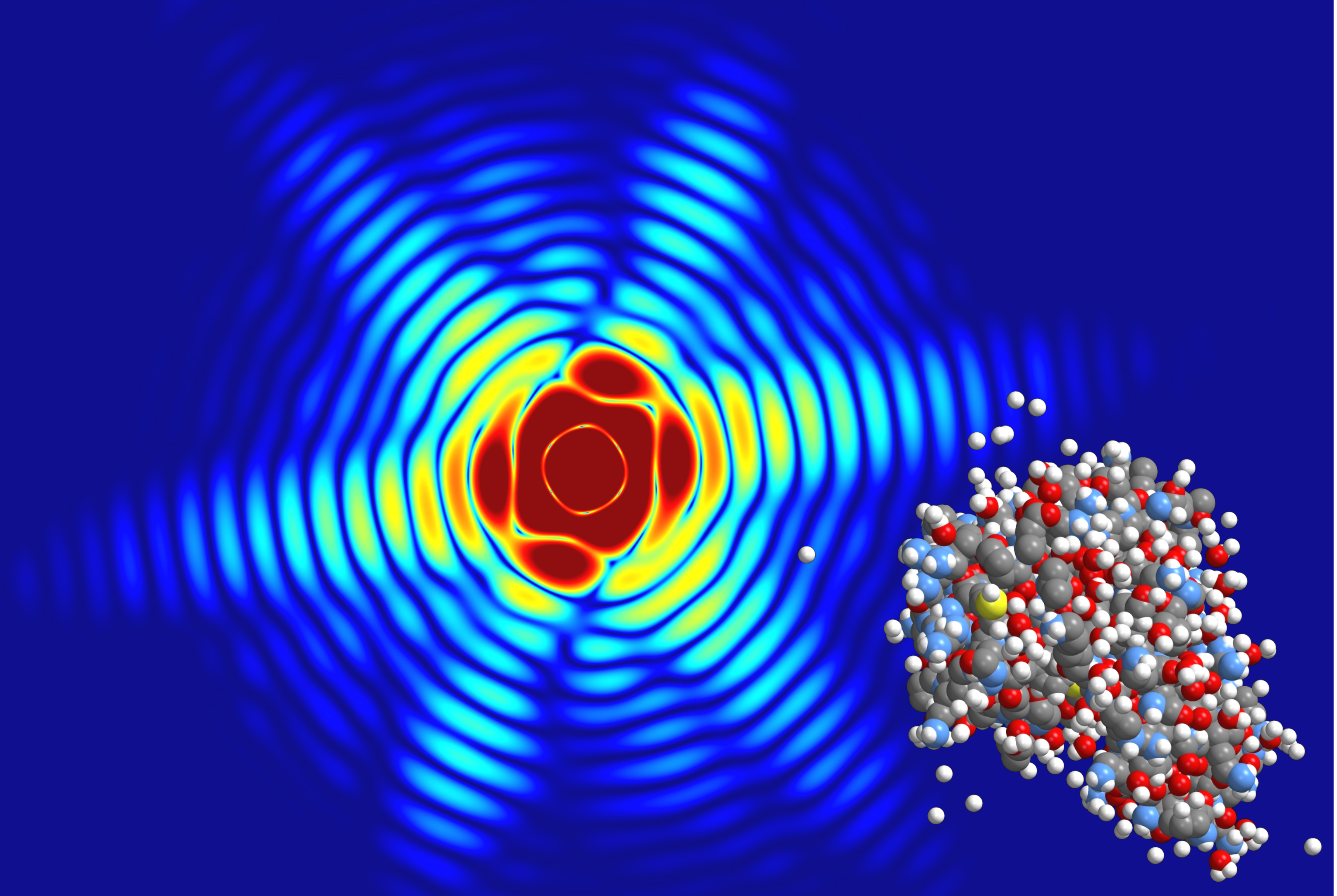
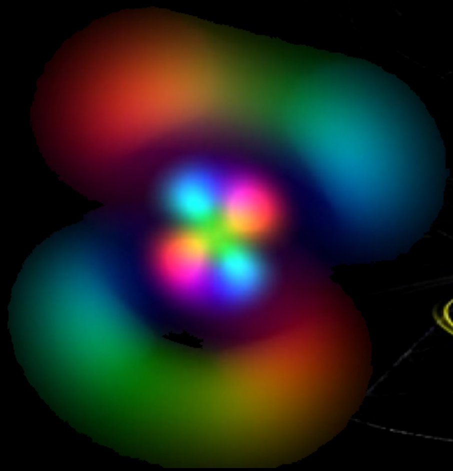
DIFFRACTION BEFORE DESTRUCTION



Janos Hajdu, Uppsala Universitet and XFEL

THE ACT OF OBSERVATION CHANGES WHAT IS BEING OBSERVED

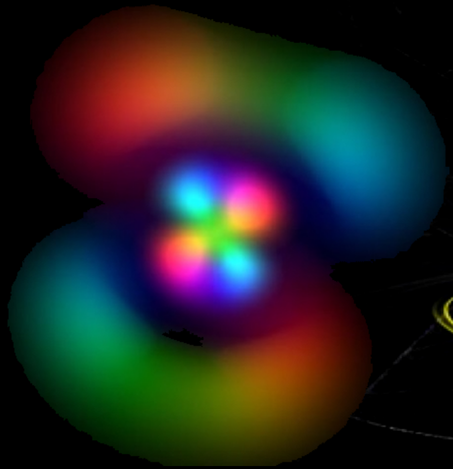


An atom

The changes can be quite dramatic

Consider the quantum world and larger systems

THE ACT OF OBSERVATION CHANGES WHAT IS BEING OBSERVED



An atom

WHEN TRYING TO IMAGE LARGE MACROMOLECULAR SYSTEMS, FUNDAMENTAL LIMITS TO OBSERVATION SEEM TO APPEAR LONG BEFORE QUANTUM LIMITS

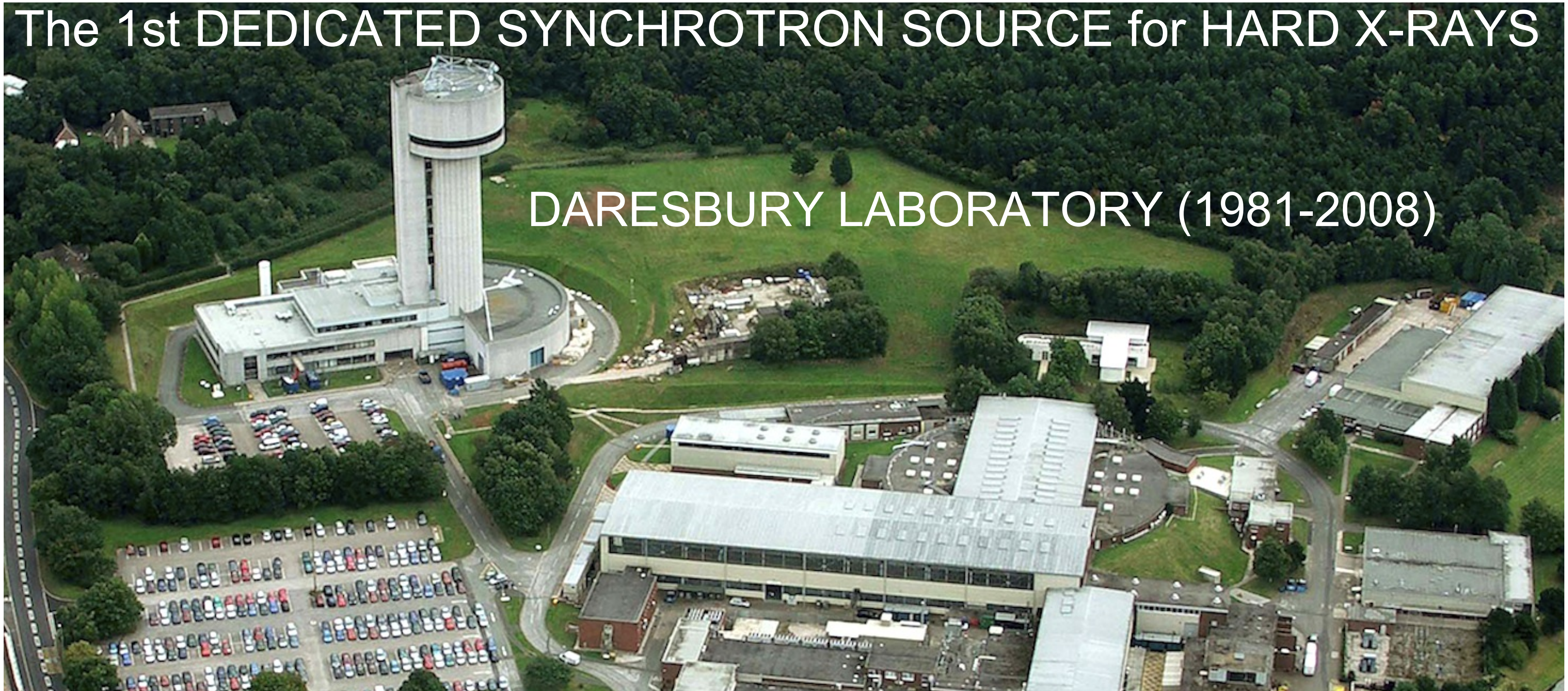
The changes can be quite dramatic

Consider the quantum world and larger systems

OXFROD 1981: Glycogen phosphorylase *b* - A large macromolecular system

Wire works by John Jenkins and Giuseppe Zanotti


The 1st DEDICATED SYNCHROTRON SOURCE for HARD X-RAYS
DARESBURY LABORATORY (1981-2008)

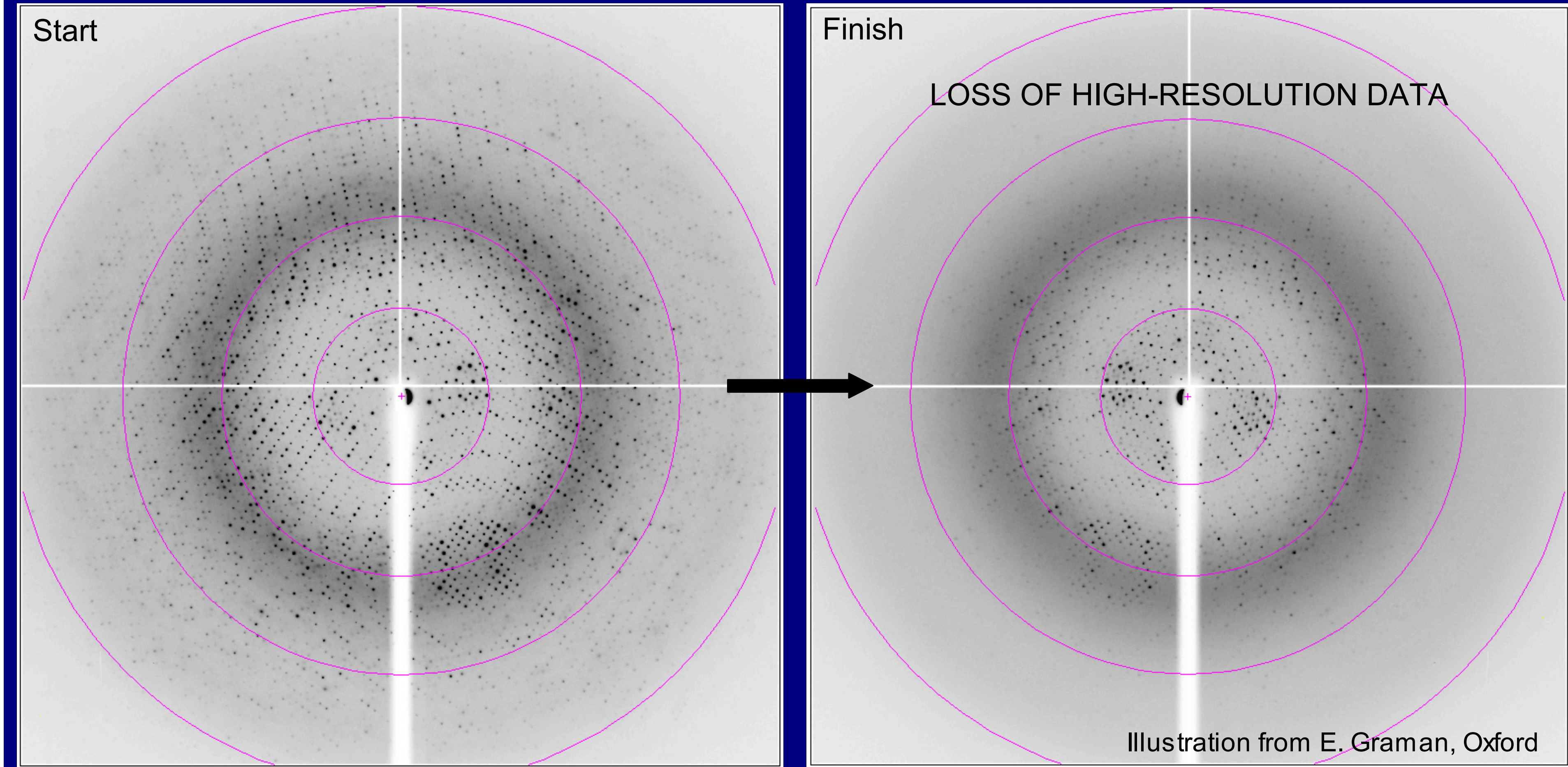

The 1st DEDICATED SYNCHROTRON SOURCE for HARD X-RAYS
DARESBURY LABORATORY (1981-2008)


From the 1st day…
Summer 1981


…to the very last
1 Aug. 2008


Marvin Seibert (UU)

RADIATION DAMAGE TO A CRYSTAL



A REVELATION FROM DARESBURY: CRYSTALS LAST

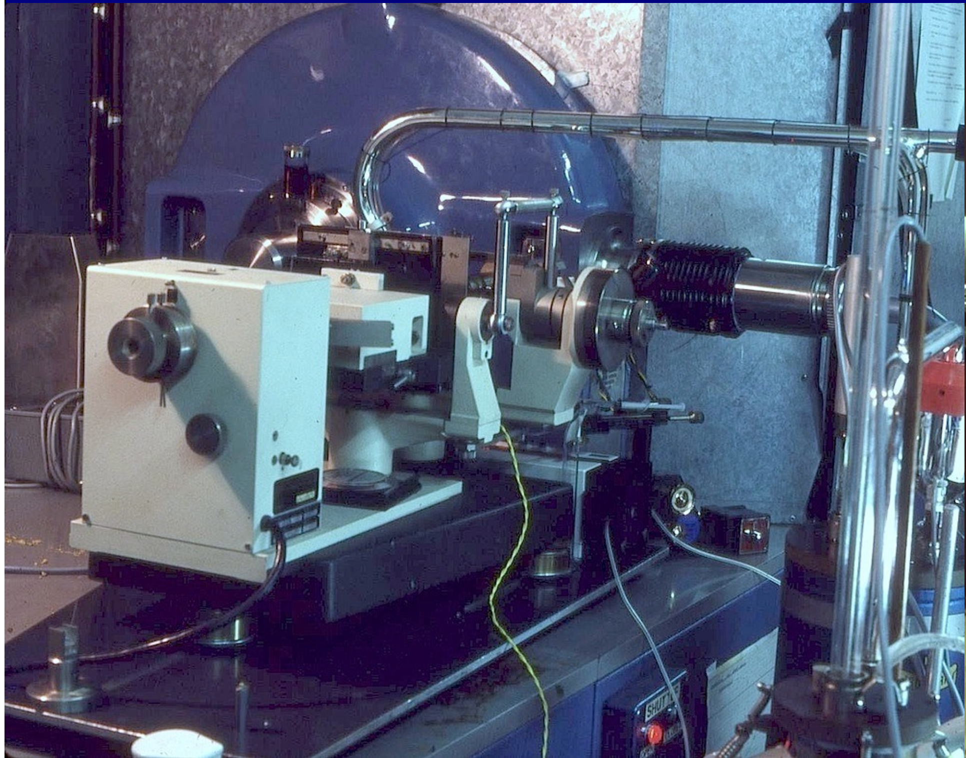
The same dose caused less damage in Daresbury than back home in Oxford.

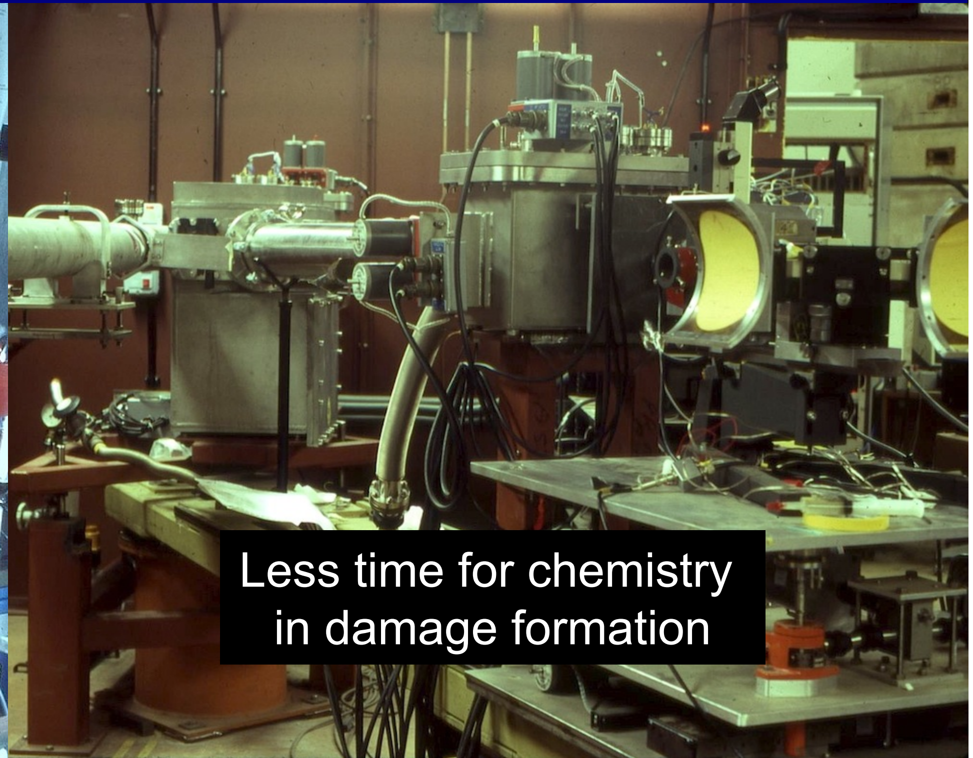
1981: The 1st hint for a significant time component in damage formation

DATA COLLECTION ON PHOSPHORYLASE CRYSTALS

OXFORD: 1-2 weeks
No. of crystals used: 5-8

DARESBURY: 20 minutes
No. of crystals used: 1

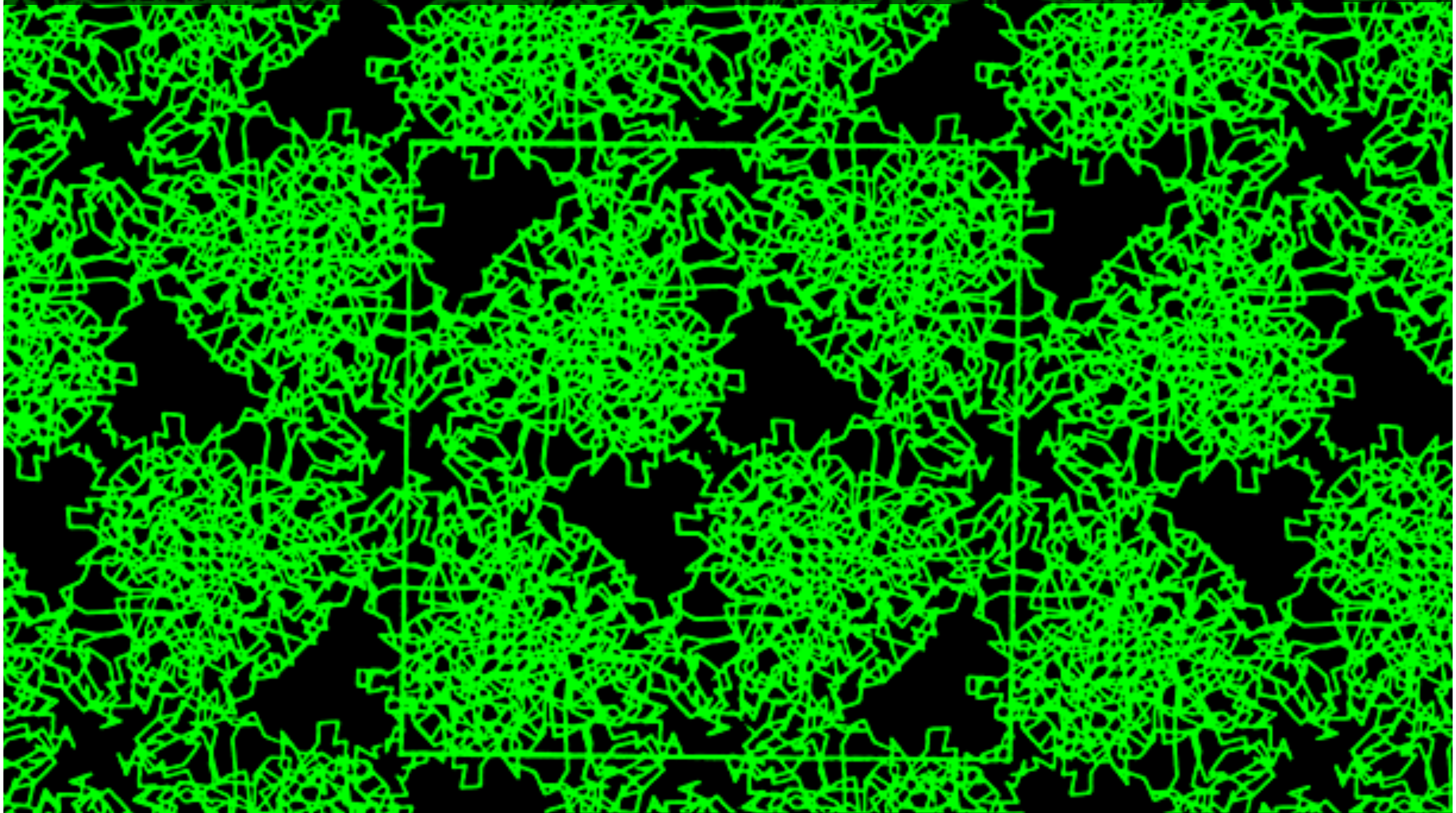




Less time for chemistry in damage formation

HIGH DATA RATES -
- NEW OPPORTUNITIES

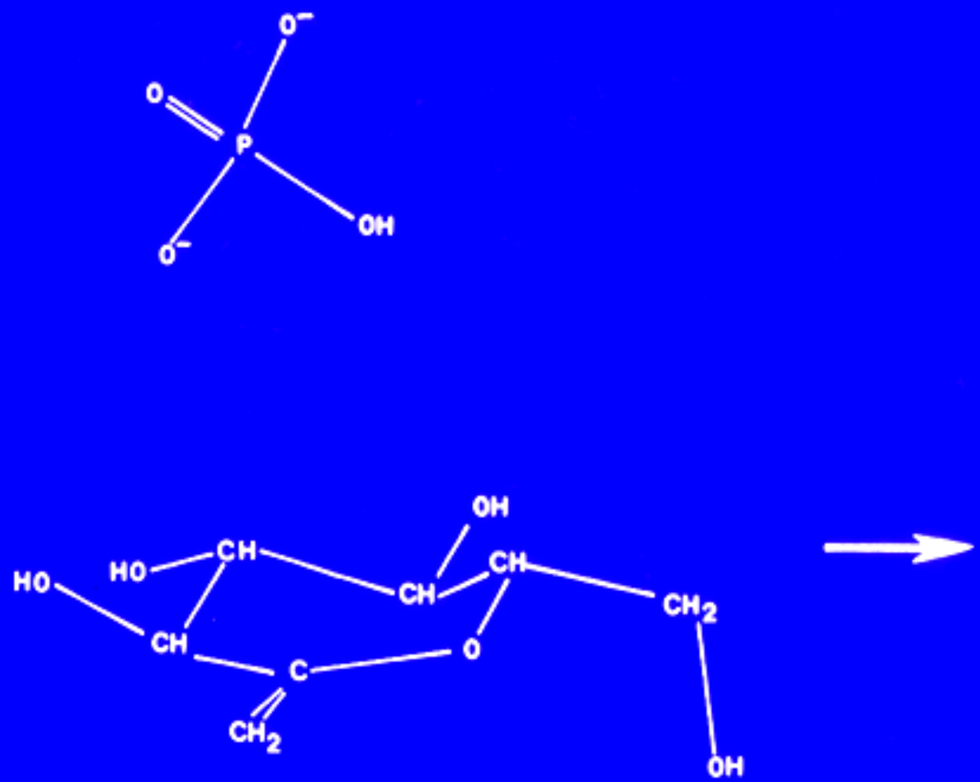
THE 1st TIME-RESOLVED EXPERIMENT IN XTALLOGRAPHY



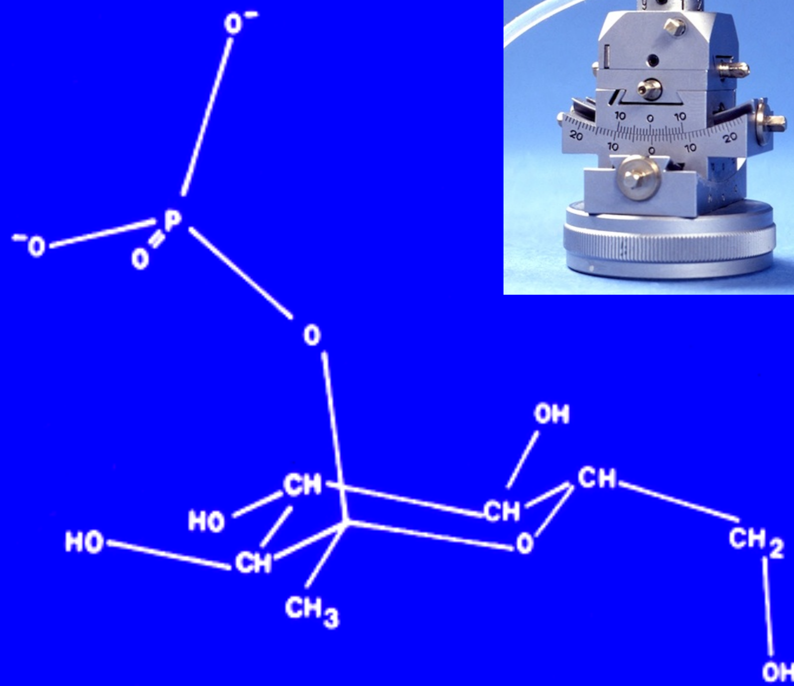
CATALYSIS IN A CRYSTAL OF PHOSPHORYLASE *b*

Hajdu, J., Acharya, K. R., Stuart, D. I., McLaughlin, P. J., Barford, D., Oikonomakos, N. G., Klein, H. & Johnson, L. N. (1987) Catalysis in the crystal: Synchrotron radiation studies with glycogen phosphorylase *b*. *EMBO J.*, 6, 539-546.

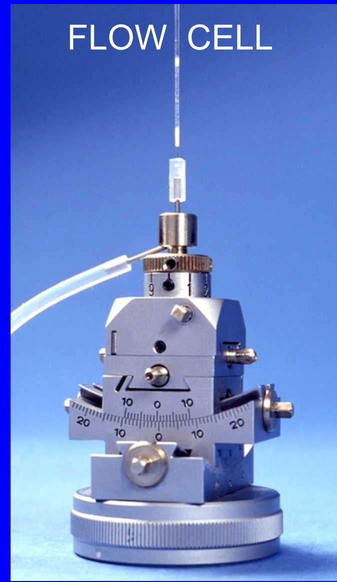
Hajdu, J., Acharya, K. R., Barford, D., Stuart, D. I., Johnson, L. N. (1988) Catalysis in enzyme crystals. *TIBS*, 13, 104-109.



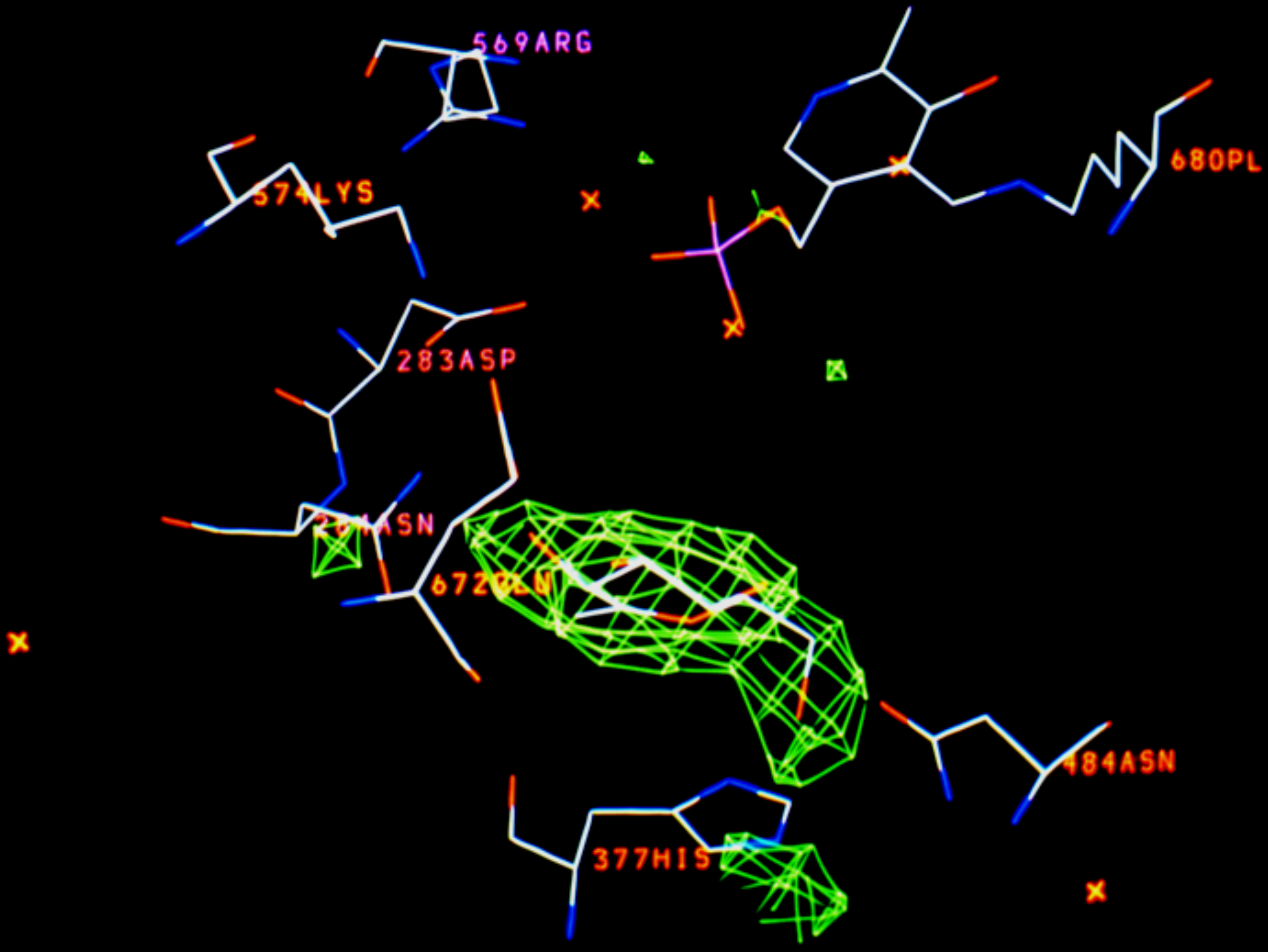
HEPTENITOL



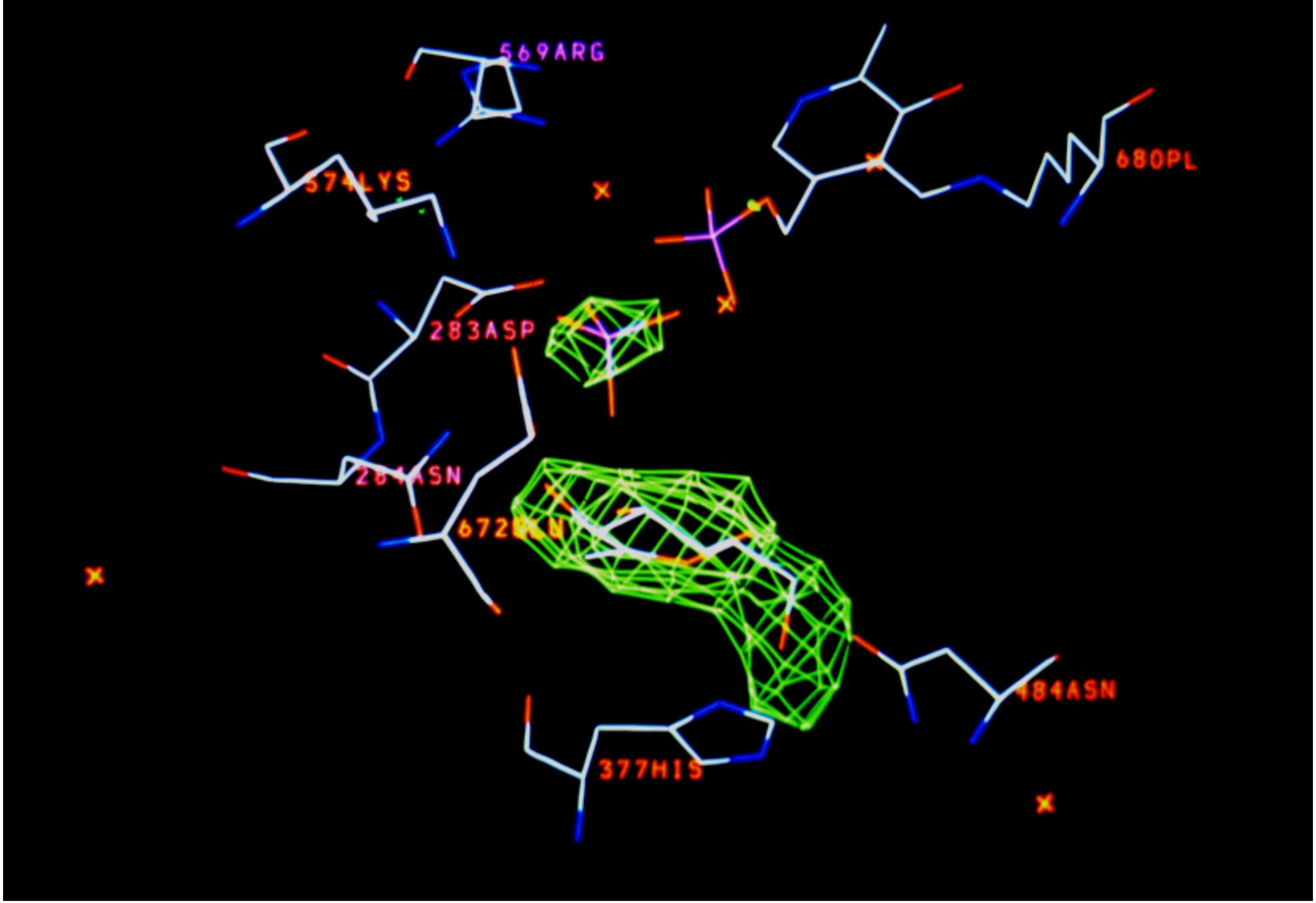
HEPTULOSE-2-PHOSPHATE



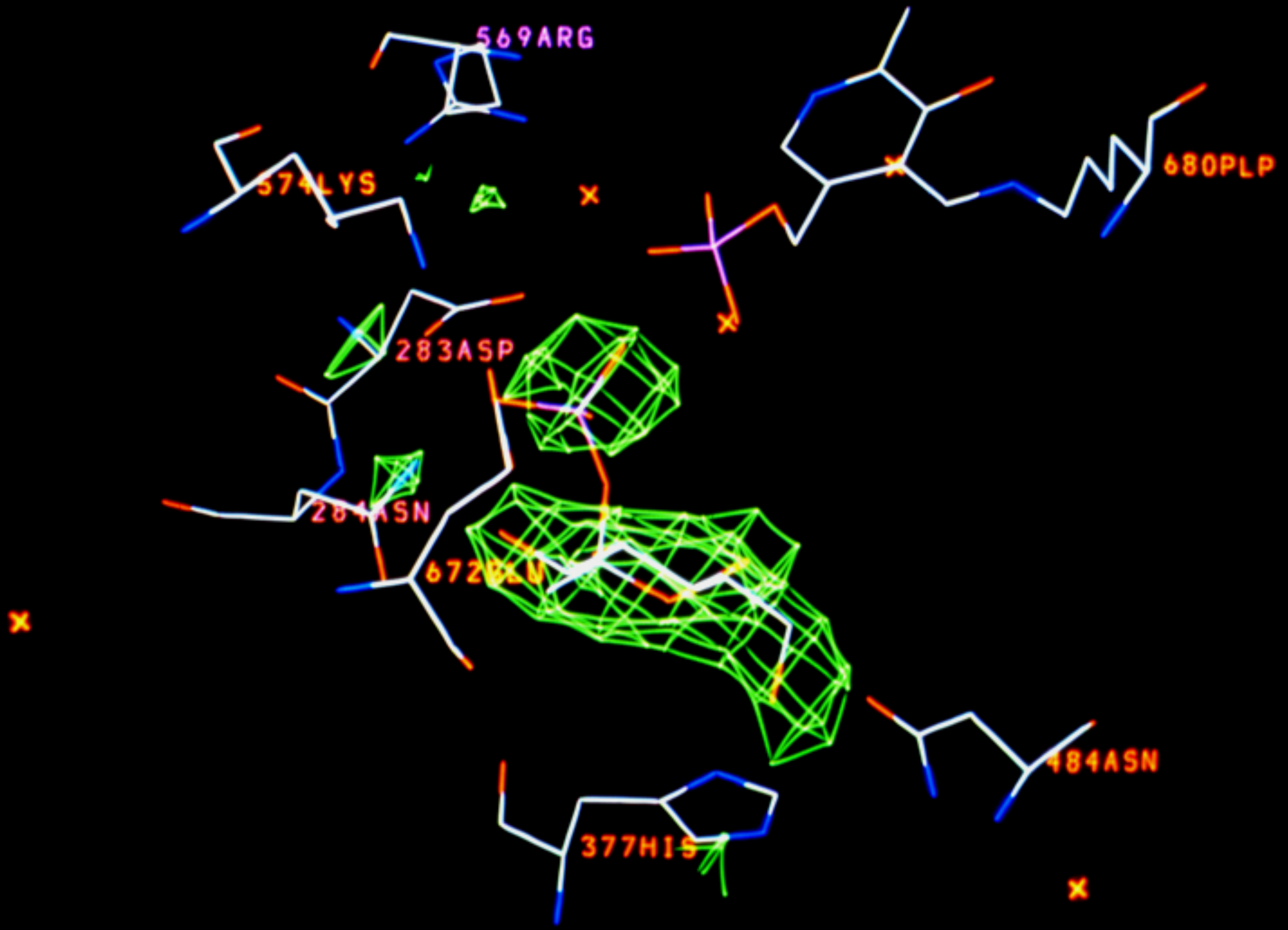
EACH DATA SET REPRESENTS A TIME & VOLUME AVERAGE



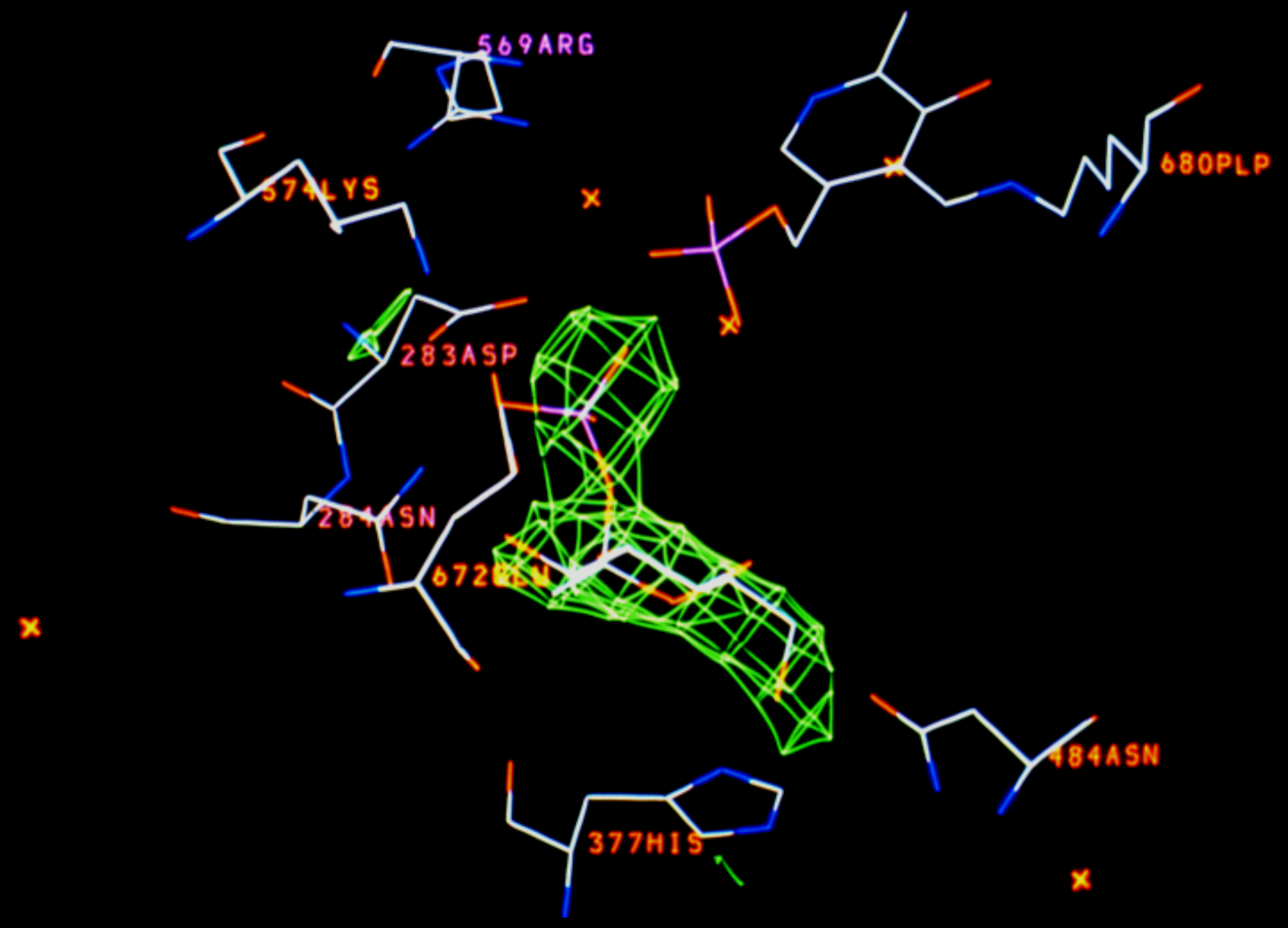
EACH DATA SET REPRESENTS A TIME & VOLUME AVERAGE



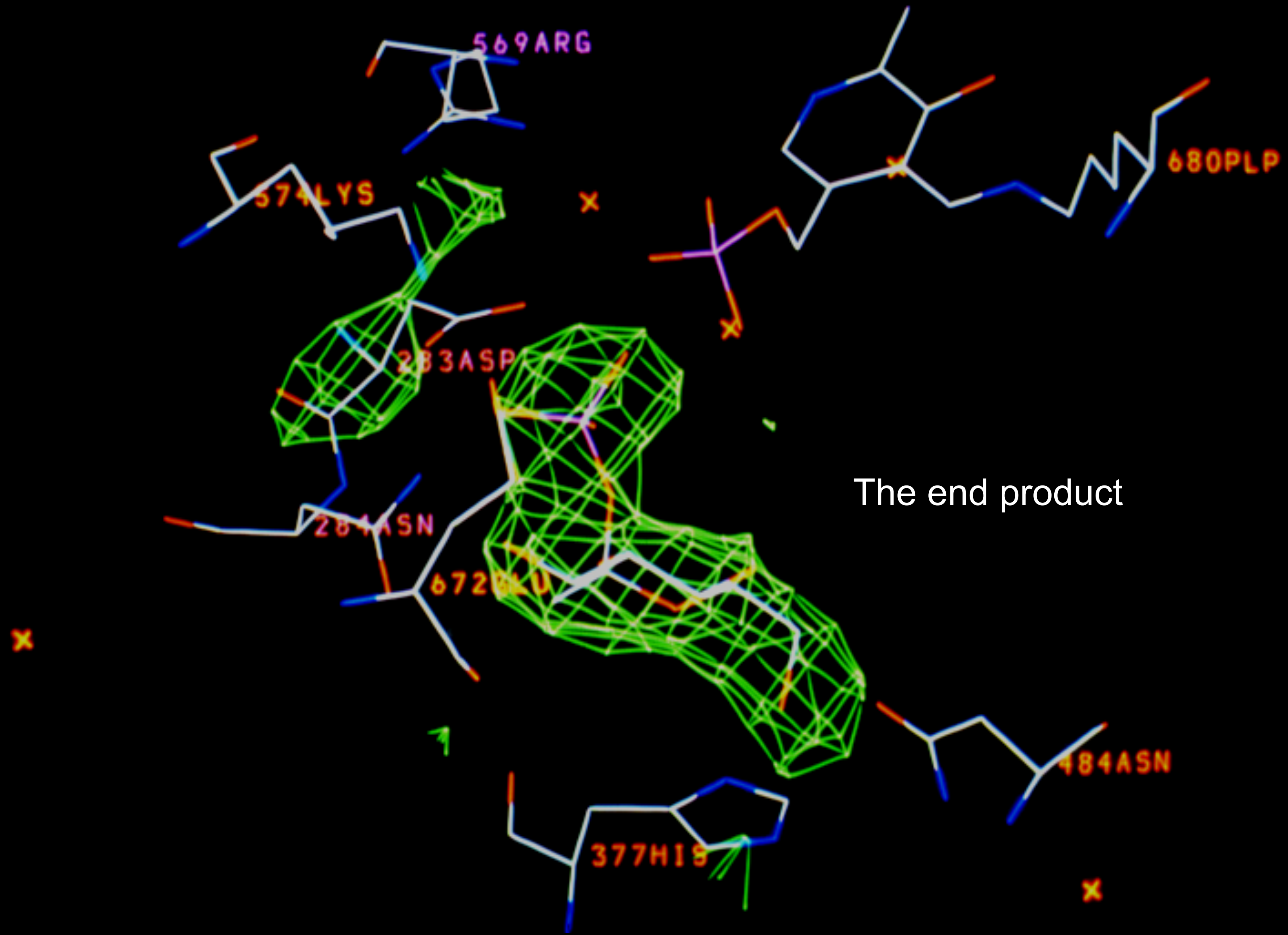
EACH DATA SET REPRESENTS A TIME & VOLUME AVERAGE



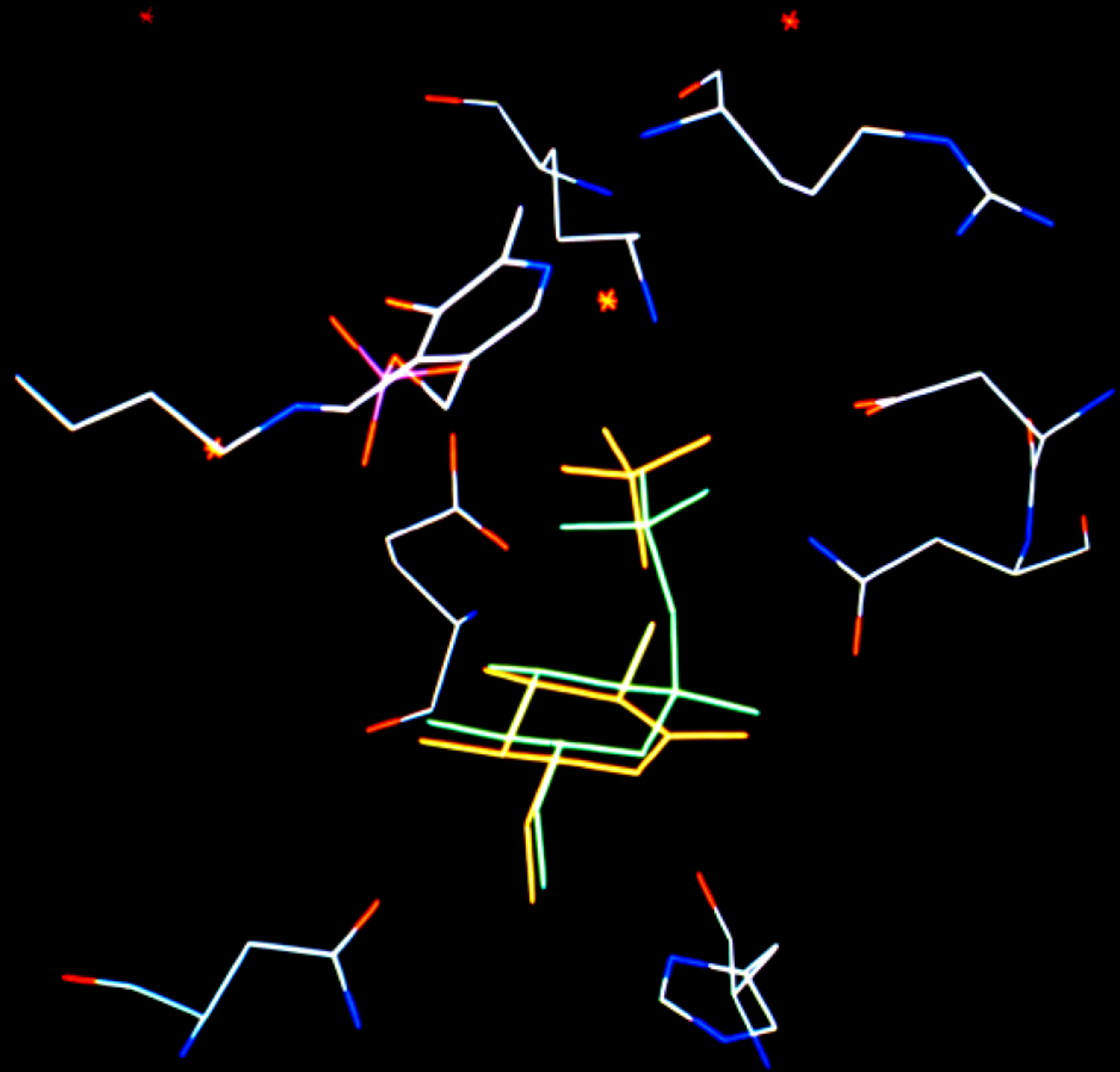
EACH DATA SET REPRESENTS A TIME & VOLUME AVERAGE



EACH DATA SET REPRESENTS A TIME & VOLUME AVERAGE

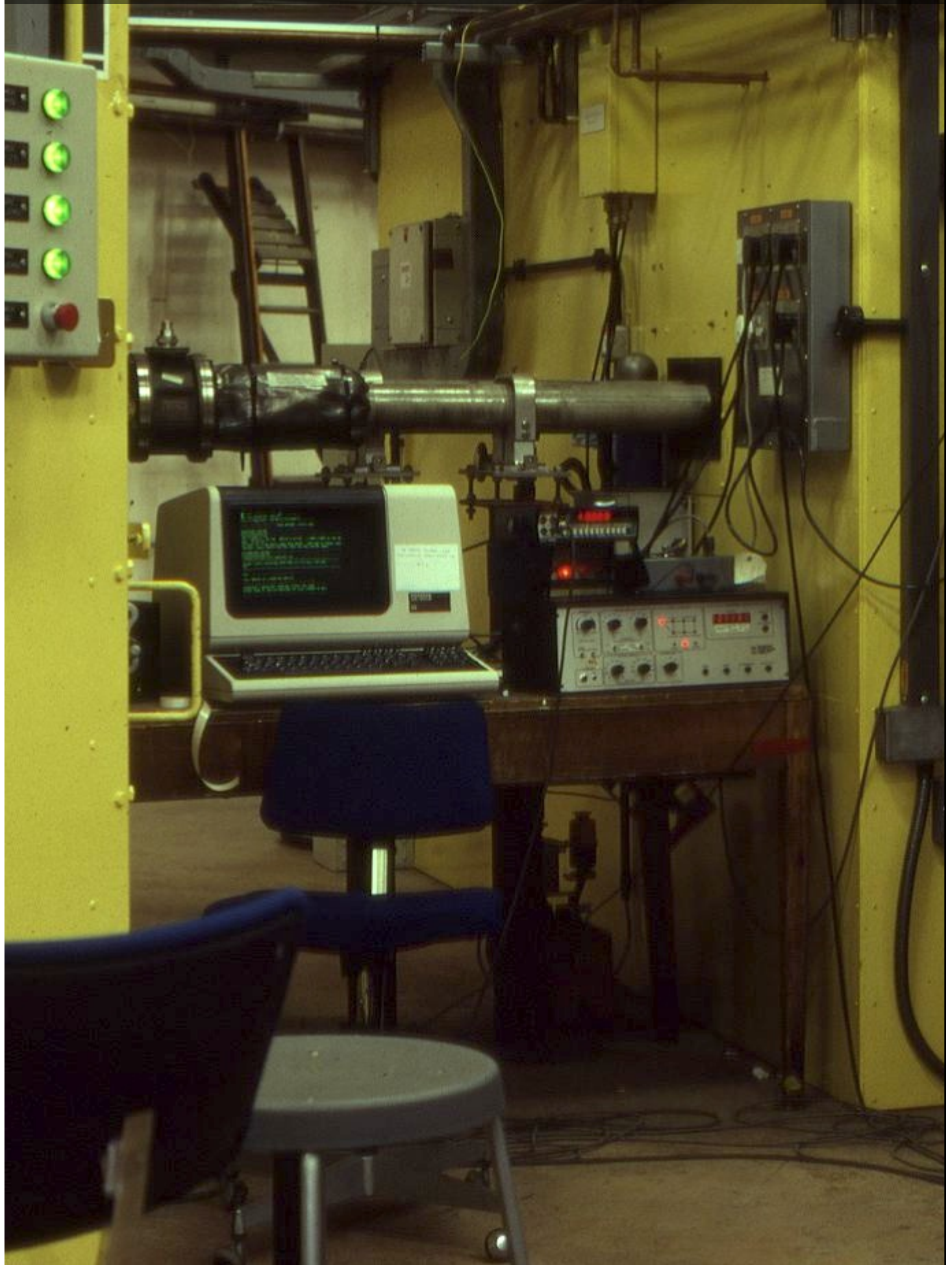


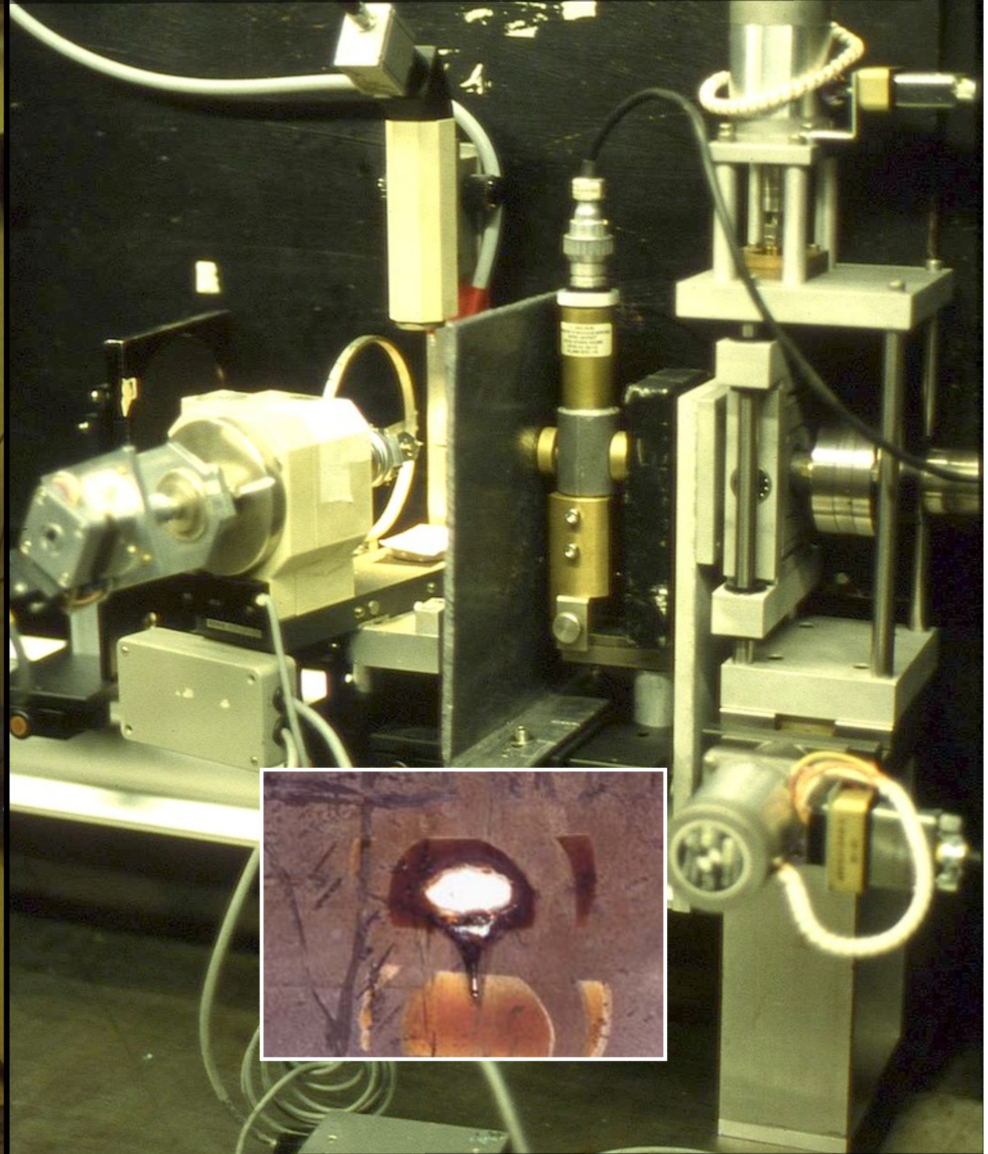
The end product



“Catalysis in the crystal: Synchrotron radiation studies with glycogen phosphorylase b”
Hajdu, J. et al. *EMBO J.* **6**, 539-546 (1987).

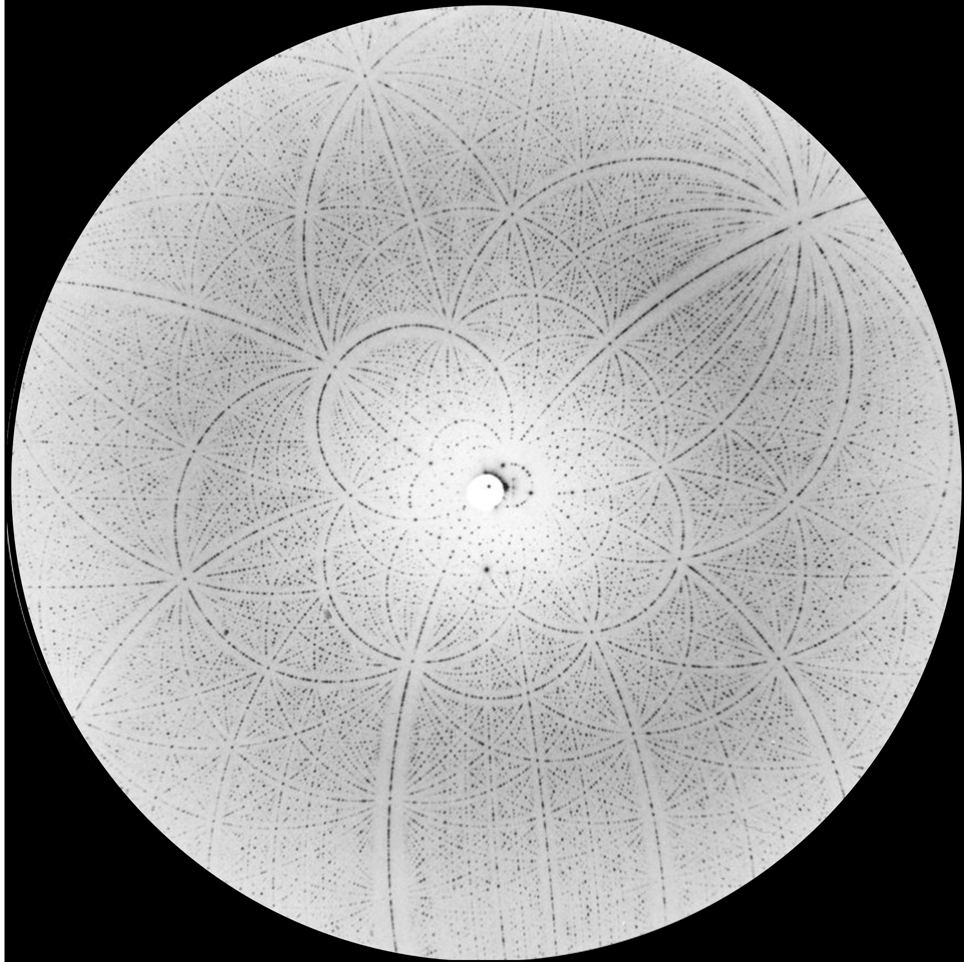
HIGHER SPEED REQUIRES HIGHER ENERGY DENSITIES
BRUTE FORCE - WHITE BEAM





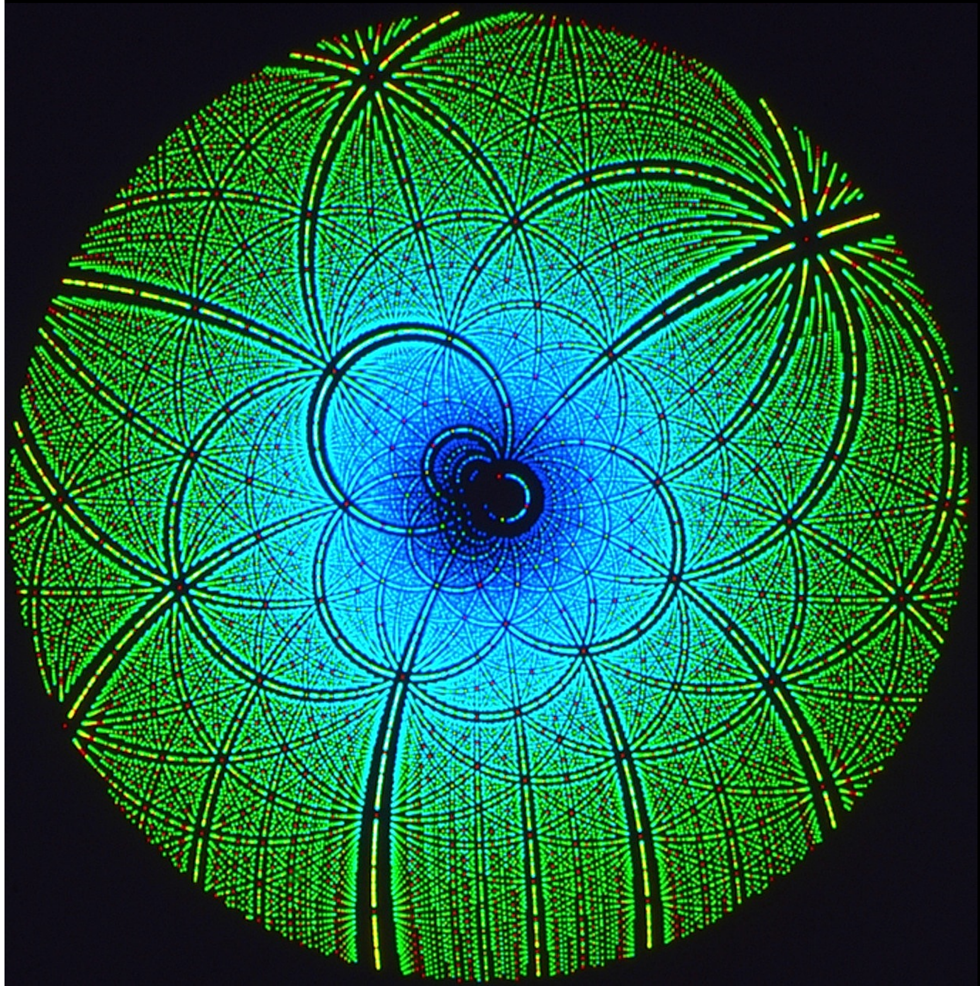
OUR LAUE DIFFRACTION STATION

"Millisecond X-ray diffraction: First electron density map from Laue photographs of a protein crystal"
Hajdu et al. *Nature* **329**, 178-181 (1987)



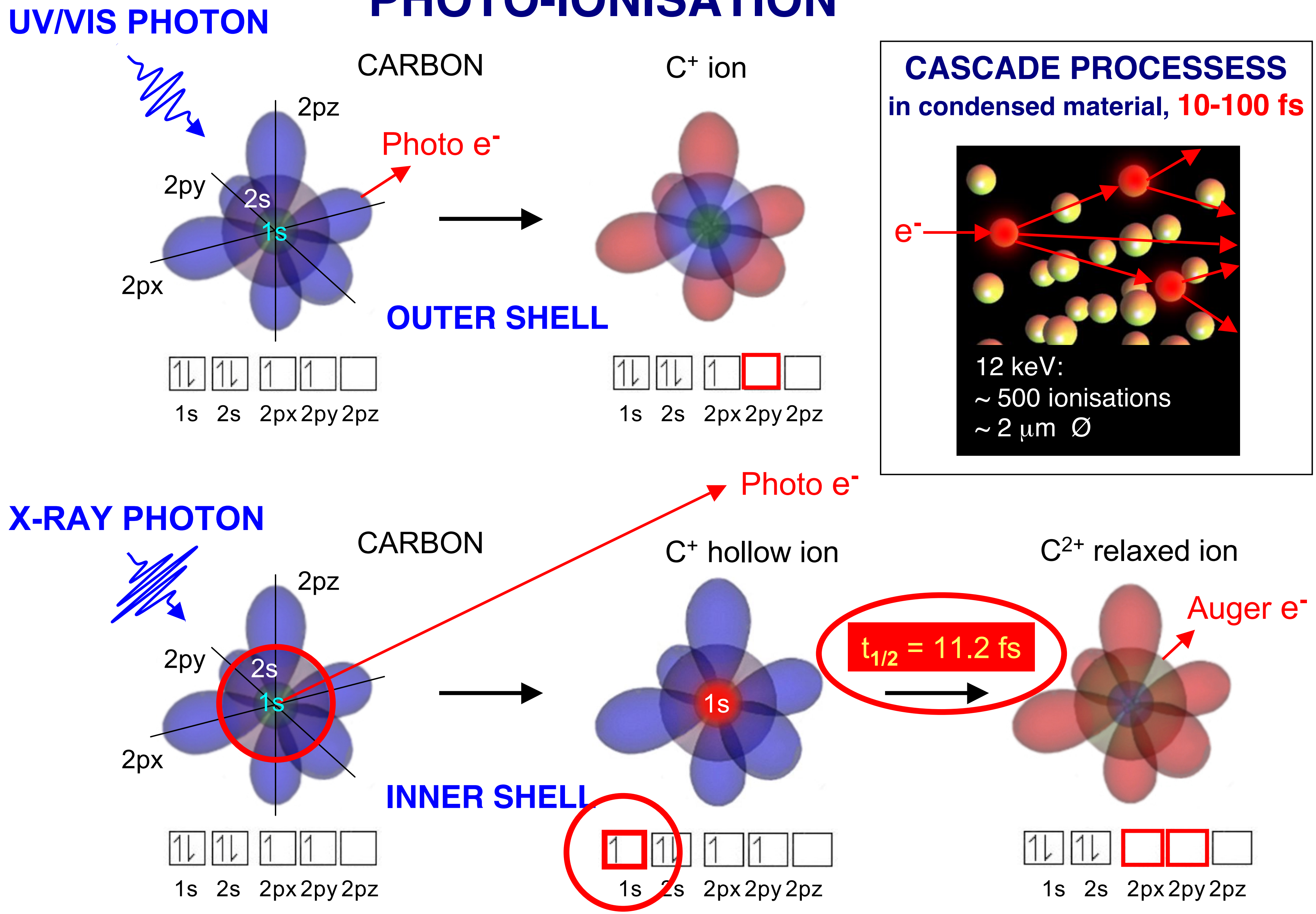
Binding of maltoheptose to glycogen phosphorylase *b*

"Millisecond X-ray diffraction: First electron density map from Laue photographs of a protein crystal"
Hajdu et al. *Nature* **329**, 178-181 (1987)



Binding of maltoheptose to glycogen phosphorylase *b*

PHOTO-IONISATION



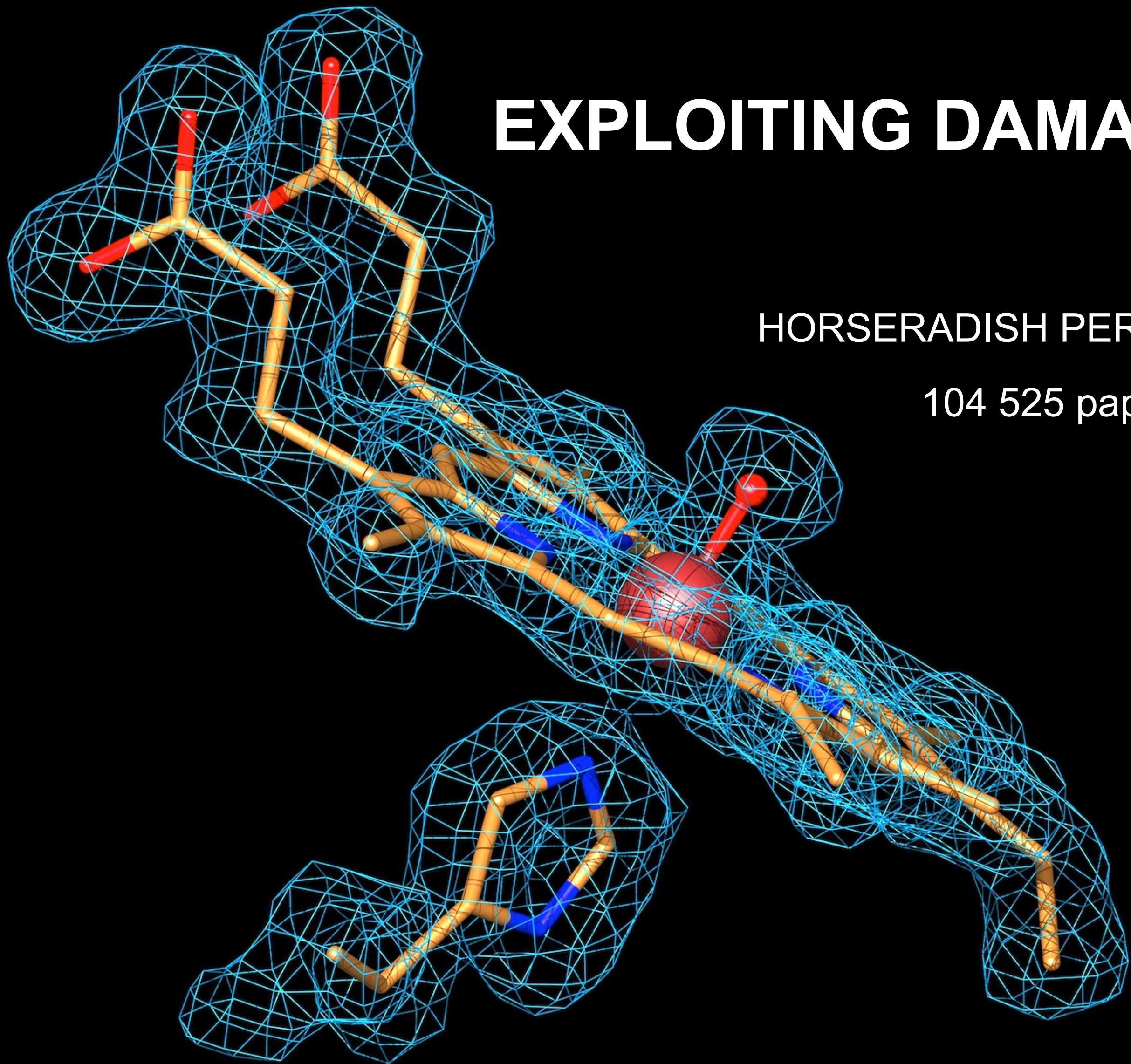
Neutze, R., Wouts, R., van der Spoel, D., Weckert, E. Hajdu, J. (2000) *Nature* 406, 752-757

X-RAY DRIVEN CATALYSIS

EXPLOITING DAMAGE PROCESSES

HORSERADISH PEROXIDASE (HRP)

104 525 papers !

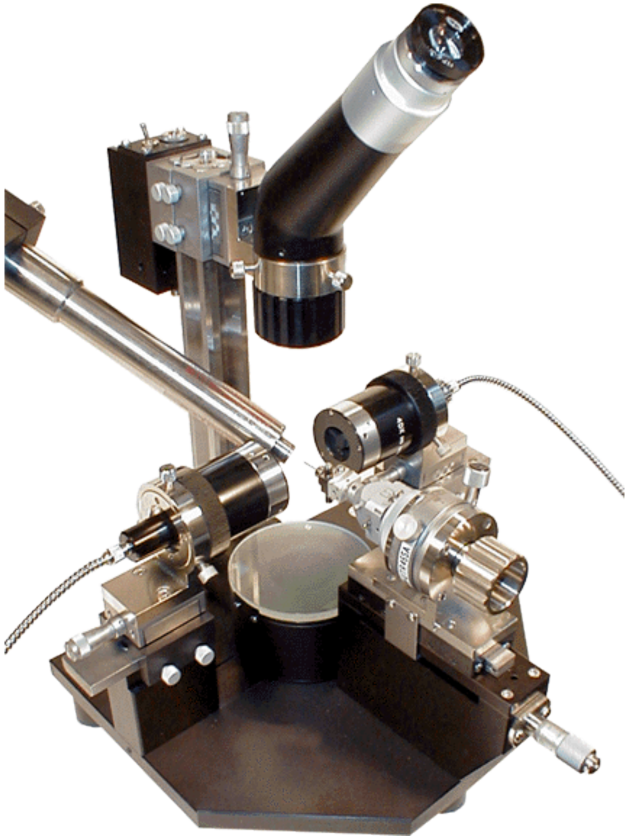


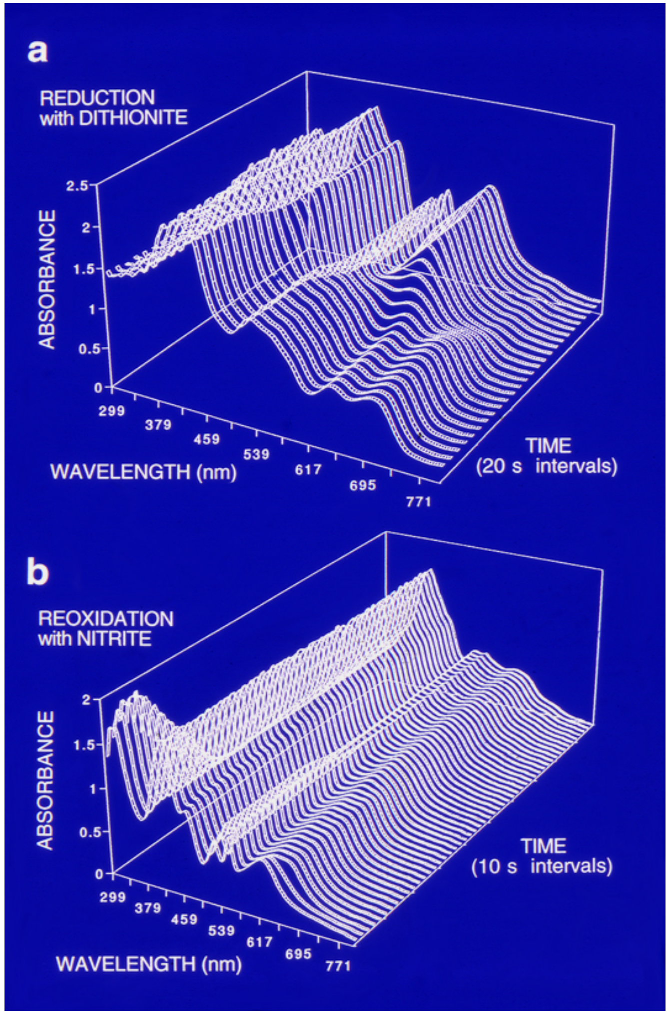
Berglund, G.I., Carlsson, G.H., Smith, A.T., Szöke, H., Henriksen, A. & Hajdu, J. (2002) *Nature* 417, 463-468.

COMBINATION OF SPECTROSCOPY WITH CRYSTALLOGRAPHY (1993)

1. ASSIGN THE CORRECT ELECTRONIC STATE TO A STRUCTURE (particularly important for redox proteins)

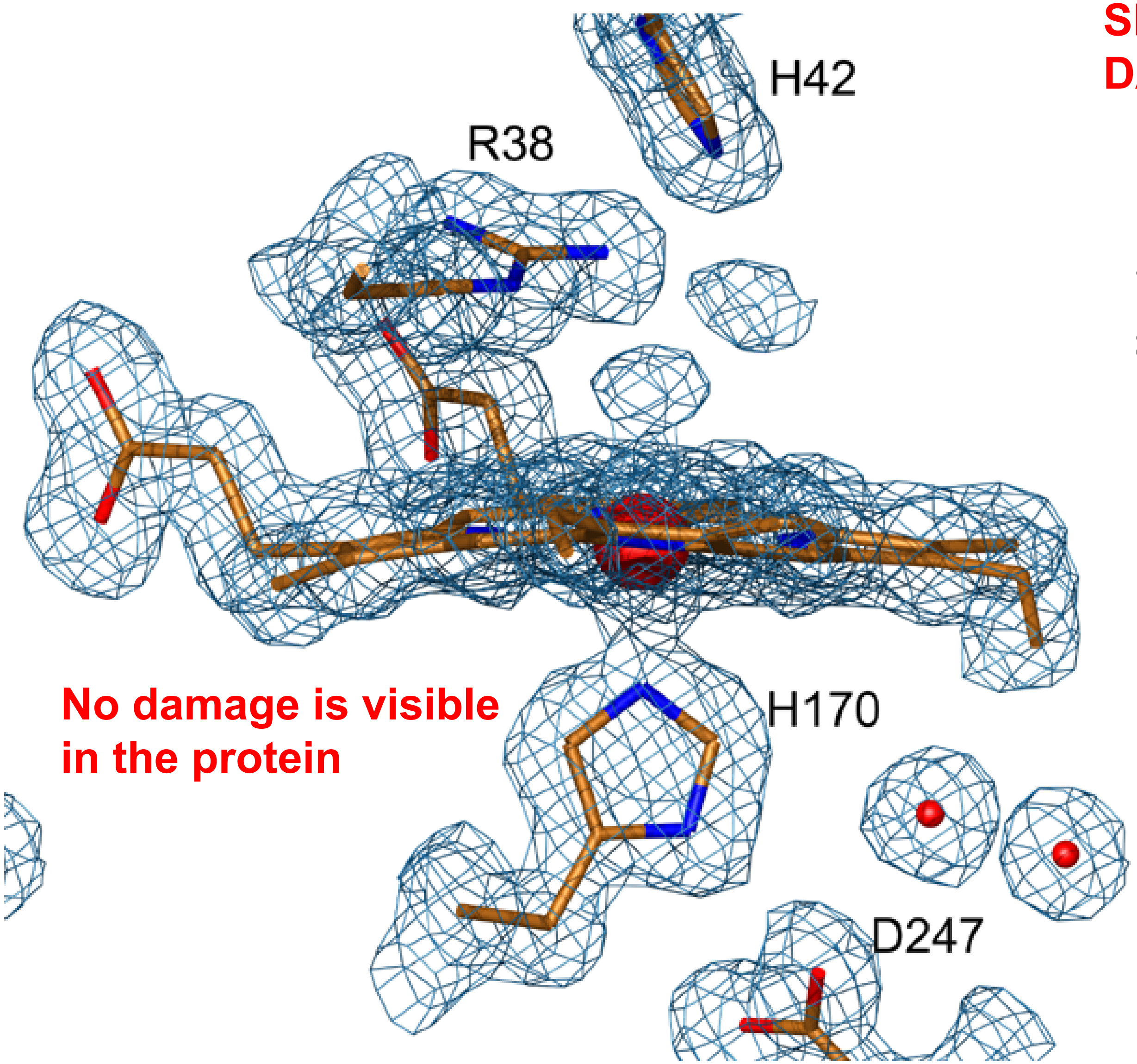
2. CORRELATE ELECTRONIC TRANSITIONS WITH STRUCTURAL TRANSITIONS





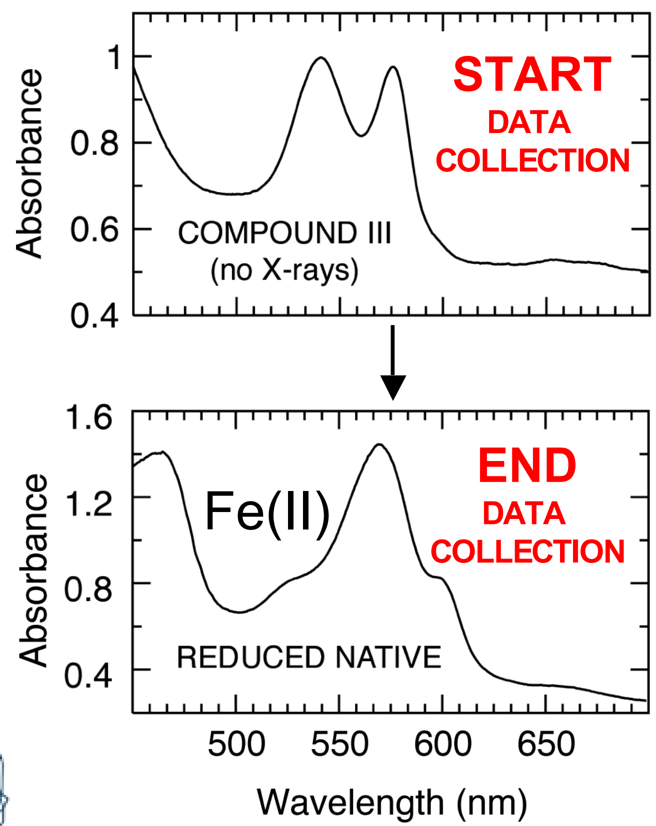
Hadfield, A. T. & Hajdu, J.: A fast and portable micro-spectrophotometer for time-resolved X-ray diffraction experiments. *J. Appl. Cryst.* **26**, 839-842 (1993).

COMPOUND III OF HRP CONTAINS A BOUND DIOXYGEN SPECIES. THE X-RAY STRUCTURE SHOWS TWO WATER MOLECULES...

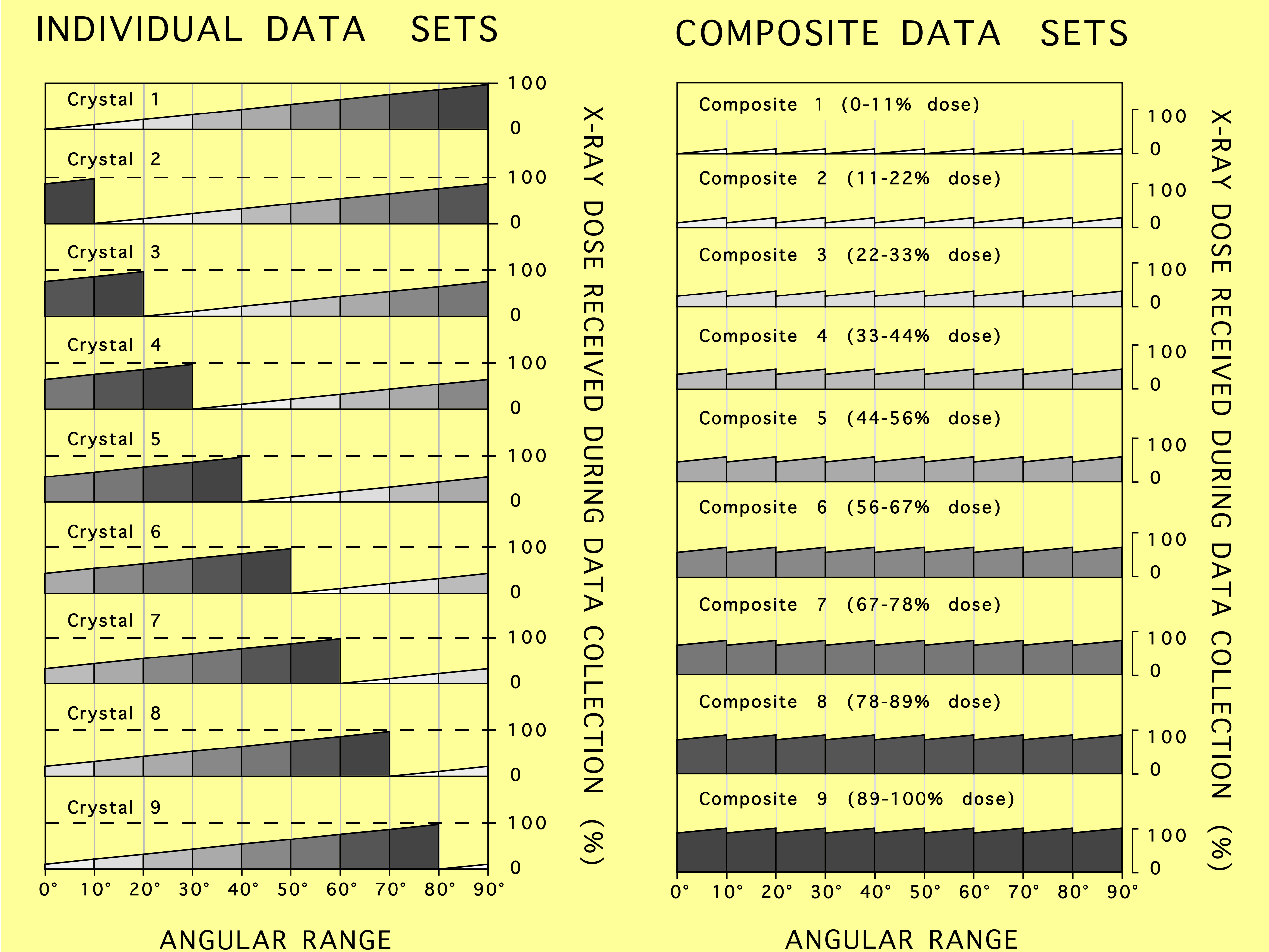


No damage is visible in the protein

SPECTRAL CHANGES DURING DATA COLLECTION:



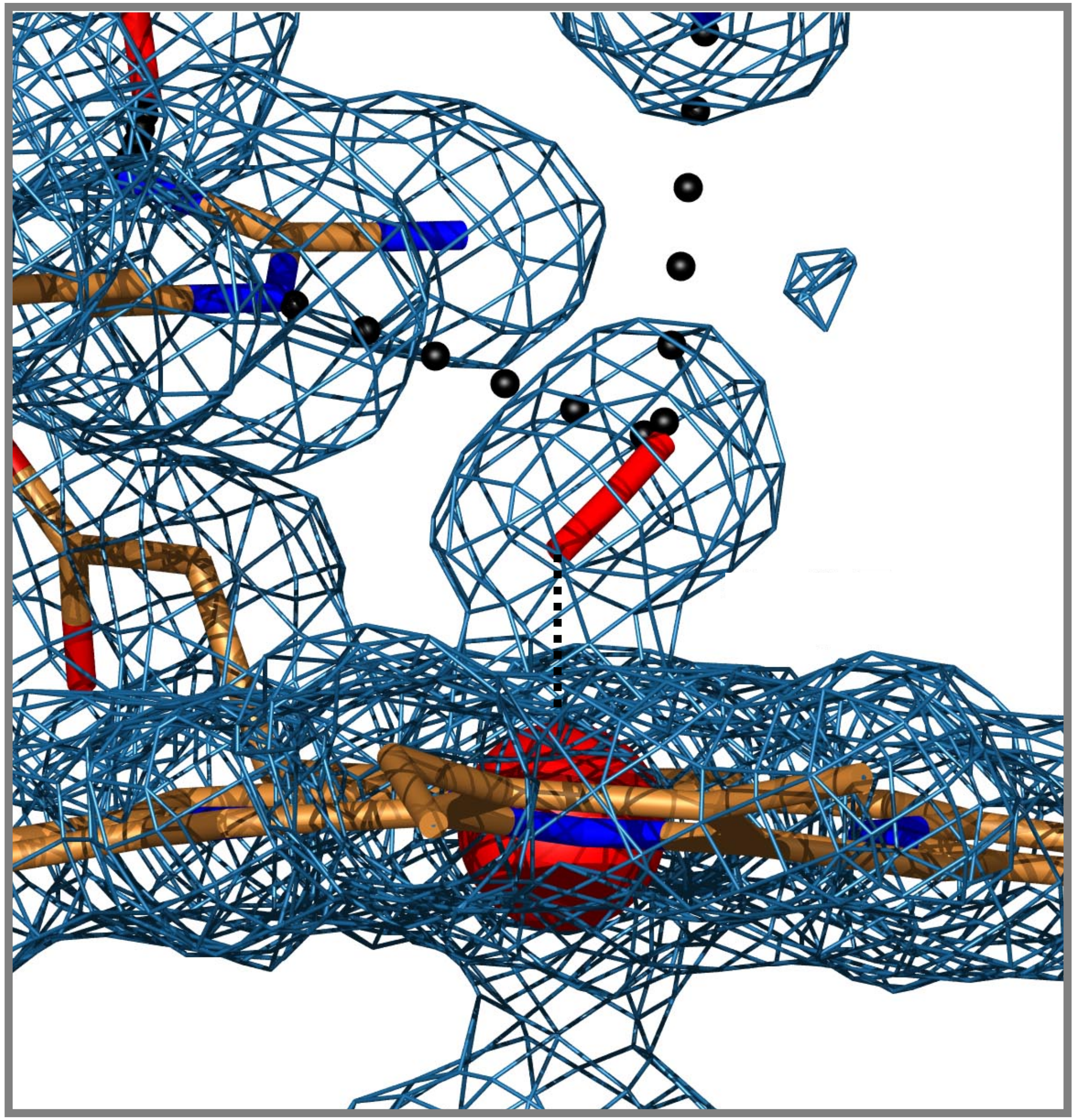
THE ENZYME GOT REDUCED

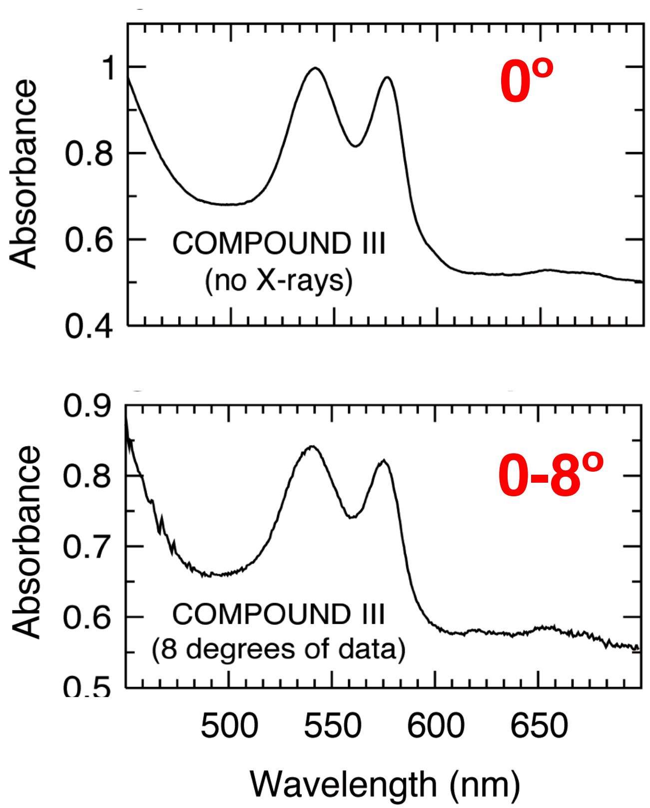


INDIVIDUAL DATA SETS
Crystal 1
Crystal 2
Crystal 3
Crystal 4
Crystal 5
Crystal 6
Crystal 7
Crystal 8
Crystal 9
100
0
0° 10° 20° 30° 40° 50° 60° 70° 80° 90°
ANGULAR RANGE
X-RAY DOSE RECEIVED DURING DATA COLLECTION (%)
COMPOSITE DATA SETS
Composite 1 (0-11% dose)
Composite 2 (11-22% dose)
Composite 3 (22-33% dose)
Composite 4 (33-44% dose)
Composite 5 (44-56% dose)
Composite 6 (56-67% dose)
Composite 7 (67-78% dose)
Composite 8 (78-89% dose)
Composite 9 (89-100% dose)
100
0
0° 10° 20° 30° 40° 50° 60° 70° 80° 90°
ANGULAR RANGE
X-RAY DOSE RECEIVED DURING DATA COLLECTION (%)

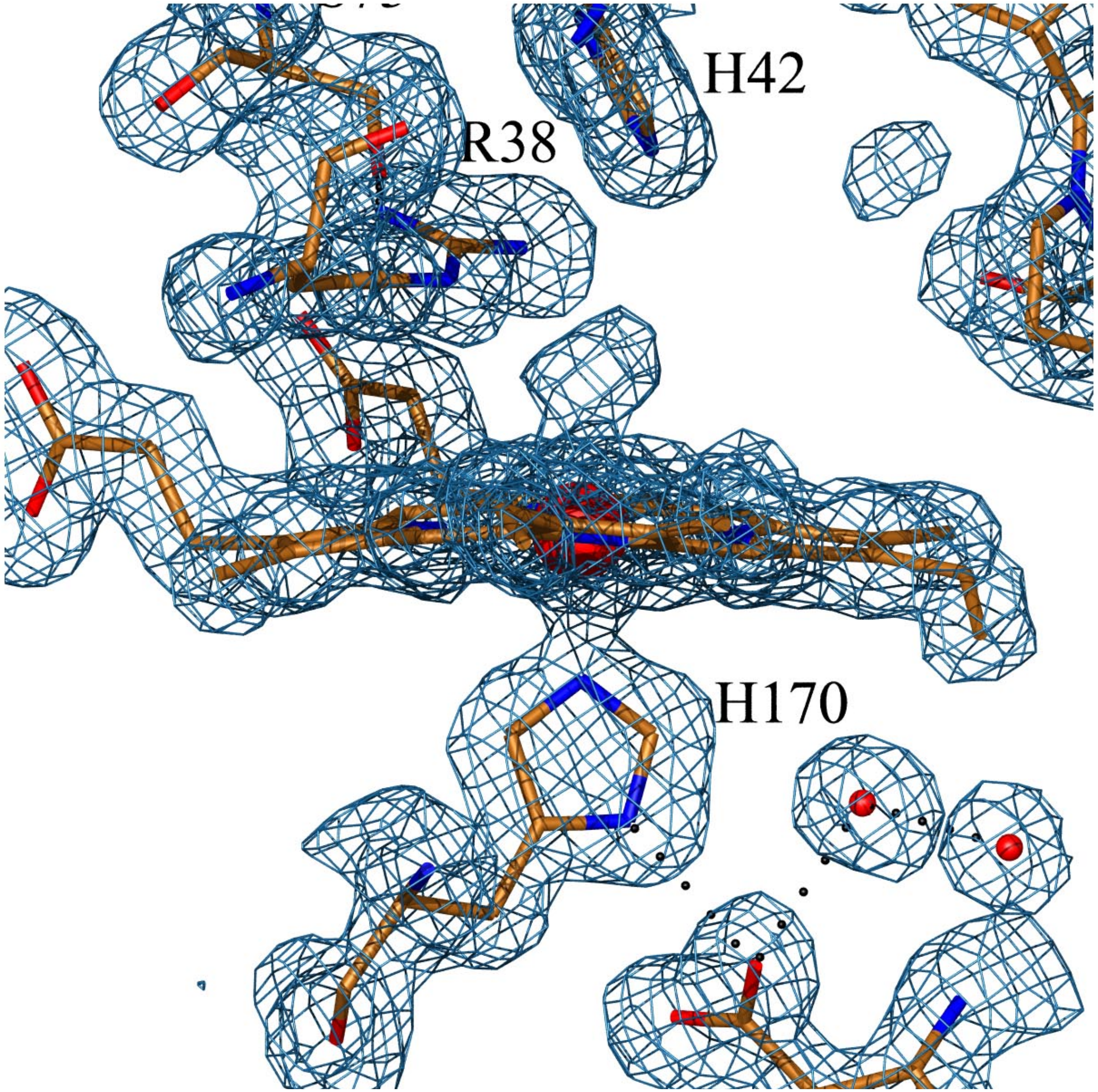
Voilà! HRP WITH BOUND DIOXYGEN SPECIES

Results from composite data set 0-8°

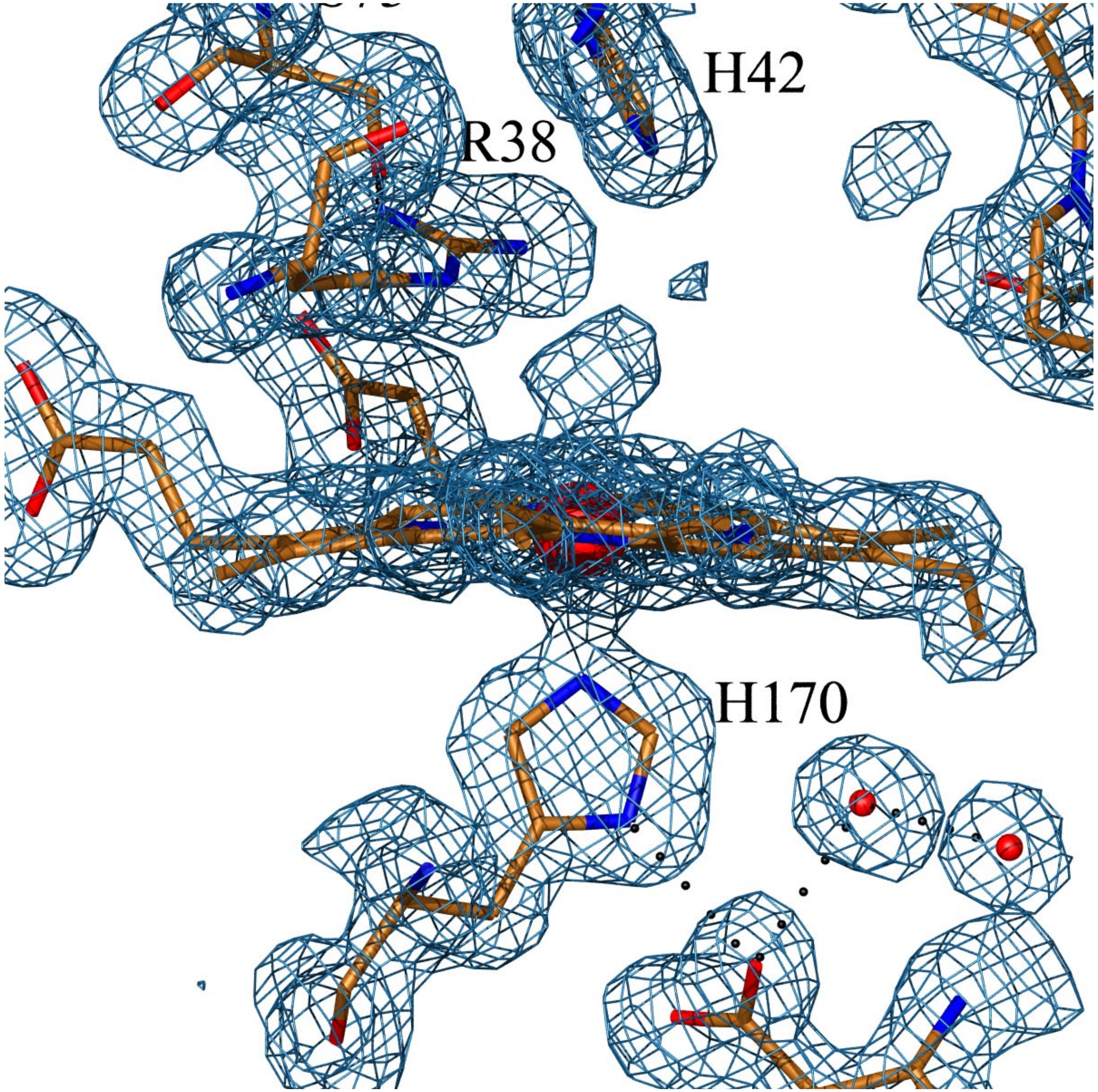




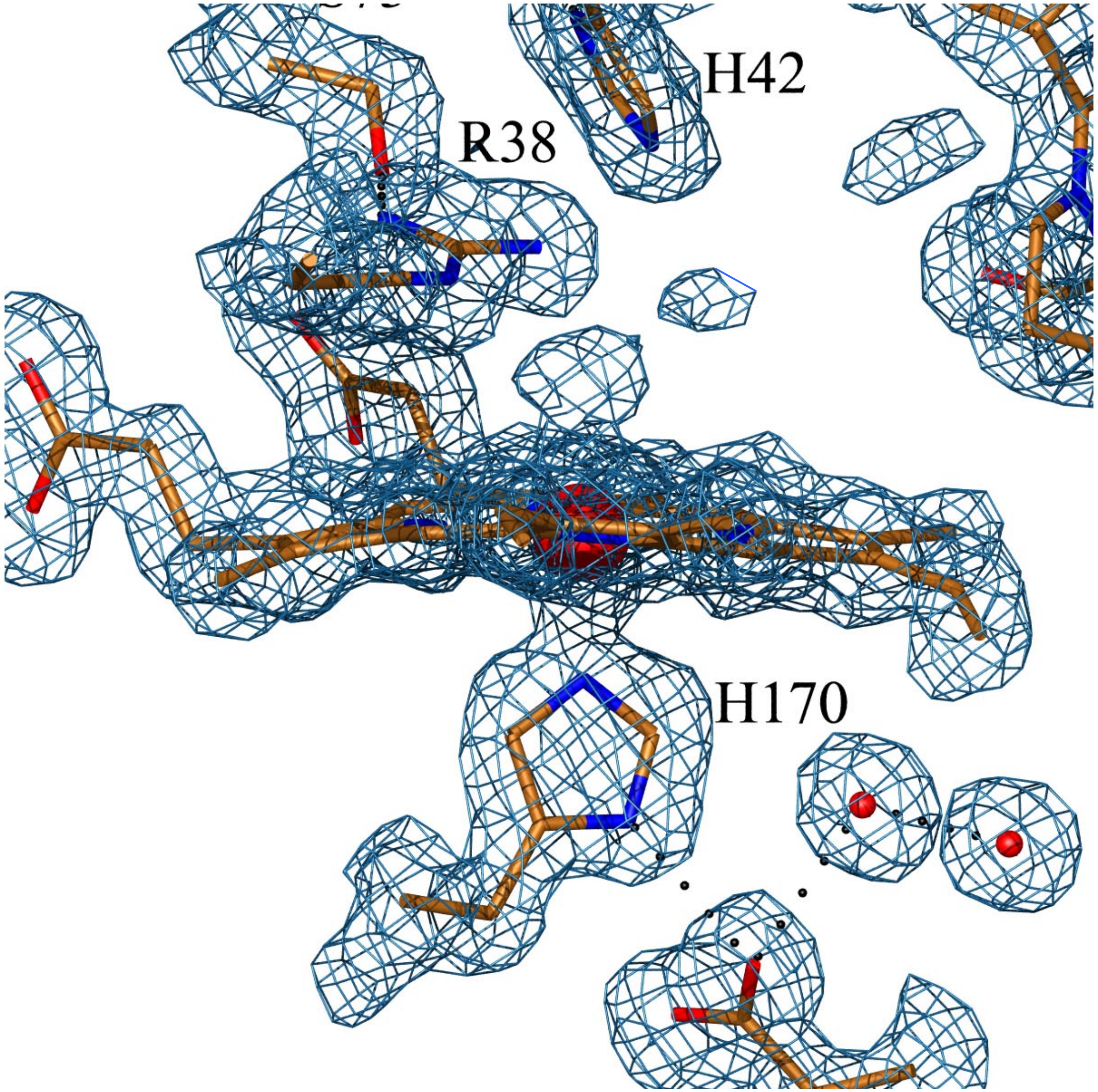
0-8°　　X-RAY-DRIVEN CATALYSIS



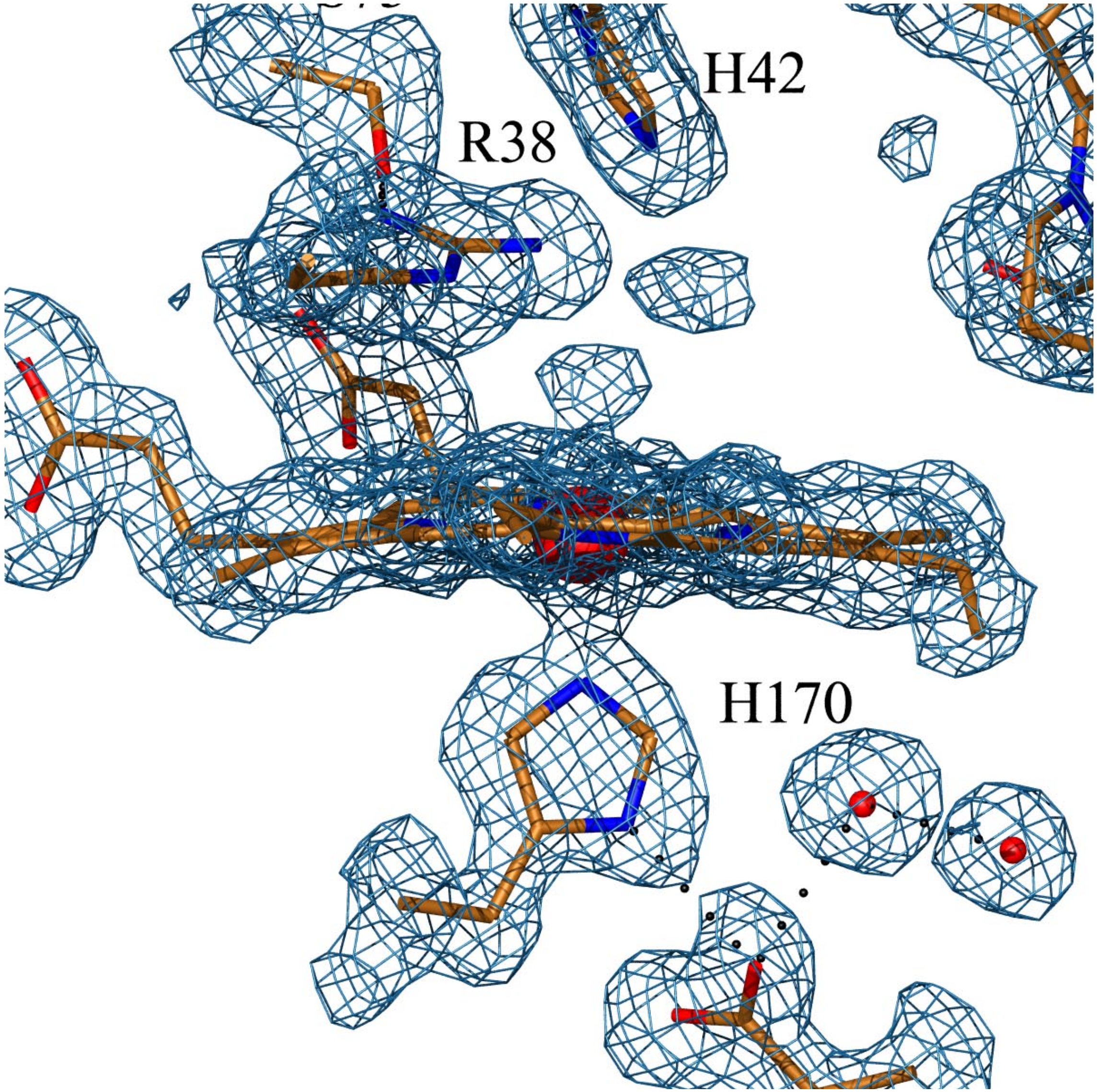
0-10°



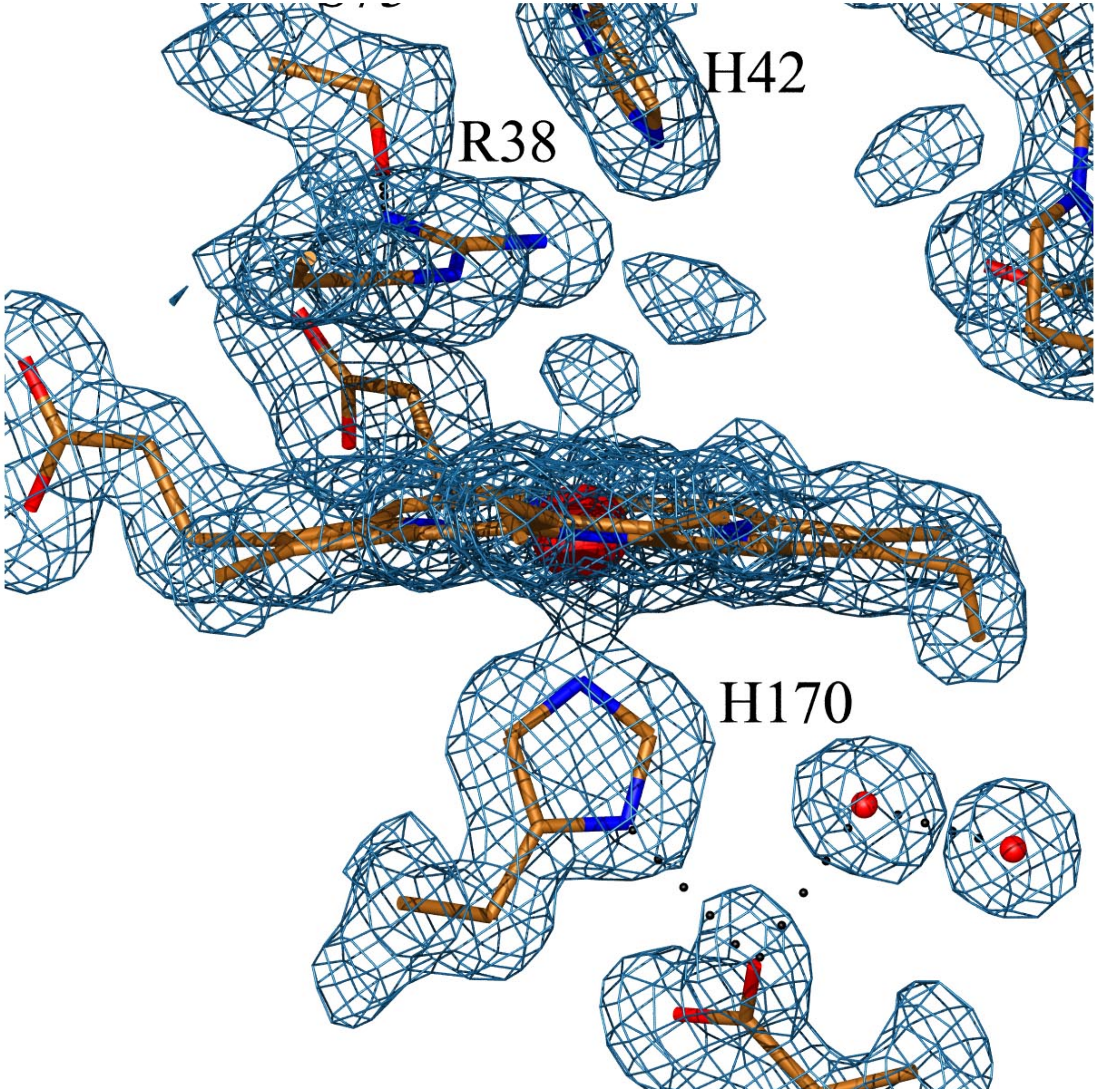
10-20°



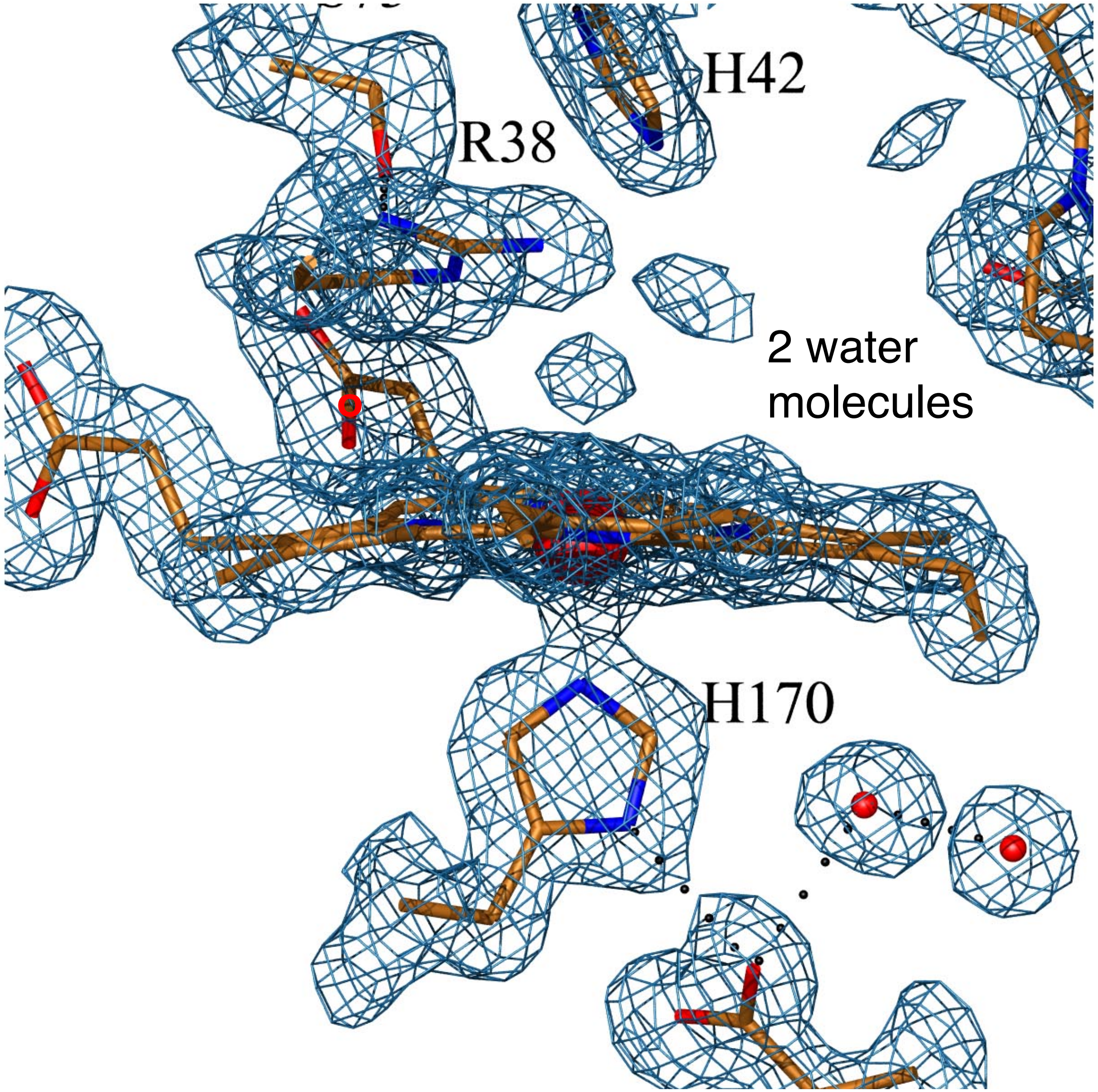
40-50°



50-60°



70-80º



“The catalytic pathway of horseradish peroxidase at high resolution”
Berglund, G.I., Carlsson, G.H., Smith, A.T., Szoke, H., Henriksen, A. & Hajdu, J., *Nature* **417**, 463-468 (2002)

(1) Less than 10 electrons were liberated per unit cell

(2) Reduction of dioxygen to 2 waters requires 4 electrons

(3) Redox enzymes evolved to channel electrons to/from active sites

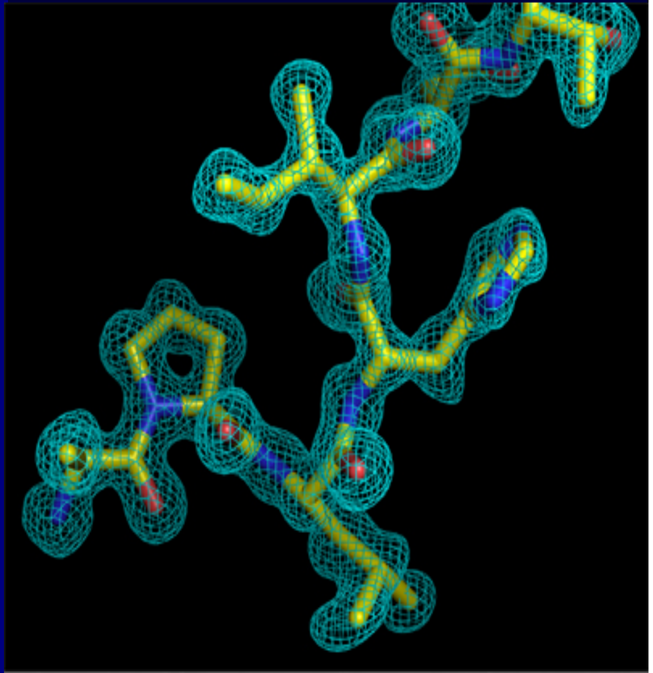
(4) No damage was visible in the protein

THIS IS ALL FINE BUT RADIATION DAMAGE IS A SERIOUS BARRIER TO OBSERVATION

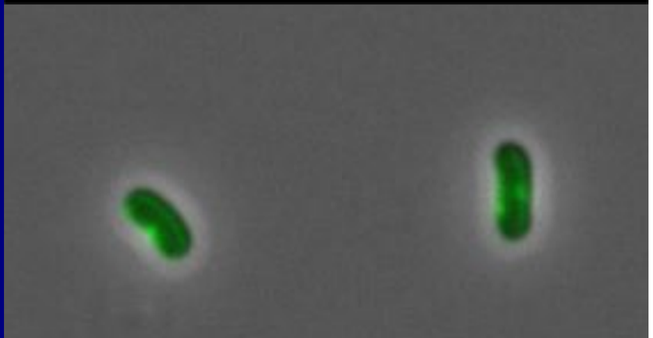
RADIATION DAMAGE KILLS

The more detail we want to see, the less is left from the sample

CRYSTAL STRUCTURE
DISTRIBUTED DAMAGE



THERE IS A TIME COMPONENT IN DAMAGE FORMATION



NO IDENTICAL COPIES
CONCENTRATED DAMAGE

Neutze, R., Wouts, R., van der Spoel, D., Weckert, E. Hajdu, J. (2000) *Nature* 406, 752-757

A GLIMMER OF HOPE:

SLAC-437
SLAC/SSRL-0066

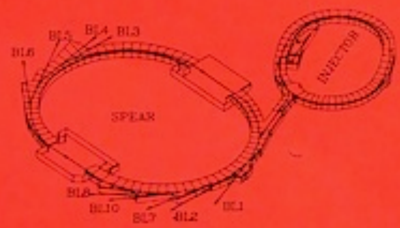
Workshop
on
Scientific Applications of Coherent X-Rays

Stanford, CA
February 12, 1994

Organization and Program
J. Arthur, G. Materlik, H. Winick

Executive Summary
R.J. Birgeneau, C.S. Fadley, G. Materlik

SLAC-Report-437



Prepared for the Department of Energy
under contract number DE-AC03-76SF00515

STANFORD LINEAR ACCELERATOR CENTER
STANFORD SYNCHROTRON RADIATION LABORATORY
Stanford University • Stanford, California

SciFi program: Explore the physical limits of imaging (1996-2000)

Carol V. Robinson
Oxford

Gyula Faigel
Budapest

Edgar Weckert
Karlsruhe

Sven Hovmöller
Stockholm

Marin van Heel
London

Janos Hajdu
Uppsala
Coordinator

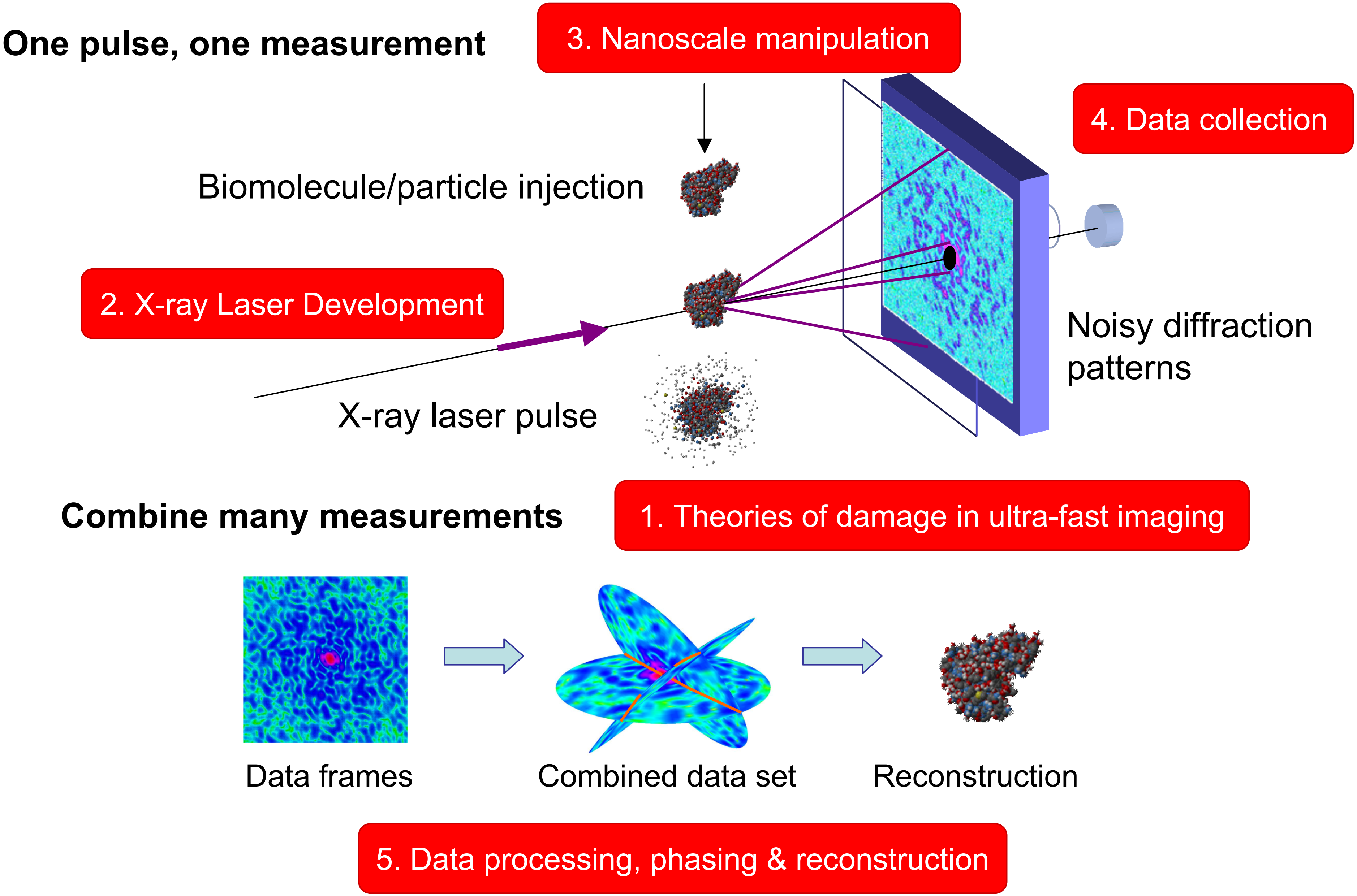
THREE LINES OF DEVELOPMENT JOIN IN THE PROJECT

1. DEVELOPMENT OF THEORIES TO EXPLORE THE PHYSICAL LIMITS OF IMAGING

2. DEVELOPMENT OF X-RAY LASERS

3. DEVELOPMENT OF PHASING ALGORITHMS (continuous diffraction patterns offer advantages)

Single Molecule X-ray Imaging



Scattering and damage by X-rays

(12 keV photons, biological samples: C, N, O, H, S, P)

X-RAYS INTERACT WITH MATTER THROUGH ABSORPTION AND SCATTERING:

(1) PHOTOELECTRIC EFFECT (~90%) followed by Auger emission, shake-up excitations, and secondary electron cascades (large samples)

(2) ELASTIC SCATTERING (~7-10%)

(3) INELASTIC SCATTERING (~3%)

We compute the effect of ionisation, changing scattering factors, and sample explosion on the diffraction pattern

Compute time-integrated diffraction intensity:

$$I(\mathbf{q}) = \Omega r_e^2 \int_{-\infty}^{\infty} I(t) \left| \sum_j f_j(\mathbf{q},t) \exp\left\{ i\mathbf{q} \cdot \mathbf{x}_j(t) \right\} \right|^2 dt$$

Radiation damage interferes with atomic scattering factors $f_j(\mathbf{q},t)$ and atomic positions $\mathbf{x}_j(t)$

Calculate "degradation (R) factor" to see how the explosion degrades the image

$$R = \sum_u \left| \frac{K^{-1}\sqrt{I_{real}(u)} - \sqrt{I_{ideal}(u)}}{\sum_{u'} \sqrt{I_{ideal}(u')}} \right| \qquad K = \frac{\sum_u \sqrt{I_{real}(u)}}{\sum_u \sqrt{I_{ideal}(u)}}$$

- $R = 0$ is ideal; larger R means larger error
- For two totally random arrays: R Å 0.67
- Typical R-values in Protein Database: 0.20

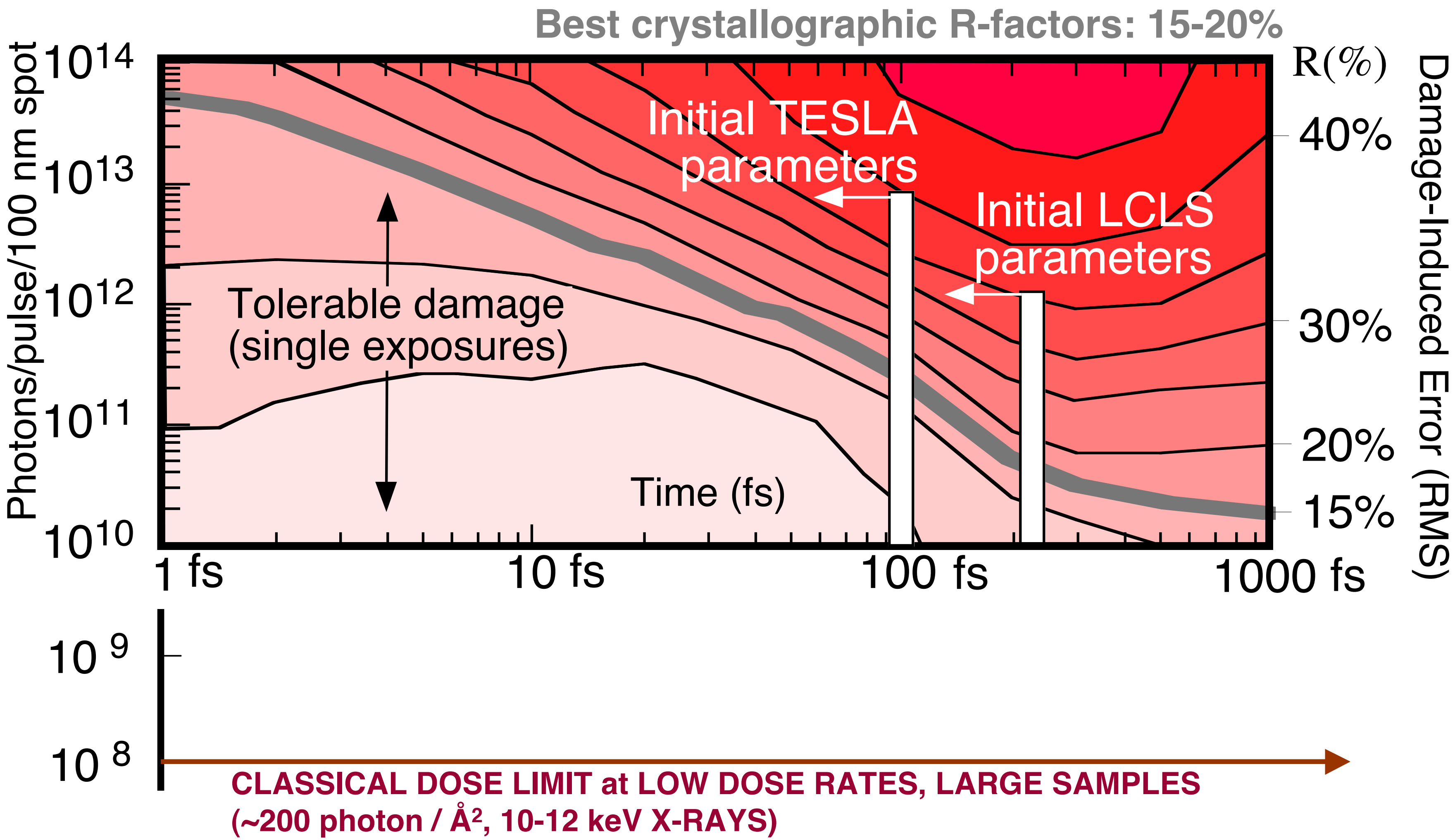
Landscape of damage tolerance from our XMD model

Neutze et al. (2000) *Nature* 406, 752-757

Ionisation and subsequent sample explosion cause diffraction intensities to change →

Damage-Induced Error

$$R = \frac{\left| \sqrt{I(t)} - \sqrt{I_o} \right|}{\sqrt{I_o}}$$



Potential for biomolecular imaging with femtosecond X-ray pulses

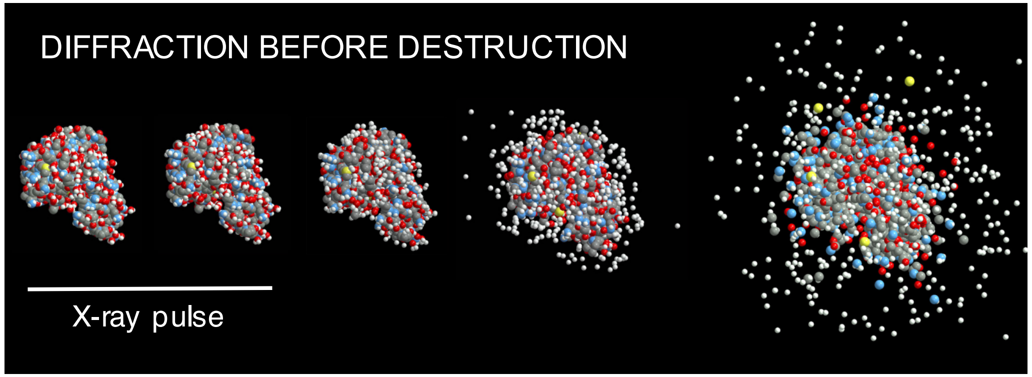
Richard Neutze*, Remco Wouts*, David van der Spoel*, Edgar Weckert†‡ & Janos Hajdu*

** Department of Biochemistry, Biomedical Centre, Box 576, Uppsala University, S-75123 Uppsala, Sweden*
† Institut für Kristallographie, Universität Karlsruhe, Kaiserstrasse 12, D-76128 , Germany

Sample damage by X-rays and other radiation limits the resolution of structural studies on non-repetitive and non-reproducible structures such as individual biomolecules or cells[1]. Cooling can slow sample deterioration, but cannot eliminate damage-induced sample movement during the time needed for conventional measurements[1,2]. Analyses of the dynamics of damage formation[3–5] suggest that the conventional damage barrier (about 200 X-ray photons per Å^2 with X-rays of 12 keV energy or 1 Å wavelength[2]) may be extended at very high dose rates and very short exposure times. Here we have used computer simulations to investigate the structural information that can be recovered from the scattering of intense femtosecond X-ray pulses by single protein molecules and small assemblies. Estimations of radiation damage as a function of photon energy, pulse length, integrated pulse intensity and sample size show that experiments using very
ort exposures may provide useful
radiation damage destroys the
ultrashort, high-intensity X-ray
[7] that are currently under devel-
container-free sample handling
echniques, will provide a new
ations with X-rays.

X-ray photons depositing energy
avelength, the photoelectric cross-
s higher than its elastic-scattering
ectric effect the primary source of
t is a resonance phenomenon in
n electron ejected[8], usually from a
ut 95% of the photoelectric events

Nature **406**, 752-757 (2000)



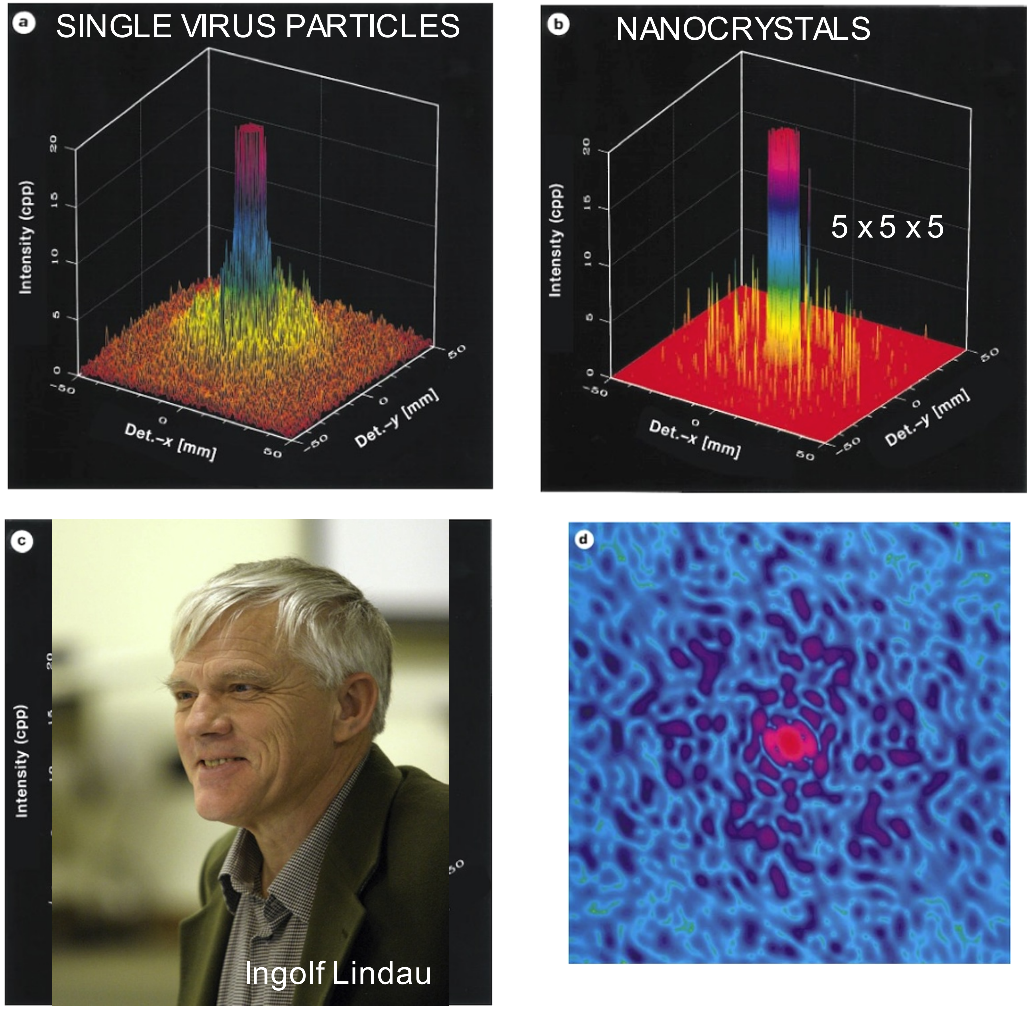
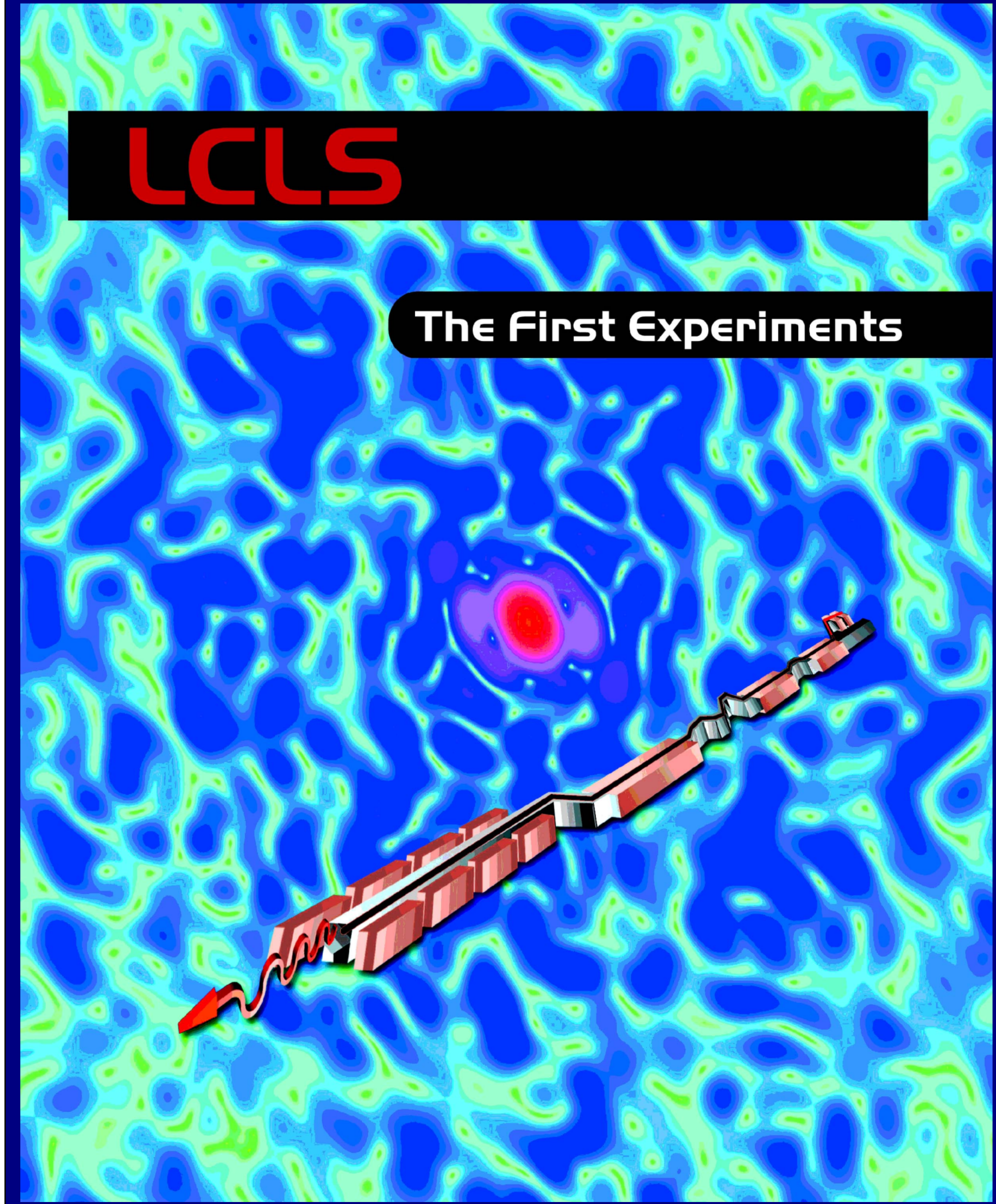


Figure 3 Elastic scattering from a variety of samples. **a–c**, Simulated diffraction images of 0.2 Å to model an imperfect lattice. **c**, Scattering from a single molecule of lysozyme. **d**,

AIMING THE BIG GUNS - The science case for X-ray FELs

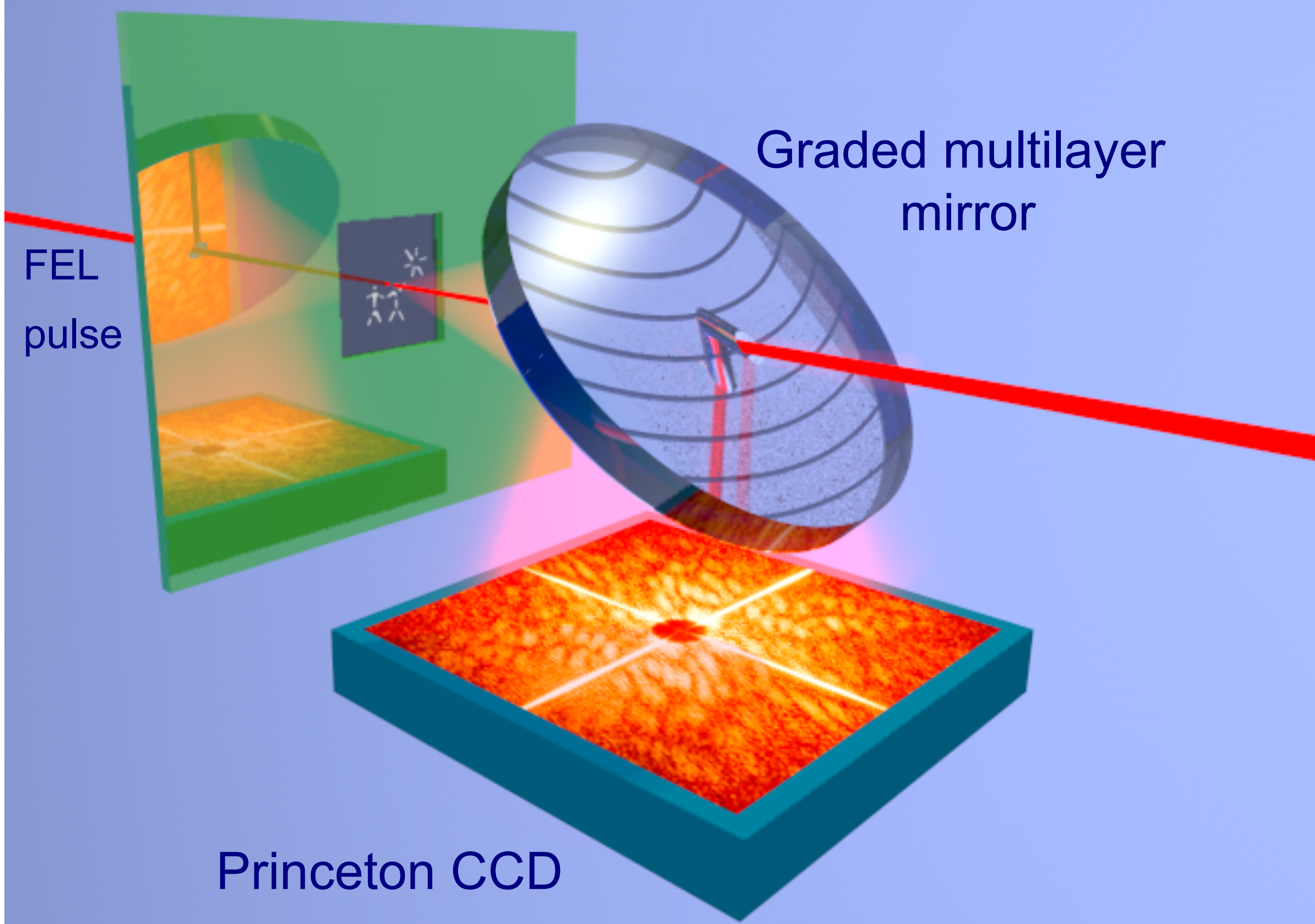


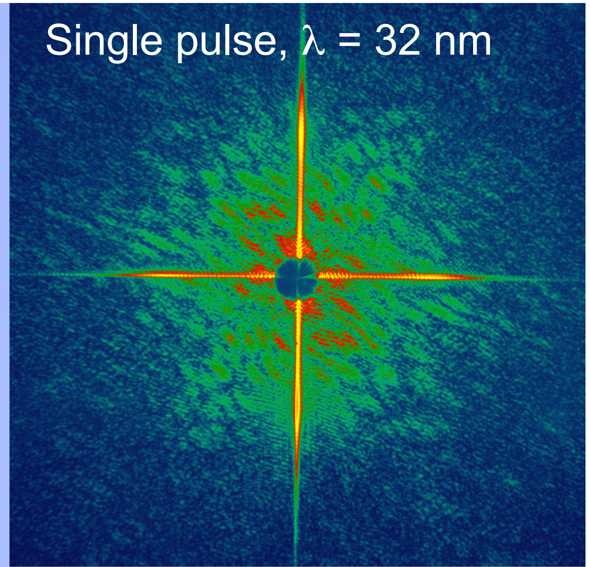
September 2000

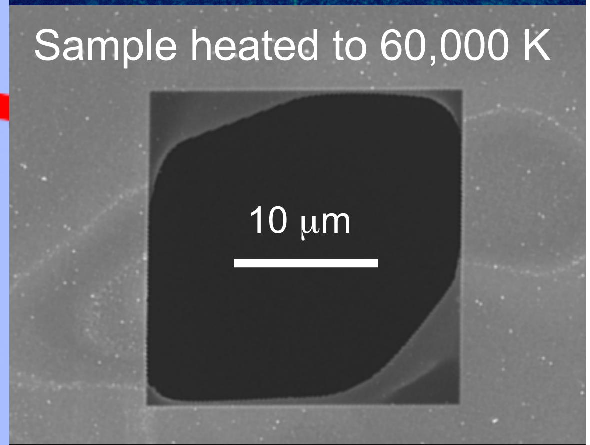
October 2002

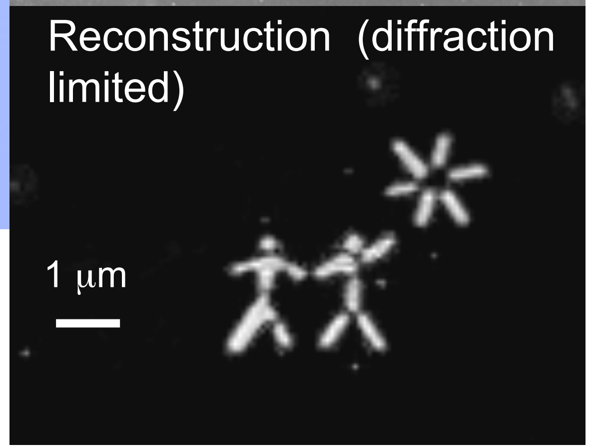
In 2002, Livermore joins our SciFi project

PROOF OF PRINCIPLE
1 February 2006





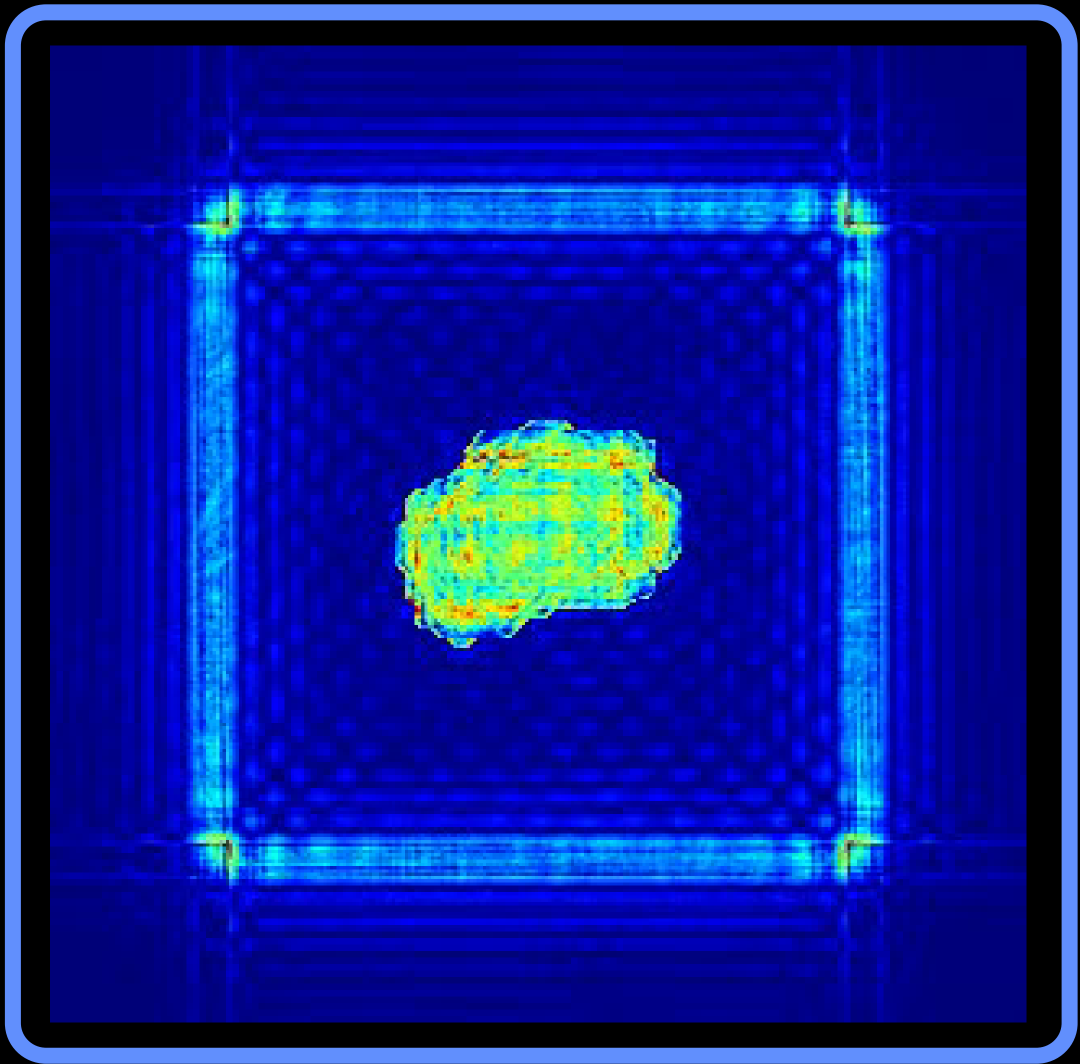




Nature Physics **2**, 839-843 (Dec. 2006)

First flash diffraction experiment at the VUV-FEL

Reconstruction from the over-sampled diffraction pattern

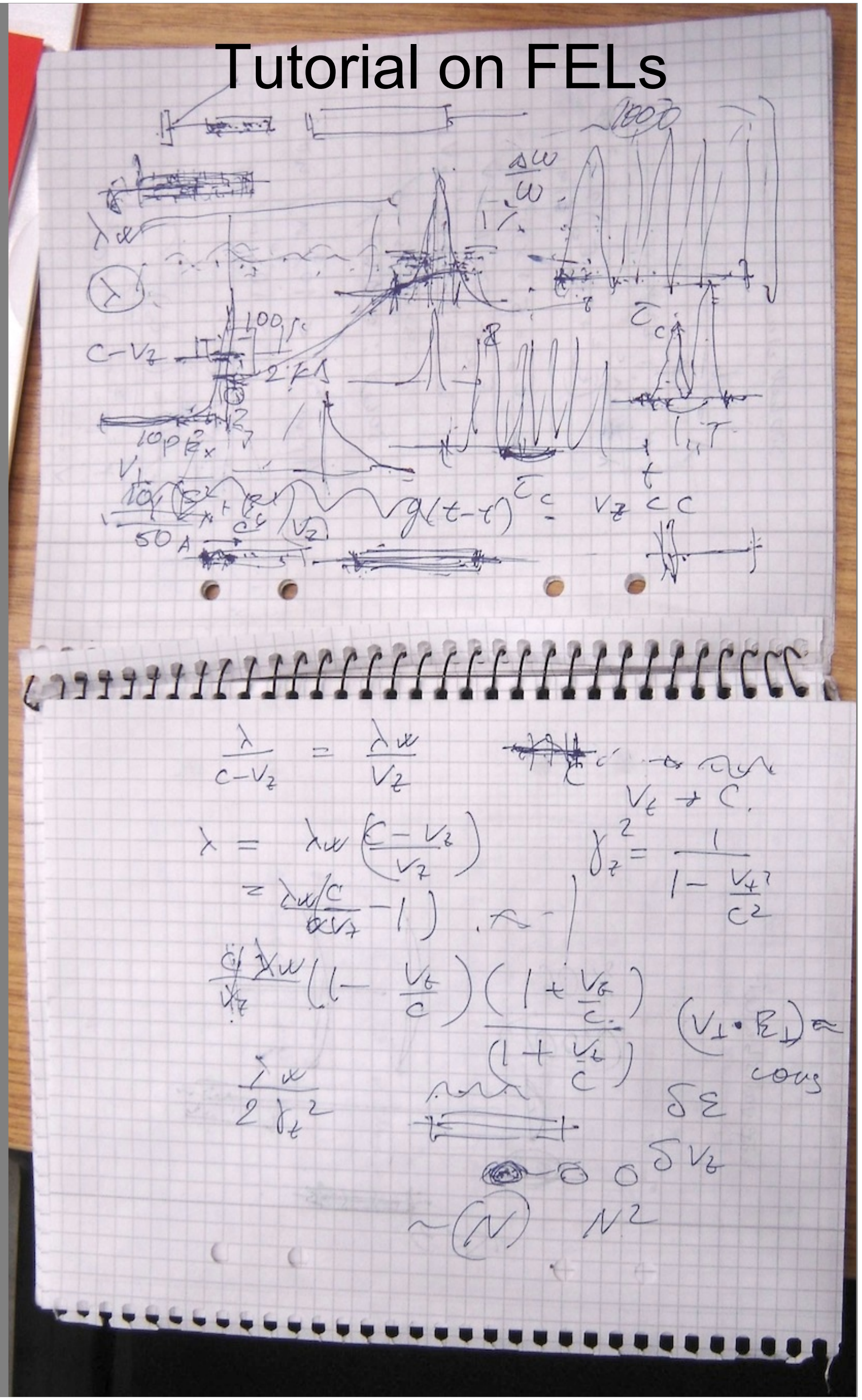


Filipe Maia (Uppsala University)

Based on the *Gerchberg-Saxton* error reduction algorithm

- ***Hybrid Input Output* as implemented in *Shrinkwrap / Hawk***
- Uses a dynamic instead of a static support.
- Uses a low resolution version of the current guess as new support.

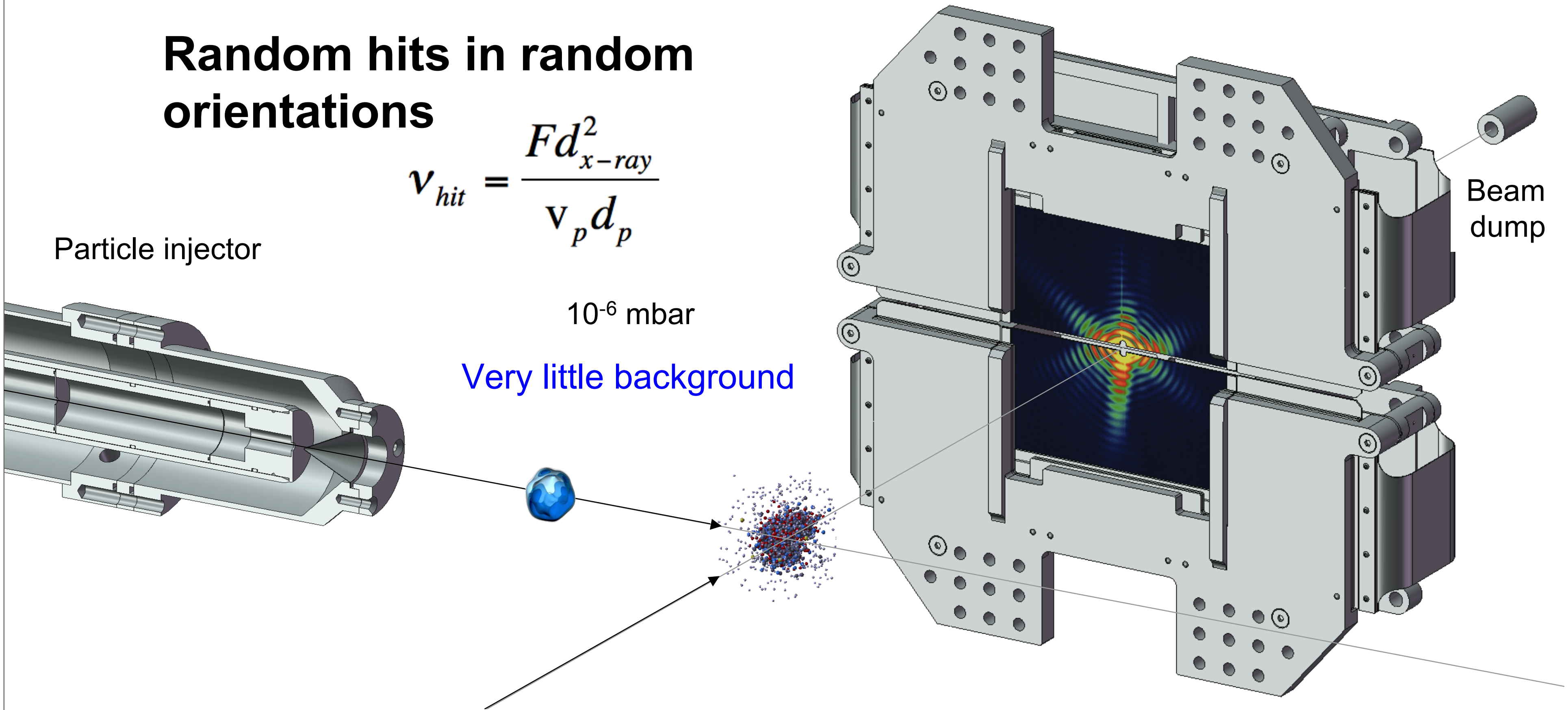

Evgeny Saldin
Mikhail Yurkov


Tutorial on FELs

LCLS: SINGLE PARTICLES AND BIOMOLECULES

Random hits in random orientations

$$\nu_{hit} = \frac{F d_{x-ray}^2}{v_p d_p}$$



One pulse, one measurement

BIOLOGY IN THE GAS PHASE

Many infectious diseases are transmitted via aerosols

Ocean sprays put out 10^{13} kg aerosol per year from jet drops formed when bubbles burst

Metabolically active cells have been captured at altidudes of 20-70 km

The Telegraph

HOME » SCIENCE » SPACE

Sea plankton 'found living outside International Space Station'

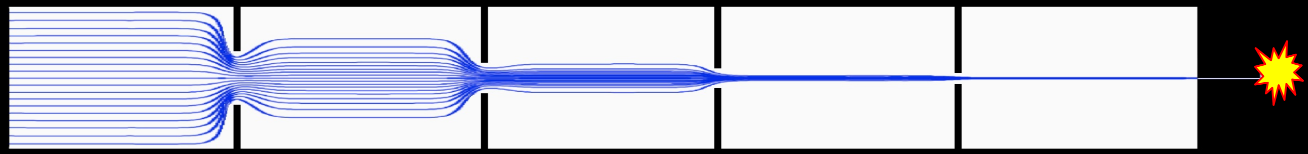
Russian cosmonauts claim to have discovered tiny marine creatures thriving in zero-gravity on the outside of the International Space Station

Russian cosmonauts claim to have found marine creatures living on the outside of the International Space Station where conditions for life are believed to be impossible

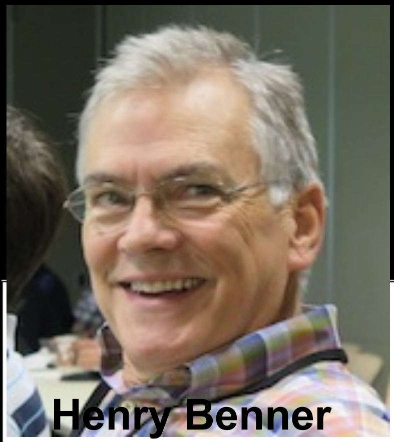
Cells sorters and tissue printing devices are based on aerosols

AEROSOL SAMPLE INJECTION

AEROSOLISATION, EQUILIBRATION

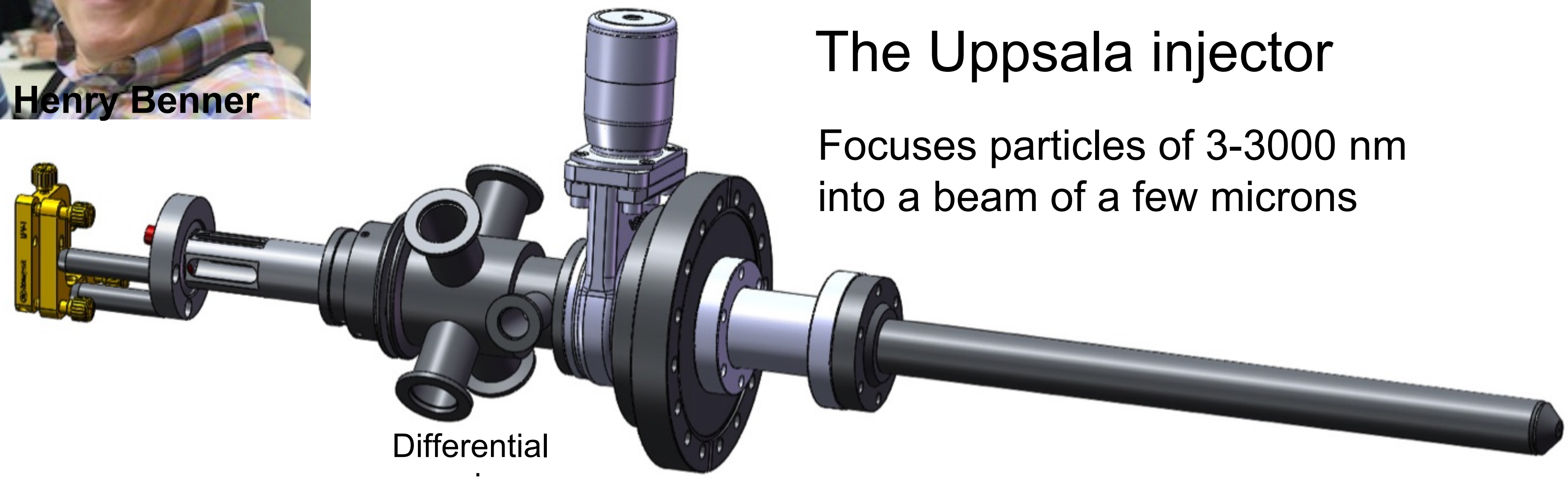


AERODYNAMIC FOCUSING: W.K. Murphy and G.W. Sears, "Production of Particulate Beams" *J. Appl. Phys.* **35**, 1986–1987 (1964).

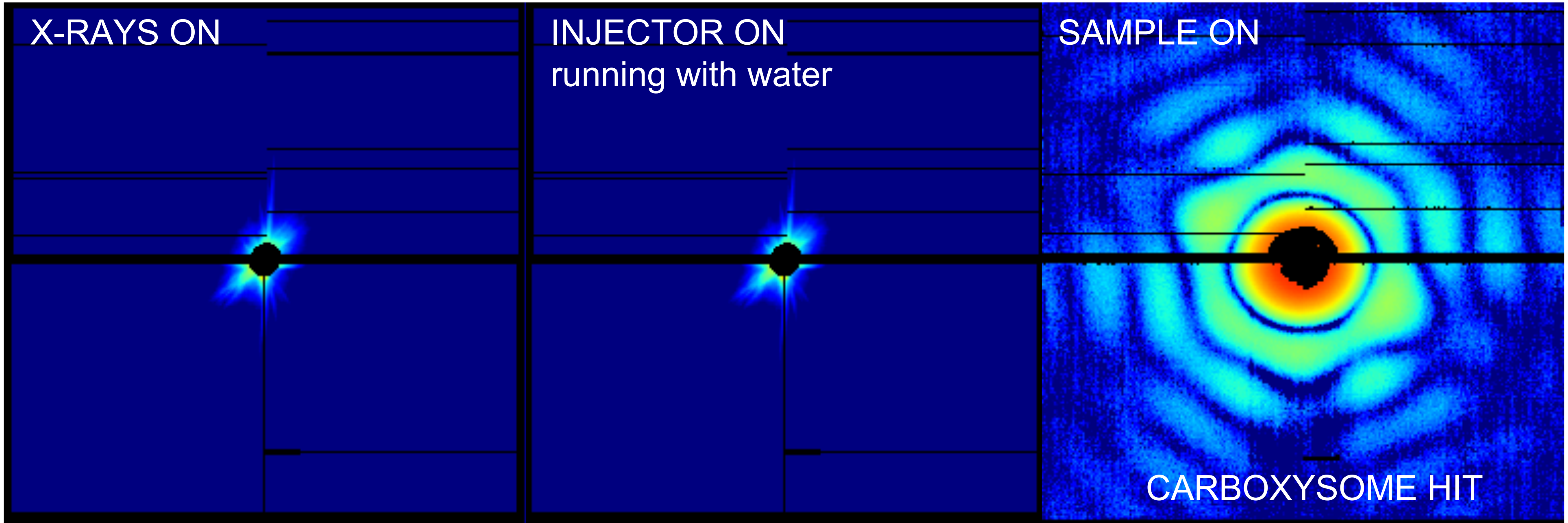


The Uppsala injector

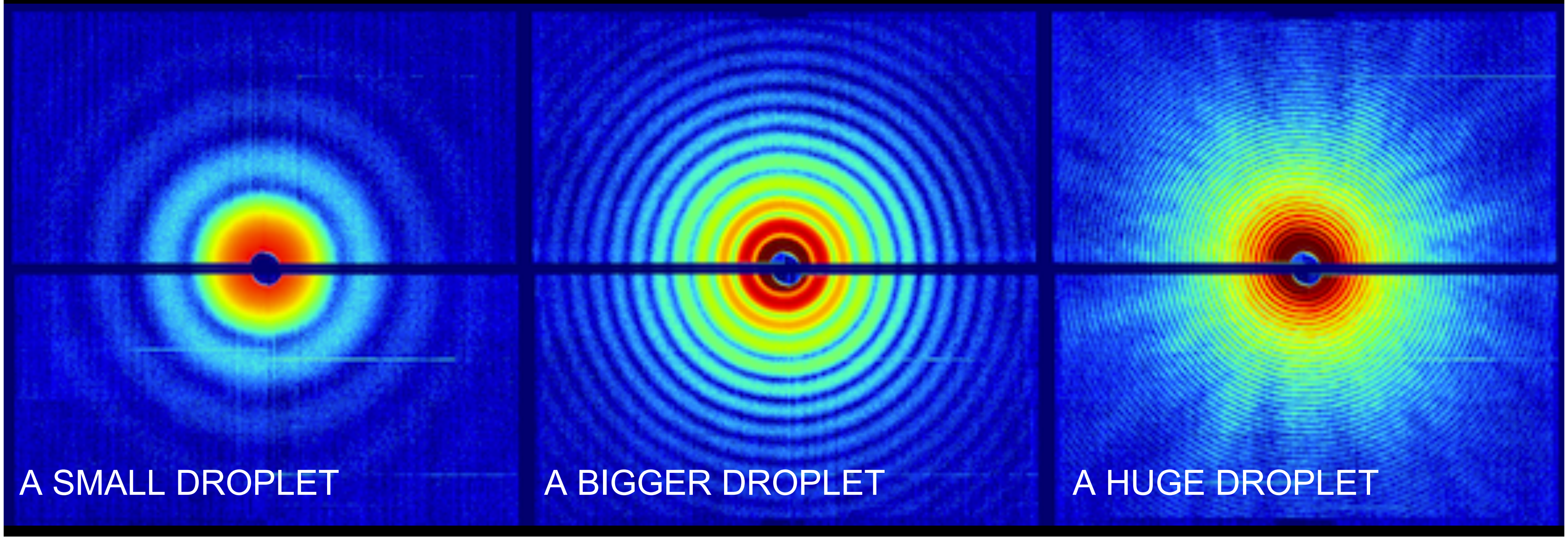
Focuses particles of 3-3000 nm into a beam of a few microns




NARROW
PARTICLE BEAM
Rick Kirian

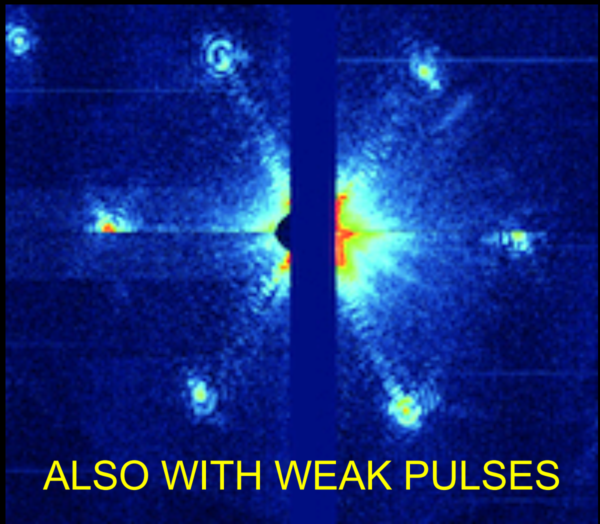


EVAPORATION CAN BE CONTROLLED BY ADJUSTING, PRESSURE, TEMPERATURE, LIQUID FLOW AND GAS FLOW. WE AVOID DROPLETS COMING THROUGH THE INJECTOR.

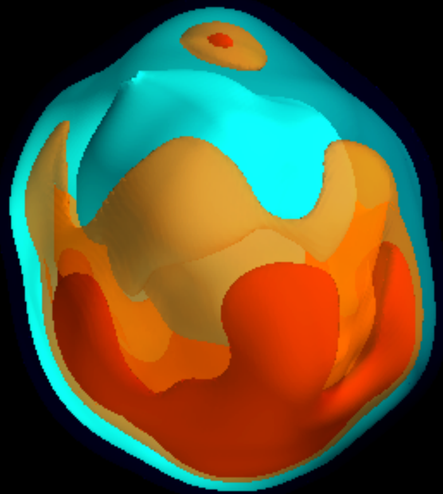


OPPORTUNITIES

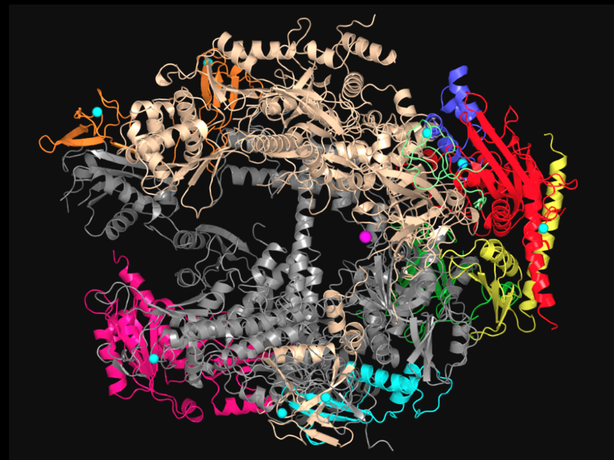
NANOCRYSTALS



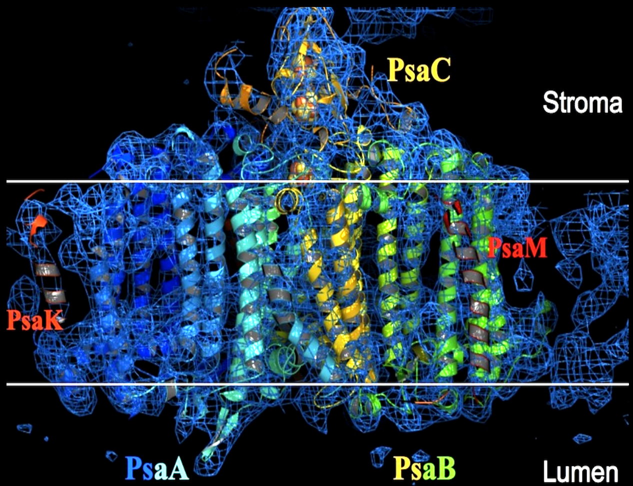
VIRUS PARTICLES



SINGLE MOLECULES

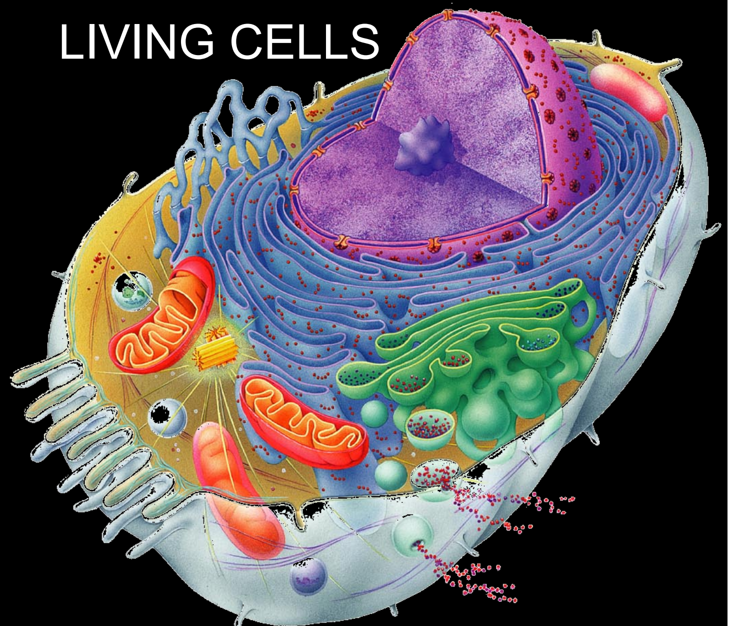


MEMBRANE PROTEINS



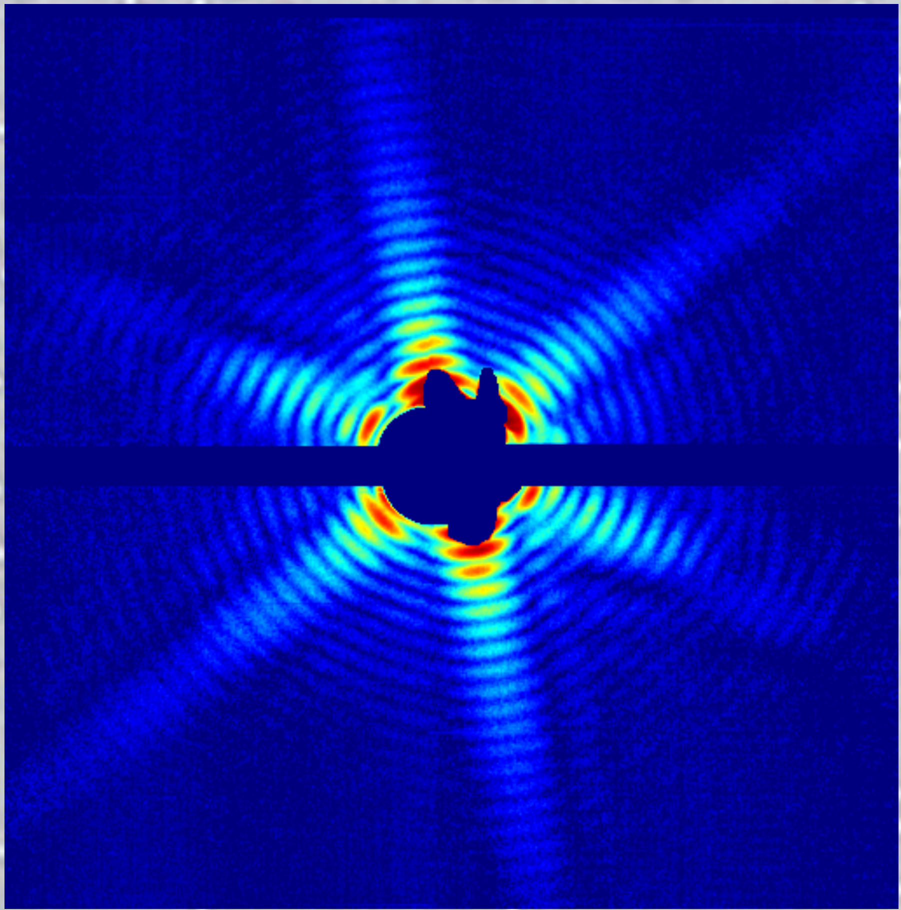
CELL ORGANELLES

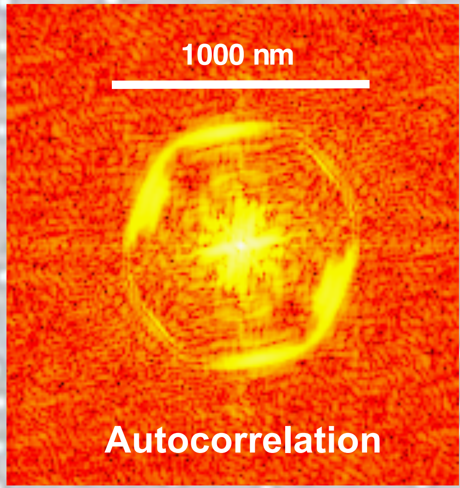
LIVING CELLS

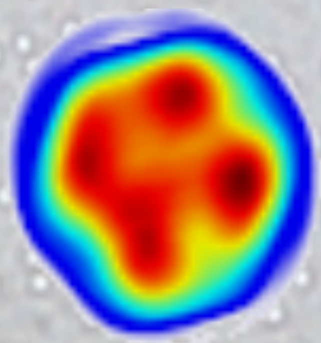


2009: 1st RESULTS - SINGLE VIRUS PARTICLES

THE GIANT MIMI VIRUS







Projection image

Photon energy: 1.80 keV
Pulse length: 70 fs (FDHM)
Focus: 10 μm (FWHM)
1.6 x 10^{10} photons/μm^2

Seibert, Ekeberg, Maia *et al*. *Nature* **470**, 78-U86 (2011).

PRL 114, 098102 (2015)

Selected for a Viewpoint in *Physics*
PHYSICAL REVIEW LETTERS

week ending
6 MARCH 2015

Three-Dimensional Reconstruction of the Giant Mimivirus Particle with an X-Ray Free-Electron Laser

Tomas Ekeberg,[1,*] Martin Svenda,[1] Chantal Abergel,[2] Filipe R. N. C. Maia,[1,3] Virginie Seltzer,[2] Jean-Michel Claverie,[2] Max Hantke,[1] Olof Jönsson,[1] Carl Nettelblad,[1] Gijs van der Schot,[1] Mengning Liang,[4] Daniel P. DePonte,[4] Anton Barty,[4] M. Marvin Seibert,[1,5] Bianca Iwan,[1,6] Inger Andersson,[1] N. Duane Loh,[7] Andrew V. Martin,[8] Henry Chapman,[4,9] Christoph Bostedt,[5] John D. Bozek,[5] Ken R. Ferguson,[5] Jacek Krzywinski,[5] Sascha W. Epp,[10] Daniel Rolles,[10,11] Artem Rudenko,[11] Robert Hartmann,[12] Nils Kimmel,[13,14] and Janos Hajdu[1,15]

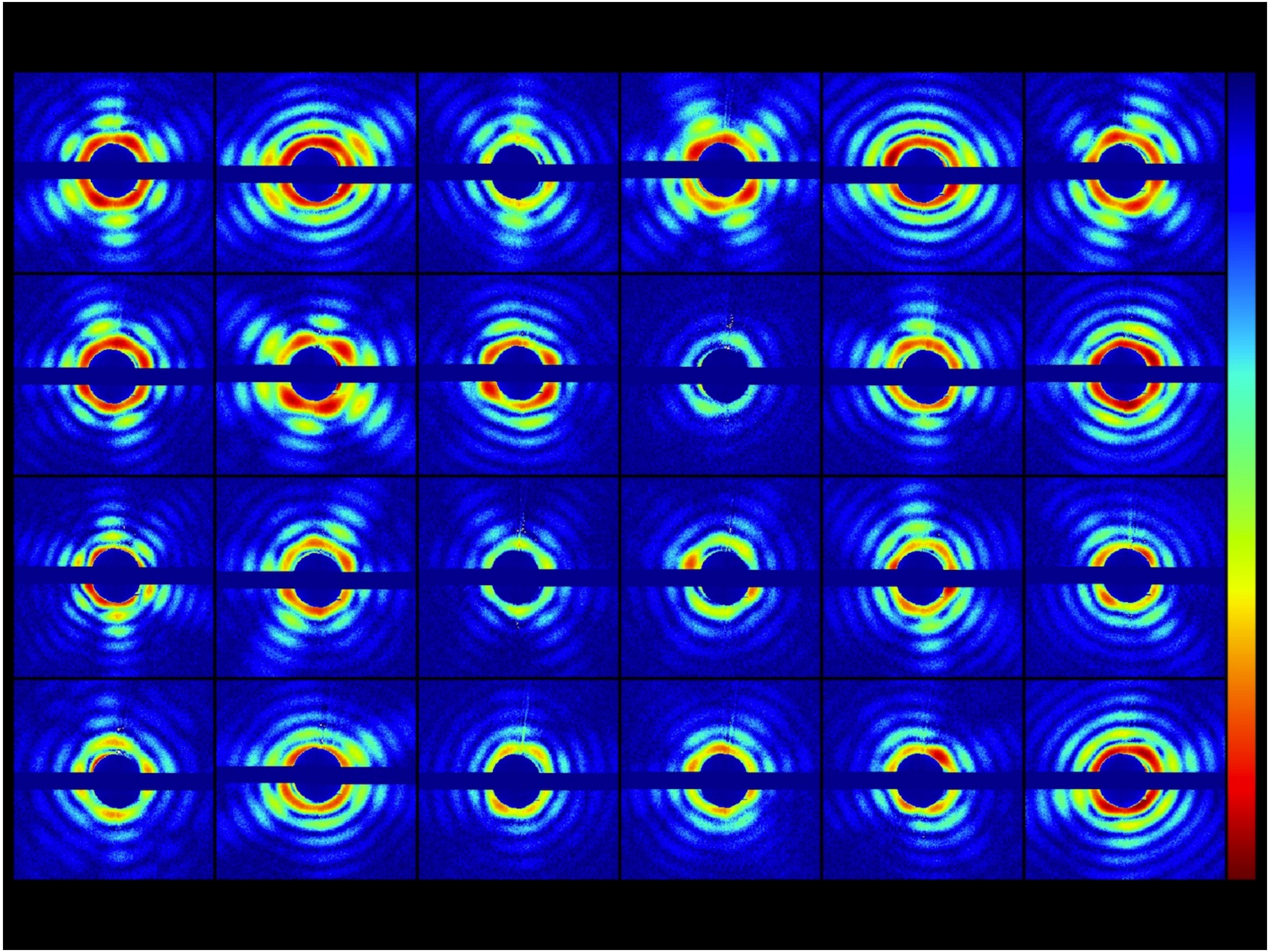
[1]*Laboratory of Molecular Biophysics, Department of Cell and Molecular Biology, Uppsala University, Husargatan 3 (Box 596), SE-751 24 Uppsala, Sweden*
[2]*Génomique & Structurale - IGS - UMR 7256, CNRS, Aix-Marseille Université, Institut de Microbiologie de la Méditerranée, Parc Scientifique de Luminy, Case 934, 13288 Marseille Cedex 9, France*
[3]*NERSC, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA*
[4]*Center for Free-Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany*
[5]*LCLS, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, California 94025, USA*
[6]*Attophysics Group, CEA-Saclay, 91191 Gif sur Yvette Cedex, France*
[7]*Centre for BioImaging Sciences, National University of Singapore, 14 Science Drive 4 Blk S1 A, Singapore 117546, Singapore*
[8]*The University of Melbourne, Parkville, 3010 Victoria, Australia*
[9]*University of Hamburg, Notkestrasse 85, 22607 Hamburg, Germany*
[10]*Max Planck Advanced Study Group, Center for Free Electron Laser Science, Notkestrasse 85, 22607 Hamburg, Germany*
[11]*J. R. Macdonald Laboratory, Department of Physics, Kansas State University, 116 Cardwell Hall, Manhattan, Kansas 66506, USA*
[12]*PNSensor GmbH, Römmerstrasse 28, 80803 München, Germany*
[13]*Max-Planck-Institut Halbleiterlabor, Otto-Hahn-Ring 6, 81739 München, Germany*
[14]*Max-Planck-Institut für extraterrestrische Physik, Giessenbachstrasse, 85741 Garching, Germany*
[15]*European XFEL, Albert-Einstein-Ring 19, 22761 Hamburg, Germany*

(Received 3 October 2014; published 2 March 2015)

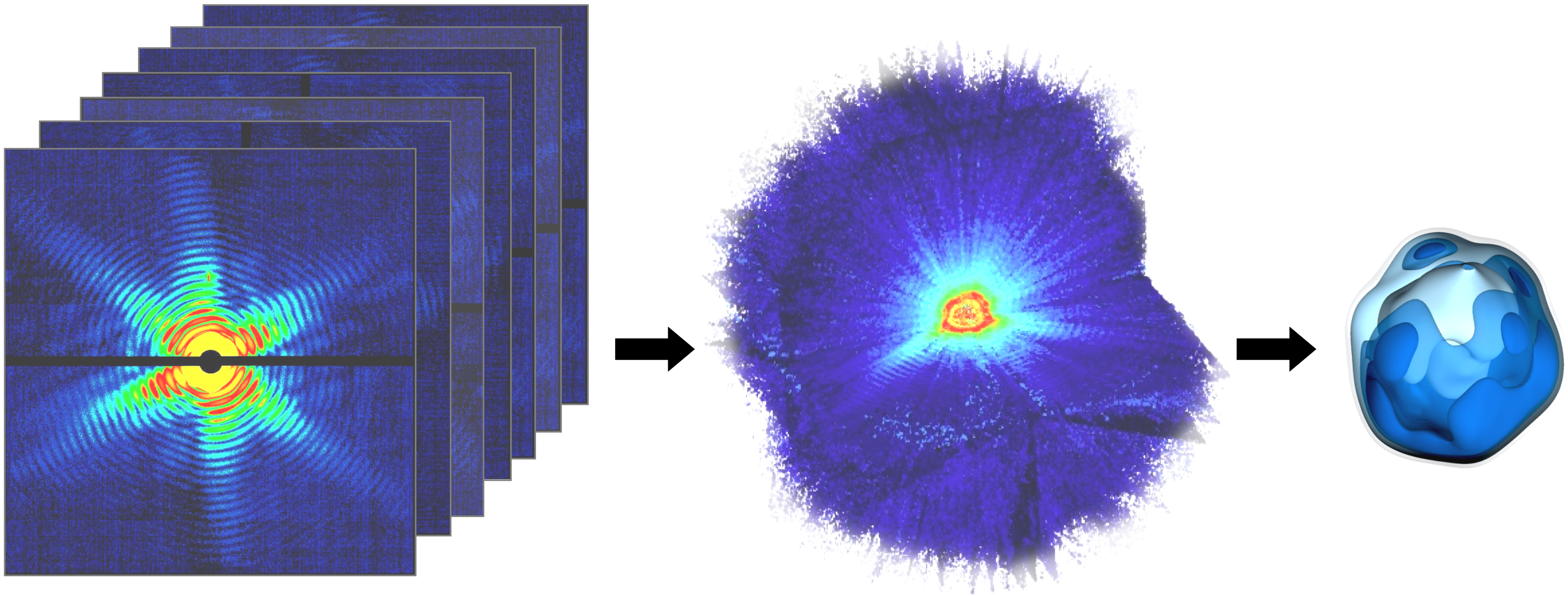
We present a proof-of-concept three-dimensional reconstruction of the giant mimivirus particle from experimentally measured diffraction patterns from an x-ray free-electron laser. Three-dimensional imaging requires the assembly of many two-dimensional patterns into an internally consistent Fourier volume. Since each particle is randomly oriented when exposed to the x-ray pulse, relative orientations have to be retrieved from the diffraction data alone. We achieve this with a modified version of the expand, maximize and compress algorithm and validate our result using new methods.

DOI: 10.1103/PhysRevLett.114.098102

PACS numbers: 42.30.-d, 87.64.-t, 87.64.Bx



From 2D to 3D structure determination

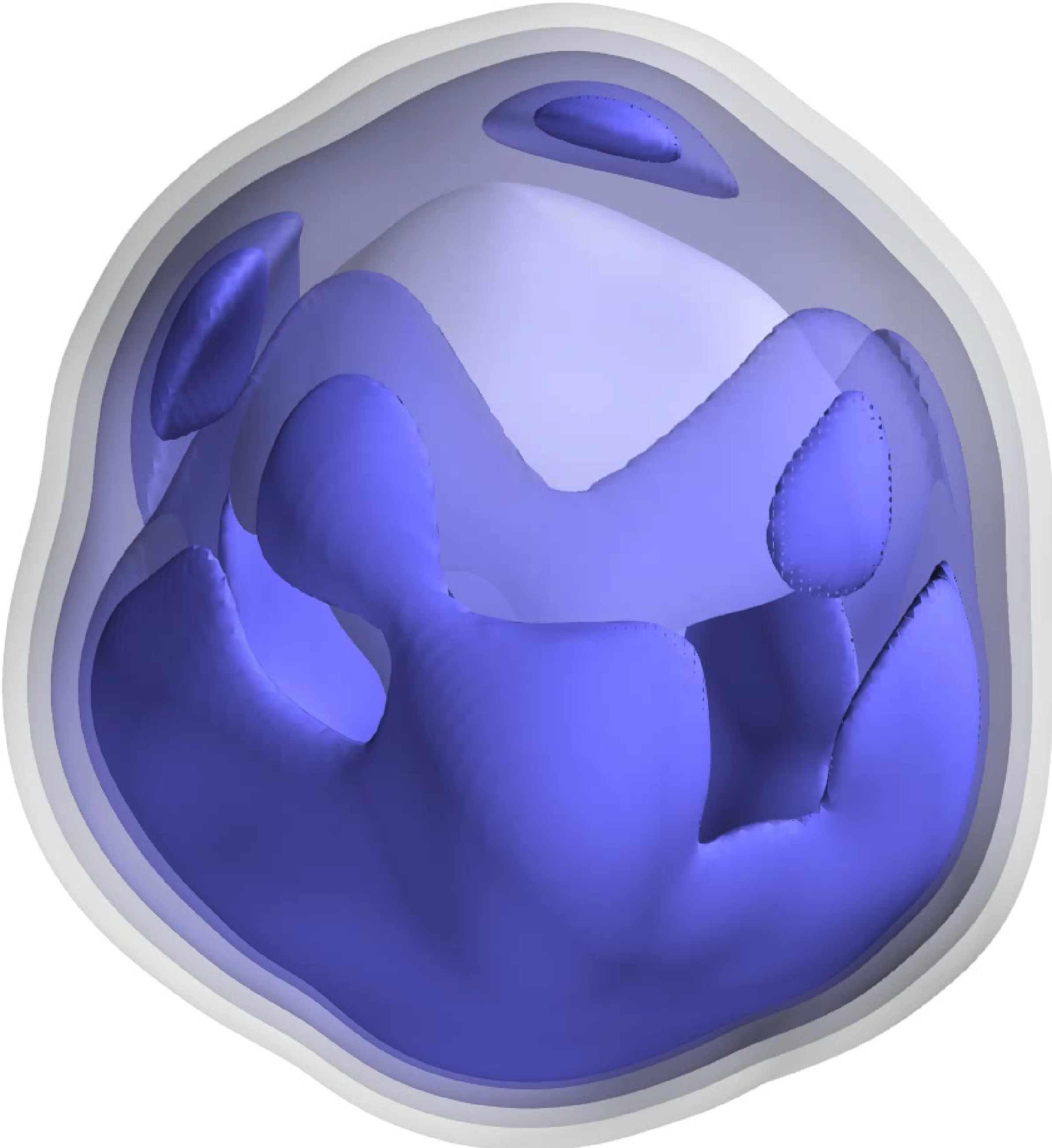


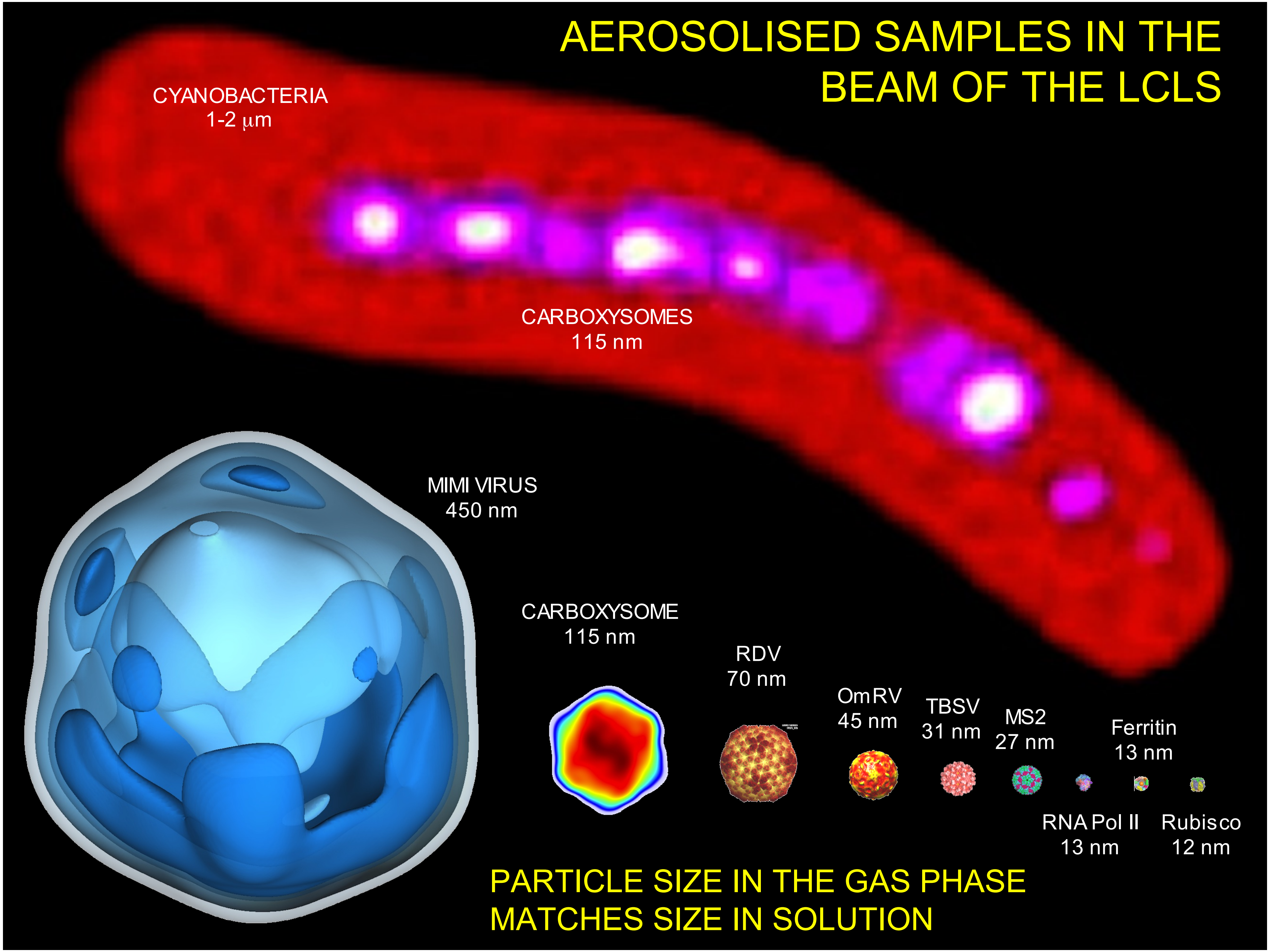
Individual exposures　　Self-consistent 3D data set　　Electron density map

EMC: Expectation Maximalisation and Compression

Based on Loh et al. *PRE* (2009)

PROOF OF CONCEPT: First 3D structure determination




AEROSOLISED SAMPLES IN THE
BEAM OF THE LCLS
CYANOBACTERIA
1-2 μm
CARBOXYSOMES
115 nm
MIMI VIRUS
450 nm
CARBOXYSOME
115 nm
RDV
70 nm
OmRV
45 nm
TBSV
31 nm
MS2
27 nm
Ferritin
13 nm
RNA Pol II
13 nm
Rubisco
12 nm
PARTICLE SIZE IN THE GAS PHASE
MATCHES SIZE IN SOLUTION

nature photonics

ARTICLES

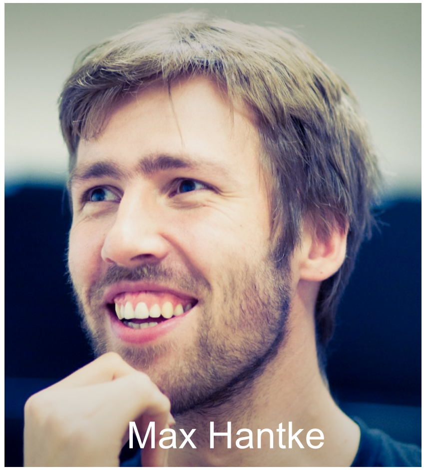
PUBLISHED ONLINE: 17 NOVEMBER 2014 | DOI: 10.1038/NPHOTON.2014.270

High-throughput imaging of heterogeneous cell organelles with an X-ray laser

Max F. Hantke, Dirk Hasse *et al.**

We overcome two of the most daunting challenges in single-particle diffractive imaging: collecting many high-quality diffraction patterns on a small amount of sample and separating components from mixed samples. We demonstrate this on carboxysomes, which are polyhedral cell organelles that vary in size and facilitate up to 40% of Earth's carbon fixation. A new aerosol sample-injector allowed us to record 70,000 low-noise diffraction patterns in 12 min with the Linac Coherent Light Source running at 120 Hz. We separate different structures directly from the diffraction data and show that the size distribution is preserved during sample delivery. We automate phase retrieval and avoid reconstruction artefacts caused by missing modes. We attain the highest-resolution reconstructions on the smallest single biological objects imaged with an X-ray laser to date. These advances lay the foundations for accurate, high-throughput structure determination by flash-diffractive imaging and offer a means to study structure and structural heterogeneity in biology and elsewhere.

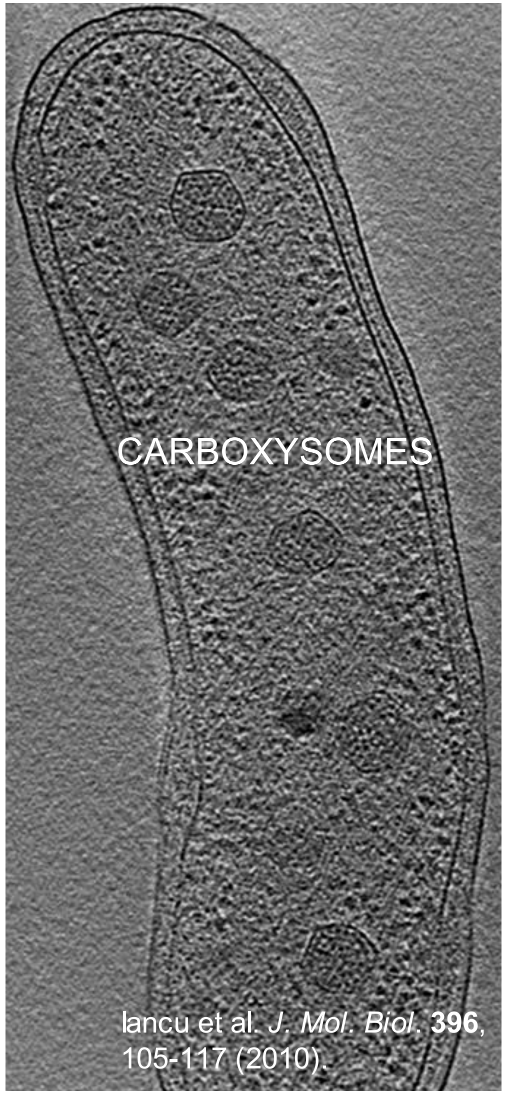
- 12 minutes of beam time, 79% hit ratio, 70 000 hits
- Sample "purification" in *silicio* after data collection



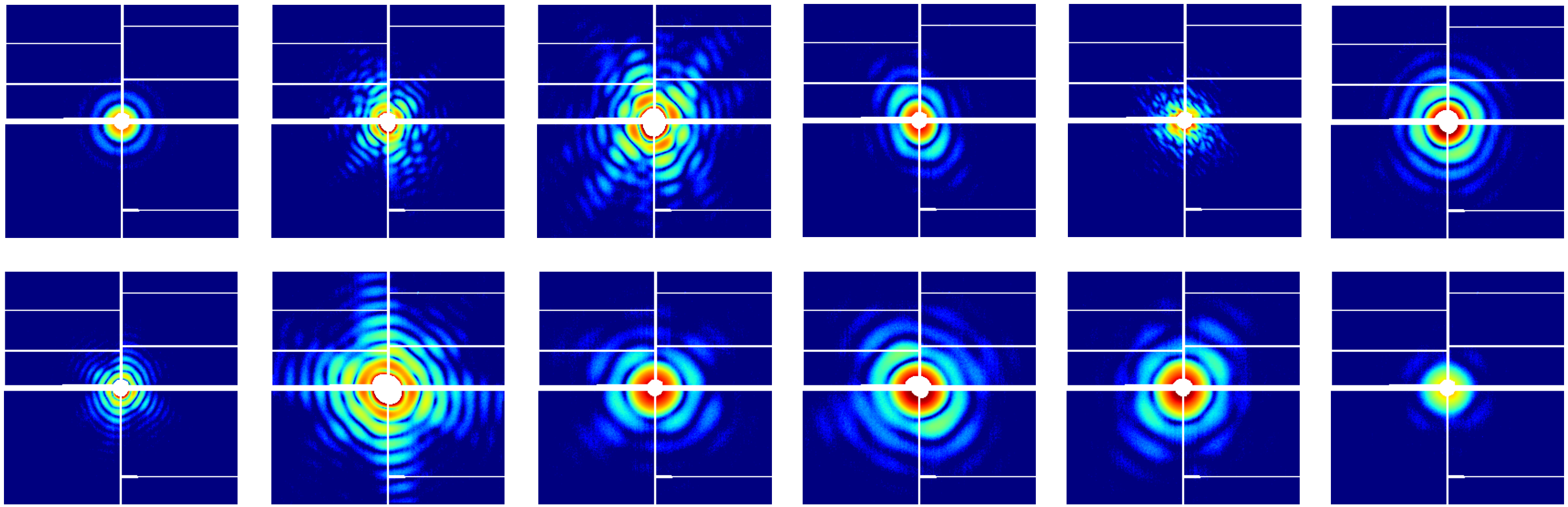
Total sample consumption:
3.4 x 10^9 particles

Only 50 000 particles go between two hits

Nature Photonics **8**, 943-949 (2014)

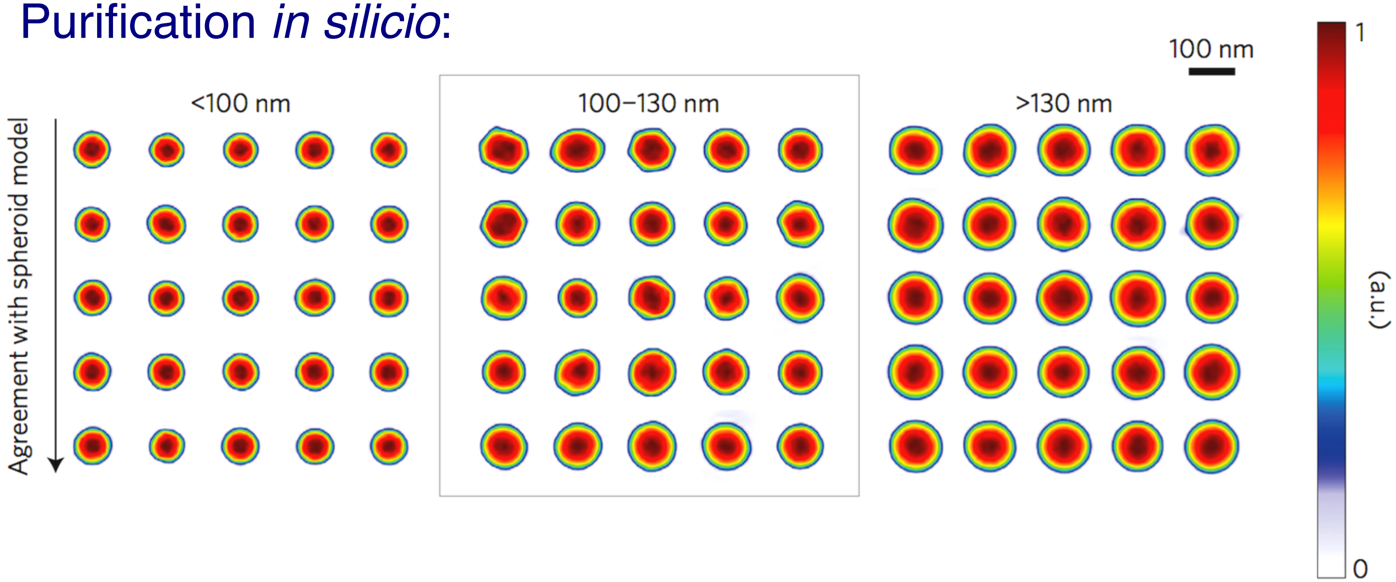


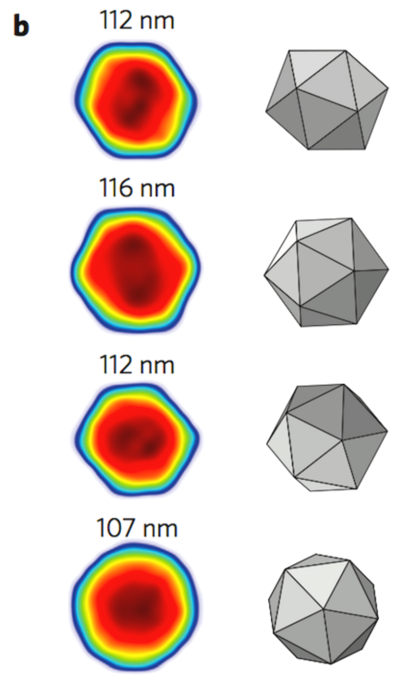
WE OBSERVE A HIGH DIVERSITY IN SHAPE AND SIZE

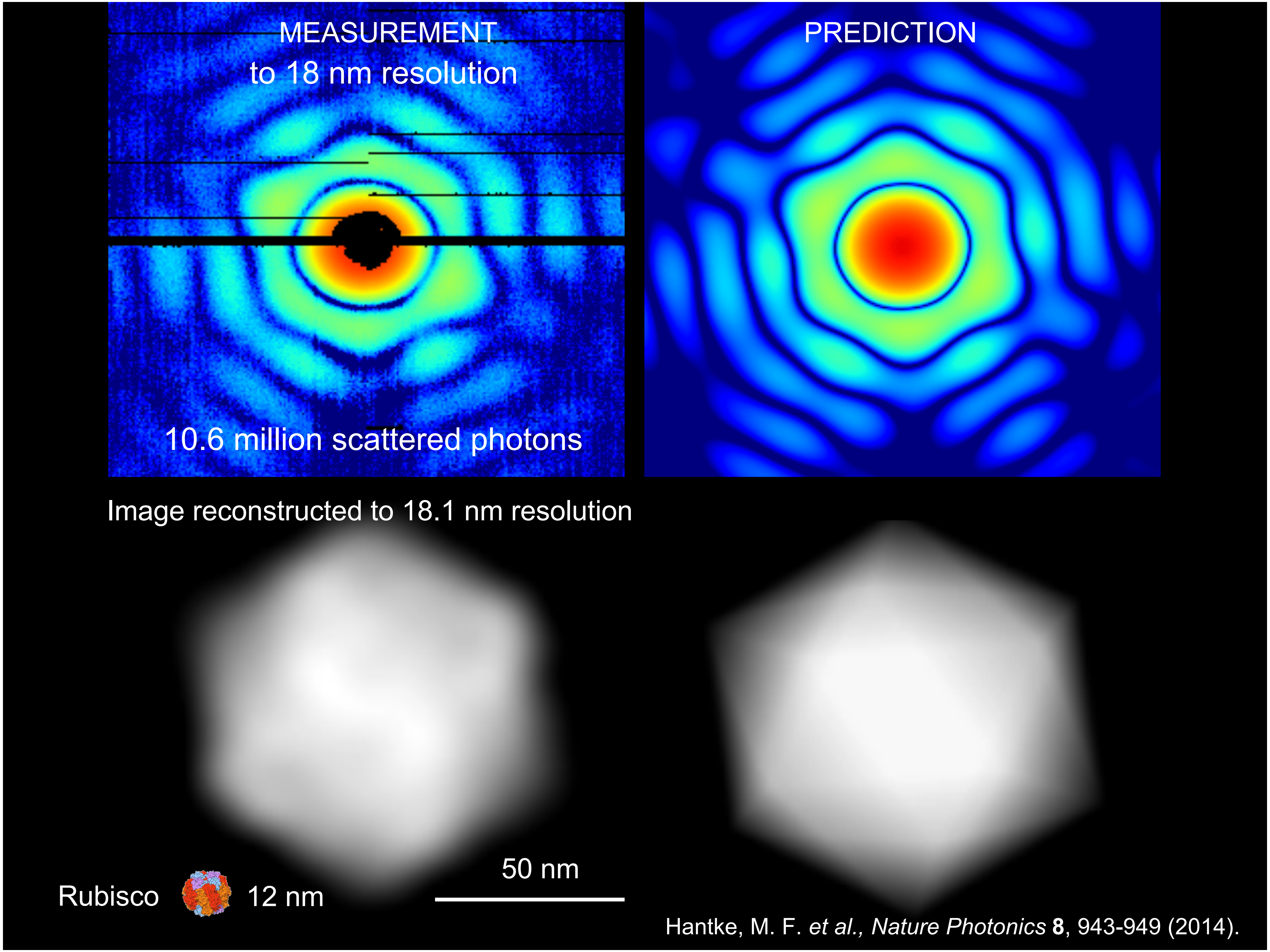


Same size distribution as in solution

Purification *in silicio*:

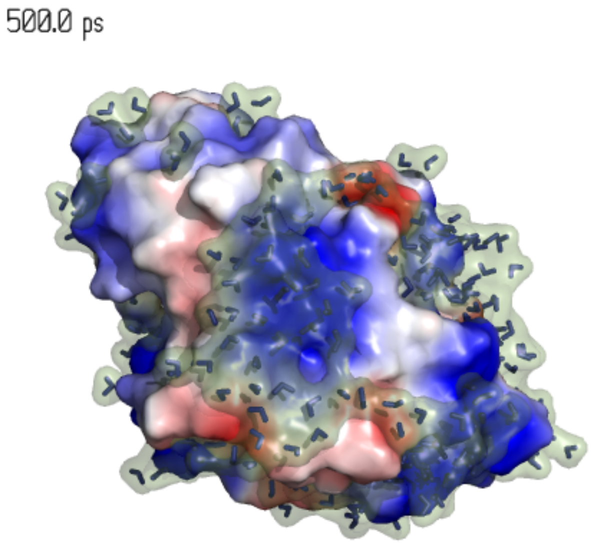


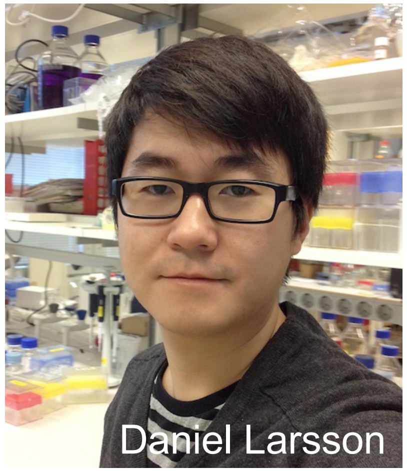



MEASUREMENT
to 18 nm resolution
10.6 million scattered photons
PREDICTION
Image reconstructed to 18.1 nm resolution
Rubisco
12 nm
50 nm
Hantke, M. F. et al., Nature Photonics 8, 943-949 (2014).

Identical samples do not exist in biology

The thermal energy at physiological temperatures is commensurate to the energy of interactions, which stabilise the structure of macromolecules, and as a consequence, macromolecules fluctuate around distinct conformers.





Strong single-shots can explore this heterogeneity

Structural variability and the incoherent addition of scattered intensities in single-particle diffraction.
Maia, F.R.N.C. et al., *Physical Reviews* **E 80**, 031905 (2009).

Identical samples do not exist in biology

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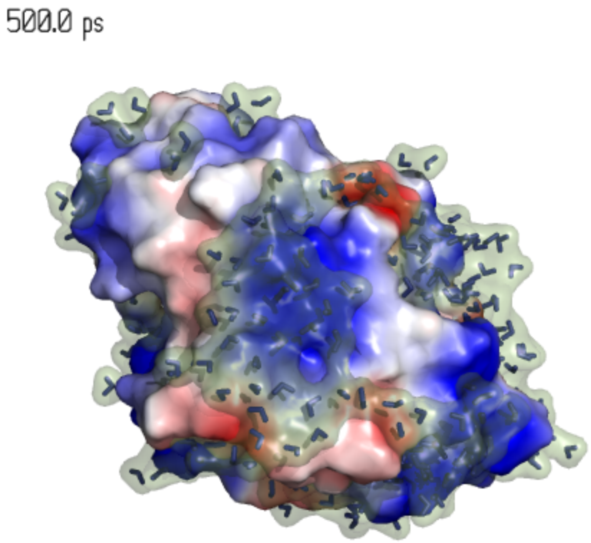


AN OPPORTUNITY: By looking at one snapshot at a time, combined with sorting procedures, we can get access to the entire *conformational space* of macromolecules (T. Ekeberg)

nature COMMUNICATIONS

ARTICLE

Received 1 Apr 2014 | Accepted 29 Oct 2014 | Published 11 Feb 2015 DOI: 10.1038/ncomms6704

Imaging single cells in a beam of live cyanobacteria with an X-ray laser

Gijs van der Schot[1,*], Martin Svenda[1,*], Filipe R.N.C. Maia[1,2], Max Hantke[1], Daniel P. DePonte[3,4], M. Marvin Seibert[1,4], Andrew Aquila[3,5], Joachim Schulz[3,5], Richard Kirian[3], Mengning Liang[3], Francesco Stellato[3,6], Bianca Iwan[1], Jakob Andreasson[1], Nicusor Timneanu[1], Daniel Westphal[1], F. Nunes Almeida[1], Dusko Odic[1], Dirk Hasse[1], Gunilla H. Carlsson[1], Daniel S.D. Larsson[1], Anton Barty[2], Andrew V. Martin[3,7], Sebastian Schorb[4], Christoph Bostedt[4], John D. Bozek[4], Daniel Rolles[3], Artem Rudenko[3,8], Sascha Epp[3], Lutz Foucar[9], Benedikt Rudek[10], Robert Hartmann[11], Nils Kimmel[11,12], Peter Holl[11], Lars Englert[13], Ne-Te Duane Loh[14], Henry N. Chapman[3,15], Inger Andersson[1], Janos Hajdu[1,5] & Tomas Ekeberg[1]

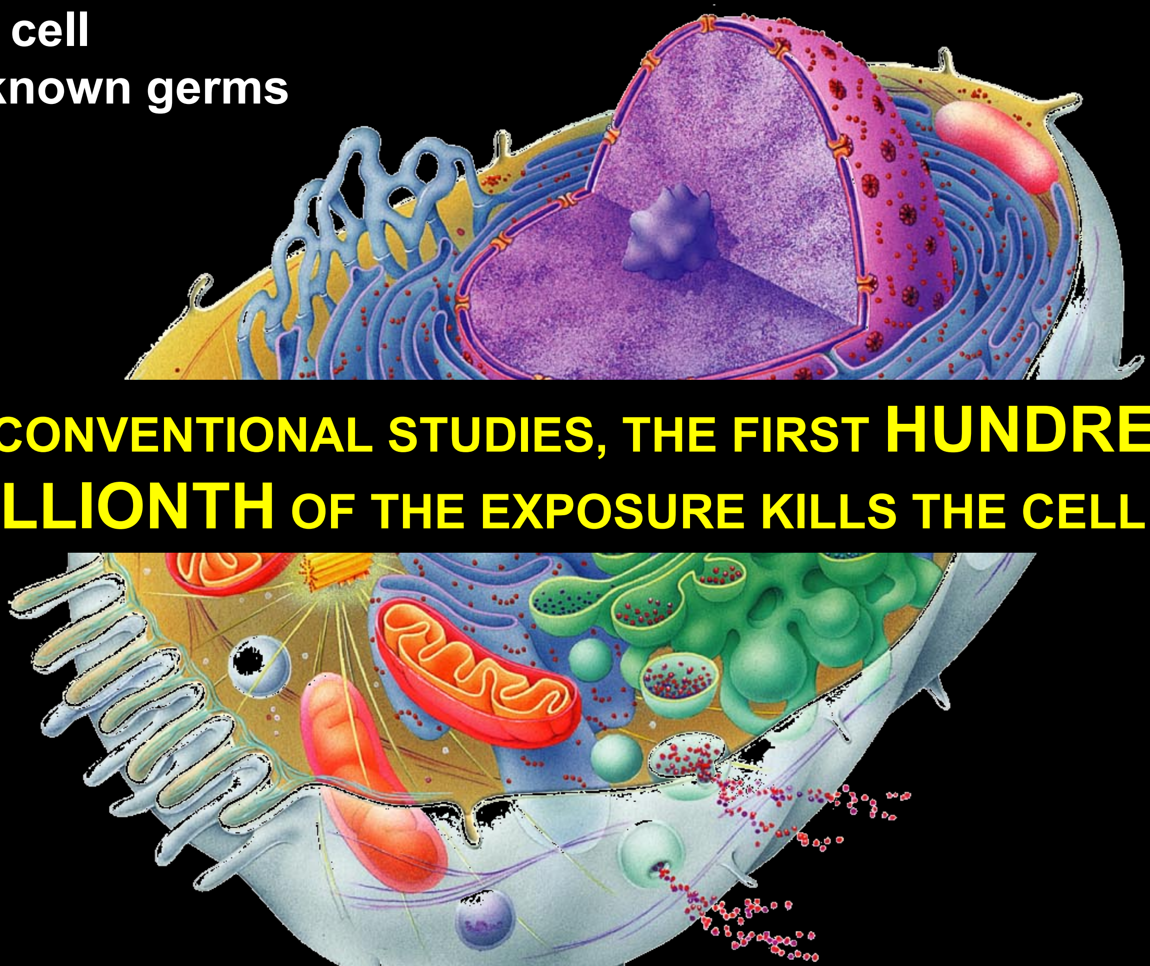
There exists a conspicuous gap of knowledge about the organization of life at mesoscopic levels. Ultra-fast coherent diffractive imaging with X-ray free-electron lasers can probe structures at the relevant length scales and may reach sub-nanometer resolution on micron-sized living cells. Here we show that we can introduce a beam of aerosolised cyanobacteria into the focus of the Linac Coherent Light Source and record diffraction patterns from individual living cells at very low noise levels and at high hit ratios. We obtain two-dimensional projection images directly from the diffraction patterns, and present the results as synthetic X-ray Nomarski images calculated from the complex-valued reconstructions. We further demonstrate that it is possible to record diffraction data to nanometer resolution on live cells with X-ray lasers. Extension to sub-nanometer resolution is within reach, although improvements in pulse parameters and X-ray area detectors will be necessary to unlock this potential.

Gijs van der Schot

DREAMS: IMAGING A LIVING CELL AT HIGH RESOLUTION

THE PROBLEM:

100,000,000 Grey is needed for a cell at 1 nm resolution
20 Grey kills a human
100 Grey kills a cell
25,000 kills all known germs



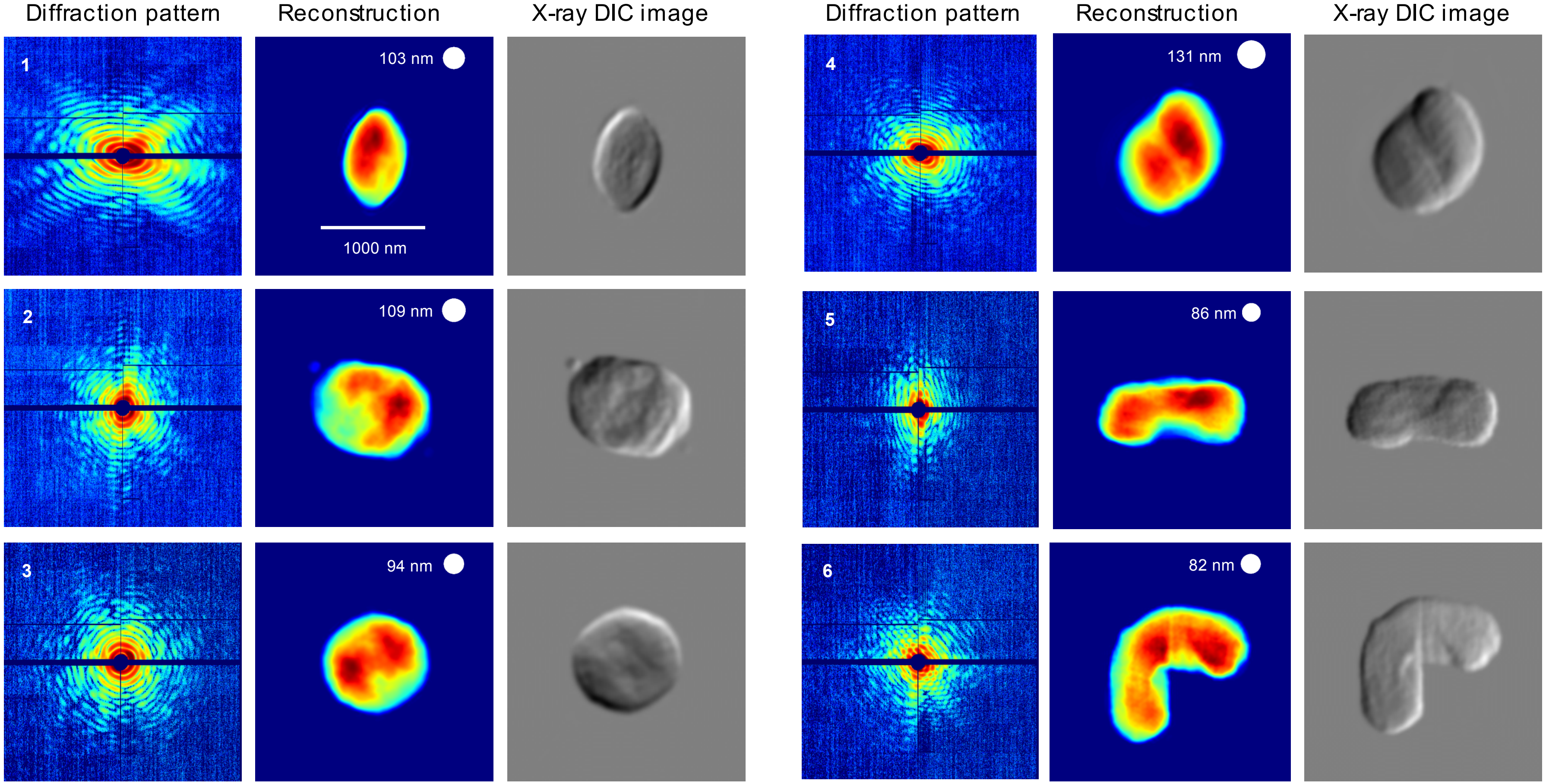
IN CONVENTIONAL STUDIES, THE FIRST HUNDRED MILLIONTH OF THE EXPOSURE KILLS THE CELL

DIFFRACTION BEFORE DESTRUCTION CAN OVERCOME THIS PROBLEM

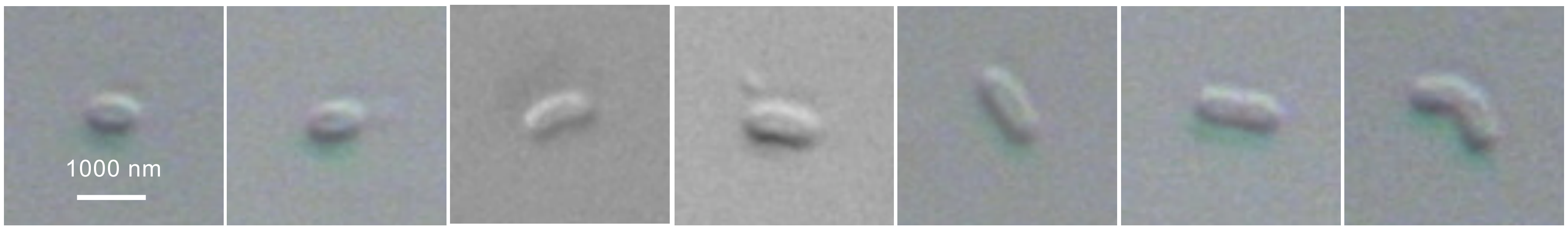
Movie at http://lmb.icm.uu.se/video-single-cells/

Snapshots of *Cyanobium gracile* cells

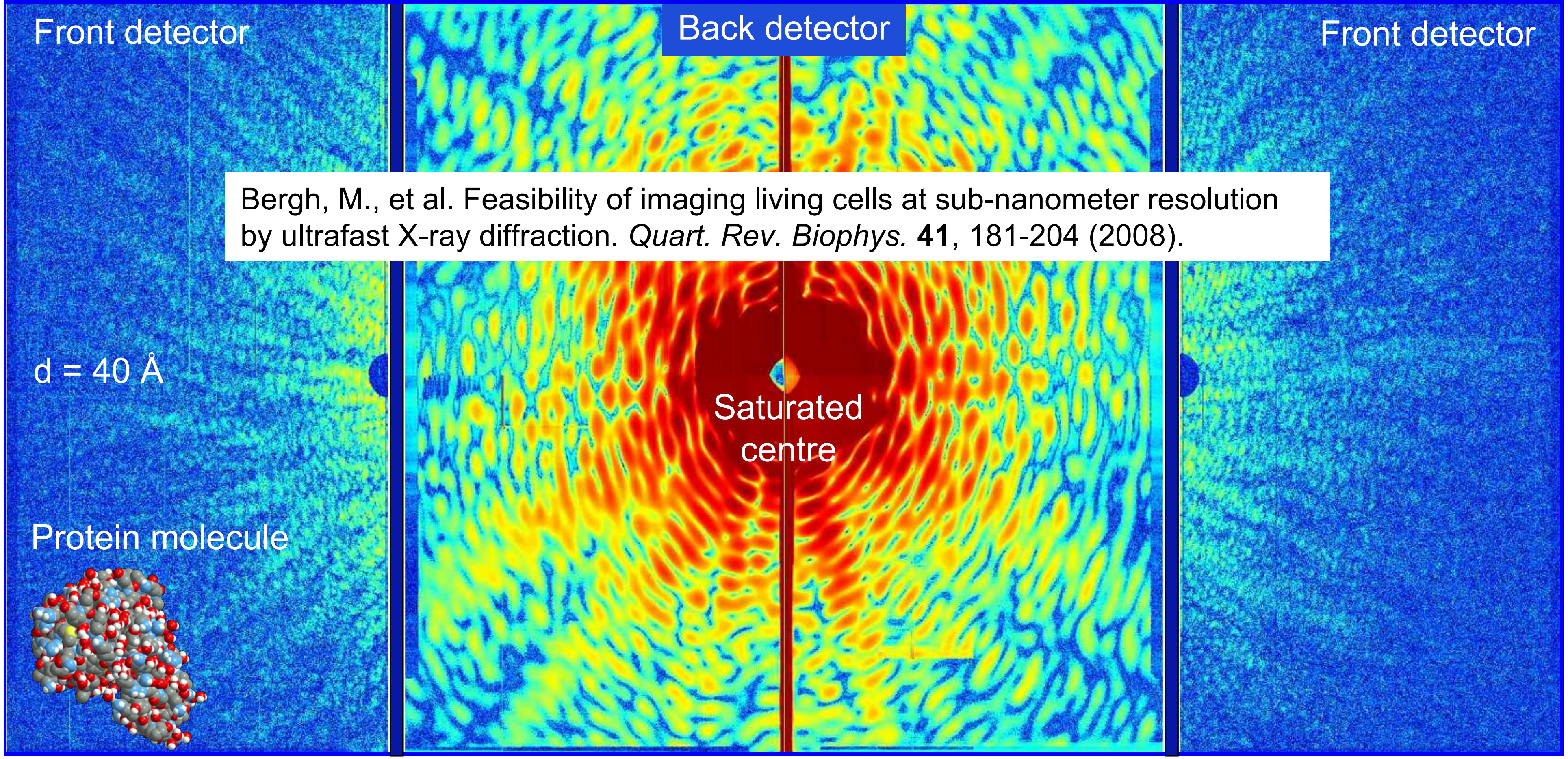
DIFFERENTIAL INTERFERENCE CONTRAST (DIC) IMAGES WITH X-RAYS



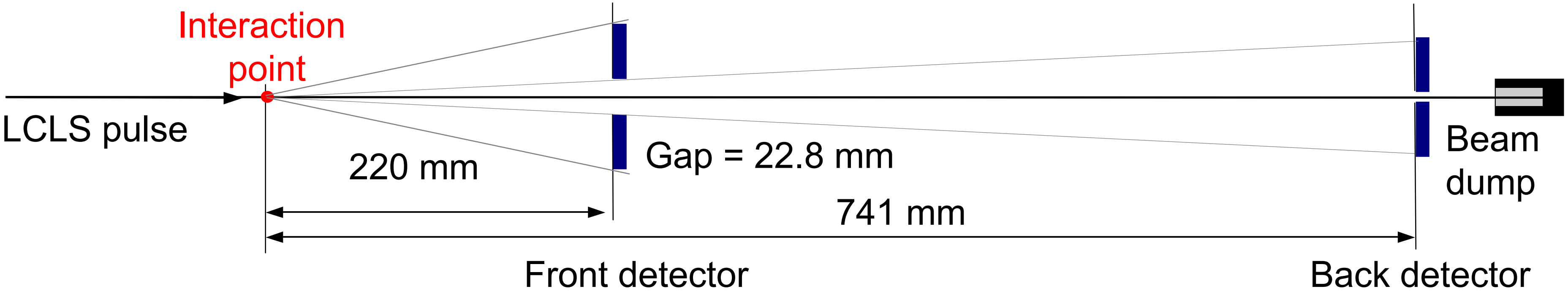
Optical DIC images of similar *Cyanobium gracile* cells:



LIVE CELLS IN MOLECULAR DETAIL?



Bergh, M., et al. Feasibility of imaging living cells at sub-nanometer resolution by ultrafast X-ray diffraction. *Quart. Rev. Biophys.* **41**, 181-204 (2008).

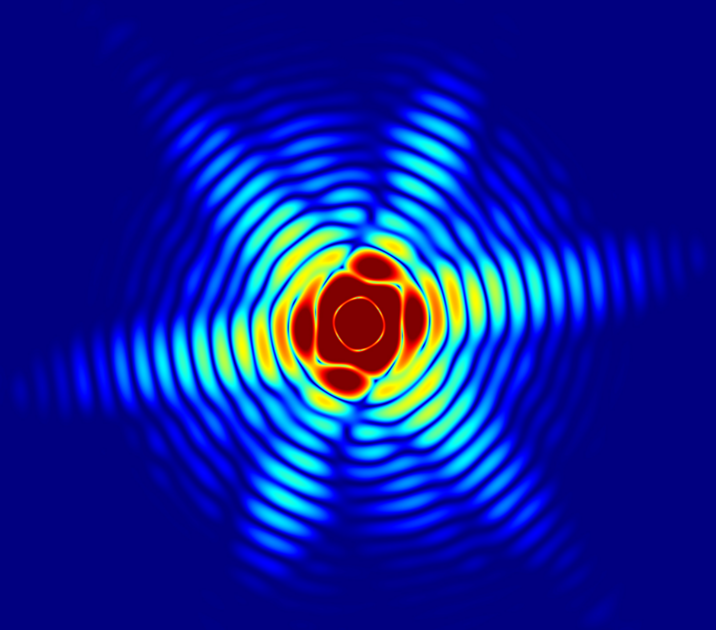



"To understand the whole you must look at the whole"
H. Kacser "On parts and wholes in metabolism" in: Welch GR, Clegg JS (eds) *The organisation of cell metabolism*, Plenum Press, New York, p 327, (1986).
BILLIONS OF SHOTS PER DAY AT XFEL…
BIG DATA and DATA DRIVEN DISCOVERY
Background image: http://biology.usf.edu/cmmb/undergrad/bio

SUMMARY - DIFFRACTION BEFORE DESTRUCTION

Outrunning damage is now routinely done in practically all applications of X-ray FELs: (nano)crystallography, spectroscopy, single particle imaging. Practically every experiment is using our effect. The CXI station is entirely based on this principle.

AMO experiments confirm our predictions and guide critical developments.



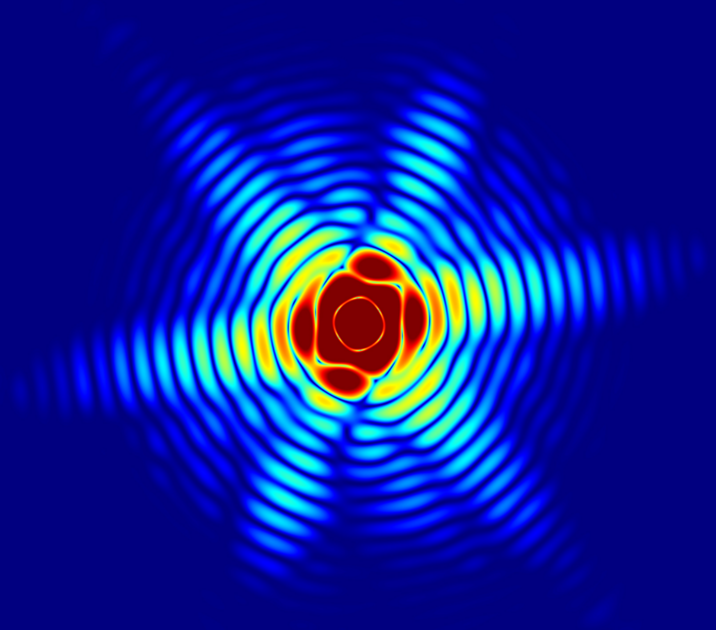
SUMMARY - DIFFRACTION BEFORE DESTRUCTION

Outrunning damage is now routinely done in practically all applications of X-ray FELs: (nano)crystallography, spectroscopy, single particle imaging. Practically every experiment is using our effect. The CXI station is entirely based on this principle.

AMO experiments confirm our predictions and guide critical developments.

No physical limit has been reached so far.

MORE INTENSE PULSES PROMISE AMAZING NEW SCIENCE



$>10^{24}$ W/cm^2
BOILING of VACUUM, etc.

ACKNOWLEGEMENTS

This community did not exist 10 years ago, neither did requisite X-ray lasers