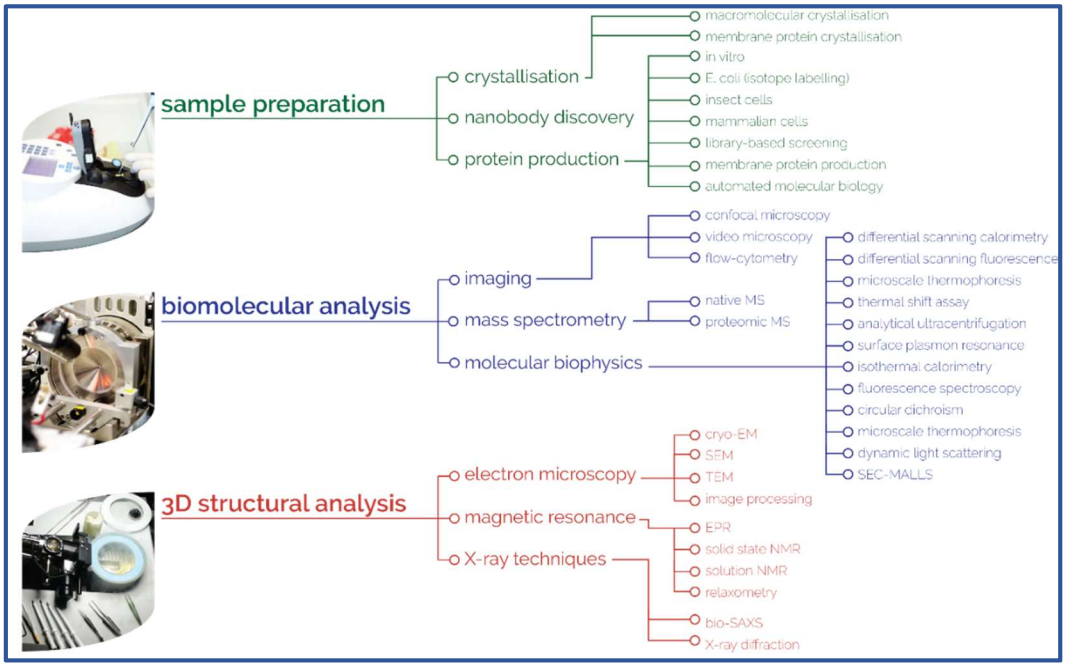
Instruct-ERIC
Catalogo dei servizi Offerti

Il catalogo dei servizi di Instruct-ERIC comprende 81 servizi disponibili nelle piattaforme tecnologiche degli 11 centri di Instruct-ERIC. Oltre al catalogo dei servizi, disponibile su https://instruct-eric.org/platform-catalogue, il sito dell'infrastruttura internazionale riporta i dettagli tecnologici di ciascun servizio, oltre alle modalità di accesso e il supporto che la comunità scientifica ha a disposizione.

Qualsiasi scienziato, accademico o industriale, può presentare una richiesta d'accesso a qualsiasi servizio disponibile presso i centri dell'infrastruttura distribuita mediante un singolo *entry point*. Questo portale permette la gestione di tutte le fasi di un progetto, dalla sottomissione, alla valutazione fra pari, dalla comunicazione con gli utenti alla gestione logistica delle visite. I ricercatori di uno Stato membro di Instruct-ERIC hanno diritto al supporto finanziario da parte dell'infrastruttura, senza nessuna limitazione di accesso transnazionale.



Instruct Centre IT, centro italiano di Instruct-ERIC, opera esclusivamente presso CERM-Unifi con il supporto del Consorzio Interuniversitario CIRMMP (CERM/CIRMMP Infrastructure). Instruct-IT offre servizi di risonanza magnetica per la caratterizzazione strutturale di proteine, servizi per la loro caratterizzazione biofisica e servizi relativi alla preparazione di campioni proteici arricchiti isotopicamente. I dettagli dei servizi sono riportati nelle pagine seguenti, estratte dal sito di Instruct-ERIC (https://instruct-eric.org/centre/instruct-centre---cermcirmmp-italy/).

Service / Technology Catalogue

Browse the services and technology available

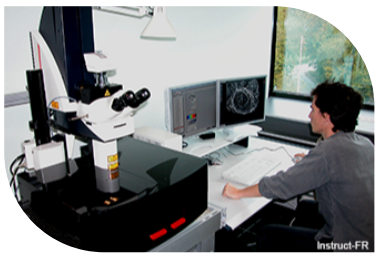
Click on the arrows to reveal more about the service/technology and the locations that offer it.

Sample Preparation

Crystallisation

Nanobody Discovery

Protein Production



Biomolecular Analysis

Imaging

Mass Spectrometry

Molecular Biophysics

3D Structural Analysis

Electron Microscopy

Magnetic Resonance Techniques

X-Ray Techniques

Filter Catalogue

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Consult the Instruct-ERIC Technology Availability List to find out which services are currently available.

Instruct Computational Services

To view a wide range of computational services for structural biology, with extensive user support, visit our Computational Services page.

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Service / Technology Instance

About Solution NMR, CERM/CIRMMP, Florence, Italy

☆ Flagship Service/Technology

View All Magnetic Resonance Techniques at Instruct

View All Solution NMR at Instruct

CERM/CIRMMP provides state of the art instrumentation and expertise to perform the most comprehensive array of experiments needed for the structure and dynamic characterisation of biological macromolecules and their complexes. All the standard pulse sequences for spectroscopic, structural and dynamical characterisation are available for attaining fundamental atomic level information. CERM/CIRMMP have developed ^{13}C direct detection protocols for "protonless" NMR experiments and for in-cell NMR spectroscopy, and tailored pulse sequences for structural determination of paramagnetic systems.

Service Availability:

Remote **Physical**

Instruments Available:

– Solution NMR at CERM/CIRMMP in Florence, Italy

The infrastructure is equipped with high and ultra-high field spectrometers equipped with cryoprobes (1.2 GHz TCI cryo, 950 MHz TCI cryo, 900 MHz TCI cryo, 700 MHz TCI cryo, 700 MHz TXO cryo, 500 MHz TCI cryo), heteronuclear detection probes at 600 MHz for low frequency nuclei to meet the most disparate experimental needs, liquid handler system and autosamplers for metabonomics and ligand screening at 400, 600 and 700 MHz. Special accessories such as high power probes (short hard pulse) at 400 and 600 MHz for paramagnetic NMR. All NMR

User Guide

To get structural information on proteins by solution NMR, isotopically labelled samples, usually ^{15}N and/or $^{15}N/^{13}C$, are required. For proteins with MW > 20 kDa, deuteration is also needed. In general, the degree of deuteration required is largely dependent on spectral quality, the size and modularity of the system studied and the types of experiment performed. However, perdeuteration (>90 %) are usually required for proteins with molecular weights of the order of 35 kDa or greater. A screening with several buffers (with different pHs, ionic strength and containing chemicals such as detergents, protease inhibitor cocktails, arginine etc.) is recommended to optimise NMR samples.

Support offered

The support might include sample preparation, instruments setup, data acquisition and data analysis. Based on the visiting scientist expertise assistance and supervision will be assigned.

For remote access, if the samples are not shipped in the NMR tube, our staff takes care to transfer it from the vial to the tube; air-sensitive samples will be handled in an oxygen-free chamber. Our staff will operate following the protocol for operations (air/light sensitivity, temperature of storage, etc...) provided together with the samples.

Contacts:

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University of Florence
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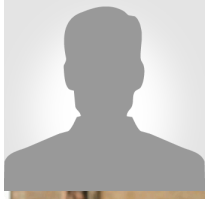

Francesca Morelli
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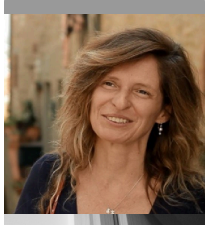

Isabella Felli
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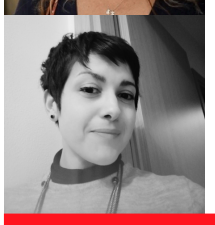

Laura Di Genova
CERM/CIRMMP
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Before the visit

The visiting scientist must communicate the scientific plan of the experiments and the contacts to arrange the visit. On average a visit is one week of instrument time and it can be arranged as a physical visit to the facility or by mailing-in the samples. Some projects might require more time, up to several weeks, depending on the complexity of the work to be performed and the experience of the visiting scientist in the field.

Contact the facility staff before submitting your proposal, so that the access request is in agreement with the scientific plan of the experiments.

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Service / Technology Instance

About Solid State NMR, CERM/CIRMMP, Florence, Italy

View All Magnetic Resonance Techniques at Instruct

View All Solid State NMR at Instruct

CERM/CIRMMP (Consorzio Interuniversitario Risonanze Magnetiche di Metallo Proteine) offers unique research capabilities in the field of solid-state NMR of biomolecules by providing state of the art instrumentation and expertise to perform, at the highest level, the most comprehensive array of experiments needed for the structure and dynamic characterisation of biological macromolecules and their complexes.

Solid-state NMR available at CERM/CIRMMP is typically applied for the determination of fibril structures and to obtain atomic-level structural information of biomolecules when they are bound to or trapped in solid matrices that lack long-range three-dimensional order. Detailed structural studies can be accomplished by exploiting the effects induced by the presence of paramagnetic metal ions. CERM/CIRMMP has a long tradition in the determination of paramagnetic effects in the solid state to access structural information.

Service Availability:

Remote Physical

Instruments Available:

– Solid State NMR at CERM/CIRMMP in Florence, Italy

Three solid state spectrometers are available:850WB Avance III-HD: PH MAS 850WB BL3.2 N/C/H DVT 3.2 mm + PH MAS 850W6 BL1.3 X/Y/F-H DVT 1.3 mm + PH MAS DVT 850W6 BL0.7 N/C/H 0.7 mm800SB Avance III-HD: PH MASDVT 800S5 BL1.3 Y/X/H TRIGAMMA 2 + PH MAS DVT 800S5 EF CNH 3.2 + PH MAS DVT 800S5 TL2 HCN 1.3700WB Avance AV: CPMAS DVT 1H/BB/BB 4 mm + CPMAS DVT 1H/BB/BB

User Guide

Solid State NMR spectroscopy measurements can be performed in virtually any kind of biological solid. CERM/CIRMMP has experience with several different kinds of biosolids including microcrystalline proteins, amyloids, fibrils and sedimented solutes. The vast majority of the experiments are based on direct heteronuclear detection, therefore ^{13}C-^{15}N labelled samples are required. Users can thus perform the following studies: assignment, determination of structural constraints; local dynamics; determination of paramagnetic effects.

Support offered

The support might include sample preparation, instruments setup, data acquisition and data analysis. Assistance and supervision will be assigned based on the expertise of the visiting scientist.

For remote access, storage instruction should be shipped together with samples. Transfer to the rotor is performed by our staff only for 3.2 and 4 mm rotors and non air/light sensitive samples. For high MAS frequencies (rotors of 1.3 mm or 0.7 mm), samples must be provided directly in the rotor, after testing that they spin properly.

Contacts:

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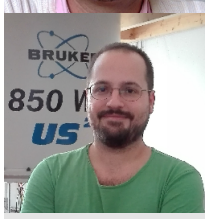

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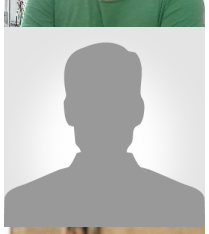

Francesca Morelli
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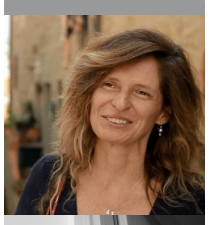

Isabella Felli
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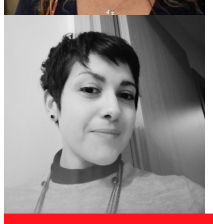

Laura Di Genova
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Before the visit

The visiting scientist must communicate the scientific plan of the experiments and the contacts to arrange the visit. On average a visit is one week of instrument time and it can be arranged as a physical visit to the facility or by mailing-in the samples. Some projects might require more time, up to several weeks, depending on the complexity of the work to be performed and the experience of the visiting scientist in the field.

Contact the facility staff before submitting your proposal, so that the access request is in agreement with the scientific plan of the experiments.

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Service / Technology Instance

About Relaxometry, CERM/CIRMMP, Florence, Italy

View All Magnetic Resonance Techniques at Instruct

Relaxometry is a technique that has been developed to obtain structural and dynamical information on nuclear spin systems. In the presence of a paramagnetic metal ion in the compound under investigation, relaxometry may provide information on the coordination of the nuclear spin with respect to the paramagnetic metal and, indirectly, information on the electron spin system. In fact, if the water proton exchange rate is fast or of the same order as the NMR timescale, the magnetic properties of the paramagnetic center are carried over from the water in bound position to the bulk.

Service Availability:

Remote Physical

Instruments Available:

– Fast Field Cycling Relaxometry at CERM/CIRMMP in Florence, Italy

Access to low-resolution instrument operating between 0.01 and 42.6 MHz, designed to measure the field dependence of the NMR longitudinal spin-lattice relaxation time. Technical specification: 1H Frequency range 0.01-42.6 MHz

User Guide

Relaxometry experiments are performed by measuring the longitudinal relaxation rate of bulk water protons as a function of the external magnetic field. In this way, Nuclear Magnetic Relaxation Dispersion (^{1}H NMRD) profiles are obtained. Programs are available for the analysis of the NMRD profiles of paramagnetic complexes with respect to these parameters. NMRD profiles can be measured also for diamagnetic molecules in water or in D_2O, to obtain information of the reorientation time of the molecules, and thus on their rigidity and aggregation.

Support offered

The support might include sample preparation, instruments setup, data acquisition and data analysis. Based on the visiting scientist expertise assistance and supervision will be assigned.

For remote access, if the samples are not shipped in the 10-mm tube, our staff takes care to transfer it from the vial to the tube; air-sensitive samples will be handled in an oxygen-free chamber. Our staff will operate following the protocol for operations (air/light sensitivity, temperature of storage, etc...) provided together with the samples.

Before the visit

The visiting scientist must communicate the scientific plan of the experiments and the contacts to arrange the visit. On average one "visit" is one week of instrument time and it can be arranged as a physical visit to the facility or by mailing-in the samples. Some projects might require more time, up to several weeks, depending on the complexity of the work to be performed and the experience of the visiting scientist in the field.

Contacts:

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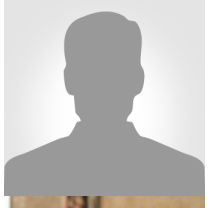

Francesca Morelli
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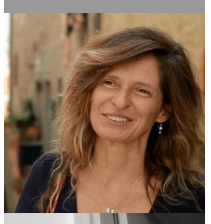

Isabella Felli
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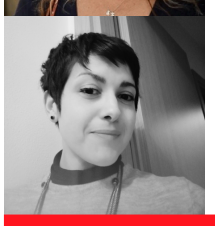

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Service / Technology Instance

About EPR, CERM/CIRMMP, Florence, Italy

View All Solution NMR at Instruct

View All Magnetic Resonance Techniques at Instruct

Electron Paramagnetic Resonance (EPR)-based methods have been used to map local dynamic and structural features of biomolecules, to explore different modes of biomolecule-ligand interaction, to obtain long-range structural restraints and to probe metal-ion-binding sites.

The Center of Magnetic Resonance (CERM) facility includes two EPR instruments (Continuous-wave X-Band and Continuous-wave/Pulse Q-Band), the necessary sample preparation wet lab (including glove-box) and the necessary processing computer. EPR measurements can be performed on biological samples containing paramagnetic metal ions (i.e. Fe, Cu, Mn etc.) or on samples opportunely labelled with paramagnetic tags (i.e. spin labels). The pulse Q-band EPR instrumentation present at CERM permit to measure DEER, HYSCORE and ESEEM experiments.

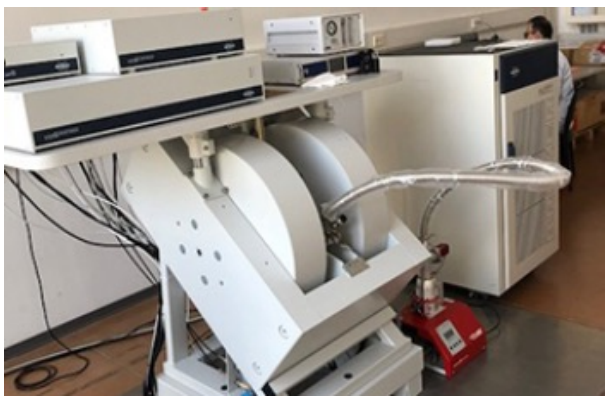
Service Availability:

Remote Physical

Instruments Available:

– EPR at CERM in Florence, Italy

Spectrometer Q-Band CW/FT-EPR ELEXSYS E580 with the possibility of operating in CW X-band



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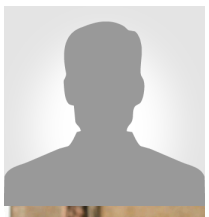

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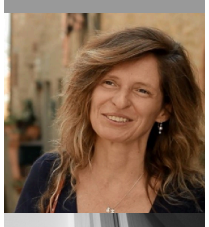

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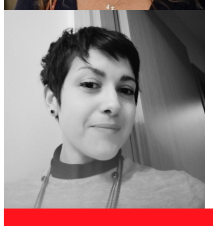

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User Guide

Sample Preparation

All tubes and capillaries should be filled to the same volume. Sample heights are in addition to the hemi-spherical tube bottom. If there are issues with the amount of sample available, we may be able to work with smaller volumes. However, we suggest to use the preferred sample heights to fill completely the EPR cavities in order to have spectra with high signal-to-noise ratio.

Preferred:

- X-band 35mm sample height
- Q-band 25mm sample height

EPR spectra can be recorded at different temperature, between 298K and 4K. For cryogenic conditions and pulsed Q-band measurements, samples must be previously frozen. Freezing is done by placing the bottom tip of the tube in liquid nitrogen until it starts to fizzle, then lower the tube into the liquid nitrogen at about 1mm/sec. This will allow for sample expansion upwards during freezing, thus preventing the tube from cracking. Rubber septa and commercial blue caps should be removed because they will admit liquid nitrogen, resulting in tube explosion and injury upon removal from storage. For aqueous samples, 30%-10% v/v glycerol or ~0.4 M sucrose can be used as a glassing agent.

Support offered

The support offers sample preparation, instruments setup, data acquisition and data analysis. Based on the visiting scientist expertise assistance and supervision will be assigned.

Before the visit

Before their visit, the visiting scientist must communicate the scientific plan of the experiments and the contacts to arrange the visit. Visits to the facilities may range from one day to weeks, depending on the complexity of the work to be performed and the experience of the visiting scientist in the field.

For remote access, if the samples are not shipped in the quartz EPR tube, our staff takes care to transfer it from the vial to the tube; air-sensitive samples will be handled in an oxygen-free chamber. Our staff will operate following the protocol for operations (air/light sensitivity, temperature of storage, etc…) provided together with the samples.

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Service / Technology Instance

About Molecular Biophysics, CERM/CIRMMP, Florence, Italy

View All Molecular Biophysics at Instruct

The CERM/CIRMMP platform provides instruments and services leading to the biophysical characterisation of biomolecules and the sample quality control using Dynamic Multi Angle Light Scattering (MALS-QELS) and circular dichroism (CD).

Service Availability:

Physical

Instruments Available:

+ Fluorimetry

+ Multi Angle / Dynamic Light Scattering at CERM/CIRMMP in Florence, Italy

+ UV-Visible Circular dichroism at CERM/CIRMMP

User Guide

Dynamic Multi Angle Light Scattering

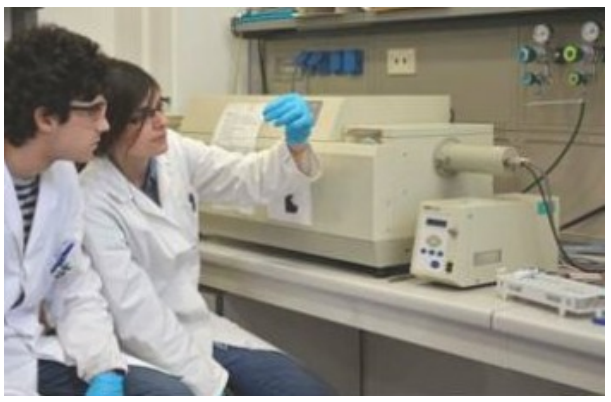
MALS-QELS is a straightforward technique to determine the accurate molar mass and the size of proteins and macromolecular complexes in solution. MALS-QELS can measure the absolute molar mass and size of molecules without the use of reference standards. One of the major application is the determination of the size and stoichiometry of tightly bound hetero-complexes, such as protein/protein, protein/DNA, protein/RNA and protein/detergent interactions.

The MALS-QELS technique available at the CERM/CIRMMP platform offer the possibility to analyse aggregation state of proteins and protein mixtures. Measurements can be performed with SEC separation column (HPLC) or in on batch mode. Samples volume range from 20 to 200 uL or more. Samples need to be deeply filtered and centrifuged to remove dust and small particles. All common buffers and additives are allowed for the measurements. The sample can be back collected after the experiments.

UV-Circular Dichroism

UV-CD is an excellent technique for studying conformational changes adopted by proteins and nucleic acids in solution as function of temperature, pH and concentration, to determine the secondary structure of proteins and peptides. Though unable to give detailed insights into the tertiary structure of a protein, UV-CD can be useful in complementing other structural techniques.

Far-Circular Dichroism



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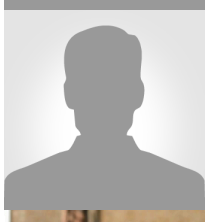

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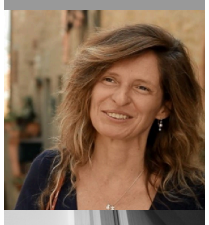

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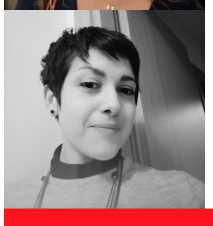

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The far-CD technique at CERM /CIRMMP platform offers several type of applications ranging from the determination of secondary structure elements, thermal stability analysis, studies of proteins unfolding caused by heat. Measurement of CD spectrum for the determination of secondary structure of protein requires 160 µl of 0.1-0.2 mg/ml protein solution; for the determination of DNA conformation requires 160 µl of 20 µM of solution or 1400 µl of 2 µM solution. For better results, the buffer alone should not have a high absorbance in the region of the spectrum (far or near-UV). For instance, high concentrations of dithiothreitol, glycerol, DMSO, histidine, imidazole etc. cannot be used in the far-UV region. The chloride ion as buffer component is not recommended with CD experiments and should therefore be kept as low as possible within the limit of acceptability for the molecule stability.

Visible-Circular Dichroism

Visible-CD is a very powerful technique to study metal protein interactions. Visible-CD can resolve the individual transitions as separate bands, particularly where the CD bands are of opposite sign.

The visible-CD spectroscopy at CERM /CIRMMP platform can be applied for studies of binding of metals to proteins/peptides/DNA. Unlike absorption spectra, CD spectra are only produced where a metal ion is in a chiral environment. In this way, the use of visible-CD has the advantage of being immune from interference by free metal ions, as only the protein-bound species are observed. The buffer can contain high concentrations of organic molecules/salts, i.e DTT, imidazole, NaCl etc.

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About Protein Production in Mammalian Cells for in-cell NMR, Florence, Italy

View All Protein Production at Instruct

The mammalian expression for in-cell NMR platform at CERM/CIRMMP is devoted to the to the production of mammalian cell samples overexpressing the protein of interest for its characterization by in-cell NMR. It relies on transient transfection in HEK293T adherent cells. The gene of interest is cloned in a vector optimized for high constitutive cytoplasmic expression. Small scale transfections are performed to determine the expression level and to assess the feasibility of in-cell NMR. Cell samples for NMR are produced in T75 flasks. Different protein labelling strategies are possible, e.g. U-15N labelling; amino acid type-selective ^{13}C,^{15}N labelling. Co-expression of two or more proteins is possible. In-cell NMR experiments are required to be performed at the Solution NMR Facility at CERM.

Service Availability:

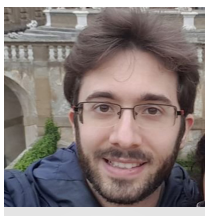
Physical

Instruments Available:

– Mammalian expression for in-cell NMR at CERM/CIRMMP in Florence, Italy

This facility is specifically dedicated to the production of mammalian cell samples overexpressing a protein of interest for characterisation by in-cell NMR. It relies on transient transfection in HEK293T adherent cells. The gene of interest is cloned in a vector optimized for high constitutive cytoplasmic expression. Small scale transfections are performed to determine the expression level and to assess the feasibility of in-cell NMR. Cell samples for NMR are produced in T75 flasks. Different protein labelling strategies are possible, e.g. U-15N labelling; amino acid type-selective 13C,15N labelling. Co-expression of two or more proteins is possible. In-cell NMR experiments are performed at the Solution NMR Facility at CERM

Contacts:



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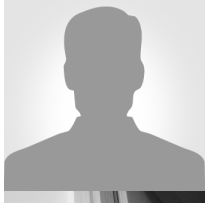

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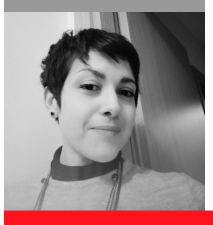

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User Guide

CERM/CIRMMP offers a platform dedicated to test and produce samples from mammalian cells overexpressing a protein of interest for its characterisation by in-cell NMR. Access to the platform allows users to perform small-scale tests to evaluate transfection and expression efficiency of their construct(s), and to produce full-scale cell samples with different labelling strategies for in-cell NMR analysis. Access to this platform is designed to be requested together with access to the Solution NMR platform at CERM/CIRMMP for sample analysis by in-cell NMR.

Proposals must include the requests for both Mammalian Expression for in-cell NMR and Solution NMR platforms.

Gene cloning

The DNA vector(s) will be provided by the facility to the user, which will clone the construct(s) of interest at his/her home institution, and will check them by gene sequencing.

Protein expression tests

Protein expression tests are required to assess the feasibility of in-cell NMR experiments. Transfection and expression tests will be performed at CERM/CIRMMP. The positive outcome (i.e. high expression levels in the soluble fraction of at least one construct) is necessary to provide access to the Solution NMR platform for in-cell NMR experiments.

Sample production for in-cell NMR experiments

Once protein expression at sufficient levels is established, the user will come at the platform for full-scale sample preparation and acquisition of in-cell NMR experiments at the Solution NMR platform.

Given the highly experimental nature of the technique, variation to the access scheme outlined above are possible, which will be discussed on a case-by case basis. The platform staff will make all efforts before and during the access to support the work of the user and ensure a successful outcome. However, successful protein expression or in-cell NMR data acquisition cannot be guaranteed.

Instruct Centre

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Service / Technology Instance

About Protein Production in E. coli with Isotope Labelling for NMR, CERM/CIRMMP, Florence, Italy

View All Protein Production at Instruct

The isotope labelling platform at CERM/CIRMMP is devoted to the large scale production of proteins uniformly enriched in stable isotopes (^{15}N, ^{13}C and ^{2}H) for biomolecular NMR spectroscopy studies. The platform is offering protein expression in bacterial cultures grown on chemically defined minimal media, as well as advanced protein purification approaches with the unique possibility of running them in anaerobicity. Users have access to the facility for large scale expression of labelled proteins in *E. coli*, under the supervision of a qualified platform engineer. Dedicated bench, optimised protocols and adequate isotopically labelled materials are available to users.

Service Availability:

Remote **Physical**

Instruments Available:

– Isotope labelling for NMR at CERM/CIRMMP

The isotope labelling platform installed at CERM/CIRMMP offers the possibility of large scale production of a 15N- and/or 15N,13C-labelled protein with labeling methodologies ranging from the standard uniform labeling to the most recent advanced selective labeling schemes. The platform benefit of the interaction with a

– Isotope labelling for NMR at CERM/CIRMMP (triple labelled)

The isotope labelling platform installed at CERM/CIRMMP offers the possibility of large scale production of a 15N,13C-,2H-labelled protein with a number of labeling methods ranging from the standard uniform labeling to the selective labeling schemes. The platform benefit of the interaction with a number of scientists from

User Guide

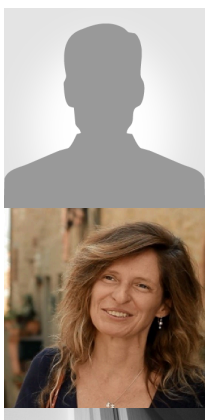
CERM/CIRMMP isotope labelling platform combines a large set of expression vectors for *Escherichia coli* and protocols, which enable a broad screening of soluble protein expression in labelled media through the use of multiple fusion proteins and various growth conditions.

CERM/CIRMMP isotope labelling expression platform offers two modes of access

1. The platform provides the expression vectors to the external users who perform cloning at their home institutions and come to CERM/CIRMMP to perform the expression assays in minimal media
2. The external users perform cloning and expression assays in minimal media at the CERM/CIRMMP platform

Support includes training and supervision, as well as help with data analysis which is especially crucial for complex systems. The facility has a long-term expertise in producing metallo-proteins.

Contacts:



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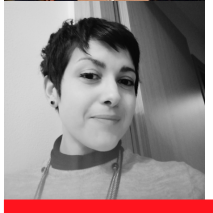

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Cost For Users

Approved Instruct Users: Access costs (Consumables, travel and accommodation) can be partially or fully covered by Instruct, depending on the general costs.

Academics: The cost of isotopic materials and consumables used for the production of proteins will be refunded by the users to the CERM/CIRMMP platform.

Private companies: quotation under request.

How to make a request

A request form should be sent to the administrators of the platform by email. Following receipt of the form, we will study the feasibility of your request within one month. If the request is accepted, we will contact you in order to propose a date for the access to the platform. Alternatively if a request cannot be satisfied, external users can have access to our protocols in order to express the protein in their lab.

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