

# MASSIF-1 beamline upgrade

Integration of the CrystalDirect harvester

## Serena Rocchio

Postdoctoral Fellow

McCarthy Team - Marquez Team

[rocchio@embl.fr](mailto:rocchio@embl.fr)



# MASSIF-1



- Fully autonomous beamline
  - no user control
  - data collection optimised for every sample

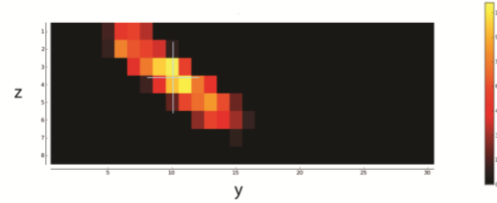
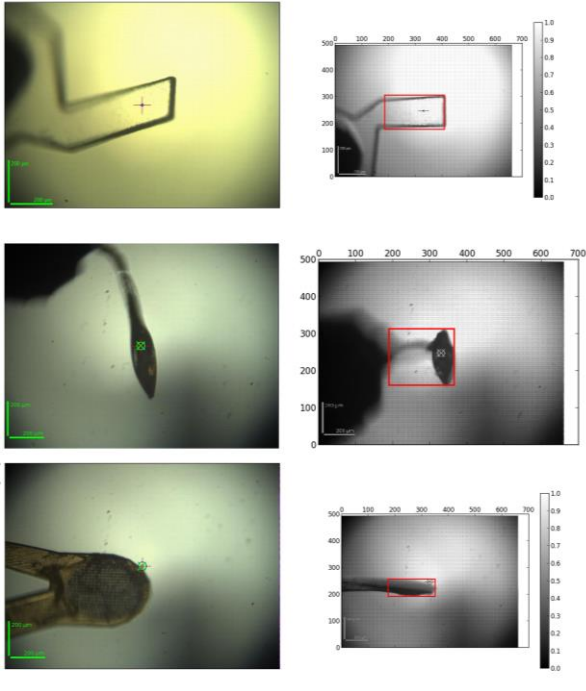
- Flexible booking, queuing system

Fixed energy: 12.84 keV  
Highly intense beam:  $5 \times 10^{12}$  ph/sec  
Flexible beamsizes: 100 to 10  $\mu\text{m}$

- Arinax MD2s diffractometer
- Flex HCD – 368 samples capacity
- Pilatus3 2M
- CrystalDirect Harvester

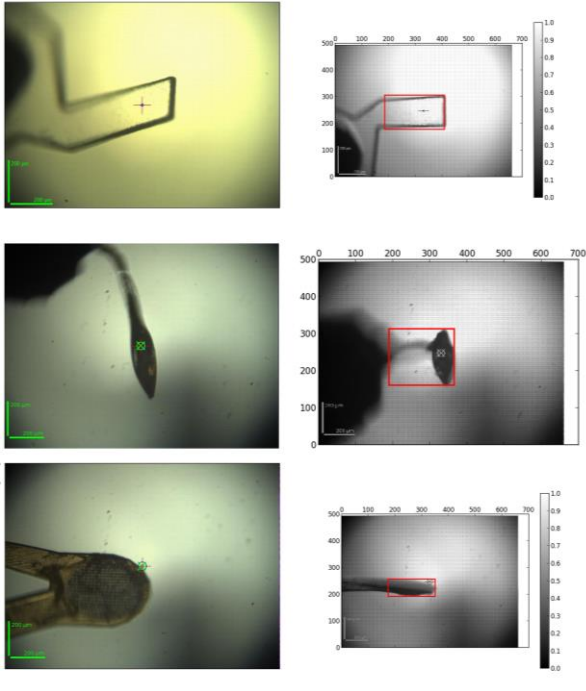
# Samples at MASSIF-1

## Diversity of sample holder (pins)

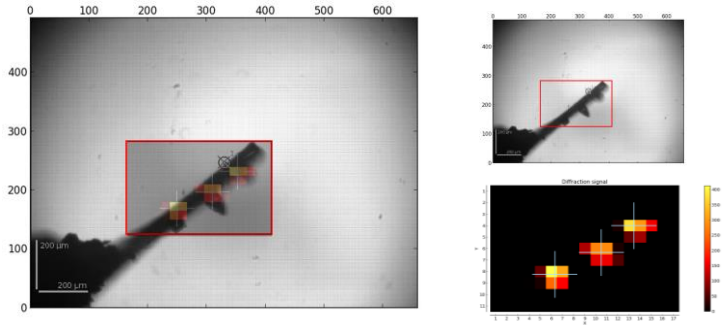


# Samples at MASSIF-1

## Diversity of sample holder (pins)

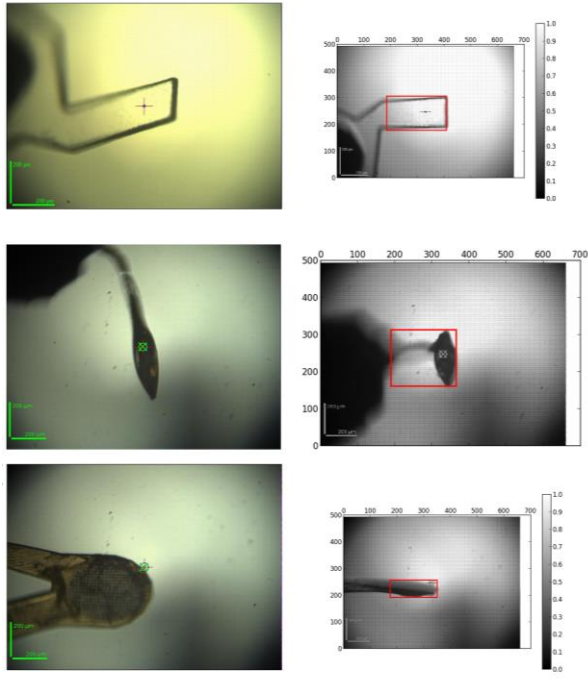


## Multiple data collection

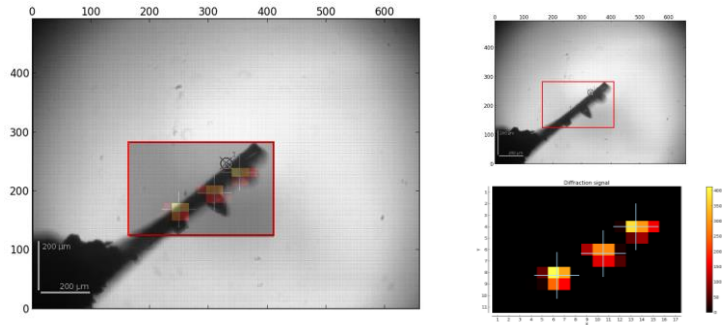


# Samples at MASSIF-1

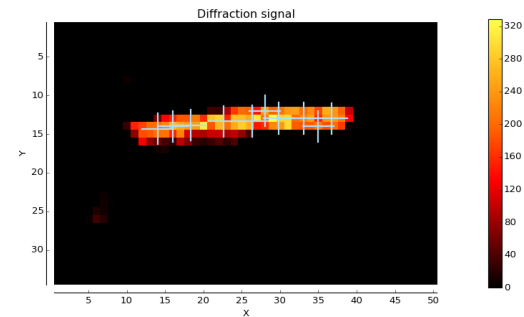
## Diversity of sample holder (pins)



## Multiple data collection



## Pseudo-helical data collection



# Diffraction Plan

## Workflows

Tutorial on Feb 7<sup>th</sup>, afternoon

ExiMX Extended ISPyB for MX<sub>BETA</sub>

Retrieved 5062 samples for selected proposal

Home Shipment Proteins and Crystals Prepare Experiment Data Explorer Offline Data Analysis SMIS

search by protein acronym

Log out mx415@mx415

Shipment

Name: AA123A Type: SPINE Beamline: #Sample Changer: Status:

Sample name field is mandatory and no special characters are allowed

Protein + sample name should be unique for the whole proposal

#	Protein Acronym	Sample Name	Pin BarCode	Crystal Form	Exp. Type	Aimed resolution	Required resolution	Beam Diameter	Number of positions	Aimed multiplicity	Aimed Completeness	Forced Space G.	Radiation Sensitivity	Smiles	Tot Rot. Angle	Observed resolution
1	LYS	z1		Undefined	MXPressO	1.7								Co1cccc1	360	
2	LYS	z2		Undefined	MXPressO	1.7							0.7		360	
3	LYS	z3		Undefined	MXPressE_SAD	1.7										
4	LYS	z4		Undefined	MXPressE_SAD			20								
5	LYS	z5		Undefined	MXPressP				7	6						
6	LYS	z6		Undefined	MXPressE				3	6	80					
7	LYS	z7		Undefined	MXPressE											
8	THAU	x234		P41212 - (57.3 , 57.3 , 148.8 - 90 , 90 , 90)	MXPressE											
9	THAU	x235		P41212 - (57.3 , 57.3 , 148.8 - 90 , 90 , 90)	MXPressE	3.2									200	
10	THAU	x236		P41212 - (57.3 , 57.3 , 148.8 - 90 , 90 , 90)	MXPressE	3.2									200	

Workflow details: <https://www.esrf.fr/MXPressWF>

# Data collection results

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/data/visitor/mx...

Summary

Beamline Parameters

Data Collections **5**

Sample

Last Collect Results **14**

Workflow **6**

Snapshots

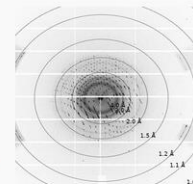
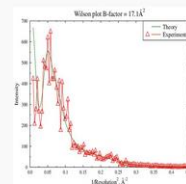
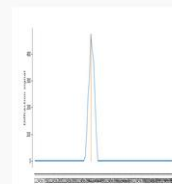
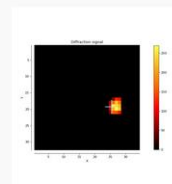
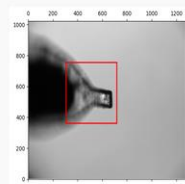
Automesh

Mesh

Line

Characterisation

Characterisation



- Automated data processing
- Structure solution
- Ligand fitting

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/data/visitor/m...

Summary

Beamline Parameters

Data Collections **5**

Sample

Last Collect Results **16**

Workflow **6**

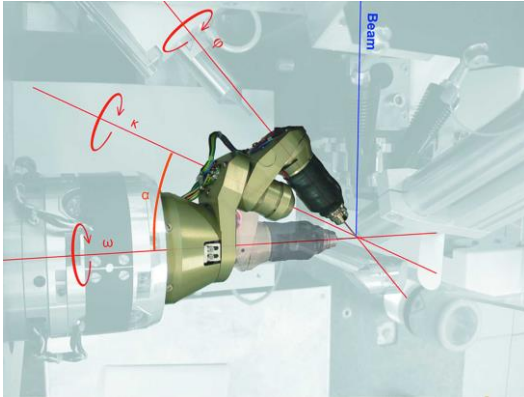
Pipeline	SpaceGroup	a,b,c (Å)	α,β,γ (°)	Shell	Resolution (Å)	Multiplicity	Completeness %	Anomalous multiplicity	Anomalous completeness %	<I>Sigma	Rmeas	Rmerge	Rpim	cc(1/2)	ccAno	sigAno	ISA	Download
ANCM tctt	XDSAPP	P 41 212	40.2 90.0	Overall	40.5-1.5	6.0	79.8	3.1	73.8	28.7	4.1	3.4	2.0	100				
				Inner	40.5-2.2	5.6	99.3	4.6	100.0	49.4	3.2	2.9	1.5	100				
				Outer	202.4 90.0	1.5-1.5	1.3	22.6	1.0	6.5	2.7	56.1	26.5	26.5	40			
ANCM	autoPROC	P 41 212	40.2 90.0	Overall	50.5-1.5	5.9	72.6	3.5	66.7	28.6	3.9	3.8	1.9	100				
				Inner	50.5-4.0	6.4	99.6	4.1	99.2	50.2	3.2	2.9	1.5	100				
				Outer	202.4 90.0	1.5-1.5	1.2	17.4	1.0	4.5	2.9	39.6	28.0	14.2	60			
ANCM	autoPROC_staranis	P 41 212	40.2 90.0	Overall	50.5-1.5	5.9	73.0	3.5	66.9	28.5	3.9	3.8	1.9	100				
				Inner	50.5-4.7	6.0	99.6	4.0	98.6	48.3	3.4	3.1	1.6	100				
				Outer	202.4 90.0	1.6-1.5	1.5	28.9	1.2	11.6	3.3	28.3	21.1	13.6	60			
ANCM	EDNA_proc	P 41 212	40.2 90.0	Overall	40.5-1.4	5.5	74.8	2.9	66.8	16.3	8.5	6.7	4.0	100				
				Inner	40.5-5.6	5.7	99.8	3.9	99.4	28.3	7.0	6.1	3.4	100				
				Outer	202.3 90.0	1.5-1.4	1.1	10.5	1.0	1.6	1.8	86.0	0.0	0.0	20			
ANCM	grenades_parallelproc	P 41 212	40.2 90.0	Overall	202.2-1.6	5.9	90.1	3.1	84.6	17.5	9.3	6.9	4.1	100				
				Inner	202.2-4.8	5.4	99.9	4.6	100.0	26.1	7.2	6.3	3.5	100				
				Outer	202.2 90.0	1.6-1.6	3.1	55.6	1.8	46.9	3.8	25.2	19.2	17.5	90			
ANCM	grenades_parallelproc	P 41 212	40.2 90.0	Overall	202.2-1.6	5.9	90.1	3.1	84.6	17.5	9.3	6.9	4.1	100				
				Inner	202.2-4.8	5.4	99.9	4.6	100.0	26.1	7.2	6.3	3.5	100				
				Outer	202.2 90.0	1.6-1.6	3.1	55.6	1.8	46.9	3.8	25.2	19.2	17.5	90			
ANCM	XIA2_DIALS	P 41 212	40.5 90.0	Overall	68.0-1.6	5.5	84.2	3.1	79.8	8.7	26.3	23.4	14.2	90				
				Inner	68.0-4.2	4.3	100.0	2.7	99.2	26.1	11.1	9.2	6.6	90				
				Outer	203.9 90.0	1.6-1.6	1.8	38.7	1.2	21.6	0.3	80.7	57.3	56.0	0			

# Data collection of low symmetry space groups

Automated crystal reorientation and additional data collection

GΦL

Global Phasing Limited



Mini-k goniometer head

Space group P1: small amount of data lost in the blind region

- Mini kappa opening
- Second data collection at different crystal orientation

Profile	Run	Images	Exposure Time	Res. (corner)	Wavelength	Transmission	Directory and image template	Time	Run status	Indicators	View Results	Phasing	Comments
me...	0	300	0.05 s	1.3 Å (1.0 Å)	0.9855 Å	100%		07:43:30	Data collection successful				
me...	1	100	0.05 s	1.3 Å (1.0 Å)	0.9855 Å	100%		07:45:09	Data collection successful				
me...	1	4	0.1 s	1.3 Å (1.0 Å)	0.9855 Å	100%		07:48:13	Data collection successful				
me...	1	1800	0.02 s	1.5 Å (1.1 Å)	0.9855 Å	85.10%		07:47:55	Data collection successful				
P 1													
Overall	Res.	Completeness	Range	Sigma	CC1/2								
Overall	47.79-1.42	95.1%	5.0	3.3	99.9								
Inner	47.79-7.77	95.2%	1.9	45.1	99.7								
Outer	1.44-1.42	97.3%	250.1	0.3	15.2								
me...	1	64	0.05 s	1.3 Å (1.0 Å)	0.9855 Å	100%		07:50:10	Data collection successful				
me...	2	15	0.1 s	1.3 Å (1.0 Å)	0.9855 Å	80.24%		07:51:35	Data collection successful				
me...	2	1800	0.02 s	1.5 Å (1.1 Å)	0.9855 Å	85.10%		07:52:30	Data collection successful				
P 1													
Overall	Res.	Completeness	Range	Sigma	CC1/2								
Overall	47.90-1.51	95.3%	4.5	11.4	99.6								
Inner	47.90-8.25	95.3%	1.8	58.0	99.9								
Outer	1.53-1.51	94.0%	245.2	0.4	15.2								



# Data collection of 1057 (pilot project: P1)

PanDDA analysis 135 datasets did not detect any binding for this ligand

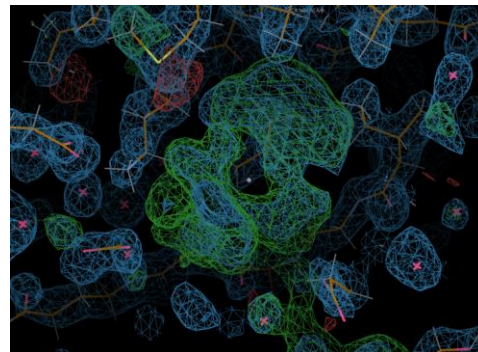
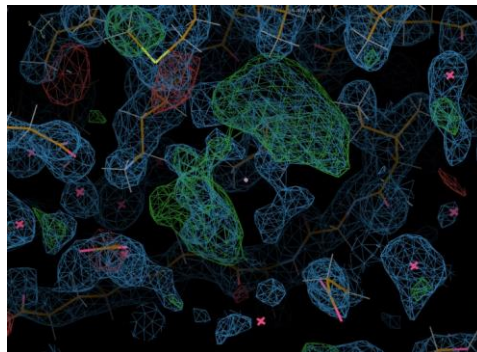
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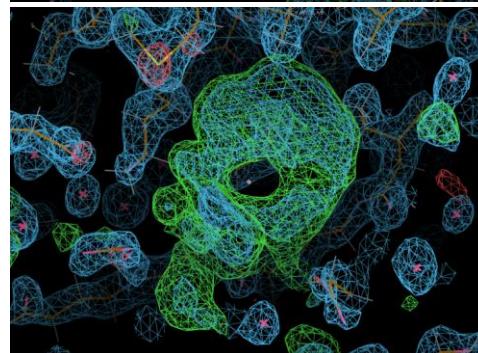
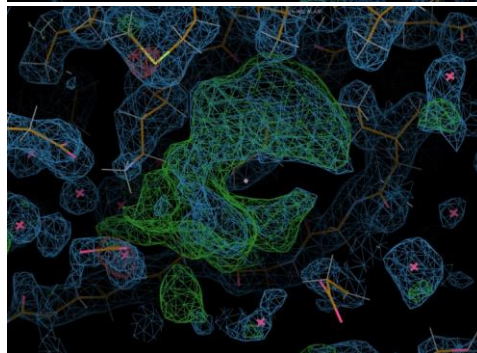
“Old” data for complex  
(Standard protocol)

“New” data for complex  
(4-sweep protocol on ID30-B)

“Old” Model  
(refined against best  
standard dataset)



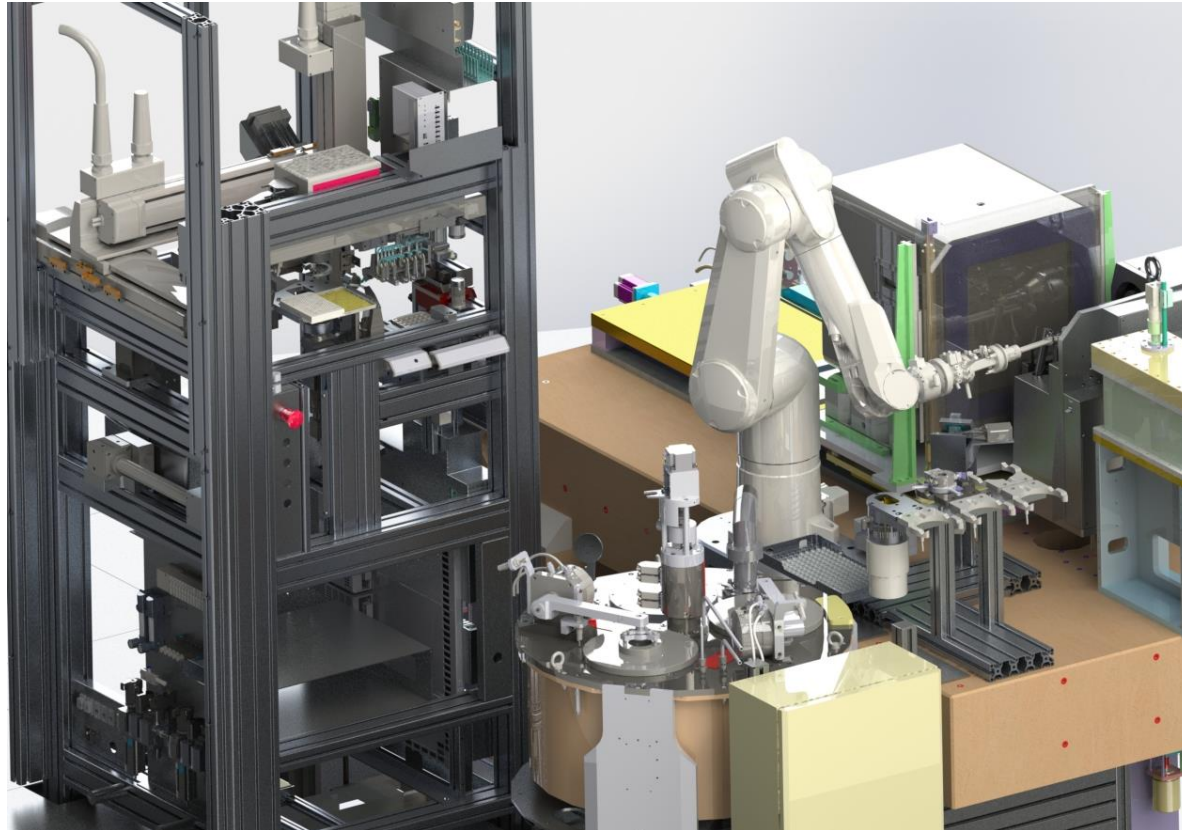
“New” Model  
(refined against a  
4-sweep DMSO  
dataset, ID30-B)



(Collaboration: Merck KGaA and Marquez Team)

# Integration of the CrystalDirect harvester

Commissioning ongoing



# CrystalDirect harvester

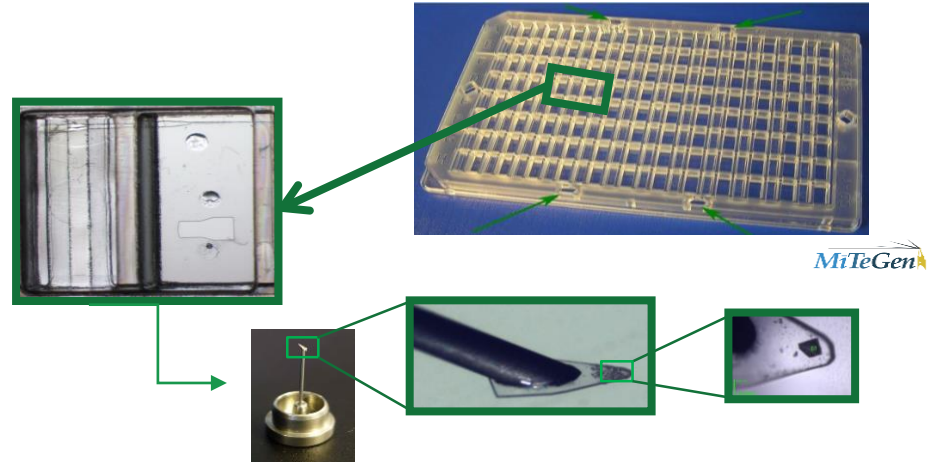
Fully automated crystallography pipeline controlled through CRIMS interface



## Crystallization facility at EMBL:

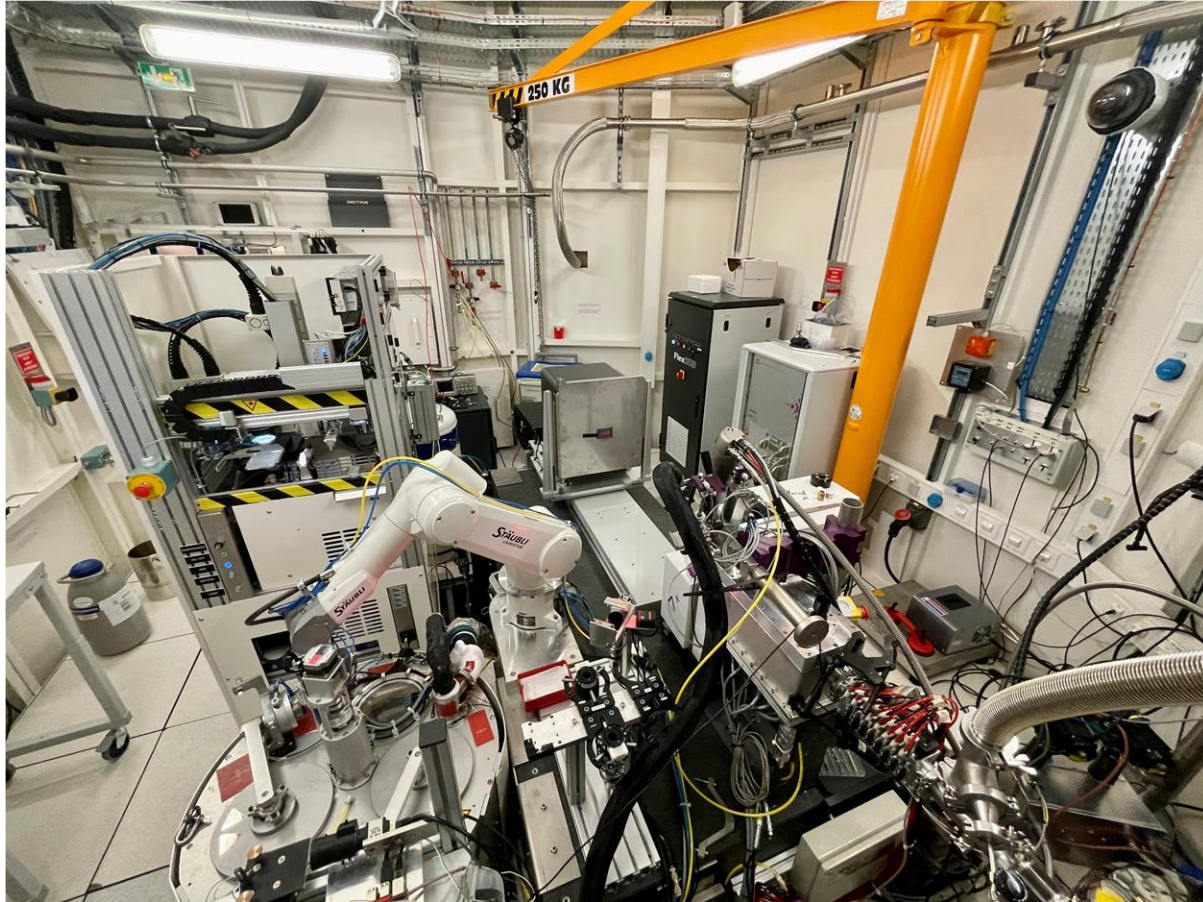
- Online Crystallography (Protein to Structure)
- Fragment Screening
- Serial crystallography for membrane proteins

Funded access:



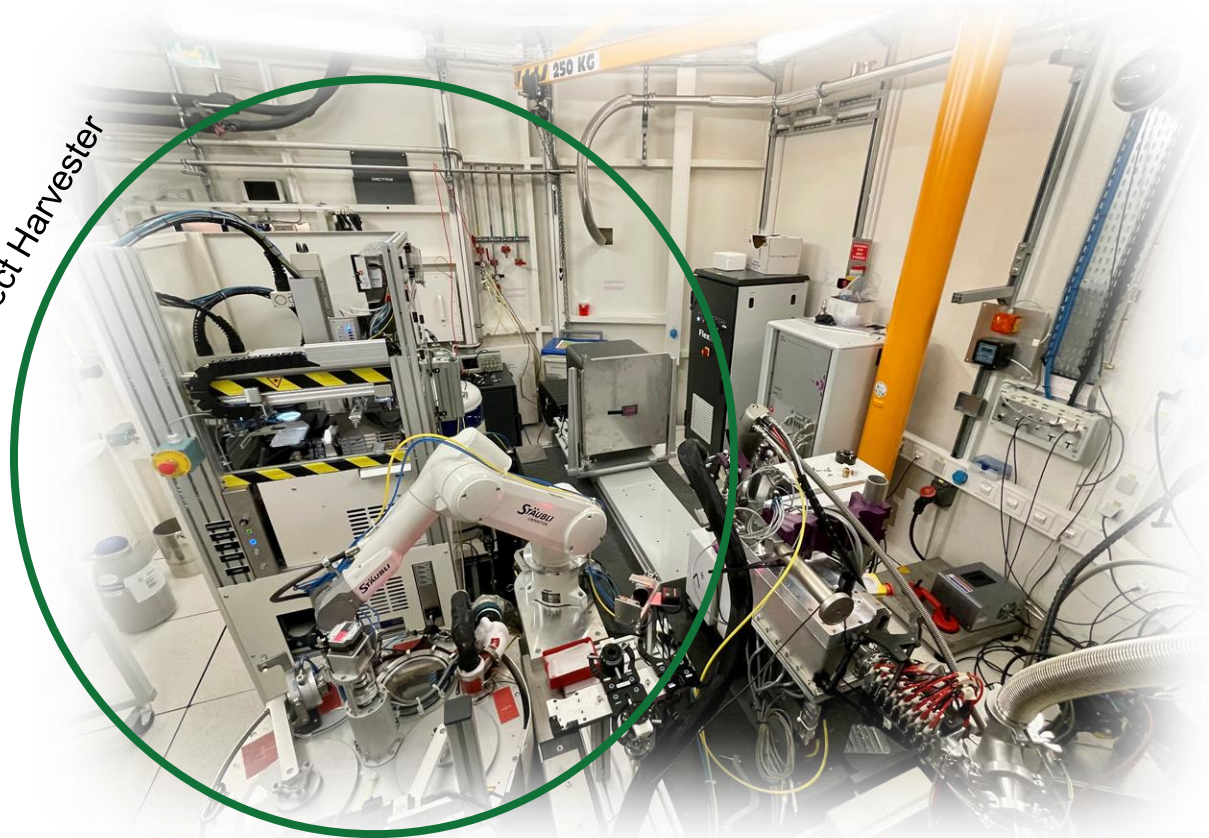


# CrystalDirect harvester at MASSIF-1



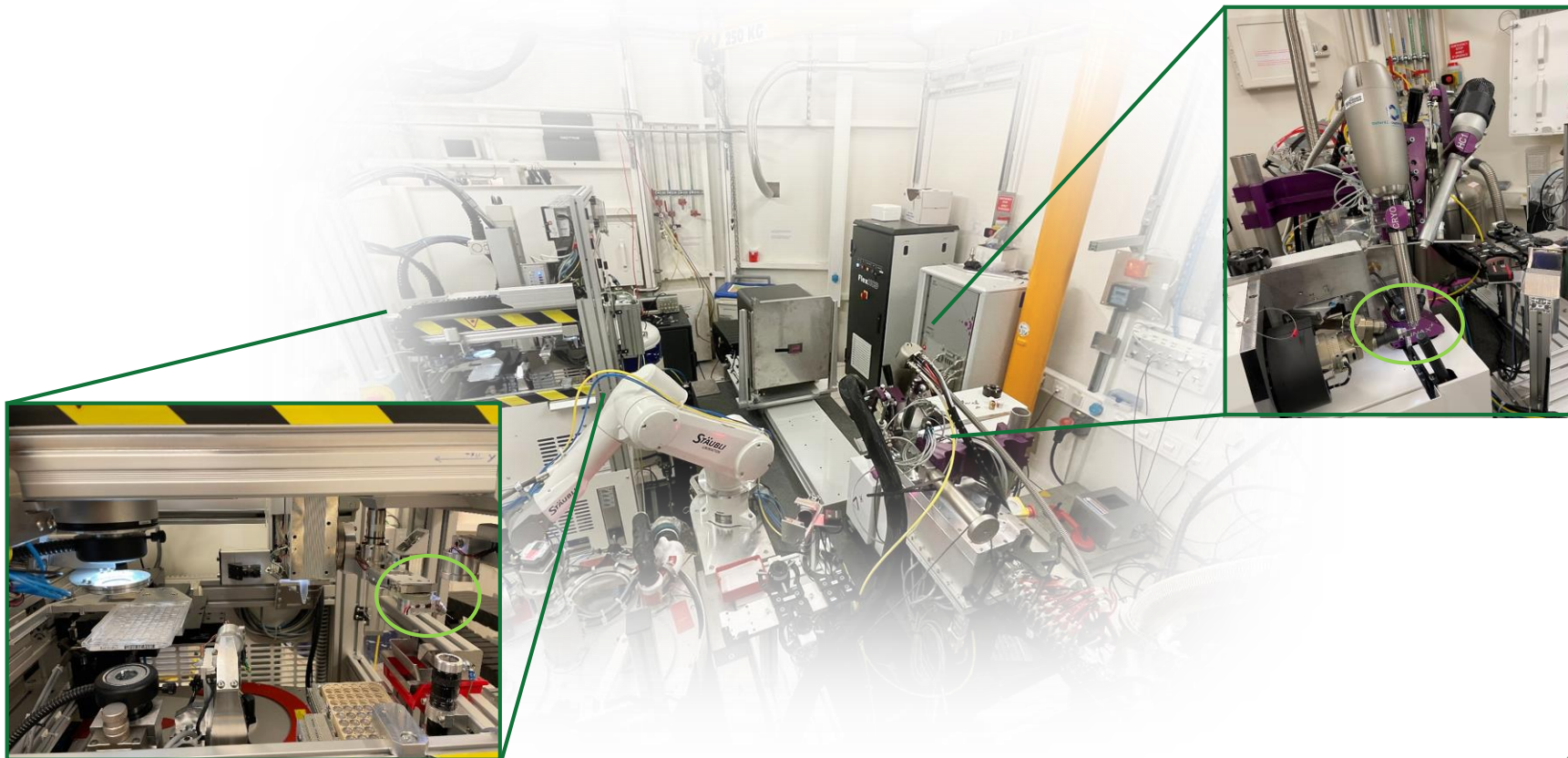
# CrystalDirect harvester at MASSIF-1

CrystalDirect Harvester





# CrystalDirect harvester at MASSIF-1



# Automated crystal harvesting and data collection pipeline

## Harvesting plan preparation in CRIMS interface

CD032398 E06-1

Thaumatococcus CDH3 20 mg/ml

rocchio

	1	2	3	4	5	6	7	8	9	10	11	12
A	█	█	█	█	█	█	█	█	█	█	█	█
B	█	█	█	█	█	█	█	█	█	█	█	█
C	█	█	█	█	█	█	█	█	█	█	█	█
D	█	█	█	█	█	█	█	█	█	█	█	█
E	█	█	█	█	█	█	█	█	█	█	█	█
F	█	█	█	█	█	█	█	█	█	█	█	█
G	█	█	█	█	█	█	█	█	█	█	█	█
H	█	█	█	█	█	█	█	█	█	█	█	█

Latest Scores

E6-1 Harvesting Plan

Save Harvesting

Crystal Harvesting Manual Harvesting Measure UV HD HD

Harvester

Cut Shapes

- Straight Edge
- 300x200
- Asymmetric
- Single-Crystal
- Full-Drop
- Small-Single-Crystal

Actions

Plate ID: **CD033242** Set Open Processing Plan

Table

Pin Storage

AD078A Cdl:4 Pos:2

13 Cycles before defreeze + 5 (Grace harvestings)

Empty Puck

Harvesting Parameters Advanced Maintenance

Harvest unmarked crystal Pause

Harvest

Pin: 500x300.svg Orientation: 145.803°

Gating Delay: 0.2 s Force Film Focusing Cut shape side cuts in advance

Cryo Protection  Auto-pause after Cryo Protection

Depressurization: Cross.lms Cryo Protection(s): Cross.lms Number of aspiration s: 1 +

Drop Aspiration: Count: 4 Time: 0.7 s

Unload Sample

Focus: 10.124 Zoom: Back Light: Diffuser

Harvest Results Logs

Day	Time	Position	Plate Id	Crystal Id	Success	Pin Bar...	Puck Ba...	Pin Posi...	Aspirat...	No Xtals	Ice?	Temper...	Message
27/01/2022	15:03:50	F06-1	CD033242	c5739e7...	true	null	AD057A	15	Auto-4x7...	1	false	20.01	
27/01/2022	15:05:12	F06-1	CD033242	9a96682...	true	null	AD057A	16	No aspir...	1	false	20.01	
27/01/2022	15:07:36	F06-3	CD033242	6f1f8a...	true	null	AD081A	11	Auto-4x7...	1	false	20.03	
27/01/2022	15:09:30	D06-1	CD033242	af5fab80...	true	null	AD081A	2	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:11:36	C06-1	CD033242	caa06f68...	true	null	AD081A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:13:40	B06-1	CD033242	e526222...	true	null	AD081A	4	Auto-4x7...	1	false	19.98	
27/01/2022	15:16:00	A06-1	CD033242	1532922...	false	null	AD081A	11	Auto-4x7...	1	false	20.0	auto-detect no harvesting shape
27/01/2022	15:17:46	A07-1	CD033242	0e3f314f...	true	null	AD081A	5	Auto-4x7...	1	false	20.0	
27/01/2022	15:20:43	B07-1	CD033242	82f56e5...	true	null	AD081A	6	Auto-4x7...	1	false	20.0	
27/01/2022	15:22:36	C07-1	CD033242	26578e...	true	null	AD081A	7	Auto-4x7...	1	false	20.0	
27/01/2022	15:24:25	D07-1	CD033242	3475604...	true	null	AD081A	8	Auto-4x7...	1	false	20.0	
27/01/2022	15:28:55	E07-1	CD033242	0375834...	true	null	AD081A	9	Auto-4x7...	1	false	20.0	
27/01/2022	15:31:44	F07-1	CD033242	142330b...	true	null	AD081A	10	Auto-4x7...	1	false	19.98	
27/01/2022	15:33:53	G07-1	CD033242	6803876...	true	null	AD081A	11	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:36:38	H08-3	CD033242	347f6e5...	true	null	AD081A	12	Auto-4x7...	1	false	20.0	
27/01/2022	15:38:41	G08-1	CD033242	0f980e9...	true	null	AD081A	13	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:40:29	F08-3	CD033242	ff4346f9...	true	null	AD081A	14	Auto-4x7...	1	false	19.97	
27/01/2022	15:42:32	E08-1	CD033242	a87199a...	true	null	AD081A	15	Auto-4x7...	1	false	20.0	
27/01/2022	15:44:05	D08-1	CD033242	ab6f738...	true	null	AD081A	16	Auto-4x7...	1	false	19.98	
27/01/2022	15:46:14	D08-1	CD033242	885c422...	true	null	AD081A	15	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:48:32	C08-1	CD033242	d8014bc...	true	null	AD021A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:52:42	A08-1	CD033242	f67ad37d...	true	null	AD021A	4	Auto-4x7...	1	false	20.0	

Monitoring Hardware Status

Laser: Ready

Smart Magnet Detection Pin mounted

Remaining Pins: 26 ( Rack: 22 - SC: 4 ) View

Temperature Control

20.00 °C

Target: 20.00 °C

Time before next draining: 82min

Cryo view PPU view TRU view

Park All Abort



Actions

Plate ID: **CD033242** Set Open Processing Plan

Table

Pin Storage

AD078A Cdl4 Fos.2

13 Cycles before defreeze + 5 (Grace harvestings)

Harvesting Parameters Advanced Maintenance

Harvest

Pin: 600x300.svg Orientation: 145.803°

Gating Delay: 0.2 s  Force Film Focusing  Cut shape side cuts in advance

Cryo Protection  Auto-pause after Cryo Protection

Depressurization: Cross.lms Cryo Protection(s): Number of aspiration sites: 1

Drop Aspirations: Count: 4 Time: 0.7 s

Unload Sample

Focus: << < 10.147 > >> Zoom: [Slider] Back Light: [Slider] Diffuser

Harvest Results Logs

Day	Time	Position	Plate Id	Crystal Id	Success	Pin Barc...	Puck Ba...	Pin Posi...	Aspirat...	No Xtals	Ice?	Temper...	Message
27/01/2022	15:03:50	F06-1	CD033242	c5739e7...	true	null	AD057A	15	Auto-4x7...	1	false	20.01	
27/01/2022	15:03:52	F06-1	CD033242	9a66692...	true	null	AD057A	16	No aspir...	1	false	20.01	
27/01/2022	15:07:26	E06-3	CD033242	46f146a...	true	null	AD081A	11	Auto-4x7...	1	false	20.03	
27/01/2022	15:09:30	D06-1	CD033242	af5fab80...	true	null	AD081A	2	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:11:36	C06-1	CD033242	caa06f68...	true	null	AD081A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:13:40	B06-1	CD033242	ea52622a...	true	null	AD081A	4	Auto-4x7...	1	false	19.98	
27/01/2022	15:16:00	A06-1	CD033242	5339c22...	false	null	AD081A	11	Auto-4x7...	1	false	20.0	auto-detect no harvesting shape
27/01/2022	15:17:46	A07-1	CD033242	0e3f314f...	true	null	AD081A	5	Auto-4x7...	1	false	20.0	
27/01/2022	15:20:43	B07-1	CD033242	82f56ea...	true	null	AD081A	6	Auto-4x7...	1	false	20.0	
27/01/2022	15:22:36	C07-1	CD033242	26578ec...	true	null	AD081A	7	Auto-4x7...	1	false	20.0	
27/01/2022	15:24:25	D07-1	CD033242	5476504...	true	null	AD081A	8	Auto-4x7...	1	false	20.0	
27/01/2022	15:28:55	E07-1	CD033242	0375834...	true	null	AD081A	9	Auto-4x7...	1	false	20.0	
27/01/2022	15:31:44	F07-1	CD033242	142330b...	true	null	AD081A	10	Auto-4x7...	1	false	19.98	
27/01/2022	15:33:53	G07-1	CD033242	6803876...	true	null	AD081A	11	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:36:38	H08-3	CD033242	547f6e6...	true	null	AD081A	12	Auto-4x7...	1	false	20.0	
27/01/2022	15:38:41	G08-1	CD033242	0f980e9...	true	null	AD081A	13	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:40:29	F08-3	CD033242	ff43446...	true	null	AD081A	14	Auto-4x7...	1	false	19.97	
27/01/2022	15:42:32	E08-1	CD033242	a87199a...	true	null	AD081A	15	Auto-4x7...	1	false	20.0	
27/01/2022	15:44:05	D08-1	CD033242	4b46738...	true	null	AD081A	16	Auto-4x7...	1	false	19.98	
27/01/2022	15:46:14	C08-1	CD033242	885422...	true	null	AD081A	15	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:48:32	B08-1	CD033242	d8014bc...	true	null	AD021A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:52:42	A08-1	CD033242	f67ad37a...	true	null	AD021A	4	Auto-4x7...	1	false	20.0	

Monitoring Hardware Status

Laser : Ready

Remaining Pins **26 ( Rack : 22 - SC : 4 )** View Smart Magnet Detection  Pin mounted

Temperature Control

20.00 °C

Target : 20.00 °C

Time before next draining : 82min

Cryo view PPU view TRU view

Actions

Plate ID: **CD033242** Set Open Processing Plan

Table

Pin Storage\*

AD078A Cdl4 Pos:2

Pucks

Puck Types

SC Rescan

Match Gripper

Defreeze Gripper

**13** Cycles before defreeze  
+ **5** (Grace harvestings)

Empty Puck

Harvesting Parameters **Advanced** Maintenance

Continue Redo Aspiration

Harvest

Pin: 600x300.svg Orientation: 145.803°

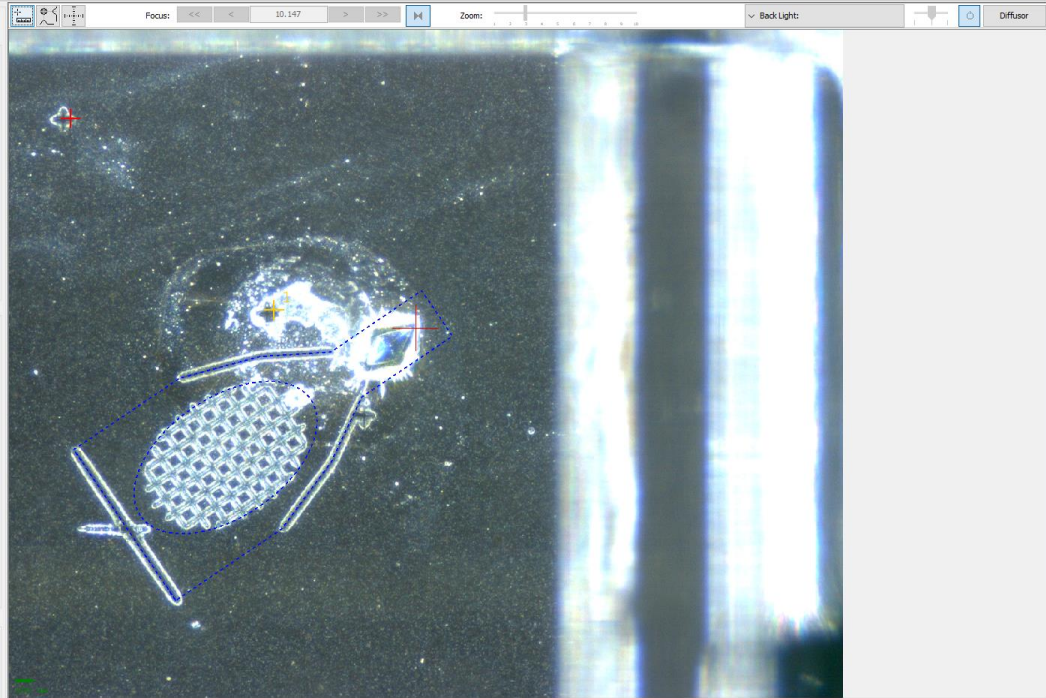
Gluing Delay: 0.2 s  Force Film Focusing  Cut shape side cuts in advance

Cryo Protection  Auto-pause after Cryo Protection

Depressurization: Cross.lms **Cryo Protection(s):** Cross.lms Number of aspiration s: 1

Drop Aspirations: Count: 4 Time: 0.7 s

Unload Sample



Harvest Results Logs

Day	Time	Position	Plate Id	Crystal Id	Success	Pin Barc...	Puck Ba...	Pin Posi...	Aspirat...	No Xtals	Ice?	Temp...	Message
27/01/2022	15:03:21	F06-1	CD033242	5739e7...	true	null	AD057A	14	Auto-4x7...	1	false	20.01	
27/01/2022	15:03:30	F06-1	CD033242	5739e7...	true	null	AD057A	15	Auto-4x7...	1	false	20.01	
27/01/2022	15:03:32	F06-1	CD033242	g9a6692...	true	null	AD057A	16	No aspir...	1	false	20.01	
27/01/2022	15:07:36	E06-3	CD033242	46116a...	true	null	AD081A	31	Auto-4x7...	1	false	20.03	
27/01/2022	15:09:30	D06-1	CD033242	af5fab80...	true	null	AD081A	2	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:11:36	C06-1	CD033242	caa06f68...	true	null	AD081A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:13:40	B06-1	CD033242	e52b622a...	true	null	AD081A	4	Auto-4x7...	1	false	19.98	
27/01/2022	15:16:00	A06-1	CD033242	532b922...	false	null	AD081A	11	Auto-4x7...	1	false	20.0	auto-detect no harvesting shape
27/01/2022	15:17:46	A07-1	CD033242	0e3f314f...	true	null	AD081A	5	Auto-4x7...	1	false	20.0	
27/01/2022	15:20:43	B07-1	CD033242	82f56ea...	true	null	AD081A	6	Auto-4x7...	1	false	20.0	
27/01/2022	15:22:36	C07-1	CD033242	26578e...	true	null	AD081A	7	Auto-4x7...	1	false	20.0	
27/01/2022	15:24:25	D07-1	CD033242	54f7604...	true	null	AD081A	8	Auto-4x7...	1	false	20.0	
27/01/2022	15:28:55	E07-1	CD033242	0375834...	true	null	AD081A	9	Auto-4x7...	1	false	20.0	
27/01/2022	15:31:44	F07-1	CD033242	142330b...	true	null	AD081A	10	Auto-4x7...	1	false	19.98	
27/01/2022	15:33:53	G07-1	CD033242	680387d...	true	null	AD081A	11	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:36:38	H08-3	CD033242	54f7604...	true	null	AD081A	12	Auto-4x7...	1	false	20.0	
27/01/2022	15:38:41	I08-1	CD033242	f0980e9...	true	null	AD081A	13	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:40:29	F08-3	CD033242	ff4346f9...	true	null	AD081A	14	Auto-4x7...	1	false	19.97	
27/01/2022	15:42:32	E08-1	CD033242	a87199a...	true	null	AD081A	15	Auto-4x7...	1	false	20.0	
27/01/2022	15:44:05	D08-1	CD033242	4b6573b...	true	null	AD081A	16	Auto-4x7...	1	false	19.98	
27/01/2022	15:46:14	C08-1	CD033242	885c422...	true	null	AD081A	15	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:48:32	B08-1	CD033242	d8014bc...	true	null	AD021A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:52:42	A08-1	CD033242	f67d37d...	true	null	AD021A	4	Auto-4x7...	1	false	20.0	

Monitoring Hardware Status

Laser: Ready

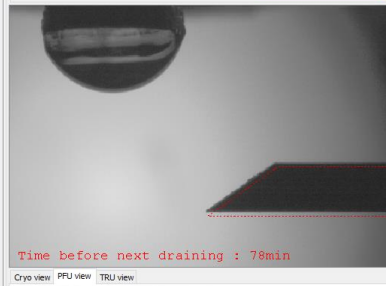
Smart Magnet Detection: Pin mounted

Remaining Pins: **26 ( Rack : 22 - SC : 4 )** View

Temperature Control

20.00 °C

Target: 20.00 °C



Cryo View PPU View TRU View

Park All Abort



Actions

Plate ID: **CD033242** Set Open Processing Plan

Table

Pin Storage\*

AD078A Cdl:4 Pos:2

Pucks

Puck Types

SC Rescan

Match Gripper

Defreeze Gripper

13 Cycles before defreeze + 5 (Grace harvestings)

Empty Puck

Harvesting Parameters Advanced Maintenance

Continue Redo Aspiration

Harvest

Pin: 600x300.svg Orientation: 145.803°

Gating Delay: 0.2 s Force Film Focusing Cut shape side cuts in advance

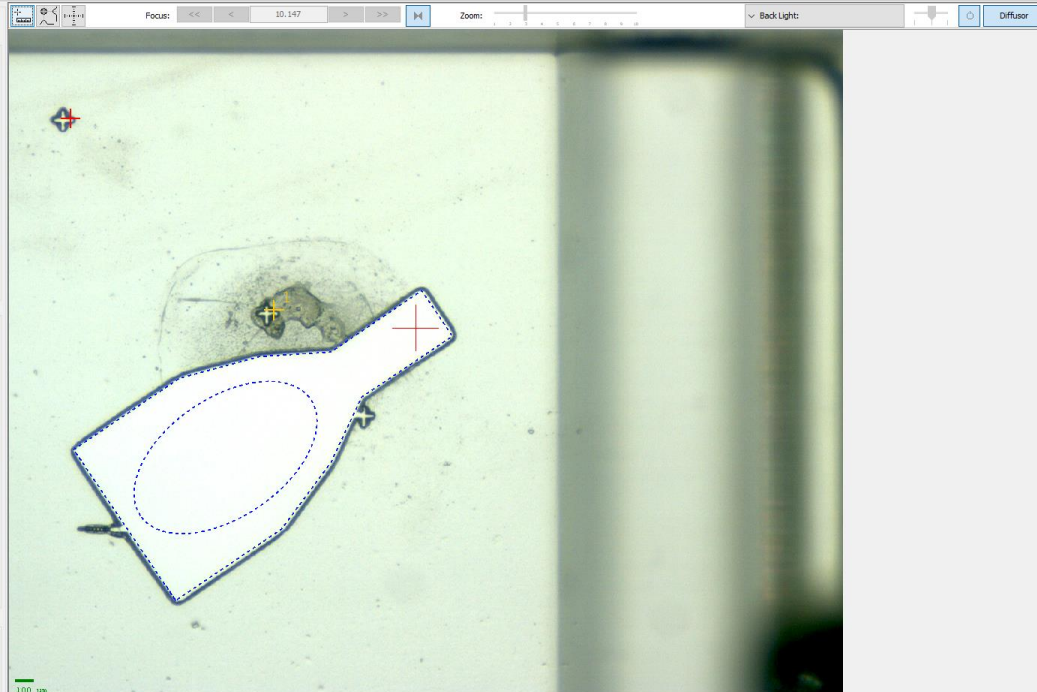
Cryo Protection  Auto-pause after Cryo Protection

Depressurization: Cryo Protection(s): Number of aspiration(s):

Cross.lms Cross.lms 1

Drop Aspiration: Count: 4 Time: 0.7 s

Unload Sample



Harvest Results Logs

Day	Time	Position	Plate Id	Crystal Id	Success	Pin Barc...	Puck Ba...	Pin Posi...	Aspirat...	No Xtals	Ice?	Temper...	Message
27/01/2022	15:03:50	F06-1	CD033242	c5739e7...	true	null	AD057A	15	Auto-4x7...	1	false	20.01	
27/01/2022	15:05:12	F06-1	CD033242	9a96682...	true	null	AD057A	16	No aspir...	1	false	20.01	
27/01/2022	15:07:26	E06-3	CD033242	461f48a...	true	null	AD081A	1	Auto-4x7...	1	false	20.03	
27/01/2022	15:09:30	D06-1	CD033242	af5fab80...	true	null	AD081A	2	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:11:36	C06-1	CD033242	caa06f68...	true	null	AD081A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:13:40	B06-1	CD033242	ea52622a...	true	null	AD081A	4	Auto-4x7...	1	false	19.98	
27/01/2022	15:16:00	A06-1	CD033242	5339c22...	false	null	AD081A	1	Auto-4x7...	1	false	20.0	auto-detect no harvesting shape
27/01/2022	15:17:46	A07-1	CD033242	0e3f314f...	true	null	AD081A	5	Auto-4x7...	1	false	20.0	
27/01/2022	15:20:43	B07-1	CD033242	82f56ea...	true	null	AD081A	6	Auto-4x7...	1	false	20.0	
27/01/2022	15:22:36	C07-1	CD033242	26578ec...	true	null	AD081A	7	Auto-4x7...	1	false	20.0	
27/01/2022	15:24:25	D07-1	CD033242	5476504...	true	null	AD081A	8	Auto-4x7...	1	false	20.0	
27/01/2022	15:28:55	E07-1	CD033242	0375834...	true	null	AD081A	9	Auto-4x7...	1	false	20.0	
27/01/2022	15:31:44	F07-1	CD033242	142330b...	true	null	AD081A	10	Auto-4x7...	1	false	19.98	
27/01/2022	15:33:53	G07-1	CD033242	6803876...	true	null	AD081A	11	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:36:38	H08-3	CD033242	547f6e6...	true	null	AD081A	12	Auto-4x7...	1	false	20.0	
27/01/2022	15:38:41	G08-1	CD033242	0f980e9...	true	null	AD081A	13	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:40:29	F08-3	CD033242	ff4346f9...	true	null	AD081A	14	Auto-4x7...	1	false	19.97	
27/01/2022	15:42:32	E08-1	CD033242	a87199a...	true	null	AD081A	15	Auto-4x7...	1	false	20.0	
27/01/2022	15:44:05	D08-1	CD033242	4b6f738...	true	null	AD081A	16	Auto-4x7...	1	false	19.98	
27/01/2022	15:46:14	C08-1	CD033242	885c422...	true	null	AD081A	15	Auto-4x7...	1	false	19.9900...	
27/01/2022	15:48:32	B08-1	CD033242	d8014bc...	true	null	AD021A	3	Auto-4x7...	1	false	20.01	
27/01/2022	15:52:42	A08-1	CD033242	f67ad376...	true	null	AD021A	4	Auto-4x7...	1	false	20.0	

Monitoring Hardware Status

Laser: Ready

Smart Magnet Detection Pin mounted

Remaining Pins 26 ( Rack : 22 - SC : 4 ) View

Temperature Control

20.00 °C

Target : 20.00 °C

13 Cycles before defreeze + 5 (Grace harvestings)

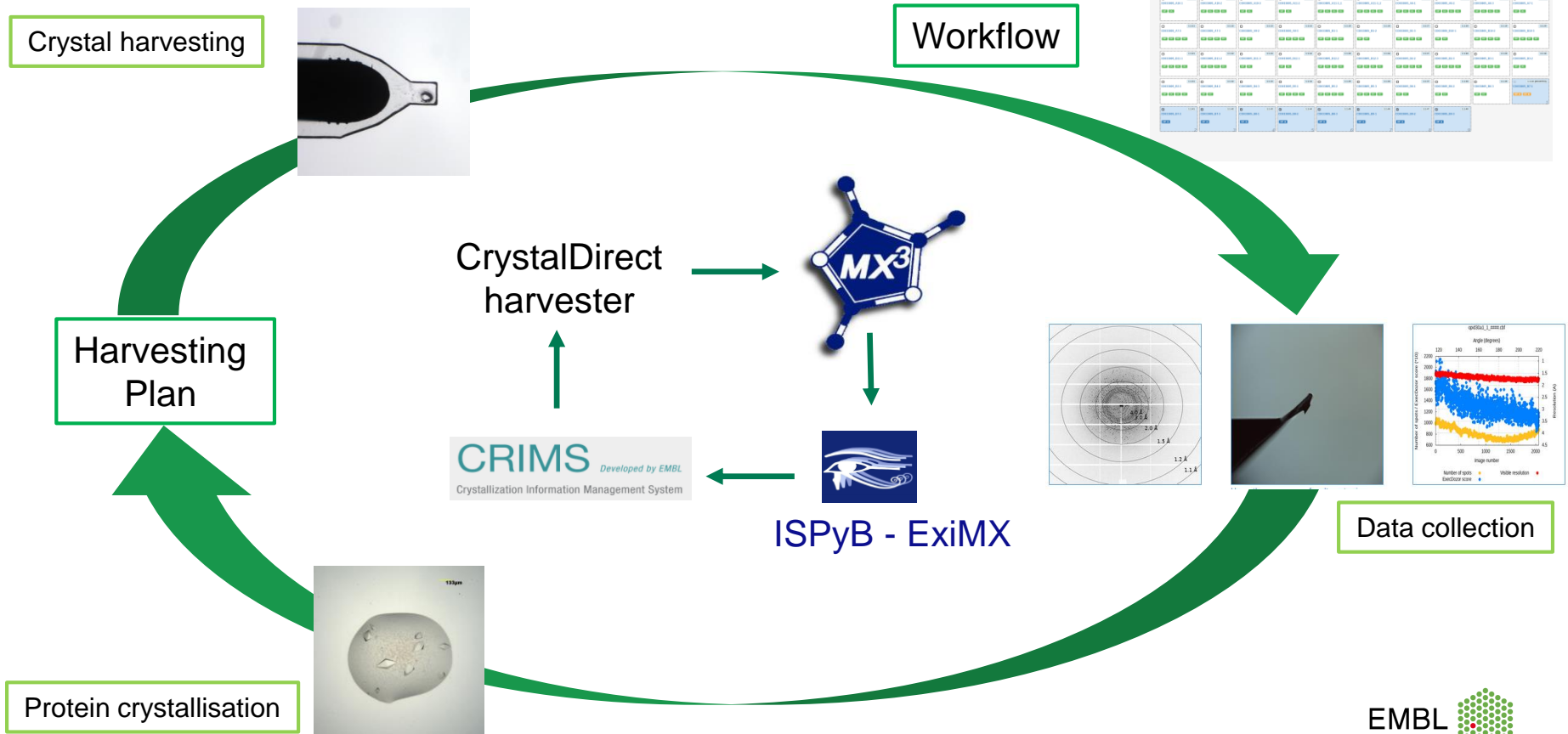
Time before next draining : 82min

Cryo view PFLU view TRU view

ParK All Abort

# Automated crystal harvesting and data collection pipeline

Software integration



# Current status

Validation of different data collection modalities

## Data collection at cryogenic temperature

- Samples harvested and collected in automated mode without user intervention
- Systematic analysis of crystallization conditions
- MXPressR: small fast mesh + EDNA

## Dehydration screening

- Improve crystal diffraction quality
- Queue system to shorten the experimental set up
- MXPressR\_dehydration: batch configurable parameter

## Data collection at room temperature

- Protein dynamic, ligand binding site interactions, protein allosteric sites
- MXPressR\_180: small fast mesh with batch configurable dose adapted to beam size

# Current status

Validation of different data collection modalities

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# Conclusion

The integration of CrystalDirect harvester at MASSIF-1 will expand the beamline automated services. The beamline upgrade will open to new experimental possibilities allowing the development of new automated and target-based data collection modalities.

## Plate-to-beam Applications

- ✓ Increased efficiency for fragment screening and high throughput applications
  - ✓ Automated dehydration experiments
    - ✓ Systematic studies at RT
    - ✓ Time course experiments
- ✓ Systematic analysis of crystallization conditions

[rocchio@embl.fr](mailto:rocchio@embl.fr)





# Conclusion

The integration of CrystalDirect harvester at MASSIF-1 will expand the beamline automated services. The beamline upgrade will open to new experimental possibilities allowing the development of new automated and target-based data collection modalities.

## Future Plans

User acces: mid 2022

- High throughput large scale RT data collection series
  - Workflow optimization (multiple target systems)
- RT serial crystallography
- In situ screening and data collection

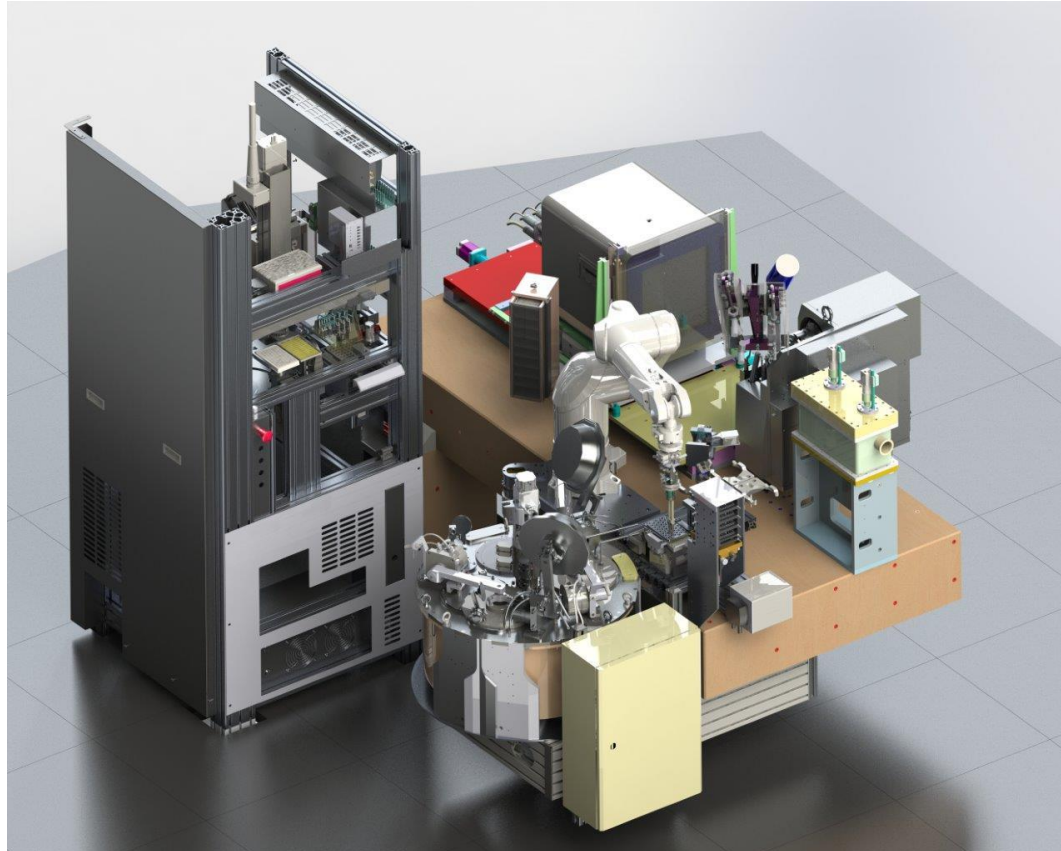
[rocchio@embl.fr](mailto:rocchio@embl.fr)



# Future development

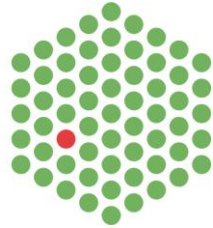
Fully automation of the entire process: plate holder and pin station

Instrumentation  
Team



# Acknowledgements

EMBL



Andrew McCarthy  
**Matthew Bowler**  
Jean Baptiste Florial  
Nicolas Foos

José Antonio Márquez  
Florine Dupeux  
Anne-Sophie Humm  
Peter Murphy



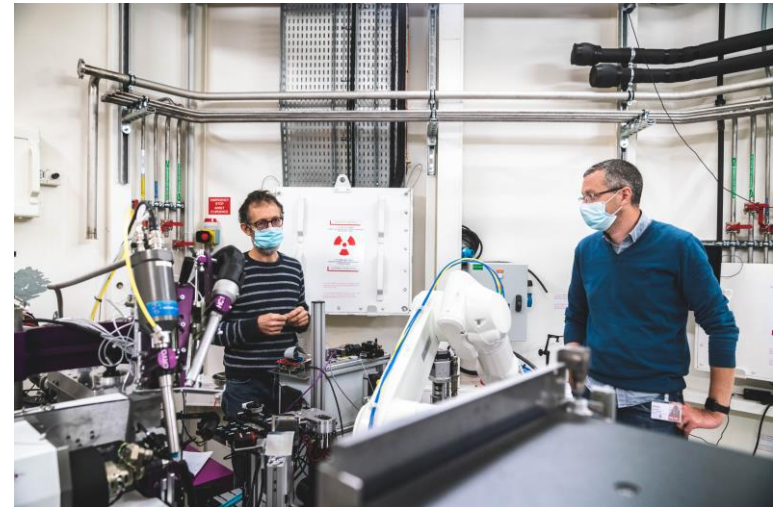
## **Instrumentation Team**

Gergely Papp  
Jeremy Sinoir  
Marcos Lopez Marrero  
Frank Felisaz



Gordon Leonard  
SB Group

Didier Nurizzo  
Olof Svensson



# Poster Session

8 Feb, 2022

13:15 – 13:45



McCarthy/Marquez Teams



Serena Rocchio

## Fully autonomous end-to-end protein to structure pipelines: the CrystalDirect harvester at MASSIF-1

S. Rocchio<sup>1</sup>, J. Sinoir<sup>1</sup>, J.B. Florial<sup>1</sup>, M. Lopez-Merraro<sup>1</sup>, F. Felisa<sup>1</sup>, P. Murphy<sup>1</sup>, F. Dupex<sup>1</sup>, AS. Humm<sup>1</sup>, D. Nurizzo<sup>2</sup>, O. Svesson<sup>2</sup>, N. Fooks<sup>1</sup>, GA. Leonard<sup>2</sup>, G. Papp<sup>1</sup>, AA. McCarthy<sup>1</sup>, JA. Marquez<sup>1</sup>, MW. Bowler<sup>1</sup>.

<sup>1</sup>European Molecular Biology Laboratory (EMBL) - Grenoble, <sup>2</sup>European Synchrotron Radiation Facility (ESRF).

Automation is changing the way in which crystallographic data are collected, allowing large amounts of high-quality data to be collected efficiently and paving the way to the development of more specific methods [1,2]. Here, the possibility to explore different data collection modalities is important to keep up with modern structural biology projects. The CrystalDirect harvester allows access to a fully automated protein crystallography workflow, integrating protein crystallization, sample harvesting and cryocooling into an automated process [3,4,5]. The combination of the CrystalDirect harvester with the automation on MASSIF-1 allows the development of different data collections modalities, aimed to respond to multiple technical and experimental challenges [5,6,7]. To date, room temperature (RT) data collection and dehydration experiments require a large number of manual steps and the set-up of the experimental conditions is time-consuming; the automation of this process will facilitate their access to non-expert users [8,9,10,11]. The beamline upgrade will open new experimental opportunities and help respond to the dynamic change of scientific needs, such as the increased interest in structural data at physiological temperatures [10,11, 12]. Commissioning is currently ongoing with full user mode access foreseen in mid 2022.

13:45 – 14:15



McCarthy Synchrotron



Nicolas Fooks

## Performing *in-situ* data collection : service provision for the most complex experiments

N. Fooks<sup>1</sup>, M. Bowler<sup>1</sup>, D. Nurizzo<sup>2</sup>, S. Rocchio<sup>1</sup>, J. Sinoir<sup>1</sup>, J. B. Florial<sup>1</sup>, C. Mueller-Dieckmann<sup>2</sup>, G. A. Leonard<sup>2</sup>, J. Márquez<sup>1</sup>, G. Papp<sup>1</sup>, A. A. McCarthy<sup>1</sup>

<sup>1</sup>EMBL Grenoble Out-Station, <sup>2</sup>ESRF

Being fast, reliable and automated is what makes X-ray crystallography efficient, popular, successful and in constant improvement. The bright beam offered by the ESRF-EBS makes possible to collect excellent data from micro to nano-crystals using the latest developments in serial approaches. Our project aims to address the issue of high throughput in structural biology projects, while at the same time pushing forward more classical experiments thanks to the implementation of the EMBL Grenoble HTX platform which offers a robust solution for automated crystal harvesting. To achieve this we are creating the convergence between the final diffraction experiment and the original crystallization assay.

Our project takes advantage of the FLEX robot and its versatility to make the missing link between the HTX high throughput crystallization platform, the Crystal-Direct harvester and the MD2-S diffractometer on the beamline. In order to create a complete pipeline we are implementing an automated in-situ screening and data-collection service. To drive the experiment and make it user friendly, we are developing an efficient workflow integrating automated decision making to achieve a fully automated data collection beginning from crystal growing devices (plates as well as microfluidic chips).



Thank you

