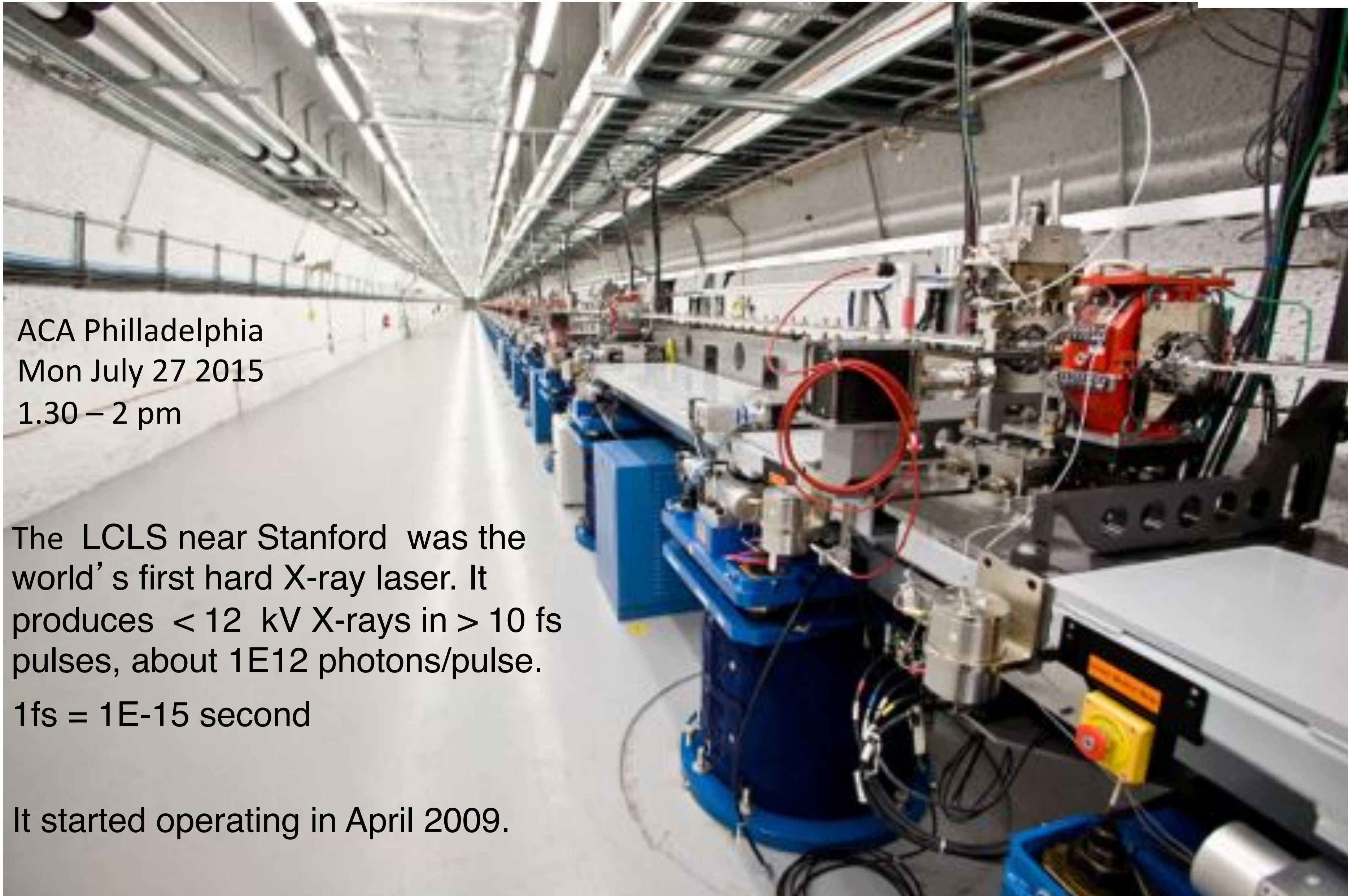
Recent advances in SFX at the NSF BioXFEL STC.

BioXFEL



ACA Philladelphia
Mon July 27 2015
1.30 – 2 pm

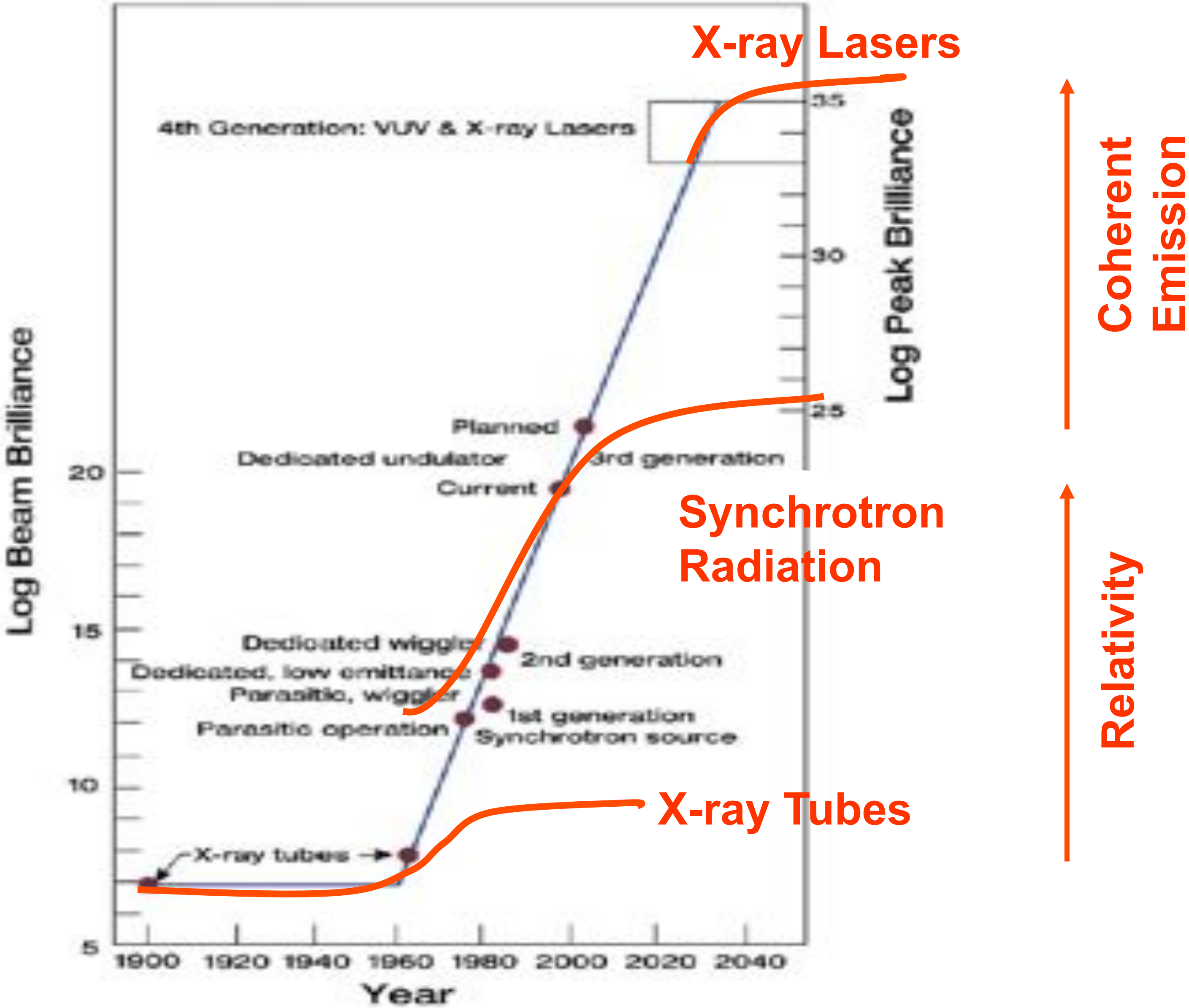
The LCLS near Stanford was the world's first hard X-ray laser. It produces < 12 kV X-rays in > 10 fs pulses, about 1E12 photons/pulse.

1fs = 1E-15 second

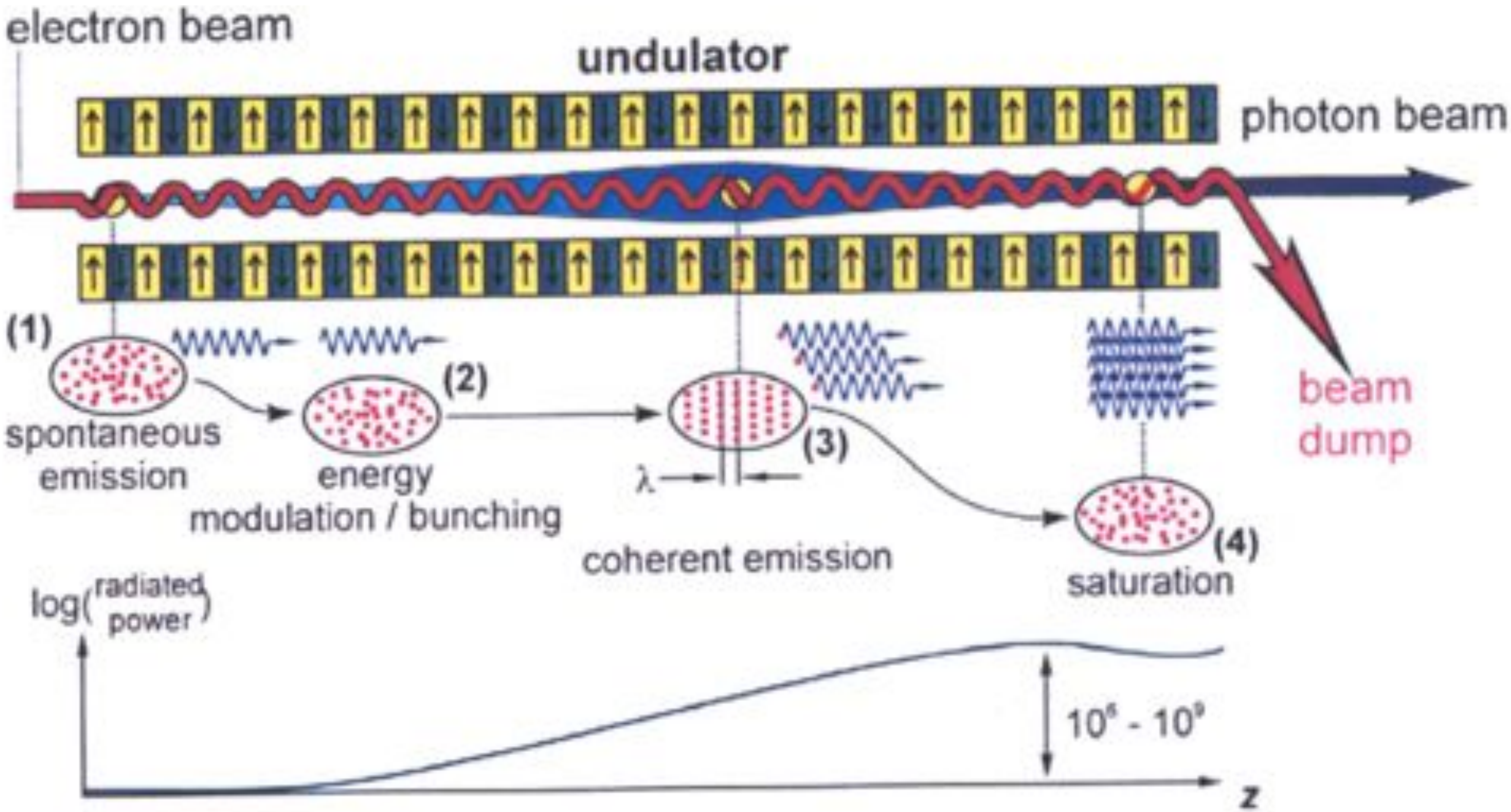
It started operating in April 2009.

Madey 1972 Stanford; Saldin,, Bonefachio, Sessler 1985 Microwaves; Pellegrini optical 1991; Leutl 1997 Argonne; Flash Matterlink 1997 ; Murphy Brookhaven 2001; (SASE mode)

Generational advances in beam brilliance



From undulator radiation to x-ray free-electron lasers (XFEL)



Self Amplified Spontaneous Emission (SASE) is powerful amplified undulator radiation with full transverse coherence

Bonifacio, R., C. Pelligrini, and L. Narducci, Opt. Commun. **50**, 373 (1984)

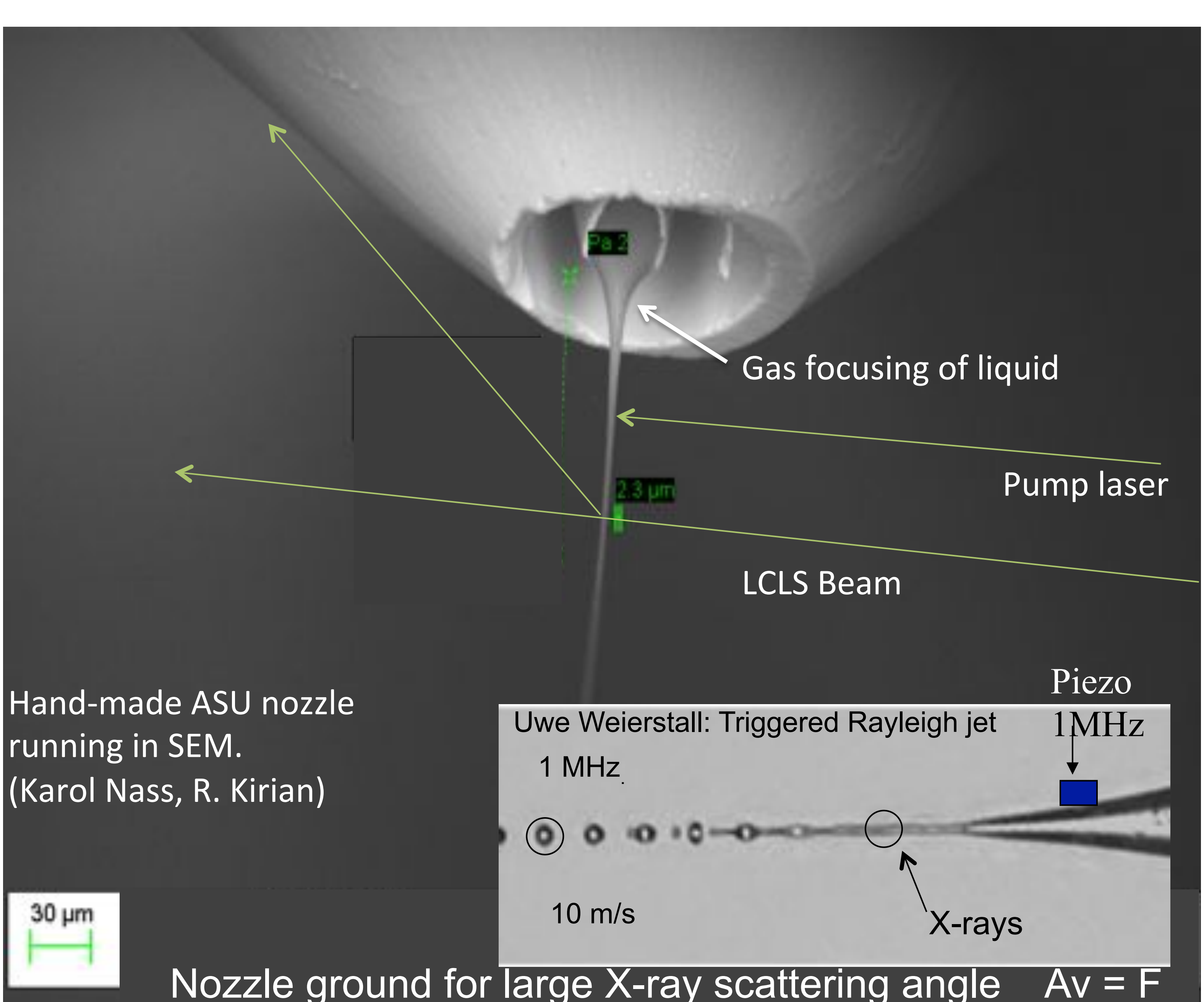
Madey 1972.

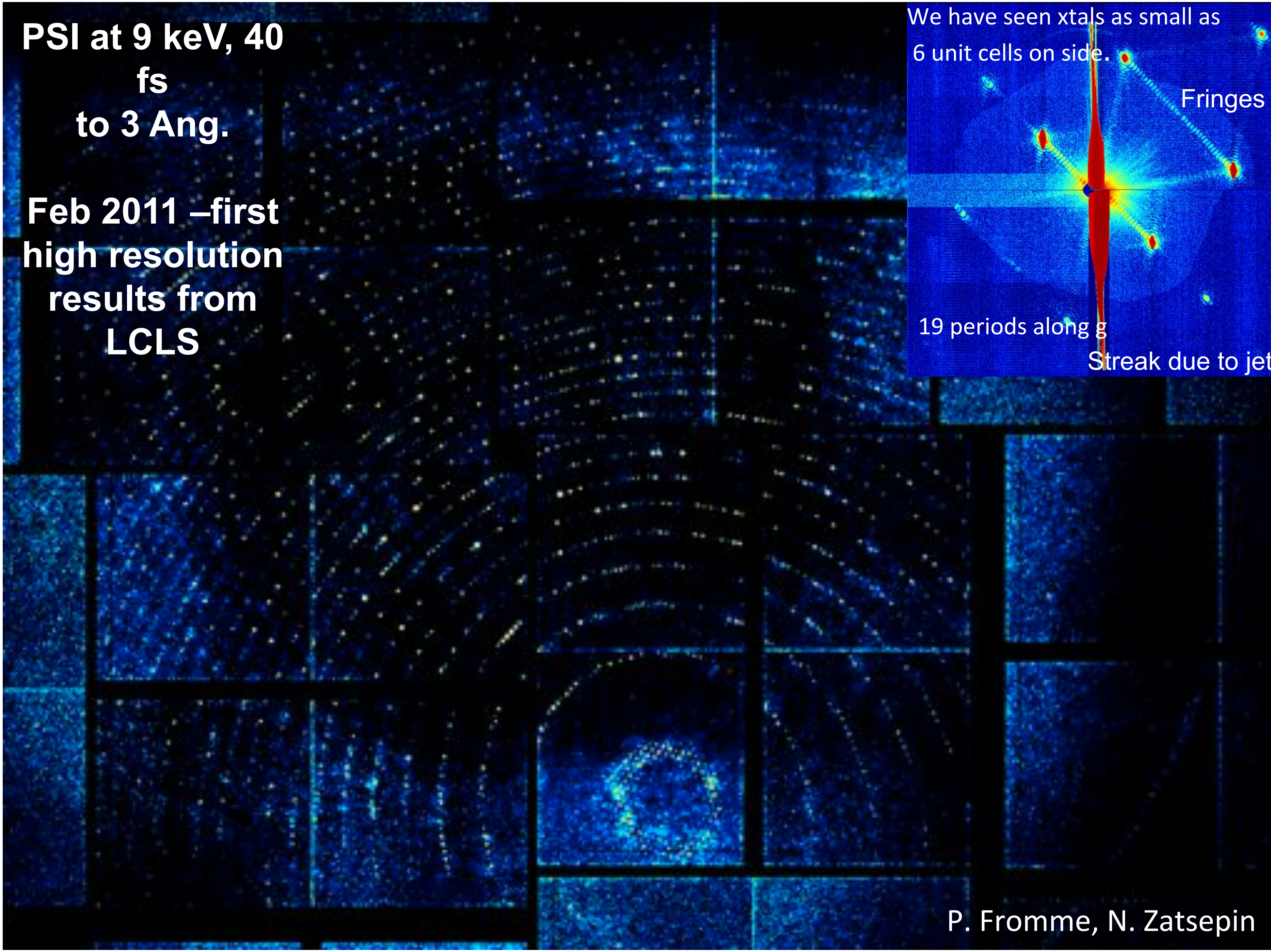
Time-resolved Protein nanoxtal sample delivery uses a liquid jet

Droplets freeze at 10^6 °/sec. in vacuum to vitreous ice if cryo protectant used

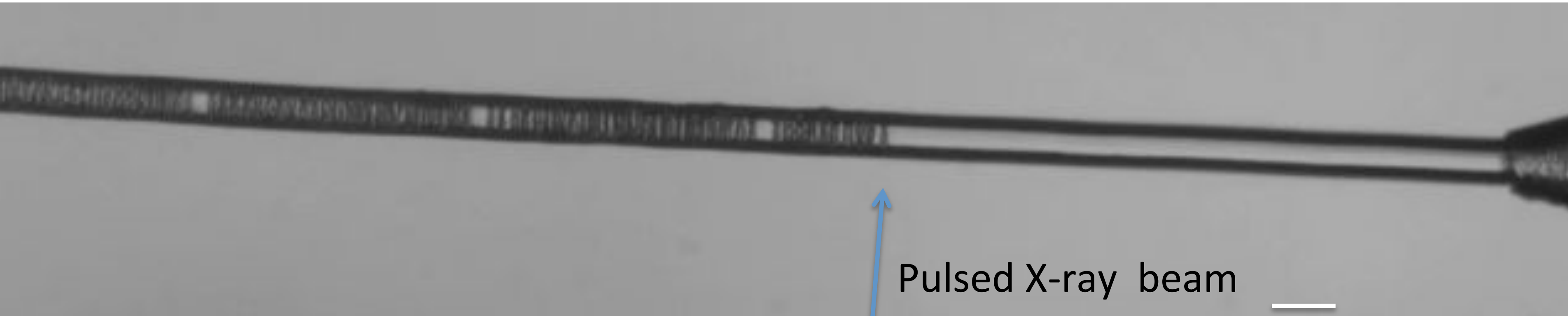
Flow velocity about 10 m/sec

Area * velocity = flow rate = constant.




PSI at 9 keV, 40 fs
to 3 Ang.
Feb 2011 –first high resolution results from LCLS
We have seen xtals as small as
6 unit cells on side.
Fringes
19 periods along g
Streak due to jet
P. Fromme, N. Zatsepin

LCP jet operating at LCLS



50 μm

Optimize conc, rep rate, viscosity, chemistry, particle size, jet size, for each sample.
V = F/A   AV = const.

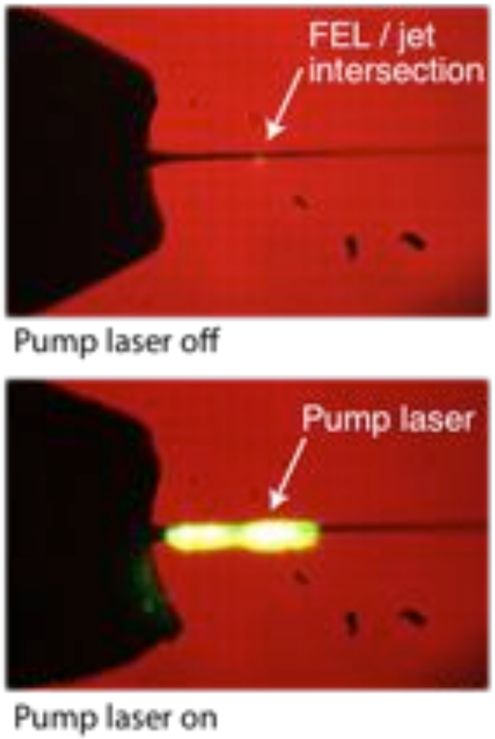
XFEL beam drills 120 holes/sec across LCP tube.
Adjust flow speed to avoid shrapnel from last hole
Gaps due to time-structure of LCLS pulses.

Agarose also works for soluble proteins.
Conrad et all IUCrJ in press. Phycocyanine to 2.6 Ang.

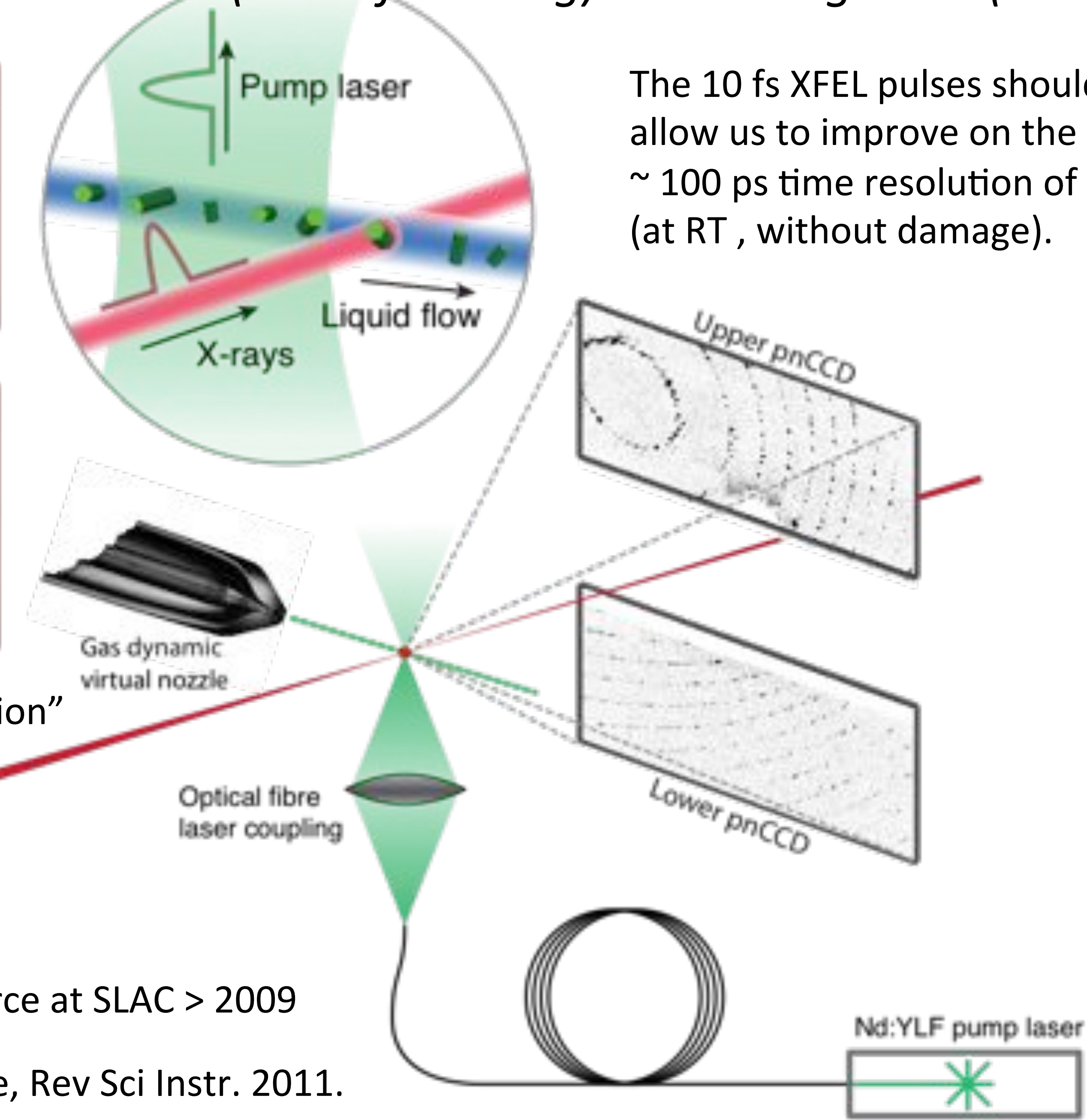
GPCR in viscous LCP at 300 picoliters per minute. LCLS at 1 Hz. 9.4 kV 7% attenuation
50 microliters total used Later 5 microliters/min.

Also works at synchrotrons ! MSX 2 Ang Resolution in bR @ ESRF – Standfuss, Schertler.

Dynamics: *Nanoxtals are sprayed across the pulsed X-ray beam. This allows Time-Resolved SFX (serial fs xtallog). Now in Agarose (PYP)*



The 10 fs XFEL pulses should allow us to improve on the ~ 100 ps time resolution of SR (at RT , without damage).



"Diffraction before destruction"

Linear Coherent Light Source at SLAC > 2009

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

Pump-probe experiments are possible with the liquid jet.

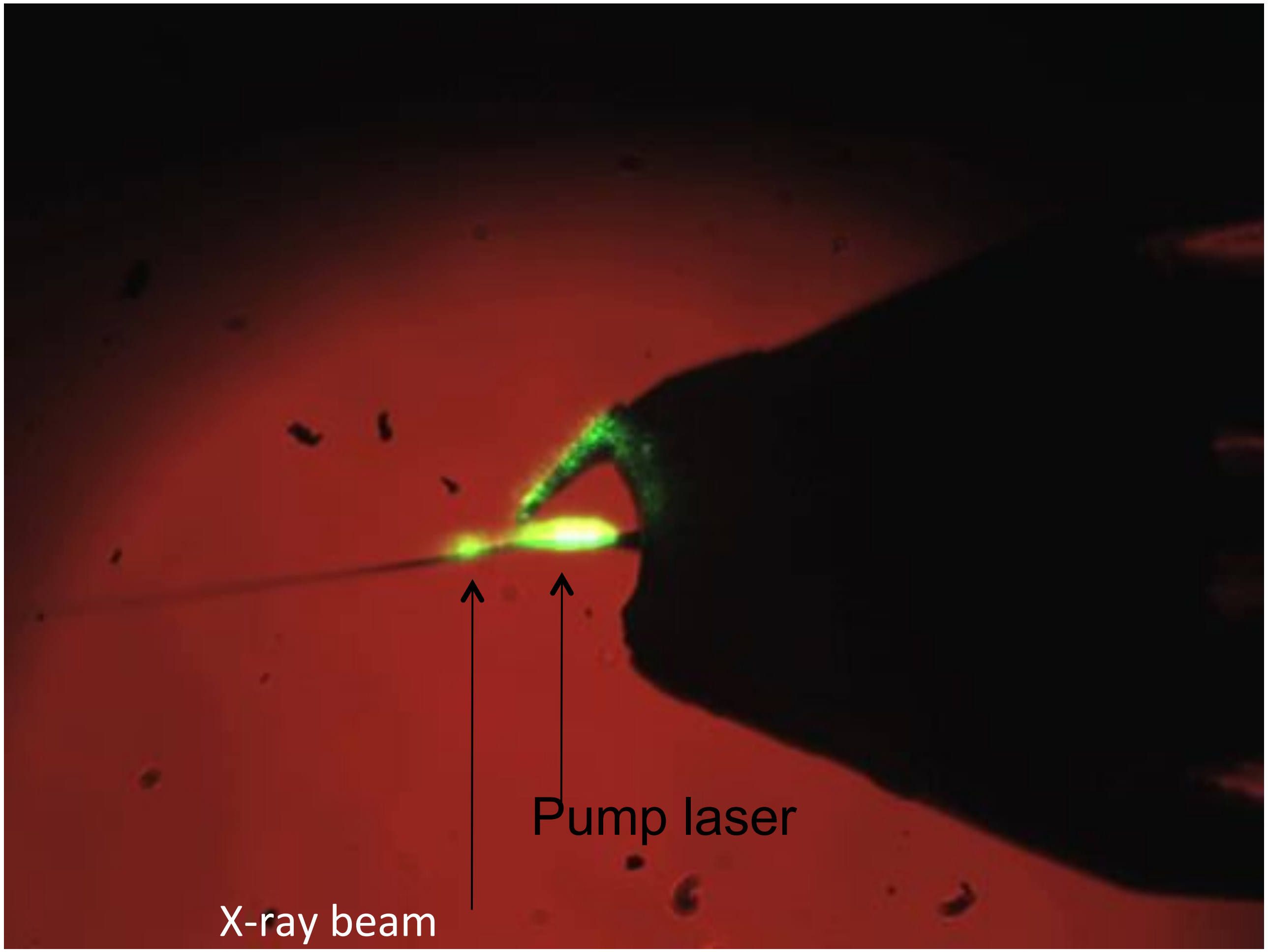
Pump laser and XFEL on jet – exploding PS I nanoxtals

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

Movie

Aquila Optics Express 2012

Kupitz, Fromme et al Nature 2014



pump laser: 532nm, 10ns pulse, 8 microjoules, focused to 380 micron spot, fiber coupled,

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

7 micron beam
0.5-2 mic. xtals
4 micron jet

To observe ***undocking of ferredoxin from PSI***, excite xtal 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.

TR-SFX of Photoactive Yellow Protein (now 1.4 Ang resolution).

Blue light photoreceptor mechanism at 1 μsec & 10 ns time delays.

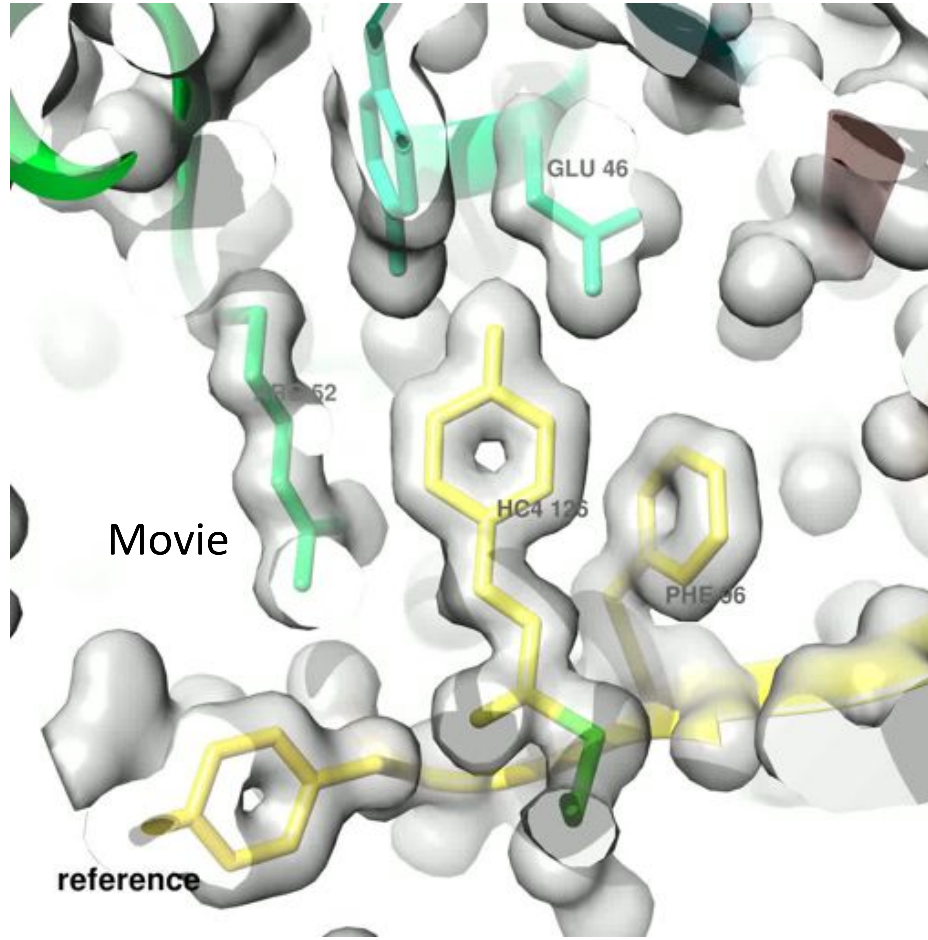
A light-sensor in purple **photosynthetic** bacteria

Pump light saturates nanoxtal

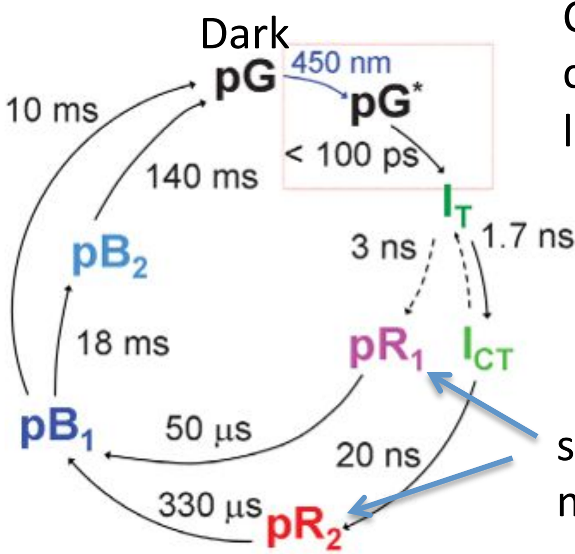
Science Dec 2014
Tenboer…. Marius Schmidt et al
group (Milwaukee) +STC

Delays 10ns and
1 microsec.
pR1 and pR2 are maximally
occupied at 1 microsec.

Red is PR1 then later PR2. Not a diff. map.



Colors on map correspond to labels on cycle



sulfur's move most

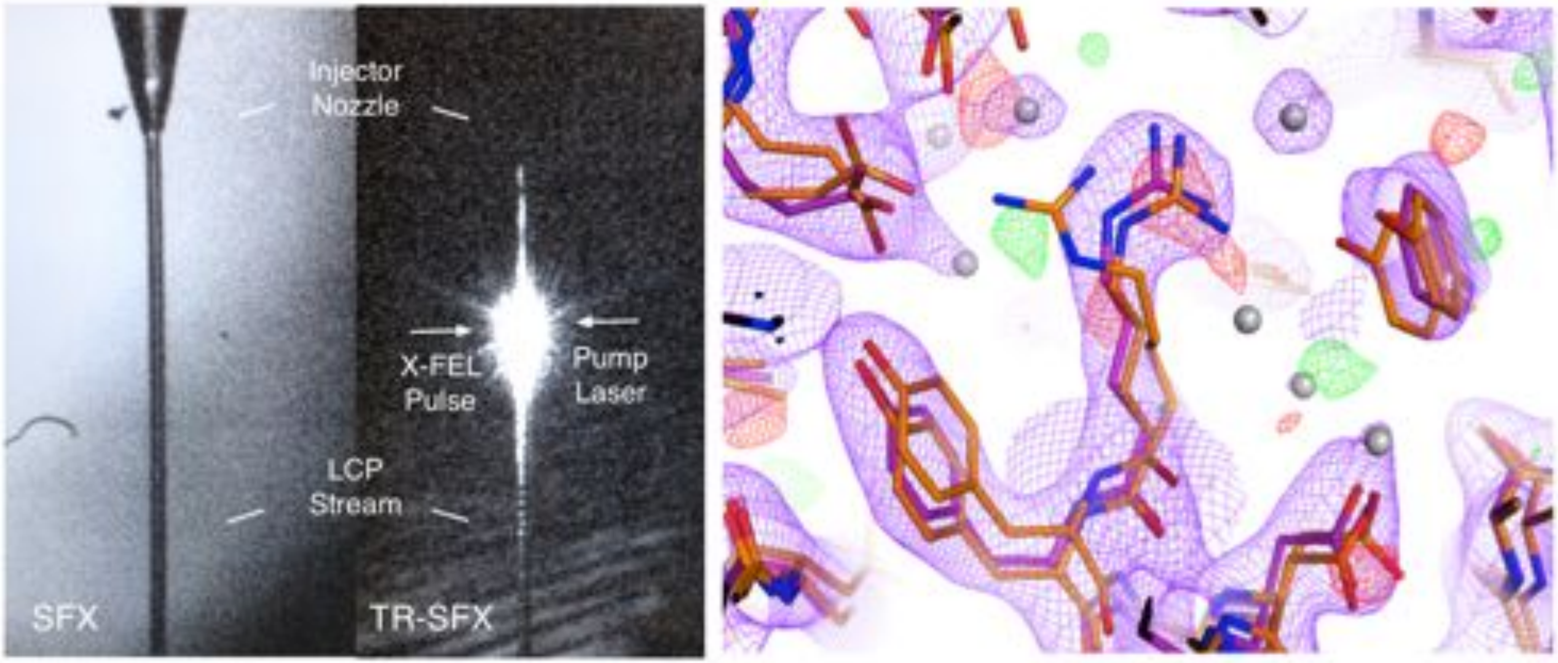
reaction rate lifetimes, exp decay. SVD
Six stable intermediates

Photoactive Yellow Protein, pR_1, pR_2, imaged at LCLS
Shows total density of intermediates that accumulate and decay during the photocycle. Chromophores.

Preliminary Time Resolved SFX-LCP data from LCLS

~ 40000 indexable patterns were collected in 12h of beamtime with 8000-10000 per time point

1 millisec time delay probes M1 state



bR

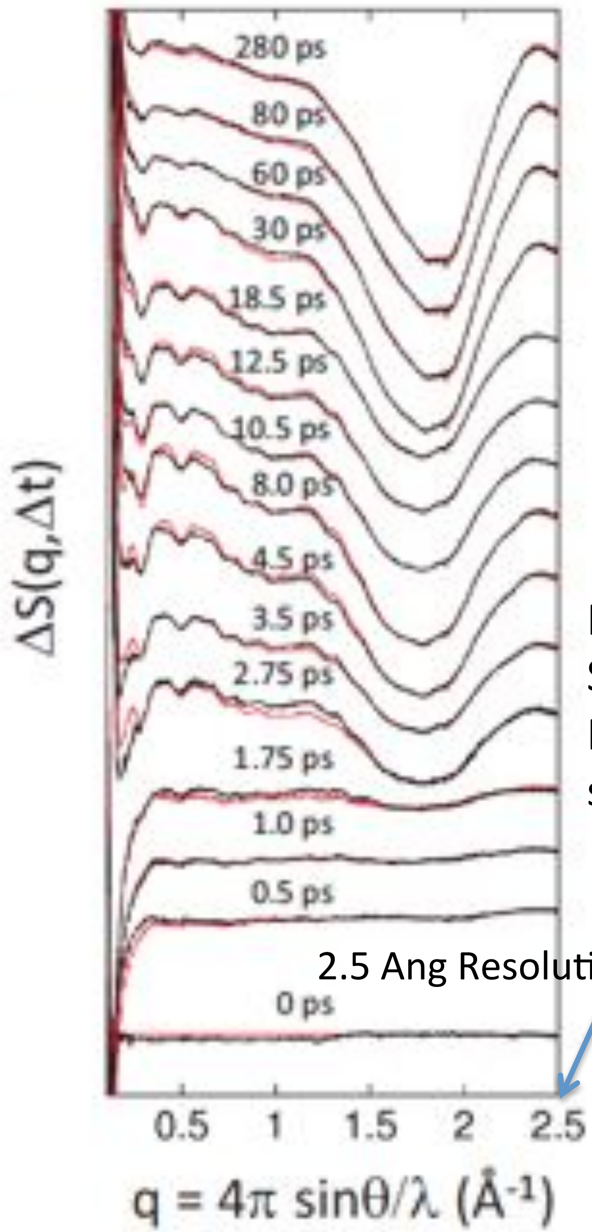
Fo-Fo difference maps at 3 sigma. Compatible with known conformational changes on bR activation for example a rotamer change of Arg82, a key residue in the proton transfer chain.

This is time-resolved (pump-probe) SFX at LCLS in LCP

Standfuss, Weierstall, Schertler et al 2015

Fast solution scattering (FSS) at EXFEL for photosynthesis. Neutze group

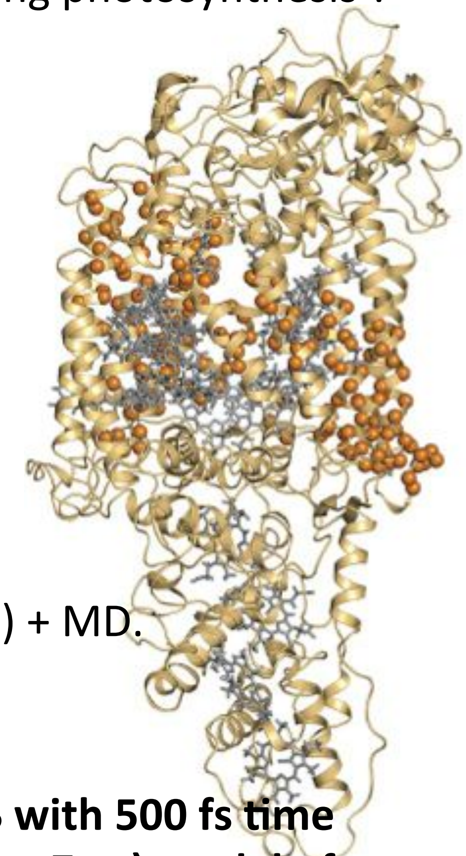
Compare change in scattering due to pump with model   "Snapshot SAXS"



1. Why don't solar photons unfold photosynthetic proteins ? (they have enough energy).
2. Do fast nuclear motions occur during photosynthesis ?

Black: model
Red: experiment.

Orange spheres are carbon atoms which move



Not possible with synchrotron (too fast)
Solution scattering, not crystals
Record time, space resolution for solution scattering ? SVD (4 basis vectors) + MD.

Conclude: 3 Ang model from TR-FSS with 500 fs time resolution supports a "quake" (low q, 7 ps) model of energy dissipation, followed by heat (high q, 14 ps) pulse.
Fast (ps) nuclear motions occur. Quake preceeds heat.

Neutze group. Nature Meth 2014 Arnlund et al .

Dynamics

The quake in Reaction Center responding to a light pulse.

Obtain by Neutze group using fast solution scattering (FSS) at LCLS, pump-probe.

This is a movie.

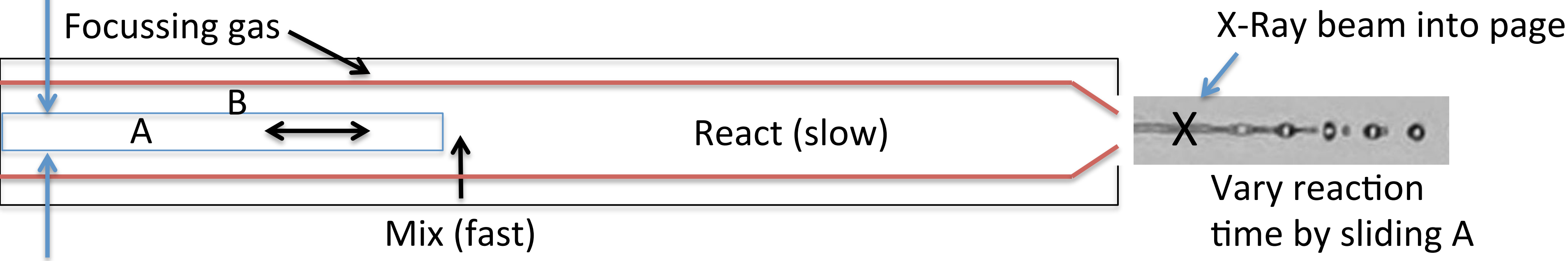
Arnlund…Neutze 2014. Nature Meth.

BioXFEL is developing a mixing jet for dynamics

(eg. study of enzyme/substrate reactions, folding of DNA, tRNA)

D.Wang, J. Spence, U.Weierstall, L.Pollack J. Synch Rad. 2014.

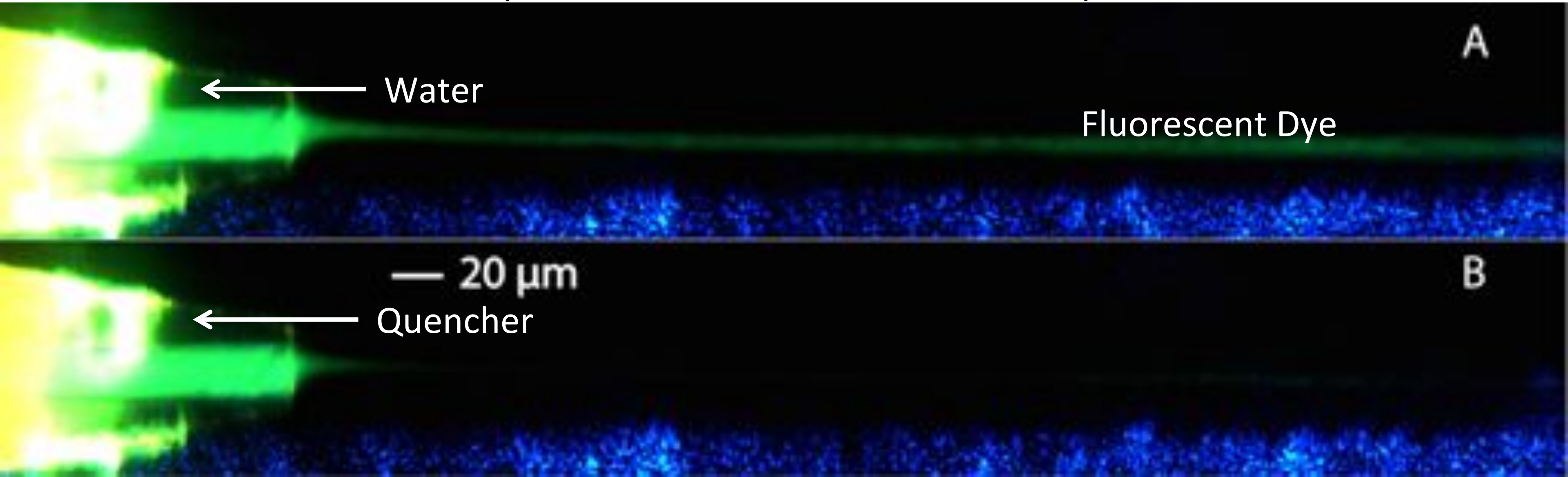
20 microns



Mixing time (time resolution) is 200 - 300 microseconds
Reaction time adjustable from 200 microsec to 1 sec.
Diffusive mixing is possible using protein nanxtals !

Outer flow rate 100 microliters/min. Inner flow rate 0.1 microliters/min.

Diffusion into nanxtals is fast : 17 microsec for glucose into 0.5 micron xtal. Ink.



BioXFEL for improved drug design – high blood pressure

G protein-coupled receptors (GPCR)

- These span the cell membrane. About human 800 GPCRs respond to many extracellular signaling molecules and transmit signals into the cell

- 40% of drugs target GPCRs. 70% of recent drug approvals were GPCRs

- Challenges: low expression yields, low receptor stability after extraction from native membranes with detergent, high conformational heterogeneity

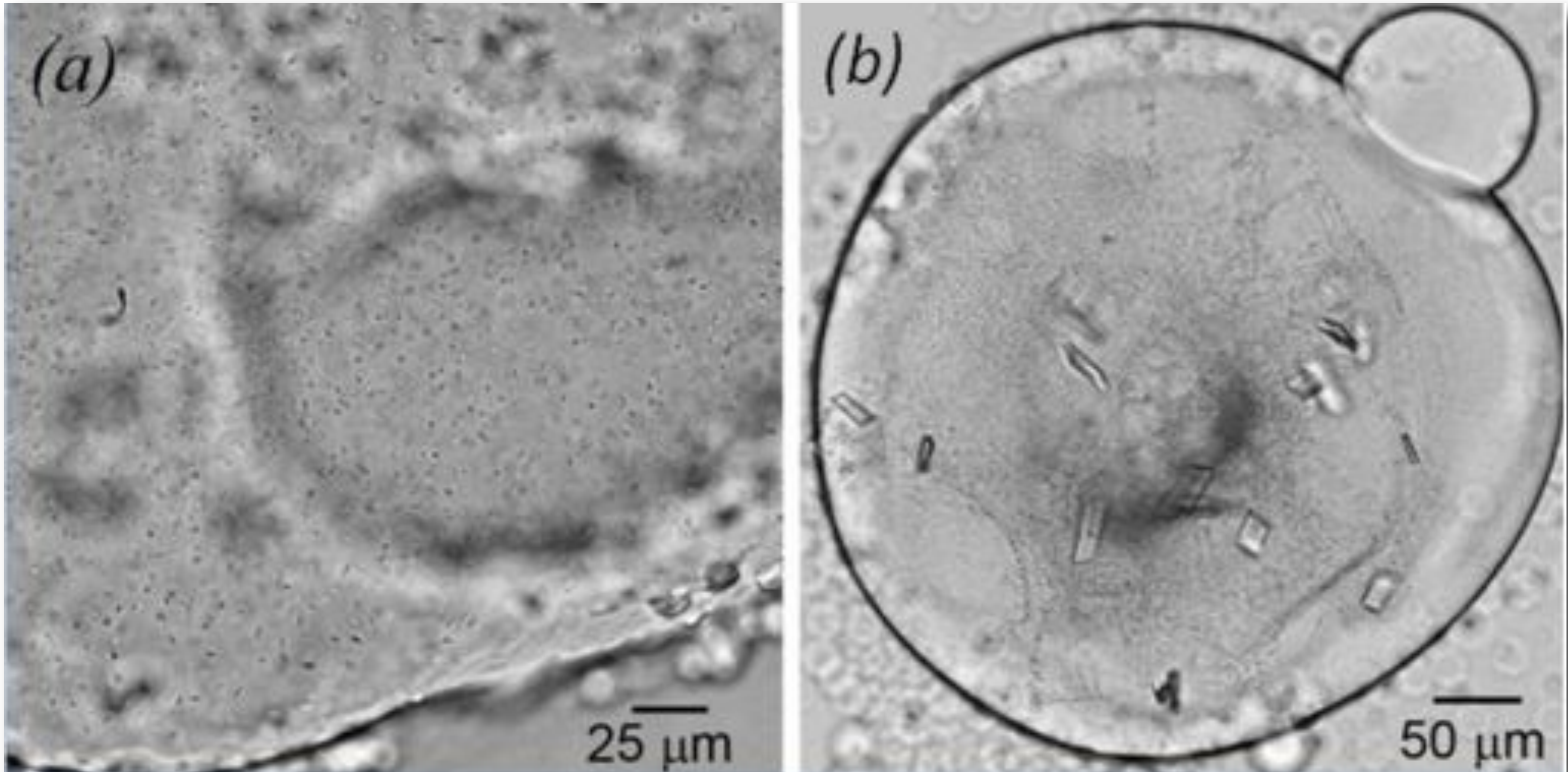
- 19 receptor structures solved so far
- most were crystallized in LCP
- crystals often limited in size, sub 10 micron

- microfocus beamlines have been used, radiation damage severe, merge data from multiple crystals.

Vadim Cherezov/Uwe Weierstall/Ray Stevens

Brian Kobilka Nobel Prize

Crystal size is a major bottlenecks in GPCR structure determination



A typical initial hit contains high-density of 3x1x1 μm^3 crystals suitable for XFEL.

Substantially larger crystals (40x20x7 μm^3), required for microfocus synchrotron data collection, were produced after one year of intensive optimization studies.

Serotonin.

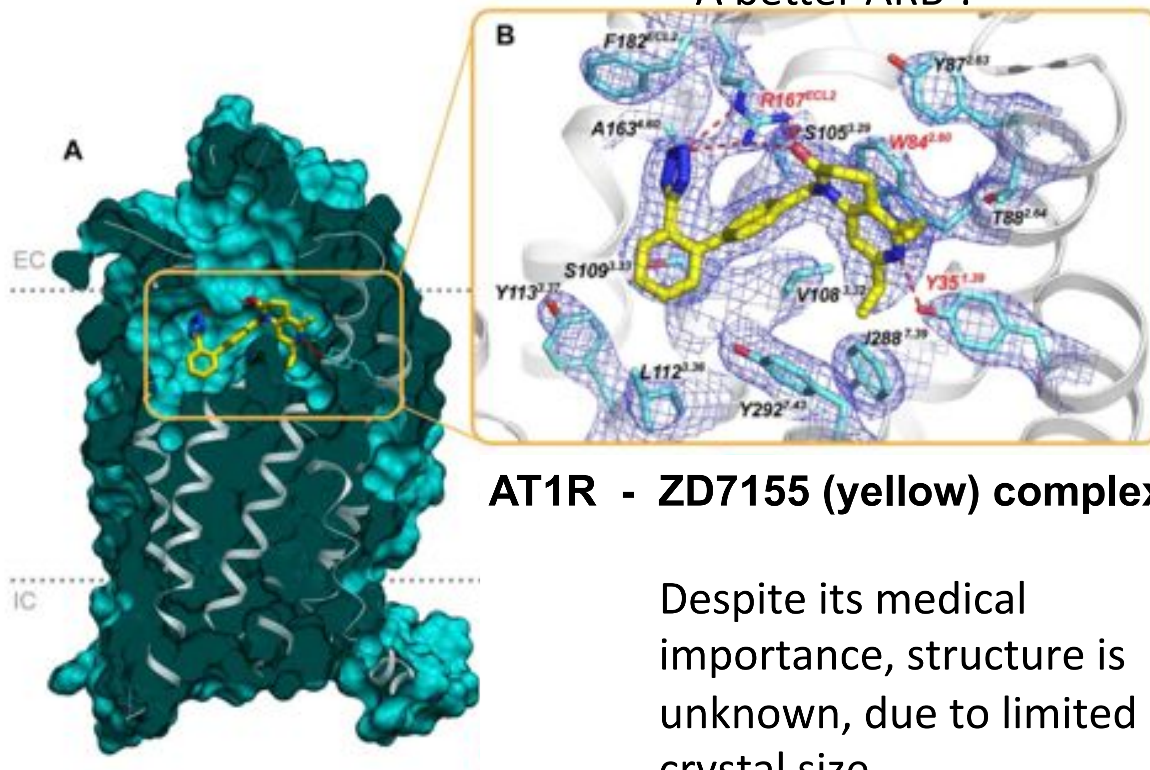
Vadim Cherezov

Structure of Angiotensin Receptor, a Blood Pressure Regulator

BioXFEL NSF SLAC

- Serves as a primary regulator for blood pressure maintenance
- In complex with a selective antagonist ZD7155
- We got 2.9 Å resolution (vs 4 Ang with SR, twinned)

A better ARB ?



AT1R - ZD7155 (yellow) complex

- **Docking simulations of the clinically used AT_1R blockers into the AT_1R structure show distinct binding modes for anti-hypertensive drugs**
- **Results provide fundamental insights into AT_1R structure-function.**
- **Ligand is also arestin-biased agonist.**

Despite its medical importance, structure is unknown, due to limited crystal size.

***First novel GPCR structure solved with SFX**

***Better res than SR.**

The Scripps Research Institute

Fusion proteins added assist crystallization.
Serotonin, δ-opiod, plus 4 other GPCRs....

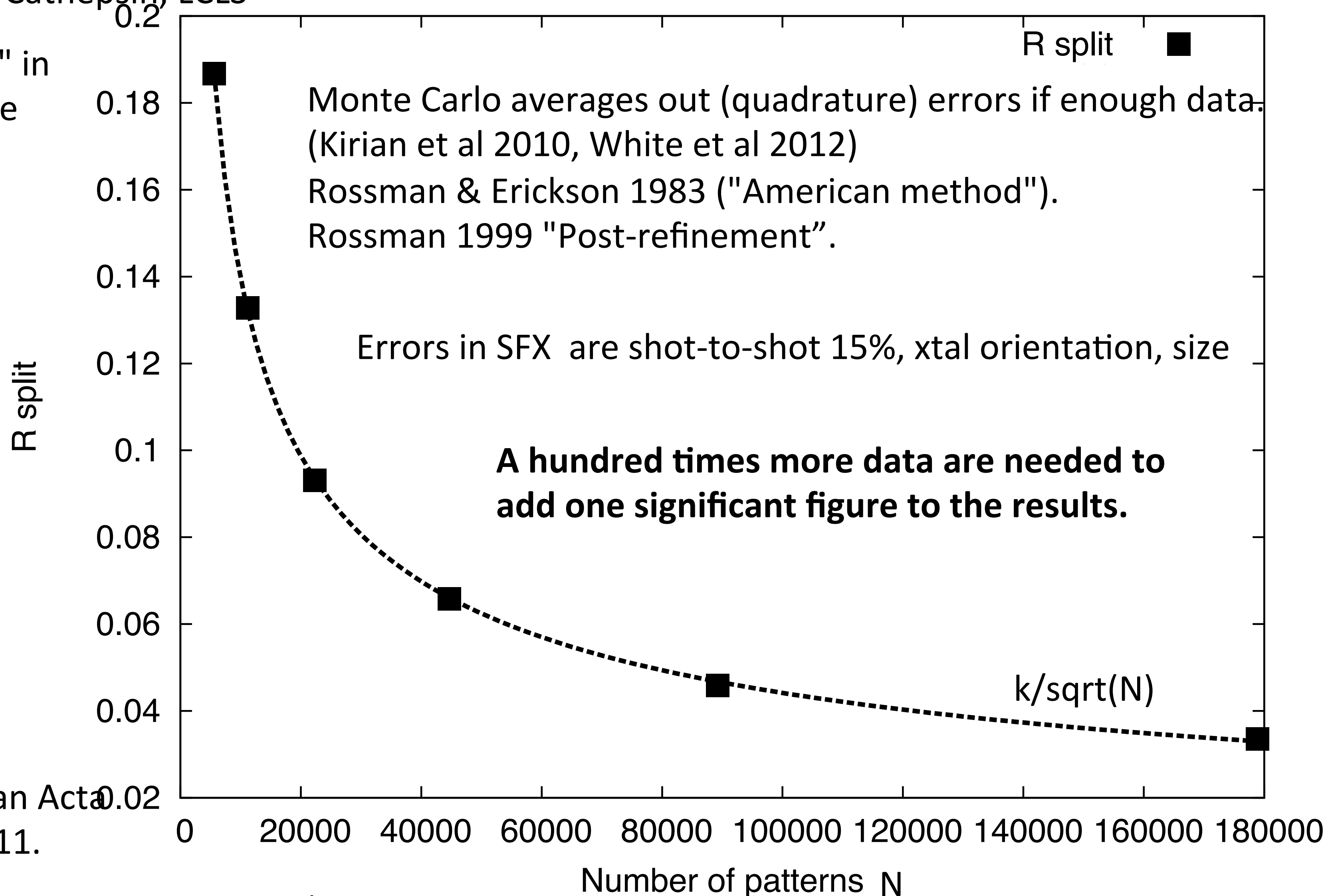
Zhang, Cherezov et al. **2015**, Cell.

BioXFEL is committed to algorithm development

The Monte Carlo method for SFX reduces error as k/sqrt(N)

Data for Cathepsin, LCLS

~ "Error" in structure factors



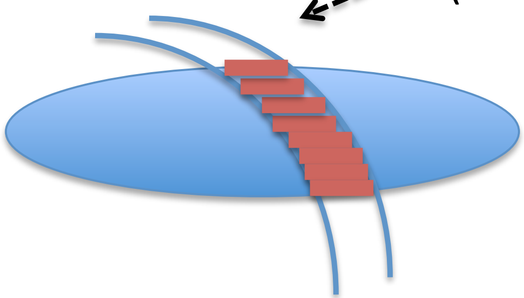
See Kirian Acta Cryst 2011.

Chufeng LI – PhD. Zatsepin/White collab.

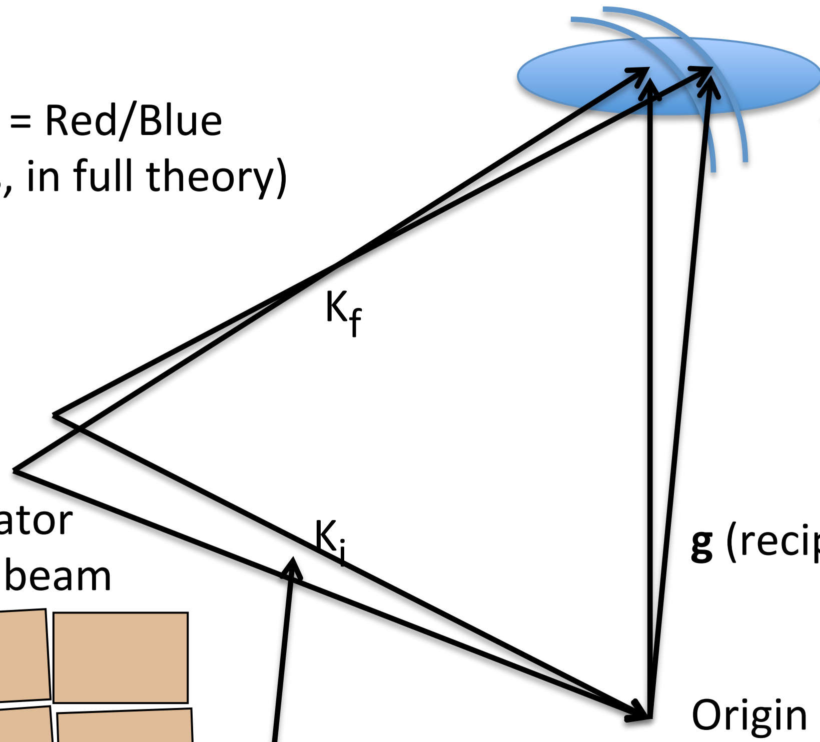
New algorithms now take account of partiality and mosaic spread.

SFX data sets now small enough to allow iterative optimization of experimental params (λ, S_g) on *every* shot – beyond Monte Carlo (MC) averaging. (White, Ginn, Sauter, Brunger, Kapsch…)

Partiality = Red/Blue
(volumes, in full theory)



Each block acts as a monochromator for a different wavelength in the beam

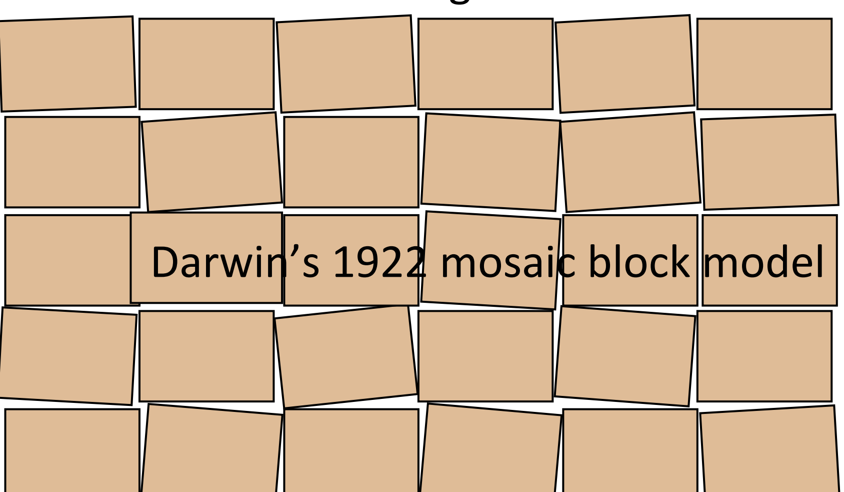


Spherical cap traced out by mosaic spread

g (reciprocal lattice vector)

Origin of reciprocal lattice

Spread of wavelengths and directions in XFEL beam

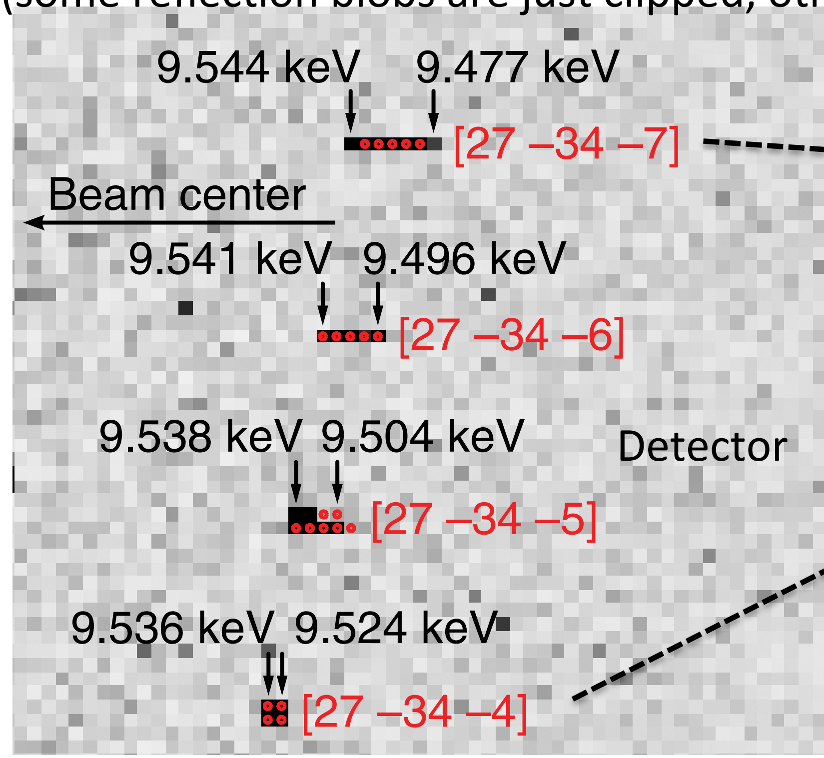


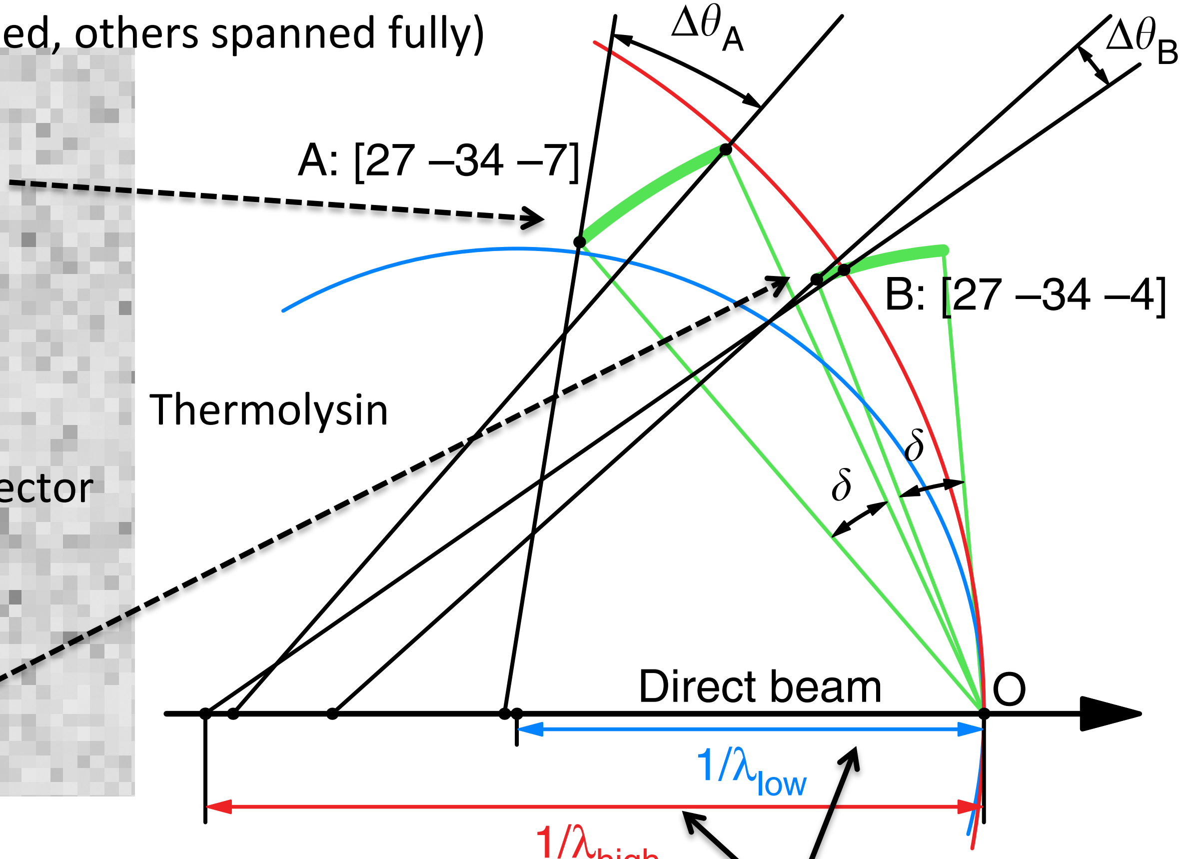
MC is first guess. Then refine each pattern against this, and update MC.

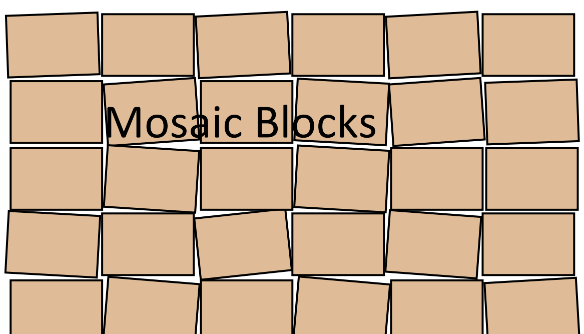
Rossman et al. (1999).

The wavelength spread in XFEL beam spans wider spots at higher angle.

so different fractions of different reflections are uncovered by the range of wavelengths.
(some reflection blobs are just clipped, others spanned fully)







Each tilted mosaic block acts as a monochromator for a different component wavelength in the beam..

From Hattne/Sauter et al Nature Methods 2014

The time for SFX data collection and analysis has been greatly reduced

Protein	2011 Cathepsin	2014 Phycocyanin
Sample injection	GVDN	LCP
Protein size	37 kDa protease	209 kDa hexameric antenna complex
Year of experiment	2011	2014
Data collection took	**5 days**	**2.5 hours**
Crystal hits	293,195	18,794
Indexed hits	**178,875**	**6,629**
Time data took to analyze	**1 year**	**3 months**
Resolution	2.1 Å	1.95 Å

The improvement is due to better detectors, algorithms, samples.

Phycocyanin: SFX results in liquid jet and LCP agreed, but differed from cryo-cooled SR.

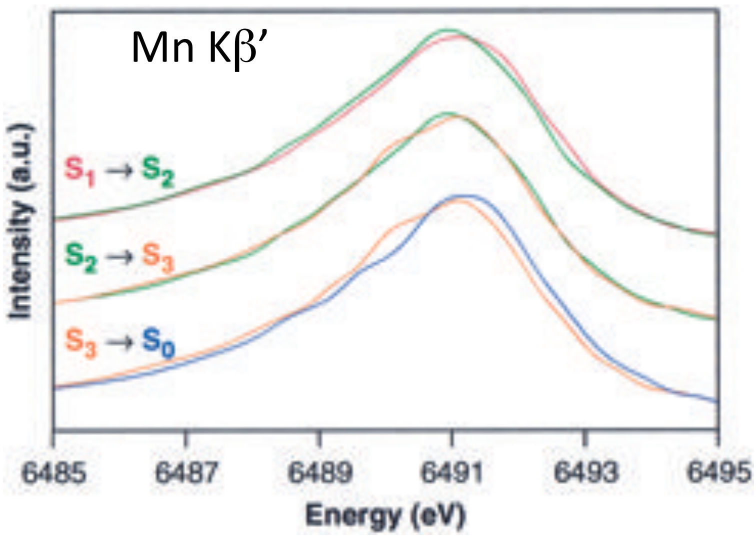
Phycocyanin in LCP: Only 6 µL of crystal suspension of globular protein into LCP.

Ginn, Stuart, et al . **used 6000 patterns (of 144K total) in 30 mins to get 1.74 Ang.** map of Polyhederin CPV17 (Aug 2014). Adjust λ for max # of spots, optimize orient matrix

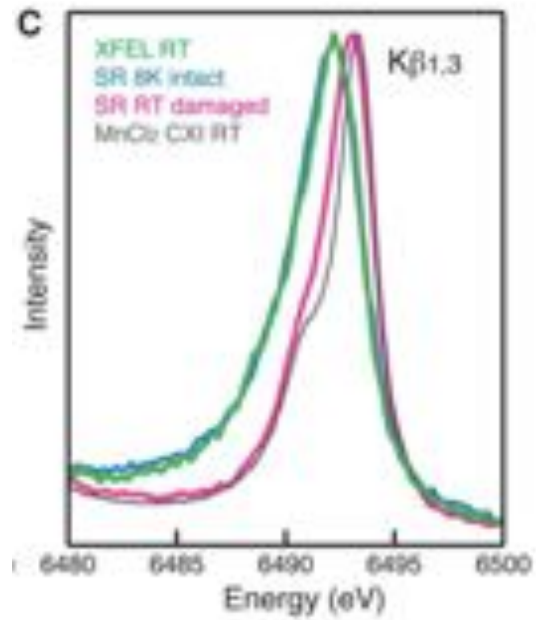
This greatly advances our goal of increased capacity for SFX work and XFEL availability for biology

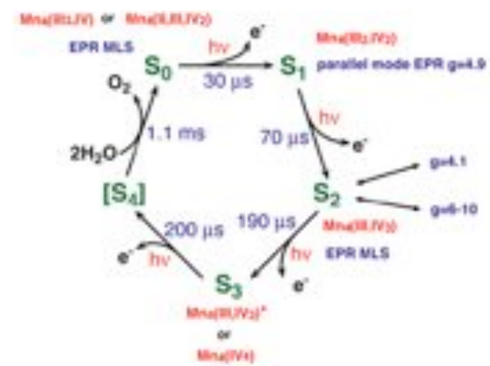
BioXFEL

Simultaneous SFX and spectroscopy will track oxidation states in time.



A different chemical shift for every oxidation state of Mn in the Kok cycle
Messinger, Bergman et al (2001)





Kern et al (2014) see absence of reduction (excess e^-) on Mn Kβ in dark S1 state using XES at LCLS (green). Synchrotron (red) shows reduction “damage”in PS II.

Mn Kβ1,3 line is a probe of the number of unpaired 3d electrons, and oxidation (e- loss) and/or spin state

STC collaborations

*** Fromme, Pushcar**
*** Scott Sayer ASU**
*** Spence/Subramanian**
(fast XAS theory, expts at APS)

Correlate oxidation states with time-resolved density maps

COMPACT XFEL LIGHT SOURCE*

W.S. Graves[#], K.K. Berggren, S. Carbajo, R. Hobbs, K.-H. Hong, W. R. Huang, F.X. Kärtner, P. D. Keathley, D.E. Moncton, E. Nanni, K. Ravi, M. Swanwick, L. F. Velásquez-Garcia, L.J. Wong, Y. Yang, L. Zapata, Y. Zhou, MIT, Cambridge MA
J. Bessuille, P. Brown, E. Ihloff, MIT-Bates Laboratory, Middleton, MA
S. Carbajo, J. Derksen, A. Fallahi, F.X. Kärtner, F.Scheiba, X.Wu, CFEL DESY, Hamburg
D. Mihalcea, Ph. Piot, I. Viti, N. Illinois University, Dekalb IL

Abstract

X-ray free electron laser studies are presented that rely on a nanostructured electron beam interacting with a "laser undulator" configured in the head-on inverse Compton scattering geometry. The structure in the electron beam is created by a nanoengineered cathode that produces a transversely modulated electron beam. Electron optics demagnify the modulation period and then an emittance exchange line translates the modulation to the longitudinal direction resulting in coherent bunching at x-ray wavelength.

The predicted output radiation at 1 keV from a 7 MeV electron beam reaches 10 nJ or 6×10^8 photons per shot and is fully coherent in all dimensions, a result of the dominant mode growth transversely and the longitudinal coherence imposed by the electron beam nanostructure. This output is several orders of magnitude higher than incoherent inverse Compton scattering and occupies a much smaller phase space volume, reaching peak brilliance of 10^{27} and average brilliance of 10^{17} photons/(mm^2 mrad^2 0.1% sec). The device is much smaller and less expensive than traditional XFELs, requiring electron beam energy ranging from 2 MeV to a few hundred MeV for output wavelengths from the EUV to hard x-rays. Both laser and THz radiation may provide the undulator fields.

laser that is within present state-of-the-art technologies and results in a very compact and inexpensive x-ray laser. A facility to test these ideas is currently under construction within the DARPA Axis program and is described below. First though the conditions for FEL gain are reviewed as is the method of producing the nanostructured electron beam.

FEL GAIN

For head-on ICS the resonance condition is given by

$$\lambda_x = \frac{\lambda_L}{4\gamma^2}\left(1+\frac{a_0^2}{2}\right) \tag{1}$$

where λ_x is the x-ray wavelength, λ_L is the laser wavelength, γ is the relativistic factor, and $a_0 = \frac{eE_L\lambda_L}{2\pi mc^2}$ where E_L is the laser electric field. The Pierce parameter that determines many of the FEL properties is given by

$$\rho_{fel} = \frac{1}{2\gamma}\left(\frac{I}{I_A}\frac{\lambda_L^2 a_0^2}{8\pi^2\sigma_x^2}\right)^{1/3} \tag{2}$$

where σ_x is the electron beam size, I is the peak current, and I_A = 17,045 A is the Alfen current. A Bessel function factor in Eq. 2 has been dropped because its value is very

MIT lab-scale XFEL based on Inverse Compton scattering and a patterned beam with emittance exchange, building for ASU. 2015.

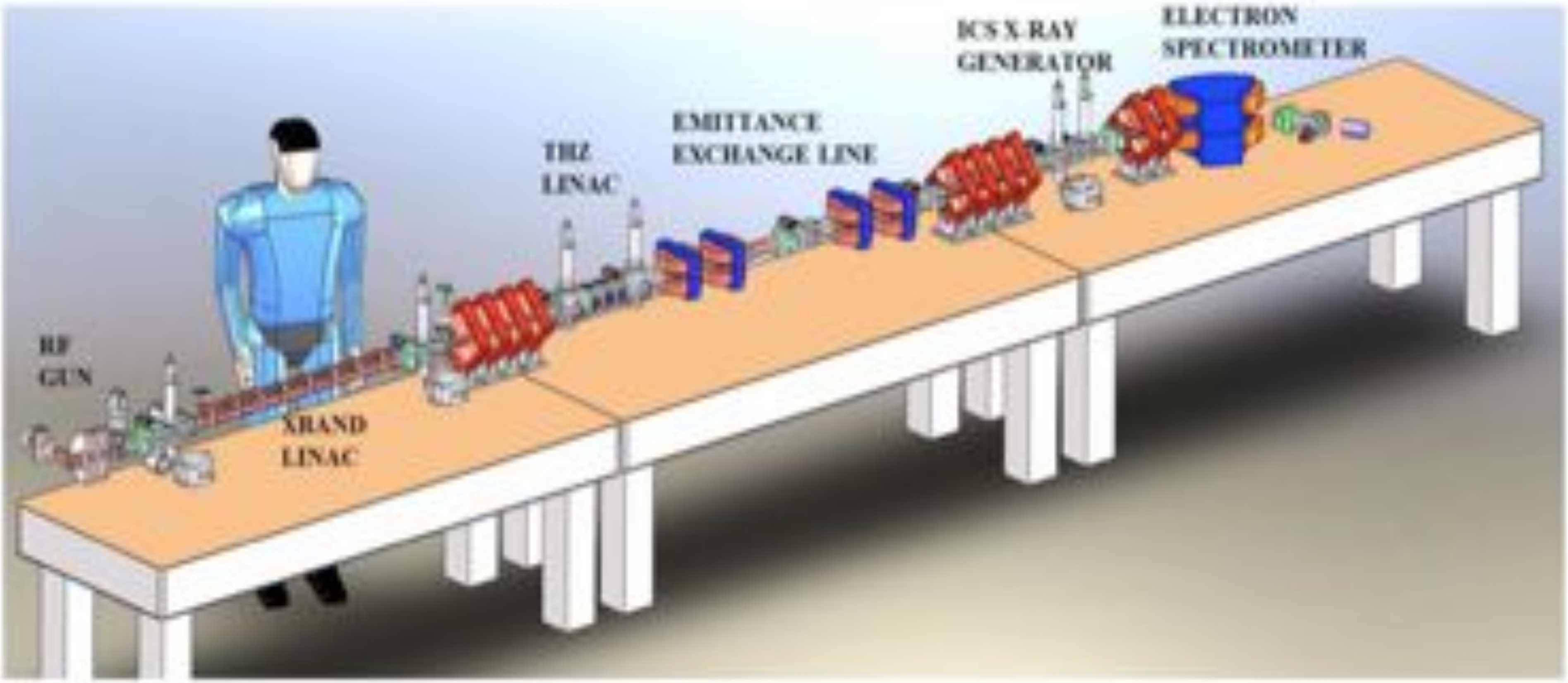


Figure 2. Layout of prototype currently under construction. Major components are labeled and shown mounted on optical tables. Structure is less than 5 m long and will be commissioned in early 2014.

Table 1: Electron beam parameters

Charge [pC]	2
Bunch length [fs]	28
Peak current [A]	70
Energy spread [%]	0.01
Spot size at IP [μm]	0.5
Emittance [nm]	10
Repetition rate [Hz]	1000
Energy [MeV]	7

Table 2: X-ray parameters

Photon energy [keV]	1
Pulse energy [nJ]	10
Photons/pulse	$6X10^{8}$
Photons/sec	$6X10^{11}$
Source size [μm]	0.5
Source divergence [μrad]	200
Peak brilliance [phot/s 0.1% mm^2 $mrad^2$]	10^{27}
Avg brilliance [phot/s 0.1% mm^2 $mrad^2$]	10^{17}

MIT (Graves et al) Compact XFEL with patterned electron beam and emittance exchange

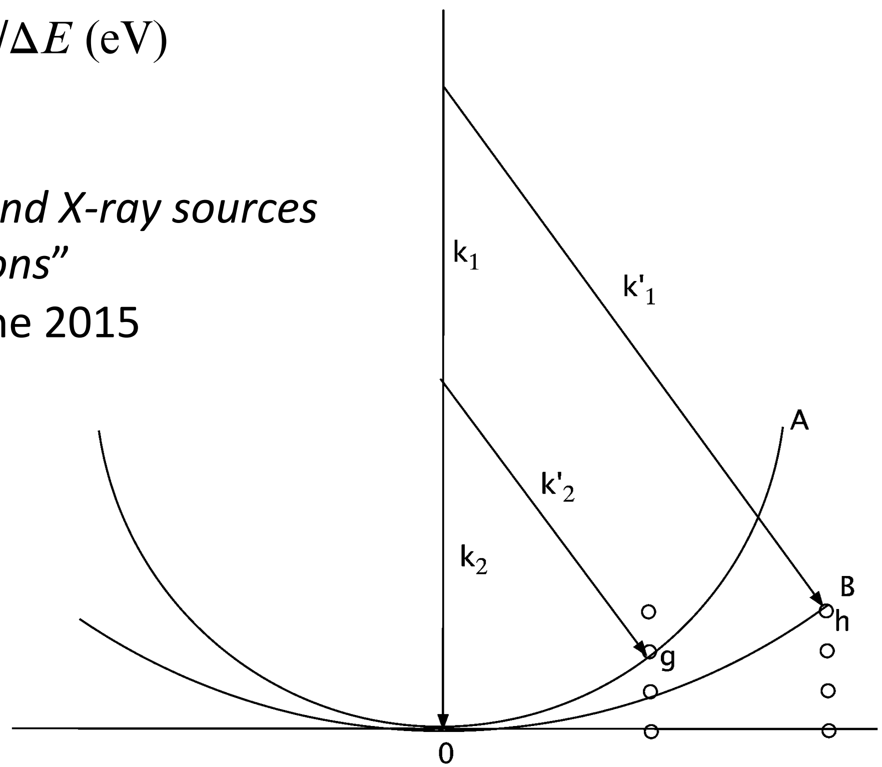
Attosecond pulses give Laue mode for TR-SFX

Attosecond XFEL avoids damage, gives full Braggs, not partials.

F

$$\Delta t\ (\text{fs}) = 4.14/\Delta E\ (\text{eV})$$

"Compact attosecond X-ray sources and their applications"
Workshop CFEL June 2015



Different wavelengths cause different Bragg reflections to interfere on same detector pixel , if pulse duration less than beat period.
This gives structure factor phase information .

Attosecond XFEL gives 300 eV = 3% bandwidth at 10 kV with 14 as pulses, hence full reflections, without damage.
Coherence length $L = \lambda E/\Delta E \sim 3$nm, less than sample thickness !
3-phase sums (as above , plus Friedel conjugate of sum) are origin independent.

Spence, Trans Faraday Soc 2015

The international XFEL context

	FEL Facility	Location	Electron Energy (GeV max)	Photon Energy Range (keV)	Rep Rate	Linac Technology (NC or SC)
	LCLS-1kHz	SLAC, USA	19	0.24-25	120 Hz	NC
2017	EXFEL	DESY, FRG	17	0.4-20	10 Hz, 2700 pulse burst	SC
	FERMI	Trieste, IT	1.25	0.01-0.4	50 Hz	NC
	FLASH	DESY, FRG	1.25	0.01-0.2	10 Hz, 800 pulse burst	SC
2020	LCLS 2	LBNL, USA	2.4	0.23-2	1 MHz	SC
2017	PALXFEL	PAL, Korea	10	0.28-20	60 Hz	NC
	SACLA	Harima, Japan	8	5-15	60 Hz	NC
2017	SwissFEL	Switzerland	6	1.8-12	100 Hz	NC

STC has two SACLA beamtimes before summer 2015.

SwissFel is like LCLS I.

Summary

1. The invention of the free-electron X-ray laser has made possible molecular movies with femtosecond time resolution. (Debye period = 100 fs = 100E-15 sec)

2. These hard X-ray pulses are so brief that they can "out-run" radiation damage*.

3. They have been used to make movies using snapshot Bragg diffraction from protein nanocrystals by serial fs X-ray diffraction (SFX) to begin to reveal the detailed atomic mechanism of photosynthesis, which supports life on earth.

4. They have been used to image small drug molecules bound to signalling membrane proteins (GPCRs). This helps refine better drugs, eg blood pressure.

Building blocks have explanatory power for understanding mechanisms in matter.
eg The α-helix in bio - need only 6-9 Ang resolution to see it.
- Atoms at 2 Ang for mat sci., cond matter. Eg kink landscape.

Current XFELs need Bragg Boost (& lasing) to see them (or modelling, Bayesian)
So either learn to make 10x10x10 xtals or build 1E6 times more powerful XFEL !

*Chapman et al Nat Meth 2006

Exciting times ahead !

*Data analysis team: Zatsepin, Grant, Messerschmidt

BioXFEL

http://www.bioxfel.org

The NSF BioXFEL STC Research Consortium

Biologists

Techniques (Methods Group)

established Oct 2013

BioXFEL STC

University@Buffalo HWI
E. Lattman, E. Snell, T. Grant*
Outreach, nanocrystals, data

ASU
J. Spence. Zatsepin*
Science, Data analysis

Center for Applied Structural Discovery (CASD) ASU

U. Wisc. Milwaukee
M. Schmidt
Energetics of Bioreactions

Rice
G. Phillips:
conformational variability

UCSF
R. Stroud
membrane proteins

ASU
P. Fromme:
membrane proteins
B. Hogue: viruses

Stanford
R. Kornberg
D. Bushnell: Pol II, S.P. transcription complex

Cornell
L. Pollack:
mixing reactions

Puerto Rico International conference,
Data Analysis Workshop,
LCP injector sales (17), Beamtimes, LCLS 2.
Crystallization workshops at HWI.

LBNL, LLNL
Unfunded collaboration
J. Holton, M. Frank, S. Hau-Riege

U. Wisc. Milwaukee
A. Ourmazd, D. Saldin:
SP data analysis

ASU
injectors
Weierstall, Kirian

DESY
Unfunded collaboration
Chapman, Messerschmidt*

The End

BioXFEL

The birth of a new field

Phil Trans BioXFEL special issue 2014. Vol 369, Spence and Chapman Eds

Structural Dynamics. Special Issue (Puerto Rico BioXFEL conference), Jan 2015, Ourmazd Ed

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

The last fifteen years has seen two important breakthroughs in imaging science :
Lensless imaging and outrunning damage..

THE END

"Physics is a problem in search of a solution; Biology a solution in search of a problem".

"The successful man adapts himself to the world, the failure tries to change it.
Therefore all progress depends on loosers". GBS