Serial femtosecond crystallography (SFX) using X-ray free electron laser (XFEL)

Katerina Dörner
Center for Free-Electron Laser Science - CFEL
Deutsches Elektronen-Synchrotron DESY

RTG 1976 Symposium on Structural Biology
Freiburg, 15th of October 2015

Outline

- Introduction
- Serial femtosecond crystallography (SFX)
- Nanocrystal characterization and growth
- Nanocrystal injection and delivery methods
- Time-resolved studies

X-ray free electron lasers (XFEL) applied to structural biology

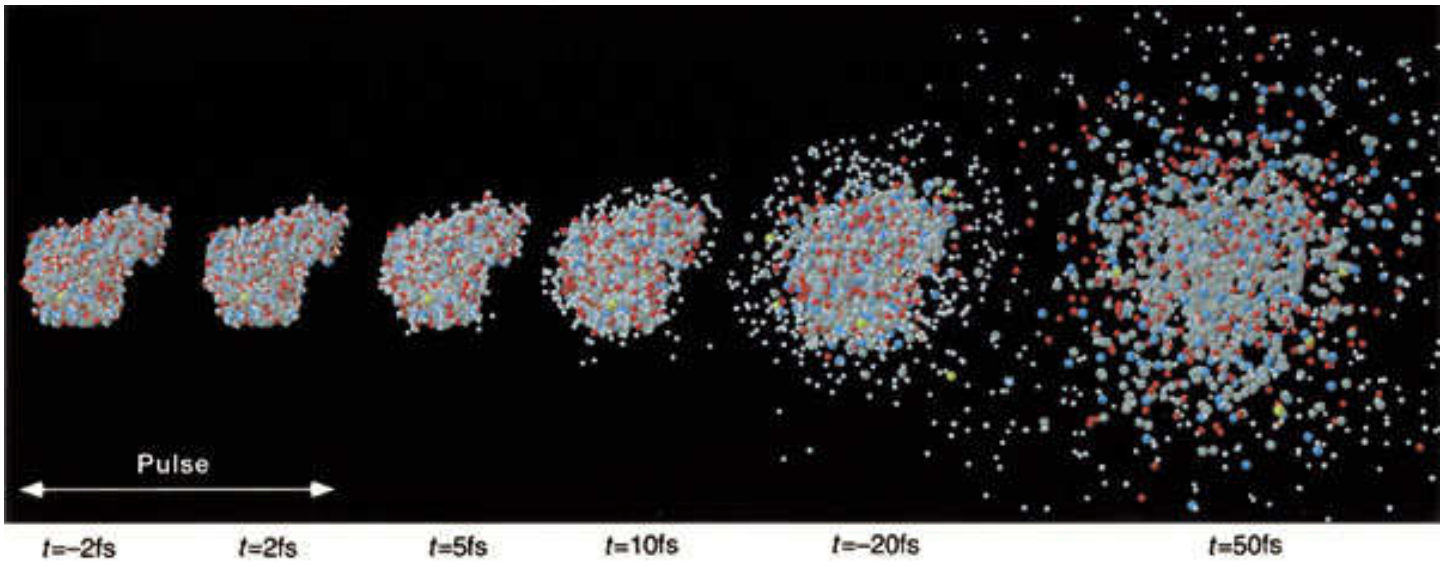
- X-ray crystallography is the most applied method for the structural analysis of proteins

- Major limitations in protein X-ray crystallography:
  - Obtaining crystals of sufficient size
  - Radiation damage

- The invention of femtosecond X-ray free electron laser opens new possibilities
  - Nano- and Microcrystals can be used
  - Femtosecond X-ray pulses outrun radiation damage processes

"diffraction before destruction" principle

Prediction of the "diffraction before destruction" principle

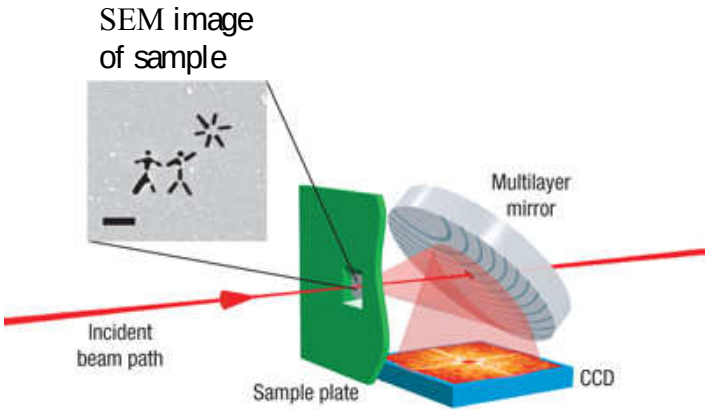


- In the year 2000 the "diffraction before destruction" principle was proposed
- Effect of ultrashort (2 fs), high-intensity (12 keV) X-ray pulses on T4 Lysozyme was simulated
- The simulation was continued for 50 fs after the pulse, the molecule explodes, but the structural information has been gathered.

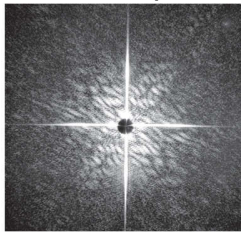
Neutze, R. et al (2000) Nature 406, 752-757

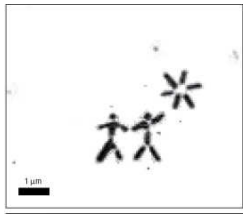
First experimental demonstration of the "diffraction before destruction" principle

Demonstrated at the first soft-X-ray free electron laser in the world (FLASH facility at DESY), published in 2006

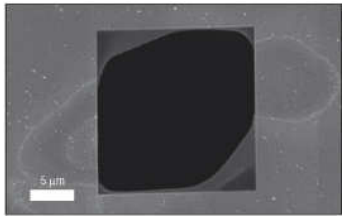


Diffraction pattern





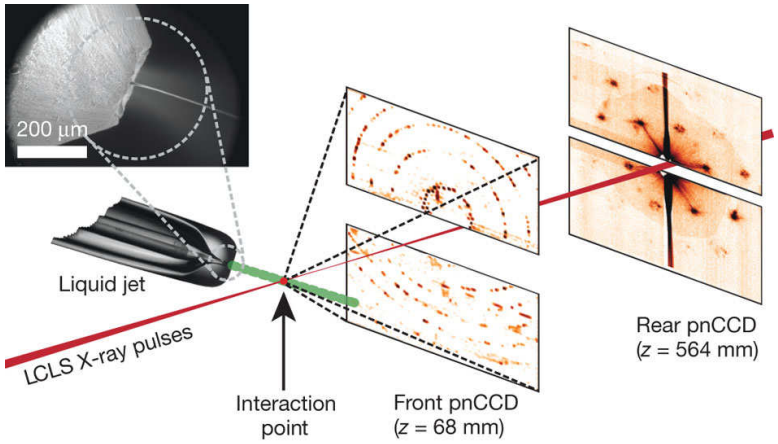
Reconstructed image

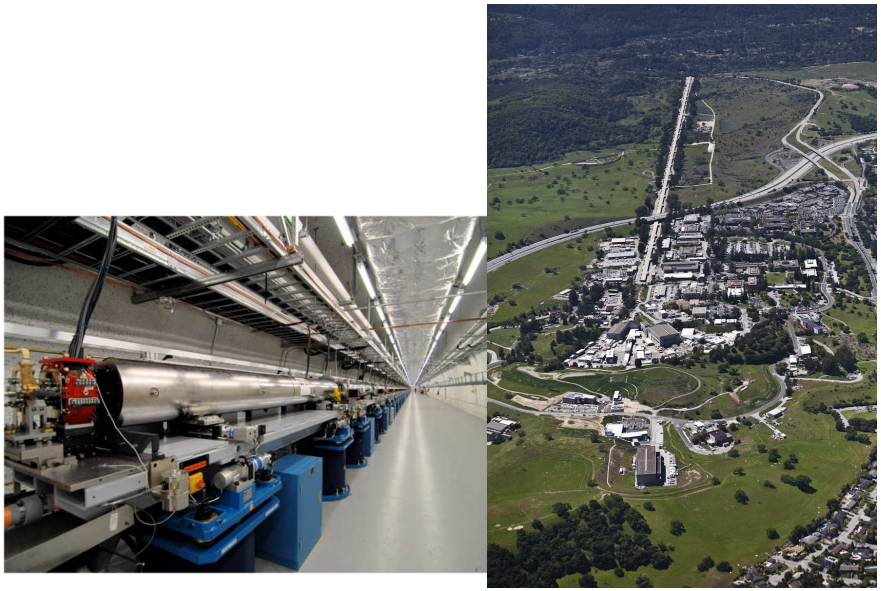


SEM image of sample after exposure to the FEL beam

Chapman, H. N. et al (2006) Nature Physics 2, 839 - 843

First demonstration of serial femtosecond X-ray protein nanocrystallography



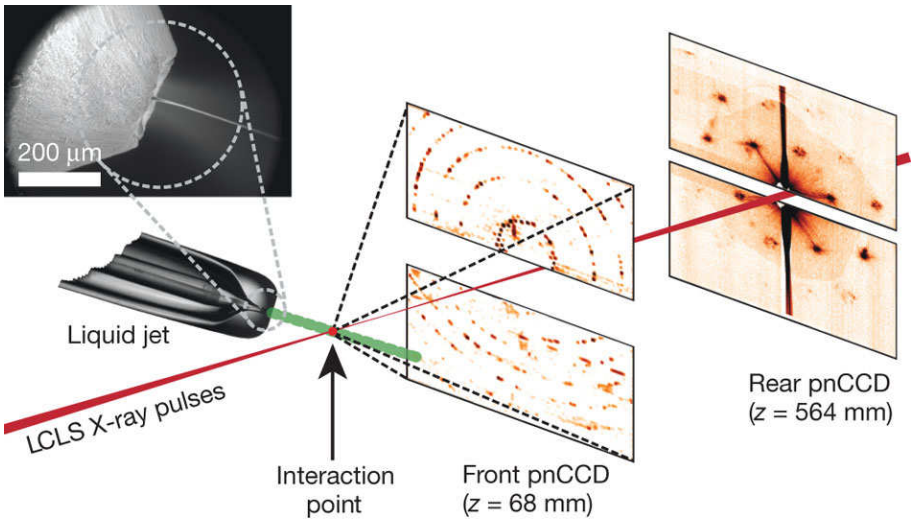


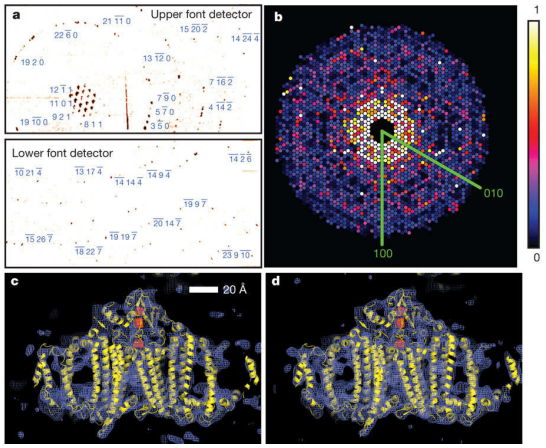
- At the hard-X-ray free-electron laser, the LCLS (Stanford University, DOE)
- Data collected from a fully hydrated stream of protein nanocrystals
- Two detectors (X-ray p–n junction charge-coupled device (pnCCD))
- Structure from Photosystem I (1 MDa membrane protein complex)

Chapman, H. N. et al (2011) Nature 470, 73-77.

First demonstration of serial femtosecond X-ray protein nanocrystallography

- Photon energy of X-ray pulse 1.8 keV (6.9 Å)
- 10^{12} photons per pulse at the sample
- pulse durations 10, 70, and 200 fs
- Data recorded with 30 Hz (1800 patterns per minute)
- Liquid jet with nanocrystals (0.2 to 2 ! m)
- Crystal hit rate 20%
- 1 850 000 X-ray pulses needed
- Diffraction patterns with 'partial' Bragg reflections analyzed with Monte Carlo approach

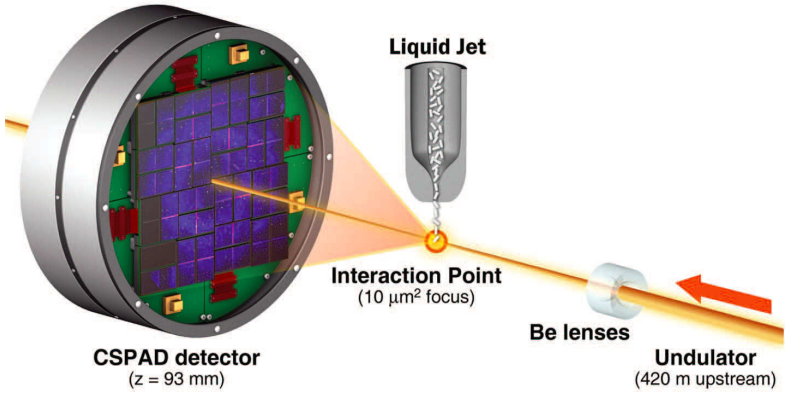




Chapman, H. N. et al (2011) Nature 470, 73-77.

Serial femtosecond X-ray protein nanocrystallography

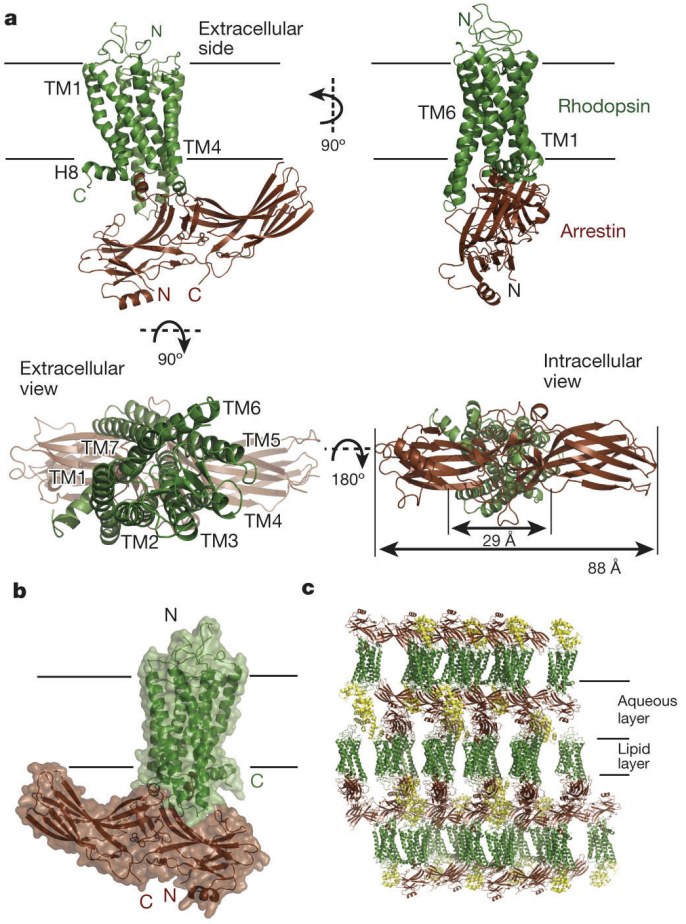
- Photon energy of X-ray pulse 9.5 keV (1.3 Å)
- 10^{11} to 10^{10} photons per pulse at the sample
- Pulse duration 40 fs or less
- Cornell-SLAC pixel array detector (CSPAD)
- Data recorded with 120 Hz (7200 patterns per minute)
- Resolution down to 1.9 Å



Boutet, S. et al. (2012) Science, 337, 362-364.

Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser

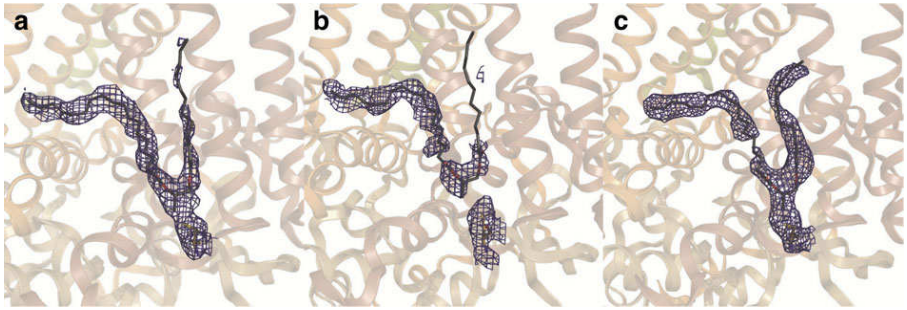
- 12 h of run time
- over 5 million detector frames
- 22,262 hits were found by the Cheetah hit-finding software
- Diffraction patterns from 18,874 crystals indexed and integrated using CrystFEL
- Structure with 3.8 Å resolution
- Only 6–8 Å at synchrotron sources



Kang, Y. et al. (2015) Nature, 523, 561-567.

‘Radiation damage free’ structures with XFEL

Thioether covalent bond
in photosynthetic reaction center from Blastochloris viridis

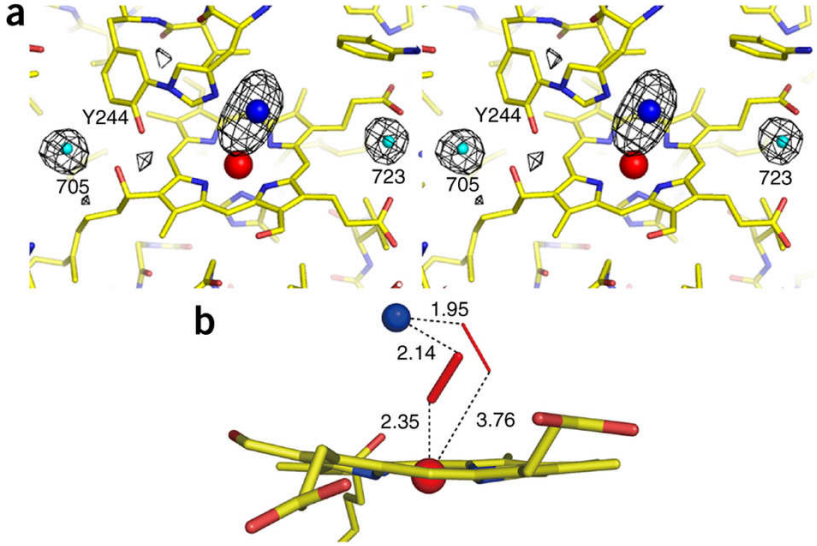


Cryogenic temperatures (4.4MGy)

Cryogenic temperatures (77MGy)

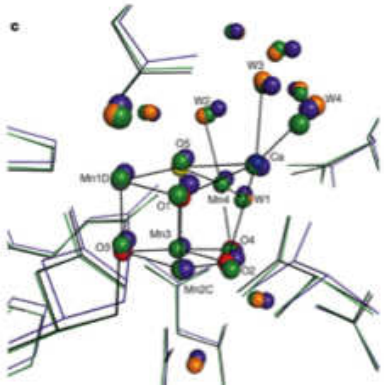
Room temperature SFX structure (33MGy)

The O2-reduction site in bovine heart cytochrome c oxidase free of radiation damage



Large single crystal exposed to femtosecond pulses of an X-ray free-electron laser (1.9-Å resolution)

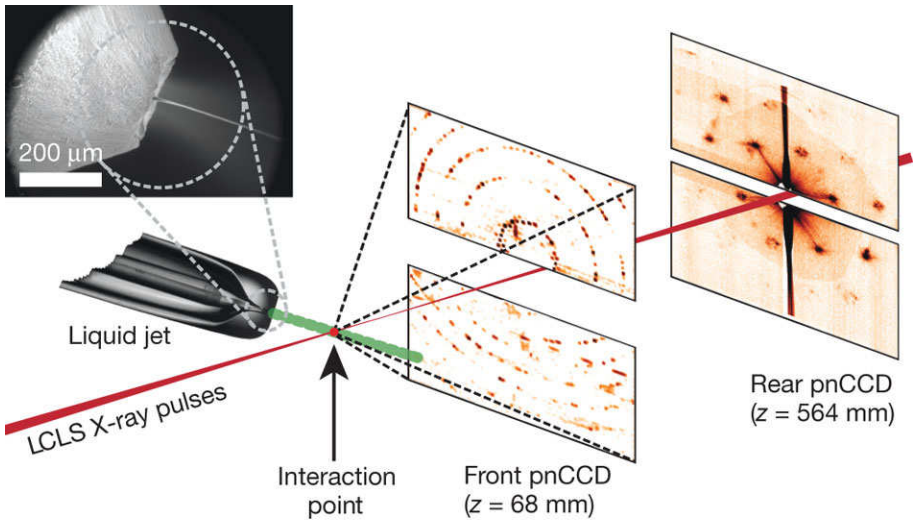
Oxygen evolving complex of Photosystem II



Overlay of structures obtained by XFEL and synchrotron radiation

Johansson, L. C. et al (2013) Nature Communications 4:2911
Hirata, K. et al (2014) Nature Methods 11, 734–736.
Suga, M. et al (2015) Nature 517, 99-103.

Serial femtosecond X-ray protein nanocrystallography



- Overcomes the radiation damage problem
- Room temperature data collection
- Fast crystal optimization
- Time resolved data collection

Chapman, H. N. et al (2011) Nature 470, 73-77.

Nanocrystal characterization

Nanocrystal characterization

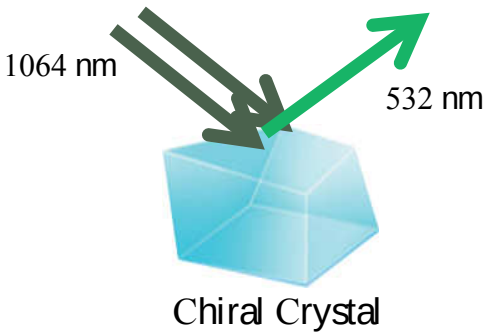
Nanocrystals are too small to be detected with an optical microscope

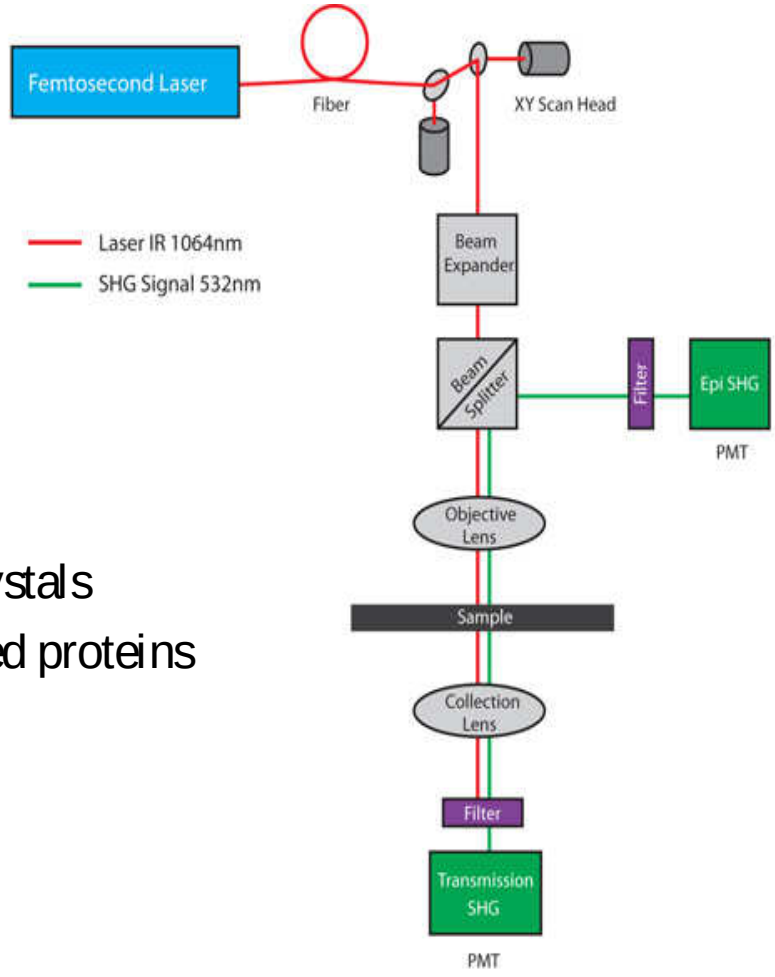
⇒ crystal growth cannot be monitored with common methods used in crystallography

Methods of choice:

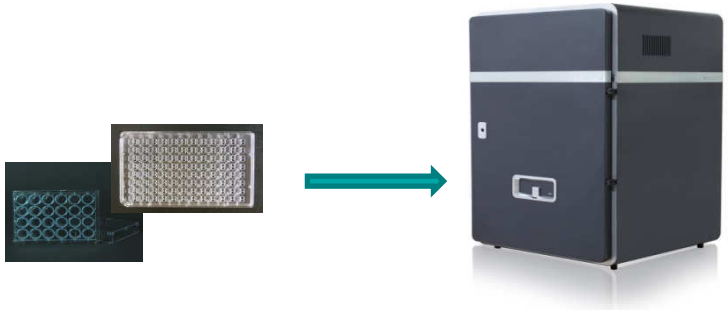
- Second order non-linear optical imaging of chiral crystals (SONICC)
- Dynamic Light Scattering (DLS)
- Nanoparticle Tracking Analysis (NTA)

SONICC –
Second order non-linear optical imaging of chiral crystals

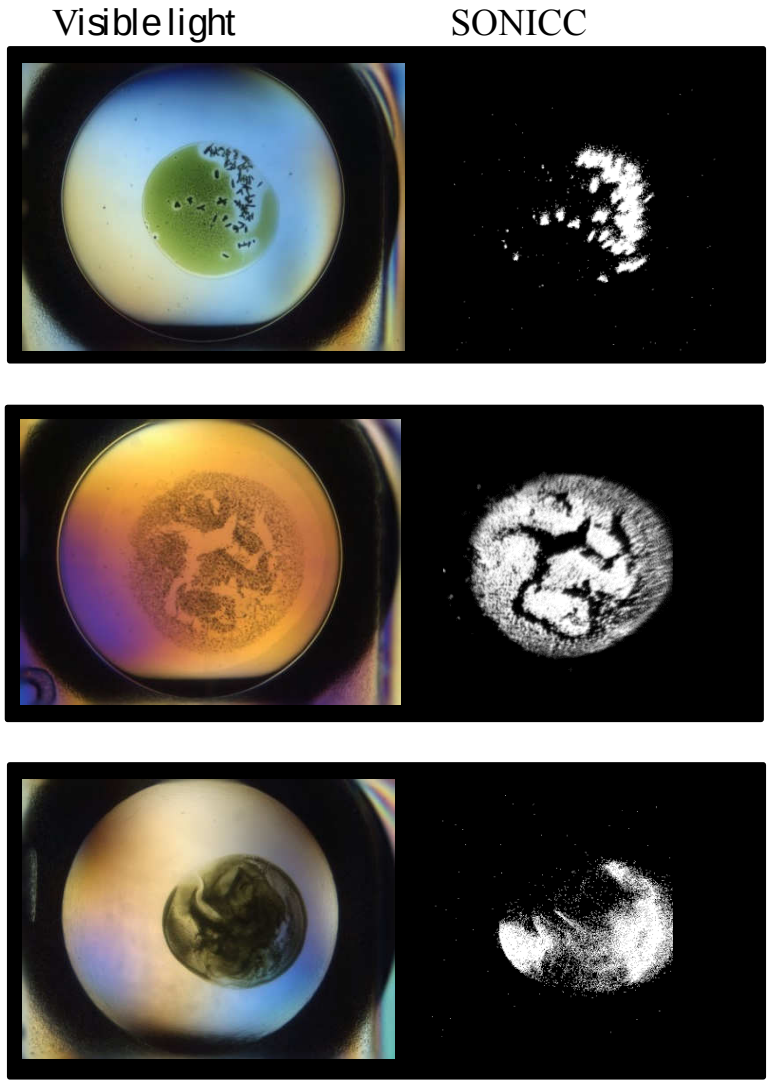


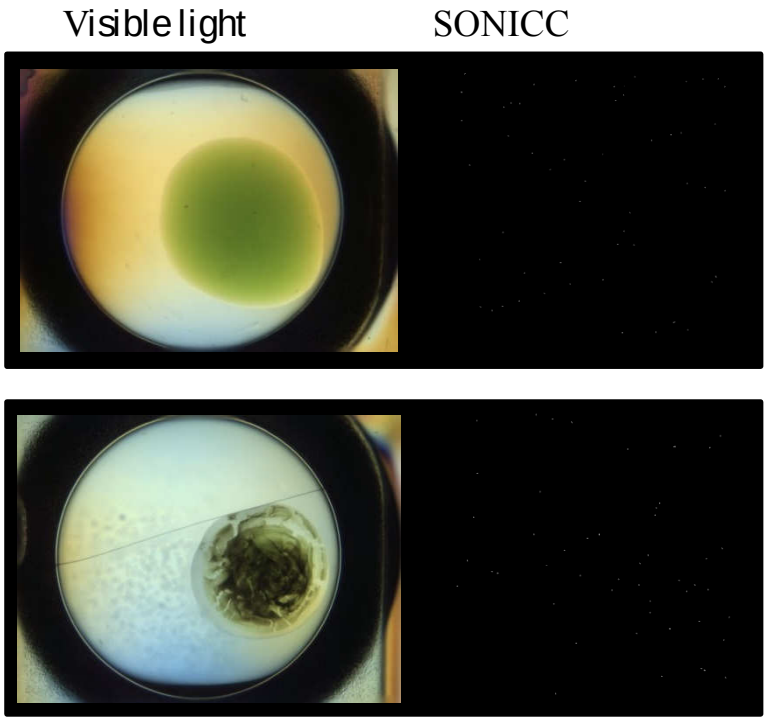


- Principle: Second Harmonic Generation (SHG)
- Detects non-centrosymmetric ordered (chiral) crystals
- no signal occurring from solubilized or aggregated proteins



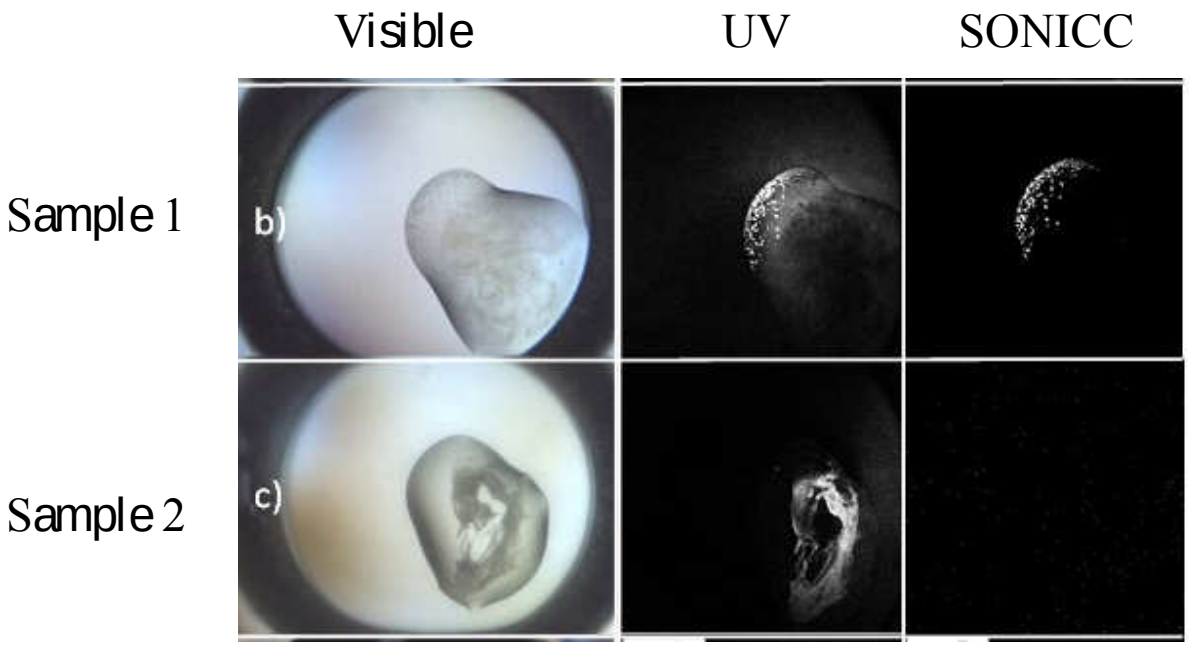
SONICC – Detection of nanocrystals





- Powerful technique for nanocrystallography

SONICC – Detection of nanocrystals



Nonlinear microscopy and principle of Second Harmonic Generation (SHG)

3 phenomena in parallel:

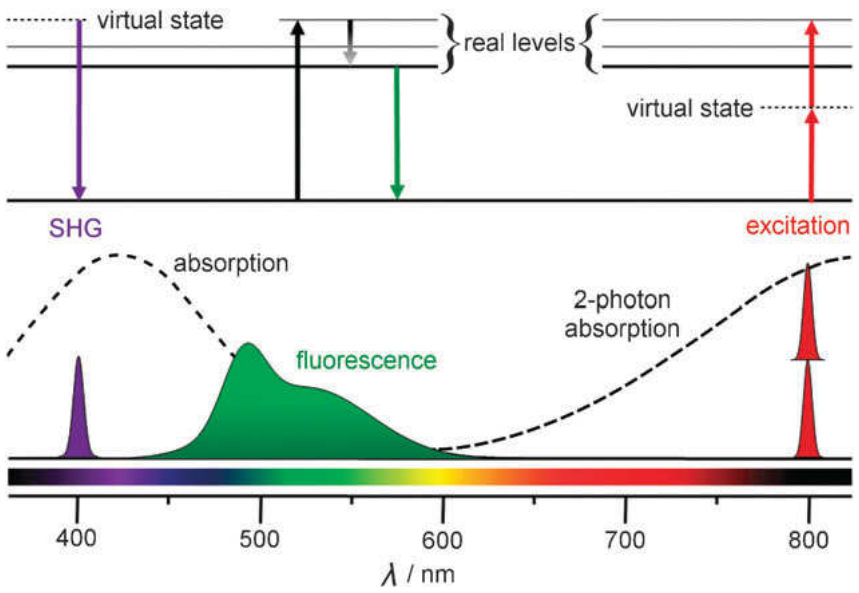
- One photon excited fluorescence (1PF)
- Two photon excited fluorescence (2PF)
- Second harmonic generation (SHG)

1PF and 2PF

- Excitation and relaxation in real states

SHG

- Parametric process
- Scattering through interaction with a virtual states
- No excited states, not photo damage
- Retains phase, polarization and directional information



Reeve, J. E. et al. (2010) Phys. Chem. Chem. Phys., 12, 13484–13498

Molecular design for SHG and therefore SONICC

1.) Medium without center of inversion

Ensemble of chiral molecules: protein crystals, collagen, dyes in a biological membrane

(No SHG: Random order and centro-symmetric crystals: protein solution, aggregated protein, most of salt crystals)

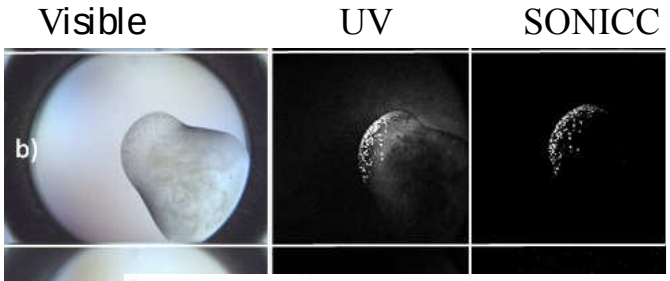
2.) Sufficient intensity of scattered light

- Intensity depends on crystal size, crystal class, amino acid sequence...
- "-conjugated systems lead to signal enhancement (resonance enhancement)

Proteins with cofactors like chlorophyll and heme groups, GFP-like proteins, proteins with high density of aromatic amino acids lead to strong SONICC signal

Reeve, J. E. et al. (2010) Phys. Chem. Chem. Phys., 12, 13484–13498

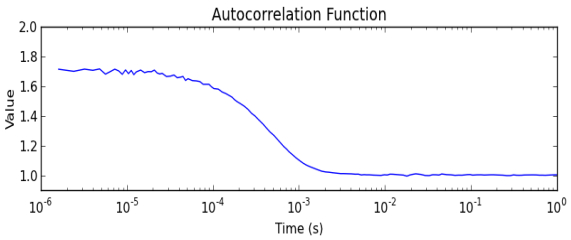
SONICC
High symmetry crystal classes not necessarily detectable

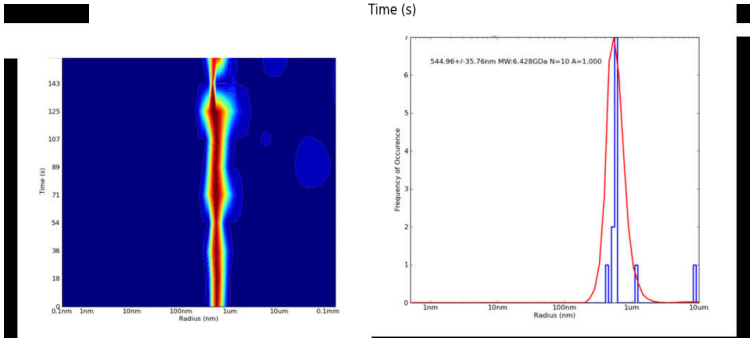


Crystal system	Point group/ Crystal Class		Space groups (international short symbol)	Generates SHG	% of known structures (A)	% of structures in the space group that do not have any tryptophan residues (B)	% of structures with no tryptophan residues (organized by space group) (=A*B)
	Intl	Schönflies					
Triclinic	1	C_1	P1	yes	4.04	18.34%	0.74%
Monoclinic	2	C_2	P2, $P2_1$, C2	yes	24.78	16.88%	4.18%
Orthorhombic	222	D_2	P222, $P222_1$, $P2_12_12$, $P2_12_12_1$, $C222_1$, C222, F222, I222, $I2_12_12_1$	yes	36.36	16.83%	6.12%
Trigonal	3	C_3	P3, $P3_1$, $P3_2$, R3	yes	4.04	20.24%	0.82%
	32	D_3	P312, P321, $P3_112$, $P3_121$, $P3_212$, $P3_221$, R32	yes	7.3	18.18%	1.32%
Tetragonal	4	C_4	P4, $P4_1$, $P4_2$, $P4_3$, I4, $I4_1$	questionable	2.36	19.26%	0.45%
	422	D_4	P422, $P42_12$, $P4_122$, $P4_12_12$, $P4_222$, $P4_22_12$, $P4_322$, $P4_32_12$, I422, $I4_122$	questionable	10.02	17.33%	1.73%
Hexagonal	6	C_6	P6, $P6_1$, $P6_5$, $P6_2$, $P6_4$, $P6_3$	questionable	3.6	19.46%	0.70%
	622	D_6	P622, $P6_122$, $P6_522$, $P6_222$, $P6_422$, $P6_322$	questionable	4.26	20.07%	0.85%
Cubic	23	T	P23, F23, I23, $P2_13$, $I2_13$	questionable	1.22	19.88%	0.24%
	432	O	P432, $P4_232$, F432, $F4_132$, I432, $P4_332$, $P4_132$, $I4_132$	questionable	.59	25.85%	0.15%

Size distribution and homogeneity of nanocrystals

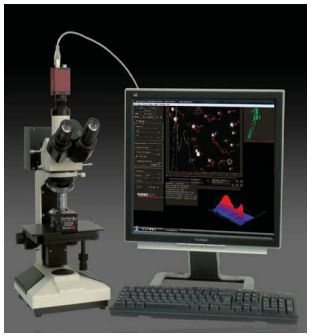
Dynamic light scattering (DLS)

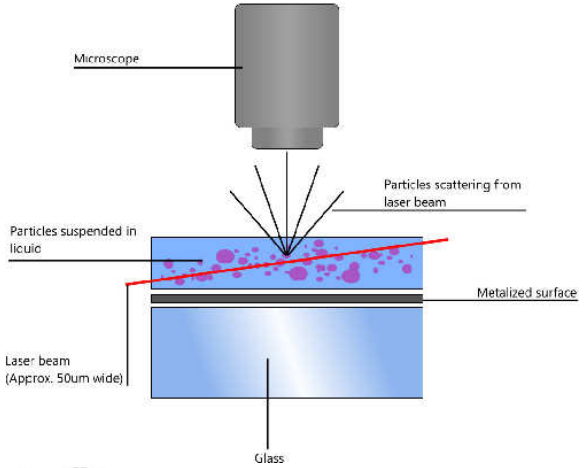


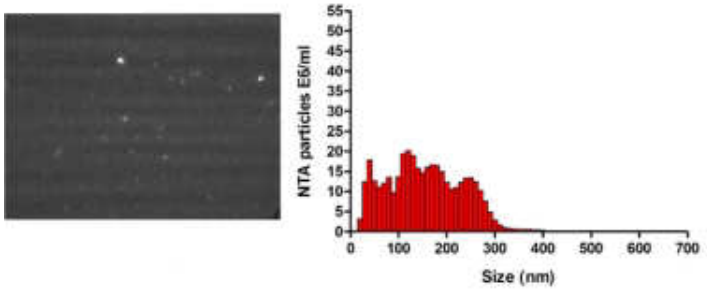


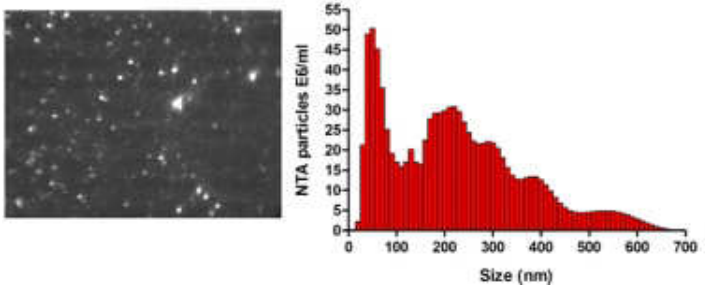
www.wyatt.com

Nanoparticle Tracking Analysis (NTA)



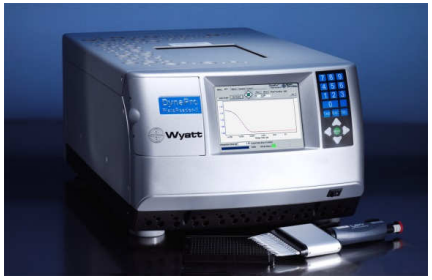


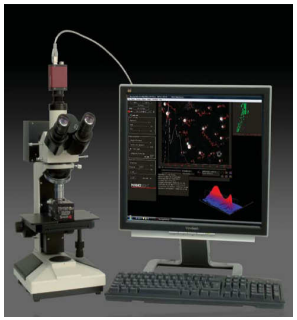




www.malvern.com

Size distribution and homogeneity of nanocrystals





	DLS	NTA by NanoSight
Size range	About 1 – 1000 nm	About 30 – 1000 nm
Size accuracy	Accurate for monodisperse samples, inaccurate for polydisperse samples	Accurate for monodisperse and polydisperse samples
Peak resolution	Low (>3 fold difference in diameter)	High (<0.5 fold difference in diameter)
Contaminations	Large particles problematic	Large particles have little influence
Reproducibility	more	less
Duration of measurement	2 – 5 min	5 min to 1 hour

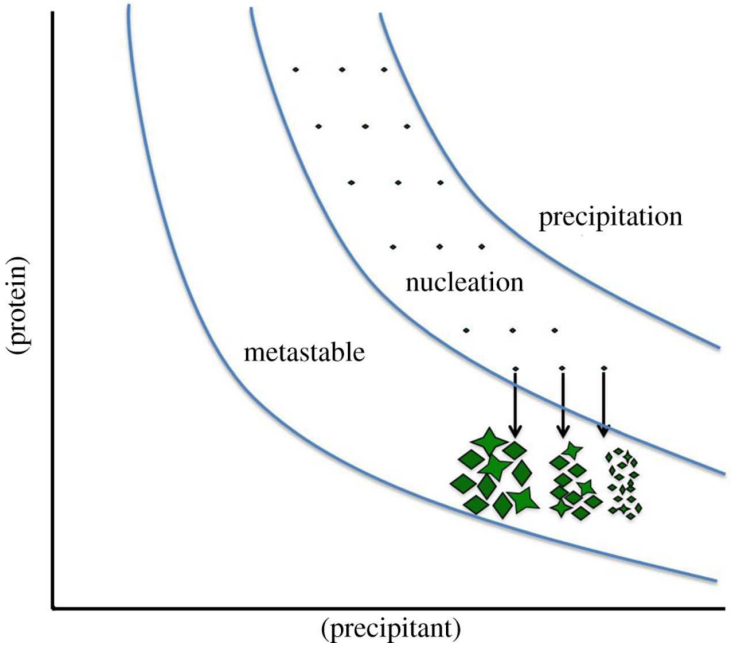
Filipe, V. et al. (2010) Pharmaceutical Research, 27, 796-810.

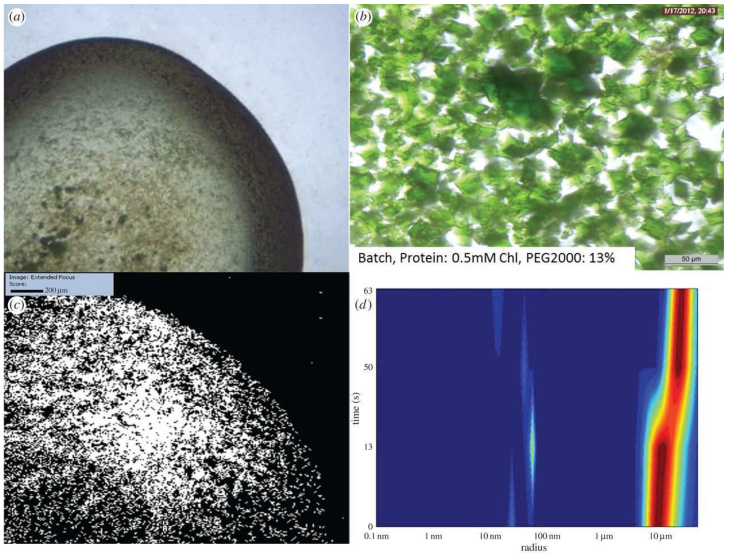
Nanocrystal growth

Nanocrystal growth

- Batch method
- Free interface diffusion (FID)
- Free interface diffusion with centrifugation

Nanocrystal growth - Batch method

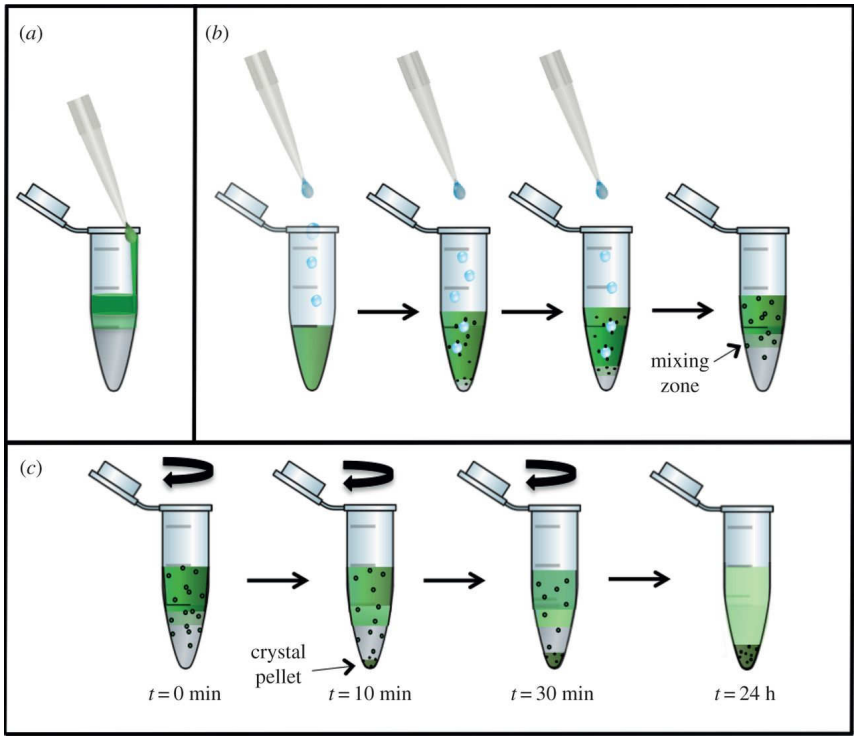


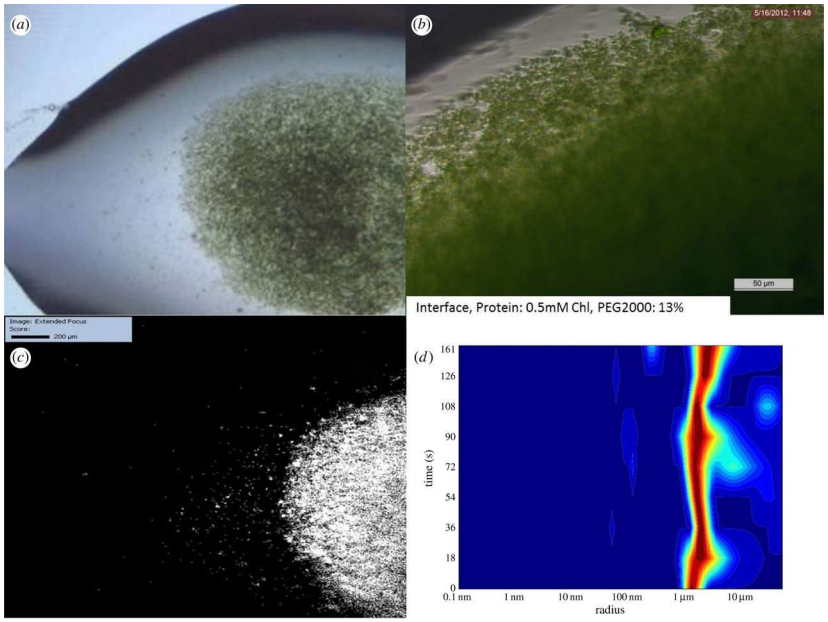


- Phase diagram should be known
- Rapid mixing of protein and precipitation buffer (Eppendorf tube, Falcon tube)
- Final concentration should be in nucleation zone
- May lead to polycrystalline samples

Kupitz, C. et al. (2014) Phil. Trans. R. Soc. B, 369, 20130316.

Nanocrystal growth - Free interface diffusion (FID)



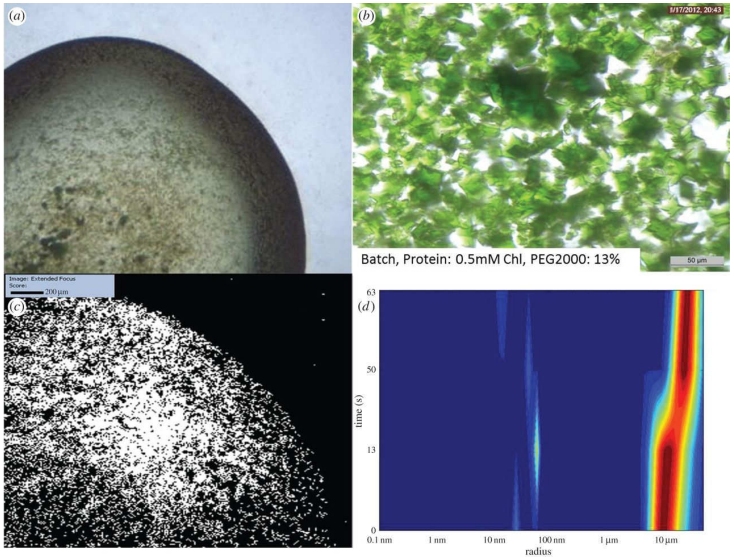


- Crystal grow at interface of high concentrated protein solution and precipitant buffer
- Precipitant solution drop wise added to protein solution
- Process can be accelerated by centrifugation

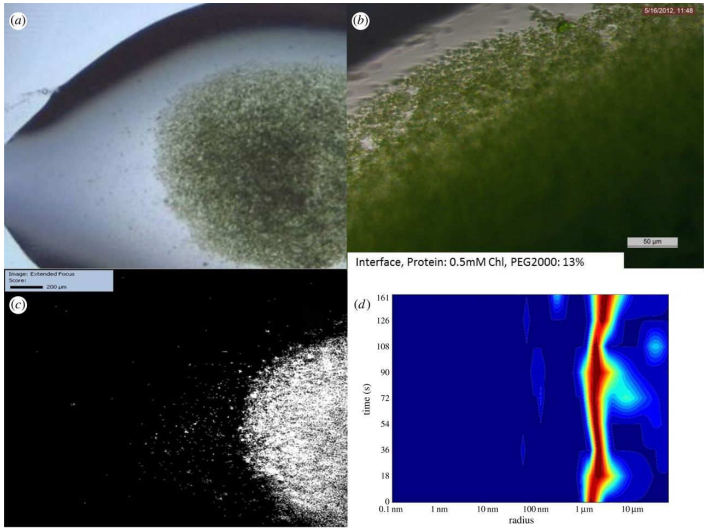
Kupitz, C. et al. (2014) Phil. Trans. R. Soc. B, 369, 20130316.

Nanocrystal growth

Batch method



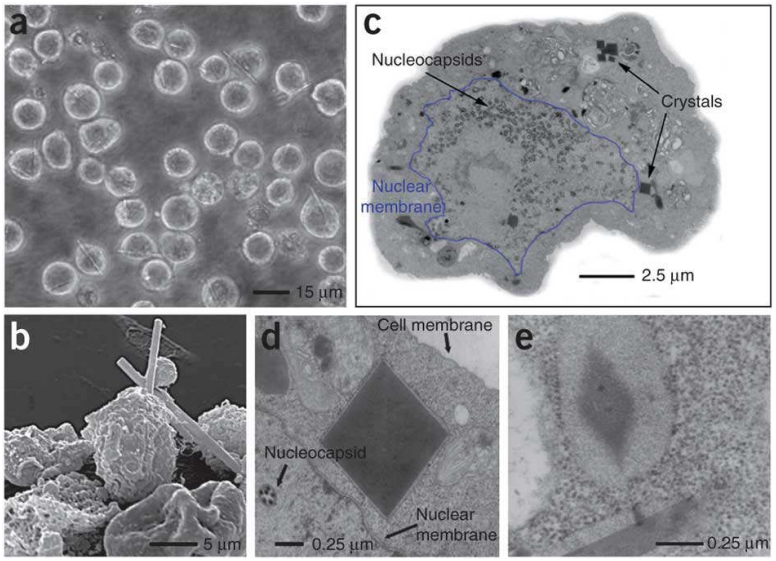
FID method

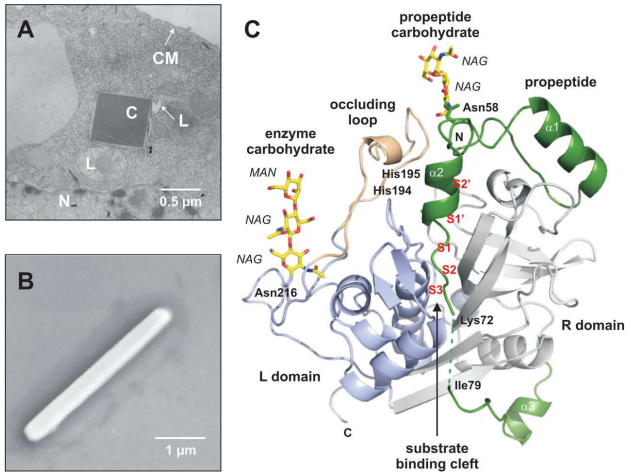


- Quenching of crystal growth may be necessary
- Transport of nanocrystals can be problematic
- Crystallization on site (LCLS, SACLA)

Kupitz, C. et al. (2014) Phil. Trans. R. Soc. B, 369, 20130316.

In vivo crystallization to generate nanocrystals





- Cysteine protease cathepsin from Trypanosoma brucei (TbCatB) crystals within Sf9 insect cells
- TbCatB is promising target for treatment of sleeping sickness
- Crystals too small for synchrotron-based experiments
- With SFX structure was solved with 2.1 Å resolution
- In the future: Imaging of crystals in cells?

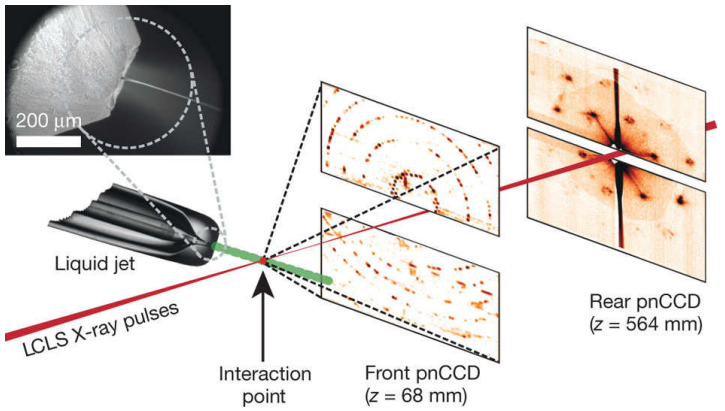
Redecke, L. et al. (2013) Science, 339, 227-230.

Nanocrystal injection/delivery methods

Nanocrystal injection methods

Requirements

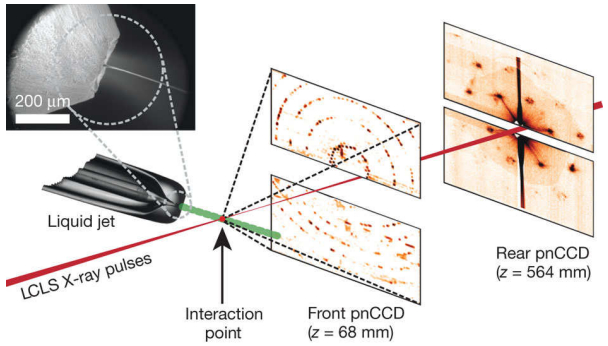
- Stable running jet
- No clocking of system
- No dissolving of crystals
- Low sample consumption
- Low background scattering

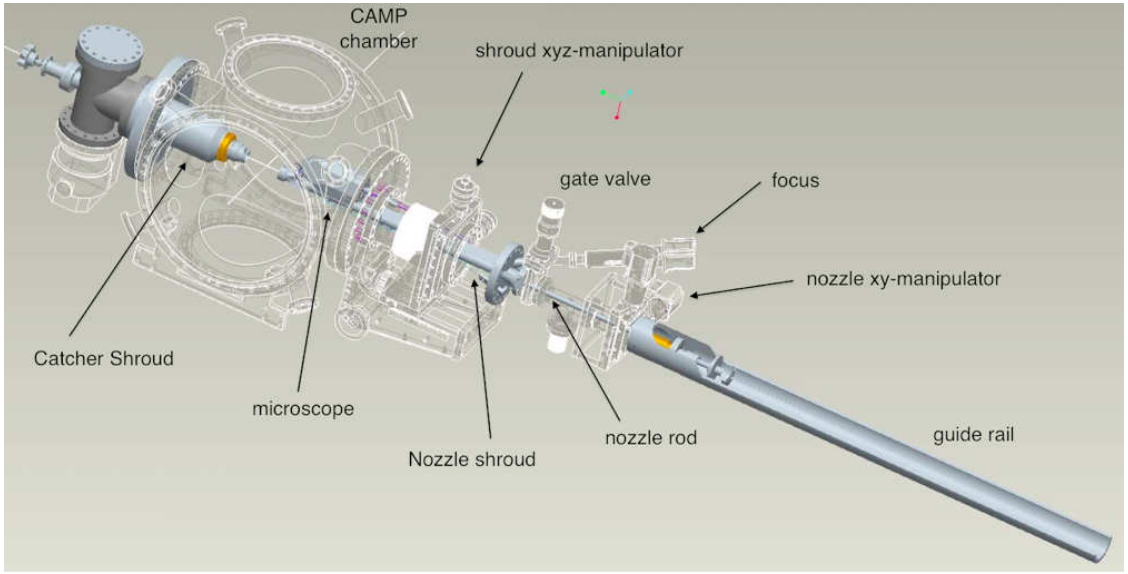


Chapman, H. N. et al (2011) Nature 470, 73-77.

Injection of crystal into X-ray beam

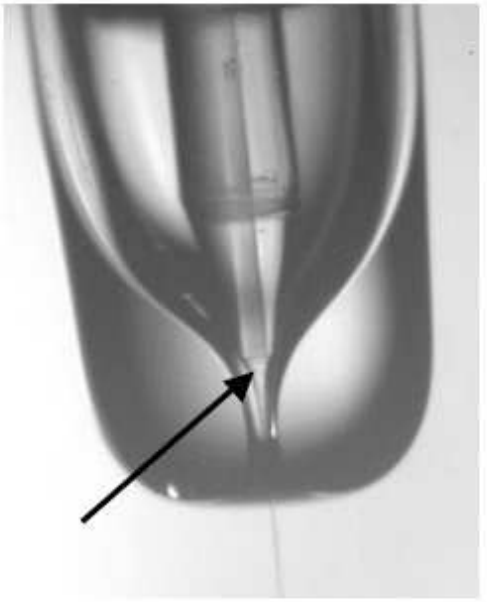
- Liquid jet injector
- Nozzle shroud and catcher shroud
- Based on Gas Dynamic Virtual Nozzle (GDVN)



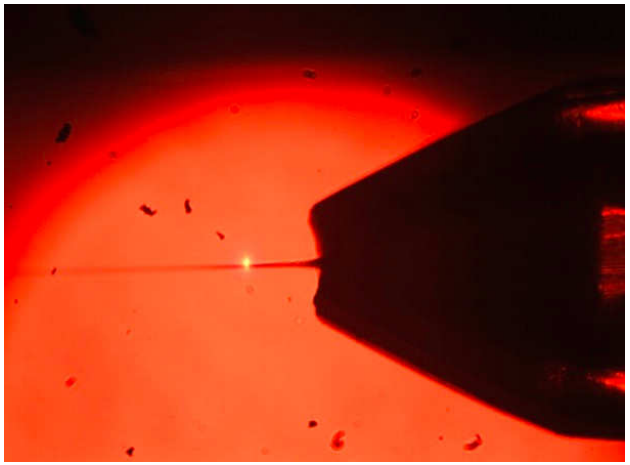


Deponte, D. P. et al. (2008) J. Phys. D: Appl. Phys. 41, 195505.

Gas Dynamic Virtual Nozzle (GDVN)

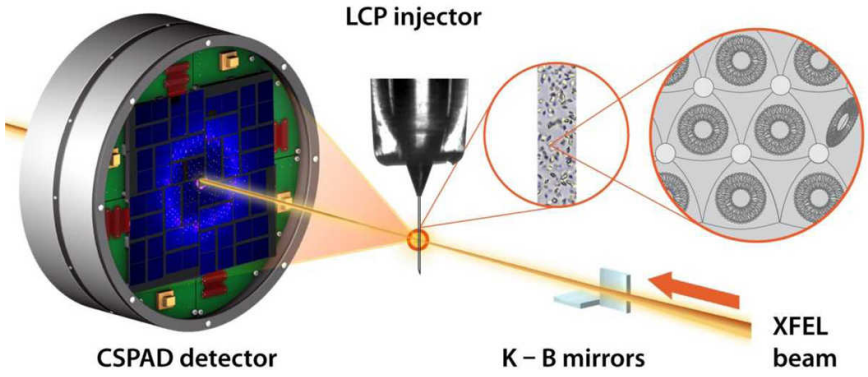


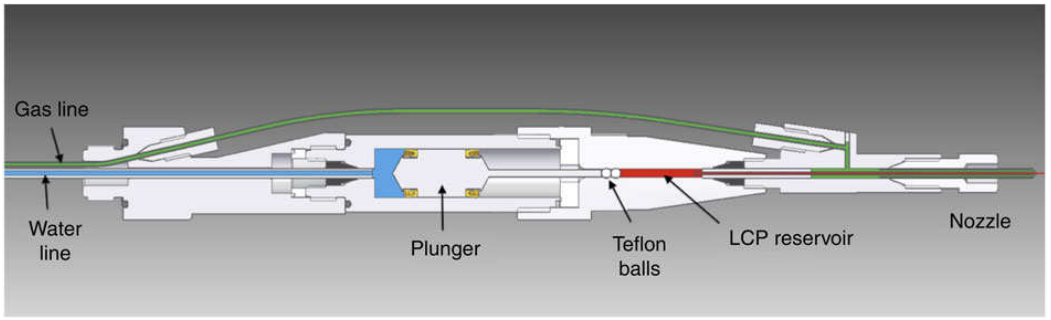
- Inner capillary (20 – 50 µm) with liquid crystal stream
- Co-flowing gas in an outer capillary reduce the diameter of the liquid jet
- Resulting jet diameter down to 4 µm
- Flow rates 10 to 20 µL min^{-1}

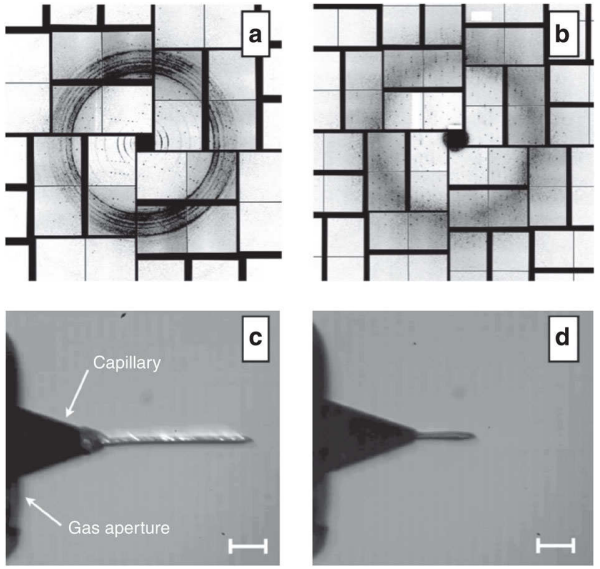


Deponte, D. P. et al. (2008) J. Phys. D: Appl. Phys. 41, 195505.

Lipid Cubic Phase (LCP) injector



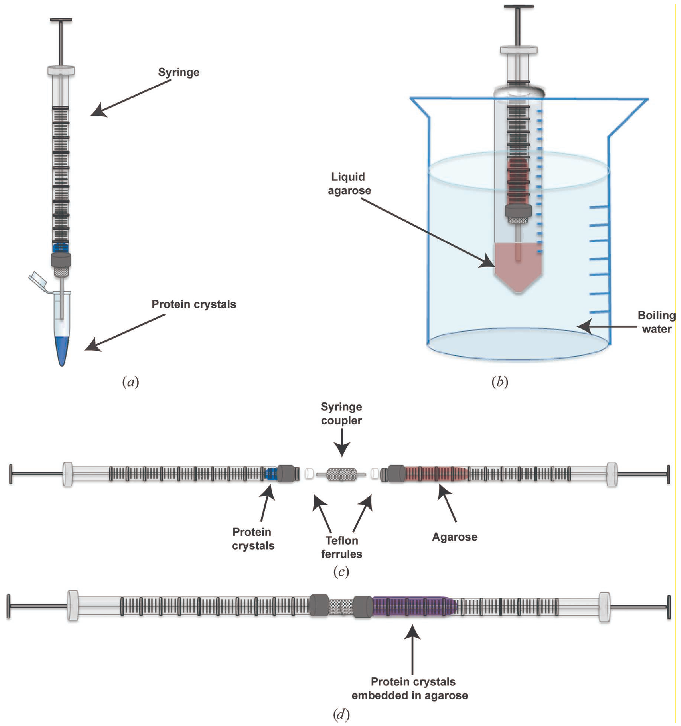


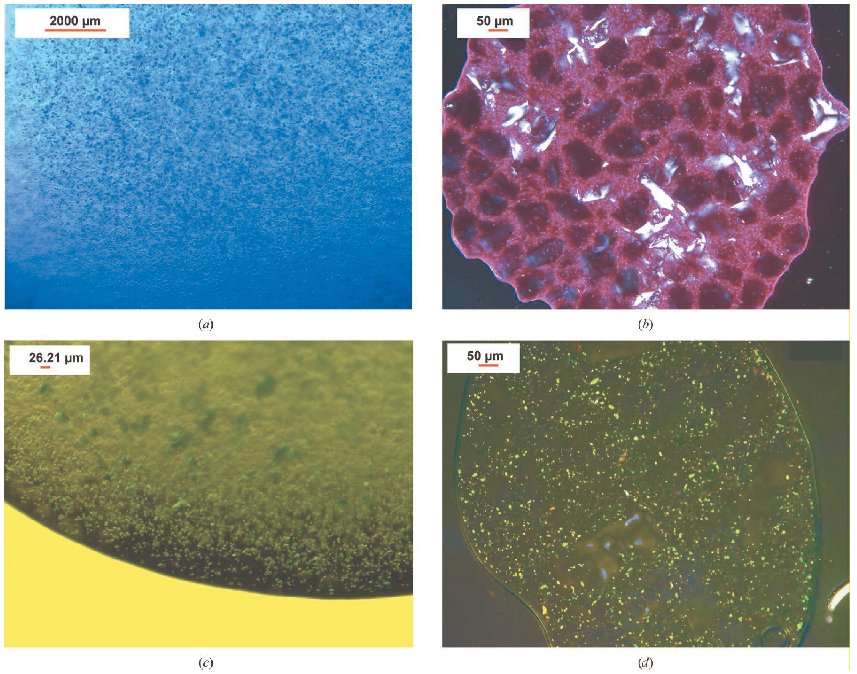


- Injection of gel-like LCP with microcrystals
- 10–50 µm diameter stream
- Protein consumption reduced by a factor of 20 compared with to GDVN nozzle

Weierstall, U. et al. (2014) Nat. Commun. 5, 3309.
Liu, W. et al (2014) Science 342, 1521-1524.

Agarose as injection medium



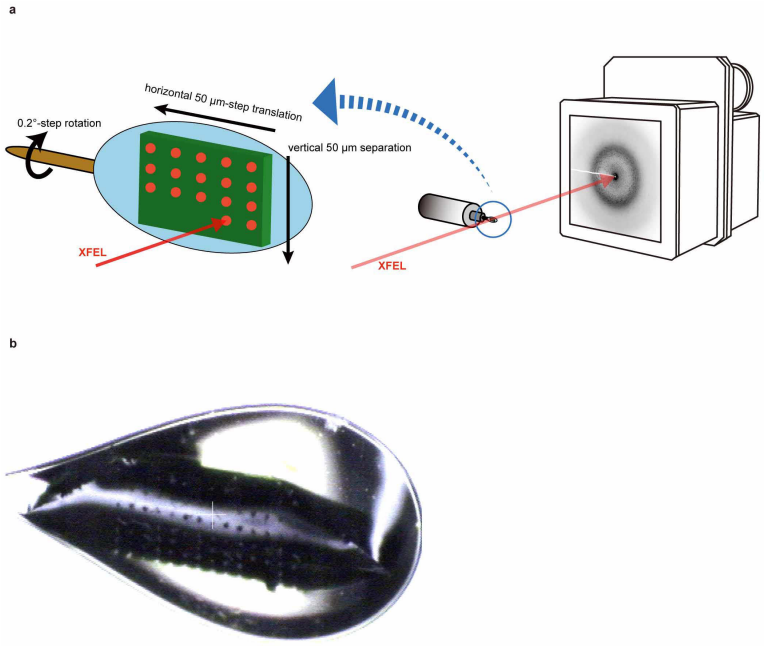


- Injection with LCP injector
- Microcrystallization via conventional methods and then mixed with agarose
- Protein consumption reduced by a factor of 100 compared with the GDVN nozzle

Conrad, C. E. et al (2015) IUCrJ, 2, 421–430.

Fixed-target delivery methods – Rotating large crystal

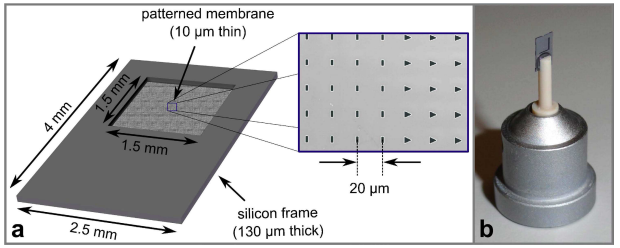
- Developed at SACLA in Japan
- Large single crystal (µm/mm range) rotated stepwise in order to record still diffraction images
- Hundreds of crystals "scanned" with the X-ray laser
- High resolution, radiation damage-free structures of Photosystem II and Cytochrome C oxidase

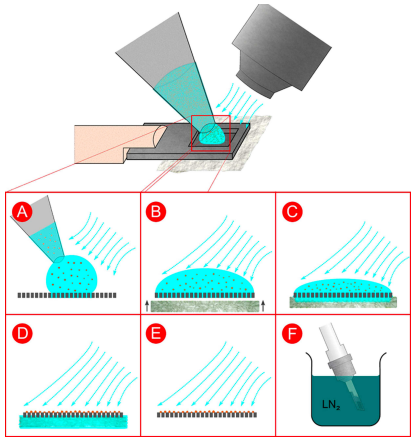


Hirata, K. et al (2014) Nature Methods 11, 734–736.

Fixed-target delivery methods – Silicon chips

- Microcrystal slurry is applied on silicon chip with micropores
- Chip is scanned with x-ray beam
- Room temperature and cryogenic temperatures
- Quick and easy sample preparation

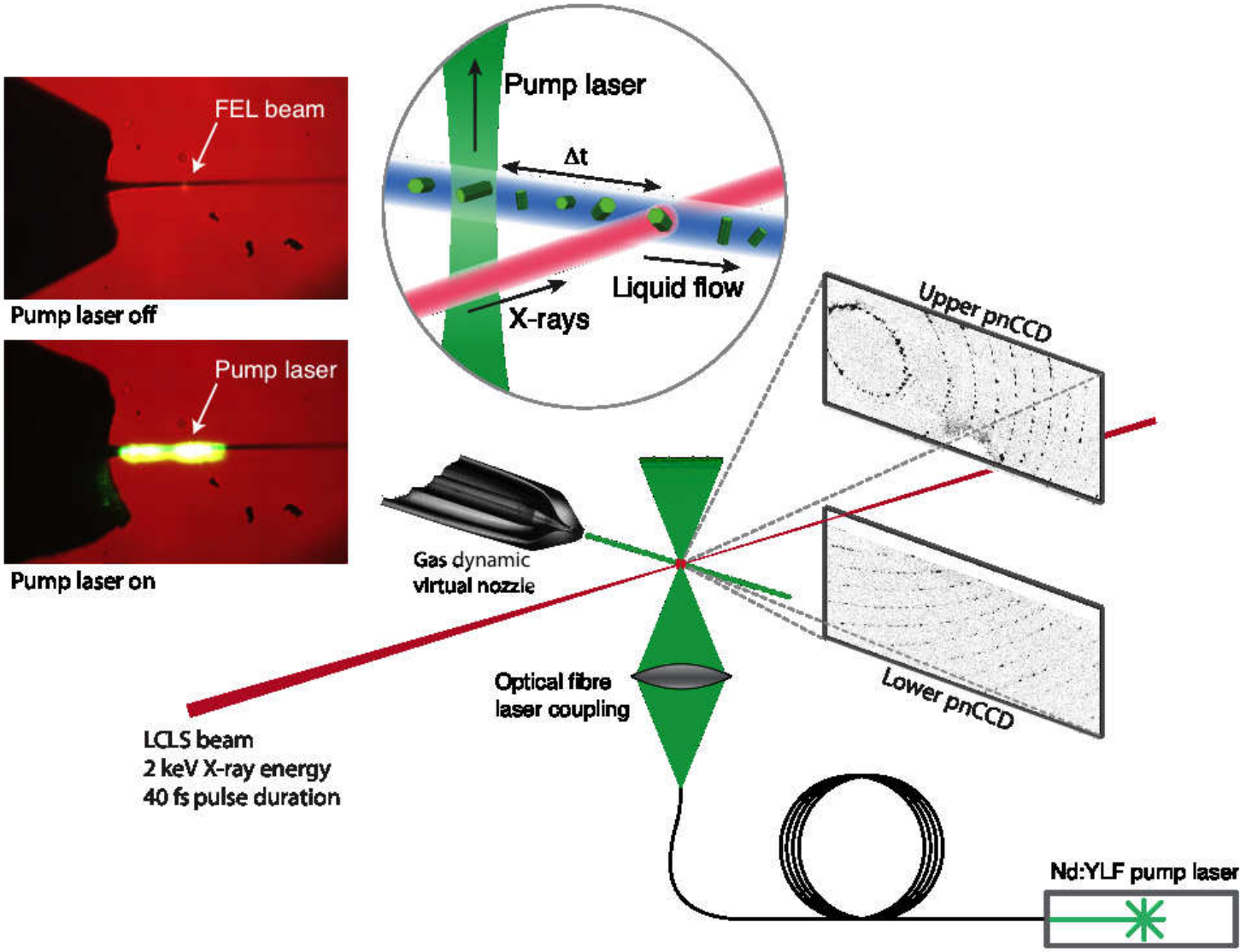




Roedig, P. et al (2015) Scientific Reports, 5:10451.

Time-resolved studies

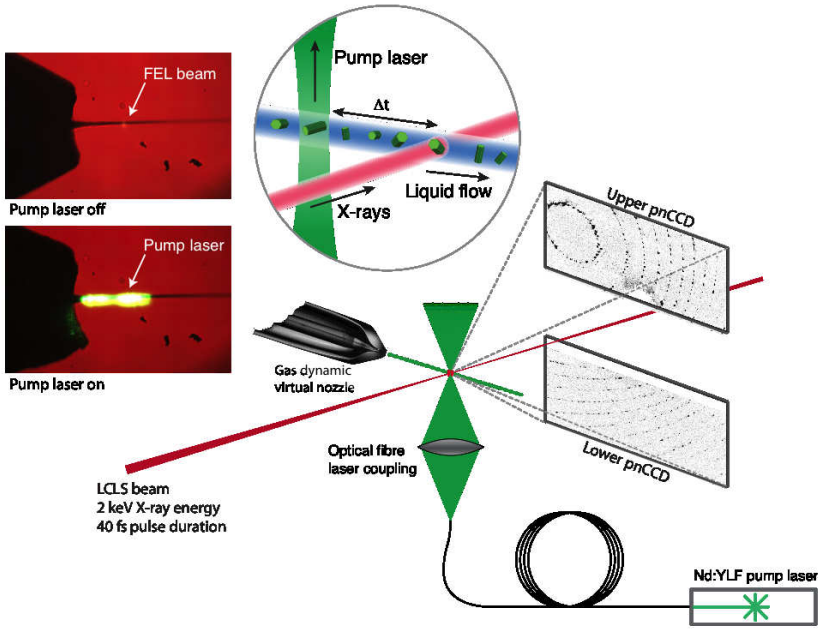
First time-resolved studies at XFEL on Photosystem I



Aquila, A., (2012) Optics Express, 20, 2706-16.

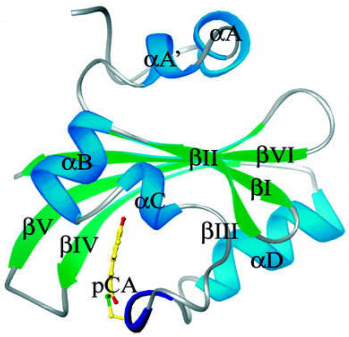
First time-resolved studies at XFEL on Photosystem I

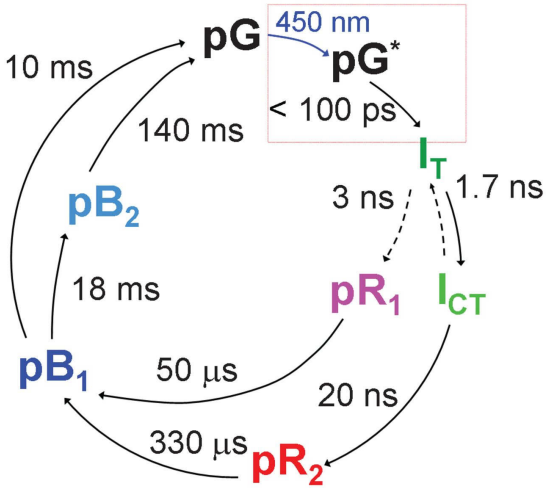
- XFEL synchronized with an optical pump laser
- Light-induced changes of Photosystem I-Ferredoxin co-crystals observed at time delays of 5 to 10 µs after excitation
- Enables study of the dynamics in hydrated single nano- to micron-sized crystals

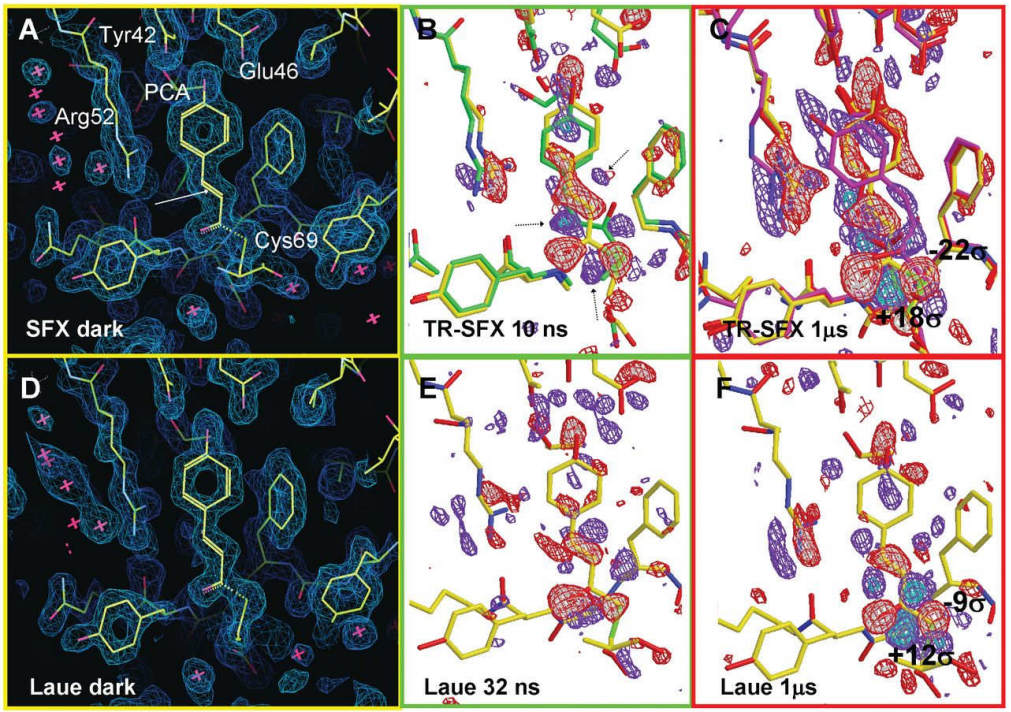


Aquila, A., (2012) Optics Express, 20, 2706-16.

Time-resolved studies on photoactive yellow protein (PYP)



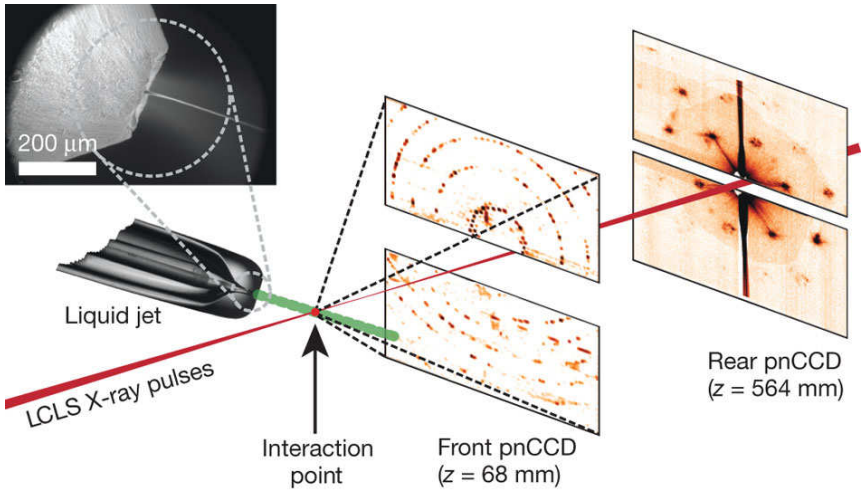




- Light-triggered dynamics in PYP (pump probe experiments)
- High-resolution (1.6 Å), time-resolved difference electron density maps
- Study of biological reactions on fs time scale possible

Tenboer, J. et al, (2014) Science, 346,1242-1246 .

Serial femtosecond X-ray protein nanocrystallography - Outlook



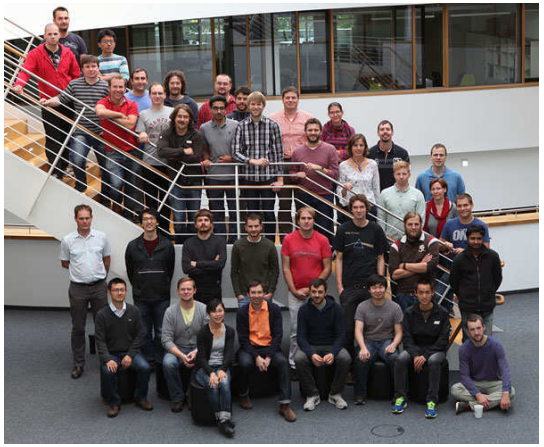
Chapman, H. N. et al (2011) Nature 470, 73-77.

- Phasing problem
- Carefully study radiation damage
- Time consumption
- Accessibility
- Mixing of proteins with substrates
- …

Acknowledgement

Prof. Henry Chapman (Center for Free Electron Laser, DESY, Hamburg)
Prof. Petra Fromme (Arizona State University, Tempe, USA)
Prof. Franz Kärtner (Center for Free Electron Laser, DESY, Hamburg)

Chapman group
Fromme group
Kärtner group
CFEL team
ASU team



European Research Council
Established by the European Commission

DESY

CFEL SCIENCE

ASU ARIZONA STATE UNIVERSITY

THANK YOU!