Future Research Infrastructure, Opportunities and Challenges,
Varenna, Italy, July 10, 2015

Structural Biology using XFEL: Status and future accelerator based infrastructure requirments

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Outline: XFEL structural biology

- Integrated structural biology
- Recent results from LCLS
- New XFEL beamlilne project: macromolecular femtosecond crystallography
- Development of new XFEL mode: Two-pulse/two-color development for phasing and radiation damage: close collaboration with accelerator physicists
- Single particle imaging consortium
- LCLS-II project

Frontier of bioscience: most challenging problems require hybrid methods with multiple length- and time-scales

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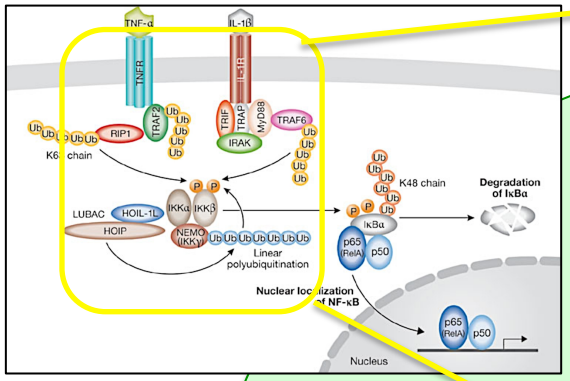
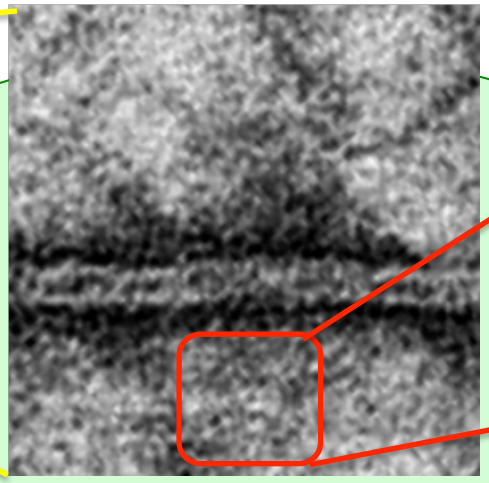
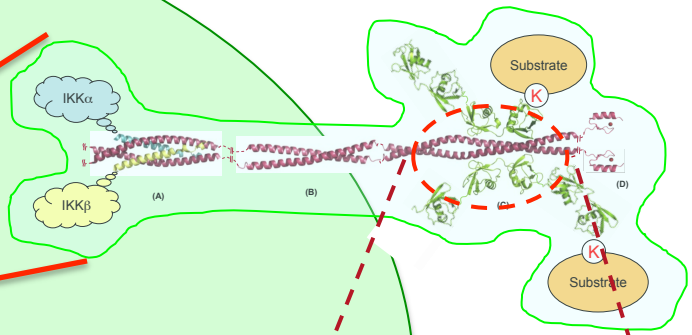


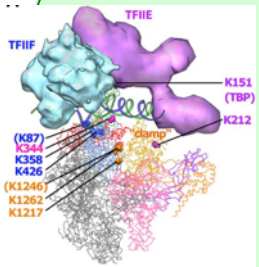


Cryo-EM

Dynamics:
NMR & SAXS

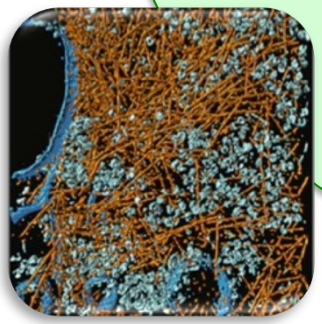


Cryo-EM single particle analysis

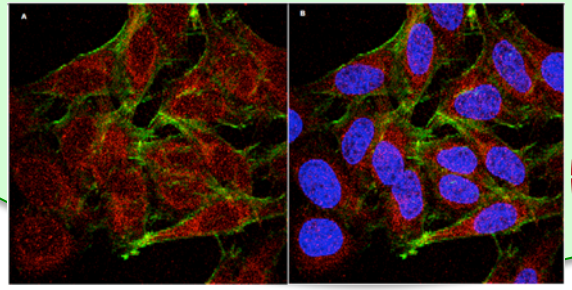


Integration

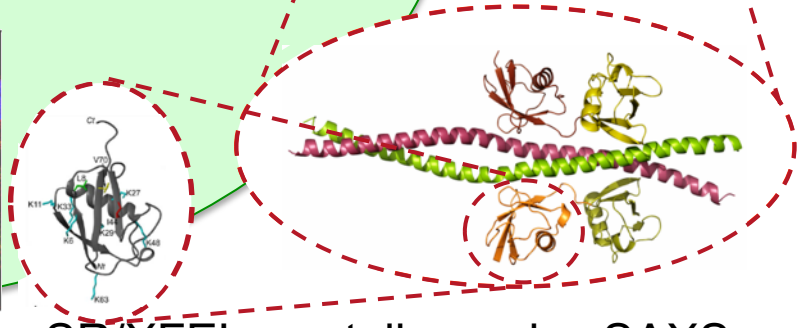
- Correlation between different scales
- Manipulate (pump) and measure



Cryo-electron tomography with focused ion beam (FIB)

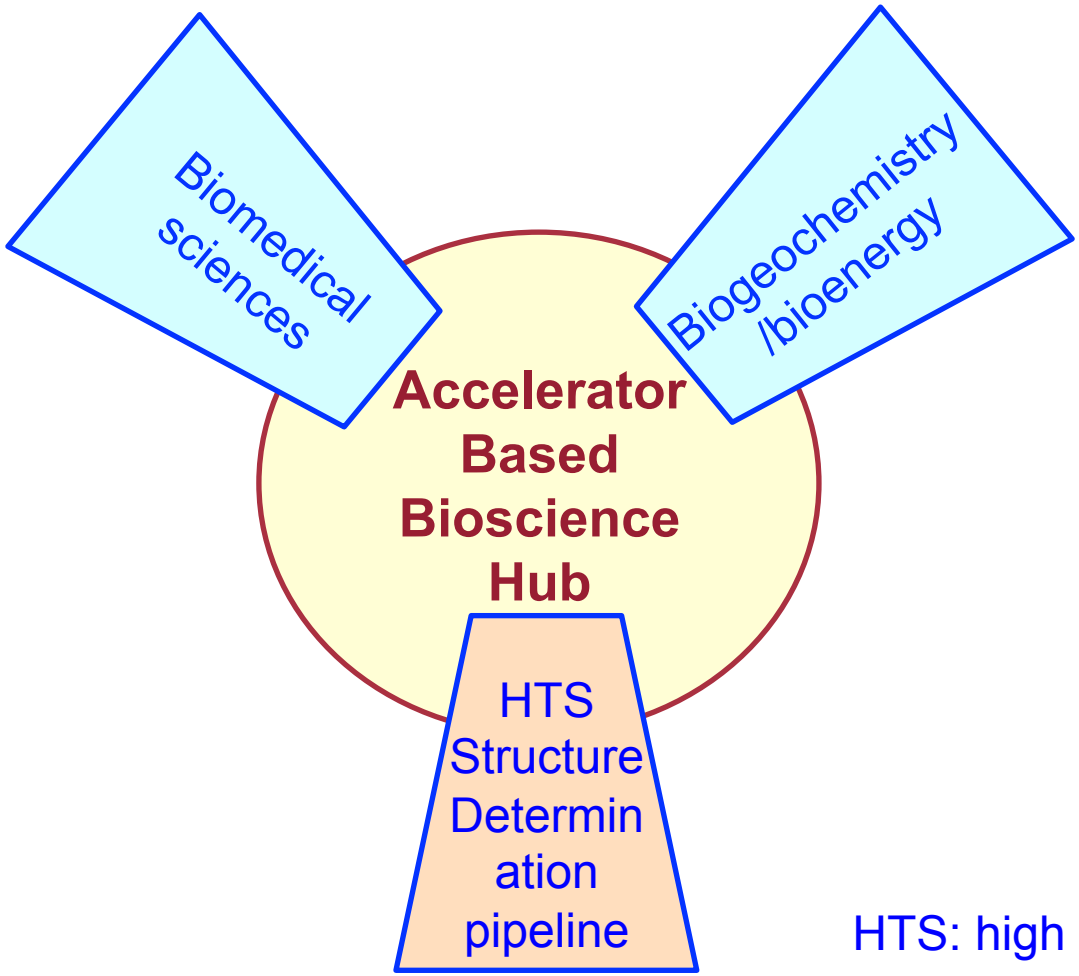


Super-resolution microscopy



SR/XFEL crystallography, SAXS, XAS, imaging (Rahighi et al.)

Accelerator based bioscience hub strongly coupled to biology problems



HTS: high throughput

Example of integrated x-ray structural biology: SLAC BioScience Strategy

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LCLS

PX, SAXS, XES, imaging

MFX (in-air)
CXI (in vacuum)

SSRL

Crystallography, SAXS, XAS, imaging

BL12-1 microfocus and other BLs

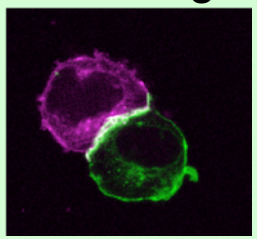
Sample delivery systems and screening

Synergy

- Instrumentation
- Computation

CryoEM core expertise and facility

Super-resolution optical imaging



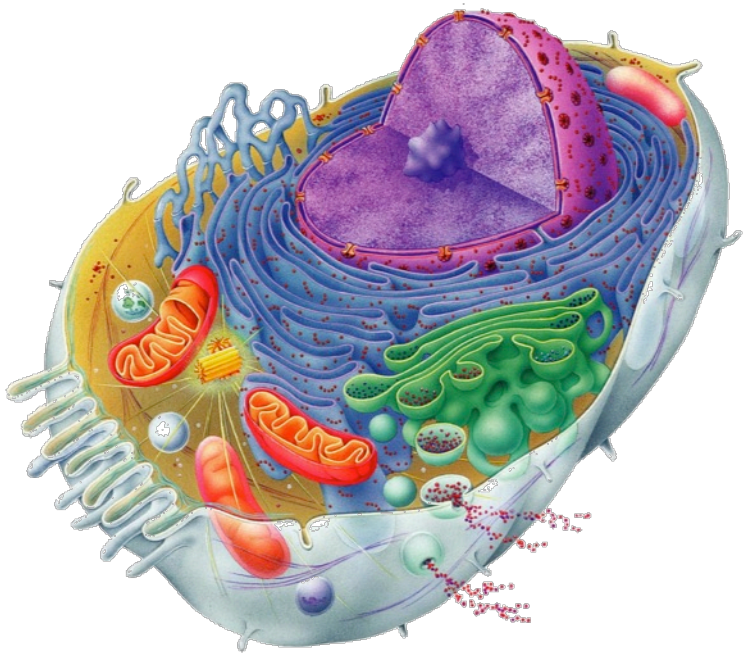
Single particle 3D reconstruction algorithm & ultra short e^- beam development (UED/UEM, damage free structures)

Multi length scale structural analysis of large complexes

Science case and XFEL tools for biology

Biology

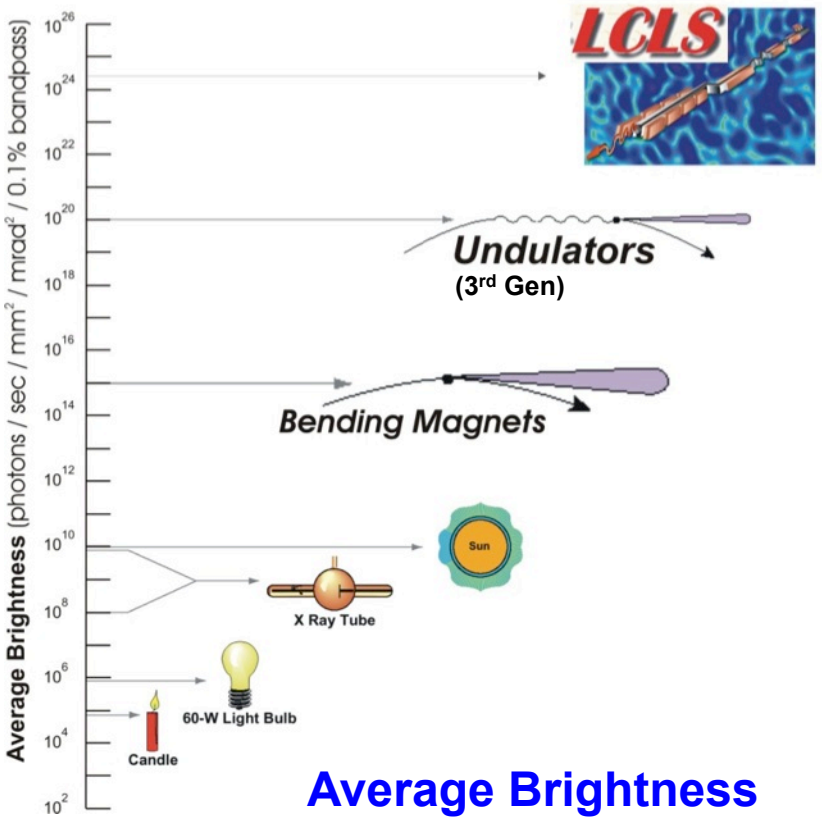
- Cells, viruses, organelles (e.g. carboxysome)
- Macromolecular complexes (kinetochore, mediator complex, CRISPR, RNA biology, etc.)
- Dynamics
- Electronic states



XFEL tools

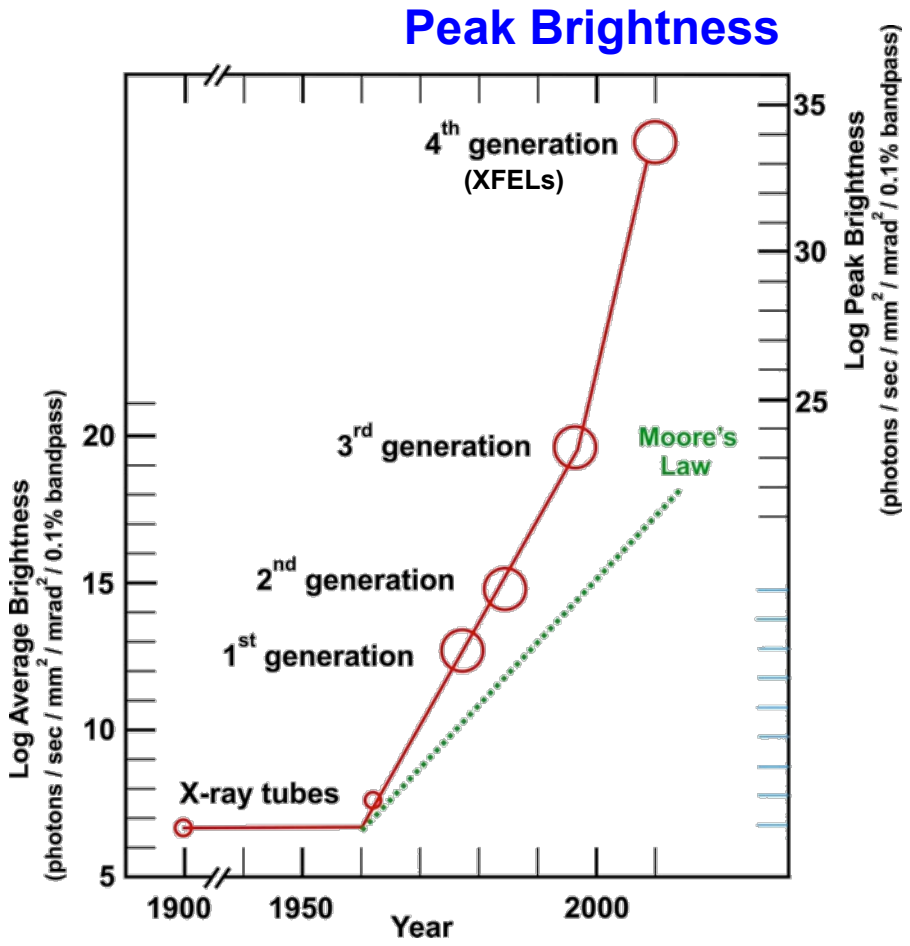
- Femtosecond crystallography
- Fluctuation (correlation) x-ray scattering
- SAXS/WAXS
- Single particle imaging

X-rays – the Brightness Impact of Synchrotron Radiation



Average Brightness

Peak Brightness



The World Scene in Hard X-Ray FELs - in Operation & Under Construction

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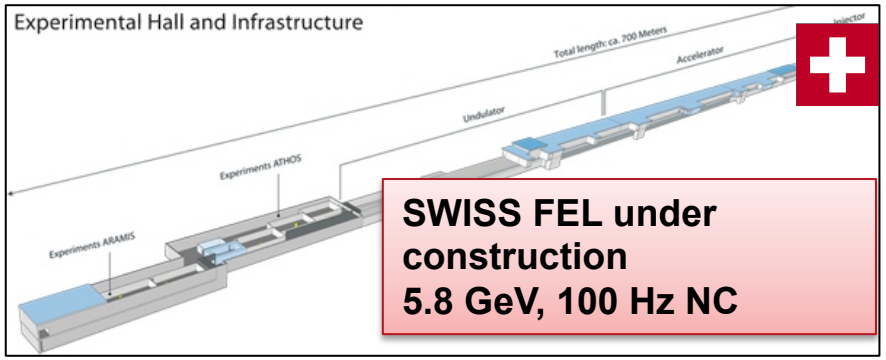
LCLS-I, II 2009, 2019
14.5 GeV, 120 Hz NC

SACLA 2011
8.5 GeV, 60 Hz NC

PAL XFEL under construction
10 GeV, 100 Hz NC

XFEL under construction
17.5 GeV, 2700 x 10 Hz SC



SWISS FEL under construction
5.8 GeV, 100 Hz NC

NC: normal conducting acceleration, SC: super conducting acceleration

Aims and goals: future of structural biology at LCLS X-ray Free Electron Laser (XFEL)

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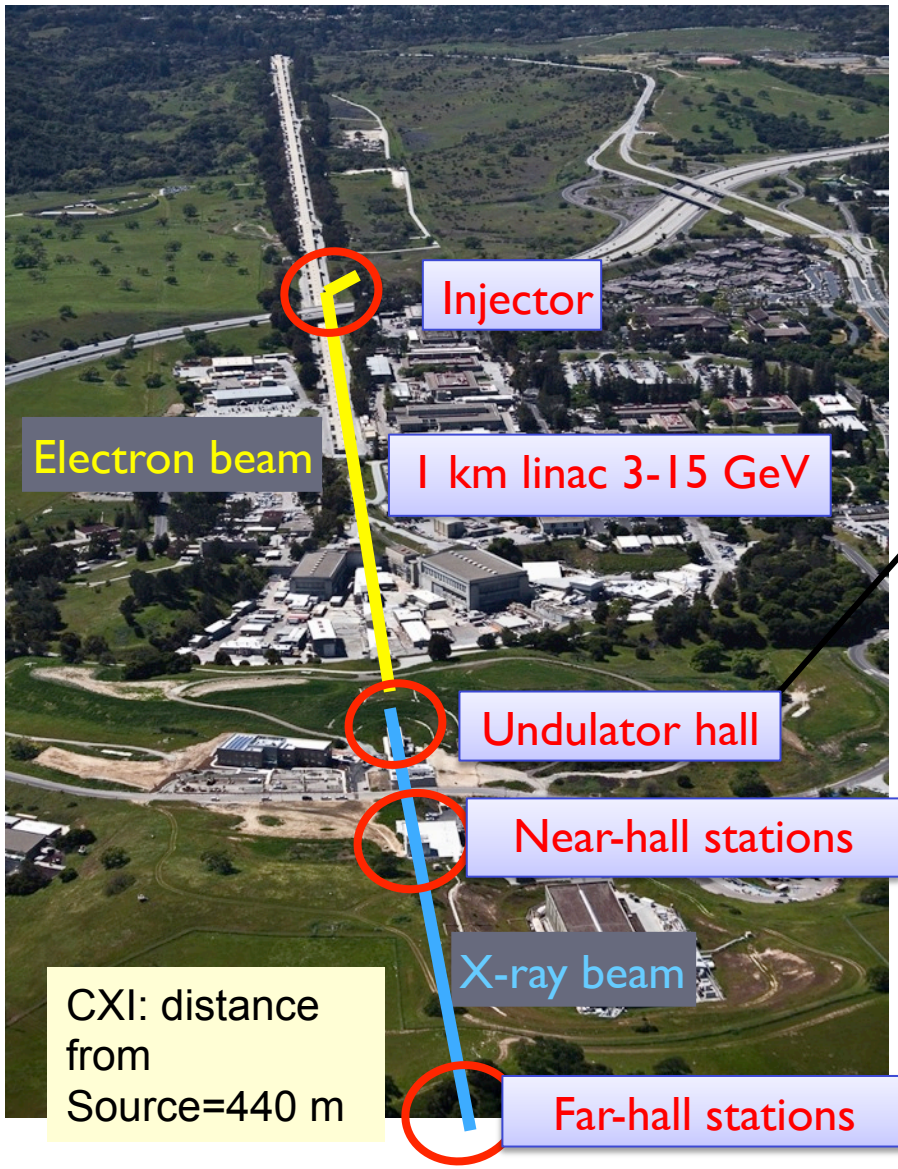
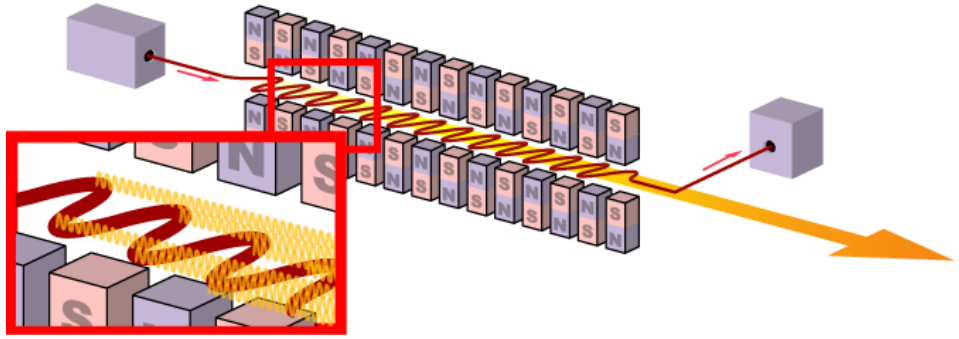
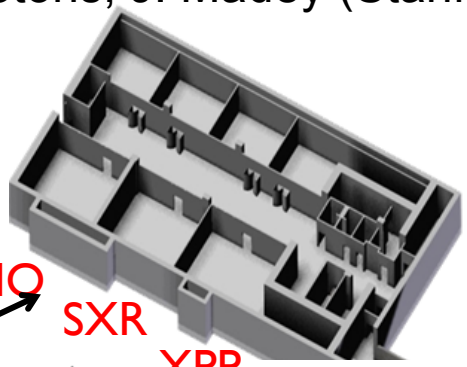
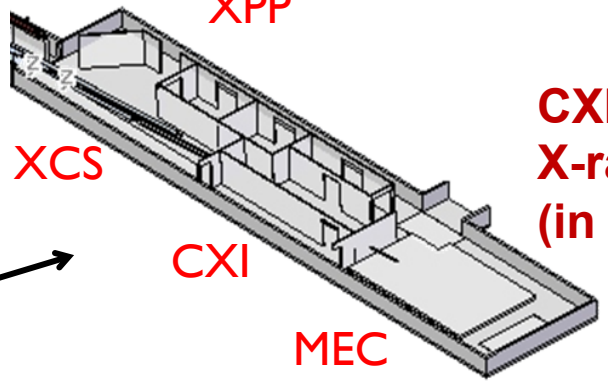




Electron motion in phase with field of emitted photons, J. Madey (Stanford, 1970)

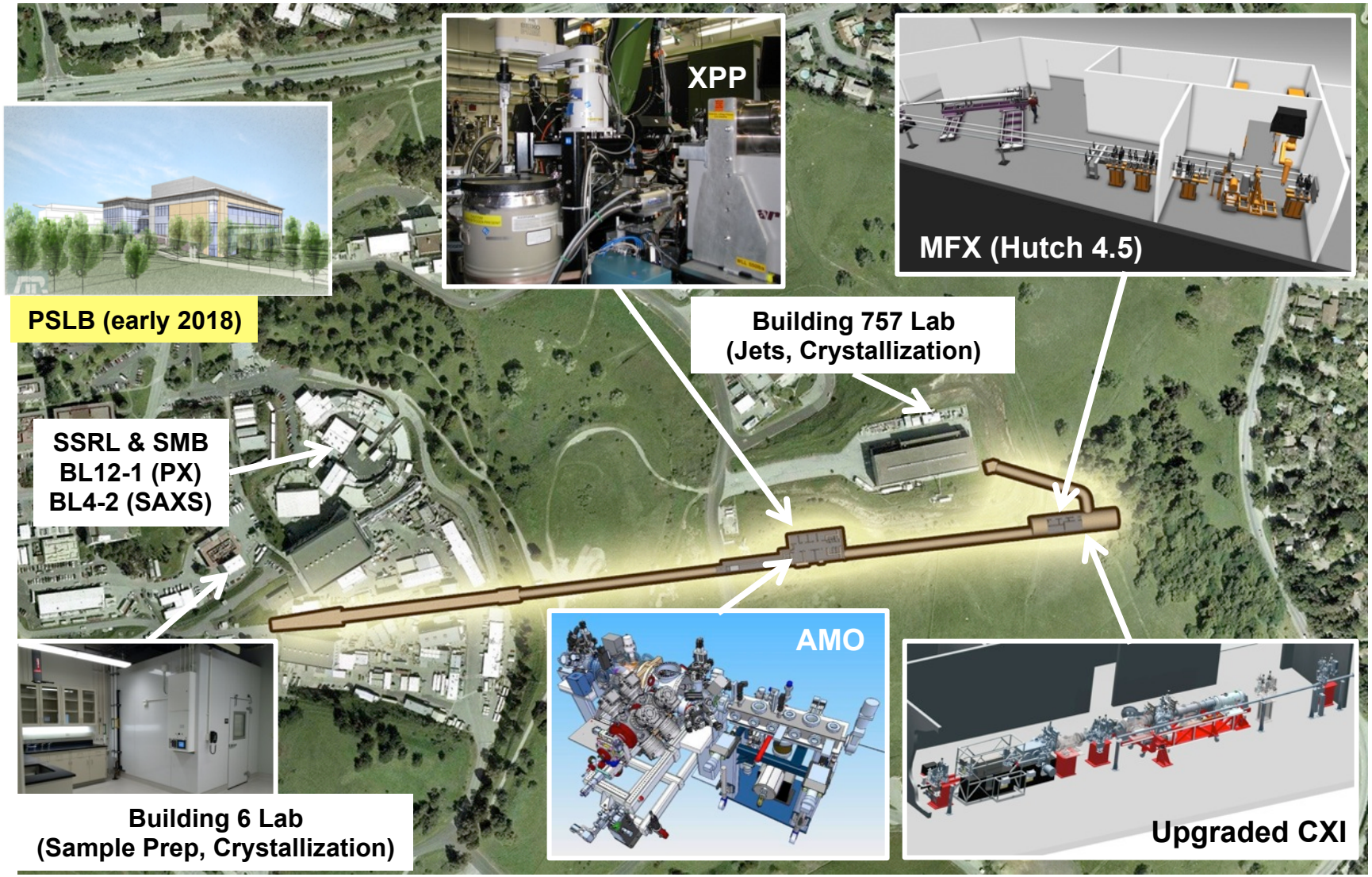


XPP: X-ray Pump-Probe (in air)



CXI: Coherent X-ray Imaging (in vacuum)

Suite of integrated structural biology facilities at SLAC related to LCLS initiatives

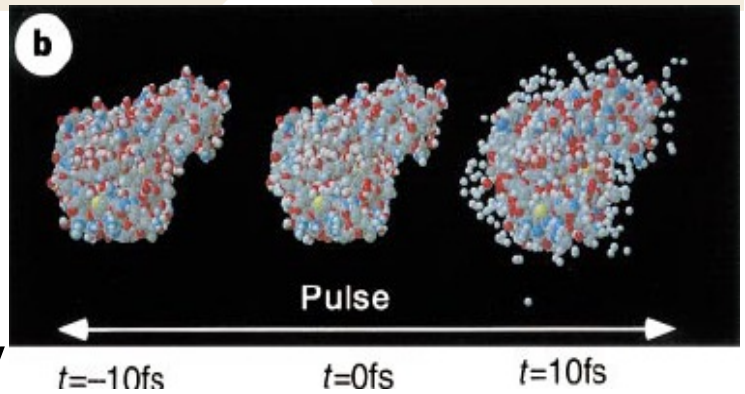


Photon Science Laboratory Building
PSLB construction started

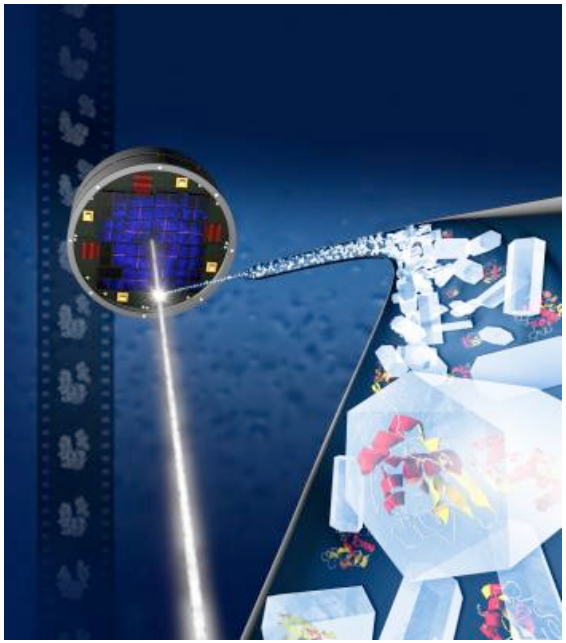
Construction site in front of SUSB (inauguration in Oct)
Moving in date expected: early 2018

Why XFEL Crystallography?

- Structural biology at LCLS and XFELs is based on the use of the diffraction-before-destruction technique
- XFEL crystallography is not likely to ever replace standard synchrotron crystallography
  - Complementary technique for unique and challenging problems
- Use LCLS and XFELs when other techniques fail
  - Can't get large crystals
    - Membrane proteins
  - Radiation damage is an issue
    - Metalloproteins
  - Dynamic measurements
    - Rapid mixing
    - Laser-induced dynamics
  - Room temperature structures



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



Serial femtosecond crystallography (SFX)

Lipidic Cubic Phase Jet Allows Structure Determination of GPCRs at Room Temperature with Small Sample Volumes

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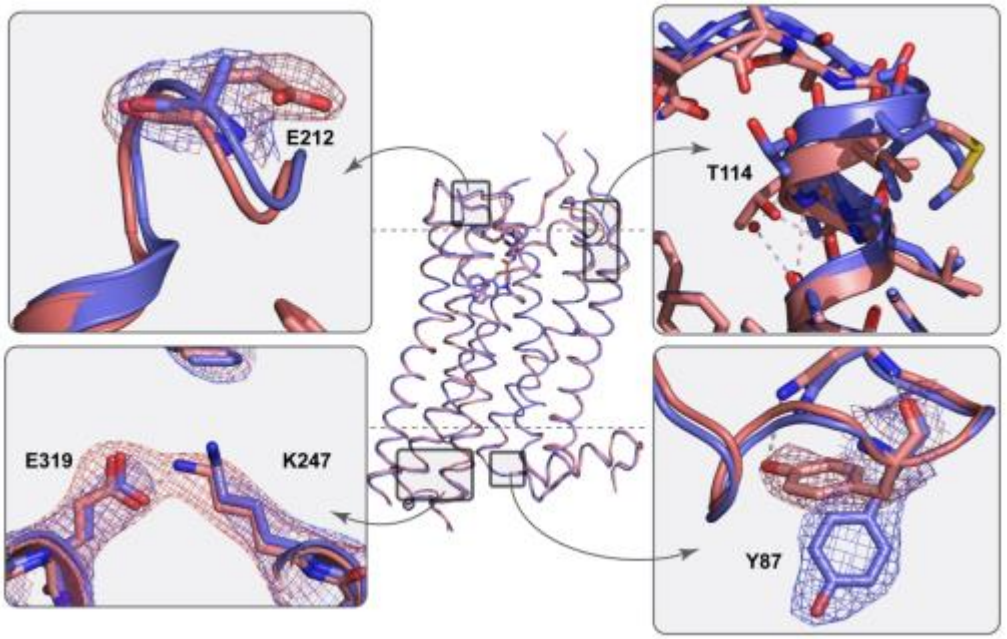
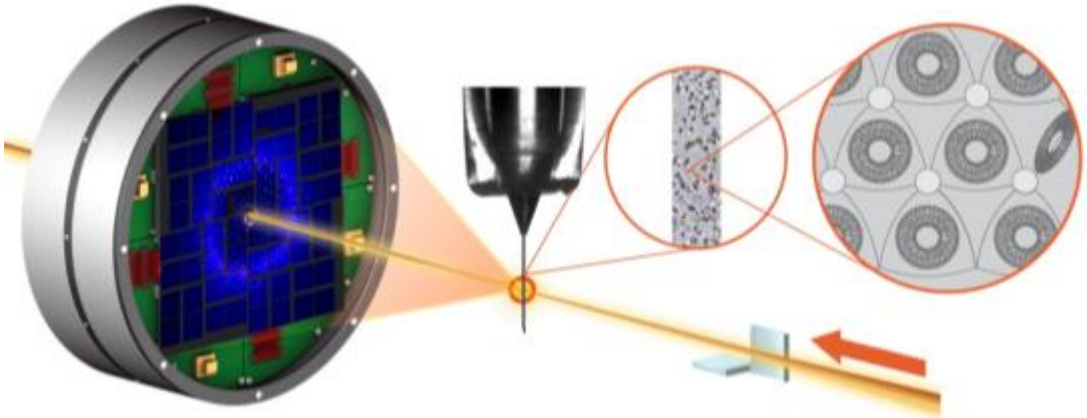
- Human serotonin receptor refined with molecular replacement to 2.8 Å
  - 5-HT_{2B}/ergotamine
    - Previously solved at synchrotron to 2.7Å
- Average crystal size
  - LCLS 5x5x5 μm^3
  - Synchrotron 80x20x10 μm^3
- LCLS room temperature structure displays a unique distribution of thermal motions and conformations of residues
- **Only 0.3 mg of protein used**
- **Many more GPCR structures**

LCP jets being purchased from ASU (technology transfer) for CXI
MFX will be a future home of LCP jets

LCLS (red) vs synchrotron (blue) comparison



Liu *et al*. Science, **342**, 1521 (2013)

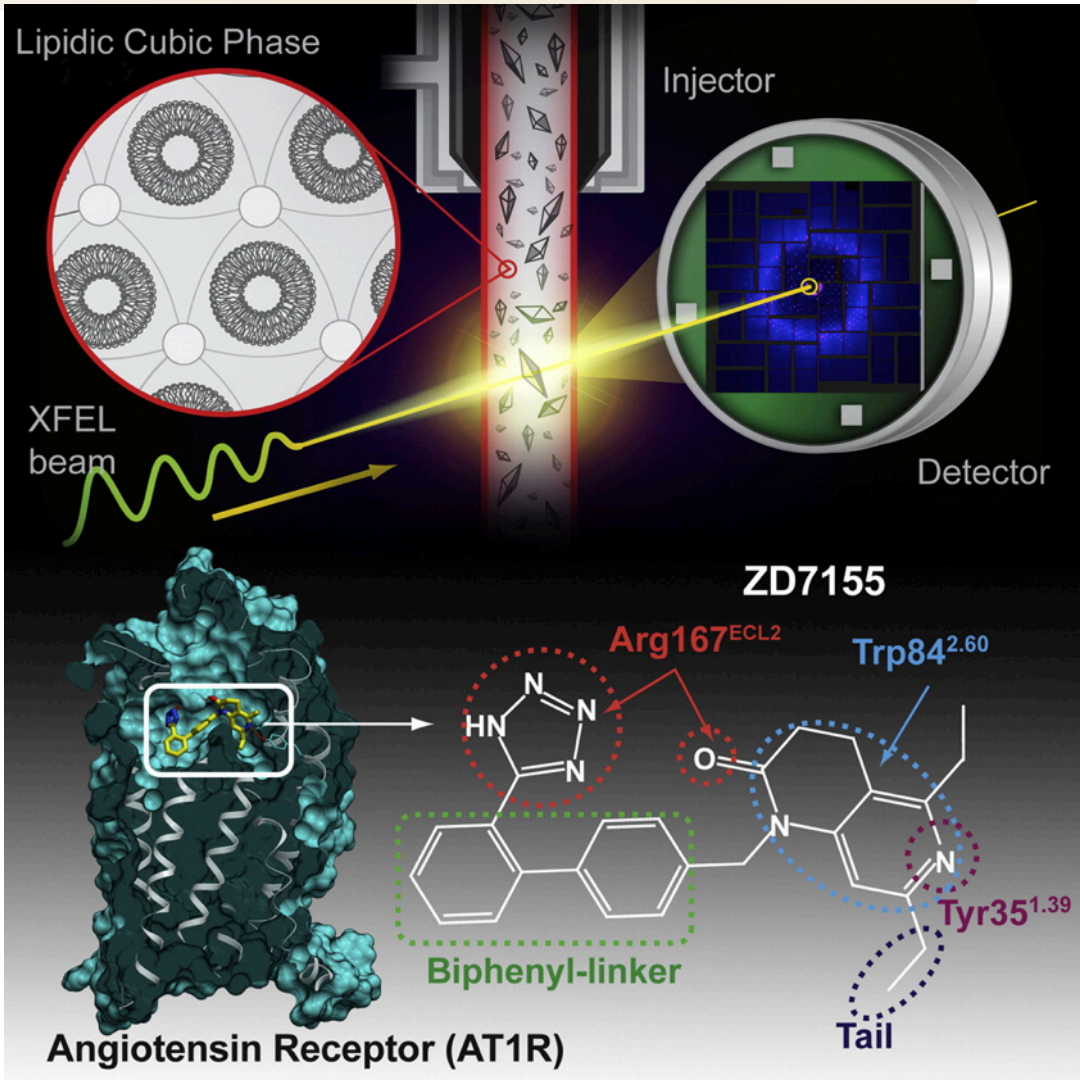


Weierstall *et al*. Nature Comm **5**, 3309 (2014)

The Scripps Research Institute

ASU Arizona State University

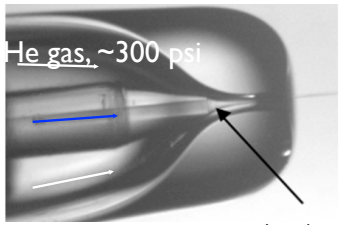
Lipidic Cubic Phase Jet Allows Structure Determination of Complex Membrane Protein Structures at Room Temperature



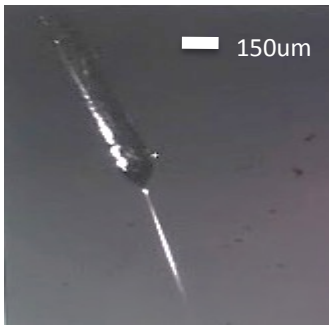
Structure of angiotensin receptor in complex with its selective antagonist Zhang et al., Cherezov, Cell, 2015

Current and future LCLS biology suite of facilities

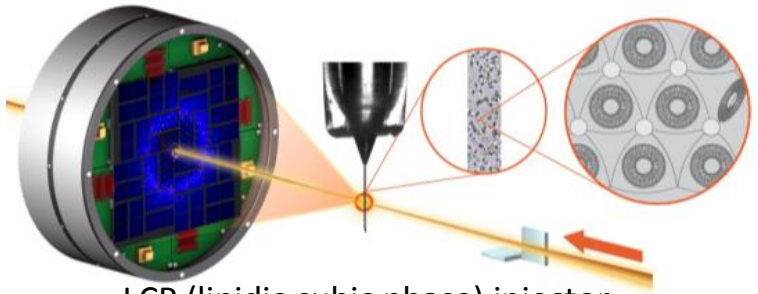
SLAC



Gas dynamic virtual nozzle (GDVN)



Electro-spinning injector



LCP (lipidic cubic phase) injector

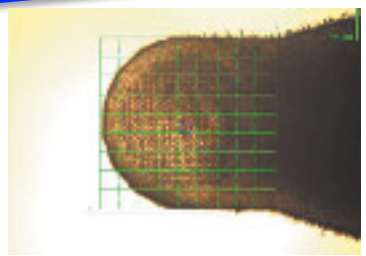
CXI station (in vacuum)

Single particle

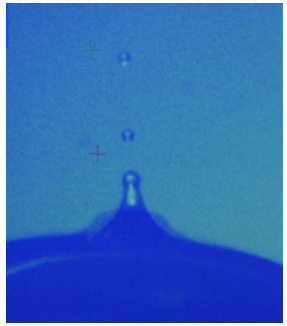
Crystal size

Number of crystals

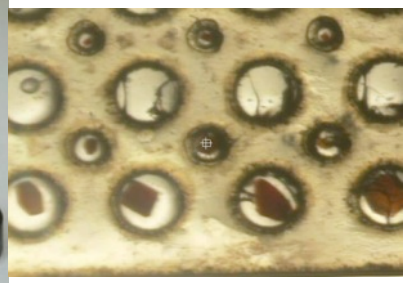
XPP station (in air)



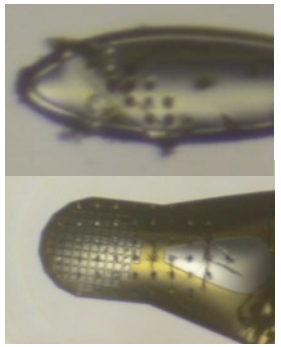
Mesh and microfluidics (future) for submicron crystals



Acoustic injector



Grid arrays

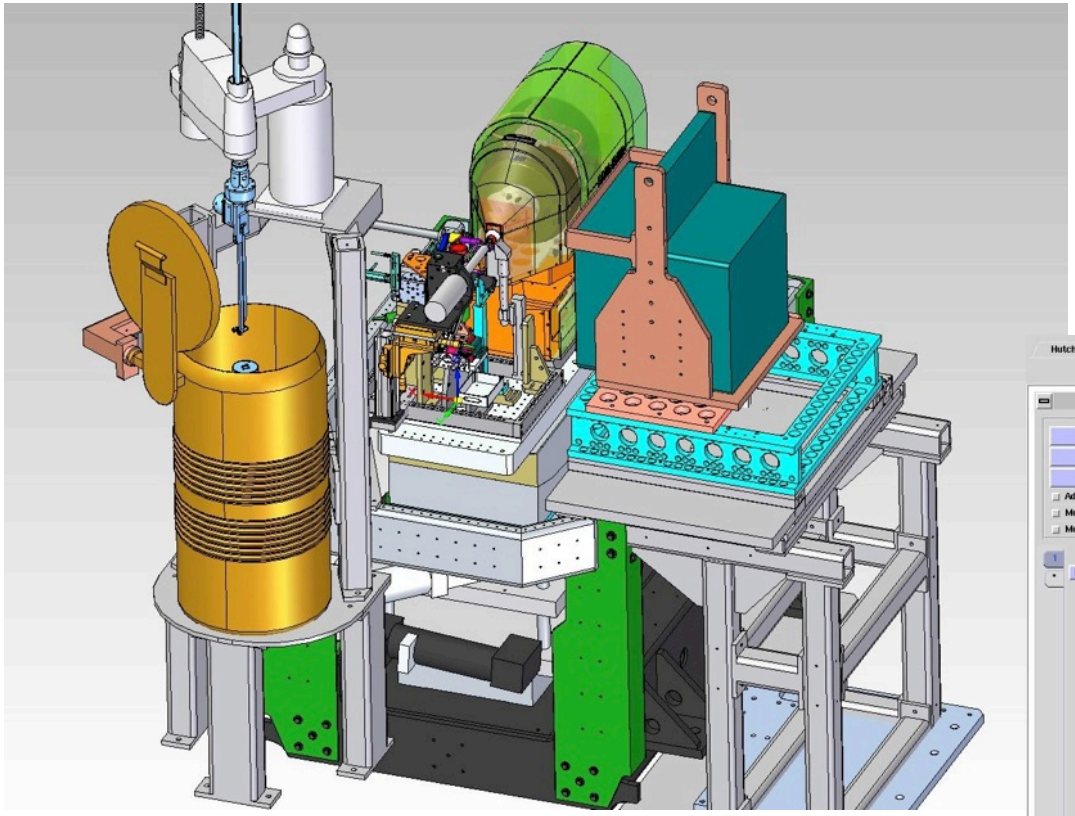


Rastering

MFX as dedicated in-air crystallography station, synergy with SSRL BL 12-1
Collaboration of LCLS, SSRL, SU, and HHMI

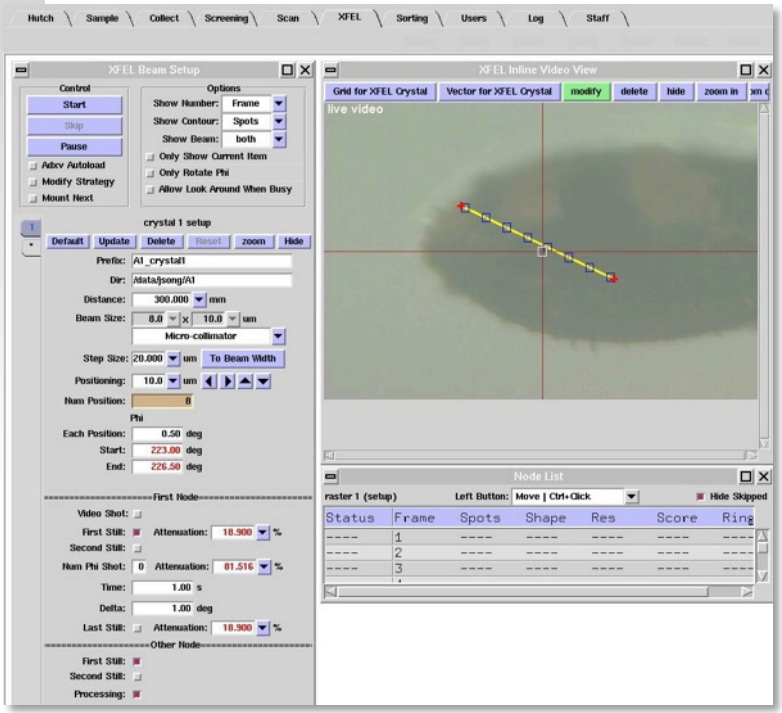
Goniometer setup at LCLS-XPP

Collaboration between LCLS-XPP and SSRL-SMB



1. Improve the resolution from weakly diffracting crystals

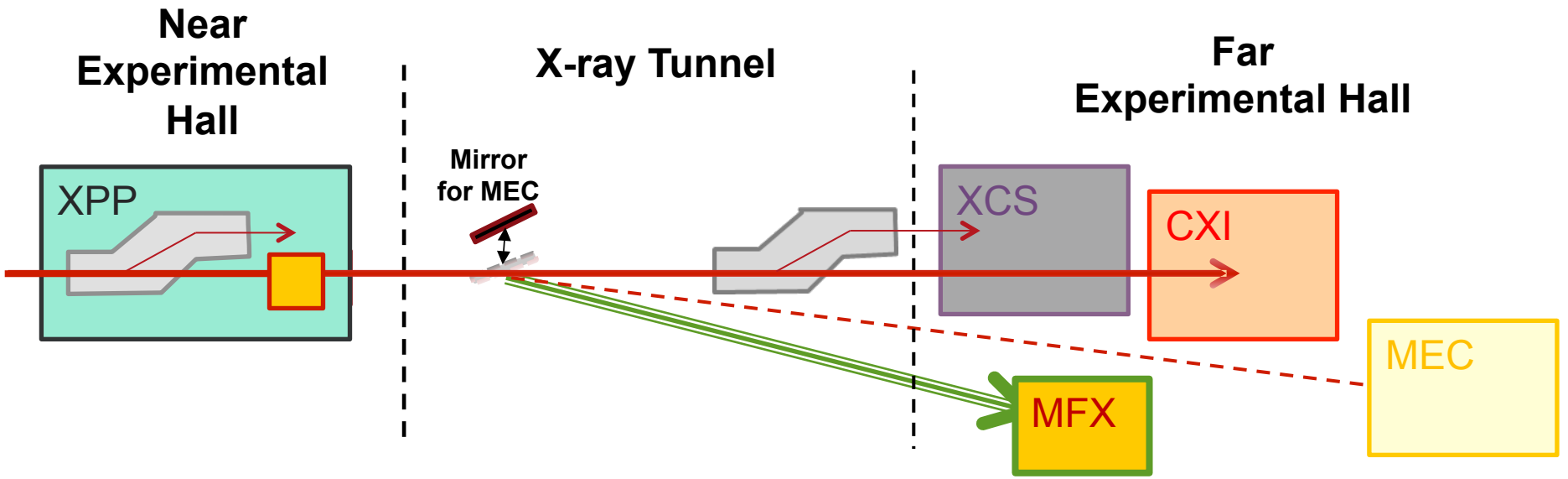
2. Chemically accurate structures of metalloenzymes



- Front-end Based on SSRL BL12-2
- Highly Automated - Blu-Ice/DCSS
- High Density Sample Holders

A. Cohen et al., PNAS 2015

Multiplexing and Ease of Use
More beamtime for biology experiments



- A dedicated setup will save setup and alignment time
- Multiplexing could increase beamtime by a factor of 2
- Switching between MFX and MEC can be ~ min
- Overall efficiency will increase

Macromolecular Femtosecond Crystallography (MFX)
MFX station concept

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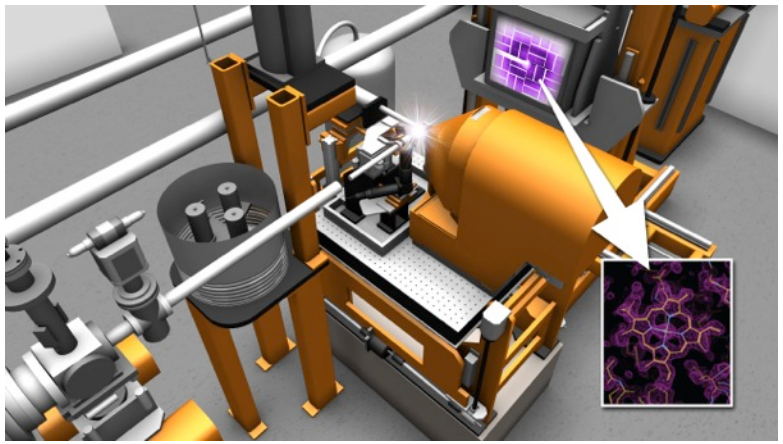
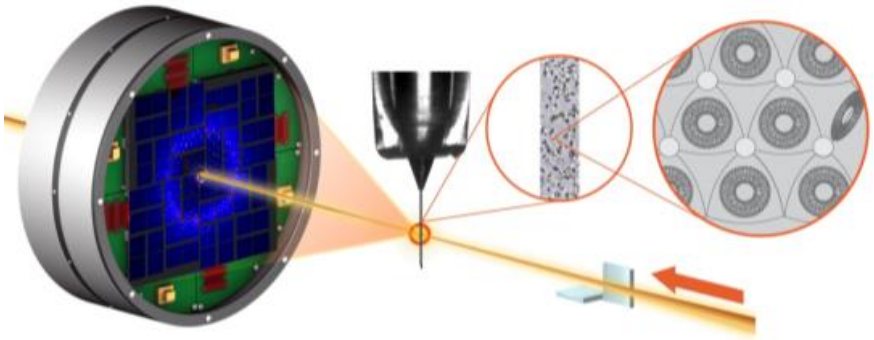
- Independent station in Far Experimental Hall of LCLS
- Increased capacity with beam multiplexing mitigating time pressure on installation/removal of exptl. apparatus
- In-air sample environment for variety of sample delivery methods
- Time-resolved MX, SAXS/WAXS and XES capabilities
- Minimum implementation can be completed in 2 years

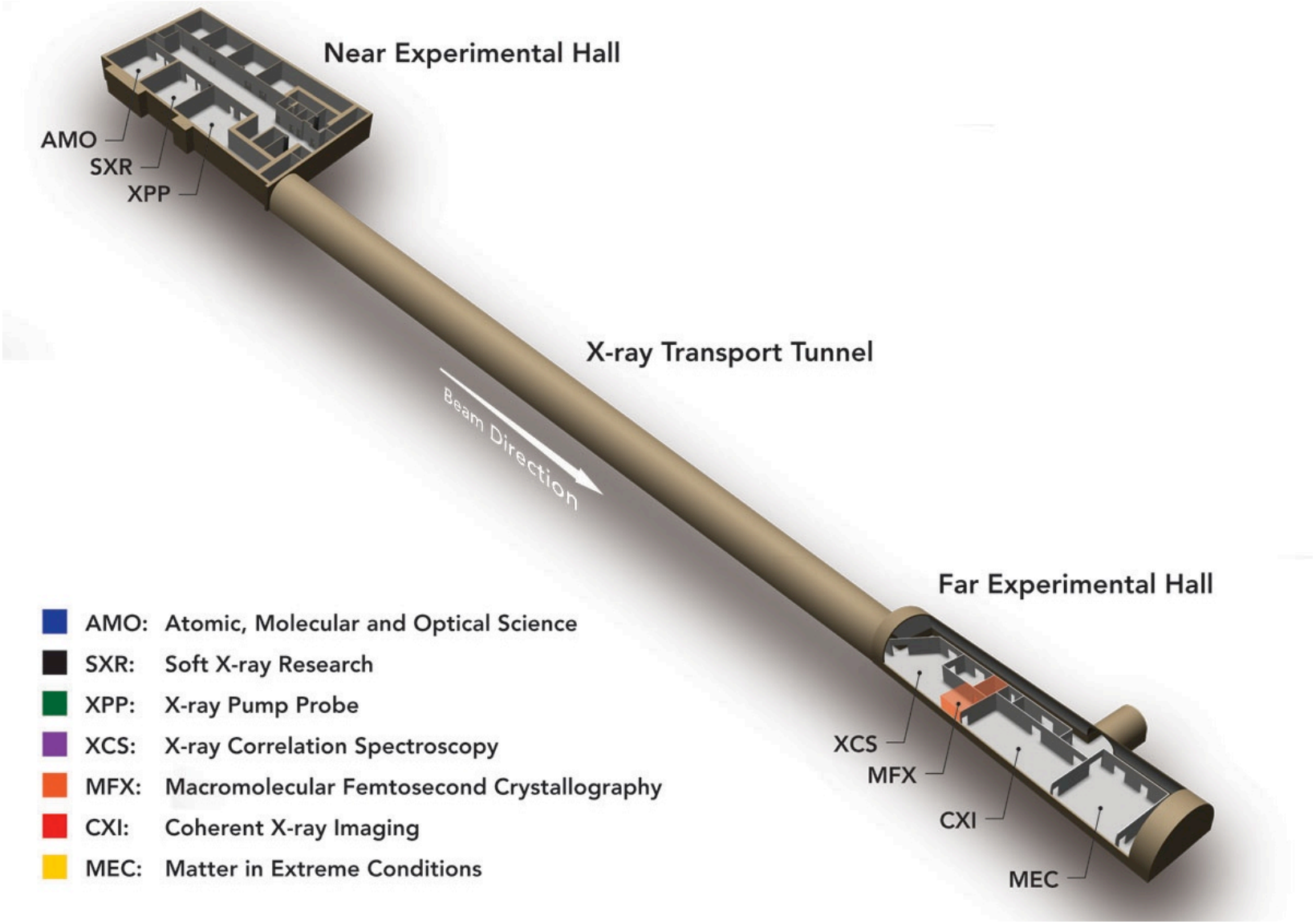


Goniometer for grids, loops etc.

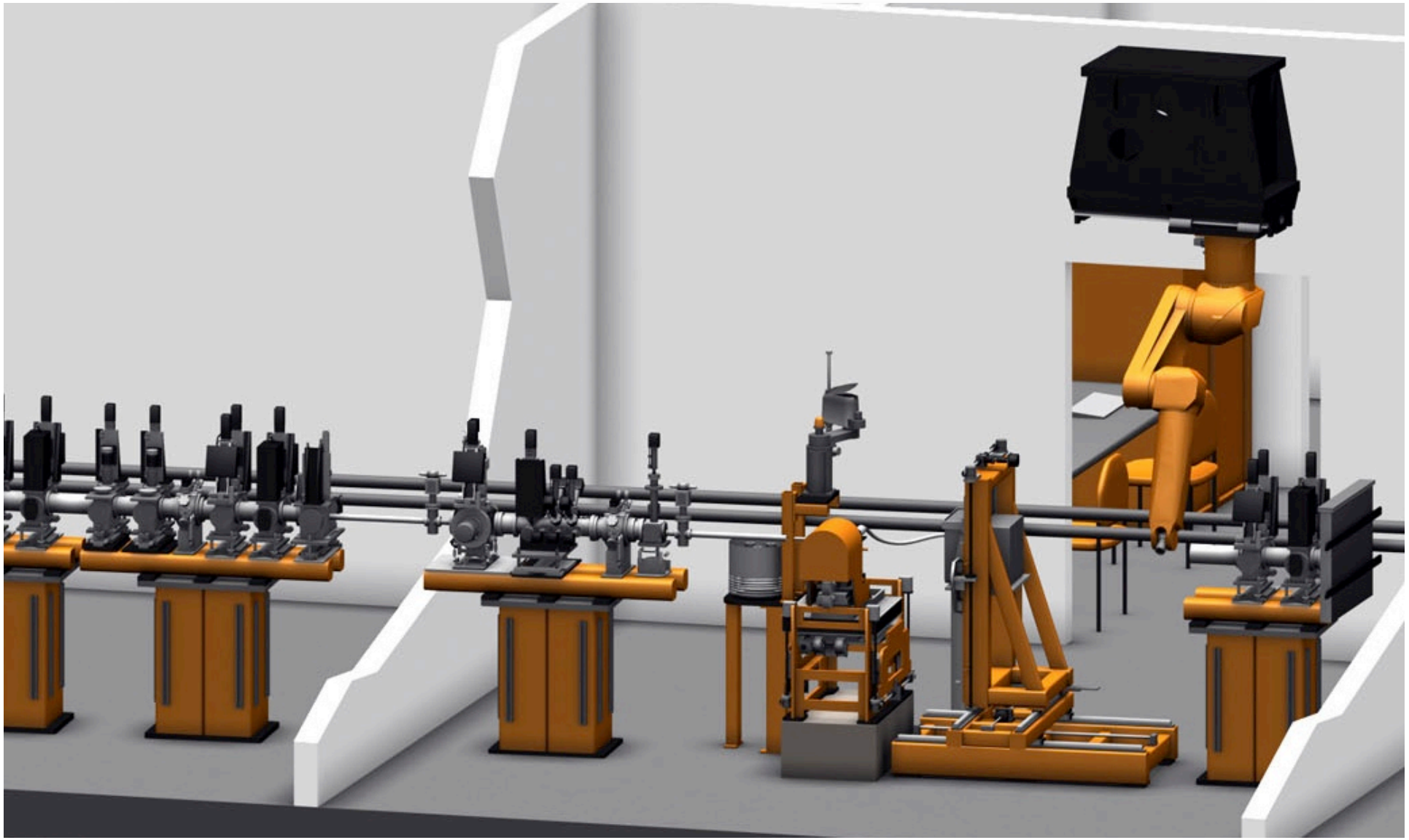


LCP (lipidic cubic phase) injector

LCLS Instruments with MFX



MFX station launched in April, 2014



XFEL: “Diffraction before destruction”

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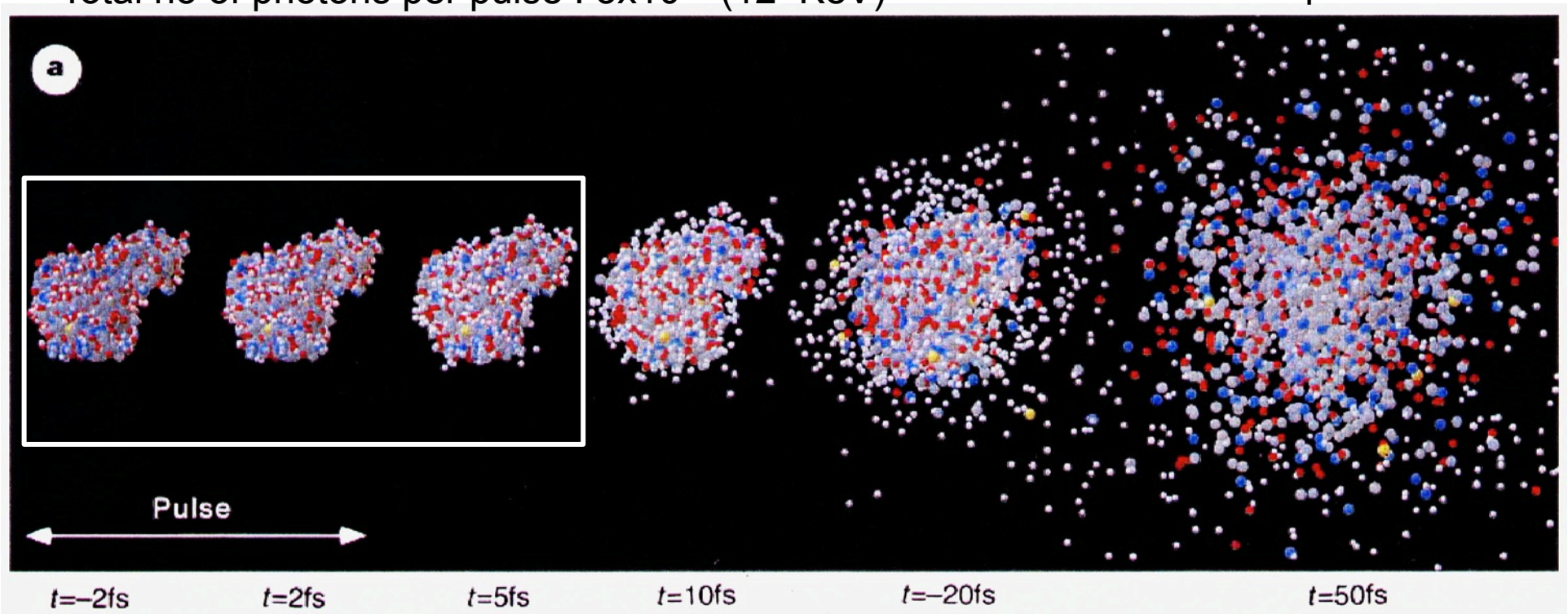
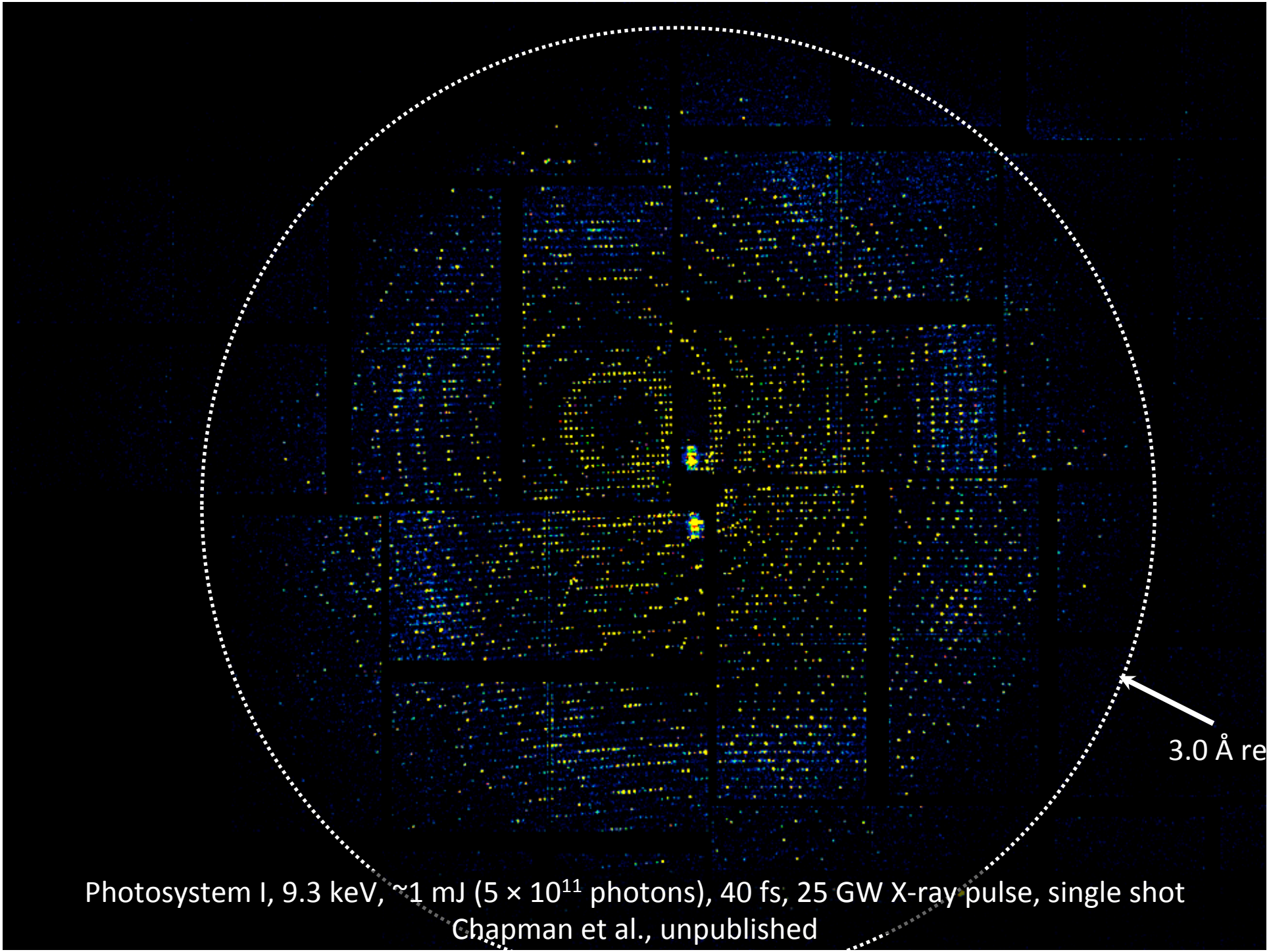
Total no of photons per pulse : 3×10^{12} (12 KeV)

20 fs pulse



Neutze et al., Nature, vol 406, 752 (2000)

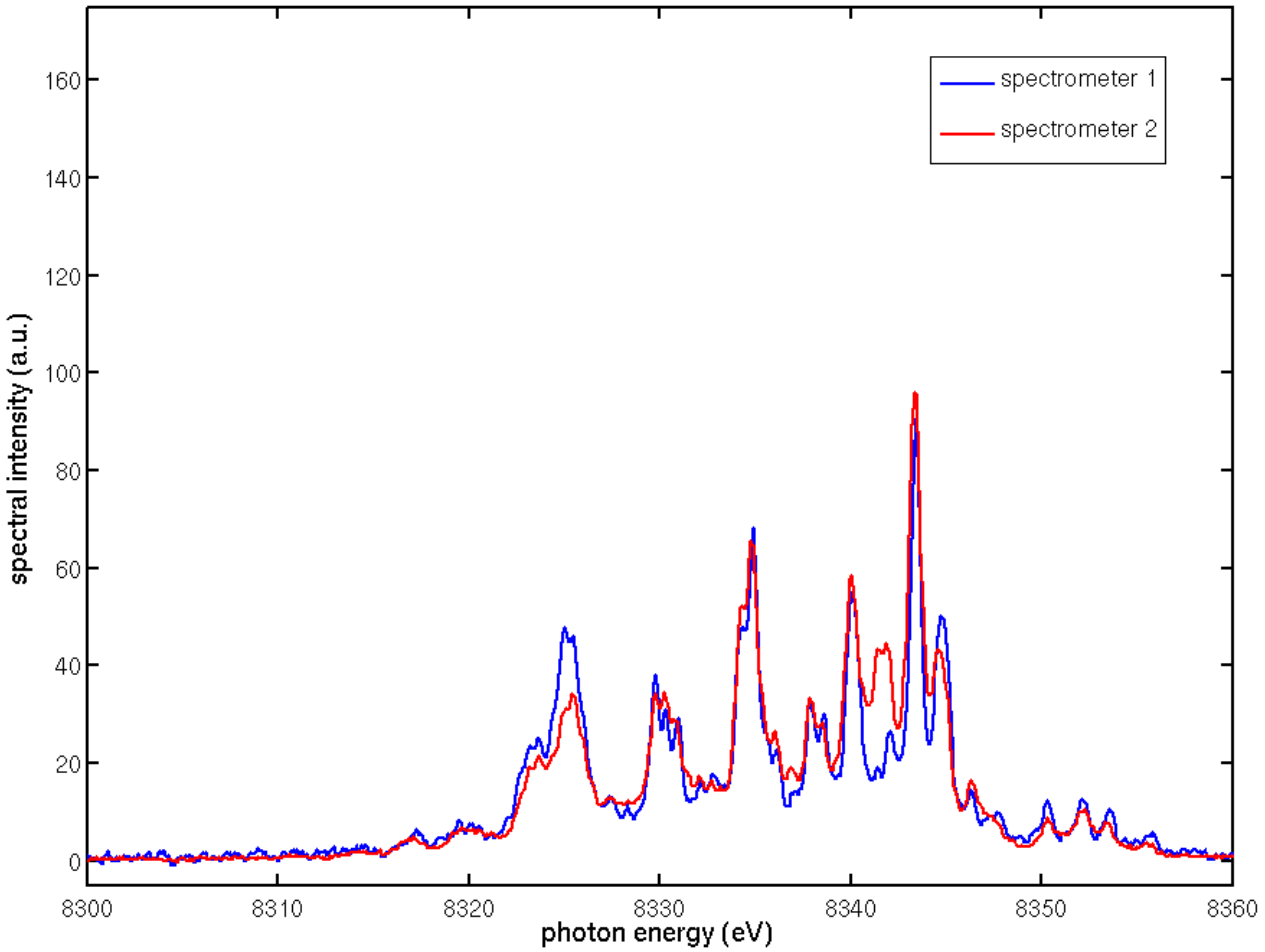
Q: Can we determine structures of challenging targets without model structures? Independent, *de novo* phasing?

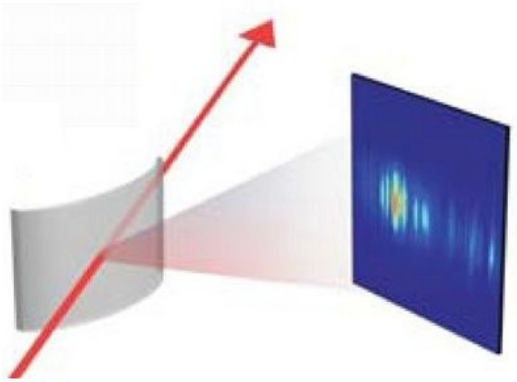


Photosystem I, 9.3 keV, ~1 mJ (5×10^{11} photons), 40 fs, 25 GW X-ray pulse, single shot
Chapman et al., unpublished

Measuring spectra of each XFEL SASE pulse

- Si(111) spectrometer resolution of 0.49±0.02 eV
- Si(333) spectrometer, the resolution lower bound 0.13 eV



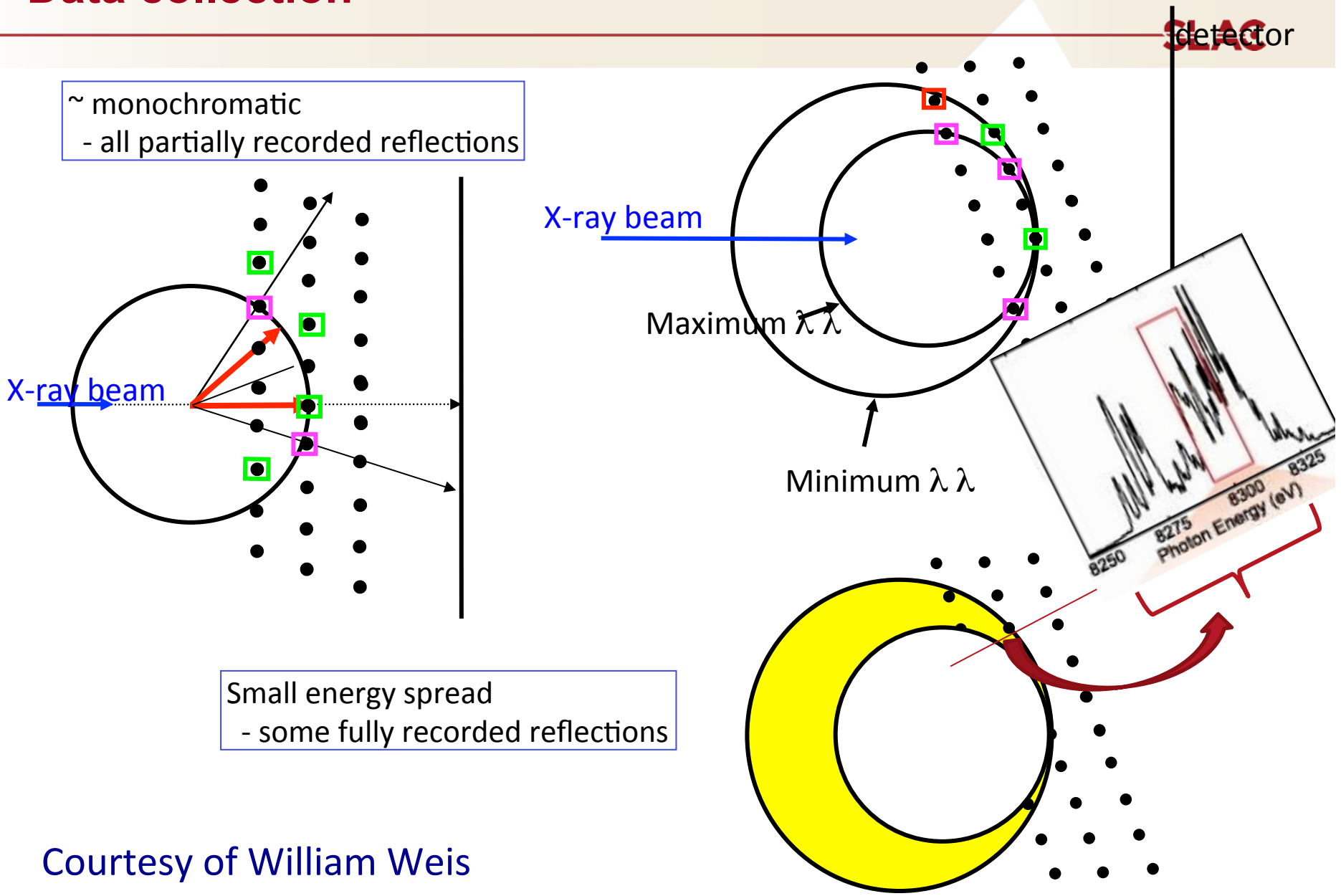


Bent Silicon Single Shot Spectrometer

D. Zhu et al., Appl. Phys Lett. 101, 034103 (2012)

Courtesy of David Fritz

Data collection



Courtesy of William Weis

Is *de novo* phasing phasing possible at XFELs?

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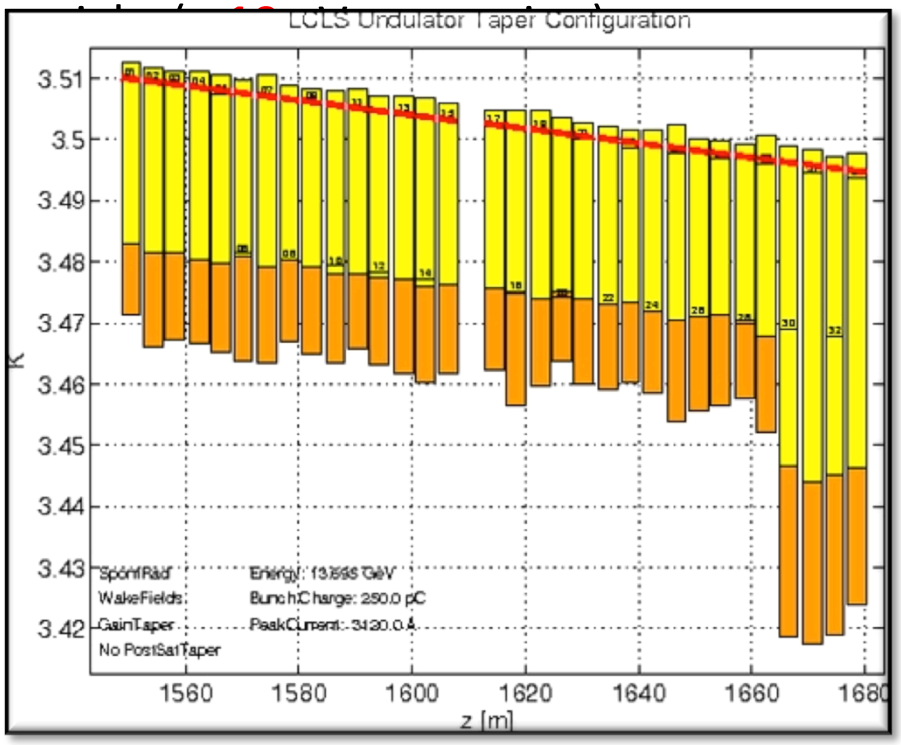
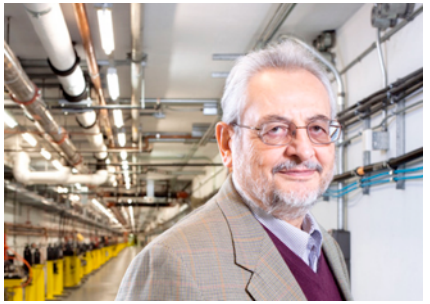
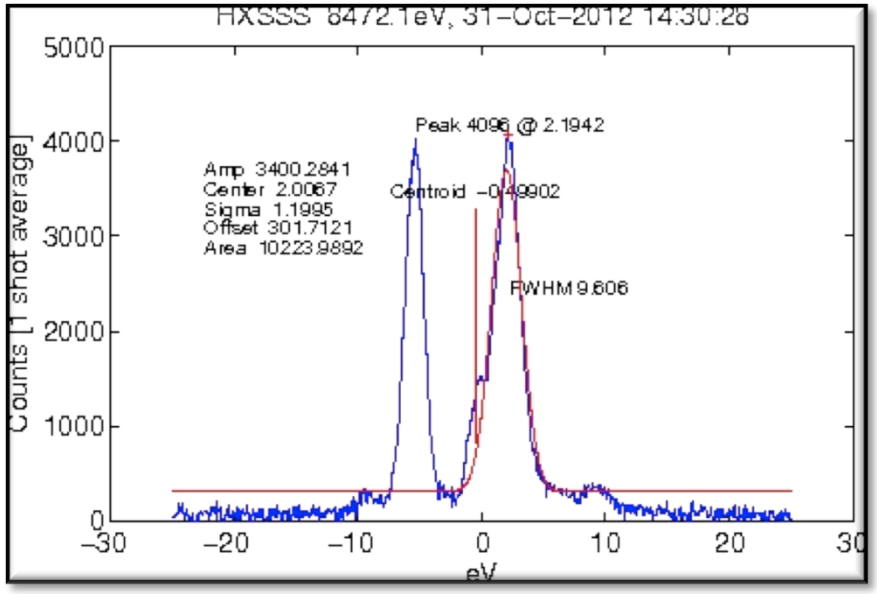
IMPROVED SASE: 10/31/2012 SHIFT

- iSASE: 2-color
  - Taper profile on left, and spectrum on



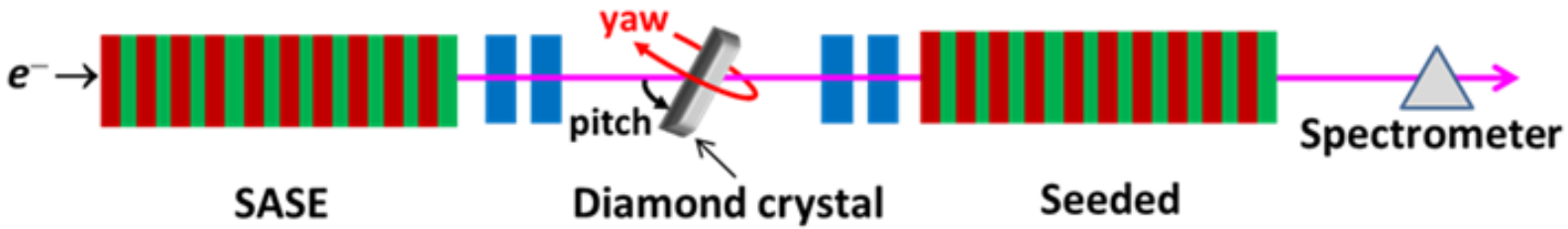


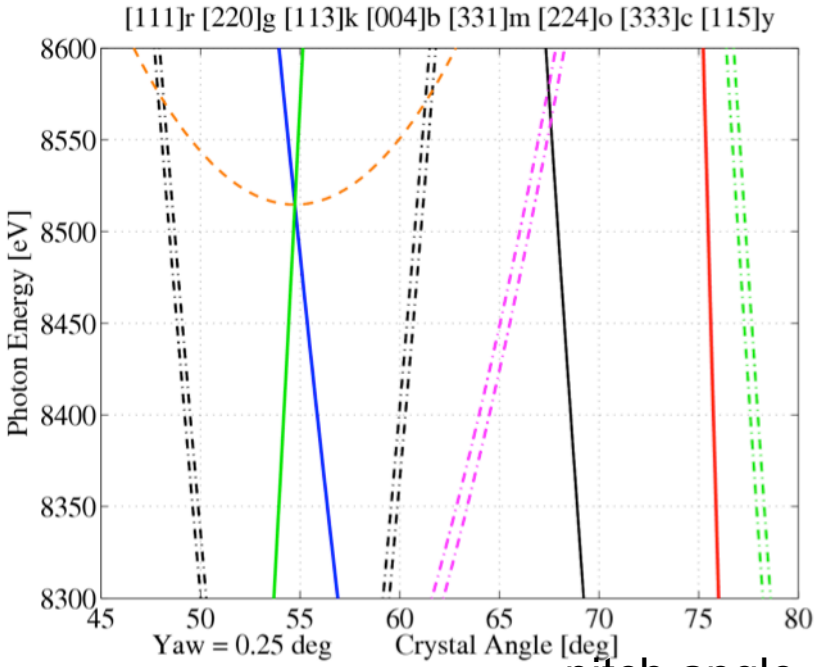
Claudio Pellegrini



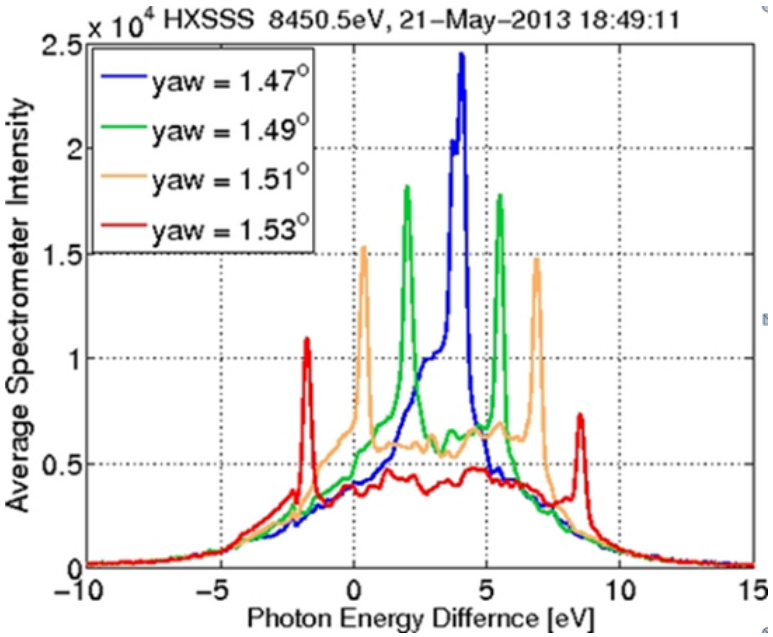
Conversation with Claudio Pellegrini on two colors Jan 8, 2013

Hard X-ray Two-Color Self Seeding





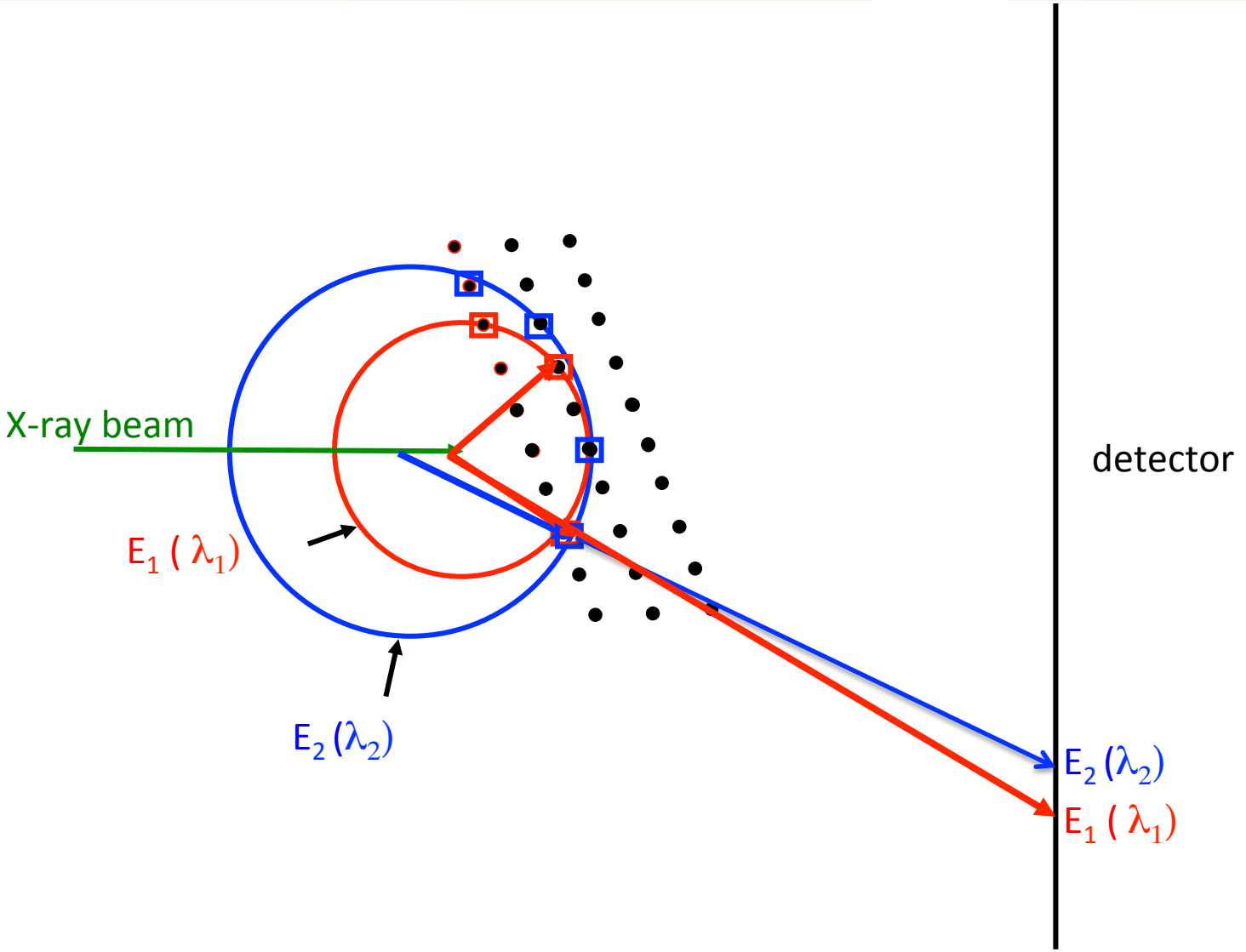
pitch angle



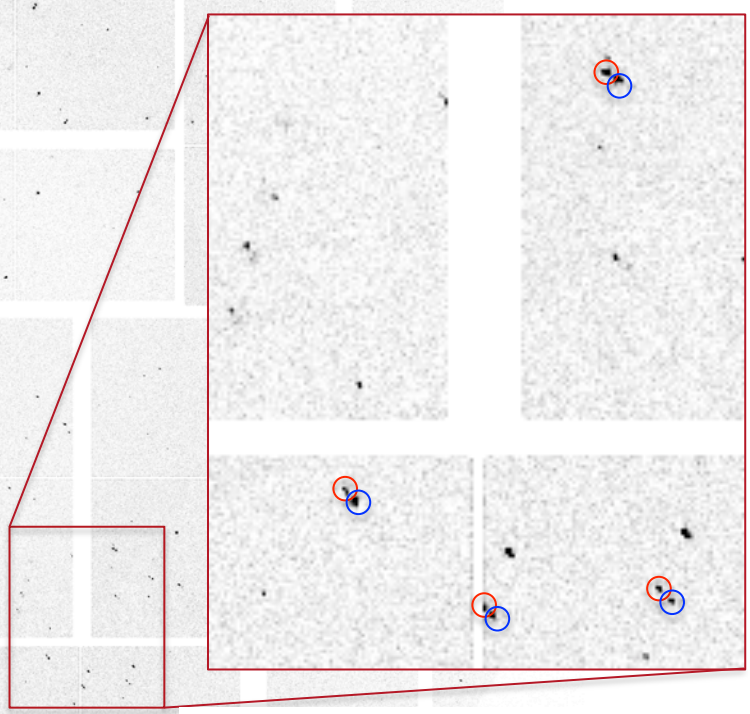
Yaw angle controls the splitting between the out-of-plane (dash dotted) reflection lines

Franz-Josef Decker et al.

Two-color data collection

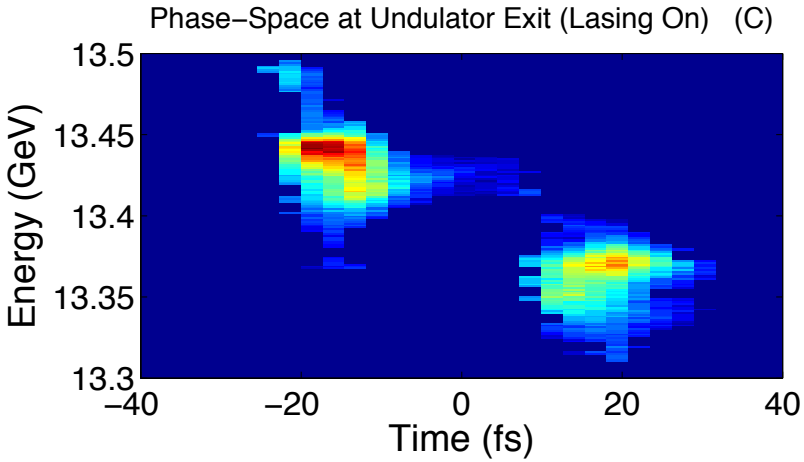


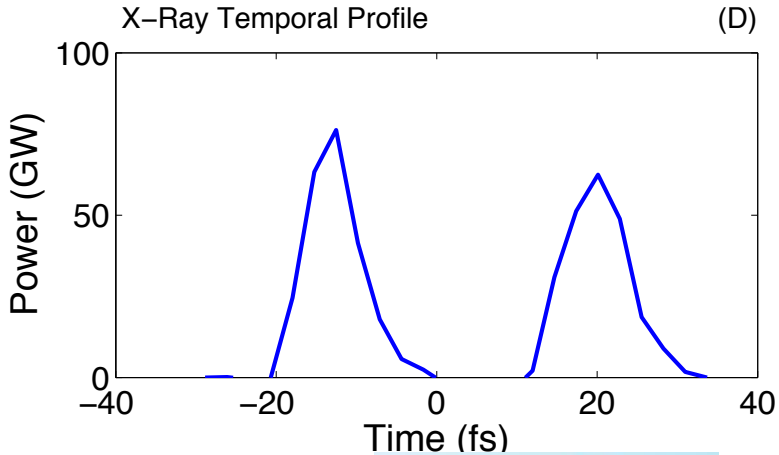
1 % energy separation give doublets on the detector



Above edge E2, t=10 fs
Inflection point E1, t=0

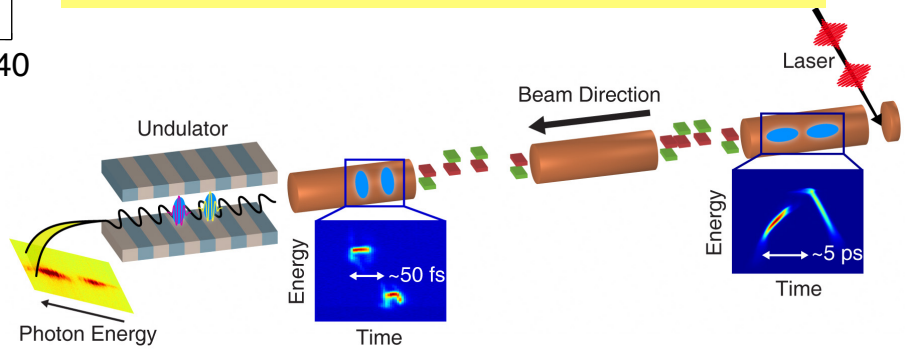
Can 2-pulse/2-color mode be used for pump and probe femtosecond crystallography?





Time delay: 0 to 150 fs
Two color ΔE/E up to 1%
Both color self-seeded

Are systematic radiation damage studies possible?



(Agostino Marinelli et al., Nature Communication 2015)

Femtosecond radiation damage

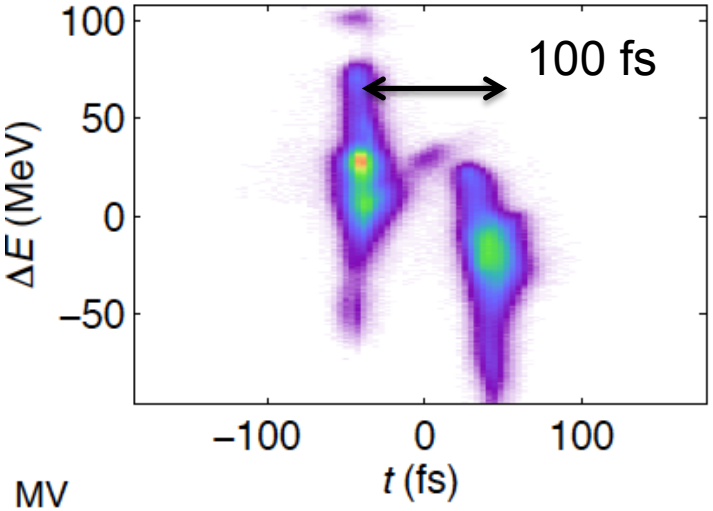
Plasma physics, not chemistry

1. Random atomic motion -> B-factor increase
2. Ionization -> Diffraction intensity reduction
3. Nonrandom atomic motion -> structural change

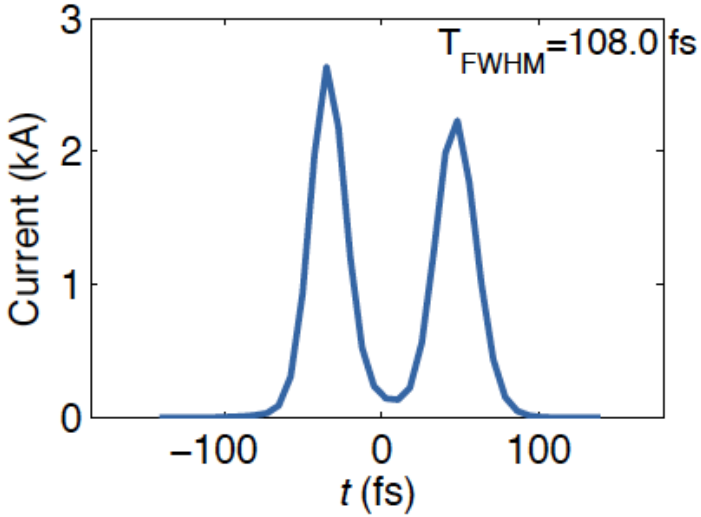
4. Localized damage

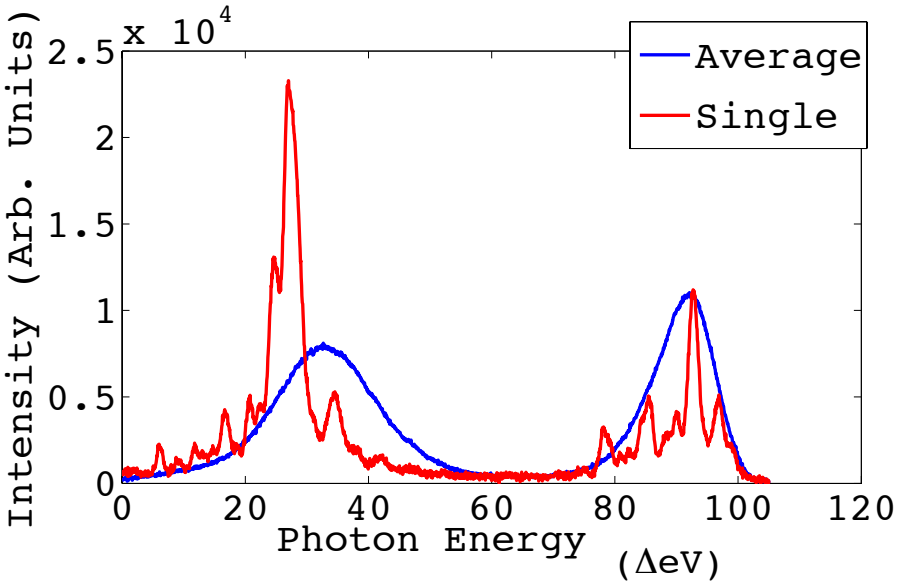
Temporal and Spectral Profiles of 2-pulse/2-color mode

60 eV energy separation around 7.1 keV iron edge

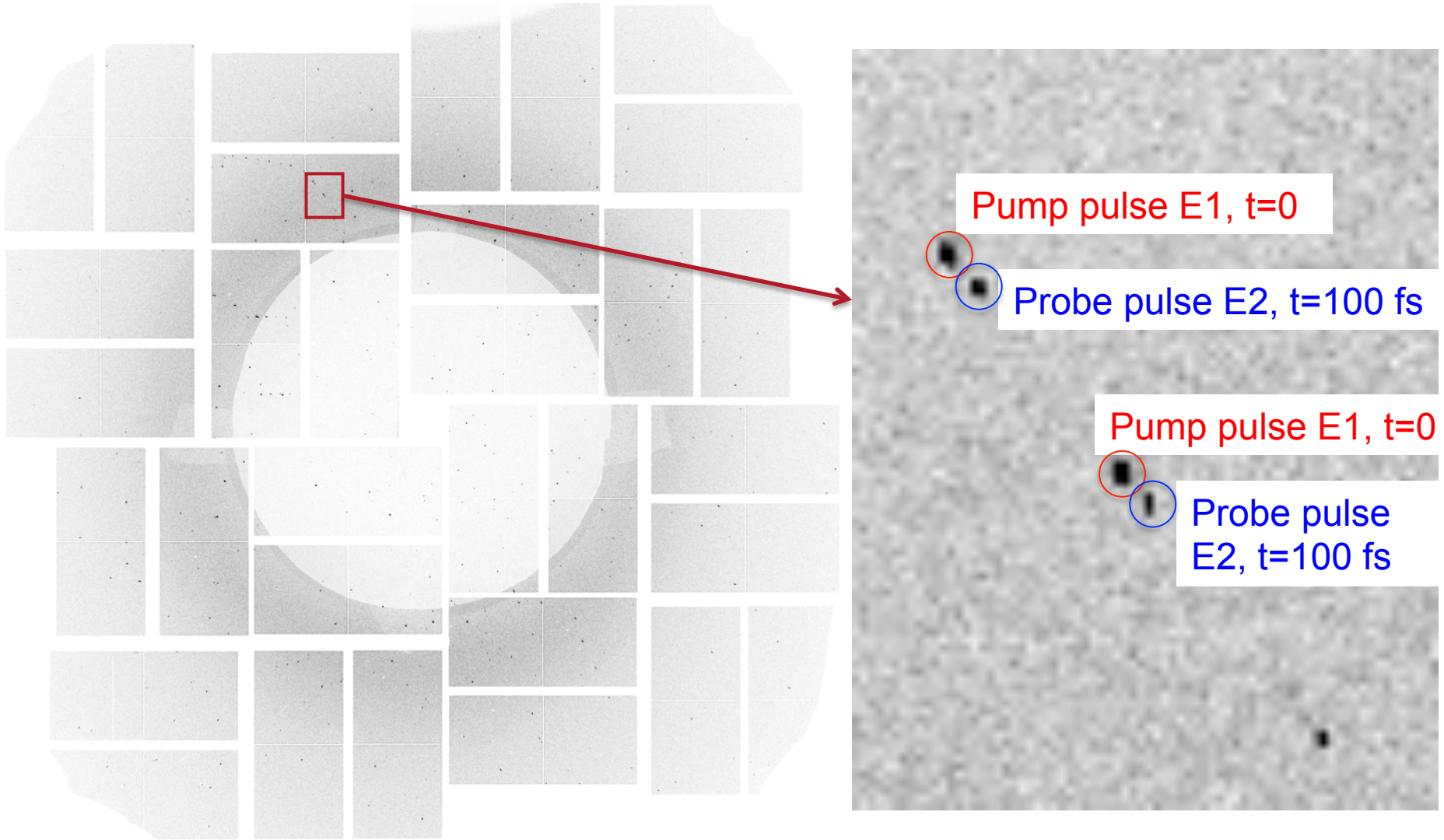


MV

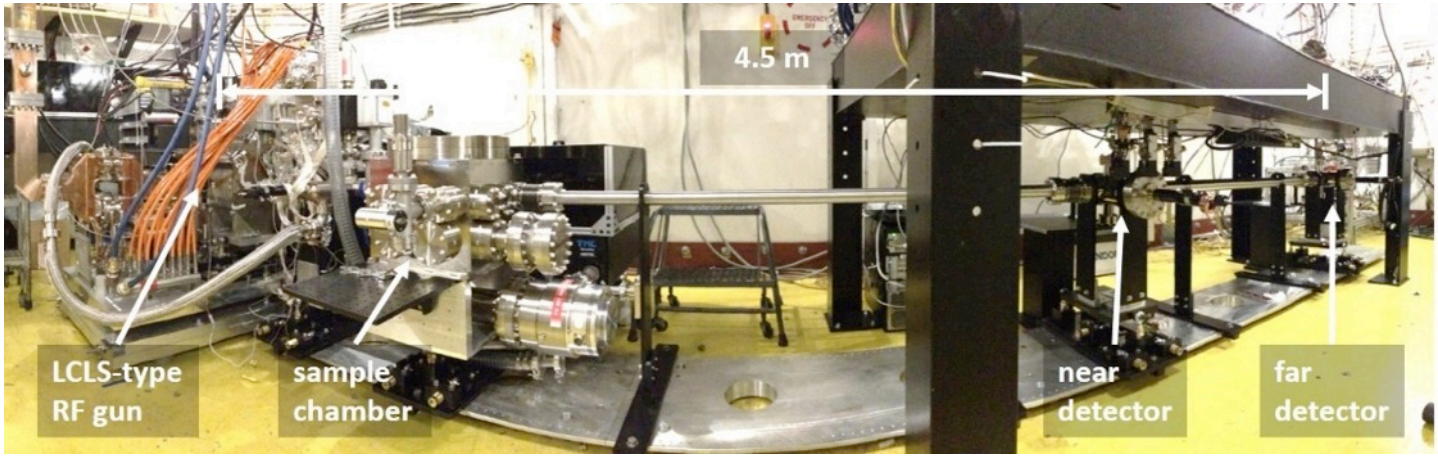




2-color diffraction from lysozyme crystals



SLAC ASTA UED: for time resolved studies



ASTA UED system

Typical operation parameters

Parameters	Values
rep. rate	120 Hz
beam energy	3-5 MeV
bunch charge	50 fC
emittance	<50 nm-rad
bunch length	~100 fs

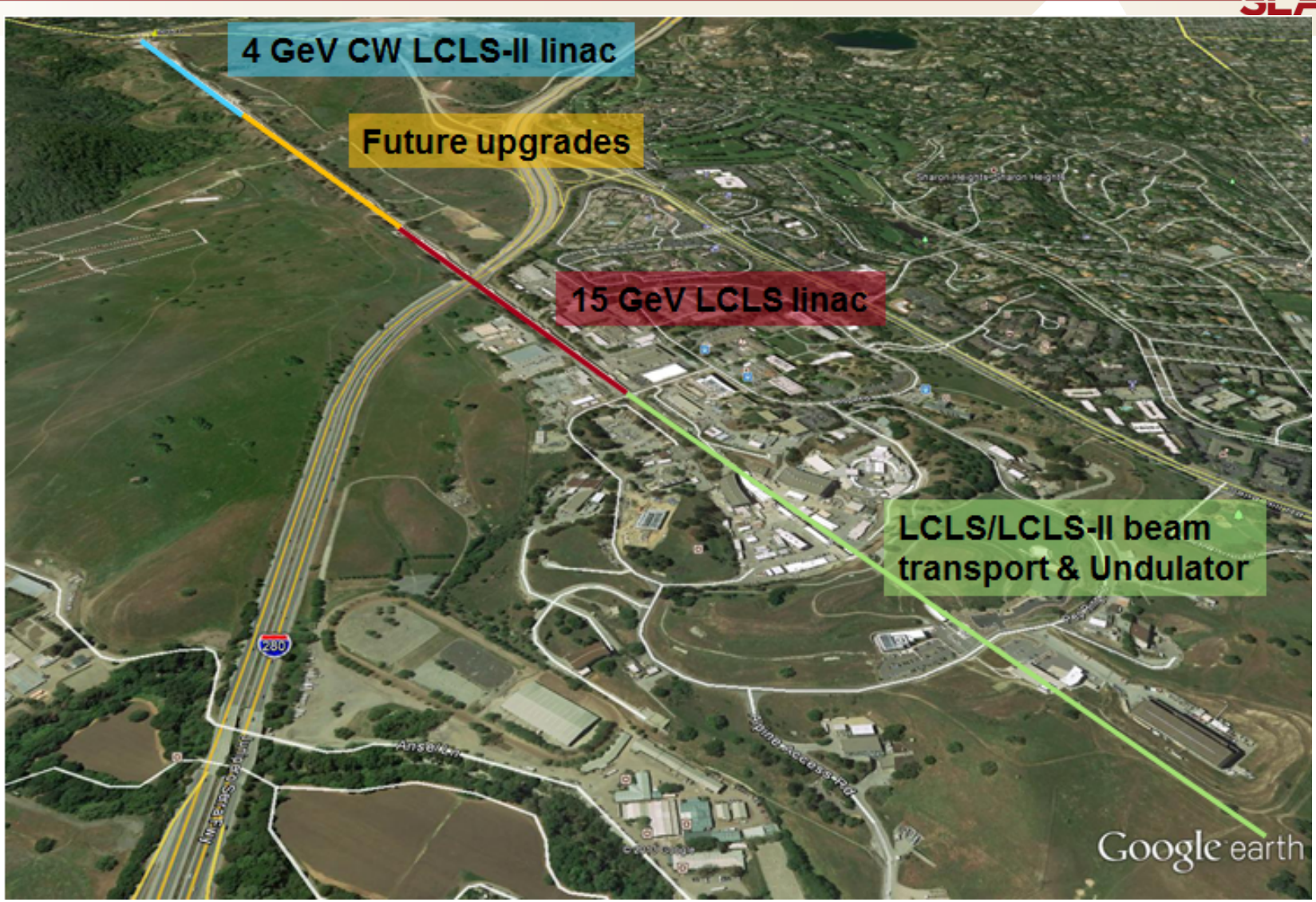
Courtesy of X.J. Wang, SLAC

Large-q of electrons allows to simultaneously measure in-plane and out-of-plane motion in metal clusters have been demonstrated.

Time resolved biology exp planned using direct detection detectors (coll. with P. Denes, LBNL)

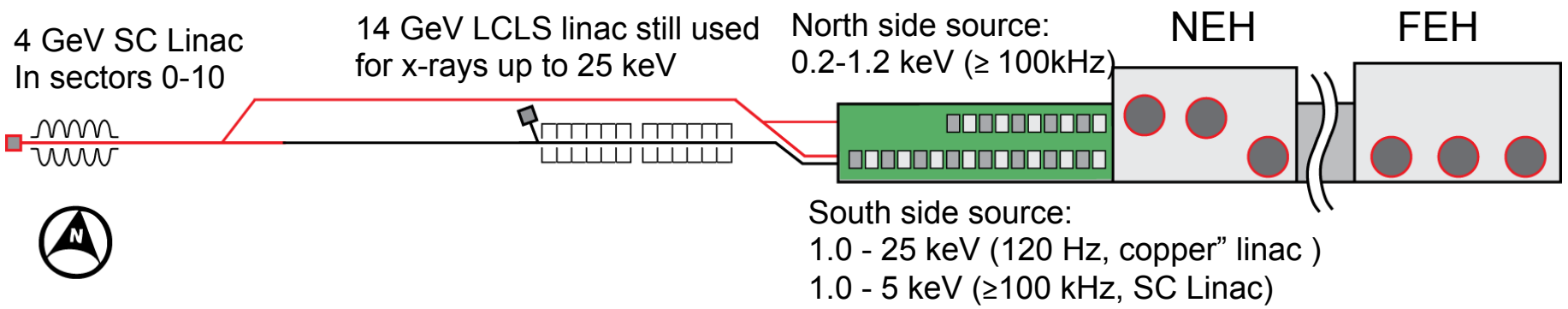
LCLS-II Concept

Use 1st km of SLAC linac tunnel for CW SCRF linac

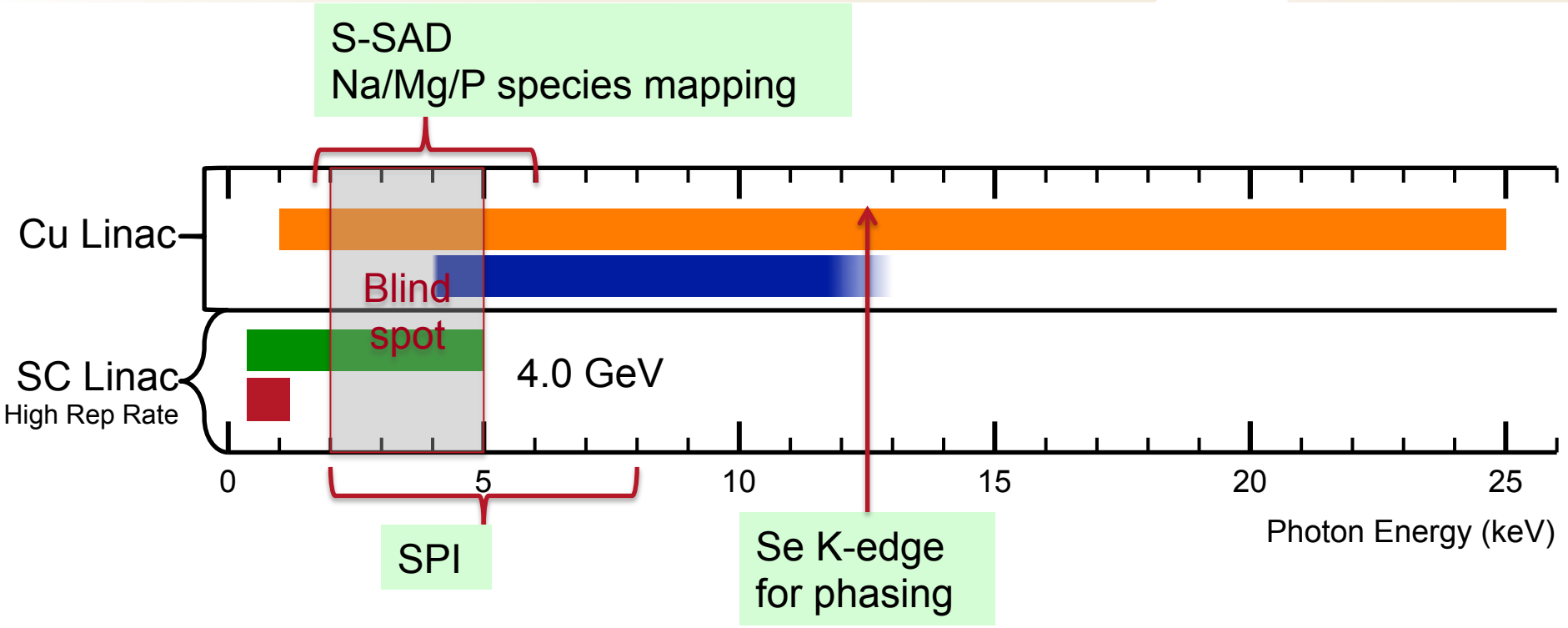


LCLS-II Project Scope

Accelerator	**<u>Superconducting linac</u>**: 4 GeV
Undulators in existing LCLS-I Tunnel	New variable gap (north) New variable gap (south), replaces existing fixed-gap und.
Instruments	Re-purpose existing instruments (instrument and detector upgrades needed to fully exploit)



LCLS-II Baseline Deliverables: energy ranges for biology



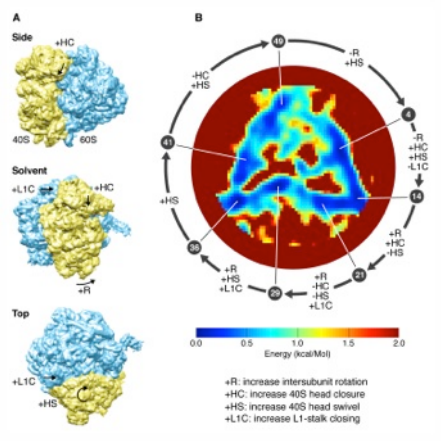
Legend

- Cu SASE
- Cu Self Seeded
- High Rep Rate SASE
- Self Seeded (Grating)

- Hard X-Ray Source:
  - 1-5 keV w/ 4 GeV SC linac
  - Up to 25 keV with LCLS Cu Linac
- Soft X-Ray Source:
  - 250 eV-1.2 keV w/ 4 GeV linac
    - 200 eV requires <4 GeV

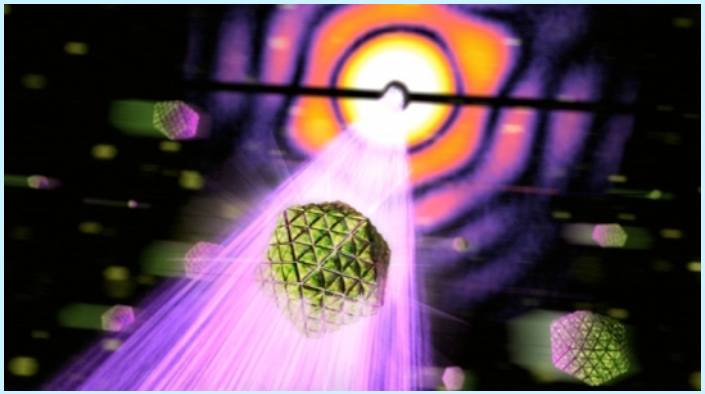
Single particle imaging: international consortium

A) Functional movies in physiological conditions (single mol.)



High repetition rate for large data sets
Clean data sets, large data volumes needed

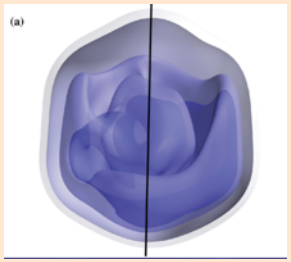
B) Proteins and molecular complexes that don't crystalize (single mol.)



2-6 keV, as many mJ as possible per pulse

LCLS led international consortium to develop SPI (Director's discretionary time)

C) Imaging of complex structures including living cells



How to group 2D projection images of individual cells and reconstruct a 3D image single cell
Maybe 3D structures of an ensemble average

Credits
Ourmazd;
(A, ribosome)
Hajdu:
(B, caroboxysomes.
C, mimi virus)

Summary

- Femtosecond crystallography: in-vacuum & in-air data collection
- Structure determination of challenging targets enabled by
  - Viscous (LCP) jets requiring much less protein material
  - Post-refinement enabling structure determination from 100~1000s images (crystals)
- New beamline, MFX, project proceeding, much higher efficiency, first test experiments early 2016
- 2-pulse/2-color mode for *de novo* phasing and pump & probe radiation damage studies: close collaboration with accelerator physicists essential
- LCLS-II: high-rep rate and expanded energy ranges

Acknowledgments

Thank you for your attention!

LCLS

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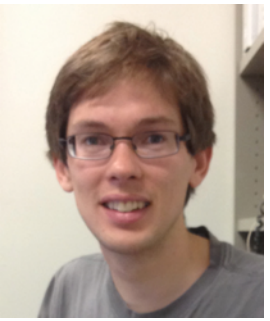
Howard Hughes Medical Institute Collaboration Innovation Award

A. Brunger, J. Berger, D. Rees, D. Eisenberg, N. Sauter, W. Weis, *et al.*



Ulf Lundstrom