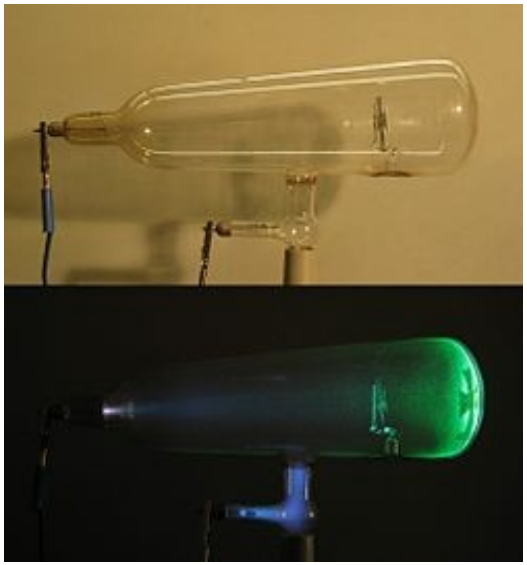
Past and present progress in X-ray sources: consequences for crystallography of biological macromolecules

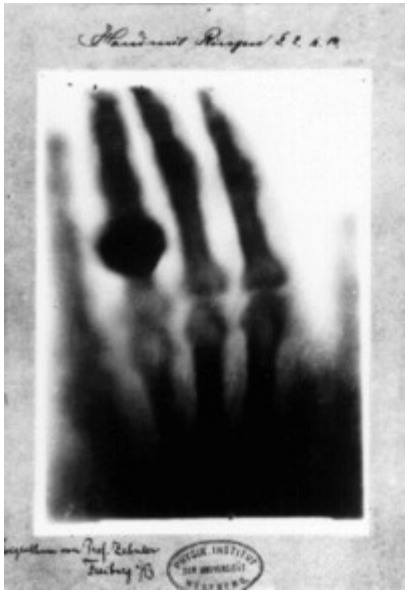
J-L Ferrer
IBS/Synchrotron Group (Grenoble, France)

1895: First X-rays



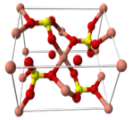
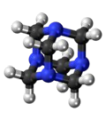
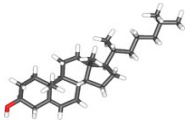
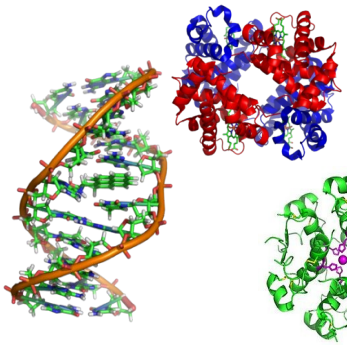
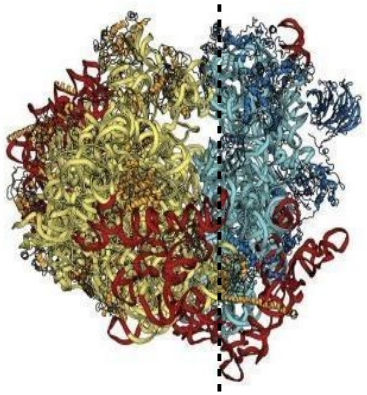
Crookes tubes are cold cathode tubes: from a few kilovolts to about 100 kilovolts is applied between the electrodes.
The Crookes tubes require a pressure from about 10-6 to 5×10-8 atmosphere.

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

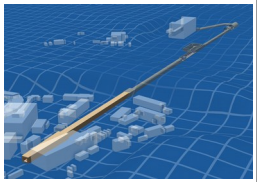
1910 1920 1930 1940 1950 1960 1970 1980 1990 2000 2010


1917





?






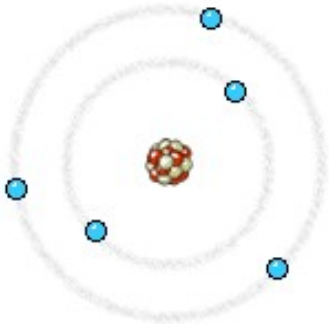
1895-...: sealed tubes / rotating anodes
1-2nd generation synchrotrons

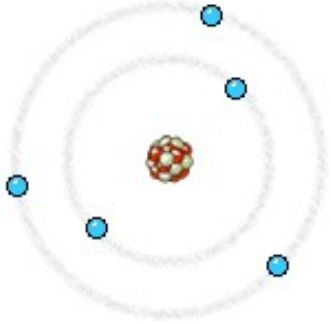
X-ray sources
Films / IP

Capillaries

Isomorphous replacement

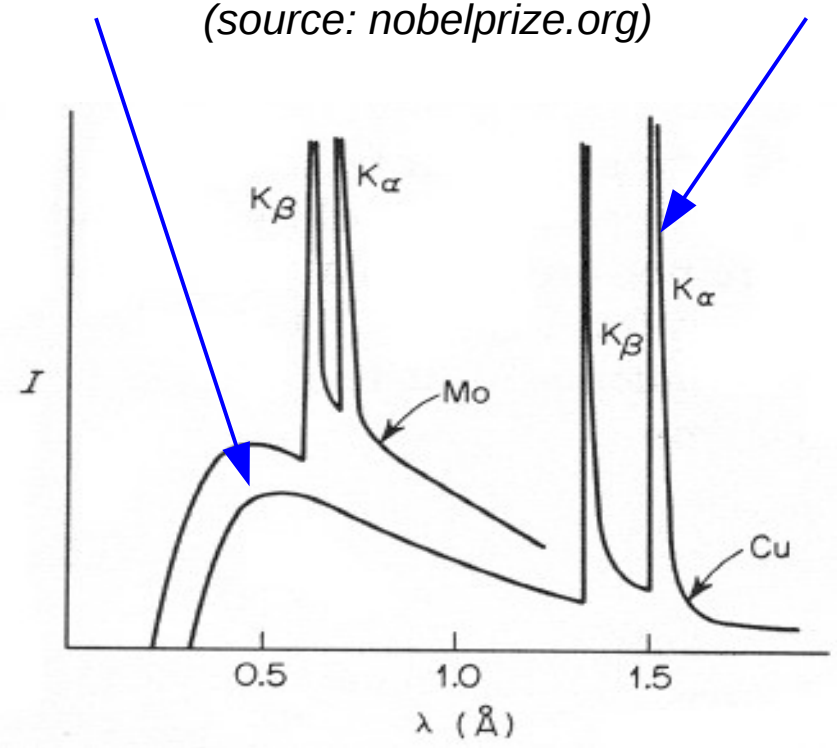
Lab sources

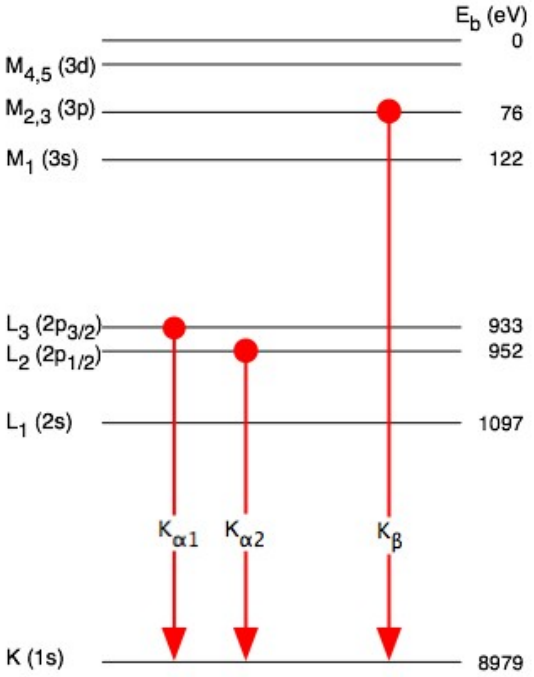




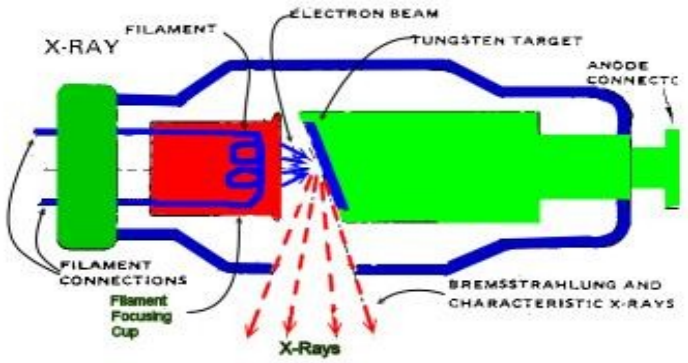
Bremsstrahlung (braking radiation) ***Characteristic lines***

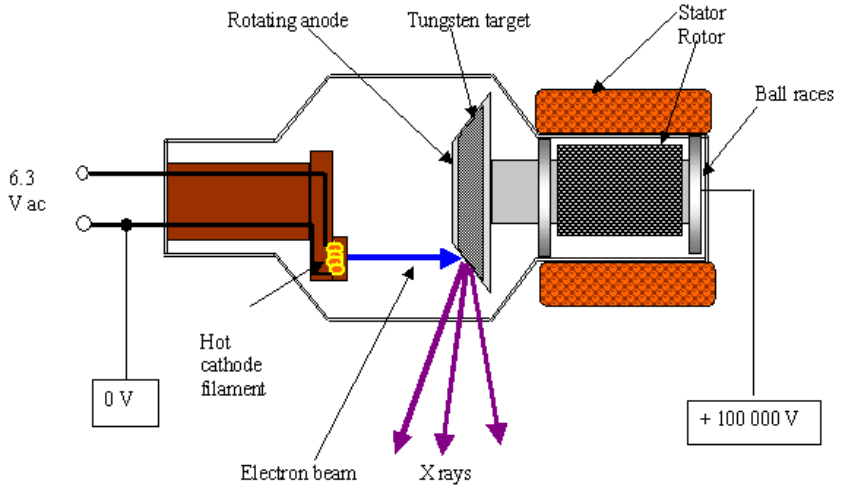
(source: nobelprize.org)

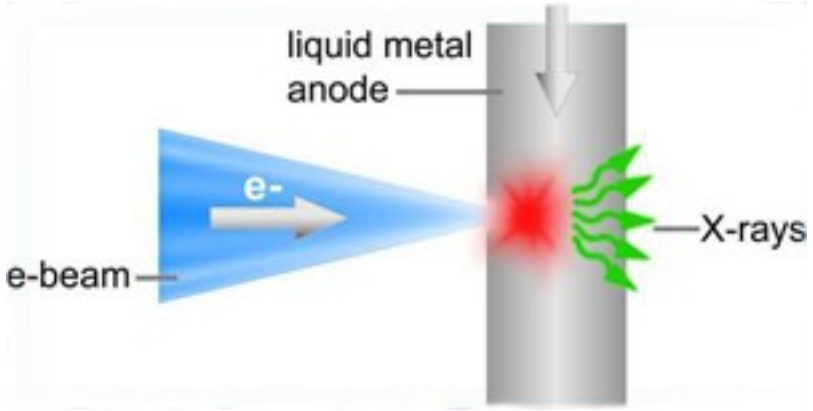




Lab sources







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

Synchroton generations

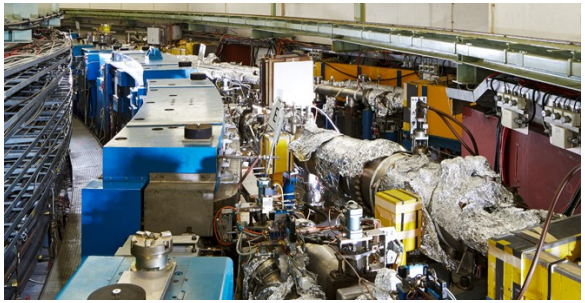
1st generation synchrotron: parasitic operation (50s to 70s)

ACO, DORIS, SPEARS...

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

2nd generation synchrotron: dedicated to SR (80s)

SRS, DORIS, NSLS, SuperACO...

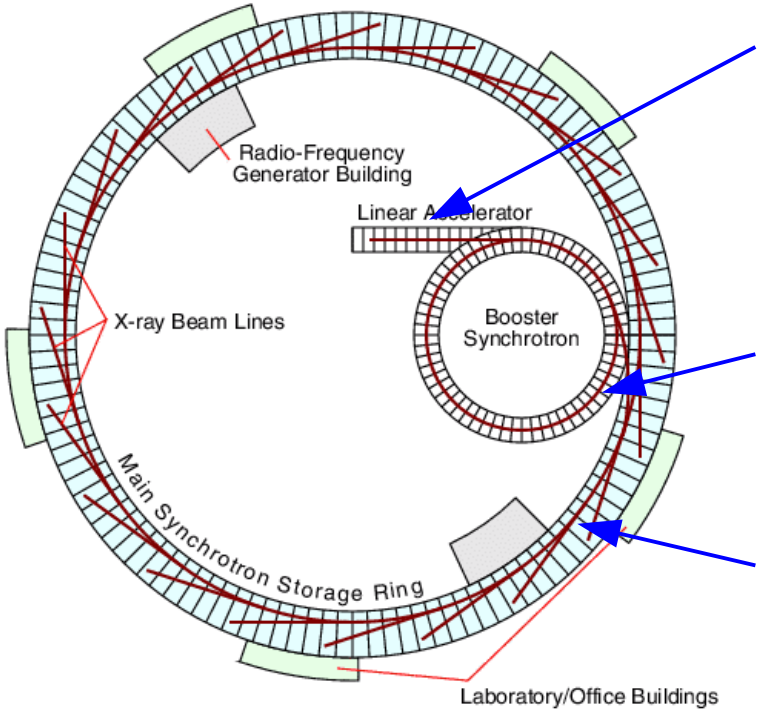


DORIS (http://www.desy.de)

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

ESRF, ALS,...

Synchrotron components



Linac

Electron beam generation
First beam acceleration

Booster

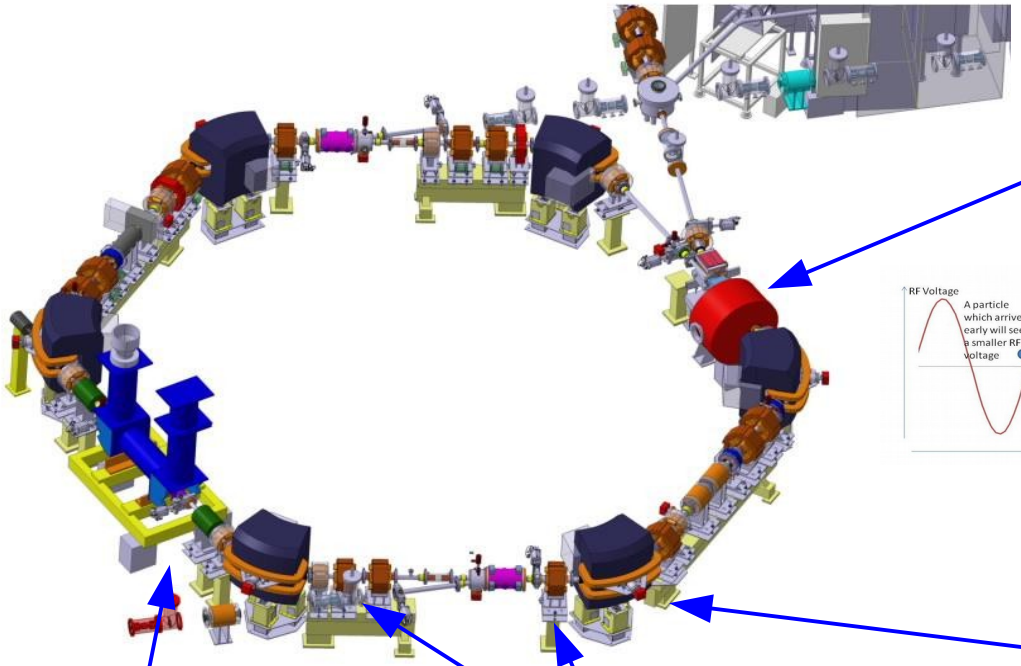
Acceleration to nominal energy

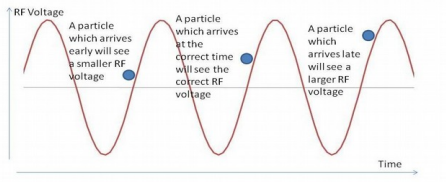
Storage ring

Beam storage for SR use

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

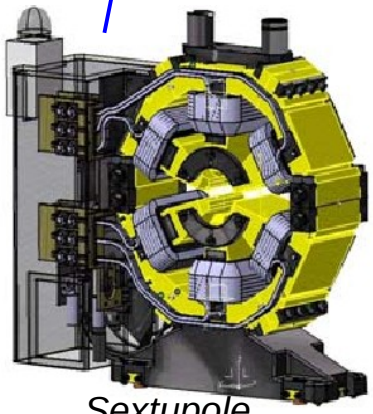
Synchrotron components



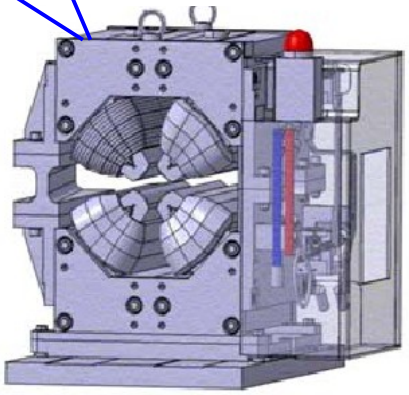


RF cavity
electron beam acceleration
(www.synchrotron-soleil.fr)

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



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



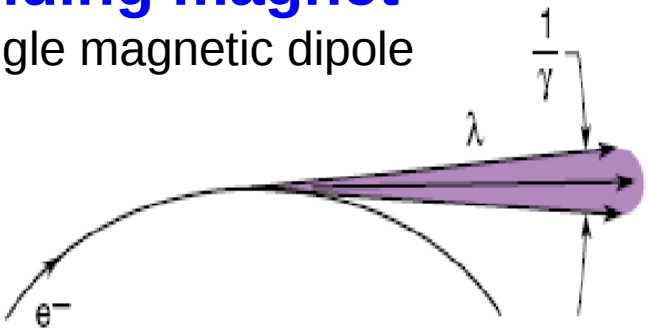
Quadripole
for electron beam focusing
(www.synchrotron-soleil.fr)

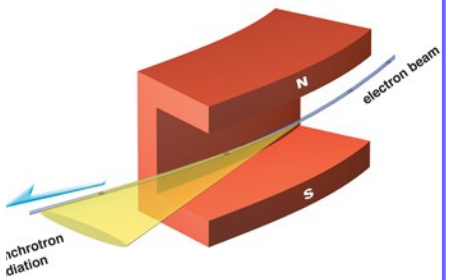
The multipole magnets refocus the beam after each deflection section, as deflection sections have a defocusing effect.

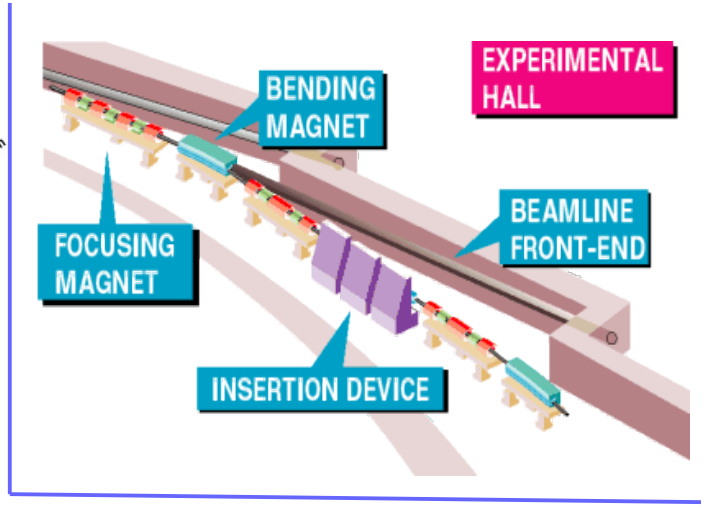
2nd generation synchrotrons

Bending magnet

A single magnetic dipole



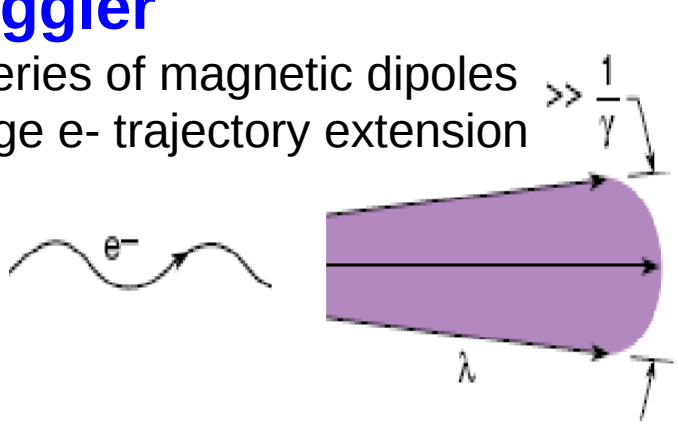


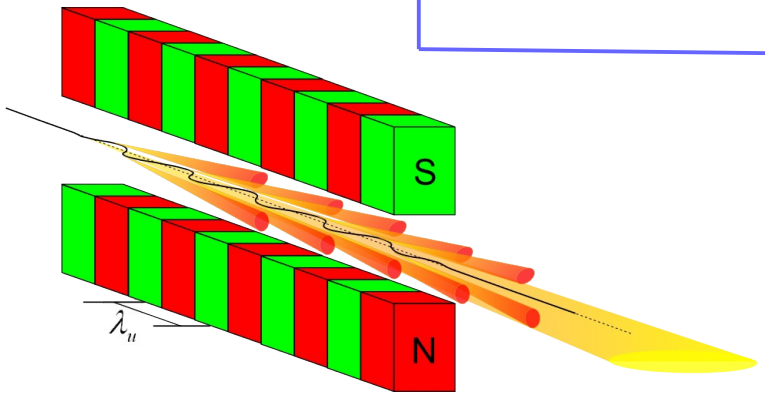


Wiggler

A series of magnetic dipoles

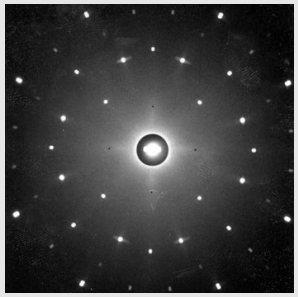
Large e- trajectory extension



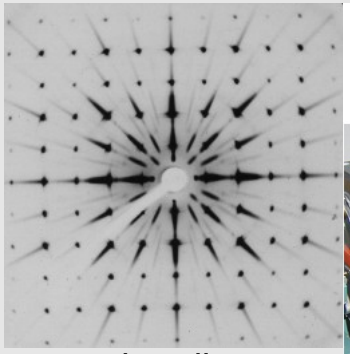


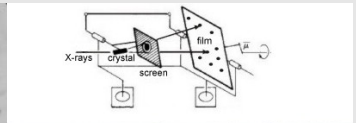
Brown et al. Nuclear Instruments and Methods, Volume 208, 1983, 65-77

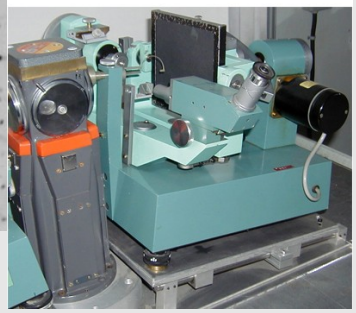
Detectors: films and IPs



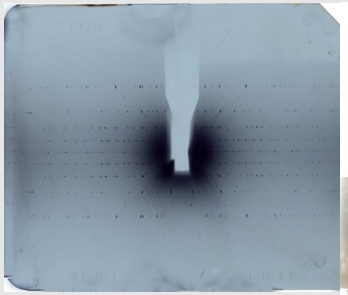
Laue diagram (www.xtal.iqfr.csic.es)

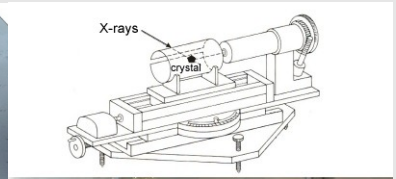






Precession diagram (www.xtal.iqfr.csic.es)





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

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

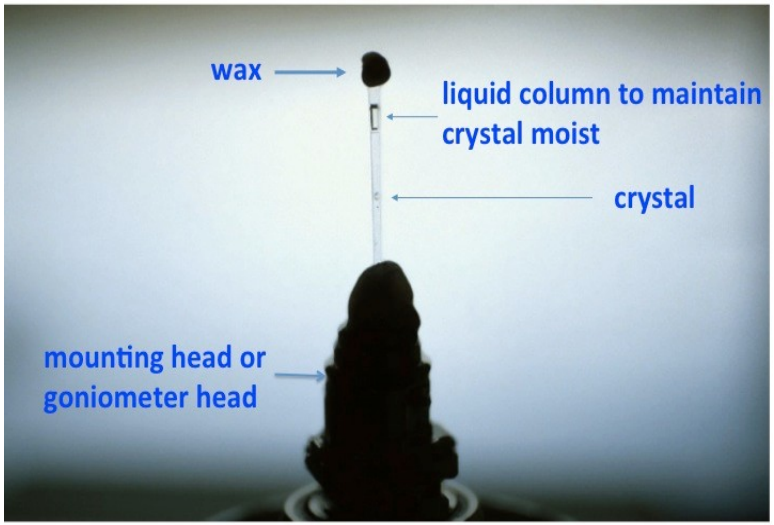
Upon readout:
Photostimulated luminescence (PSL) releases the stored energy within the phosphor by stimulation with visible light, to produce a luminescent signal.

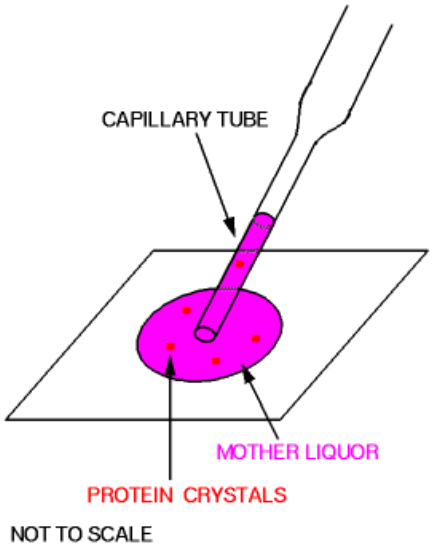
A multi-wire chamber at LURE (1974-1992)

1st MAD structure!

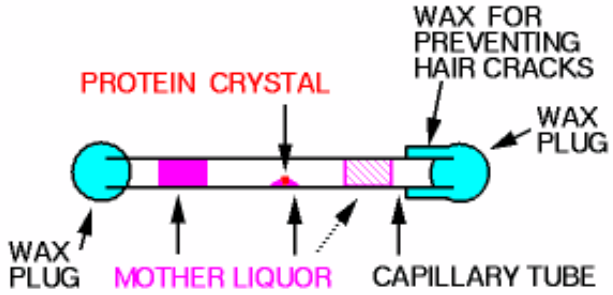
LURE:
R. Kahn, R. Bosshard,
A.Bahri, G. Bricogne,
A. Bentley, R. Fourme
CERN:
R. Bouclier, R. Million
J.C. Santiard, G. Charpak

Samples in capillaries





NOT TO SCALE



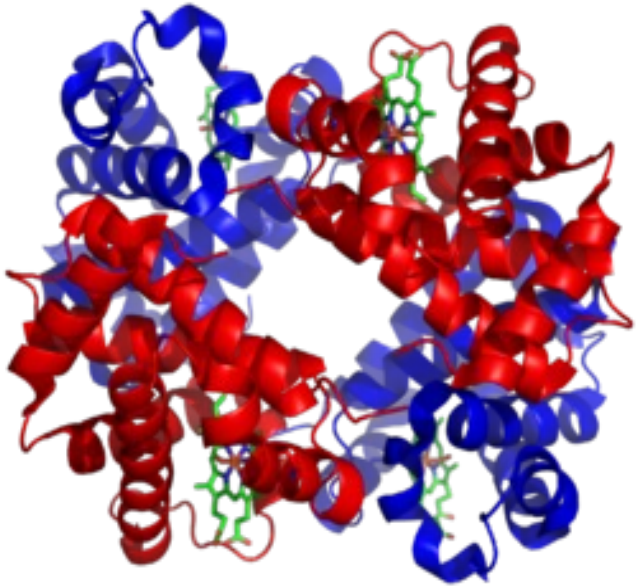
NOT TO SCALE

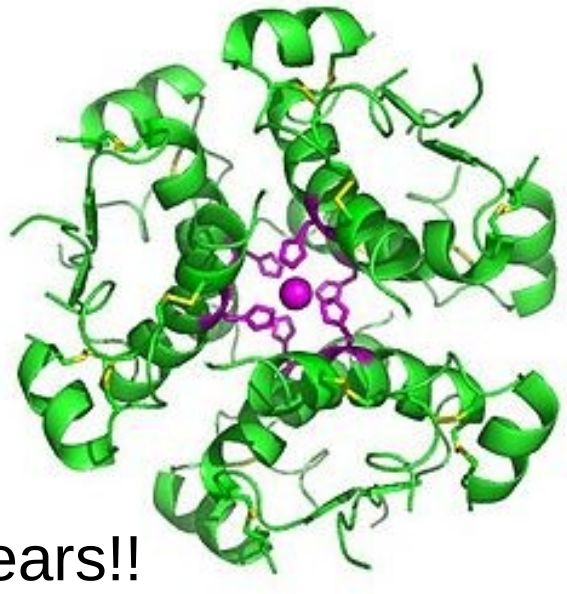
(www.mitegen.com/products/micrort/micrort.shtml)

1959: First protein structures

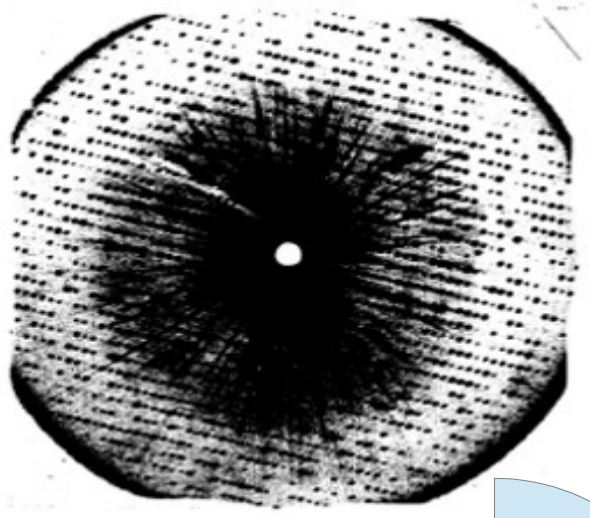
In 1953 **Max Perutz** showed that diffracted X-rays could be phased by comparing the patterns with and without heavy atoms attached.
In 1959 he determined the structure of hemoglobin

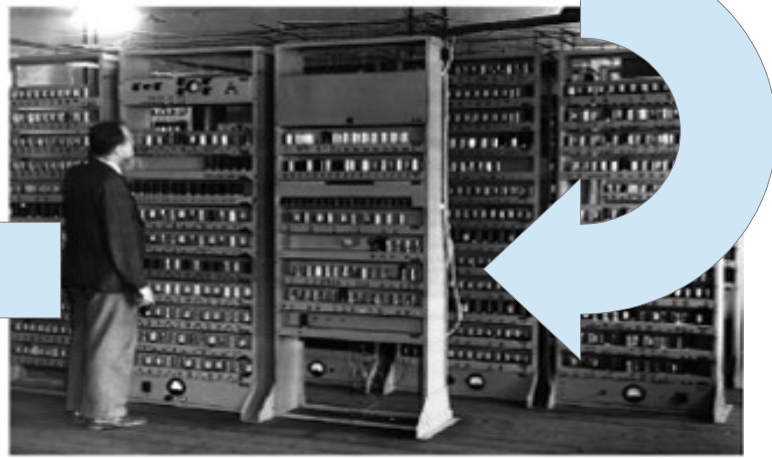
Max Perutz and **John Kendrew** shared the 1962 Nobel Prize for Chemistry for the structures of hemoglobin.

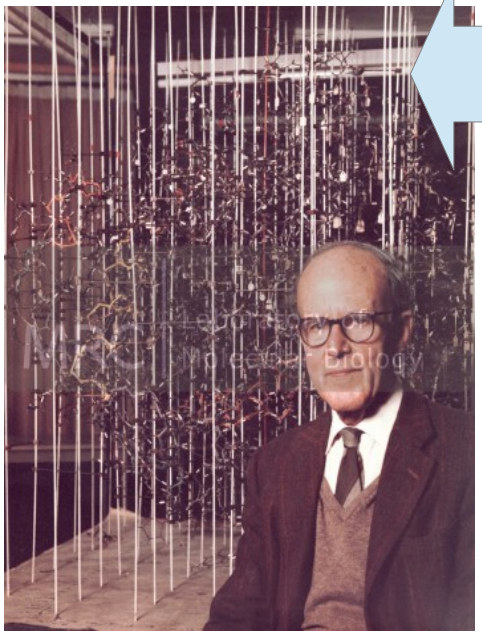


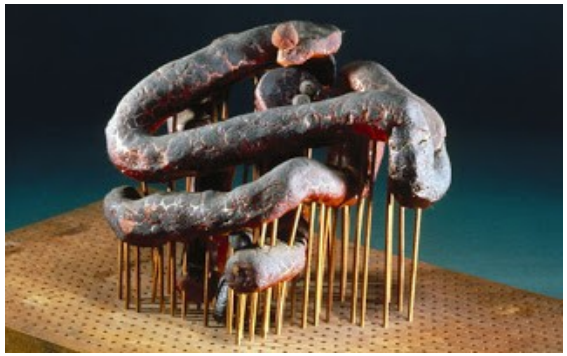


1969, **Dorothy Crowfoot Hodgkin** solved the 3D structure of insulin, on which she worked for over thirty years!!









Myoglobin (1957)

Haemoglobin model 1957

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

1990s: 3rd generation synchrotrons

X-ray sources
CCD detectors / SS detectors

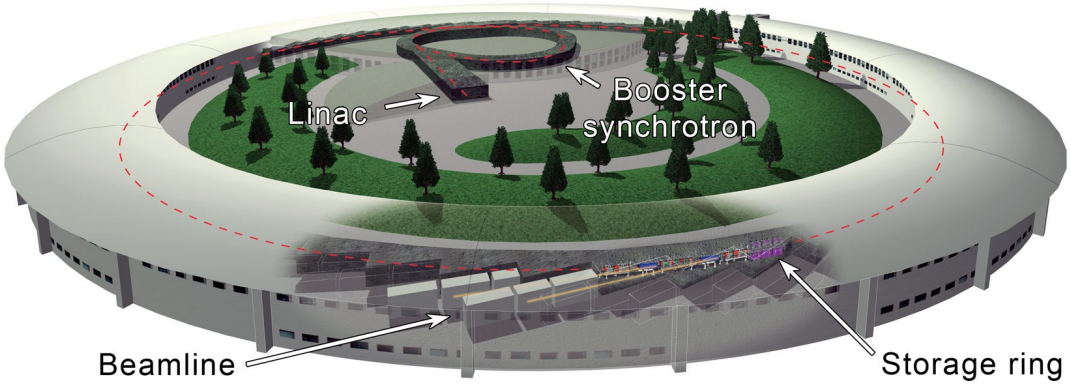
Freezing

Anomalous diffraction

3rd generation synchrotrons

ESRF

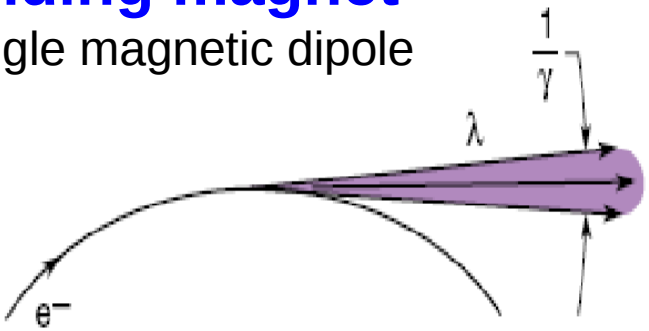
SOLEIL

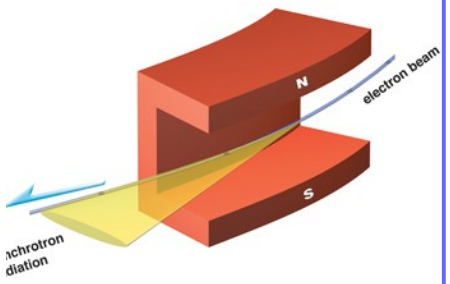


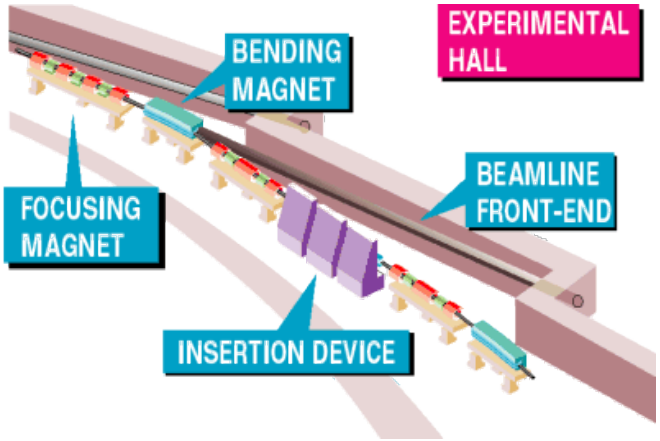
3rd generation synchrotrons

Bending magnet

A single magnetic dipole



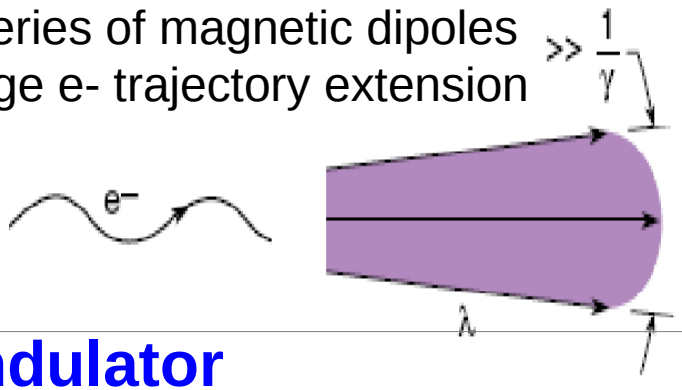


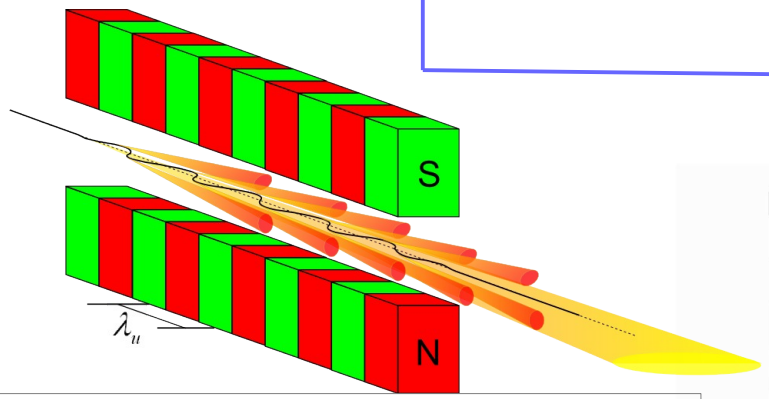


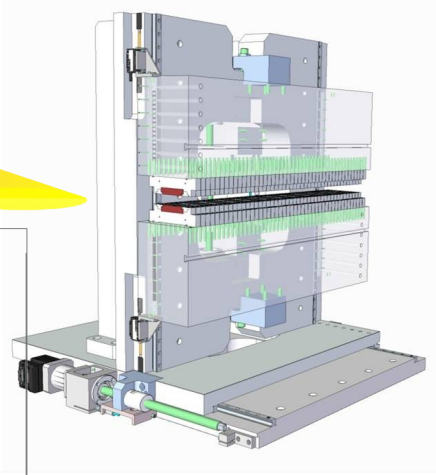
Wiggler

A series of magnetic dipoles

Large e- trajectory extension



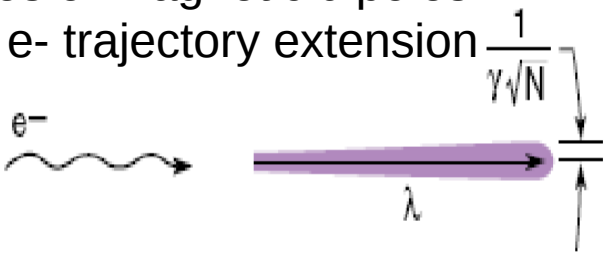


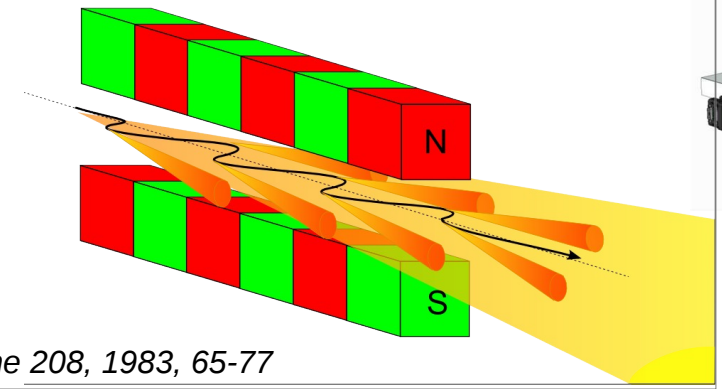


Undulator

A series of magnetic dipoles

Small e- trajectory extension





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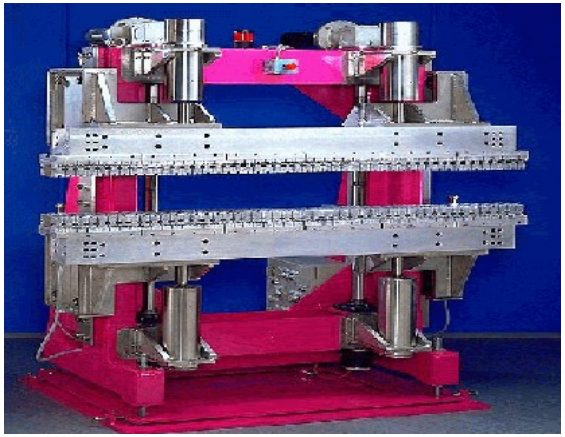
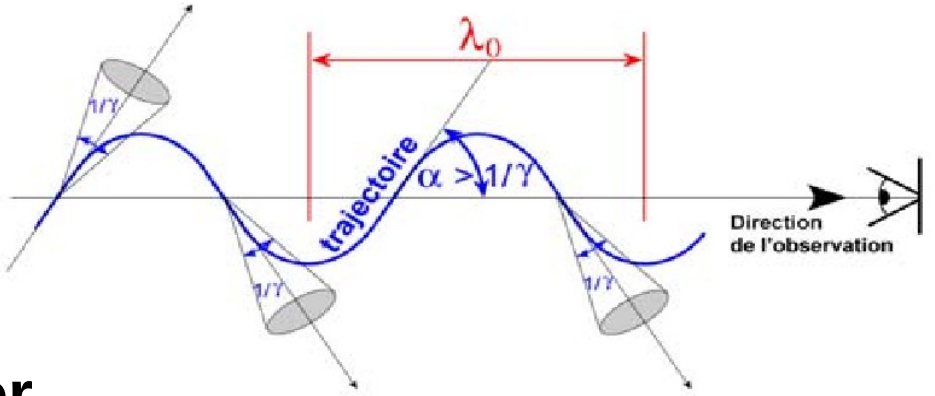
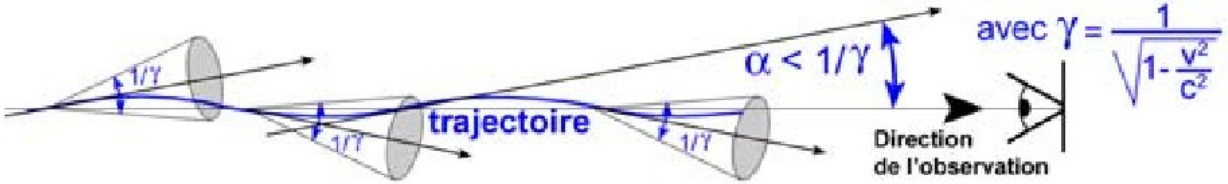
Wiggler *vs* undulator



Wiggler



Undulator



α : angular extension of the e- traj.

γ : emission cone aperture

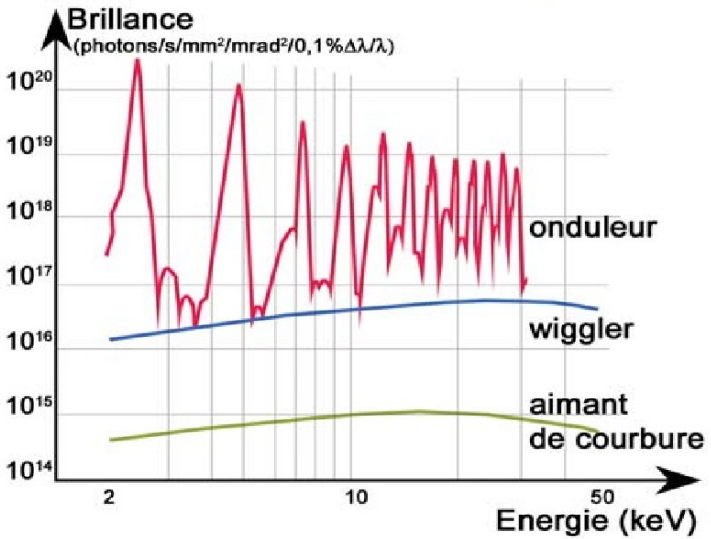
In an undulator: α < 1/γ

=> emission in presence of the beam

=> constructive interferences

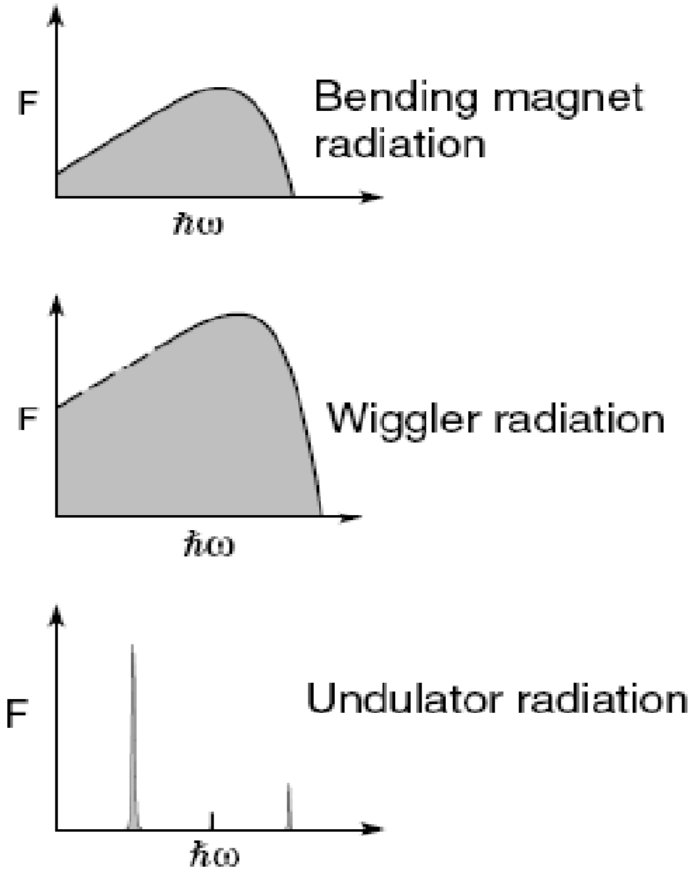
=> spectral lines (brightness in N^2)

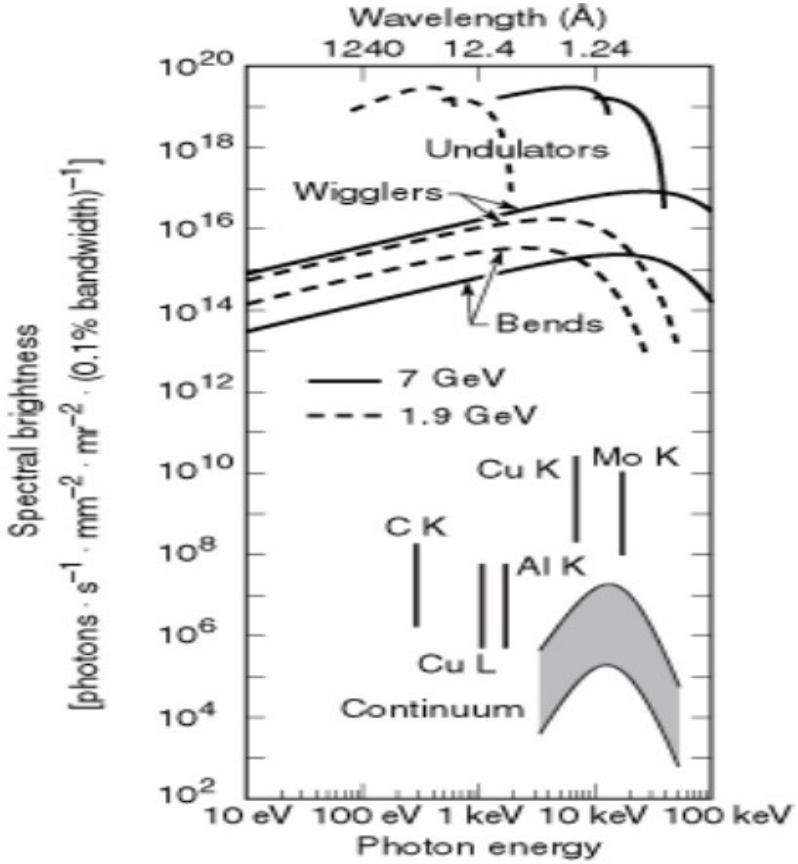
=> beam divergence in $N^{-1/2}$



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

3rd generation synchrotrons



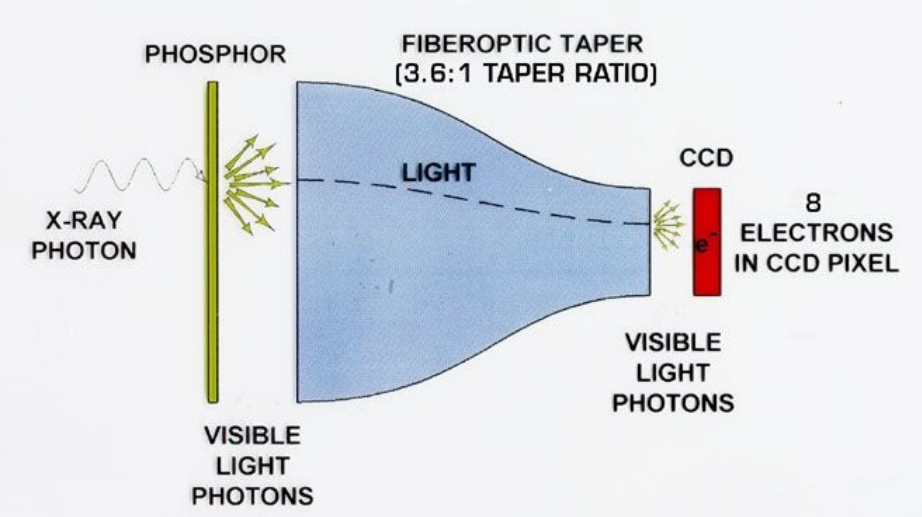


http://xdb.lbl.gov/Section2/Sec_2-1.html

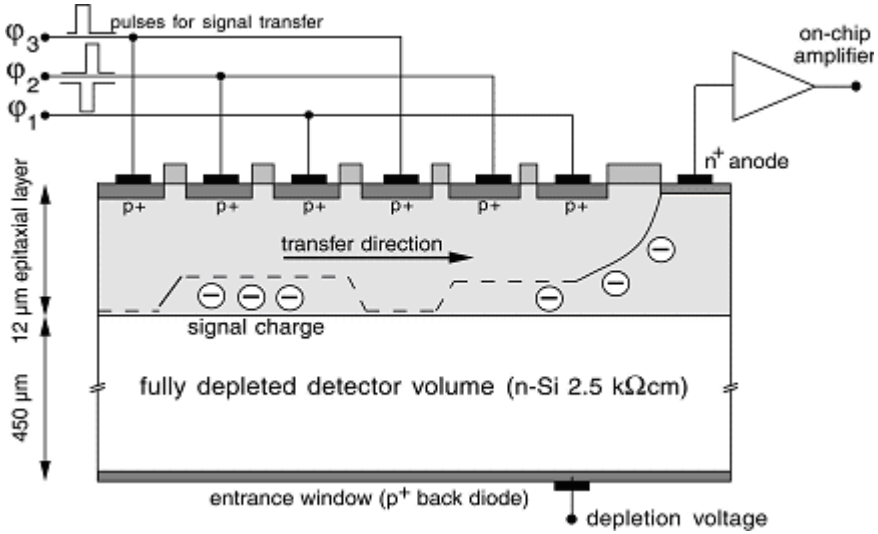
Jim Clarke, ASTeC, SRS

					Bending magnet		**Wiggler**		**Undulator**	
Ring	Energy (GeV)	ρ (m)	I_b (mA)	P_{total} (kW)	$dP/d\theta$ (W/mrad)	$dP/d\Omega$ (W/mrad^2)	$dP/d\theta$ (W/mrad)	$dP/d\Omega$ (W/mrad^2)	$dP/d\theta$ (W/mrad)	$dP/d\Omega$ (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

CCD detectors



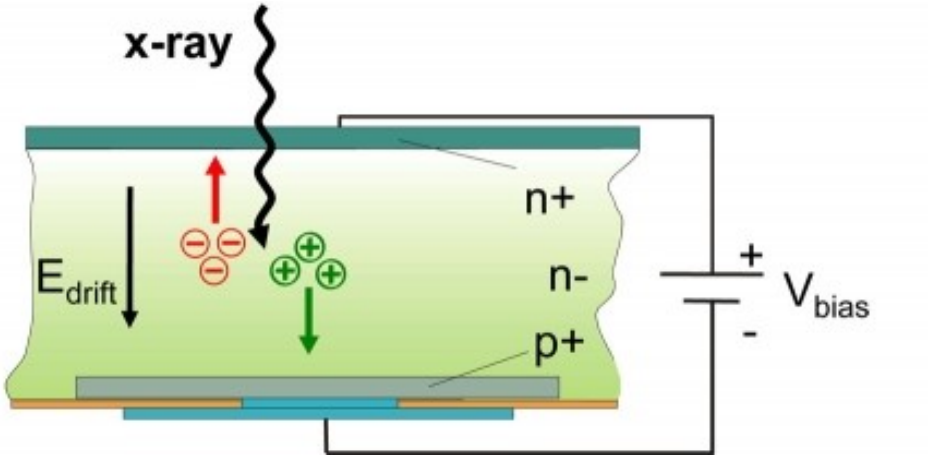
http://proteincrystallography.org



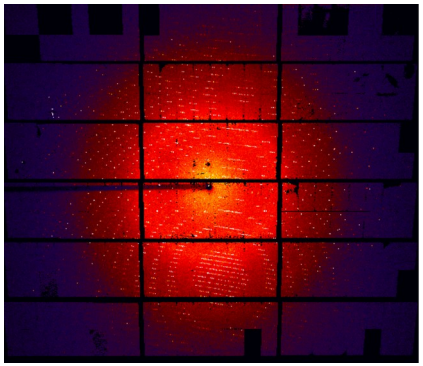
A scintillator converts X-ray photons to visible light photons. The image is demagnified to match the CCD size.

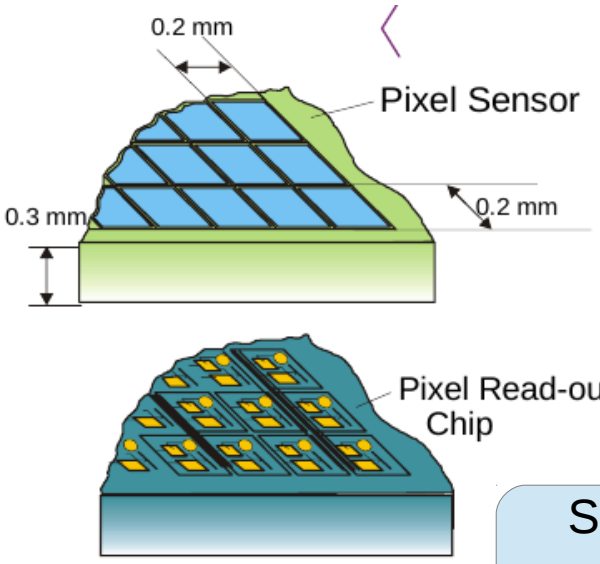
Readout time compatible with synchrotron exposure time + high dynamic

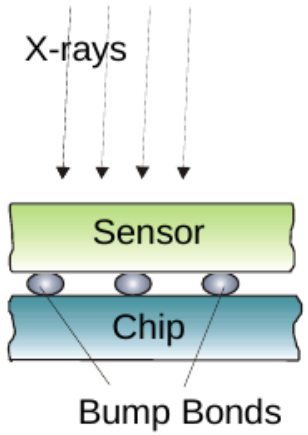
Pixel detectors



http://www.dectris.com







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

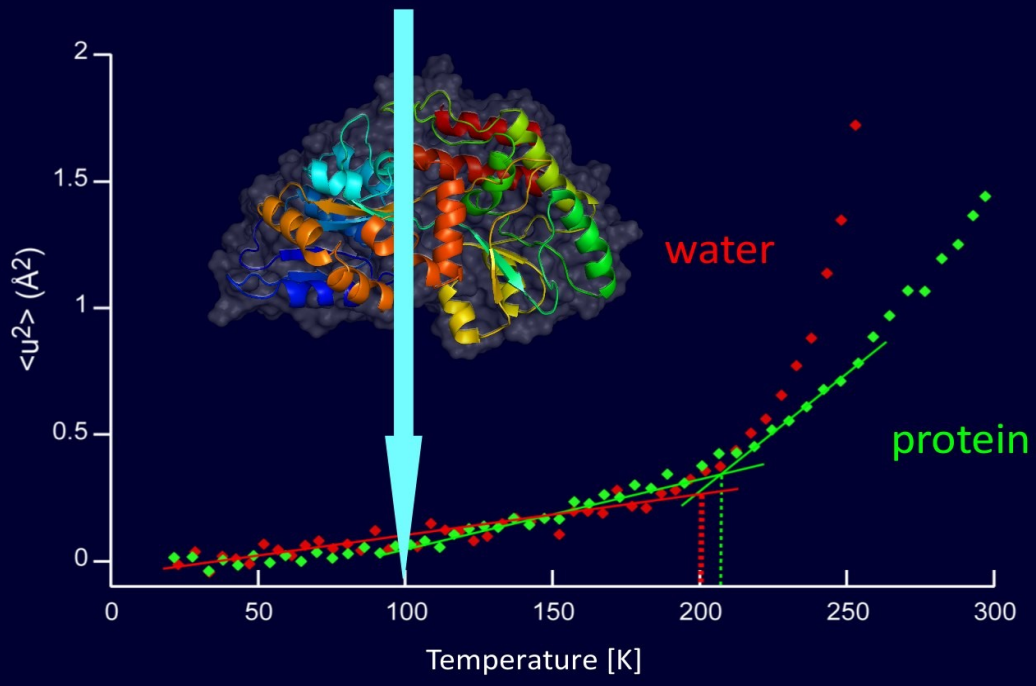
Short readout time
→ shutterless mode
→ fine slicing

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

Cryo-cooling

Temperature-dependent side-chain flexibility from neutron scattering

Cryo X-ray data collection

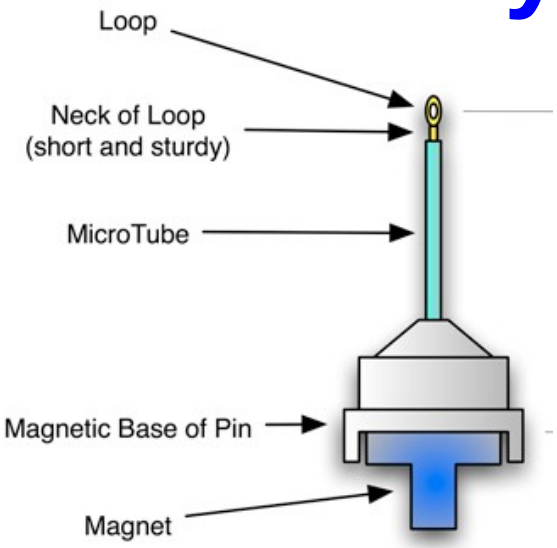


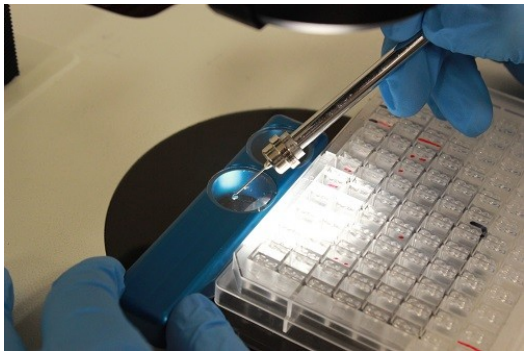
Wood, Frölich, Gabel, Moulin, Haertlein, Paciaroni, Zaccai, Tobias & Weik (2008) JACS 130, 4586

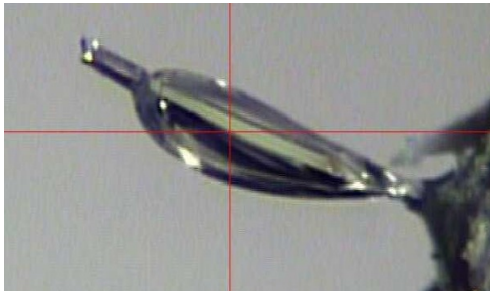
Cryo-cooling at 500 K / s : protein conformational changes quenched at 200 K

Halle (2004) PNAS 2004, 4793

Crystal flash-freezing







Possible improvements:

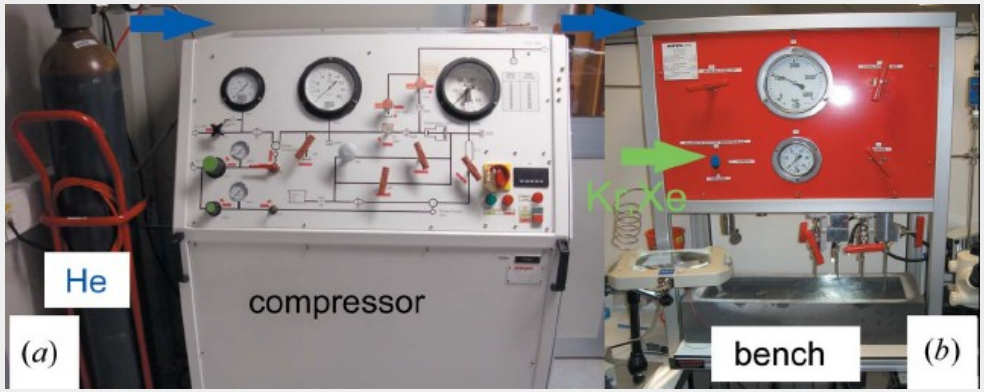
Optimized cryo-protectant

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

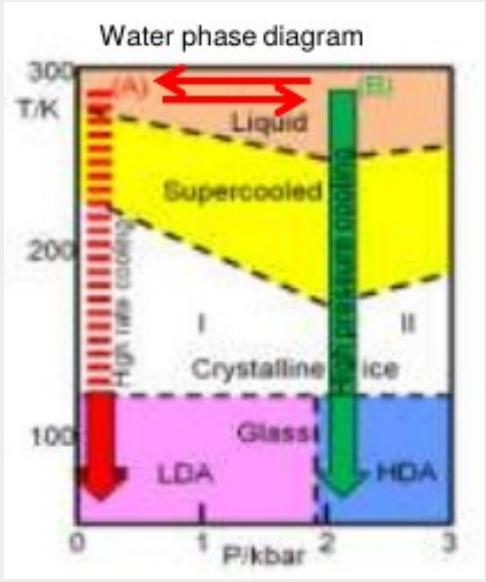
High speed freezing (Warkentin et al., J Appl Cryst. (2006) 39, 805–11)

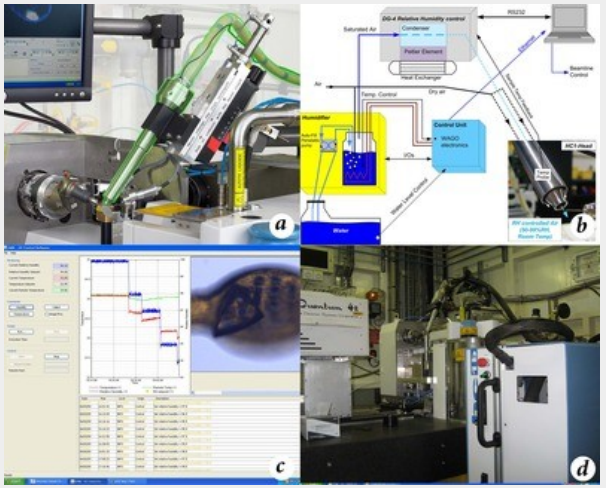
Freezing in propane, etc...

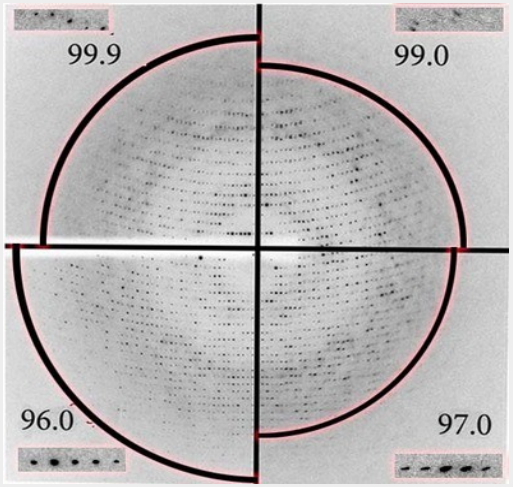
Crystal flash-freezing
Last improvements



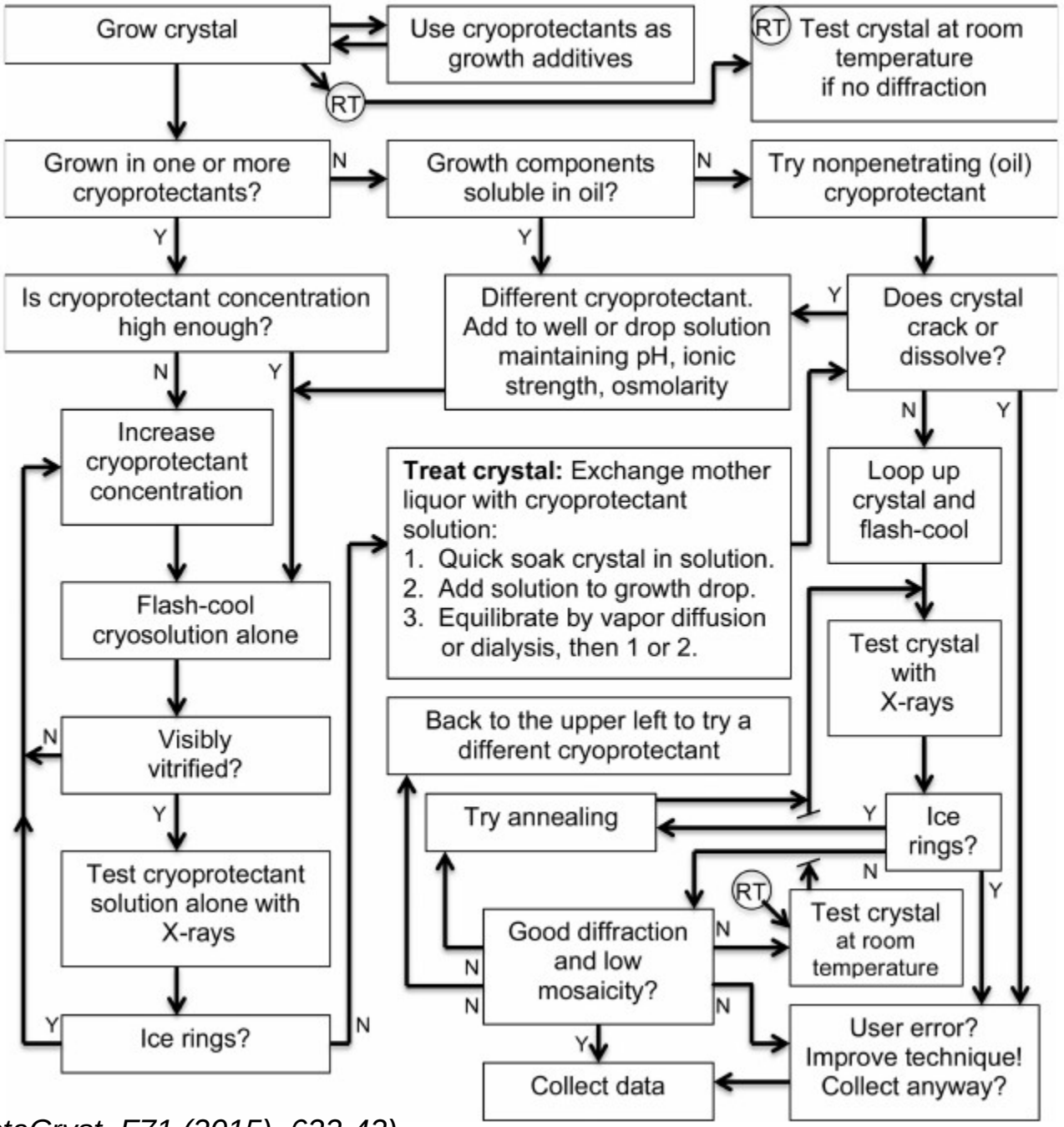
P. Carpentier, ESRF







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



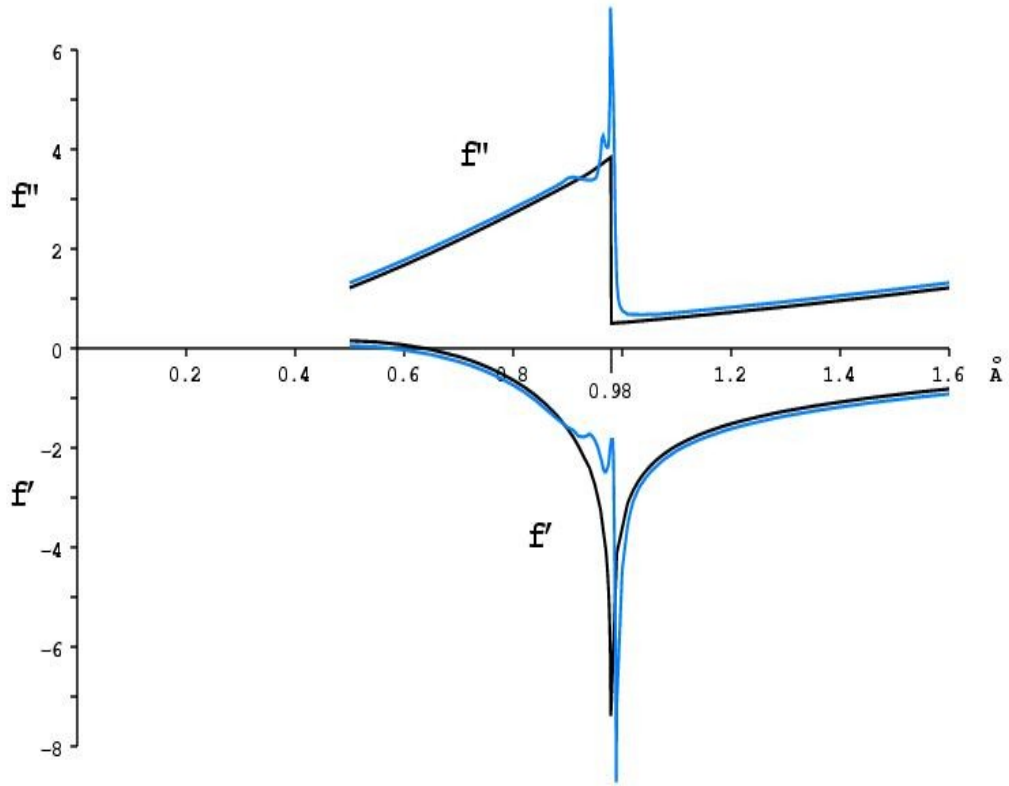
(J.W. Pflugrath, ActaCryst. F71 (2015), 622-42)

The anomalous signal

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

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

Anomalous correction f" is proportional to absorption and fluorescence and f' is its derivative



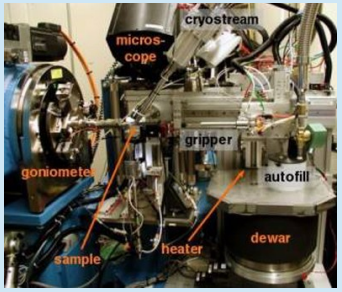
2000s: Automation

Crystallization / nanodrops

Sample changers / sample holder standard

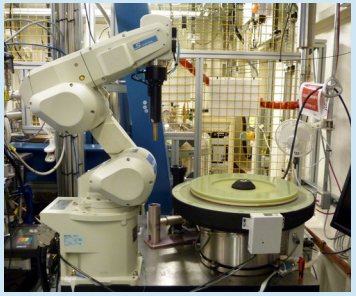
Automation: Sample changer

Manual handling

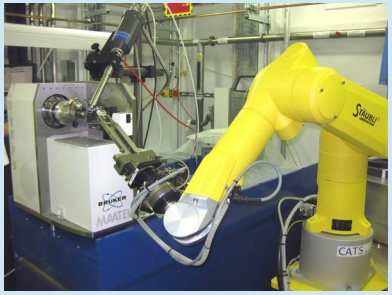


ALS

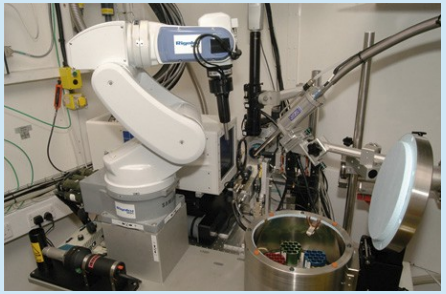
EMBL



Rigaku



FIP



APS

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

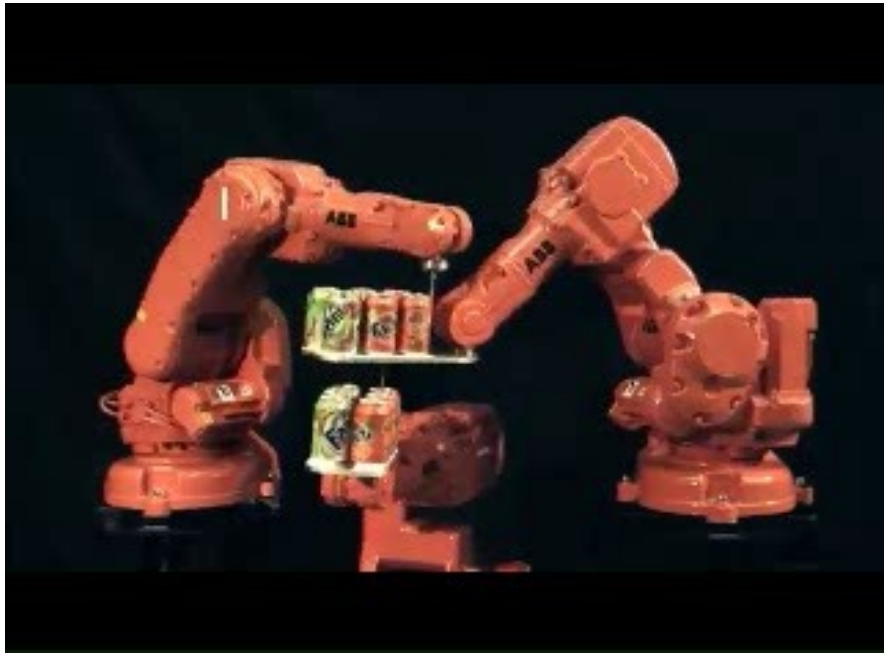
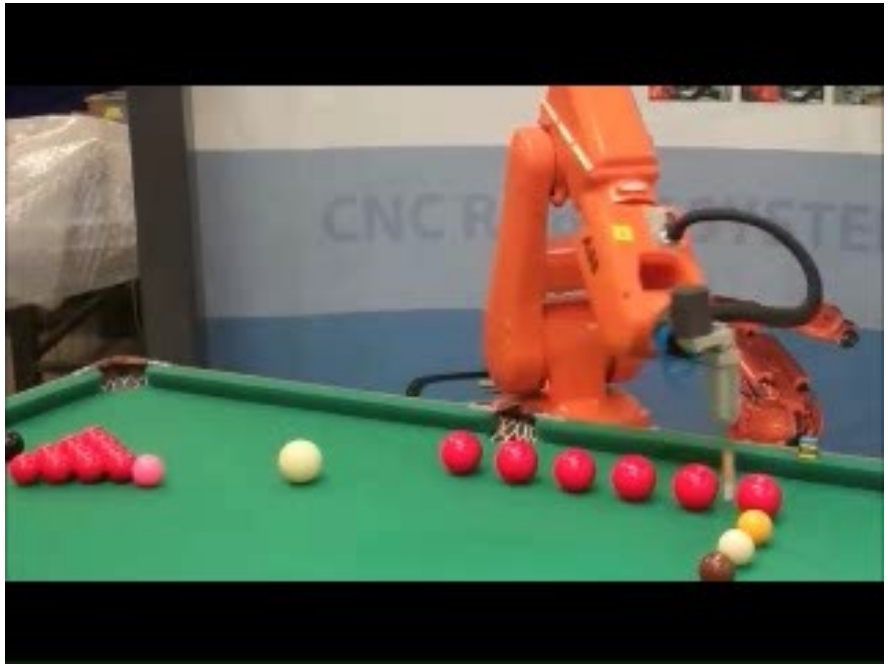
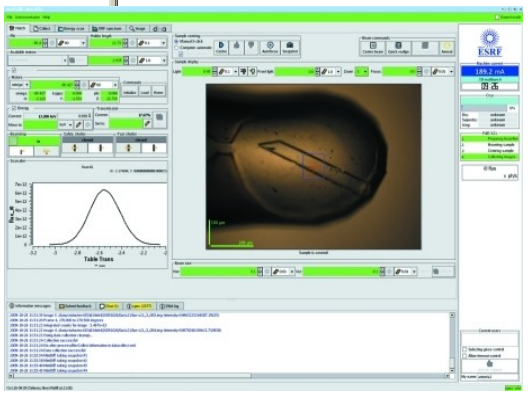


ABB
ABB



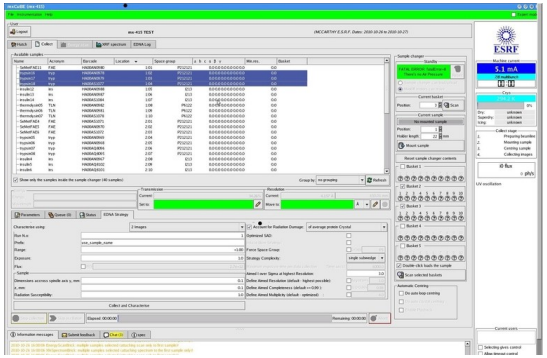
Automation
Software

- MxCube



- ISPyB

- EDNA / xdsadp, meXDS, etc.



RT + ensemble

Flash cooling of protein crystals

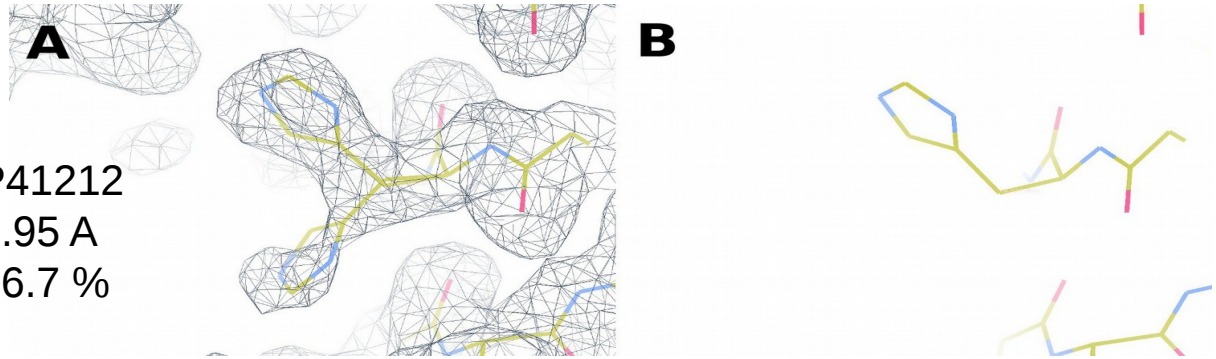
- biases structural collective motions;
- remodels > 35% of side chains;
- induces bias toward smaller, overpacked, and unrealistically unique models.

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

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

Automated *in situ* experiment on cyclophilin D

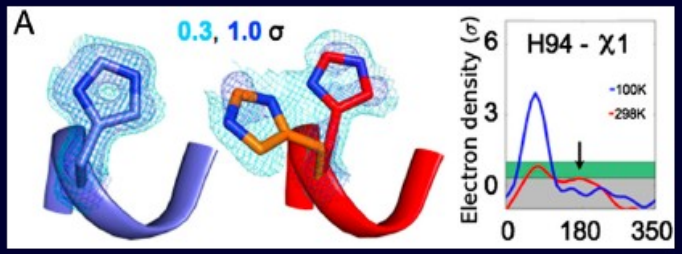
Spacegroup:	P41212
Resol:	1.95 A
Completness:	86.7 %



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

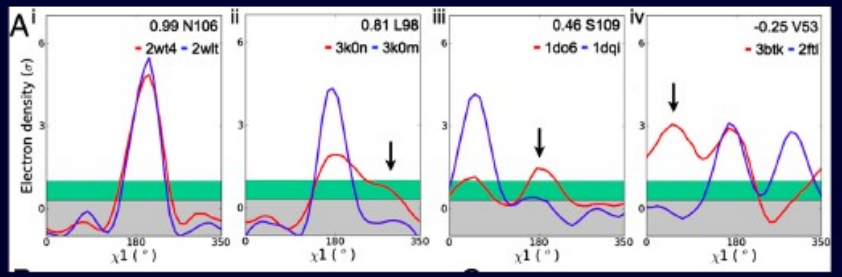
Fraser, van den Bedem, Samelson, Lang, Holton, Echols & Alber (2011) PNAS 108, 16247



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)

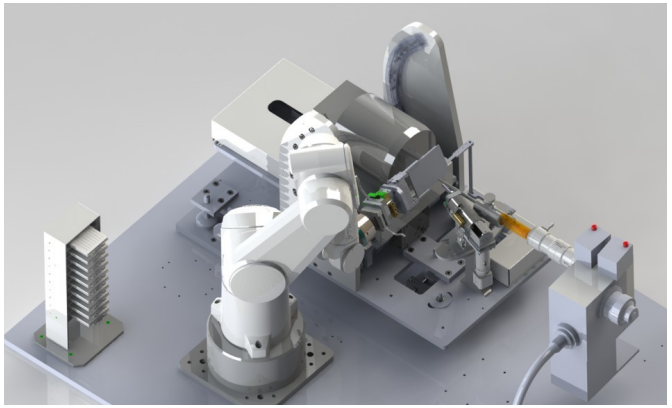
- END, RAPID: place e- density maps on absolute scale and calculate noise at each position in the map (Lang *et al.* (2014) PNAS 111, 237)

In situ screening / data collection

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

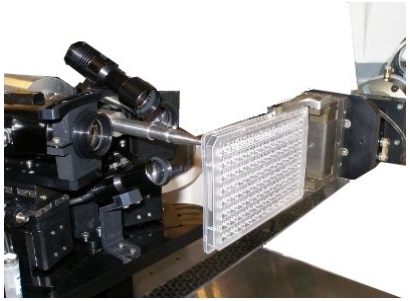
in situ screening & data collection

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

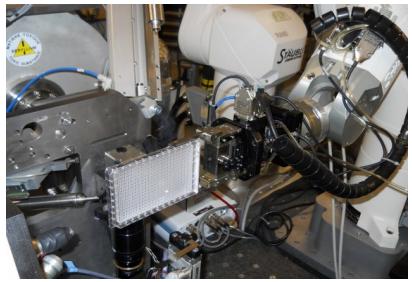


Applications

- 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



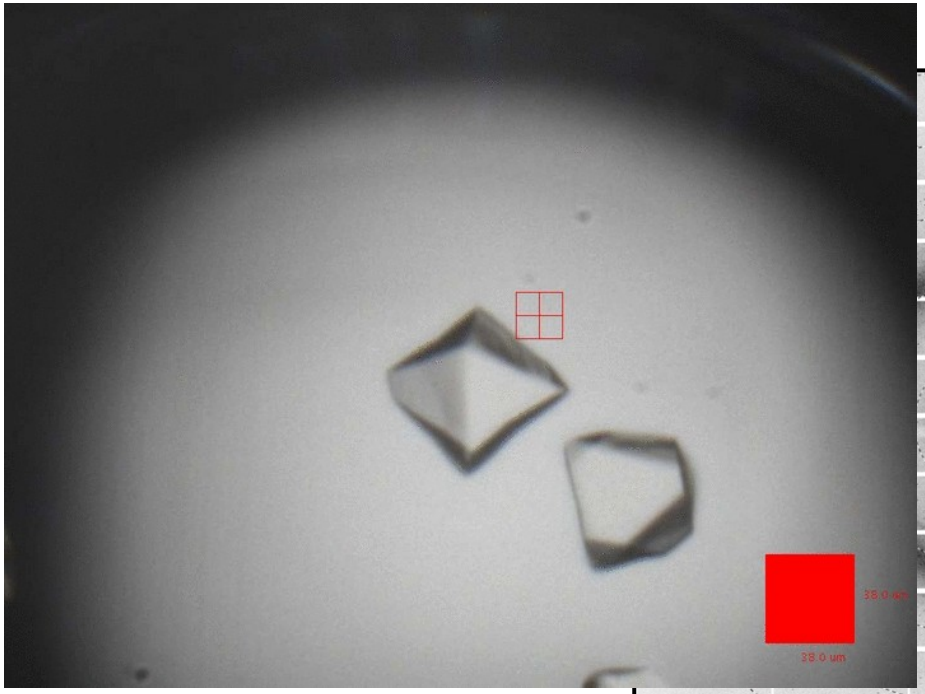
96-well crystallization plate

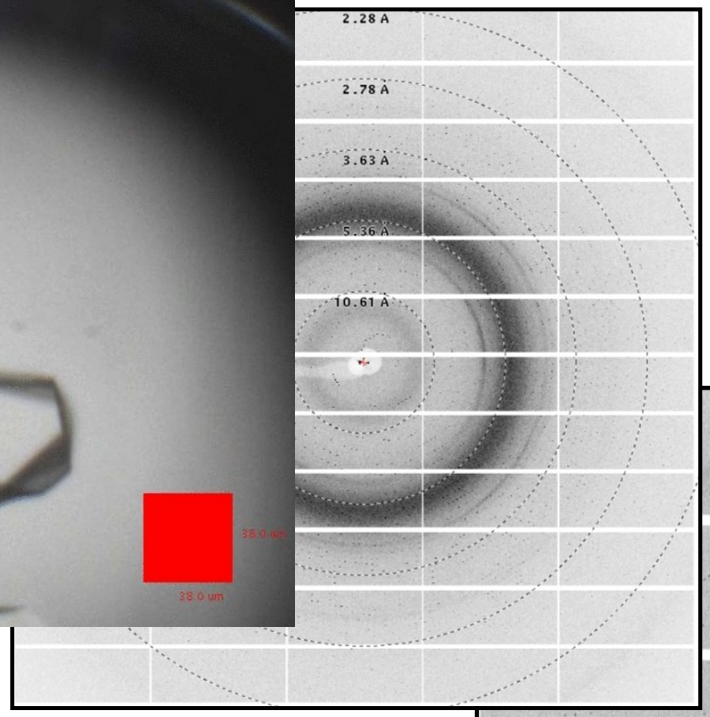


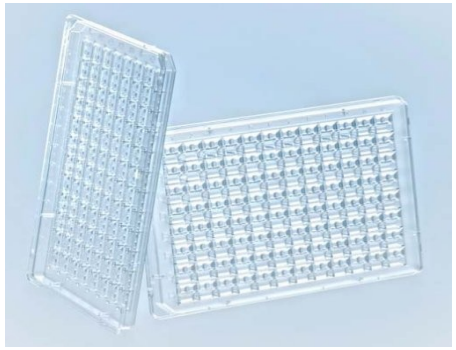
1536-well micro-batch plate

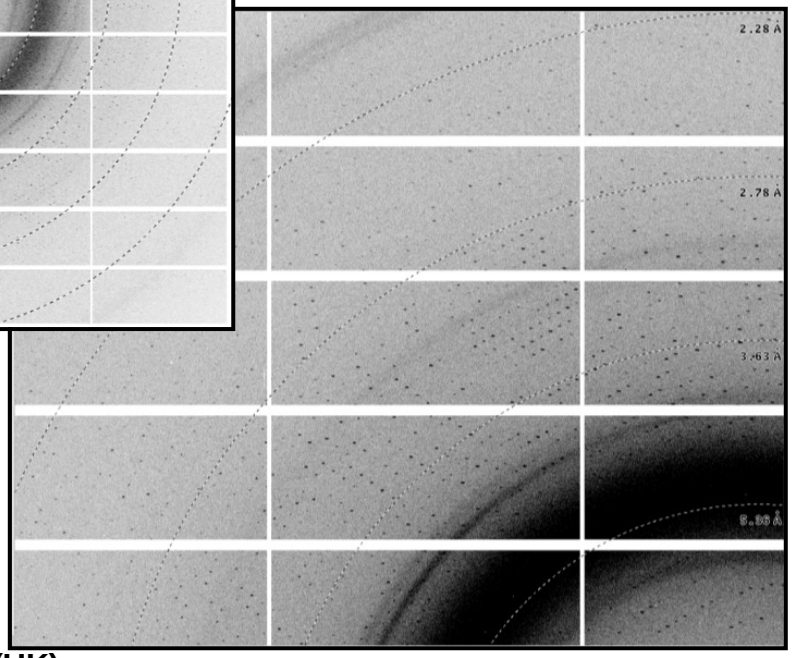
FIP-BM30A (ESRF)
CBS (Montpellier)

Bovine enterovirus 2
Crystallization plate screening on I24





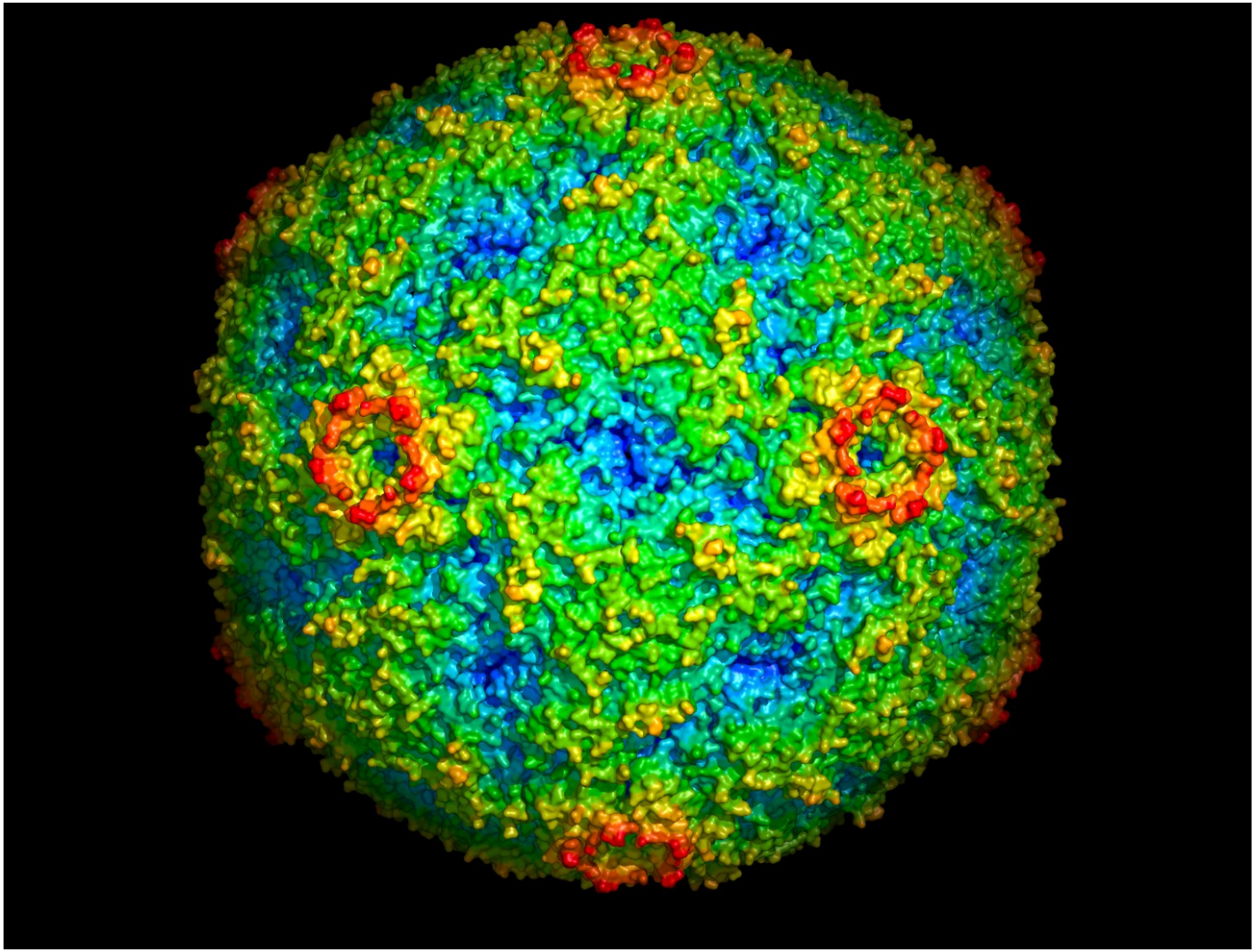




Data collected at DLS, I24
Beam size 20 microns, focus at detector (P6M)
exposure time 0.1 sec, 0.1º oscillation,
detector distance = 480 & 645 mm,
resolution at edge of detector 2.28 & 2.97 Å

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)

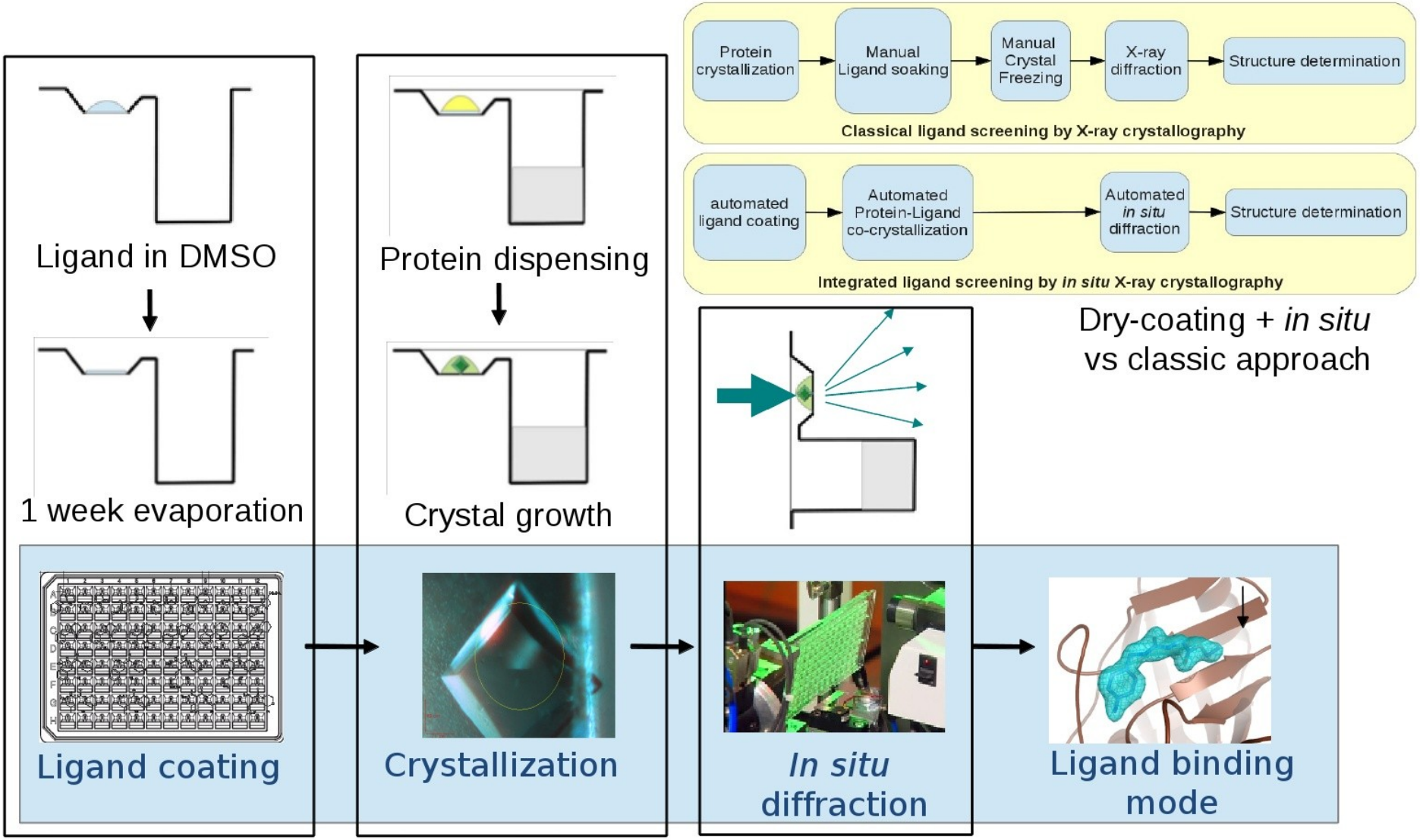
A new virus structure: Bovine enterovirus 2
Crystallization plate screening on I24 (DLS)



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)

In situ PX for FBDD

Automated screening of fragment libraries at room temperature



le Maire et al., Acta Cryst. D67 (2011), 747-755.
Gelin et al., in revision.

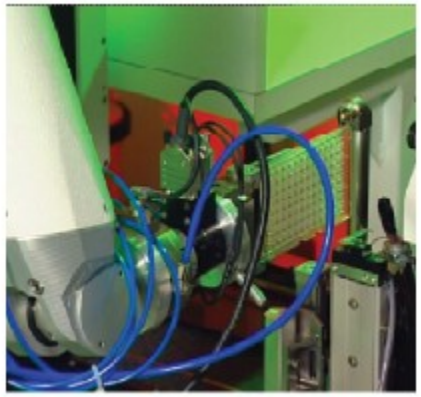
MXIS 2015

17-19 Nov

IBS-ESRF

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

G-Rob at the CBS (Montpellier)

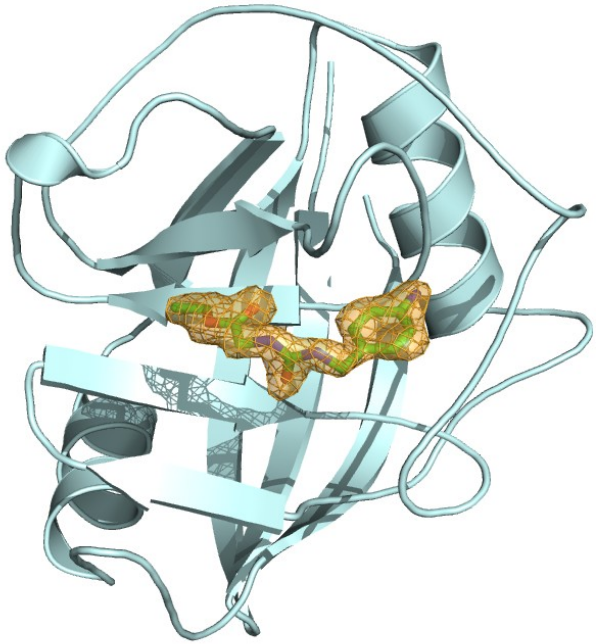


G-Rob on FIP (ESRF)

« 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

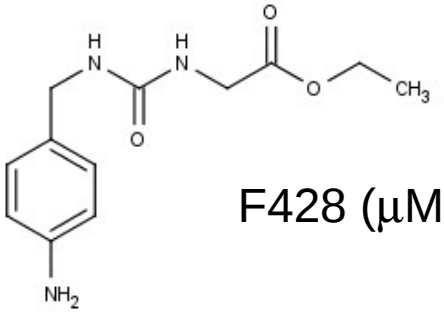
- micromolar range
- MW: 251 and XlogP: 0.37

Further chemical derivations

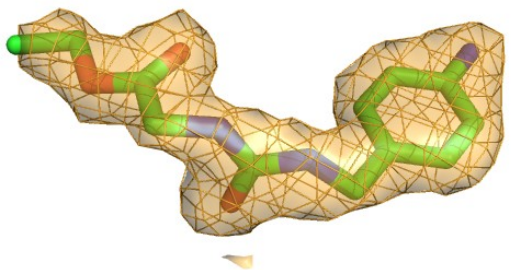
=> nanomolar inhibitors

=> pre-clinical trials

(Guichou et al., 2011).



F428 (µM)



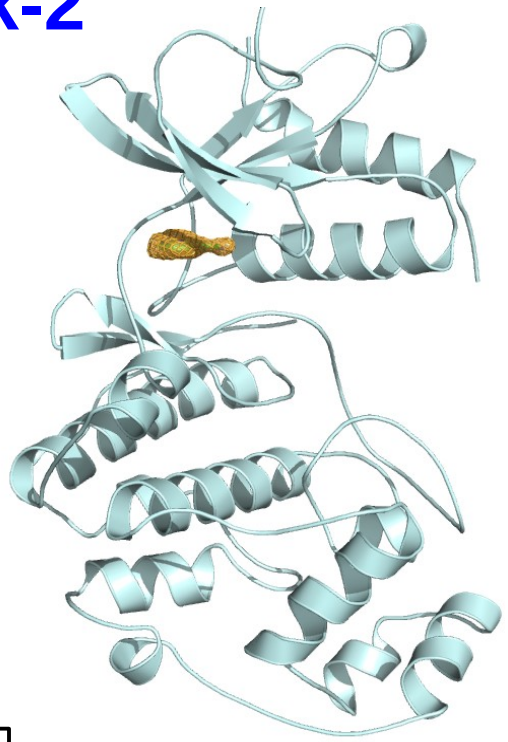
In situ
In house
(2.23 Å)
2 crystals

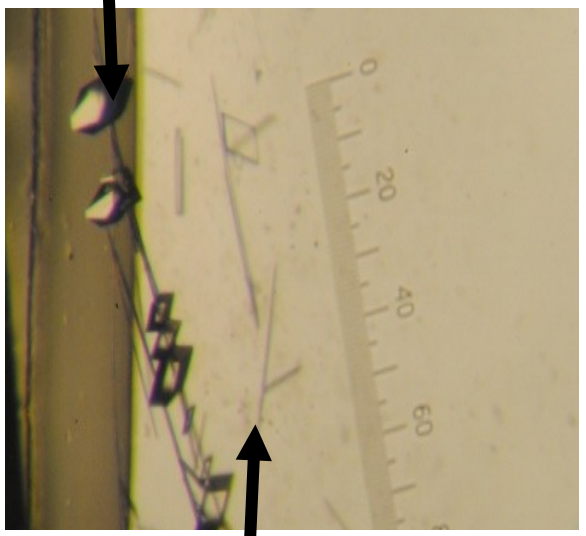
Labesse G, Gelin M, Guichou J-F, CBS Montpellier)

« Dry co-crystallization » of Erk-2

Protein-kinase Erk-2

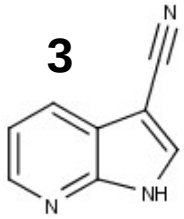
MAP kinase
- involved in important signaling pathways
- mis-functioning is linked to inflammation and cancer (Wortzel and Seger 2011)
- crystallized in $P2_1$

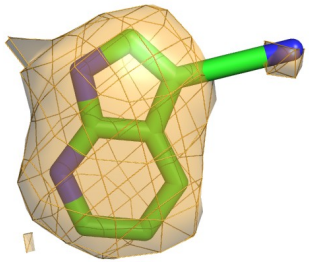




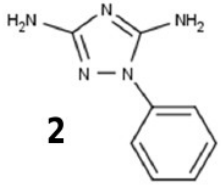
Labesse G, Gelin M, Guichou J-F, CBS Montpellier)

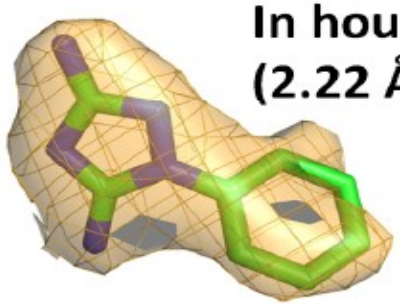
=> 3 new binders





In situ
In house
(2.55 Å)
2 Crystals (88%)





In situ
In house
(2.22 Å)

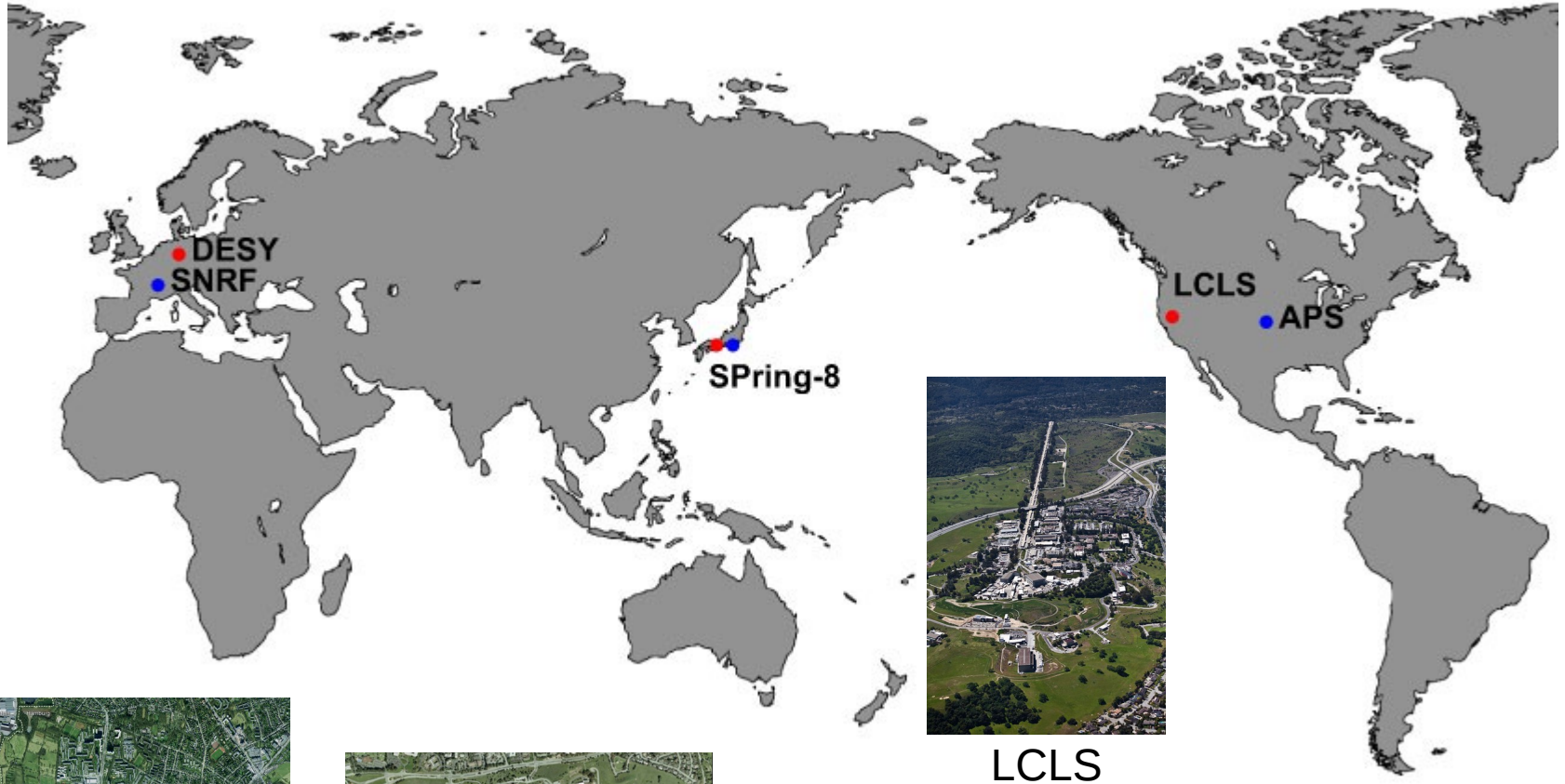
2010s: 3rd+/4th generation sources

X-ray sources
Fast SS detectors

Micro/nano crystals

Room temperature / serial data collection

XFELs: 4th generation X-ray sources



LCLS
(Stanford)

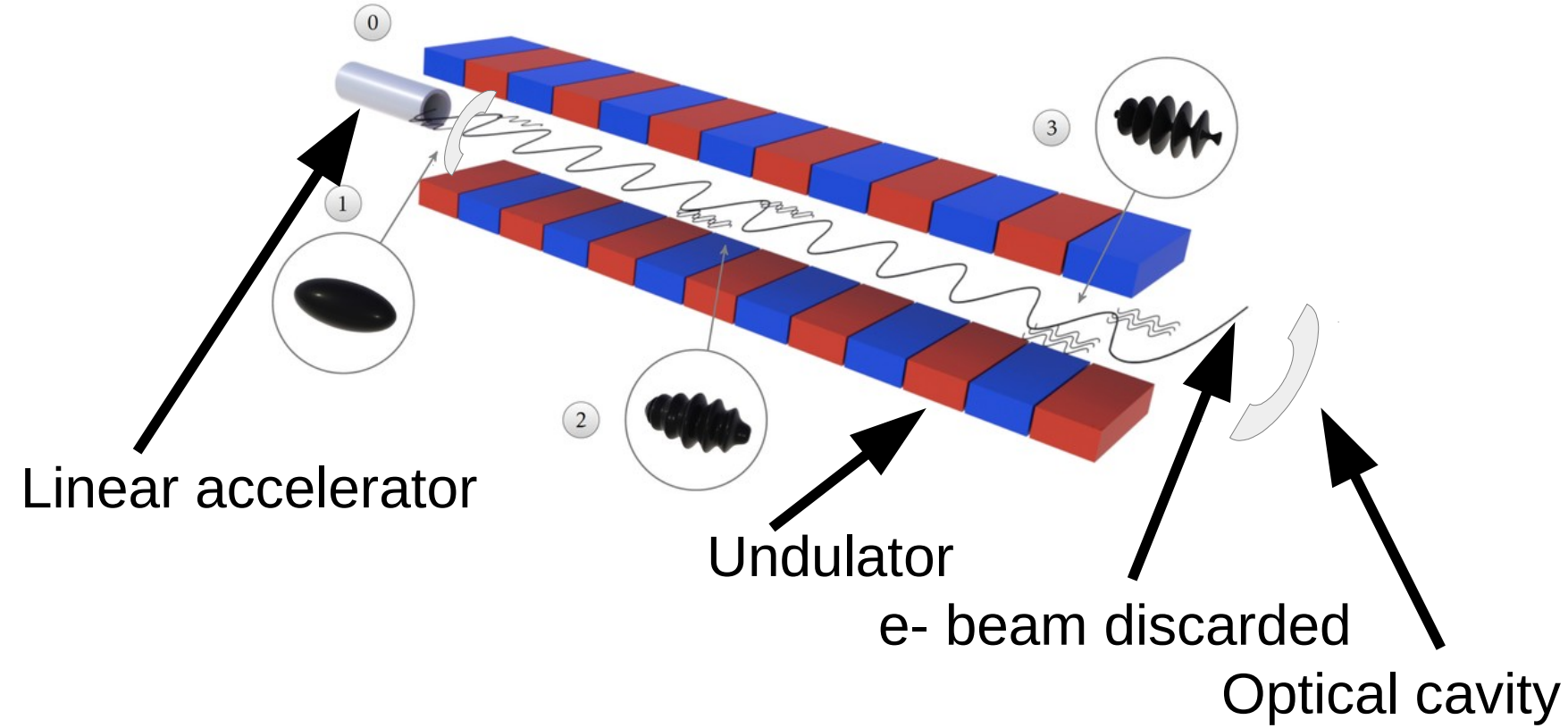
European XFEL
(Germany)

SwissFEL
(Viligen)

SACLA (Japan)

XFEL-O (Argonne)

XFELs: 4th generation X-ray sources

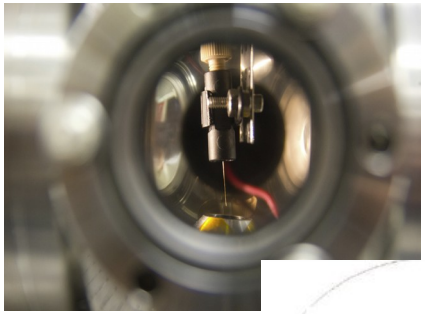


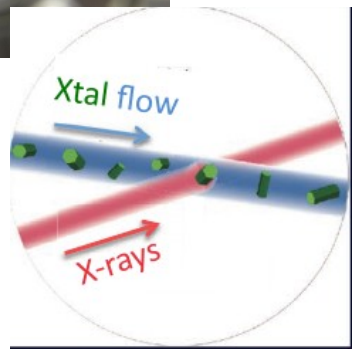
Long undulator => **micro-bunching** of the electron beam
=> **self amplificating spontaneous emission**
e- in undulator field → X-ray beam
e- in X-ray beam field → X-ray beam exponentially
Transverse and longitudinal coherent beam

4th generation X-ray sources: Sample dispensing

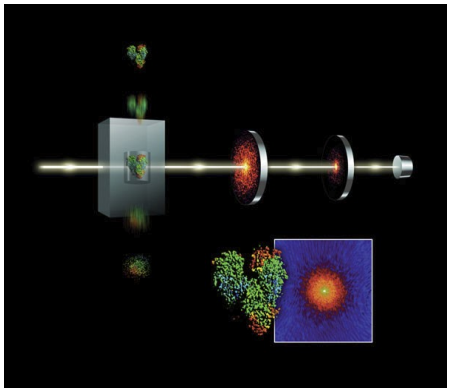
Sample destroyed upon exposure to the beam (1 frame /sample)
→ samples to intercept the beam at an high frequency
→ merging of many randomly collected diffraction frames

Crystals in droplets ejected with sonic waves





Continuous stream of nano-crystals solution



Up to single particules analysis ?

4th generation X-ray sources: Detectors

Present fast detectors

dead time ~1 msec

MH-HS (Rayonix)

Pilatus (Dectris)

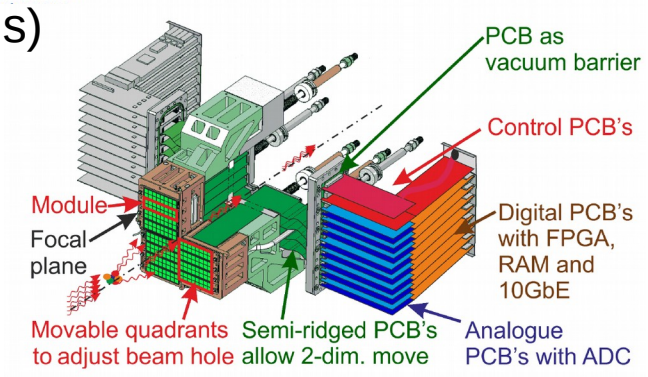
Starting operation

dead time ~3 usec

Eiger (Dectris)

To come…

3.5 MHz frame rate!



AGIPD (DESY, PSI)

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

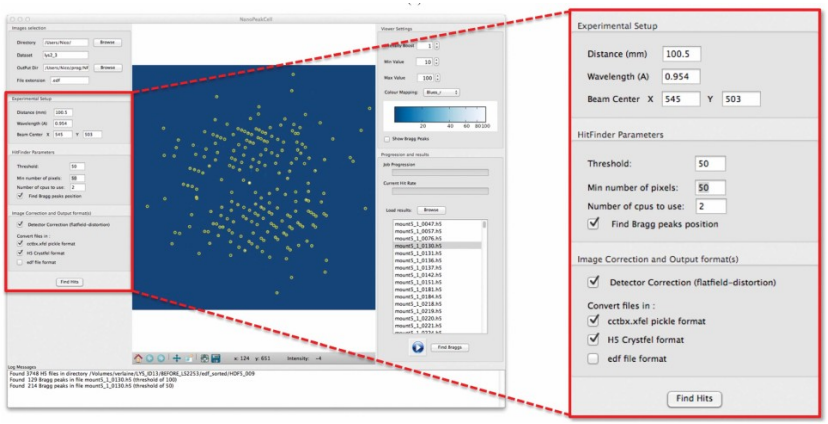
3 and 3+rd generation X-ray sources: Serial data collection

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

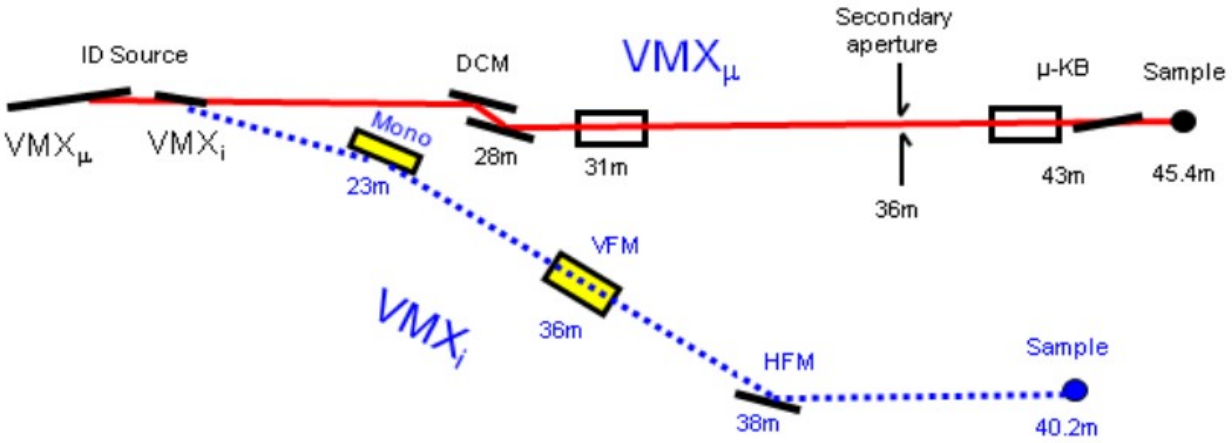
→clustering and merging data



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

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

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
	ID30A1	100x65um/0.968Å	RoboDiff/Pilatus6M	single wav.
	ID30A3	15um/0.984Å	MD2/SC3/Pilatus2M	single wav.
	ID30B	20x20um/0.62-2.1Å	MD2/SC3/Pilatus6M	SAD, MAD
	BM14	???/0.7-1.8Å	MD2/G-Rob/Mosaic225	in situ/SAD/MAD
	BM30A	300um/0.7-1.8Å	MD2/G-Rob/ADSC315	in situ/SAD/MAD
SOLEIL				
	Proxima1	100um/0.84-2.5Å	Kappa/CATS/Pilatus6M	SAD, MAD
	Proxima2A	5um/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
MAXII	BLI711	???/0.9-1.5 Å	SAD/MAD
ELETTRA	XRD1	200um/0.6-3.15Å	SAD/MAD
ALBA	BL13-XALOC	50x10um/0.6-2.4Å	SAD/MAD