To understand proteins

Network brought together 20 groups that defined the structure and function of 200 molecules that are essential to organisms

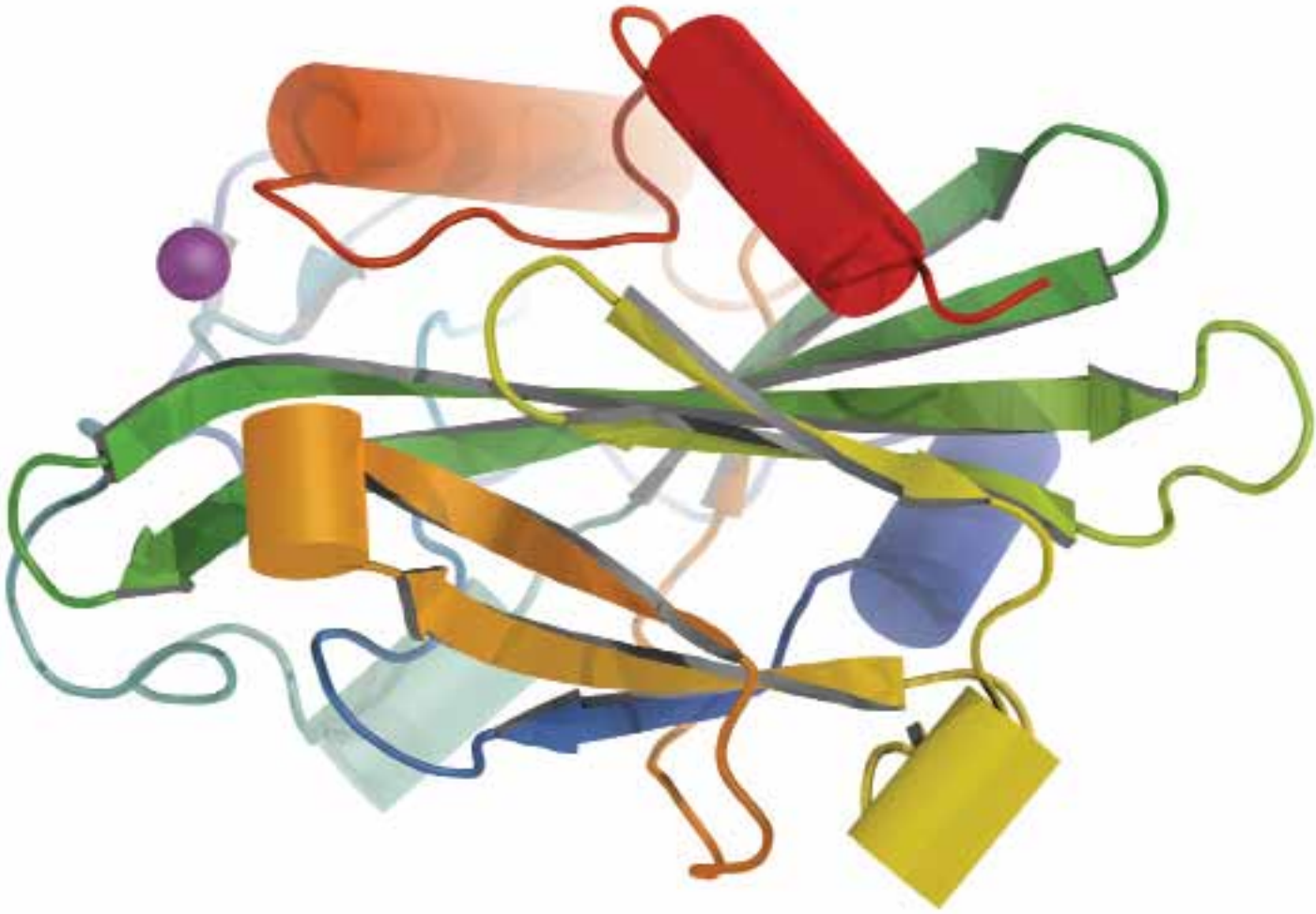
In 1989, *Moniliopthora perniciosa*, a fungus from the Amazon basin, appeared in the state of Bahia and infected cocoa plantations in the Ilhéus and Itabuna region. During the following decade, annual production fell from 320,000 tons to around 100,000 tons, knocking down Brazil's share of the international market from 15% to 4%. The effect of the disease is devastating: the fungus invades the cells of the cocoa plant, secretes proteins that interact with other proteins in the plant, and the branch hypertrophies and dries out. This physiological process exhausts the plant, and in the case of Bahia, also exhausts the livelihoods of more than two million people. From a scientific standpoint, the solution to stop the progress of this disease is to understand the interaction between the fungus and the plant.

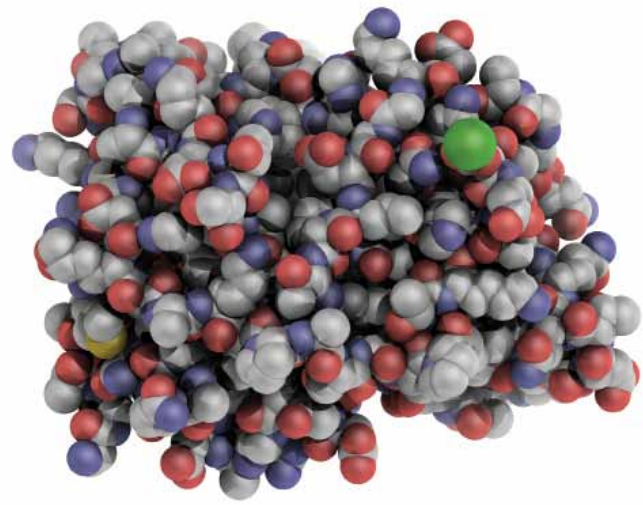
In 2000, a consortium led by the University of Campinas (Unicamp), with support from the Bahia state government and the National Council on Scientific and Technological Development (CNPq), mapped the genome of *Moniliopthora perniciosa*. During the following years, a group of researchers from Unicamp, including Gonçalo Amarante Guimarães Pereira, and Jorge Mondego, from Campinas Institute of Agronomy (IAC), identified 27 fungus proteins with the potential to reduce or halt the effects of this disease on the plant.

These 27 target proteins are currently the focus of the project *Structural studies of key proteins for fungal cocoa diseases: witch's broom and moniliasis: developing strategies to control and understand the pathogenicity models* , approved under the scope of the Structural Biology Network on Advanced Topics in Life Sciences, SMOLBnet 2.0, launched by FAPESP in 2010.

Strictly speaking, this research network began to be formed in 2001, when FAPESP supported

ANDRE AMBROSIO/LNBIO





Representations of the three-dimensional structure of the protein MpNEP2 (*necrosis-and ethylene-inducing peptide 2*), extracted from the fungus that causes witch's broom, obtained using a special beam line at the LNLS. In the larger image, the shades of colors indicate the sequence of envelopment of the protein structure, from the beginning, in blue, to the end, in red. In the smaller imate, the colored spheres correspond to carbon, nitrogen and oxygen atoms, which make up the molecule

the creation of the Structural Molecular Biology Network (SMOLBnet), which was then composed of twenty research groups, with the mission to increase knowledge about the genes mapped in the Human Cancer and Sugarcane Genome Projects and in the genomes of the bacteria *Xylella fastidiosa* and *Xanthomonas citri*, and others. The mission involved studying the three-dimensional structure and function of 200 proteins, in order to compare their manifestations in normal cells and in diseased cells, and to look for clues to find new diagnoses and treatments.

The SMOLBnet network brought together teams of laboratory researchers that had already cloned the gene up to its expression in proteins. After the program was implemented, they were also able to discover its three-dimensional structure. "The structure of a protein is directly linked to its function. In fact, it is the structure that defines the function, and this is a crucial piece of information to understanding how it acts in living organisms," explains Rogério Meneghini, who coordinated the SMOLBnet network from its inception until 2004, when he took over scientific coordination of the SciELO Program, which is also supported by FAPESP.

These groups have a powerful tool available to them: the Brazilian Synchrotron Light Laboratory (LNLS), in Campinas, the only such lab in Latin America, which is open to the scientific community. At that time, in addition to an X-ray diffraction synchrotron beam line dedicated to macromolecule crystallography, it had a laboratory specialized in this type of investigation, the Center for Structural Molecular Biology (Cebime), inaugurated in 1999 and run by Mengenhini at that time. This Center was equipped with nuclear magnetic resonance apparatus and mass spectrometers for protein crystallization.

The network enabled specialists to be trained in structural biology, a strategic area in world science

Cebime trained biologists, biochemists, chemists and physicians from the twenty laboratories in São Paulo in the techniques used. "Our objective was to teach the investigation process into the three-dimensional shapes of the proteins to the teams of researchers, who frequently needed to know the structure of these molecules," Meneghini told reporter Ricardo Zorzetto, in a story published in issue 113 of the magazine *Pesquisa FAPESP* from July 2005.

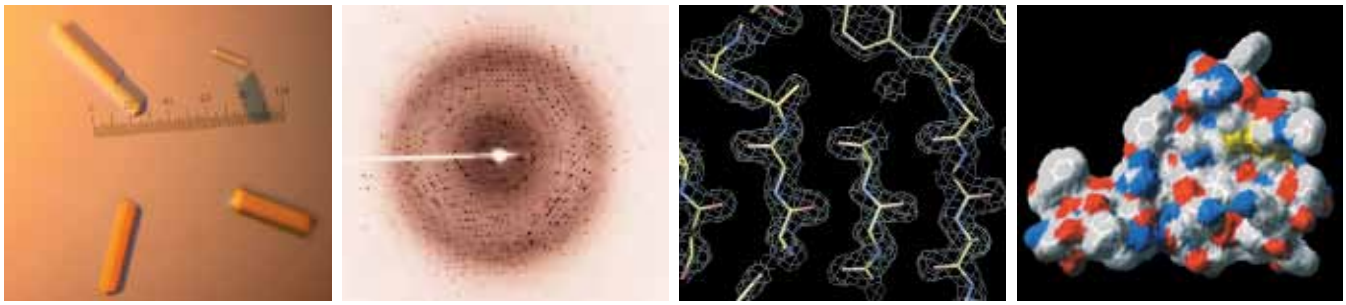
According to Marie-Anne Van Sluys, from the Biosciences Institute of USP and adjunct coordinator of the life sciences area at FAPESP, creation of this network led to the formation of "critical mass" in structural biology, an area of research that has gained recognition throughout the world, principally after the results of the Genome projects. "SMOLBnet has become a network of contacts that little by little began to take on a life of its own," she says.

Shaker Chuck Farah, a biochemist from the Chemistry Institute of USP, studied the proteins involved in secretion and signaling systems between bacteria. "I incorporated structural biology

Proposals selected for SMOLBnet 2.0

PROJECT	COORDINATION	INVESTMENT
Structural studies of key proteins for fungal diseases in cocoa: witch's broom and moniliasis: developing strategies to control and understand the pathogenicity models No. 2010/51884-8) (2011-2012)	André Luis Berteli Ambrósio, ABTLuS	R$382,051.42
Determination of three-dimensional structure of protease inhibitors and anti-hemostatic molecules identified in disease vector hemotophage animals – No. 2010/51868-2 (2011-2013)	Aparecida Sadae Tanaka, Unifesp	R$155,542.56
Structural studies on proteins related to trypanosomatids infection and to complexes of these proteins with molecules, which are involved in the infection process – No. 2010/51867-6 (2011-2012)	Eduardo Horjales Reboredo, IFSC/USP	R$281,738.33
Combining genetics and NMR to dissect fundamental protein-protein interactions for the bacterial division complex – No. 2010/51866-0 (2011-2012)	Frederico José Gueiros Filho, IQ/USP	R$280,305.09
Functional and structural studies of protein kinases involved in cancer and neglected diseases: towards the development of new inhibitors – No. 2010/51730-0 (2011-2012)	Jorg Kobarg, ABTLuS	R$642,928.67
Structural characterization of alpha-importin protein and of importin proteic complexes – transcription factors of the fungus Neurospora crassa – No. 2010/51889-0 (2011-2013)	Marcos Roberto de Mattos Fontes, IB/Unesp Botucatu	R$307,249.38
Structural studies of transcription factors involved in the regulation of hydrolytic enzyme genes and swollenin from Aspergillus niger and Aspergillus fumigatus – No. 2010/51890-8 (2011-2012)	Mario Tiago Murakami, ABTLuS	R$341,802.40
Structural study of protein components of the exosome and some of its regulatory factors from Archaea and yeast – No. 2010/51842-3 (2011-2014)	Carla Columbano De Oliveira, IQ/USP	R$693,392.06
Determination by crystallography and nuclear magnetic resonance of the structure of proteins in the NEP families (Necrosis and Ethylene inducing Peptides) and thaumatin, as well as alternative oxidase – No. 2010/51891-4 (2011-2012)	André Luis Berteli Ambrósio, ABTLuS	R$148,691.20

Four scenes from a long journey



Stages of identification of the spatial structure of an anti-coagulant, the inhibitor of factor XII: from left to right, crystals seen under a magnifying glass (scale, 100 =1 mm) and by X-ray diffraction before atom by atom reconstitution of the molecule

IMAGES SERGIO SCHENKMAN, APARECIDA TANAKA E IVAN CAMPOS/UNIFESP

techniques into my line of research, I received orientation from experienced researchers and I trained people," he says.

Sérgio Schenkman, together with his team from the Federal University of São Paulo (Unifesp), decided to investigate the spatial shape of the proteins he had been working with for years. He detailed the structure of two proteins of the insect that transmits the protozoan *Trypanosoma cruzi*, which causes Chagas disease. The proteins act in different phases of blood coagulation: one inhibits the action of thrombin and the other prevents the action of factor XII. Both have potential uses in treating the problems caused by increased blood coagulation, such as thrombosis (*see illustration above*).

In October 2004, an international evaluation of results from SMOLBnet, conducted by an independent commission composed of specialists from the Pasteur Institute, in France, the University of Oxford, in England, and Brookhaven National Laboratory, in the United States, showed that the effort paid off. "The success rate, ranging from obtaining clones to determining the structures is comparable to that of international projects," the specialists said. They recommended installation of another synchrotron beam line dedicated to crystallography of macromolecules for experiments that require the use of SAD/MAD (Single and Multiple-wavelength Anomalous Diffraction), open to users in 2006.

As a result of this network, specialists were trained, which enabled the SMOLBnet 2.0 network to be created in 2010. In this second call, the objective was to establish partnerships between research groups in structural biology and in molecular biology research. Twenty-three proposals were approved, and this time there is no general coordination. "The groups are independent and research can be linked to the other programs of the institution," explains Van Sluys.

Research on the fungus *Moniliopthora perniciosa,* which attacks cocoa trees, is proof of this. The project is coordinated by André Ambrósio, of the Brazilian Biosciences National Laboratory (LNBio). Together with Sandra Dias and Ana Zeri, also from LNBio, Ambrósio is responsible for investigations focused on structural molecular biology, through a combination of crystallography, nuclear magnetic resonance, biochemistry and biophysics technique to understand the pathogenicity of this disease. The project began with the study of the 27 target proteins identified by Gonçalo Guimarães Pereira and Jorge Mondego and has already resulted in the publication of an article in the journal *Biochemistry* in 2011. The structures of several of the other proteins originally proposed have been discovered and their function is now being studied. Other publications are under preparation.

In fact, the LNBio originated from Cebime. It was created as a national laboratory in 2009, and today is a part of the Brazilian Center for Research in Energy and Materials (CNPEM). It operates the two LNLS macromolecular crystallography beam lines, as well as equipment that supports research in structural molecular biology.

Having determined the structures of the proteins and how they act, the next step in the project is the rational design of molecules that make it possible to inhibit the action of these proteins. This will be done with support from Rafael Guido, from the São Carlos Physics Institute, and Ronaldo Pilli, from Unicamp, respectively specialists in the identification and synthesis of small molecules, explains Ambrósio. "We are also trying to study, from a structural standpoint, a protein associated with fungal mitochondrial membranes. This is a challenging process, and as far as we know, it is the only study of its kind that has ever been conducted in Brazil," says Ambrósio. ■

PROJECTS

Structural Molecular Biology Network (SMOLBnet)

COORDINATOR
Rogerio Meneghini – LNLS

INVESTMENT
R$13,036,329.12

SCIENTIFIC ARTICLE

Zaparoli, G. *et al.* The Crystal Structure of Necrosis- and Ethylene-Inducing Protein 2 from the Causal Agent of Cacao's Witches Broom Disease Reveals Key Elements for Its Activity. **Biochemistry**. v. 50, p. 9901-10, 2011

FROM OUR ARCHIVES

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Issue No. 113 – July 2005