UPPSALA UNIVERSITET

Ultrafast biological imaging without crystals

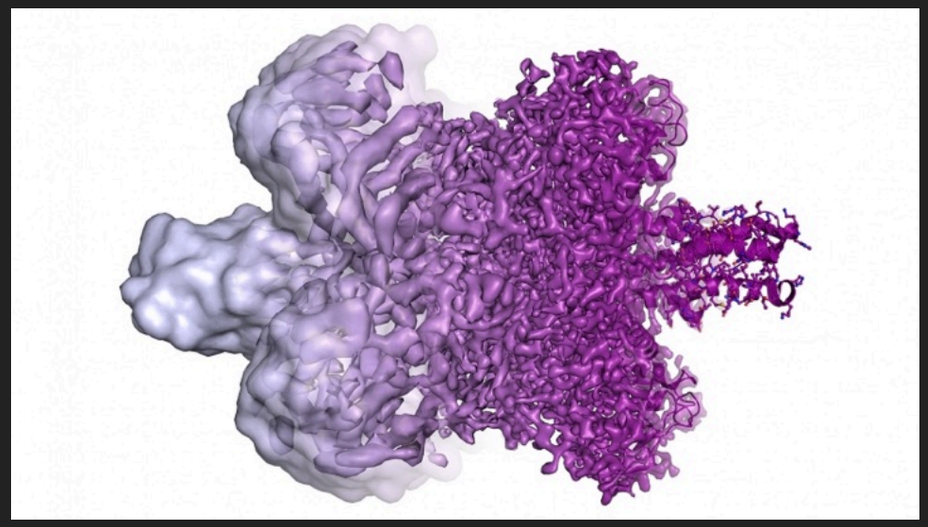
XI AUSE Conference and VI ALBA Users Meeting

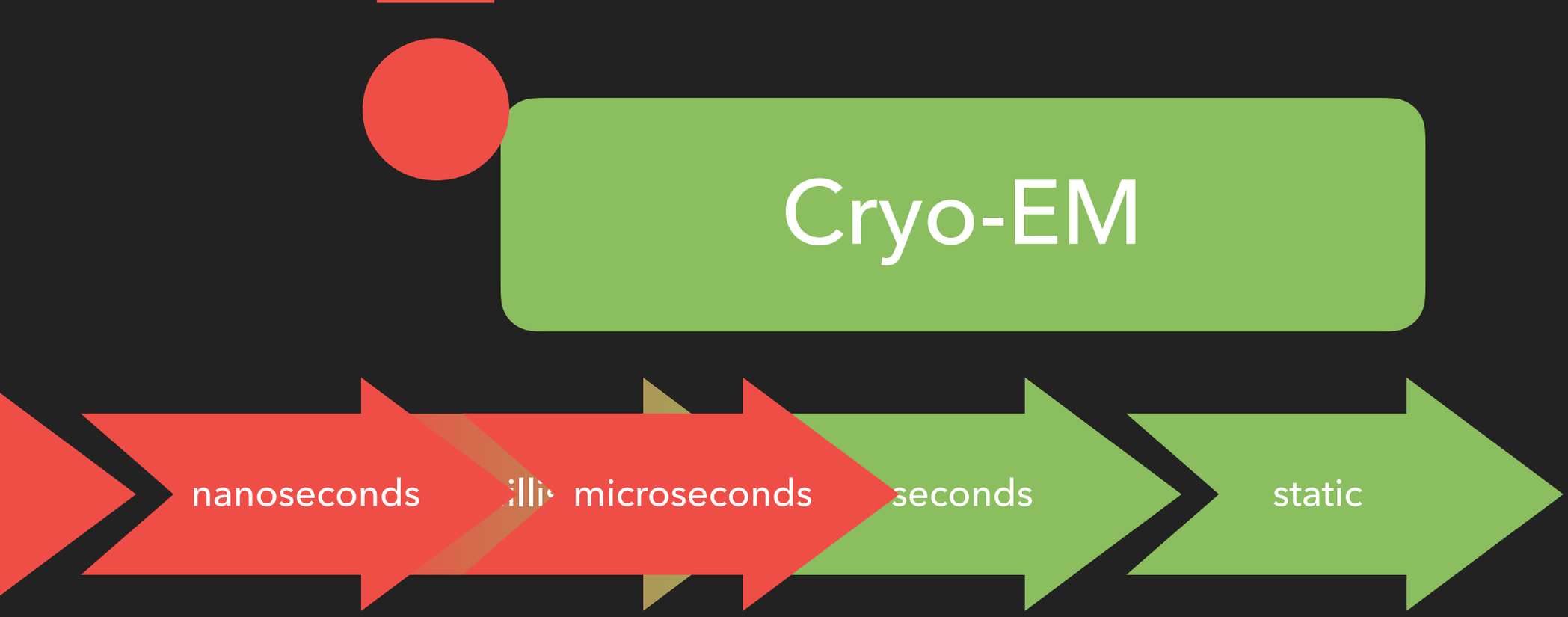
2024-09-05

Filipe R N C Maia

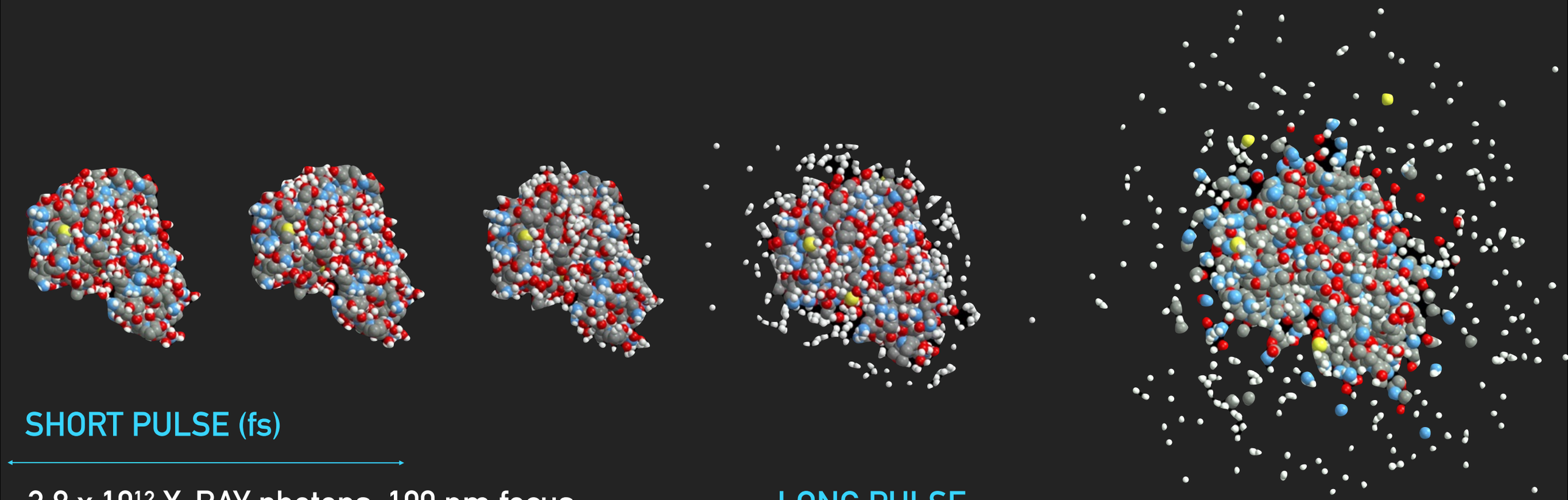
Lab. of Mol. Biophysics
Uppsala University

TIME-RESOLVED SINGLE-PARTICLE IMAGING





DIFFRACTION BEFORE DESTRUCTION!



SHORT PULSE (fs)

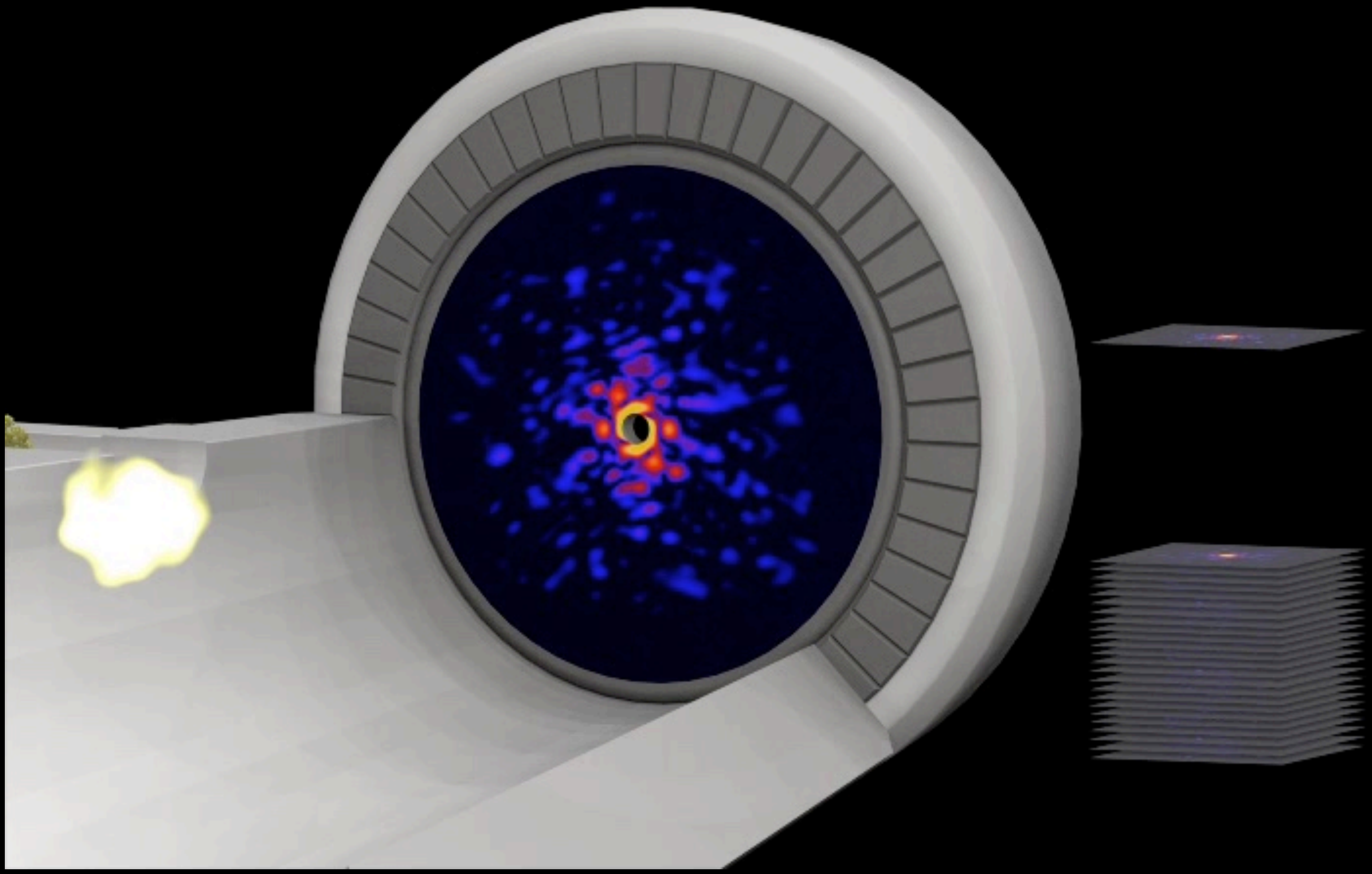
3.8 x 10^{12} X-RAY photons, 100 nm focus

LONG PULSE

SPEED OF LIGHT vs. SPEED OF A SHOCK WAVE

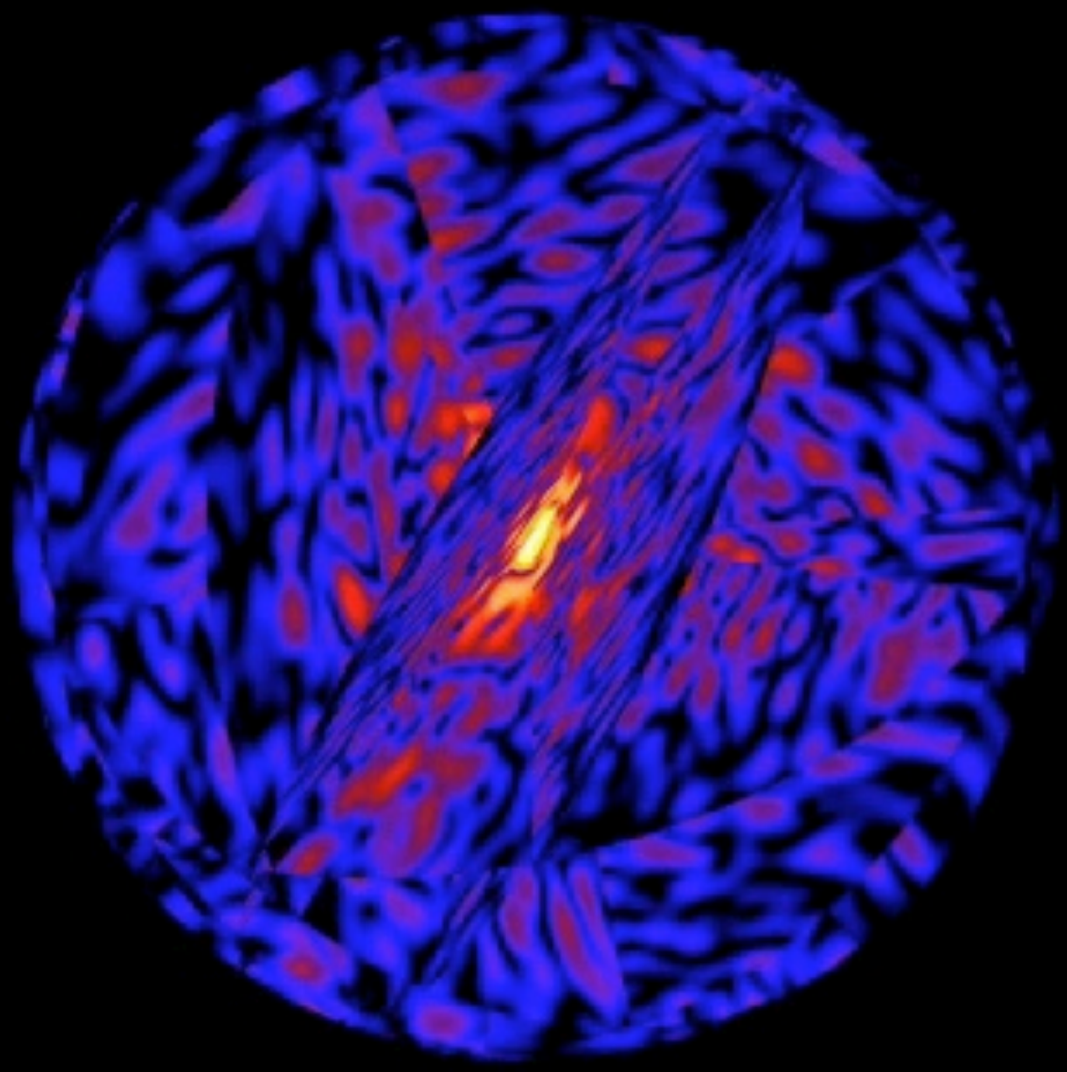
Neutze, R., Wouts, R., van der Spoel, D., Weckert, E., Hajdu, J., *Nature* 406, 752-757, (2000).

The Dream…



The Dream…

The Dream…



The First Sample

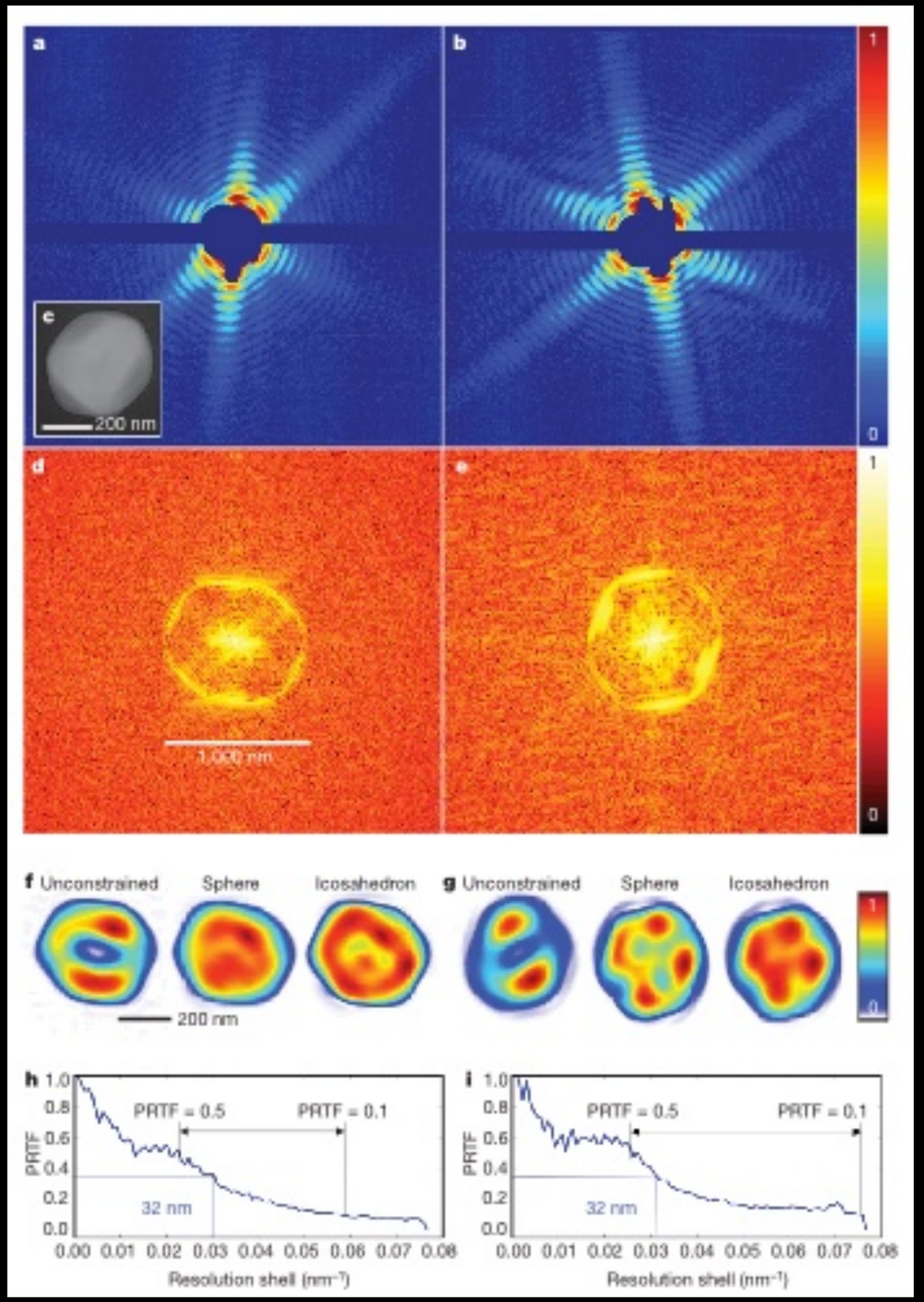
Mimivirus diffraction patterns from LCLS

EM

Autocorrelations

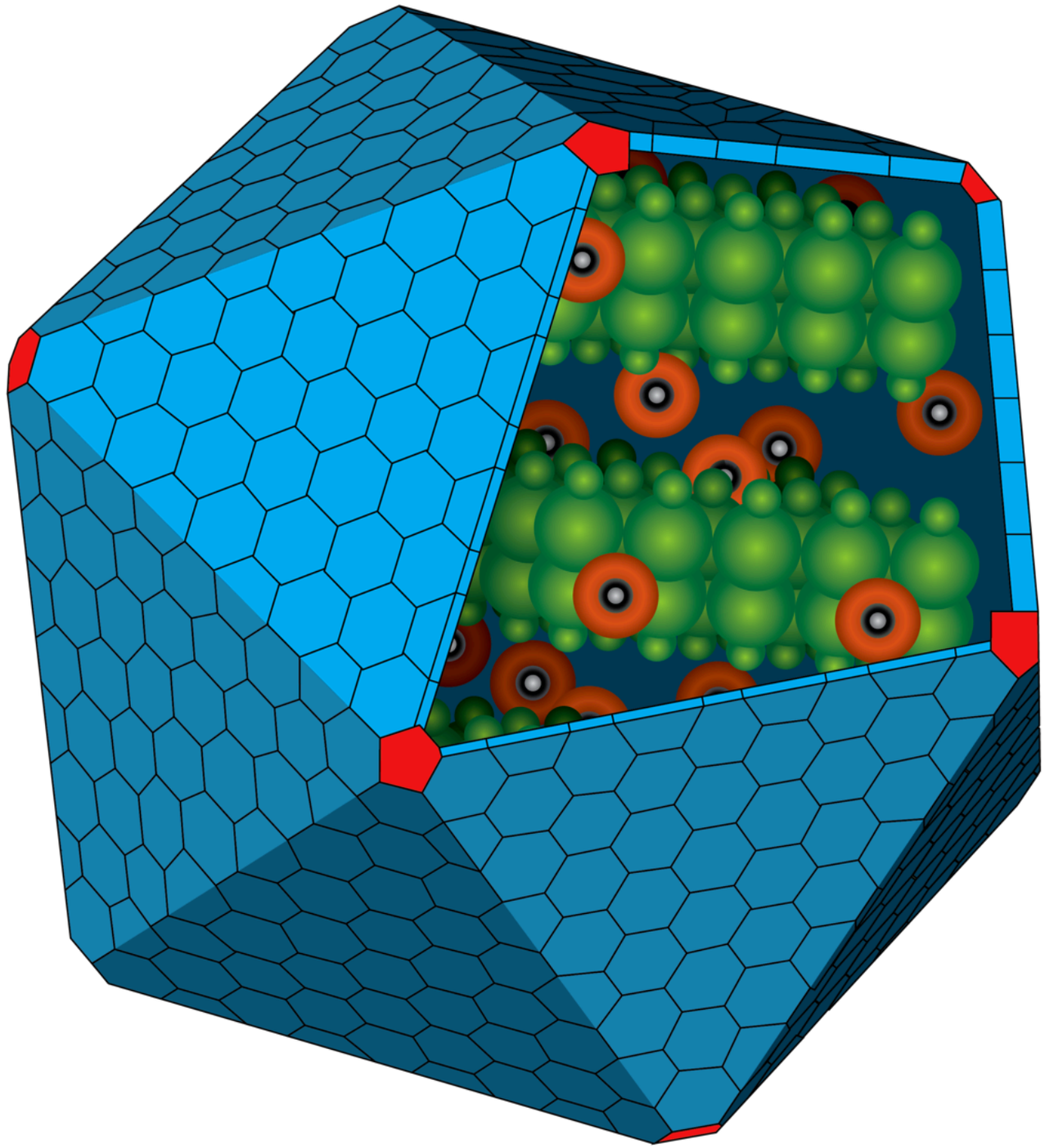
Reconstructions

PRTFs



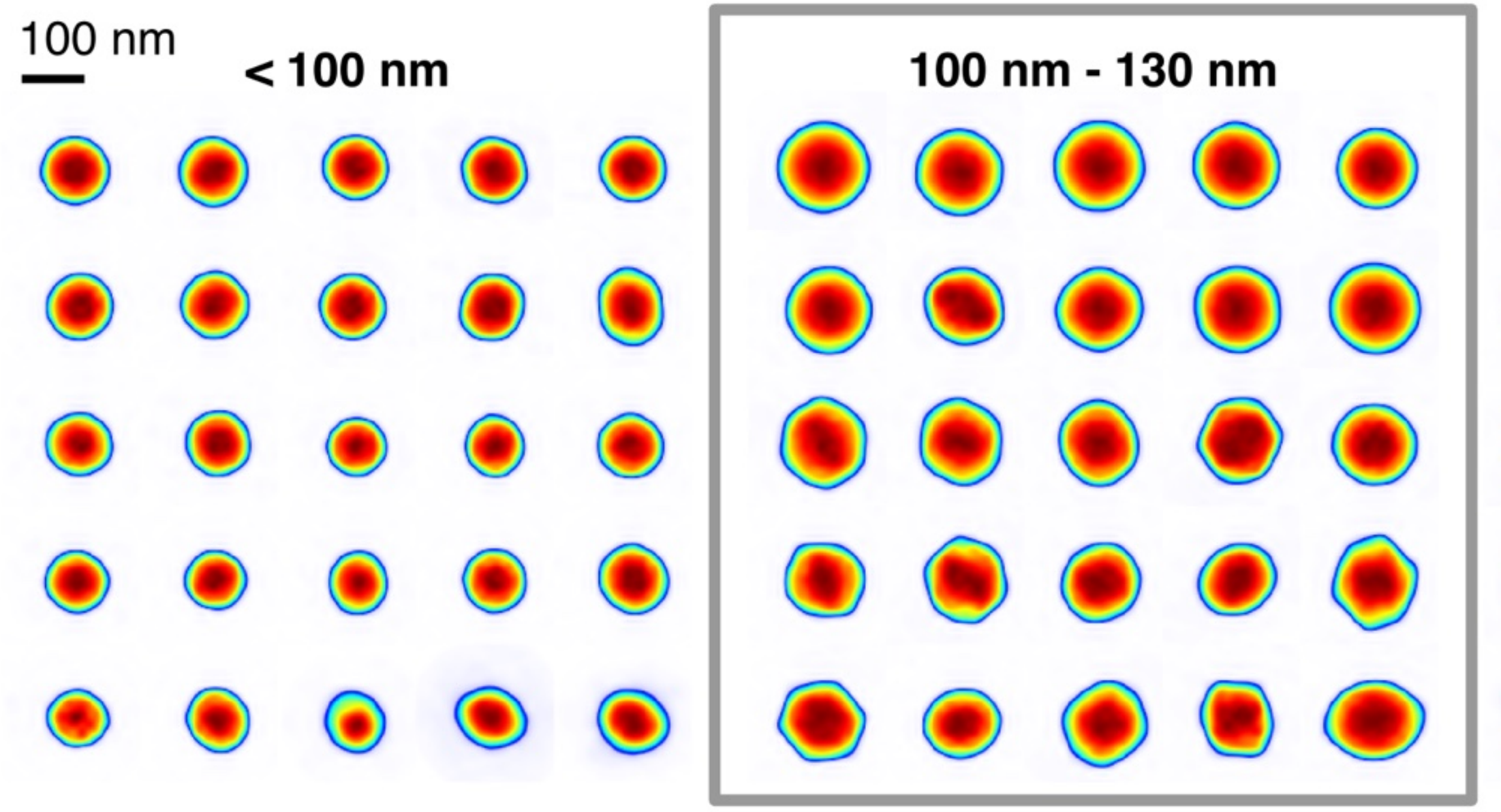
Seibert, Ekeberg, Maia et al. ***Nature*** (2011)

IMAGING 100 NM PARTICLES – CARBOXYSOMES



Hantke, M. F. et al. Nature Photonics 8, 943–949 (2014).

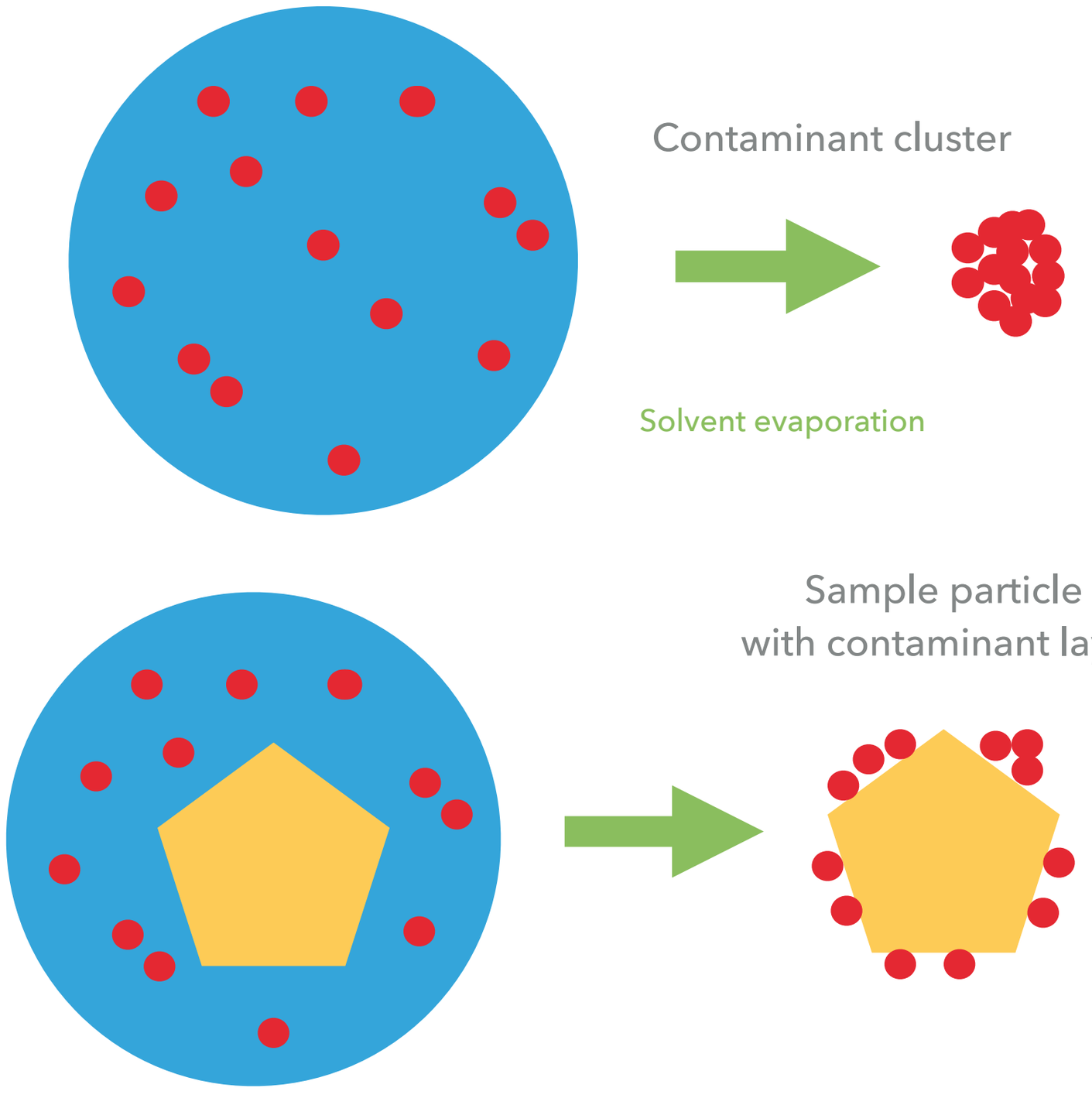
IMAGING 100 NM PARTICLES – CARBOXYSOMES



WE JUST GOT SPHERES!

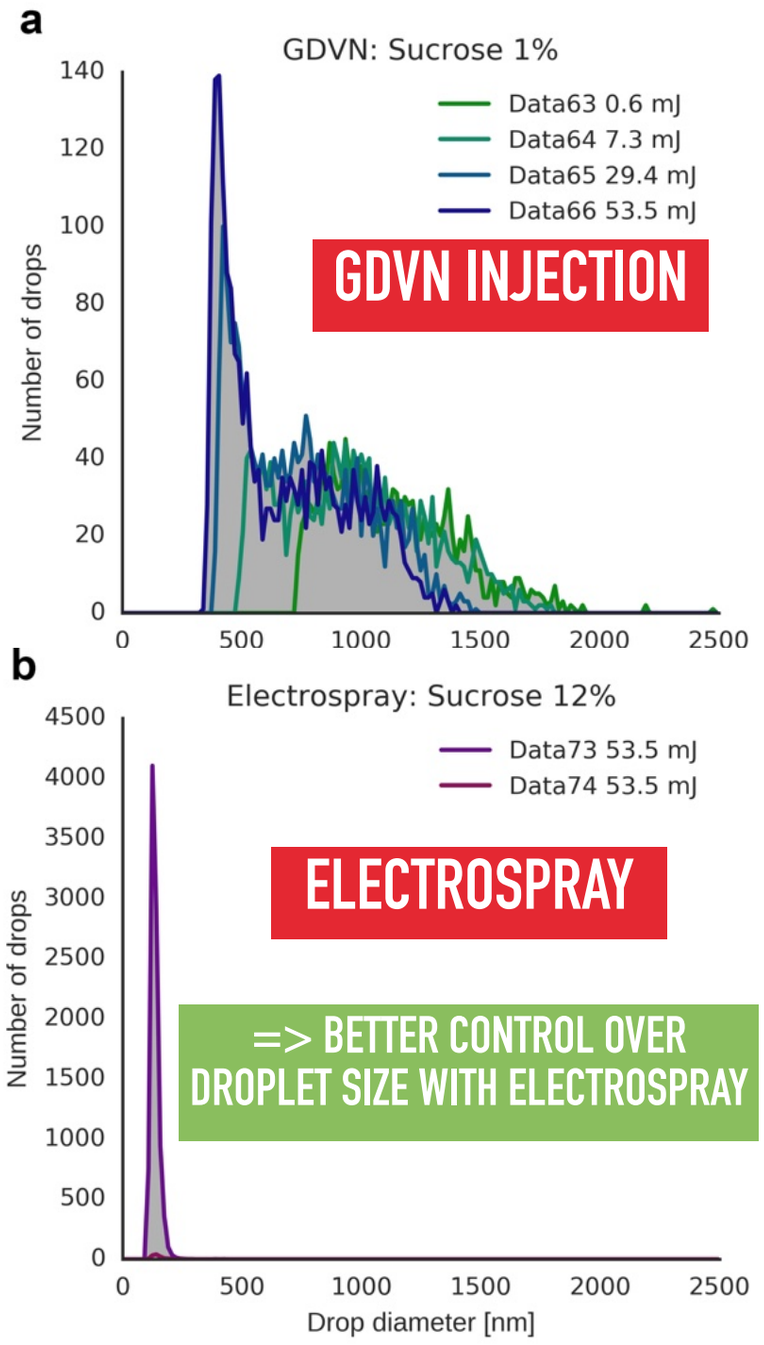
Hantke, M. F. et al. Nature Photonics 8, 943–949 (2014).

LIKELY EXPLANATION: SAMPLE CONTAMINANTS

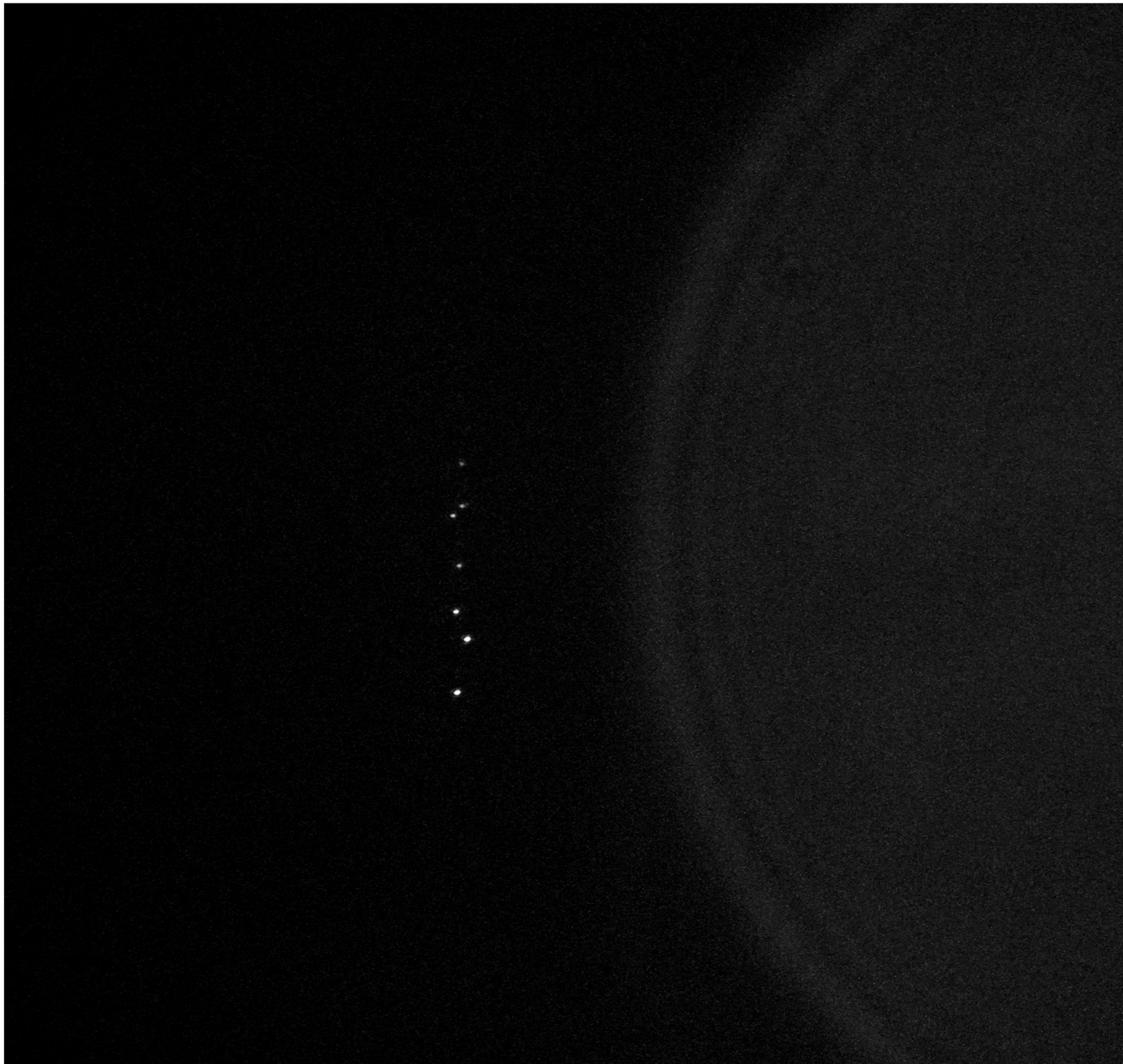


MEASURE INITIAL DROPLET SIZE BY MIE SCATTERING IMAGING

DROPLET SIZE DISTRIBUTIONS



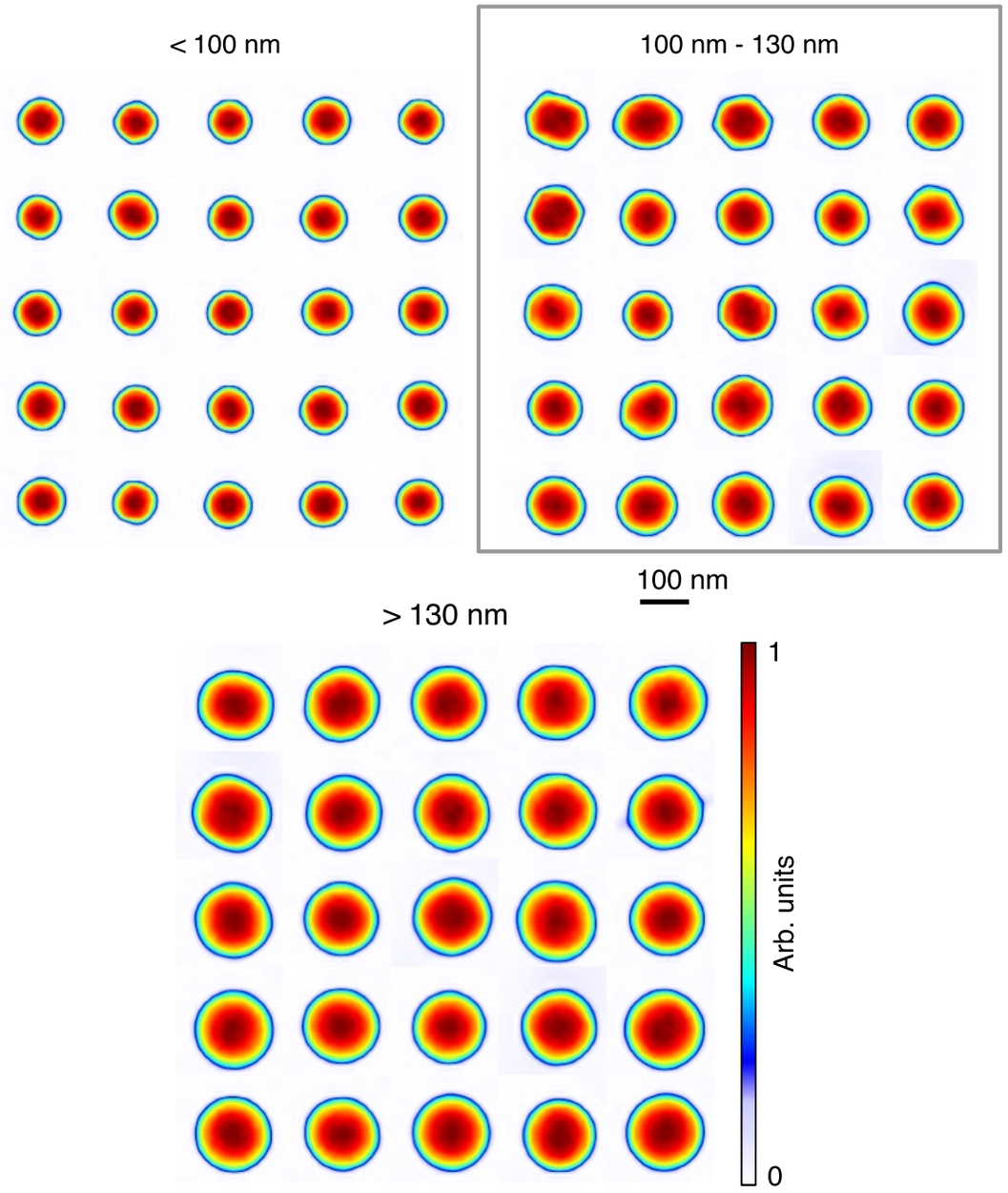
(Thesis Fig. 5.6)



ELECTROSPRAY INJECTION

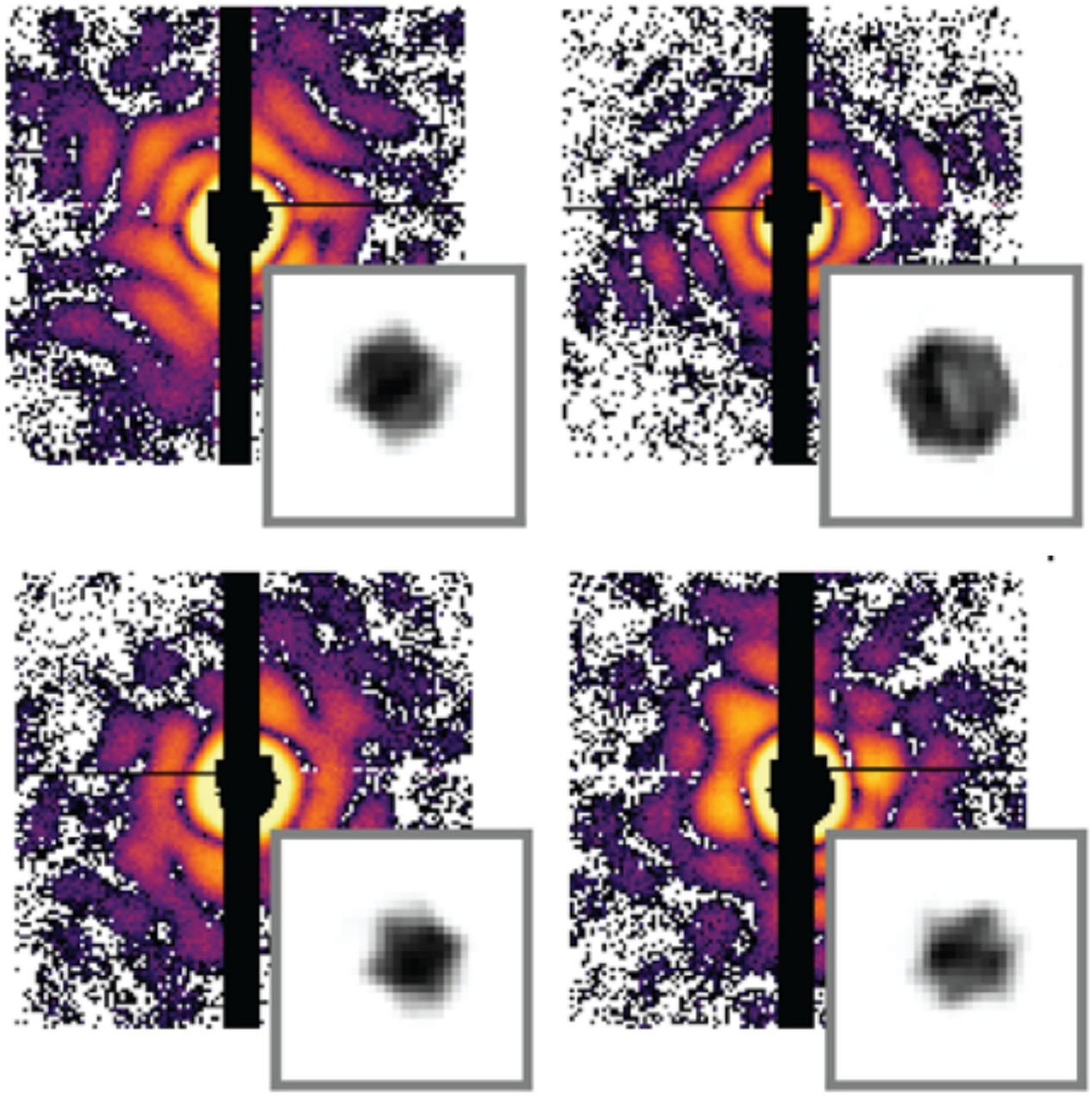
MOSTLY ROUND PARTICLES

GDVN DATA



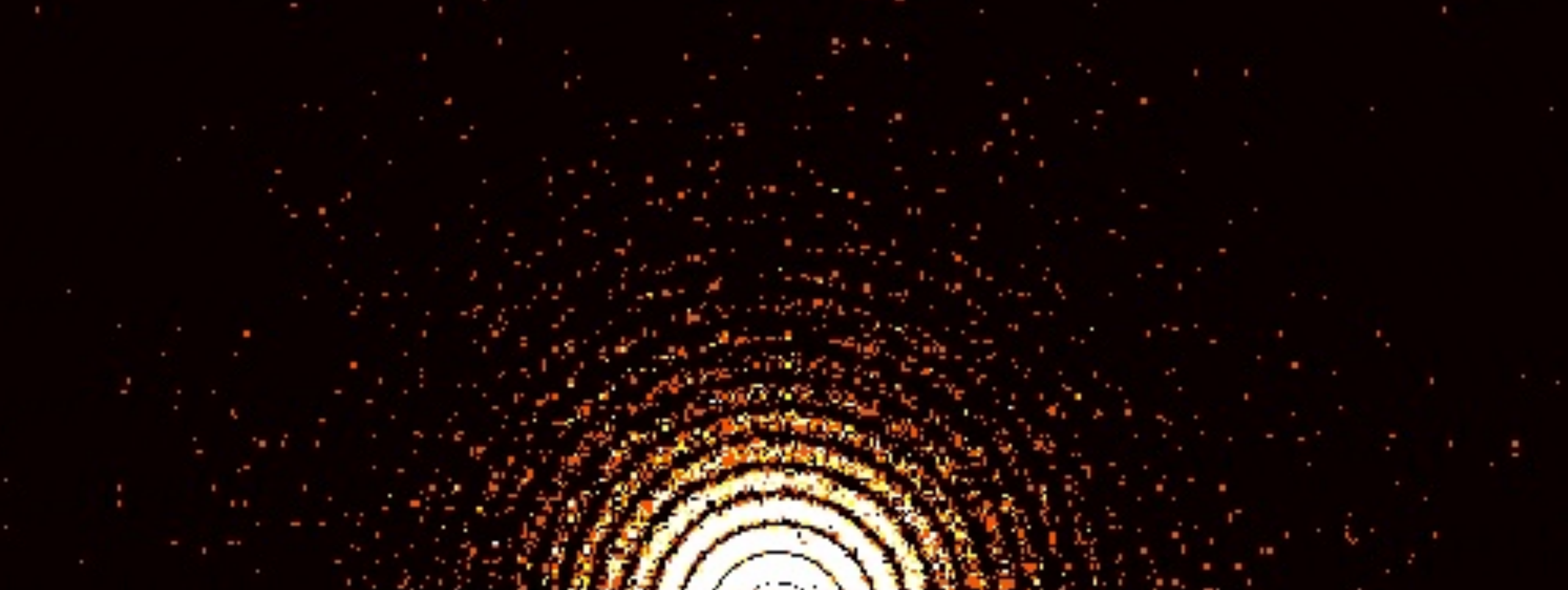
NO ROUND PARTICLES

ELECTROSPRAY DATA



Bielecki, J et al. *Science Advances* 5, (2019).

SINGLE PARTICLE EXPERIMENTS AT THE EUROPEAN XFEL



Flash X-ray Imaging of Single Proteins

European XFEL - Experiment SQS 2146

PI: Filipe Maia (Uppsala University)

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Philipp Schmidt
Yevheniy Ovcharenko
Tommaso Mazza
Sergey Usenko
Daniel Rivas
Alberto De Fanis
Robin Schubert
Adrian Mancuso
Johan Bielecki

Tomas Ekeberg (Uppsala University)
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Henry Chapman (CID CFEL)
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Tamme Witte

Jochen Küpper (CMI CFEL)
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Elena Rodina (Moscow State University)

Victor Lamzin (EMBL)
Egor Sobolev

Davide Emilio Galli (University of Milan)

Jonas Sellberg (KTH Stockholm)
Jayanath Koliyadu

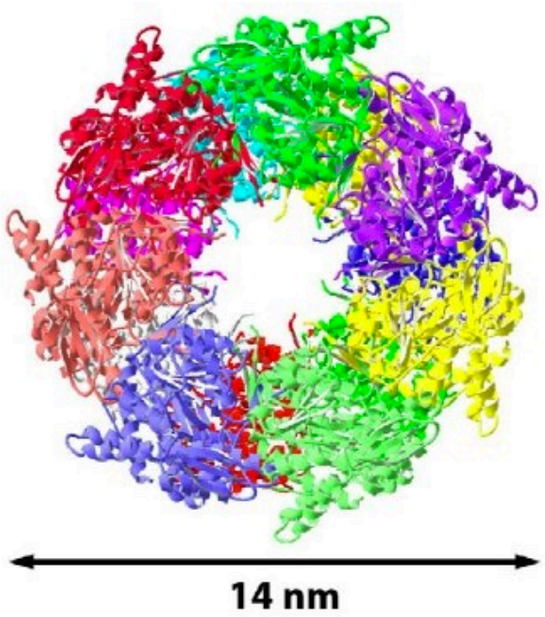
Ivan Vartaniants (DESY)
Sergei Zolotarev
Sergey Bobkov
Jerome Carnis

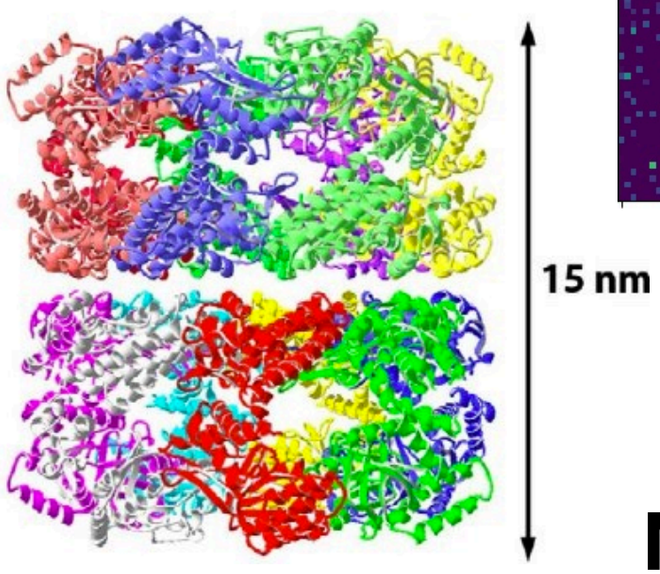
Duane Loh (National University of Singapore)
Benedikt Daurer

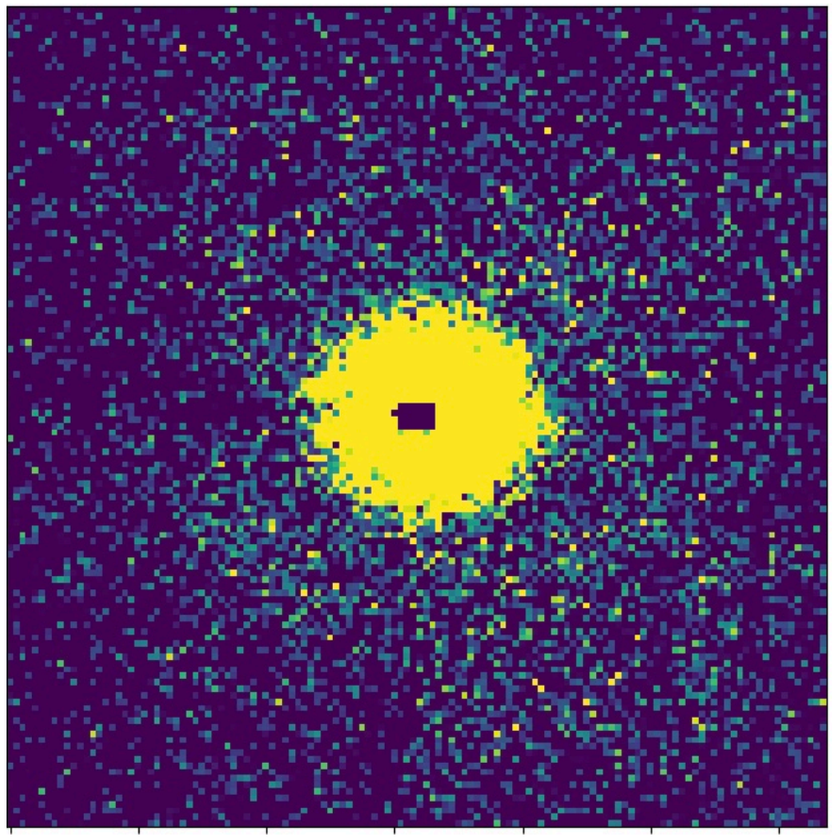
Flash X-ray Imaging of Single Proteins

European XFEL - Experiment SQS 2146

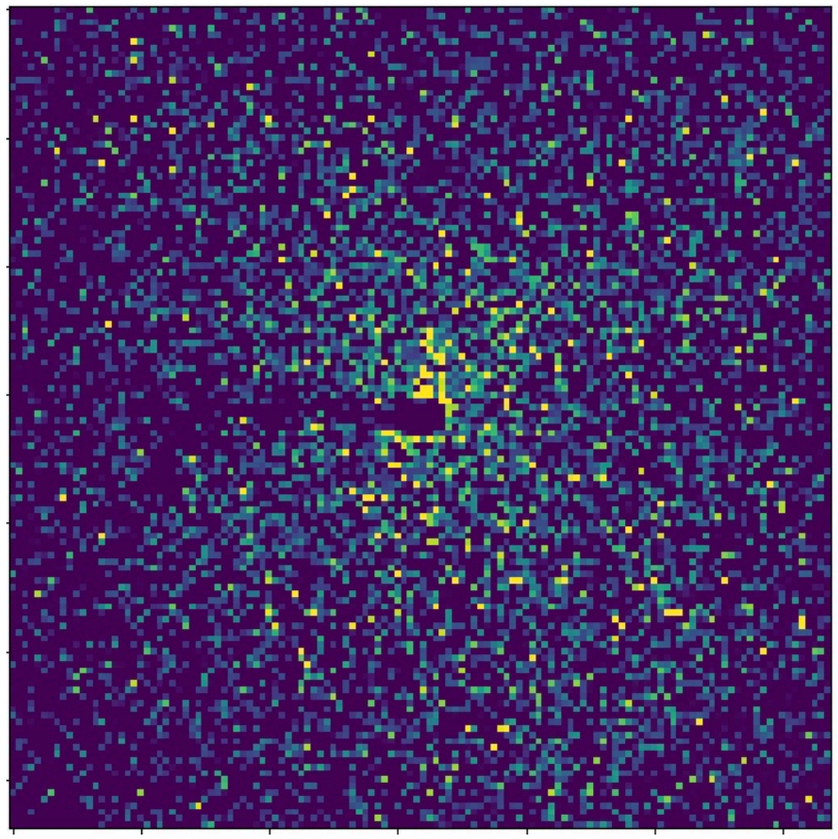
Chaperonin 60 from *Escherichia coli* (GroEL)







GroEL signal from SQS (8x downsampled)



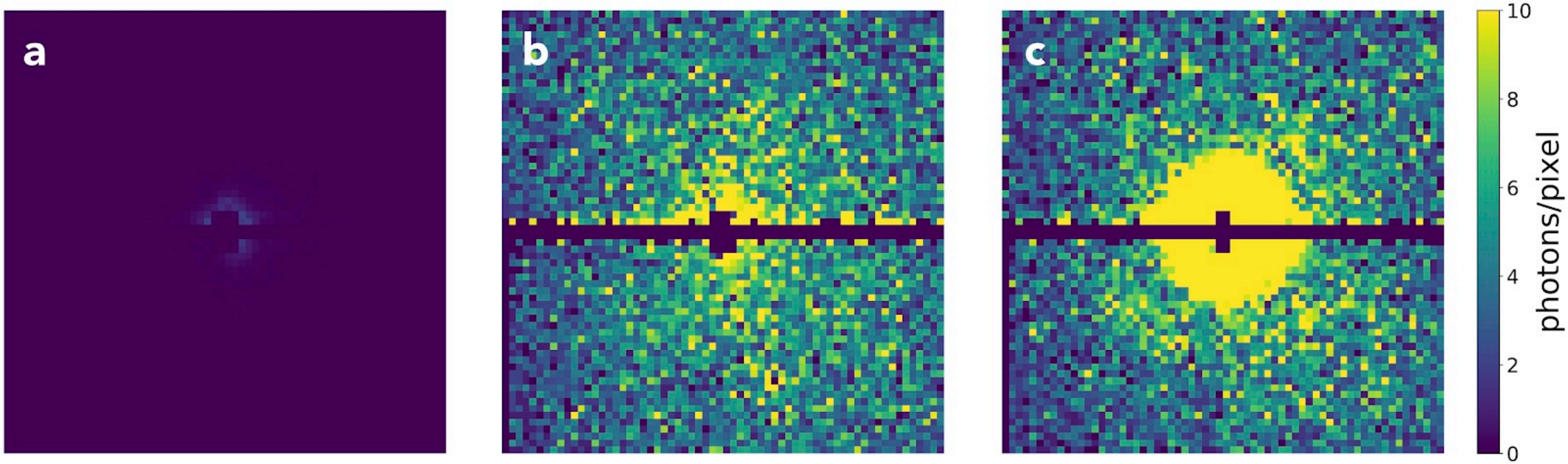
Gas background from SQS (8x downsampled)

Mass: 840 kDa

Ekeberg, T. *et al.* Observation of a single protein by ultrafast X-ray diffraction. ***Light Sci. Appl.*** **13, 15 (2024).**

Flash X-ray Imaging of Single Proteins

European XFEL - Experiment SQS 2146



BEAMLINE BACKGROUND
65 PHOTONS

INJECTION BACKGROUND
17 000 PHOTONS

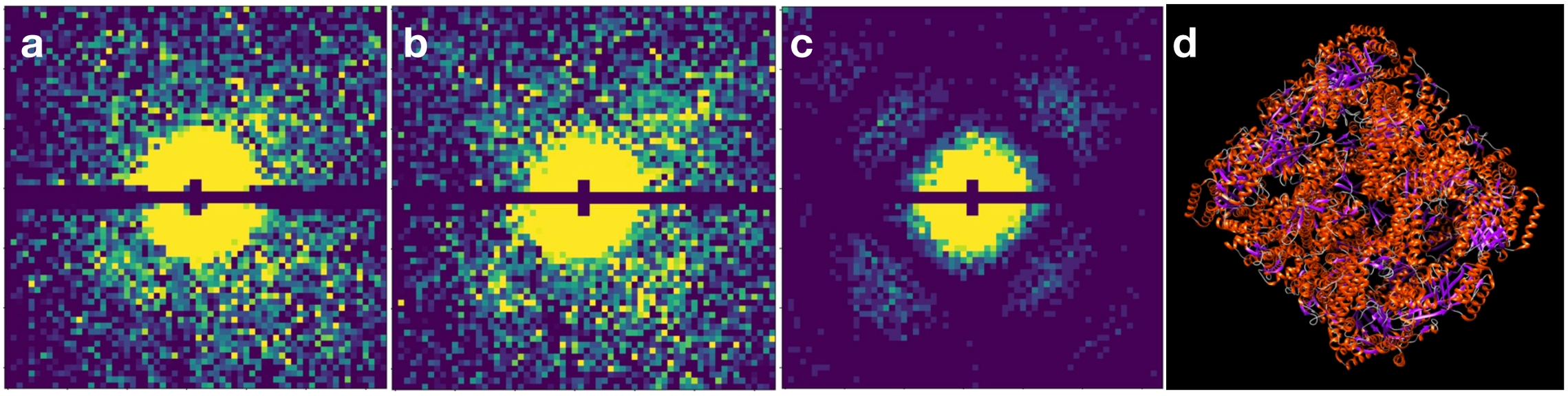
SAMPLE HIT
32 000 PHOTONS

Ekeberg, T. *et al.* Observation of a single protein by ultrafast X-ray diffraction. ***Light Sci. Appl.*** **13, 15 (2024).**

Flash X-ray Imaging of Single Proteins

European XFEL - Experiment SQS 2146

Chaperonin 60 from *Escherichia coli* (GroEL)



GroEL data from SQS

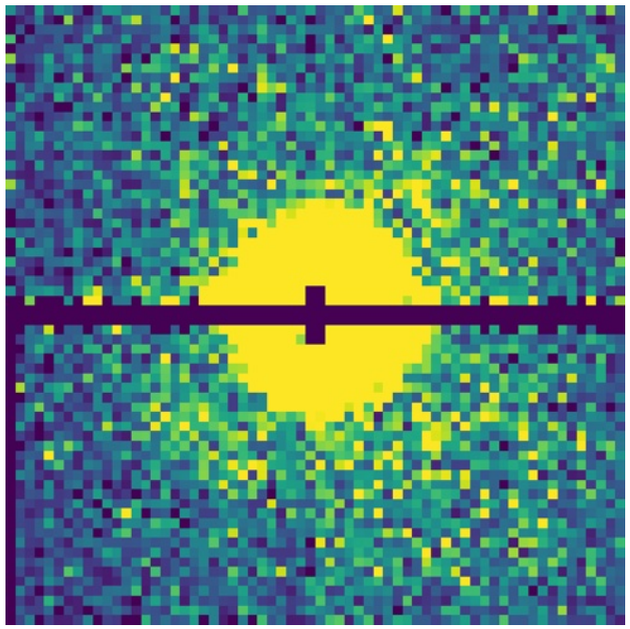
Model from PDB plus background

Model from PDB alone

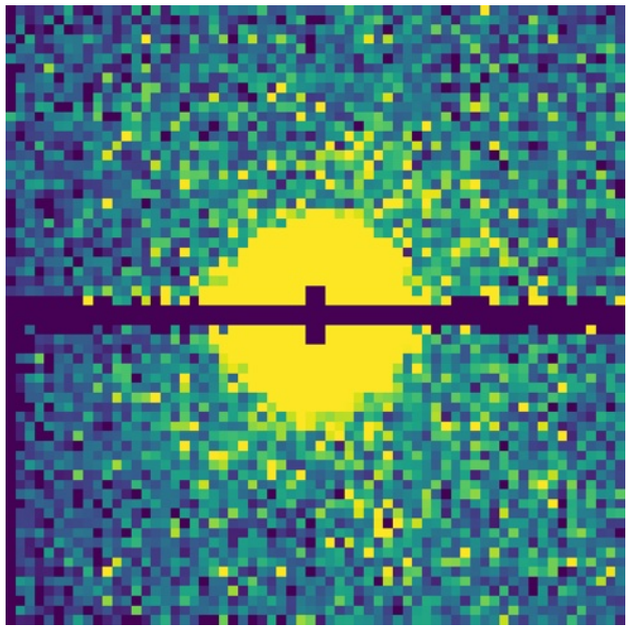
Real space image of the model

Ekeberg, T. *et al.* Observation of a single protein by ultrafast X-ray diffraction. ***Light Sci. Appl.*** **13, 15 (2024).**

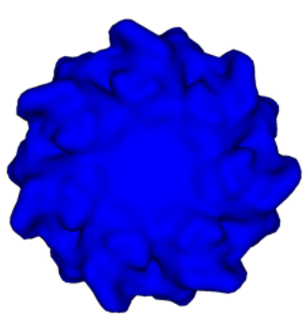
And we see some stuff in the middle!

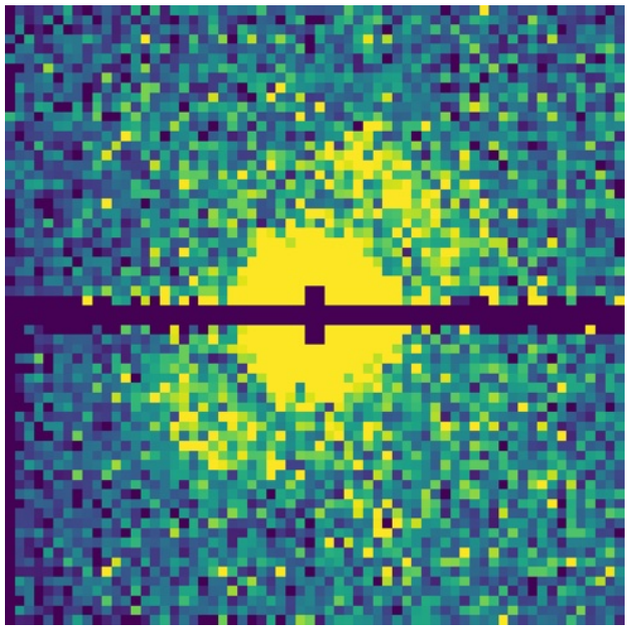


Experimental Pattern

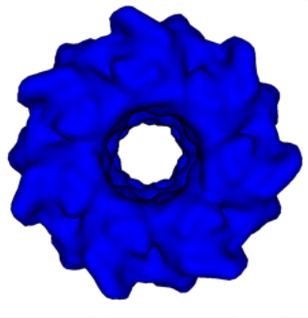


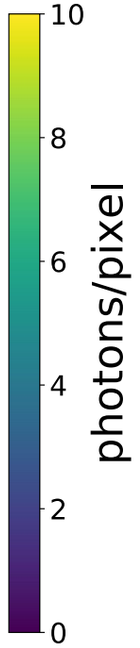
Simulation with filled GroEL

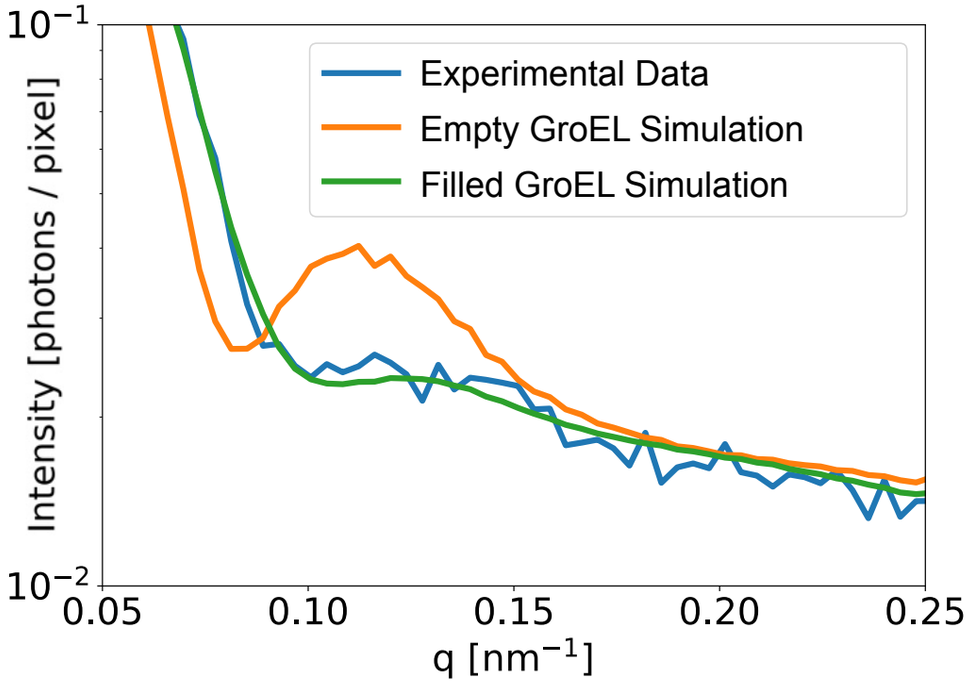




Simulation with empty GroEL



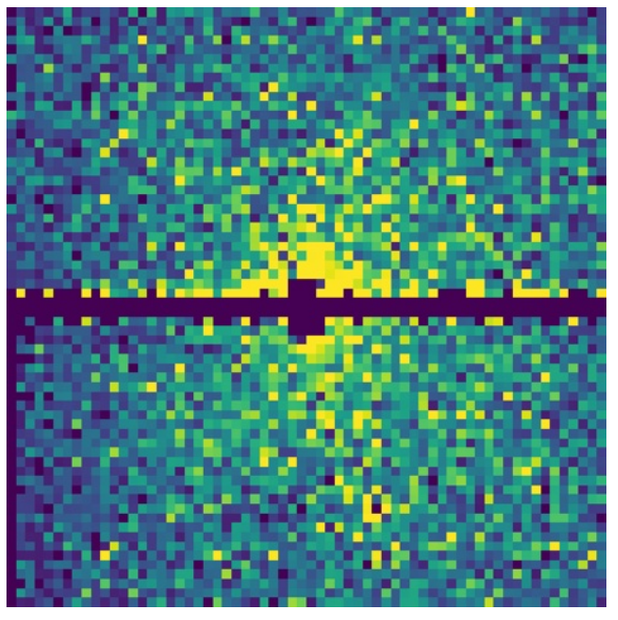


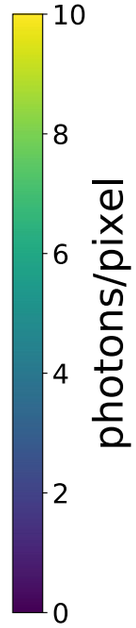


GroEL (14 nm diameter) Diffraction from SQS!

The hole through GroEL seems to be fully filled

6 mJ and 1.2 keV

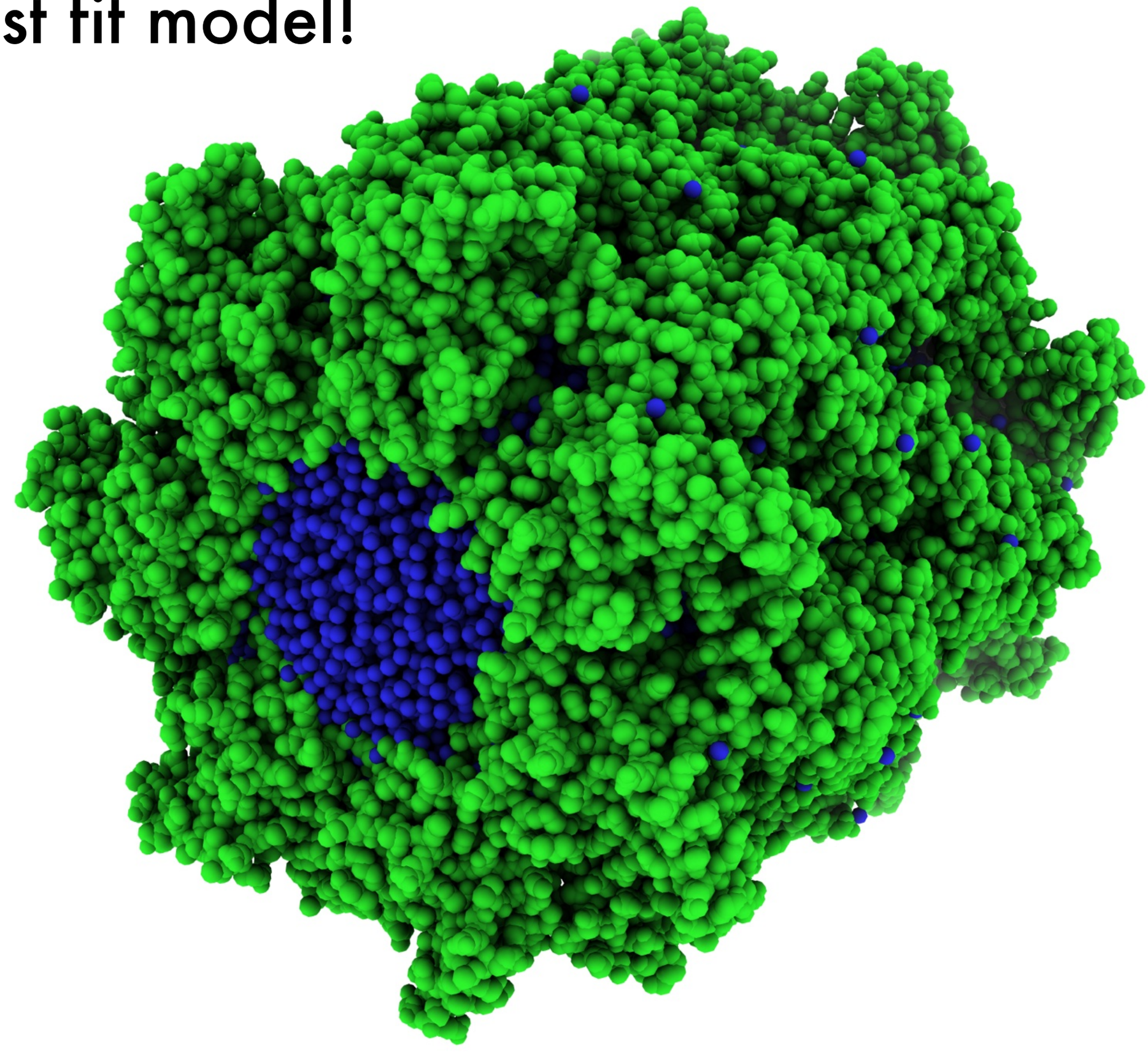




Background Scattering

Ekeberg, T. *et al.* Observation of a single protein by ultrafast X-ray diffraction. ***Light Sci. Appl.*** **13, 15 (2024).**

Our best fit model!



Ekeberg, T. *et al.* Observation of a single protein by ultrafast X-ray diffraction. ***Light Sci. Appl.*** **13, 15 (2024).**

TIME-RESOLVED SOLUTION SCATTERING AT THE EUROPEAN XFEL

AsLOV2 TR-WAXS (SPB/SFX)

acknowledgements

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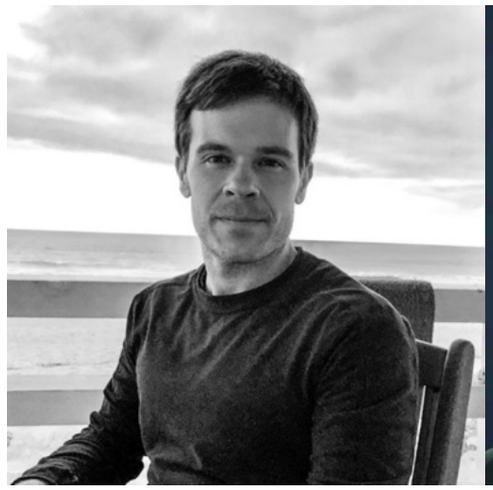
Biochemistry,
Uppsala University

Filipe Maia
Molecular Biophysics,
Uppsala University

Helmut Grubmüller
Max Planck Institute for
Multidisciplinary Sciences

Leonardo Monrroy



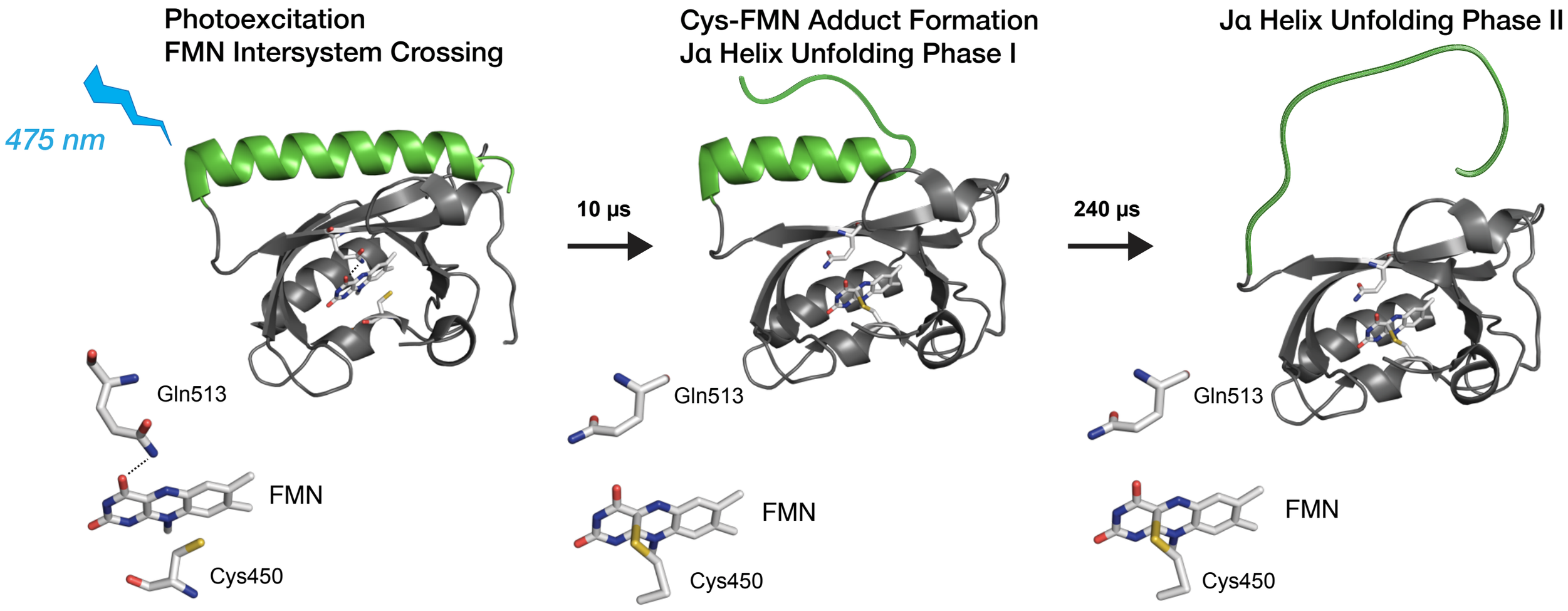
Patrick Konold

Alfredo Belissario

Michael Maihoefer

AsLOV2 TR-WAXS (SPB/SFX)

intro



AsLOV2 TR-WAXS (SPB/SFX)

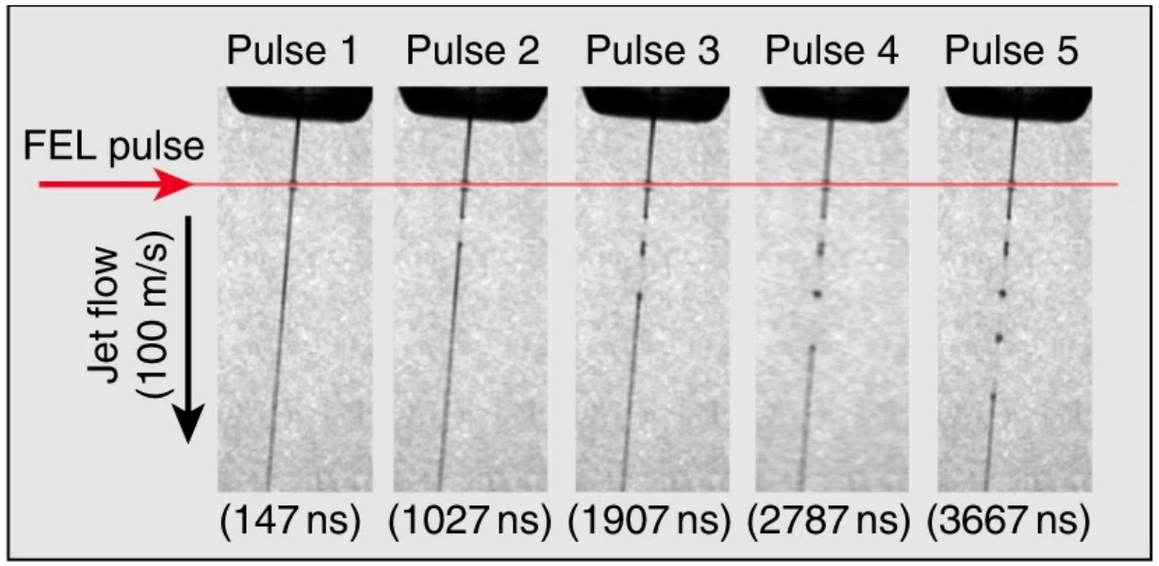
experimental setup

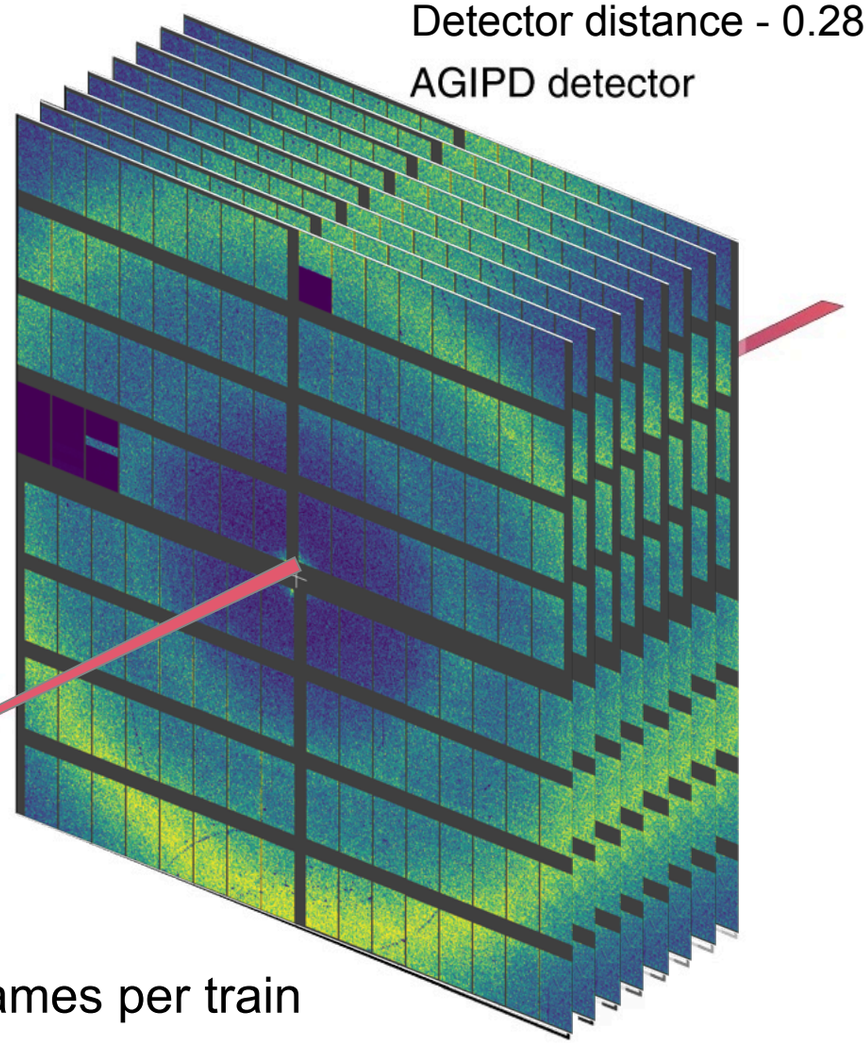
Photon Energy - 8 keV

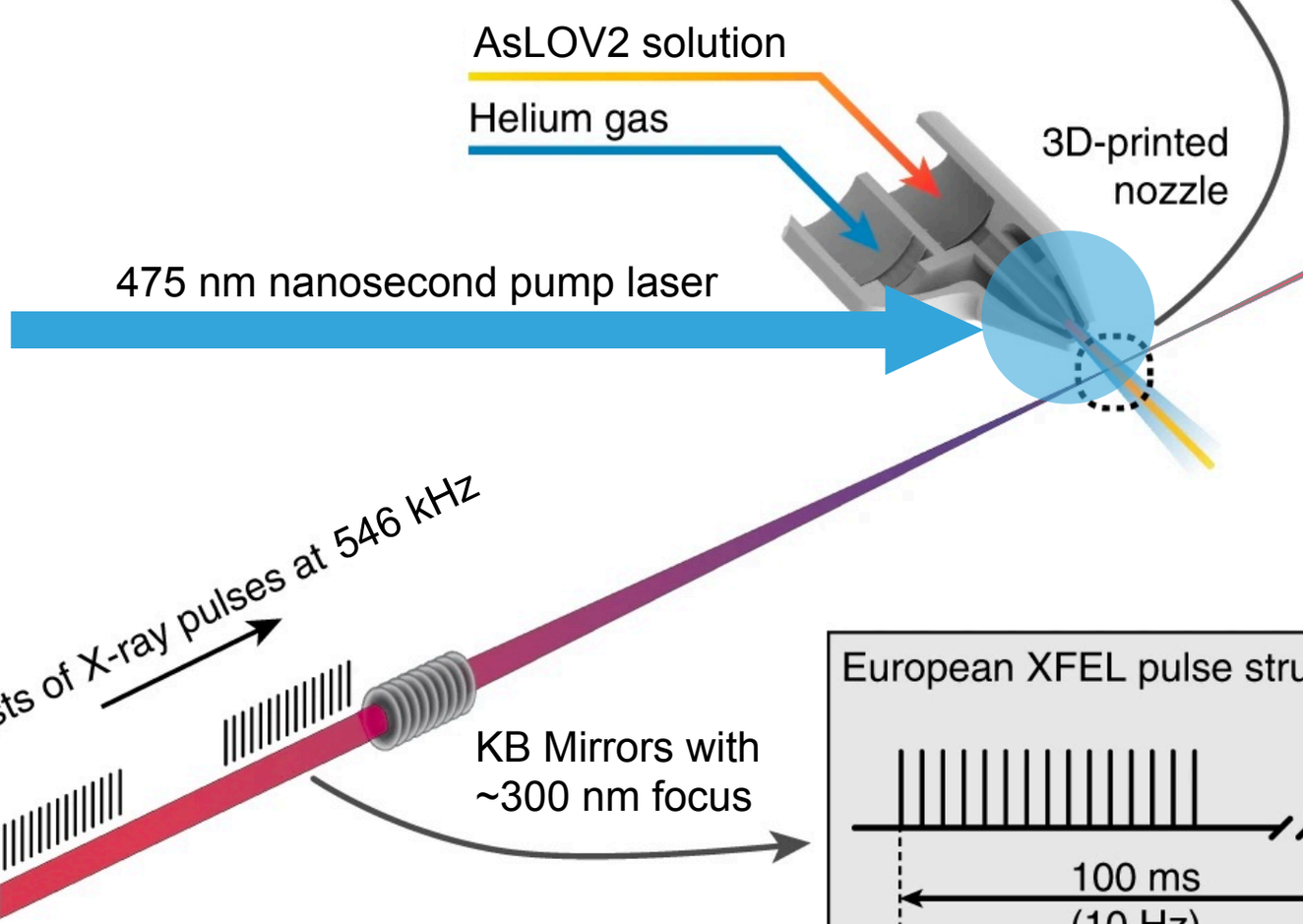
Pulse Energy - ~2mJ

Detector distance - 0.28 m

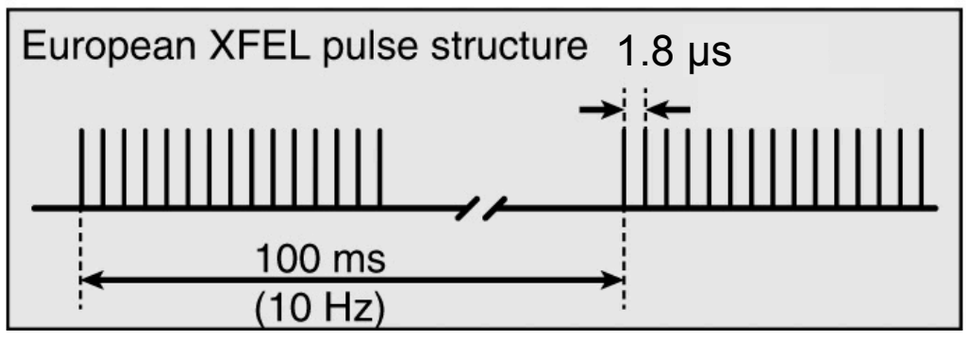
AGIPD detector







176 frames per train

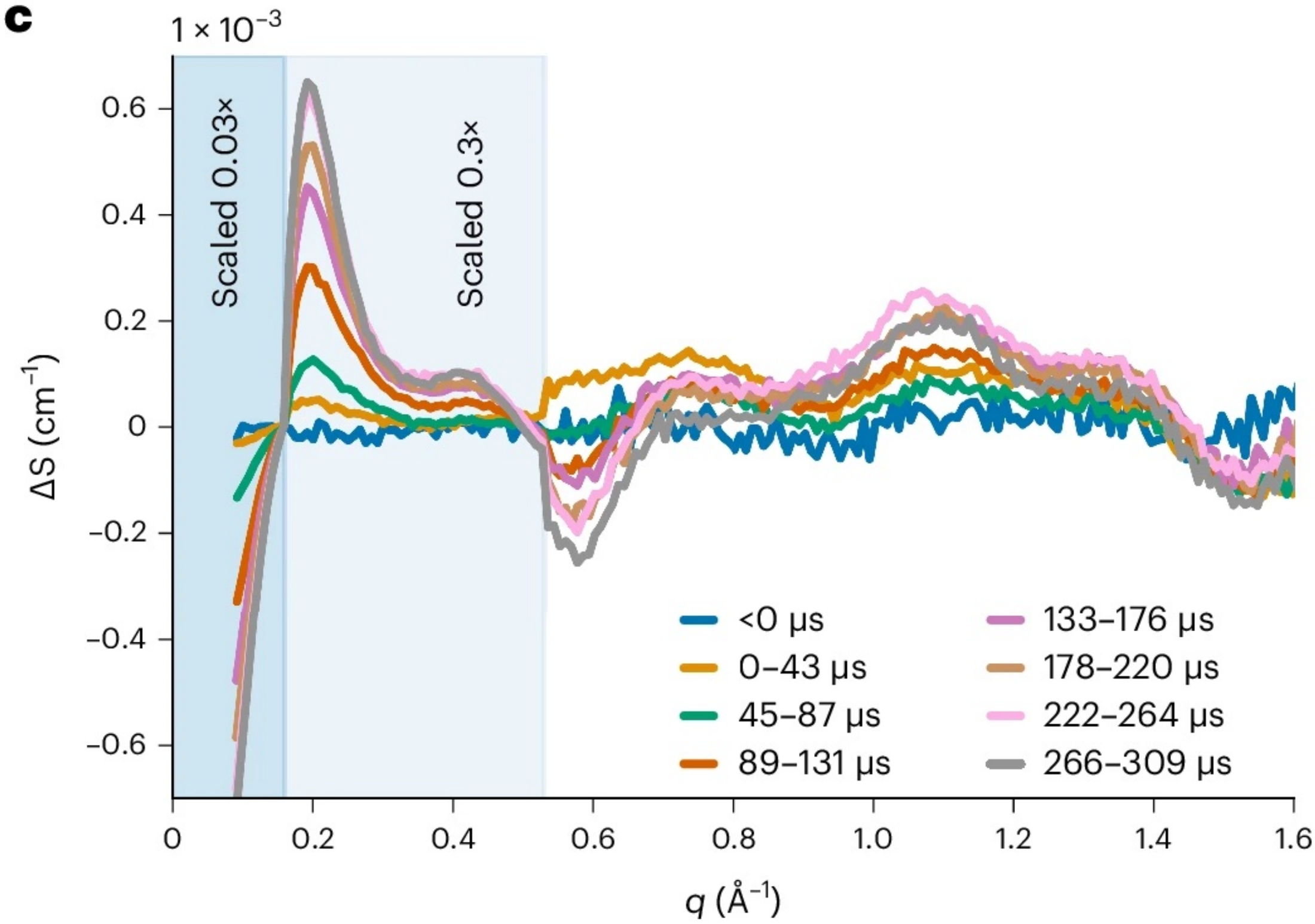


Pump laser excites enough volume to last the entire train

Each pulse is a different time delay

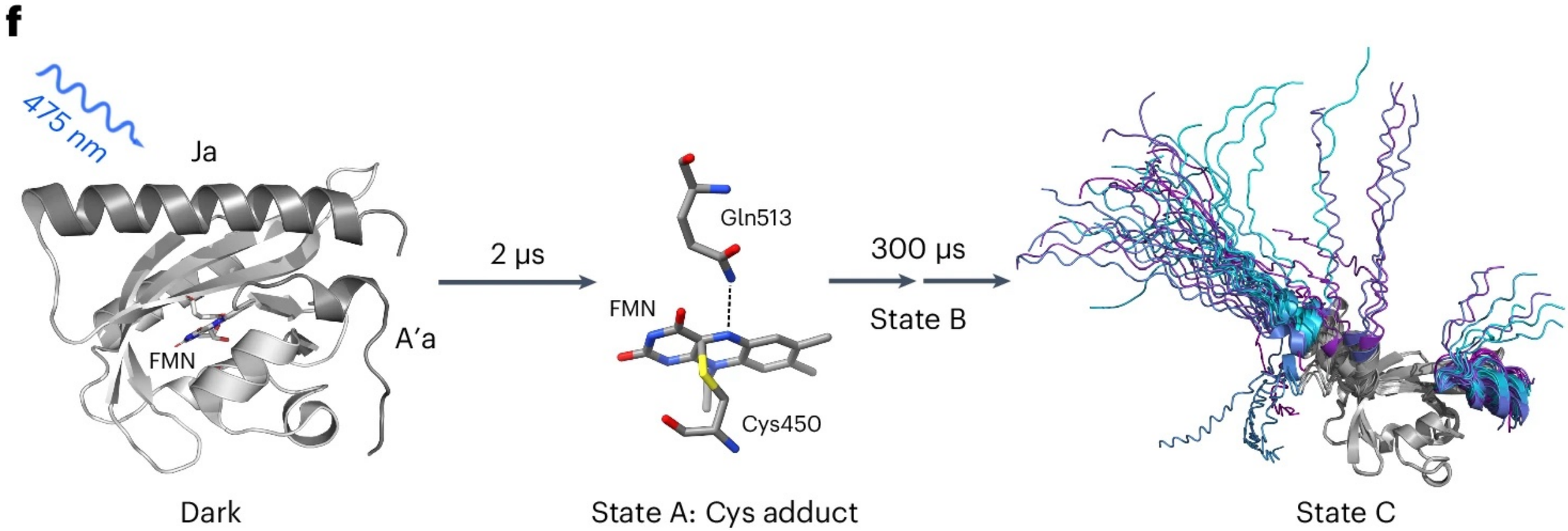
Shamelessly adapted from Wiedorn, M. O. et al. Megahertz serial crystallography. Nat. Commun. 9, 4025 (2018).

AsLOV2 TR-WAXS (SPB/SFX)



Konold, P. E. *et al.* Microsecond time-resolved X-ray scattering by utilizing MHz repetition rate at second-generation XFELs. ***Nat. Methods*** **(2024)**

AsLOV2 TR-WAXS (SPB/SFX)



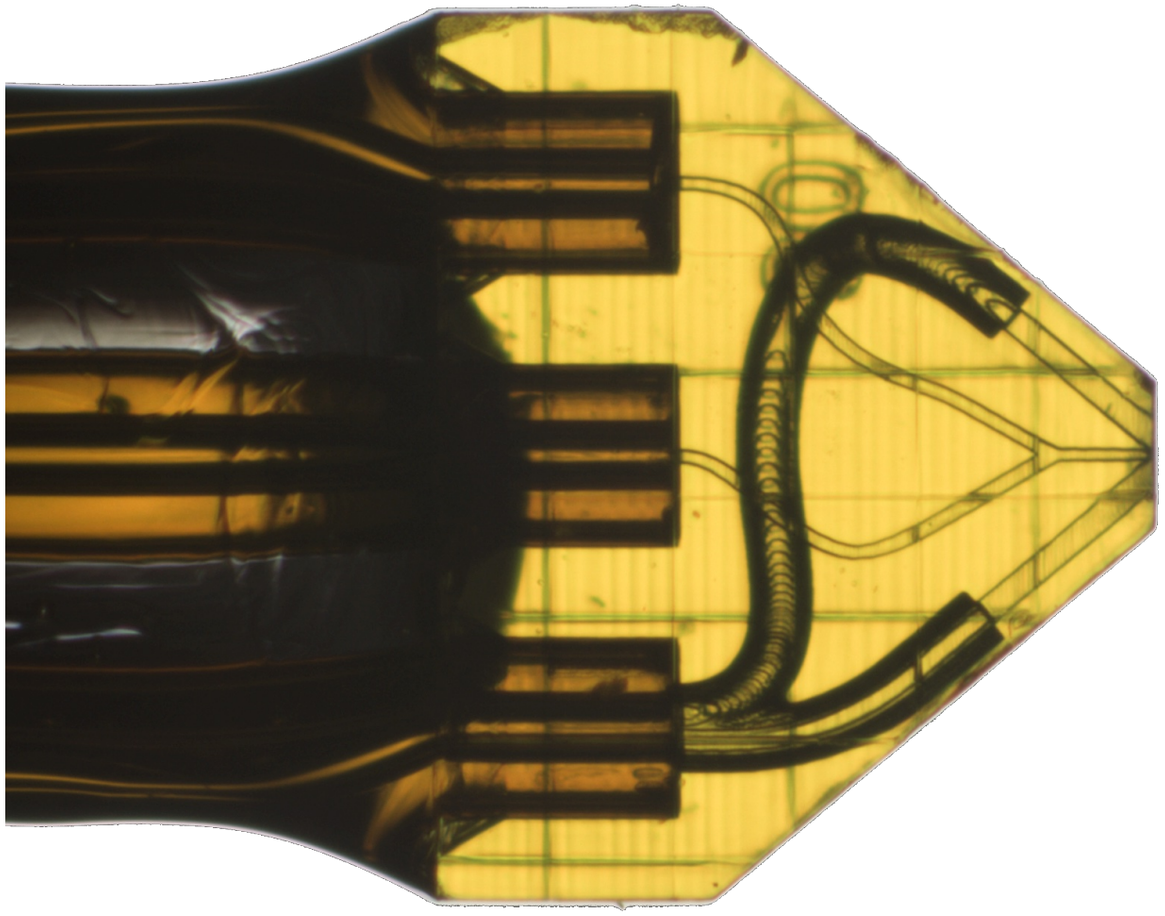
Konold, P. E. *et al.* Microsecond time-resolved X-ray scattering by utilizing MHz repetition rate at second-generation XFELs. ***Nat. Methods*** **(2024)**

Ultra-thin Liquid Sheets

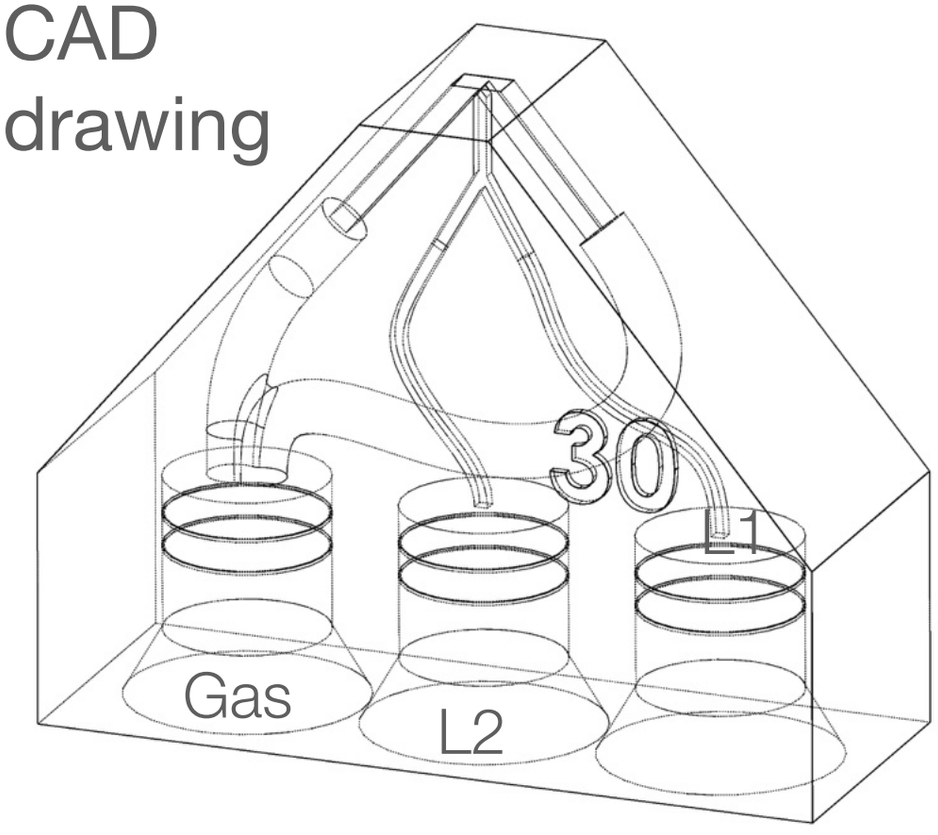
design

Patrick Konold

Printed nozzle with capillaries



CAD drawing

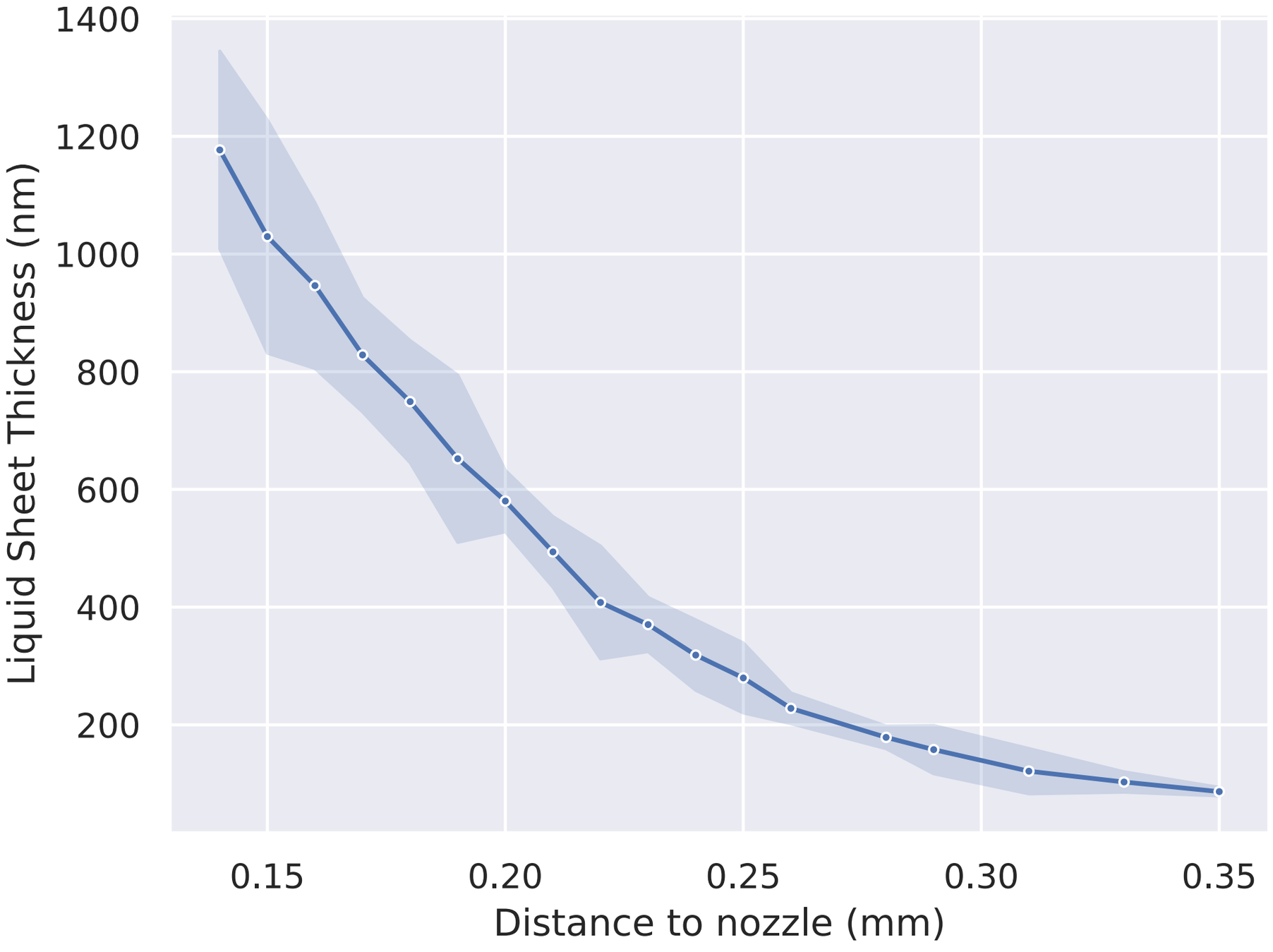


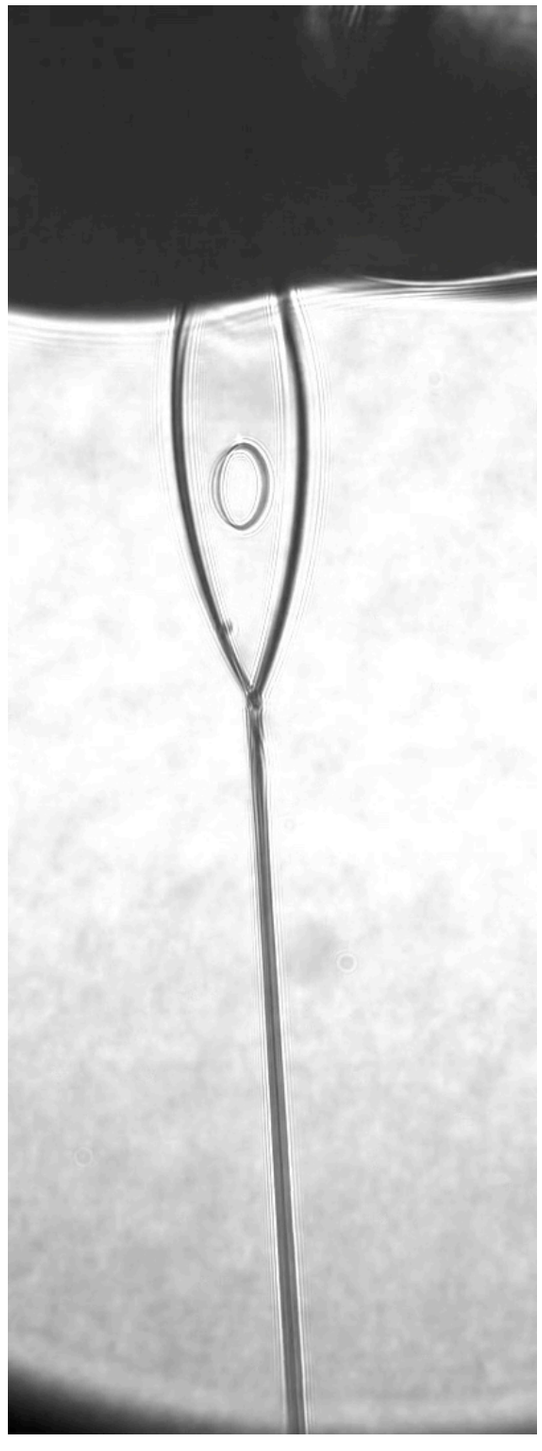
Liquid - 30x30 µm
Gas - 50x50 µm

3D-printed using two photon polymerization
UpNano NanoOne
Printing time ~ 10 minutes / development in PGMEA

Ultra-thin Liquid Sheets (SPB/SFX)

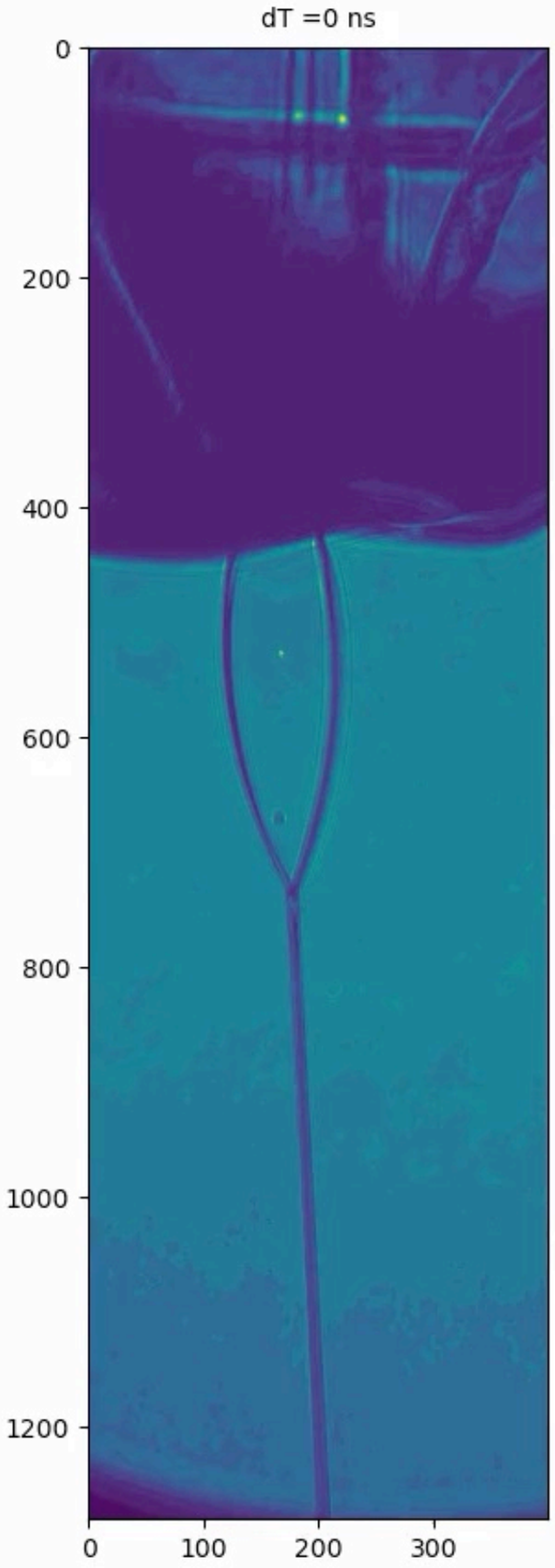
thickness





Ultra-thin Liquid Sheets (SPB/SFX)

stability



Stroboscopic measurement of the liquid sheet a certain delay after the X-rays

Note there's no explosion above the interaction point

Konold, P. E. *et al.* 3D-printed sheet jet for stable megahertz liquid sample delivery at X-ray free-electron lasers. ***IUCrJ* 10, 662–670 (2023)**

From here

Mimivirus

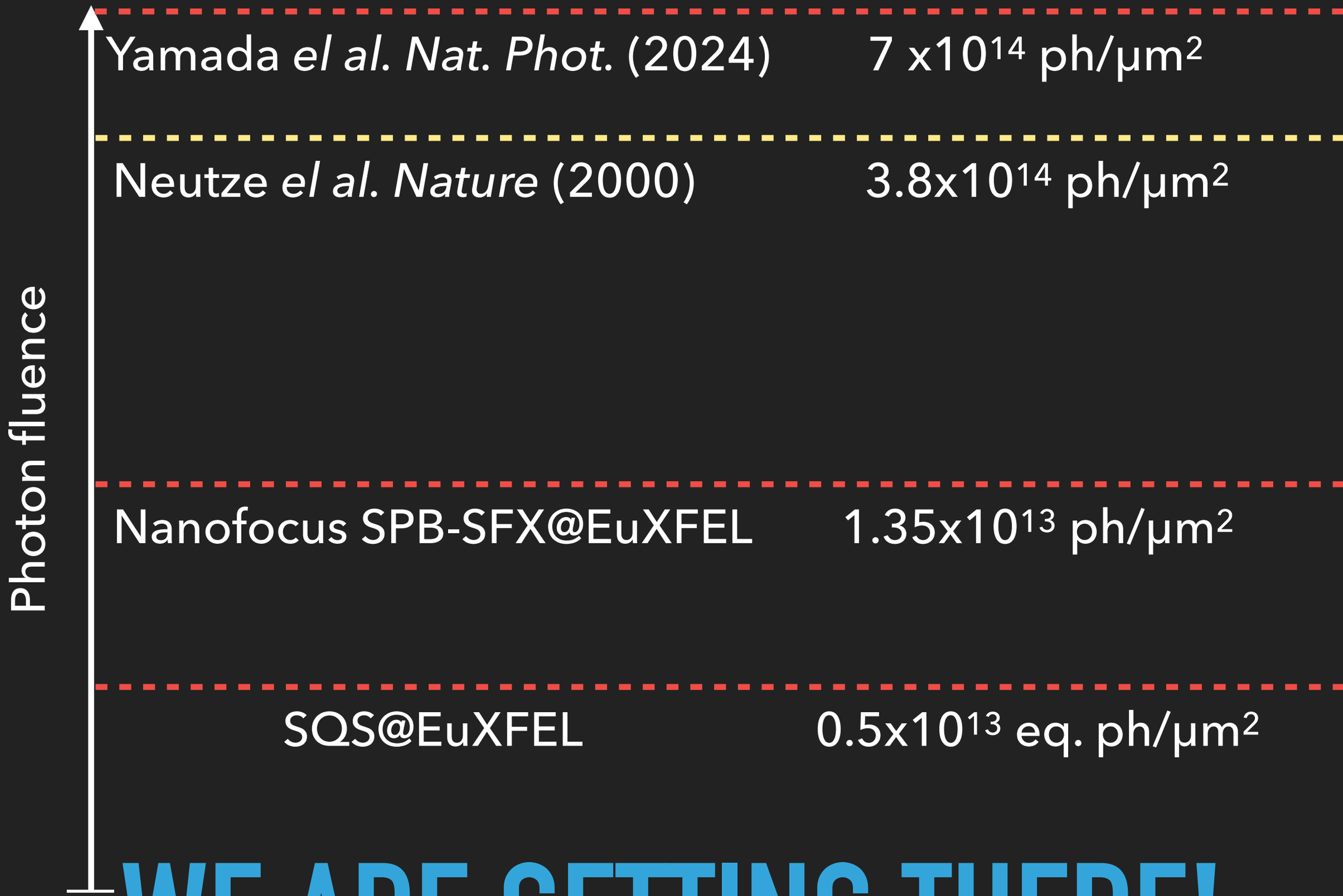
Rhinovirus

Single protein molecule

To here

HIV

HOW MANY PHOTONS DO WE REALLY NEED?



WE ARE GETTING THERE!

Acknowledgments

Funded by the European Union

European Research Council
Established by the European Commission

Swedish Research Council

CARL TRYGGERS STIFTELSE
FÖR VETENSKAPLIG FORSKNING

Röntgen Ångström Cluster

Thank you!