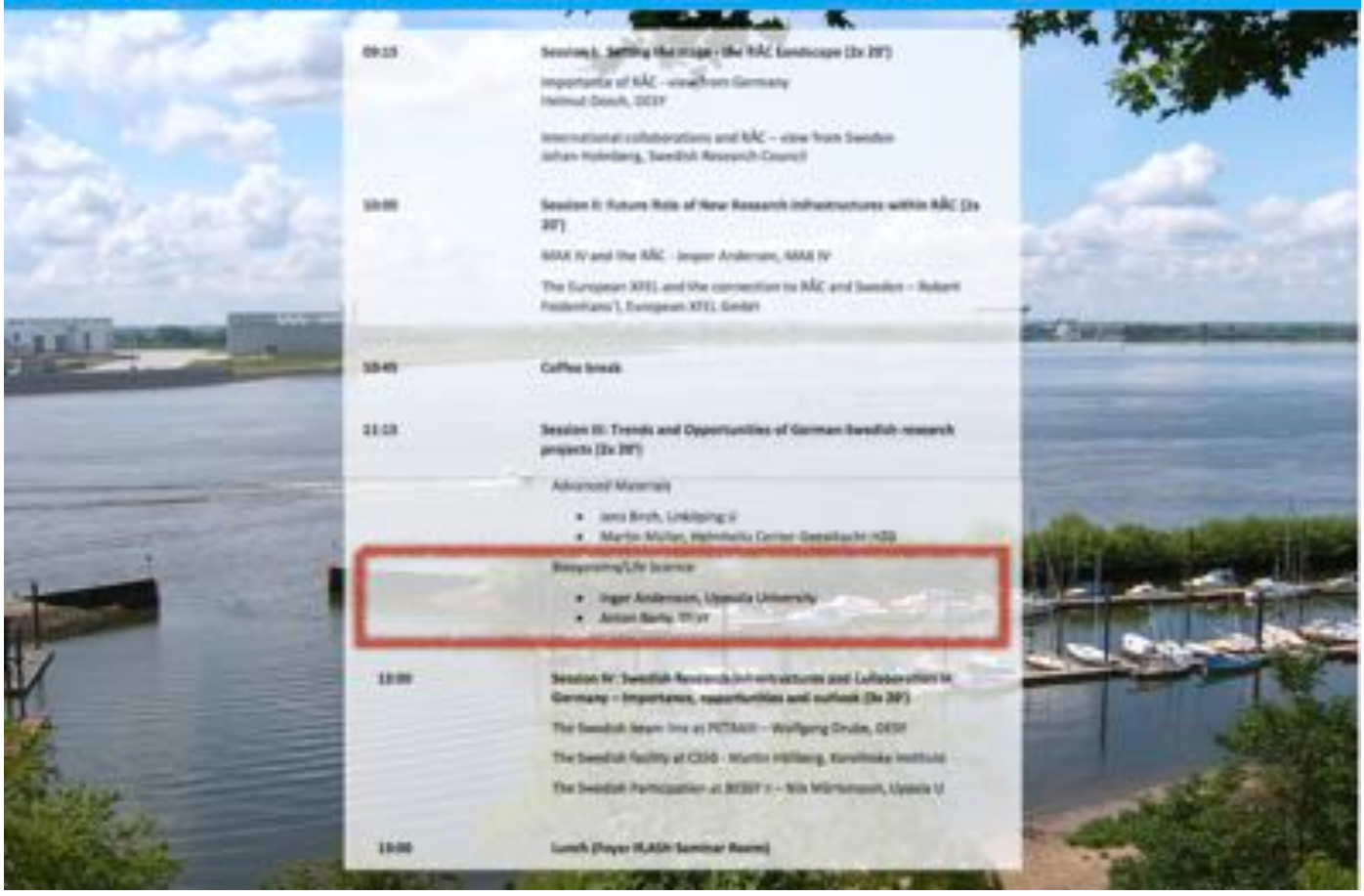
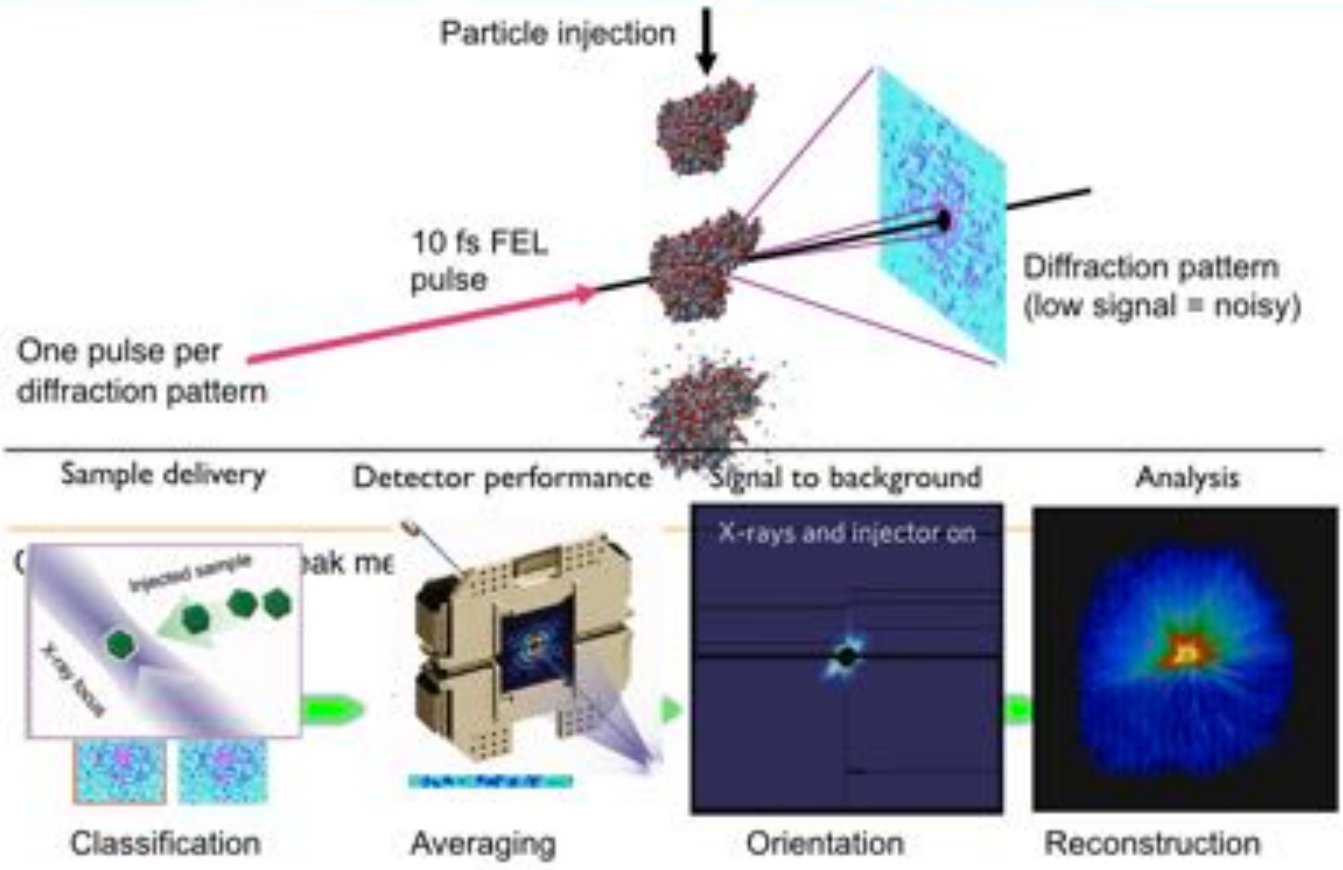
RÅC Perspective Workshop
09 June 2017, FLASH Seminar room, DESY Hamburg

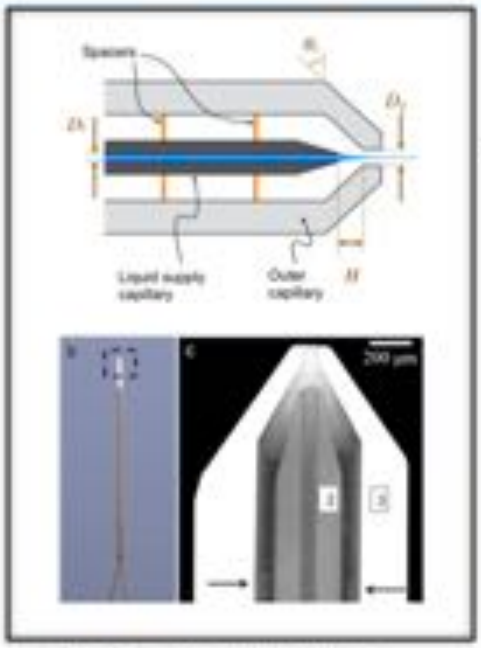
Röntgen Ångström Cluster



The ultimate goal remains atomic-resolution imaging of macromolecules without the need to grow large crystals

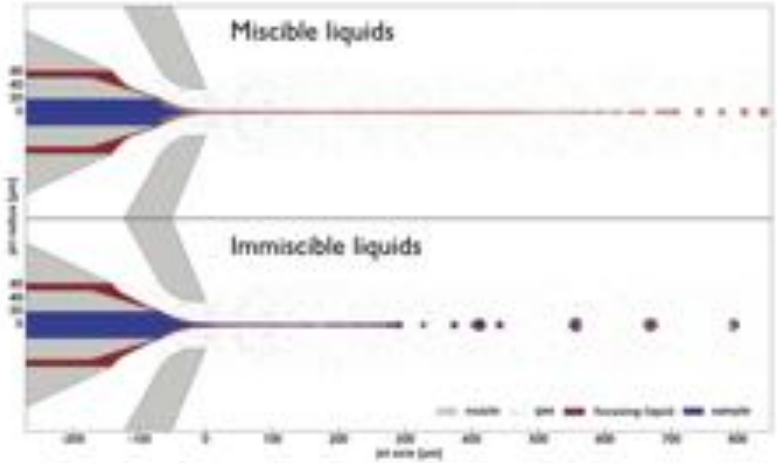


Gas dynamic virtual nozzles aerosolised carboxysomes for introduction to the focal region through an aerodynamic lens



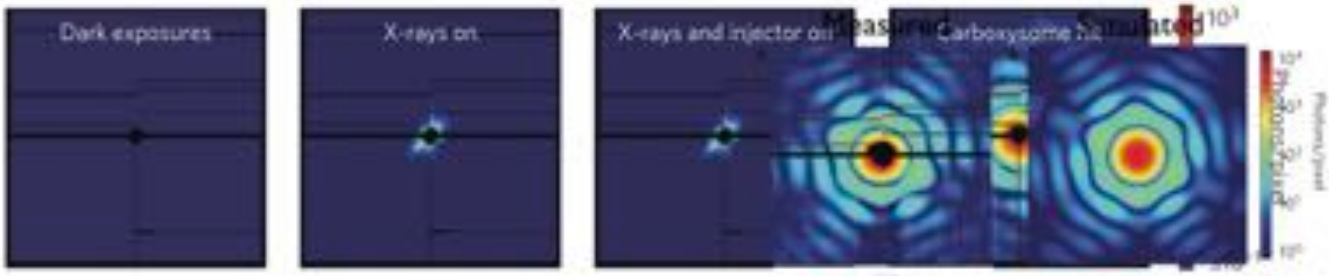
"Ceramic micro-injection molded nozzles for serial femtosecond crystallography sample delivery"
Beyerlein et al. *Rev Sci Instrum* **86**, 125104–11 (2015).

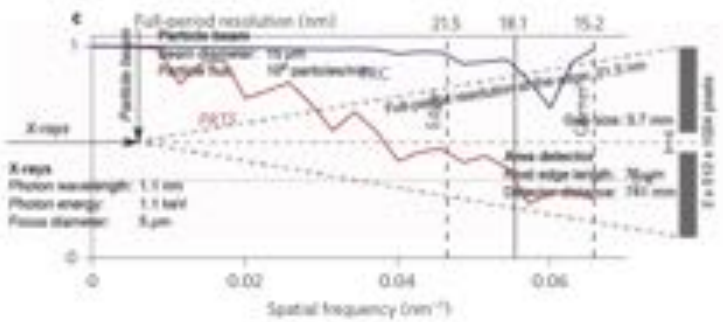
"Double-flow focused liquid injector for efficient serial femtosecond crystallography"
Oberthuer et al. *Sci Rep* **7**, 1–10 (2017).



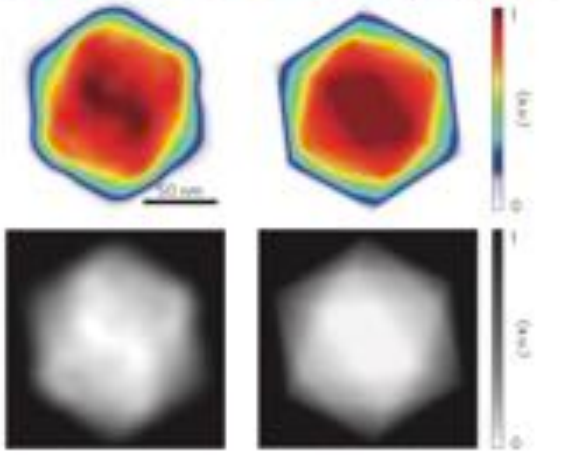
Simulation: Božidar Šarler, Grega Bellak, Marjan Maček

We achieved sufficiently low photon background for carboxysome measurements at the AMO instrument, LCLS



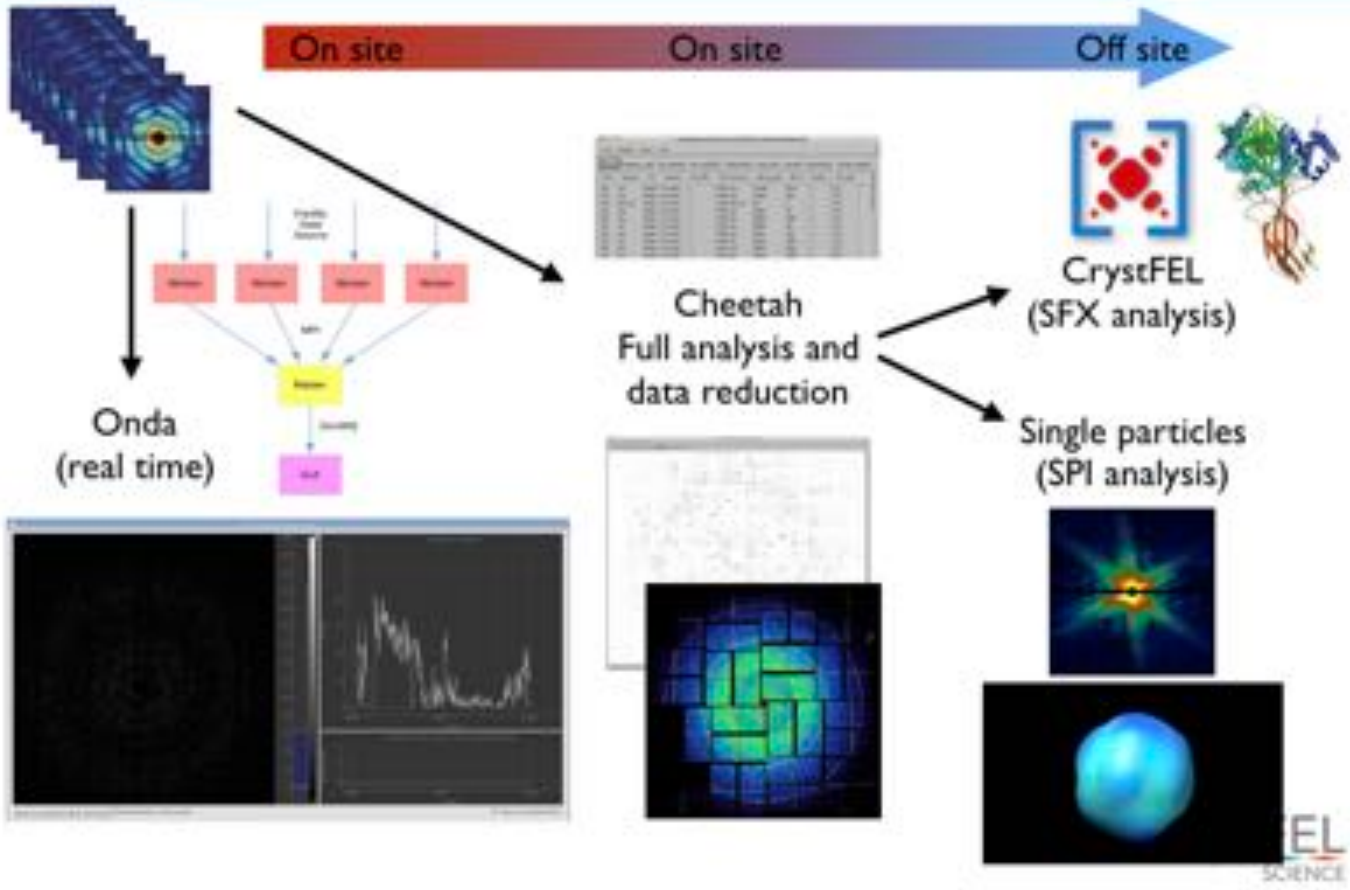


"A data set from flash X-ray imaging of carboxysomes"
Hantke et al. *Sci Data* **3**, 160061 (2016)

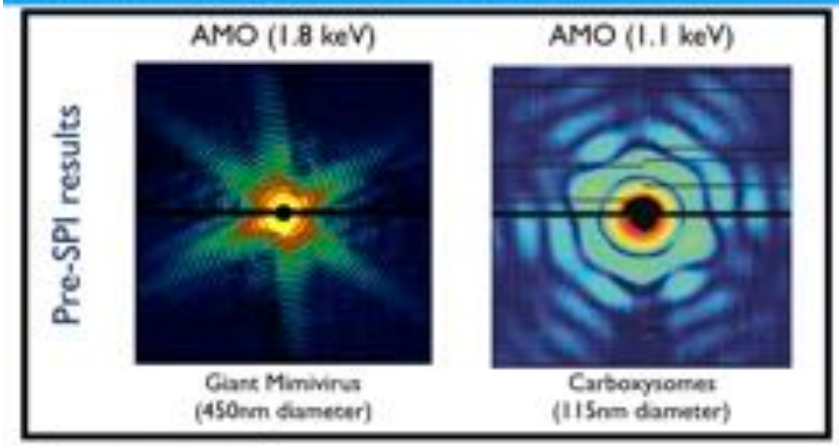


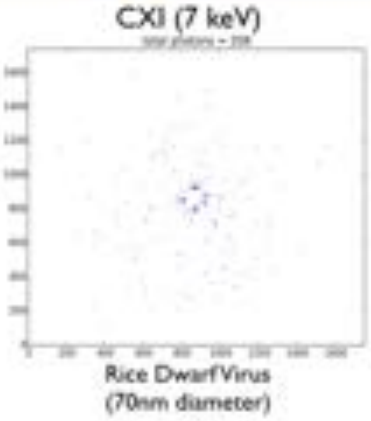
The diameter of a Rubisco molecule is ~11 nm and these molecules cannot be resolved at 18.1 nm resolution.

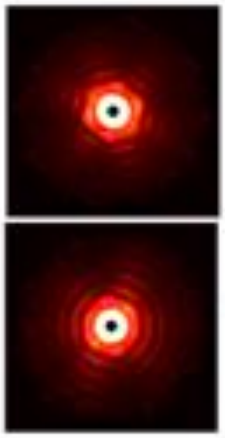
The CFEL data reduction pipeline is divided into online, offline and offsite stages



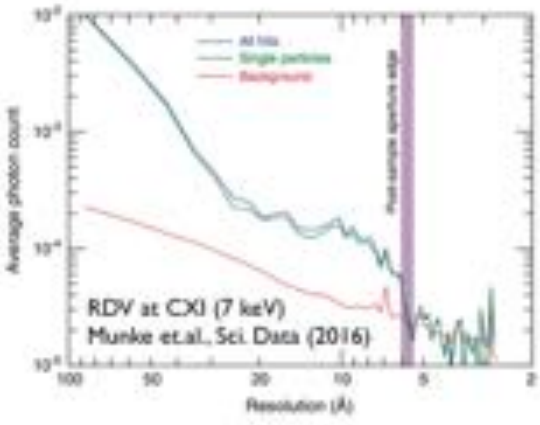
The Single Particle Imaging initiative of LCLS has facilitated recent experiments in which Uppsala and DESY are major players



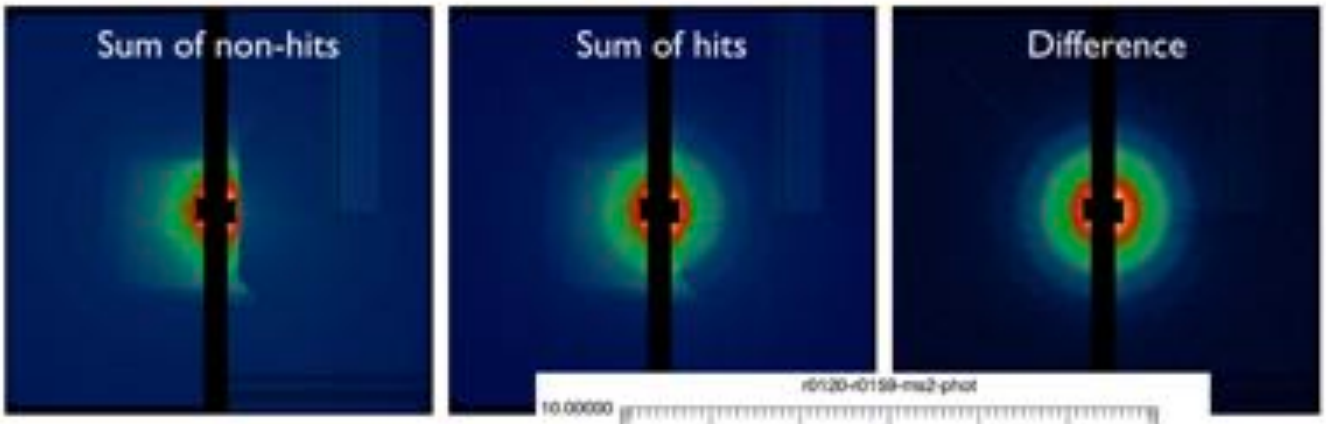




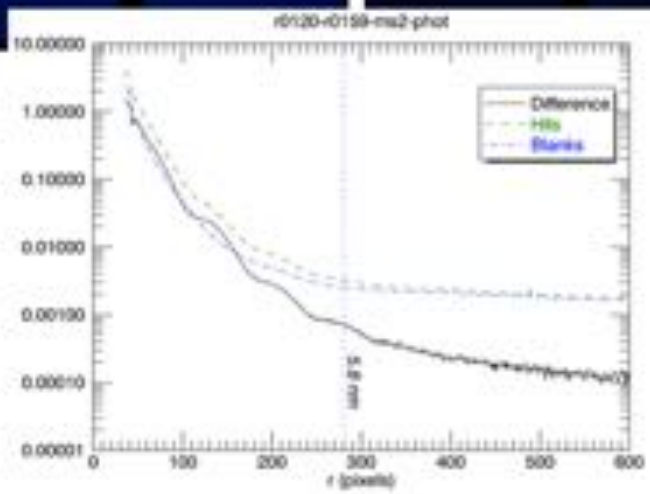
PR772 at AMO (1.6 keV)
(65-70nm diameter)
Kartik Ayyer and Andrew Morgan



MS2 virus aerosolised using electrospray at AMO highlight the importance of controlling background levels



MS2 bacteriophage
26.8 nm



LCLS is used to solve difficult to crystallise and radiation sensitive proteins, and for time resolved structural dynamics

Lysozyme
4Z3X, 5C6I, 5C6J, 5C6L, 4RW1, 4RW2, 4NSR

Thermolysin
4QW3, 4TNL, 5DLH

ATPase
4XOU

30s ribosome
5IR8

SNARE complex
5CCG

Rhodopsin-arrestin
4ZWJ

Angiotensin
4YAY

A2a
5K2D, 5K2C, 5K28, 5K2A

5-HT2B
4IAQ

Smoothened
4O9R

DgkA
4UYO

d-opioid
4RWD

Granulovirus
5GOZ

CathepsinB
4HWY

Cry toxin
4QX3, 4QX2, 4QX1, 4QX0

Phytochrome
5MG8, 5MG1, 5L8M

BinAB
5F0X, 5F0Z, 5G37,

Phycocyanin
4Q70, 4Z8K, 4Z1Z

CPV17
4ZQX

PAS-GAF
5L8M

Photosystem I+II (x16)
3PCQ, 4F8Y, 4XXR, 4XXQ, 4TNK, 4TNJ, 4TNI, 4TNH, 4PBU, 4RVF, 5E7C, 5T15, 5KAI, 5KAF,

Myoglobin (x15)
4PNJ, 5CNG, 5CNF, 5CNE, 5CND, 5CNC, 5CNB, 5CN9, 5CN8, 5CN7, 5CN6, 5CN5, 5CN4, 5CMV, 5JOM

PYP
4WLA, 4WL9, 5HD3, 5HD5, 5HDD, 5HDC, 5HD5,

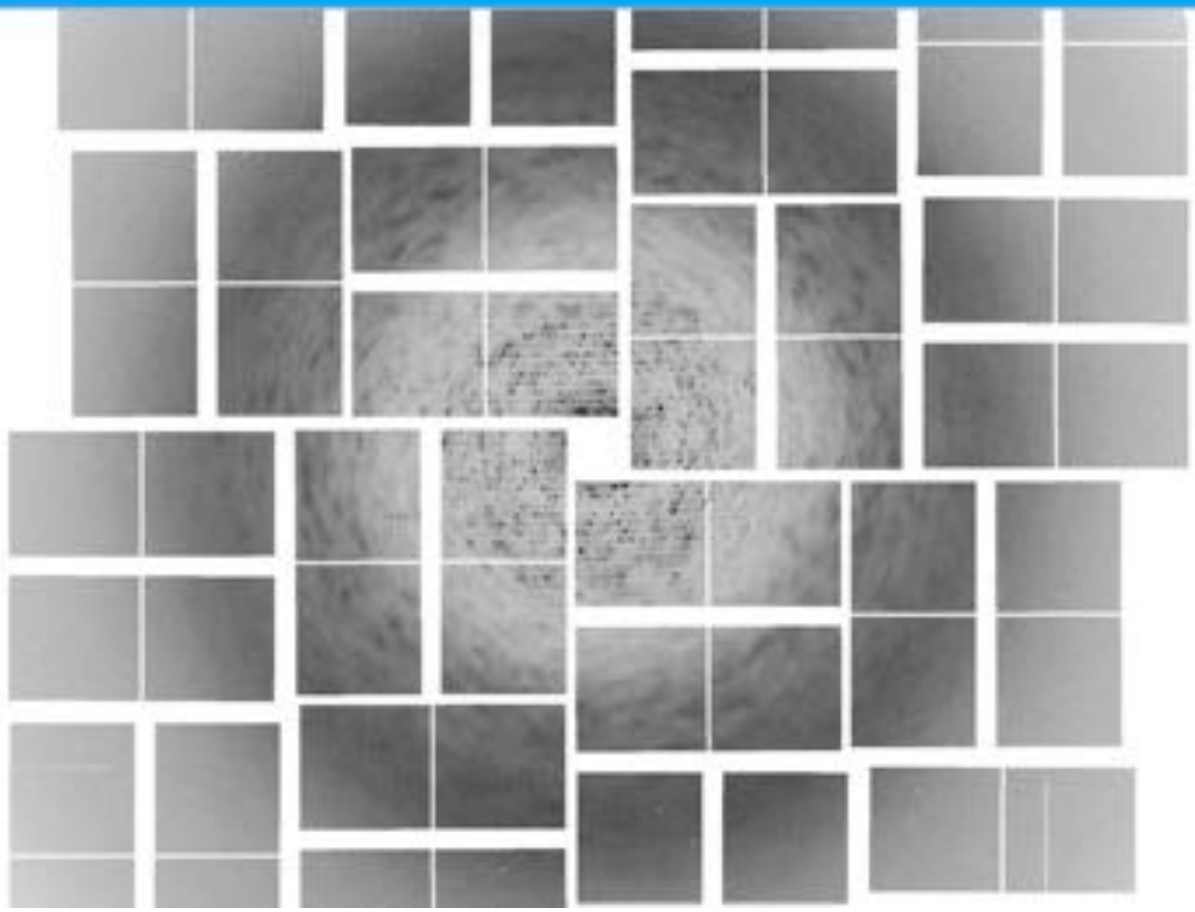
Reaction centre
4ACS, 4CAS, 5M7K, 5M7J

Bacteriorhodopsin
5JTA

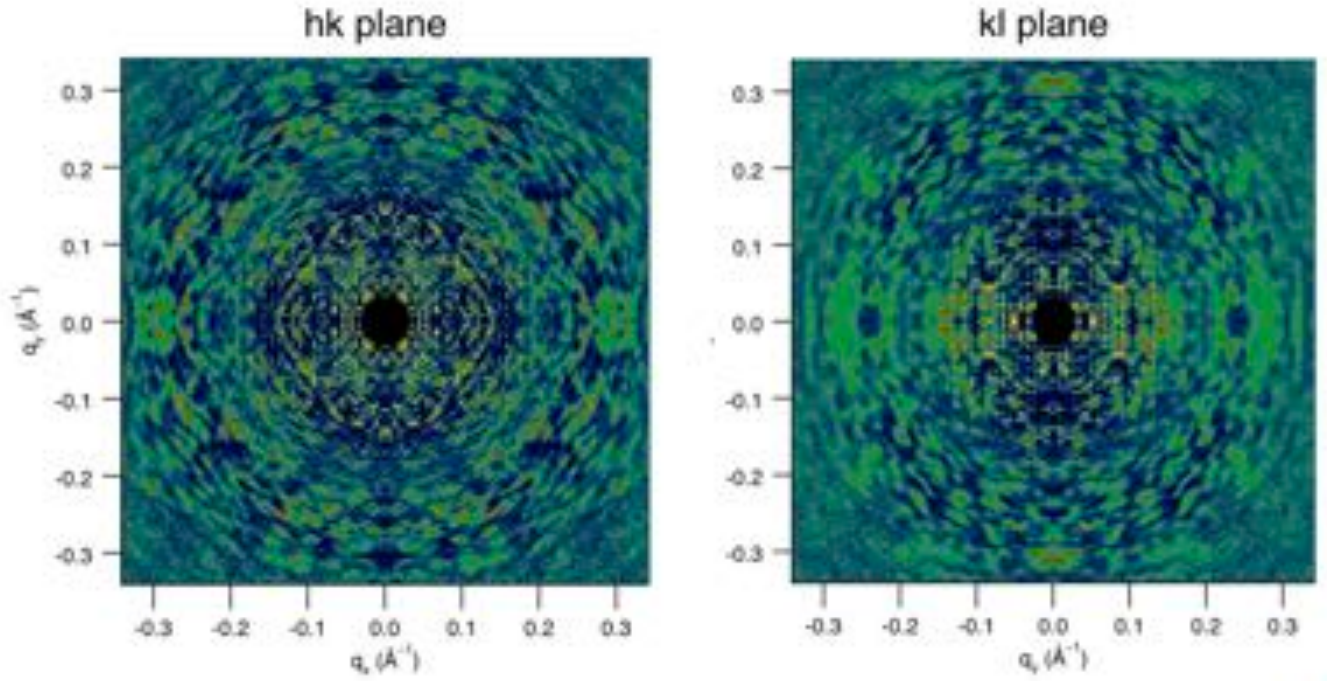
Riboswitch
5DWE, 5DWD, 5E54

From LCLS: >90 From SACLA: >42

Single frame diffraction from Photosystem-II diffraction at LCLS



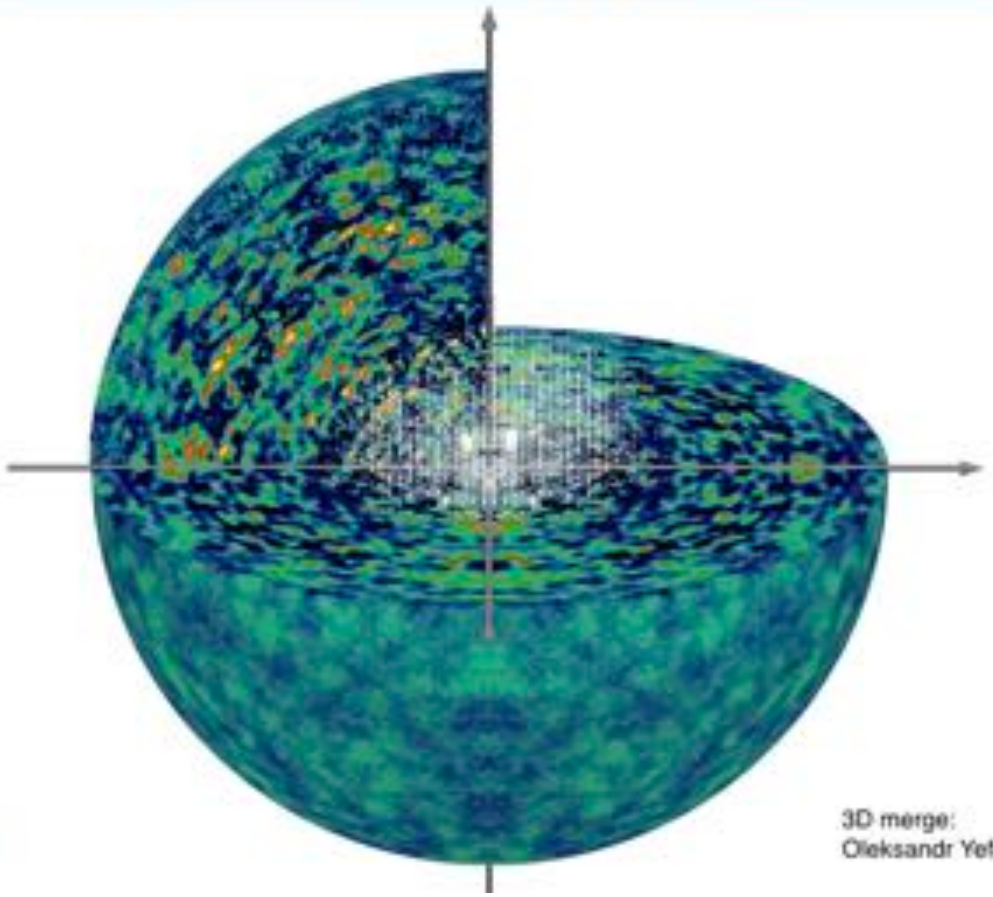
Merging intensities in 3D reveals continuous diffraction beyond the Bragg peak resolution



Merge of 2848 strongest patterns

3D merge:
Oleksandr Yefanov

The 3D intensity distribution resembles single molecule diffraction to the edge of the detector, with Bragg peaks in the core



CFEL SCIENCE

3D merge:
Oleksandr Yefanov

DESY

Resolution is improved by treating the continuous diffraction as single particle data

Bragg only
(4.5 Å)

Bragg and continuous
(3.5 Å)

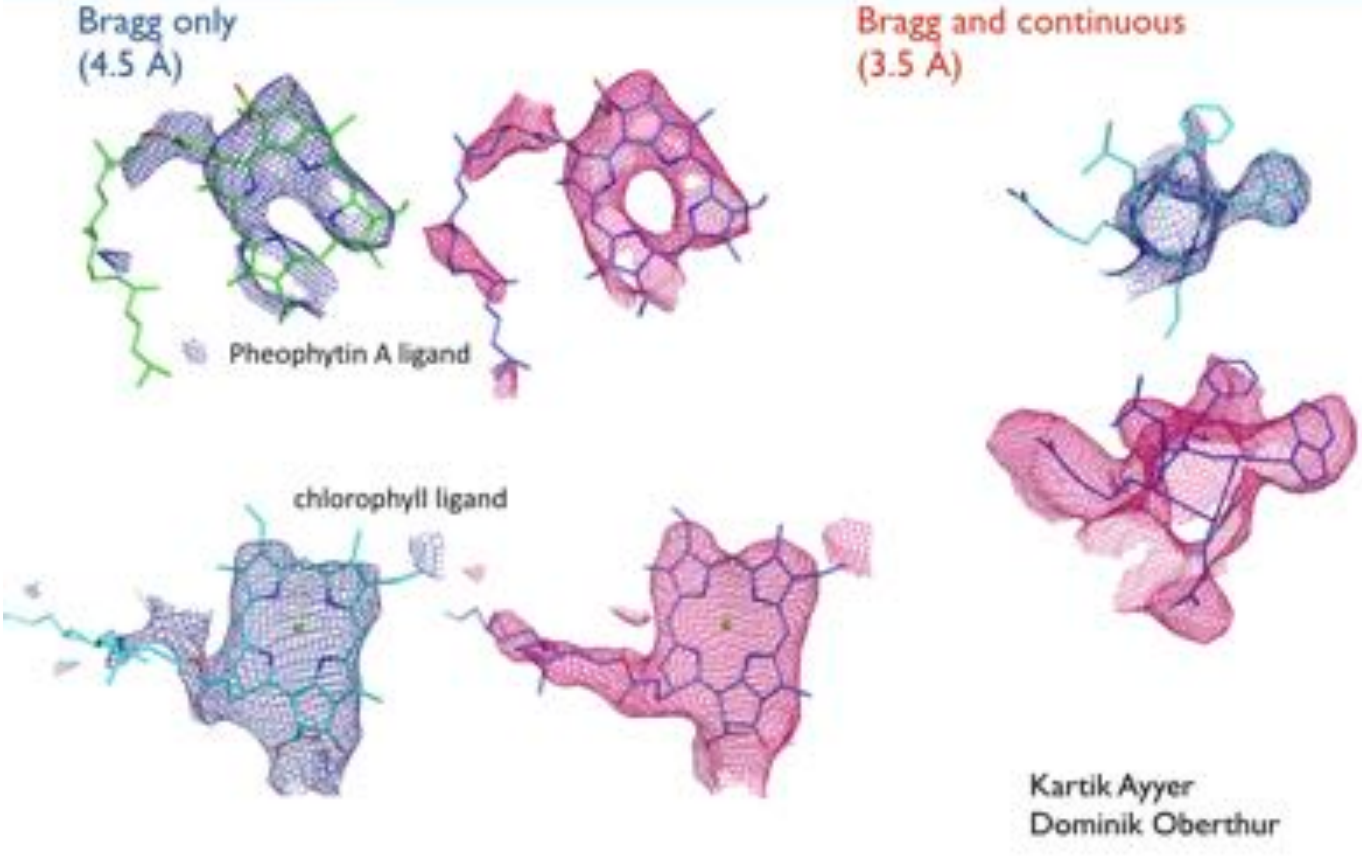
Number of molecules per shot: 1 $\mu m^3 \times 4 / (9.2 \times 10^6 Å^3) = 4 \times 10^5$

Resolution not limited by the crystal, just detector extent and shots

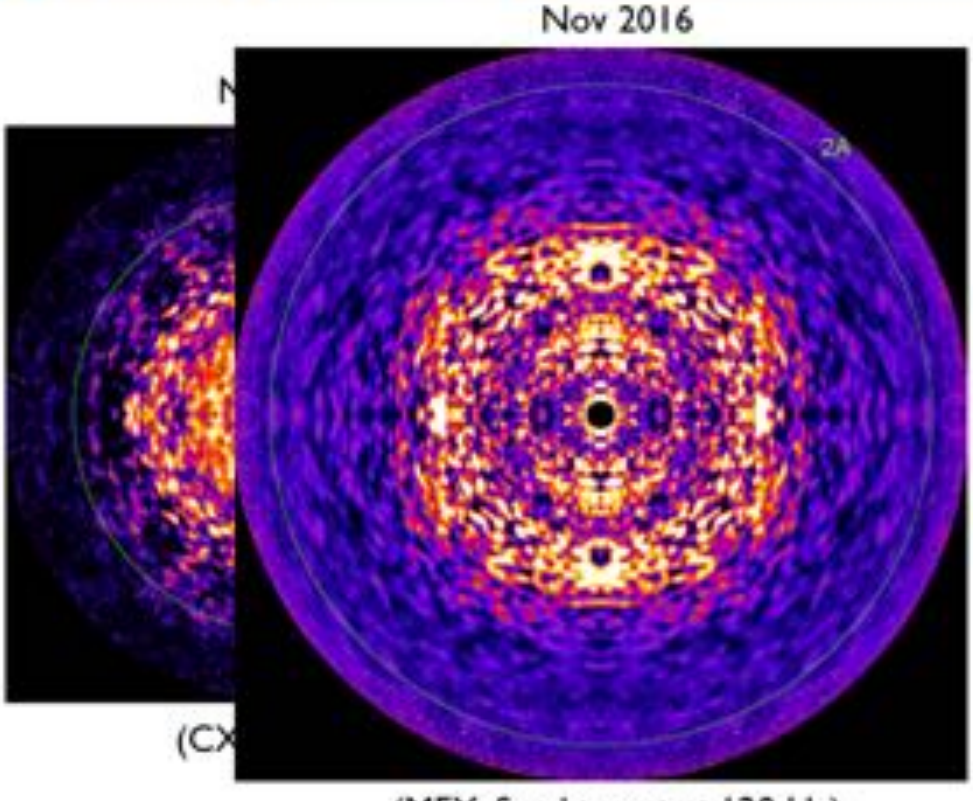
Improved diffraction sampling — more reliable structure determination
— potential for model free phasing

Kartik Ayyer
Dominik Oberthur

Resolution is improved by treating the continuous diffraction as single particle data



Continuous diffraction signal goes to even higher resolution using improved sample delivery to reduce background



(MFX, fixed target at 120 Hz)

The phasers


Henry Chapman


Kartik Ayyer


Oleksandr Yefanov


Dominik Oberthür


Lorenzo Galli


Anton Barty


Tom White


Valerio Mariani

Röntgen Ångström Cluster

DESY

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Filipe Maia
Tomas Ekeberg
Benedikt Daurer
Gijs van der Schot
Janos Hajdu & team

Röntgen Ångström Cluster

LCLS

European XFEL

DESY

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Ken Beyerlein
Richard Bean
Richard Kirian
Holger Fleckenstein
Sasa Bajt
Miriam Barthelmess
Oleksandr Yefanov
Dominik Oberthür
Cornelius Gati
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Kartik Ayyer