Structural biology with synchrotron radiation and free electron lasers

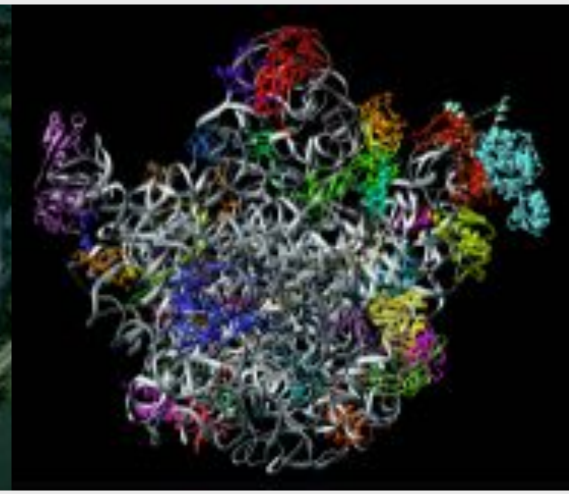
Matthias Wilmanns,

Head of EMBL-Hamburg

BioStruct-X course, Budapest, August 31, 2013

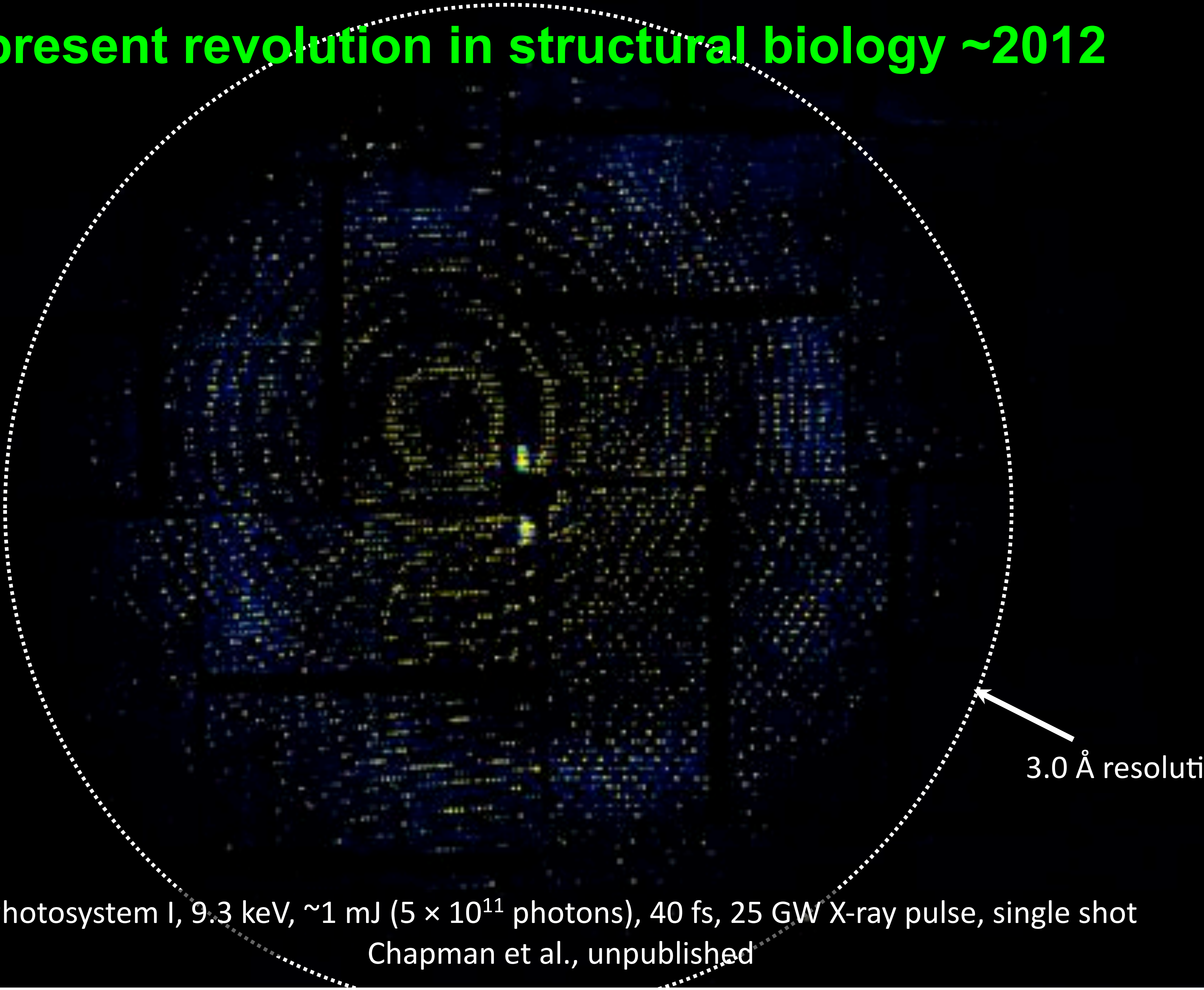
EMBL

The revolutions in Structural Biology



1970 **1990** **2000** **2009**

The present revolution in structural biology ~2012



Photosystem I, 9.3 keV, ~1 mJ (5 × 10^{11} photons), 40 fs, 25 GW X-ray pulse, single shot
Chapman et al., unpublished

EMBL's portfolio

The Five Branches of EMBL

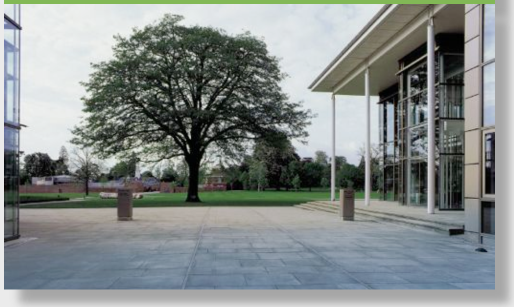
Heidelberg

Basic Molecular Biology Research Laboratory
Central Administration
EMBO

Hamburg

Structural Biology
DESY

Hinxton



European Bioinformatics Institute (EBI)
Sanger Centre

>1600 staff
>70 nationalities

Grenoble

Structural Biology
ILL, ESRF, IBS, UVHCI

Monterotondo

Mousebiology
EMMA, CNR


CSSB
EMBL
EMBL@PETRA III

DESY Accelerators and Photon Facilities

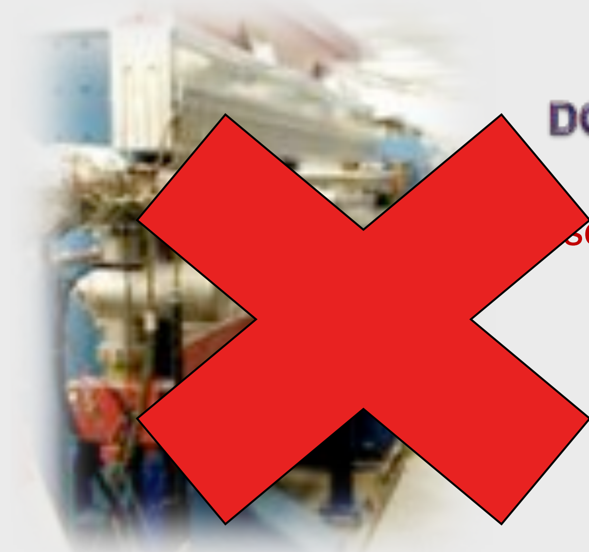
XFEL

European X-Ray Laser
„Life Reports from Nanospace“

„Nanoscience“

FLASH

1st (soft) X-Ray Laser
„Ultrashort Science“



DORIS III

se out 2013

German Workhorse Synchrotron radiation

Petra-III: opportunity for state-of-the-art experiments

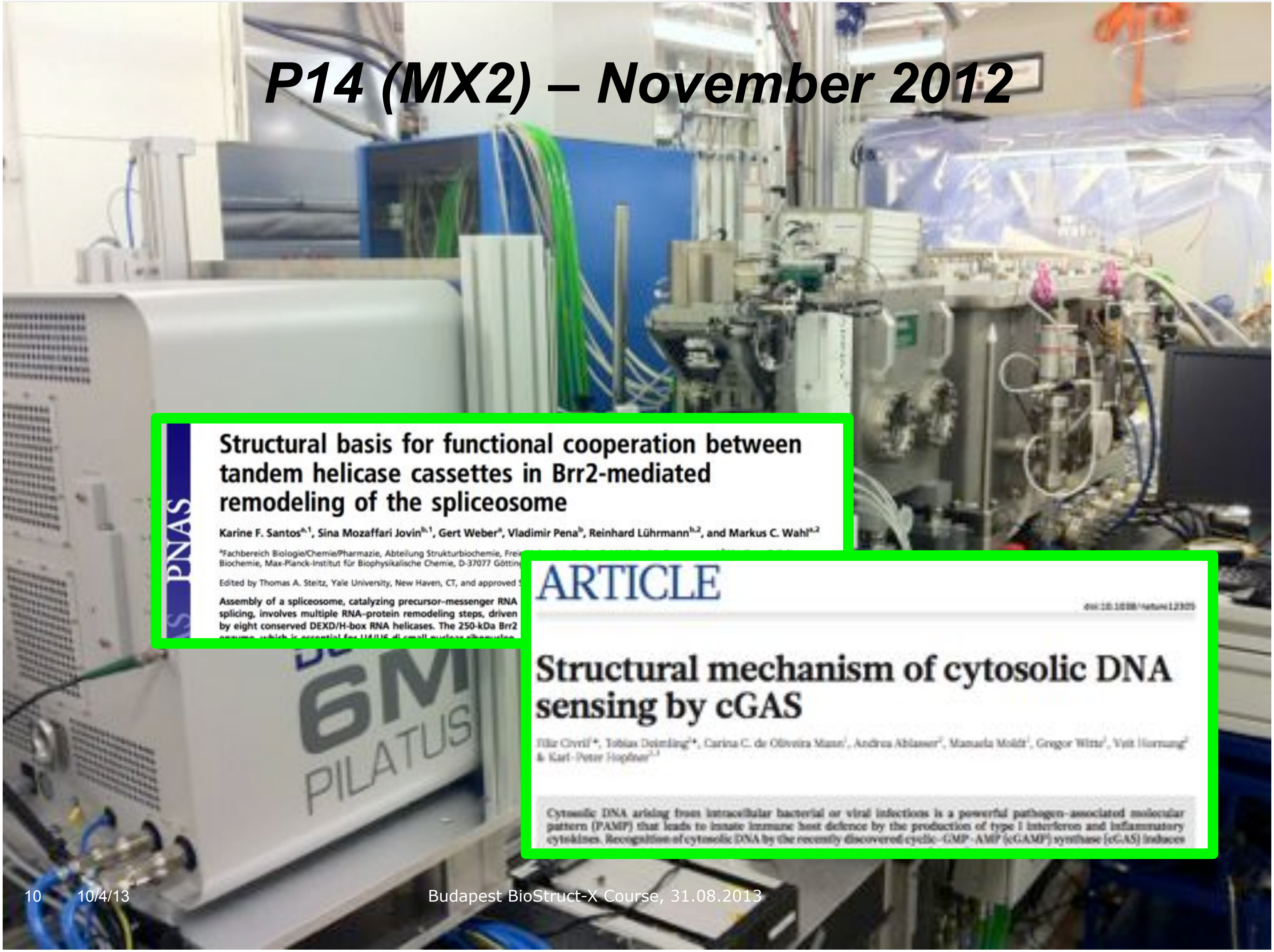
One of the most brilliant storage rings on earth

Investment ~ 300 M EUR

Space for 14 SR beamlines, 3 by EMBL (life sciences)

P14 (MX2) – July 2012

P14 (MX2) – November 2012



PNAS

Structural basis for functional cooperation between tandem helicase cassettes in Brr2-mediated remodeling of the spliceosome

Karine F. Santos[a,1], Sina Mozaffari Jovin[b,1], Gert Weber[a], Vladimir Pena[b], Reinhard Lührmann[b,2], and Markus C. Wahl[a,2]

[a]Fachbereich Biologie/Chemie/Pharmazie, Abteilung Strukturbiochemie, Frei... Biochemie, Max-Planck-Institut für Biophysikalische Chemie, D-37077 Göttin...

Edited by Thomas A. Steitz, Yale University, New Haven, CT, and approved ...

Assembly of a spliceosome, catalyzing precursor–messenger RNA splicing, involves multiple RNA–protein remodeling steps, driven by eight conserved DEXD/H-box RNA helicases. The 250-kDa Brr2

ARTICLE

doi:10.1038/nature12305

Structural mechanism of cytosolic DNA sensing by cGAS

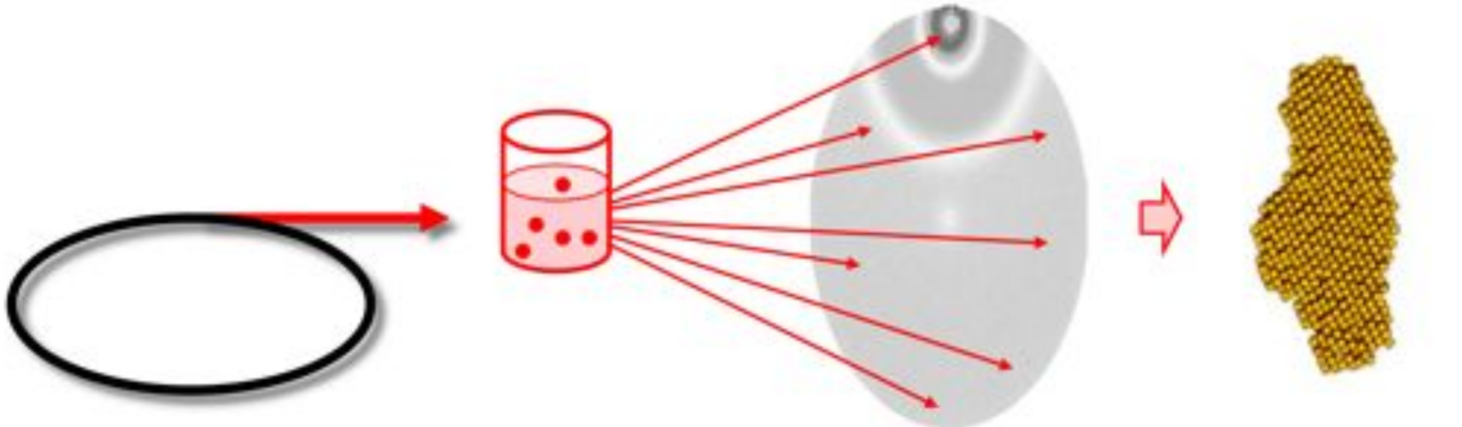
Filiz Civril[1]*, Tobias Deimling[1]*, Carina C. de Oliveira Mann[1], Andrea Ablasser[2], Manuela Moldt[1], Gregor Witte[1], Veit Hornung[2] & Karl-Peter Hopfner[1,3]

Cytosolic DNA arising from intracellular bacterial or viral infections is a powerful pathogen-associated molecular pattern (PAMP) that leads to innate immune host defence by the production of type I interferon and inflammatory cytokines. Recognition of cytosolic DNA by the recently discovered cyclic-GMP-AMP (cGAMP) synthase (cGAS) induces

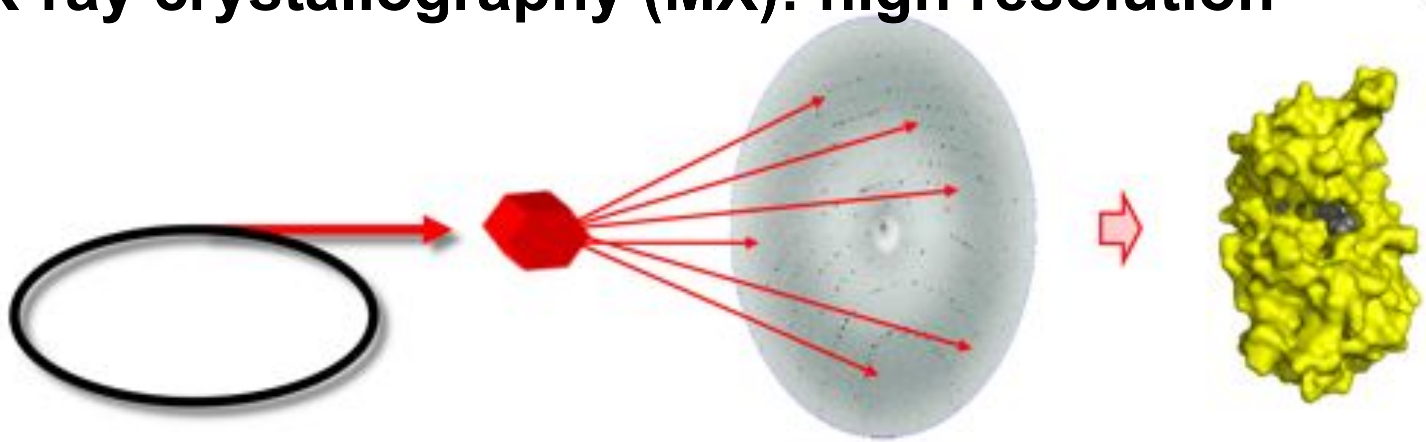
What is he talking about?

X-ray based structural biology methods

Small angle X-ray scattering (SAXS): low resolution



X-ray crystallography (MX): high resolution



X-ray source *Sample* *Detection* *Structure*

There are more …

X-ray imaging / X-ray microscopy

X-ray absorption spectroscopy

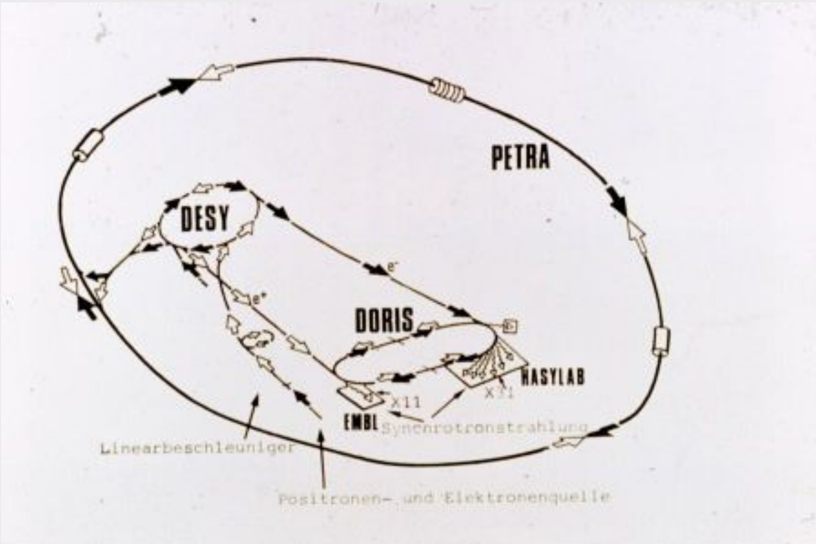
X-ray Raman spectroscopy

X-ray based circular dichroism

etc.

(not further covered in this lecture)

Why synchrotron radiation ?



Synchrotron Radiation as a Source for X-ray Diffraction

G. ROSENBAUM & K. C. HOLMES
Max-Planck-Institut für Medizinische Forschung, Heidelberg
J. WITZ
Laboratoire des Virus des Plantes, Institut de Botanique de la Faculté des Sciences de Strasbourg, Strasbourg

Some preliminary results have been obtained with synchrotron radiation from the 7.5 GeV electron synchrotron Deutsches Elektronen - Synchrotron (DESY) in Hamburg as a source for X-ray diffraction.

When an electron is accelerated it emits radiation. At the very high energies used in DESY, the emitted radiation is confined to a narrow cone about the instantaneous direction of motion of the electron. Thus the synchrotron radiates tangentially. Synchrotron radiation is polychromatic, with a peak in the X-ray region for an electron energy of 7.5 GeV (see ref. 1 for the original theoretical description and refs. 2-4 for experimental details).

The DESY synchrotron uses bursts of 50 pulses/s and each 10 ms pulse contains 6×10^{10} electrons (10 mA average beam current). The injection energy is relatively low and the electrons are accelerated up to 7.5 GeV in the 10 ms.

Most of the X-radiation is emitted during the last 3 ms of each pulse: little radiation is produced at the lower electron energies, and so the time averaged intensity at 1.5 Å is about 30% of the peak value.

Table 1 Data for Quartz Monochromator in Synchrotron Radiation Beam

Synchrotron	7.5 GeV, 10 mA beam current
Electron beam diameter	approximately 4 mm (= effective X-ray source diameter)
Distance	37 m from synchrotron to monochromator
Cross-fire of the incident beam	approximately 10^{-4} rad
Polarization	85% at 1.5 Å in the eighth ms of the cycle, polarized in the plane of the synchrotron
Be-window	0.5 mm (96 mg cm^{-2})
Crystal	quartz cut at α = 8° 30' to the 1011 planes, dimensions 45 × 13 × 0.3 mm^3
Bender	pins: outer pair 40.5 mm, inner pair 39.5 mm; radius of curvature of crystal, 9 m
Wavelength	1.51 Å (θ = 13° 15')
Wavelength spread	Δλ = 3 × 10^{-3} Å (due to deviation from Johann focusing and to finite source size)
Focus	1.5 m from crystal; line focus 180 µm wide
Angular aperture of reflected beam	horizontal: 2 mrad (convergence); vertical: 3–4 mrad (divergence)
Measured flux in line focus	1.8 × 10^{9} photons s^{-1} mm^{-1} (of focal length) (at the eighth ms of the cycle)

Table 2 Biological Applications

Specimen	Elliott fine-focus X-ray tube*	DESY synchrotron with Bereman point-focusing monochromator‡
Single crystal	Standard collimator 0.5 mm diameter	
a = 0.5 mm, b = 0.5 mm, L = 7.5 cm	A = 12.5 cm, d = 0.7 mm, P = 10^{8} photons s^{-1}, I = 2 × 10^{8} photons s^{-1} mm^{-2}	D = 1 m, d = 120 µm, P = 4 × 10^{8} photons s^{-1}, I = 2.5 × 10^{11} photons s^{-1} mm^{-2}
Tobacco mosaic virus gel	Double-crystal focusing monochromator†	
a = 0.6 mm, b = 1 mm, L = 12 cm	d = 80 µm, P = 10^{7} photons s^{-1}, I = 2 × 10^{9} photons s^{-1} mm^{-2}	D = 0.8 m, d = 100 µm, P = 1 × 10^{9} photons s^{-1}, I = 3 × 10^{11} photons s^{-1} mm^{-2}
Insect muscle	Double-crystal focusing monochromator†	
a = 3 mm, b = 0.3 mm, L = 40 cm	d = 300 µm, P = 5 × 10^{7} photons s^{-1}, I = 5 × 10^{7} photons s^{-1} mm^{-2}	D = 1.5 (3) m, d = 180 (350) µm, P = 5 × 10^{8} (2 × 10^{9}) photons s^{-1}, I = 1.5 × 10^{10} photons s^{-1} mm^{-2}

a, Width of specimen; b, height of specimen; L, specimen film distance; A, anode specimen distance; D, focal length, that is, monochromator film distance; d, spot or focus diameter on film; P, X-ray power reaching the specimen; and I, flux density at the focus.

* Loaded with 40 kV, 50 mA into a 0.2 × 2 mm^2 electron focus at the anode in the first case, and 40 kV, 15 mA into a 0.14 × 0.7 mm^2 focus in the other two cases. This set is the most powerful fine-focus X-ray tube currently available.

† The setting of this Johann-type[5] monochromator is optimized for each type of specimen.

‡ Conditions of the synchrotron are as in Table 1, computed for 1.5 Å radiation.

We have evaluated the spectral luminance (that is, the power in photons per second radiated per unit area, solid angle, and wavelength interval) of both the synchrotron and a fine-focus rotating anode X-ray tube (see Table 2). The values are 2 × 10^{14} (time averaged) and 3 × 10^{10} photons s^{-1} steradian^{-1} cm^{-2} Å^{-1} respectively at 1.54 Å, showing clearly that the synchrotron is, relative to present X-ray tubes, a very bright source. The actual advantage to be gained in a diffraction experiment depends critically on the optical system necessary to focus and monochromate the radiation. Three types of focusing mono-

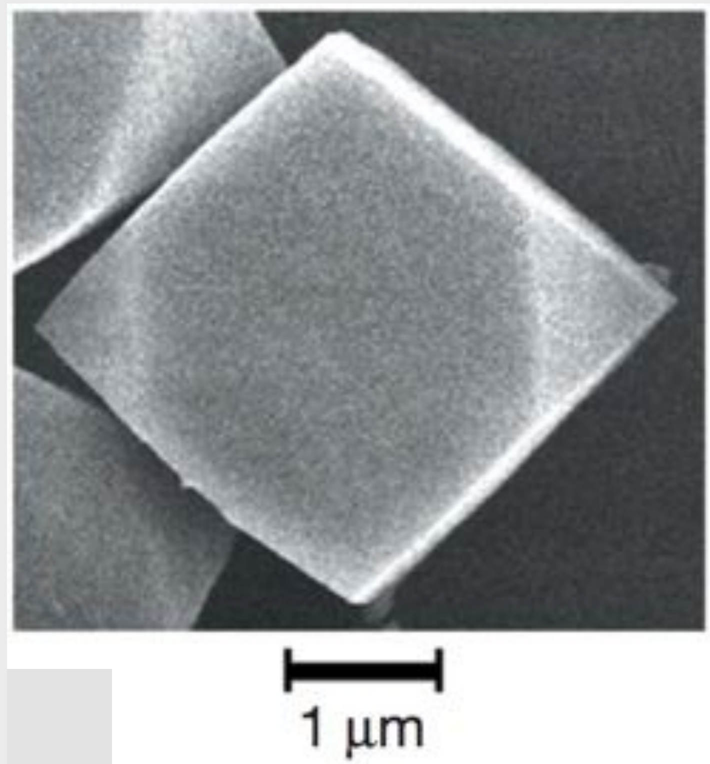
Crystals

- Are difficult to grow
- Are difficult to reproduce
- Are often very small (microns)
- Are often inhomogeneous
- Are mechanically fragile
- Are radiation sensitive

Here: Cueva de los Cristales,
Chihuahua region, Mexico

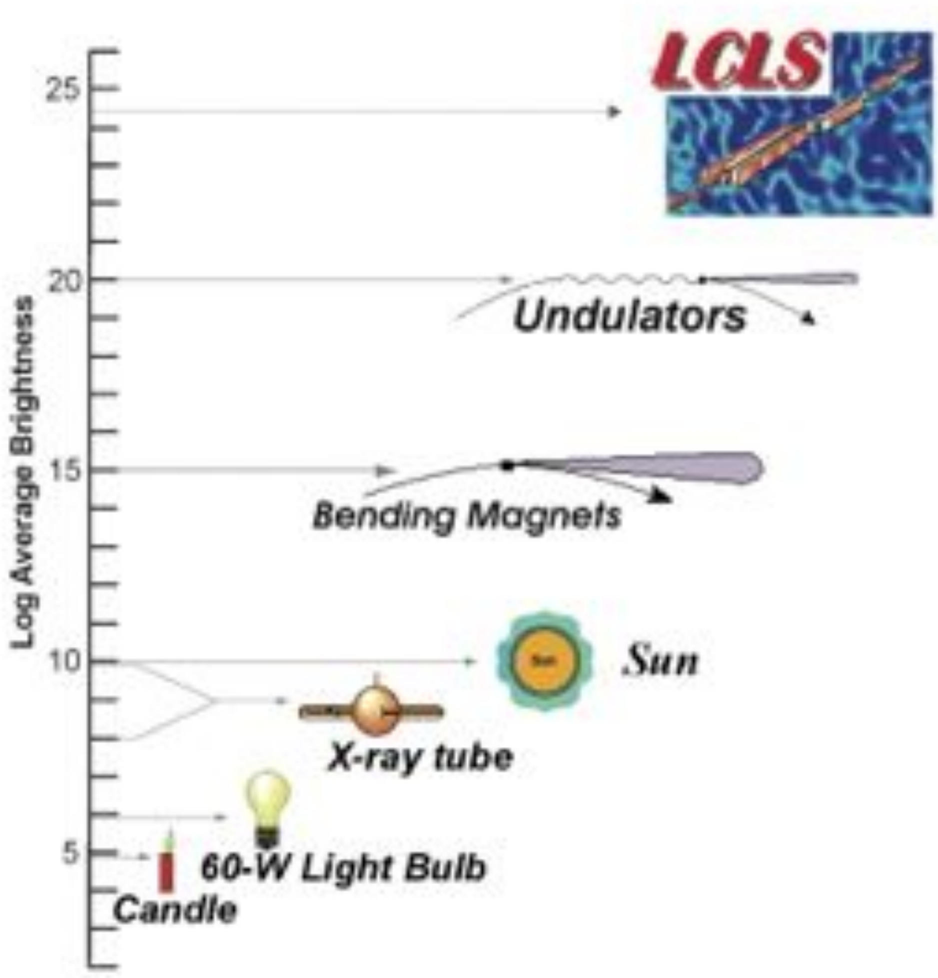
Small Crystals may produce better data

- Crystal dimensions of 1-10 µm are not rare.
- Often these crystals have superior diffraction properties.
- Very small and very parallel beams needed.

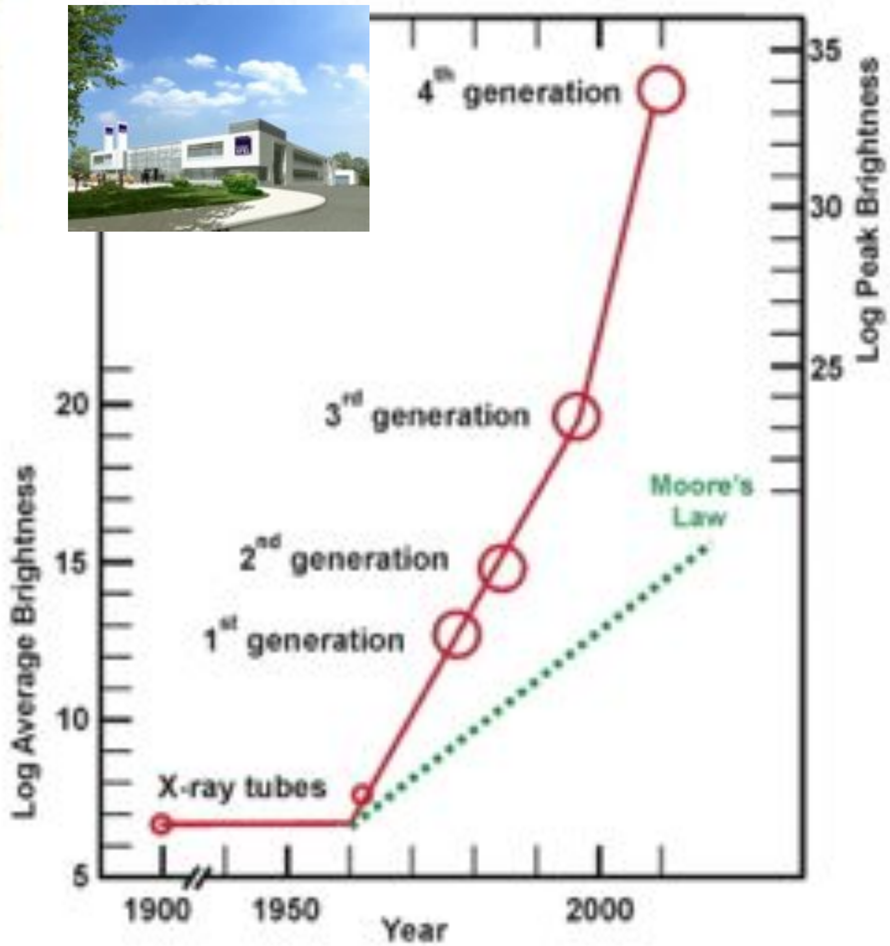



hair Ø ≈ 50 µm

Light Sources and their Brightness

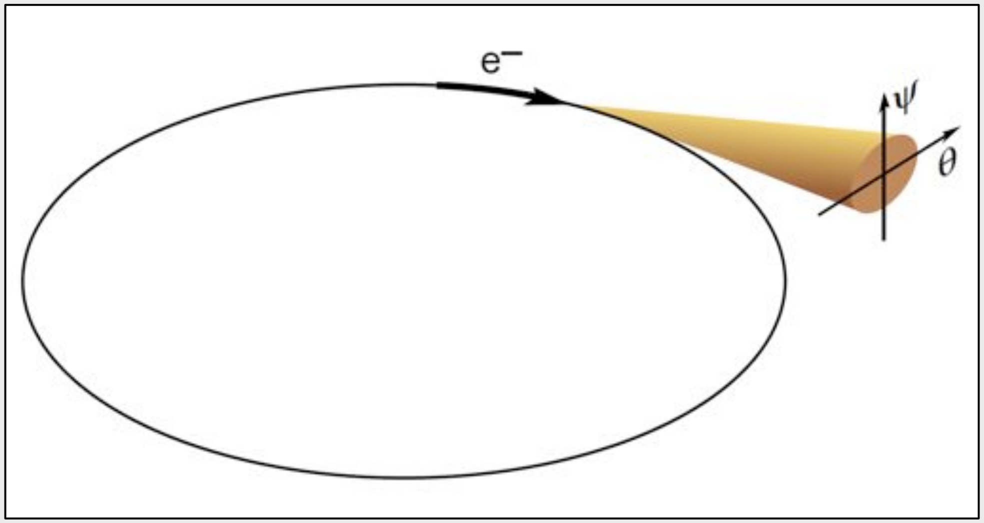


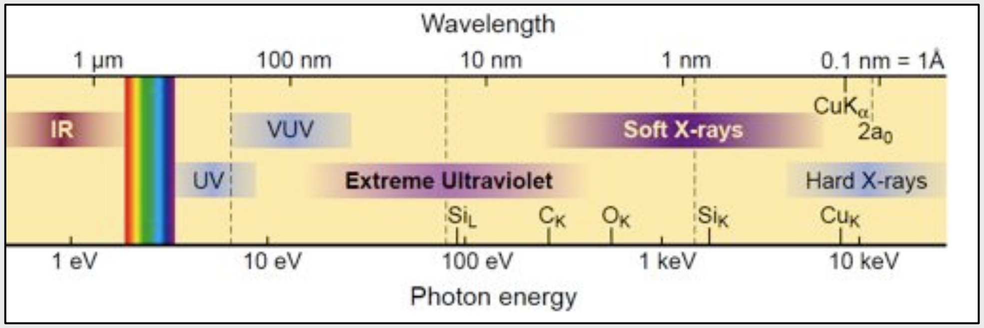
XFEL
X-ray laser



Synchrotron Radiation

- When bend around a curve, electrons at relativistic speed emit synchrotron radiation in a narrow forward cone.
- The spectrum of the emitted radiation is **continuous**.
- Synchrotron light is extremely **bright** (brightness is measured in photons/s/mm^2/ $mrad^2$/0.1%BW)





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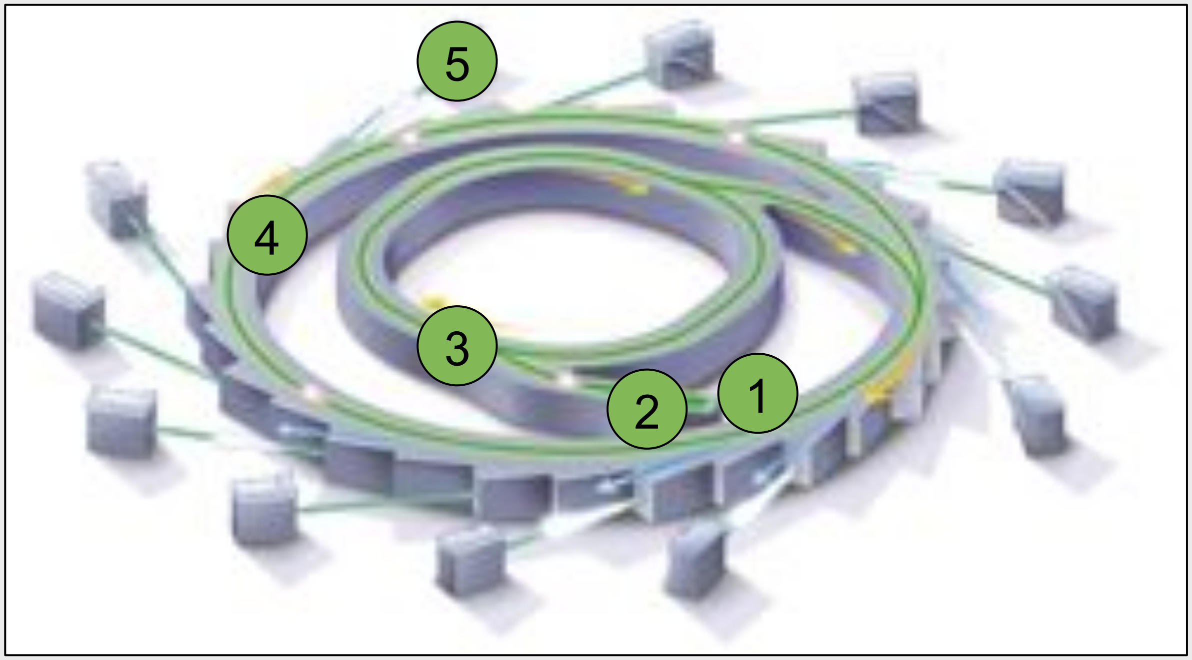
Setup of a synchrotron storage facility

1. Electron gun
2. Linear accelerator
3. Booster ring
4. Storage ring
5. Beamline & experimental hutch



SOLEIL, Paris, F

APS, Chicago, U.S.A.

BESSY, Berlin, D

ESRF, Grenoble, F

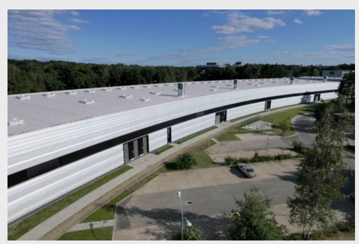
The SR champions league

SPring-8, JP

DIAMOND, Oxford, UK

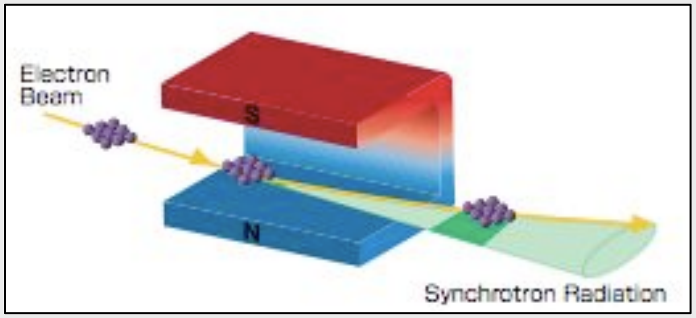
Shanghai SRS, CN

SLS, Villigen, CH

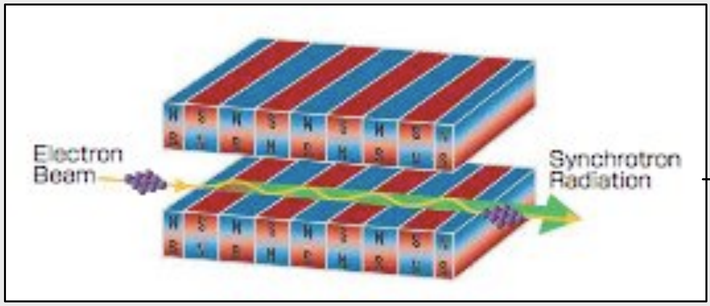


PETRA III, Hamburg, D

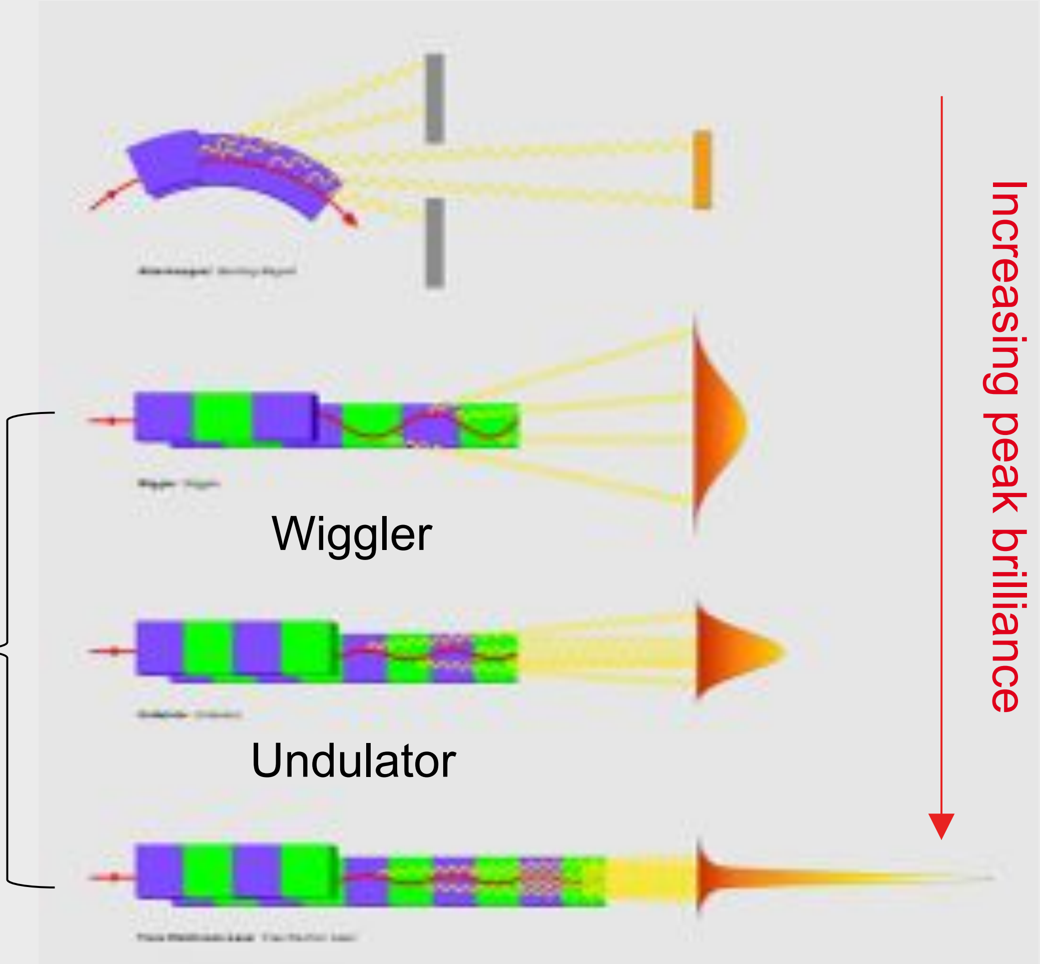
Bending magnets and insertion devices



Bending magnet



Insertion device



SASE Undulator (X-FEL)

Key properties of synchrotron radiation

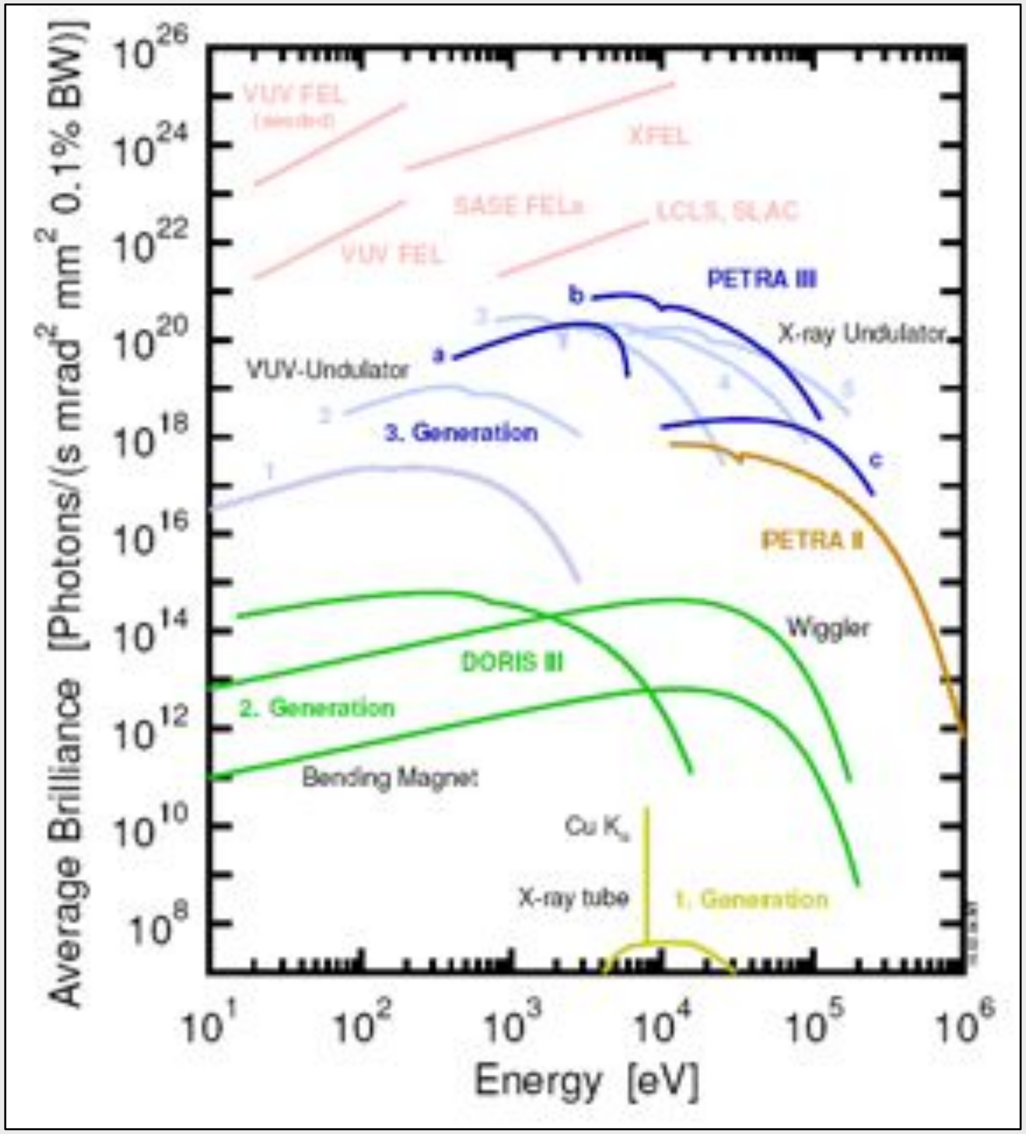
- **Small source size**
  PETRA III @ 0m:
  12 µm x 300 µm (FWHM)

- **Collimation**
  PETRA III @ 70m:
  700 µm x 1500 µm

- **Wide energy spectrum**

- **Time-structure**

- **Polarization**



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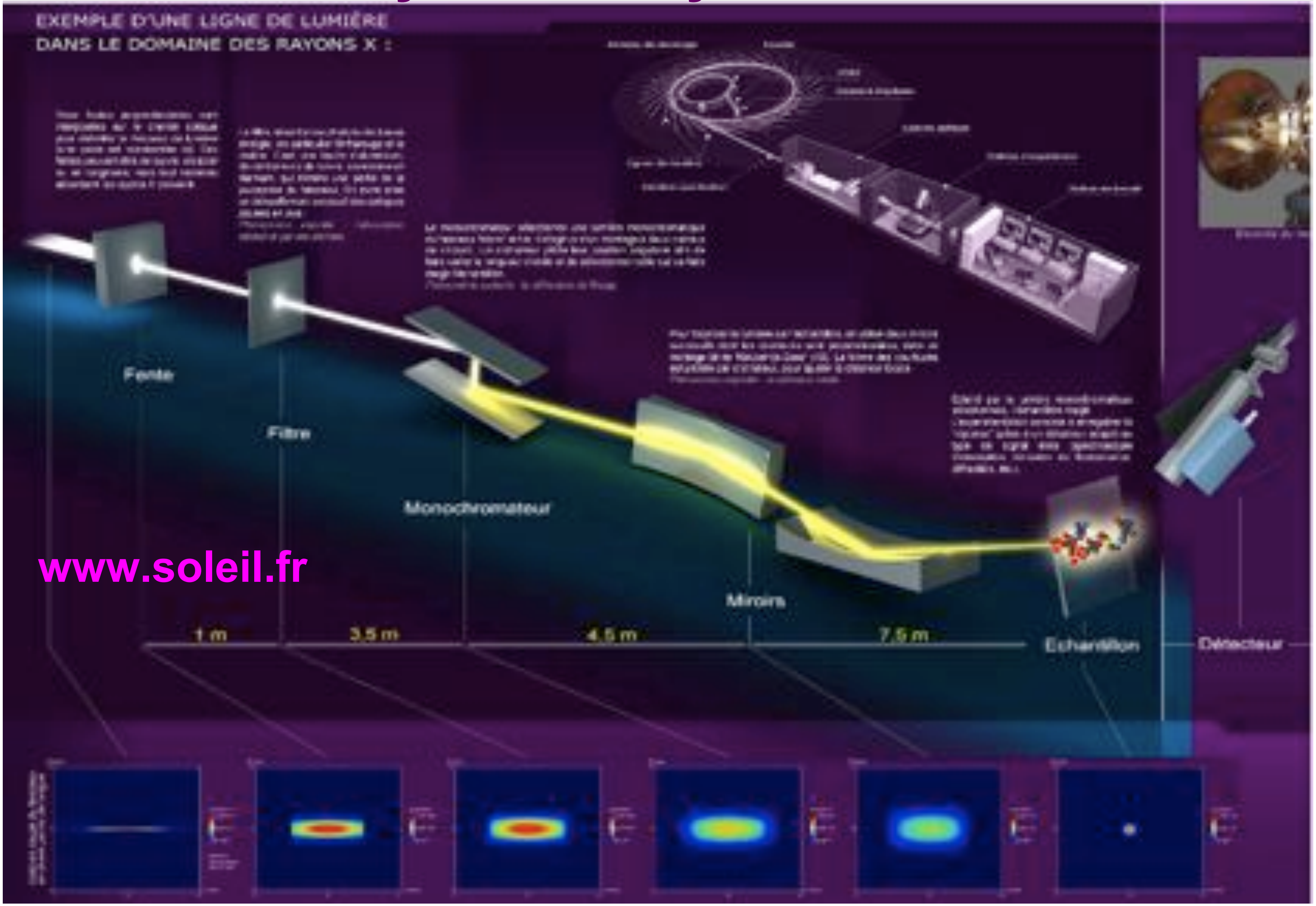
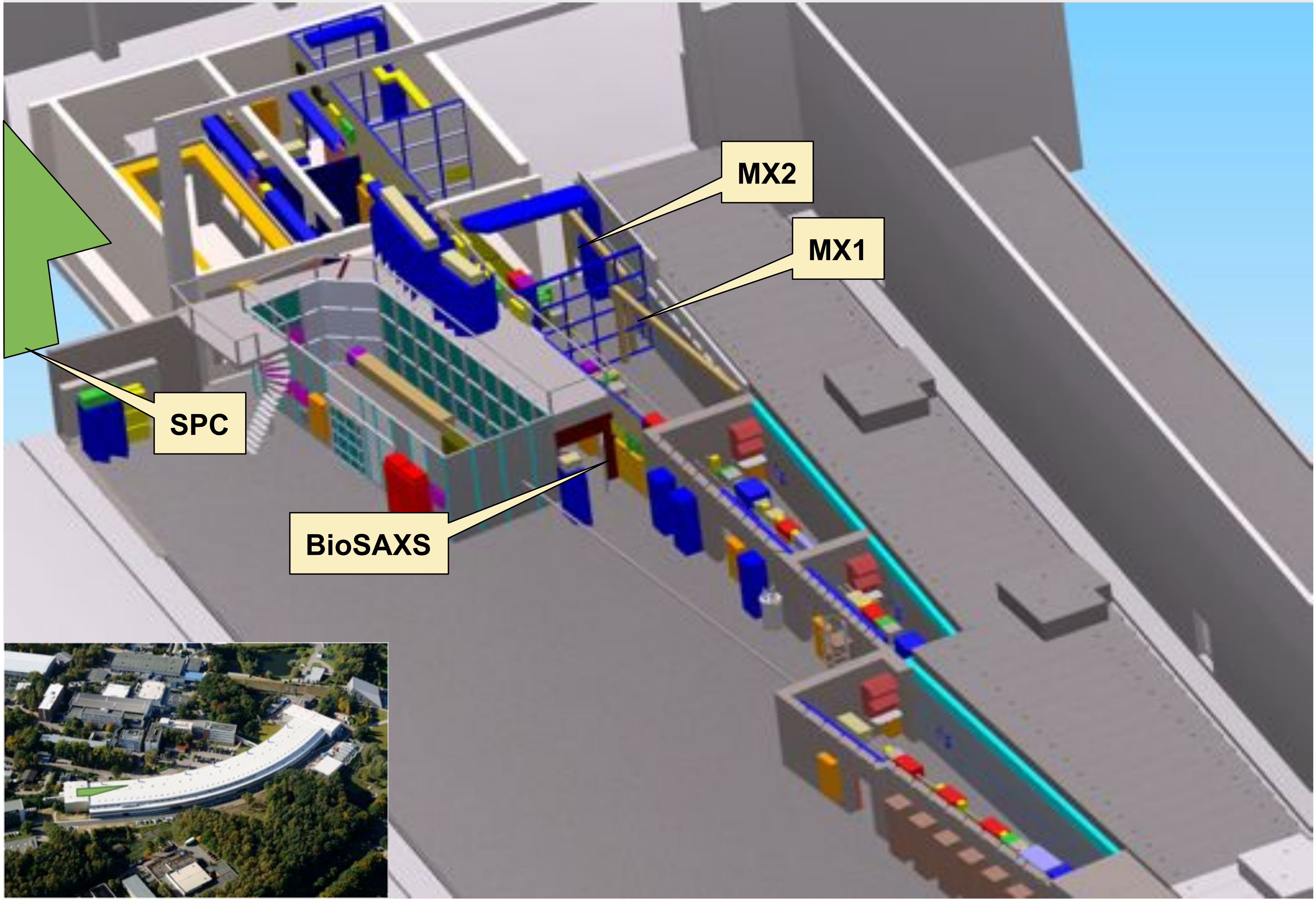
Schematic layout of a synchrotron beamline

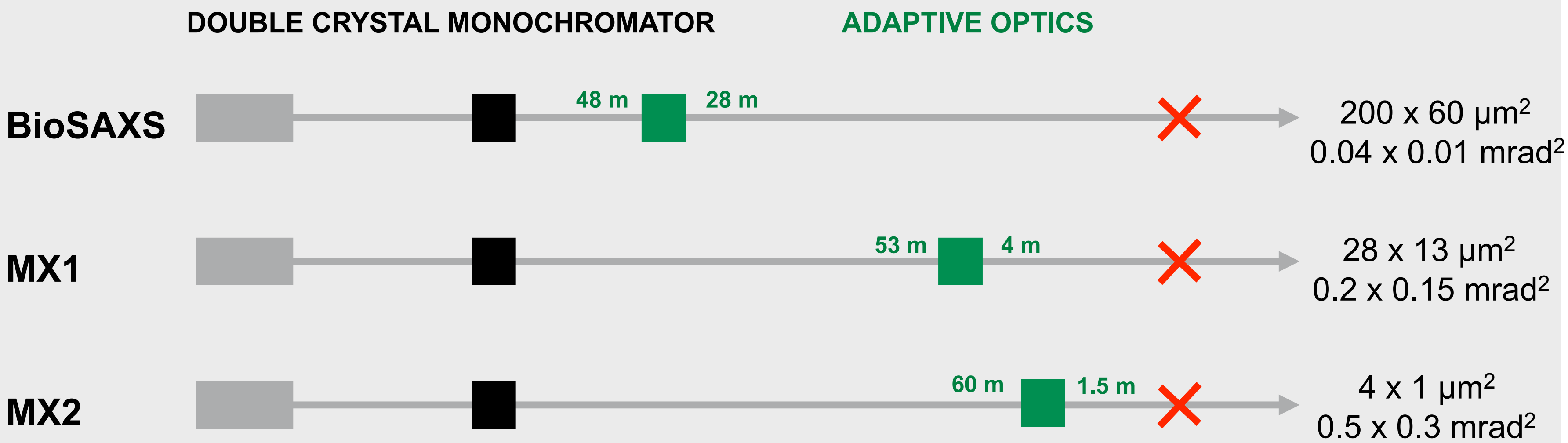


www.soleil.fr

Schematic layout of Petra III SB beamlines



Schematic layout of Petra III SB beamlines



	BioSAXS	MX1	MX2
Energy [keV]	4-20	5(4)-17	7-35
Monochromators	Si(111)	Si(111)	Si(111)
Beam size H x V [μm^2]	200 x 60	28 x 13	4 x 1
Divergence H x V [mrad]	0.04 x 0.01	0.2 x 0.15	<0.5 x <0.3
Demagnification H / V	1:1.4 / 1: 1.2	1:12 / 1:15	1:60 / 1:40
Intensity [ph/sec]	10^{13}	10^{13}	10^{12}

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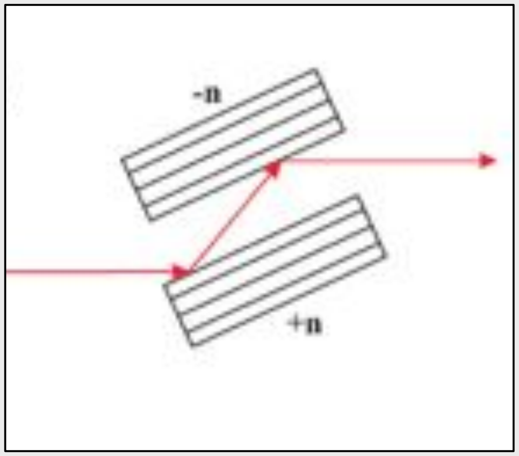
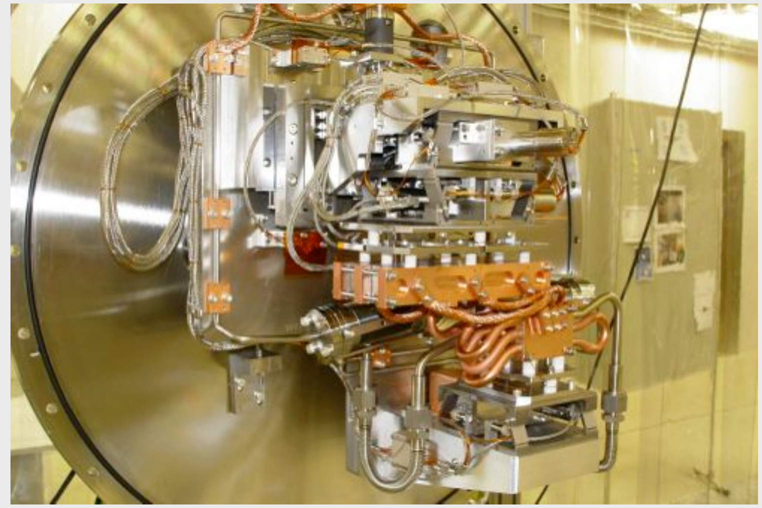
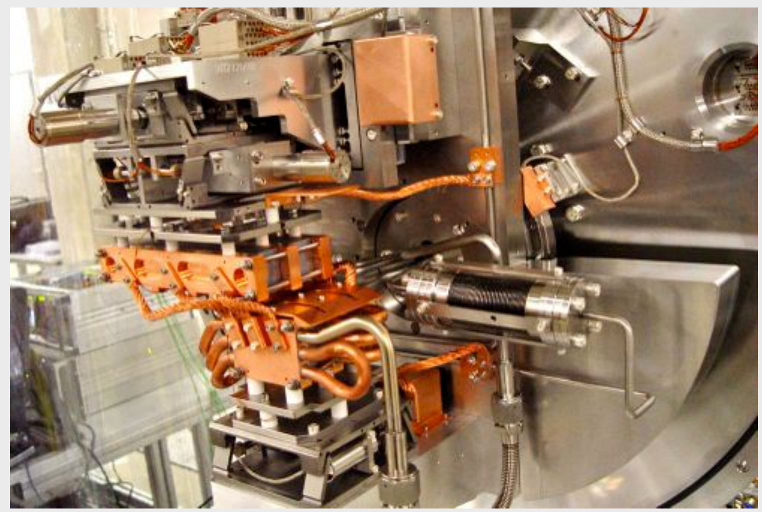
Double Crystal Monochromators



$$2\, d \sin \theta = n\, \lambda$$

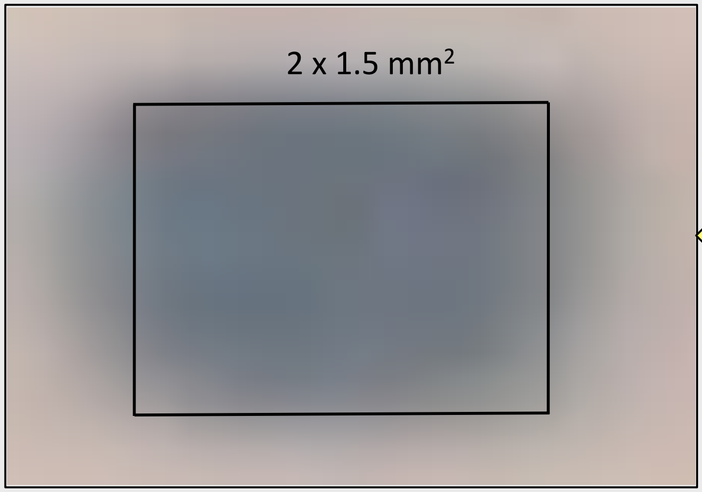
Use of **Si(111)** monochromators, due to excellent performance in relevant X-ray energy regime (4-15 keV)

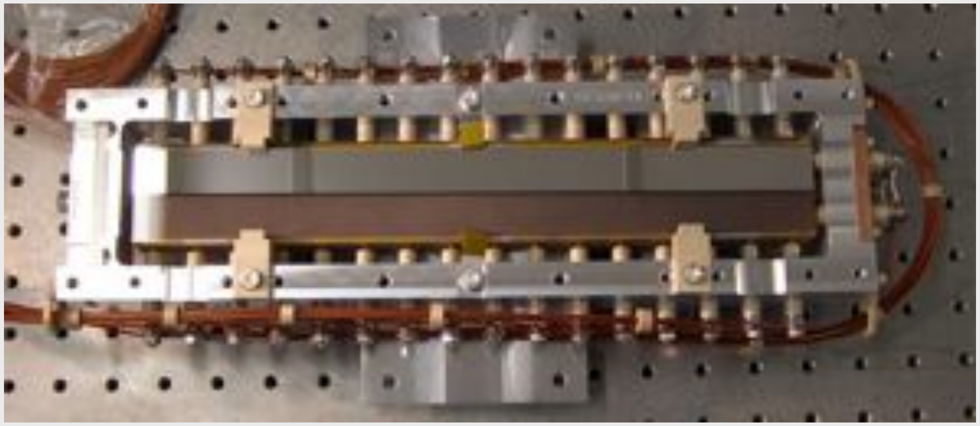




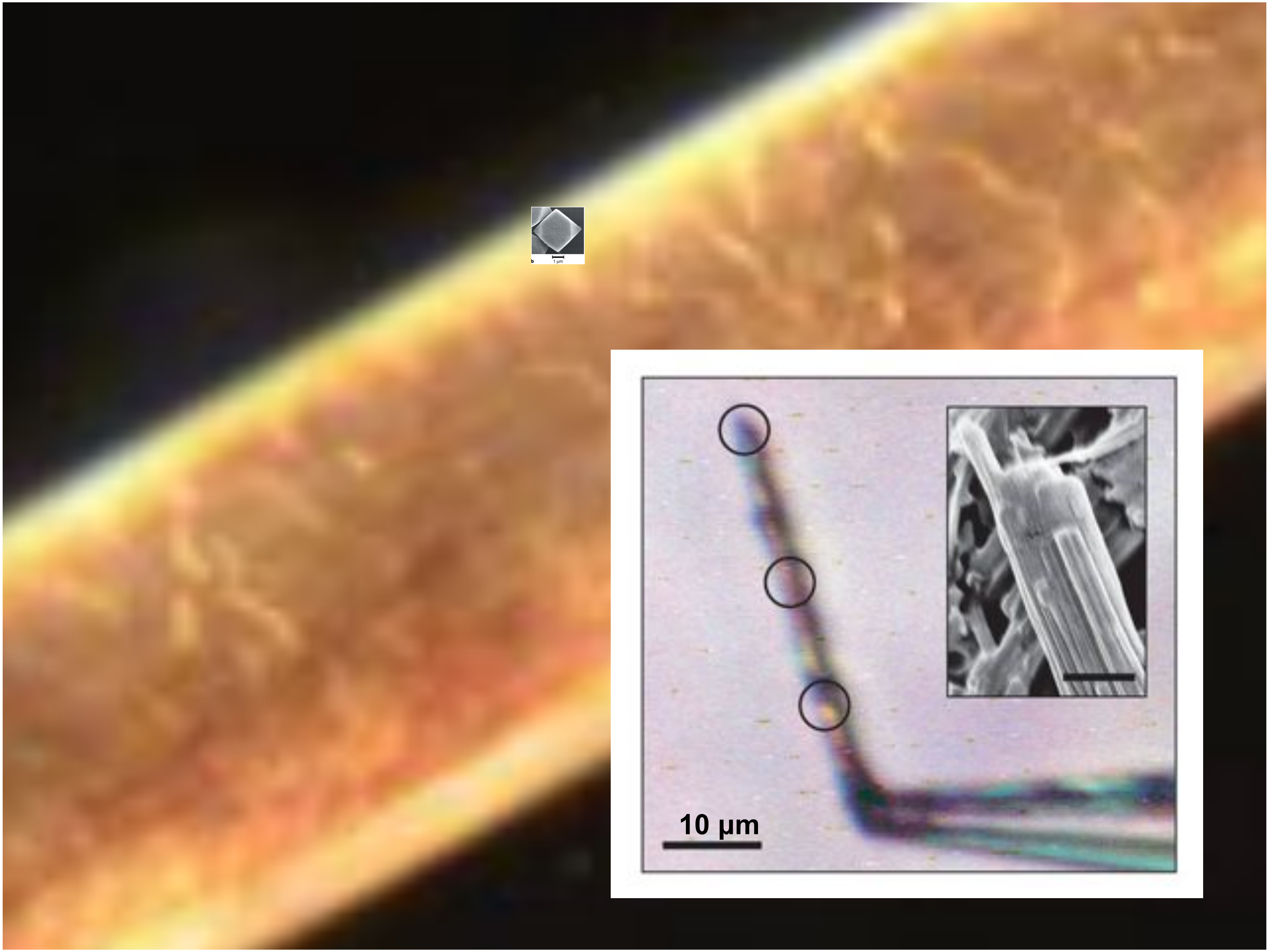
X-ray mirrors

- Purpose: **beam focusing**
- **Bimorphic mirrors** with Kirkpatrick-Baez (KB) geometry
- Typical specification: **slope error < 1 µrad.**
- Very few companies around then world with the ability to produce high quality KBs

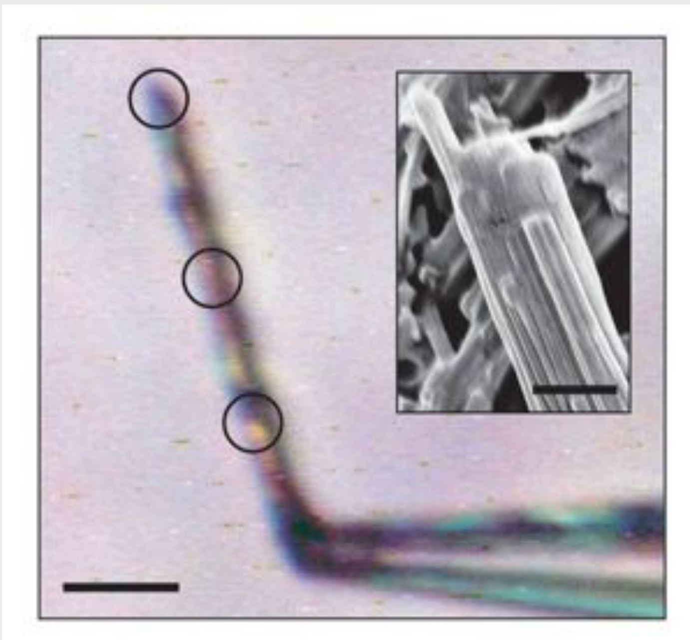


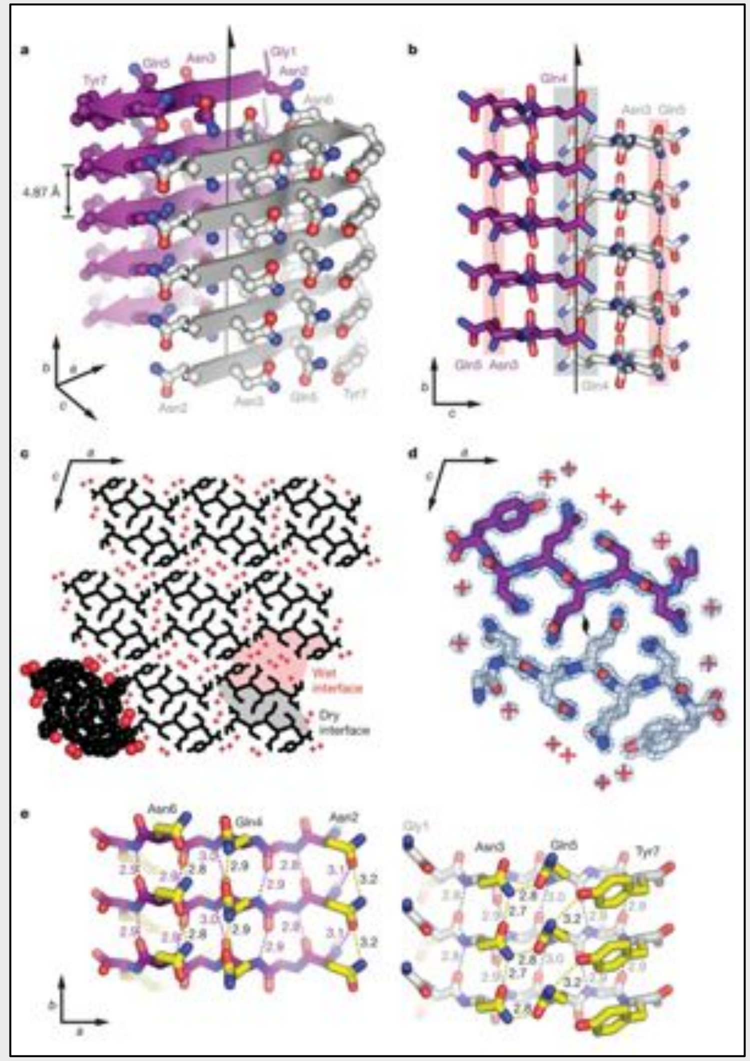


So what can we do will all this ?

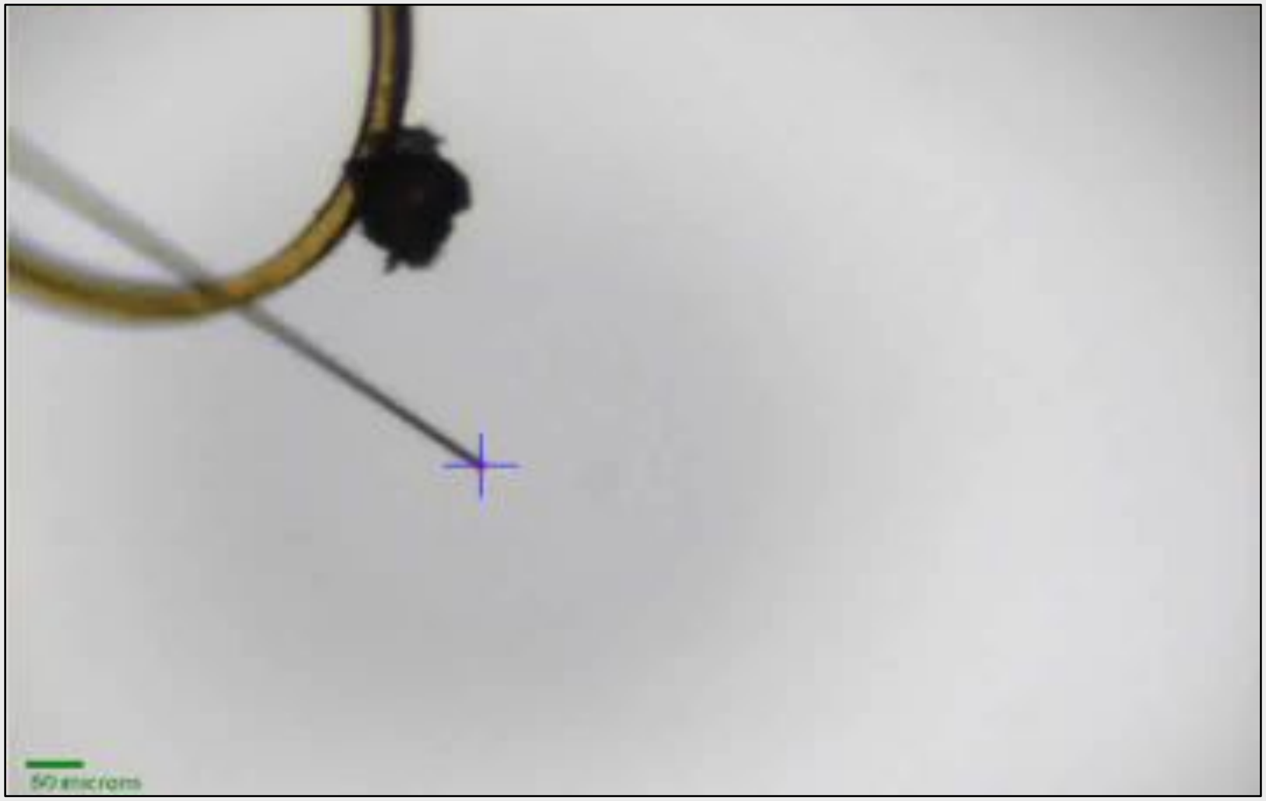

10 µm

High-resolution structures of amyloid fibrils





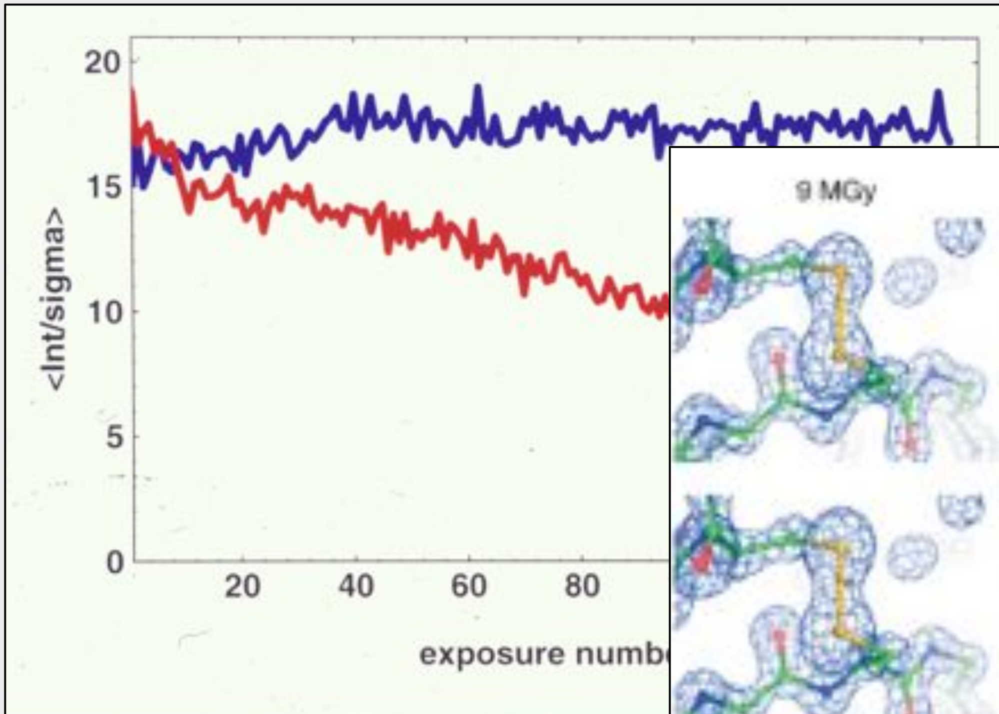
4D-Scan to control precise crystal position for data collection



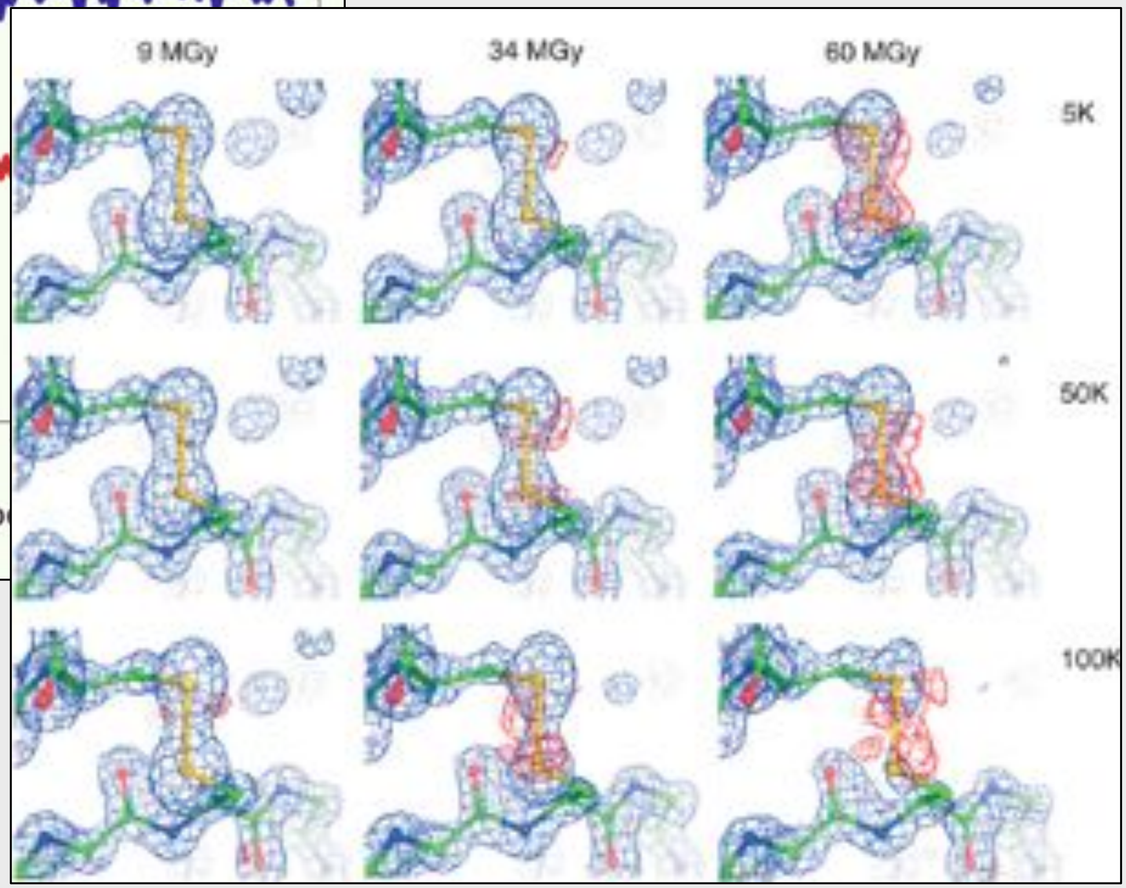
EMBL


Radiation Damage !

Effects of radiation damage



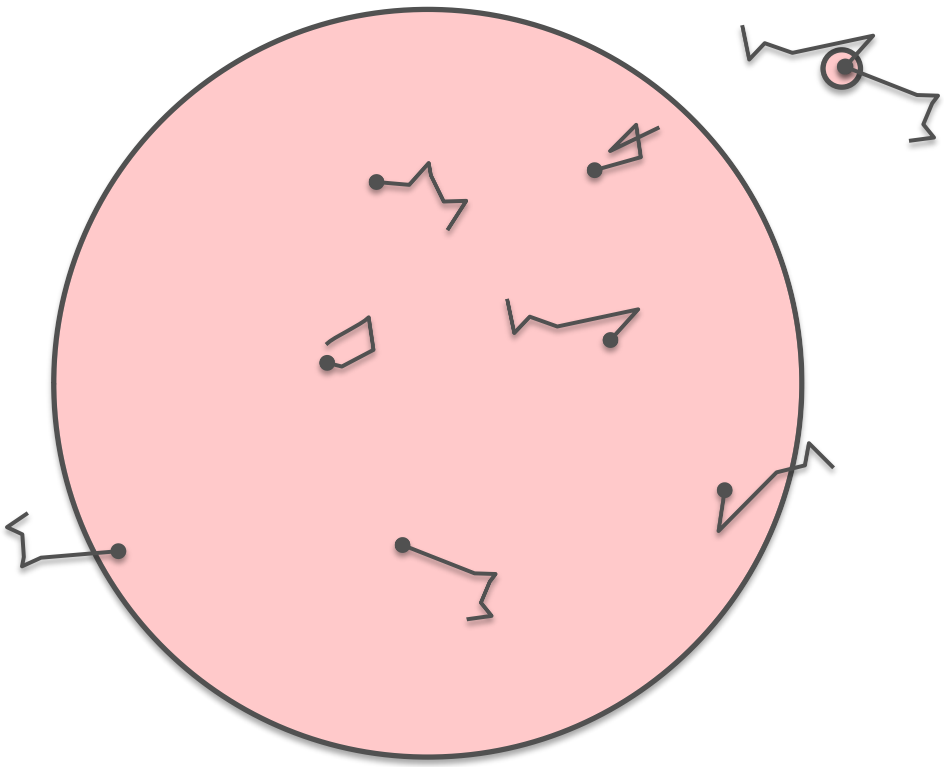
Lysozyme data set
120 K *vs.* 300 K

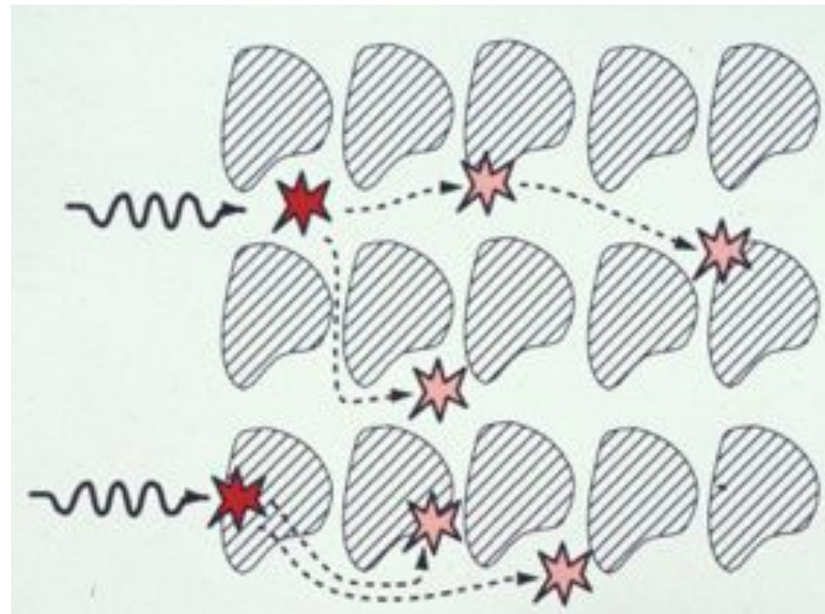


Fate of disulphide bridges

Ments *et al.* (2010) PNAS

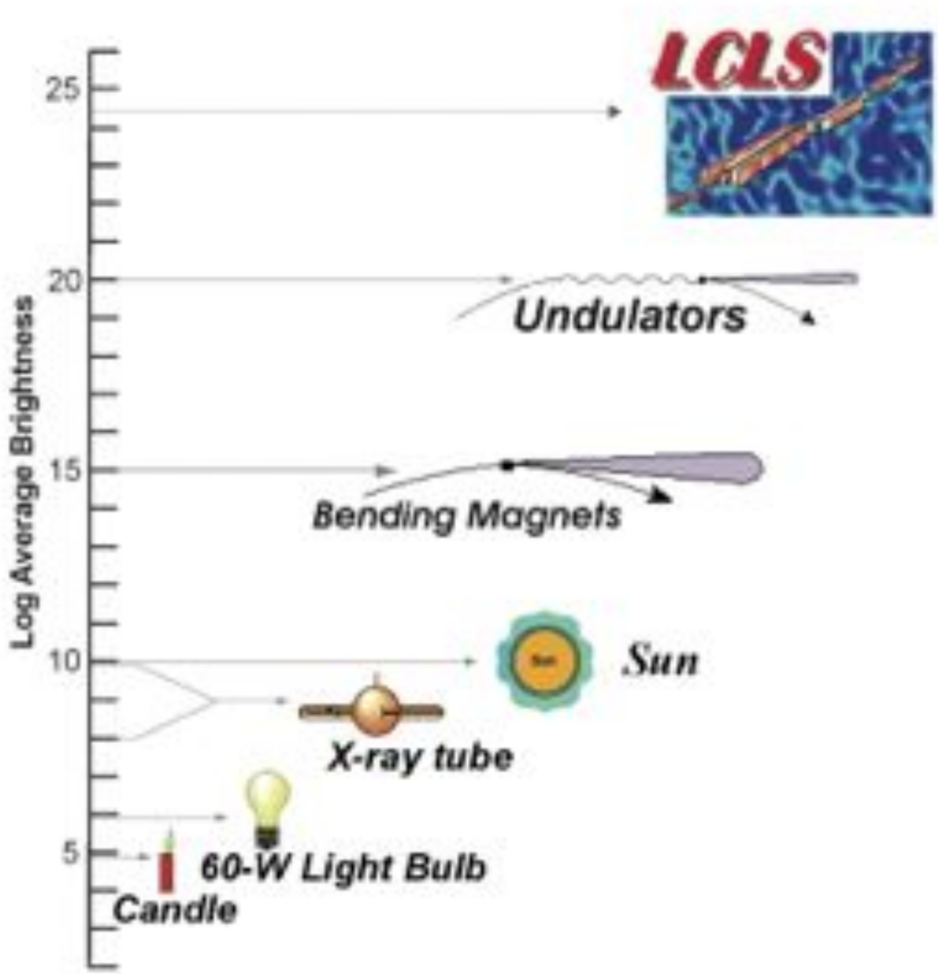
Intense radiation leads photo-electron escape



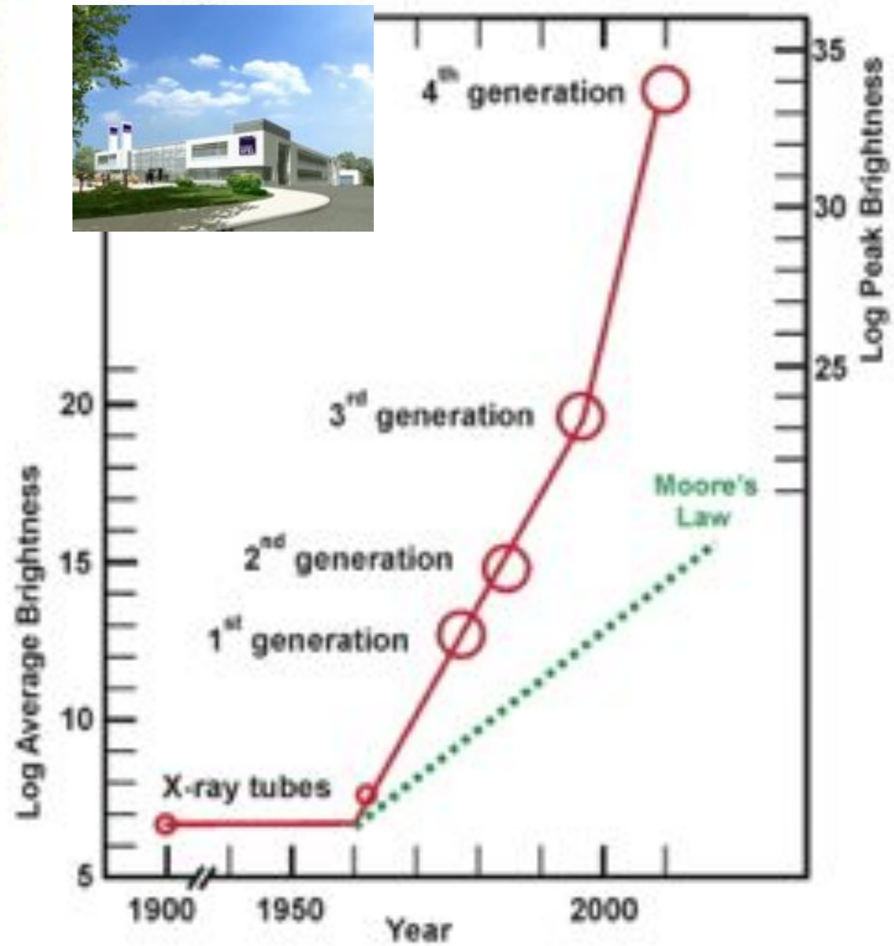


- For small volumes a large part of the energy of the photo-electrons is dissipated outside the irradiated volume
- This effect becomes more pronounced for higher energy X-rays.
- For higher energy X-rays the diffraction becomes weaker.
- Whether this effect can be exploited remains to be seen.

Light Sources and their Brightness



XFEL
X-ray laser



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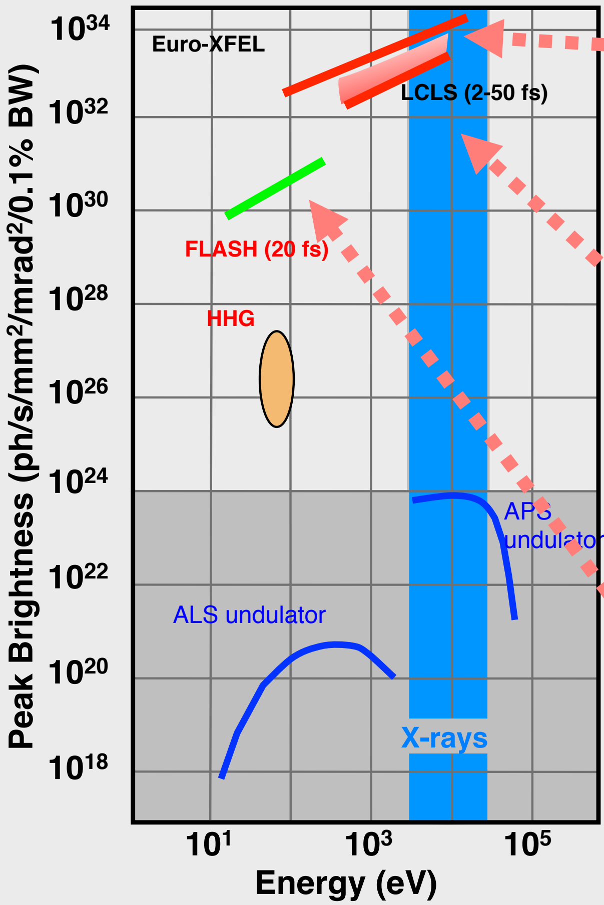
X-FELs provide pulses that are very intense, short duration, short wavelength, and coherent

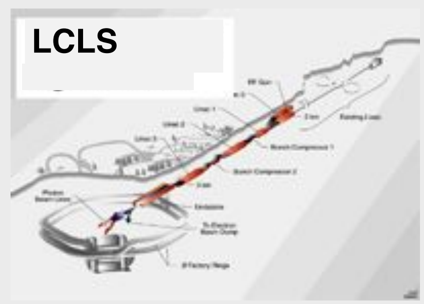


APS=Advanced Photon Source (ANL)
ALS=Advanced Light Source (LBNL)

operational 2015

12 keV, 50 fs, 10^{13} photons

European X-ray FEL, DESY, Hamburg



operational now

800 eV to 2 keV in 2009
2 to 50 fs, up to 10^{13} photons
8 keV, 10^{12} photons

Linac Coherent Light Source, SLAC, Stanford

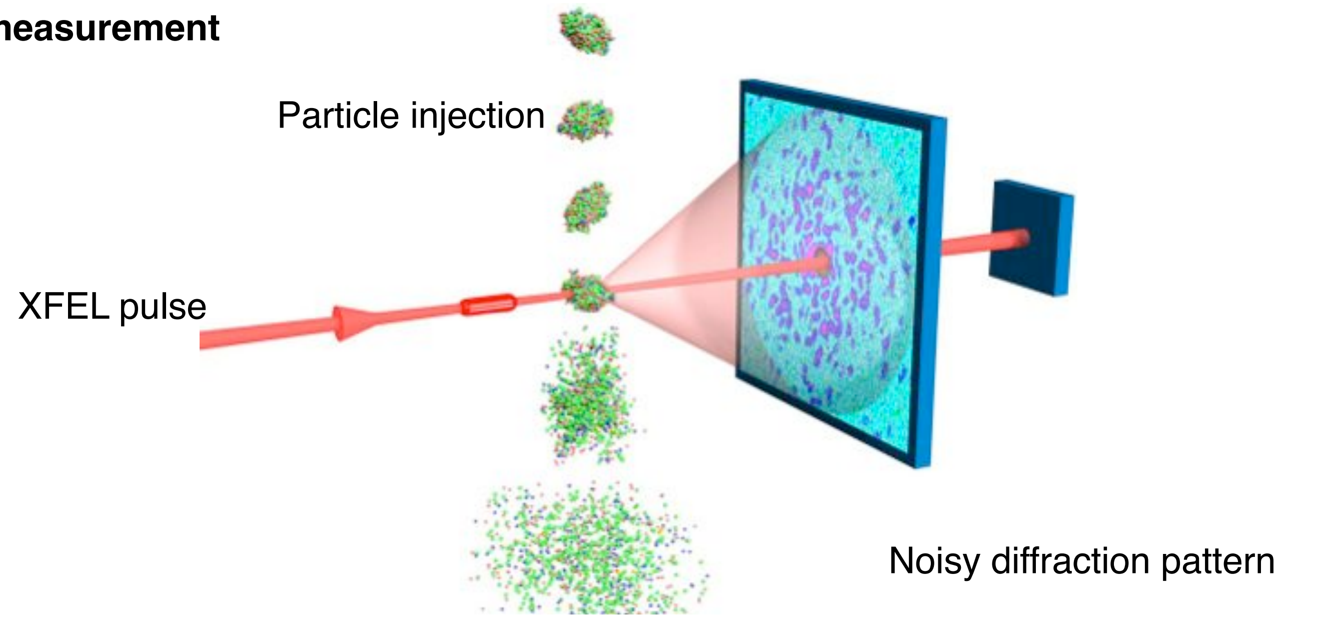
operational now

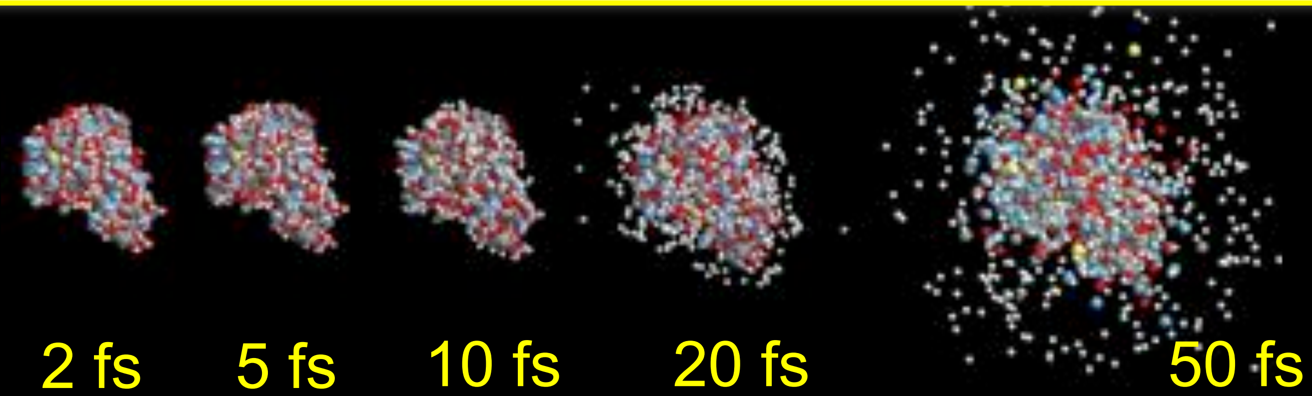
200 eV, 25 fs, 10^{12} photons
upgrade to 300 eV, 400 fs

FLASH
DESY, Hamburg

X-ray free-electron lasers may enable atomic-resolution imaging of biological macromolecules

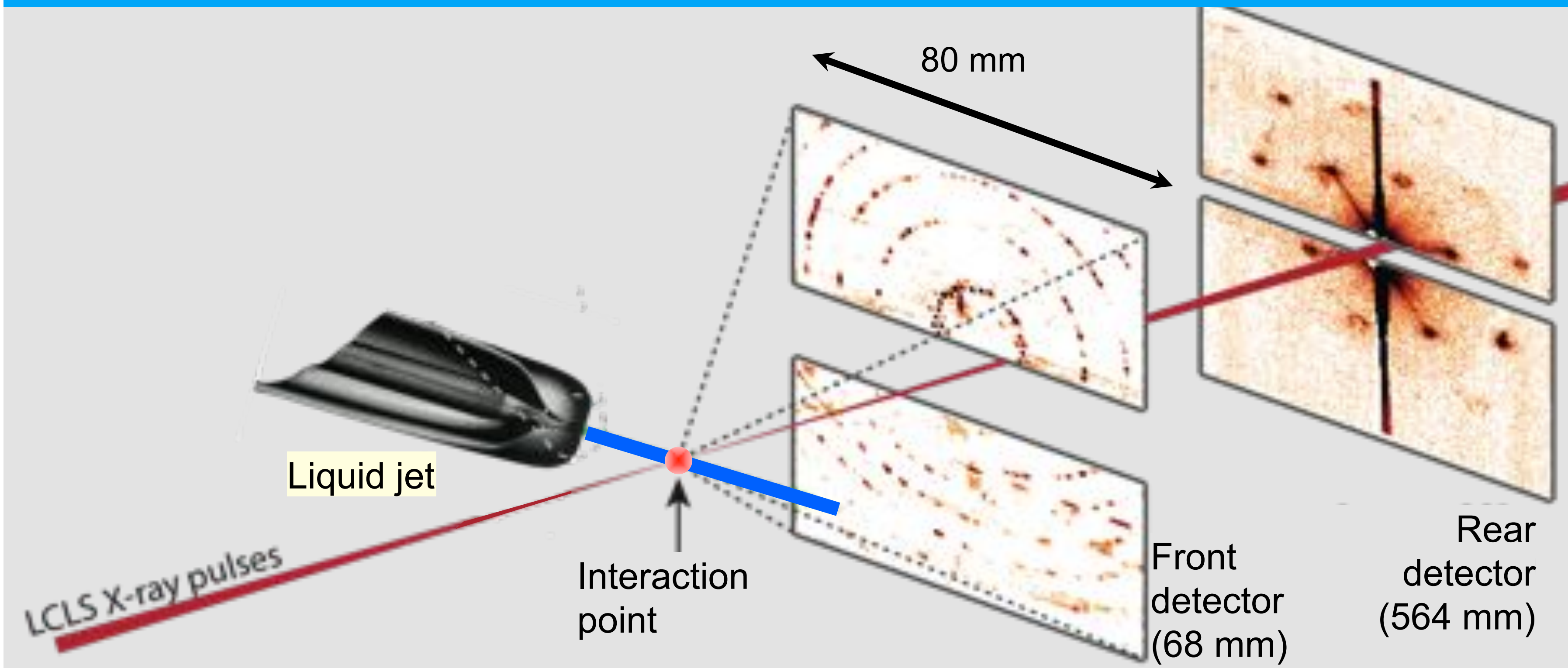
One pulse, one measurement

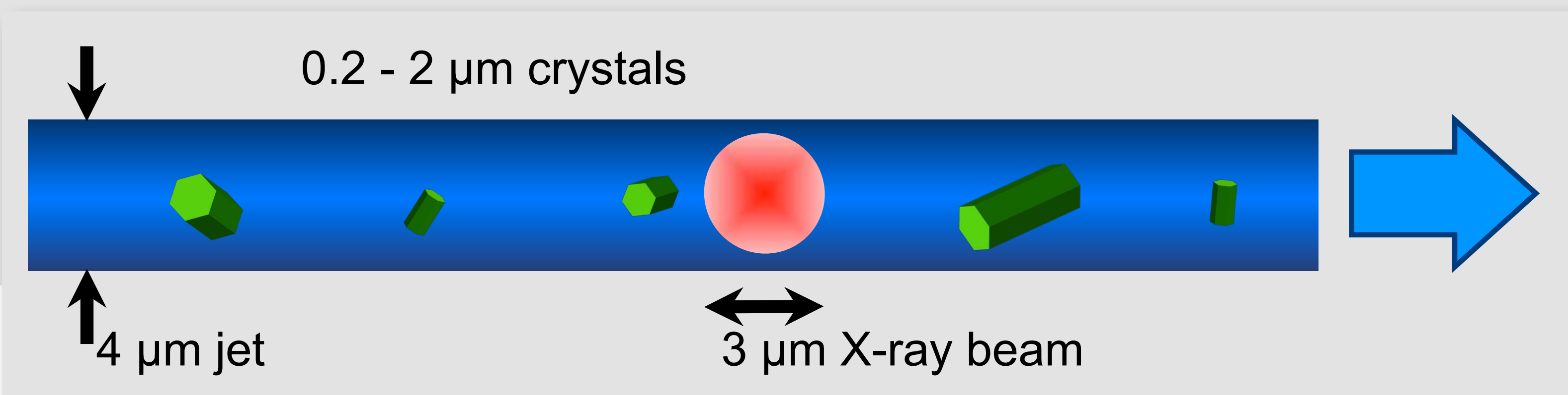




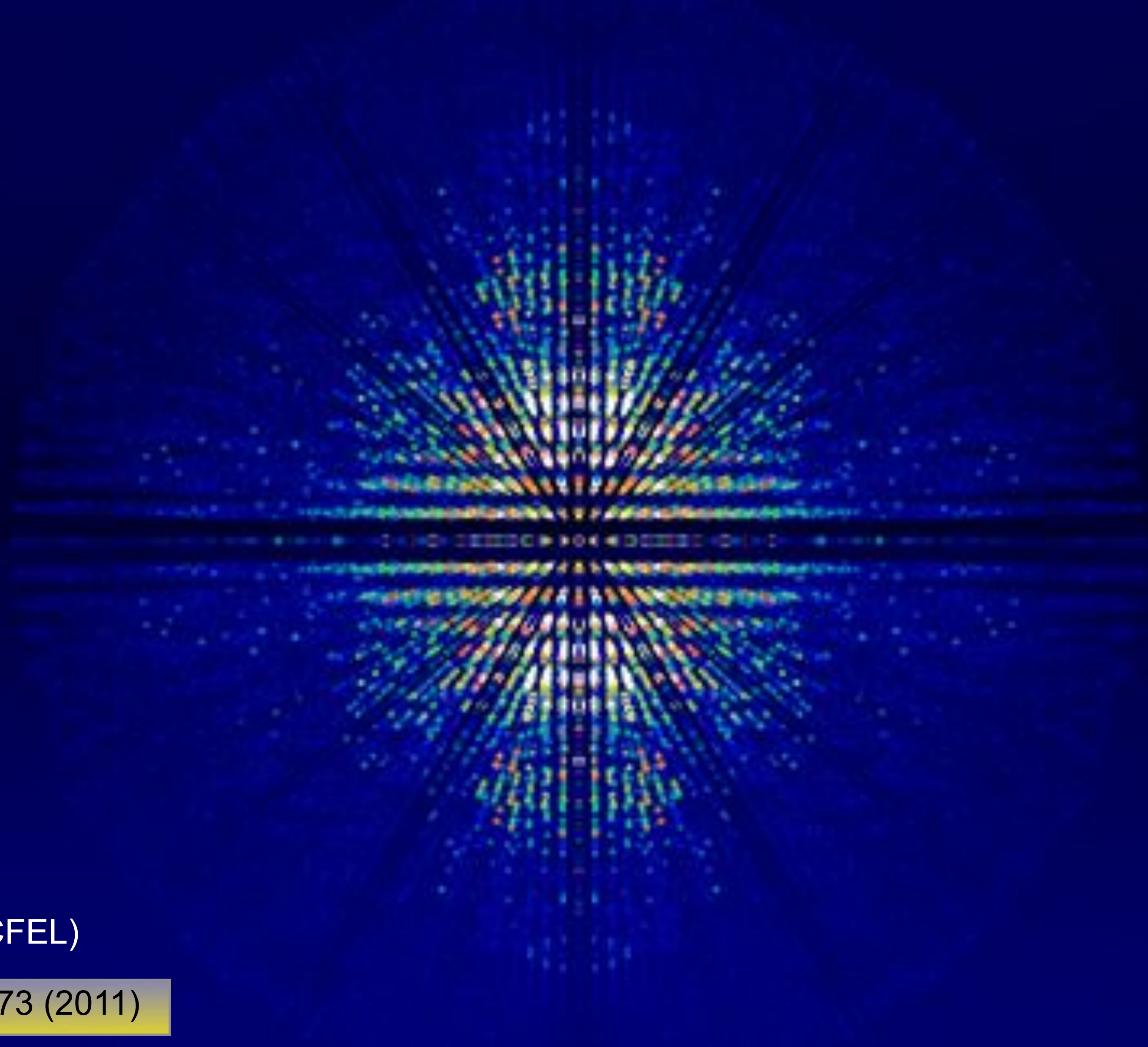
R. Neutze, R. Wouts, D. van der Spoel, E. Weckert, J. Hajdu, Nature **406** (2000)

Nanocrystallography is carried out in a flowing water microjet





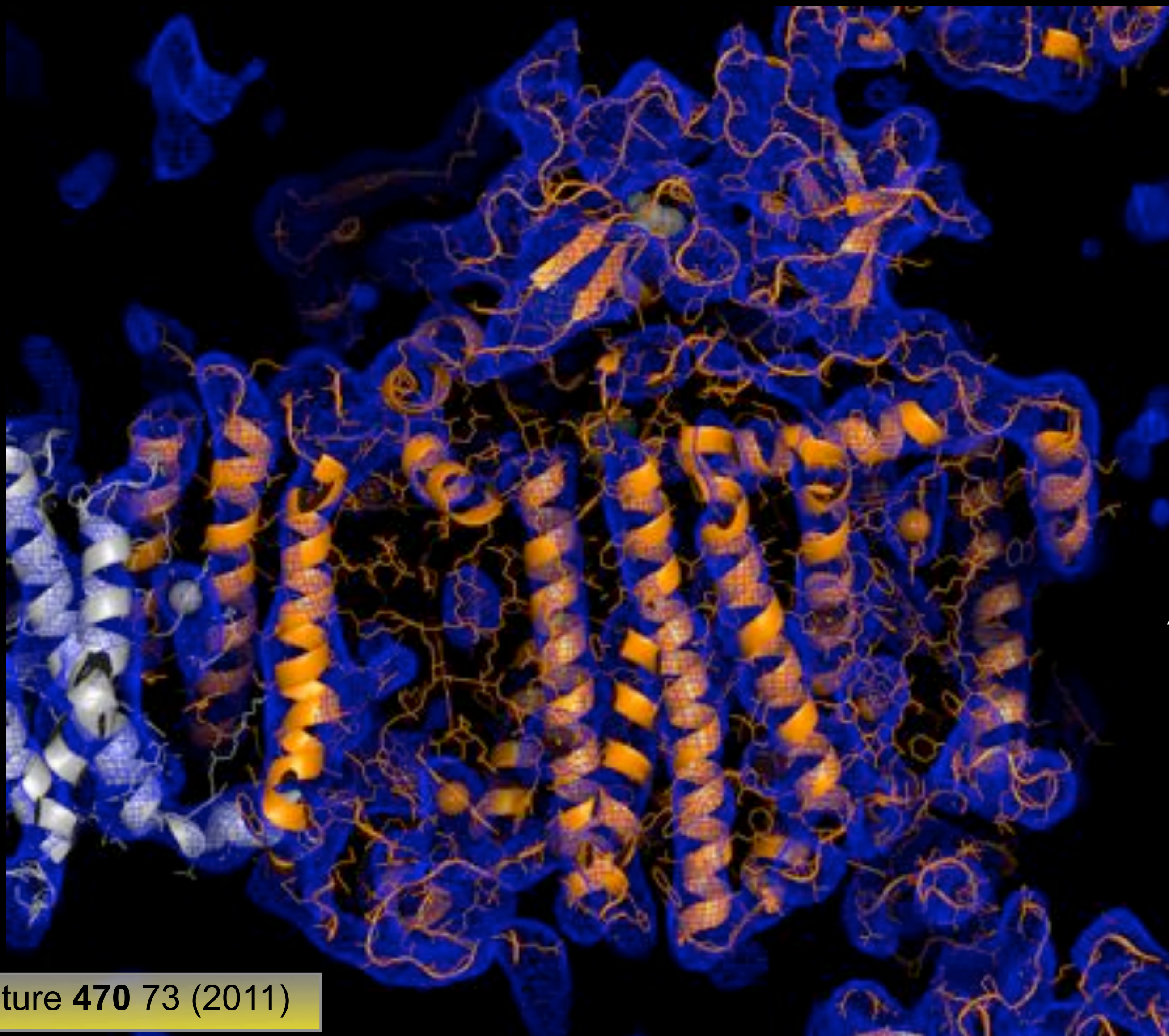
We have merged tens of thousands of snapshot patterns into a set of 3D structure factors



Tom White (CFEL)

Nature **470** 73 (2011)

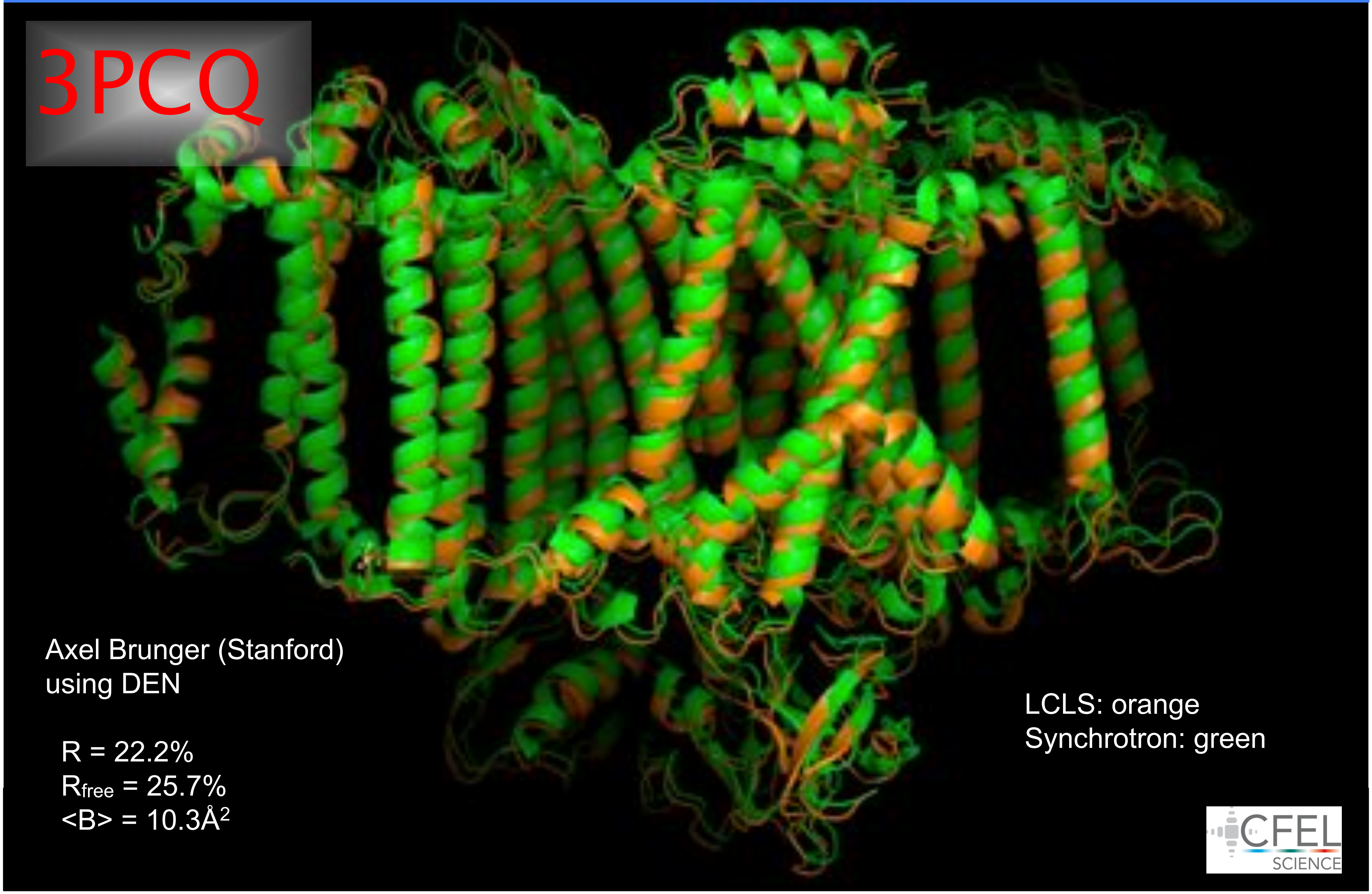
Molecular replacement reconstructs the 8.5 Å structure



Axel Brunger (Stanford)

Nature **470** 73 (2011)

The difference between the synchrotron and FEL structures might be due to temperature



The need bridge different resolutions

Atomic Imaging

1-20 Å

X-ray

Scatt.

NMR

Macro-molecular Imaging

4-200 nm

EM

Tomography

LM (FRET)

Cellular Imaging

0.2 - 2 µm

LM

functional

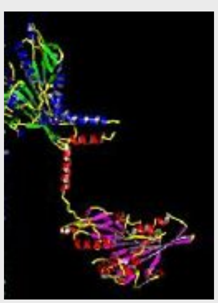
probes

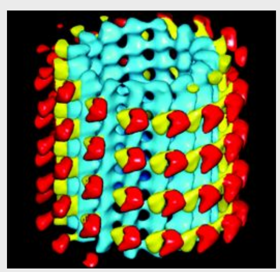
Organismal Imaging

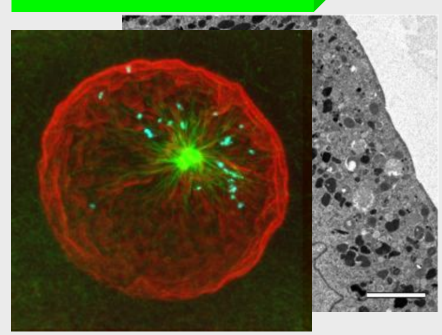
1 µm - 5 mm

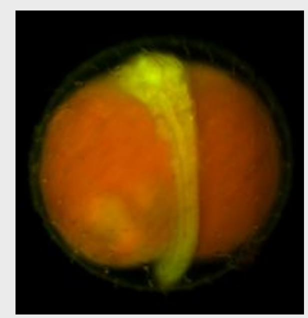
LM

MRI





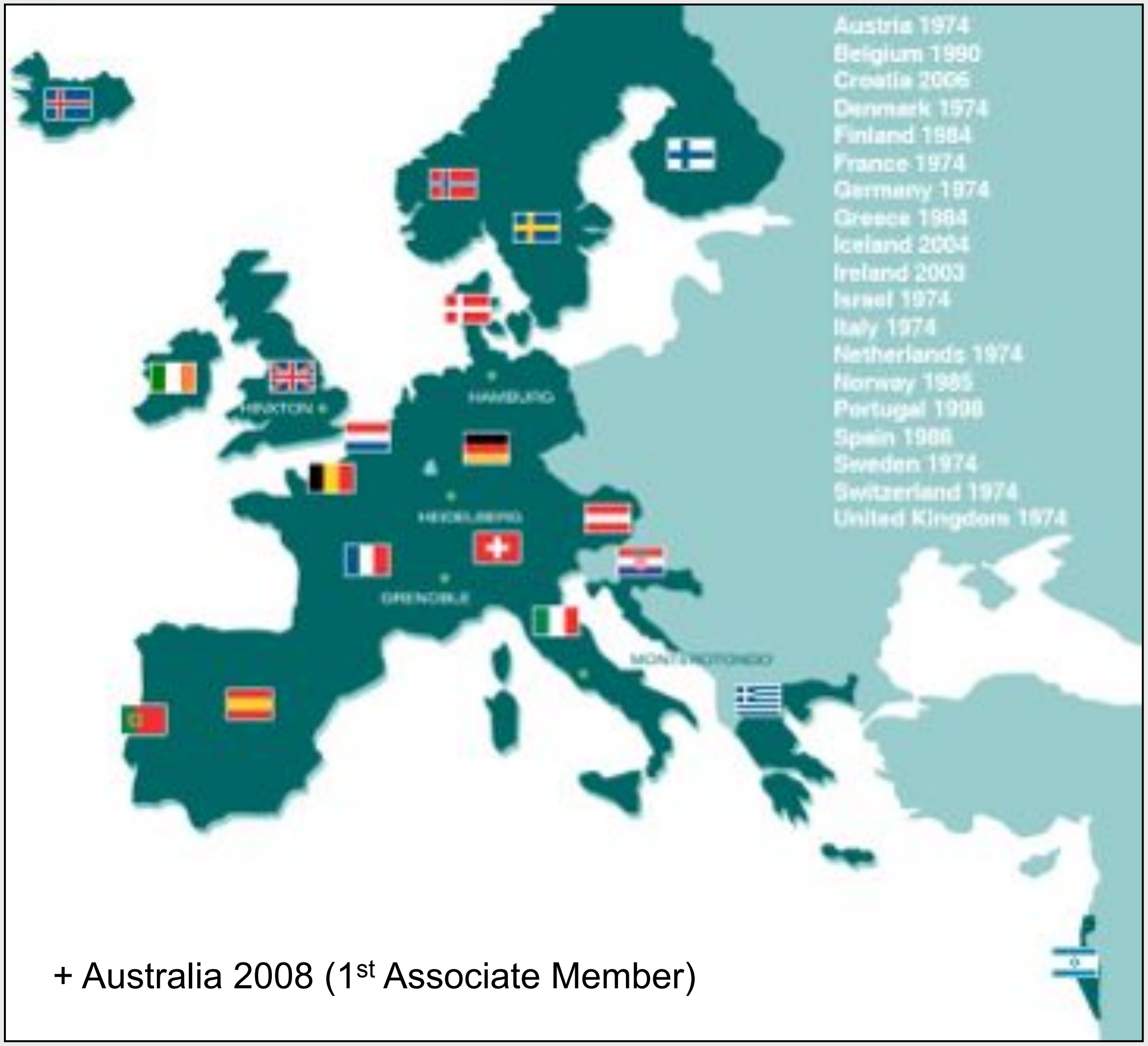


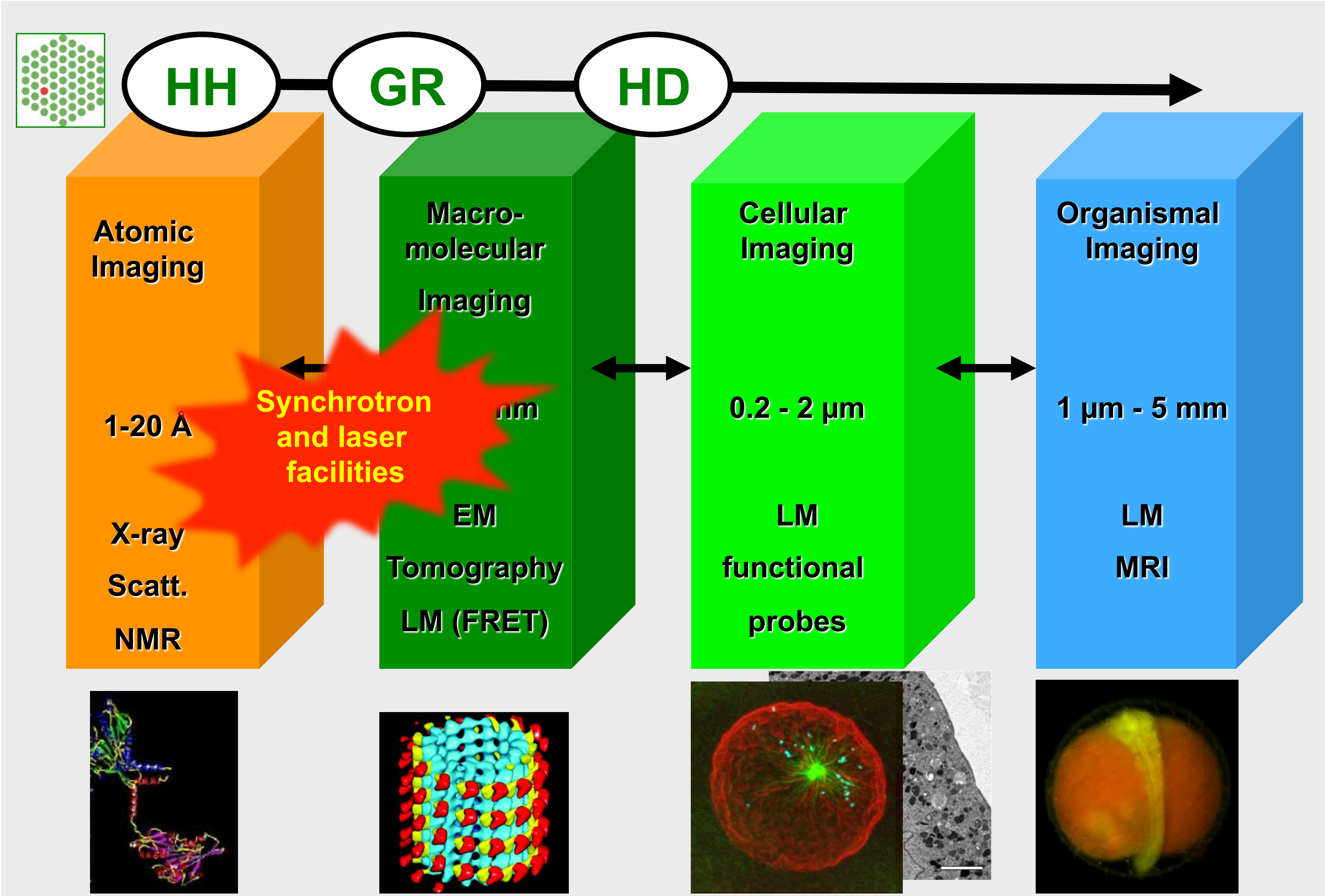


Courtesy Jan Ellenberg, EMBL

EMBL

3 EMBL Units with complementary structural biology activities




HH
GR
HD
Atomic Imaging
1-20 Å
X-ray
Scatt.
NMR
Macro-molecular
Imaging
nm
EM
Tomography
LM (FRET)
Cellular Imaging
0.2 - 2 µm
LM
functional
probes
Organismal Imaging
1 µm - 5 mm
LM
MRI
Synchrotron and laser facilities

Center for Structural Systems Biology

CSSB
Centre for Structural Systems Biology

Universitätsklinikum Hamburg-Eppendorf

Medizinische Hochschule Hannover

EMBL

HELMHOLTZ GEMEINSCHAFT

HPI

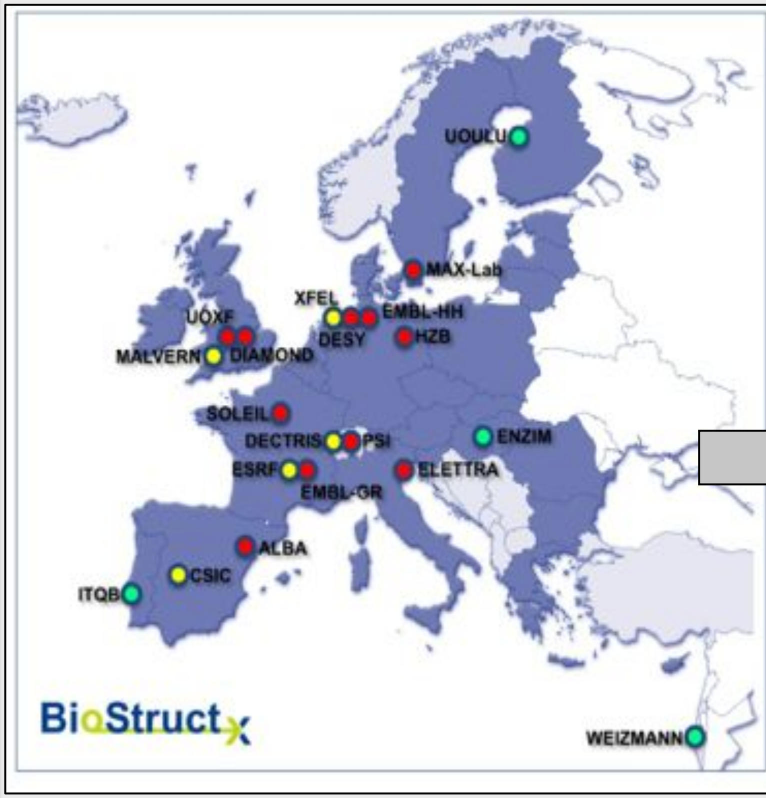
Inflammation at Interfaces

Research Center Borstel

EMBL Hamburg faculty

Missing: Johanna Kallio, Christoph Hermes, Gleb Bourenkow; seven faculty members departed 2007-2011.

BioStruct-X Project Partners and Status



no.	Participant short name	Participant organisation name	Country
1a Coord.	EMBL-HH	European Molecular Biology Laboratory	DE
1b	EMBL-GR	European Molecular Biology Laboratory	DE
2	ALBA	CONSORCIO PARA LA CONSTRUCCION, EQUIPAMIENTO Y EXPLOTACION DEL LABORATORIO DE LUZ DE SINCROTRON	ES
3	DESY	Stiftung Deutsches Elektronen-Synchrotron	DE
4	DIAMOND	Diamond Light Source Ltd	UK
5	ELETTRA	SINCROTRONE TRIESTE SCPA	IT
6	HZB	HELMHOLTZ-ZENTRUM BERLIN FÜR MATERIALIEN UND ENERGIE GMBH	DE
7	MAX-Lab	Lund University	SE
8	PSI	PAUL SCHERRER INSTITUT	CH
9	SOLEIL	Société Civile Synchrotron SOLEIL	FR
10	UOXF	The Chancellor, Masters & Scholars of the University of Oxford	UK
11	CSIC	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	ES
12	DECTRIS	Dectris Ltd.	CH
13	ESRF	Installation Européenne de Rayonnement Synchrotron	FR
14	XFEL	European X-ray Free Electron Laser Facility GmbH	DE
15	UOULU	Oulun yliopisto (University of Oulu)	FI
16	ITQB	Instituto de Tecnologia Química e Biológica – Universidade Nova de Lisboa	PT
17	WEIZMANN	Weizmann Institute of Science	IL
18	ENZIM	Magyar Tudományos Akadémia Enzimológiai Intézet	HU
19	MALVERN	Malvern Instruments Ltd.	UK

Caption: TNA/JRA/NA partners, red; JRA/NA partners, yellow; NA partners, green.

- Partner Categories: experiment facilities (red), only R&D (yellow), TID (green)

BioStruct-X

BioStruct-X Project Tasks

TNA support for 44 installations:

- Biological small angle X-ray scattering (5)
- macromolecular X-ray crystallography (26)
- Biological X-ray imaging (4)
- Protein production and HTP crystallisation (9)

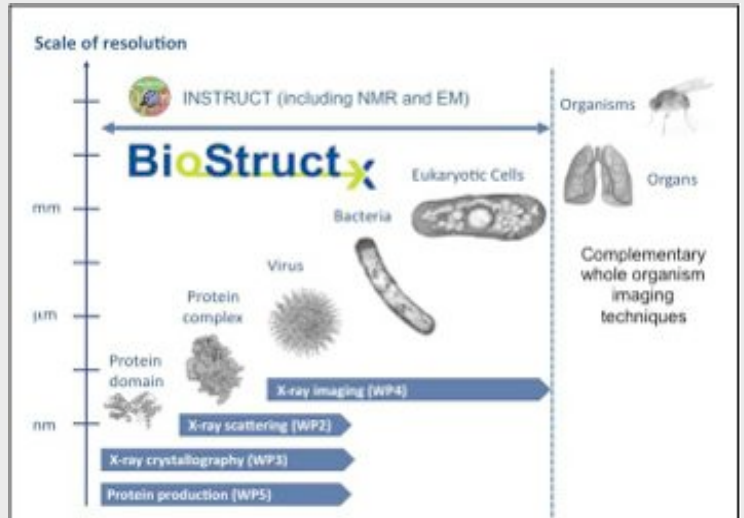
<u>Level of funding: 60%</u>

4 selected Joint Research Activities:

- To enhance methods integration
- Integration of emerging facilities (XFELs) and emerging methods (X-ray imaging)

<u>Level of funding: 28%</u>

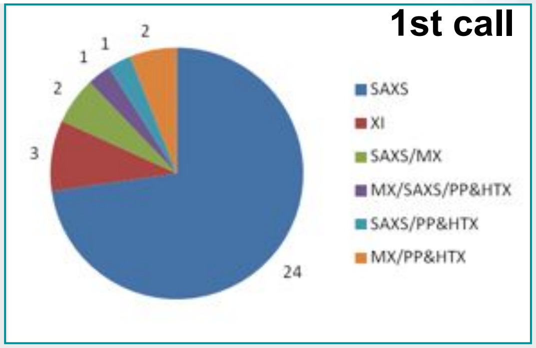
- **Centralised** (via providing facilities) and **decentralised** (via TID centres) training and networking activities.

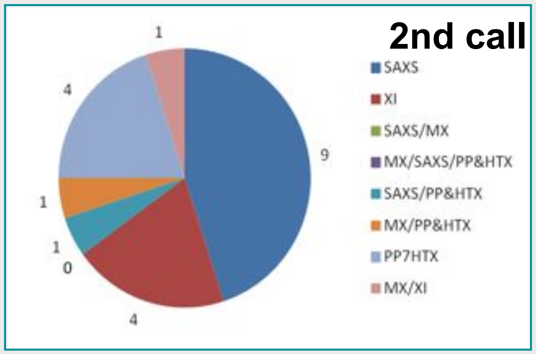


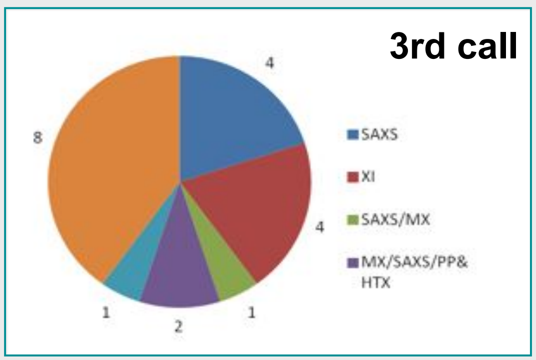
TNA Facilities	WP2 (SAXS)	WP3 (MX)	WP4 (XI)	WP5 (PP + HTP-X)	Total
EMBL-HH	2	5		1	8
EMBL-GR		1		3	4
ALBA		1	1		2
DESY		1	1		2
DIAMOND	1	5		1	7
ELETTRA		2			2
HZB		3	1		4
MAX-Lab	1	3			4
PSI		3		2	5
SOLEIL	1	2	1		4
UOXF				2	2
Total	5	26	4	9	44

Project visions – Novelty of the project

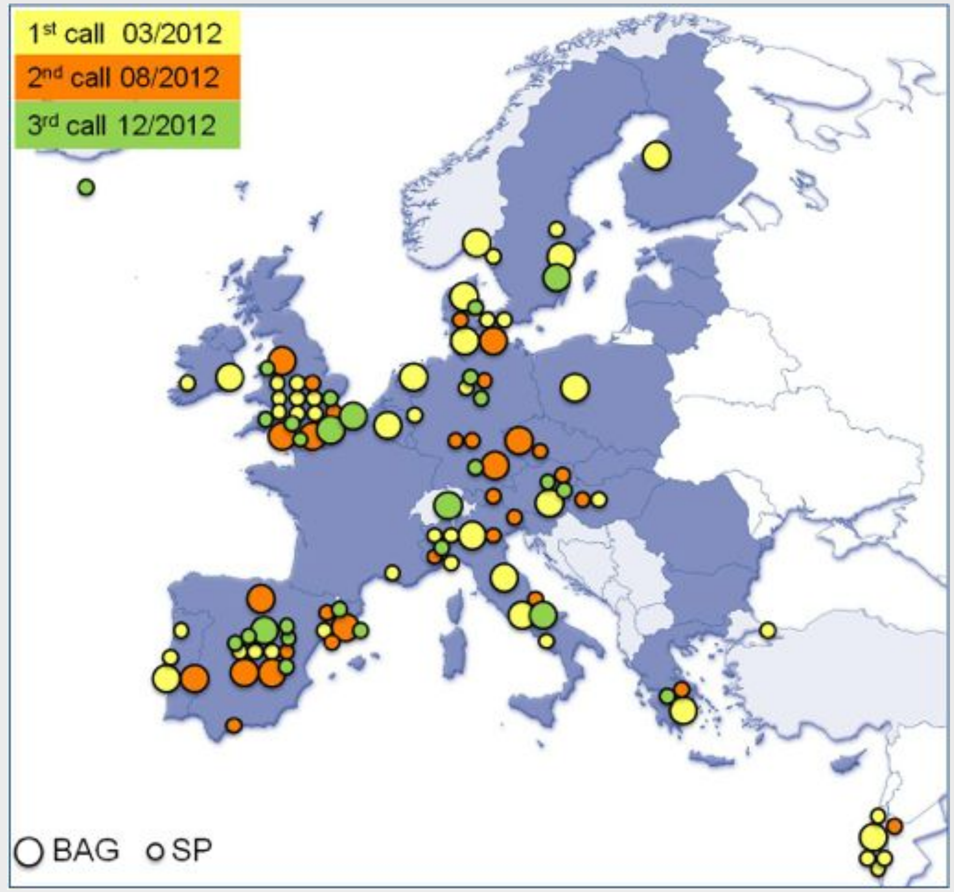
- To provide **integrated support for X-ray based structural biology applications**, plus protein production and HT crystallization.
- To participate in an **integrated provision for all infrastructure-based applications in structural biology** across Europe (INSTRUCT).
- The definition of **needs are user-driven**: strong user bottom-up elements.
- To establish a **unified, transparent and simple-to-use project portal and proposal application procedure.**
- **Training, Information & Dissemination**: structural biology community, overall scientific community, public.







Applications from 22 countries



BioStruct-X Project Evaluation Committee

- **Chair/Deputy**: Joel Sussman, Tassos Perrakis
- Asked for **revision of the application procedure**, approved revision on January 19, 2012; call for applications was opened in February 2012.
- **Rules for BAGs and SPs** were established
- **3-4 calls per year**, allowing comparative assessment, face/face meetings. **Next call by the end of 2013.**
- Proposals from **5 calls in 2012/13** have been evaluated
- **Ongoing improvements on technical aspects of evaluation procedures**, in collaboration with EMBL HH IT group.

Further questions?

Ivana Custic, project manager

biostructx@embl-hamburg.de

www.biostructx.org


Thank You