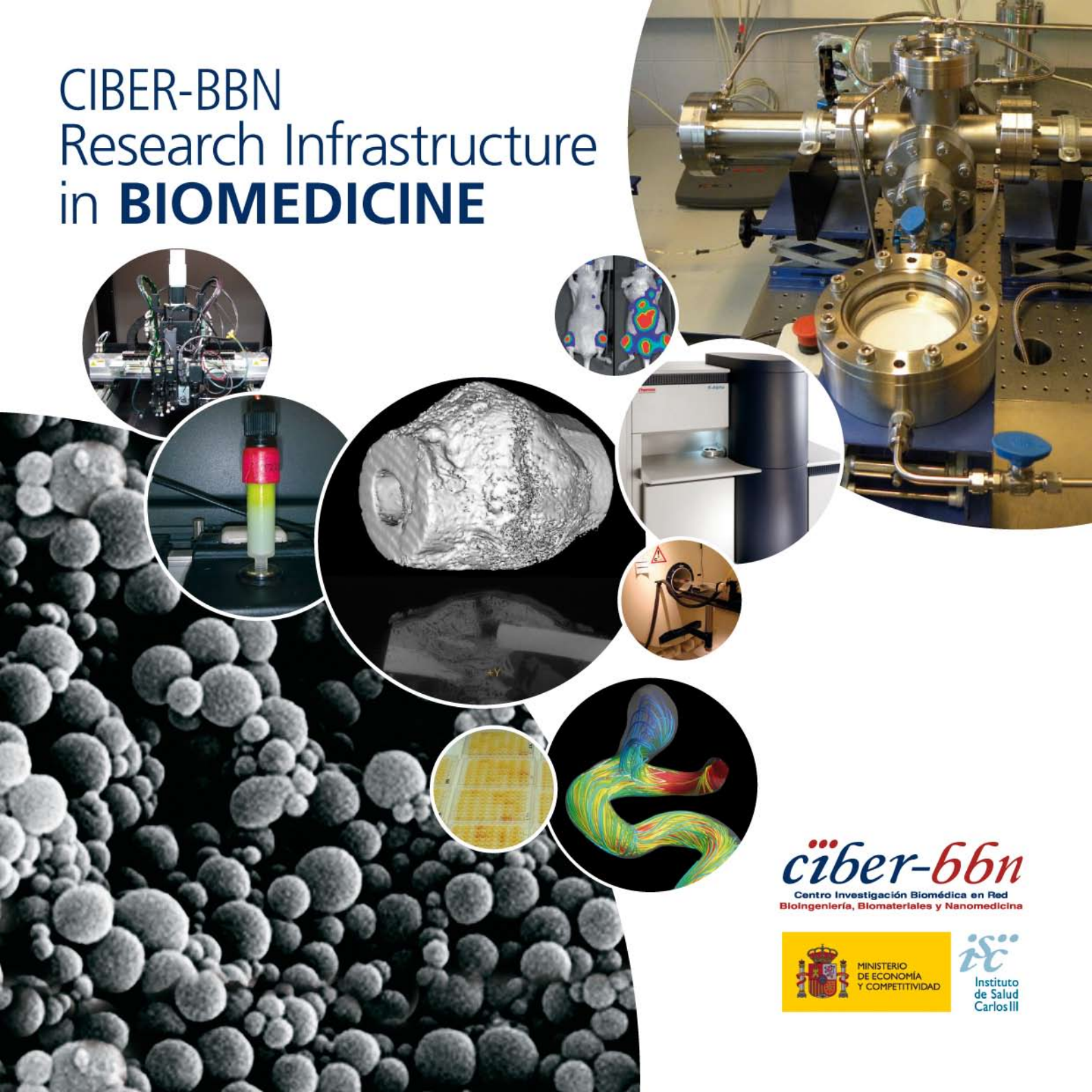
CIBER-BBN
Research Infrastructure in **BIOMEDICINE**



ciber-bbn
Centro Investigación Biomédica en Red
Bioingeniería, Biomateriales y Nanomedicina

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Instituto de Salud Carlos III

ciber-bbn

"Una manera de hacer Europa"

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CIBER-BBN

CIBER-BBN Research Infrastructure in **BIOMEDICINE**

The CIBER-BBN Research Infrastructure in Biomedicine is constituted as clusters of existing technical-scientific equipment which complement or are complemented by that equipment provided by CIBER-BBN. These clusters offer top-level technological resources to the CIBER-BBN research groups, external groups and companies, under established conditions.

This Infrastructure includes 19 Units which are distributed through the Spanish geography as follows: 13 units are located in Barcelona, 2 in Zaragoza, 1 in Badajoz, 1 in Valencia, 1 in Madrid and 1 in Alcalá de Henares (Madrid). Considering the purpose and functionality of each of them, these units are grouped in 5 Complementary Platforms.

WHAT IS THE RESEARCH INFRASTRUCTURE ON **BIOMEDICINE?**

19 Units distributed through the Spanish geography and grouped in 5 Complementary Platforms.

Clusters of state-of-the-art technical-scientific equipment to support your research in:

› Therapeutic Nanoconjugates and Drug delivery

› Regenerative Medicine and Tissue Scalffolding

› Biosensors and IVDs

› Diagnosis and contrast agents for MRI

› *In vivo* imaging

WHICH KIND OF SERVICES OFFERS THE PLATFORM?

› Top-level technological resources in the fields with the support and advice of international recognized researchers.

› You can subcontract the required services or we can work under a collaborative frame.

WHO CAN USE THESE SERVICES?

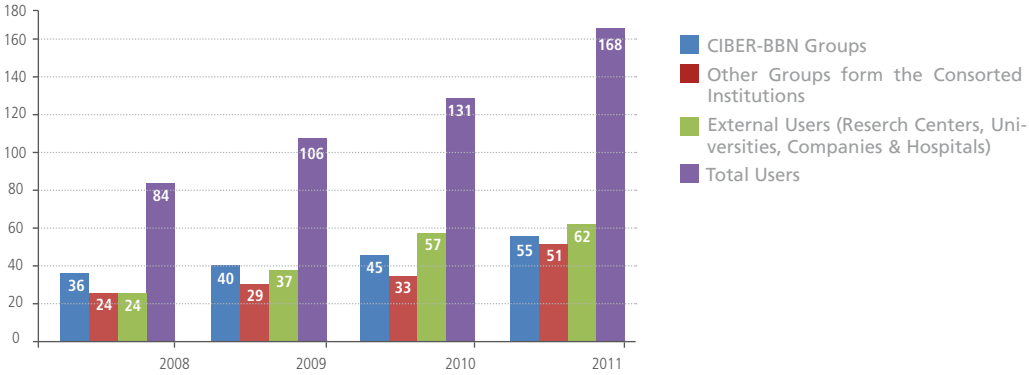
› CIBER-BBN Research Groups

› External Research groups

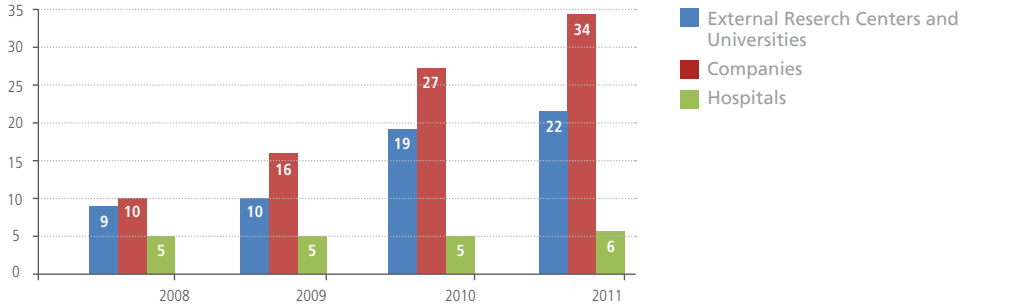
› Companies

› Hospitals

Users



External Users



COMPANIES THAT HAVE WORKED WITH THE CIBER-BBN RESEARCH SERVICES

I WOULD LIKE TO BE ORIENTED

Coordination of the required units for the development of the research project is offered under a "Single Contact Point Model" and like one only partner in both, subcontracting or in collaboration.

Research projects proposals for collaborative competitive calls, such as INNPACTO, FPVII, etc, like one only partner under the umbrella of CIBER-BBN.

Support in the design and the strategy to develop your project as advised by international recognized researchers in the matter.

More information about the CIBER-BBN research infrastructure and the selected services through CIBER-BBN website (www.ciber-bbn.es/en/servicios-de-investigacion).

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1. Production of Biomolecules Platform
2. Production of Biomaterials and Nanoparticles Platform
3. Tissue, Biomaterial and Surface Characterization Platform
4. Bioimaging Platform
5. High-performance Computing Platform

Aragon

(2) Synthesis of Nanoparticles. U. de Zaragoza.

(3) Tissue and Scaffold Characterization Unit*. Inst. Aragonés de Ciencias de la Salud /U. de Zaragoza.

Catalonia

(1) Protein Production Platform (PPP). U. Autónoma de Barcelona.

(1) Customized Antibody Service (CAbS). Inst. de Química Avanzada de Cataluña - CSIC.

(1) Synthesis of Peptides. Parc Científic de Barcelona.

(2) Biodeposition and biodetection. Centro de Investigación en Nanociencia y Nananotecnología de Barcelona (ICN2-CSIC).

(2) Rapid Prototyping. Inst. Bioingeniería de Cataluña.

(2) Biomaterial processing and nanostructuring. Inst. de Ciencia de Materiales de Cataluña (ICMAB-CSIC)

(2) Nanolithography / E-beam. Parc Cientific de Barcelona

(2) Micro-Nano Technologies. Centro Nacional de Microelectrónica, Inst. de Microelectrónica de Barcelona

(3) Nanostructured Liquid Characterization. Inst. de Química Avanzada de Cataluña (IQAC-CSIC)

(3) Nanotoxicology. Hospital Sant Pau de Barcelona.

(4) *In vivo* Experimental. Hospital Vall d'Hebrón.

(4) NMR: Biomedical Applications I. U. Autónoma de Barcelona.

(5) Equipment for High Performance Computing, massive storage and software for Biomedical Applications. U. Pompeu i Fabra.

Extremadura

(3) Surface Characterization and Calorimetry. U. de Extremadura.

Madrid

(3) Functional Characterization of Magnetic Nanoparticles. U. Politécnica de Madrid.

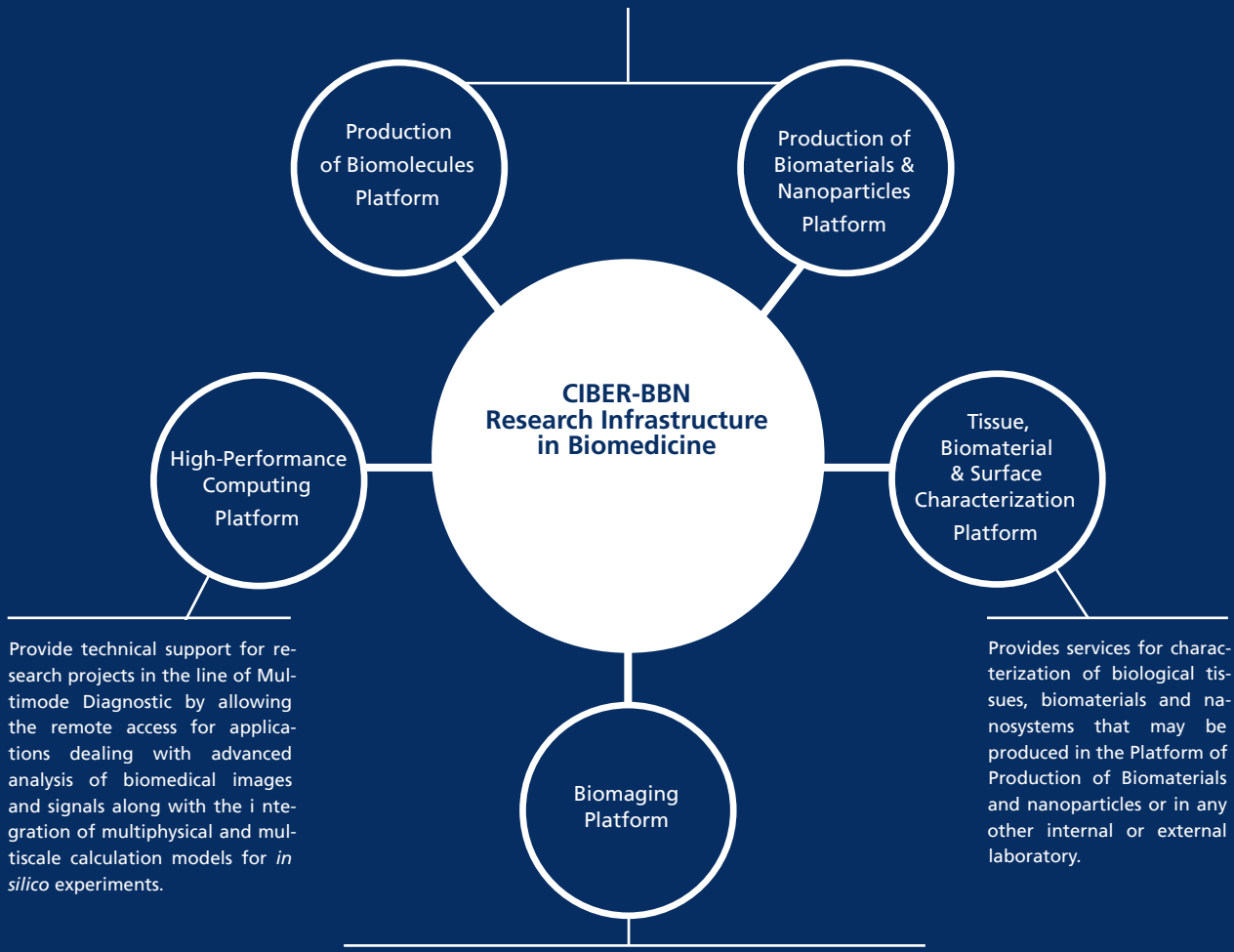
(3) Confocal Microscopy Service*. U. Alcalá de Henares.

Valencia

(4) NMR: Biomedical Applications II. U. de Valencia.

*These Units can also provide support for the Bioimaging Platform

Supply biological molecules, 2D and 3D materials and constructs for the development of research projects framed in the strategic lines of Tissue Engineering, Intelligent Devices, Implants, Therapeutic Nanoconjugates and Biosensors by providing customized design and production services.



Provide technical support for research projects in the line of Multimode Diagnostic by allowing the remote access for applications dealing with advanced analysis of biomedical images and signals along with the i ntegration of multiphysical and multiscale calculation models for *in silico* experiments.

Provides services for characterization of biological tissues, biomaterials and nanosystems that may be produced in the Platform of Production of Biomaterials and nanoparticles or in any other internal or external laboratory.

Give support in the preclinical development of new therapeutic compounds, nanosystems, materials and/or contrast agents and validate new therapeutic targets and/or nanotherapies by using optical image techniques and NMR.

Platform1

PRODUCTION OF BIOMOLECULES

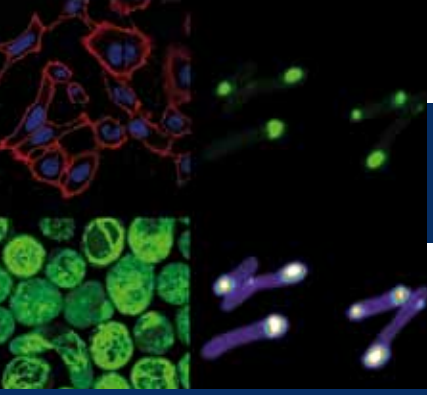
CIBER-BBN Research Infrastructure in **BIOMEDICINE**

1. PRODUCTION OF BIOMOLECULES PLATFORM

Supply biological molecules needed to the development of research projects framed in the strategic lines of Tissue Engineering, Intelligent Devices, Implants, and specially Therapeutic Nanoconjugates and Biosensors by providing customized design and production services for applications such as:

- Functionalization of nanosystems.
- Functionalization of implants and prosthesis.
- Tissue regenerative processes.
- Surfaces functionalization.

UNITS	OBJECTIVE
PROTEIN PRODUCTION PLATFORM (PPP)	Customized design, production and purification of recombinant proteins
CUSTOMIZED ANTIBODY SERVICE (CAbS)	Customized design and production of antibodies.
SYNTHESIS OF PEPTIDES	Synthesis and purification at large scale of peptide with difficult sequence and/or special modifications.



Protein Production Platform (PPP)

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www.ciber-bbn.es/es/programas/89-plataforma-de-produccion-de-proteinas-ppp

This facility is coordinated by the Applied Microbiology Group, leaded by Prof. Antonio Villaverde. It has both highly specialized personnel and the necessary equipment to offer an "a la carte" service for the design, production and purification of recombinant protein using both prokaryotic and eukaryotic expression systems. Prokaryotic expression systems, such as *Escherichia coli*, has low cost and high efficiency, but it is limited when posttranslational modifications are required. Besides, eukaryotic expression systems, such as those based on insect cells-Baculovirus and mammalian cell lines, are highly efficient introducing posttranslational modifications.

Many biomedical applications, including imaging diagnosis, nanoparticle functionalization, the incorporation of receptors for biosensing, the use and guided delivery of therapeutic enzymes and the development of artificial viruses for molecular therapy, require high amounts of recombinant proteins. Moreover, in many cases it may be helpful to add useful domains, not present in the natural versions (e.g., histidine-, lysine- or arginine-rich tails, and proteins fused to reporter proteins or solubilization tags (Green Fluorescent Protein -GFP- and Glutathione-s-transferase -GST-, respectively). This service relies on the experience of the Applied Microbiology group in design, molecular cloning, mutagenesis, gene expression, production and purification of proteins and characterization of the product obtained.

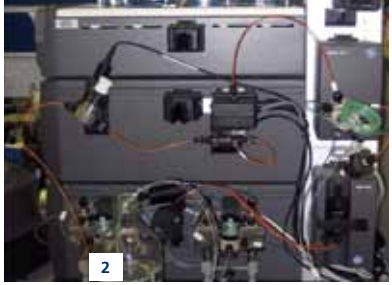
The members of this research group have an interdisciplinary background, covering basic and applied microbiology fields, molecular biology, cell culture, protein production and protein purification. In addition, incorporation of senior researchers from pharmaceutical and biotechnological companies provides added value to this platform.

SERVICES

The aim of this platform is to offer the design, production and purification of recombinant proteins, as well as the optimization of the technical processes, adding functional domains, purification tags, protease cleavage sites and reporter proteins when needed. The offered services are:

› Molecular cloning and bioproduction of recombinant proteins:

- Identification of the most appropriate system, selecting between *Escherichia coli*, insect cells (with expression systems based on infection with Baculovirus) and mammalian cells, according to the characteristics of the desired protein in terms of its size, modifications, etc.
- Cloning of the gene that codifies for the protein of interest in the chosen expression vector; selection of the positive genes and their characterization by sequencing.
- Production of the plasmid DNA that contains the gene of the relevant protein to be incorporated into the producing cells by transformation/transfection.
- Selection of the optimal protein production conditions using small and medium-scale expression; confirmation of the expression using Western-blot and SDS-PAGE analysis and quantification by densitometry.
- Measurements of the solubility/aggregation of the protein under study.
- Scale-up of the upstream processing in bioreactors or incubator shaker according to requirements.



- Protein purification:
  - Identification of the most appropriate strategy for purification, given the physical and chemical properties, opting either for purification modules or, in the case of fusion proteins, elements which enable purification by affinity.
  - Purification (downstream processing) using FPLC chromatography and/or tangential filtration.
  - Quality control of the purified protein in collaboration with the UAB Proteomics and Bioinformatics Service (SepBio) and the Crystallography Service:
    - Measurement of the molecular weight and purity using mass spectrometry (MS) and SDS-PAGE.
    - Amino acid sequencing of terminal amino groups by Edman degradation.
    - Assessment of the biological activity.
    - Infrared spectroscopy (FTIR) and dynamic light scattering (DLS) to determine the conformation of the protein.
- Cryopreservation
- Consultancy
- Training courses: (e.g. Course on "Strategies for optimization of recombinant protein production")

EQUIPMENT

- 2 and 7 liter bioreactors
- 10 liter WAVE bioreactor for insect cells
- Incubator shaker with cooling unit for insect cell suspension cultures
- Biosafety level 2 laboratory
- FPLC AKTA Chromatography systems for purification
- Tangential filtration Unit

Furthermore, the fact that the PPP Unit is at the Autonomous University of Barcelona (UAB), allows this service to be linked with other complementary services available within the university (Cell Culture, Cytometry, Production of Antibodies, Microscopy, Proteomic, and Bioinformatic, Cristalization, and the Microarray and Sequencing Services), as well as facilitating the management of subsequent uses of the produced protein.

1. **Purified Green Fluorescent Protein.**
2. **FPLC AKTA** system for purification.
3. **7 liter bioreactor.**

Customized Antibody Service Unit (CAbS)

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www.ciber-bbn.es/es/programas/90-unidad-de-produccion-de-anticuerpos-cabs

The CAbS (Custom Antibody Service) offers the production of monoclonal (MAbs) and polyclonal (PAbs) antibodies to CIBER-BBN member groups , to CSIC and to other research groups at public and private institutions and companies.

The purpose of this unit is to produce polyclonal and monoclonal antibodies with tailored affinity and selectivity features, following customized and standard protocols and using various experimental strategies including: the design of haptens for immunization and competition, number of fusions (MAbs), screening systems (MAbs) and degree of antibody purification.

The unit benefits from the experience of the Nabiotechnology for Diagnostics group (Nb4D-IQAC), led by Prof. M.-Pilar Marco, regarding production of antibodies against a wide variety of biomarkers and target analytes of interest for different applications. These include, for example, their use in various procedures related to the evaluation of carcinogenic processes, administration of drugs, delivery of therapeutic agents and food quality control.

All the experimental protocols are regulated and reviewed by the Bioethics Committee of the IQAC-CSIC.

SERVICES

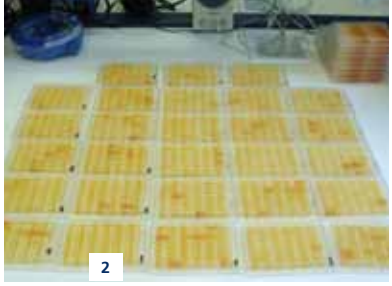
The CAbS provides a high quality service. Every project is examined by the Scientific Committee which produces a feasibility report. Once each project is completed, the antibody generated is delivered with a report including data related to its characterization, as well as the aspects related to its production.

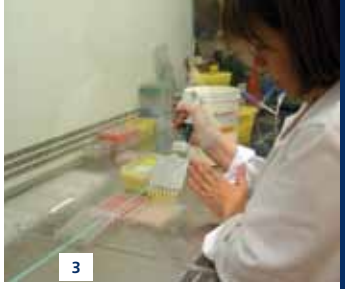
The services offered by the facility are:

› **Production of monoclonal antibodies:** Monoclonal antibodies are produced "*in vitro*" and result from the Synthetical activity of a single cell clone isolated from hybridoma cells that are kept in culture in the laboratory. The process includes various steps.

- Immunization of Balb/c mice aged between 6 and 8 weeks. Every mouse is administered with 4 injections of immunogen. Ten days after the last inoculation, ELISA (Enzyme-Linked Immuno-Sorbent Assay) is used to select the animal that shows the best immune response.
- Cell fusion of spleen lymphocytes from selected mice with mouse myeloma cells and screening of the antibody production from resulting hybridomas. Next, antibody-producing hybridomas are selected and cloned.
- Cryopresevation of selected hybridomas.
- Growth and culture of hybridomas for the production of monoclonal antibodies.
- Characterization of selected clones.

› **Production of polyclonal antibodies:** polyclonal antibodies are produced in White New Zealand rabbits. Each animal is administered with 4 injec-





EQUIPMENT

tions of immunogen. The antisera obtained is assessed by ELISA to evaluate antibody production. Immunogen characteristics and Immunization protocols are tailored to the needs of the user.

The unit also offers other services complementary to the production of antibodies which include:

- Advice on the design and preparation of immunogens.
- Preparation of immunogens and immunoreagents.
- Production of bioconjugates: biotinylation, fluorophore and enzymatic labeling, etc.
- Advice on setting up immunochemical methods of analysis.
- Characterization of polyclonal antibodies by ELISA.
- Isotyping of the purifed antibodies obtained.
- Purification of antibodies.
- Antibody fragmentation.
- Antibody production from frozen hybridoma clones.
- Mycoplasma testing.
- Cryopreservation of hybridomas.

The facility is located in the Institute of Advanced Chemistry of Catalonia (IQAC-CSIC; Barcelona). It is equipped with a laboratory for the development and production of monoclonal antibodies, which is kept under positive pressure to maintain sterile conditions. The laboratory is fully equipped for obtaining, selecting and storing monoclonal antibody producing hybridomas. The equipment consists in an inverted phase contrast microscope with a camera, two Class II laminar flow biological safety cabinets, two CO_2 incubators, a bioreactor for hybridoma culture, and tangential filtration unit for purification of the antibodies produced in the culture. In addition, the facility has infrastructure for the production of polyclonal antibodies, as well as laboratories for the synthesis of bioconjugates, haptens, the purification of antibodies and the performance of immunoassays.

Among the items of available equipment it should be highlighted a spotter for selection of high-producing cell lines and an automated ELISA microplate washer, plus standard equipment (centrifuges, fridges, liquid nitrogen containers, autoclave, baths, and vacuum equipment) and common laboratory apparatus.

In addition to the above mentioned equipment, the IQAC-CSIC provides animal husbandry facilities and a unit for the synthesis of organic molecules.

1. **Laboratory,** under positive pressure to maintain sterile conditions.
2. **Screening of the hybridomas.**
3. **Characterization** of antibodies by ELISA.

Synthesis of Peptides Unit

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www.ciber-bbn.es/es/programas/91-unidad-de-sintesis-de-peptidos

This is coordinated by the Peptides and Nanoparticles Group at the Parc Científic de Barcelona (PCB) and has the equipment necessary to provide services of synthesis of peptides at different scales (mg to g), purification, characterization, and post-synthesis modification, such as, conjugation to proteins and fluorescent labels.

This facility benefits from the wide experience of Dr. Fernando Albericio in the design and synthesis of peptides with specific biological activity and the introduction of modifications necessary for these peptides to be bound to therapeutic nanoconjugates and other molecules, either to take advantage of the pharmacological activity of the peptide itself or to facilitate the introduction of nanoconjugates or other molecules into the cells in order to reach the therapeutic target.

This group places especial emphasis on the synthesis of antitumor peptides of marine origin such as Kahalalide F, which are particularly difficult to synthesize and require the optimization of specific protocols, and on cell penetrating peptides with diverse chemical characteristics. The latter may be especially useful for treatments focusing on intracellular targets, such as gene and mitochondrial therapies. In addition and directly related to the Unit, this group also develops techniques for production of peptides and scale up in collaborative research projects with various companies including Lonza, PharmaMar and Infinitec.

SERVICES

- **Synthesis of peptides at various different scales (100 mg to grams).**
- **Purification and characterisation of these peptides using HPCL and HPLC-MS.**
- **Modification of the peptides during and post synthesis to meet user requirements:**
  - Binding to fluorophores and other types of molecules.
  - Conjugation to KLH protein or other carriers for immunization.
- **Synthesis of libraries of medium-sized peptides, their characterisation and purification.**


1


2


3

EQUIPMENT

With the exception of various peptides, which are notably difficult to work with, and certain modifications, which require synthesis to be carried out in liquid state, the technique used usually is the solid-phase synthesis. This consists of building the peptidic chain from a first amino acid (the C-terminal one) anchored to an insoluble polymer substrate. The advantage of this approach is that with the peptide bound to a solid substrate, an excess of reagents can be used to ensure that the reaction occurs quantitatively and the fraction which has not reacted together with any by-products formed can be removed by filtration.

After synthesis, the peptide is liberated from the substrate and can then be analysed using HPLC-MS and amino acid analysis before purifying by preparative HPLC. The post-synthesis modifications, such as, for example, the formation of disulfide bridges, fluorphore binding and conjugation to KLH-type proteins for immunizations to trigger antibody production, and those required for anchoring on nanoparticles or for conjugation with therapeutic nanoconjugates, can be carried out either while they are still on the solid substrate or once they have been liberated.

The instruments available include:

› Automatic synthesizer which can be operated at a range of scales (0.1-0.5 mmol) and with different types of chemistry (Fmoc and Boc) and coupling agents.

› Analytical high-performance liquid chromatography (HPLC) system with a diode array detector.

› Preparative high-performance liquid chromatography (HPLC) system with dual-wavelength UV detector.

› Analytical high-performance liquid chromatography (HPLC-MS) system with a diode array detector and coupled to a mass spectrometer.

› Bohdan miniblocks to generate libraries of medium-sized peptides.

› Lyophilizers and SpeedVac evaporators.

› System for acidolactic cleavage of the peptide resin boundby anhydrous HF (Boc/Bzl strategy).

In addition, this unit has access to:

› Amino acid analyzer and MALDI-TOF for characterization.

› Microwave peptide synthesizer.

1. **Automatic synthesizer** which can be operated at a range of scales (0.1-0.5 mmol).
2. **System for acidolactic cleavage** of the peptide resin boundby anhydrous HF (Boc/Bzl strategy).
3. **Analytical high-performance liquid chromatography (HPLC-MS)** system with a diode array detector and coupled to a mass spectrometer.

Platform2

PRODUCTION OF BIOMATERIALS AND NANOPARTICLES

CIBER-BBN Research Infrastructure in **BIOMEDICINE**

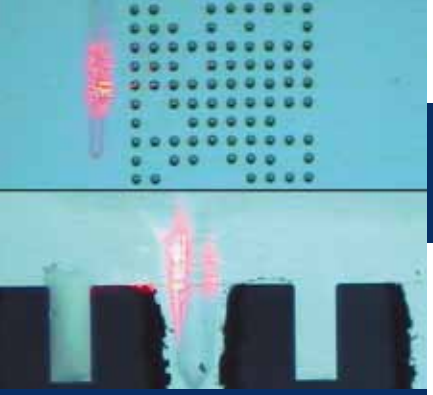
2. PRODUCTION OF BIOMATERIALS AND NANOPARTICLES PLATFORM

Supply 2D and 3D materials and constructs for the correct development of research projects dealing with the strategic lines of Tissue Engineering, Implants and with the Area of Nanomedicine. Some of the possible applications are:

- Functionalization of biosensors and devices by biodeposition.
- Surface treatment to improve the integration of implants and prosthesis.
- Creation of 3D matrices made of polymers, ceramics or composites; elaboration of scaffolds and prototypes for experimental assays, and functionalization of scaffolds with stem cells.
- Production and characterization of pure active pharmaceutical ingredients with nanoscale size, vesicular systems, nanosuspensions or composites of a biopolymer with an active ingredient.
- Synthesis of functionalized nanoparticles and surfaces.
- Preparation at lab-scale of molecular materials with controlled micro-, nano- and supramolecular structure.
- Growing of materials on surfaces to improve the bio-electronic interphases to enhance the signals biomonitoring.

UNITS	OBJECTIVE
BIODEPOSITION AND BIODETECTION	Controlled and reproducible microdeposition of biomaterials on different sensor areas and surfaces, and study of biomolecular interactions in real-time.
RAPID PROTOTYPING	Automated dispensing equipment for preparing scaffolds by using the Rapid Prototyping technique.
BIOMATERIAL PROCESSING AND NANOSTRUCTURING	Large-scale nanomedicine preparation through the use of compressed fluids.
NANOLITHOGRAPHY / E-BEAM	Manufacture of nanoelectrodes and nanostructured surfaces for implementing nanobiosensors and devices for cell growth and differentiation applicable to tissue engineering.
MICRO-NANO TECHNOLOGIES	Carbon nanotube growth. Prototyping and manufacture of micro/nanostructures and micro/nano-electrode arrays.
SYNTHESIS OF NANOPARTICLES	Synthesis of nanoparticles with applications in biomedicine , such as drug delivery and contrast agents to MRI.

Biodeposition and Biodetection Unit



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This Unit is coordinated by Prof. Laura Lechuga from the Nanobiosensors and Bioanalytical Applications Group (nanoB²A). It is constituted by two systems: the Nano eNabler™ system and a Surface Plasmon Resonance (SPR) fully automated biosensor (Sensia ß-SPR).

The Nano eNabler™ system consists of a highly flexible and automated molecular printer that can dispense minute volumes of liquids at defined positions to create patterns of spots or lines with high spatial resolution and high reproducibility. The printing process is based on the direct deposition of any solution onto the desired surface by the Surface Patterning Tool (SPT™), which is a microcantilever-based microfluidic device, with a reservoir which contains the solution and a microchannel with an opening gap at the end. The system is highly versatile and allows both the deposition of a wide variety of materials and over all kind of surfaces. It is especially suitable in the field of micro/nanoarrays, to create user-defined patterns, with clear advantages in terms of versatility, speed, and ability to deposit over a big surface area. Moreover, the process can be monitored through a high resolution optical microscope with video capture controlled by the NanoWare™ software.

On the other hand, the Surface Plasmon Resonance is a powerful technique to measure biomolecular interactions in real-time and under label-free configurations. The SPR biosensors rely on detection of changes in refractive index which are related to mass changes at the sensor surface. SENSIA ß-SPR is a dual channel SPR instrument which integrates computer controlled pumps, valves and injection fluidics. The biosensor allows the detection of the interaction between the target molecules and their specific receptors, previously immobilized on the gold surface. It has been applied for the detection of a wide range of target analytes with interest in many areas such as:

- Analysis of antibody-antigen interactions
- Drug design
- Clinical diagnosis
- Monoclonal antibody characterization
- Nucleic acids detection
- Virus-protein interactions

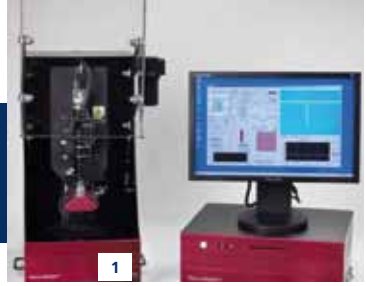
SERVICES

The Nano eNabler™ service offered is endorsed by the scientific and technical support of the members of the Unit of Biodeposition and Biodetection. Some of the possible applications of this service are:

- **Biosensor functionalization:**
  - Microarrays
  - Biomedical devices
- **Surface patterning for cell culture or virus detection**
- **Low-volume bioasays**
- **Molecular screening, e.g. biomarkers**

The Surface Plasmon Resonance (SPR) is offered as a self-service, however a specific training by the scientific and technical members of the nanoB²A group will be provided.
Some examples of applications developed in the nanoB2A group using the SENSIA ß-SPR are:

- **Genomics:** Detection of DNA and RNA sequences, either synthetic and real samples, and screening of point mutations.
- Environmental Quality Control: Detection of small molecules like pesticides using competitive inhibition immunoassays.
- Clinical diagnosis and drug design: Detection of biomolecules (proteins, antibodies, organic compounds...) in biological fluids (urine, serum...) using direct or indirect immunoassays.



EQUIPMENT

1.Nano eNabler™ System equipped with an outer enclosure to completely control de environment (Humidity range: 25-80% RH). Optical Microscope (150X to 1000X) with Video Capture controlled with the NanoWare™ software. A Bioforce UV Tip Cleaner for cleaning of the SPT™ for further reuse is also available

› System specifications:

- Delivery of attoliter (10^{-15} L) to femtoliter (10^{-18} L) volumes
- Printing of spots and lines from 1 to 60 µm
- 20 nm stage resolution, 50 mm XY travel range
- 100 msec printing cycle
- 25 to 80% Relative Humidity (RH) work range
- High control of the dispensed volume and the size of the formed drop
- Printing in any user defined-pattern
- Multiplexing ability

› Compatible materials for deposition:

- Antibodies and other proteins
- Nucleic acids
- Viruses
- Cells, bacteria
- Nanoparticles (e.g. Quantum dots, colloidal nanoparticles…)
- Etchants, solvents, adhesives

› Compatible surfaces:

- Glass, silicon, silanes
- Alkanethiol, SAMs
- Gold and other metals
- Hydrogels
- Nitrocellulose
- PDMS
- Plastic and other polymers

2. Surface Plasmon Resonance (SPR) (Sensia ß-SPR).

› System specifications:

- 300 nl each flow cell
- Angular resolution of 0.01°
- Detection limit of 10^{-5} in refractive index
- Disposable sensor glass slides of 10 mm x 10mm coated with a 50nm Au layer
- Monitoring changes in refractive index as a function of time allows real-time analysis of the binding events occurring at the sensor surface.

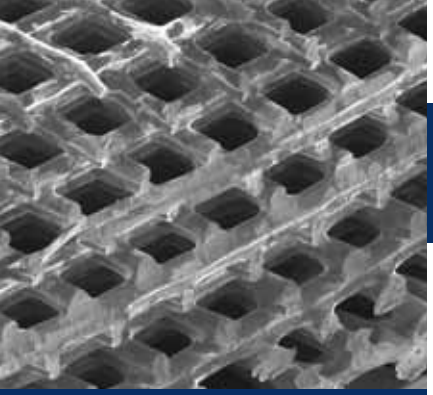
› Compatible targets for detection:

- Antibodies and other proteins
- Nucleic acids
- Viruses
- Cell, bacteria
- Organic and inorganic compounds

› Compatible media:

- Aqueous buffers
- Biological fluids (serum, urine…)

1. **Nano eNabler™ System.**
2. **Surface Plasmon Resonance (SPR).**
3. **Nano eNabler™ System** with Surface Patterning Tool (SPT™).



Rapid Prototyping Unit

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This facility is headed by Dr. Josep A. Planell, PI of the Biomaterials for Regenerative Therapies Group at the Institute for Bioengineering of Catalonia (IBEC). It consists of a rapid prototyping set-up based on an automated dispensing system that produces 3D structures by the layer-by-layer deposition of different materials. This system allows the fabrication of well-defined, computer designed 3D structures with predetermined geometry in a reliable and highly reproducible manner. The tool allows a high control of the structure porosity, pore distribution and morphology. The rapid prototyping facility enables fabrication of 3D structures using polymers and ceramics with a wide range of viscosities; it can also dispense proteins and cell suspensions.

The main application of this system is the fabrication of 3D porous scaffolds of defined geometry and porosity for tissue engineering applications. Dispensing and locating cells in specific positions on defined substrates is also possible due to the high precision levels of this tool.

Some examples of this application include:

› Fabrication of polymer, ceramic and composite (polymer reinforced using ceramics and/or glass) scaffolds for tissue engineering.

› Incorporation of biomolecules, such as proteins and peptides, within these 3D structures.

› Fabrication of scaffolds of defined characteristics to study the effect of scaffold design variables, such as porosity, geometry and pore size, on the biological response.

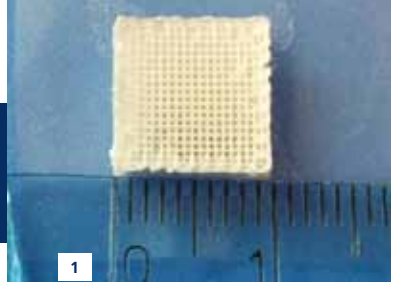
› Design of scaffolds for finite element modeling and simulation of their mechanical behavior under physiological conditions

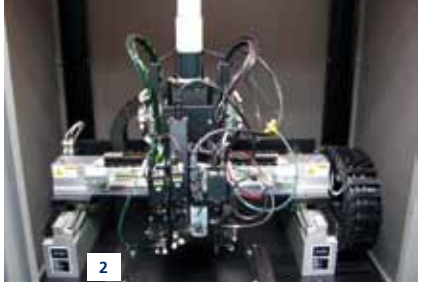
SERVICES

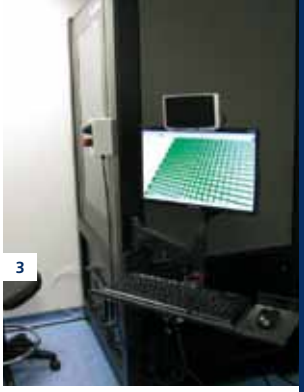
The services offered by this unit consist of the use of the equipment to:

› **Fabrication of 3D porous scaffolds for tissue engineering with defined geometry and porosity using polymers, ceramics, glass, proteins and cell suspensions. There are existing predefined protocols for the production of polymer scaffolds based on polylactic acid (PLA) and chitosan and also PLA/calcium phosphate glass composite scaffolds.**

› **Dispensing groups of cells on specific sites on defined substrates.**







1. PLA/PEG/calcium phosphate glass composite scaffold.
2. and 3. Tissue Engineering Tool 3D-300.

EQUIPMENT

The system is a Tissue Engineering Tool 3D-300 series (nScript Inc.) for rapid prototyping with the following specifications:

› Three heads for injection of different materials:

- Polymers
- Ceramics
- Living cells

› Allowed viscosities ranging from 1 to 10^6 centipoises.

› System for temperature and humidity control to enable the printing conditions to be adjusted. It is possible to create a microenvironment appropriate for each mixture of materials and proteins and to achieve high rates of cell viability.

› Controlled by Software .

› Visual monitoring system based on a digital camera, firewire cameras and high magnification lenses, with detection and measurement of colormetric/monochromatic variations.

› Mapping system using a laser sensor for control of normal and extended working distances. It includes Target, Grid and Path mapping.

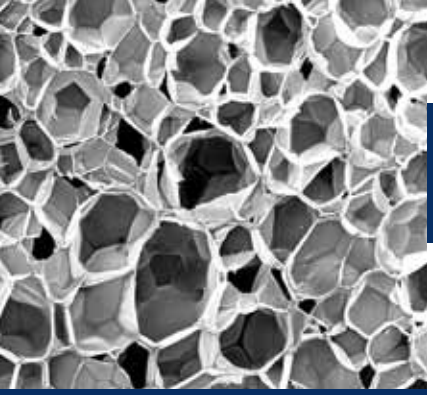
› High-precision dispensing pump for dynamic control of the flow rate.

› Position control system:

- X/Y/Z accuracy: ±10 µm
- X/Y reproducibility: ±2 µm
- X/Y resolution: 0.5 µm
- X/Y velocity: 304 mm/s (12”/s)
- X/Y displacement: 304X 152 mm (12”X6”)
- Z displacement: 101 mm (4”)

In addition, it should be noted that the system is sited in a clean room with a controlled environment and supply of the gases required for these tests.

Biomaterial Processing and Nanostructuring Unit



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Under the coordination of Professor Jaume Veciana, current director of NANOMOL Group, the mission of this facility is the large-scale production and characterization of molecular biomaterials of therapeutic or biomedical interest, with controlled micro-, nano- and supramolecular structure. This unit is composed of the equipment and the scientific and technical personnel to develop and apply efficient, robust and green technologies based on the use of supercritical fluids (or dense gases), such as compressed CO_2. By this approach, it is possible to produce solid or liquid-dispersed particulate materials:

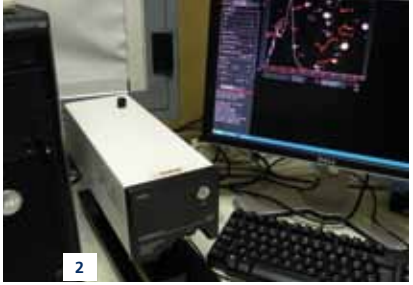
- in a single step;
- with high control in structural characteristics (particle size, polymorphism, supramolecular structure, morphology);
- in highly reproducible and easy scalable processes.

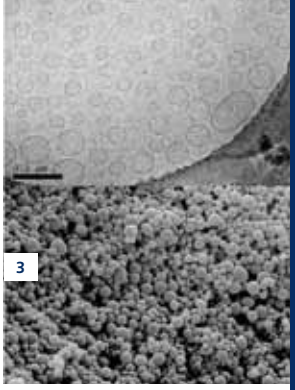
In relation to conventional processes, these technologies are less energy consuming, achieve higher batch-to-batch consistency and produce lower environmental impact. The total or partial replacement of organic solvents by compressed fluids in the processing of molecular materials for nanomedicine or biomedical applications, enables to reach the purity levels required by regulatory agencies (e.g. FDA) in the production of active principles of interest for pharmaceutical industry.

Therefore, the main activity of this unit is focused on the production of pure micro- and nanoparticulate active therapeutic ingredients and passive or active therapeutic nanoconjugates (vesicles, nanosuspensions, active-biopolymer nanocomposites), as well as porous matrices of biocompatible polymers for tissue engineering or implants. The unit has also instruments and the necessary know-how to undertake nanostructuring of molecular biomaterials in films and surfaces, with application in the development of new biomedical devices.

To complement materials processing facilities this unit has equipment for the characterization of micro- and nanostructured materials:

- Particle size distribution measurements by dynamic light scattering (DLS).
- Statistical particle size and shape characterisation from 0.5 microns to several millimeters (Morphologi G3).
- Studies of crystallinity and polymorpism by differential scanning calorimetry (DSC).
- Visual observation using an optical microscope.
- Chemical analysis using spectroscopic techniques.





1. High-pressure laboratory-scale plant.
2. Nanosight LM20 Particle Size Analyser.
3. Vesicles, nanosuspensions and nanoparticles.

SERVICES

› Preparation of micro- and nanostructured materials, using compressed fluids as Green solvents (high pressure plants from 30 to 300 mL).

› Preliminary assessments of scalability and economic viability of the processes.

› Characterization of molecular biomaterials:

- Analysis of particle size and shape distributions (Dynamic Light Scattering, DLS, and DLS + image processing, optical microscopy).
- Thermal analysis of solid materials (differential scanning calorimetry, DSC).
- High-pressure phase analysis.
- Visual observation by an optical microscope with a heating place and fluorescence source.
- Fluorescence and UV-Vis spectroscopic analysis.
- Measurements of specific density using a helium pycnometer and of packed (tapped) density using an Autotap analyzer.
- Treatment of sample by ultrasound probe.

In general, the services provided by this facility may be subcontracted or used in collaboration. The biomaterial characterisation tools can be used on a self-access basis by qualified users.

EQUIPMENT

› High-pressure laboratory-scale plant with 50, 100 and 300 ml reactors for the processing of biomaterials.

› High-pressure phase analyzer for studying the thermodynamic behaviour of materials.

› Malvern Zetasizer Nano ZS particle size analyzer.

› Nanosight LM-20 particle size analyzer.

› Perkin-Elmer DSC 8500 differential scanning calorimeter.

› Olympus BX51 optical microscope with heating plate and fluorescence accessories.

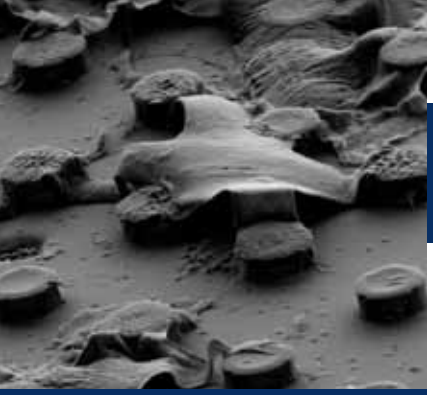
› Perkin-Elmer LS 45 fluorescence spectrometer.

› Varian Cary 5 UV-Vis spectrometer.

› Quantachrome Ultrapyc 1200e helium pycnometer.

› Ultrasound probe and Autotap instrument for packed density measurements of powders.

› Malvern Morphologi G3 for advanced particle morphologi characterization.



Nanolithography/e-Beam Unit

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This platform is coordinated by Dr. Josep Samitier, PI of the Nanomedicine Group of the Instituto de Bioingeniería de Cataluña (Bioengineering Institute of Catalonia) (IBEC). This unit is made up of an Ultra High Resolution (UHR) field emission scanning electron microscopy (SEM) system coupled to an Electron Beam Lithography (EBL) system.

The SEM currently has a wide range of detectors which, when combined with the two available operating modes: High Vacuum-HV mode (conductive materials) and the Low Vacuum-LV mode (semiconducting or insulating materials without needing metal coatings), offers the possibility of obtaining topographic and composition information of a very wide range of different inorganic and organic samples.

According to the detector used, its applications are different:

› The ETD (Standard) and TLD (High Resolution) detectors allow, in High Vacuum mode, detecting the Secondary Electrons (SEs) on a sample to obtain high-resolution images. Some examples achieved already are:

- Images of silicon masters with different topographic conformations.
- Images of bismuth sulfide nanoparticles of the order of 50nm x 8nm (magnifications of the order of 120,000x).
- Images of bacteria, with a size of the order of microns, coated with gold (magnifications of the order of 160,000x).
- Images of peptides forming fibers with gold nanoparticles (magnifications of the order of 100,000x).

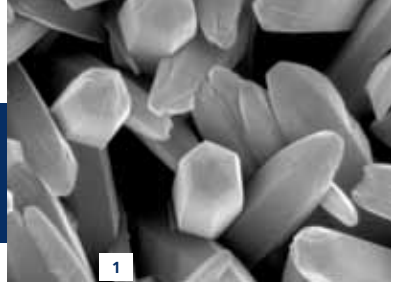
› The vCD detector (HV and LV mode) allows detecting the Backscattered Electrons (BSEs) to obtain images of the composition and topography of the surface. The combination of this detector with the Beam Deceleration (BD) option allows working at very small voltages (500V-2KV), providing high contrast and resolution. Example:

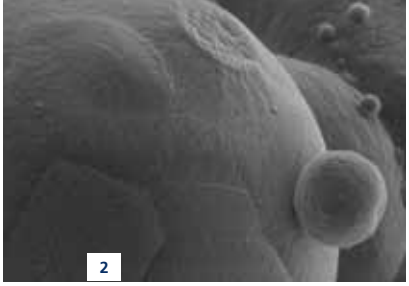
- Images of nanoparticles (magnifications of the order of 160,000x).

› The LVD detector (LV mode) offers the possibility of obtaining topographic images of semiconducting or insulating samples without prior treatment of the sample, widely used in biological samples: bacteria, cells and nanoparticles.

In addition, EBL is based on the selective exposure of a material sensitive to electrons (resin) by means of a focused electron beam which allows performing lithography with a current resolution of about 100 nm. The polymeric materials most used for this purpose are polymethylmethacrylate (PMMA). Lithography is a technique which is used to manufacture micro/nano-structures such as those used, for example, in microelectronic devices such as integrated circuits.

The main application of the group responsible for this platform is focused on the manufacture of nanoelectrodes and nanostructured surfaces for implementing nanobiosensors and devices for cell growth and differentiation applicable to tissue engineering.





SERVICES

The services provided in this Unit are managed by the Parc Cientific de Barcelona (Barcelona Science Park) (PCB) together with the rest of the services of the "Nanotechnology Platform" of the PCB where the techniques necessary for manufacturing and characterizing structures ranging from one millimeter to a few nanometers are located under clean room conditions (classes 10000/100).

EQUIPMENT

Nova NanoSEM 230:

› High and Low Vacuum field emission Scanning Electron Microscope (SEM) incorporating:

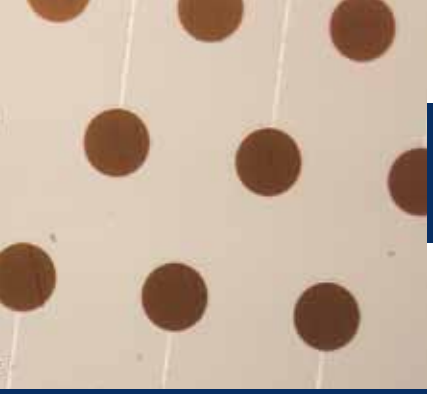
- Resolution: 1.6 nm at 1 kV (ETD and TLD detectors) in High Vacuum.
- Resolution: 1.8 nm at 3 kV (LVD detectors) in Low Vacuum.
- Field emission filament -> High-resolution images even of non-conductive samples.
- Backscattered electron detector (vCD detector): High-resolution images of backscattered electrons.
- 6-channel preamplifier for detectors of solid state.
- Compressor.
- Cryo Can, anti-contamination system.
- Thermoflex closed water circuit.

› Electron beam lithography system with:

- High writing resolution (maximum speed of 6 MHz and control of the dwell time < 2 ns).
- Electrostatic Beam Blanker.
- Picoammeter exchanger box.
- Keithley picoammeter.
- Advanced nanolithography system for microscope.
- Beam Blanker amplification electronics
- Software for correcting the Proximity Effect.
- Universal sample carrier for Lithography.
- EBL Starter kit.

1. **ZnO nanowire.** Courtesy of Fan Jiandong (UB).
2. **Microparticles of Polyethylene Glycol.** Courtesy of Gemma Vilar (IRB)
3. **Human hair.** Courtesy of Infinitec Activos S.L.

LEFT: Morphology of cells growing on membranes with smooth/posts interface. Tejeda-Montes E, Smith KH, Poch M, Lopez-Bosque MJ, Martín, L, Alonso M, Engel E, Mata A. Engineering membrane scaffolds with both physical and biomolecular signaling. Acta Biomaterialia 8:998-1009, 2012.



Micro-Nano Technologies Unit

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This Micro-Nano Technologies Unit is located in the Microelectronic Institute of Barcelona, (IMB-CNM, CSIC) which is the largest installation with micro-nano technologies facilities in Spain. Its 1500 m² Clean Room classes 10 to 10,000 are considered as Singular Scientific-Technical Infrastructure (ICTS) by the Ministry of Economy and Finance of Spain. The platform is coordinated by Dr. Jordi Aguiló, PI of the Biomonitoring Group of the CIBER-BBN.

The unit is based on a CVD (chemical vapor deposition) equipment for the controlled growth of carbon nanotubes (CNTs). Because of the specificities of the CNTs concerning biocompatibility, high conductivity, very low impedance and its possibilities of surface functionalization, the Micro-Nano Technologies Unit is enhancing the CNM facilities towards the R+D in technology and devices focusing biomedical applications.

The CVD machine grows both single and multi-walled carbon nanotubes, using both plasma-enhanced CVD (PECVD) and/or thermal CVD. It enables the production of advanced CNT Micro-Nano-Bio Systems (MNBS) for biological, chemical or biochemical analysis. As the suitability of the interface of any MNBS device is a critical point, the selective growth of CNT improves the electrode-electrolyte interface enhancing the biomonitoring.

It is worth noting that the integration of the unit in the clean room facilities of the IMB-CNM enables a complete range of processes (thermal processes and CVD, metallization, photolithography, dry and wet etching) for the complete fabrication of MNBS devices. So, from the platform it is supported the whole MNBS fabrication steps: the customization of the technology needed, the design of the devices, the complete fabrication process, their encapsulation, the characterization and test up to the technological support on the experimental uses.

SERVICES

The services provided by this Unit are mainly focused on growing CNTs on substrates, but it also provides facilities for the development of micro/nanostructures and devices where the CNTs could be incorporated.

› **Carbon nanotube growth service.**

› **"Large Installation Program" Service (priority for CIBER-BBN groups).**

The Unit is channeling access to the facilities of the "Integrated Micro and nanofabrication Clean Room", which allows processes and technologies as well as the support of highly qualified researchers and technicians to help in the design and fabrication.

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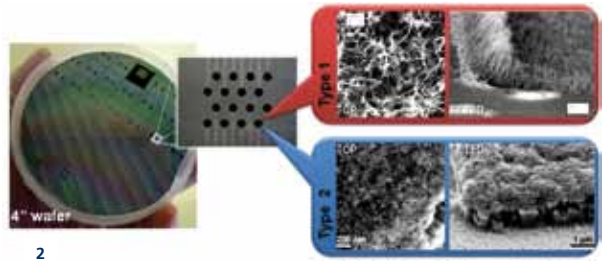


2

EQUIPMENT

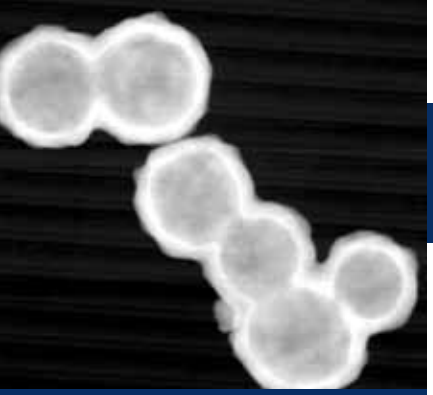
1. Chemical vapor deposition (CVD) equipment for growth of CNTs, Black Magic Pro 4-inch System (AIXTRON Ltd) with:

- Heat processing control up to 900°C, with controlled ramps of up to 300°C/min.
- Plasma control; completely configurable source. Possibility of working without plasma.
- Camera for 4" wafers.
- Camera for following the process.
- Possibility of processes in high vacuum (5 mBar) and at atmospheric pressure (800 mBar).
- **Process gases:** hydrogen, methane, acetylene, argon, ammonium and air.

Furthermore, in the clean room (1500 m², Classes 10 to 10,000) in which this equipment is located there is:

- Thermal oxidation process equipment.
- Platinum and gold deposition equipment.
- Optical photolithography equipment.
- CVD nitride and oxide passivation equipment.
- Chemical banks for micro/nanotechnologies.
- RIE and DRIE equipment.
- Nanolithography equipment.
- Optical (SEM) and electrical (Impedance) characterization equipment.
- Encapsulation equipment.

1. **Chemical vapor deposition (CVD) equipment.**
2. **MWNT nanotubes grown selectively in MEAS (multielectrode array system) and in devices in the form of a needle:**
   - type 1: diameter of platinum nanoparticle 4 nm, spaghetti growth, distance between nanotubes ~60 nm
   - type 2: diameter of platinum nanoparticle 2 nm, vertical growth, bundles of nanotubes.



Synthesis of Nanoparticles Unit

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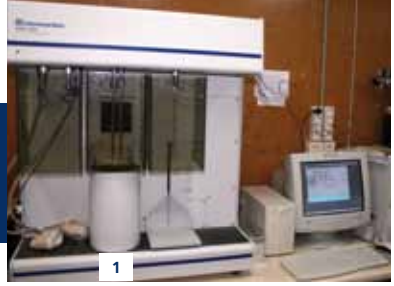
This facility is coordinated by Dr. Jesús Santamaría of the Nanostructured Films and Particles Group at the Institute of Nanoscience of Aragon (INA). It corresponds to an automated system for the synthesis of nanoparticles using laser-induced pyrolysis of chemical precursors in gas and/or aerosol phase, which enables either individual nanoparticles or biocompatible hybrid nanostructures to be produced in large quantities (between 1 and 10 grams per hour). In addition, the unit provides a wide range of nanoparticle fabrication techniques based on the decomposition of chemical precursors in the liquid phase, such as: hydrothermal synthesis systems, equipment for nanoparticle synthesis by microemulsions, microwave-induced synthesis, synthesis reactors using non-hydrolytic decomposition of organometalic precursors at high temperatures, etc. In addition, this facility is able to draw on a wide range of experimental techniques, as well as having the necessary specialized personnel, to undertake exhaustive characterization of the microstructure, chemical composition, particle size and distribution of sizes, as well as magnetic, optical and colloidal properties and degree of biological functionality of the synthesized material.

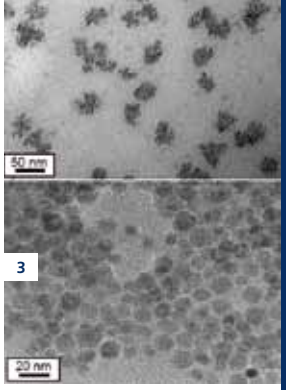
The unit can produce a wide range of inorganic, polymer and nanocomposite-hybrid nanomaterials with controlled porosity, microstructure, optical, magnetic and/or surface properties. Such materials may be used directly as platforms for building more complex nanostructures or derivatized by biomolecules for uses in biomedicine. The potential applications include:

- scaffolds for use in regenerative medicine and implants,
- contrast agent for diagnosis using molecular imaging techniques
- vectorization and drug delivery
- destruction of tumor cells using magnetic hyperthermia and/or phototherapy.

SERVICES

- **Synthesis of nanoparticles and nanostructures using laser-induced pyrolysis from precursors in the vapor phase, aerosols and controlled gas and liquid mixtures; the final product can be supplied as either a solid form (powders) or a stable colloidal dispersion;**
- **Production of nanoparticles using wet synthesis;**
- **Characterization of nanoparticles, nanostructures and/or colloidal suspensions of nanoparticles:**
  - Composition (chemical analysis)
  - Structure (UV-Vis, NIR, XPS, FTIR, TEM)
  - Surfaces (Gas adsorption)
  - Magnetic properties (VSM, SQUID)
- **Study of the capacity of materials for controlled adsorption and drug delivery;**
- **Advice on the synthesis of nanoparticles.**





EQUIPMENT

› Reactor for laser-induced pyrolysis for controlled synthesis of nanoparticles from vapor phase precursors, aerosols and vaporized mixtures of gases and liquids. This reactor is composed of the following components:

- Infrared CO_2 laser resonator.
- RF and DC three-phase radiofrequency supply at 360 and 440 V.
- External cooler unit.
- Laser power meter Unit by Ophir Optronics Ltd.
- Reaction chamber for working under vacuum (10-6 mbar).
- System for mixing and vaporization of gases and liquids to produce aerosol and gas mixtures at known compositions (Brooks Instrument).

› System for measuring of adsorption and drugs delivery consisting of an UV-Visible spectrometer (Agilent) with flow cell and diode array detector (DAD).

› Two instruments for liquid-phase synthesis of nanoparticles by high-temperature decomposition of organometallic precursors.

› Two photoelectron correlation spectrometers (PCS) (a Malvern Instruments ZETASIZER 3000 HS and a Brookhaven 90PLUS PCS) to obtain measurements of average hydrodynamic diameter, aggregate size distribution and isoelectric point of colloidal dispersions from nanoparticles.

› Micromeritics ASAP 2020 analyzer to measure the specific surface area and porosity of nanoparticles and nanostructures by gas adsorption.

› System for the thermal and optical characterization of core-shell metallic nanoparticles by surface plasmon resonance.

› Software for data acquisition and processing from PCS, BET (for the interpretation of gas adsorption) and thermogravimetry.

In addition to the equipment allocated to the synthesis of nanoparticles unit, there is immediate access to a wide range of state-of-the-art characterization facilities available at the Nanoscience Insitute of Aragon (INA), where this platform is located. These include advanced electron microscopies (SEM, TEM, HRTEM, UHRTEM), as well as other surface analysis and characterization equipment (XPS, XRD, FTIR, magnetic characterization).

1. **Micromeritics ASAP 2020 analyzer.**
2. **Laser-induced pyrolysis experimental reactor** for controlled synthesis of nanoparticles from vapor phase precursors, aerosols and vaporized mixtures of gases and liquids.
3. **Tri-ethylene-glycol-coated iron oxide nanoparticles synthesized** using non-hydrolytic decomposition of iron acetylacetonate in the presence of tri-ethylene glycol, taken using Transmission Electron Microscopy.
   Iron oxide nanoparticles embedded in a silicon matrix synthesized using a combined hydrothermal process, taken using Transmission Electron Microscopy.

Platform 3

TISSUE, BIOMATERIAL AND SURFACE CHARACTERIZATION

CIBER-BBN Research Infrastructure in **BIOMEDICINE**

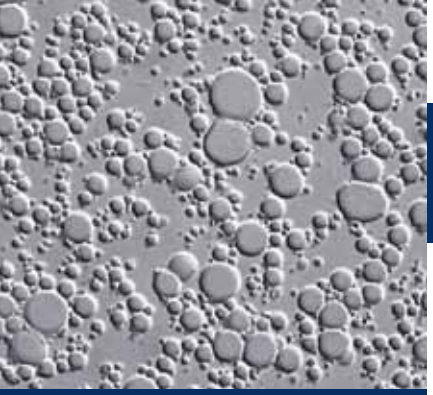
3. TISSUE, BIOMATERIAL AND SURFACE CHARACTERIZATION PLATFORM

Provides services for characterization of biological tissues, biomaterials and nanosystems that may be produced in the Platform of Production of Biomaterials and nanoparticles or in any other internal or external laboratory. The services are focused on:

- Characterization of the structural, physicochemical and functional behavior of 2D and 3D scaffolds.
- Characterization of the histological and mechanical behavior of biological tissues.
- Compositional, surface and colloidal characterization of materials and biological fluids.
- *In vitro* and *ex vivo* real time study of cells and tissue behavior in presence of potential therapeutic agents and identification and localization of therapeutic targets.
- Intramolecular interactions characterization and control of functional properties of materials.
- Characterization and control of the process of internalization of therapeutic agents in cells.

UNITS	OBJECTIVE
NANOSTRUCTURED LIQUID CHARACTERIZATION	Characterization of size and morphology of nanostructured liquids and colloidal dispersions. Interfacial properties. Rheology under flow and deformation regimes.
TISSUE AND SCAFFOLD CHARACTERIZATION UNIT*	Structural and physical characterization of biological tissues and tissue scaffolding.
FUNCTIONAL CHARACTERIZATION OF MAGNETIC NANOPARTICLES	Characterization of the magnetic and mechanical behavior of magnetic nanoparticles against the action of external magnetic fields in environmental conditions compatible with life.
SURFACE CHARACTERIZATION AND CALORIMETRY	Conducting liquid-phase analyses. Obtaining information of the behavior of surfaces of biomaterials and devices developed in contact with biological fluids, microorganisms or cells.
CONFOCAL MICROSCOPY SERVICE*	Confocal microscopy of live cells and tissues for characterizing their 3D morphology and their interaction with biomaterials.
NANOTOXICOLOGY	*In vitro* and *in vivo* evaluation of the toxicity of new active pharmaceutical ingredients, therapeutical nanoconjugates, nanoparticles and biomaterials to identify the most promising lead compounds for clinical trials.

*Due to their objectives, purpose and functionality, these Units can also provide support for the Bioimaging Platform.



Nanostructured Liquid Characterization Unit

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Most biological fluids have a nanostructure which determines their functionality. Specifically, drug controlled-release systems and diagnosis materials are suspensions of nanoparticles, nanocapsules, liposomes, etc. Preparing nanostructures in solutions or dispersions implies a huge increase in surface area, and accordingly interfaces play a key role in their properties. Therefore, physico-chemical characterization of nanostructured liquids is of fundamental importance. Indeed, the study of their properties is crucial for progress in the field of biomedicine, and consequently, many research groups focus their attention on the characterization of solids, solid/solid, solid/liquid and liquid/liquid dispersions and interfaces. However, there are a very limited number of laboratories who are specialized in the characterization of nanostructures in liquids and liquid/liquid interfaces. In this context, this unit for characterization of nanostructured liquids was created, being coordinated by the Colloidal and Interfacial Chemistry Group at the Institute of Advanced Chemistry of Catalonia (QCI-CSIC), led by Dr. Concepción Solans, pioneering in Spain in research in this field.

The QCI Group has specific techniques for the study of nanostructured liquids. Specifically, using both laser light and small-angle X-ray scattering, the researchers are able to measure the size distribution, morphology and aggregate number in colloidal dispersions such as micelles, vesicles, micro- and nano-emulsions, as well as particle dispersions. A particularly important tool is the 3D cross-correlation dynamic light scattering (DLS) spectrometer, which enables to characterize more turbid colloidal dispersions than those which can be measured using conventional laser light scattering spectrophotometers, avoiding possible structural changes due to sample dilution.

On the other hand, tensiometry techniques, such as electrobalance and "spinning drop" interfacial tensiometer, enable characterization of extremely small surface tensions occurring in colloidal systems and interfacial tensions at liquid/liquid interfaces. It is also possible to characterize rheological properties of colloidal dispersions under deformation and flow regimes using an AR-G2 rheometer.

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SERVICES

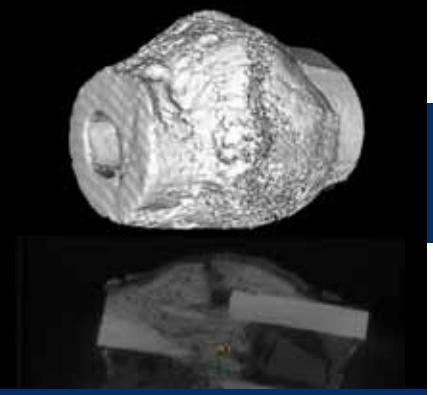
The services provided by this facility are the following, in which the service manager will give advice on scientific and technical aspects:

- Determination of particle size and size distribution, morphology of aggregates, radius of gyration and aggregation number.
- Study of phase behavior of surfactant systems.
- Characterization of liquid crystals (type, repeat distance, etc.).
- Characterization of the rheological properties of fluids and soft matter under deformation and flow regimes (viscosity, shear-thinning, shear-thickening, elastic and viscous modulus, relaxation times).
- Measurements of surface and interfacial tension of pure liquids, solutions and colloidal dispersions (adsorption properties of molecules at the liquid-air and liquid-liquid interfaces).
- Measurements of contact angles of wetting liquids on different solid materials.
- Measurements of differential refraction index .
- Measurements of liquid densities.

EQUIPMENT

- 3D cross-correlation DLS Spectrometer for static and dynamic light scattering (SLS and DLS) (LS Instruments).
- AR-G2 Rheometer for characterization of rheological properties under deformation and flow regimes (TA Instruments).
- Malvern 4700 photon correlation spectrometer (Malvern Instruments).
- Mastersizer 2000 light diffraction particle size analyzer (Malvern Instruments).
- S3 Micro SAXS/WAXS small- and wide-angle X-ray scattering spectrometer (Hecus).
- SITE "spinning drop" interfacial tensiometer (Krüss).
- K12 tensiometer with Wilhelmy plate and du Noüy ring (Krüss).
- Sigma 700 surface tensiometer for advancing and receding contact angles (KSV Instruments)
- Optilab rEX differential refractometer (Wyatt).
- DMA-46 densitometer (Anton Paar).
- Reichert Polyvar 2 optical microscope (Leica).
- Turbiscan Lab backscattering stability analyzer (Formulaction).
- Avanti J30I ultracentrifuge (Beckman Coulter).

1. **Characterization** using an optical microscope and visual inspection of an emulsion and a nanoemulsion.
2. **GmbH 3D DLS Spectrometer PRO** for static and dynamic light scattering (SLS and DLS) (LS Instruments).
3. **AR-G2 Rheometer.**



Tissue and Scaffold Characterization Unit

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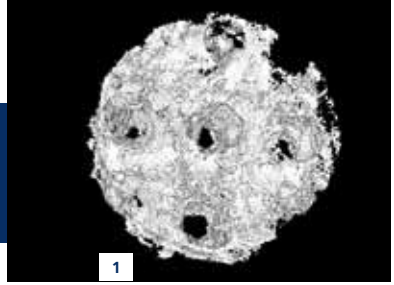
This platform is coordinated by Dr. E. Peña member of the Group of Structural Mechanics and Materials Modelling of the Aragón Institute of Engineering Research (I3A) at the University of Zaragoza, whose Principal Investigator is Prof. Manuel Doblaré. The objective of this unit is to provide serviced for structural and physical characterization of biological tissues and tissue scaffolding, including microstructure, histology and mechanical tests among others. Several tests are now protocolized, including those for determining the mechanical properties of musculoskeletal tissues (bone, ligaments, cartilage, muscles) cardiovascular ones (heart and blood vessels) and scaffolds for tissue engineering.

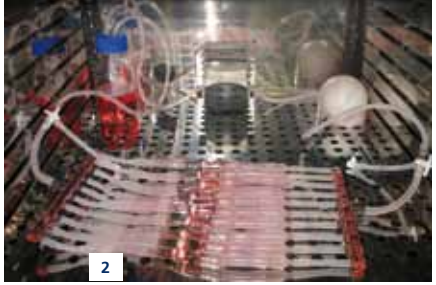
It has several types of INSTRON testing machines specialized in biological tissues, for the characterization of soft tissues and fibers, as well as an X-ray microCT which allows quantitatively characterizing the microstructure of *in vitro* samples of hard tissues and of biomaterials for scaffolds. It also has the entire system necessary for performing high-quality histologies which would complete the structural characterization of the previously cited types of tissues (staining of preparations, immunohistochemistry in paraffin with EnVision, laser microdissection, etc.). As additional tests, equipment for determining the permeability of biological tissues and scaffolds under culture conditions, monitoring their variation over time is also available. Finally, the platform is also composed of different types of bioreactors, including perfusion or direct compression on seeded scaffolds for stimulating cell cultures for tissue engineering applications.

This platform, unique in Spain, has a clear and direct translational impact, especially in regenerative medicine, implant design and evaluation, as well as in the study of the mechanical behavior of living tissues (especially in the musculoskeletal and cardiovascular systems). Its relationship with the Instituto Aragonés de Ciencias de la Salud (Health Sciences Institute of Aragon), and through it with the Tissue and Blood Bank of Aragon and with the School of Veterinary Science at the Universidad de Zaragoza, allows an easy provision of tissues both from animals (mice, rabbit, sheep, pig) and humans, following in their handling the recommendations of the different ethics committees.

SERVICES

- **Image Acquisition and Processing:** X-ray microtomography with image analysis software.
- Mechanical characterization of living tissues and biomaterials: Axial-torsional, uniaxial tensile, confined and unconfined compression tests, fatigue and dynamic tests.
- Histological characterization of biological tissues. Sample carving and processing, cutting of frozen or paraffin blocks, laser microdissection, staining with routine and special techniques, direct or indirect labeling by means of immunohistochemistry and preparation of studies and digital microphotography.





1. Microstructure and morphology of tissue scaffolds.
2. Perfusion System.
3. Tensile test of a rat muscle.

EQUIPMENT

1. MicroCT GE eXplore Locus SP (GE): Computed X-ray Axial Tomograph for the non-destructive study of the microstructure and *in vitro* tomography:

› Samples of up to 40 mm in diameter; resolution of 10 µm.

› Different types of tissues, implants, scaffolds, etc.

› Complete "section-by-section" imaging with a single rotation (cone beam volumetric technology); excellent image quality and "signal-to-noise" ratio.

› "Bone Analysis Tool" software provided with:

- BMD (Bone Mineral Density): It calculates the density of minerals in the bone, the bone volume fraction and the mineral content in the bone.
- Anisotropy: It determines the degree of symmetry and the orientation of the trabecular structure.
- Cortical Analysis: It determines "section-by-section" the width, surface area and the BMD value of the cortical bone.
- Stereology: It provides the bone volume fraction, the ratio between the surface of the bone and its volume and the width of the trabecular plate.
- Direct Measures: It determines the local trabecular width of a bone and allows obtaining a visual representation of this local width.

2. Machines for mechanical tests:

› Instron MicroTester 5848 and 5548: electromechanical machines for displacement or load control tests:

- load range from 1mN to 2kN at full scale.
- load cells of:+/- 5, 50, 500 and 1000N.
- synchronous data capture in all the channels up to 500 Hz.

› Biaxial Instron MicroTester 8874: axial-torsional, servohydraulic testing machine:

- load capacity of 25 kN and 100 Nm.
- fatigue tests at a low number of cycles.

› Software for data control and analysis.

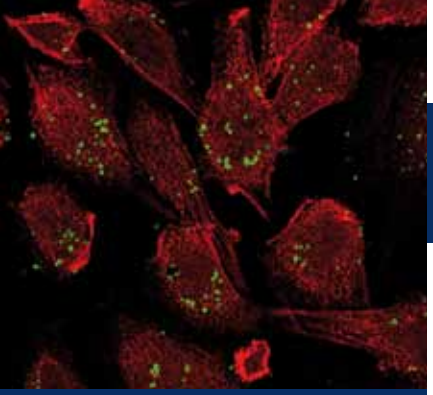
› Accessories and tools especially designed by the group, which allow a wide flexibility in designing tests, allowing the verification and/or validation of theoretical models.

› AVE video extensometer connected to the machines; resolution of 0.5 µm. To determine the axial and transverse deformation without contact (tension, dynamic properties and viscoelasticity) of the samples.

› E1000 Test Instrument: Dynamic systems to offer slow-speed static testing and high-frequency dynamic fatigue testing with hundreds of Hertz capability: Load range: 250 N to 2.0 KN.

› Nano BIONIX MTS: For nanomechanical characterization of biomaterials. Load range to 0.001N to 0.5N.

3. Complete histology equipment.



Functional Characterization of Magnetic Nanoparticles Unit

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This facility is coordinated by Dr. Francisco del Pozo, PI of the Biomedical Engineering and Telemedicine Centre at the Centre for Biomedical Technology (CTB), Technical University of Madrid (UPM). The set-up is dedicated to the characterization of the magnetic and mechanical behavior of magnetic nanoparticles at temperatures and other environmental conditions compatible with physiological conditions. Currently, it is already possible to characterize magnetic materials (e.g., magnetic nanoparticles) in terms of their magnetic moment under applied external magnetic fields. Specifically, technical equipments are available to undertake the following measurements:

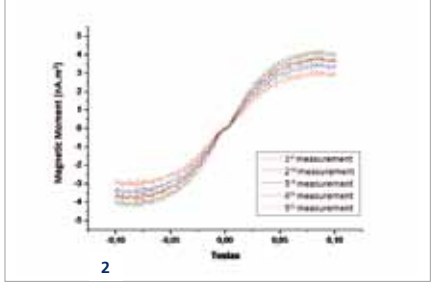
- Characterization of magnetic nanoparticles and nanothreads, even immersed in biological fluids or embedded in tissues.
- Characterization of biological samples which exhibit a magnetic response.
- Determination of the differences between the behavior of nanoparticles phagocytosed and non-phagocytosed by cells.
- Characterization of interactions between systems of particles, such as those between nanoparticles and certain types of target cells.
- Characterization of contrast agents for Magnetic Resonance Imaging (MRI) over a continuous spectrum of frequencies, without being limited to a fixed frequency. Maximum magnetic field strength of 1.9 T.

Shortly, it will also be possible to measure other characteristics including, for example, the capacity of nanoparticles embedded in biological media to produce a heating effect in a specific area under the influence of non-invasive external fields (hyperthermia), which would have diverse applications in cancer, drug-delivery and other therapies.

In addition, for the longer term, technology is being developed to enable controlled movement (guiding) and concentration (focusing) of nanoparticles in specific locations within biological fluids using external magnetic fields.

SERVICES

- **Magnetometry on nanoparticles in solid form, ultra thin films, powders, liquids and even slurries.**
  - Magnetization curves.
  - Coercitivity (normal and remanent).
  - Magnetization vs. time curves.
  - First Order Reversal Curves (FORC) diagrams.
  - Diamagnetic and paramagnetic susceptibility.
  - S* (measurement of the gradient in the second quadrant).
  - Remanent and saturation magnetization.
  - Initial permeability.
- **Relaxometry**
  - Measurement of the relaxation times in aqueous solutions and biological samples containing superparamagnetic nanoparticles as contrast agents for MR images. For T1 in the continuous range 10 kHz to 80MHz; for T2 in the range 10 MHz to 80MHz.
  - Characterization of contrast agents for MR imaging, ascertaining their relaxivity and the dominant effect.



- Measurement of the Nuclear Magnetic Relaxation Dispersion (NMRD) profile of samples in aqueous solutions and biological media.

Once the techniques for the characterization of the hyperthermic response and, on a longer timescale, those for guiding/focussing have been optimized, they will be added to the list of services.

EQUIPMENT

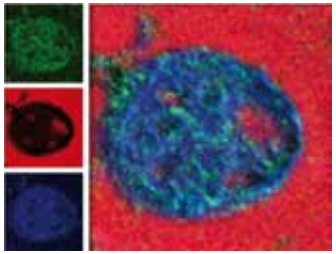
› **Alternating Gradient Magnetometer:** MicroMag M2900-4 AGM (Princeton Measurements Corporation, USA) for the magnetic and mechanical characterization of nanoparticles in various different media:

- Magnetic Moment Range: 1nA.m^2 to 5mA.m^2 full scale (1μemu to 5emu).
- Resolution: 0,005% of full scale with 60% overrange capability.
- Speed of measurement: 100 ms/point.
- High sensitivity: 10 pA.m^2 (10 nemu) of standard deviation at room temperature and with 1 second of averaging time.
- High performance with samples of very small magnetization and dimensions (few nanometers).
- The AGM can accommodate a large range of samples with widely different properties.

› **T1 and T2 Nuclear Magnetic Resonance Relaxometry:** Stelar SmarTRACER (Italy) + Bruker (Germany) 2 T electromagnet. Fast Field Cycling designed to measure longitudinal nuclear magnetic relaxation as a function of the magnetic field intensity. Measurements of the longitudinal (T1) and transverse (T2) time constants as a function of the Larmor frequency.

- Measurement range: continuous measurement from 10 kHz (almost null field) to 10 MHz (0.25T) to obtain T1.
- Measurement range: measurement from 10 MHz (0.25T) to 80MHz (1.9T) at desired intervals to obtain T1 and T2.
- Inhomogeneity lower than 150 PPM.
- Main pulse sequences implemented with the possibility of modifying parameters to the design and programming of new sequences.
- Temperature control from -120°C to +140°C with accuracy and stability of 0.1°C.

1. **T1 and T2 Nuclear Magnetic Resonance Relaxometry.**
2. **Magnetization curves and hysteresis loops** of magnetic nanoparticles in presence of triethylene glycol (TREG).
3. **Alternating Gradient Magnetometer.**



Surface Characterization and Calorimetry Unit

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This platform is coordinated by Dr. M. Luisa González, PI of the Microbial Adhesion Research Group at the University of Extramadura. The purpose of this unit is to obtain valuable information concerning the behaviour of the surfaces of the biomaterials and devices being developed when they come into contact with biological fluids, microorganisms and cells, to guide and optimize their design. The response of an implant or prosthesis to biological media such as a proteins, cells and microorganisms is determined by the characteristics of its surface, specifically the composition and structure. While behavior of given materials can be ascertained by *in vitro* and *in vivo* tests, such experiments do not tend to provide a comprehensive understanding of the fundamental bases of the response of the biomaterial and still less predictive data, and this type of information is fundamental for optimization in the design process.

On the other hand, using calorimetry it is possible to study generic binding processes (adhesion/adsorption/retention/reaction) through the associated heat/enthalpy changes, and thereby obtain data in real time and continuously as the process happens. It is possible to study:

- changes in surface coating with the aim of adding functionality.
- adsorption on a surface of biological molecules, such as proteins.
- adhesion of microorganisms and the cell response.
- binding of growth factors to their ligands.
- metabolic activity of cells and bacteria placed in different environments, such as internalization of nanoparticles and adhesion to substrates on open surfaces or within scaffolds.
- degradation of biomaterials and their coatings.
- interaction between proteins and of proteins with biomaterial coatings.

Other techniques such as XPS and ToF-SIM make it possible to characterize the surface on which the interaction between cells, proteins and microorganisms takes place and also to analyze changes which occur following these interactions.

All of this is complemented by ellipsometry which provides information concerning the configuration, packing, orientation and restructuring of films immersed in liquids, as occurs in biological media, and this is very useful for studying biodevices, surface functionalization and absorption of biomolecules.

SERVICES

1. Characterization of the composition and surface structure of materials, to build up predictive data concerning their functional behavior:

- **Calorimetry tests in real time to measure:**
  - Molecule-molecule interactions:
    - Protein-protein
    - Receptor-ligand
    - Antibody-antigen
  - Biomaterial-molecule interactions.
  - Biomaterial-cell interactions.
  - Microbial growth.
  - Cell metabolism.
- **X-ray photoelectron spectroscopy to measure quantitative elemental composition of surfaces (%) (except H and He).**

1. K-Alpha X-ray photoelectron spectroscopy (XPS) system.
2. UVISEL ellipsometer.
3. TAM III isothermal nanocalorimeter system.

› **Time-of-flight secondary-ion mass spectrometry to measure the qualitative composition of the surface.**

2. Physical characterization of the coatings of materials including in liquid phase (in biological media):

› **Ellipsometry to measure the thickness of layers, and the composition, porosity and roughness of materials on a surface.**

EQUIPMENT

› **TAM III isothermal nanocalorimeter system including options for titration and perfusion. Also solution quasi-adiabatic calorimetry is available. (TA Instruments).**

Thermostatic bath

- Working temperatures between 15 and 150 °C
- Temperature scan < 2 °C/h
- Stability: +/- 10 µK on short timescales and +/- 100 µK drift over 24 hours

Calorimetric response

- Precision better than 100 nW
- Reproducibility better than 1%
- Stability: < 10 nW on short timescales and < 40 nW drift over 24 hours

Solution

- Maximum temperature 80°C
- Quasi-adiabatic mode of operation

› **UVISEL ellipsometer (Horiba JovinYvon), range 190-2100 nm, based on phase modulation with cell for liquid media:**

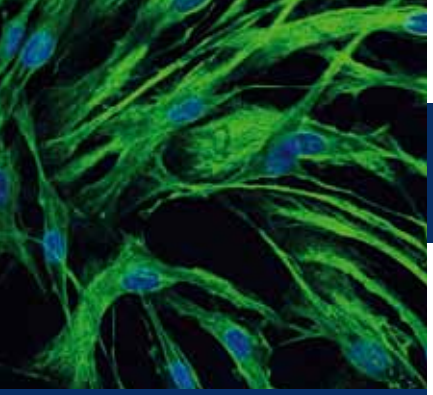
- Measurement of thicknesses from 0.1 nm to 30 µm.
- Measurement of thickness of various superimposed layers.
- Simultaneous measurement of the proportion of various different materials present on a surface.
- Estimation of the porosity as percentage of pores vs. deposited material.
- Measurement of roughness of coating between 2.5 and 15 nm.

› **K-Alpha X-ray photoelectron spectroscopy (XPS) system (Thermo):**

- Lateral resolution ~ 30-400 µm (extension of surface area analyzed).
- Depth resolution ~ 10 nm (thickness of surface analyzed).
- Depth profiles.

› **ToF-SIMS 5 Time-of-flight secondary-ion mass spectrometry system (IONTOF).**

- Sensitivity < 100 ppm.
- Lateral resolution ~ 100 nm (extension of surface area analyzed).
- Depth resolution ~ 1 nm (thickness of surface analyzed).
- Identification of molecules up to 10000 amu.



Confocal Microscopy Service

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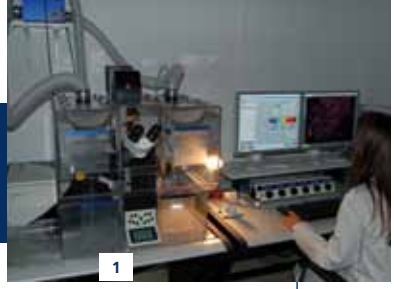
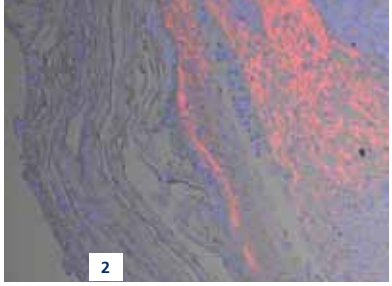
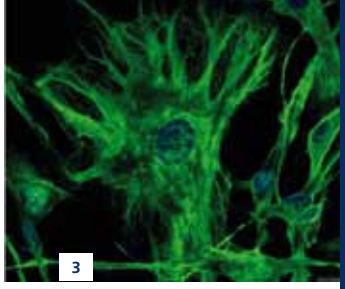
This Unit is coordinated by Dr. Juan Manuel Bellón, PI of the Translational Research and Tissue Engineering Group at the University of Alcalá de Henares. The system has the following main features:

- System for spectral confocal microscopy based on a fully motorized and automated DMI6000 inverted microscope with incubator chamber to suit most live-cell imaging applications.
- Acousto Optical Beam Splitter (AOBS) to coordinate the simultaneous use of various wavelengths to excite the sample, replacing the traditional dichroic filters. This is a fully automated system enabling the laser beams to be modulated using acoustic frequencies incident on a crystal which reflects or transmits the excitation and emission wavelengths with high transmission of these signals (meaning that a lowest laser intensity used on the sample is necessary).
- Capacity for simultaneous acquisition of one to three confocal channels (fluorescence) and the potential to add a channel for light transmitted or reflected.
- Compact scanning unit with spectral detection system based on a prism which separates the light emitted from the sample and disperses it into the various spectral wavelengths. These beams are then incident on a set of shutters that have a reflective surface and open or shut allowing light to reach the detector (depending on the range selected by the user), while reflecting the light not admitted to other sets of shutters and detectors, enabling detection of the full range required.
- Four laser beams.
- Field Programmable Gate Array (FPGA) allowing automatic control of the scanning module.
- System for digital recording of the photomultiplier signal, with no dead time, at a frequency of 40 MHz.
- Real optical path with a software-controlled variable size pinhole.
- 200º real rotation of the image with no interruption or even slowing down of the rate of acquisition.
- Selective attenuation of the laser beams using a computer-controlled Acousto Optical Tunable Filter (AOTF).

All of these features make this set-up ideal for studying interactions between cells/tissues and materials. Indeed, the experience of the research group in charge of this Unit makes this a unique service for the study of cells and tissues and the interactions between various materials and cell components as well as between implants/scaffolds and tissues of the recipient organism. For example, it makes it possible to characterize in detail the specific markers of particular cell populations for tissue engineering applications and *in vitro* tests of biocompatibility of new materials, allowing the process of cell colonization of surfaces to be explored and simultaneous analysis of the cells or subcellular structures and materials. The combined use of fluorescence and reflection makes it possible to study the tissue-implant interface, with up to 8 fluorophores, at wavelengths from 405 to 633 nm allowing histological studies of a wide range of tissue types which would be restricted at certain fixed wavelengths due to autofluorescence phenomena.







SERVICES

The users of the facility should provide the prepared sample and use the equipment under the terms of the service, accompanying a member of the technical staff of the Unit throughout the process. If sample preparation is required, the contact person from the Unit should be consulted in advance.

The services offered by this facility are principally focused on the following applications:

› **Morphological and functional characterization of cell and living tissues in real time.**

- Studies of inter- and intermolecular localization.
- Three-dimensional reconstruction of the cell and tissue morphology.
- Measurements of cell transport and endo/exocytosis.

› **Characterization of intermolecular and intercellular interactions, as well as between cells/tissues and other materials, from surfaces to nanoparticles.**

- Tests of biocampatibility of materials.
- Identification of bioactive topography.
- Measurements of intracellular transport (endocytosis of nanoparticles).
- Monitoring of the quality of the functionalization of biomaterials.
- Tests of tissue behavior under guided cell therapy.

EQUIPMENT

Leica TCS-SP5 confocal microscope with:

› **DMI6000 inverted microscope with 4 objectives:** 10X (dry); 20X; 40X and 63X(for immersion).

› Confocal module:

- 3 channels for spectral detection
- AOBS (Acousto Optical Beam Splitter)
- Resonant scanning system.

› **4 lasers:**

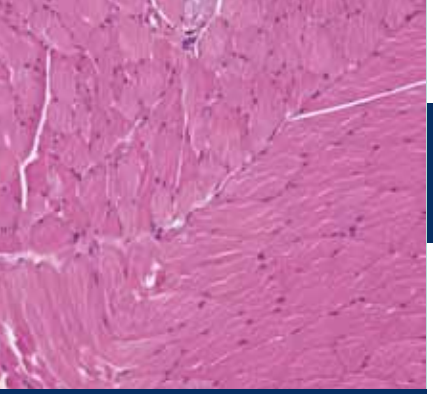
- Argon laser (excitation at 458, 477, 488, 496 and 514 nm)
- He/Ne laser (excitation at 633 nm)
- DPSS (diode-pumped solid-state) laser (excitation at 561 nm)
- UV laser (excitation at 405 nm)

› **Incubation chamber on a motorized stage.**

› **Workstation with 4 types of software for data acquisition and processing:**

- 3D imaging
- Colocalization analysis
- FRAP (Fluorescence Recovery After Photobleaching)
- FLIP (Fluorescene Loss In Photobleaching)
- FRET (Fluorescence Resonance Energy Transfer)

1. **Leica TCS-SP5 confocal microscope.**
2. **Section of abdominal prosthesis.** Nuclear staining with Dapi (UV 405 nm) and collagen with rhodamine (DPSS 561 nm) for differential interference contrast (DIC).
3. **Vimentin in human muscle cells.** Nuclear staining with Dapi (UV 405 nm) and cytoskeleton with FITC (488 nm).



Nanotoxicology Unit

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This platform is coordinated by Dr. Ramón Mangues, PI of the Oncogenesis and Antitumor Dr. Group of the Hospital de la Santa Cruz y San Pablo Research Institute, in collaboration with Dr. Simó Schwartz, of the CIBBIM-Nanomedicine of the Hospital Universitario Valle de Hebrón, in Barcelona.

The preclinical development of new compounds requires an exhaustive toxicological evaluation, which is possibly more rigorous and exhaustive than the demonstration of its efficacy. This evaluation is even more relevant when new biomaterials or nanoparticles are evaluated. In this context, the main objective of the Nanotoxicology Unit is to assess the toxicity of new drugs, nanoparticles or biomaterials in *in vitro* and *in vivo* systems, with the goal of optimizing lead compounds and identifying those with the highest probability of success in the preclinical programme due to their greater safety and tolerability or reduced toxicity. Other applications of this unit are:

› Collaborating in the determination of the distribution of the compound by performing a pharmacokinetic and pharmacodynamic study to assess the biodistribution in blood and tissues of the new products, coupled to a toxicological study and targeting capacity in relation to the efficacy.

› Collaborating in the evaluation of the metabolism of the new substance, including the possible identification of toxic byproducts, as well as the study of the biopharmaceutical properties of the new compounds regarding their industrial production, which will enable its development as a pharmaceutical formulation usable in clinical practice.

› Advising clinicians on the expected toxicities and on the co-development of possible biological markers which could be used in clinical practice to monitor, both, the possible toxicity and the therapeutic or diagnostic efficacy of the new agents.

SERVICES

***IN VITRO* TOXICOLOGY:**

› **Assays of cell viability/toxicity by XTT, release of LDH and similar indicators.**

› **Study of toxicity in normal human cells immortalized by viral infection or tTERT, by means of metabolic XTT test:**

- Hepatocytes for detecting liver toxicity.
- Vascular endothelial cells for detecting vascular toxicity.
- Tubular epithelial cells for detecting nephrotoxicity.
- Myelocytes for detecting hematotoxicity.
- Neuronal and glial cells for detecting neurotoxicity.
- Cardiomyocytes for detecting cardiotoxicity.

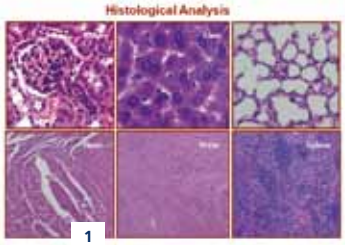
› **Production of reactive oxygen species (ROS) by fluorimetry.**

› **Internalization assays:** toxicity mediated by specific receptor, by evaluation of ligand internalization and competition.

› Study of the mechanism of toxic action: activation of apoptotic death, caspase-independent death, autophagia or programmed necrosis pathways.

› **Analysis of hemocompatibility in blood:**

- Hemolysis study.
- Red blood cell aggregation studies.
- Platelet aggregation studies.
- Coagulation test and platelet activation studies.
- Neutrophil activation studies.



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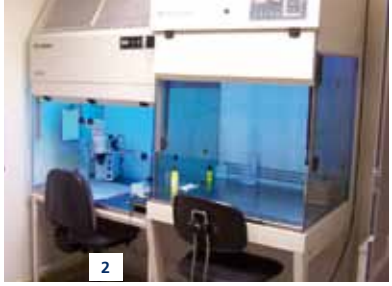
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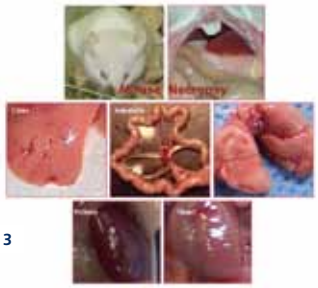
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IN VIVO TOXICOLOGY:

› **Complete necropsy**
- Macroscopic findings.
- Histopathology: Search for possible inflammation, formation of granulomas, thrombosis, apoptosis, necrosis, fibrosis, hypertrophy and metaplasm.
- Immunohistochemistry of markers of interest.

› **Hematology:** Blood count.

› Blood biochemistry: electrolytes, hormones, cytokines.

› ADME/Tox of nanoparticles
- Blood pharmacokinetics.
- Half-life.
- Volume of distribution.
- Main degradation products.
- Urinary clearance.
- *Ex vivo* tissue distribution.
- Correlation between pharmacokinetic and pharmacodynamic parameters and toxicity.

› **5. Study of distribution of markers of interest by immunohistochemistry.**

› **6. Study of the mechanism of toxic action (apoptosis, caspase-independent death, autophagia, necrosis).**

EQUIPMENT

› **Cell culture rooms with:** cell culture hoods, CO_2 incubators, automatic cell counters, inverted fluorescence and phase contrast microscopes.

› Autoclaves, refrigerators, freezers and liquid nitrogen tanks.

› Dissection hoods.

› Equipment for paraffin processing: tissue processors and inclusion stations.

› Equipment for cutting and staining histological sections: cryostats, microtomes, baths, heating plates, fume hoods etc.

› Dako AS10030 immunostainer.

› Tip and bath sonicators.

› Plate readers (for spectrometry and fluorimetry).

› Electrophoresis systems for DNA and proteins.

› Access to flow cytometers, sorters, confocal microscope and other equipment of the Hospital de la Santa Creu i Sant Pau and Hospital Universitario Valle de Hebrón Research Institutes.

› Facilities for housing small rodents (rats and mice).

1. **Histological Analysis.**
2. **Dissection hoods.**
3. **Mouse necropsy.**

Platform4

BIOIMAGING

CIBER-BBN Research Infrastructure in **BIOMEDICINE**

4. BIOIMAGING PLATFORM

Give support in the preclinical development of new therapeutic compounds by validating new therapeutic targets and/or nanotherapies by using optical image techniques and NMR. Some of the functions of this platform are:

- Analysis of fagocytic behavior and interactions of cells in presence of magnetic nanoparticled therapeutic agents.
- Spectroscopies for *in vivo* applications and for applications in biofluids, tissues and biomaterials.
- *In vivo* Validation, visualization and quantification of new therapeutic agents of the induced tissue regeneration and of the cellular and genetic activities dealing with pathologies at real time.
- Validation of new therapeutic targets.

UNITS	OBJECTIVE
IN VIVO EXPERIMENTAL	Preclinical and biomedical research of new therapeutic compounds under development and their clinical transfer. (Development of basic preclinical studies –toxicology, histopathology and treatment efficacy–).
NMR: BIOMEDICAL APPLICATIONS I	Acquisition, processing and/or interpretation of NMR data, structural characterization, chemical analysis and resolution of analytical problems.
NMR: BIOMEDICAL APPLICATIONS II	Microimaging, molecular imaging and metabolomic applications by increased spatial resolution and extended possibilities of generating molecular images.

In vivo Experimental Unit

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www.ciber-bbn.es/es/programas/123-unidad-de-experimentacion-in-vivo

This unit is headed by Dr. Simó Schwartz, the coordinator of Nanomedicine at CIBBIM-Vall d'Hebron University Hospital in Barcelona. Its mission is to offer products and services for the preclinical *proof-of-concept* validation of therapeutic compounds and biomarkers with potential clinical applications.

The early implementation of *in vivo* models significantly saves time and costs in the preclinical development of drugs and novel nanomedicines. For this purpose the ***In vivo* Experimental Unit** has established a complete integrative service platform, from the design of the *in vivo* assays, to their execution, histopathological analysis and final evaluation. One of the distinguishing features of this unit is that basic preclinical studies including toxicology, histopathology and efficacy treatments are complemented with non-invasive optical imaging technologies. *In vivo* bioluminescence and fluorescence imaging allows monitoring of living mice longitudinally, offering real-time insight into treatment efficacy, whole-body biodistribution and target mechanisms.

Currently, we are carrying out *in vitro* and *in vivo* studies to evaluate new therapeutic targets and/or nanotherapies in the fields of oncology and rare diseases, either for research groups in public institutions or for private companies.

Furthermore, the ***In vivo* Experimental Unit** collaborates closely with the **Nanotoxicology Unit**, so the efficacy evaluation of therapeutic compounds is run altogether with their safety and toxicological evaluation.

PRECLINICAL SERVICES

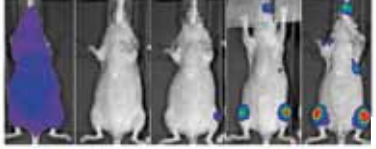
Our comprehensive services include:

› **Experimental consultancy:** We provide preclinical model design, support and advice for research projects, including selection and use of the appropriate imaging system and evaluation and interpretation of the data obtained.

› **Preclinical animal models in oncology and rare diseases:** In the field of oncology, we offer a core battery of tumor cell lines and experimental mouse models for studying tumor progression (see **Table**), monitorable by means of non-invasive optical imaging techniques (see **Figure 1**). Alternative animal oncology models may be developed upon request. In addition to tumor models, we can also provide disease specific models such knockout mice for studying Fabry disease.

Tumor Model
› Subcutaneous models
› Orthotopic models:
• Intraprostatic
• Intramammary (i.m.f.p.)
• Stereotactic
• Ceccum
• Intrapulmonar
› Experimental metastases:
• Intracardiac (bone metastases)
• Intravenous (lung metastases)
• Intraportal (liver metastases)
› Spontaneous metastases

(*Table 1)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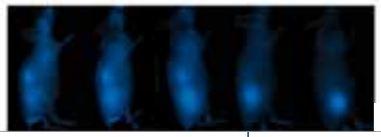
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1. **Longitudinal *in vivo* BLI** monitoring of tumor growth.
2. **Whole-body biodistribution** by *in vivo* fluorescence imaging.
3. **Preclinical histology.**
4. **Xenogen IVIS® Spectrum.**

› **Non-invasive optical imaging (bioluminescence and fluorescence):** The services in this section include the use of singular equipment for optical *in vivo* imaging (IVIS® Spectrum and Macrofluo) for the acquisition and quantification of bioluminescent and/or fluorescent images *in vitro*, *in vivo* or *ex vivo* (see **Figure 1**). Support on the analysis of obtained images by specialized personnel is also included.

› **Preclinical histology:** We count with conventional histology equipments exclusively devoted to preclinical tissue processing. All the biological material provided by the customer or collected by ourselves is processed rapidly and reliably, including standard or immunohistochemical stainings. In addition, when required, histopathological assessments carried out by specialized veterinary personnel are provided.

KEY APPLICATIONS

The key applications are focused on:

› Validation of new biomarkers and therapeutic targets *in vivo*

› Efficacy of new therapies under development using specific animal models *in vitro* and *in vivo*

› *In vivo* tumor-accumulation and whole-body biodistribution of new therapeutic agents (see **Figure 2**)

› Non-invasive monitoring of functional activities *in vivo*

› Preclinical histopathology

EQUIPMENT

1. Optical imaging equipment:

› **Xenogen IVIS® Spectrum:** (see **Figure 4**) is a high sensitivity, low background noise system for non-invasive optical imaging which enables bioluminescence and fluorescence imaging of cells (*in vitro*), entire animals (*in vivo*) and organs (*ex vivo*).

› **Leica MacroFluo™:** is a precision macroscope with filters for multiple fluorochromes, a Z16 APO (16:1) zoom system and a CCD camera to monitor and record the fluorescence of entire organs and tissues at high resolution with no need to process them histologically.

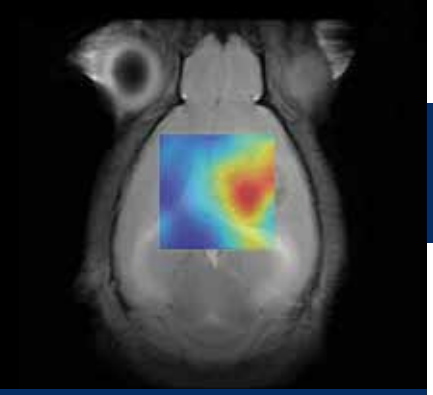
2. Equipment for histological processing

The facility has the equipment necessary for fixation (chemical or by freezing), preparations of blocks, slicing and staining using various techniques, from conventional to immunohistochemical stains. The set-up includes everything from the automatic tissue processor to a paraffin embedding station, a microtome and automated staining systems (see **Figure 3**).

3. Infrastructure

The Unit is supported by facilities provided by the Vall d'Hebron University Hospital Research Institute Foundation (VHIR), specifically a 683 m² animal house with areas for rodents, rabbits, pigs and sheep. In the case of rodents, it includes:

- **3 quarantine and isolation rooms** with a capacity to house 380 rats and 960 mice.
- **7 holding rooms** for 1,200 rats and 4,000 mice.
- **6 handling areas** for carrying out experimental and surgical procedures with supplies of gases and inhalable anesthesia as well as surgical microscopes.
- **The Molecular Imaging Platform Room** where the molecular imaging equipment is located.



NMR: Biomedical Application I

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www.ciber-bbn.es/programas/plataformas/equipamiento/bioimagen

UAB
Universitat Autònoma de Barcelona

The main objective of the Platform of Biomedical Applications of Nuclear Magnetic Resonance at the Universitat Autònoma de Barcelona (UAB) is the acquisition, processing and/or interpretation of Nuclear Magnetic Resonance (NMR) data. This platform is coordinated by Prof. Carles Arús, PI of the Nuclear Magnetic Resonance Biomedical Applications Group (GABRMN) (http://gabrmn.uab.es/). The platform is divided between the Servei de Ressonància Magnètica Nuclear (Nuclear Magnetic Resonance Facility) (SeRMN) (http://sermn.uab.cat/) and the Institut de Biotecnologia i Biomedicina (IBB) for bioinformatics applications of the UAB . The platform is equipped with 8 spectrometers operating at magnetic fields between 5.8 and 14.1 Teslas (T), including a MRI scanner, Bruker BioSpec, equipped with a 7T horizontal magnet for preclinical trials.

The unit also hosts a computational platform at the IBB, with a total storage capacity of 12TB, which is accessible through the UAB network (agarcia@gabrmn.uab.es for access).

The computational platform also hosts two multicentre databases (INTERPRET and eTUMOUR), with NMR and clinical data for more than 1000 human brain tumour patients.

Likewise, the SeRMN participates actively in the TRANSACT project (http://homes.esat.kuleuven.be/~sistawww/ibbt/project.php?prid=654), dedicated to the development of offline processing routines of MR data formats from different manufacturers (GE, Siemens, Philips, Bruker, Varian) for their integration in the MRUI programme (http://www.mrui.uab.es/mrui/), which has more than 1200 users around the world, e.g research groups or hospitals.

Furthermore, the platform has the scientific support of the GABRMN, with a recognized research track record in the use of NMR as a tool for biomedical applications (http://gabrmn.uab.es/).

SERVICES

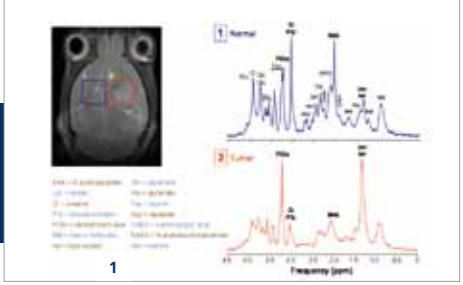
Some examples of the applications of the biomedical NMR facility are provided below:

- Identification of specific biomarkers of human pathologies by means of techniques of magnetic resonance spectroscopy (MRS), in its single volume (SV) mode, or in its multi-volume mode, spectroscopic imaging (MRSI). Development of protocols for early diagnosis of pathologies, specifically at brain level, monitoring the progress of disease or evaluating response during the development of new drugs.
- Development of MRSI protocols in preclinical brain pathology models, with future translational potential.
- Consultancy in processing and mathematical analysis of MRSI data, preclinical and clinical.

Several imaging protocols are currently available at the platform:

- proton density-weighted,
- proton longitudinal relaxation time T1-weighted,
- proton transverse relaxation time T2-weighted,
- proton transverse relaxation time T2*-weighted ,
- diffusion-weighted images using spin echo sequences or echo-planar imaging sequences,
- DCE (Dynamic Contrast Enhancement),
- cardiac images,
- localized single-voxel and MRSI (Magnetic Resonance Spectroscopic Imaging) proton spectroscopy and 31P spectroscopy,
- angiography, magnetic resonance technique in which the arteries and veins can be viewed in a non-invasive manner or with the use of contrast agent in 2 and 3 dimensions,
- functional parameter maps, such as, for example:
  - maps of relaxation time values T1, T2 and T2 *,
  - apparent diffusion coefficient (ADC) maps,
  - diffusion tensor maps (calculation of the typical values and vectors of the diffusion tensor, trace of the tensor and fractional anisotropy),
  - cerebral blood flow maps.

Even though there are already standardized protocols, each application may require a specific optimization of those protocols by a specialized technician. At the request of the different BioSpec users, the protocols mentioned above have been optimized to obtain rat and mouse brain, ear, knee, elbow, shoulder, hip, abdomen, larynx, snout, eye, spinal cord and genital images.



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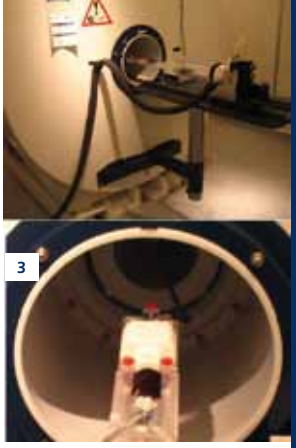

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1. **Left: T2-weighted axial image of mouse brain** with the areas delimited for single-volume spectroscopy, Right: MRS at short echo time (12ms) of normal parenchyma (blue voxel), compared with a tumor area (red voxel).
2. **AVIII 400 MHz (9.4 T) spectrometer** suitable for High Resolution Magic Angle Spinning (HRMAS) experiments.
3. **BioSpec 70/30 300 MHz (7T) spectrometer** suitable for *in vivo* applications.

EQUIPMENT

› **Two AVANCE 250 MHz (5.8 T)** spectrometers, one of them with an autosampler.

› **An AVANCE 360 MHz (8.4 T)** spectrometer for the high-resolution analysis of samples in liquid state. Suitable for the structural determination of chemical molecules.

› **AVII 400 MHz (9.4 T)** spectrometer - with a narrow bore, equipped with a solid state accessory for working under Cross Polarization Magic-Angle-Spinning (CPMAS) conditions with materials under solid conditions. It has rotors with a variable capacity between 12-40 µl, 4 mm-diameter and a spinning rate up to 15000 Hz.

› **AVIII 400 MHz (9.4 T)** spectrometer - with a narrow bore, equipped with a High Resolution Magic Angle Spinning (HRMAS) probe for analyzing semi-solid/semi-liquid samples such as biopsies, cell extracts or biofluids. It has rotors with a variable capacity between 12-40 µl, 4 mm-diameter and a spinning rate up to 15000 Hz.

› **AVANCE 500 MHz (11.7T)** spectrometer with a 5 mm TCI cryoprobe with a high sensitivity increase (4X). It is connected to a high pressure liquid chromatography (HPLC), mass spectrometry (MS) and Diode Array (DAD) detectors. It has the possibility of using solid-phase extraction (SPE). It is unique for metabolomic studies and analysis of samples with limited mass and for the characterization of complex samples with compounds at a low concentration (µg), for biomedical and pharmacological applications, and analysis of natural products.

› **AVIII 600 MHz (14T)** spectrometer for high-resolution spectroscopy studies. It is equipped with a Triple Broad Band Inverse (TBI) detection-type probe which makes it suitable for proteomic and metabolomic studies, allowing the analysis of complex mixtures of very different origins (urine, plasma, serum, extracts, biopsies, etc).

› **BioSpec 70/30 300 MHz (7T)** spectrometer, suitable for *in vivo* applications, both spectroscopy and magnetic resonance imaging and microimaging applications. It is suitable for working with small animals, especially rats and mice, although *in vitro* analyses can also be carried out.

› **Focused Microwave Fixation System,** to carry out the immediate sacrifice of small animals (rats, mice), preventing changes occurring in the metabolic pattern of the tissue due to post-mortem metabolism.

› **Hypersense sample polarizer,** to increase the sensitivity in *in vitro* and *in vivo* applications of the AV600 and BioSpec spectrometers. This method not only facilitates the detection of the hyperpolarized substrate in vivo, but also the rapid imaging of cellular metabolism

In addition to the indicated equipment, it has:

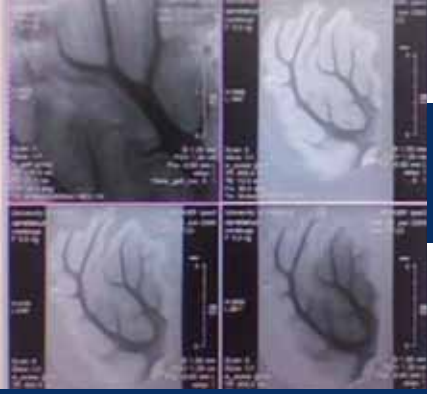
› **Animal housing room and animal preparation area equipped** with a vital constants monitoring system, 2 full sets of anesthesia equipment, water bath, infrared lamp, injection pump, etc.

› **Software packages for data acquisition and processing:** Paravision, Topspin and Amix. Users will have access to the database of spectra of diffe¬rent substances for metabolomics through this ac¬cess http://sermn.uab.cat/wiki/doku.php?id=bbiorefcode. This database is installed in a SeRMN computer, fully accessible to facility users. Reservations are made through the NMR facility webpage (http://sermn.uab.cat/reserves/).The AMIX software page (http://www.bruker-biospin.com/amix. html) contains more information about its different applications: analysis of mixtures, metabolomic stu-dies, etc.

Other packages include the INTERPRET decision-support system for human brain tumour diagnosis based on MRS and SpectraClassifier, for pattern recognition of in vivo MRS data single and multivoxel are developed and distributed through the platform (http://gabrmn.uab.es).

› **Databases of human brain tumour data.** For requesting access to them, address an e-mail to carles.arus@uab.es.

NMR: Biomedical Application II



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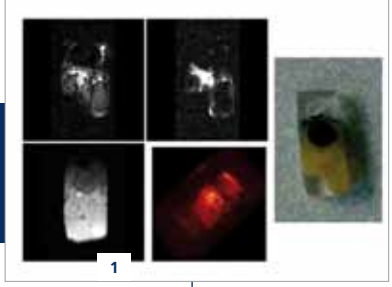
www.ciber-bbn.es/es/programas/125-unidad-de-rmn-aplicaciones-biomedicas-ii

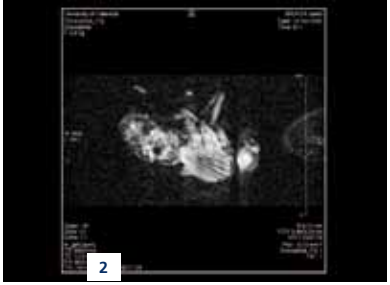
This platform is coordinated by Dr. Bernardo Celda, PI of the Physical-Chemistry Department of the Universidad de Valencia. The principal equipment of this platform is a 14T NMR system, singularly equipped for the automatic and thermostated acquisition at low temperatures of metabolic profiles of biological samples (biofluids, cell lines and tissues) (up to 4 x 96 samples), which is essential for the development of metabonomic clinical applications with use in pharmacology studies in different phases. Likewise, the 14T NMR equipment has: i) HR-MAS probe for metabolomic studies in tissues and cell lines; ii) NMR microscopy system for imaging, functional and metabolic studies in cells, tissues and animal models; iii) micro-probe for dealing with biomedical systems with limited amount of biofluid samples; iv) tetranuclear liquid probe for structural studies of biological molecules and complexes. The applications of this Unit include:

- Obtaining spectra of molecules present in a certain tissue;
- Follow-up of the effect of therapies by checking:
  - the re-establishment of the morphology, and
  - the molecular components and other conditions typical of a healthy tissue,
  - the decrease of the pathology indicators.
- Applications in regenerative medicine and in implants, e.g.:
  - analysis of phagocyte behavior and cell interactions against magnetic nanoparticulate therapeutic agents;
  - establishment of possible infections by magnetotactic bacteria;
  - performance of angiographs to determine the vascularization of the tissues in response to the treatments applied,
  - in device and implant integration studies, offering metabolomic spectroscopy studies to control the evolution thereof.

It also has a (patented) completely transparent microcamera (lab-on-chip), developed from an intramural project of the CIBER-BBN, which may be used in high-field (>11T) NMR equipment, in standard optical, confocal and electron microscopes. This makes it a high throughput validation tool for therapeutic targets and/or nanotherapies by using organotypic tissue or cell line model systems. The differential characteristic of this "lab-on-chip" is that it enables the longitudinal study of the same model system and under the control of fundamental variables such as temperature, pH and pO_2 and/or pCO_2. It allows high-throughput identification since nanogram amounts are required, which makes it a singular tool for:

- seeking biomarkers for clinical diagnosis and prognosis;
- verifying molecular imaging properties;
- checking various targeted nanotherapeutic effects and drug delivery.





1. **Study of interactions biomaterial/bone** (leg rabit/scaffold) of samples embedded in PMMA.
2. **Animal model for pharmacological studies** in preclinical phases.
3. **DRX-600 Bruker NMR equipment (14 T).**

SERVICES

The services can be summarized in:

- use of the mentioned equipment,
- preparation of samples
- the design of non-standardized experiments for specific projects,

The services offered by the platform are supported by its experience in assays performed in samples of patients from hospitals, in European projects of the 7th FP (Central Nervous System tumors), of small and medium-sized health-related enterprises (Instituto Valenciano de Infertilidad) for *in vitro* fertilization, and CENIT projects (neurodegenerative diseases such as Alzheimer's disease). The most common applications of this service are:

› **Support for the diagnosis and prognosis of various diseases such as:**

- cardiovascular pathologies,
- neurodegenerative pathologies (Alzheimer's, Multiple Sclerosis …),
- neonatal diseases,
- breast, central nervous system and prostate cancer;
- diffuse liver diseases and
- obesity

through the metabolic profiles of different types of samples such as:

- biofluids (serum, urine, cerebrospinal fluid, follicular fluid, vesicle fluid, …);
- cell lines;
- pulmonary exhalations and
- tissues.

› **Animal model for pharmacological studies in preclinical phases.**

› **Applications in clinical areas such as *in vitro* fertilization.**

EQUIPMENT

› **DRX-600 Bruker NMR equipment (14 Teslas):**

- 4-channel electronic systems with applications in fluids, tissues, biomaterials and microimaging
- Thermostating system for fluids and tissues, and for molecular imaging and microimaging
- Set of coils: fluids 5 mm, micro-fluids 1 mm, tissues (High Resolution Magic Angle Spinning, HR-MAS) + pneumatic and microimaging system for 10, 5 and 2 mm.
- 60 Amp GREAT unit for each X/Y/Z axis of the field gradients
- 13C selective device with 1H decoupling.
- 2D microcoil for 500 µm and 100 µm microsamples.
- BACS autosampler with temperature control for 60 samples

› **Microcamera for its use in the NMR equipment for:**

- the simultaneous determination of structural images (dimensions and sections of 50 µm and less)
- metabolic images (voxel sizes < 200 µm) in model systems.

› **Furthermore, there is access to an 11T NMR equipment with applications in tissues and cell lines, installed in the central research support services of the Universidad de Valencia for applications of NMR microscopy, molecular imaging and metabolomics. It consists of the following probes:**

- HR-MAS,
- Cryoprobe, and
- multinuclear probe with service for metabolomics for tissues, cell lines and biofluids.

Platform 5

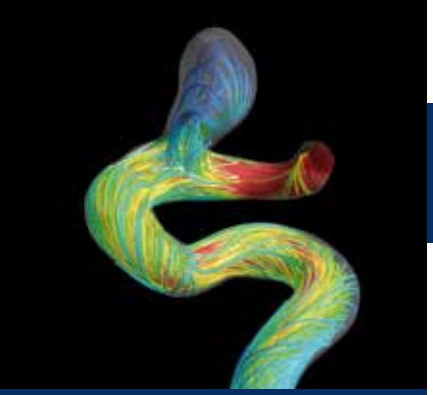
HIGH-PERFORMANCE COMPUTING

CIBER-BBN Research Infrastructure in **BIOMEDICINE**

5. HIGH-PERFORMANCE COMPUTING PLATFORM

Provide technical support for research projects in the line of Multimode Diagnostic by allowing the remote access for applications dealing with advanced analysis of biomedical images and signals along with the integration of multiphysical and multiscale calculation models for in silico experiments.

UNIT	OBJECTIVE
COMPUTATION	System for the management of computational resources and calculation tasks in a distributed environment which allows a large number of local and remote users to access the system and perform intensive calculations simultaneously and independently.



Equipment for High Performance Computing, massive storage and software for Biomedical Applications

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This Unit is hosted by the CISTIB group (Computational Imaging and Simulation Technologies in Biomedicine) led by Dr. Alejandro Frangi. It consists of a high performance computational platform with high availability for work in parallel. The cluster has a computational capacity of more than 2 Teraflops and a centralized data storage system with a capacity greater than 15 Tb. Also, a scalable, replicable and multiprotocol14 TB storage system is provided additionally to serve as the final repository for the medical database.

There are several examples of application areas that benefit from HPC in general, and also many of researchers that utilise the resources of the CIBER-BBN HPC Platform in particular. These range from the modelling of Anatomically-Based Cardiac Conduction, to experiments in artificial intelligence, the simulation of cerebral aneurysms, the development of innovative applications under the framework of the "Virtual Physiological Human" project.

Communication technologies and shared software applications are essential to fully capitalize on the scientific knowledge gathered over the years by different technological areas. For this, it is necessary to have access to and be able to use shared data stored in clinical and research databases as well as have a great distributed computation capacity continuously available. Both these requirements (for calculation and storage) are met by the current platform.

Following the technology trends in HPC, the last year has been incorporated into the CIBER-BBN Plataform a new HPC Cloud Cluster named "NEPHOS". In addition to our current clusters, ARGO/ORION/FORNAX, now we have access to a new set of high performance calculation resources. Elasticity is the main benefit, we can add and remove compute resources to meet the size and time requirements for the workloads.

SERVICES

The services offered include:

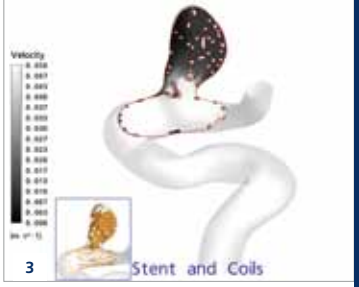
- **Calculation**
- **Mass storage.**
- **HPC Cloud computing**
- **Special configurations and services.**
- **Spreading awareness of the facility and training.**
- **Other services: Technical Support, Management of users, HPC consulting.**

Remote access to the platform enables calculation intensive numerical models to be run for use in applications including:

- **Processing of meshes.**
- **Image segmentation.**
- **Data and image registry.**
- **Model simulations.**

The use of computer models, numerical simulations and IT tools for biomedical image and signal processing makes it possible to:

- **Understand the physiological processes at various levels.**
- **Analyze the response of the body to certain therapies and drugs.**



1. **ARGO (SGI ALTIX ICE 8200).**
2. **ARGO (SGI ALTIX ICE 8200),** detail.
3. **Aneurism model.**

- **Carry out personalized monitoring of each patient.**
- **Design new drugs and new therapeutic techniques.**
- **Develop models of illnesses to help with diagnosis.**

Some real examples of these applications include:

- **Simulation of Physiological systems:**
  - Electromechancial models of heart.
  - Pathophysiological model of renal function.
  - Model of nerve signal transmission.
- **Systems for identifying chains in DNA sequences.**
- **Creation of an 3D atlas based on medical imaging data.**
- **Simulation of cell models to study tumor progression.**

EQUIPMENT

- **NEPHOS:** is a cluster in the AWS Cloud. Using NEPHOS' instances, our users can expedite their HPC workloads on elastic resources as needed and save money by choosing from low-cost pricing models that match utilization needs. "Each" NEPHOS instance includes:2 Intel Xeon processors, with 8 cores each one 60.5 GB of RAM, 3.37 TB of instance storage Hyper-Threading is enabled, so you can execute up to 32 threads in parallel. We can set as much instances as needed!.

- **ARGO :** (SGI ALTIX ICE 8200) is a cluster composed of 32 diskless computing nodes: 2 INTEL XEON 5355 Quad-Core processors, 16 Gb RAM with a total of 256 computational cores. There is one administration node (ALTIX XE 240, 4 Intel Xeon 5150 Dual-Core 2 processors, 16 Gb RAM and a 224 Gb hard drive), one login node (ALTIX XE 240, 4 Intel Xeon 5150 Dual-Core 2 processors, 16 Gb RAM and a 64 Gb hard drive) and one compilation node (ALTIX XE 250, with Intel Xeon 6420 processors, 16 Gb RAM and five 146 Gb hard drives). In addition, this cluster has two 16 TB INFINITE STORE 4000 disks and an InfiniBand-dual-plane as primary connection.

- **ORION:** (Altix XE 240) is a cluster composed of 20 dual nodes (80 cores). There is one main node and two SGI Tezrix 240 file servers, with 2 Xeon Woodcrest Dual-Core processors, 8 Gb RAM and two 250 Gb SATA internal hard drives. It has a 6 Tb SGI Infinite Storage 4000 system and primary interconnect: a 48-port Gigabit switch.

- **FORNAX:** (SGI ALTIX 350 SERVER) is a shared memory cluster of 32 nodes each comprising 1 Intel titanium processor and 2 GB RAM. This cluster has a total of 1.4 TB external storage and 250 Gb SATA internal storage.

- **STORAGE:** (NetApp FAS3140) is an expandable and scalable 14 Tb device for mass storage, which offers multiprotocol support.

CIBER-BBN
Research Infrastructure in
BIOMEDICINE

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