Femtosecond X-ray diffraction at megahertz repetition rates

Anton Barty

Center for Free Electron Laser Science (overview talk presenting results on behalf of many, many people)

The European XFEL in Hamburg is 3 kilometres long





Scientific Background





Photosynthesis transformed our planet 2.5B years ago producing oxygen, capturing solar energy and CO₂, that slowly converted to fossil fuels.



Source: Petra Fromme, ASU

Why?



Source: Petra Fromme, ASU

Rhodopsin-Arrestin complex solved at LCLS



Y. Kang, ... E. Xu et.al.

"Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser.," Nature, vol. 523, no. 7562, pp. 561–567, Jul. 2015.

Conit Cell: a=b=109.2 A, C=452.6 A Resolution: 3.8 Å (a*/b*) and 3.3 Å (c*) physically twinned, pseudo-merohedrally twinned diagonal pseudo-translation in *a/b* plane two fold rotational pseudo-symmetry parallel to *a*

δ -opioid receptor crystals delivered in LCP showed superior diffraction at room temperature compared to synchrotron data

Structural basis for bifunctional peptide recognition at human δ-opioid receptor





Crystal size: 1 to 10 μ m (average size of 5 × 2 × 2 μ m) Dose: 46 MGy/crystal (room temperature) Flow rate: 0.17 μ L/min Resolution (synchrotron): 3.3 Å (cryo) Resolution (LCLS): 2.7 Å (room temperature)

G. Fenalti, ... V. Cherezov et al. (2015). Structural basis for bifunctional peptide recognition at human delta-opioid receptor Nature Structural & Molecular Biology, 22(3), 265–268.

Data is merged from thousands of separate crystals



X-ray radiation destroys protein crystals during exposure



Axford et.al. *Acta Cryst (2012). D68, 592-60,* pp. 1–9, Apr. 2012.

Crystal of Bovine enterovirus 2 (BEV2) after subsequent exposures of 0.5 s, at $6x10^8$ ph/ μ m² 300 kGy dose, room temperature

Cryogenic cooling extends dose limit to 30 MGy

Gy = 1 J/kg				
MGy ≈1eV/Da absorbed				
≈ 0.16 eV / atom!				
≈2×10º ph/µm²				
(about one ionisation per 10 amino-acid residues)				

The gain in peak brightenss at an FEL is several orders of magnitude





Source: Robert Feidenhans'l

X-ray free electron lasers are a unique class of X-ray source





operational 2017 1-12 keV 20-50 fs 10¹³ photons/pulse

European X-ray FEL, DESY, Hamburg

Operational 2009 0.8-8 keV 100 fs 10¹² photons/pulse

Linac Coherent Light Source, SLAC, Stanford



operational 2006

50-400 eV (7-50 nm) 25 fs 10¹² photons/pulse

FLASH DESY, Hamburg



Source: Thomas Tschentscher

The European XFEL free electron laser facility in Hamburg opened for users in 2017







17.5 GeV 27,000 pulses per second

A brief overview of the EuXFEL accelerator







Source: Thomas Tschentscher

800 Nb cavities, 100 cryo modules, ~1000m long accelerator, 17.5 GeV,

Experimental Hall





Source: Thomas Tschentscher

First user experiments at the European XFEL started on 14 September 2017



SFX at the European XFEL





beam and need to recover before the

5.5 µs 10.7 µs

200 µm

Linuid a

nature

physics

ARTICLES

Liquid explosions induced by X-ray laser pulses

Claudia A. Stan¹*, Despine Milathianaki², Hartawan Laksmono¹, Raymond G. Sierra¹, Trevor A. McQueen³, Marc Messerschmidt²², Garth J. Williams²³, Jason E. Koglin², Thomas J. Lane², Matt J. Hayes², Serge A. H. Guillet², Mengning Llang², Andrew L. Aquila², Philip R. Willmott^{2,4}, Joseph S. Robinson², Karl L. Gumerlock³, Sabine Botha⁴⁴, Karol Nass⁴, Ilme Schlichting⁴, Robert L. Shoeman⁶, Howard A. Stone⁴ and Sébastien Boutet³



Shock waves propagate even faster

Claudiu Stan, SLAC, Nature Physics, 2016

Fast jets recover in time for the next pulse at 1.1 MHz repetition rate



9.3 keV
580 mJ XFEL pulses
10 µm FWHM focus (or smaller)
1.1 MHz repetition rate

Max Wierdorn, Claudio Stan

Fast jets recover in time for the next pulse at 1.1 MHz repetition

t = 37 ns





Max Wierdorn, Claudio Stan

rate

Fast jets recover in time for the next pulse at 1.1 MHz repetition

t = 37 ns







Max Wierdorn, Claudio Stan

rate

CFEL-designed fast jets recover in time for the next pulse at I.I MHz repetition rate

t = 37 ns







Max Wierdorn, Claudio Stan

The Adaptive Gain Integrating Pixel Detector (AGIPD) can read out 3520 frames per second with MHz pulse spacing

1 M Pixels 4.5 MHz pulse rate 3520 frames per second 7 GB per second 25 TB per hour

Energy range	3keV-18keV
Frame rate	> 4.5MHz (burst)
Memory depth	352 frames
Dynamic range	1 to 10 ⁴ photons/pixel/frame at 12.4 keV
Pixel size	(200µm)²
Operating principle	Charge integrating
Dynamic gain switching	Yes (3 gains)
Single Photon sensitivity	Yes



Pixel circuit overview



We can extract reasonable looking diffraction patterns with low noise and high dynamic range thanks to dynamic gain switching



Crystal hits are evenly distributed through the pulse train



Wiedorn et.al. "Megahertz serial crystallography" Nat Commun. 9, 4025 (2018).

Lysozyme data confirms an accurate structure can be measured to I.76Å resolution using MHz pulse trains



We solved the unknown structure of Beta-lactamase CTX-M-14 to 1.7 Å using data from 12,500 indexed lattices

PDB: 6GTH



2Fo-Fc map (at 1.5 σ) over the corresponding part of the model of β -lactamase CTX-M14 from multidrug resistant *Klebsiella pneumoniae*.

P3221 Unit Cell: 41.8 41.8 233.3 90 90 120

Detailed view of the binding of the ß-lactamase inhibitor avibactam to Ser70. The inhibitor is bound covalently to the protein (hemiacetal).

Wiedorn et.al. "Megahertz serial crystallography" Nat Commun. 9, 4025 (2018).

Sample: Christian Betzel, UHH

Over 125 authors from 38 institutions contributed to this work



NATURE COMMUNICATIONS | DOI: 10.1038/s41467-018-06156-7

ARTICLE

DOI: 10.1038/s41467-018-06156-7 OPEN

Megahertz serial crystallography

Max O. Wiedorn b al.#

Max O. Wiedorn ^{1,2,3}, Dominik Oberthür¹, Richard Bean⁴, Robin Schubert^{3,5,6}, Nadine Werner⁵, Brian Abbey ⁷, Martin Aepfelbacher⁸, Luigi Adriano⁹, Aschkan Allahgholi⁹, Nasser Al-Qudami⁴, Jakob Andreasson^{10,11,12}, Steve Aplin¹, Salah Awel^{1,3}, Kartik Ayyer¹, Saša Bajt⁹, Imrich Barák¹³, Sadia Bari⁹, Johan Bielecki⁴, Sabine Botha^{3,5}, Djelloul Boukhelef⁴, Wolfgang Brehm¹, Sandor Brockhauser⁰, ^{4,14}, Igor Cheviakov⁸, Matthew A. Coleman¹⁵, Francisco Cruz-Mazo¹⁶, Cyril Danilevski⁴, Connie Darmanin⁷, R. Bruce Doak¹⁷, Martin Domaracky ¹, Katerina Dörner⁴, Yang Du¹, Hans Fangohr^{4,18}, Holger Fleckenstein¹, Matthias Frank ¹⁵, Petra Fromme¹⁹, Alfonso M. Gañán-Calvo¹⁶, Yaroslav Gevorkov^{1,20}, Klaus Giewekemeyer⁴, Helen Mary Ginn^{21,22}, Heinz Graafsma^{9,23}, Rita Graceffa⁴, Dominic Greiffenberg²⁴, Lars Gumprecht¹, Peter Göttlicher⁹, Janos Hajdu^{10,11}, Steffen Hauf⁴, Michael Heymann²⁵, Susannah Holmes⁷. Daniel A. Horke^{1,3}, Mark S. Hunter²⁶, Siegfried Imlau¹, Alexander Kaukher⁴, Yoonhee Kim⁴, Alexander Klyuev⁹, Juraj Knoška ^{1,2}, Bostjan Kobe ²⁷, Manuela Kuhn⁹, Christopher Kupitz²⁸, Jochen Küpper ^{1,2,3,29}, Janine Mia Lahey-Rudolph^{1,30}, Torsten Laurus⁹, Karoline Le Cong⁵, Romain Letrun¹,⁴, P. Lourdu Xavier^{1,31} Luis Maia⁴, Filipe R.N.C. Maia ^{10,32}, Valerio Mariani¹, Marc Messerschmidt⁴, Markus Metz¹, Davide Mezza ²⁴, Thomas Michelat⁴, Grant Mills⁴, Diana C.F. Monteiro³, Andrew Morgan¹, Kerstin Mühlig¹⁰, Anna Munke¹⁰, Astrid Münnich⁴, Julia Nette³, Keith A. Nugent⁷, Theresa Nuguid⁵, Allen M. Orville²², Suraj Pandey²⁸, Gisel Pena¹, Pablo Villanueva-Perez¹, Jennifer Poehlsen⁹, Gianpietro Previtali⁴, Lars Redecke^{8,30}, Winnie Maria Riekehr³⁰, Holger Rohde⁸, Adam Round⁴, Tatiana Safenreiter¹, Iosifina Sarrou¹, Tokushi Sato^{1,4}, Marius Schmidt²⁸, Bernd Schmitt²⁴, Robert Schönherr³⁰, Joachim Schulz⁴, Jonas A. Sellberg³³, M. Marvin Seibert¹⁰, Carolin Seuring ^{1,3}, Megan L. Shelby¹⁵, Robert L. Shoeman¹⁷, Marcin Sikorski⁴, Alessandro Silenzi⁴, Claudiu A. Stan³⁴, Xintian Shi²⁴, Stephan Stern^{1,4}, Jola Sztuk-Dambietz⁴, Janusz Szuba⁴, Aleksandra Tolstikova¹, Martin Trebbin^{3,35,36}, Ulrich Trunk⁹, Patrik Vagovic^{1,4}, Thomas Ve³⁷, Britta Weinhausen⁴, Thomas A. White¹, Krzysztof Wrona⁴, Chen Xu⁴, Oleksandr Yefanov¹, Nadia Zatsepin³⁸, Jiaguo Zhang²⁴, Markus Perbandt^{3,5,8}, Adrian P. Mancuso⁴, Christian Betzel^{3,5,6}, Henry Chapman ^{1,2,3} & Anton Barty¹

A tale of two papers



Experiments: September 2017, March 2018

Published: 2 Oct 2018

ARTICLE

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Megahertz serial crystallography

Max O. Wiedorn et al.[#]

Max O. Wiedorn (2^{1,2,3}, Dominik Oberthür¹, Richard Bean⁴, Robin Schubert^{3,5,6}, Nadine Werner⁵, Brian Abbey (2⁷, Martin Aepfelbacher⁸, Luigi Adriano⁹, Aschkan Allahgholi⁹, Nasser Al-Qudami⁴, Jakob Andreasson^{10,11,12} Steve Aplin¹, Salah Awel^{1,3}, Kartik Ayyer¹, Saša Bajt⁹, Imrich Barák¹, Sadia Bari⁹, Johan Bielecki⁴, Sabine Botha^{3,5}, Djelloul Boukhelef⁴, Wolfgang Brehm¹, Sandor Brockhauser ^{4,14}, Igor Cheviakov⁸, Matthew A. Coleman¹⁵, Francisco Cruz-Mazo¹⁶, Cyril Danilevski⁴, Connie Darmanin⁷, R. Bruce Doak¹⁷, Martin Domaracky^[6], Katerina Dörner⁴, Yang Du¹, Hans Fangohr^{4,18}, Holger Fleckenstein¹, Matthias Frank^{[6] 15}, Petra Fromme¹⁹, Alfonso M. Gañán-Calvo¹⁶, Yaroslav Gevorkov^{1,20}, Klaus Giewekemeyer⁴, Helen Mary Ginn^{21,22}, Heinz Graafsma^{9,23}, Rita Graceffa⁴, Dominic Greiffenberg²⁴, Lars Gumprecht¹, Peter Göttlicher⁹, Janos Hajdu^{10,11}, Steffen Hauf⁴, Michael Heymann²⁵, Susannah Holmes⁷, Daniel A. Horke 1, Mark S. Hunter²⁶, Siegfried Imlau¹, Alexander Kaukher⁴, Yoonhee Kim⁴, Alexander Klyuev⁹, Juraj Knoška ^{1,2}, Bostjan Kobe ²⁷, Manuela Kuhn⁹, Christopher Kupitz²⁸, Jochen Küpper ^{1,2,3,29}, Janine Mia Lahey-Rudolph^{1,30}, Torsten Laurus⁹, Karoline Le Cong⁵, Romain Letrun⁶, P. Lourdu Xavier^{1,31}, Luis Maia⁴, Filipe R.N.C. Maia ^{10,32}, Valerio Mariani¹, Marc Messerschmidt⁴, Markus Metz¹, Davide Mezza ²⁴, Thomas Michelat¹, Grant Mills⁴, Diana C.F. Monteiro³, Andrew Morgan¹, Kerstin Mühlig¹⁰, Anna Munke¹⁰, Astrid Münnich⁴, Julia Nette³, Keith A. Nugent⁷, Theresa Nuguid⁵, Allen M. Orville²², Suraj Pandey²⁸, Gisel Pena¹, Pablo Villanueva-Perez¹, Jennifer Poehlsen⁹, Gianpietro Previtali⁴, Lars Redecke^{8,30}, Winnie Maria Riekehr³⁰, Holger Rohde⁸, Adam Round⁴, Tatiana Safenreiter¹, Iosifina Sarrou¹, Tokushi Sato^{1,4}, Marius Schmidt²⁸, Bernd Schmitt⁶²⁴, Robert Schönherr³⁰, Joachim Schulz⁴, Jonas A. Sellberg⁵³, M. Marvin Seibert¹⁰, Carolin Seuring^{1,3}, Megan L. Shelby¹⁵, Robert L. Shoeman¹⁷, Marcin Sikorski⁴, Alessandro Silenzi⁴, Claudiu A. Stan³⁴, Xintian Shi²⁴, Stephan Stern^{1,4}, Jola Sztuk-Dambietz⁴, Janusz Szuba⁴, Aleksandra Tolstikova¹, Martin Trebbin^{3,35,36}, Ulrich Trunk⁹, Patrik Vagovic^{1,4}, Thomas Ve³⁷, Britta Weinhausen⁴, Thomas A. White¹, Krzysztof Wrona⁴, Chen Xu⁴, Oleksandr Yefanov¹, Nadia Zatsepin³⁸, Jiaguo Zhang²⁴, Markus Perbandt^{3,5,8}, Adrian P. Mancuso⁴, Christian Betzel^{3,5,6}, Henry Chapman ^{1,2,3} & Anton Barty¹

Experiments: June 2018

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ARTICLE

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Megahertz data collection from protein microcrystals at an X-ray free-electron laser

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The future of MHz SFX is very promising

MHz SFX is a success

The XFEL2012 data sets could be collected in 3 minutes and 15 minutes assuming no improvements other than 352 pulses per train (3520 frames per second)



Wiedorn et.al. "Megahertz serial crystallography" Nat Commun. 9, 4025 (2018).

We have measured 190,000 crystals in under 30 minutes at 1200 frames per second

EuXFEL 2120: September 2018

Data collection:

120 pulses per train / 1200 per second856,000 hits1.4M Indexed crystals

11k-12k Indexed patterns per/pulse



Refinement:

R_{free} for single pulse: 19%, R_{work} 16% R_{free}/R_{work} for pulses: 1 - 18.4/16.2 2 - 18.9/16.1 3 - 18.5/16.2 20 - 19/16.1 119 - 19.6/16.5 120 - 19.1/16.3 R_{free}/R_{work} for all data: 15.4/13.9

Res. range for refinement: I5A - I.6A B-factors: 25

>130,000 indexed lattices in <30 minutes has been achieved

3 runs (r0096-r0098)
627,228 patterns per run, each approx. 9 minutes long
= 1.9M patterns in total measurement time of approx. 30min.
9-11% hitrate = ~ 200,000 hits in total.

Equivalent to 13,000 indexed lattices in 3 minutes Enough for the BLAC structure in 3 minutes Flow rate at 100 m/s: 13 µL / min 40µL in 3 mins

> Oleksandr Yefanov CFEL

Data across the pulses appears self-consistent



Electron densities appear quite similar between pulses 1 and 2



Blue: Pulse I

Red: Pulse 2

Exploiting the EuXFEL pulse trains for time resolved studies



Pandey et.al. "Time-resolved serial femtosecond crystallography at the European XFEL." Nature Methods 101, 1–11 (2019).

Exploiting the EuXFEL pulse trains for time resolved studies



Photosynthetic yellow protein (PYP)

Difference electron density



Pandey et.al. "Time-resolved serial femtosecond crystallography at the European XFEL." Nature Methods 101, 1–11 (2019).

Membrane proteins: Photosystem I at the European XFEL





Gisriel et.al. Membrane protein megahertz crystallography at the European XFEL. Nat Comms 10, 1–11 (2019)

The majority of biochemical dynamics are initiated by chemical triggers, such as the RNA riboswitch measured at LCLS



What about imaging without crystals?



Combine 10⁵-10⁷ weak measurements



Coherent diffractive imaging is lensless

In conventional microscopy a lens is used to 'phase' the light and form a (magnified) real-space image of the object



Coherent diffractive imaging is lensless

In conventional microscopy a lens is used to 'phase' the light and form a (magnified) real-space image of the object



We detect light at the 'lens' plane and use a computer to recreate the image

Rotating an object results in the Ewald sphere intersecting different parts of diffraction space





Example: simple molecule (Lysozyme, 1108 atoms, 51Å diameter, mol.wt. = 7250) 1.5Å X-rays, 84mm square detector 50mm from focus Diffraction from a single monomer of photosystem I shows continuous coherent speckle extending to the edge of detector

8.2 keV 4 mJ (3x10¹² photons) Single shot pattern 1.2 Å resolution at edge Diffraction from a single monomer of photosystem I results in single photon counting at high resolution

8.2 keV
4 mJ (3x10¹² photons)
Single shot pattern
1.2 Å resolution at edge

The Single Particle Imaging initiative of LCLS is making steady progress towards realising single particle X-ray imaging at FELs



Single molecule X-ray imaging is making steady progress



Diffraction from MS2 virus is weak compared to background



J. Mol. Biol. (1963) 7, 43-54



Electrons interact more strongly with matter



Henderson, R."The Potential and Limitations of Neutrons, Electrons and X-Rays for Atomic-Resolution Microscopy of Unstained Biological Molecules." Quart. Rev. Biophys. 28, 171–193 (1995).

We have demonstrated serial crystallography in an ordinary TEM



 10^{-2}

0-10

5

Resolution (nm⁻¹)

0.0

20

15

10

Time (ms)

Bücker et.al., N.Comms (submitted)

The femtosecond MeV electron diffraction facility at SLAC



Source Parameters

Parameter	Value
Electron beam energy	2 - 4 MeV
Repetition rate	Single shot → 360 Hz
Charge per pulse	1 - 100 fC (10 ⁴ - 10 ⁶ electrons)
Beam emittance	2 - 20 nm-rad
Bunch length	<150 fs FWHM*
Momentum resolution	<0.17Å ⁻¹
Beam spot size	100-200 um (typical), 10 um (FWHM) focused

"Mega-electron-volt ultrafast electron diffraction at SLAC National Accelerator Laboratory" Weathersby et.al. Rev.Sci.Instrum. **86**, 073702 (2015)

Estimate for single shot protein diffraction:

 10^{6} - 10^{7} electrons per pulse into a ~ $1 \mu m$ focus (crystal size)

transverse coherence ~10-20nm (unit cell size)

> $I C \sim 6.2 \times 10^{18}$ electrons I fC ~ 6×10^{3} electrons I pC ~ 6×10^{6} electrons

First results from femtosecond MeV electron diffraction





N₂ density: 2×10^{17} cm³ and effective thickness of 300 μ m.

Total number of incident electrons is 2×10^9 Total exposure time of 300 s is required at 10-fC bunch charge and 120 Hz repetition rate

Spot size can be reduced by focussing at the expense of flux



UED diffraction from 10 μm paraffin crystals



Shen et.al. "Femtosecond mega-electron-volt electron microdiffraction" Ultramicroscopy **184**, 172–176 (2018).

The European XFEL injector is designed for higher bunch charge





W. Decking, H. Weise, "Commissioning of the European XFEL accelerator" <u>https://accelconf.web.cern.ch/AccelConf/ipac2017/papers/moxaa1.pdf</u>

Table 1: Injector Parameters					
Parameter	Design	Achieved			
RF pulse rep. rate [Hz]	10	10			
RF flat top [µs]	650	650			
Bunches/RF pulse	2700	2700			
Bunch charges [pC]	20-1000	20-1000			
Proj. emittance @ 500 pC		1.2			
[mm mrad]					
Slice. emittance @ 500 pC	0.6	0.6^{1}			
[mm mrad]					

European XFEL design: Nominal bunch charge: 20-1000 pC at 2-100 fs 10⁷-10⁹ electrons



Fig. 3 REGAE scheme. Indicated with numbers are: (1) gun cavity, (2) solenoids, (3) collimators, (4) rebunching cavity, (5) dipole for electron energy measurements, (6) target interaction chamber, and (7) detector.

100 fC/pulse (<1e6 electrons)

3 MeV electron microscopes do exist

3 MeV TEM Osaka University





13th Users Meeting European XFEL

Robert Feidenhans'l 23 January 2019

Acknowledgement to the fantastic staff at European XFEL



European XFEL

Source: Robert Feidenhans'l

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13th Users Meeting European XFEL

Robert Feidenhans'l 23 January 2019

And to the fantastic Accelerator Staff





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European XFEL

Source: Robert Feidenhans'l

The first EuXFEL experiment at SFX/SPB was an open collaboration with over 100 participants

Scientists Adrian Mancuso Richard Bean Klaus Giewekemeyer Marjan Hadian Yoonhee Kim Romain Letrun Marc Messerschmidt Grant Mills Adam Round Tokushi Sato Marcin Sikorski Stephan Stern Patrik Vagovic	Dominik Oberthuer Carolin Seuring Imrich Barak Sadia Bari Christian Betzel Matthew Coleman Chelsie Conrad Connie Darmanin XY Fang Petra Fromme Raimund Fromme S. Holmes Inari Kursula 김경현	Max Wiedorn Saša Bajt Jakob Andreasson Salah Awel Miriam Barthelmess Anja Burkhardt Francisco Cruz-Mazo Bruce Doak Yang Du Holger Fleckenstein Matthias Frank Alfonso Gañán Calvo Lars Gumprecht Janos Hajdu	 Anton Barty Steve Aplin Andrew Aquila Kartik Ayyer Wolfgang Brehm Aaron Brewster Henry Chapman Florian Flachsenberg Yaroslav Gevorkov Helen Ginn Rick Kirian Filipe Maia Valerio Mariani Andrew Morgan 	Technology and Data <u>Krzysztof Wrona</u> Djelloul Boukhelef Illia Derevianko Jorge Elizondo Kimon Filippakopoulos Manfred Knaack Siriyala Kujala Luis Maia Maurizio Manetti Bartosz Poljancewicz Gianpietro Previtali Nasser Al-Qudami Eduard Stoica
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FS-CFEL-I group photo at DESY



A fly through the European XFEL tunnels



The end

Backup slides from here on