a novel class of integrin/MDM inhibitors, which might be especially useful in subpopulations of patients with glioblastoma expressing a functional p53 concomitantly with a high level of α5β1 integrin.[2] Inspired by DHTS structure, we have designed an array of *ortho*-quinones (tanshinone mimics). Two of these compounds show a nanomolar K_i and disrupting HuR binding to RNA in cells. A combined approach of NMR titration and molecular dynamics (MD) simulations suggests that this compound stabilises HuR in a peculiar closed conformation, which is incompatible with RNA binding.[3]

Structure-based drug design is an approach that can be combined well with solution NMR spectroscopy and biophysical methods to identify new targets and to develop new candidate drugs. This strategy has been pursued here to find new ligands and inhibitors of pharmaceutically relevant proteins.



Residues of MDM2 showing the largest CSP (blue sticks) and residues (isolated signals) exhibiting the largest decreases (red sticks).

References:

(1) Baggio C, Cerofolini L, Fragai M, Luchinat C, Pellecchia M. *ACS. Med. Chem. Lett.* **2018**, *9*, 137-142.

(2) Merlino F, Daniele S, La Pietra V, Di Maro S, Di Leva FS, Brancaccio D, Tomassi S, Giuntini S, Cerofolini L, Fragai M, Luchinat C, et al. *J Med Chem.* **2018**, *61*, 4791-4809

(3) Manzoni L, Zucal C, Maio DD, D'Agostino VG, Thongon N, Bonomo I, Lal P, Miceli M, Baj V, Brambilla M, Cerofolini L, Elezgarai S, Biasini E, Luchinat C, Novellino E, Fragai M, Marinelli L, et al. *J Med Chem.* **2018**, *61*, 1483-1498

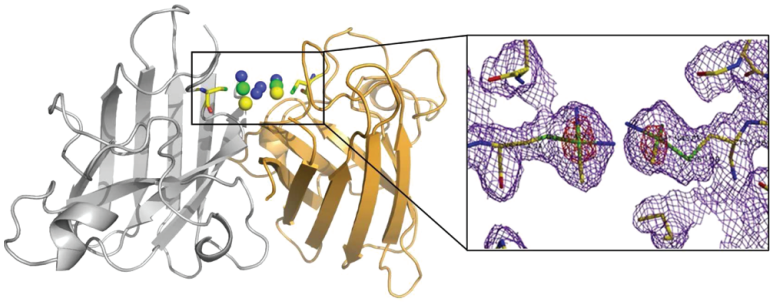
Amyloid Formation, Protein Aggregation Studied with Solution and and Solid-State NMR

We are continuously developing new solid-state NMR-based methodologies for providing the structural characterisation at atomic level of proteins have opened new perspectives to study systems in their in-operando *conditions.*

Resonance assignment and structural characterisation of pharmacologically relevant proteins promise to improve understanding and safety of these proteins by 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. We demonstrated that it is possible to obtain the resonance assignment and a reliable structural model of large PEGylated proteins through an integrated approach encompassing NMR and X-ray crystallography.[1]

In another work, solution NMR has been used to monitor the aggregation kinetics in Abeta1-40. By NMR it is possible to monitor the consumption of the monomeric Abeta1-40 during aggregation, and it provides complementary information with respect to ThT fluorescence, which is sensitive to fibril formation. Through NMR it has been possible to obtain information on the early stages of the aggregation process, with clear indications about the kinetics of the oligomers formation, which are suspected to be the most toxic species in the Alzheimer disease.[2]

We have demonstrated that *cis*-diamminedichloroplatinum(II) (Cisplatin) is able to interact with human superoxide dismutase (hSOD1) in the disulfide oxidised apoform exhibiting a dissociation constant of 37±3 μM through binding cysteine 111 located at the edge of the subunit interface. This molecule is able to dissolve and monomerize oxidized hSOD1 oligomersin *vitro*, and *in cell,*thus indicating its potential role in the treatment of amyotrophic lateral sclerosis.



Interaction of *cis*-platin with hSOD1

References:

(1) Cerofolini L, Giuntini S, Carlon A, Ravera E, Calderone V, Fragai M, Parigi G, Luchinat C. ***Chemistry***. 2018, doi: 10.1002/chem.201804488.

(2) Bellomo G, Bologna S, Gonnelli L, Ravera E, Fragai M, Lelli M, Luchinat C. *Chem Commun* **2018,** *54,* 7601-7604.

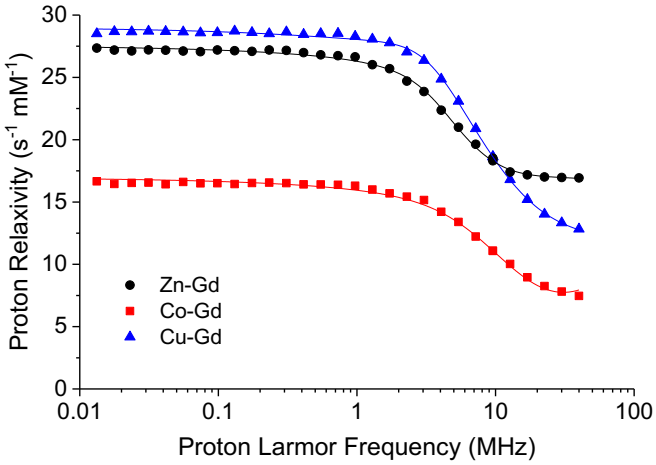
(3) Cantini F, Calderone V, Di Cesare Mannelli L, Korsak M, Gonnelli L, Francesconi O, Ghelardini C, Banci L, and Nativi C *ACS Med. Chem. Lett.* **2018** *9*: 1094−1098.

FFC Relaxometry and Overhauser DNP

The field dependence of the nuclear relaxation rates, from 0.01 MHz to tens or hundreds of MHz, contain information on structural and dynamic features of a molecule and, in the case of paramagnetic systems, on electron relaxation. It can be used to monitor association processes and hydration in complex systems, like the polyion complex micelles formed from the electrostatic interaction between oppositely charged polymers.

FFC relaxometry is used for characterising the efficacy of paramagnetic complexes as contrast agents for MRI. Through relaxometry measurement, it was demonstrated that, when Gd(III) is coupled to a paramagnetic transition metal ion, intramolecular magnetic exchange coupling provides a mean to modulate the electron relaxation time of Gd(III).

DNP signal enhancements are strongly linked to the spin relaxation properties of the paramagnetic molecule used as DNP polariser. Relaxometry can thus be used to understand the physical processes at the origin of Overhauser DNP in solution



FFC relaxometry profiles for bimetallic (Zn-Gd, Co-Gd or Cu-Gd) complexes. The relaxivity of the complexes is strongly dependent on the electron relaxation time of the Gd ion, which is modulated by magnetic exchange coupling with the paramagnetic transition metal ion.

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 optimization of contrast agents for MRI, for the characterization of polimeric systems, and for sheding light onto the mechanisms responsible for Overhauser DNP.

References:

(1) Lilley, L.M.; Du, K.; Krzyaniak, M.D.; Parigi, G.; Luchinat, C.; Harris, T.D.; Meade, T.J. *Inorg. Chem.* **2018**, 57, 5810-5819

(2) Gineste, S.; Di Cola, E.; Amouroux, B.; Till, U.; Marty, J.-D.; Mingotaud, A.-F.; Mingotaud, C.; Violleau, F.; Berti, D.; Parigi, G.; Luchinat, C.; Balor, S.; Sztucki, M.; Lonetti, B. *Macromolecules* **2018**, 51, 1427-1440

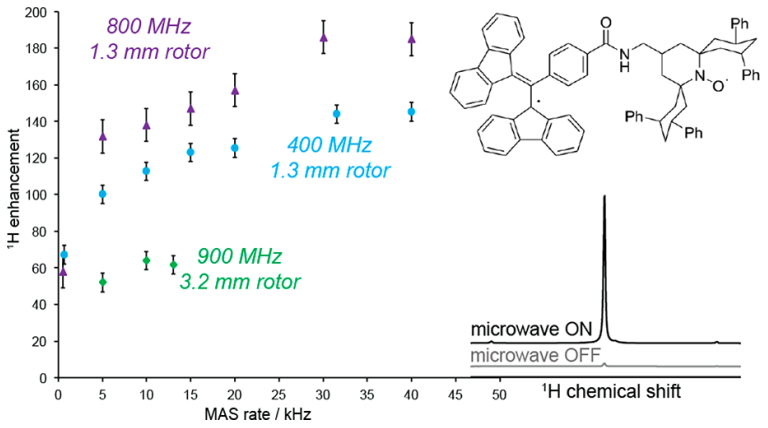
(3) Parigi, G.; Ravera, E.; Bennati, M.; Luchinat, C. *Mol. Phys.* **2018**, doi: 10.1080/00268976.2018.1527409

(4) Fedeli, L. et al. *Phys Med.* **2018**, 55, 135-141

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 structural determination of very diluted species like surface functionalities.

Solid-state NMR (ssNMR) is the method of choice for the characterisation of many liquid crystals, solid chemicals, and materials. For example, we have prepared a protein-hydroxyapatite composite and we have found through ssNMR that the protein is able to promote the formation of crystalline hydroxyapatite, which is surrounded by an amorphous layer of calcium hydrogen phosphate, which in turn is in contact with the protein component.[1]
As another example, EPR and ssNMR were used to investigate the arrangement of copper ions in a doped perovskite. These data indicate that the copper ions tend to be paired and weakly coupled to one another.[2]
The main limitation of ssNMR is due to the intrinsically low sensitivity of NMR, for this reason hyperpolarisation techniques like DNP, are rapidly expanding the application field of ssNMR. Nevertheless, DNP is strongly loosing efficiency at high magnetic field and fast MAS. In 2018, we introduced a new generation of hybrid biradical, combining together a narrow line BDPA moiety and a bulky nitroxide radical, that show DNP enhancements at 18.8 T (800MHz, 527 GHz) around 200 that increases with MAS frequency.[3] This opens the way to high-field fast-MAS high-sensitivity solid-state NMR The impact high-field DNP[3] in the investigation of solid biomolecules and material science is enormous.



Trends of increasing DNP enhancement with MAS frequency in Hybrid Biradical (HyTEK2) for different magnetic field and different rotor sizes.

References:

(1) Louka A, Matlahov I, Giuntini S, Cerofolini L, Cavallo A, Pillozzi S, Ravera E, Fragai M, Arcangeli A, Ramamoorthy A, Goobes G, Luchinat C. *Phys Chem Chem Phys.* **2018,** *20,* 12719-12726.

(2) Karmakar, A.;Dodd,MS;Agnihotri,S.; Ravera,E.; Michaelis,VK; *Chemistry of Materials,* **2018,** doi *10.1021/acs.chemmater.8b03755.*

(3) Wisser, D.; Karthikeyan, G.; Lund, A.; Casano, G.; Karoui, H.; Yulikov, M.; Menzildjian, G.; Pinon, A.C.; Purea, A.; Engelke, F.; Chaudhari, S.R.; Kubicki, D.; Rossini, A.J.; Moroz, I.B.; Gajan, D.; Copéret, C.; Jeschke, G.; Lelli, M.; Emsley, L.; Lesage, A.; Ouari, O.; *J Am Chem Soc.* **2018,** *140,* 13340-13349.

Metabolomics in Biomedicine

Metabolomics is focused on the analysis of intermediates and end products of metabolism in the form of endogenous (gene-derived metabolites), exogenous (environmentally derived metabolites) and gut microbiota-derived metabolites. For this reason, metabolites correlate with the phenotype and act as direct signatures of biochemical activity. This makes metabolomics a perfect instrument to investigate and understand the molecular mechanisms of human health and disease.[1-2]

NMR has been used for different applications such as the definition of the metabolic signature of novel diseases and to validate previously-defined signatures for diagnostic and prognostic purposes; the evaluation of the effect of medical intervention, the influence of life-style on the individual metabolome.

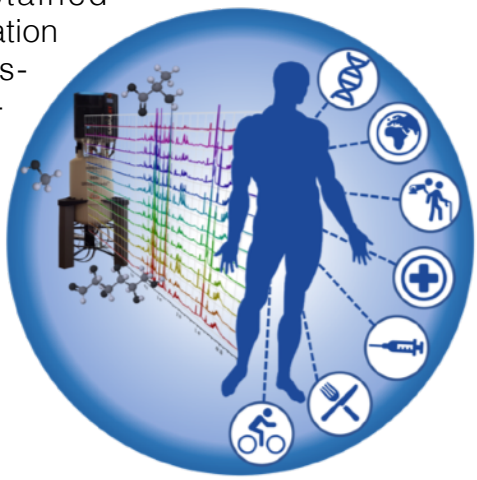
A clear signature of Down syndrome emerged from the comparison of sera and urine from diseased subjects and healthy control subjects recruited among Down syndrome normal siblings.[3]

Sera and urine were also used to investigate the possible metabolic association between primary biliary cholangitis and celiac disease.[4] As a main result, the primary biliary cholangitis was found to display a unique metabolomic fingerprint, suggesting and impaired energy metabolism, probably associated with an altered gut microbiota.[4]

Saliva was instead analysed to characterize chronic periodontitis and generalised aggressive periodontitis and to evaluate the impact of their medical treatment.[4-5.]

Insights into the individual susceptibility to drug administration have been obtained through the characterisation of exhaled breath condensate in patients with Chronic Obstructive Pulmonary Disease.[6]

Beside humans, metabolomics was employed in a veterinary study to analyse calves with severe sepsis.[7]



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

The contribution of ^{1}H NMR metabolomics to precision medicine includes the characterisation of the metabolic signature of diseases for diagnostic and prognostic purposes as well as the definition of individual susceptibility to medical and influence of environmental factors.

References:

(1) McCartney, A.; *et al. Cancer Treat. Rev.* **2018,** *67,* 88-96.

(2) Takis, PG.; *et al. Trends in Analytical Chemistry,* **2018** doi: 10.1016/j.trac.2018.10.036

(3) Caracausi, M. *et al. Sci Rep.* **2018,** *8,* 2977.

(4) Romano, F. *et al. ;J Periodontol.* **2018.** *89,* 1452-1460.

(5) Romano, F. *et al. Arch. Oral. Biol.* **2018,** 10.1016/j.archoralbio.2018.10.023.

(6) Montuschi, P.; *et al. Front. Pharmacol,* **2018;** *9, 258.*

(7) Basoglu, A. *et al.. Mediators Inflamm.* **2018;** 8016510.

NMR in Metabolomic Fingerprinting

Fingerprinting by NMR is the global evaluation of the whole NMR spectrum, viewed as a snap-shot of all (assigned or unassigned) detectable metabolites present in the sample. Fingerprinting is the preferred approach to provide fast sample classification (e.g. disease vs. healthy) using multivariate statistical techniques and could be the basis for metabolomic application to large population screening.

Metabolomics, as the "omic" sciences focused on measuring the ensemble of metabolites from bio-specimens, provides a holistic overview of the complex biochemistry underlying life, perfectly fitting with the spirit of systems biology and systems medicine[1], because of its ability to detect in real time the response of the organisms to pathological stressors. The two main approaches to metabolomics are fingerprinting and profiling[2]. We believe that Nuclear Magnetic Resonance (NMR) spectroscopy is a unique approach to provide the metabolic fingerprint of a subject through the fast analysis of biofluids that can be collected non-invasively. In the fingerprinting approach, the entire spectrum is analysed by considering all the spectral features, independently of signal assignment. This is usually achieved through a bucketing procedure, where the spectrum is subdivided into several bins. Multivariate techniques are then applied to build predictive models. However, due the large amount of data that the technique generates (and will keep generate in the future), there is a need for dedicate computational infrastructures[3]. NMR fingerprinting has application in areas such as: individual monitoring (to assess the individual metabolic changes after a severe physiological or pathological stress)[4,5], food traceability and nutritional studies[6,7], in vitro characterisation of bacteria strains[8]

With only one-shot analysis, metabolomics can fingerprint the evolution of an individual.

References:

(1) Rosato, A. *et al. Metabolomics* **2018**, *14* (4), 37.

(2) Vignoli, A. *et al. Angew. Chem. Int. Ed Engl.* **2018.** *doi: 10.1002/anie.201804736.*

(3) Peters, K.; *et al. GigaScience* **2018.** *doi:10.1093/gigascience/giy149.*

(4) Rittweger, J. *et al. NPJ Microgravity* **2018,** *4*, 18.

(5) Vignoli, A. *et al. J. Proteome Res.* **2018,** *17,* 97–107.

(6) Tenori, L. *et al. Food Res. Int.* **2018,** *113,* 131–139.

(7) Trimigno, A. *et al. Mol. Nutr. Food Res.* ***2018.*** ***doi:****10.1002/mnfr.201800216.*

(8) Checcucci, A. *et al ACS Synth. Biol.* ***2018****, 7,* 2365–2378.

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 cell 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.

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 2018 CERM/CIRMMP provided overall 591 days of NMR access to external users. A more detailed analysis shows that the access in the frame of INSTRUCT-ERIC, CORBEL and iNEXT, increased from total of 254 days in 2017 up to 465 days in 2018.

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 2018, thanks to the freshly inaugurated centre CERM TT and the BIO-ENABLE project, the pay-for-services access to industries was overall 73 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

Cloud Centurion Srls

Italmatch Chemicals

Glaxo Smith Kline

Giotto Biotech Srl

Menarini Srl

Valagro S.p.a.

Abiogen S.p.a.

Infineum

Dompé Pharmaceutical

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

Fondazione FIORGEN ONLUS

The FIORGEN ONLUS Foundation is a non-profit foundation that promotes studies in predictive and personalised medicine. The Foundation conducts research in the application of genomic and molecular data to help finding new drugs or to determine the individual susceptibility to a particular disease or disorder. CERM and FIORGEN work together on the main lines of structural biology and metabolomics.

The study of diseases through the holistic approach of metabolomics can be very useful to obtain new information about their biological mechanisms and their impact on human health. At the same time, new emerging applications of metabolomics in the field of food analysis and human nutrition were explored.

The FIORGEN Biobank da Vinci European Biobank (daVEB) has been donated to the University of Florence in October 2017.

http://www.fiorgen.net/

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 ser-

vice of samples and the associated data, following protocols in accordance with international standards.

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

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

Giotto Biotech Srl

GIOTTO BIOTECH S.r.l. is a SME founded in 2011 as a spin-off of CERM that aims at contributing to the biomedical sciences by providing enabling products and services, with a particular focus on complementary technologies in the field of NMR. GIOTTO BIOTECH provides a full range of compounds and custom manufacturing to supply research needs in the field of biomedical sciences, consulting and services. The company is active in various fields including protein production and isotope labelling, organic synthesis, services for NMR, and information technology. The services include NMR metabolomics and statistical analysis.

In 2018 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 AEGIS, FP7, Accelerated Early staGe discovery; 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, funded by Piemonte Region).

GIOTTO BIOTECH research activity is carried out in synergy with CERM scientists. As an outcome of this collaboration, in 2018 GIOTTO BIOTECH and CERM researchers co-authored seven 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 honor 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 2018 has been awarded to Prof. Wolfgang Lubitz, Max Planck Institute for Chemical Energy Conversion, Mülheim a. d. Ruhr, Germany, with a lecture titled "The Water Oxidation Cycle in Photosynthesis Probed by Magnetic Resonance Techniques".

The Sacconi Medal Lecturer 2018 has been awarded to Prof. Clare Grey, University of Cambridge

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

Cloud Centurion Srls

Cloud Centurion is a start-up company that originates from the experience of some researchers at CERM/CIRMMP in the field of Information Technology. It deals with web security and cloud services. Web Security, also known as Cyber Security, involves protecting information by preventing, detecting, and responding to attacks.

The main aspects of Cyber Security are: Confidentiality (keeping your information private); Integrity (knowing that the information has not been changed); Authenticity (knowing who sent the information).

The objective of this Company is to provide a security platform where users can define, visualise, and modify the properties of any uniform resource identifier (URI) element, thereby achieving a full control on how the files hosted on public cloud services are shared. More specifically, the platform allows users to protect their on-line life when exploiting services to share any file type.

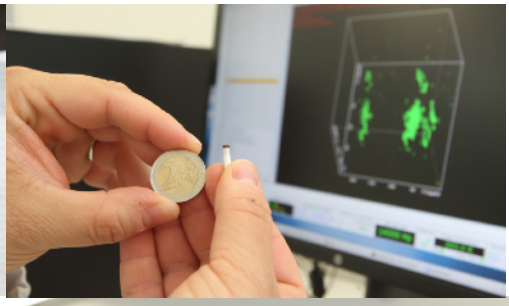
The main goal of the platform is to enable its users to safely exchange files over the internet, in an easy manner that does not involve setting up a sophisticated IT infrastructure.

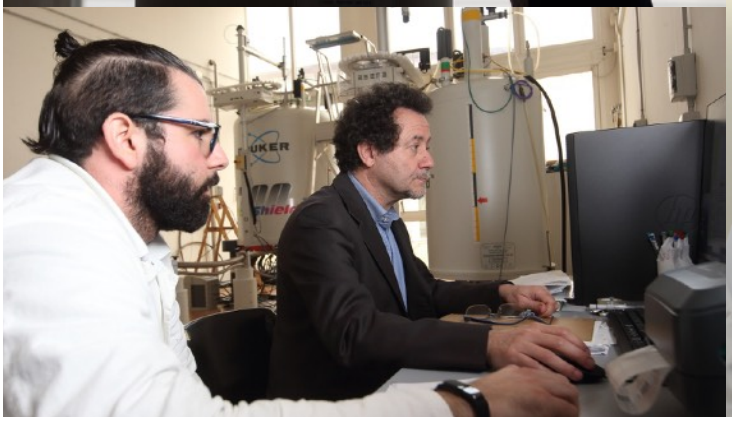
http://cloudcenturion.eu

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.





INSTRUMENTATION

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 (AgilentTechnologies) for routine in-house data collections. Regular access to synchrotron beam time slots in Europe facilities is also possible.

Biological and Biophysical Facilities and Services

Molecular and Cellular Biology

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

EPR

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

Multi Angle/Dynamic Light Scattering

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

Isothermal Calorimetry (ITC)

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

Optical Spectroscopy

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

Computational Structural Biology Tools

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

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

Electronic infrastructure (e-infrastructure)

The grid and cloud-based services of CERM/CIRMMP are currently being provided *via* the West-Life Virtual Research Environment (VRE). West-Life (http://about.west-life.eu/) leverages on the success of the WeNMR e-Infrastructure and aims to provide the application-level services specific to different cases in Structural Biology. West-Life covers all experimental techniques of Structural biology (X-ray, cryo-EM, NMR, SAXS), enabling researchers in this field to benefit fully of many computational services being provided by collaborative initiatives at the European level, including but not limited to the EGI-ENGAGE and INDIGO-DataCloud projects already mentioned. Some services specific for NMR data included also in the MoBrain competence centre of the EGI-ENGAGE project.

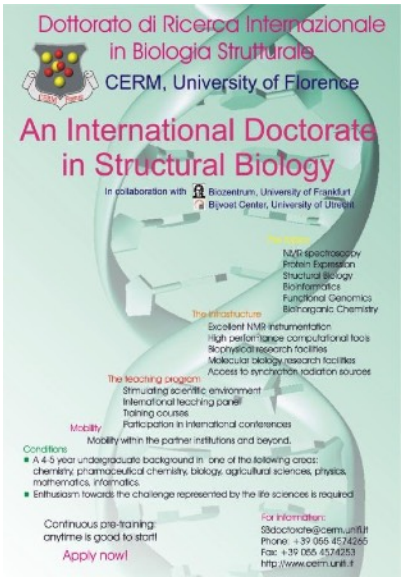
CERM/CIRMMP maintains a node of the European Grid Initiative. The available hardware comprises two clusters with 80 and 1024 CPU-cores respectively, and four TB of shared storage. A cluster with six Nvidia Tesla K20 GPGPU cards is also available.

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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List of publications

1. Luchinat E, Banci L. In-Cell NMR in Human Cells: Direct Protein Expression Allows Structural Studies of Protein Folding and Maturation. **Acc Chem Res.** (2018), 51, 1550-1557. doi: 10.1021/acs.accounts.8b00147 (IF 20.955)

2. Gourdoupis S, Nasta V, Calderone V, Ciofi-Baffoni S, Banci L. IBA57 Recruits ISCA2 to Form a [2Fe-2S] Cluster-Mediated Complex. **J Am Chem Soc.** (2018);140, 14401-14412 (IF 14.357)

3. Wisser D, Karthikeyan G, Lund A, Casano G, Karoui H, Yulikov M, Menzildjian G, Pinon AC, Purea A, Engelke F, Chaudhari SR, Kubicki D, Rossini AJ, Moroz IB, Gajan D, Copéret C, Jeschke G, Lelli M, Emsley L, Lesage A, Ouari O, BDPA-Nitroxide Biradicals Tailored for Efficient Dynamic Nuclear Polarization Enhanced Solid-State NMR at Magnetic Fields up to 21.1 T, **J Am Chem Soc.** (2018), 140, 13340-13349, (IF 14.357)

4. Rumpel S, Ravera E, Sommer C, Reijerse E, Farès C, Luchinat C, Lubitz W, 1H NMR Spectroscopy of [FeFe] Hydrogenase: Insight into the Electronic Structure of the Active Site **J Am Chem Soc.** (2018), 140, 131-134. doi: 10.1021/jacs.7b11196 (IF 14.357)

5. Capper, M. J.; Wright, G. S. A.; Barbieri, L.; Luchinat, E.; Mercatelli, E.; McAlary, L.; Yerbury, J. J.; O'Neill, P. M.; Antonyuk, S. V.; Banci, L. & Hasnain, S. S. The Cysteine-Reactive Small Molecule Ebselen Facilitates Effective SOD1 Maturation. **Nat Commun,** 9, 1693 (2018). (IF: 12.124)

6. 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.** (2018), doi: 10.1002/anie.201804736 (IF 11.994)

7. N. Rezaei-Ghaleh, G. Parigi, A. Soranno, A. Holla, S. Becker, B. Schuler, C. Luchinat, M. Zweckstetter, Local and Global Dynamics in Intrinsically Disordered Synuclein, **Angew.Chem.Int.Ed.** (2018) 57, 15262-15266. (IF 11.994)

8. Putignano, V., Rosato, A., Banci, L. & Andreini, C. MetalPDB in 2018: a database of metal sites in biological macromolecular structures. **Nucleic Acids Res** doi: 10.1093/nar/gkx989 (2018). (IF 10.162)

9. Karmakar, A.;Dodd,MS;Agnihotri,S.; Ravera,E.; Michaelis,VK; Cu(II)-Doped Cs2SbAgCl6 Double Perovskite: A Lead-Free, Low-Bandgap Material, , DOI 10.1021/acs.chemmater.8b03755 **Chemistry of Materials**,(2018) 30, 8280-8290 (IF 9.890)

10. McCartney A, Vignoli A, Biganzoli L, Love R, Tenori L, Luchinat C, Di Leo A Metabolomics in breast cancer: A decade in review. **Cancer Treat Rev.** (2018); 67:88-96. doi: 10.1016/j.ctrv.2018.04.012. (IF 8.122)

11. Saponaro A, Cantini F, Porro A, Bucchi A, DiFrancesco D, Maione V, Donadoni C, Introini B, Mesirca P, Mangoni ME, Thiel G, Banci L, Santoro B, Moroni A, A synthetic peptide that prevents cAMP regulation in mammalian hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, **Elife.** (2018) pii: e35753. doi: 10.7554/eLife.35753 (IF 7.725)

PUBLICATIONS

12. Peters,K.; Brandbury,J, Bergmann,S, Capuccini,M, Cascante,M, Rosato,A. et al. PhenoMeNal: Processing and analysis of Metabolomics data in the Cloud **GigaScience**, giy149, (2018) https://doi.org/10.1093/gigascience/giy149 (IF 7.267)

13. PG Takis, V Ghini, L Tenori, P Turano, C Luchinat Uniqueness of the NMR approach to metabolomics, TrAC **Trends in Analytical Chemistry**, (2018) https://doi.org/10.1016/j.trac.2018.10.036 (IF 7.030)

14. Schober D, Jacob D, Wilson M, Cruz JA, Marcu A, Grant JR, Moing A, Deborde C, de Figueiredo LF, Haug K, Rocca-Serra P, Easton J, Ebbels TMD, Hao J, Ludwig C, Günther UL, Rosato A, Klein MS, Lewis IA, Luchinat C, Jones AR, Grauslys A, Larralde M, Yokochi M, Kobayashi N, Porzel A, Griffin JL, Viant MR, Wishart DS, Steinbeck C, Salek RM, Neumann S nmrML: a community supported open data standard for the description, storage, and exchange of NMR data. **Anal. Chem** 90, 649-656 (2018). (IF 6.320)

15. Szekely O, Olsen GL, Felli IC, Frydman L. High-Resolution 2D NMR of Disordered Proteins Enhanced by Hyperpolarized Water. **Anal Chem**.; 90, 6169-6177 (2018) doi: 10.1021/acs.analchem.8b00585 (IF 6.320)

16. Bellomo G, Bologna S, Gonnelli L, Ravera E, Fragai M, Lelli M, Luchinat C. Aggregation kinetics of the Aβ1-40 peptide monitored by NMR. **Chem Commun**. (2018) 7601-7604. doi: 10.1039/c8cc01710g. (IF 6.290)

17. Merlino F, Daniele S, La Pietra V, Di Maro S, Di Leva FS, Brancaccio D, Tomassi S, Giuntini S, Cerofolini L, Fragai M, Luchinat C, Reichart F, Cavallini C, Costa B, Piccarducci R, Taliani S, Da Settimo F, Martini C, Kessler H, Novellino E, Marinelli L. Simultaneous Targeting of RGD-Integrins and Dual Murine Double Minute Proteins in Glioblastoma Multiforme, **J Med Chem.** (2018) 61, 4791-4809. (IF 6.253)

18. B. Wienen-Schmidt, H.R.A. Jonker, T. Wulsdorf, H.-D. Gerber, K. Saxena, D. Kudlinzki, S. Sreeramulu, G. Parigi, C. Luchinat, A. Heine, H. Schwalbe, G. Klebe, Paradoxically, Most Flexible Ligand Binds Most Entropy-Favored: Intriguing Impact of Ligand Flexibility and Solvation on Drug-Kinase Binding, **J. Med. Chem**. (2018) 61, 5922-5933.(IF 6.253)

19. Manzoni L, Zucal C, Di Maio D, D'Agostino VG, Thongon N, Bonomo I, Lal P, Miceli M, Baj V, Brambilla M, Cerofolini L, Elezgarai S, Biasini E, Luchinat C, Novellino E, Fragai M, Marinelli L, Provenzani A, Seneci. Interfering with HuR-RNA Interaction: Design, Synthesis and Biological Characterization of Tanshinone Mimics as Novel, Effective HuR Inhibitors, **J. Med. Chem.**, (2018), 61, 1483–1498, (IF: 6.253).

20. Gineste,S., E. Di Cola, B. Amouroux, U. Till, J.-D. Marty, A.-F. Mingotaud, C. Mingotaud, F. Violleau, D. Berti, G. Parigi, C. Luchinat, S. Balor, M. Sztucki, B. Lonetti, "Mechanistic insights into polyion complex associations", **Macromolecules** (2018) 51, 1427-1440. (IF 5.835)

21. Checcucci A, diCenzo GC, Ghini V, Bazzicalupo M, Becker A, Decorosi F, Döhlemann J, Fagorzi C, Finan TM, Fondi M, Luchinat C, Turano P, Vignolini T, Viti C, Mengoni A. Creation and Character-

ization of a Genomically Hybrid Strain in the Nitrogen-Fixing Symbiotic Bacterium Sinorhizobium meliloti. **ACS Synth Biol.** (2018), 7:2365-2378. doi: 10.1021/acssynbio.8b00158. (IF 5.316)

22. Cutrin JC, Alberti D, Bernacchioni C, Ciambellotti S, Turano P, Luchinat C, Crich SG, Aime S. Cancer cell death induced by ferritins and the peculiar role of their labile iron pool. **Oncotarget.** 46, 27974-27984. (2018) doi: 10.18632/oncotarget.25416. (IF 5.168)

23. 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 **Chemistry.** (2018), doi: 10.1002/chem.201804488. [Epub ahead of print] (IF 5.16)

24. Marra A, Dong J, Ma T, Giuntini S, Crescenzo E, Cerofolini L, Martinucci M, Luchinat C, Fragai M, Nativi C, Dondoni A Protein Glycosylation via Sulfur Fluoride Exchange (SuFEx) Chemistry: The Key Role of a Fluorosulfate Thiolactoside. **Chemistry** (2018) 24, 18981-18987 (IF 5.16)

25. Chatzikonstantinou, A, M. Chatziathanasiadou, E. Ravera, M. Fragai, G. Parigi, I. Gerothanassis, C. Luchinat, H. Stamatis, A. Tzakos, Enriching the biological space of natural products, through real time biotransformation monitoring: the NMR tube bioreactor, **BBA - General Subjects** (2018) 1862, 1-8. (IF 4.702)

26. Maione V, Cantini F, Severi M, Banci L. "Investigating the role of the human CIA2A-CIAO1 complex in the maturation of aconitase", **BBA - General Subjects** (2018) 1862 1980-1987. (IF 4.702)

27. Lilley, L.M, .K. Du, M.D. Krzyaniak, G. Parigi, C. Luchinat, T.D. Harris, T.J. Meade, "Effect of magnetic coupling on water proton relaxivity in a series of transition metal Gd3+ complexes", **Inorg. Chem.** (2018) 57, 5810-5819 (IF 4.700)

28. Montuschi P, Santini G, Mores N, Vignoli A, Macagno F, Shoreh R, Tenori L, Zini G, Fuso L, Mondino C, Di Natale C, D'Amico A, Luchinat C, Barnes PJ, Higenbottam T, Breathomics for Assessing the Effects of Treatment and Withdrawal With Inhaled Beclomethasone/Formoterol in Patients With COPD, **Frontiers in pharmacology** (2018); 9: 258 (IF 4.40)

29. 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.** (2018) e1800216. (IF 4.323)

30. Vignoli, A., Tenori, L., Luchinat, C., Saccenti, E. Age and sex effects on plasma metabolite association networks in healthy subjects. **J. Proteome Res** (2018)17, 97-107 (IF 4.268)

31. Caracausi M, Ghini V, Locatelli C, Mericio M, Piovesan A, Antonaros F, Pelleri MC, Vitale L, Vacca RA, Bedetti F, Mimmi MC, Luchinat C, Turano P, Strippoli P, Cocchi G., Plasma and urinary metabolomic profiles of Down syndrome correlate with alteration of mitochondrial metabolism, **Sci Rep**. 8(1):2977. doi: 10.1038/s41598-018-20834-y. (2018). (IF 4.122)

PUBLICATIONS

32. Sturlese M, Manta B, Bertarello A, Bonilla M, Lelli M, Zambelli B, Grunberg K, Mammi S, Comini MA, Bellanda M. The lineage-specific, intrinsically disordered N-terminal extension of monothiol glutaredoxin 1 from trypanosomes contains a regulatory region. **Sci Rep.** (2018) 8, 3716 (IF 4.122)

33. 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.**, (2018), Advance Article doi: 10.1039/C8DT03090A (IF 4.099)

34. Ciofi-Baffoni, S., Nasta, V., Banci, L. Protein networks in the maturation of human iron-sulfur proteins. **Metallomics** 10, 49-72, (2018) (IF 3.975)

35. Andreini C, Putignano V, Rosato A, Banci L.The human iron-proteome, **Metallomics.** (2018) doi: 10.1039/c8mt00146d. 10, 1223-1231 (IF 3.975)

36. Wang Y, Weisenhorn E, MacDiarmid CW, Andreini C, Bucci M, Taggart J, Banci L, Russell J, Coon JJ, Eide DJ The cellular economy of the Saccharomyces cerevisiae zinc proteome. **Metallomics**. (2018). doi: 10.1039/c8mt00269j. [Epub ahead of print] (IF 3.975)

37. A. Vignoli, B. Orlandini, L. Tenori, M. R. Biagini, S. Milani, D. Renzi, C. Luchinat, A. Calabrò. "The metabolic signature of Primary Biliary Cholangitis and its comparison with Coeliac Disease", **J.Proteome Res.** (*2019*) doi: 10.1021/acs.jproteome.8b00849 , (IF 3.950)

38. Gigli L, Andrałojć W, Dalaloyan A, Parigi G, Ravera E, Goldfarb D, Luchinat C. Assessing protein conformational landscapes: integration of DEER data in Maximum Occurrence analysis. **Phys Chem Chem Phys.** (2018), 43, 27429-27438. doi: 10.1039/c8cp06195e. (IF 3.906)

39. Louka A, Matlahov I, Giuntini S, Cerofolini L, Cavallo A, Pillozzi S, Ravera E, Fragai M, Arcangeli A, Ramamoorthy A, Goobes G, Luchinat C. Engineering l-asparaginase for spontaneous formation of calcium phosphate bioinspired microreactors. **Phys Chem Chem Phys** (2018), 20, 12719-12726. doi: 10.1039/c8cp00419f. (IF 3.906)

40. Luchinat, E., Chiarella, S., Franceschini, M., Di Matteo, A., Brunori, M., Banci, L., & Federici, L. Identification of a novel nucleophosmin-interaction motif in the tumor suppressor p14arf. **The FEBS journal**, 285, 832-847 (2018). (IF: 3.902)

41. Mitri, E., Barbieri, L., Vaccari, L. & Luchinat, E. 15N isotopic labelling for in-cell protein studies by NMR spectroscopy and single-cell IR synchrotron radiation FTIR microscopy: a correlative study. **Analyst** 143, 1171-1181 (2018). (IF: 3.885)

42. Cantini F, Calderone V, Di Cesare Mannelli L, Korsak M, Gonnelli L, Francesconi O, Ghelardini C, Banci L, and Nativi C Interaction of half oxa-/half cis-platin complex with human superoxide dismutase and induced reduction of neurotoxicity **ACS Med. Chem. Lett.** 9: 1094−1098. (2018). (IF 3.794)

43. Baggio, C., Cerofolini, L., Fragai, M., Luchinat, C. & Pellecchia, M. HTS by NMR for the Identification of Potent and Selective Inhibitors of Metalloenzymes. **ACS Med. Chem Lett** 9, 137-142 (2018) (IF 3.746).

PUBLICATIONS

44. Tenori, L., Santucci, C., Meoni, G., Morrocchi, V., Matteucci, G., Luchinat, C. NMR metabolomic fingerprinting distinguishes milk from different farms, **Food Research Int.,** 113, (2018), 131-139. (IF 3.520)

45. Rosato A, Tenori L, Cascante M, De Atauri Carulla PR, Martins Dos Santos VAP, Saccenti E. From correlation to causation: analysis of metabolomics data using systems biology. **Metabolomics** (2018) doi: 10.1007/s11306-018-1335-y 14, 37 (IF 3.511)

46. Cerofolini L, Fragai M, Luchinat C. Mechanism and Inhibition of Matrix Metalloproteinases **Current Medicinal Chemistry,** (2018), DOI: 10.2174/0929867325666180326163523, (IF: 3.469)

47. Romano F., Meoni G., Manavella V. , Baima G., Tenori L., Cacciatore S. , Aimetti M., Analysis of salivary phenotypes of generalized aggressive and chronic periodontitis, through nuclear magnetic resonance-based metabolomics. **J Periodontology** (2018) doi: 10.1002/JPER.18-0097 (IF 3.392)

48. Valasatava Y, Rosato A, Furnham N, Thornton JM, Andreini C, To what extent do structural changes in catalytic metal sites affect enzyme function?, **J.Inorg.Biochem.** 179, (2018) 40-53, (IF 3.348)

49. Basoglu A, Sen,I., Meoni,G., Tenori,L.,Naseri A., NMR-Based Plasma Metabolomics at Set Intervals in Newborn Dairy Calves with Severe Sepsis, **Mediators of Inflammation**, doi. 10.1155/2018/8016510, (2018) (IF 3.232)

50. Silva JM, Cerofolini L, Giuntini S, Calderone V, Geraldes CFGC, Macedo AL, Parigi G, Fragai M, Ravera E, Luchinat C. Metal centers in biomolecular solid-state NMR .**J Struct Biol** (2018), S1047-8477 (IF 3.231)

51. Cerofolini L, Staderini T, Giuntini S, Ravera E, Fragai M, Parigi G, Pierattelli R, Luchinat C Long-range paramagnetic NMR data can provide a closer look on metal coordination in metalloproteins. **J. Biol. Inorg. Chem** 23, 71-80 (2018) (IF 2.894)

52. Banci L, Camponeschi F, Ciofi-Baffoni S, Piccioli M. Correction to: The NMR contribution to protein-protein networking in Fe-S protein maturation. **J Biol Inorg Chem.** (2018), 687. doi: 10.1007/s00775-018-1573-5. (IF 2.894)

53. Barbieri, L., Luchinat, E., Banci, L. Intracellular metal binding and redox behavior of human DJ-1. **J. Biol. Inorg. Chem,** 23, 61-69 (2018). (IF: 2.894)

54. 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? DOI: 10.1007/s00775-018-1630-0 **J Biol Inorg Chem** (2018) doi: 10.1007/s00775-018-1630-0. [Epub ahead of print] (IF 2.894)

55. Murrali, MG, Piai,A., Bermel, W., Felli,IC, Pierattelli,R., Prolines' fingerprint in intrinsically disordered proteins, **ChemBioChem,** (2018), 19, 1625-1629. doi: 10.1002/cbic.201800172 (IF 2.774)

PUBLICATIONS

56. Mateos B, Konrat R, Pierattelli R, Felli IC. NMR characterization of long-range contacts in intrinsically disordered proteins from paramagnetic relaxation enhancement in 13C direct-detected experiments. **ChemBioChem.** (2018) doi.10.1002/cbic.201800539

57. Murrali MG, Schiavina M, Sainati V, Bermel W, Pierattelli R, Felli IC., 13 C APSY-NMR for sequential assignment of intrinsically disordered proteins. **J Biomol NMR.** (2018); 70, 167-175, (IF 2.534)

58. Carlon A, Ravera E, Parigi G, Murshudov GN, Luchinat C. Joint X-ray/NMR structure refinement of multidomain/multisubunit systems. **J Biomol NMR.** (2018) doi: 10.1007/s10858-018-0212-3. (IF 2.534)

59. Sala, D., Musiani, F., Rosato, A. Application of Molecular Dynamics to the Investigation of Metalloproteins Involved in Metal Homeostasis **Eur. J. Inorg. Chem.** (2018) (43), 4661-4677 (IF 2.507)

60. Ravera,E., P.G. Takis, M. Fragai, G. Parigi, C. Luchinat, "NMR Spectroscopy and Metal Ions in Life Sciences", **Eur. J. Inorg. Chem.** (2018) https://doi.org/10.1002/ejic.2018.00875, (IF 2.507)

61. Ciambellotti S, Turano P. Structural biology of iron-binding proteins by NMR spectroscopy, (2018). https://doi.org/10.1002/ejic.201801261 (online accepted article) **Eur. J. Inorg. Chem** (IF 2.507)

62. Fedeli L, Belli G, Ciccarone A, Coniglio A, Esposito M, Giannelli M, Mazzoni LN, Nocetti L, Sghedoni R, Tarducci R, Altabella L, Belligotti E, Benelli M, Betti M, Caivano R, Carni' M, Chiappiniello A, Cimolai S, Cretti F, Fulcheri C, Gasperi C, Giacometti M, Levrero F, Lizio D, Maieron M, Marzi S, Mascaro L, Mazzocchi S, Meliado' G, Morzenti S, Noferini L, Oberhofer N, Quattrocchi MG, Ricci A, Taddeucci A, Tenori L, Luchinat C, Gobbi G, Gori C, Busoni S. Dependence of apparent diffusion coefficient measurement on diffusion gradient direction and spatial position - A quality assurance intercomparison study of forty-four scanners for quantitative diffusion-weighted imaging.; Italian Association of Physics in Medicine (AIFM) Working Group on MR Intercomparison. **Phys Med.** (2018) Nov;55:135-141. doi: 10.1016/j.ejmp.2018.09.007 (IF=2.240)

63. 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.** (2018) doi: 10.1016/j.archoralbio.2018.10.023. (IF=2.050)

64. Calderone, V., Fragai, M. Luchinat, C. When molecular replacement has no trivial solution: the importance of model editing in humanS100Z X-ray structure solution. **Inorg Chim Acta** 470, 402-406 (2018) (IF 2.002)

65. Jörn Rittweger, Kirsten Albracht, Martin Flück, Severin Ruoss, Lorenza Brocca, Emanuela Longa, Manuela Moriggi, Olivier Seynnes, Irene Di Giulio, Leonardo Tenori, Alessia Vignoli, Miriam Capri, Cecilia Gelfi, Claudio Luchinat, Claudio Francheschi, Roberto Bottinelli, Paolo Cerretelli, Marco Narici, "Sarcolab pilot study into skeletal muscle's adaptation to long-term spaceflight", **Microgravity**, 4:18. doi: 10.1038/s41526-018-0052-1 (IF 2.00)

PUBLICATIONS

66. G. Parigi, E. Ravera, M. Bennati, C. Luchinat, “Understanding Overhauser Dynamic Nuclear Polarisation through NMR relaxometry”, **Mol. Phys**. (2018) DOI 10.1080/00268976.2018.1527409 (IF 1.774)

67. Luchinat, E., Banci, L. New structural and functional insights from in-cell NMR. **Emerging Topics in Life Sciences,** 2, 29-38 (2018). (IF: n.d.)

68. E. Ravera, A. Carlon, M. Fragai, G. Parigi, C. Luchinat, “Paramagnetic NMR as a new tool in structural biology”, **Emerging Topics in Life Sciences** (2018) 2, 19-28.

69. Sala, D., Giachetti, A., Rosato, A., Molecular dynamics simulations of metalloproteins: A folding study of rubredoxin from Pyrococcus furiosus AIMS **Biophysics** (2018) 5, 77-96

Meetings and Events Organised by CERM

EMBO Workshop on Challenges for Magnetic Resonance in Life Sciences, Principina Terra (Grosseto), Italy, May 27 - June 1, 2018

GIDRM day "Metabolomics in cancer", Florence, November 28th 2018

*CHANCES: **C**rystallograp**H**y **A**nd **N**MR in **C**ompl**E**mentary **S**tructural investigations,* Florence, September 4th 2018.

Seminars Held at CERM

Tuesday, November 6th 2018, 12:00 pm, **Raymond Joseph Turner,** PhD, Department of Biological Sciences, Faculty of Science, University of Calgary, Alberta, Canada, *"Characterization of a system specific chaperone for complex iron-sulfur molybdo-enzyme maturation"*

Friday, 26th October 2018, 6:00 pm, **Beatriz Jiménez,** Ph.D., Imperial Phenome Centre, Imperial College London, *"Towards standardisation of metabolic profiling approaches for molecular phenotyping"*

Monday, 22nd October 2018, 6:00 pm, **Gaetano T. Montelione,** Center for Advanced Biotechnology and Medicine, Rutgers, The State University of New Jersey, *"Proteins Flex to Function: Conformational Plasticity in Molecular Recognition"*

Friday, 11th October 2018, 18.00, Dr. **Lukas Trantirek**, CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czech Republic, *"Monitoring of nucleic acids structure and interactions in living human cells using in-cell NMR spectroscopy"*

Friday, 12th October 2018, 18.00, Dr. **Elizaveta A. Suturina,** Centre for Sustainable Chemical Technologies (CSCT), University of Bath, UK, *"Theory and modelling of lanthanide induced paramagnetic shift and relaxation enhancement"*

Thursday, October 4th 2018 at 6:00 pm, **Brian J Goodfellow**, Departamento de Química, Universidade de Aveiro, Portugal, *"Structural and functional aspects of the heme-binding protein p22HBP"*

Wednesday, 3rd October 2018 at 2:00 pm, Prof. **Alejandro J. Vila,** Instituto de Biologia Molecular y Celular de Rosario, CONICET - Universidad Nacional de Rosario, *"Metallo-beta-lactamases: a Tug of War between Bacteria and the Immune System for the available Zn(II)".*

Tuesday, July 17th 2018 at 11:00 am, Webinar – Metabolomics in Systems Biology

Friday, July 6th 2018 at 6.00pm, Dr. **Vladimir K. Michaelis** (Department of Chemistry - University of Alberta, Canada), *"Deciphering Local- and Medium-range Structure in Next-Generation Materials with Solid-state NMR Spectroscopy"*

Friday, May 11th 2018 at 10.00 am, Prof. **Sheref Mansy**, CIBIO - Centre for Integrative Biology, Università di Trento, *"Prebiotic synthesis and activity of iron-sulfur peptides"*

Friday, 2nd March, 2018 at 1:30 pm, Dr. **Chiara Falciani** (Università di Siena), *"Branched Peptides as Drugs"*

Monday April 23rd 2018 at 6:00 pm, Dr. **Luciano Abriata**, École polytechnique fédérale de Lausanne, Lausanne, Switzerland, *"Prospect for interactive, immersive molecular modelling"*

Thursday April 19th 2018 at 12:00 pm, Prof. **Martino Bolognesi**, Department of BioSciences, University of Milano, Italy, *"Structural Biology @UNIMI: now and then"*

19th-23rd February, 2018, West-Life Training Course "Advanced Methods for the Integration of Diverse Structural Data"

Monday, 5th February, 2018 at 6:00 pm, Prof. **Claus Seidel,** (Heinrich-Heine-Universitaet Duesseldorf, Lehrstuhl fuer Molekulare Physikalische Chemie), *"Optimally fluoresence-assisted structural modeling for dynamic biomolecules"*

Wednesday, 17th January, 2018, at 6:00 pm, Dr. **Chiara Bruckmann,** (IFOM, Foundation FIRC -Italian Foundation for Cancer Research- Institute of Molecular Oncology, Milan, Italy), *"Targeting TALE transcription factor function in cancer therapy: the competition between PREP1 and MEIS1 transcription factors in human cancer"*

Group Meetings

10/01 **Spyridon Gourdoupis** "Molecular aspects of iron sulfur protein biogenesis"

24/01 **Gaia Meoni** "Metabolomic investigation of food matrices by ^{1}H NMR spectroscopy"

31/01 **Tim Campbell** "Developing bioreactors for in-cell NMR spectroscopy"

07/02 **Panagis Polykretis** "SOD1, Cd and MTs… Lost Found"

28/02 **Eriberto Natali** "Synergic complement-mediated bactericidal activity of monoclonal antibodies with distinct specificity"

07/03 **Giovanni Bellomo** "Diagnostic applications of α-synuclein aggregation assays"

21/03 **Federica Bianchi** "Characterization of the human immune response to fHbp after vaccination with Bexsero through structural and functional studies"

28/03 **Alessio Bonucci** "SITE DIRECTED SPIN LABELING EPR SPECTROSCOPY: protein dynamics and distance measurements"

MEETINGS & EVENTS

04/04 **Matteo Cremonini** "Segmental labelling approach applied to the characterization of CCS D3 domain"

18/04 **Cristina Licari** "1H-NMR-based metabolomic applications in biomedical research"

02/05 **Veronica Ghini** "1H-NMR-based metabolomics: applications in biomedicine"

09/05 **Nihar Ranjan Prusty** "Expression and Characterization of Human Cystolic Fe-S Cluster Binding Scaffold Protein"

23/05 **Dafne Suraci** "Functional and structural elucidation of the mitochondrial Fe/S protein network"

06/06 **Silvia Ciambellotti** "L-ferritin iron-biomineralization: a step forward"

03/06 **Maria Grazia Murrali** "Functional interaction studies of intrinsically disordered proteins"

27/06 **Timothy Campbell** "Developing bioreactors for in-cell NMR spectroscopy"

04/07 **Valeria Putignano** "hMeProt database"

11/07 **Stefano Giuntini** "Preparation of protein-based bioconjugates for NMR studies: An overview about differently modified ANSII systems"

18/07 **Davide Sala** "Molecular Dynamics Studies of zinc(II) Diffusion Through the YiiP Transporter"

05/09 **Sara Bologna** "Production of Recombinant Alpha-Synuclein and its Application for the Development of New Strategies for the Diagnosis of Synucleophaties"

19/09 **Maxime Denis** "Design and optimization of Paramagnetic Tags for NMR"

26/09 **Vincenzo Maione** "IOP1: another brick in the (cytosolic iron-sulfur assembly) wall"

04/10 **Letizia Barbieri** "Recent advances in in-cell NMR spectroscopy @CERM"

17/10 **Lucia Gigli** "Representing conformational preferences of flexible proteins"

24/10 **Linda Cerofolini** "Carbonic anhydrase II: a model system for (paramagnetic) solution and solid-state NMR applications"

31/10 **Veroinca Nasta** "Protein networks in the maturation of human iron-sulfur proteins"

14/11 **Alessia Vignoli** "Serum NMR Metabolomics in Breast Cancer: a step further"

12/12 **Pantaleimon Takis** "NMR metabolomics of pancreatic "juice""

19/12 **Enrico Luchinat** "Monitoring protein-ligand interactions in human cells by NMR"

JOURNAL CLUBS

MEETINGS & EVENTS

17/01 - D. Sala, S. Bologna

14/02 - S. Giuntini, V. Putignano

14/03 - S. Gourdoupis, L. Gigli

11/04 - P. Polykretis, G. Bellomo

16/05 - G. Meoni

20/06 - F. Bianchi, E.N. Natali

21/11 - M. Cremonini, C. Licari

28/11 - D. Suraci, N. Prusty

05/12 - M. G. Murrali, M. Denis

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