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Science Applications with XFELs: structural biology

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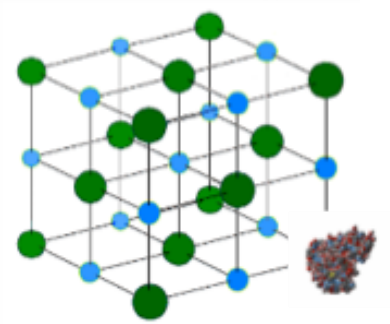
Outline

1. Problem of radiation damage in structural biology
2. The concept of lensless imaging – coherent diffractive imaging
3. Serial Femtosecond Crystallography (reminder)
4. Extension to time-resolved SFX
5. MHz (time-resolved)SFX at European XFEL
6. SPB/SFX Instrument
7. Conclusions and outlook

Non-crystalline matter is very important but scatter less…

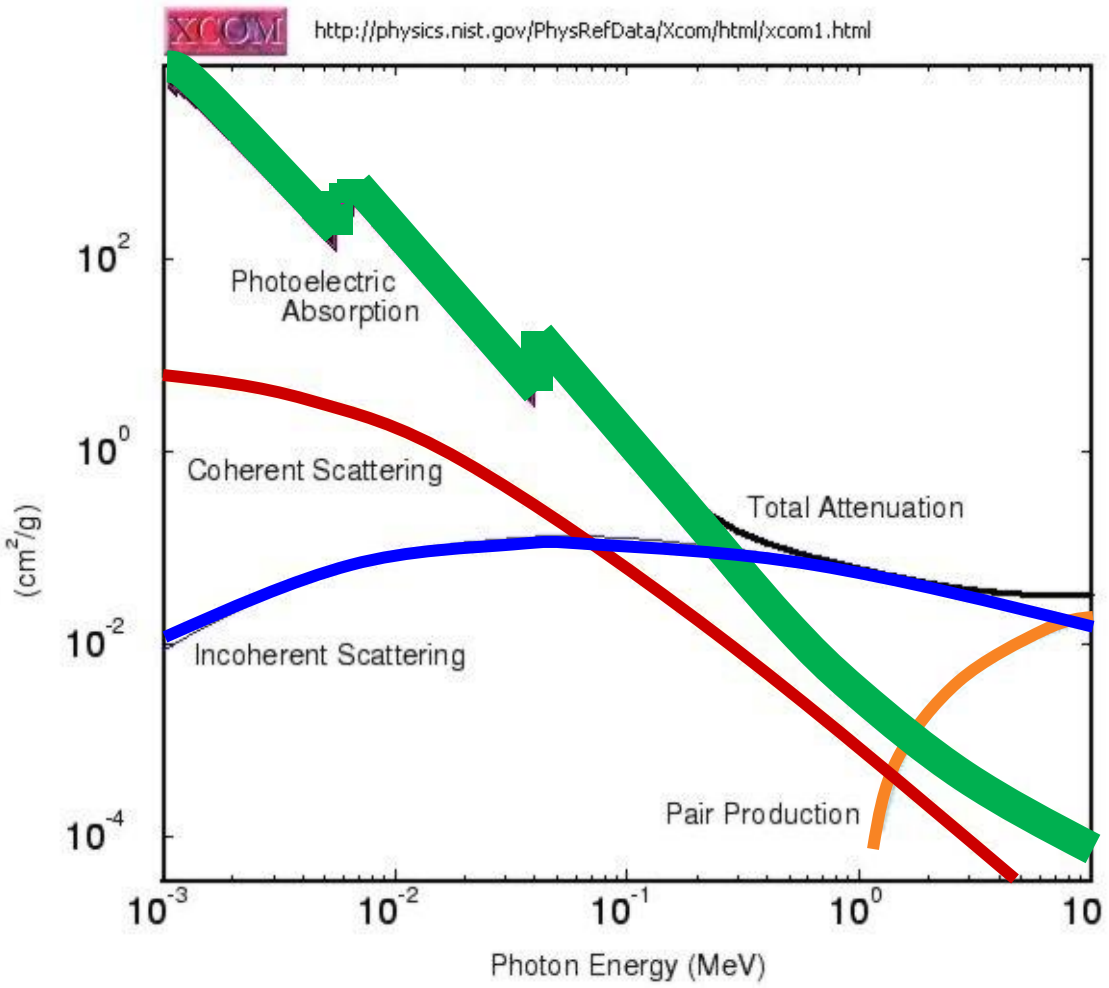


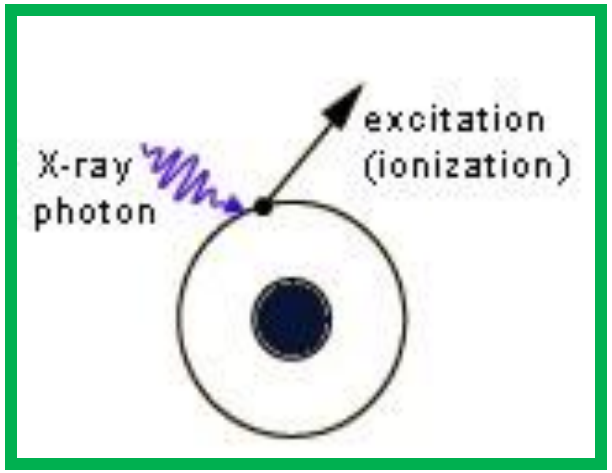
Scattered x-rays is proportional to N^2
(~ 100 x 100 x 100 elements)

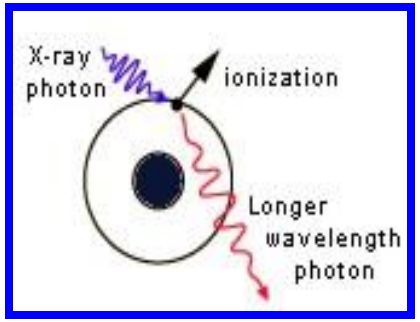
One guy scatters like... 1
(~ a million times less than above)

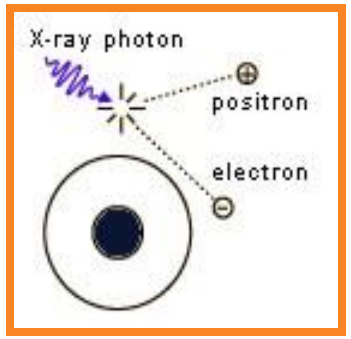
Conclusion: Need a lot more x-rays to see a single particle

X-ray interaction with matter



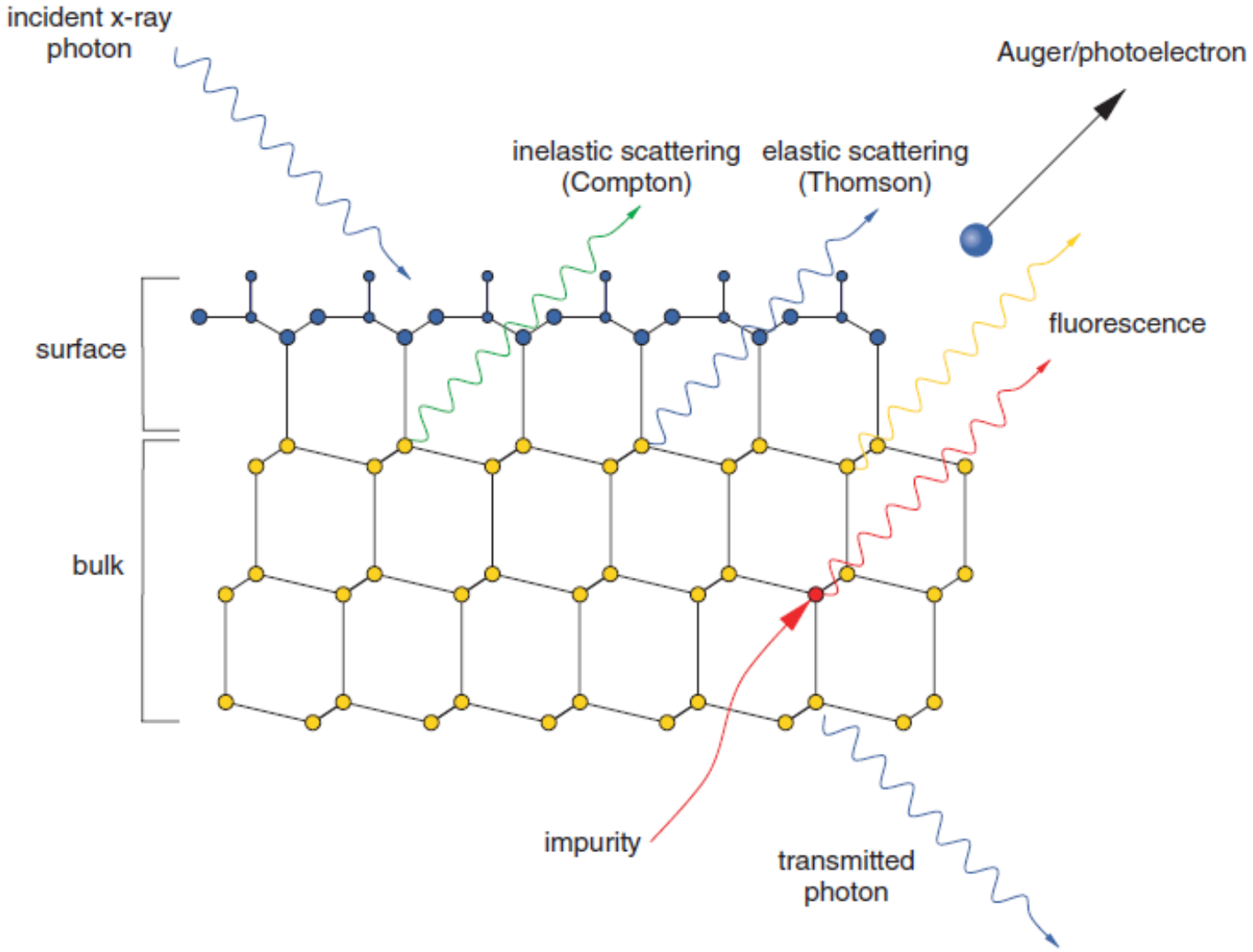






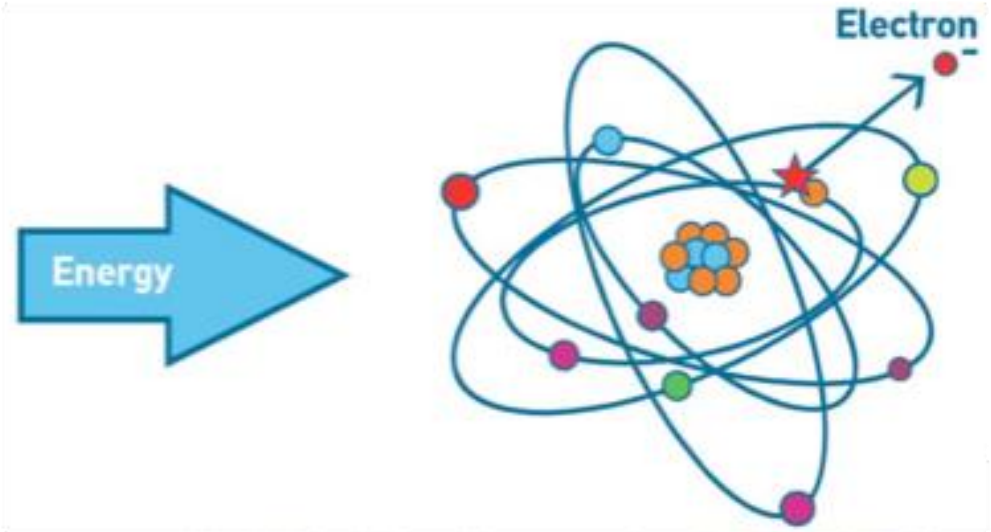
X-ray interactions mechanisms

source: [2]



The problem of radiation damage

Absorption of x-rays is much stronger than x-ray scattering



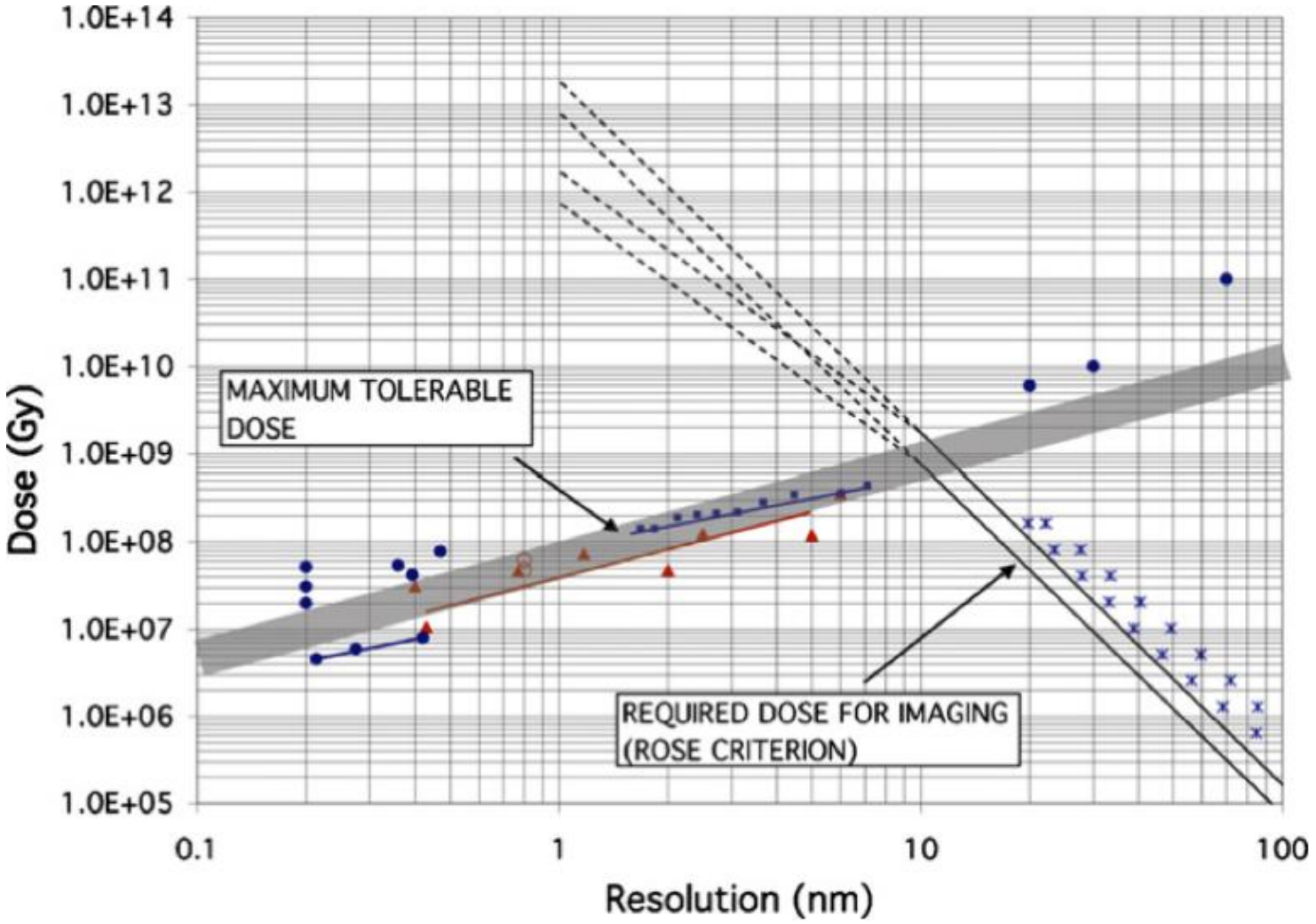
$$I_{scatter} << I_{absorb}$$

- At ~12 keV for bio-samples (C, H, N, O) $I_{scatter}$ ~0.1 I_{absorb}

Ionization of electrons 'destroys' the molecule

Photo-ionization → 1st electron (primary)
Generation of Auger-electrons → 2nd electron (primary)
Inelastic electron scattering → xth electrons (secondary)

Overcoming the damage using ultrashort X-rays

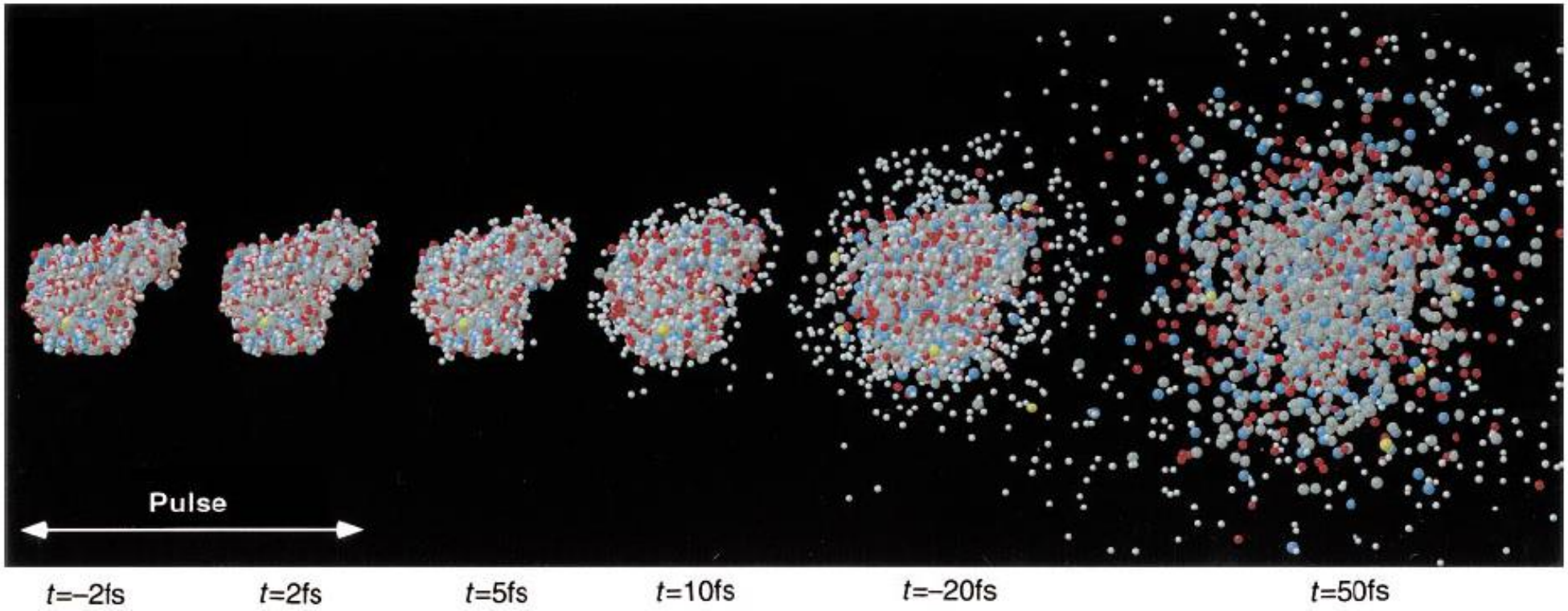


"The principal conclusion of this paper is that for unique, frozen hydrated biological objects with only natural X-ray contrast the resolution of XDM at Rose-criterion image quality will be limited by radiation damage to be not better than 10 nm."

"We have made a case that the 10-nm limit is not insurmountable..."

M.R. Howells *et al.*,
J. Elec. Spec. Rel. Phenom. **170**, 4 (2009)

Overcoming the damage using ultrashort X-rays

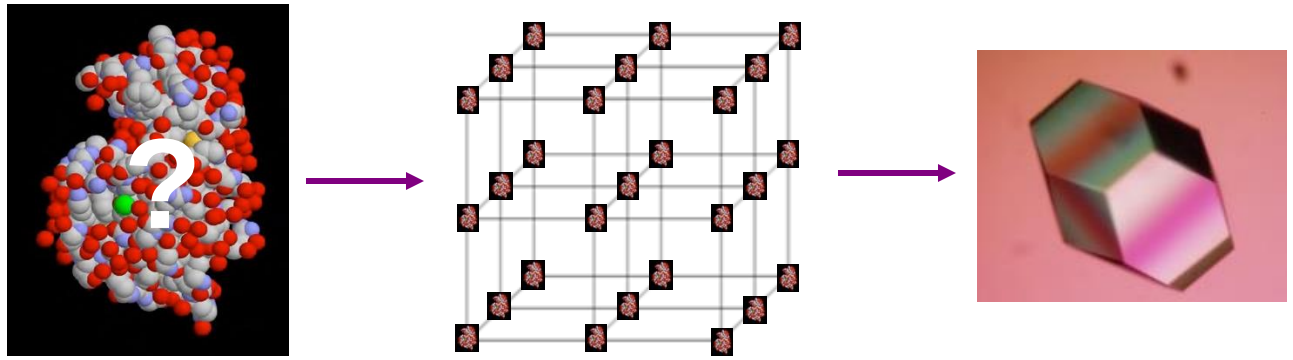


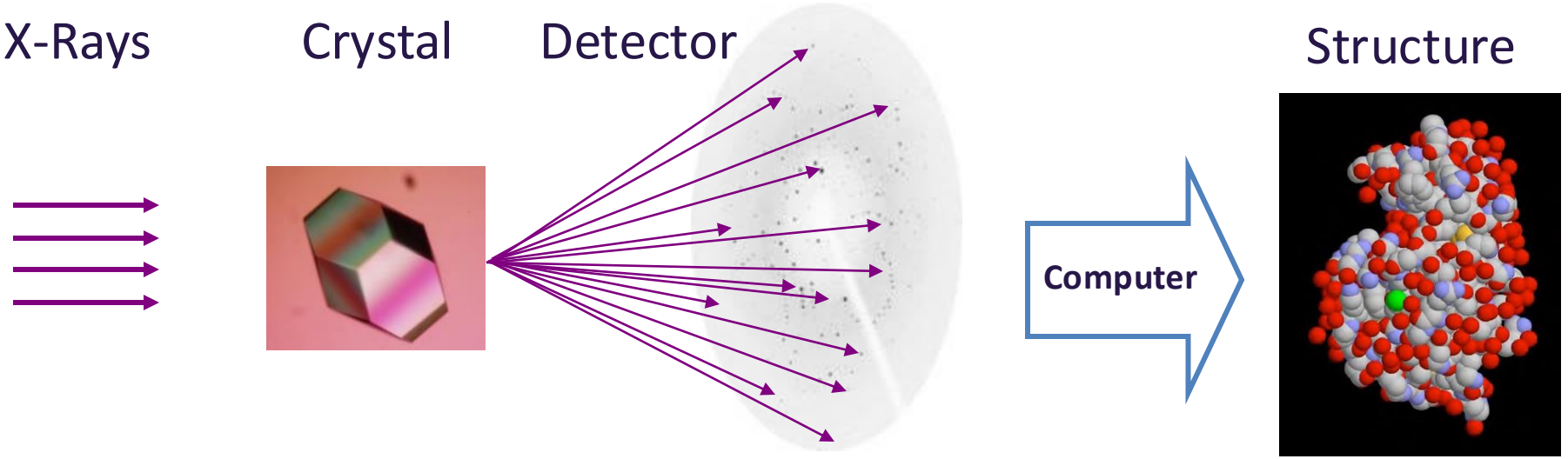
This concept gave birth to a new methodology,
also referred to as “diffract before destroy”

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

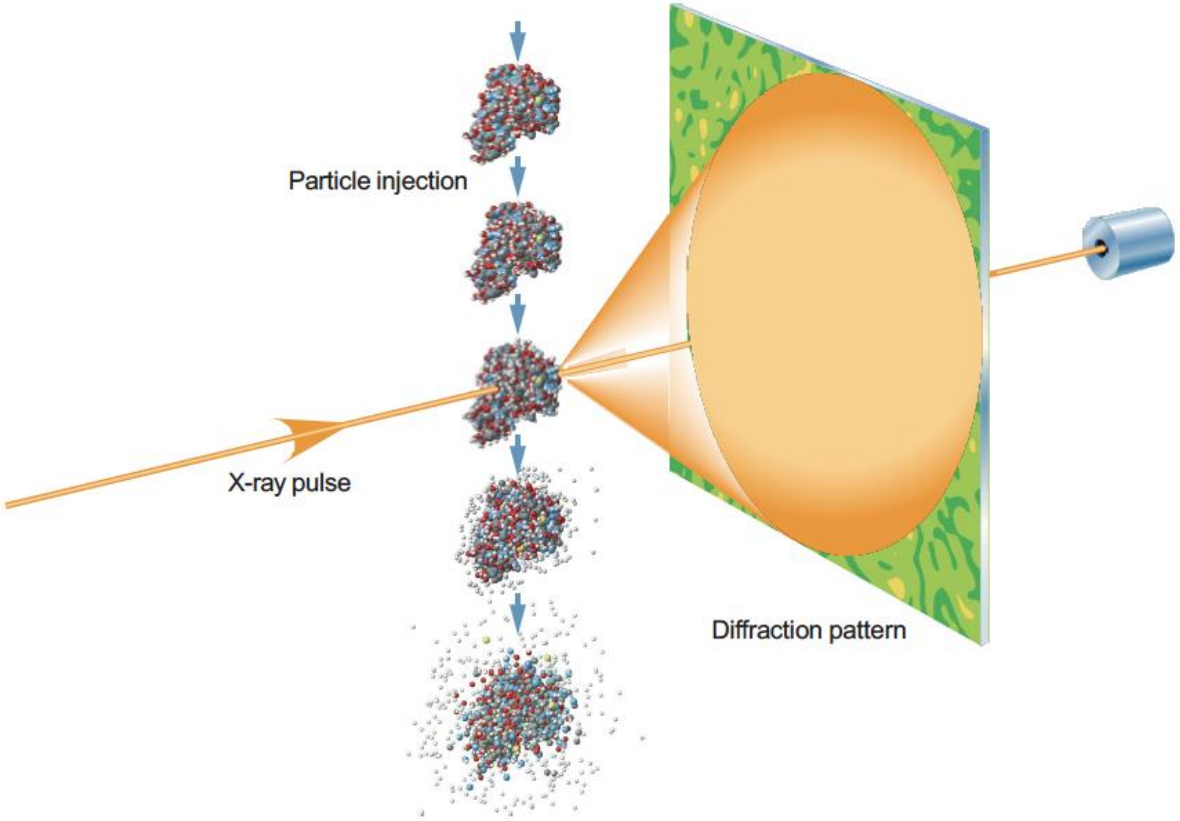
New directions in biomolecular imaging: no crystals needed!

Lysozyme
(Enzyme from chicken eggs)



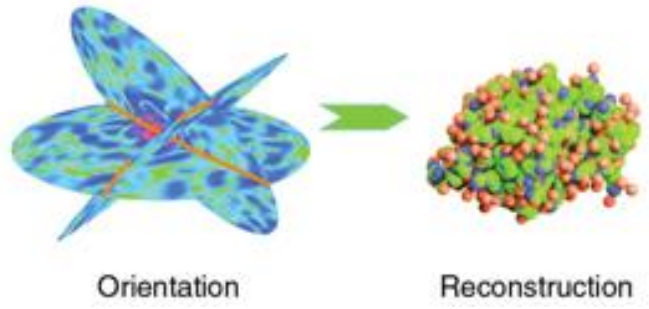


Coherent Diffractive Imaging (CDI): high resolution imaging without the lens!



No lenses needed: resolution is not limited by X-ray optics (rather by signal-to-noise ratio)

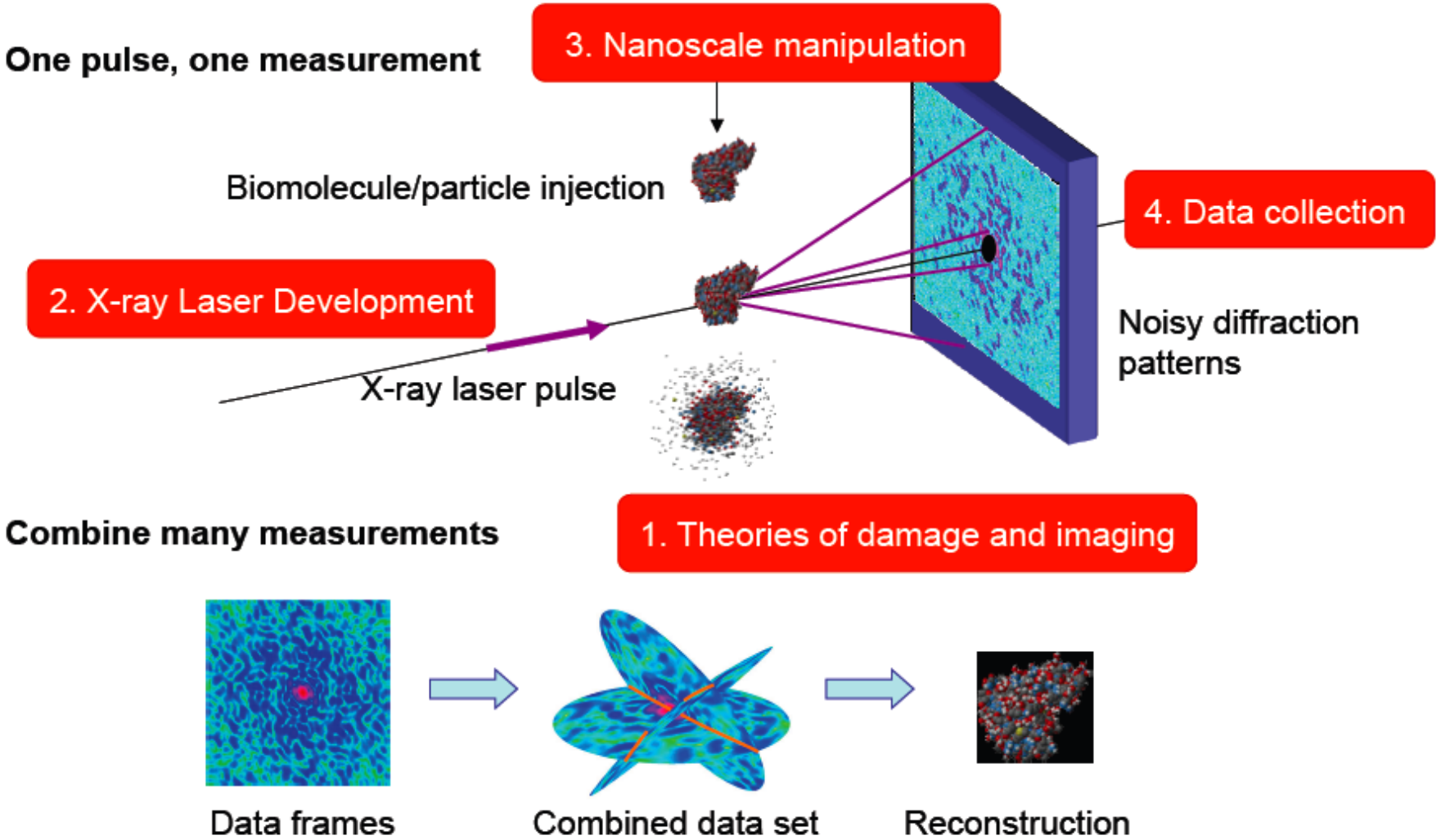
Measured data are not images! They must be inverted to yield real-space images



*K.J. Gaffney and H.N. Chapman, Science, **316**, 1444 (2007)*

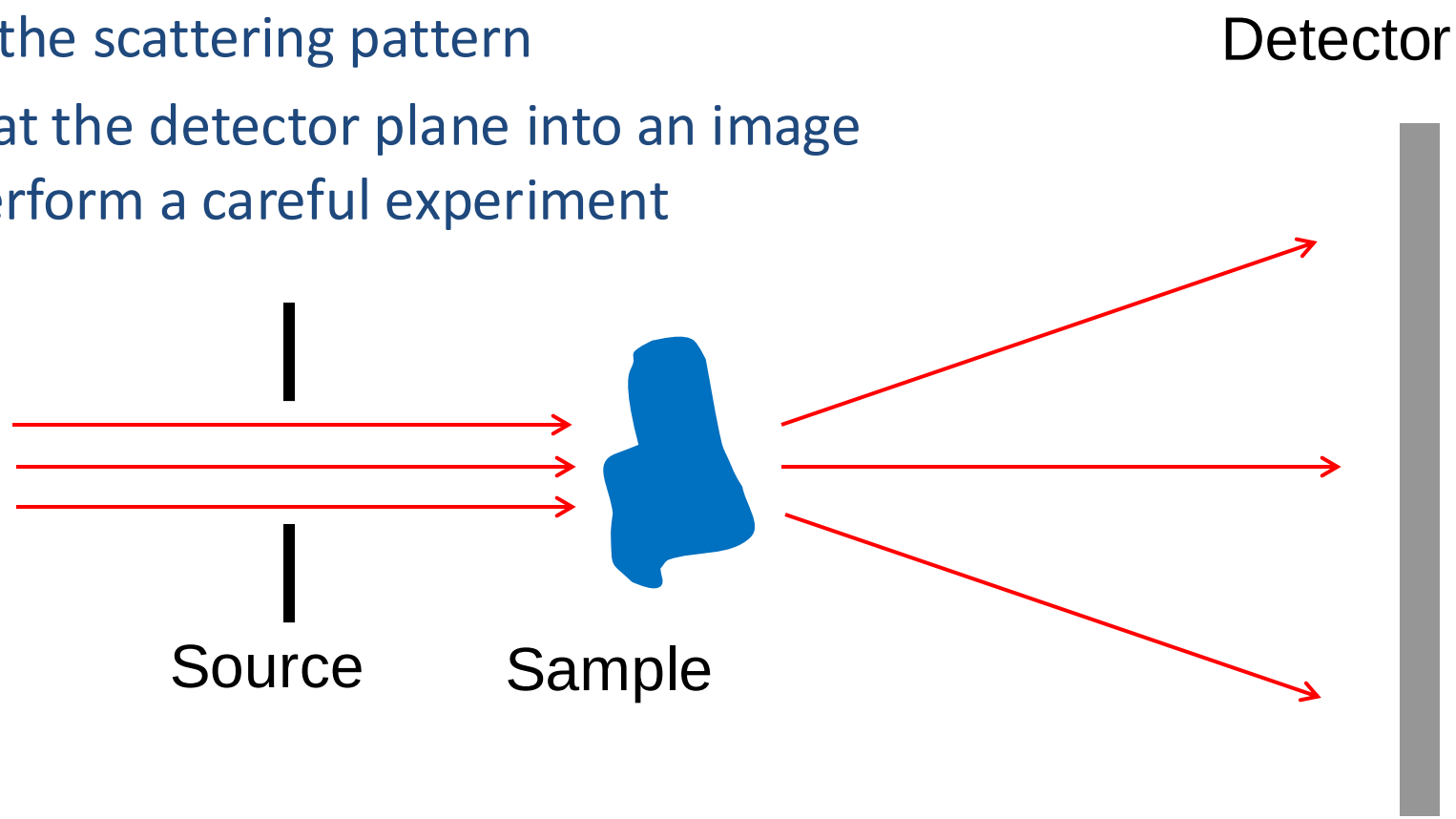
The experiment is conceptually rather simple and in practice is not too hard to realize. However, there is lots of physics to understand!

Coherent diffractive imaging

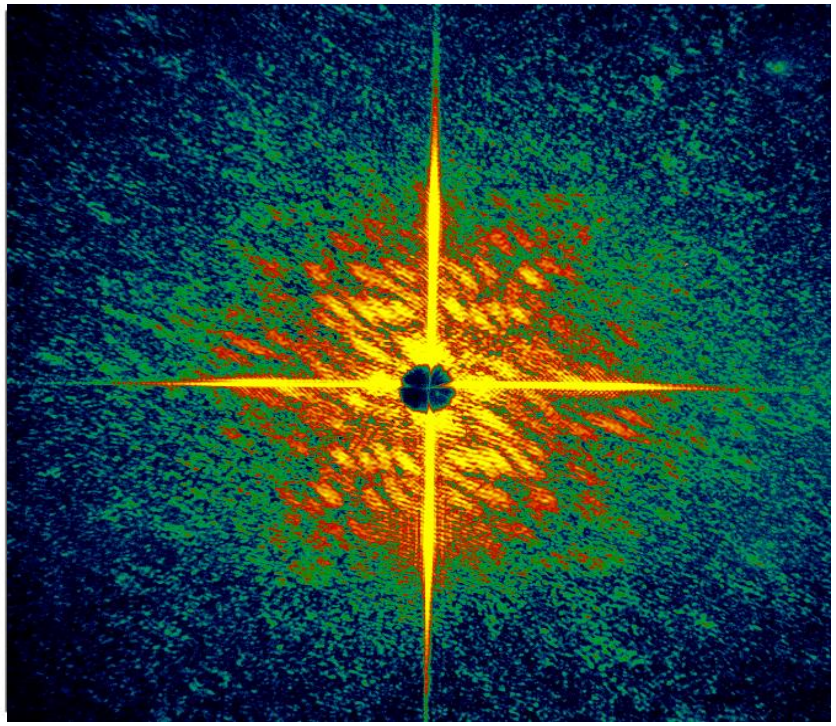


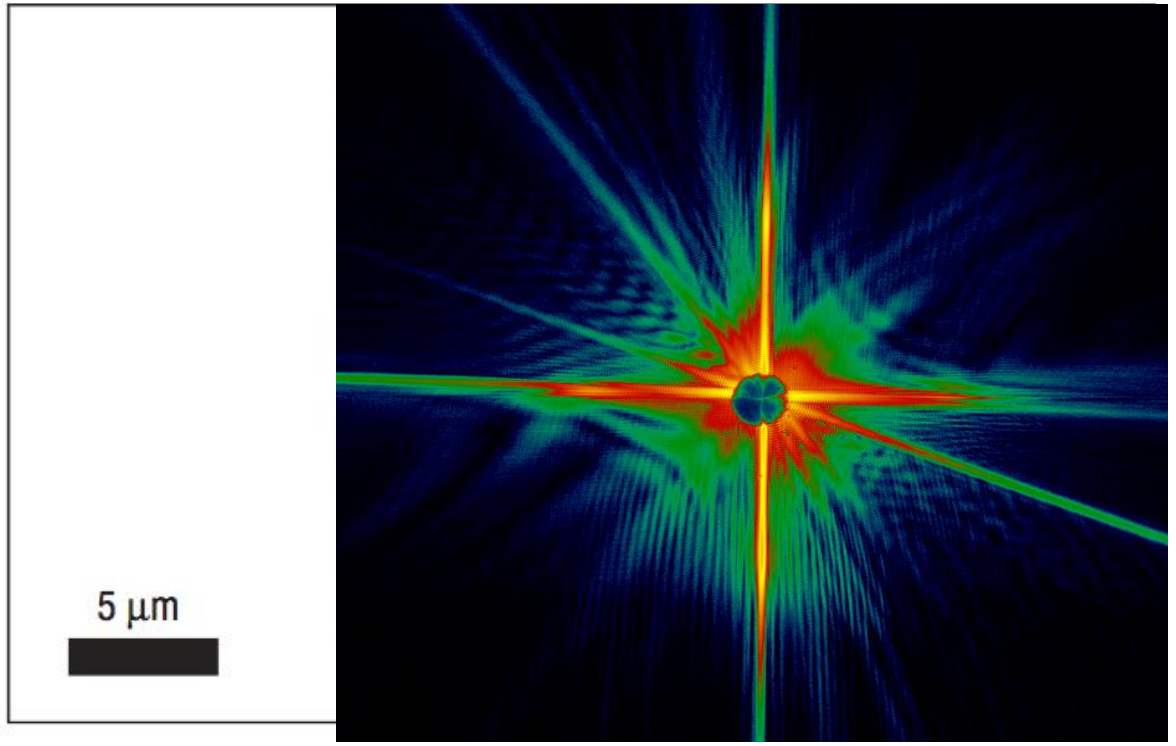
Principle of CDI

1. “Lensless” imaging
2. Determine density modulations from coherent scattering pattern
3. Requires to retrieve phase of the scattering pattern
4. Can invert the measurement at the detector plane into an image of the sample provided we perform a careful experiment



A little bit of history:
Diffract & Destroy - Proof of Principle



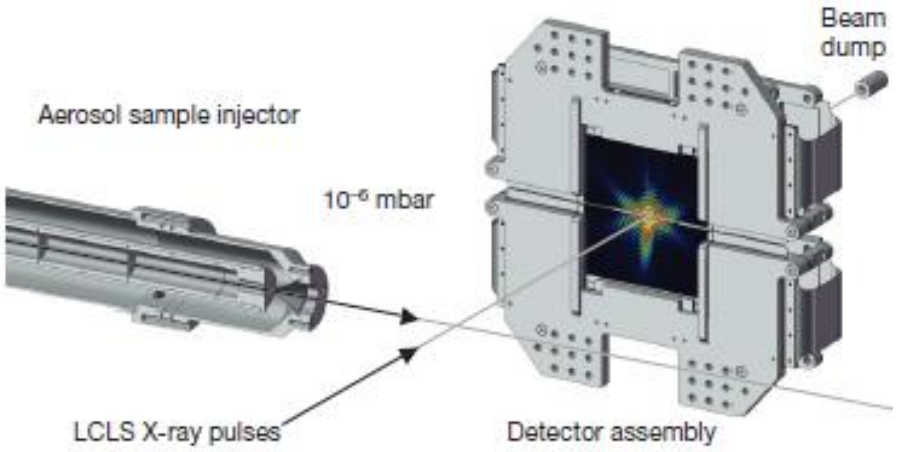


H. N. Chapman, et al, *Nature Physics* (2006)

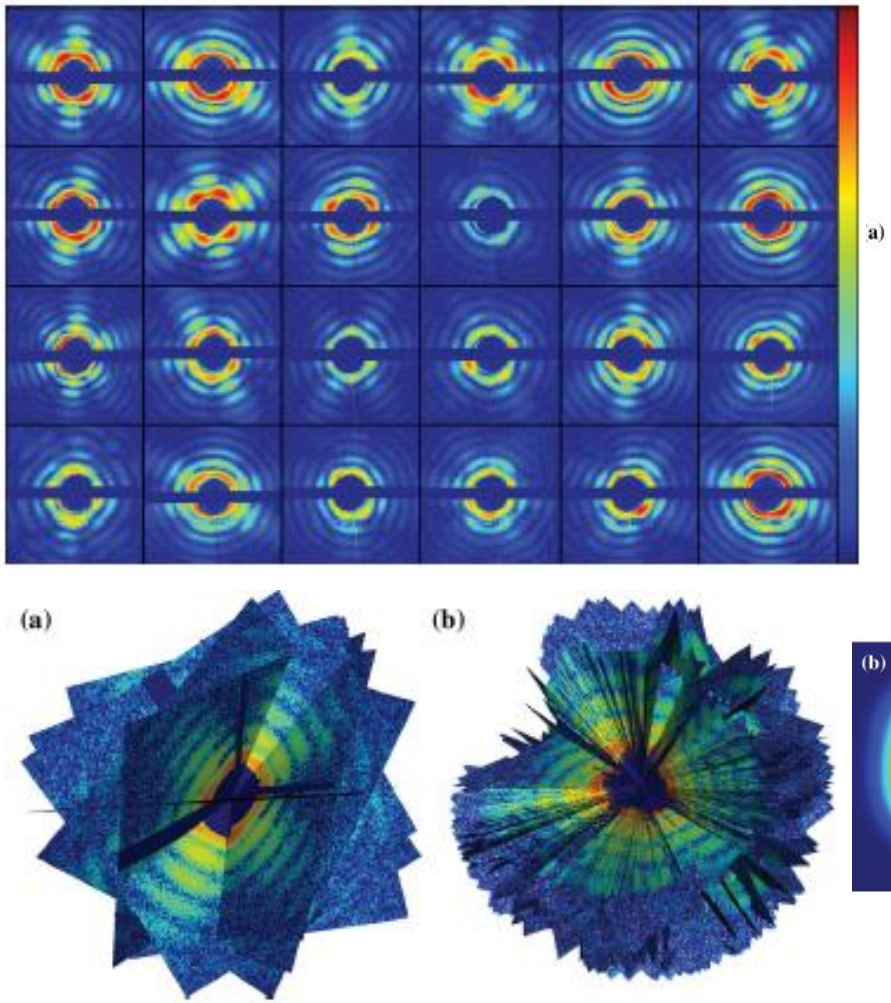
Biology – structure determination with atomic resolution

Single particle imaging

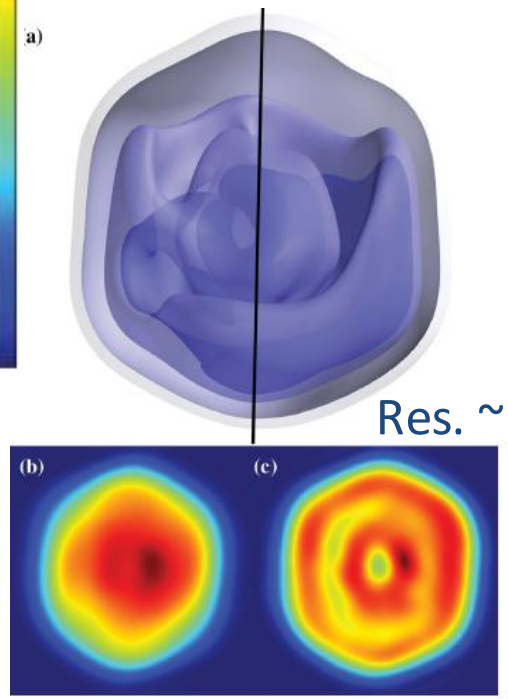
Avoid crystallization completely



T. Ekeberg et al., PRL 114, 098102 (2015)



Application to e.g.
Giant Mimivirus particles

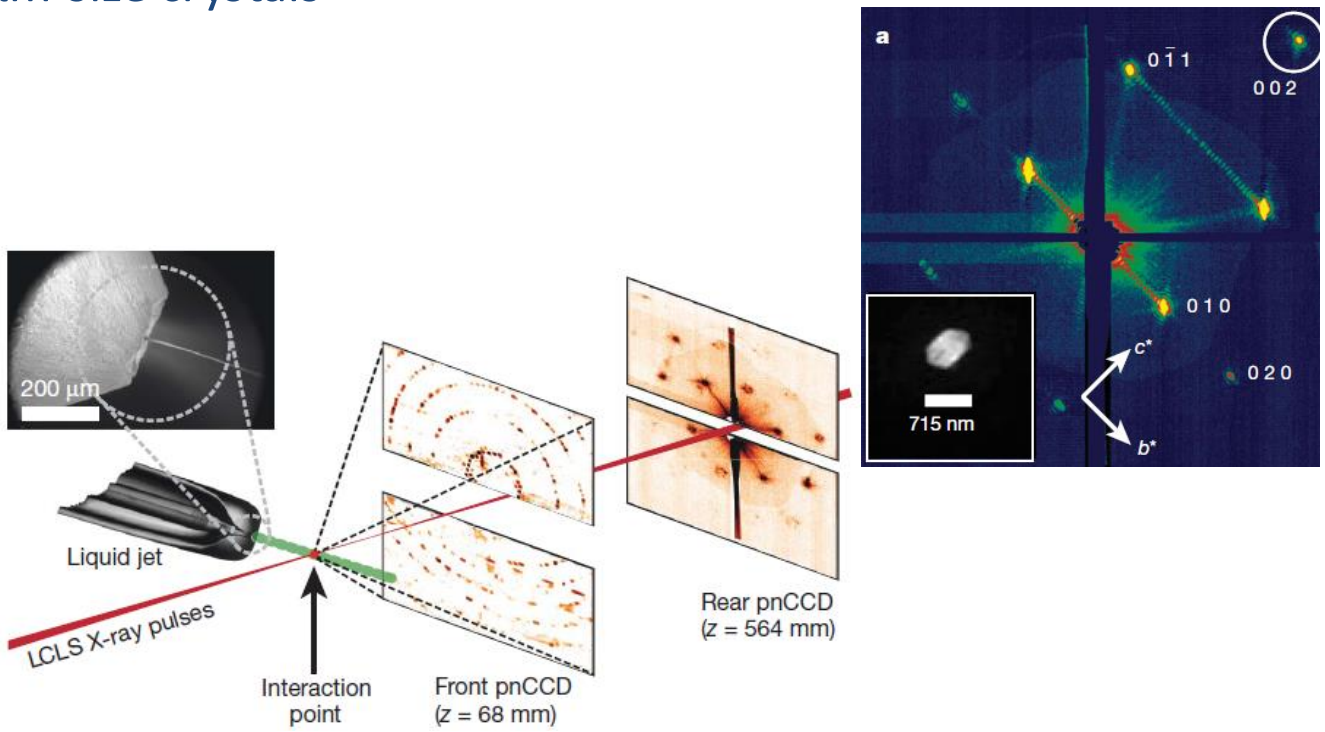


Res. ~125 nm

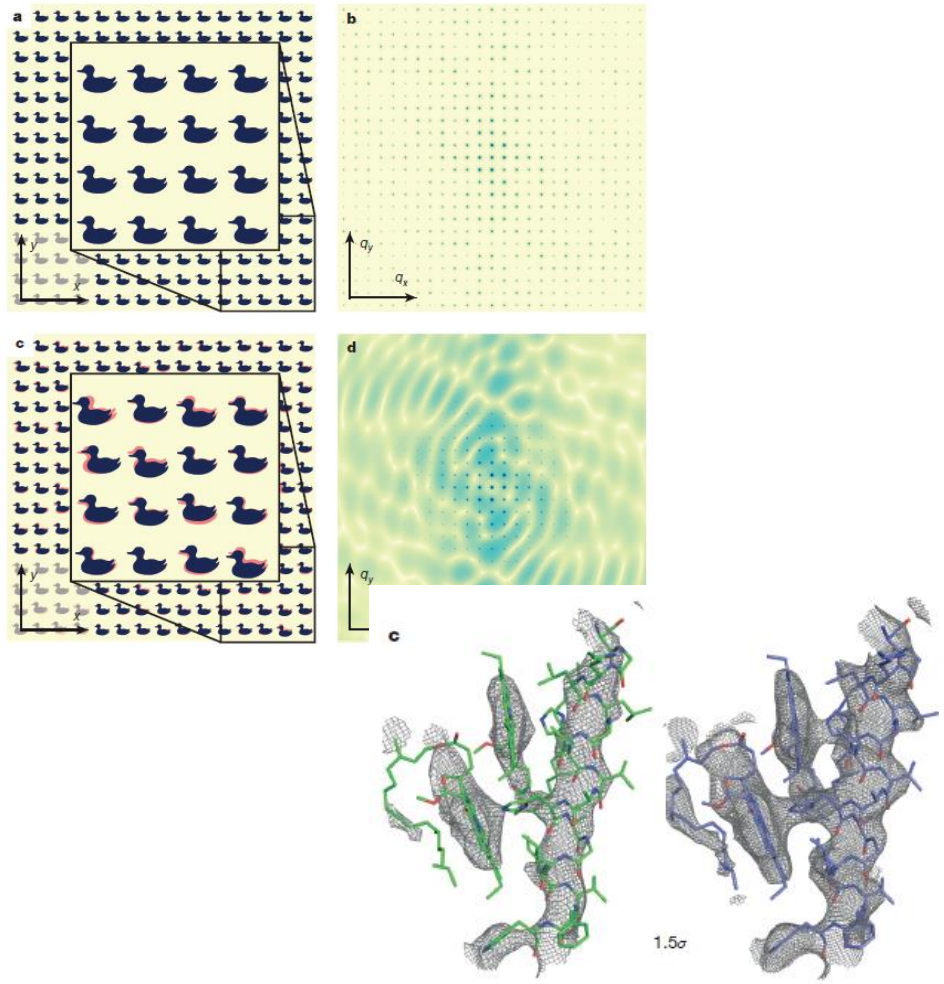
Biology – structure determination with atomic resolution

Serial femtosecond crystallography (SFX)

µm-size crystals



H.N. Chapman et al., Nature 470, 73 (2011)



K. Ayyer et al., Nature 530, 202 (2016)

Entering the era of structural dynamics in Biology!

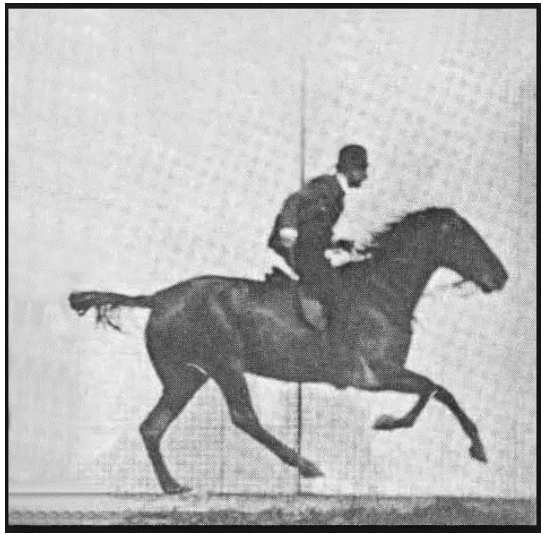
$$E + S_1 \rightleftarrows E{\bullet}S_1 + S_2 \rightleftarrows E{\bullet}S_1{\bullet}S_2 \rightarrow [\,I_1 \rightarrow I_2 \rightarrow I_3 \rightarrow \ldots] \rightarrow E{\bullet}P \rightleftarrows E + P$$

In 19th Century

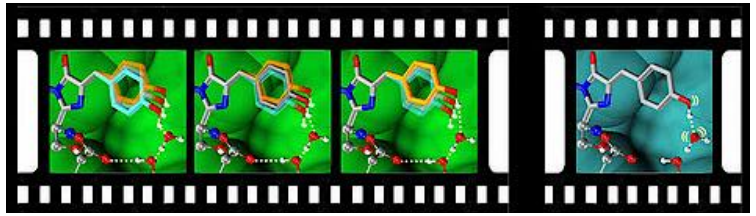
Traditional MX @ synchrotrons:
macro-crystals, 100K, **E**, **E•S$_1$**, **E•P**;
lacks function and dynamics

In 21st Century

Race-horse first film ever by Edward Muybridge (1878)



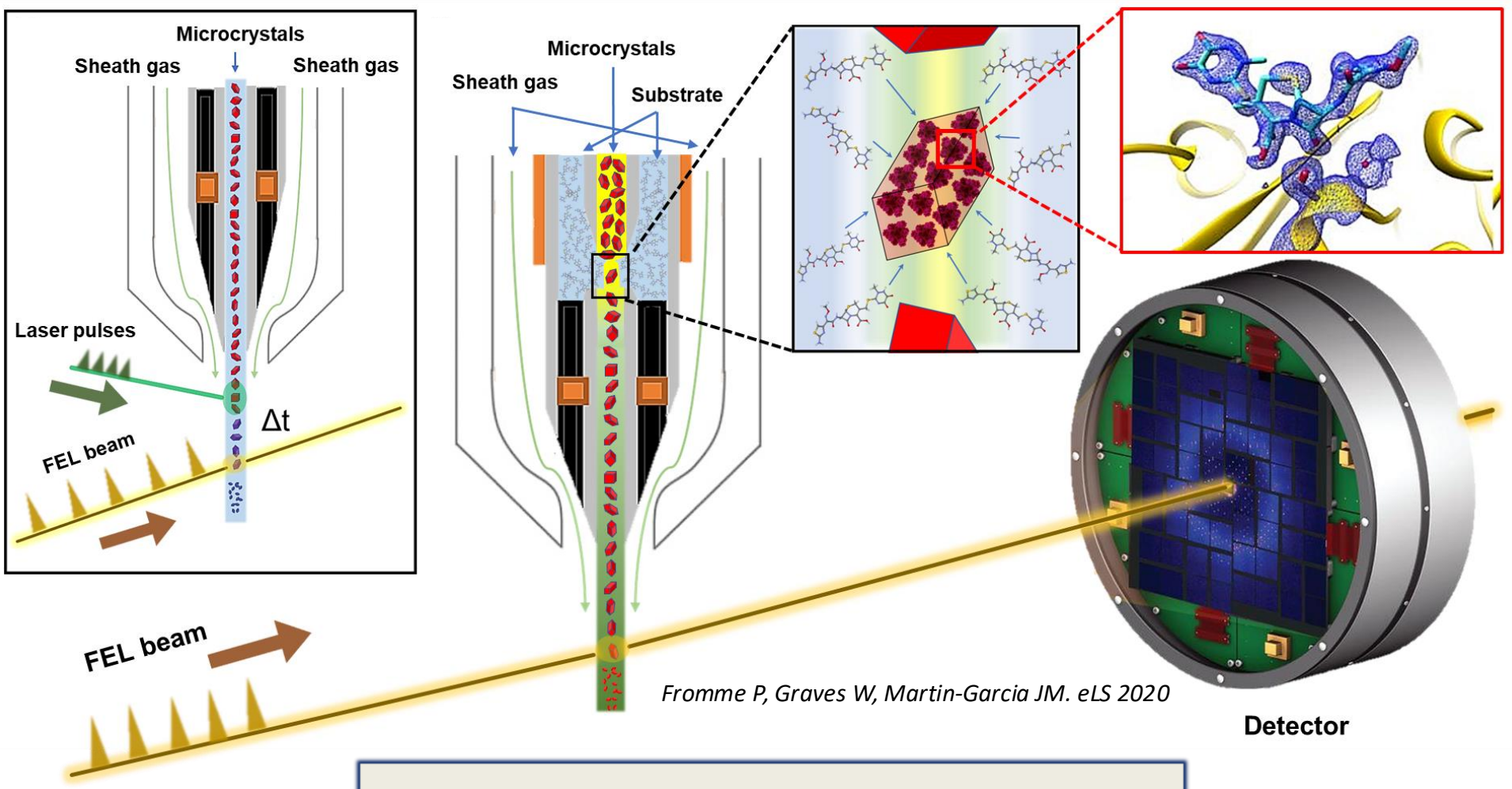
XFELs => TR-SFX



Atomic resolution molecular movies of macromolecules

XFELs´ time resolution, **fs**
Synchrotrons´ time resolution, **µs**

Typical setup for time-resolved SFX at XFELs

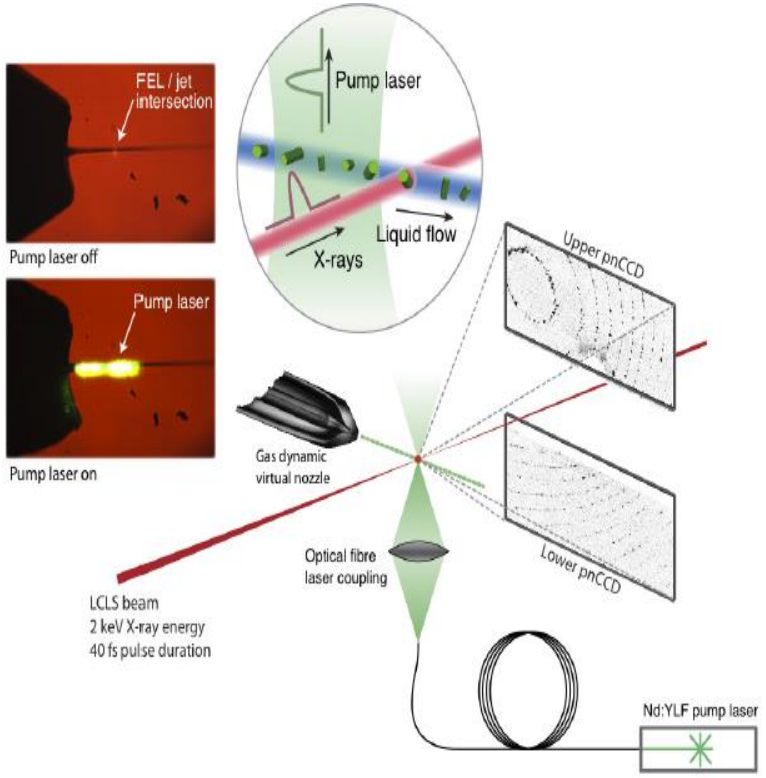


Fromme P, Graves W, Martin-Garcia JM. eLS 2020

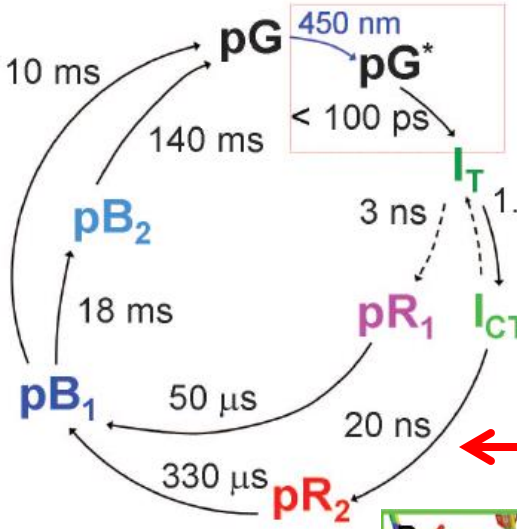
For light activated reactions => Δt = fs - μs

For reactions induced by rapid mixing => Δt = μs - s

Biology – structure determination with atomic and temporal resolution



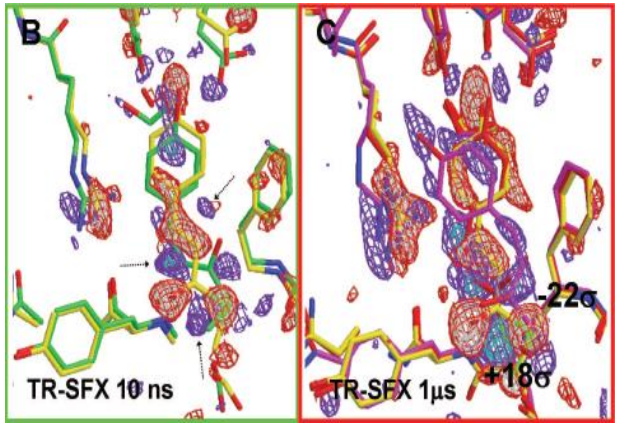
A. Aquila et al., Opt. Exp. 20, 2706 (2012)



Application to e.g. PYP, a bacterial photo-receptor

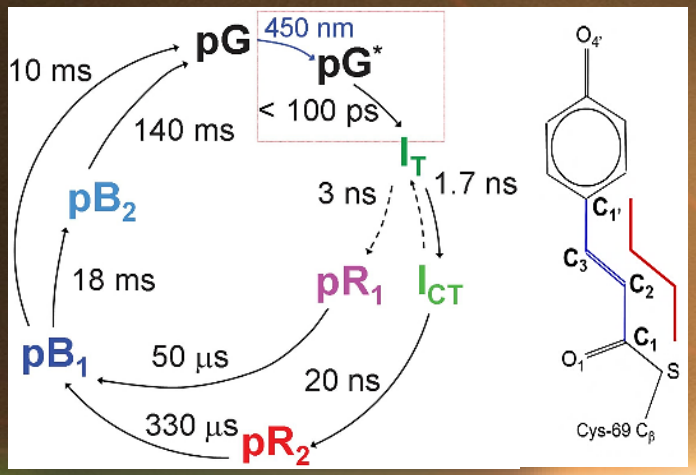
Δt=10 ns

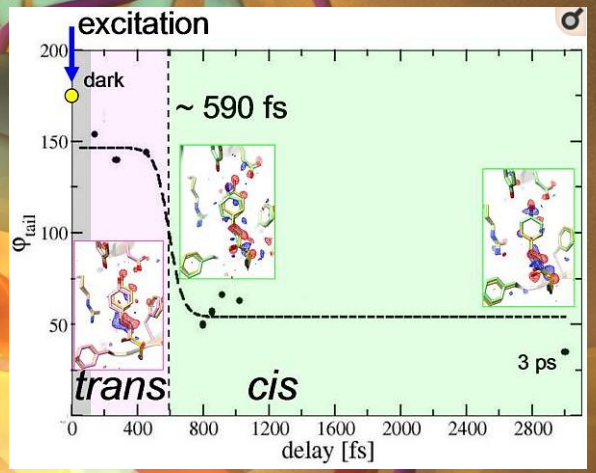
⊗t=1 µs



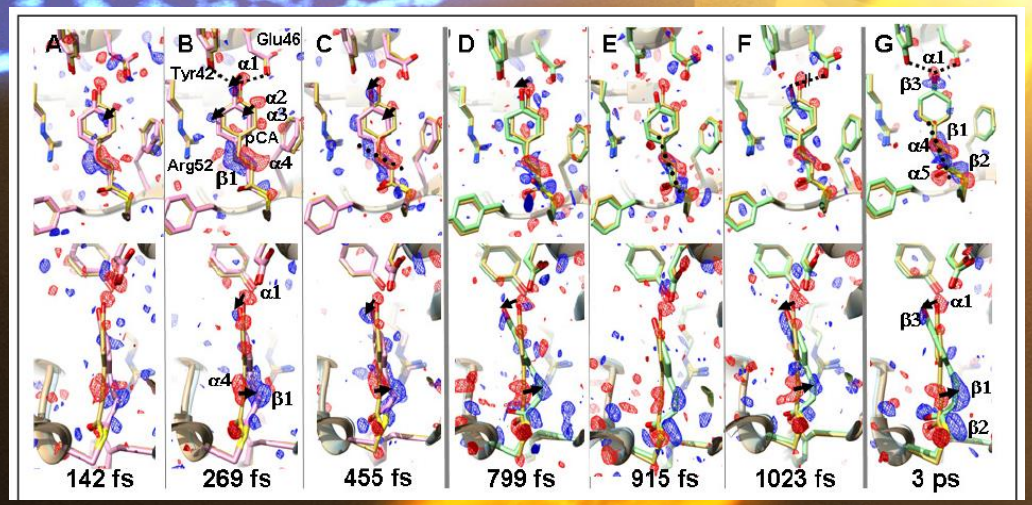
J. Tenboer et al., Science 346, 1242 (2014)

SFX to Make the Molecular Movie of the Trans/Cis Isomerization in Photoactive Yellow Protein





Marius Schmidt
(UWM)



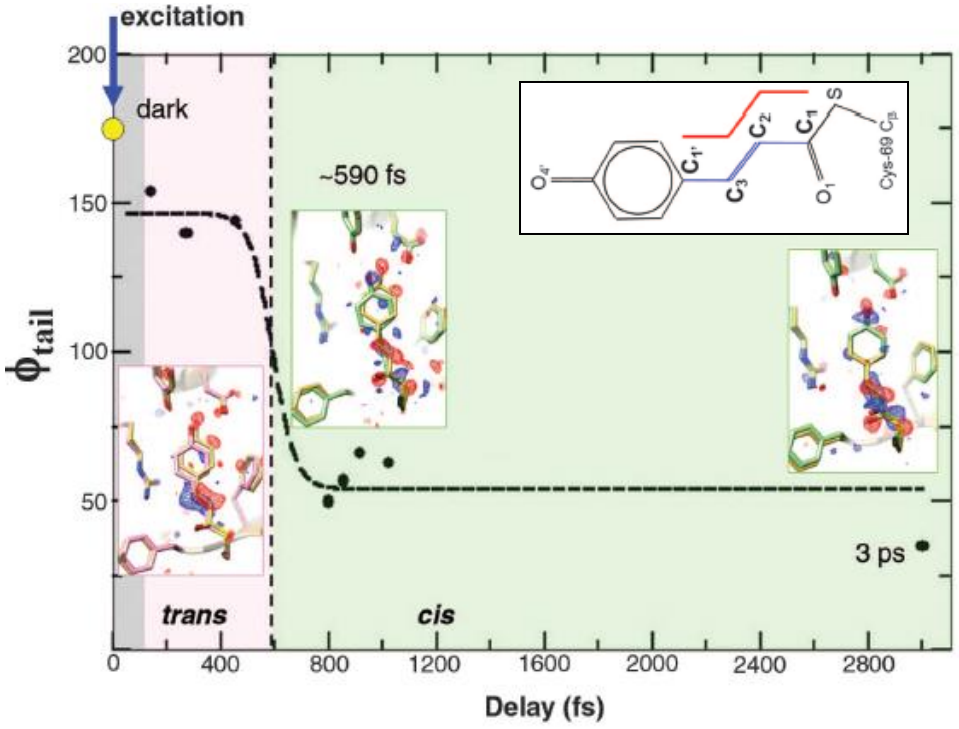
Molecular movie available @ https://www.youtube.com/watch?v=b-CyE1f08Uk

Tenboer *et al.*, Science 2014

Pande *et al.*, Science. 2016

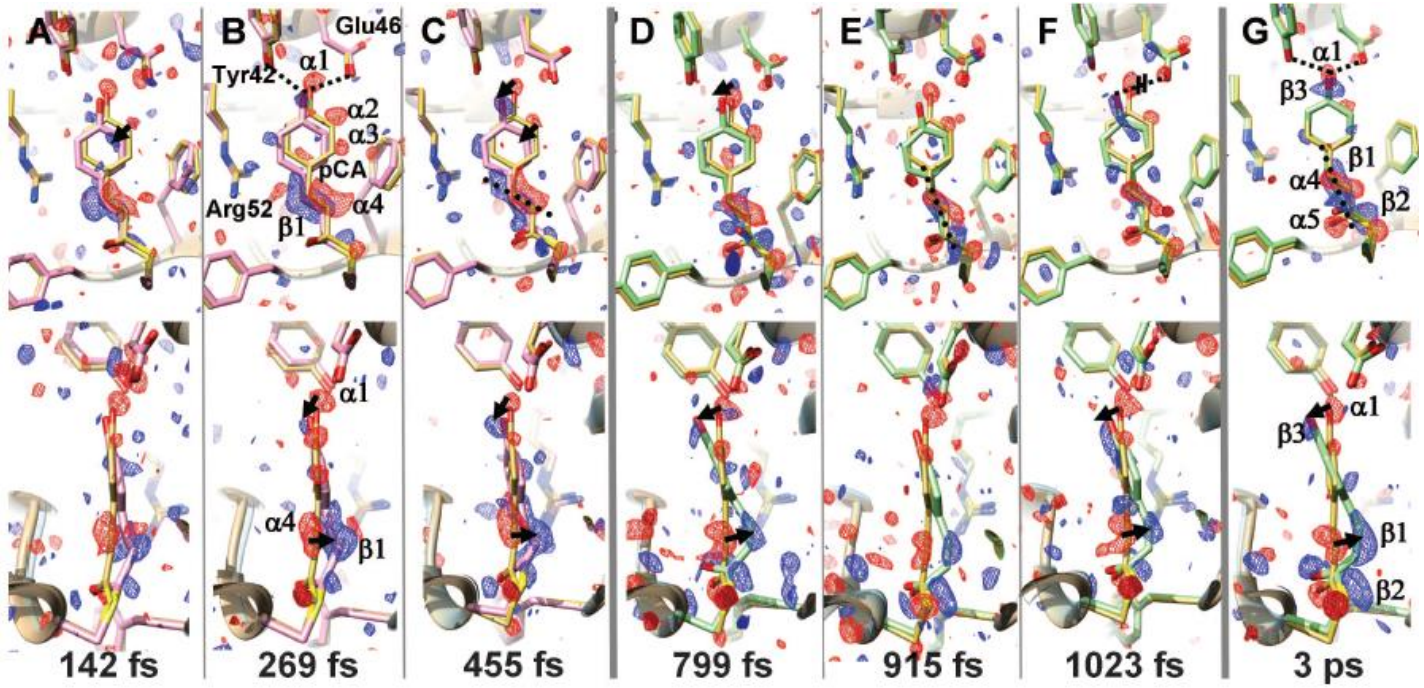
Image credits by SLAC

Molecular movies taking using x-ray FELs



Nango et al., Science 354, 1552 (2016)

Photo-active Yellow Protein (100 fs to 3 ps):



K. Pande et al., Science 352, 725 (2016)

Ultrafast studies of solvated proteins

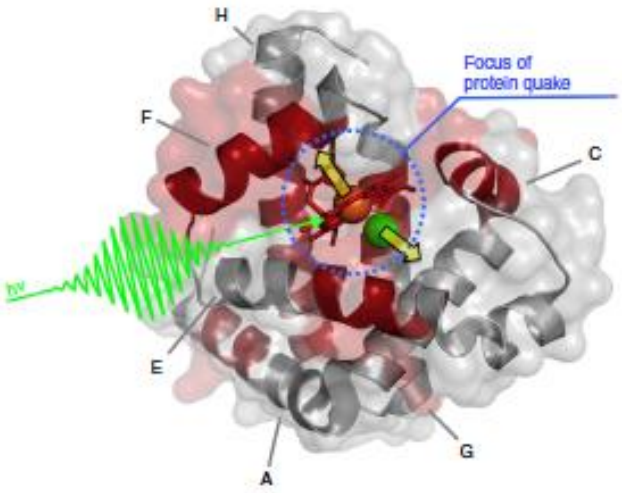
nature COMMUNICATIONS

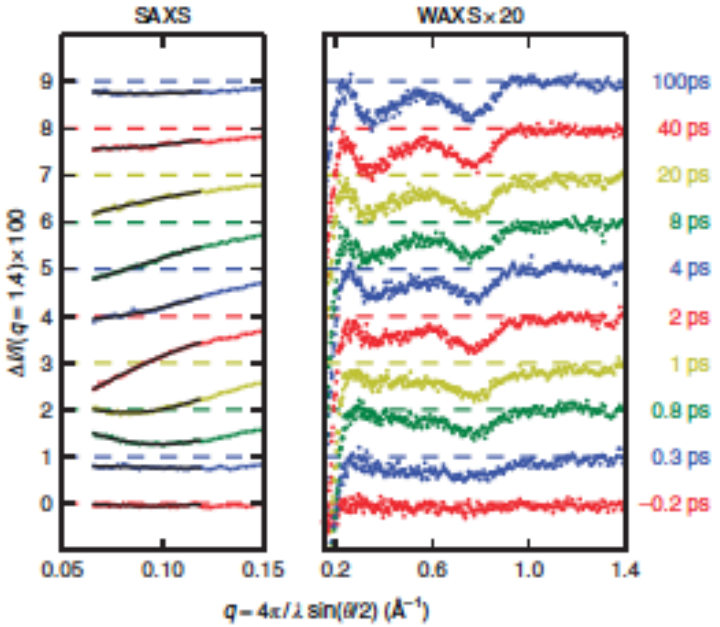
ARTICLE

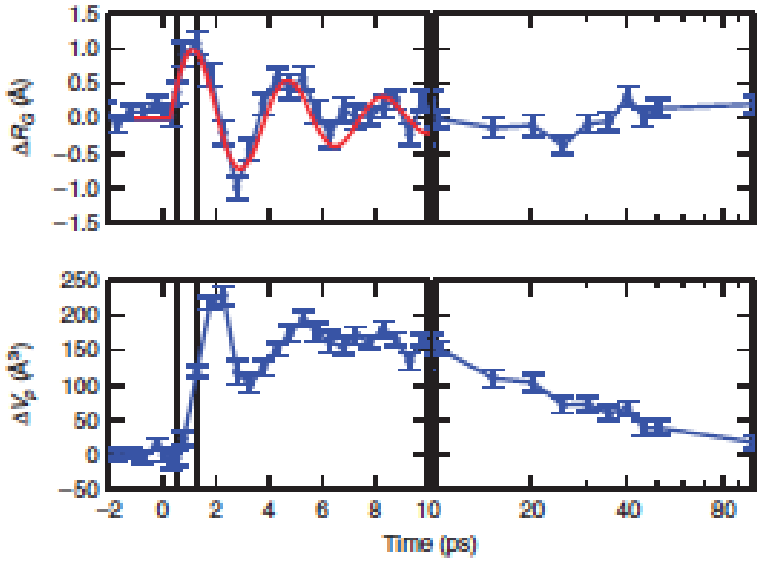
Received 3 Nov 2014 | Accepted 25 Feb 2015 | Published 2 Apr 2015 DOI: 10.1038/ncomms7772 OPEN

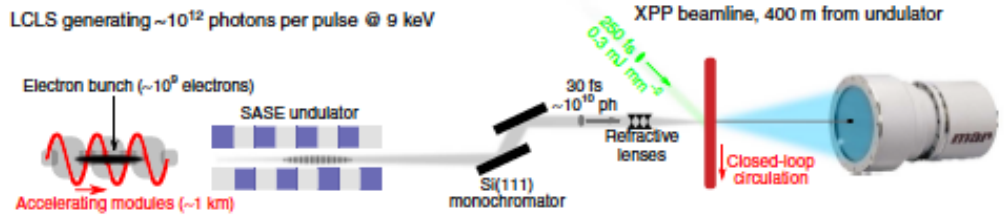
Ultrafast myoglobin structural dynamics observed with an X-ray free-electron laser

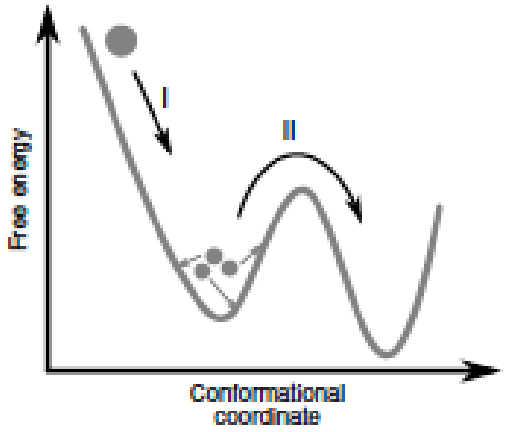
Matteo Levantino[1,*], Giorgio Schirò[2,*], Henrik Till Lemke[3], Grazia Cottone[1], James Michael Glownia[3], Diling Zhu[3], Mathieu Chollet[3], Hyotcherl Ihee[4,5], Antonio Cupane[1] & Marco Cammarata[6]











Correlated spin and structural dynamics in myoglobin

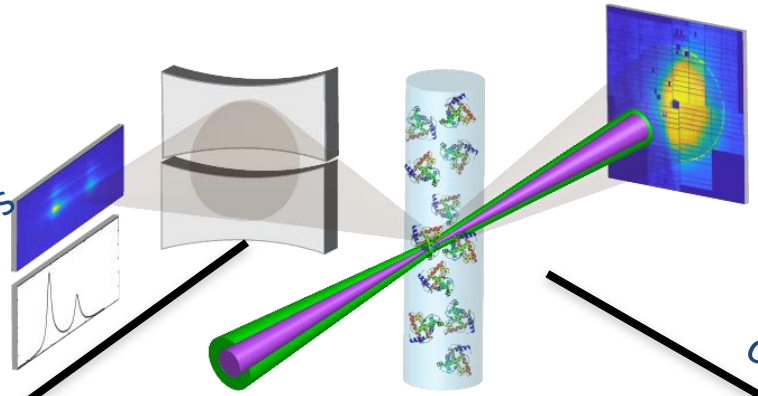
ARTICLE

https://doi.org/10.1038/s41467-020-17923-w OPEN

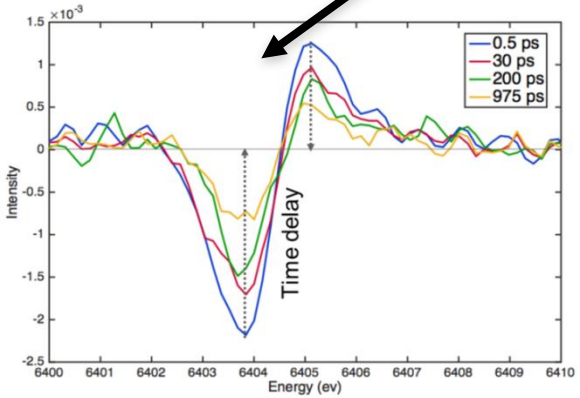
Femtosecond X-ray emission study of the spin cross-over dynamics in haem proteins

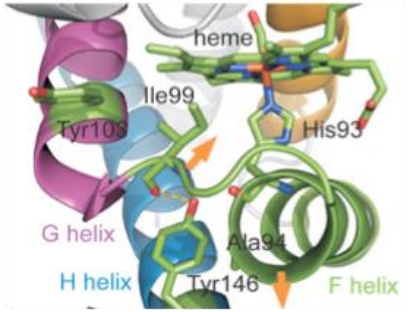
Dominik Kinschel[1], Camila Bacellar[1], Oliviero Cannelli[1], Boris Sorokin[1], Tetsuo Katayama[2], Giulia F. Mancini[1], Jérémy R. Rouxel[1], Yuki Obara[3], Junichi Nishitani[4], Hironori Ito[3], Terumasa Ito[3], Naoya Kurahashi[5], Chika Higashimura[4], Shotaro Kudo[4], Theo Keane[6], Frederico A. Lima[7], Wojciech Gawelda[7,8], Peter Zalden[7], Sebastian Schulz[7], James M. Budarz[1], Dmitry Khakhulin[7], Andreas Galler[7], Christian Bressler[7], Christopher J. Milne[9], Thomas Penfold[6], Makina Yabashi[2], Toshinori Suzuki[4], Kazuhiko Misawa[3] & Majed Chergui[1]✉

X-ray Emission Spectroscopy



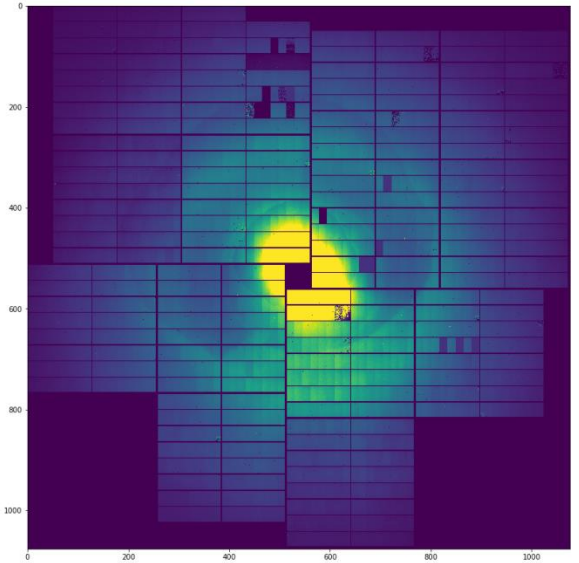
Electronic changes



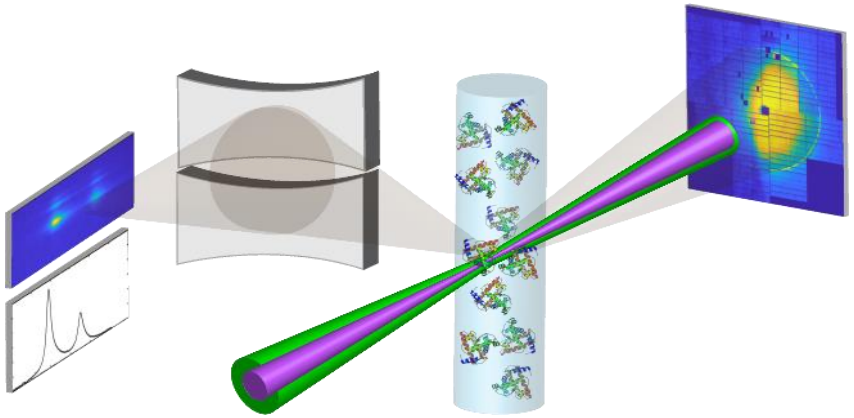


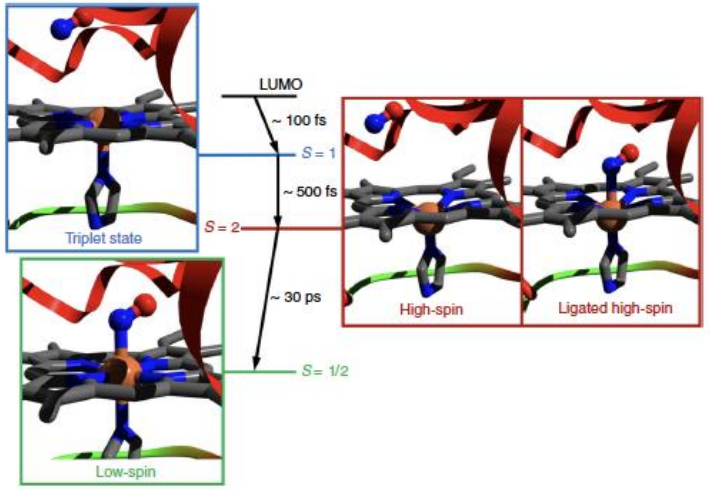
Conformational changes

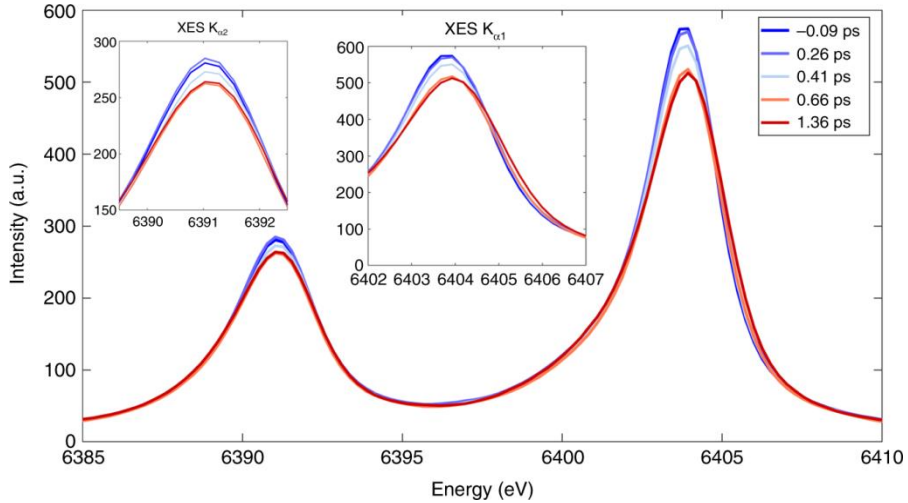
X-ray Solution Scattering (WAXS)

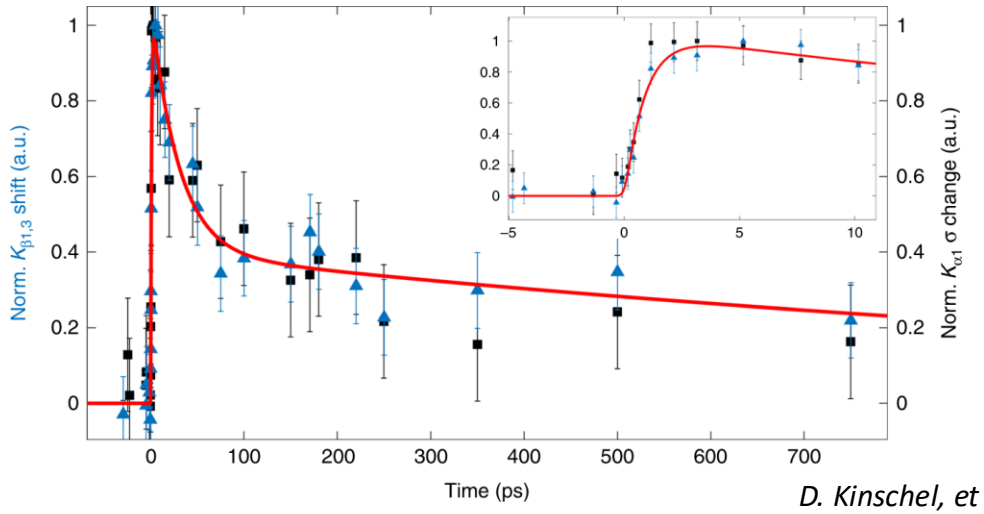


Correlated spin and structural dynamics in myoglobin



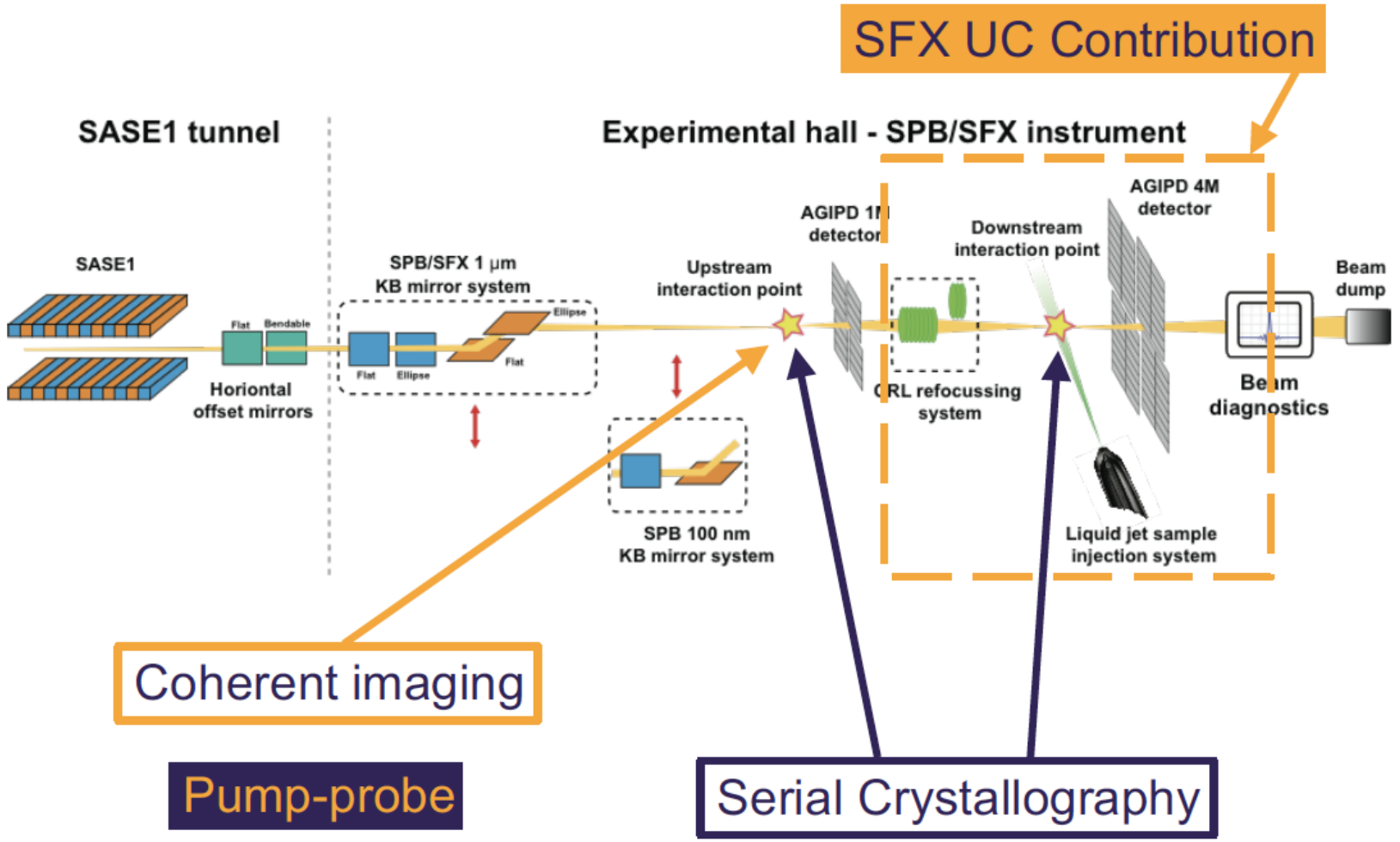




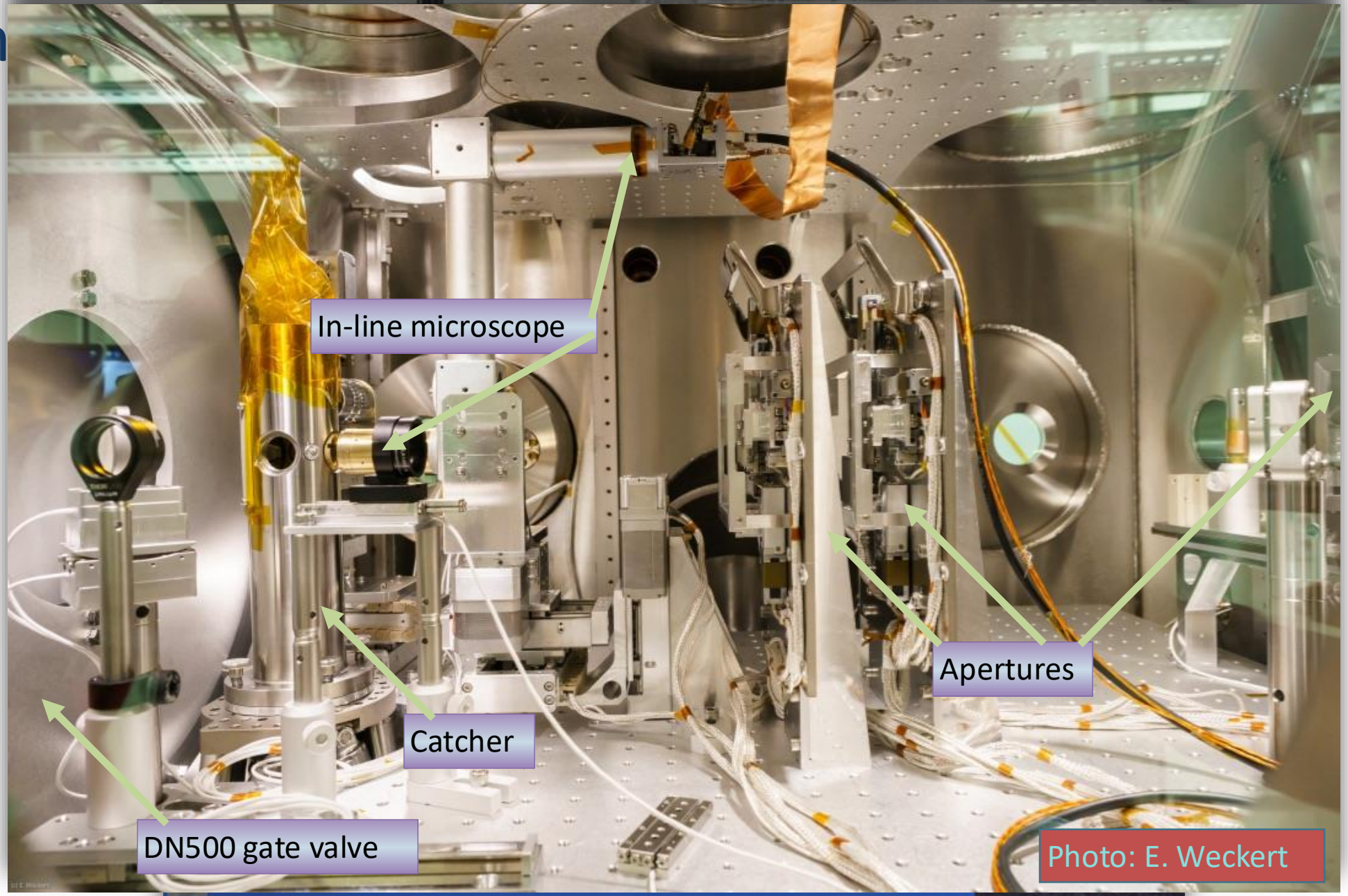


D. Kinschel, et al., Nature Communications (2020)

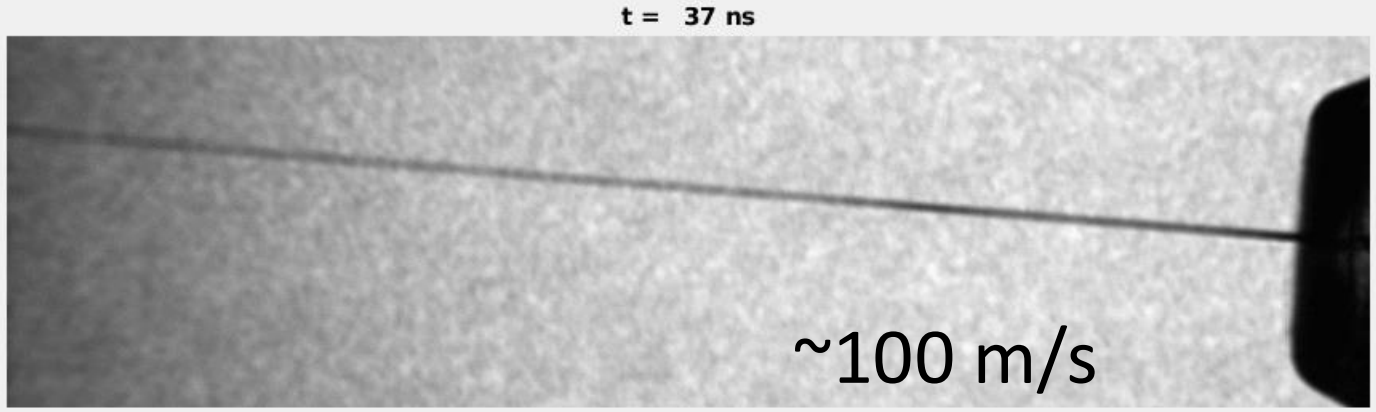
Single **P**article and **B**iomolecules/SFX Instrument at EuXFEL

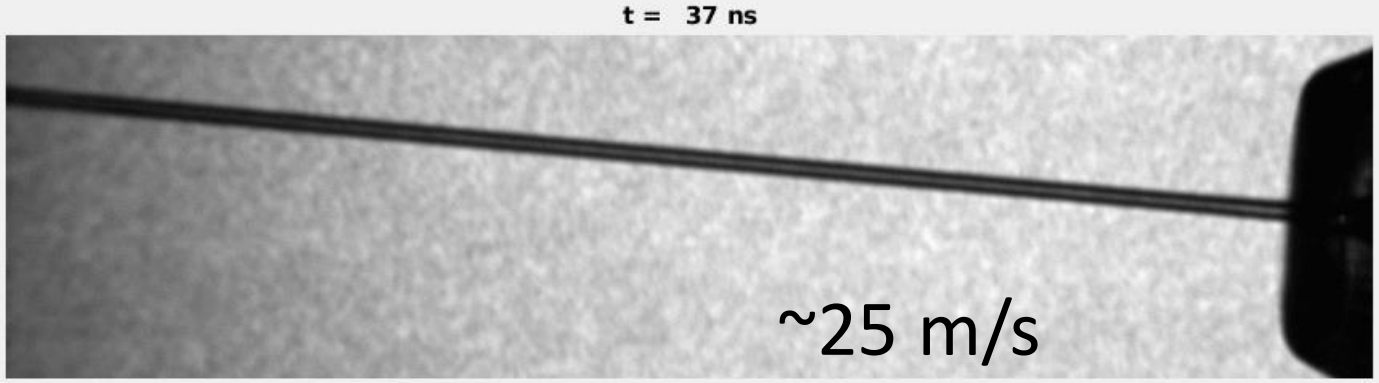


Sam


In-line microscope
Apertures
Catcher
DN500 gate valve
Photo: E. Weckert

Serial crystallography works at MHz rates

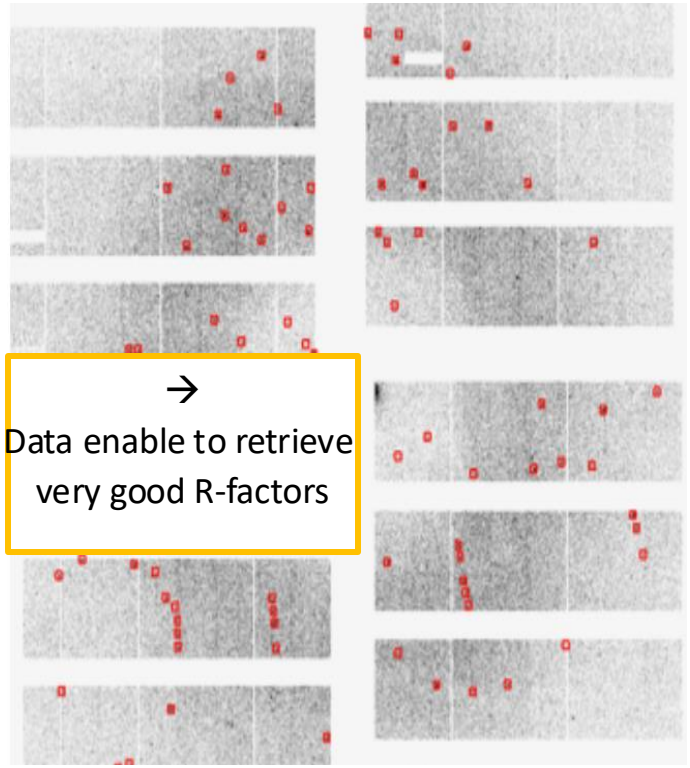




Wiedorn et al, Nat. Comms., 2018.

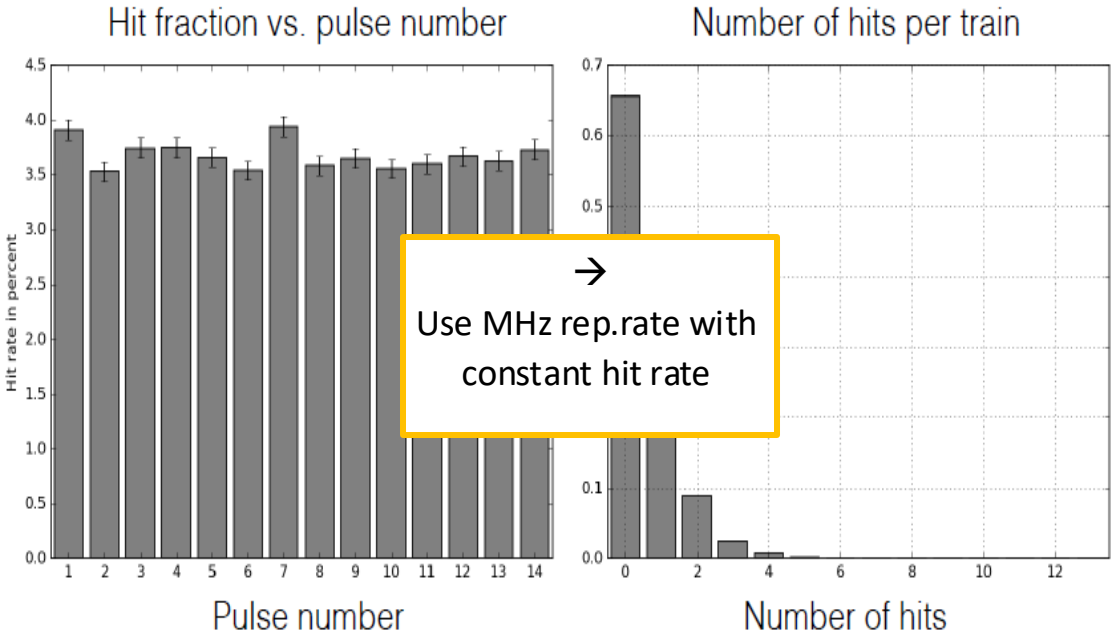
MHz Serial Crystallography at EuXFEL

Single pulse diffraction pattern



→
Data enable to retrieve very good R-factors

9.3 keV, 30 pulses, 100µJ, 12 µm focus

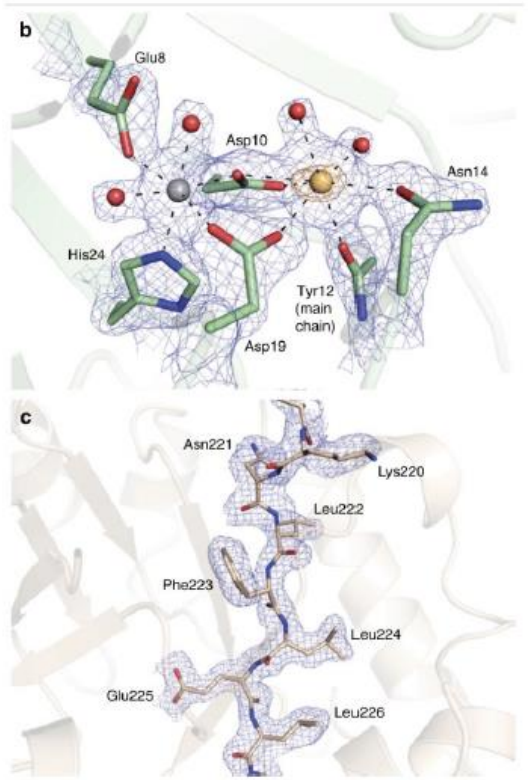


→
Use MHz rep.rate with constant hit rate

Could make use of 14 pulses only (AGIPD/DAQ issue). Still need to show that this hit rate can be transferred to full AGIPD capability of (3500 frames/s) (~30 times faster than LCLS)

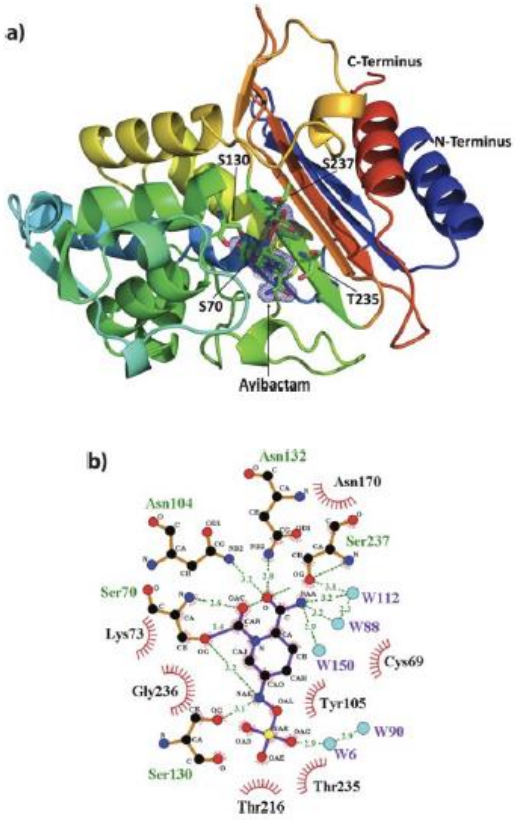
MHz Serial Crystallography at EuXFEL

Jack bean protein



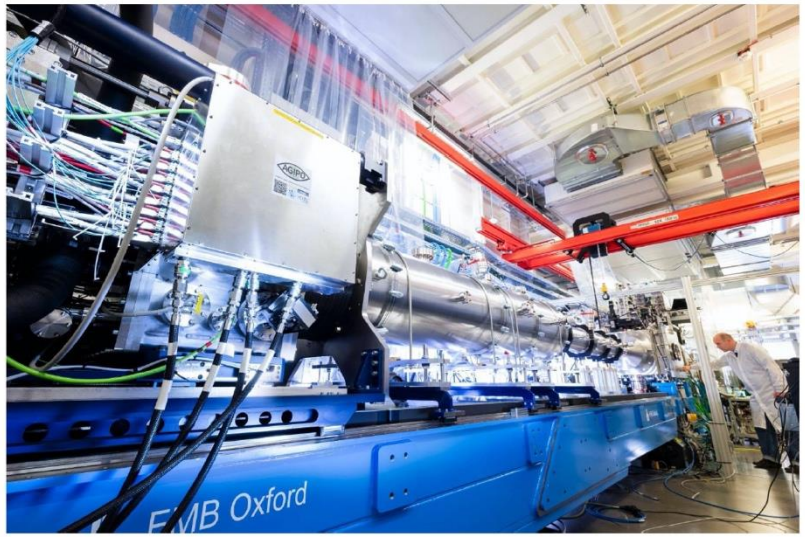
Grünbein et al, *Nat. Commun.* 9, 3487 (2018)

CTX-M-14 β-lactamase



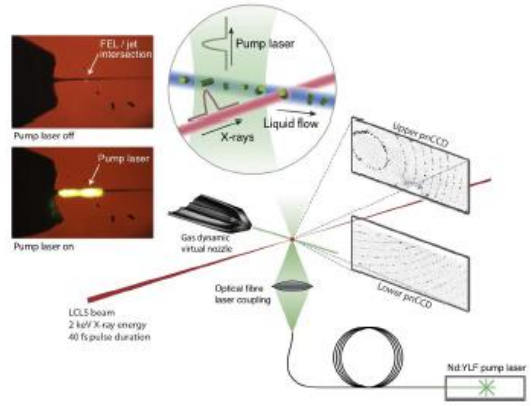
Wiedorn et al, *Nat. Commun.* 9, 4025 (2018)

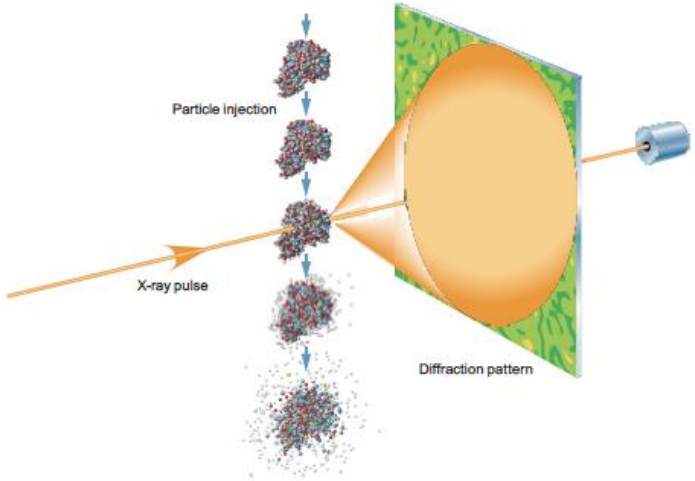
The SPB/SFX Instrument



Future Science Opportunities

- ✓ Crystalline samples that can't be seen with synchrotrons
  - but see C. Gati, et al, IUCrJ, 2014.
- ✓ Fast, ultra-fast, or complex dynamics
  - In crystals (see A. Aquila et al, Opt Express, 2012)
  - In solution (see D. Arnlund et al, Nat. Methods, 2014)
- ✓ Reproducible single particles
  - Close to realising "larger" samples
  - Smaller samples TBD
- ✓ Radiation damage sensitive samples
  - see K. Hirata, et al, Nature Methods, 2014.





Conclusions

- Diffractive imaging experiments using coherent and ultrashort XFEL radiation have started and promise to provide very good contrast and highest time resolution
- Single nanoscale objects could be imaged, and ultrafast structural dynamics of nanocrystals of proteins and solvated proteins could be recorded
- The ultimate goal of coherent imaging of single bio-molecules is not yet achieved → very challenging goal
- MHz serial crystallography of small crystals works!
- The field of structural biology using XFELs is in constant development and is expected still to see many new applications