

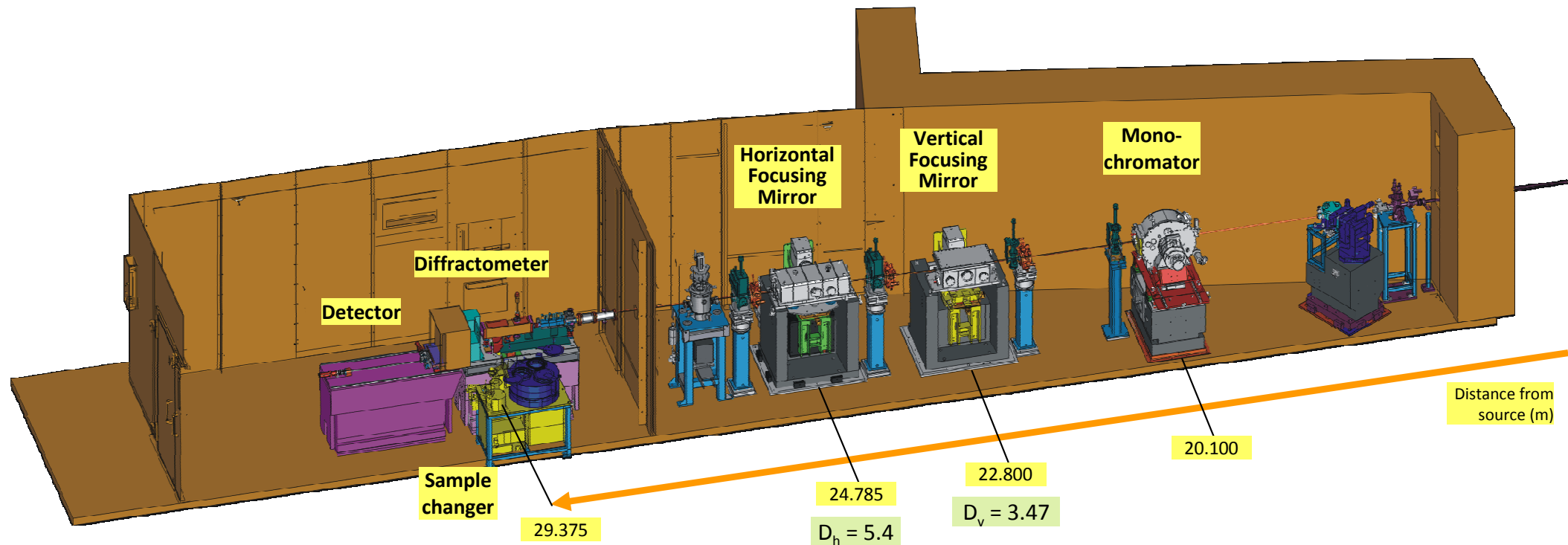


Status of BL13-XALOC Macromolecular Crystallography beamline



Jordi Juanhuix

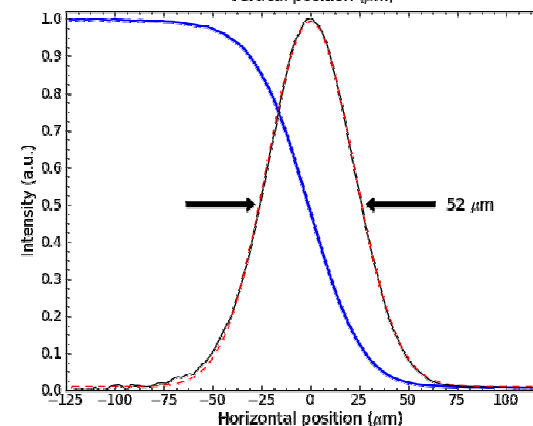
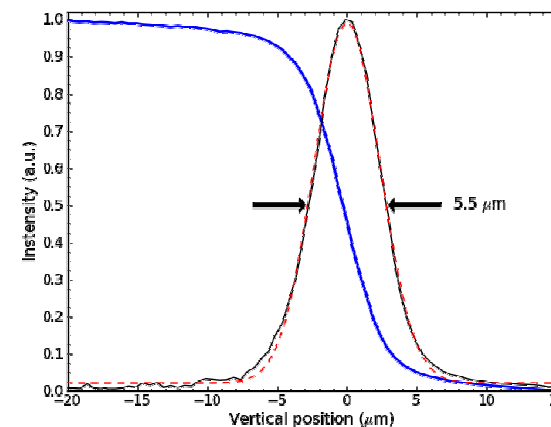
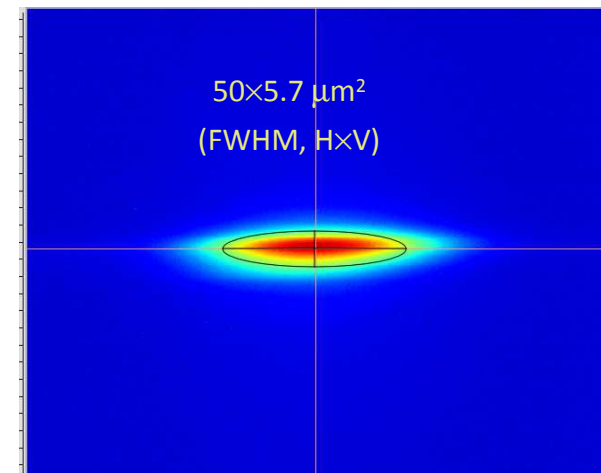
Beamline concept : In-vacuum Undulator IVU21.6 + Channel-cut Si(111) monochromator + KB focusing mirrors



Experimental
Hutch

MD2M
CATS
PILATUS 6M

- Typical beam size at sample is $50 \times 5.7 \mu\text{m}^2$ FWHM (H×V) as measured with a W knife edge
Constant over 2 years
- Measured beam flux is $\sim 2 \cdot 10^{12}$ ph/s/250mA
Constant over 1 year
- Very reliable beam optics
(alignment done daily in 10')
- Beam fairly insensitive after injections in top up mode
- The beam at sample drifts vertically
→ Contributed by beam decay and daily period
→ See Top Up tests
- Main source of LC interventions:
 - Robot
 - Control issues
 - Mechanical Failures



Last year two new BL scientists and two new engineers have joined XALOC

Daniel Fullà
Roeland Boer
(Albert Castellví)

Nahikari González
Gabriel Jover

	Construction Phase	Current
BL scientists	Jordi Benach Jordi Juanhuix	Roeland Boer (Jun.14) Daniel Fullà (Sep.13) Fernando Gil Jordi Juanhuix
PhD Student		Albert Castellví (RadDam)
Control Engineer	Guifré Cuní	Gabriel Jover
Mechanical Engineer	Carles Colldelram Eva Boter	Nahikari González
Electronics Engineer	Julio Lidón	José Ávila
Mechanics Technician	Álex Enrique	(Álex Enrique) Robert Oliete
Electronics Technician	Xavi Fariña	Xavi Fariña
PLC Control Engineer	Alberto Rubio	Alberto Rubio

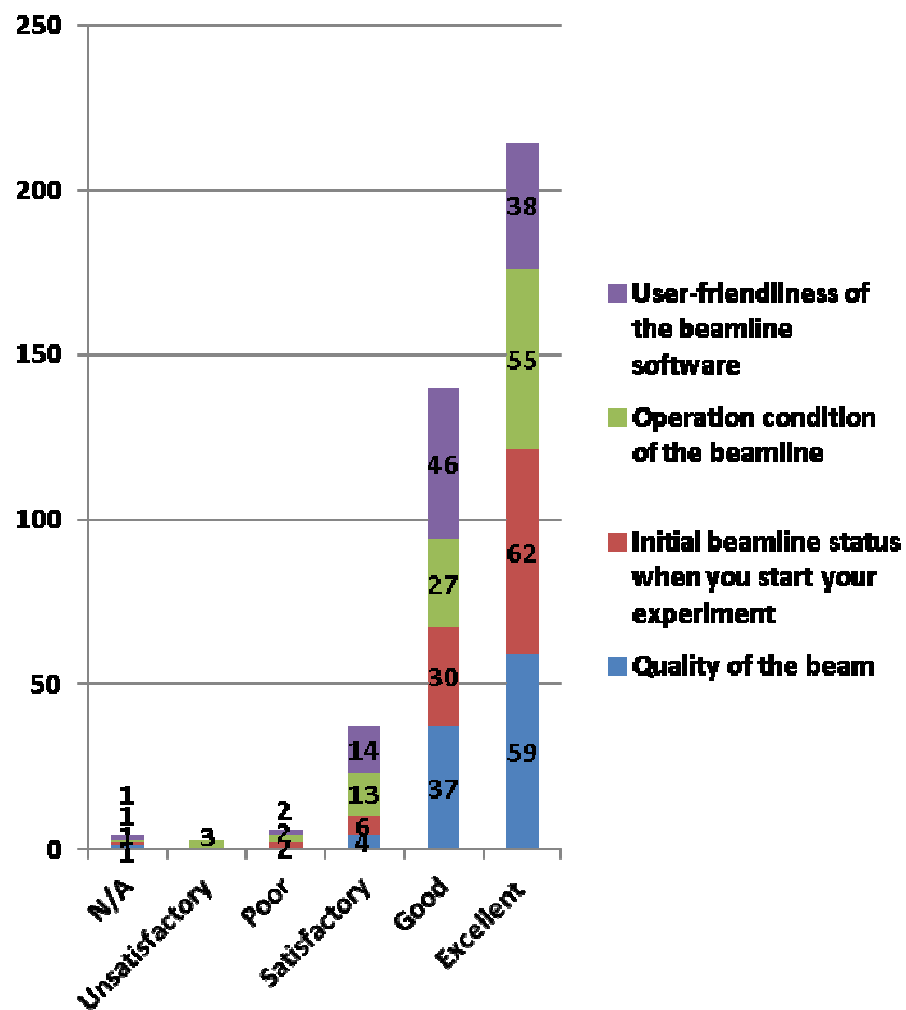
Till 29 June 2014:

- **~60 user groups**
- Tested **~10k crystals** & collected **~4k datasets** (> 90 images)
- **~55 crystals/day**, **~34 datasets/day**
- Collected native & MAD/SAD data sets
- Used K and L3 edges: S, Cd, Mn, I, Cu, Zn, Co, Os, Ir, Hg, Fe, Ta, Se, Br, Pt, Au, Ni, Gd, Sm, Ho
- BL failures are recovered in few minutes due to the intensive local contacts

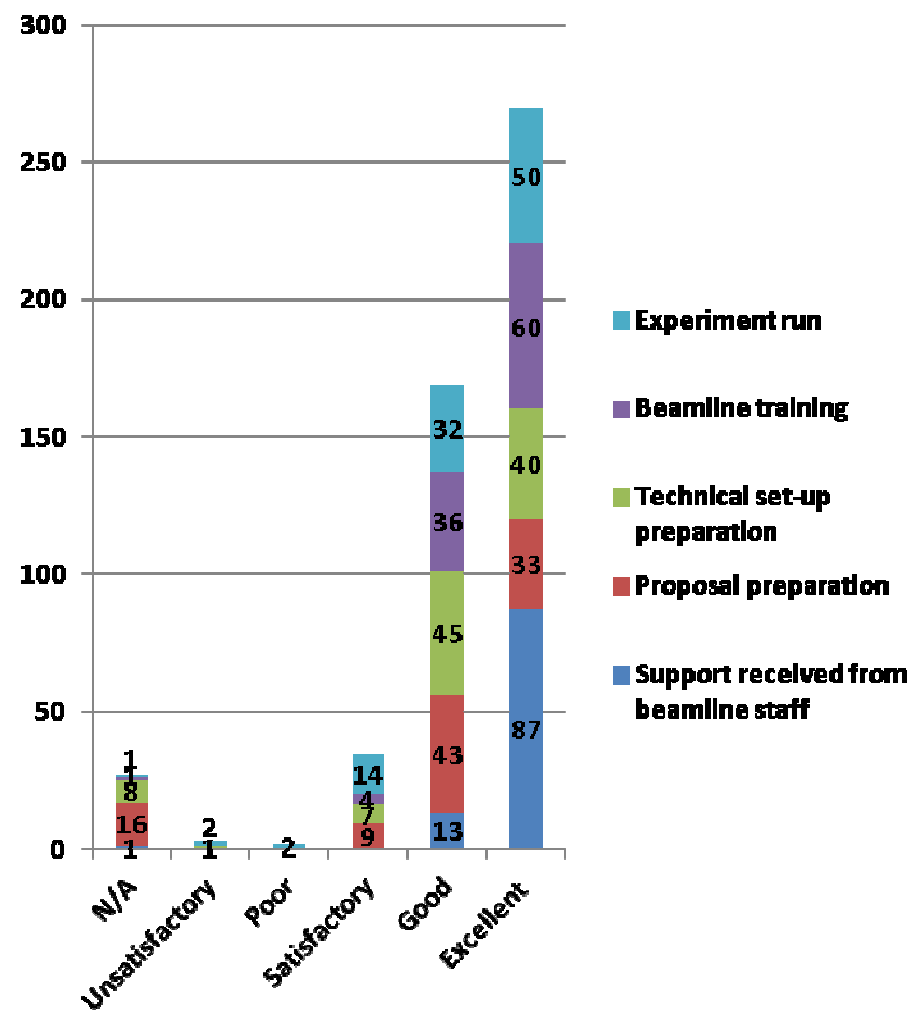
Cycle	Images	Crystal tests	Datasets	Success rate
2012	1.40M	4604	1796	39.0%
2013	1.14M	3783	1485	39.2%
2014	0.64M	1180	562	47.6%
TOTAL	3.20M	9567	3843	40.2%

PDB entries		
SAC16	17 June 2013	4
SAC18	2 July 2014	14

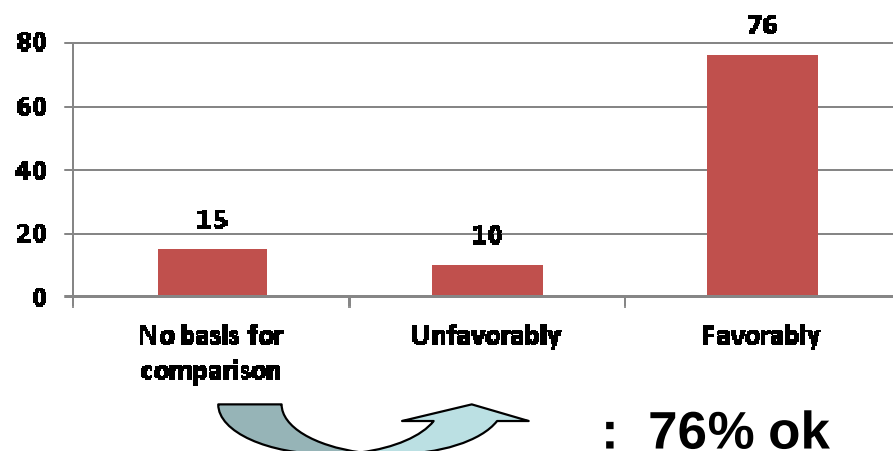
XALOC Beamline



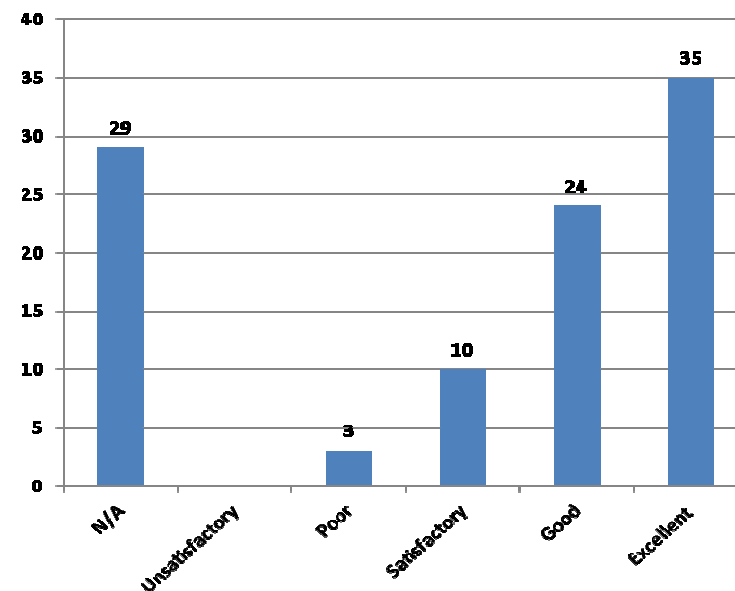
XALOC Beamline Staff



How does this beamline compare to others elsewhere in the world?



Access and support of present laboratories



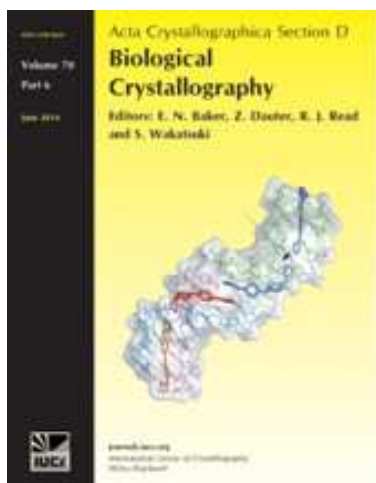
User feedback:

- Very dedicated beamline staff, very high satisfaction
- Problems found: Air conditioning, robot failures, smaller beam stop, faster operation and analysis
- Safety problems: user training tough with no feedback (just repeat) and unclear wording, cumbersome paperwork without added value, non-uploaded requested forms

Interaction of an AT-rich DNA with drug CD27 research papers



FJ Acosta-Reyes, et al. & L. Campos, 2014,
IQM-CSIC (Madrid), U Glasgow and UPC (Barcelona)



Cover in Acta Cryst D

Acta Crystallographica Section D
Biological
Crystallography
ISSN 1399-0047

In and out of the minor groove: interaction of an AT-rich DNA with the drug CD27

Francisco J. Acosta-Reyes,^a
Christophe Dardonville,^b
Harry P. de Koning,^c Manal
Natto,^c Juan A. Subirana^a and
J. Lourdes Campos^{a*}

^aDepartament d'Enginyeria Química, Universitat Politècnica de Catalunya, Diagonal 647, 08028 Barcelona, Spain, ^bInstituto de Química Médica, IQM-CSIC, Juan de la Cierva 3, 28006 Madrid, Spain, and ^cInstitute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, 120 University Place, Glasgow G12 8TA, Scotland

The DNA of several pathogens is very rich in AT base pairs. Typical examples include the malaria parasite *Plasmodium falciparum* and the causative agents of trichomoniasis and trypanosomiasis. This fact has prompted studies of drugs which interact with the minor groove of DNA, some of which are used in medical practice. Previous studies have been performed almost exclusively with the AATT sequence. New features should be uncovered through the study of different DNA sequences. In this paper, the crystal structure of the complex of the DNA duplex d(AAAATTTT)₂ with the dicationic drug 4,4'-bis(imidazolinylamino)diphenylamine (CD27) is presented. The drug binds to the minor groove of DNA as expected, but it shows two new features that have not previously been described: (i) the drugs protrude from the DNA and interact with neighbouring molecules, so that they

Received 4 February 2014
Accepted 28 March 2014

PDB reference:
d(AAAATTTT)₂-CD27, 4ood

Binding specificity of plant ARF Transcription factors

R Boer, et al. & M. Coll, 2014,
IBMB-CSIC, IRB (Barcelona), U Wageningen, U Leeds, CNB (Madrid)

Structural Basis for DNA Binding Specificity by the Auxin-Dependent ARF Transcription Factors

D. Roeland Boer,^{1,2,6} Alejandra Freire-Rios,^{3,6} Willy A.M. van den Berg,^{3,6} Terrens Saaki,³ Iain W. Mantfield,⁴ Stefan Kepinski,⁴ Irene López-Vidriero,⁵ Jose Manuel Franco-Zorrilla,⁵ Sacco C. de Vries,³ Roberto Solano,⁵ Dolf Weijers,^{3,7,*} and Miquel Coll^{1,2,7,*}

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<http://dx.doi.org/10.1016/j.ccell.2013.12.027>

Cell

The paper of the beamline: Journal of Synchrotron Radiation (2014) **21**:679-689



research papers

Journal of
Synchrotron
Radiation

ISSN 1600-5775

Received 27 January 2014

Accepted 11 April 2014

Developments in optics and performance at BL13-XALOC, the macromolecular crystallography beamline at the Alba Synchrotron

Jordi Juanhuix,^{a,*} Fernando Gil-Ortiz,^a Guifré Cuní,^a Carles Colldelram,^a Josep Nicolás,^a Julio Lidón,^{a,b} Eva Boter,^{a,c} Claude Ruget,^a Salvador Ferrer^a and Jordi Benach^a

^aALBA Synchrotron, BP 1413, km 3.3, Cerdanyola del Vallès, Spain, ^bMAX IV Laboratory, Ole Rømers väg 1, 223 63 Lund, Sweden, and ^cFusion for Energy, Josep Pla 2, 08019 Barcelona, Spain. *E-mail: juanhuix@cells.es

The authors wish to acknowledge the whole ALBA staff, and in particular those mostly involved in the construction of the beamline: X. Fariña and A. Enrique (technicians), F. Rey (alignment group), L. Campos (administration), R. Ranz and A. Rubio (controls), D. Beltrán, A. Ruz, O. Matilla and J. Ávila (electronics), M. Quispe, L. Nikitina and N. González (engineering), P. Lukiyonov and Y. Nikitin (cryogenics), I. Sics and J. C. Martínez (metrology and diagnostics), I. Ramos (User Office), M. Díaz and S. Pusó (systems) and J. Bordas. We also wish to acknowledge A. Cámara-Artigas (U. Almería, Spain), G. Montoya (CNIO, Spain) and N. Verdaguer (IBMB, Spain) for the test crystals.

Substrate binding and catalysis of the *O. iheyensis* macrodomain (Fernando Gil)

Macrodomains are evolutionarily conserved ubiquitous protein modules:

- Transcriptional regulation, chromatin remodelling, and development.
- Two hypothesis for the catalytic activity
- 3 different mutants of the active center have been crystallized: **G37V** (Diffraction to 1.35 Å), **G37A**, **N30A**

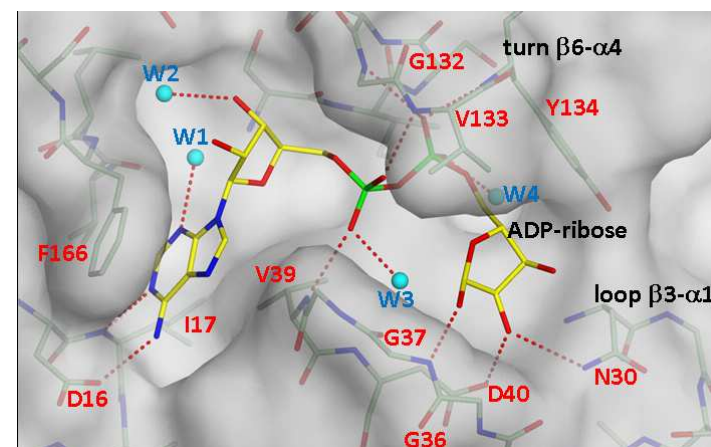
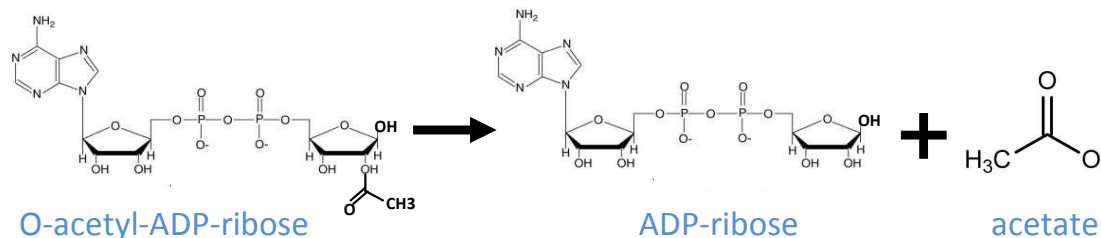
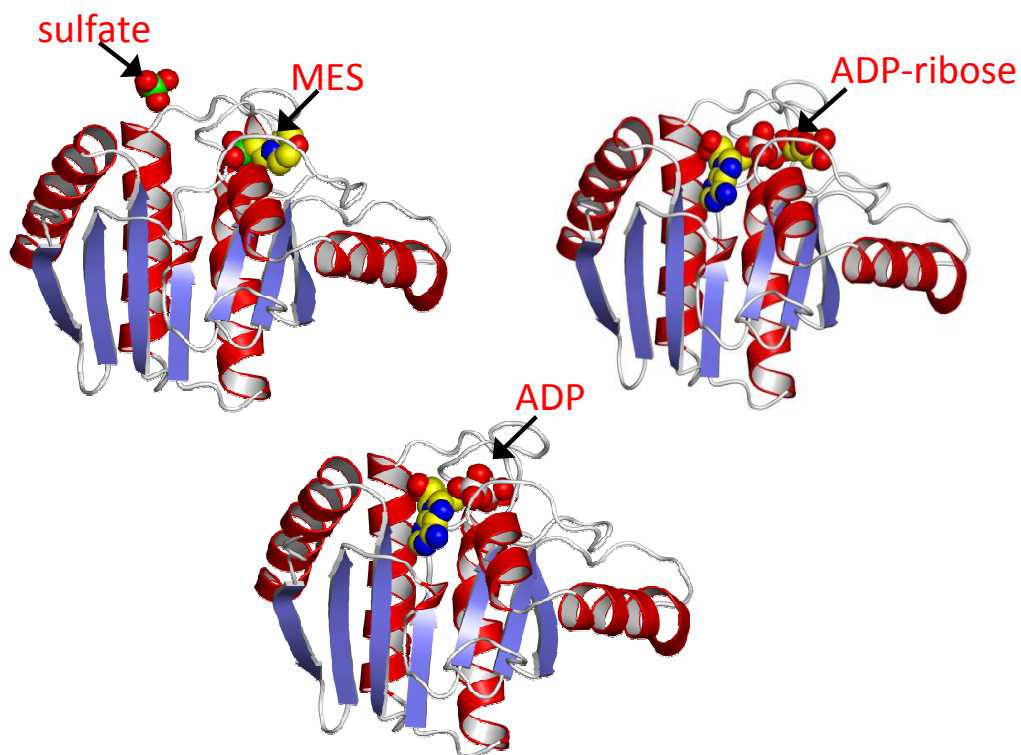


Table 1. Data collection statistics

	Native	ADP-ribose	ADP
Parameter	Data collection		
Beamline	XALOC (ALBA)	XALOC (ALBA)	XALOC (ALBA)
Wavelength (Å)	0.9794	0.9794	0.9794
Space group	$P2_1$	$P2_1$	$P2_1$
Unit cell a, b, c (Å); α, β, γ (deg)	47, 96.7, 54.4; 90, 115.7, 90	47.4, 96.9, 54.4; 90, 115.8, 90	47.4, 95.8, 54.7; 90, 115.6, 90
Resolution range (Å) (Highest resolution shell)	49.0-1.9 (2.0-1.9)	49.0-2.0 (2.1-2.0)	48.0-2.7 (2.85-2.70)
Reflections (Total/ Unique)	96581/33155	100849/29679	21326/10977
I/σ_I	6.3 (1.9)	3.1 (6.9)	7.8 (11.7)
R_{sym} (%)	9.8 (35.6)	7.8 (23.0)	5.3 (9.1)
Completeness (%)	96.3 (93.8)	99.4 (99.4)	90.2 (90.2)

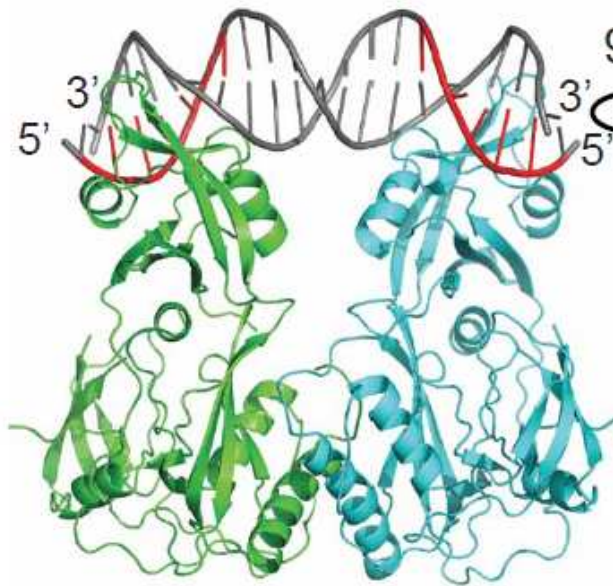


DNA binding specificity of ARF members (Roeland Boer)

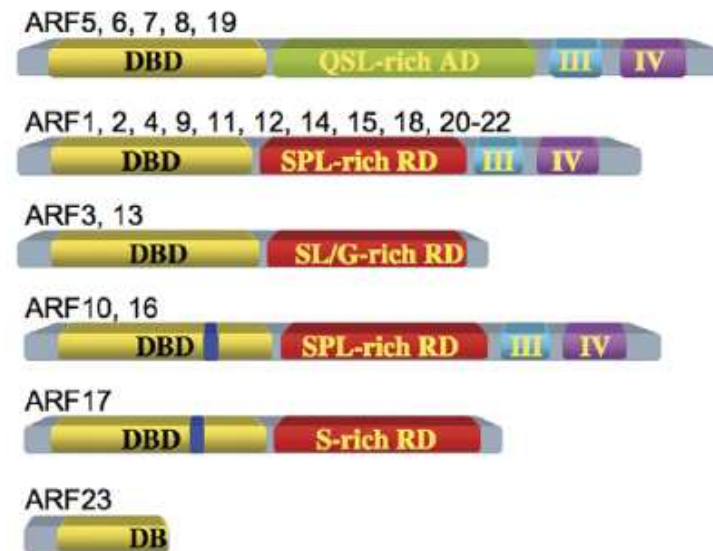
The structure of the plant transcription factor ARF1 in complex with DNA revealed how a homodimer binds to two separated binding sites on the DNA. We are now interested in determining the DNA specificities of other repressor and activator members of the ARF family. We also aim to determine structures of ARFs bound to DNA fragments with varying distance between binding sites.

Collaborator: Dolf Weijers (Wageningen University, Netherlands)

5' - T**TGTCTC**CCAAAGG**GAGACAA** - 3'
 3' - AACAGAGGTTTCC**CTCTGT** - 5'



Green central domain represents activators, Red domains repressor



Current Opinion in Plant Biology

Guilfoyle et al., Curr Opin Plant Biol, 10, p.453 (2007)

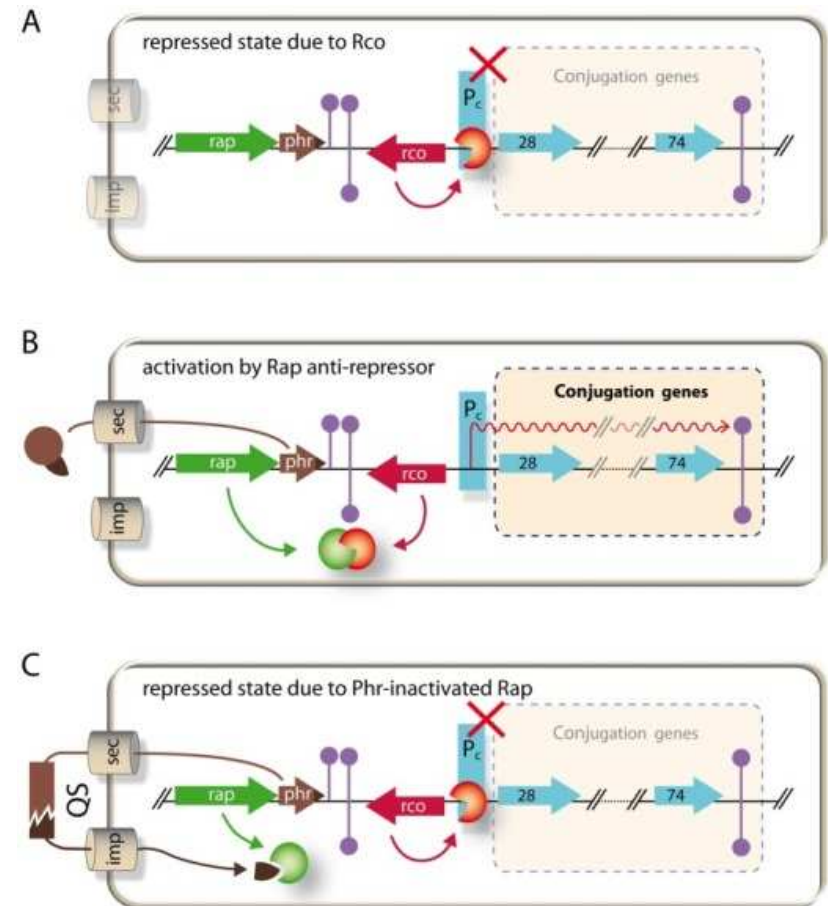
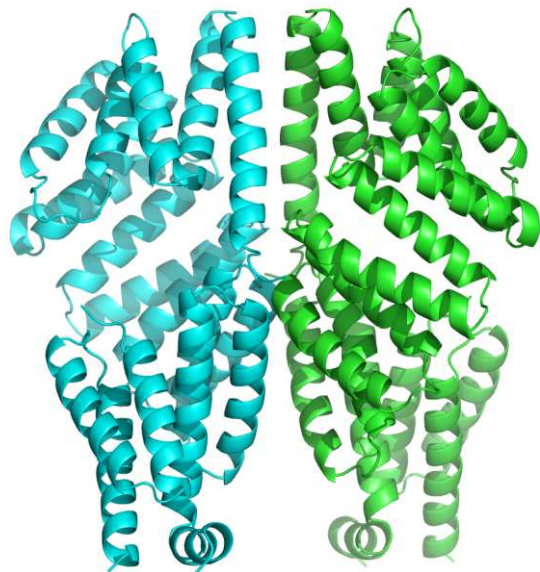
Regulation of the mobility of the native *Bacillus subtilis* conjugative plasmid pLS20 (Roeland Boer)

The expression of the conjugation machinery of the *B. subtilis* plasmid pLS20 is regulated by extracellular and intracellular signals.

We are investigating the structural basis of this regulatory mechanism, aiming to solve the structures of the proteins involved and determine how they affect the DNA conformation in the promoter region.

Collaboration with Wilfried Meijer (CBMSO, CSIC-UAM, Madrid)

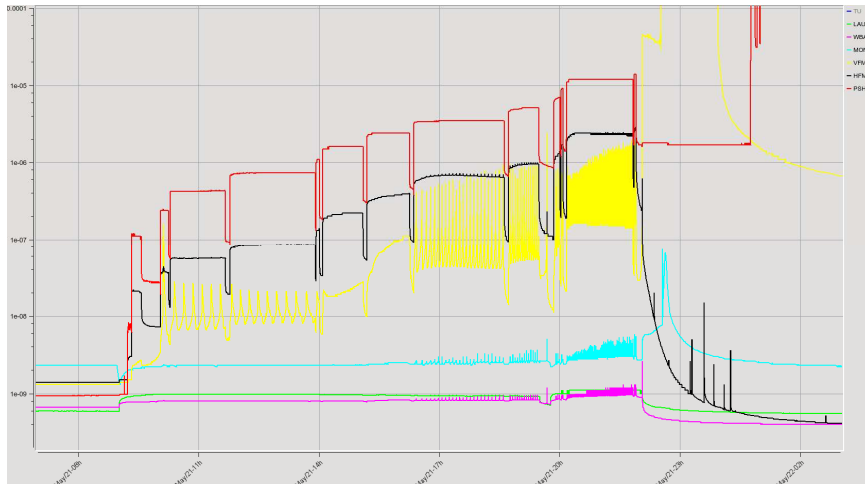
Structure of the activator (Rap) dimer



Praveen et al., *PLoS Genet.* 9 (2013)

Photon Shutter

- The bellows actuating the Bremsstrahlung stop had a vacuum leak
- Very rapid reaction of Engineering Div and technicians, only 2 days were lost
- The bellows was obtained from the spare PS. No more spares are available



Jose Ferrer
Nahikari González
Marta Llloch
Alex Enrique
Edmundo Fraga
Carles Colldelram
Lluís Ginés
Raquel Monge
Francesc Farré
Jordi Navarro

...



Robot collisions

- Humidity is no longer a concern
- Only collisions due to internal failures of the robot (particularly the gripper)

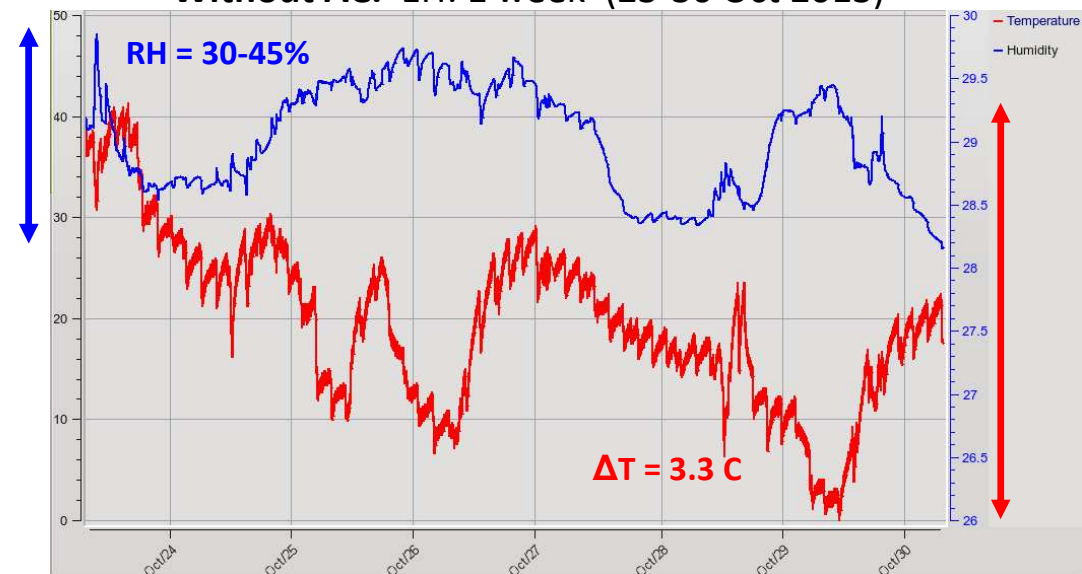
Broken sample lighting LED

- Diffractometer needs continuous upgrading and maintenance: lighting led, controls, omega axis metrology, new apertures, new beam stops

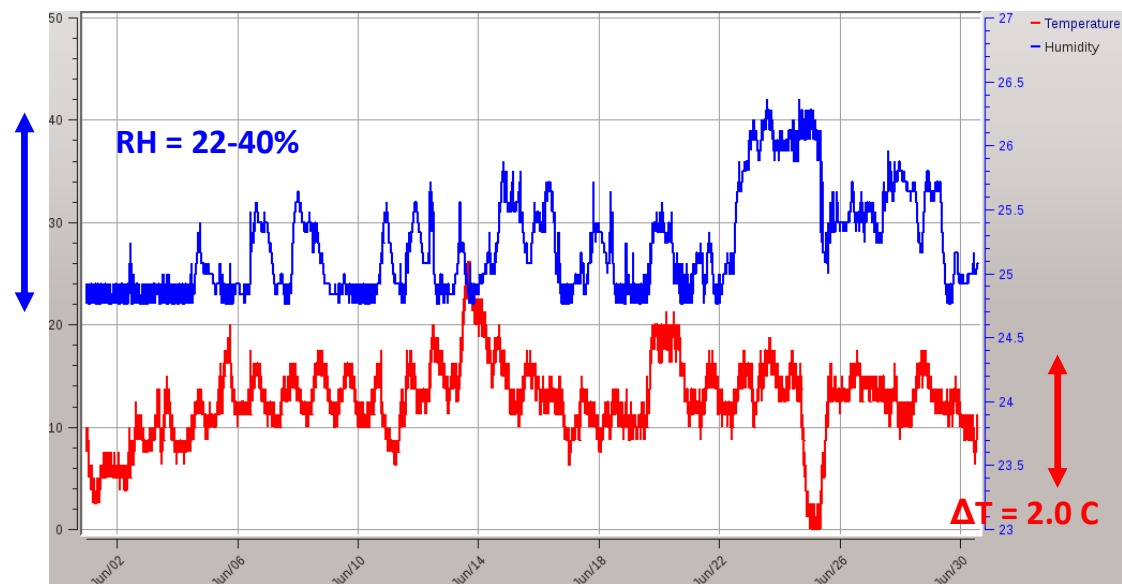
- A **Temperature/Humidity** control has been installed for the Experimental Hutch
- Big effort from N. González and C. Colldelram



Without AC: EH: 1 week (23-30 Oct 2013)



With AC: EH: Whole June 2014



- Already in Summer. However the robot has not been suffering from humidity
- Set points need to be optimized (still in tests)
- To be finally set up (final set point, maintenance, etc) to face increase of ambient T

Cryostream actuation (manufacturing phase)

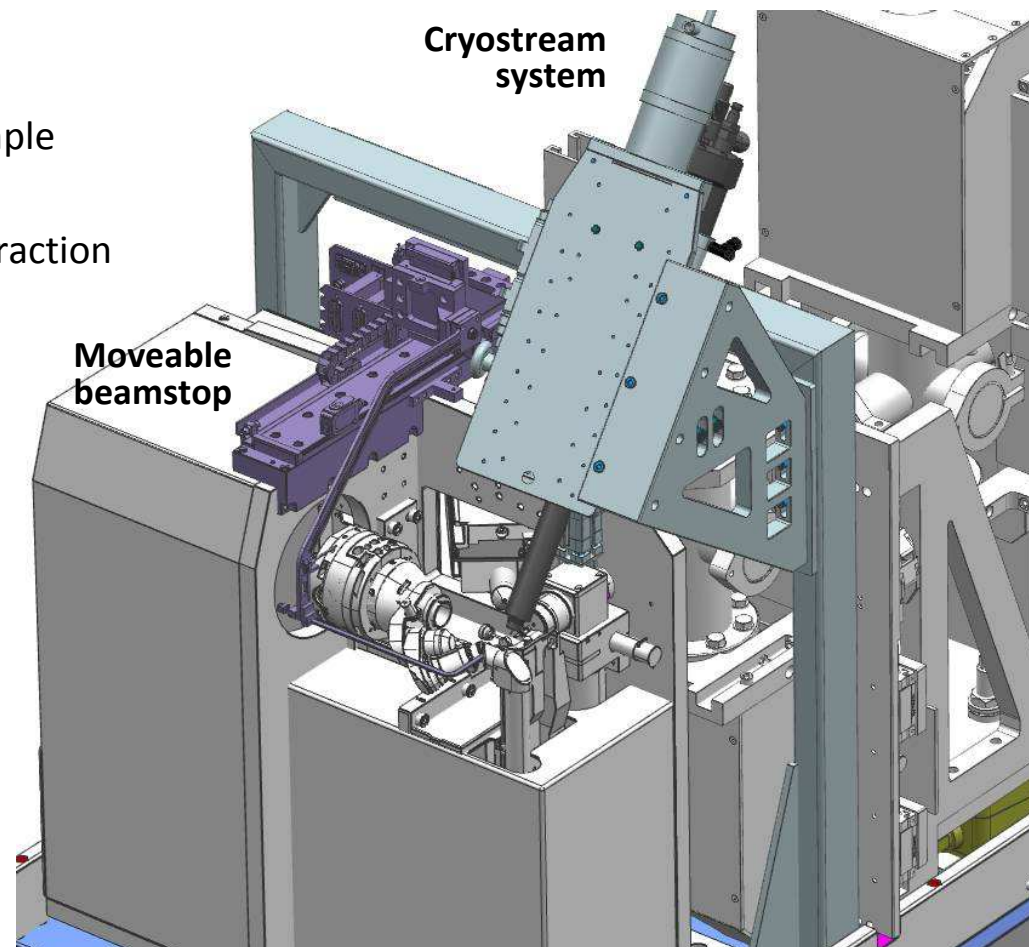
- Translation table for IN/OUT positions needed for sample mounting (Stepper motor)
- Rotation for FAR position, needed for plate in-situ diffraction and commissioning operations (Pneumatic actuator)
- Controlled from a PLC, integrated in EPS

Moveable beamstop (manufacturing phase)

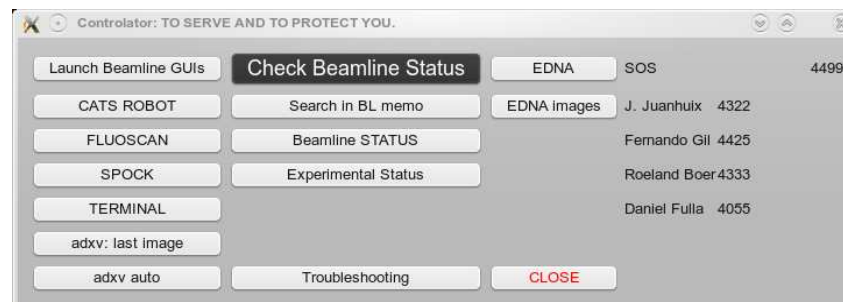
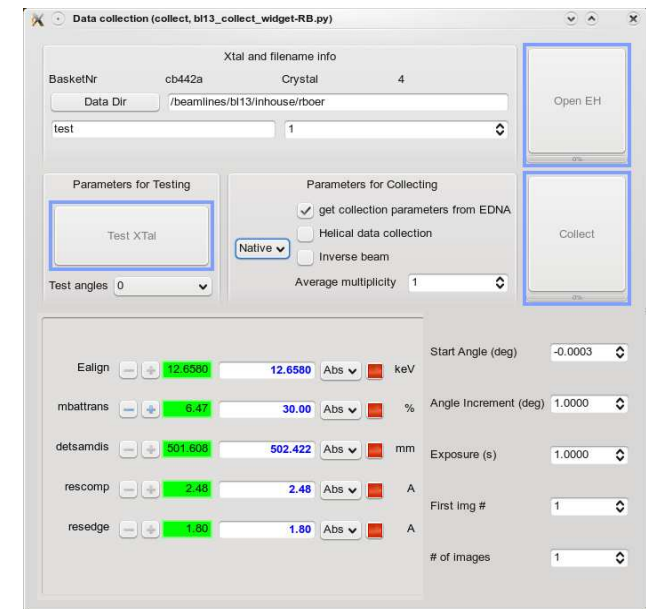
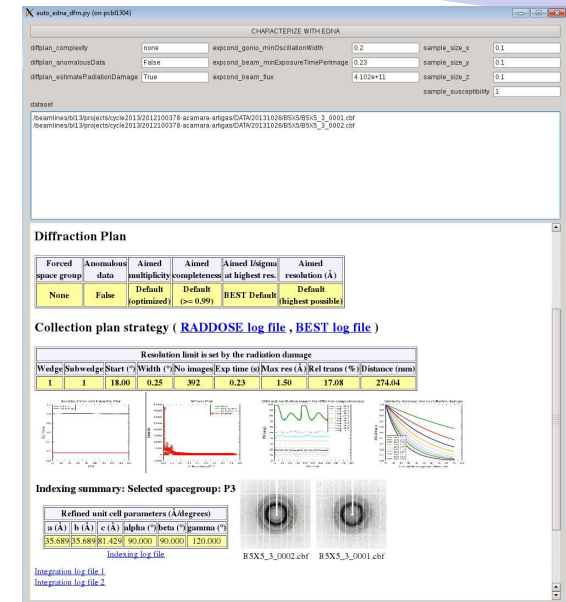
- Needed for crystallization plate scanning and for better low resolution data
- Movements are using stick-slip positioners (SmarAct)
- Equipped with a optical proximity sensor and a magnetic holder
- Different beamstops (with or without diode, different diameters)

Other

- Refurbishment apertures in OH – slow progress
- Manufacturing of a transmissive diode (collaboration with CNM) – FIRST TESTS



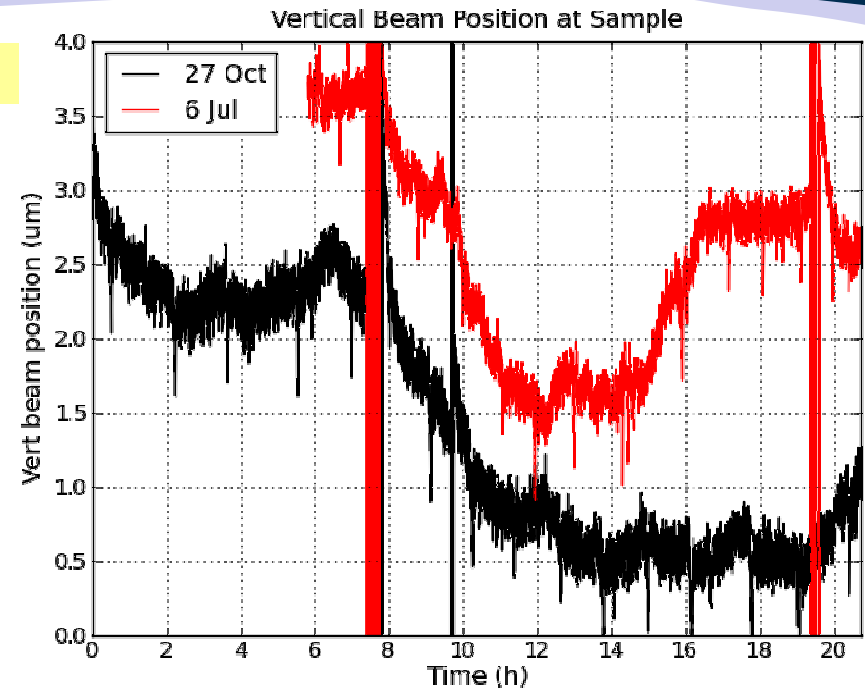
- **EDNA** and **AutoProc** implemented
 - Need to progress in the data processing pipeline!
- **Flux at sample** is measured routinely
- **Automatic alignment** to be available as soon as fluorescence screen can be automatically placed at sample position
- Diffraction **data queues** ready. Need to be implemented to use in
 - Inverse beam (wedged) anomalous data collection
 - Multiple crystal position data collection: crystal cartography
 - Helical data collection
 - MAD data collection
 - Multiple kappa angle data collection
- Improvements on widget lay outs
- **Beamline diagnostic tool**
- Agreement with **ESRF-DESY-MaxLab IV** to discuss common developments in Taurus GUIs and macros using MacroServer



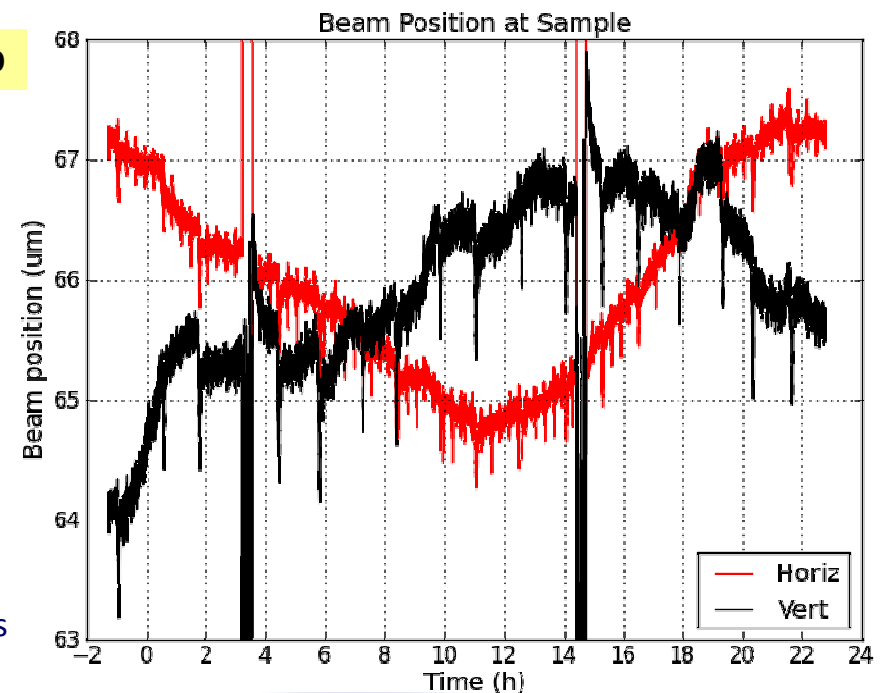
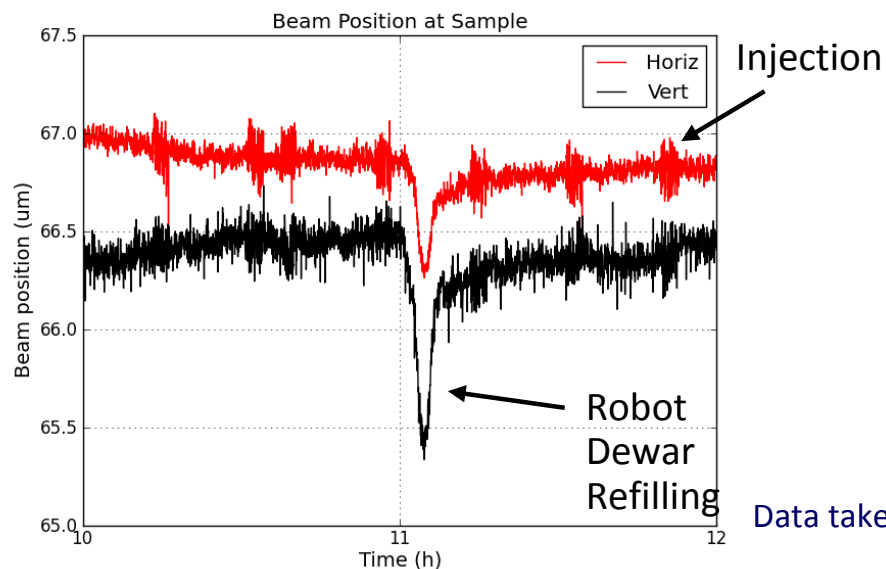
- **Slow data processing speed**
 - Local machines take 45 sec. to run EDNA and ca. 10 min to process a single dataset (AutoPROC)
 - Need to have much higher processing speed using the DPC
 - Delivery expected in few weeks
- **Remote Data Collection**
 - Delivery expected in few months, to be tested
- **sftp service**
 - Users are experiencing troubles uploading the data via vpn. No sftp service is available yet.
 - Expected in some weeks
- **User Scheduling**
 - Low priority
- **Data mining**
 - Low priority
- **Improving backlight system**
 - Tests with new Bassler camera. Results pending
 - Need to integrate color cameras into control system
- **Improve image processing**
 - Autocentering of the loop
 - Select rastering zones to locate the crystal
 - Select points upon which the helical data collection will be done

- Beam position is slightly more stable than in decay mode
- Beam position follows day-night pattern
- Vertical beam size does not change significantly
- Beam at sample blows $2\text{ }\mu\text{m}$ horizontally and $0.3\text{ }\mu\text{m}$ vertically just after injection
- The beam reduces size by $0.1\text{ }\mu\text{m}$ in both directions during decay (standby) phase
- Tests with crystals are inconclusive, but seem to indicate that top up is not affecting the data

Decay



Top Up



Life Science and Condensed Matter College

(nb the term 'College' was not used by ALBA Management but was a term introduced by the Chairman as a hopefully more evocative descriptor than the term 'Grouping')

Overall:

- Life sciences is already a big topic and its synergies academically with soft condensed matter are not so obvious;
 - The BLs that can usefully be grouped under Life Sciences are XALOC, bio-NCD, bio-MISTRAL and bio-CLAESS. I.e the NCD, MISTRAL and CLAESS staff could be split between Life Sciences and Chemistry with Materials in terms of their academic interests.
-
- I like *College*
 - Sinergies are being found between XALOC, NCD and MISTRAL:
 - New **research project on Radiation Damage** to study the same damage from XALOC, NCD and functional points of view: PhD XALOC (Albert Castellví) and Post-Doc NCD (Eva Crosas) – **See poster**
 - Apply to grants with projects based upon XALOC, NCD and MISTRAL
 - From the scientific perspective, grouping splitted BLs into Scientific fields is the soundest way...
 - However, pragmatically most of our time is devoted to instrumentation and user support. Splitting staff in different Scientific Colleges would probably not ease BL operation

- There is clearly a high productivity of crystals measured and number of users served. The publications are now starting in a healthy flow. The first high impact publication in eg Nature, Science or Cell is eagerly awaited.
- The vertical beam drift is significant in percentage of vertical beam size when the V beam is finely focused to 4.7 microns minimum. The top up is very likely the ultimate answer but meanwhile to defocus the beam somewhat, where very weak signals eg cases of very dilute anomalous scatterers, is a pragmatic approach.
- The significantly reduced number of data sets measured by users overnight is an impairment of productivity which is regrettable. The introduction of remote access will help address this challenge. In house monitoring of the robotic sample changing and changing sample dewars would be needed though.



- First Cell paper: Boer *et al.*, 2014, Cell **30**:577-589



- Using top up the beam drift has been reduced, but not eliminated. The probable origin is the daily thermal cycle.



- The reduced productivity during night shifts is not solved yet. Remote access project is ongoing

Engineering

Carles Colldelram
Marta Llonch
Yuri Nikitin
Nahikari González

Mech. Engineer
Alignment
Cryogenics
Mech. Engineer

Computing

Gabriel Jover
Guifré Cuní
José Ávila
Xavi Fariña

Software Engineer
Controls Engineer
Electronics Engineer
Electronics Tech.

Experiments

(Jordi Benach)
Fernando Gil
Daniel Fullà
Roeland Boer
Jordi Juanhuix
Josep Nicolás
Igors Sics
Alex Enrique
Ricardo Valcárcel
Robert Oliete

BL scientist
Post-doc
BL scientist
BL scientist
BL responsible
Mirrors, Metrology
Diagnostics
Mech. Technician
Mech. Technician
Lab Technician

Inma Ramos
Laura Campos

User Office
Administration



Thank you for your attention