Projector color

Bragg Centenial Adelaide. Dec 6   30 mins 2012

Six Surprises For Willie

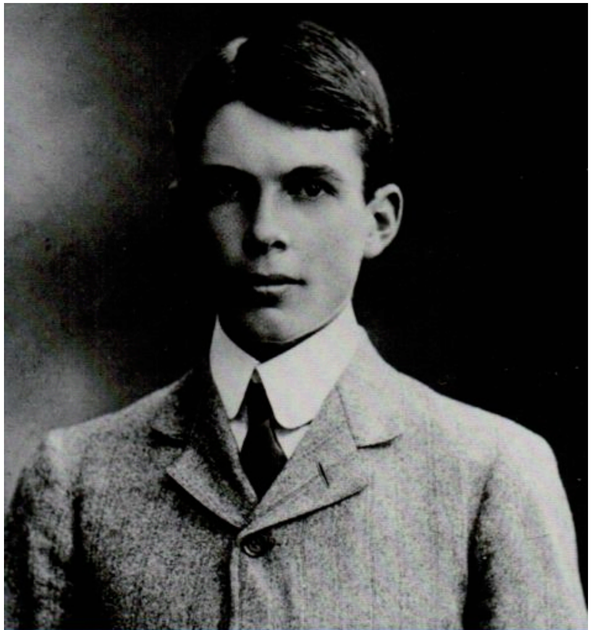
John C H Spence*

ASU Physics/LBNL

*and many others, especially

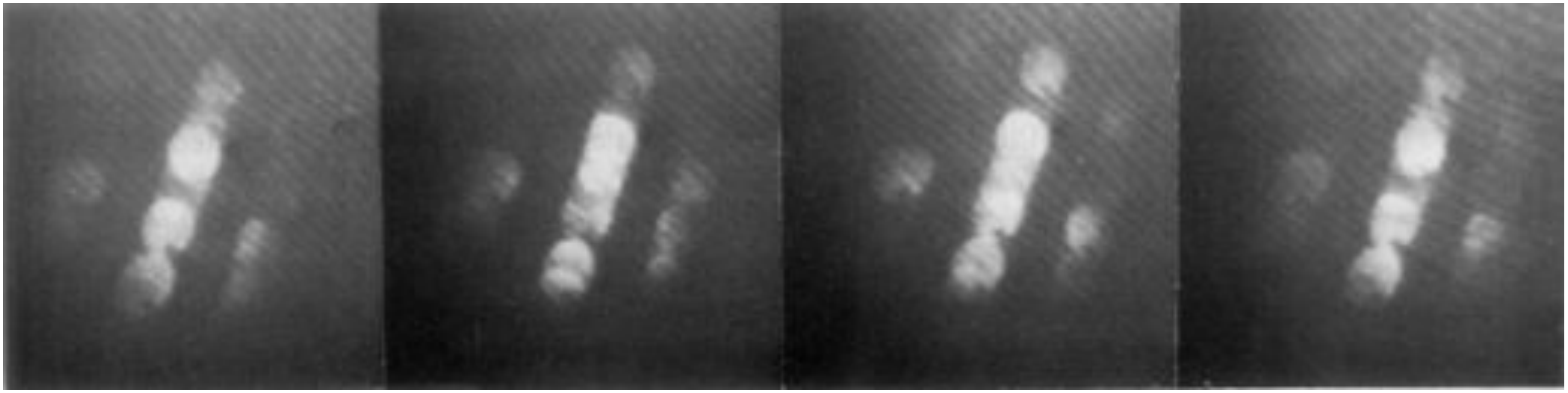
Henry Chapman, David Shapiro, Uwe Weierstall, B. Doak, A. Barty, Ilme Schlichting, Janos Hajdu
Zuo, James Holton, Garth Williams, Mike Bogan, Petra Fromme, Joachim Ullrich, S. Marchesini….

William Lawrence Bragg.
1890-1971

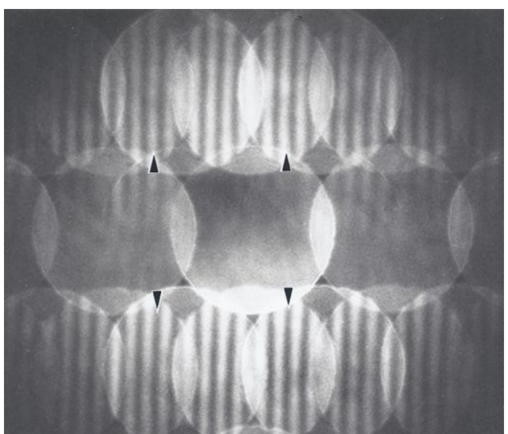


6 surprises for Willie.

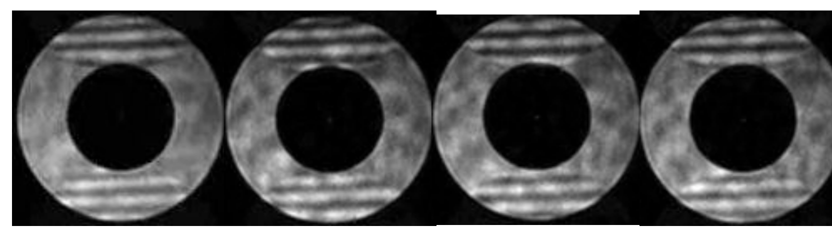
1. Bragg diffraction is possible with a beam smaller than a unit cell !



S1. Transmission electron microdiffraction patterns from a mineral using STEM to give a 0.5nm probe, smaller than the unit cell. The pattern is seen to repeat as the probe is scanned across a single unit cell, from left to right. (From Cowley, 1981).



Coherent CBED pattern from FeS_2 with beam along [100] (d_{001} = 0.54 nm, 200 kV) (Tanaka et al 2002). Ptychography.



1 kV X-rays on 80nm period grating 30nm diam beam. (ALS 2011, Marchesini. See also Chapman 1999).

2. Bragg's law is an approximation !

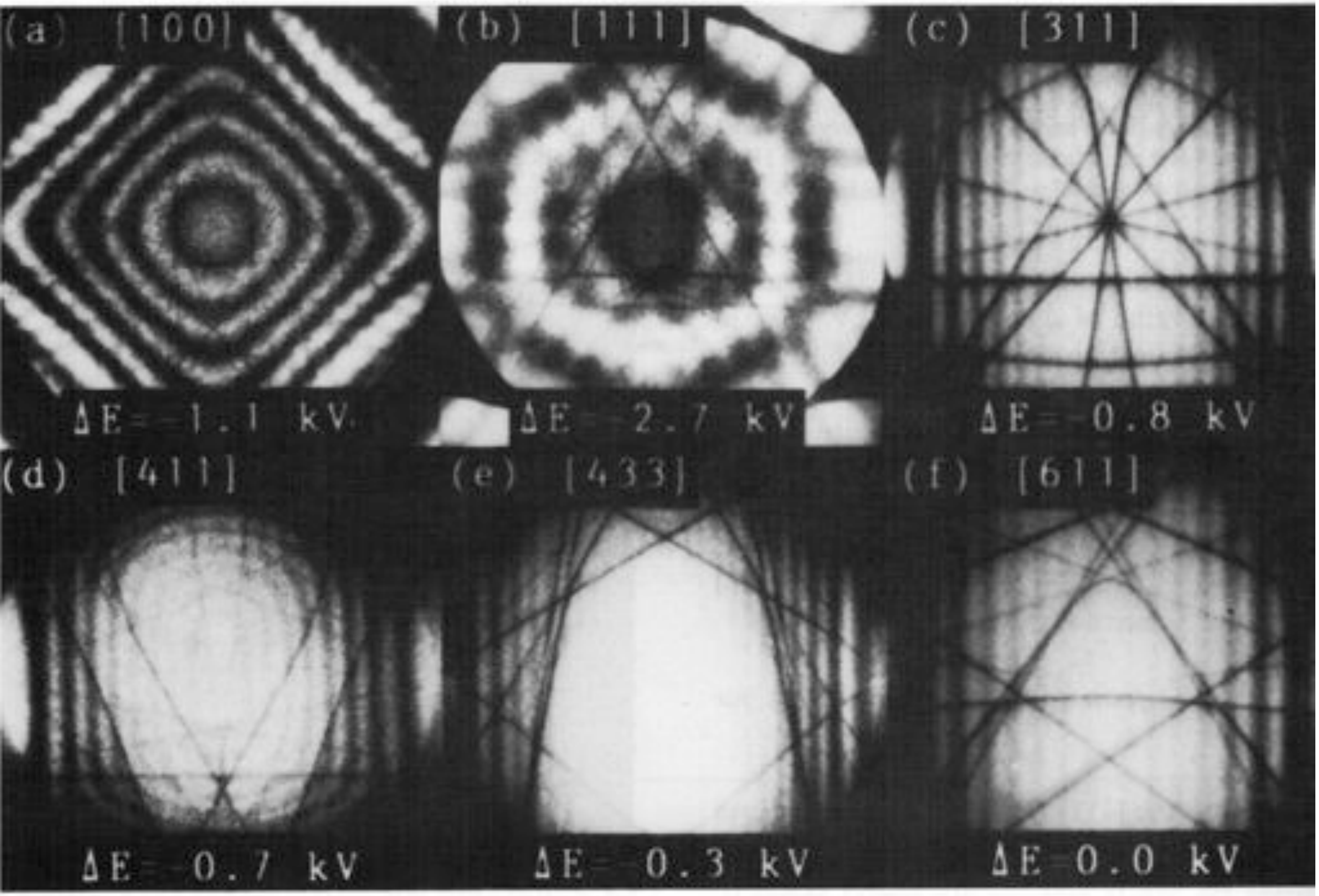


Fig. 5. Various zone axis HOLZ patterns and the estimated values of ΔE.

Okuyama et al 1989.

The position of the Bragg maximum depends on the strength of interaction for both XRD and TED.

See CJH Mss for another example (critical voltage)

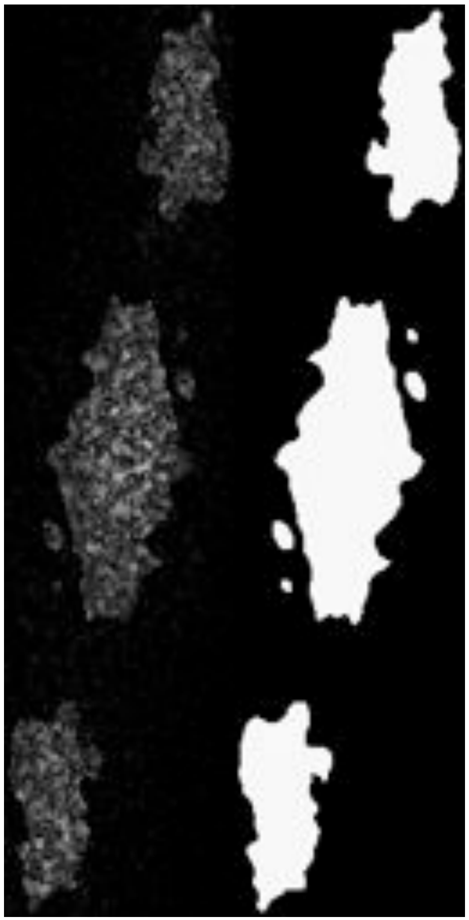
"The persistent rediscovery of the failure of Bragg's law" due to dynamical shifts.

Use of Braggs law to derive electron wavelength from these six different orientations of the same Si xtal gives six different beam energies (differing by ΔE shown) ! (V_c at any V !)

3. The phase problem can be solved for non-periodic samples - crystallography without crystals !

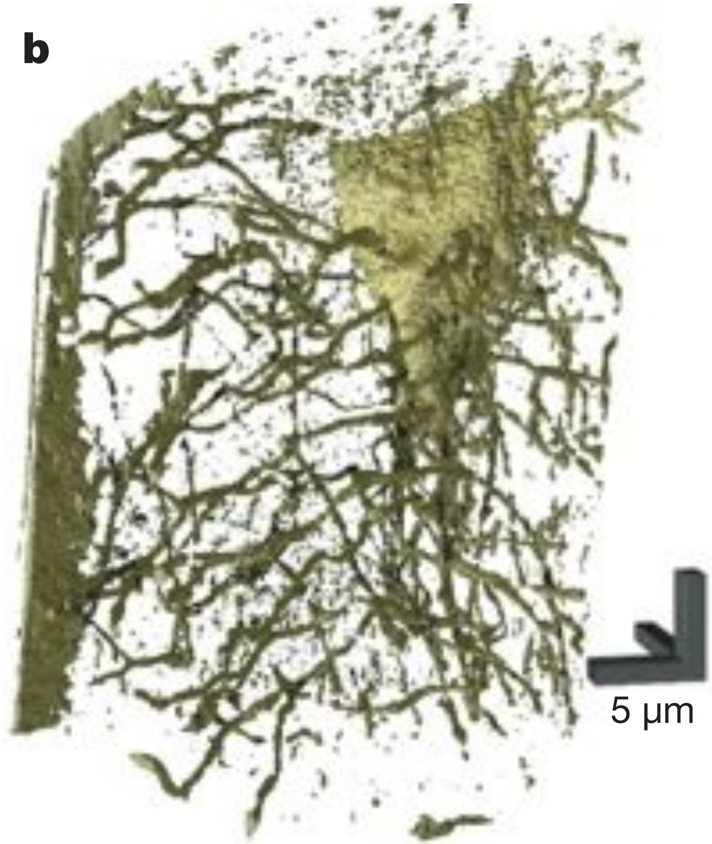
Object estimate

Support estimate



50nm Gold Balls

λ=2.1nm



65 nm resolution
Mouse femur Dierolf
Nature 2010. Rodenburg.

Marchesini,He,Howells,Chapman,Weierstall,Spence.
Phys Rev B. 2003 .

3. Atomic-resolution image reconstructed from microdiffraction pattern.
Computational phasing. First atomic-resolution diffractive image reconstruction.

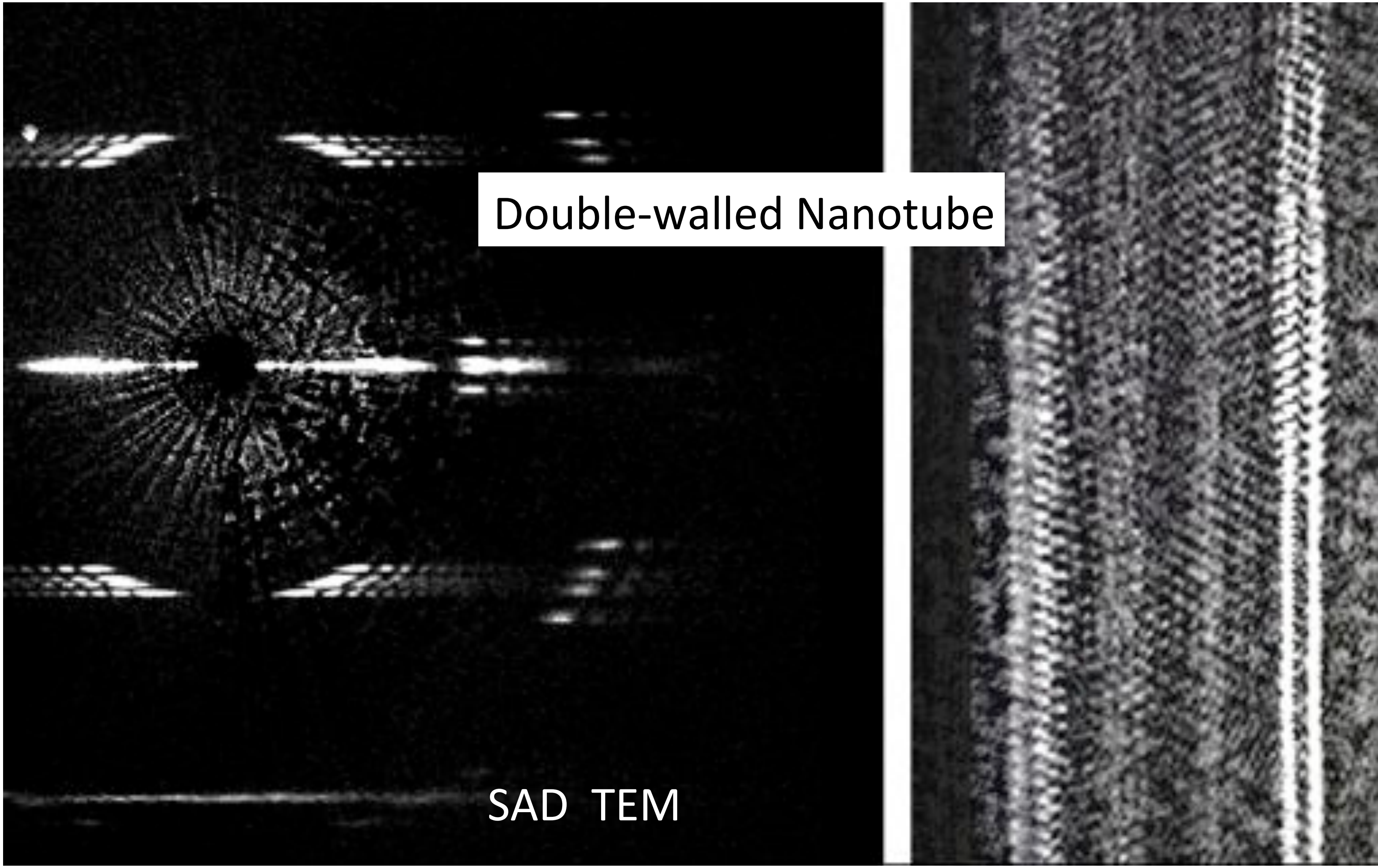
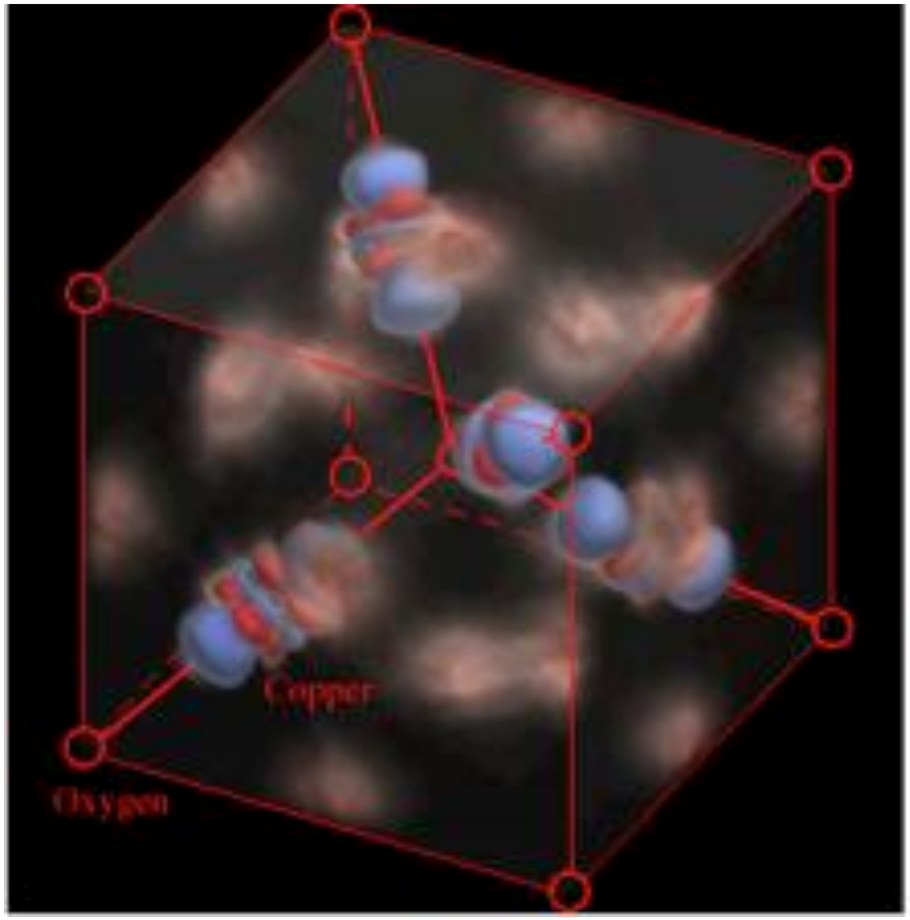
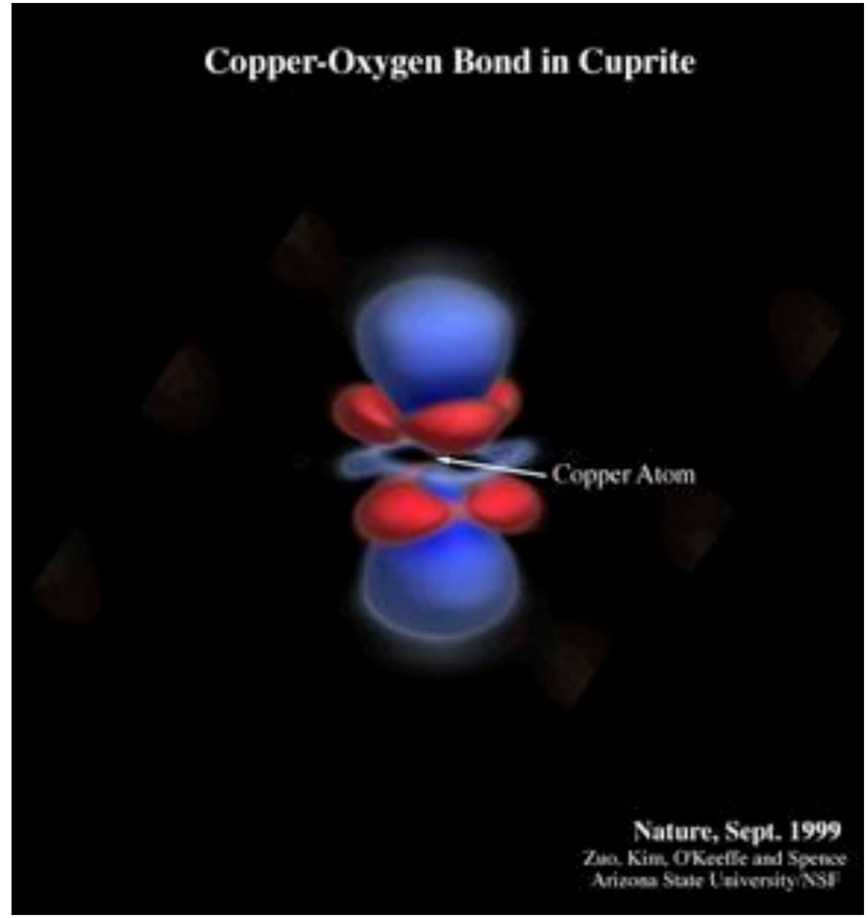


Image reconstructed from electron-diffraction pattern by HiO
J.M.Zuo et al Science 300, 1420 (2003).

4. Low-angle electron diffraction sees bonds, avoids extinction.





Surface of constant charge density difference in Cu_2O.
Total measured charge density minus calculated spherical ions on lattice sites. If cuprite were purely ionic (Cu^+O^{--}) this picture would be blank. Blue is less charge than MCDF ions (making "holes" in the shape of d_z^2 orbitals), red is more. Coppers form FCC lattice. Image shows deviations from textbook ionic bonding, e.g dz^2 hole, covalent metal-metal bonding. 0.22 electrons of charge move from the hole into this bond.
Copper-copper bonds seen experimentally are not predicted by FLAPW/LDA theory.
Results are accurate enough to distinguish between many-e approxs (LDA, GGA).

*Zuo,Kim, O' Keefe, Spence, Nature, 401, p.49 (1999)

Cu^+ $[Ar]3d^{10}$ 4s O^{--} $1s^2$ $2s^2$ $2p^6$ (2 unpaired)

5.Diffraction of a beam of viruses ?

Matter-wave interferometery.

Zeillinger et al diffract a beam of buckyballs Nature 1999.

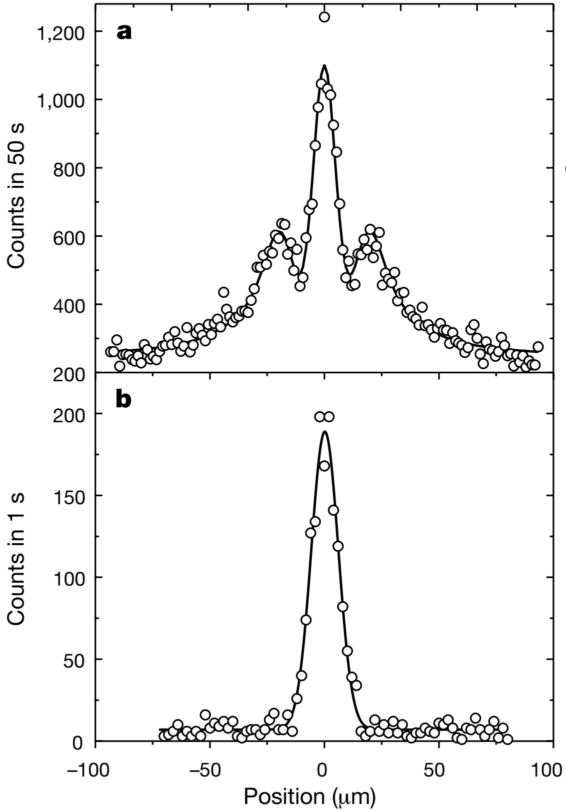


Figure 2 Interference pattern produced by C_{60} molecules. **a**, Experimental recording (open circles) and fit using Kirchhoff diffraction theory (continuous line). The expected zeroth and first-order maxima can be clearly seen. Details of the theory are discussed in the text. **b**, The molecular beam profile without the grating in the path of the molecules.

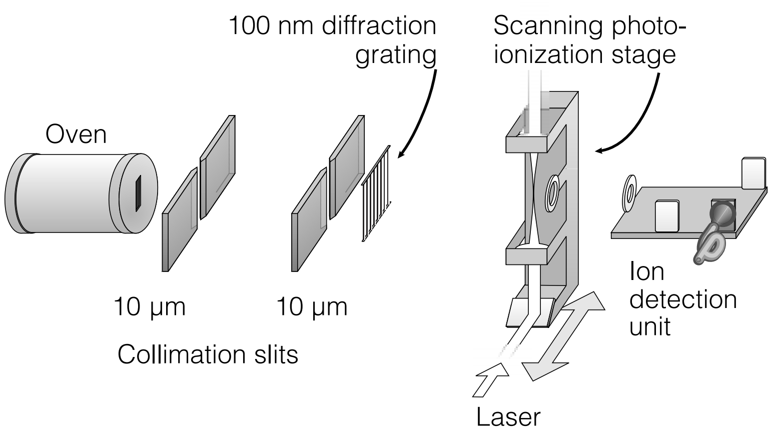


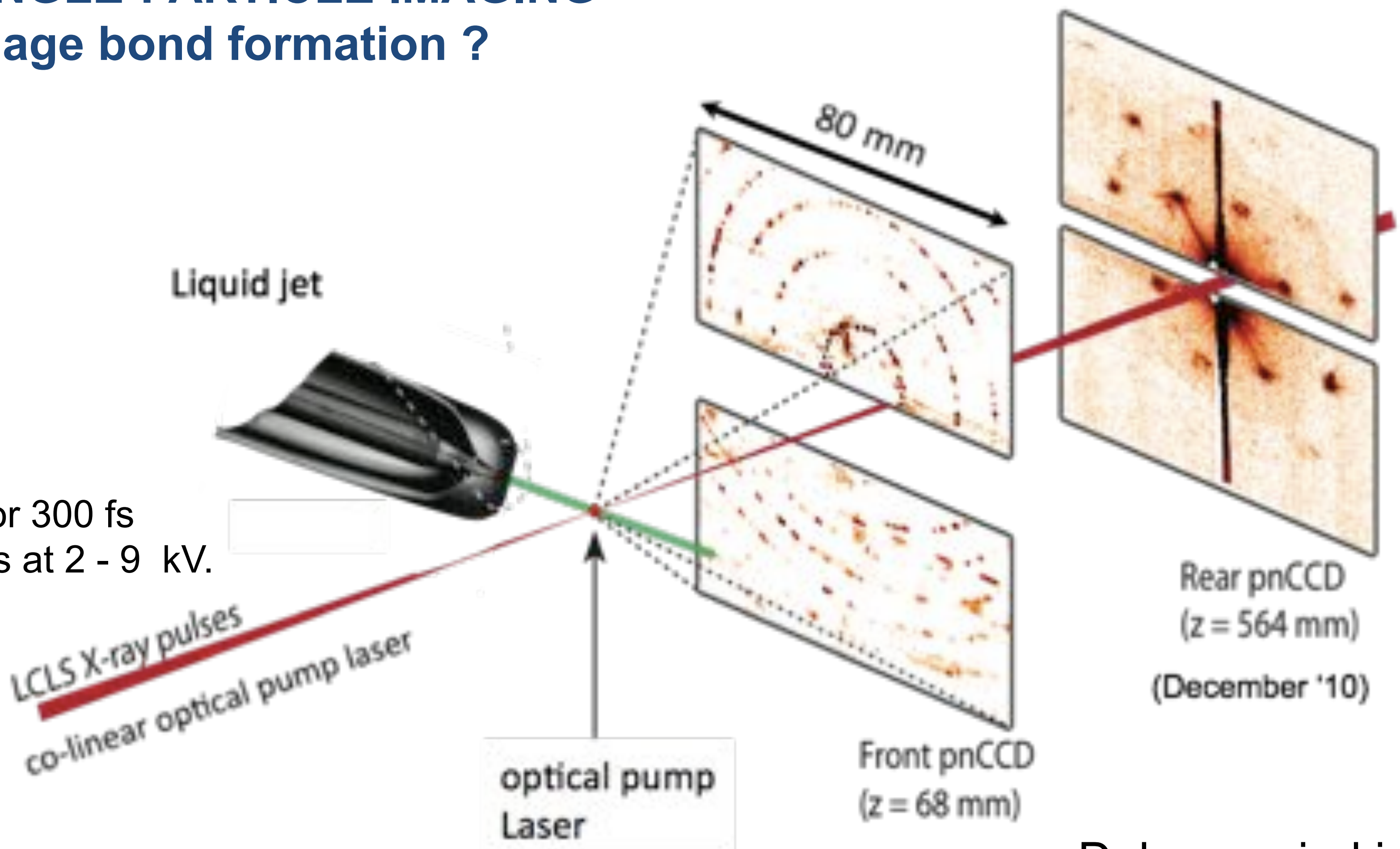
Figure 1 Diagram of the experimental set-up (not to scale). Hot, neutral C_{60} molecules leave the oven through a nozzle of 0.33 mm × 1.3 mm × 0.25 mm (width × height × depth), pass through two collimating slits of 0.01 mm × 5 mm (width × height) separated by 1.04 m, traverse a SiN_x grating (period 100 nm) 0.1 m after the second slit, and are detected via thermal ionization by a laser 1.25 m behind the grating. The ions are then accelerated and directed towards a conversion electrode. The ejected electrons are subsequently counted by a Channeltron electron multiplier. The laser focus can be reproducibly scanned transversely to the beam with 1-μm resolution.

6. Fast pulses avoid damage

In 2009, the LCLS near Stanford became the world's first hard X-ray laser. It produces < 10 kV X-rays in 70 fs pulses, ~ 1E12 photons/pulse.

AIMS:

- OUTRUN DAMAGE ? Currently PX limited by damage and xtal quality.
- SOLVE SUBMICRON ("invisible") XTALS ?
- REAL-TIME SNAPSHOT CHEMISTRY AT ROOM TEMPERATURE ?
- PUMP-PROBE WITH VERY HIGH TIME RESOLUTION.
- SOLVE PHASE PROBLEM ?
- SINGLE-PARTICLE IMAGING
- Image bond formation ?



5, 70 or 300 fs
pulses at 2 - 9 kV.

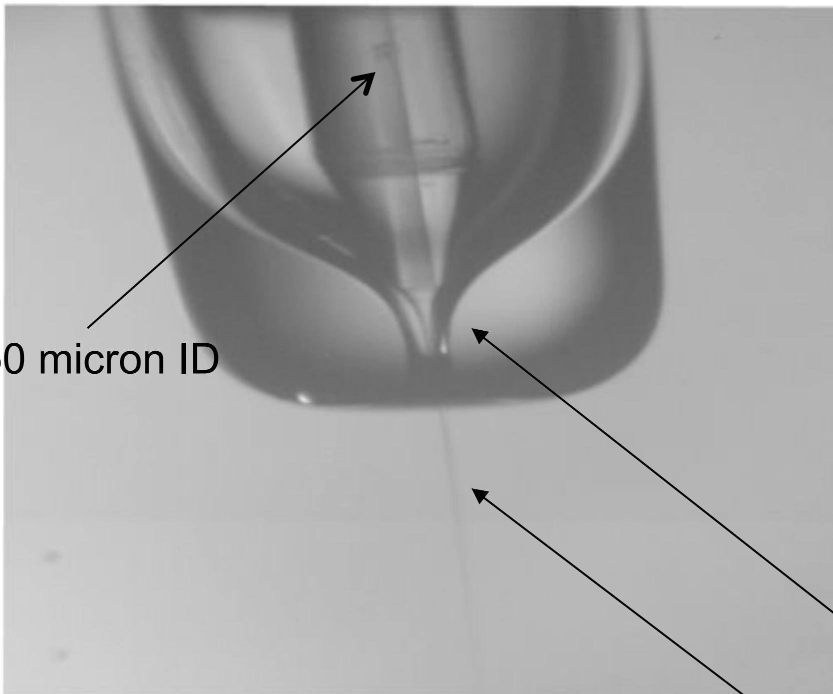
Henry

Debye period is ~100fs

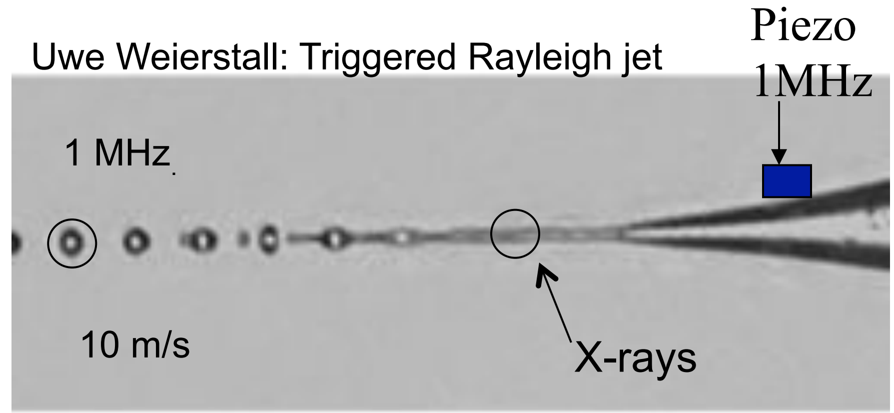
Hydrated particle delivery

Our Liquid jet uses gas focusing to make a micron jet from bigger nozzle.

Gas focusing prevents clogging - get *submicron droplets* from a 15 micron nozzle
Absence of fields (electrospray) prevents charge artifacts on proteins.
Liquid feed ! No goniometer !



Uwe Weierstall: Triggered Rayleigh jet



LCLS Rep Rate ~100 Hz
Droplet frequency 1 MHz. v = 10 m/s
Droplet diam 1 micron
LCLS beam diam 1 micron.
Flow rate F= 10 microL per minute. v = F/A

Liquid cone enhances flow alignment
(from HPLC syringe pump)

Av = const
Gas accelerates liquid
Making cone as shown.
Higher pressure for smaller droplets

~1 MHz single-file micron-sized droplet beam. Big nozzle makes Small droplets

M. Frank, Mike Bogan, have gas-phase jet

Allows study of *irreversible* processes in *fully hydrated* biomolecules

Agilent, Medical Rayleigh 1890

Spence, Doak, Phys Rev Letts. 92, 198102 (2004). Weierstall, Doak, Spence Rev Sci Instr. (2012)

We can sum the Bragg spots as they arrive (virtual powder pattern)

Lysozyme, 1.8 kV.
6.9 Ang wavelength

With 6 x10^6 patterns, human examination of the data is impossible !

I ~ N^6 = 1E6 for N=10

Each 2kV shot is 70 fs, 2 mJ
1E12 photons per shot
900 J cm^{-2} fluence
1E16 W/cm^2
7 micron diam beam
Dose 700 MGy per pulse

Ilme Schlichting, Bob Shoeman et al

Milestones in bioXFEL over past 3 years

1. Can "diffract-and-destroy" (sFX) give atomic resolution ?

Can diffract-and-destroy give atomic resolution ?

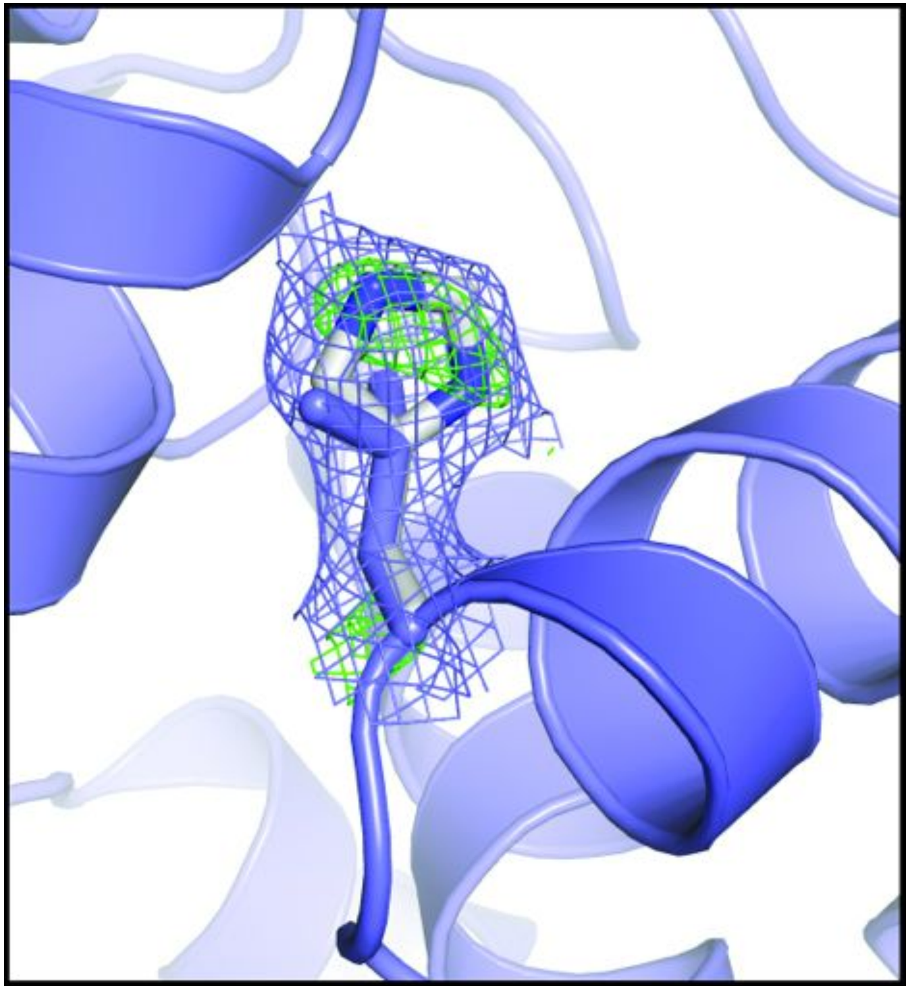


Fig. S3

Quality of the molecular replacement map. The region around Leu15 in the turkey egg white lysozyme model (blue carbon atoms) is shown. In the actual hen egg white lysozyme structure obtained of the presented SFX data, this is a histidine (white carbon atoms). Both the $2mF_{obs}$-DF_{calc} (blue, 1.5 σ) and Fobs-DFcalc (green, +/- 3 σ) maps *(10)* clearly show the difference between the search model and the actual structure obtained from the SFX data.

XFEL data can distinguish Turkey from Hen.

Hen Lysozyme, 1 x 1 x 3 microns xtallites at 9.4 kV with 40 fs pulses, Dose 33 Mgy. CrystFEL . **RESOLUTION 1.9 ANGSTROMS**.

Serial Femtosecond Crystallography
SFX

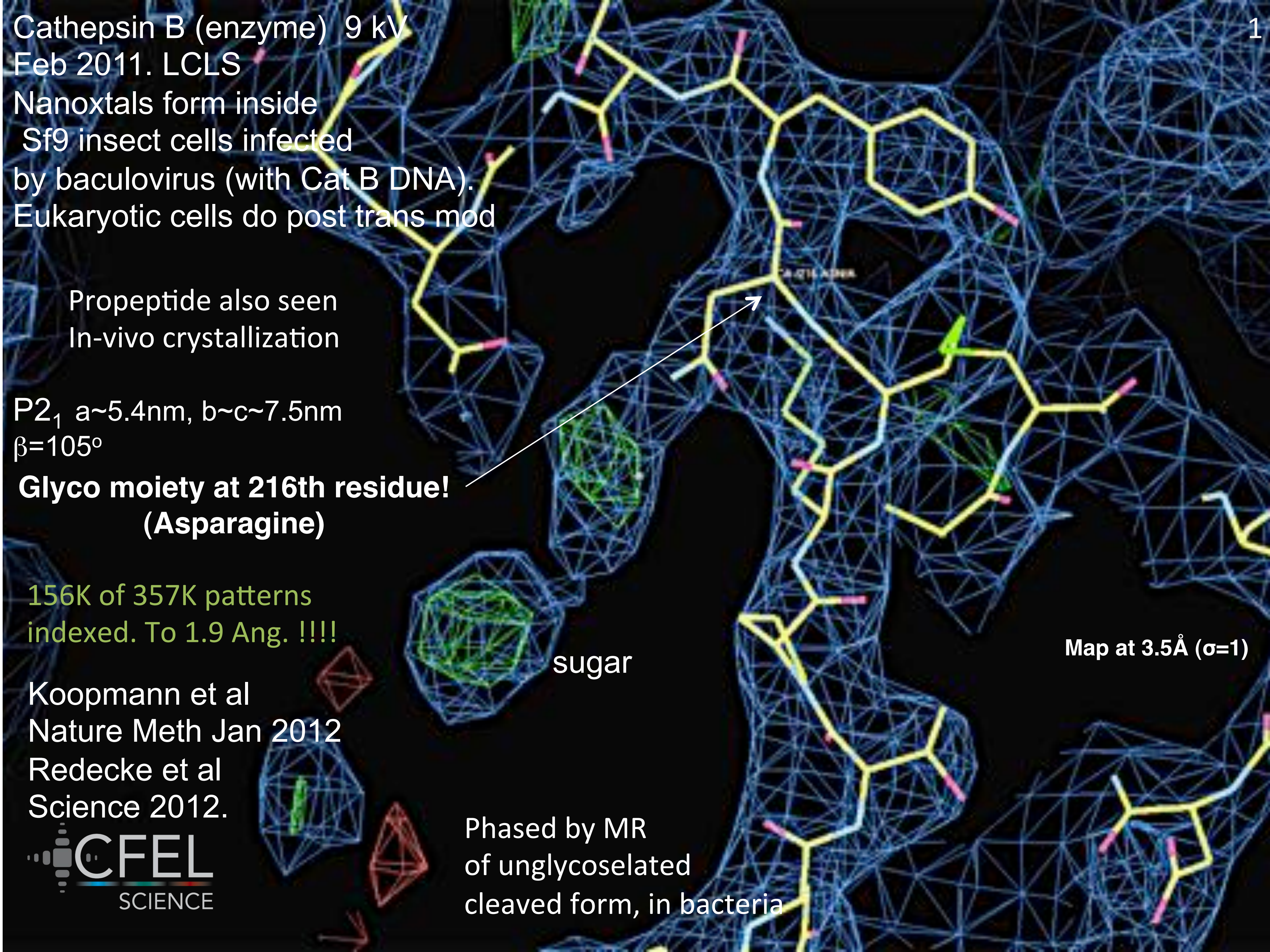
Weighted R-factor between LCLS and SLS data is about 10%

D.C.Phillips, L.N. Johnson, 1965 first enzyme solved.

S. Boutet, G. Williams………I. Schlichting. Science 2012

Milestones

2. Can "diffract-and-destroy" (sFX) give New Biology ?



Cathepsin B (enzyme) 9 kV
Feb 2011. LCLS
Nanoxtals form inside
Sf9 insect cells infected
by baculovirus (with Cat B DNA).
Eukaryotic cells do post trans mod

Propeptide also seen
In-vivo crystallization

$P2_1$ a~5.4nm, b~c~7.5nm
β=105°

Glyco moiety at 216th residue! (Asparagine)

156K of 357K patterns indexed. To 1.9 Ang. !!!!

Koopmann et al
Nature Meth Jan 2012
Redecke et al
Science 2012.

CFEL SCIENCE

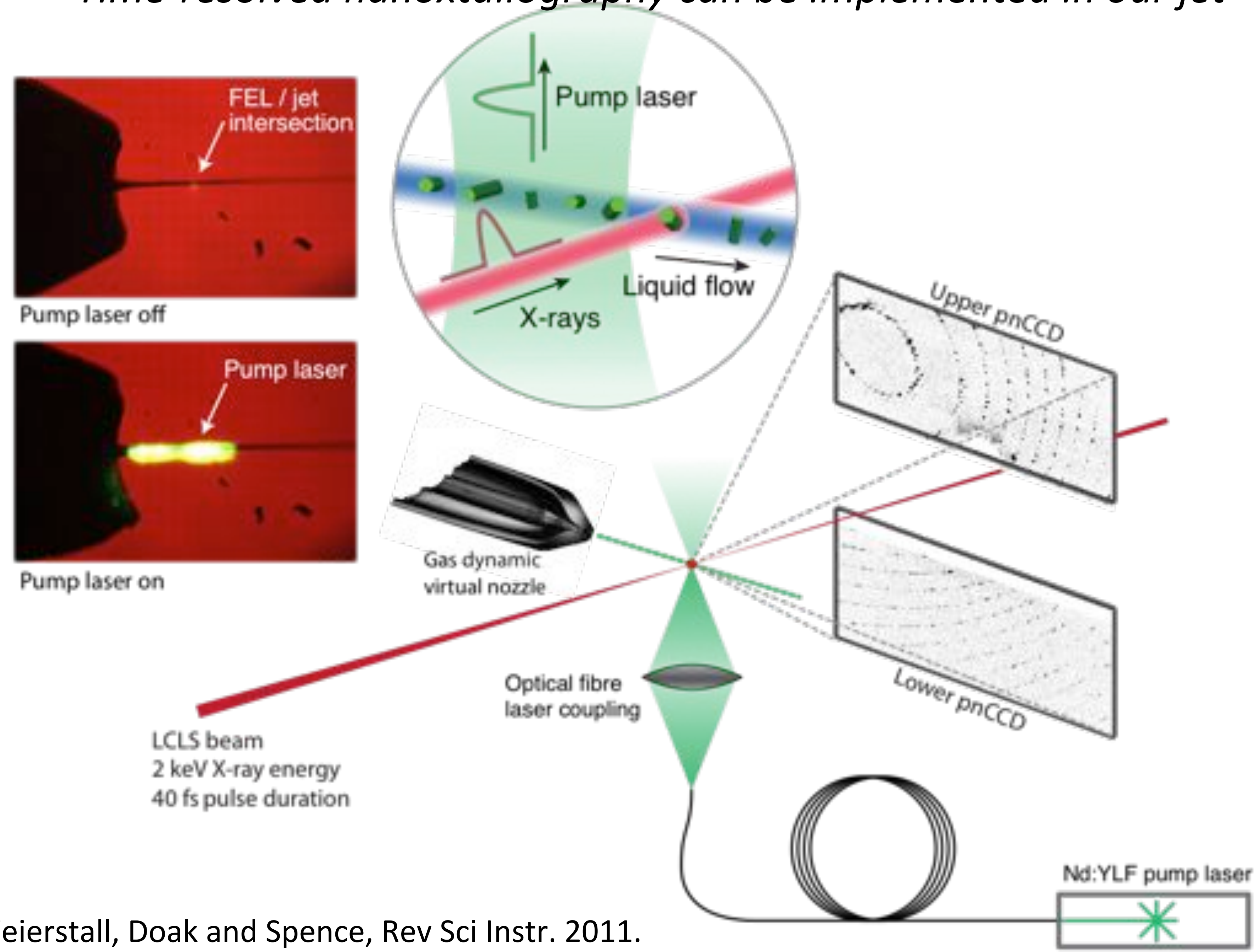
Phased by MR
of unglycoselated
cleaved form, in bacteria

Milestones

3. Time-resolved nanocrystallography : pump-probe in a jet.

Earlier work… Moffat, Anfinrud, Neutze…..

Time-resolved nanoxtallography can be implemented in our jet



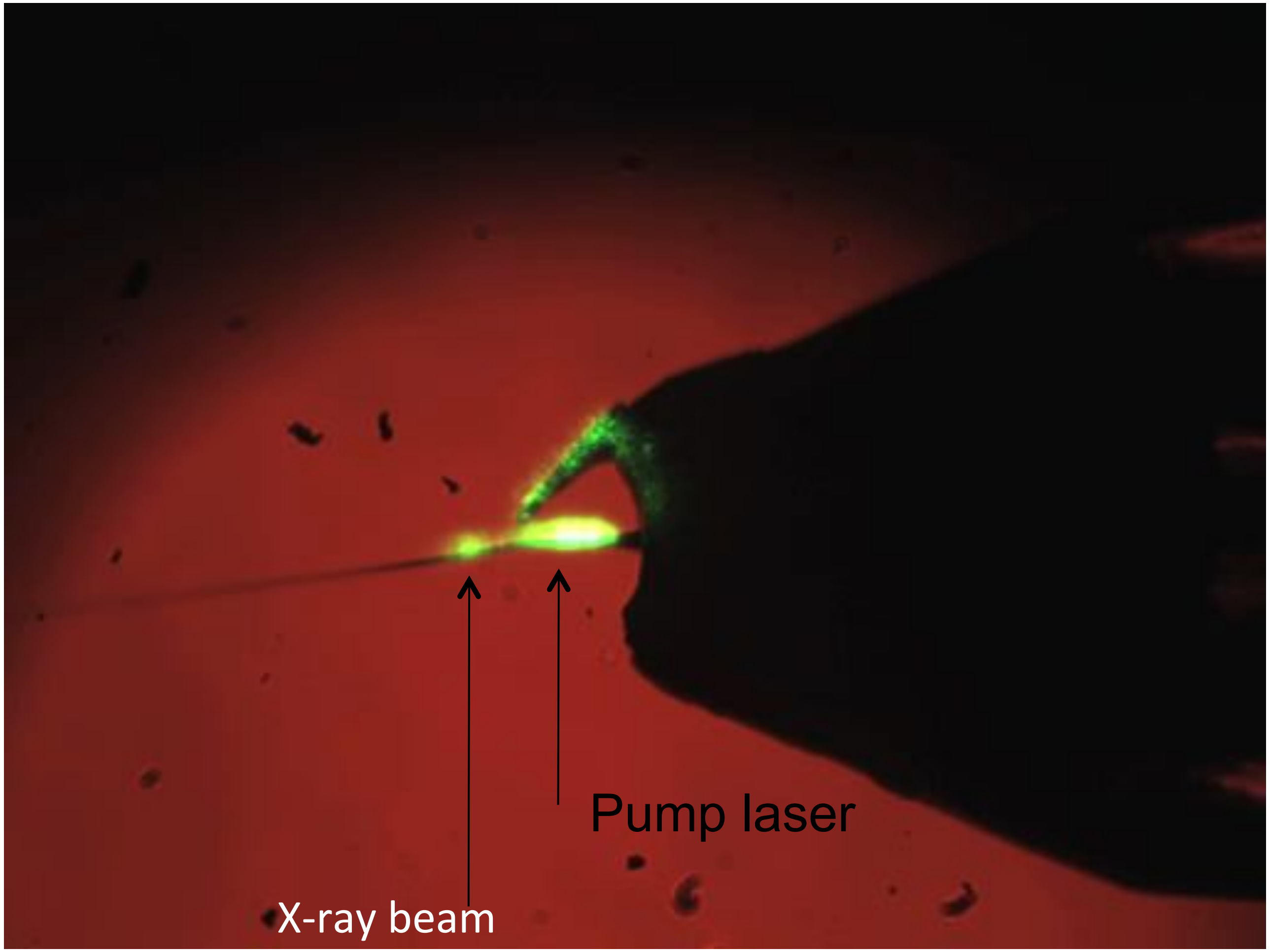
Weierstall, Doak and Spence, Rev Sci Instr. 2011.

Pump-probe experiments are possible with the liquid jet. PSI-ferre.

Pump laser and XFEL on jet - exploding nanoxtals

Like sunlight on a leaf….snapshots of the excited state density

Movie



pump laser: 532nm, 7ns pulse, 10 microjoules, focused to 100 micron spot, fiber coupled,

Time delay between 70 fs X-ray pulse and laser 0 - 10μs

7 micron beam
2 micron xtals
4 micron jet

To observe undocking of ferredoxin from PSI, excite nanoxtal 10 microseconds before XRD snapshot
Travelling at 10 m/s, nanoxtals go 100 microns, less than width of 400nm doubled Jedai fs beam
Flow rate 10 microliters/min.

Time-resolved pump-probe XRD of ferredoxin undocking from PSI

Electron transfer causes undocking. Ferre is responsible for entire redox regulation in plants. PSI-ferre solved by Fromme at 3.8 A -3KCD

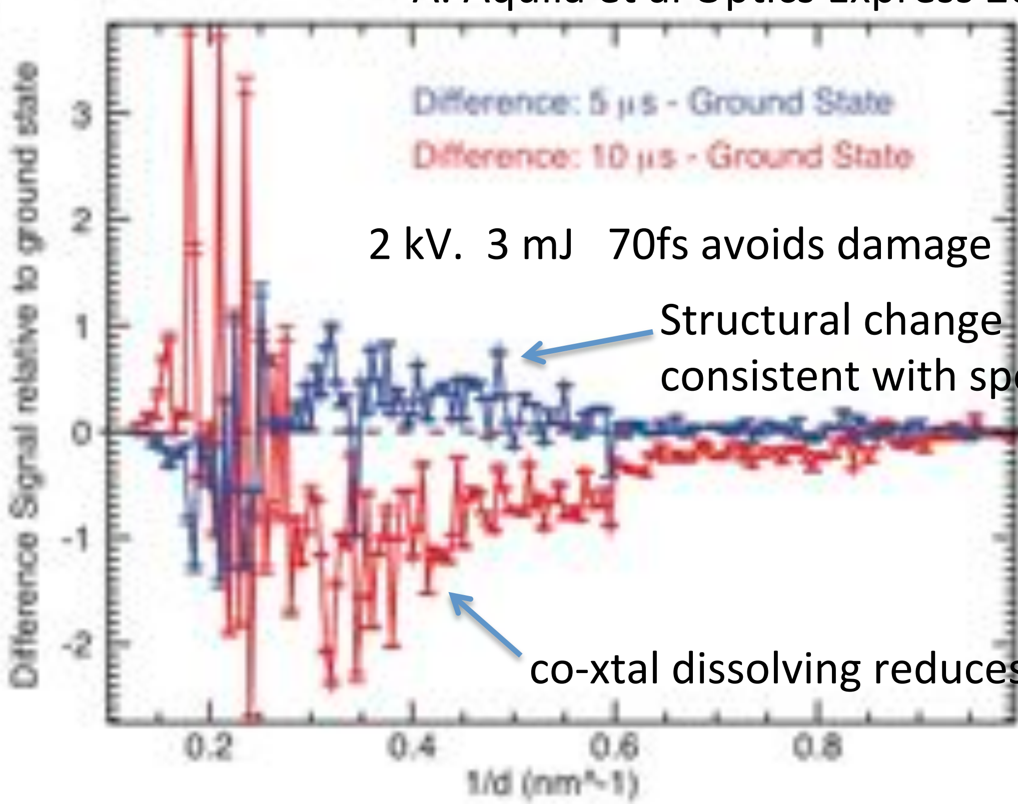
Any LCLS damage effect is the same for all.

Takes CO_2 to sugar

Irreversible changes

A. Aquila et al Optics Express 2011

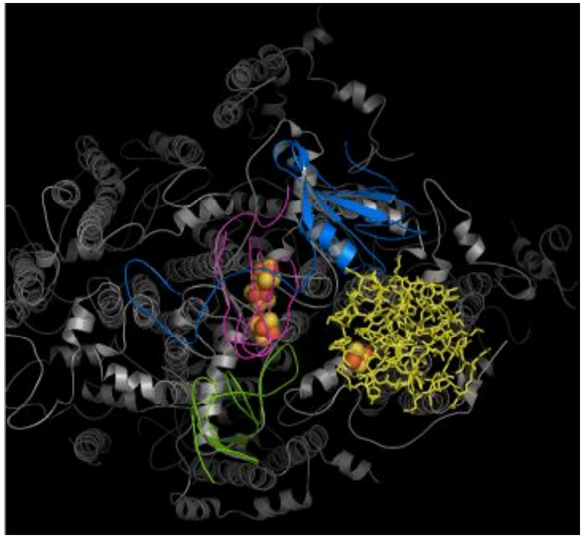
c)

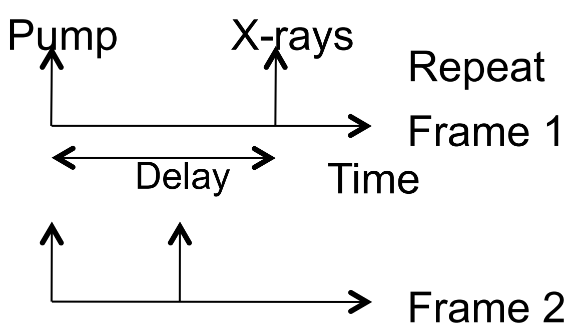


2 kV. 3 mJ 70fs avoids damage

Structural change consistent with spectros.

co-xtal dissolving reduces Braggs





Scaled to minimize RMS difference between virtual powders

219,960 frames at 5 microsec of which 7% indexable

Snapshot structure factors measured for 3 delays between pump laser and 70 fs X-ray pulse. Pump laser causes electron transfer, which causes undocking. Then entire xtal falls apart (but not the mol). Small change in lattice constants consistent with 3deg temp change, up to 10 microsecs, indicating no serious damage.

Milestones

4. Single Particle Imaging.

SINGLE-PARTICLE IMAGING OF A VIRUS AT THE LCLS X-RAY LASER

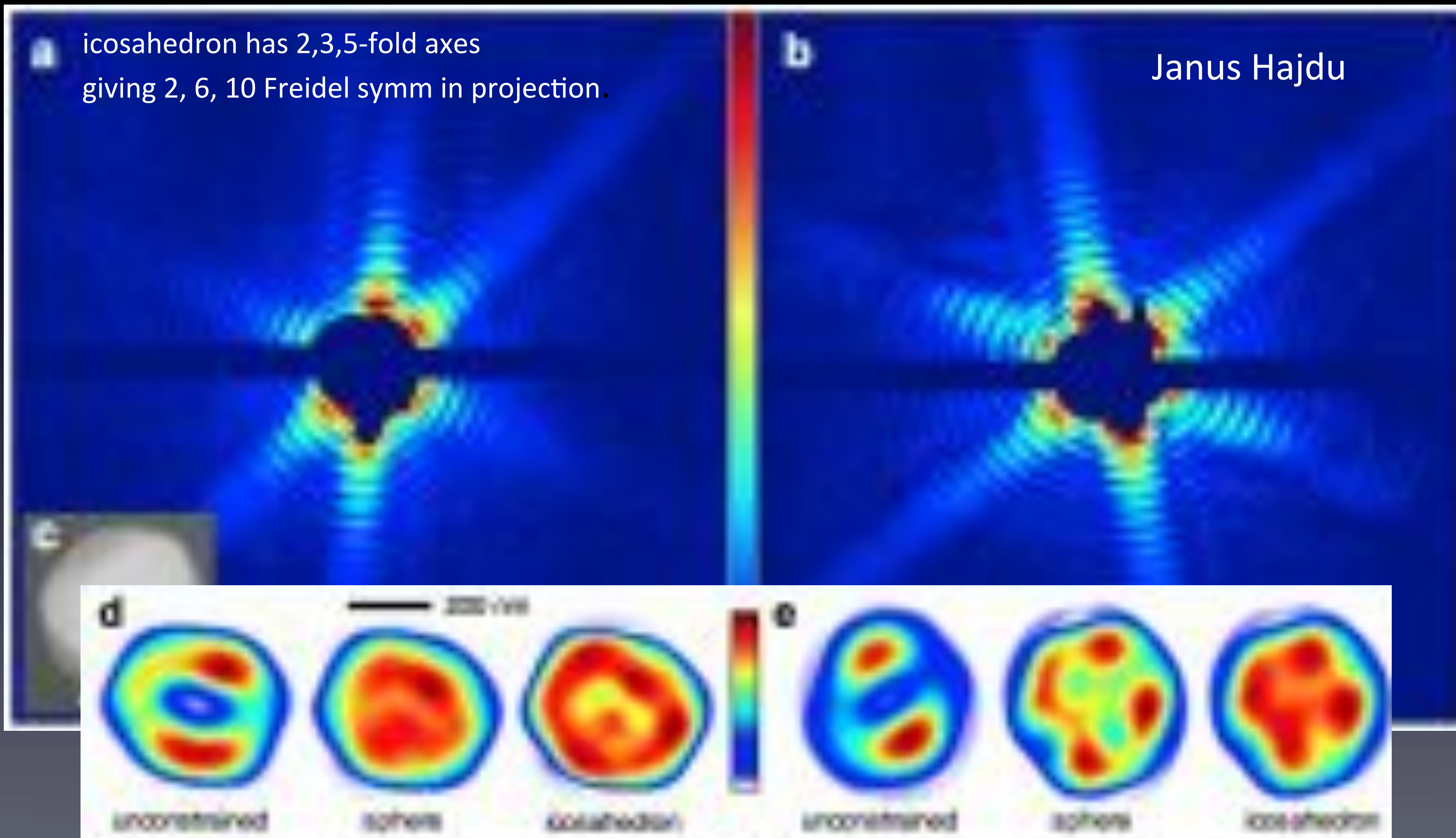
Mimivirus diffraction from LCLS reconstructs in 2D

"A crystal is a protein graveyard"

Get 1E6 photons/pulse from 1 virus

<1 ph/pulse at 1nm resolution

Reconstruction from single 40fs pulse, 1E12 ph, 1.8 kV X-rays on one virus.



Seibert *et al.* Nature **470** 78–81 (2011)

Resolution 30 nm ~ 300 (size)/10 (fringes)

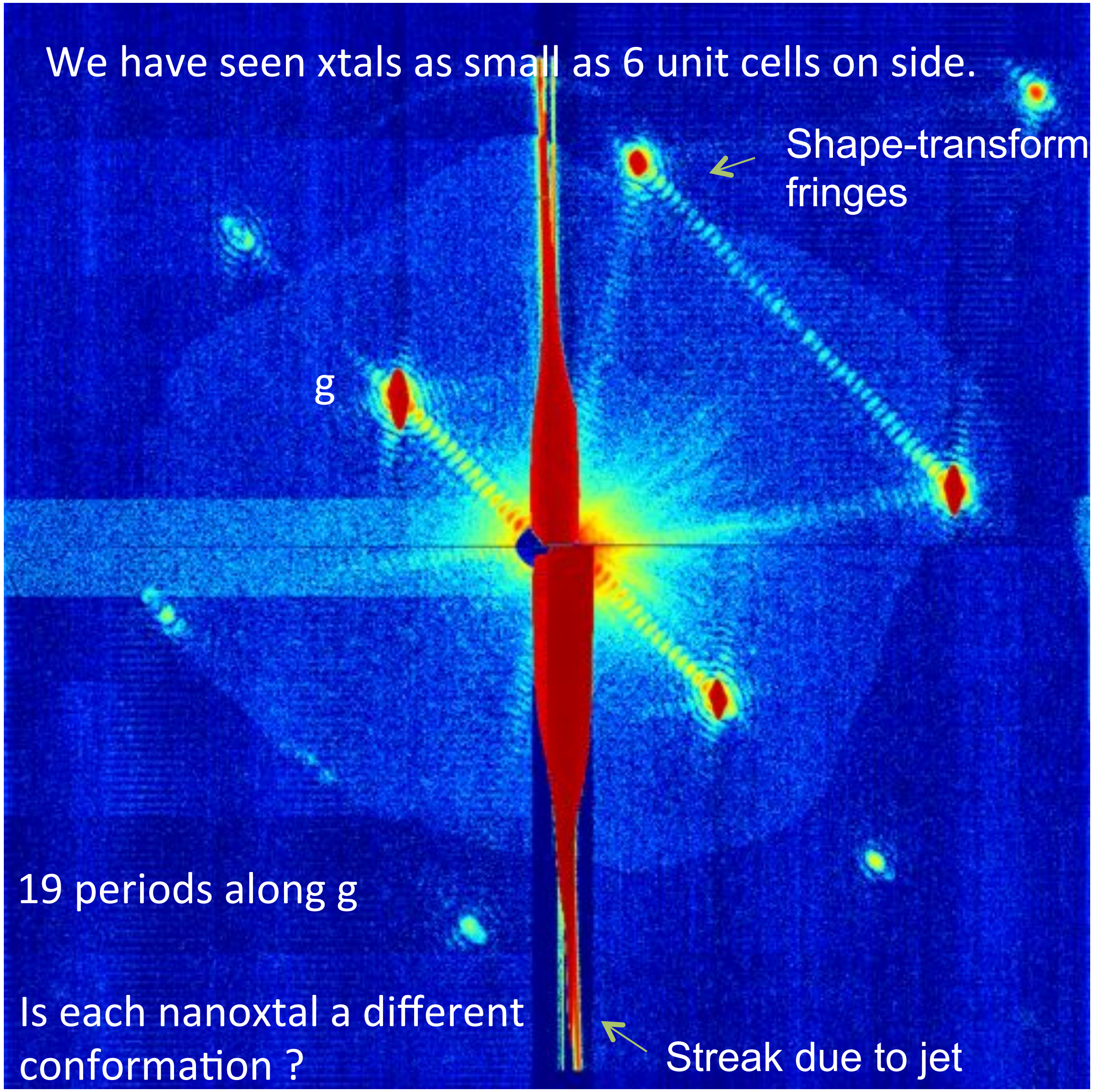
Milestones

5. 2D Xtals.

6. New phasing methods.

A new solution to phase problem – ab initio
Image nucleation and xtal growth ?.

Fast MAD/SAD

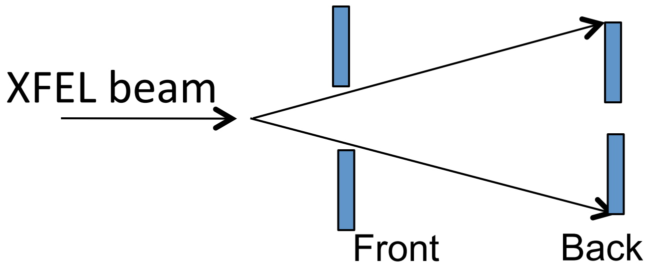


Phasing by shape transforms
- Does not need atomic res.
- No chemical mods to sample
- Ab initio, PDB not used, "a new measurement"

Fast SAD also possible ?

To phase, "divide out" particle size distribution.

Every nanoxtal is in a different (random) orientation.



Photosystem I nanocrystals at 2 kV (6.9 Ang wavelength).
Single Shot (10^{12} photons incident).

See Spence et al Optics Express, 2011

Improved sample delivery devices

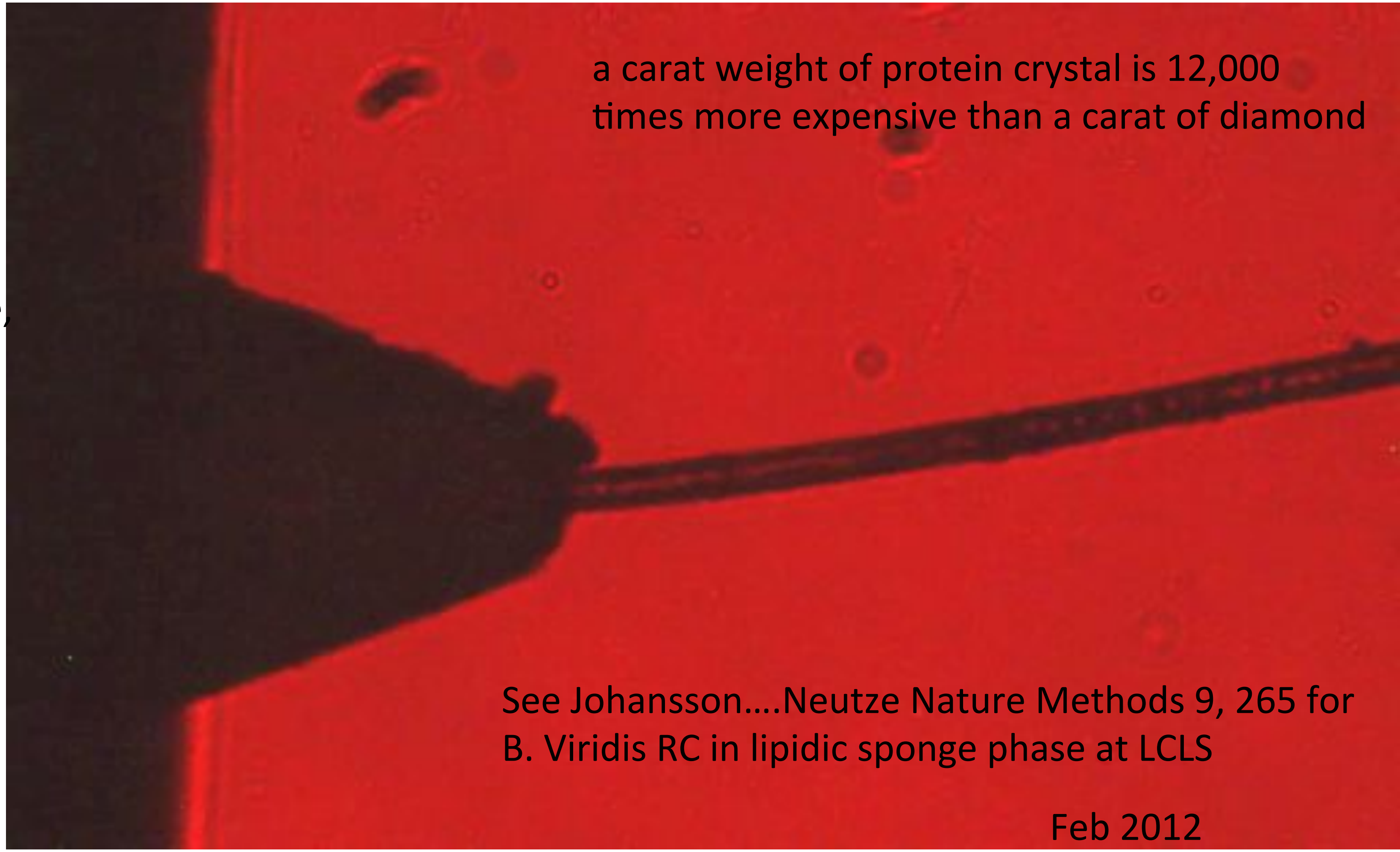
Slow LCP "toothpaste" jet wastes less protein, good for GPCRs

GPCR in viscous LCP at 300 picoliters per minute. LCLS at 1 Hz. 9.4 kV 7%
50 microliters total used Adenosine A2A

Uwe Weierstall

Optimize
conc, rep rate,
viscosity,
particle size,
jet size,
for each
sample.

V = F/A
AV = const.



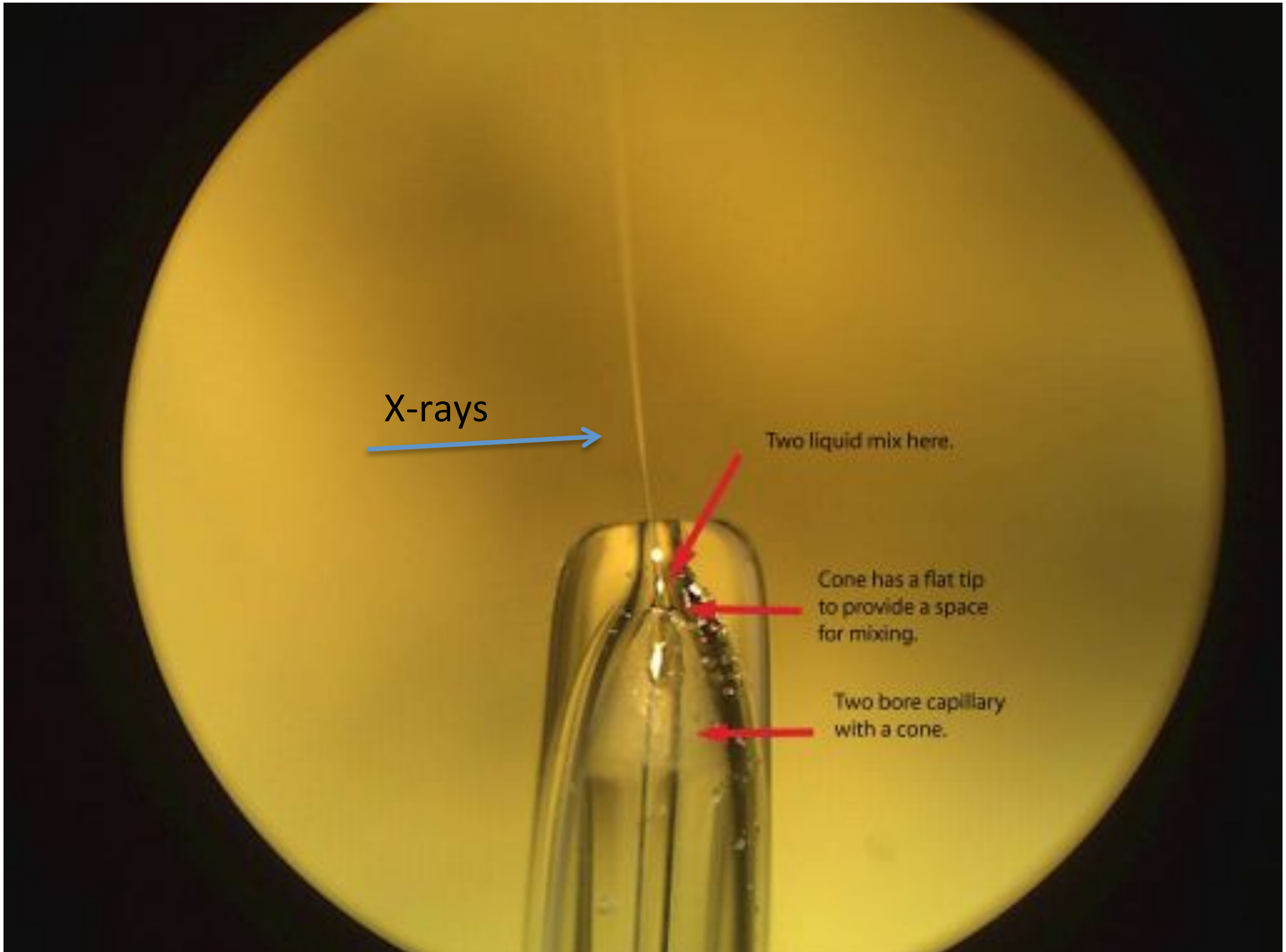
Vadim Cherezov, Ray Stevens, Bob Stroud. High rep rate of NGLS helps

New hydrated bioparticle injectors being developed at ASU

The dual-bore mixing jet, with gas focusing . Doniac/Pandy. Enzyme chemistry

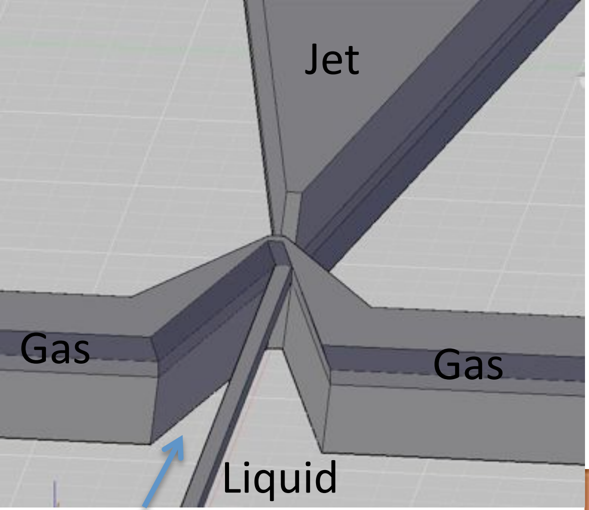
Wang
Doak
Weierstall
Spence
Pollack
Segei Pletnev

Red Fluor protein RFP. cis-trans due to pH.



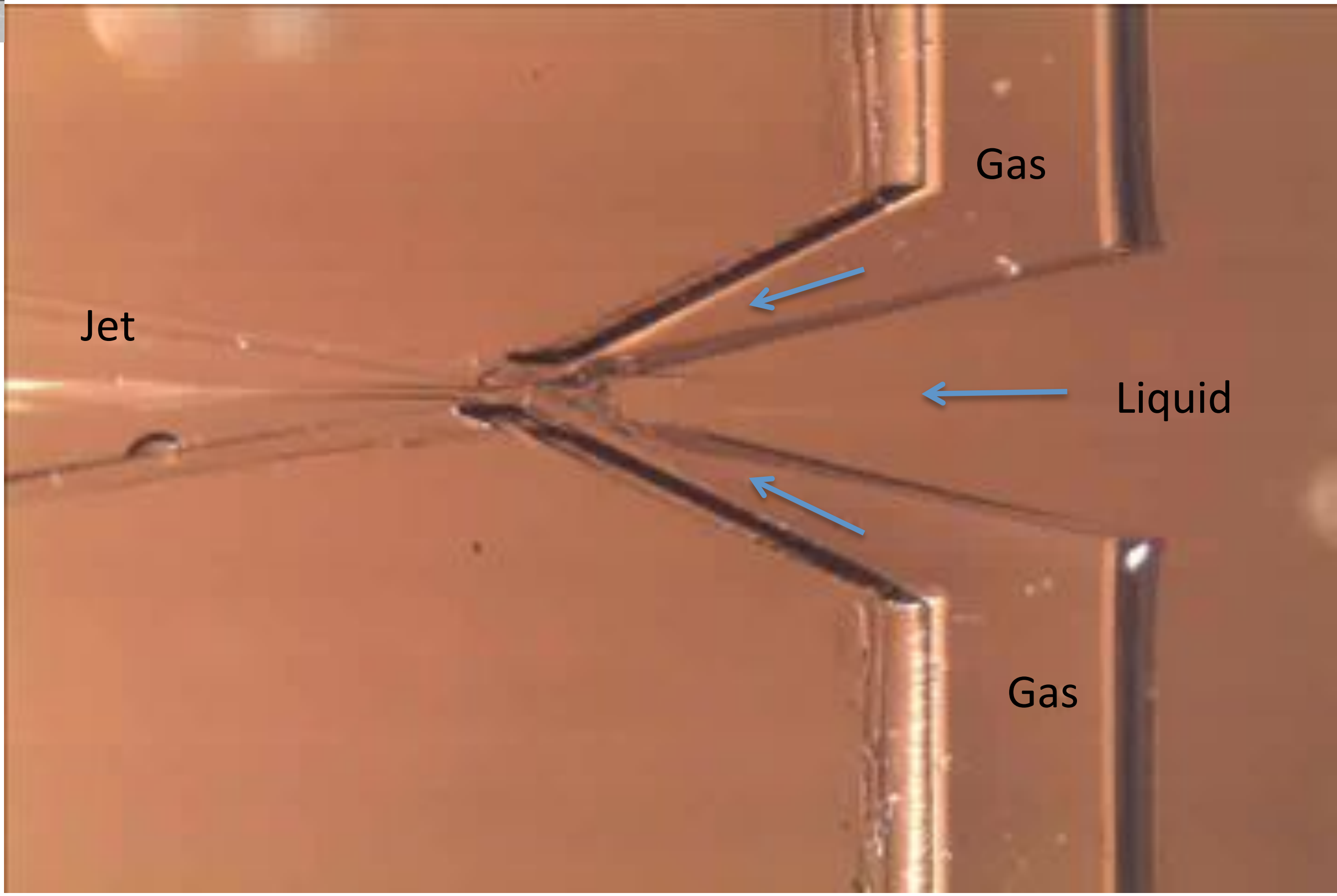
"Snapshot chemistry" – watch molecules react.

Dingjie Wang. June 2011



All-PDMS nozzle (movie). Nov 6 2012. Prelim.
Garret Nelson.
Liquid supplied in 50x50 micron square "capillary"
produces 25 micron diam jet.
Final version will make 5 micron jet.

Fabrication of injection nozzles by lithography



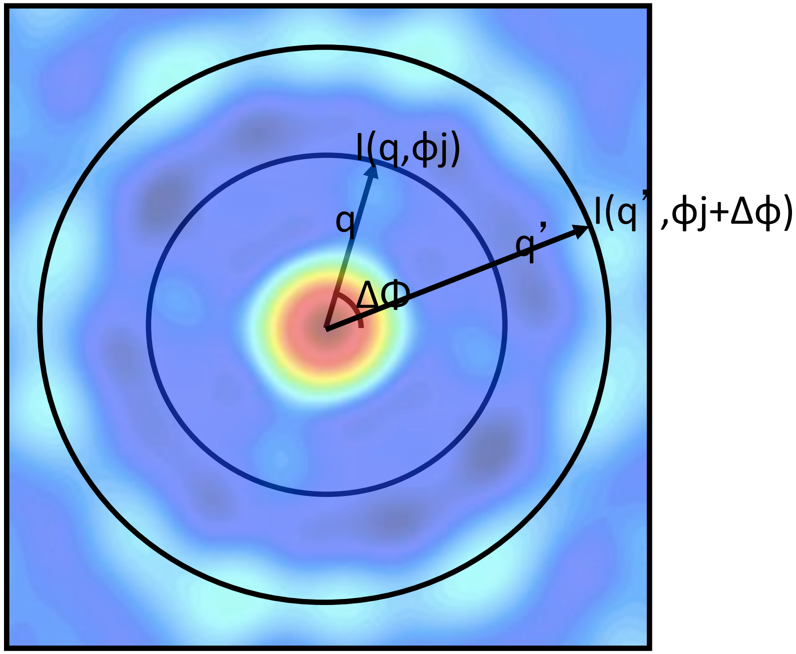
"Snapshot SAXS"

* Extracting an image of one particle
  using the scattering from many copies in soln*

* If particles are frozen in space or time, the normally isotropic
  WAXS pattern becomes 2D

* Hit rate 100% ! No orientation determination !

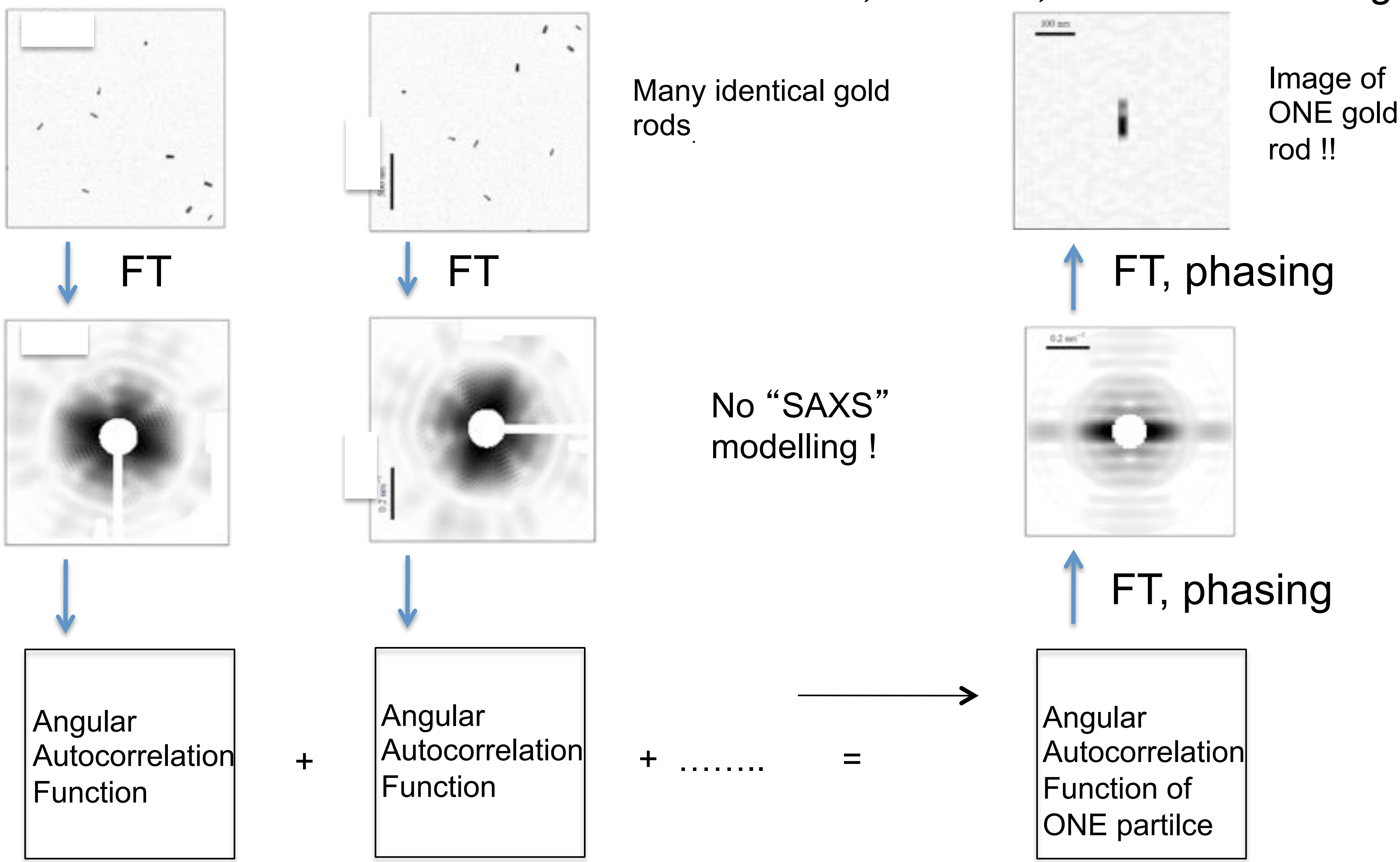
$$C_2(q,q',\Delta\phi) = \left\langle I(q,\phi) I(q',\phi+\Delta\phi) \right\rangle_\phi$$



*identical and randomly oriented. By summing the angular correlation functions we unscramble the orientational disorder.

For identical, randomly oriented particles frozen in space or time we can reconstruct an image of one particle, using the scattering from many…

Kam's method unscrambles orientational disorder, ab-initio, without modelling.



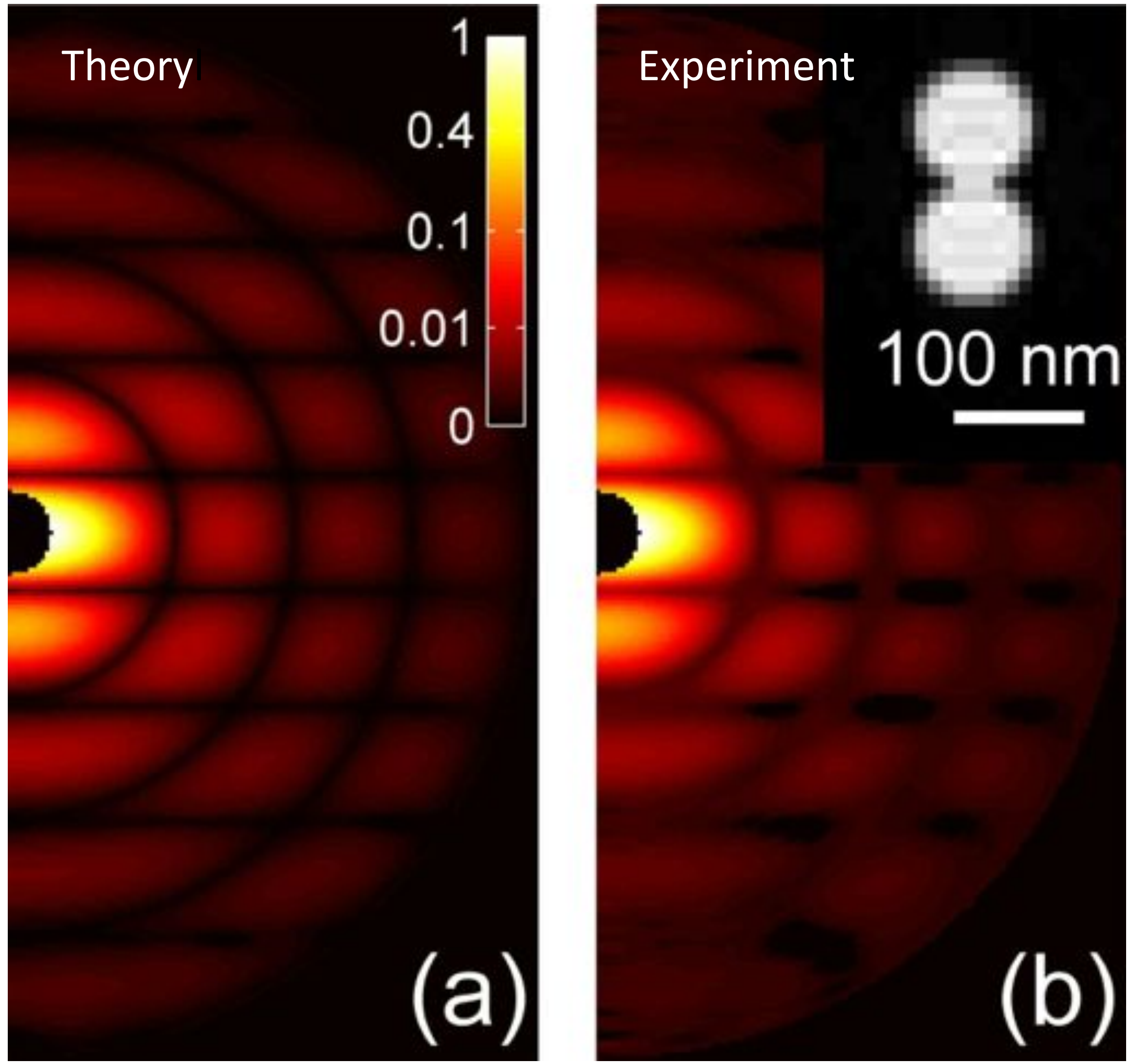
Kam, 1978 ; Saldin, Spence, Kirian PRL 2011, Phys Rev B81, 174175 (2010) Starodub et al 2011.

Pattern for one dumbell reconstructed from angular correlations of many patterns,
Each diffraction pattern comes from one particle per shot in solution.

Two angles required
to define orientation.
(unlike god rods with one).

Plastic Dumbells

Hit rate is 100% !!!!



For proteins (with modelling)
- Neutze, time-resolved WAX of B. Viridis
- Doniac, time-resolved WAX of enzyme.

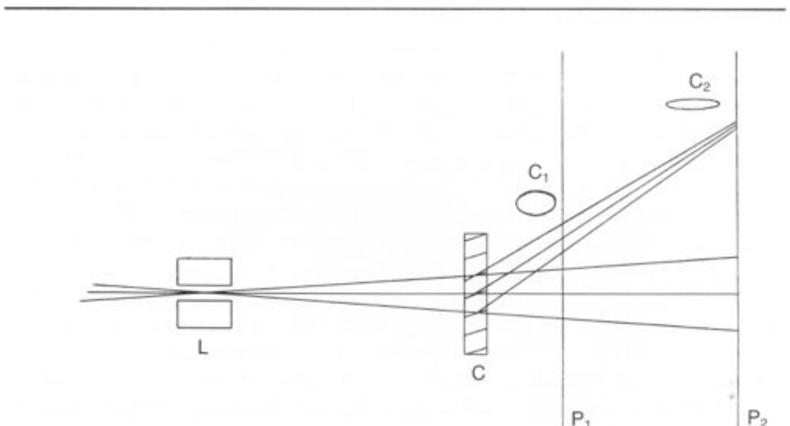
Starodub, Bogan et al 2012

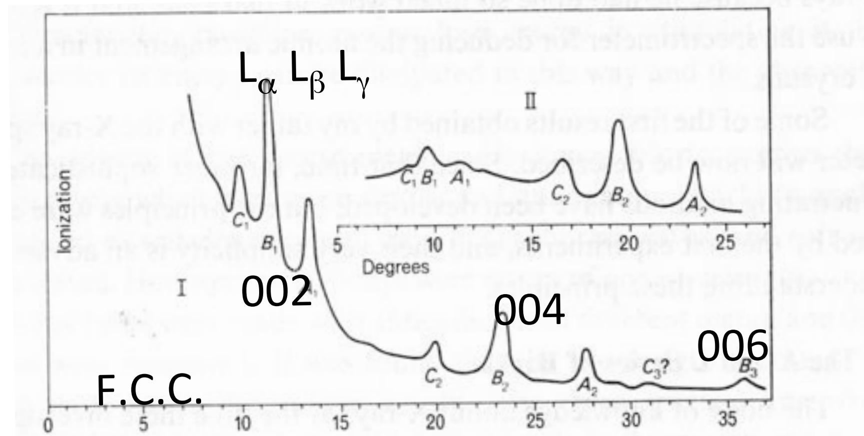
Some History –

1912 Waves or channelling particles ? Xtal structure ? Wavelength ? 3D or 2D ? Polychromatic ?

Friederich, Knipping & Laue (June 1912) – Spots (20 hrs), wrong lattice, 4 λ frm sample inner shell*

Bragg senior "If one supposed X-rays to be waves, it would be as if a plank, dropped from a height of 100 ft into the sea, were to cause far away another plank to be jerked 100 ft up !"





X-ray spectrometer measurements of X-rays reflected from a sodium chloride crystal. I, from the (100) face; II, from the (111) face.

Bragg realised, if the reflection was specular, polychromatic rays in a cone would be focussed vertically, but not horizontally. **A stack of half-silvered mirrors; specular and λ selective**. Since specular, incident angle defines scattering angle.

$2 d \sin \theta = n \lambda$. A wavelength-filtering mirror.

All became clear in Leeds with his dad's "optical" spectrometer** where orders split into L line components. Pope, Barlow helped on structure.

From his optics lectures with Wilson, Bragg, aged 22, used the expression for optical interference from a thin film as the model for Bragg's Law. But this gives colors with polychromatic radiation. 3D diffraction does not. Uncertainty principle, intensity analysis of Ewald and Darwin.

Modern meaning of Laue and Bragg cases.

*Barkla (1905)

**Preserves specular condition

Summary

*X-ray snapshots, without damage, at atomic resolution. Short pulses, instead of freezing allows RT work, hence dynamics, with x100 dose !

*Serial Crystallography solves nanoxtals (eg grown in cells). More perfect.

* Correlated fluctuations in snap-shot SAXS give ab-initio inversion. One from many.

***"**On-demand", "Toothpaste" (LCP, Sponge) injectors improve hit rate, less protein.

* New spurting mode combined with electrostatic size sorter (A. Ros) saves protein.

*Both snapshot SAD and "Shape Transforms" will be tested soon for phasing.

* 2D xtals, not otherwise possible, have given 8 Ang (Frank, Evans, Hunter…)

Conclude: Many exciting possibilities remain in fSAX, static, time-resolved, single particle, 2D xtals, and nanoxtal serial crystallography .

Amount of protein needed will be greatly reduced.

Single particle injection, particle homogeneity are challenging.

For a review, see Spence, Weierstall and Chapman, Rep Prog Phys, 75, 102601 (2012).

The End

With thanks for many collaborators from CFEL, MPI, ASU,SLAC, Uppsala.

'The passage of time, the advancing "now", converts a wavy future into a particle past". L. Bragg.

"The muse of science flees from the busy man..
The fatal enemy of research is a full engagement book". L. Bragg
"A busy life is wasted" : Senaca
All that is needed for a happy life is a project one is enthusiastic about.