



X-RAY DIFFRACTION @ ELETTRA SYNCHROTRON: ATOMIC INSIGHT OF YOUR BIOMEDICAL TARGET

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Abstract — Crystallography is an invaluable tool to understand atomic structure. The knowledge at atomic scale of small and large molecules is crucial for many biomedical and biotechnological applications, ranging from drug design and optimization to enzyme functional studies and engineering. The physical properties of the solid state seen in crystals and powders of both drugs and pharmaceutical excipients are of interest because the nature of the crystalline form of a drug substance, due to polymorphism, may affect both the production and formulation as well as its stability in the solid state, its solution properties and its absorption. We offer a comprehensive access to state of the art X-ray diffraction tools and methodologies, supporting all the steps, from sample preparation to 3D structure analysis.

Index Terms — X-Ray diffraction, Macromolecular Crystallography, Alzheimer's disease, CK2 kinase, drug polymorphism, XRD1, Structure Based Drug Design, Active Pharmaceutical Ingredients

1 BACKGROUND

X-ray diffraction from crystalline samples of macromolecules, like proteins or enzymes, allows to get atomic level details about their 3D structure, active site details, charge distribution, substrate binding features. These are the foundations - aided by quantum-mechanics and molecular dynamics calculations - of rational design of new molecules, acting as potential drugs (Structure Based Drug Design). Molecular structure is also the starting point for industry related projects based on molecular engineering for technological applications, like enzymes optimization or immobilization. Furthermore, the packing analysis of solid state crystal forms contains invaluable information for pharmaceutical industry about polymorphism in APIs (Active Pharmaceutical Ingredients), connected to solubility and bioavailability.

Thanks to the high brilliance in the X-Ray spectral range, the synchrotron light sources played since the beginnings a pivotal role in the development and success of structural biology related sciences. They represent today the ideal tool to investigate biological samples.

2 OBJECTIVES

Elettra-Sincrotrone Trieste provides access to atomic insights of biomedical targets via powerful biocrystallography tools and methodologies that requires time and cost demanding platforms for sample preparation and sample screening. Elettra offers a convenient way to exploit cutting edge technologies and proven long term experience in molecular structure solution and characterization.

3 APPROACH & METHODS

General approach

1. Target production and purification. 2. Target crystallization. 3. Crystal screening. 4. Data acquisition. 5. Data analysis and target model building.

Methods

Steps involved in macromolecular crystallography are well established [1]. Brief graphical summary of the main pathway is shown in Figure 1.

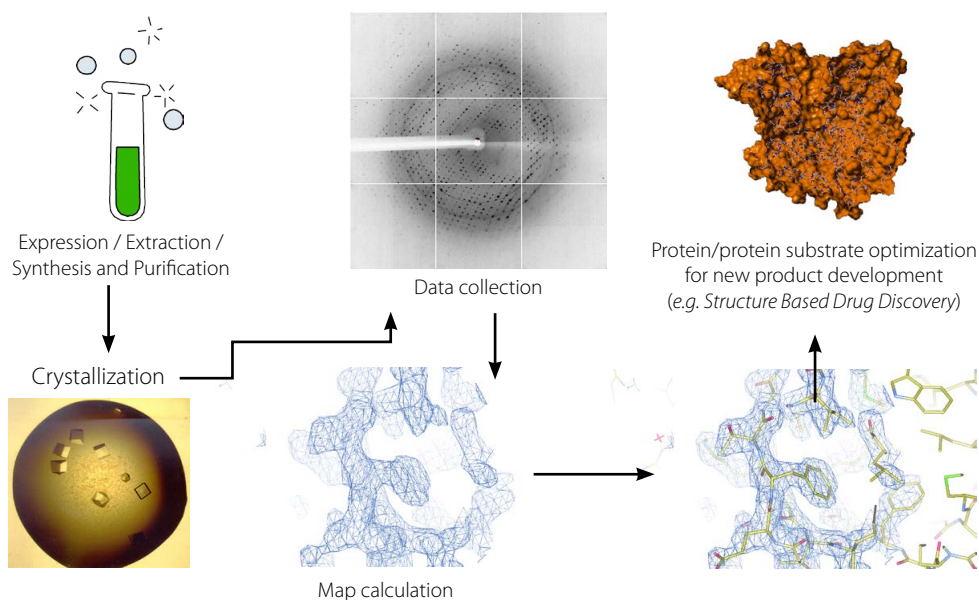


Figure 1: Schematic presentation of a macromolecule structural characterization work flow, starting from gene expression to the 3D structure determination. Any of these steps are offered to customers to accelerate structure-based drug discovery, with competitive timelines and scientific excellence. Pictures refer to structural characterization of acetylcholinesterase from *Torpedo californica*, a protein template for the development of inhibitors for Alzheimer's disease [2].

The macromolecule of interest has to be produced and purified in milligram amounts and characterized by biochemical and biophysical methods. Suitable physico-chemical parameters have then to be assessed aiming to obtain ordered solid aggregates (crystals). Crystallization is achieved by screening different conditions exploiting buffer pH, precipitant concentrations, additives, etc. Elettra crystallization lab aims to obtain single crystals of the bio-molecular system under investigation. In order to find the optimal crystallization conditions, thousands of experiments are carried out in parallel, using computer-assisted robotic pipelines that are capable of cocktail preparation, crystallization plate setup, and inspection and interpretation of results. Robots are used to prepare, dispense, inspect, evaluate, handle volumes of solutions down to 50 nL and monitor in time the evolution of the crystallization process. The whole work flow is tracked with unique identification bar-codes, assigned to each crystallization trial. High quality crystalline samples are then tested at the XRD1 beamline, where data are collected from best diffracting crystals.

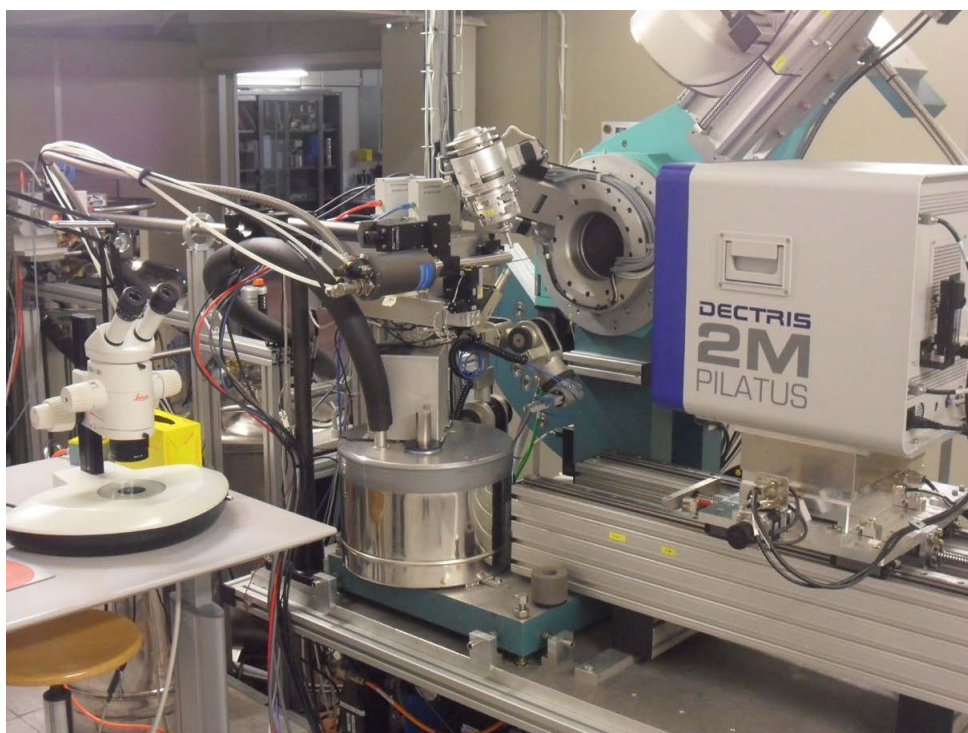


Figure 2: Current Elettra XRD1 setup showing also a robotic sample changer suitable to store and quickly screen up to 50 cryogenically cooled (Liquid nitrogen) crystals.

XRD1 is operative since 1994 and since then it contributes to the resolution of several important bio-organic-inorganic systems, ranging from small molecules (drugs, vitamins, catalysts), to protein and enzymes involved in several physiological and pathological states. The beamline is characterized by cutting edge instrumentation for automatic sample mounting and diffraction data collection, as well as up-to-date software and high performance computing capabilities for data analysis.

4 RESULTS: SOME EXAMPLES

Several examples of small and large molecules successfully determined at XRD1 have been published by the worldwide user community. In-house activities have produced important results in the characterization of enzymes involved in neurodegenerative diseases.

Alzheimer's disease (AD) is thought to be the leading cause in senile dementia, hence the development of an effective therapeutic treatment of this disease is of primary importance and would have a great social impact. The improvement of old, and the development of new drugs targeting the cholinergic system is still pursued. In collaboration with pharmaceutical companies as well as academic research groups, we contributed to the screening process for new drugs showing a better pharmacokinetic as well as a pharmacodynamic profile, through the determination of the X-ray crystal structure of complexes with derivatives carefully selected among several others.

The results obtained clearly pinpointed that in order to help to direct the rational design of new inhibitors and to elucidate the molecular determinants in enzyme inhibition, the crystallographic structure determination of molecular complexes at certain milestones along the elucidation of an enzymatic process or the development of interacting inhibitory drugs based on molecular modeling, appeared to be a tool of choice.

In this context, the experimental AD therapeutic bisnorcymserine (Figure 3), a synthetic carbamate, shows an interesting activity and selectivity for burrylcholinesterase [2], and its clinical development is currently being pursued by QR Pharma Inc.

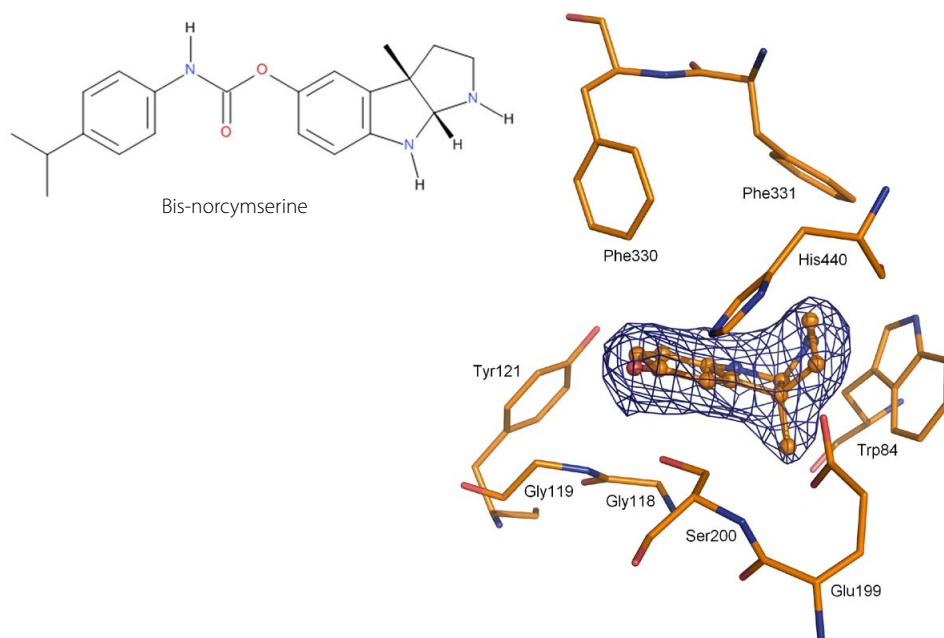


Figure 3: Close-up view of the of active site of TcAChE in complex with bis-noreseroline, the leaving product of the enzyme catalyzed reaction with bis-norcymserine [2].

Several systems have been also characterized by XRD1 users.

CK2 is an example of important tumor targets successfully tackled [3]. CK2 is a ubiquitous, acidophilic Ser/Thr protein kinase. It has been known for a long time that kinase activity tends to be particularly high in cancer cells compared to that of their “normal” counterparts and an ample spectrum of cancer cells derived from tumors of different origin share abnormally high levels of CK2. These tumors cells have been shown to undergo apoptosis after treatments, either genetic or pharmacological, that reduce CK2 catalytic activity.

CX-4945 is the first clinical stage (clinical stage 2) inhibitor of protein kinase CK2 for the treatment of cancer. It is representative of a new class of CK2 inhibitors with K_i values in the low nanomolar range and unprecedented selectivity versus other kinases. Figure 4 presents the crystal structure of the complex of CX-4945 with the catalytic subunit of human CK2. Consistent with their ATP-competitive mode of inhibition, all three compounds bind in the active site of CK2 (type I inhibitors).



Figure 4: Crystal structure of human protein kinase CK2 with CX-4945, a clinical stage inhibitor for the treatment of cancer (shown with pink sticks).

Another research line deals with small molecules powder diffraction analysis in particular drugs. Indeed, drug polymorphic characterization is one of the most critical point for pharmaceutical industries. Dosing solid state drug in fact is the most common way to supply APIs (Active Pharmaceutical Ingredient); different solid state forms have different bioavailability. This property of solid state is so crucial that different drug forms are patented and are tightly monitored by national institution responsible for regulating, protecting and promoting public health. Measurements in this field are

routinely performed at XRD1 beamline to characterize polymorphic contamination, exploiting synchrotron brightness to detect amount, unreachable with conventional X-ray sources. During these industrial committed activities, procedures compliant with Good Manufacturing Practices (GMP) are applied. Ad hoc protocols and methodologies (International patent n. PCT/EP2012/059127) have been especially developed for these purposes.

5 POTENTIAL NEW PRODUCTS & SERVICES

Service: Tailor-made solutions in protein production, crystallization, data acquisition and data analysis for the molecular target of interest. The structural information could be applied to:

- Drug discovery solutions for global pharma, biotech and research organizations: lead compound characterization and improvement through crystallographic screening of target-ligand complexes.
- Macromolecules engineering: to suit new biotechnological applications or for functional studies among others.
- Polymorphic characterization for pharmaceutical industry: from process control to final product compliance.

6 CURRENT COLLABORATIONS

6.1 Accademic collaborations

The service of molecular structural and functional characterization is offered by a joint Elettra-Sincrotrone Trieste and the Istituto di Cristallografia – CNR - Trieste Outstation, collaborative research group.

6.2 Industrial collaborations

The polymorphic characterization is offered as a collaboration between Elettra-Sincrotrone Trieste and Zach System S.p.A. (Zambon group).

7 COMMUNICATION TOOLS

- The high level of expertise in the field of Structural and Functional Biology as well as the high level of applicability, quality and performance of the presented methods is disseminated through the high quality scientific publications.
- Dissemination of scientific results using media for the general public ([HTTPS://WWW.YOUTUBE.COM/USER/SINCROTRONTRIESTE](https://www.youtube.com/user/sincrotrontrieste), [HTTP://WWW.ELETTRA.EU/ELETTRA-BEAMLINES/XRD1.HTML](http://www.elettra.eu/elettra-beamlines/xrd1.html), <http://www.ic.cnr.it/icnew/site/index.php>).

8 CONCLUSION

The 3D model obtained from the crystallographic experiment show details on topology and “hot” binding sites of a macromolecular target. This model is the starting point for quanta-mechanics and molecular dynamics calculations aimed to investigate enzymes mechanisms of action, to design new molecules interacting with the target, to adapt enzymes for industrial applications.

The XRD1 beamline hosts national and international academic users, selected on the base of scientific and technological impact via a peer review process as well as industrial proprietary research. Italian and European specific programs (BiostructX) support academic national and international access to the facility. A second highly automated X-ray diffraction beamline (XRD2) is under construction in partnership with the Indian Institute of Sciences (IIS). Users' activities are expected to start in the beginning of 2015.

Accessing Elettra Structural Biology Laboratory and X-ray Diffraction Beamlines is a unique opportunity to thoroughly characterize your biomedical target and to start a collaborative project that will open new or expand your scientific horizons.

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