Serial Femtosecond Crystallography

technical journal club
02.06.2015

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outline

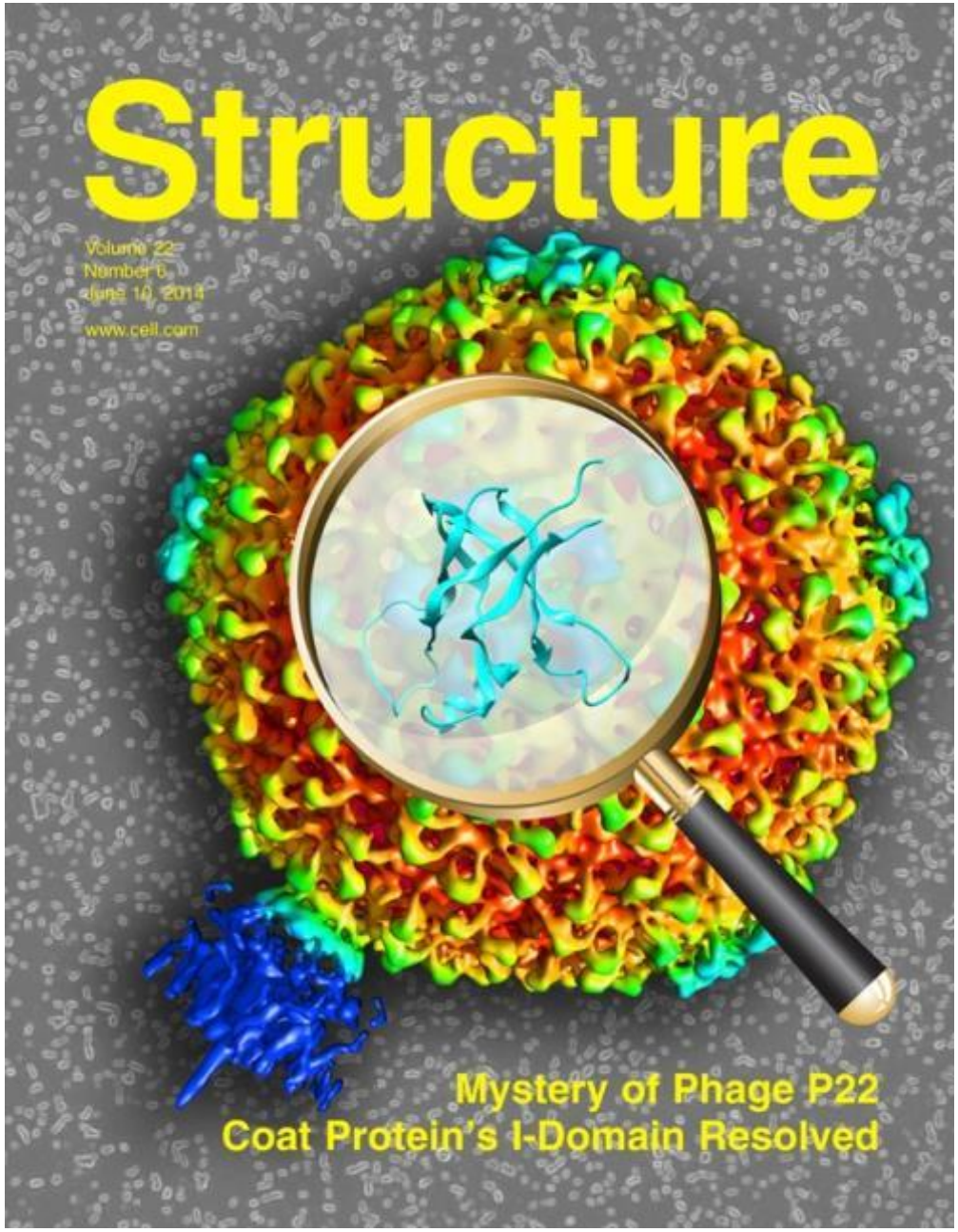
principle of x-ray free electron laser (XFEL)

serial femtosecond crystallography (SFX) *Chapman et al., Nature, 2011*

lipid cubic phase injector facilitates membrane protein SFX *Weierstall et al., Nat Commun, 2014*

serial femtosecond crystallography of G protein-coupled receptors *Liu et al., Science, 2013*

conclusion & outlook



why structure determination?

protein function

▲

interactions

○

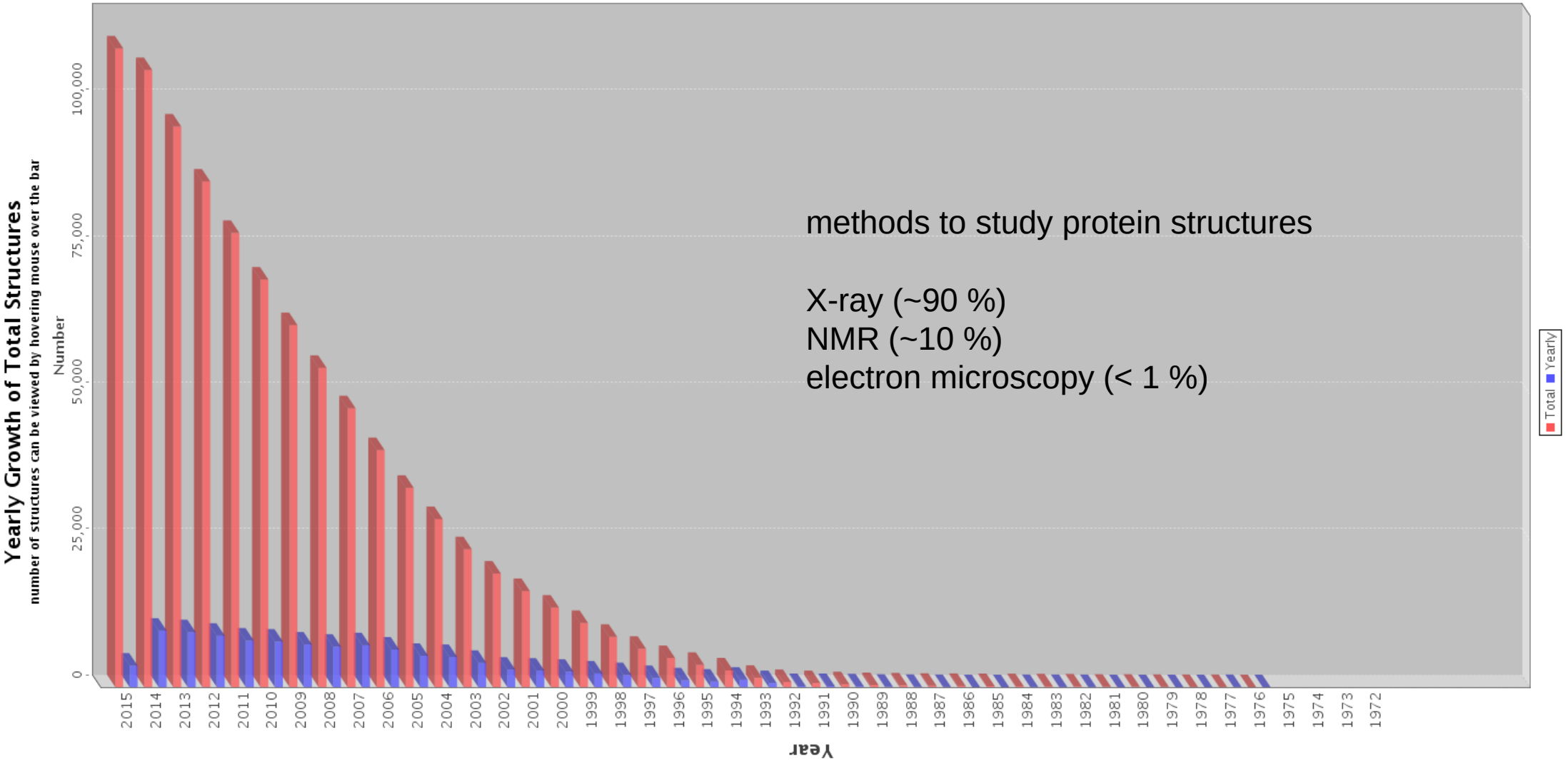
dynamics

▲

structure

elucidate **structures** of macromolecules to understand the **chemical mechanisms** underlying **biological function**

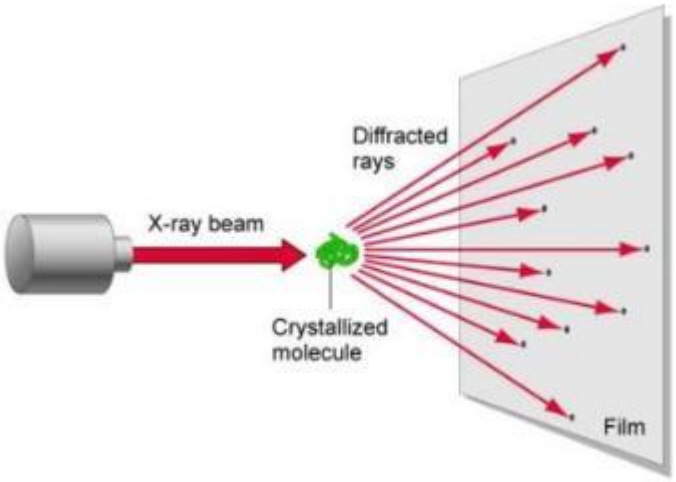
methods for protein structure determination



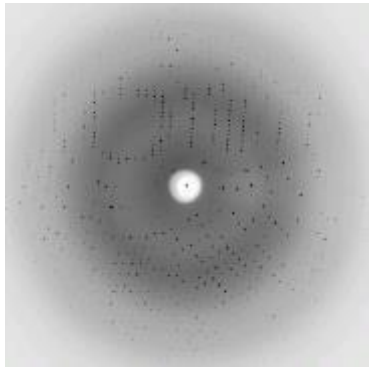
methods to study protein structures

X-ray (~90 %)
NMR (~10 %)
electron microscopy (< 1 %)

principle of x-ray crystallography



diffraction pattern

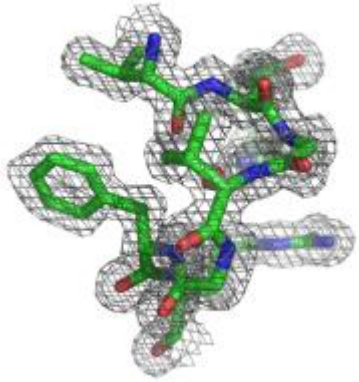


– synchrotron radiation

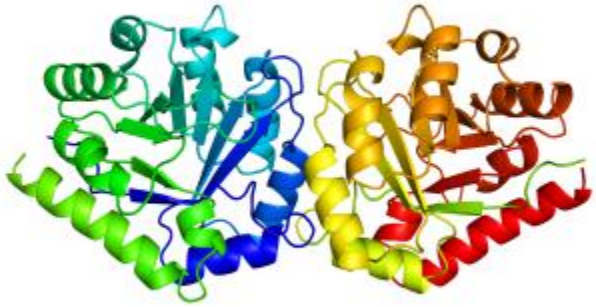
– cryo-cooled
well-ordered
well-diffracting
macroscopic crystals

- ☝ membrane proteins
- ☝ glycosylated proteins
- ☝ complexes

electron density map



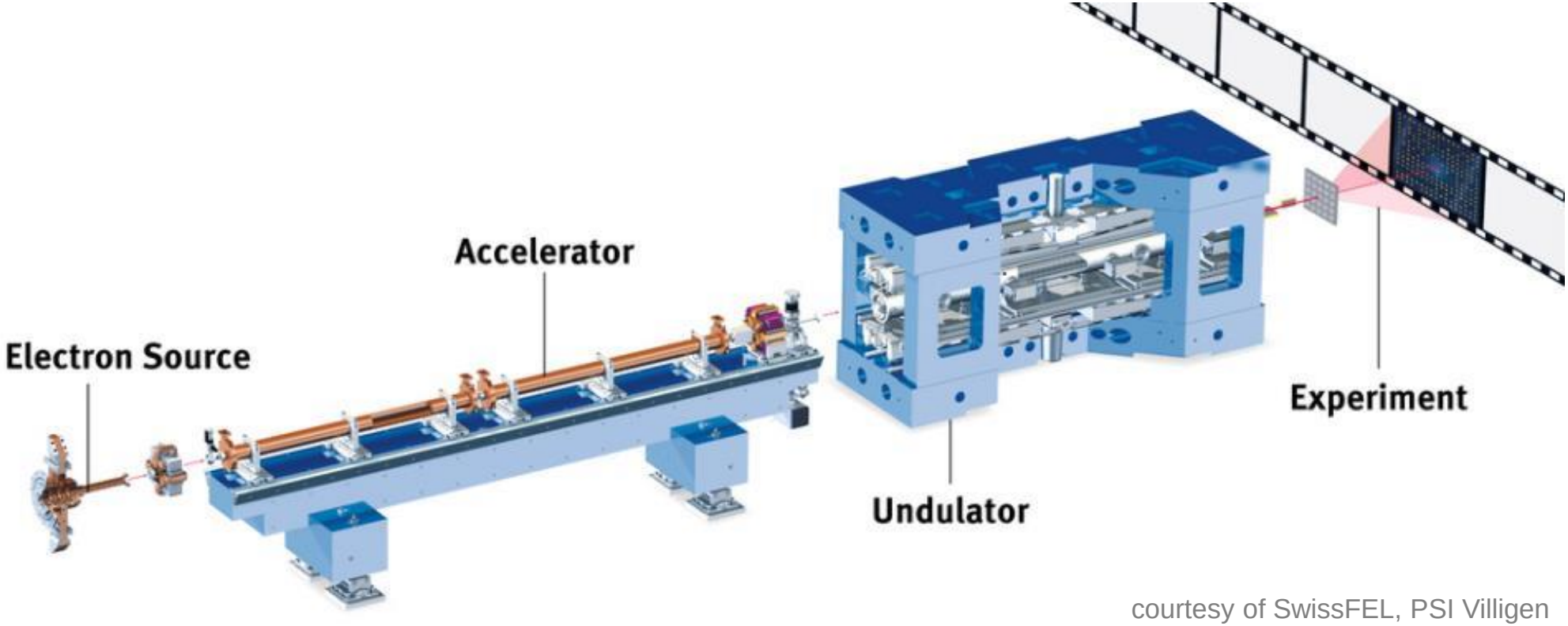
crystal structure



–> x-ray free electron laser

– short light pulses
– peak brillance

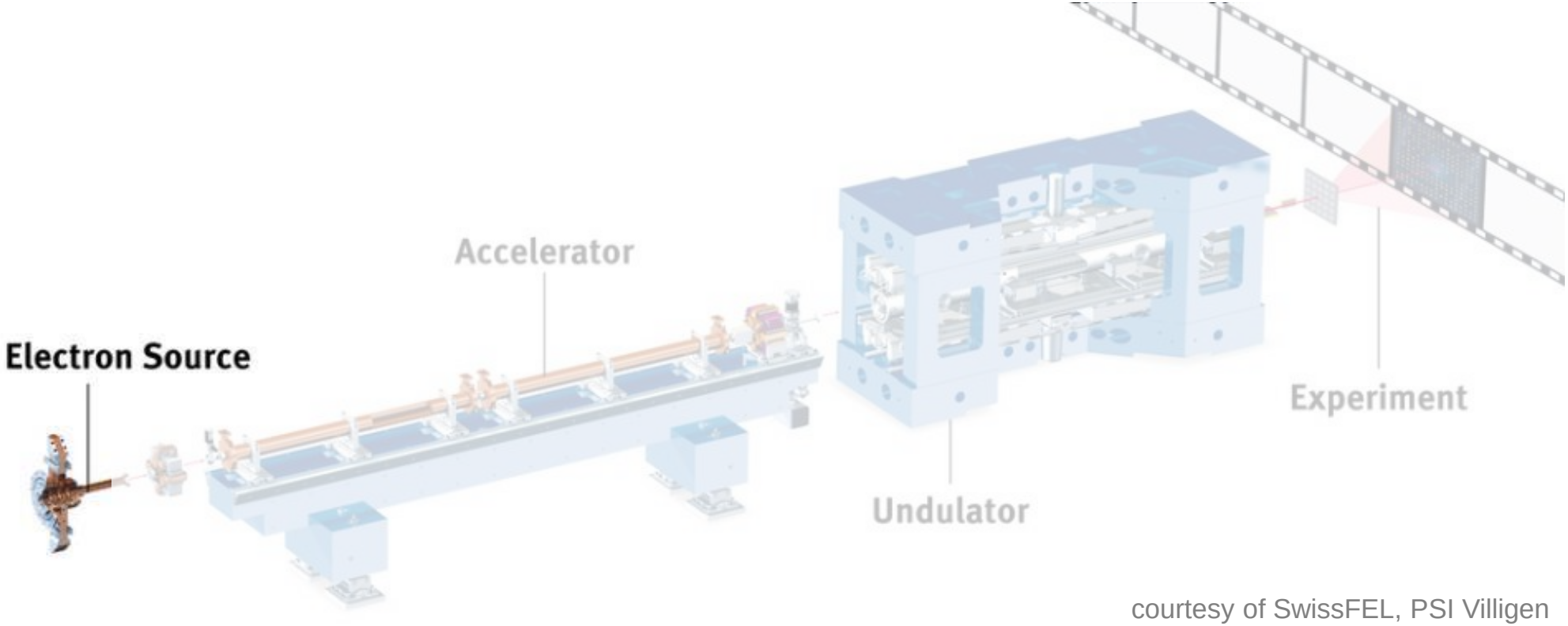
setup of the x-ray free electron laser



courtesy of SwissFEL, PSI Villigen

(Illustration not to scale)

electron source

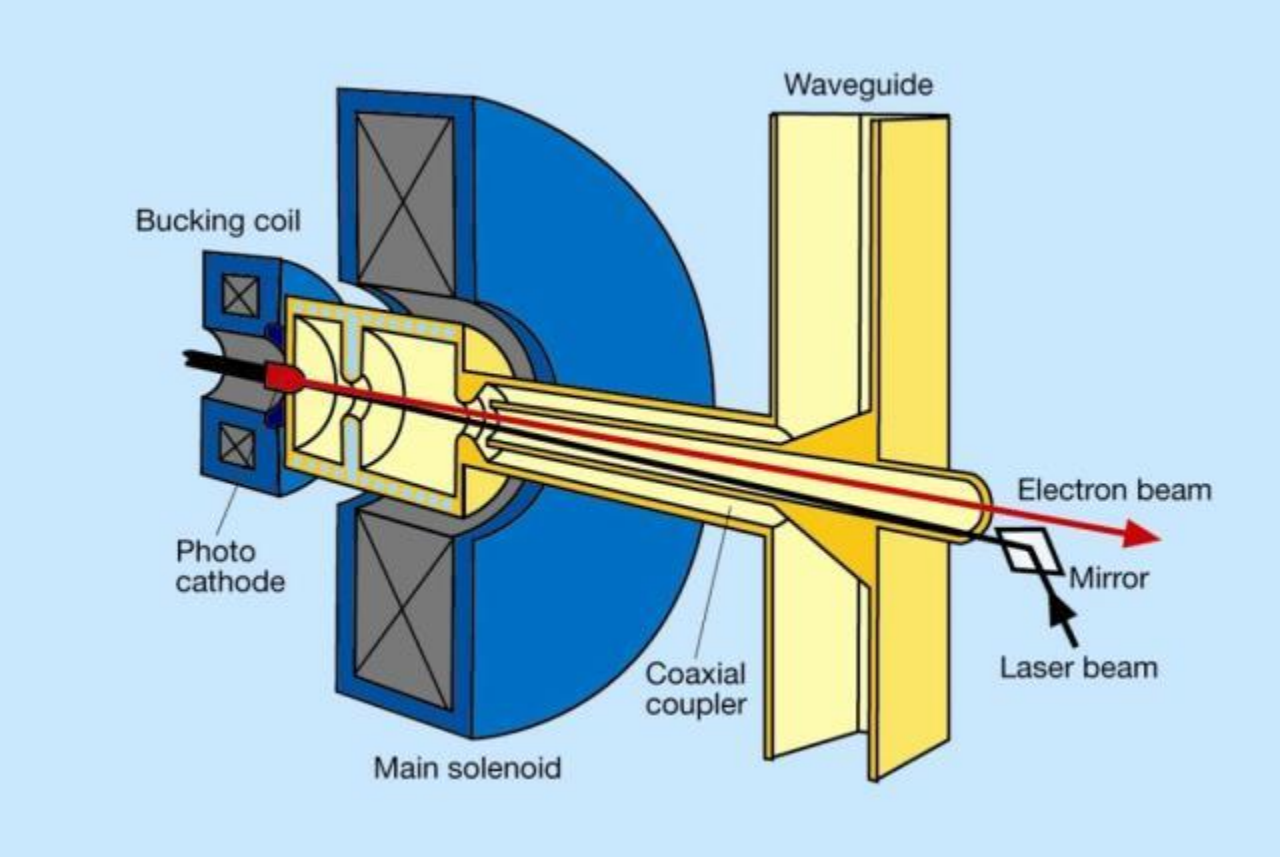


courtesy of SwissFEL, PSI Villigen

(Illustration not to scale)

electron source

electron gun photocathode (Cs_2Te) illuminated with short ultraviolet laser pulses



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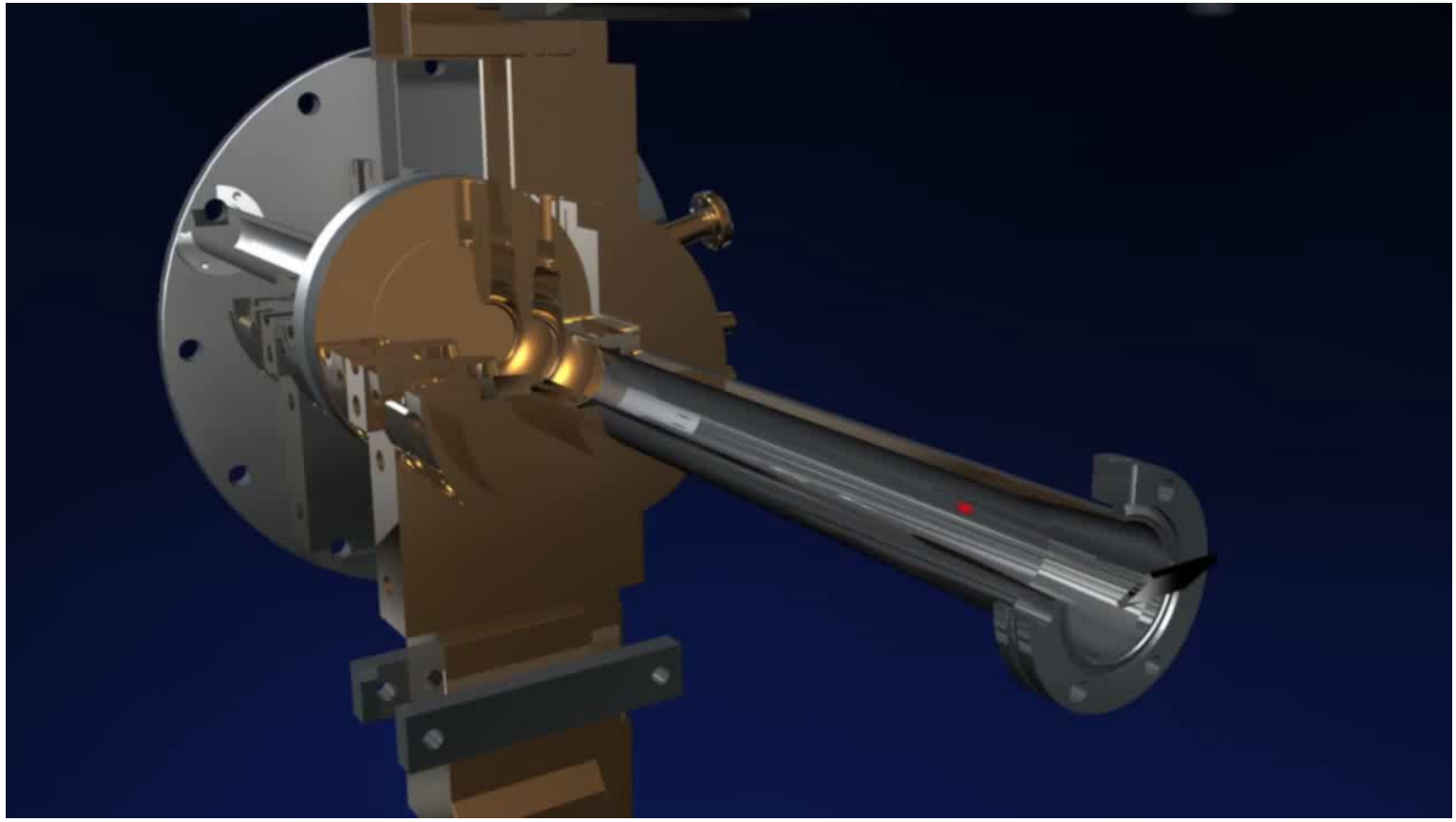
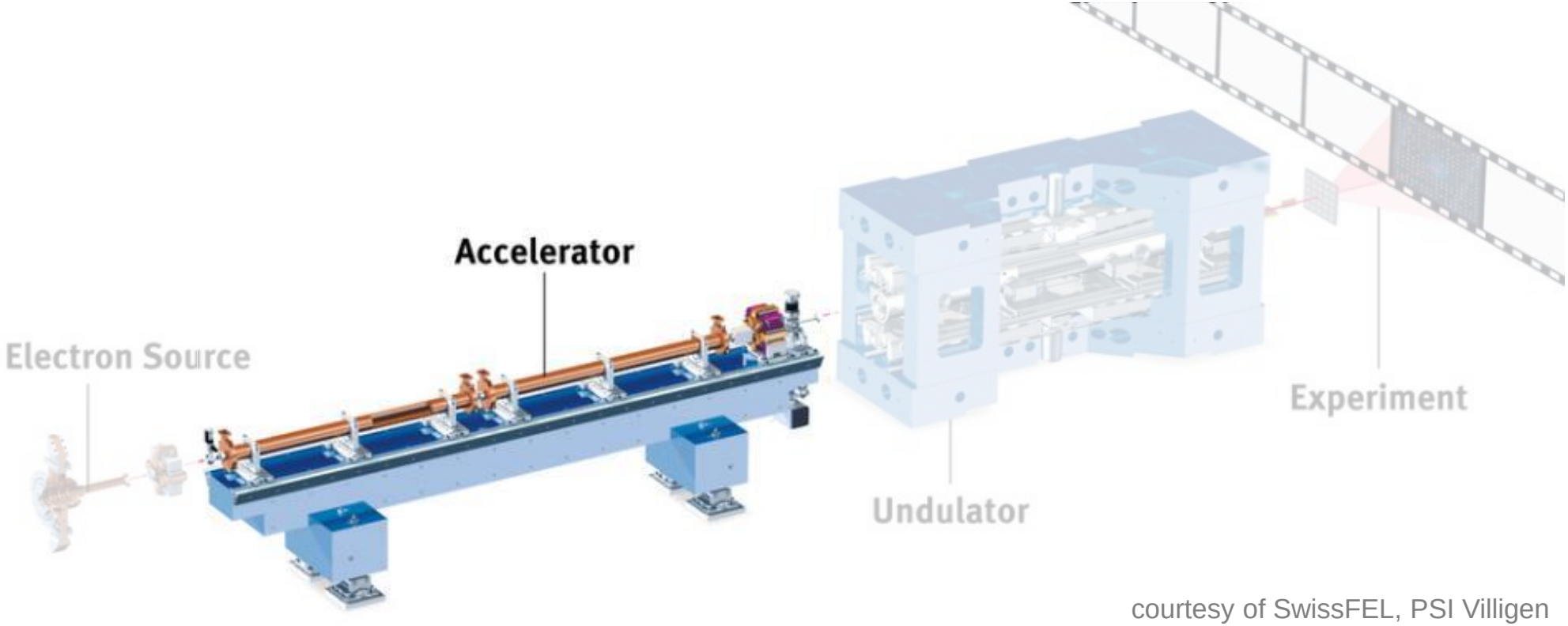


courtesy of SwissFEL, PSI Villigen

linear particle accelerator (LINAC)

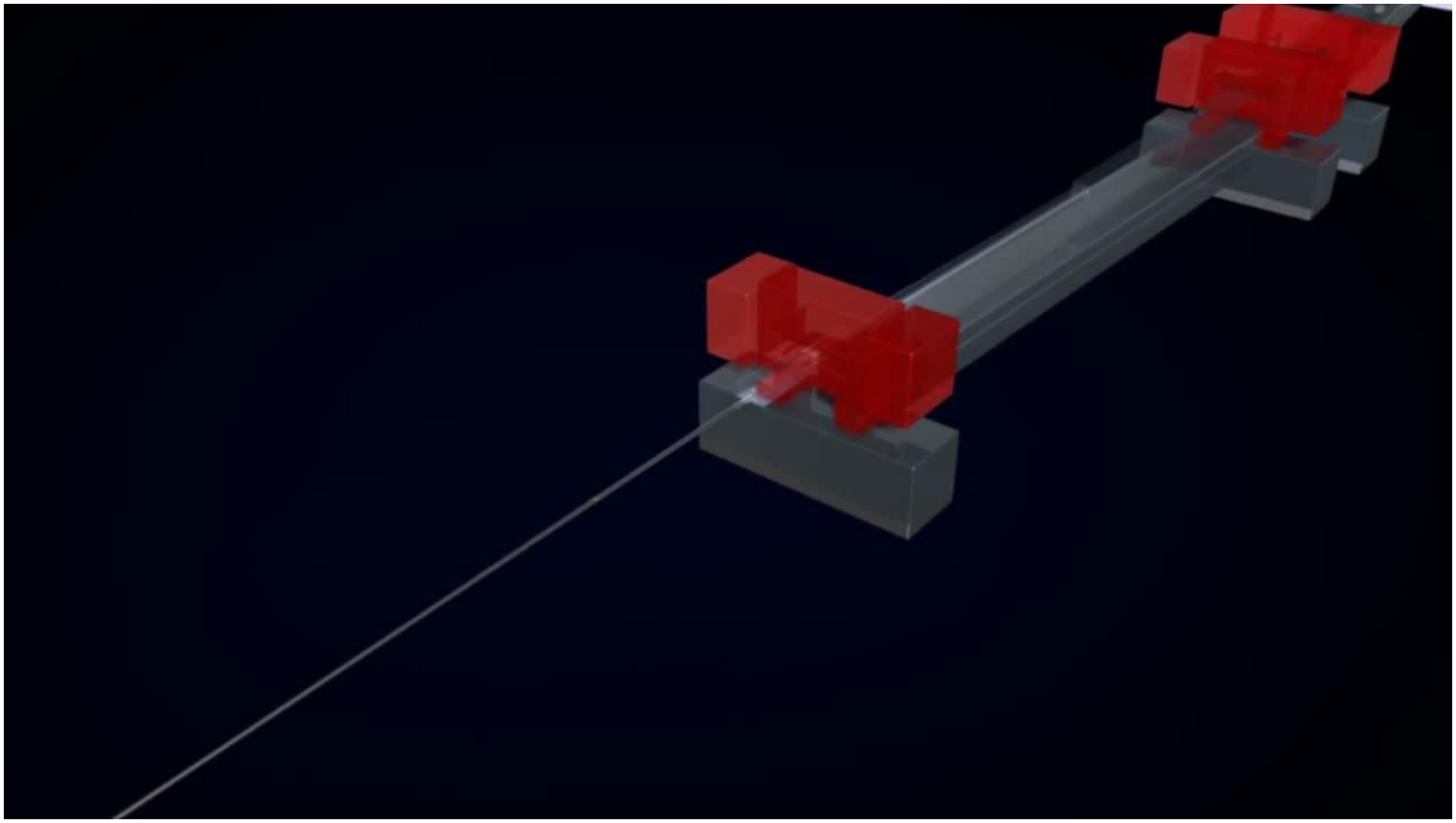


courtesy of SwissFEL, PSI Villigen

linear particle accelerator (LINAC)

bunch compressor magnetic chicanes that compress the bunch of electron

- very short pulse length
- very high charge density



courtesy of SwissFEL, PSI Villigen

linear particle accelerator (LINAC)

bunch compressor magnetic chicanes that compress the bunch of electron

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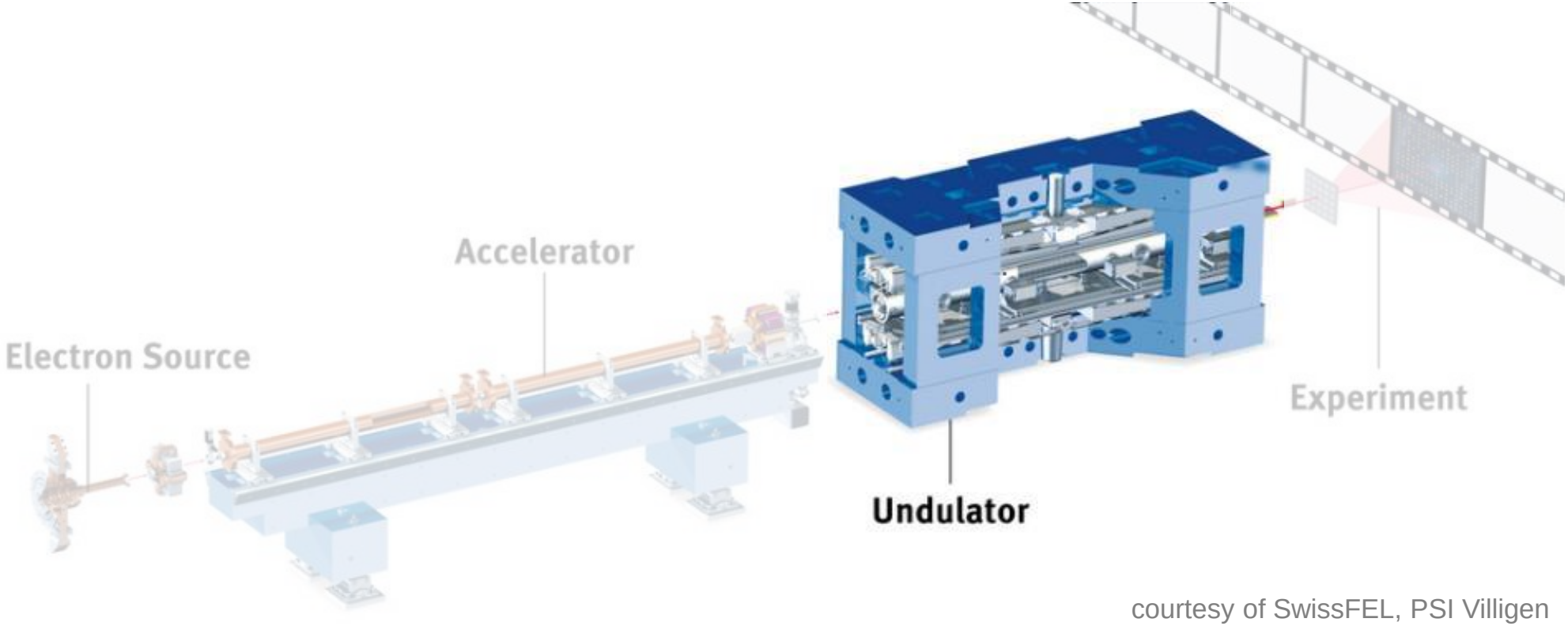
linear accelerator radiofrequency (RF) cavities with alternating electric fields
–> accelerate the electrons

courtesy of SwissFEL, PSI Villigen

undulator



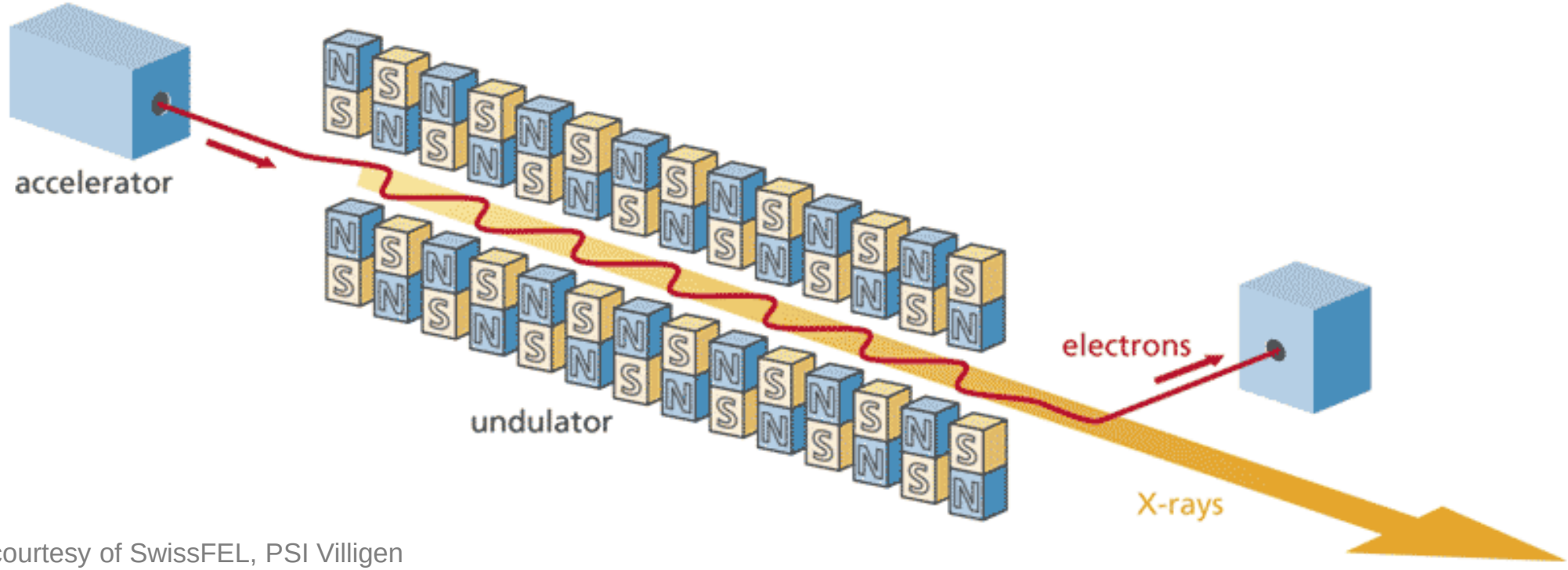
courtesy of SwissFEL, PSI Villigen

undulator

undulator periodic arrangement of short dipole magnets with alternating polarity

deflects electrons into a wavy path

–> radiation of x-rays



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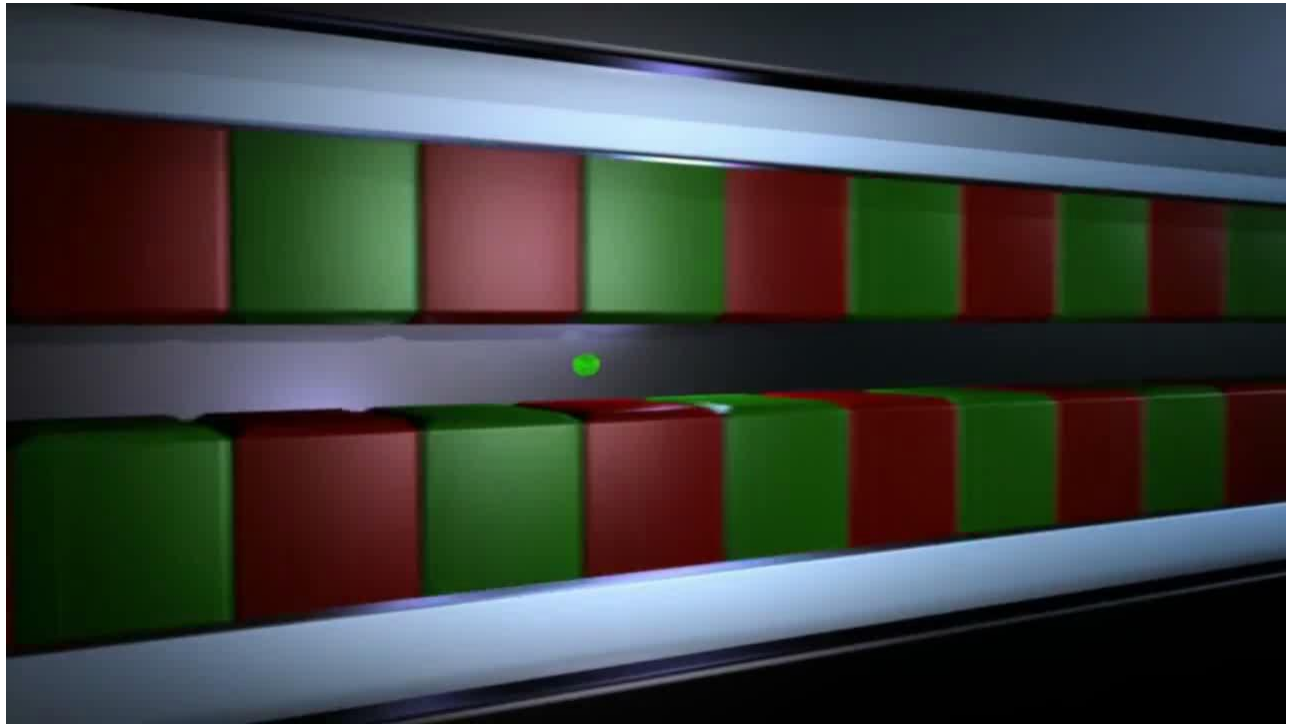


courtesy of SwissFEL, PSI Villigen

experiment

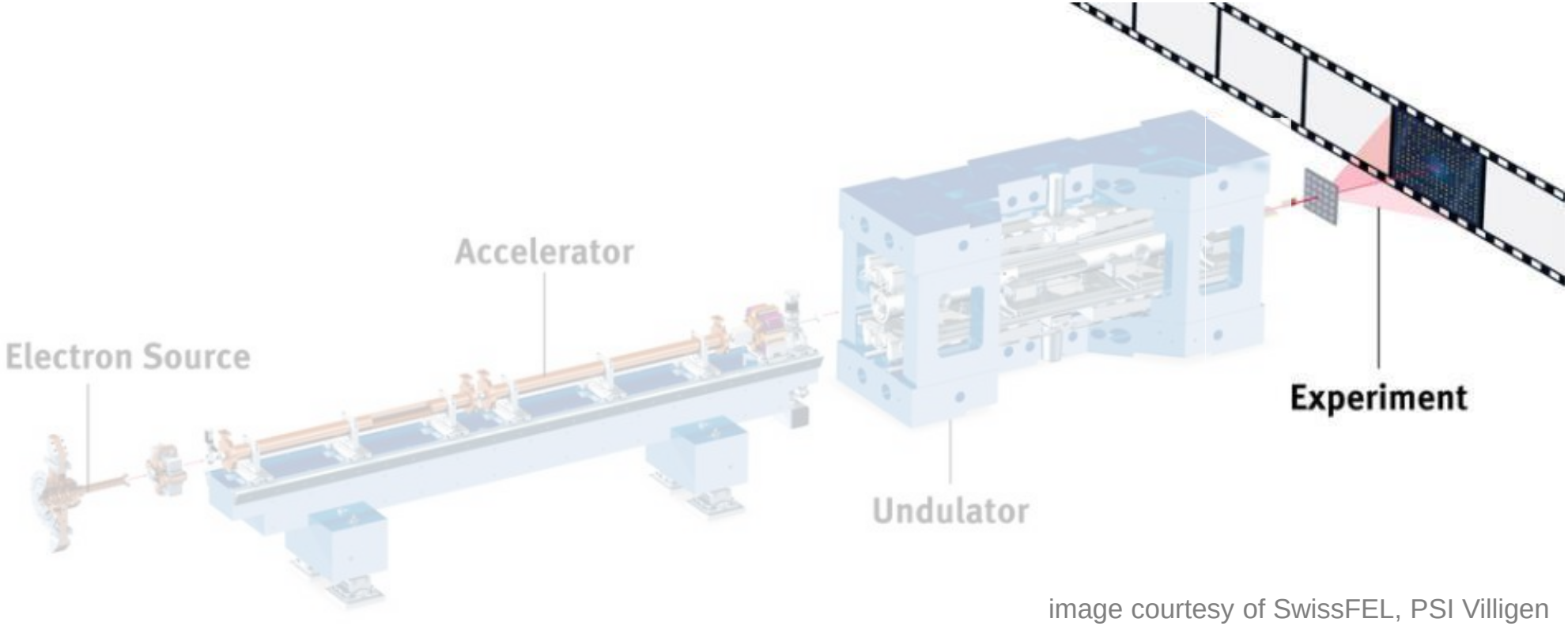
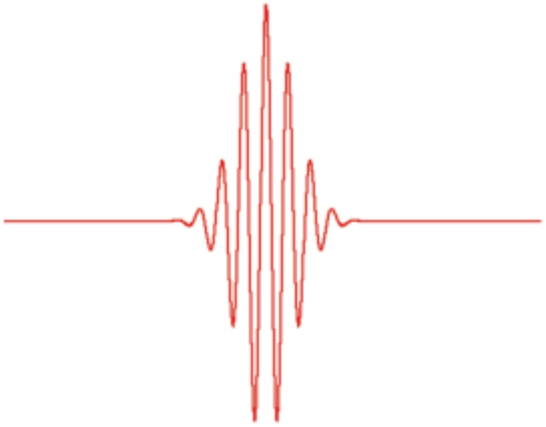


image courtesy of SwissFEL, PSI Villigen

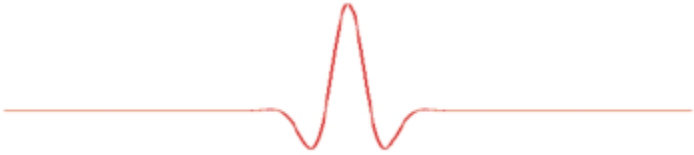
experiment / beamline

XFEL



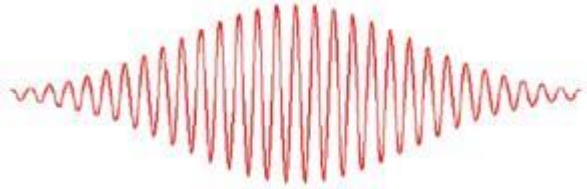
high spatial resolution
high temporal resolution

laser



low spatial resolution
(long wavelength)

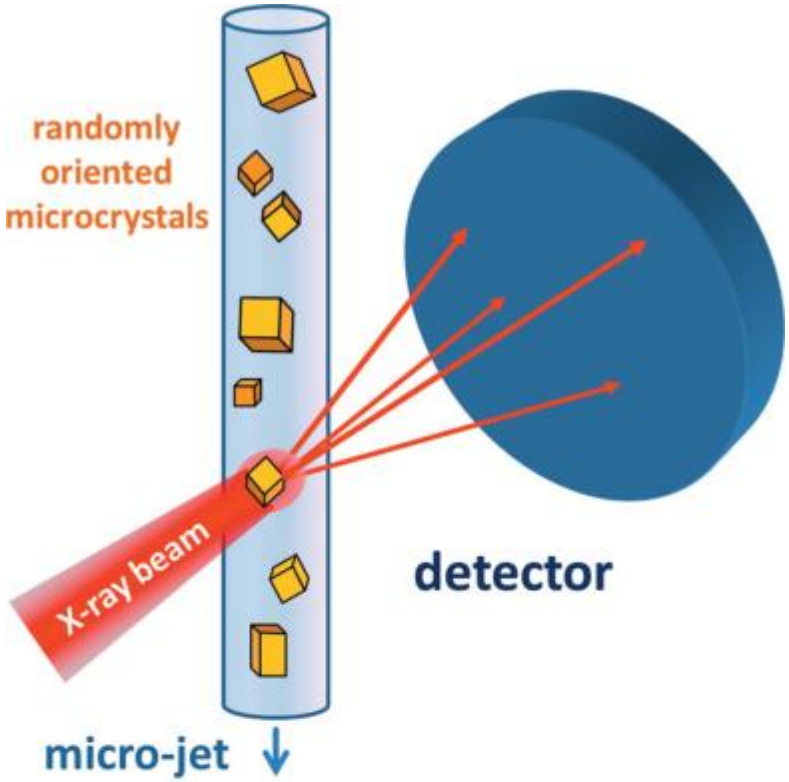
synchrotron



low temporal resolution
(long pulse-length)

highly intense and **ultrashort-pulsed** light allows for imaging of **microcrystals**

serial femtosecond crystallography (SFX)



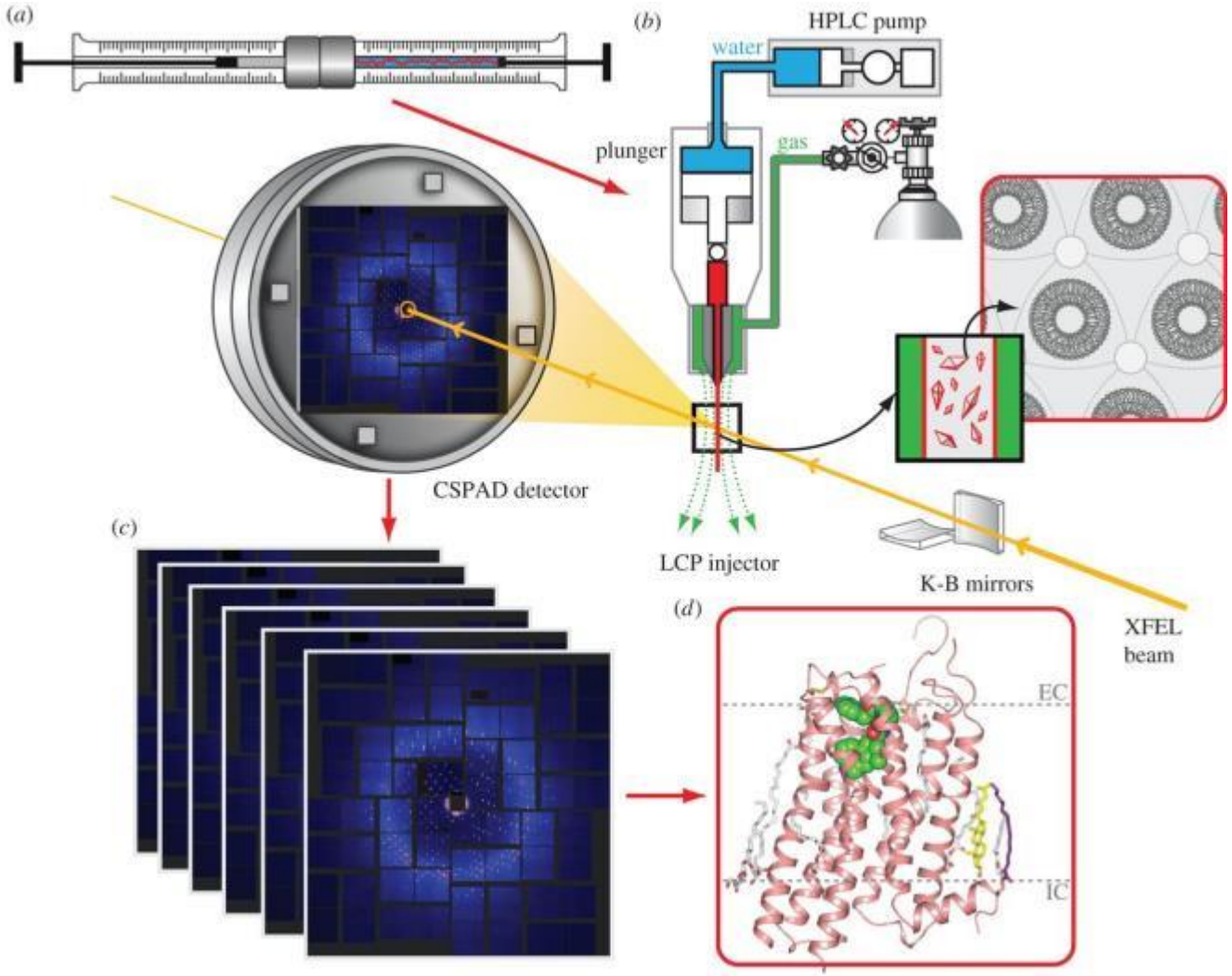
Schlichting, IUCrJ, 2015

“diffraction before destruction”

continuous supply of randomly oriented microcrystals

single crystal exposure

serial femtosecond crystallography (SFX)



lipidic cubic phase (LCP) injector

Weierstall et al., Nat Commun, 2014

Liu W et al., Philos Trans R Soc Lond B Biol Sci, 2014

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]*

Science
AAAS

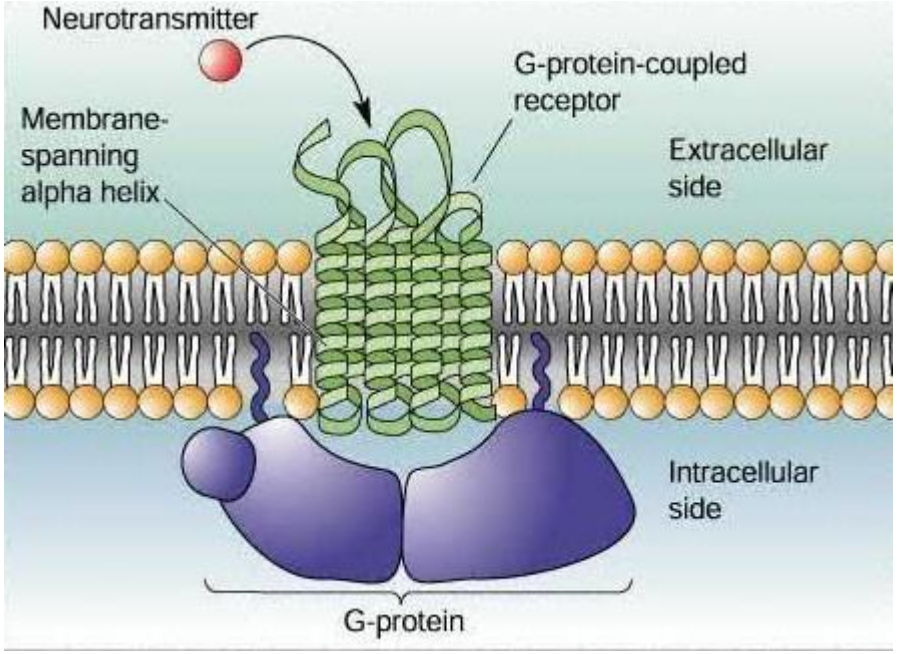
G protein-coupled receptors (GPCRs)

largest family of cell surface proteins

7 transmembrane helices

mediate cellular communication / signal transmission:
ligand binding –> conformational change (GEF)
G protein: exchange GDP for GTP –> downstream signaling cascades

>60 % of current drug targets in humans



challenges for crystallisation of GPCRs:

– low expression yields

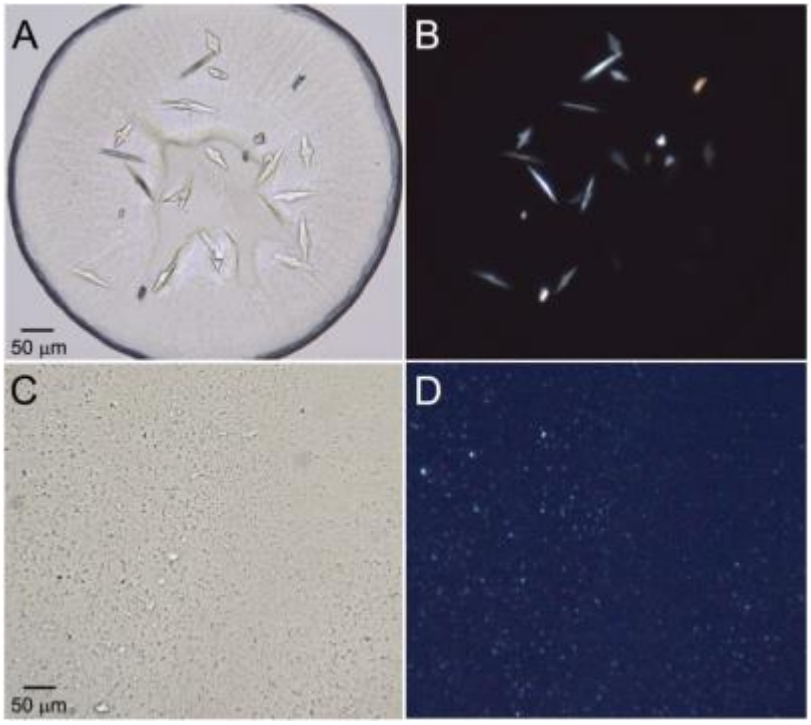
– low receptor stability

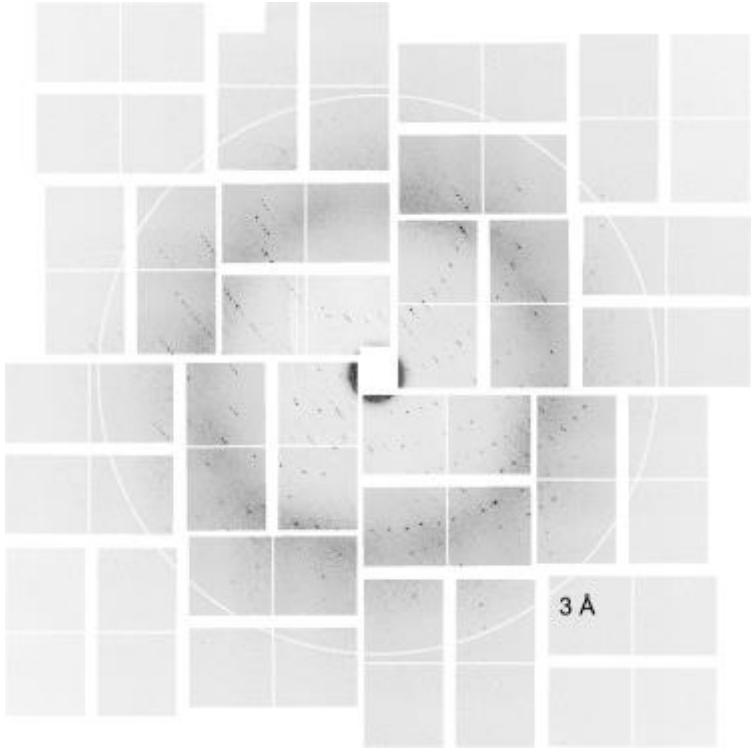
– high conformational heterogeneity

crystallisation of human serotonin 5-HT2B receptor

SYN

XFEL

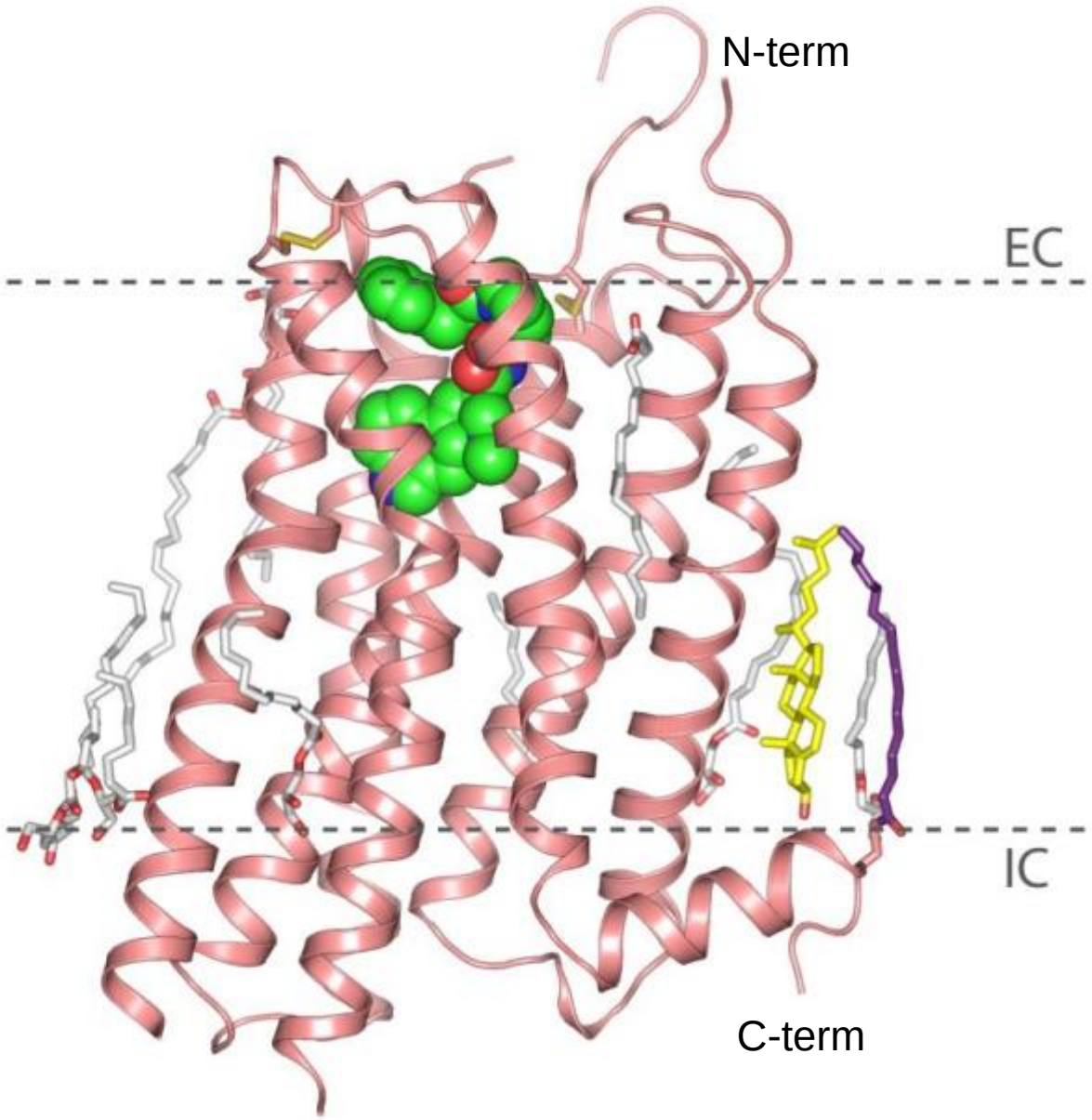




XFEL single diffraction snapshot

Liu W et al., Science, 2013

human serotonin 5-$HT_{2B\text{-}XFEL}$ receptor XFEL structure



ergotamine

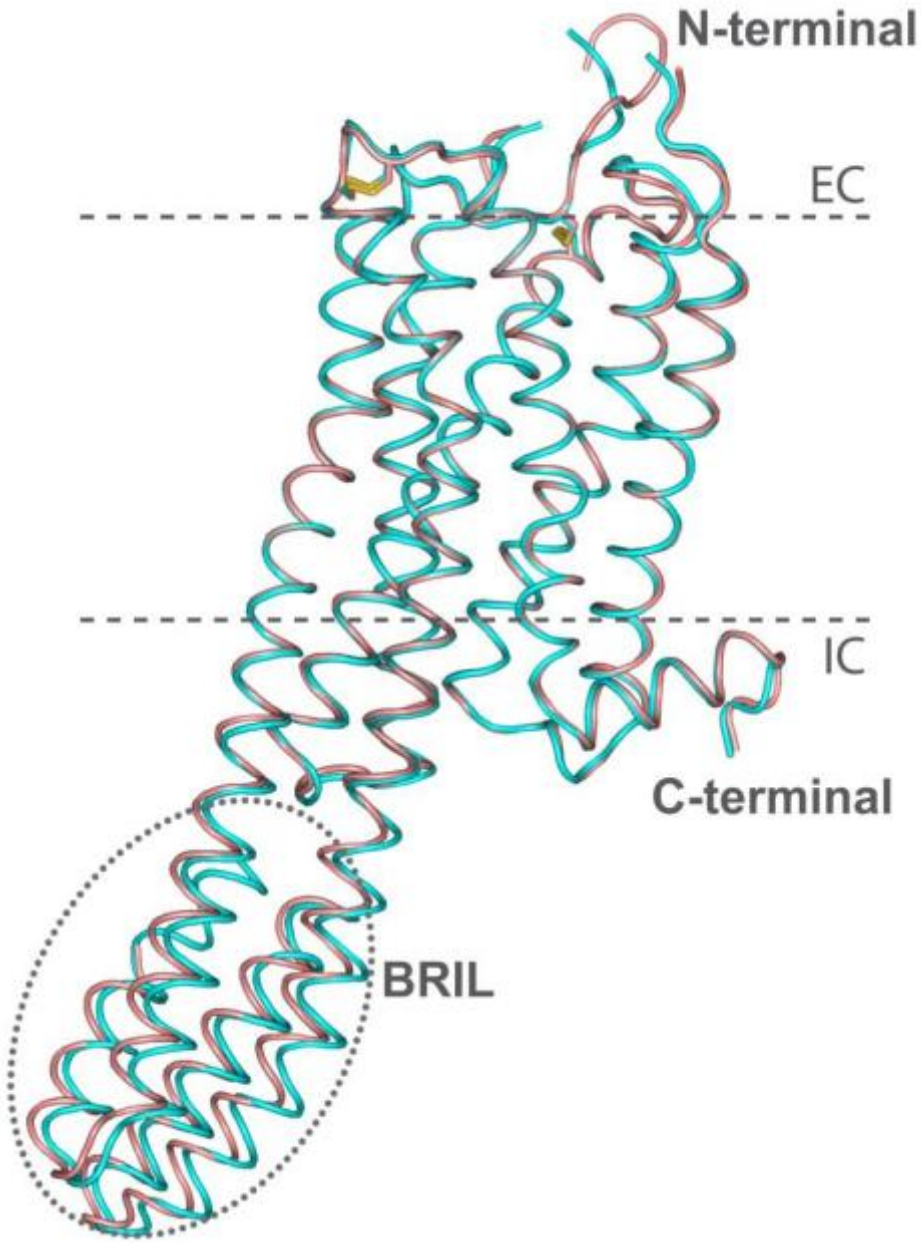
cholesterol

palmitoyl

lipid molecules

Liu W et al., Science, 2013

structure comparison 5-$HT_{2B\text{-}XFEL}$ and 5-$HT_{2B\text{-}SYN}$



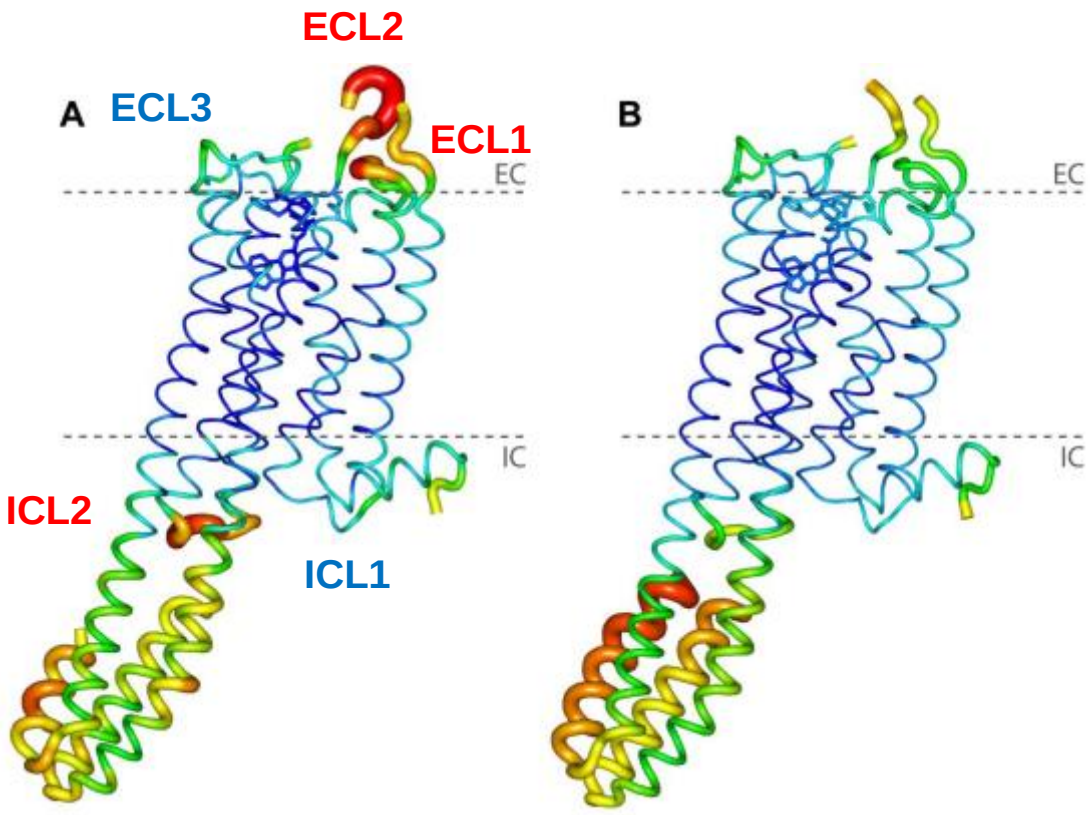
main differences:

N-terminal residues
ECL 1
ECL 2
ICL 3 (BRIL fusion domain)

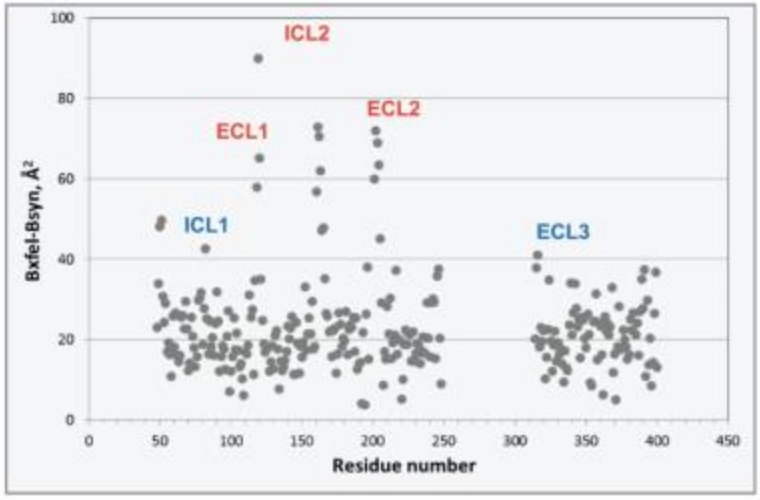
Liu W et al., Science, 2013

B-factor comparison 5-HT$_{2B\text{-}XFEL}$ and 5-HT$_{2B\text{-}SYN}$

B factor measure of how much an atom oscillates around the position specified in the model



5-HT$_{2B\text{-}XFEL}$ 5-HT$_{2B\text{-}SYN}$



kinetics of ligand binding?

interactions with intracellular binding partners?

Liu W et al., Science, 2013

comparison of data collection statistics

Data collection	5-HT$_{2B\text{-}XFEL}$	5-HT$_{2B\text{-}SYN}$
Temperature, K	294[a]	100
Wavelength, Å	1.3	1.032
Beam size, µm	1.5	10
Average crystal size, µm	5 × 5 × 5	80 × 20 × 10
Number of crystals	32,819	17
Flux	$3 \cdot 10^{10}$ ph/pulse	10^{11} ph/s
Max dose per crystal, MGy	25	20
Space group	$C222_1$	$C222_1$
Unit cell, Å	61.5, 122.2, 168.5	60.57, 119.75, 170.61
Oscillation / exposure	0° / 50 fs	1.0° / 1.0-3.0 s
No. collected images	4,217,508	91
No. hits / indexed images	152,651 / 32,819	91 / 91
No. total / unique reflections	18,515,376 / 16,052	51,559 / 16,041
Resolution, Å	35 – 2.8 (2.9 – 2.8)	50 – 2.7 (2.8 – 2.7)
Completeness, %	100 (100)	90.5 (92.2)
Multiplicity	1,150 (1035.6)	3.2 (3.1)
I/σ(I)	5.9 (0.64)	8.7 (1.7)
CC* [b]	0.998 (0.74)	0.992 (0.77)
R_{split} [c] (XFEL) or R_{merge} (SYN), %	9.5 (161.9)	15.0 (91.4)
Refinement		
No. reflections / test set	16,025 / 814	15,818 / 823
R_{work} / R_{free}, %	22.7/ 27.0	22.7 / 26.6
No. atoms		
Protein	2,856	2,854
Ligand	43	43
Lipids and other	224	170
B-factors, Å^2		
Wilson B/ Overall B	115.7 /98.7	72.1 / 80.0
Receptor / BRIL	88.4 / 133.7	67.2 / 126.0
Ergotamine	68.1	57.7
Lipids and other	110.4	82.5
R.m.s bonds, Å / angles, °	0.002 / 0.60	0.009 / 0.98
Ramachandran plot stats, %		
Favored	96.4	98.1
Allowed	3.6	1.9
Disallowed	0.0	0.0

measurement at room temperature

measurement of microcrystals

high resolution

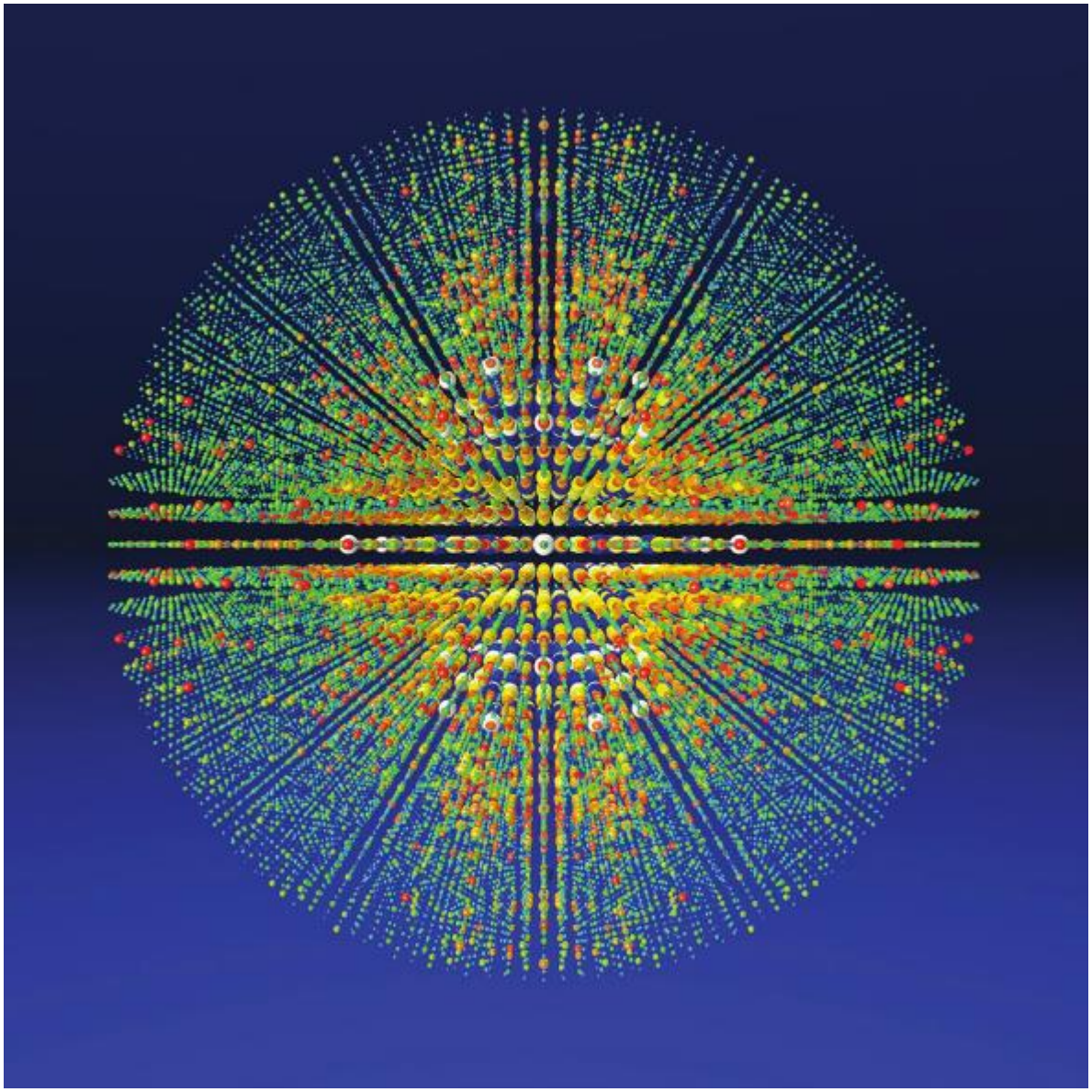
Liu W et al., Science, 2013

conclusions and outlook

+ high-resolution data
+ microcrystals
+ no radiation damage
+ room-temperature
+ track dynamics

– method under development
– large, expensive factilities

outlook



3D-merge of XFEL diffraction patterns of thousands of crystals.

Liu W et al., Science, 2013

nano-crystallography

–> membrane proteins, …

‘time-resolved’ x-ray crystallography

–> visualise protein functions in 4D

…

questions?