The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2332-25

School on Synchrotron and FEL Based Methods and their Multi-Disciplinary Applications

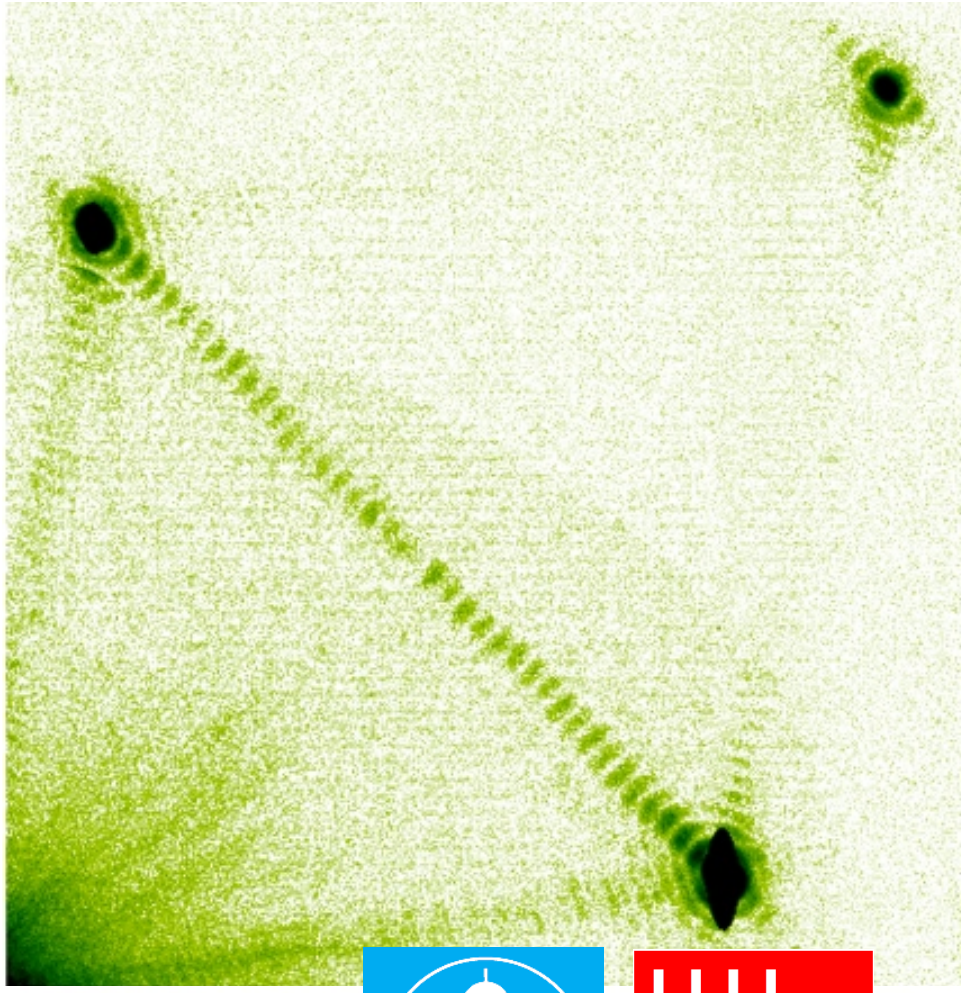
19 - 30 March 2012

Coherent Diffractive Imaging with FELs: methods and applications in life sciences

Henry Chapman

DESY and University of Hamburg
Germany

Coherent Diffractive Imaging with FELs: methods and applications in life sciences



Henry Chapman
Center for Free-Electron Laser Science
DESY and University of Hamburg

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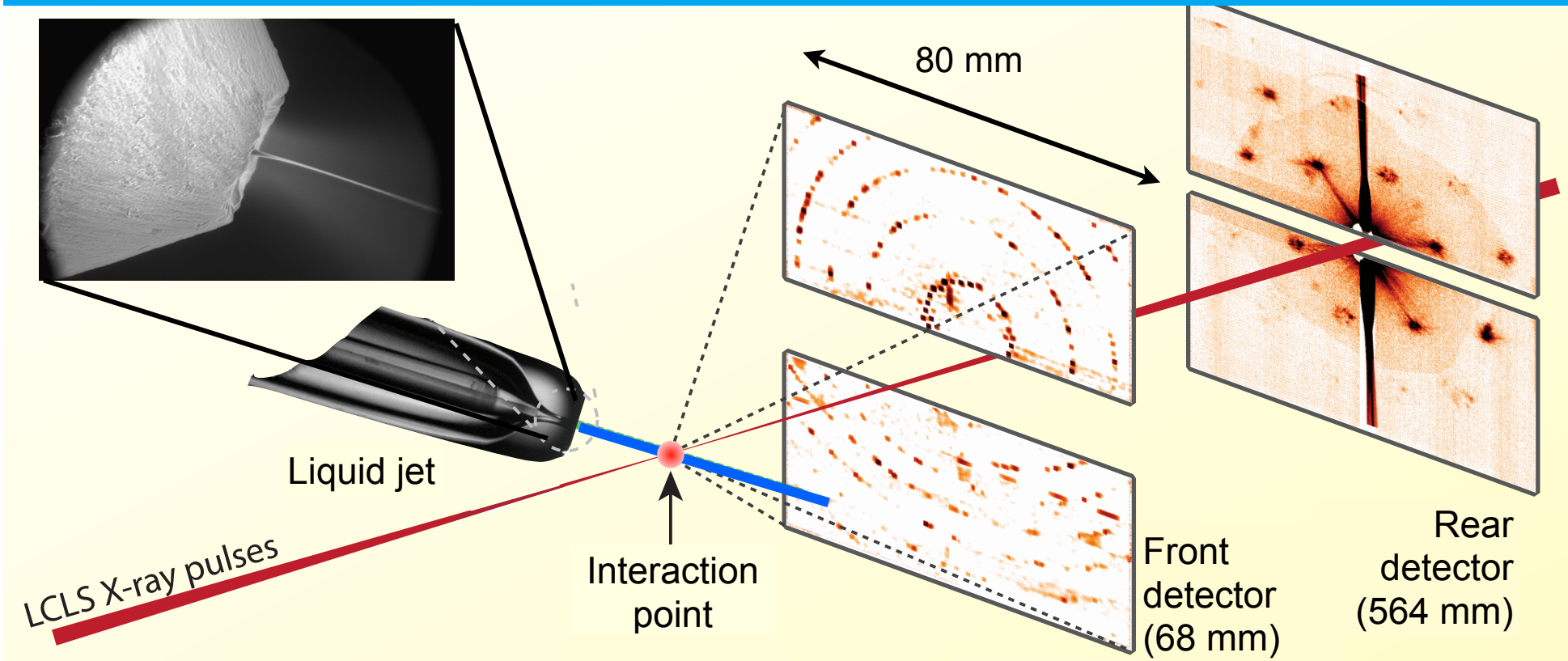
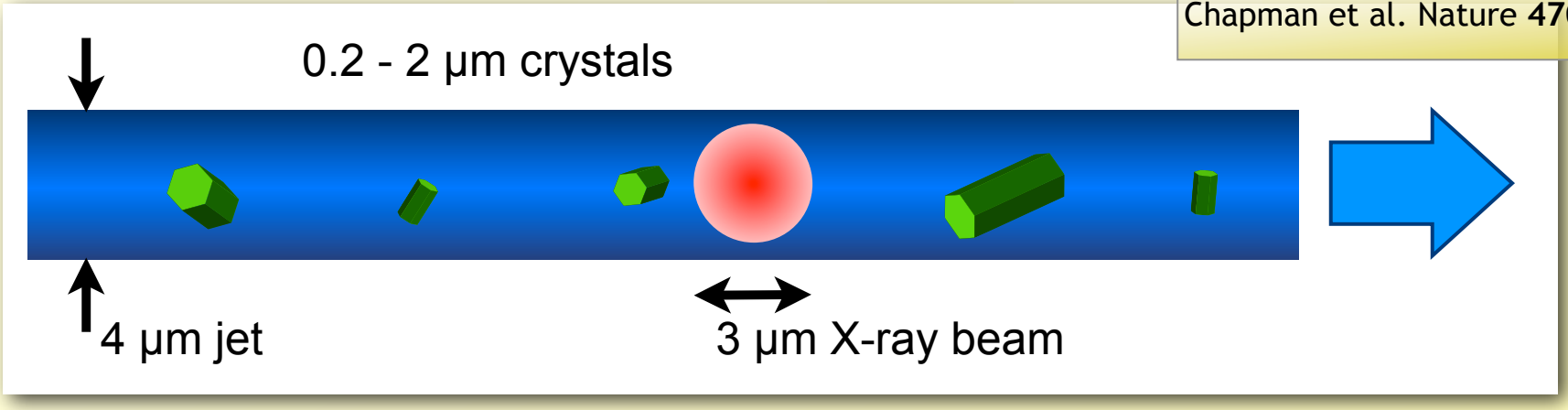
Trieste School, March 2012

Nanocrystallography is carried out in a flowing water microjet



Chapman et al. Nature **470**, 73 (2011)

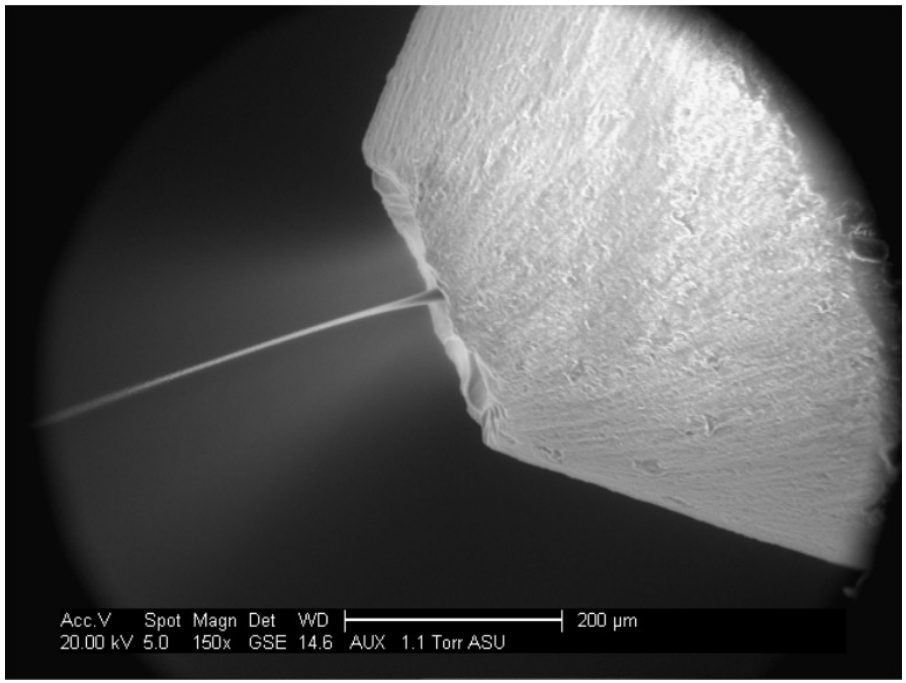


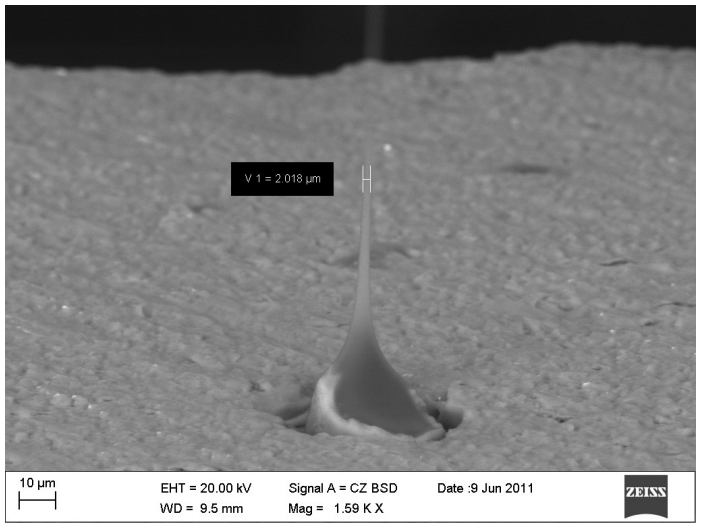
Samples are delivered to the beam in a liquid jet

> Sample delivery (“injection”) technology is **critical** to the success of serial crystallography and many other FEL experiments

Gas dynamic nozzle creates liquid streams with diameters down to 200 nm.

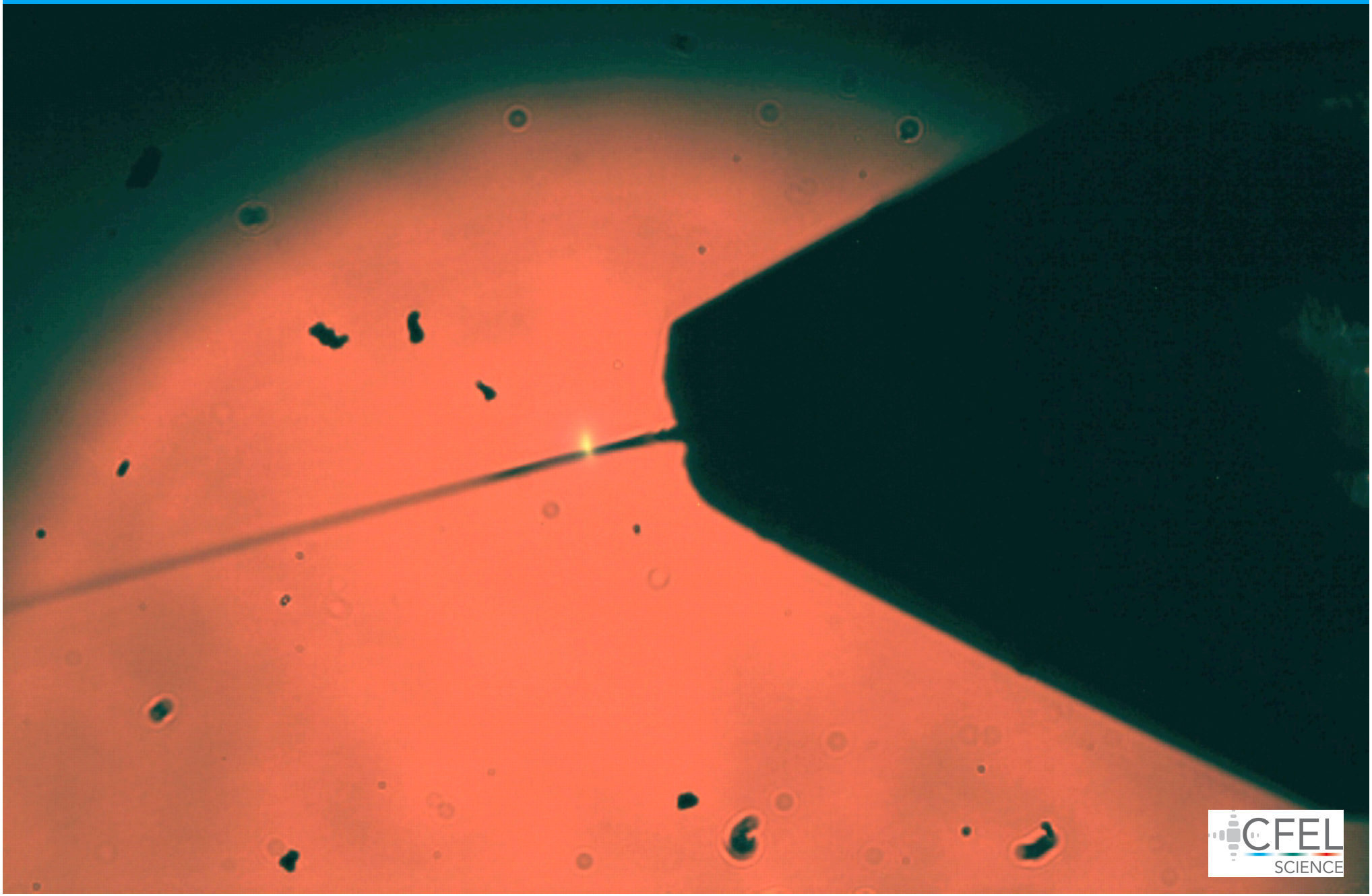
“Droplet on demand” offers potential reductions in sample consumption of an **order of magnitude**.



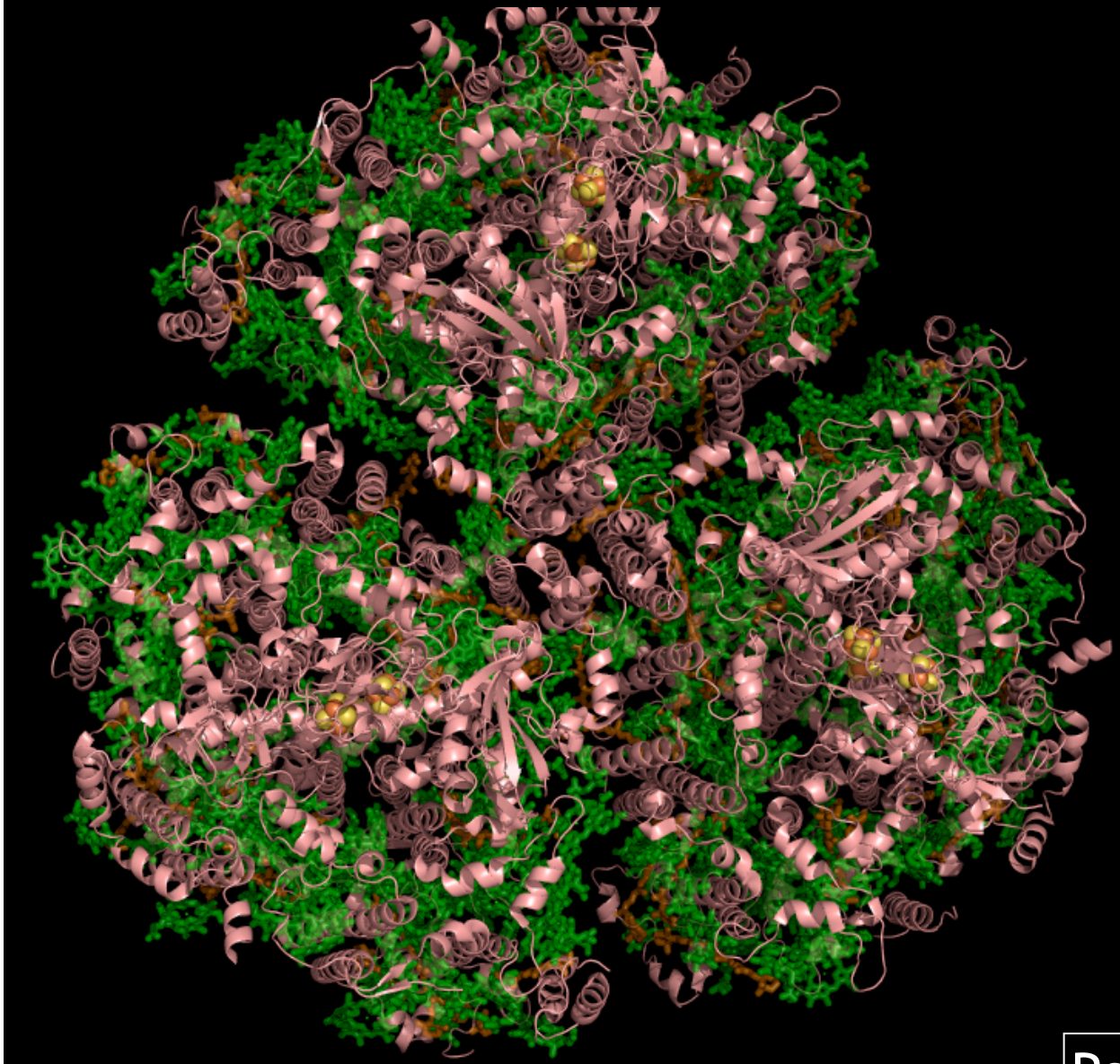


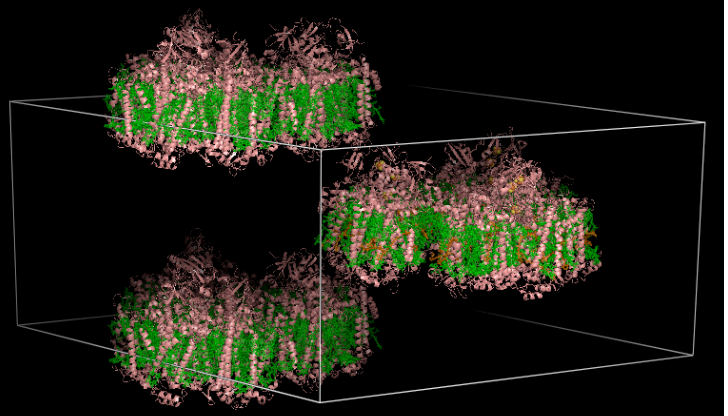
Dan DePonte, CFEL

Optical emission is observed for dose rates above about 20 MGy/fs

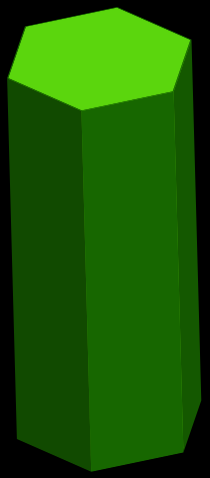


First experiments were carried out on Photosystem I



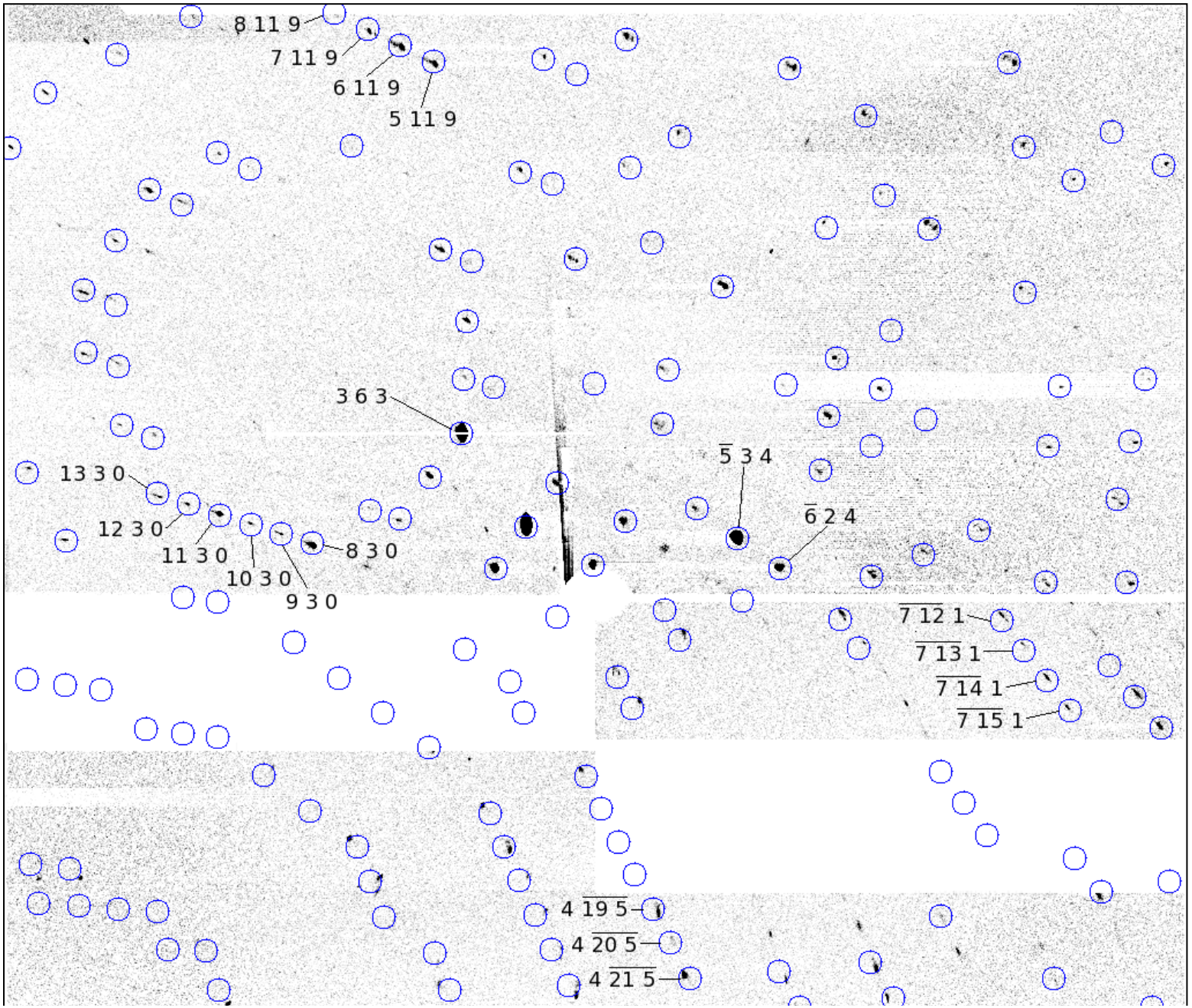


$a = b = 288$ Å
$c = 167$ Å

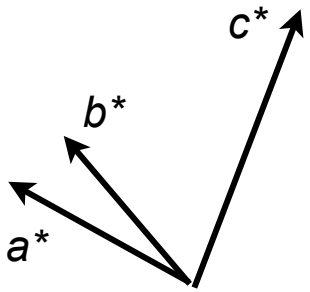


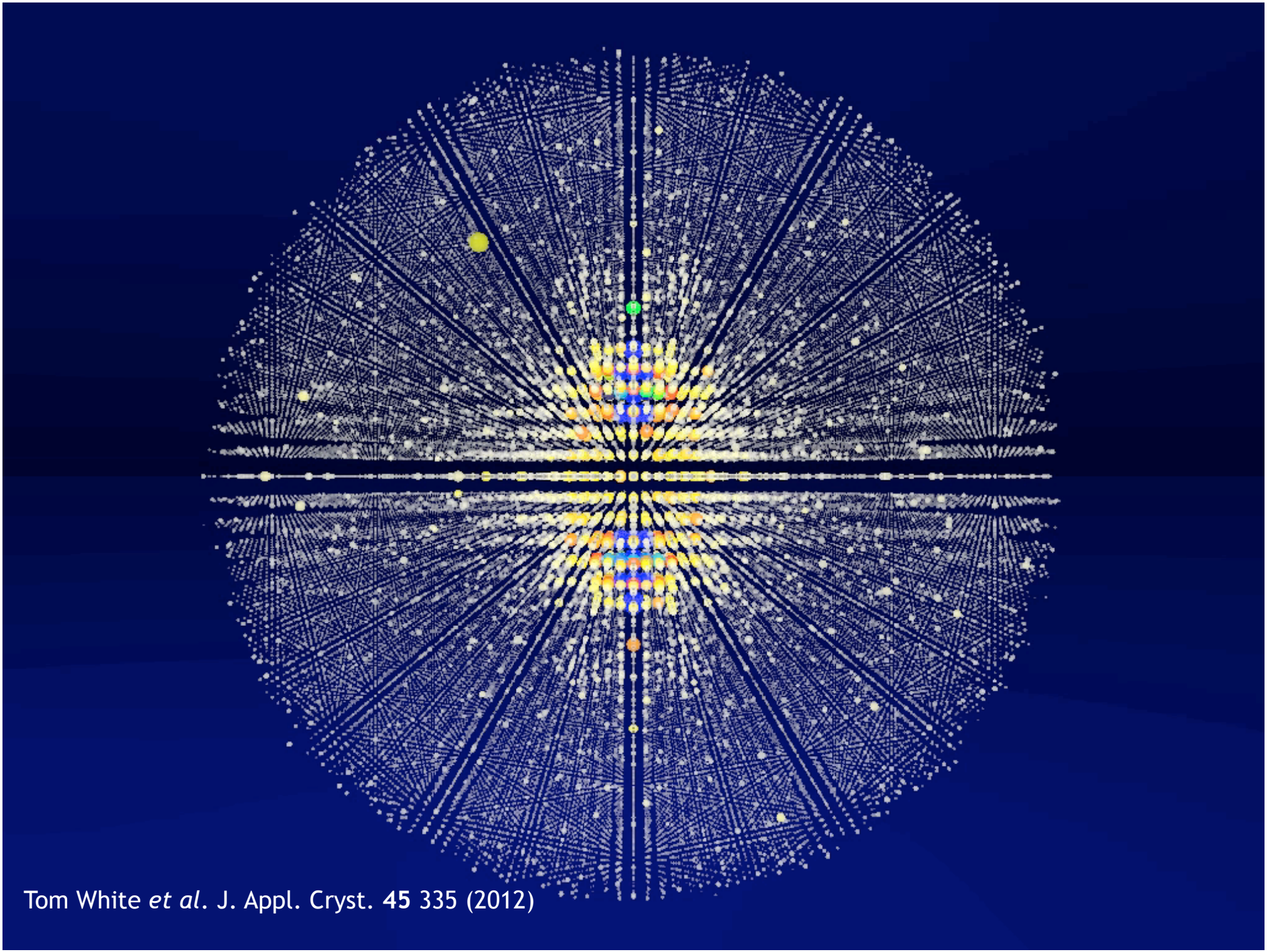
Petra Fromme, ASU

Each pattern is indexed



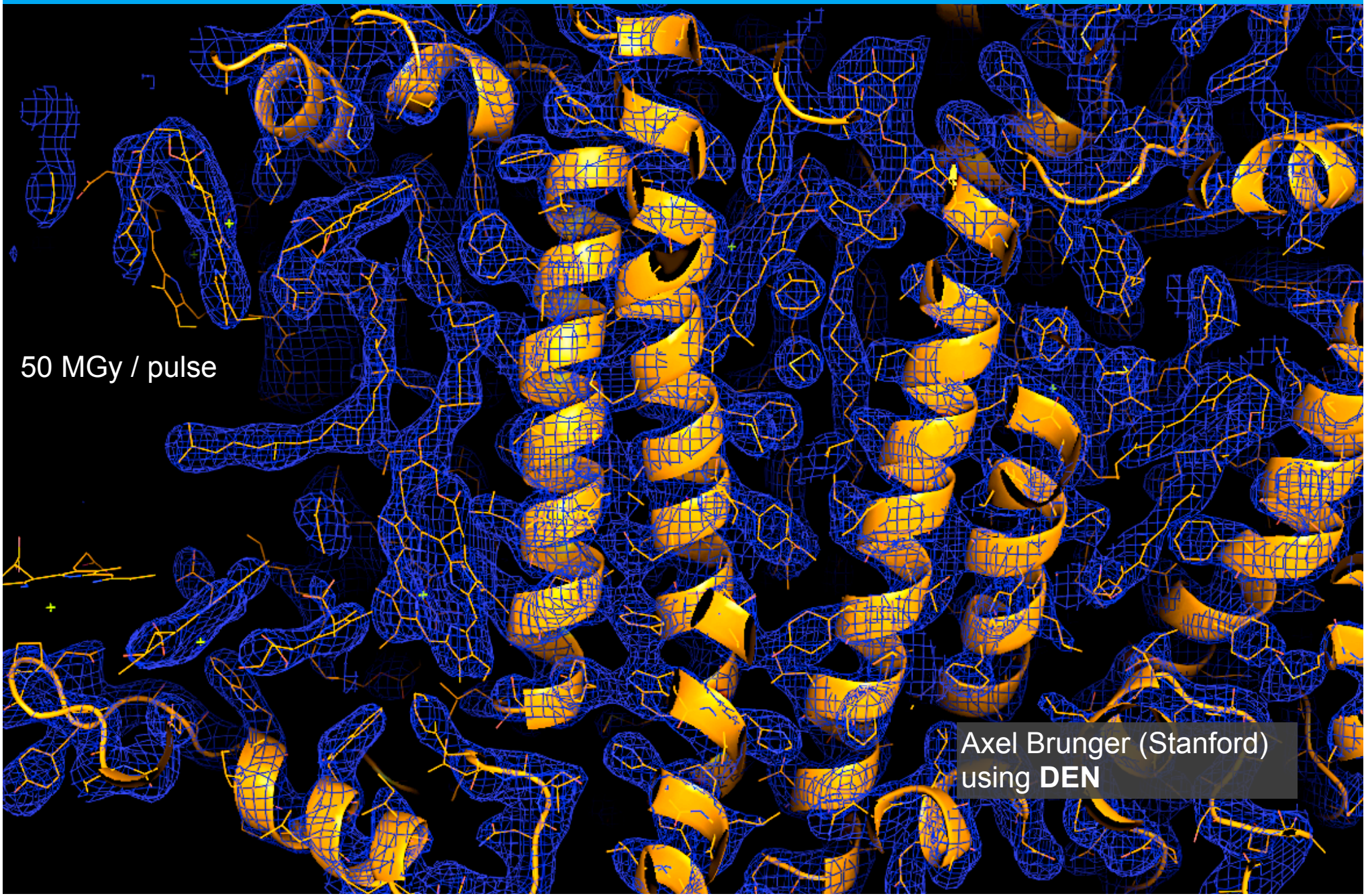
Tom White (CFEL)
Rick Kirian (ASU)



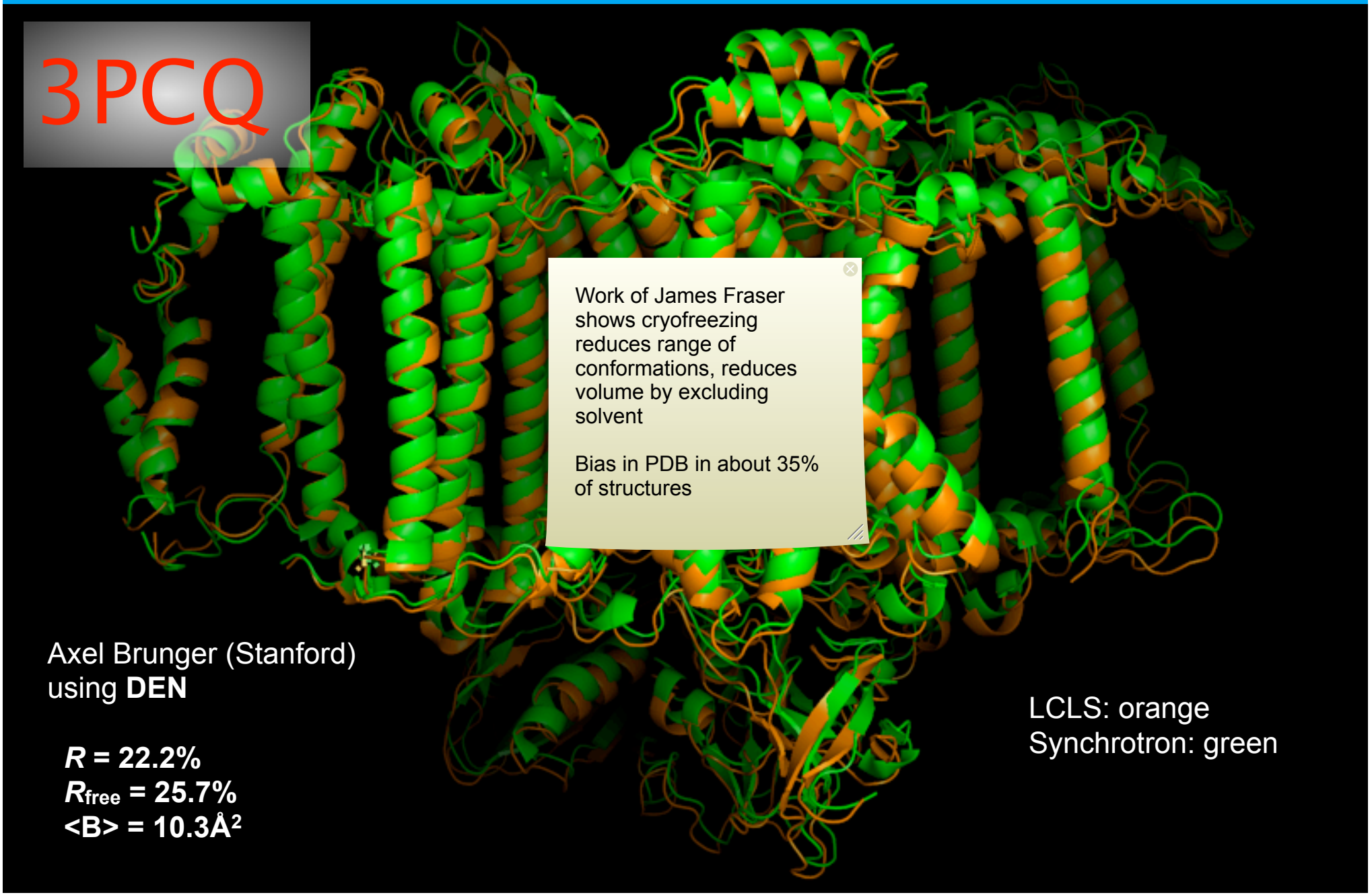


Tom White *et al*. J. Appl. Cryst. **45** 335 (2012)

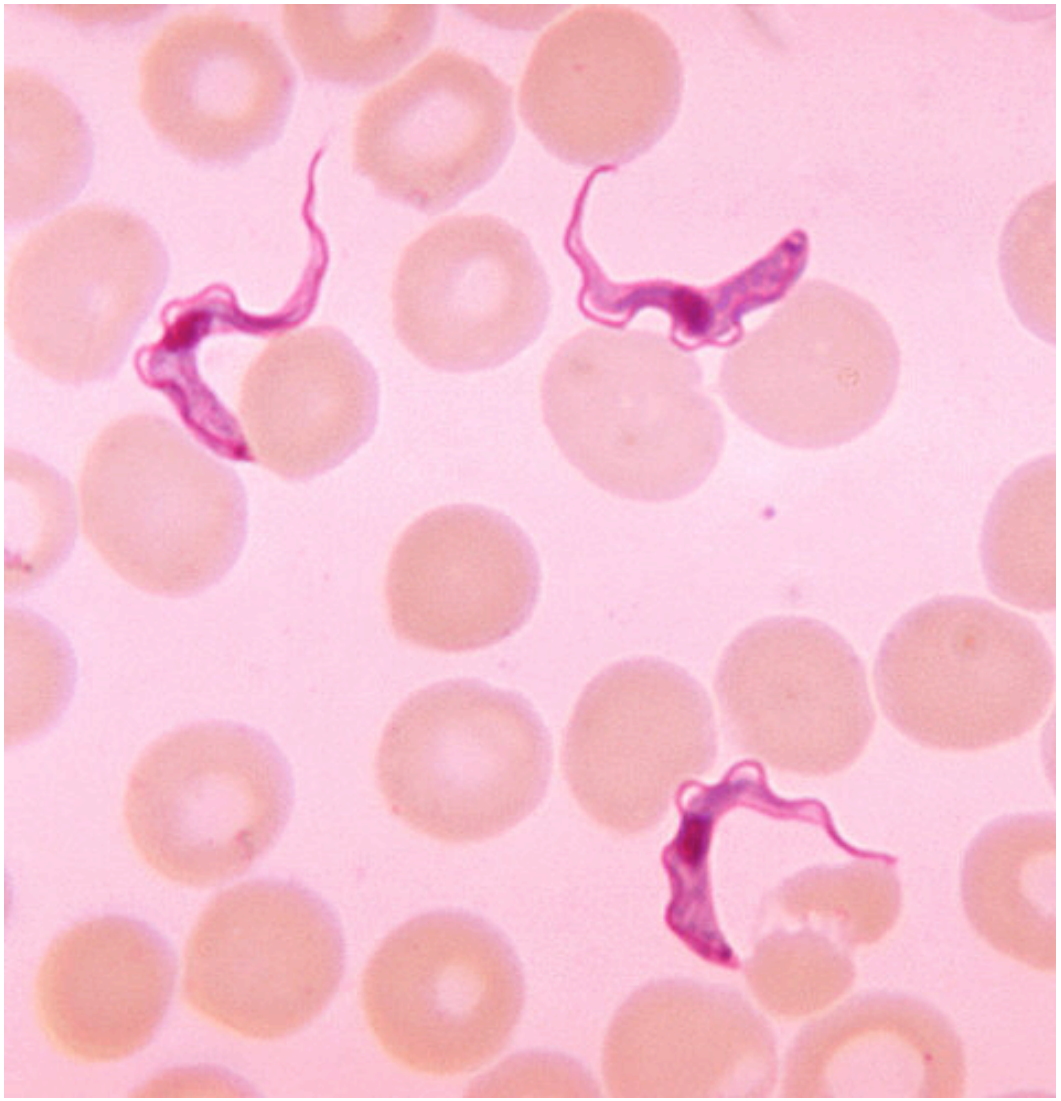
Molecular replacement reconstructs the photosystem I structure



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



Trypanosoma brucei Cathepsin B (TbCatB) is a potential target to treat sleeping sickness



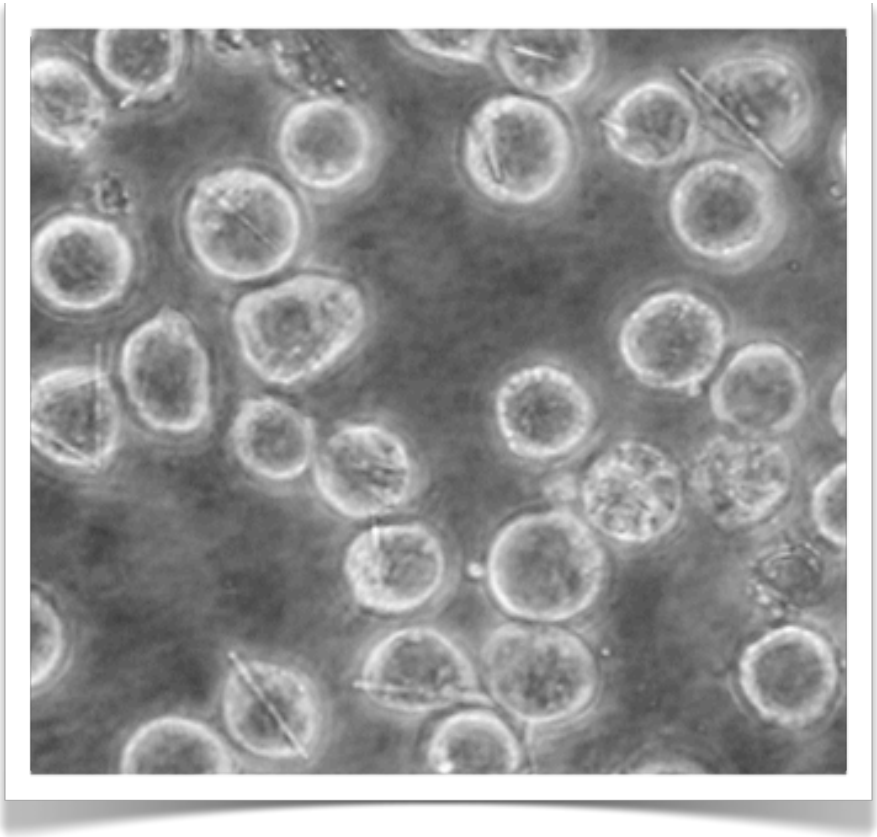
The protozoan parasite Trypanosoma brucei is the cause of Human African Trypanosomiasis (HAT, sleeping sickness).

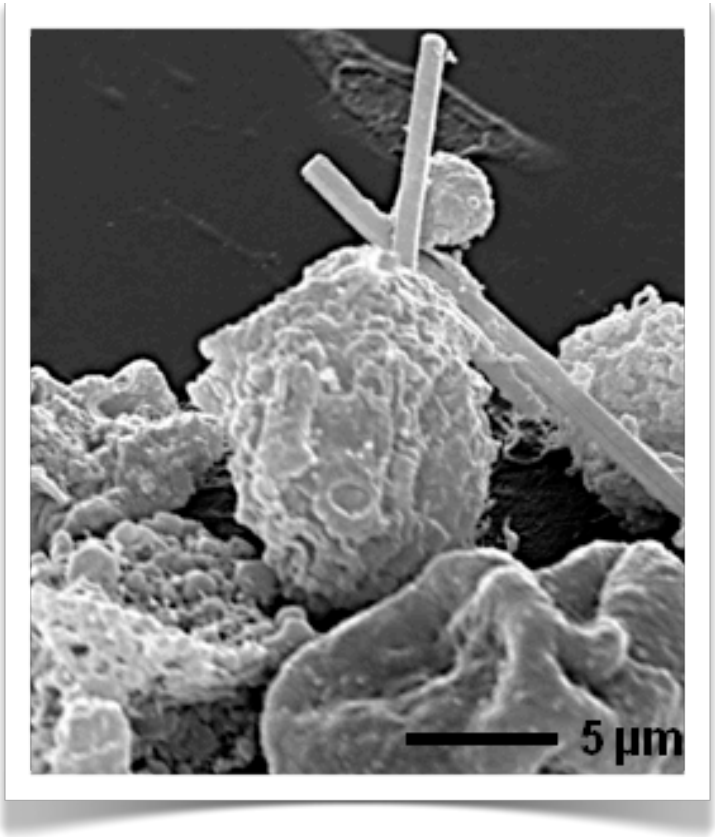
60 million people are at risk and ~50,000 infected yearly. New drugs are required to control the spread of disease and associated mortality.

Bloodstream T. brucei parasites express two proteases: a cathepsin L-like enzyme and a cathepsin B-like enzyme.

Cysteine proteases play central roles during the lifecycle of many parasitic organisms and have been established as effective drug targets in treating many parasitic diseases.

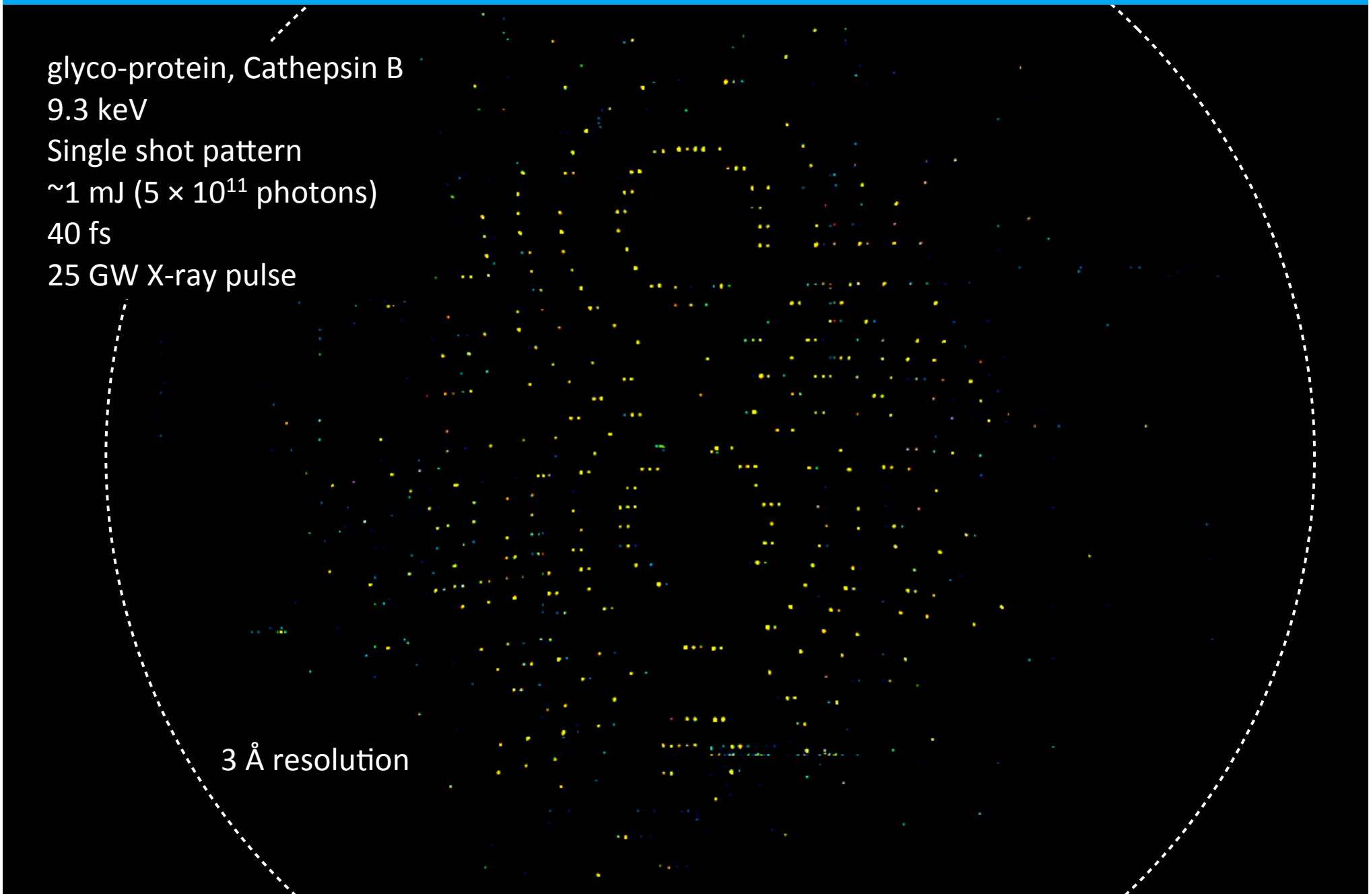
Needle-shaped crystals can be grown in vivo by infection of cells by a modified baculovirus



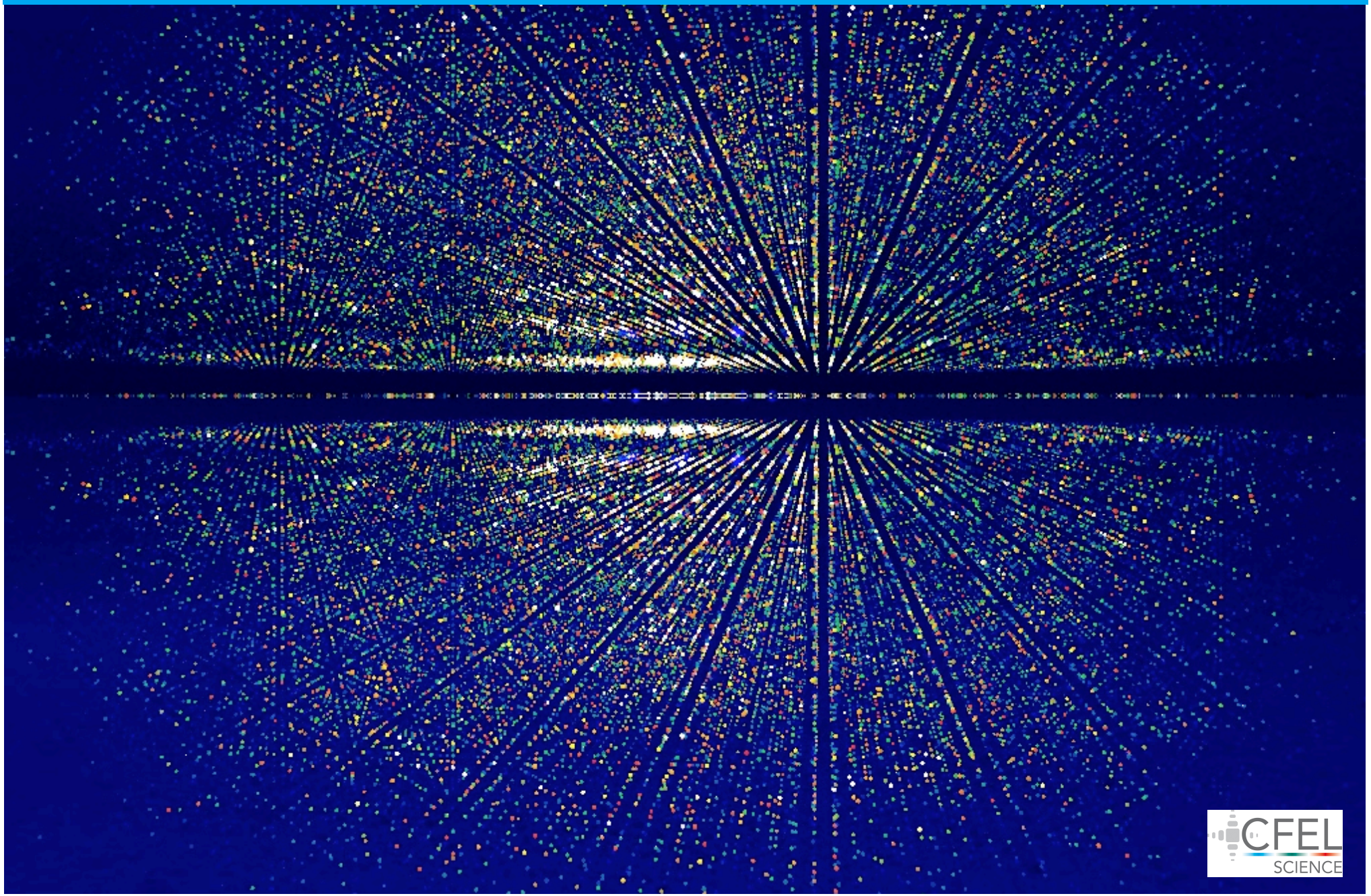


Rudolph Koopman, Karolina Cupelli, Michael Duszenko, *U Tübingen*
Lars Redecke, Dirk Rhedes, *U Lübeck & Hamburg*
Christian Betzel, *U Hamburg*

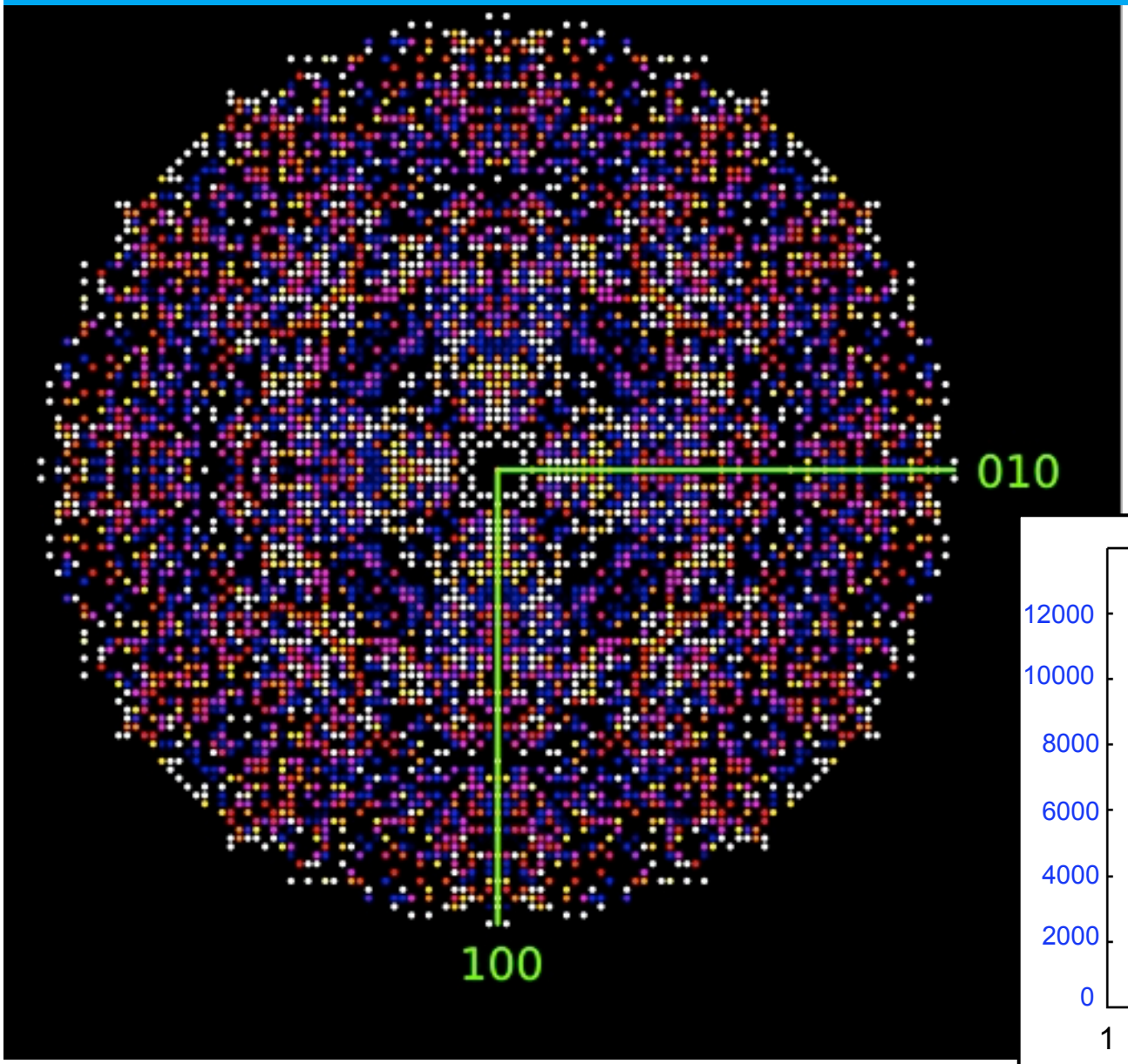
We have collected 3Å resolution diffraction from an unsolved glyco-protein, Cathepsin B



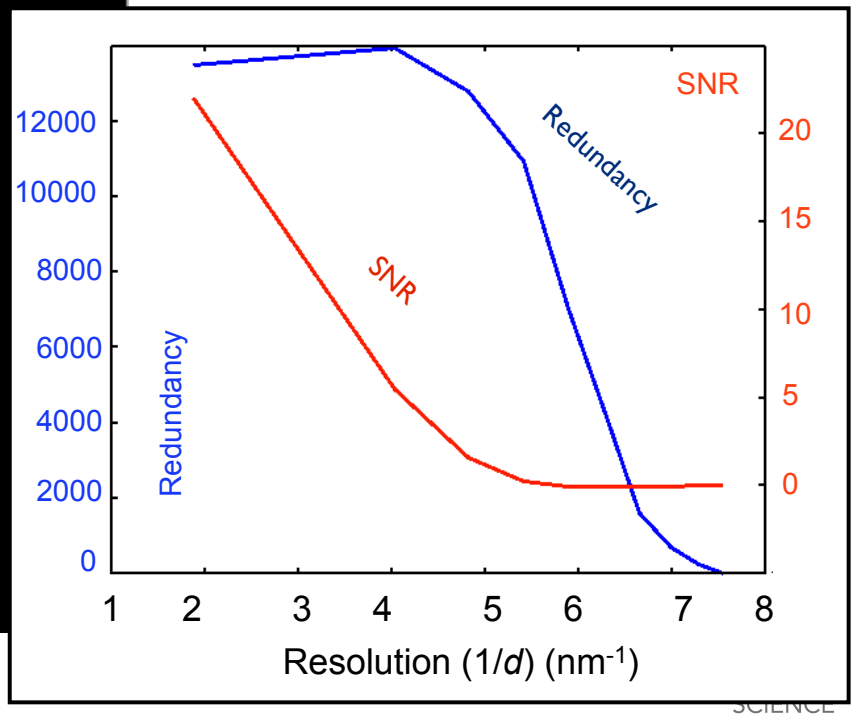
We record over 30,000 reflections at 1.7 Å resolution



We have indexed 1.8 Å resolution diffraction from an unsolved glyco-protein, Cathepsin B



- 3,953,201 frames collected with 40 fs pulses
- 8 hours of data collection
- 357,555 “crystal hits” detected (9%)
- 156,565 indexed (44%)



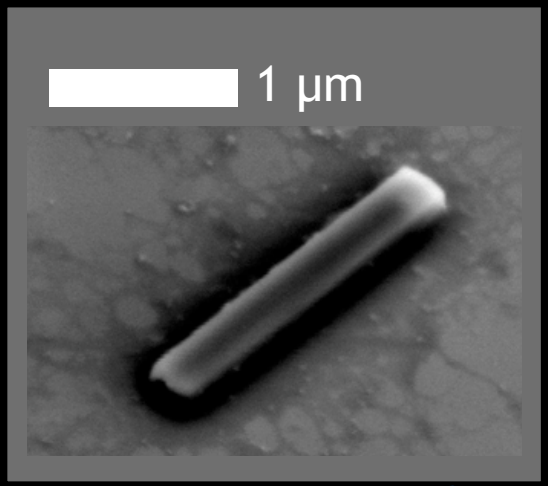
We solved the TbCatB structure to 1.8 Å resolution

3,953,201 frames collected with 40 fs pulses

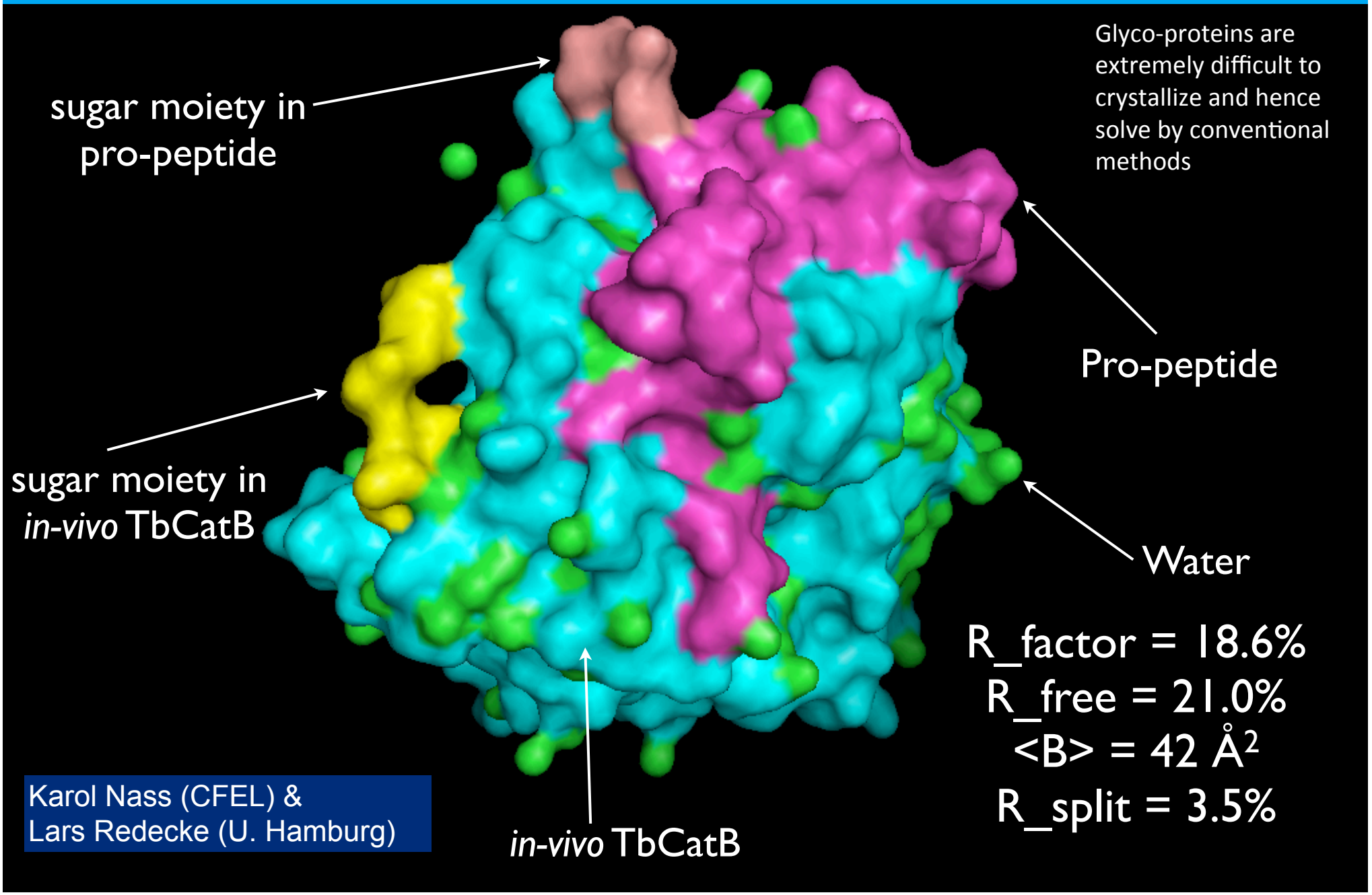
8 hours of data collection

357,555 “crystal hits” detected

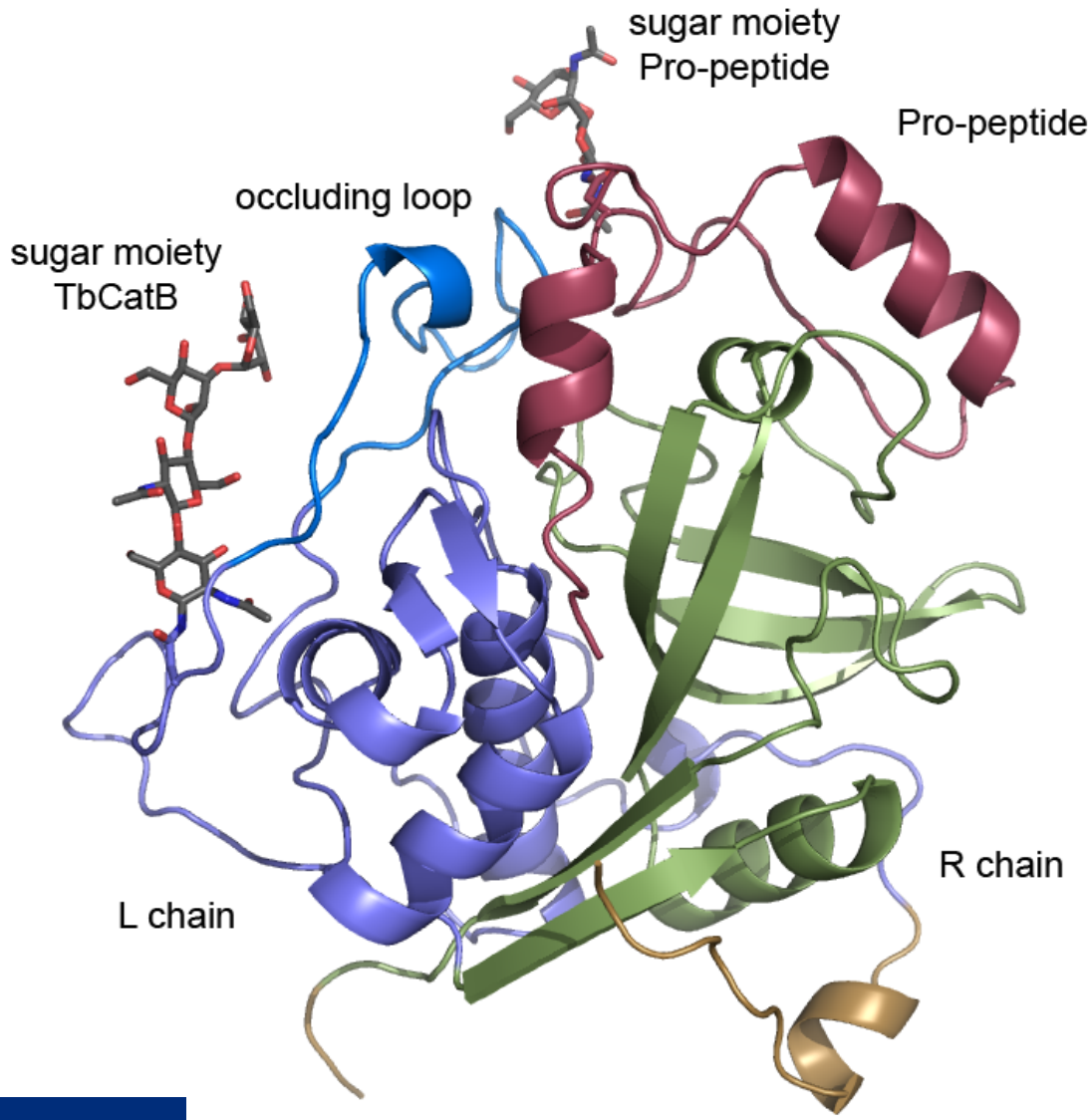
156,565 indexed



Structure determination of TbCatB by molecular replacement reveals a complex with a pro-peptide

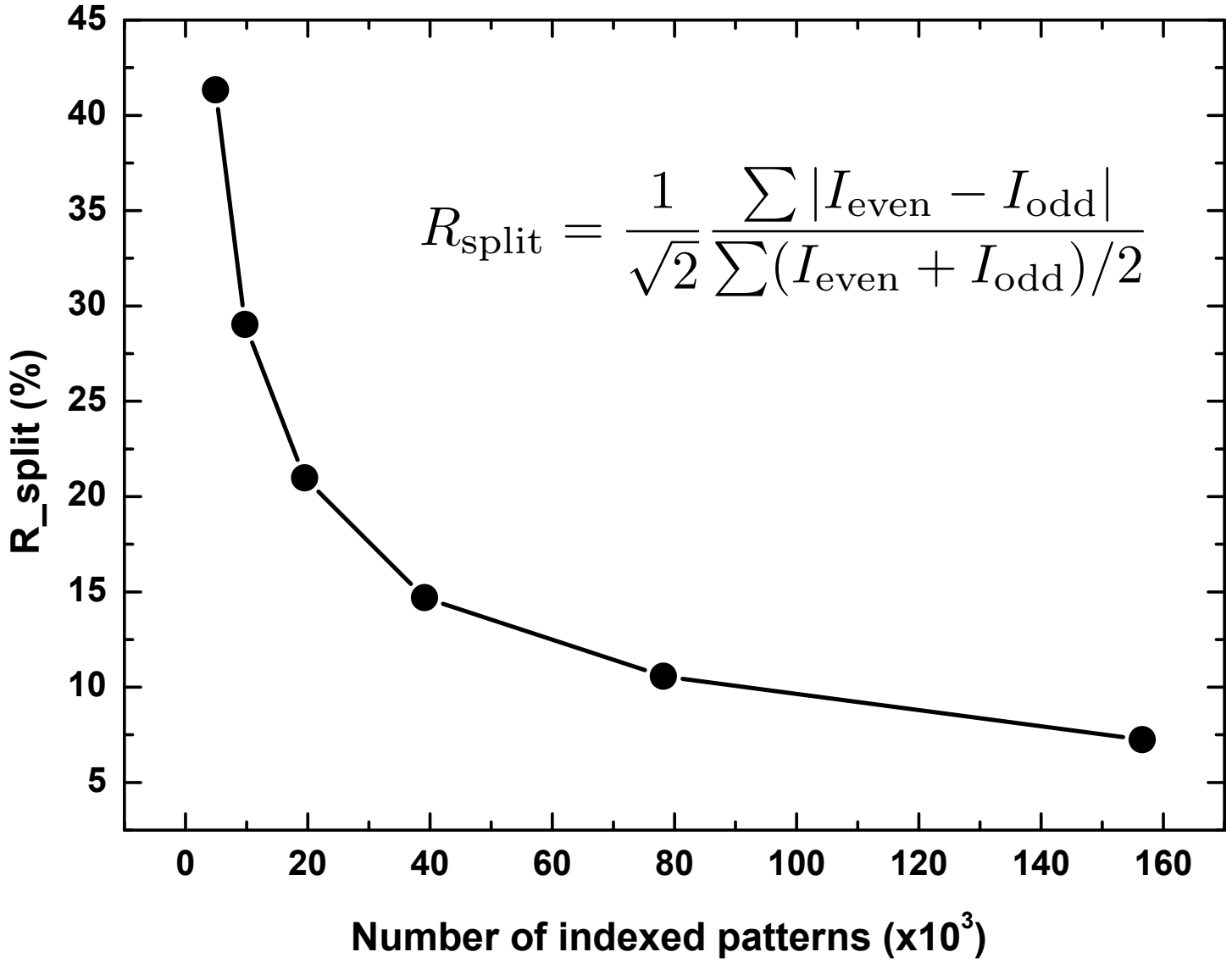


Structure determination of TbCatB by molecular replacement reveals a complex with a pro-peptide

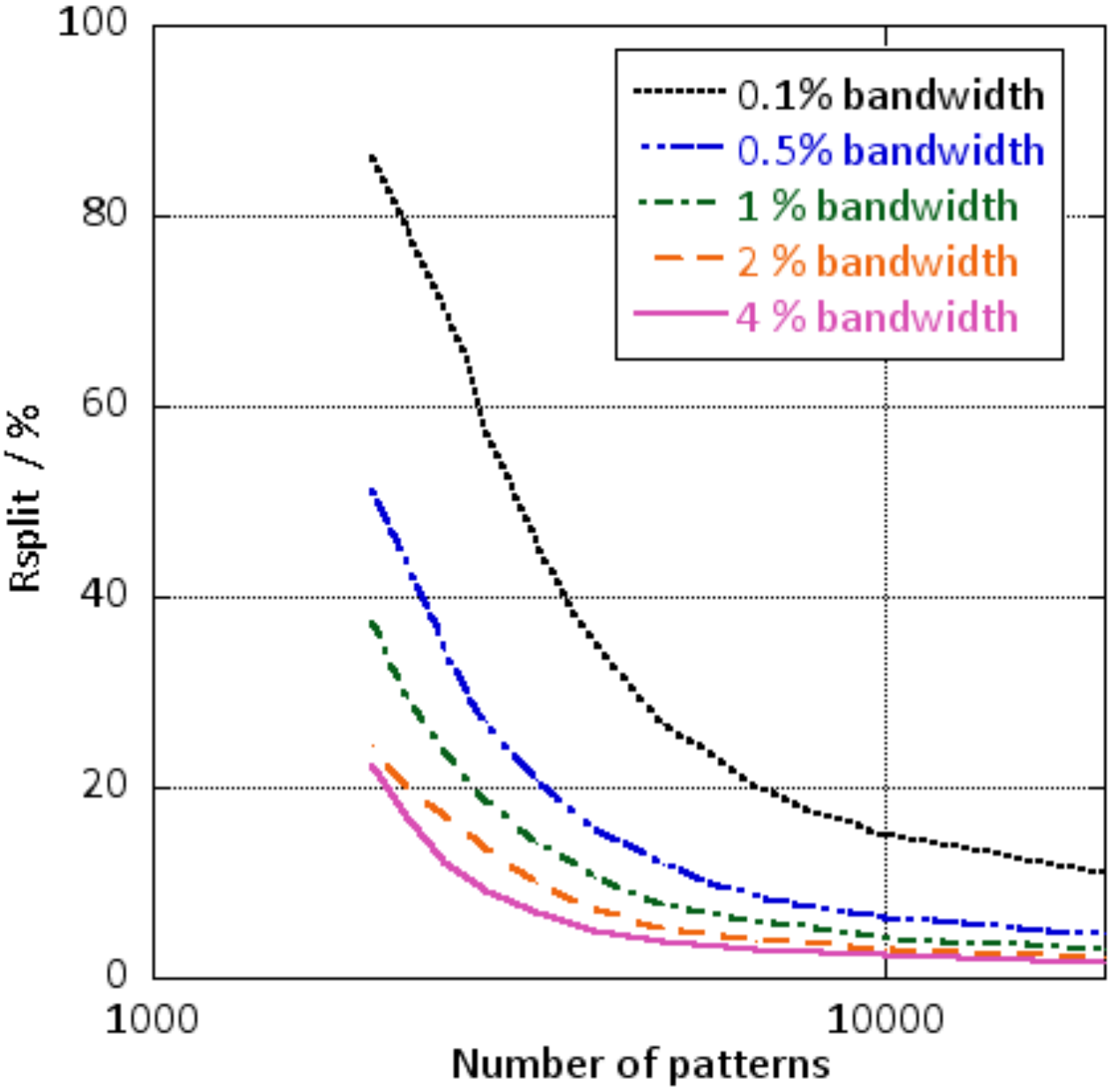


Karol Nass (CFEL) &
Lars Redecke (U. Hamburg)

We need 10,000 to 100,000 indexed patterns



Data collection efficiency is increased by increasing the bandwidth



Francesco Stellato
Thomas White

We have a new DESY system for processing and storage

LCLS Data

A typical run at
120 Hz generates
>200 TB of data

~ 1 Petabyte
collected from our
experiments

SGI Altix
72 physical cores
360GB RAM
Shared memory
Direct connected storage

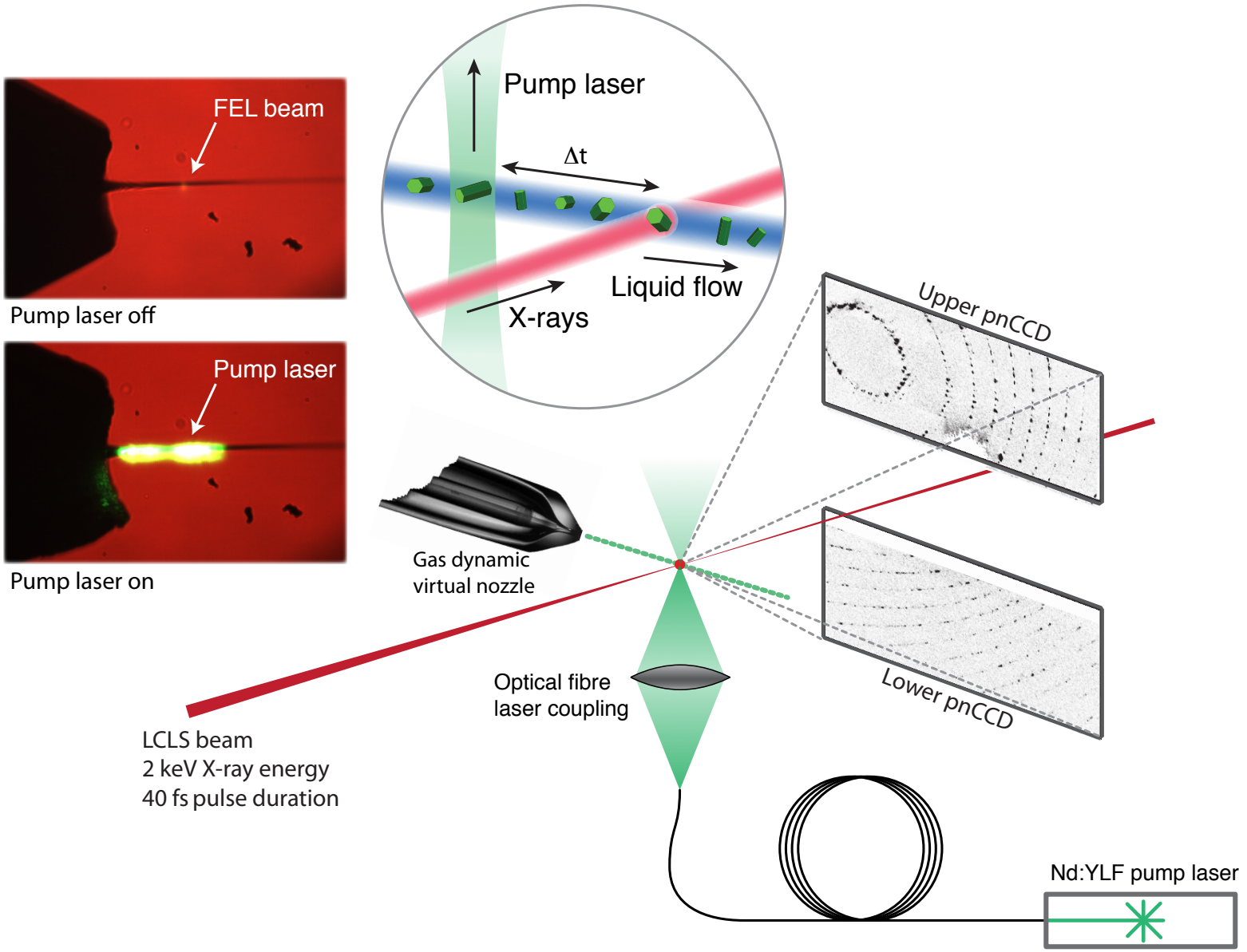
Data Direct Networks SFA10000
60-bay HDD / 4U unit
~1 PB/rack (formatted)
(600 x 2 TB HDDs)

Can process 30 patterns / second

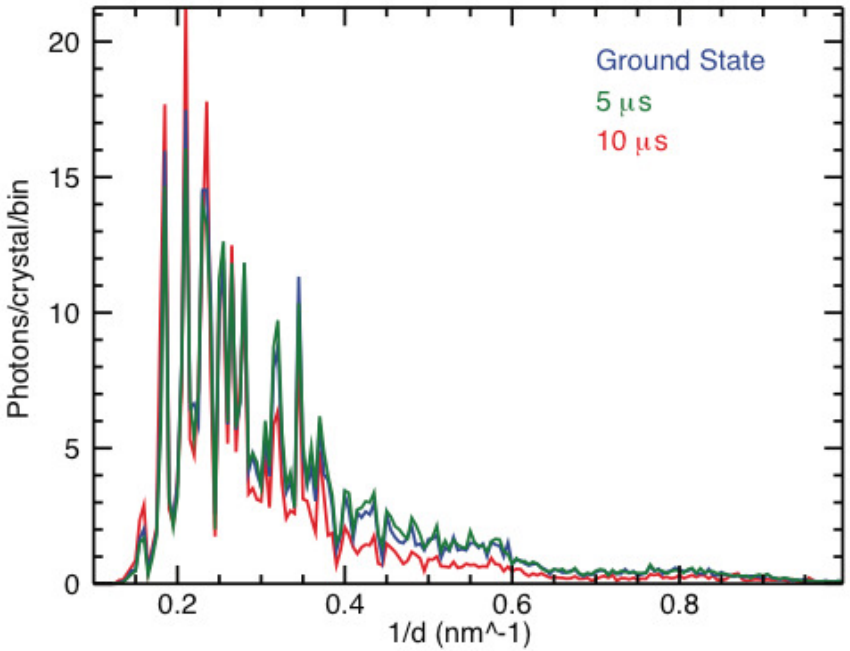
Anton Barty, Tom White, and DESY IT

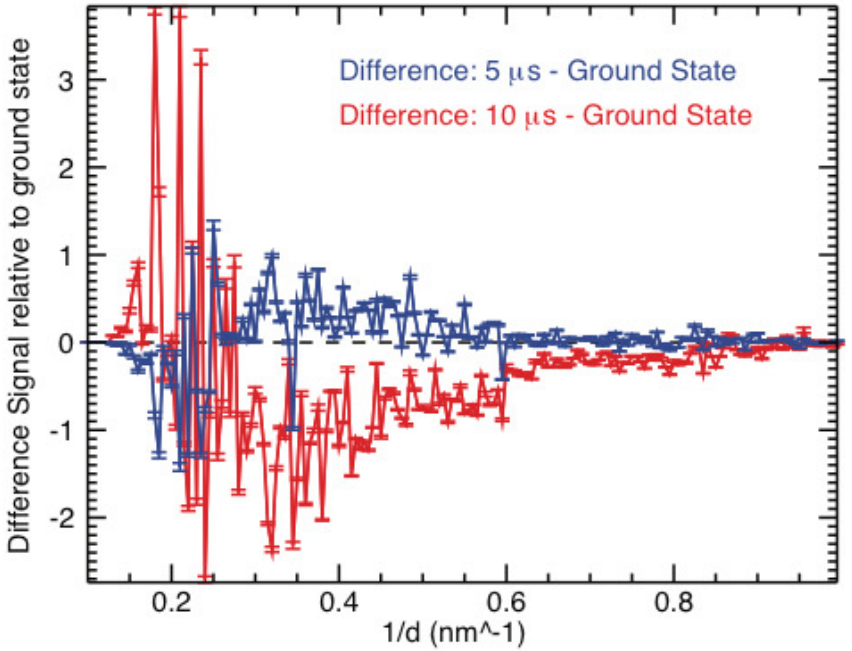
Tom White *et al*. J. Appl. Cryst. **45** 335 (2012)

Irreversible reactions can be studied



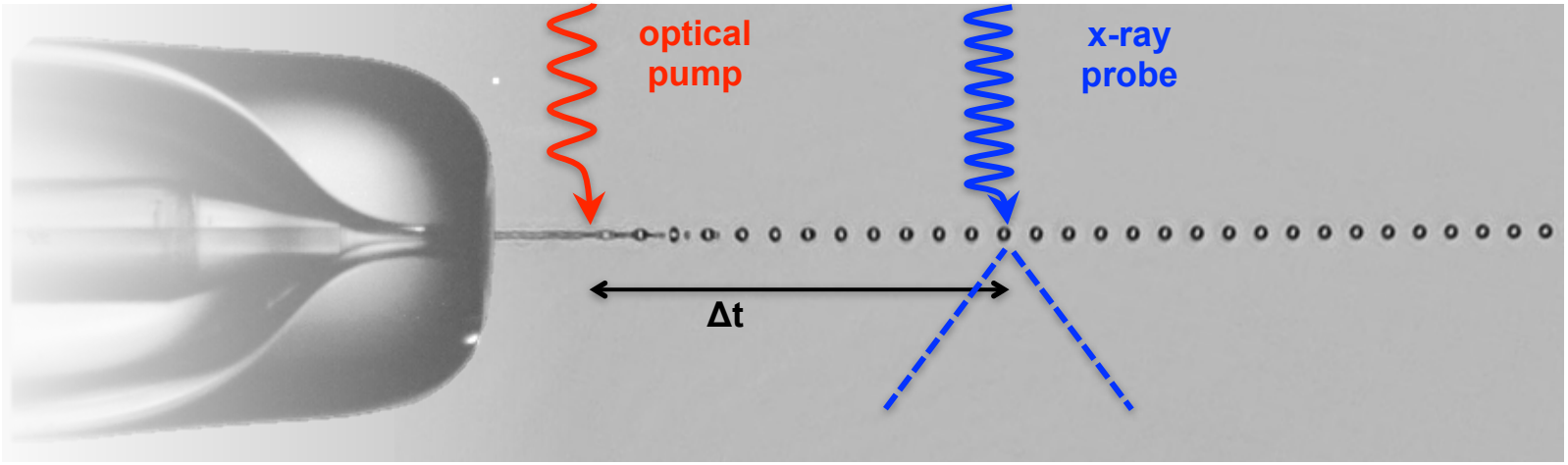
Irreversible reactions can be studied

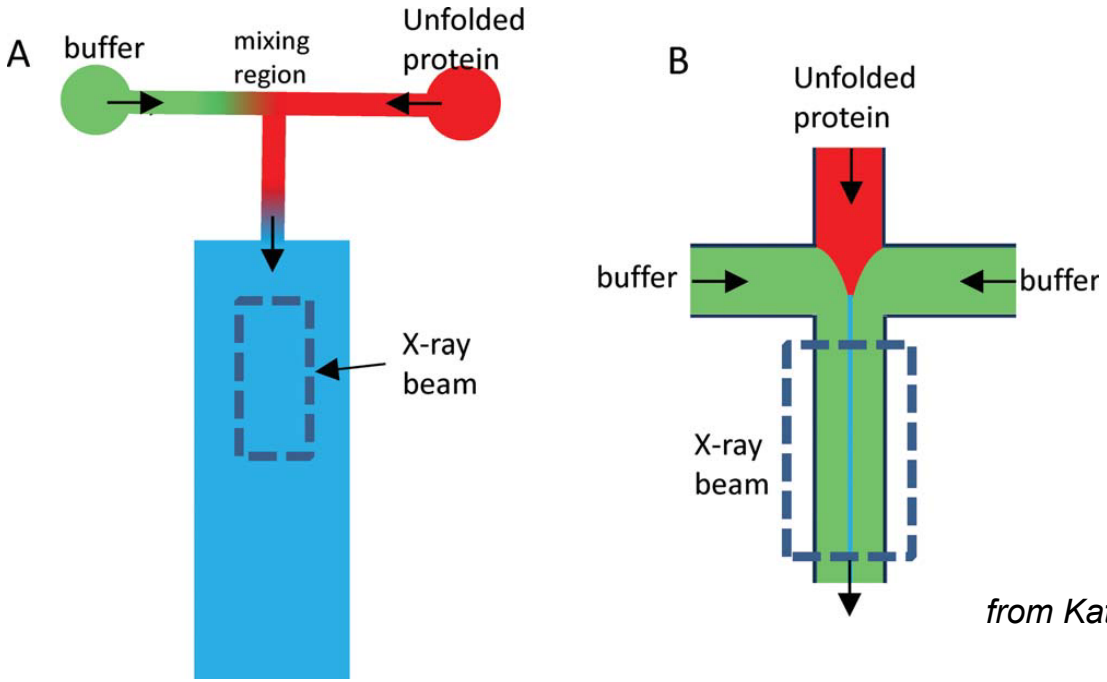




Aquila *et al*. Opt. Express **20**, 2706 (2012)

We will develop mixing and time-resolved methods for XFEL-based diffraction





from Kathuria et al. Biopolymers 95 (2011)

Opportunities could be tremendous

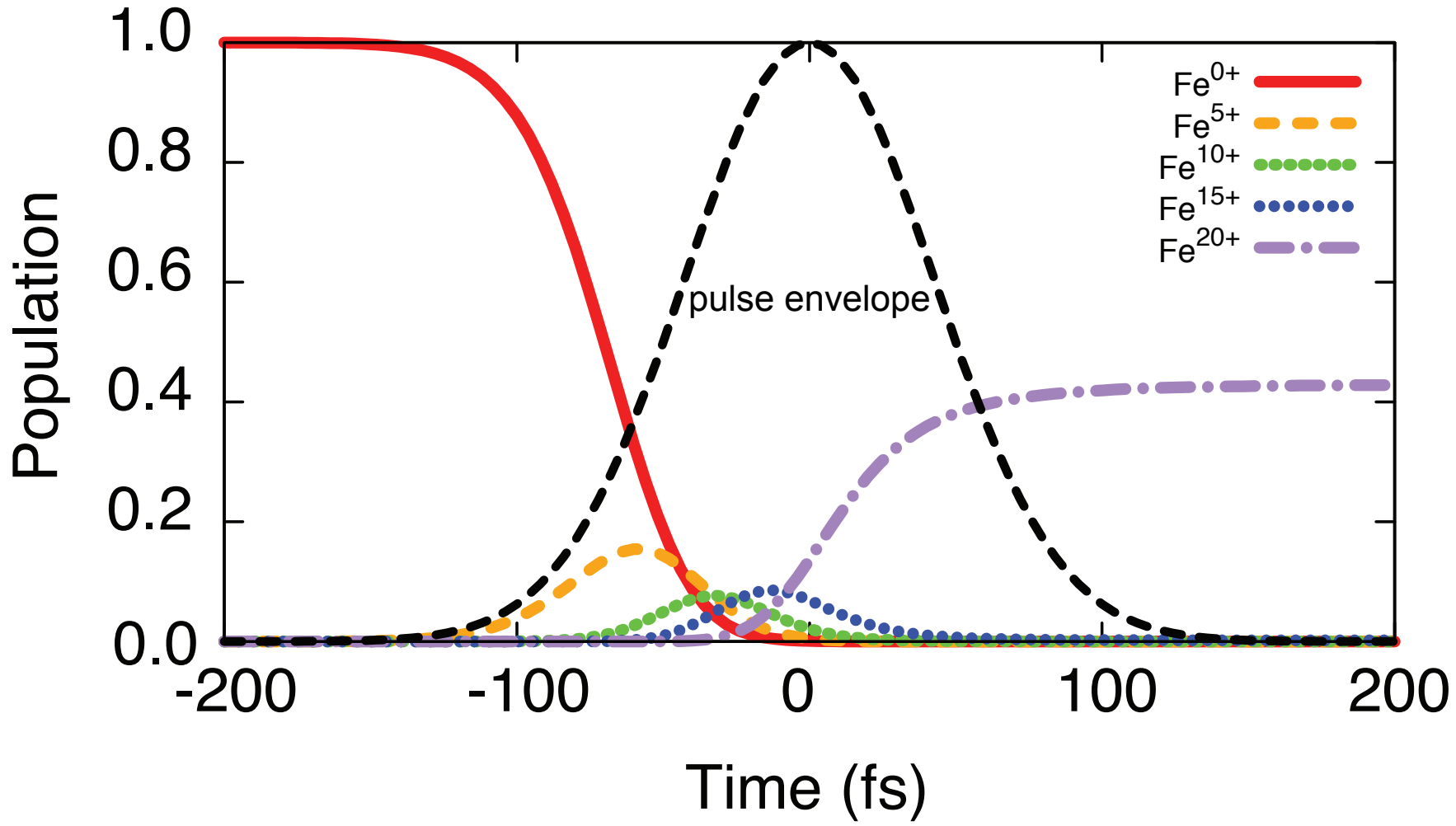
We need about 10,000 oriented patterns for a structure

This could be achievable with <100,000 shots (with 10^{10} particles/ml)

The continuous flowing jet consumes about 10 µl/minute

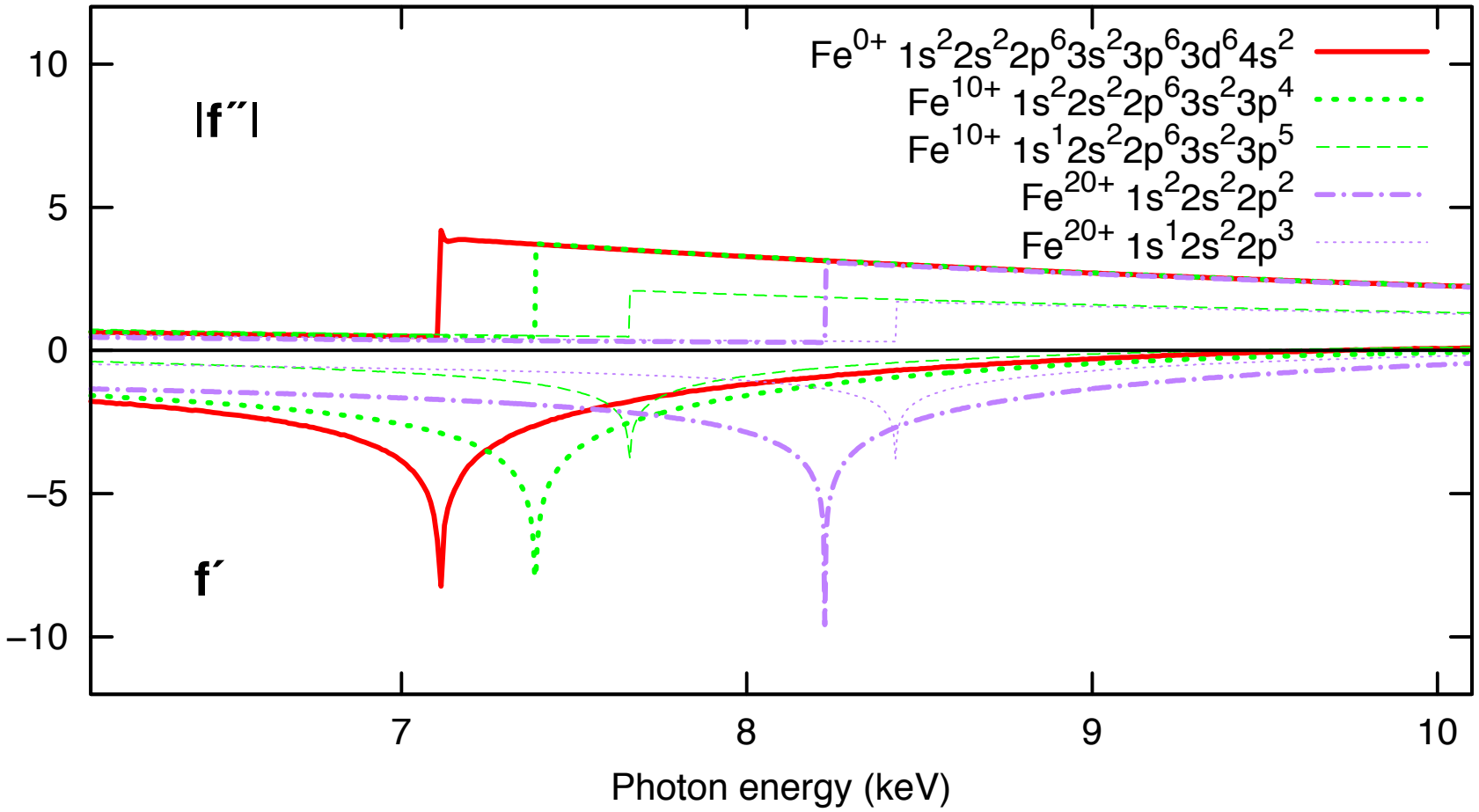
	LCLS at 120 Hz	XFEL at 27,000 pulses/second	XFEL at 3,000 pulses/second
Measurement time	14 minutes	3 seconds	31 seconds
Number of structures per day (1 minute exchange)	96	1370	950
Volume of suspension	140 µl	0.5 µl	5 µl
Amount of protein	1.4 mg	5 µg	50 µg
Amount of protein with pulsed jet	10 µg	0.5 µg	5 µg

Heavy elements ionize a lot during the pulse



Robin Santra and Sang-Kil Son
CFEL DESY

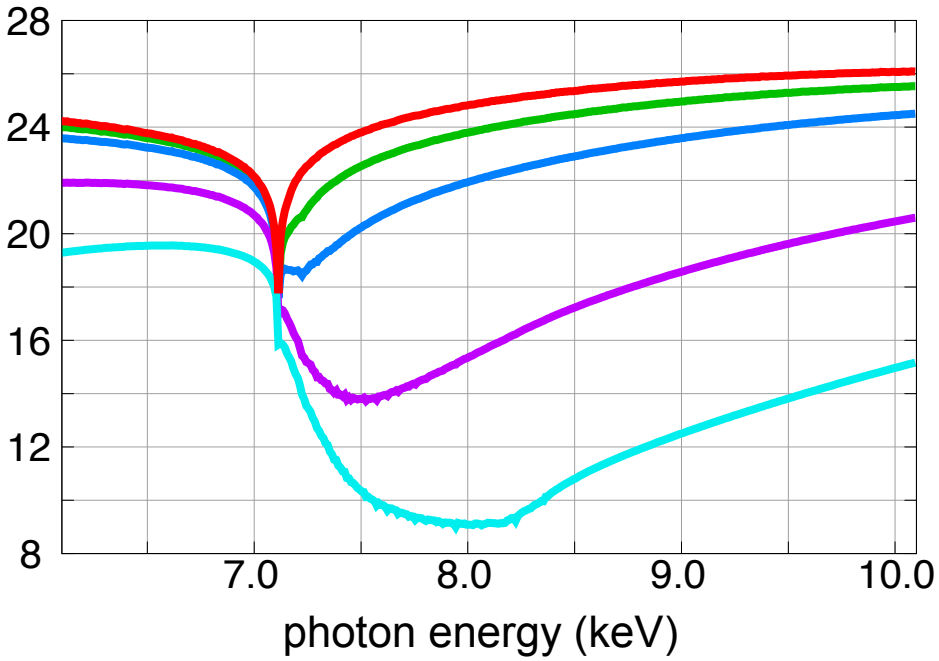
Ionized states have higher binding energies



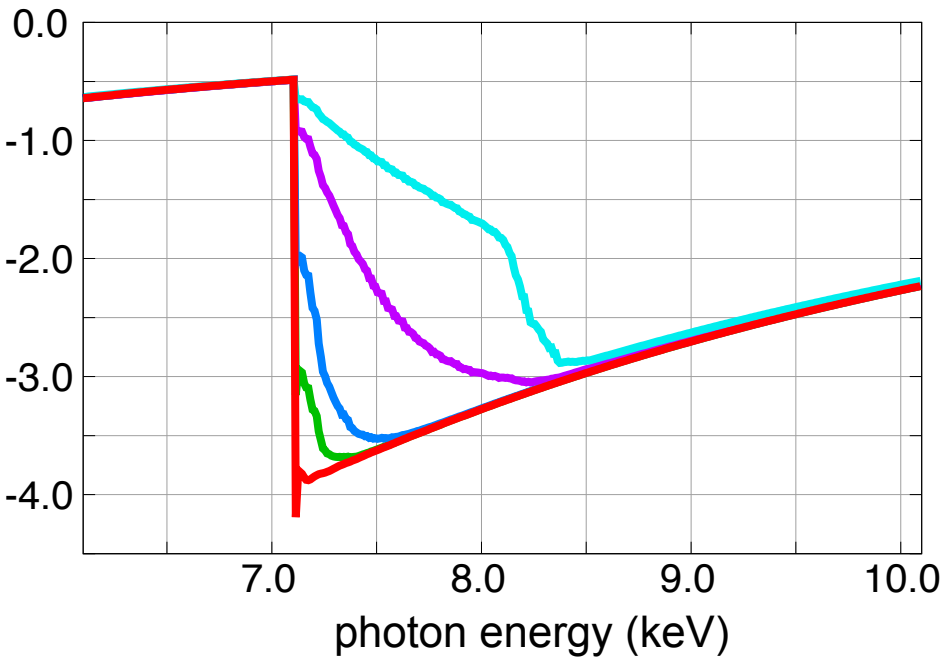
Calculations show that anomalous signals are enhanced by high X-ray intensity

Effective scattering factors for Fe with 2 mJ pulse
Average ionization by end of pulse is +14 for highest fluence

b(λ) → f^0+f'



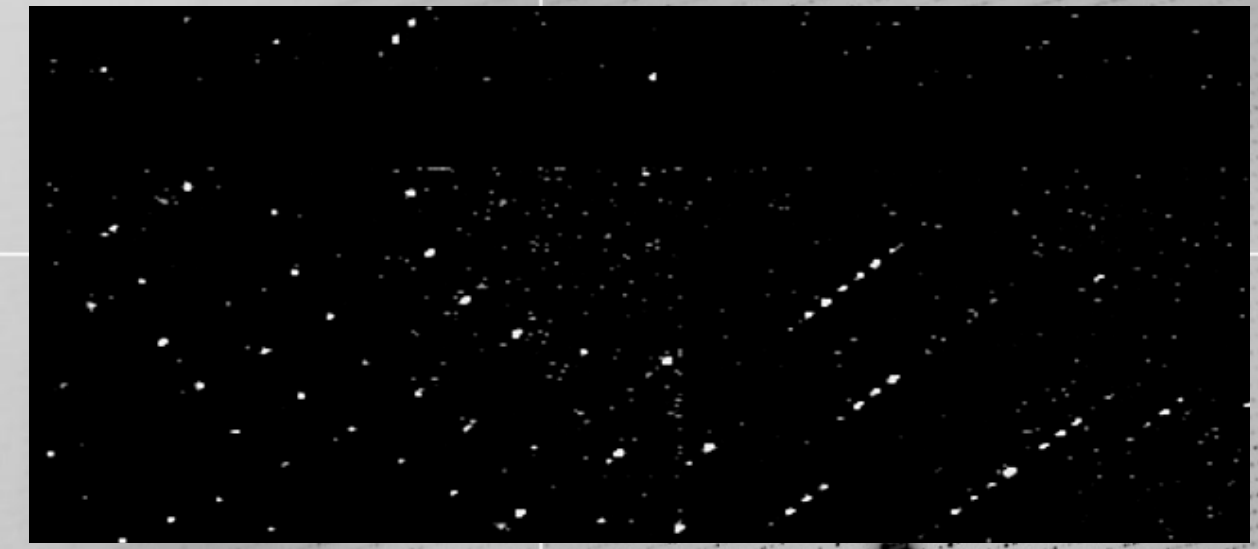
c(λ) → f''

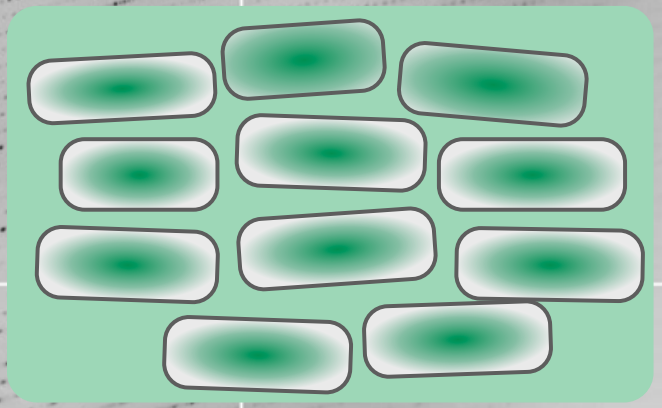


Undamaged	
1.6×10^{17} W/cm^2	1.6 MGy/fs
5×10^{17} W/cm^2	5 MGy/fs
2×10^{18} W/cm^2	20 MGy/fs
2×10^{19} W/cm^2	200 MGy/fs

S.-K. Son, H.N.C., R. Santra,
PRL **107**, 218102 (2011).

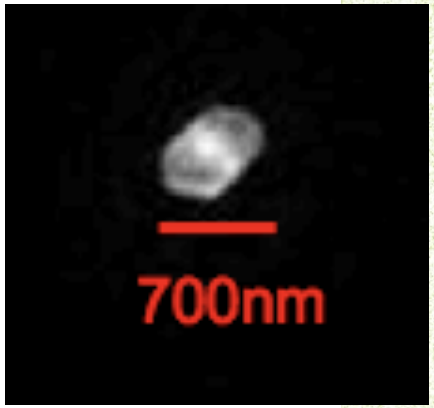
Femtosecond-pulse nanocrystal data appears better than diffraction from large crystals

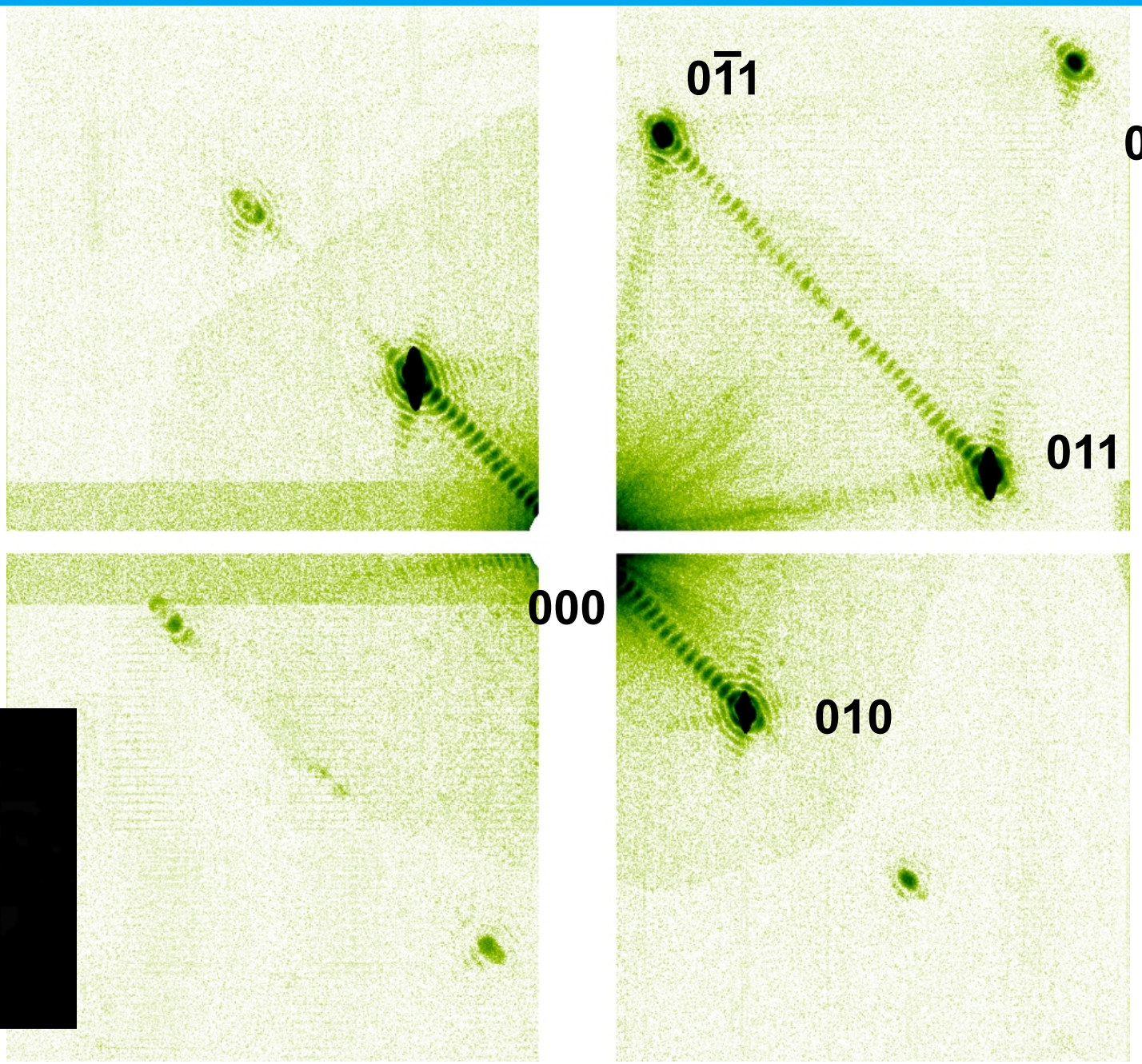




Petra Fromme,
Mark Hunter, ASU

The crystal shape can be used to obtain additional information about the molecular transform



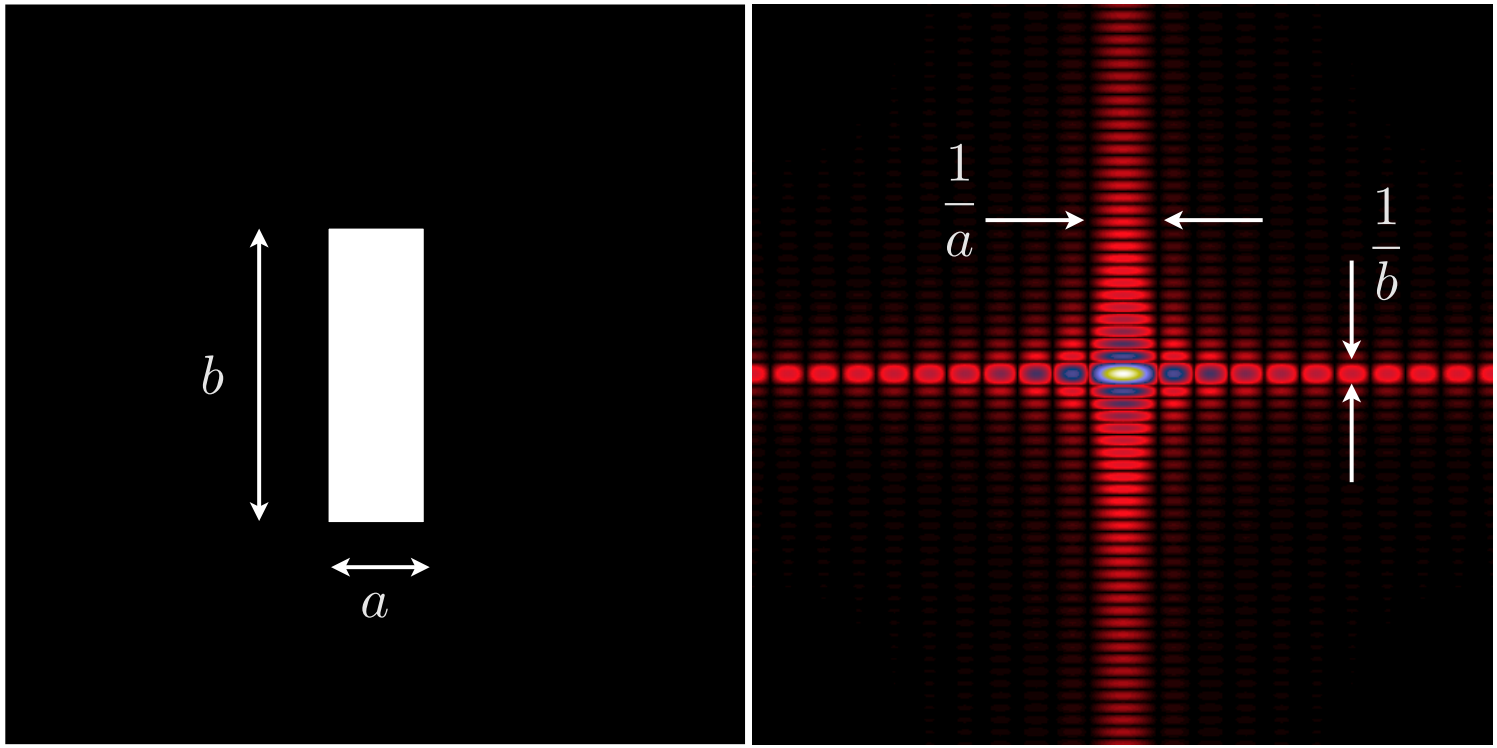


Diffraction pattern of a rectangular aperture

$$f(x,y) = \mathrm{rect}(x/a)\,\mathrm{rect}(y/b)$$

$$F(u,v) = ab\,\mathrm{sinc}\,(au)\,\mathrm{sinc}\,(bv)$$

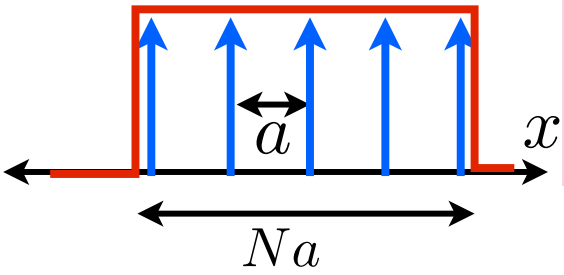
$$I(u,v) = |F(u,v)|^2 = (ab)^2\,\mathrm{sinc}^2\,(au)\,\mathrm{sinc}^2\,(bv)$$



The truncated lattice

$$f(\mathbf{x}) = m(\mathbf{x}) \otimes \{l(\mathbf{x}) \cdot s(\mathbf{x})\}$$

$$F(\mathbf{u}) = M(\mathbf{x}) \cdot \{L(\mathbf{u}) \otimes S(\mathbf{u})\}$$



$$s(x) = \text{rect}\,(\frac{x}{Na})$$

$$S(u) = Na\,\text{sinc}\,Nau$$

$$l(x) = \sum_{n=-\infty}^{\infty} \delta(\mathbf{x} - n\mathbf{a}) \qquad L(u) = 1/a \sum_{h} \delta(u - h/a)$$

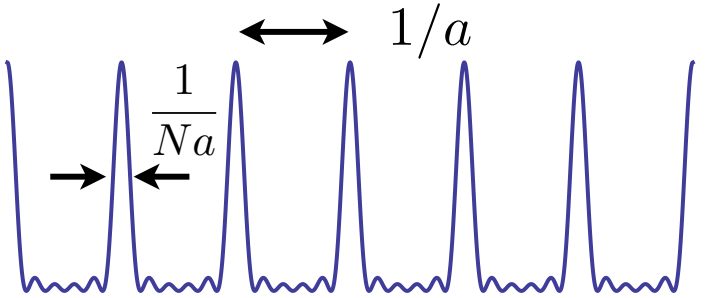
$$S(u) \otimes L(u) = Na\,\text{sinc}\,Nau \otimes \frac{1}{a}\sum_{h} \delta(u - \frac{h}{a})$$

$$= N \sum_{h} \text{sinc}\,Nau \otimes \delta(u - \frac{h}{a})$$

$$= Na \sum_{h} \text{sinc}\,N(au - h)$$

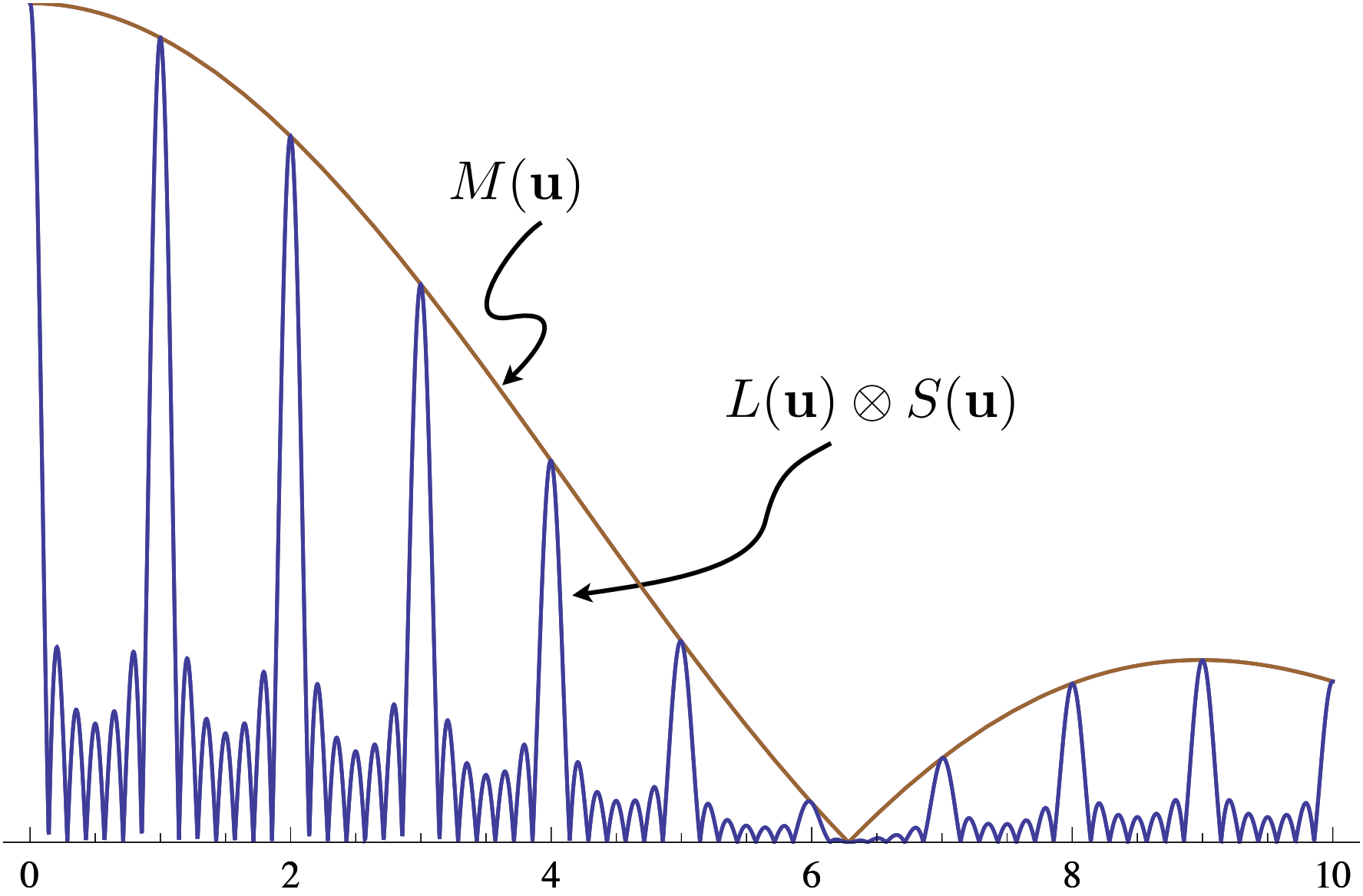
$$= \frac{\sin(\pi Nau)}{\sin(\pi au)}$$

Exercise to reader
Cowley p 44



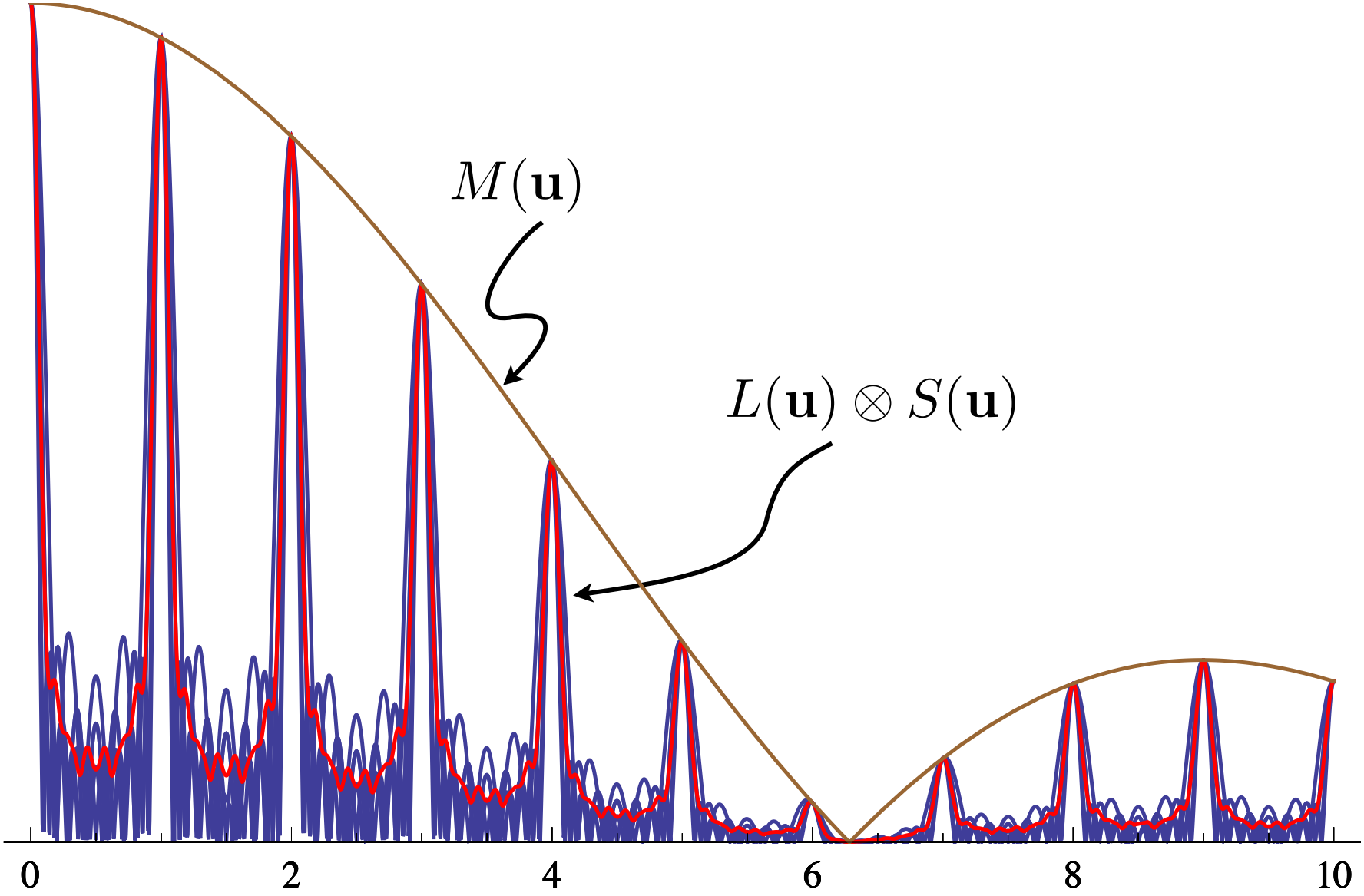
N wiggles including the two Bragg peaks

The finite crystal shape gives access to finer sampling of the molecular transform



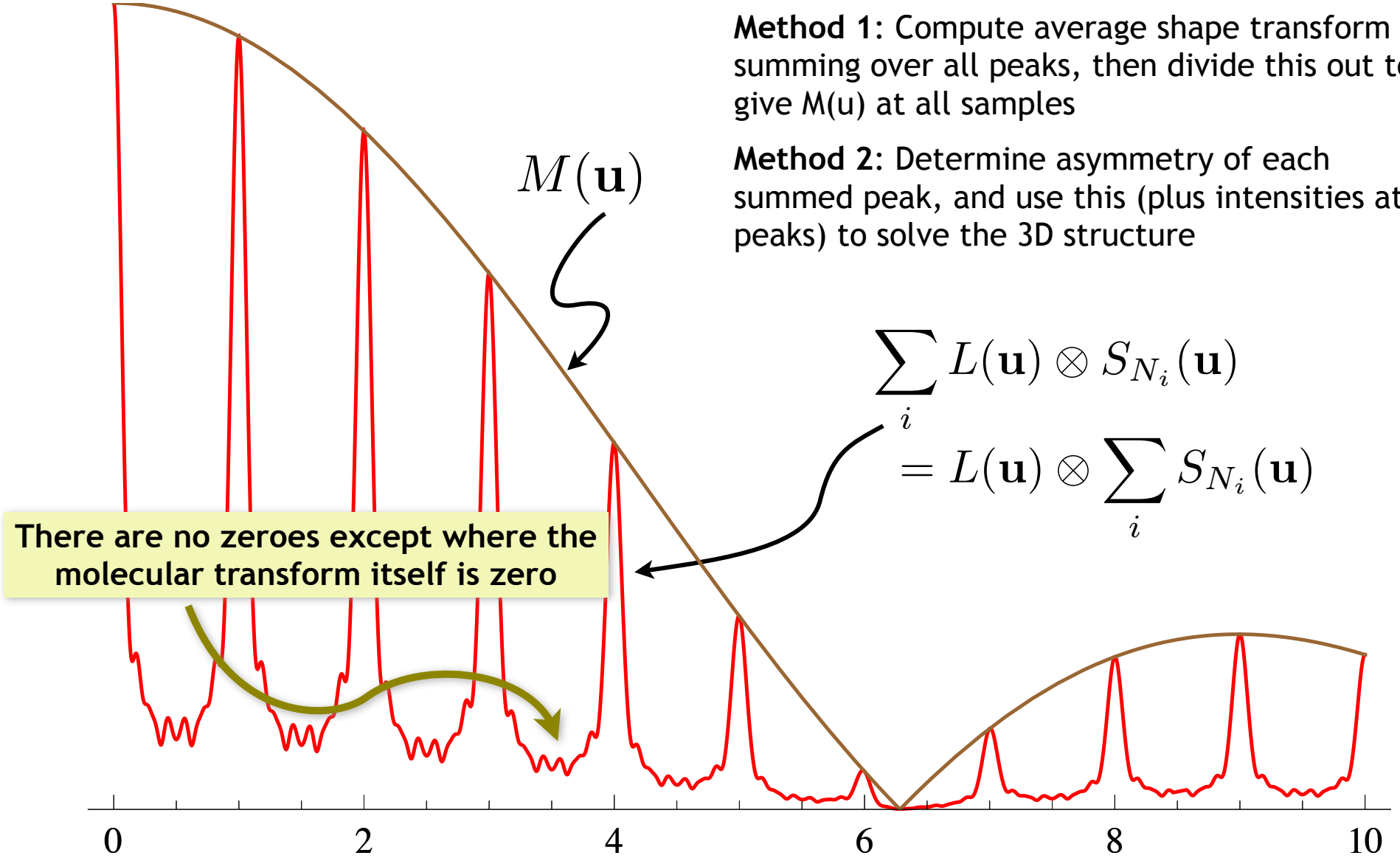
John Spence *et al*. Opt. Express **19** 2866 (2011)

The finite crystal shape gives access to finer sampling of the molecular transform



John Spence *et al*. Opt. Express **19** 2866 (2011)

The finite crystal shape gives access to finer sampling of the molecular transform

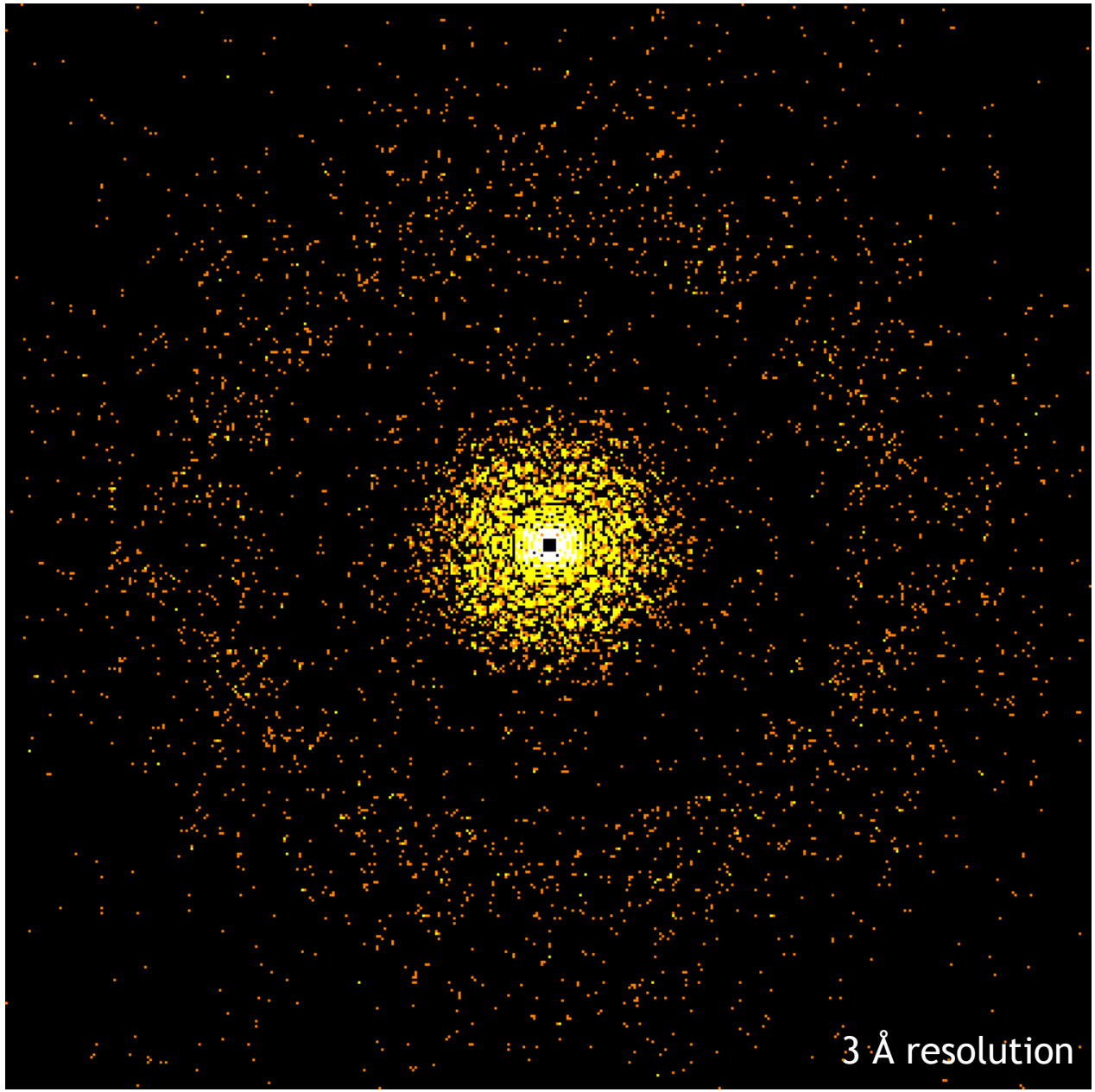


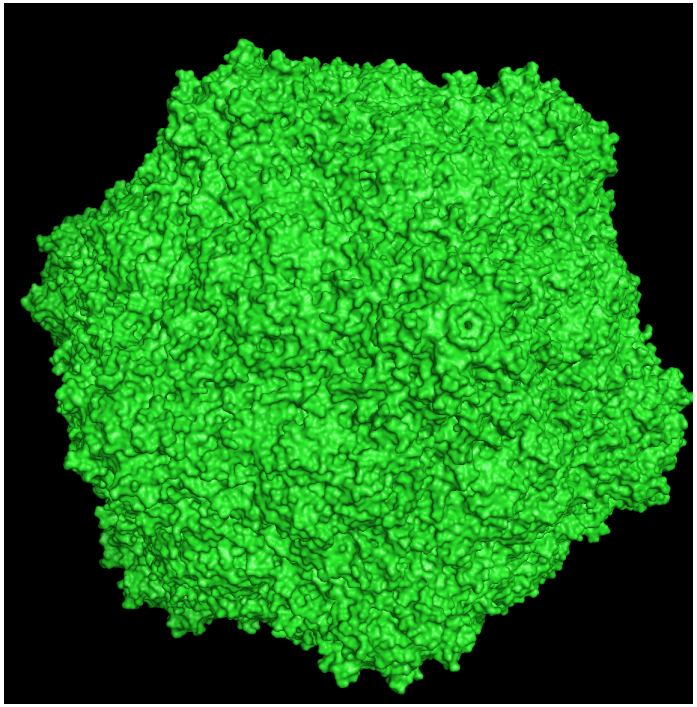
Method 1: Compute average shape transform by summing over all peaks, then divide this out to give M(u) at all samples

Method 2: Determine asymmetry of each summed peak, and use this (plus intensities at peaks) to solve the 3D structure

John Spence *et al*. Opt. Express **19** 2866 (2011)

Atomic-resolution diffraction from single particles should be possible with 10^{14} ph/μm^2



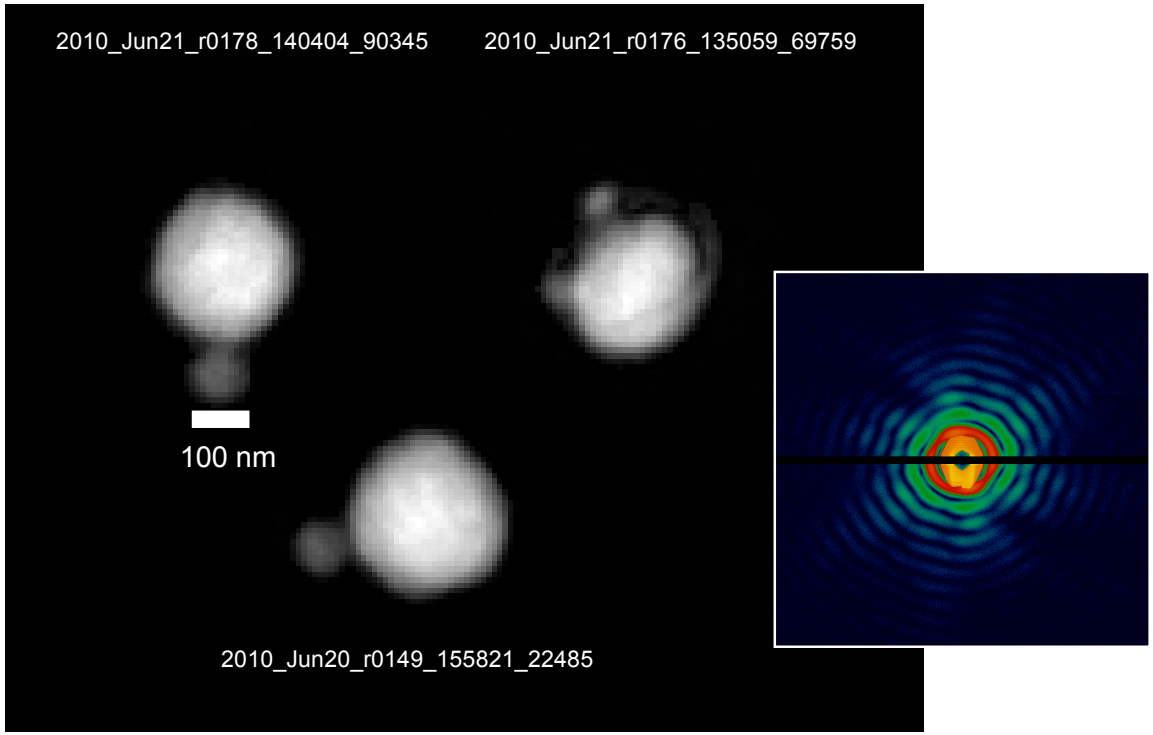


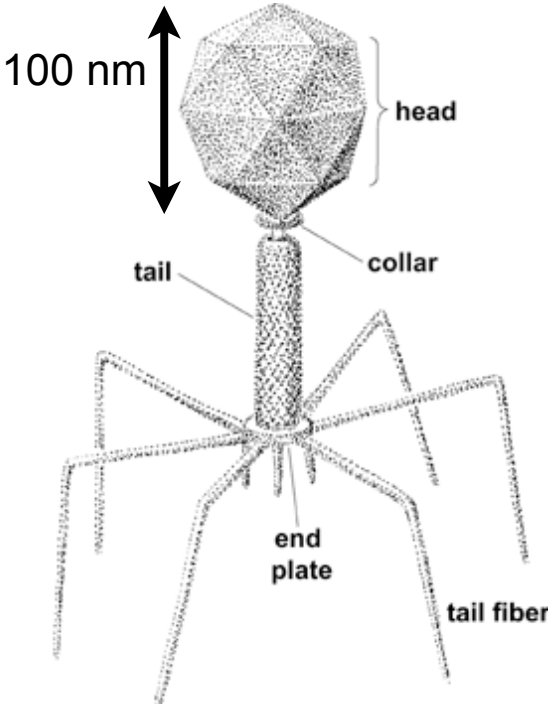
28 nm

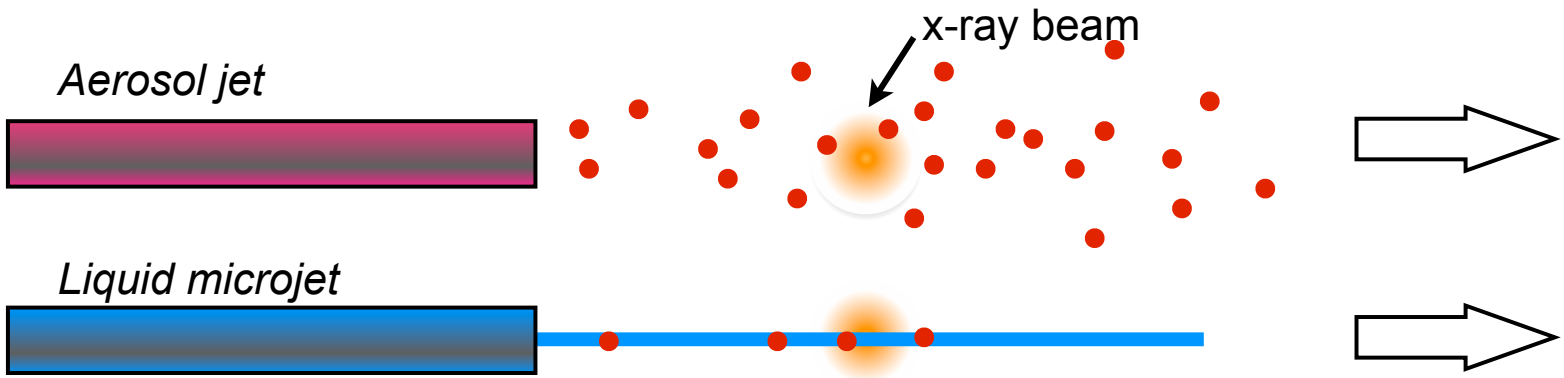
10^{14} ph/μm^2
60 GGy
6000 MGy/fs × 10 fs

RMS displacement: 0.5Å
half electrons ionized

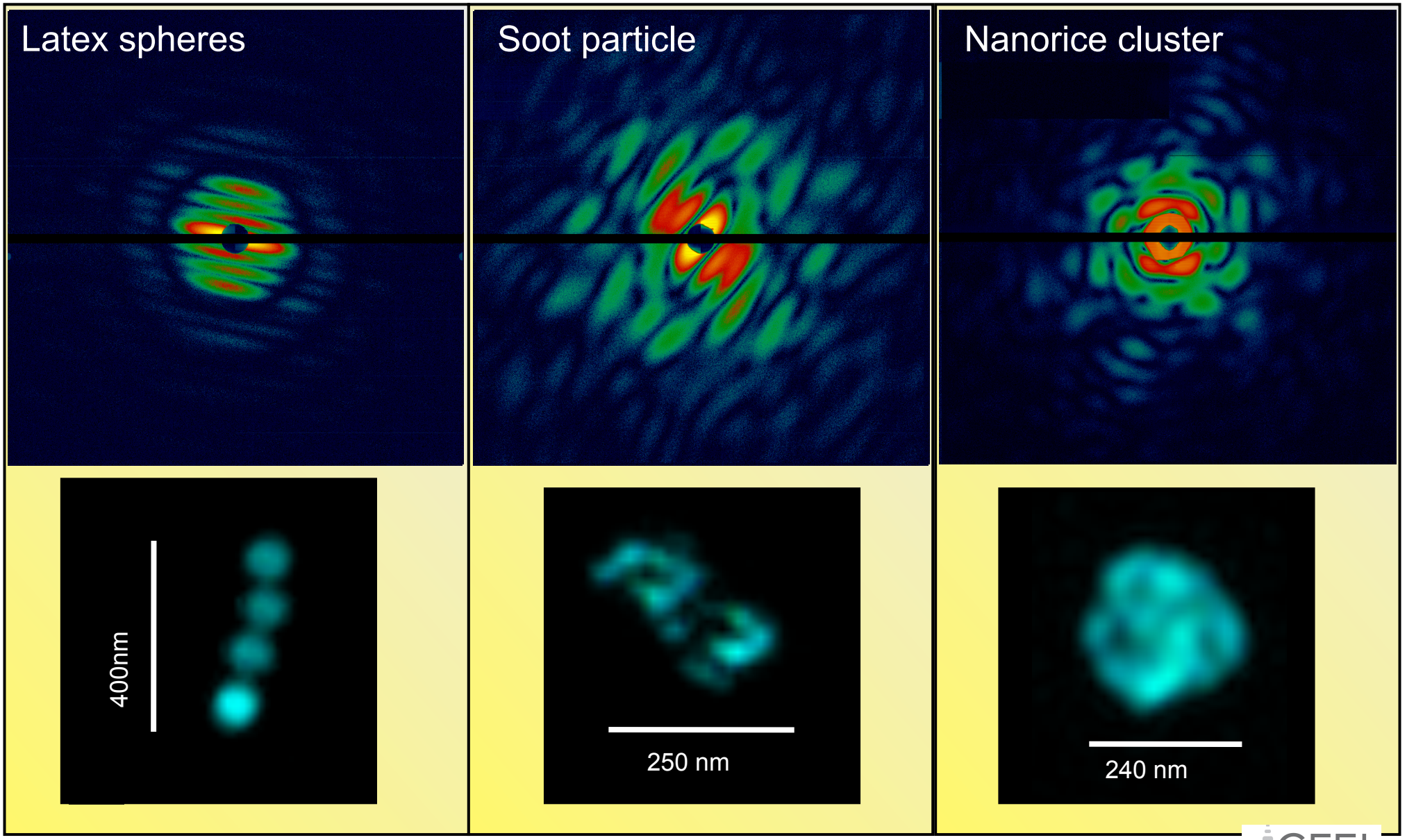
Aerosol beams are diffuse and samples are not completely dry





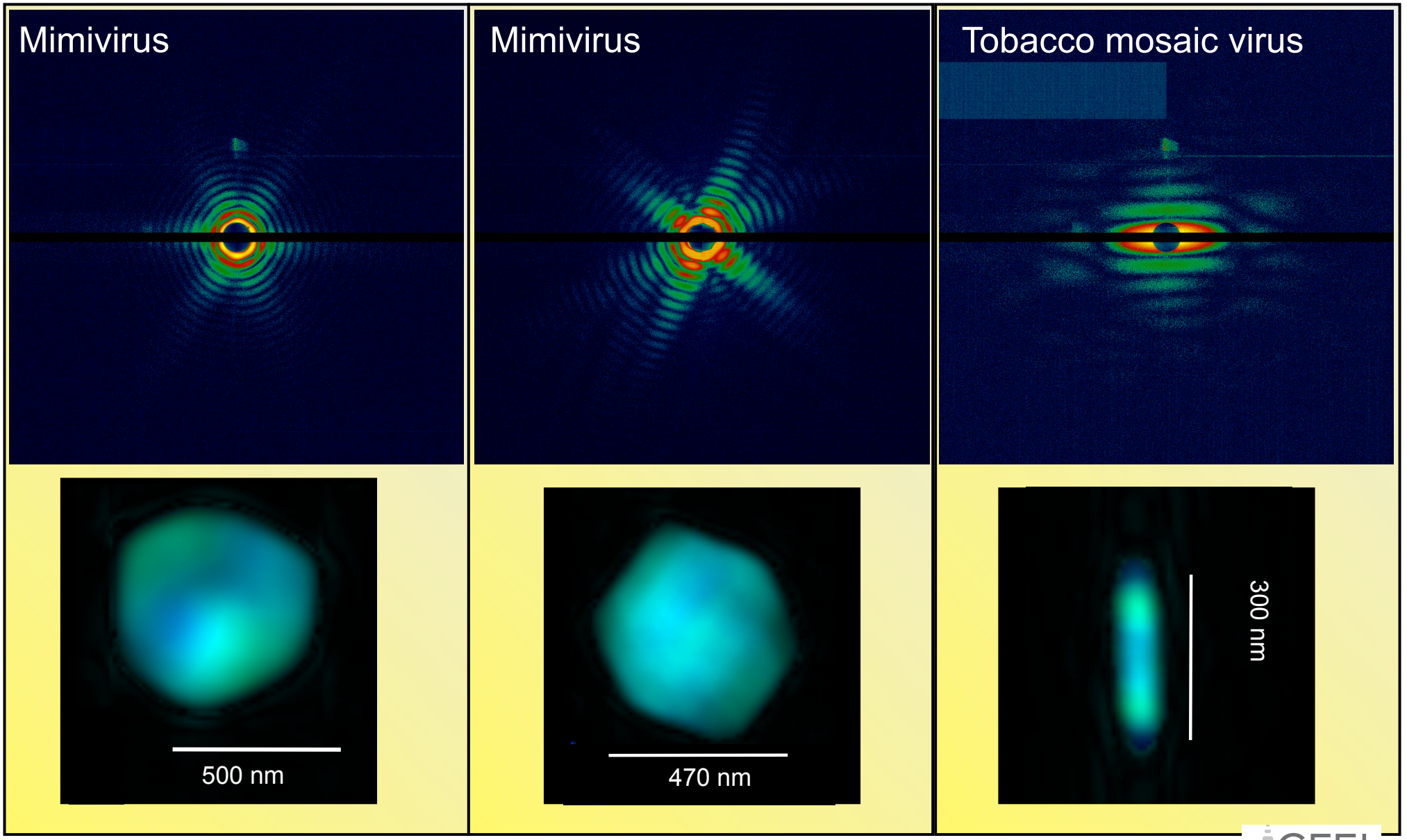


We can reconstruct images of soot and nanoparticles particles



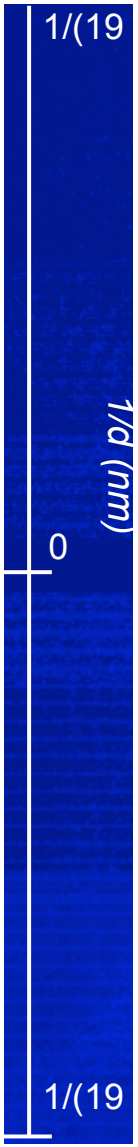
Andrew Martin, CFEL

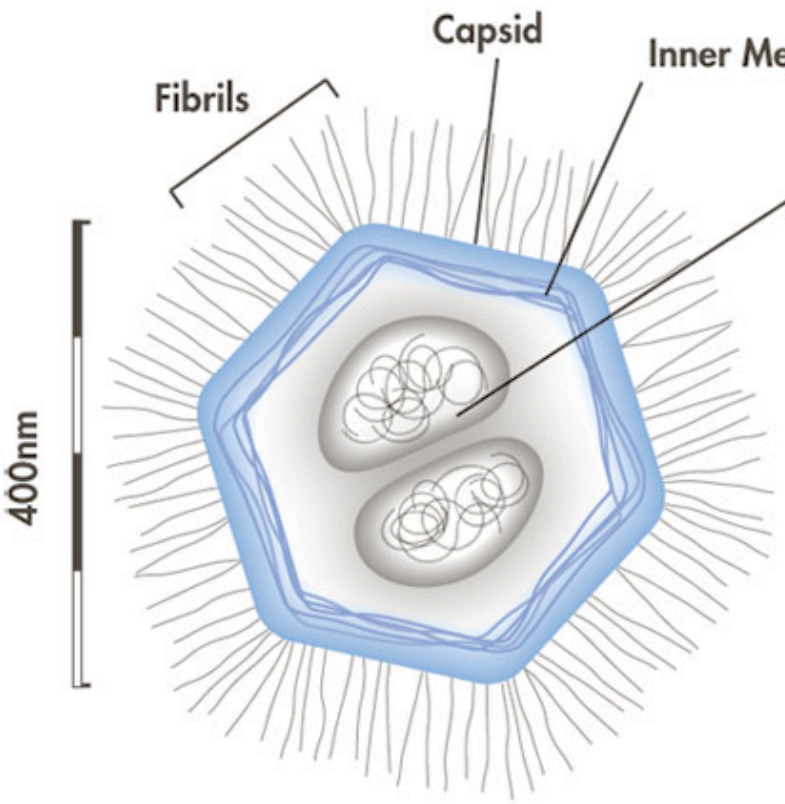
We can reconstruct images of virus particles



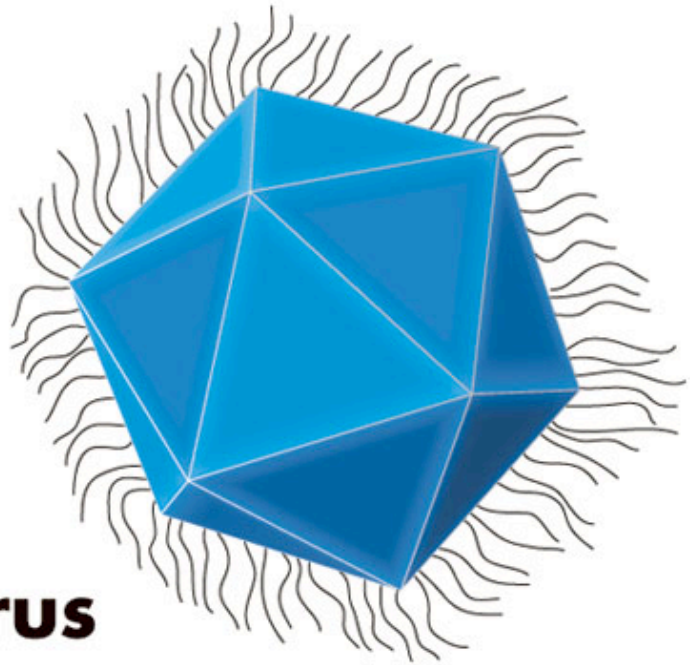
Andrew Martin, CFEL

Clear diffraction is measured from individual mimivirus





- dsDNA virus
- 90% coding capacity
- 10% Junk DNA
- 1.2 million base pairs
- ~911 protein coding genes
- additional genes (inc. aminoactyl tRNA synthetases; sugar, lipid, and amino acid metabolism)



3 mJ
ım)

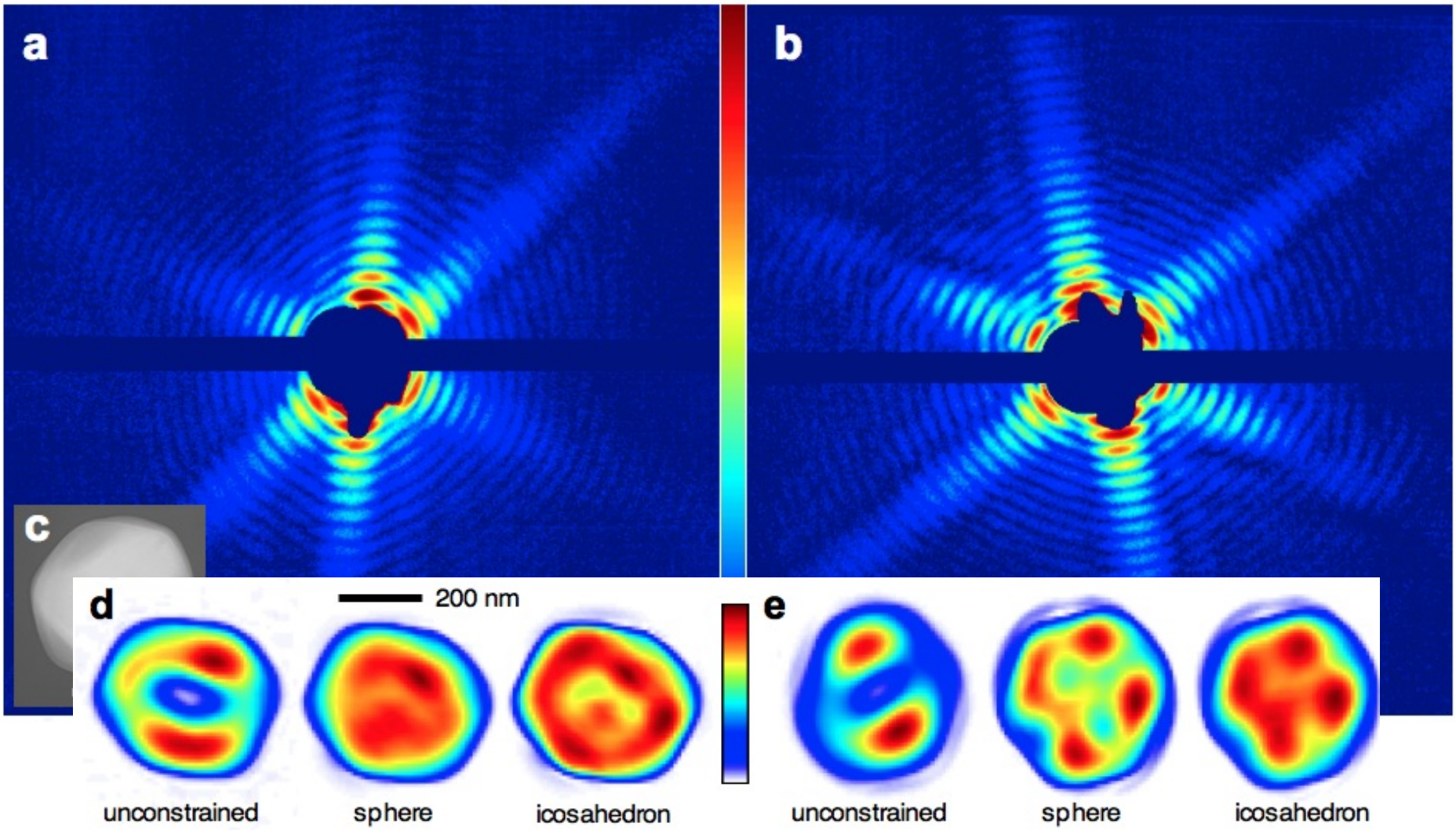
ıtion of

acanthamoeba polyphaga mimivirus

Single particles at 20 nm resolution

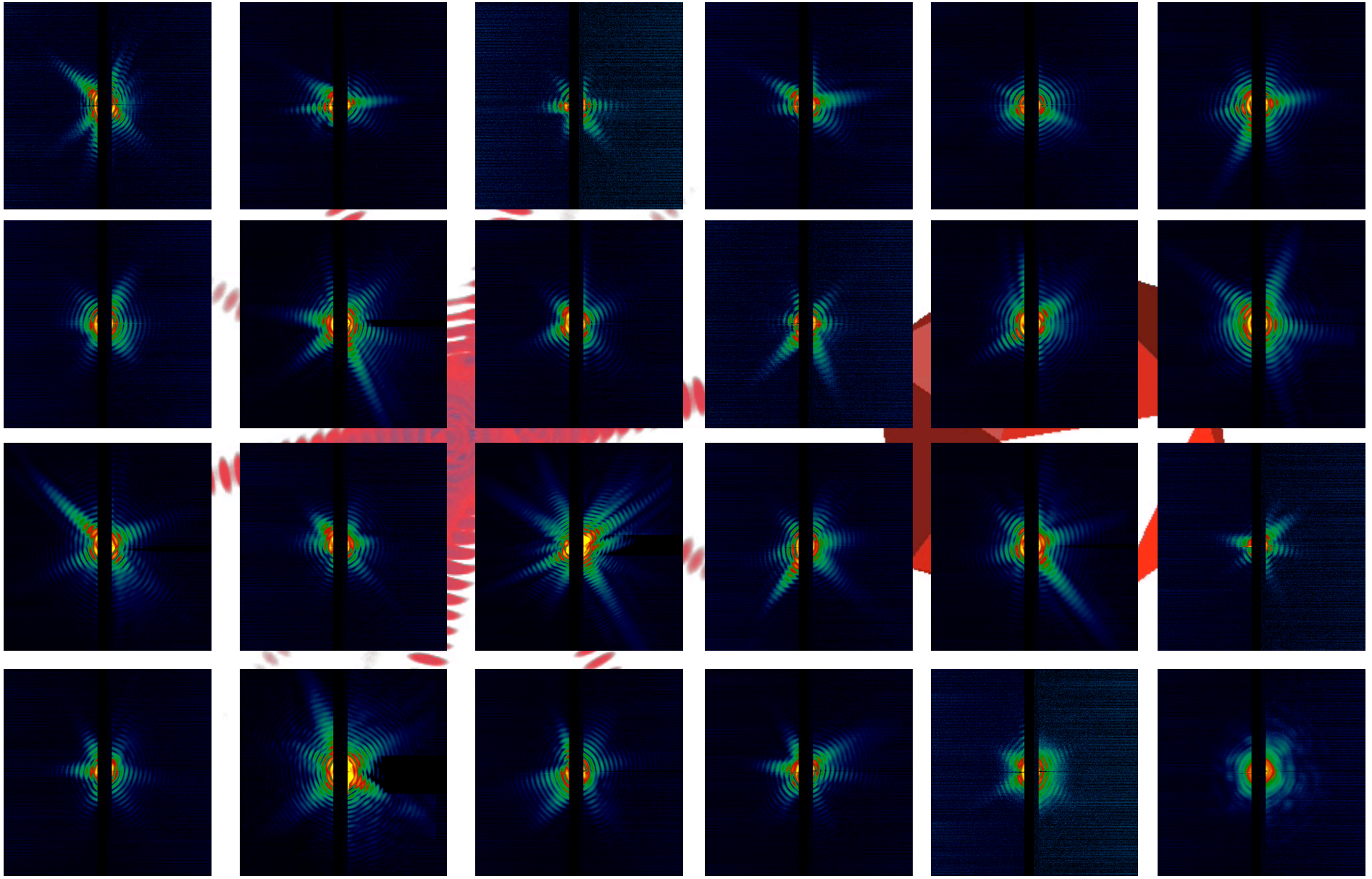
Samples Janos Hajdu, Uppsala University, CNRS Marseille

Mimivirus diffraction from LCLS successfully reconstructs in 2D

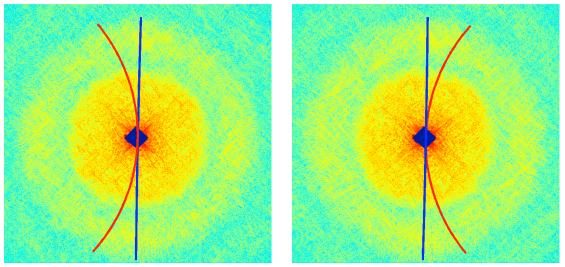


Janos Hajdu, Filipe Maia, Tomas Ekberg - Uppsala

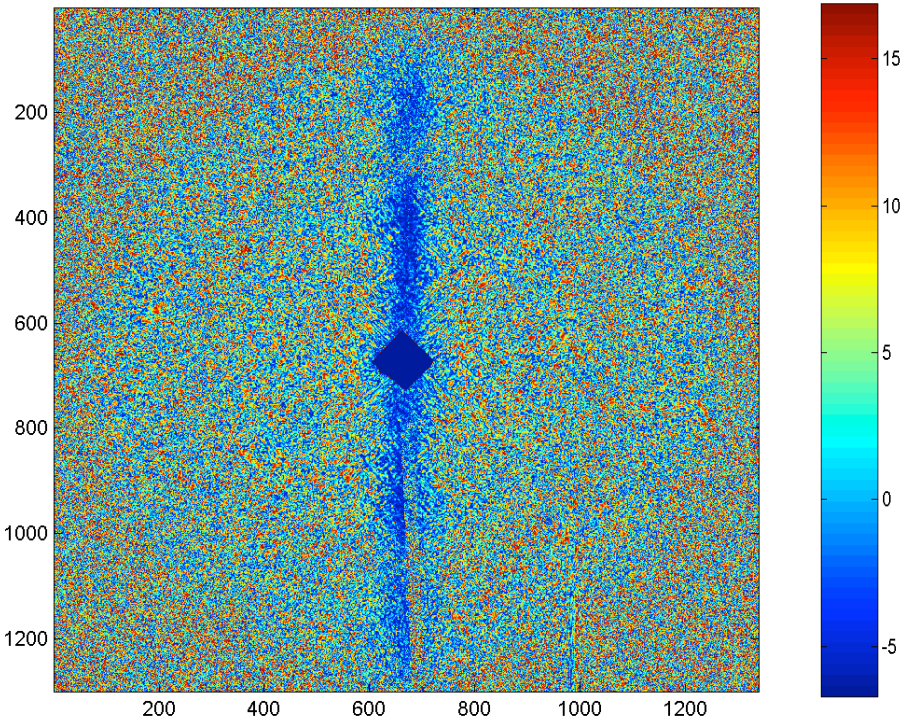
The randomly oriented diffraction patterns will be assembled to recover a 3D image

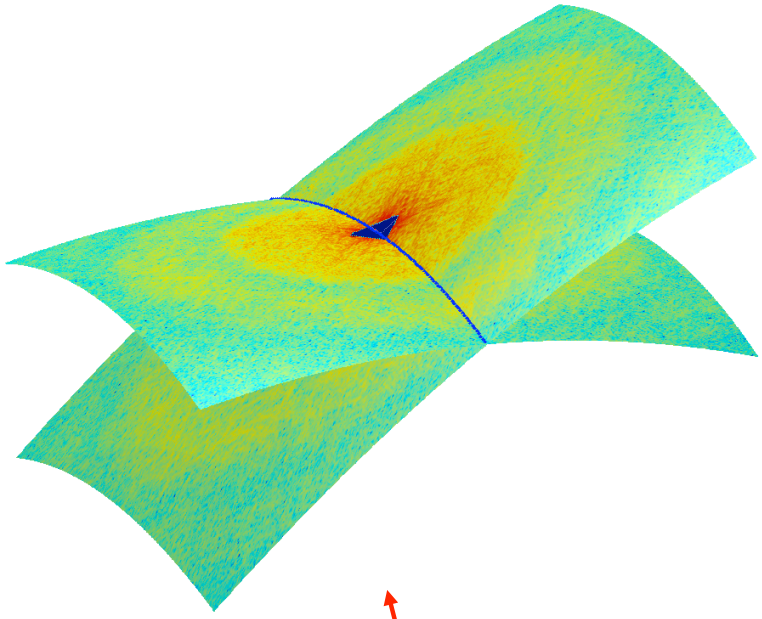


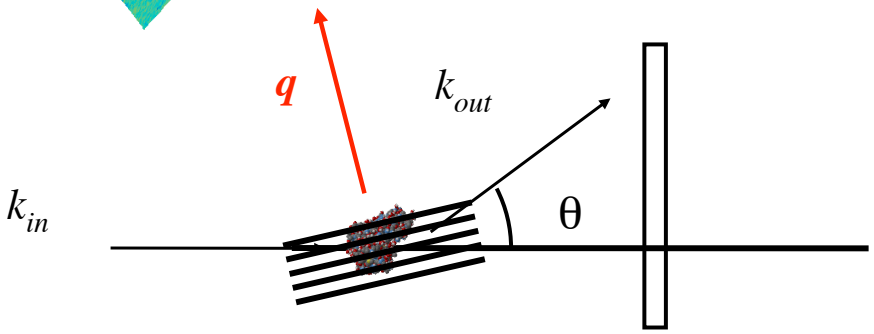
Orientation of diffraction data can be found from the intersection of common lines



Difference of patterns at two object orientations





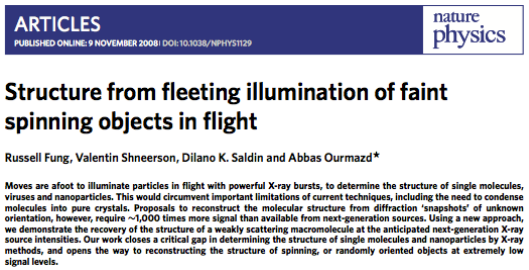


Experimental X-ray diffraction data of a 3D test object, measured at 1.6 nm wavelength

Gösta Huldt, U. Uppsala

Manifold embedding in a nutshell (GTM = Generative Tomographic Mapping)

Beyesian optimisation

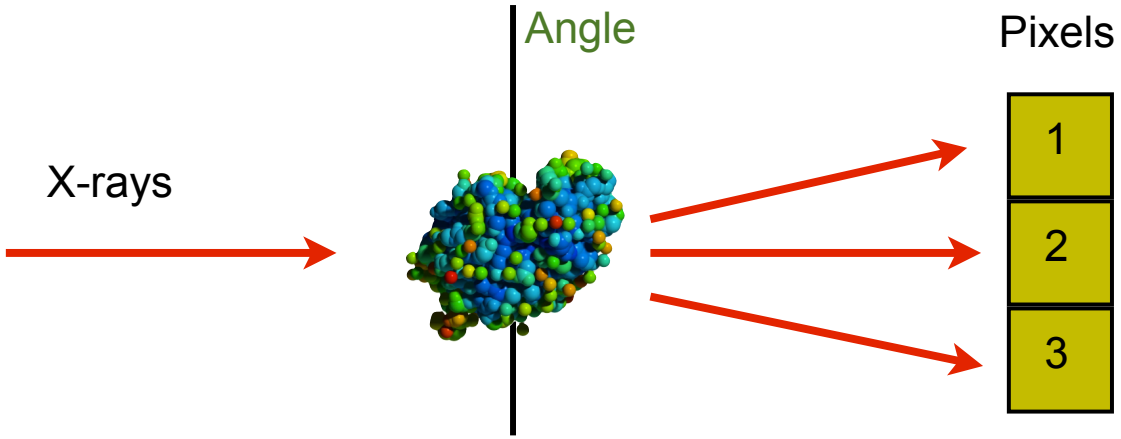

ARTICLES
PUBLISHED ONLINE: 9 NOVEMBER 2008 | DOI: 10.1038/NPHYS1129

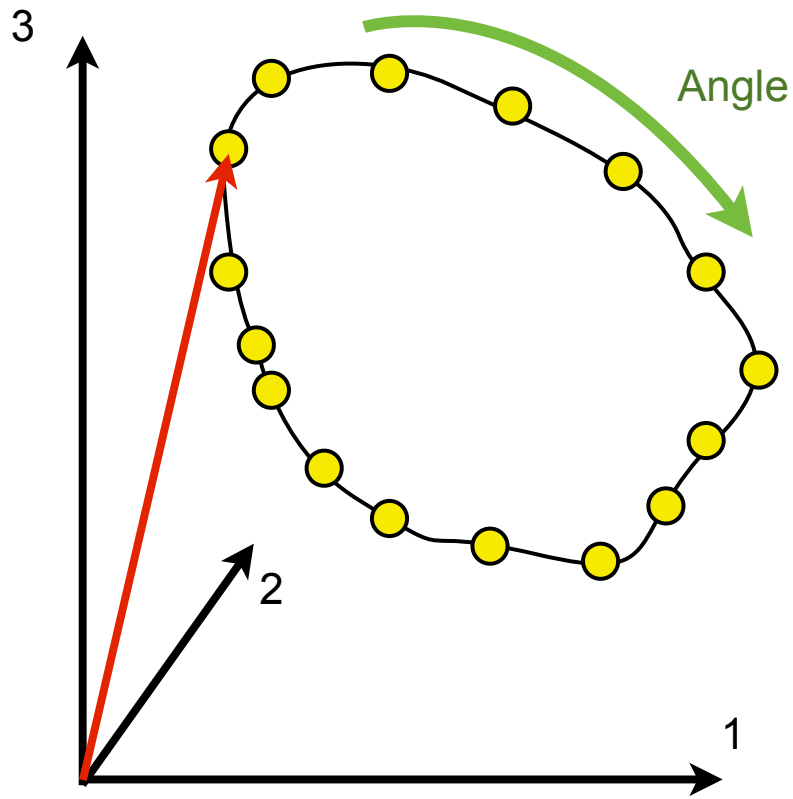
nature physics

Structure from fleeting illumination of faint spinning objects in flight

Russell Fung, Valentin Shneerson, Dilano K. Saldin and Abbas Ourmazd*

Moves are afoot to illuminate particles in flight with powerful X-ray bursts, to determine the structure of single molecules, viruses and nanoparticles. This would circumvent important limitations of current techniques, including the need to condense molecules into pure crystals. Proposals to reconstruct the molecular structure from diffraction 'snapshots' of unknown orientation, however, require ~1,000 times more signal than available from next-generation sources. Using a new approach, we demonstrate the recovery of the structure of a weakly scattering macromolecule at the anticipated next-generation X-ray source intensities. Our work closes a critical gap in determining the structure of single molecules and nanoparticles by X-ray methods, and opens the way to reconstructing the structure of spinning, or randomly oriented objects at extremely low signal levels.





1. Pixels form an n-dimensional hyperspace

2. Each diffraction pattern is a unique point in n-space

3. Points move in n-space as the sample rotates

4. Euclidean metric links adjacent points into a smooth manifold encoding sample rotation

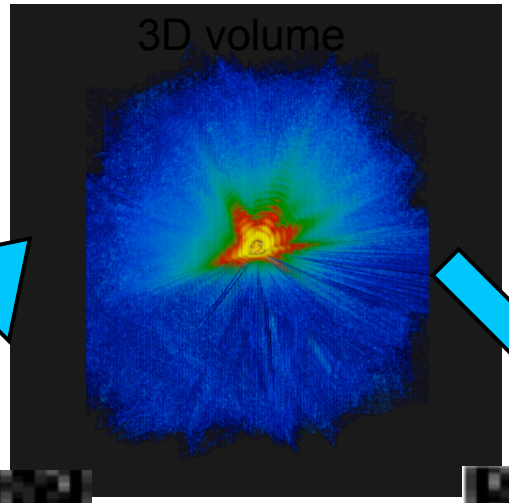
Ourmazd *et.al.* (Wisconsin)
Nature Physics, 5, 64 (2009)

EMC (Expansion, Maximisation, Compression)

PHYSICAL REVIEW E 80, 026705 (2009)

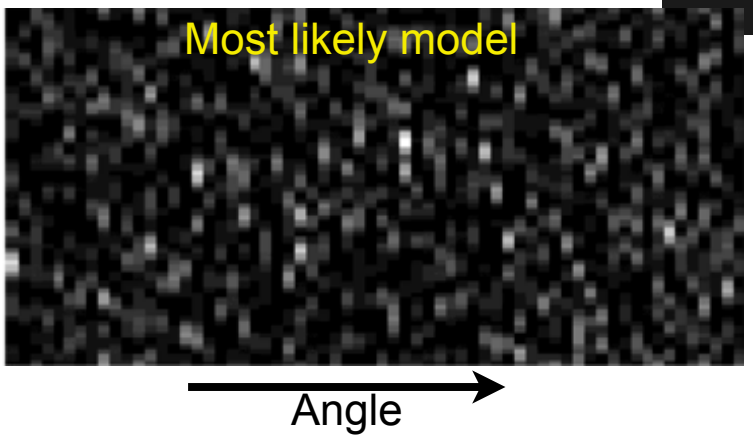
Reconstruction algorithm for single-particle diffraction imaging experiments

Ne-Te Duane Loh and Veit Elser
Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, New York 14853-2501, USA
(Received 4 May 2009; published 24 August 2009)

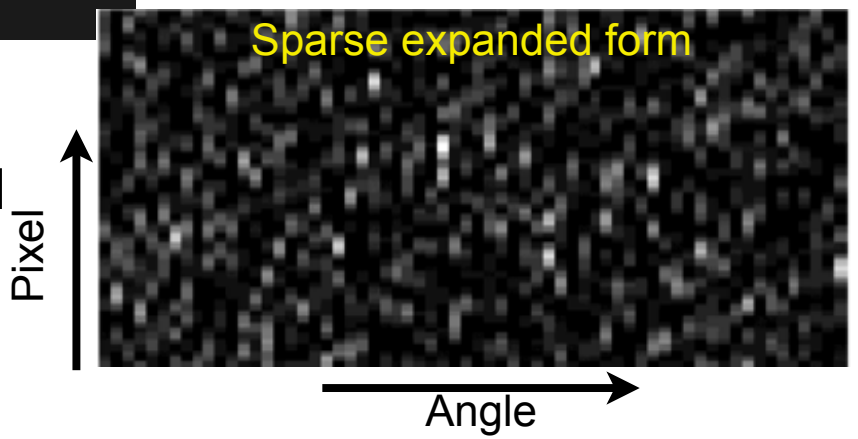


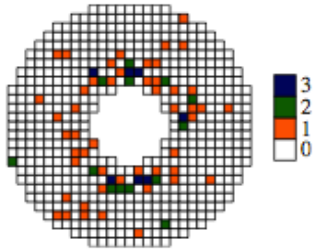
Compress

Expand

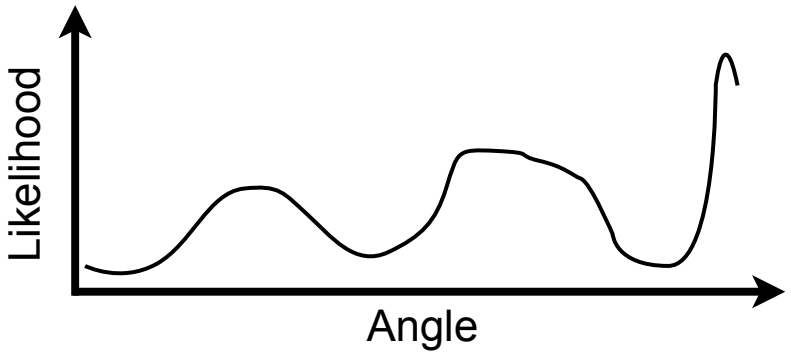


Maximise



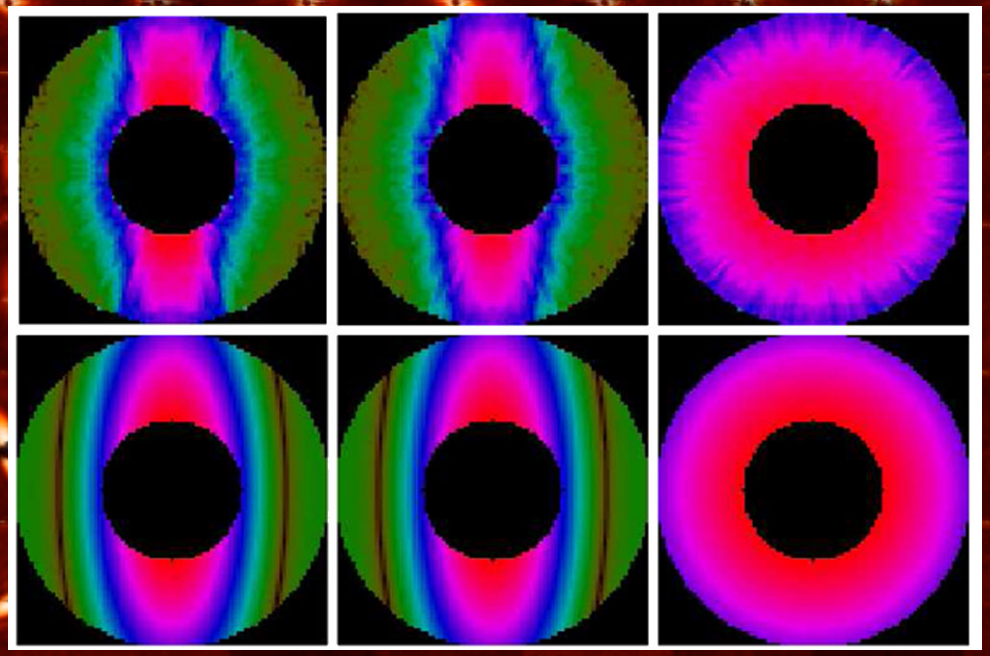


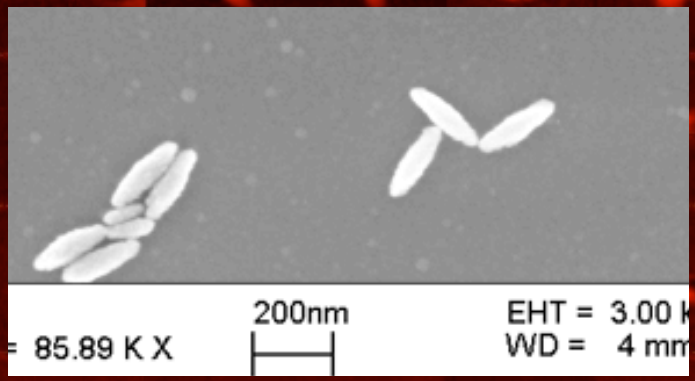
Compute log-likelihood probabilities



Loh & Elser (Cornell)
Loh & Elser, PRE 80, 026705 (2009)

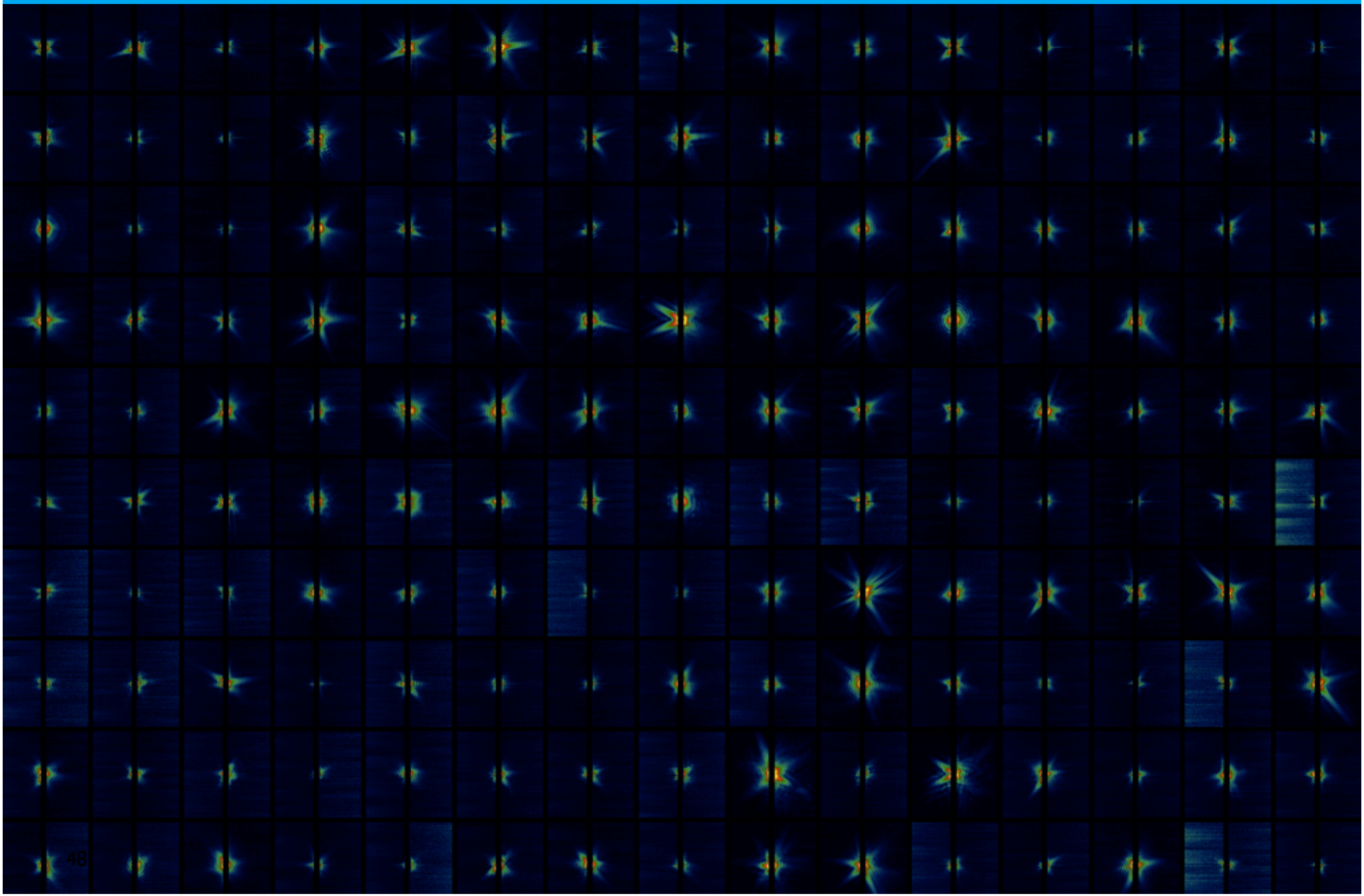
The EMC algorithm has been applied to FEL data



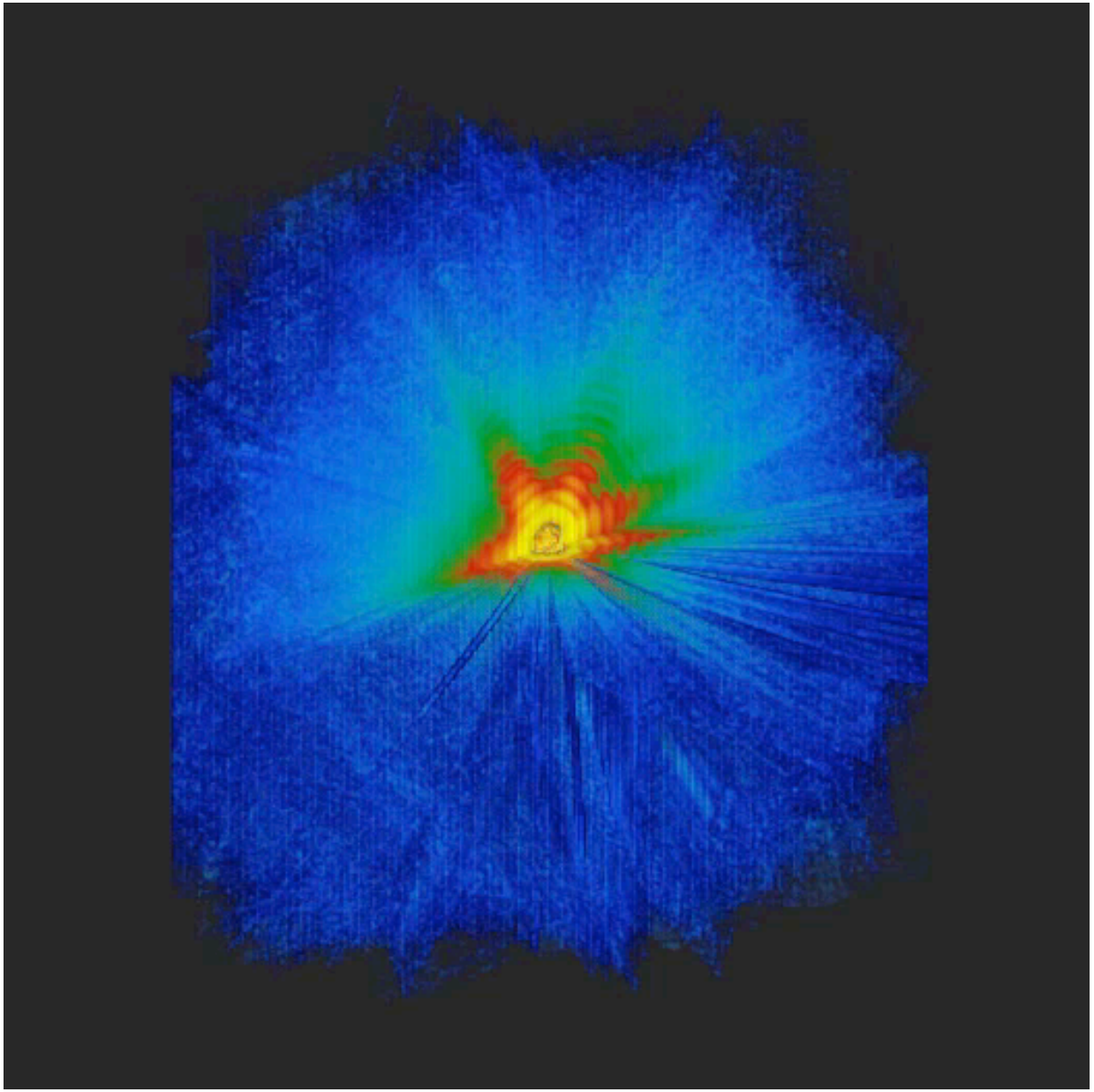


Loh et al Phys Rev Lett 104 225501 (2010)

We will assemble the randomly oriented diffraction patterns to recover a 3D image



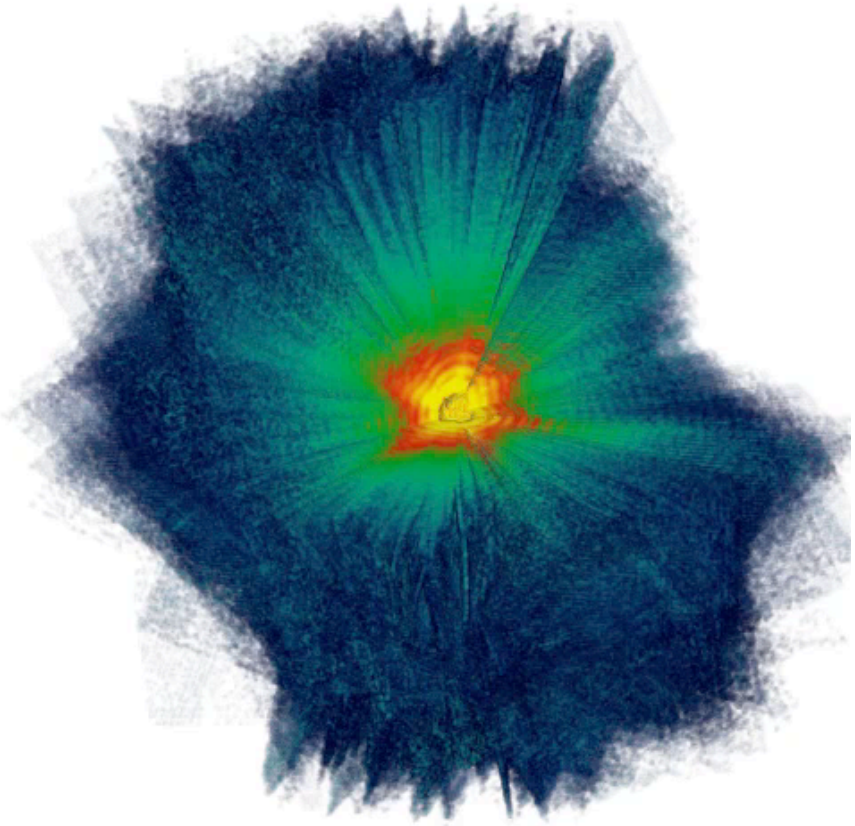
We can assemble individual snapshots in 3D



Anton Barty, CFEL

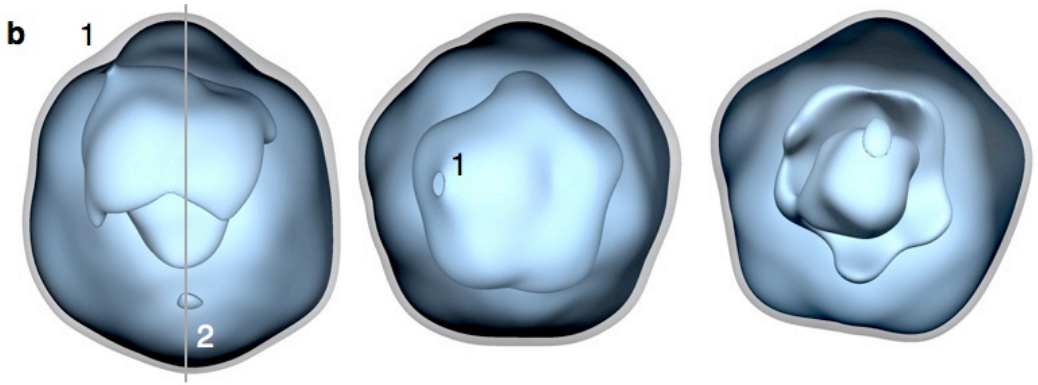
We are developing 2D and 3D imaging of non-periodic objects

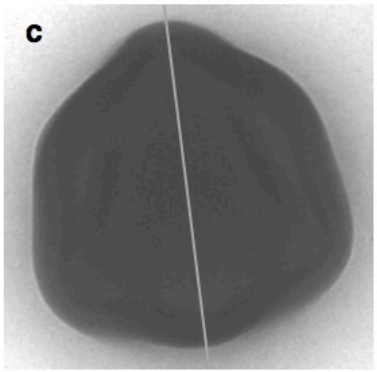
Resolution ~50 nm



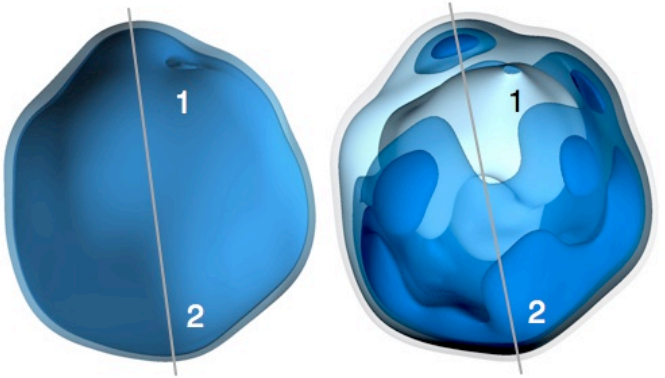
Anton Barty, CFEL

LCLS reconstruction





Electron micrograph



LCLS reconstruction

Tomas Ekeberg, Uppsala, Sweden
Chantal Abergel, CNRS, France
Janos Hajdu, Uppsala

Outlook

The steps to carry out serial snapshot 3D diffractive imaging and nanocrystallography have been demonstrated

Phase retrieval of non-periodic objects is much easier than for the case of crystallography. Nevertheless, there are an abundance of crystallographic tools and methods that could be applied to single particle imaging

Theoretical tools and experimental methods still need to be improved and refined to work at extremely low noise levels, to give us the highest resolution

3D reconstruction may be possible even if there is some sample inhomogeneity -- but methods must be developed to account for that

There is a world of structures to be explored...both in time and space