

# A DECADE OF CUBAN PRESENCE IN SYNCHROTRON LIGHT SOURCE FACILITIES

UNA DÉCADA DE PRESENCIA CUBANA EN INSTALACIONES DE FUENTES DE LUZ SINCROTRÓN

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In this contribution, a simple description of a synchrotron light laboratory is offered, highlighting the basic elements comprising it. The fundamental features of the emission spectrum, that distinguishes it from other conventional sources, are described. We also describe the types of experiments that can be performed at such facilities. Finally, we review the most important pieces of research performed by various groups of Cuban scientists using Synchrotron Light Source facilities, in particular at the Brazilian Synchrotron Light Laboratory (LNLS).

Se ofrece una descripción sencilla de un laboratorio de luz sincrotrón, destacándose los elementos básicos que lo conforman y las características fundamentales del espectro de emisión que lo distingue de otras fuentes de radiación convencionales. Se hace también referencia a los tipos de experimentos que pueden ser realizados en este tipo de facilidades. Finalmente, se hace un recuento de las investigaciones más importantes hechas por varios grupos de trabajo de científicos cubanos en instalaciones de fuentes de luz sincrotrón, en particular en el Laboratorio Nacional de Luz Sincrotrón (LNLS) en Brasil.

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## INTRODUCTION

The first time a group of Cuban researchers performed experiments at a synchrotron laboratory was in 2000. That marked the beginning of a long series of experiments made at the Brazilian Synchrotron Light Laboratory (LNLS), as well as other sources around the world. The LNLS, for more than 10 years, has become a focal point for Cuban researches working in areas as diverse as physics, chemistry, materials science and biotechnology, among others. With this report, we deservedly acknowledge the LNLS for all its support over the past decade. We would also like that this article contributes to catalyze the use of synchrotron sources by Cuban researchers.

## A LIGHT FACTORY

The use of electromagnetic radiation has been an essential tool for understanding the world around us albeit of being essential in improving our living standards. Usually, we are accustomed to dealing with equipment or processes that generate electromagnetic radiation in a narrow range of the spectrum. Thus, a source of radio or television signals generates electromagnetic waves with relatively small frequencies allowing communication, a laser emits coherent radiation mainly in the range of the visible spectrum, and we use X-ray radiation in several applications.

A synchrotron light source, unlike the former examples, is a machine able to produce electromagnetic radiation in a wide range of energies, from the infrared to the X - rays. It can be said that *it is like a light factory*. Such facilities have their origins in particle accelerators, dedicated primarily to the study of high energy phenomena and interaction between particles. It was not until the 60's of the last century, when scientists realized how useful would be to dedicate a particle accelerator only with the goal to produce electromagnetic radiation. Several material characterization techniques could then be employed using a single radiation source, a breakthrough idea.

The first generation synchrotrons sources were built in the 60's, and were dedicated basically to X-ray diffraction experiments. In the 80's, with the construction of second generation light sources, they began to gain popularity among the international scientific community: in fact, they are now an indispensable tool for scientific research. In the last decade, the so-called third generation synchrotrons have been constructed, whose essential characteristic is that the intensity of the emitted radiation exceeds approximately in 10 orders of magnitude the intensity of conventional X-ray tubes. Today there are more than 40 synchrotron light facilities around the world, mostly concentrated in the U.S., Europe and Asia. By contrast, the LNLS is located in Sao Paulo, Brazil.

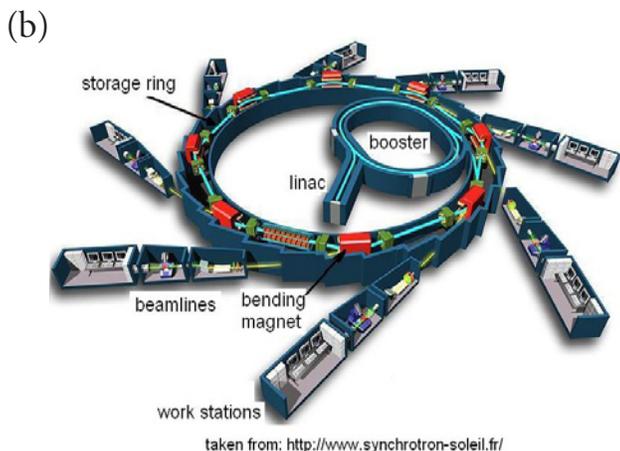


Figure 1. (a) General view of the LNLS (taken from [www.lnls.br](http://www.lnls.br)). (b) Simplified schematic representation of a synchrotron light source (taken from [www.synchrotron-soleil.fr/](http://www.synchrotron-soleil.fr/)).

## WHY WE NEED A SYNCHROTRON LIGHT SOURCE?

The inherent properties of any material, organic or inorganic, depend on its crystal structure, i.e., how atoms are distributed spatially; what kind of atoms and what kind of chemical bonds exist between them. For example, once the 3D crystal structure of a protein molecule is known, it allows us to identify their active part, so we can inhibit or potentiate its action. The former is fundamental in the design of new drugs. The possibility to make changes at the atomic level, in order to develop new materials requires special tools: synchrotron light facilities perhaps stand at the top of the list.

The synchrotron is a large plant producing electromagnetic radiation in a wide range of energies. Figure 1 shows a simplified schematic representation of a synchrotron light source. It shows three main parts: the linear accelerator (linac), the injector (booster) and the storage ring. The linear accelerator is where electrons are accelerated initially before being transferred into the injector. Once the electrons reach their maximum acceleration using radio frequency (RF) cavities, they are transferred to the storage ring, where they travel at relativistic velocities.

In the storage ring (with a diameter of several meters depending on the synchrotron machine), with curved and straight

sections, the electrons remain in orbit for a large period of time (up to 12 hours) thanks to an array of permanent magnets, RF cavities and a high vacuum ( $10^{-9}$  torr). After this time electrons are injected again in the storage ring, and in some cases the injection process occurs continuously. Permanent magnets or bending magnets are placed on the curved sections of the ring (Fig. 1).

These make it possible to maintain the electrons on their orbit while they emit electromagnetic radiation along the tangent to the curve. In the second generation synchrotrons new insertion devices are located in the straight sections of the storage ring. These elements are called wiggler and undulators [1]. Both are magnetic dipoles arrangements with the goal of changing the properties of the emitted radiation compared to that emitted from the bending magnets. At the bending magnets, beamlines are placed. At the end of the beamlines, the work stations are installed (Fig. 1).

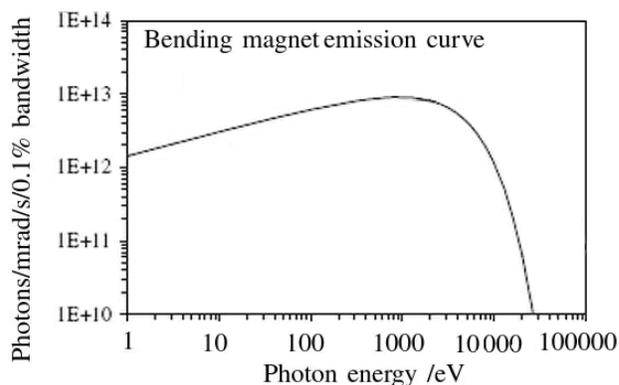


Figure 2. Emission spectrum of the electromagnetic radiation emerging from a bending magnet.

## CHARACTERISTICS OF THE EMITTED SPECTRUM

If we were able to “see” the electromagnetic radiation that emerges along the tangent of the storage ring (at the bending magnets) and along the beamlines, we would view it as a flash or a periodic series of flashes. This temporary structure, short pulses separated by intervals, is due to the packaging structure of the electrons (bunches) orbiting in the storage ring [1]. This time structure allows us to “tune” the emitted radiation at the desired time scale, in which the phenomena to study occur.

The time scales range from  $s$  to  $ps$ . As the light pulses are very short timed and inversely proportional to the cube of the electron energy (of the order of  $GeV$ ) [1], it results in a continuous distribution of the emitted electromagnetic energy, as shown in Figure 2.

Hence, the main feature of a synchrotron is its continuum spectrum extending from the infrared to the X-rays. As suggested before, its *high intensity* (brightness) is a central feature that distinguishes it from conventional X-rays sources. It is also a *highly collimated* beam of *polarized* electromagnetic radiation.

## BEAMLINES AND WORK STATIONS

The beamlines are located at the exit of the bending magnets (or wigglers and undulators). Along the beamlines, complex optical systems (including monochromator arrangements), guarantee the energy (or the energy range) required for performing specific experiments, which take place at the work stations (experimental hutch).

## WHAT KIND OF EXPERIMENTS CAN BE CONDUCTED AT A SYNCHROTRON?

In general, different kinds of diffraction and spectroscopy experiments can be performed at a synchrotron. These experiments can be used to characterize single crystals, polycrystalline, or amorphous materials with high space and time resolution, and with a signal/background ratio much better than in conventional equipments (for example, tabletop X-ray diffractometers). For example, using 3<sup>rd</sup> generation synchrotrons, X-ray diffraction experiments at a nanometer scale can be performed while scanning the sample surface with a resolution of about  $50\text{ nm}$ .

## HOW TO APPLY FOR BEAMTIME AT A SYNCHROTRON?

In general, there are two proposal application calls per year, in first and second semester. When you write a proposal the following items should be included: background, motivation for the proposal, experimental plan, explain why this work calls for access to a synchrotron facility and some references should be given in order to help the reviewer to understand what you want to do. The next step is the proposal evaluation: the access to a synchrotron facility is provided to scientists on the basis of the proposal scientific merit. To this purpose, the synchrotrons usually have independent, external proposal review panels to receive advice about the scientific value of the proposals and about the viability of the experiment to be executed at a specified beamline. Once your proposal has been accepted for execution, beamtime is located for a specific date. Financial support from specific programs or agreements between institutions are possible in order to cover partial or total reimbursement of travel and living expenses during your stay at a synchrotron.

## USING SYNCHROTRON LIGHT SOURCES BY CUBAN SCIENTISTS

For more than 10 years, several groups of Cuban scientists have been making use of this type of facilities. Figure 3 shows an estimation of the number of projects that have been executed by different Cuban groups at the LNLS.

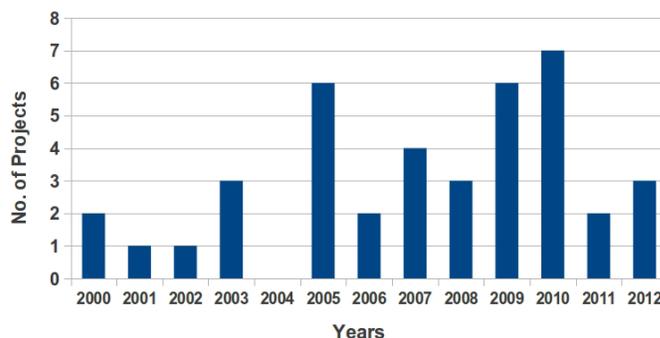


Figure 3. Number of synchrotron projects per year executed by Cuban scientists during the last decade at the LNLS (Brazil).

The projects reported in Fig. 3 have been linked to specific research topics, which in turn has resulted in an important number of publications. Fig. 4 shows the number of publications per year related to measurements made at synchrotrons by Cuban scientists. There is also an important number of Master and PhD degrees which have been supported by synchrotron experimental data.

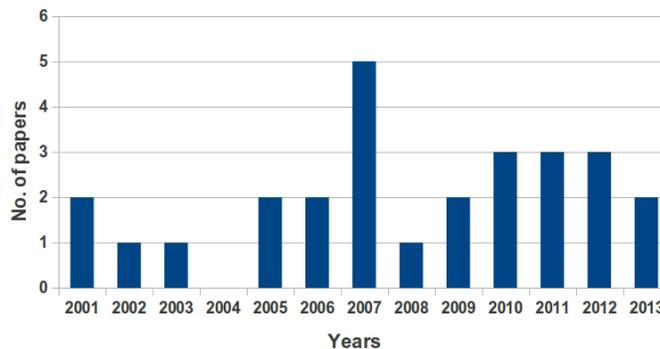


Figure 4. Number of scientific papers per year authored by Cuban scientists based on data taken at synchrotron sources (mostly from the LNLS (Brazil)) during the last years.

## EXAMPLES OF CUBAN RESEARCH PROJECTS INVOLVING SYNCHROTRON SOURCES

1. *Study of order-disorder phase transitions.* The study of “real structures” by X-ray diffraction has become an important research field in X-ray diffraction. Real crystals do not exhibit perfect periodic ordering of the atoms but instead several kinds of defects can be found which lead to a departure from the ideal case. During the last years, it has been one of the main research topics by Cuban synchrotron users. This topic has two essential venues: on one hand a theoretical model for the analysis of crystal defects, in particular stacking faults, have been elaborated; on the other, experimental diffraction data in order to test the proposed model have been collected using synchrotron facilities.

Order-disorder phase transitions in crystalline layer structures are a good example of loss of perfect periodic ordering due to the occurrence of stacking faults. Understanding these structural transformations sheds light on the relationship between structure and properties.

A direct approach to find a solution to the quantitative characterization of layer ordering from the diffraction pattern of a layered structure, has been developed and tested [2-4]. The model known as *direct solution* of the diffraction pattern from a crystal structure with planar faulting, has been used to quantify layer disorder in  $\text{RE}_2\text{Co}_{17}$  (RE rare earth) alloys as function of RE [5,6]. In the framework of the proposed model, the probability correlation function  $P_0(\Delta)$  [2-4] of the stacking arrangement in layer structures (like in  $\text{RE}_2\text{Co}_{17}$ ) has been directly determined from high resolution powder X-ray diffraction patterns measured at the LNLS (Fig.5). The probability correlation function is essential in describing the disorder – order transition in layer structures.

The use of the term “direct solution” must be understood in the sense that quantitative information on layer ordering is derived directly from the kinematical equations of diffraction, avoiding the need of any prior assumption about the kind of stacking disorder occurring in the crystal, and making no use of particular models of faulting with associated faulting probabilities. In this sense the formalism is of general use for any layer structure as long as it is considered that the planar faulting does not alter the interlayer distance.

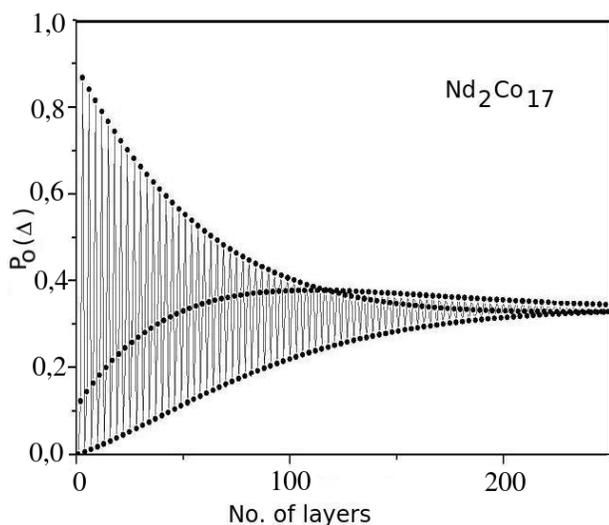


Figure 5. Probability correlation function of  $\text{Nd}_2\text{Co}_{17}$  calculated directly from the experimental diffraction pattern. Data taken at LNLS.

**2. Location of extra-framework cations in natural modified clinoptilolite.** Natural zeolites, in particular clinoptilolite, have emerged as promising materials for several applications such as environmental ones. That work has focused on the detailed structural characterization of the natural and modified clinoptilolite by ion exchange processes and dealumination. The influence of these modifications on the carbon dioxide adsorption properties of the clinoptilolite has been investigated.

The increase of the effectiveness of zeolites in adsorption applications can be improved on the basis of the detailed knowledge of the structural features associated to the extra-framework cations. The combination of computational assisted structural approaches and synchrotron characterization techniques, like high resolution X-ray diffraction experiments and X-ray absorption spectroscopy experiments (XANES and EXAFS executed at Elettra synchrotron, Italy) allows us overcoming difficulties arising from the limitations of obtaining zeolite single crystals and due to the strong peak overlap in zeolites powder diffraction patterns.

As result of this investigation it is possible to determine the positions of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  cations within the modified clinoptilolite channels with potential application in catalysis and gas adsorption [7]. The geometrical features of the clinoptilolite framework and the small ionic radii of these cations rule out any direct coordination of the metals to the framework, and thus only octahedral coordination to water molecules was observed. The metals are located in two different extraframework sites, in the center of channels A and in the window of the B channel. Figure. 6 shows the case of  $\text{Ni}^{2+}$  within the channels of the modified clinoptilolite [7].

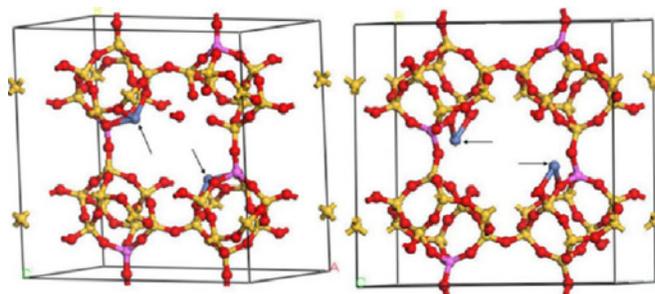


Figure 6. Sections of the structure of the natural Cuban zeolite clinoptilolite. Extra-framework  $\text{Ni}^{2+}$  positions marked with arrows. Their precise localization (established with the help of synchrotron radiation experiments) allows explaining catalytic and gas adsorption properties of clinoptilolite.

### 3. Materials for Hydrogen Storage: Cation Mobility in Zeolite-Like Zinc Hexacyanometallates.

The hydrogen molecule can be adsorbed through electrostatic interactions, van der Waals type forces and by formation of a coordination bond to transition metal sites. The electrostatic interactions are related to the permanent quadrupole moment of the hydrogen molecule and to the polarization of its electron cloud by the presence of a charge center. From this fact, the hydrogen storage in porous solids can be enhanced by the presence of extra-framework ions within cavities. Such possibility has been considered for  $\text{H}_2$  storage in zeolites. However, in zeolites the ion polarizing power is partially shielded by the framework oxygen atoms electron cloud, behaviour not expected for zeolite-like zinc hexacyanometallates [8]. This last family of coordination polymers has been considered for  $\text{H}_2$  storage.

HR-XRD patterns using synchrotron radiation (LNLS) were recorded for  $\text{Zn}_3\text{K}_2[\text{Fe}(\text{CN})_6]_x \cdot x\text{H}_2\text{O}$  for different amount (x) of water molecules accompanying the metal K within the

cavity. The occurrence of a structural change on the crystal water removal is evident. The patterns for the hydrated and anhydrous samples are quite different [8]. The anhydrous phase crystallizes with a monoclinic unit cell (P2/c space group) (Figure 7). This structure contains six positions for the metal, within different occupation factor and with K-K average distances of 5.75, 6.01 and 6.13 Å, which are different from the distance in the hydrated phase (5.23 Å). Figure 7 (Right) shows the cavity corresponding to the monoclinic phase. It is slightly deformed and with a significant reduction for the unit cell volume per formula unit, from 732 to 672 Å<sup>3</sup>. On cooling the original structure is restored, even for the anhydrous samples.

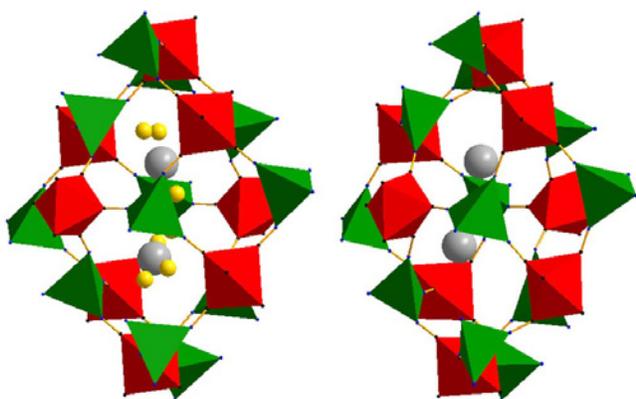


Figure 7. Porous framework for  $Zn_3K_2[Fe(CN)_6]_2 \cdot xH_2O$  as hydrated (left) and anhydrous under vacuum (right) samples determined using synchrotron radiation experiments. Indicated are the potassium atom positions including the water of coordination for the hydrated phase.

4. Study of the protease-inhibitor interactions: a contribution to structural knowledge of proteins from marine invertebrates. X-ray crystallography is currently the most favored technique for structure determination of biological macromolecules, such as proteins. Among them, serine proteases and their natural protein inhibitors are one of the most intensively studied, mainly due to their importance in several physiological and pathological processes [9]. High-resolution crystal structures, accessible through synchrotron-based diffraction experiments, are indispensable for elucidating the structural principles of such protein-protein interaction.

The related research performed by Cuban scientists has focused on the structural characterization of the interaction between ShPI-1, a protease inhibitor from the sea anemone *Stichodactyla helianthus* and the serine proteases trypsin [10] and chymotrypsin, as well as between a mutant of ShPI-1 with leucine at the reactive site (P1 site) and pancreatic elastase (PDB codes: 3M7Q, 3T62 and 3UOU, respectively). As assessed by the crystal structures, the overall folds of the three complexes are highly similar to homologous complexes with mammalian inhibitors [11]. The inhibitor P3-P3' sites (amino acids Arg11- Phe16) adopt the canonical conformation and most of the atomic interactions previously observed in homologous inhibitors are conserved. Thus, these structures represent an interesting case of how a particular inhibitory function is conserved throughout evolution.

Analyzing the molecular contacts by comparison with trypsin complexes of mammalian homologous (BPTI-Kunitz type) inhibitors indicated a prominent role not only for Lys13 at a reactive site, but also for Arg11 at P3 site of ShPI-1 in the stabilization of this complex that was so far not described. The Arg11 side chain, in contrast to equivalent amino acid (Pro13) of bovine pancreatic trypsin inhibitor (BPTI) points directly into a enzyme pocket and forms new H-bonds and several other contacts (Fig. 8a). These interactions have been proposed to partially compensate the absence of important contacts at other sites within ShPI-1 binding loops [10].

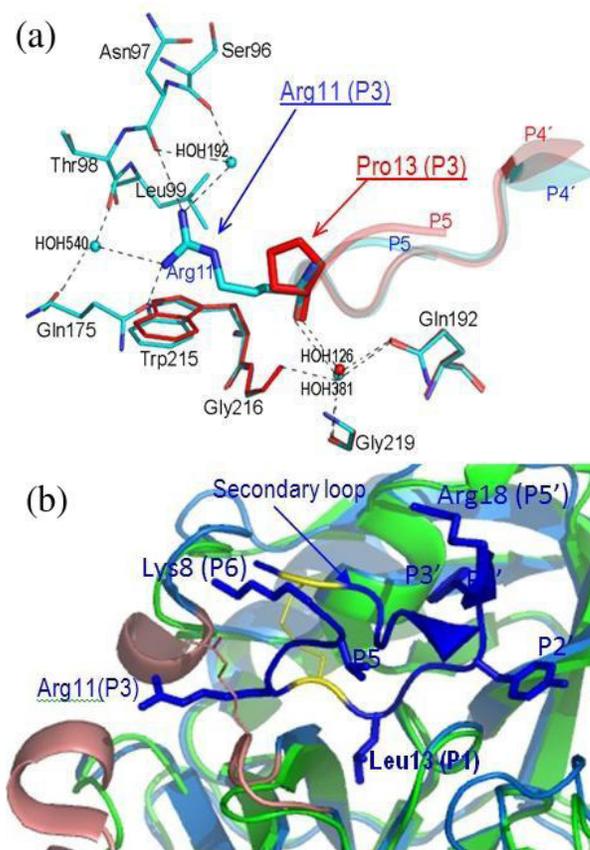


Figure 8. (a) Close view of the interactions between trypsin and amino acids at position P3 of the ShPI-1 (blue) and BPTI (red). The side chain of Arg11 in ShPI-1 is involved in several direct and water-mediated contacts that are not established by Pro13 of BPTI. (b) Interface between pancreatic elastase (green) and primary (amino acids Lys8-Arg18) and secondary binding loops of rShPI-1/K13L (blue), which are linked by a disulphide bridge (yellow). As observed, the enzyme loops near Arg11 at P3 site of the inhibitor are different from those of neutrophil elastase (light red) in complex with another inhibitor (elafin, PDB: 1FLE). This feature suggests that P3 site could contribute to differences in selectivity towards these enzymes.

The details of the interaction between the mutant ShPI-1/K13L and pancreatic elastase (PPE) were also obtained. The crystal structure revealed that besides P1 site, amino acid at P3 site could be important in increasing the selectivity towards elastase-like enzymes, such as human neutrophil elastase (HNE), which has strong implications in chronic inflammatory lung diseases [12]. A remarkable difference was found around the PPE pocket that interacts with Arg11 at P3 site of

ShPI-1/K13L (Fig 8b), compared to a similar pocket of HNE in reported complexes with other inhibitors following a similar mechanism. This information supports previous functional reports [13] and could contribute to further mutagenesis experiments of ShPI-1 in order to obtain a more selective molecule.

*5. Structure of the Fab fragment of the anti-murine EGFR antibody 7A7 and exploration of its receptor binding site.* The Epidermic Grow Factor (EGF) receptor is an important target of cancer immunotherapies. The 7A7 monoclonal antibody has been raised against the murine EGFR, but it cross-reacts with the human receptor. As result, experiments using immune-competent mice can be extrapolated to the corresponding scenario in humans. The crystal structure of the 7A7 Fab at an effective resolution of 1.4 Å has been resolved using synchrotron radiation [14]. The antibody binding site comprises a deep pocket, located at the interface between the light and heavy chains, with major contributions from CDR loops H1, H2, H3 and L1. As shown in binding experiments, the 7A7 recognizes a site on the EGFR extracellular domain that is not accessible in its most stable conformations, but that becomes exposed upon treatment with a tyrosine kinase inhibitor. It exhibits a recognition mechanism similar to that proposed for mAb 806 [14].

## CONCLUSIONS

After ten years we believe that the number of Cuban researchers that make use of synchrotron facilities is still limited to a very small number, namely, those coming from the University of Havana and the Center for Molecular Immunology. From this experience, it is necessary to focus our efforts in increasing the number of scientists in our country that potentially could make use of these tools in their research. People working in our country in other research areas such as alternative energy, archeology, paleontology, petroleum, construction materials, etc., may be potential users of synchrotron light facilities with the goal of solving specific problems.

## ACKNOWLEDGMENTS

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