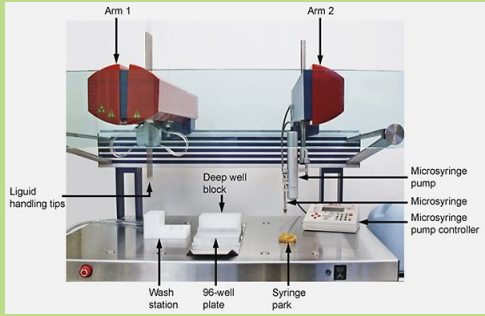
The Future of automation with G-ROB: A 6-axis robotic-arm-based system for crystallography experiments

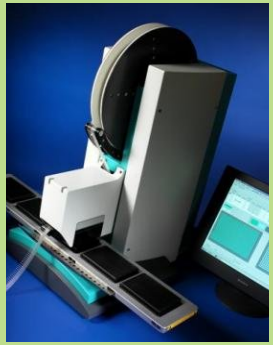
J-L Ferrer, IBS (Grenoble)

Crystallization robot

Phoenix/Gryphon
(Art Robbins)



Cherezov robot for membrane proteins
(Scripps, IBS)



Mosquito
(TTP Labtech)

Cartesian
(Cartesian Inc.)

Sample changer

SAM (SSRL)

Actor (Rigaku)

Marcsc
(MarResearch)

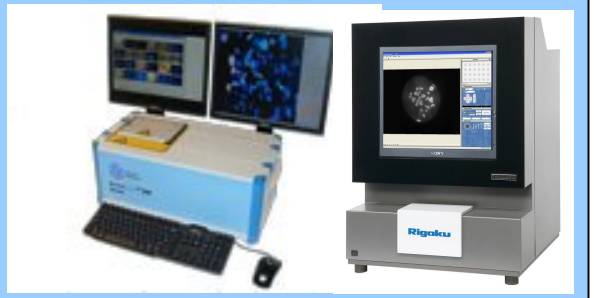
SC3 (EMBL/Maatel)

CATS (FIP/Irelec)

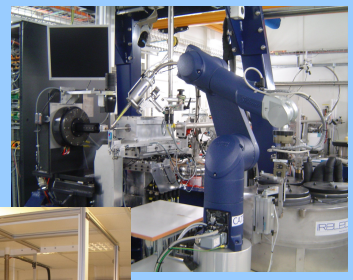
Visible light screening
(Molecular Dim., Etc.)

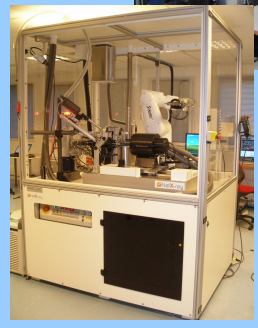
Crystallization + Visible light screening
(Bruker, Etc.)

UV screening
(Rigaku, Molecular Dimension, Etc.)

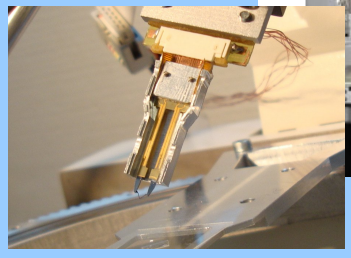


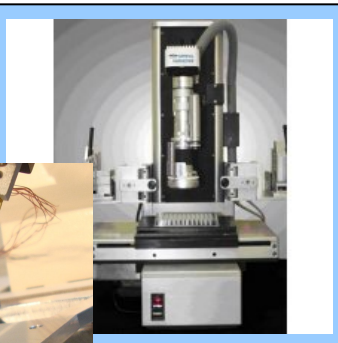
X-ray screening
(Oxford Diffraction)





X-ray screening + data collection
(NatX-ray / Irelec)





Automated/assisted harvesting
(commercial: Bruker, projects: FIP, EMBL)

Freezing ?!

Crystallization + visible light screening

EMBL Grenoble
Etc.

Crystallization + UV screening

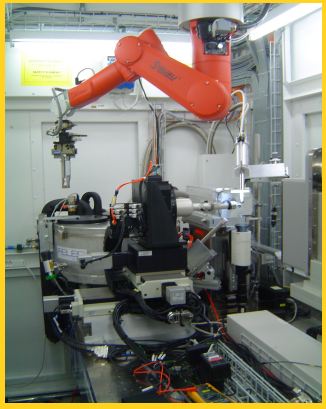
Rigaku, Germany
Scripps Inst., USA

X-ray screening + data collection

BM14.1, BESSY, Germany
LS-CAT, APS, USA
DLS, UK; MASSIF, ESRF ?

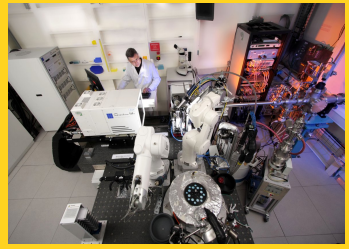
X-ray screening + data collection

Stewart Cole Lab., EPFL



Crystallization + X-ray screening

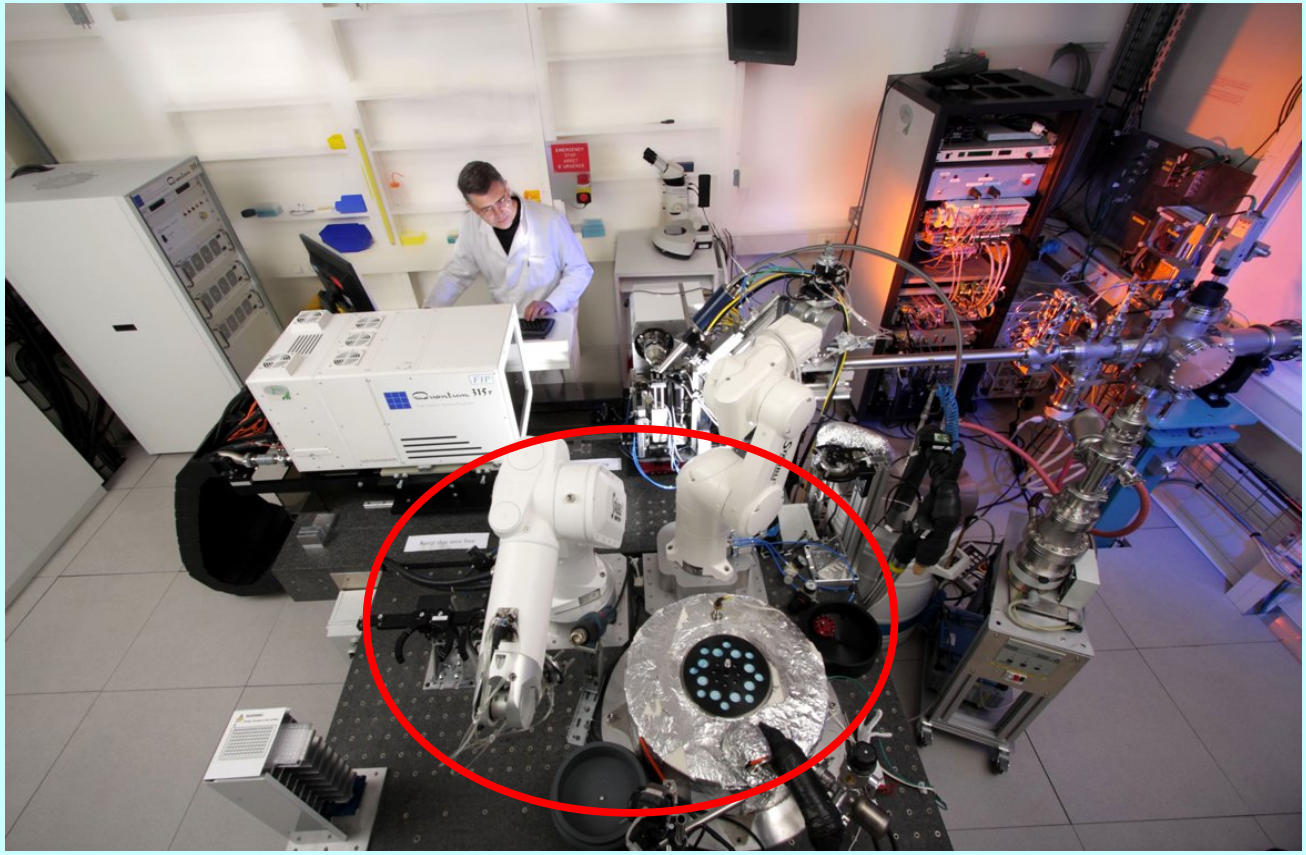
SLS, Switzerland



Crystallization + vis./X screening + data collection

EMBL Grenoble + FIP-BM30A

Beamline FIP-BM30A at the ESRF, Grenoble

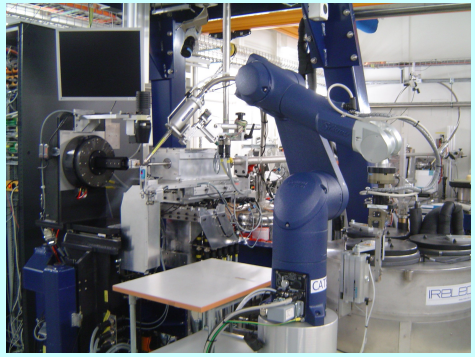


CATS: Development started in 2000.
CATS in operation on FIP in 2004.
Commercialized at end of 2006.

12 CATS already ordered

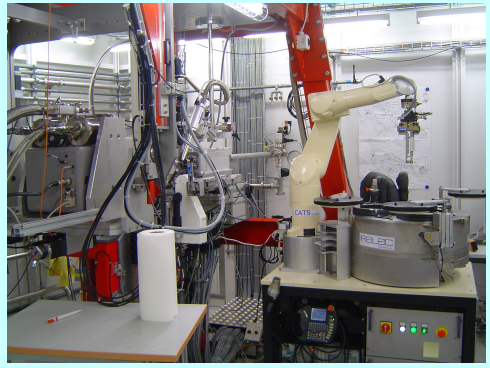
SLS

- X06SA (March 2008)
- X06DA (March 2008)
- X10SA (March 2008)



DLS

- I04.1 (Feb. 2008)
- I24 (Dec. 2007)

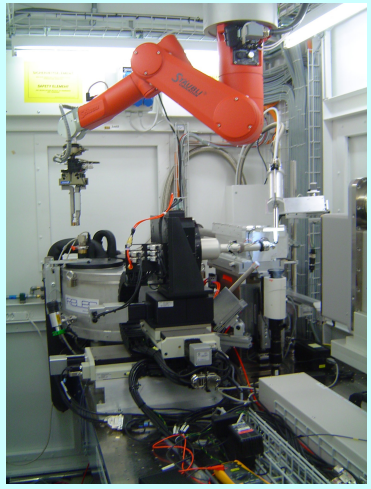


BESSY

- BL 14.1 (Jan. 2008)

APS

- 21-ID-D (March 2008)
- 21-ID-E (March 2008)
- 21-ID-F (March 2008)
- 21-ID-G (March 2008)
- Eli Lilly (June 2009)



ALBA

- XALOC (Nov. 2008)

IRELEC

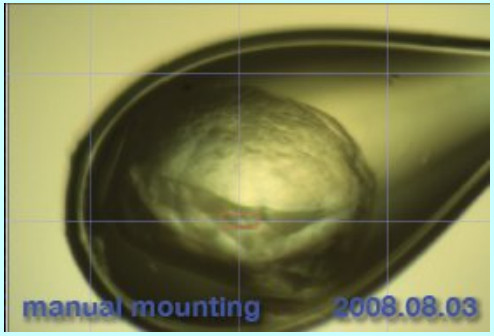
CATS tested on X06DA (SLS)

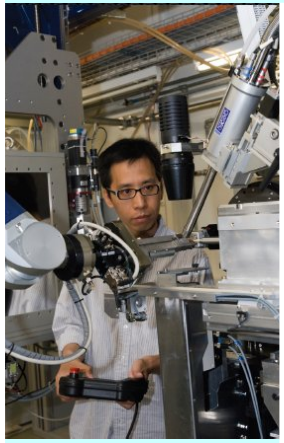
X06DA (PXIII): a high-throughput beamline

PILATUS 6M Pixel Detector

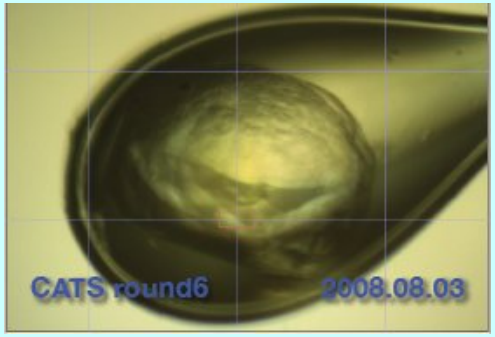
Fast automatic data processing

Automatic sample changing at cryogenic temperature





6 mounting/unmounting cycles

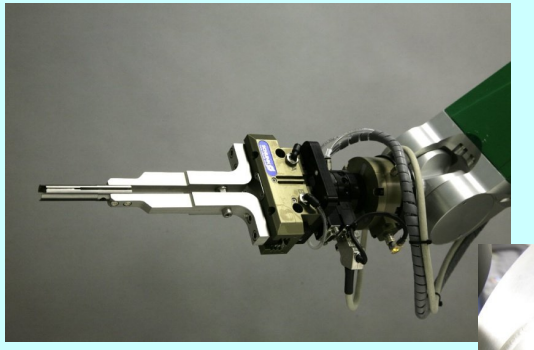


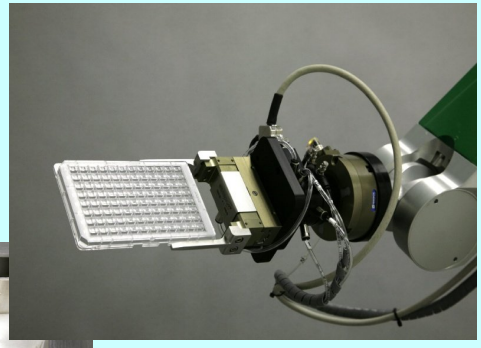
Icing test on lysozyme with 8 x 10_9 phs/sec, 1deg/sec, 90 deg per data set

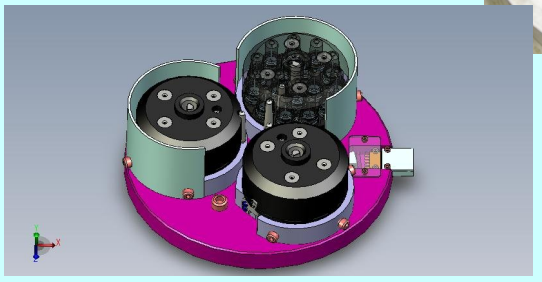
manual mounting (Mosaicity: 0.052 deg)	1.20	37365	11132	11206	99.3%	51.2%	59.7%	37123	1.99	61.2%	69.2%	1% 0.683 5168
CATS round1 (0.053):	1.20	37433	11170	11224	99.5%	50.7%	58.8%	37189	2.01	60.7%	69.1%	1% 0.681 5178
CATS round2 (0.053):	1.20	37524	11182	11219	99.7%	50.1%	57.2%	37289	2.06	59.9%	68.4%	2% 0.701 5194
CATS round3 (0.053):	1.20	37557	11189	11227	99.7%	49.6%	56.5%	37320	2.10	59.3%	66.5%	2% 0.707 5198
CATS round4 (0.055):	1.20	37469	11144	11185	99.6%	50.0%	57.2%	37244	2.11	59.7%	65.3%	-1% 0.680 5183
CATS round5 (0.052):	1.20	37510	11210	11236	99.8%	49.8%	56.9%	37275	2.09	59.5%	66.2%	1% 0.690 5216
CATS round6 (0.053):	1.20	37457	11120	11194	99.3%	52.6%	59.4%	37246	2.01	62.9%	69.8%	2% 0.703 5182

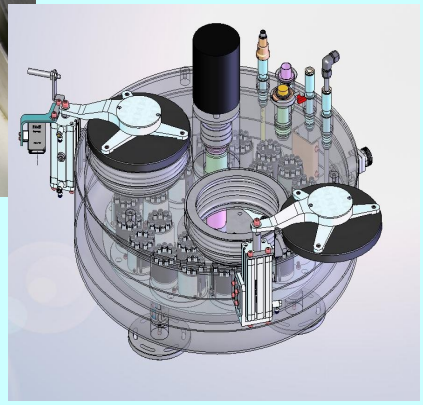
CATS Sample Changer

- CEA-CNRS licence
- Automatic Dewar - Barcode reader
- Compatibility with most magazine and sample holder standards
- Open source programs and high level API for easy integration in control system









Supported standards

So called “SPINE” standard

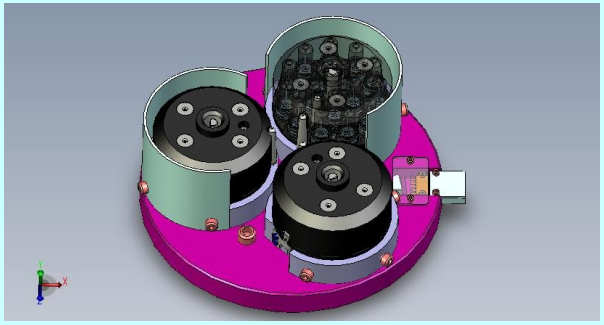
Actor (Rigaku) format

- Same caps as SPINE, no vial
- A different puck

Universal Puck standard,

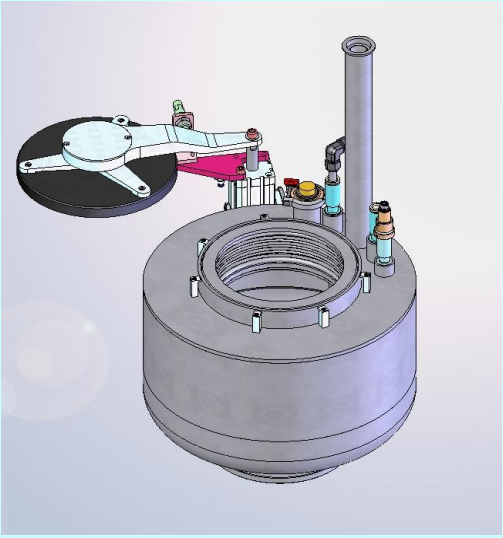
recently tested at DLS (UK)

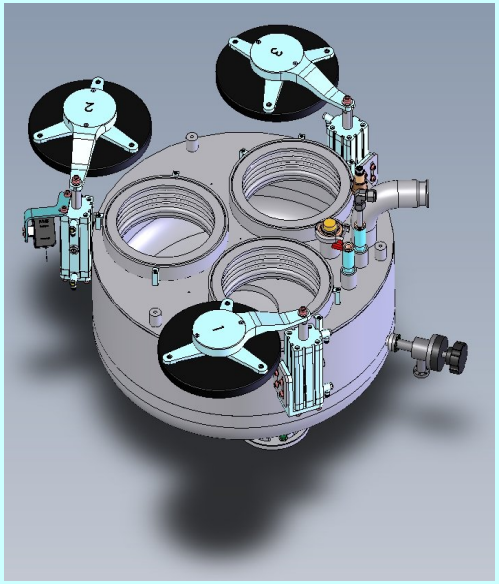
- Same caps as SPINE, no vial
- A different puck



Small Dewar

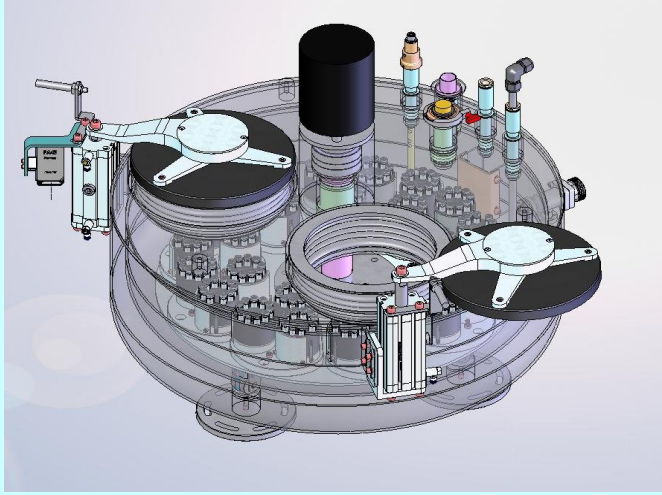
30 samples storage





Medium size Dewar

90 samples storage (SPINE based)



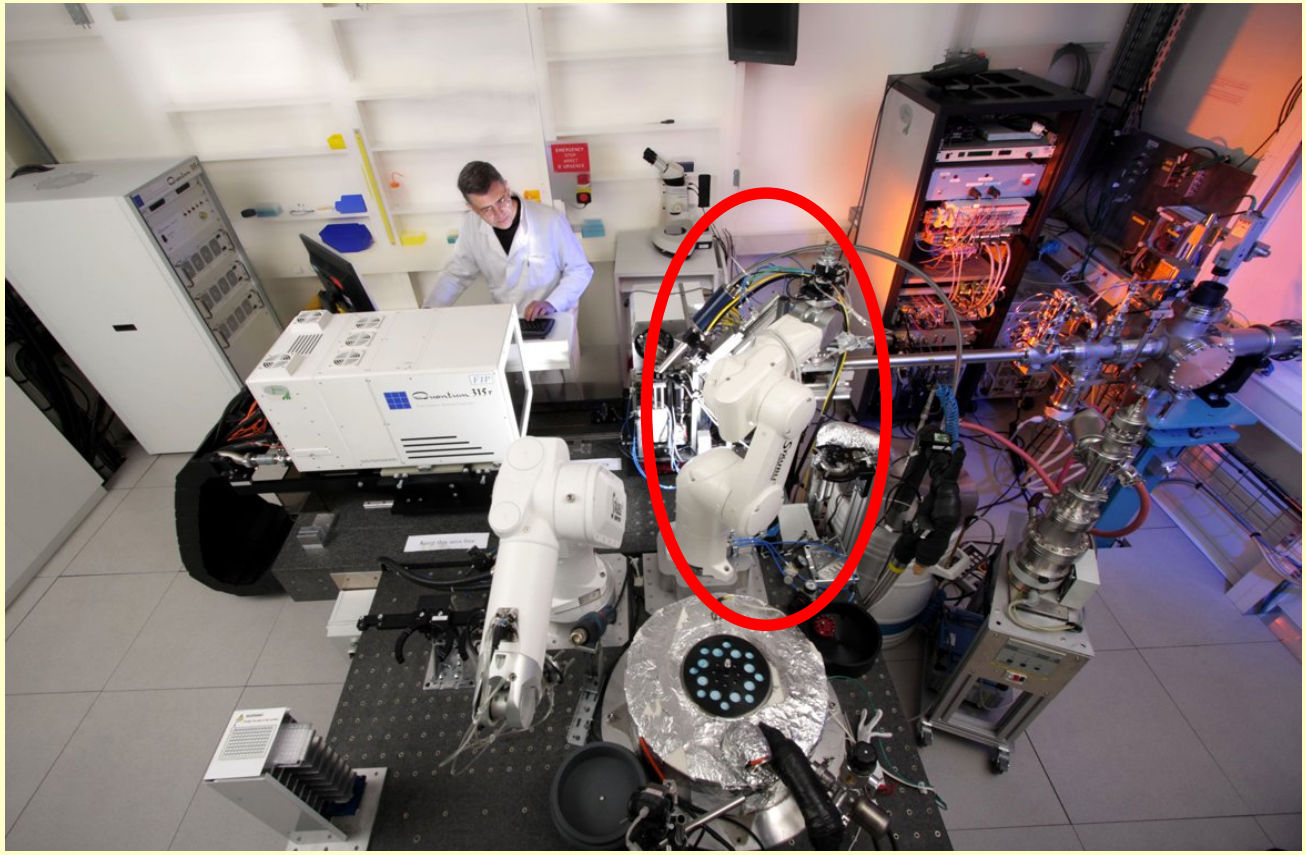
High capacity Dewar

270 samples storage

Advantages/drawbacks of the SPINE standard

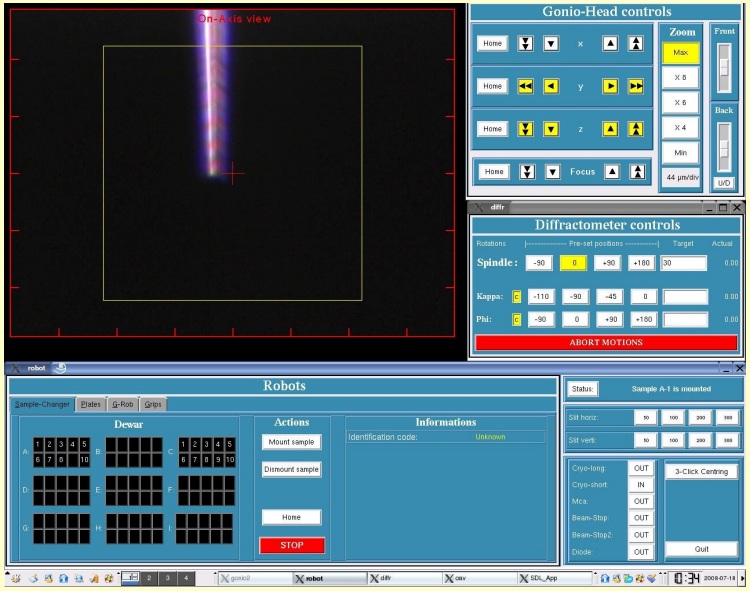
Positive	Negative
Manual manipulation easy (do not make it shorter)	Not compact (see pucks Actor)
Faster transfer with the vial	No accurate positioning
Safer transfer with the vial	Dot matrix not reliable
OK for both manual and robot transfer	Holes in the vial: 90° better than 180°
	Quality control on the geometry!
	Degradation (mostly the vial)

Beamline FIP-BM30A at the ESRF, Grenoble

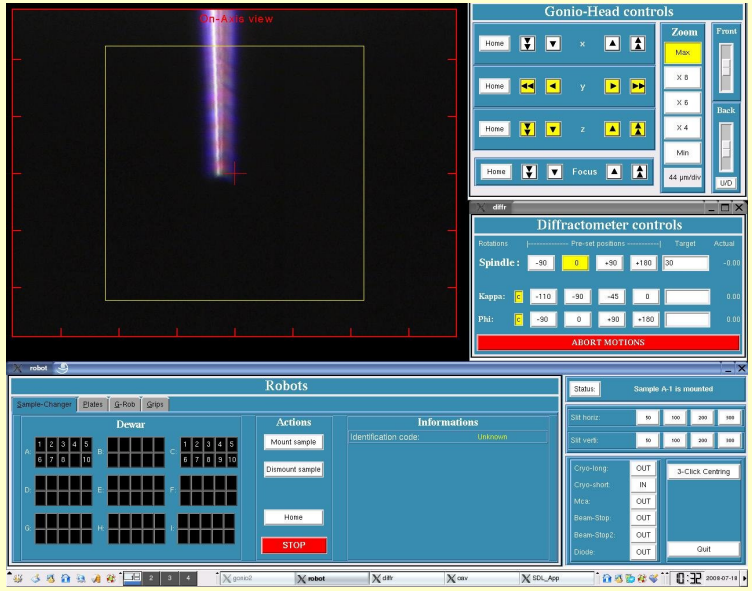


G-Rob: Development started in 2004.
1st tests of G-Rob 2D in 2006.
In operation on FIP since 2008.
Commercialized in 2009.

High accuracy sample oscillation, using axis #6



Phi = -180°

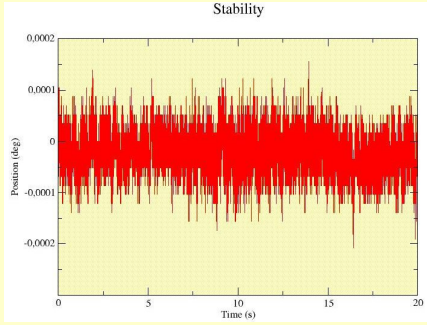


Phi = +180°

Sphere of confusion: ~12 µm (radius)

Reproducibility: 0-4 µm

Stability (jitter): +/-0.0002 deg



Quality of rotation: following error <= 5 mdeg

(difference at each time between the actual angle and the theoretical angle)

G-Rob 1D

Data collection on frozen samples, capillaries, etc...

Single axis data collection: 24 µm ➔ 15 µm SOC?

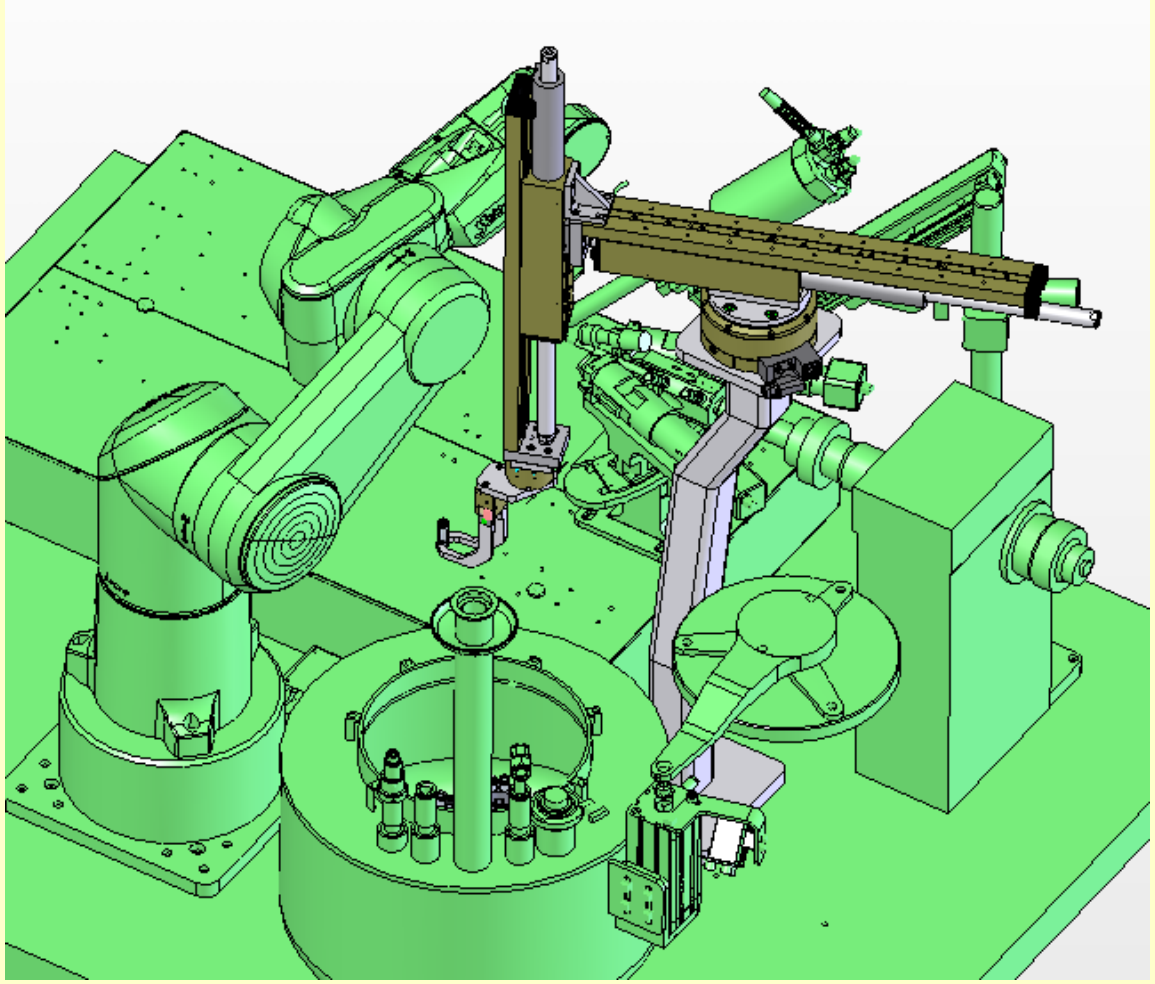
- beam down to 0.07 mm diam.
- Phi data collection strategy

Automated fast sample transfer: 20s ➔ 10s ?

More room, flexibility

Reduced maintenance…

Fast transfer with G-Rob (G-Rob 1DT)

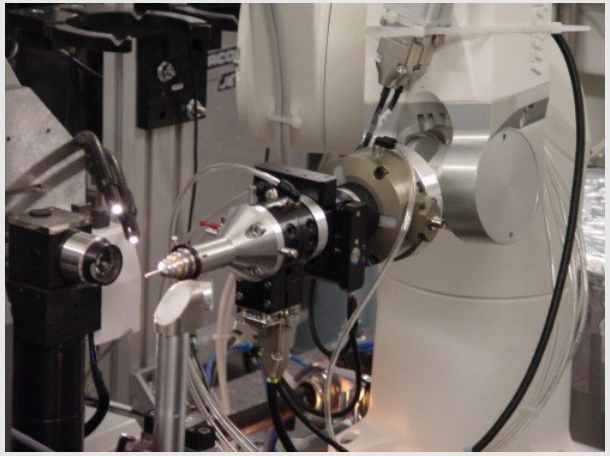


G-Rob, a multi-purpose, automated robotized system for protein crystallography

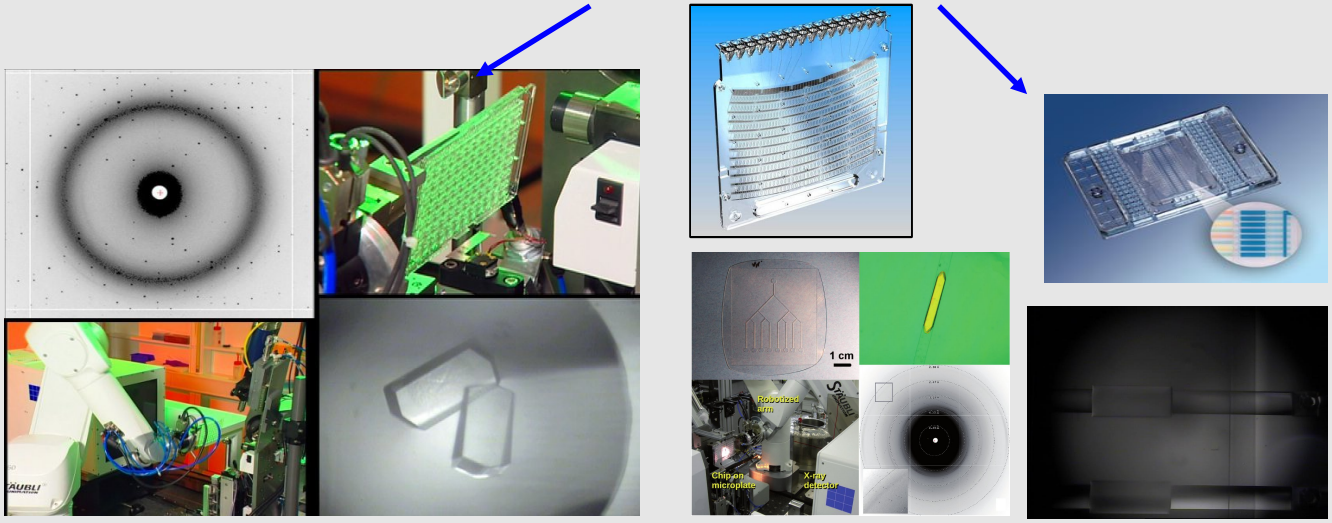
In the continuity of CATS (available at the ESRF, SLS, APS…), it integrates more functions:

- Picking up and transferring frozen crystals from a Dewar to the beam (~sample changer)
- Screening and collecting data on frozen crystals / crystals in capillaries (~goniometer)
- Screening of crystallization plates/µChips, collecting data on crystals still in the drop!!!
- Collecting data with special set-up/environment (capillaries, high pressure, …)

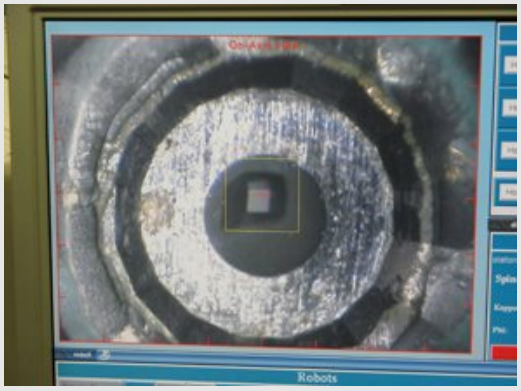
Transfer, rapid screening and data collection on frozen sample

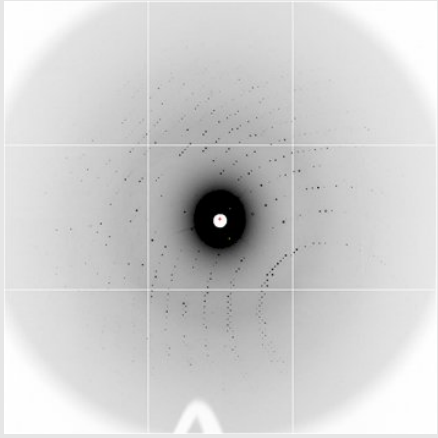


Screening of crystallization plates and µChips, data collection…



Data collection with high pressure cell



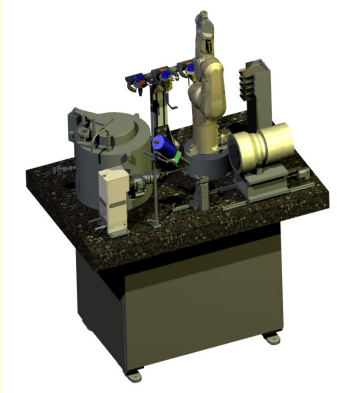


G-Rob was developed on FIP-BM30A at the ESRF

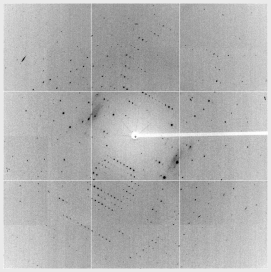
Lab and synchrotron setup commercialized by NatX-ray & IRELEC

www.natx-ray.com

Email: contact@natx-ray.com



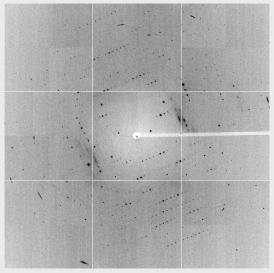
High pressure cell
(R. Kahn *et al*., IBS, Grenoble)

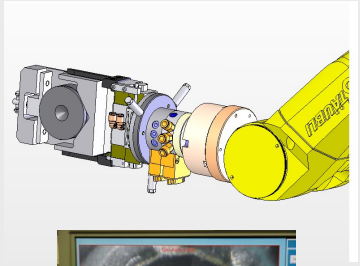


Z-DNA

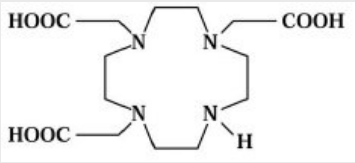
0 Mpa

540 MPa



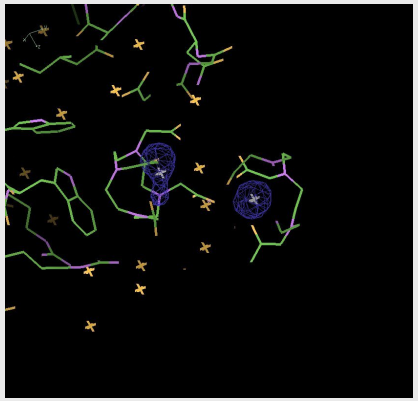


Anomalous data of Yb-DO3A / lysozyme complex solved "in the drop"



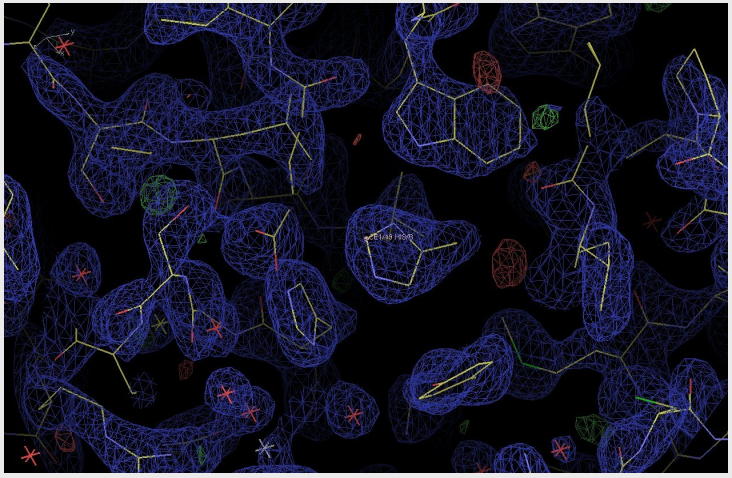
resolution: 1.8 Å
Complet.: 90 %
(anom.)

Anomalous diff. map (threshold: 6 σ)



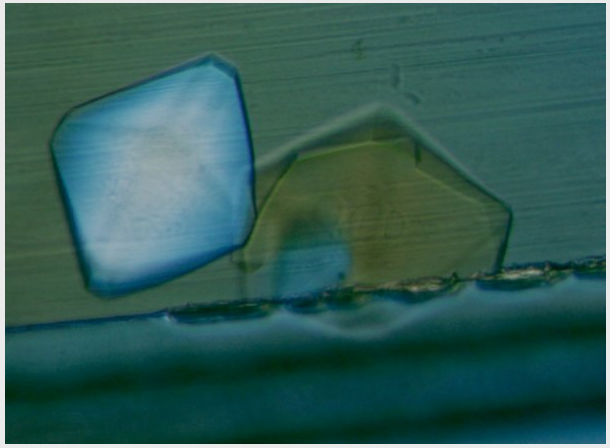
Structure of NDK from *A. Polyphaga* Mimivirus solved "in the drop"
(C. Abergel, CNRS)

space group: p6(3)
a/b/c: 70.8/70.8/106.3
resolution: 2.3 Å
Completness: 80 %, I/σ(I): 3.3
Rsym: 19.6 %, Rfree: 27.4 %



G-Rob 2D

Extra-cellular domain of a membrane protein
(A. Haouz, Inst. Pasteur, Paris)



>300 um crystals
No diffraction when frozen

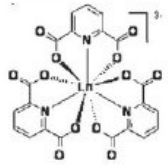
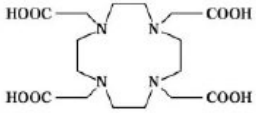
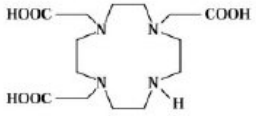
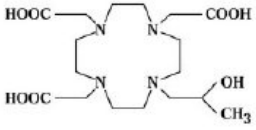
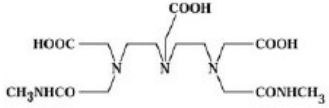
Beamline: FIP-BM30A
Plate: X-ray plate
Resolution: ~ 10 Å

Lanthanide complexes for anomalous phasing:

- for soaking/co-crystallization
- a huge signal
- less toxic

NatX-ray

The present kit contains 10 complexes, obtained from five different chelators:

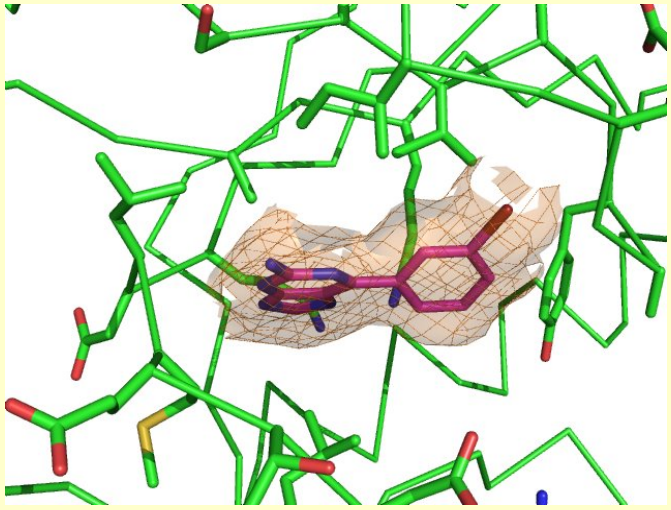
Acronym	Name	Formula
$(DPA)_3$	tris(pyridine-2,6-dicarboxylate) or tris(dipicolinate)	
DOTA	1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (CAS # 60239-18-1)	
DO3A	1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid	
HPDO3A	10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid	
DTPA-BMA	N,N-bis[2-[(carboxymethyl)[(methylcarbamoil)methyl]amino]ethyl]glycine	

Each of these chelators being provided with two different lanthanide atoms:

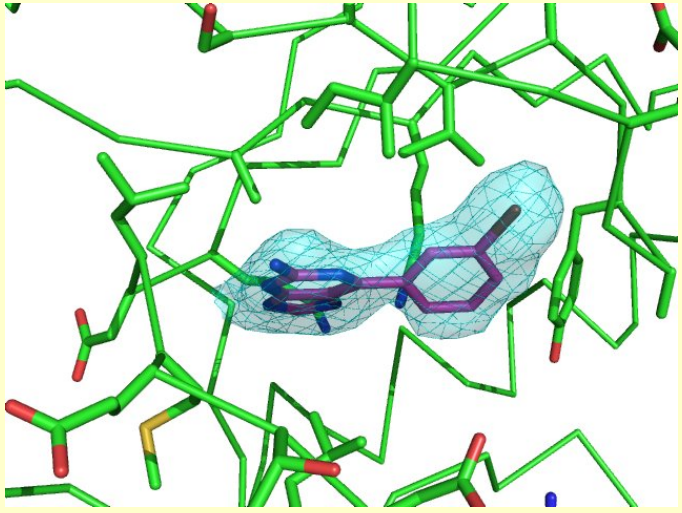
Atom	LIII edge	f'' at L_{III} edge (e-)	f'' at 1.0 Å (e-)	f'' at 1.54 Å (e-)
Eu	1.7761 Å / 6980 eV	28-30	6.52	**11.26**
Yb	1.3862 Å / 8944 eV	28-30	**9.69**	4.43

Test of ERK in P21 with bromated ligand.
Completness of 83%
By merging 3 dataset (50+50+41 frames)
Rsym ~5.4 at 2.15 A.
Refinement with the structure of ERK with no bromo-phenyl.

ERK (6PB)
Refinement: Refmac/Coot
Without ligand (R/Rfree ~ 20.4/25.5)

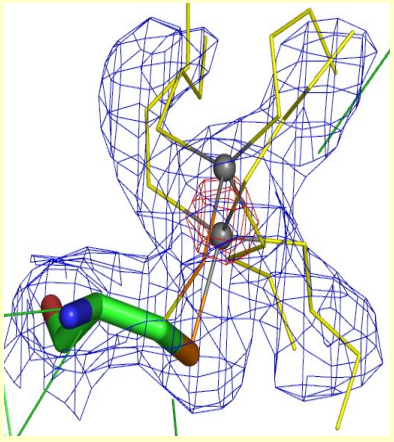


fo-fc (orange) 0.9 sigma

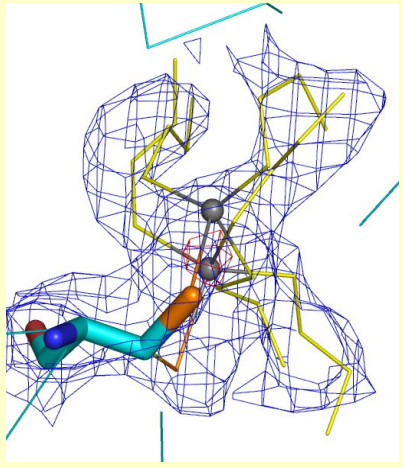


2fo-fc (blue) 0.9 sigma

Test of RXR in $P3_12_12$ with ligand organo-Sb.



3E94 (RXR-TBT-Tif2)
Rfact=0.506
contrast=5.08
rigid body: Rfact=0.36
fo-fc (red) 9.0 sigma
2fo-fc (blue) 1.0 sigma

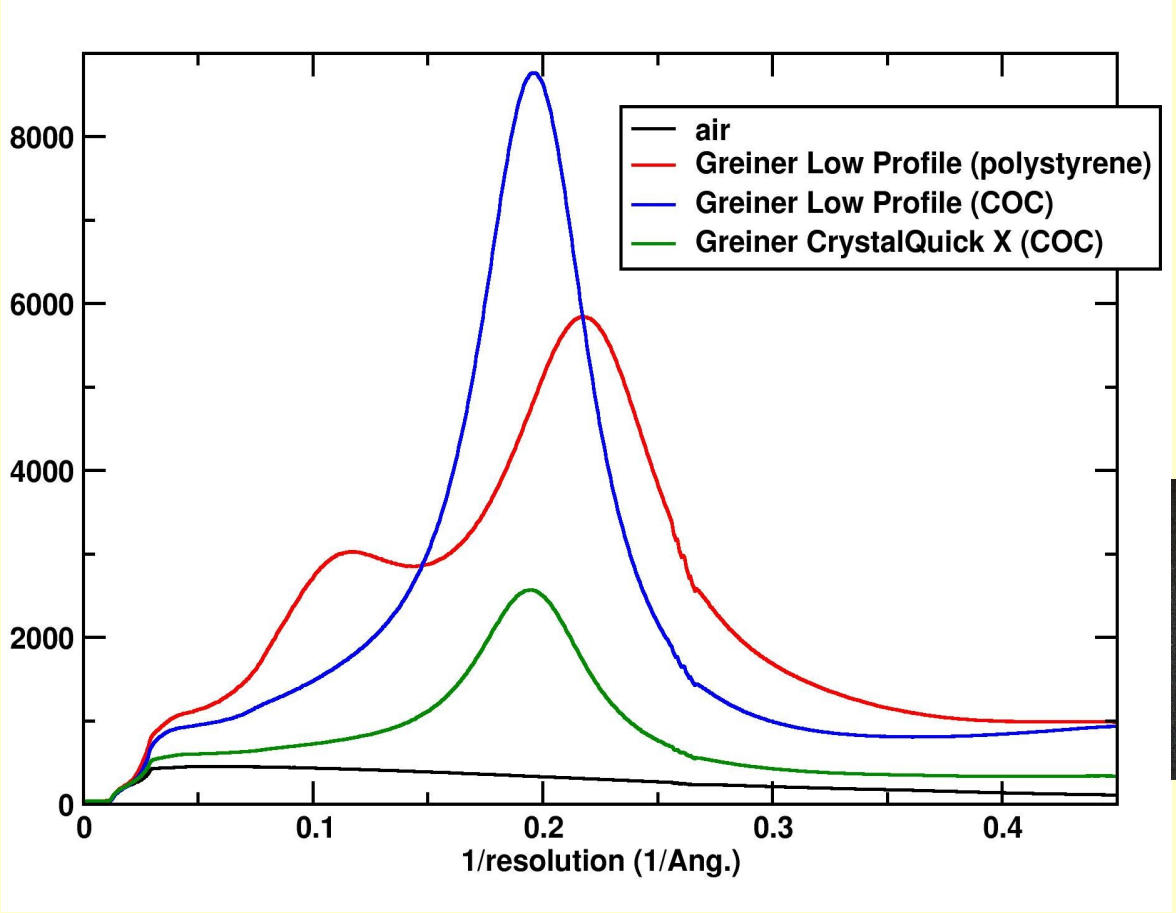


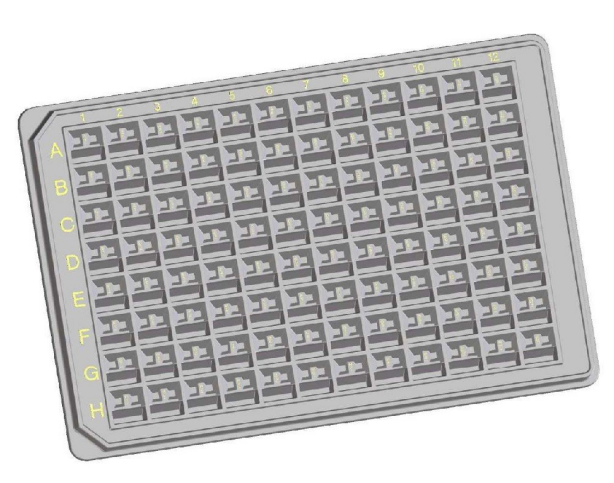
1K74 (RXR-9cisRA-SRC-1)
Rfact=0.519
contrast=4.57
rigid body: Rfact=0.41
fo-fc (red) 9.0 sigma
2fo-fc (blue) 1.0 sigma

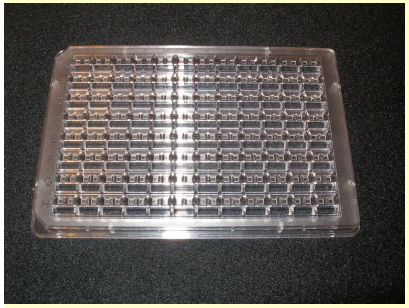
A new X-ray plate for *in situ* screening

Co-development of a new plate (shape, materials):

- better geometry (>80 deg oscillation range)
- better I/sigma (scattering significantly reduced)







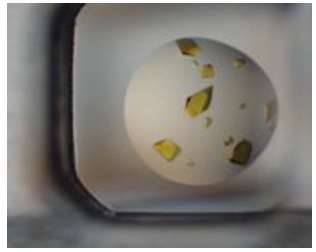
greiner bio-one

EMBL/HTX → FIP-BM30A pipeline

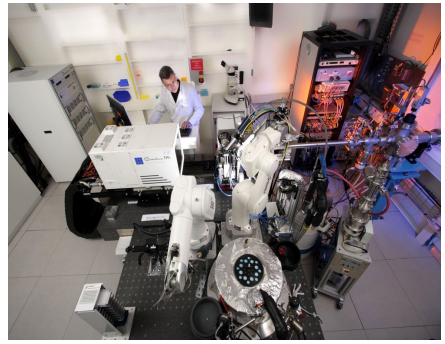
Nanodrop dispenser

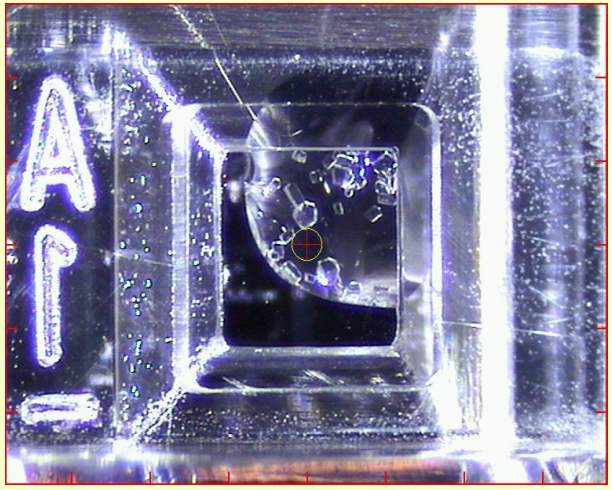
plate hotel

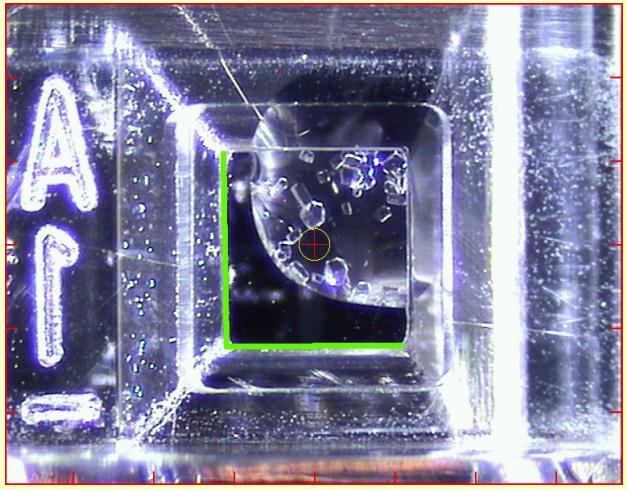
automated microscope

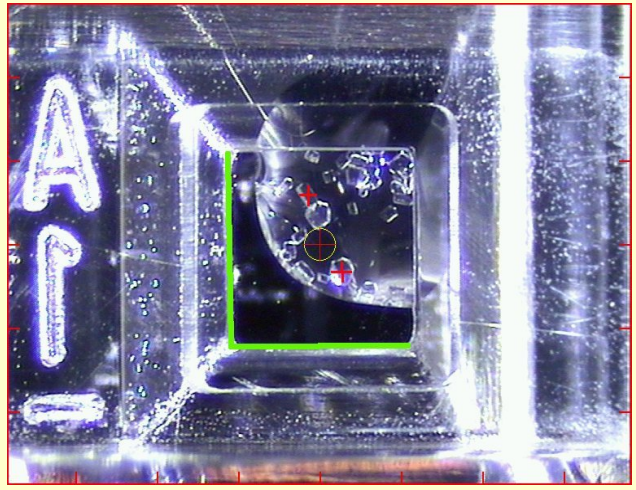


FIP-BM30A



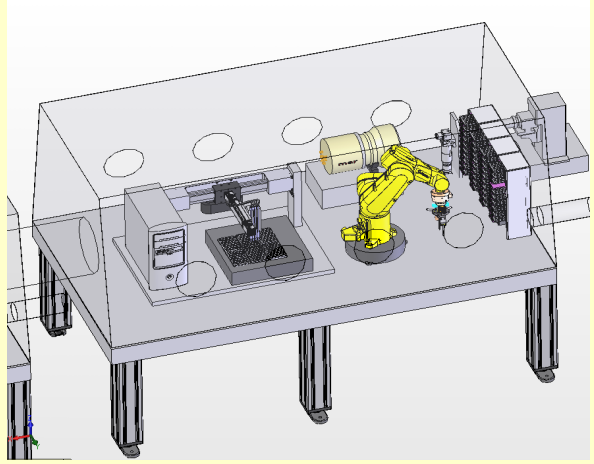




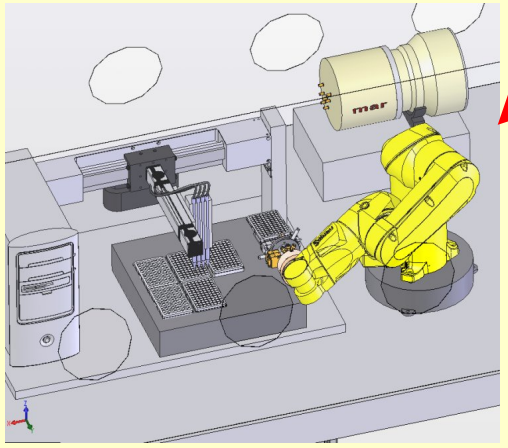


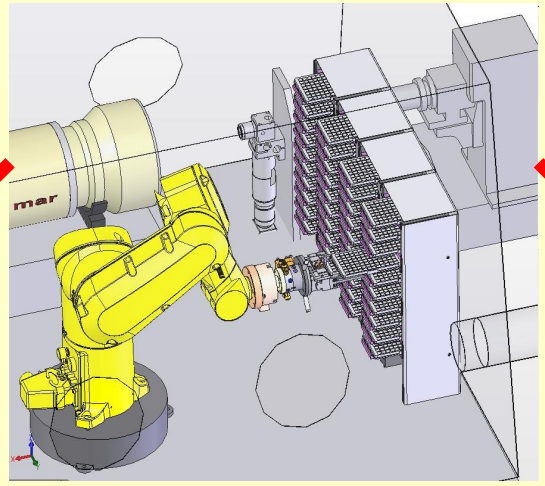
A G-Rob system in glove-box for the IBS

Controlled atmosphere, Bio-hazard (P3?)

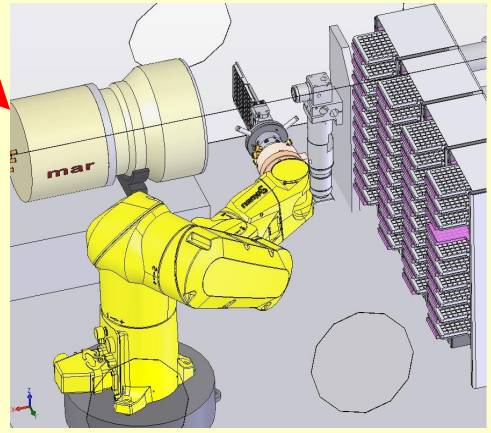


Crystallization robot





Screening



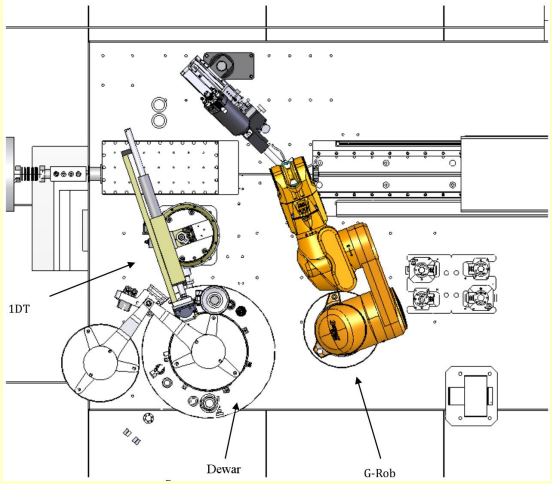
2 G-Rob already ordered, one more soon...

EPFL

- S. Cole Lab. (March 2009)

ESRF

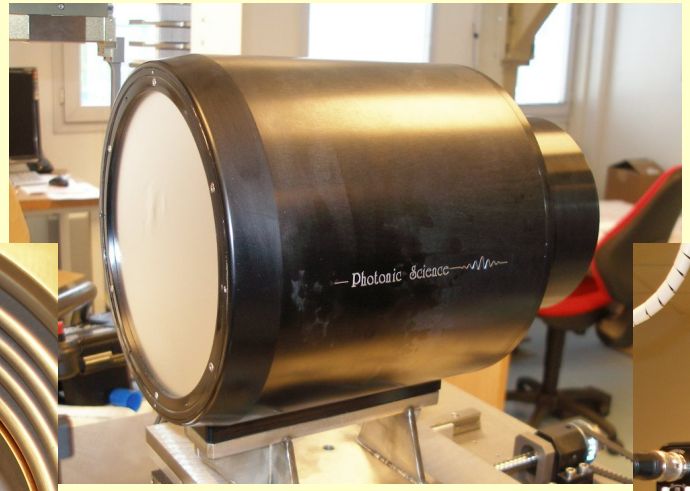
- MASSIF (Dec. 2009)

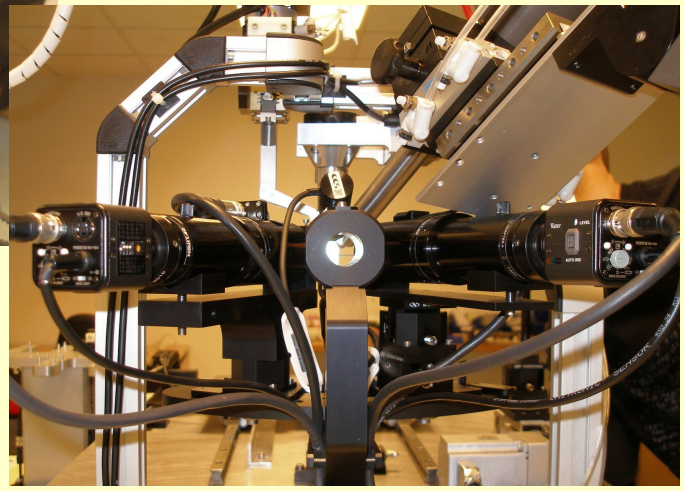


????????

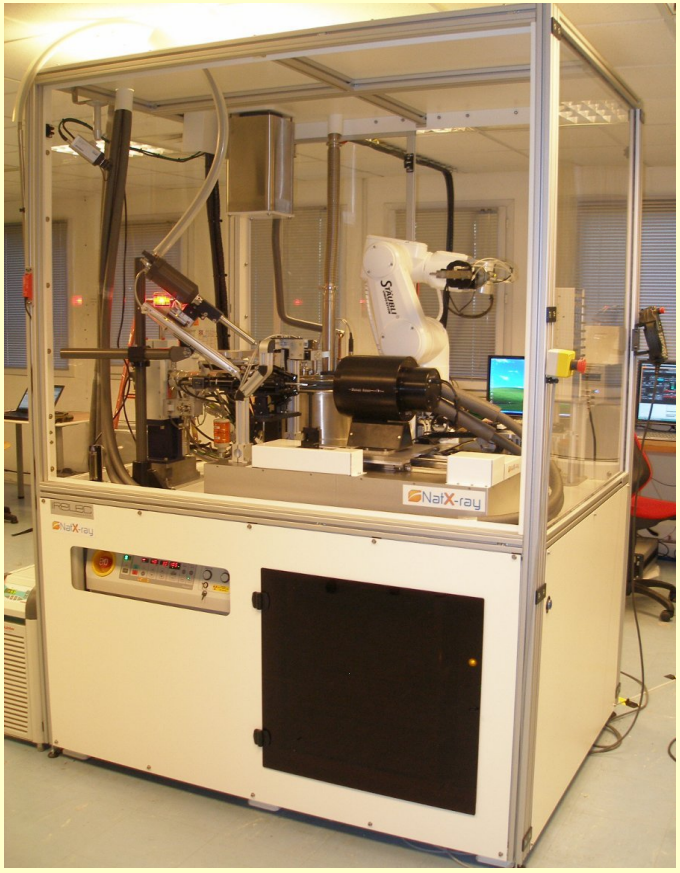
- PX wiggler beamline (in progress)

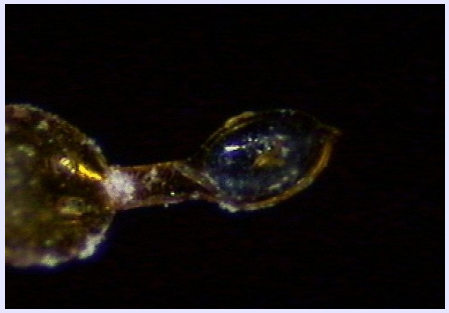
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

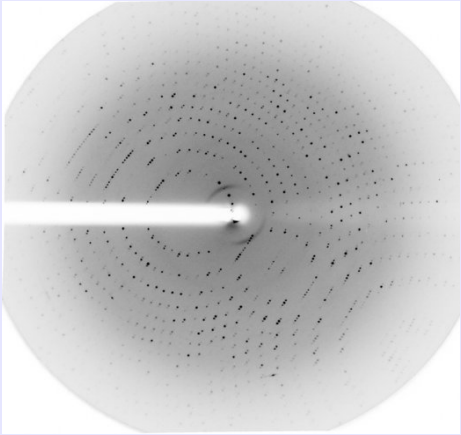




ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

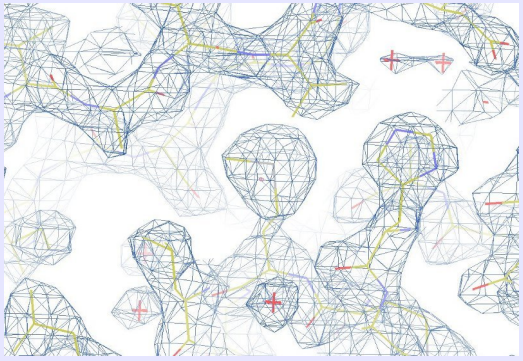


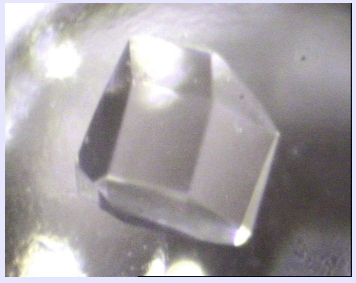


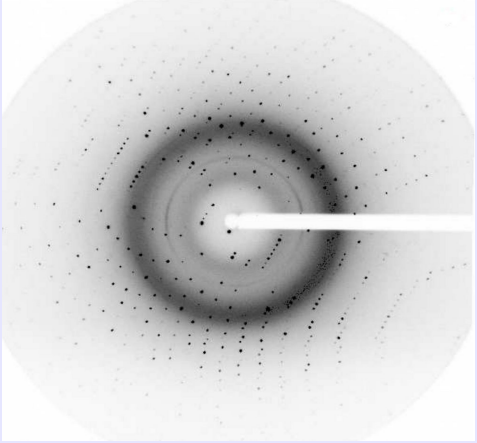


***E. coli* ADC lyase**
180 deg (0.5 deg per frame)
2 min exposure time

16.7% Rsym and 8.6 I/sigma in the last resolution shell (2.45-2.3 A)
(courtesy of D. Cobessi, IBS).





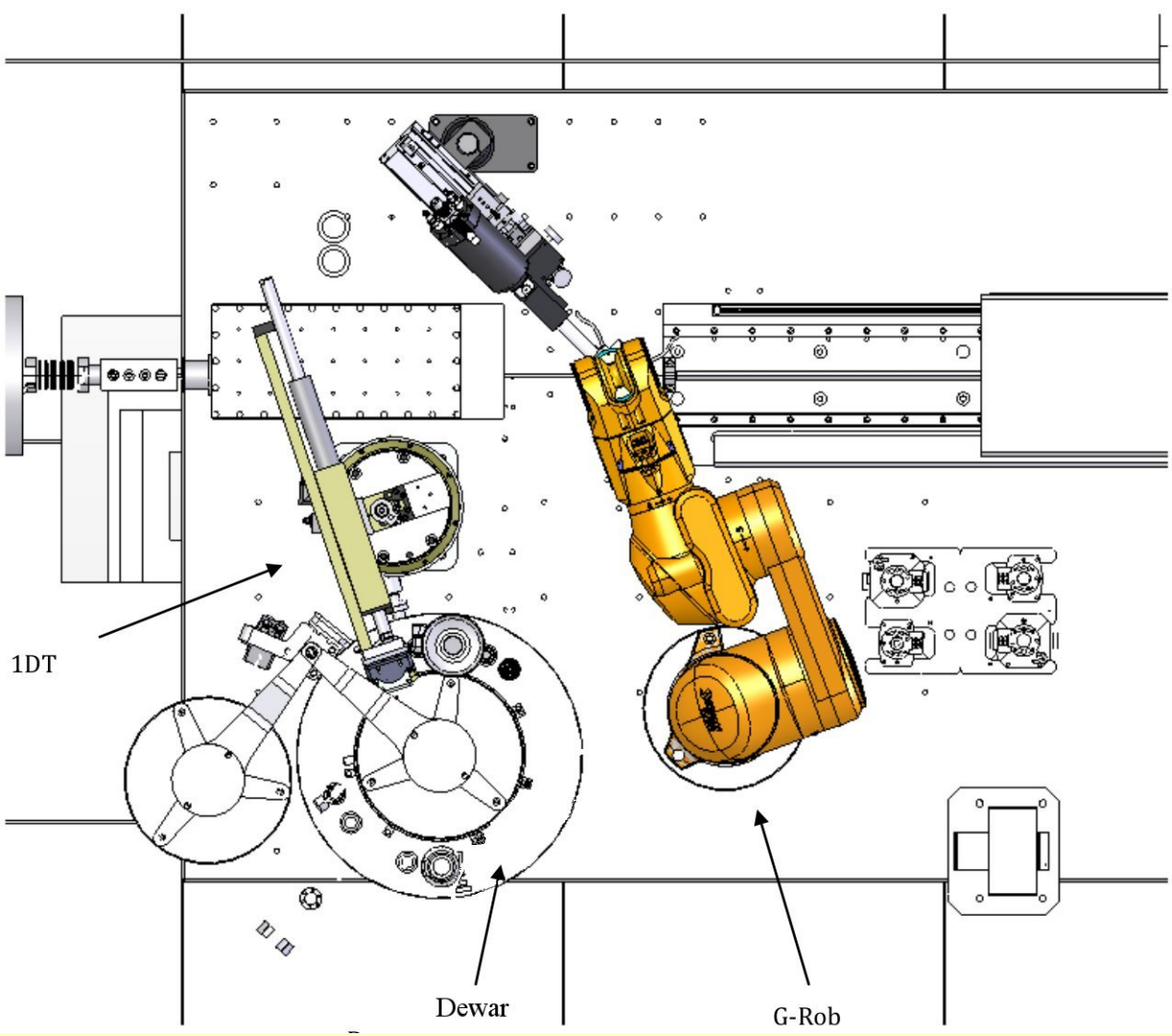


Lysozyme crystal
20 deg (1.0 deg per frame)
30 sec exposure time

<Rsym>: 2.9 % (up to 2.0 A), <I/s>: 13.4, completness: 54%

G-Rob to be tested on ID14-eh2 at the ESRF, Grenoble

- G-Rob 1D (2 microns SOC on 1 deg)
- G-Rob 1DT (20 s transfer, down to 10 s?)



IBS/GSy

L. Jacquamet
J. Joly
X. Vernede
M. Pirrochi
F. Bouis
J. Ohana
P. Charrault
G. Pagel
J. Quilez

NatX-ray

P. Mazel
P. Rogues
N. Larive-Ferrer