LIFE SCIENCES PLATFORM

A system of open research facilities for applied research

SISTEMA ARGO is an industrial development iniziative supported by:

Ministero dell'Università e della Ricerca

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coordination of activities:

AREA SCIENCE PARK

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COMPETENCES AND FACILITIES FOR INDUSTRIAL RESEARCH

Scientific and technological platforms are places where **open research infrastructures** - based upon core facilities and skills - create specialized functions, capable of providing know-how and services to carry out experimental tests as well as applied and industrial research projects.
These platforms are **a relevant asset to support research and development**, since they provide companies with optimal scientific and technological conditions to **carry out their experimental activities**. They do so by granting access to research laboratories with core scientific expertise as well as equipment and instrumentation hardly found elsewhere.

The **Life Sciences Platform** integrates equipment and expertise available at several different sites, such as Elettra synchrotron light source, FERMI free electron laser, Elettra laboratories of Structural Biology, Infrared Spectroscopy and Nanotechnologies, CNR-IOM laboratories of microscopy, microfluidics, micro and nanofabrication and mechanobiology, CNR-IC structural biology laboratory. The platform thus provides companies with **a system of expertise and facilities** dedicated to the application of imaging techniques, structural analysis as well as biochemical and biophysical characterization. This leads to a very wide range of possible **applications in the pharmaceutical, biotechnological, nutraceutical and diagnostic fields**.

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INSTITUTES AND LABORATORIES

ELETTRA SINCROTRONE TRIESTE | www. elettra.trieste.it

Elettra Sincrotrone Trieste S.C.p.A is a company of national interest managing an international and multidisciplinary research centre of excellence. Elettra's hallmark is the study of materials done using the light generated by two sources, Elettra and FERMI. The high brilliance and wide energy range of the light produced by Elettra and the extremely brilliant and ultrashort pulses produced by FERMI (in the order of one millionth of a billionth of a second) allow to unveil the most hidden secrets of matter. In the biomedical field, both Elettra and FERMI develop cutting-edge technologies to study tissues, organs and small animals using micro and nanometric resolution. In the structural biology field, it is possible to perform analyses including studies on DNA replication, genome stability, cell signalling and protein characterization. Moreover, small molecules can be investigated for their therapeutic effect and their interactions with target enzymes or proteins on an atomic scale.

ISTITUTE OF MATERIALS (CNR-IOM) | www.iom.cnr.it

The BioMed division of IOM applies to biology and medicine those methodologies, materials and instrumentation usually employed in physics and chemistry. The BioMed division in particular investigates the physical interaction and the dynamics of single cells, as well as of single subcellular structures down to the biomolecular level. To that purpose, it is possible to take advantage of existing expertise in optical microscopy, electron microscopy, scanning probe microscopy, material science and micro and nanofabrication.

IC CRYSTALLOGRAPHY INSTITUTE (CNR-IC) | www.ic.cnr.it

CNR-IC manages the Structural Biology laboratory of the National Research Council (CNR) at Area Science Park's Basovizza Campus. CNR-IC uses innovative methodologies for the study and characterization of intermolecular interactions and of molecular structure, for nutraceuticals and for the development of new pharmacologically active molecules.

AREA SCIENCE PARK | www.areasciencepark.it

Area Science Park - a public national research organization - promotes the development of innovation processes. For 40 years, its mission has been to boost connections between research and enterprise, public administration and the private sector, supporting national and international initiatives and fostering territorial development. Area coordinates the Life Sciences Platform.

MAIN APPLICATIONS

The Life Sciences Platform integrates and systematizes the participating institutes' and laboratories' equipment and competences for the study and development of structural, cellular and molecular biology, biophysics and microscopy techniques. That is in particular:

- Elettra synchrotron light source and FERMI free electron laser, Elettra Structural Biology, infrared spectroscopy and nanotechnology laboratories;
- CNR-IOM microscopy, microfluidics, micro and nanofabrication and mechanobiology laboratories;
- CNR-IC structural biology laboratory.

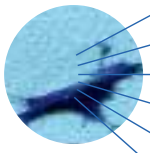
The Life Sciences Platform is available to companies and developers of new products in the pharmaceutical, biotechnological, nutraceutical and diagnostic fields. It is furthermore possible to test the biological activity of new molecules and preparations, diagnostic and prosthetic devices, and to carry out several types of studies, at molecular, single cell or organoid level. The main feasible activities include:

- Resolution of molecular structure at atomic level;
- Measurement of molecule affinities;
- Study of biological activity at cellular level;
- Screening of new biologically active compounds (e.g., drugs, nutraceutical products);
- Measurement of cell-cell and cell-substrate interaction forces;
- Measurement and analysis of cellular and tissue biomechanical profiles;
- Development of substrates and scaffolds for cell growth and differentiation;
- Development of organoid systems;
- Study of interaction between nanoparticles, nanostructures, viruses, bacteria with cells and tissues using electronic cryomicroscopy;
- Study of proteins and protein complexes involved in fundamental cell mechanisms and useful for the formulation of new drugs;
- Morphological study of microorganisms, cells and tissues, to obtain information on proteins, lipids, carbohydrates and nucleic acids with micrometric and nanometric resolution;
- Study of biological tissues and organs in animal models with 3D imaging (in vivo and ex vivo) and sub-micrometric resolutions, useful to understand morphological variations in different pathological conditions;
- Development and optimization of miniaturized sensors on a nano-micrometric scale for real-time analysis of biomarkers (proteins, miRNAs, exosomes);
- Design and prototyping of diagnostic instrumentation.

© Elettra Sincrotrone Trieste, photo by Massimo Goina, *Instrumentation of SISSI (Synchrotron Infrared Source for Spectormicroscopy And Imaging) Beamline - Elettra*

TECHNOLOGIES AND LABORATORIES

1. IMAGING 2. STRUCTURAL BIOLOGY 3. BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION



1

- Scanning electron microscopy – SEM and FIB
- Atomic force microscopy - AFM
- Fluorescence microscopy
- Soft X-ray transmission and emission microscopy using synchrotron light radiation
- X-ray micro-computed tomography using synchrotron light radiation – MicroCT
- Infrared microscopy using synchrotron light radiation

2

- Structural Biology Laboratory and Protein Facility
- UV Resonant Raman spectroscopy using synchrotron light radiation - UVRR
- Circular Dichroism spectroscopy – CD
- Small Angle X-Ray Scattering using synchrotron light radiation – SAXS
- X-ray Diffraction using synchrotron light radiation - XRD

3

- Energy dispersive X-ray spectrometry - EDS
- RAMAN spectroscopy
- Mechanobiology laboratory
- Isothermal titration microcalorimetry - ITC
- Grating Coupled Interferometry - GCI
- Terahertz Pulse Spectroscopy - TPS
- Fourier-transform infrared spectroscopy using synchrotron light radiation – FTIR

1. IMAGING

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Scanning electron microscopy – SEM and FIB

Similar in many ways to transmission electron microscopy (TEM), scanning electron microscopy (SEM) has reached over the years extremely interesting and promising resolution levels and application potentials. Coupled with STEM detectors, the quality of images in transmission mode is comparable to those of TEM images. Coupled with in situ ultramicrotomes or combined with focused ion beam systems (FIB), SEM allows to create a 3D reconstruction of cell samples. SEM also allows the study of interactions between samples and growth surfaces and scaffolds, which is very important in the field of prosthetics and regenerative medicine. When combined with cryogenic systems, SEM allows to study how cells and tissues interact with pathogens, such as viruses and bacteria. Finally, coupled with an X-ray

energy dispersive detector, SEM can be used to identify the nature of contamination and calcification in tissues. Finally, in combination with supercritical dehydration, SEM allows to observe the real cell and tissue morphology, avoiding the deformations typically induced by standard sample preparation procedures.

Atomic force microscopy - AFM

Unlike electron microscopy, atomic force microscopy (AFM) can operate on samples in physiological conditions (i.e., living cells), and allows the morphological and functional characterization of cellular and subcellular structures.
Due to the specific physical properties of the probes used, AFM also allows to study mechanical properties of the systems under investigation, and, in combination with optical fluorescence microscopy, the cellular response to mechanical, electrical or chemical stimuli. Finally, when used in FluidFM mode, this microscopy can act as a micropipette to induce mutations, specifically colour or collect the content at a single cell level.

Fluorescence microscopy

Fluorescence microscopy is an optical microscopy technique, used as support for cell investigation techniques, that allows to identify the spatial distribution of specific proteins, labelled with fluorescent molecules with micrometric resolution.

Infrared microscopy using synchrotron light radiation

Infrared microscopy produces spatial information, at micrometric and nanometric resolution levels of the main constituents of cells and tissues - including proteins, lipids, carbohydrates and nucleic acids – and of pharmaceutical formulations. A new multipurpose platform for the chemical characterization of biological samples with nanometric resolution is also available.

X-ray micro-computed tomography using synchrotron light radiation – MicroCT

X-ray imaging techniques using synchrotron light radiation find several applications in different biomedical and biological fields, from preclinical to clinical studies. Particularly, in diagnostics, the use of synchrotron light radiation allows important improvements in imaging quality associated with a decrease in radiation exposure. The technique is typically used for studies in bone tissues aimed at understanding bone regeneration processes and morphological variations that occur in different pathological conditions. In some cases, it is possible to combine morphological and functional information referred to specific organs or tissues, or it is possible to extract quantitative parameters such as sample density, composition or porosity.

Soft X-ray transmission and emission microscopy using synchrotron light radiation

X-ray transmission microscopy allows to analyse sample morphology, revealing cellular and sub-cellular organisation with a resolution of less than 100 nm. This technique allows to simultaneously determine in the sample both the distribution and the quantification of chemical elements distribution. Additionally, the availability of different detection methods (absorption and phase contrast, low-energy X-ray fluorescence) enables to perform cells and tissues analyses without the support of staining processes.

2. STRUCTURAL BIOLOGY

- Structural Biology Laboratory and Protein Facility
- X-ray Diffraction using synchrotron light radiation - XRD
- Infrared microspectroscopy using synchrotron light radiation
- UV Resonant Raman spectroscopy using synchrotron light radiation - UVRR
- Circular Dichroism spectroscopy – CD
- Small Angle X-Ray Scattering using synchrotron light radiation – SAXS

Structural Biology Laboratory and Protein facility

The laboratory of Structural Biology provides state-of-the-art instrumentations and competences to perform studies in the field of structural and molecular biology. The laboratory staff has great competences in the study of proteins, macromolecular protein-protein or protein-DNA complexes regulating cell proliferation processes (e.g., DNA replication and repair, signal transduction and autophagy). In collaboration with other academic and industrial research laboratories, the lab performs structural and functional studies of proteins which are pharmacological targets or biomarkers for the treatment or diagnosis of complex diseases such as cancer, neurodegenerative diseases and inflammation. A Protein Facility is also active in the laboratory: it is dedicated to the production and characterization of recombinant proteins and offers scientific and industrial communities the opportunity to closely cooperate with experts of the field, granting support and services.

X-ray diffraction using synchrotron light radiation – XRD

This technique allows to obtain structural details on ordered materials that form single crystals, including crystal structure, intermolecular interactions and charge density studies. In particular, high-performance macromolecular crystallography experiments can be carried out on crystals - of proteins, DNA, RNA or complexes - thus providing their 3D structures. This information is fundamental to design potential pharmaceutical drugs, since it allows to understand crystal functions, to identify the interaction sites of interest and to define the existing interactions with small molecules.

Infrared microspectroscopy using synchrotron light radiation

IR microspectroscopy is a non-invasive and label- and contrast-free technique requiring a very limited sample amount to produce its biochemical fingerprint, providing information on the content and structure of its main biomolecules (e.g., protein secondary structure), as well as on their chemical modifications. Examples of an alteration of chemical structure that can be monitored by infrared spectroscopy are protein modifications, like phosphorylation and establishment of supramolecular interactions.

UV Resonant Raman spectroscopy using synchrotron light radiation - UVRR

Raman spectroscopy is a vibrational technique widely used to reveal structures of biological macromolecules in a label-free manner. In particular, Ultraviolet Resonance Raman (UVRR) spectroscopy has emerged as a powerful tool for selective enhancing of Raman signal of molecules with absorption band in the UV region.
The use of synchrotron light radiation for UVRR enables highly-selective analysis of specific functional group vibrational bands, leading to the chemical and conformational studies of essential biomolecules, e.g., proteins and DNA.

Circular Dichroism spectroscopy – CD

CD is a spectroscopic technique used to study macromolecular conformation. It is very often used in protein studies since it allows the evaluation of the secondary structure of proteins in solution. Furthermore, CNR-IC uses advanced diffraction techniques to study the three-dimensional structure of macromolecules and of their complexes.

Small Angle X-Ray Scattering using synchrotron light radiation – SAXS

Small angle X-ray scattering (SAXS) is a powerful technique for the structural characterization of biomolecules in solution. It does not require labelling and, through the high brightness of X-rays from synchrotron light, it requires relatively small amounts of sample, such as protein solutions with low mg/ml concentrations.

3. BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION

- Energy dispersive X-ray spectrometry - EDS
- RAMAN spectroscopy
- Mechanobiology laboratory
- Isothermal titration microcalorimetry - ITC
- Grating Coupled Interferometry - GCI
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Energy dispersive X-ray spectrometry - EDS

EDS is a technique associated with SEM scanning electron microscopy and allows microanalysis of chemical elements, also in the form of a bi-dimensional map of selected chemical elements. SEM coupled with EDS is a powerful tool for obtaining microscopic chemical maps.

RAMAN spectroscopy

It is a molecular spectroscopy analysing molecules' rotational and vibrational energy levels, commonly used in chemical analyses and studies on the structure of complex chemical compounds such as organic compounds. In due conditions, the tool recognises the presence of specific molecular groups, even in very low concentrations, allowing the definition of sample composition. Unlike infrared spectroscopy, it is not sensitive to the water signal so it can be easily applicable in the medical and biological fields

Mechanobiology laboratory

The laboratory combines micro- and nano-fabrication techniques, optical microscopy and AFM microscopy to study the mechanical response of cells and tissues. Indentation, adhesion, creep and stress-relaxation measurements are usually performed.
Cell and tissue mechanical properties play a key role in many crucial aspects of health, e.g., fertilization and embryonal development, tumoral invasion and metastasis, cardiac dysfunction, tissue differentiation and regeneration.

Isothermal titration microcalorimetry - ITC

ITC is a technique which determines the heat developed during the interaction between two molecules in volumes below a millilitre and with micro-millimolar concentrations. The strength and thermodynamics of the interaction between the two molecules can thus be assessed. This technique is widely used to develop new pharmacologically active molecules.

Grating Coupled Interferometry - GCI

It is a very sensitive and versatile technique that determines association and dissociation rates between two interacting molecules, and consequently their affinity. This technique is often used to screen libraries of chemical compounds to identify molecules potentially active on a specific biological target.

Terahertz Pulse Spectroscopy - TPS

Terahertz pulsed spectroscopy is a technique that provides several types of information useful to investigate amino acids, proteins, DNA, bioactive compounds, pharmaceutical formulations and dynamic systems. The low energy used in TPS minimizes the risk of sample degradation. For such reason, TPS is used as a complementary tool of X-ray diffraction techniques for the investigation of pharmaceutical active molecule crystals and polymorphisms.

TPS also allows time-resolved studies on the sub-picosecond time scale, potentially providing insight into dynamic systems, such as biologic ones.

Small Angle X-Ray Scattering using synchrotron light radiation – SAXS

Small Angle X-Ray Scattering is an analytical technique used to identify density differences within a sample with nanoscale resolution. It works by analysing the elastic scattering angles of X-rays. SAXS is used for the investigation of new composite nano-systems and of biomacromolecules, providing information related to their dimension, shape, distribution and surface-to-volume ratio.

X-ray diffraction using synchrotron light radiation - XRD

The technique allows to obtain atomic structural details of ordered materials that form single crystals. In particular, it is possible to perform:

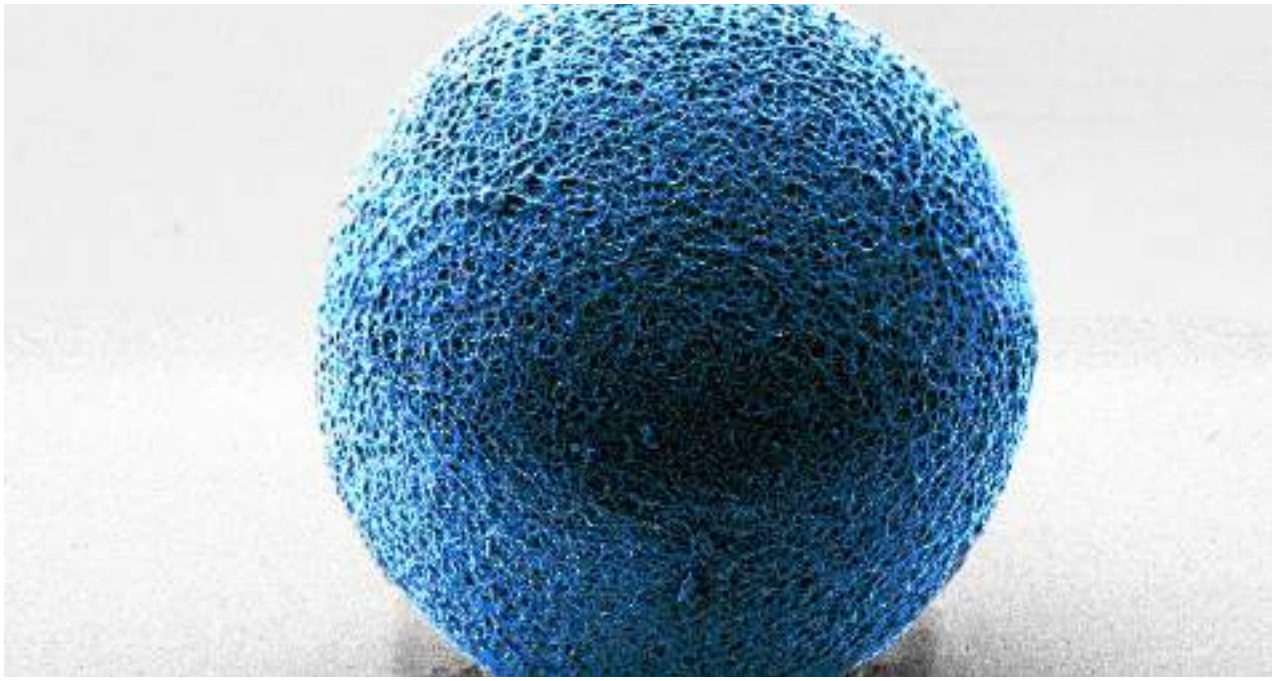
- high performance macromolecular crystallography experiments on crystals of proteins, DNA, RNA or complexes
- a wide range of non-single crystal diffraction experiments such as phase identification, structural and kinetic studies, diffraction and grazing incidence reflectivity.

The technique also allows to perform polymorph screening, structure determination and microstructural analysis of pharmaceutical systems. Different types of materials can be investigated, ranging from organic to inorganic materials, as powder in capillaries or as ready-to-use formulations such as gels, tablets or blisters.

Fourier-transform infrared spectroscopy using synchrotron light radiation – FTIR

IR spectroscopy uses energy levels associated with the vibrational transitions of molecules. It provides useful information on secondary structures of macromolecules and it can be used in life sciences field to study cells, tissues, and small microorganisms, to obtain biochemical information on their main constituents, e.g., proteins, lipids, carbohydrates and nucleic acids.

MECHANOBIOLOGY IN REPRODUCTIVE MEDICINE



© Institute of Materials, photo by Alice Battistella, *Image of an oocyte obtained by atomic force microscopy*

Mechanobiology in reproductive medicine in vitro fertilization (IVF) represents a pervasive approach to counterbalance the progressive age increase in developed countries' populations. The average maternal age at first birth in Italy is above 31 years, with **an increasing need for medically assisted procreation**.

Among the two main approaches adopted, **intra cytoplasmic sperm injection (ICSI)** involves the retrieval of maternal oocytes, their manipulation, selection through visual inspection and, finally, the syringe assisted injection of one spermatozoon. The fertilized cell is then implanted in the woman, letting nature run the rest of the process.

Although the mechanical process appears to be rather safe and simple, **success rate** is still very low, **below 10%** for women above 40 years of age [1]. One of the main sources of uncertainty in the whole process is the **proper selection of the competent oocytes**, which should be performed through non-invasive techniques, in a short time and in a physiological environment.

We used a **mechanobiological approach** to develop a non-invasive, independent evaluation strategy capable of providing oocyte quality score, which is strongly correlated to oocyte maturation.

The **oocytes' mechanical properties** can provide information on the maturation phase. In particular, they can discriminate between immature metaphase I (MI) and mature metaphase II (MII), and we demonstrated that the pregnancy outcome can be associated with the mechanical properties of the oocyte's zona pellucida [2].

We also investigated the evolution of the mechanical score with post ovulatory ageing, showing how these values can be used to **monitor oocyte ageing**. This allows to reject those cells that will undergo a degradation during the ICSI cycle, although at an initial visual inspection these do not show detectable differences from the good counterparts [3].

All the measurements were performed using **AFM and microfabricated tools**. Commercial AFMs are not designed and are thus not suitable to be used in clinical practice, however the information can be used to design a micromechanical sorter suitable for clinical practice [4].

[1] Eur J Obstet Gynecol Reprod Biol . 2000, 9, 1177-82. doi: 10.1016/s0301-2115(99)00260-2.
[2] Integr. Biol., 2016, 8, 886 doi:10.1039/c6ib00044d
[3] Bioengineering & Translational Medicine in press
[4] Micromachines 2015, 6, 648-659; doi:10.3390/mi6050648

MICROMECHANICAL ANTIBIOGRAMS

© Resistell AG, © M Oeggerli (Micronaut 2021), supported by Pathology Uni Hospital Basel, *Image of the cantilever and its deflection following interaction with antibiotic-resistant bacteria*

At least 700.000 people die every year from infections caused by bacteria capable to survive any antibiotic treatment. If no action is taken, the death toll may reach 10 million a year by 2050.

Antibiotic resistance is the ability of a microorganism to resist antibiotic treatment. As an effect, standard therapies become ineffective, and **infections propagate**, leading to the patient's death. Such resistance is spreading, with the terrifying prospect that even routine operations will become impossible to perform.

Currently, the **antimicrobial susceptibility test**, the so called antibiogram, requires growing a culture for 1-2 days in order to detect the pathogen. The precise timescale depends on the type of bacteria. After this, 12–24 additional hours are needed to screen existing antibiotics for their specific efficacy. In the case of slow-growing bacteria, e.g., M. tuberculosis, such a test may take up a full month to complete.

It is urgent to find an **alternative to traditional antibiograms**, in order to obtain an answer in few hours, or, even faster, in real time.

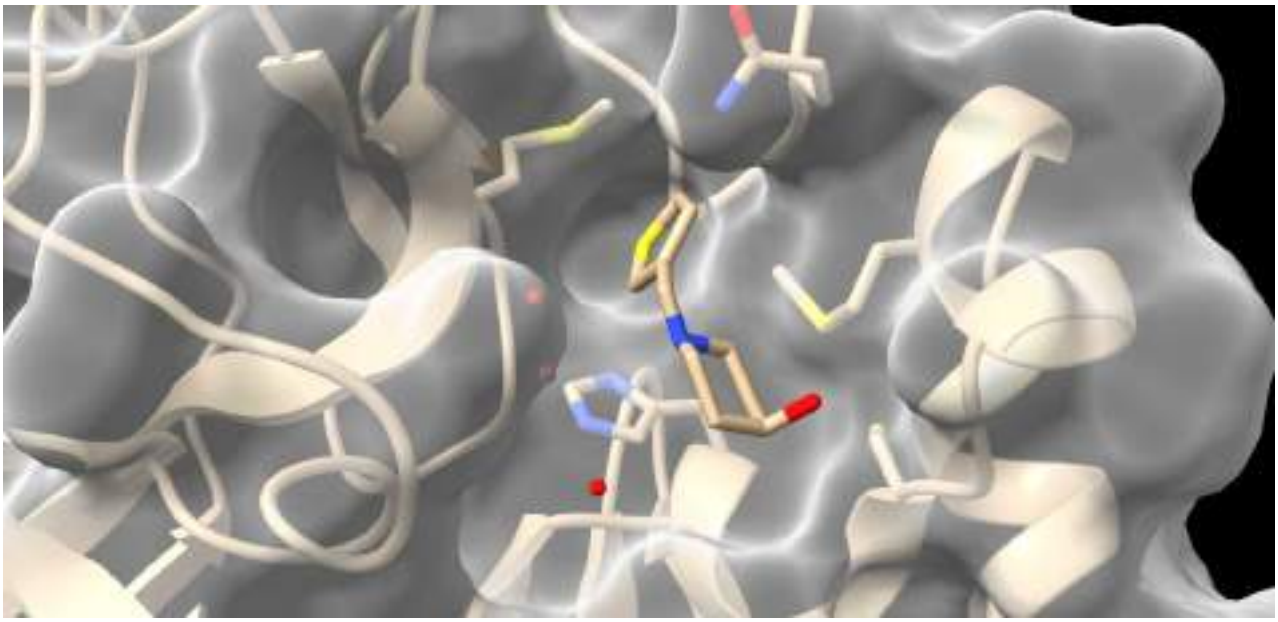
Using the **Atomic Force microscope technology** some scientists at EPFL in Lausanne (CH), developed a very fast drug screening approach, published in Nature Nanotechnology less than 10 years ago [1]. The authors demonstrated that the **fluctuations of highly sensitive atomic force microscope cantilevers** can be used to detect low concentrations of bacteria, characterize their metabolism and **quantitatively screen their response to antibiotics** within few minutes.

Since then, many other applications of AFM technology in the measurements of the dynamics, and thus the vitality of living organisms, were investigated [2].

In 2018 a startup, **Resistell AG**, was funded with the purpose to exploit this idea in a set of instruments for the medical diagnostic market. In three years, the company grew to 14 employees, and total funding exceeds 7M CHF.

[1] Nature Nanotechnology volume 8, pages 522–526 (2013) https://doi.org/10.1038/nnano.2013.120
[2] J Mol Recognit. 2020;33: e2849. https://doi.org/10.1002/jmr.2849

CRYSTALLOGRAPHY WITH SYNCHROTRON LIGHT AND DRUG DISCOVERY



© IC Crystallography Institute (CNR-IC), photo by Alberto Cassetta, *SARS-CoV-2 Mpro protease complex with its inhibitor*

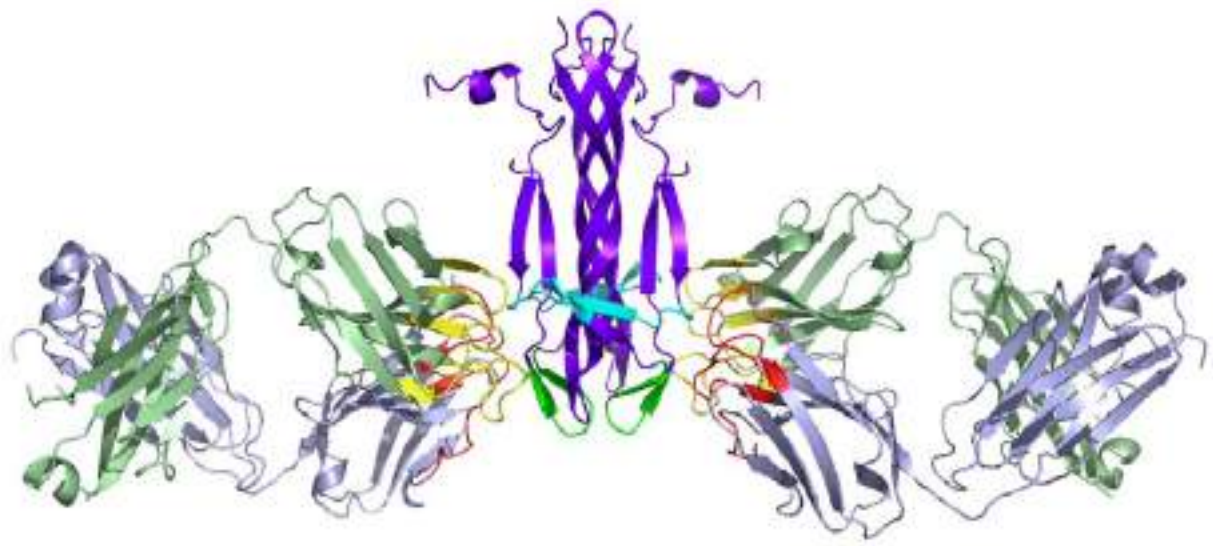
In the research for new drugs, a great amount of time and resources is dedicated to identifying new pharmacologically active molecules for specific target macromolecules. In this sense, **structural biology** is one of the most used strategies. It is based on the analysis of the target's **three-dimensional structure**, for example a protein involved in the pathological process, and of how this target molecule interacts with specific molecules, such as a specific antibody or a synthetic drug. This approach is commonly referred to as "Structure-based Drug Discovery". **X-ray crystallography** is one of the methods typically used to investigate the **molecular structure** of the complexes involving target molecule and potential drugs, although this kind of studies usually take a long time, since hundreds of **crystallographic structures** need to be analysed. The use of **synchrotron light** as an X-ray source, due to its very high intensity, allows for a significant reduction in investigation times.

A health emergency, such as the COVID-19 pandemic crisis, requires an immediate and timely response, both in terms of vaccine and pharmacological research. For this purpose, some proteins of the SARS-CoV-2 betacoronavirus, responsible for COVID-19 infections, have been identified as potential targets for specific drugs. Mpro-protease protein, in particular, involved in SARS-CoV-2 replication, is considered one of the most promising pharmacological targets.

For this reason, as early as February 2020, an English research team started to develop a "Fragment Based Drug Discovery" study in which a limited number of low-molecular-weight compounds have been studied to identify possible targets on Mpro protease's surface [1]. The subsequent processing of some molecules found to be active in molecules with higher molecular weight, can lead to the development of new molecules with higher pharmacological efficacy. In less than two months, the use of **X-ray crystallography with synchrotron light** permitted to analyse more than 1500 crystals. As a result, 96 low molecular weight compounds were identified as well as their interactions with Mpro protease. After a subsequent first processing, it was possible to obtain more complex molecules that were able to even more effectively interact with the Mpro protease, blocking its activity. **The incredible speed with which this result was achieved is essentially attributable to the use of a high intensity synchrotron light source**, which reduced - from many months to few days - the time required for the acquisition of diffractometric data.

[1] Douangamath A. et al. (2020) "Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease" Nat. Commun. 11, 5047

STRUCTURE-BASED HUMANIZATION OF THERAPEUTIC ANTIBODIES



© IC Crystallography Institute (CNR-IC), photo by Sonia Covaceuszach, *Model of neuro-antibody Fab – NGF complex, obtained by crystallographic and SAXS data*

Since their introduction in 1986, due to their high specificity and low toxicity, monoclonal antibodies (mABs) have been recognised as one of the most promising classes of pharmacologically active molecules. Out of the new drugs which appeared on the market in the last five years, monoclonal antibodies constitute the most important class, due to their use for the treatment of various diseases such as cancer, osteoarthritis, autoimmune and metabolic diseases. To reduce their immunogenicity - i.e., the ability to induce adverse immune reactions in the patient - humanized animal antibodies have been developed. In these antibodies, the key regions for antigen recognition, called Complementary Determining Regions (CDRs), are grafted on a human antibody, thus ensuring at the same time antigen recognition and low immunogenicity. Antibody engineering is not a simple technique, and it very often results in a long and expensive operation, requiring subsequent attempts to ensure the same affinity and specificity degrees of the original antibody.
At the Structural Biology laboratories in Trieste, the team led by A. Cattaneo, D. Lamba and S. Covaceuszach developed a new and simpler humanization strategy, based on a-priori knowledge of the molecular structure of the murine antibody's Fab, i.e., the fragment responsible for binding with the antigen [1]. The grafting of the CDRs parental antibody onto the human antibody was optimized by combining the **structural information**, acquired through **crystallography**, with an in-depth analysis of the human and humanized **antibody structures** available at the Protein Structure Database (PDB). This way, the resulting antibody maintains high **affinity for the antigen**, combined with a low immunogenicity.
This strategy has been successfully applied to the **rational design** of two neutralizing neuro-antibodies: aD11, an anti-Nervous Growth Factor (NGF) rat Fab and MNAC13, a murine Fab targeted against the TrkA receptor. The molecular structures of aD11 and MNAC13 were determined **by X-ray crystallography with synchrotron light** and the application of the described protocol resulted in the maturation of two neuro-antibodies interfering with the NGF / TrkA system. The disruption of the NGF-TrkA signal is a widely recognised approach for the treatment of inflammatory conditions and neuropathic pain, as demonstrated by preclinical and clinical studies that clearly showed the strong anti-NGF and anti-TrkA analgesic actions.
Overall, the new humanization protocol, used in this study and based on a **bio-structural approach**, represents an important improvement as compared to the previously applied protocols. Moreover, it has allowed to design on a rational basis,two new highly efficient humanized antibodies.

[1] D A. Cattaneo, S. Covaceuszach, D. Lamba "Methods for the humanization of antibodies and humanized antibodies thereby obtained" Patent WO/2005/061540

OPTIMIZATION OF THE FORMULATION AND THE MANUFACTURING CONDITIONS FOR THE EFFICIENT DEVELOPMENT OF COATED TABLETS

Coating of solid pharmaceutical dosage forms is considered a key part in pharmaceutical production. The most important functions of tablet coatings are protection of tablet core, containing the active pharmaceutical ingredients, and control of drug dissolution properties (which may involve rapid, timed, or sustained release). Manufacturing conditions during the coating process can affect the performance of coatings by inducing alterations in their thickness, structural uniformity, and defects. Inspections of coatings' thickness are necessary to guarantee quality standard. However, they are not trivial to perform.

Terahertz (THz) spectroscopy available at the Life Science platform provides a suitable analytical tool to **perform a non-destructive evaluation of tablet coating thickness**, measuring the time delay between the signal from the surface and the signal from the coating-core interface. The direct measure of the thickness of the coating layer of the tablet obtained by means of ThZ allows to monitor the coating process and to evaluate the success of scale-up [1].

[1] M. Naftaly et al., "Industrial Applications of Terahertz Sensing: State of Play", Sensors, 2019, 19, 4203.

DEFINITION OF THE RIGHT EXCIPIENTS IN A FORMULATION FOR ACTIVE PHARMACEUTICAL INGREDIENTS (APIS) STABILITY

Physical and chemical instability of APIs is the reason of many market recalls of products, due to unacceptable levels of their decomposition, which alter content uniformity of the final formulation. Excipients' properties, such as hygroscopicity and microenvironmental acidity, can have a significant impact on API stability in a drug product. Incompatibility or interaction between API and excipients, during manufacture or shelf storage, need detailed investigation.

Synchrotron X-ray diffraction can be used to assess the physical stability of an API in powder blends, tablets (even in blister), gel capsules, and creams providing information on the presence of API different physical forms. API quantification can be accomplished based on the intensities of one or more of its characteristic peaks. By quantifying the API in the dosage form, it is possible to readily assess the influence of formulation composition on API stability.

Most of these studies have been performed with companies under non-disclosure agreements. However, an example of a similar study can be found here:

N. Kaur et al., "Investigating the Influence of Excipients on the Stability of Levothyroxine Sodium Pentahydrate", Mol. Pharmaceutics, 2021, 18, 2683.

HOW TO ACCESS THE LIFE SCIENCES PLATFORM

A dedicated channel is made available to companies for them to be able to submit technical issues or innovation needs, thus initiating a process which, starting from an idea, a need or an identified opportunity, leads to the provision of services or the development of collaborative research projects.

STAGE	ACTIVITY	TIMING
First Contact	**Send an email** to: openlab@areasciencepark.it	Recall within **1** working **week**
Need definition	**Request and acquisition of technical information** to focus on the company's needs and **identify the best-suited technical and scientific competences** to address them	**1** working **week** (from need definition)
Scientific and technical analysis	**Follow-up meetings** (which may also take place by teleconference) with the involvement of the most suited technical and scientific experts to further **analyze the proposed topic**	
Feasibility checks	**Testing and experiments** are conducted - free of charge for companies - to ensure the feasibility of the identified techniques **to address the proposed topic**	Initiation within **2** working **weeks** (from follow up meeting)
Definition of a work-plan for experimental activities	**Results of feasibility checks are shared** and a **work-plan elaborated for experimental activities**, including detailed descriptions, machine time for the required instrumentation, definition of goals and milestones, estimated timing and costs	**2** working **weeks** (from completion of feasibility checks)
Contractual agreements	**Definition and signing of contract** (research project or collaborative project agreement) including clauses for the **management of know-how and intellectual property** either pre-existing or resulting from the implementation of the work-plan	**2** working **weeks** (net of negotiations)
Project implementation	**Implementation of experimental activities work-plan** compliant to contractual agreements	n/a

For more information

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