latin american summit meeting

IYCr2014

biological crystallography and
complementary methods

book of abstracts

pages.cnpem.br/iycr2014-lasummit

Funding Agencies

CNPq

CAPES

FAPESP

Sponsors

Molecular Dimensions

BRUKER

nano TEMPER technologies

Agilent Technologies

GE Healthcare

Rigaku

DAIRIX

FORMULATRIX

astex pharmaceuticals

ttplabtech natural innovators

INCOATEC innovative coating technologies

Institutional Partnerships

UNESCO

2014 international year of crystallography IUCr

IUCr-UNESCO Campinas, 21-24 Sept 2014 summit meetings

IFSC

ABCr

LNLS sirius

LNBio

CNPEM

The International Year of Crystallography (IYCr2014) and the Latin American Summit Meeting on Biological Crystallography and Complementary Methods

The year of 2014 has been designated the International Year of Crystallography (IYCr2014) under the auspices of the United Nations Educational, Scientific and Cultural Organization (UNESCO) and the International Union of Crystallography (IUCr). It celebrates not only the centennial of X-ray diffraction, which allowed the detailed study of crystalline materials, but also the 400th anniversary of Kepler's observation in 1611 of the symmetrical form of ice crystals, which began the wider study of the role of symmetry in matter.

The major objectives of the IYCr2014 are:

- To increase public awareness of the science of crystallography and how it underpins most technological developments in our modern society;
- To inspire young people through public exhibitions, conferences and hands-on demonstrations in schools;
- To illustrate the universality of science;
- To intensify the programme Crystallography in Africa and create similar programmes in Asia and Latin America;
- To foster international collaboration between scientists worldwide, especially North–South cooperation;
- To promote education and research in crystallography and its links to other sciences;
- To involve the large synchrotron and neutron radiation facilities worldwide in the celebrations of IYCr2014, including the SESAME project set up under UNESCO auspices.

As part of IYCr2014, three summit meetings are being organized across the world, in Pakistan, South Africa and Brazil, which will bring together scientists and other interested parties from the region. The principal objective of the summit meetings is to promote a forum for the exchange of ideas and experiences between scientists in the region and to consolidate the use of crystallography and related techniques for regional scientific development.

Structural Biology has been chosen as the theme for the Latin American summit, in Campinas, Brazil. The event to be held in Campinas aims to gather distinguished researchers in the field from Latin-American countries and around the world to evaluate the progress achieved in recent years on crystallography applied to biological phenomena. It also aims to pursue greater integration among researchers in the field and with those in complementary fields, such as Nuclear Magnetic Resonance and Electron Microscopy, as well as to seek solutions to bottlenecks that hinder the achievement of competitive scientific research in developing countries.

The "Latin American Summit Meeting on Biological Crystallography and Complementary Methods" is organized in association with the CNPEM (Brazilian Center for Research in Energy and Materials), the Brazilian Synchrotron Light Laboratory (LNLS), the Brazilian National Biosciences Laboratory (LNBio), the Physics Institute of São Carlos (IFSC - USP), the Brazilian Crystallographic Association (ABCr) and The United Nations Educational, Scientific and Cultural Organization (UNESCO).

Crystallography in Latin American Countries and the Latin American Crystallographic Association, LACA

On August 08, 2014, during the 23rd Congress and General Assembly of the International Union of Crystallography (IUCr) in Montreal, Canada, the newly created Latin American Crystallographic Association (LACA) was accepted as a Regional Associate of the IUCr. This fact consolidated the old aim of gathering crystallographers from Latin American countries from all groups and associations in the region. The North, Central and South American countries, historically bound by the Luso/Hispanic heritage and language, have constantly made efforts to work together promoting the advance of science and education.

Renowned crystallographers from Spain and Portugal originally visited the Latin American countries and shared with us their knowledge and made efforts to create the Ibero-American Crystallographic Group. During the second half of the 1900s, very famous scientists from France, Switzerland, England and other Eu-

- In 1959 an Advanced Course on Pure and Applied Crystallography was organized and financed by UNESCO in Santiago de Chile.

Santiago de Chile - September 21st, 1959.

The Spanish crystallographers Julio Palacios and Julio Garrido, were among the organizers.

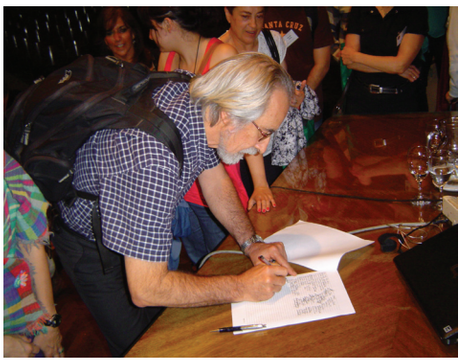
ropean countries came to South America and were mentors of our pioneering crystallographers (students and researchers) using X-ray diffraction techniques in chemistry, physics, biology and related areas.

Thanks to those enthusiastic efforts, the science of crystallography was successfully developed in our countries, in spite of reduced investment in some of them. The mere existence of this very dedicated and productive crystallographic community in the Latin American countries contributed significantly to inspire and support the construction of a synchrotron facility in Campinas, Brazil, in operation since 1997. On that occasion, the first macromolecular crystallography X-ray diffraction beamline in the Southern hemisphere, D03B-MX1, was made available to the scientific community. Ten years later, in 2007, LNLS inaugurated a newer, tunable wiggler beamline, W01B-MX2, dedicated to the same purposes. Both are still in operation and Latin American crystallographers represent the majority of its users.

The region involved in the LACA initiative comprehends important scientific communities and well-established crystallographic societies, and consolidated crystallographic groups. Starting with México (SBCr), Venezuela (consolidated groups), Brazil (ABCr), Chile (consolidated groups), Argentina (AACr), Colombia (consolidated groups), Peru (consolidated groups) and Uruguay (consolidated groups). Most of the existing groups have started their own national societies. Costa Rica (UCRC) recently established collaborative projects in order to create a united group of countries. All of them subscribed the Foundation Act of the LACA and the rest are being invited to join the LACA as single country members or as a United Group of Latin American Countries.

Through its association with the IUCr as a Regional Associate the LACA expects to increase the international interaction among the countries of the LA region with the rest of the world. Many of our LA scientists have easy transit between European, Asiatic or North American countries. Many have worked abroad for many years and never came back to their countries of origin. Many only come for a visit. But many come and go frequently when opportunities arise, to teach courses, participate in symposiums, or do temporary work in Latin American universities or facilities. As a Regional Associate, LACA will configure the consolidation of the science of crystallography in the LA countries and acquire an international institutional level that will greatly help the development of new laboratories, student exchanges, and financial support from governmental institutions in all countries of the region. The future is in the hands of the young researchers of the LA region.

This Summit Meeting is considered part of such an important initiative, symbolically happening during 2014, the International Year of Crystallography, carrying a lot of hope for its successful development.



CNPEM: A cutting-edge research center for Latin America

Welcome to the **Brazilian Center for Research in Energy and Materials (CNPEM)**, host of the 2014 Latin American Summit Meeting on Biological Crystallography and Complementary Methods. CNPEM is a world-class center for the development of science, composed of four laboratories: The **Brazilian Synchrotron Light Laboratory (LNLS)**, The **Brazilian National Biosciences Laboratory (LNBio)**, The **Brazilian Bioethanol Science and Technology Laboratory (CTBE)** and The **Brazilian Nanotechnology National Laboratory (LNNano).**

Located in Campinas (SP), CNPEM is a private nonprofit organization qualified by the Ministry of Science, Technology and Innovation (MCTI), whose laboratories have open facilities to the scientific and industrial communities across the country and abroad.

The choice of CNPEM to host the "Latin American Summit Meeting on Biological Crystallography and Complementary Methods" took into account the fact that this Center houses the only synchrotron light source in Latin America, which offers unique conditions for the progress of crystallographic techniques in this region.

The beginning of operation of the Brazilian Synchrotron Light Laboratory (LNLS), in 1997, was a milestone for the development of Latin American crystallographic activities. As part of CNPEM, the LNLS is responsible for operating the synchrotron light source and its 18 experimental stations (beamlines), where proposals for analysis of organic and inorganic materials can be conducted.

Two of the 18 available beamlines at LNLS are dedicated to X-ray diffraction of biological macromolecules. The operation of these LNLS beamlines is performed in partnership with the Brazilian National Biosciences Laboratory. The LNBio is dedicated to research in biotechnology and drug development and provides the necessary skills for crystallization of biological macromolecules.

Moreover, LNBio brings together expertise, state of art equipment and a team of world-class researchers focused on conducting studies in the areas of structural biology, proteomics, genomics, metabolomics, development of genetically modified organisms (GMO), bioinformatics and biological imaging, amongst others.

The Latin American scientific community will soon get a major reinforcement for researches that rely on synchrotron light. This is because LNLS is currently engaged in the development and construction of **Sirius**, a latest generation synchrotron light source that will open new experimental possibilities in several areas of research, including macromolecular crystallography.

Designed to be one of the most advanced light sources in the world, it will open up new perspectives for research in many fields, such as material science, structural biology, nanoscience, physics, earth and environmental science, cultural heritage and many others.

Sirius has the potential to elevate the Latin American countries to a higher and more competitive level in scientific research, creating possibilities for the region to become a major player in structural biology in the future.

Delegates

Ada Yonath (Israel)
Adela Rodríguez-Romero (Mexico)
Adriana Rojas (Spain)
Alejandro Buschiazzo (Uruguai)
Alejandro Vila (Argentina)
Alessandro Nascimento (Brazil)
Alexei Soares (USA)
Ana Carolina Zeri (Brazil)
Ana Paula Valente (Brazil)
Andre L. B. Ambrosio (Brazil)
Andrea Balan (Brazil)
Andrea Dessen (Brazil)
Antonio José Roque da Silva (Brazil)
Artur Cordeiro (Brazil)
Carlos Aguilar (Argentina)
Claude Lecomte (IUCr)
Cristiane Guzzo Carvalho (Brazil)
Daniel Guerra (Peru)
Eduardo Horjales (Brazil)
Enrique Rudino-Pinera (Mexico)
Felipe Trajtenberg (Uruguay)
Glaucius Oliva (Brazil)
Gonzalo Prat Gay (Argentina)
Harry Westfal (Brazil)
Iris Torriani (Brazil)
Jerson Lima da Silva (Brazil)
João Alexandre Barbosa (Brazil)
João Renato Muniz (Brazil)
Jorge Iulek (Brazil)
Jose Antonio Martinez Oyanedel (Chile)
Juan Fontecilla-Camps (France)
Kleber Franchini (Brazil)
Lidia Brito (UNESCO)
Luis Gabriel Brieba de Castro (Mexico)
Luis Mauricio Trambaioli (Brazil)
Marcelo Costabel (Argentina)
Marcio Bertacine Dias (Brazil)
Marcos Fontes (Brazil)
Marcos Navarro (Brazil)
Maria Cristina Nonato (Brazil)
Marta Cecilia Del Ca Bunster Balocchi (Chile)
Marvin Hackert (IUCr)
Mauricio Andres Baez Larach (Chile)
Miguel Angel Martínez Cabrera (Paraguay)
Otavio Thiemann (Brazil)
Pedro Alzari (France)
Plínio Delatorre (Brazil)
Rafael Guido (Brazil)
Rafael Radi (Uruguay)
Raul Alejandro Padrón Crema (Venezuela)
Ricardo Aparico (Brazil)
Ricardo Cabrera (Chile)
Richard Garratt (Brazil)
Roberto Salinas (Brazil)
Rodrigo Portugal (Brazil)
Ronaldo Nagem (Brazil)
Samar Hasnain (IUCr)
Sebastian Klinke (Argentina)
Sergio Pantano (Uruguay)
Shaker Chuck Farah (Brazil)

Early Career Researchers (ECRs)

Ariel Mechaly (Uruguay)
Carlos Trasviña-Arenas (Mexico)
Juliana Barbosa Gonçalves (Brazil)
Lisandro Otero (Argentina)
Lucia Chemes (Argentina)
Matias Fuentealba (Chile)
Matias Machado (Uruguay)

September, 22nd 2014

08:00 - 08:45 **Registration**
at the LNLS entrance hall

08:45 - 09:00 **Kleber Franchini**
Welcoming remarks

Introduction - Chair: Glaucius Oliva

09:00 - 09:20 **Claude Lecomte (IUCr)**
IYCr2014, open labs and the Africa Initiative

09:20 - 09:40 **Latin America UNESCO representative**
UNESCO and the International Year of Crystallography

Historical Perspective on Protein Crystallography in Latin America - Chair: Richard Garratt (BRA)

09:40 - 10:10 **Glaucius Oliva (IFSC/USP)**
From Laue to present Latin America

10:10 - 10:40 **Antonio José Roque (LNLS)**
The future in Latin America: Sirius Project

10:40 - 11:10 **Kleber Franchini (LNBio)**
Structural Biology and multidisciplinarity at the LNBio

11:10 - 11:30 **Coffe Break**

Biological Crystallography 1 - Chair: Adela Rodríguez-Romero (MEX)

11:30 - 12:00 **Alejandro Buschiazzo (URU)**
Novel insights into bacterial signaling:
the 'one sequence - multiple structures' perspective

12:00 - 12:12 **Sebastian Klinke (ARG)**
S-SAD phasing of monoclinic histidine kinase from Brucella abortus
at low resolution using true redundancy and multiple crystal data collection

12:30 - 13:00 **Chuck Farah (BRA)**
The Physiological Function of the Xanthomonas Type IV Secretion System

13:00 - 14:30 **Lunch at the campus**

Biological Crystallography 2 - Chair: Marcos Fontes (BRA)

14:30 - 15:00	**Luis Brieba (MEX)** Crystallographic and protein engineering studies of Triosephosphate isomerase
15:00 - 15:30	**Marta Bunster (CHI)** How different techniques such as Spectroscopy, proteomics, and in silico approaches, are associated to X-ray crystallography to obtain structural information in a complex system, the Phycobilisome
15:30 - 16:00	**Rafael Radi (URU)** Oxidative Postranslational Modifications in Proteins: Structural Biology Studies on Tyrosine Nitration
16:00 - 16:30	**Coffee Break**

Chair: Glaucius Oliva (BRA)

16:30 - 17:00	**Marvin Hackert (IUCr President)** Tautomerase Superfamily – good things come in small packages
17:00 - 18:00	**Keynote Lecture: Ada Yonath** Can structures lead to advanced therapeutics?
18:00 - 20:30	**Poster Session 1** Presentation of odd numbered posters + Social Gathering

September, 23rd 2014

A View From Abroad: Latin Americans Working Overseas - Chair: Samar Hasnain (IUCr)

09:00 - 09:30	**Pedro Alzari (FRA)** Some structural insights into mycobacterial signalling mechanisms
09:30 - 10:00	**Alexei Soares (USA)** X-rays and sound pulses: acoustic methods in crystallography and drug discovery
10:00 - 10:30	**Adriana Rojas (ESP)** Structural and biophysical studies of human Methionine Adenosyltransferases
10:30 - 11:00	**Juan Fontecilla-Camps (FRA)** Structure-function relationships of anaerobic metalloenzymes
11:00 - 11:30	**Coffee Break**
11:30 - 13:00	**Round-table mediated by Samar Hasnain (IUCr)** Participants: Pedro Alzari, Alexei Soares, Adriana Rojas and Juan Fontecilla-Camps
13:00 - 14:00	**Lunch at the campus**

Biological Crystallography 3 - Chair: Sergio Pantano (URU)

14:00 - 14:30 **Maria Cristina Nonato (BRA)**
Structural and functional characterization of human tandem-repeat galectins

14:30 - 15:00 **Miguel Angel Martínez Cabrera (PAR)**
Progress in the field of Chemistry of Natural Products in the Faculty of Natural Sciences at Nacional University of Asunción

15:00 - 15:30 **Ricardo Cabrera (CHI)**
Different ways to bind cofactors: allosteric ATP inhibition and NAD(P) binding selectivity

15:30 - 16:00 **Ronaldo Nagem (BRA)**
Structural characterization of the enzymes in the naphthalene degradation pathway: filling the gaps

16:00 - 16:30 **Coffee Break**

Complementary Techniques 1 - Chair: Roberto Salinas (BRA)

16:30 - 17:00 **Jerson Lima da Silva (BRA)**
Unraveling Problems in Protein Misfolding Diseases using Structural Biology and Bioimaging

17:00 - 17:30 **Alejandro Vila (ARG)**
Protein dynamics as an essential trait in evolution: interplay of NMR and crystallographic studies

17:30 - 18:00 **Daniel Guerra (PER)**
Structure and dynamics of protein-DNA interactions in transcription initiation complexes of Escherichia coli

18:00 - 18:30 **Raul Padrón (VEN)**
The molecular basis of the relaxation and activation of tarantula striated muscle thick filament is revealed by the disclosure of the myosin interacting-heads motif structure

18:30 - 21:00 **Poster Session 2**
Presentation of even numbered posters + Social Gathering

September, 24th 2014

Biological Crystallography 4 - Chair: Jose Antonio Oyanedel (CHI)

09:00 - 09:30	**Marcos Navarro (BRA)** Insights into c-di-GMP-mediated transcriptional mechanisms involved in biofilm formation of Pseudomonas aeruginosa
09:30 - 10:00	**Enrique Rudino-Pinera (MEX)** Using X-rays to describe electronic fluxes in REDOX enzymes
10:00 - 10:30	**Andrea Dessen (BRA)** Bacterial mimicry of eukaryotic innate immunity
10:30 - 11:00	**Gonzalo Prat Gay (ARG)** Intrinsic disorder and unstructural biology
11:00 - 11:30	**Coffee Break**

Complementary Techniques 2 - Chair: Marcelo Costabel (ARG)

11:30 - 12:00	**Ana Carolina Zeri (BRA)** NMR in structural biology: Beyond the structures
12:00 - 12:30	**Rodrigo Portugal (BRA)** A Cryo-Electron Microscopy Facility for Single Particle Analysis at the National Center for Energy and Materials Research
12:30 - 12:45	**Alejandro Buschiazzo (URU)** Centro de Biología Estuctural del Mercosur (CeBEM)
12:45 - 13:00	**Iris Torriani** Latin American Crystallography Association (LACA)
13:00 - 13:10	**Samar Hasnain** Closing Remarks
13:10 - 14:30	**Lunch at the campus**

Attention:

Participants whose abstracts are preceded by **odd numbers** should present their posters during **Poster Session 1** (on September 22nd), and those whose abstract are preceded by **even numbers** should present their works during **Poster Session 2** (on September 23rd)

Summary

Abstracts

1) Ca2+-binding modulates the inter-domain dynamics between the two Ca2+-binding domains of the Na+/Ca2+ exchanger

Abiko, Layara A.1; Vitale, Phelipe M.1; Favaro, Denize C.1; Bruschweiler-Li, Lei2; Brüschweiler, Rafael2; Salinas, Roberto K. 1
1Instituto de Química, Universidade de São Paulo, São Paulo, Brazil; 2Department of Chemistry and Biochemistry, the Ohio State University, Columbus, USA.

Na+/Ca2+ exchangers (NCX) use the electrochemical gradient from Na+ to extrude Ca2+ from the cells. Besides transporting Na+ and Ca2+, the exchangers are regulated by these ions. Thus, binding of Ca2+ to two cytosolic calcium-binding domains (CBD1 and CBD2) activates the NCX. The exchanger of *Drosophila melanogaster*, CALX, displays an anomalous behavior because it is inhibited by calcium binding to CBD1, and the CALX-CBD2 domain does not bind Ca2+. In this seminar I will briefly describe solution NMR studies of the CBD1 and CBD2 domains of the CALX. Measurements of 15N relaxation rates suggest that Ca2+-binding to CBD1 modulates the CBD1-CBD2 inter-domain dynamics similarly as observed previously for the mammalian NCX. It is intriguing that similar structural and dynamic changes induced by Ca2+-binding to CBD1 leads to opposite physiological responses by the exchanger.

2) Alternative Structural Solution for [4Fe-4S]2+ cluster in Adenine Glycosylases

Arenas, Carlos H. Trasviña; Castro, Luis G. Brieba
National Laboratory of Genomic for Biodivesity

MutY is an adenine glycosylase that belongs to Base Excision Repair pathway (BER). MutYs are in charge of the repairing 8oG:A lesion. MutY hydrolyzes the N-glycosydic bond between deoxyribose and adenine, leaving an apuric base that is repaired by others BER's enzymes. [4Fe-4S]2+ cluster is a widespread motif among MutYs and others DNA glycosylases whose function has been related with protein stability and substrate recognition. Interestingly, we found that MutYs from Entamoeba histolytica (EhY) and Lactobacillus brevis (LbY) do not contain [4Fe-4S]2+ cluster, in those organisms the [4Fe-4S]2+ cluster has been lost by the loss of a region involved in coordination or by substitution of the cysteinyl ligands. Moreover, our results show that EhY and LbY are monofunctional glycosylases. EhY is more active over 8oG:A than G:A, but, LbY has the same activity over those substrates. LbY, is a more promiscuous glycosylase acting over 8oG:G, 8oG:T, G:T. Additionally, we carried out a structure homology-modelling, where we found that MutYs without [4Fe-4S]2+ cluster are stabilized by amino acids such as Arg, Phe and Tyr, suggesting a steric stabilization. Thus, This work sheds light on understanding new molecular ways to deal with 8-oxoG:A mismatch with 4Fe-4S cluster dispensability.

3) How different techniques such as Spectroscopy, proteomics, and in silico approaches, are associated to X-ray crystallography to obtain structural information in a complex system, the Phycobilisome

Balocchi, Marta Cecilia Del Ca Bunster
Universidad de Concepción, Chile

In order to understand the energy transfer process through phycobilisomes towards the photoreaction center in *Gracilaria chilensis*, we have used X-ray crystallography to determine the three dimensional structure of Phycoerythrin and Phycocyanin and we are in the process of crystalize protein complexes. This information together with *in silico*, proteomic and spectroscopic approaches allowed us to propose main pathways for the energy flow in this system. The rods of Phycobilisomes contain phycoerythrin, phycocyanin and linker proteins. In this moment we are studying the association of the g33 linker with phycoerythrin and studying the position of the chromophores in order to understand the role of chromophorylated linkers in the energy transfer process. The advances in this aspect will be also reported.

4) Crystal Structure and Functional Mechanism of the Human Dermcidin and its Splice Variant

Belizário, José E.1; Souza, Diorge P.2; Farah, Shaker C.2
1Department of Pharmacology of Institute of Biomedical Sciences and 2Department of Biochemistry of Institute of Chemistry - University of São Paulo, SP, Brazil

Human Dermcidin (DCD) and a splice variant (DCD-SV) are 48- and 59-residues, anionic (-2) and neutral, respectively, helical peptides antibiotics for Gram positive and -negative bacteria. A previous X-ray study has shown that two antiparallel monomeric 48-DCD peptides assemble as hexameric channel in the presence of Zn2+. It is proposed that this barrel-stave-like channel may integrate into membrane and promote permeability to water and ions. To study the possible structural and functional interaction of DCD and DCD-SV, we have been optimizing the conditions to grow co-crystals formed by heterodimer peptides. Based on the previous study, we prepared 72 well plate drops containing 10 mM of synthetic peptides in 0.2 M Zn2+-acetate, 0.1 M Na-cacodylate and 12 to 18% (wt/vol) PEG8000, at pH 5.7 to 7.1 and 18oC. We will fine-tune the conditions to generate diffraction-grade crystals for each peptide and their combination. We will collect X-ray diffraction data using a Rigaku MicroMax-007 micro focus rotating anode generator with VariMax HR (high resolution) optics. We will present and discuss crystal screening and data collection from interested parties regarding possibilities for scientific collaboration.

5) Urocanate Hydratase from Trypanosoma cruzi: Crystallization and Circular Dichorism Studies

Boreiko, Sheila1; Iulek, Jorge1; Barison, Barison, Maria Julia2; Melo, Raissa2; Silber, Ariel Mariano2; Silva, Marcio3
1 Department of Chemistry, State University of Ponta Grossa, Ponta Grossa, Brazil. 2 Department of Parasitology, University of São Paulo, São Paulo, Brazil. 3 Department of Education, Federal Technological University of Paraná, Ponta Grossa, Brazil.

Urocanate hydratase from Trypanosoma cruzi (TcUH) participates in the catabolic pathway of histidine. It acts on converting urocanate to 4-imidazolone-5-propionate. Studies confirmed that TcUH is present in the epimastigote and metacyclic trypomastigota forms of T. cruzi. Here we show some results of crystallization assays and highlight some structural data obtained by the circular dichroism technique. Preliminary crystallization trials were performed both at UEPG (50 drops) and at LNLS (1284 drops). From these assays, it was possible to determine the conditions and concentrations more favorable for protein crystallization. Accordingly, a refinement with surrounding 96 conditions were performed. The best protein crystals, observed through ultraviolet flourescence, according to their size, were formed when the protein was in 5 mg mL-1 solution and had PEG 8000 and glycerol as precipitants. Data collection should still be performed. The structural study by circular dichroism indicated that the protein has the characteristic spectrum of proteins composed mainly of α-helices. A thermal denaturation study at elevated temperatures (from 20 to 100 °C) showed that TcUH starts its denaturation process at 50 °C, but after full denaturation the process is irreversible.

6) Novel insights into bacterial signaling: the 'one sequence - multiple structures' perspective

Buschiazzo, Alejandro
Institut Pasteur de Montevideo, Unit of Protein Crystallography, Montevideo, Uruguay

We have focused on the study of two-component systems (TCSs), key players in bacterial signaling, to better understand signal-transmission with molecular detail. The TCS DesK/DesR controls fatty acid desaturation in *Bacillus subtilis* in response to cold shock and other membrane-altering effectors. We had previously put forward a model of signal-dependent allosteric control of the sensor kinase catalytic activity [1,2].
We have now turned our attention to the response regulator DesR. A canonical activation pathway has been widely accepted to explain phosphorylation-mediated control of response regulator function, allosterically coupling the phosphorylation site to the α4β5α5 surface. However, the structural evidence supporting the main hypotheses is still highly fragmentary.
We are now reporting the crystal structure of full-length DesR, in complex with a phosphoryl-mimetic, showing the activated state [3]. Several crystal forms of the receiver domain were determined in the active and inactive configurations, revealing molecular details of the activation switch. Comparative small angle X ray scattering of full-length constructs, structure-guided point mutagenesis, as well as *in vitro* and *in vivo* functional analyses, allow us to propose an integral model of DesR activation. The phosphorylation of the receiver domain is allosterically coupled not to one, but two exposed surfaces, independently controlling its dimerization and tetramerization. Notably, the α1α5 surface is shown to be essential for a non-canonical dimerization and activation mechanism, shared by NarL/LuxR regulators. This surface is further involved in cognate histidine kinase binding, disclosing a novel view of response regulator allosteric control.

Bacterial signaling has become a good example of the 'new view' in protein structure/function principles, with 'old roots' in the MWC model of allostery and proteins' conformational equilibria. One sequence allows proteins to adopt more than one structure, and these alternate structures are frequently the basis of switching and transmission of information.

[1]Albanesi *et al.* (2009) *PNAS* 106:16185
[2]Trajtenberg *et al.* (2010) *JBC* 285:24892
[3]Trajtenberg *et al.* (2014) *submitted*

7) The energetic cost to knot the unfolded state of a protein determined by optical tweezers

Bustamante, Andrés1; Reyes, Javiera1; Wilson, Christian A. M.1; Guerra, Daniel2; Bustamante, Carlos3,4,5; Baez, Mauricio1.
1 Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile, 2Universidad. Peruana Cayetano Heredia, Lima, Peru, 3QB3 California Institute for Quantitative Biosciences, Berkeley, CA, USA, 4Jason L. Choy Laboratory of Single-Molecule Biophysics, Berkeley, CA, USA, 5Howard Hughes Medical Institute, Berkeley, CA, USA.

The unfolded state of a protein is considered as a highly fluctuating state of the polypeptide chain, but its tendency to form entangled structures like knots has not been quantified. In this work, we determined the energetic cost of threading a designed knotted protein. The bacteriophage P22 ARC repressor is a homodimer, which can be converted into a single-chain monomer (mARC) adding a 15-residue glycine-rich linker to connect both subunits. Structural models show that flexible glycine richlinker have the potential to create a knotted or not knotted chain by movements around the protein structure. Using optical tweezers, we explored the folding mechanism of mARC pulling their C and N-terminal extremes at the single molecule level. Analysis of 537 unfolding events obtained from eleven molecules of mARC show two populations of proteins characterized by contours lengths of 37 (7 molecules) and 43 nm (4 molecules). These values agree with the expected size of the polypeptide being stretching from a knotted (37 nm) or unknotted (43 nm) conformation. Furthermore, thermodynamic analysis obtained from the knotted or unknotted conformation of mARC indicates that the unfolded knotted conformation is destabilized by 6 kcal/mol with respect to the unknotted fully unfolded conformation. This situation contrasts with respect to naturally-occurring knotted proteins where knots prevail in the unfolded state. Fondecyt 11110534.

8) Different ways to bind cofactors: allosteric ATP inhibition and NAD(P) binding selectivity

Cabrera, Ricardo
Department of Biology, University of Chile – Santiago, Chile

ATP, NAD and NADP are by far the most connected molecules in the metabolic networks. In this work, I describe the use of X-ray protein complexes showing interactions with these metabolites to explore the underlying structural basis of the widespread enzymatic features, regulation and ligand discrimination.
In the case of enzyme regulation, we analyzed the substrate inhibition of *E. coli* phosphofructokinase-2 by ATP. This inhibition impedes the futile hydrolysis of ATP when the bacterium is feeding over gluconeogenic sources. Different crystallographic structures show both, the structure of the inhibited specie and the conformational changes associated to the whole kinetic mechanism.
In regard to ligand selectivity, we studied the fact that most dehydrogenases are specific to use either NAD or NADP to perform redox reactions on carbon substrates. The presence or absence of the 2'-phosphate allows discrimination at the NAD(P) binding pocket, ultimately resulting in their different metabolic functions such as energy generation, biomass formation or oxidative stress. The protein complexes with these ligands were examined through all over the PDB. Statistical potentials were used to compare the distinctive features of the binding pockets for NAD and NADP, in order to find fold-specific and fold-independent traits, related to interactions beyond the 2'-phosphate.
FONDECYT 1121170

9) Progress in the field of Chemistry of Natural Products in the Faculty of Natural Sciences at Nacional University of Asunción

Cabrera, Miguel Angel Martínez

Paraguay is a country that has been through unfortunate wars, which have caused its death in two occasions, and had to be reborn twice. This has been a great obstacle for the scientific and technological development of the country, which is still felt to this day. Having lost 82% of its territory, the country nevertheless possesses rich biodiversity, within which plant resources (medicinal plants) are potential reservoirs of bioactive molecules that the paraguayan population still uses to treat diseases, even those that become clinically complicated. The Area of Organic Chemistry of Natural Products of the Laboratory of Analysis of Plant Resources (LAREV, for its acronym in Spanish) of the Department of Biology of the Faculty of Exact and Natural Sciences (FACEN, for its acronym in Spanish) of the National University of Asunción (UNA, for its acronym in Spanish) was born four and a half years ago with the goal of isolating, purifying and characterizing bioactive molecules —primarily of plant origin— with which it aims to develop new efficient alternative drugs to meet the needs of the paraguayan population at a lower cost. In addition, LAREV is beginning a new line of research dedicated to Biological Control of Pathogens in Plants; and the most remarkable achievement brought about by the hard, uninterrupted work in research and academic support offered by LAREV is the initiation of the Master's Program in Organic Chemistry with Emphasis on Medicinal Phytochemistry and Bioactive Synthetic Compounds, which is unique in Paraguay. This program will form the first local organic chemists, with the great commitment of training qualified human resources that will strengthen the work in organic synthesis and structural biology in the country, which since 2012 is a member of the Center for Structural Biology of MERCOSUR (CEBEM, for its acronym in Spanish), and also of a FOCEM project to create the first school of Structural Biology in Paraguay.

10) Crystallization and preliminary X-ray studies of a Carbohydrate binding module (CBM_E1) derived from sugarcane soil metagenomics

Campos, Bruna Medéia1; Alvarez, Thabata Maria1; Liberato, Marcelo Vizoto2; Zeri, Ana Carolina de Mattos1; Squina, Fabio M.2
1Laboratório Nacional de Biociências, Centro Nacional de Pesquisa em Energia e Materiais, Campinas, SP, CP6192, Brazil; 2Laboratório Nacional de Ciências e Tecnologia do Bioetanol, Centro Nacional de Pesquisa em Energia e Materiais, Campinas, SP, Brazil

In recent years, due to the growing global demand for energy, the dependence on fossil fuels and the limited natural resources and environmental pollution, biofuels have attracted great interest as source of renewable energy. However, the production of biofuels from plant biomass is still considered an expensive technology and with low efficiency. In this context, the study of carbohydrate binding modules (CBMs) is attracting attention. CBMs are protein modules involved on guiding catalytic modules to the polysaccharidases. Aiming the identification of new CBMs, a sugarcane soil metagenomics library was analyzed and a Cellulase 5 (CelE1) with an uncharacterized CBM (CBM_E1) was identified. In this study, the CBM_E1 was expressed, purified nd crystallized. X-ray diffraction data were collected to 1.95Å resolution. The crystal belonged to the space group I23, with unit-cell parameters a = b = c = 88,07Å and $\alpha = \beta = \gamma = 90°$ and was obtained by sitting-drop vapour diffusion. Structure refinement and model building are in progress.

11) Crystallographic studies with Bothropstoxin-I complexed to inhibitory cinnamic compounds

Cardoso, Fábio F.; Fontes, Marcos R. M.
Departamento de Física e Biofísica, Instituto de Biociências de Botucatu, UNESP

Snakebite envenoming is an important public health problem in many tropical and subtropical countries. Bothrops snakes are main responsible for snakebites that happen in Latin America and the phospholipases A2 (PLA2) are among major compounds of their venoms. PLA2 are enzymes that promote cellular membrane disruption through Ca2+-dependent hydrolysis of phospholipids. A class of these proteins (Lys49-PLA2) does not show catalytic activity but can exert a pronounced local myotoxic effect which is not neutralized by serum therapy. However, there are evidences that cinnamic compounds neutralize various activities of snake venoms. Structure studies with Lys49-PLA2 and their potential inhibitors may contribute to elucidate its mechanism of action and the regions involved in biological effects observed. We noted that PrTX-I, a Lys49-PLA2 isolated from Bothrops pirajai venom, is completely and partially neutralized, respectivity, by cinnamic compounds rosmarinic acid and caffeic acid. With these results we suggest that membrane destabilization promoted by the toxin is the probable cause of the observed effects, well as well reiterated the involvement of the C-terminal region and hydrophobic channel. To better understand these myotoxins (Lys49-PLA2) and the discovery of new inhibitors more effective than the previous two, we performed the X-ray crystallographic studies with BthTX-I and other cinnamic compounds.

12) Crystallographic and protein engineering studies of Triosephosphate isomerase

Castro, Luis Gabriel Brieba
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, México

Triosephosphate isomerase (TIM) is one of the enzymes more studied to date and terms like the "perfect enzyme" or an "obligated dimer" are used frequently for this enzyme. We found that the protozoan parasite *Trichomonas vaginalis* contains two TIM copies (TvTIM) in its genome. Those TIMs only differ in their character in four amino acids: E/Q 18, I/V 24, I/V 45, and P/A 239. TvTIMs differ with other TIMs in that the energy that is need for dissociation is minor than the energy required for monomer unfolding. The energies required for dissociation are 35.7 and 19.1 kJ mol−1 for TvTIM1 and TvTIM2, and the monomeric intermediates have a stability of 86.2and 65.3 kJ mol−1 respectively.
Crystal structures of TvTIMs indicate that the character of residue 45 (Ile or Val) is determinant for dimer stability by a ball and socket interaction composed of residue 45 of subunit A against a hydrophobic pocket formed by alpha-helices 2 and 3 of subunit B and by swapping of loop 3 between monomers. Single amino acid substitutions in residue 45 controls the dimer-monomer fate of TvTIMs. Unexpectedly, monomeric variants of TvTIM1 are highly stable and one of them (Ile45Ala) is only 8 fold less active than wild-type TvTIM1. Cross-linking and protease sensitivity experiments indicate that monomeric TvTIMs dimerized to a catalytic competent species assisted by substrate binding. We propose that TvTIMs represent a window to the evolutionary process of an ancestral stable monomeric TIM that evolved towards a dimeric enzyme.

13) Structural context, intrinsic disorder and binding properties of linear motifmediated viral and cellular retinoblastoma-target protein interactions

Chemes, Lucía B.1; Foutel, Nicolás González1; Sánchez, Ignacio E.2; Prat Gay, Gonzalo1
1 Protein Structure Function and Engineering Laboratory, Fundación Instituto Leloir, Buenos Aires, Argentina. 2 Protein Physiology Laboratory and Departamento de Química Biológica FCEN-UBA, Buenos Aires, Argentina

Many proteins from pathogenic viruses have intrinsically disordered (IDP) domains harboring short linear motifs that target cellular functions. Within the IDP E7N domain from the human papillomavirus (HPV) E7 oncoprotein, the LxCxE and E2F motifs mediate high-affinity binding to the retinoblastoma (Rb) tumor suppressor. Binding of E7 to Rb induces dissociation of the E2F transcription factor-Rb complex and expression of cell cycle regulated genes, allowing for efficient replication of the viral genome. However, increased E7 expression levels in persistent infections can lead to cell transformation and cancer. The presence of disordered regions harboring Rb-interaction motifs is a common feature of many pathogenic viruses. However, while understanding how viral proteins interfere with host functions has a direct relevance for human disease, current insight into structure-sequence-function relationships and binding properties of these proteins is still scarce, with few affinity measurements and binding mechanisms reported so far. Both motifs have been shown to bind to conserved surface grooves in the structured RbAB domain, acquiring either extended (LxCxE motif) or alpha helical (E2F motif) conformations in a folding-upon-binding process. We undertook a comprehensive database and literature search, which revealed the presence of 58 experimentally validated examples of the LxCxE motif in 37 cellular and 21 viral Rb targets, and five instances of the E2F motif in three cellular and two viral targets. Structure-based analysis revealed that 90% of instances were located in disordered domains or in exposed loop regions of target proteins. A few examples were located within well-structured domains, suggesting that they may undergo order-to-disorder transitions upon binding. Scoring

of sequence determinants that lie outside the canonical LxCxE motif-defining residues and have been reported to increase binding affinity revealed a subset of features that was overrepresented in viral targets and in disordered structural contexts, suggesting that optimization of affinity based on these positions may have been evolved by viral proteins to allow for effective competition with cellular interactions. In order to gain insight into structure-function relationships, we performed in-solution binding studies of the LxCxE and E2F motifs from four additional cellular and viral Rb targets. The 18-mer motif from the cellular E2F2 transcription factor bound to Rb with high affinity (KD = 12 nM). However, opposed to the two-state behavior previously reported for the LxCxE site, two association and dissociation phases present may reflect conformational rearrangements or sequential binding of two discrete binding surfaces present in the E2F2 motif. The individual LxCxE and E2F Rb-binding motifs from the adenovirus E1A protein presented KD values in the 200 nM range and bound by a two state route, suggesting that the full-length protein harboring both motifs has sub-nanomolar affinity comparable to that of HPV E7 and that both viral proteins compete effectively for binding with the histone deacetylase (HDAC) protein, which bound to Rb with low affinity (KD = 10 µM). Circular dichroism (CD) studies revealed a prevalence of disorder within all motif-containing peptides, but revealed large differences in secondary structure (PII and alpha helix) propensities for the same motif from different target proteins. Taken together, our results suggest that intrinsic disorder may favor the evolution of both conformational as well as sequence features within viral linear motifs, allowing for strong binding and effective interference with cellular interactions, and stress the need for a combined structure-function and evolutionary analysis for understanding pathogenic virus-host interactions mediated by udisordered viral proteins.

14) Imperfect coordination chemistry facilitates metal ion release in the Psa permease

Couñago, Rafael M.1,2; Ween, Miranda P.3; Begg, Stephanie L.3; Bajaj, Megha2; Zuegg, Johannes2; O'Mara, Megan L.1,4; Cooper, Matthew A.2; McEwan, Alastair G.1; Paton, James C.3; Kobe, Bostjan1,2; McDevitt, Christopher A.3
1 School of Chemistry and Molecular Biosciences and Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, Australia. 2 Institute for Molecular Bioscience, University of Queensland, Brisbane, Australia. 3 Research Centre for Infectious Diseases, School of Molecular and Biomedical Science, University of Adelaide, Adelaide, South Australia, Australia. 4 School of Mathematics and Physics, University of Queensland, Brisbane, Australia.

The relative stability of divalent first-row transition metal ion complexes, as defined by the Irving-Williams series, poses a fundamental chemical challenge for selectivity in bacterial metal ion acquisition. Here we show that although the substrate-binding protein of Streptococcus pneumoniae, PsaA, is finely attuned to bind its physiological substrate manganese, it can also bind a broad range of other divalent transition metal cations. By combining high-resolution structural data, metal-binding assays and mutational analyses, we show that the inability of open-state PsaA to satisfy the preferred coordination chemistry of manganese enables the protein to undergo the conformational changes required for cargo release to the Psa permease. This is specific for manganese ions, whereas zinc ions remain bound to PsaA. Collectively, these findings suggest a new ligand binding and release mechanism for PsaA and related substrate-binding proteins that facilitate specificity for divalent cations during competition from zinc ions, which are more abundant in biological systems. Reference: Nat Chem Biol. 2014; 10(1):35-41.

15) Salicylaldehyde Dehydrogenase from Pseudomonas putida is Highly Efficient to Aromatic Aldehydes: Structural and Kinetics Studies

Coitinho, Juliana Barbosa.1; Brandao, T. A. S.2; Nagem, R. A. P.3
1Departamento de Ciências Fisiológicas, UFES, ES, Brazil; 2Departamento de Química, UFMG, MG, Brazil; 3Departamento de Bioquímica e Imunologia, UFMG, MG, Brazil.

Naphthalene is the most abundant toxic compound found in oil and the exposure to this compound has been associated with several toxic manifestations in humans and laboratory animals. The enzyme NahF (salicylaldehyde dehydrogenase) belongs to the naphthalene degrading pathway in P. putida, converting salicylaldehyde to salicylate.This enzyme belongs to the aldehyde dehydrogenase (ALDH) superfamily which members oxidize aliphatic and aromatic aldehydes using NAD(P)+ as cofactor. Despite of general similarities between the members this superfamily, studies which reveal the slight differences between its members can be useful to improve the knowledge of the relation structure-function in this family and also on the creation of more effective naphthalene degrading enzymes to use on bioremediation process. To understand the structural determinants of activity of this enzyme, the recombinant protein NahF (NahFr) was expressed in E. coli Arctic Express at 12 oC, purified by affinity chromatography and gel filtration, and had its 3D structure solved.
After gel filtration, NahFr eluted in three fractions. The main fraction contained the protein in its dimeric form and was monodisperse (data obtained by Dynamic Light Scattering). This fraction was used in the kinetic and structural studies. NahFr was most active at pH 8.5 and 60 oC. and showed high values of kcat/KM to aromatic followed by long chain aliphatic aldehydes. The structure showed an α/β folding with the three domains – oligomerization, cofactor binding, and catalytic, well defined. The salicylaldehyde was present in a deep pocket in the structure where the catalytic Cys284 and Glu250 were seen. Moreover, the residues Arg157, Gly150 and Trp96 were important to differentiate the specificity of aromatic and aliphatic aldehyde dehydrogenases.
The understanding of NahF aspects responsible for its activity and specificity and also its differences to other ALDHs from other organisms may open possibilities for improving characteristic as stability, substrate preference and affinity by systematic mutations, making possible its use and other related enzymes in biotechnological processes.

16) Crystal structure of *Canavalia maritima* lectin (ConM) in complex with a dinucleotide

Delatorre, Plinio; Vieira, Derek Barroso Holanda Asp; Teixeira, Claudener Souza; Silva Filho, José Caetano; Nóbrega, Raphael Batista; Rocha, Bruno Anderson Matias; Cavada, Benildo Sousa
Universidade Federal da Paraíba (UFPB), João Pessoa, Brasil

Lectins can be classified according to its ability to bind to simple sugars or large complex carbohydrates. Many biological reactions involve protein interaction with nucleic acids, such as those related to the expression of the genetic message. Certain plant lectins have mitogenic characteristics and are able to stimulate DNA synthesis in T lymphocytes. Despite the similar structures, lectins have different mitogenic properties due to their different carbohydrate specificities. The ConM/2-AMP cocristal showed orthorhombic space group I222. The 2-AMP electron density contoured at 3σ was found in the polar central cavity of the ConM/2-AMP tetramer structure.
The lectins ability to form tetramers can be physiologically relevant in receptor binding. The dimer-tetramer equilibrium also depends on the conformation of His or Asn residues in position 131, which are determinant to permit the interactions between the dimers and the tetramer assembly. Delatorre and colleagues (2013) showed that in the symmetric region of the ConM lectin, four

IAA molecules bind to the tetramer in the central region, and this arrangement helps to stabilize through hydrophobic interactions between these molecules. We suggest that the presence of the dinucleotide also helps stabilize the structure of the tetramer. When molecules were aligned, it was shown that the responsible amino acids for these interactions, kept the same position as the native form.

17) Development of cholesterol oxidase as a biocatalyst towards industrial production of steroids

Fagundes, Michelle; Ranzani, Americo; Cordeiro, Artur
Brazilian Biosciences National Laboratory (LNBio); Brazilian Center for Research in Energy and Materials (CNPEM), Campinas – Brazil.

Cholesterol oxidase (ChOx) is a flavoenzyme that catalyzes the oxidation and isomerization of cholesterol into cholest-5-en-3-one, the first step in the cholesterol degradation pathway. There are two classes of ChOx´s: type I, with non-covalently bound FAD and type II in which this cofactor is covalently bound to at a conserved histidine residue of the protein. ChOx´s I and II differ in overall structure and substrate specificity. ChOx-II are known to oxidize a broader variety of steroids, including chemical intermediates currently used at large scale production of synthetic drugs. High-resolution crystallography structures are available for both ChOx classes. There are 17 PDB entries for class I ChOx´s, including a complex with a substrate analogue, and only 2 entries for class II, both without substrate analogue ligands and missing electron density for a gating loop that would close the active site. Our objective are to elucidate the first crystallographic structure of ChOx-II in complex with a steroid substrate and reveal missing structural details of the active site. To achieve our goals, site-direct mutants with reduced catalytic active have been designed, characterized and are currently being used in co-crystallization and soaking experiments. In the poster, we present the strategy for mutants design and some preliminary results.

18) Fumarate hydratase: a highly oxygen sensitive enzyme from Leishmania major

Feliciano, P.R.; Drennan, C.L.; Nonato, M.C.
Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil. Departments of Chemistry and Biology and the Howard Hughes Medical Institute, Massachusetts Institute of Technology, USA

Leishmaniases, classified as neglected tropical diseases, are caused by different species of the parasite Leishmania and affect 12 million people worldwide. Fumarate hydratase (FH) catalyzes the stereospecific reversible hydration of fumarate to malate. Eukaryotes express two isoforms of FH: the mitochondrial isoform (FH-1), which performs this reaction as part of the TCA cycle and as such is central to aerobic respiration, and the cytosolic isoform (FH-2), which is thought to be involved in the metabolism of fumarate. EPR approach has identified the presence of an Fe-S cluster, and the catalytic efficiency under aerobic and anaerobic environments suggests that FHs from Leishmania major (LmFH) are highly oxygen sensitive. The crystal structure of LmFH-2 was solved by SAD techniques using iron as the anomalous scatter. To our knowledge this is the first crystal structure of class I FH to be reported. LmFH-2 an Fe–S cluster-containing homodimeric protein, had its structure solved in complex with substrates and inhibitors. Structural analysis, kinetic characterization and site-directed mutagenesis of LmFH-2 have provided insights into its catalytic mechanism. The structural characterization of LmFH will not only significantly contribute to the evaluation of FH as a potential drug target against Leishmaniases, but also other trypanossomatid-related diseases such as Chagas'disease and Sleeping sickness.

19) A structure-based proposal for a comprehensive myotoxic mechanism of phospholipase A2-like proteins from viperid snake venoms

Fernandes, Carlos A. H.1; Lomonte, Bruno2; Fontes; Marcos Roberto de Mattos1
Department of Physics and Biophysics - Biosciences Institute - UNESP1, Clodomiro Picado Institute, University of Costa Rica2

Envenoming due to snakebites is an important public health problem in tropical and subtropical countries, which, in addition to mortality, may result in permanent sequelae because of local tissue damage. One the most abundant components of venom from snakes of Viperidae family is the phospholipases A2 (PLA2s). These proteins target the sarcolemma and induce degeneration of skeletal muscle fibers and the rapidity of their action represents a major challenge to antivenom therapy, which often results in limited effectiveness to prevent tissue damage. A subgroup of PLA2s, the Lys49-PLA2s, a PLA2-like which is catalytically inactive however conserves PLA2 scaffold, and still induce an acute local myonecrosis. In this work, based on an extensive structural analysis with all Lys49-PLA2s available on PDB and by recent results obtained by our group data, we propose an integrated hypothesis of the myotoxic mechanism. According to our proposition, this mechanism is composed by five steps that includes an allosteric transition and two independent sites of interaction with target membrane. In fact, recent functional and structural studies with these toxins complexed to inhibitory ligands supports our hypothesis and reveals novel neutralization mechanisms. These results could provide a useful molecular basis for the search of new neutralizing strategies to improve the treatment of envenomings by viperid snakes.

20) Structure Of The Complex Between The Peptide PTRY and Trypsin

Fernandes, J.P.C.; Oliveira, S.A.; Freitas, S.M.; Barbosa, J.A.R.G.
Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, Brasil.

Serine proteases are enzymes that catalyse the cleavage of peptide bonds in proteins and thus have extensive functions in the biological systems. For instance, some of these proteases are involved in cancer-associated activities. Inhibition of such enzymes could benefit patients with this disease as indicated by initial tests. Amongst the serine protease inhibitors, the Bowman-Birk family appears as one of the best characterized. The structure of the Bowman-Birk inhibitor extracted from Vigna unguiculata (Blackeyed pea Trypsin and Chymotrypsin Inhibitor – BTCI) in complex with bovine trypsin was solved (PDB code 2G81). According to the solved structure, the loop between the residues Cys24 and Cys32 of BTCI, named here PTRY, could be clearly identified as the region responsible for the high binding affinity and consequent inhibition of trypsin. In the current study we have used this PTRY synthetic 9 amino acids peptide (CTKSIPPQC) in crystallization trials with bovine trypsin. Co-crystallization was successful in seven different conditions. The crystals were taken to the LNLS synchrotron beamline MX2 and data collection was conducted. Data processing of the best crystal allowed the determination of space group P212121 with unit cell parameters a=60.40, b=63.99 and c=69.66 and a maximum resolution of 1,38 Å. Molecular replacement has been used in phasing and the refined structure will be presented.

21) The human Liver-type Glutaminase catalytic domain structure, kinetic chracterization and importance in the redox balance within the cell

Ferreira, Igor M; Vollmar, Melanie; Krojer, Tobias; Strain-Damerell, Claire; Burgess-Brown, Nicola; Delft, Frank von; Yue, Wyatt; Dias, Sandra M. G.; Ambrosio, Andre L. B
Brazilian Bioscience National Laboratory-Brazil; Structural Genomics Consortium-United Kingdom

The glutaminases are enzymes which hydrolyse glutamine to form glutamate and ammonia. Mammalians have three different glutaminases isozymes. The Kidney-type glutaminase (KGA) and GAC (Glutaminase C) are isoforms from alternative splicing of the gls1 gene and the Liver-type Glutaminase (LGA) is coded by de gls2 gene. We have recently reported that the catalytic differences among the three isoforms – with LGA being the less active in the presence of the activator inorganic phosphate – and that the glutaminase activaty is directly related with the oligomerization of the proteins. To find the difference in the structure that leads to this discrepant enzymatic activity we got the structure of the LGA catalytic domain, since the KGA and GAC catalytic domain were already known. And we also measured the catalytic efficiency of some mutants in catalytic domain and in the gating loop to find key residues.The crystallographic structure of the three isoforms are very similar, with less than 0,4 Å of rmsd. And the mutants didn't show big differences in the catalytic efficience, what led us to suppose that the difference is in other regions of the protein.We are also evaluating the importance of the LGA in the balance redox in cells, once this enzyme was recently related with the control of GSH levels in normal and cancer cells.

22) Structure-function relationships of anaerobic metalloenzymes

Fontecilla-Camps, Juan C.
Metalloproteins Unit - Institut de Biologie Structurale CEA, CNRS, UGA, Grenoble, France

Many biological reactions, especially those related to gas metabolism, are depend on enzymes containing metallic cofactors and metal-containing active sites. This is so because these reactions cannot be catalyzed by the organic functions found in the 20+ amino acids. The sometimes stunning resemblance of cofactors such as the 4Fe-4S clusters to pyrite and other inorganic iron sulfides has led some authors to postulate that life originated at or near Fe-S structures that provided catalysts, reducing power for carbon fixation and even encapsulation. This is the so-called autotrophic theory of the origin of life (as opposed to the heterotrophic "primordial soup" theory). Regardless the role that transition metals such as Fe, Ni and W may or may not have played at the onset of life it is clear that most basic biological processes are based on metalloproteins: cellular respiration, photosynthesis, nitrogen fixation and many metabolic pathways are examples of this.
In my laboratory, we have been especially interested in the structure-function relationships of hydrogen metabolism by microorganisms both by determining the crystal structures of the two types of enzymes responsible for this process and those of ancillary proteins. Because one of the hydrogenases is a Ni-Fe enzyme we have extended our studies to Ni-containing anaerobic acetyl-CoA synthase/CO dehydrogenase and to the Ni-transporting protein Ni-A. By the same token, besides determining the structure of the FeFe- hydrogenase, we have also become interested in understanding how its site active is put together. Both CO and CN- iron ligands and a small dithiolate-containing organic molecule are components of this active site, which are made by radical *S*-adenosyl-L-methionine (SAM)-depending enzymes. So far, we have determined the structures of the two radical SAM FeFe-hydrogenase maturases and have elaborated mechanistic proposals for their catalysis.
Work with oxygen-sensitive enzymes like the ones mentioned above has required a major effort in setting up a series of glove boxes covering all aspects of a crystallographic analysis: protein expression, purification and crystallization, both manual and automated. Anaerobic crystallization using elements of this platform -we have called "AnoXtal"- is open to the international scientific community (http://www.isbg.fr/platforms/other-facilities-developments/crystallisation-under-anaerobic/). All the points addressed above will be addressed during my talk.

23) Proposition Of Novel Comprehensive Model For Myotoxic Activity On Bothropic Phospholipases A2

Fontes, Marcos R. M.
Dep. de Física e Biofísica, Inst. de Biociências, UNESP – Univ Estadual Paulista, Botucatu-SP, Brazil

Phospholipases A2 are enzymes responsible for cellular membrane disruption through Ca2+-dependent hydrolysis of phospholipids. A class of these proteins (Lys49-PLA2s) does not show catalytic activity but can exert a pronounced local myotoxic effect that is not neutralized by serum therapy. After more than 20 years of structural, biochemical and biological studies with this class of proteins, its biological mechanism still remain not totally understood. Here, based in a comprehensive study including over than 30 crystallographic structures, Small Angle X-ray Scattering, Dynamic Light Scattering, Isothermal Titration Calorimetry, Biochemical, Bioinformatics, Phylogenic and Myografic experiments, we proposed a complete myotoxic mechanism. This work confirms the biological dimer indicated by recent studies in which both C-termini are in the dimeric interface. In this configuration, we propose that the myotoxic site of these toxins is composed by the Lys 20, Lys115 and Arg118 residues. The extensive structural analysis also include: (i) the function of hydrophobic long-chain molecules as Lys49-PLA2s inhibitors, (ii) the role of Lys122, previously indicated as being responsible for Lys49-PLA2s catalytic inactivity, (iii) a structural comparison of the Ca2+-binding loop region between Lys49 and Asp49-PLA2s, (iv) the importance of Tyr119 residue, (v) the role of different classes of inhibitors and (vi) the role of hydrophobic knuckle. Taking into account all these issues we were able to propose a complete mechanism of action of these proteins and also proposed the different ways to inhibit them. These results may be useful to guide new experiments that can definitively clarify the action mechanism of snake venom PLA2s and lead to the design of structure-based inhibitors to complement the serum therapy.

24) Structures of the serine protease inhibitor BTCI in complex with proteases and the birth a new crystallography lab in central Brazil

Freitas, S. M., Ventura, M. M., Teles, R. C. L., Esteves, G. F., Silva, L. P., Azevedo, R. B., Cavalcante, N. S., Neves, D., Barbosa, J. A. R. G.
Molecular Biophysics Lab, Department of Cell Biology, University of Brasília

The roles played by serine proteases in organisms are great in importance and number. These enzymes catalyse the cleavage of peptide bonds in proteins and thus needs to be tightly controlled. One of the controlling mechanisms is the use of protein inhibitors. The Blackeyed pea Trypsin and Chymotrypsin Inhibitor (BTCI) from Vigna unguiculata is one of these molecules, being able to inhibit both trypsin and chymotrypsin at the same time. This inhibitor is a member of the Bowman-°©-Birk family, characterized by having several disulphide bonds and two inhibitory sites on opposite sides of the molecule. BTCI has been studied for decades at the biophysics lab of the university of Brasília. Some years ago, finally, BTCI had its structure solved in two different macromolecular complexes: one with trypsin (2G81, 1.55 Å, P212121, Rfree 0.169) and the other with trypsin and chymotrypsin (3RU4, 1.68 Å, P1, Rfree 0.192). The later is the first ternary complex with different enzymes for this family. We shall discuss and compare both structures and relate the work with the new crystallography lab that is being formed in Brasília.

25) Statistical potentials as a tool to characterize NAD(P) binding sites and propose mutations to change the specificity for these dinucleotides in enzymes

Fuentealba, M.; Cabrera, R.
Departamento de Biología, Facultad de Ciencias, Universidad De Chile.

The nicotinamide adenine dinucleotides, NAD and NADP (NAD(P)), are cofactors that mediate production and consumption of reducing power in a huge variety of reactions of the metabolic network in all organisms. Despite the structural similarity of these cofactors, the NAD(P) binding proteins display an impressive capacity of molecular recognition, since they are capable to discriminate for the presence of the 2'-phosphate group. Given that the enzymes present different levels of specificity for NAD or NADP depending on the residues in the binding sites of these cofactors. We aimed for a better understanding of the recognition determinants in NAD(P) binding protein through the derivation of statistical potentials generated from the whole repertory of known NAD(P) complexes. A protein database was build by selecting a non-redundant and high resolution structures hat binds NAD(P), showing a complete ligand in extended conformations. We present cases in which these potentials can be used to predict the preference for NAD or NADP in enzymes allowing to propose mutations that invert the specificity.

26) Relations between structure and function of the beta-galactosidase from Xanthomonas campestris

Godoy, Andre Schutzer; Camilo, César Moises; Polikarpov, Igor
Instituto de Fisica de Sao Carlos

Glycosyl hydrolases are an important group of enzyme with the potential of catalyze the glycosil bond between two carbohydrates. Nowadays there are more than 120 families of glycosyl hydrolases, dispersed in all phyla from life. Beta-galactosidases are a special class of enzymes that can be found on families 1, 2, 35 and 42. These enzymes are responsible for the hydrolysis of beta one-four bond between a galactosyl and another carbohydrate. In addition, those enzymes are also responsible for the synthesis of galacto-oligosaccharides, an important class of prebiotics, commonly found in yogurts. Furthermore, the natural substrate for those enzymes is usually lactose, which makes them a viable target for biotechnology applications in food industry. Here we want to explore in a structural level, both synthetic and catalytic capacities of a beta-galactosidases from Xanthomonas campestris, expressed by the gene Xcc_1754. For that, we cloned all three genes in pET_Trx expression vector, and overexpressed all enzymes in E. coli. Then, all enzymes pure enzymes were crystallized, and x-ray data was collected in National Synchrotron Light Source. Structural reveals a double conformation on loop 12, which may be involved in the reaction mechanism of the enzyme.

27) Nucleoside Diphosphate Kinase Oligomerization: Crystallographic And Biological Analyses

Gómez Barroso J.A.1, Miranda M.R.2, Pereira C.A.2, Garratt R.C.3 and Aguilar C.F.1
1Lab. de B. Estruct. (UNSL-IMIBIO SL-CONICET); 2Dpto. de B. Molec. de *T. cruzi* (LBMTC-IDIM-CONICET-UBA); 3IFSC (USP-Brasil)

Nucleoside diphosphate kinases (NDPK) are enzymes involved in cell nucleotide homeostasis by the interconversion of nucleoside di- and tri-phosphates. TcNDPK1 is the canonical isoform of *Trypanosoma cruzi*, the causative agent of Chagas' Disease, and like eukaryotic NDPKs forms active hexamers. In this work we study TcNDPK1 oligomerization through molecular biology and X-ray crystallography techniques. The three dimensional structure at 3Å resolution showed that the 24 hexamers in the asymmetric unit are arranged into a helix-like oligomeric formation. The oligomerization observed in the crystalline structure was also detected in the parasite by over-expressing the NDPK gene fused to GFP, in order to reduce the intermolecular distance by the formation of weak dimers. Transgenic parasites showed a granular organization localized mainly in the anterior part of the cell that could be destabilized by changes in the intracellular salt concentration. Electron microscopy analysis indicated that these granules were filled structures without membranes. This work represents the first report of a NDPK assembled into an organized arrangement with a physiological relevance.

28) Protein dynamics as an essential trait in evolution: interplay of NMR and crystallographic studies

Gonzalez, Mariano; Gonzales, Javier; Meini, Maria-Rocio; Buschiazzo, Alejandro; Vila, Alejandro J.
Institute for Molecular and Cellular Biology (IBR), University of Rosario, Rosario, Argentina

Conformational variability, or dynamism, is an inherent property of proteins, involving dynamic events at different time scales and distances. However, the role of protein dynamics in evolution is still matter of debate. Recent evidence has shown that alternative conformational substates can mediate new functions, which led to the hypothesis that active site flexibility can favor protein evolvability, facilitating the development of new functions. We decided to explore this hypothesis by exploring the conformational dynamics in an in vitro evolved variant of a metallo-beta-lactamase. NMR data show that evolution indeed exploits conformational dynamics to elicit fitness in a biological context. Crystal structures of different lactamase variants indeed support this notion, being able to capture some snapshots of this dynamics.

29) Structure and dynamics of protein-DNA interactions in transcription initiation complexes of *Escherichia coli*

Guerra, Daniel
Universidad Peruana Cayetano Heredia, Lima, Peru

Transcription initiation complexes (RPO) were formed by incubating *Escherichia coli* RNA polymerase-sigma 70 holoenzyme (RNAP) with bacteriophage lambda pR or ribosomal promoters. AFM images showed different levels of DNA compaction corresponding to DNA wrapping on the surface of RNAP enzyme in a specific way for each kind of promoter. In the presence of the bacterial stringent-response modulators, ppGpp and DksA, the DNA-protein contacts were partially lost in ways that are reminiscent of different transcription initiation intermediates, thus offering a new model for transcription regulation. The highly-stable wrapped conformation of the pR promoter was further characterized by reversible mechanical denaturation with optical tweezers. By holding and pulling a single DNA molecule between two polystyrene beads, we were able to observe a transition that corresponds to the breaking of some of the protein-DNA interactions that occur in RPO complexes. Importantly, the change in extension determined in this way resulted in 20nm, which is closely compatible to the extension of the compaction observed by AFM images. The kinetic and thermodynamic characterization of this system offers new insights on the energetics of transcription initiation as it is influenced by extended specific contacts between RNAP and enhancer DNA elements.

30) Structural biology toward rational drug and agrochemical design: Applications in infectious diseases

Guido, Rafael
Universidade de São Paulo (USP), São Carlos, Brasil

Drug discovery has moved toward more rational strategies based on our increasing understanding of the fundamental principles of protein–ligand interactions. Structure-(SBDD) and ligand-based drug design (LBDD) approaches bring together the most powerful concepts in modern chemistry and biology, linking medicinal chemistry with structural biology. The definition and assessment of both chemical and biological space have revitalized the importance of exploring the intrinsic complementary nature of experimental and computational methods in bioactive compounds design. This talk will outline the utility and applications of structural biology toward rational ligand design aimed at the discovery of new small-molecule as drug and agrochemical candidates.

31) Tautomerase Superfamily - *good things come in small packages*

Hackert, Marvin
University of Texas at Austin

The X-ray structure of Cg10062 from Corynebacterium glutamicum reveals it to be a member of the tautomerase superfamily. The members of this family share two distinguishing features - a beta-alpha-beta structural motif of typically 60 to 80 amino acid residues and a catalytically important N-terminal proline. These proteins also exhibit a trait known as “catalytic promiscuity”, brought about by altering the active site environment around the N-terminal proline. This alters the pKa of the N-terminal proline residue enabling it to play different mechanistic roles - acting as a general acid (pKa ~9) in some cases or a general base (pKa ~ 6) in others, resulting in tautomerase, isomerase, dehydratase, decarboxylase, dehalogenase, etc. activities. Many of these bacterial proteins are found in relatively recently evolved catabolic pathways that can degrade aromatic or halogenated hydrocarbons. Cg10062 is closely related by sequence to cis-CaaD (cis-3-chloroacrylic acid dehalogenase). However, it is a poor dehalogenase lacking catalytic efficiency and isomer specificity, but exhibiting consecutive hydratase and decarboxylase activities on its initial substrate. The range of activities exhibited from this common fold support evidence for divergent evolution from a common ancestor, and illustrate how catalytic promiscuity can lead to the valuable emergence of new enzyme activities.

32) Crystallization and preliminary crystallographic analysis of the transcriptional activator ElrR from Enterococcus fecalis in apo form and in complex with DNA and with possible allosteric ligands

Horjales, Eduardo; De Groote, Michel; Salcedo, David Palomino
Instituto de Física de São Carlos/USP

Enterococcus faecalis is a common nosocomial pathogen widespread all over the world. The protein ElrR is a positive transcriptional regulator considered as belonging to Rgg family, it acts as a dimer and have a HTH N-terminal domain which binds the DNA.
The protein was concentrated in Amicon (Millipore) filter up to 10, 20 and 30mg/mL and crystallization tests were performed, using MORPHEUS (Molecular Dimensions) and SaltRx (Hampton Research) crystallization kits.
Both crystallization screen assays presented crystals of good size and diffraction quality but in MORPHEUS kit, crystals appeared after 24h. Two types of crystals were obtained. Some with octahedral form and others with plate form. Plate crystals appeared preferentially in lower protein concentration (20mg/mL) and octahedral crystals in higher concentration (30mg/ml).
These two types of crystals were X-rays diffracted at the Diamond Light Source in England, and two different datasets were obtained. Octahedral crystals presented space group P41212 and the dataset was collected at 3,2 Å resolution. Plate shaped crystals presented large anisotropic diffraction with space group P1 and we obtained a dataset at 2,7 Å resolution.
Low homologous structures could not provide phases (through molecular replacement) to solve the structure. We recently obtained crystals of the SeMet protein.
We also crystaliized ElrR in the presence of four possibleallosteric ligands, known to activate the expression of ElrA, one of the proteins coded at the operon regulated by ElrR. We also detected the DNA binding site. We also set co-crystallization experiments of ElrR with a 23 pairs double-helix DNA.

33) Protein Structural Studies at Universidade Estadual de Ponta Grossa

Iulek, Jorge
Laboratório de Estudos Estruturais de Proteínas, Departamento de Química - Universidade Estadual de Ponta Grossa (UEPG), Brasil

We could implement, few years ago at UEPG, the basic structure for heterologous protein expression, and since then we are engaged into expressing Trypanosoma cruzi proteins which have already been characterized as or have the potential to be targets for the development of drugs, through a rational and structural approach. Therefore, at the meeting, we intend to present the ongoing work at the lab with these proteins, which have as a central activity the structure determination by means of x-ray crystallography, but also include other experimental and computational techniques such as SAXS, kinetic and in vitro assays, homology modeling, docking and molecular dynamics.

34) Structural analysis by X-ray crystallography of Glutathione S-transferase ffrom shrimp Litopenaeus vannamei

Juárez-Martínez, Ariadna B.1,2; Rudiño-Piñera, Enrique1; Sotelo-Mundo, Rogerio R.3
Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Avenida Universidad 2001, Chamilpa, 62210 Cuernavaca, MOR, México 1; Facultad de Ciencias , Universidad Autónoma del Estado de Morelos (UAEM), Av. Universidad 1001, Chamilpa , 62209, Cuernavaca MOR, México 2; Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera a la Victoria Km. 0.6, Hermosillo, Sonora, México 3.

Glutathine S-transferase is ubiquitously distributed in the nature, are found in organisms as well as diverse microbes, insects, plants, fish, birds and mammals. These enzymes can catalyse the conjugation of reduced glutathione (GSH) with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulphur atom of GSH and the substrate. Experimental data suggest that sublethal concentration of metals may affect the capacity of the organism to detoxification of pesticides or xenobiotics. Our work is focused on the structural description of a Mu class GST from Litopenaeus vannamei (leg white shrimp -LvGST). Members of the same group of Cytosolic GSTs have been described as dimers form, with a molecular weigh of 25-kDa. Mu-GST have a glutathione (GSH)-binding site 0(G site) and an hydrophobic substrate-binding site (H site). We purified this protein and then we obtained crystals at different condition , the which were diffracted in the APS in Chicago, at the moment we are trying to solve the native structure and also we are really interested in test the binding of GST protein with heavy metals and then evaluate the possible effects that could have over the catalytic site and look forward which amino acids are interacting with those metals.

35) S-SAD phasing of monoclinic histidine kinase from Brucella abortus at low resolution using true redundancy and multiple crystal data collection

Klinke, Sebastian
Fundación Instituto Leloir, Buenos aires, Argentina

The structure of the histidine kinase associated with the light-oxygen-voltage domain from Brucella abortus, containing 968 amino acids in the asymmetric unit, was solved using the “off edge” anomalous signal from sulfur to a resolution of 2.9 Å, by combining data from several isomorphous crystals (spacegroup P21) as well as data collected in multiple orientations of the same crystal (true redundancy). A shorter construct, designed with the aid of the low resolution structure, enabled the production of improved crystals and subsequent refinement of the structure to 2.51 Å resolution. The importance of true redundancy in “off edge S-SAD” will be discussed.

36) The interaction of plant secondary metabolite (quercetin) with PLA2 from Bothrops brazili: spectroscopy and computational assays

Kumar, Reetesh1,2; Caruso, Ícaro P. 1,2; Ullah, Anwar1,2; Fossey, Marcelo Andres1,2; Souza, Fátima Pereira1,2; Cornélio, Marinônio Lopes1,2; Arni, Raghuvir Krishnaswamy1,2; Carvalho, Sidney Jurado1; Araújo, Alexandre Suman1
Instituto de Biociências, Letras e Ciências Exatas (IBILCE) – UNESP 1; Centro Multiusuário de Inovação Biomolecular (CMIB), IBILCE – UNESP 2

The interaction of flavonoid Quercetin with Phospholipase A2 isolated from snake venom Bothrops brazili (MTX-II) was investigated by fluorescence spectroscopy, molecular dynamic and modeling calculations. From the fluorimetric titrations conducted at 288, 298 and 308 K and at pH 8.0, the Stern-Volmer quenching constant (K_SV) and binding constant (K_b) were calculated along with the corresponding thermodynamic parameter ΔG, ΔH and ΔS at 288 and 298 K. From these analysis evidences of complex formation in between MTX-II and QCT are found. Besides that modified Stern-Volmer plot show evidences for two types of intrinsic fluorophores with different accessibilities at 308 K. The mean distance between the donor (MTX-II) and acceptor (QCT) was determined by fluorescence resonance energy transfer (FRET). The optimized structure of QCT was obtained by ab initio calculation, which geometry was performed in its ground states by using DFT/B3LYP functional with 6-311+G (d,p) basis set. Molecular dynamic and modeling studies of compacted and extended forms of MTX-II and MTX-II/QCT verified the stabilization of the dimmer and the complex in solution, respectively. These results compared with the intrinsic fluorescence of Trp and Tyr residues provide information about the forces that driven the interaction and the residues that are partaking.

37) Toward Understanding of Septin´s "G Interfaces"

Leonardo, Diego A.; Silva, Sabrina M. O.; Garrat, Richard C.
Instituto de Física de São Carlos, Universidade de São Paulo

Septins comprise a family of GTPases which can divided into 4 groups based on their amino acid sequences, and which are involved in various biological processes. A determining factor for their function is the ordered assembly of individual septins into oligomeric complexes (filaments, rings or networks). Filament formation is mediated by two interfaces: the G interface and the NC interface. Structural determinants for the correct assembly of a filament in vivo are poorly understood. The aim of this study was to characterize in silico, the G interface formed by SEPT5-SEPT8 and SEPT7-SEPT9. We aligned the amino acid sequences of the 13 human septins together with that of Septin 10 from Schistosoma mansoni and used it to identify conserved (characteristic) residues for each of the four groups. These were defined as residues present in all members of a given group but absent from all others. Using representative septins for the four groups, all ten possible G-interface dimers were modeled using MODELLER, employing SmSEPT10 as the template. The conserved residues identified above were subsequently mapped into the resulting models. Considering possible interfacial contacts between these characteristic residues, we observed interactions between Thr19-SEPT8 and Phe131-SEPT5 at the G interface of the SEPT5-SEPT8 heterodimer while at the G interface of SEPT7-SEPT9, Gln18-SEPT9 and Ser131-SEPT7 interact.

38) Fact or artifact: beyond macromolecular crystallography

Lima, Luis Mauricio T. R.
School of Pharmacy, Federal University of Rio de Janeiro – UFRJ, Brazil.

Macromolecular crystallography has long been assisting the biological and health sciences in the detailed understanding of the machinery of life and addressing the development of new biotech products. The technological advances in the physics and computing science have been pushing the modern crystallography to the ultra-high resolution level and beyond. What are the limitations for extracting significant structural information? What is the likely need for ultra-high resolution information for biomolecular crystallography? How to get the best out of the crystallographic information in association with other physico-chemical information in solution?
Support: CNPq, FAPERJ, CAPES, INMETRO, CNPEM, LNLS

39) The protein kinase PknG from Mycobacterium tuberculosis: what do we know about mechanism and substrate binding?

Lisa, Maria Natalia; Biondi, Ricardo; Alzari, Pedro
Unité de Microbiologie Structurale, Institut Pasteur, Paris, France; Research Group PhosphoSites, Medizinische Klinik I, Universitätsklinikum Frankfurt; Unité de Microbiologie Structurale, Institut Pasteur, Paris, France

Mycobacterium tuberculosis (Mtb) possesses eukaryotic-like S/T protein kinases that regulate metabolic processes and the interaction with the host. Among them, protein kinase G (PknG) is essential for mycobacterial virulence and controls the glutamate metabolism by regulating downstream partners. Then, PknG is an attractive drug target in Mtb, and we are conducting studies to better understand the molecular determinants of the enzyme activity. We will present several high resolution X-ray crystal structures of PknG together with the biochemical study of an extensive set of mutants. Notably, we have obtained PknG variants with higher catalytic activity than the wild type enzyme. These results allowed a better understanding of the role of different protein domains and motives in the regulation of the kinase activity. Also, we have been able to engineer a phosphorylation site in a normally non phosphorylatable portion of the protein. Indeed, it was found that this motif is phosphorylated in vitro, and we will present structural evidence about how this event occurs, providing information about the binding of the peptide substrate in the kinase active site. Finally, we will present evidence about how some “generally accepted ideas” in the frame of S/T protein kinases are not fulfilled by PknG, highlighting the need of considering a broader picture regarding this family of enzymes.

40) Functional and structural analysis of the putative glutamine ATP-Binding Cassette transporter from Mycobacterium tuberculosis

Lusa, Ana Letícia Gori1; Ferraz, Guilherme1; Bird, Louise2; Moraes, Isabel; Balan, Andrea1
1Brazilian National Laboratory of Biosciences, LNBio, Center of Research in Energy and Materials, CNPEM, Campinas, São Paulo, Brazil. 2Oxford Protein Production Facility, OPPF, Harwell Campus, Didcot, UK. 3Membrane Protein Laboratory (MPL), Diamond Light Source, Harwell Campus, Didcot, UK.

Tuberculosis caused by M. tuberculosis is responsible for approximately 2 million deaths each year and remains a major health challenge. The nutritional requirements for the growth of mycobacteria have been extensively studied since the discovery of M. tuberculosis. ATP binding cassette (ABC) transporters, found in eukaryotes and prokaryotes, constitute a large superfamily of multi-subunit permeases that transport various molecules across biological membranes. The glutamine is essential for growth of M. tuberculosis since it is involved with the nitrogen metabolism and its depletion also affects the pathogenesis. In this work we show the functional and preliminary structural data from the putative glutamine transporter from M. tuberculosis. In M. tuberculosis genome, Rv2563 and Rv2564 genes encode, respectively, the permease and the ATPase proteins. In order to obtain the transporter for functional and structural analysis, the Rv2563 and Rv2564 genes were cloned and submitted to expression tests in different conditions. Membrane extracts containing permeases Rv2563 were solubilized and purified using n-Decyl-β-D-Maltopyranose (DM). The ATPase Rv2564 was expressed as a soluble protein and attempts to reconstitute the transporters in liposomes have been carried out. In addition, we obtained crystals of Rv2563 that diffracted at 7 Å at the microfocus beamline X24, Diamond Light Source.

41) Crystallization assays of the long isoform of Alanine Racemase from Trypanosoma cruzi

Machado, Agnes Thiane Pereira1; Iulek, Jorge1; Silva, Marcio2
Universidade Estadual de Ponta Grossa1; Universidade Tecnológica Federal do Paraná2

Alanine Racemase, long isoform (TcAR) (EC 5.1.1.1), from Trypanosoma cruzi is an enzyme involved in the production of amino acids, more specifically, involved in the interconversion between L-alanine and D-alanine. TcAR was overexpressed in soluble form in E.coli, which was then submitted to affinity purification on a nickel column. The fractions containing the purified TcAR were concentrated by ultrafiltration up to 10 mg/mL and then assayed for crystallization by the vapor diffusion method. In all 96 drops prepared, only amorphous precipitate was observed. Beside the experimental work, TcAR was modeled by homology with the program Modeller.

42) Three-dimensional structure determination of the catalytic domain of PlpD, a virulence factor in Pseudomonas aeruginosa

Madeira, P. V. M; Neves, D; Dessen, A
Brazilian National Laboratory for Biosciences (LNBio), CNPEM, Campinas, São Paulo, Brazil; Brazilian National Laboratory for Biosciences (LNBio), CNPEM, Campinas, São Paulo, Brazil; Bacterial Pathogenesis Group, Institut de Biologie Structurale (IBS), Université Grenoble I, 41 rue Jules Horowitz, 38027 Grenoble, France

According to the World Health Organization, infectious diseases are the second leading cause of deaths worldwide. P. aeruginosa is an opportunistic pathogen, multidrug-resistant and the main Gram negative cause of nosocomial infections, this pathogen may lead to death due to pneumonia and septcemia immunocompromised patients, especially patients with cystic fibrosis, AIDS and burn victims. The Patatin-Like Protein D (PlpD) founded in P. aeruginosa strains (PA01 and PA14) shows a catalytic domain that is secreted by the bacterium and shows lipase activity, important for the infection process. As P. aeruginosa, as well as other pathogens have become multidrug resistant, the search for new therapeutic targets is being encouraged. The understanding of the structural components involved in the infective process is essential for the development of new therapeutic agents. The structure of the secreted portion of PlpD protein which has catalytic activity was resolved at 2.14 angstroms resolution. The protein analysis showed interesting differences between its structure and its homologous ExoU, providing evidences to the characterization of its mechanism of action at a structural level. This work was developed as part of the characterization of protein PlpD performed by the Macromolecular Engineering Systems Laboratory group in Marseille, France under the coordination of Dr Sophie Bleves.

43) DNA allostery: Atomistic insights into the signal transduction mechanisms

Machado, Matias R.1; Pantano, Sergio1; Dans, Pablo D.2
1Institut Pasteur de Montevideo, Mataojo 2020, 11400, Montevideo, Uruguay
2Institute for Research in Biomedicine, Baldiri Reixac 10, 08028, Barcelona, Spain
Corresponding author: spantano@pasteur.edu.uy

The DNA is a molecule able to store a lot of information in its structure ranging from protein sequences to regulatory elements. Its transcription is tightly controlled by an orchestra of proteins which have to play in a coordinated order. By far the direct interaction between transcription factors (TFs). However, recent evidence suggests a more active role for DNA in which part of the signal could be mechanically transmitted though its structure as perturbations1. Several questions about this mechanism, which is referred as distortion transmission or allosteric communication, remain to be answered such as: What atomistic details give rise to the transmission of information? Which structural patterns are altered during protein binding and how far do the perturbations travel along the sequence? To address these questions, we applied state of the art molecular dynamic simulations on five different viral core promoter sequences of 100bp long. We evaluated their structural behavior in the presence and absence of bounded TFs at the beginning of the sequence. To afford the computational cost of simulating large systems for long time scales, we used the SIRAH forcefield [http://www.sirahff.com], which is a coarse grained potential developed in our group for treating DNA in either a simplified or multiscale representation within an implicit or explicit solvent environment2-4. The possibility to reach multi-microsecond time scales allowed us to explore the cross talk between DNA and TFs. The results showed that the base pair opening is affected up to 10bp away from the TFs binding site in a clear sequence dependent way. On the other hand, distortions on helical twist and groove sizes reached 50bp (170Å) independently of the sequence. Moreover, the oscillatory pattern found for minor and mayor grooves support the proposed role of DNA mechanics in allostery1. From that observations we derived a physical model to explain and predict the protein-protein communication trough the DNA. These findings supports the idea that the symmetry of the DNA and the location of regulatory boxes are linked properties.

References

[1]Kim S, et al. Science 339:816-9, 2013.
[2]Dnas PD, et al. J Chem Theory Comput 6:1711-25, 2010.
[3]Darré L, et al. J Chem Theory Comput 6:3793-807, 2010.
[4]Machado MR, et al. Phys Chem Chem Phys 13:18134-44, 2011.

44) Synthesis and characterization of substituted (N-phenylamino)oxoacetic acids as PTP1B inhibitors

Mandapati, Kishore R.; Fonseca, Emanuella M. B.; Scorsato, Valéria; Ferreira-Halder, Carmen V.; Pilli, Ronaldo A.; Aparício, Ricardo
Institute of Chemistry; Institute of Biology

Cancer emerges today as a major public health problem in developing countries, with both incidence and mortality increasing at alarming rates. Over the last few decades, it has become extremely important the developing of new therapeutic strategies. Protein tyrosine phosphatases (PTPs) have gained importance and emphasis due to their role in various cell signaling pathways, and have emerged as potential targets for developing new drugs against cancer. In particular, the protein tyrosine phosphatase 1B (PTP1B), which is overexpressed in many types of malignant tumors, has been identified as an important target for new anticancer therapies. Based on docking studies, we have selected and synthesized a focused library of molecules displaying a substituted (N-phenylamino)oxoacetic acid moiety, which have been assayed against PTP1B. Compounds MKR -10a/-17a/-23a/ and 20a were quite efficient in inhibiting PTP1, which displayed IC50 values in a micromolar range. In this study, we present preliminary results including kinetic assays and computational analysis of PTP1B and the selected compounds. Crystallographic studies are in course to provide structural information on the interaction site of ligand and enzyme. Acknowledgements: TWAS/CNPq, FAPESP, OpenEye Scientific Software.

45) Understanding the behavior of the 6aJL2 amiloidogenic protein

Martinez, Roberto C. Maya; Tello, Carlos Amero
Universidad Autonoma del Estado de Morelos; Universidad Autonoma del Estado de Morelos

Primary amyloidosis is a disease characterized by the amyloid fibril formation of immunoglobulin light chains proteins. We have used NMR to characterize the lambda 6a germline protein and its single point mutation at position 24. Whereas structurally both proteins are very similar, they have different amyloid fibril formation propensities. To get a better understanding of the correlation between movements and the amyloid formation, we have investigated their dynamics. At fast time scales both proteins are very similar, nevertheless, we have found that at slower scales (relaxation-dispersion and H/D exchange) the single point mutation affects different parts of the protein. These differences in specific regions may allow the R24G mutants to reach amyloid fibers-competent states more efficiently than the germline protein.

46) Introducing Protein Structure In Undergraduate Courses And In Outreach Activities To High School

Martínez–Oyanedel, José; Marta, Bunster Marta
Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Biológicas, Universidad de Concepción

Proteins are a major component of all the cells and are responsible for carrying out many of the functions of living organisms. Understanding the function these complexes molecules is crucial to appreciate these entities as three-dimensional objects because all molecular interactions are mediated through the surfaces of the interacting components.
Most of the biochemistry courses included the structure and function of proteins, teachers similar to biochemistry books are present and discussed these topics in two-dimensional and static. This drawback has been partially solved with the use of websites that allow you to moving and interactively visualize molecules and using molecule visualization software. In the case of protein crystallography, this technique is only given for their potential and most important steps.
In our department we have added two strategies recommended by various authors. Incorporation of 3D visualizations using bicolor stereo images and visualization with red and cyan lenses, and the use of kit to build proteins and DNA models.
Programs like Chimera to prepare images and using DeepView for display molecules in guided sessions on computers, where students can rotate and examine molecules have allowed students not only be excited about the classes but also incorporate the concept of three-dimensionality. Additionally the use of educational kit of LAM, have allowed to the students have better understood of the folding processes and stabilization of protein conformation.
Also DeepView has allowed to our students to develop activities of adjustment of the side chains of some amino acids to electron density maps, recreating the work of crystallographers.
To introduce the protein crystallography to the high school we develop a activity supported by the Biophysical Society where 50 high school students attend for a day to lectures and practical on protein structure, spectroscopy and microscopy.

47) Crystallization and preliminary crystallographic analysis of LipC12, a true lipase isolated through a metagenomics approach

Martini, V.P.; Glogauer, A.; Iulek,, J.; Souza ,E. M.; Pedrosa, F.O.; Krieger, N.
Federal Institute of Parana; Federal University of Parana; State University of Ponta Grossa

The lipases are among the most widely used biocatalysts in organic chemistry. The search for new lipases and esterases that are able to adapt to non-conventional biocatalysis is active. Approaches have been used to obtain biocatalysts that can better adapt to non-physiological conditions. Among these is the screening of metagenomic libraries. This study aimed at screening a new functional lipase from a metagenomic library, built from an oil contaminated soil for use in biocatalysis. LipC12, a true lipase from family I.1 of bacterial lipases, which was previously isolated through a metagenomics approach, contains 293 amino acids. Compared to lipases of known three-dimensional structure, it has a sequence identity of 47% to the lipase from Pseudomonas aeruginosa PAO1. Recombinant

N-terminal His6-tagged LipC12 protein was expressed in Escherichia coli, purified in a homogenous form and crystallized in several conditions, with the best crystals being obtained using 2.0 M sodium formate and 0.1 M bis-tris propane pH 7.0. X-ray diffraction data were collected to 2.70 A ° resolution. The crystals belonged to the tetragonal space group P4122, with unit-cell parameters a = b = 58.62, c = 192.60 A °. Its 3D structure should provide the basis for the application of protein engineering techniques aimed at obtaining a modified LipC12 with improved characteristics for use in biocatalysis.

48) Mechanism of Autophosphorylation in Histidine Kinases: Crystallographic Snapshots of the Reaction

Mechaly, Ariel E.*; Sassoon, Nathalie; Betton, Jean-Michel; Alzari, Pedro M.
Institut Pasteur, Unité de Microbiologie Structurale & CNRS UMR, France; *Present address: Institut Pasteur de Montevideo, Unit of Protein Crystallography, Uruguay

Histidine kinases (HKs) are dimeric receptors that participate in most adaptive responses to environmental changes in prokaryotes. In prototypical HKs, an extra-cytoplasmic sensor region couples to two highly conserved catalytic modules -termed DHp and CA domains- at the cytoplasm, through an adaptor HAMP domain. Although it is well established that stimulus perception induces HK activation via its autophosphorylation, little is known on how input signal propagates through HAMP, and how DHp and CA domains transiently interact during this reaction. Recently, we reported crystal structures of the full cytoplasmic region of CpxA, a HK involved in E. coli response to envelope stress and resistance to some antibiotics. An ensemble of asymmetric conformations captured in these structures, unveil HK activation as a highly dynamic process that requires rupture the cytoplasmic transmitter core symmetry. Our biochemical and structural data also support the notion that it proceeds through concerted segmental movements of HAMP and DHp α-helices that facilitate the formation of the autophosphorylating Michaelis complex. Previously elusive, this conformational state, in which one of the CA domains transiently approaches its bound ATP to the phosphorylatable histidine residue in the DHp domain, has been trapped in some of the structures.

49) Expression, Purification and Crystallization Trials of Histidine Ammonia-Lyase from Trypanosoma cruzi

Miranda, R. R.; Iulek, J.; Barison, M. J.; Melo, R.; Silber, A. M.; Silva, M.
Department of Chemistry, State University of Ponta Grossa, Ponta Grossa, Brazil; Department of Parasitology, University of São Paulo, São Paulo, Brazil; Department of Education, Federal Technological University of Paraná, Ponta Grossa, Brazil

The objectives of this research were to express, purify and perform crystallization trials of TcHAL. The enzyme was cloned into vector pET-28a(+) fused to a tail of 6 histidines at the N-terminus and expressed in soluble form in E. coli. The expression was carried out at 25 °C for 16 hours, after induction with IPTG 0.5 mmol L-1. Purification was performed on affinity chromatography on nickel-agarose column and the protein was eluted at circa 200 mmol L-1 in an increasing imidazole gradient. After electrophoretic analysis, the protein TcHAL pure fractions were pooled and concentrated and monitored by the Bradford assay to reach 10 mg mL-1. Crystallization trials were performed with hanging drops, containing 500 µL precipitant solution were in the reservoir and the drops were prepared with 3 µL of protein solution with 3 µL of reservoir solution. The crystallization kits used were Morpheus Screen and JCSG + Suite. The crystals were obtained under the following conditions, as described for the Morpheus Screen kit: i) 10 % w/v PEG 8000, 20 % v/v ethyleneglycol, 0.12 M etyleneglycols, 0,1 M buffer system 1, pH 6,5; ii) 10 % w/v PEG 8000, 20 % v/v ethyleneglycol, 0.12 M monosaccharides, 0.1 M buffer system 1, pH 6,5 and iii) 10 % w/v PEG 8000, 20 % v/v ethyleneglycol, 0.12 M monosaccharides, 0.1 M buffer system 2, pH 7,5. The best crystals will be subjected to analysis by X-ray diffraction.

50) Structural characterization of the enzymes in the naphthalene degradation pathway: filling the gaps

Nagem, Ronaldo Alves Pinto
Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG)

Polycyclic aromatic hydrocarbons (PAHs) are a group of aromatic hydrocarbons composed of two or more fused rings and include naphthalene, anthracene, phenanthrene, pyrene and benzo[a]pyrene amongst others (Haritash & Kaushik, 2009). A number of studies have attested to the carcinogenic and mutagenic properties of PAHs (Park et al., 2006; Spink et al., 2008; Topinka et al., 2008), suggesting the need for further work on the elimination of these compounds from the environment.
The Gram-negative bacterium *P. putida* G7 is among the best studied naphthalene-degrading species. In *P. putida* G7 the naphthalene-oxidation genes are organized into two operons under salicylate control. The first operon, which is associated with the upper naphthalene-degradation pathway, includes the genes nahAaAbAcAdBCDEF, which code for the conversion of naphthalene to salicylate, while the second operon includes the genes nahGTHINLOMKJ coding for the lower pathway that includes the oxidation of salicylate via the catechol meta-cleavage pathway (Yen & Gunsalus, 1985). In the last years, our group focused on the structure elucidation and the kinetic characterization of the *P. putida* G7 enzymes involved in the naphthalene degradation pathway. We intend to present these results which basically describe the 3D structures of NahB, NahF, NahG, NahI, NahK and NahK/NahL complex and some kinetic data of these enzymes and their mutants.
Haritash, A. K. & Kaushik, C. P. (2009). J. Hazard. Mater. 169, 1–15.
Park, S.-Y., Lee, S.-M., Ye, S.-K., Yoon, S.-H., Chung, M.-H. & Choi, J. (2006). Toxicol. Lett. 167, 27–33.

Spink, D. C.,Wu, S. J., Spink, B. C., Hussain, M. M., Vakharia, D. D., Pentecost, B. T. & Kaminsky, L. S. (2008). Toxicol. Appl. Pharmacol. 226, 213–224.
Topinka, J., Marvanova´ , S., Vondra´ cek, J., Sevastyanova, O., Nova´kova´, Z., Krcma´ r, P., Pencı´kova´ , K. & Machala, M. (2008). Mutat. Res. 638, 122–132.
Yen, K.-M. & Gunsalus, I. C. (1985). J. Bacteriol. 162, 1008–1013.

51) Expression, crystallization and structure determination of the surface protein SdrE from Staphylococcus aureus

Neves, David; Job, Viviana; Trindade, Daniel Maragno; Dessen, Andrea
University of Brasilia, Institut de Biologie Structurale Jean-Pierre Ebel, Brazilian Biosciences National Laboratory, Institut de Biologie Structurale Jean-Pierre Ebel/Brazilian Biosciences National Laboratory

Staphylococcus aureus represents a major problem for public health authorities being the cause of nosocomial infections and acquiring resistance for several antibiotics. The complement cascade is responsible for controlling bacterial infections and is triggered in the first moments of bacterial's entry. The major fluid-phase complement regulator Factor H (fH) is plasma protein that accelerates the decay of the alternative pathway C3 convertase, avoiding opsonization of self cells. However, S. aureus has evolved several methods for interfering in the complement cascade avoiding its effector phase. The surface protein SdrE from S. aureus has been shown to sequester fH from plasma and disrupts the alternative pathway convertase. In order to provide structural insight in how is the interaction, the important residues to form the complex we have expressed, purified and crystallized the SdrE in its apo form. The crystals were diffracted and the structure was determined by molecular replacement. As next step we are going to find which part of the fH binds to SdrE and try to crystallize the complex.

52) Protein PXRD in Brazil: features and challenges

Nunes, Rafael S.; Paiva-Santos, Carlos O.
IQ-Araraquara

Protein x-ray powder diffraction in Brazil still does not exist; however, Protein Crystallography and Powder x-ray diffraction are very engaged in this country, working separately up to this moment. Since Von Dreele and co-workers work (Von Dreele et al., 2000), Protein PXRD (powder x-ray diffraction) has become very important to provide additional and/or complementary information about protein crystallography. Scientific community, in these 14 years, has dedicate time to improve beamlines at Synchrotrons Facility around the World (Margiolaki et al., 2007). In view of some contributions as discovery of proteins polyphasic, the advantage, in general, to make powder materials and the unexplored protein materials crystallography (Von Dreele, 2013), this work brings to Brazilian community a discussion about how are our possibilities to adapt the actual availability of equipment at LNLS to start this application.

Von Dreele, R. B., Stephens, P. W., Smith, G. D., Blessing, R.H. (2000). Acta Cryst. D. 56, 1549-53. Von Dreele, R. B. (2013). Introduction to Protein Powder Diffraction: Overview, International Workshop: Powder & Electron Crystallography, in press. Margiolaki, I., Wright, J. P., Fitch, A. N., Fox, G. C., Labrador, A., Von Dreele, R. B., Miura, K., Gozzo, F., Schiltz, M., Besnard, C., Camus, F., Pattison, P., Beckers, D., Degen, T. (2007). Z. Kristallogr. Suppl., 26, 1-13.

53) Investigating Membrane Structure with Small Angle Scattering

Oliveira, Cristiano L. P.
Institute of Physics, University of São Paulo

In this work it is presented a new modeling method [1] in which the electron density profile of planar bilayers is described by a set of Gaussian functions. The main advantage of this new approach is the fact that the scattering intensity is adjusted directly, without the need of the pair distance distribution function and also without any previous assumptions for the profile and sample composition. By the use of an automatic optimization procedure based on partial constrained least squares routine coupled with the point of inflexion method, it is possible to optimize simultaneously the form factors, structure factors and several other parameters that describe the model. The applicability of this method was already demonstrated in several applications in the literature [2,3,4,5] permitting a detailed characterization on the membrane structure.

[1] Oliveira, C. L. P. et al (2012). Journal of Applied Crystallography 45, 1278-1286. [2] Trevisan, J. E. et al (2011). Non-Viral Gene Therapy, edited by X. Yuan, pp. 267-292: InTech. [3] Balbino, T. A. et al, (2012). Langmuir 28, 11535-11545. [4] Balbino, T. A. et al, (2013). Chemical Engineering Journal 226, 423-433. [5] Gerbelli, B. B. et al, (2013). Langmuir 29, 13717-13722.

54) Crystal structure of BA42, a protein from the Antarctic bacterium Bizionia argentinensis comprised of a stand-alone TPM domain

Otero, Lisandro H.1,3; Klinke, Sebastián1,3; Aran, Martin1; Smal, Clara1; Pellizza, Leonardo1; Goldbaum, Fernando A.1,3; Cícero, Daniel O.2
1Fundación Instituto Leloir, IIBBA-CONICET, Patricias Argentinas 435 (C1405BWE) Buenos Aires, Argentina. 2Dipartimento di Scienze e Tecnologie Chimiche, Università di Roma "Tor Vergata", via della Ricerca Scientifica SNC (00133) Rome, Italy. 3Plataforma Argentina de Biología Estructural y Metabolómica PLABEM, Patricias Argentinas 435 (C1405BWE) Buenos Aires, Argentina
e-mail: lotero@leloir.org.ar

The structure of the BA42 protein belonging to the Antarctic flavobacterium Bizionia argentinensis was determined by X-ray crystallography. This is the first structure of a member of the PF04536 family comprised of a stand-alone TPM domain. The structure reveals a new topological variant of the four β-strands constituting the central β-sheet of the αβα architecture and a double metal binding site stabilizing a pair of crossing loops, not observed in previous structures of proteins belonging to this family. BA42 shows structural differences in the presence or absence of bound metals. The affinity for divalent metal ions is close to that observed in proteins that modulate their activity as a function of metal concentration, anticipating a possible role for BA42.

55) The molecular basis of the relaxation and activation of tarantula striated muscle thick filament is revealed by the disclosure of the myosin interacting-heads motif structure

Padrón, Raúl
Centro de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC), Apdo. 20632, Caracas 1020A, Venezuela.

Muscle contraction involves interactions of thin and thick filaments. The myosin molecule, which assembles forming thick filaments, has a tail with two heads. The tails pack forming the filament backbone with heads protruding helically. Relaxation is crucial for muscle function, and thick filaments are essentially involved in it; consequently the determination of thick filament structure is fundamental.

We determined the structure of the 4-5µm long tarantula thick filaments. Cryo-EM studies of 2D-crystals of chicken smooth muscle myosin suggested that the relaxed state was achieved by an asymmetric, intramolecular interaction between the acting -binding region of one myosin head and the converter region of the other, switching both heads off. By cryo-EM and 3D-reconstruction we found that this myosin interacting-heads motif underlies the relaxed state also on thick filaments from tarantula striated muscle (Nature 436: 1195-1199, 2005). The motif is present in species separated by at least 850 million years of independent evolution, both in thick filaments from striated muscle (cnidaria, arthropods, mollusks, vertebrate cardiac muscle) and from smooth muscle (platyhelminthes). The uniqueness of the motif, widely present in striated skeletal or cardiac, smooth and in nonmuscle cells, highly conserved since vertebrates and invertebrates diverged, implies that it is of fundamental functional importance as being the conserved structural mechanism that explains the relaxed state in muscle.

The motif is only established in relaxing conditions when both heads are bent producing the required conformations for establishing five intramolecular interactions as well as two intermolecular interactions allowing the filament formation. Intramolecular interactions are conserved and are the basis for a general mechanism for inducing muscle relaxation and for switching off myosin II-based motile activity in both muscle and nonmuscle cells.

The tarantula striated muscle motif structure (PDB 3DTP) together with *in vitro* motility assays, sequence analysis, mass spectrometry, western blots, immunofluorescence, all-atom molecular dynamic simulations and flexural rigidity measurements led us to propose a molecular mechanism for the cooperative phosphorylation-based thick filament activation and a model for activation, potentiation and post-tetanic potentiation in tarantula striated muscle; controlled by a disorder-to-order transition in the N-terminal extension of the myosin regulatory light chain.

In one hand, the striated muscle motif (PDB 3DTP) is involved in the so-called super-relaxed state proposed for relaxed vertebrate skeletal and cardiac muscle fibers, characterized by a slow ATP turnover rate, particularly slower in animals like tarantulas that spend long periods completely immobile but ready to quickly capture a prey. In the other hand, the smooth muscle motif structure (PDB 1i84) has helped understanding its mechanism of activation and the role of the myosin essential light chain on its activation.

The motif structure is important for understanding the role of mutations in human hypertrophic cardiomiopathy, which affect the human motif intramolecular interactions; the adaptive thermogenesis by human skeletal muscle myosin, playing a role in calories dissipation during overfeeding and regulated by pharmaceuticals targeting the human motif; and for understanding the action mechanism of praziquantel, the used to treat Schistosomiasis, possibly targeting the myosin regulatory light chains of the schistosome motif.

Supported by the Howard Hughes Medical Institute (HHMI), U.S.A.

56) SIRAH: a new Coarse-Grained Force Field for Multiscale Simulations

Pantano, S.
Institut Pasteur de Montevideo, Uruguay

Despite the robusness and reliability achievable by molecular simulations, a direct comparison with experimental data is often difficult owing to the large size and long time scales needed for a proper description of biological systems. This have motivated the development of coarse grained (CG) methods aimed to bridge the gap between experiments and simulations. SIRAH is a CG force field for molecular dynamics simulations of biomolecular systems, which includes parameters for water and electrolytes [1], DNA [2] and proteins [3]. Interactions are treated using a typical Hamiltonian for classical molecular dynamics simulations, allowing for a rigorous treatment of long-range electrostatics via PME. Furthermore, the CG parameters can be straightforwardly combined with atomistic force fields to perform dual-resolution simulations, in which i) solutes (i.e., proteins or membranes) can be treated at fully atomistic detail while the bulk water can be simulated at a supramolecular level [4]; or ii) DNA filaments can be simulated at CG resolution with regions of interest including atomistic nucleobases [5] (www.sirahff.com).
This contribution will present some of the latest developments of this force field applied to the study of cAMP driven allosteric transitions in proteins and its use to design novel fluorescent sensors for cAMP in living cells.

References

[1] Darré L, Machado MR, Dans PD, Herrera FE, Pantano S. JCTC, **6**:3793. (2010)
[2] Dans PD, Zeida A, Machado MR, Pantano S. JCTC, **6**:1711. (2010)
[3] SIRAH : A structurally unbiased force field for Coarse-Grained simulation of proteins. Submitted 2014
[4] Darré L, Tek A, Baaden M and Pantano S. JCTC, **8**, 3880. (2012)
[5] Machado MR, Dans, PD and Pantano S. PCCP. **13**:18134. (2011)

57) Understanding the interaction between kidney-type glutaminase (KGA) and peroxisome proliferator-activated receptor gamma (PPARg)

Pascoal, Camila
Laboratório Nacional de Biociências

Several experiments from our research group have demonstrated that KGA and PPARg seem to be interaction partners. In a cellular context, confocal microscopy and Fluorescence Resonance Energy Transfer (FRET) assays confirmed that KGA and PPARg are co-localized in the cytoplasm. Moreover, two-hybrid assays have shown that the interacting regions are the N-terminal and the LBD of KGA and PPARg, respectively. The interaction hypothesis was also supported by heterologous expression and purification of both proteins followed by fluorescence anisotropy assays, which revealed low dissociation constants. Therefore, we decided to use X-ray protein crystallography for the structural resolution of the complex formed between KGA and PPARg. So far, we have performed a large screening of crystallization conditions followed by the refinement of the promising ones.

58) A Cryo-Electron Microscopy Facility for Single Particle Analysis at the National Center for Energy and Materials Research

Portugal, Rodrigo Villares
Brazilian Nanotechnology National Laboratory – LNNano – CNPEM (rodrigo.portugal@lnnano.cnpem.br)

The study of vitrified solutions of biological macromolecules by transmission electron microscopy (Cryo-EM) is a powerful structural biology technique that is now reaching maturity. Technological advances of computer-controlled cryogenic electron microscopes, of camera technology allowing the direct detection of electrons, and of data processing methodology brought cryo-EM to a new level of resolution. Recent successes include the elucidation of multiple conformations of macromolecular complexes at near-atomic resolution. The Brazilian Nanotechnology National Laboratory (LNNano) at the National Center for Energy and Materials Research (CNPEM) is establishing the necessary infrastructure to provide single particle cryo-EM as one of the available techniques at the Electron Microscopy Laboratory (LME/LNNano), an open national facility. In this presentation, I will discuss the implementation of this infrastructure as well as discuss the first results obtained at the new facility.

59) Intrinsic disorder and unstructural biology

Prat-Gay, Gonzalo
Fundación Instituto Leloir, Buenos Aires, Argentina

The indisputable fact that the paradigm one structure-one function is no longer a dogma in biology places protein intrinsic disorder at the center of the scene. Over 30% of eukaryotic genomes are composed of disordered regions, and these are particularly overrepresented in both physiology (signaling networks) and pathology (viruses). Understanding structural biology of intrinsically disordered proteins requires the analysis of ensembles of discrete but multiple conformations in solution and requires a collection of complementary approaches such as biophysics, bioinformatics, computational analysis and protein engineering. We use a paradigmatic viral intrinsically disordered oncoprotein, papillomavirus E7, to address fundamental questions in the era of “unstructural biology”.

60) Oxidative Postranslational Modifications in Proteins: Structural Biology Studies on Tyrosine Nitration

Radi, Rafael
Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Oxidative postranslational modifications in proteins occur continuously as part of aerobic life under normal metabolic conditions. Oxidized proteins accumulate during the aging process and also under inflammatory and degenerative cellular and tissue conditions. Oxidized proteins represent biomarkers of oxidative stress and, in some cases, they undergo structural and functional changes of pathophysiological relevance. Among the various oxidative modifications in amino acid residues, we have focused on tyrosine nitration, a free radical process that yields protein-3 nitrotyrosine with the participation of nitric oxide-derived oxidants such as peroxynitrite. I will present recent structural biology studies that help to rationalize at the molecular level why tyrosine nitrated proteins can undergo changes in structure and function. The cases of cytochrome c and Mn/Fe-superoxide dismutases will be examined in detail. The current studies show the relevance of structural biology approaches to unravel how oxidative postranslational modifications in proteins may impact in cell physiology and pathology.

61) Vascular smooth muscle relaxation induced by a mannose binding lectin from Cymbosema roseum: evidence of endothelial NOS pathway

Rocha, Bruno Anderson Matias; Barroso-Neto, Ito Liberato; Teixeira, Claudener Souza; Pires, Alana de Freitas; Delatorre, Plinio; Assreuy, Ana Maria Sampaio; Cavada, Benildo Sousa
Universidade Federal do Ceará; Universidade Estadual do Ceará; Universidade Federal da Paraíba

Lectins comprise a large family of proteins able to specifically and reversibly recognize carbohydrates via the carbohydrate-recognition domain (CRD). Here, the three-dimensional structure of the mannose-binding lectin isolated from Cymbosema roseum (CRLI) (1.65 Å) was determined with X-Man molecule modeled into the carbohydrate recognition domain. CRLI relaxant activity in thoracic rat aorta was also investigated, and based on the results, a molecular docking of CRLI with heparan sulfate (HS) was performed to investigate the possible interaction with mechanoreceptors involved in vasorelaxation. CRLI elicited vasorelaxant response in endothelialized rat aorta contracted with phenylephrine. Endothelium-derived relaxant factors, extracellular calcium (Ca2+e) and muscarinic receptors were also evaluated as putative participants in the CRLI relaxant effect. CRLI relaxant effect was blocked by L-NAME, a nonselective inhibitor of nitric oxide synthase (NOS), and partially inhibited in a calcium-free solution (0Ca) and by atropine, but it remained unchanged in the presence of indomethacin and TEA. In summary, our data suggest interaction between CRLI and muscarinic receptors located in vascular endothelial cells leading to NOS activation triggered by a mechanism that involves Ca2+e along with the ability of CRLI to interact with heparan sulfate, a highly rated mechanoreceptor involved in eNOS activation.

62) Structural and functional characterization of plant pathogenesis-related proteins implicated in allergic diseases

Rodríguez-Romero, Adela
Instituto de Química, Universidad Nacional Autónoma de México. Circuito Exterior, CU. México, D.F 04510.

Allergic diseases mediated by IgEs are increasing worldwide, particularly in low and middle-income countries. Natural rubber from *Hevea brasiliensis* contains several proteins involved in this type of allergy. In general, these proteins participate in plant defense mechanisms and are also implicated in cross-reactivity reactions with allergens from fruits, pollen and insect venoms. Scarce structural information exists about these proteins and the events that lead to the symptoms of allergy. Natural rubber contains defense proteins, which are also allergens, such as endo-β-1,3-glucanase (Hev b2), lectins (Hev b 6.02) and chitinase-like proteins (Hev b 11). We have determined the three-dimensional structure of an endogenous glycosylated isoform of Hev b 2. The electron density maps show three post-translational modifications that include two glycosylation sites. We showed that the glycosylation pattern is important in IgE recognition by patient sera. In addition, a dimer was found in each asymmetric unit that could reflect a regulatory mechanism of this plant defense protein.
Furthermore, we have isolated two isoforms of a chitinase-like protein from *H. brasiliensis* latex, one with a larger chitin-binding domain. Both proteins were reverted back to active chitinases by substituting an Ala residue by a catalytic Glu at the active site. The crystal structure of the catalytic domain showed evidence that even when the architecture of the active site groove is similar to those in active chitinases, there are minor differences in some residues that could account for differences in binding-specificity and affinity. Our results suggest an adaptive functional diversification of chitinases, or chitinase-like protein families, involved in defense mechanisms in plants. Finally, several proteins from rubber gloves have been characterized corroborating the high stability of at least two allergens. All this information contributes to develop diagnostic strategies.

This work was supported by DGAPA-UNAM (Grant IN207613) and CONACYT (Grants 82947 and 166472). We thank M. Sc. Georgina Espinosa Pérez from Instituto de Química-UNAM for technical assistance. Latex and leaves from rubber trees were obtained from the Experimental Camp “El Palmar”, Tezonapa, Ver.

63) Structural and biophysical studies of human Methionine Adenosyltransferases

Rojas, Adriana
Parque Tecnológico de Bizkaia, Espanha

Transmethylation, the transfer of a methyl group between molecules, plays a central role in fundamental biological processes such as, cell growth, gene expression and apoptosis. S-adenosylmethionine (SAMe) molecule, which is synthesised by methionine adenosyltransferase (MAT), is the main source of methyl groups in all living organisms. MAT enzymes are conserved from bacteria to mammals, thus highlighting its essential regulatory function in maintaining the appropriate levels of SAMe. In humans, there are 3 genes, MAT1A, MAT2A and MAT2B, which encode MAT enzymes. MAT2A and MAT2B transcribe MATα2 and MATβ enzymes subunits, respectively, with catalytic and regulatory roles. MATα2 expression confers a cell growth advantage and is considered increasingly important for differentiation and apoptosis, for instance, in human hepatocellular carcinoma (HCC), colon cancer and leukemic cells. Despite the central role of MATα2β complex, its nature has remained elusive and is still unclear how MATβ regulates the activity of MATα2. Recently we have solved the structure of the 258kDa MATα2β complex. Our structural and biophysical studies shed light on SAMe production and its regulation, providing clues for potential anticancer-therapies.

64) Structural analysis of Leishmania major Adenylosuccinate Lyase

Rosa e Silva, Ivan; Mantovani, Monique; Reboredo, Eduardo Horjales; Thiemann, Otavio Henrique
Physics and Informatics Department, Physics Institute of Sao Carlos, University of Sao Paulo - USP, Sao Carlos, SP, Brazil

Leishmania major is dependent on purine recycling and host purines due to the lack of a de novo biosynthetic pathway. We characterized the three-dimensional structure of L. major Friedlin adenylosuccinate lyase (ASL), a key enzyme in the conversion of adenylosuccinate into AMP. Crystals grown by the hanging drop vapor diffusion method adopted I4122 space group, with unit-cell parameters of: a = 130.0 Å, b = 130.0 Å, c = 316.8 Å and α = β = γ = 90.0º. L. major ASL structure was determined by molecular replacement phasing using Trypanosoma brucei ASL crystallographic structure (PDB 4EFC – Wernimont et al, 2013) as a search model and refined to a Rwork of 20.59 % and an Rfree of 22.63 % at 2.2 Å resolution. The asymmetric unit consists of two subunits of the ASL molecule and a functional tetramer was created by a twofold symmetry operation. L. major ASL conserved regions from L36-A45 and A93-S101, which are spatially separated from each other in the monomer, come together from three different subunits in the tetramer to form the active site. The catalytic flexible loop was not observed in L. major ASL structure, although sulfate ions were identified in the substrates sites. Taken together, our data give new insights into the catalytic mechanism of ASL from trypanomatids.

65) Using X-rays to describe electronic fluxes in REDOX enzymes

Rudino-Pinera, Enrique; De la Mora, Eugenio; Serrano-Posada, Hugo; Rojas-Trejo, Sonia P.; Cardona-Felix, Cesar S.; Rodriguez-Almazan, Claudia
Instituto de Biotecnología, UNAM, Avenida Universidad 2001, Cuernavaca, Morelos, México.

X-ray radiation induces two main effects at metal centres contained in protein crystals: radiation-induced reduction and radiolysis and a resulting decrease in metal occupancy. In blue multicopper oxidases (BMCOs), the geometry of the active centres and the metal-to-ligand distances change depending on the oxidation states of the Cu atoms, suggesting that these alterations are catalytically relevant to the binding, activation and reduction of O_2. In this work, the X-ray-determined three-dimensional structure of laccase from bacterial and fungus, are described. By combining spectroscopic techniques (UV-Vis, EPR and XAS) and X-ray crystallography, structural changes at and around the active copper centres were related to pH and absorbed X-ray dose (energy deposited per unit mass). Depletion of two of the four active Cu atoms as well as low occupancies of the remaining Cu atoms, together with different conformations of the metal centres, were observed at both acidic pH and high absorbed dose, correlating with more reduced states of the active coppers. These observations provide additional evidence to support the role of flexibility of copper sites during O_2 reduction. This study supports previous observations indicating that interpretations regarding redox state and metal coordination need to take radiation effects explicitly into account.

66) Structural and functional characterization of human tandem-repeat galectins

Rustiguel, Joane K.; Kamugai, Patricia; Baruffi, Marcelo; Costa Filho, Antonio José da; Nonato, Maria Cristina
Faculdade de Ciências Farmacêuticas de Ribeirão Preto – USP, Ribeirão Preto, Brasil

From the broad spectrum of macromolecules, the lectins correspond to a proteic group related to the ability to recognize carbohydrates and glycoconjugates. From this group of proteins, the galectins display specific affinity for b-galactosides. Galectins are found in a variety of cell types and are involved in several biological phenomena including cell adhesion, tumor progression and metastasis. The structural and functional studies available for the lectins reveal that the ability of binding to carbohydrates is dependent on a domain defined as carbohydrate recognition domain (CRD). Based on the number and organization of the CRDs, members of the galectin family are classified in three subgroups: proto, chimera and tandem-repeat. The latter is composed of proteins with two CRDs with difference in terms of ligand specificity, located at the N- (CRD-1) e C- (CRD-2) terminal regions of the proteins connected by a peptide link. In our project we develop a multidisciplinary study on galectins from the tandem-repeat group, using the human proteins galectin-4 and galectin-12 as models. We will present the newest results obtained through the application of spectroscopic, crystallographic techniques associated to biological and cell location studies, to investigate the carbohydrate-dependent mechanisms that confer functional diversity for this class of proteins.

67) Crystallization and Structural studies with two Lysine 49 - Phospholipases A2s from Bothrops moojeni venom

Salvador, Guilherme H. M.; Fernandes, Carlos A. H.; Fontes, Marcos R. M.
Departamento de Física e Biofísica, Instituto de Biociências de Botucatu, Universidade Estadual Paulista – UNESP

Many biophysical experiments demonstrate the bothropic Lys49-PLA2s are dimeric in solution. Various studies with their crystal structures discussed the dimer conformation, when two possibilities in asymmetric unit are observed: “conventional” and “alternative”. The alternative dimer conformation presents a higher interfacial area with more negative free energy compared to the conventional dimer. Futhermore, the alternative conformation is also important to Lys49-PLA2s myotoxic activity, and a specific myotoxic site for bothropic Lys49-PLA2s was proposed. More recently, a complement of the myotoxic site was presented: i) the membrane docking site (MDoS) and ii) the membrane disruption site (MDiS). We present a comparison of MjTX-I and MjTX-II to others Lys49-PLA2s of Bothrops genus. MjTX-I and MjTX-II crystals were obtained by hanging-drop vapor-diffusion method. Crystal data were collected using a synchrotron-radiation source (MX beam lines – LNLS, Brazil). Refinement of MjTX-I structure reveals a tetrameric conformation, a very distinct were compared to other bothropic Lys49-PLA2s, leading us to suppose that this reason for its reduced myotoxic activity. MjTX-II structure presents 4 PEG4K molecules in structure and an alternative dimeric conformation. A mutation (Leu32Gly) and an insertion of the residue of Asn in position 120 causes structural differences, but this does not affect their myotoxicity.

68) Structural studies of the human TIPRL protein, a regulator of protein phosphatase 2A

Scorsato, V.[1], Zanchin, N. I. T.[2], Smetana, J. H. C.[3], Aparicio, R.[1]
[1] Institute of Chemistry – UNICAMP; [2] FIOCRUZ; [3] Brazilian Biosciences National Laboratory, CNPEM

Protein phosphatase 2A (PP2A) regulates important signaling and metabolic pathways. PP2A holoenzyme is a heterotrimer formed by a catalytic, a scaffold and a regulatory subunit. The assembly of the active holoenzyme is a tightly regulated process which requires a series of protein-protein interactions and post-translational modifications of the catalytic subunit. TIPRL possibly plays a role in the biogenesis of PP2A but its function remains unclear. Several regulators of PP2A had their structures solved in recent years, which allowed for an in depth characterization of the biogenesis and mechanism of this phosphatase. TIPRL is one of the last missing pieces of this puzzle. TIPRL was recombinantly expressed and purified by standard techniques. SAXS data collected at the SAXS-1 beamline (LNLS/CNPEM) indicated a well-folded protein present either as a monomer or a dimer in solution, corresponding to different elution peaks in size exclusion chromatography. TIPRL crystals were obtained by the vapor diffusion method. Crystallographic data extending up to ~ 2.8 Å resolution were collected both in a Bruker Kappa APEX II Duo diffractometer and at the MX2 beamline (LNLS/CNPEM). Exhaustive attempts to solve the structure by Molecular Replacement failed due to the lack of homologues in the PDB. We are currently working to obtain selenomethionine-labeled derivatives.
Acknowledgments: FAPESP, CNPq, LNLS/CNPEM.

69) Single-crystal X-ray Crystallography at the Institute of Chemistry/UNICAMP

Simoni, Deborah de Alencar[1]; Aparicio, Ricardo[2]
[1] Laboratory of Single-crystal X-ray Crystallography, dsimoni@iqm.unicamp.br
[2] Laboratory of Structural Biology and Crystallography, aparicio@iqm.unicamp.br
Institute of Chemistry, UNICAMP, CP6154, CEP 13083-970, Campinas-SP, Brazil

The Institute of Chemistry at the University of Campinas, Campinas-SP, Brazil, was founded on 1967 and, although young, is considered a center of excellence and one of the most prominent national institutions of teaching and research in different areas of Chemistry. The Institute has facilities providing access to techniques which include NMR, mass spectrometry, optical and electron microscopy, thermal, spectroscopic and powder diffraction analyses. Powder diffractometers have been in use since the 1980s but single-crystal structure determination has become available for routine use only in 2011, after the acquisition of a single-crystal X-ray diffractometer through a joint effort of professors from various departments and support from the São Paulo Research Foundation (FAPESP; Proc. 09/51602-5). The equipment is a dual-source Bruker Kappa APEX II Duo, with Mo radiation generated by a sealed tube combined with a curved graphite crystal monochromator (Triumph) and a Cu X-ray source consisting of an Incoatec microfocus Iμs with a Quazar MX (Montel multilayer optics). Its configuration allows for structure determination of inorganic compounds, organometallics and complexes, small molecules (both synthetic and natural products), absolute configuration of chiral molecules containing only light atoms and protein structures. This flexibility was essential to meet the needs of research groups in different areas and establish a crystallography facility which has filled in a gap in the analytical instrumentation available at the Institute. The presence of a single-crystal diffractometer was a key strategy for the nucleation of a productive and growing community of users. Besides, continuous effort to offer training and specific courses in both graduate and undergraduate levels has also contributed to spread Crystallography within the Institute. As a result, a number of users has already incorporated structural studies in their daily work and benefit from structural data, which help increasing the impact of current research projects, whose outcome is usually reported as scientific papers, PhD thesis and master dissertations. Further details and examples of applications will be included in this presentation.

70) X-rays and sound pulses: acoustic methods in crystallography and drug discovery

Soares, Alexei
Brookhaven National Laboratory, New York

Acoustic droplet ejection (ADE) is a touch-less technology for growing, improving, and harvesting protein crystals. We describe a fragment screening strategy that uses acoustic droplet ejection (ADE) for high throughput screening of fragment libraries (using soaking or co-crystallization) either on plates or on MiTeGen micro-meshes. The high speed of specimen preparation (>1/s) and the low consumption of fragment + protein (2.5 nL/screen) make single fragment screening viable (avoiding cocktails). The high accuracy for the trajectory of the ejected components allows multiple co-crystallization experiments to be discretely positioned on each specimen holder (hence the goniometer acts as an 'auxiliary robotic automounter' by rapidly translating between different screening experiments). All components are transferred through small apertures so that the crystallization tray is at equilibrium with the reservoir before, during, and after the transfer of protein + precipitant + fragment to the location where crystallization will occur. After crystallization, additional components can be added to the in situ experiment, and excess fluid can be removed, still in an equilibrium environment. This strict control of the specimen environment means that crystallization trials remain identical as working volumes are decreased from the few microliter level to the few nano-liter level. This technology pushes towards a much faster, more automated, and more flexible strategy for structure based drug discovery using as little as 2.5nL of each major component.

71) Structural Characterization and Affinity Analysis of a Pro-Lectin from the seeds of Dioclea grandiflora

Sousa, Bruno Lopes; Silva Filho, José Caetano; Rocha, Bruno Anderson Matias; Delatorre, Plínio; Bezerra, Gustavo Arruda; Nagano, Celso Shiniti; Gruber, Karl; Cavada, Benildo Sousa
Federal University of Ceará; Federal University of Ceará; Federal University of Ceará; Federal University of Paraíba; Max F. Perutz Laboratories; Federal University of Ceará; Universit of Graz; Federal University of Ceará

Legume lectins are the most extensively studied group of plant lectins and among them species from the Diocleinae subtribe present a sophisticated lectin post-translational processing. The role of this processing remains elusive and there is still no agreement about the precise point where Diocleinae lectins become active. Here we present high resolution structures of a pro-lectin from the seeds of Dioclea grandiflora, recombinant expressed in Escherichia coli. The current data attest the protein functionality before chain permutation, showing the binding site structuration upon metal binding through comparison between the de-metallized and metallized structures, as well as the carbohydrate binding capability through complexed structures with mannose and the dimmannoside M1-3M. The structural data is supported by Isothermal Titration Calorimetry (ITC) measurements. Small Angle X-Ray Scattering (SAXs) analysis were performed to analyse the pH dependency of the this pro-lectin in different pH and also to acess the protein envelope in solution. The oligomerization profile was analyzed and compared to the pH-independent native lectin from this specie. This work configures as the first report about the structure of an unprocessed legume lectin.

72) The Physiological Function of the Xanthomonas Type IV Secretion System

Souza, D.P.[1];; Oka, G.U.[1]; Alvarez-Martinez, C.E.[1,2], Bisson-Filho, A.W.[1];
Dunger, G.[1]; Hobeika, L.[3]; Cavalcante, N.S.[4]; Barbosa, L.R.[5];
Salinas, R.K.[1]; Guzzo, C.R.[1,6]; Farah, C.S.[1]
[1]Dept. Bioquímica, IQ-USP, SP, Brazil. [2]Dept. Genética e Evolução, IB-Unicamp, Campinas, Brazil. [3]ENS Cachan, France. [4]IF-USP, São Carlos, Brazil. [5]Dept. Física Geral, IF-USP, SP, Brazil. [6]Dept. Microbiologia, ICB-USP, SP, Brazil.

Xanthomonas citri subsp. citri (Xac) is a Gram-negative bacterial phytopathogen that infects citrus. Xac codifies a Type IV Secretion System (T4SS) of unknown function. T4SSs are multiprotein macromolecular complexes involved with the transport of DNA to bacterial cells (conjugation) and effector proteins to eukaryotic cells, thus contributing directly to bacterial pathogenicity. Key to understanding the function of a secretion system is the identification and characterization of the substrates or effectors that it exports. We previously identified a set of 12 probable substrates with a conserved C-terminal domain which interact with VirD4, the T4SS substrate receptor. One of these proteins, XAC2609 specifically interacts with XAC2610. Through the characterization of the structure and function of XAC2610, we have gained insight into the physiological role of the Xac T4SS.
Keywords: *Xanthomonas*, Type IV Secretion System, X-ray diffraction.
Supported by: FAPESP and CAPES.

73) Schistosome Muscles Contain Striated Muscle-like Myosin Filaments in a Smooth Muscle-like architecture

Sulbarán, Guidenn1,2; Alamo, Lorenzo1; Pinto, Antonio1; Marquez, Gustavo1; Méndez, Franklin1; Padrón, Raúl1; Craig, Roger2.
1Centro de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas 1020A, Venezuela 2Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01655, USA.

Schistosomes are parasitic worms infecting 200 million humans worldwide. Schistosomiasis is treated with the drug praziquantel, which affects the parasite muscle, possibly by binding to myosin light chains. To understand the molecular functioning of Schistosome muscles, we have studied their cellular and molecular makeup. EM sections of adult worms reveal exclusively smooth muscle throughout the body wall (thick filaments not in register, dense bodies instead of Z-lines). We carried out 3D-reconstruction of negatively stained, relaxed Schistosome thick filaments. Surprisingly, the reconstruction was indistinguishable from those obtained previously for arthropod striated muscles, showing a 4-fold helical arrangement of myosin interacting-head motifs. Published data show that only one myosin heavy chain (MHC) gene is expressed in Schistosoma mansoni. By proteomic-Mass Spectrometry (MS) analysis of myosin from the adult parasite stage we showed that this was similar to a striated, not smooth, MHC. Analysis of published protein sequences supports this finding. A percentage-of-identity distance tree, and alignment of multiple diverse MHC sequences, showed two main branches: one for vertebrate and invertebrate striated muscles, and a smooth and nonmuscle branch. The Schistosome MHC lies in the striated muscle group, completely separate from the smooth/nonmuscle group. The in vitro motility assay showed movement of Schistosome thin filaments over unregulated tarantula striated myosin filaments independent of calcium and similar to rabbit F-actin motility, suggesting the absence of thin filament regulation. In agreement with this result, proteomic-MS analysis of Schistosome filament homogenates demonstrated the expression of the thin filament components tropomyosin and actin, but no signal for troponin was detected. We conclude that Schistosome muscles are hybrids, containing striated muscle like thick filaments (with striated muscle MHC) and smooth muscle-like thin filaments (no troponin), arranged in a smooth muscle-like architecture.

Supported by grants from the National Institutes of Health (R01 AR034711 to R.C.) and the Howard Hughes Medical Institute (HHMI) to R.P.

74) Structural basis of ConM binding with resveratrol, an anti-inflammatory and antioxidant polyphenol

Teixeira, Claudener Souza1; Rocha, Bruno Anderson Matias1; Silva Filho, José Caetano2; Chaves, Beatriz1; Guerra, Dayane Lima1; Sampaio, Silvana Saker2; Alencar, Daniel Barroso2; Delatorre, Plinio3; Cavada, Benildo Sousa1; Sampaio, Alexandre Holanda1
1Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, BioMol-Lab, Campus do Pici S/N, 60440-970, Fortaleza, Ceará, Brazil; 2 Departamento de Engenha de Pesca, Universidade Federal do Ceará, BioMol-Group, Campus do Pici S/N, Fortaleza, Ceará, Brazil; 3 Departamento de Biologia Molecular, Universidade Federal da Paraíba, Campus I, Cidade Universitária, 58059-900, João Pessoa, Paraíba, Brazil

Resveratrol can also inhibit the activation of proinflammatory mediators and cytokines at the early gene expression stage. It is well known that lectins are sugar-binding proteins that act as both pro- and anti-inflammatory molecules. Thus, the objective of this work was to verify the binding of a polyphenol compound with a lectin of Canavalia maritima (ConM) based on their ability to inhibit pro-inflammatory processes. To accomplish this, ConM was purified and crystallized, and resveratrol was soaked at 5 mM for 2 hours of incubation. The crystal belongs to the monoclinic space group C2, the final refinement resulted in an Rfactor of 16.0% and an Rfree of 25.5%. Resveratrol binds in the rigid β-sheet through H-bonds and hydrophobic interaction with amino acids that compose the fifth and sixth β-strands of the rigid β-sheet of ConM. The ConM and resveratrol inhibited DPPH oxidation, showing synergic activity with the most effective ratio of 2:3 and carbohydrate binding site is not directly related to antioxidant activity. It is the interaction between ConM and resveratrol that indicates the synergism of these two molecules in acting as free radicals scavengers and in reducing the inflammatory process through the inhibition of many pro-inflammatory events.

75) Novel flagellar proteins from Leptospira are key structural elements

Trajtenberg, Felipe; Wunder, Elsio; Mechaly, Ariel; Picardeau, Mathieu; Ko, Albert and Buschiazzo, Alejandro
Institut Pasteur de Montevideo, Uruguai

Motility in bacteria is an essential process, not only linked to chemotaxis and nutrition, but also coupled to host penetration and dissemination in pathogenic species. In Leptospira there are two periplasmic flagella, sub-terminally attached to each end of the cell, responsible for its unique hook-shaped cell morphology. The protein composition of the flagella appears to be much more complex than the well- known models studied to date (e.g. E. coli, Salmonella spp.). Two novel proteins, named Flagellar-coilling protein 1 (Fcp1) and Flagellar-coilling protein 2 (Fcp2), were identified as part of the flagellar assembly in Leptospira. Fcp1 is essential for translational motility and fcp2 knockout strain shows a motility reduce phenotype and both show a loss of cellular hook-shaped ends. These proteins form the sheath of the flagella and appears to be involved in the spontaneous flagellar coiling, a pathognomonic feature of Leptospira flagella. We solved the structure of both proteins, revealing two novel protein fold. Their structures and functional characterization start to unveil a whole new family of proteins from Leptospira flagella, which are involved in structuring the flagella filaments, as well as interacting with others cellular components. The combination of cryoelectron microscopy and subsequent fitting of crystal sructures, will allow us to generate a structural model of the flagellar filament seeking for a molecular explanation of the coiling inducing property for both proteins.

76) Insights into the structure of Triatoma Virus (TrV) capsid. Combining crystallography data with computational simulations

Viso, J.F.[1]; Zamarreño, F.[1], Amundarain, M. J.[1], Guérin Aguilar, D. M. A.[2], Costabel, M.D.[1]
[1]Grupo de Biofísica. Departamento de Física, Universidad Nacional del Sur (UNS), IFISUR, (UNS-CONICET), Bahía Blanca, Argentina. [2]Unidad de Biofísica, Universidad del País Vasco (UPV/EHU) – Fundación Biofísica Bizkaia, Leioa, España.

Triatoma Virus (TrV) is an insect virus that belongs to the Dicistroviridae family and infects several species of triatomine insects which are the vectors for human trypanosomiasis, commonly known as Chagas disease. Because of this, TrV is proposed as a biological control against this vector.
The crystal structure of TrV was solved recently, but an omit map of the structure, in the region of the icosahedral 5-fold axis of the capsid, shows an interesting electronic density.
In this work we study the 5-fold symmetry axis of the icosahedral capsid of TrV, because may be responsible of the interaction between the interior and the exterior of the virus capsid, across a putative pore. Using molecular dynamics simulations, we have observed that the pore formed in this axis remains without water molecules in the region surrounded by a ring of Valines which created a supposed hydrophobic gate. Also, we have found certain conditions where the axis is completely full of water molecules, even in the hydrophobic region. The complete hydration of the channel may lead to their “opening”, and the interaction between the interior and the exterior of the capsid.
Combining different results we could explain the characteristics of the omit map.

77) Preliminary Structural Analysis of the Glycosyltransferase GTFB in Complex with UDP-6F-Glucose, an Analog of Substrate

[1,2,3]Wilson, Carolina; [2,3]Dias, Marcio Vinicius Bertacine

[1]Instituto de Biociências, Letras e Ciências Exatas, IBILCE, Unesp, Campus São José do Rio Preto – SP, Brasil[2]Laboratório Nacionacioal de Biociências, LNBio, CNPEM, Campinas, SP, Brazil/ [3]Instituto de Ciências Biomédicas, ICB, Departamento de Microbiologia, USP, São Paulo – SP, Brasil

Glycopeptide antibiotics are applied in the treatment of diseases caused by Gram-positive bacteria, including infections caused by Methicillin-Resistant *S. aureus* (MRSA). Vancomycin is a first-line drug to treat infections that provide high risk of life. However, due to its intensive and indiscrimate use there is the explosive increase in the resistance of pathogenic enterococci, causing high morbidity and mortality, especially in immune-compromised patients. Thus, there is the interest in developing new glycopeptide derivatives that are effective against these resistant pathogens. However, is necessary to know the structure of the antibiotics and the processes of their biosynthesis. Vancomycin has a heptapeptide core (aglycone) decorated with several amino sugars and halogens, and the glycosyltransferases are responsible for adding the glycosides to aglycone. The GtfB is responsible for transferring glucose to UDP-glucose donor to acceptor aglycone. This enzyme is considerate a promising tool for generating chemical diversity of vancomycin through of synthetic biology and biotechnology using mutant enzymes. However, basic questions about this enzyme are still need as the binding mode of substrates. The objective of this work was the purification, crystallization and preliminary analysis of X-ray data of GtfB in complex with the non-hydrolyzable analogue of substrate, UDP-6F-glucose. The gene identified in the vancomycin gene cluster from *A. orientalis* was cloned in pET28a (+) and its expression was carried out using BL21(DE3). Expression of GtfB was successful with addition of 0.2 M of IPTG at 16ºC. GtfB was purified using affinity and gel filtration columns. Crystallization was performed using hanging drop and the crystals obtained were incubated in the presence of 65mM of UDP-6F-Glucose substrate analogue. These crystals diffracted about 2.8Å. As the structure of this enzyme in its apo form was obtained, it is expected to solve the structure with the substrate analogue because little is known about the factors that determine the identification or selectivity of these molecules and the binding mode of the acceptor and donor. This knowledge is crucial for the biotechnological application into the generation chemical diversity of novel analogues of glycopeptides by synthetic biology.

Keywords: vancomycin; glycosyltransferase; crystallization.

Acknowledgements: CAPES, FAPESP

78) Bacterial mimicry of eukaryotic innate immunity

Wong, Steve, Dessen, Andréa
Institut de Biologie Structurale, Grenoble, França (CNRS/CEA/UJF) e LNBio (CNPEM)

Alpha-2-macroglobulins (A2Ms) are plasma proteins that trap and inhibit a broad range of proteases and are major components of the eukaryotic innate immune system. Surprisingly, A2M-like proteins were identified in pathogenically invasive bacteria and species that colonize higher eukaryotes. Bacterial A2Ms are located in the periplasm where they are believed to provide protection to the cell by trapping external proteases through a covalent interaction with an activated thioester. We have been able to structurally characterize the A2M from *Salmonella typhimurium* in different states of thioester activation. Our structures reveal thirteen domains whose arrangement displays high similarity to proteins involved in eukaryotic immune defense. A structural lock mechanism maintains the stability of the buried thioester, a requirement for its protease trapping activity. These findings indicate that bacteria have developed a rudimentary innate immune system whose mechanism mimics that of eukaryotes.

79) Can structures lead to advanced therapeutics?

Yonath, Ada
Department of Structural Biology, Weizmann Institute, Rehovot 76100, Israel

The incredible global increase in resistance to antibiotics that we are witnessing recently, is a serious medical threat. It seems that the world is approaching a post-antibiotic era, in which common infections and minor injuries, which have been treatable for decades, could become fatal once again. Ribosomes, the universal cellular machines that translate the genetic code into proteins, are paralyzed by almost half of the clinically useful antibiotics that bind to their functional sites. By investigating the three dimensional structures of ribosomes from non-pathogenic bacteria as models for genuine pathogens, common features were identified. Thus, the antibiotics binding modes, inhibitory actions and synergism pathways have been determined for almost all ribosomal antibiotics. These indicated the principles of differentiation between patients and pathogens and suggested common principles of mechanisms leading to bacterial resistance.
However, as species specific diversity was detected in susceptibility to infectious diseases and in developing specific resistance mechanisms, our structural studies have been extended to ribosomes from genuine pathogens.
By determining the high resolution structure of the first and only ribosomal particle from a genuine pathogen with several antibiotics, we identified subtle, albeit highly significant structural elements that can account for the species specificity in resistance, thus could paved ways for improvement of existing antibiotics as well as for the design of advanced therapeutics capable of minimizing antibiotics resistance.

80) Characterization Of Archaeological Pigments By X-Ray Diffraction With Synchrotron Radiation

Zeballos-Velásquez, Elvira L.1; Wright, V.2; Prieto, O. G.3; Suescun, A.4; Suchomel, M.5
1 Laboratorio de Cristalografía de Rayos-X, Facultad de Ciencias Físicas, Universidad Nacional Mayor de San Marcos, Ap. Postal 14-0149, Lima, Perú. 2 Instituto Francés de Estudios Andinos, Avenida Arequipa 4500, Casilla 18-1217, Lima, Perú. 3 Department of Anthropology, Yale University, 10 Sachem Street, New Haven, CT 06511, USA. 4 Facultad de Química, Universidad de la República, Montevideo, Uruguay. 5 Advanced Photon Source, Argonne National Laboratory 9700 S. Cass Ave, Bldg. 433-D003, Lemont, IL 60439, USA.

In this study, the investigated samples are pigments from the Pampas Gramalote archaeological site located in Trujillo city, Peru. Phase composition of the samples was studied by powder X-ray diffraction using beamline 11-BM of the Advanced Photon Source (APS), Argonne National Laboratory. Quantitative phase analysis of the crystalline portion of the samples was performed by the Rietveld method using Bruker AXS Topas program. We have found that all samples contain significant amounts of quartz. The red color of the pigments could be associated with the presence of hematite and magnetite, since these phases are among the main components of the samples, with additional contributions coming from anorthite and jarosite phases. The research work being carried out on this site will allow us to understand the process of social complexity from the perspective of communities. These results will contribute directly to the conservation and/or restoration of the historic architecture, in order to preserve it.

81) Structural Study Of The Bacterial Macroglobulin MagD from Paseudomonas aeruginosa

Zouhir, Samira; Attree, Ina; Dessen, Andrea
Laboratório Nacional de Biociências (LNBio); Institut de Recherches en Technologies et Sciences pour le Vivant (iRTSV-France); Laboratório Nacional de Biociências (LNBio) & Institut de Biologie Structurale (IBS-France)

Human pathogens frequently use protein mimicry to manipulate host cells in order to promote their survival. Pseudomonas aeruginosa is a Gram-negative bacterium that inhabits most available environments and is a major opportunistic human pathogen responsible for nosocomial infections with high mortality rates. Recently, it has been shown that the bacterium synthesizes a 167kDa structural homolog of the human large spectrum protease inhibitor α2-macroglobulin and named MagD. MagD synthesis is co regulated at the posttranscriptional level with other virulence determinants, suggesting that is has a role in bacterial pathogenicity and/or in defense against the host immune system. The bacterial protein is localized in the periplasm and is associated with the inner membrane through the formation of a multimolecular complex involving three other proteins: MagA, MagB and MagF. The aim of this work is to investigate the molecular mechanisms of MagD through its biochemical characterization and its structural study using crystallography.

Notes

Funding Agencies

CNPq

CAPES

FAPESP

Sponsors

Molecular Dimensions

BRUKER

TEMPER technologies

Agilent Technologies

GE Healthcare

Rigaku

DAIRIX

FORMULATRIX

astex pharmaceuticals

ttplabtech natural innovators

INCOATEC innovative coating technologies

Institutional Partnerships

UNESCO

2014 international year of crystallography IUCr

summit meetings

IFSC

ABCr

LNLS

LNBio

CNPEM