

Biological applications of ultrafast X-rays - basics

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A structural biologist's dreams

Cells are the basis of life

Wanted -

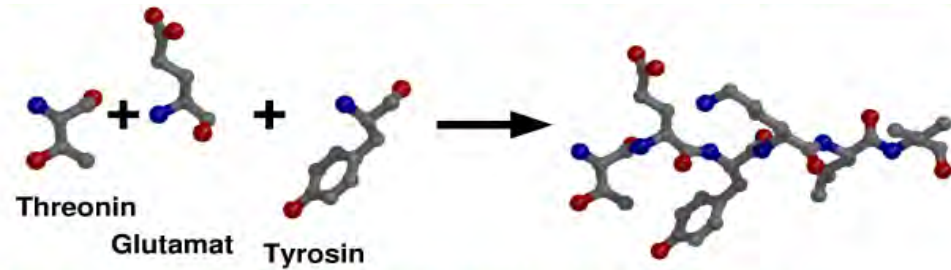
High resolution temporal & spatial inventory of cells

Who is where, when, why

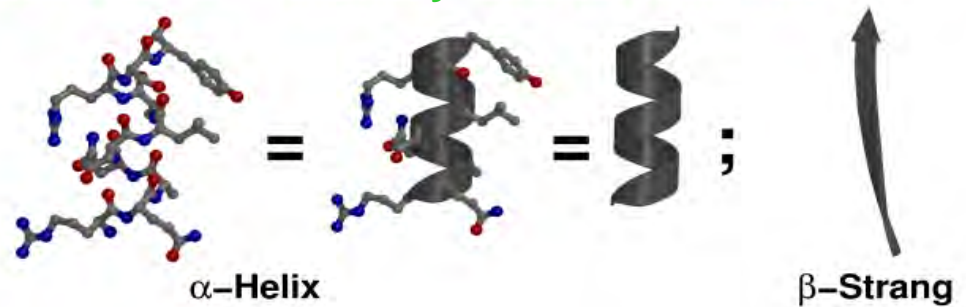
Comprehensive characterization of the players

- 3D structures of macromolecules & assemblies
- how do they function, structural changes
- how do they acquire their structures, i.e. fold

Linear chains of 20 different building blocks, the amino acids



Fold into secondary structure elements

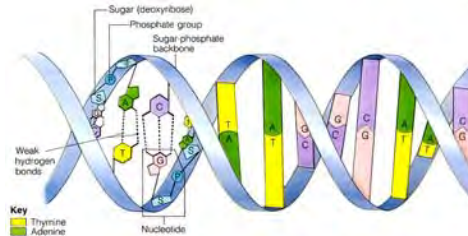


Arranged in 3-dimensional structures



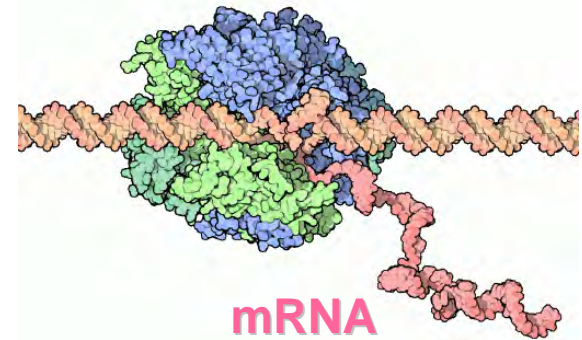
Proteins
linear biopolymers

Cells are the basis of life

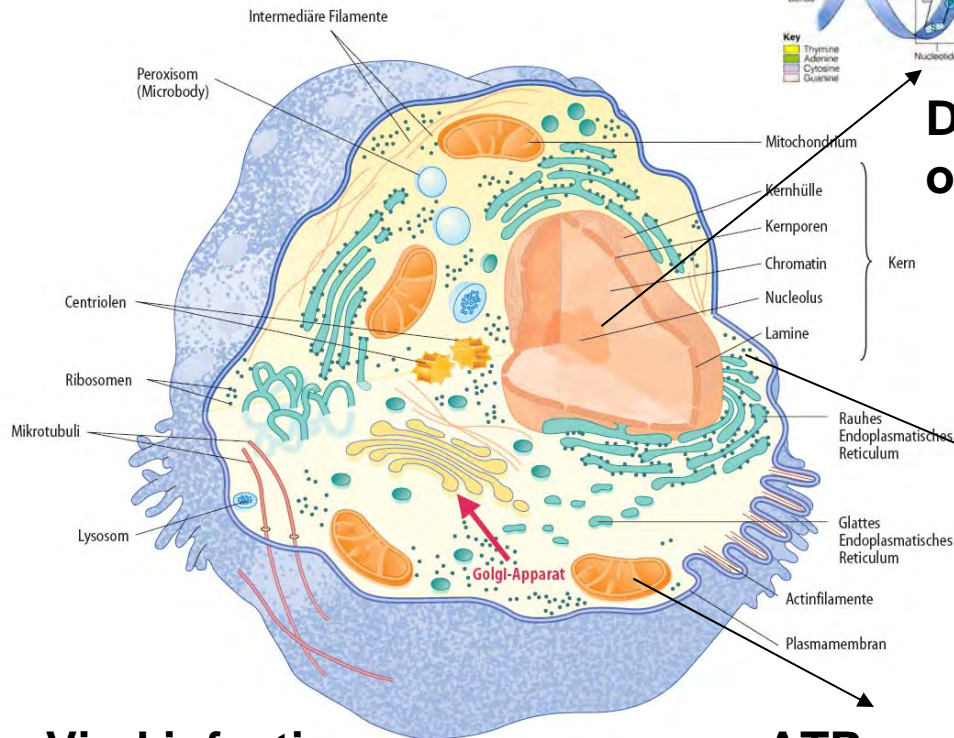
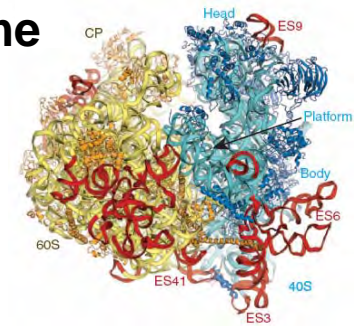


DNA: storage of genetic info

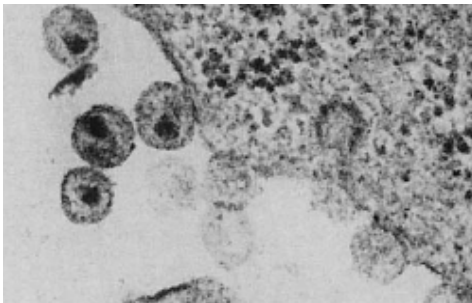
Transcribed by RNA polymerase



Translated and synthesized in protein: ribosome



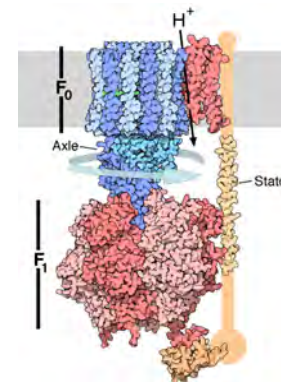
Viral infection



ATP synthesis



F₁/F₀ ATP synthase



Structural Biology

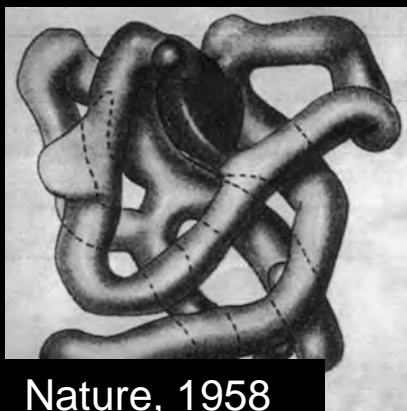
Elucidation of structures of macromolecules with the aim of understanding the chemical mechanisms underlying biological function.

Applications

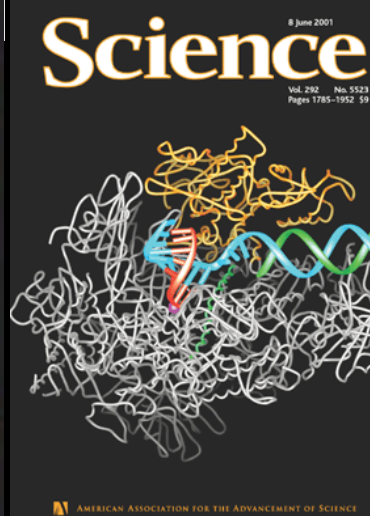
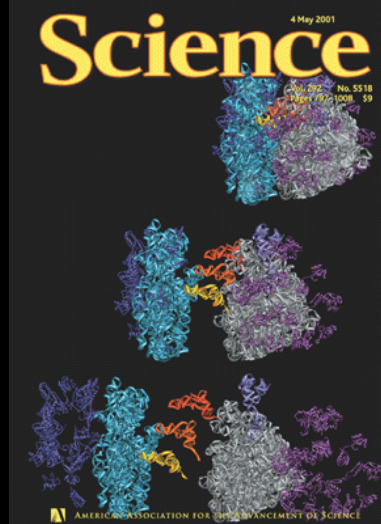
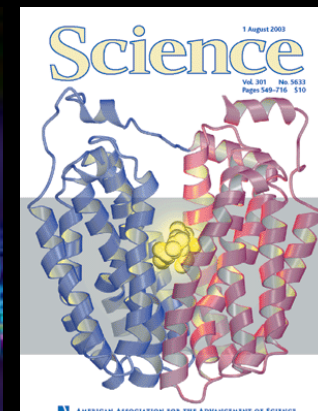
1. Cell & Molecular Biology
2. Chemistry & Chemical Physics
3. Drug Discovery

Crystallography

1. Mature discipline that continues at a high level of achievement.
X-ray structures: 60,252
(NMR structures: ~ 7,797)
2. No molecular size limit
(> 10⁶ Daltons)
3. Facilitated by synchrotron sources



Nature, 1958



Can crystallography be improved?

1. Do away with the need for **Crystals**.
2. Avoid the **Phase Problem** by direct measurement.
3. **Radiation damage**

“Cryo-Electron Microscopy” with “Single Particle Reconstruction”
bypasses these requirements.

General problem of radiation damage still exists.

Can crystallography be improved?

1. Do away with the need for (large) **Crystals**.
2. Avoid the **Phase Problem** by direct measurement.
3. **Radiation damage**



LD₅₀ doses

species	Dose [Gy]*
human	4.5
rat	6
<i>Escherichia coli</i>	50
Herpes virus	2500
<i>Micrococcus uranus</i>	18x10 ³
protein crystal (100K)	3 x 10 ⁷

*Gy (Gray) J kg⁻¹

Can crystallography be improved?

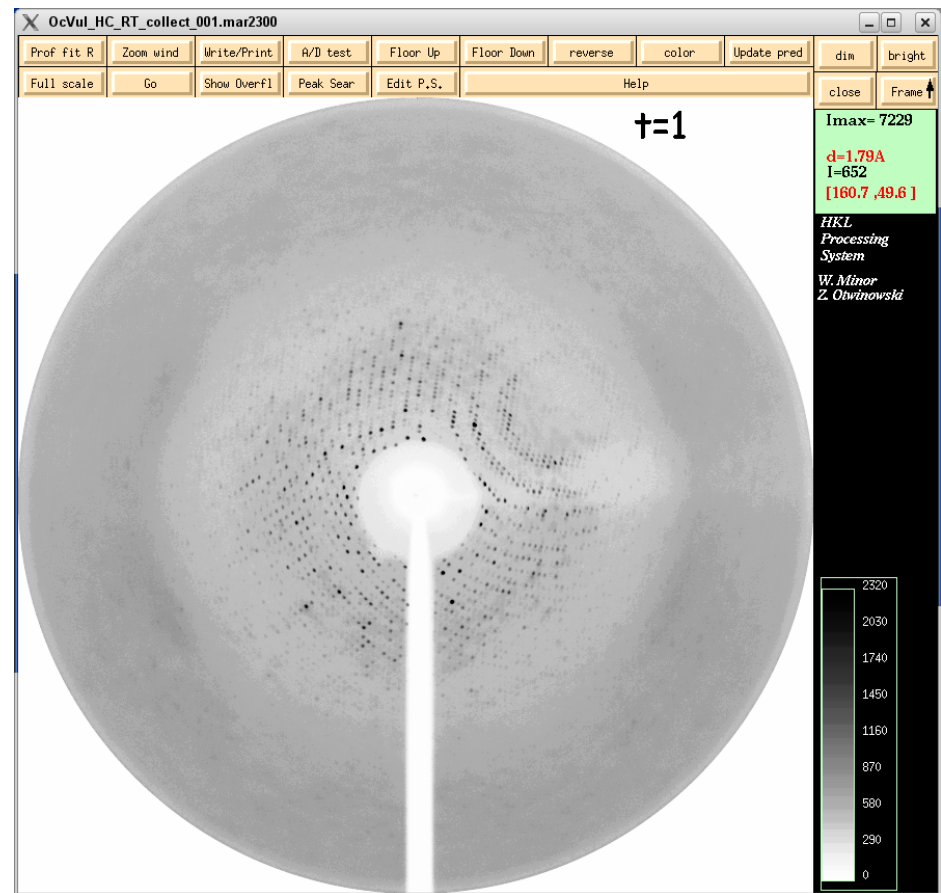
1. Do away with the need for (large) **Crystals**.
2. Avoid the **Phase Problem** by direct measurement.
3. **Radiation damage**

Global damage

- Loss of resolution
- Increase of mosaicity
- Change in unit cell constants

Local damage

- Decarboxylations, S-S bond breakage
- Photo-reduction of redox systems, e.g. metal centers



Loss of resolution due to global damage

Can crystallography be improved?

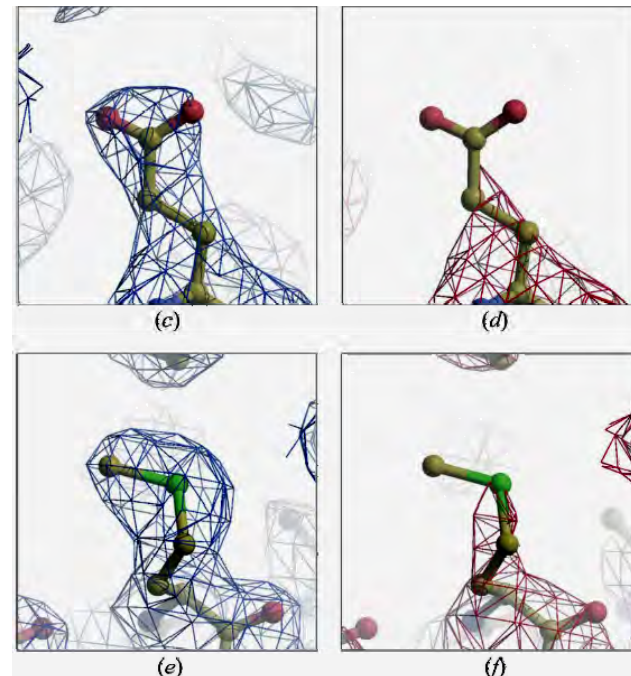
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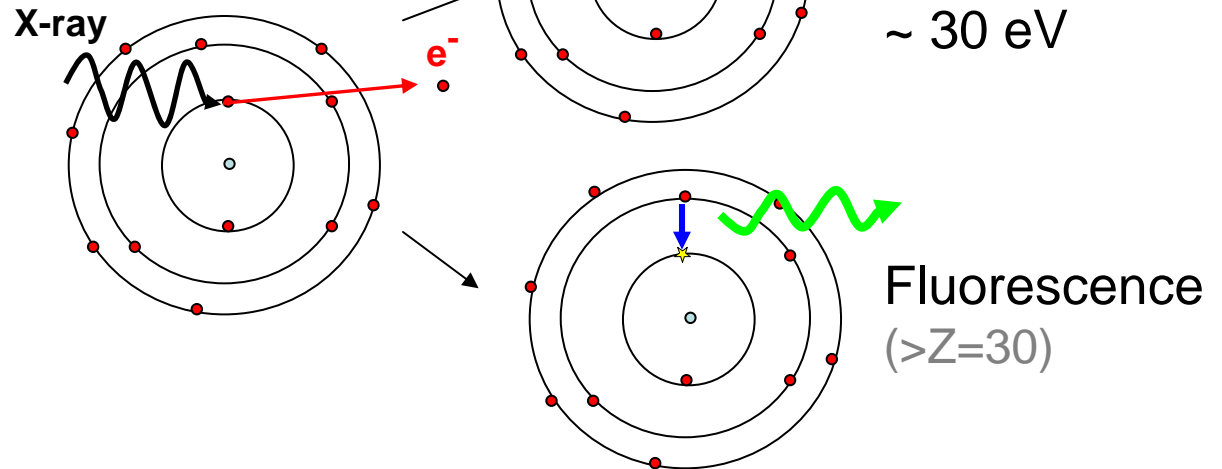
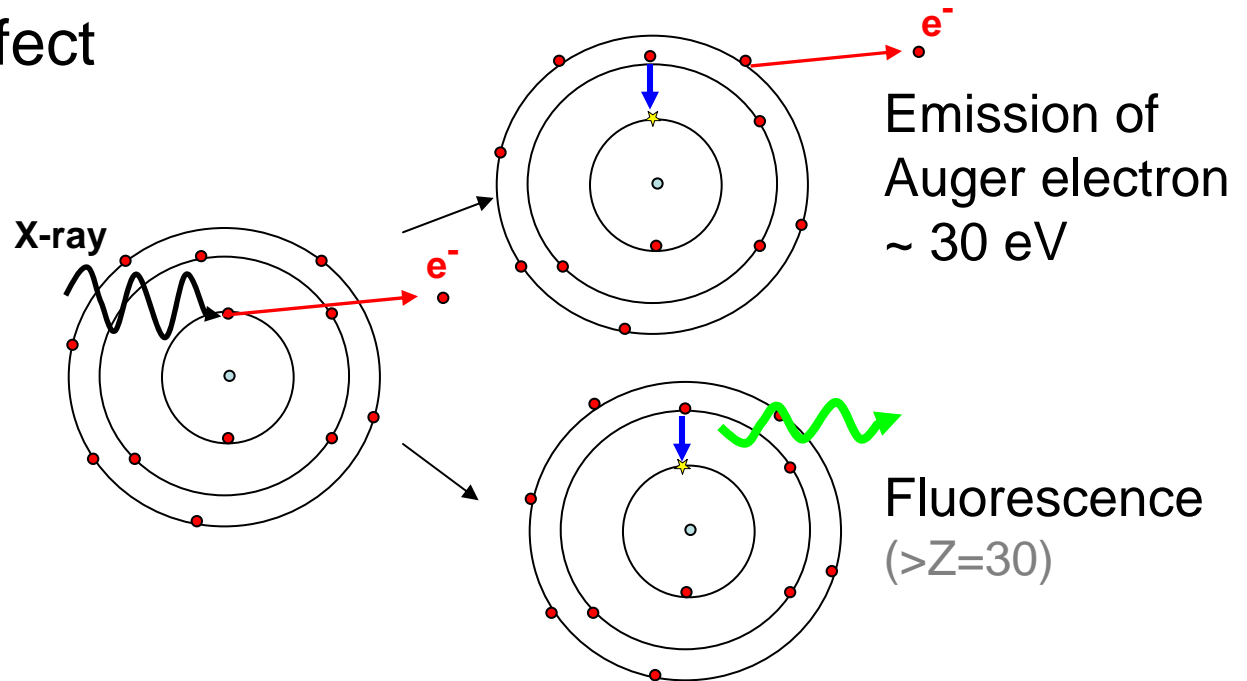
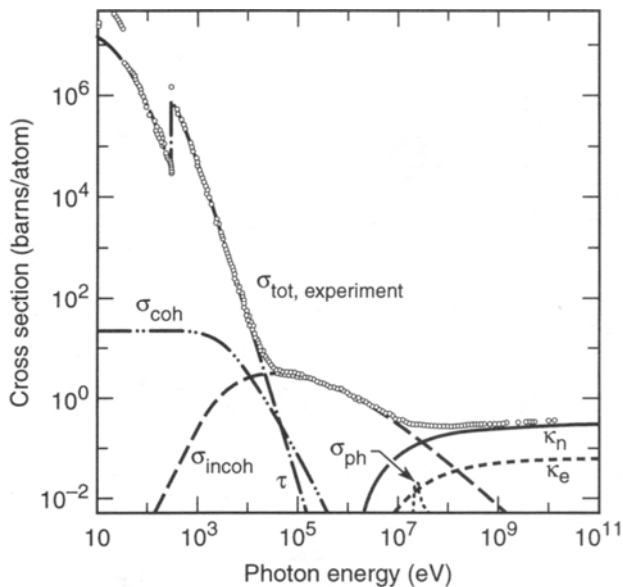
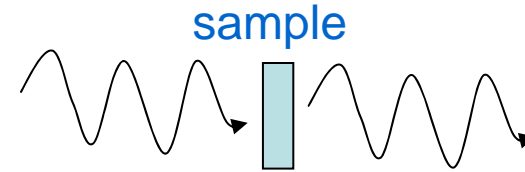
Local damage to structure

From: Garman and Owen (2005), *Acta Cryst. D*62, 32-47

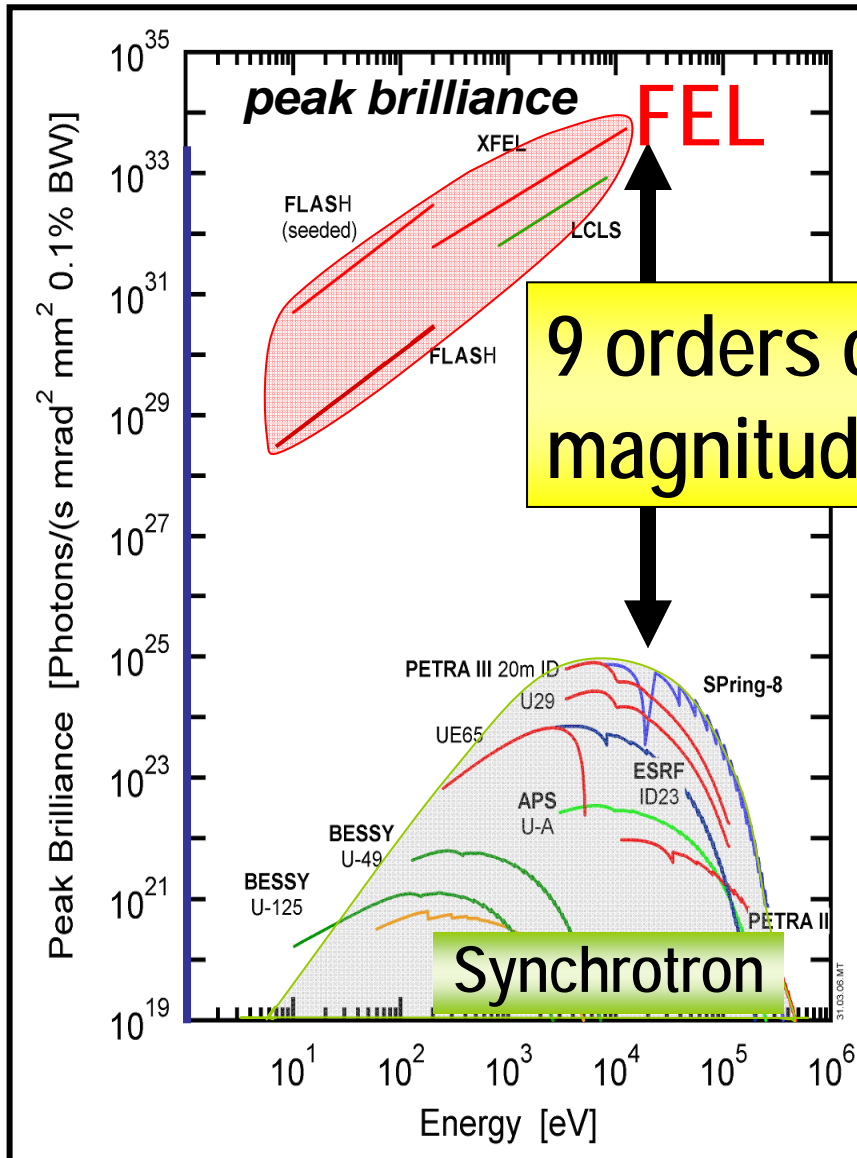
Nine out of ten X-ray photons cause radiation damage

At 12 keV ($\lambda=1.03 \text{ \AA}$)

- 10% Thomson scattering
- 10% Compton effect
- 80% Photoelectric effect



Free-Electron Lasers



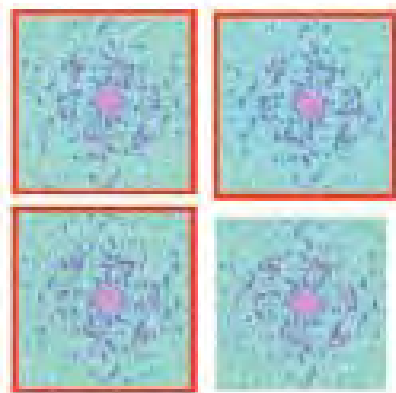
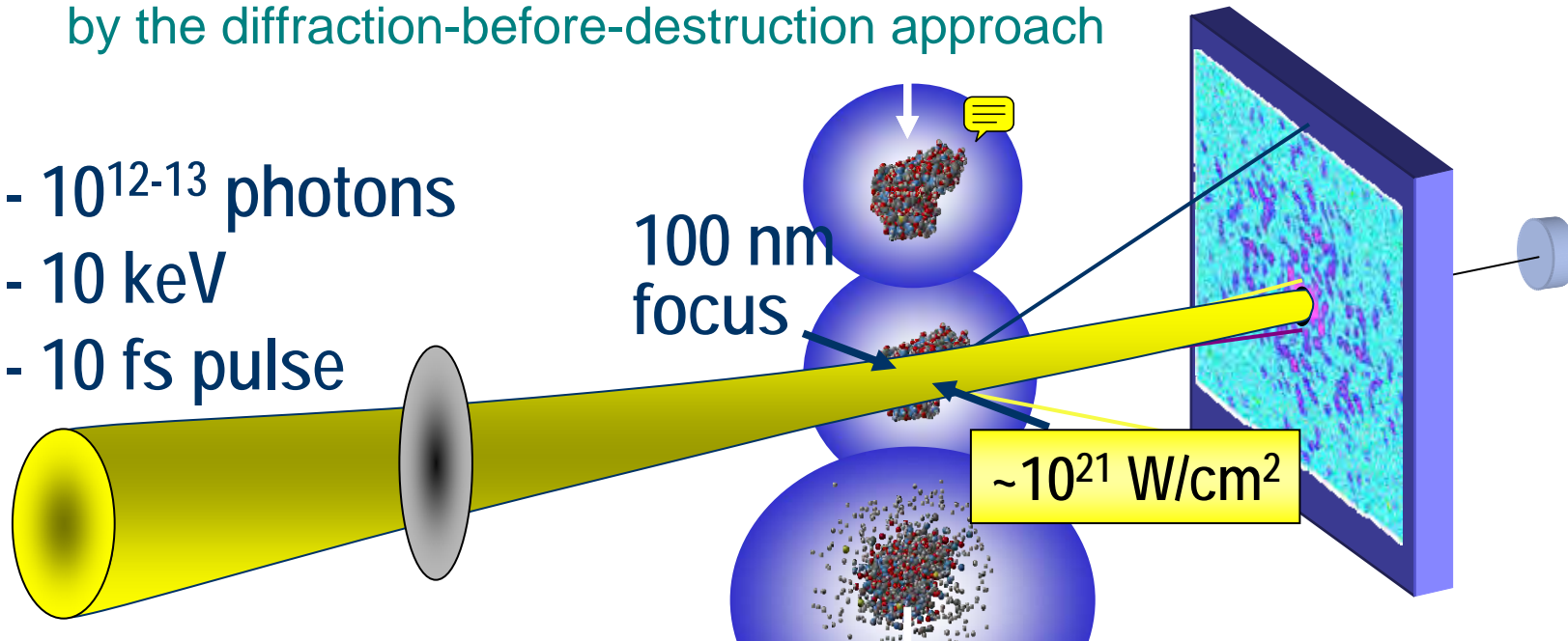
- FLASH: 2005
- Fermi: 2009
- LCLS: 2009
- SCSS/SACLA: 2011-14
- Fermi 2011
- XFEL: 2016
- PSI, LBNL, KVI, Shanghai,...

- 10^{12-13} photons \sim 3-400 fs pulses
- repetition rate: 120 Hz
- photon energy: 540 eV-10 keV
- transversally: fully coherent

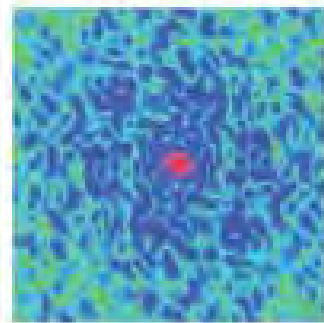
Coherent diffractive imaging of single particles

by the diffraction-before-destruction approach

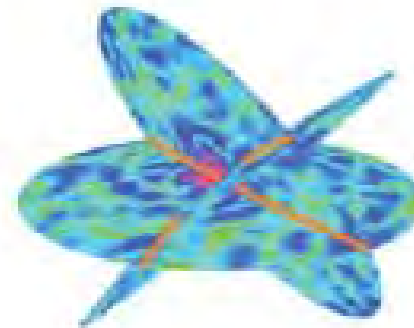
- 10^{12-13} photons
- 10 keV
- 10 fs pulse



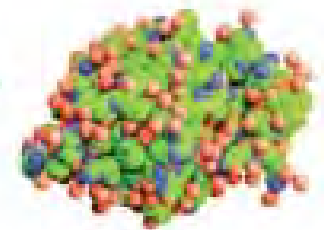
Classification



Averaging

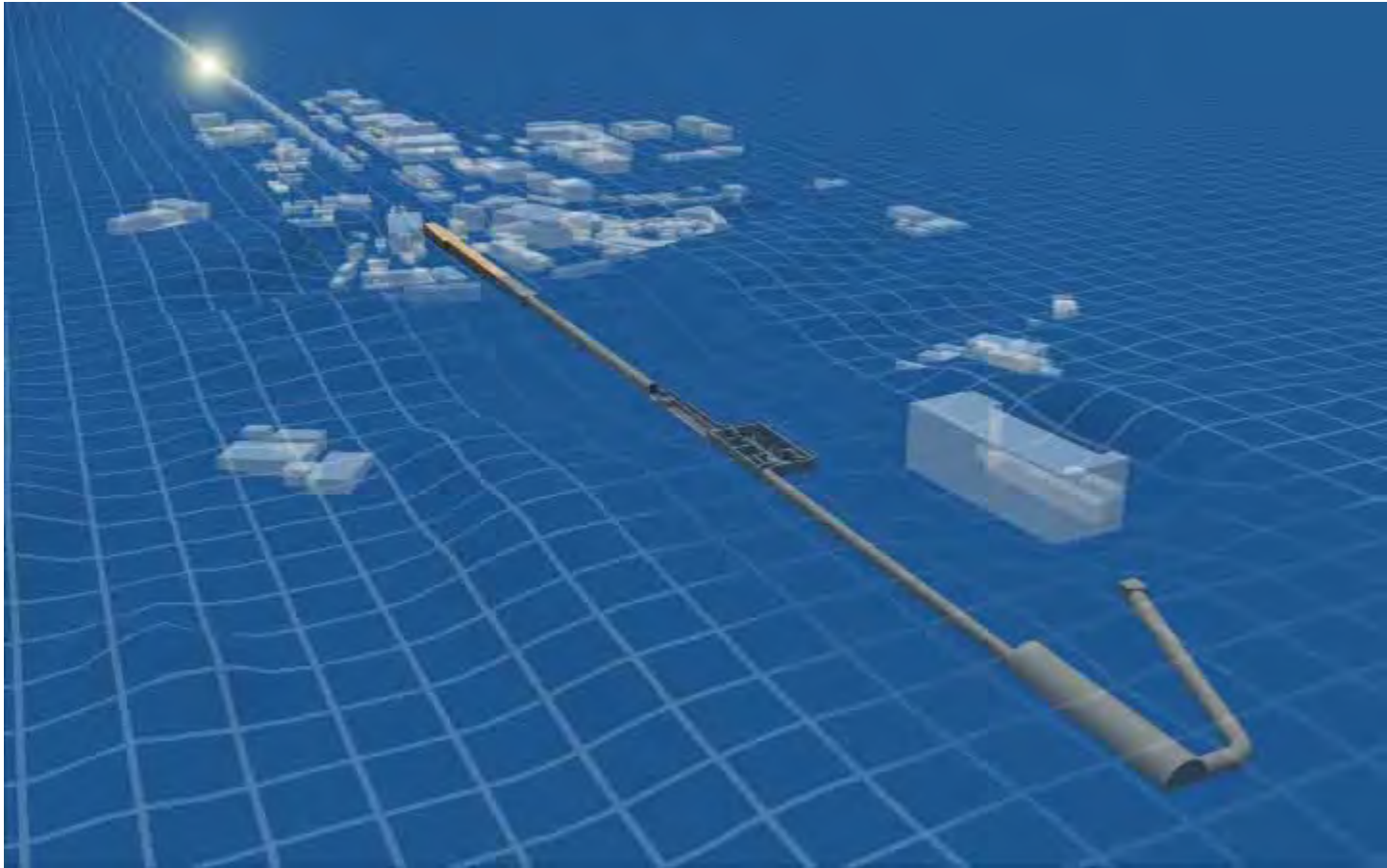


Orientation



Reconstruction

Calculations. in vacuum Neutze et al., Nature 2000 Chapman, Gaffney Science 2007

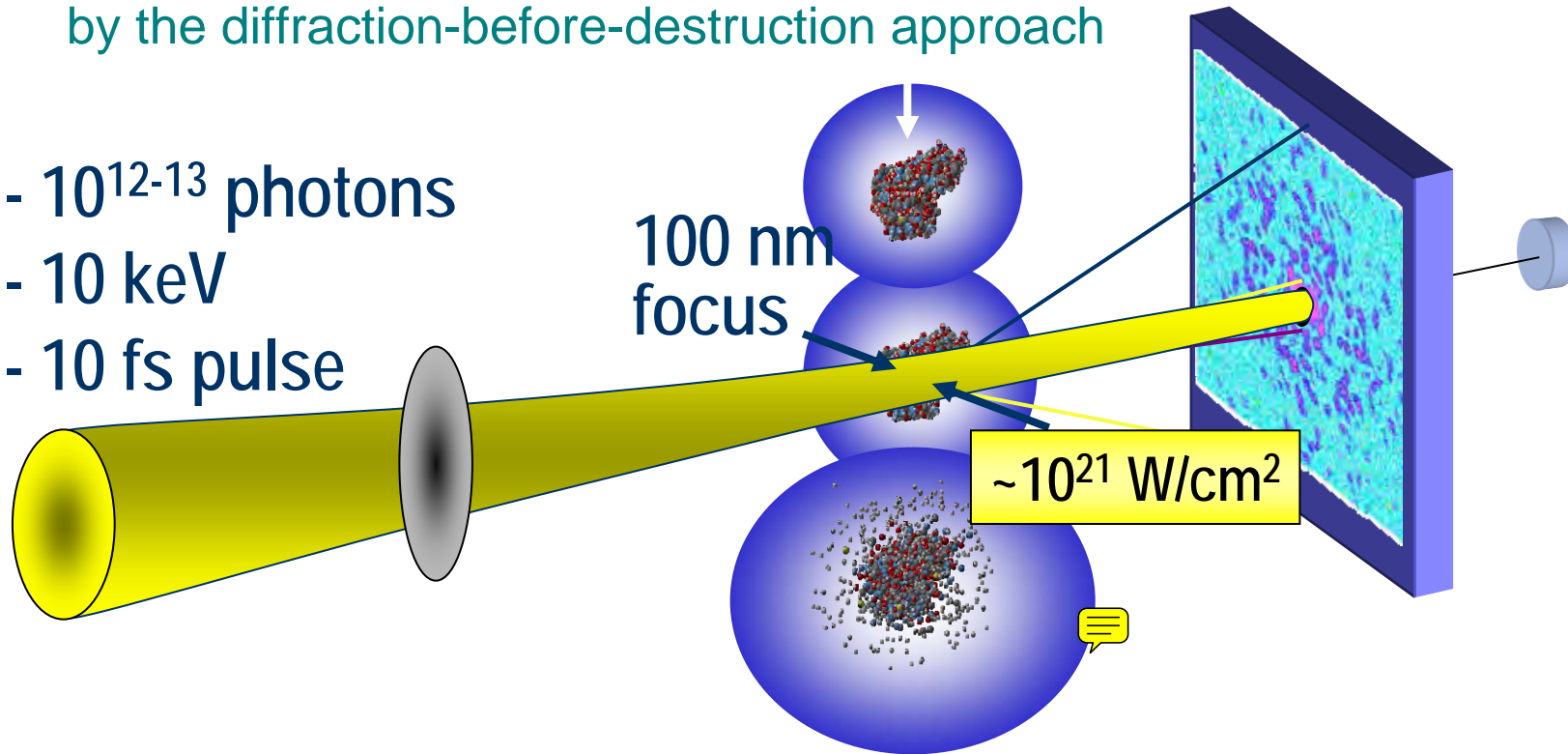


Coherent X-ray imaging at LCLS

Coherent diffractive imaging of single particles

by the diffraction-before-destruction approach

- 10^{12-13} photons
- 10 keV
- 10 fs pulse



- Which questions can be addressed ?
What resolution is required/useful?
- What is needed to make this work?
- Where do we stand?

Macromolecules are often difficult to crystallize

- Limited range of stability
- Conformational flexibility
- Tendency to aggregate

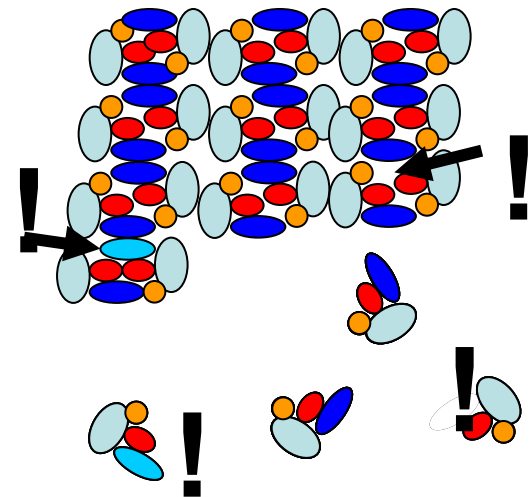
Supramolecular complexes are even more challenging

- big, multicomponent
- transient in cells
- often weakly bound
- often biochemically unstable
- often low abundance

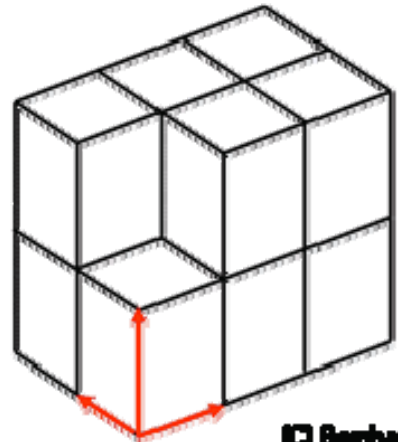


Characterization,
purification challenging
Structure determination difficult,
in particular crystallization

Probability for growth defects lower
for small crystals?



Crystal lattice

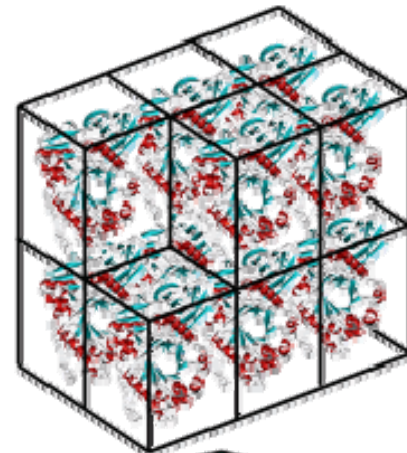


© Bernhard Rupp 2010

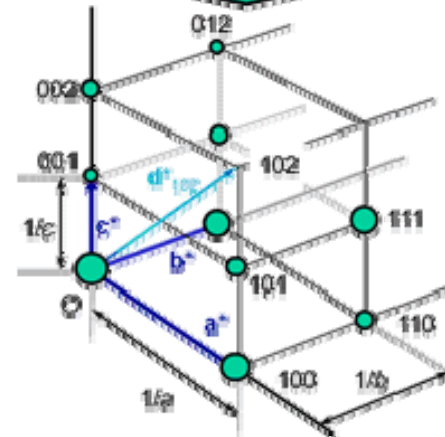
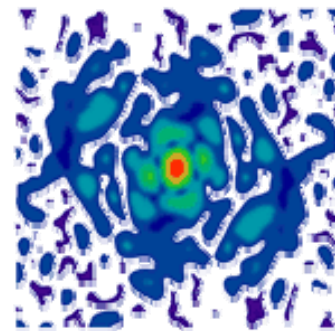
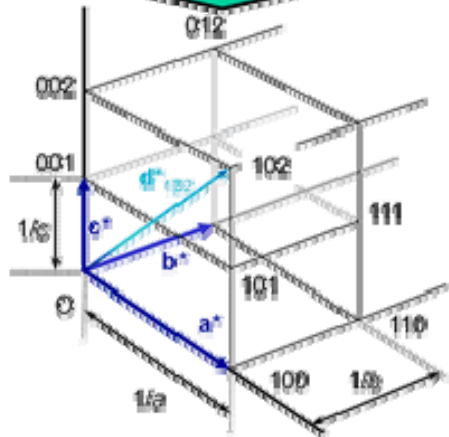
motif



crystal



Crystal Space
Real space



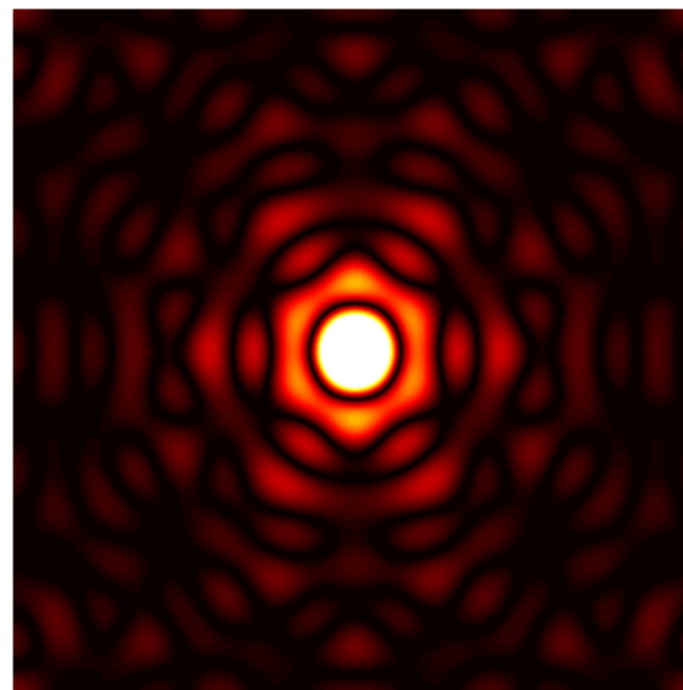
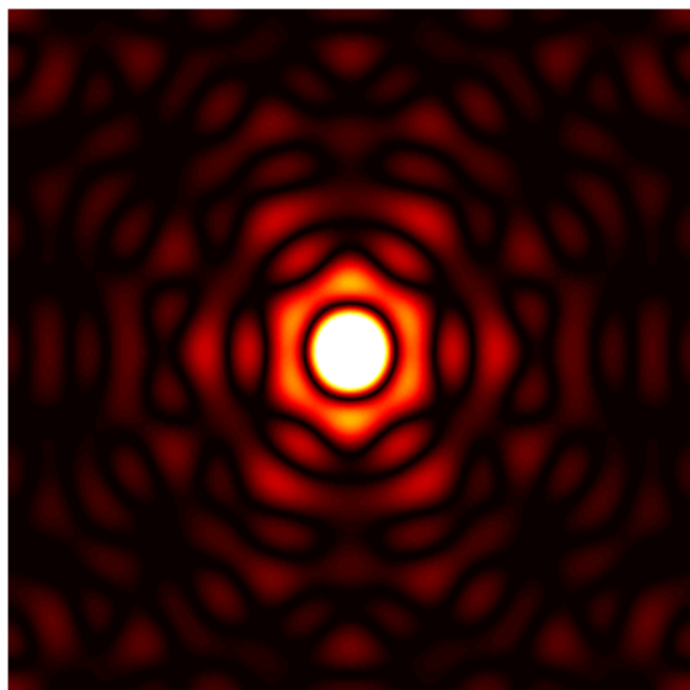
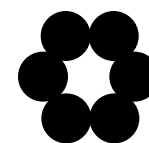
Diffraction Space
Reciprocal space

reciprocal lattice

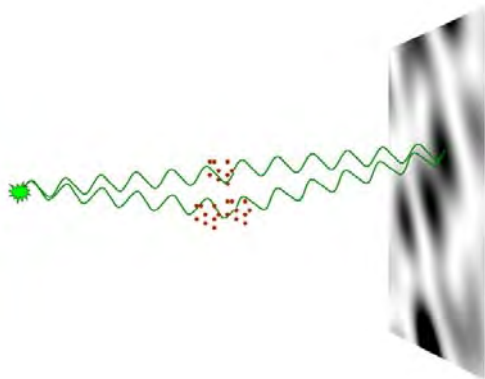
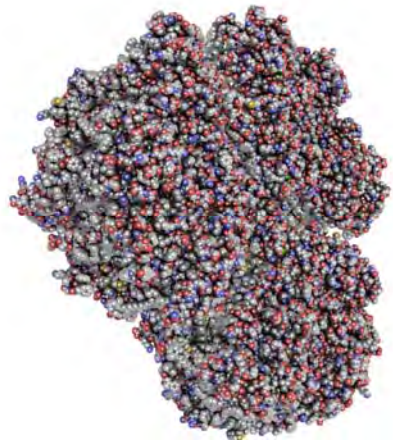
structure factors

From Rupp, Biomol. Crystallography

Benzene molecule(s)



Photosystem I 1x1x1 molecule

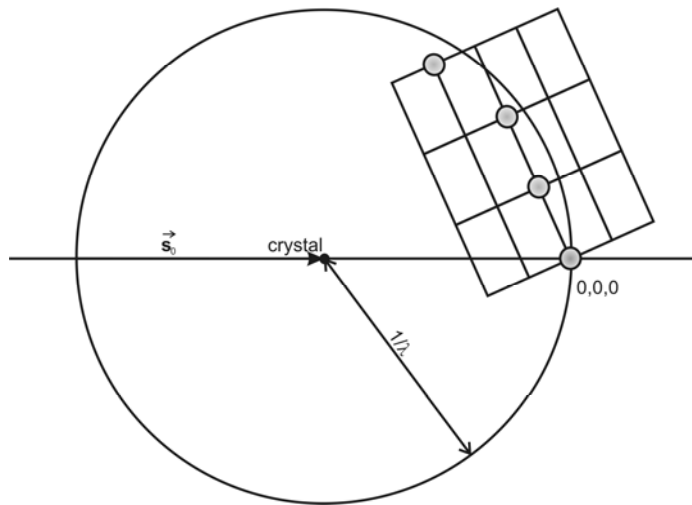


See NearBragg website

<http://bl831.als.lbl.gov/~jamesh/nearBragg/>

Indexing and integrating reflections: conventional methods

Ewald sphere



Rotation method

- rotate xtal over finite range
- calculate orientation matrix from observed spot positions

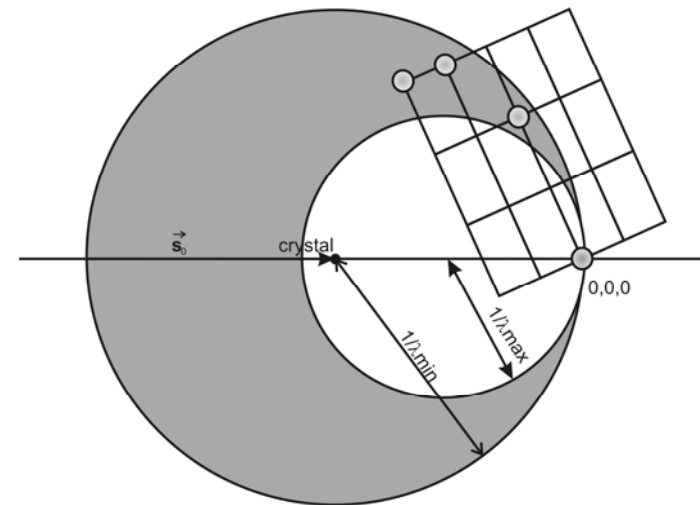
Can fully integrate whole reflections!

Powder method:

- Rotate powder of many xtals
- assign hkl from scattering angle of reflections (*if unique!*)

Fully integrates whole reflections!

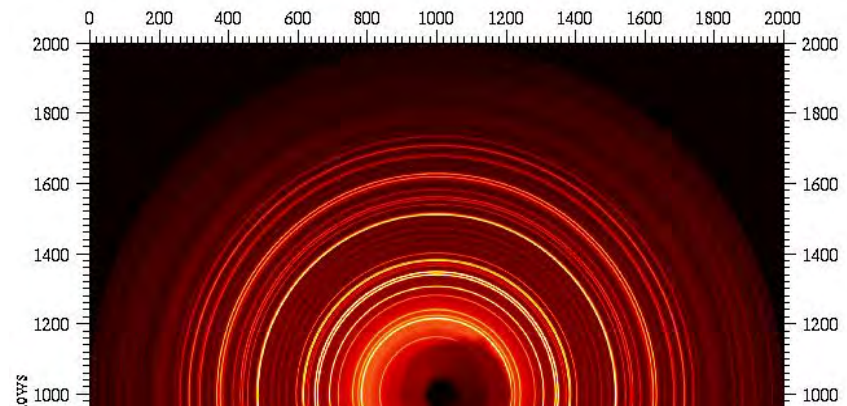
Ewald sphere



Laue method

- use polychromatic radiation
- calculate orientation matrix from observed spot positions

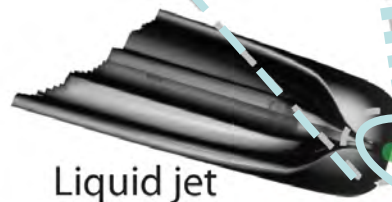
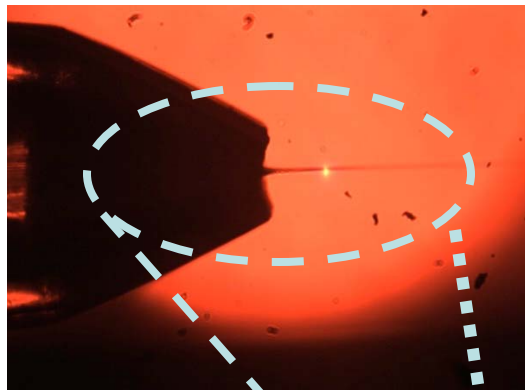
Can fully integrate whole reflections!



First serial femtosecond crystallography experiments at LCLS/AMO/CAMP

Chapman et al
Nature 470: 73 (2011)

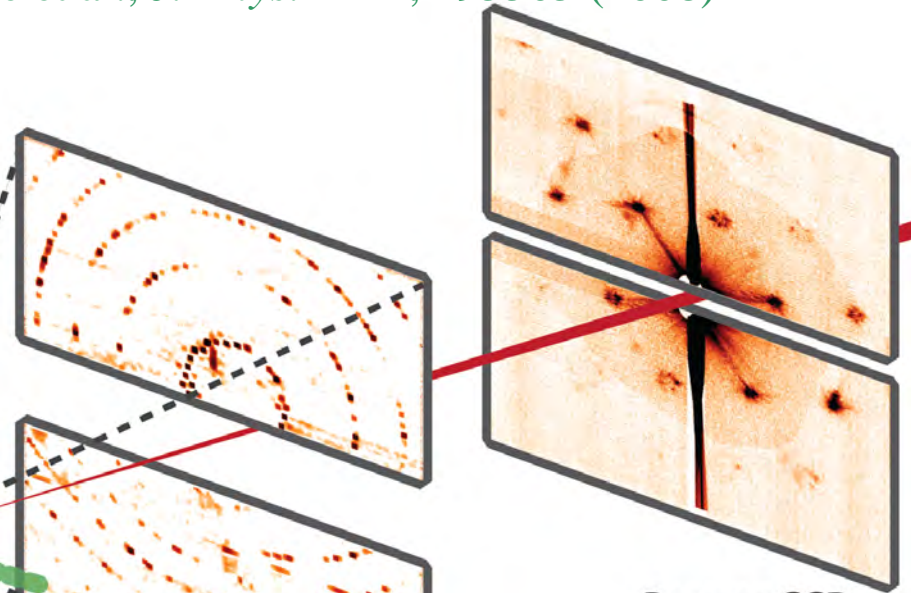
Gas focussed liquid jet:
4 μ m diam., flow rate 10- 14 μ l min, 10 ms/s
De Ponte et al., *J. Phys. D* 41, 195505 (2008)



$\lambda = 6.2-6.9 \text{ \AA}$, 1.8-2.0 keV
10-300 fs pulse duration
30 (60) Hz
LCLS X-ray pulses

10^{12} photons/pulse
900 J/cm²
Dose/pulse: 300-700 MGy

Interaction point
~ 7 μ m diam.
~ 4 μ m diam.



Front p-n CCD
(z = 68 mm)

Rear p-n CCD
(z = 564 mm)

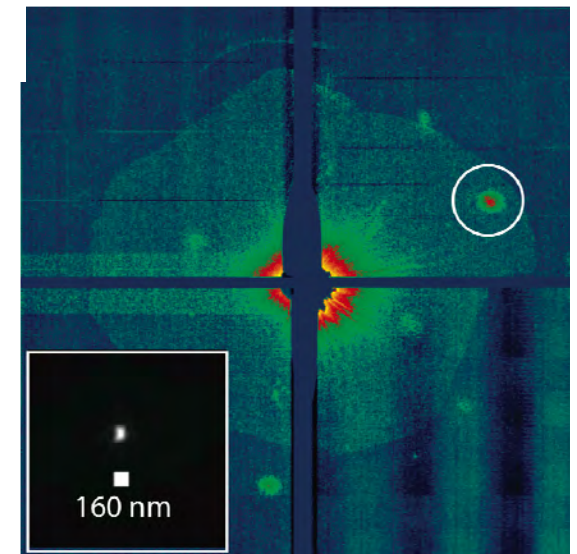
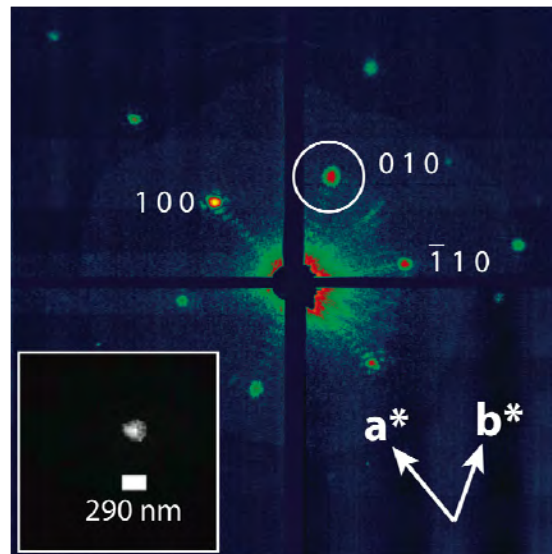
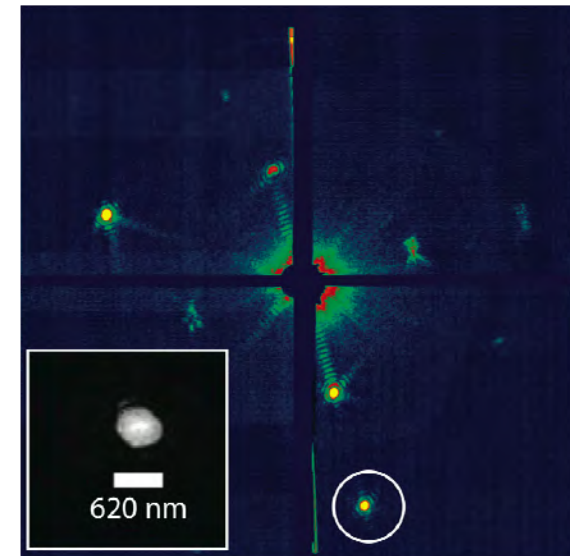
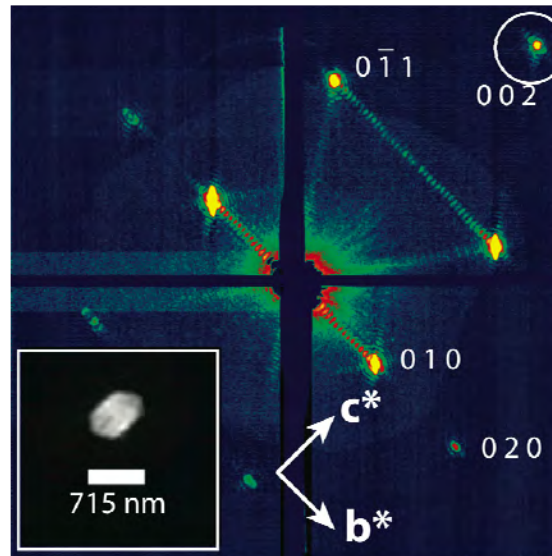
1800 (3600) patterns/min
5 TB in one night

Coherent features allow sizing and phasing of nanocrystals

N unit cells

N-2 fringes,

Intensity $\frac{\sin^2(N \pi \theta \lambda / b)}{\sin^2(\pi \theta \lambda / b)}$



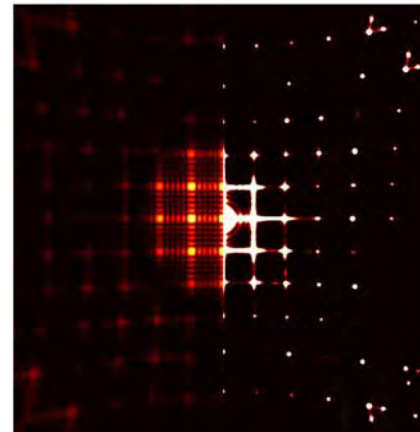
Chapman et al
Nature 470: 73 (2011)

Serial femtosecond crystallography

- Numerous shots of different crystals with possibly different sizes
- No *a priori* control over orientation
- Crystals effectively stand still during a 300 fs pulse
- Only part of reflection intersects Ewald sphere (“partials”, no “fullies”)
- Fringes rather than neat spots

6x6x6
unit cells

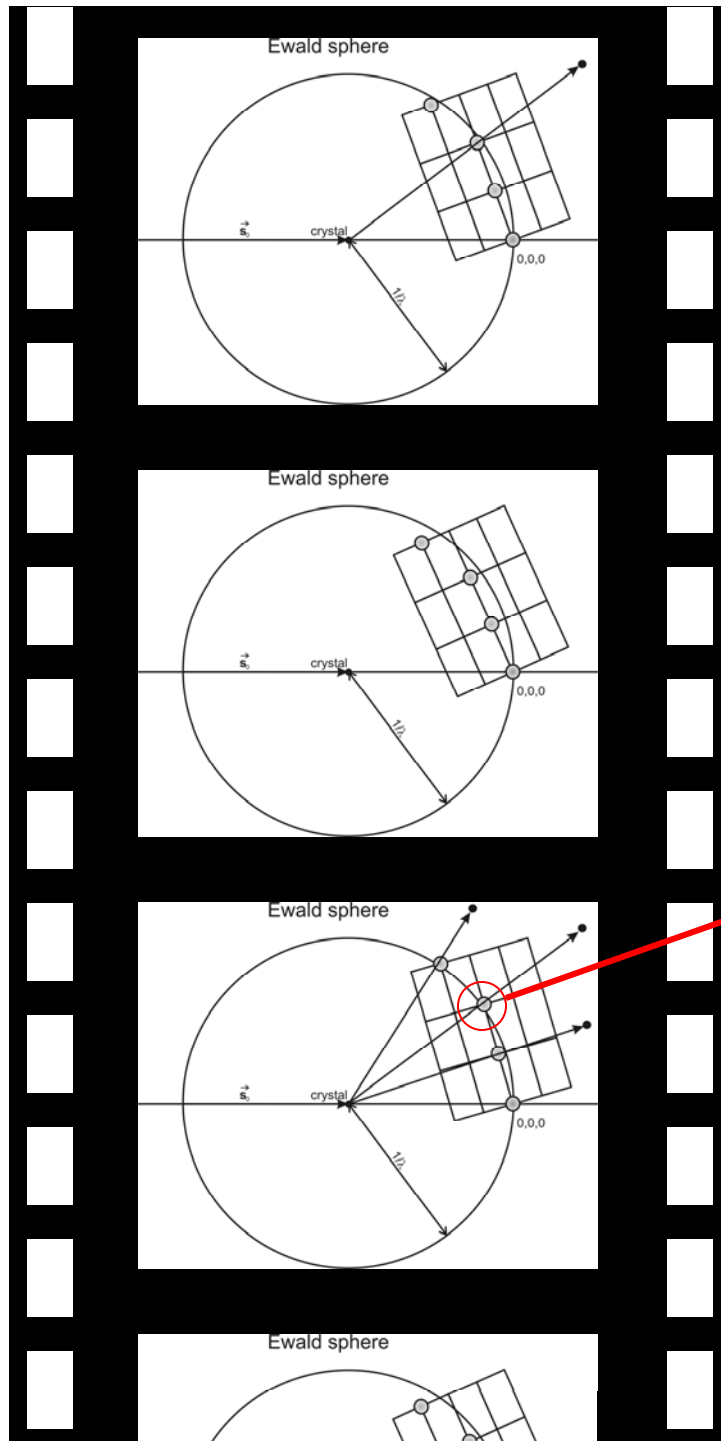
200x200x200
unit cells

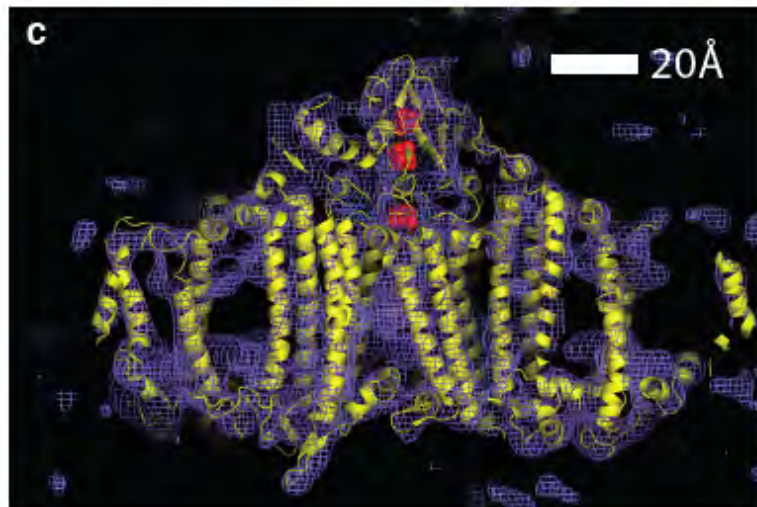
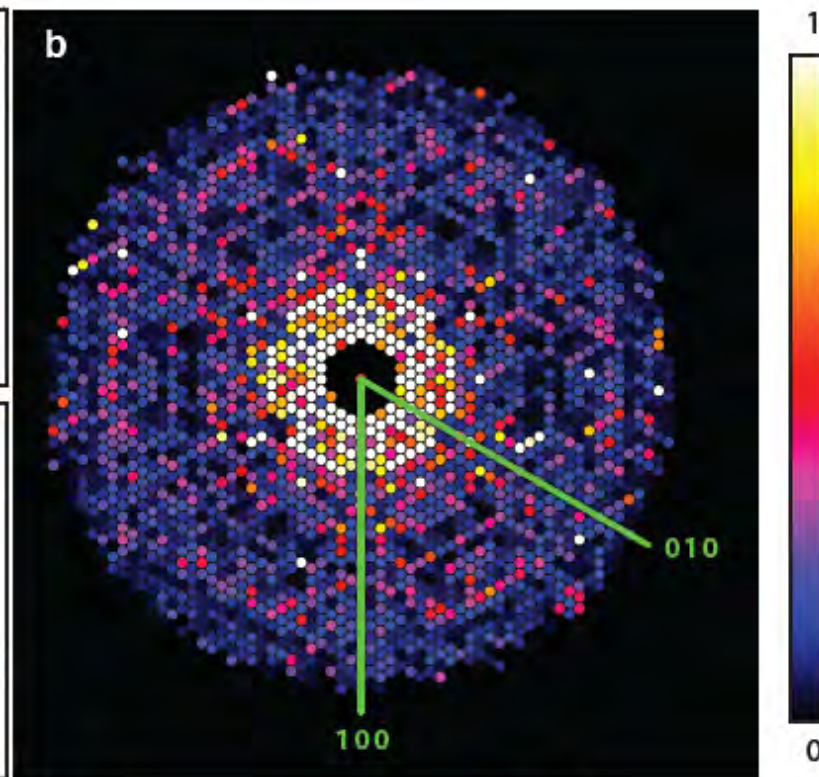
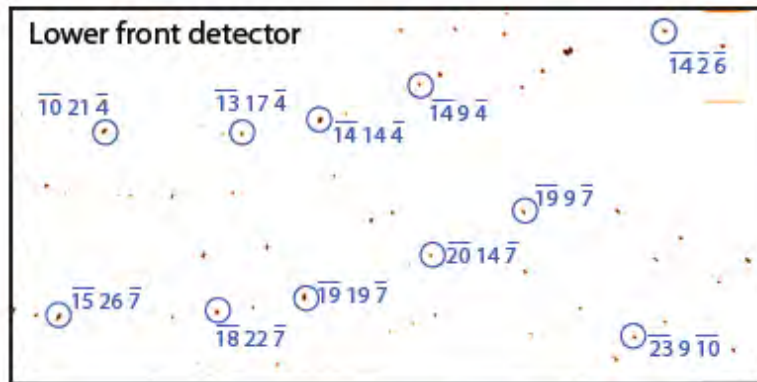
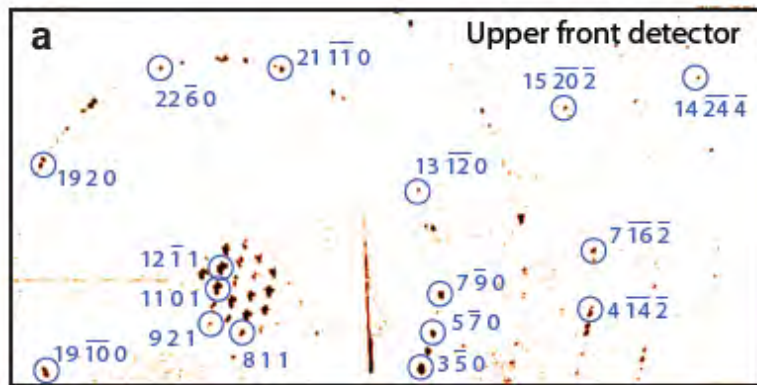


(Simulation software by Wolfgang Kabsch)

It is possible to do a *Monte Carlo* integration over multiple *indexed* femtosecond images and obtain a dataset of fully integrated reflections

Kirian *et al* (2010), *Optics Express*, **18**, 5713-5723:





Chapman et al
Nature 470: 73 (2011)

Coherent imaging experiments

Nanocrystals

Bragg reflections

at discrete angles

Bragg reflections

Quick check by calculation
of virtual powder patterns

Liquid jet

Single particles

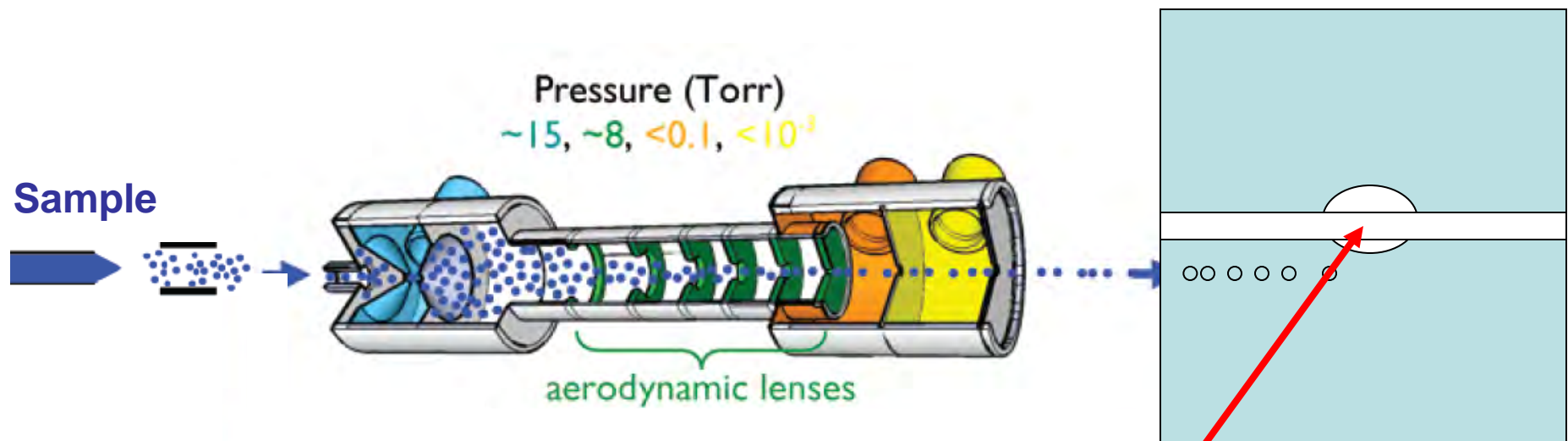
(Cells, viruses,
macromolecular assemblies)

Continuous transform

Aerosol injector

Imaging single non-crystalline particles

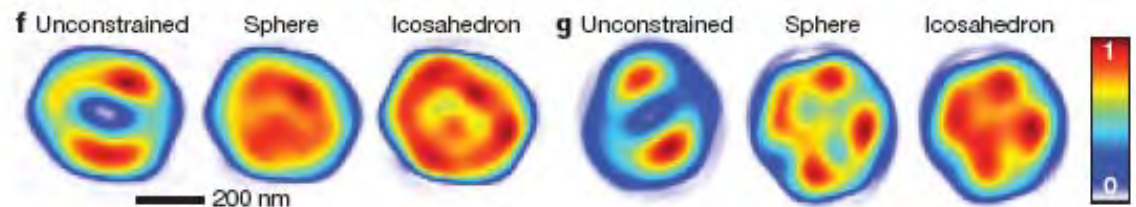
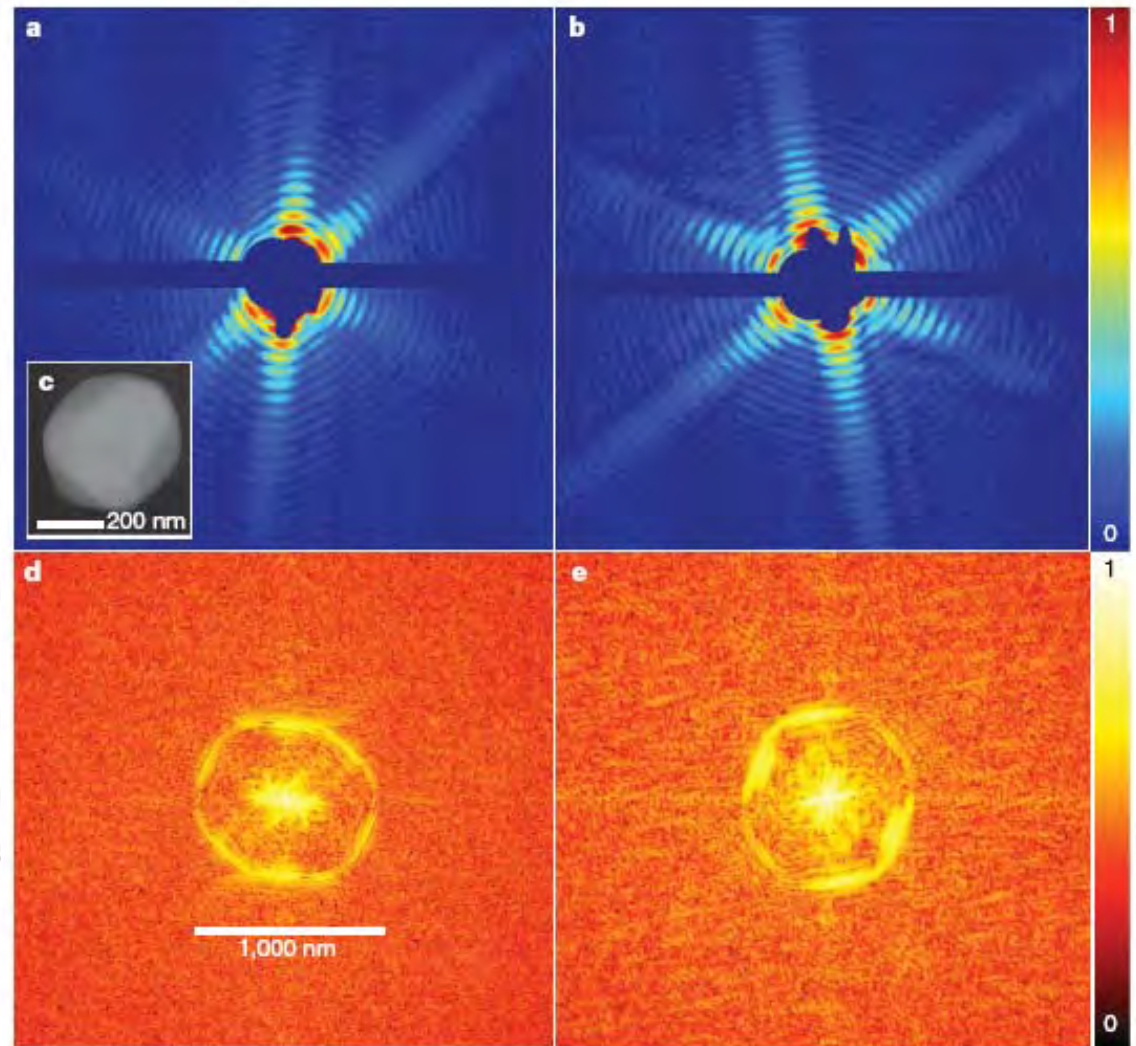
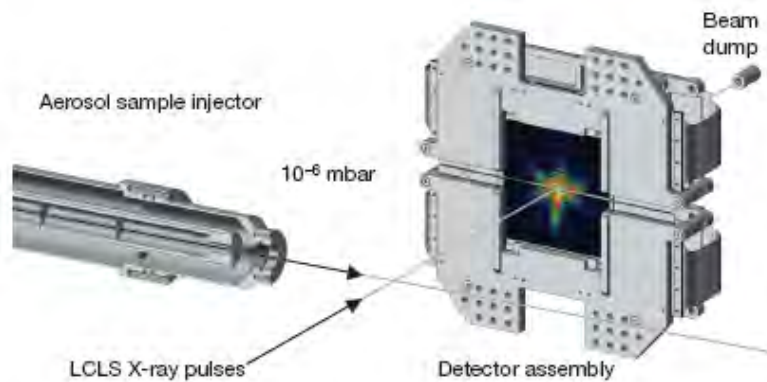
Aerodynamic lens stack for injection (Bogan et al., *NanoLetters*, **8**, 310-316 (2008))



Challenges

- Efficient aerosol generation
- Focusing / confinement of aerosol
- Hit identifier !

Single mimivirus particles intercepted and imaged with an X-ray laser



Seibert et al
Nature 470: 78 (2011)

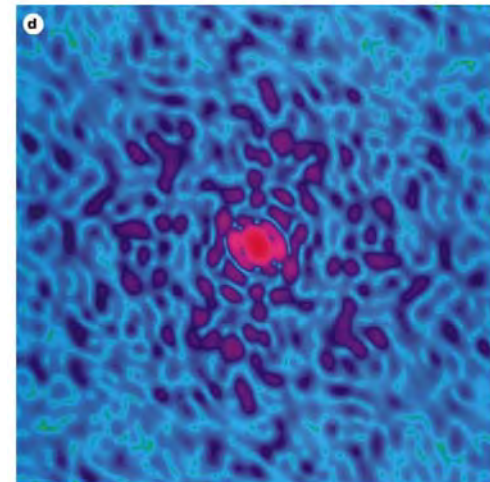
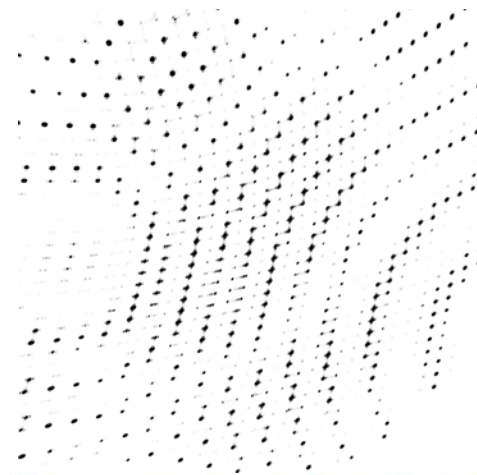
Potential for biomolecular imaging with femtosecond X-ray pulses

Diffraction patterns

Strong enough scatterer:

Nano-crystal
Bragg reflections

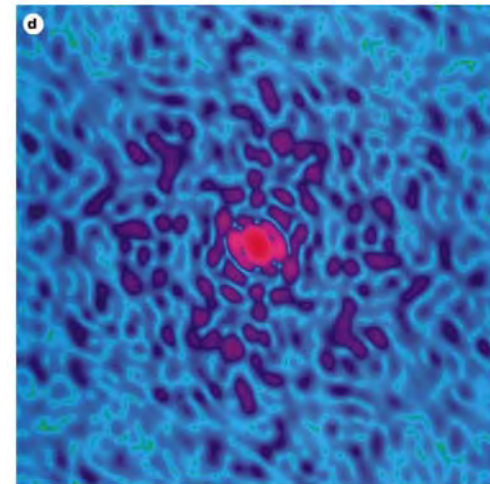
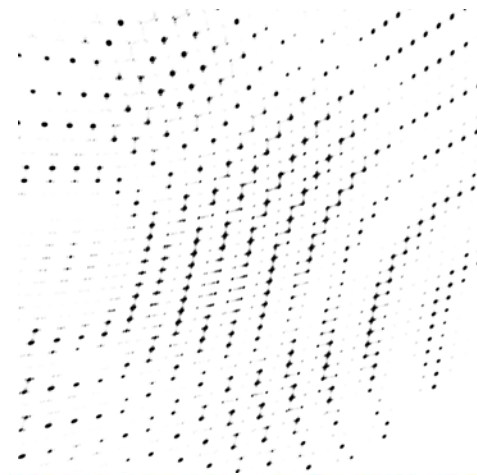
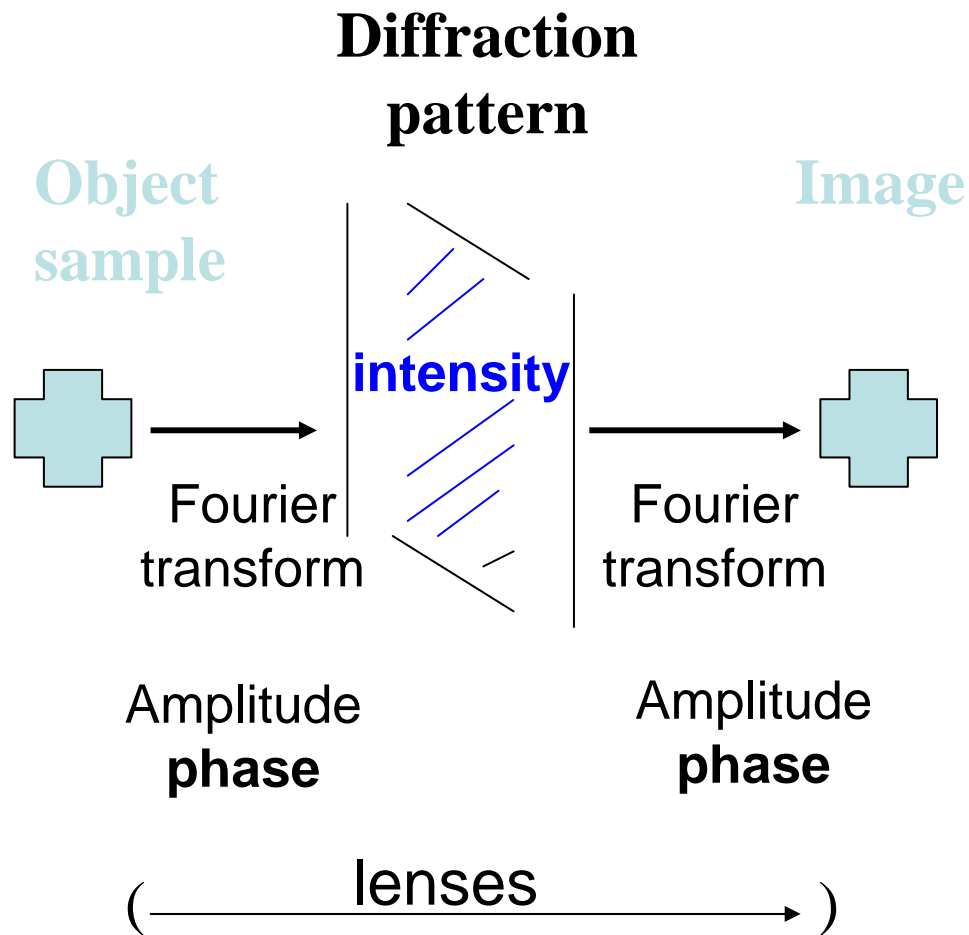
Single particle
Continuous transforms



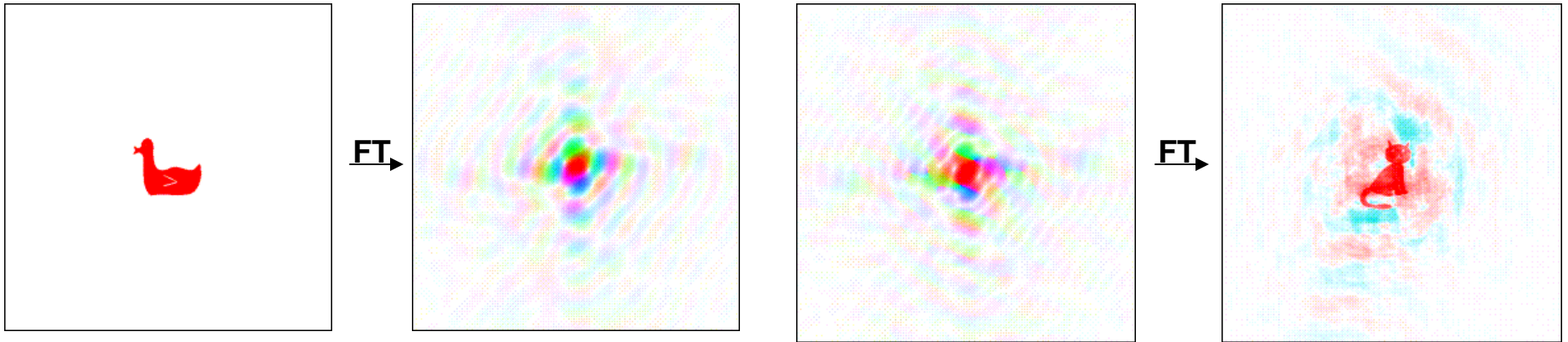
ature 406:752ff

Structure determination, imaging - basics

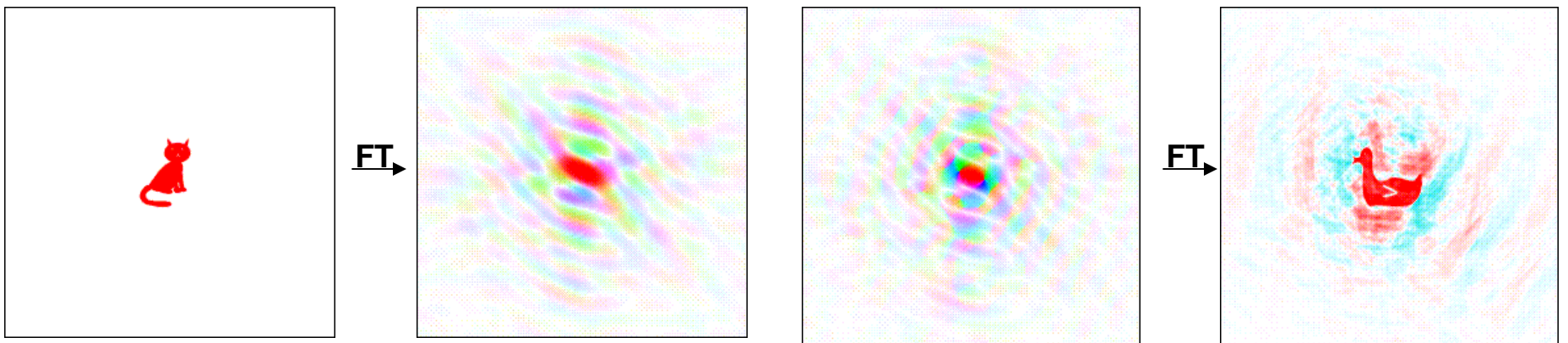
Diffraction patterns



Fourier Transforms

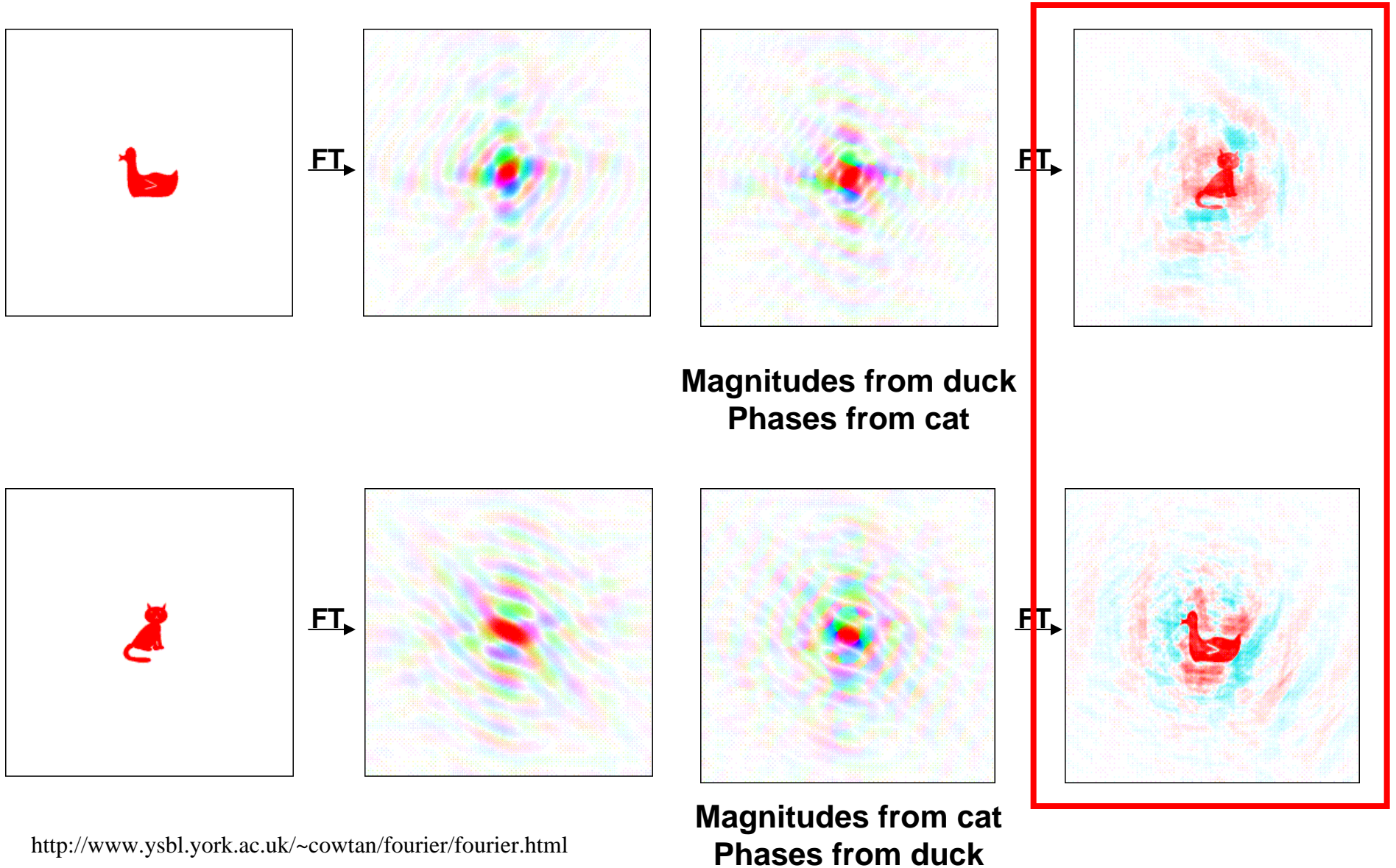


**Magnitudes from duck
Phases from cat**



**Magnitudes from cat
Phases from duck**

Since phases cannot be measured directly, structure determination by convent. X-ray crystallography not straight forward



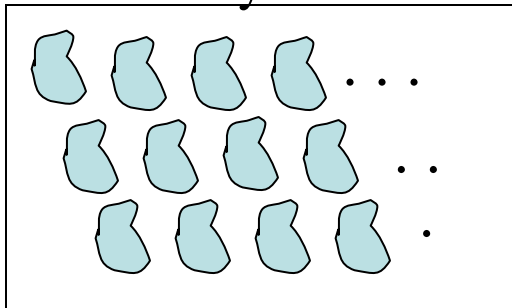
Structure determination by X-ray Crystallography

“large enough” single crystals

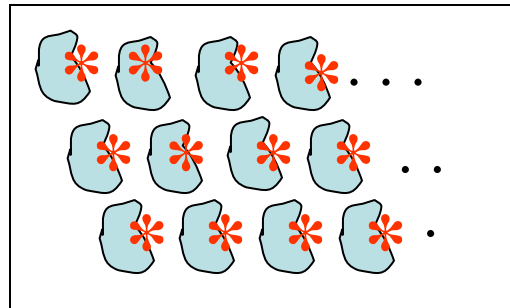
Phase problem

- Bragg peaks
- “Reference wave” via special scatterer

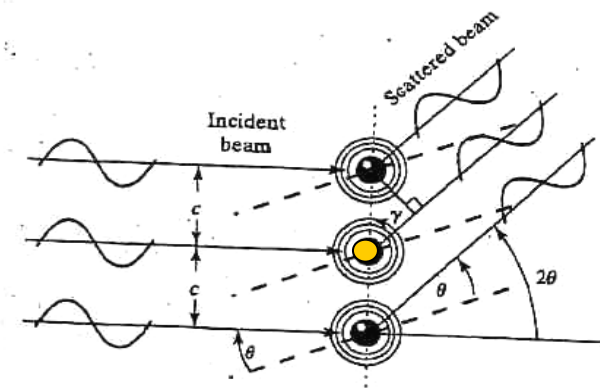
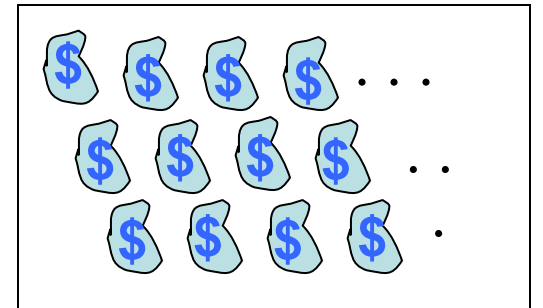
Native crystal



Crystal with reporter H_1 *



Crystal with reporter H_2 \$



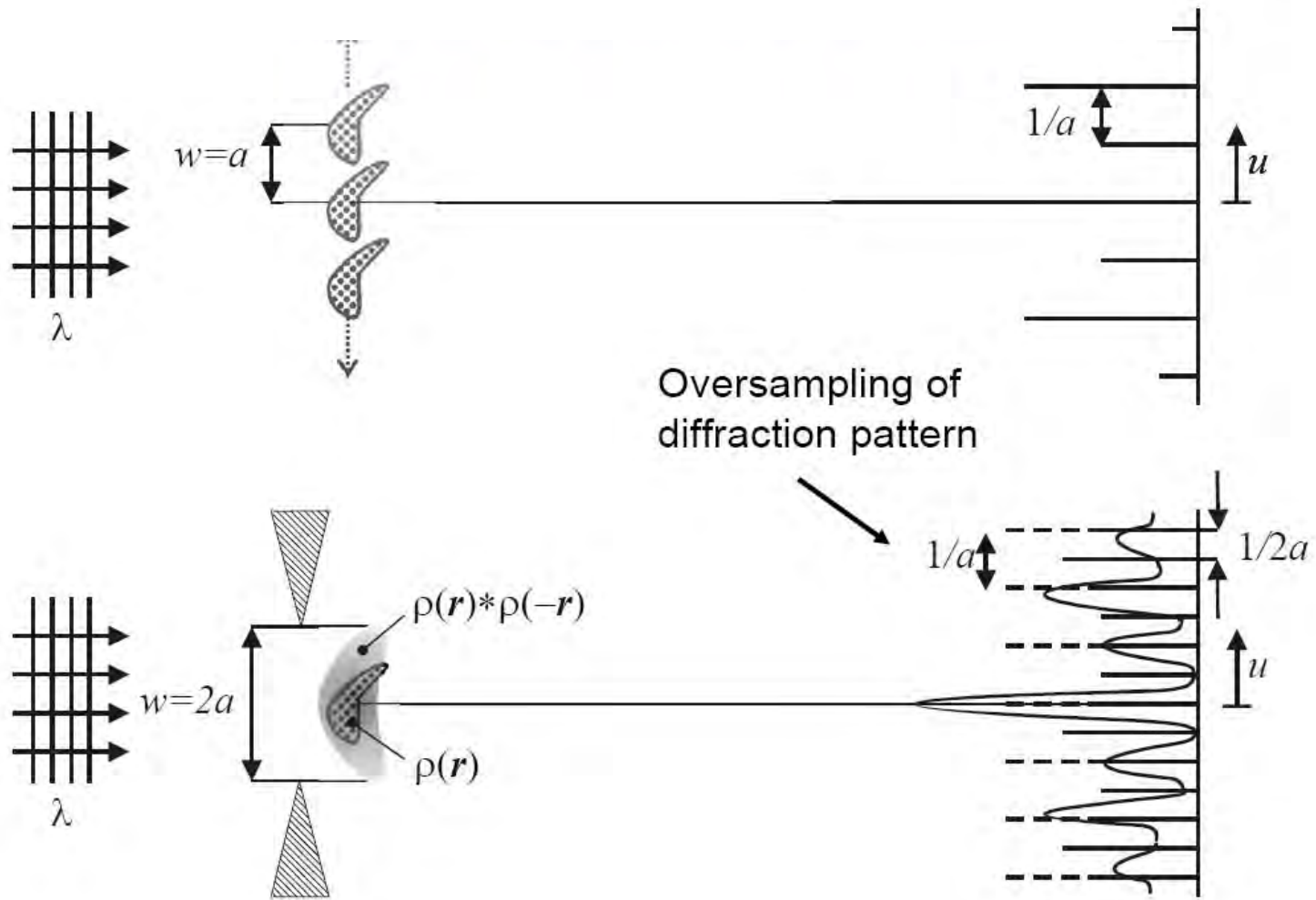
Chemical: \rightarrow scattering of atoms with high Z

Physical: \rightarrow absorption edges

Phasing approaches

- “large enough” single crystals
 - Bragg peaks
 - “Reference wave” via special scatterer
- Single particle or nanocrystal
 - Lens
 - holographic approach
 - continuous transforms
 - phasing by oversampling (lensless imaging)

'Solving the phase problem' by direct inversion of diffraction patterns

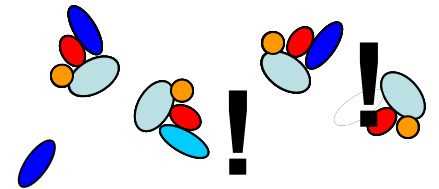


Many interesting biological problems around ...

... almost all challenging / limited in terms of sample availability ...
heterogeneity (composition, conformations)

Excellent, established methods for structure determination exist...

- nothing beats a crystal in terms of S/N, handling etc
- EM (cross sections!) is pretty good for single particle reconstruction:
 - excellent input/output ratio (sample efficiency)
 - problem of identifying and treating heterogeneity
 - radiation damage limiting
- FEL-based CDI promises to beat the radiation damage problem
 - problem of identifying and treating heterogeneity remains
 - needs to become competitive in terms of sample consumption:



Target preparation, cryogenic mount for fixed targets
Efficient delivery of sample to interaction point
Need higher repetition rates, more photons (better focusing)

Some challenges ...

- Samples
- Establishment of optimization of injection conditions for each sample (e.g. humidity for aerosols,)
- Focusing of X-ray beam, molecular beam
- Efficiency of hitting the sample, in particular for 100 nm beam
- Orientation of sample (single particle, ensemble), pre- and post interaction with X-ray beam
- Classification of the weak patterns, inherent flexibility of bio-samples for averaging and determination of rel. orientation
- Phase retrieval, 3D reconstruction
- Radiation damage (bond geometries, ionization states, making chemical sense of structures)
- Identification of appropriate “reliability factor” - how do we know the structure is correct, to what resolution?

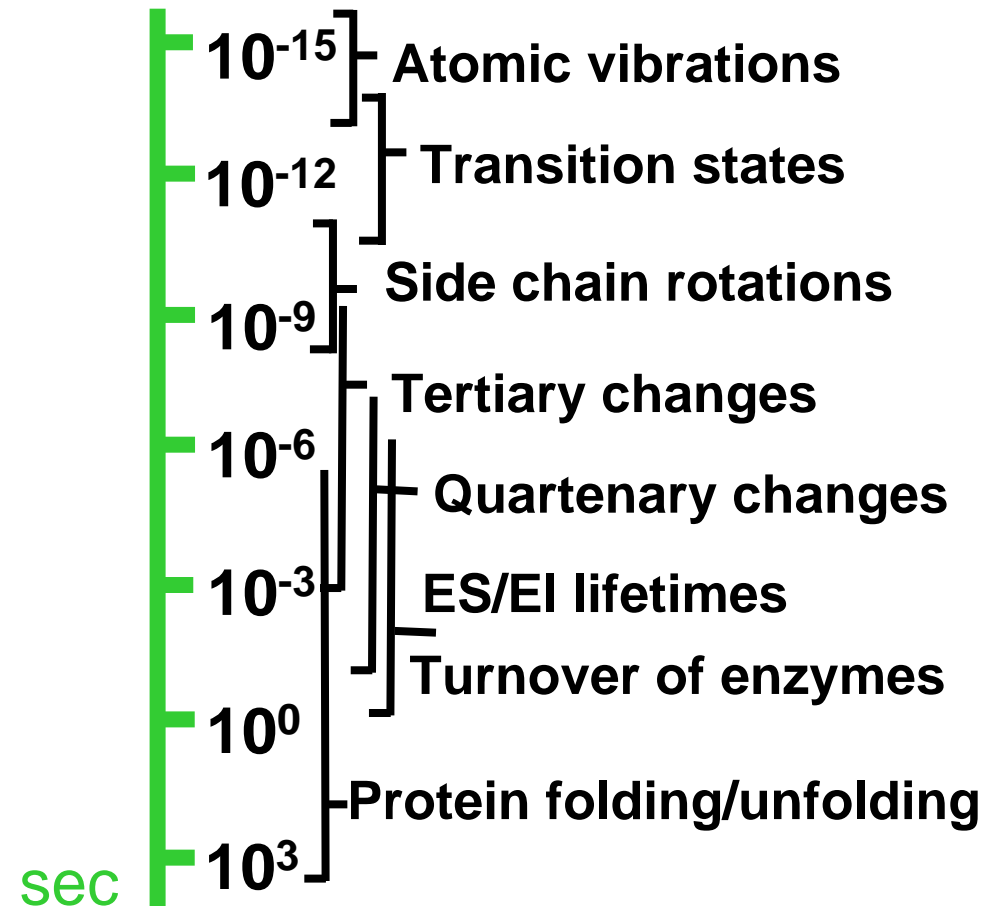
Biological applications of **ultrafast X-rays**

- Used time-resolution of data collection to compete with time-scale of **radiation damage** reactions
- How about study of other reactions? **Biochemical** reactions?

Time scales

Reaction initiation

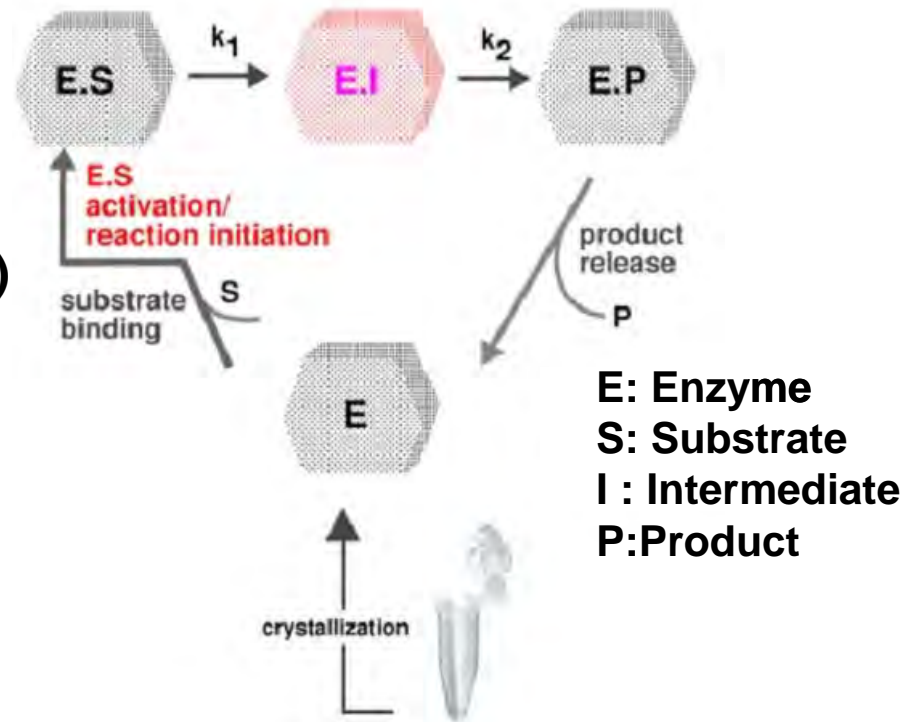
Crystallization takes “forever”
Data collection takes “forever”
Temporal & spatial average



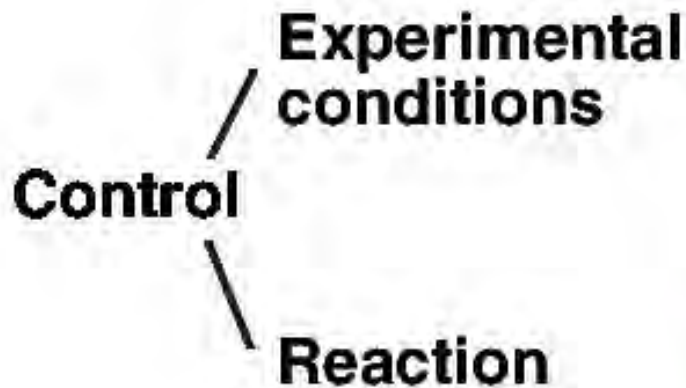
Crystallography – a static method?

Crystallization takes forever - yes, so don't crystallize active complex, find reaction trigger

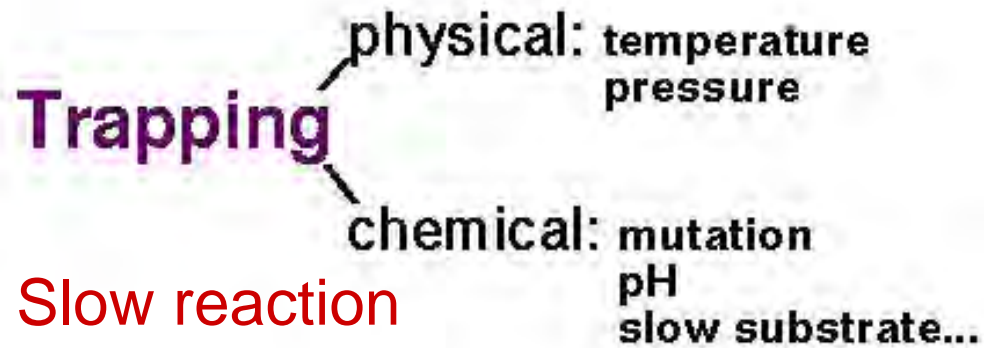
Trigger
Diffusion (Substrate, cofactor, pH jump ...)
Photolysis (caged compounds, naturally light sensitive systems)
Radiolysis
...



Data collection approaches



- Time-resolved crystallography



Slow reaction

- Kinetic crystallography

Data collection approaches

Fixed wavelength
Rotating crystal

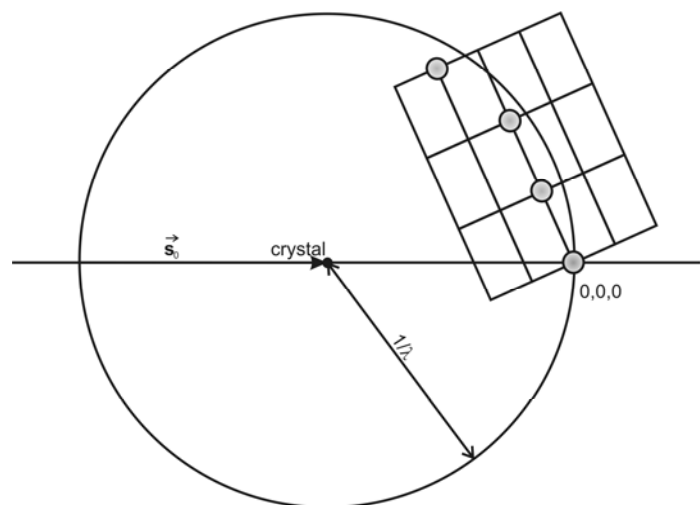
Monochromatic
X-ray diffraction

Wavelength range,
Stationary crystal

Polychromatic
X-ray diffraction

$$\leftarrow 2 \cdot d \cdot \sin \theta = n \cdot \lambda \rightarrow$$

Ewald sphere

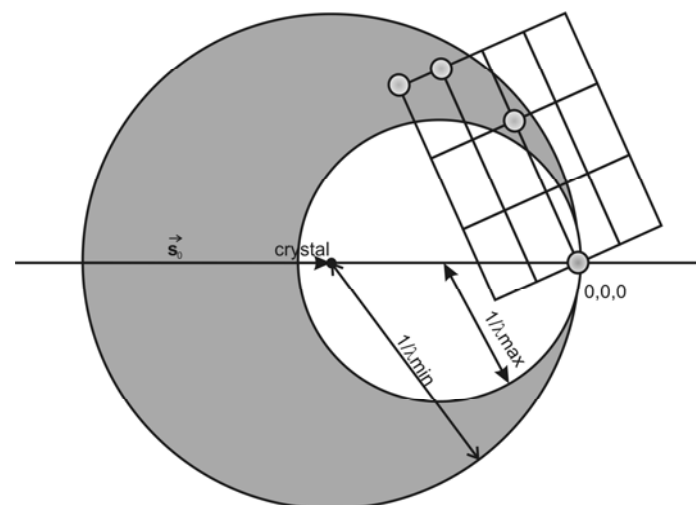


Rotation method

- rotate xtal over finite range
- calculate orientation matrix from observed spot positions

Can fully integrate whole reflections!

Ewald sphere



Laue method

- use polychromatic radiation
- calculate orientation matrix from observed spot positions

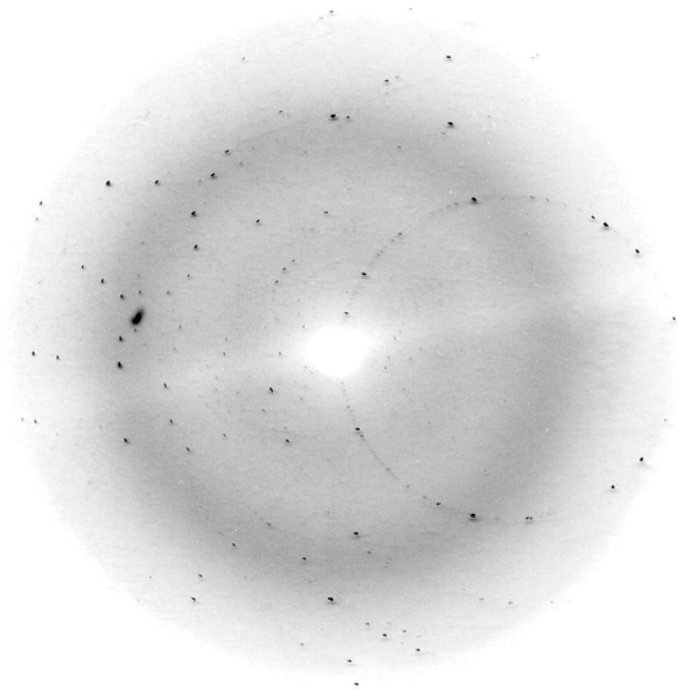
Can fully integrate whole reflections!

Data collection approaches

Fixed wavelength

Rotating crystal

Monochromatic
X-ray diffraction

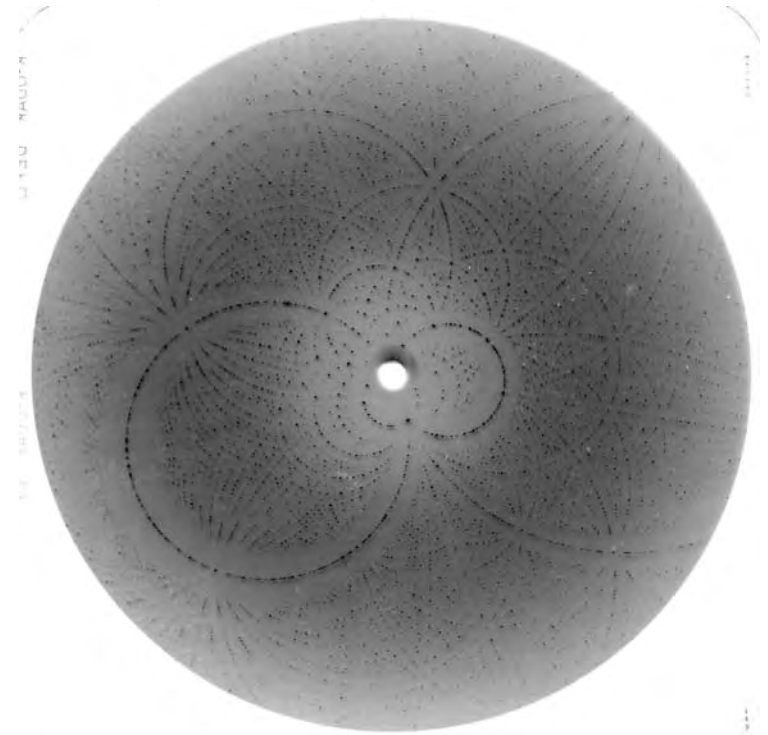


$$\leftarrow 2 \cdot d \cdot \sin \theta = n \cdot \lambda \rightarrow$$

Wavelength range,

Stationary crystal

Polychromatic
X-ray diffraction



Synchrotron storage rings Intensive X-ray sources



Synchrotron storage rings

e.g. European Synchrotron Radiation Facility

Energy: 6 GeV

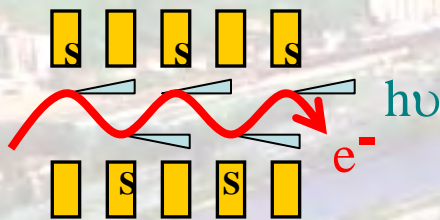
Max. current: 200 mA

Revolution frequency: 355 kHz

Number of bunches: 1-992

Time between bunches: 2816-2.8 ns

- Intensity
- Time-structure
- Coherence



Insertion device



$$P(\lambda, \gamma, \psi_0, \rho, \Delta\lambda, I_B, \Delta\psi, \Delta\theta) = \int_{-\psi_0 + \Delta\psi}^{+\psi_0 + \Delta\psi} \frac{2}{3} \frac{e_0 \Delta\lambda \Delta\theta I_B \rho^2}{\epsilon_0 \beta \lambda^4 \gamma^4} [1 + (\gamma\psi)^2]^2$$

$$\times \left[K_{2/3} [\xi(\lambda, \psi)]^2 + \frac{(\gamma\psi)^2}{1 + (\gamma\psi)^2} K_{1/3} [\xi(\lambda, \psi)]^2 \right]$$

150 ps

Schwinger equation

Synchrotron Radiation in Crystallography

- very intense
- Highly collimated

> Weakly diffracting crystals

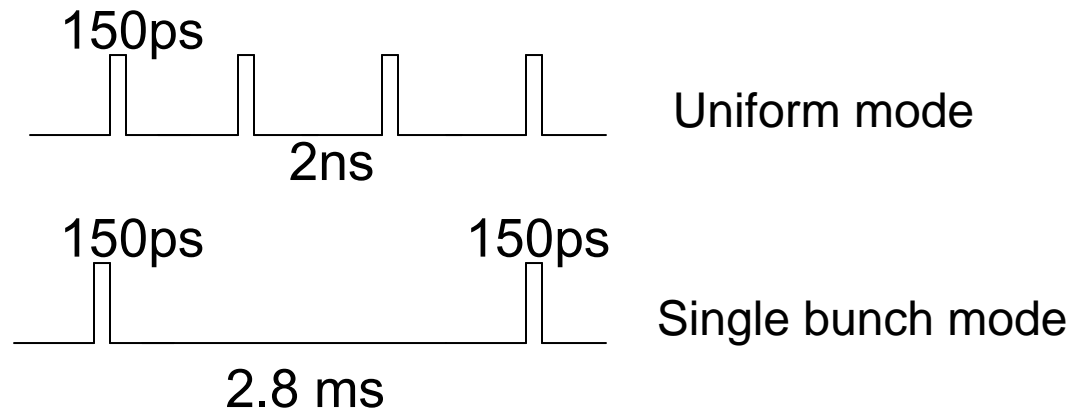
- Polarized

- Polychromatic, tunable
(Hard x-rays → IR)

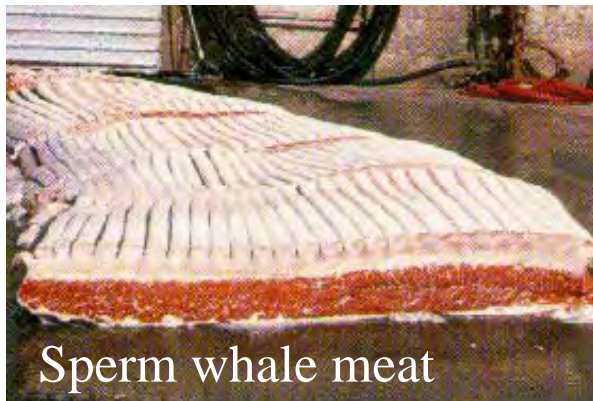
< Absorption
Laue diffraction (0.4-2Å)

- Time-structure

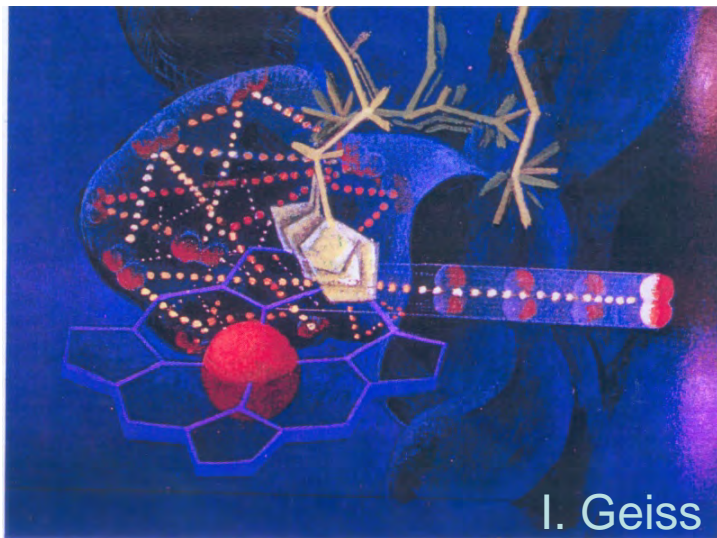
Time-resolved experiments



Light-triggered ligand dissociation from myoglobin is a model reaction to study protein dynamics



Small heme protein (18kD) found in muscle binds oxygen O₂, carbon monoxide (CO) and nitric oxide (NO) reversibly
Follow the initial events upon iron CO bond cleavage in carbonmonoxy myoglobin by time resolved crystallography

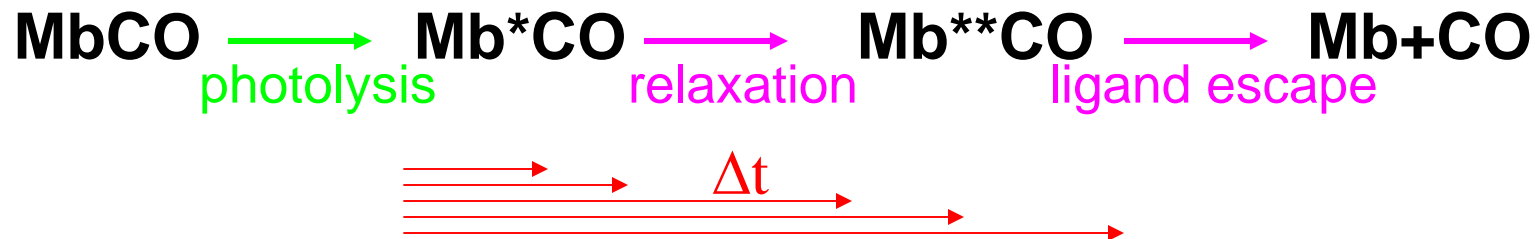


- Prompt, laser-triggered structural change photodissociation of bound CO
- Quantum efficiency ~1
- Fast, fully reversible recovery
CO rebinds in ~ 2 ms
- Diffracts to high resolution at room temperature
- Dynamics studied by spectroscopy, comput. modelling, theory, including characterization by kinetic & time-resolved crystallography

Watching a Protein as it Functions with 150-ps Time-Resolved X-ray Crystallography

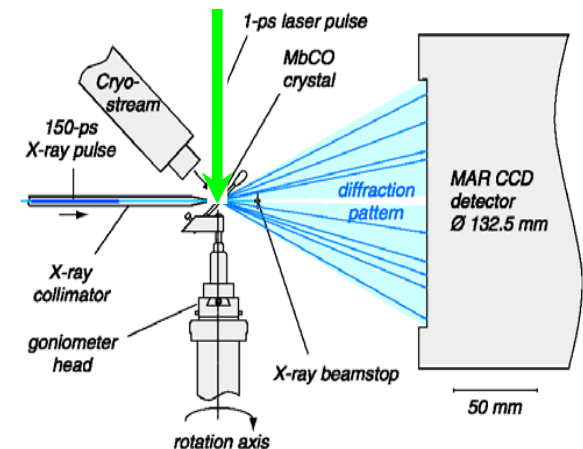


Friedrich Schotte,¹ Manho Lim,² Timothy A. Jackson,³
Aleksandr V. Smirnov,¹ Jayashree Soman,⁴ John S. Olson,⁴
George N. Phillips Jr.,⁵ Michael Wulff,⁶ Philip A. Anfinrud¹

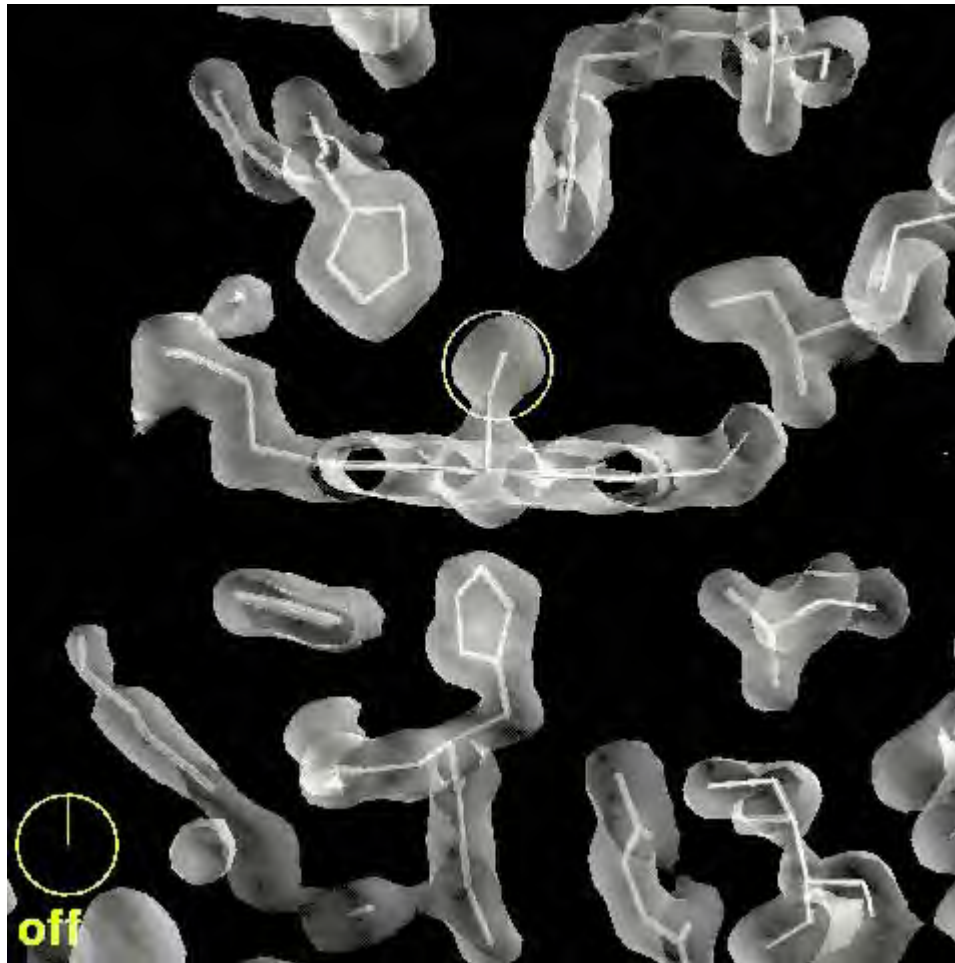


Pump-Probe Experiment:

Variable delay Δt between
ps **pump** laser pulse and
150 ps X-ray **probe** pulse



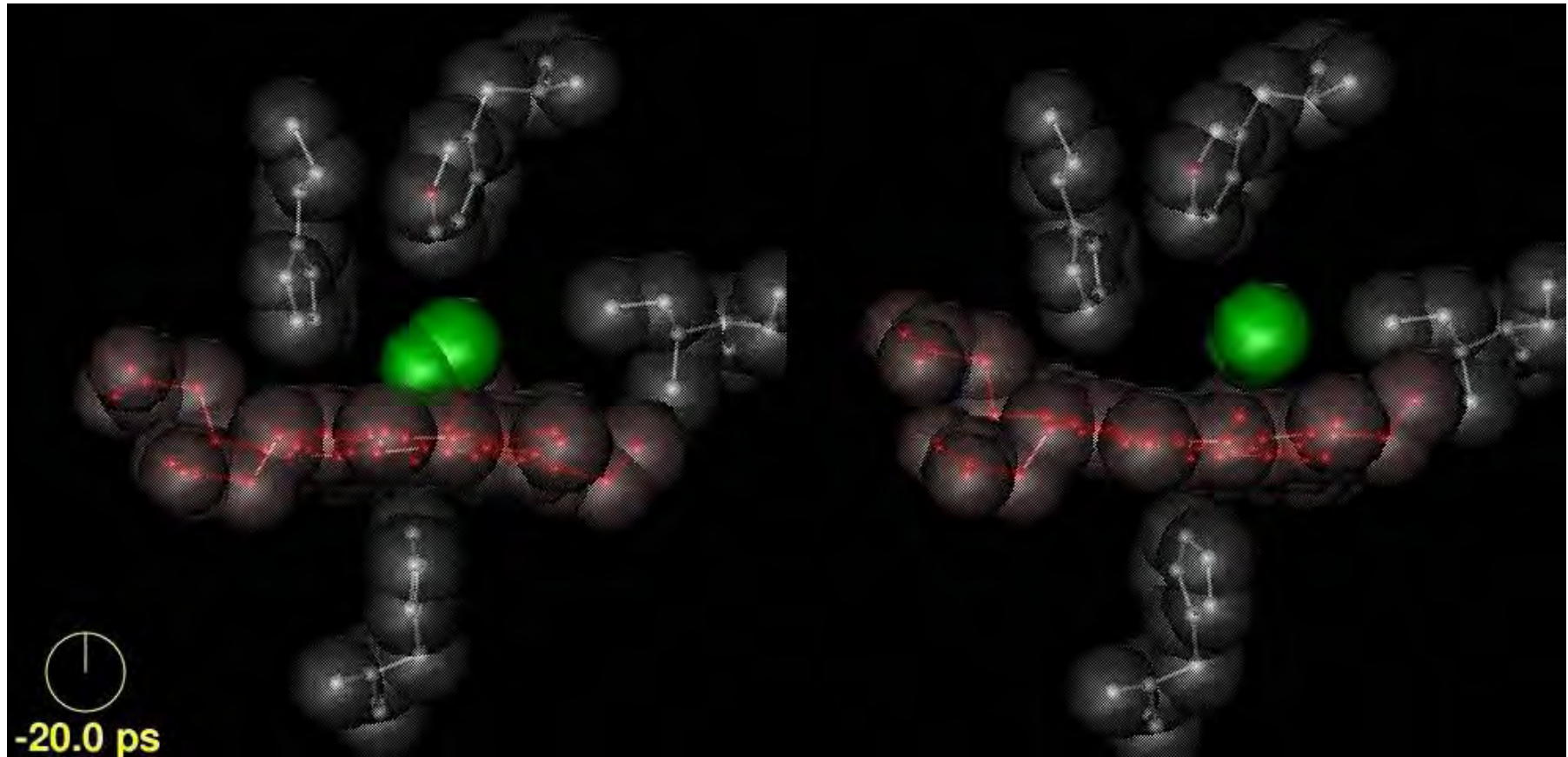
Watching CO-dissociation in Myoglobin L29F with 150 ps Time-resolved X-ray Crystallography



Electron density:
— before
— Δt after
photolysis

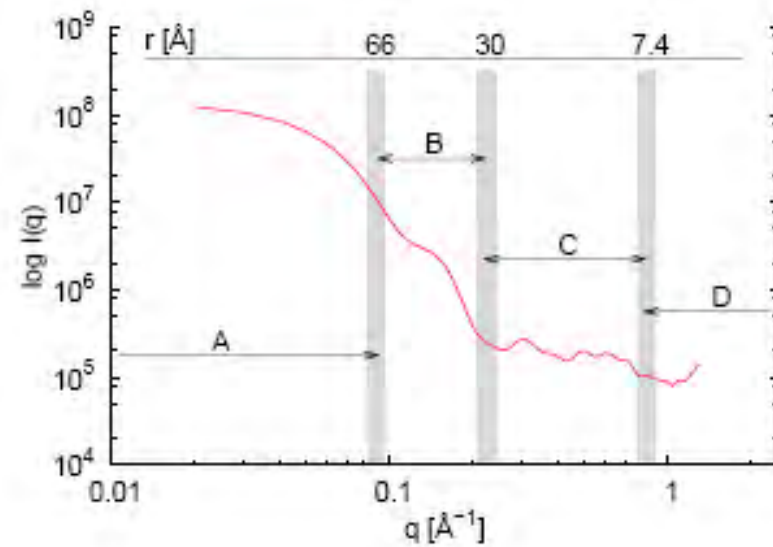
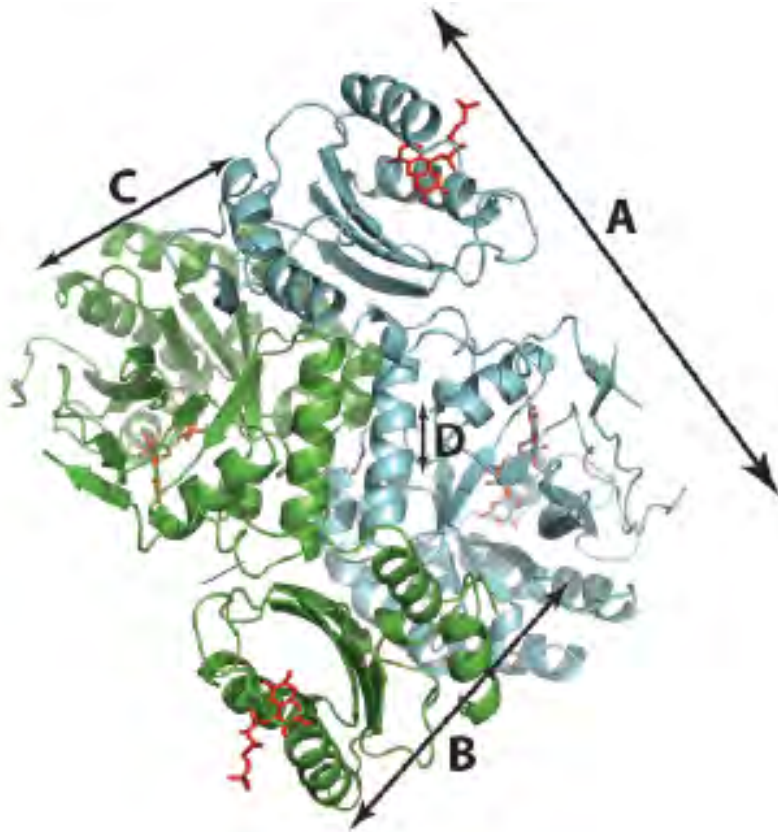
Schotte et al. Science **300**, 1944, 2003

Unveiling functional protein motions with picosecond x-ray crystallography and molecular dynamics simulations



Gerhard Hummer*, Friedrich Schotte, and Philip A. Anfinrud* PNAS: 101:15330 (2004)

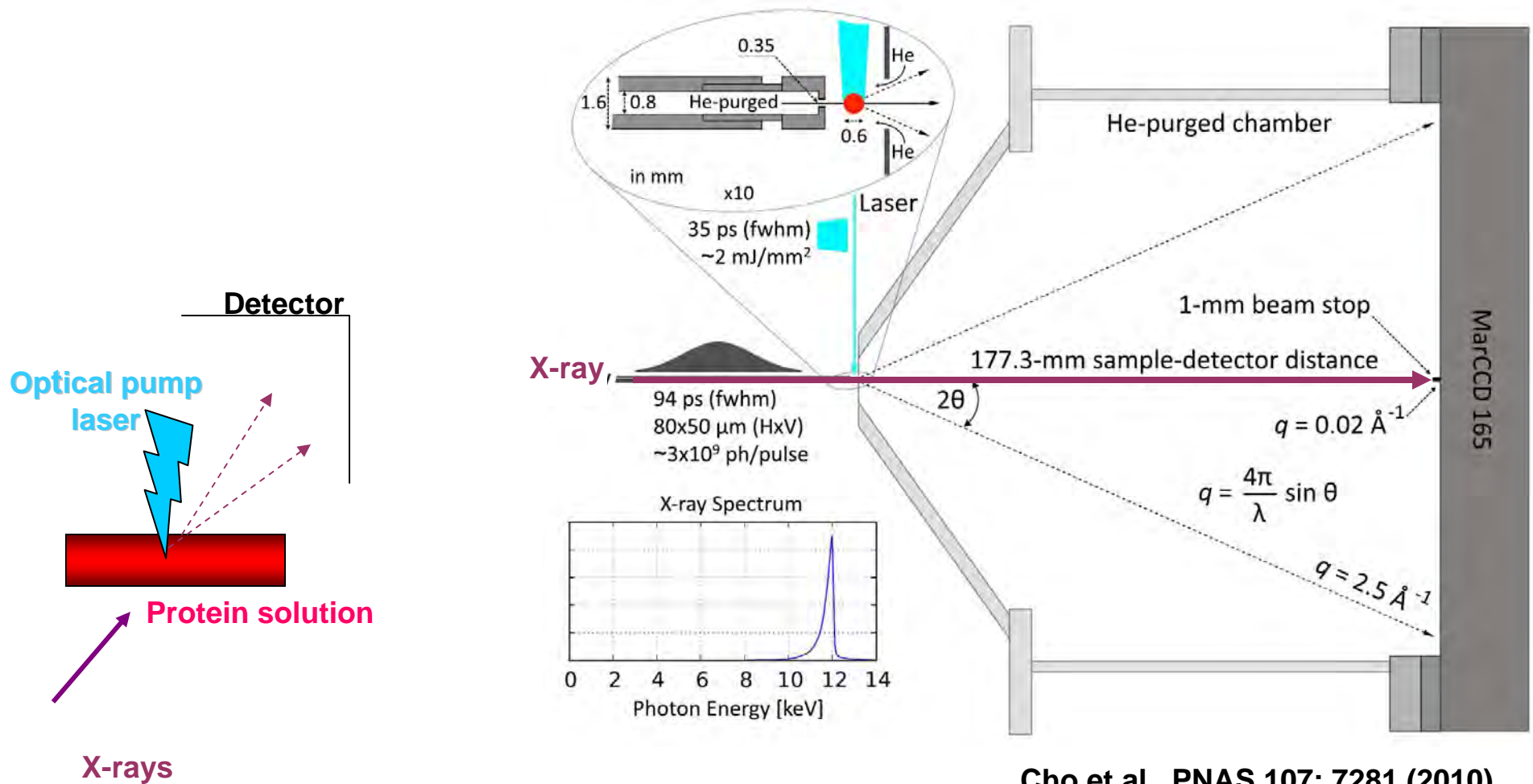
Small and wide angle X-ray scattering studies on solutions provide structural information on many length scales



Protein structural dynamics in solution unveiled via 100-ps time-resolved x-ray scattering

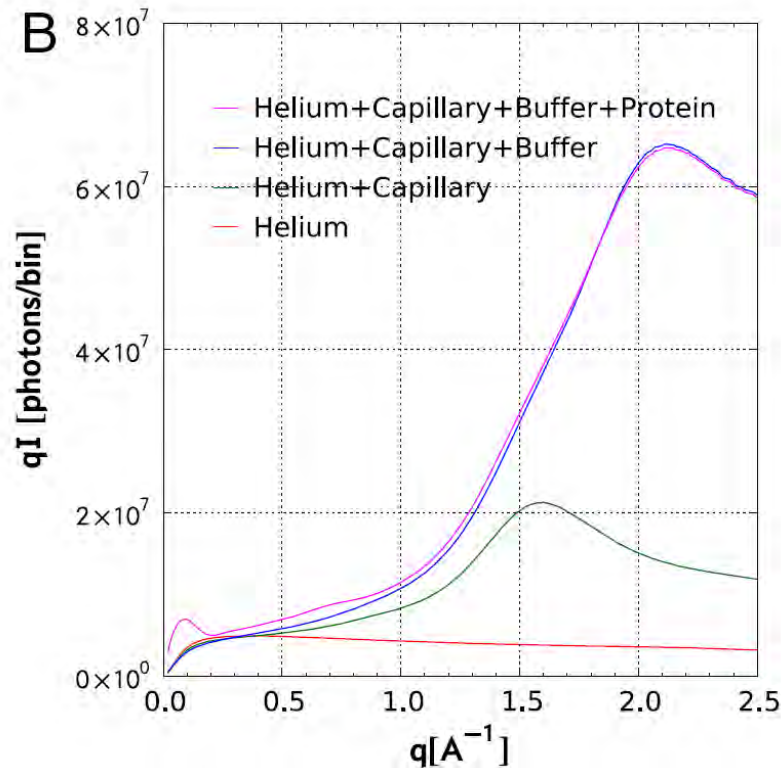
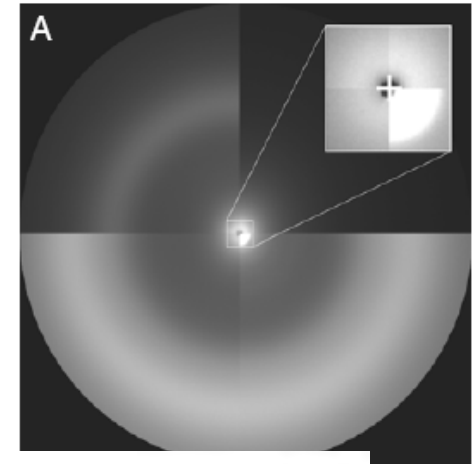
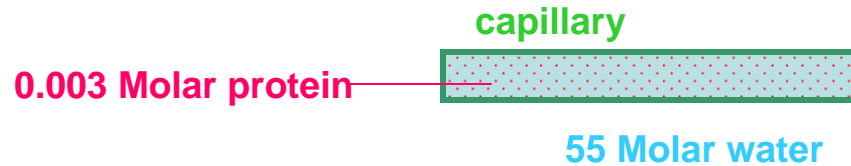
Hyun Sun Cho^{a,1}, Naranbaatar Dashdorj^{a,1}, Friedrich Schotte^{a,1}, Timothy Graber^b, Robert Henning^b, and Philip Anfinrud^{a,2}

Pump probe experiments on carbonmonoxy myoglobin solutions

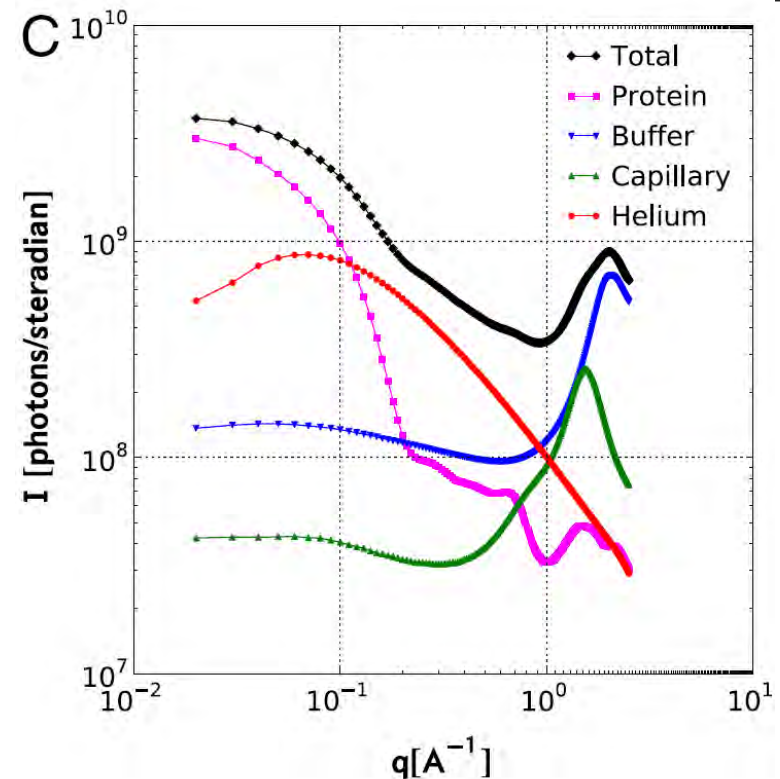


Cho et al., PNAS 107: 7281 (2010)

SAXS/WAXS signals of proteins are small



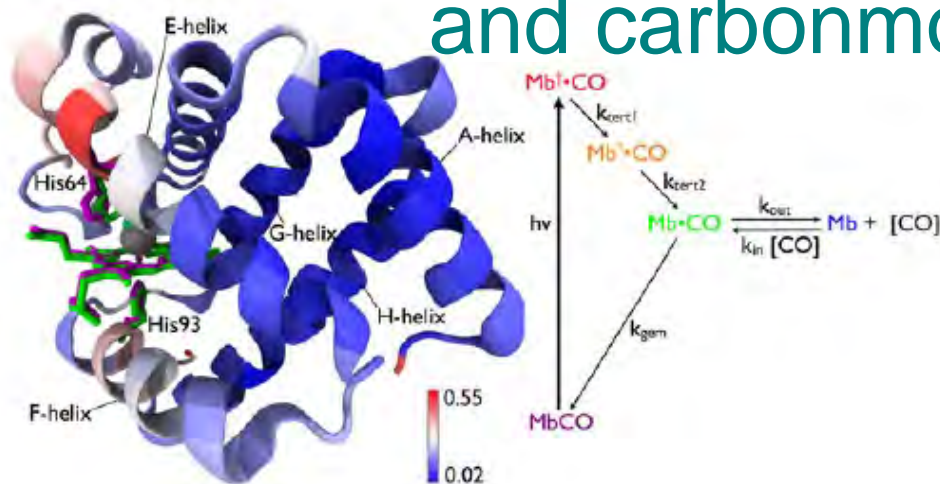
Angular integration of experimental scattering images in A with the detector pixels binned into annular rings spaced by 0.01 \AA^{-1} .



Decomposition of the protein solution scattering pattern into its respective contributions. Note the log-log scale

Cho et al., PNAS 107: 7281 (2010)

Time-resolved structural differences: unligated and carbonmonoxy myoglobin



A strong, negative-going feature in the SAXS region appears promptly, corresponding to a sudden $>22 \text{ \AA}^3$ volume expansion of the protein. The ensuing conformational relaxation causes the protein to contract to a volume $\sim 2 \text{ \AA}^3$ larger than MbCO within $\sim 10 \text{ ns}$.

Global analysis yields time-independent spectra and species

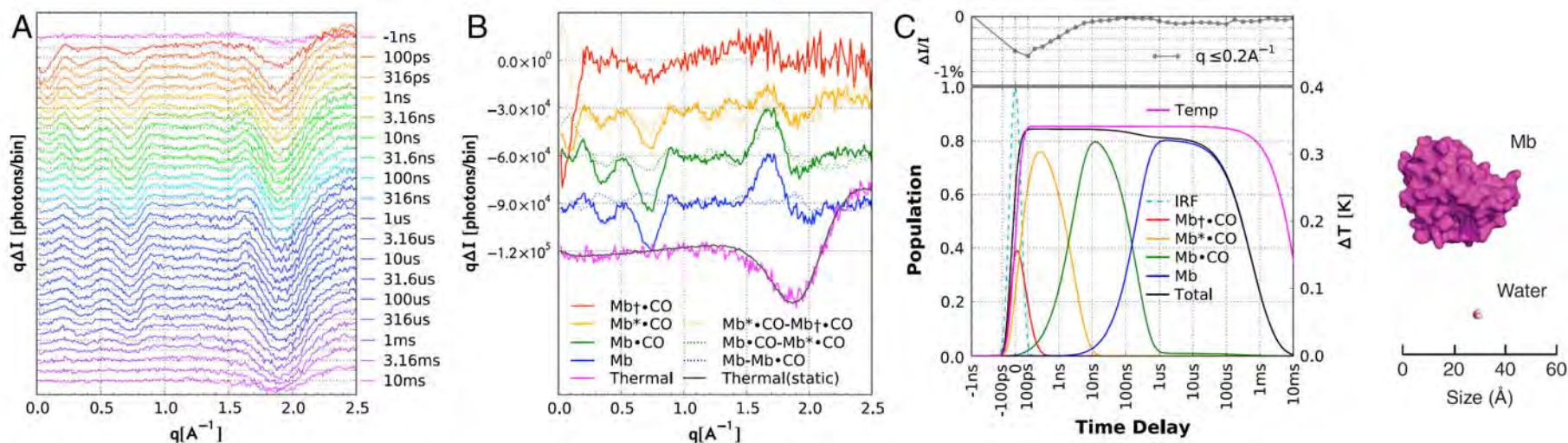
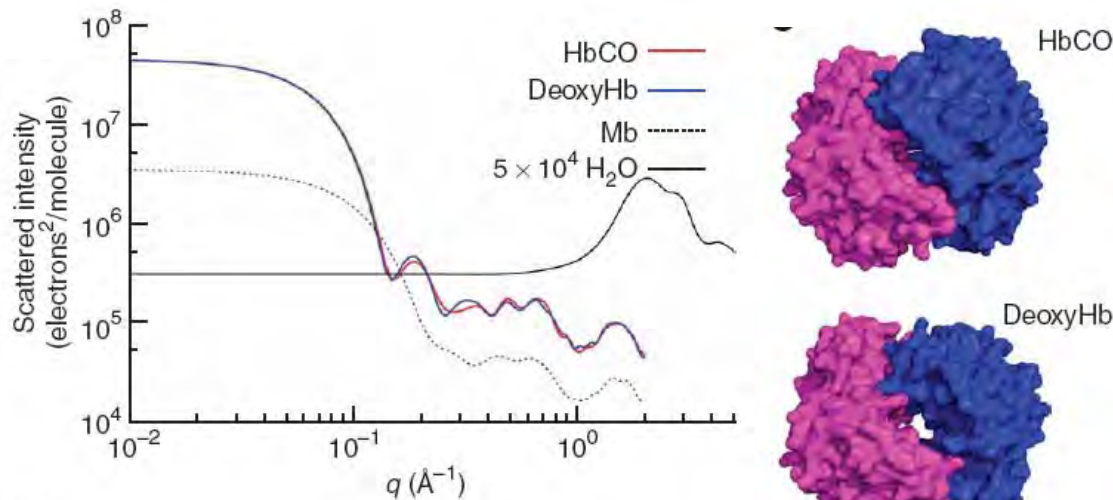


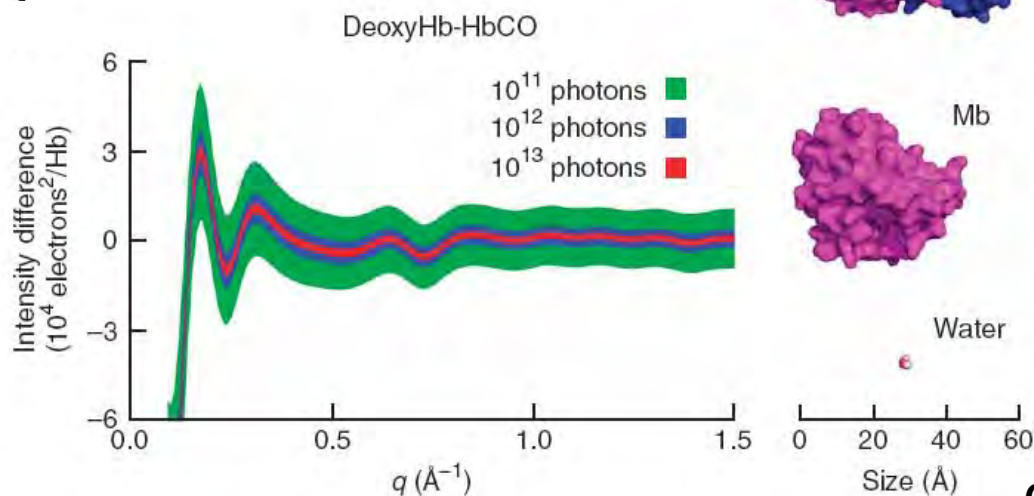
Fig. 4. (A) Time-resolved SAXS/WAXS differences. For clarity, the curves are color-coded according to the model in Fig. 1B and offset from one another. (B) Time-independent scattering fingerprints extracted from global analysis of the time-resolved scattering data in A. Scattering differences between each intermediate state and the ground state (MbCO) are plotted as solid lines, whereas differences between each state and the state that precedes it are plotted as dotted lines (three-point smoothing has been applied to the dotted lines). For clarity, the curves are offset vertically from one another. A scaled thermal signal from static measurements (gray) is plotted on top of the thermal signal recovered from global analysis of the time-resolved scattering patterns. (C, Upper) Time-dependence of the integrated SAXS signal. (Lower) Time-dependent population of states in B. The dashed line labeled IRF (cyan) represents the instrument response function (convolution of the laser and x-ray pulses).

Cho et al., PNAS 107: 7281 (2010)

SAXS/WAXS measurements allow analysis of large structural changes, e.g. ligand binding to hemoglobin



Calculated scattering curves for myoglobin, unligated and carbonmonoxy ligated hemoglobin



Calculated difference pattern for 3 different photon fluxes

Biological applications of **ultrafast X-rays**

Use time-resolution of data collection to

- compete with time-scale of **radiation damage** reactions taking place upon exposure to X-rays
- study **biochemical** reactions in crystals and solutions

Some open issues in structural biology

Structures of big (weakly binding) complexes
membrane proteins
transient intermediates, folding
chromatin/ genome structure
cellular organization at high resolution

Current limitations of crystallography,
electron microscopy
X-ray microscopy

Sample preparation (biochemistry)

Crystals for crystallographic approaches

Data collection, **radiation damage**

Computational approaches dealing with disorder

FELs – useful for structural biology?

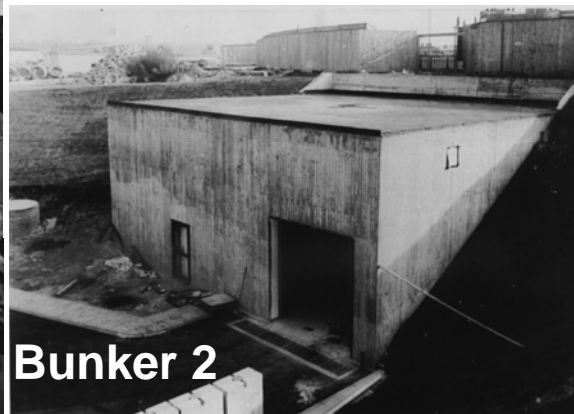
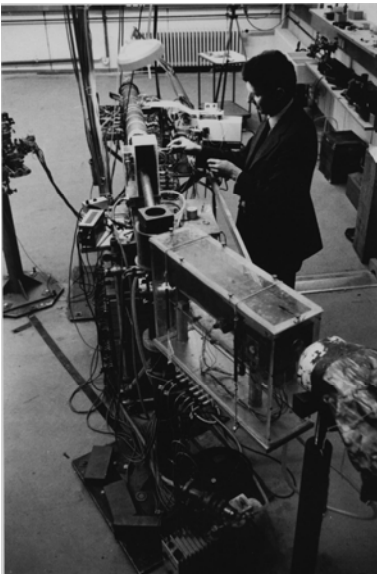
Need to solve sample delivery issue (and others) ...

- May prevent radiation damage issues, thus allowing study of
- very radiation sensitive samples, e.g. metalloenzymes
- nanocrystals
- single particles, for example nuclear pores (correlations!)
- Time-resolved studies
- ...

Thinking back in time ...

Synchrotron Radiation as a Source for X-ray Diffraction

G. ROSENBAUM*, K. C. HOLMES* & J. WITZ *Nature* 230, 434-437 (16 April 1971)



FELs – useful for structural biology?



<https://sites.google.com/a/lbl.gov/biology-with-fels/>