X-ray sources and detectors: past and present evolution

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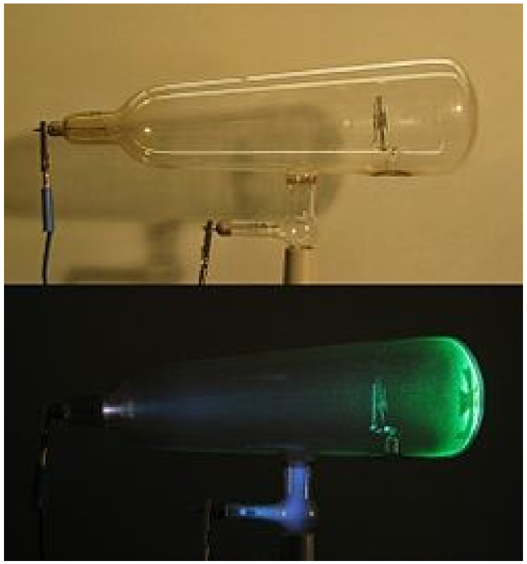
4ème École de Biologie Structurale Intégrative
RéNaFoBiS
Oléron – du 16 au 23 juin 2017

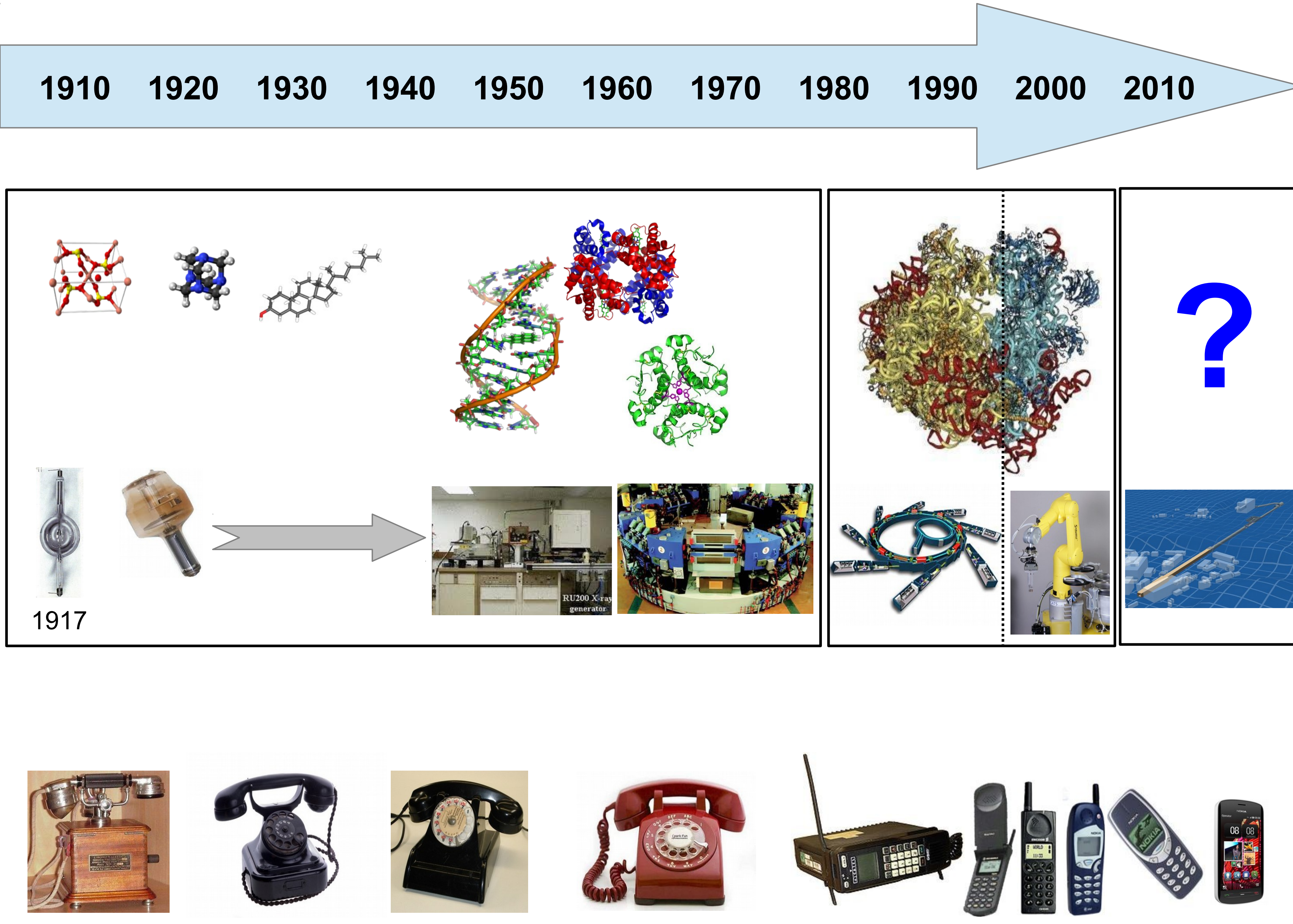
1895: First X-rays



Crookes tubes are cold cathode tubes: from a few kilovolts to about 100 kilovolts is applied between the electrodes.
The Crookes tubes require a pressure from about 10-6 to 5×10-8 atmosphere.

German physicist Wilhelm Röntgen, credited as the discoverer of X-rays in 1895

Wilhelm Röntgen's first "medical" X-ray, of his wife's hand, taken on 22 December 1895 and presented to Ludwig Zehnder of the Physik Institut, University of Freiburg, on 1 January 1896


1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
2010
1917
RU200 X-ray generator
?

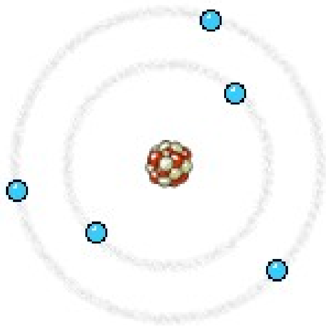
1895-...: sealed tubes / rotating anodes
1-2nd generation synchrotrons

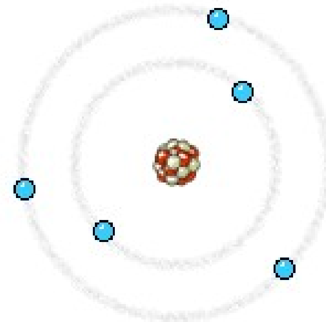
X-ray sources
Films / IP

Capillaries

Isomorphous replacement

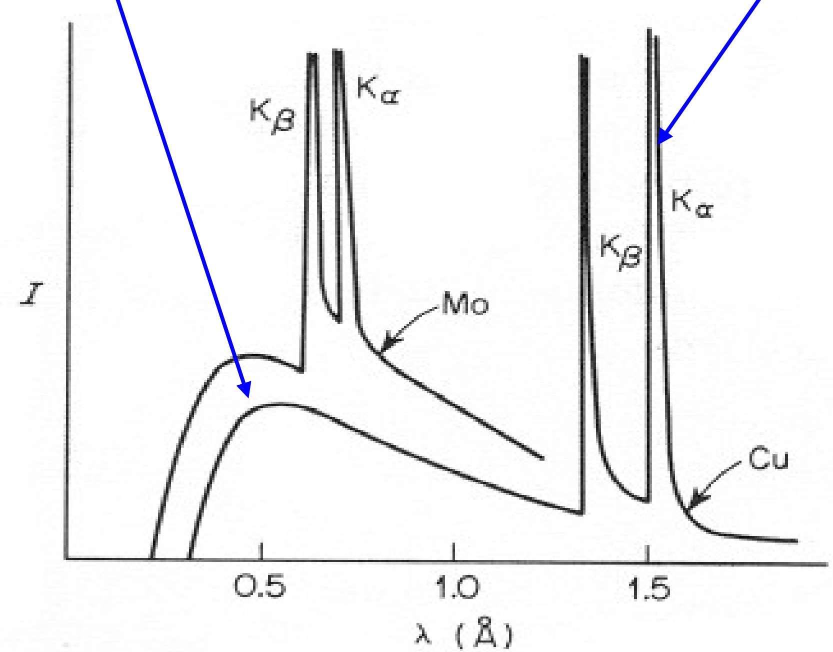
Lab sources

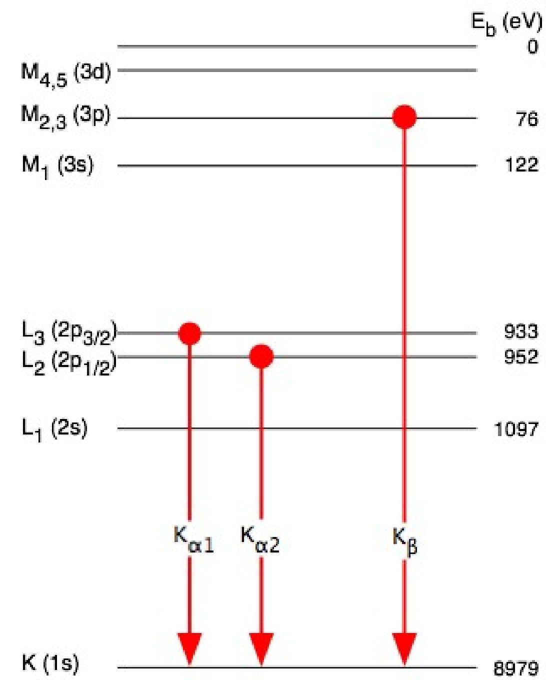




Bremsstrahlung (braking radiation) ***Characteristic lines***

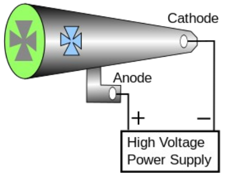
(source: nobelprize.org)



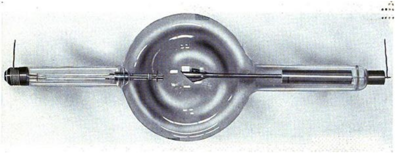


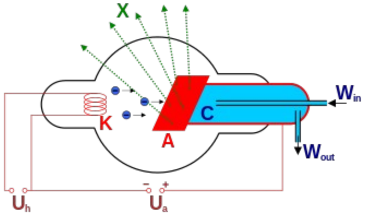
X-ray generation

Crookes (1869)
(cold cathode)
Ionization → e- from cathode to anode

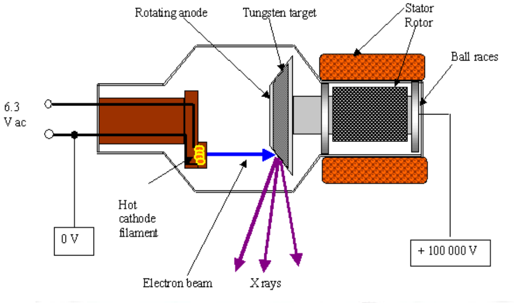


Coolidge (1917)
(hot cathode)
Cathode : heated filament

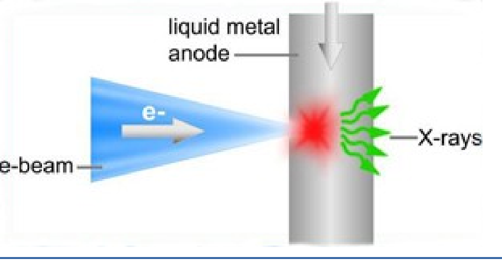




Moving target
Cathode: heated filamen
Anode: rotating/liquidt



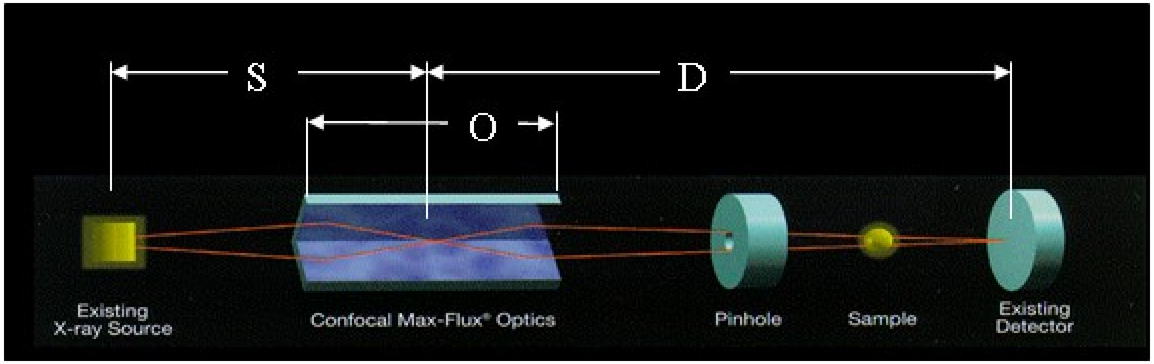
Siemens, 1933



Optics

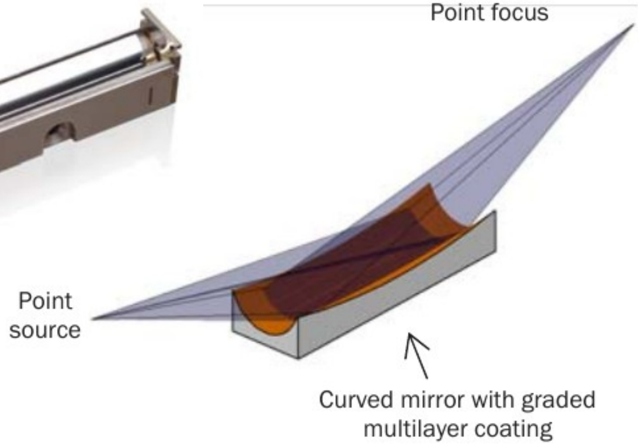
Osmic confocal optics / Montel optics

2 multi-layer mirrors at 90°



Ellipsoidal mirror

1 multi-layer mirror



Lab sources

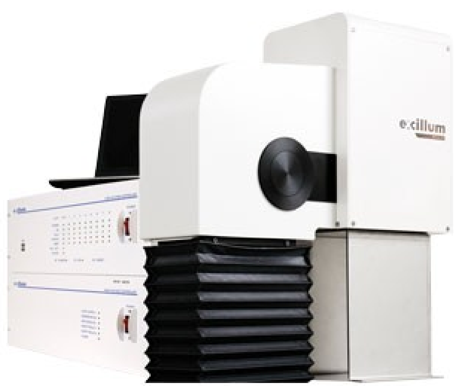
xenocs
Exploring the very small

BRUKER

Rigaku
oxford diffraction

excillum

70/160 kV
Ga target



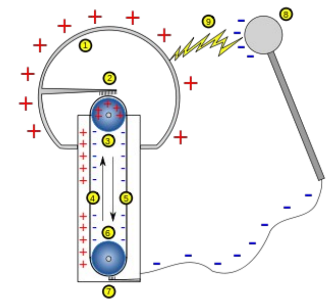
BRUKER

Rigaku
oxford diffraction

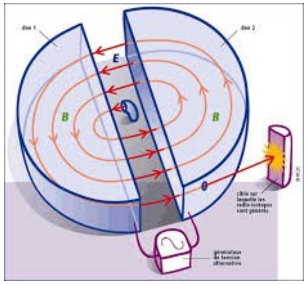
2-5 10e11 X-rays/mm²/sec
70 um source size
(www.rigaku.com)

Particules accelerators

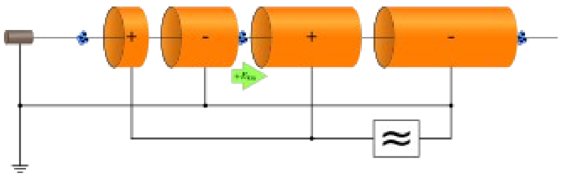
Electrostatic accelerator



Cyclotron



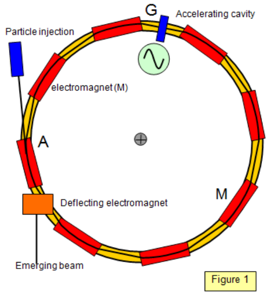
Linear accelerator



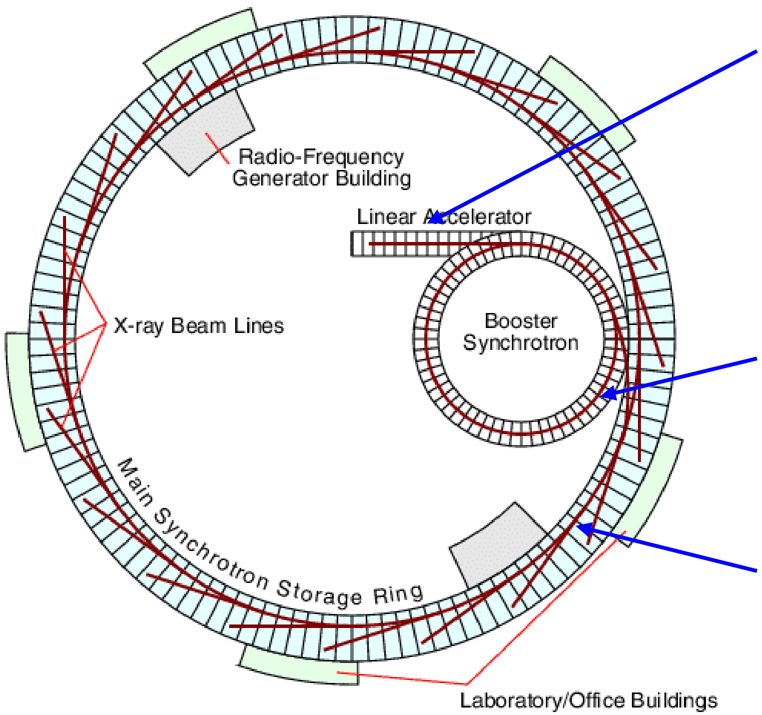
Synchrotron

→ high energy

→ store high current



Synchrotron components



Linac

Electron beam generation

First beam acceleration

Booster

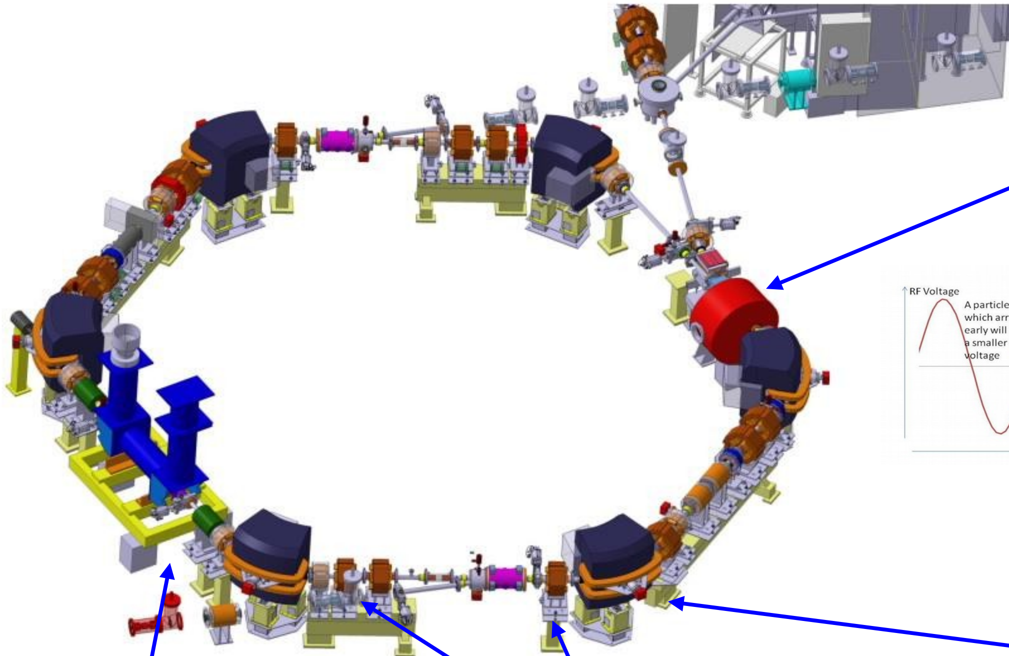
Acceleration to nominal energy

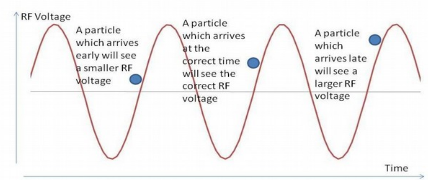
Storage ring

Beam storage for SR use

(http://pd.chem.ucl.ac.uk/pdnn/inst2/work.htm)

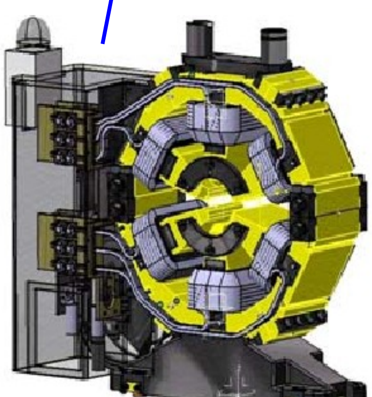
Synchrotron components



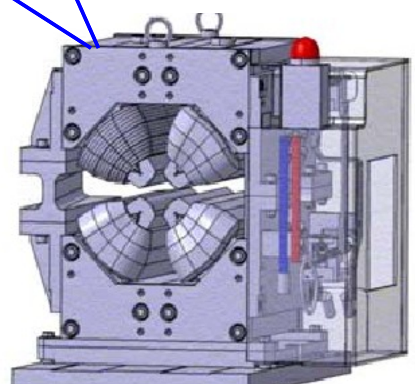


RF cavity
electron beam acceleration
(www.synchrotron-soleil.fr)

Dipole
for electron beam deviation
(www.synchrotron-soleil.fr)



Sextupole
for electron beam stability
(www.synchrotron-soleil.fr)



Quadripole
for electron beam focusing
(www.synchrotron-soleil.fr)

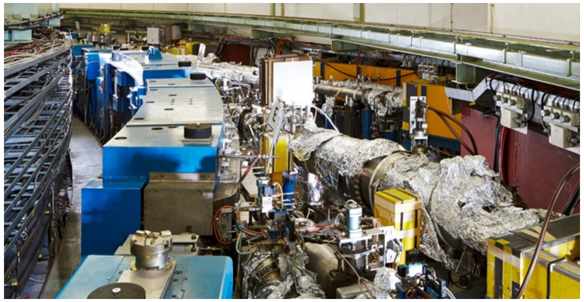
The multipole magnets refocus the beam after each deflection section, as deflection sections have a defocusing effect.

Synchroton generations

1st generation synchrotron: parasitic operation (50s to 70s)
ACO, DORIS, SPEARS...

ACO (www.media-paris-saclay.fr)

2nd generation synchrotron: dedicated to SR (80s)
SRS, DORIS, NSLS, SuperACO...



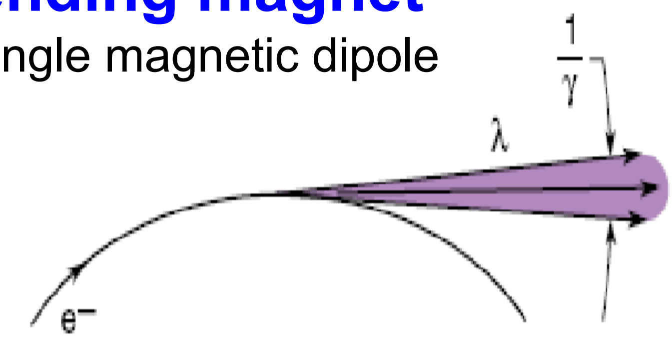
DORIS (http://www.desy.de)

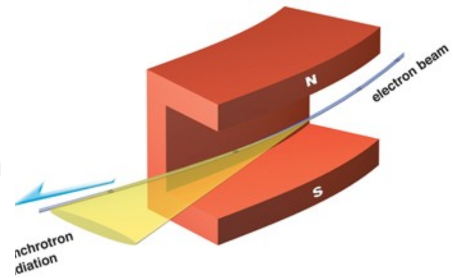
3rd generation synchrotron: ID with high brightness, low emittance
ESRF, ALS,...

1^{st} generation synchrotrons

Bending magnet

A single magnetic dipole





Brown et al. Nuclear Instruments and Methods, Volume 208, 1983, 65-77

Synchroton generations

1st generation synchrotron: parasitic operation (50s to 70s)
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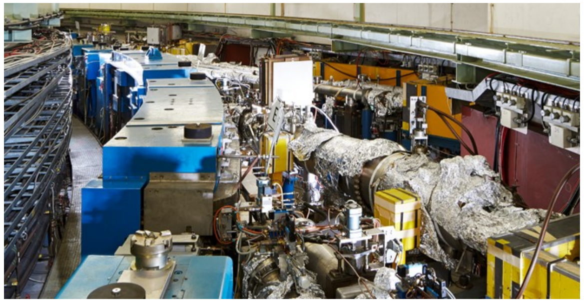
2nd generation synchrotron: dedicated to SR (80s)
SRS, DORIS, NSLS, SuperACO...



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2nd generation synchrotrons

Bending magnet

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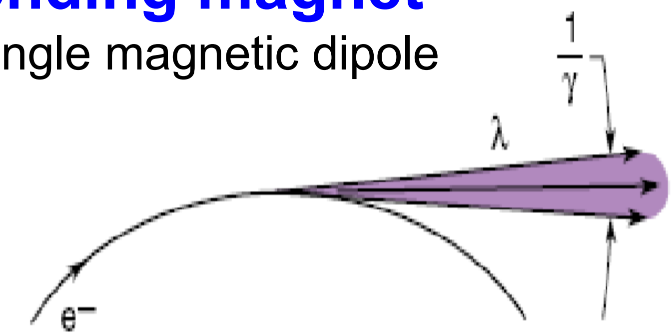
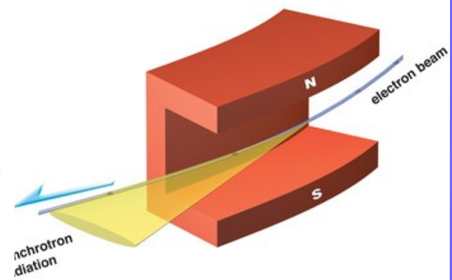
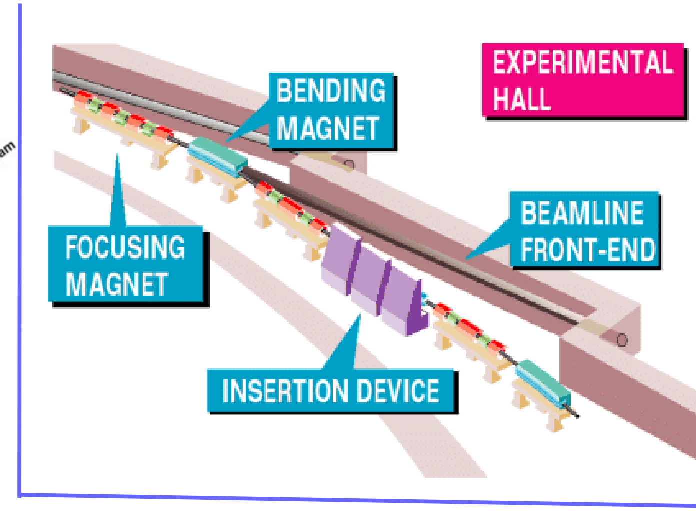
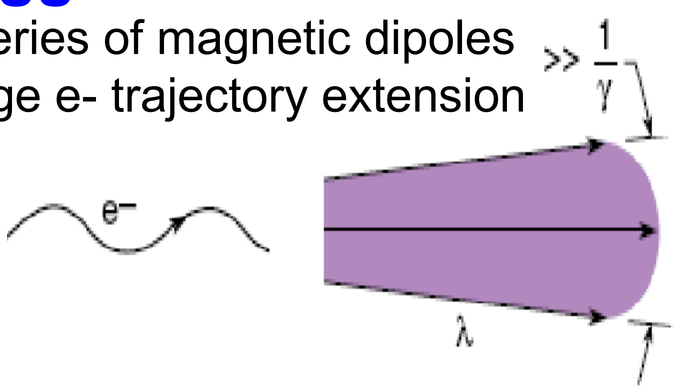


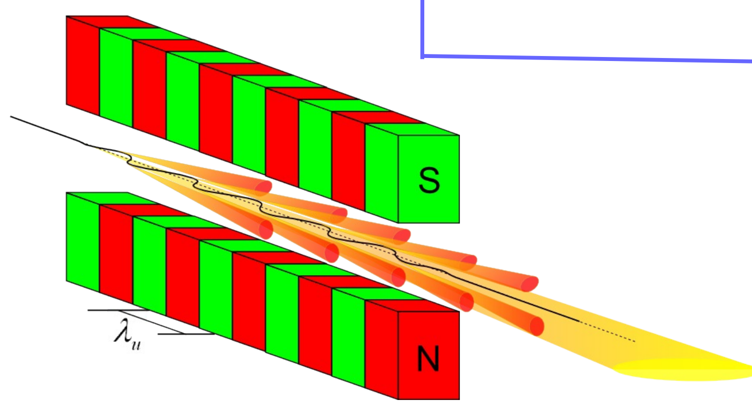




Wiggler

A series of magnetic dipoles
Large e- trajectory extension





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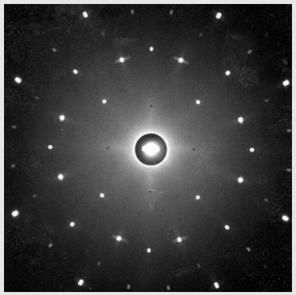
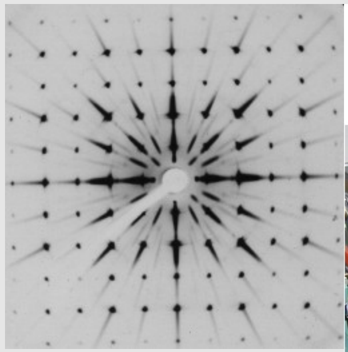
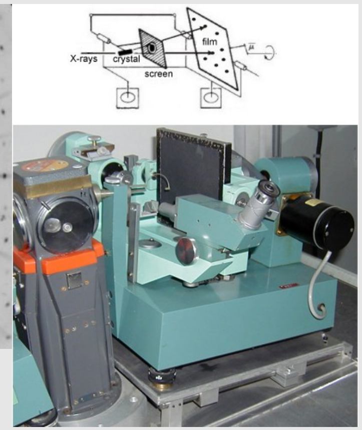
Detectors: films and IPs

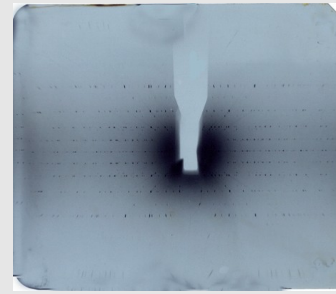


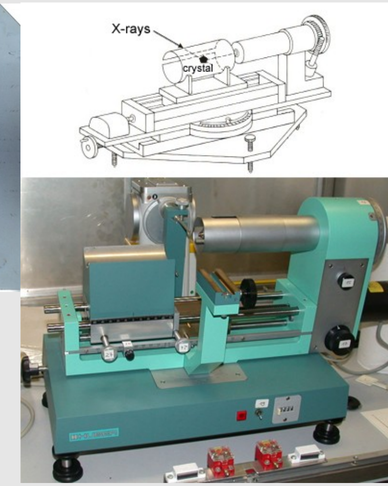
Laue diagram
(www.xtal.iqfr.csic.es)





Precession diagram
(www.xtal.iqfr.csic.es)





Weissenberg diagram
(www.xtal.iqfr.csic.es)

Upon exposure to X-ray:
Storage of the signal in the phosphor plate over a prolonged period,

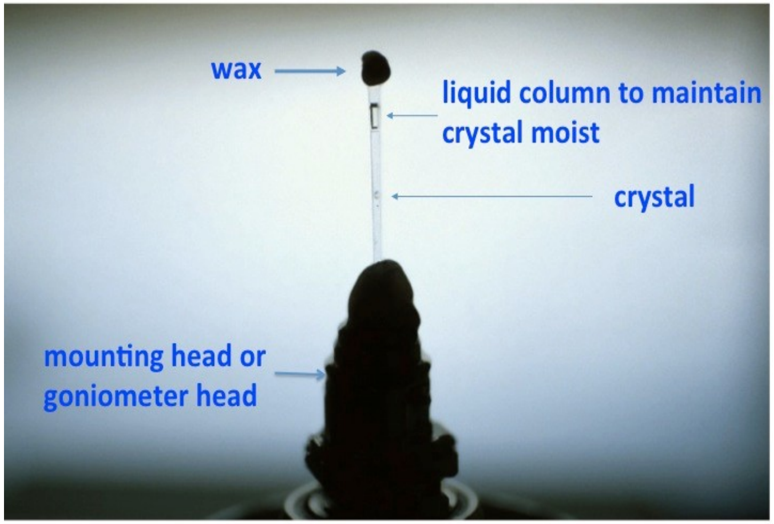
Upon readout:
Photostimulated luminescence (PSL) releases the stored energy within the phosphor by stimulation with visible light, to produce a luminescent signal.

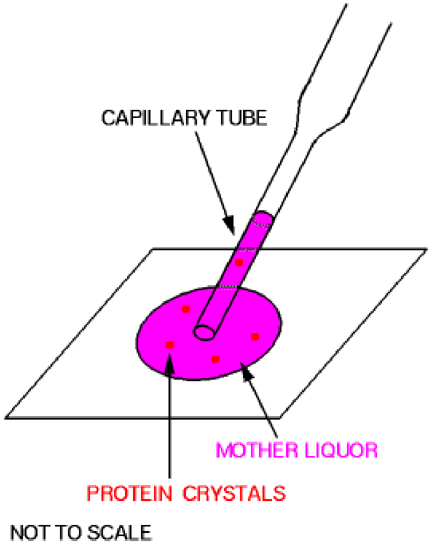
A multi-wire chamber at LURE (1974-1992)

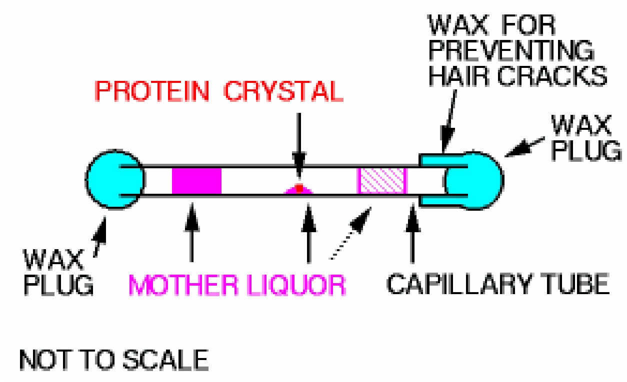
1st MAD structure!

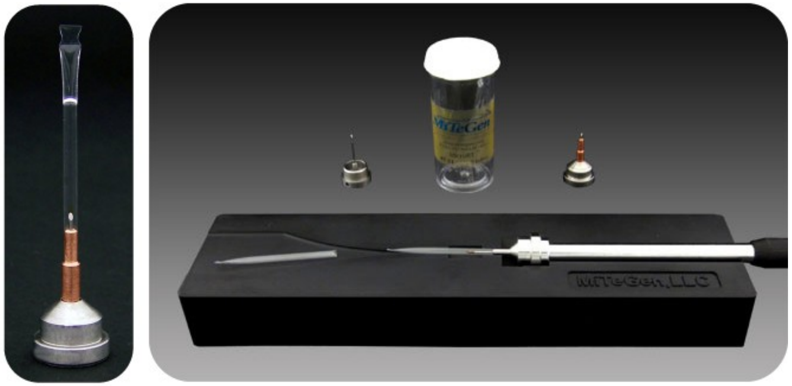
LURE:
R. Kahn, R. Bosshard,
A.Bahri, G. Bricogne,
A. Bentley, R. Fourme
CERN:
R. Bouclier, R. Million
J.C. Santiard, G. Charpak

Samples in capillaries







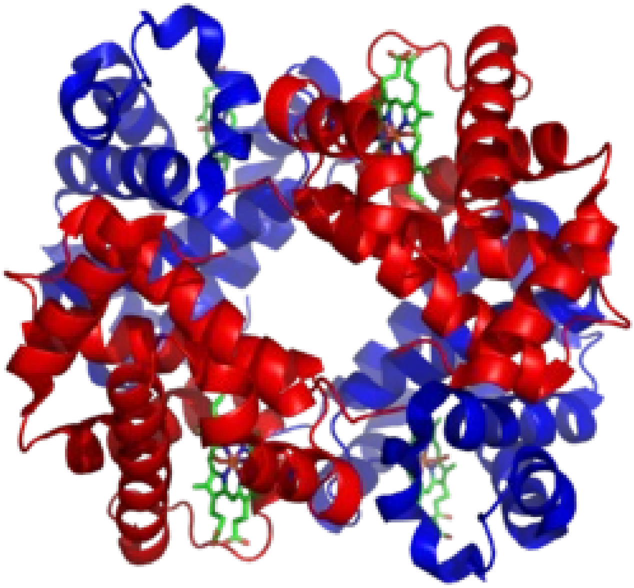


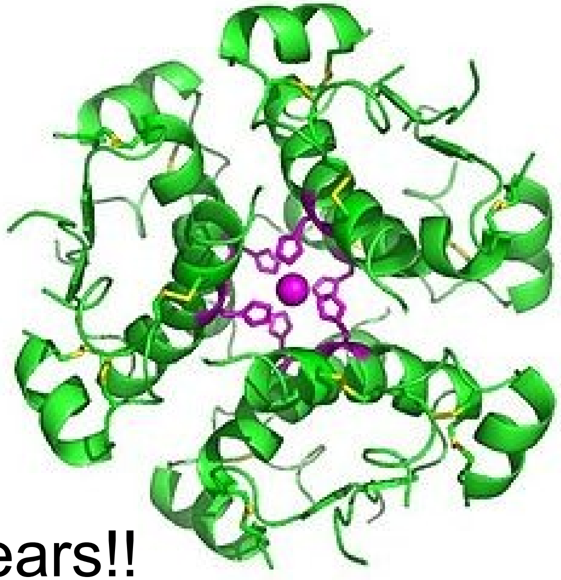
(www.mitegen.com/products/micrort/micrort.shtml)

1959: First protein structures

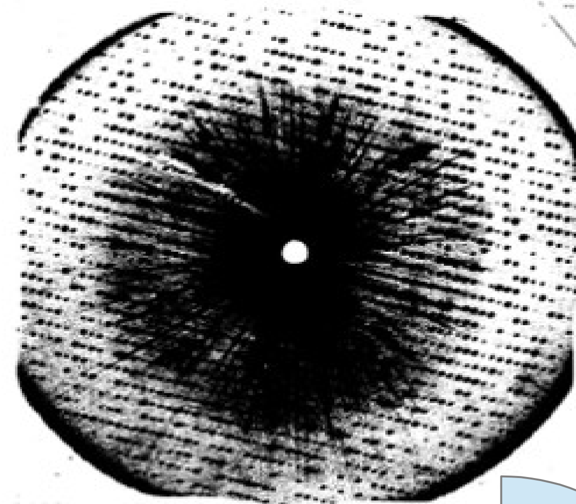
In 1953 **Max Perutz** showed that diffracted X-rays could be phased by comparing the patterns with and without heavy atoms attached. In 1959 he determined the structure of hemoglobin

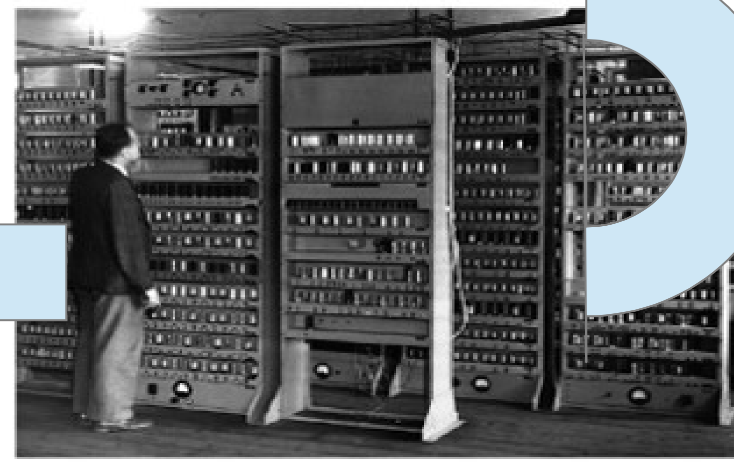
Max Perutz and **John Kendrew** shared the 1962 Nobel Prize for Chemistry for the structures of hemoglobin.

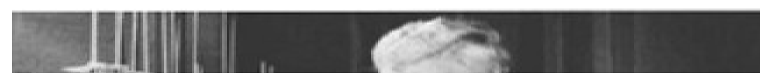




1969, **Dorothy Crowfoot Hodgkin** solved the 3D structure of insulin, on which she worked for over thirty years!!

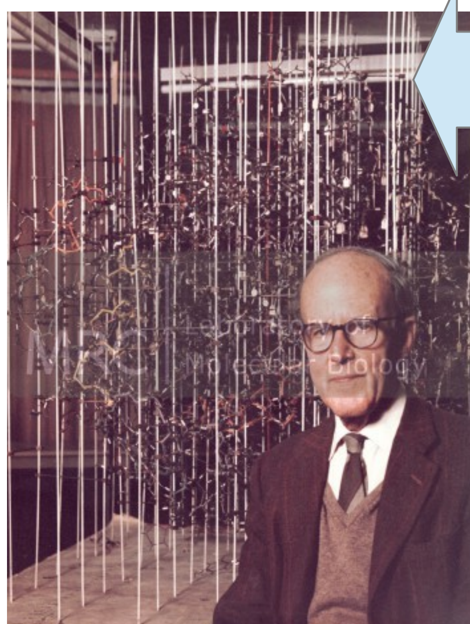






To analyse the 25,000 reflections of haemoglobin data, Perutz and Kendrew used the EDSAC I computer introduced in 1949

Myoglobin (1957)



Haemoglobin model 1957

1990s: 3rd generation synchrotrons

X-ray sources
CCD detectors / SS detectors

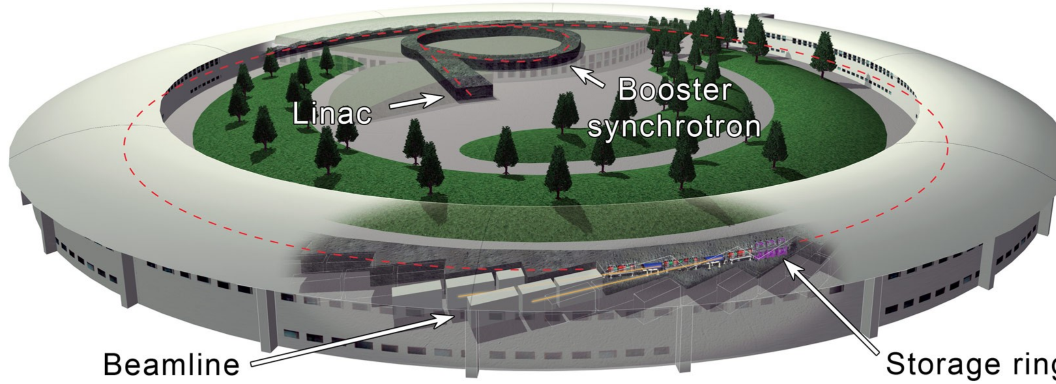
Freezing

Anomalous diffraction

3rd generation synchrotrons

ESRF

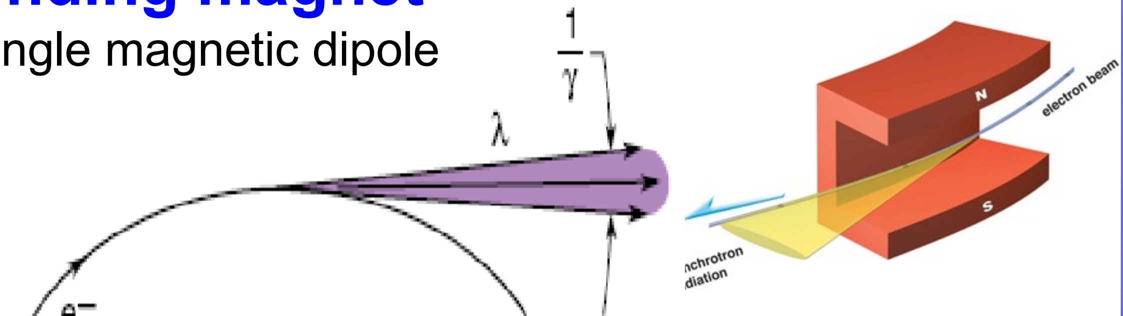
SOLEIL

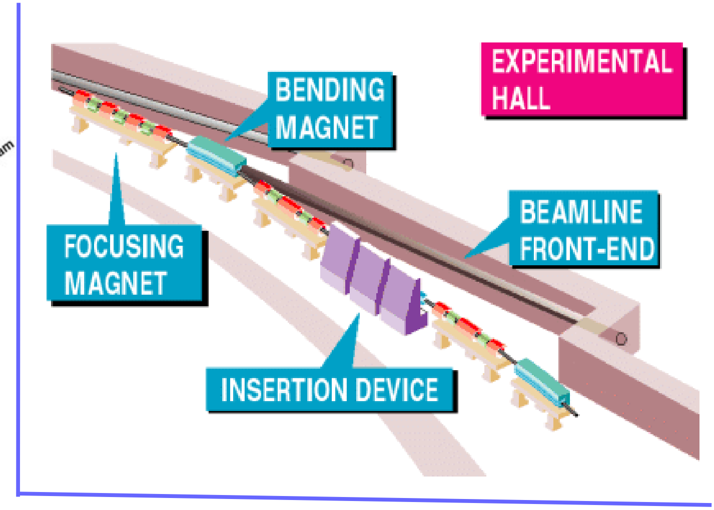


3rd generation synchrotrons

Bending magnet

A single magnetic dipole

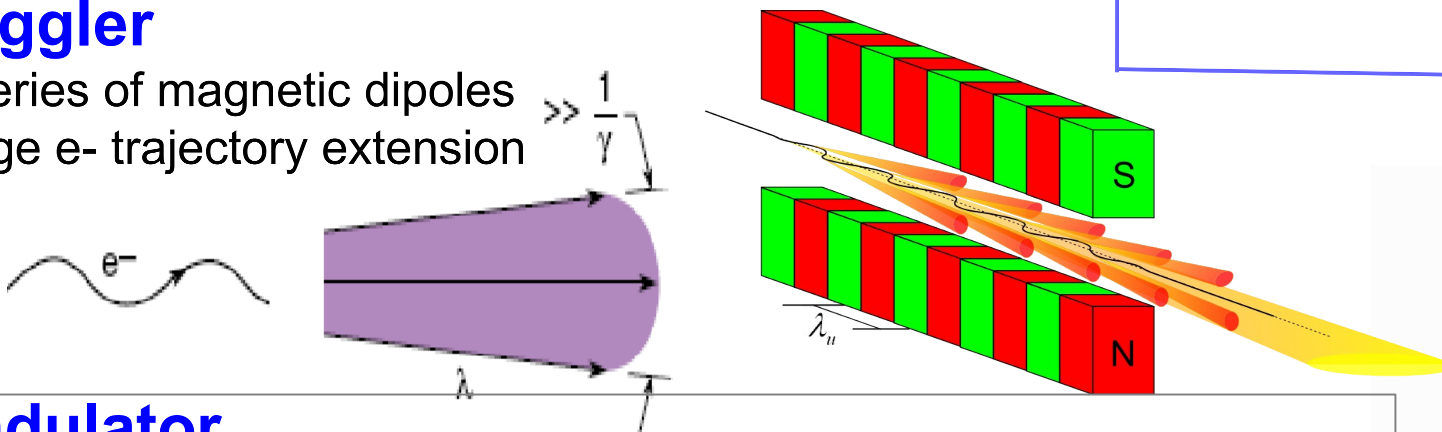


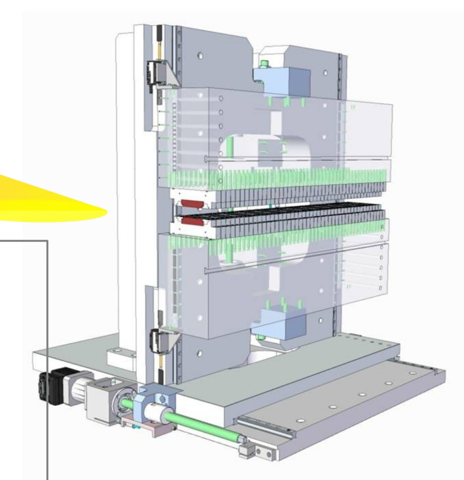


Wiggler

A series of magnetic dipoles

Large e- trajectory extension

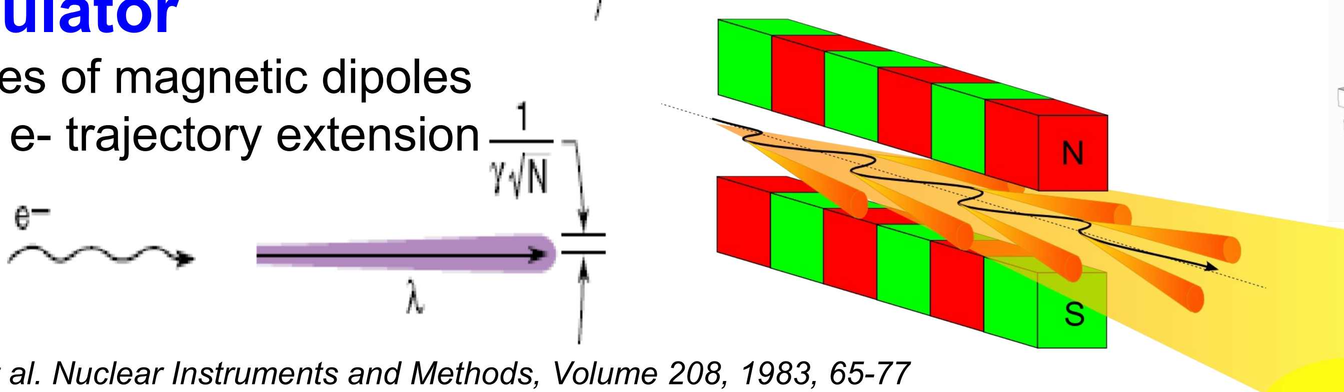




Undulator

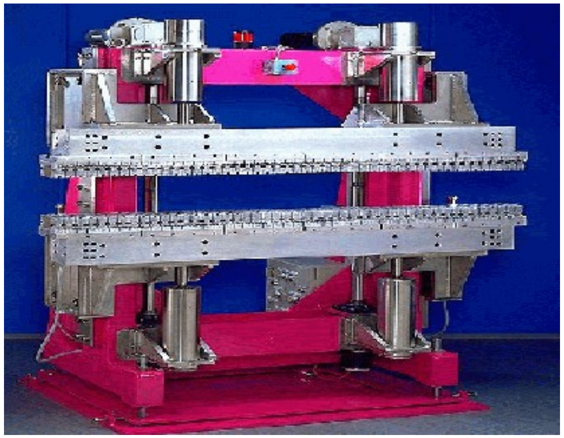
A series of magnetic dipoles

Small e- trajectory extension

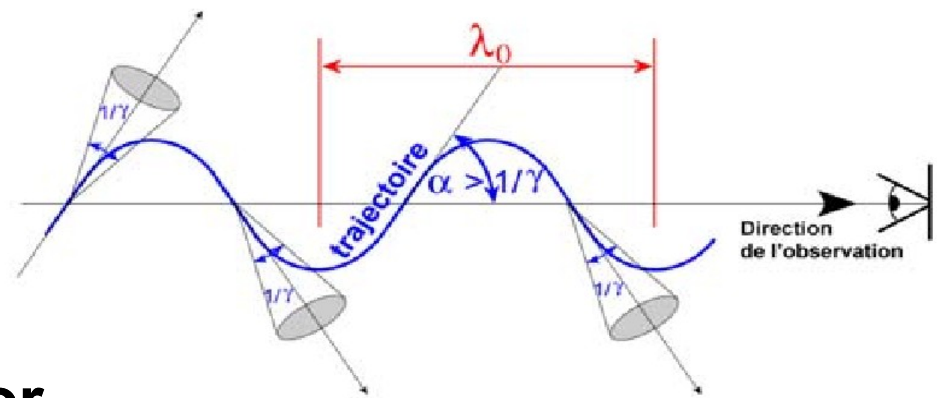


Brown et al. Nuclear Instruments and Methods, Volume 208, 1983, 65-77

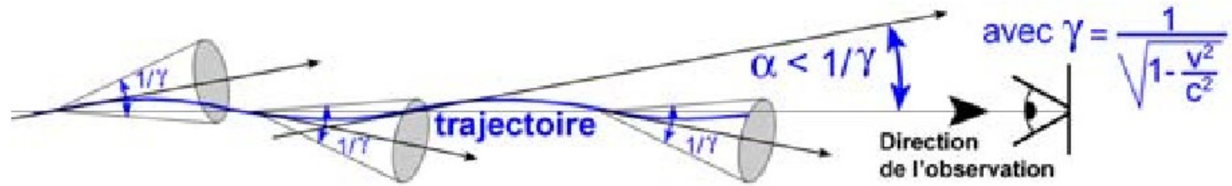
Wiggler *vs* undulator



Wiggler



Undulator



α : angular extension of the e- traj.

γ : emission cone aperture

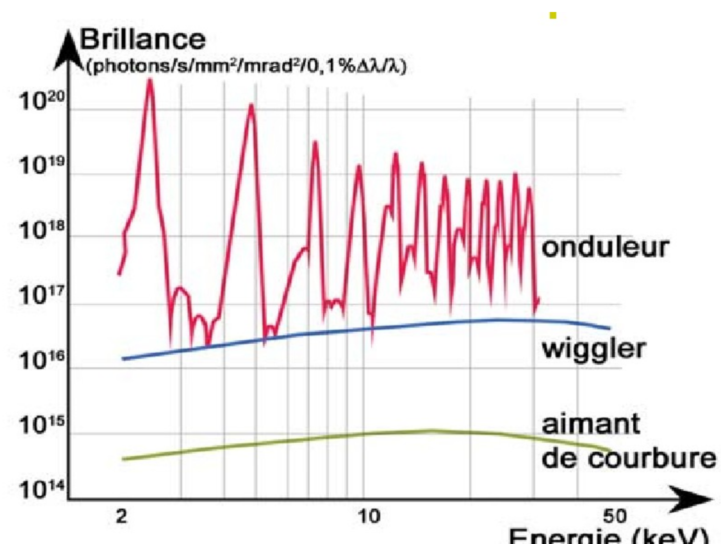
In an undulator: $\alpha < 1/\gamma$

=> emission in presence of the beam

=> constructive interferences

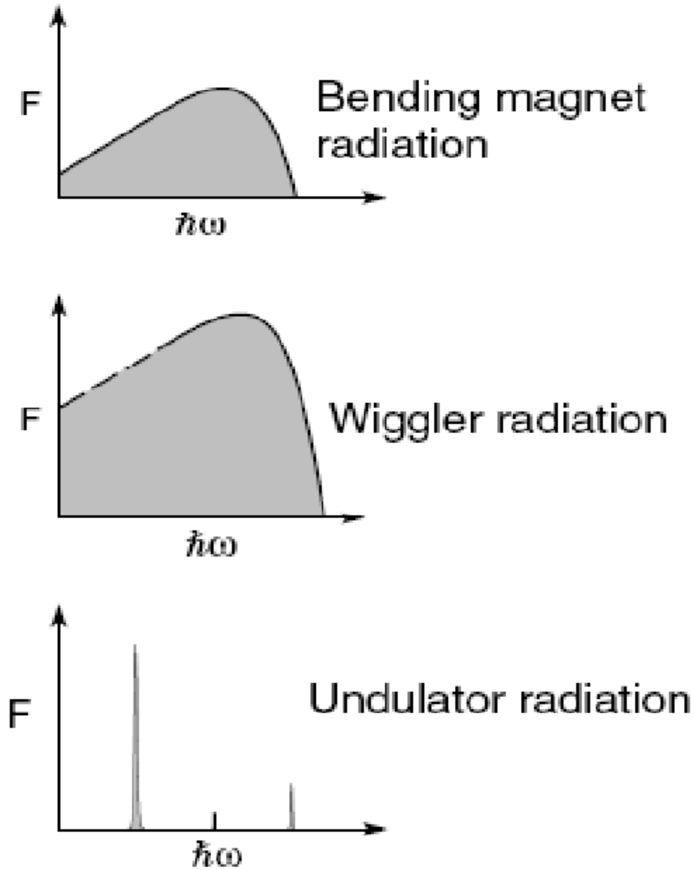
=> spectral lines (brightness in N^2)

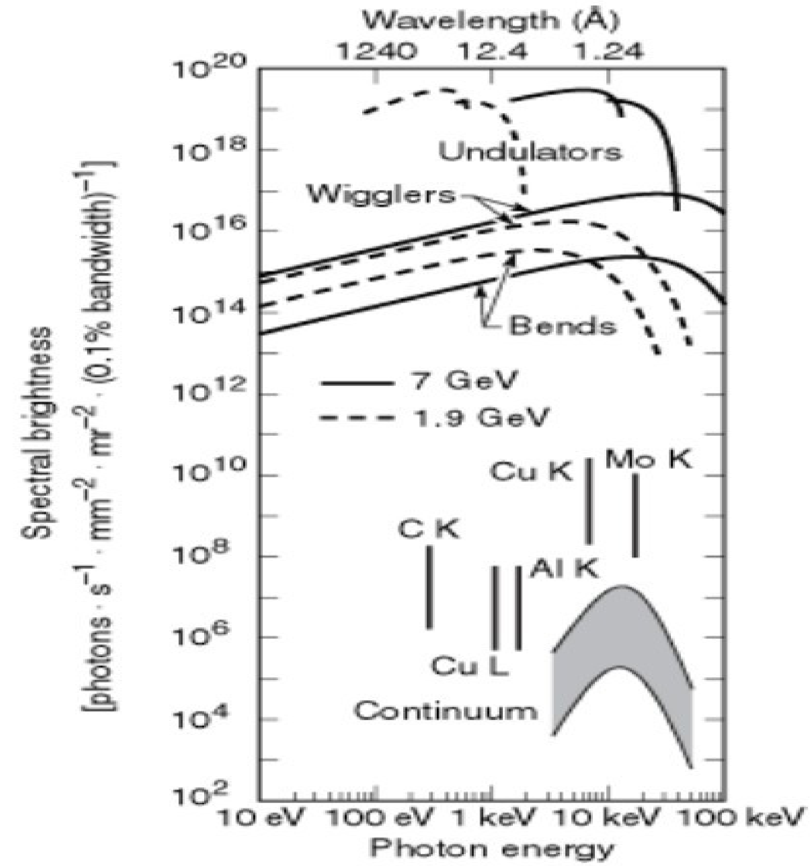
=> beam divergence in $N^{-1/2}$



(www.synchrotron-soleil.fr/images/File/RessourcesPedagogiques/Documentation/Machine-FicheEnseignant.pdf)

3rd generation synchrotrons



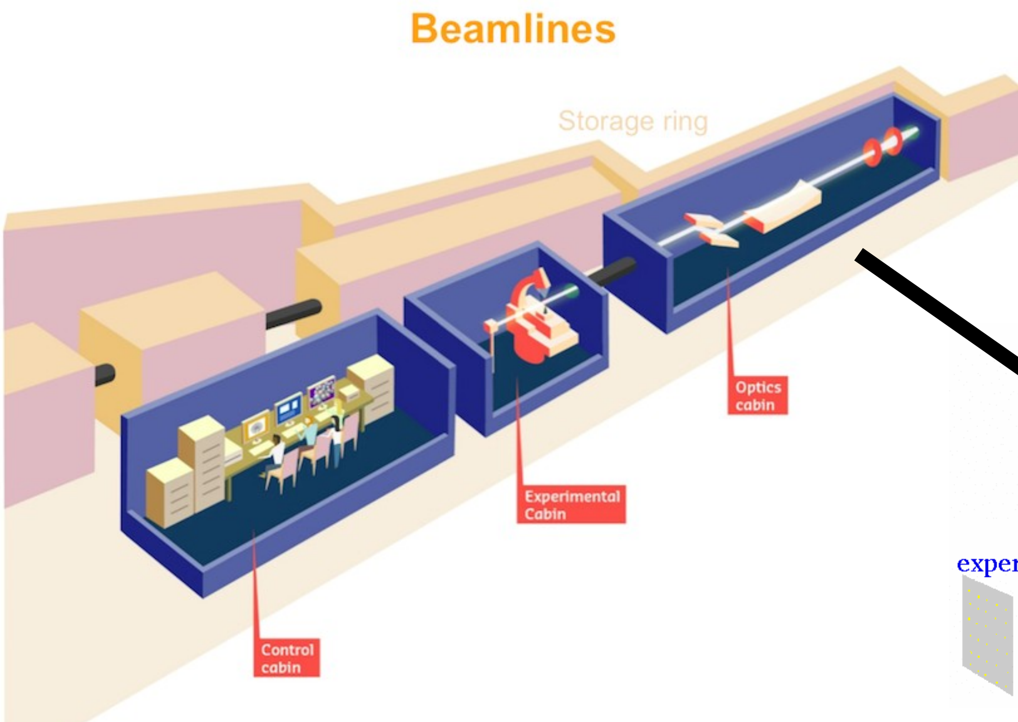


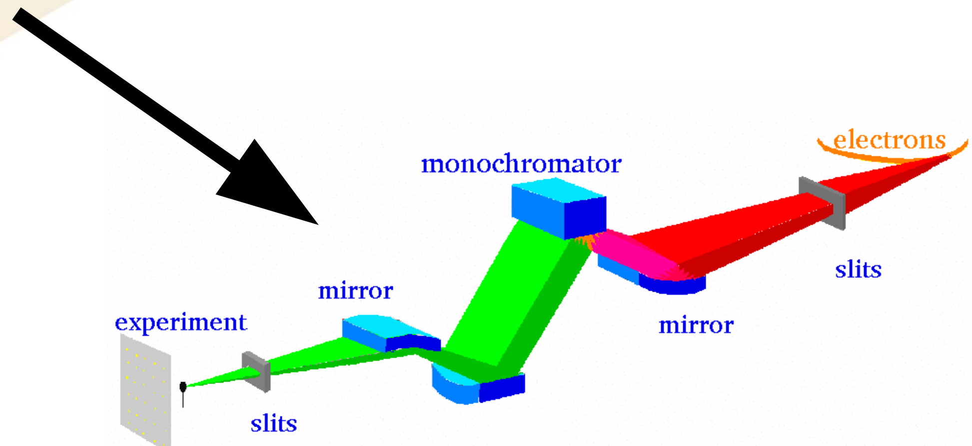
http://xdb.lbl.gov/Section2/Sec_2-1.html

Jim Clarke, ASTeC, SRS

Ring	Energy (GeV)	ρ (m)	I_b (mA)	P_{total} (kW)	Bending magnet $dP/d\theta$ (W/mrad)	Bending magnet $dP/d\Omega$ (W/mrad^2)	Wiggler $dP/d\theta$ (W/mrad)	Wiggler $dP/d\Omega$ (W/mrad^2)	Undulator $dP/d\theta$ (W/mrad)	Undulator $dP/d\Omega$ (W/mrad^2)
SRS (2nd generation)	2	5.56	200	50.9	8.1	20.8	4.0	0.6	1.0	2.2
DIAMOND	3	7.15	300	300.7	47.9	184.4	13.7	4.9	3.5	16.8
ESRF	6	25.0	200	916.5	145.9	1124.0	36.4	52.5	9.3	179.1

Beamline optics





Reflecting elements

→ collimating / focusing

→ high energy cutoff (harmonic rejection, …)

Refracting elements

→ energy selection

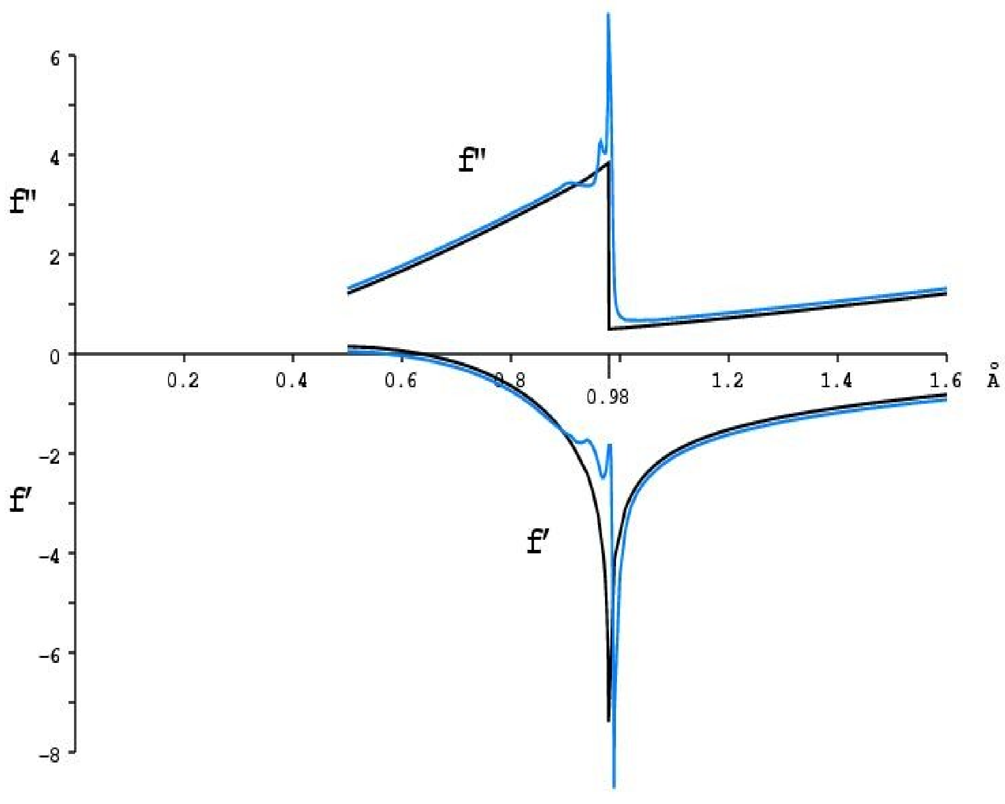
→ focusing

The anomalous signal

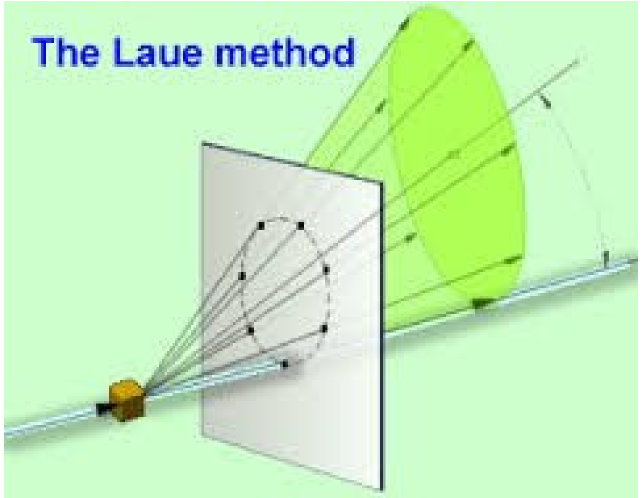
$$F(h) = \Sigma_j\, f_j \cdot \exp\,(2\pi i h \bullet r_j)$$

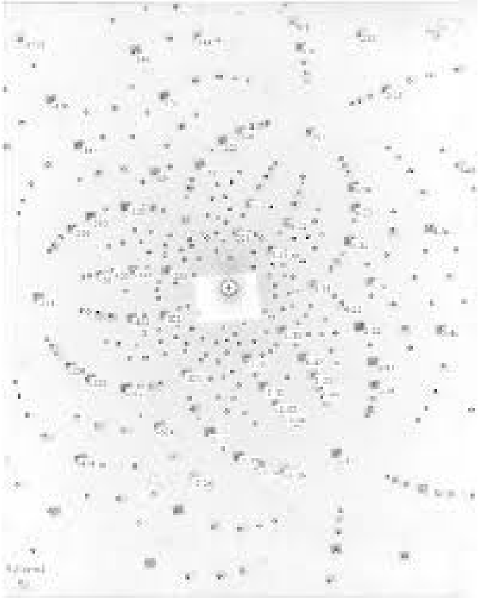
$$f_j = f^{\circ}_j(\theta) + f'_j\,(\lambda) + i \cdot f''_j\,(\lambda)$$

Anomalous correction f″ is proportional to absorption and fluorescence and f′ is its derivative



Laue



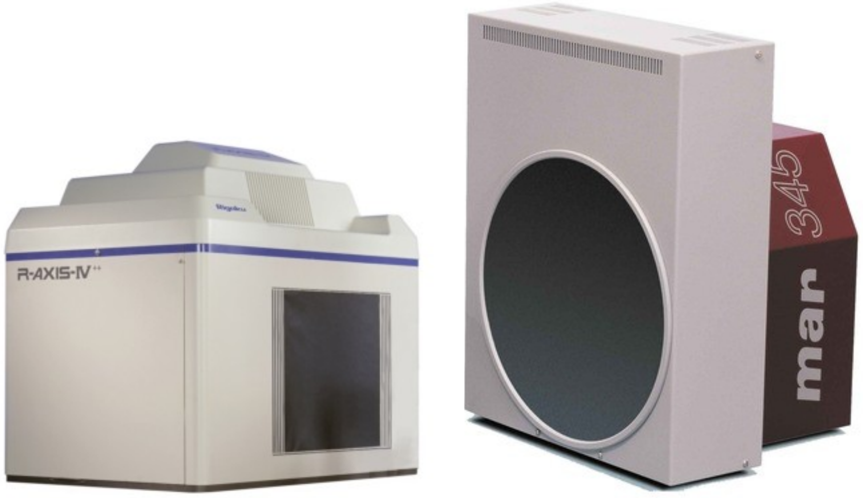


Complete dataset in 1 frame
→ real time experiment

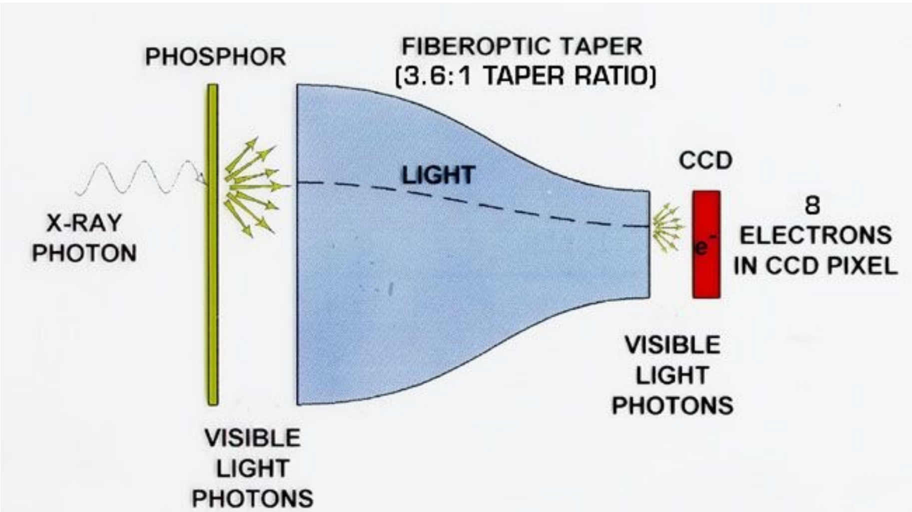
Detectors: automated IPs

Upon exposure to X-ray:
Storage of the signal in the phosphor plate over a prolonged period,

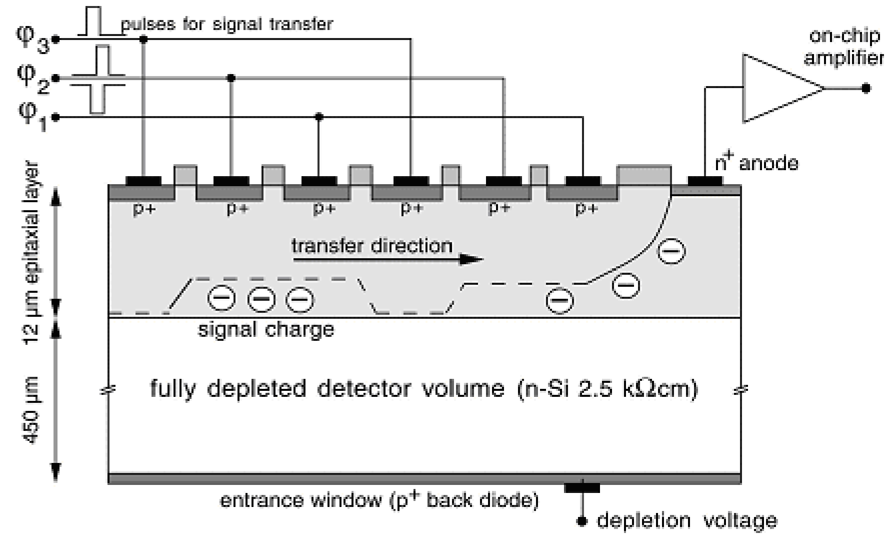
Upon readout:
Photostimulated luminescence (PSL) releases the stored energy within the phosphor by stimulation with visible light, to produce a luminescent signal.



CCD detectors



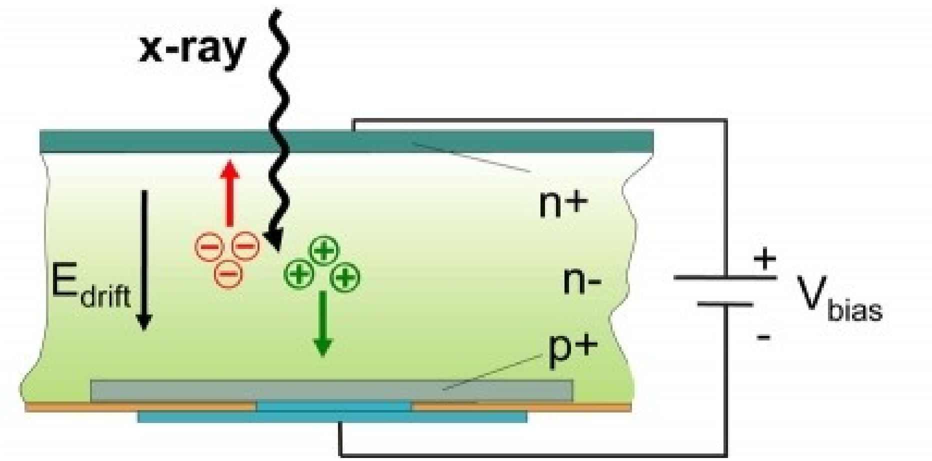
http://proteincrystallography.org



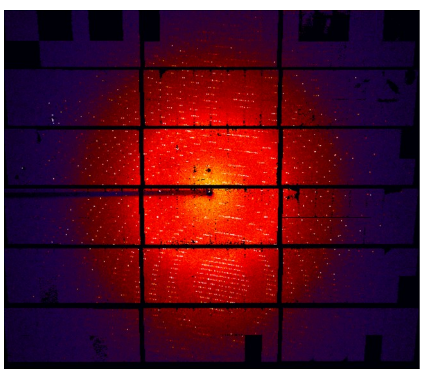
A scintillator converts X-ray photons to visible light photons. The image is demagnified to match the CCD size.

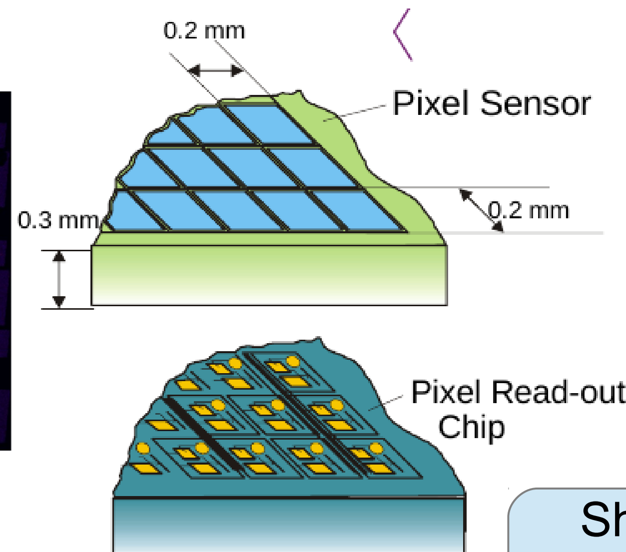
Readout time compatible with synchrotron exposure time + high dynamic

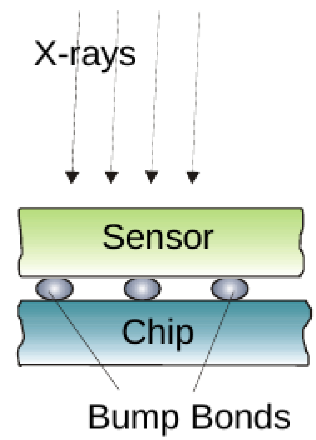
Pixel detectors



http://www.dectris.com







Short readout time
→ shutterless mode
→ fine slicing

Direct detection of photons in the sensor (no need for a scintillator for conversion to visible light photons).

Pflugrath, J. W. (1999). The finer things in X-ray diffraction data collection, *Acta Cryst. D* **55**, 1718-1725.

Last evolutions

Present fast detectors

dead time ~1 msec

Pixel : 75 um
Frame rates : 25 Hz
1 ms readout time

Pilatus (Dectris)

Eiger (Dectris)

Pixel : 75 um
Frame rates : 10^3 Hz
3 us readout time

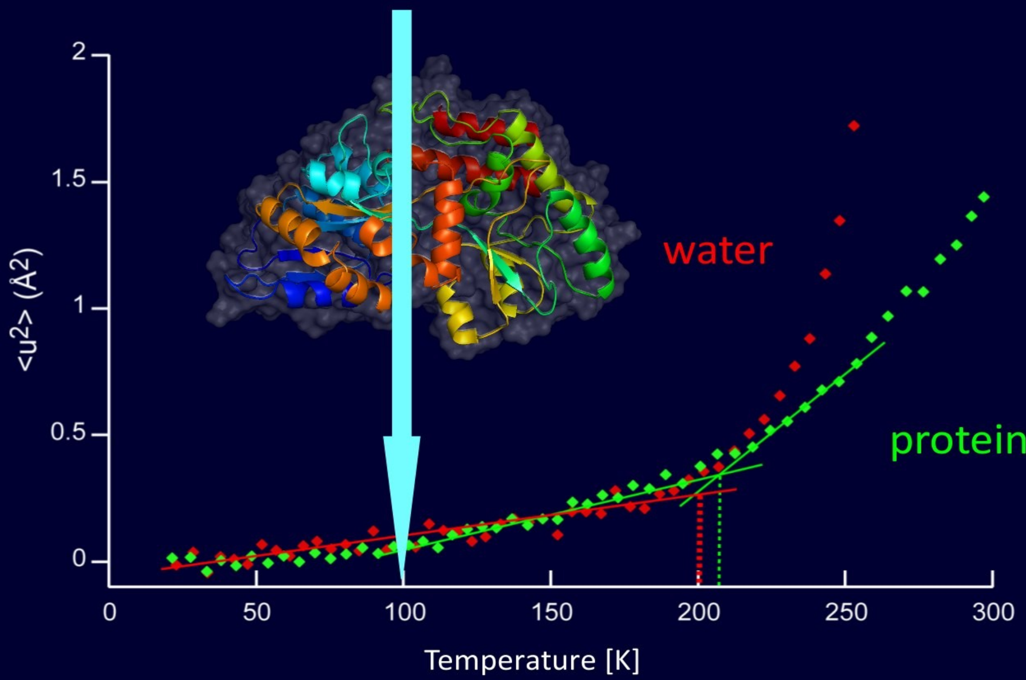
Last generation

dead time ~3 usec

Cryo-cooling

Temperature-dependent side-chain flexibility from neutron scattering

Cryo X-ray data collection

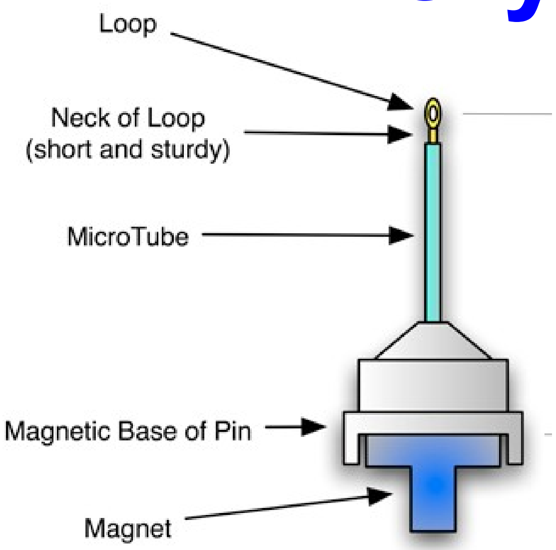


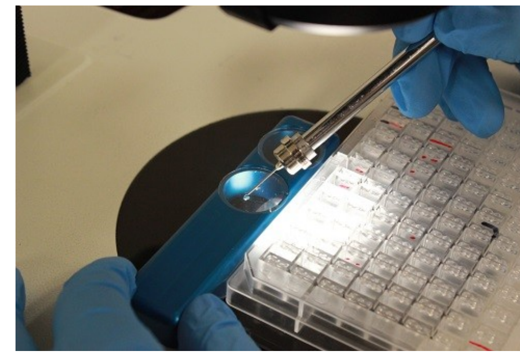
Wood, Frölich, Gabel, Moulin, Haertlein, Paciaroni, Zaccai, Tobias & Weik (2008) JACS 130, 4586

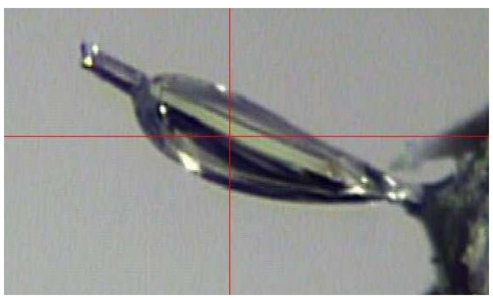
Cryo-cooling at 500 K / s : protein conformational changes quenched at 200 K

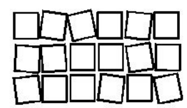
Halle (2004) PNAS 2004, 4793

Crystal flash-freezing









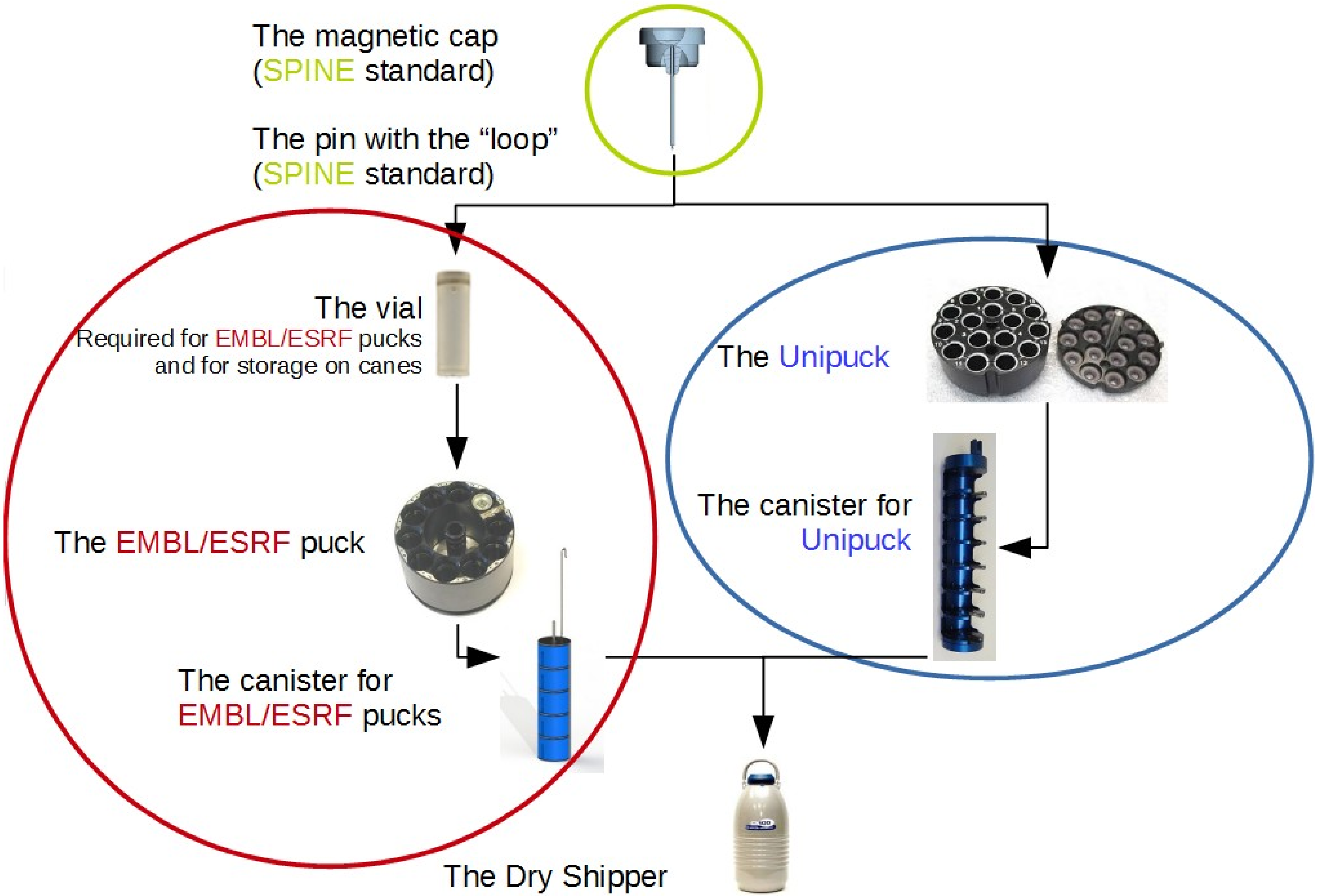
Possible improvements:

Optimized cryo-protectant

Absence of liquid (Pellegrini et al., Acta Cryst. (2011). D67, 902-6)

High speed freezing (Warkentin et al., J Appl Cryst. (2006) 39, 805–11)

Freezing in propane, etc...



EMBL/ESRF pucks are required for example on:

- ESRF (BM30A, and all ID MX beamlines)
- BESSY (BM14.1-14.2)
- SLS (all MX beamlines)
- ALBA (XALOC)

Unipucks are required for example on:

- SOLEIL (Proxima1 & 2)
- ESRF (BM30A, ID30B)
- BESSY (BM14.2)
- DLS (all MX beamlines)

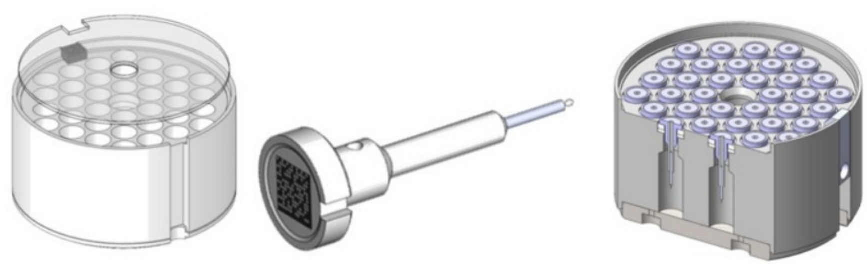
Other formats:

SSRL: “pineapple”

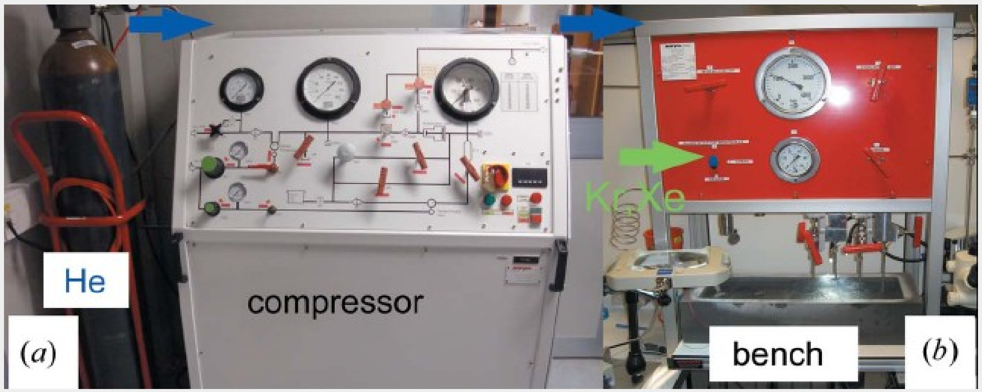
ALS: similar to Unipuck

Rigaku: used by the Actor

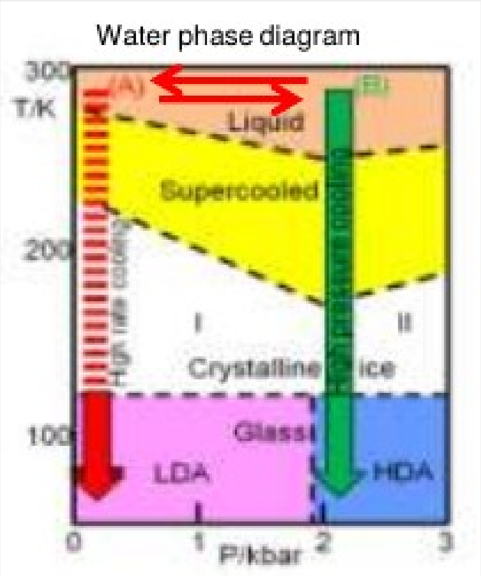
MiniSpine: new format, under evaluation at the ESRF

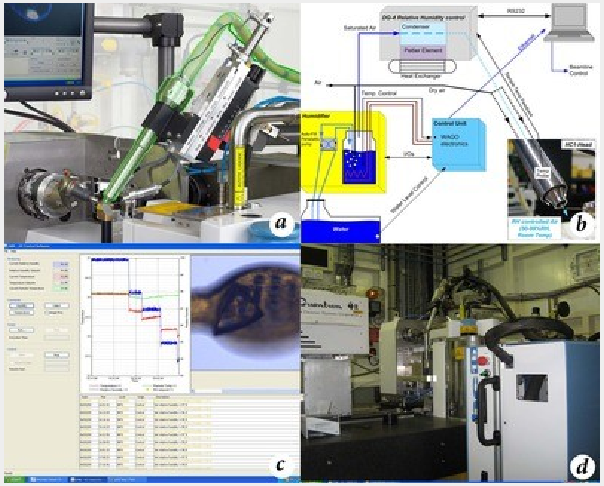


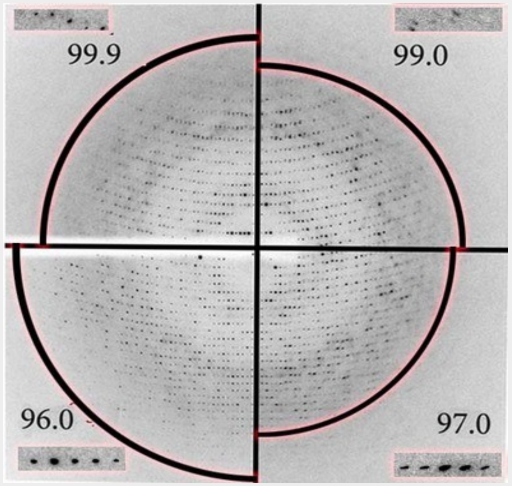
Crystal flash-freezing
Last improvements



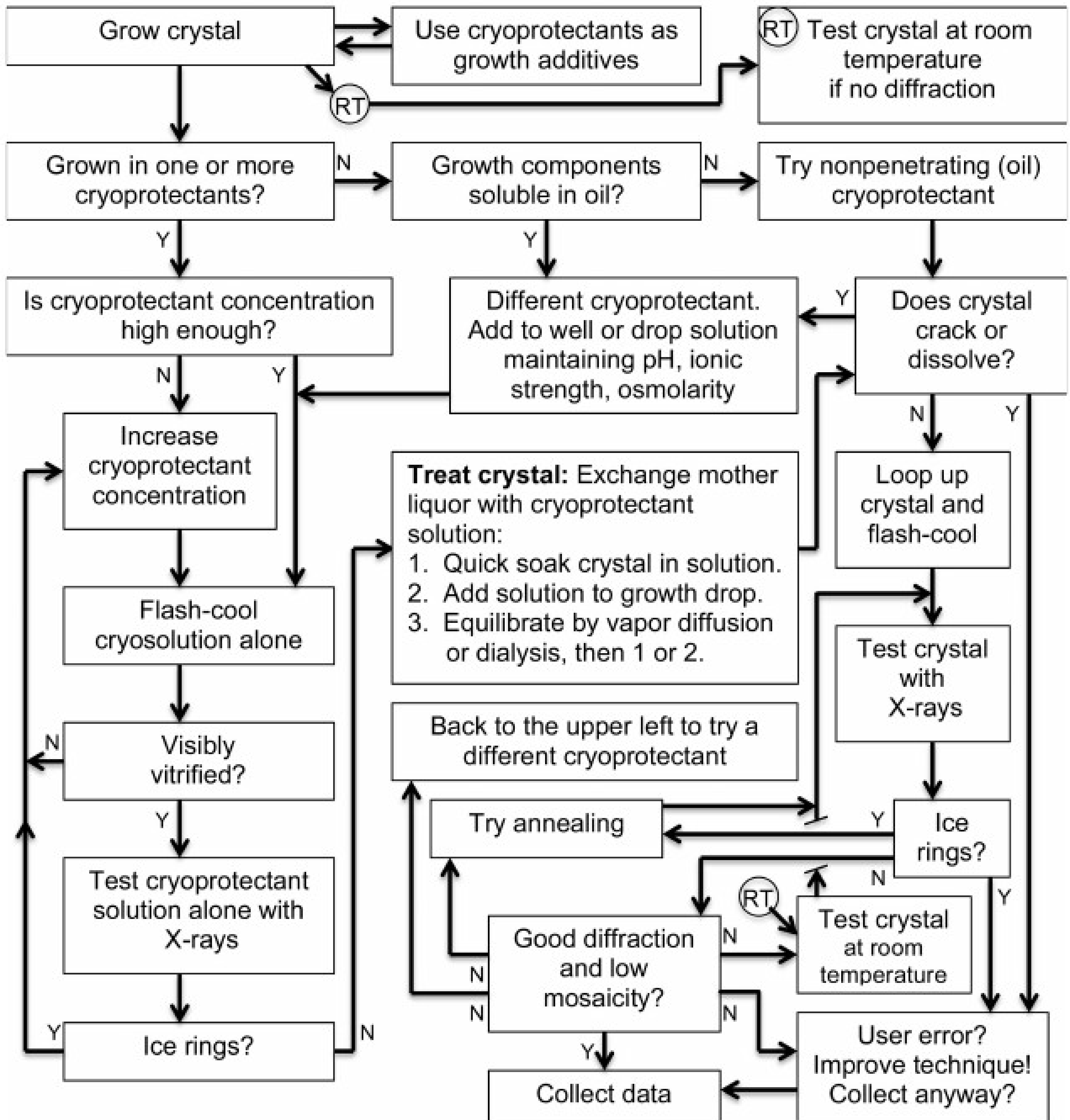
P. Carpentier, ESRF







(Bowler et al., Cryst. Growth Des., (2015) 15, 1043−1055)



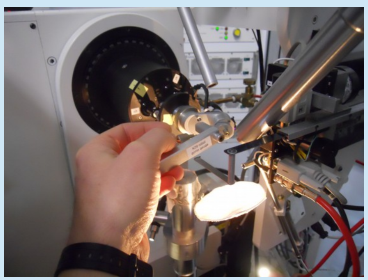
(J.W. Pflugrath, ActaCryst. F71 (2015), 622-42)

2000s: Automation

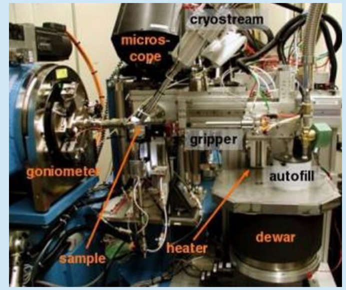
Crystallization / nanodrops

Sample changers / sample holder standard

Automation: Sample changer

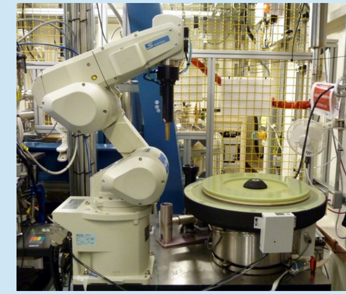


Manual handling

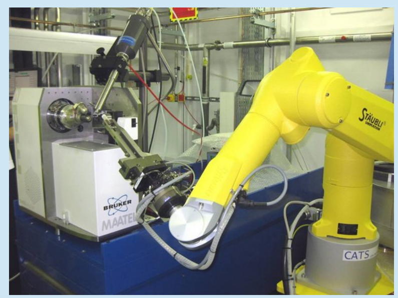


ALS

EMBL



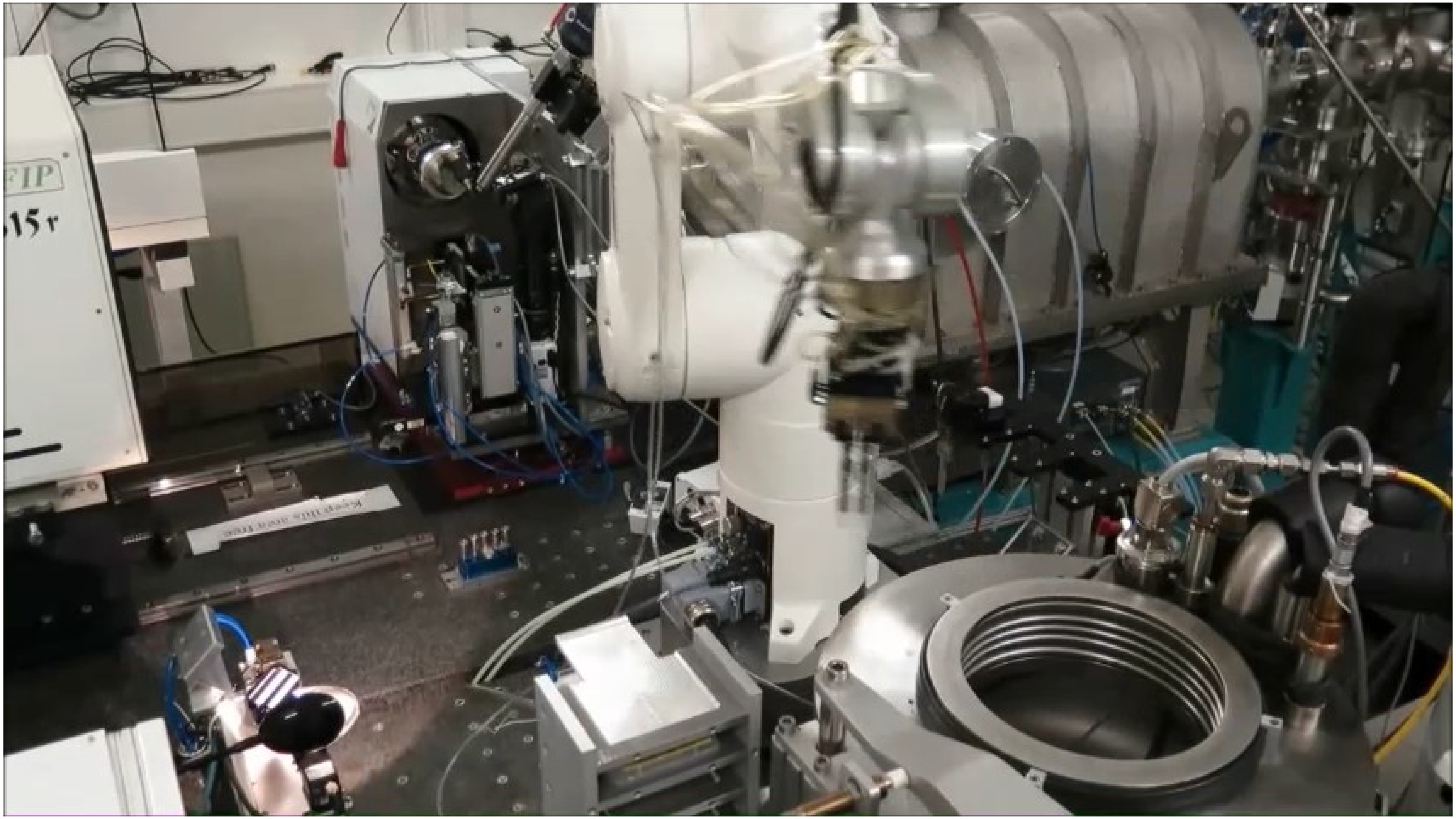
Rigaku



FIP

APS

Higher reliability
Better reproducibility
=> screening, to find the best crystal

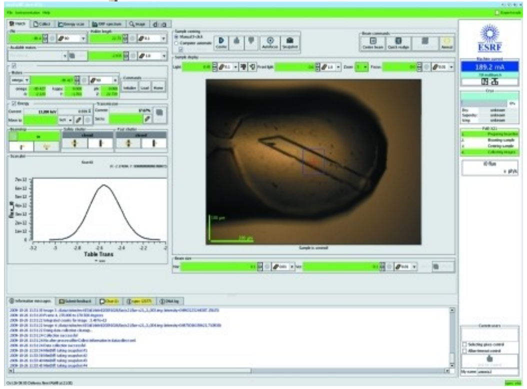


Automation

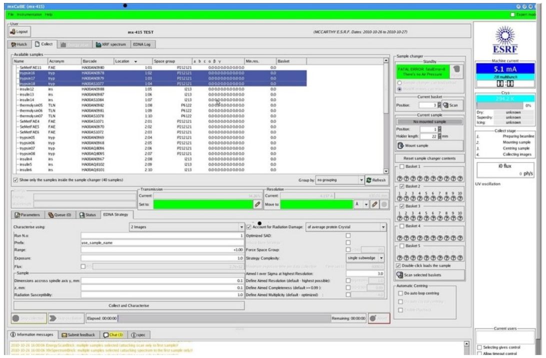
Software

- MxCube

- ISPyB



- EDNA / xdsadp, meXDS, etc.



RT experiments

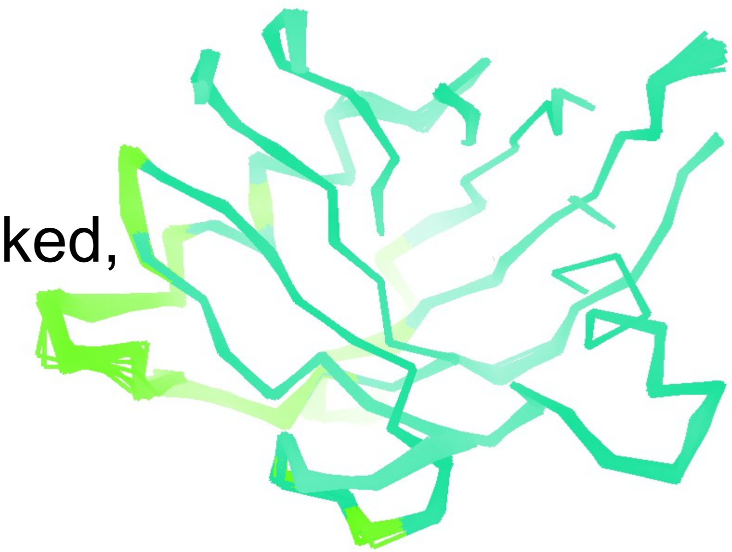
RT + ensemble

Flash cooling of protein crystals

- biases structural collective motions;
- remodels > 35% of side chains;
- induces bias toward smaller, overpacked, and unrealistically unique models.

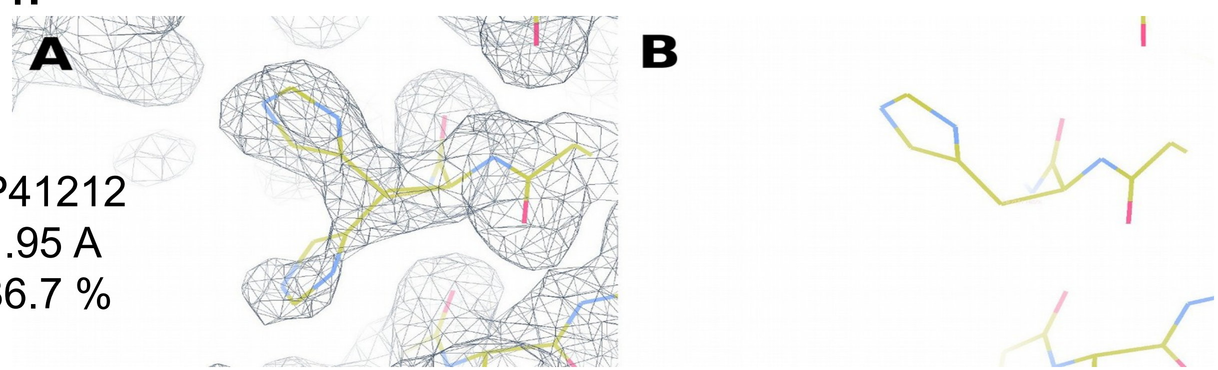
Instead, **room-temperature** X-ray crystallography helps in revealing

- motions crucial for catalysis,
- ligand binding,
- allosteric regulation.



Automated *in situ* experiment on cyclophilin D

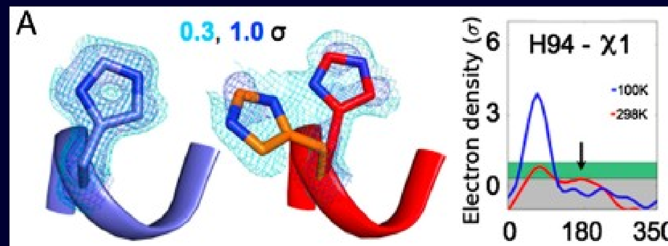
Spacegroup:	P41212
Resol:	1.95 A
Completness:	86.7 %



Structure of cyclophylin D at room temperature (A) and with flash-frozen crystals (B). When His173 exhibits a single conformation in the later, clear density is observed for a double conformation at room temperature (unpublished data).

Protein conformational heterogeneity greater in RT than in 100 K structures

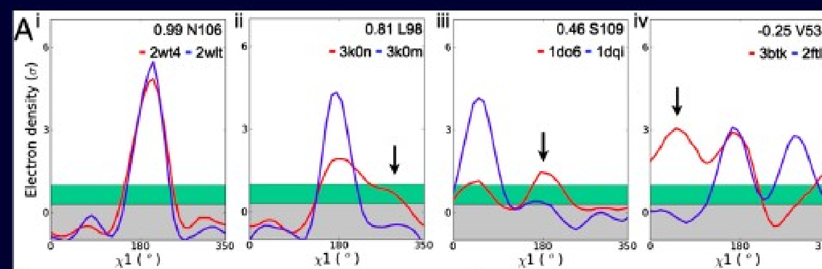
Fraser, van den Bedem, Samelson, Lang, Holton, Echols & Alber (2011) PNAS 108, 16247



100 K RT

Alternate conformation of H94
In H-Ras at RT, but not at 100 K

Cryo-cooling remodels conformational distributions in 35% of all protein side-chains



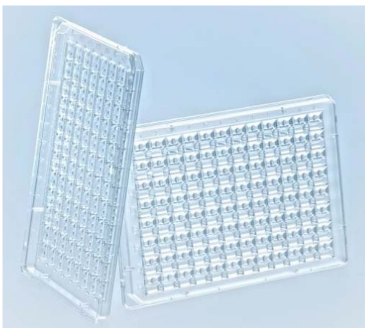
Tools to analyse conformational heterogeneity in crystal structures:

- RINGER: samples e- density around side-chain dihedrals below 1σ level (Lang *et al.* (2010) Protein Sci. 19, 1420)
- qFit: automates building of alternative polypeptide conformations (van den Bedem *et al.* (2009) Acta Cryst. D65, 1107)
- Time-averaged crystallographically restrained MD refinement of ensembles (Burnley et al. (2012) eLife 1, e00311)

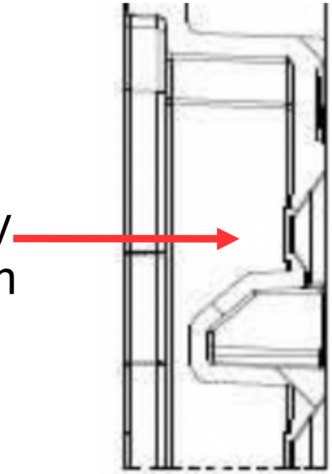
- END, RAPID: place e- density maps on absolute scale and calculate noise at each position in the map (Lang *et al.* (2014) PNAS 111, 237)

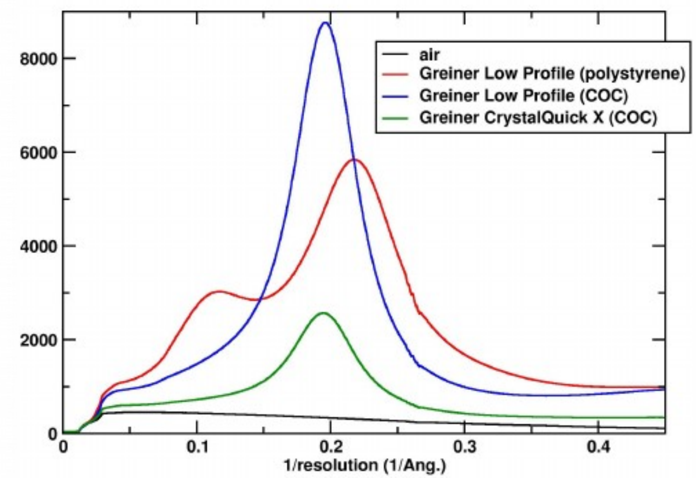
Optimized plates for *in situ* X-ray diffraction

CrystalQuick X from Greiner
- molding technique
- 2 drops location per condition



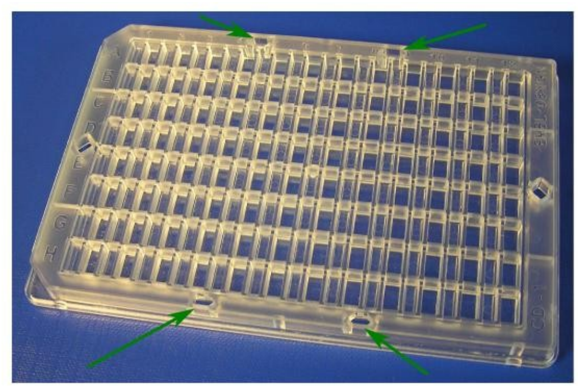
X-ray beam

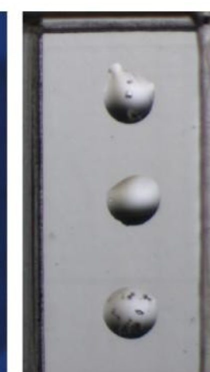




CrystalDirect from EMBL
- tapes on both side
- positioning marks

In-situ-1 from Mitegen
- tapes on both side
- small volume for the reservoir



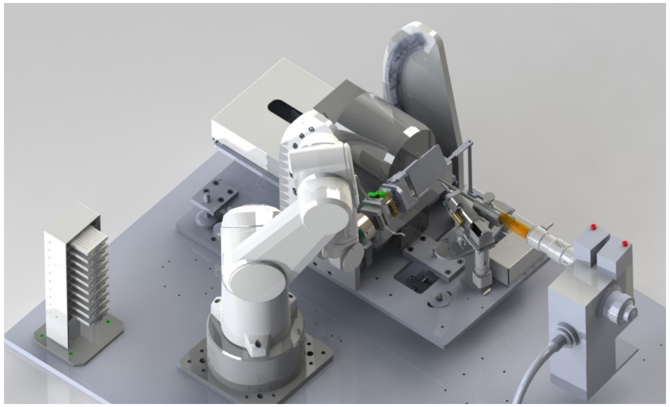


In situ screening / data collection

Diffraction "in the plate"
=> no crystal handling
Great for fragile crystals (larges complexes...), RT, ligand screening

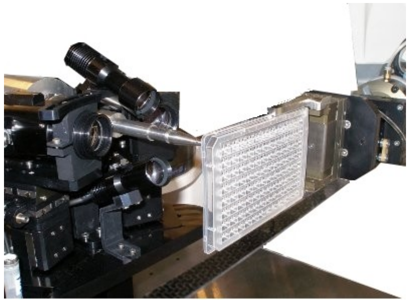
in situ screening & data collection

- SBS micro-plates (sitting/hanging drops)
- SBS high density batch plates
- micro-chips
- high pressure cells

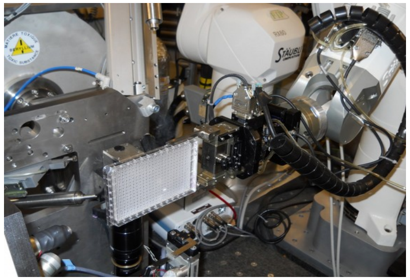


Applications

- rapid crystallization screening
- data collection on fragile crystals, significantly degraded upon freezing
- data collection at room temperature on series of crystals
- automated screening of compounds, fragments, heavy atoms



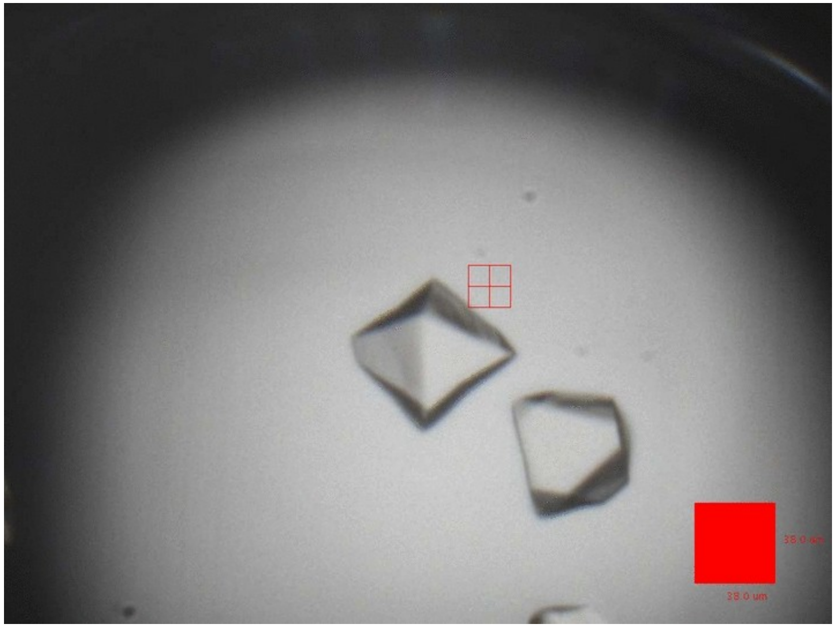
96-well crystallization plate

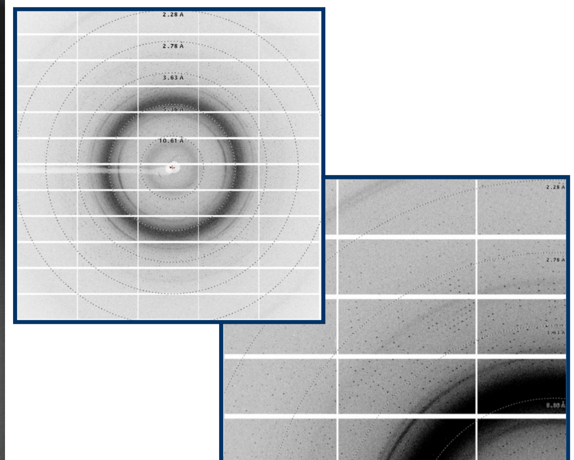


1536-well micro-batch plate

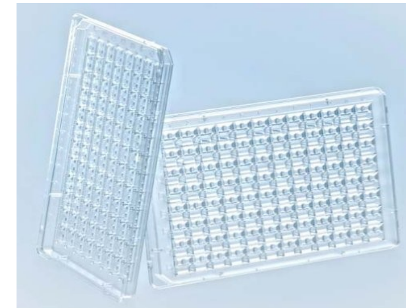
FIP-BM30A (ESRF)
CBS (Montpellier)

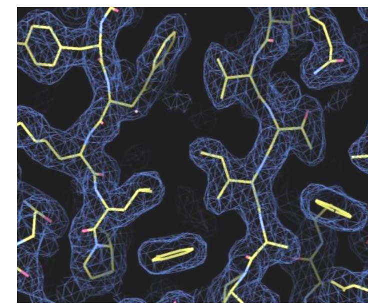
A new virus structure: Bovine enterovirus 2 crystallization plate screening on I24 at DLS



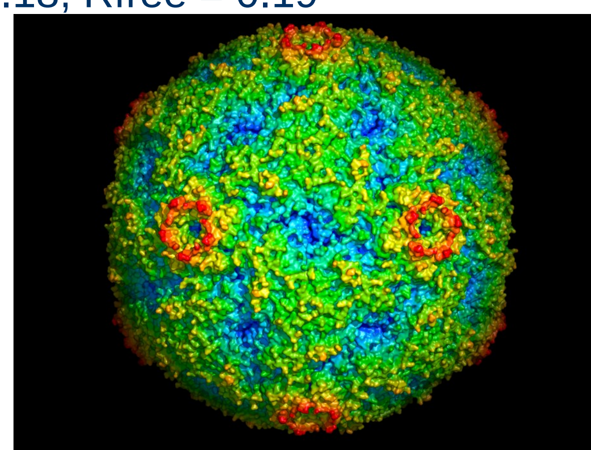


CrystalQuick X plate





2.2 Å resolution
R= 0.18, Rfree = 0.19



Data collected at DLS, I24
Beam size 20 microns, focus at detector (P6M)
exposure time 0.1 sec, 0.1º oscillation,
detector distance = 480 & 645 mm,
resolution at edge of detector 2.28 & 2.97 Å

E.E. Fry, J.S. Ren, A. Kotecha, T.S. Walter, C. Porta, D.I. Stuart, The Wellcome Trust Centre for Human Genetics, University of Oxford (UK),
D.J. Rowlands, Institute of Molecular and Cellular Biology, University of Leeds (UK) and
Gwyndaf Evans, Robin Owen, Danny Axford, Jun Ashima, I24, Diamond Light Source (UK)

MXIS

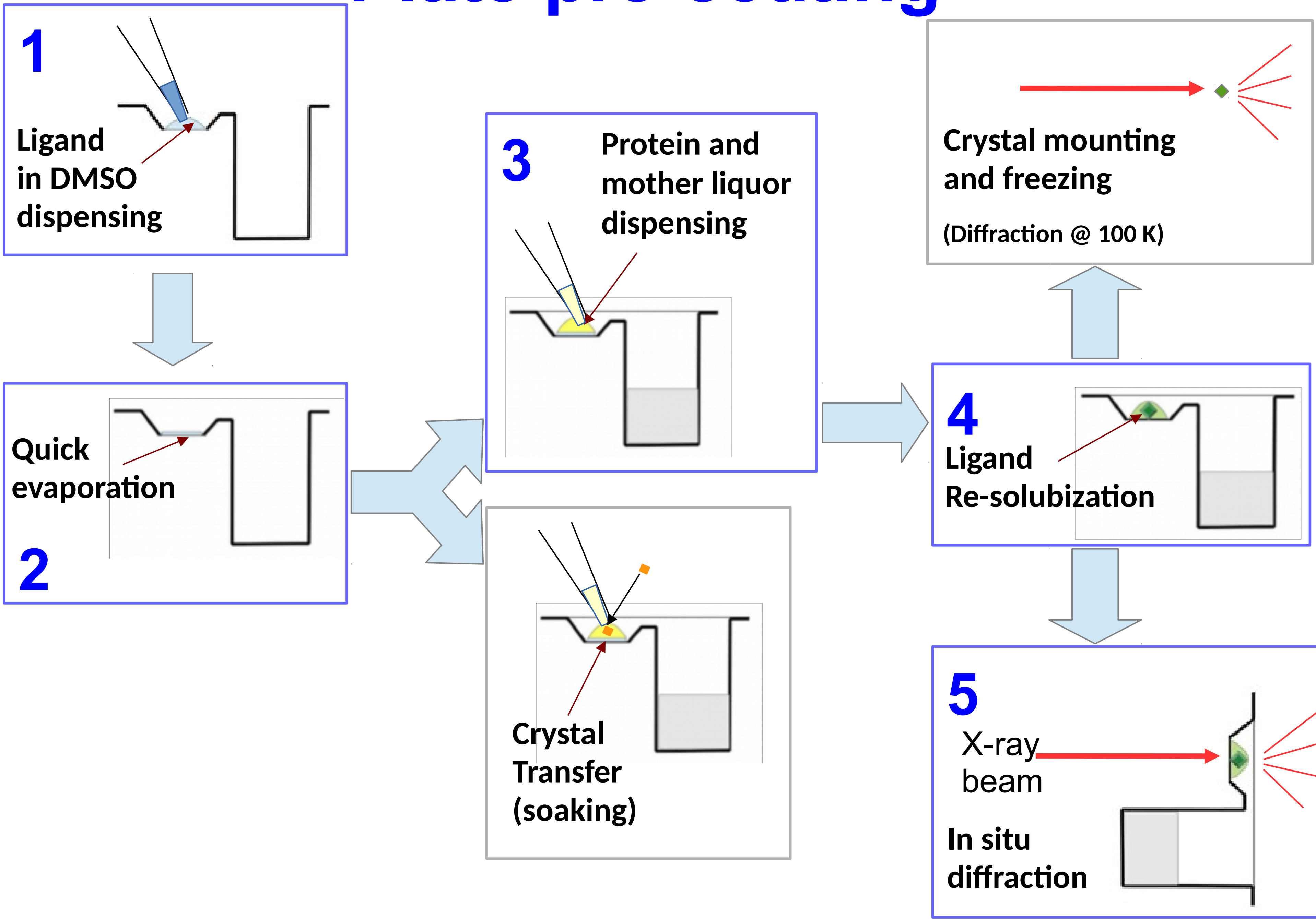
Nov.-Dec. 2017

IBS-ESRF-CBS-EMBL

A workshop on in situ technique
With special focus on small molecules screening



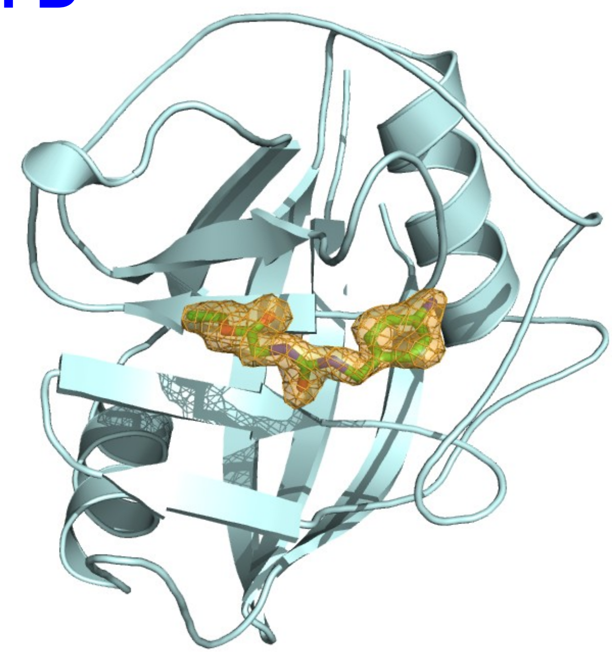

Plate pre-coating



« Dry co-crystallization » of CypD

- proline isomerase
- crystallized in $P4_12_12$
- validated target in ischemia (Alam et al., 2015)

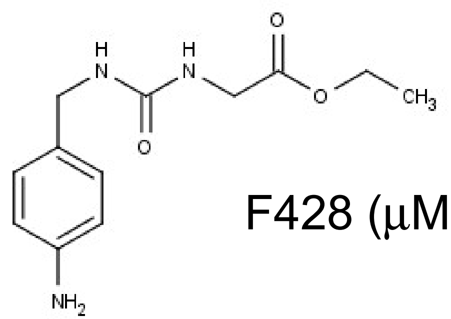
Cyclophilin D (CypD)



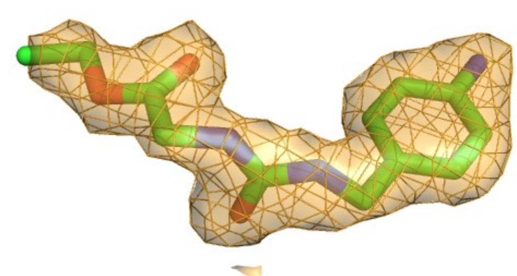
=> new inhibitor detected

- micromolar range
- MW: 251 and XlogP: 0.37

Further chemical derivations
=> nanomolar inhibitors
=> pre-clinical trials
(Guichou et al., 2011).



F428 (µM)



In situ
In house
(2.23 Å)
2 crystals

Labesse G, Gelin M, Guichou J-F, CBS Montpellier)

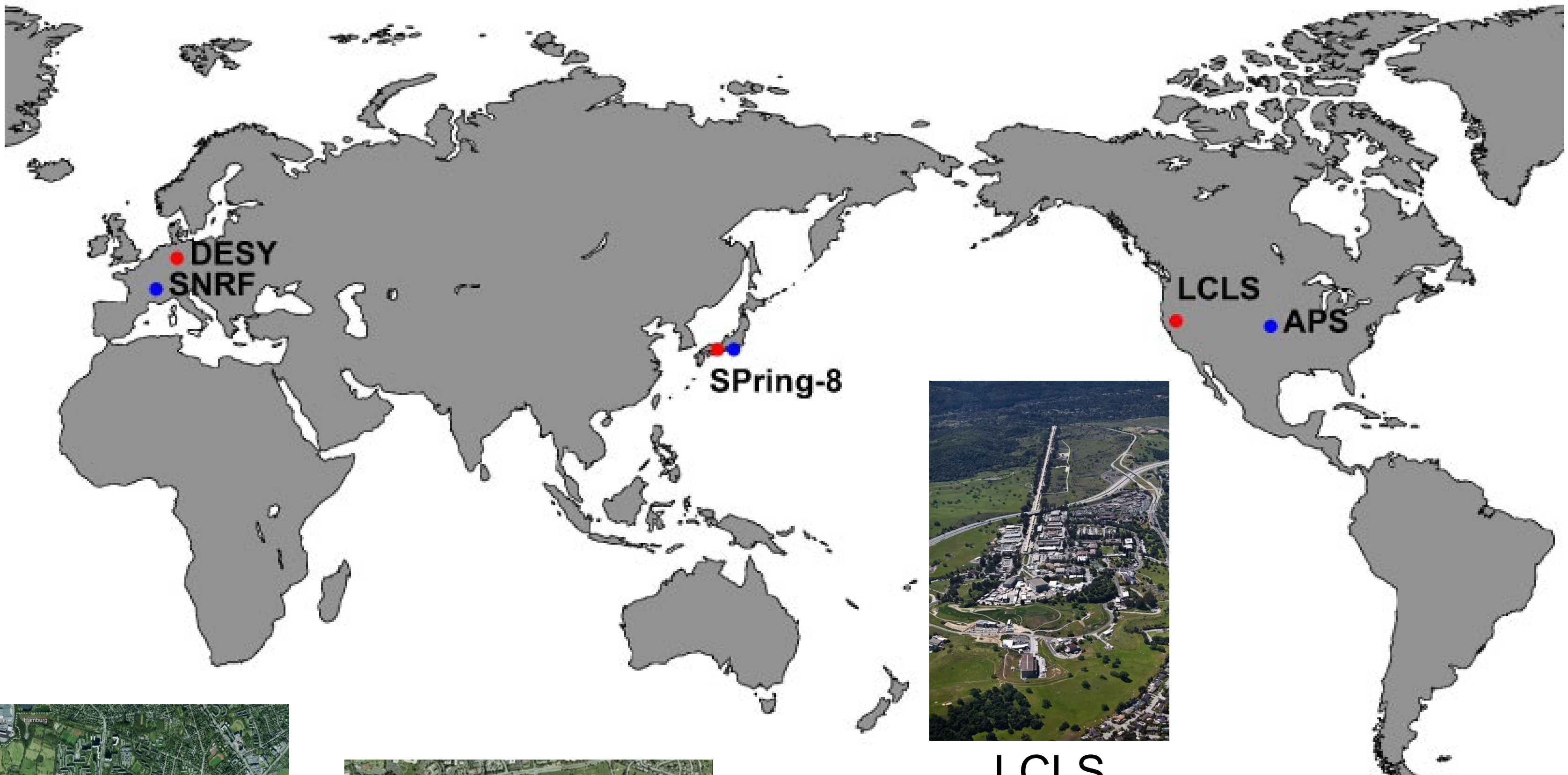
2010s: 3rd+/4th generation sources

X-ray sources
Fast SS detectors

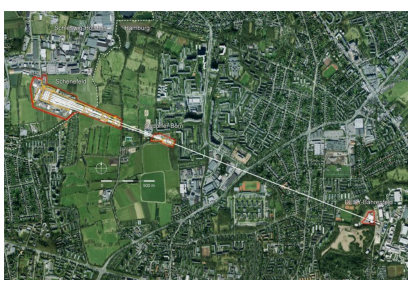
Micro/nano crystals

Room temperature / serial data collection

XFELs: 4th generation X-ray sources



LCLS
(Stanford)



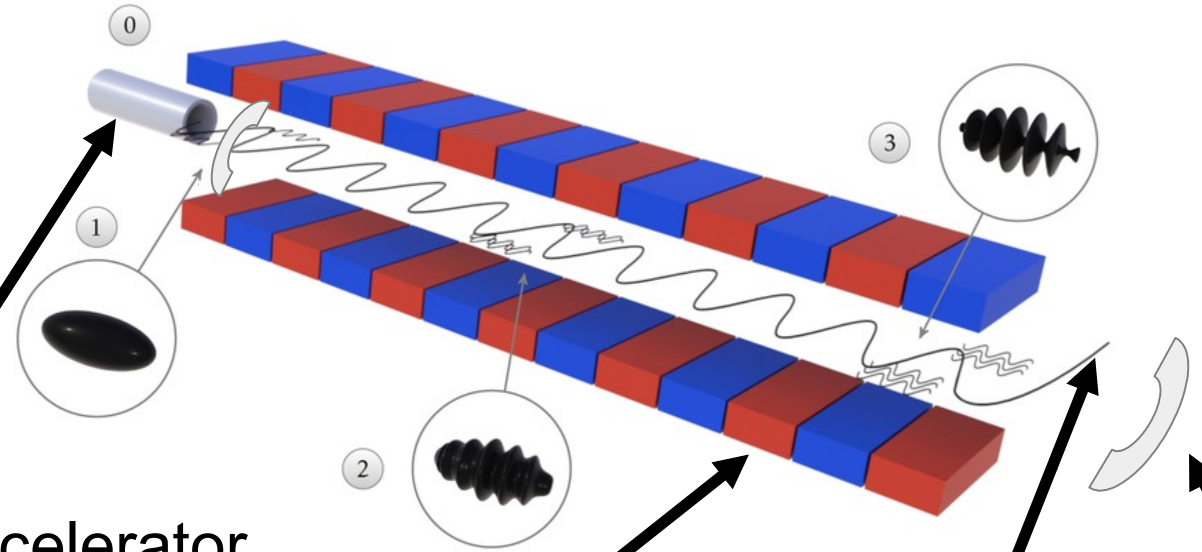
European XFEL
(Germany)

SwissFEL
(Viligen)

SACLA (Japan)

XFEL-O (Argonne)

XFELs: 4th generation X-ray sources



Linear accelerator
Undulator
e- beam discarded
Optical cavity

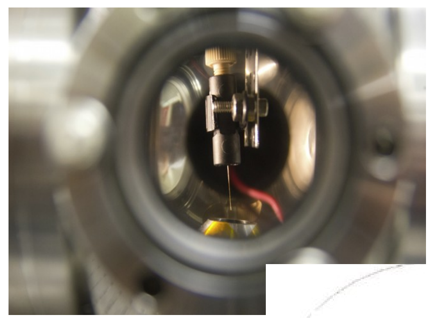
Long undulator => **micro-bunching** of the electron beam
=> **self amplificating spontaneous emission**

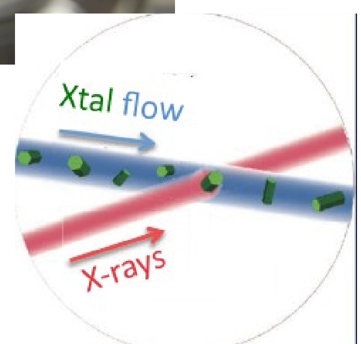
e- in undulator field → X-ray beam
e- in X-ray beam field → X-ray beam exponentially
Transverse and longitudinal coherent beam

4th generation X-ray sources: Sample dispensing

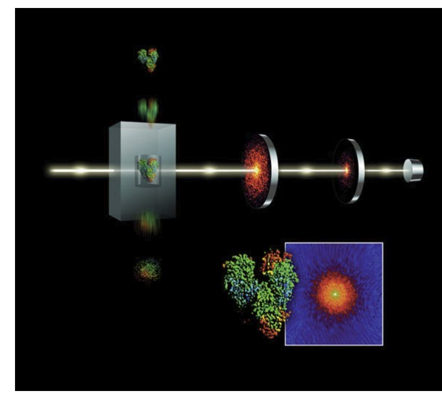
Sample destroyed upon exposure to the beam (1 frame /sample)
→ samples to intercept the beam at an high frequency
→ merging of many randomly collected diffraction frames

Crystals in droplets ejected with sonic waves





Continuous stream of nano-crystals solution

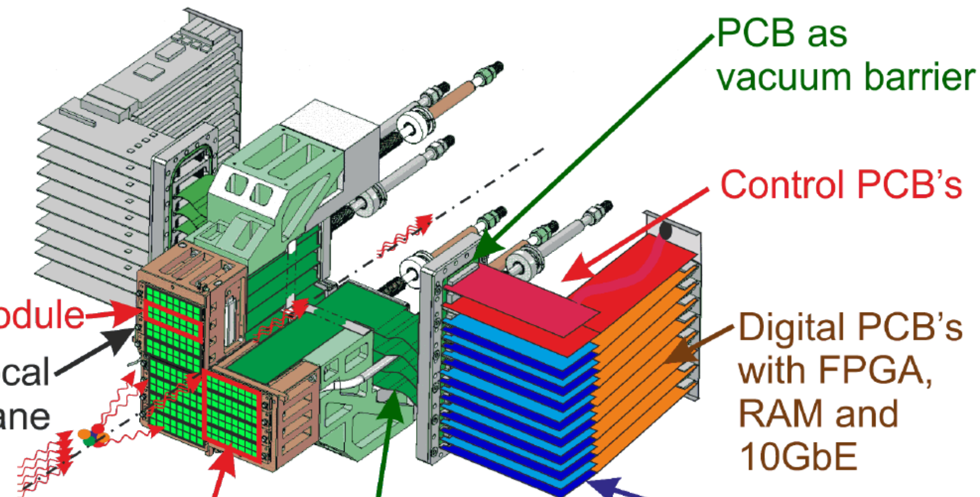


Up to single particules analysis ?

4^{th} generation X-ray sources: Detectors

To come…

3.5 MHz frame rate!



AGIPD (DESY, PSI)

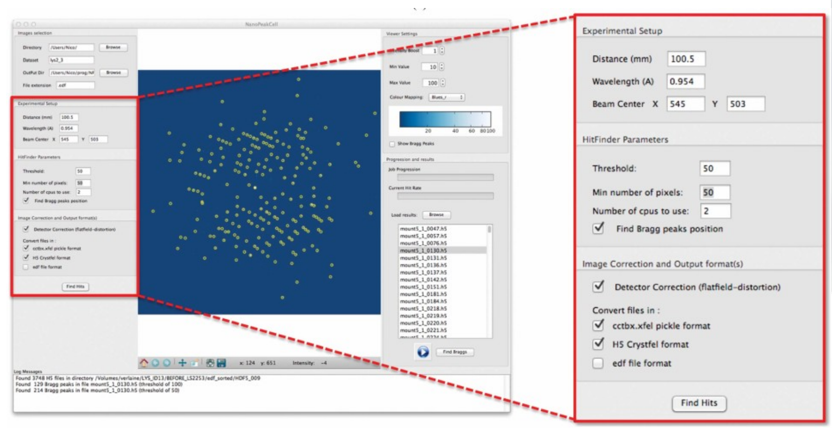
(Allahgholi et al., Journal of Instrumentation, 10 2015)

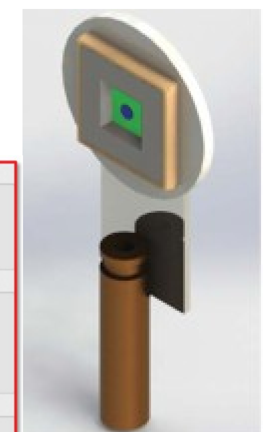
3 and 3+rd generation X-ray sources: Serial data collection

Convergence between in situ approach on 3rd gen. sources and high rate sample dispensing on X-FELs

A large number of small crystals used to collect partial dataset at room temperature
→multiple crystals on a single support
→clustering and merging data

Spectra quality
Small crystals
RT
Access





Raster-scanning serial protein crystallography using micro- and nano- focused synchrotron beams. Coquelle et al.. Acta Crystallogr D 71(Pt 5), 2015:1184-96

The X-ray offer on large facilities

Synchrotron beamlines in France

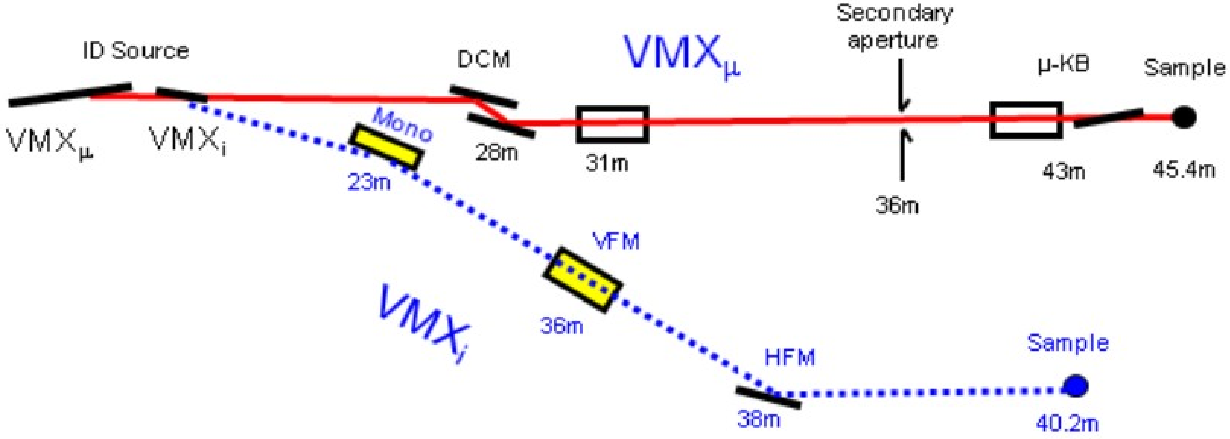
Synchrotron / Station	Beam	Main equipments	Experiments
ESRF			
ID23-1	40x30um/0.6-2.5Å	MD2/SC3/Pilatus6M	SAD, MAD
ID23-2	8x6um/0.873Å	MD2/SC3/Mosaic225	single wav.
ID29	10x75um/0.7-2.1Å	MD2/SC3/Pilatus6M	SAD, MAD
ID30A-1	100x65um/0.968Å	RoboDiff/Pilatus2M	single wav.
ID30A-2	~100um/0.984Å	?	single wav.
ID30A-3	15um/0.984Å	MD2/SC3/Eiger4M	single wav.
ID30B	20x20um/0.62-2.1Å	MD2/SC3/Eiger?	SAD, MAD
BM30A	300um/0.7-1.8Å	MD2/G-Rob/ADSC315	in situ/SAD/MAD
SOLEIL			
Proxima1	40x20um/0.84-2.5Å	Kappa/CATS/Pilatus6M	SAD, MAD
Proxima2A	10um/0.84-2.5Å	MD2/CATS/ADSC315	SAD, MAD

Synchrotron beamlines in Europe

Synchrotron	Station	Beam	Experiments
SLS	PXI-X06SA	10x40um/0.72-2.2Å	SAD/MAD
	PXII-X10SA	50x10um/0.62-2.07Å	SAD/MAD
	PXIII-X06DA	80x45um/0.71-2.07Å	SAD/MAD/in situ
DLS	I02	80x20um/0.5-2.5Å	SAD/MAD
	I03	80x20um/0.5-2.5Å	SAD/MAD/in situ
	I04-1	???/0.92Å	single wav./in situ
	I04	10x5um/0.88-2.07Å	SAD/MAD
	I23	*1.5-4Å*	*sulphur SAD*
	I24	10x10um/0.7-2.0Å	SAD/MAD/in situ
	VMXi / VMXu…	*(I02)*	
BESSY	MX14-1	40-30um/0.8-2.5Å	SAD/MAD
	MX14-2	180x70um/0.8-2.5Å	SAD/MAD
	MX14-3	180x110um/0.91Å	single wav.
PETRAIII	P13	30x20um/0.7-2.7Å	SAD/MAD
	P14	5x5um/0.6-2.1Å	SAD/MAD
MAX IV	commissioning		
ELETTRA	XRD1	200um/0.6-3.15Å	SAD/MAD
ALBA	BL13-XALOC	50x10um/0.6-2.4Å	SAD/MAD

3+rd generation X-ray sources and sub-micron beams

Project of sub-micron beams, such as VMXu at DLS, ...



High flux, very small beam size

→ small crystals

→ short exposure

makes possible **complete data collection at RT** before decay

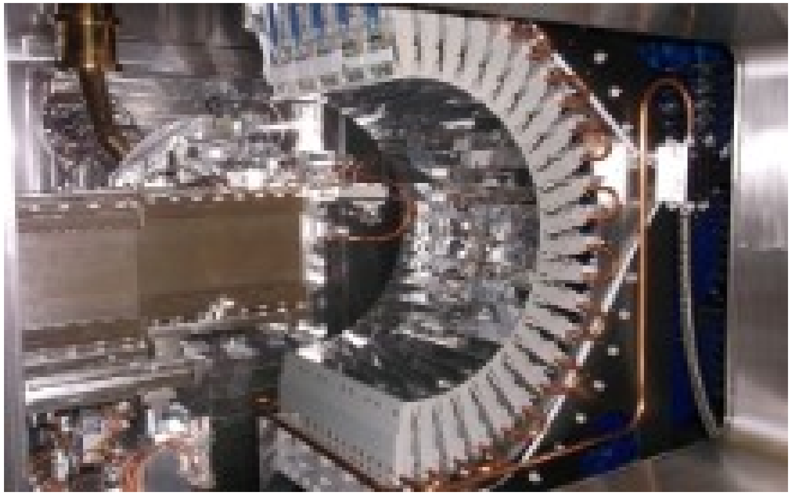
Ultrafast (ms) data collection with ultra-high dose rate at RT could reduce radiation sensitivity to the one at 100 K

Warkentin et al. (2013) JSR 20, 7

Owen et al. (2012) Acta Cryst D68, 81

Large wavelength beamline at DLS

ID23 is dedicated to anomalous phasing at large wavelength (S/P-SAD, anom. from Ca, S, … atoms)



Up to 4 Ang

Experiment under vacuum !
Cylindrical pixel detector