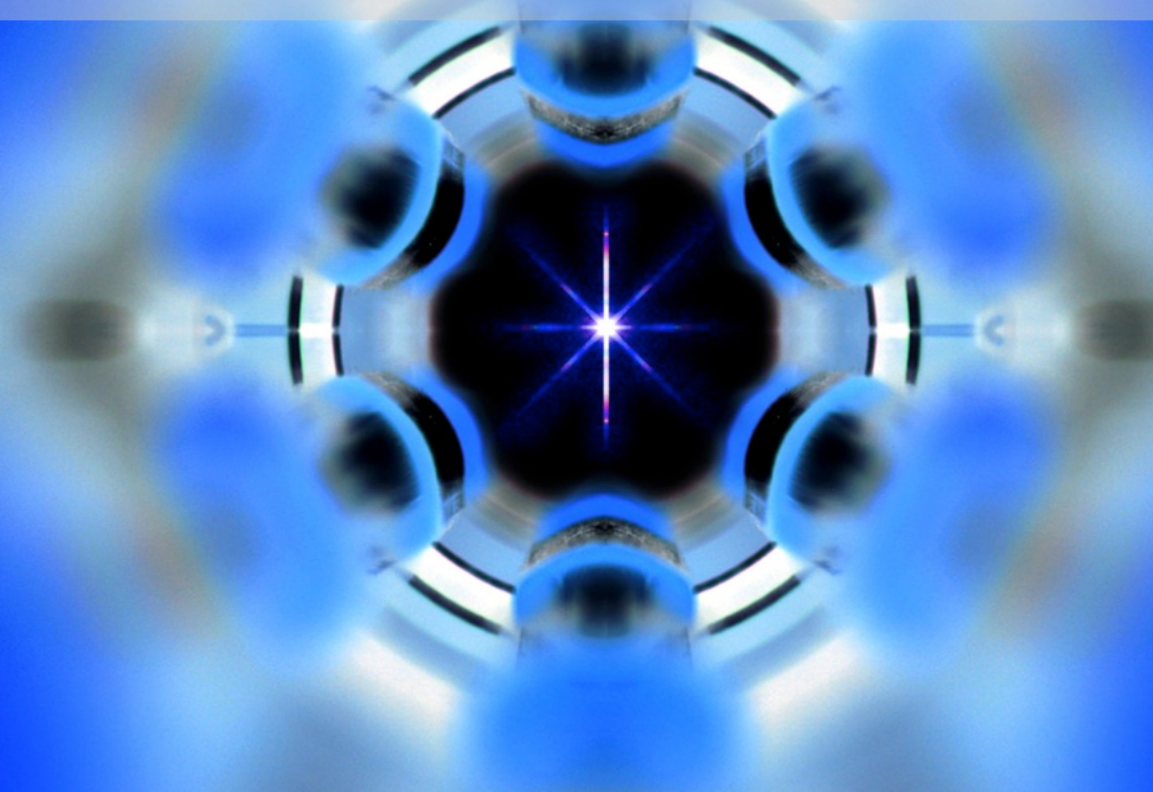
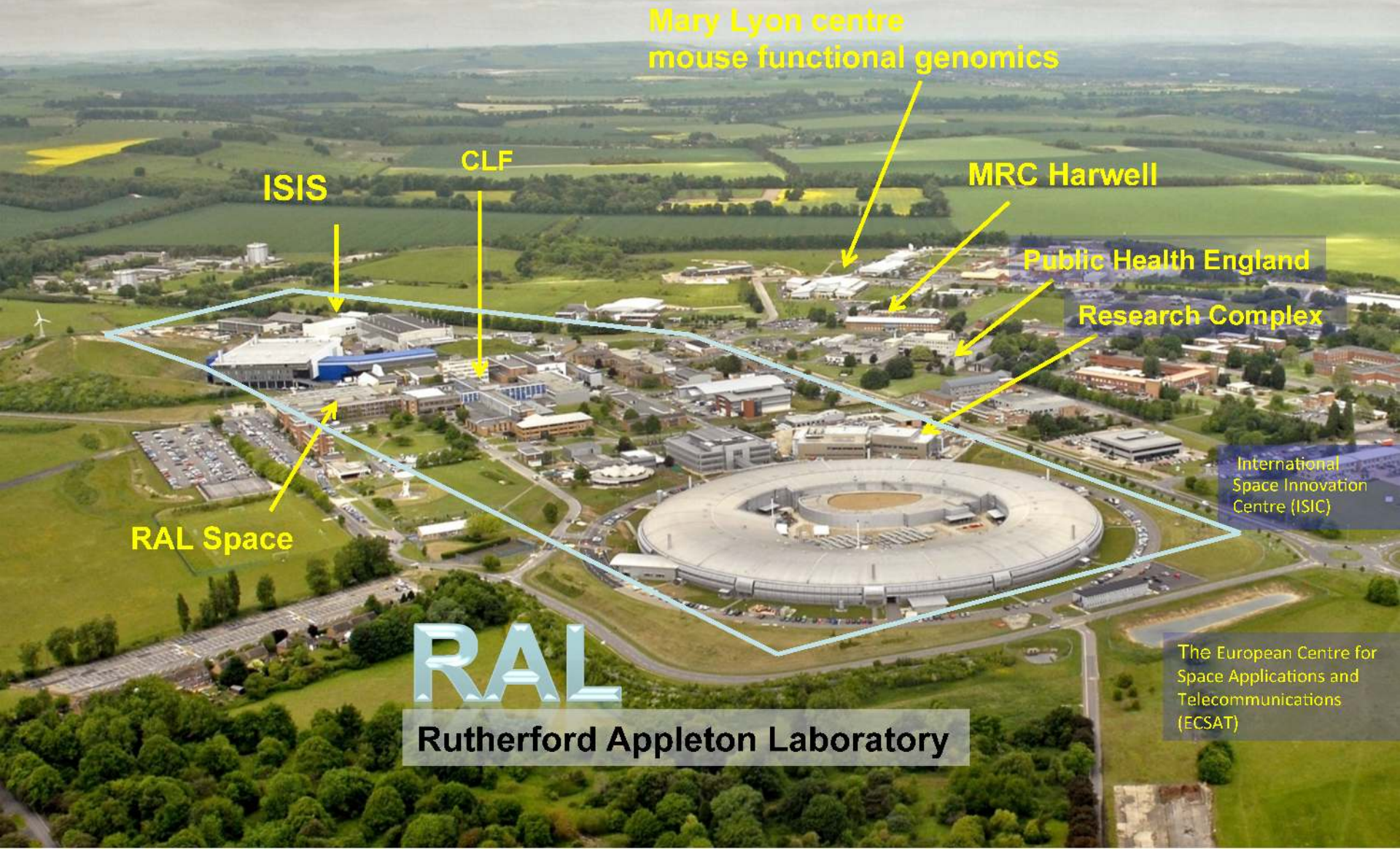


Snapshot of Structural Biology at Diamond



Martin Walsh

Harwell Campus



Mary Lyon centre
mouse functional genomics

ISIS

CLF

MRC Harwell

Public Health England

Research Complex

International
Space Innovation
Centre (ISIC)

The European Centre for
Space Applications and
Telecommunications
(ECSAT)

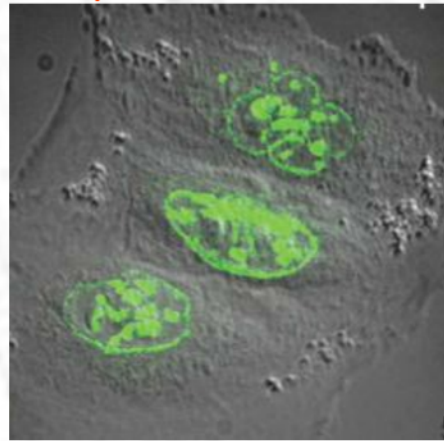
RAL Space

RAL

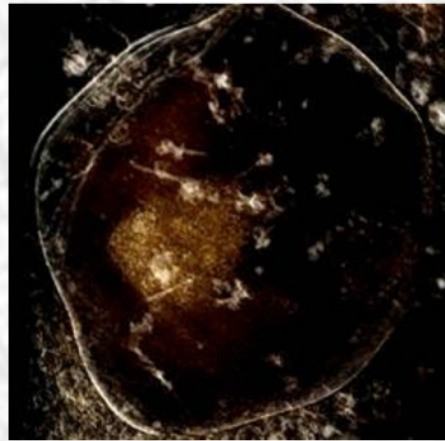
Rutherford Appleton Laboratory

One major player – integrated Structural biology

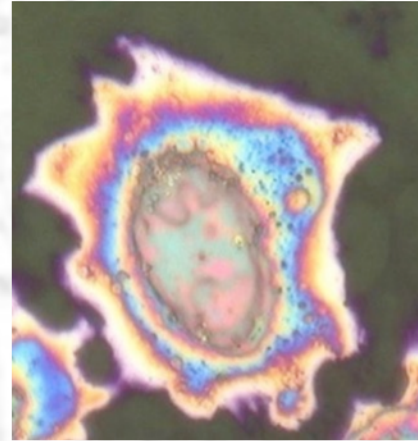
Increasing biological complexity and integrity



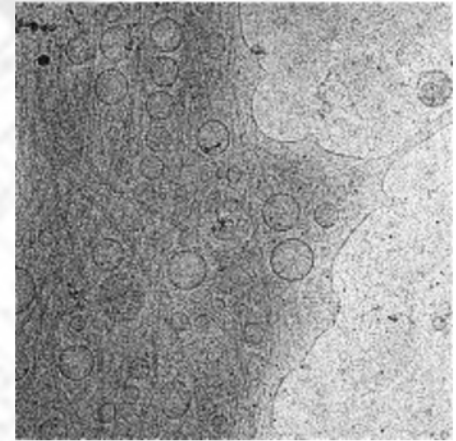
Fluorescence microscopy (B24/CLF)



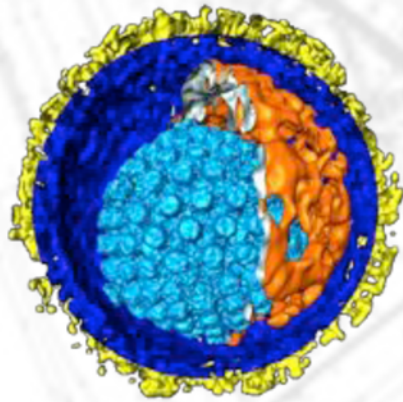
B24 X-ray microscopy



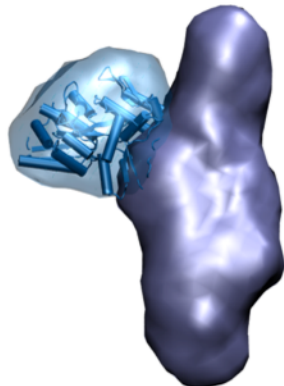
B22 - Infrared microspectroscopy



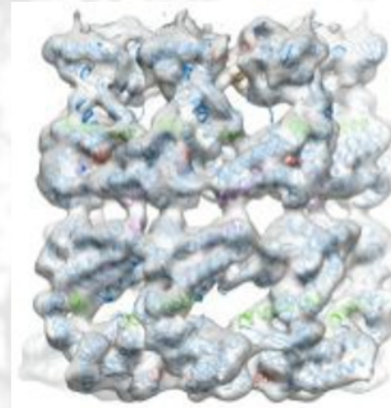
Cellular cryo-electron tomography



Cryo-electron tomography



B21 X-ray Solution Scattering



Single particle cryo EM



X-ray crystallography

Increasing resolution



The Diamond Project

*At its base electrons...
encompassing SR, EM and X-FEL*

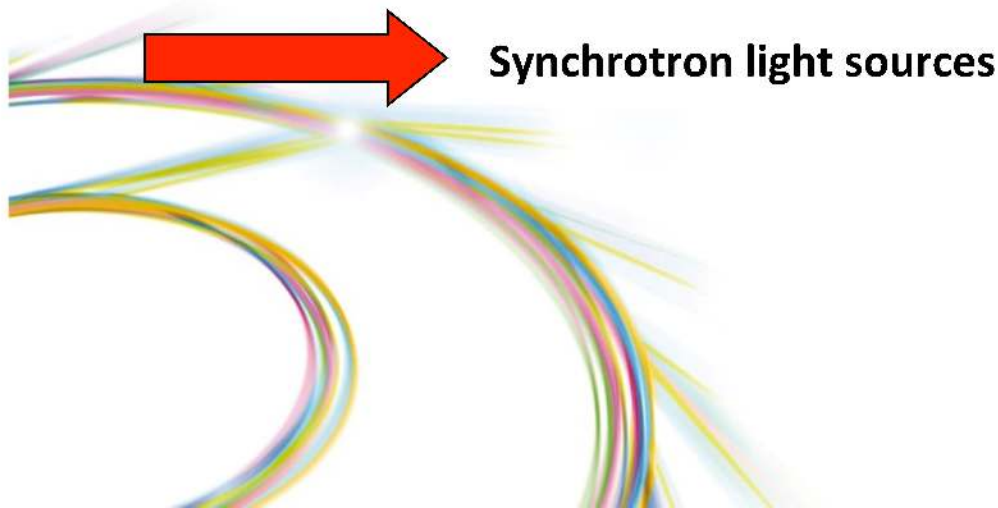
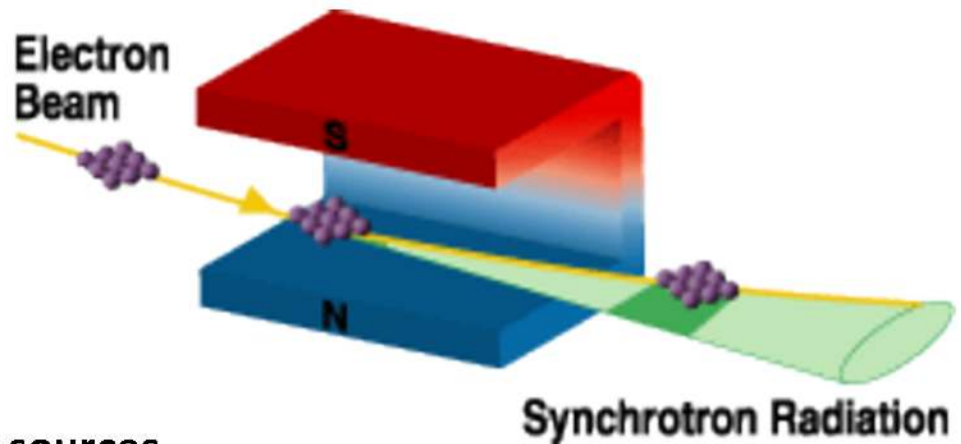
About the Diamond Project ...

- The largest single scientific investment in the UK for 45 years ~ £500 million on completion;
- A source of extreme intensity light;
 - Synchrotron light provides a brilliant source of light spanning from the Infrared to Hard X-rays!
- Funded by Our Shareholders – The UK Government through the STFC (86%) and the Wellcome Trust (14%)
- A **Scientific** USER Facility
 - First scientific users from 2007....
 - Project celebrated first 10 years in autumn of 2012!

What is Synchrotron Radiation?

Synchrotron light is the name given to the electromagnetic radiation emitted by charged particles when accelerated in an external magnetic field.

This could be from any source (e.g. astrophysical), but is generally thought of as coming from a man-made relativistic beam of electrons.



What is a synchrotron?

- A synchrotron is a particle accelerator (typically electrons) which provides a source of extreme intense light
- Can be compared to a series of 'super microscopes' or a gigantic x-ray machine;
- Diamond is the UK's new synchrotron light facility that provides intense light ranging from Infrared to hard X-rays



Origin of synchrotron radiation



1947

**First observation
of synchrotron
radiation at
General Electric
(USA).**

**Although first considered as a nuisance
by particle physicists, synchrotron
radiation has now been fully recognised
as having exceptional properties to
explore matter.**

EVOLUTION

1930

**First particle
accelerators**

1947

**First observation
of synchrotron
radiation**

**More and
more energetic
particles,**

**Bigger and
bigger
machines**

1980

**Construction
of the first
dedicated
machines**

**Particle
physics**

**Synchrotron
radiation**

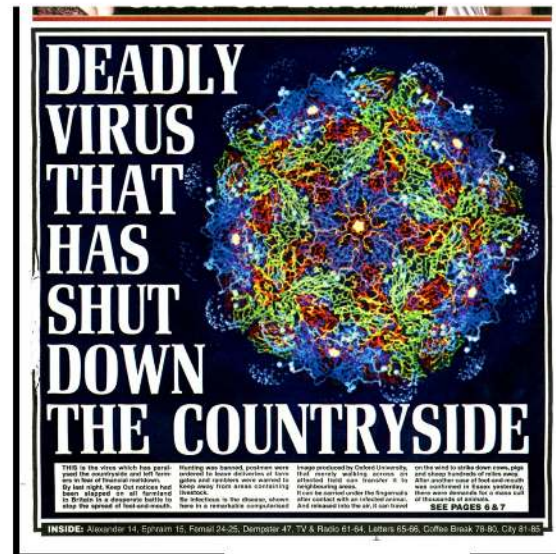
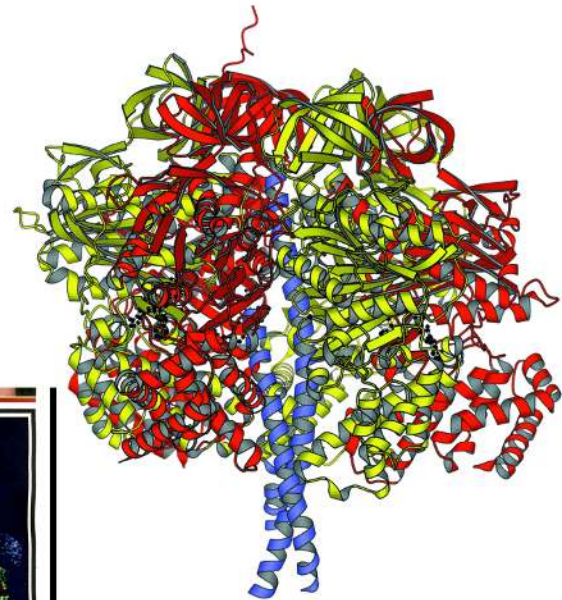


SRS Daresbury, UK

The first dedicated synchrotron facility!

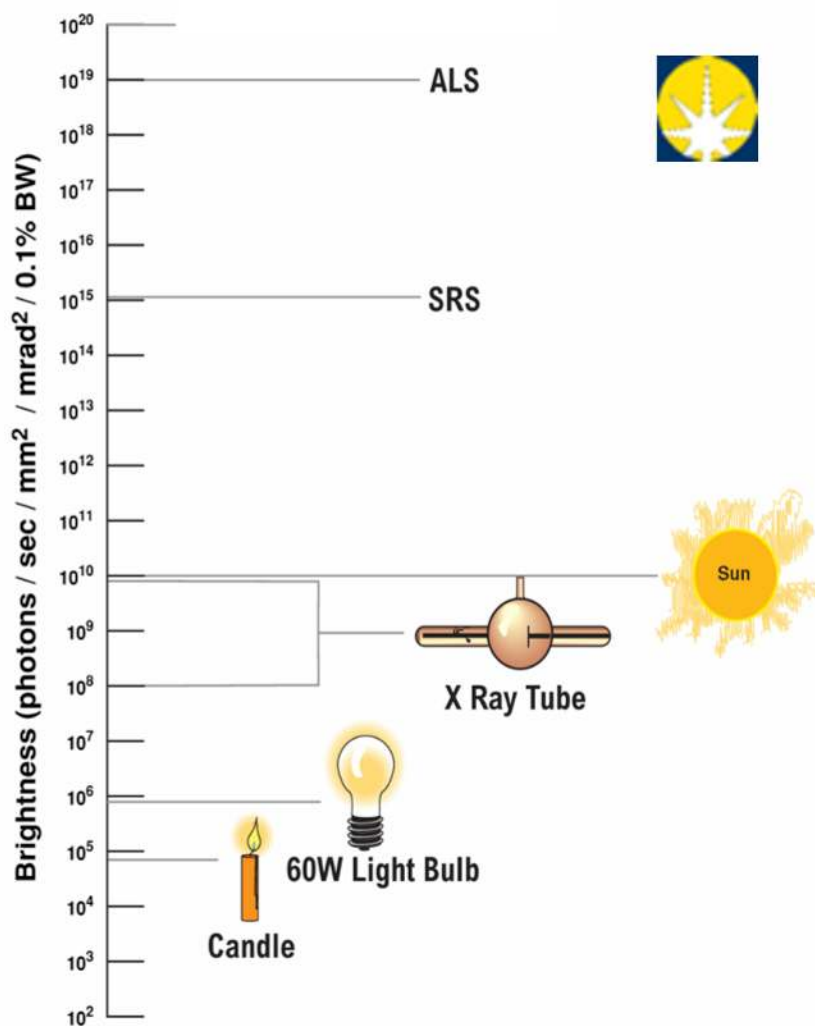
- First synchrotron source dedicated to producing radiation
- 2 GeV 200 mA Circumference; 96 m; emittance 100 nm rad
- Commissioned 1981; closed 4th August 2008
- 11000 users; 5000 research papers; 12000 protein structures
- F1 ATPase (J. Walker, A. Leslie et al 1994)
- FMDV (D. Stuart, R. Acharya et al 1989)

F1-ATPase
A 'molecular machine' for the production of ATP



Feb 2001

In this business, it's all about brightness !

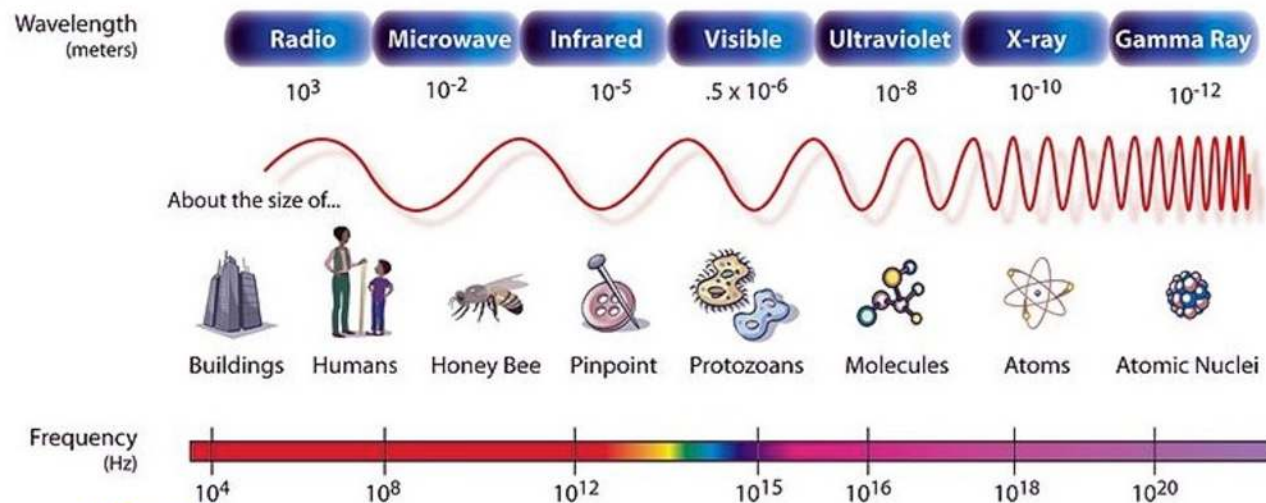


- X-rays from Diamond are 100 billion times brighter (10^{11}) than from an X-ray tube
- Or 10 billion times brighter (10^{10}) than the Sun
- $>10^5$ times brighter than the previous UK Synchrotron, the SRS !

Synchrotron Radiation - Properties

Synchrotron light is useful because it:

- **Covers a wide spectrum (infra-red through to x-ray)**
- **Small spot size**
- **Pulsed time structure**
- **Tuneable**
- **High stability**
- **Intense**
- **Polarised**



Foundations – Sept 2003



1500 piles anchored into the Chilton chalk at a depth of 12-15 meter.
600 mm diameter and about 3 m apart

Not the first in the area....?



November 2004

..on time and on budget



Diamond vital Statistics



- Construction completed 2006
- Machine commissioned from June 2006
- First Users Jan 2007

1.75+ million man-hours
2,100 tons of steel
35,000 m³ of concrete
33,000 m² of roofing
(football pitch is 7,150m²)

Electron Beam Energy

3 GeV

Circumference

561.6 m

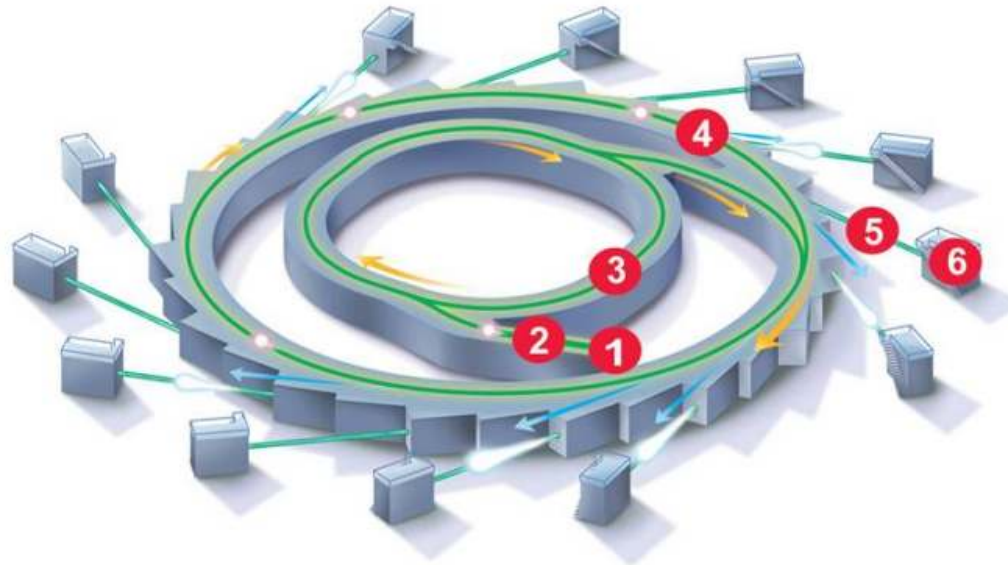
Diameter of outer wall

235 m

Beam current

300 (500) mA

Synchrotron – main components



1 Electron Gun: produces electrons

2 Linear Accelerator (LinAc): accelerates electrons to near the speed of light

3 Booster Ring: further increases the energy of the electrons

4 Storage Ring: maintains the electron energy and produces radiation

5 Beamline: set up of radiation for experiments

6 Experimental Hutch: User area

Types of Beamlines

Synchrotron light is the name given to the electromagnetic radiation emitted by charged particles when accelerated in an external magnetic field.

There are three main types of synchrotron radiation source at Diamond:

Bending magnet



Wiggler

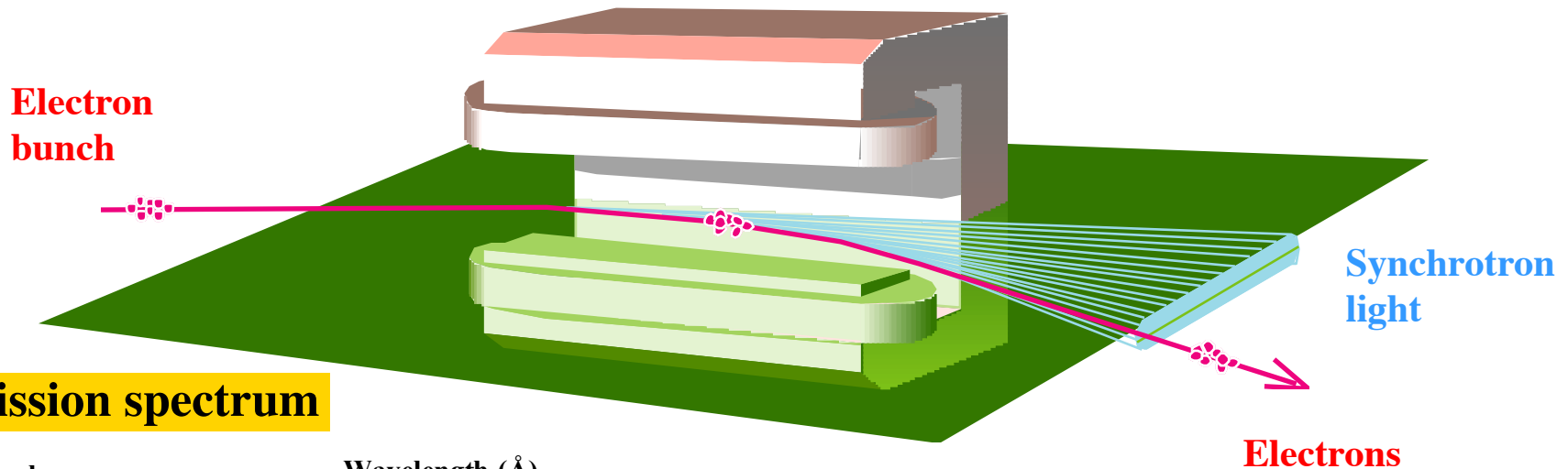


Undulator

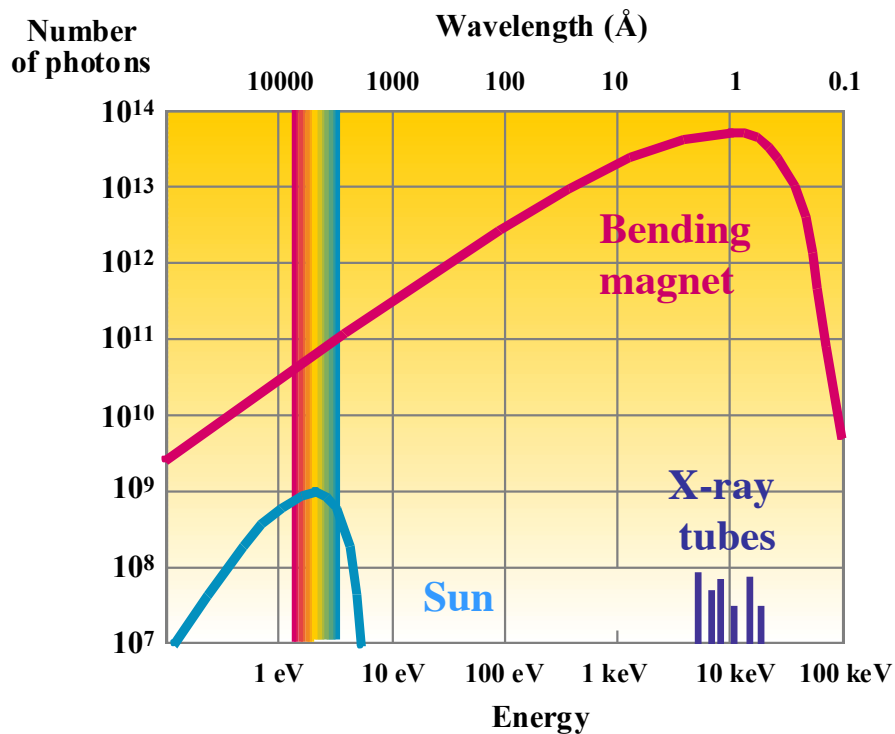


Each of these produces light with different characteristics

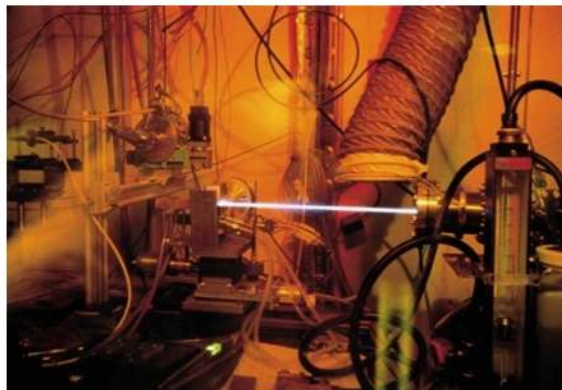
Bending Magnet



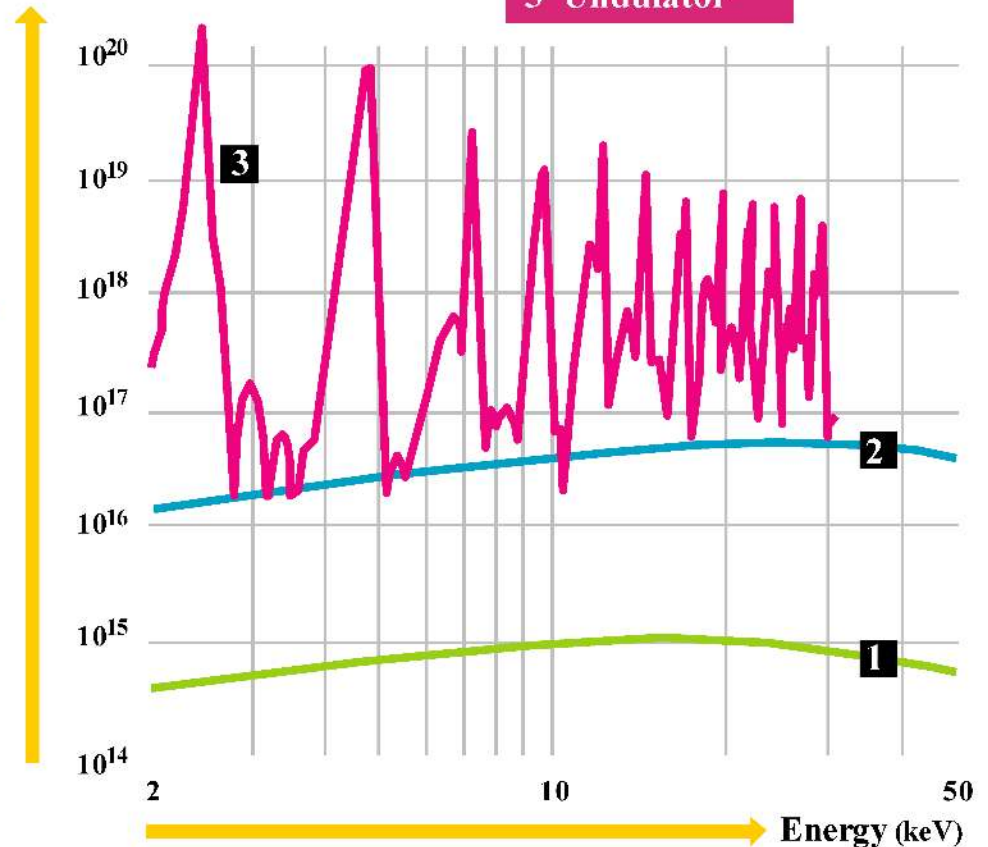
Emission spectrum



Insertion devices



Brilliance
(photons/s/mm²/mrad²/0.1%BW)



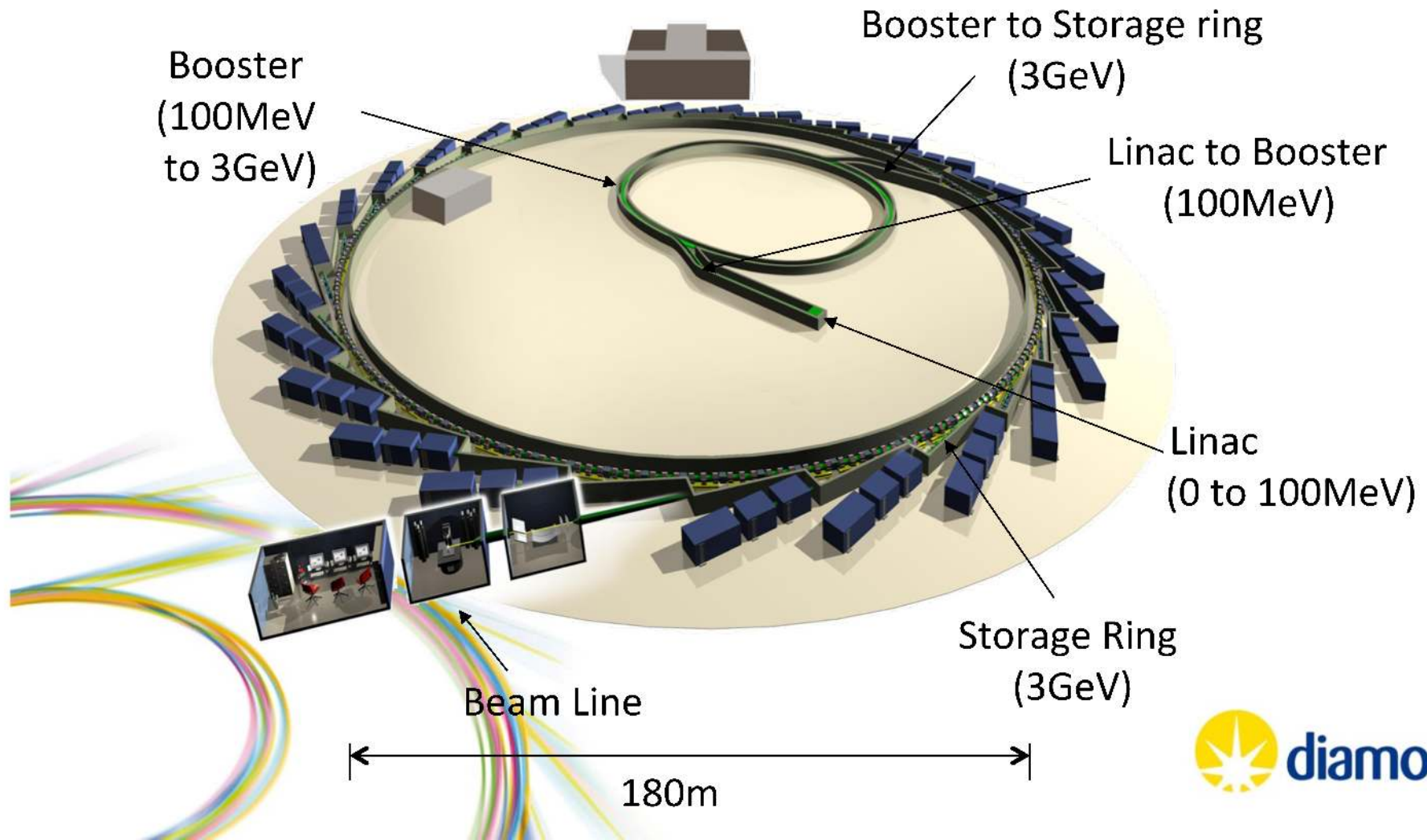
Bending magnet

2 Wiggler

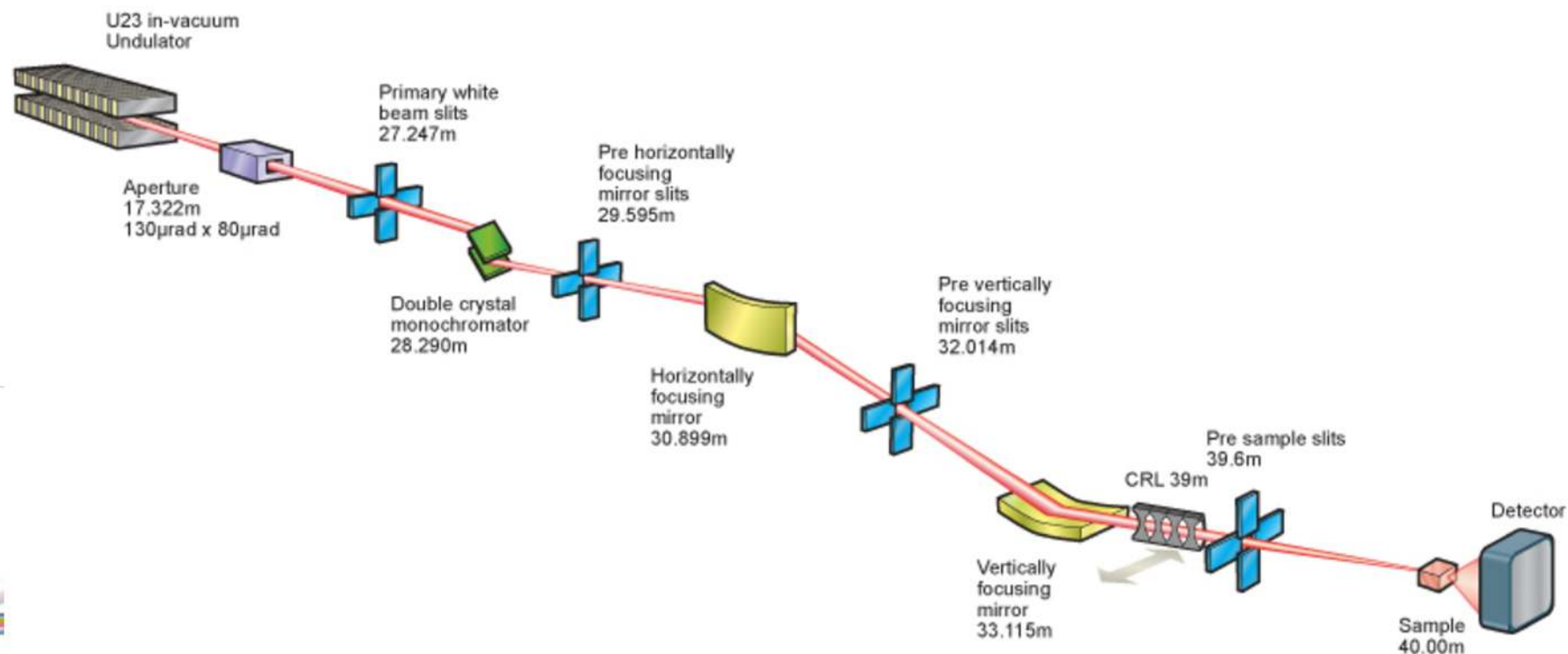
3 Undulator

Beamlines

- Experimental stations



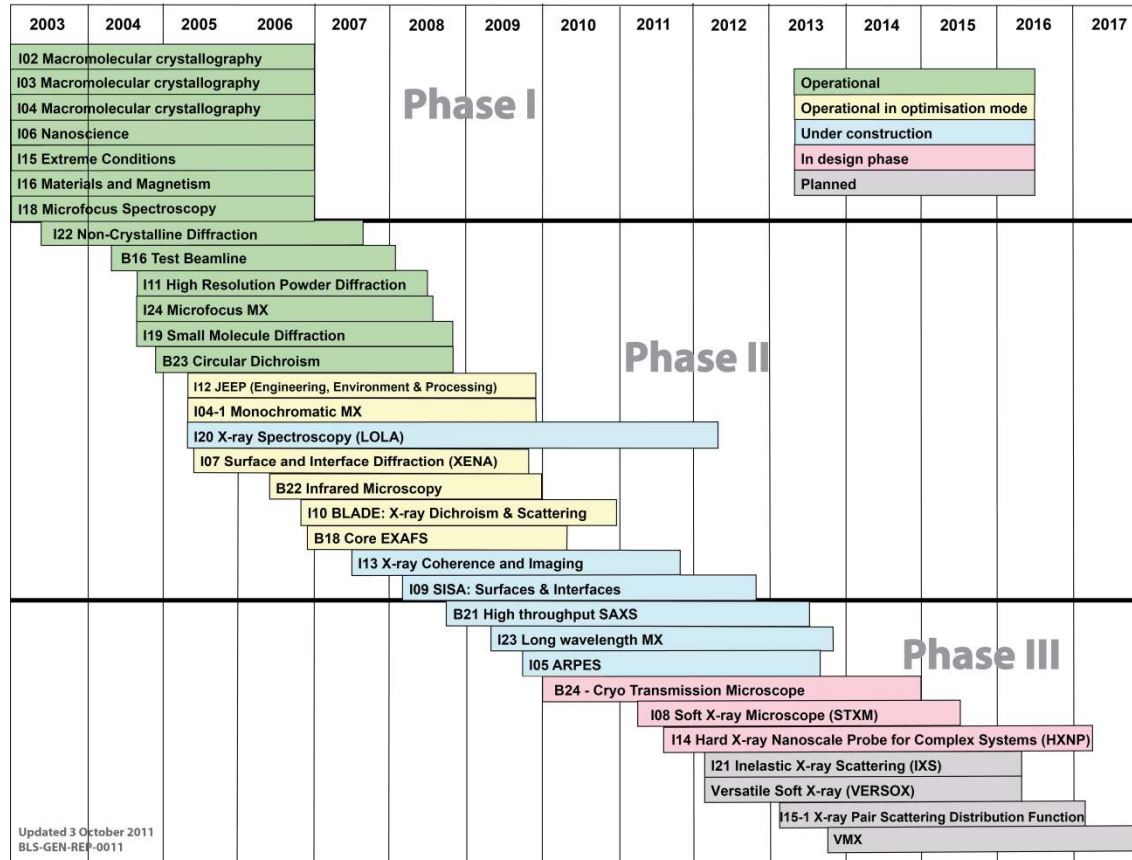
MX Beamline Optics Layout



Some Numbers for 2014-15

- Total number of User experiments: 642 proposals
- 7,964 experimental shifts
- Number of User Visits: ~4988 (2,708 remote; total 7,577)
- Total number of Unique Users: 2,947
- Total number of Unique PhD Student Users: 857
- Total Journal Papers Published by Staff and Users:
>3,883 (677 in 2014 alone)

Diamond Build Phase



Construction phases:

Phase I (completed 2007)

- 7 beamlines (£235M)

Phase 2 (approved 2004)

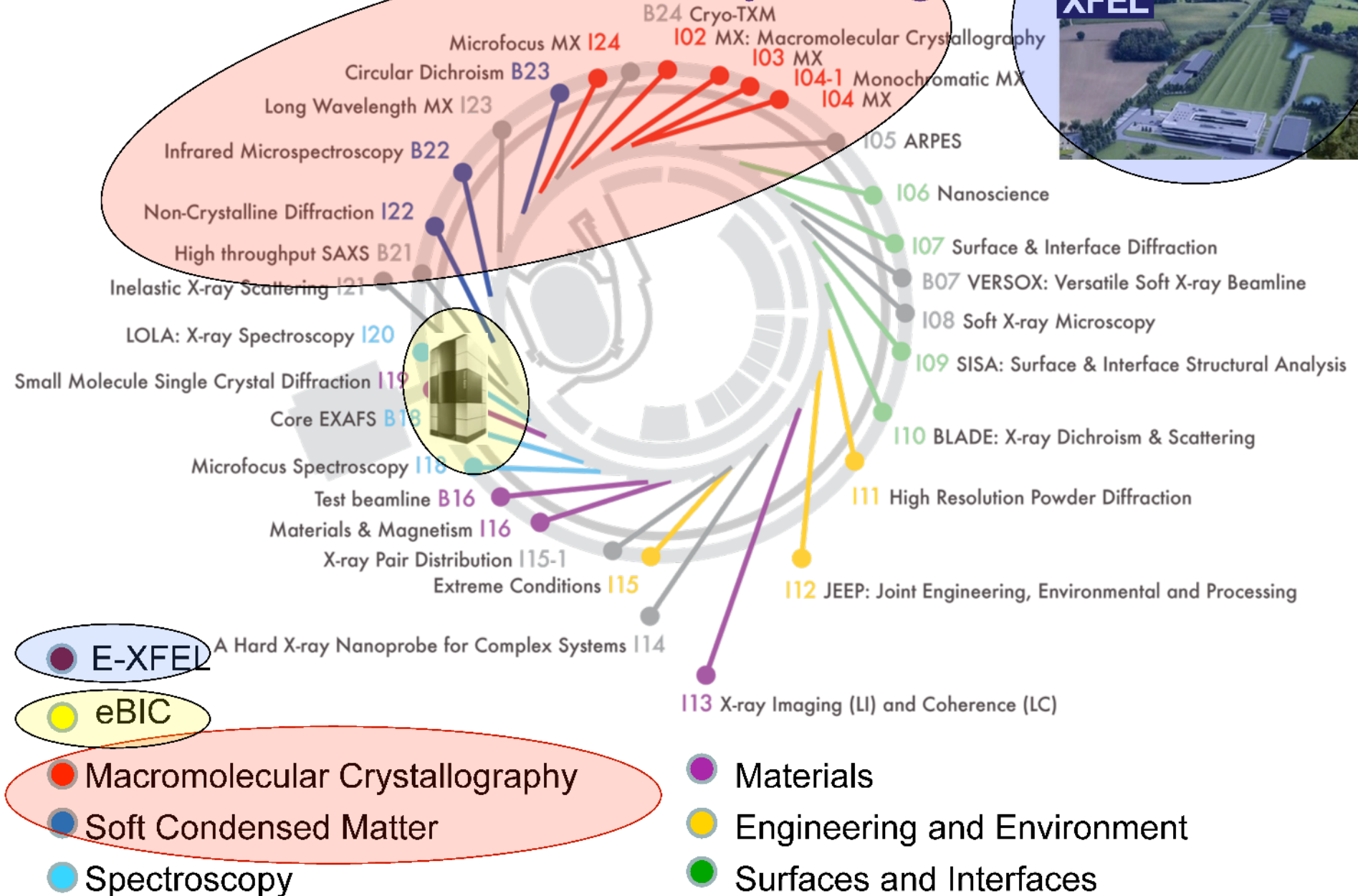
- 15 additional beamlines
- 11 in operation (£120M)

Phase 3 (approved 2010)

- final 10 beamlines (£100M)

33 Beamlines on completion of phase III ...future beamlines & Diamond Phase IV – ‘diamond II ...~2022...’

Beamlines by Village

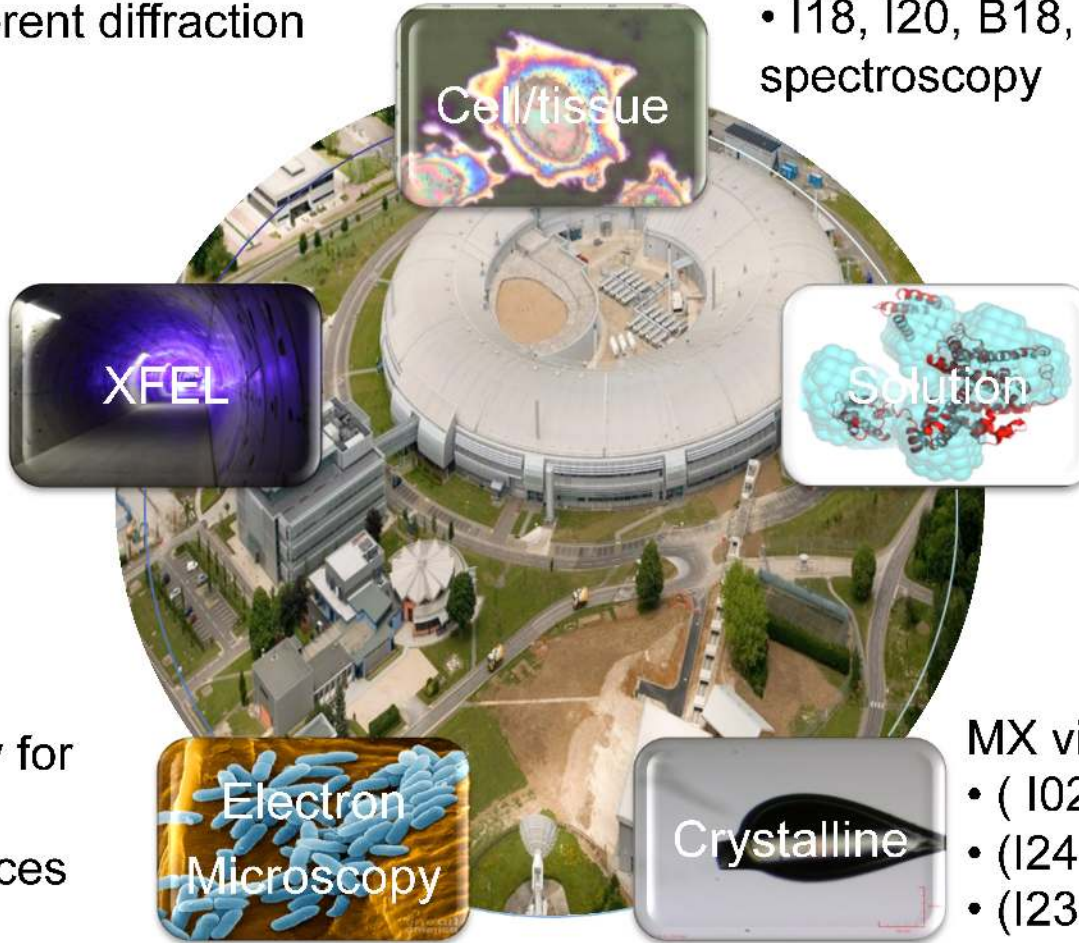


Life Science & DLS

- I08 X-ray STXM
- I13 X-ray tomography & coherent diffraction

- B22 Infrared
- B24 Cryo X-ray microscopy
- I18, I20, B18, I14 X-ray spectroscopy

UK Hub for
XFEL sample
and software
developments



- I22/B21 SAXS
- B23 CD
- Spectroscopy

National facility for
EM in life &
Physical Sciences

MX village

- (I02, I03, I04)
- (I24, I04-1)
- (I23, VMX)

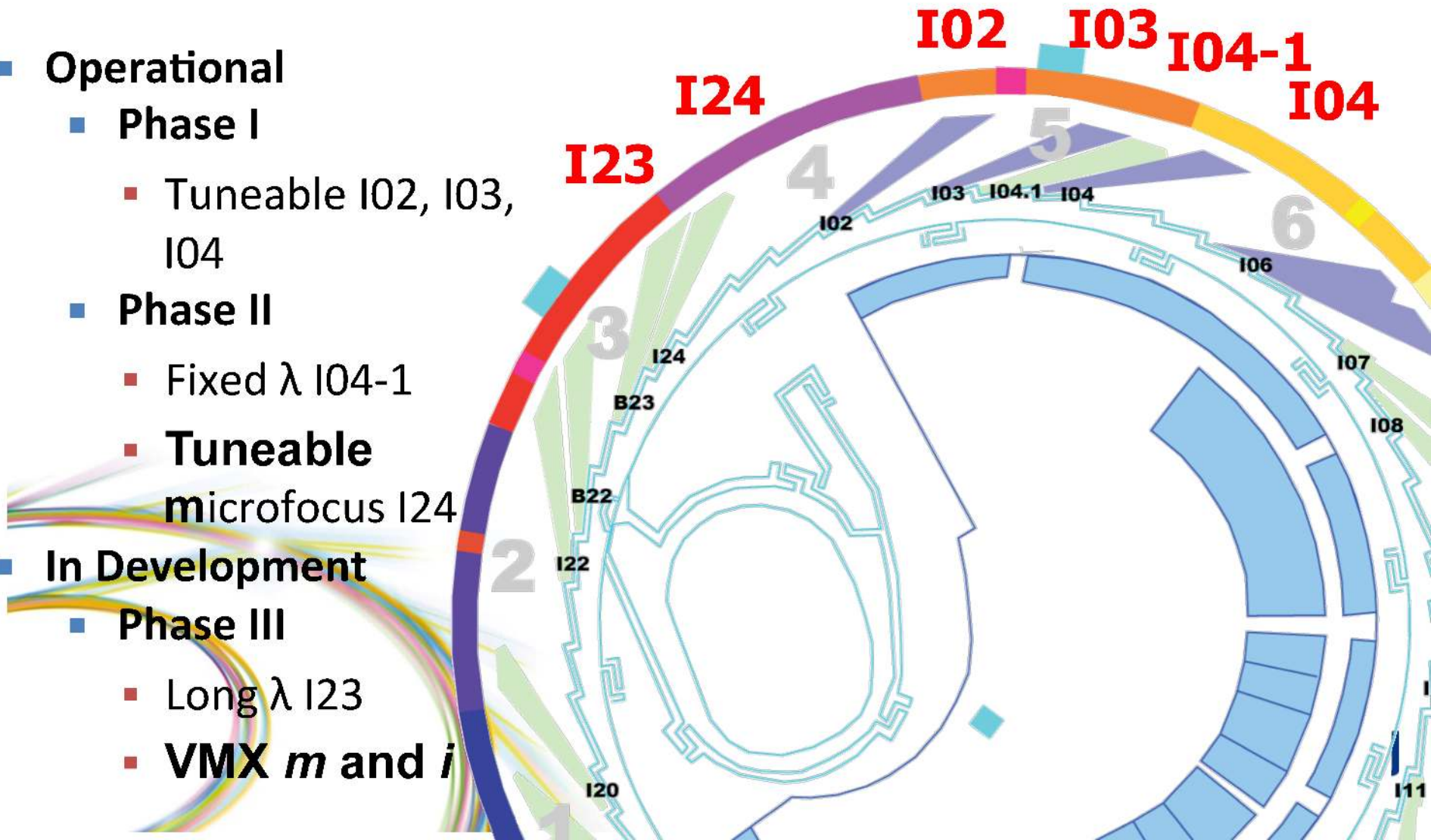


MX@DLS

I02 & VMXi Thomas Sorensen I03 Katherine McAuley
I04 Dave Hall I04-1 Frank von Delft I24 Robin Owen
I23 Armin Wagner & VMXm Gwyndaf Evans

MX Village

- Operational
 - Phase I
 - Tuneable I02, I03, I04
 - Phase II
 - Fixed λ I04-1
 - Tuneable microfocus I24
- In Development
 - Phase III
 - Long λ I23
 - VMX *m* and *i*



New era for MX - VMX Project & I23

- Versatile Macromolecular Crystallography (VMX) is....

Two next generation MX beamlines that will provide:

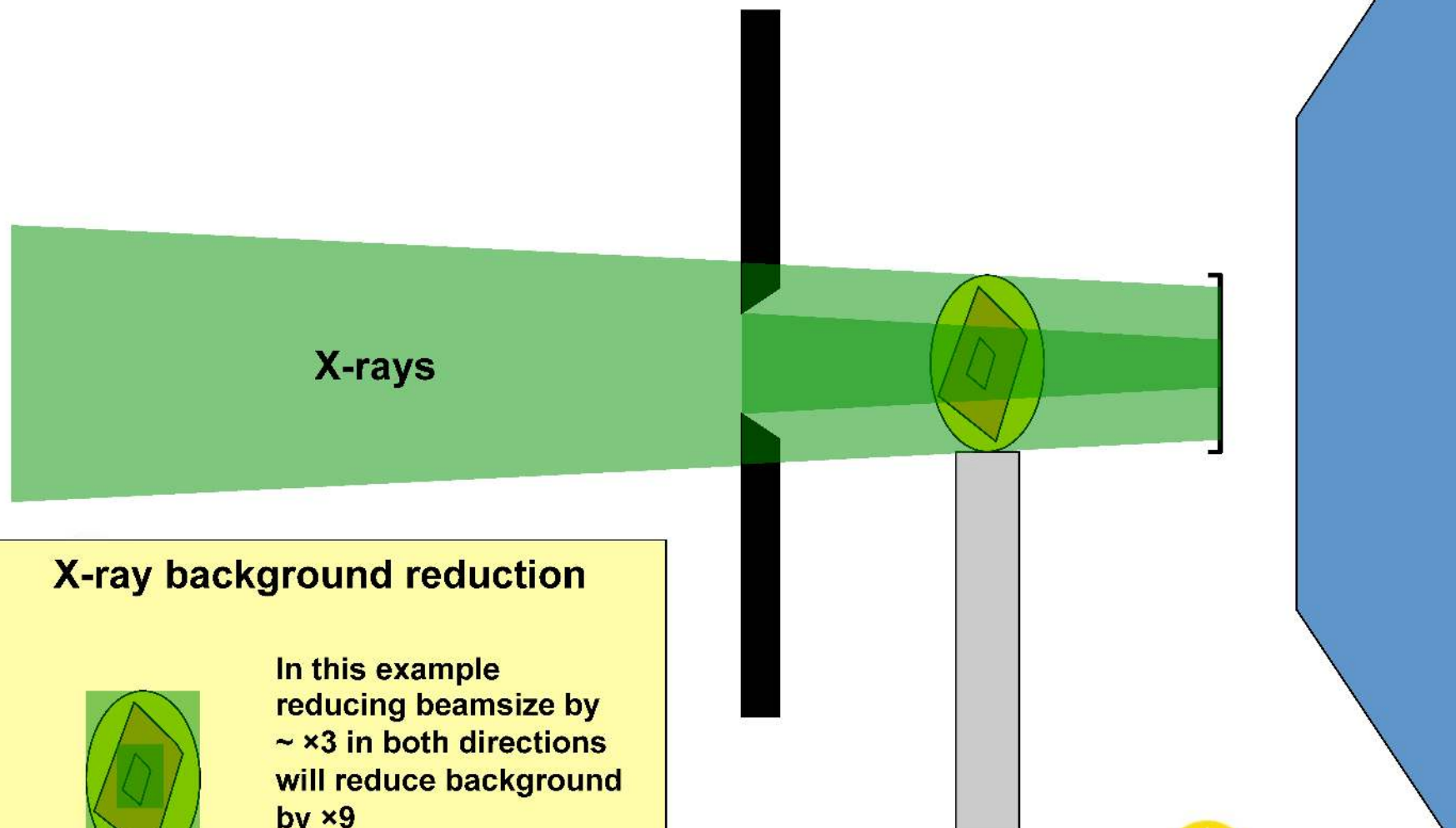
- A sub micron variable focus beam in the 0.5 – 5 micron range
- A dedicated *in-situ* data collection facility

- VMX will deliver....

- Capabilities to solve the toughest crystallographic problems that are beyond the reach of existing experiments
- Revolutionise the way the community collect data on many samples through use of *in-situ* diffraction

Matching the beam to the crystal is critical

This is the key premise of microfocus MX



X-ray background reduction



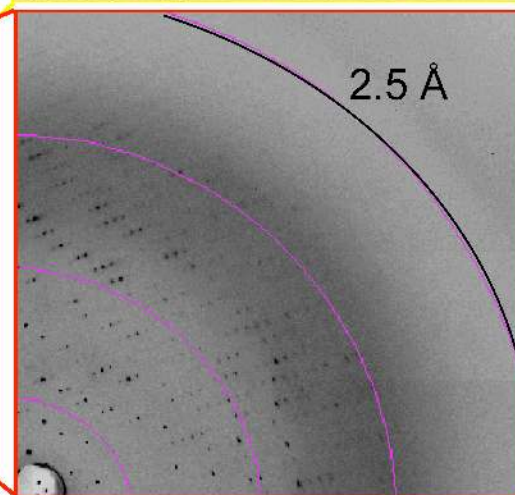
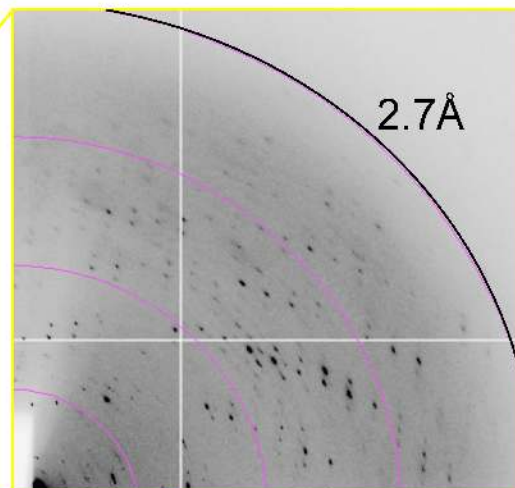
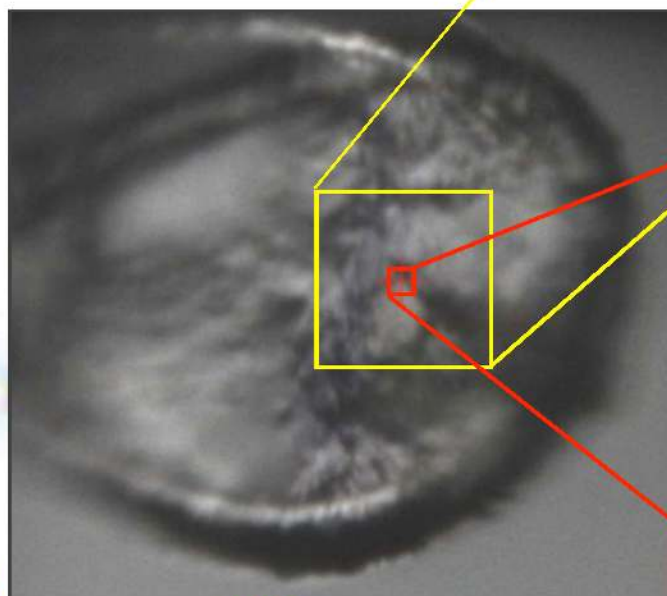
In this example
reducing beamsize by
 $\sim \times 3$ in both directions
will reduce background
by $\times 9$

Example: crystal of Adenosine A2a receptor (GPCR) complexed with antibody Fab fragment.

	Full beam flux (ph/s)	Exposure time (s)	Transmission (%)	Beam size (μm^2)
I03	10^{12}	5	50	70×70
I24	10^{12}	1	30	10×10

Normal Beam
($70\mu\text{m} \times 70\mu\text{m}$)

Microbeam
($10\mu\text{m} \times 10\mu\text{m}$)



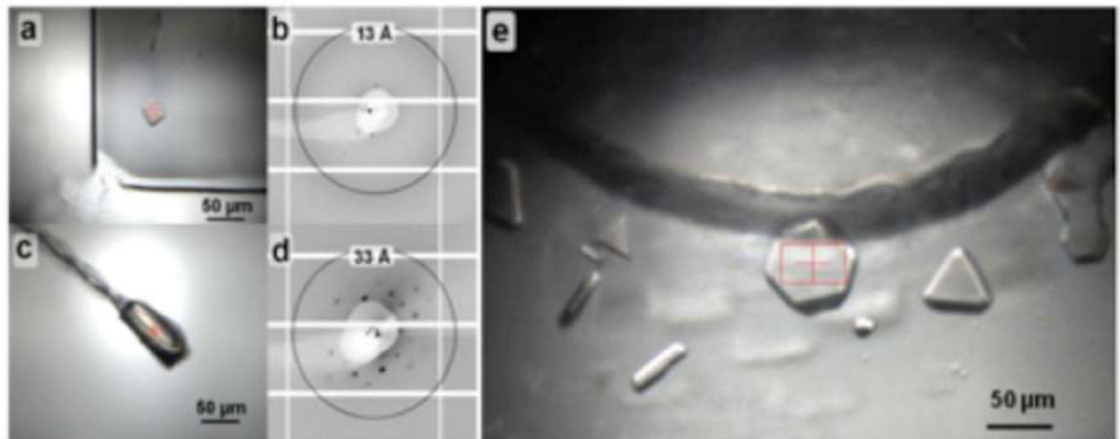
- Science-wise
 - enable the use of micro/nanocrystals in MX thereby avoiding the need for the optimization of crystal size upwards where this is problematic
 - permit data collection from the smallest number of available samples – maximize data:sample ratio (while other developments currently seem to be minimizing it)
- Technology-wise
 - take advantage of recent advances in multi-crystal data analysis
 - develop and leverage technology at the interface of synchrotron, FELs crystallography and cryoEM/microED
 - Explore use of polychromatic radiation for increasing flux and better exploiting serial snapshot crystallography

VMXm Specifications

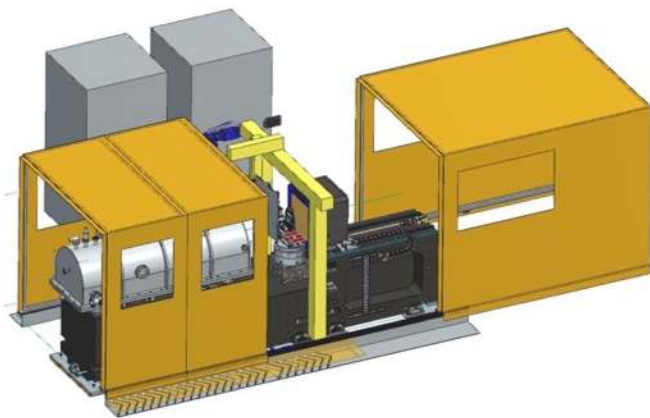
- 7 – 25 keV energy range (0.62 – 1.77 Å)
- Beam size
 - 0.3 – 5 μm (v)
 - 0.5 – 4 μm (h)
- Flux
 - $\sim 10^{13}$ ph/s in $0.3 \times 4 \mu\text{m}$
 - $> 10^{11}$ ph/s $0.3 \times 0.5 \mu\text{m}$
- initially monochromatic beam
- polychromatic beam as an upgrade path

In-situ data collection

- All samples grown in plates/microfluidic devices
- Sample mounting
 - overhead time/ lack of experimental control
 - Mechanical & osmotic damage
- Not only does handling / cryo-protection frequently lead to a dramatic increase in mosaic spread but it can seriously compromise diffraction - *in situ* provides a gold standard.



VMXi



Compact hutch design

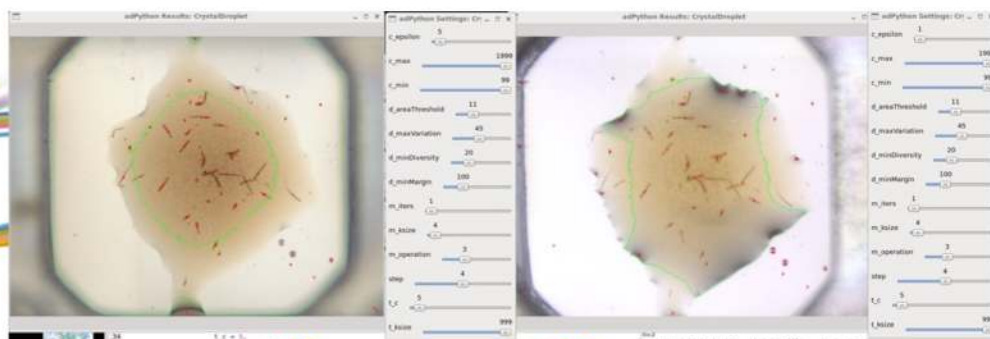


Image-based alignment



FORMULATRIX



Automatic sample handling



123 – Long wavelength Macromolecular Crystallography Beamline

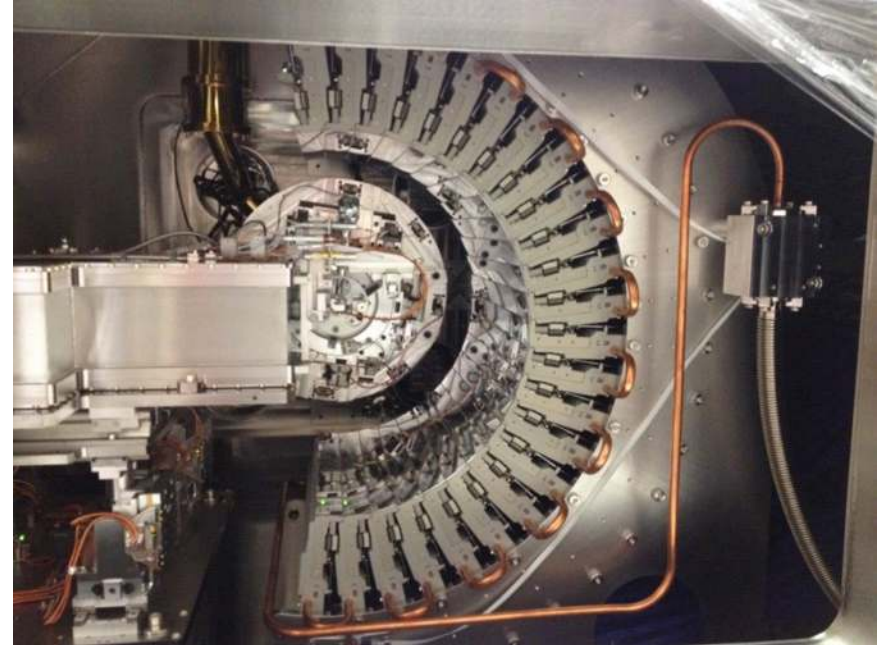
123 will be the first MX beamline optimized for the long-wavelength region (1.5 – 4 Å). It will provide a unique tool to fully exploit the potential of experimental phasing from native protein and DNA/RNA crystals.

Main challenges:

- **In-vacuum MX to reduce air absorption and scattering.**
- **Large area detector to cover increased Bragg angles at long wavelengths.**
- **Multi-axis goniometer to re-orient crystals for complete diffraction data.**

Current status

- Insertion device, monochromator and focussing optics installed.
- Pilatus 12M detector installed.
- Day-1 single-axis goniometer installed.
- Sample changer to transfer frozen crystals into vacuum chamber in commissioning.
- Strategy software under development in collaboration with Global Phasing.
- First Users before end 2015.



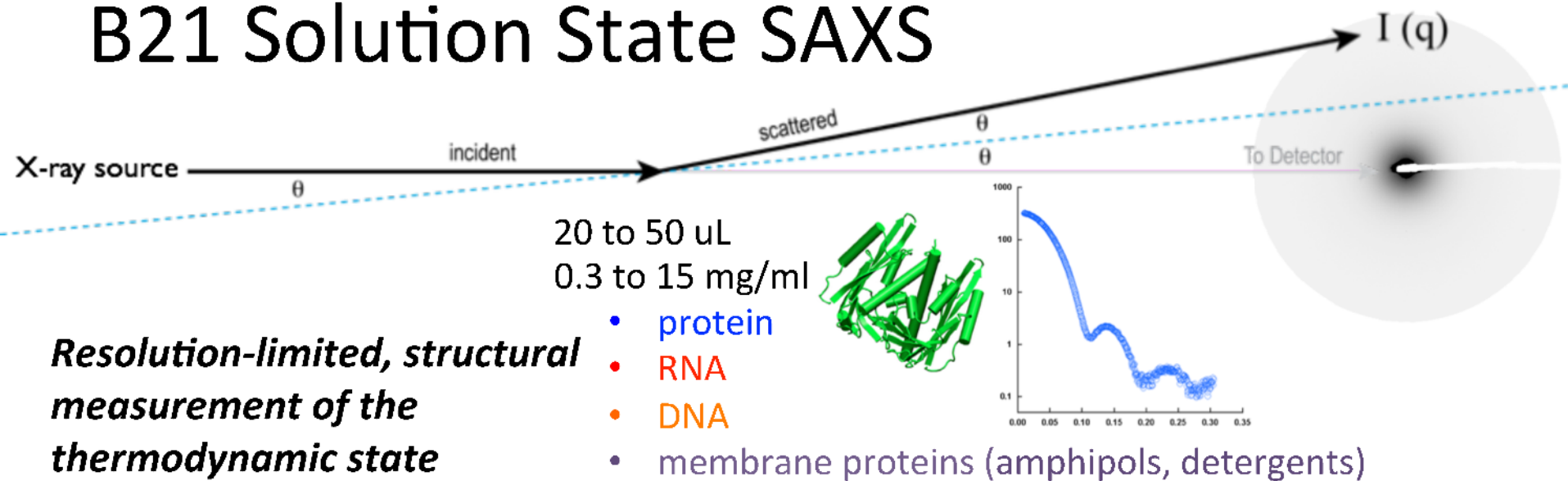


X-ray Solution Scattering -B21

Rob Rambo (Principal Scientist: robert.rambo@diamond.ac.uk)

Katsuaki Inoue, Nathan Cowieson, Nikul Khunti & Mark Tully

B21 Solution State SAXS



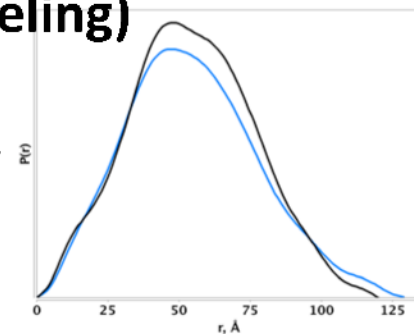
Resolution-limited, structural measurement of the thermodynamic state

Why do SAXS?

- Validate structural hypothesis
 - does MX structure represent solution state?
- Determine architecture of complexes (multi-phase modeling)
- Screen ligand binding (8 to 96-well strips)

Detecting structural change in a membrane protein by SAXS via the $P(r)$ -distribution

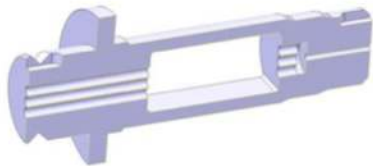
- Observe nano-crystal formation
- Observe filament formation (SAXS covers a large size and mass range)
- Assess flexibility of the particle



B21 SAXS

Main Experimental Configuration: Batch mode SAXS

1. bioSAXS robot for 96-well plates
2. SEC-coupled HPLC SAXS
 - Flow (perform SAXS during SEC run, 30 min)
 - Static capture (isolate 17 μ L fraction during SEC) for extended exposures (15 min)



Offer specialize sample cells, CAD design and print
(3 capillary holder)

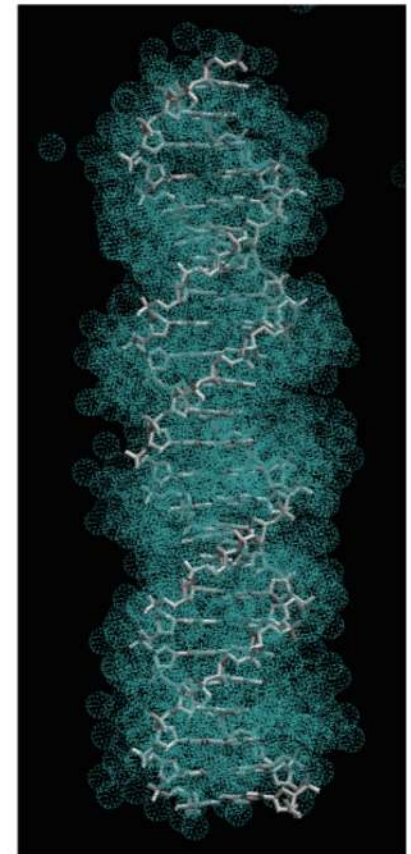
Access:

1. Peer-review (onsite)
2. Rapid access (onsite)
3. Mail-in services (rapid access)

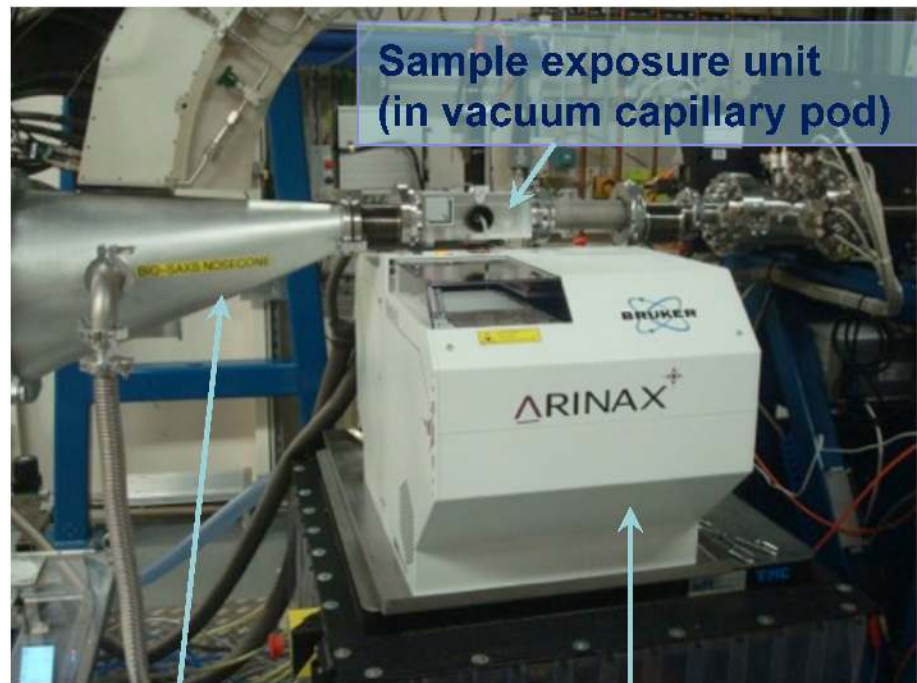
*B21 has an exceptionally low instrumentation background:
40 kDa protein at 0.3 mg/ml and 200 mAmps required 12 minute
exposure using our specialized SAXS cell*

IKETAMA (modeling algorithm)

- Developed our own density modeling algorithm
- Exploits Information Theory and targets $P(r)$ -distribution
- Modeling scales with resolution!



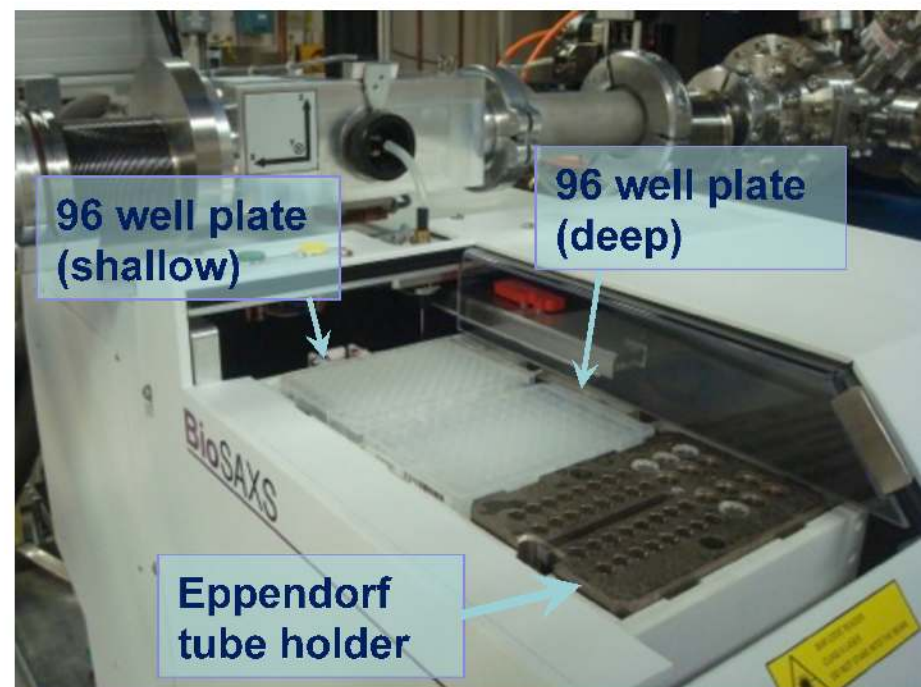
BIOSAXS automation – EMBL robot



Sample exposure unit
(in vacuum capillary pod)

SAXS flight tube

Sample storage unit
(temperature controlled)

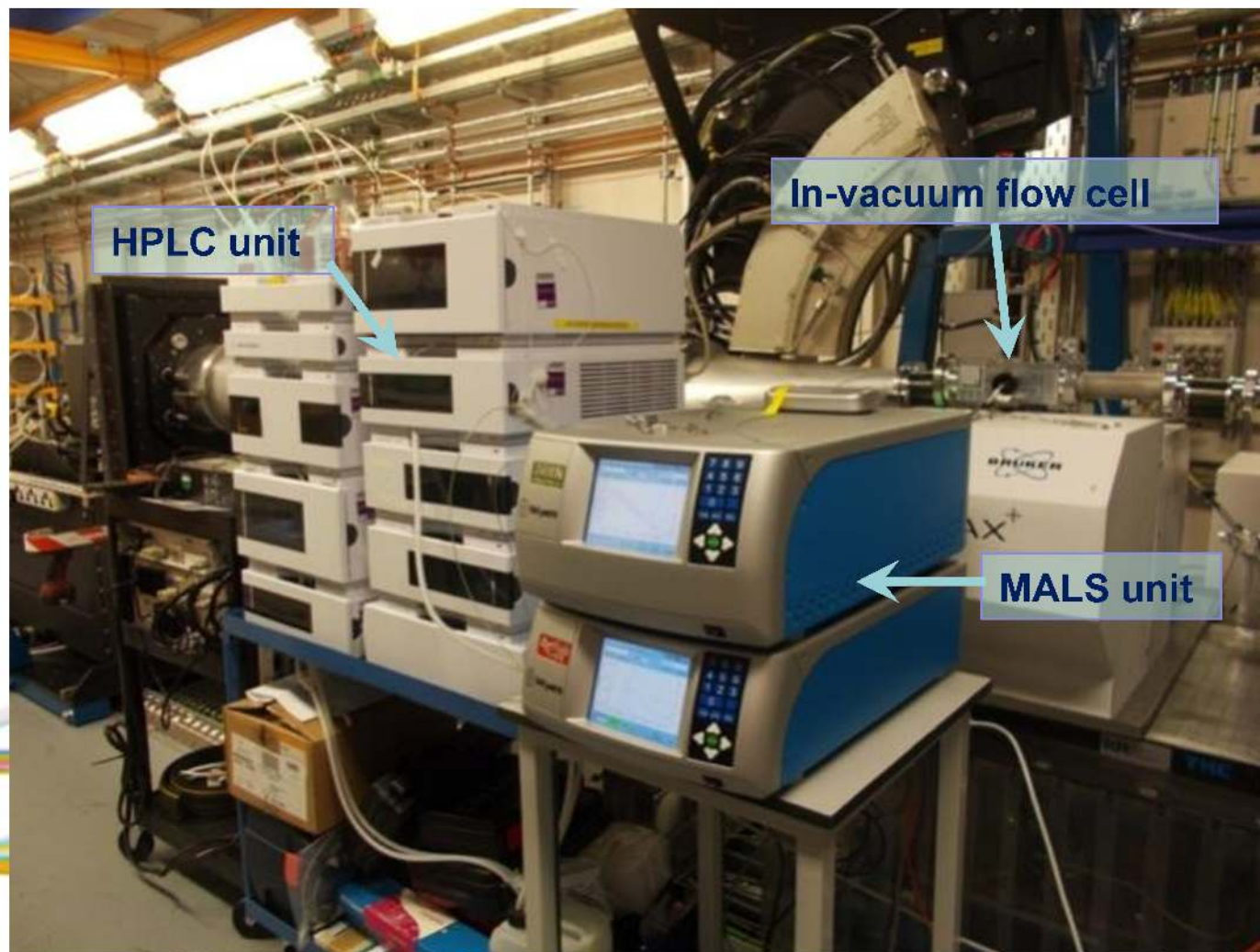


96 well plate
(shallow)

96 well plate
(deep)

Eppendorf
tube holder

In-line HPLC/MALS system



ab initio Modeling

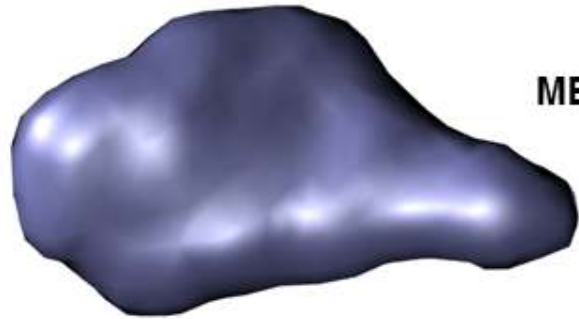
At low resolution, $q_{max} < 0.18 \text{ \AA}$, model the macromolecule as a collection of **Dummy Atoms** (DAMMIN/F and MONSA developed by Svergun's group EMBL)

SMARCL1 protein with dsDNA (25 bp+10nt ss)

in collaboration with A. Mason, B. Eichman and D. Cortez (Vanderbilt U.)

DAMMIN/F (volume constrained)

MEASUREMENT TO INITIAL MODEL ~ 30 MINUTES

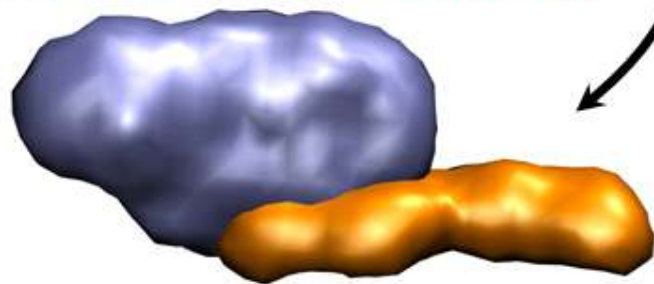


Measure Scattering of component(s) and complex

Two-phase system:

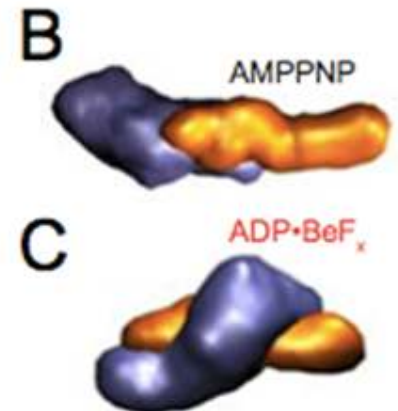
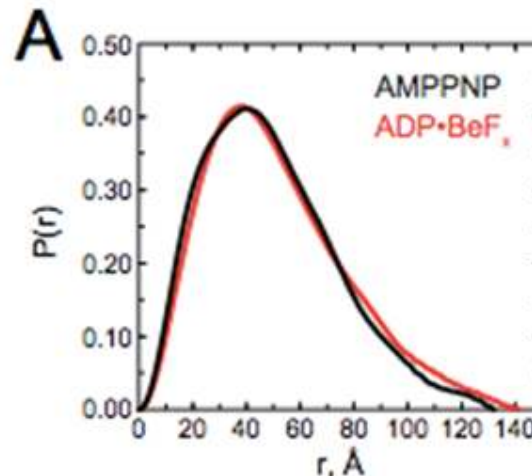
1. DNA (orange)
2. protein (purple)

MONSA (multi-phase)



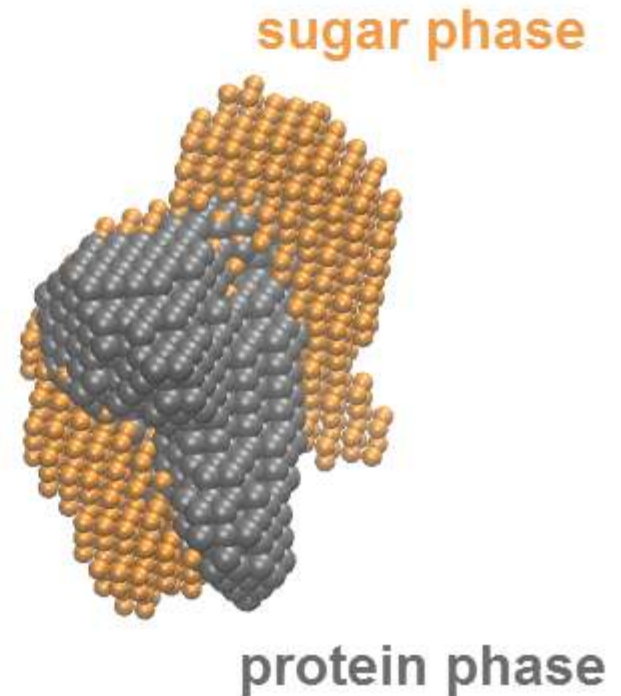
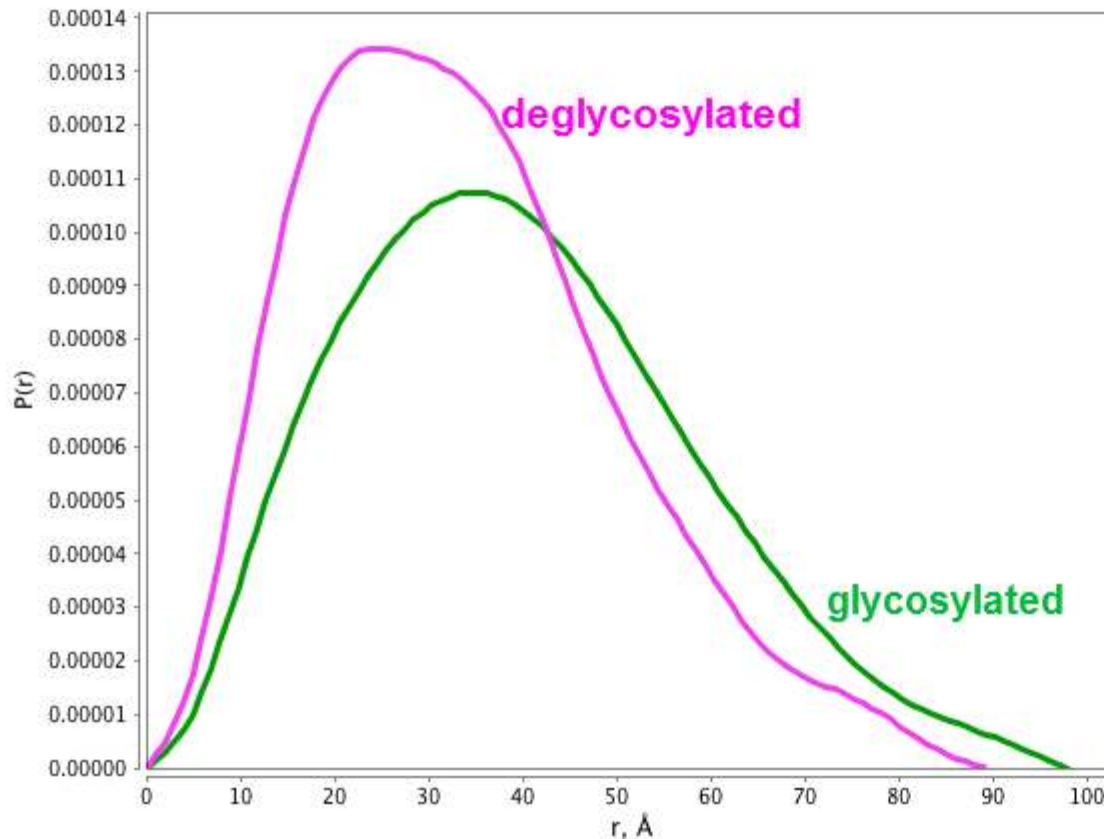
Good for:

- establishing overall architecture
- following conformational changes

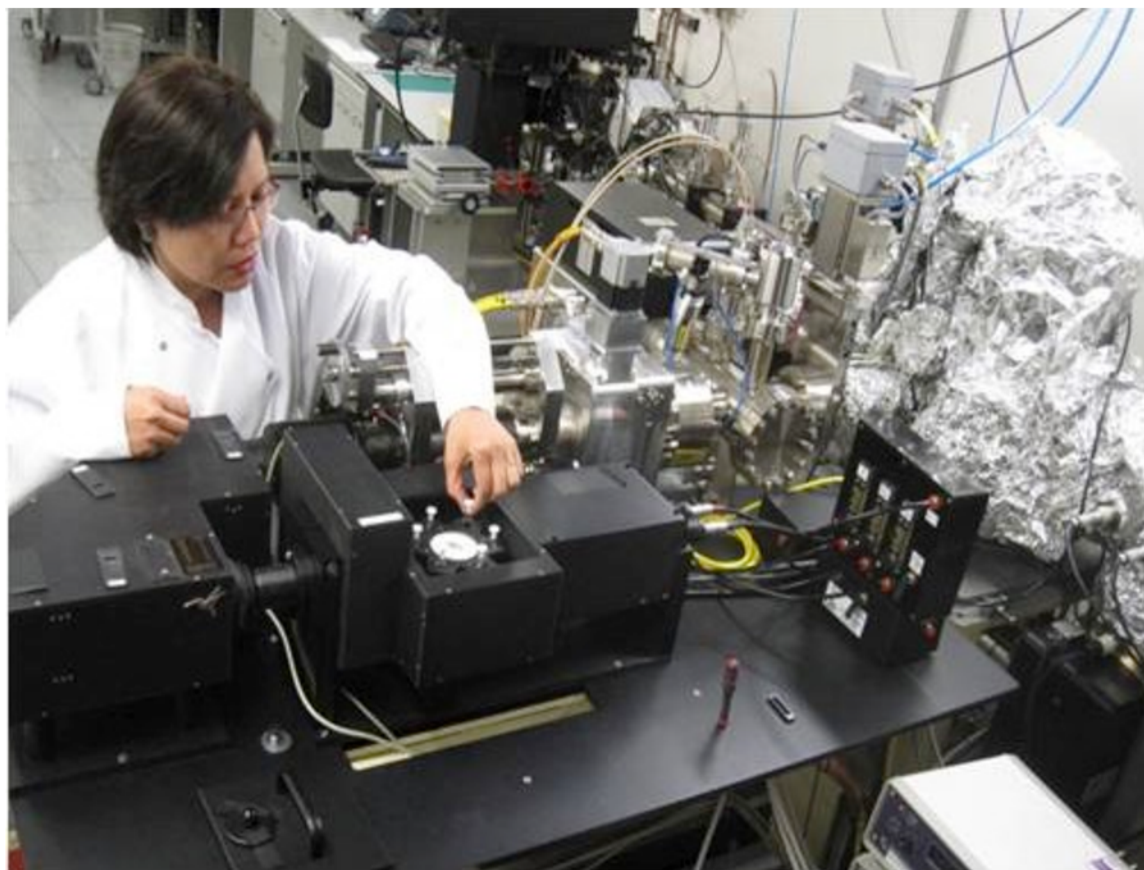


SAXS and Glycosylations

P(r)-distribution



- Everything scatters in SAXS, thus amorphous modifications like glycosylations are full observed
- Using SAXS of +/- glycosylation, we can model the distribution of modifications

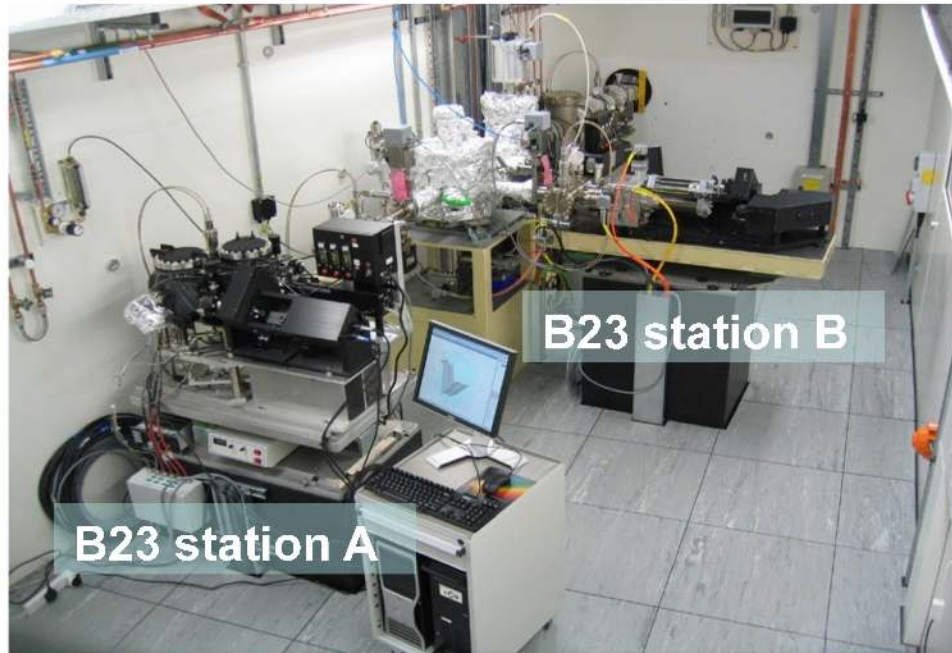


B23 Circular Dichroism

Giuliano Siligardi @diamond.ac.uk

Rohanah Hussain, Tamas Javorfi

B23 - applications



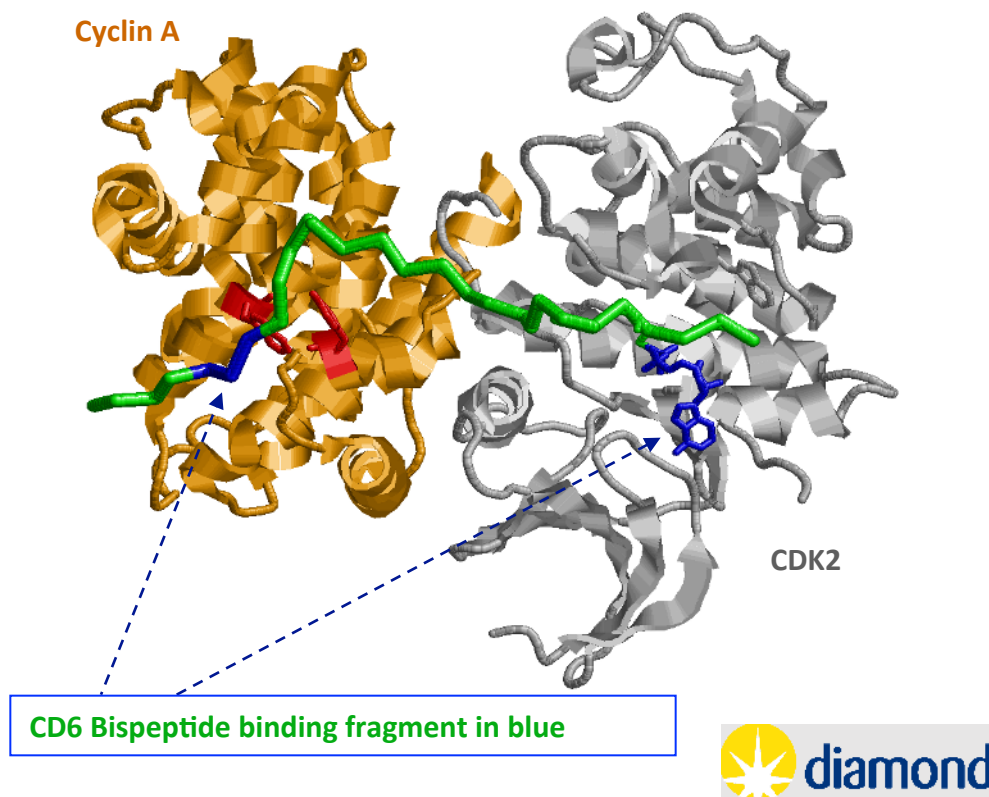
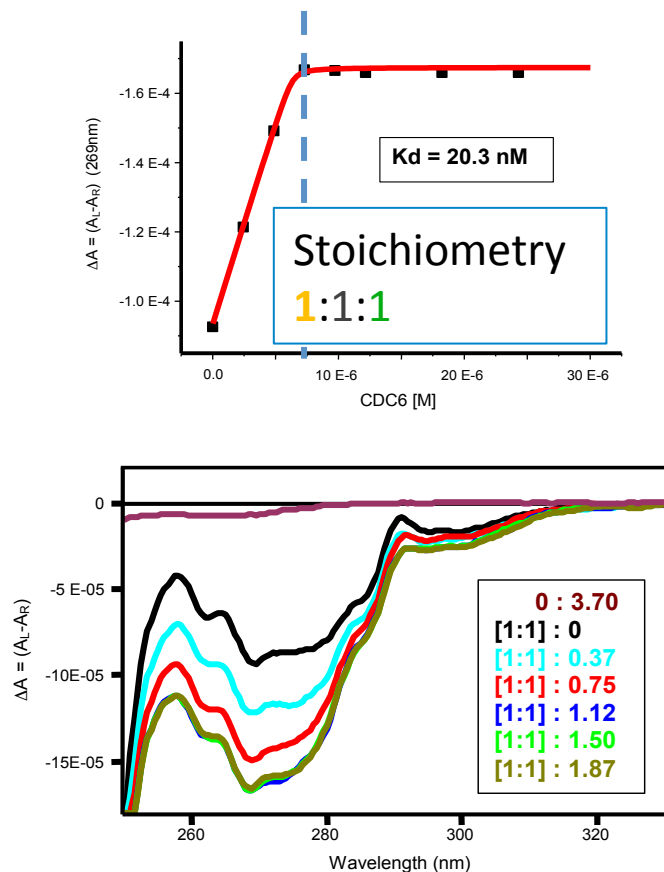
- Qualitative and quantitative analysis of protein-protein and protein-ligand interactions
- Characterisation of IMP's
- Protein stability
- Quality control /drug formulations and delivery

Experimental cabin of B23 beamline. B23 consists of two stations with wavelength range of 125-550nm for B23-A, and 170-650nm for B23-B. Insert)

Module A for high throughput SRCD (HT-SRCD) and CD Imaging

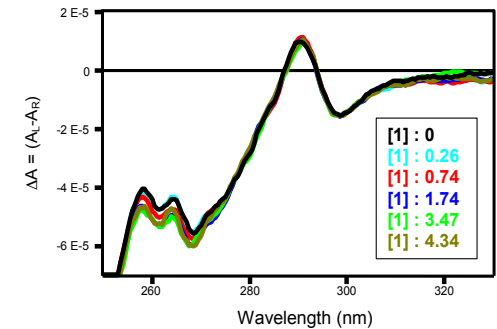
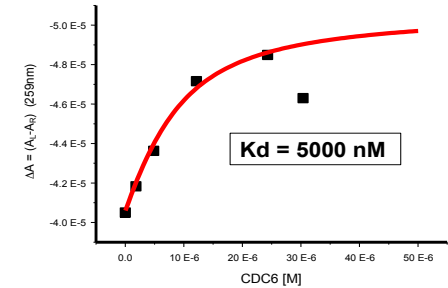
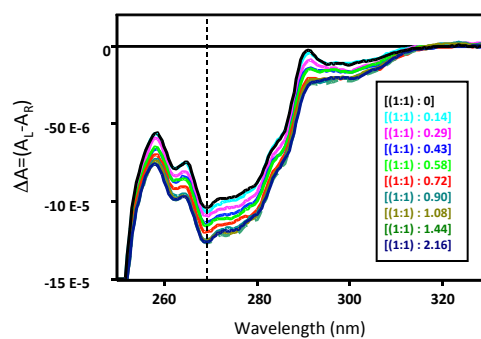
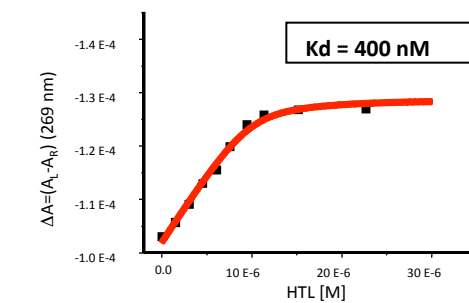
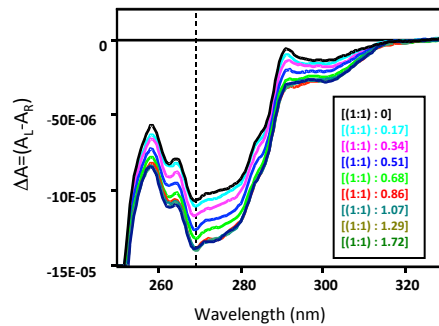
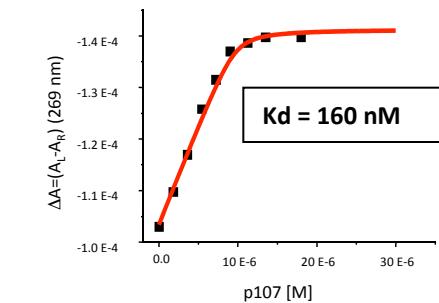
Module B for ligand titrations, temperature studies, UV-denaturation assay for photo-stability and Stopped-Flow for kinetic studies

Peptide-Protein-Protein Interactions (Ternary complex)

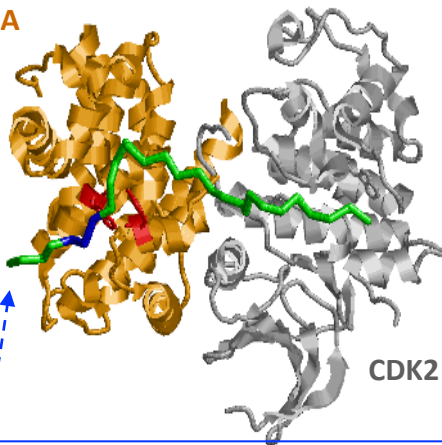


The crystal structure of the ternary complex Cyclin A:CDK2:CD6 peptide revealed only the bound C and N terminal portions of the ligand peptide. The central part was not visible due to its high mobility. The stoichiometry of the ternary complex could not be solved by the crystal structure. Was it 1:1:2 or 1:1:1? The stoichiometry was solved by CD spectroscopy revealing to be 1:1:1

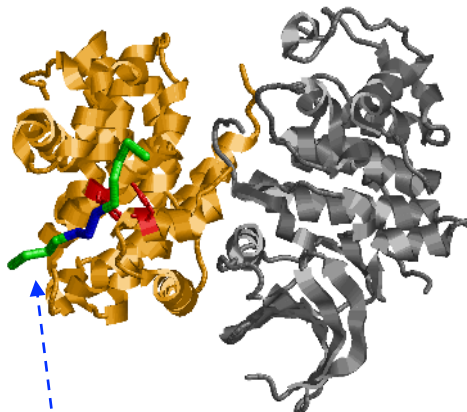
Binding affinity of peptide derivatives and role of binary complex



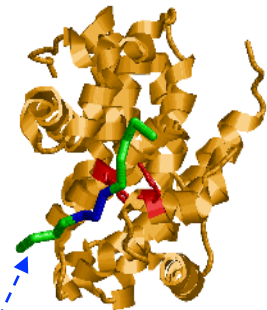
Cyclin A



Only the peptide binding residues (blue) remain available to secure the interaction. The ADP moiety is not present.



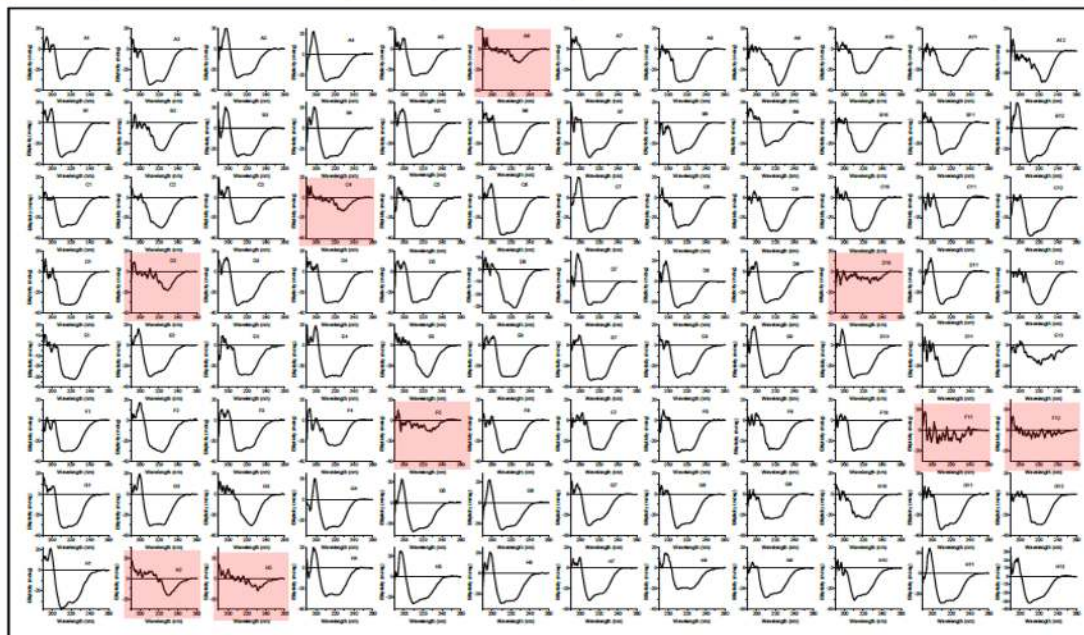
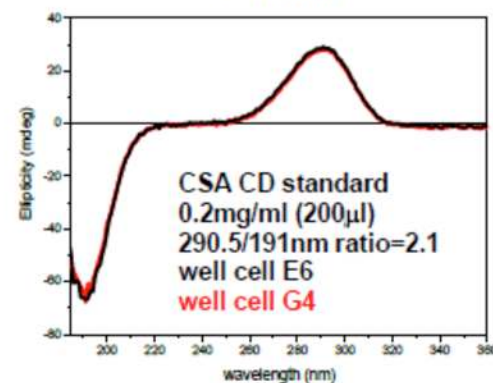
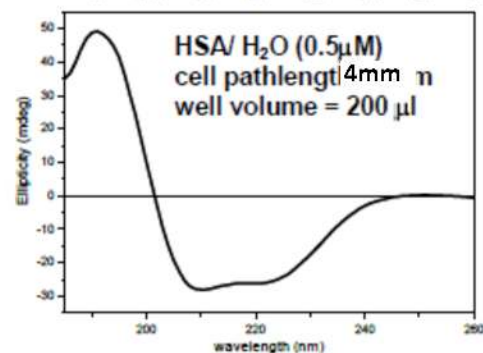
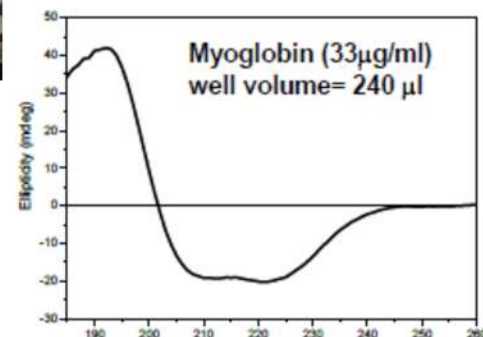
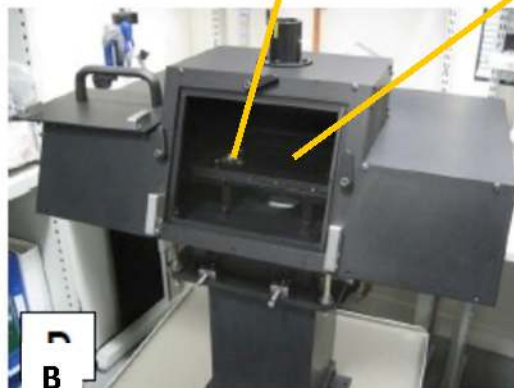
Shorter peptide sequence that interact only with Cyclin A of the preformed Cyclin A:CDK2 complex



Shorter peptide sequence that interact only with Cyclin A.

Vertical sample Chamber

For 96-well plate of B23 module A



B23 HT-SRCD

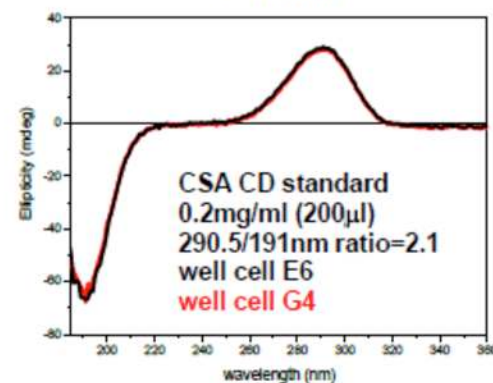
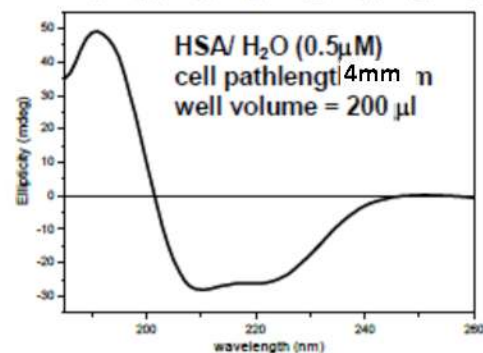
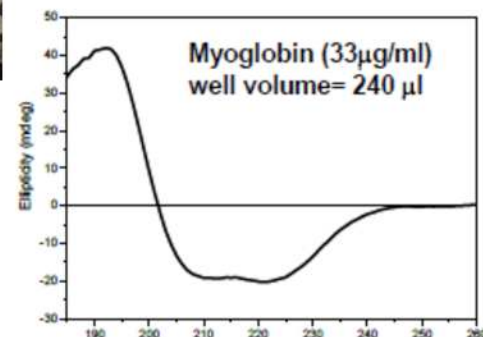
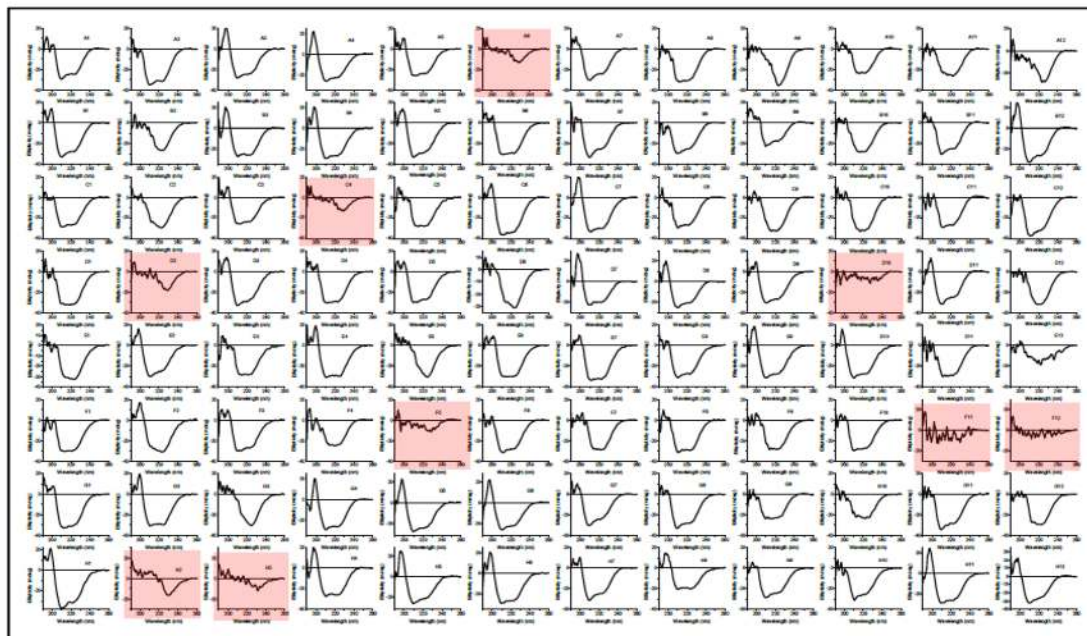


Questions that could be addressed:

Does the protein change its conformation under different solvent conditions?

Do the conditions that generate good crystals correlate with CD data ..are data similar or different than those that do not crystallise

We are open to collaborations





Cryo transmission X-ray microscopy

B24 Liz Duke @diamond.ac.uk

B24: Soft X-ray Microscope

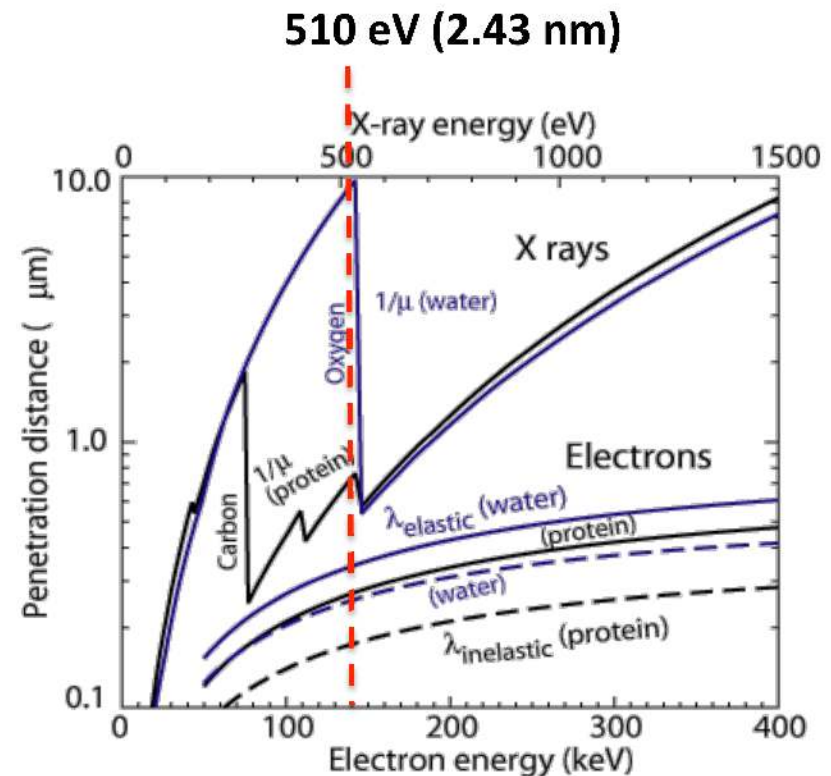
- Soft X-ray tomography allows to image whole hydrated cells in 3-D to resolutions of 50 nm or better.
- Provides for imaging of thick samples ~up to 10 μm
- Contrast provided by the differential absorption of X-rays hence imaging of cells in a close-to-native state possible
- Amiable to high-throughput
- Correlated imaging possible with light microscopy -allowing tagged molecules to be localized in the high resolution 3-D image of a cell
- **Visualisation of 3D structural details**
 - Complementary to cryo-EM
 - Correlative light microscopy

First Users this month!

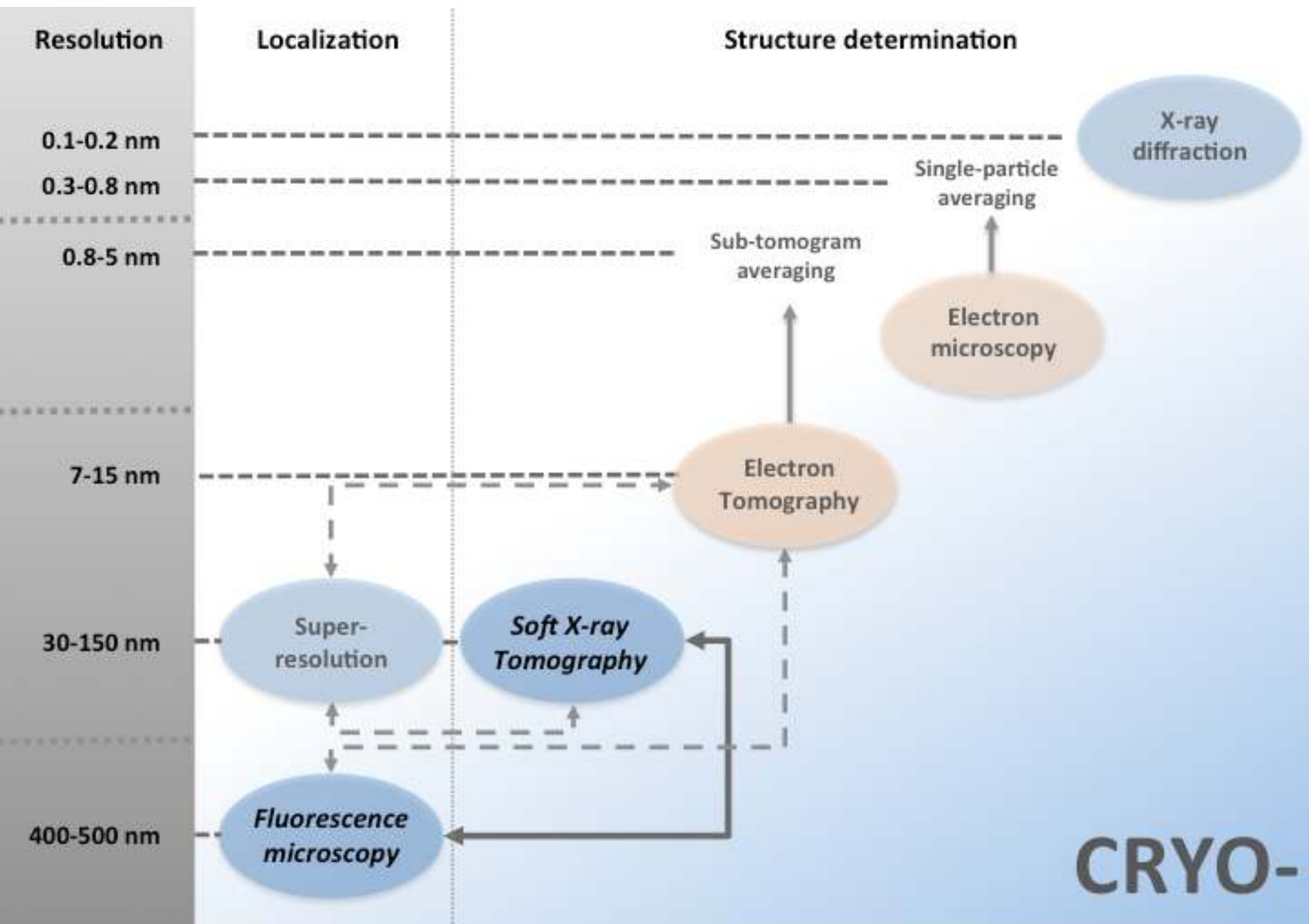
Overview

'A beamline for correlative soft X-ray/light cryo-microscopy'

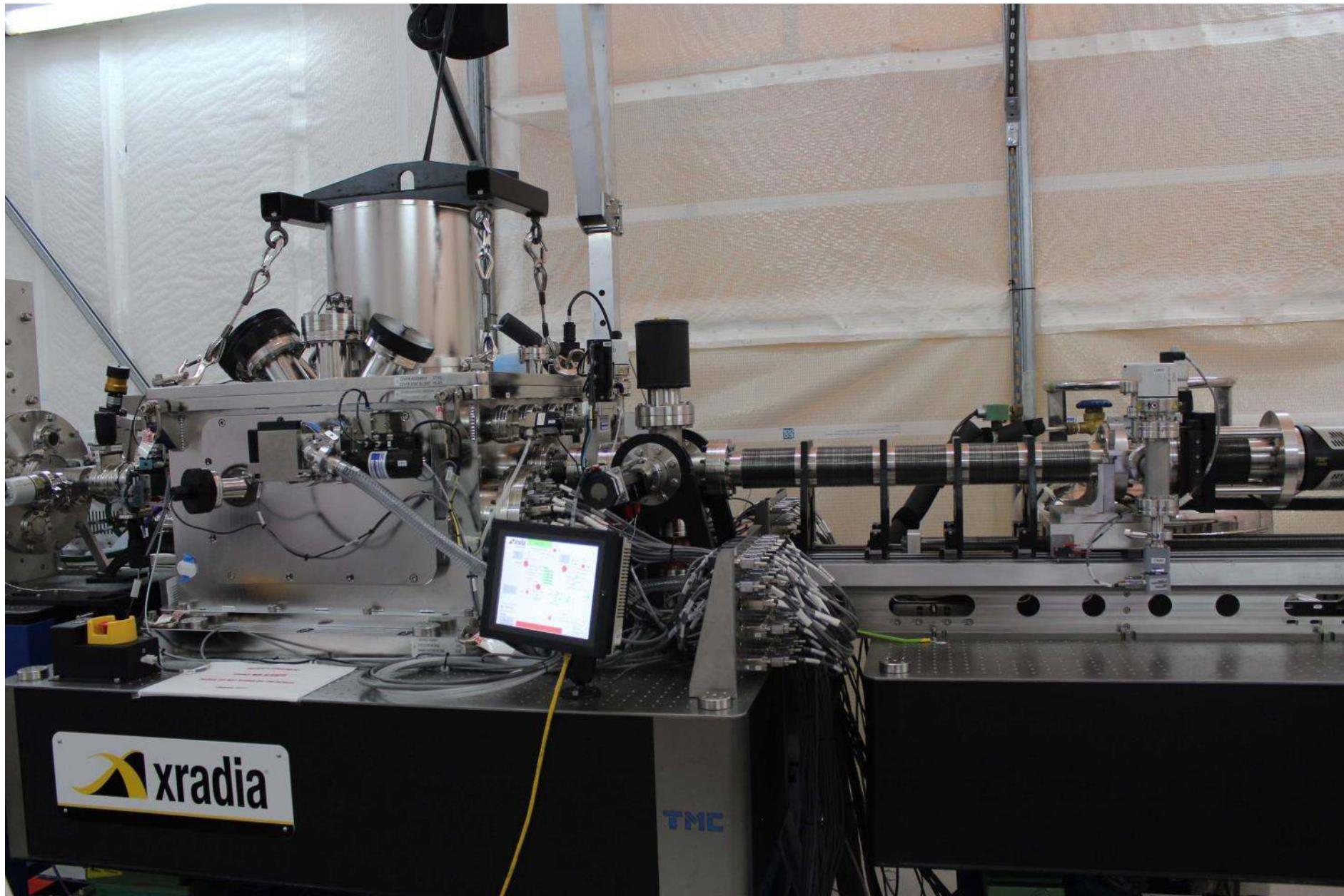
- Image specimens up to 10 μm thick
- 'Water-window' imaging
 - *Absorption contrast*
- Readily reveals biological membranes and heavy metal labels
- Tailored to study intact adherent mammalian cells
- Fluorescence correlation fundamental for elucidation of biological mechanisms



Integrated Structural Biology and Correlative Imaging



Soft X-ray Microscope (Endstation)



Soft X-ray Transmission Optics – The Endstation (Microscope)



B24 Cryo soft x-ray tomography

From data collected in-house with plasma source

N: Nucleus

C: Cytoplasm

Nuclear envelope

Nucleoli

N

C

Cell membrane

Cytoskeleton

Endoplasmic reticulum

Mitochondria

Lysosomes

A National User Facility for Biological Electron Cryo-microscopy (eBIC)

Wellcome Trust Strategic Award/MRC/BBSRC, applicants:
Helen Saibil, Kay Grünewald, David Stuart, Gerhard Materlik

- **Funded by the Wellcome Trust, MRC and BBSRC at level of £15.6 M over 5 years**
- **The planned facility** will include:
 - 2 high-end 300kV automated cryo EMs (Titan Krios FEI)
 - 200 keV automated feeder instrument (Talos Arctica)
 - Cryo focussed ion beam instrument
 - Sample prep incl. vitreous sectioning
 - Correlative fluorescence/EM
 - FEI Polara @ Oxford for CAT 3 samples

Electron Microscopy facility



eBIC today...





Aims

- Establish state-of-the art facilities for single particle analysis and cryo-tomography
- Provide cost-effective access, based on scientific excellence, to high-end Microscopes and auxillary equipment, incl. containment facilities at OPIC, Oxford
- Run 24/7 aka synchrotron beamline supported by expert staff
- Provide focus for future hardware/software developments
- Grow and train user community – especially structural biologists and cell biologists with facilities advanced sample preparation
- Retain cutting edge with strong in-house research program
- Key part of vision for integrated approach to structural and cellular biology

Cryo-EM & Cryo-Tomo

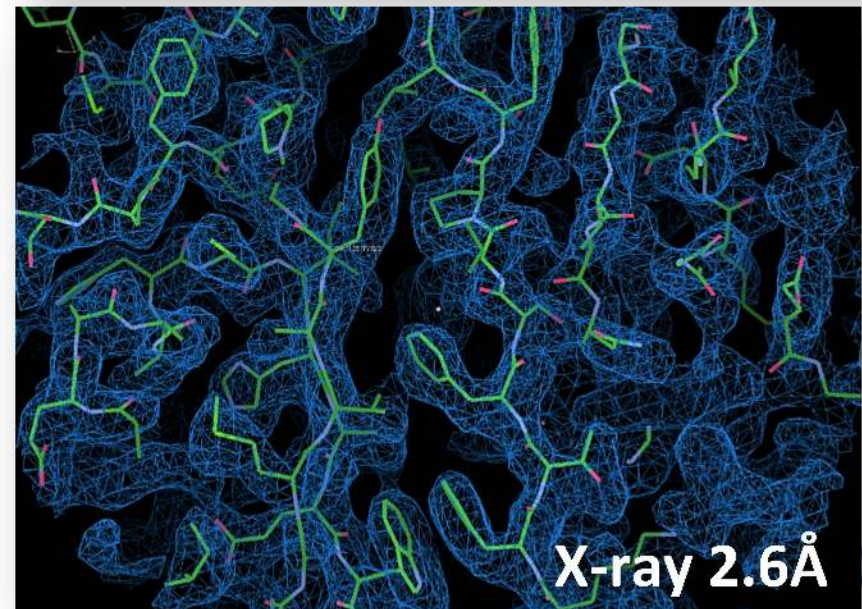
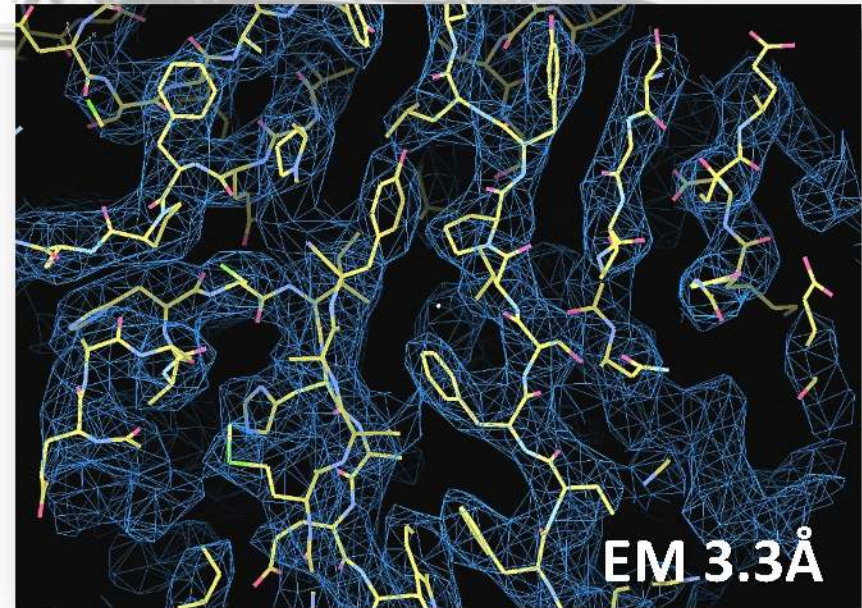
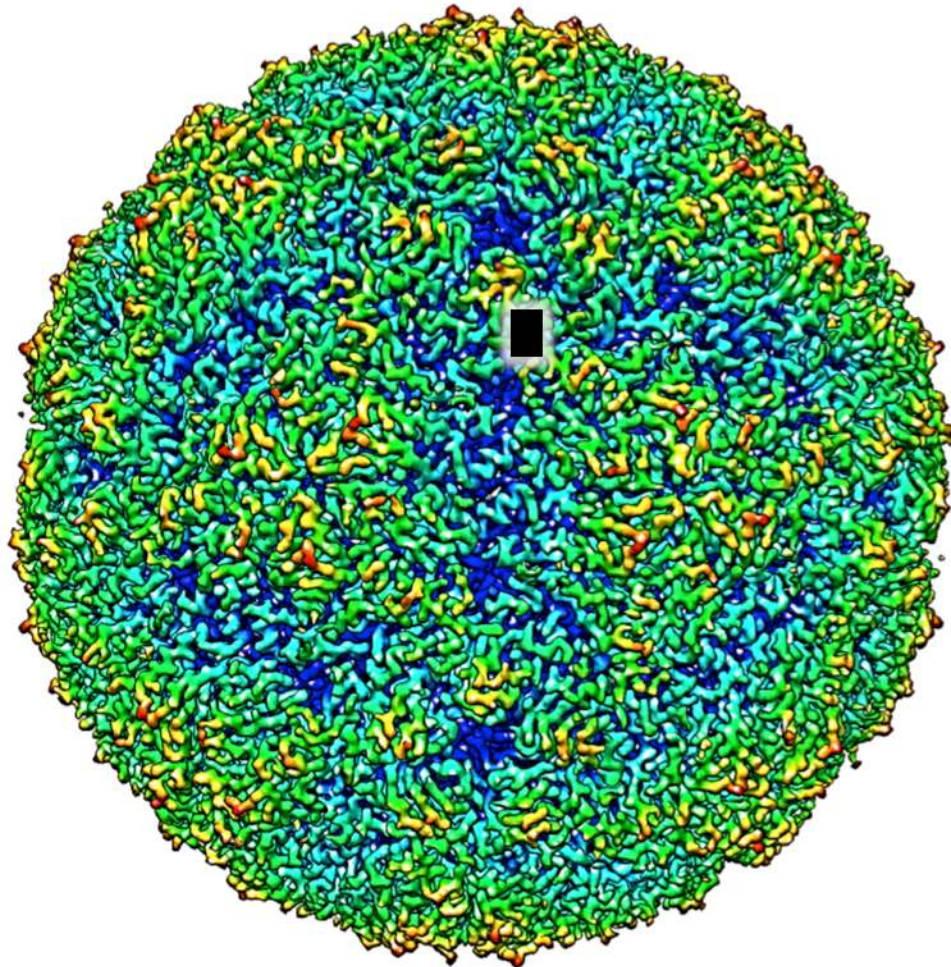
- Single particle analysis requires similar high quality samples to X-ray crystallography
- Massive advances in microscope, detectors (movies), software.
- Still much to come with improved sample presentation, automation, hardware/software.
- Already pretty efficient (data output > MX beamline, computational demands circa 1000-fold more).



Example from foot-and-mouth disease virus

electron microscopy and X-ray structures

(data collection time *circa* 1/2 day)

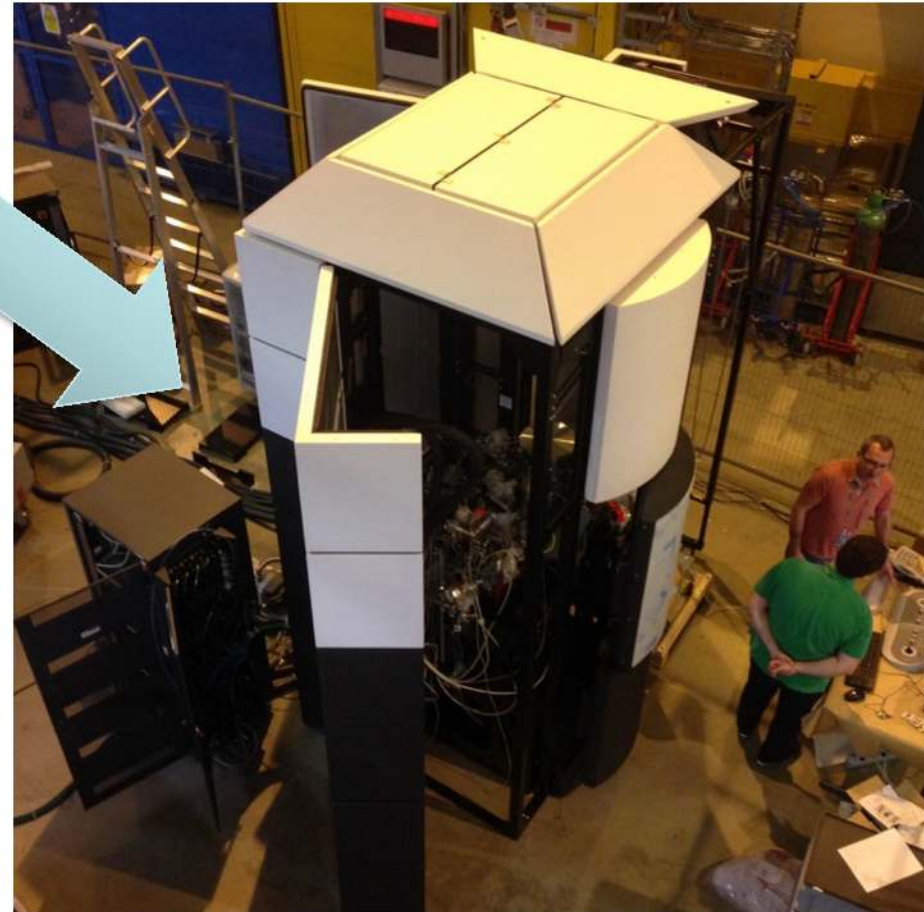


Krios I Diamond

Installation, on the Diamond hall floor, commenced 28th April 2015 (really like a beamline!)

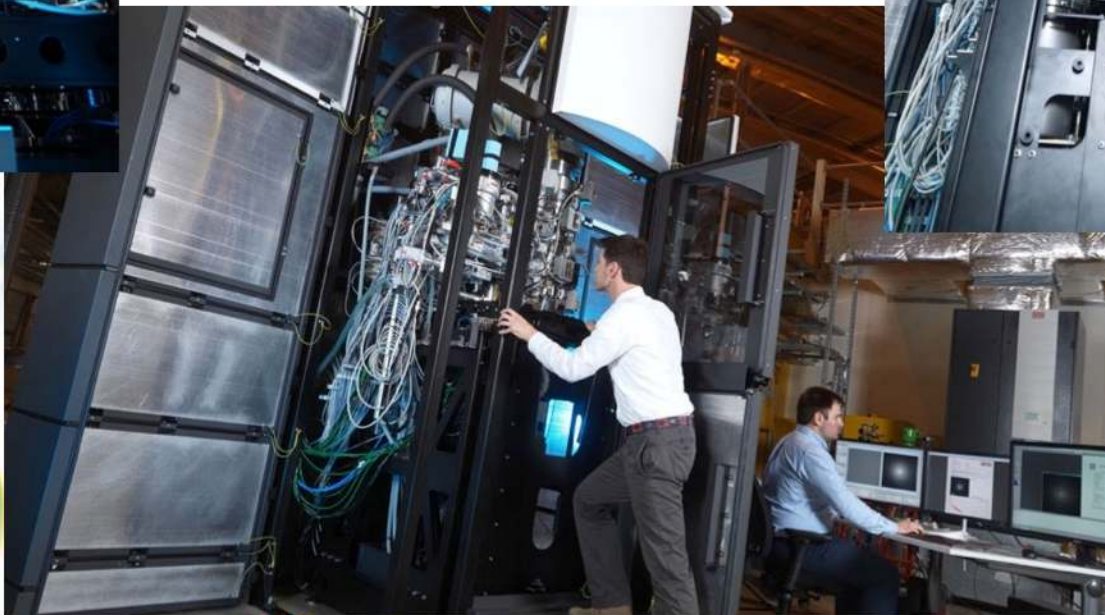
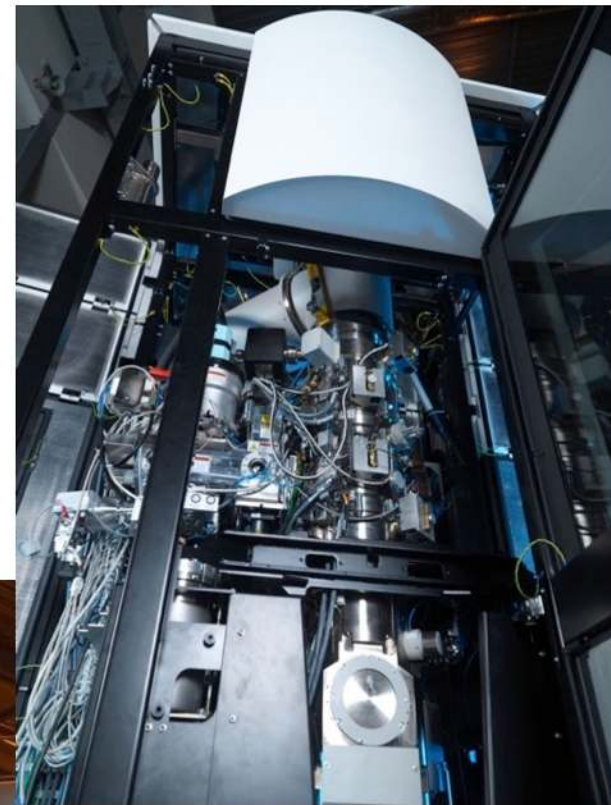
2nd Titan krios microscope will be delivered at end of 2015

CL2 and CL3 work:
Polara with direct electron counting detector (K2 Gatan) available at Oxford Particle Imaging Centre OPIC (<http://www.opic.ox.ac.uk>)

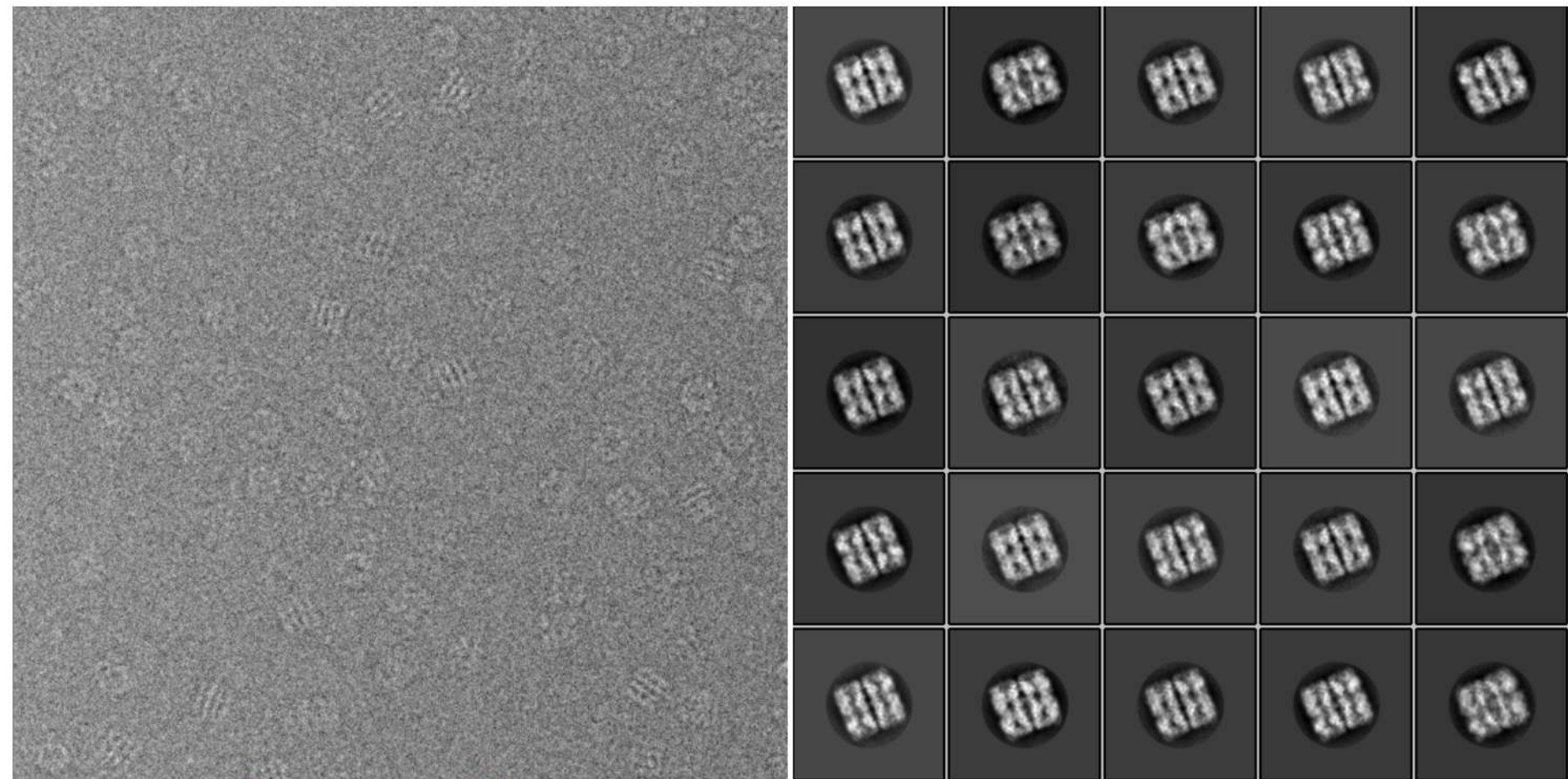


Ready for users.....

- 38 Proposals so far
- 95 TB of data !
- Averaging 2.7 TB /48 hours
- Only facility in world at Present to have automated High resolution Microscope (since Oct 2015!)

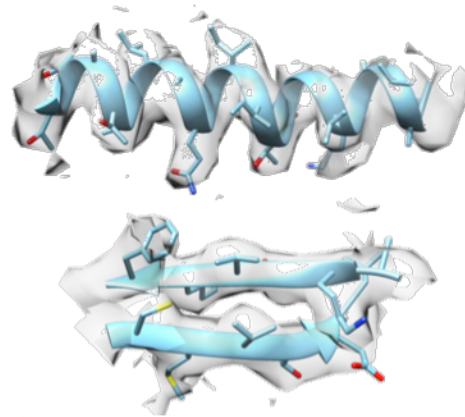
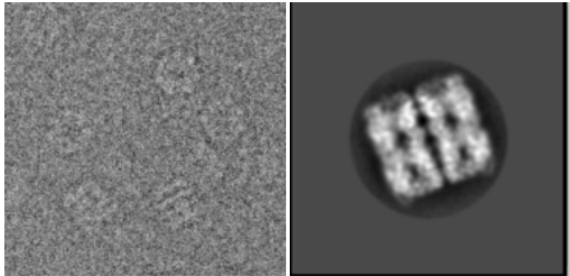


1st Sample GroEL

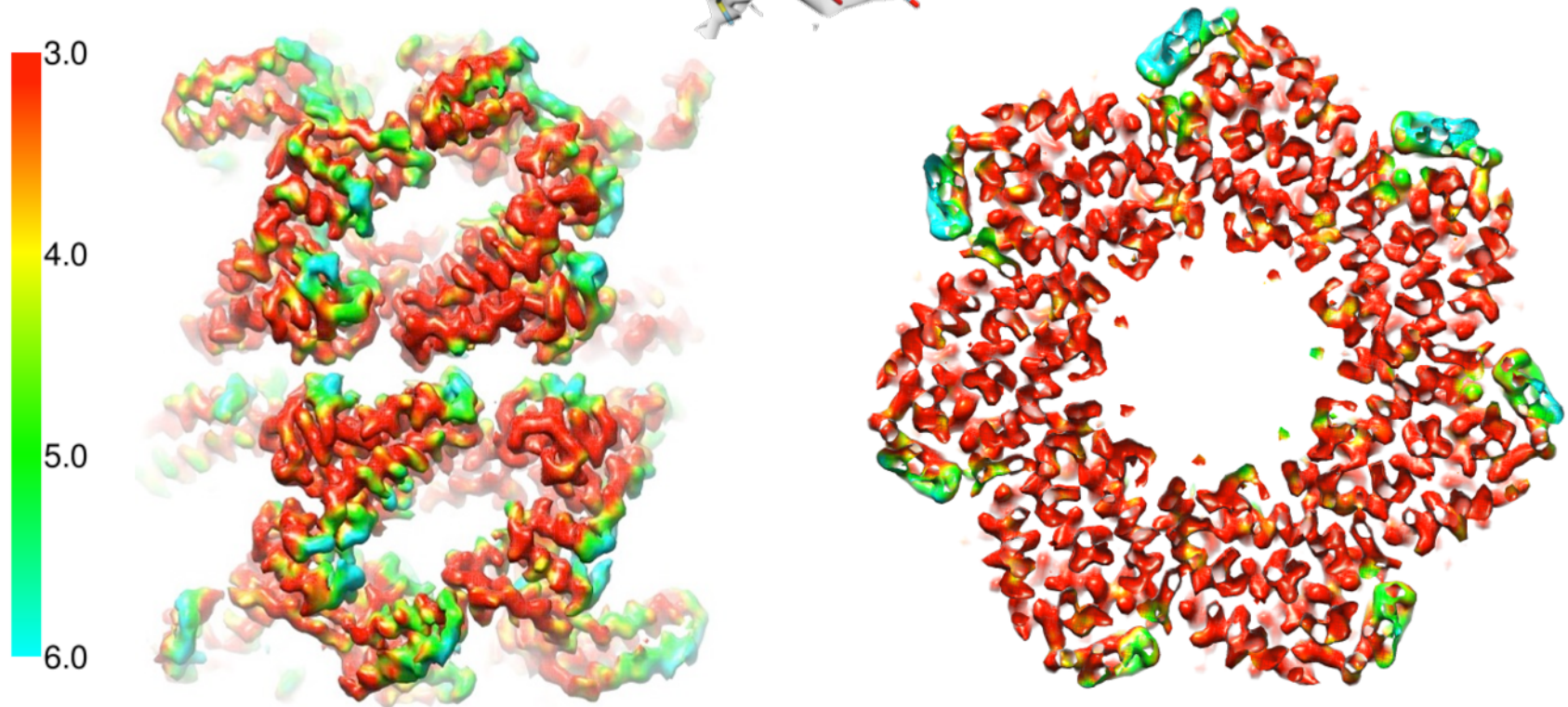


Raw image and 2D classes from Relion

Commissioning test I: GroEL

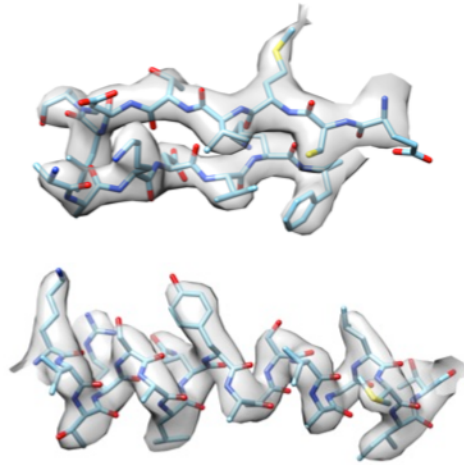
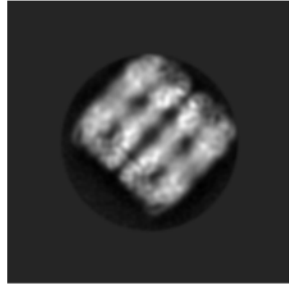
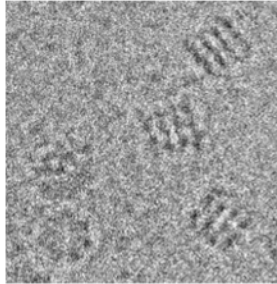


33,000 particles
15 hours data collection
Average resolution 4.1 Å
Rigid regions 3 Å
Processed with Relion

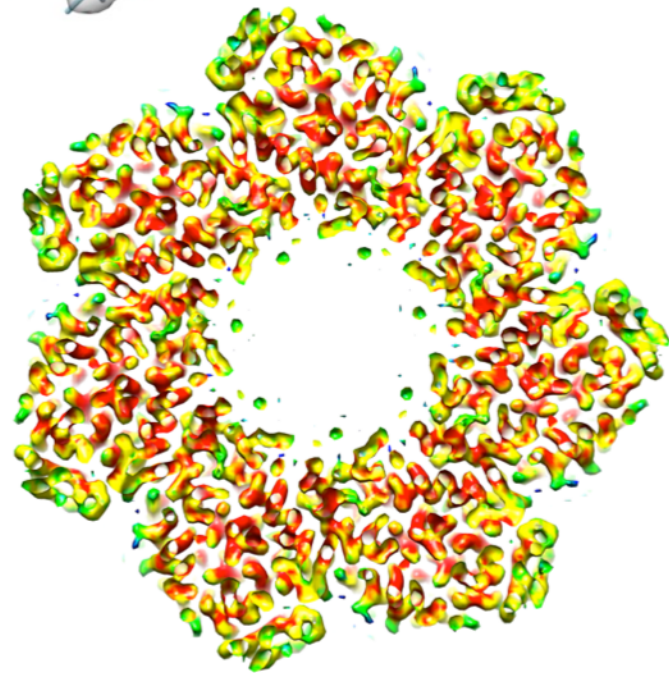
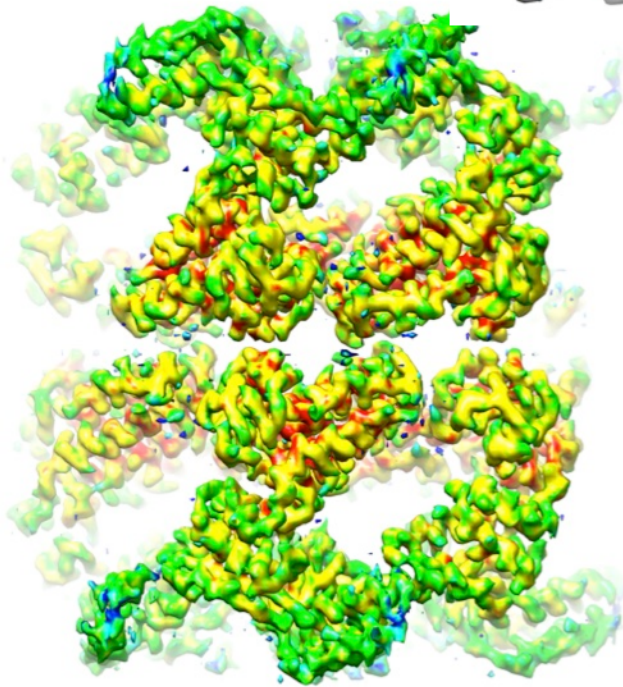


Dan Clare Diamond, Sonja Welsch FEI

Commissioning automated: GroEL EPU-K2

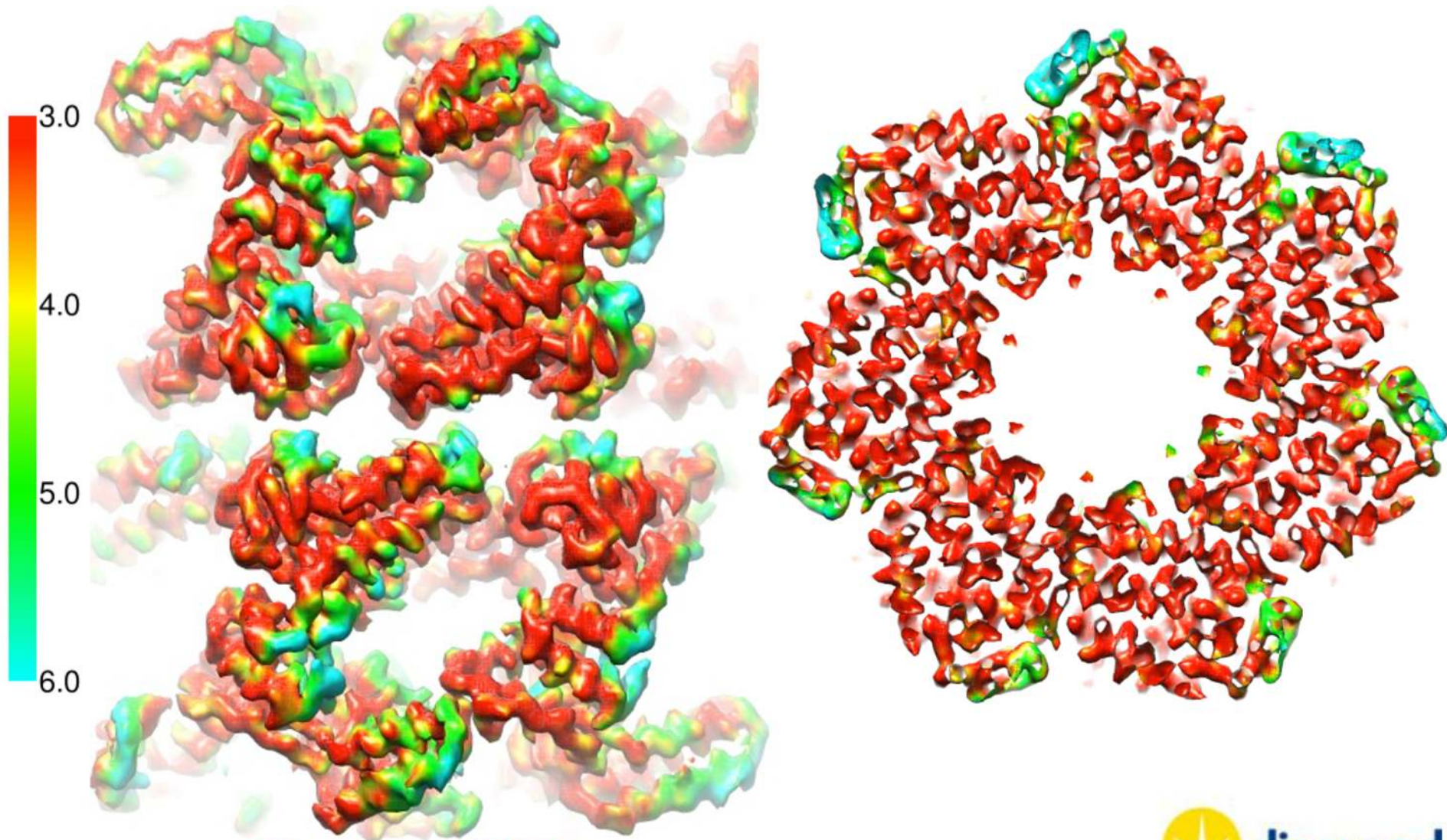


17,500 particles
10 hours data collection
Average resolution 3.2 Å
processed with Relion



Dan Clare Diamond, Sonja Welsch FEI

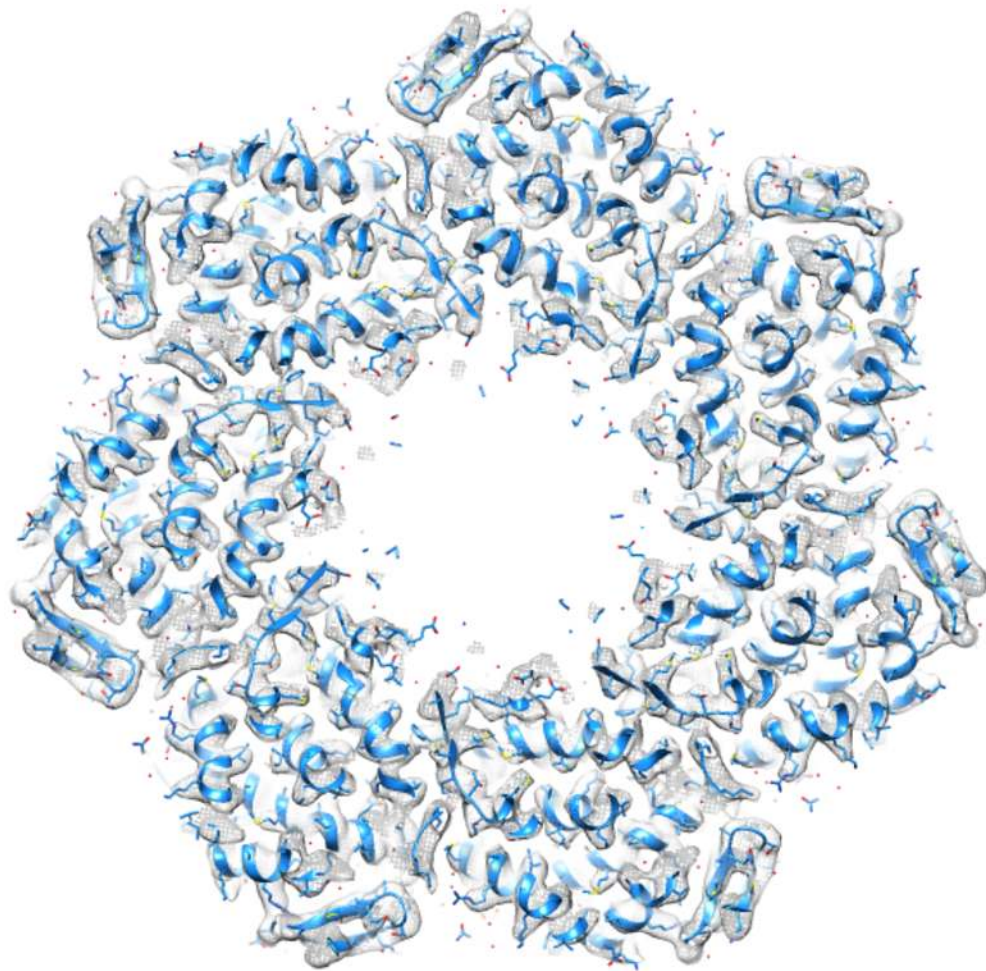
GroEL D7 symmetry overall resolution 4.1 Å



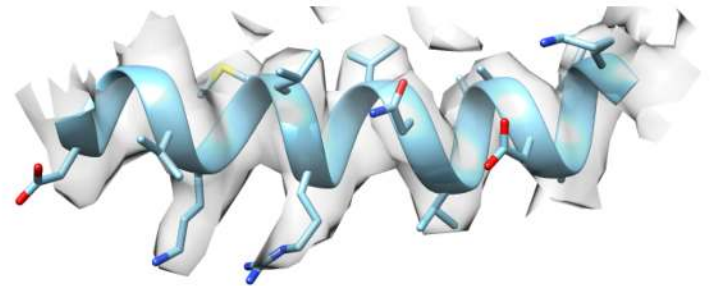
Resmap shows that the local map resolution varies from 3 to 6 Å

GroEL equatorial domain

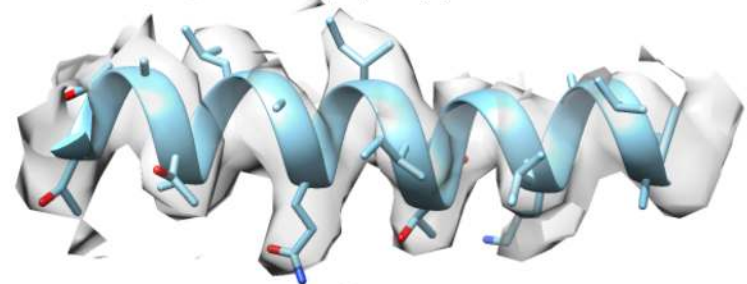
Atomic coordinates docked



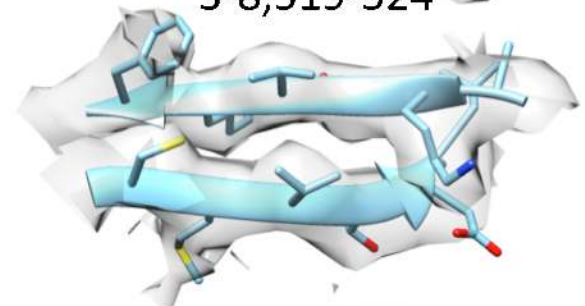
11-28



90-109



3-8,519-524



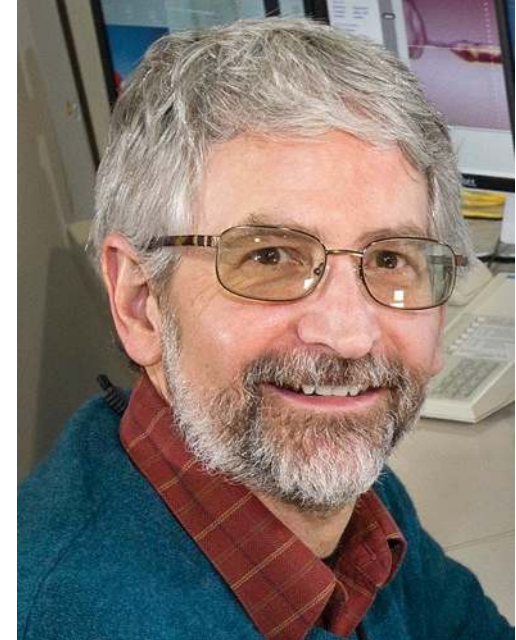


UK and femtosecond crystallography at the European X-FEL - SFX

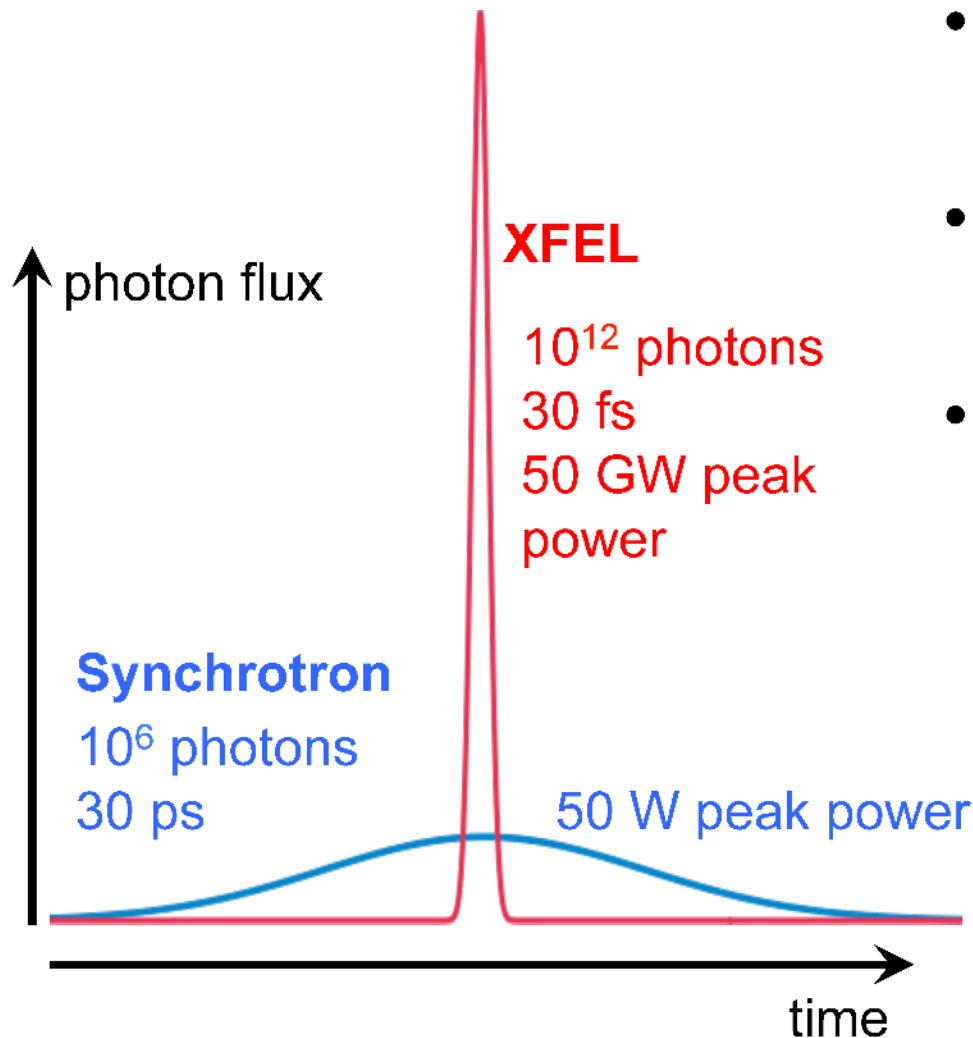
James Naismith David I Stuart Jan Löwe Martin A Walsh
Henry N Chapman

On behalf of the UK Structural Biology Community
via Instruct & CCP4

- Funded by Wellcome Trust, BBSRC and MRC to make the UK the largest partner in the consortium at the European XFEL (<http://www.xfel.eu>) for this dedicated crystallography instrument
- Enable diffraction from nanocrystals....time resolved experiments...ultimate goal image single particles...
- Allen Orville now leading from Diamond



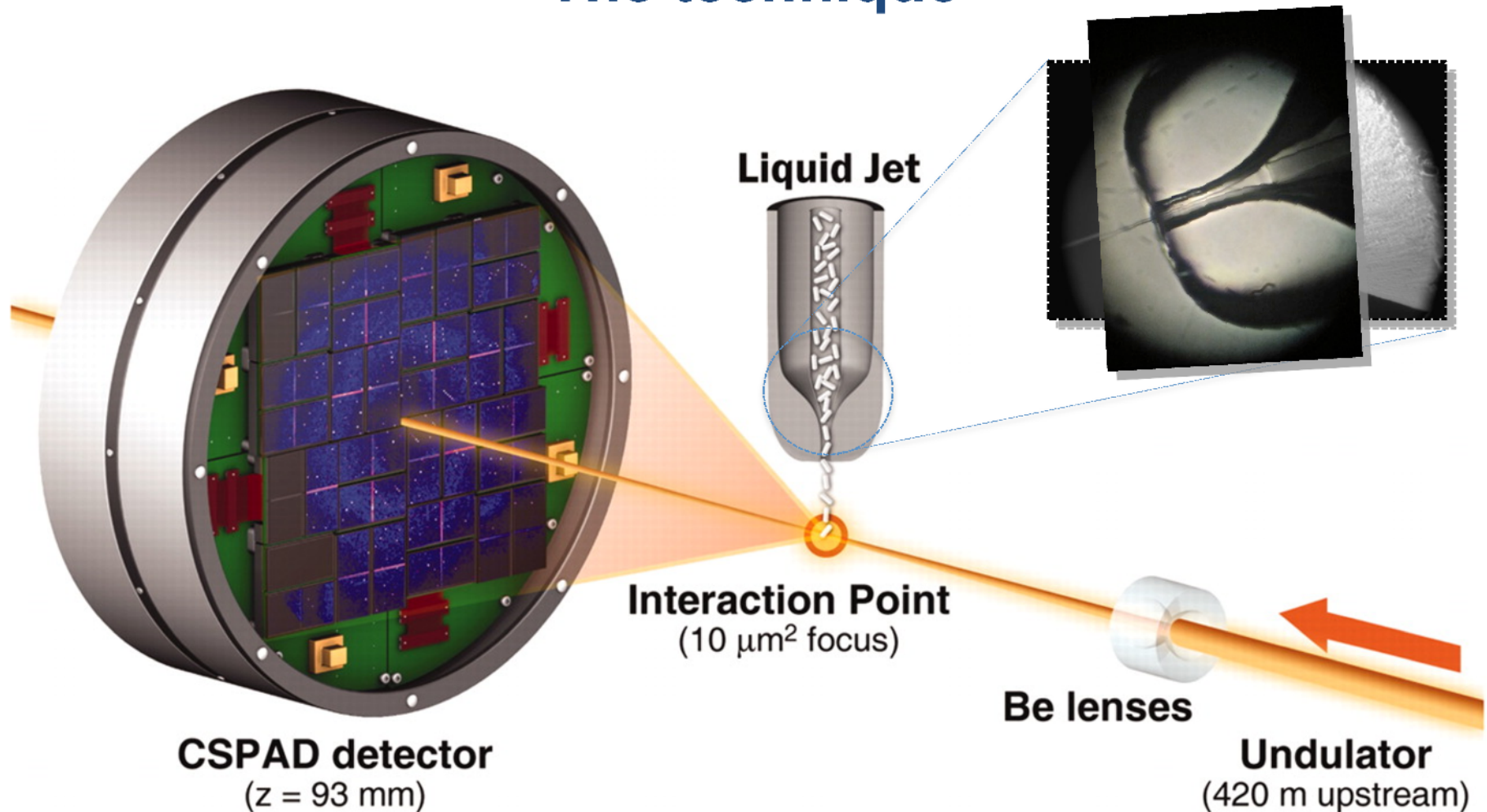
Potential of XFEL



- 30 fs pulse, X-ray doses exceeding 1 GGy
- **Unique: diffraction before sample destruction**
- Structures from smaller & sensitive crystals not currently tractable: paradigm shift for
 - Membrane proteins
 - Protein protein complexes

Serial Femtosecond Crystallography

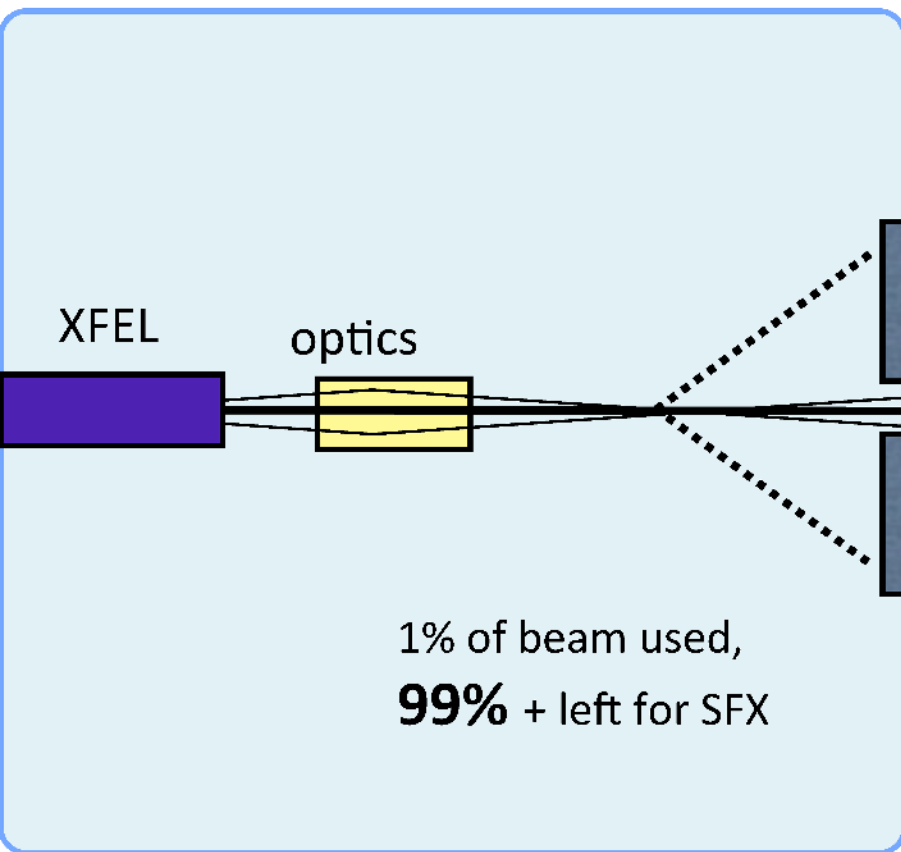
The technique



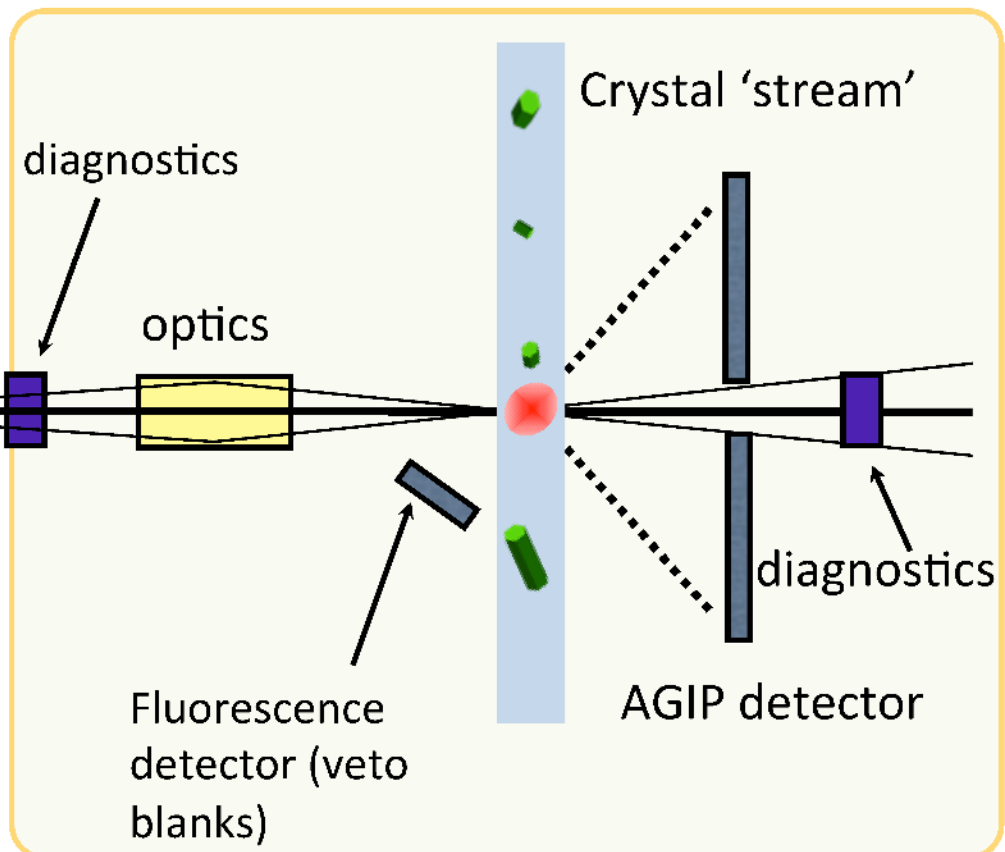
Single shot diffraction patterns are obtained from a stream of fully hydrated nano-crystals injected at room temperature into the intense X-ray pulses from the XFEL source. Each intense pulse lasts for ~30 Femtoseconds and allows the diffraction data to be collected before the crystal is destroyed

Serial Femtosecond Crystallography

SPB Upstream

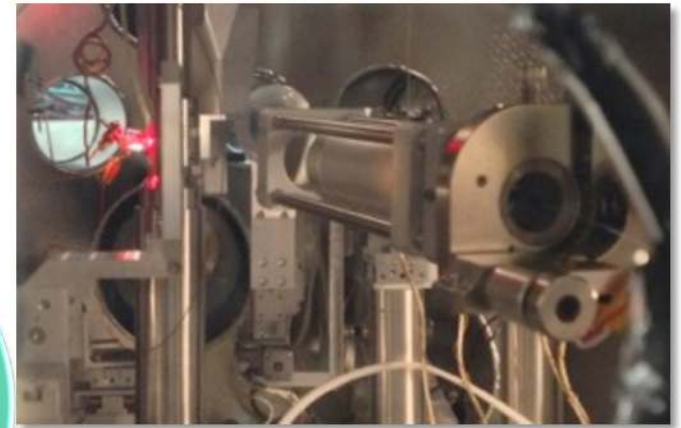


SFX Downstream



UK Hub at Diamond

- establishing an open XFEL user programme



Software Developments for automated data analysis

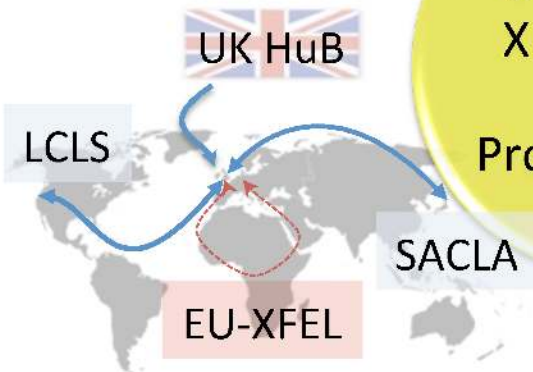
Development of Sample delivery & Environments

High Performance Computing and Data curation

UK XFEL HuB

Facilitate access to XFELS/UK User Program for SFX

Integrated Structural Biology Infrastructure @RAL



Users

Timelines

SPB/SFX experimental hutch



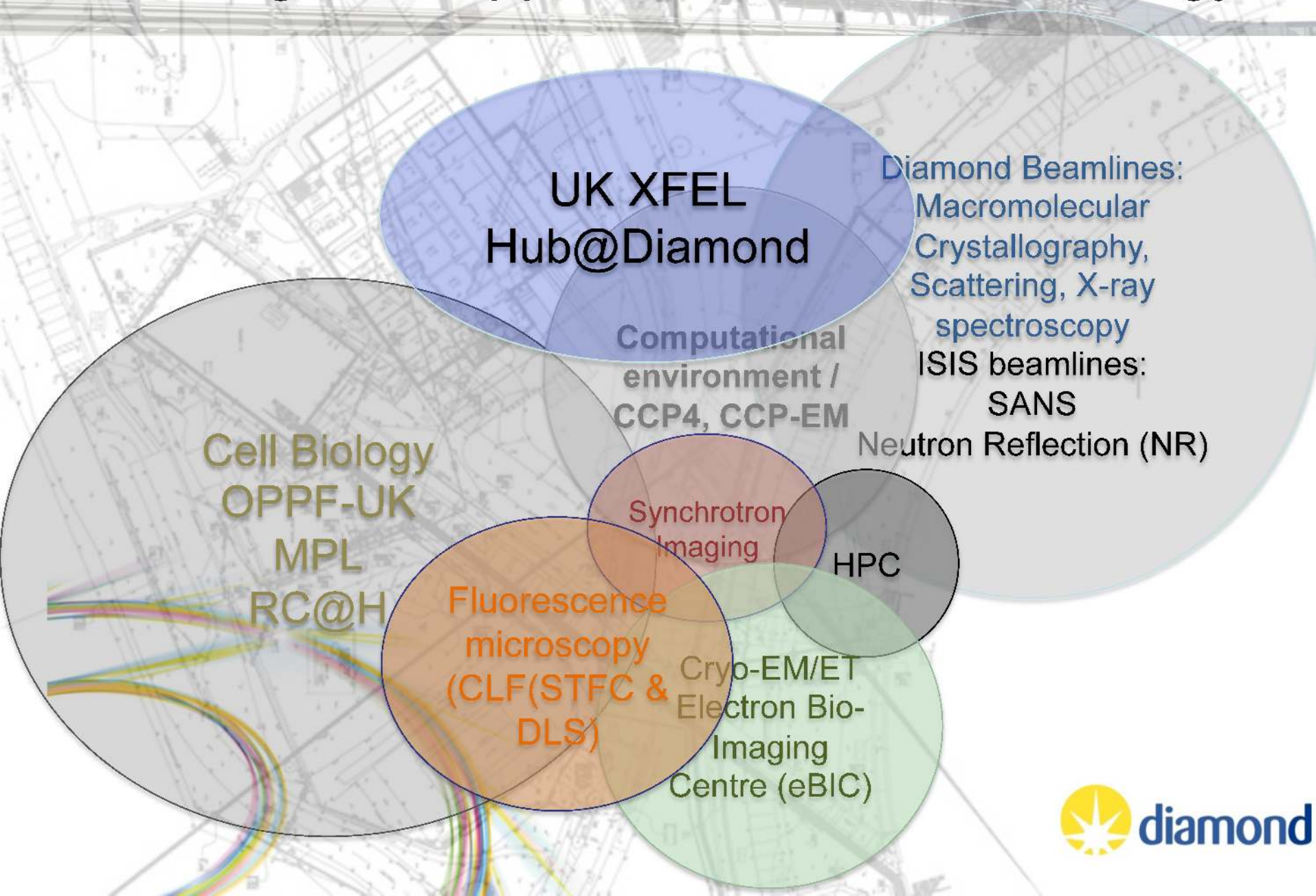
Commissioning of XFEL injector from November 2015

Instrument commissioning from mid 2017

Expecting 1000 hours of beamtime in 2017 (first call Q4 2016)

Ramp up to 2000 hours (2018) and full operation 2019 (4000 hrs)

An integrated Approach to Structural Biology



**Thanks for your
attention**

