Wir schaffen Wissen – heute für morgen

Paul Scherrer Institut

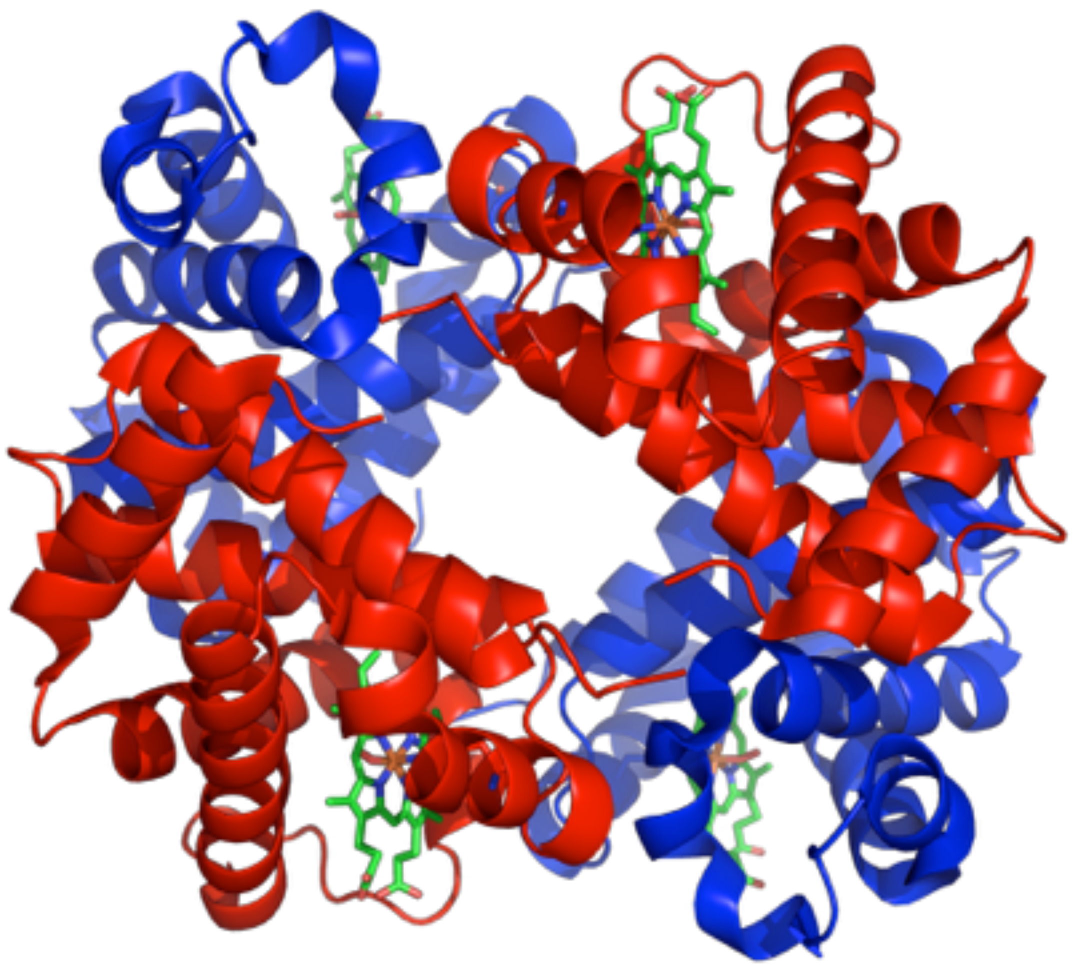
Chris Milne

Using X-ray free electron lasers to understand biological function and how it can be disrupted (or initiated)

Is function structure or dynamics ?

Structure

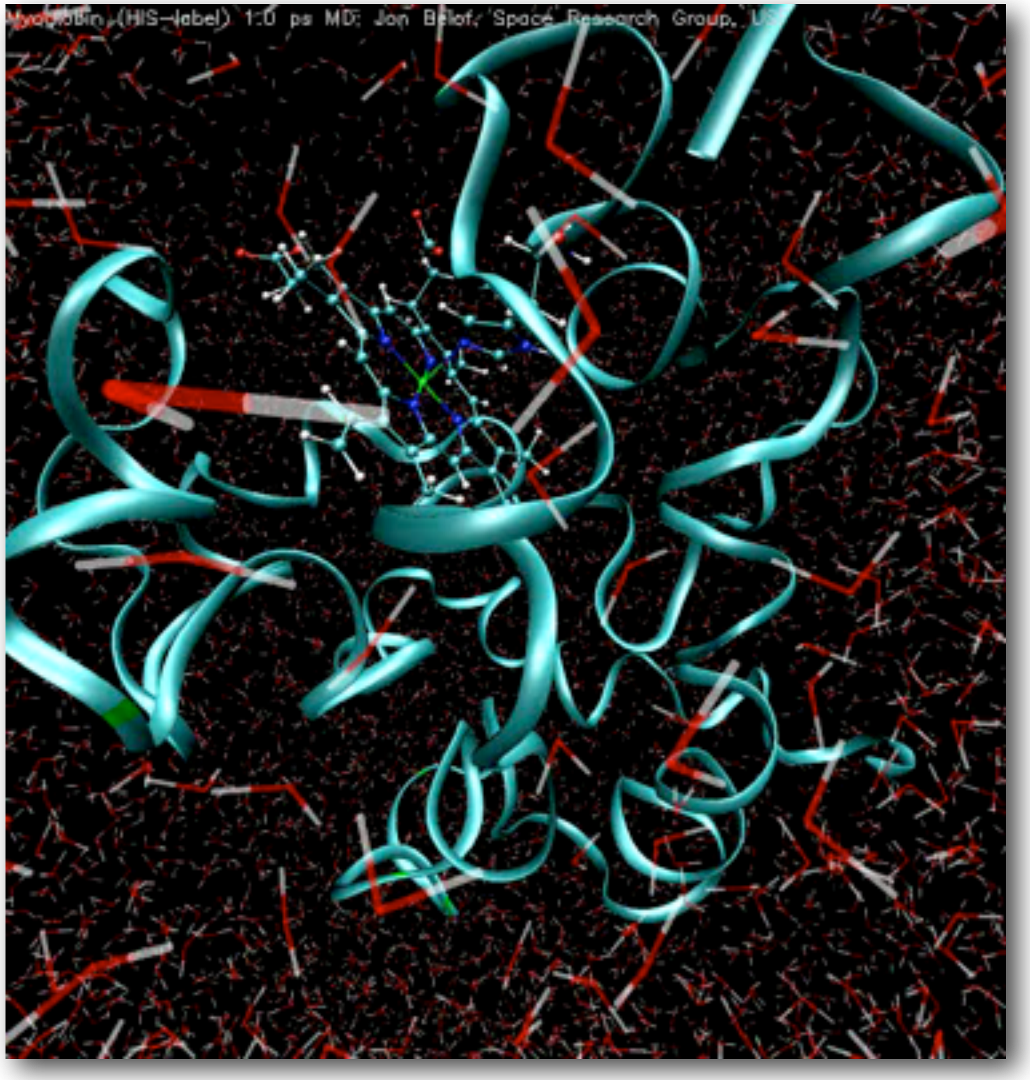
- X-ray crystallography
- electron microscopy
- atomic force microscopy
- electron diffraction
- X-ray absorption spectroscopy
- NMR



Protein structure of human hemoglobin in the T-state with oxygen bound at all 4 hemes (from PDB 1GZX Wikipedia)

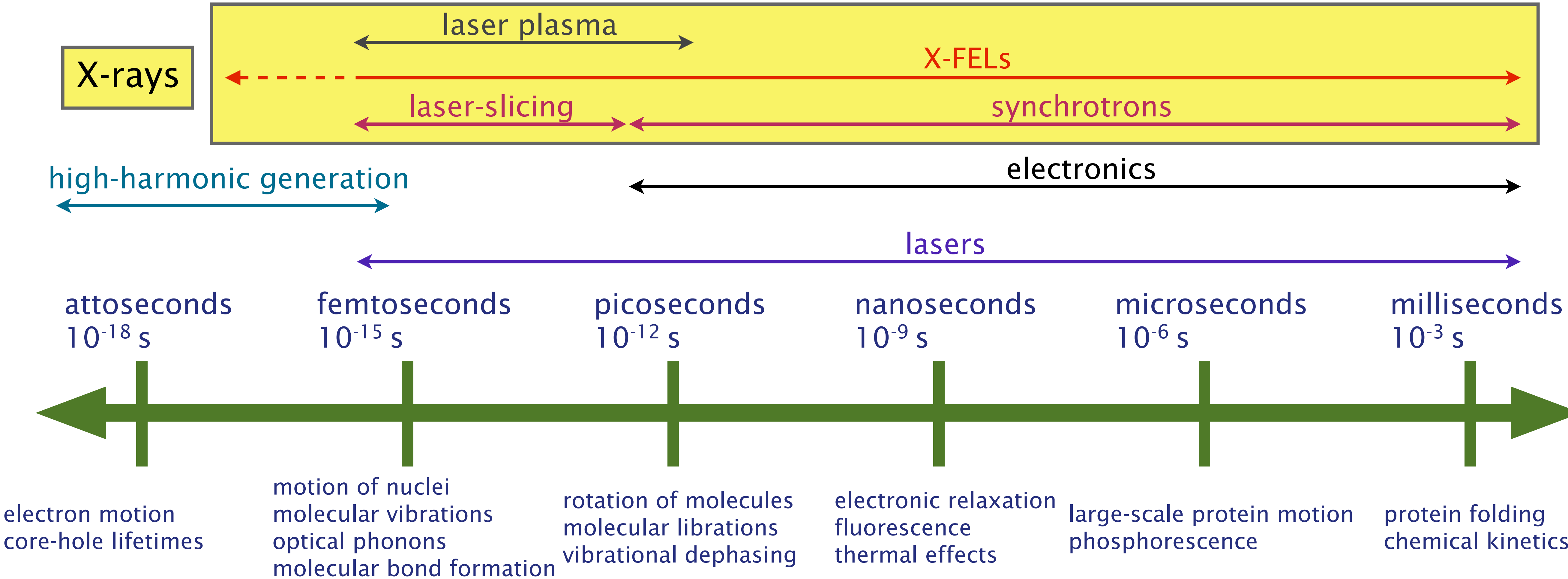
Dynamics

- Laser spectroscopy
- NMR
- time-resolved diffraction
- X-ray absorption spectroscopy



Rotating hydrated myoglobin molecule
http://uweb.cas.usf.edu/chemistry/faculty/space/
B. Space & J. Belof (University of South Florida)

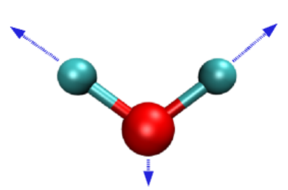
On what timescale do we want to measure structure ?

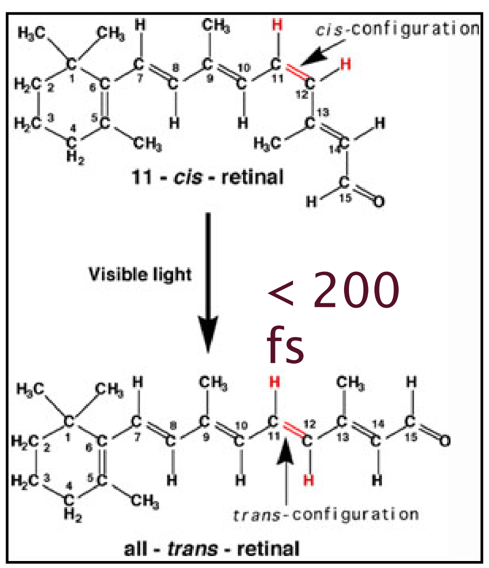


110 as delay between electron emission from conduction band and lower-lying states in Tungsten upon irradiation

The Fe K-edge core-hole lifetime is 4 fs

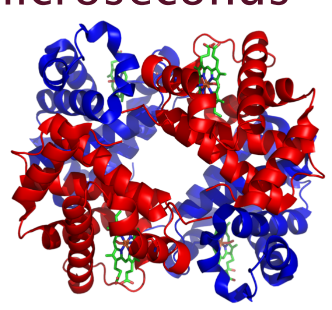
period of the symmetric stretch in H_2O is 10 fs





< 200 fs

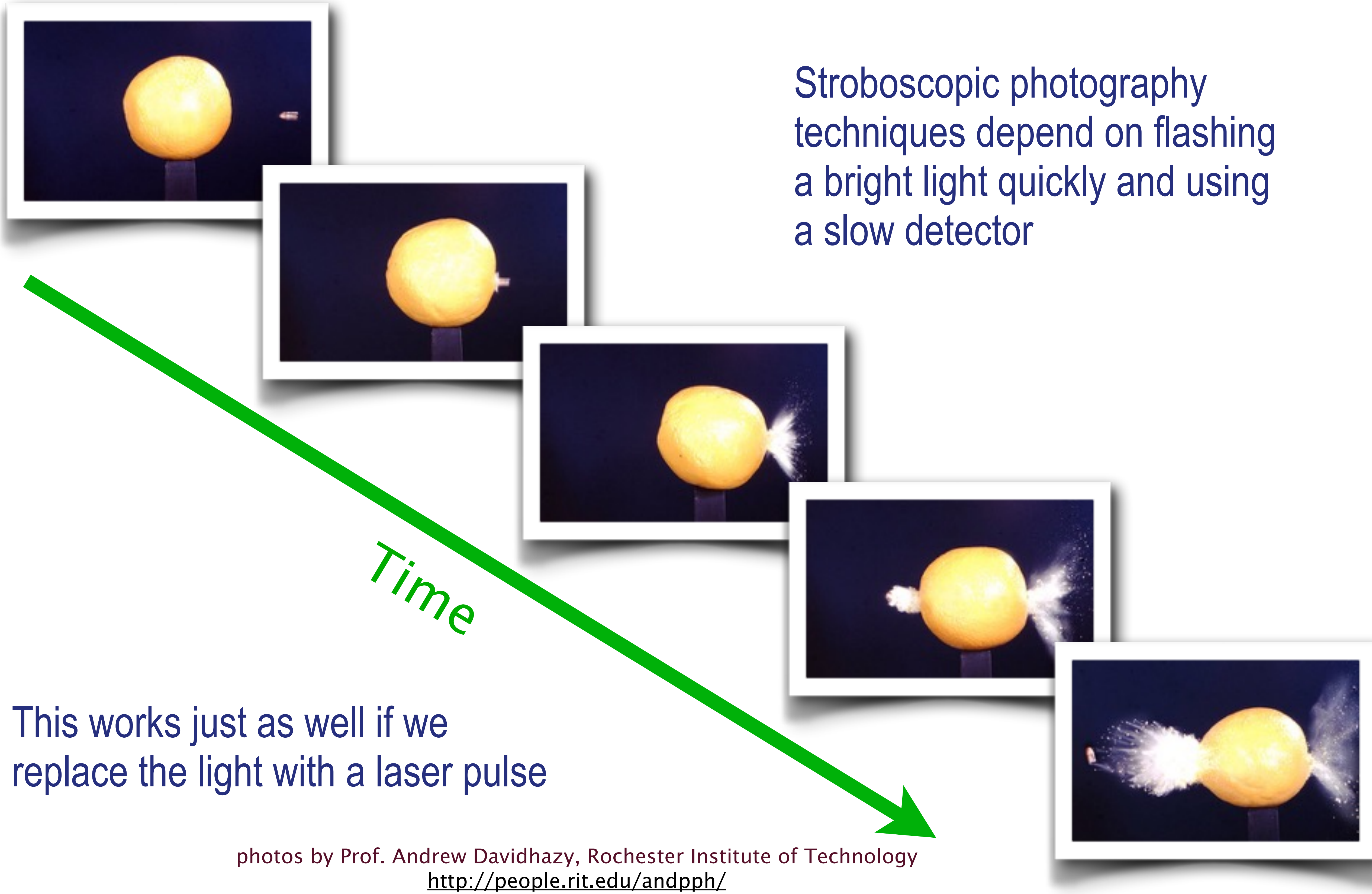
Hemoglobin R->T transition takes microseconds



Camera shutter speeds range from ms through to seconds

How do we measure fast things ?

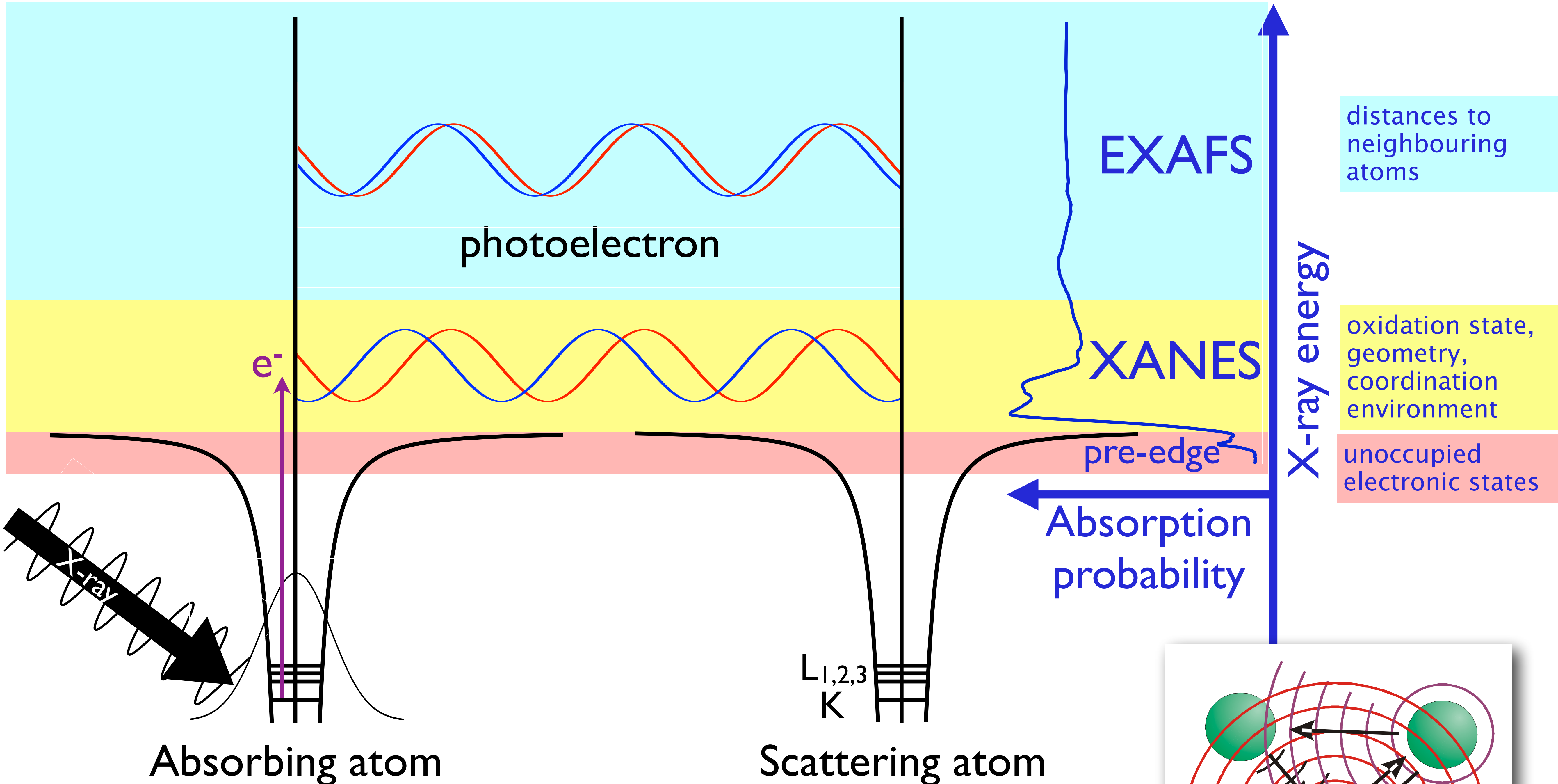
Stroboscopic photography techniques depend on flashing a bright light quickly and using a slow detector



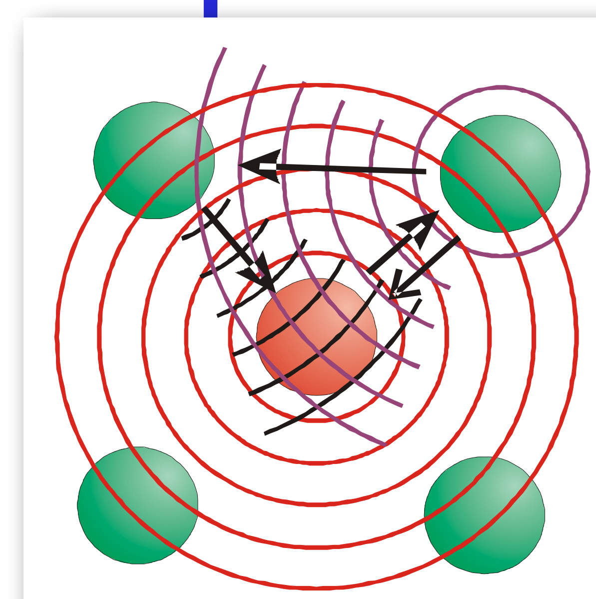
This works just as well if we replace the light with a laser pulse

photos by Prof. Andrew Davidhazy, Rochester Institute of Technology
http://people.rit.edu/andpph/

X-ray absorption: Retrieving local structure

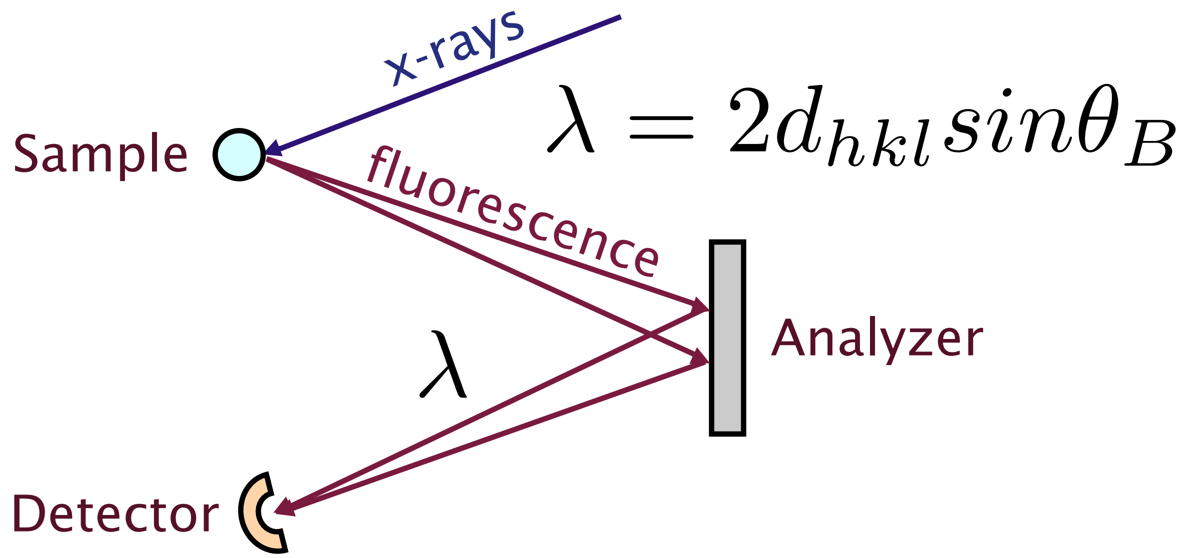


X-ray absorption gives information on the unoccupied electronic states and the local structure

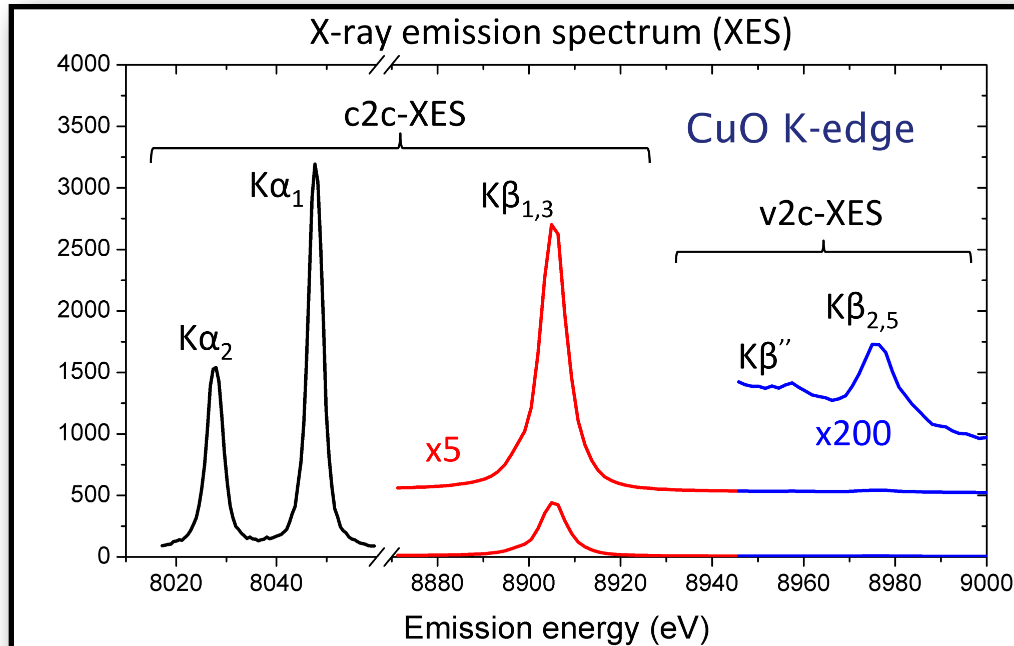


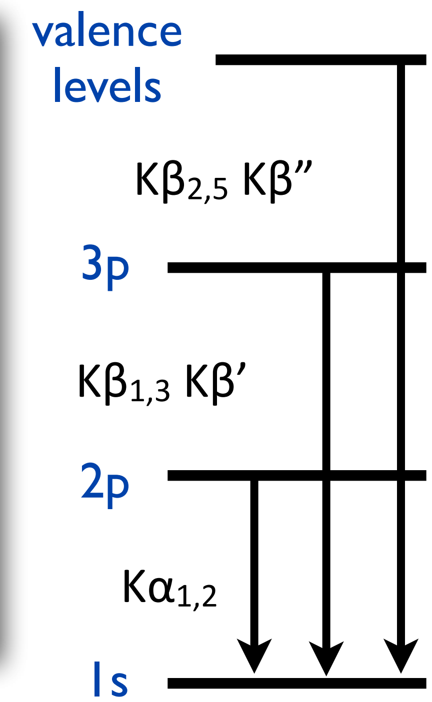
C.J. Milne, T.J. Penfold & M. Chergui *Coord. Chem. Rev.* **277**, 44 (2014)

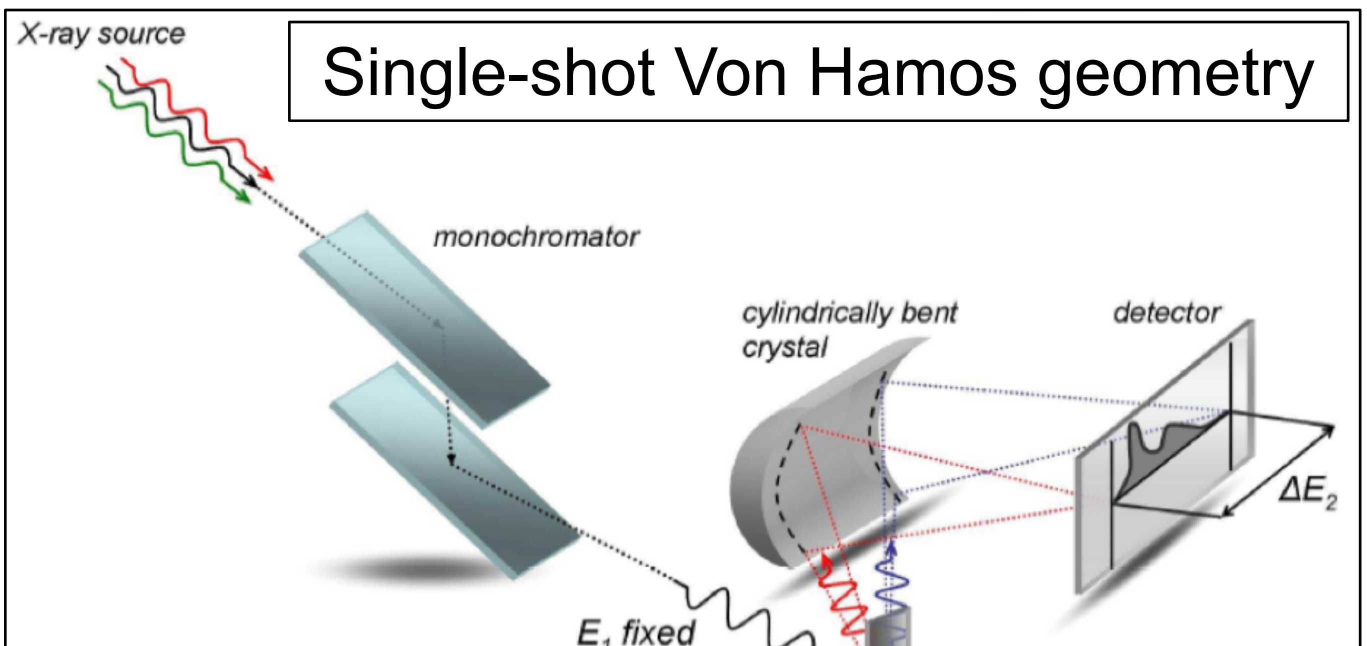
X-ray emission: Retrieving electronic information



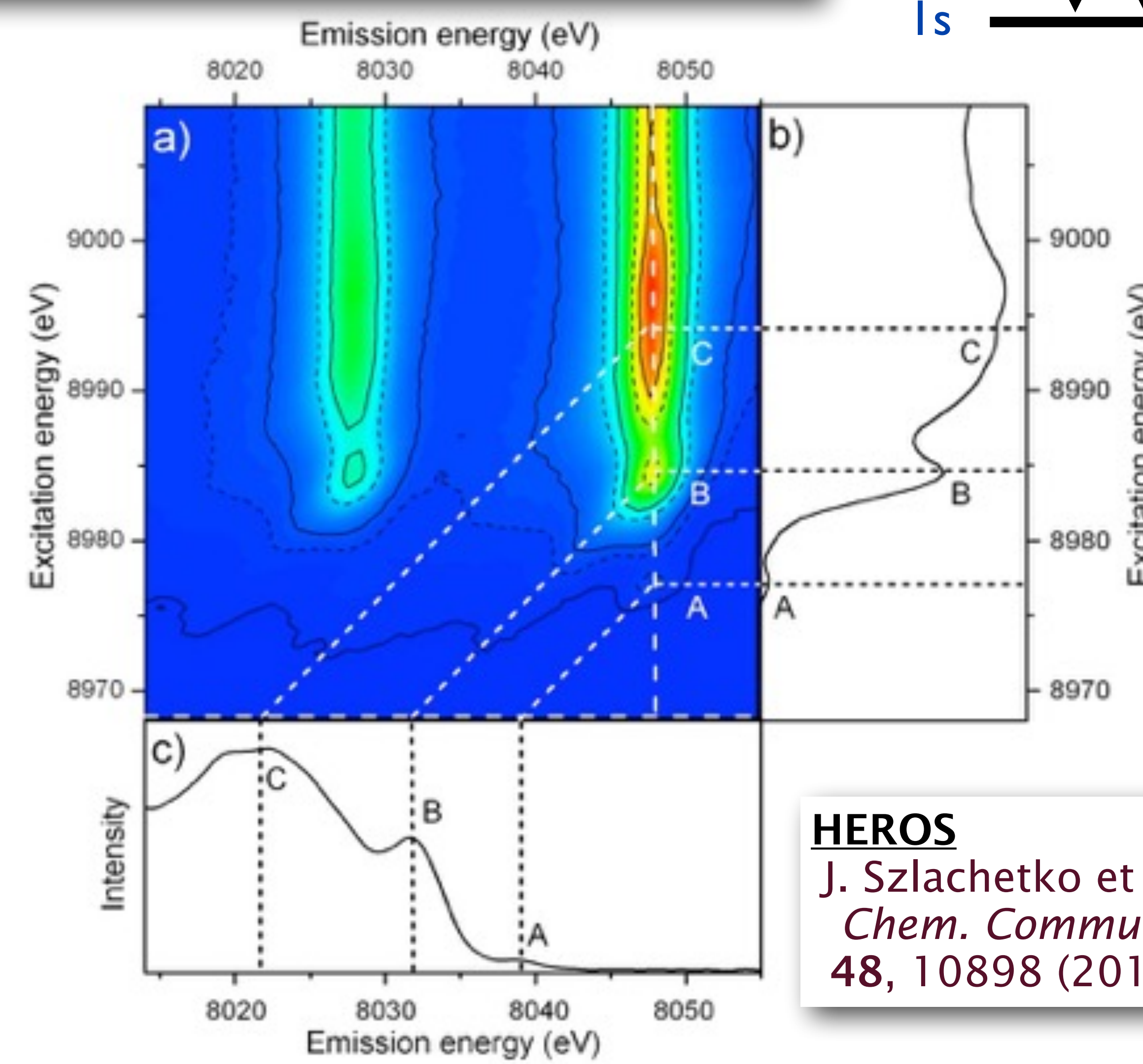
P. Glatzel et al. *Coord. Chem. Rev.* **249**, 65 (2005)
G. Vankó et al. *JPCB* **110**, 11647 (2006)







J. Szlachetko et al. *Rev. Sci. Instr.* **83**, 103105 (2012)



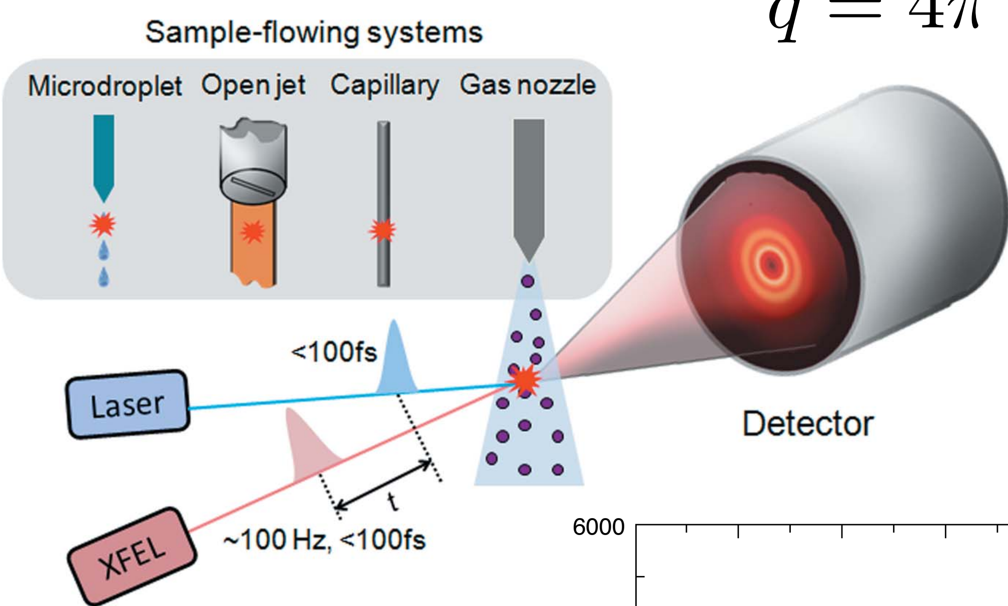
HEROS
J. Szlachetko et al. *Chem. Commun.* **48**, 10898 (2012)

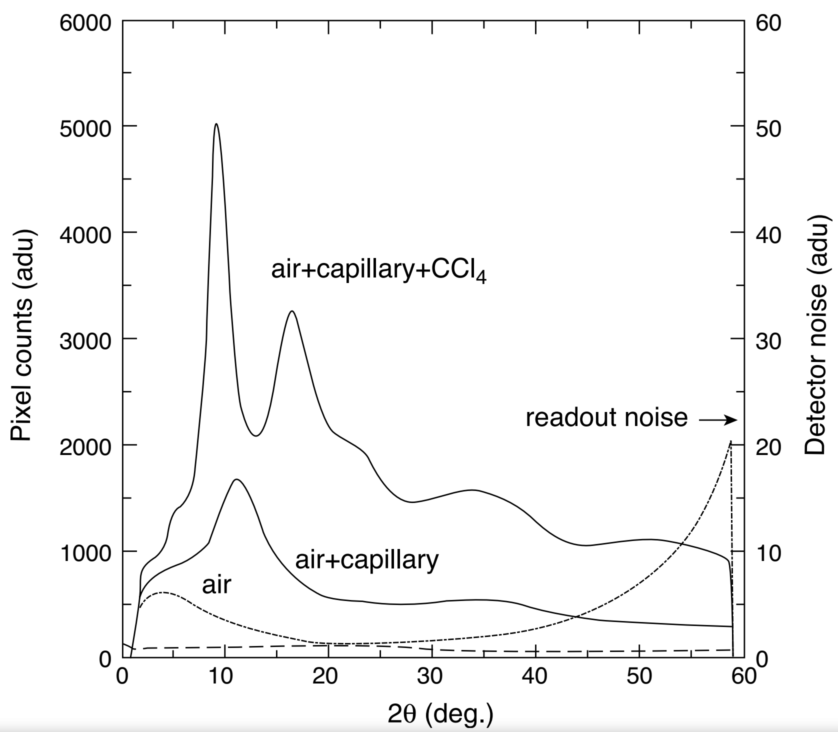
X-ray emission gives information on the occupied electronic states

X-ray scattering: Retrieving local and global structure

X-ray scattering is sensitive to all the atoms in the sample

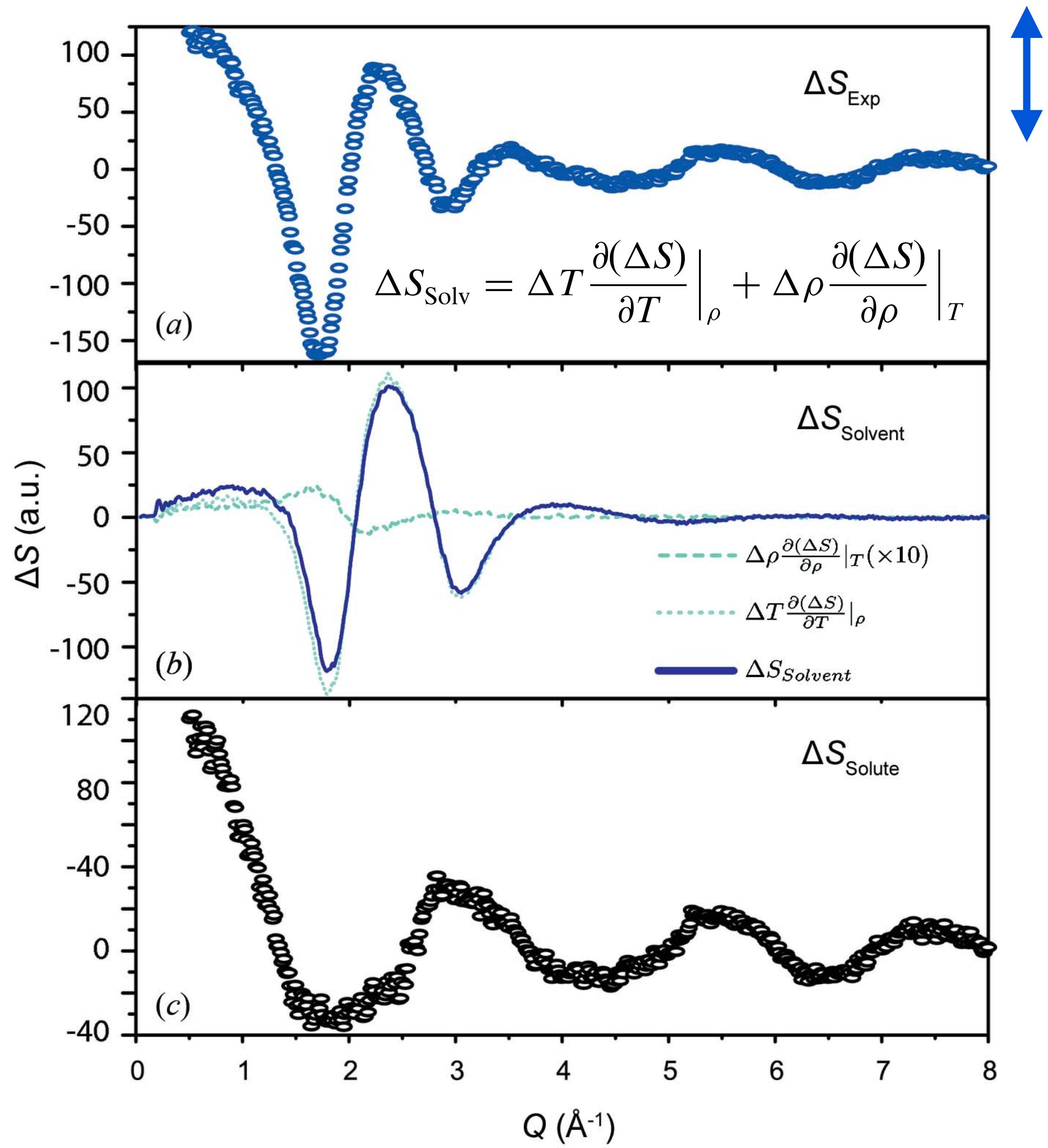
$$q = 4\pi \sin\theta/\lambda$$





- S. Bratos and M. Wulff *Adv. Chem. Phys.* **131**, 1 (2008)
- J. Kim et al. *Acta. Cryst. A* **66**, 270 (2010)

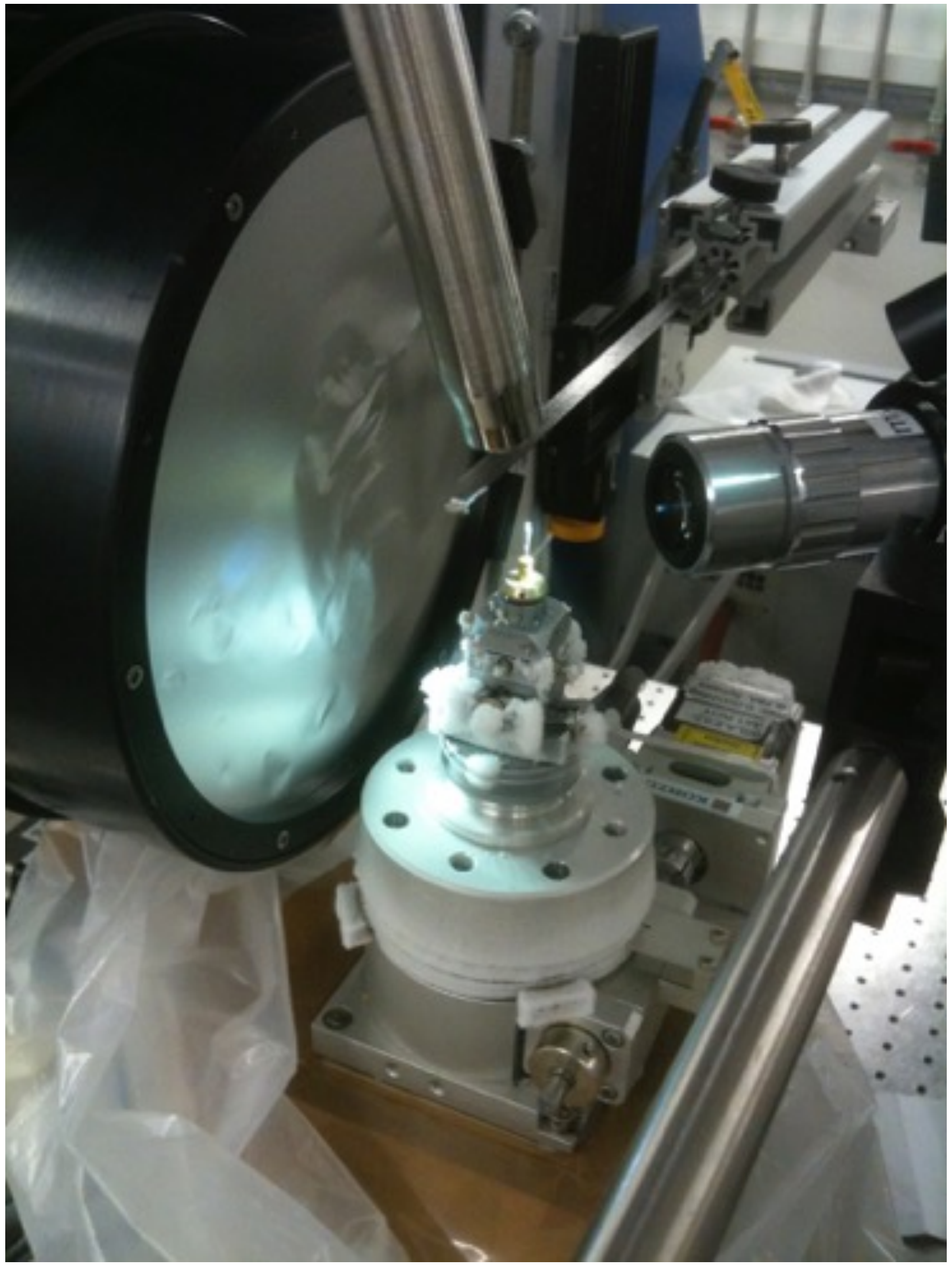
K. Haldrup et al. *Acta. Cryst.* **A66**, 261 (2010)

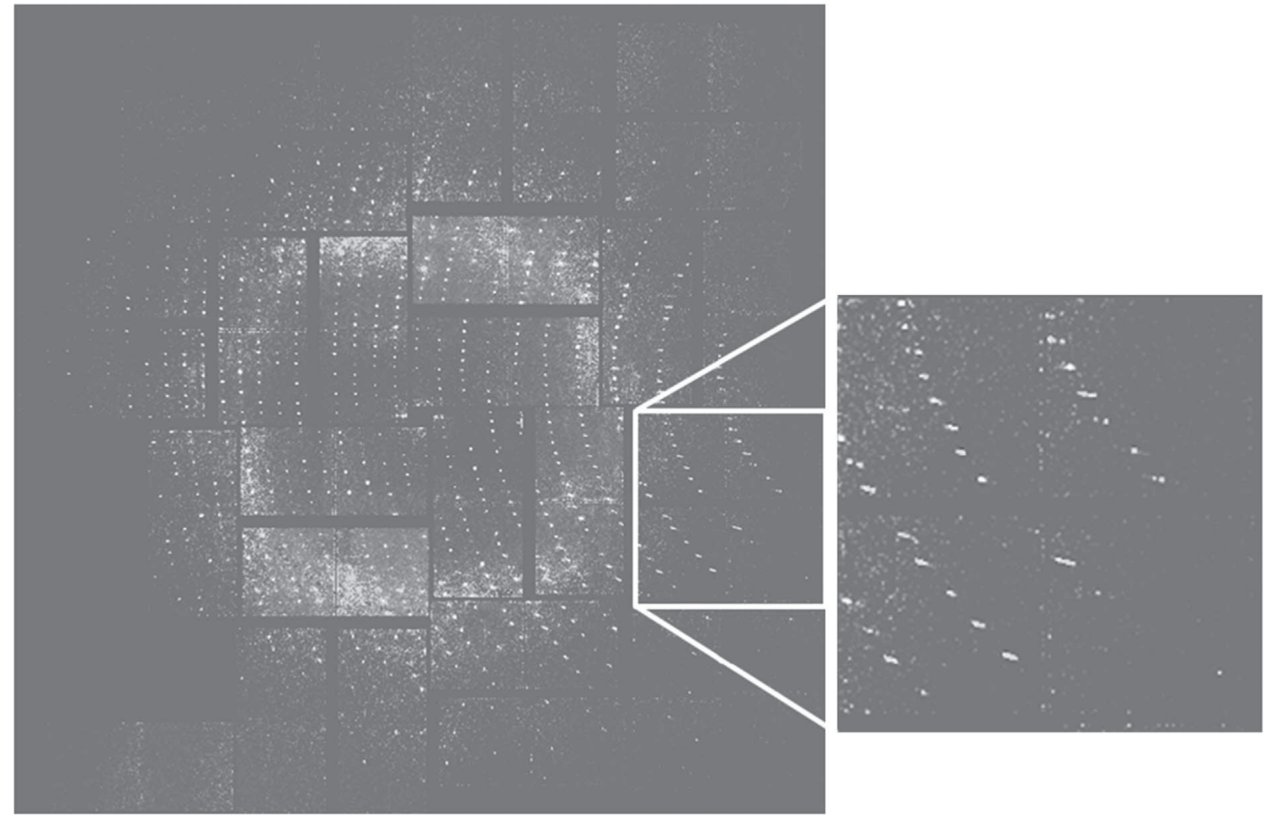


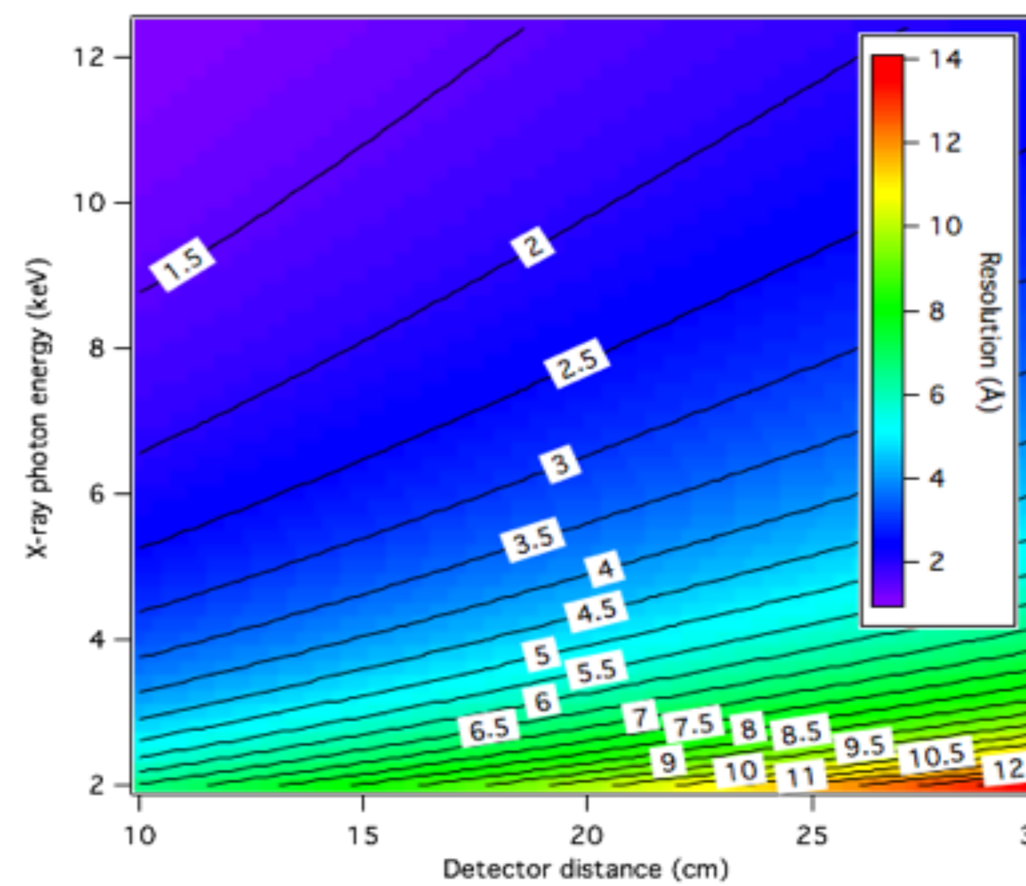
X-ray scattering gives information on relative atomic positions of all atoms in the sample

You can retrieve the pair distribution function, giving the distances between two atoms in the sample

X-ray diffraction: Retrieving atomic-scale structure

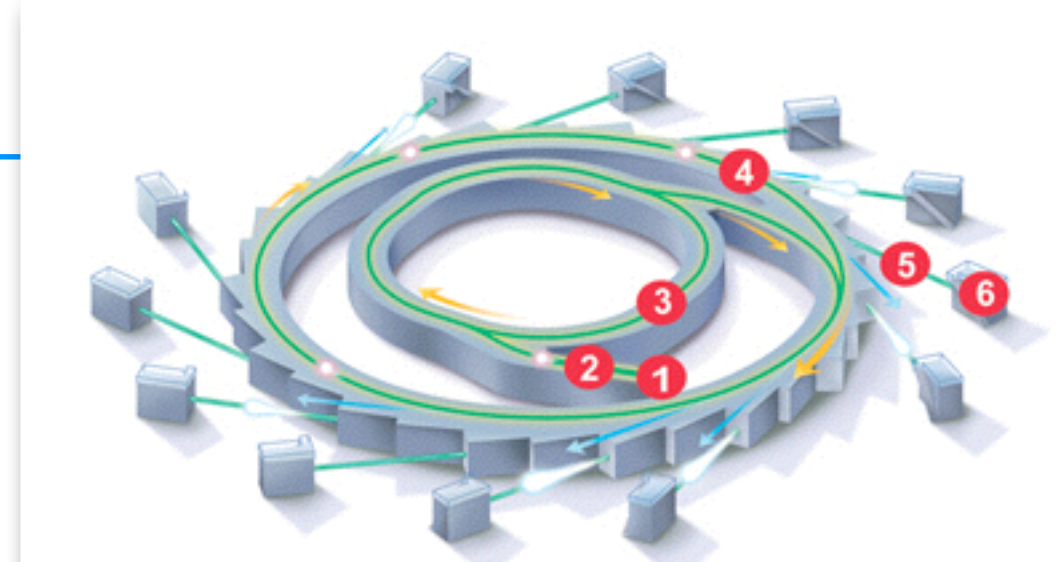






X-ray diffraction gives information on the atomic positions of all atoms in the crystal with Ångstrom accuracy

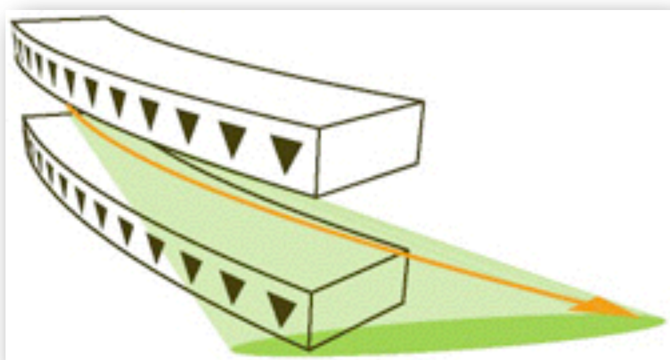
Swiss Light Source

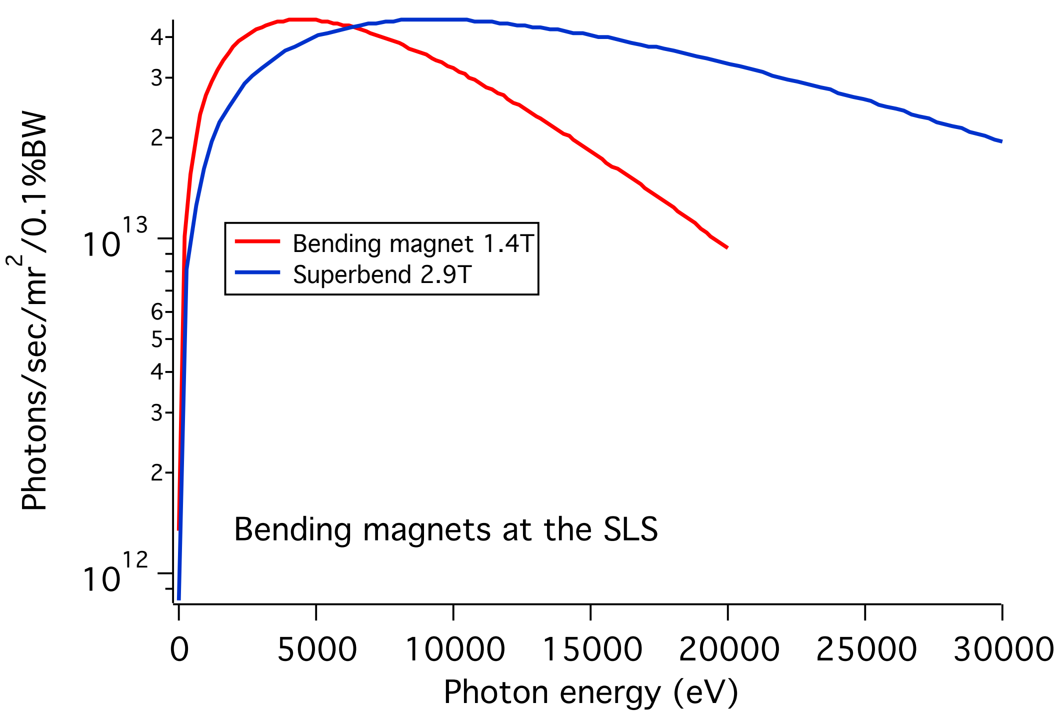


http://www.synchrotron.org.au/content.asp?Document_ID=97

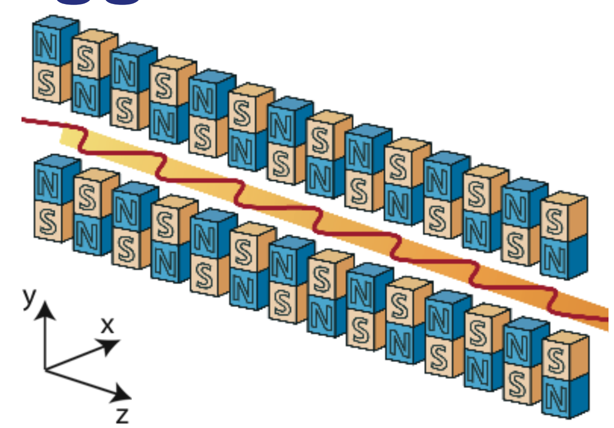
There are two methods of generating light at a 3rd-generation storage ring source (synchrotron)

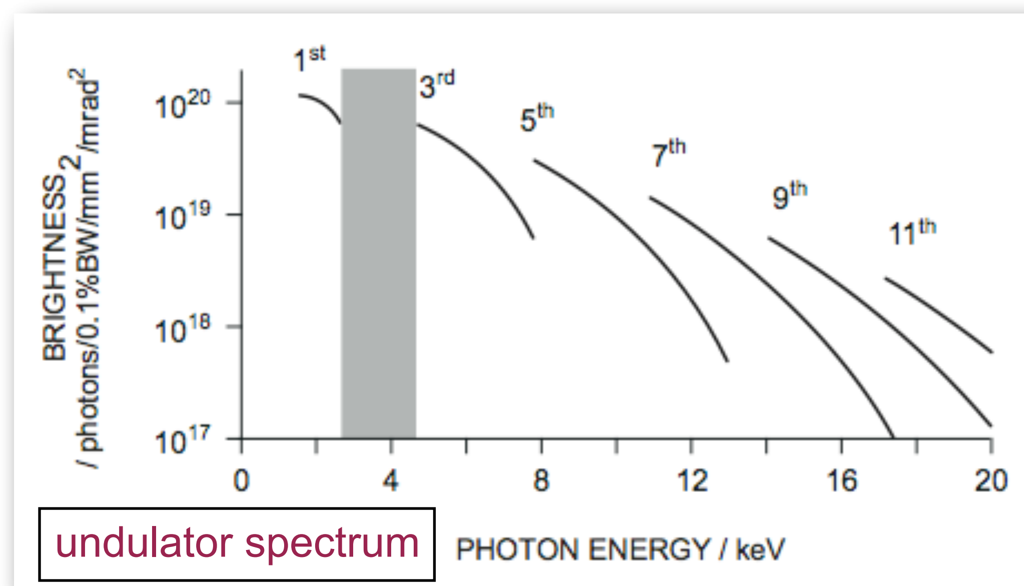
Bending magnet





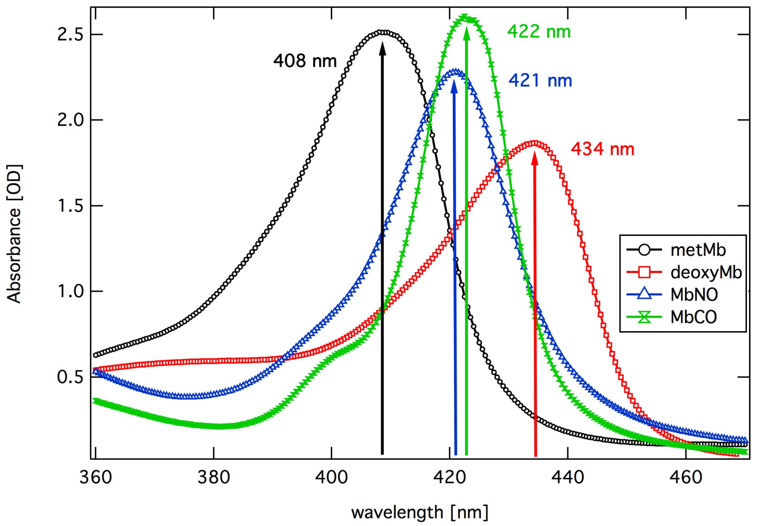
Wiggler/Undulator



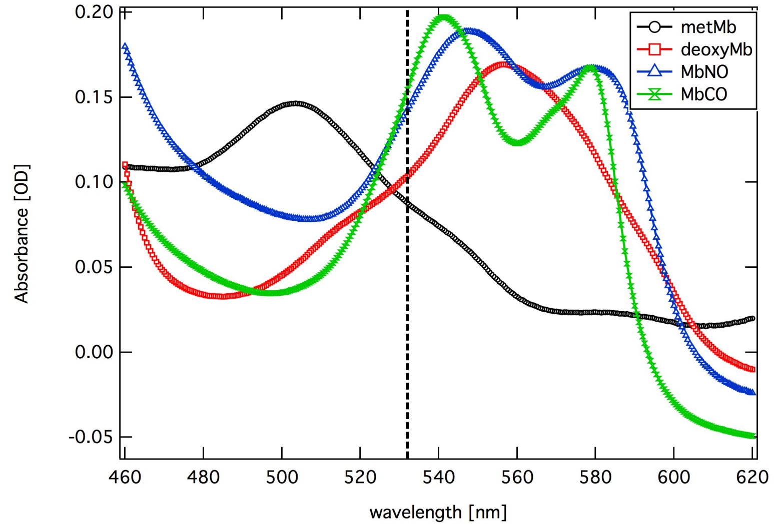


Myoglobin: Ground-state X-ray absorption spectroscopy

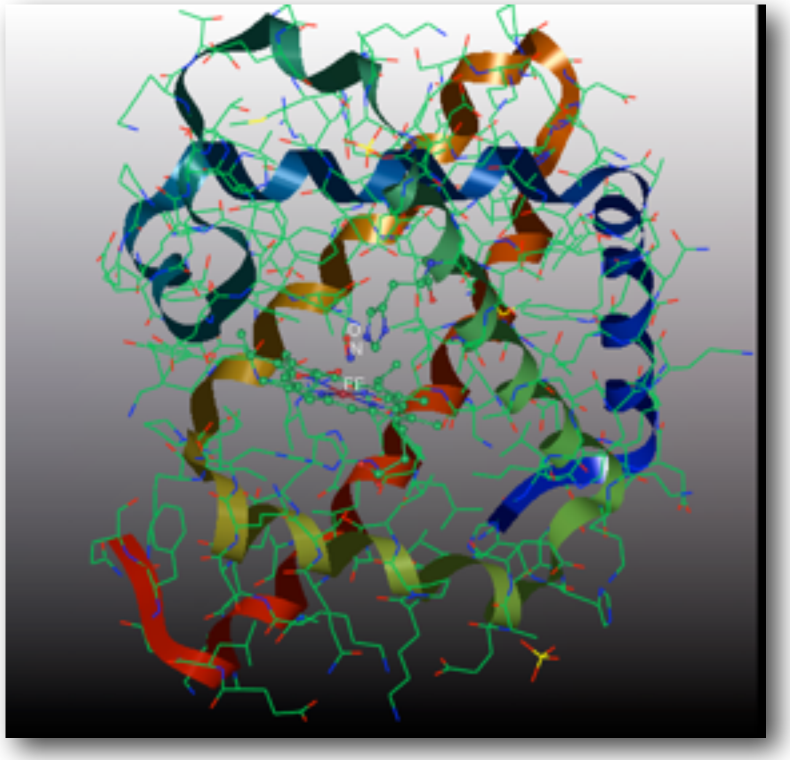
Myoglobin is an oxygen transport protein that has the ability to bind small molecules such as O_2, CO, NO and CN

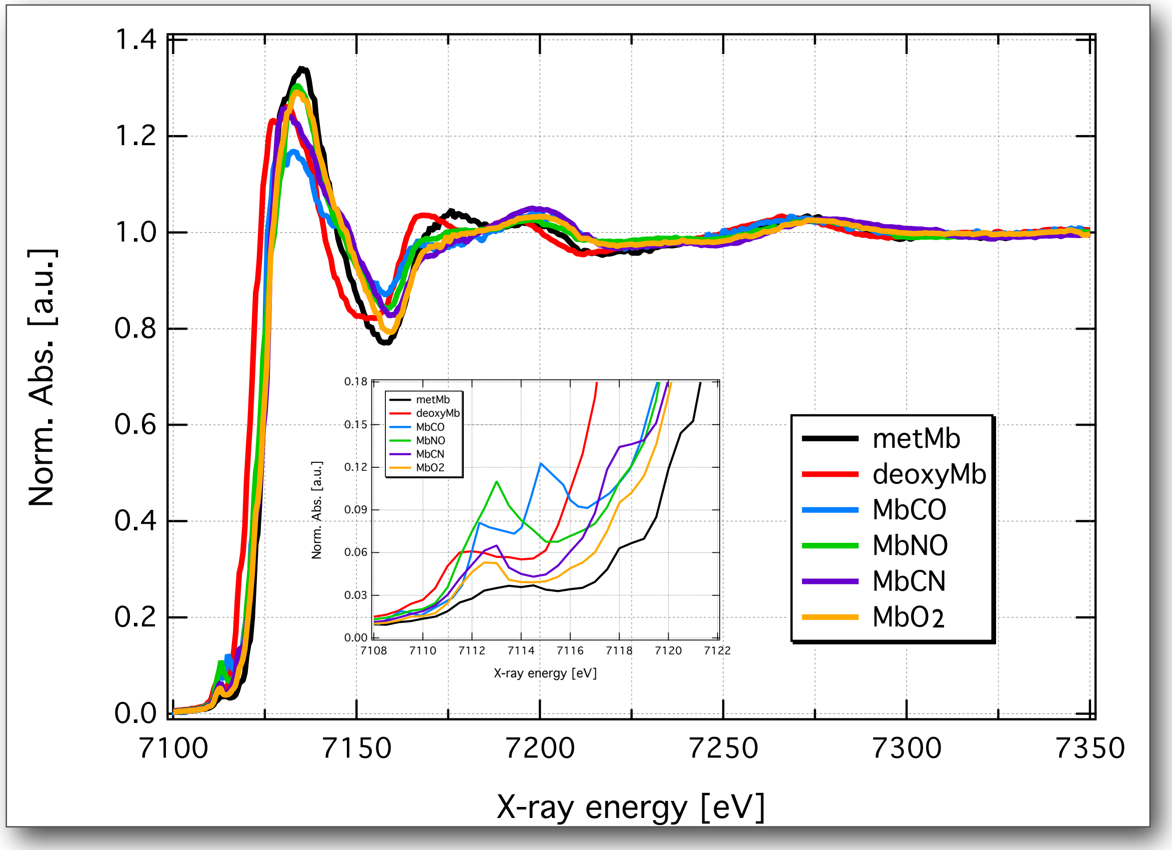


(a) Soret band region



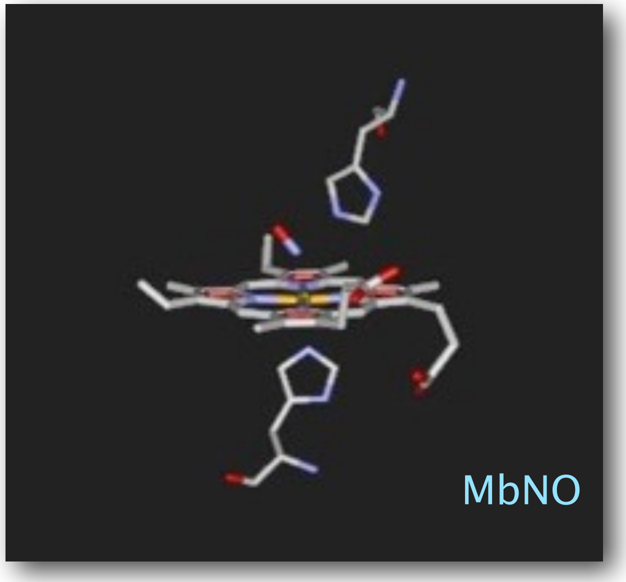
(b) Q-bands region



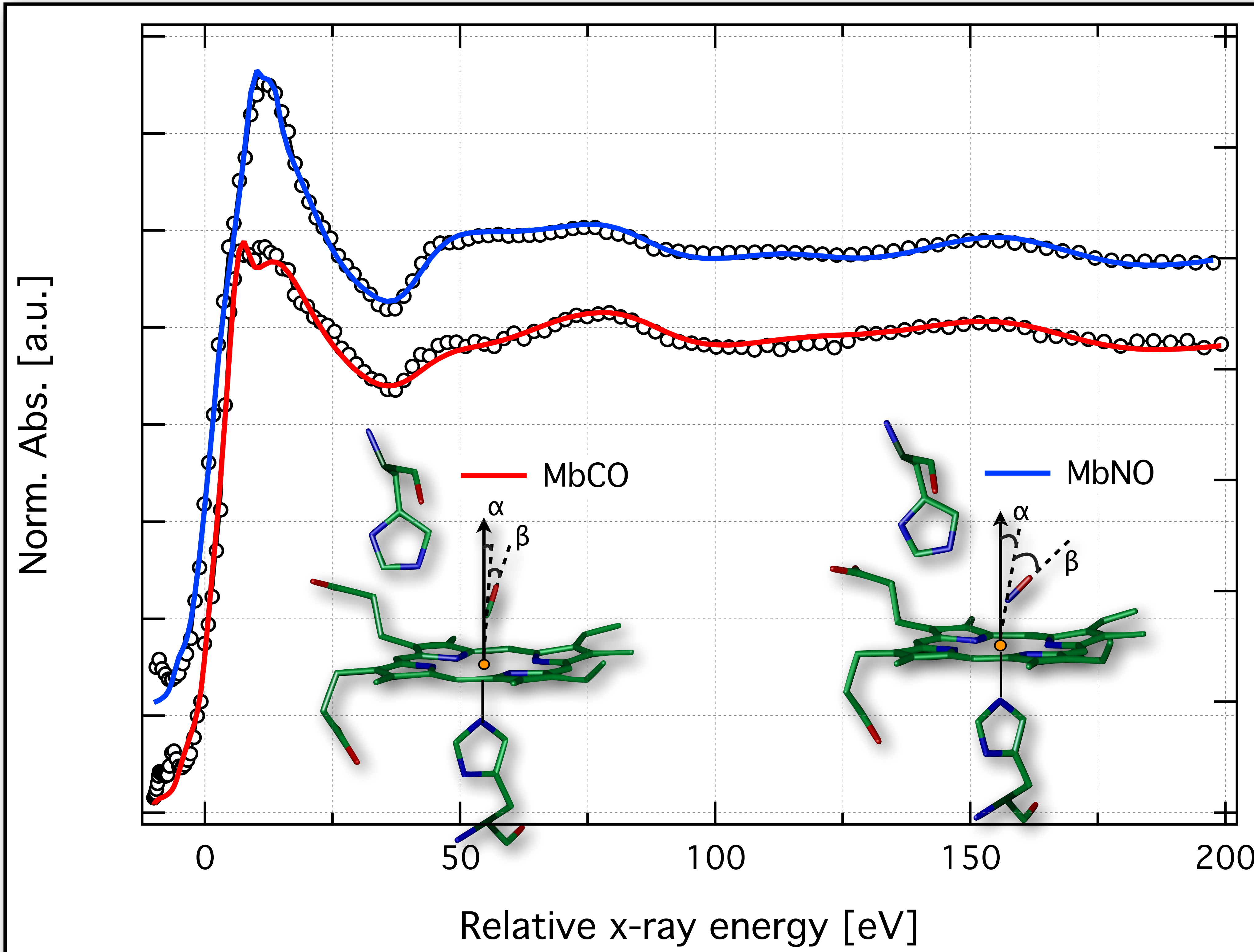


Small changes in the ligand character have profound spectroscopic effects

We can knock this ligand off with a photon of green or blue light

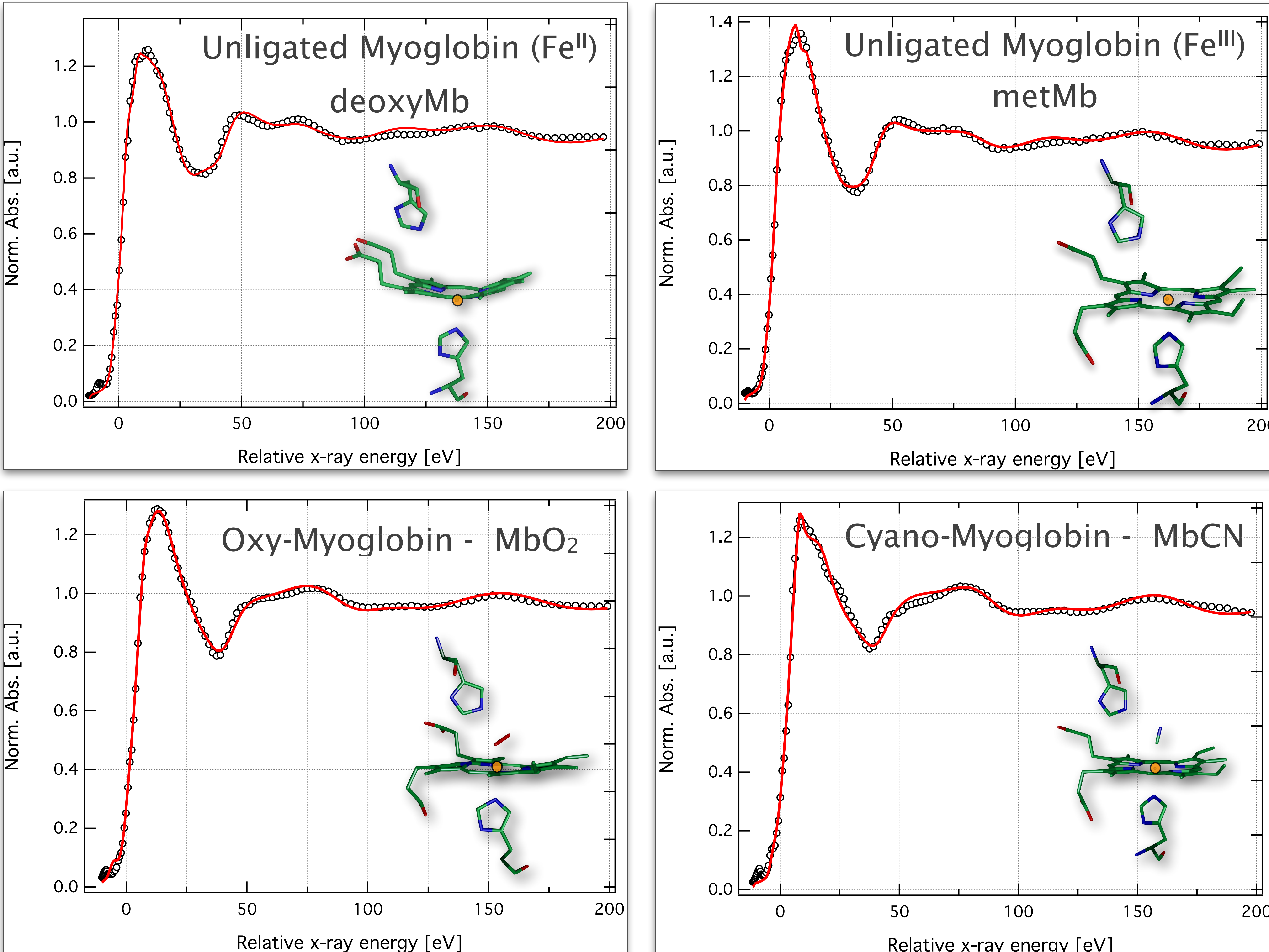


Myoglobin: Ground-state structures using MXAN



Resulting optimized MXAN structure gives good agreement with crystallography with better precision

F.A. Lima, T.J. Penfold et al., *PCCP* **16**, 1617 (2014)

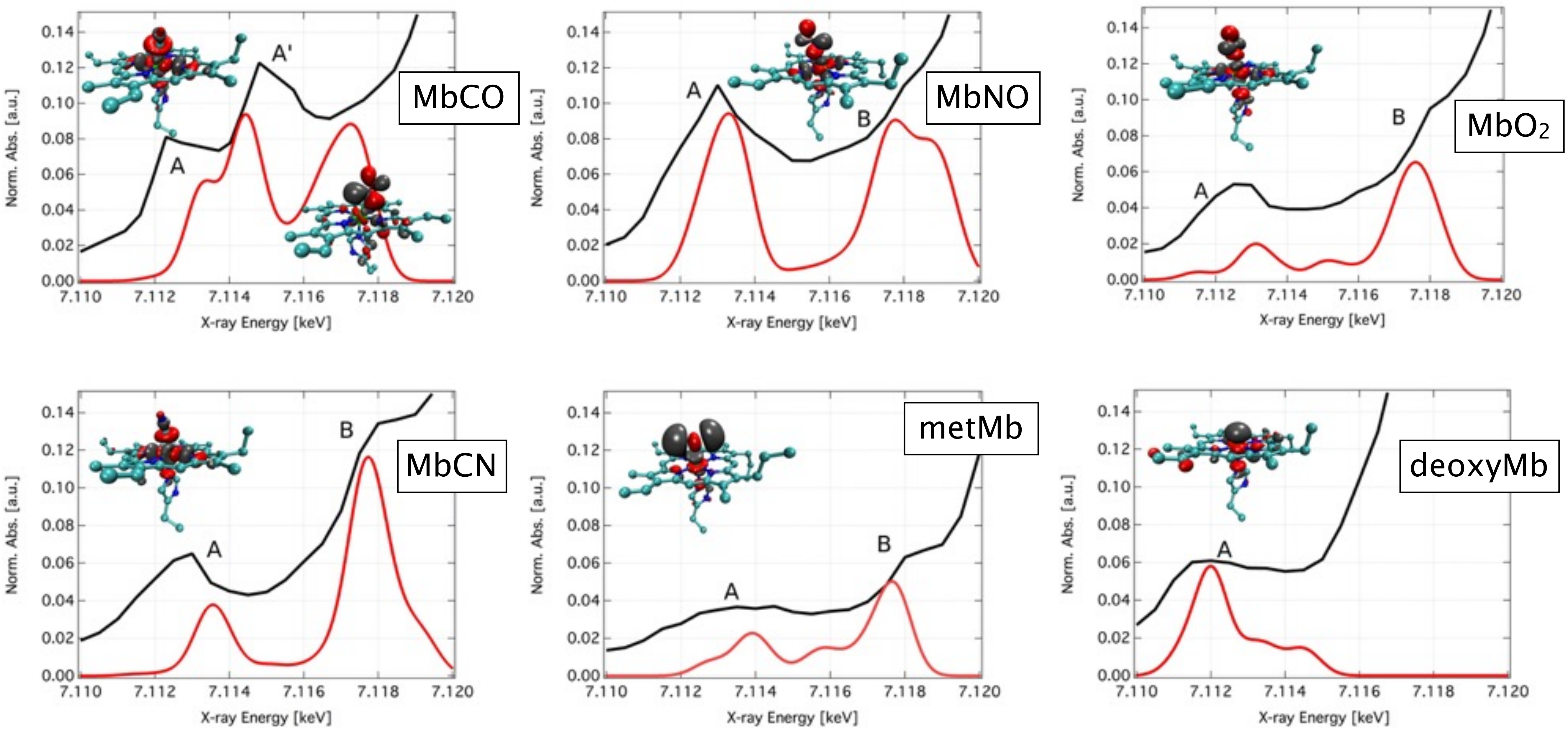


Unligated Myoglobin (Fe^{II})
deoxyMb
Unligated Myoglobin (Fe^{III})
metMb
Oxy-Myoglobin - MbO_2
Cyano-Myoglobin - MbCN
Norm. Abs. [a.u.]
Relative x-ray energy [eV]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
50
100
150
200

Myoglobin: Pre-edge transitions using TD-DFT

The pre-edge peaks are primarily 1s to 3d transitions with core-to-ligand contributions only to the CO/NO/CN/O_2

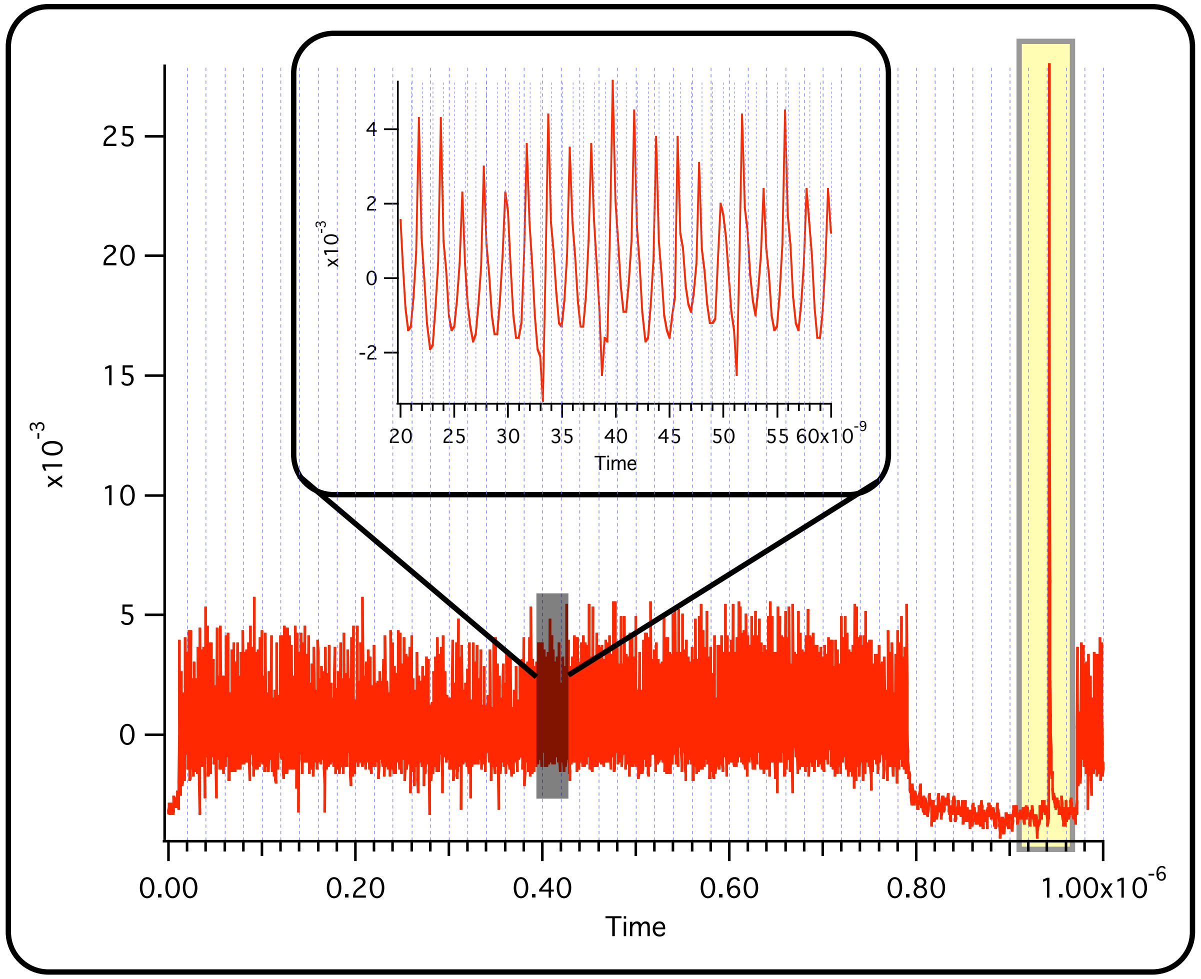
ORCA: F. Neese, WIREs Comput Mol Sci 2012, 2: 73-78



F.A. Lima, T.J. Penfold et al., *PCCP* **16**, 1617 (2014)

How do we apply this to time-resolved experiments ?

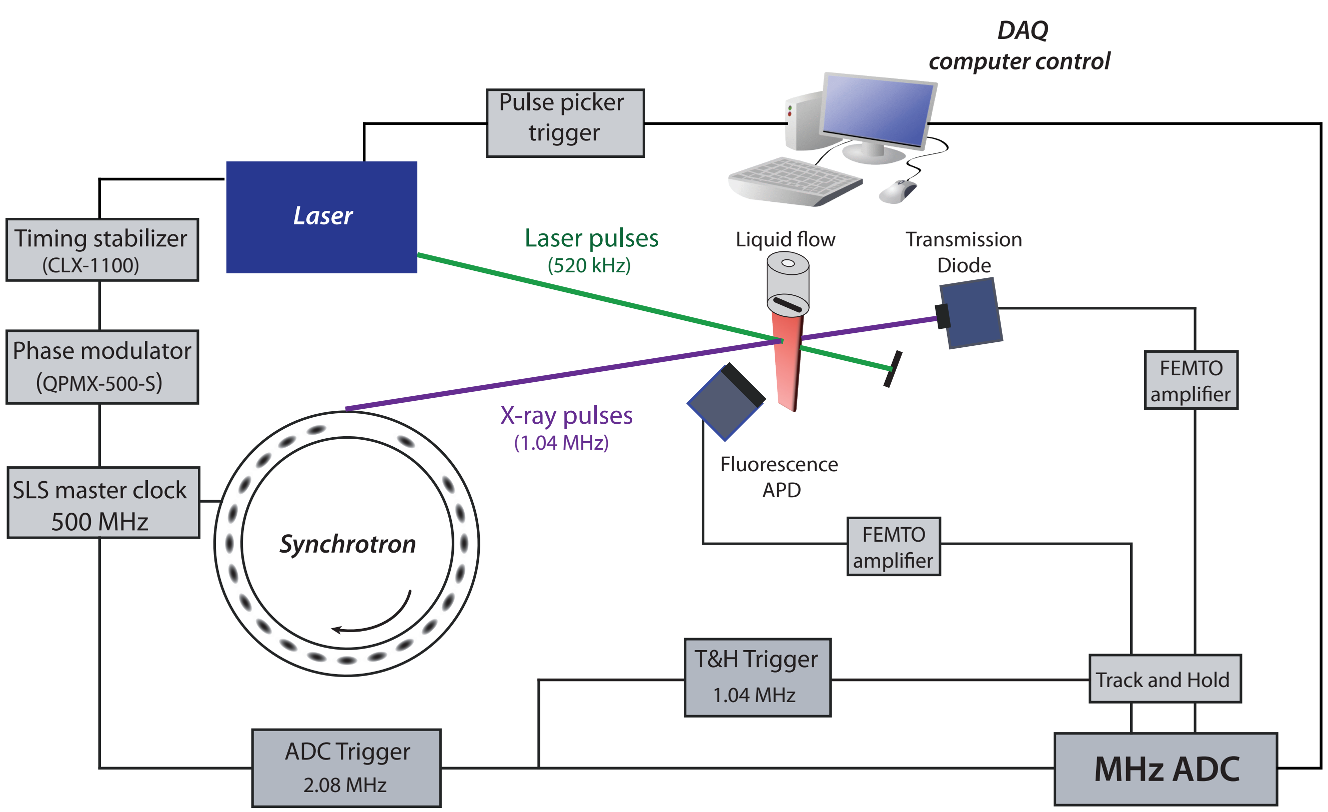
High-repetition rate pump-probe X-ray experiments



microXAS beamline
- tuneable hard x-ray in-vacuum undulator (4-20 keV)
- Si (111), Ge(111) & Si(311) monochromator crystals
- micro-focus capability (< 1μm^2)
- 10^{12} photons/second

PHOENIX beamline
- tuneable 'tender' x-ray in-vacuum undulator (0.8-8 keV)
- Si (111), KTP, Be, InSb monochromator crystals
- micro-focus capability (< 1μm^2)
- 10^{11}-10^{12} photons/second

Using fast avalanche photodiodes and either boxcar integrators or track-and-hold circuits we can selectively measure using only the camshaft pulse giving us **100 ps** time resolution



F.A. Lima, C.J. Milne et al. *Rev. Sci. Instr.* **82**, 063111 (2011)

Myoglobin: Unresolved dynamics & structural issues

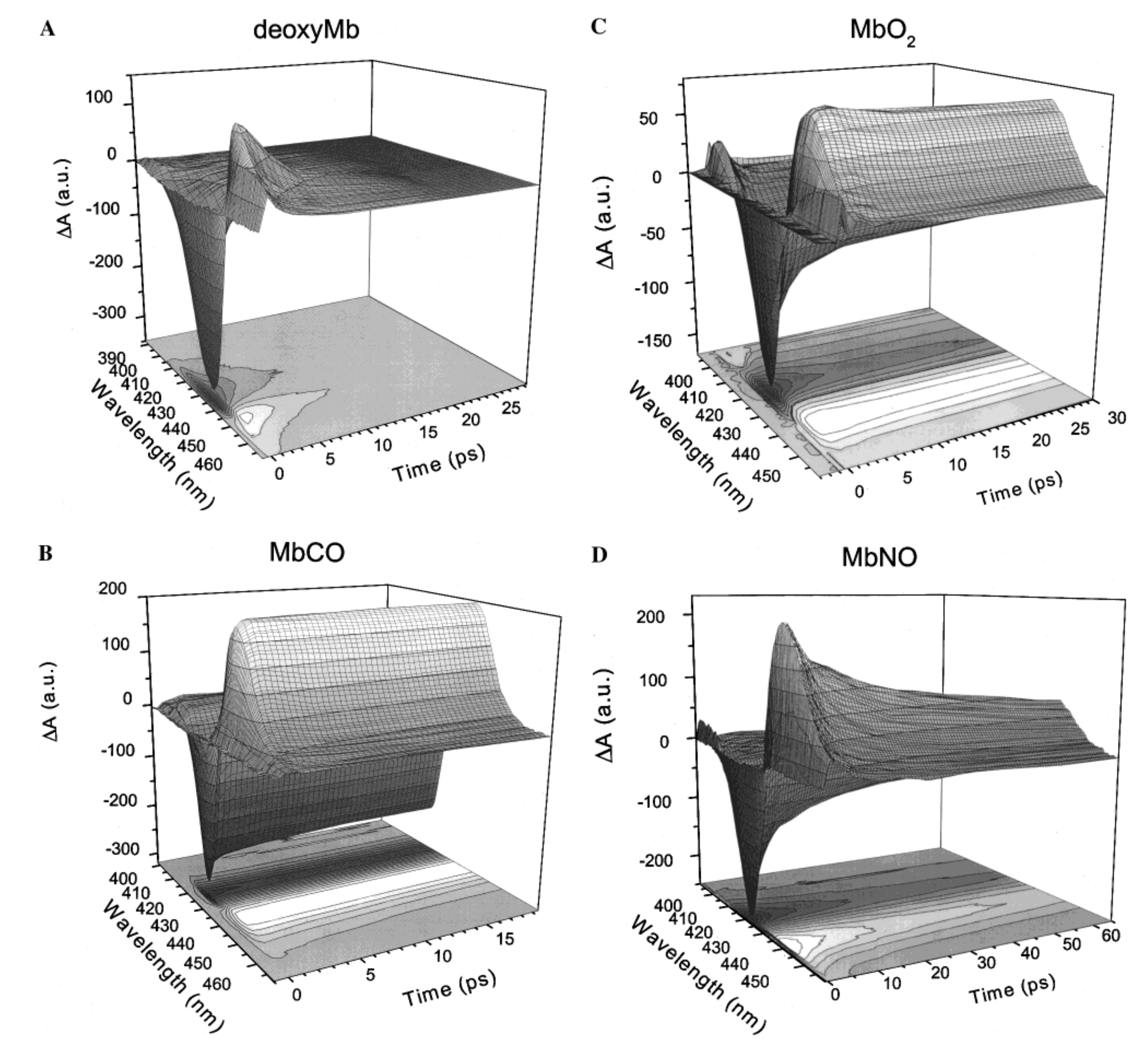


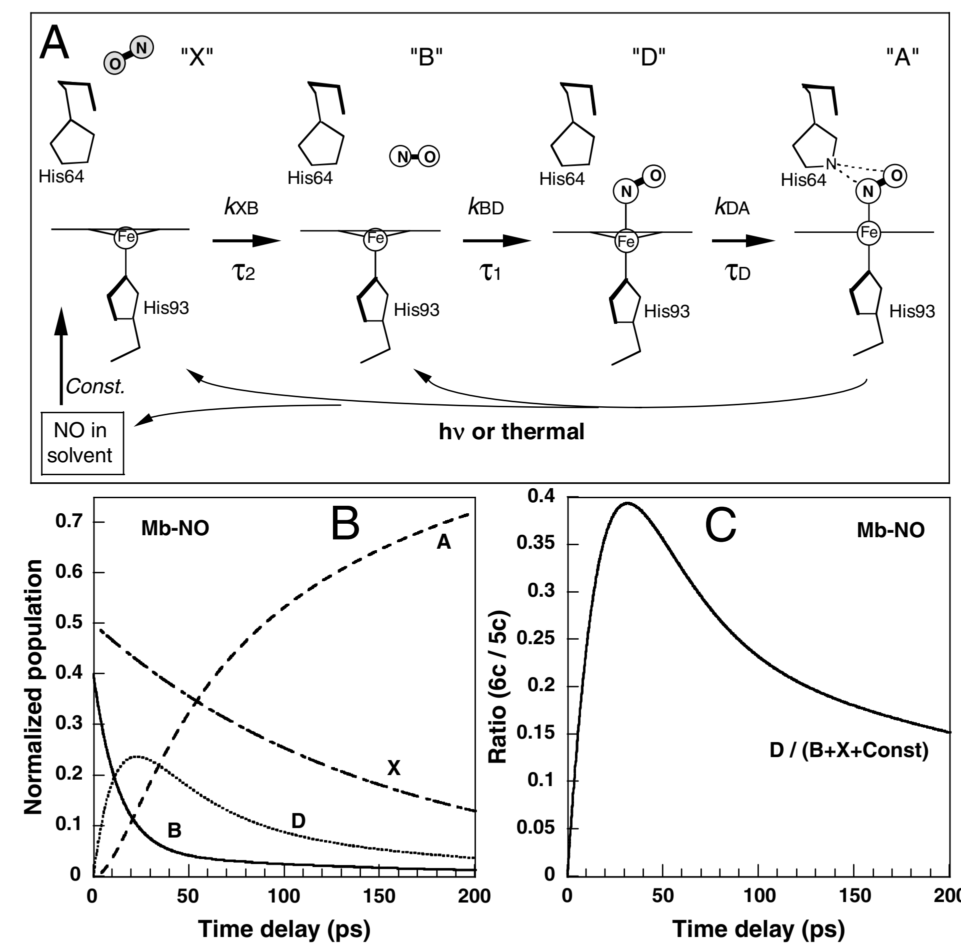
Figure 1. (A−D) Transient spectra of various myoglobin samples pumped at 580 nm. In this and the following figures, au stands for arbitrary units. In this figure, the ordinate values are proportional to the lock-in amplifier read-out in microvolts, while in the following figures, the ordinate has been renormalized to accommodate the fitting procedure.

	MbO_2	MbCO	MbNO
Quantum yield	0.28	1	0.5
Hot 6-coordinate relaxation	1 ps	-	1 ps
Geminate recombination	4–5 ps	-	13 & 200 ps
Geminate probability	0.3	-	0.5 & 0.5
Binary recombination	>10 μs	>10 μs	-
Binary probability	0.7	1	-

X. Ye et al. *JACS* **124**, 5914 (2002); E. Scott et al. *J. Biol. Chem.* **276**, 5177 (2001)

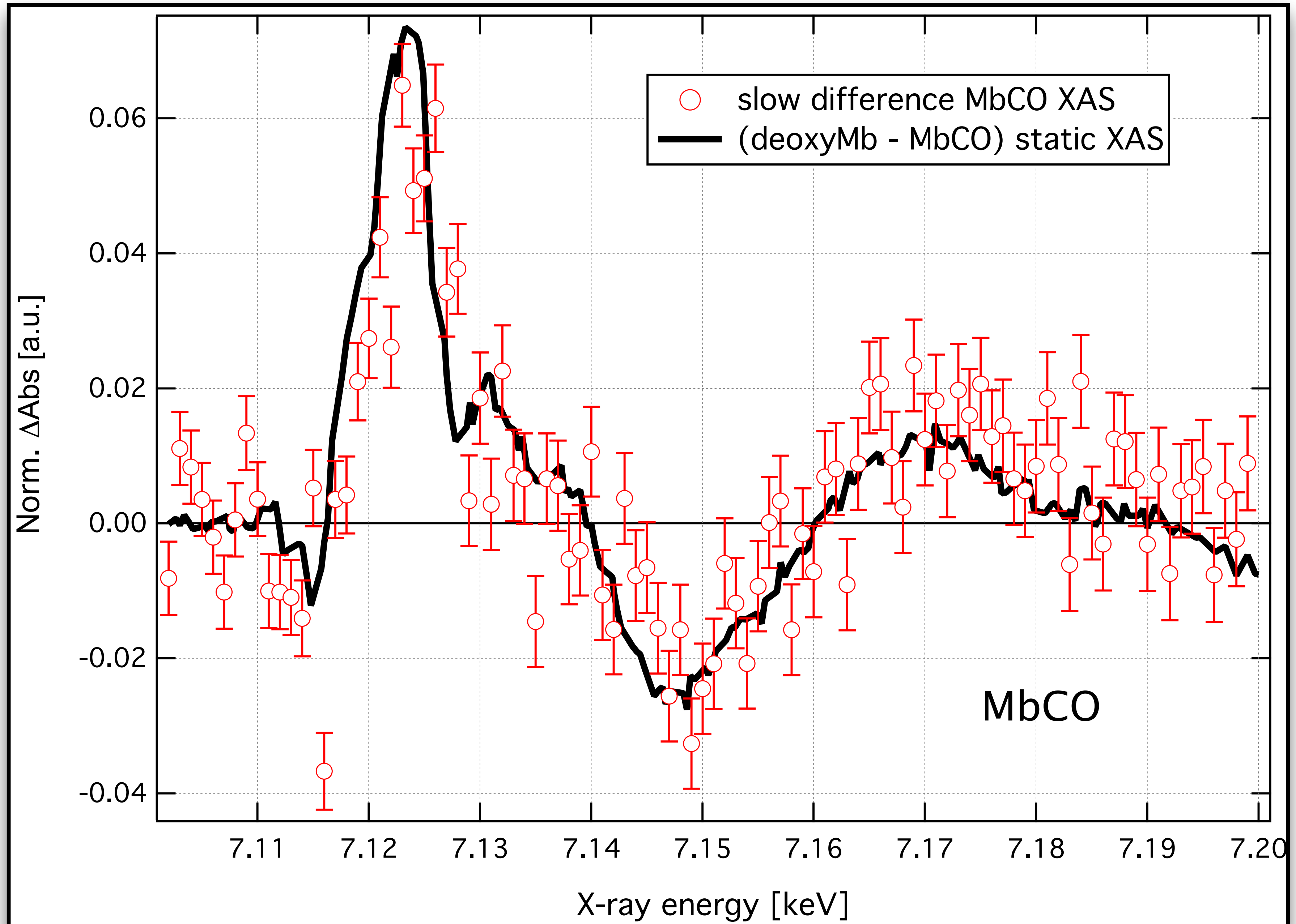
Of the ligands, MbNO is the most interesting but still poorly understood

- NO rebinds very quickly
- Geminate recombination occurs on two timescales (13 & 200 ps)
- There's an indication of a 6-coordinate domed structure
- MbNO & MbO_2 have similar binding geometries but very different affinities
- The geminate recombination has an excitation wavelength-dependence



S. Kruglik et al. *PNAS* **107**, 13678 (2010)

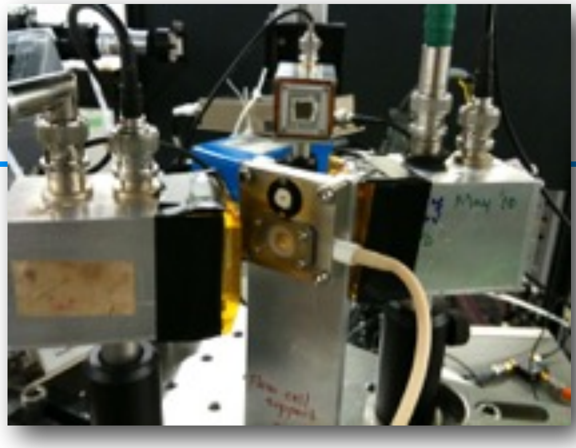
Myoglobin: MbCO pump-probe XAS



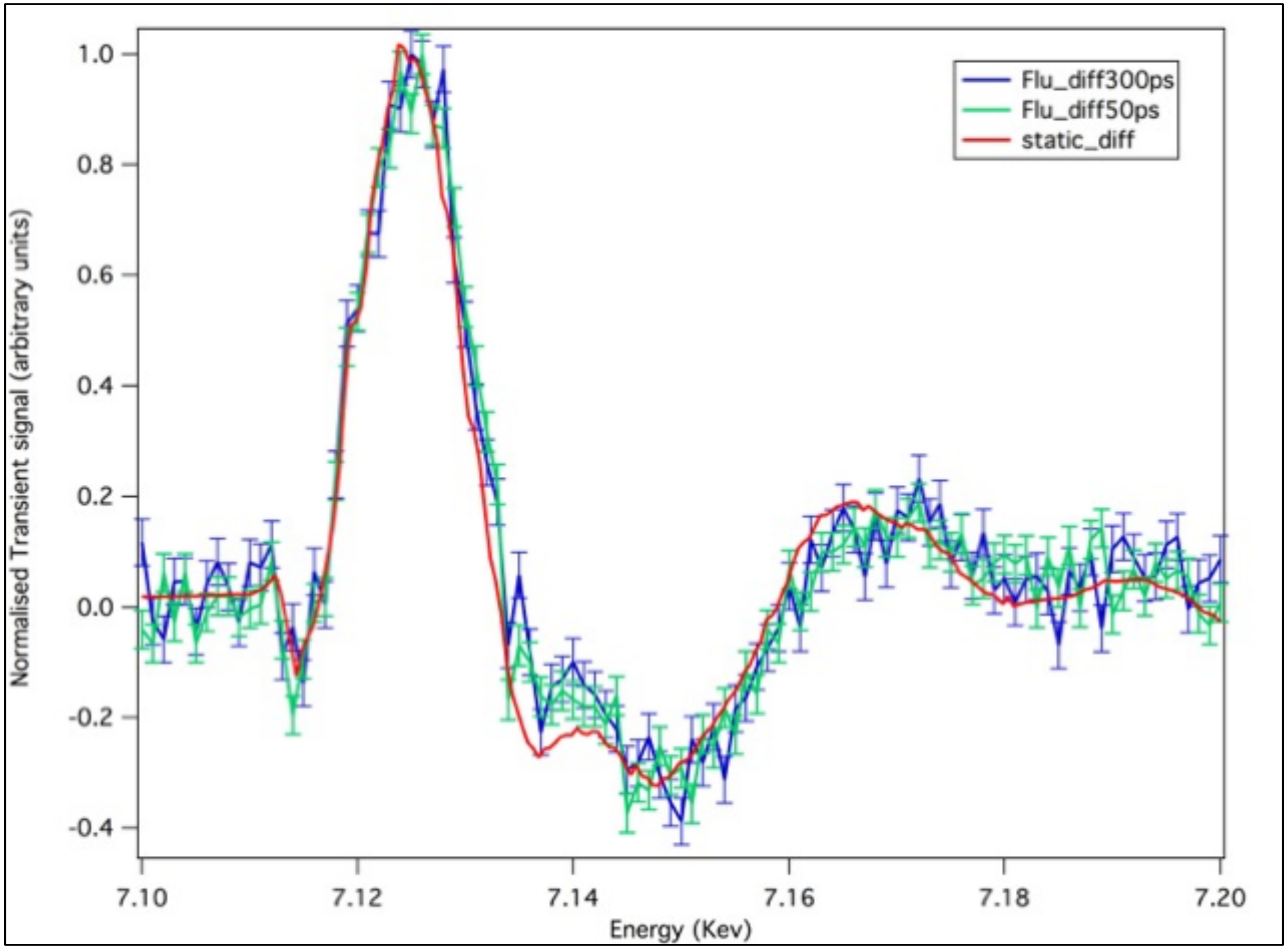
As expected quasi-continuous excitation of MbCO shows formation of deoxyMb

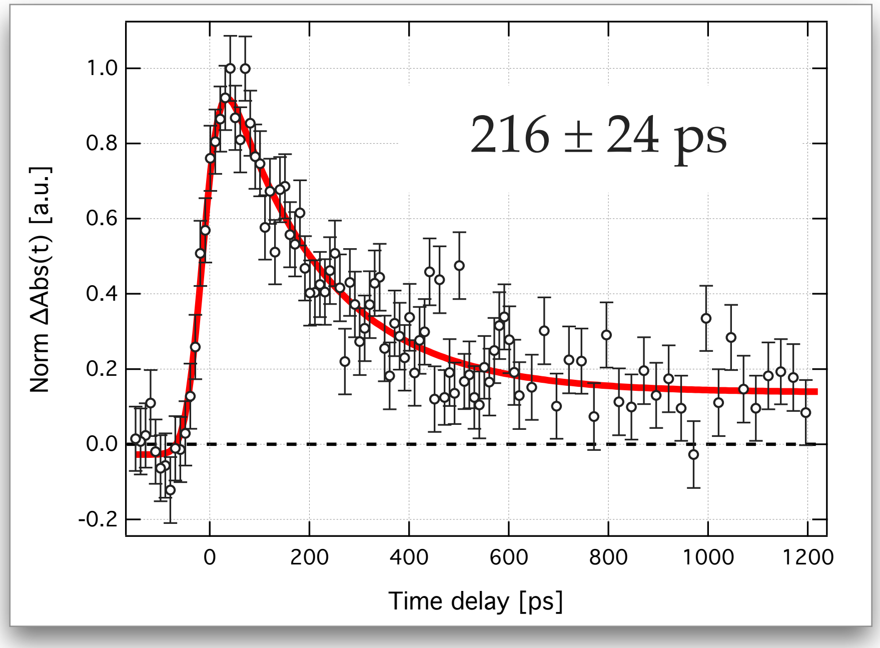
F.A. Lima, C.J. Milne et al. *Rev. Sci. Instr.* **82**, 063111 (2011)

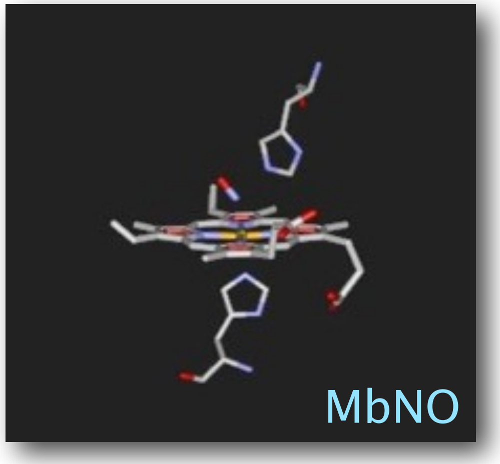
Myoglobin: MbNO pump-probe XAS



4 mM MbNO excited at 532 nm and probed at the Fe K-edge



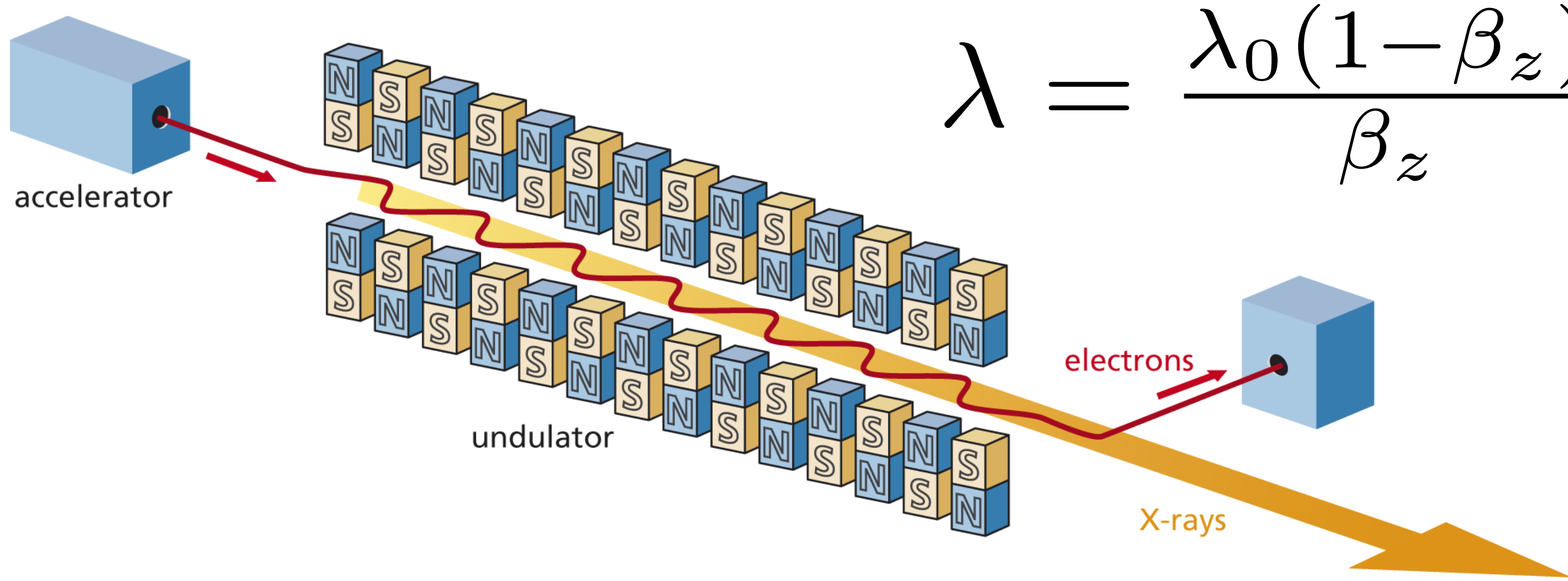




Can we extract further information ?

Yes, but we need better time resolution

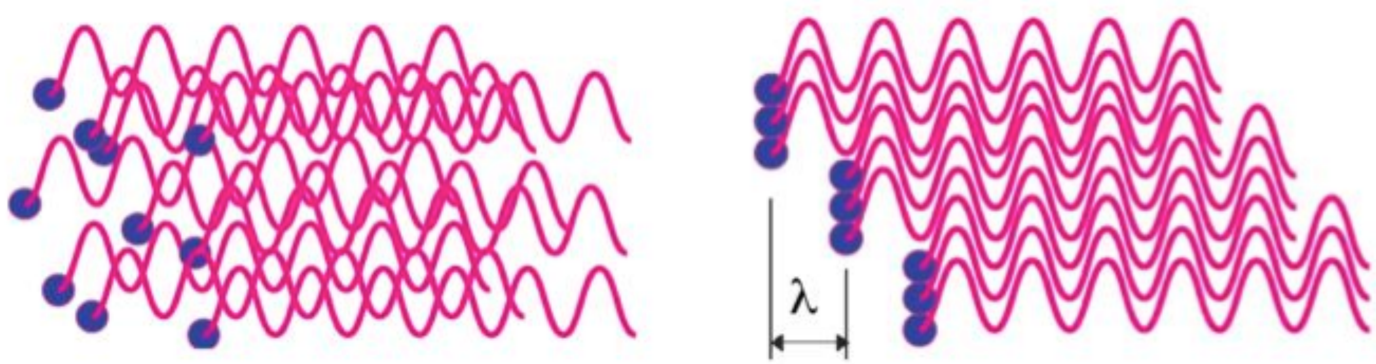
How are we going to get better time-resolution ?



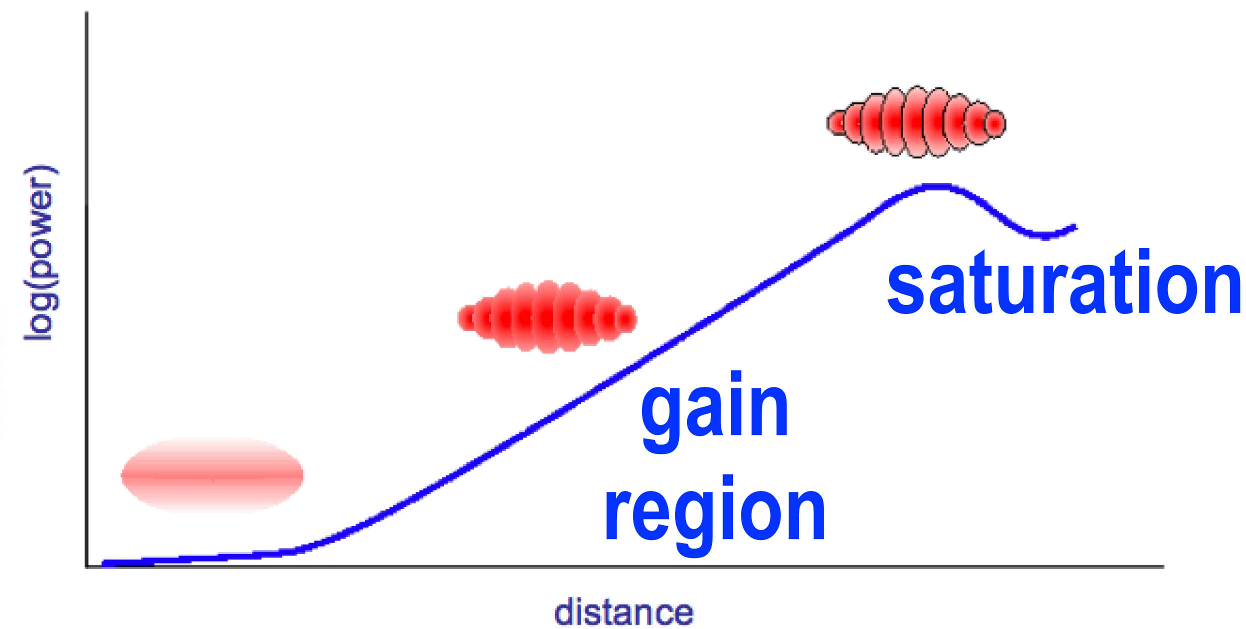
$$\lambda = \frac{\lambda_0(1-\beta_z)}{\beta_z}$$

If the electrons and light are resonant they can exchange energy as they propagate through the undulators

$I \propto N_e$ $\qquad$ $I \propto N_e^2$



Which leads to microbunching and the electrons emit light in phase



which leads to exponential gain than saturation

So we have a single-pass, noise-seeded free electron laser

C. Pellegrini and S. Reiche, in *Digital Encyclopedia of Applied Physics*, Wiley (2003).

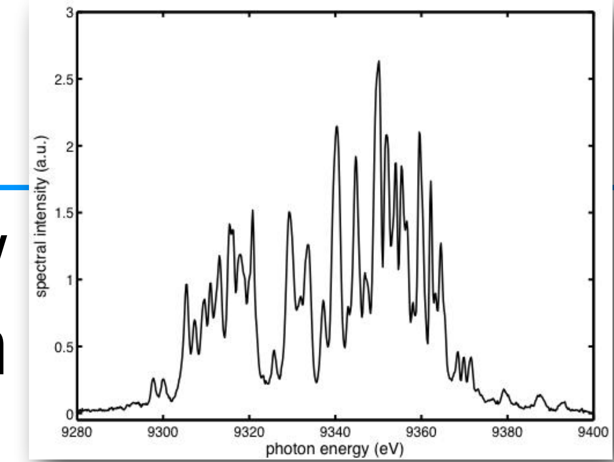
Moving FELs into the X-ray regime

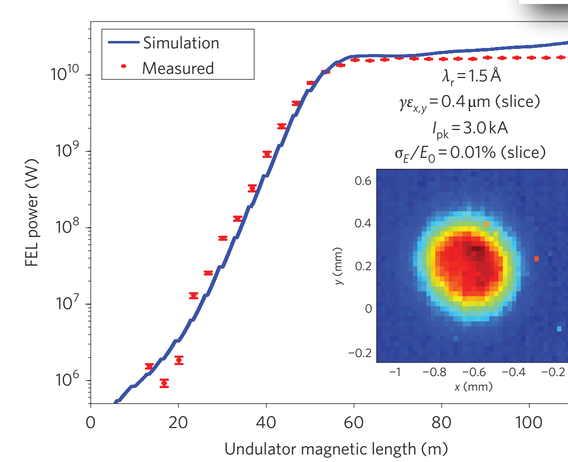
Table 1 | Design and typical measured parameters for both hard (8.3 keV) and soft (0.8–2.0 keV) X-rays. The 'design' and 'hard' values are shown only at 8.3 keV. Stability levels are measured over a few minutes.

Parameter	Design	Hard	Soft	Unit
Electrons				
Charge per bunch	1	0.25	0.25	nC
Single bunch repetition rate	120	30	30	Hz
Final linac e^- energy	13.6	13.6	3.5–6.7	GeV
Slice† emittance (injected)	1.2	0.4	0.4	µm
Final projected† emittance	1.5	0.5–1.2	0.5–1.6	µm
Final peak current	3.4	2.5–3.5	0.5–3.5	kA
Timing stability (r.m.s.)	120	50	50	fs
Peak current stability (r.m.s.)	12	8–12	5–10	%
X-rays				
FEL gain length	4.4	3.5	~1.5	m
Radiation wavelength	1.5	1.5	6–22	Å
Photons per pulse	2.0	1.0–2.3	10–20	10^{12}
Energy in X-ray pulse	1.5	1.5–3.0	1–2.5	mJ
Peak X-ray power	10	15–40	3–35	GW
Pulse length (FWHM)	200	70–100	70–500	fs
Bandwidth (FWHM)	0.1	0.2–0.5	0.2–1.0	%
Peak brightness (estimated)	8	20	0.3	10^{32} *
Wavelength stability (r.m.s.)	0.2	0.1	0.2	%
Power stability (r.m.s.)	20	5–12	3–10	%

*Brightness is photons per phase space volume, or photons s^{-1} mm^{-2} $mrad^{-2}$ per 0.1% spectral bandwidth.
†'Slice' refers to femtosecond-scale time slices and 'projected' to the full time-projected (that is, integrated) emittance of the bunch.

spiky X-ray spectrum





shows power saturation profile

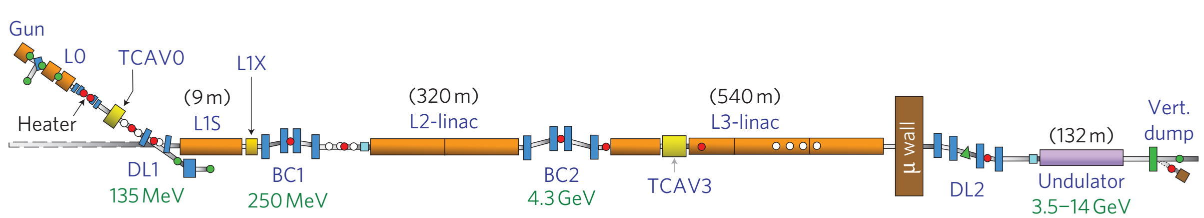
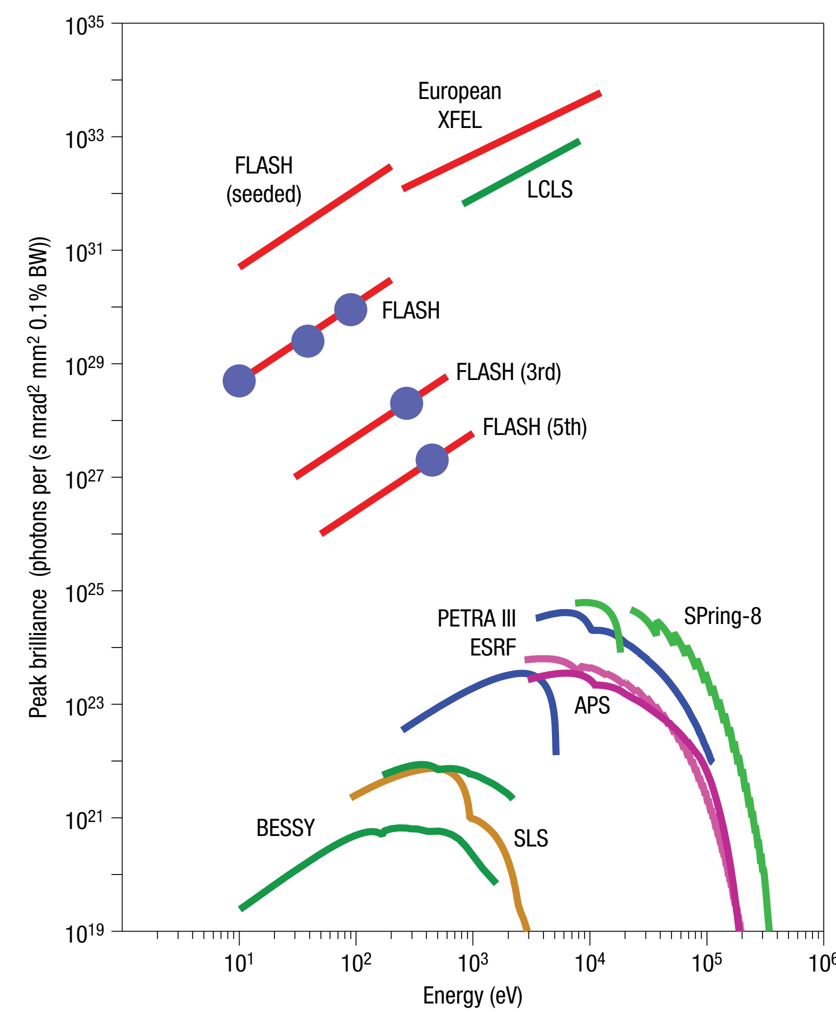
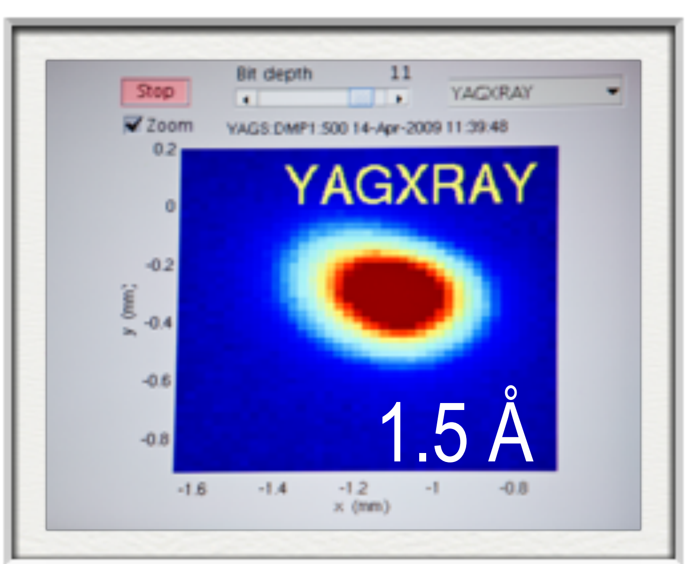


Figure 1 | LCLS machine layout. Layout from the electron gun to the main dump, with two bunch compressors, BC1 and BC2, and a 132-m-long undulator.

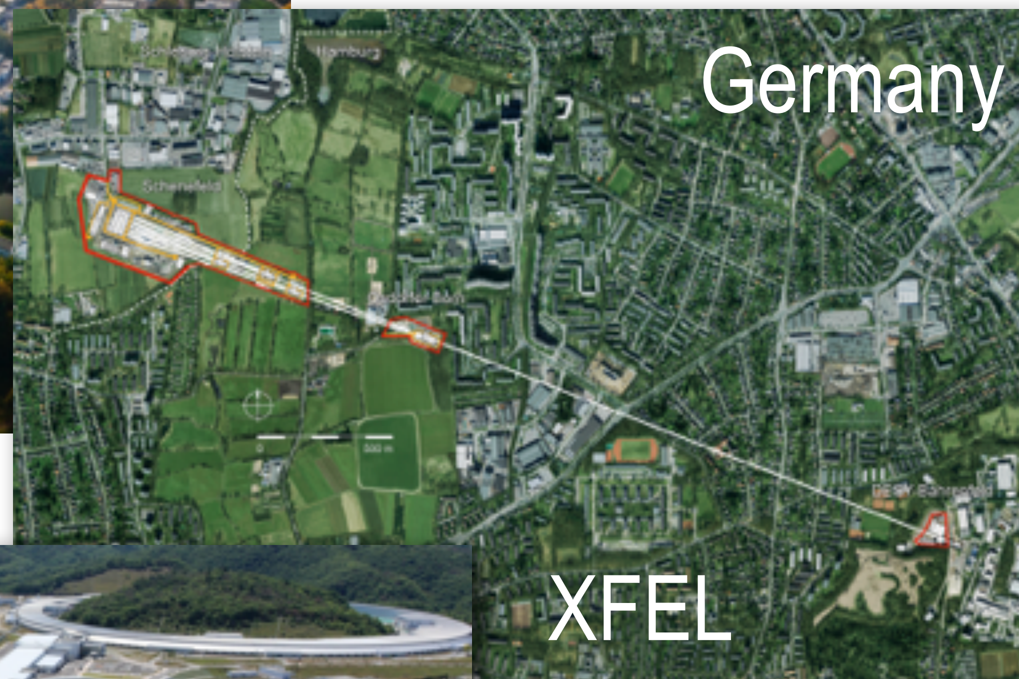


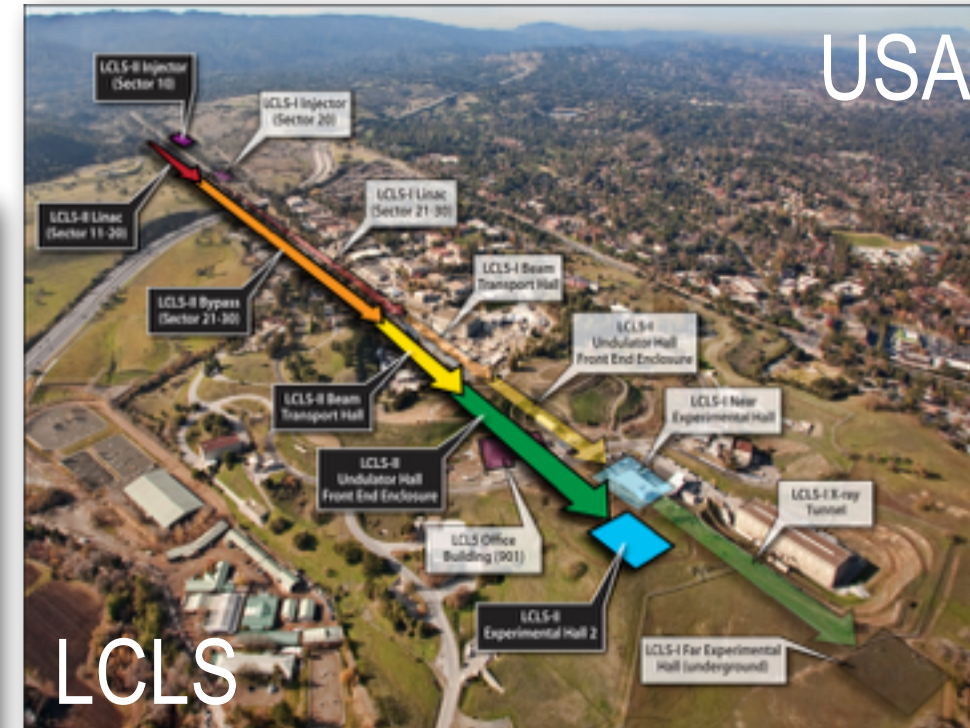


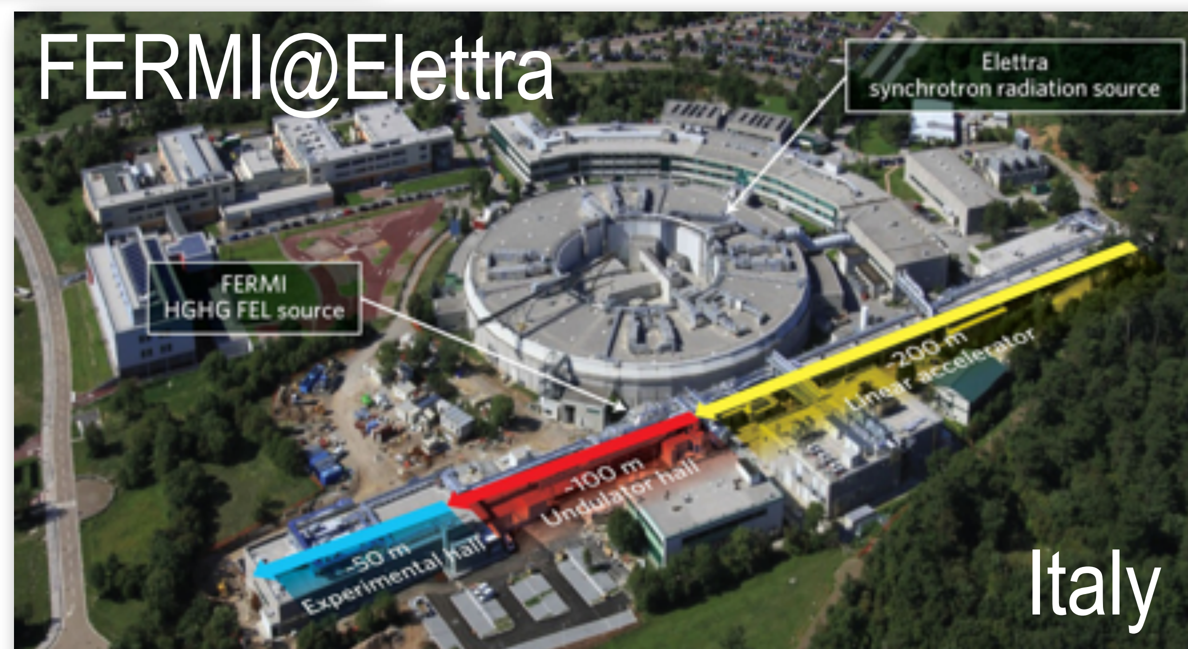
2009: LCLS first achieved lasing at hard X-ray wavelengths

P. Emma et al., *Nat. Phot.* **4**, 641 (2010)

Now there are XFEL projects everywhere…







What is an XFEL going to be good at ?

What types of experiments are XFELs good at ?

XFELS are defined by lots of photons in a very short pulse but the average flux isn't that different from a 3rd-generation synchrotron

Only ***three****** types of experiments benefit from the high peak flux from an XFEL:

1. **Single-shot experiments** that need lots of photons in a short pulse
2. **Pump-probe measurements** where the short pulse allows measurement of fast dynamics
3. **Nonlinear X-ray experiments** that depend nonlinearly on the number of incident X-ray photons

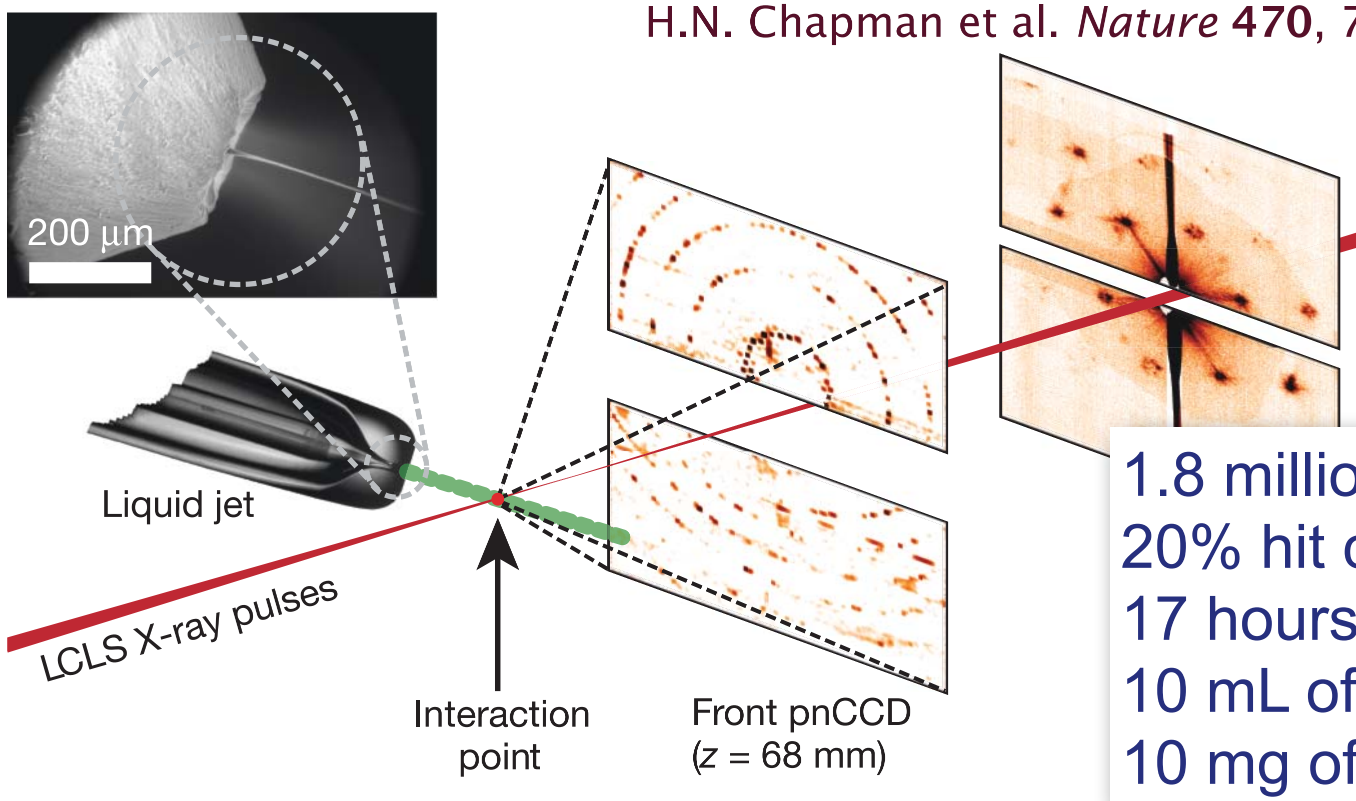
Not all experiments are going to automatically be better at an XFEL

*I'm ignoring the transverse coherence properties

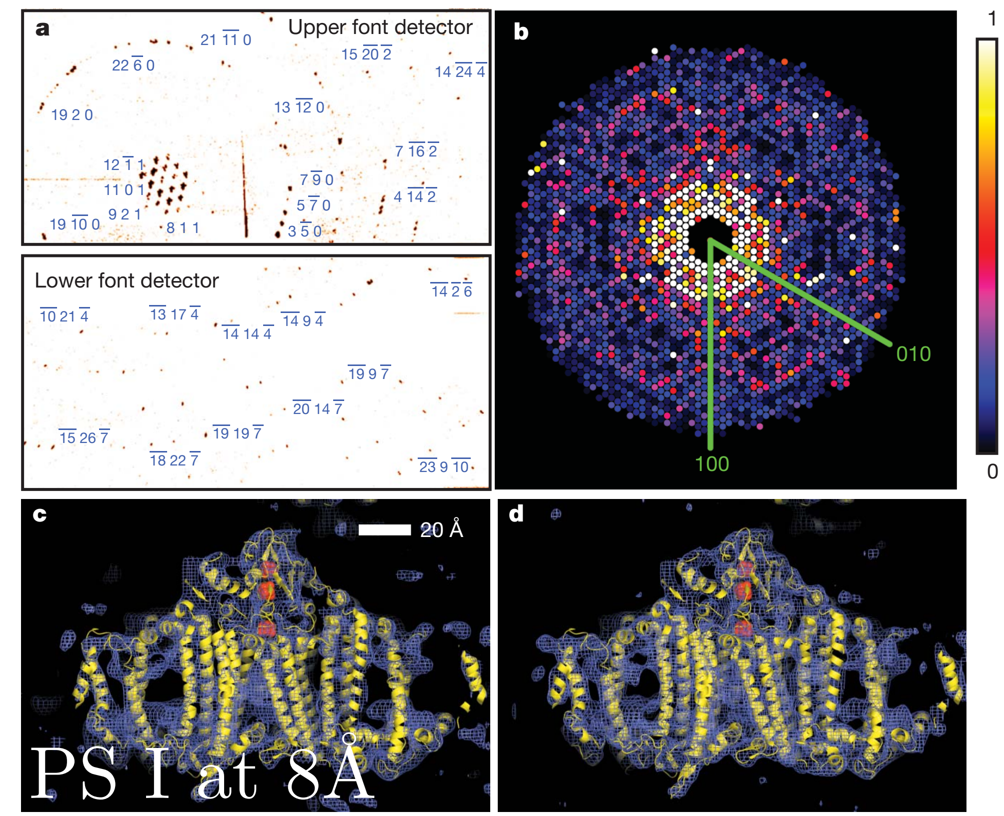
How is this relevant to biology ?

Serial Femtosecond Crystallography (SFX)

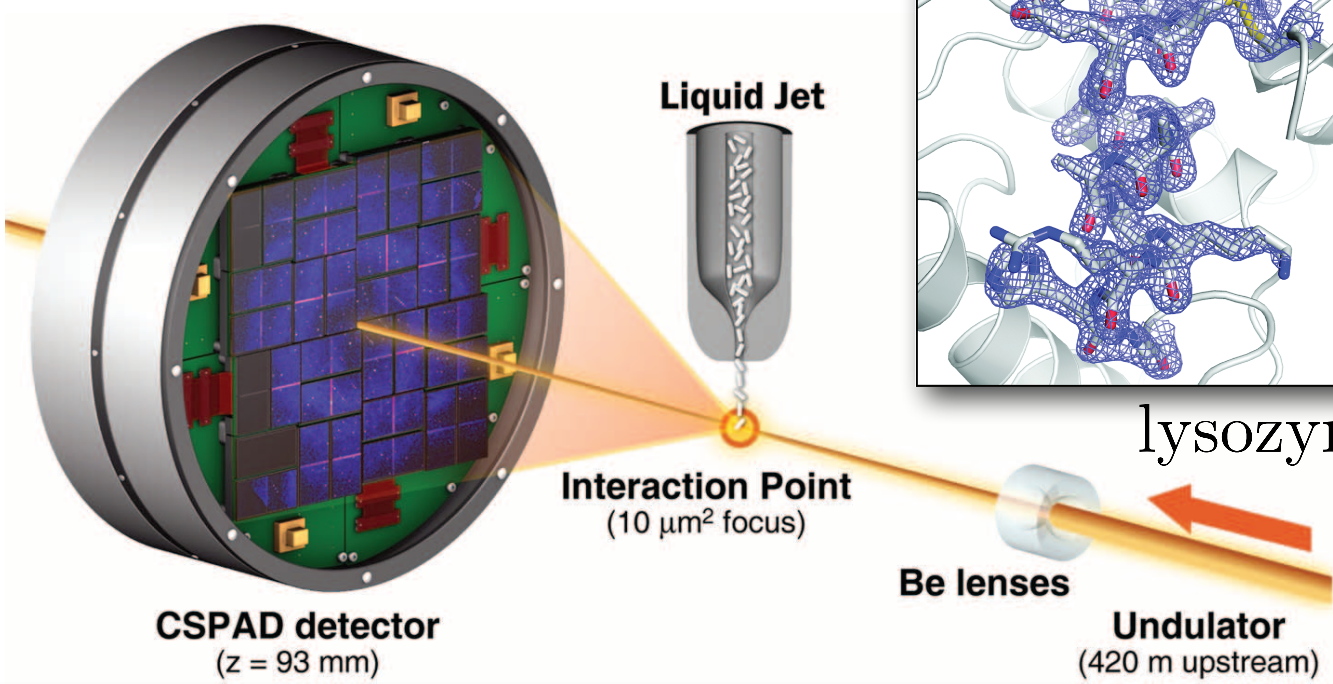
H.N. Chapman et al. *Nature* **470**, 73 (2011)

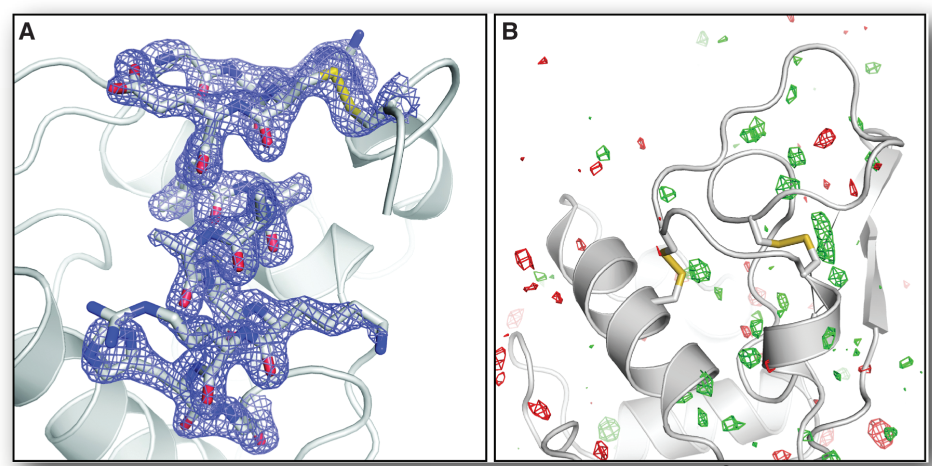


1.8 million shots
20% hit crystals
17 hours
10 mL of sample
10 mg of protein



S. Boutet et al. *Science* **337**, 362 (2012)





lysozyme at 1.9Å

1.5 million shots
4% hit crystals
0.007% were useable
3-4 hours

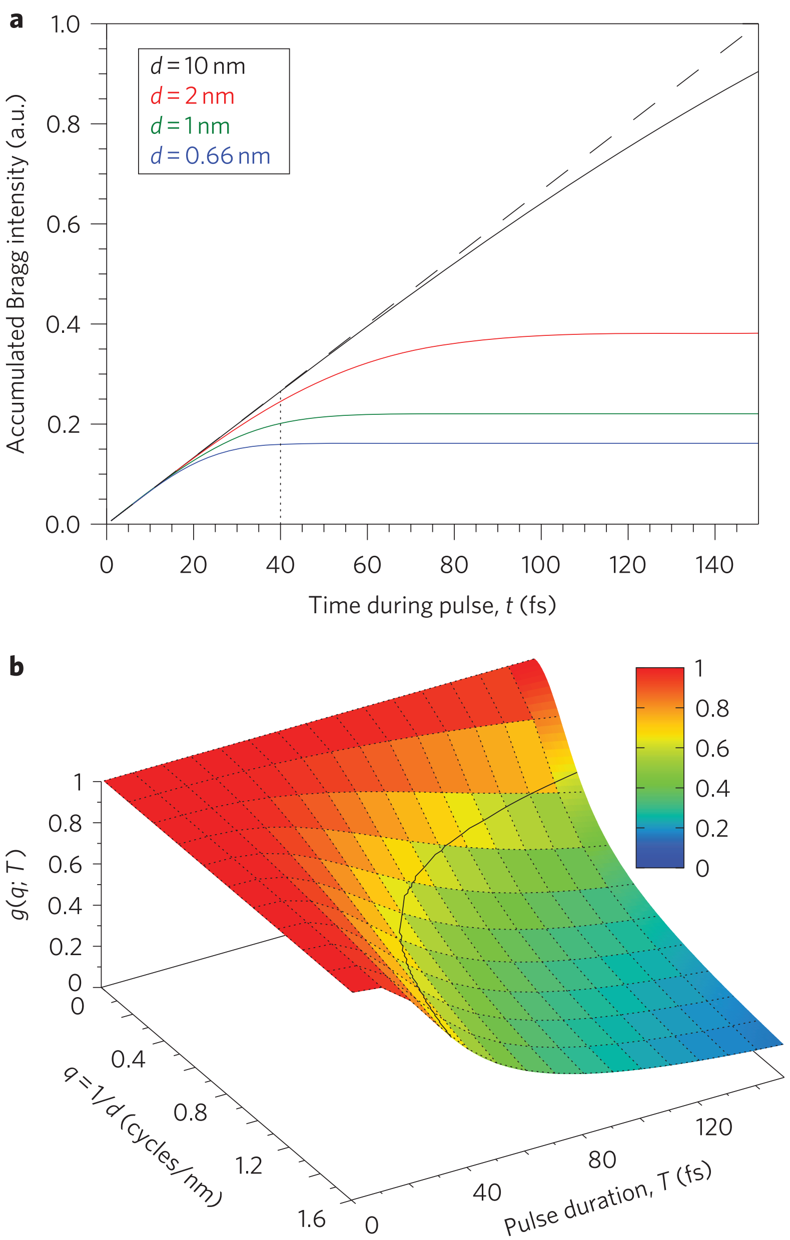
J.C.H. Spence et al. *Rep. Prog. Phys.* **75**, 102601 (2012)

X-ray diffraction gives information on the positions of all the atoms in the crystal

SFX Highlight: High-resolution damage-free measurements

S. Boutet et al. *Science* **337**, 362 (2012)

lysozyme at 1.9Å
measured with liquid jet



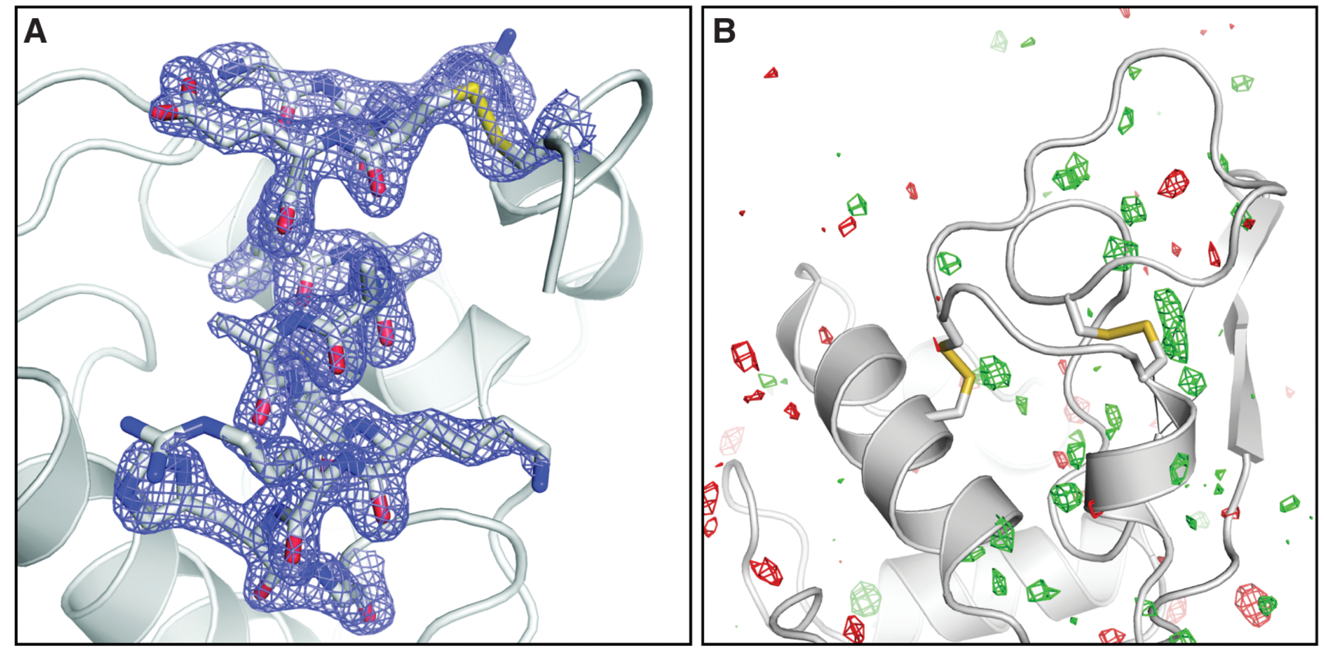
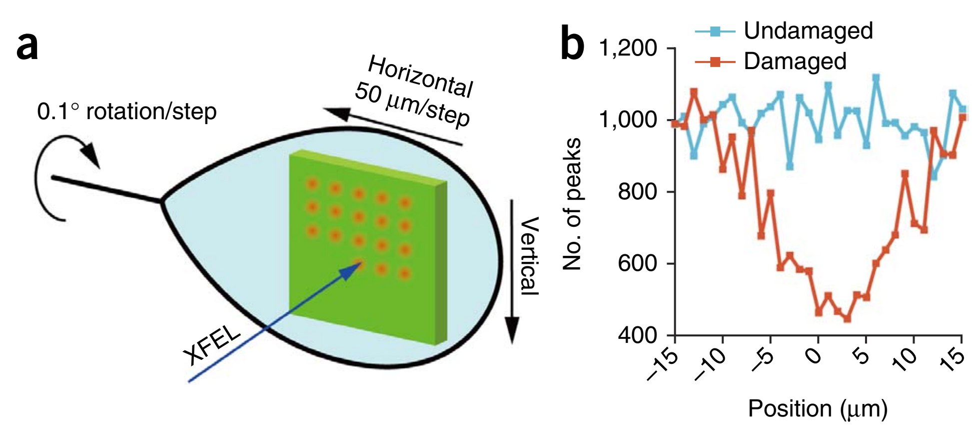
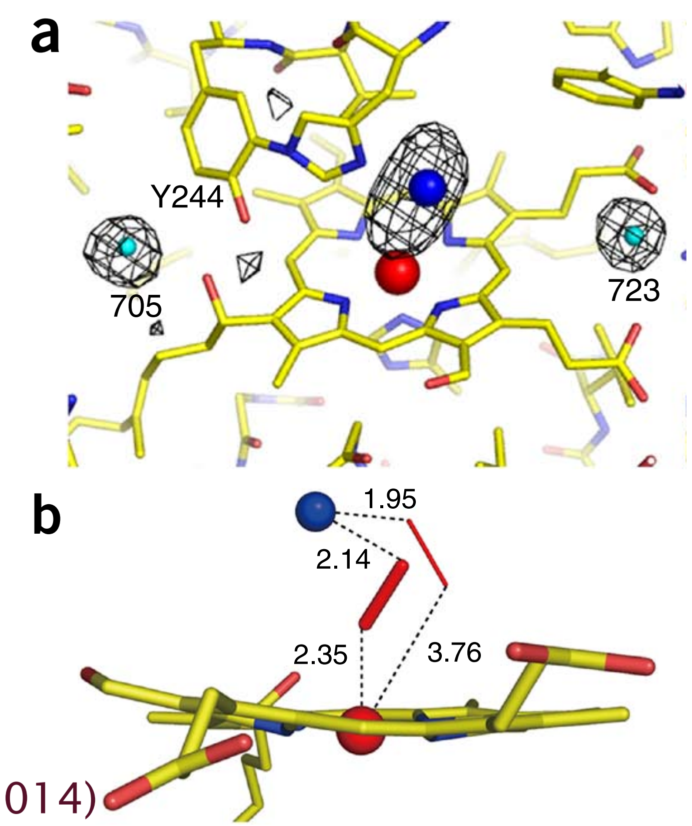


Fig. 2. (**A**) Final, refined $2mF_{obs} - DF_{calc}$ (1.5σ) electron density map (*17*) of lysozyme at 1.9 Å resolution calculated from 40-fs pulse data. (**B**) F_{obs}(40 fs) − F_{obs} (synchrotron) difference Fourier map, contoured at +3 σ (green) and −3 σ (red). No interpretable features are apparent. The synchrotron data set was collected with a radiation dose of 24 kGy.





self-terminating diffraction

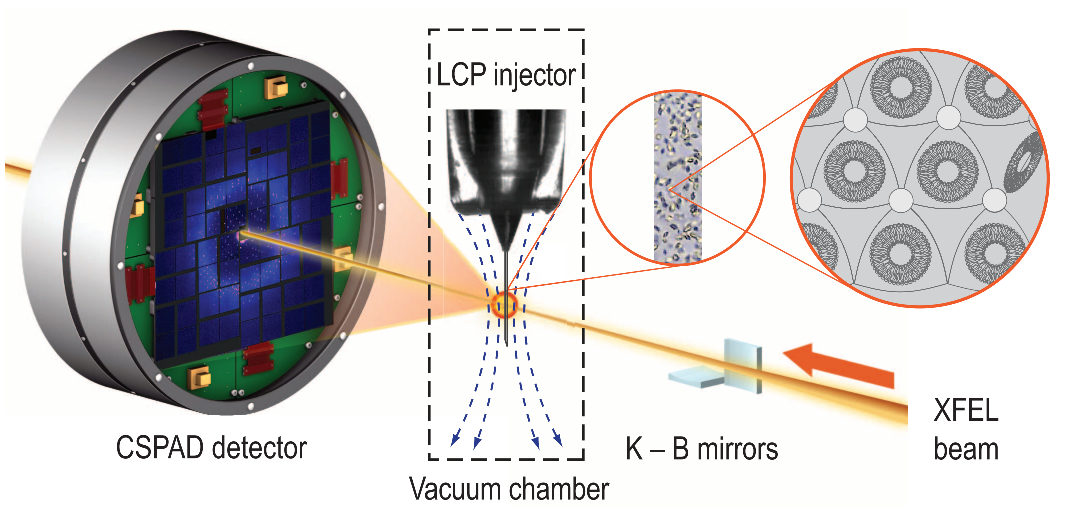
A. Barty et al. *Nat. Phot.* **6**, 35 (2012)

cytochrome *c* oxidase
measured with a
large crystal

K. Hirata et al. *Nat. Meth.* (2014)

SFX Highlight: G protein coupled receptors (GPCRs)

GPCRs are membrane proteins that mediate cellular communication but are difficult to grow in large crystals



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

But GPCRs can be grown into small crystals in lipidic-cubic phase media which can be injected into SFX experiments

AT_1R blockers are anti-hypertensive drugs but structure has been difficult due to inability to grow large crystals

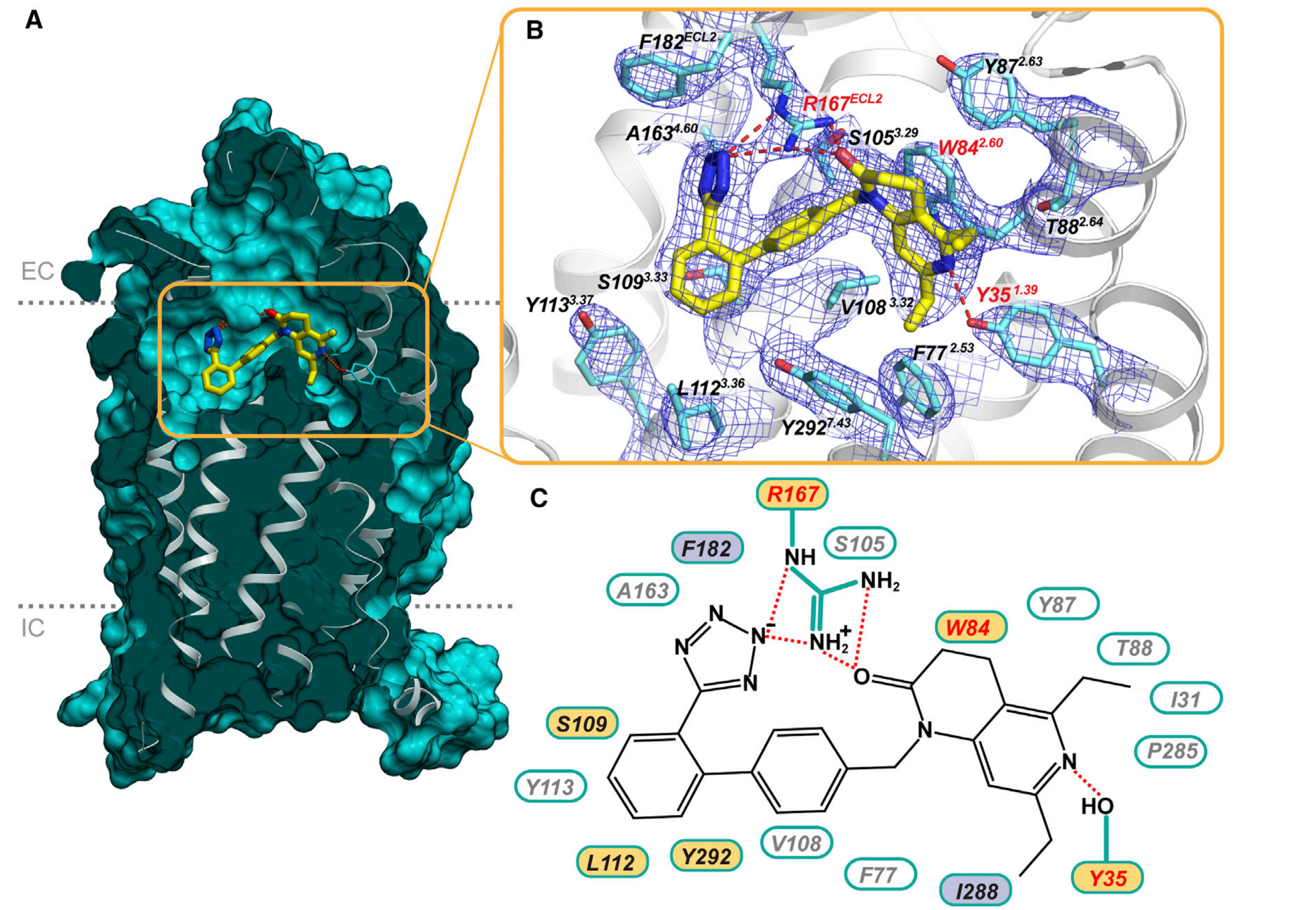


Figure 3. Interactions of ZD7155 with AT_1R

(A) Cross-section view of AT_1R highlighting the shape of the ligand binding pocket.

(B) Zoomed-in view of the ligand binding pocket showing all residues within 4 Å from the ligand ZD7155, along with the 2mFo-DFc electron density (blue mesh) contoured at 1 σ level. In (A) and (B) ZD7155 is shown as sticks with yellow carbons.

(C) Schematic representation of interactions between AT_1R and ZD7155. Hydrogen bonds/salt bridges are shown as red dashed lines. The residues shown by mutagenesis to be critical for ligand binding are labeled red, those that are important for either peptide or non-peptide ligands binding are labeled in yellow, and the residues that discriminate between peptide and non-peptide ligands are labeled in purple.

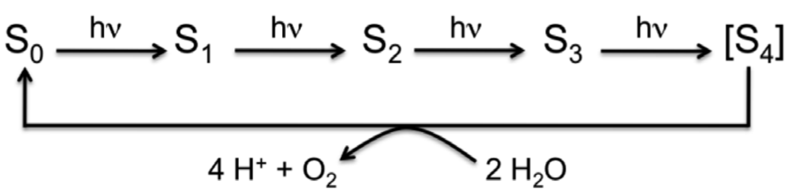
See also Figure S2 and Table S2.

human Angiotensin II type 1 receptor at 2.9Å

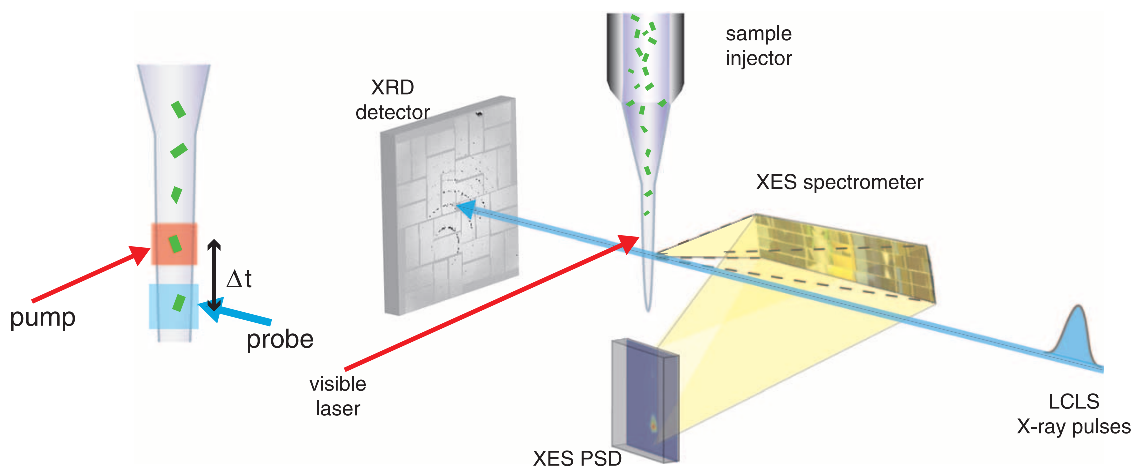
H. Zhang et al. *Cell* **161**, 1-12 (2015)

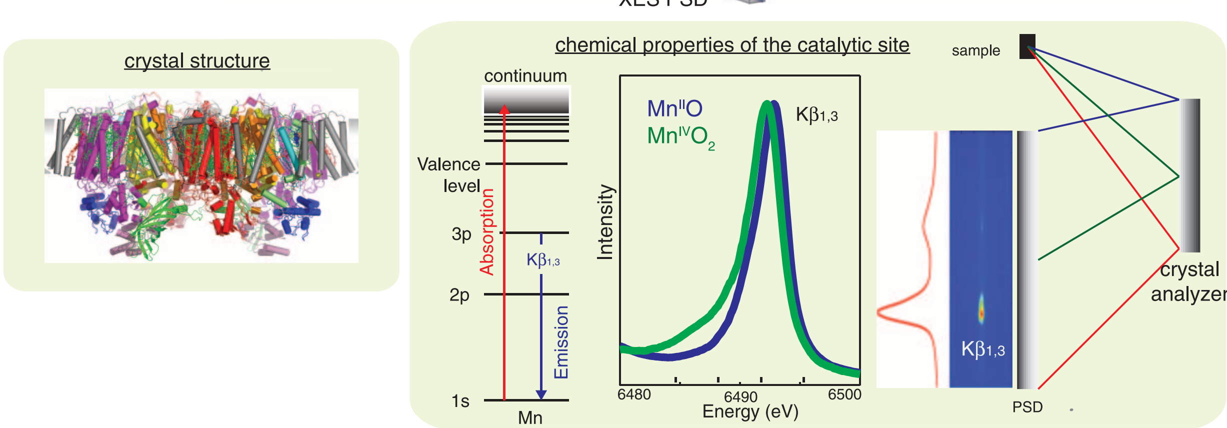
SFX Highlight: Time-resolved SFX on Photosystem II

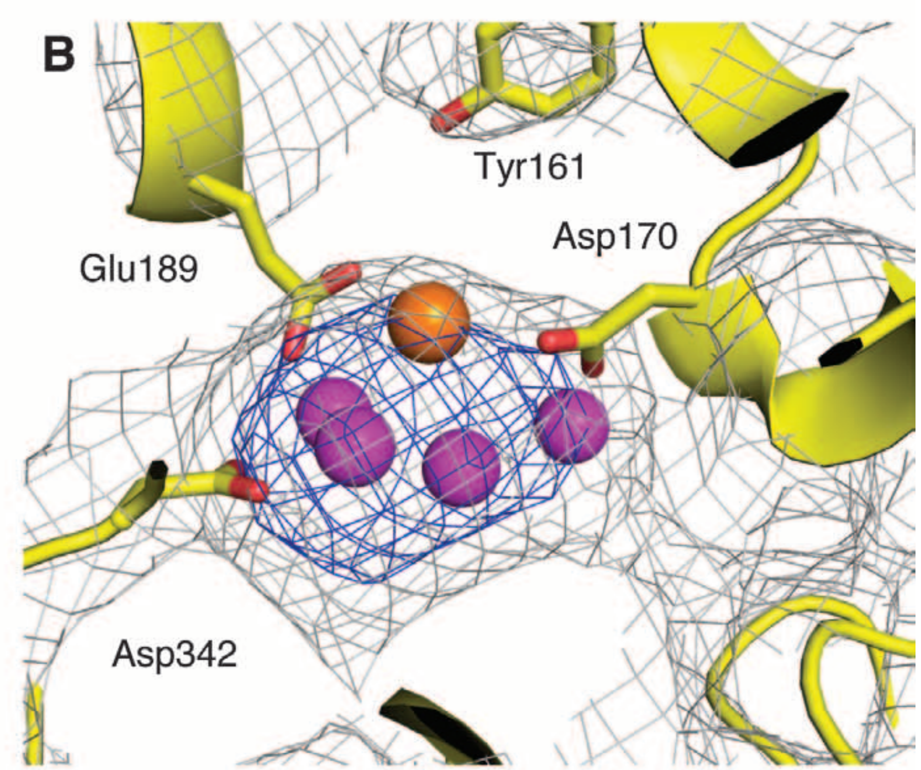
C. Kupitz et al. *Nature* **513**, 261 (2014)
J. Kern et al. *Science* **340**, 491 (2013)

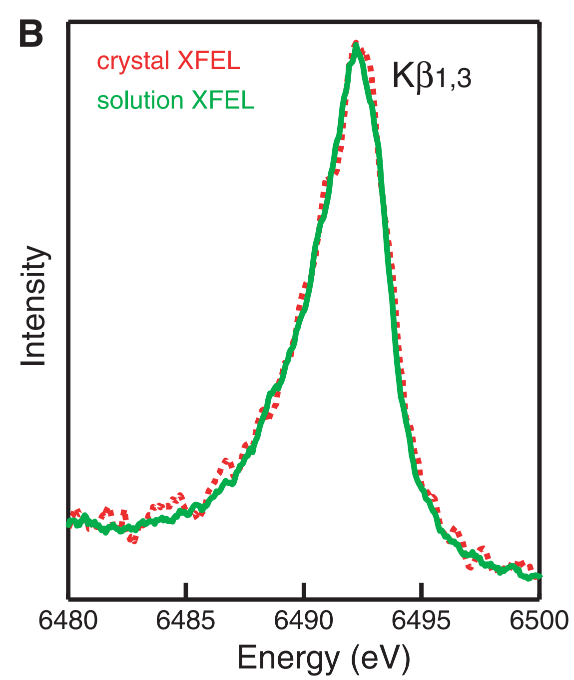


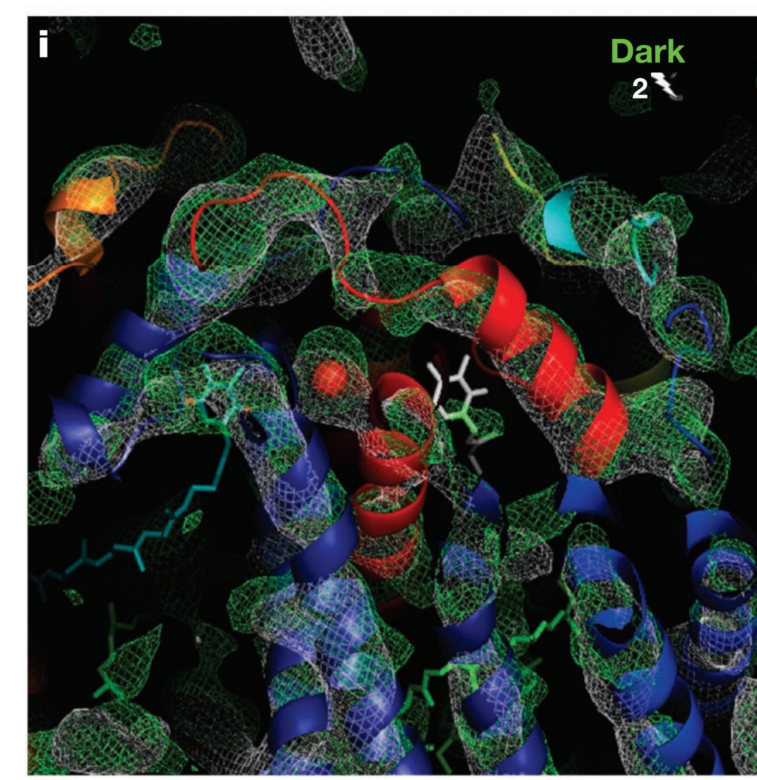
Scheme 1. Reaction cycle of water oxidation at the Mn_4CaO_5 cluster in PS II. h, Planck's constant; ν, frequency.











combined X-ray emission
and diffraction experiment

SFX has proven extremely successful

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]†

LETTER

doi:10.1038/nature13453

Serial time-resolved crystallography of photosystem II using a femtosecond X-ray laser

Christopher Kupitz[1]*, Shibom Basu[1]*, Ingo Grotjohann[1], Raimund Fromme[1], Nadia A. Zatsepin[2], Kimberly N. Rendek[1], Mark S. Hunter[1,3], Robert L. Shoeman[4], Thomas A. White[5], Dingjie Wang[2], Daniel James[2], Jay-How Yang[1], Danielle E. Cobb[1], Brenda Reeder[1], Raymond G. Sierra[6], Haiguang Liu[2], Anton Barty[5], Andrew L. Aquila[5,7], Daniel Deponte[5,8], Richard A. Kirian[2,5], Sadia Bari[9,10], Jesse J. Bergkamp[1], Kenneth R. Beyerlein[5], Michael J. Bogan[6], Carl Caleman[5,11], Tzu-Chiao Chao[1,12], Chelsie E. Conrad[1], Katherine M. Davis[13], Holger Fleckenstein[5], Lorenzo Galli[5,14], Stefan P. Hau-Riege[3], Stephan Kassemeyer[4,9], Hartawan Laksmono[6], Mengning Liang[5], Lukas Lomb[4], Stefano Marchesini[15], Andrew V. Martin[5,16], Marc Messerschmidt[8], Despina Milathianaki[8], Karol Nass[4,5,14], Alexandra Ros[1], Shatabdi Roy-Chowdhury[1], Kevin Schmidt[2], Marvin Seibert[8,17], Jan Steinbrener[4], Francesco Stellato[5], Lifen Yan[13], Chunhong Yoon[5,7], Thomas A. Moore[1], Ana L. Moore[1], Yulia Pushkar[13], Garth J. Williams[8], Sébastien Boutet[8], R. Bruce Doak[2], Uwe Weierstall[2], Matthias Frank[3], Henry N. Chapman[5,14,18], John C. H. Spence[2] & Petra Fromme[1]

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]

Determination of damage-free crystal structure of an X-ray–sensitive protein using an XFEL

Kunio Hirata[1,2,9], Kyoko Shinzawa-Itoh[3,9], Naomine Yano[2,3], Shuhei Takemura[3], Koji Kato[3,8], Miki Hatanaka[3], Kazumasa Muramoto[3], Takako Kawahara[3], Tomitake Tsukihara[2–4], Eiki Yamashita[4], Kensuke Tono[5], Go Ueno[1], Takaaki Hikima[1], Hironori Murakami[1], Yuichi Inubushi[1], Makina Yabashi[1], Tetsuya Ishikawa[1], Masaki Yamamoto[1], Takashi Ogura[6], Hiroshi Sugimoto[1], Jian-Ren Shen[7], Shinya Yoshikawa[3] & Hideo Ago[1]

ARTICLE

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Structure of a photosynthetic reaction centre determined by serial femtosecond crystallography

Linda C. Johansson[1], David Arnlund[1], Gergely Katona[1], Thomas A. White[2], Anton Barty[2], Daniel P. DePonte[2], Robert L. Shoeman[3,4], Cecilia Wickstrand[1], Amit Sharma[1], Garth J. Williams[5], Andrew Aquila[2], Michael J. Bogan[6], Carl Caleman[2], Jan Davidsson[7], R. Bruce Doak[8], Matthias Frank[9], Raimund Fromme[10], Lorenzo Galli[2,11], Ingo Grotjohann[10], Mark S. Hunter[10], Stephan Kassemeyer[3,4], Richard A. Kirian[8], Christopher Kupitz[10], Mengning Liang[2], Lukas Lomb[3,4], Erik Malmerberg[1], Andrew V. Martin[2], Marc Messerschmidt[5], Karol Nass[2,11], Lars Redecke[12], M. Marvin Seibert[5], Jennie Sjöhamn[1], Jan Steinbrener[3,4], Francesco Stellato[2], Dingjie Wang[8], Weixaio Y. Wahlgren[1], Uwe Weierstall[8], Sebastian Westenhoff[1], Nadia A. Zatsepin[8], Sébastien Boutet[5], John C.H. Spence[8], Ilme Schlichting[3,4], Henry N. Chapman[2,11], Petra Fromme[10] & Richard Neutze[1]

Serial Femtosecond Crystallography of G Protein–Coupled Receptors

Wei Liu,[1] Daniel Wacker,[1] Cornelius Gati,[2] Gye Won Han,[1] Daniel James,[3] Dingjie Wang,[3] Garrett Nelson,[3] Uwe Weierstall,[3] Vsevolod Katritch,[1] Anton Barty,[2] Nadia A. Zatsepin,[3] Dianfan Li,[4] Marc Messerschmidt,[5] Sébastien Boutet,[5] Garth J. Williams,[5] Jason E. Koglin,[5] M. Marvin Seibert,[5,6] Chong Wang,[1] Syed T. A. Shah,[4] Shibom Basu,[7] Raimund Fromme,[7] Christopher Kupitz,[7] Kimberley N. Rendek,[7] Ingo Grotjohann,[7] Petra Fromme,[7] Richard A. Kirian,[2,3] Kenneth R. Beyerlein,[2] Thomas A. White,[2] Henry N. Chapman,[2,8,9] Martin Caffrey,[4] John C. H. Spence,[3] Raymond C. Stevens,[1] Vadim Cherezov[1]*

Structure of the Angiotensin Receptor Revealed by Serial Femtosecond Crystallography

Haitao Zhang,[1] Hamiyet Unal,[2] Cornelius Gati,[3] Gye Won Han,[4] Wei Liu,[5] Nadia A. Zatsepin,[6] Daniel James,[6] Dingjie Wang,[6] Garrett Nelson,[6] Uwe Weierstall,[6] Michael R. Sawaya,[7] Qingping Xu,[8] Marc Messerschmidt,[9] Garth J. Williams,[10] Sébastien Boutet,[10] Oleksandr M. Yefanov,[3] Thomas A. White,[3] Chong Wang,[11] Andrii Ishchenko,[4] Kalyan C. Tirupula,[2] Russell Desnoyer,[2] Jesse Coe,[5] Chelsie E. Conrad,[5] Petra Fromme,[5] Raymond C. Stevens,[1,4,12] Vsevolod Katritch,[1] Sadashiva S. Karnik,[2] and Vadim Cherezov[4,*]

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]

nature photonics LETTERS

PUBLISHED ONLINE: 18 DECEMBER 2011 | DOI: 10.1038/NPHOTON.2011.297

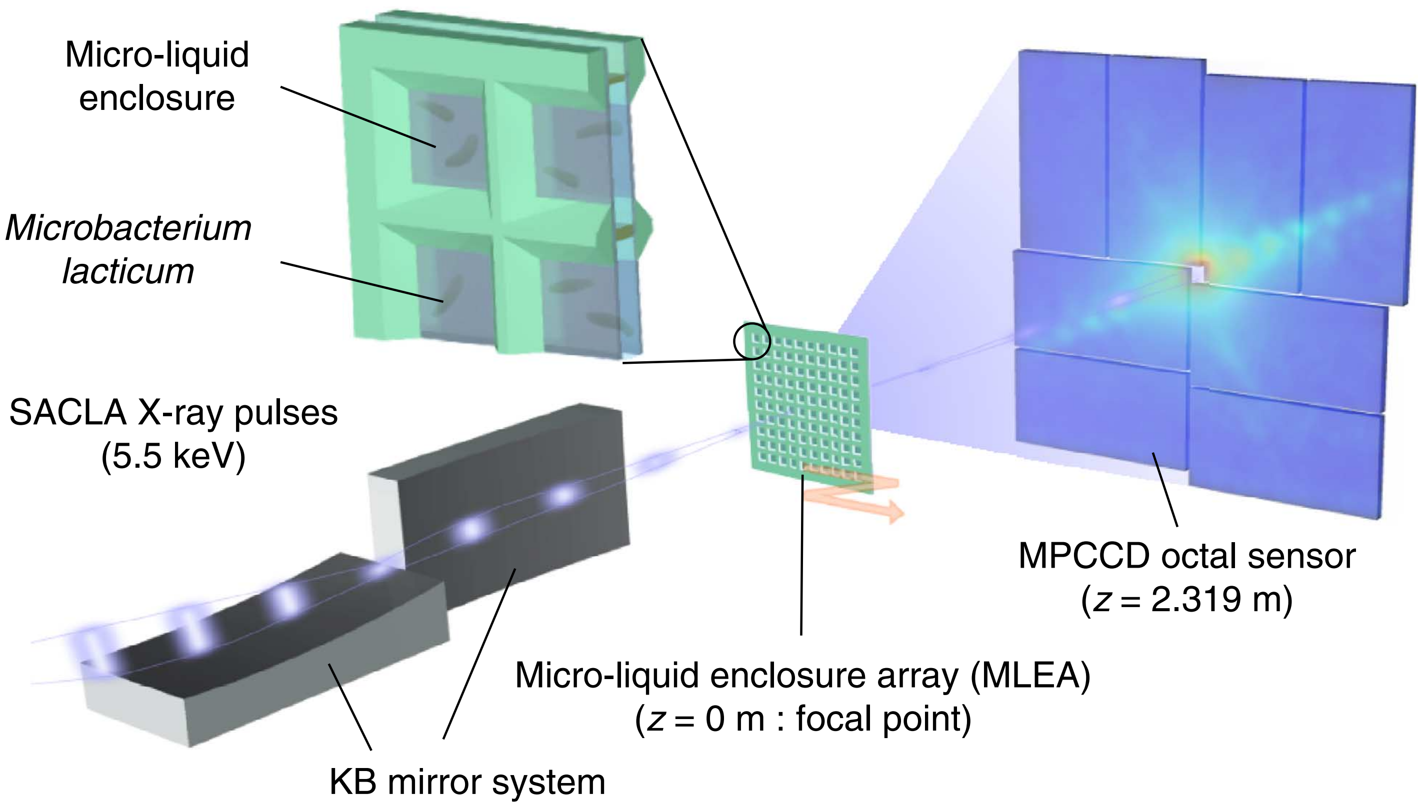
Self-terminating diffraction gates femtosecond X-ray nanocrystallography measurements

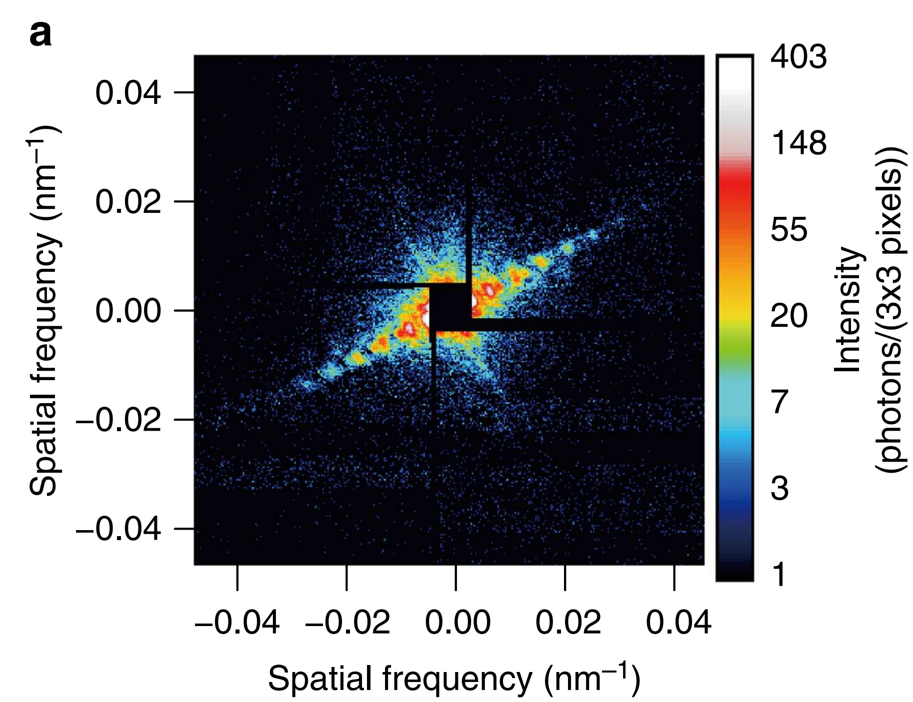
Anton Barty, Carl Caleman and Henry N. Chapman *et al.**

Wow, so all we've managed to do is some fancy protein crystallography ?

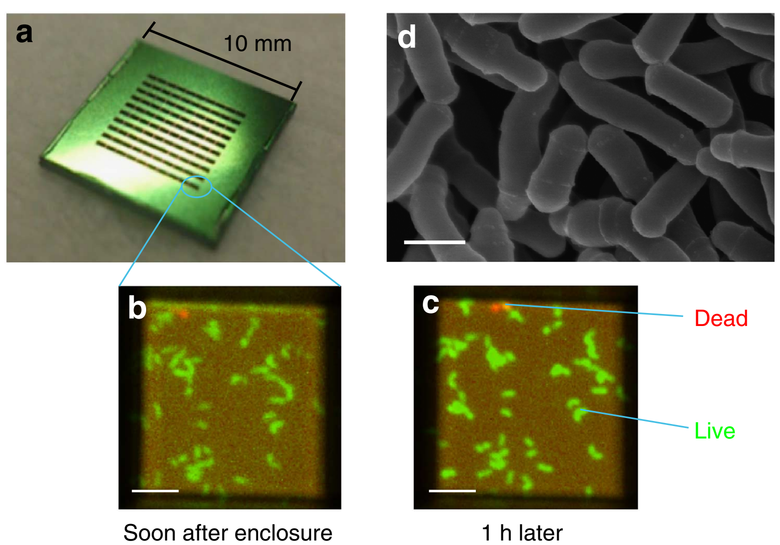
Live-cell imaging using an XFEL

T. Kimura et al. *Nat. Comm.* **5**, 3052 (2014)

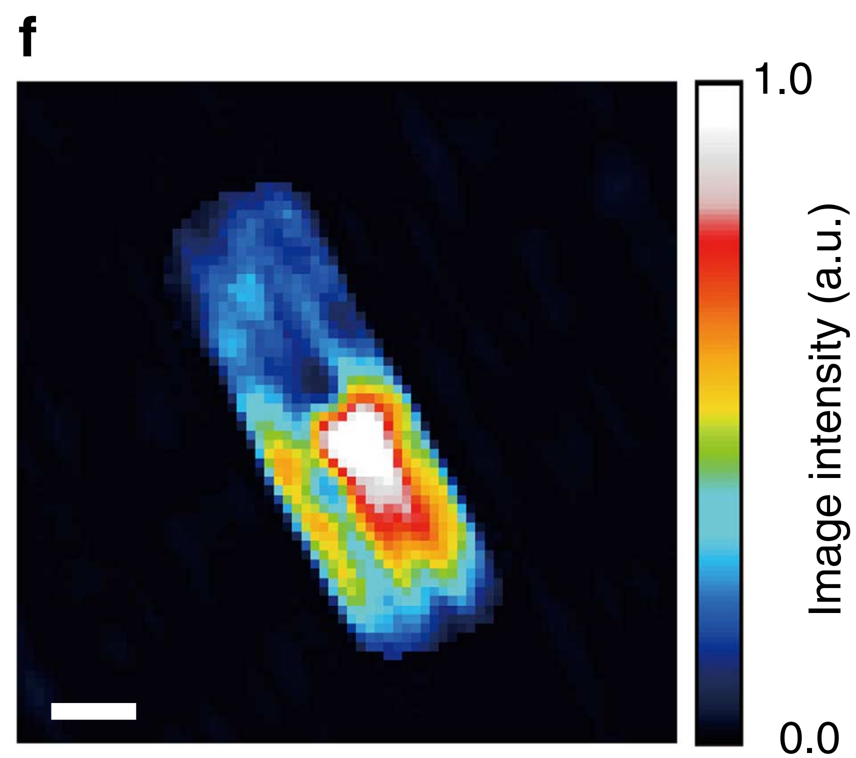




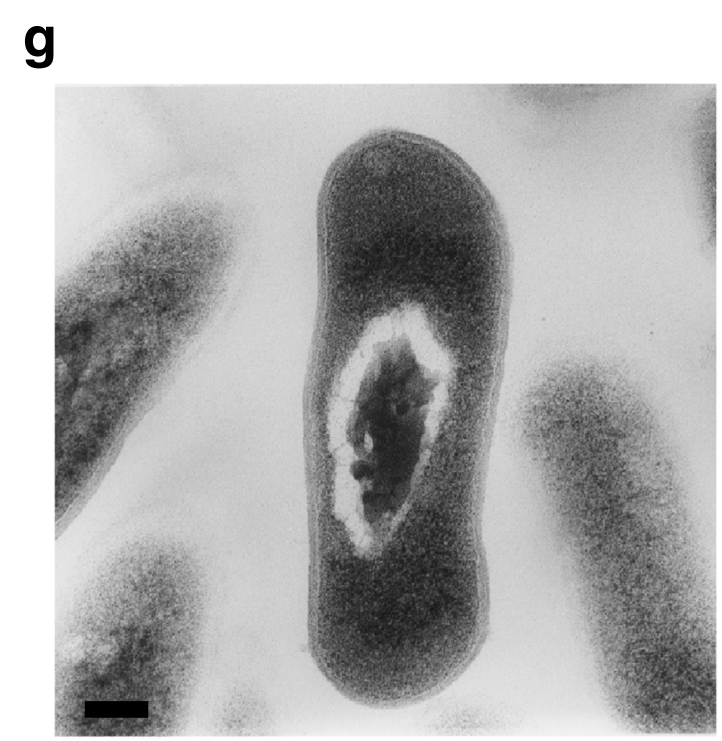
coherent diffraction pattern from live cell



28 nm resolution



reconstructed image

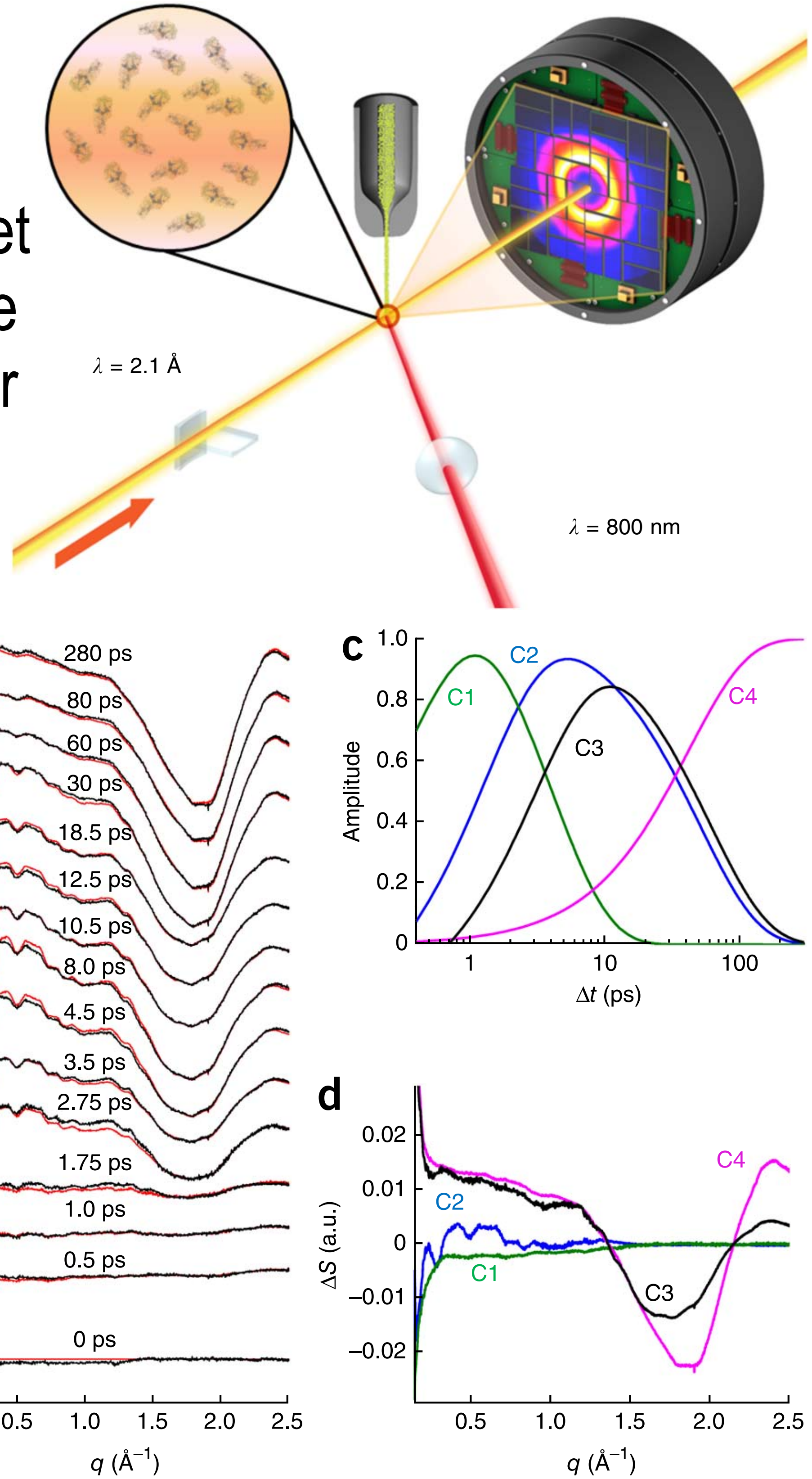


TEM image

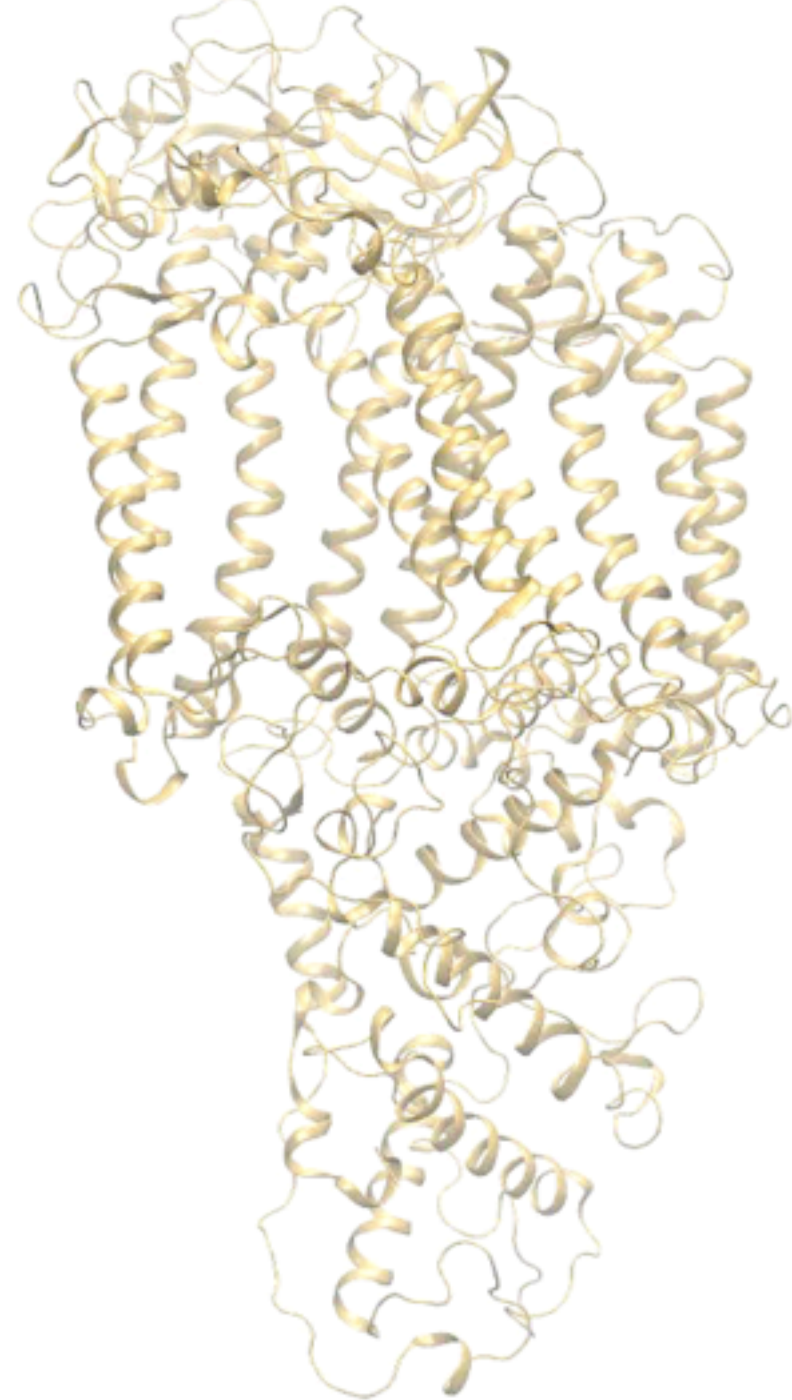
Time-resolved Wide-Angle X-ray Scattering (WAXS)

D. Arnlund et al. *Nat. Meth.* **11**, 927 (2014)

liquid jet sample injector



Blastochloris viridis
photosynthetic reaction centre

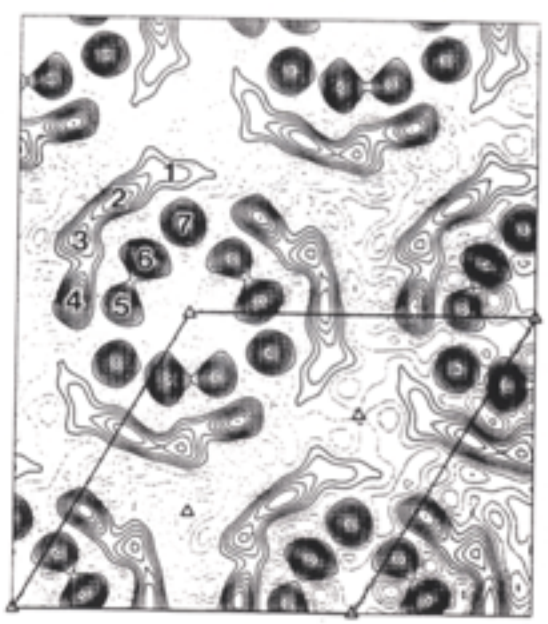


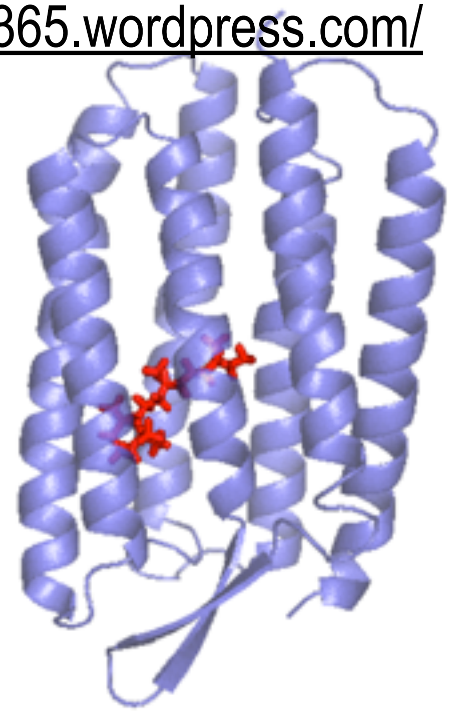
shows evidence of rapid dissipation of energy through a ‘protein quake’

2D crystallography at an XFEL

https://crystallography365.wordpress.com/

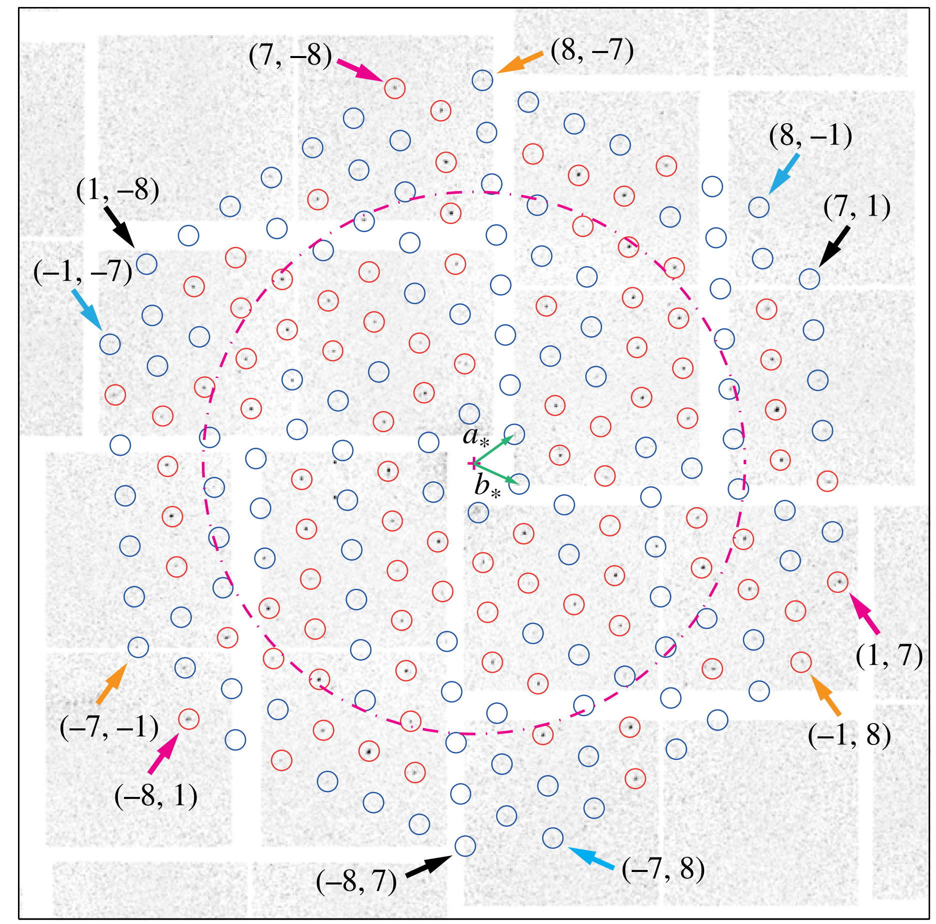
B. Pedrini et al. *Phil. Trans. Roy. Soc B* **369**, 20130500 (2014)
M. Frank et al. *IUCrJ* **1**, 1-6 (2014)

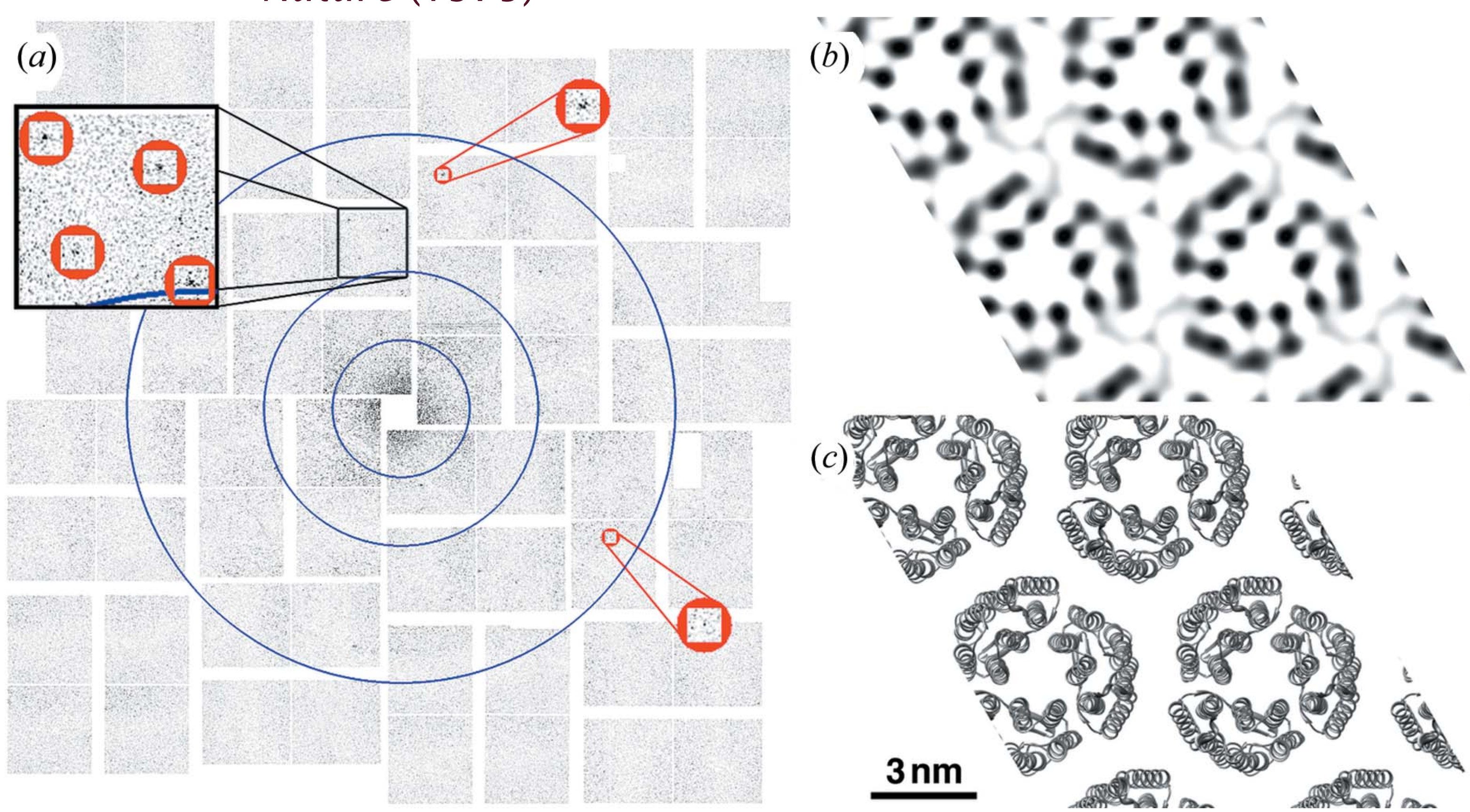




Unwin & Henderson
Nature (1975)

bacteriorhodopsin diffraction out to 7Å



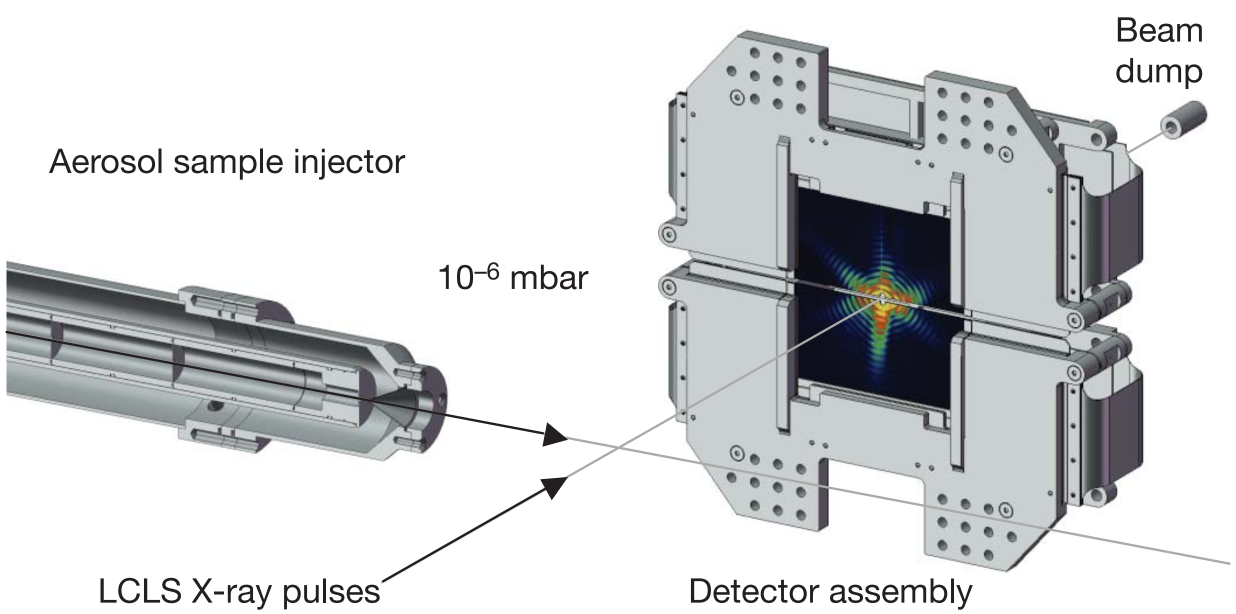


diffraction from 2D crystals of bacteriorhodopsin

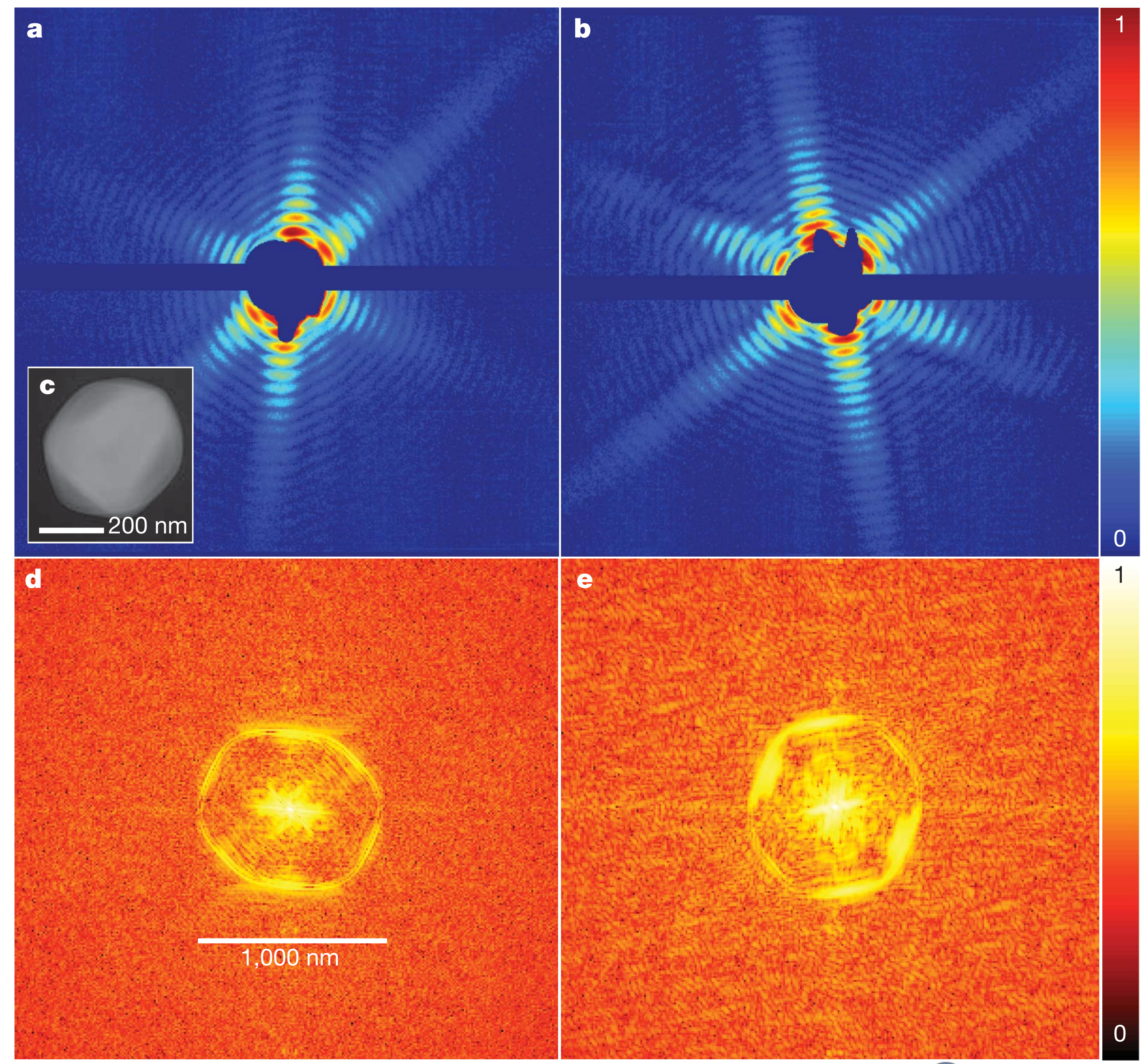
these experiments require lots of X-rays and a tight focus (100 nm)

Single-particle imaging at an XFEL

M.M. Seibert et al. *Nature* **470**, 78 (2011)



Single shot scattering from *Acanthamoeba polyphaga* mimivirus



single-shot scattering pattern gives enough information to reconstruct the structure of the mimivirus

STRUCTURAL DYNAMICS **2**, 041601 (2015)

Single-particle structure determination by X-ray free-electron lasers: Possibilities and challenges

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STRUCTURAL DYNAMICS **2**, 041702 (2015)

Perspectives for imaging single protein molecules with the present design of the European XFEL

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STRUCTURAL DYNAMICS **2**, 041701 (2015)

The linac coherent light source single particle imaging road map

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Conclusion: Biology is well represented at XFELs

Structural Dynamics
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Volume 2, Issue 4, July 2015

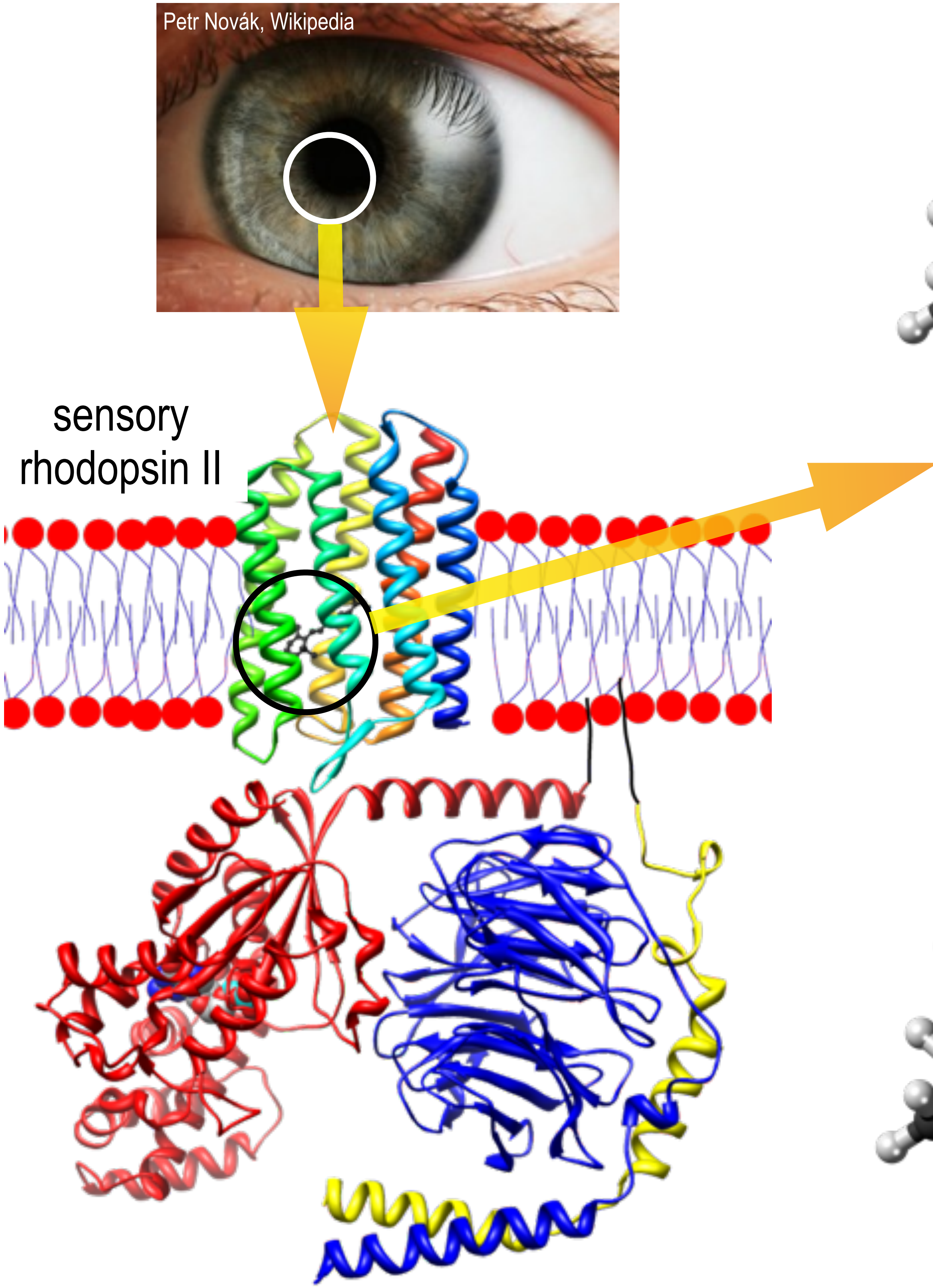
http://scitation.aip.org/content/aca/journal/sdy/

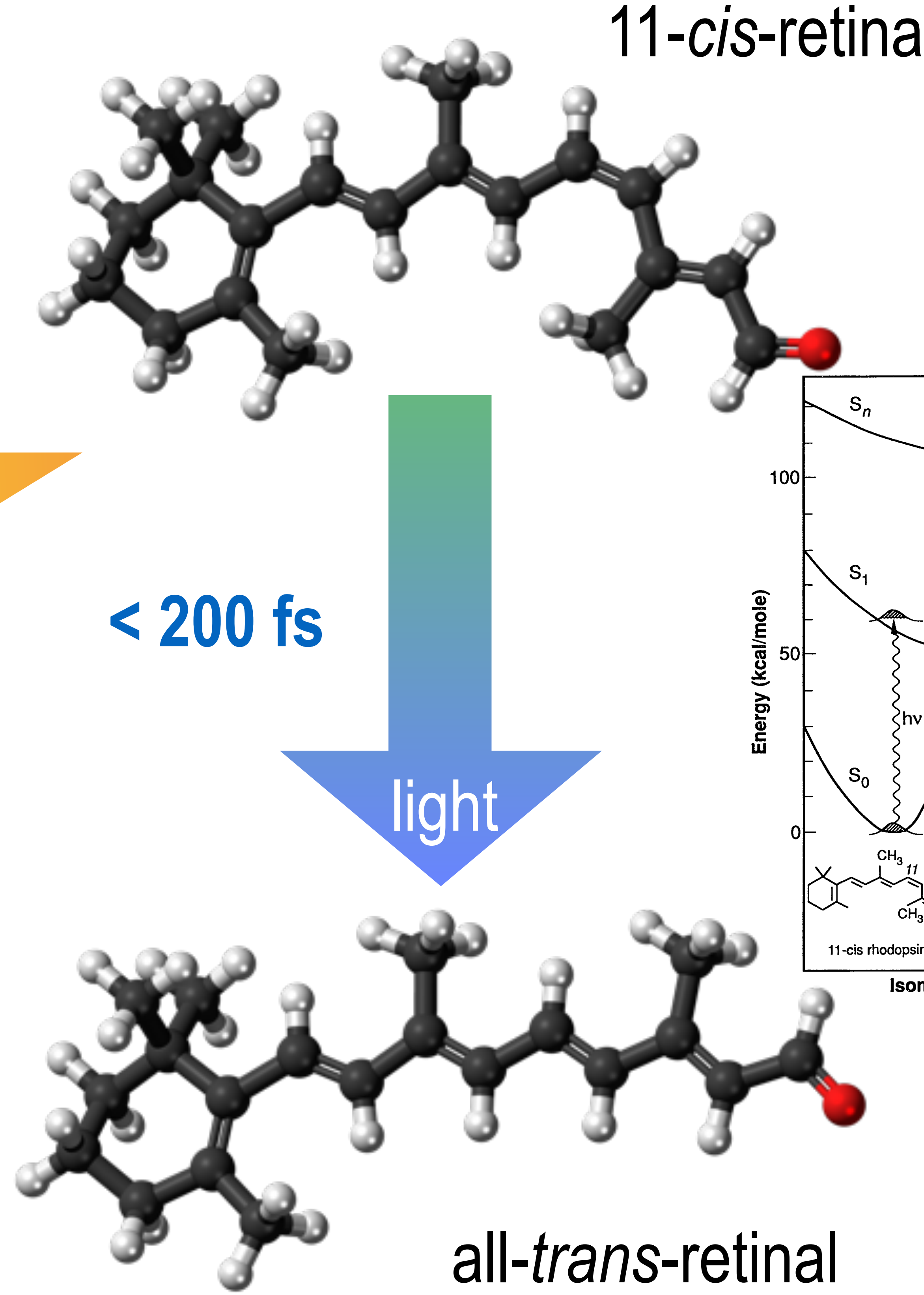
PHILOSOPHICAL TRANSACTIONS B

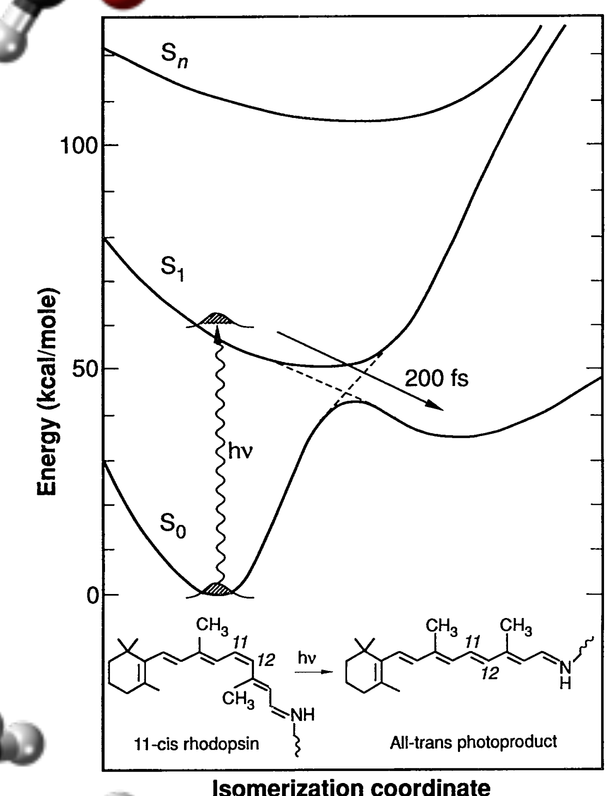
Discussion Meeting Issue 'Biology with free-electron X-ray lasers'
17 July 2014; volume 369, issue 1647

http://rstb.royalsocietypublishing.org/content/369/1647

The future: Ultrafast events in vision



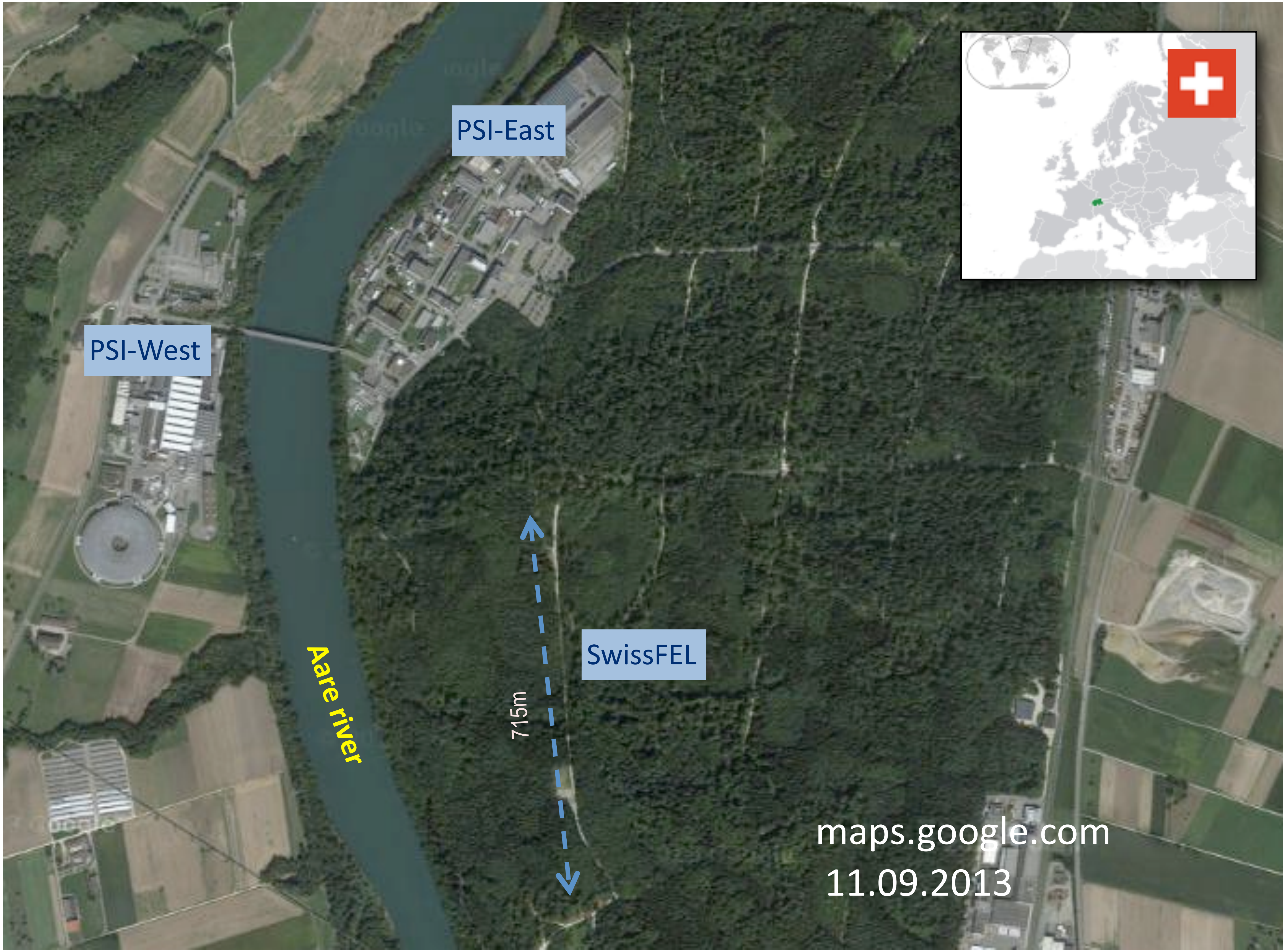




Schoenlein et al. *Science* **254**, 412 (1991)

Now that you're convinced how awesome they are, let's build one !

SwissFEL location at the Paul Scherrer Institute

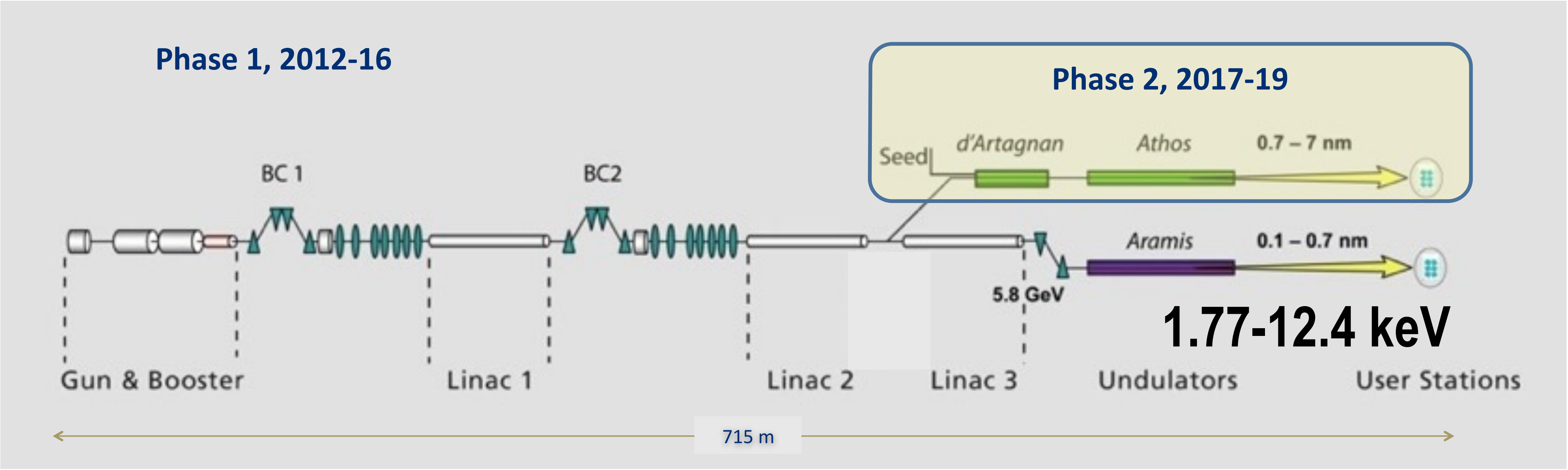


SwissFEL: June 27, 2013

SwissFEL under construction

ESA hutch 23.01.2015

What are we going to put into this building ?



2012-2017

Aramis: 1-7 Å (2-12.4 keV) hard X-ray SASE FEL,
In-vacuum , planar undulators with variable gap.
User operation from mid 2017

after 2017

Athos : (2nd phase) 7-70 Å soft X-ray FEL for SASE & Seeded operation .
APPLE II undulators with variable gap and full polarization control.
To be implemented after 2017

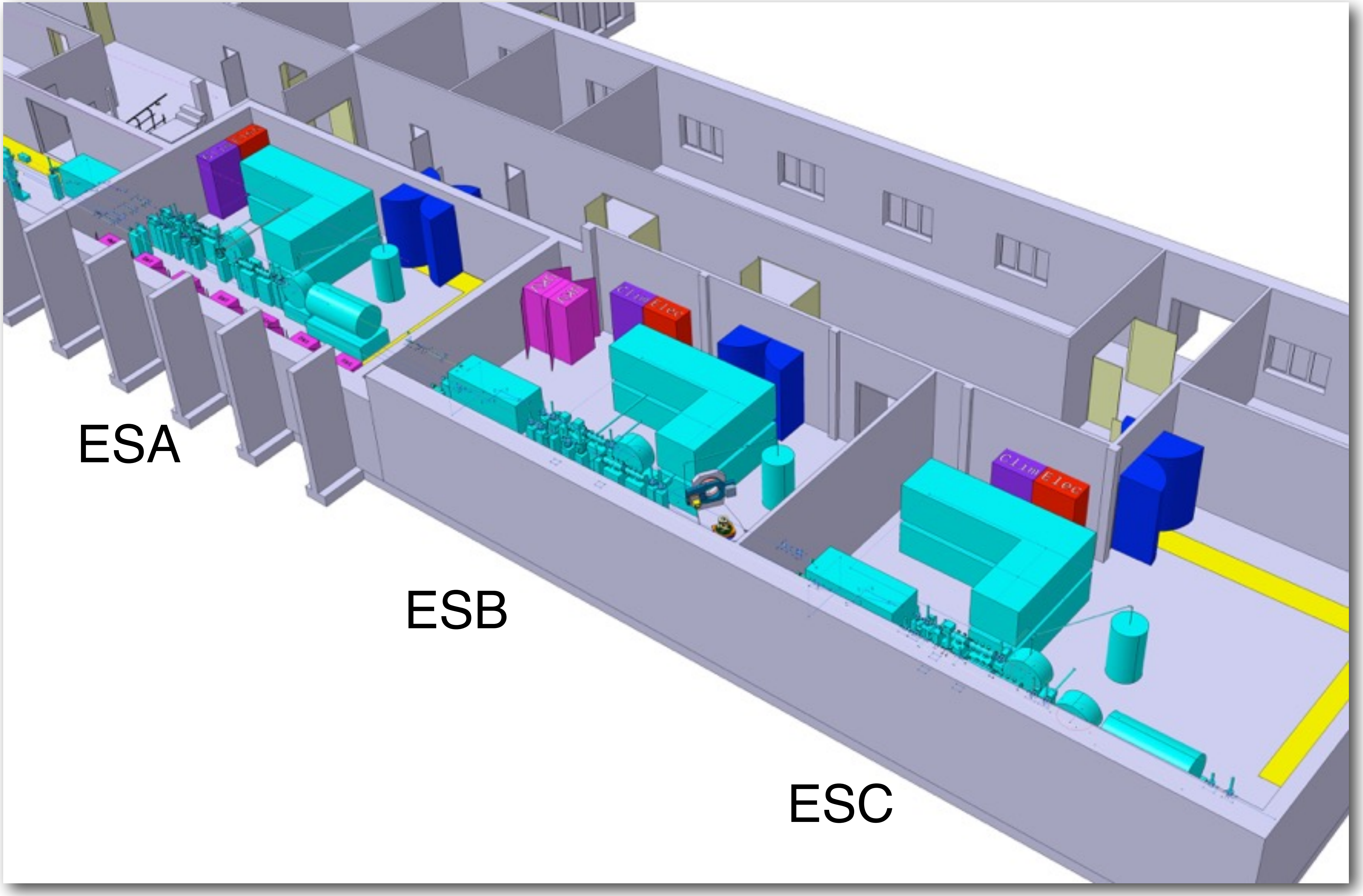
SwissFEL parameters	
Wavelength from	1 Å - 70 Å
Photon energy	0.2-12 keV
Photon / pulse (1Å)	7.3E+10
Pulse duration	1 fs - 20 fs
Energy bandwidth	0.05-0.16%
e^- Energy	5.8 GeV
e^- Bunch charge	10-200 pC
Repetition rate	100 Hz

SwissFEL design parameters at 1 Å

Sven Reiche
and co-workers

FEL Beam Design Parameters	Nominal Operation Mode		Special Operation Mode	
	Long Pulses	Short Pulses	Large Bandwidth	Ultra-Short Pulses
Undulator period (mm)	15	15	15	15
Undulator parameter	1.2	1.2	1.2	1.2
Energy spread (keV)	350	250	17000 (FW)	1000
Saturation length (m)	47	50	50	50
Saturation pulse energy (μJ)	150 (*)	3	100	15
Effective saturation power (GW)	2.8	0.6	2	50
Photon pulse length (fs, rms)	21	2.1	15	0.06
Beam radius (μm)	26.1	17	26	17
Divergence (μrad)	1.9	2	2	2.5
Number of photons	$7{,}3.10^{10}$	$1{,}7.\ 10^{9}$	5.10^{10}	$7.5.\ 10^{9}$
Spectral Bandwidth, rms (%)	0.05	0.04	3.5 (FW)	0.05
Peak brightness (# photon/mm^2.$mrad^2$.s^1.0.1% bandwidth)	7.10^{32}	1.10^{32}	8.10^{30}	$1{,}3.10^{33}$
Average brightness (# photon/mm^2.$mrad^2$.s^1.0.1% bandwidth)	$2{,}3.10^{21}$	$5{,}7.10^{18}$	3.10^{19}	$7{,}5.10^{18}$

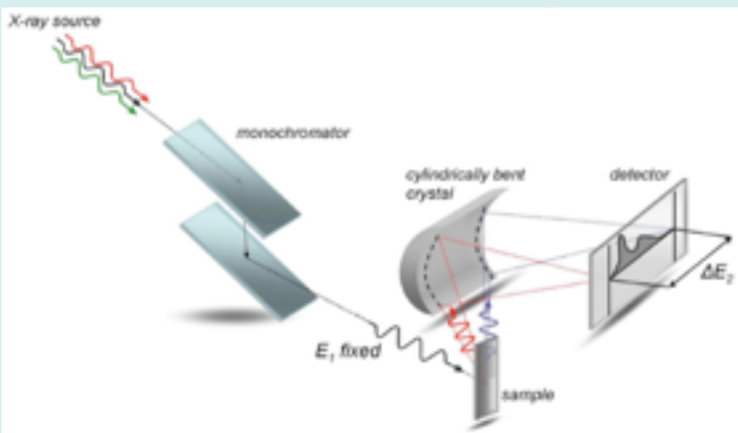
Aramis Experimental Hutches

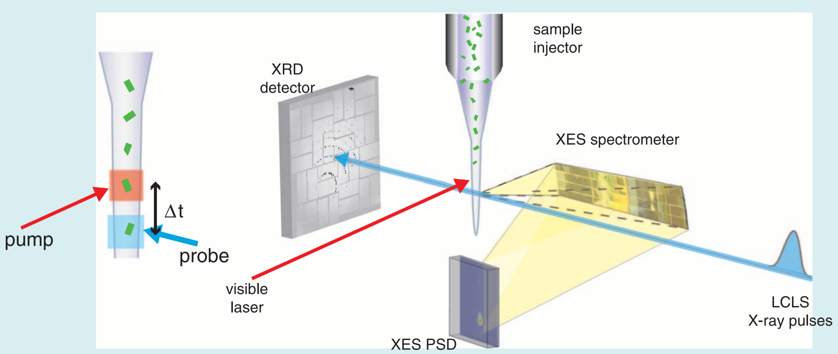


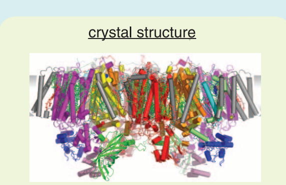
SwissFEL Experimental Stations

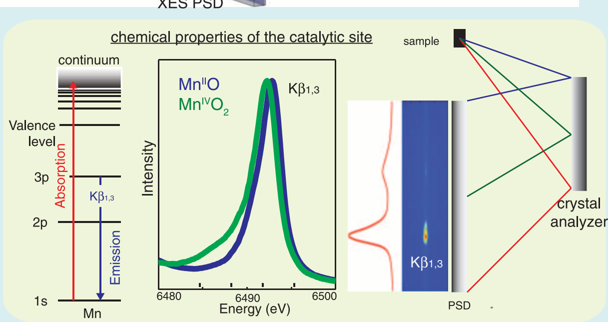
Bruce Patterson
and co-workers

ESA:
Ultrafast photochemistry and photobiology

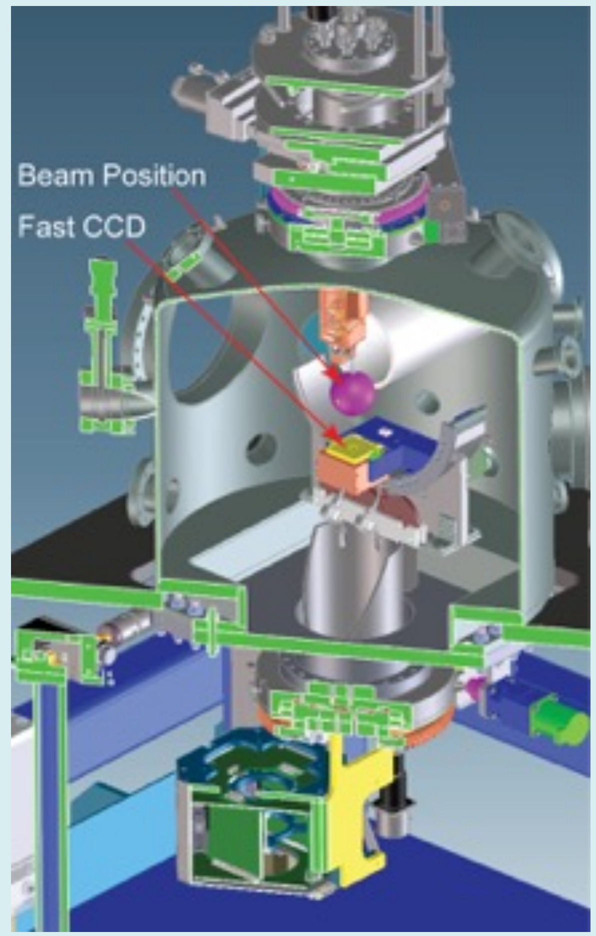






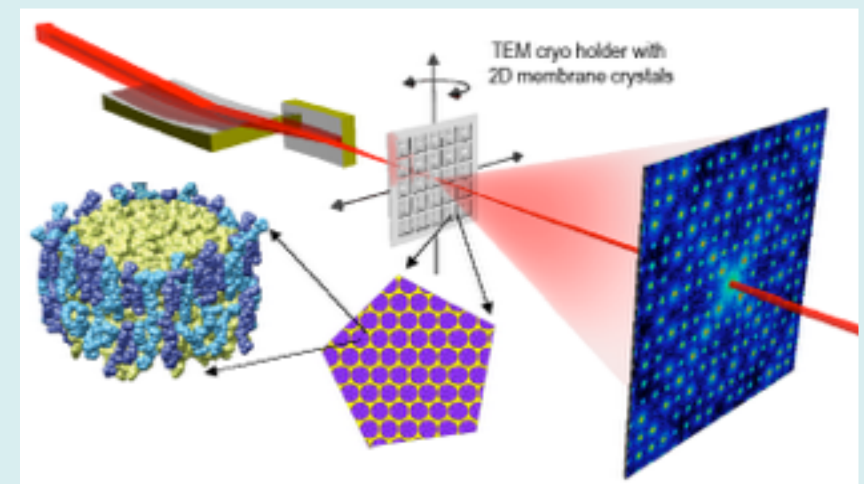


ESB:
Pump–probe crystallography

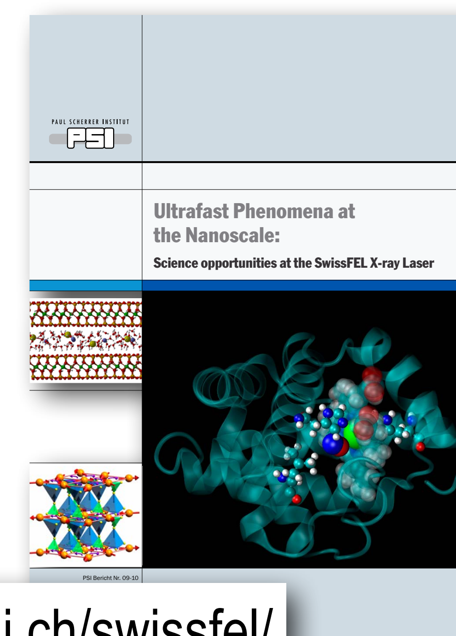


Phase I:
Ready by 2017

ESC: **Phase II:** >2017
Materials science and nanocrystallography



Scientific Case
B. Patterson
editor

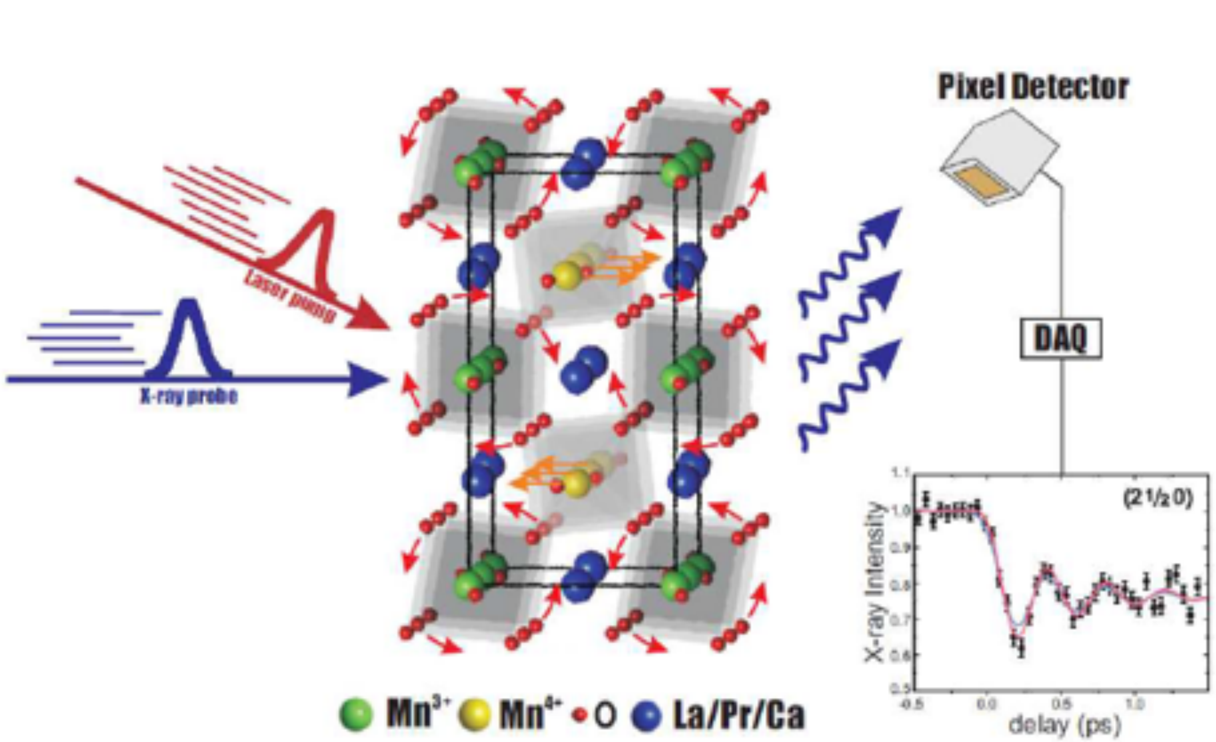


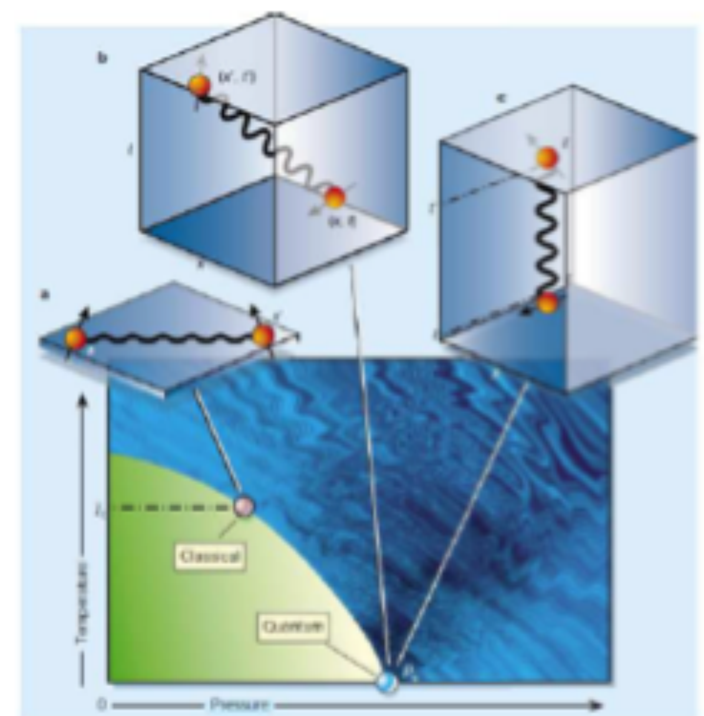
http://www.psi.ch/swissfel/

ESB: Probing Correlated Coupled Dynamics & Fluctuations

→ pump: launch coherent excitation
(phonon, spin wave, charge wave, orbital wave, ...)

→ tune system close to critical point
(apply static pressure or B-field at low T)





[P. Coleman, Nature 413 (2001)]

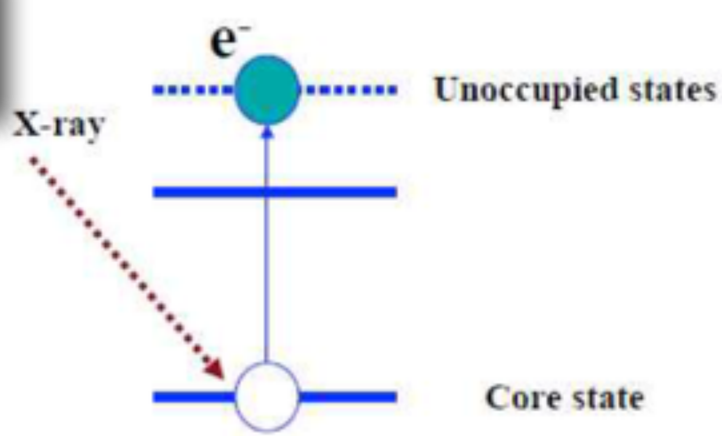
→ X-ray probe: how does the (coherent) excitation evolve in time ?

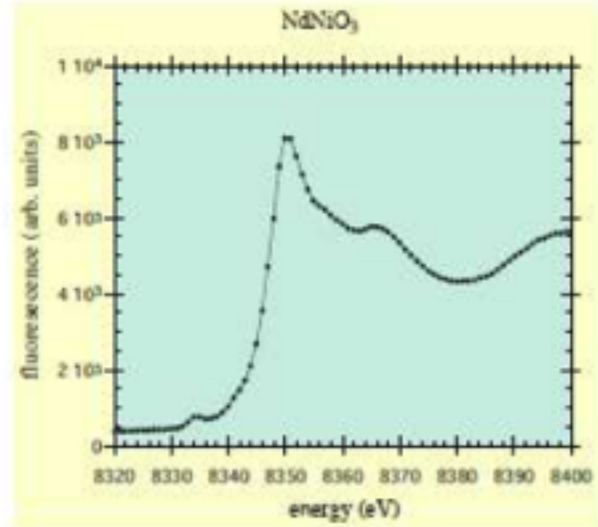
↔ tr-XRD: measures changes in lattice constants & symmetry
↔ tr-RXRD: sensitive to coupling of charge-, orbital- and spin-order (↔ polarization)
↔ tr-(N)TDS: measures S(**q**,ω = 0) & fluctuating coherence length ξ_F
↔ tr-(R)IXS: measures S(**q**,ω) & change of momentum dispersion

ESB: The X-ray probe techniques

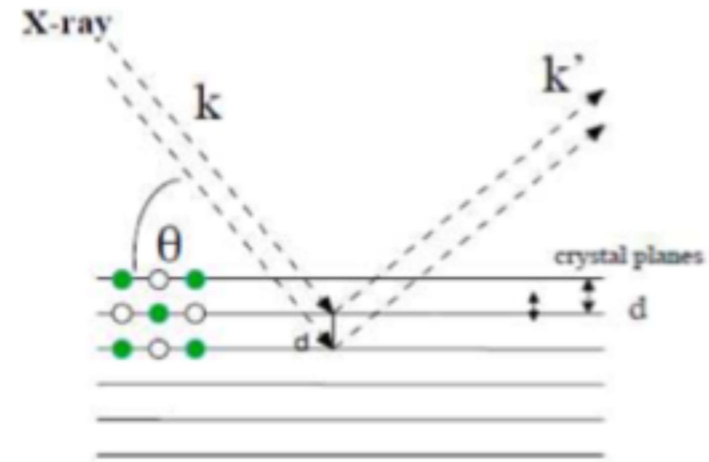
Gerhard Ingold and Paul Beaud

Resonant X-ray Diffraction



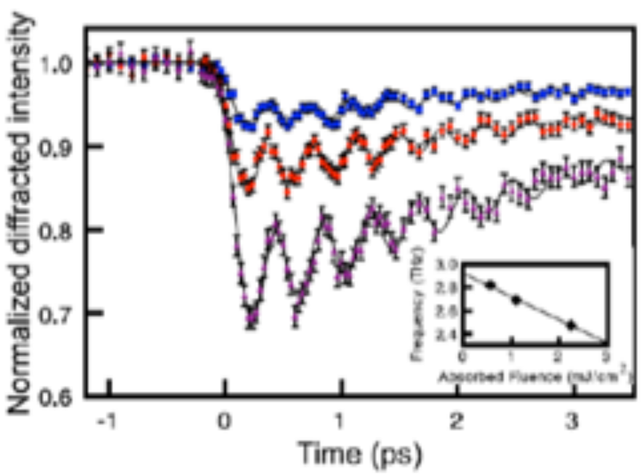


+



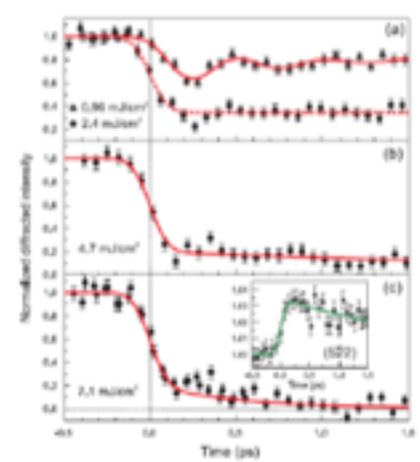
X-ray Diffraction

Peierls system: coherent phonons (Bi)



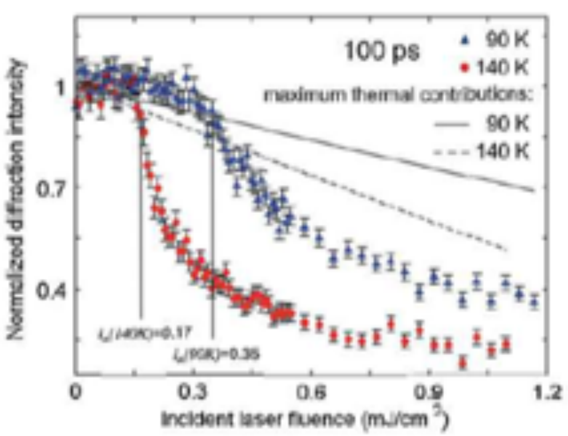
[Johnson PRL 2008]

Jahn-Teller dynamics (LCMO)



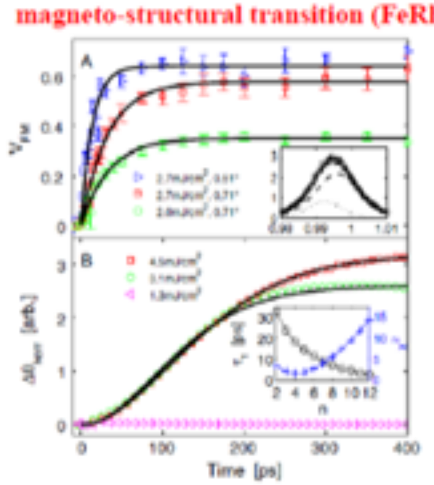
[Beaud PRL 2009]

excitonic CDW system ($TiSe_2$)



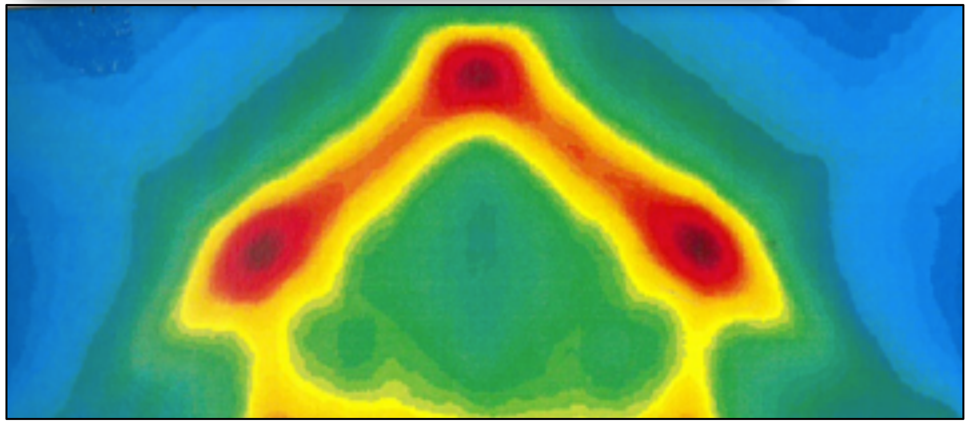
[Vorobeva PRL 2011]

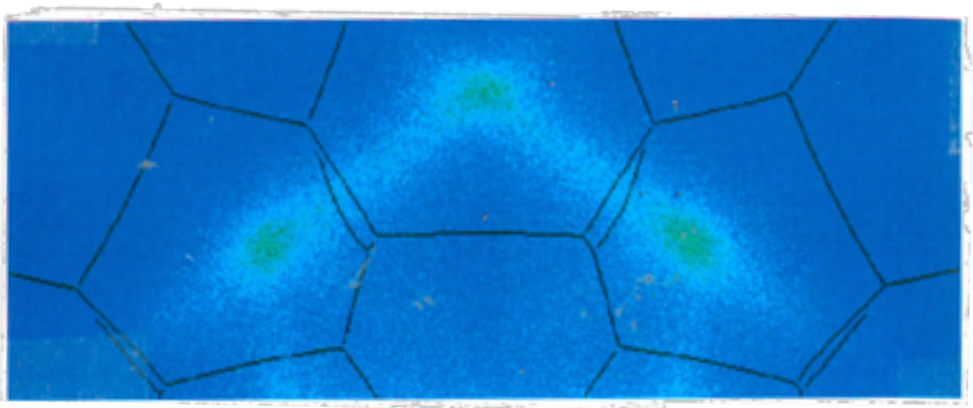
magneto-structural transition (FeRh)



[Mariager PRL 2012]

Elastic X-ray Diffuse Scattering





TDS from Si(100) measured at the SLS (courtesy J. Johnson)

ESA: Ultrafast photochemistry and photobiology

Chris Milne and Jakub Szlachetko

Electronic relaxation in nanoparticles

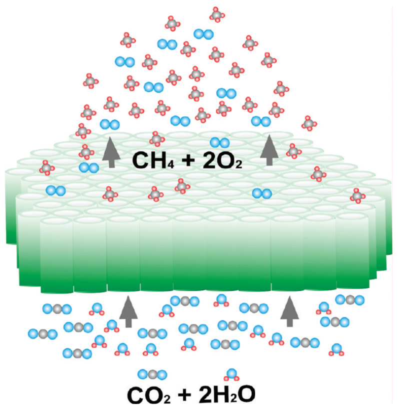
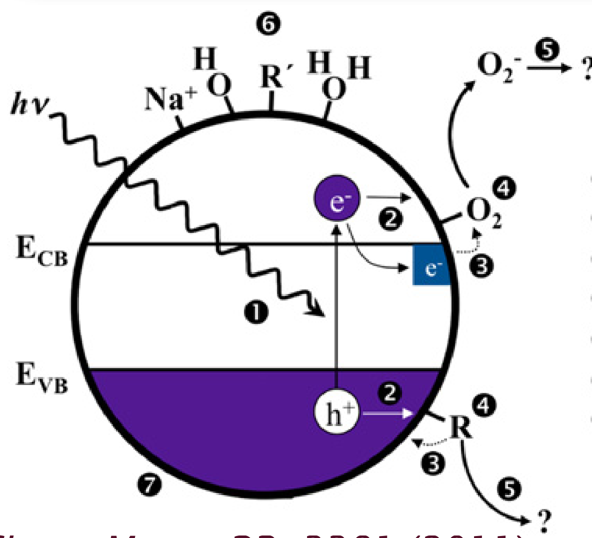


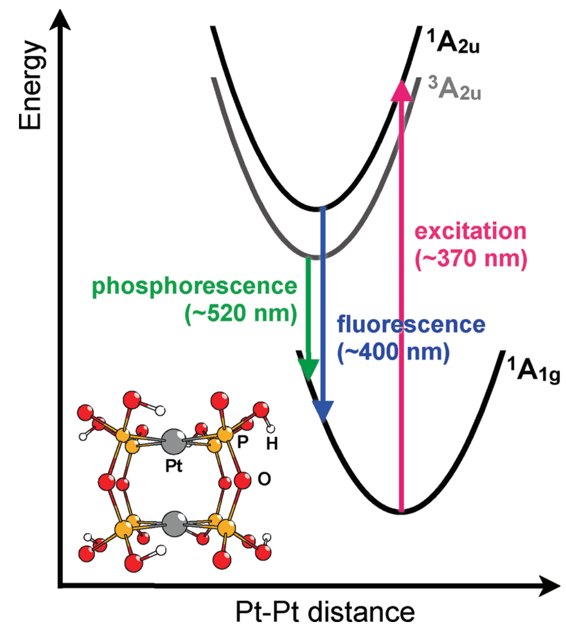
Figure 16. Depiction of flow-through photocatalytic membrane for CO_2 conversion.

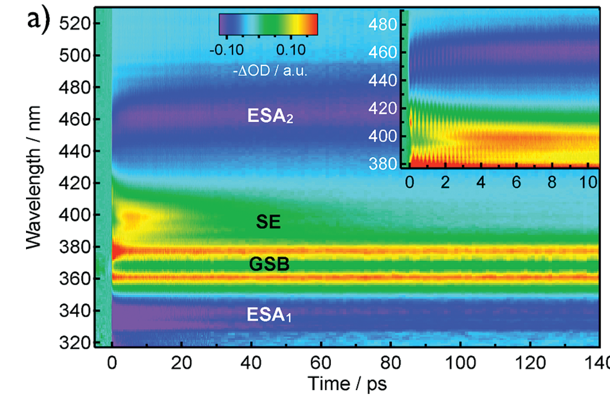


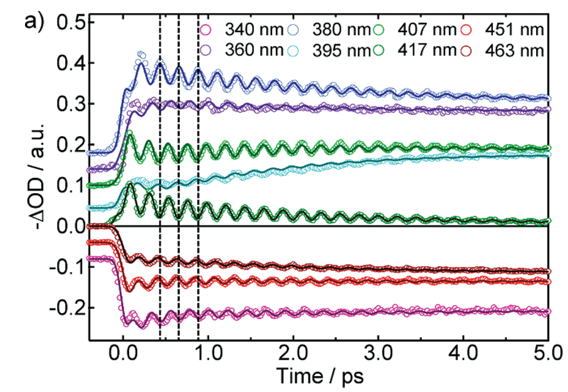
Important issues
- ❶ excitation
- ❷ charge transport and trapping
- ❸ charge transfer
- ❹ molecular adsorption
- ❺ reaction mechanisms
- ❻ poisons and promoters
- ❼ surface and material structure

A. Listorti et al. *Rev. Chem. Mater.* **23**, 3381 (2011);
A. Hagfeldt et al. *Chem. Rev.* **110**, 6595 (2010);
M. Henderson *Surf. Sci. Rep.* **66**, 185 (2011);
S.C. Roy et al. *ACS Nano* **4**, 1259 (2010)

Photochemistry

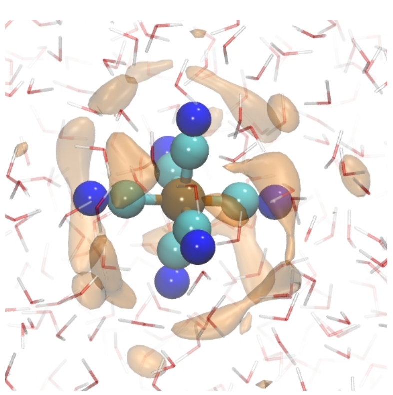


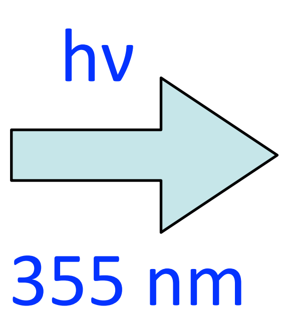


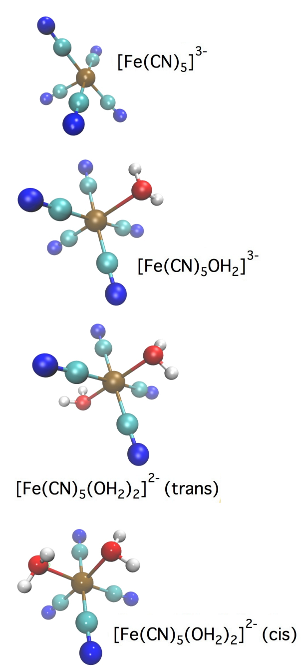


R. van der Veen et al. *JACS* **133**, 305 (2011)

Bond breaking and bond making

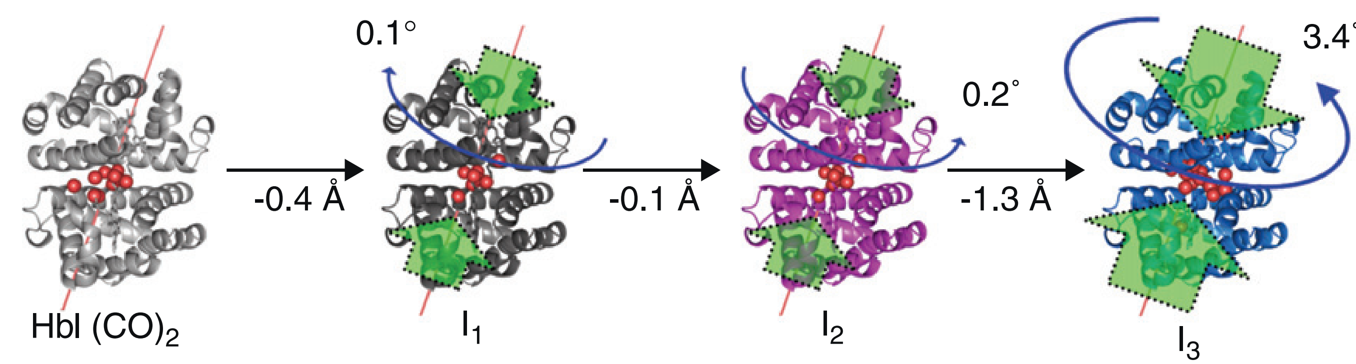

$[Fe(CN)_6]^{4-}$





Protein function

Intermediate states of homodimeric hemoglobin (HbI) ligated with CO

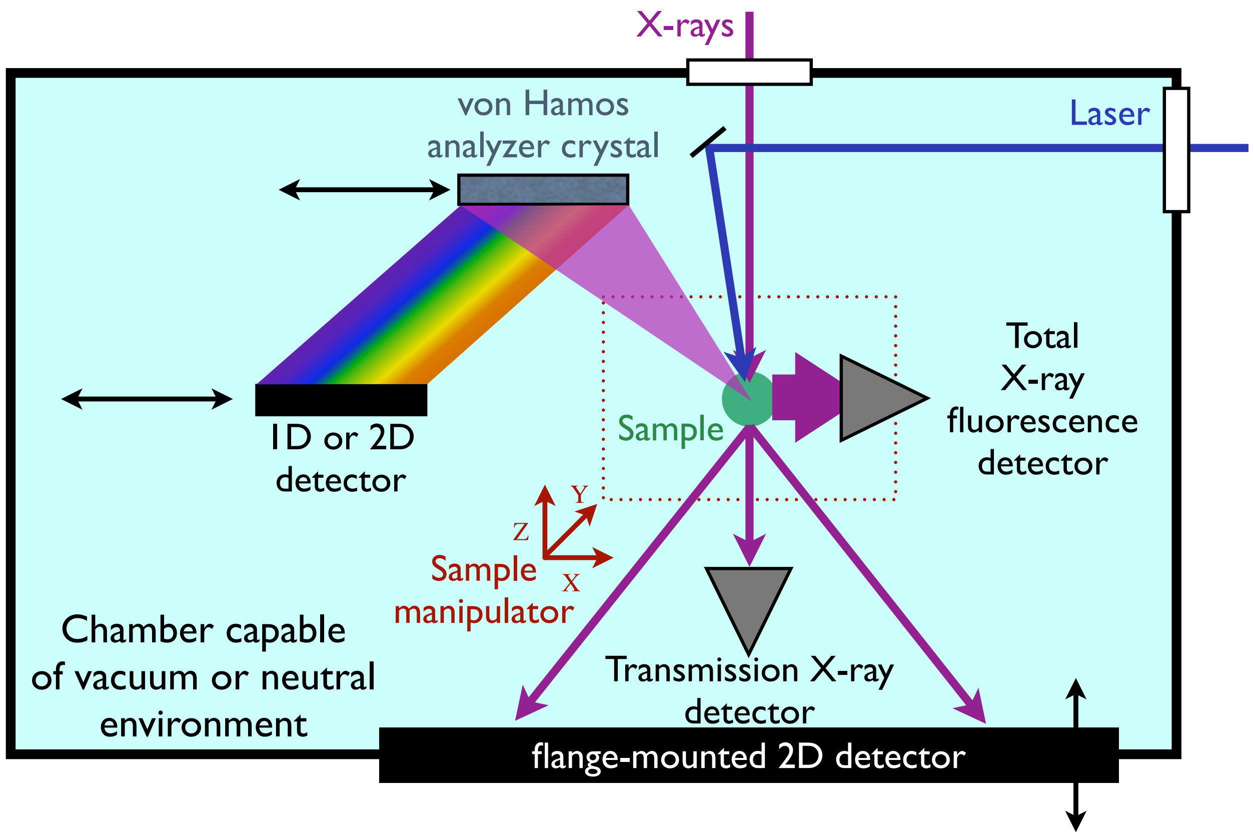


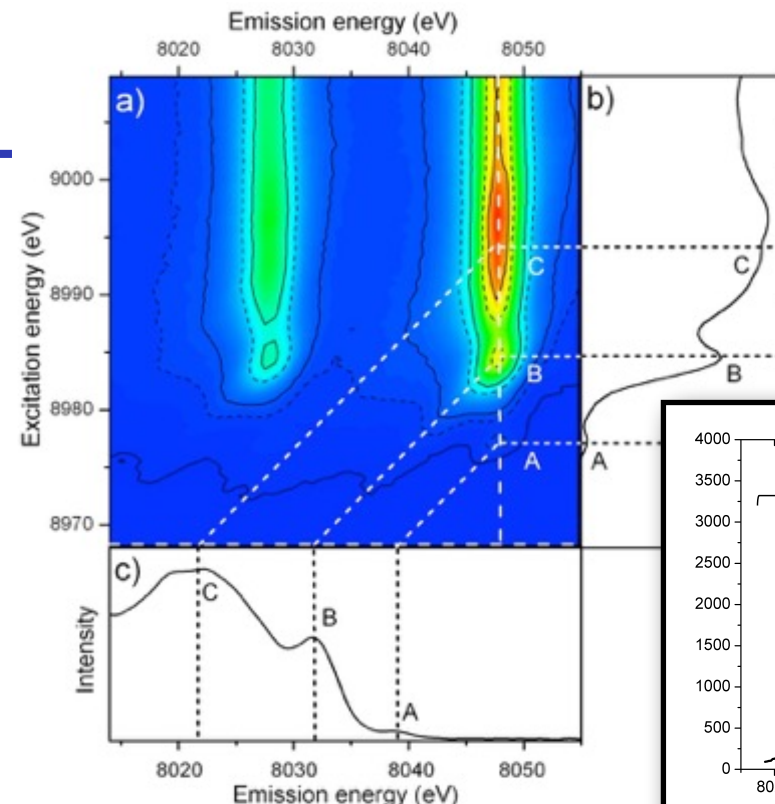
R. Neutze and K. Moffat *Curr. Op. Struc. Bio.* **22**, 651 (2012)

We want time-resolved electronic and structural information on these systems as they evolve

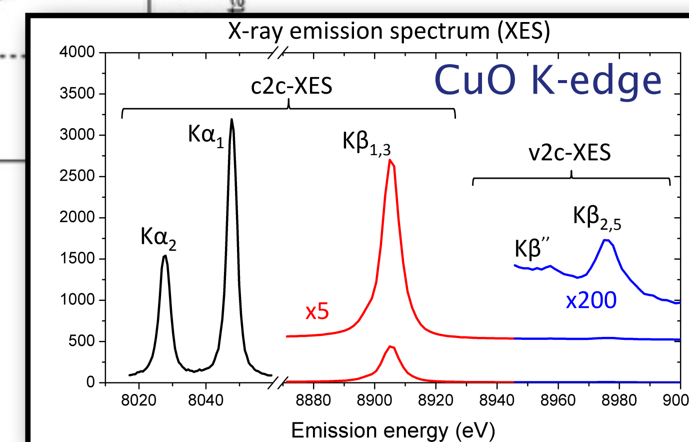
ESA: The X-ray probe techniques

Chris Milne, Gregor Knopp and Jakub Szlachetko

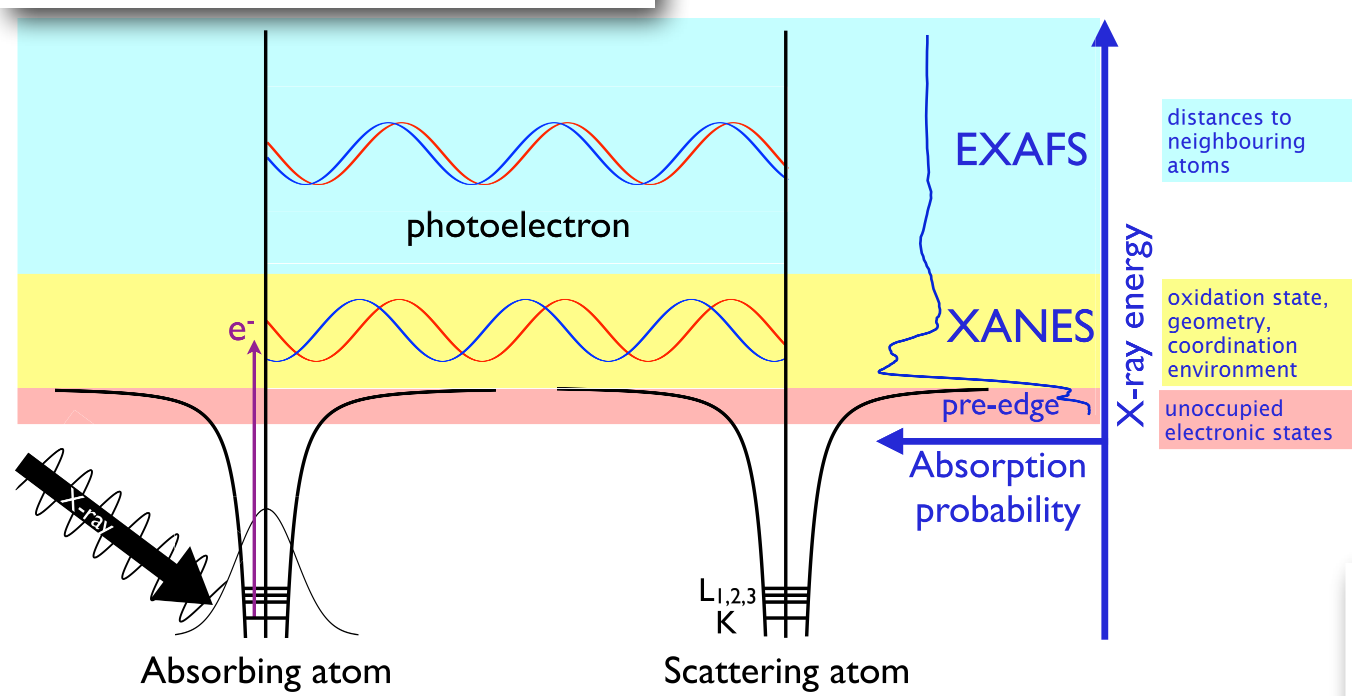




(Resonant) X-ray emission spectroscopy

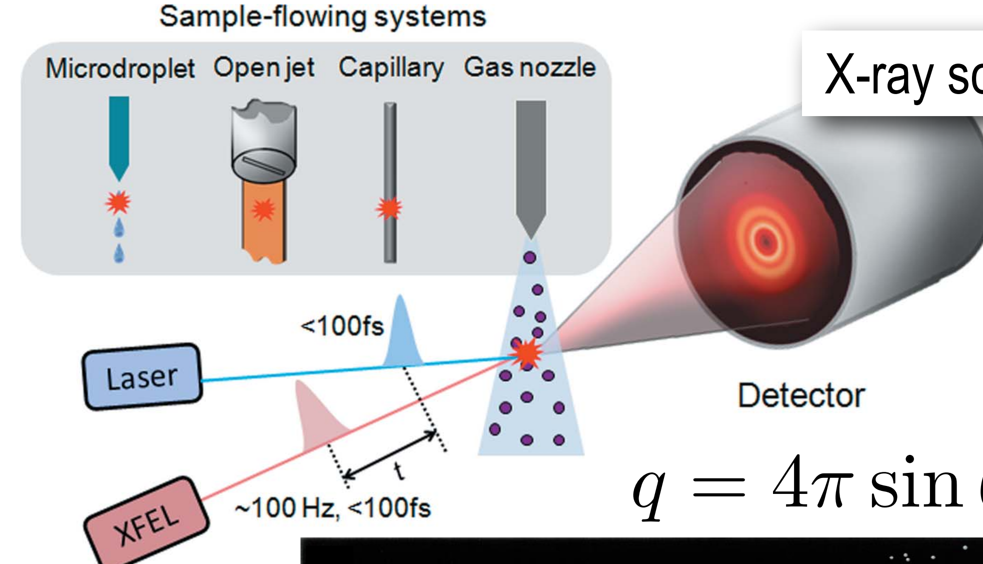


X-ray absorption spectroscopy



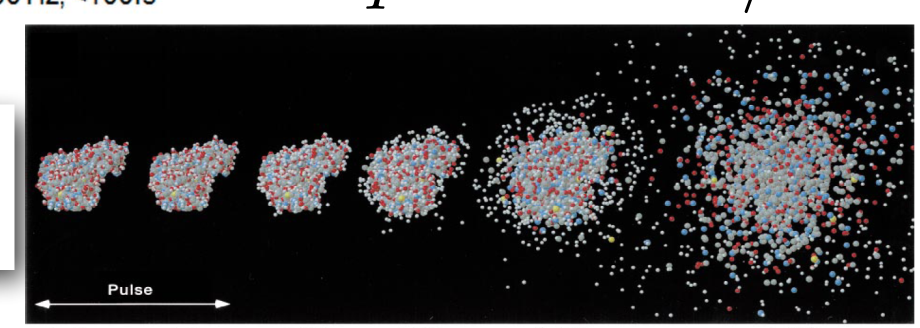
T.J. Penfold et al. *Adv. Chem. Phys.* **143** 1-41 (2013)

X-ray scattering



$$q = 4\pi \sin\theta / \lambda$$

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



PAUL SCHERRER INSTITUT PSI

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Detectors: A. Mozzanica, J. Smith, B. Schmitt

BIO Department J. Standfuss, P. Nogly, G. Schertler, V. Panneels

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Uni. Fribourg: J-C. Dousse, J. Hoszowska, W. Błachucki, F. Zeeshan

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