MX automation:
few perspectives from ESRF

MXCUBE UI

Present	Future
Unique interface (user/BL scientist)	
Manual puck assignment	Auto puck assignment

SHIPMENT ASSIGNMENT

Shipment is always linked to a session with date

Immutable session date is part of the folders structure

89 shipments candidates for FX89

☐ **Display only shipments scheduled for future sessions or in processing status**

Shipment			Experiment		
Name	Status	Created on	Start on	beamline	
ESRF FX23 15...	PROCESSING	08-11-2024	15-11-2024	ID30A-1	1 parcels / 2 containers (25 samples)
ESRF FX23 8 ...	SENT TO USER	01-11-2024	08-11-2024	ID30A-1	1 parcels / 4 containers (62 samples)
ESRF FX23 25...	SENT TO USER	17-10-2024	25-10-2024	ID30A-1	1 parcels / 3 containers (38 samples)
ESRF FX23 4 ...	AT_ESRF	26-09-2024	04-10-2024	ID30A-1	2 parcels / 3 containers (16 samples)
ESRF FX23 13...	SENT TO USER	05-09-2024	13-09-2024	ID30A-1	1 parcels / 2 containers (20 samples)
ESRF FX23 6 ...	SENT TO USER	29-08-2024	06-09-2024	ID30A-1	1 parcels / 2 containers (32 samples)
ESRF FX23 30...	SENT TO USER	22-08-2024	30-08-2024	ID30A-1	1 parcels / 5 containers (64 samples)
ESRF FX23 26...	SENT TO USER	18-07-2024	26-07-2024	ID30A-1	1 parcels / 1 containers (6 samples)
ESRF FX23 28...	SENT TO USER	20-06-2024	28-06-2024	ID30A-1	1 parcels / 3 containers (69 samples)
ESRF FX23 21...	SENT TO USER	13-06-2024	21-06-2024	ID30A-1	1 parcels / 2 containers (32 samples)
ESRF FX23 14...	SENT TO USER	06-06-2024	14-06-2024	ID30A-1	1 parcels / 3 containers (39 samples)
ESRF FX23 7 ...	SENT TO USER	30-05-2024	07-06-2024	ID30A-1	1 parcels / 7 containers (112 samples)
ESRF FX23 12...	SENT TO USER	04-04-2024	12-04-2024	ID30A-1	1 parcels / 3 containers (33 samples)
ESRF FX23 5 ...	SENT TO USER	26-03-2024	06-04-2024	ID30A-1	1 parcels / 2 containers (18 samples)
ESRF FX23 28...	SENT TO USER	22-03-2024	29-03-2024	ID30A-1	1 parcels / 6 containers (81 samples)
ESRF FX23 4 ...	SENT TO USER	27-02-2024	08-03-2024	ID30A-1	1 parcels / 3 containers (48 samples)
ESRF FX23 16...	SENT TO USER	09-02-2024	16-02-2024	ID30A-1	1 parcels / 1 containers (12 samples)
ESRF FX23 5 ...	SENT TO USER	01-02-2024	09-02-2024	ID30A-1	1 parcels / 3 containers (48 samples)

PUCK ASSIGNMENT

89 shipments candidates for FX89

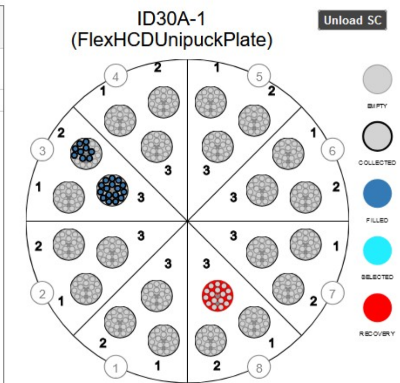
☐ **Display only shipments scheduled for future sessions or in processing status**

Shipment			Experiment		
Name	Status	Created on	Start on	beamline	
ESRF FX23 15...	PROCESSING	08-11-2024	15-11-2024	ID30A-1	1 parcels / 2 containers (25 samples)
ESRF FX23 8 ...	SENT TO USER	01-11-2024	08-11-2024	ID30A-1	
ESRF FX23 25...	SENT TO USER	17-10-2024	25-10-2024	ID30A-1	
ESRF FX23 4 ...	AT_ESRF	26-09-2024	04-10-2024	ID30A-1	
ESRF FX23 13...	SENT TO USER	05-09-2024	13-09-2024	ID30A-1	
ESRF FX23 6 ...	SENT TO USER	29-08-2024	06-09-2024	ID30A-1	
ESRF FX23 30...	SENT TO USER	22-08-2024	30-08-2024	ID30A-1	
ESRF FX23 26...	SENT TO USER	18-07-2024	26-07-2024	ID30A-1	
ESRF FX23 28...	SENT TO USER	20-06-2024	28-06-2024	ID30A-1	
ESRF FX23 21...	SENT TO USER	13-06-2024	21-06-2024	ID30A-1	
ESRF FX23 14...	SENT TO USER	06-06-2024	14-06-2024	ID30A-1	
ESRF FX23 7 ...	SENT TO USER	30-05-2024	07-06-2024	ID30A-1	
ESRF FX23 12...	SENT TO USER	04-04-2024	12-04-2024	ID30A-1	
ESRF FX23 5 ...	SENT TO USER	26-03-2024	06-04-2024	ID30A-1	
ESRF FX23 28...	SENT TO USER	22-03-2024	29-03-2024	ID30A-1	
ESRF FX23 4 ...	SENT TO USER	27-02-2024	08-03-2024	ID30A-1	
ESRF FX23 16...	SENT TO USER	09-02-2024	16-02-2024	ID30A-1	
ESRF FX23 5 ...	SENT TO USER	01-02-2024	09-02-2024	ID30A-1	

Loaded or to be Loaded on MxCube

Shipment	Parcel	Container	Container type	Beamline	Cell	Position
ESRF FX23 15 Nov 2024	dewer1	gsk1007 *(16 samples)*	UNIPUCK	ID30A-1	3	3
ESRF FX23 15 Nov 2024	dewer1	cps0801 *(9 samples)*	UNIPUCK	ID30A-1	3	2

Unload all

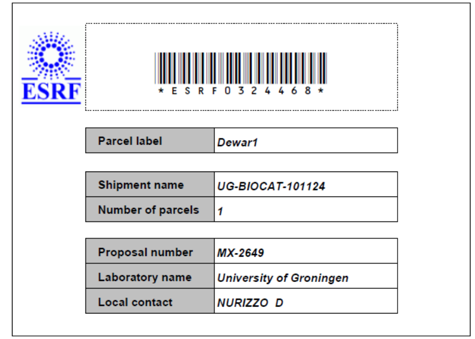


AUTOMATED SESSSION/PUCK ASSIGNMENT

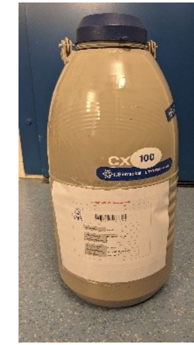
Barcode reader for the dewar

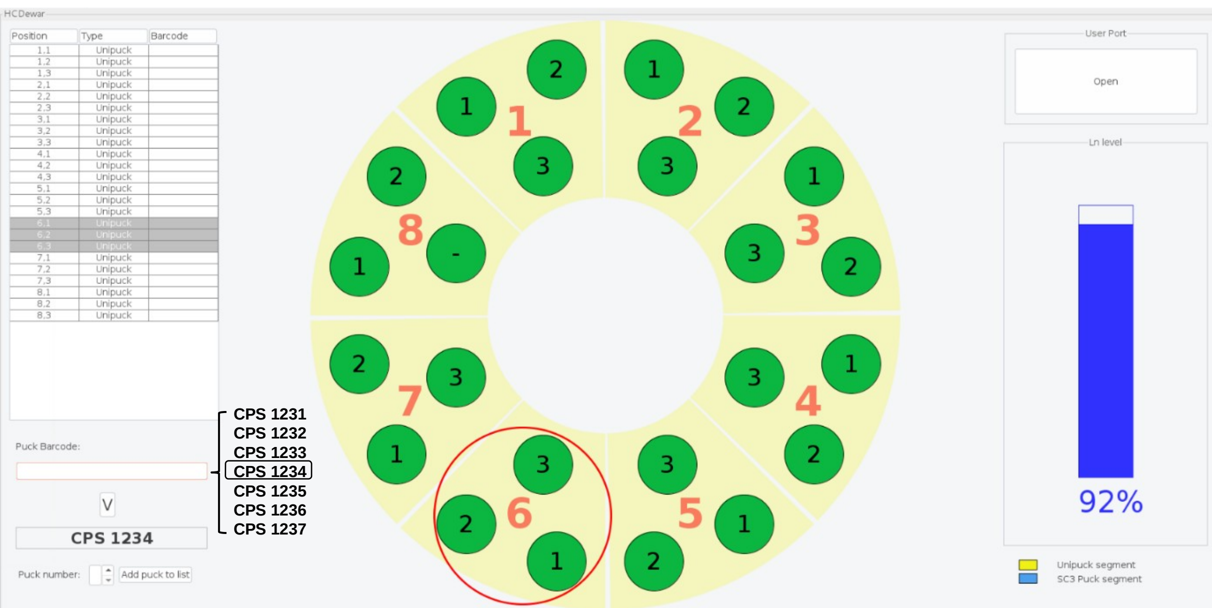
Connection between sample changer and LIMS

Puck detection



Parcel label	Dewar1
Shipment name	UG-BIOCAT-101124
Number of parcels	1
Proposal number	MX-2649
Laboratory name	University of Groningen
Local contact	NURIZZO D

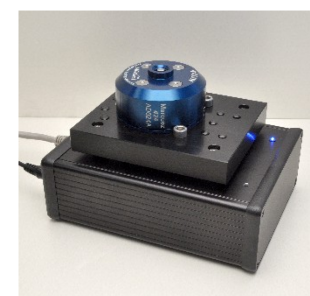




Barcode reader for the dewar

Puck detection

Connection between sample changer and DRAC

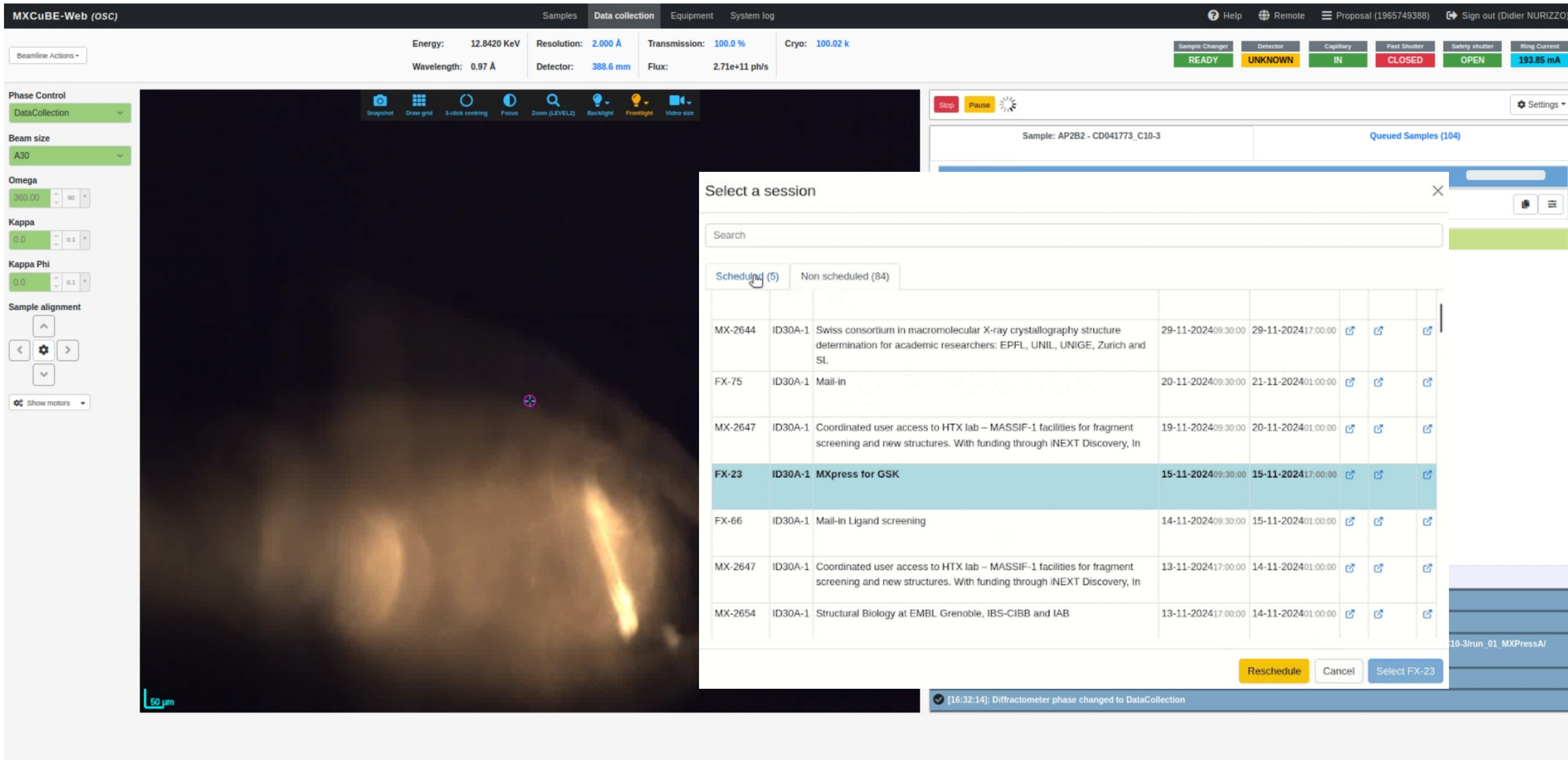


MXCUBE UI

Present	Future
Unique interface (user/BL scientist)	
Manual puck assignment	Auto puck assignment
Single user	Unique operator (SSO)

SINGLE SIGN-ON

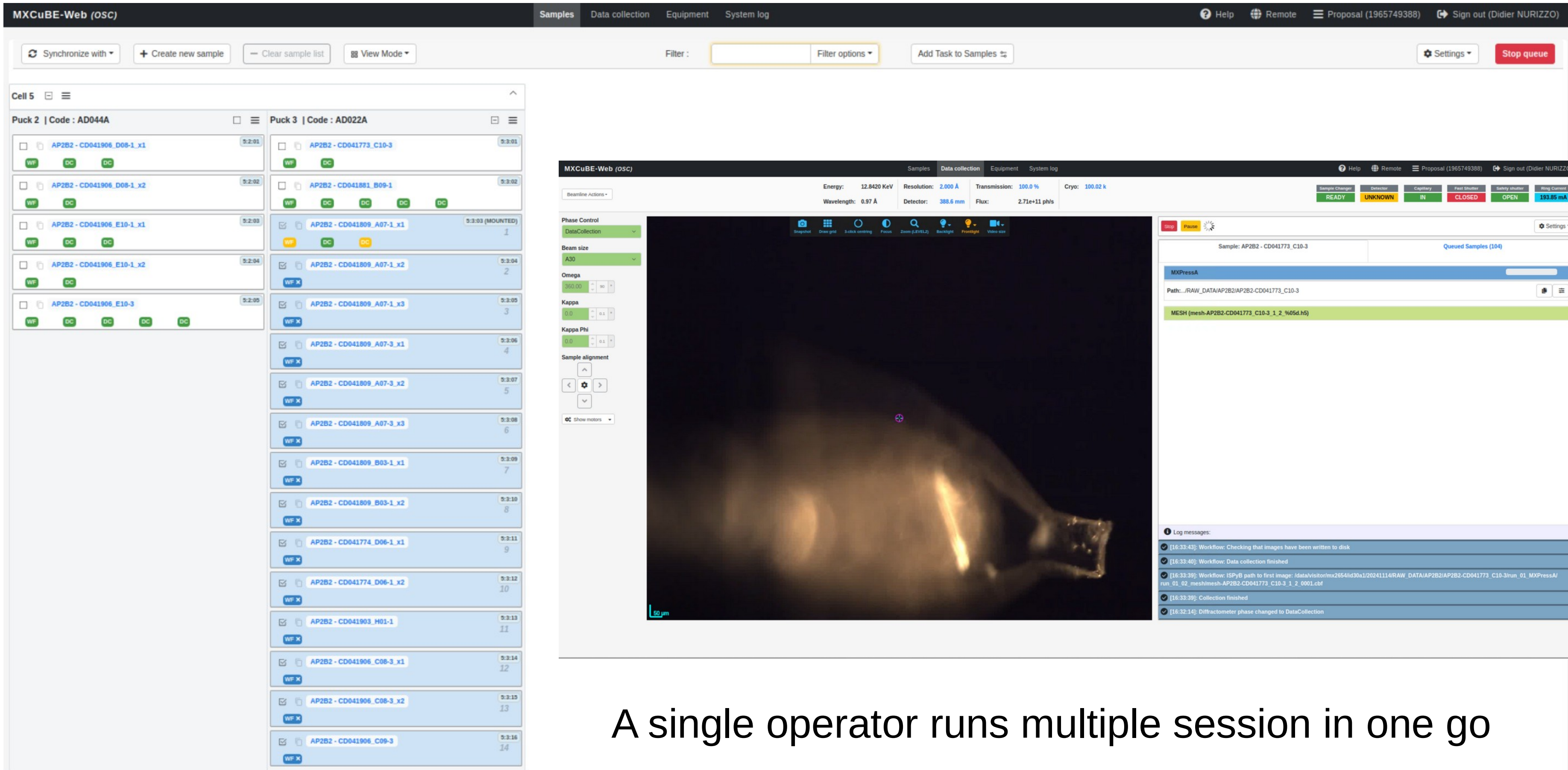
On-time and early/late session selection



MXCUBE UI

Present	Future
Unique interface (user/BL scientist)	
Manual puck assignment	Auto puck assignment
Single user	Unique operator (SSO)
Single session	Multiple sessions

MULTIPLE SESSIONS



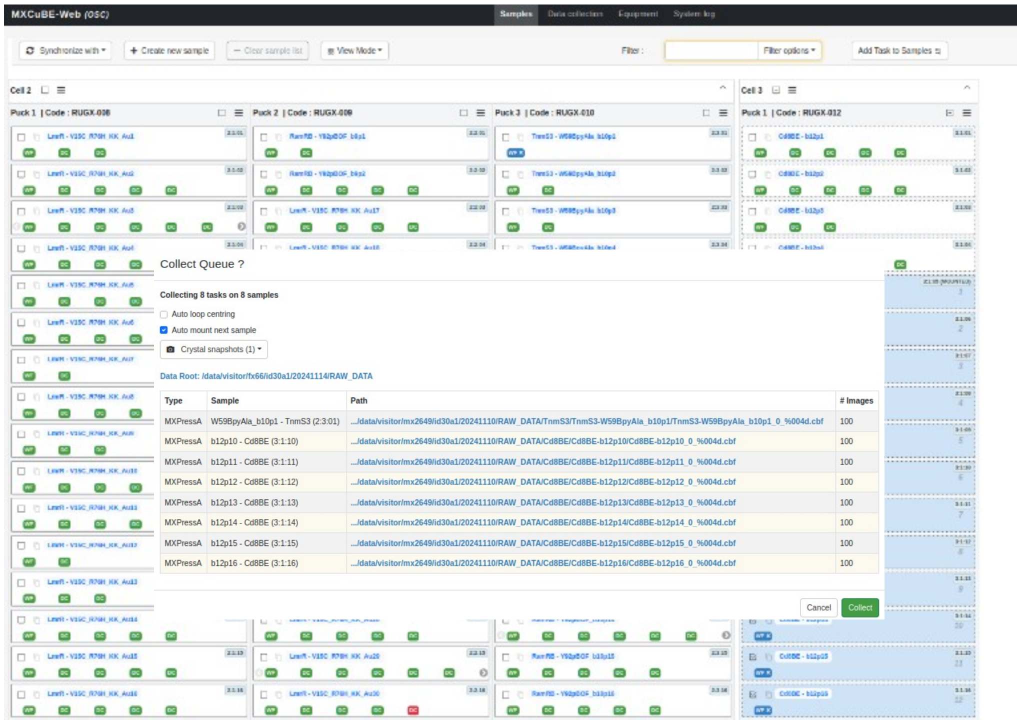
A single operator runs multiple session in one go

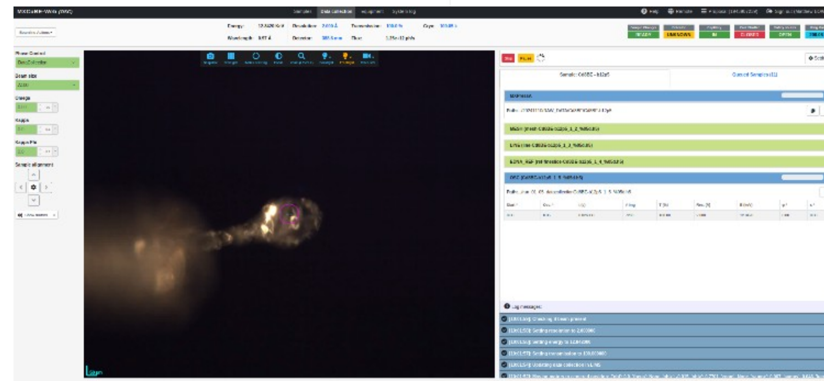
MX-CUBE UI

Present	Future
Unique interface (user/BL scientist)	
Manual puck assignment	Auto puck assignment
Single user	Unique operator (SSO)
Single session	Multiple sessions
Immutable queue	On-the-fly mutable (+/-) queue order

MX-CUBE QUEUING

Diffraction Plan including the processing plan in LIMS



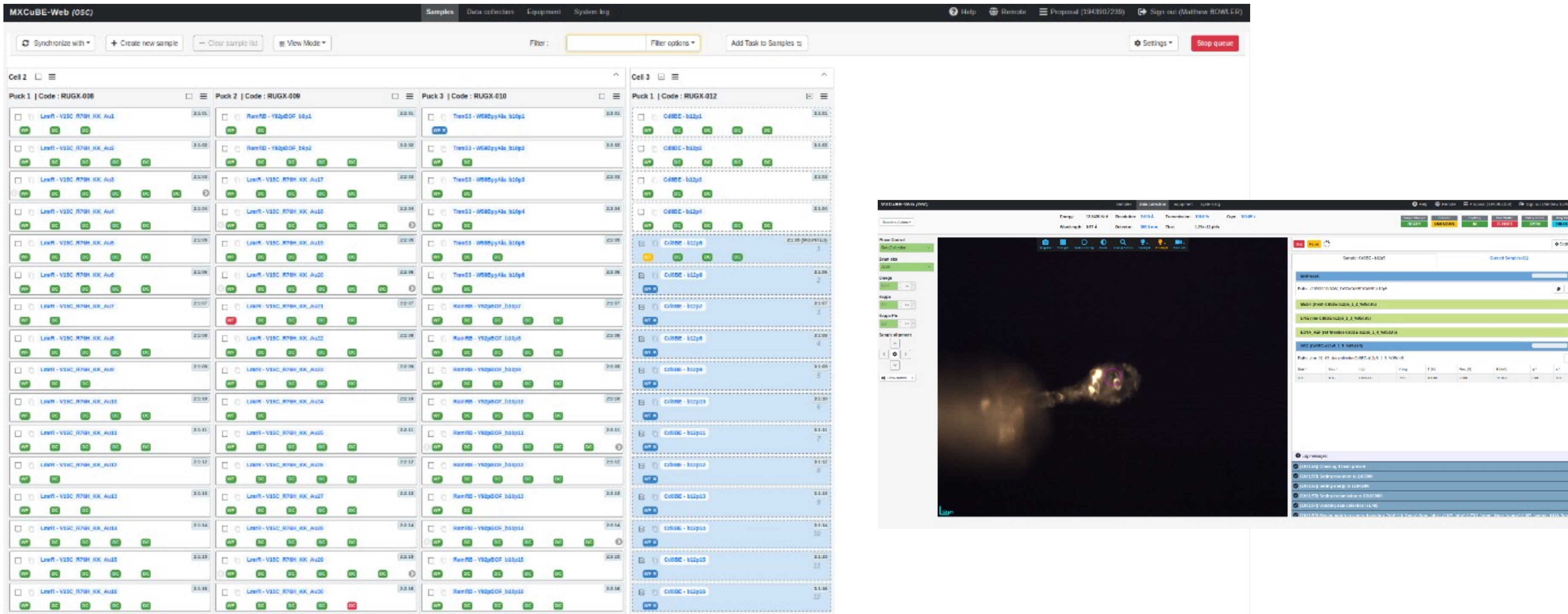


MX-CUBE UI

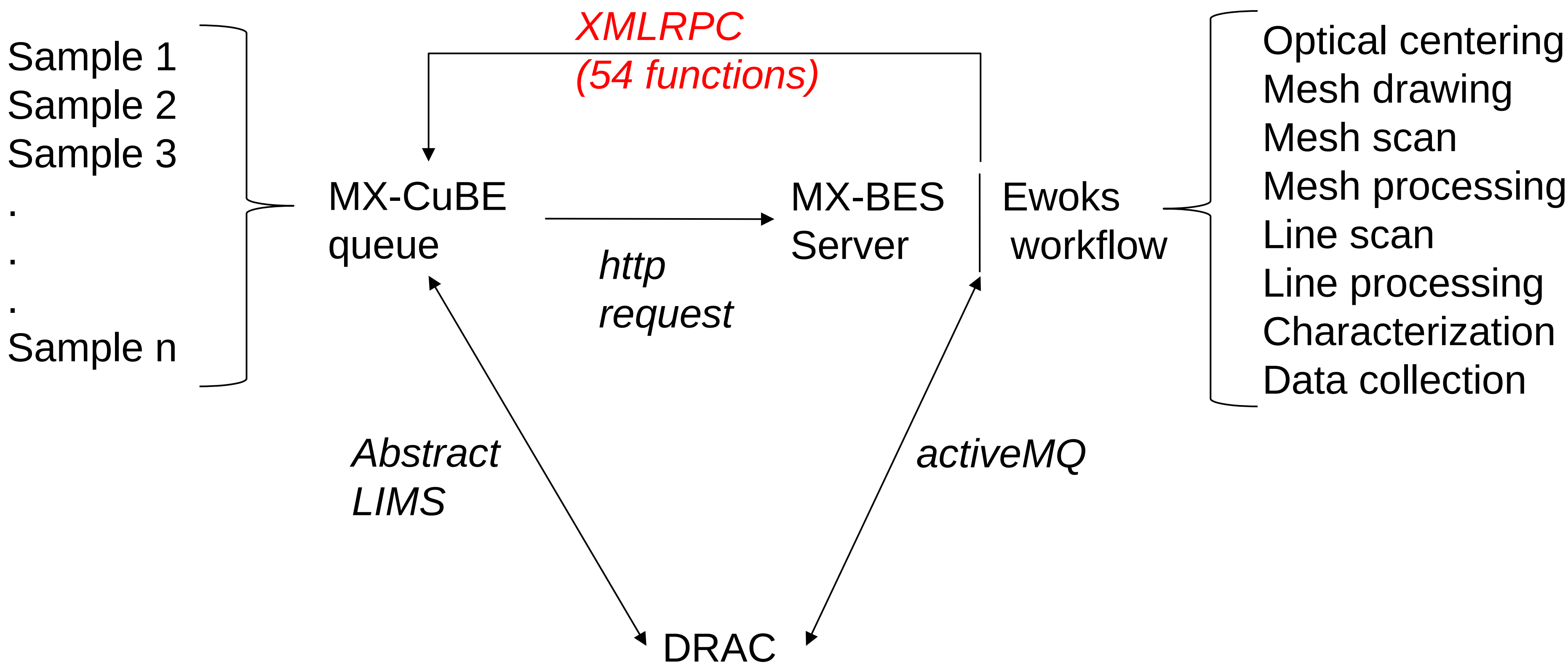
Present	Future
Unique interface (user/BL scientist)	
Manual puck assignment	Auto puck assignment
Single user	Unique operator (SSO)
Single session	Multiple sessions
Immutable queue	On-the-fly mutable (+/-) queue order
Per sample queuing list	Sample changer / Puck queuing overview

MX-CUBE QUEUING

Design a Sample Changer overview / Puck overview

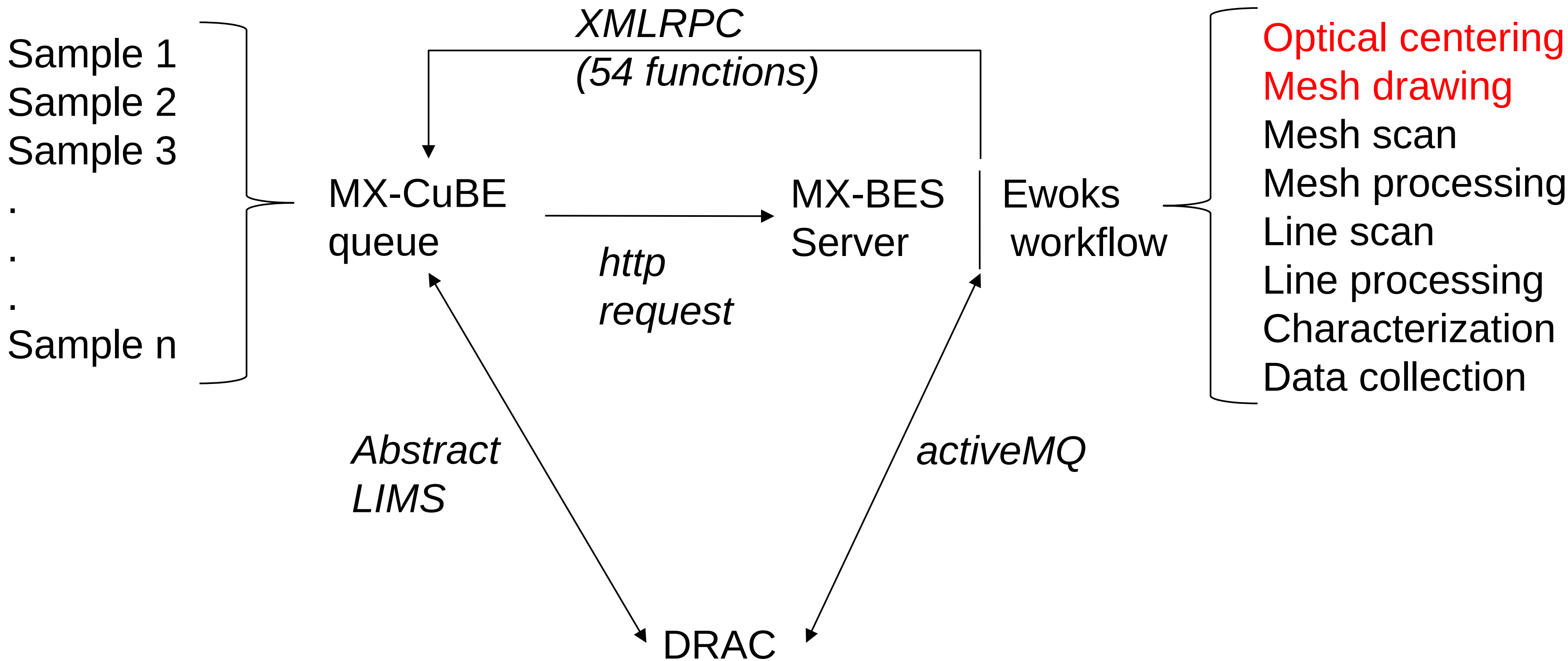


WORKFLOW / MXCUBE REFURBISHMENT



The European Synchrotron | ESRF

SOFTWARE REFURBISHMENT



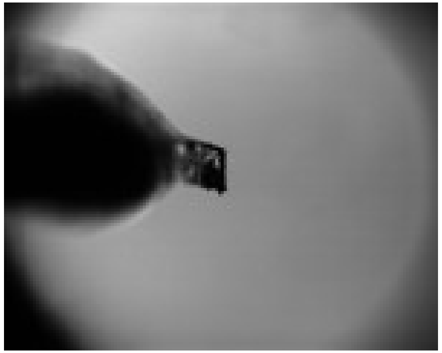
OPTICAL ANALYSIS

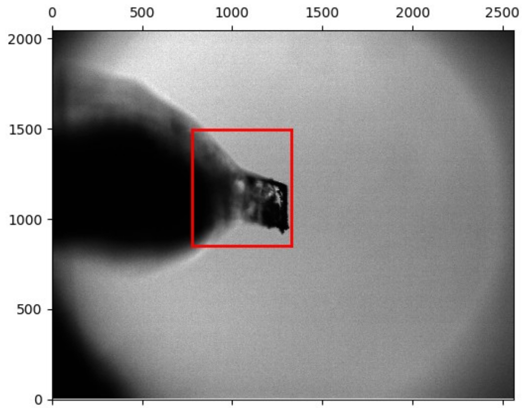
A set of optical images

optical centering

MURKO / LUCID

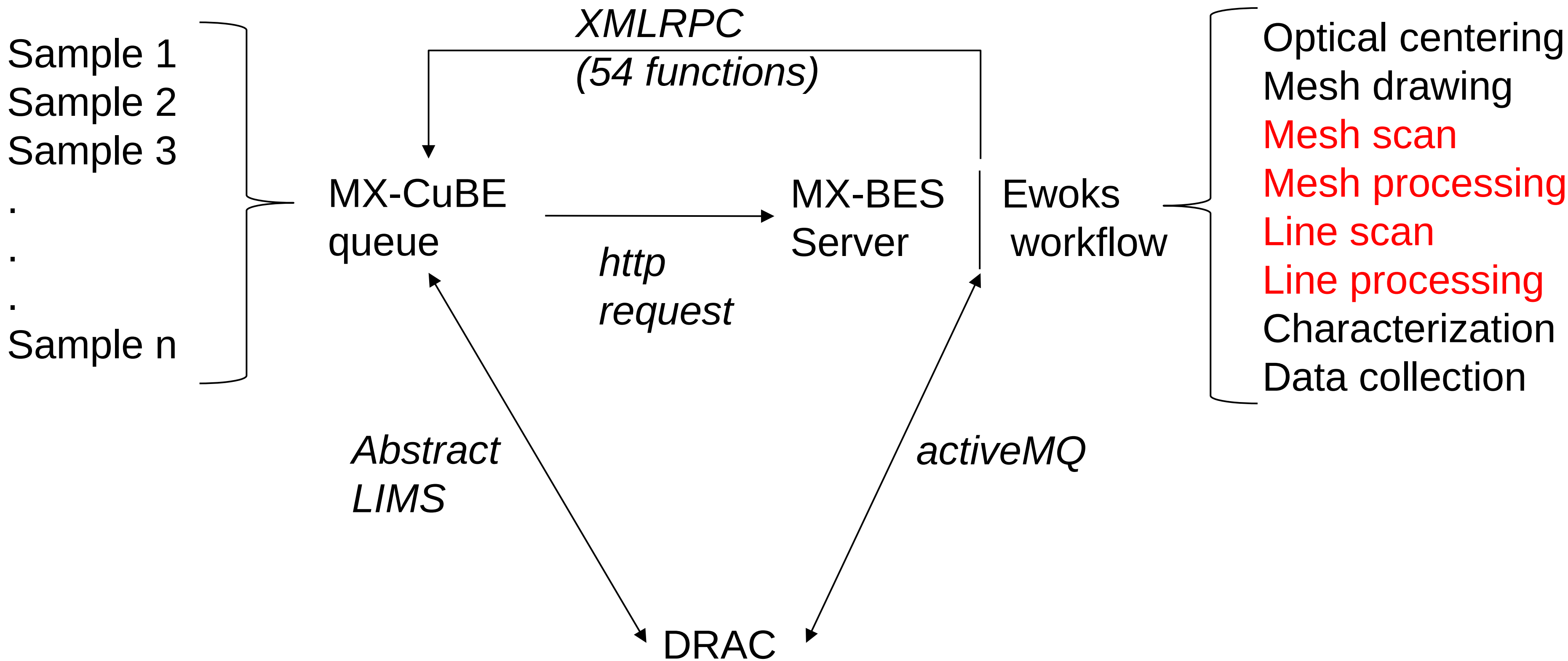
- Loop centering
- Crystal recognition
- Mesh drawing





Single object returning the optical features of the crystal inside the loop (center of mass, position of the crystal, size of the loop, etc…)

SOFTWARE REFURBISMENT

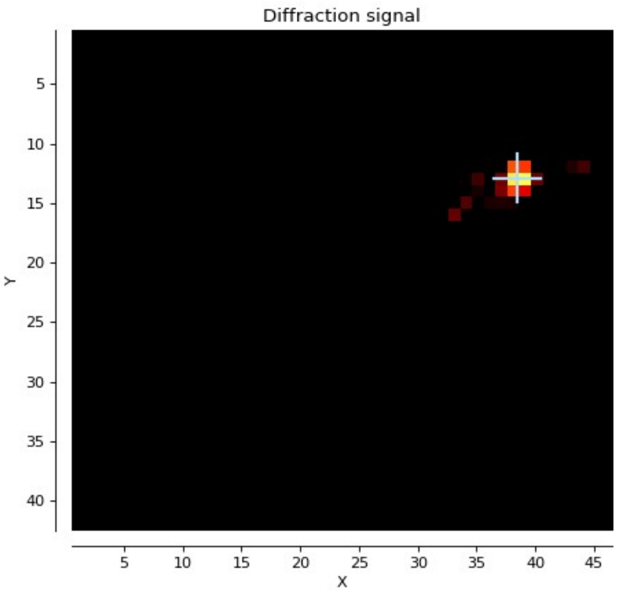


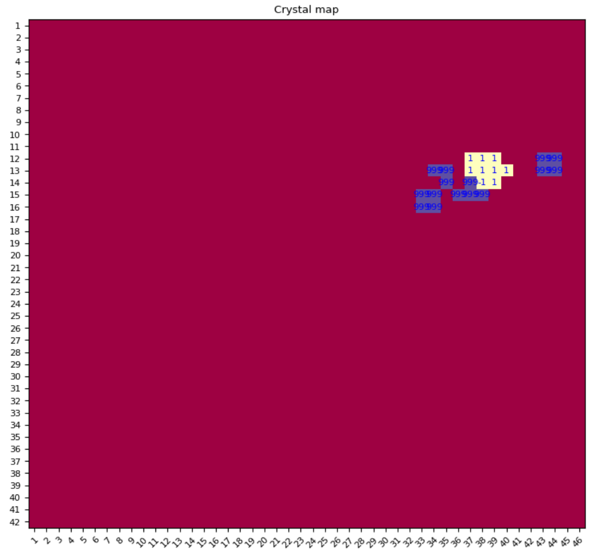
X-RAY CENTERING

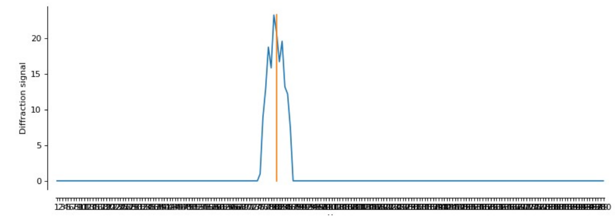
Diffraction alignment

A set of diffraction images

- Best diffraction centering
- Crystal size
- Crystal homogeneity
- Resolution limit







Single object returning the diffraction features of the crystal
(best diffracting volume and position, size of the crystal, , etc…)

ACKNOWLEDGEMENTS

Thoroughly discussed :
During our bi-weekly Automation Task Force meetings
and
In our MXCuBE automation WG:

Matthew Bowler
Antonia Beteva
Marcus Oskarsson
Estelle Mossou
Daniele de Sanctis
Andrew McCarthy

Johannes Kamps
Max Nanao
Ludovic Broche
Romain Talon
Wout de Nolf
Yan Walesh

And many more ….

Questions still under discussion:

- Common definition of automated/unattended DC
- Long term perspective for the DC model
- Granularity of the abstraction

Macromolecular Crystallography
And
Structural Biology at synchrotron

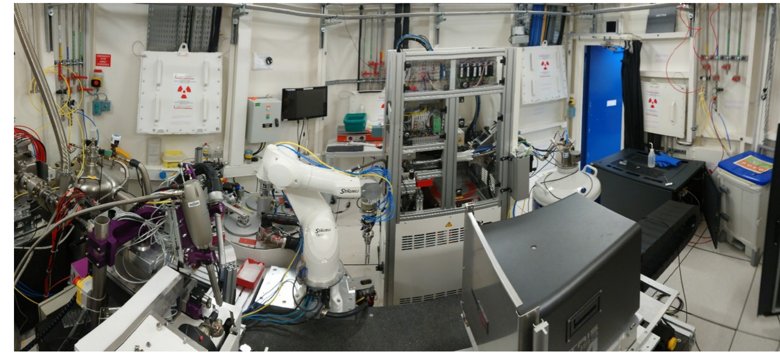
MASSIF-1 AUTOMATED DATA COLLECTION

Automation for Room Temperature experiments

- Crystal Direct Harvester

- In-Situ data collection

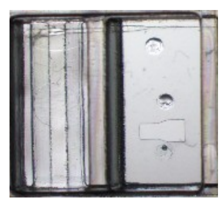
MASSIF-1 AUTOMATED DATA COLLECTION

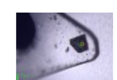


Automation for Room Temperature experiments

- Crystal Direct Harvester

- In-Situ data collection



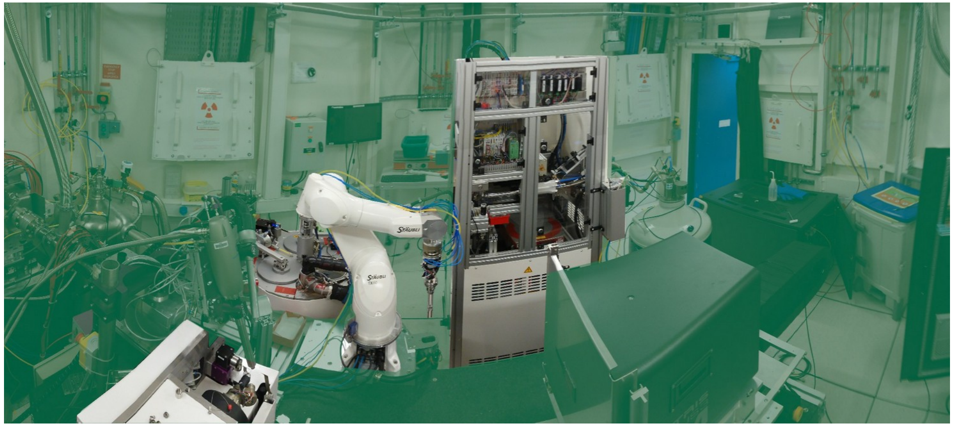


Crystal Direct technology

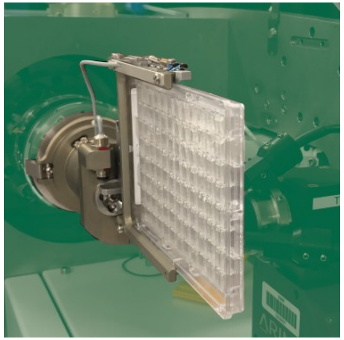
MASSIF-1 AUTOMATED DATA COLLECTION

Automation for Room Temperature experiments

- Crystal Direct Harvester



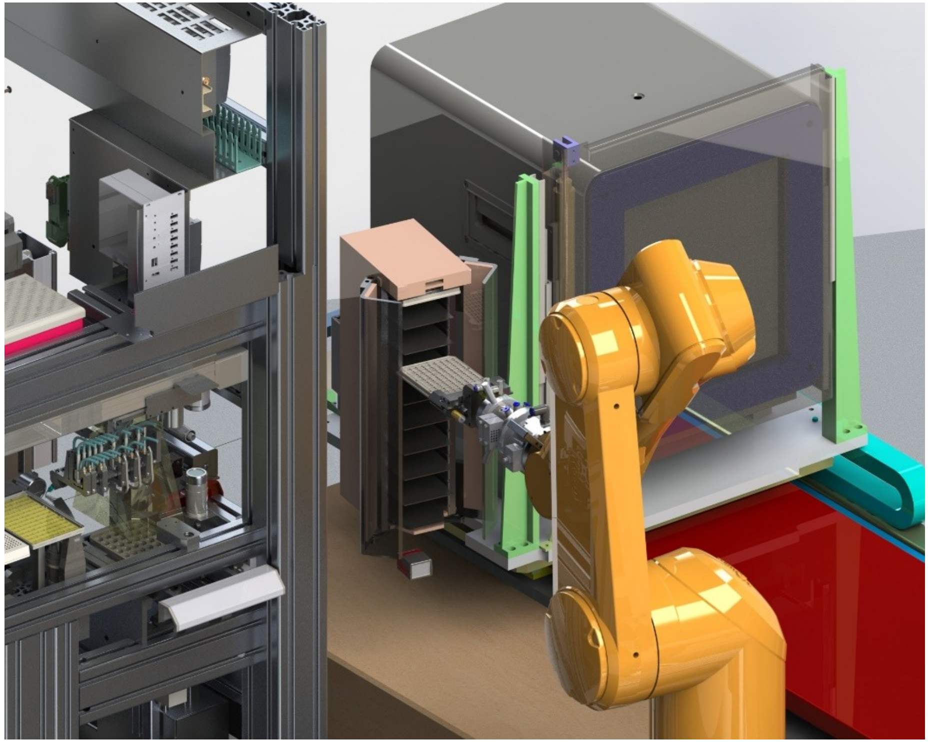
- In-Situ data collection



MASSIF-1 AUTOMATED DATA COLLECTION

Automation for Room Temperature experiments

- Crystal Direct Harvester



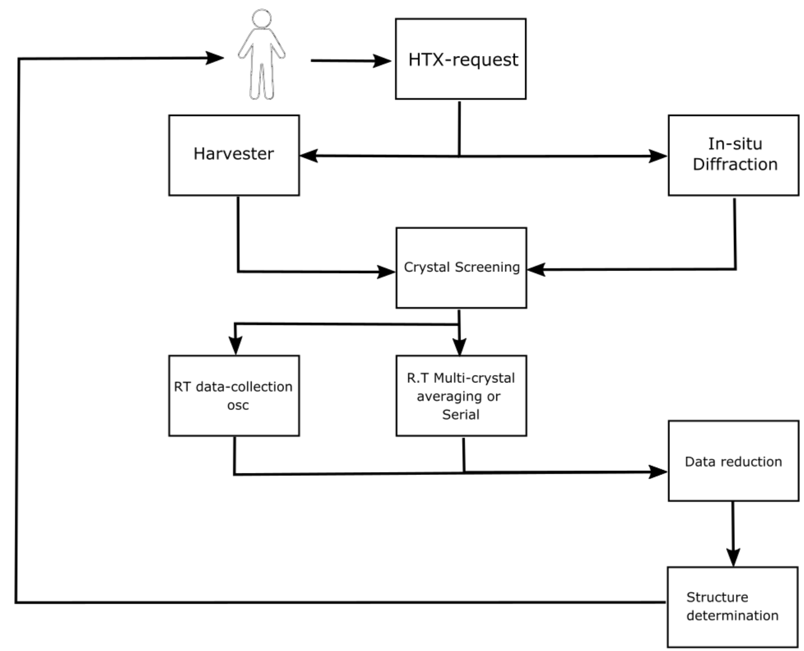
- In-Situ data collection

MASSIF-1 AUTOMATED DATA COLLECTION

Automation for Room Temperature experiments

- Crystal Direct Harvester

- In-Situ data collection



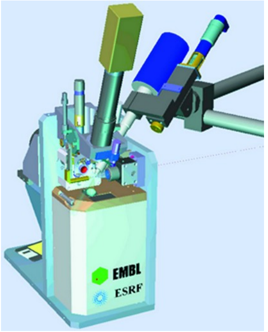
CURRENT FEATURES

Main:

Ω axis (SOC<1µm)
Centering table
Alignment table
Apertures
Capillary
Beamstop
smartMagnet
OAV camera
Scintillator
Fluo det translation

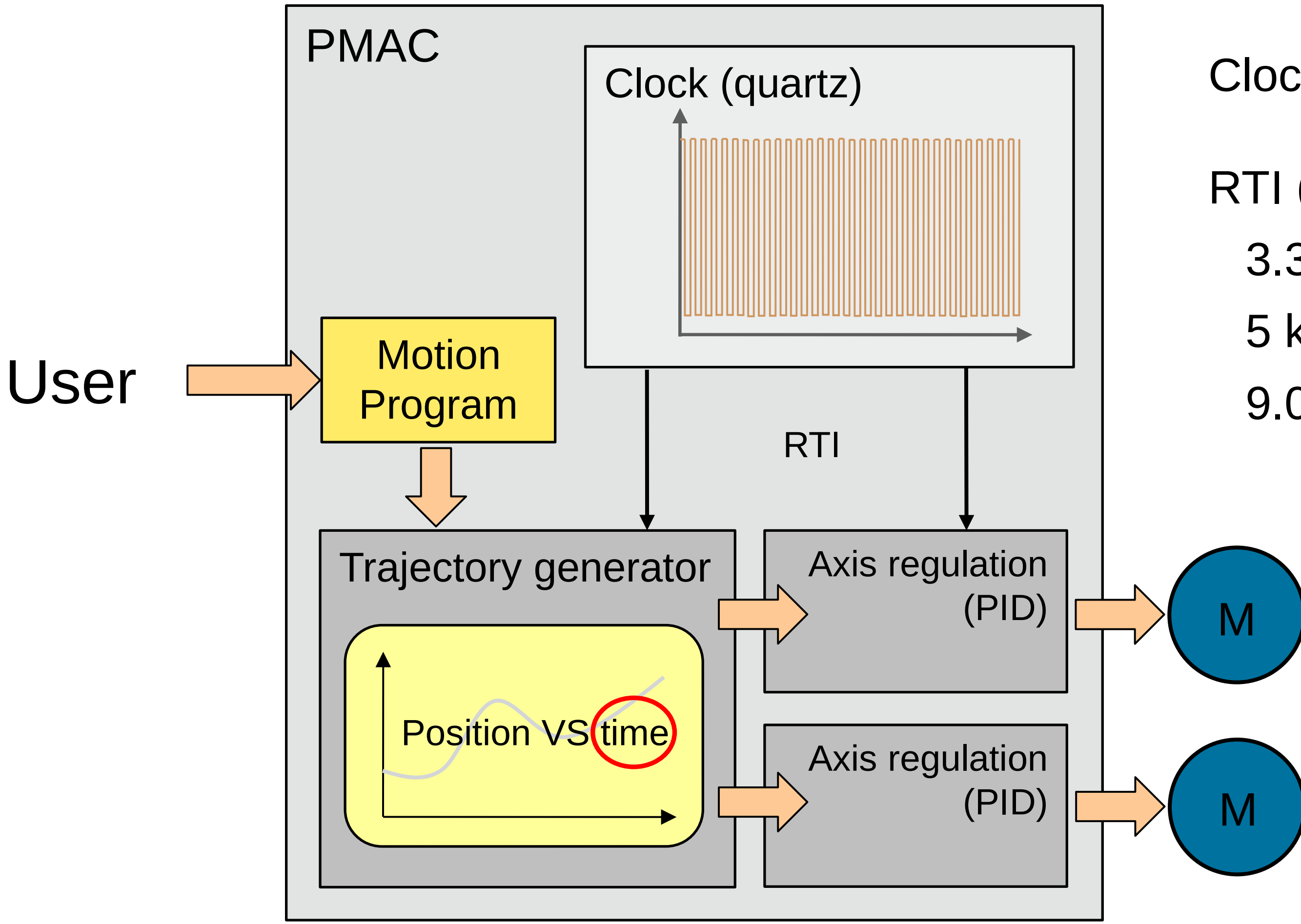
Ancillary:

Mini-Kappa
Plate manipulator
Fluo det translation
Luciole control
REX control
Shutter control
Flex communication



Protein microcrystals and the design of a microdiffractometer: current experience and plans at EMBL and ESRF/ID13
Perrakis, A., Cipriani, F., Castagna, J.-C., Claustre, L., Burghammer, M., Riekel, C. & Cusack, S. (1999). Acta Cryst. D55, 1765-1770.

PMAC – DELTA TAU

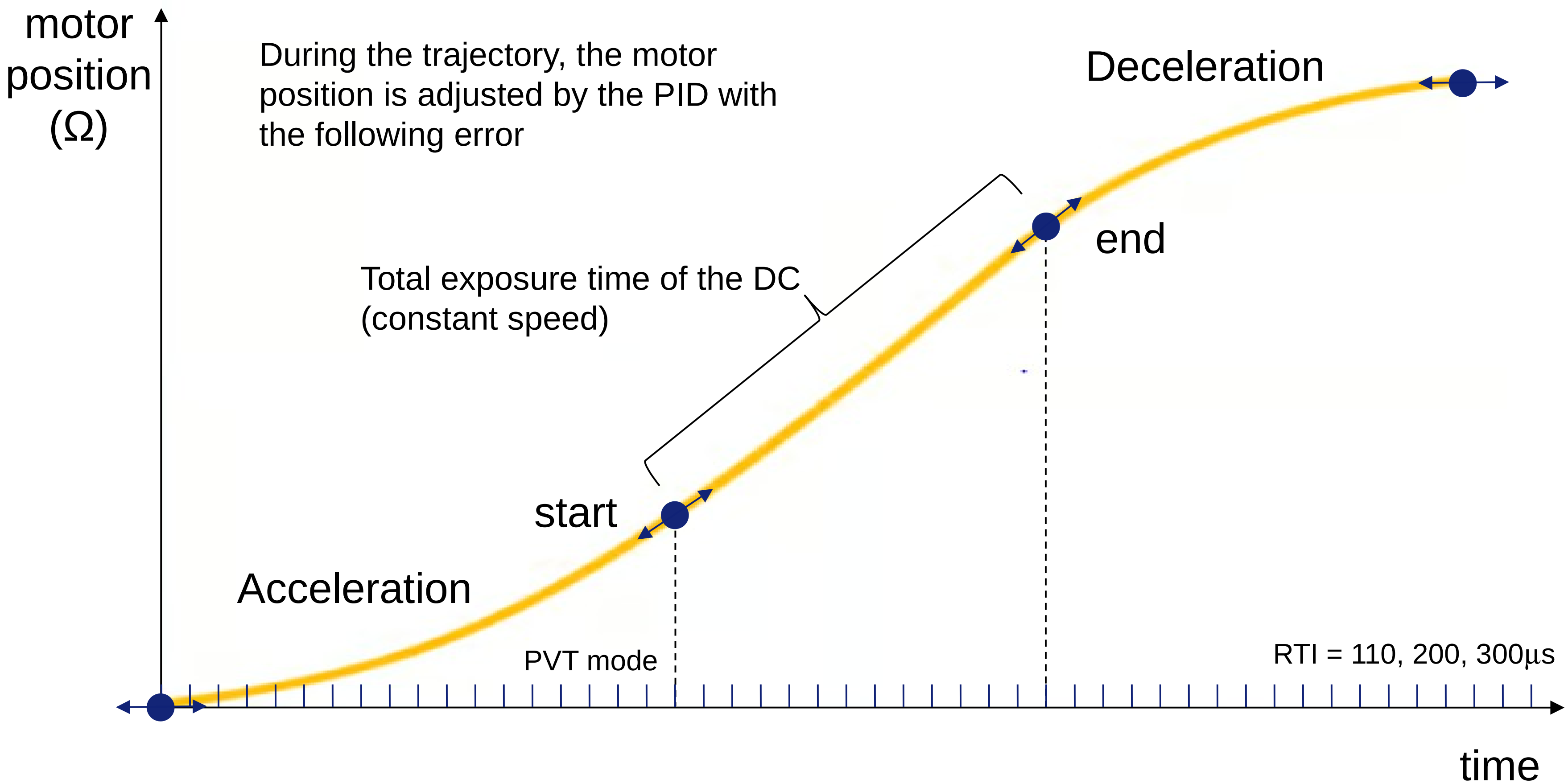


Clock at 39MHz (25.64ns)

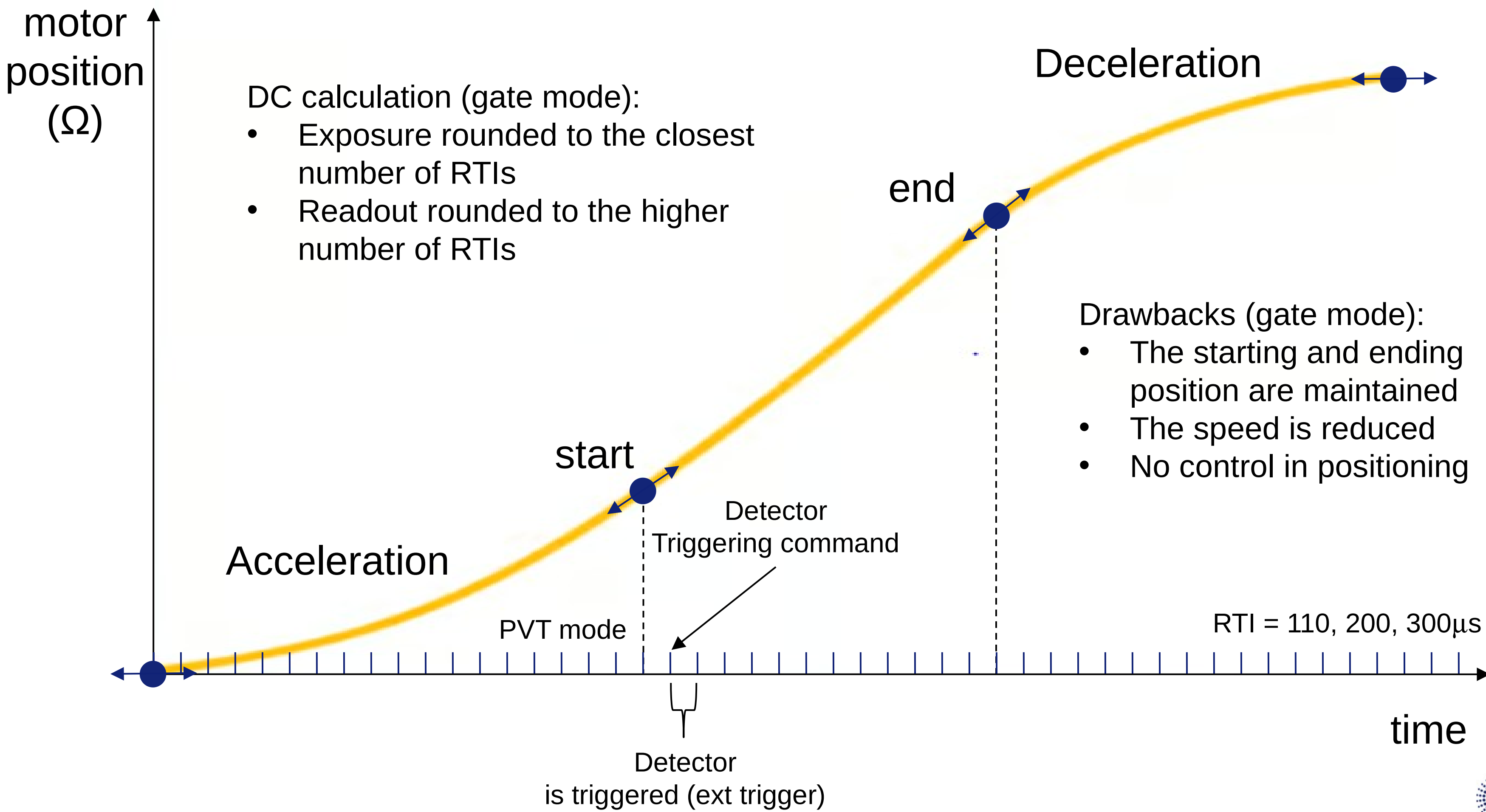
RTI (servo cycle

- 3.33 kHz (300µs)
- 5 kHz (200µs)
- 9.09 kHz (110µs)

MOTION PROGRAMME



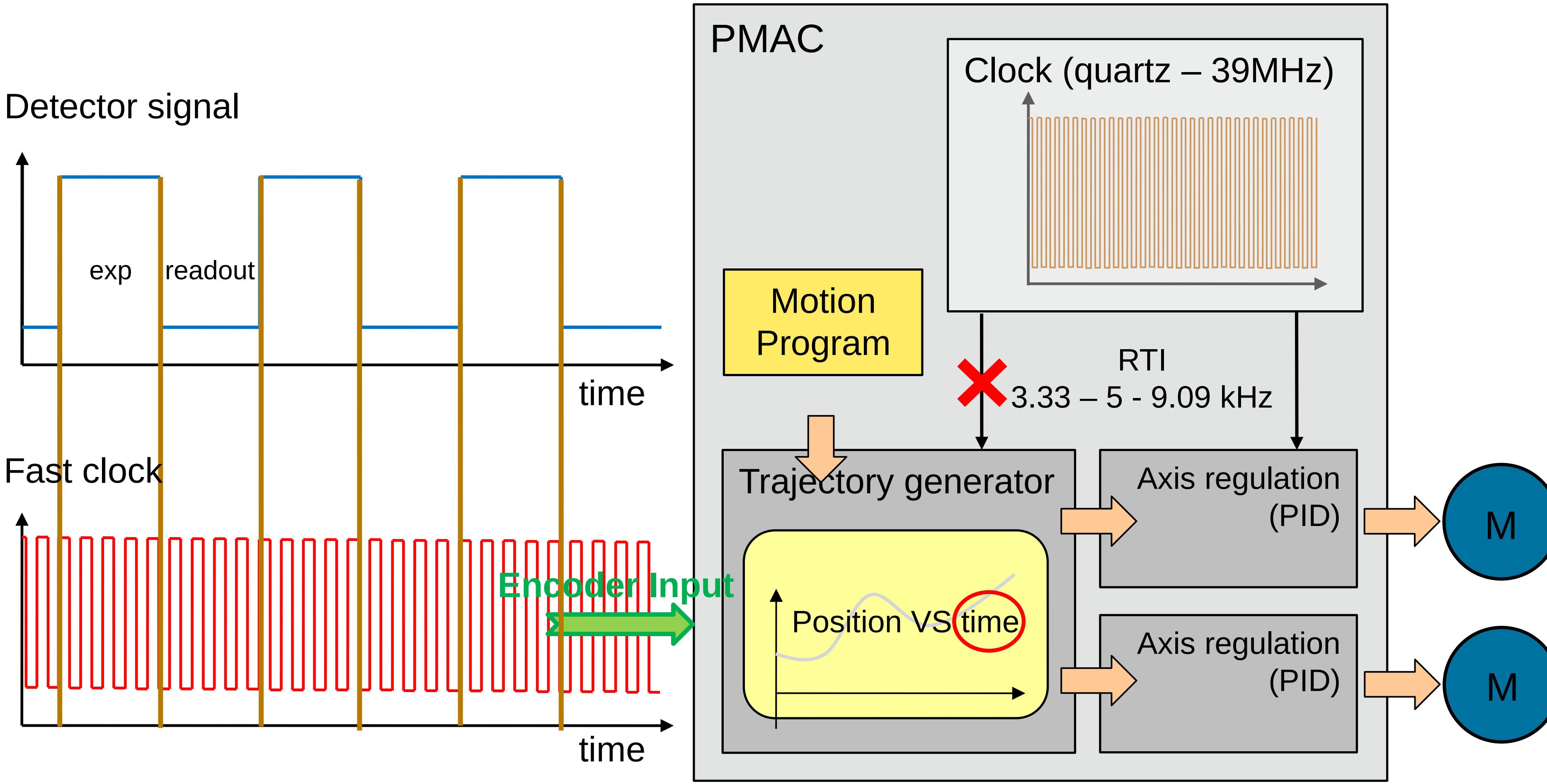
MOTION PROGRAMME



SSX CONTROL UNIT ON ID29 (FPGA)

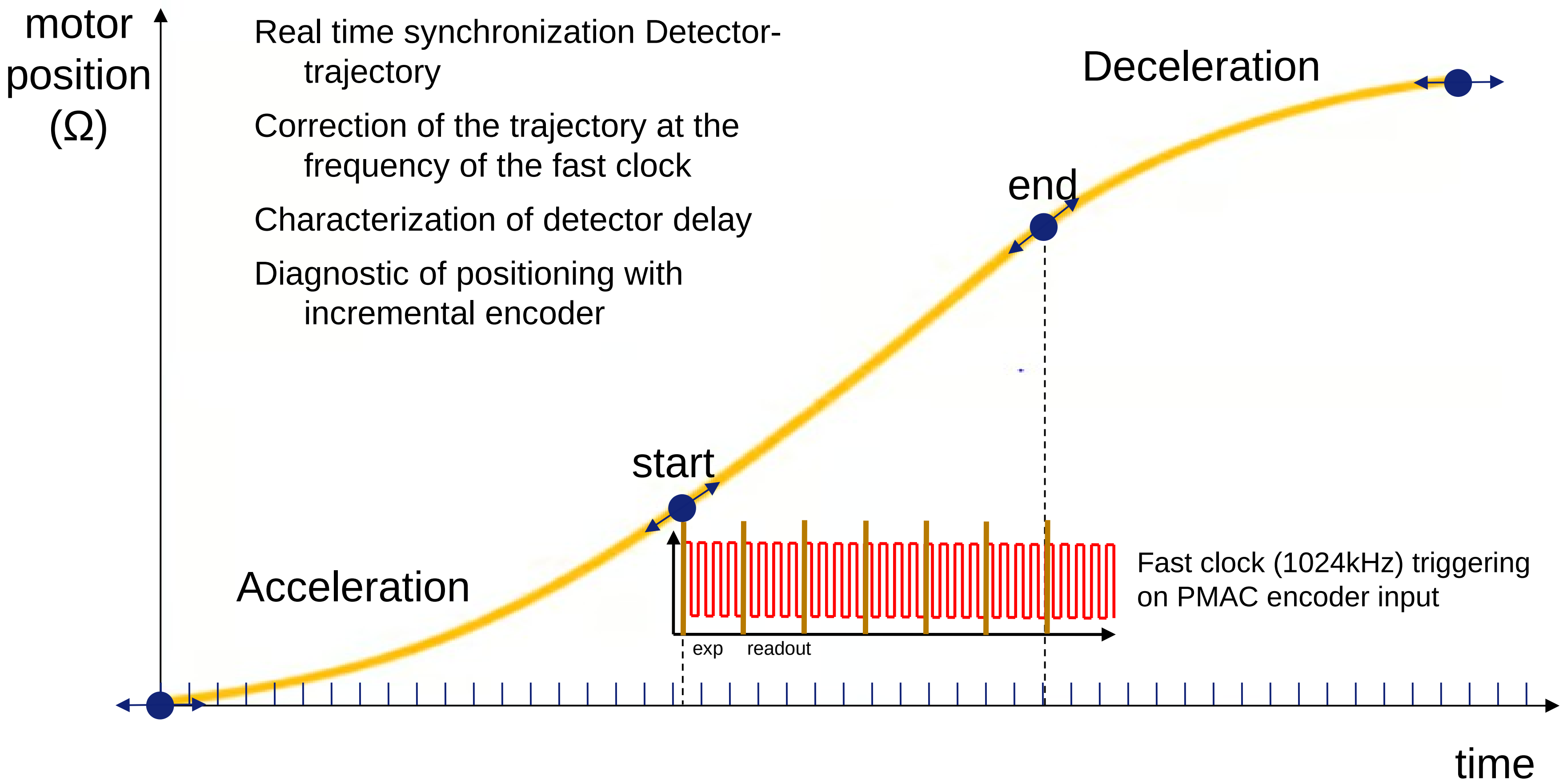
Clock at 50MHz (20-ns)

TRIGGERED TIME BASE



TRIGGERED TIME BASE

- Real time synchronization Detector-trajectory
- Correction of the trajectory at the frequency of the fast clock
- Characterization of detector delay
- Diagnostic of positioning with incremental encoder



Macromolecular Crystallography
And
Structural Biology at synchrotron

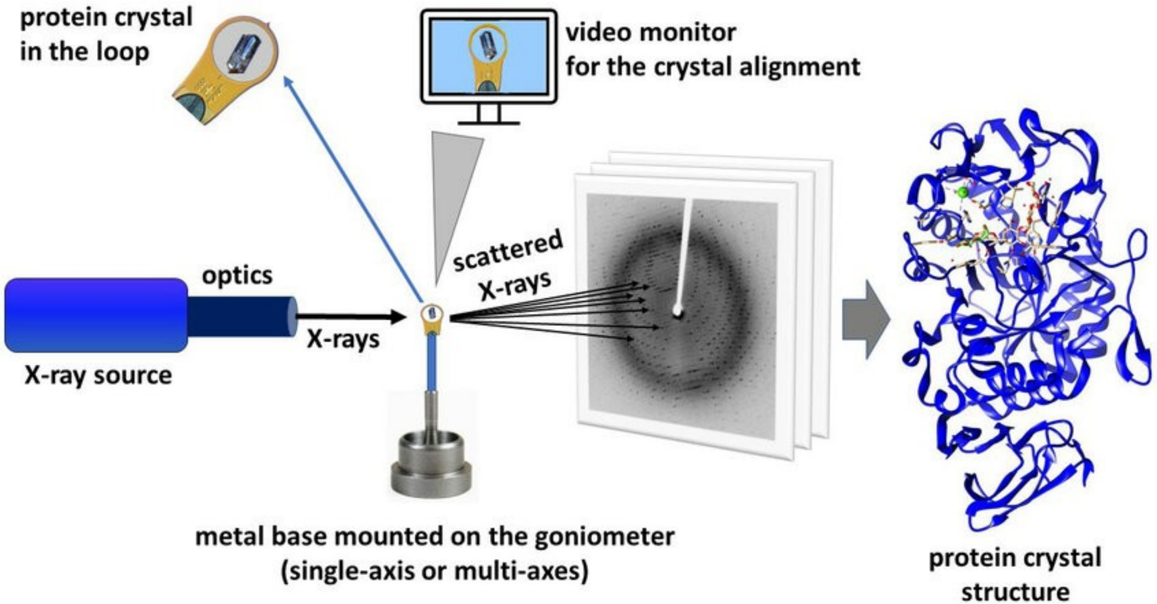
CONTENTS

Theory

1. *Crystallogenesis*
2. *Crystal symmetries*
3. *X-rays*
4. *Diffraction*
5. *Phase problem*

Practice

6. *Crystal harvesting*
7. *Data collection*
8. *Data processing*
9. *Solving structures*
10. *Examples*



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Theory

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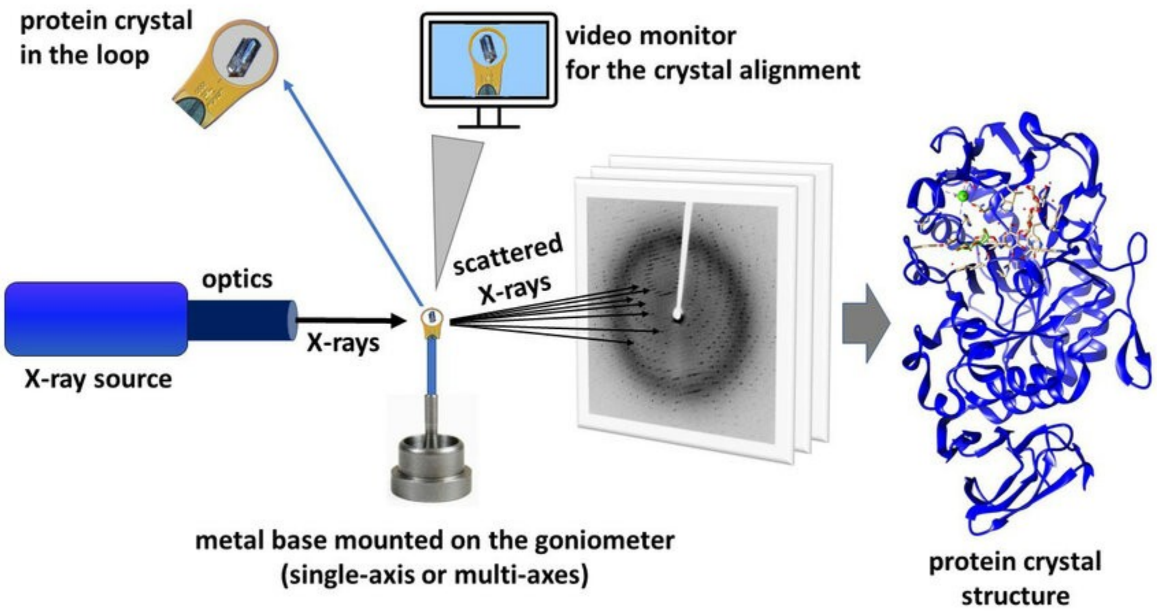
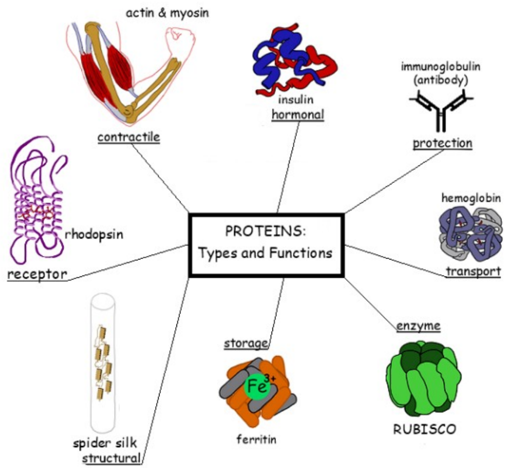
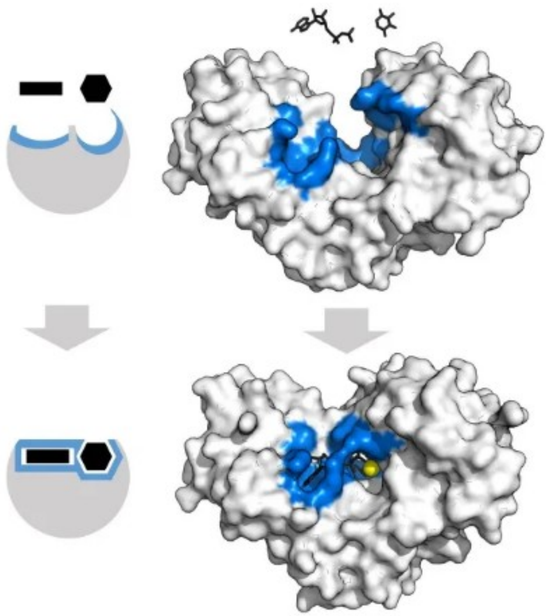
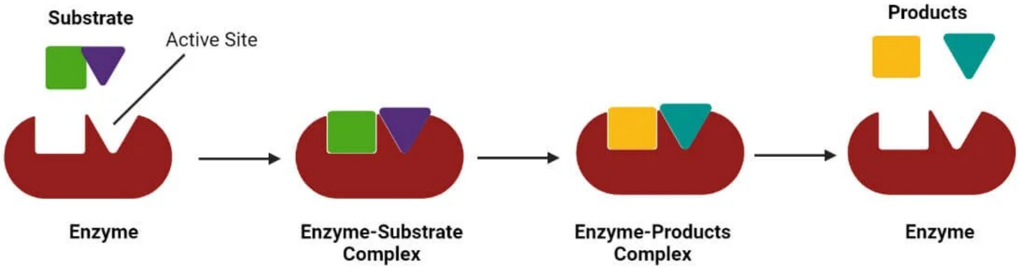


LOCK-AND-KEY MODEL (1894)



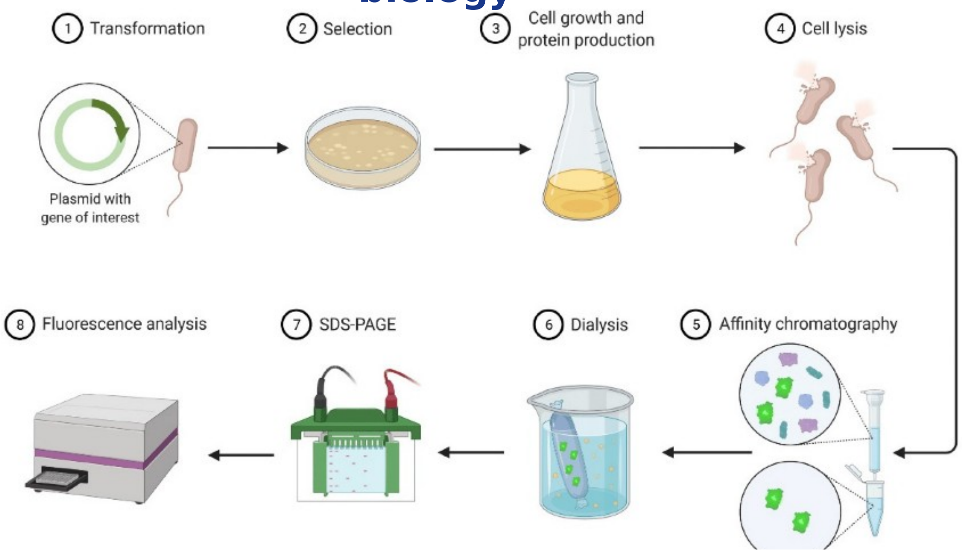
Emil Fischer (1852-1919)
Nobel prize in Chemistry(1902)



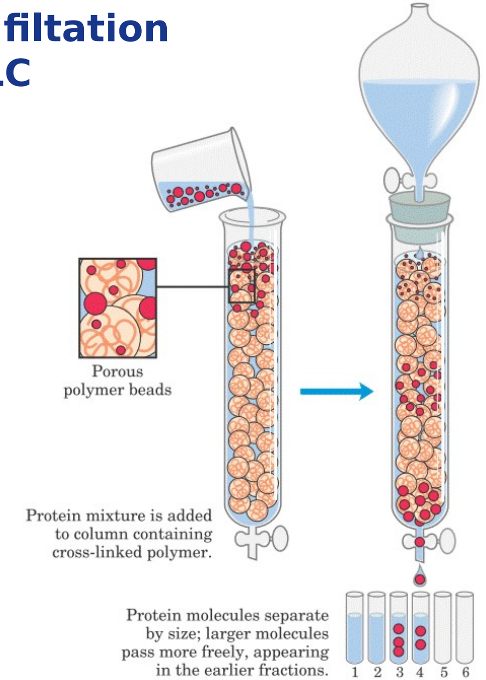


PURIFICATION

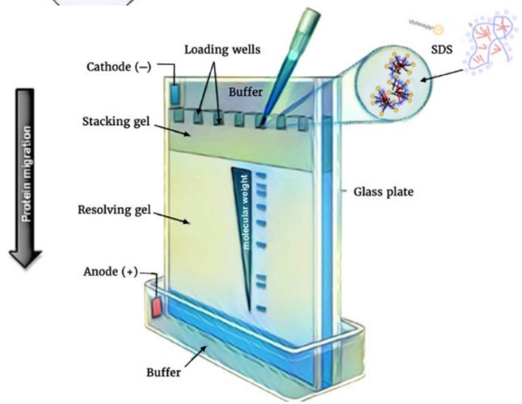
Molecular biology



Gel filtation HPLC

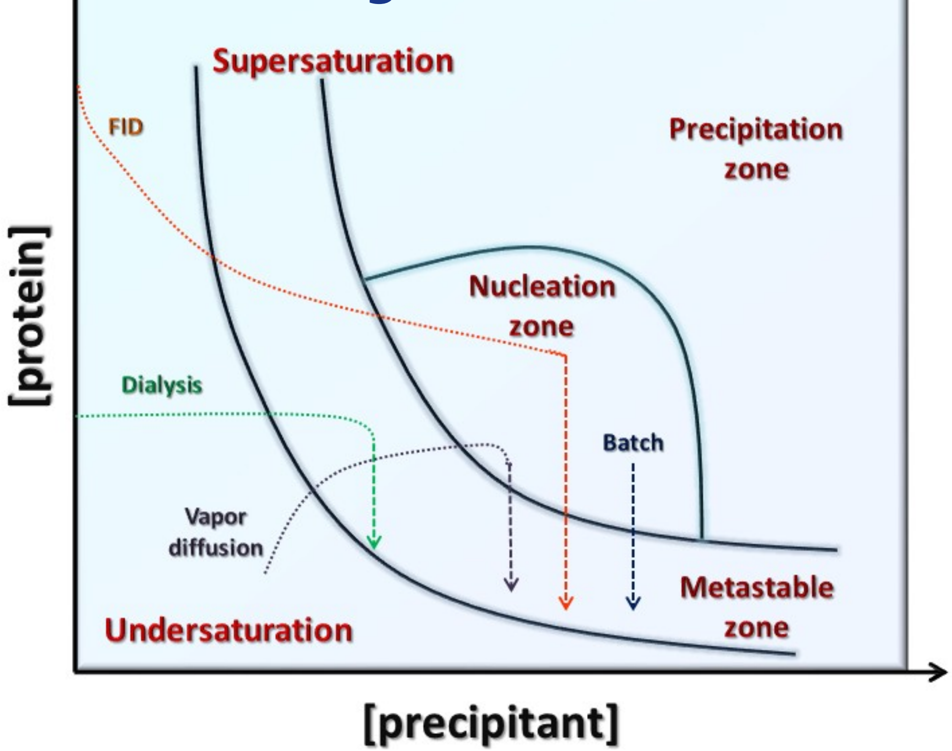


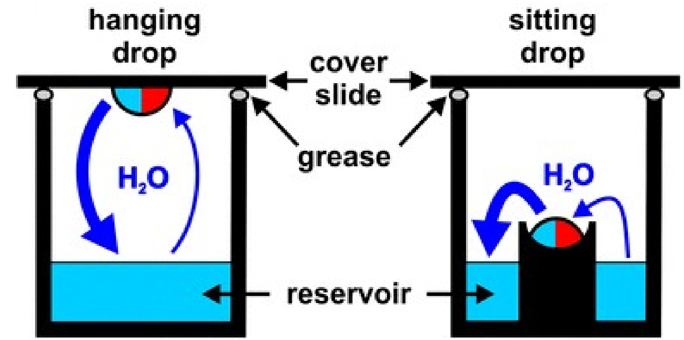
SDS-PAGE electrophoresis

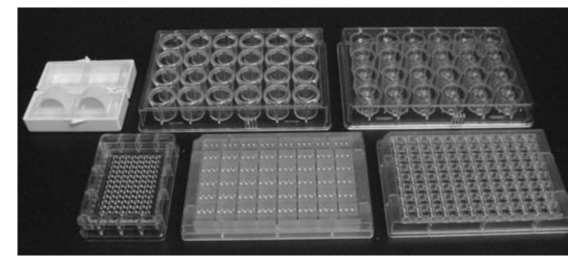


CRYSTALLOGENESIS

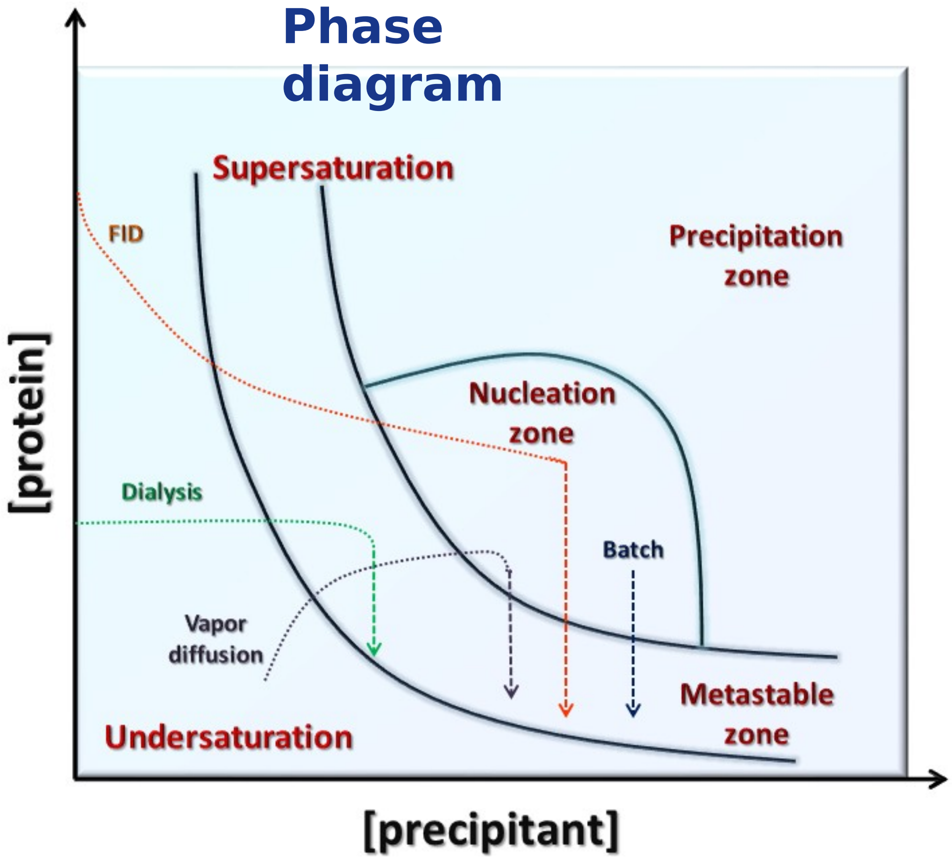
Phase diagram

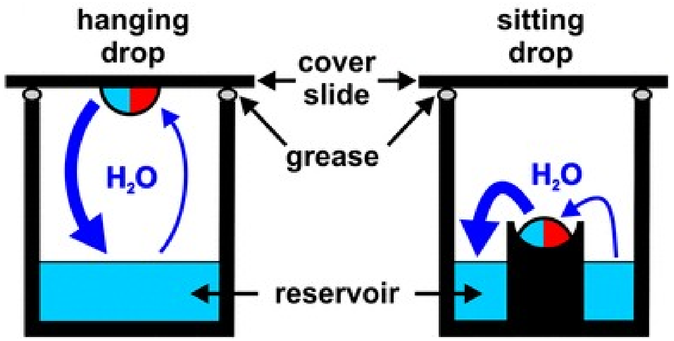


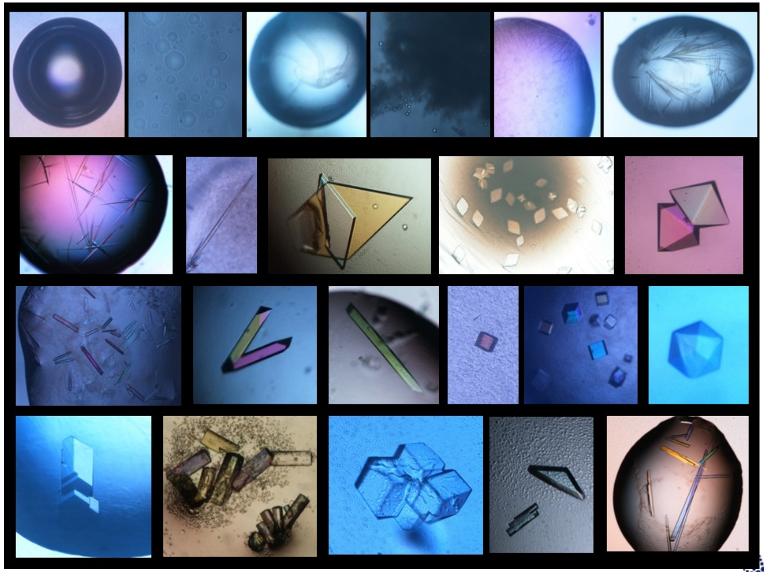




CRYSTALLOGENESIS







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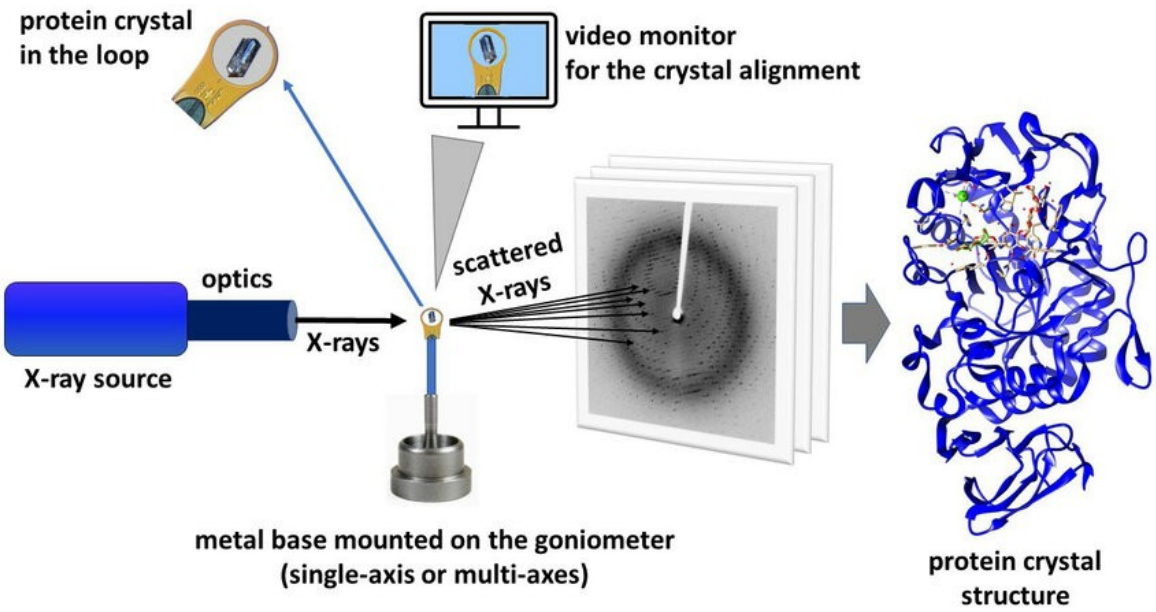
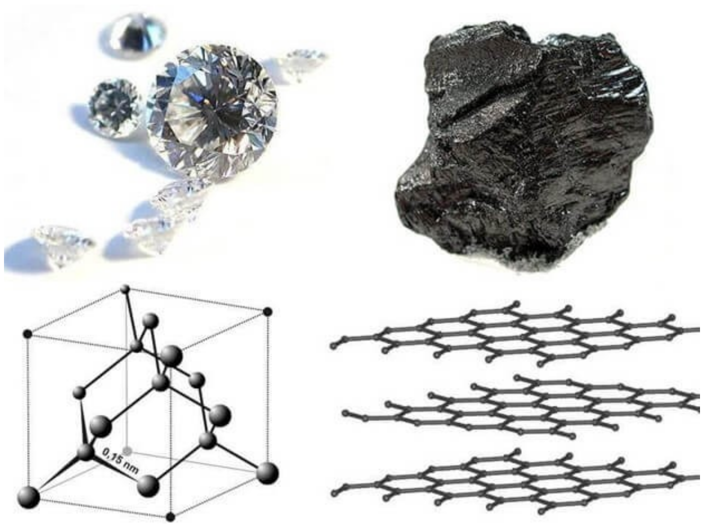
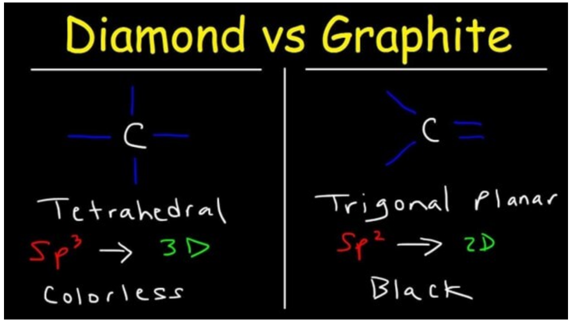


protein crystal
in the loop
video monitor
for the crystal alignment
optics
scattered
X-rays
X-ray source
X-rays
metal base mounted on the goniometer
(single-axis or multi-axes)
protein crystal
structure

CRYSTALS



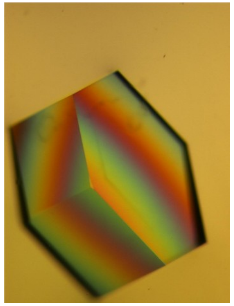


Steno's Law (1669)

The angles between corresponding faces on crystals of any solid chemical or mineral species are constant and are characteristic of the species. The interfacial angle is measured between face normals.

The law constant of interfacial angles holds for any crystals of a given species, whether they are natural or hand-made, regardless of size or provenance.

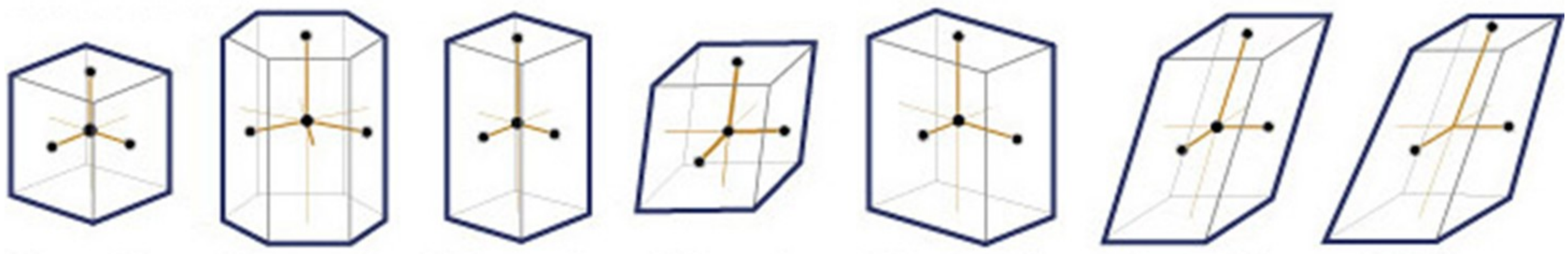

NICOLAI STENONIS
DE SOLIDO
INTRA SOLIDVM NATVRALITER CONTENTO
DISSERTATIONIS PRODROMVS.
AD
SERENISSIMVM
FERDINANDVM II.
MAGNVM ETRVRIÆ DVCEM.
GRATIA ... OMNIA VNTIO ... QVASITA
FLORENTIÆ
Ex Typographia sub signo STELLÆ MDCLXIX.
SVPERIORVM PERMISSV.



The European Synchrotron | ESRF

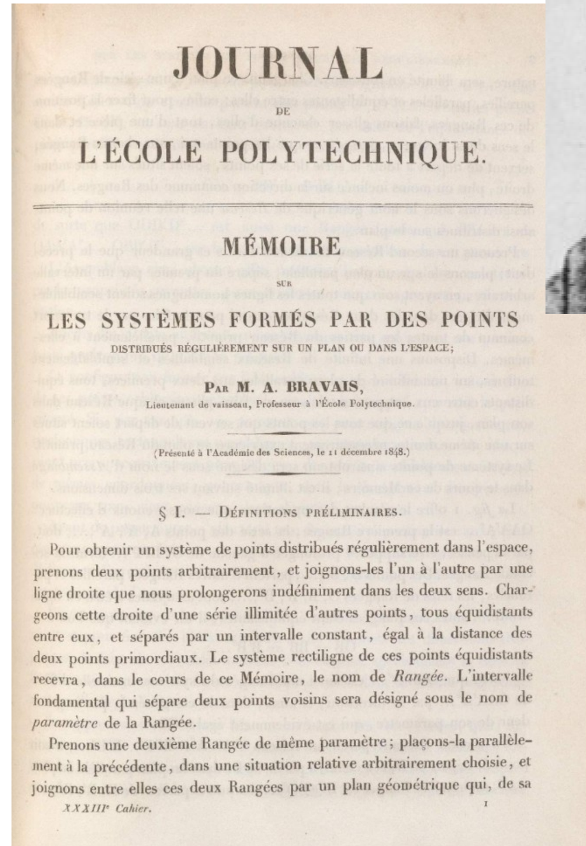
7 LATTICE SYSTEMS

Cub ic | **Hexago nal** | **Tetrago nal** | **Rhombohe dral (or** | **Orthorho mbic** | **Monocli nic** | **Tricli nic**



14 BRAVAIS LATTICES

Crystal family	Lattice system	Point group (Schönflies notation)	14 Bravais lattices			
			Primitive (P)	Base-centered (S)	Body-centered (I)	Face-centered (F)
Triclinic (a)		C_i	aP			
Monoclinic (m)		C_{2h}	mP	mS		
Orthorhombic (o)		D_{2h}	oP	oS	oI	oF
Tetragonal (t)		D_{4h}	tP		tI	
Hexagonal (h)	Rhombohedral	D_{3d}	hR			
	Hexagonal	D_{6h}	hP			
Cubic (c)		O_h	cP		cI	cF



JOURNAL
DE
L'ÉCOLE POLYTECHNIQUE.

MÉMOIRE
SUR
LES SYSTÈMES FORMÉS PAR DES POINTS
DISTRIBUÉS RÉGULIÈREMENT SUR UN PLAN OU DANS L'ESPACE;

PAR M. A. BRAVAIS,
Lieutenant de vaisseau, Professeur à l'École Polytechnique.

(Présenté à l'Académie des Sciences, le 11 décembre 1848.)

§ I. — DÉFINITIONS PRÉLIMINAIRES.

Pour obtenir un système de points distribués régulièrement dans l'espace, prenons deux points arbitrairement, et joignons-les l'un à l'autre par une ligne droite que nous prolongerons indéfiniment dans les deux sens. Chargeons cette droite d'une série illimitée d'autres points, tous équidistants entre eux, et séparés par un intervalle constant, égal à la distance des deux points primordiaux. Le système rectiligne de ces points équidistants recevra, dans le cours de ce Mémoire, le nom de *Rangée*. L'intervalle fondamental qui sépare deux points voisins sera désigné sous le nom de *paramètre* de la Rangée.

Prenons une deuxième Rangée de même paramètre; plaçons-la parallèlement à la précédente, dans une situation relative arbitrairement choisie, et joignons entre elles ces deux Rangées par un plan géométrique qui, de sa

XXXIIIe Cahier. 1

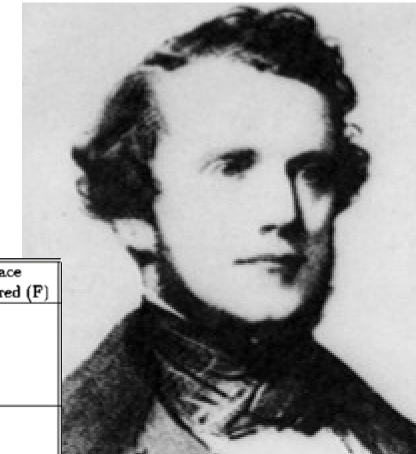
Auguste Bravais(1811-1863)

14 BRAVAIS LATTICES

$$\mathbf{R} = n_1\mathbf{a}_1 + n_2\mathbf{a}_2 + n_3\mathbf{a}_3$$

Crystal family	Lattice system	Point group (Schönflies notation)	14 Bravais lattices: Primitive (P)	Base-centered (S)	Body-centered (I)	Face-centered (F)
Triclinic (a)		C_i	aP			
Monoclinic (m)		C_{2h}	mP	mS		
Orthorhombic (o)		D_{2h}	oP	oS	oI	oF
Tetragonal (t)		D_{4h}	tP		tI	
Hexagonal (h)	Rhombohedral	D_{3d}	hR			
	Hexagonal	D_{6h}	hP			
Cubic (c)		O_h	cP		cI	cF

Bravais lattice	Parameters	Simple (P)	Volume centered (I)	Base centered (C)	Face centered (F)
Triclinic	$a_1 \neq a_2 \neq a_3$ $\alpha_{12} \neq \alpha_{23} \neq \alpha_{31}$				
Monoclinic	$a_1 \neq a_2 \neq a_3$ $\alpha_{23} = \alpha_{31} = 90°$ $\alpha_{12} \neq 90°$				
Orthorhombic	$a_1 \neq a_2 \neq a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} = 90°$				
Tetragonal	$a_1 = a_2 \neq a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} = 90°$				
Trigonal	$a_1 = a_2 = a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} < 120°$				
Cubic	$a_1 = a_2 = a_3$ $\alpha_{12} = \alpha_{23} = \alpha_{31} = 90°$				
Hexagonal	$a_1 = a_2 \neq a_3$ $\alpha_{12} = 120°$ $\alpha_{23} = \alpha_{31} = 90°$				



Auguste Bravais(1811-1863)

32 POINT GROUPS

A group of point symmetry operations leave at least one point unmoved. Lattice translation is not considered in point group.

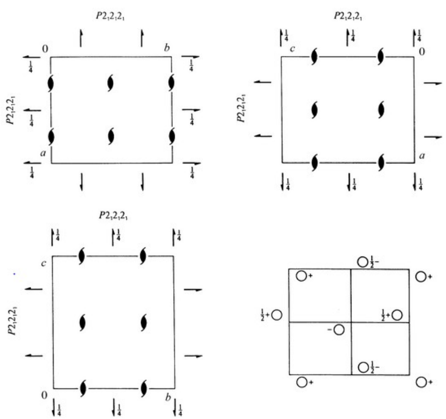
Johann Hessel (1796-1872)

Crystal family	Crystal system	Group names						
Cubic		23	m$\bar{3}$		432	$\bar{4}$3m	m$\bar{3}$m	
Hexagonal	Hexagonal	6	$\bar{6}$	$6/m$	622	6mm	$\bar{6}$m2	6/mmm
	Trigonal	3	$\bar{3}$		32	3m	$\bar{3}$m	
Tetragonal		4	$\bar{4}$	$4/m$	422	4mm	$\bar{4}$2m	4/mmm
Orthorhombic					222		mm2	mmm
Monoclinic		2		$2/m$		m		
Triclinic		1	$\bar{1}$					

230 SPACE GROUPS

Hermann-Mauguin symbol (internat. Table vol. A)

$P2_12_12_1$	D_2^4	222	Orthorhombic
No. 19	$P2_12_12_1$		Patterson symmetry *Pmmm*



Origin at midpoint of three non-intersecting pairs of parallel 2_1 axes

Asymmetric unit $0 \le x \le 1/2;\ 0 \le y \le 1/2;\ 0 \le z \le 1$

Symmetry operations

(1) 1 (2) $2(0,0,\frac{1}{2})$ $\frac{1}{4},0,z$ (3) $2(0,\frac{1}{2},0)$ $0,y,\frac{1}{4}$ (4) $2(\frac{1}{2},0,0)$ $x,\frac{1}{4},0$

Generators selected (1); $t(1,0,0)$; $t(0,1,0)$; $t(0,0,1)$; (2); (3)

Positions

Multiplicity, Wyckoff letter, Site symmetry	Coordinates				Reflection conditions
4 a 1	(1) x,y,z	(2) $-x+1/2,-y,z+1/2$	(3) $-x,y+1/2,-z+1/2$	(4) $x+1/2,-y+1/2,-z$	General: $h00$: $h=2n$ $0k0$: $k=2n$ $00l$: $l=2n$

Evgraf Fedorov (1853-1919)

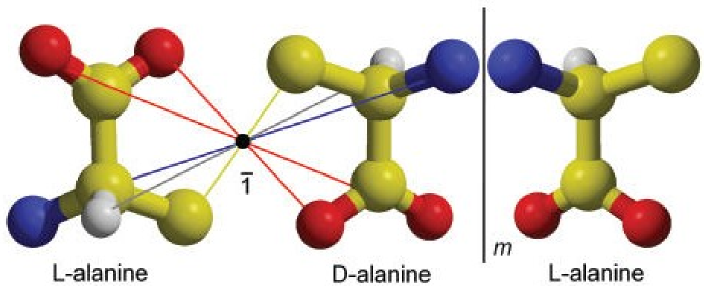
Triclinic	None		
Monoclinic*	[010] ('unique axis *b*') [001] ('unique axis *c*')		
Orthorhombic	[100]	[010]	[001]
Tetragonal	[001]	{[100] [010]}	$\{[1\bar{1}0]\ [110]\}$
Hexagonal	[001]	$\{[100]\ [010]\ [\bar{1}\bar{1}0]\}$	$\{[1\bar{1}0]\ [120]\ [\bar{2}\bar{1}0]\}$
Rhombohedral (hexagonal axes)	[001]	$\{[100]\ [010]\ [\bar{1}\bar{1}0]\}$	
Rhombohedral (rhombohedral axes)	[111]	$\{[1\bar{1}0]\ [01\bar{1}]\ [\bar{1}01]\}$	
Cubic	{[100] [010] [001]}	$\{[111]\ [1\bar{1}\bar{1}]\ [\bar{1}1\bar{1}]\ [\bar{1}\bar{1}1]\}$	$\{[1\bar{1}0]\ [110]\ [01\bar{1}]\ [011]\ [\bar{1}01]\ [101]\}$

SPACE GROUPS IN MX

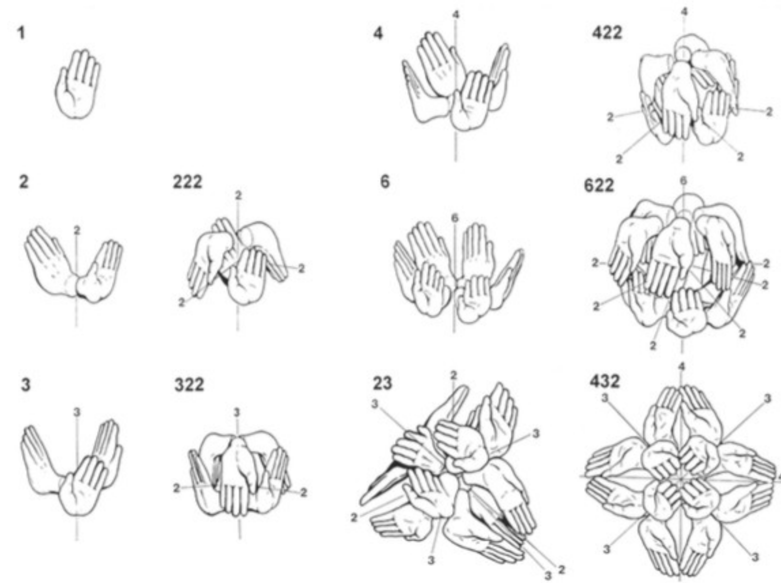
11 point group in proteins

Crystal family	Crystal system	Group names						
Cubic		23	$m\bar{3}$		432	$\bar{4}3m$	$m\bar{3}m$	
Hexagonal	Hexagonal	6	$\bar{6}$	$^6/_m$	622	6mm	$\bar{6}m2$	6/mmm
	Trigonal	3	$\bar{3}$		32	3m	$\bar{3}m$	
Tetragonal		4	$\bar{4}$	$^4/_m$	422	4mm	$\bar{4}2m$	4/mmm
Orthorhombic					222		mm2	mmm
Monoclinic		2		$^2/_m$		m		
Triclinic		1	$\bar{1}$					

Combination of point groups and Bravais lattices leaves 65 space groups for protein crystals (chiral objects)

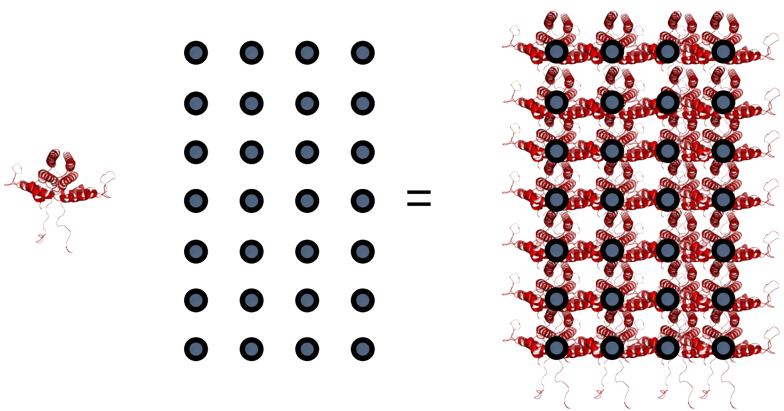


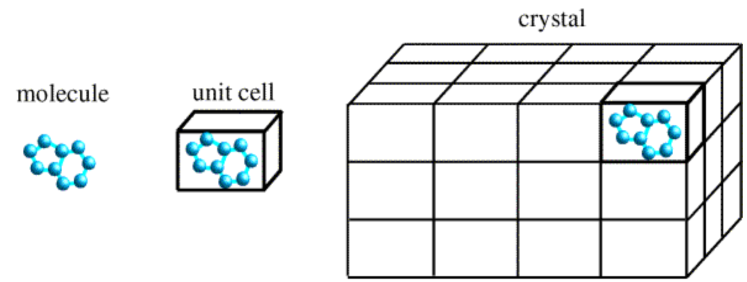
Combination of all point symmetry operations generates *32 point groups but for proteins, only 11* are allowed

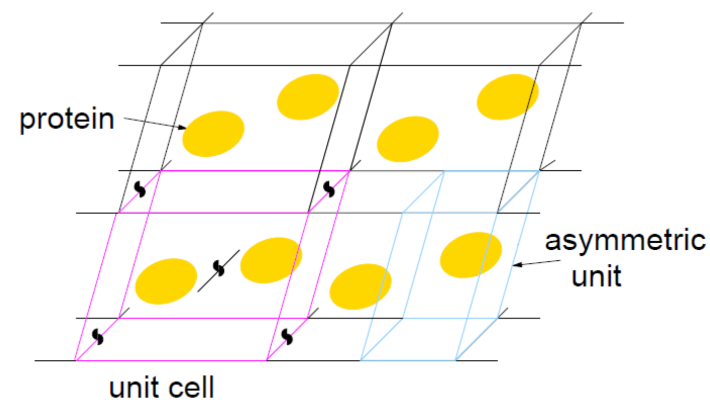


PROTEIN CRYSTAL

Crystal is a convolution of the molecule over the lattice







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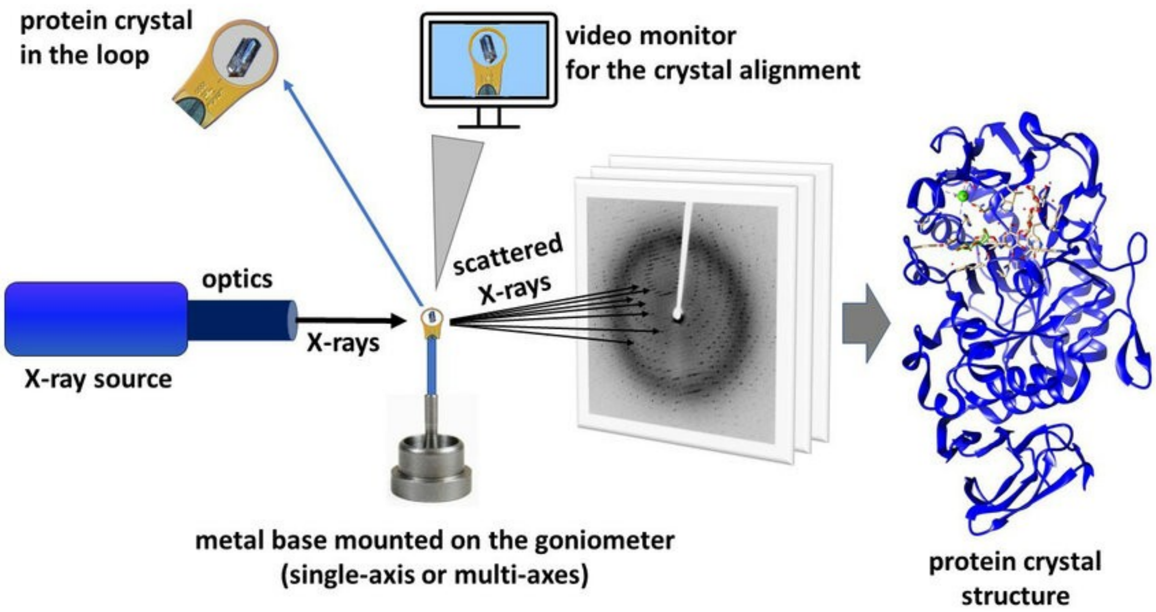
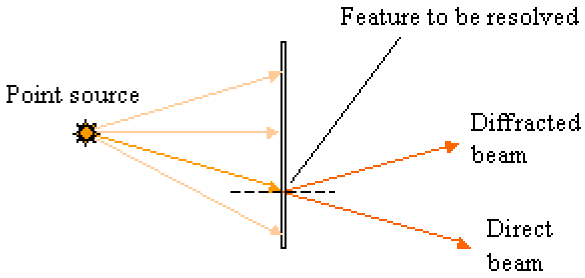
4. *Diffraction*
5. *Phase problem*

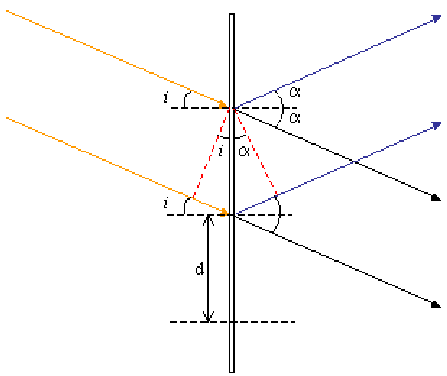
Practice

6. *Crystal harvesting*
7. *Data collection*
8. *Data processing*
9. *Solving structures*
10. *Examples*

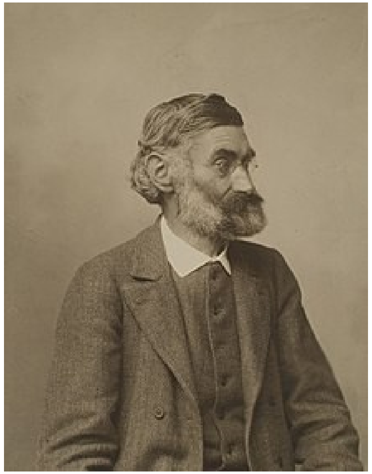


HOW TO PROBE A CRYSTAL?





d_{min}

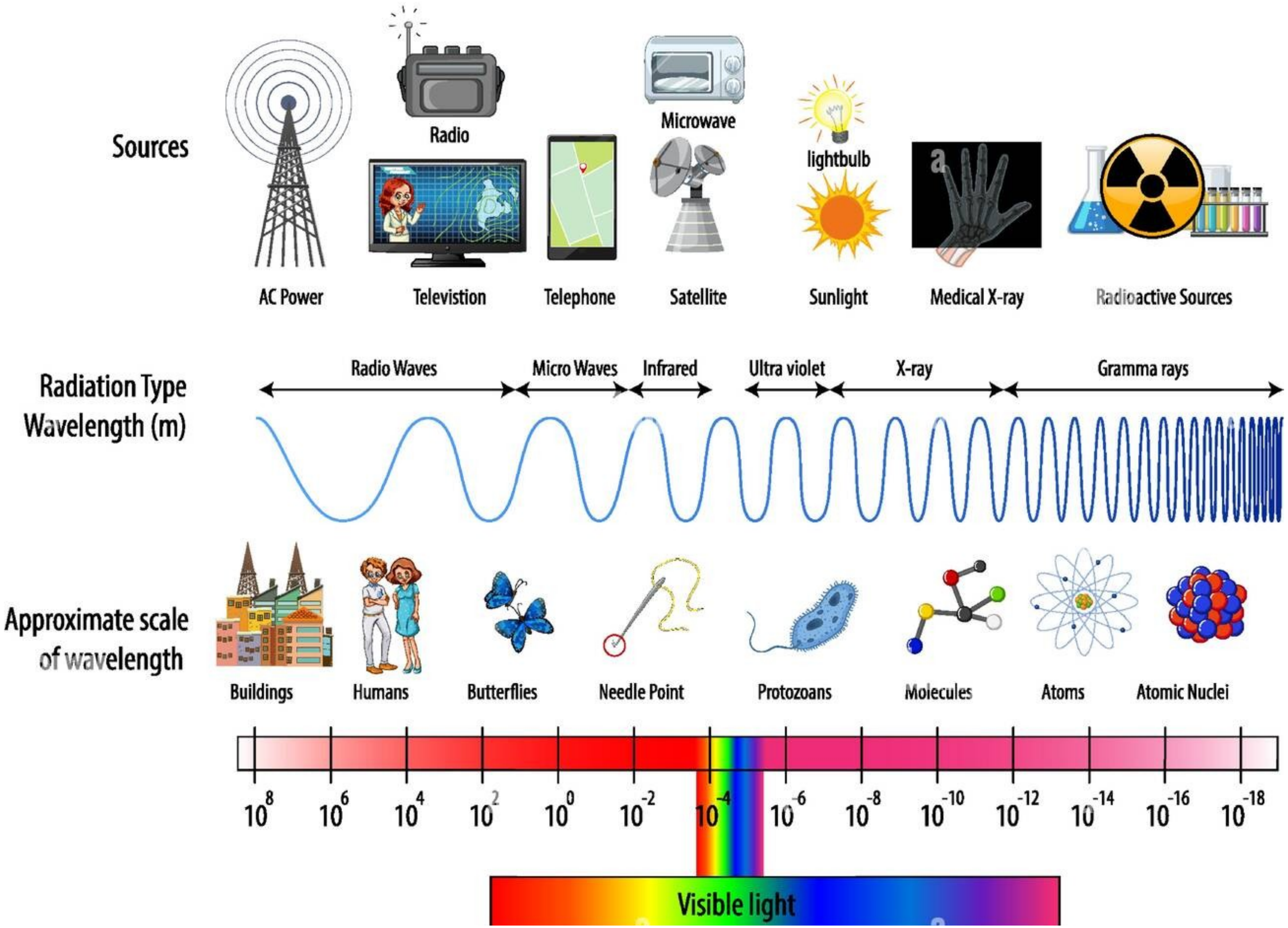


Ernst Abbe (1840-1905)

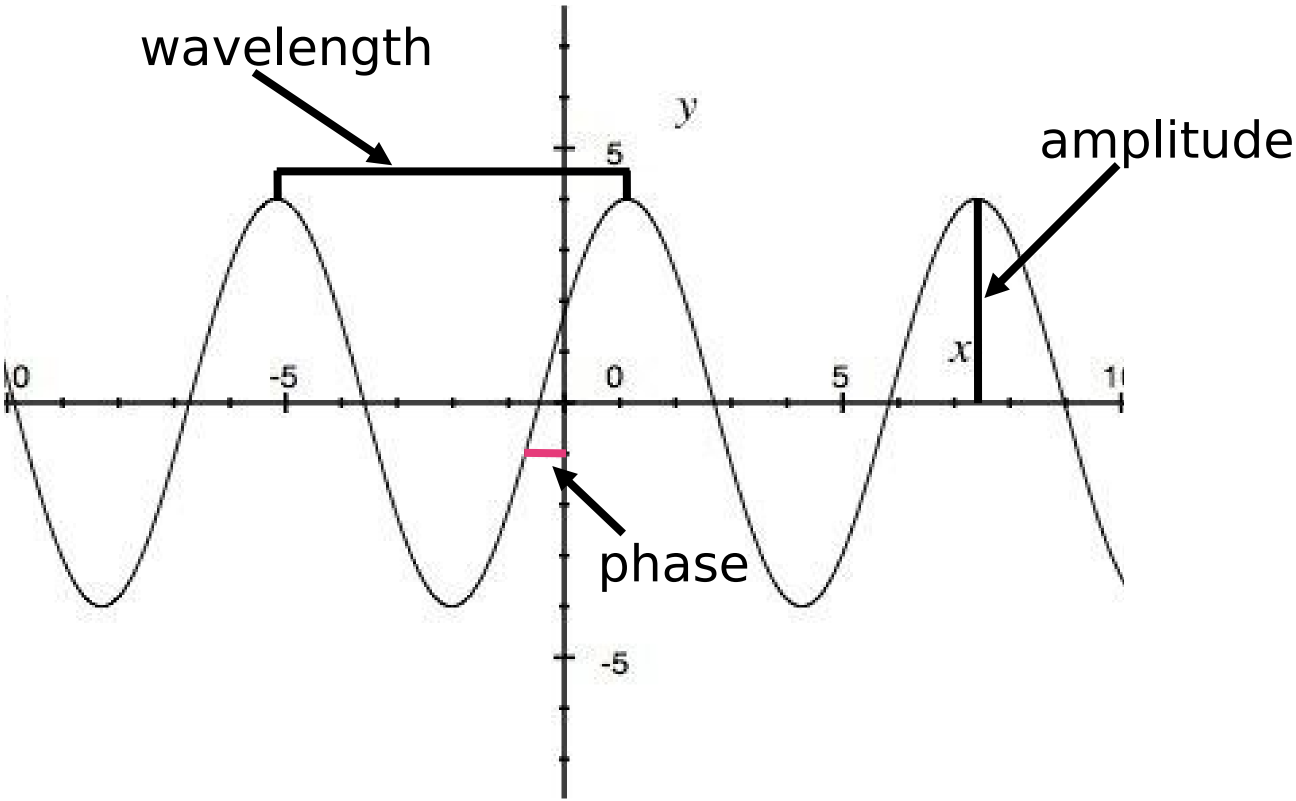
The limit of resolution (or resolving power) is a measure of the ability of the objective lens to separate in the image, adjacent details of the object.

n is the refractive index.

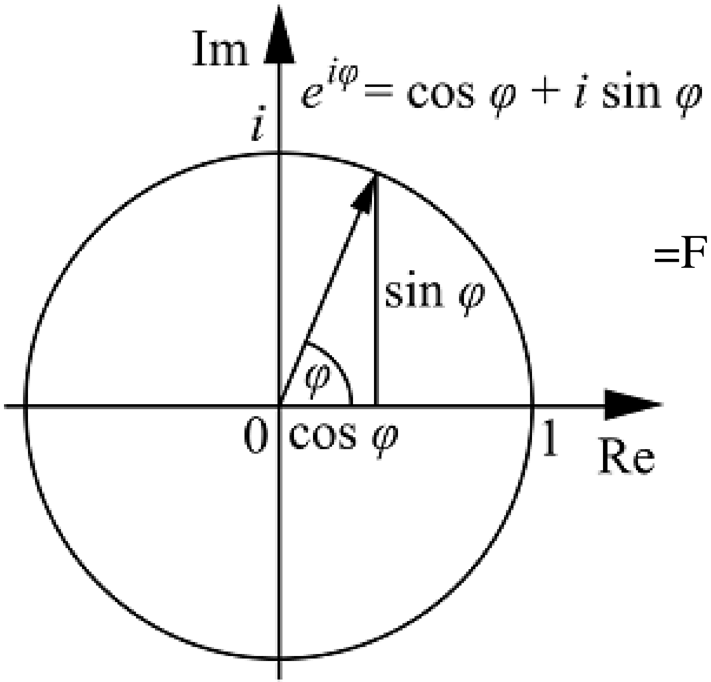
HOW TO PROBE A CRYSTAL?



X-RAY AS A WAVE

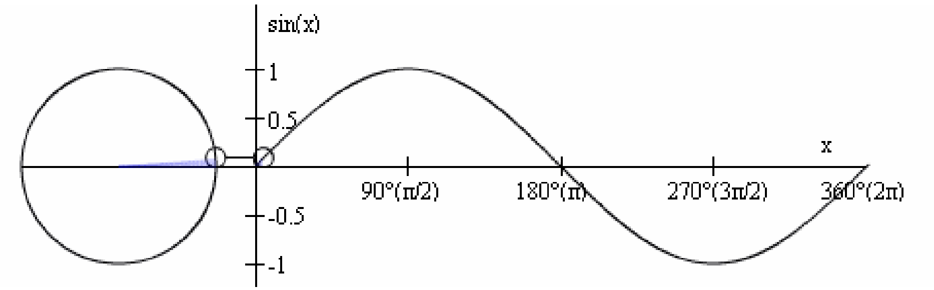


XRAY AS A WAVE



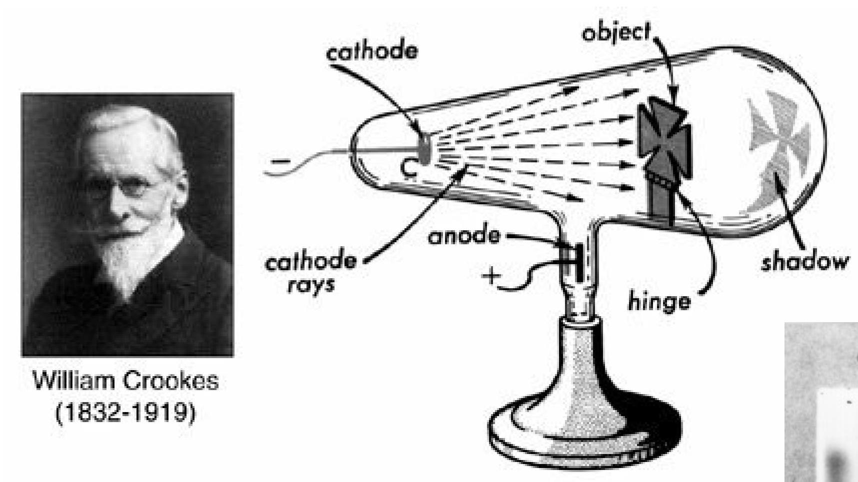
=F

A complex number may represent a wave, defined by an Amplitude and a Phase (Euler and trigonometric functions)

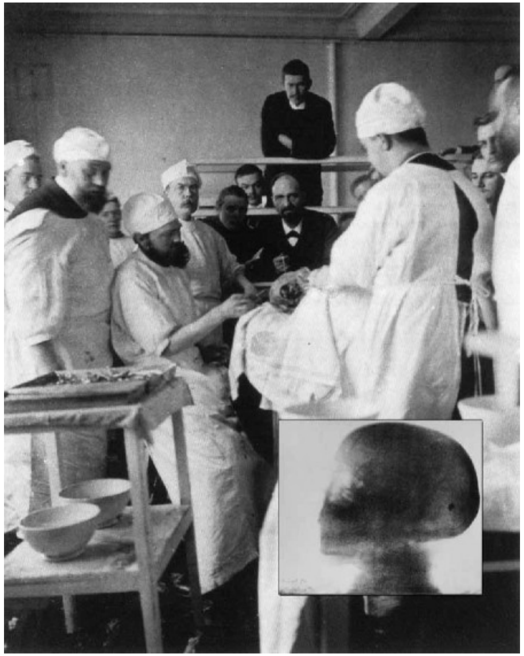


X-RAY PRODUCTION

Vacuum
tube



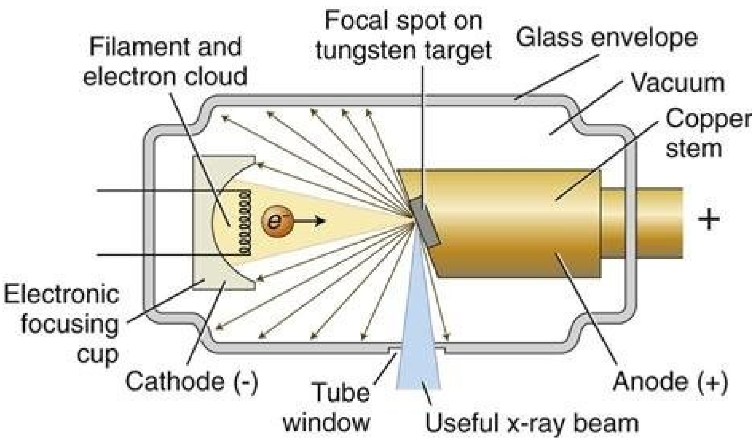
Wilhelm Röntgen is usually credited as the discoverer of X-rays in 1895



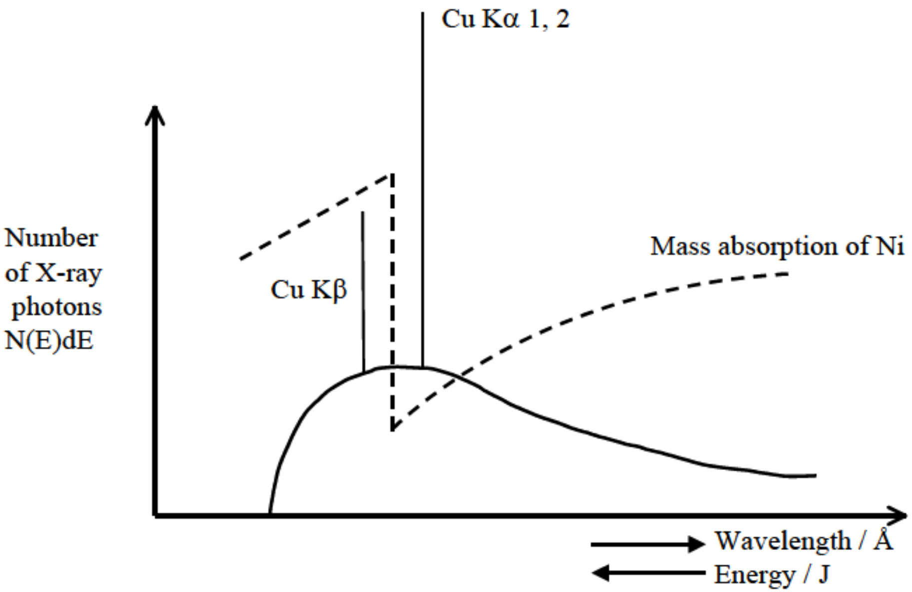
Medical applications of X-rays

MONOCHROMATIC BEAM

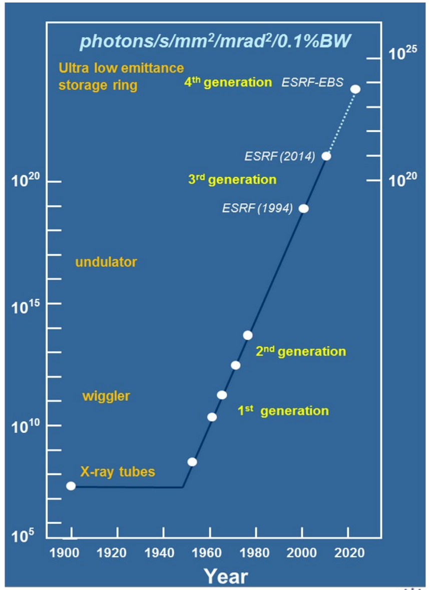
Rotating anode



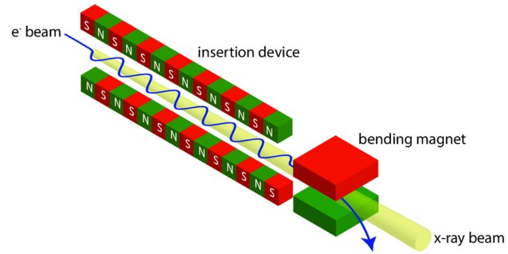
~50% of the the Cu Kα is transmitted ($\sim 10^{10}$ ph/s/mm^2)

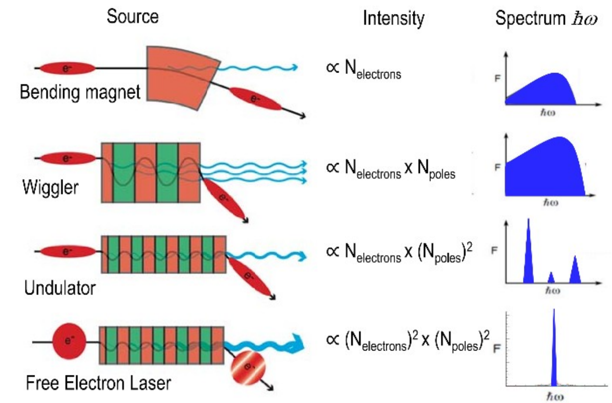


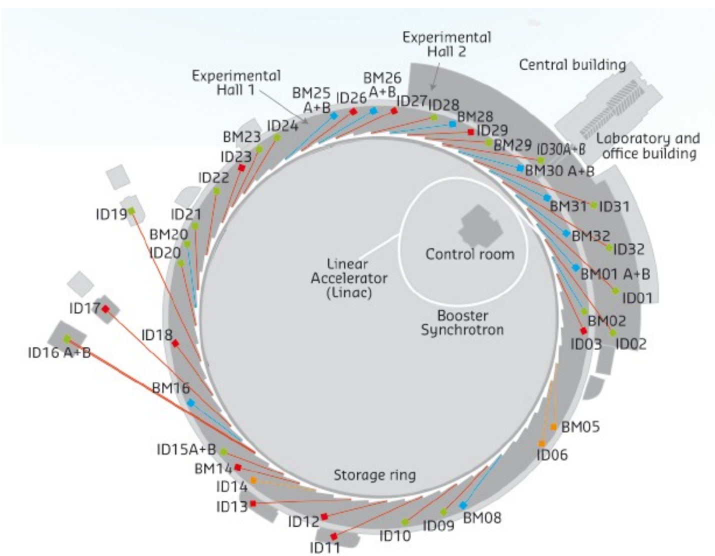
WHAT IS A SYNCHROTRON ?

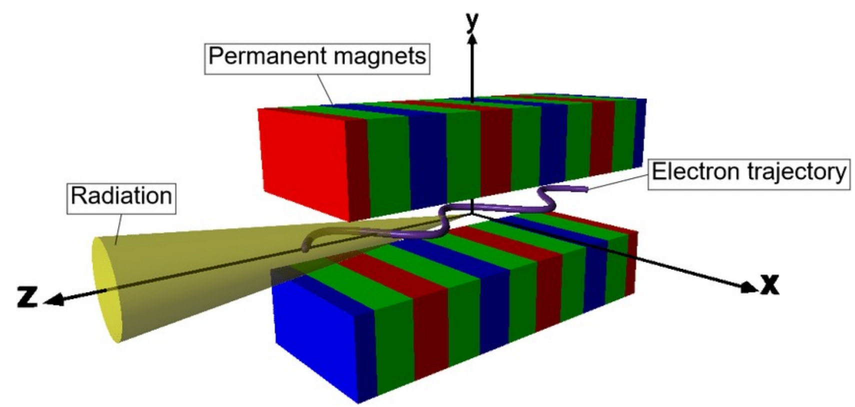


HOW DOES IT WORK?

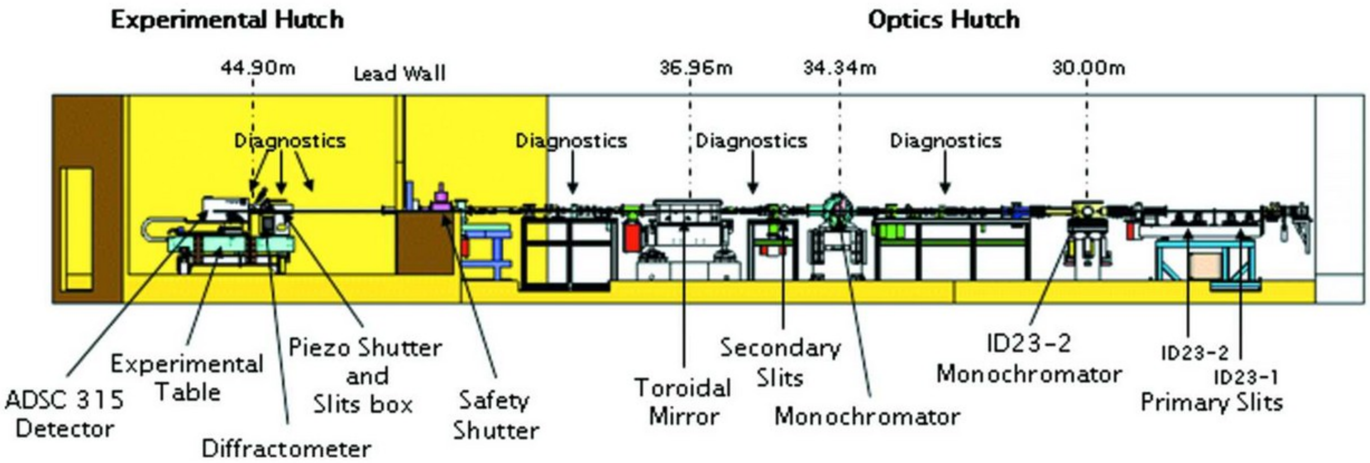








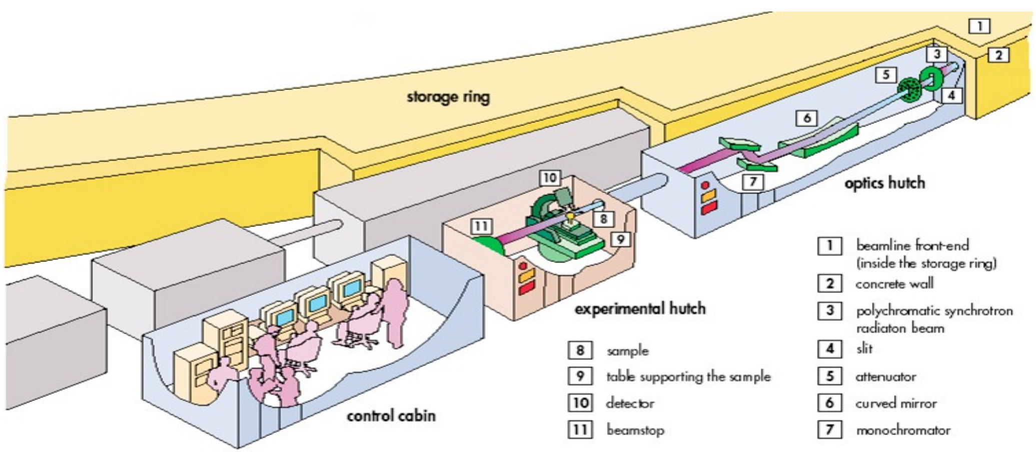
WHAT IS A BEAMLINE?

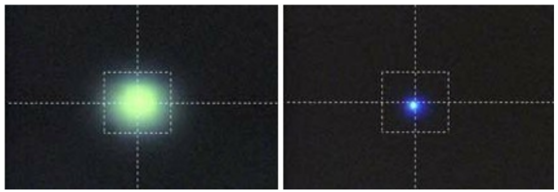


Size
<30µm Ø

Flux
> 10^{12} ph/s

Energy resolution





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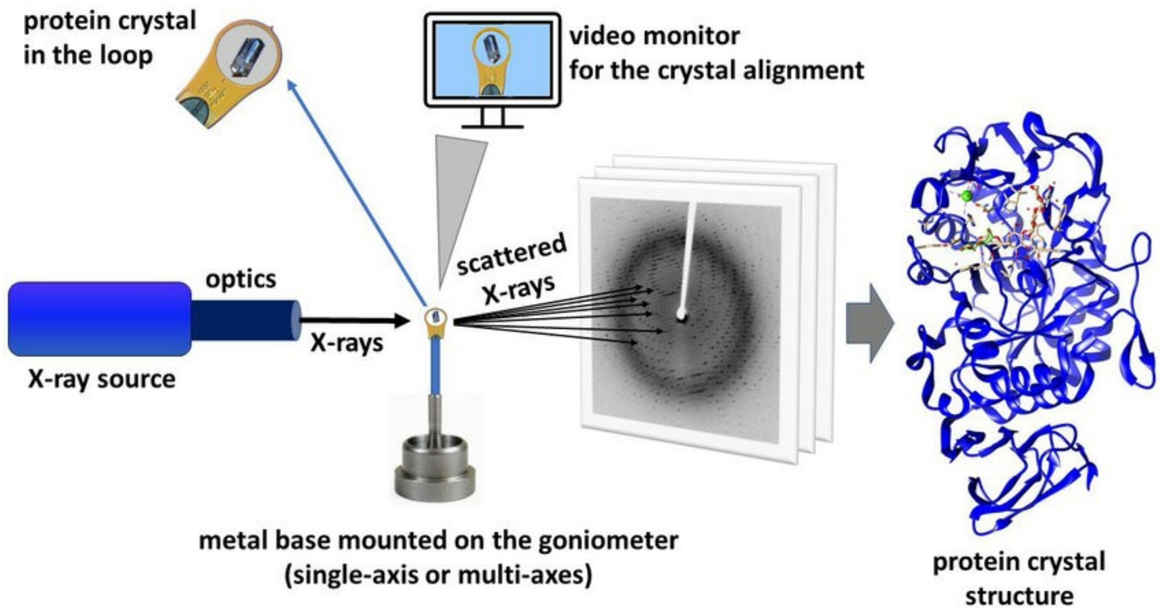
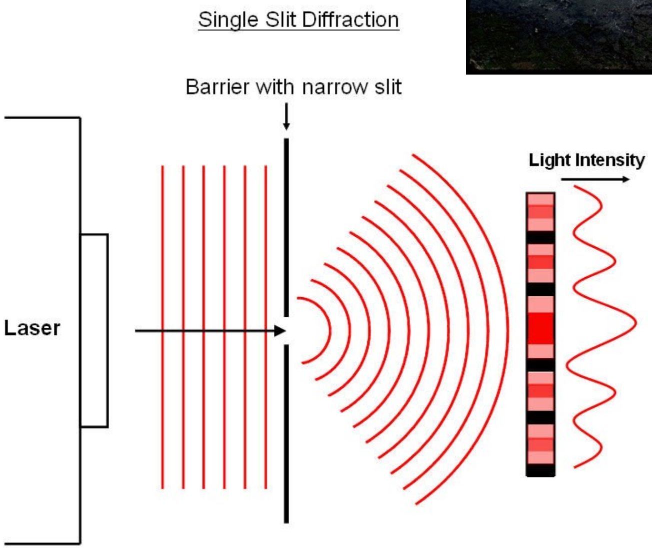
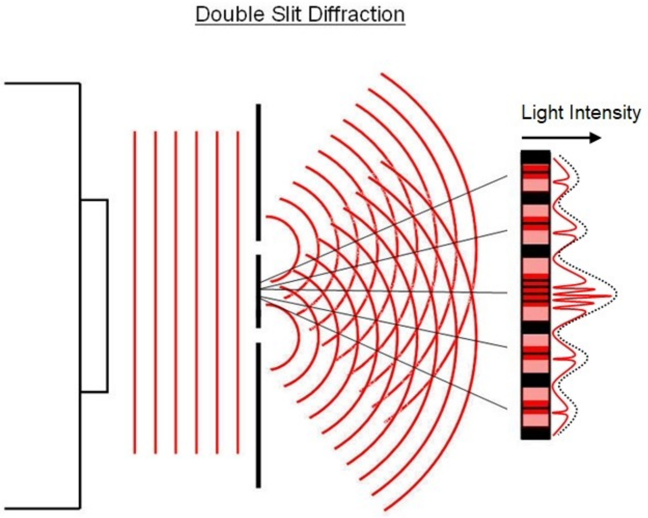


protein crystal
in the loop
video monitor
for the crystal alignment
optics
scattered
X-rays
X-rays
X-ray source
metal base mounted on the goniometer
(single-axis or multi-axes)
protein crystal
structure

DIFFRACTION

Waves interfere constructively in specific directions

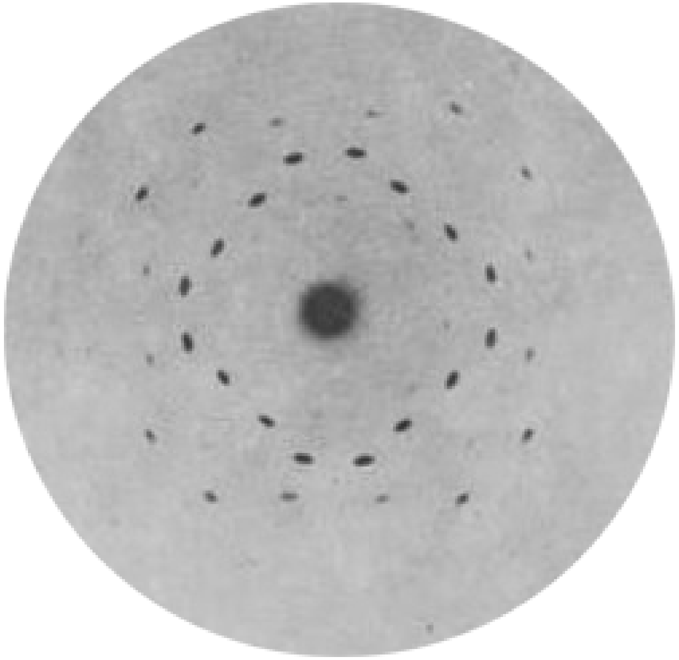




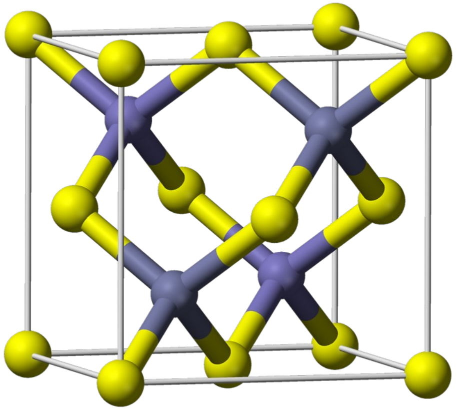
Plane waves through a slit -
slit becomes source of spherical waves

X-RAY DIFFRACTION

Max Laue's photo of X-ray diffraction from Zinc blende ZnS (Zn sulphide) cubic face centered (F3m)

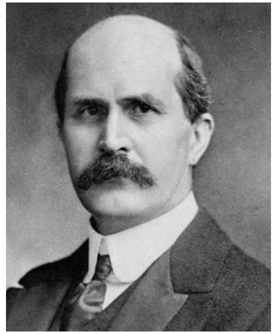


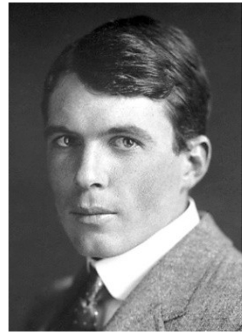
Max von Laue (1879-1960)
Nobel Prize 1914



BRAGG'S LAW

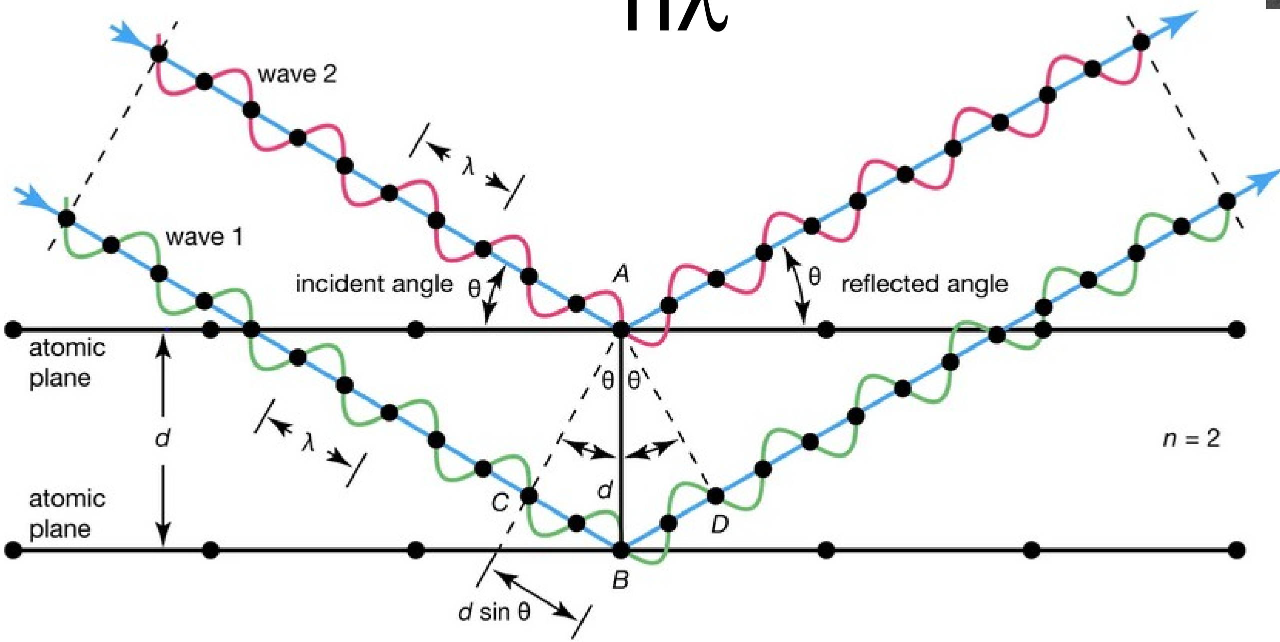
Bragg diffraction describes the condition for constructive interference from monochromatic waves, with amplitude and phase, reflected by planes in the crystalline material





W. H & W. L Bragg
(1862-1942) – (1890-1971)
Nobel prize 1915

$n\lambda$



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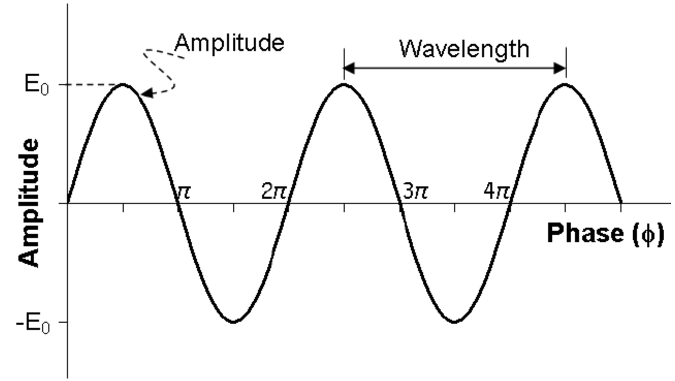
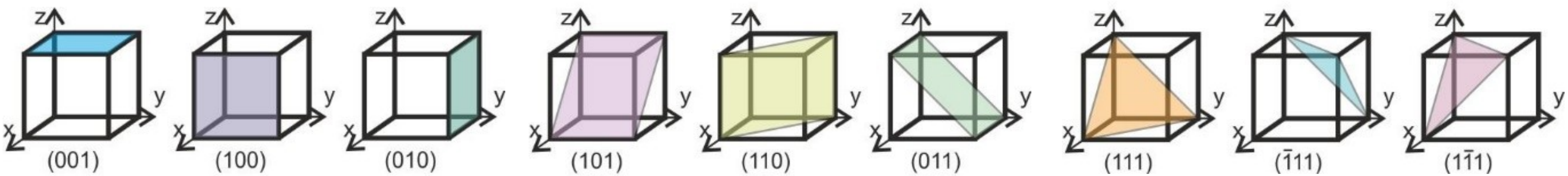
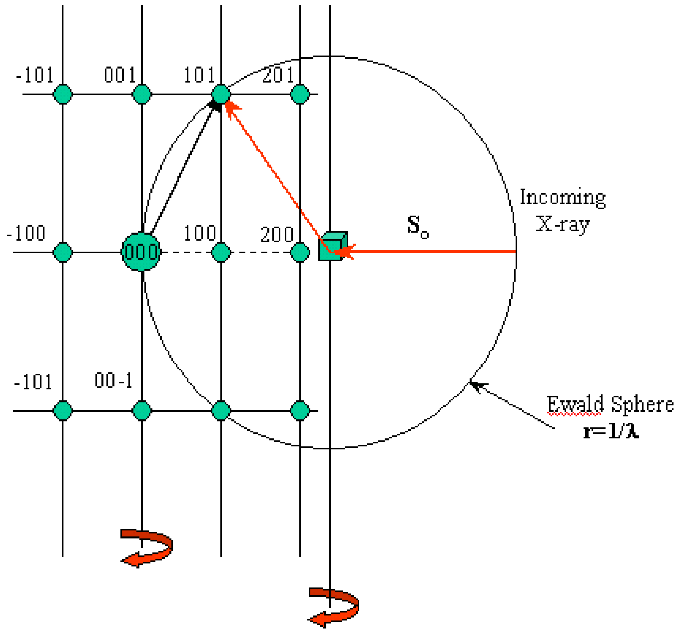


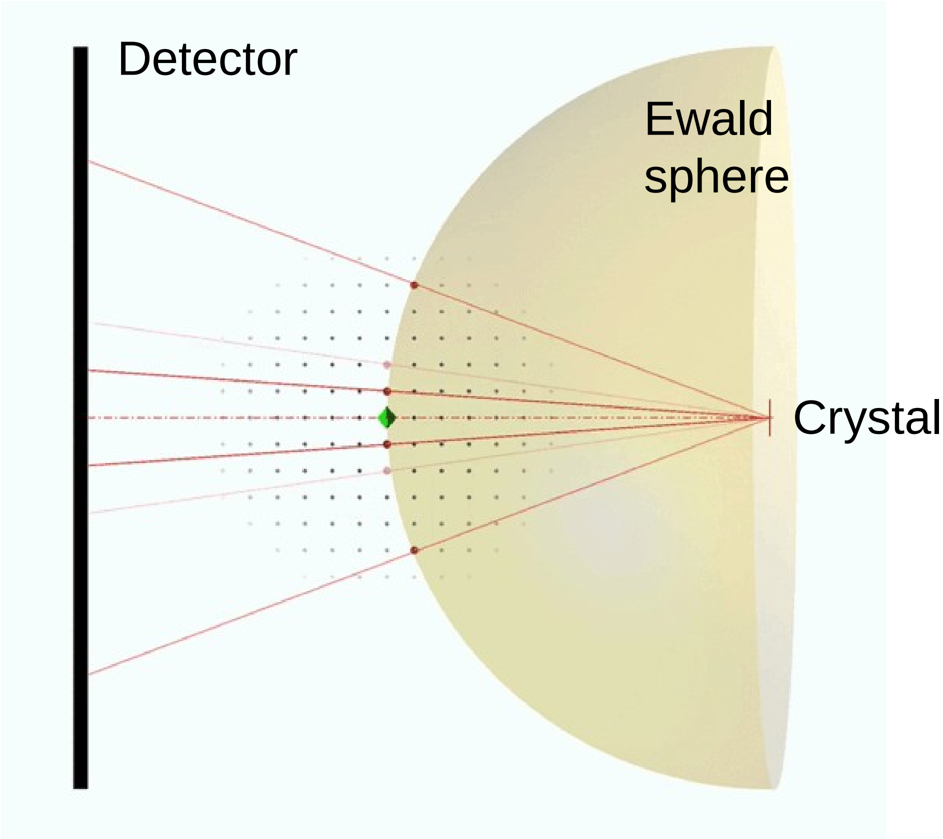
$n\lambda$



Each plane gives a spot of diffraction and this spot is defined by a triplet of integer *hkl* (Miller indices) in the reciprocal space

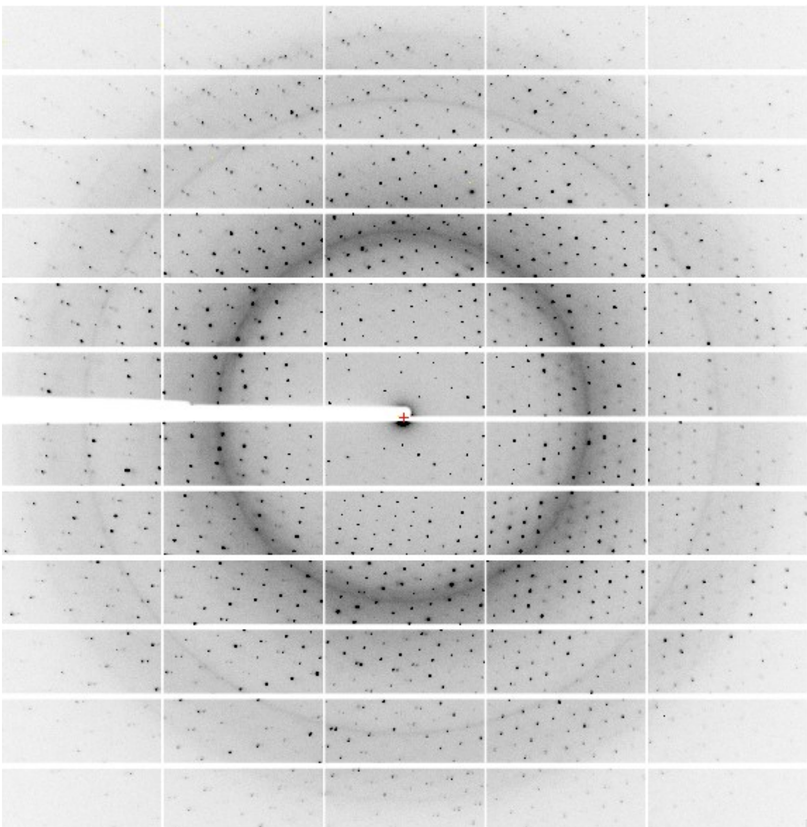
EWALD SPHERE

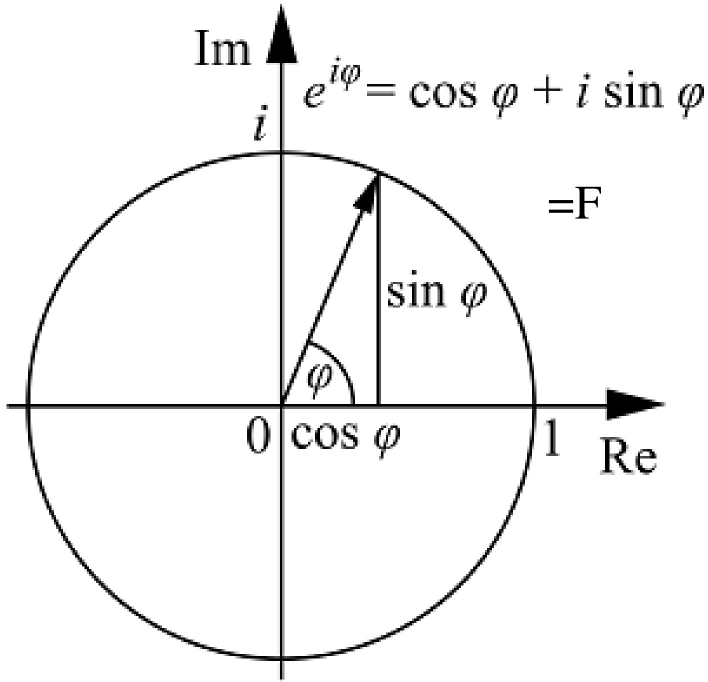




Each diffraction plane is represented as node

CRYSTAL DIFFRACTION





The 2D detector measures Intensities of the diffraction peaks BUT ....

DATASET STATISTICS

	Overall	Inner shell	Outer shell
Resolution (Å)	50-1.40	50 - 5.7	1.47 – 1.40
R_{merge}	0.052	0.022	1.047
R_{meas}	0.065	0.027	1.314
R_{pim}	0.038	0.015	0.785
$CC_{1/2}$	0.997	0.995	0.474
# observations	571512	9792	43402
# unique	127131	2224	9827
<I>/σ(I)	12.8	50.8	1.2
Completeness	0.929	0.88	0.745
Multiplicity	4.5	4.4	4.4

$CC_{1/2}$ the intensities of two randomly sets of reflections correlates

<I>/σ(I) Intensity of the reflection over the error

Completeness, ratio between the # of unique observations and the total # theoretical reflections

Multiplicity, ratio between # of observed and unique reflections

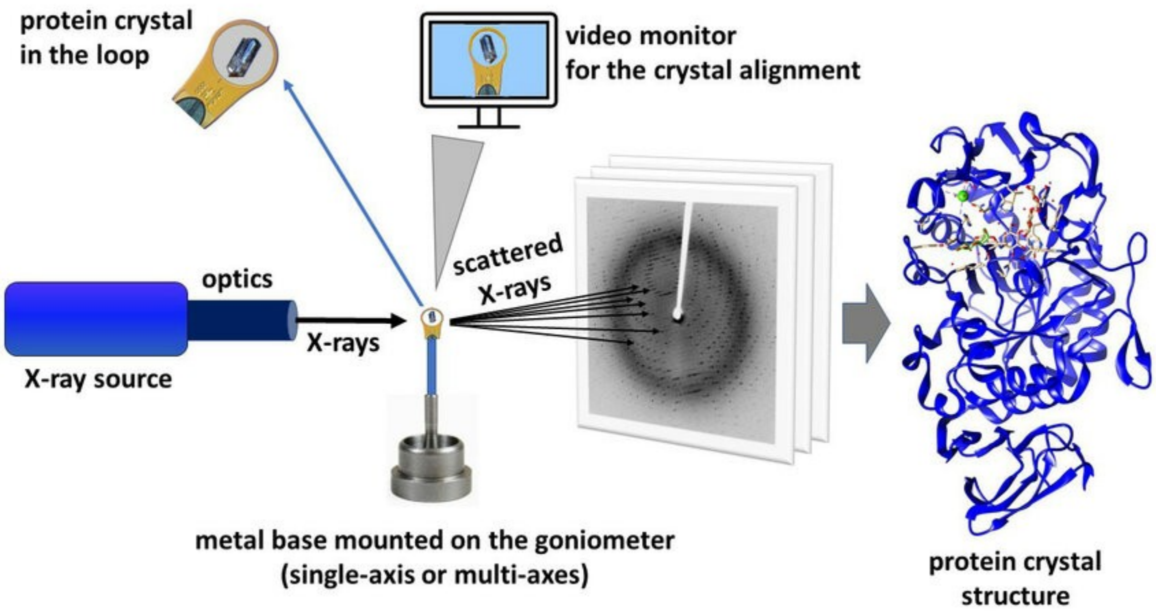
$$R_{merge} = \frac{\sum_{hkl} \sum_{i=1}^{n} |I_i(hkl) - \bar{I}(hkl)|}{\sum_{hkl} \sum_{i=1}^{n} I_i(hkl)}$$

$$R_{meas} = \frac{\sum_{hkl} \sqrt{\frac{n}{n-1}} \sum_{i=1}^{n} |I_i(hkl) - \bar{I}(hkl)|}{\sum_{hkl} \sum_{i=1}^{n} I_i(hkl)}$$

$$R_{pim} = \frac{\sum_{hkl} \sqrt{1/n-1} \sum_{i=1}^{n} |I_i(hkl) - \bar{I}(hkl)|}{\sum_{hkl} \sum_{i=1}^{n} I_i(hkl)}$$

merged data

CONTENTS

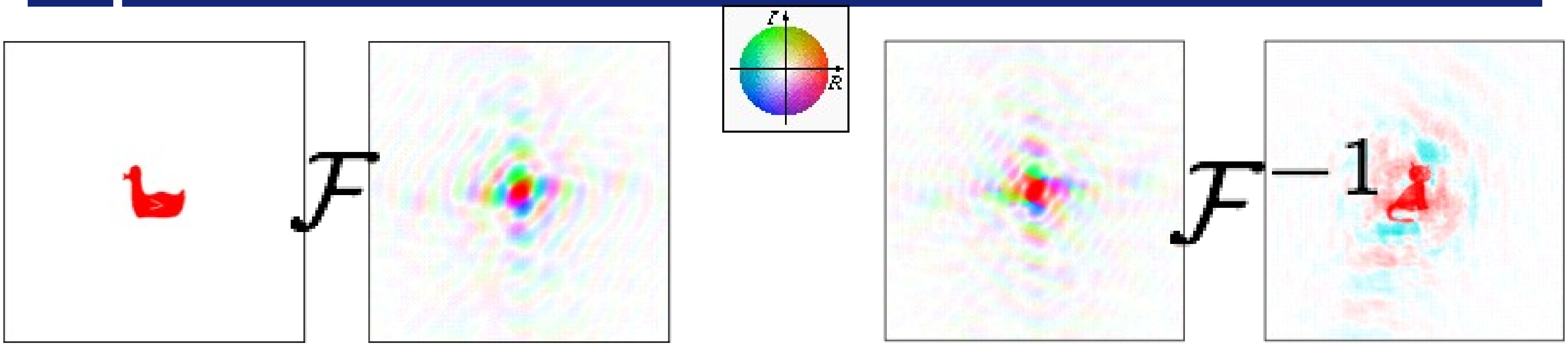


protein crystal in the loop
video monitor for the crystal alignment
optics
scattered X-rays
X-rays
X-ray source
metal base mounted on the goniometer (single-axis or multi-axes)
protein crystal structure

WHY RETRIEVING PHASES

ne electron density over the volume of the ce
'ourier transform)

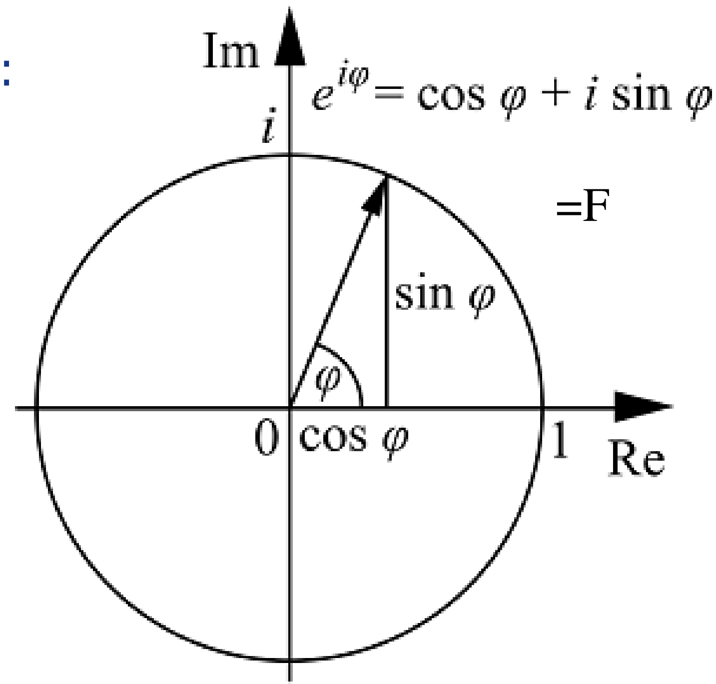
WHY RETRIEVING PHASES



Phases are the most important information to obtain

THE PHASE PROBLEM

The electron density over the volume of the cell:



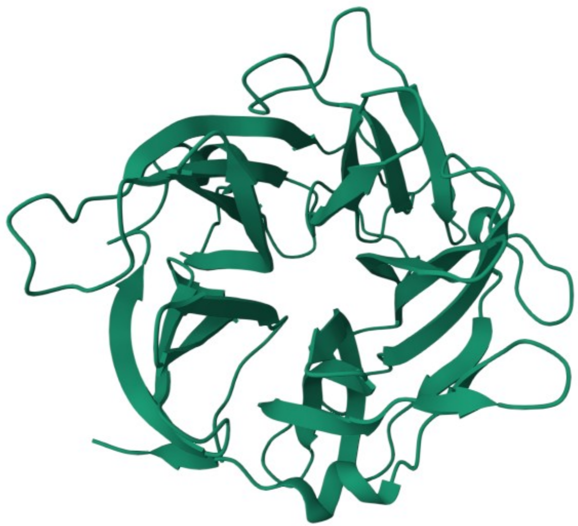
How to retrieve the phase?

Method
Direct (< 1000 non hydrogen / ua)
SIR, MIR
SIRAS, MIRAS
MAD, SAD
Molecular Replacement

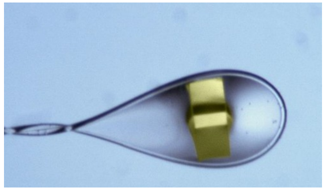
Isomorphous Replacement (SIR, MIR; SIRAS, MIRAS)

The 2D detector measures Intensities of the diffraction peaks BUT ….

HEAVY ATOM DERIVATIVE

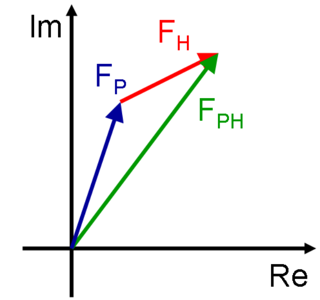


+



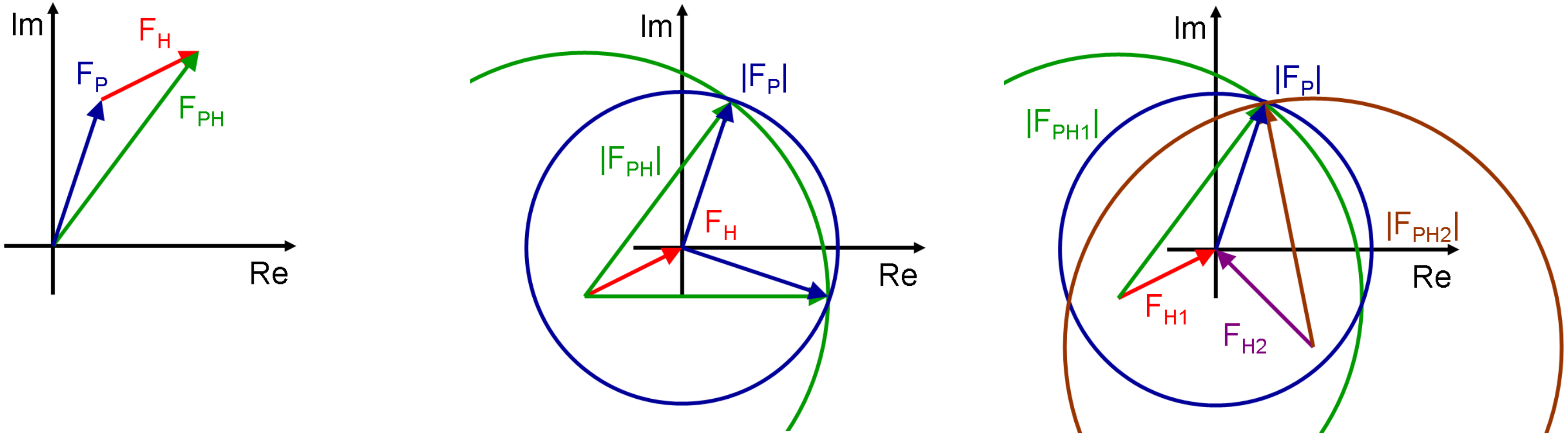
=

F_{ph}



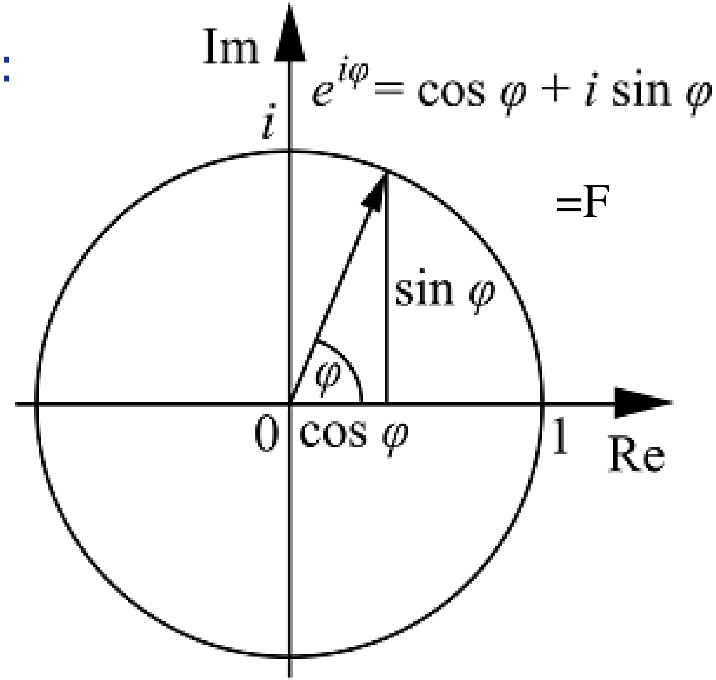
Soaking, noble gazes
co-crystallization, Se-Met derivative, ….

HARKER CONSTRUCTION



THE PHASE PROBLEM

The electron density over the volume of the cell:



How to retrieve the phase?

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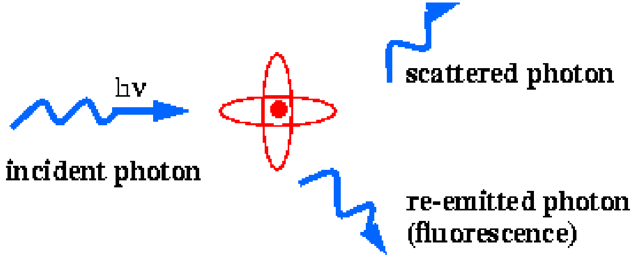
SIR, MIR	***Isomorphous Replacement***
SIRAS, MIRAS	
MAD, SAD	
Molecular Replacement	

The 2D detector measures Intensities of the diffraction peaks BUT ….

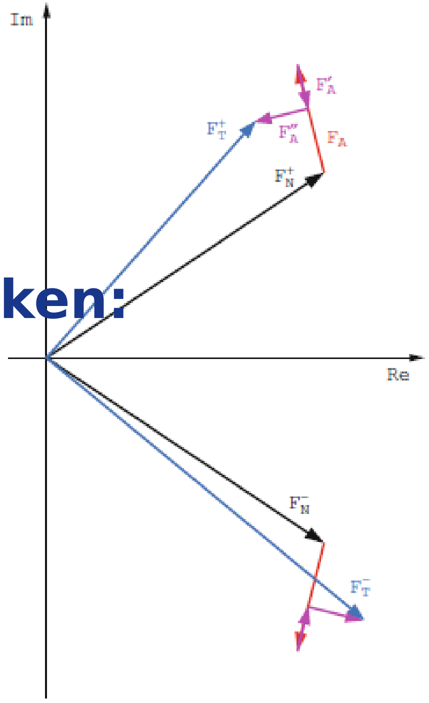
FRIEDEL'S LAW

When incident X-ray energy matches the binding energy of the electrons of the heavy atom, the absorption of X-rays increases.

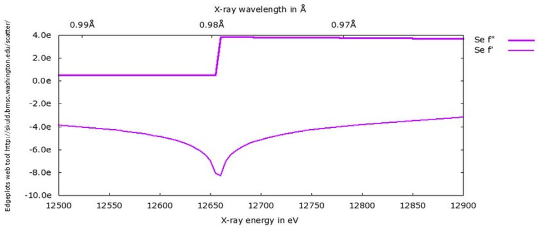
The scattering factor of the HA takes the form of :



Friedel's law is broken:
F(hkl) ≠ F(-h-k-l)

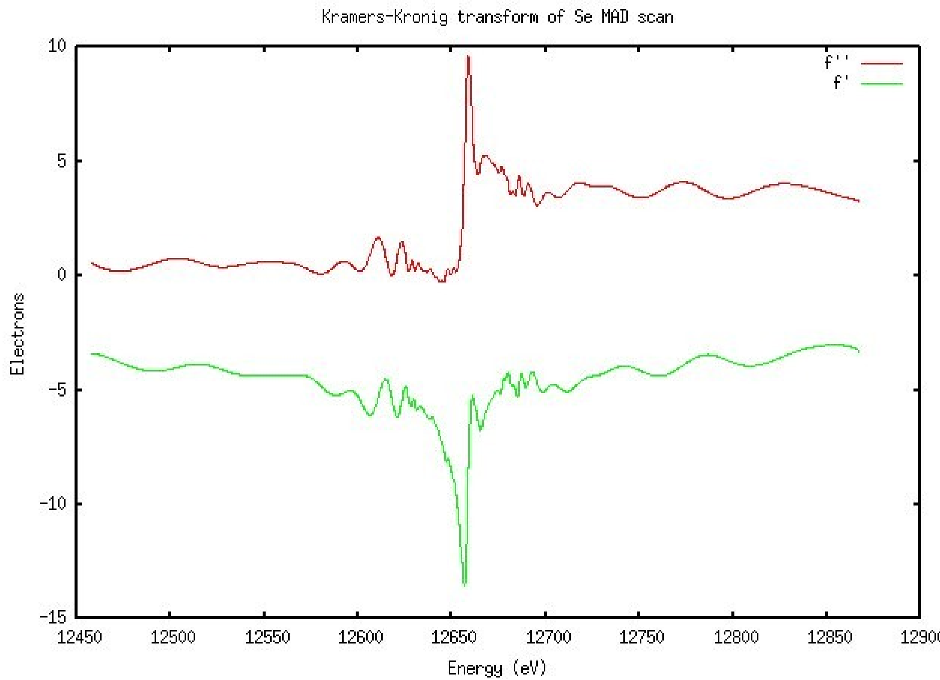


ENERGY SCAN

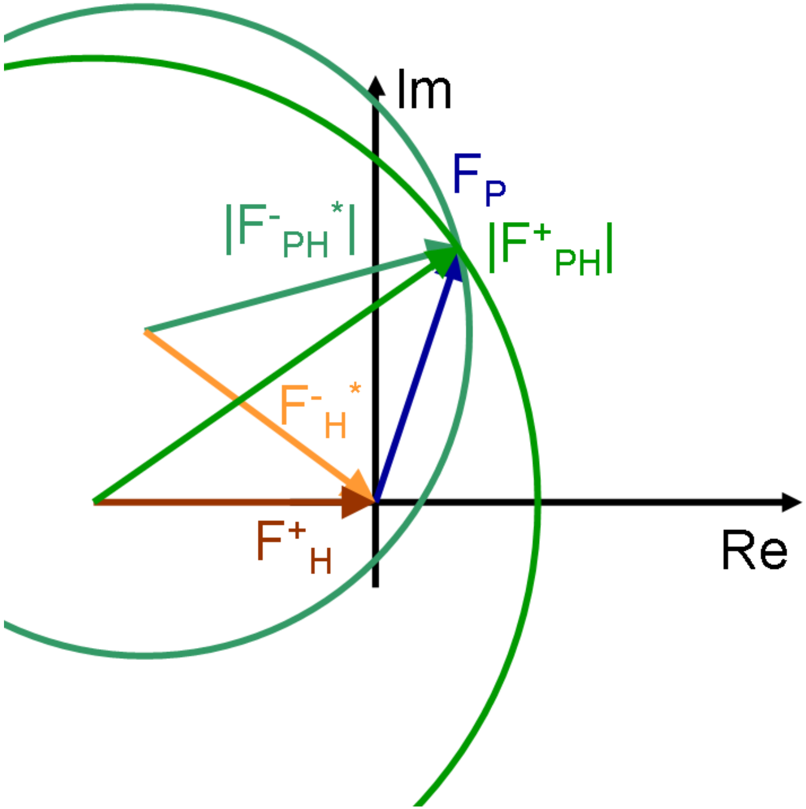


SAD is a single wavelength data collection (high E remote, peak or inflection)

MAD is a 3 or 4 wavelength data collection



SOLVING THE PHASE PROBLEM IN SAD/MAD



Anomalous differences are smaller than for HA.

However, the great advantage of anomalous phasing is that we can use a single crystal (no need for a native data set).

Seleno-methionine has been the silver bullet for the last two decades

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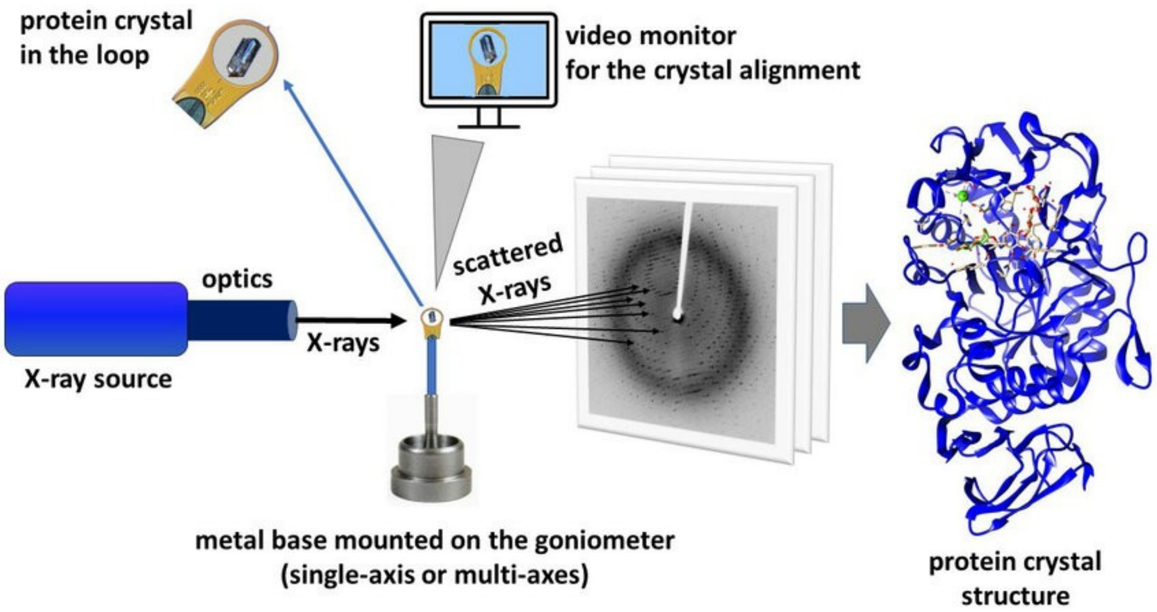
1. *Crystallogenesis*
2. Crystal symmetries
3. *X-rays*
4. *Diffraction*
5. *Phase problem*
    1. *SAD*
    2. *MR*

Practice

6. *Crystal harvesting*
7. *Data collection*
8. *Data processing*
9. *Solving structures*
10. *Examples*



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The electron density over the volume of the cell:

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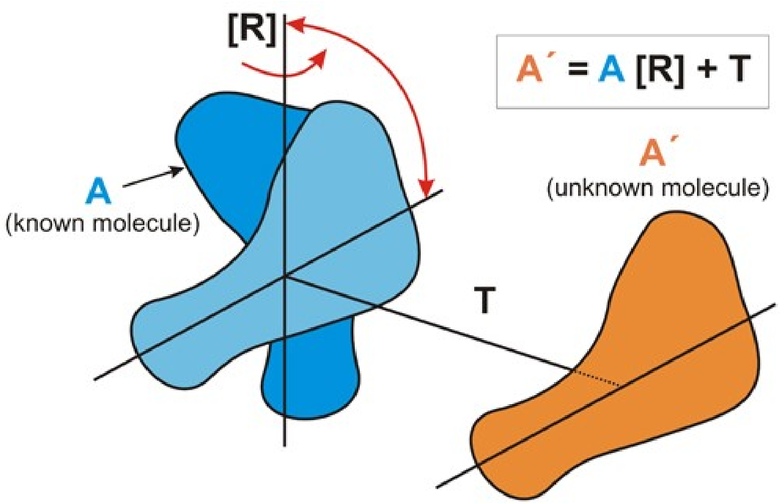
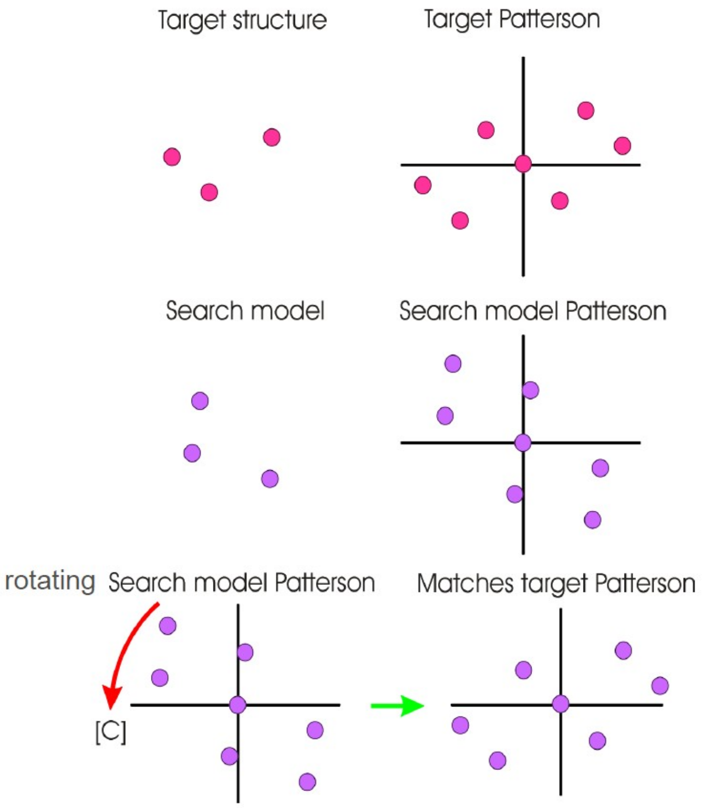


THE ROTATION-TRANSLATION FUNCTION

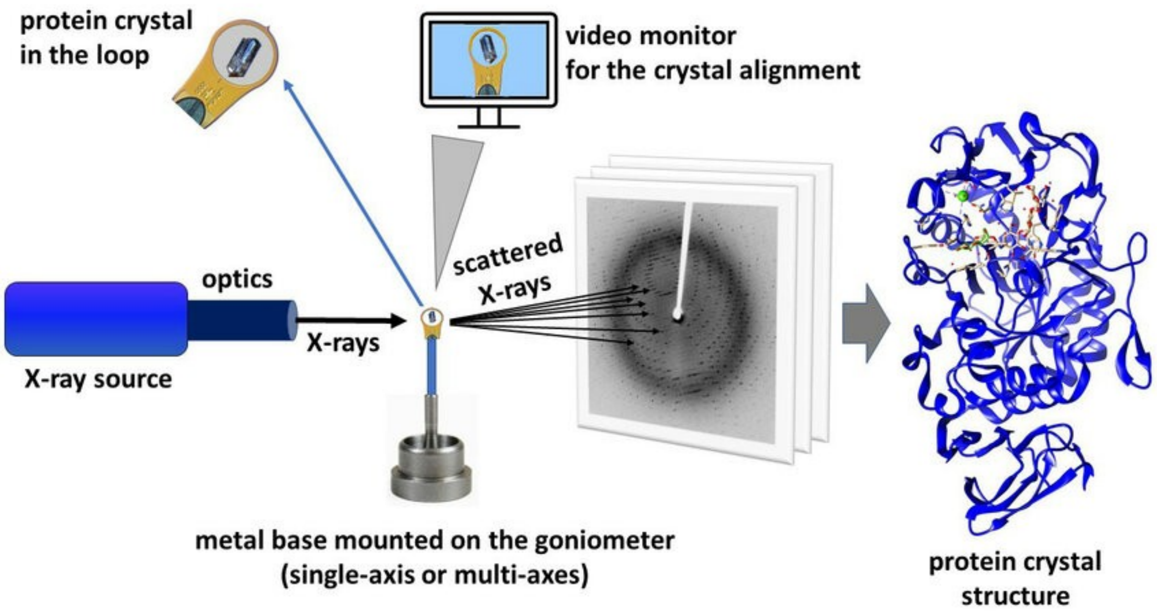
The Patterson function
(phase zero inverse Fourier):

P

R-factor for the translation function:

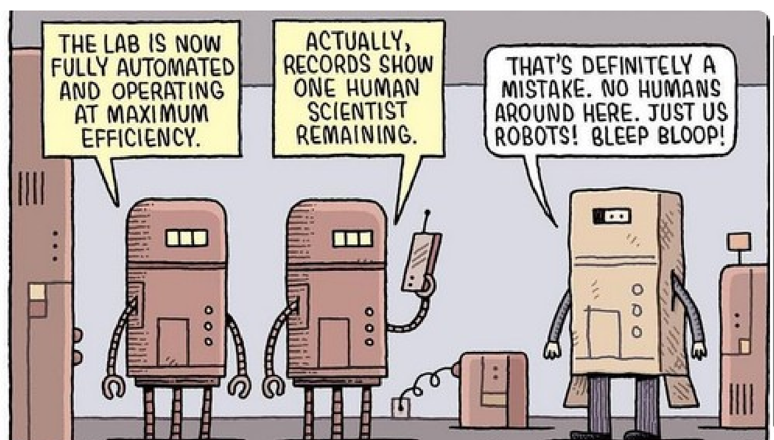


CONTENTS

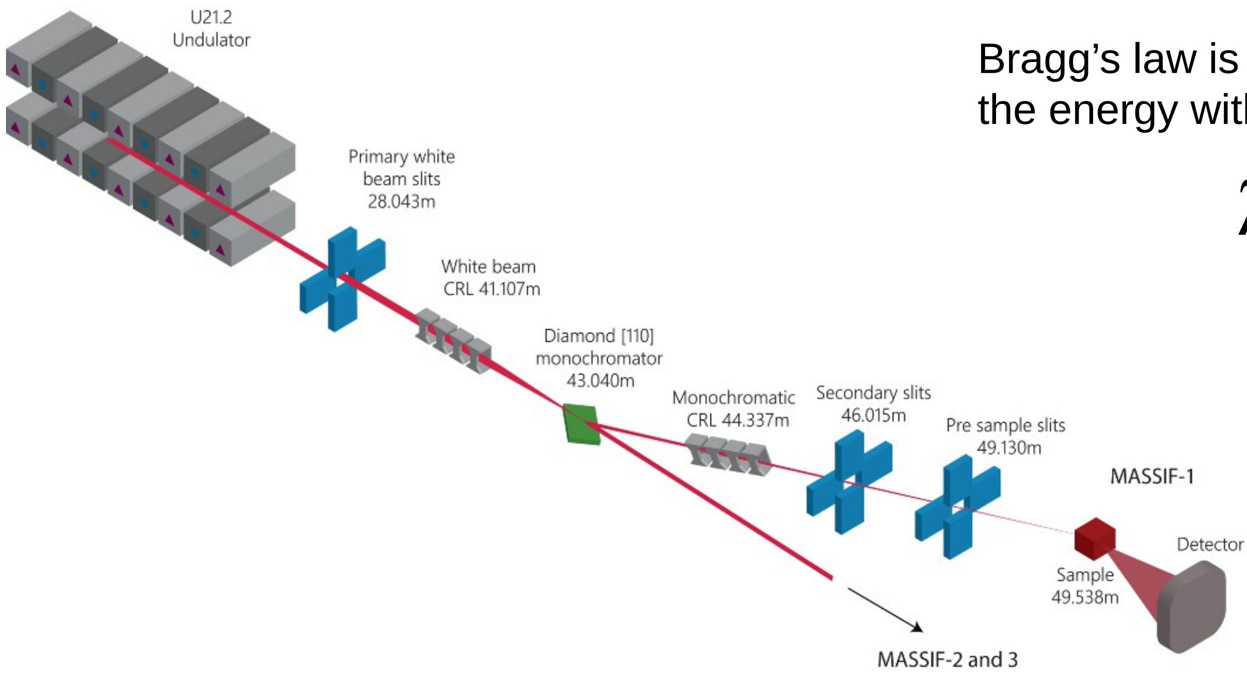


protein crystal
in the loop
video monitor
for the crystal alignment
scattered
X-rays
optics
X-rays
X-ray source
metal base mounted on the goniometer
(single-axis or multi-axes)
protein crystal
structure

Automated synchrotron STRUTURAL BIOLOGY at MASSIF-1



MASSIF-1 LAYOUT

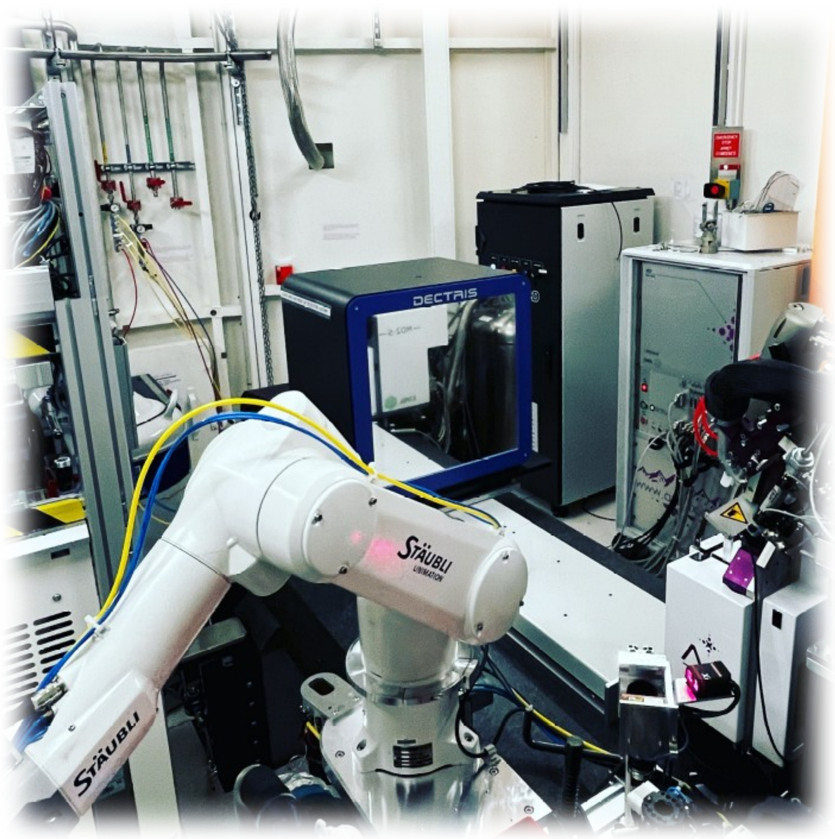


Bragg's law is used also for defining the energy with the monochromator:

$$\lambda$$

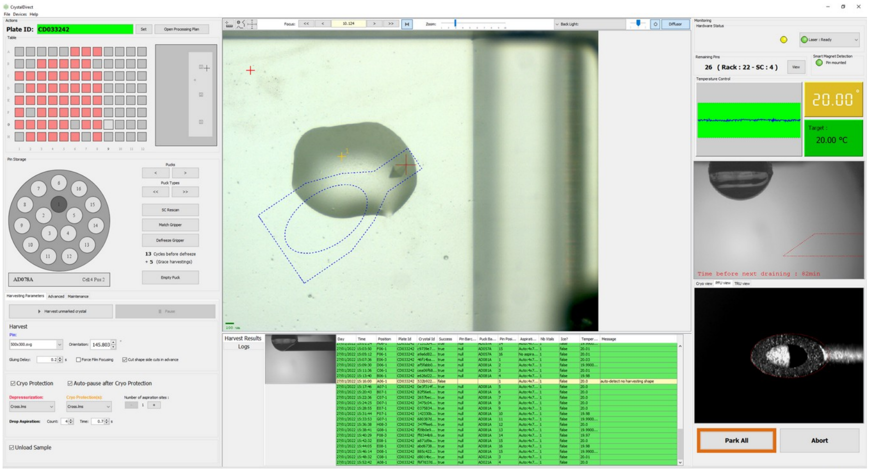
Bowler, MW, Nurizzo, D *et al.* **JSR**, Volume 22 | Part 6 | November 2015 | Pages 1540–1547 |

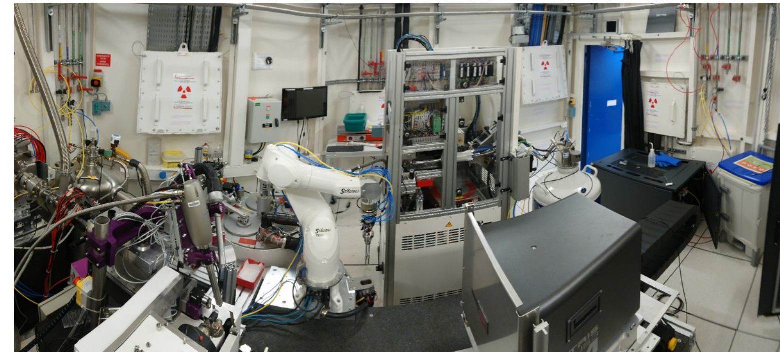
EXPERIMENTAL HUTCH

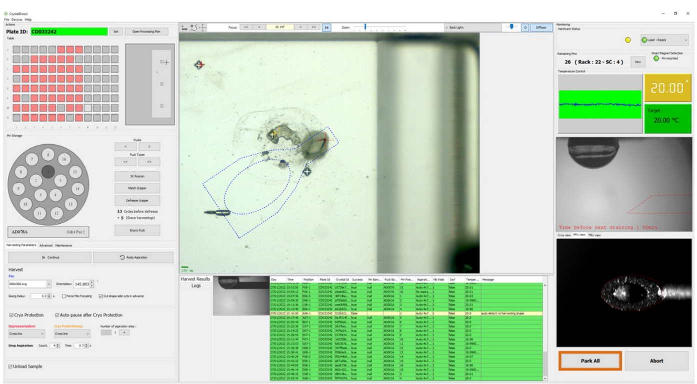


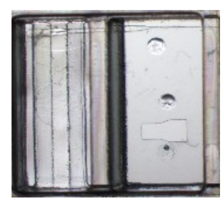
- Fully autonomous beamline
  - no user control
  - data collection optimized for every sample
- Flexible booking, queuing system
- Fully automated data collection from any sample either room or cryogenic temperatures with complex strategies and optimized parameters for each sample
- **Flex HCD Sample changer – 396 samples capacity**
- **Pilatus3 6M**
- **Arinax MD2S diffractometer with mini-Kappa**
- **CrystalDirect Harvester**

AUTOMATED CRYSTAL HARVESTING

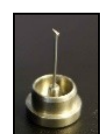


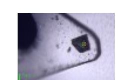






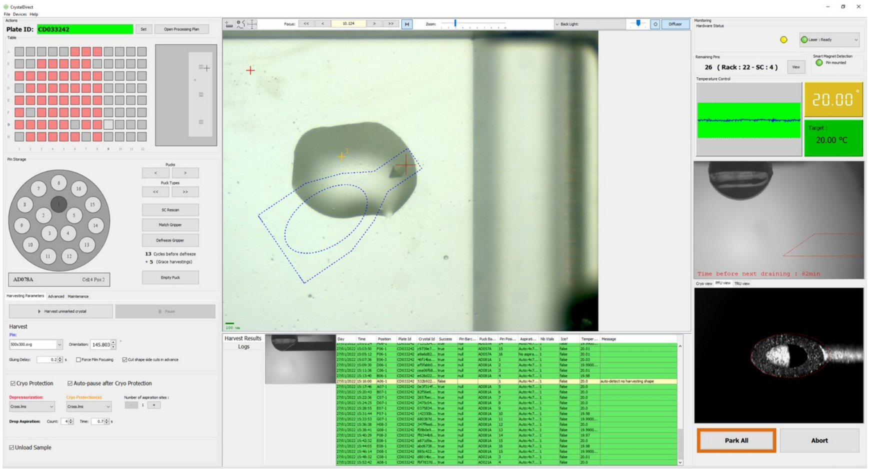
MiTeGen

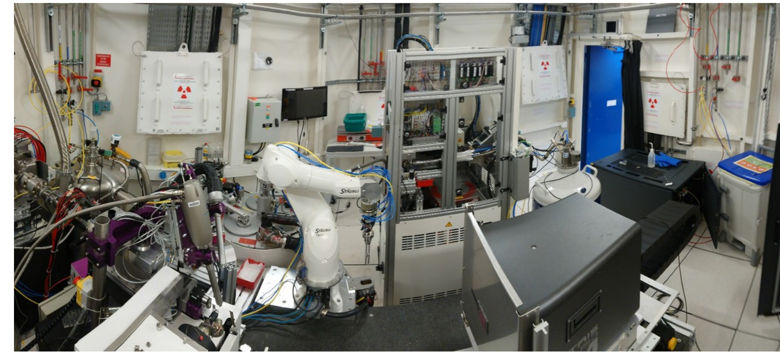


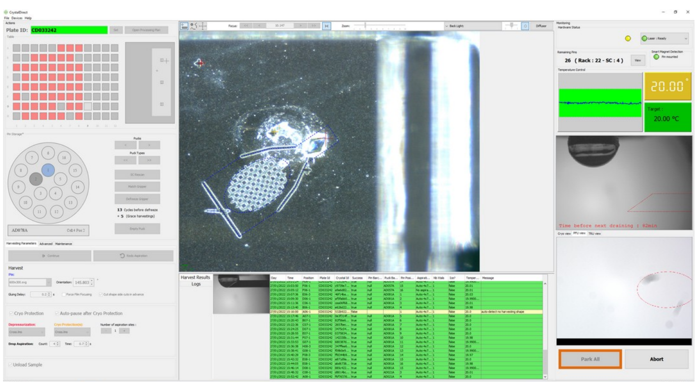


Crystal Direct technology

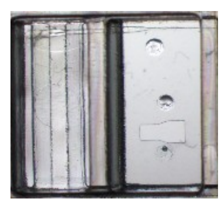
AUTOMATED CRYSTAL HARVESTING

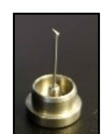


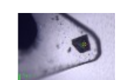




MiTeGen







Crystal Direct technology

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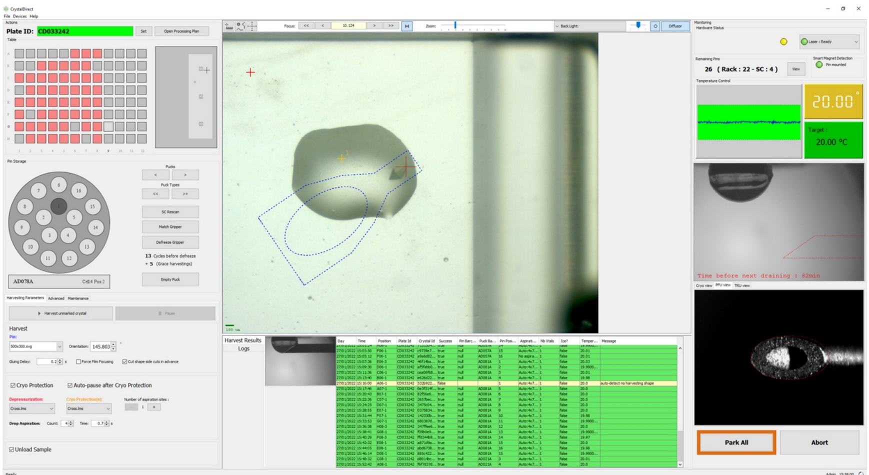
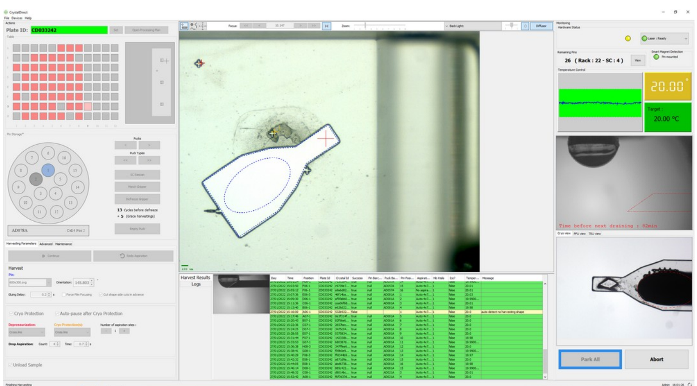
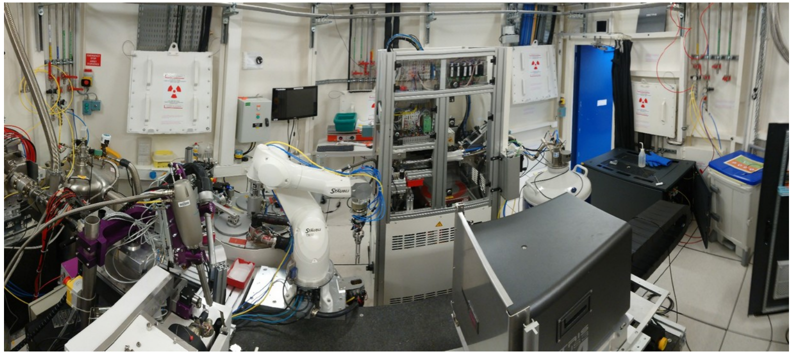
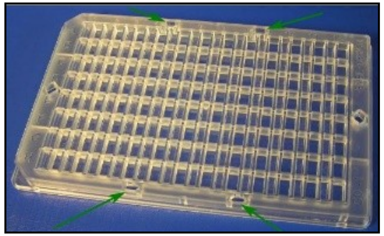
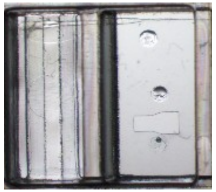


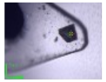




MiTeGen







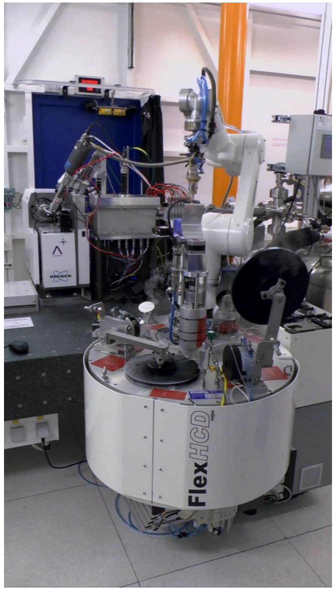
Crystal Direct technology

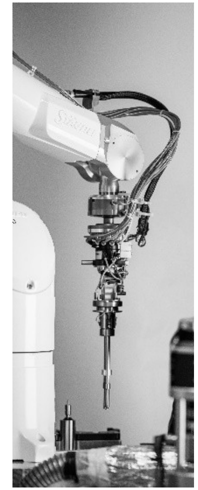
COURIER DELIVERY

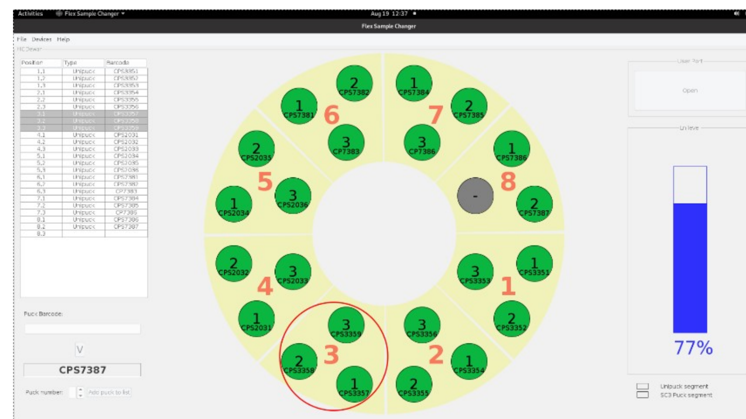
Dry shippers are delivered daily at the beamline as part of our queuing system

Delivery if free of charge for Academic Users

CRYSTAL TRANSFER







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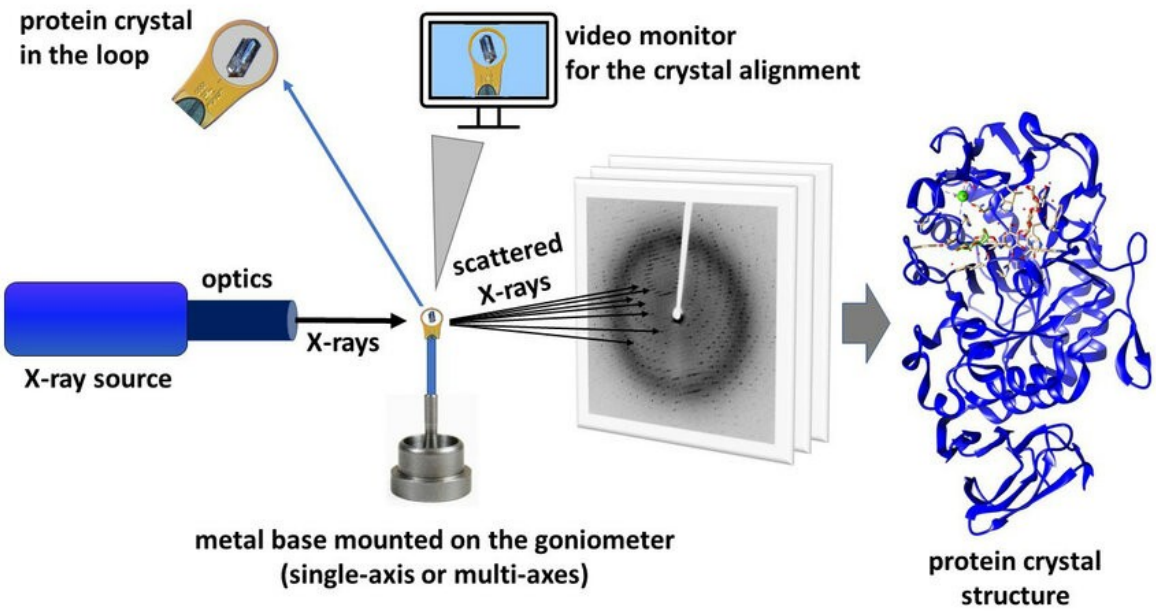
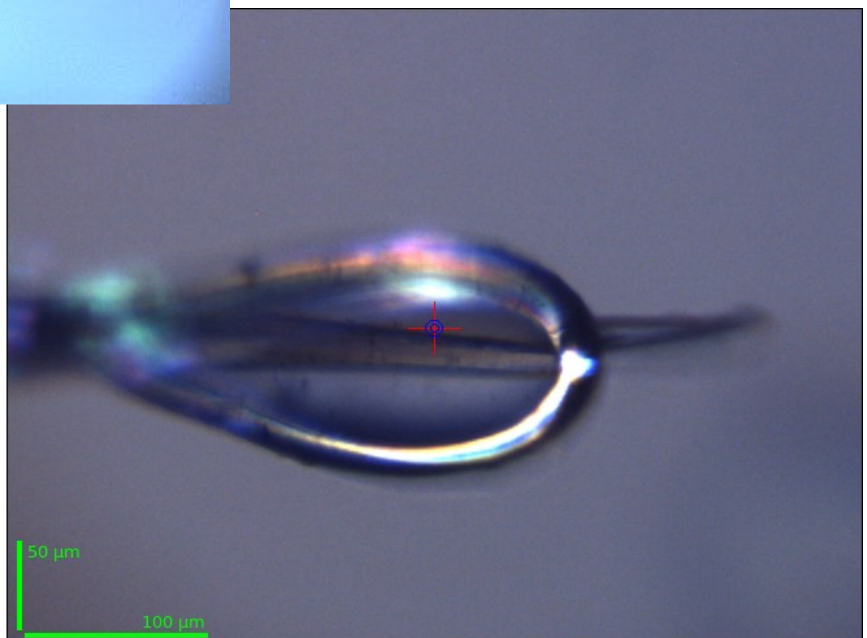
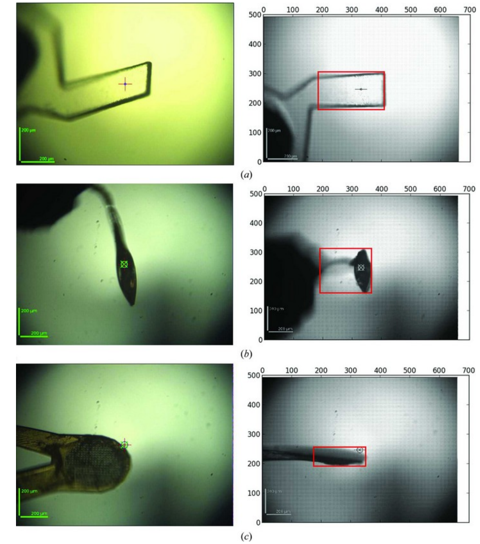


OPTICAL LOOP ALIGNMENT



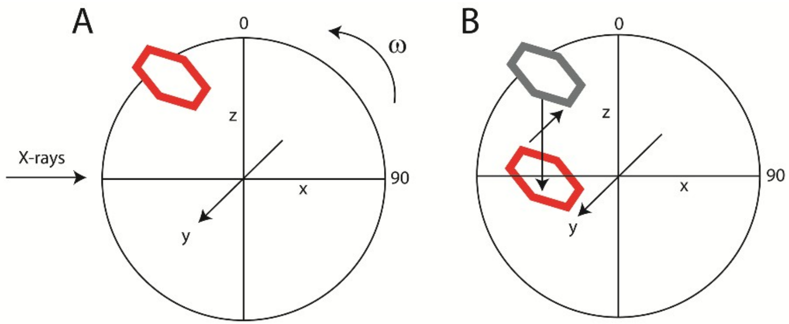
No restrictions on the mounting system

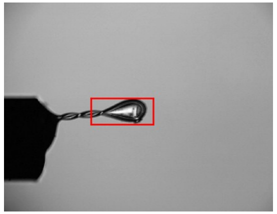


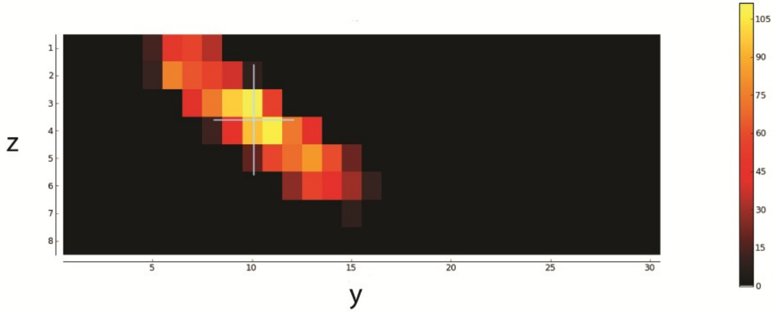
Optical defects might be disastrous

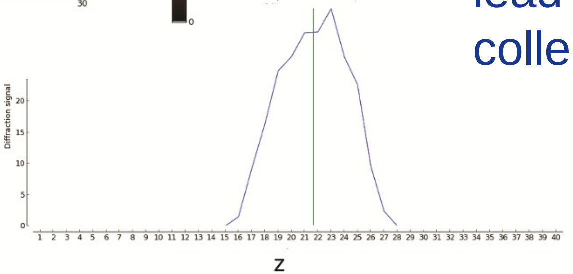
Svensson, O., Malbet-Monaco, S., Popov, A., Nurizzo, D. & Bowler, M. W. (2015). Acta Cryst. D71, 1757-1767.

X-RAY ALIGNMENT





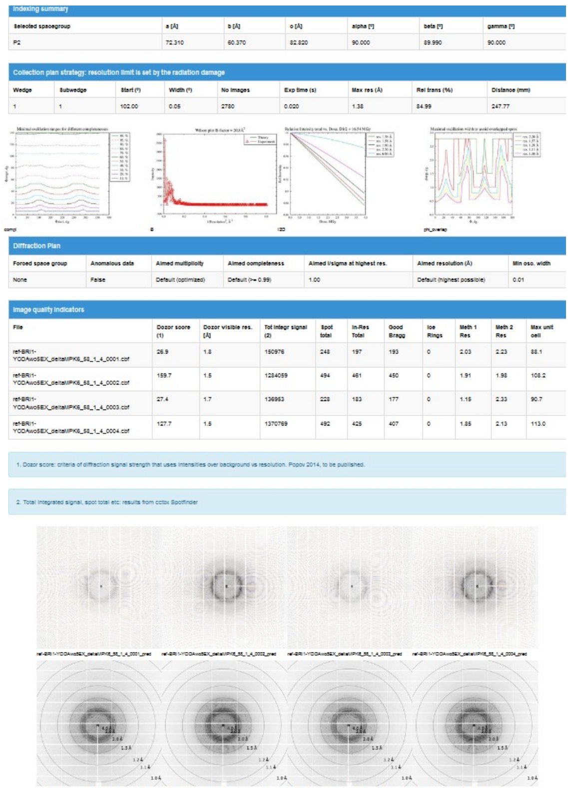




The centering table fitted on the ω axis brings the sample at the center of the camera and in the X-ray beam

Characteristics such as beam size and flux as well as crystal volume lead to highly optimised data collection

CHARACTERIZATION



Indexing summary

Selected spacegroup	a [Å]	b [Å]	c [Å]	alpha [°]	beta [°]	gamma [°]
P2	72.310	60.370	82.820	90.000	89.990	90.000

Collection plan strategy: resolution limit is set by the radiation damage

Wedge	Subwedge	Start (°)	Width (°)	No images	Exp time (s)	Max res (Å)	Rel trans (%)	Distance (mm)
1	1	102.00	0.05	2780	0.020	1.38	84.99	247.77

compl B I2D phi_overlap

Diffraction Plan

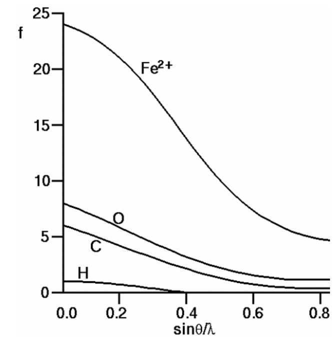
Forced space group	Anomalous data	Aimed multiplicity	Aimed completeness	Aimed I/sigma at highest res.	Aimed resolution (Å)	Min osc. width
None	False	Default (optimized)	Default (>= 0.99)	1.00	Default (highest possible)	0.01

Image quality indicators

File	Dozor score (1)	Dozor visible res. [Å]	Tot integr signal (2)	Spot total	In-Res Total	Good Bragg	Ice Rings	Meth 1 Res	Meth 2 Res	Max unit cell
ref-BRI1-YODAwo5EX_deltaMPK6_58_1_4_0001.cbf	26.9	1.8	150976	248	197	193	0	2.03	2.23	88.1
ref-BRI1-YODAwo5EX_deltaMPK6_58_1_4_0002.cbf	159.7	1.5	1284059	494	461	450	0	1.91	1.98	108.2
ref-BRI1-YODAwo5EX_deltaMPK6_58_1_4_0003.cbf	27.4	1.7	136953	228	183	177	0	1.15	2.33	90.7
ref-BRI1-YODAwo5EX_deltaMPK6_58_1_4_0004.cbf	127.7	1.5	1370769	492	425	407	0	1.85	2.13	113.0

1. Dozor score: criteria of diffraction signal strength that uses intensities over background vs resolution. Popov 2014, to be published.

2. Total integrated signal, spot total etc: results from cctbx Spotfinder



Cell parameters a,b,c, α,β,γ

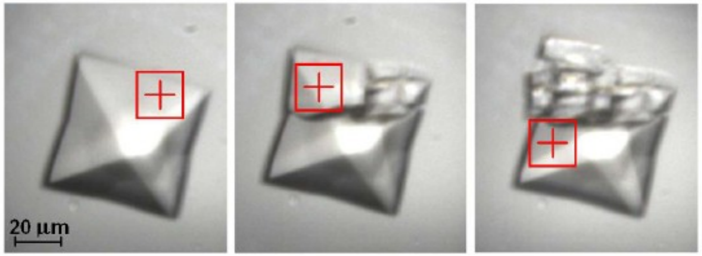
Point group (Bravais type)

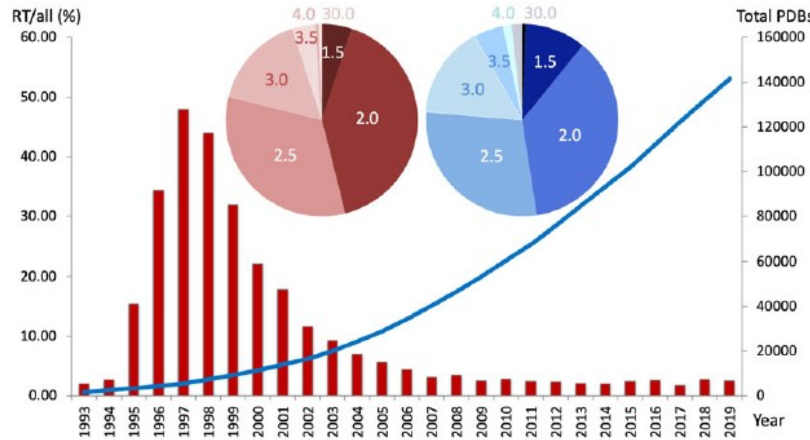
The total rotation angle to collect the entire reciprocal space

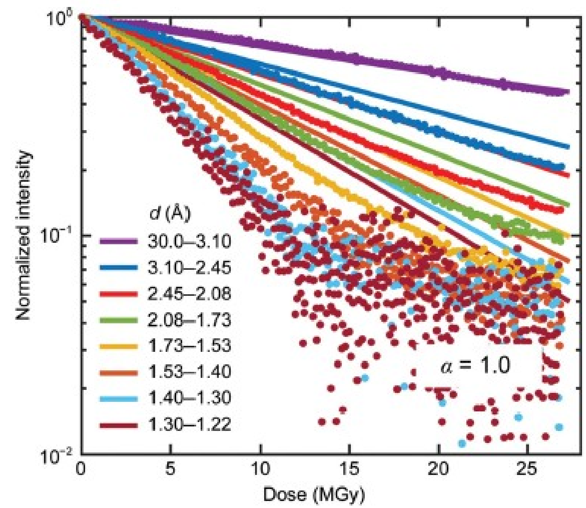
The expected resolution according to the crystal size and beam size

Check the quality of X-ray centering (feedback loop)

DC STRATEGY – RADIATION DAMAGE





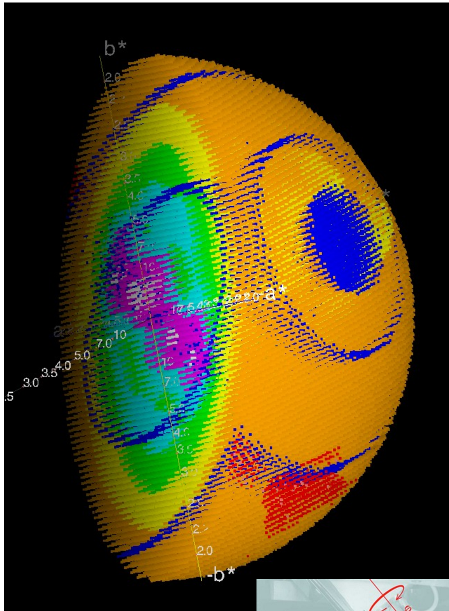


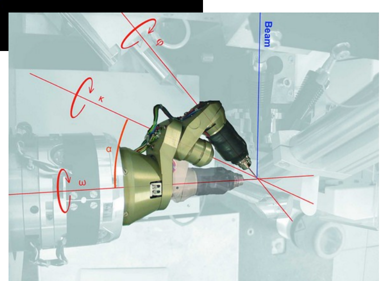
DC is mainly done (>90%) at cryo-temperature

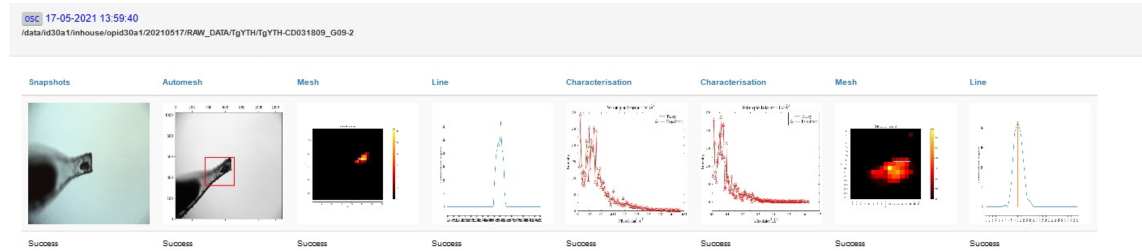
RD is visible even at cryo-temperature

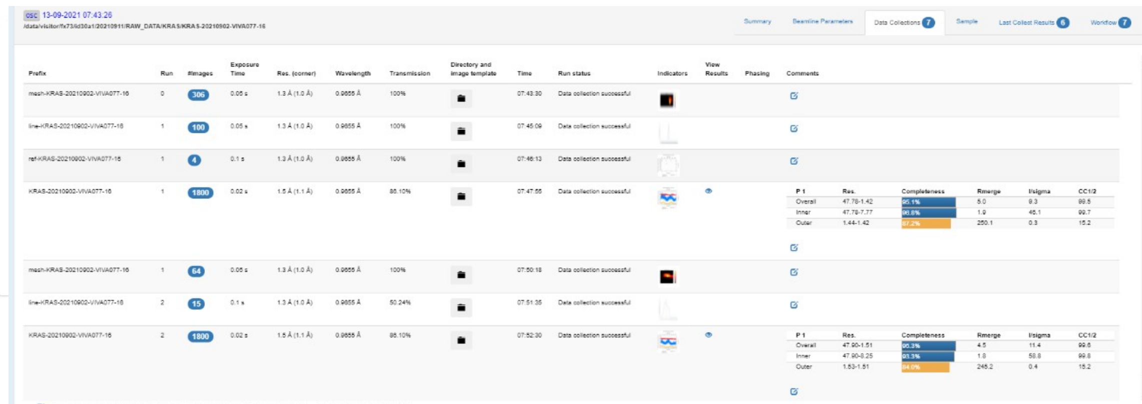
Exposure time / transmission is adjusted to collect a **complete** data set without **theoretical** RD (Henderson limit = 20MGy)

CUSP IN P1









In triclinic and monoclinic SG some volume of reciprocal space is not accessible with a rotation around a single axis (mini-κ)

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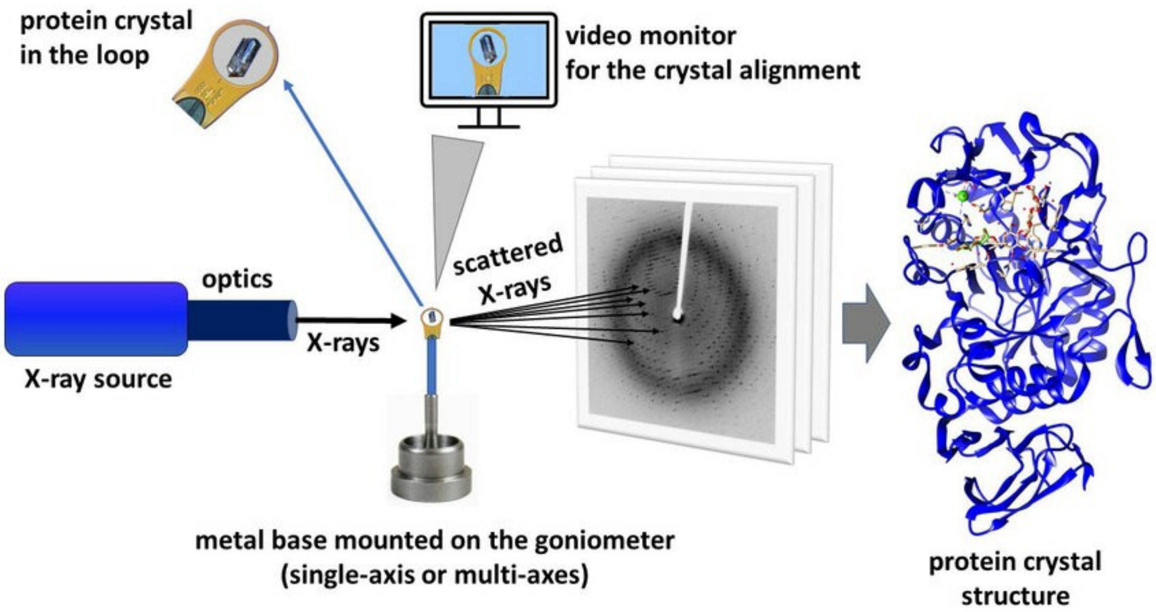
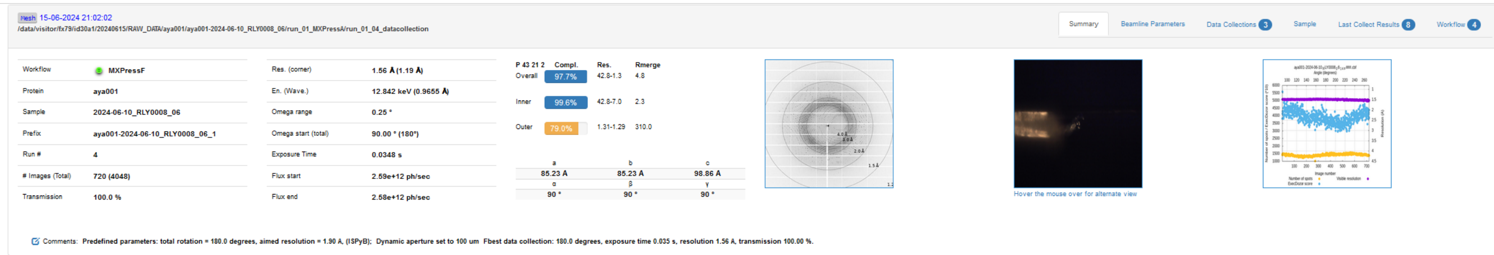


protein crystal
in the loop
video monitor
for the crystal alignment
optics
scattered
X-rays
X-rays
X-ray source
metal base mounted on the goniometer
(single-axis or multi-axes)
protein crystal
structure



Mesh 15-06-2024 20:56:52
/data/visitor/fx79/id30a1/20240615/RAW_DATA/aya001/aya001-2024-06-10_RLY0008_05/run_01_MXPressA/run_01_04_datacollection

Summary | Beamline Parameters | Data Collections 3 | Sample | Last Collect Results 8 | Workflow 4

	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
ANOM BEST	XDSAPP	P 43 21 2	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	49.5-1.3 49.5-7.1 1.3-1.3	9.8 10.3 2.1	96.8 99.7 69.2	4.8 6.5 1.4	90.0 99.7 33.3	15.7 67.3 0.1	6.5 4.4 596.6	5.9 3.8 360.1	2.5 1.6 321.0	100 100 10				
ANOM	XIA2_DIALS	P 41 21 2	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	84.9-1.4 85.1-3.7 1.4-1.4	10.8 12.1 3.7	99.8 100.0 95.5	5.7 6.9 2.1	97.4 100.0 74.1	16.3 78.0 0.3	6.5 3.7 216.1	5.9 3.4 166.4	2.5 1.4 127.8	100 100 40				
ANOM	EDNA_proc	P 41 21 2	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	49.5-1.5 49.5-5.8 1.6-1.5	12.5 10.8 10.0	100.0 99.8 99.9	6.3 6.4 4.9	100.0 100.0 99.8	20.7 63.4 0.9	6.2 3.9 258.7	5.6 3.5 232.9	2.3 1.5 112.1	100 100 60				
ANOM	grenades_parallelproc	P 41 21 2	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	99.0-1.6 99.0-8.5 1.6-1.6	12.8 9.7 11.1	99.7 99.9 97.3	6.4 6.4 5.5	99.7 100.0 97.2	23.4 67.7 1.0	5.9 4.2 240.7	5.4 3.8 215.0	2.2 1.6 97.7	100 100 60				
ANOM	grenades_fastproc	P 4 2 2	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	99.0-1.5 99.0-8.3 1.5-1.5	12.6 9.9 9.5	99.9 99.7 97.4	6.3 6.4 4.7	99.8 100.0 97.2	22.2 69.9 0.8	6.1 4.1 272.2	5.6 3.6 243.2	2.3 1.5 119.4	100 100 60				
ANOM	grenades_parallelproc	P 4 2 2	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	99.0-1.6 99.0-8.5 1.6-1.6	12.8 9.7 11.1	99.7 99.8 97.3	6.4 6.4 5.5	99.7 100.0 97.3	23.1 68.3 1.0	6.0 4.2 256.4	5.5 3.7 228.8	2.3 1.6 103.9	100 100 60				
ANOM	grenades_parallelproc	P 4	84.9 84.9 99.0	90.0 90.0 90.0	Overall Inner Outer	99.0-1.6 99.0-8.7 1.6-1.6	6.7 6.0 6.7	99.6 99.9 96.4	3.3 3.1 3.3	99.1 95.8 96.3	18.7 57.1 1.1	5.7 4.0 182.3	4.7 3.1 151.2	3.0 2.0 97.1	100 100 60				
ANOM	grenades_parallelproc	P 1	84.9 84.8 99.0	90.0 90.0 90.0	Overall Inner Outer	99.0-1.7 99.0-9.2 1.7-1.7	1.8 1.8 1.8	96.0 98.6 91.4	0.6 1.0 1.0	60.3 72.8 54.1	11.8 33.4 1.0	5.5 4.7 109.6	4.4 2.4 102.3	4.4 2.4 102.3	100 100 50				

Comments: Predefined parameters: total rotation = 180.0 degrees, aimed resolution = 1.90 A, (ISPyB); Dynamic aperture set to 100 um Fbest data collection: 180.0 degrees, exposure time 0.037 s, resolution 1.63 A, transmission 100.00 %.

Mesh 15-06-2024 20:51:45
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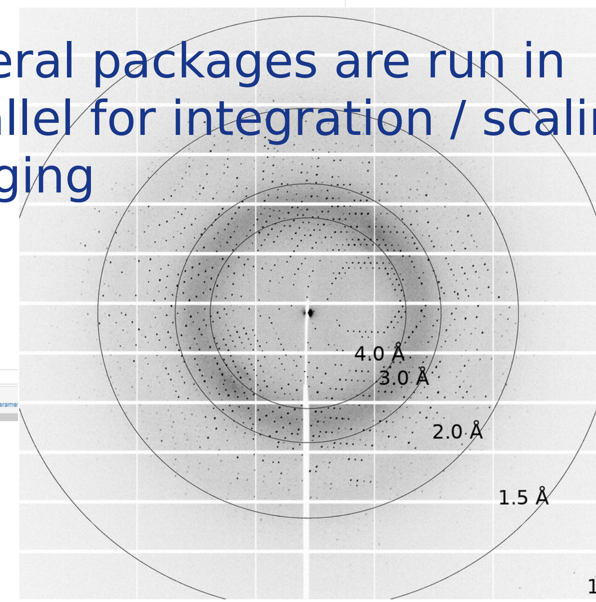
Summary | Beamline Parame

Several packages are run in parallel for integration / scaling / merging



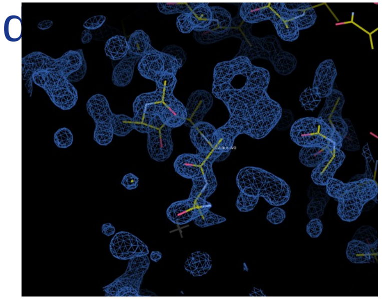
SOLVING THE PHASE PROBLEM

SAD

look for anomalous signal comparing F(hkl) and F(-h-k-l) in XDS

Find the heavy atom site(s) with SHELXD

Run density modification (NCS, solvent flattening, histogram matching) in both hand

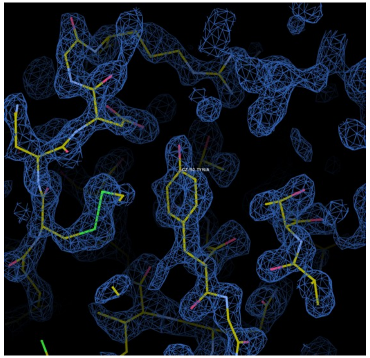


MR

look for a model in the PDB with same cell or use the model provided by the user

Run the molecular replacement (Rotation and translation function)

Refine the MR model



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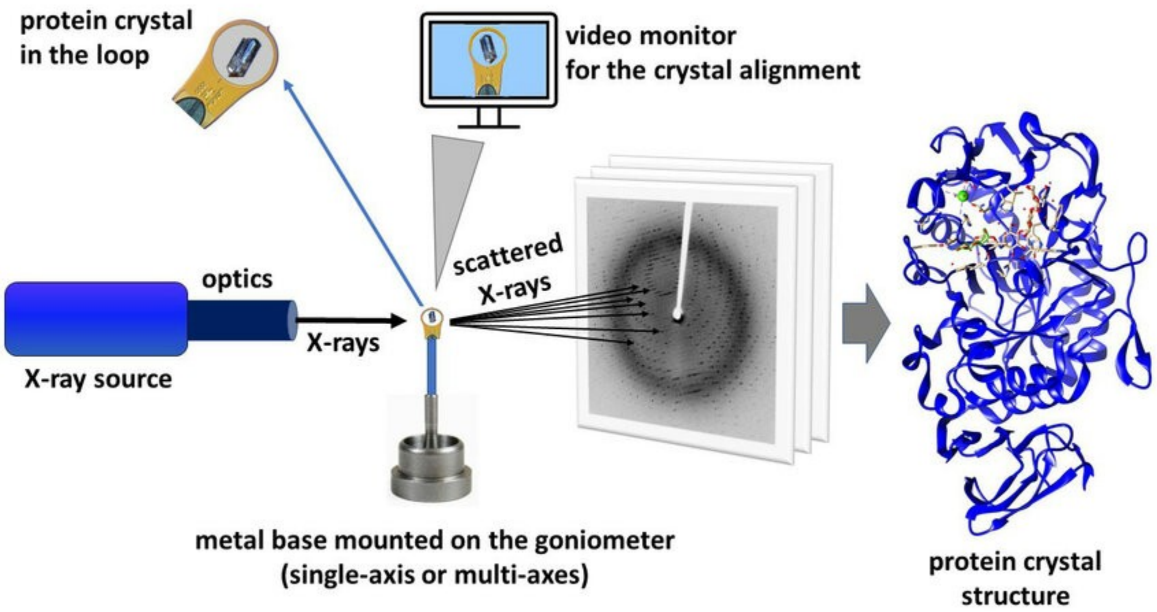
5. *Phase problem*

Practice

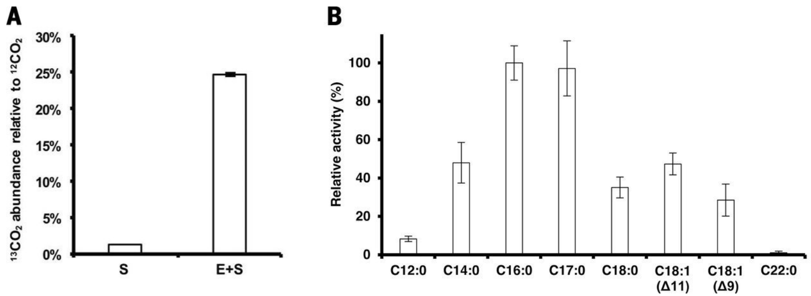
6. *Crystal harvesting*
7. *Data collection*
8. *Data processing*
9. *Examples*

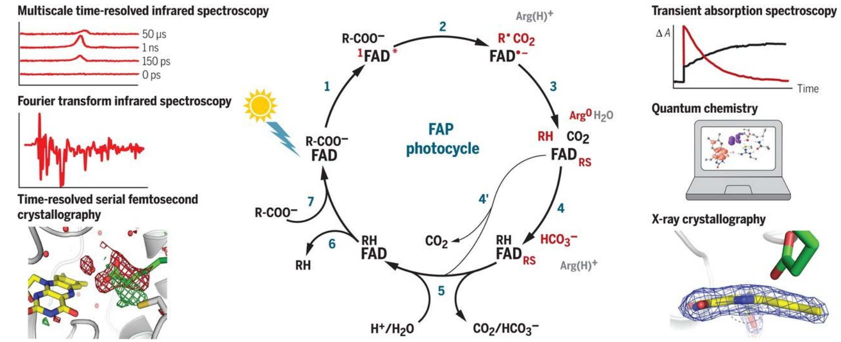


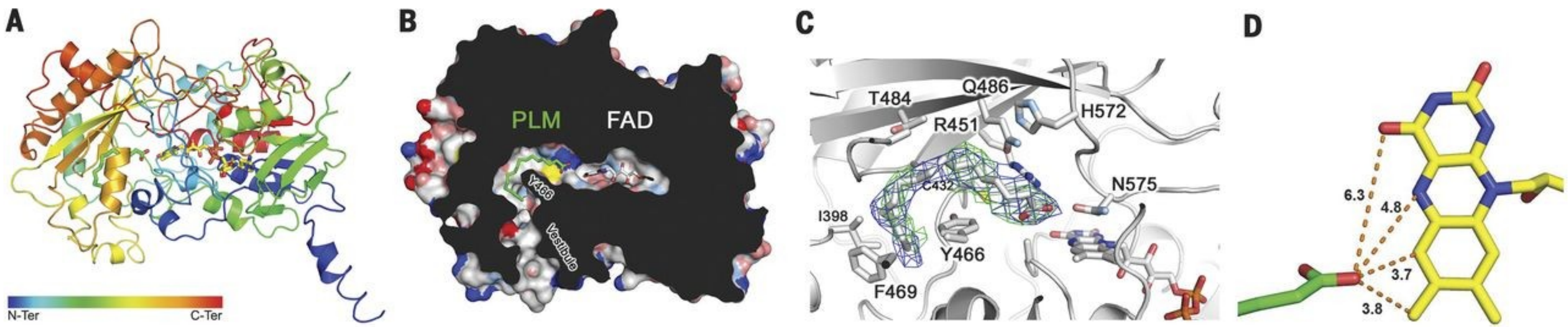
DESIGN OF MEMBRANE PROTEINS ANALOGUES

Computational design of soluble and functional membrane protein analogues. Casper et *al*. Nature DOI: 10.1038/s41586-024-07601-y

FATTY ACID PHOTODECARBOXYLASE







An algal photoenzyme converts fatty acids to hydrocarbons. Sorrigué et *al*. Science. DOI: 10.1126/science.aan6349

During a protein crystallography experiments, most of the X-ray photons that interact with the sample...

a. damage the sample, and only a small part contributes to the diffraction pattern

b. contribute to the diffraction pattern, and only a small part damages the sample

c. cause fluorescence in the sample

How can synchrotron radiation be advantageous for protein crystallography studies?

a. Because of its high level of automation

b. Because of its high intensity

c. Because of its high stability

Why do we collect diffraction data at 100K?

a. Crystal quality is higher at 100K than at room temperature

b. To reduce the size of the cell

c. To reduce radiation damage

d. Crystals are easier to carry at 100K

Why proteins crystalize only in 65 space groups out of the 230?

a. Because of the chirality of the amino-acid Cα

b. Because their molecular weight being too high

c. Because of the water molecules surrounding the protein in the mother liquor

What is mathematical relation between the electron density and the amplitudes?

a. A linear regression

b. A Fourier transform

c. The square root