



## **CeBEM / RIIP / Amsud-Pasteur International Workshop**

### **“Macromolecular Crystallography: Introduction and Applications”**

#### **Title of the course**

“Macromolecular Crystallography: Introduction and Applications”

#### **Date and place**

April 26 to May 7, 2010 – Institut Pasteur de Montevideo - URUGUAY

#### **Organizers**

Alejandro Buschiazzi – IP Montevideo  
Pedro Alzari – IP París  
William Shepard – Synchrotron SOLEIL - France

## Timetable

### Sunday 25

**18:30** Meeting at Hotel Hermitage - Registration

**19:30** Welcome cocktail

### Monday 26

8:30-9:00 Poster installation

9:00-9:30 General overview of the course - Alejandro Buschiazzi

9:30-10:30 The crystallographic approach in Biology - Pedro Alzari

**10:30-11:00 coffee break**

11:00-12:00 Theoretical aspects of macromolecular crystallization - Terese Bergfors

12:00-13:00 The protein sample: quality considerations, tips & tricks - Mario Murakami

**13:00-14:30 lunch**

14:30-16:30 Experimental: Protein crystallization (Group A)  
Instruments & robotics (Group B)

**16:30-17:00 coffee break**

17:00-19:00 Experimental: Instruments & robotics (Group A)  
Protein crystallization (Group B)

**19:00-20:00 Posters & Beer Session**

### Tuesday 27

9:00-10:00 The highways, biways, and detours of crystallization: what strategy to use - Terese Bergfors

**10:00-10:30 coffee break**

10:30-11:30 Theory of X ray diffraction - Pedro Alzari

**11:30-12:00 coffee break**

12:00-13:00 Reciprocal space - Bob Sweet

**13:00-14:30 lunch**

14:30-16:30 Experimental: Crystal Seeding - Terese Bergfors

**16:30-17:00 coffee break**

17:00-19:00 Experimental: Crystal cryocooling - Terese Bergfors

**19:00-20:00 Posters & Beer Session**

### Wednesday 28

9:00-10:00	Symmetry & space groups - Bob Sweet
<b>10:00-10:30</b>	<b>coffee break</b>
10:30-11:30	X ray generators & detectors - Bob Sweet
<b>11:30-12:00</b>	<b>coffee break</b>
12:00-13:00	Data collection : strategies, good & bad practices - Bob Sweet
<b>13:00-14:30</b>	<b>lunch</b>
14:30-16:30	Experimental: (Group A) X ray diffraction: taking your first shot - Bill Shepard (Group B) Protein preparation for crystallization : light scattering & other quality probes - Horacio Botti
<b>16:30-17:00</b>	<b>coffee break</b>
17:00-19:00	Experimental: (Group A) Protein preparation for crystallization : light scattering & other quality probes - Horacio Botti (Group B) X ray diffraction: taking your first shot - Bill Shepard
19:00-19:30	Demo: In-house data collection - Alejandro Buschiazzo

#### Thursday 29

9:00-10:00	Students' presentations
10:00-11:00	Data processing: indexing and integration - Bill Shepard
<b>11:00-11:30</b>	<b>coffee break</b>
11:30-12:30	Burning questions
12:30-13:00	X ray diffraction data : the mtz format - Bill Shepard
<b>13:00-14:30</b>	<b>lunch</b>
14:30-16:30	Tutorial: Data integration: Mosflm - Alejandro Buschiazzo
<b>16:30-17:00</b>	<b>coffee break</b>
17:00-19:00	Tutorial: Data integration: XDS - Bill Shepard

#### Friday 30

9:00-10:00	Students' presentations
10:00-11:00	Data processing: scaling and merging - Bill Shepard
<b>11:00-11:30</b>	<b>coffee break</b>
11:30-12:30	X ray data quality : statistics - Bill Shepard
12:30-13:30	Demo: Remote data collection at the ALS (beamline 5.0.2) – Alejandro Buschiazzo
<b>13:30-14:30</b>	<b>lunch</b>
14:30-16:30	Tutorial: Data scaling: Scala - Alejandro Buschiazzo
<b>16:30-17:00</b>	<b>coffee break</b>
17:00-19:00	Tutorial: Data scaling: XSCALE - Bill Shepard

#### Saturday 01

9:00-10:00	Fourier Theory: the diffraction experiment revisited - Mario Amzel
<b>10:00-10:30</b>	<b>coffee break</b>
10:30-11:30	The Fourier transform: concepts & properties; related functions - Mario Amzel
<b>11:30-12:00</b>	<b>coffee break</b>
12:00-13:00	The phase problem : introductory concepts - Pedro Alzari
<b>13:00-14:30</b>	<b>lunch</b>
14:30-15:30	Demo: Remote data collection at the ALS (beamline 5.0.2) - Alejandro Buschiazzo
<b>15:30-16:00</b>	<b>coffee break</b>
16:00-17:00	Phasing methods: general overview - Isabel Usón
<b>17:00-17:30</b>	<b>coffee break</b>
17:30-19:00	Tutorial: Heavy atoms substructure determination & refinement - Isabel Usón

### Sunday 02

8:30	Excursion to Punta del Este (all day)
<b>13:00</b>	<b>Lunch</b>

### Monday 03

9:00-10:00	Phasing : isomorphous replacement and anomalous diffraction - Isabel Usón
<b>10:00-10:30</b>	<b>coffee break</b>
10:30-12:00	Demo: Protein phasing with SHELXC/D/E - Isabel Usón
<b>12:00-13:30</b>	<b>lunch</b>
13:30-15:00	Discussion of selected papers
<b>15:00-15:30</b>	<b>coffee break</b>
15:30-17:00	Molecular replacement - Pedro Alzari
<b>17:00-17:30</b>	<b>coffee break</b>
17:30-19:30	Tutorial: Lysozyme structure determination (LSD) I: molecular replacement - Pedro Alzari / Alejandro Buschiazzo

### Tuesday 04

9:00-10:00	The electron density function: Fourier maps, difference Fourier - Mario Amzel
<b>10:00-10:30</b>	<b>coffee break</b>
10:30-11:30	Density modification & real space refinement - Igor Polikarpov
<b>11:30-12:00</b>	<b>coffee break</b>
12:00-13:00	Electron density interpretation: model building - Paul Emsley
<b>13:00-14:30</b>	<b>lunch</b>
14:30-16:30	Tutorial: LSD II: SAD - Alejandro Buschiazzo
<b>16:30-17:00</b>	<b>coffee break</b>

- 17:00-19:00 Tutorial: LSD III: DM & model building - Pedro Alzari/Alejandro Buschiazzo
- 19:00-19:30 Demo: Arp/wArp : automatic electron density interpretation - Alejandro Buschiazzo

### Wednesday 05

- 9:00-10:00 Refinement(I): introduction; restraints and constraints - João Barbosa
- 10:00-10:30 coffee break**
- 10:30-11:30 Refinement(II): Target functions in macromolecular refinement (least squared residuals, maximum likelihood) - Paul Emsley
- 11:30-12:00 coffee break**
- 12:00-13:00 Burning questions
- 13:00-14:30 lunch**
- 14:30-16:30 Tutorial: Model building: Coot - Paul Emsley
- 16:30-17:00 coffee break**
- 17:00-19:00 Tutorial: Refinement I: Refmac5 - Marcelo Costabel

### Thursday 06

- 9:00-10:00 Refinement(III): minimization methods - Marcelo Costabel
- 10:00-10:30 coffee break**
- 10:30-11:30 Model quality: concepts & statistics - Swanand Gore
- 11:30-12:00 coffee break**
- 12:00-13:00 Resolution: implications in refinement - Swanand Gore
- 13:00-14:30 lunch**
- 14:30-16:30 Tutorial: Refinement II: Refmac5 - Marcelo Costabel
- 16:30-17:00 coffee break**
- 17:00-19:00 Tutorial: Model analysis: Coot - Mario Amzel

### Friday 07

- 9:00-10:00 Structure databases: the PDB - Swanand Gore
- 10:00-10:30 coffee break**
- 10:30-11:30 Tutorial: Model visualization: PyMol - Felipe Trajtenberg
- 11:30-12:00 coffee break**
- 12:00-13:00 Structure analysis : what information can be drawn from an atomic model? - Richard Garrat
- 13:00-14:30 lunch**
- 14:30-17:00 Tutorial: Model validation - Swanand Gore
- 17:00-17:30 coffee break**
- 17:30-19:00 Final discussions
- 20:00 FIESTA**

# Program overview

	Sun 25	Mon 26	Tue 27	Wed 28	Thu 29	Fri 30	Sat 1	Sun Mon 3	Tue 4	Wed 5	Thu 6	Fri 7	
8:00		Poster installation											
9:00		Introduction Crystallography: Overview	Tough crystallizations	Theory of X ray diffraction	Students' presentations	Students' presentations	Fourier Theory I		Phasing	Electron density function	Refinement I	Refinement III	Structure databases
10:00		coffee break	coffee break	coffee break	Data processing I	Data processing II	coffee break		coffee break	coffee break	coffee break	coffee break	coffee break
11:00		Crystallization theory	Symmetry & space groups	Reciprocal space	coffee break	coffee break	Fourier Theory II		Phasing	Density modification	Refinement II	Model quality	Model visualization: PyMol
12:00		Protein sample preparation	X ray generators & detectors	Data collection	Burning questions	X-ray data quality	Intro: the phase problem			Model building	Burning questions	Resolution: implications in refinement	Structure analysis
13:00													
14:00									Discussion of selected papers				
15:00		Protein crystallization Instruments & robotics	Crystal Seeding	Crystal mounting Protein quality	Data integration: Mosflm	Data scaling: Scala	Data collection		coffee break	LSD II: SAD	Model building: Coot	Refinement II: Refmac5	Model validation
16:00		coffee break	coffee break	coffee break	coffee break	coffee break	Isomorphous replacement			coffee break	coffee break	coffee break	coffee break
17:00		Instruments & robotics Protein crystallization	Crystal cryocooling	Protein quality Crystal mounting	Data integration: XDS	Data scaling: XSCALE	Anomalous diffraction		coffee break	LSD III: DM & model building	Refinement I: Refmac5	Model analysis: Coot	Final discussions
18:00		Meeting	Posters & Beer	Posters & Beer	Data collection				Lysozyme structure determination (LSD) I: molecular replacement	Automatic model building			
19:00													FIESTA
20:00		DINNER											

	Lecture
	Experimental / tutorial
	Seminar / discussion
	Demonstration
	Lunch

## Invited Professors

Pedro M Alzari	Department of Structural Biology & Chemistry - Institut Pasteur - Paris, France
William Shepard	Soleil Synchrotron - Saint Aubin, France
Joao Barbosa	
Mario Amzel	Department of Biophysics & Biophysical Chemistry – Johns Hopkins University – Baltimore, USA
Robert Sweet	Brookhaven National Laboratory - Upton, New York, USA
Swanand Gore	EMBL Outstation – Hinxton, European Bioinformatics Institute – Cambridge, UK
Paul Emsley	
Terese Bergfors	Department of Cell and Molecular Biology - Biomedical Center -Uppsala University- Uppsala, Sweden
Isabel Usón Finkenzeller	Institut de Biologia Molecular de Barcelona – Barcelona, Spain

Igor Polikarpov	Instituto de Física de Sao Carlos –Universidade de São Paulo – Brazil
Marcelo Costabel	Departamento de Física - Universidad Nacional del Sur - Bahía Blanca, Argentina
Alejandro Buschiazzo	Unit of Protein Crystallography – Institut Pasteur de Montevideo – Montevideo, Uruguay
Mario Murakami	Laboratorio Nacional de Luz Sincrotron LNLS – Campinas- Brasil
Richard Garrat	Instituto de Física de Sao Carlos –Universidade de São Paulo – Brazil

### Students

<b>Group One</b> ( <i>full attendance: lectures and experimental tutorials</i> )		
Albanesi Daniela	Instituto de Biología Molecular y Celular de Rosario	albanesi@ibr.gov.ar
Alvarez Clarisa	Centro de Estudios Fotosintéticos y Bioquímicos	alvarez@cefobi-conicet.gov.ar
Chavez Daniela	Universidade Federal do Paraná	dani_chaves@ufpr.br
De Almeida Ivo	Institute of Physics, San carlos-University of São Paulo,	ivo@ursa.ifsc.usp.br
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Rinaldi Jimena	Ciencias Exactas – Universidad de Buenos Aires	jrinaldi@leloir.org.ar
Rosconi Federico	Inst de Investigaciones Biologicas Clemente Estable - Montevideo	federh@iibce.edu.uy
Tosar Juan Pablo	IP Montevideo	jptosar@pasteur.edu.uy
<b>Group Two</b> <i>(only lectures and special discussion tables with speakers)</i>		
Chiappini Santiago	IDEHU-Universidad de Buenos Aires	santiago.chiappini@gmail.com
Esteves Adriana	Facultad de Ciencias – Univ de la Republica, Uruguay	aesteves@fcien.edu.uy
Graña Martín	IP Montevideo	mgrana@pasteur.edu.uy
Hugo Martín	Fac. de Medicina Univ de la Republica, Uruguay	hugo0811@gmail.com
Sasen Natalia	Facultad de Química - Univ de la Republica, Uruguay	nanisas@gmail.com



## **Information on the general organisation of the course and how it was held**

### Overall Brief Description:

DURATION : 12 days

TYPE : theoretical and experimental / hands-on intensive experimental work is a central aim

SITE : Institut Pasteur de Montevideo - Unit of Protein Crystallography + Informatics and wet lab classrooms

NUMBER OF STUDENTS : 20

LEVEL OF STUDENTS: PhD, postdoctoral and Research Assistant.

LANGUAGE : English

Diffusion and information was made public four months before the application deadline (March 7<sup>th</sup>). Pertinent material was set in the form of a dedicated website ([www.pasteur.fr/mxcourse](http://www.pasteur.fr/mxcourse)), a poster (both as pdf and mailed to local and regional nodes), and E-mail brochures. Mailing lists included local and regional networks related to biological and biomedical disciplines, as well as international bulletin boards related to macromolecular crystallography (CCP4, Phenix). The Int'l Network of Pasteur Institutes (RIIP) also relayed the information through their www site.

### EQUIPMENT AND INFRASTRUCTURE

#### *Crystallization room (40m<sup>2</sup>) :*

- 96-well nanovolume robotic platform (Honeybee963)
- Automatic visualisation microscope (Cryscam)
- Cryocrystallography setup
- 2 stereomicroscopes and xtal manipulation/storage tools

#### *X ray diffraction room (40m<sup>2</sup>) :*

- Microsource rotating Cu anode generator (Rigaku Micromax007-HF)
- Multilayer mirrors (Varimax-HF)
- Image plate detector (Mar345)
- In-house liquid nitrogen generation to feed a Cryostream 700 cryo system

#### *Computer teaching room (40m<sup>2</sup>) :*

- 20 PCs under Linux (CentOS5), local disk capacity and NFS exportable central software facilities
- Two simultaneous projectors for teacher guided tutorials

#### *Wet lab teaching room (~60m<sup>2</sup>) :*

- Bench protein preparation, manipulations and crystallization experiments
- Refrigerated bench top centrifuge, ultrafiltration devices and standard laboratory facilities
- Access to a visible-light scattering instrument and UV spectrophotometer

#### *Lectures, seminars, poster halls:*

- auditorium (100 persons)
- seminar room (30 persons)

large entrance hall (~350 m<sup>2</sup>) for poster installation

Dedicated secretary & logistics support

Dedicated computing facilities (5 Linux workstations, >1Tb central storage disks, central computing services, 100Mbps Ethernet connections, NFS/CIFS comm protocols)

State-of-the-art core of academic software for computational crystallography, MOSFLM and XDS (for X ray data integration and processing); the general suites CCP4 6.1, CNS 1.2 and Phenix 1.5; Phaser, BnP, SHELXC/D/E and SHARP (molecular replacement, heavy atom substructure solution and protein phasing); Pirate, Resolve and ARP/wArp (density modification and electron density interpretation); Coot and O (model building and structure analysis & validation); PyMol, Indonesia, Raster3D (visualization, structure analysis and figure rendering); among others.

Invited speakers : 8 international, 5 regional

Local teaching staff : A Buschiazzo (Assistant Professor); S Horjales (PhD student); N Larrieux (Technician); H Botti (Postdoc); F Trajtenberg (PhD student)

At the end of the course, the students gained basic abilities to:

- ✓ Setup a crystallization assay to grow macromolecular crystals;
- ✓ Setup a typical single crystal X ray diffraction experiment;
- ✓ Collect and process X ray diffraction data;
- ✓ Understand the current methods to solve the phase problem;
- ✓ Use refinement and structure validation programs to properly fit atomic models into experimental electron density maps;
- ✓ Use visualization programs to analyze the atomic models in terms of protein folding, interatomic distances and surface interactions.

Taking into account the vast extension of the subject, topics were focused in Protein Crystallization; Data collection & Processing; and, Model refinement, Validation & Analysis.

#### Structure, organization and important teaching strategies

The course was structured chronologically, following the real-life steps in the crystallographic process

Determining the structure of lysozyme (Hen Egg White Lysozyme) was selected to be used as a "unifying" common thread along the course (...and students had an accomplishment to achieve!)

We compared molecular replacement and single anomalous diffraction (quick iodide soaking) of HEWL, as basic structure determination strategies.

Demonstrations aided in filling up "missing" steps, or limiting resources (robotic crystallization, data collection, protein phasing, etc)

Some interspersed 'Discussion' and 'Burning questions' sessions were useful for ongoing evaluation and to facilitate interaction with invited speakers

We had two remote data collection sessions (April 30 & May 1), to collect MAD data at the ALS synchrotron (thanks to Peter Zwart). Students sent their own samples beforehand!

Posters were installed very early, with early poster sessions, remaining displayed for discussion during the entire course.

Students' presentations (5' each - 2 ppt slides max) were aimed at presenting their structural hypotheses within their ongoing research (addressing the question «how MX will help assessing my ongoing hypotheses?»)

One-day, fully organized excursion to Punta del Este, just in the middle of the course as a means to relax.

A final discussion session included a survey to receive the students' evaluation and suggestions, as well as a multiple choice test concerning the main topics (as described in the course's specific aims). Both were analyzed and results are available. Overall students' evaluation was highly positive.

All the material and exchange between students and speakers was organized through the web using MOODLE ([www.pasteur.edu.uy/moodle](http://www.pasteur.edu.uy/moodle)), material that remains available to support and improve future courses.

### **Procedure for selecting participants**

Applicants were requested to present • Curriculum Vitæ, •letter of motivation and •letter of reference from the applicants' supervisor.

Documents presented before deadline, were analyzed by the organizing committee, in order to prepare a prioritized list of merits. The committee had already set a core of criteria to evaluate applications, in order of relative importance:

1. Identify and prioritize students needing crystallographic approaches for their ongoing research projects (not a 'curiosity' or general background type of course) : letters of intent and supervisor reference.
2. Introduce a weight for different backgrounds according to CV and current research team: favor applications ensuring to some extent the future use of crystallographic approaches.

3. Take into account a certain degree of equilibrium among countries (as a general rule of thumb, 30% Brazil, 30% Argentina, 20% Uruguay, 20% other regional and then international applications).
4. Favor the inclusion of diverse teams rather than several applicants from the same team (no exclusion criteria in this sense holding for same institute/center).

- Overall appraisals of the level of the participants

Everything else being equal, priority was given to PhD students at advanced levels of their graduate studies, taking into account the rather high level of the course in terms of information and methodological complexity. Postdoctoral level students were welcome. We also considered young investigators (research assistant levels). More experienced researchers were only considered for the theory-only mode.

- Method for appraising the participants

20 students to attend the full course (theory and practicals; lodging covered by the course; exceptional travel support was given to some candidates that applied separately to this possibility, special arguments for this need were requested)

15 more slots were to be offered to next priority level, to follow a theory-only mode (with no funding to support lodging, nor traveling expenses).

The organizing committee worked as evaluation and decision-making jury. The

### Second week's group picture



- |                                 |                                 |                                    |                               |
|---------------------------------|---------------------------------|------------------------------------|-------------------------------|
| 1 Mario Amzel, USA              | 11 Natalia Ruétalo, Uruguay     | 21 Natalia Lisa, Argentina         | 30 Ivo de Almeida, Brasil     |
| 2 Marcelo Costabel, Argentina   | 12 Pedro Alzari, France         | 22 Nicole Larrieux, Uruguay        | 31 Isabel Usón, España        |
| 3 Swanand Gore, UK              | 13 Patricia Feliciano, Brasil   | 23 Clarisa Alvarez, Argentina      | 32 Igor Polikarpov, Brasil    |
| 4 Horacio Botti, Uruguay        | 14 Federico Rosconi, Uruguay    | 24 Livia Faim, Brasil              | 33 Matias Musumeci, Argentina |
| 5 Agustín Correa, Uruguay       | 15 Bill Shepard, France         | 25 Ana Puhl, Brasil                | 34 Gonzalo Mardones, Chile    |
| 6 João Barbosa, Brasil          | 16 Daniela Marchi, Brasil       | 26 Gonzalo Obal, Uruguay           | 35 Guilherme Razzera, Brasil  |
| 7 Felipe Trajtenberg, Uruguay   | 17 Marisol Fassolari, Argentina | 27 Daniele Chaves, Brasil          | 36 Paul Emsley, UK            |
| 8 Myra Flores, Peru             | 18 Jimena Rinaldi, Argentina    | 28 Ariel "Chapa" Chaparro, Uruguay |                               |
| 9 Alejandro Buschiazzo, Uruguay | 19 Sofia Horjales, Uruguay      | 29 Daniela Martínez, Uruguay       |                               |
| 10 Cecilia Ortiz, Uruguay       | 20 Melisa Jacobs, Argentina     |                                    |                               |

three members worked first independently, then gathered the three lists of merits, agreeing in a final common list, which was communicated to the applicants one week after application deadline. All applicants for the full course mode accepted to attend, and ultimately attended the workshop. As for the second group (theory only mode), only 5 students accepted the offer, among which only one was non Uruguayan.

## **Photos or Graphic material about the course**

We include CD and original program. All the material is also available to the participants through the interactive Moodle page.