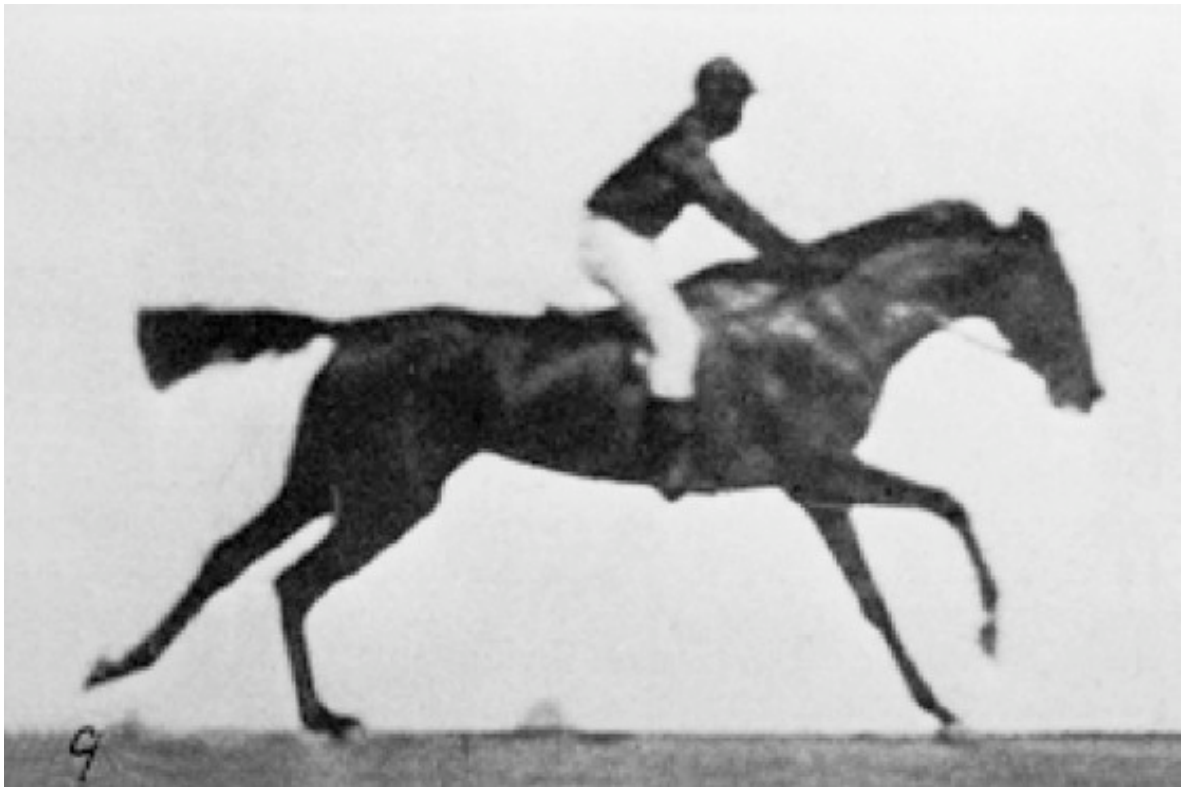
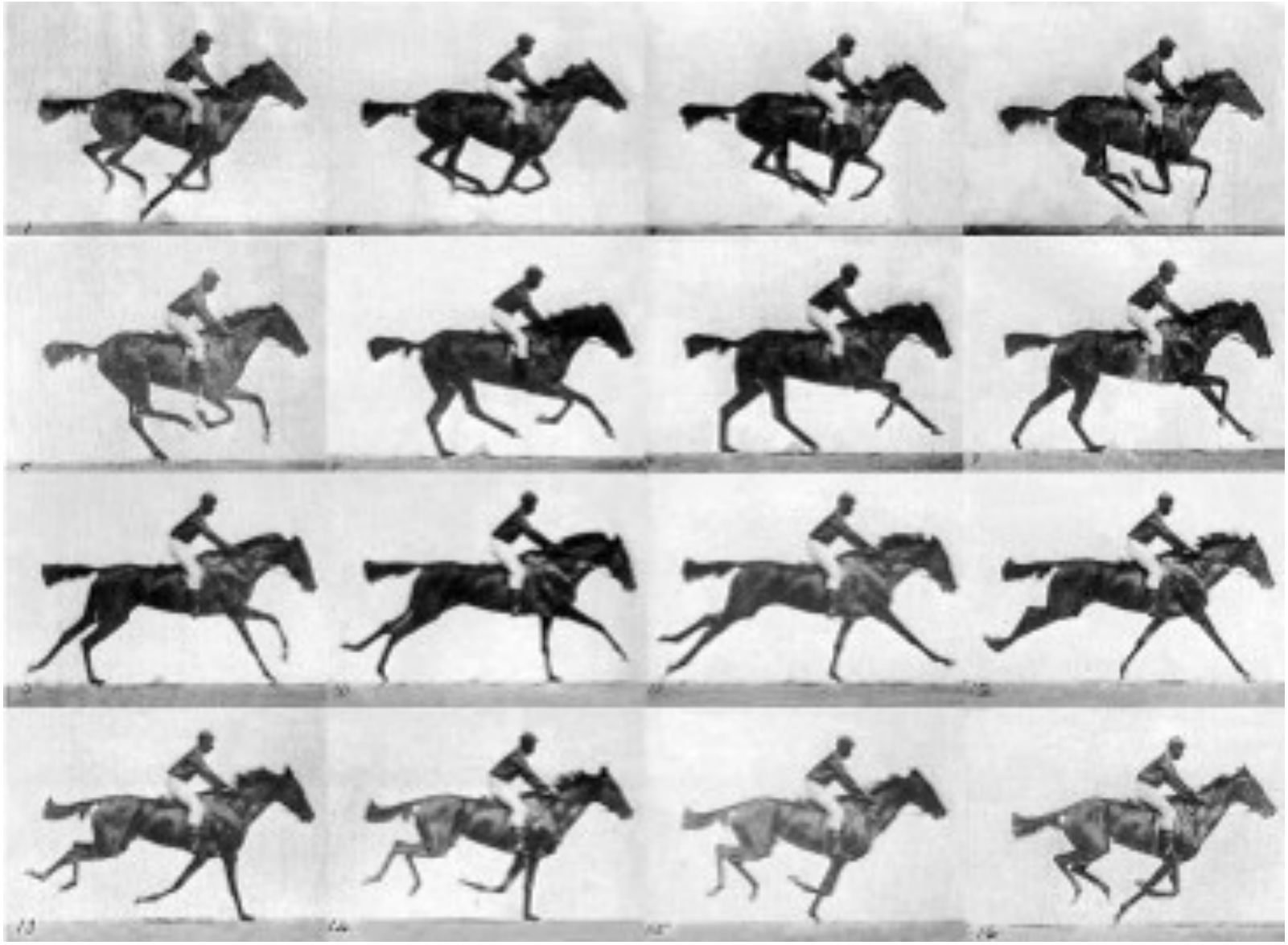
Challenge in 1878:

Structure and dynamics of a horse in motion



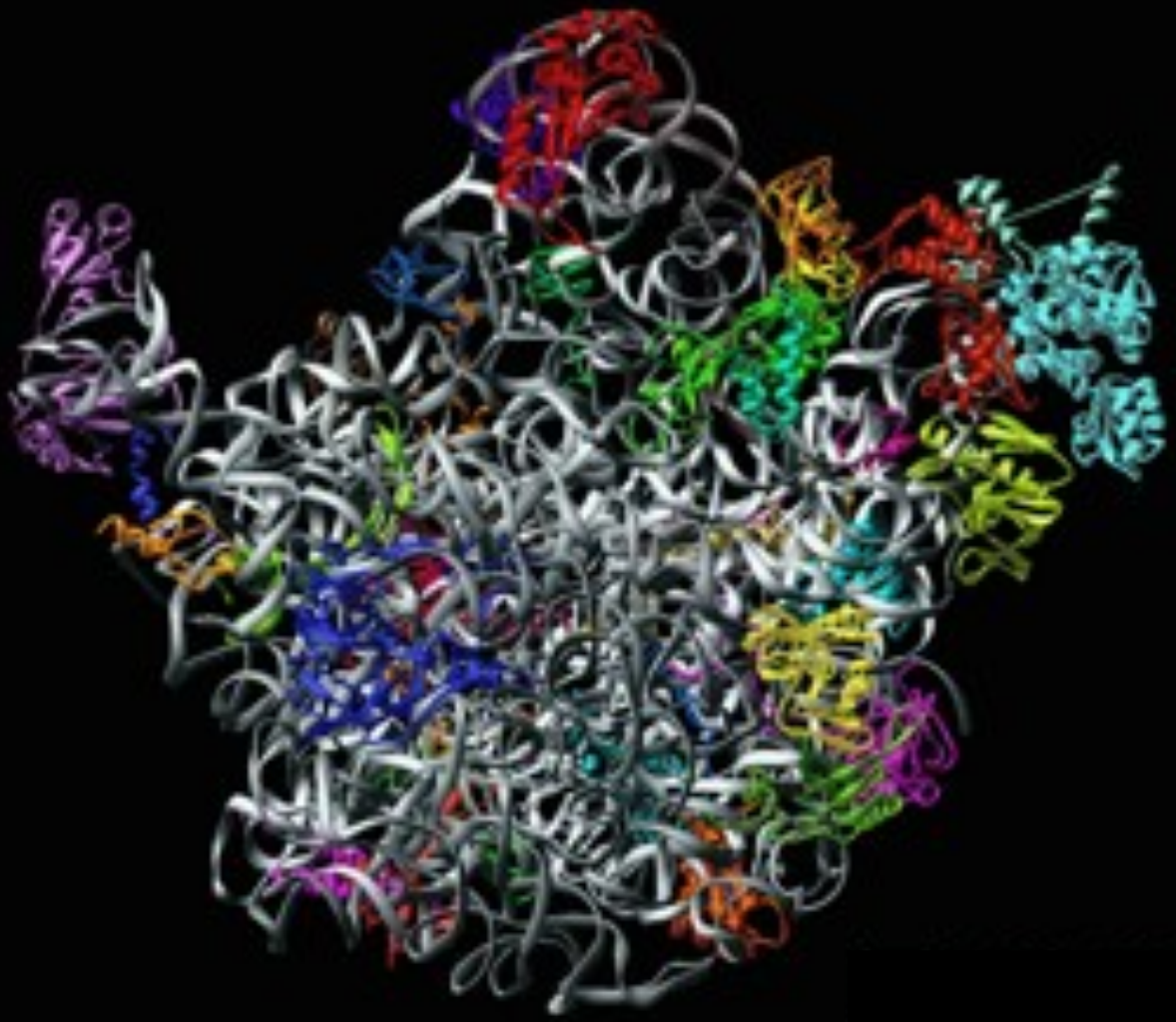
Are all four of a horse's hooves off the ground at the same time ?

Eadweard Muybridge, Sacramento, California



Eadweard Muybridge, *The Horse in Motion*

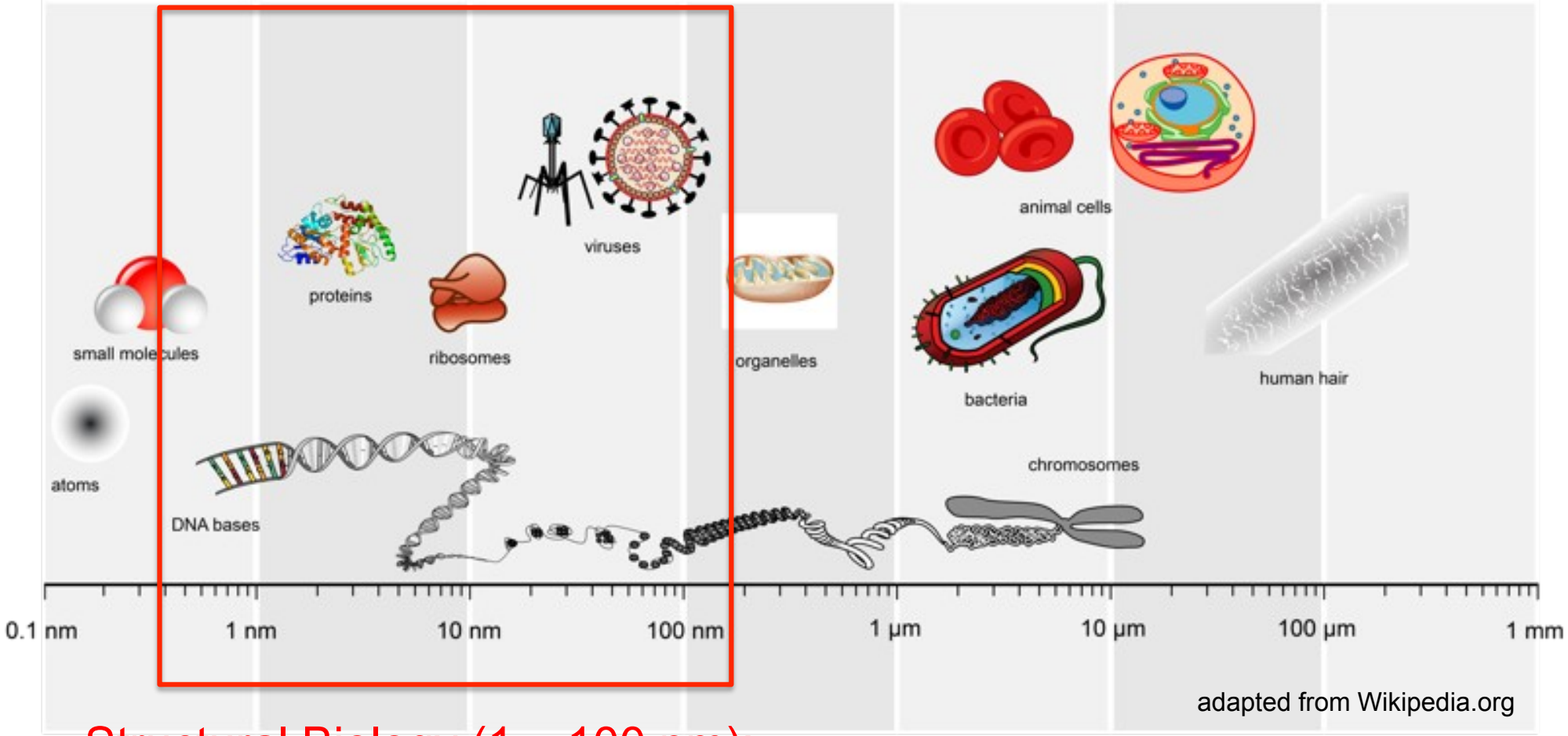
Today's Challenge: Structure and dynamics of single biomolecules



Ribosome: Harms, …. & Yonath (2001) Cell 107, 679

Opportunities for using X-ray Free electron Lasers in Structural Biology

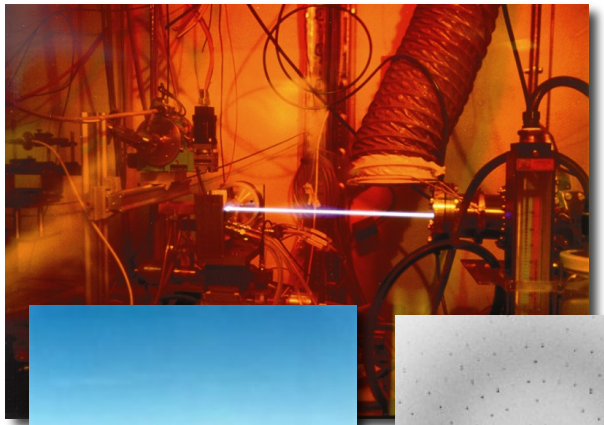
Length scales in biology



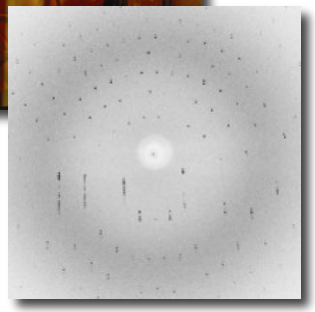
Structural Biology (1 – 100 nm):

The study of the structure and dynamics of biological macromolecules (proteins, DNA, …) and their mutual interactions to understand their function in a biological cell

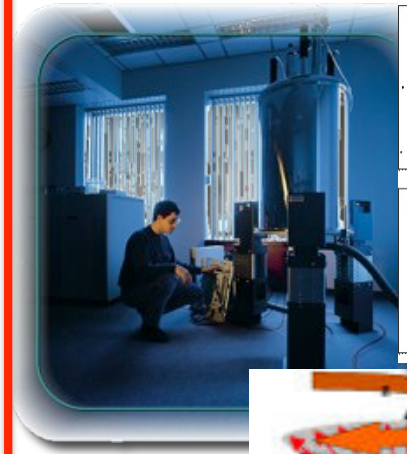
Protein structures can be determined at atomic resolution by:

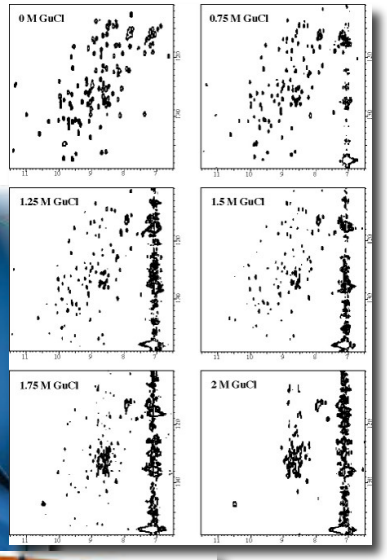


X-ray crystallography

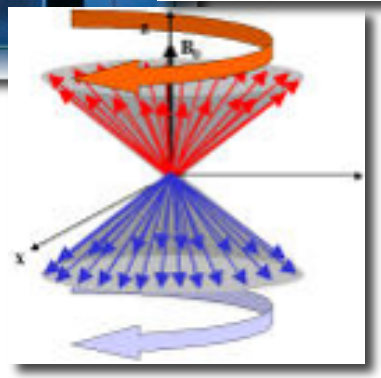


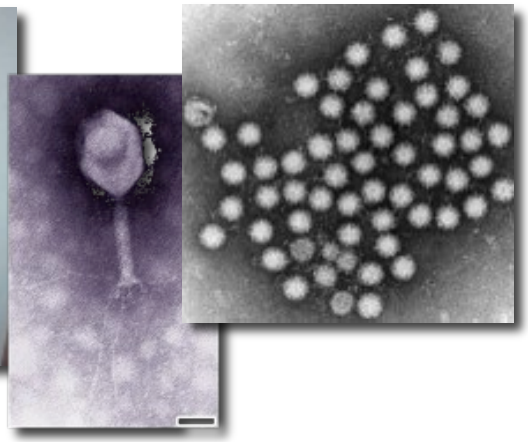
90% of the
100 000 structures
in the Protein Data Bank





NMR





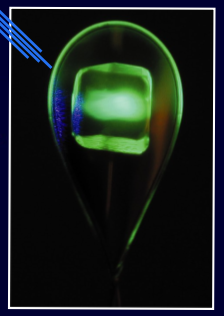
Electron microscopy

courtesy by J.-P. Colletier

CONVENTIONAL MACROMOLECULAR X-RAY CRYSTALLOGRAPHY

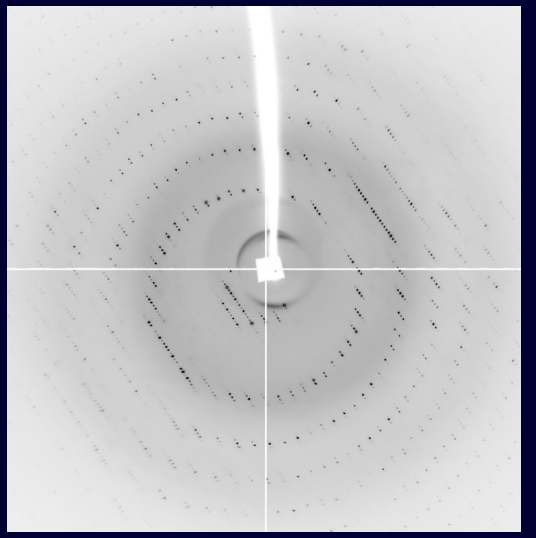
10 keV
1 Å

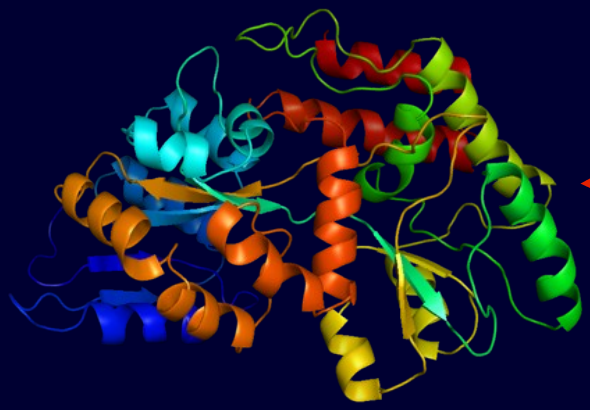
Cryo (100 K)

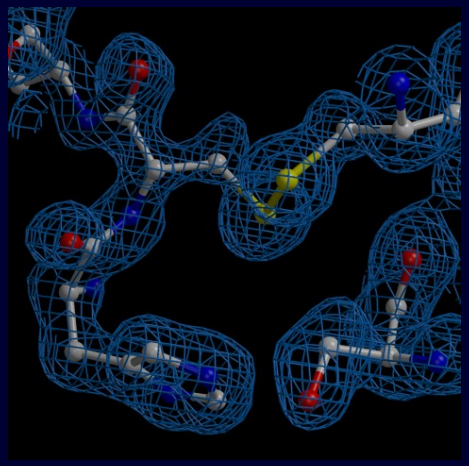


100 μm

Collect 100 diffraction images
on a single crystal
in a few minutes





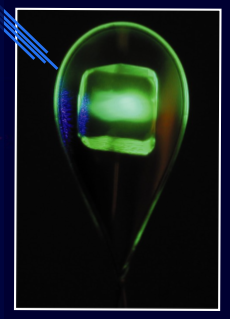


+ phase info

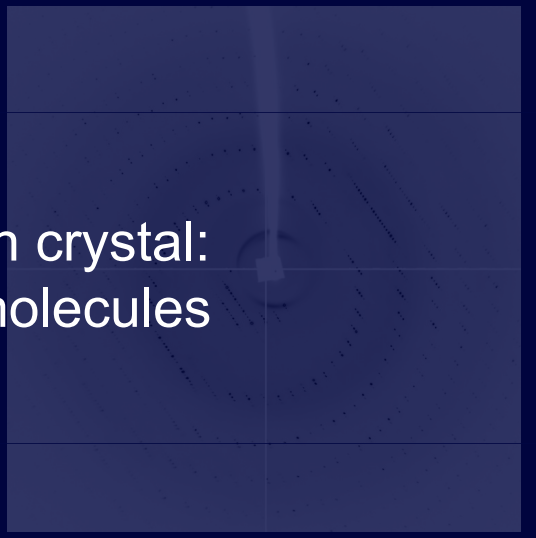
... CONVENTIONAL X-RAY CRYSTALLOGRAPHY

10 keV
1 Å

Cryo (100 K)

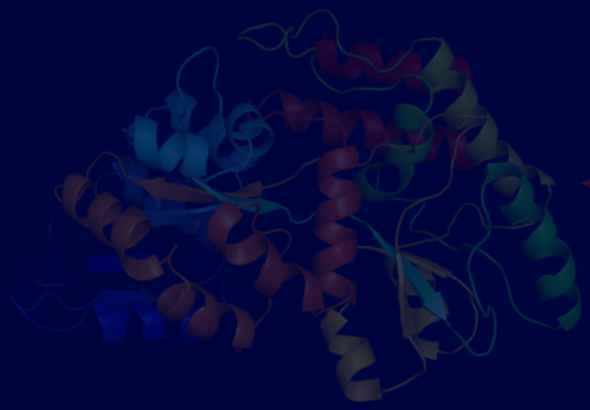


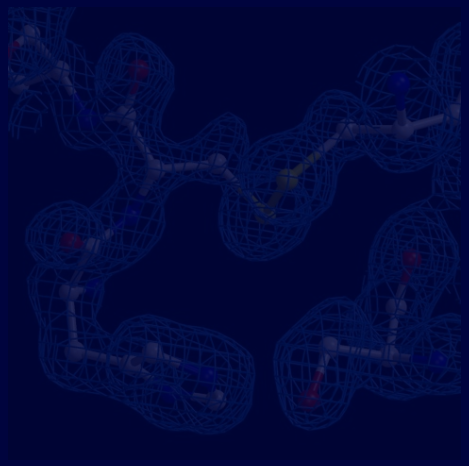
100 μm



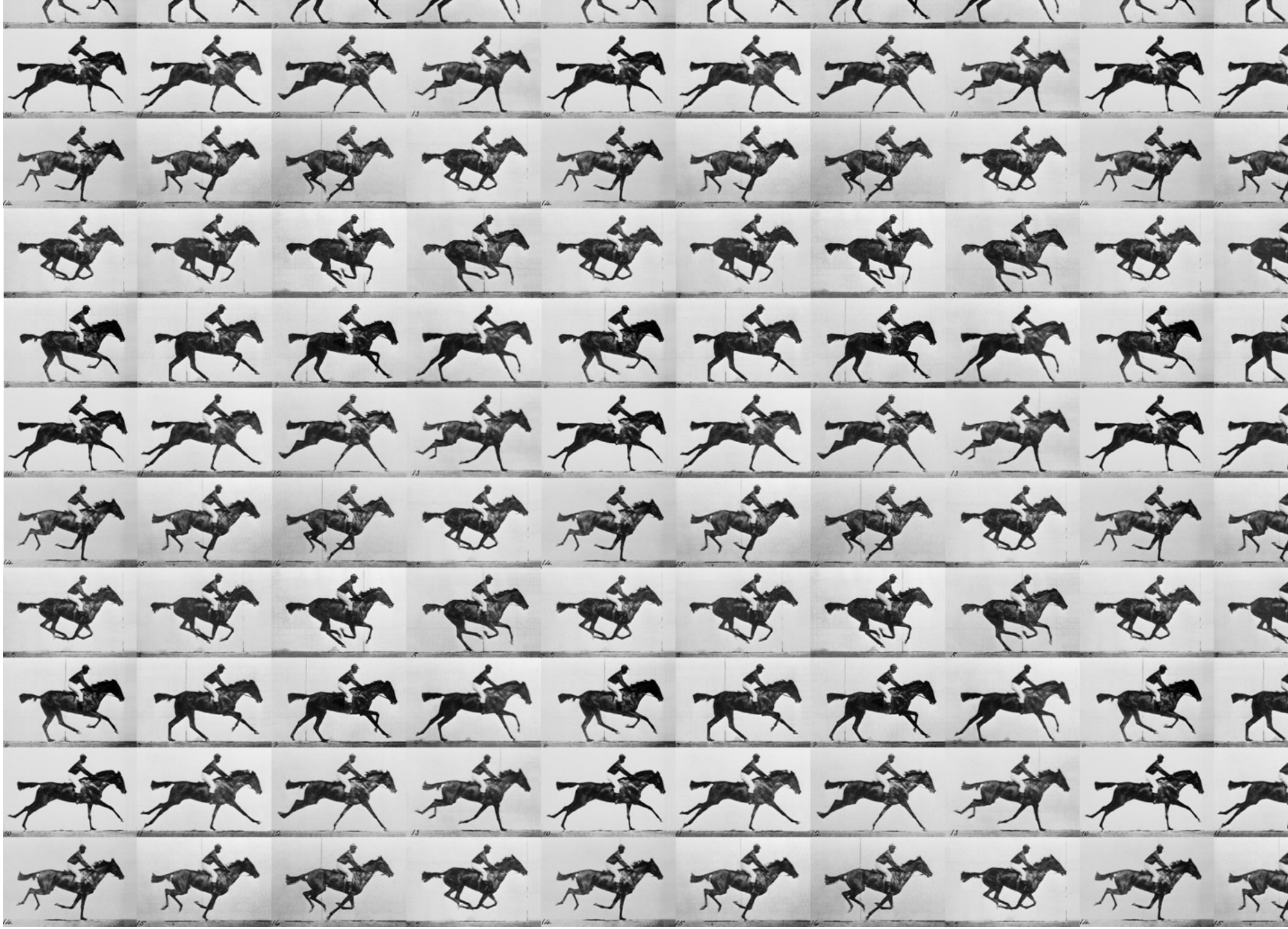
Protein crystal:
10^{15} molecules

- provides average structure





+ phase info

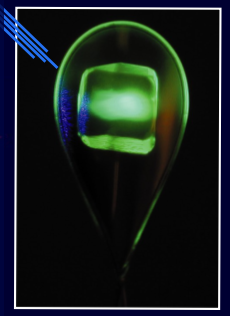


refined model: average structure

... CONVENTIONAL X-RAY CRYSTALLOGRAPHY

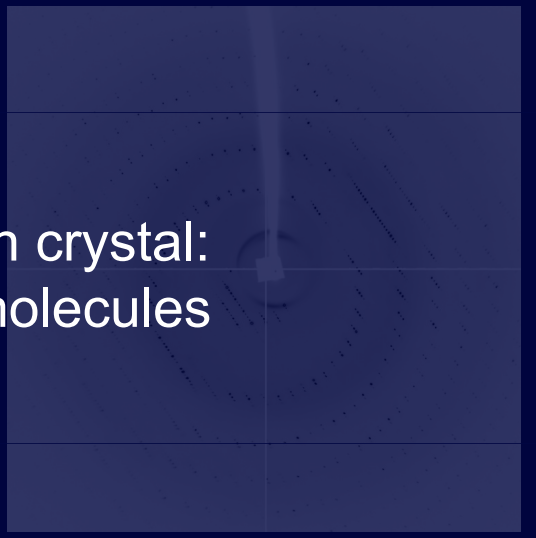
10 keV
1 Å

Cryo (100 K)



100 μm

Protein crystal:
10^{15} molecules



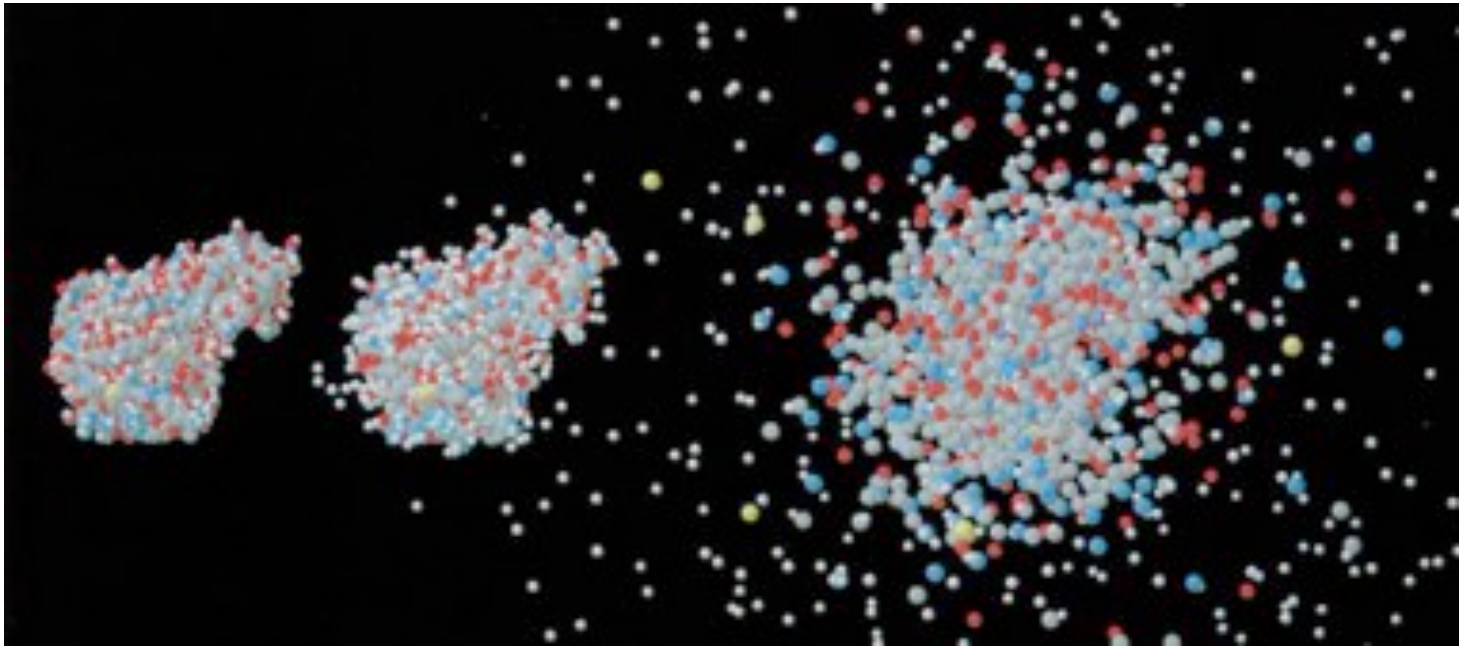
+ phase info

- provides average structure
- needs (large) crystals: problematic for complexes, membrane proteins
- structure determined at non-physiological temperature (100 K)
- chemical and structural X-ray radiation damage
- time-resolved crystallography limited to 100 ps resolution

Would be great to obtain atomic resolution structures from single molecules - no crystals needed

Coherent diffraction imaging (CDI) on single molecules using ultra-short intense X-ray pulses

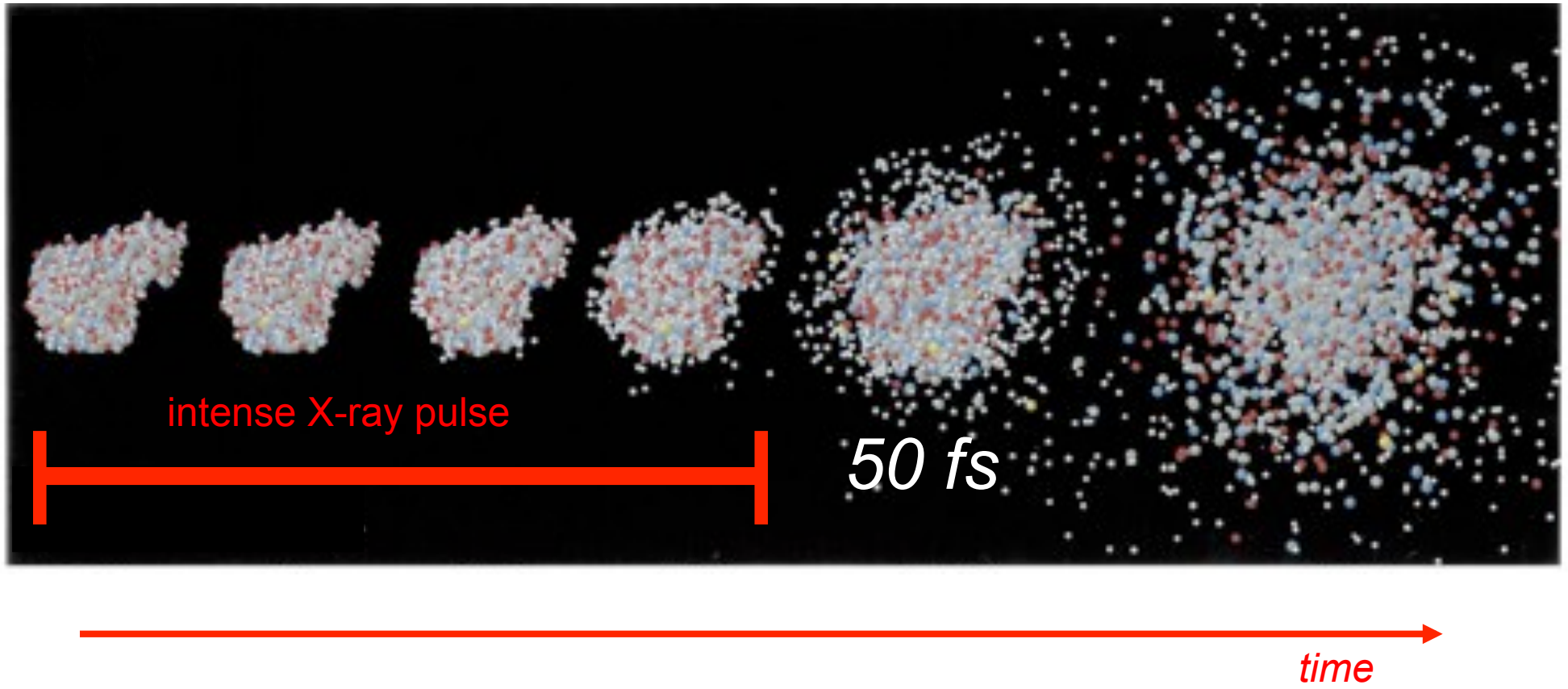
Single biomolecule hit by intense X-ray pulse



1) X-ray photons ionize atoms
2) Photoelectrons leave biomolecule
3) Protein gets more and more positively charged
4) Coulomb explosion after about 50 fs

Diffraction-before-Destruction Imaging

Idea: Neutze et al. (2000) Nature 406, 7524

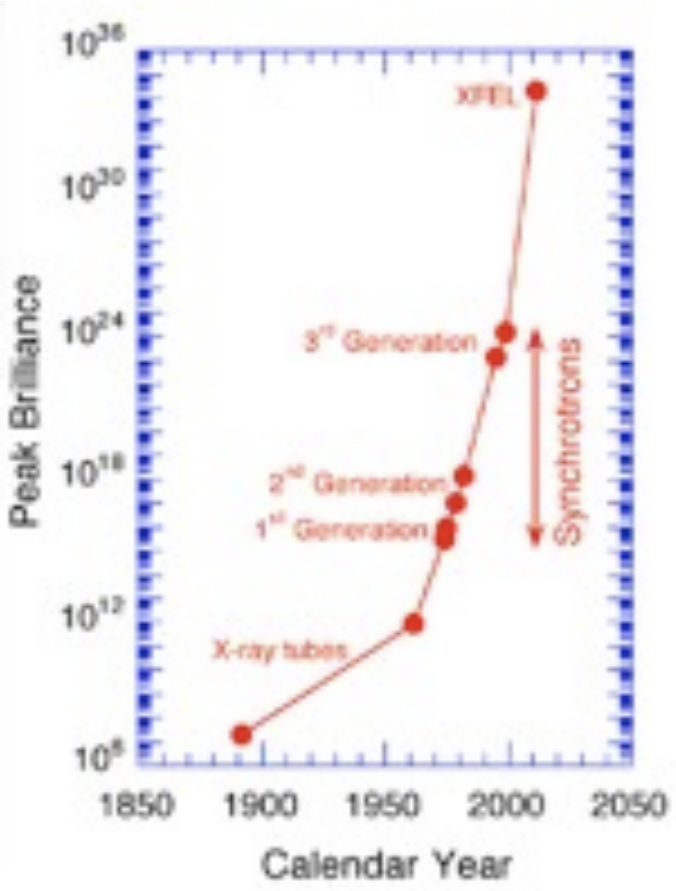


Collect data before the sample has time to respond (explode)

Diffraction-before-Distruction Imaging

needs

enormeous X-ray flux (brilliance)



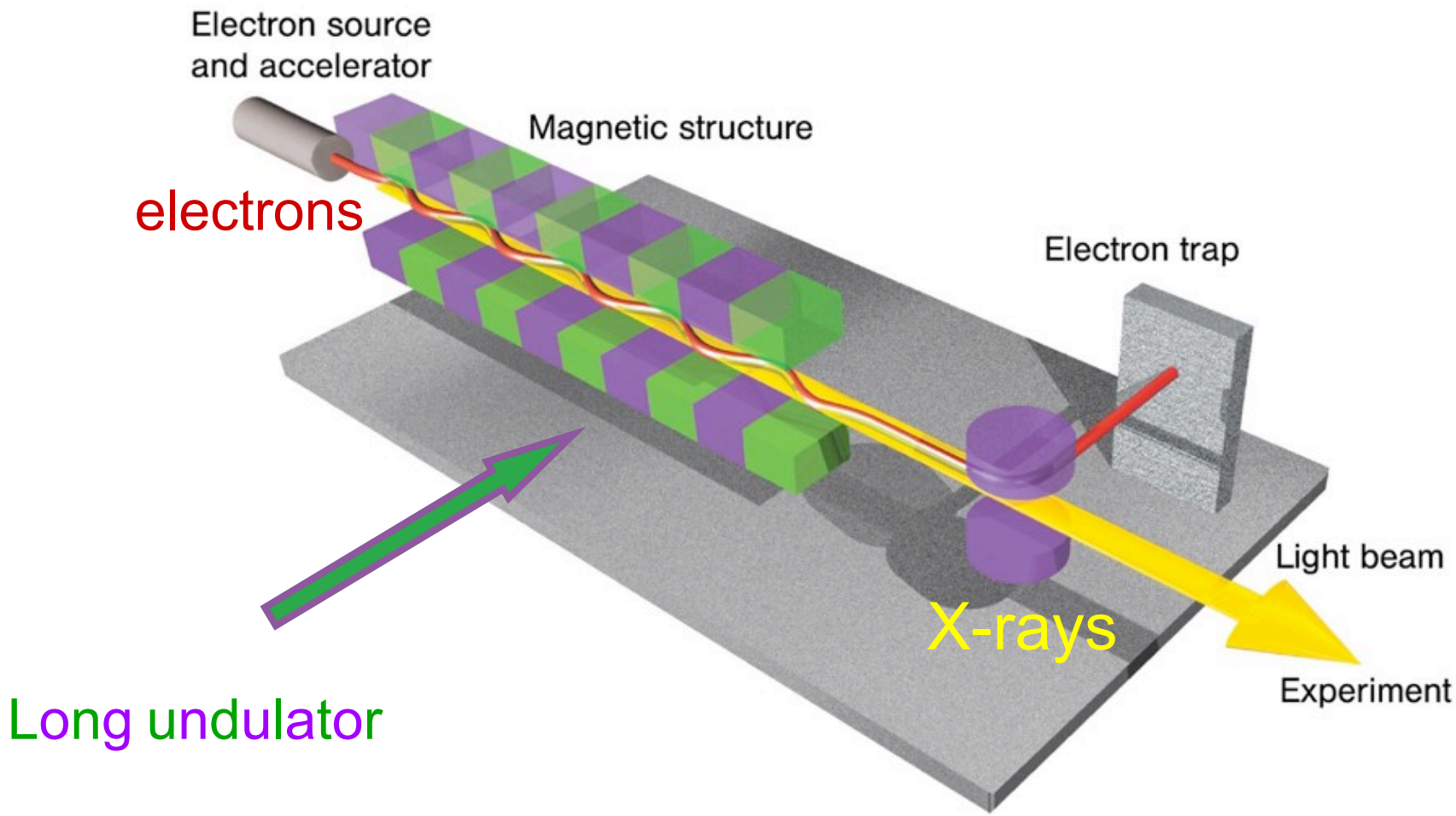
Solution:

X-ray Free Electron Lasers

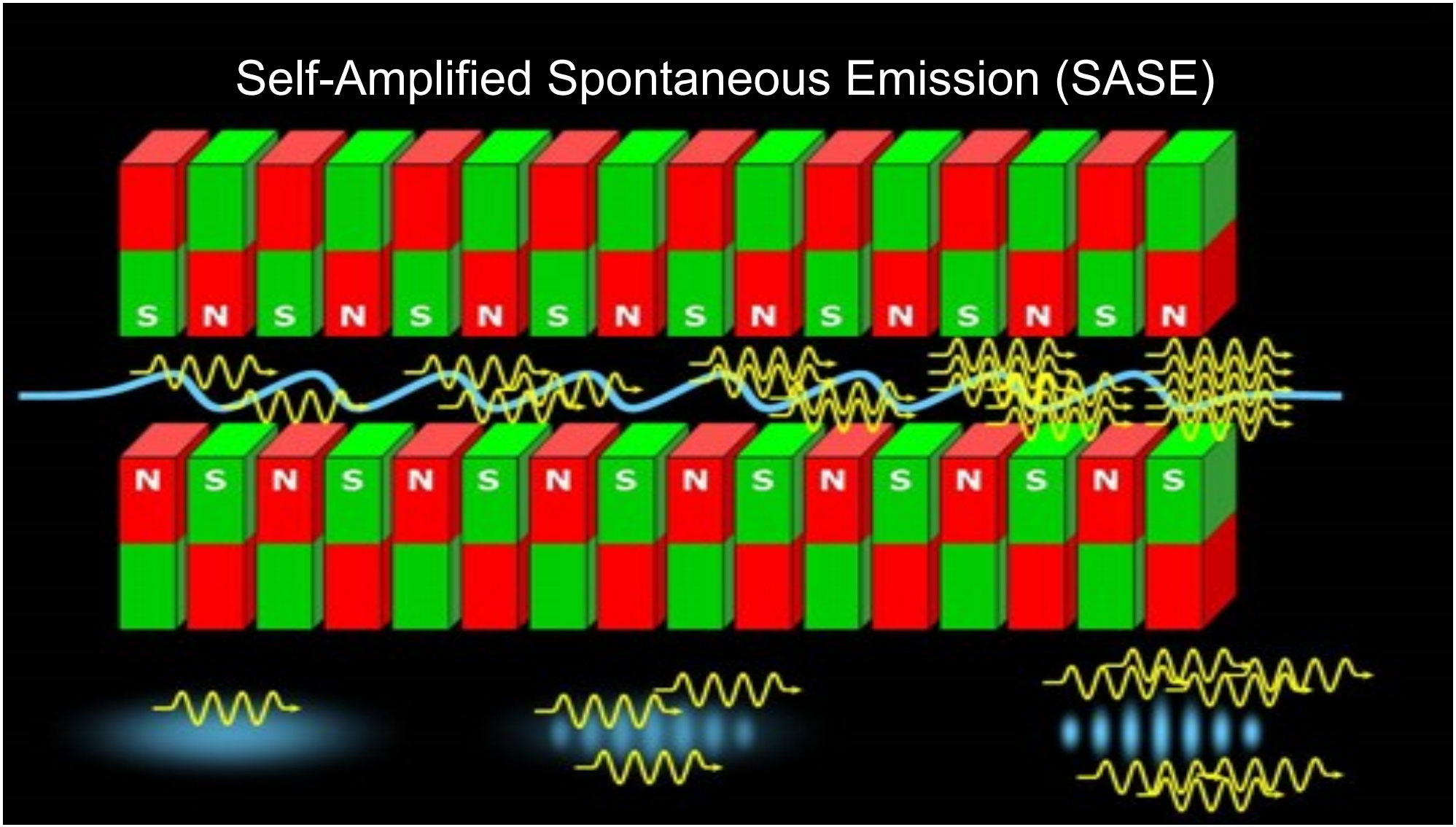
XFEL have 10^{10} times more peak brilliance than ESRF

Free Electron Laser

Idea of FEL: J. M. J. Madey (1971) J.Appl. Phys.42, 19061



Simplified description of XFEL: Margaritondo & Rebernik (2011) JSR 18, 101

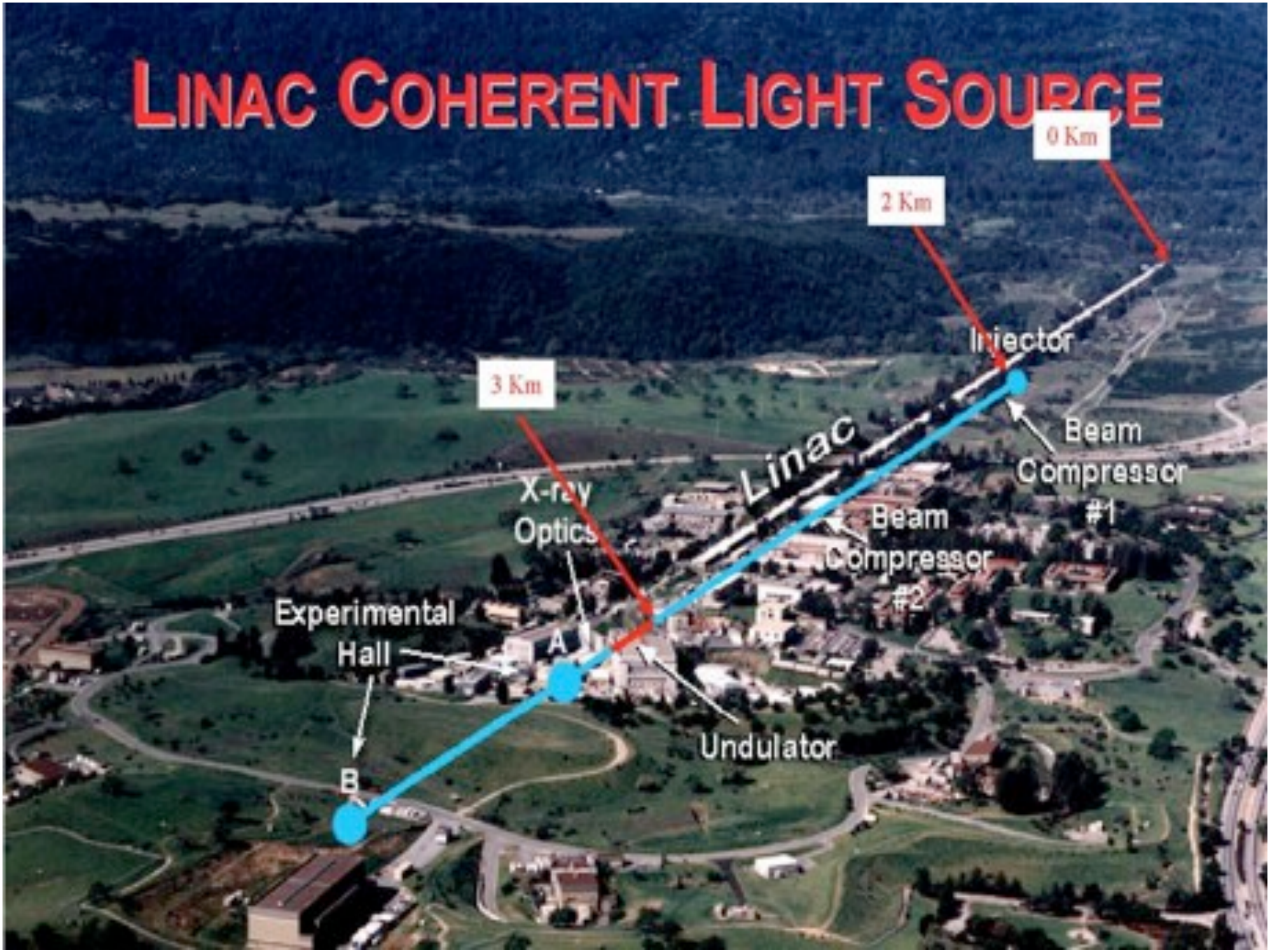


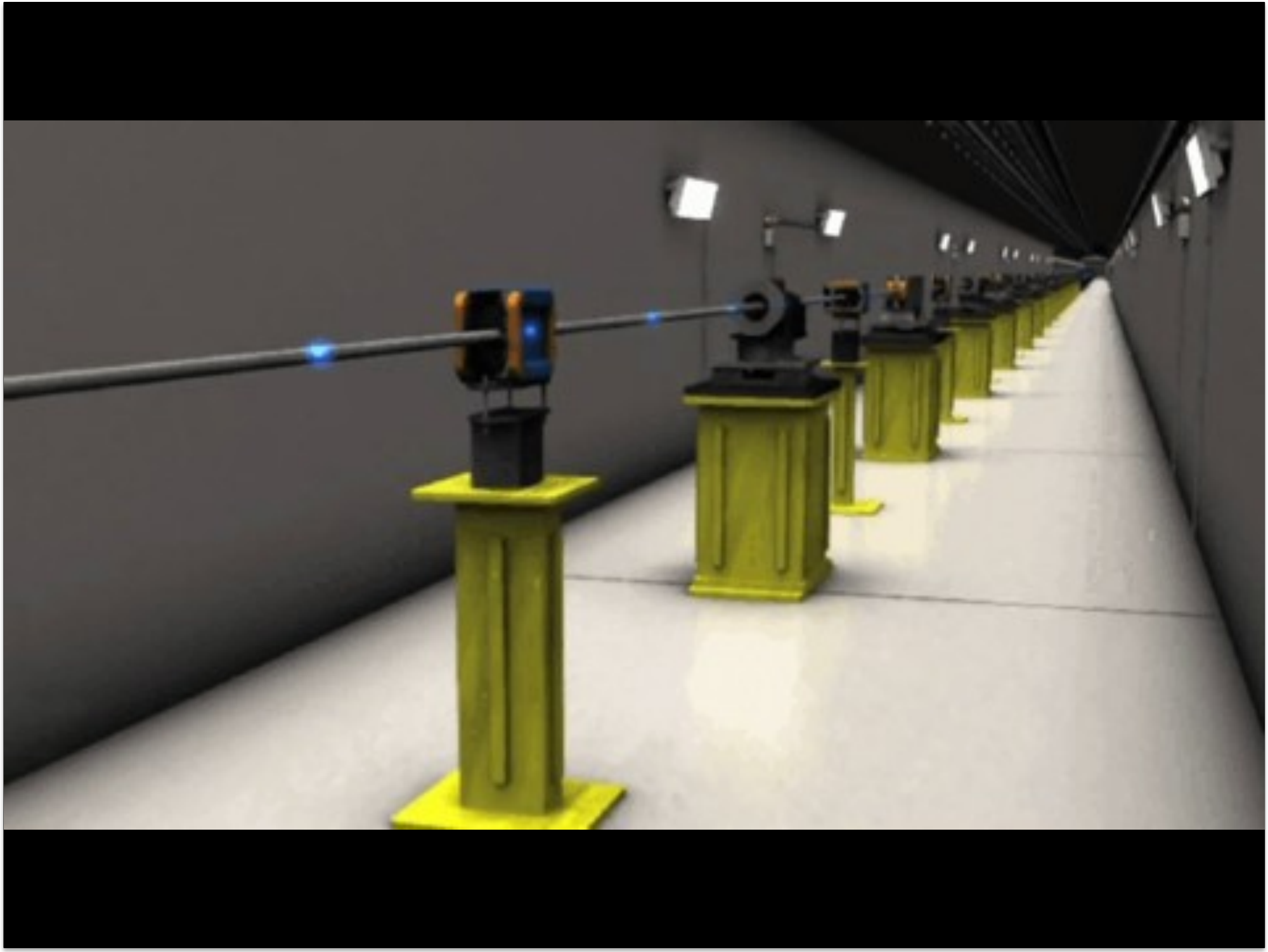
SASE in long undulator (100 m) leads to micro-bunching of electron macro-bunches

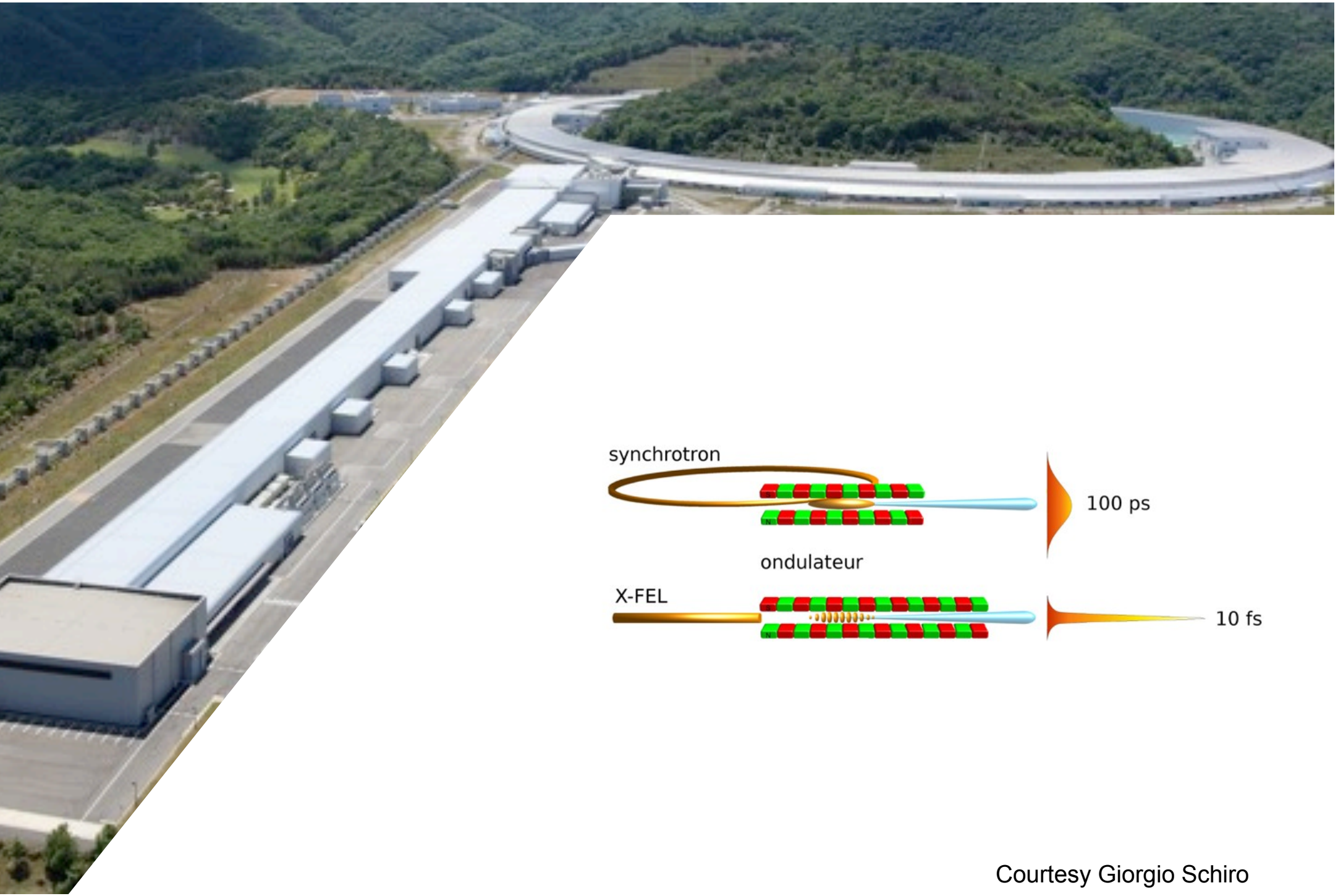
Micro-bunches are separated by a distance equal to one radiation wavelength:

Coherent wave is emitted with very high brilliance


SLAC


LINAC COHERENT LIGHT SOURCE
0 Km
2 Km
3 Km
Injector
Linac
Beam
Compressor
#1
Beam
Compressor
#2
X-ray
Optics
Experimental
Hall
A
B
Undulator



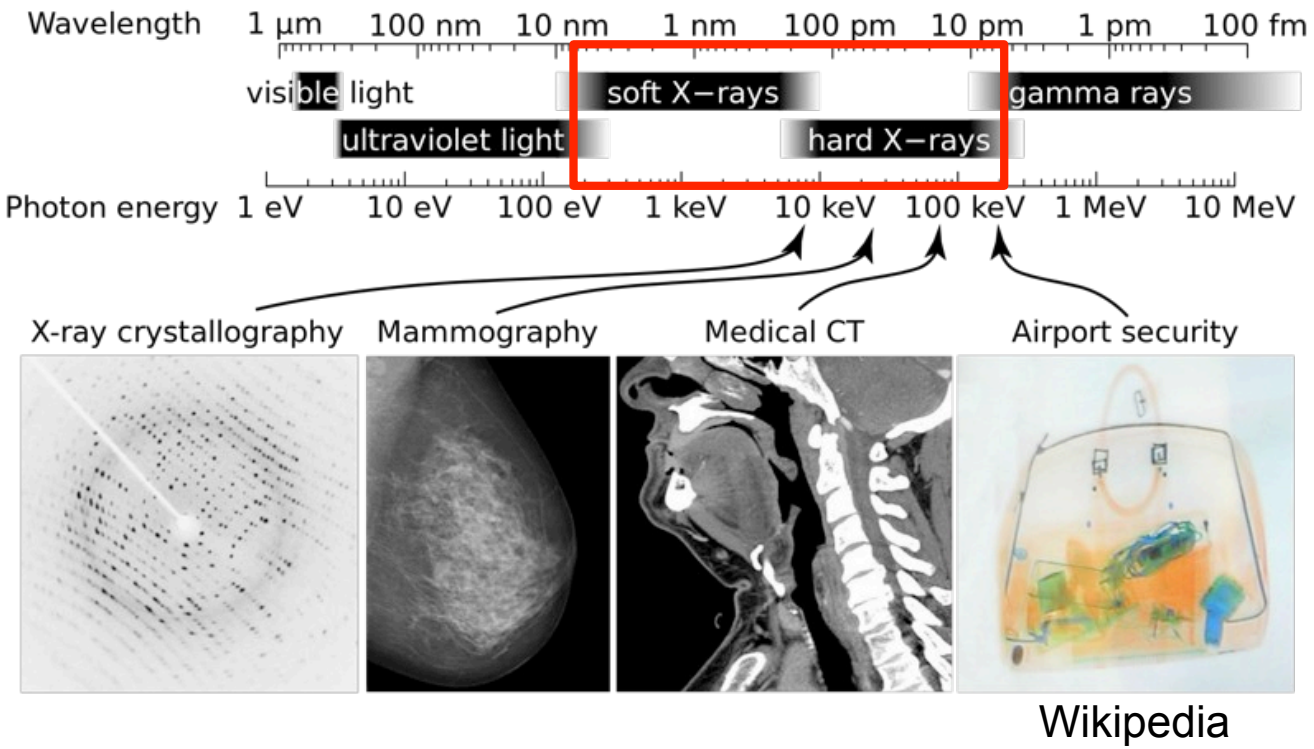


Courtesy Giorgio Schiro

X-ray Free Electron Lasers

+ PAL-XFEL (Korea, 2017 ?); Lund (2020 ?); Shanghai; UK?

X-ray free-electron LASER



Wikipedia

X-rays: wavelength 0.1 – 100 Å

X-ray **free-electron** LASER

as opposed to electrons bound to an atom

X-ray free-electron **LASER**

Similar properties than optical Laser:

- high power
- narrow bandwidth (monochromatic)
- spatial coherence

X-ray free-electron LASER
=
XFEL

- 10 orders of magnitude higher peak brilliance than synchrotrons

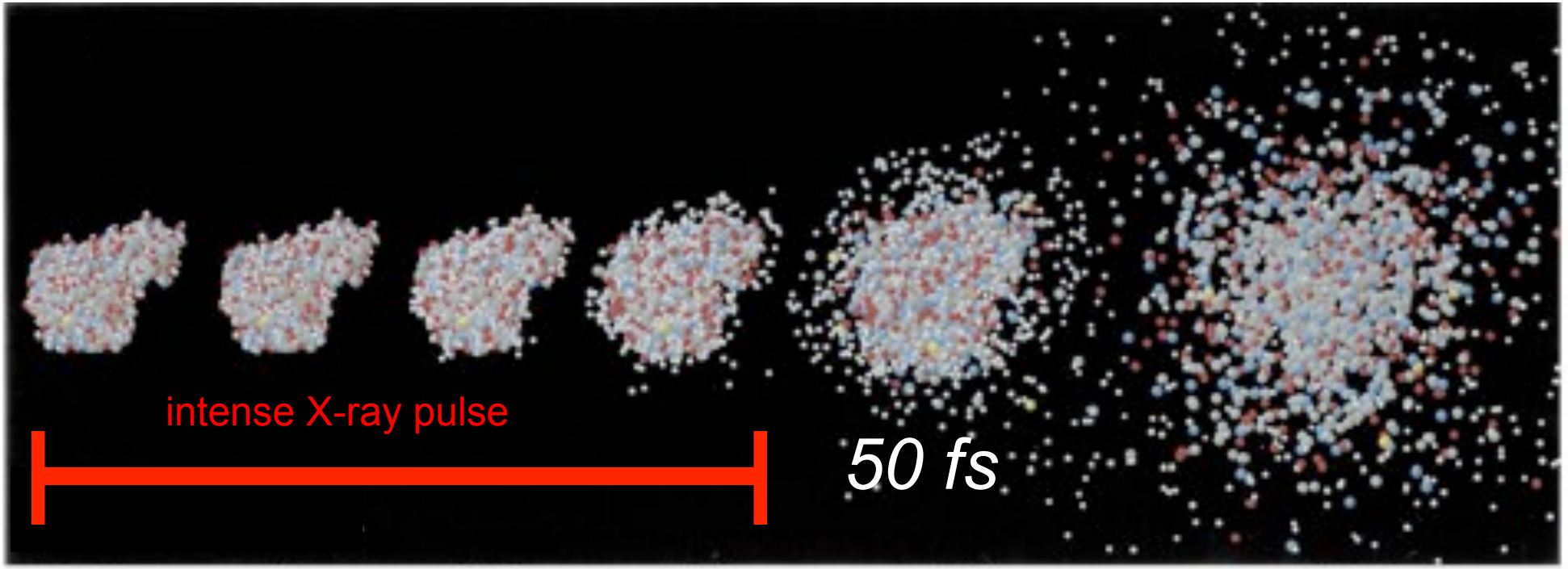
- short pulses (5 – 100 fs)

- repetition rate: 30 - 120 Hz (LCLS, SACLA, SwissFEL)
  27000 Hz (European XFEL)

- X-ray beam size : 100 nm – several microns

Diffraction-before-Destruction Imaging

Neutze et al. (2000) Nature 406, 7524



Promises of XFELs for Structural Biology:

- coherent diffraction imaging of single particles

(Review Miao et al. (2015) Science 348, 530)

- serial femtosecond crystallography (SFX)

(Review Schlichting (2015) IUCrJ 2)

Diffraction-before-Destruction Imaging

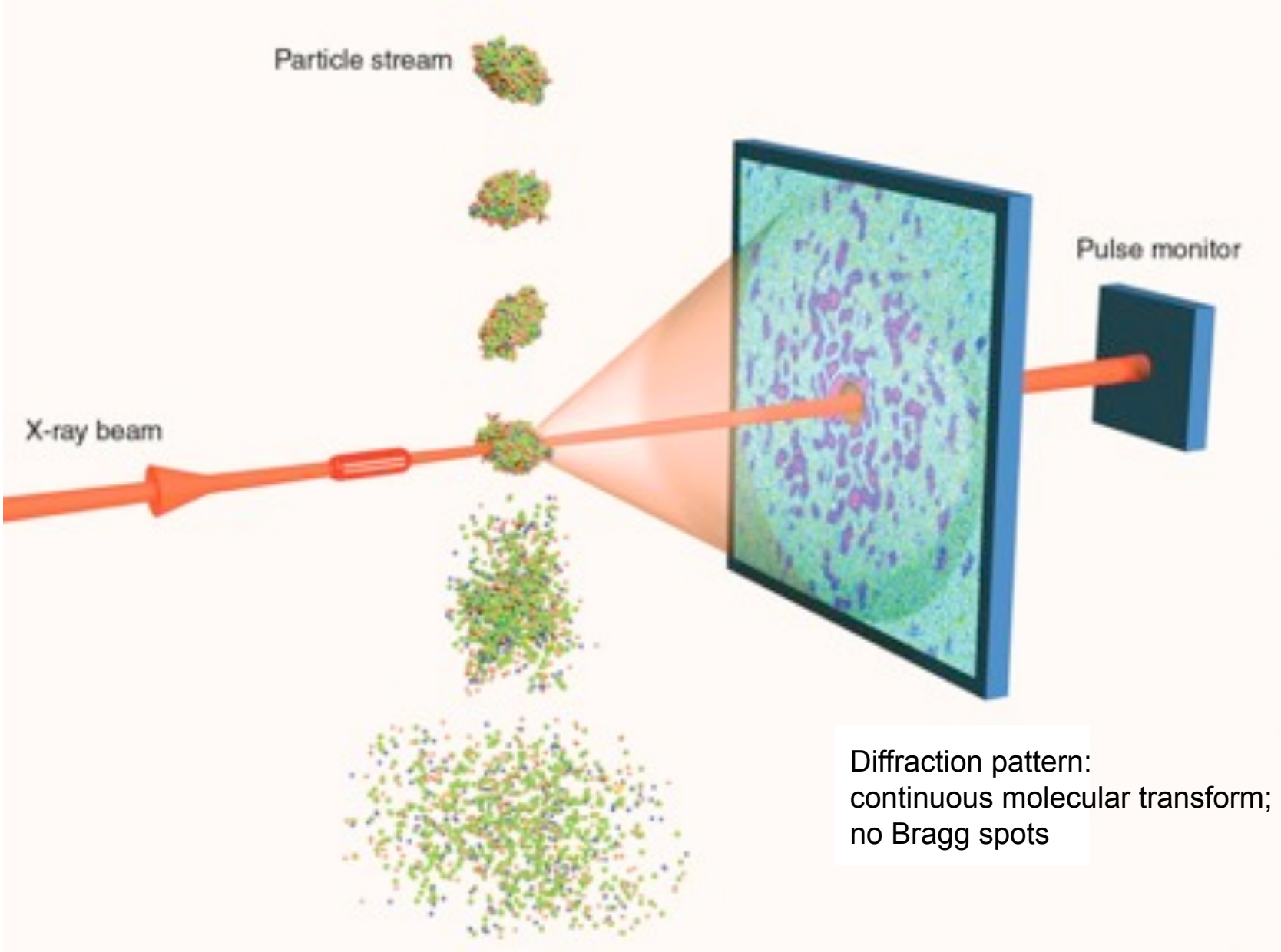
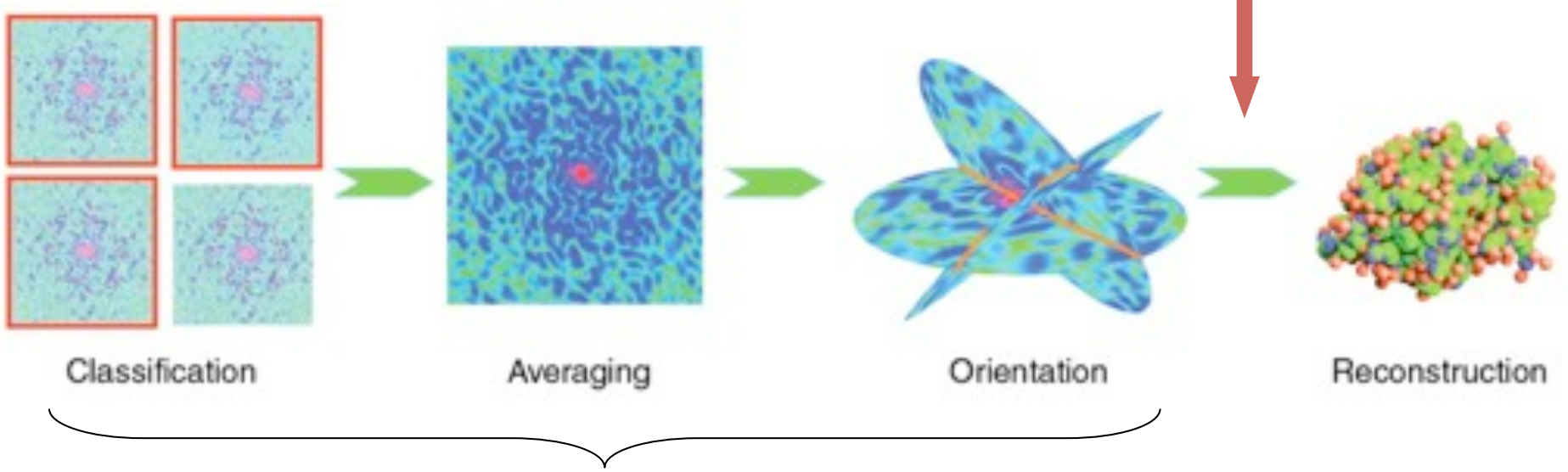


Image: Gaffney & Chapman (2007) Science 316, 1444

Diffraction-before-Distruction Imaging: data processing

Essential: Direct phase retrieval by the oversampling technique (Miao et al/ (2001) PNAS 98, 6641)

10^7 single shots necessary



Essential: Methods from single particle EM

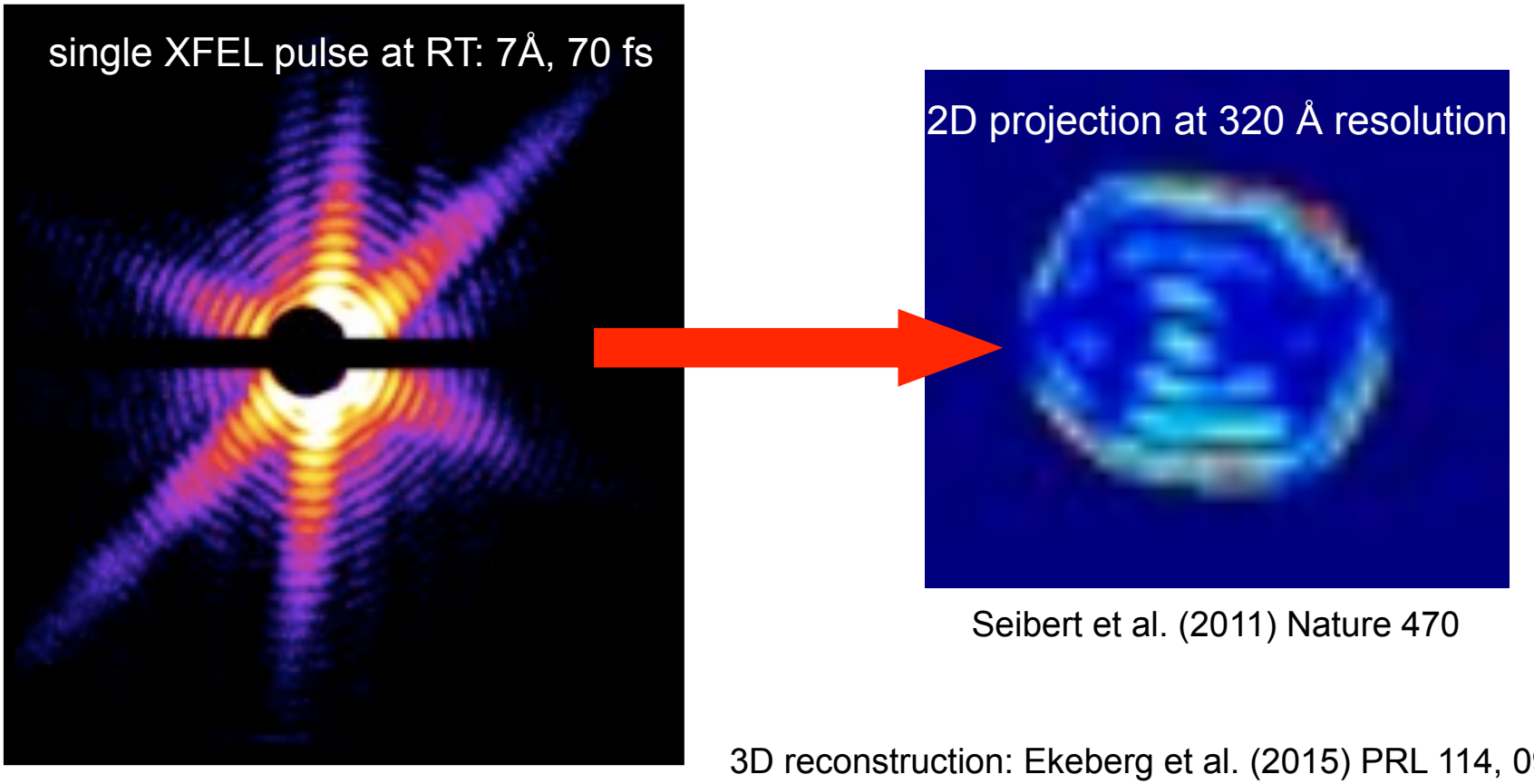
Gaffney & Chapman (2007) Science 316, 1444

LETTER 3 FEBRUARY 2011 | VOL 470 | NATURE | 79

doi:10.1038/nature09748

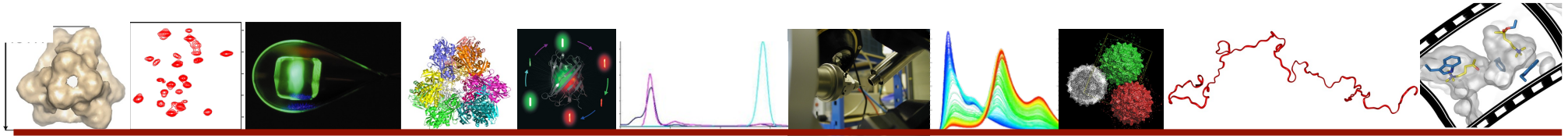
Single mimivirus particles intercepted and imaged with an X-ray laser

M. Marvin Seibert[1]*, Tomas Ekeberg[1]*, Filipe R. N. C. Maia[1]*, Martin Svenda[1], Jakob Andreasson[1], Olof Jönsson[1], Duško Odić[1], Bianca Iwan[1], Andrea Rocker[1], Daniel Westphal[1], Max Hantke[1], Daniel P. DePonte[2], Anton Barty[2], Joachim Schulz[2], Lars Gumprecht[2], Nicola Coppola[2], Andrew Aquila[2], Mengning Liang[2], Thomas A. White[2], Andrew Martin[2], Carl Caleman[1,2], Stephan Stern[2,3], Chantal Abergel[4], Virginie Seltzer[4], Jean-Michel Claverie[4], Christoph Bostedt[5], John D. Bozek[5], Sébastien Boutet[5], A. Alan Miahnahri[5], Marc Messerschmidt[5], Jacek Krzywinski[5], Garth Williams[5], Keith O. Hodgson[6], Michael J. Bogan[6], Christina Y. Hampton[6], Raymond G. Sierra[6], Dmitri Starodub[6], Inger Andersson[7], Saša Bajt[8], Miriam Barthelmess[8], John C. H. Spence[9], Petra Fromme[10], Uwe Weierstall[9], Richard Kirian[9], Mark Hunter[10], R. Bruce Doak[9], Stefano Marchesini[11], Stefan P. Hau-Riege[12], Matthias Frank[12], Robert L. Shoeman[13], Lukas Lomb[13], Sascha W. Epp[14,15], Robert Hartmann[16], Daniel Rolles[13,14], Artem Rudenko[14,15], Carlo Schmidt[14,15], Lutz Foucar[13,14], Nils Kimmel[17,18], Peter Holl[16], Benedikt Rudek[14,15], Benjamin Erk[14,15], André Hömke[14,15], Christian Reich[16], Daniel Pietschner[17,18], Georg Weidenspointner[17,18], Lothar Strüder[14,17,18,19], Günter Hauser[17,18], Hubert Gorke[20], Joachim Ullrich[14,15], Ilme Schlichting[13,14], Sven Herrmann[17,18], Gerhard Schaller[17,18], Florian Schopper[17,18], Heike Soltau[16], Kai-Uwe Kühnel[15], Robert Andritschke[17,18], Claus-Dieter Schröter[15], Faton Krasniqi[13,14], Mario Bott[13], Sebastian Schorb[21], Daniela Rupp[21], Marcus Adolph[21], Tais Gorkhover[21], Helmut Hirsemann[8], Guillaume Potdevin[8], Heinz Graafsma[8], Björn Nilsson[8], Henry N. Chapman[2,3] & Janos Hajdu[1]



Seibert et al. (2011) Nature 470

3D reconstruction: Ekeberg et al. (2015) PRL 114, 098102



Integrative structural biology in space and time

1 Å
3 Å
10 Å
5 nm
20 nm
200 nm

SAXS, mass spectrometry

single-particle
cryo EM

super-resolution
fluorescence microscopy

X-ray, neutron
crystallography,
NMR, MD, QM/MM

cryo
electron
tomography

light
microscopy

correlative microscopy

XFEL CDI

XFEL CDI

ps

Neutrons, MD, QM/MM

NMR

Kinetic crystallography
Mass spectrometry

s

Coherent diffraction imaging on single molecules

using ultra-short intense XFEL pulses …

… limited today to molecules 1μm – 100 nm

(too small: hemoglobin: 5 nm; ribosome 25 nm)

< 100 nm: not enough X-ray flux

> 1μm : saturation of detector at low angle

Still science fiction: structure of single proteins (-complexes),

but OK for viruses (> 100 nm) at low resolution

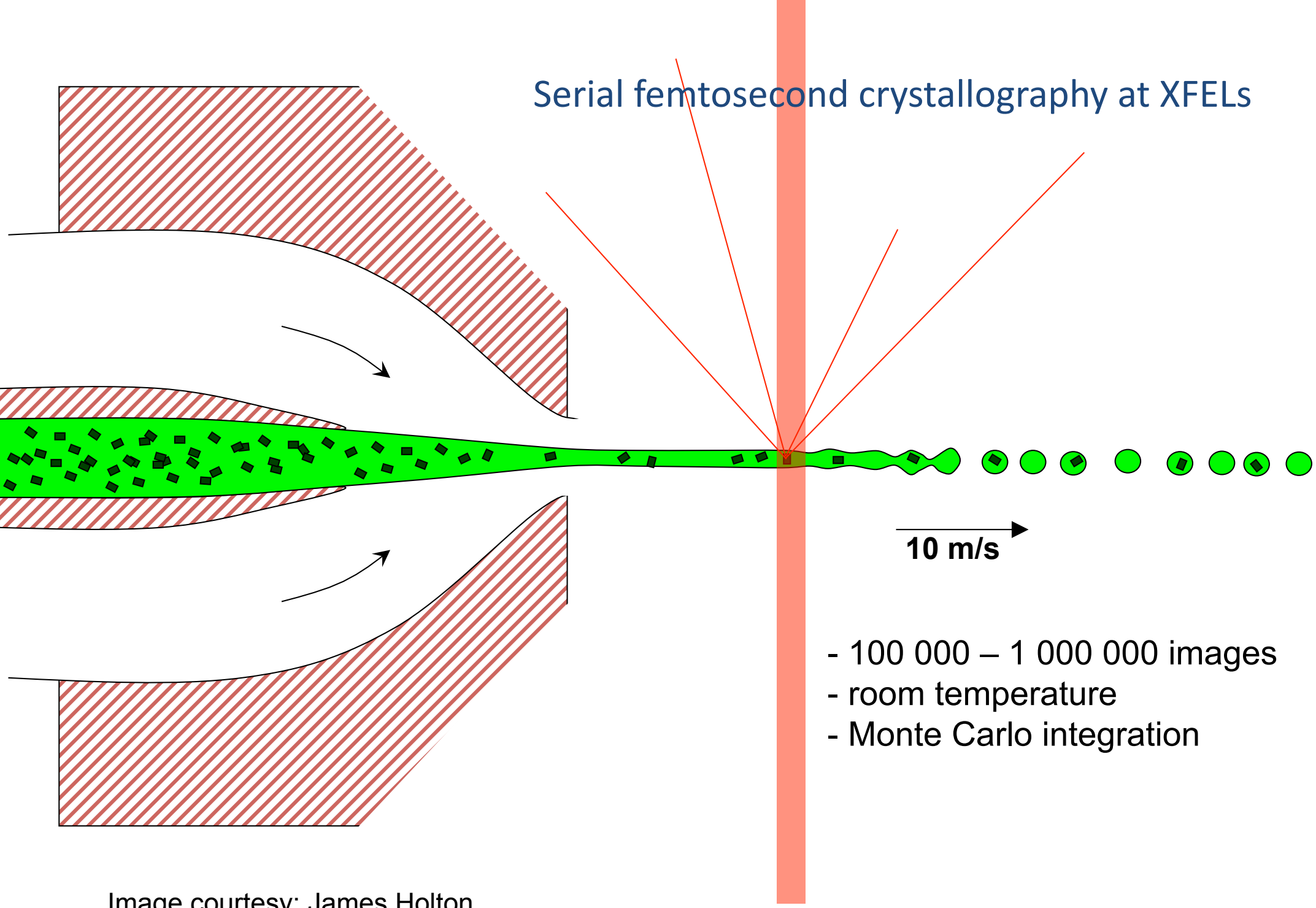
But :

Serial femtosecond crystallography (SFX)

using XFELs

shows great promise

Serial femtosecond crystallography at XFELs



- 100 000 – 1 000 000 images
- room temperature
- Monte Carlo integration

Image courtesy: James Holton

Question :

Does diffraction-before-destruction work in

serial femtosecond crystallography (SFX) at XFELs ?

Question :

Does diffraction-before-**destruction** work in

serial femtosecond crystallography (SFX) at XFELs ?

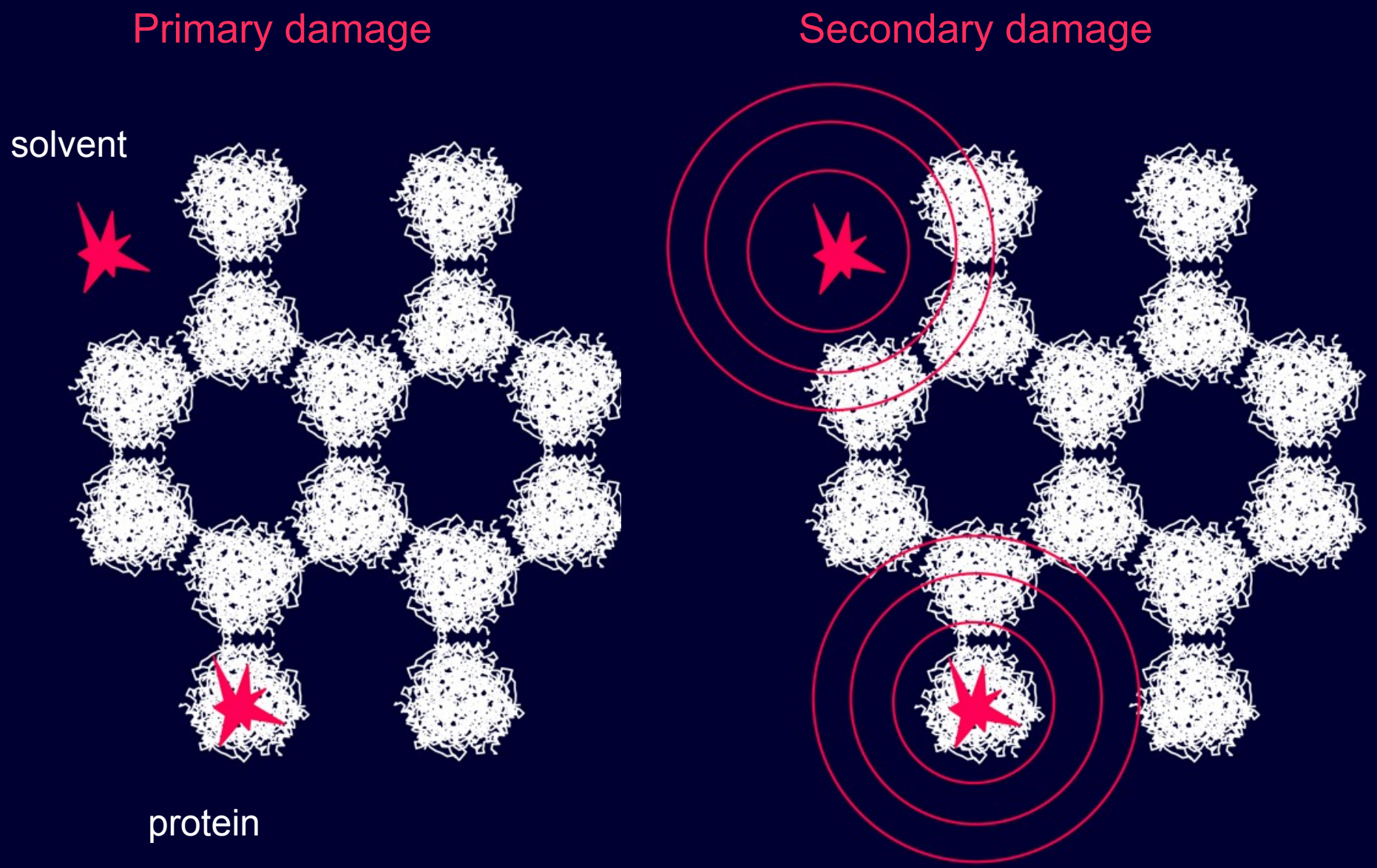
X-ray radiation damage to crystalline proteins

produced by intense **synchrotron** radiation

Courtesy James Holton

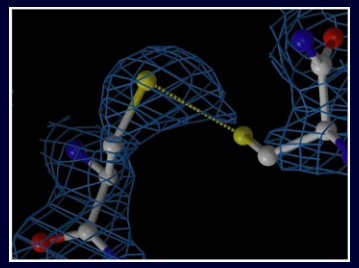
X-ray radiation damage (synchrotron)

Primary and secondary damage

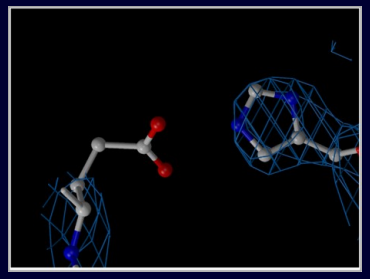


Secondary radicals produce specific radiation damage

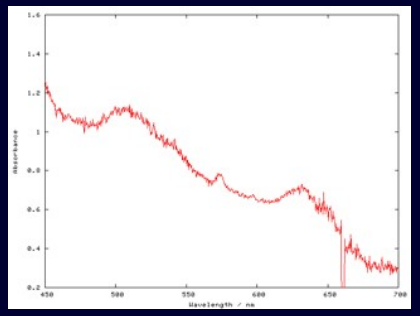
Disulfide bond cleavage



Decarboxylation



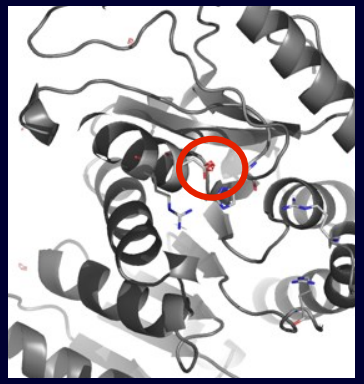
Metal center reduction …



… within seconds,

before full data set is collected

Active site damage



in PDB: many protein structures with reduced redox centers

Dose (Garman) limit in synchrotron-based protein crystallography at 100 K : **30 MGy**

Owen *et al.* (2006) *PNAS 103, 4912*

Question :

Does diffraction-before-destruction work in

serial femtosecond crystallography (SFX) at XFELs ?

Serial femtosecond crystallography (SFX) using XFELs

Six hallmark papers

Chapman et al. (**2011**) Nature 470, 73
Femtosecond X-ray protein nanocrystallography

Boutet et al. (**2012**) Science 337, 362
High-resolution protein structure determination by serial femtosecond crystallography

Kern et al. (**2013**) Science 340, 491
Simultaneous femtosecond X-ray spectroscopy and diffraction of photosystem II at room temperature

Redecke et al. (**2013**) Science 339, 227
Natively inhibited trypanosoma brucei cathepsin B structure determined by using an X-ray laser

Barends et al. (**2014**) Nature 505, 244
De novo protein crystal structure determination from X-ray free-electron laser data

Tenboer et al. (**2014**) *Science **346: 1242***
Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein

LETTER

doi:10.1038/nature09750

Femtosecond X-ray protein nanocrystallography

Henry N. Chapman[1,2], Petra Fromme[3], Anton Barty[1], Thomas A. White[1], Richard A. Kirian[4], Andrew Aquila[1], Mark S. Hunter[3], Joachim Schulz[1], Daniel P. DePonte[1], Uwe Weierstall[4], R. Bruce Doak[4], Filipe R. N. C. Maia[5], Andrew V. Martin[1], Ilme Schlichting[6,7], Lukas Lomb[7], Nicola Coppola[1]†, Robert L. Shoeman[7], Sascha W. Epp[6,8], Robert Hartmann[9], Daniel Rolles[6,7], Artem Rudenko[6,8], Lutz Foucar[6,7], Nils Kimmel[10], Georg Weidenspointner[11,10], Peter Holl[9], Mengning Liang[1], Miriam Barthelmess[12], Carl Caleman[1], Sébastien Boutet[13], Michael J. Bogan[14], Jacek Krzywinski[13], Christoph Bostedt[13], Saša Bajt[12], Lars Gumprecht[1], Benedikt Rudek[6,8], Benjamin Erk[6,8], Carlo Schmidt[6,8], André Hömke[6,8], Christian Reich[9], Daniel Pietschner[10], Lothar Strüder[6,10], Günter Hauser[10], Hubert Gorke[15], Joachim Ullrich[6,8], Sven Herrmann[10], Gerhard Schaller[10], Florian Schopper[10], Heike Soltau[9], Kai-Uwe Kühnel[8], Marc Messerschmidt[13], John D. Bozek[13], Stefan P. Hau-Riege[16], Matthias Frank[16], Christina Y. Hampton[14], Raymond G. Sierra[14], Dmitri Starodub[14], Garth J. Williams[13], Janos Hajdu[5], Nicusor Timneanu[5], M. Marvin Seibert[5]†, Jakob Andreasson[5], Andrea Rocker[5], Olof Jönsson[5], Martin Svenda[5], Stephan Stern[1], Karol Nass[2], Robert Andritschke[10], Claus-Dieter Schröter[8], Faton Krasniqi[6,7], Mario Bott[7], Kevin E. Schmidt[4], Xiaoyu Wang[4], Ingo Grotjohann[3], James M. Holton[17], Thomas R. M. Barends[7], Richard Neutze[18], Stefano Marchesini[17], Raimund Fromme[3], Sebastian Schorb[19], Daniela Rupp[19], Marcus Adolph[19], Tais Gorkhover[19], Inger Andersson[20], Helmut Hirsemann[12], Guillaume Potdevin[12], Heinz Graafsma[12], Björn Nilsson[12] & John C. H. Spence[4]

Chapman et al. (2011) Nature 470, 73

Novelty:

First XFEL SFX ever

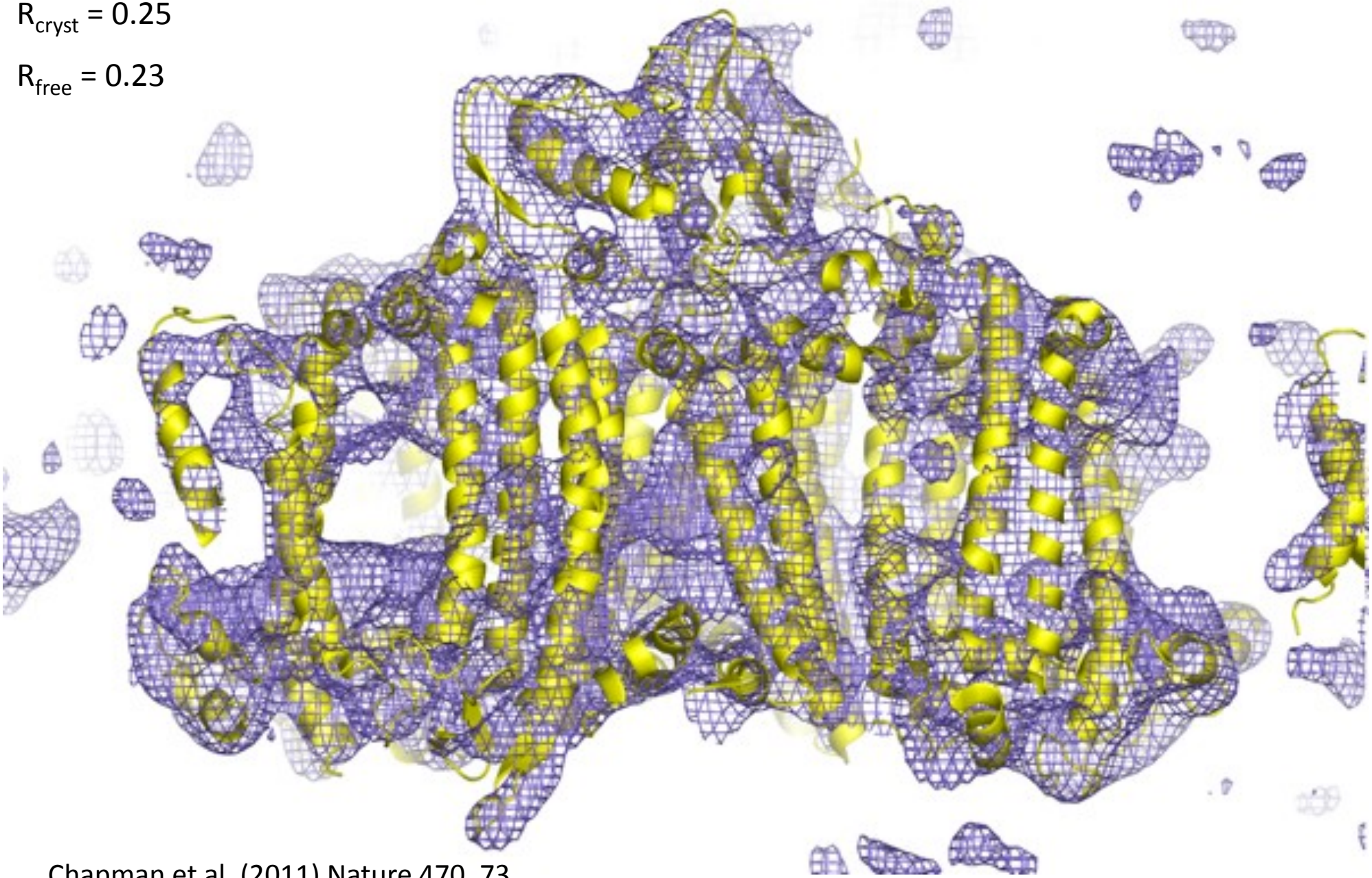
Diffraction-before-destruction works

- nanocrystals (200 nm - 1 μm) of photosystem I (membrane protein)
- XFEL pulse at room temperature: 7Å, 70 fs
- 1.85 million XFEL shots > 112725 hits (6%) > 15445 indexed images (14%)
- no global radiation damage
  after single 70 fs shot at 670 MGy / pulse (20x Garman limit – 30 MGy)
  - molecular replacement: maps at 8 Å

Electron density of PSI at 8Å

R_{cryst} = 0.25

R_{free} = 0.23

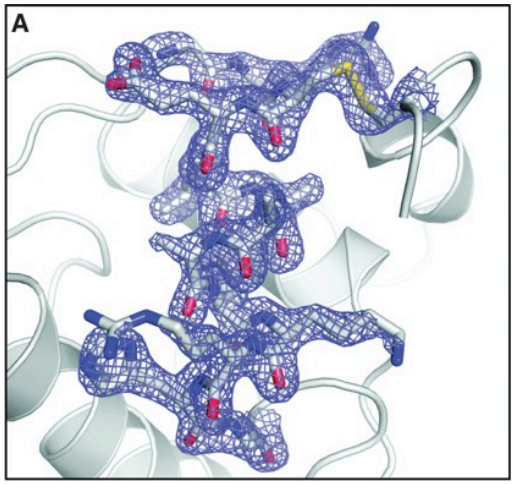


Chapman et al. (2011) Nature 470, 73

High-Resolution Protein Structure Determination by Serial Femtosecond Crystallography

Sébastien Boutet,[1*] Lukas Lomb,[2,3] Garth J. Williams,[1] Thomas R. M. Barends,[2,3] Andrew Aquila,[4] R. Bruce Doak,[5] Uwe Weierstall,[5] Daniel P. DePonte,[4] Jan Steinbrener,[2,3] Robert L. Shoeman,[2,3] Marc Messerschmidt,[1] Anton Barty,[4] Thomas A. White,[4] Stephan Kassemeyer,[2,3] Richard A. Kirian,[5] M. Marvin Seibert,[1] Paul A. Montanez,[1] Chris Kenney,[6] Ryan Herbst,[6] Philip Hart,[6] Jack Pines,[6] Gunther Haller,[6] Sol M. Gruner,[7,8] Hugh T. Philipp,[7] Mark W. Tate,[7] Marianne Hromalik,[9] Lucas J. Koerner,[10] Niels van Bakel,[11] John Morse,[12] Wilfred Ghonsalves,[1] David Arnlund,[13] Michael J. Bogan,[14] Carl Caleman,[4] Raimund Fromme,[15] Christina Y. Hampton,[14] Mark S. Hunter,[15] Linda C. Johansson,[13] Gergely Katona,[13] Christopher Kupitz,[15] Mengning Liang,[4] Andrew V. Martin,[4] Karol Nass,[16] Lars Redecke,[17,18] Francesco Stellato,[4] Nicusor Timneanu,[19] Dingjie Wang,[5] Nadia A. Zatsepin,[5] Donald Schafer,[1] James Defever,[1] Richard Neutze,[13] Petra Fromme,[15] John C. H. Spence,[5] Henry N. Chapman,[4,16] Ilme Schlichting[2,3]

Boutet et al. (2012) Science 337, 362



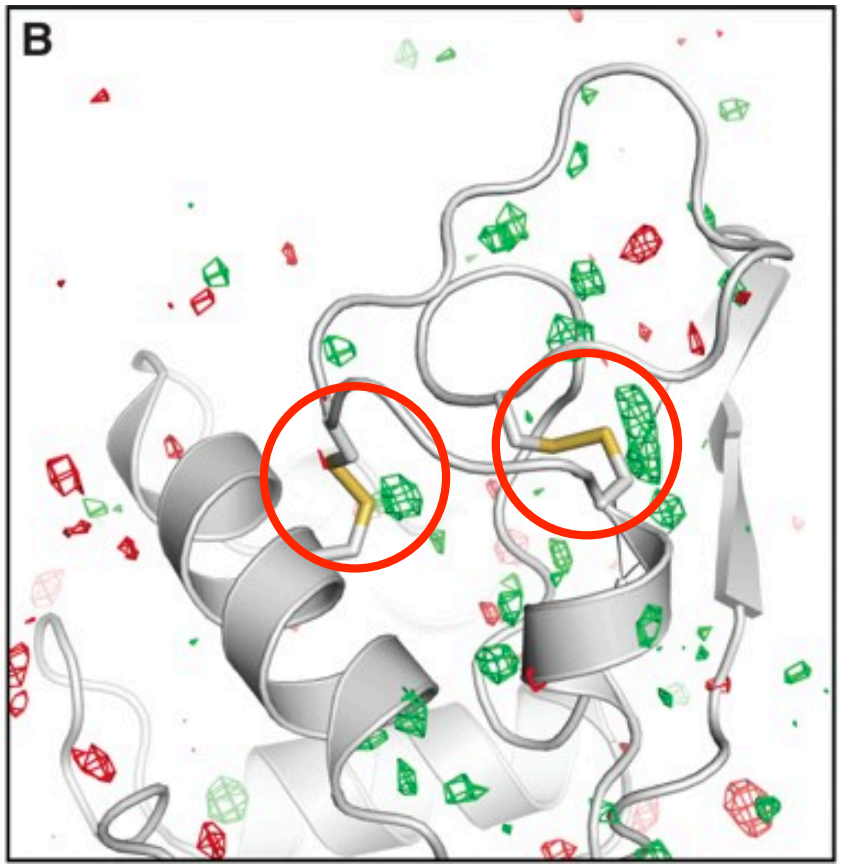
- microcrystals (1 μm) of HEWL
- XFEL pulse at room temperature: 1.3 Å, 40 fs, 33 MGy / pulse
- 1.5 million XFEL shots > 4.5% hit rate > 18% indexing rate (12500 images)
- molecular replacement: maps at 1.9 Å

Novelty:

First high-resolution SFX

Fobs (XFEL, 33 MGy) - Fobs (synchrotron, 24 kGy)

Boutet et al. (2012) Science 337, 362



No specific structural radiation damage

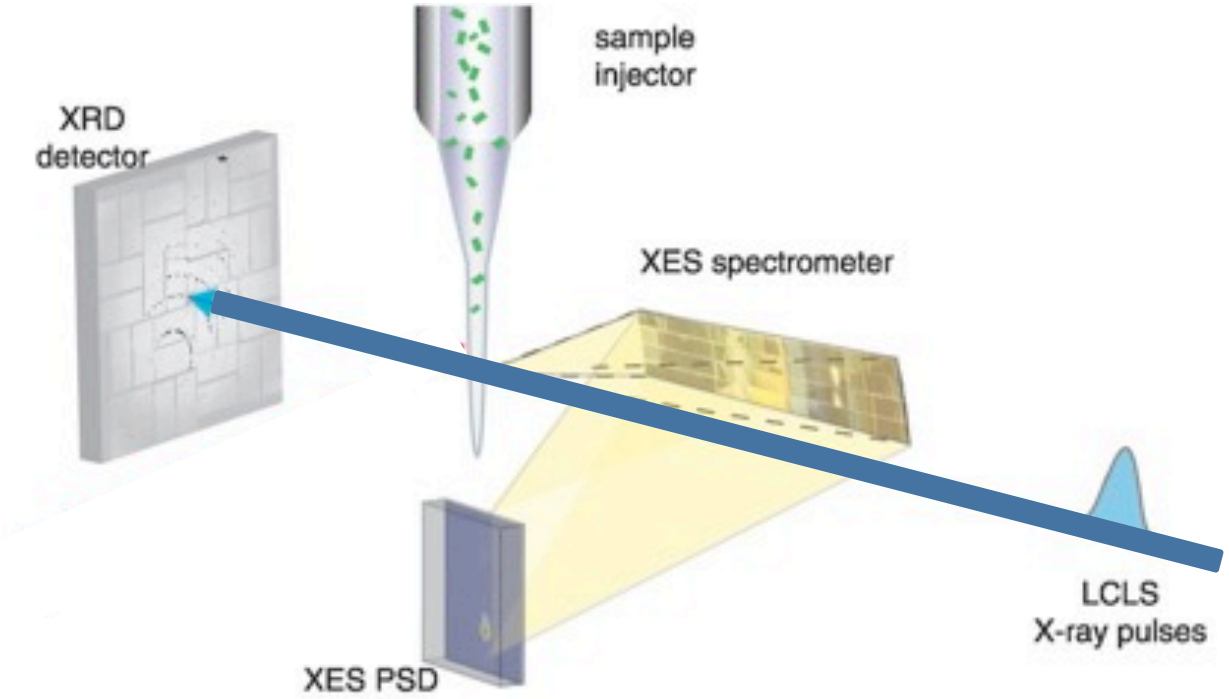
Simultaneous Femtosecond X-ray Spectroscopy and Diffraction of Photosystem II at Room Temperature

Jan Kern,[1,2] Roberto Alonso-Mori,[2] Rosalie Tran,[1] Johan Hattne,[1] Richard J. Gildea,[1] Nathaniel Echols,[1] Carina Glöckner,[3] Julia Hellmich,[3] Hartawan Laksmono,[4] Raymond G. Sierra,[4] Benedikt Lassalle-Kaiser,[1]* Sergey Koroidov,[5] Alyssa Lampe,[1] Guangye Han,[1] Sheraz Gul,[1] Dörte DiFiore,[3] Despina Milathianaki,[2] Alan R. Fry,[2] Alan Miahnahri,[2] Donald W. Schafer,[2] Marc Messerschmidt,[2] M. Marvin Seibert,[2] Jason E. Koglin,[2] Dimosthenis Sokaras,[6] Tsu-Chien Weng,[6] Jonas Sellberg,[6,7] Matthew J. Latimer,[6] Ralf W. Grosse-Kunstleve,[1] Petrus H. Zwart,[1] William E. White,[2] Pieter Glatzel,[8] Paul D. Adams,[1] Michael J. Bogan,[2,4] Garth J. Williams,[2] Sébastien Boutet,[2] Johannes Messinger,[5] Athina Zouni,[3] Nicholas K. Sauter,[1] Vittal K. Yachandra,[1]† Uwe Bergmann,[2]† Junko Yano[1]†

Kern et al. (2013) Science 340, 491

Novelty:

Combining SFX and spectroscopy:
no electronic radiation damage



Metal center (here Mn_4CaO_5 cluster of PS II)
retains intact electronic structure in XFEL SFX

LETTER

doi:10.1038/nature12773

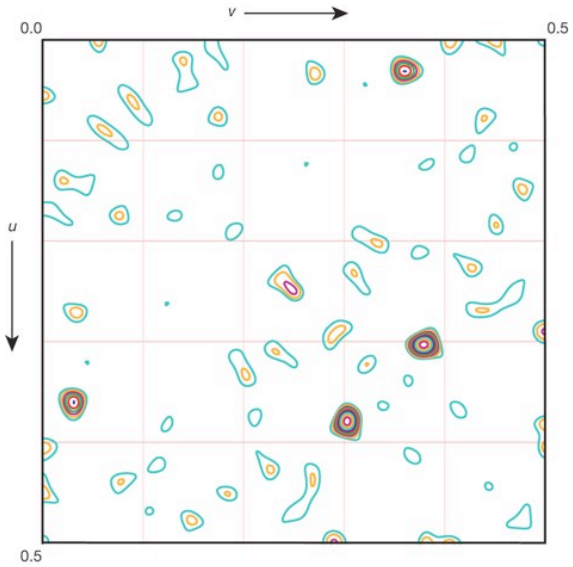
De novo protein crystal structure determination from X-ray free-electron laser data

Thomas R. M. Barends[1], Lutz Foucar[1], Sabine Botha[1], R. Bruce Doak[1,2], Robert L. Shoeman[1], Karol Nass[1], Jason E. Koglin[3], Garth J. Williams[3], Sébastien Boutet[3], Marc Messerschmidt[3] & Ilme Schlichting[1]

Barends et al. (2014) Nature 505, 244

Novelty:

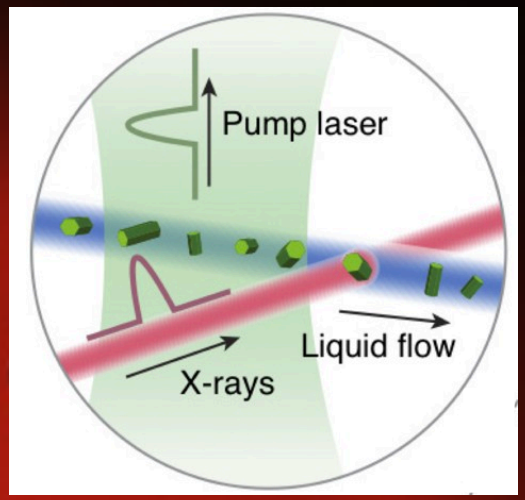
First *de novo* phasing of XFEL SFX data



- microcrystals (1 μm) of HEWL
- HEWL in complex with lanthanide compound (Girard et al. (2002) Acta D58, 1)
- 2.5 million XFEL shots > 8 % hit rate > 31% indexing rate (60000 images)
- SAD phasing: maps at 2.1 Å

Pump-and-probe SFX

Aquila *et al* (2012) Optics Express 20, 2706



STRUCTURAL BIOLOGY

Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein

Jason Tenboer,[1] Shibom Basu,[2] Nadia Zatsepin,[3] Kanupriya Pande,[1] Despina Milathianaki,[4] Matthias Frank,[5] Mark Hunter,[5]* Sébastien Boutet,[4] Garth J. Williams,[4] Jason E. Koglin,[4] Dominik Oberthuer,[6] Michael Heymann,[7] Christopher Kupitz,[2]† Chelsie Conrad,[2] Jesse Coe,[2] Shatabdi Roy-Chowdhury,[2] Uwe Weierstall,[3] Daniel James,[3] Dingjie Wang,[3] Thomas Grant,[8] Anton Barty,[7] Oleksandr Yefanov,[7] Jennifer Scales,[1] Cornelius Gati,[6,7] Carolin Seuring,[6] Vukica Srajer,[9] Robert Henning,[9] Peter Schwander,[1] Raimund Fromme,[2] Abbas Ourmazd,[1] Keith Moffat,[9,10] Jasper J. Van Thor,[11] John C. H. Spence,[3] Petra Fromme,[2] Henry N. Chapman,[6,7] Marius Schmidt[1]‡

Tenboer et al. (2014) Science 346, 1242

Novelty:

First time-resolved SFX (limited to nanoseconds)

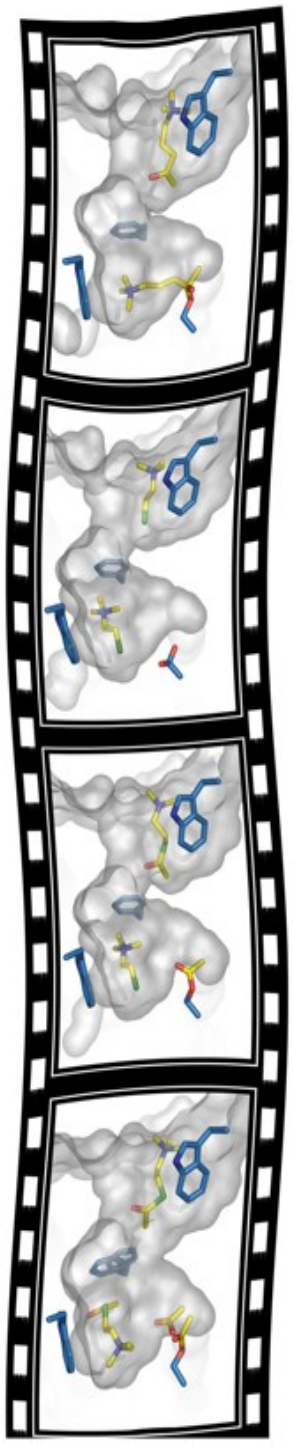
X

Pump laser XFEL

Image courtesy: James Holton

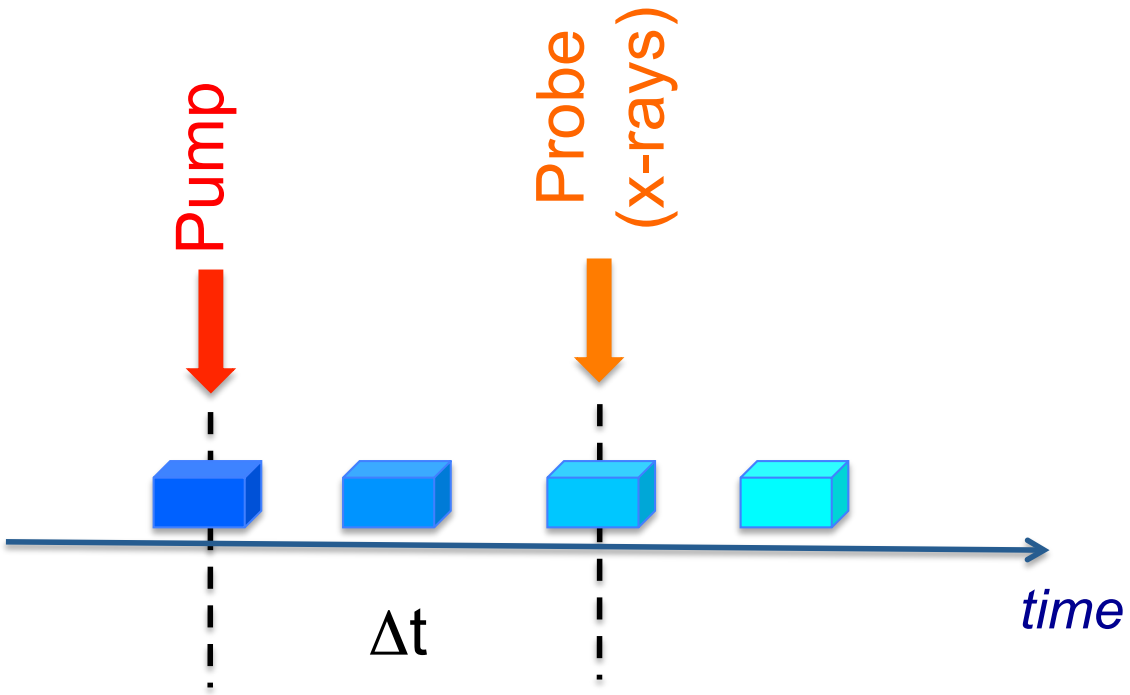
Time-resolved pump – probe crystallography: synchronization needed

Courtesy James Holton



Time-resolved crystallography: pump-probe

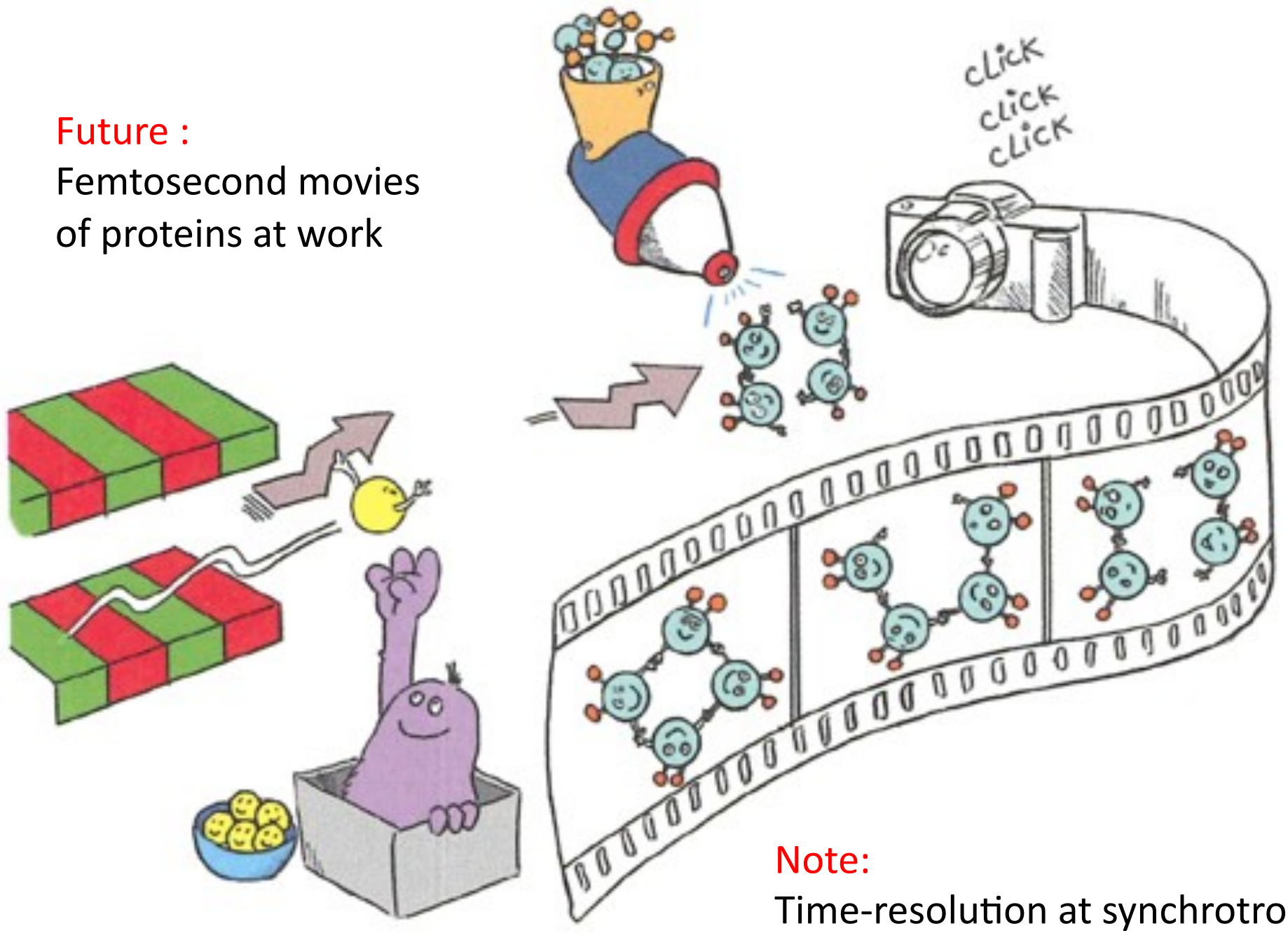
Aim: visualize structural changes in crystalline proteins
(e.g. enzymatic intermediate states)



Triggering (pump): - UV-vis light illumination
(inherent light sensitivity or caged compounds)
- diffusion of small molecules
- pH jump
- X-irradiation
- THz irradiation

Future :

Femtosecond movies of proteins at work



Note:

Time-resolution at synchrotrons limited to 100 ps

XFEL SFX – progress so far:

- SFX works - high resolution protein structures can be obtained

- micron-sized protein crystals can be used (macrocrystals are often very difficult to obtain)

- data collection at physiological (room) temperatures (100 K in most synchrotron experiments)

- de novo phasing is possible

- radiation-damage free protein structures can be determined

- proof-of-principle for time-resolved SFX

Challenges :

- generation and detection of protein microcrystals

- microcrystal injection

- protein sample consumption

- data processing (CrystFEL, cctbx.xfel): Monte Carlo integration, no estimate of partiality, requires lots of images

- phasing (only SAD with lanthanides worked so far – why ?)

- specific radiation damage still occurs (attenuate XFEL beam)

- time-resolved experiments faster than 1 ns

- BEAM TIME (only one beamline at LCLS and SACLA, cost: 400 kUSD per 12h)

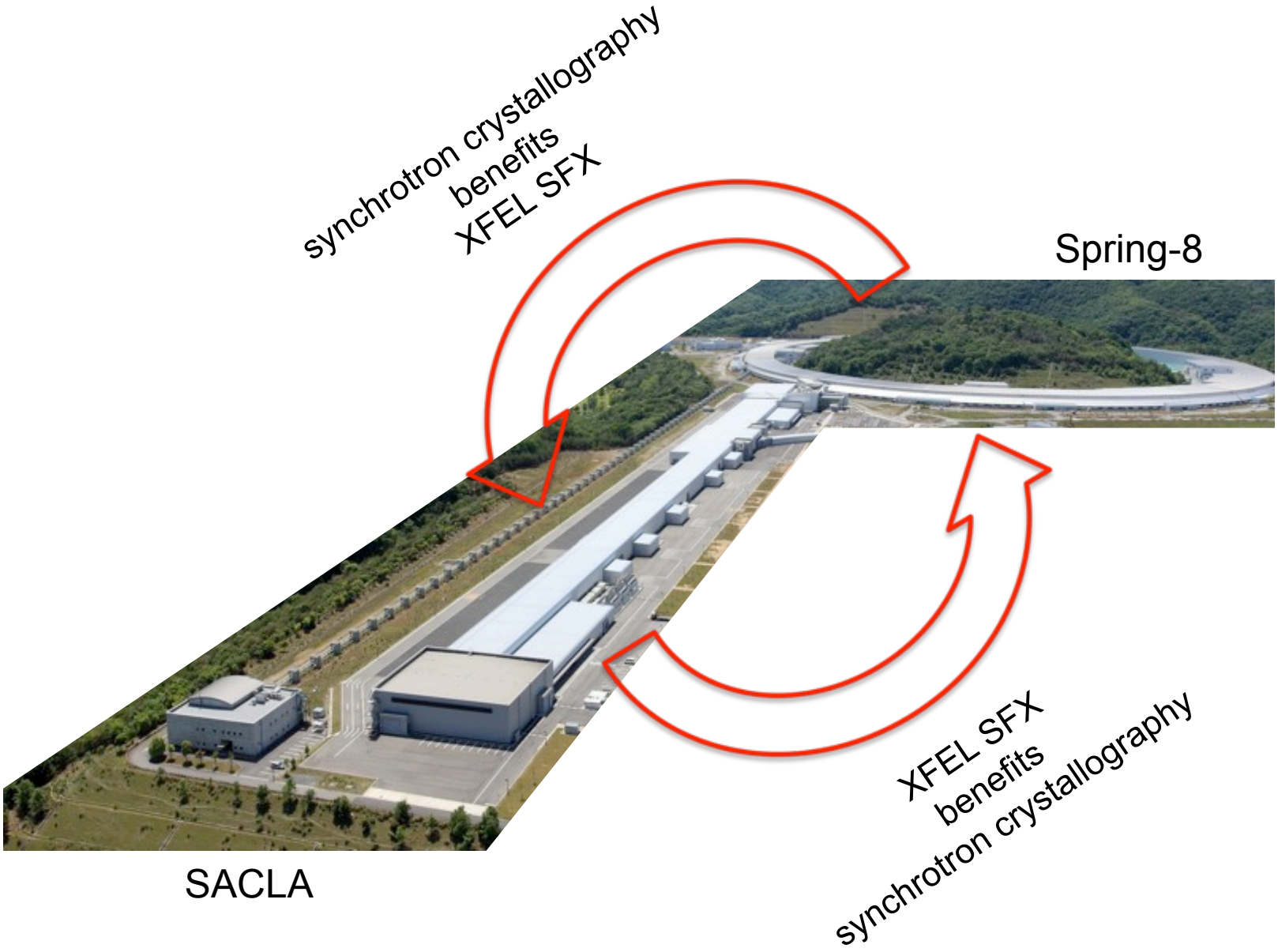
Review:
Schlichting. (2015) *IUCrJ* 2

Discussion Meeting Issue 'Biology with free-electron X-ray lasers' organized and edited by John C. H. Spence and Henry N. Chapman

July 17, 2014; 369 (1647)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

XFEL SFX does not replace, but complements synchrotron crystallography



Serial synchrotron crystallography

Gati *et al.* (2014) IUCrJ 1, 78

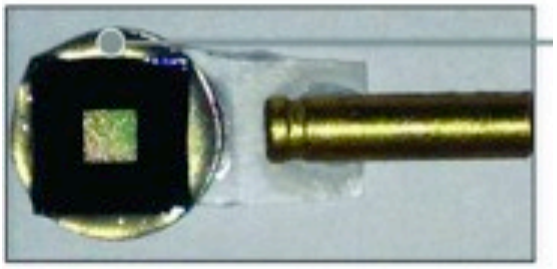
Stellato *et al.* (2014) IUCrJ 1, 204

Heyman *et al.* (2014) IUCrJ 1, 349

Nogly *et al.* (2015) IUCrJ 2

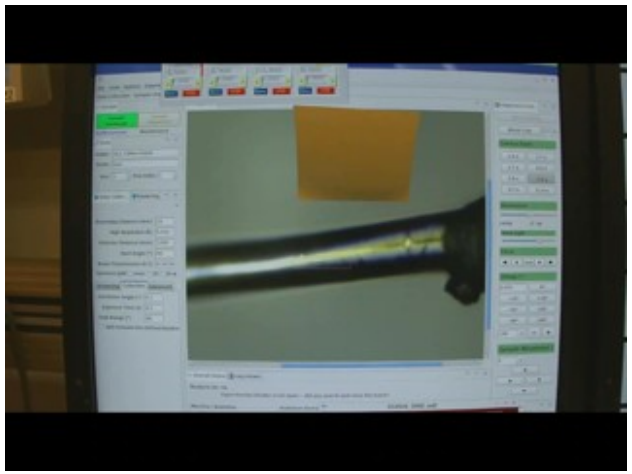
Botha et al. (2015) ActaD71, 387

Coquelle et al. (2015) ActaD71, 1184



Solid support at ESRF

Coquelle et al. (2015) ActaD71, 1184



Slow extrusion injector at SLS

Botha et al. (2015) ActaD71, 387

Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins (membrane proteins, protein complexes, …)
- *in vivo* crystallography
- Room temperature crystallography (preserves conformational heterogeneity)
- Damage-free structures of radiation sensitive proteins (metalloproteins, chromophore-containing proteins, …)
- Time-resolved experiments on ps-fs time sales

Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins
- *in vivo* crystallography
- Room temperature crystallography
- Damage-free structures
- Time-resolved experiments

G protein coupled receptors (GPCR) are targeted by 30% of known drugs

Microcrystals of GPCR (5-HT2B) too small for synchrotron crystallography

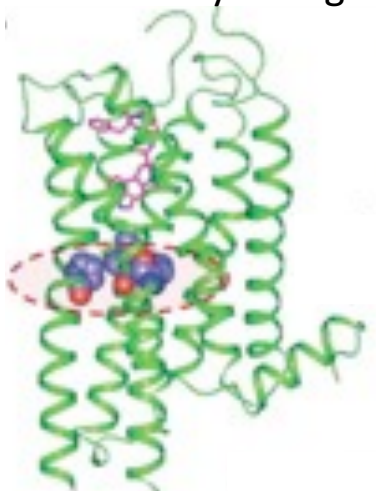
⇩

Structure solved by **XFEL SFX**

Liu et al. (2013) Science 342, 1521

⇨ 6 y ars (V. Cherezov)

Structure of GPCR (5-HT2B) solved from **macrocrystals** by **synchrotron** crystallography

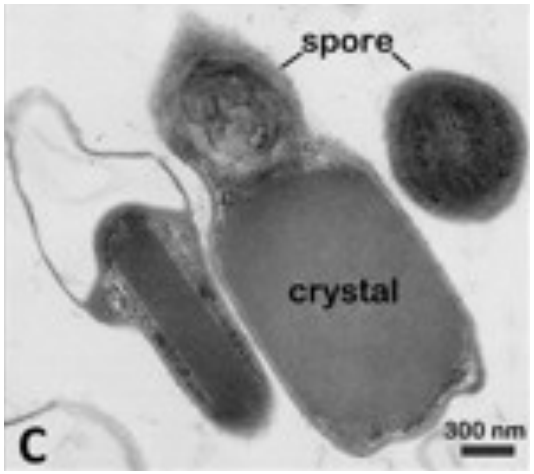


Wacker et al. (2013) Science 340, 615

Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins
- *in vivo* crystallography
- Room temperature crystallography
- Damage-free structures
- Time-resolved experiments

Naturally occurring nanocrystals of the cry3A toxin from bacillus thuringiensis

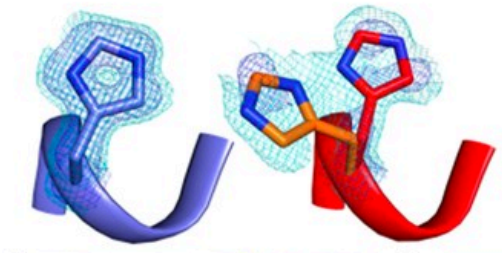


Sawaya et al. (2014) PNAS 111, 12771

Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins
- *in vivo* crystallography
- Room temperature crystallography
- Damage-free structures
- Time-resolved experiments

Room temperature crystallography preserves physiological conformational heterogeneity



100 K Room T

Fraser et al. (2011) PNAS 108, 16247

Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins
- *in vivo* crystallography
- Room temperature crystallography
- Damage-free structures
- Time-resolved experiments

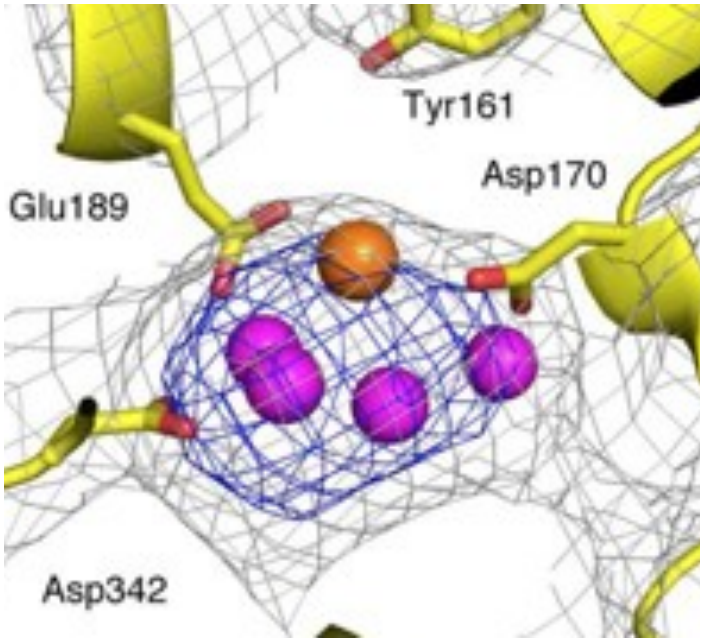
Mn_4CaO_5 cluster of crystalline photosystem II retains intact electronic structure

- 150 MGy / xtal
  (5x Garman limit in synchrotron MX at 100 K)

- short XFEL pulse even outruns electronic damage

- good news for radiation sensitive proteins
(metalloproteins, chromophore containing, …)

See also Hirata *et al.* (2014) Nat Methods 11, 734
Suga *et al.* (2015) Nature 517, 99

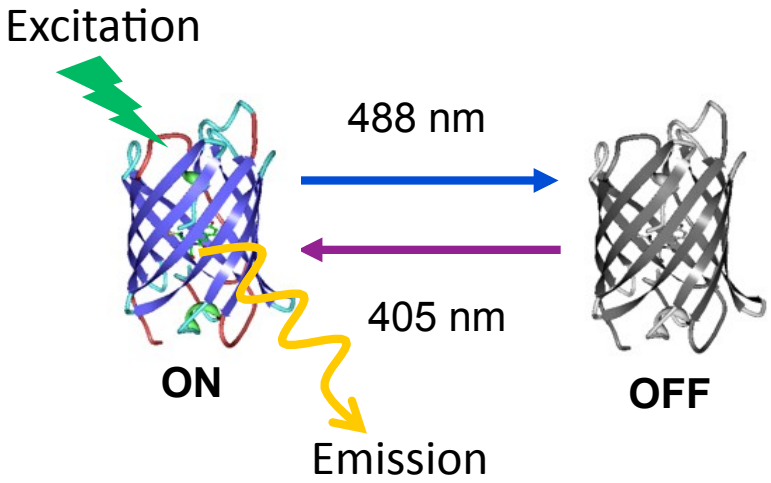


Kern et al. (2013) Science 340, 491

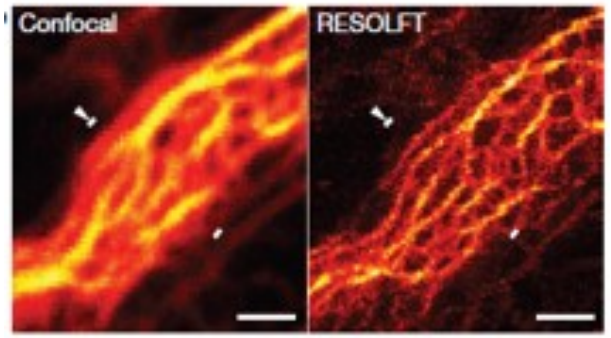
Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins
- *in vivo* crystallography
- Room temperature crystallography
- Damage-free structures
- Time-resolved experiments

French project: Femtosecond movies of reversibly photoswitchable fluorescent proteins



Photoswitchable fluorescent proteins:
Markers in super-resolution microscopy

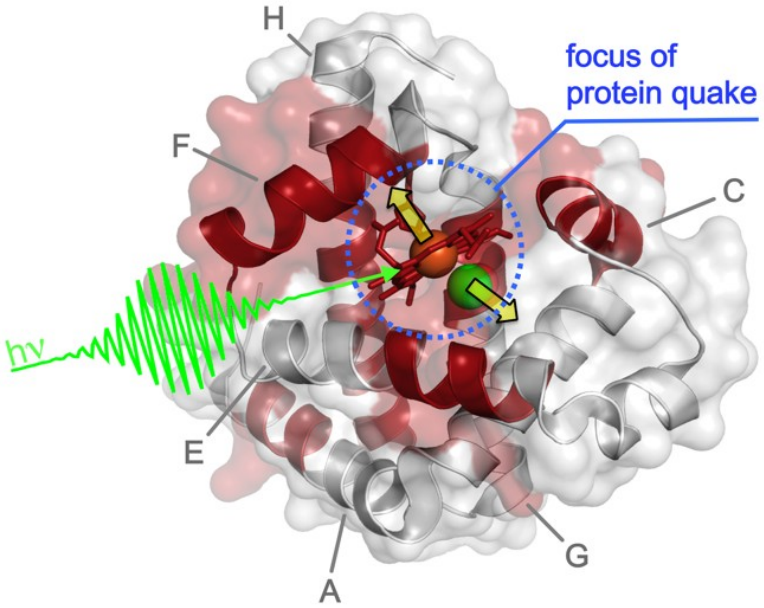


Hell, Nature 2011

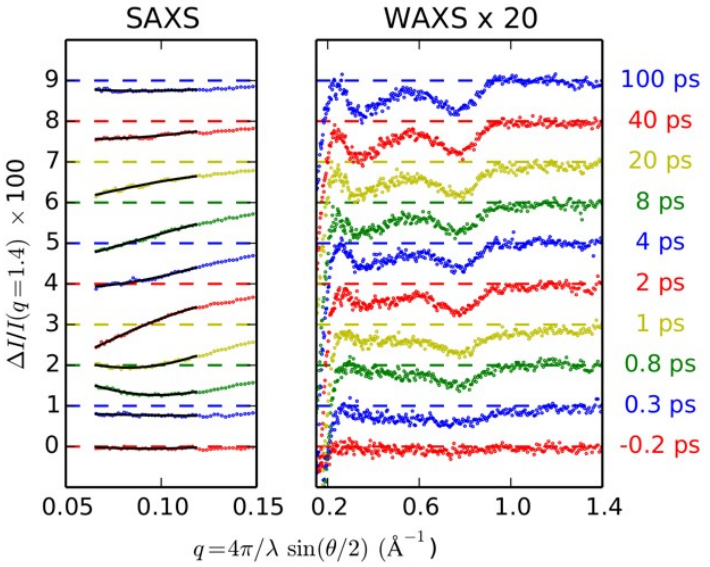
Goal: Understand photoswitching mecanism to optimize fluorescent proteins for super-resolution microscopy

Tracking ultrafast protein structural dynamics with time-resolved WAXS

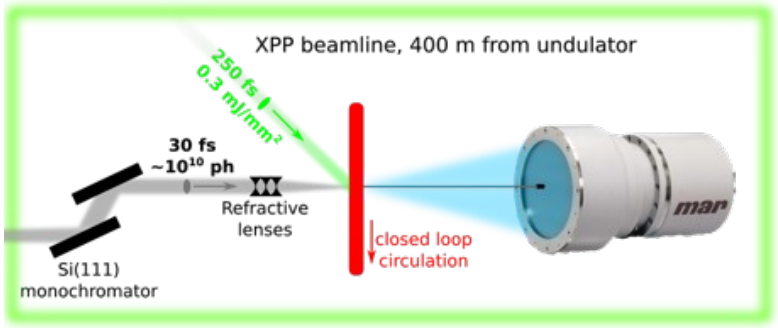
Levantino, Schirò, …, Cammarata (2015) *Nature Communications* 6, 6772



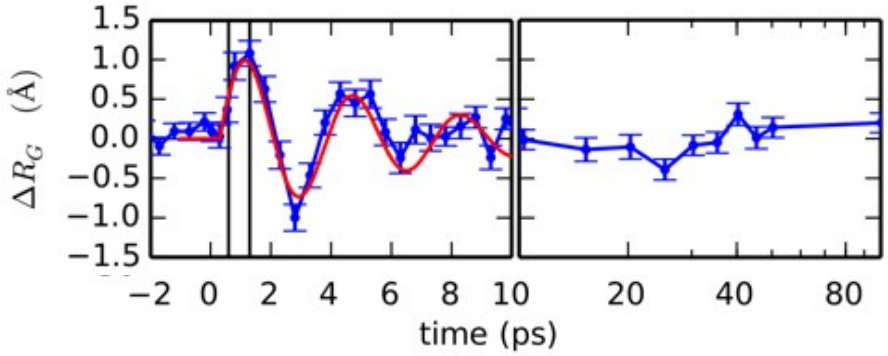
Protein motions on the ps time scale



Combining X-ray and optical lasers at LCLS/XPP

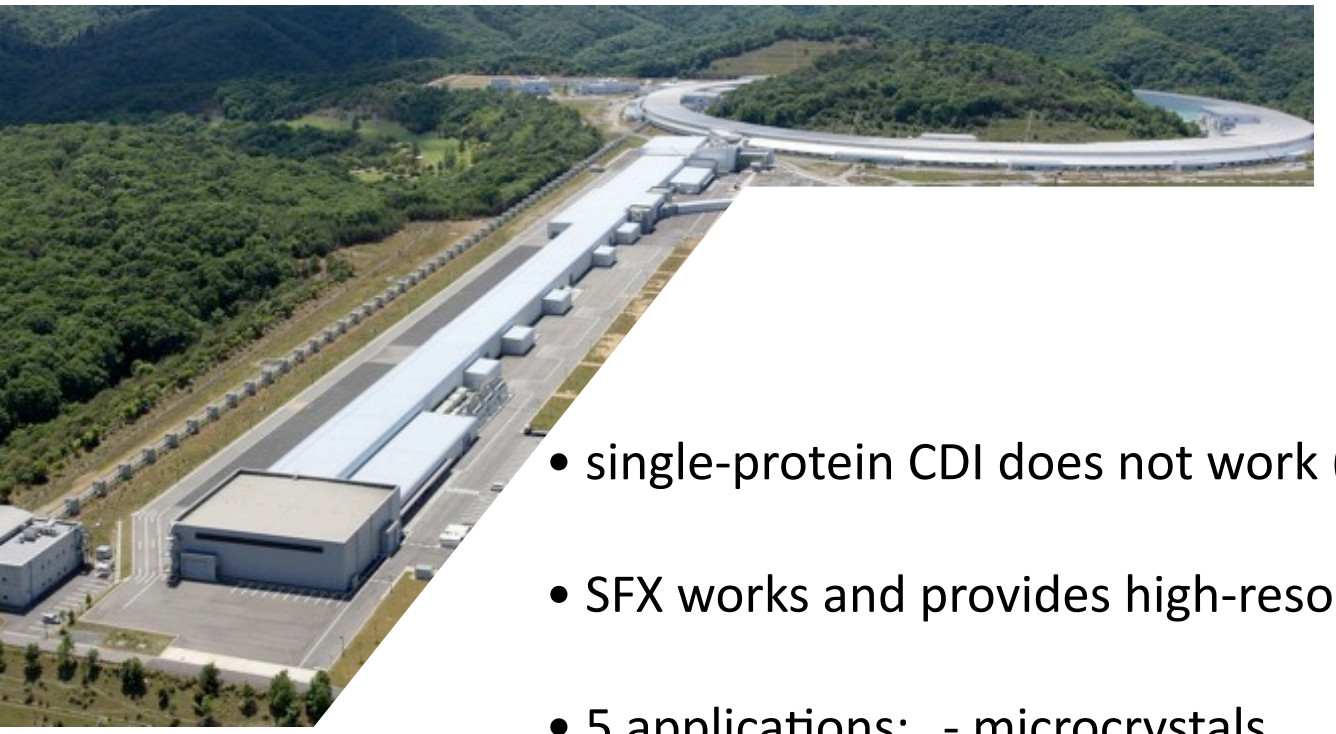


Myoglobin response (volume) after a photoexcitation



Five applications of XFEL SFX that can't (easily) be done at a synchrotron

- Microcrystals of fragile proteins (membrane proteins, protein complexes, …)
- *in vivo* crystallography
- Room temperature crystallography (preserves conformational heterogeneity)
- Damage-free structures of radiation sensitive proteins (metalloproteins, chromophore-containing proteins, …)
- Time-resolved experiments on ps-fs time sales



Summary

- single-protein CDI does not work (yet ?)
- SFX works and provides high-resolution protein structures
- 5 applications:
  - microcrystals
  - *in vivo* xtallo
  - room temperature
  - damage-free structures
  - femtosecond movies
- XFEL SFX and synchrotron crystallography are complementary
- French structural biology needs to catch the accelerating XFEL train