Femtosecond diffraction from protein nanoxtals using an X-ray laser (LCLS).

Toward the molecular movie

and..... Pump-probe in a Jet
New solution to the phase problem
Single particle (eg virus) imaging with an X-ray laser.
Imaging one particle using scattering from many.
An international collaboration

CFEL-DESY


ASU


SLAC

M. Bogan, S. Boutet, G. Williams, D. Starodub, R. Sierra, C. Hampton, J. Kryzwinski, C. Bostedt, M. Messerschmidt, J. Bozek

Uppsala U.


LLNL

S. Hau-Riege, M. Frank

LBNL

S. Marchesini, J. Holton

Max Planck CFEL Advanced Study Group

I. Schlichting, R. Shoeman, L. Lomb, M. Bott, D. Rolles, S. Epp, A. Rudenko, L. Strüder, R. Hartmann, L. Foucar, N. Kimmel, J. Ullrich
AIMS:
- OUTFRUN DAMAGE?
- SOLVE SUBMICRON XTALS?
- MAKE MOVIES?
- REAL-TIME SNAPSHOT CHEMISTRY AT ROOM TEMPERATURE?
- PUMP-PROBE WITH VERY HIGH TIME RESOLUTION.

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

Debye period is ~100fs
This is the experimental diffraction camera we used. Note gaps in both detectors.

PLAN (2004): Go down from nanoxtal to single molecule by filtering – $n^6$ – ‘03

The Camp Chamber (MPI ASG Heidelberg, Munich, DESY)
Fs Nanoxtalog offers potential solutions to three problems in MX

1. Radiation damage reduction.


3. Avoid need to grow large xtals.

If smaller xtals are more perfect, then the combination fewer defects and less damage could produce higher resolution

In addition, it offers new possibilities for time time-resolved MX and snapshot chemistry experiments.
Why use nanoxtals?

• Get $n^6$ intensity enhancement over single molecule.
• Indexing solves the molecular orientation problem.
• Solve proteins which won’t grow big xtals.

$N=6^3 = 216$
$I \sim N^2 = 50,000$
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.

\[ \text{Av} = \text{const} \]

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

Allows study of irreversible processes in fully hydrated biomolecules.

Uwe Weierstall: Triggered Rayleigh jet

Piezo 1 MHz

LCLS Rep Rate 30 Hz

Droplet frequency 1 MHz. \( v = 10 \text{ m/s} \)

Droplet diam 1 micron

LCLS beam diam 3 microns, \( v = c \)

Flow rate \( F = 12 \text{ microL per minute} \). \( v = F/A \)

Liquid cone enhances flow alignment (from HPLC syringe pump)

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

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


DePonte, Doak, Weierstall Spence
GDVN fluid calculations (Injection into free-jet)

Flow Streamlines

Temperature

Liquid

gas at 150 K


Solve Navier-Stokes equation
Rayleigh's experiments with tuning fork and spark-gap flash photography 1878

Necking instability has period $= 4.5$ D. (consider KE and Surf En of liquid column)

Rate of droplet formation given by buzzer.
An environmental SEM image of our operating protein-beam injector.

Nozzle ground to provide large X-ray diffraction angle

The use of short pulses to prevent damage instead of freezing allows us to do real-time snapshot chemistry at room temperature.

Droplets freeze at $10^6 \, \text{o/sec.}$ in vacuum to vitreous ice if cryoprotectant added.

Flow rate 12 microliters per minute. Applications to analytical chemistry, electronic printing.

New ASU Doak RR jet for June 2010 had microscope, retract nozzle, pump laser.

ASU Liquid Jet Injector, Pumping Shroud, and Microscope/Pump Laser
For Installation on CAMP Chamber, June 2010
RB Doak, r.doak@asu.edu, 480-965-0640

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

Droplets freeze in vacuum at $10^6 \, ^\circ\text{K/s}$

B. Doak, U. Weierstall
We can screen for invisible (submicron) nanoxtals in growth medium.

**Motivation:** This system allows uninterrupted flow to nozzle even when switching solutions and thereby completely avoids the turn-on/turn-off transients in liquid jet that can disrupt the liquid jet. It also provides the means to fill and/or rinse a nozzle supply reservoir such that the liquid contains no gas/air bubbles, which can also be detrimental to reliable operation of the liquid jet nozzle.

The dual reservoir system used to feed the injector. This is supplied by Autosampler, allowing control of buffer chemistry in real time.

Instead of using freezing to reduce damage, use short pulses. Then one can do controlled “snap-shot” chemistry at RT.

**Mixing jet for enzyme cycle**

Typical flow rate is 12 microliters/min. ~SAX conc. For several hours. (1 liter in 24 hours).

Bruce Doak, Bob Shoeman
PHOTOSYSTEM I.  

$P_6$ (sixfold screw along c, advance by half) 
Maintains biosphere, Eats CO2. PSII splits water. Generates oxygen. Energy via ATP

CO2 is reduced to carbohydrate 
water is oxidized into molecular oxygen. 
From cyanobacteria 12 proteins, cofactors

72,000 non-H atoms. 
PDB 1JB0

13 years from first microxtals to big xtals, to structure!
We got diffraction patterns from nanocrystals of Photosystem 1.
Maintains biosphere, Eats CO2. PSII splits water. Generates oxygen. Energy via ATP.
Photosystem 1 Protein was extracted from Thermosynechococcus Elongatus bacteria by HPLC, and crystallized in detergent micelles. Two-micron filter used.

PDB 1JB0

Crystal Packing
1 MegaDalton
72,000 atoms

Space group P6₃
solvent content 78 %

CO₂ is reduced to carbohydrates
Water is oxidized into molecular oxygen (PSII)

\[ a=b=288 \text{ Å} \]
\[ c=167 \text{ Å} \]

Two trimers per unit cell.
Crystallite aspect ratio 5:1

P. Fromme, M. Hunter.
PSI down c, 70 fs, 2kV, 7E12 photons, single pulse
Note “particle-size broadening” or shape-transforms
seen on back high-angular res. detector, corresponding
to front, high angle detector, pattern.
Hit rate >50%. 5Tb/ night. Continuous water jet
What the back (high angular resolution) detector data looks like.

Photosystem I nanocrystals at 2 kV (6.9 Ang wavelength).

Single Shot ($10^{12}$ photons incident)

Streak due to jet

Shape-transform fringes

How can structure-factors be extracted from this data?

Every snap-shot comes from a nanoxtal of different size, with different fine structure on each spot.

Every nanoxtal is in a different (random) orientation.

Coherence width spans entire nanoxtal, not cell.

Photosystem I nanocrystals at 2 kV (6.9 Ang wavelength).

Single Shot ($10^{12}$ photons incident)
The crystals are sub-micron in size.

Distance between xtal facets along direction $g=1/d$ is $N+2$ periods $d$, for $N$ subsidiary fringes.

Distance between 14 fringes = 400 nm

Distance between 9 fringes = 220 nm

$Lattice transform$: $I(q) \sim \frac{\sin^2(Nq \cdot a)}{\sin^2(q \cdot a)}$

Photosystem 1
70 fs
$7 \times 10^{12}$ incident photons
6.9 Angstrom X-rays (1.8 kV)
The shape transforms can be inverted by iterative phasing.

Note: If not at exact Bragg condition, this is “conditional transform”.
Use transforms around all spots to get complete 3D shape.

Count fringes (include both Braggs) to get number of mols on a side in xtal!
We can sum the Bragg spots as they arrive (virtual powder pattern)

Lysozyme

Ilme Schlichting et al.

With $6 \times 10^6$ patterns, human examination of the data is impossible!
Data analysis

How can structure-factors be extracted from this data?

Conventional PX: rotate sample through Bragg condition, Structure factors are proportional to integrated intensities.

Here, every snap-shot is a random slice through Bragg reflections (stills, partials) from a nanocrystal of different size, with different fine structure on each spot, in different (random) orientation. Size effects dominate mosaicity.

Rossman’s partiality assumes spherical mosaicity limit.
Diffraction patterns can be indexed using software such as MOSFLM and DirAx

A total of 1.85E6 patterns were recorded at 70 fs, of which 112,000 contained more than 10 Bragg peaks. Of these, about 30% could be indexed, so that in total we were able to use about 2% of the data collected at 30 Hz.

A. Leslie, Acta 2006
**Shape-transform effects.** PSI nanoxtals at 2 kV, random orientation.

In XRD the Ewald sphere takes a SLICE through the 3D FT of the nanoxtal shape. The structure factors are proportional to the VOLUME of the shape-transform.

Confuse autoindexing programs!

Ewald sphere

**OR**

Count fringes to get size

No scattering in this Bragg direction

Important quantity for data analysis!

This is a pattern simulated for one PS1 needle in random orientation showing sinc function shape transforms at each lattice point. J. Holton

Wavelength ~6.88 Angstroms
How can we extract structure factors from this new kind of data?

To extract structure factors from partials we must sum over shape-transform volume – over xtal orientation and size. Add Braggs from different xtals with same index.

Does this sum converge? Test using Monte-Carlo integration.
(It must converge, because Powder diffraction exists).

Diffraction from one crystallite is

\[ I_n(\mathbf{k}, \mathbf{k}_o, \ldots, N_i) = J_0 |F(\mathbf{k})|^2 r_e^2 P(\mathbf{k}_o) \frac{\sin^2(N_1^1)}{\sin^2(1)} \frac{\sin^2(N_2^2)}{\sin^2(2)} \frac{\sin^2(N_3^3)}{\sin^2(3)} \]

One crystallite, smaller than a mosaic block. X-tals too small for extinction. Assume no twins are euler angles between xtal and \( \Delta k \).

Sum over size and orientation from many is

\[ I_{hkl}^{\text{exp}}(m, t) = \sum_{n=1}^{m} \sum_j I_n(\mathbf{k}_j) \]

Subtract water background

\[ I_1 = 2a \sin(\hat{q}) \cos(\hat{q})/ \]
\[ I_2 = 2b \sin(\hat{q}) \cos(\hat{q})/ \]
\[ I_3 = 2c \sin(\hat{q}) \cos(\hat{q})/ \]

Sum over pixels around Bragg

This is similar to Powder diffraction, so must converge.
Use average, not sum, to avoid flow alignment, Lorentz factor. Kirian et al, Optics Express (2010).
What is the quality of the LCLS data (at 8 Angstroms)?
How many nano crystals are needed for given accuracy in structure factors?
How many pixels around Bragg spot should be included in sum (δ)?

**R (ALS/LCLS)**

\[ R = 2 \frac{\left( \sum_{\{\text{hkl}\}} ||F^{1}_{\text{hkl}}|| - \eta ||F^{2}_{\text{hkl}}|| \right)}{\left( \sum_{\{\text{hkl}\}} ||F^{1}_{\text{hkl}}|| + \eta ||F^{2}_{\text{hkl}}|| \right)} \]

Different numbers of pixels included around lattice point. Mean xtal size 1 micron

\[ \delta (\text{nm}^{-1}) \]

- 0.003
- 0.004
- 0.005
- 0.006
- 0.007
- 0.008

\[ * R_{\text{int}}(\text{odd vs even frames}) = 5\% \]

\[ * S/N \ I/\sigma(I) = 45 > 2 \text{ at edge.} \]

\[ * R(\text{shell; odd/even}) = 4\% \text{ at 9 A.} \]

\[ * R(\text{LCLS vs Rigid-Body refine of PDB) using REFMAC gives} \]

\[ R/R_{\text{free}}(\text{LCLS}) = 0.284/0.327. \]

\[ * R(\text{LCLS vs ALS}) = 20.5\% \]

But – different temps
- different solvents
account for most of this.

**R-factor (GOF) comparison between LCLS data and single-crystal ALS data.**

R is plotted as a function of the number of patterns merged.
The patterns are ordered from left to right in increasing number of Bragg spots per pattern.
Compare LCLS Monte-Carlo merged data with that from a single xtal of PS I.

Single Xtal, ALS – plane normal to c

LCLS, 16,500 nanoxtals

The individual xtals are not twinned, but the merge will impose 50% twinning.

|Fhkl| from ALS single xtal (50% twinning).

PSI at 2 kV, 70 fs.

From 16,500 nanoxtals, 70 fs LCLS (6, inversion enforced; twinned merge)

R(LCLS vs ALS) = 20.5%

Different temps, solvents account for most of this.

Tom White, Rick Kirian.

It takes weeks to transfer TBytes of data by ftp.
A 3D tour of the merged structure factors....
PS I density map from LCLS. 12 proteins (cyanobacteria)

After MR phasing, rigid-body relaxation (1JB0) using REFMAC. 8 Angstrom res.
Damage and dose.

3 Gy (energy/mass) kills a hampster!

1. Global measure, indep. of resolution (Henderson, Garman “safe dose”)

“Safe” PX dose is 30 MGy (energy/mass) $D_{1/2}$ when all Braggs drop by half (Owen et al)

LCLS dose is 670 MGy (3mJ pulse, 60fs, 2Kv, 44 micron$^2$ beam, = 900 J/cm$^2$; $\mu = 12.8$ microns (780 cm$^{-1}$) PSI, density = 1.05 gm/cm$^3$

Since we see no change between 3fs and 300 fs, our $D_{1/2}$ dose > 670 MGy!

Conclude:
Snapshot diffraction allows 22 times greater dose.

2. Resolution-dependent dose.

We observe no damage effects to 8 Ang resolution up to 70 fs pulse.
If this holds up at atomic resolution, the resolution for FEL is limited only by noise and wavelength, not damage.
(cf Rose criteria)

Howells et al 2006 showed dose ~ resolution$^{-4}$
Femtosecond damage - two mechanisms.

*At 2 kV, C 1s ionization occurs 360 times more frequently than elastic*. But only 2% of atoms are ionized in 70fs.

*Most damage is done by PE and Auger electron cascades (after pulse ends).*

*It takes about 50 fs for a photoelectron to lose all its energy to secondary electrons. ($V_{pe} = 50$nm/fs, 2.5 microns in 50 fs)*

*Each PE produces about 100 secondary ionizations. Secondaries $e^-$ from water jacket damages protein nanoxtal.*

But $I_{Bragg} = \sigma_{el} N^2$ for N unit cells.

$\int_{1s} I_{abs} = N$

and $N^2 \rightarrow N$ for high q. (Wilson stats)

Long pulses will diffract from exploding debris giving background. (not seen at 300 fs)

Valence electrons imaged by XRD will be disturbed by cascade before nuclei move.

*at 8 kV, 30 times

PE = Photoelectron. Beam energy-Binding energy (C,O,N)
A = Auger electron < Ionization energy.

But $I_{Bragg} = \sigma_{el} N^2$ for N unit cells.

$\int_{1s} I_{abs} = N$

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Long pulses will diffract from exploding debris giving background. (not seen at 300 fs)

Valence electrons imaged by XRD will be disturbed by cascade before nuclei move.

*at 8 kV, 30 times
Preliminary analysis shows degradation of the sample at longer pulse durations.

Photosystem I radial average of diffracted intensity

Andrew Aquila, Anton Barty, Tom White (CFEL)
Rick Kirian (ASU)
There is currently no method for ab-initio digital phasing which does not require atomic resolution data. (Few protein crystals diffract to atomic resolution)

LCLS data opens new possibilities for phasing, needed to emerge from the swamp of model-bias in the PDB.

“A genuinely new structure is one whose phases are measured” I.S.

As David Sayer suggested in 1951, any intensity between Bragg reflections can be used to solve the phase problem.

This became the basis of new iterative phasing methods for single particles (Gerchberg-Saxton, Fienup, Shrinkwrap, Elser)

Nanocrystals provide this inter-Bragg intensity.

The many pixels within central max of shape transform needed to extract structure factors is also sufficient for phasing. MAD, SIR, MR
Phasing methods. General.

Which can be used with an FEL?
(no mono, high fields, short-pulse).
Assume no PE damage

• Direct methods. \( \mathcal{T}^{\mathcal{M}}(r) = \mathcal{T}^{\mathcal{M}}(r) \) Need Atomic res.
Fails if too few or too many atoms (assumes Wilson stats).

• MAD (Anomalous diffraction). Hollow atoms?
Need time-dependent absorption coefficients. Santra.

• MIR (Isomorphous replacement). Not possible?

• MR (Molecular replacement) Model bias?

• FEL is coherent across entire nanoxal –gives new information for a new phasing method.
XRD from an acentric nanoxtal

\[
I_n(\mathbf{k}, \mathbf{k}_o, \ldots, N_i) = J_o |F(\mathbf{k})|^2 r_{c}^2 P(\mathbf{k}_o) \frac{\sin^2(N_1)}{\sin^2(1)} \frac{\sin^2(N_2)}{\sin^2(2)} \frac{\sin^2(N_3)}{\sin^2(3)}
\]

\[
= c |F(\mathbf{k})|^2 S_n(\mathbf{k})
\]

Since \(I(\Delta k)\) is known, and interference fn. \(S(\Delta k)\) is known (count fringes for \(N_i\)), we can find the continuous molecular transform \(F(\otimes K)\).....

\[
|F(\mathbf{k})|^2 = \frac{I_n}{S_n} = \sum_n \frac{I_n}{S_n}
\]

where sum avoids problems due to division by zero.

Then phase \(F\) iteratively. Reconstruct density for one molecule.

*The shape transform is the same around every lattice point for one xtal.*

*Every xtal has the same molec transform, independent of size.*

This allows them to be disentangled.

- Don’t need atomic resolution. Molecular (unit cell) density is real. Support is unit cell.
- Normal to 2,4,6-fold axis, phases are signs for origin on axis.
Phasing Nanoxtals . 2.

*Index & assemble data in 3D diffraction volume, including inter-bragg intensities (use fractional indexing)

For one xtal..

\[ I_n(\mathbf{k}, N^{(n)}) = c |F(\mathbf{k})|^2 S_n(\mathbf{k}, N^{(n)}) \]

*Now sum over particle size at each voxel….but Mol Transform is indep. of size.

\[ < I_n(\mathbf{k}, N^{(n)}) >= c |F(\mathbf{k})|^2 < S_n(\mathbf{k}, N^{(n)}) > \]

\[ c |F(\mathbf{k})|^2 = F^2 = \frac{< I_n(\mathbf{k}, N^{(n)}) >}{< S_n(\mathbf{k}, N^{(n)}) >} = \frac{Ex}{Av} \]

*To get denom, sum corresponding voxels around each recip lattice point. (molecular transform then washes out)

* Particle size distribution was extracted from shape transforms in patterns. Self-normalizing !!!!!

Now use iterative phasing on molec transform.

Spence et al Optics Express (2011)
Phasing simulation for 1AKG : $F^2 = \text{Ex/Av}$. $10^{13}$ © incident/shot, 0.5 $\int$ beam, $1.46 \times 10^6$ scattered ©per shot, Poisson noise

One molecule $F^2$

Sum of $10^6$ nanoxtals : Ex

Modulus of Molecular Transform
$|F(Dk)|$ for 1AKG protein on [001] in q-space, resolution 0.523 nm at side of the pattern, 6 samples between Braggs. Alpha-conotoxin cubic, a=5.84nm.

Sum of oriented patterns : molec transform modulates lattice. (20 mols on side, $\int = 2$)
Dividing out the particle size distribution $F^2 = \frac{E_x}{A_v}$

$10^{13}$ ©/shot, 0.5 micron beam, $1.46 \times 10^6$ scat © per shot, $10^6$ shots (nanoxtals)

Av, the average shape transform. (Add shape transforms together around different bragg spots, different xtals).

Conclude: Method works in simulation

Recovered molecular transform $F^2 = \frac{E_x}{A_v}$. Phase this iteratively.
Now phase molecular transform in doubled cell by iterative methods (Gerchberg, Saxton, Fienup, Marchesini’s shrinkwrap) cf Solvent flattening, Density modification, with feedback!

This method will fail if ….

1. xtal is too big (no inter-Bragg scattering)
2. xtal too small (can’t index and merge data)
3. dynamic range insufficient (related to 1)
4. pixel density too low (sampling for Monte-Carlo is sufficient)

(maximum would be 4L/d on a side for largest xtal width L, res d).

Structure factors require the converging sum of the volumes of the shape transforms Phasing requires 3D mapping of the continuous scattering from one molecule
Some new ASU injectors

History

10 micL/min

Now add pump-probe to any of these. (June 2010)

Strobe microscope from above sees flash, to synchronize

Uwe Weierstall, Mark Hunter

Mixing jet: Very short deadtime (shortest delay).

For velocity 10 m/s, 10 microsec is 0.1 mm
1 millisecond is 1 cm

Protein in LCP
1500 psi.

Lipid cubic phase (toothpaste): no settling, flow rate 35 nL/min, no synchronization needed. At 100 Hz with 5 micron X-ray beam, all solution is hit at this flow rate.

Bruce Doak, Uwe Weierstall, JS ASU
Drop-on-Demand (DoD) – Typical Specifications

Droplet velocity: 0.1 to 5 m/s
Fluid flow rate: 240 nl/min, e.g. (40 micron droplets at 120 Hz)
Droplet diameter: 40 to 60 micron (smaller possible)
Temperature: RT (near nozzle) to 200 K (droplets in vacuum)
Triggerable: Yes (can be run as triggered Raleigh Droplet Beam)
Environment: Gas only, no vacuum (freezes up)
Clogging: Usually not

Figure 4: Drop-on-demand type ink-jet device generating 50• m
   diameter drops at 2kHz.
Droplet-on-demand

LCLS Beam 3 micron diameter

V = 5 m/s

Dimensions

Timing synchronization with LCLS

30 microns droplet

2-micron nanoxtal

V = 0.0003 m/s

No synchronization

Next shot will be here

30 microns LCP tube

LCP “Toothpaste” injector

wasted protein in LCP
Pump-probe in a jet

Bio times are 10 microsec to 1 millisecond. At $V = 10 \text{ m/sec}$ this is 100 microns to 1 cm. DoD droplets go 1 cm in air at 1-3 m/s, dispersed after a few mm.

$L = \text{Breakup length} = 7.3 \text{ mm for 25 micron stream at 13 m/s (560 microsecs)}$

$= 1.8 \text{ mm for 10 micron stream}$

$= 650 \text{ microns (~0.5 mm) for 5 micron stream (50 microsec)}$

Notes:
* Particle hit by LCLS must have been within pump laser illumination circle at earlier time $\Delta T < X/V$ before hit. Longest delay (0.5 millisecond) needs big stream to keep within breakup length. Use cylindrical lens for more pump intensity.
  * <3 micron LCLS beam may be smaller than stream diameter. Long pathlength.
  * Alternative method using flow times, off-center laser, has worse timing error.
  * First experiments done in June 2009 (PSI-ferre).
  * Pump laser can be aligned by jet lateral motions while viewing microscope.
  * Jet height is aligned with LCLS by viewing vaporization flashes on microscope.
  * Use X-ray, Pump, X-ray on same particle in toothpaste mode, 200 ns readout
Lipid Xtal Phase jet takes 10 millisecs for protein to traverse a 3micron beam diam. Hence plenty of time to read out two diffraction patterns from same nanoxtal if dose below damage threshold. Use larger xtals, eg 2 micron.
Pump-probe experiments are possible with the liquid jet. PSI-ferre.

Pump laser on jet

Pump laser:
- 532nm, 7ns pulse, 10 μJoules,
- focused to 100 micron spot, fiber coupled,

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

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
We aim to make a movie of the ferredoxin molecule undocking from PSI.

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

Snapshot structure factors measured for 3 delays between pump laser and 70 fs X-ray pulse. Visible-light (photosynthesis) pump laser causes electron transfer, which causes undocking. Then entire xtal falls apart (but not the molecule).
“Fluctuation WAXS”

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

(ab-initio, without SAXS modelling)

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

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

\[ \text{Angular Autocorrelation Function} + \text{Angular Autocorrelation Function} + \ldots \ldots = \text{Angular Autocorrelation Function of ONE particle} \]

Fluctuation cross correlations

The experiment consists of detecting scattered intensity at TWO points on a ring, and multiplying these together. Then summing around the ring.

Averaged pair correlations are azimuthally symmetric, they depend only on $\Delta \phi$. We measure the angular cross correlation functions in the intensity fluctuations:

$$C_2(q, q', D_f)$$

$$C_2(q, q', D_f) = \langle I'(q, f) I'(q', f + D_f) \rangle$$

$$I'(q, f) = I(q, f) - \langle I(q, f) \rangle$$

intensity fluctuation (mean subtracted ring intensity)
Experimental demonstration – in two dimensions.

Diffraction patterns were collected from 90nm gold rods at ALS using coherent 2nm X-rays. Single-axis alignment

TEM Image

Reconstruction from XRD

In 3D, both beam noise and Kam fluctuations are proportional to the number of particles, so the ratio isn’t. Hence if one particle doesn’t work, many won’t. Kirian et al submitted. Elser. Wochner.

Single-particle imaging works at LCLS. Mimivirus patterns reconstructed in 2D.

- **Mimivirus patterns**: Reconstructed in 2D with a wavelength of 7 nm and 1 CCD pixel = 30 µm. Data nx = 1024. q_max is at 15.4 mm. CCD z = 30 mm, NA ~ 0.51.

Summary

• “Diffract-and-destroy” works at high resolution (0.3 nm) for delicate membrane proteins, fully hydrated. (Catpsin B, PSI, reaction center, Lyz)

• Solve invisible xtals in mother liquor? Reduce crystallization bottleneck in protein crystallography. (SONICC). More perfect xtals for better resolution?

• Use **short pulses instead of freezing** to reduce damage, **work at RT**.

• Hence control chemical conditions in jet at room temp, integrated with microfluidics, HPLC. Snap-shot chemistry.

• Dynamic studies - pump-probe for irreversible processes.

• The 50% hit rate of the ASU could allows sufficient data for 3D reconstruction of single-particles. 2D so far from virus at 32nm resolution.

• **No evidence of increased damage** between 3 – 70 fs at 8 Angstroms

• **A new solution to the phase problem**? Direct method at low resolution.

• Need better injectors – higher hit rate, waste less protein! Injector, not detector or source, limits data acquisition rate

Nature - 2 papers – single particles, nanoxtals. Phasing PRL.
Recent advances in free-electron laser technology have demonstrated the ability to collect millions of "snap-shot" hard X-ray diffraction patterns from single bioparticles (e.g., viruses, cells) or hydrated protein nanocrystals. Other modes, such as Laue diffraction, fast (correlation) SAXS, and pump-probe experiments aimed at observing protein dynamics are also under development.*

The aim of this small workshop is to determine what opportunities the invention of the FEL offers to biologists.

For a brief review, see Chapman, Nature Materials, 8, 299 (2009)

Program Committee
M. Rossman (Purdue), D. Reese (Caltec), Dame Louise Johnson (Oxford), K. Moffat (Chicago), Jack Johnson (Scripps), Bill Cramer (Purdue), K. Hodgson (SLAC), I. Schlichting (MPI Heidelberg), R. Neutze (Gothenburg), G. Simpson (Purdue), P. Fromme (ASU), H. Chapman (DESY), M. Frank (LLNL), Adam Arkin (UCB), R.M. Stroud (UCSF), A. Bruenger (Stanford), G. Phillips (Wisc), S. Lane (CBST), J.C.H.Spence (ASU)

Program
Tentative plans include talks on the following topics: The current status; Single Particles; Protein Nanocrystals; Dynamics - pump-probe possibilities; The phase problem; Instrumentation - (Sources -more photons ? Detectors; Injection devices), In-situ experiments, Relationship to Cryo-em.

A web page for registration will be established shortly.

Inquires to J.C.H. Spence at spence@asu.edu.

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https://sites.google.com/a/lbl.gov/biology-with-fels/
The End

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

“Biology is a solution in search of a problem; physics a problem in search of a solution”