The Portuguese Beam Allocation Group at the ESRF: a 14-year old success history (1999-2013)

Pedro M. Matias
Industry and Medicine Applied Crystallography
ITQB-UNL, Oeiras

2nd ENURS
15.02.2013

The ESRF at a glance

Founded in 1998

Began operation in 1994

Annual budget: ca. 80 M€

Members (minimum 4% shares, full voting rights)**:**

France, Germany, Italy, United Kingdom, Spain, Switzerland, Benesync (Belgium & The Netherlands), Nordsync (Denmark, Finland, Norway, Sweden)

Scientific Associates (< 4% shares, limited voting rights)**:**

*Austria, **Portugal**, Israel, Poland, Centralsync (Czech Republic, Hungary, Slovakia).*

Portugal, MX and the ESRF

The first Portuguese MX users of the ESRF were given access through a collaboration with the EMBL Grenoble Outstation in 1995-6

*Portugal joined the ESRF as a **scientific associate** in 1997 (1%).*

Novel statute created to accommodate the Portuguese membership, later allowed other small European countries to join the ESRF.

In 1999 the MX BAG scheme was created to promote a more efficient and productive use of the beamlines:

1 shift = 8 hours = MANY experiments

In recognition of their excellent work, the Portuguese MX groups were invited to form one of the first BAGs

Today, BAG use of the ESRF MX beamlines accounts for more than 90% of the available beamtime

The Portuguese MX BAG in 1999

ITQB – Universidade Nova de Lisboa

Maria Arménia Carrondo

REQUIMTE/FCT – Universidade Nova de Lisboa

Maria João Romão

IBMC – Universidade do Porto

Ana Margarida Damas

The Portuguese MX BAG in 2013

ITQB – Universidade Nova de Lisboa
Margarida Archer
Maria Arménia Carrondo
Carlos Frazão
Pedro Matias

IGC – Fundação Calouste Gulbenkian
Alekos Athanasiadis

REQUIMTE/FCT – Universidade Nova de Lisboa
Maria João Romão

IBMC/IMEB - Porto
Luís Gales Pinto
Sandra Macedo-Ribeiro
João Morais-Cabral
Pedro Pereira

The BAG scheme in Practice

Beam allocation ***every 6 months***

Yearly Report*: alternating* ***Progress*** *Report and* ***Full*** *Report*

Report evaluation by Review Panel determines maintenance of BAG status and overall beam allocation

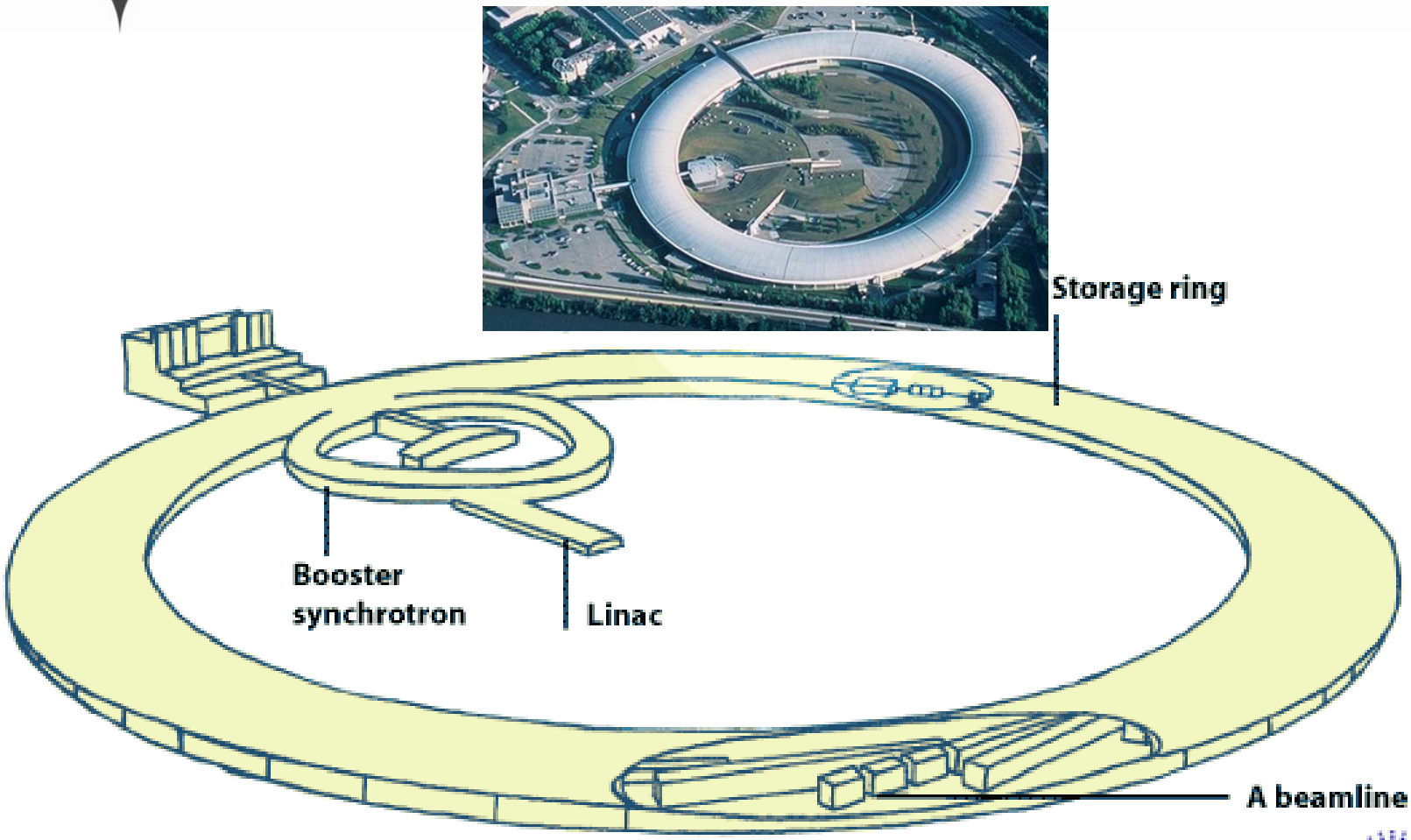
Our scores have been "good";

To improve them to "excellent" in order to increase beam allocation, we need:

- Work in more challenging projects (e.g., membrane proteins)

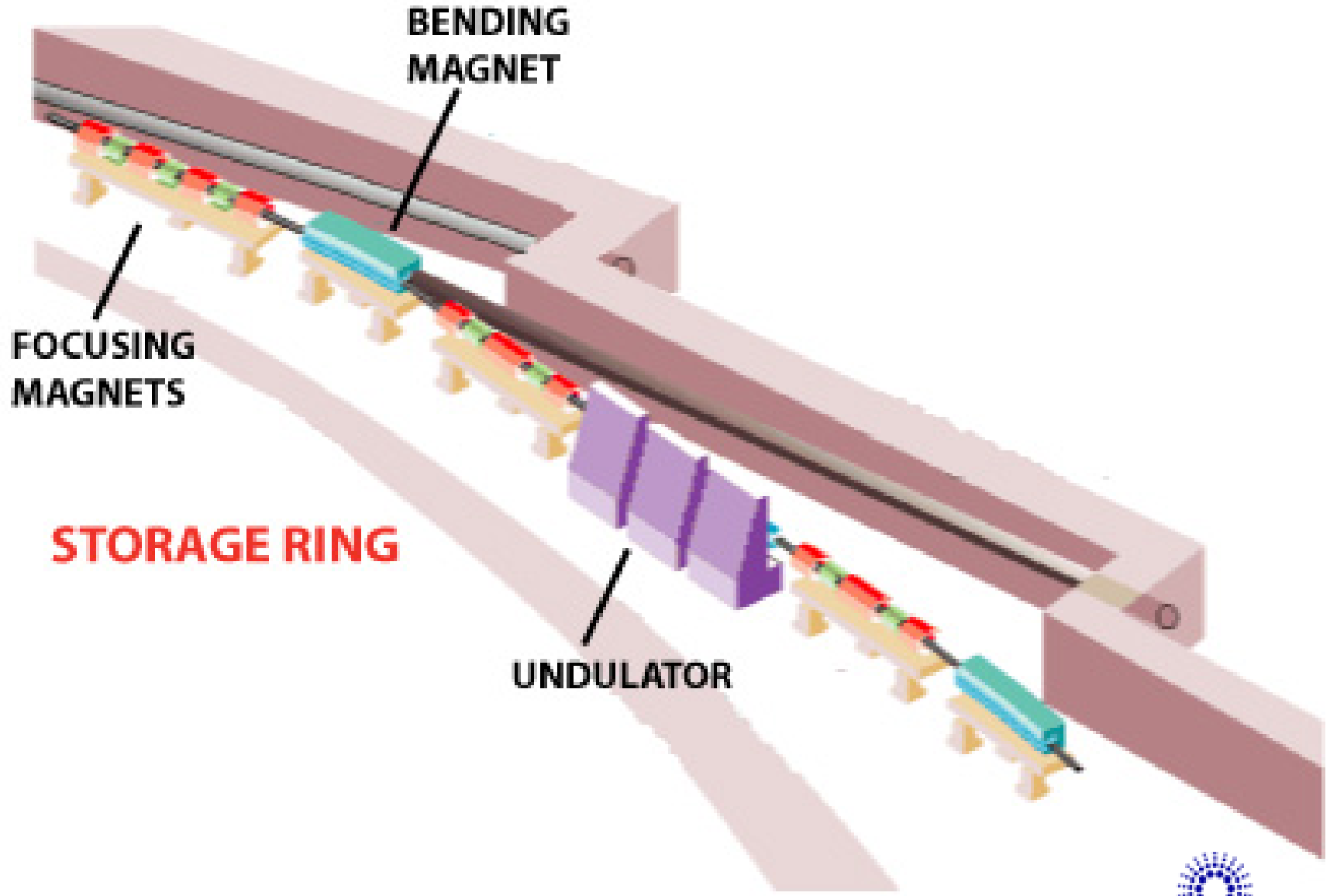
- More publications in high IF journals (e.g., Science, Nature, etc.)

An Overview of the ESRF



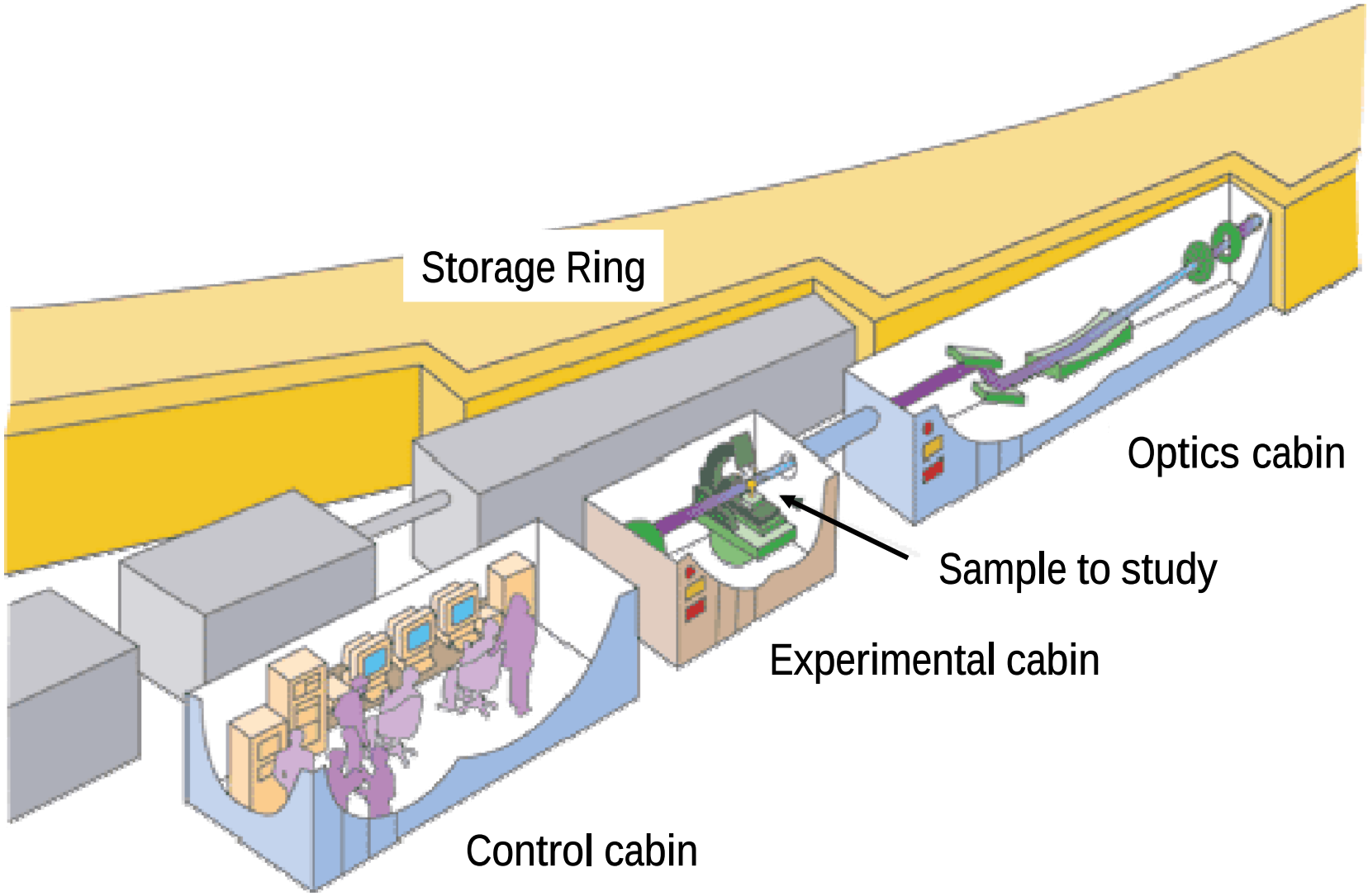
Source: http://www.esrf.fr/AboutUs/GuidedTour/Animation

ESRF



Source: http://www.esrf.fr/AboutUs/GuidedTour/Animation

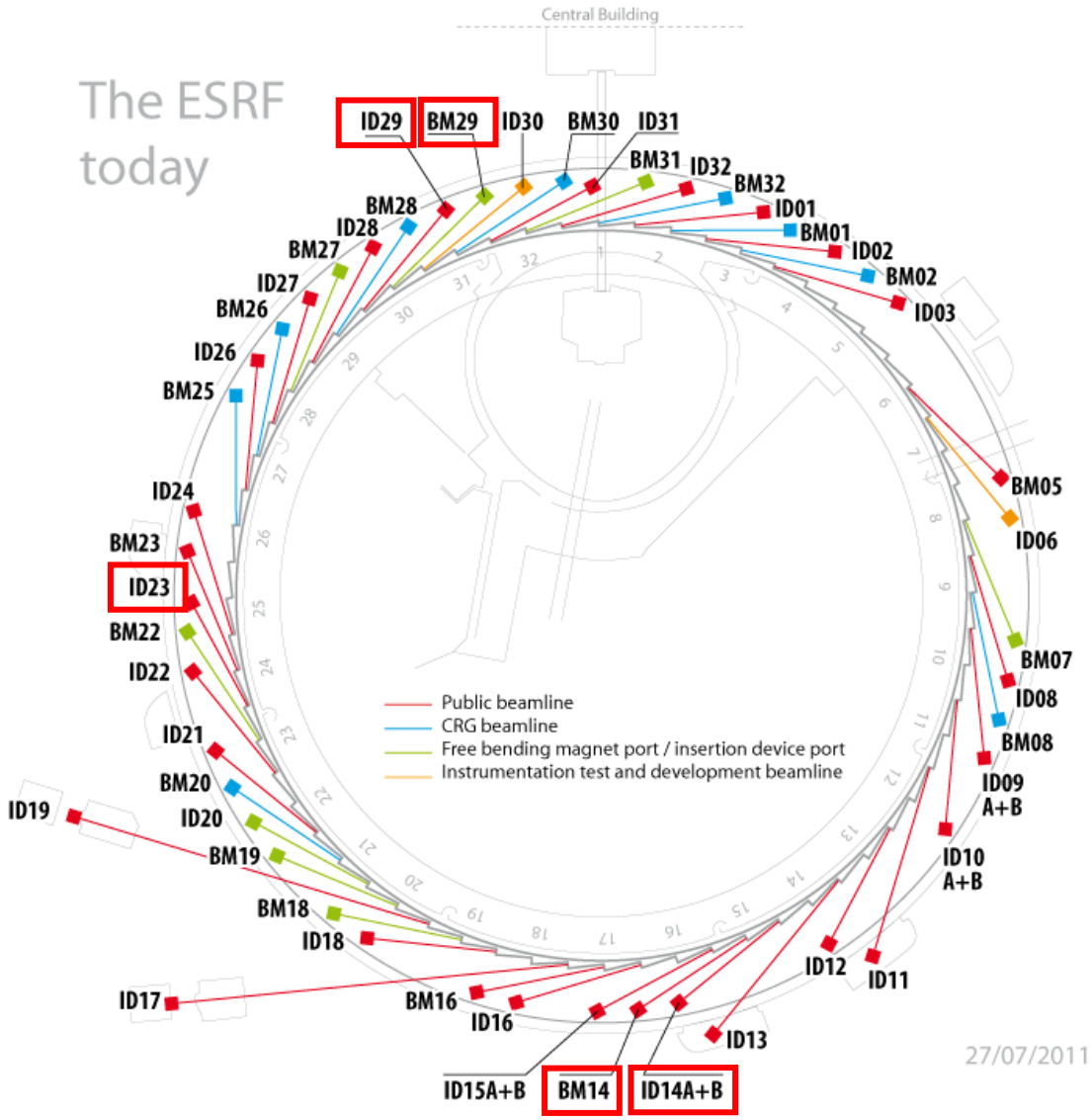
ESRF



Source: http://www.esrf.fr/AboutUs/GuidedTour/Animation

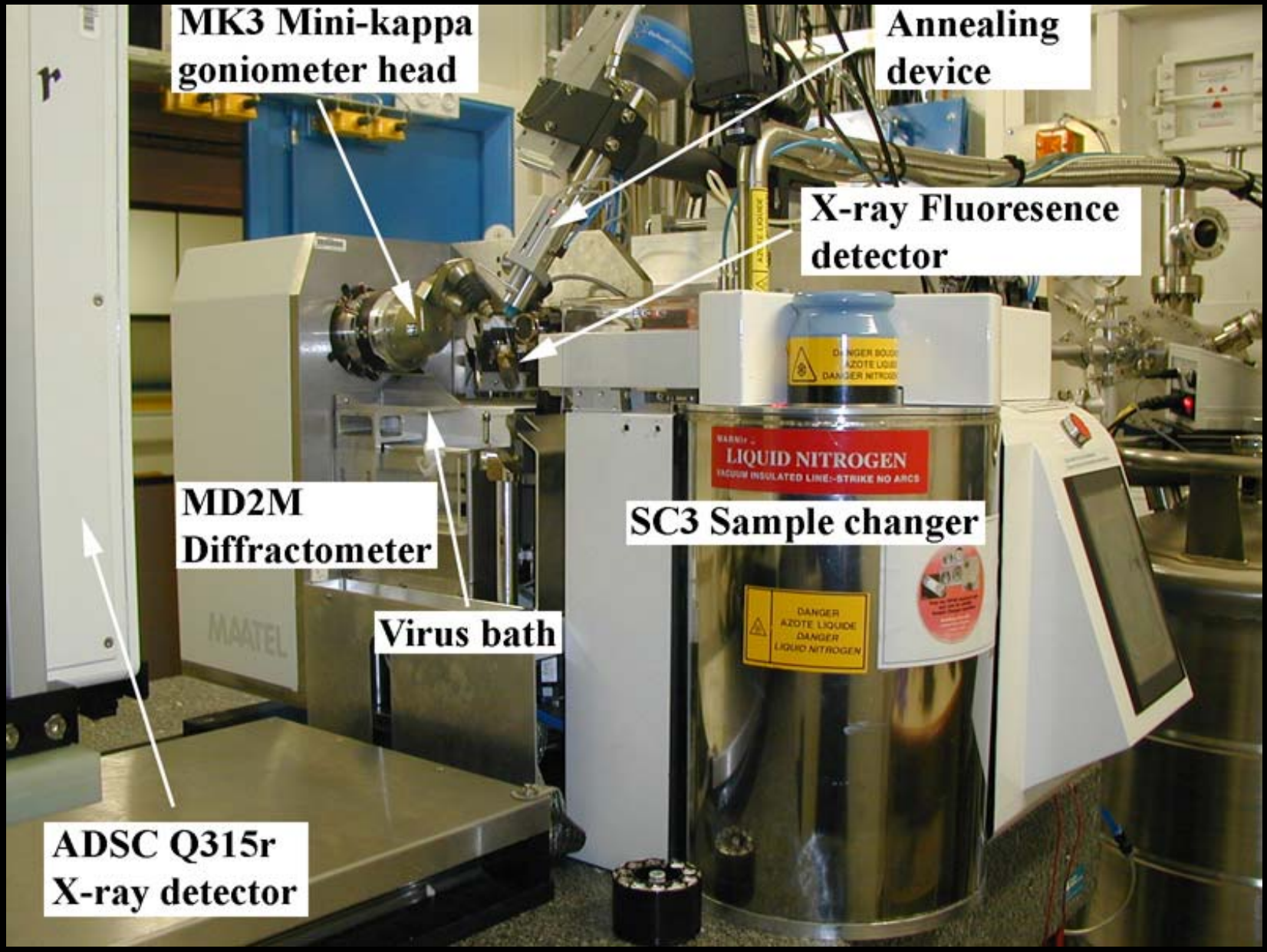
ESRF

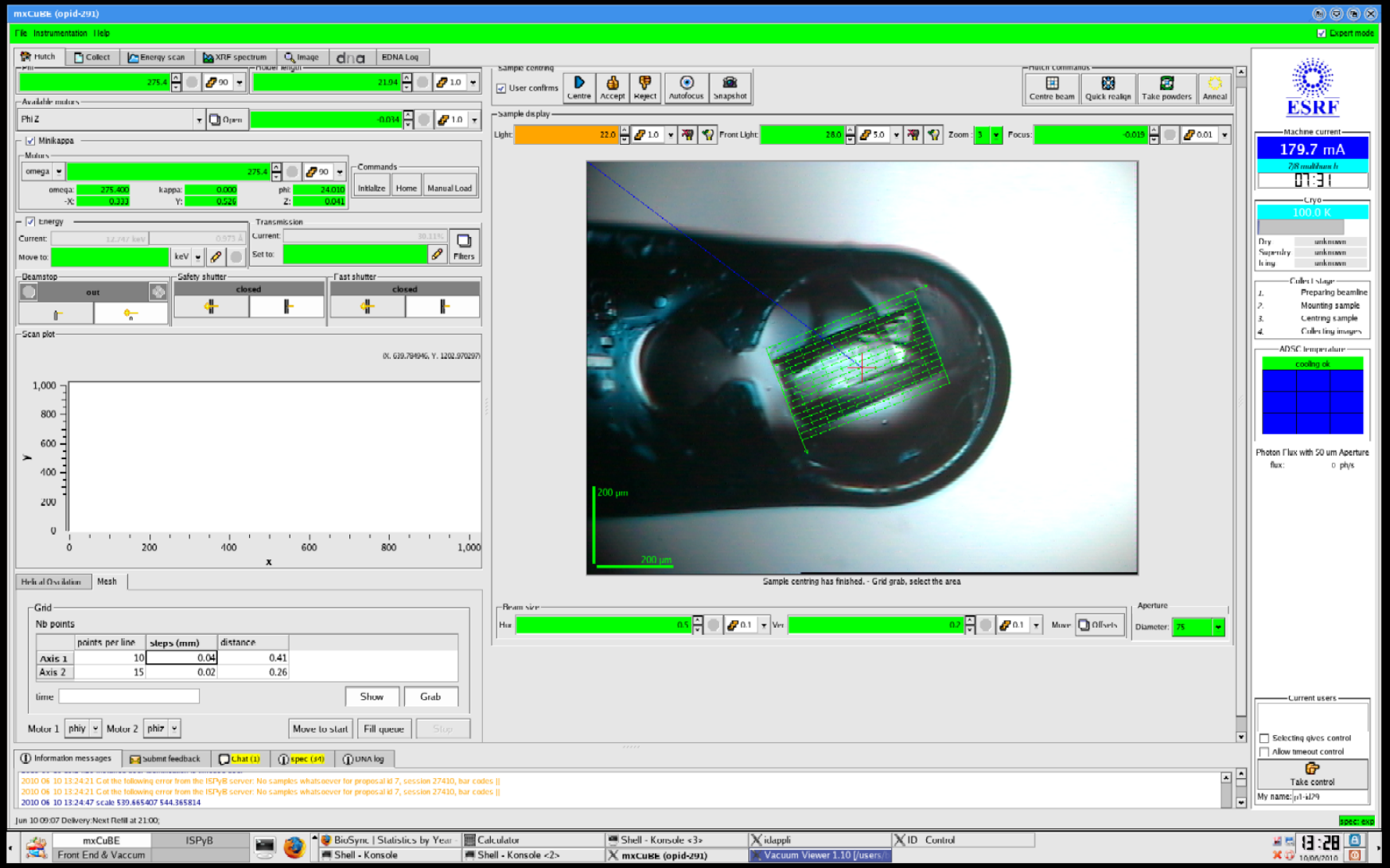
The MX beamlines at the ESRF

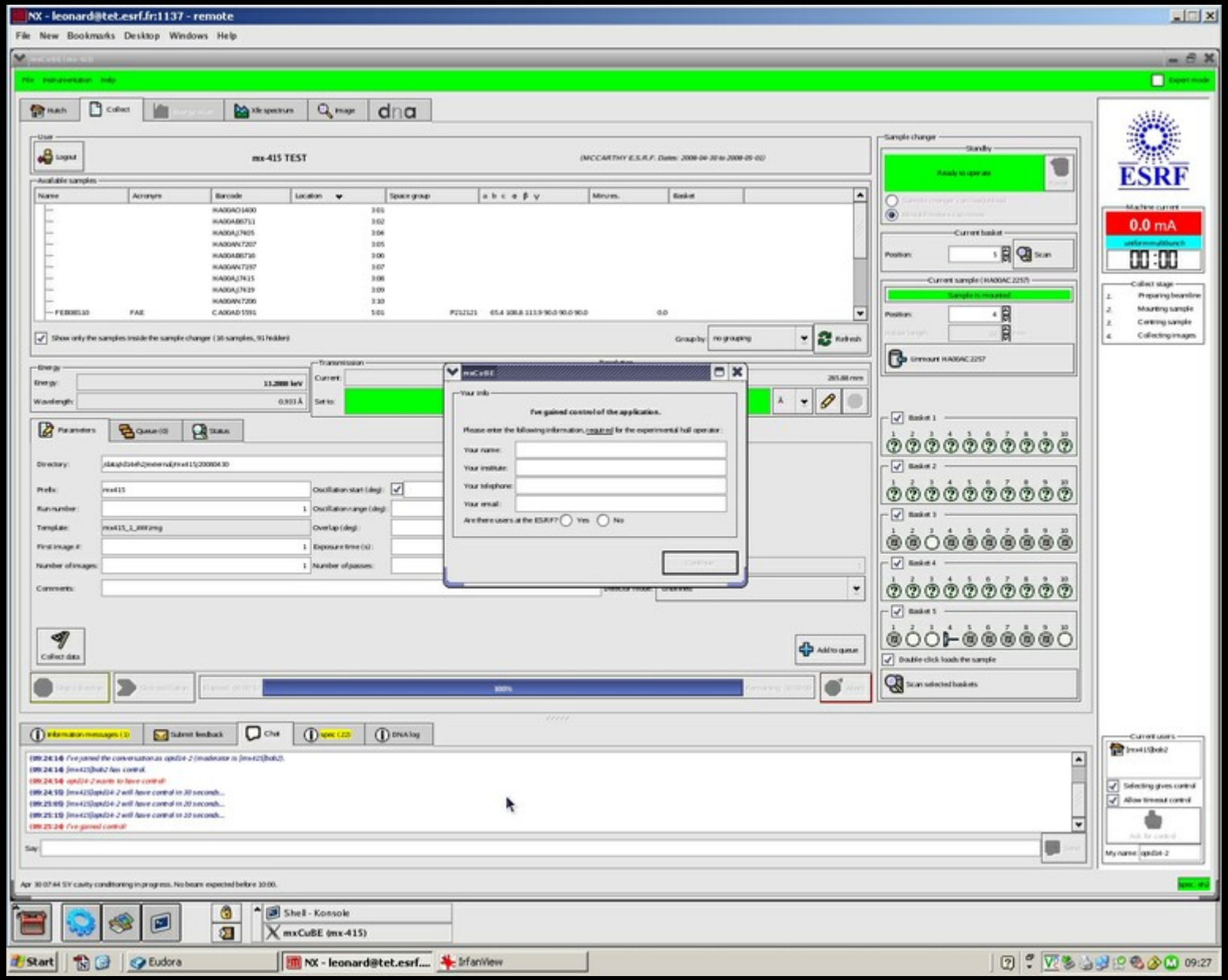


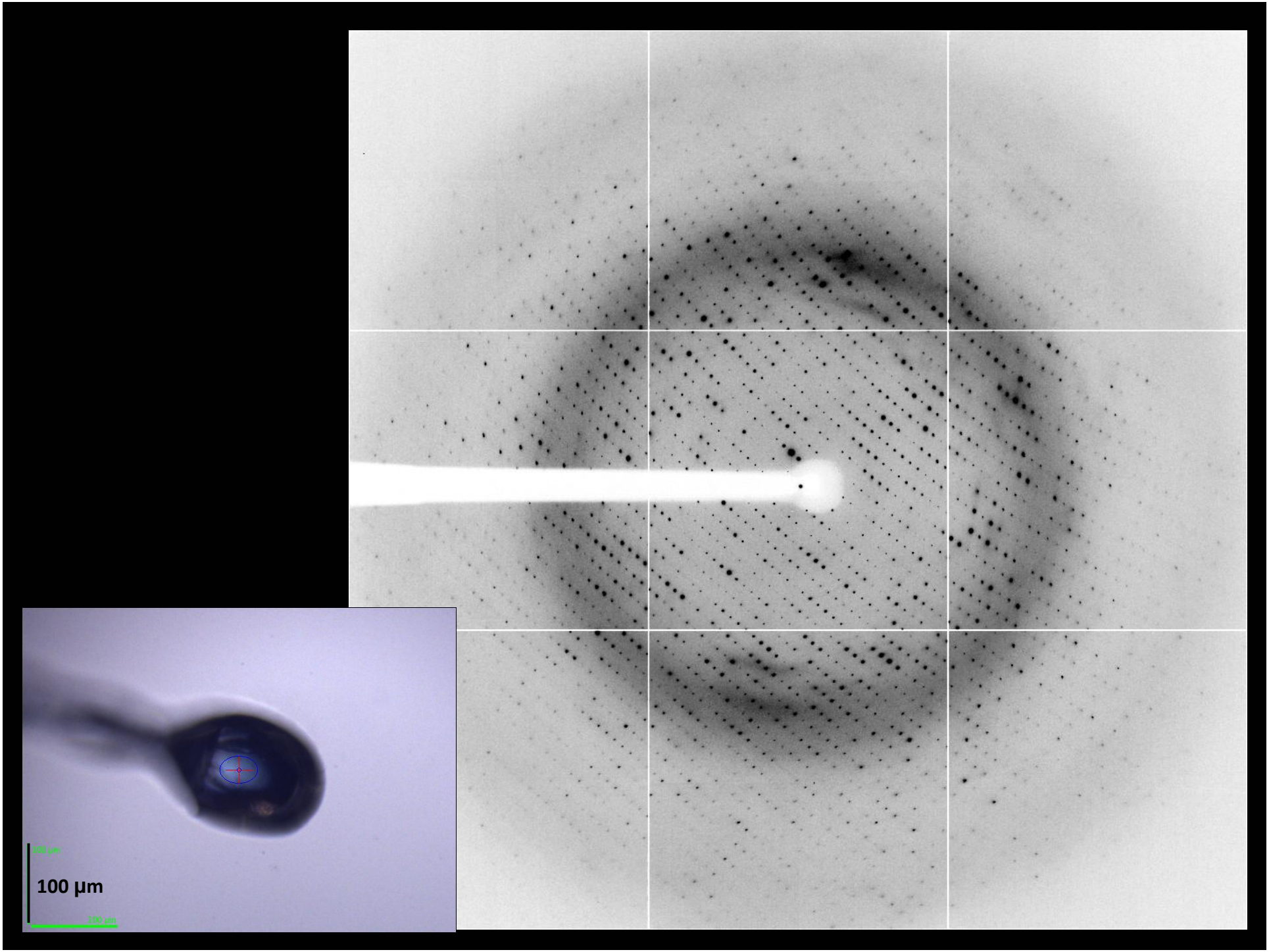

TEST OK
13·6·2011
ITRB
1
DS 1
CX100
TAYLOR-WHARTON

DEWAR I


MK3 Mini-kappa goniometer head
Annealing device
X-ray Fluoresence detector
MD2M Diffractometer
Virus bath
SC3 Sample changer
ADSC Q315r X-ray detector
LIQUID NITROGEN
DANGER AZOTE LIQUIDE DANGER LIQUID NITROGEN


mxCuBE (opid-291)
File Instrumentation Help
Expert mode
Hutch
Collect
Energy scan
XRF spectrum
Image
EDNA Log
Sample centring
User confirms
Centre
Accept
Reject
Autofocus
Snapshot
Hutch commands
Centre beam
Quick realign
Take powders
Anneal
Sample display
Light:
Front Light:
Zoom:
Focus:
Available motors
Phi Z
Open
Minikappa
Motors
omega
Commands
Initialize
Home
Manual Load
Energy
Current:
Move to:
keV
Transmission
Current:
Set to:
Filters
Beamstop
out
Safety shutter
closed
Fast shutter
closed
Scan plot
1,000
800
600
400
200
0
y
x
Helical Oscillation
Mesh
Grid
Nb points
points per line
steps (mm)
distance
Axis 1
10
0.04
0.41
Axis 2
15
0.02
0.26
time
Show
Grab
Motor 1
phiy
Motor 2
phiz
Move to start
Fill queue
Stop
200 µm
Sample centring has finished. - Grid grab, select the area
Beam size
Hor
Ver
Move
Offsets
Aperture
Diameter:
ESRF
Machine current
179.7 mA
Cryo
100.0 K
Collect stage
1. Preparing beamline
2. Mounting sample
3. Centring sample
4. Collecting images
ADSC temperature
cooling ok
Photon Flux with 50 um Aperture
flux:
phys
Current users
Selecting gives control
Allow timeout control
Take control
My name:
Information messages
Submit feedback
Chat (1)
spec (14)
DNA log
2010 06 10 13:24:21 Got the following error from the ISPyB server: No samples whatsoever for proposal id 7, session 27410, bar codes ||
2010 06 10 13:24:21 Got the following error from the ISPyB server: No samples whatsoever for proposal id 7, session 27410, bar codes ||
2010 06 10 13:24:47 scale 539.665407 544.365814
Jun 10 09:07 Delivery:Next Refill at 21:00;
spec: exp
mxCuBE
Front End & Vaccum
ISPyB
BioSync | Statistics by Year
Calculator
Shell - Konsole <3>
idappli
ID Control
Shell - Konsole
Shell - Konsole <2>
mxCuBE (opid-291)
Vacuum Viewer 1.10 [/users/
13:28


NX - leonard@tet.esrf.fr:1137 - remote
File New Bookmarks Desktop Windows Help
Hutch
Collect
dna
mx-415 TEST
ESRF
0.0 mA
Shell - Konsole
mxCuBE (mx-415)
Start
Eudora
NX - leonard@tet.esrf....
IrfanView
09:27


100 µm

Firefox | MXcube - Pesquisa do Google | Resultados da pesquisa de htt... | View results

https://wwws.esrf.fr/ispyb/user/viewResults.do?reqCode=display&dataCollectionId=1070465&rmerge=10.0&isigma=1.0&anomalous=false

Mais visitados | Anomalous Scattering C... | Best of the Web | Channel Guide | Customize Links | Free Hotmail | Internet Start | Is Your Operating Syste... | Microsoft | MSN.com | Windows Marketplace | Marcadores

Norton | Safe Web | Compartilhar | Configurar cofre | Assistente de Login

Selected Session		Selected DataCollectionGroup		Selected DataCollection	
Start Date	28-01-2013	Start Time	29-01-2013 06:02:00	Start Time	29-01-2013 06:02:00
BeamLine	ID14-4 +33476882322	Experiment Type	SAD	Image Prefix	ref-A32
	Back to this session		Back to this data collection group	Run Number	1
	Back to sessions				Back to this data collection

Experiment parameters | Beamline parameters | Characterisation parameters | **Characterisation results** | Autoprocessing

Characterisation Results (EDNA log file)

Data collection info

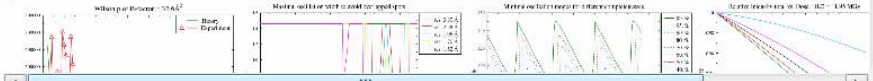
Data collection date	Tue Jan 29 06:02:34 2013
Image prefix	ref-A32_1
Directory	/data/visitor/mx1428/id14eh4/20130127/RAW_DATA/IBMC/AMPPNP06b

Diffraction Plan

Forced space group	Anomalous data	Aimed multiplicity	Aimed completeness	Aimed I/sigma at highest res.	Aimed resolution (Å)
None	False	Default (optimized)	Default (>= 0.99)	2.00	Default (highest possible)

Collection plan strategy (RADDOSE log file , BEST log file)

Resolution limit is set according to the given max.time								
Wedge	Subwedge	Start (°)	Width (°)	No images	Exp time (s)	Max res (Å)	Rel trans (%)	Distance (mm)
1	1	131.00	0.20	705	0.10	1.87	67.40	267.64



Strategies Wedge

Wedge number	Resolution Å	Completeness	Multiplicity	Total dose	Number of images	Phi °	Kappa °	Wavelength	Comments
1	1.87	99.3	3.0		705			1.0	

14:03
11-02-2013

Firefox | MXcube - Pesquisa do Google | Resultados da pesquisa de htt... | View results

https://wwws.esrf.fr/ispyb/user/viewResults.do?reqCode=display&dataCollectionId=1070465&rmerge=10.0&isigma=1.0&anomalous=false

Mais visitados | Anomalous Scattering C... | Best of the Web | Channel Guide | Customize Links | Free Hotmail | Internet Start | Is Your Operating Syste... | Microsoft | MSN.com | Windows Marketplace | Marcadores

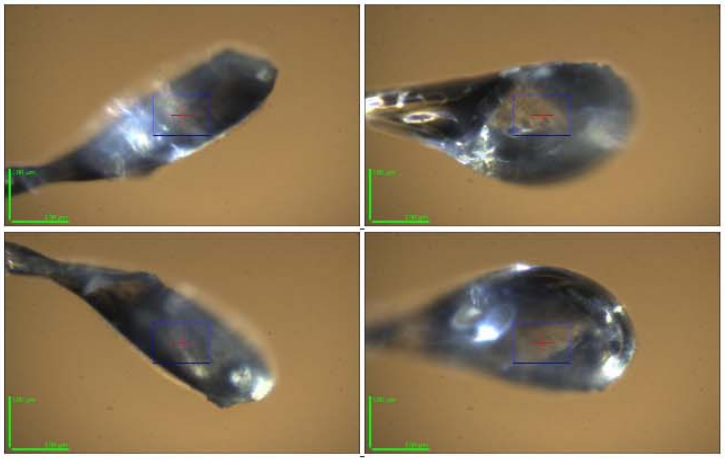
Norton | Safe Web | Compartilhar | Configurar cofre | Assistente de Login

Strategies Wedge

Wedge number	Resolution A	Completeness	Multiplicity	Total dose	Number of images	Phi °	Kappa °	Wavelength	Comments
1	1.87	99.3	3.0		705			1.0	

Sub Wedge number	Rotation axis °	Axis start °	Axis end °	Exposure time s	Transmission %	Oscillation range	Completeness	Multiplicity	Total dose	Number of images	Comments
1		131.0	272.0	0.1	67.4	0.2				705	

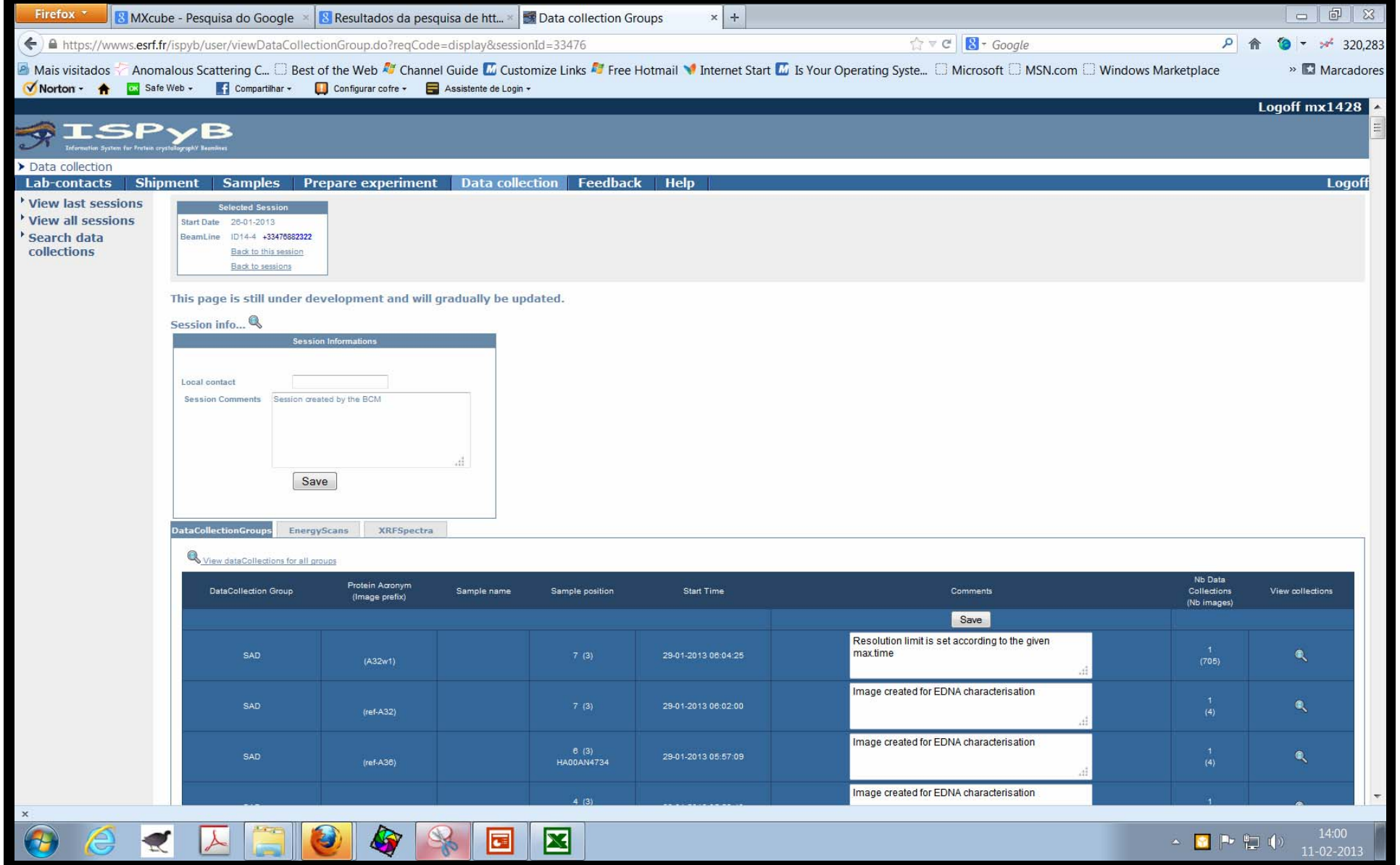
Crystal Snapshots

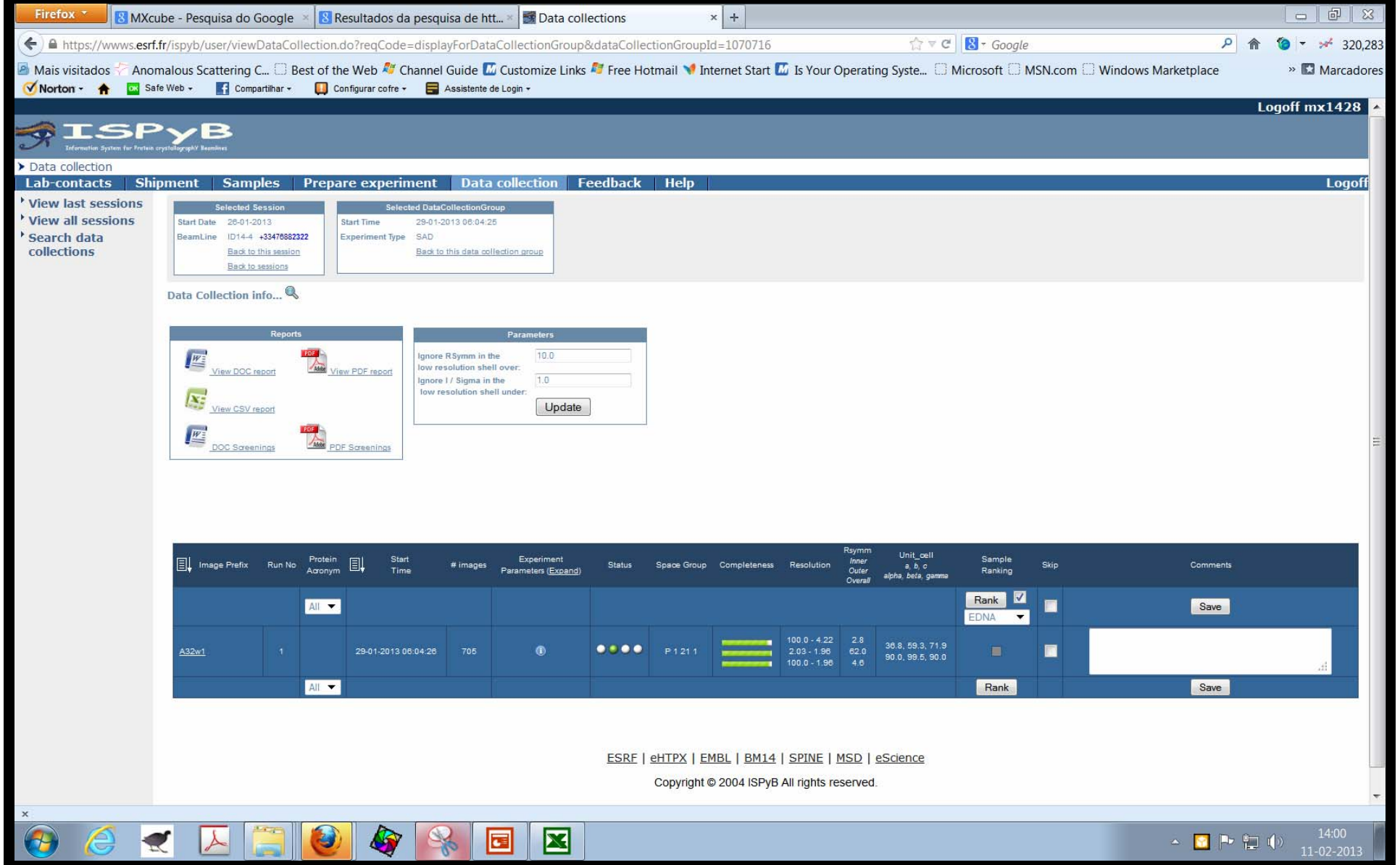


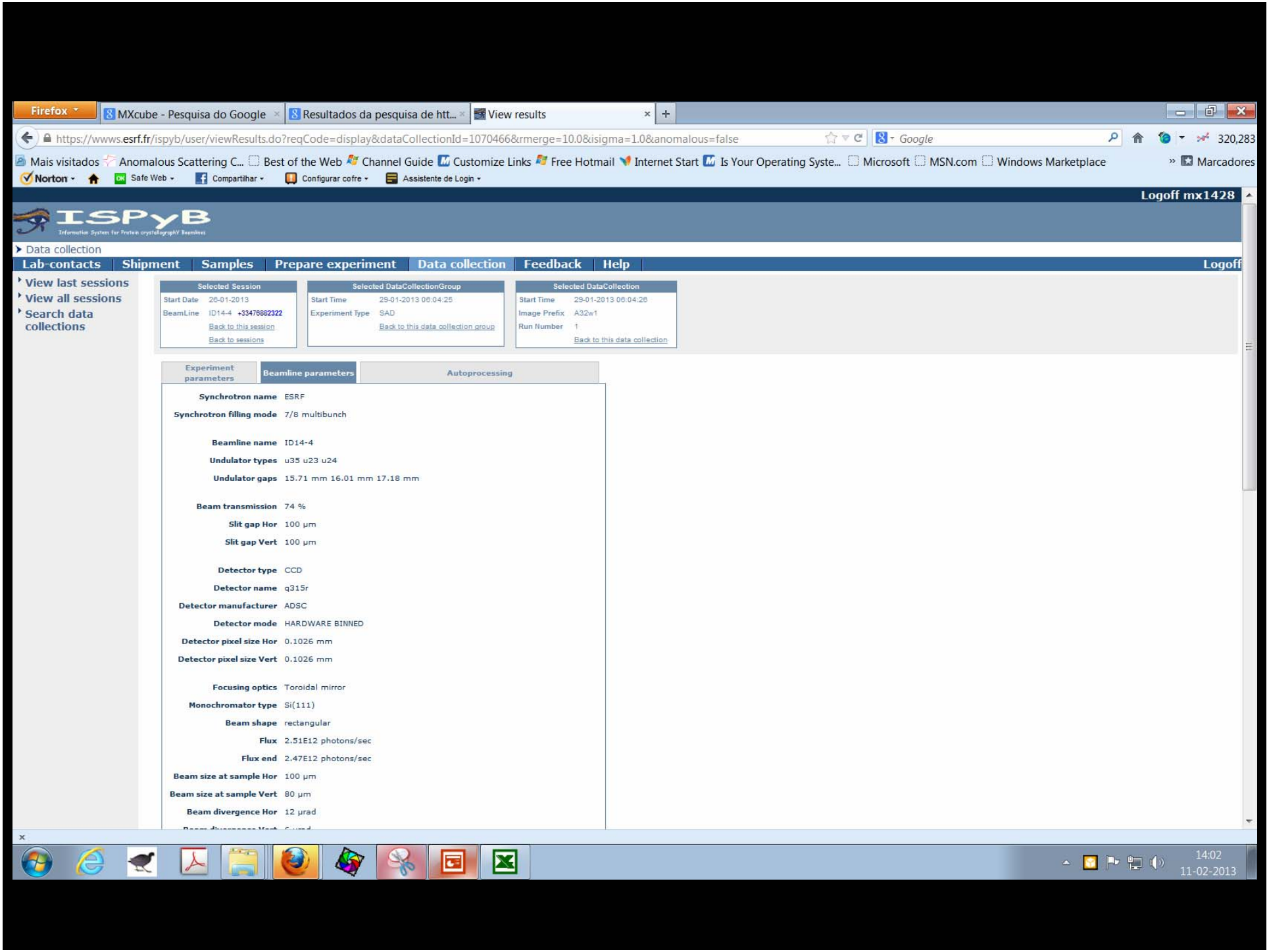
Expected Snapshots location: /data/pyarch/id14eh4/mx1428/20130127/RAW_DATA/IBMC/AMPPNP06b/ref-A32_1_1.snapshot.jpeg

Images collected

14:04
11-02-2013


Firefox
MXcube - Pesquisa do Google
Resultados da pesquisa de htt...
Data collection Groups
https://wwws.esrf.fr/ispyb/user/viewDataCollectionGroup.do?reqCode=display&sessionId=33476
Logoff mx1428
ISPyB
Data collection
Lab-contacts | Shipment | Samples | Prepare experiment | Data collection | Feedback | Help | Logoff
View last sessions
View all sessions
Search data collections
Selected Session
Start Date 26-01-2013
BeamLine ID14-4 +33476882322
Back to this session
Back to sessions
This page is still under development and will gradually be updated.
Session info...
Session Informations
Local contact
Session Comments
Session created by the BCM
Save
DataCollectionGroups
EnergyScans
XRFSpectra
View dataCollections for all groups
DataCollection Group
Protein Acronym (Image prefix)
Sample name
Sample position
Start Time
Comments
Nb Data Collections (Nb images)
View collections
Save
SAD
(A32w1)
7 (3)
29-01-2013 06:04:25
Resolution limit is set according to the given max.time
1 (705)
SAD
(ref-A32)
7 (3)
29-01-2013 06:02:00
Image created for EDNA characterisation
1 (4)
SAD
(ref-A36)
6 (3) HA00AN4734
29-01-2013 05:57:09
Image created for EDNA characterisation
1 (4)
Image created for EDNA characterisation
14:00
11-02-2013


Firefox
MXcube - Pesquisa do Google
Resultados da pesquisa de htt...
Data collections
https://wwws.esrf.fr/ispyb/user/viewDataCollection.do?reqCode=displayForDataCollectionGroup&dataCollectionGroupId=1070716
Logoff mx1428
ISPyB
Information System for Protein crystallography Beamlines
Data collection
Lab-contacts | Shipment | Samples | Prepare experiment | Data collection | Feedback | Help | Logoff
View last sessions
View all sessions
Search data collections
Selected Session
Start Date 26-01-2013
BeamLine ID14-4 +33476882322
Back to this session
Back to sessions
Selected DataCollectionGroup
Start Time 29-01-2013 06:04:25
Experiment Type SAD
Back to this data collection group
Data Collection info...
Reports
View DOC report
View PDF report
View CSV report
DOC Screenings
PDF Screenings
Parameters
Ignore RSymm in the low resolution shell over: 10.0
Ignore I / Sigma in the low resolution shell under: 1.0
Update
Image Prefix | Run No | Protein Acronym | Start Time | # images | Experiment Parameters (Expand) | Status | Space Group | Completeness | Resolution | Rsymm Inner Outer Overall | Unit_cell a, b, c alpha, beta, gamma | Sample Ranking | Skip | Comments
Rank
EDNA
Save
A32w1 | 1 | 29-01-2013 06:04:26 | 705 | P 1 21 1 | 100.0 - 4.22 2.03 - 1.96 100.0 - 1.96 | 2.8 62.0 4.6 | 36.8, 59.3, 71.9 90.0, 99.5, 90.0
All
Rank
Save
ESRF | eHTPX | EMBL | BM14 | SPINE | MSD | eScience
Copyright © 2004 ISPyB All rights reserved.
14:00
11-02-2013


Firefox
MXcube - Pesquisa do Google
Resultados da pesquisa de htt...
View results
https://wwws.esrf.fr/ispyb/user/viewResults.do?reqCode=display&dataCollectionId=1070466&rmerge=10.0&isigma=1.0&anomalous=false
Logoff mx1428
ISPyB
Information System for Protein crystallography Beamlines
Data collection
Lab-contacts | Shipment | Samples | Prepare experiment | Data collection | Feedback | Help | Logoff
View last sessions
View all sessions
Search data collections
Selected Session
Start Date 26-01-2013
BeamLine ID14-4 +33476882322
Back to this session
Back to sessions
Selected DataCollectionGroup
Start Time 29-01-2013 06:04:25
Experiment Type SAD
Back to this data collection group
Selected DataCollection
Start Time 29-01-2013 06:04:26
Image Prefix A32w1
Run Number 1
Back to this data collection
Experiment parameters
Beamline parameters
Autoprocessing
Synchrotron name ESRF
Synchrotron filling mode 7/8 multibunch
Beamline name ID14-4
Undulator types u35 u23 u24
Undulator gaps 15.71 mm 16.01 mm 17.18 mm
Beam transmission 74 %
Slit gap Hor 100 µm
Slit gap Vert 100 µm
Detector type CCD
Detector name q315r
Detector manufacturer ADSC
Detector mode HARDWARE BINNED
Detector pixel size Hor 0.1026 mm
Detector pixel size Vert 0.1026 mm
Focusing optics Toroidal mirror
Monochromator type Si(111)
Beam shape rectangular
Flux 2.51E12 photons/sec
Flux end 2.47E12 photons/sec
Beam size at sample Hor 100 µm
Beam size at sample Vert 80 µm
Beam divergence Hor 12 µrad
14:02
11-02-2013

Firefox | MXcube - Pesquisa do Google | Resultados da pesquisa de htt... | View results

https://wwws.esrf.fr/ispyb/user/viewResults.do?reqCode=display&dataCollectionId=1070466&rmerge=10.0&isigma=1.0&anomalous=false

Mais visitados | Anomalous Scattering C... | Best of the Web | Channel Guide | Customize Links | Free Hotmail | Internet Start | Is Your Operating Syste... | Microsoft | MSN.com | Windows Marketplace | Marcadores

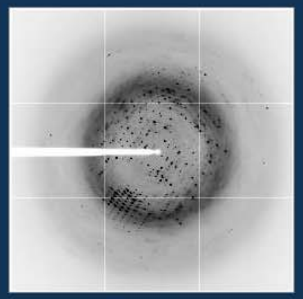
Norton | Safe Web | Compartilhar | Configurar cofre | Assistente de Login

Crystal Snapshots

Expected Snapshots location:

Images collected

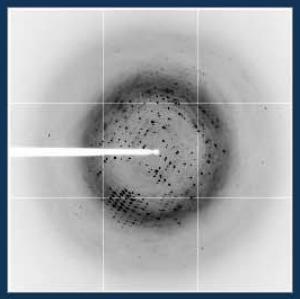
A32w1_1_0001.img



Machine message: Jan 28 21:05 No scheduled refill until tomorrow 8h00. (M.D.T.)

Comments:

A32w1_1_0002.img



Machine message: Jan 28 21:05 No scheduled refill until tomorrow 8h00. (M.D.T.)

Comments:

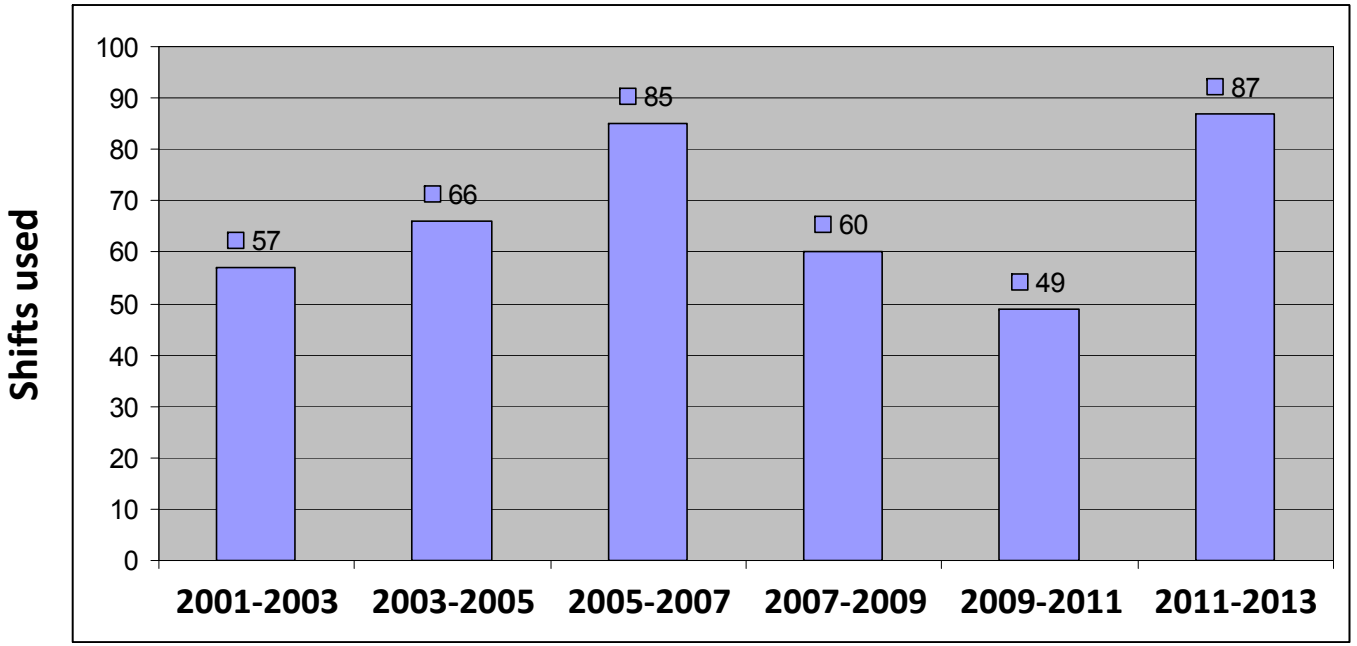
Location /data/visitor/mx1428/id14eh4/20130127/RAW_DATA/IBMC/AMPPNP06b

View as Image wall

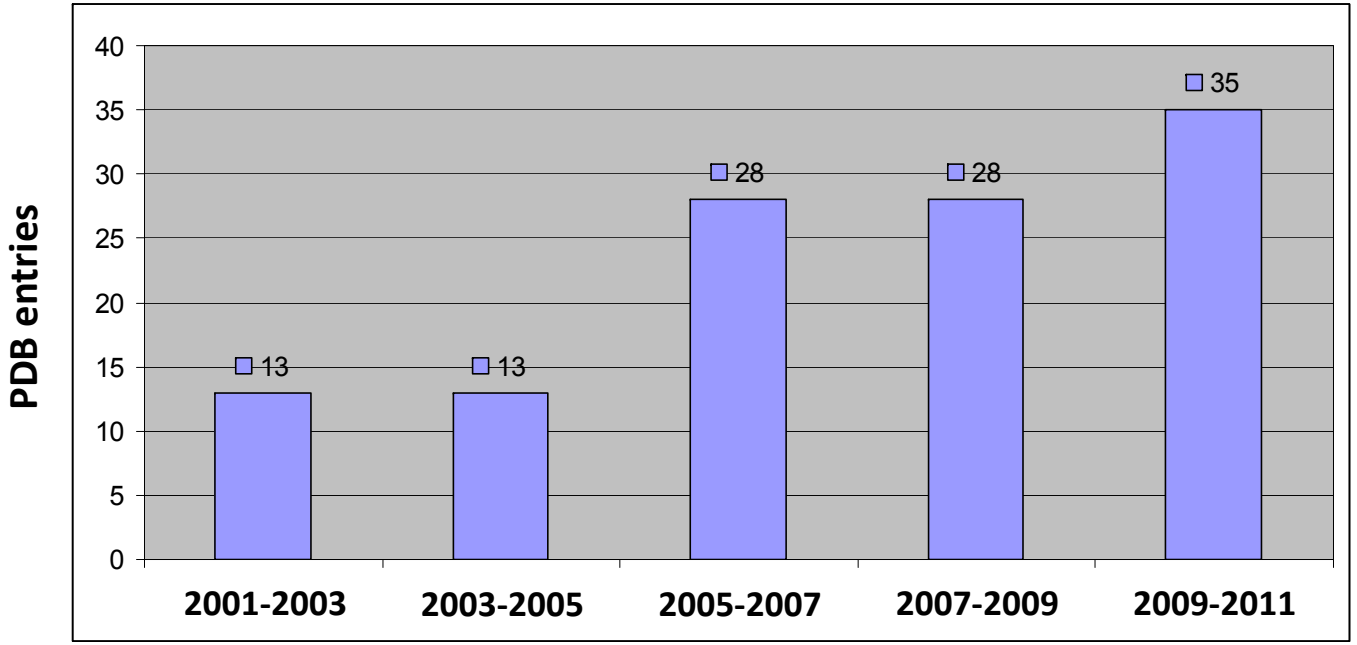
ESRF | eHTPX | EMBL | BM14 | SPINE | MSD | eScience

14:02
11-02-2013

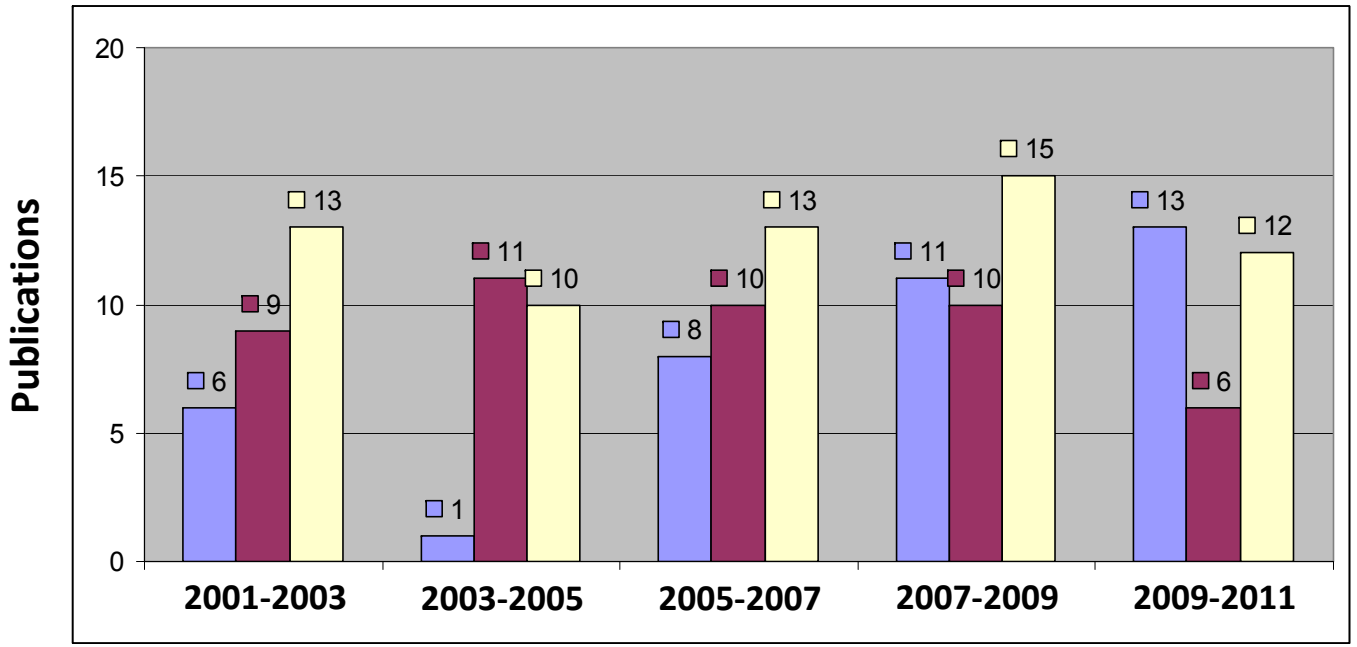
BAG statistics – shifts used



BAG statistics – PDB depositions



BAG statistics - Publications

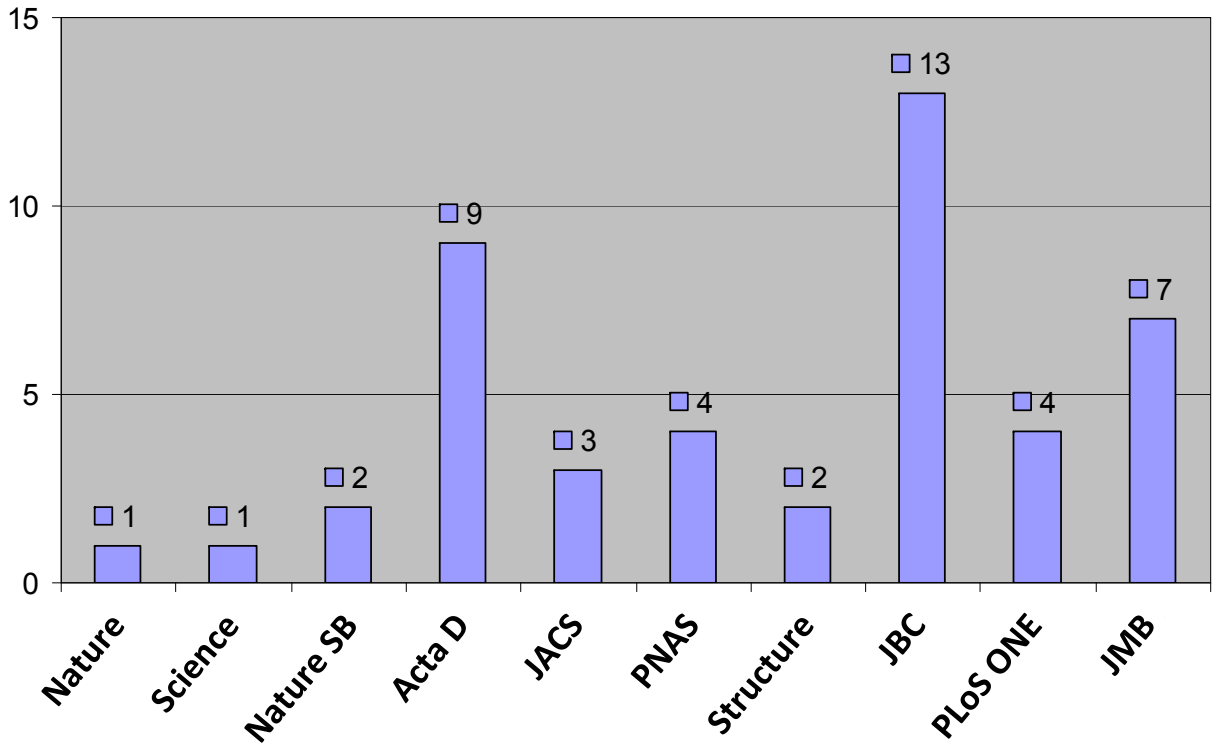


Crystallization Reports

Journals with IF > 4

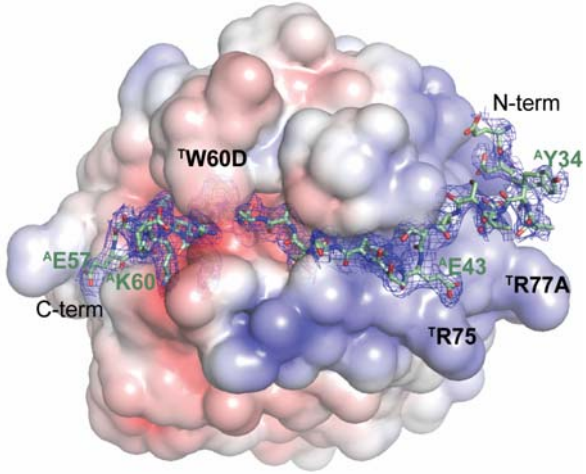
Journals with IF < 4

Publications with IF>4: 2001-2011



IBMC INEB
Laboratório Associado . Associate Laboratory

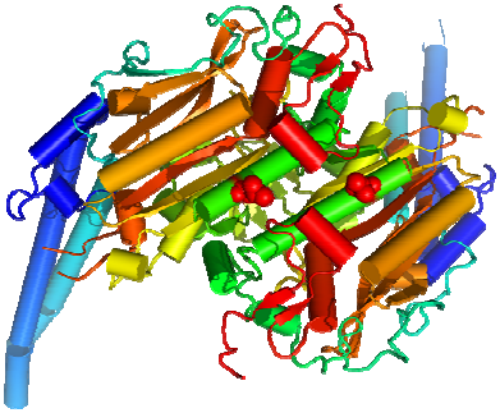
Novel anticoagulant mechanism in the malaria mosquito



- **Anophelin binds to thrombin in the reverse direction of *bona fide* substrates disrupting the catalytic triad**
- **Its compact size and resistance to proteolysis might the design of novel antithrombotics**

Figueiredo *et al*. (2012) *Proc. Natl. Acad. Sci. USA* **109**, E3649-58

SerRS, the main player in *C. albicans* genetic code alteration



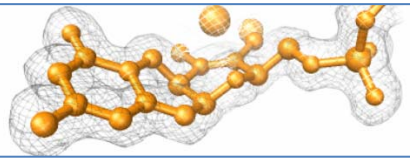
- **Crystal structures of the two natural isoforms of *Candida albicans* seryl-tRNA synthetase (SerRS-Leu / SerRS-Ser)**
- **Ambiguous codon localization tailored to minimize protein misfolding events**

Rocha *et al*. (2011) *Proc. Natl. Acad. Sci. USA* **108**, 14091-6

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

requimte
rede de química e tecnologia

xtal
Crystallography Group @ REQUIMTE



Main Research lines & Highlights

1- Molybdopterin Enzymes

• Aldehyde Oxidases:

The first **mammalian aldehyde oxidase** structures (mouse and human)

• Detailed **mechanistic studies and novel mechanisms** based on atomic-resolution structures (MOP, NAP)

2- Drug Design

• CO Releasing Molecules (CORM) binding mechanism to plasma proteins

• Adducts of Human Transferrin and Vanadium as anti-diabetic agents

• Design of potent and specific inhibitors of proteases based on Trypsin studies

3- Cellulosome: a megaDalton complex for cellulose degradation:

• Carbohydrate binding Modules

• Glycoside hydrolases

• Cohesin-dockerin complexes

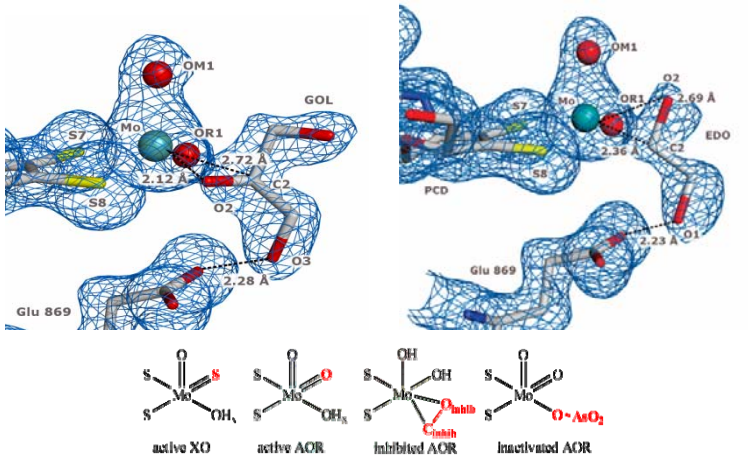
FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Molybdopterin Enzymes

requimte
rede de química e tecnologia

- **Detailed mechanistic studies**
- **Ligand identification and novel mechanisms**

Ethylene-glycol & Glycerol inhibited AOR @1.7 Å

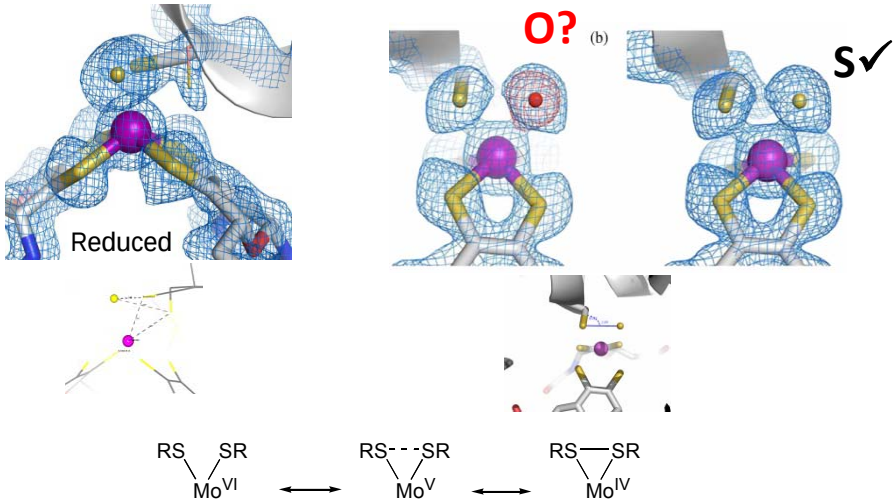


- **First structural evidence for a Mo-C bond in a biological system**
- Possible to distinguish Mo-O and Mo-C bonds
- Substrates may also bind to the protein by a η2 coordination

Romão, **Dalton Trans, 2009**
Santos-Silva et al, ***JACS*, 2009**
Correia et al, submitted

POSTER & Oral communication

Oxidized & Reduced Nitrate Reductase @1.5 Å



Mo and ligand-based redox chemistry

- **New mechanism for nitrate reduction: Ligand- based redox chemistry**
- Unanticipated sulfur ligand
- Partial disulfide bond

Coelho et al**, J Mol Biol, 2011**

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

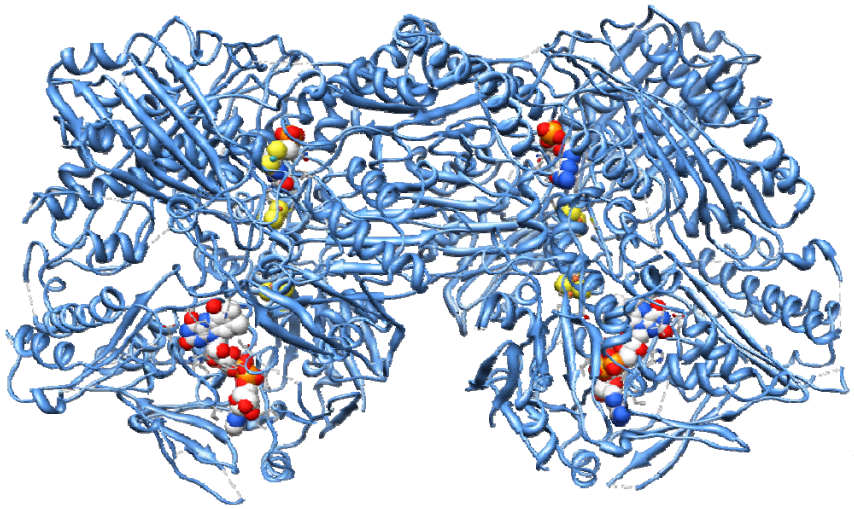
Molybdopterin Enzymes

requimte rede de química e tecnologia

- **First Crystal Structures of mammalian Aldehyde Oxidases**
- **Identification of new metallic clusters**

Mouse Aldehyde Oxidase 3.0 Å

@ ID14-1 & ID23-1



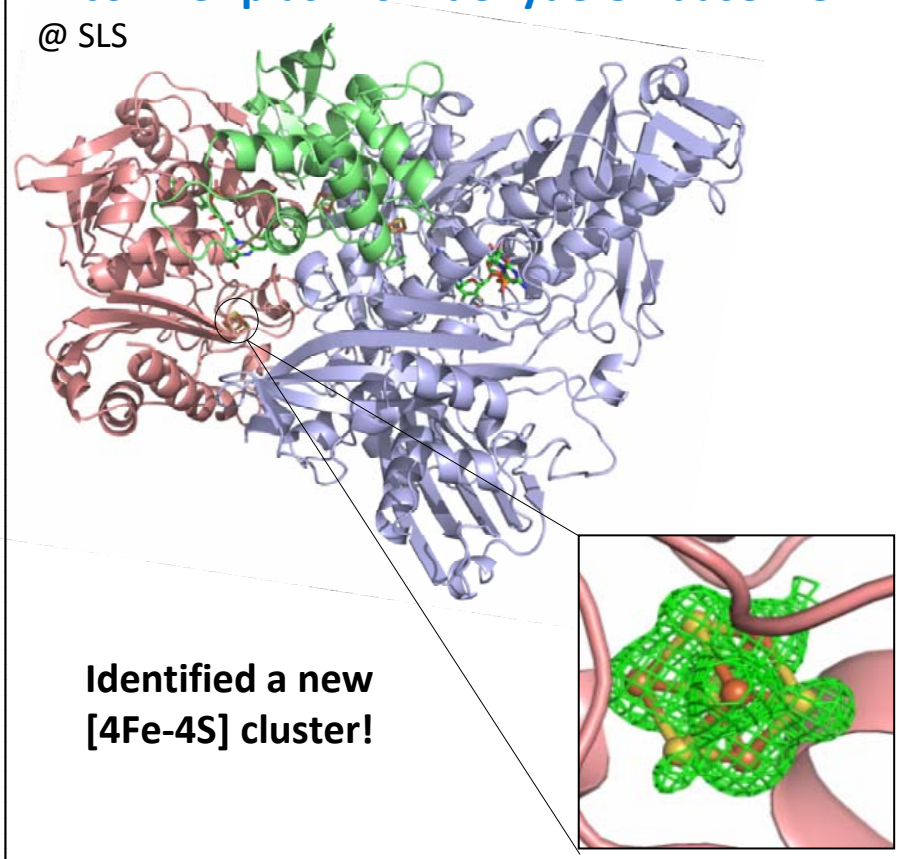
Extremely poor crystals and weak diffraction!
(P1 (4 mols/au; 1300 aa))

- Insights into substrate specificity
- Important in drug metabolism & increasing **importance in recent drug design programs** (Pfizer)

Coelho et al, **Drug Metab Dispos. 2011**
Coelho et al, **J Biol. Chem. 2012**

E. coli Periplasmic Aldehyde Oxidase 1.8 Å

@ SLS



Identified a new [4Fe-4S] cluster!

- The first monomeric XO-related enzyme
- Unique member of the XO family

POSTER

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

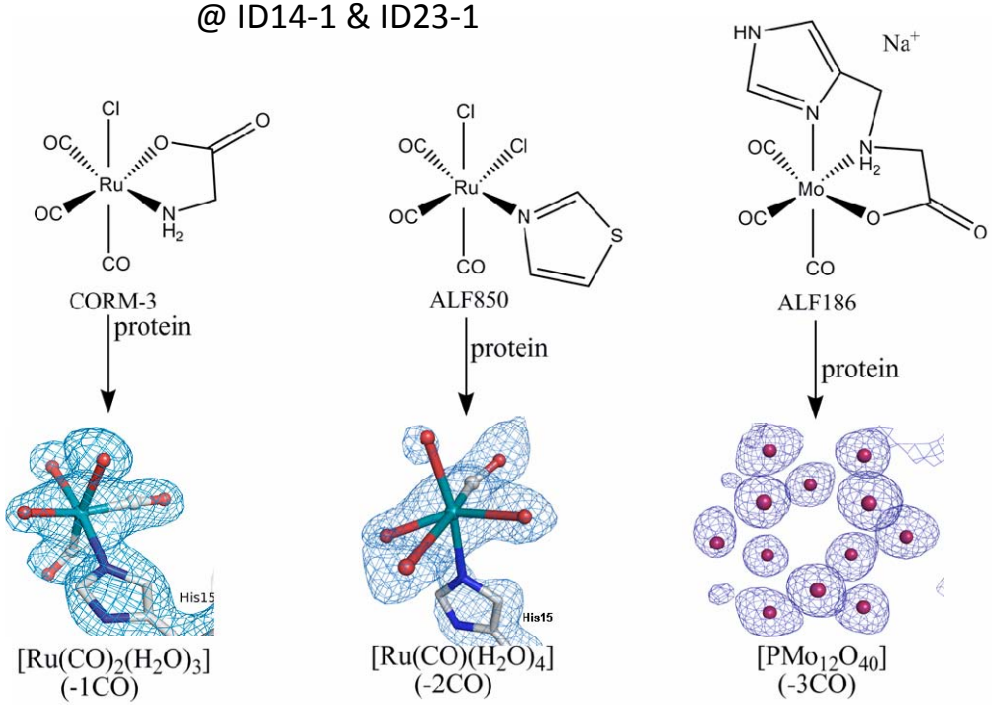
Drug Design

requimte rede de química e tecnologia

- **CO Releasing Molecules (CORM) binding mechanism to plasma proteins**
- **Vanadium – Transferrin adducts**
- **Trypsin – Inhibitors binding**

CORM – Lysozyme adducts 1.7 Å

@ ID14-1 & ID23-1



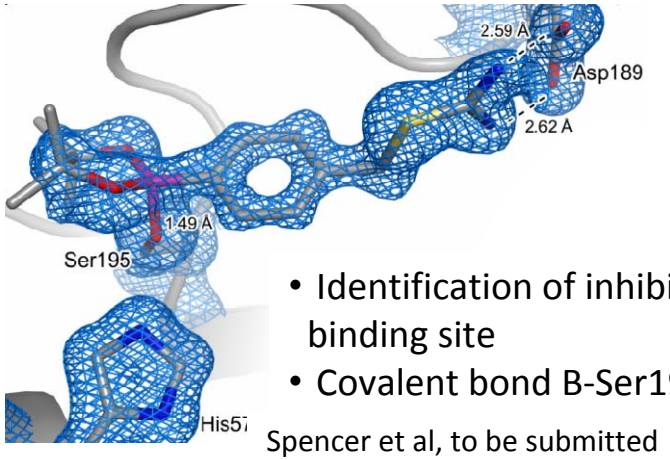
- Structural characterization of protein – CORM interactions
- Insights into CO release and polyoxometallate formation

Santos-Silva et al, **JACS, 2011; Curr Med Chem, 2011**
Santos et al **, JIB, 2012**
Seixas et al, **Dalton T, 2012**

POSTER

Trypsin and inhibitors of Urokinase 1.5 Å

@ ID29



- Identification of inhibitors binding site
- Covalent bond B-Ser195

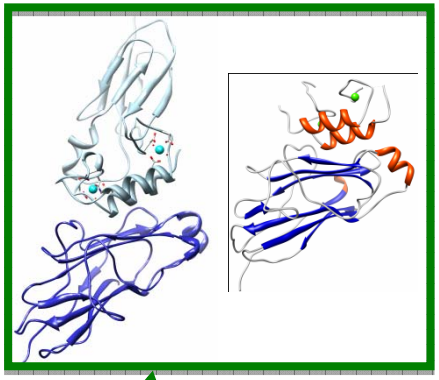
Spencer et al, to be submitted

Vanadium - Human Transferrin adducts

- Identification of the binding sites of V compounds
- Conformational changes upon binding (SAXS)
- Urea Gel Electrophorese to control protein modifications upon binding

Mehtab et al, **J Inorg Biochem**, in press

Cellulosome: a megaDalton complex for cellulose degradation

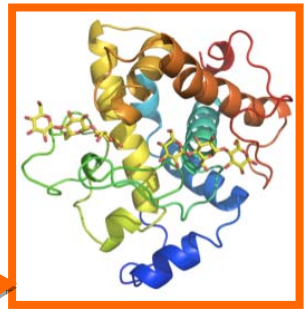


Type II Cohesin-Dockerin complex

Crystal structures of the type II interaction in *Clostridium thermocellum*

Carvalho et al, *JMB*, 2005
Alves et al, in prep

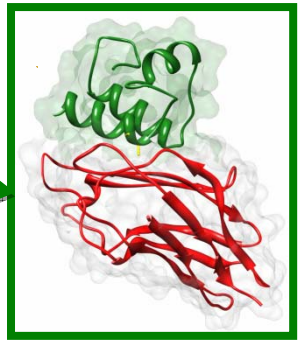
POSTER & Oral communication



Family 124 Glycoside Hydrolase

First structure of a representative member

Brás et al, *PNAS*, 2011

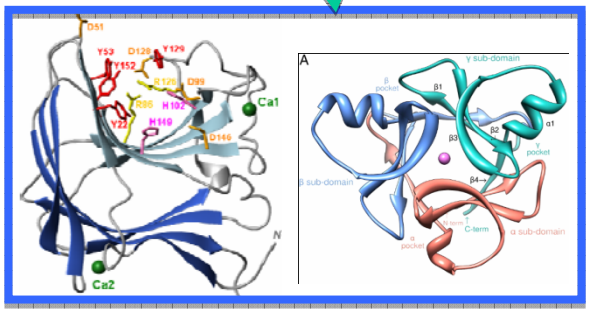


Type I Cohesin-Dockerin complex

First crystal structure of a type I interaction

Carvalho et al, *PNAS*, 2003
Carvalho et al, *PNAS*, 2007
Brás et al, *JBC*, 2012

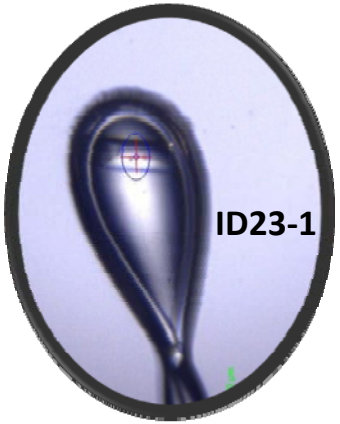
POSTER

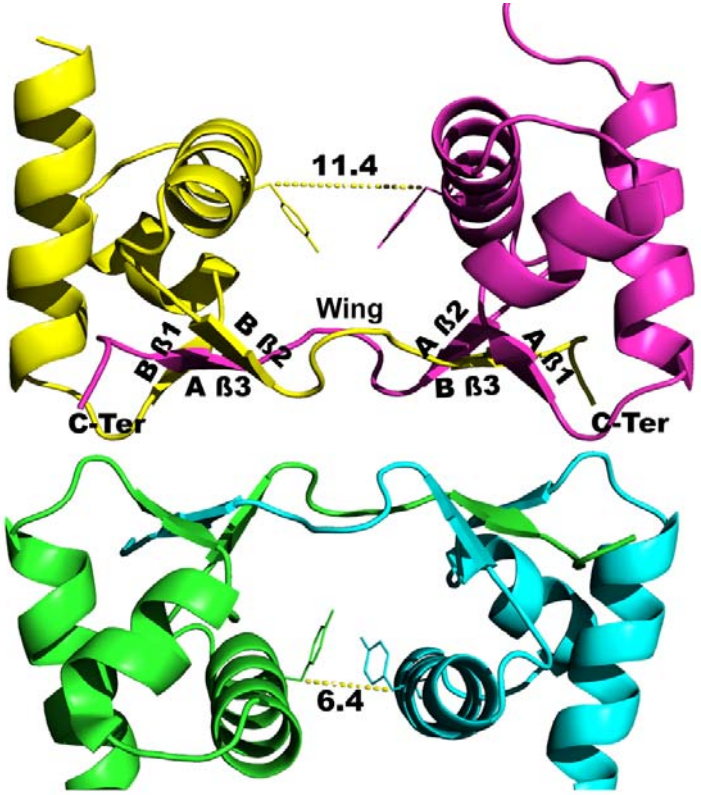


Carbohydrate Binding Modules types B and C

Carvalho et al, *JBC*, 2004
Najmudin et al, *JBC*, 2006
Viegas et al, *FEBS J*, 2008
Santos-Silva et al, *BBA*, 2010

Cyprinid Herpes Virus 3 Orf122
Crystal Structure - **A poxvirus-like Zalpha domain**

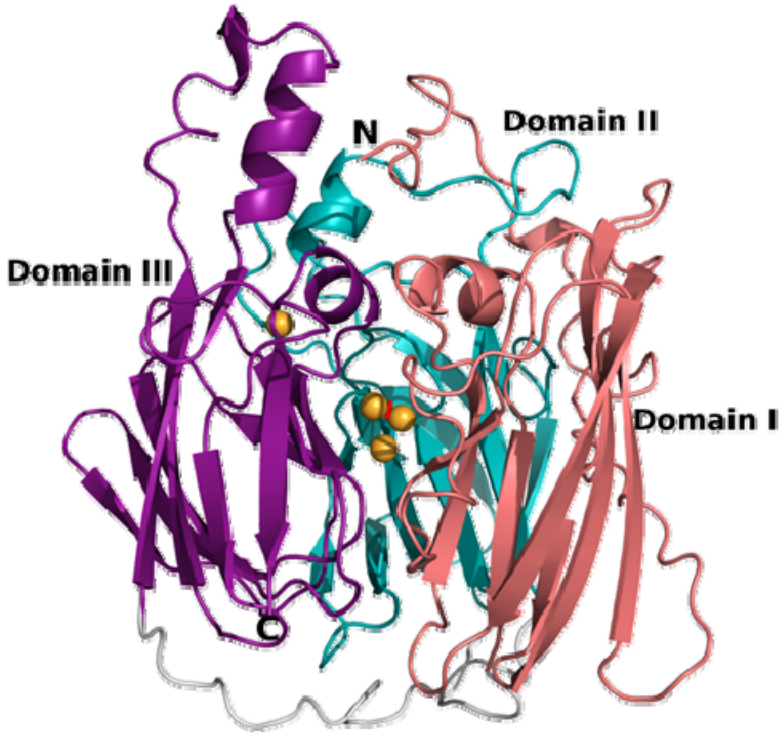




Unlike other Zalpha domains, ORF112 forms a dimer through a unique domain-swapping mechanism.

Tomé AR, Kuś K., Correia S., Paulo L., Zacarias S., de Rosa M., Figueiredo D., Parkhouse RME. and Athanasiadis A. (2013) J. Virology in print

Structure Characterization of a multicopper oxidase from *Campylobacter jejuni* CGUG11284



Resolution 1.95 Å

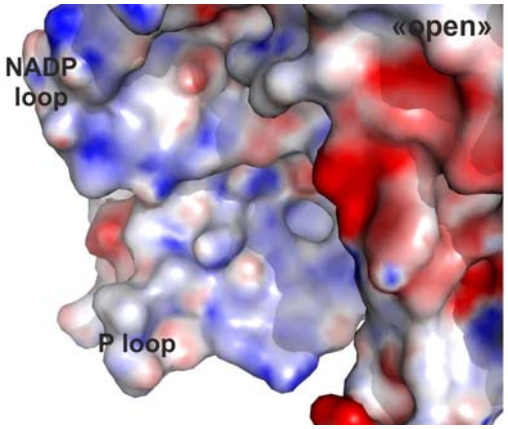
Campylobacter jejuni is a Pathogenic, Gram-negative bacterium, that is the most common cause of human gastroenteritis and bacterial food poisoning.

McoC – is a periplasmic multicopper oxidase thought to be involved in copper homeostasis.

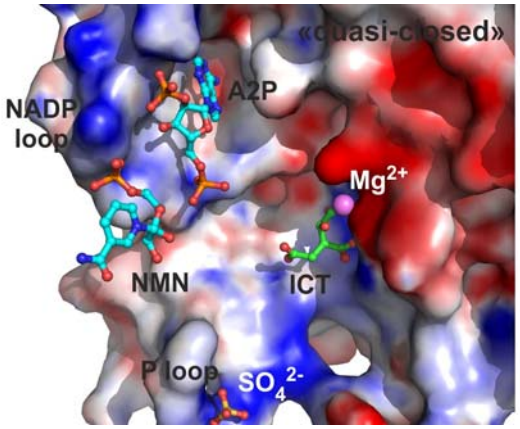
The McoC structure displays a characteristic laccase-like fold, with three cupredoxin domains and two types of copper centres: a **T1** copper centre in domain III and a tri-nuclear center, with two **T3** and one **T2** copper atoms, localised between domain I and III.

C. Silva *et.al.* **Metallomics**, 2012;4(1):37-47.

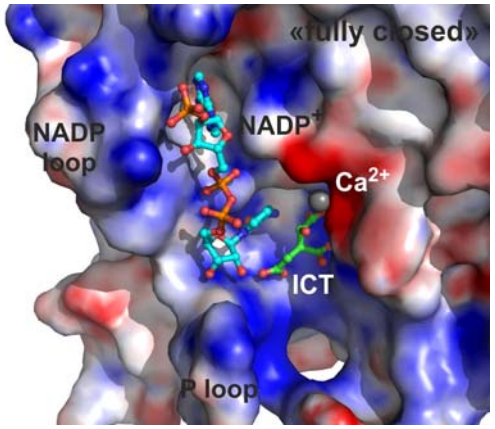
Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase



PDB 1sjs
Finer-Moore et al. (1997)



PDB 4ajs, ESRF ID23-1
Gonçalves et al. (2012)



PDB 4aj3, *in-house*
Gonçalves et al. (2012)

Changes in the electrostatic potential landscape of the *E. coli wt* IDH active site from the **open** (left) to the **quasi-closed** (centre) and **fully-closed** (right) conformations.

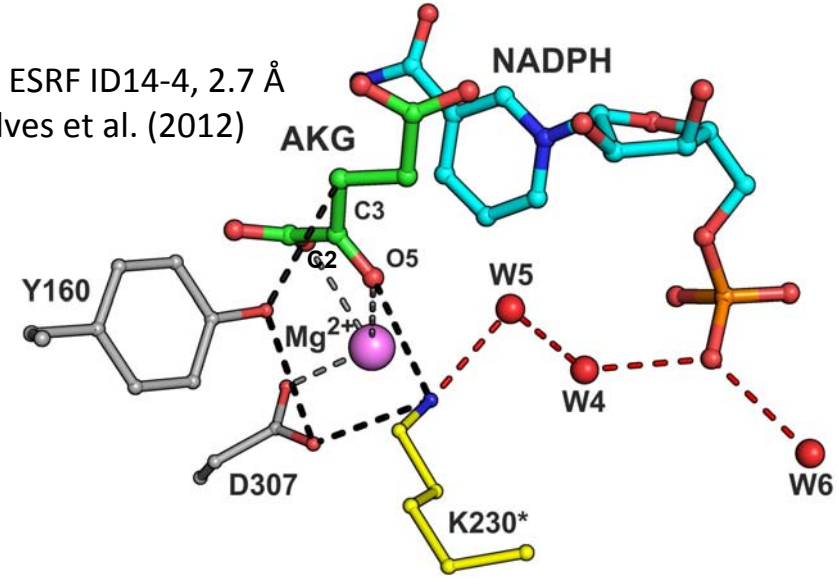
The motion of the “NADP loop” and “P loop” is also evident.

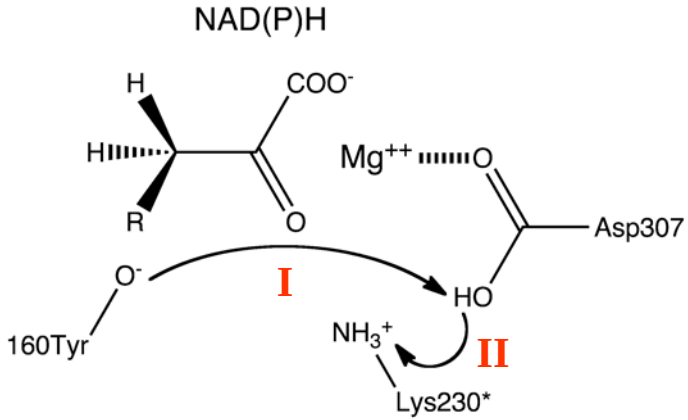
POSTER

Induced Fit and the Catalytic Mechanism of Isocitrate Dehydrogenase

The product complex **K100M IDH:NADPH:AKG:Ca^{2+}**
was obtained by ICT turnover *in crystallum*

PDB 4ajs, ESRF ID14-4, 2.7 Å
Gonçalves et al. (2012)





Tautomerization of the enol intermediate

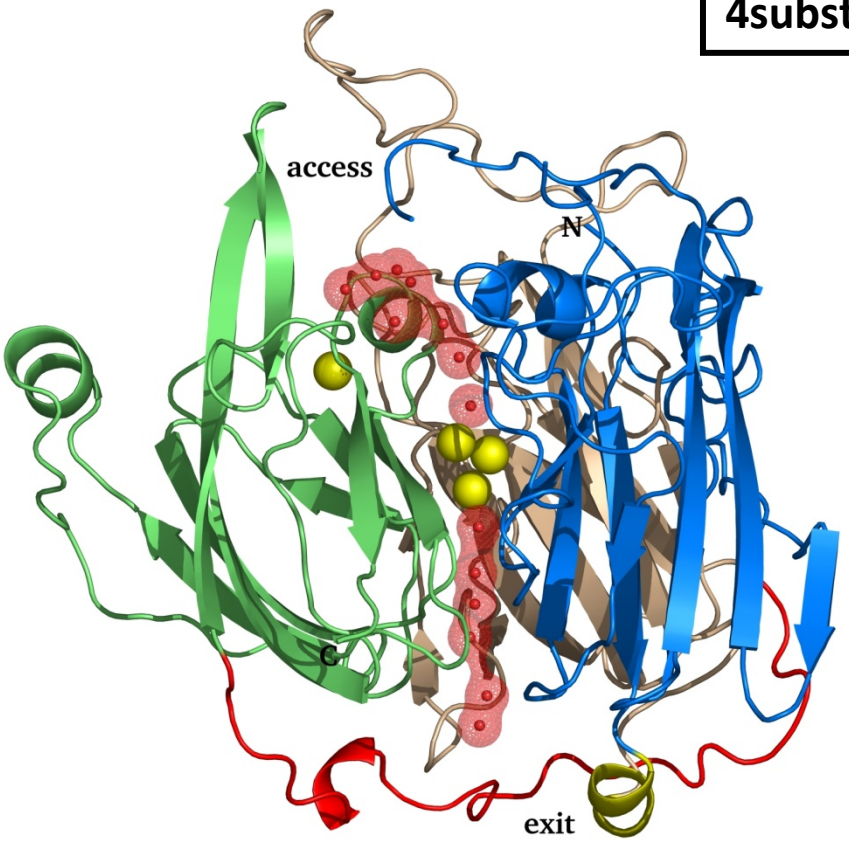
Y160 after C3 protonation (d = 3.4 Å)

K230* after C2 hydroxyl deprotonation (O5 in AKG) (d = 3.3 Å)

Y160 re-protonation: D307 or a proton relay from bulk solvent (I, II)

Gonçalves et al., (2012) Biochemistry 51:7098-7115

Dioxygen reduction by Multicopper oxidases



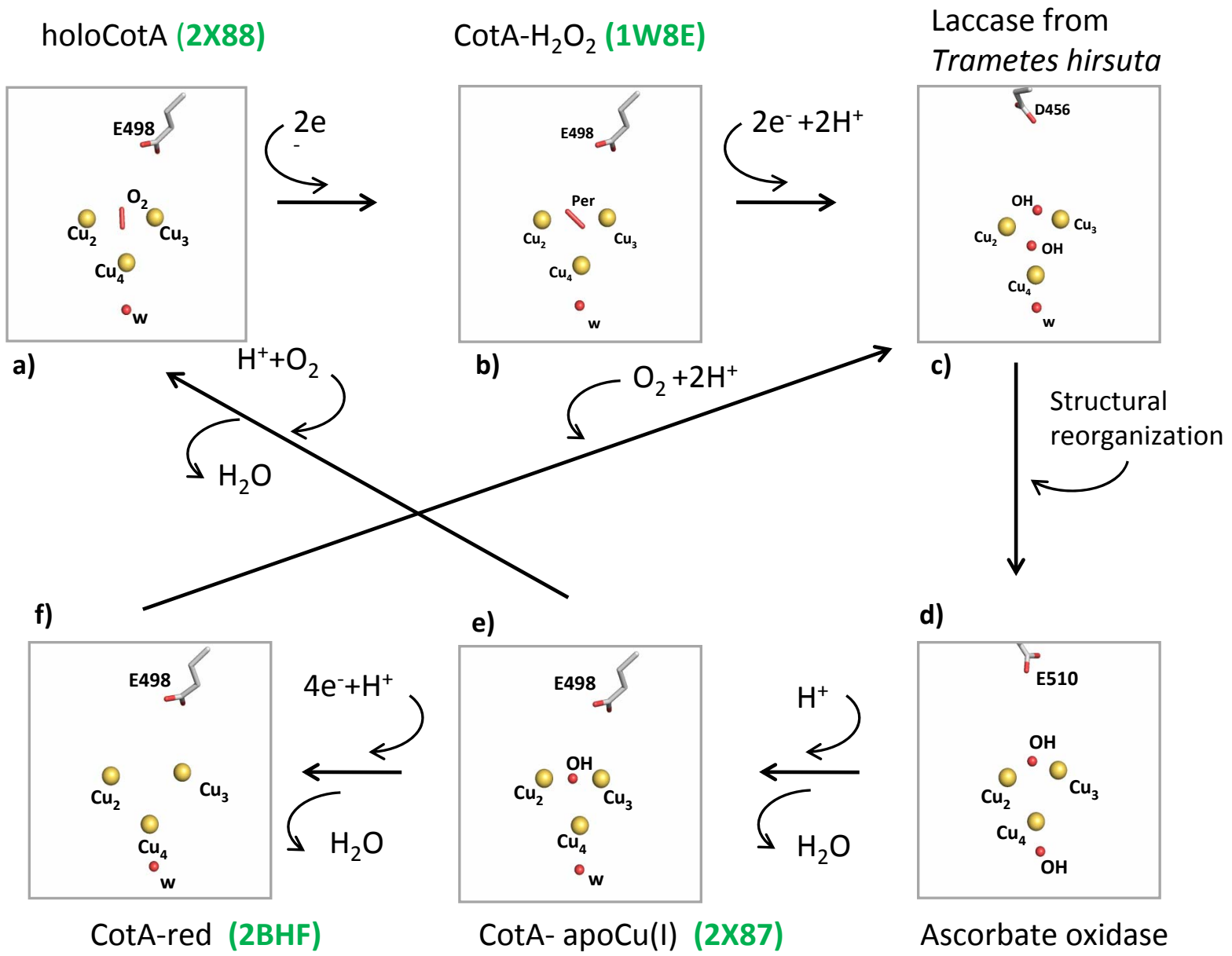
$$4substrates\_red + 4H^+ + O_2 \longrightarrow 4substrates\_ox + 2H_2O$$

$$4e^- + 4H^+ + O_2 \longrightarrow 2H_2O$$

- Which oxygen species are present in the mechanism?
- Which residues are involved in the proton transfer events that occur during the mechanism?

CotA laccase from *Bacillus subtilis* used as a model system

Proposed mechanism for dioxygen reduction by multicopper oxidases

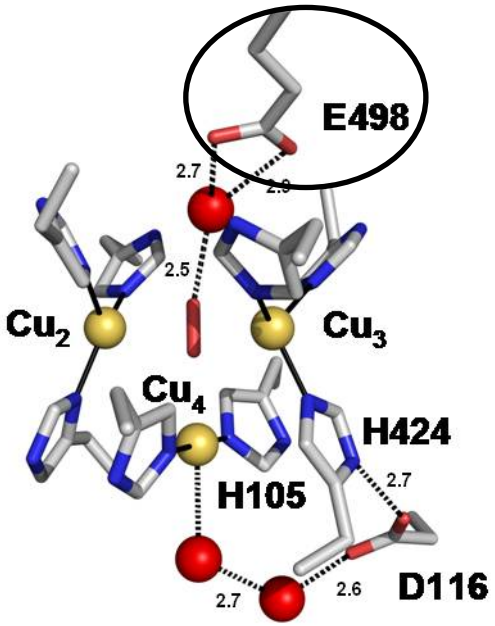


In the absence of O_2

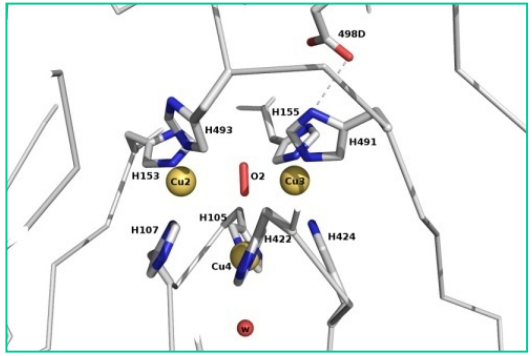
Bento I., *et al.*, Dalton Trans. (2005) 21:3507-13;
Bento I., *et al.* BMC Struct. Biol. (2010) 10:1-14.

Which residues are involved in proton transfer?

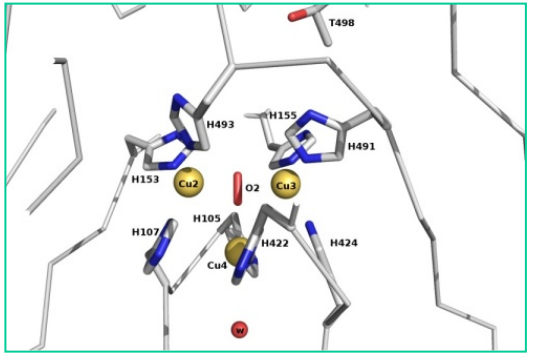
Site-directed mutagenesis of E498



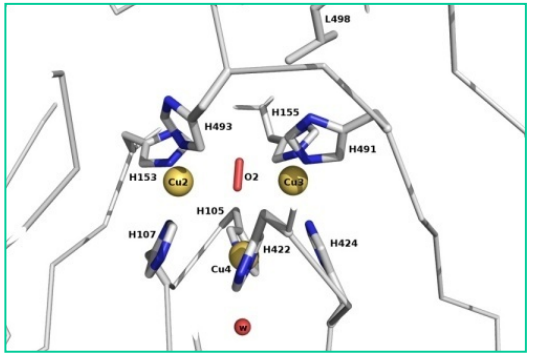
E498D (4AKQ)



E498T (4AKP)



E498L (4AKO)



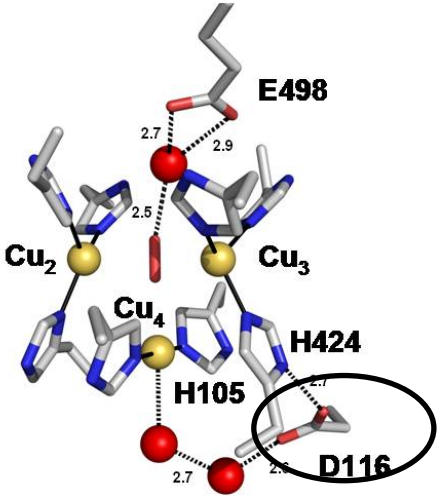
Important differences were located at the copper centres and in their neighbourhood.

498T and 498L mutants were inactive and 498D retained part of the catalytic activity showing that Glu498 plays an important role in channelling the protons to the mechanisms of dioxygen reduction and that no other alternative pathway is observed.

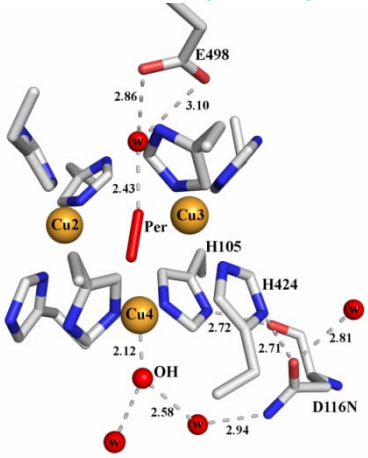
Chen Z, *et.al.* Dalton Trans. 2010 Mar 21;39(11):2875-82.

Which residues are involved in proton transfer?

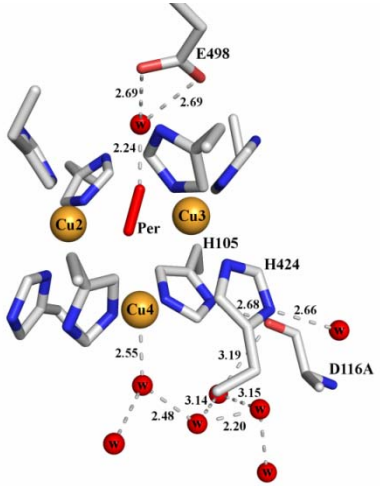
Saturated mutagenesis of D116



D116N (4A68)



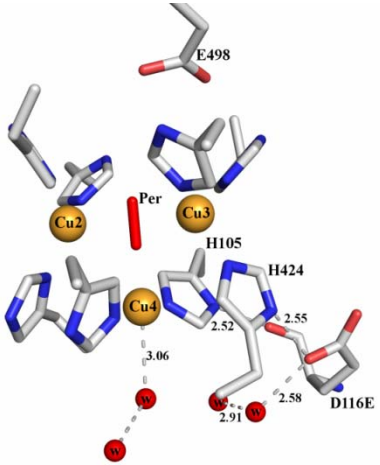
D116A (4A66)



The structures of the mutant enzymes D116N, D116A and D116E, were used in equilibrium protonation simulations in order to further assess the role of Asp116 during the protonation process.

The results indicate that D116 is important for catalysis, either by modulating the protonation events through E498 or by maintaining the local geometry and water connectivity at the trinuclear copper site.

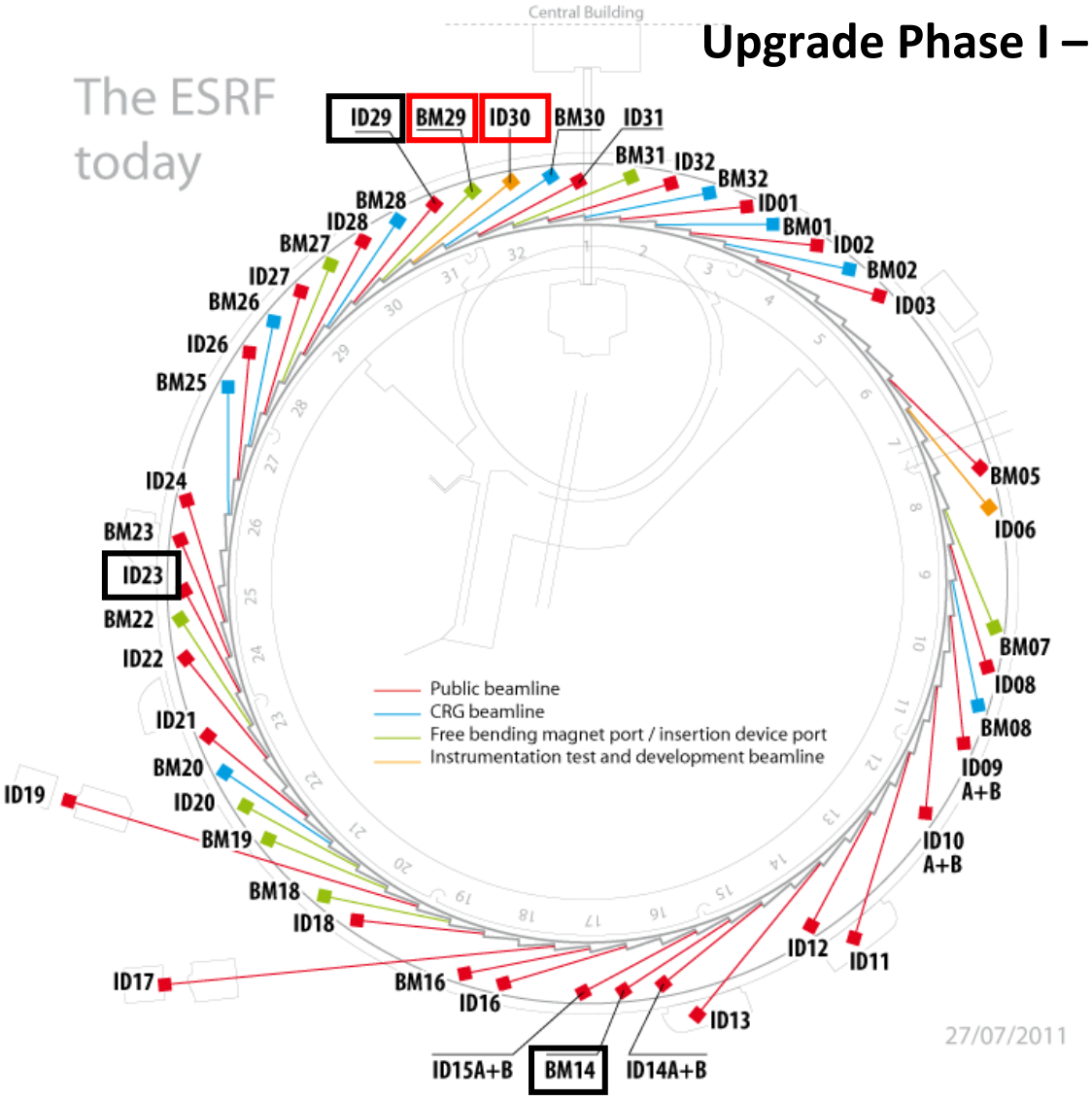
D116E (4A68)



Silva C.S., *et al.* (2012) Acta Crystallogr., D **68:**186-193

Things to come at the ESRF

Upgrade Phase I – new beamlines



Things to come at the ESRF

Upgrade Phase II:

– Architecture of the straight sections will be changed to reduce beam horizontal emittance → Long (1-year) shutdown in 2018-2019

- Increase in X-ray brilliance/flux:

- 10-fold at the BM beamlines

- Up to 40-fold at the ID (Undulator) beamlines

- Radiation damage problems increased → crystal lifetime may be as low as a few miliseconds!

- New methods for data collection and sample handling are in development

Things to come at the ESRF

User input and participation at the Users Meeting and Associated Workshops is very important!