PAUL SCHERRER INSTITUT

PSI BIO

Wir schaffen Wissen – heute für morgen

RAMC : Le Bischenberg 2013

Membrane protein crystallisation for X-ray free-electron laser (XFEL) applications

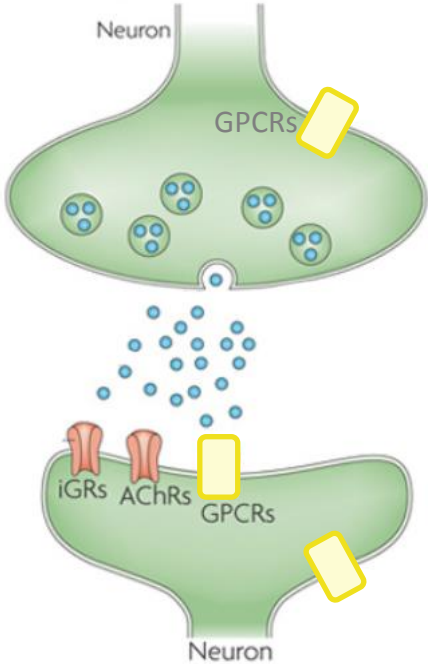
Valérie Panneels – Schertler Group - LBR – Paul Scherrer Institute

G Protein-coupled Receptors (GPCRs)

intro

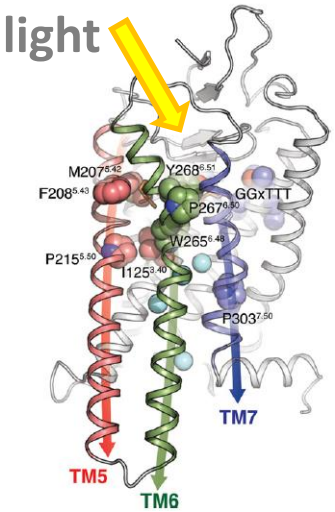
incoming signal

Neuronal Synapse



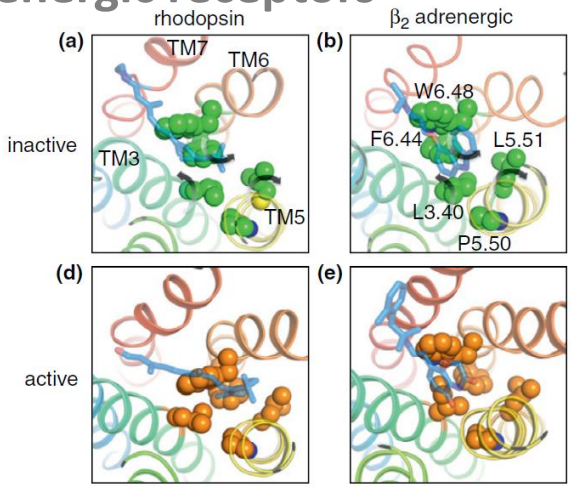
output

rhodopsin, the light receptor



Review: Deupi & Schertler, Biochem.Soc.Trans. 2012

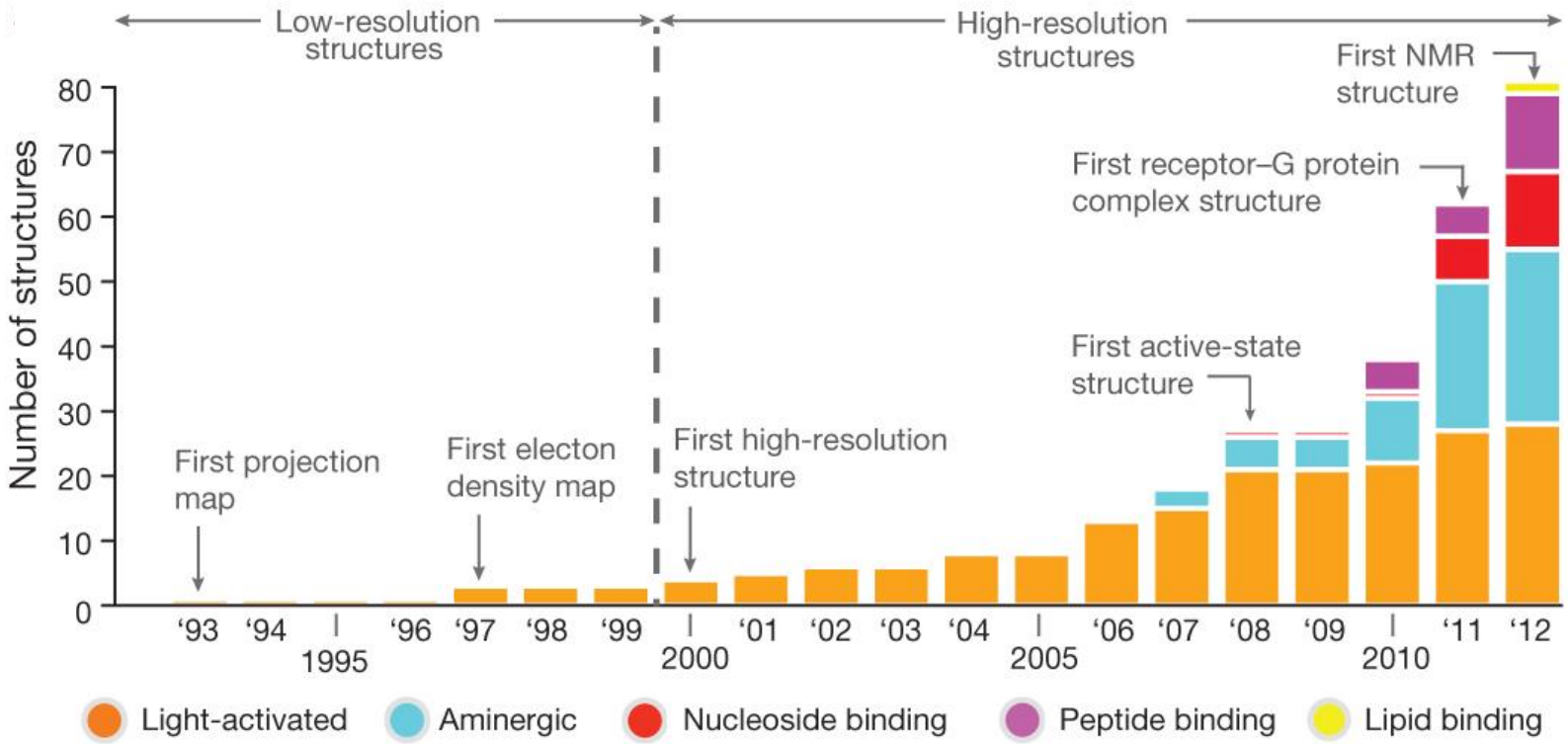
β-adrenergic receptors



Review: Deupi & Schertler, Curr.Op.Struct.Biol. 2011

Time-line of GPCR structures

intro



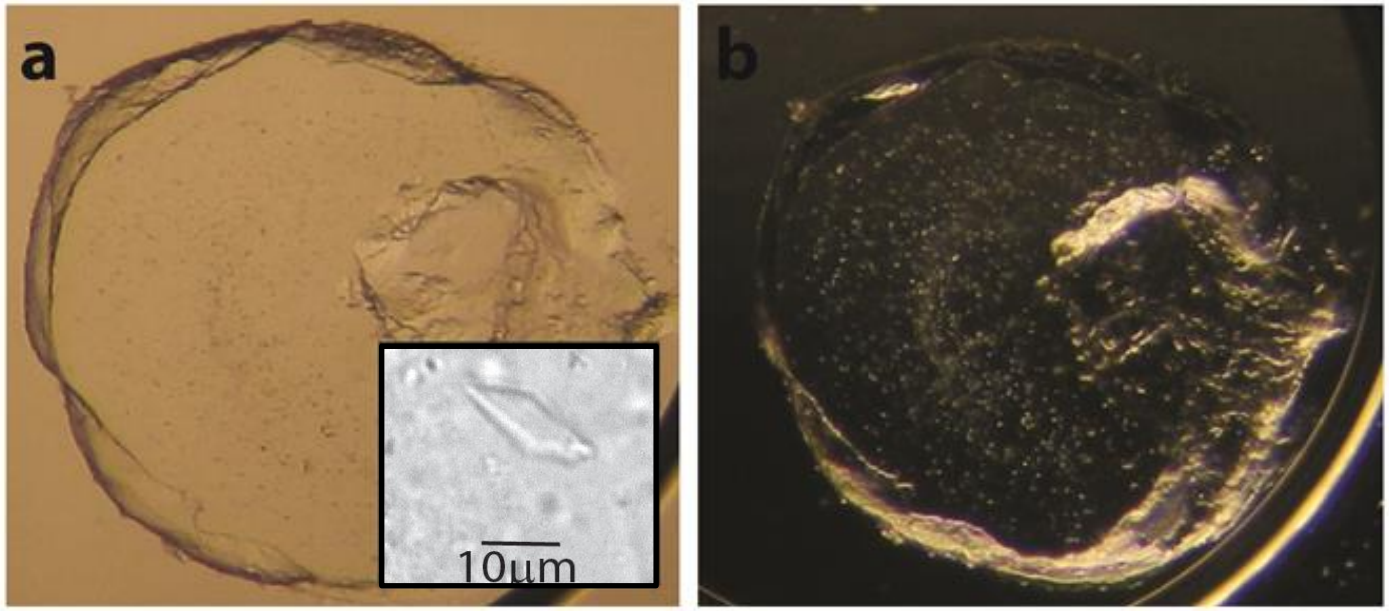
Venkatakrishnan *et al. Nature* 2013

Routine GPCRs Crystallisation

De novo Structure Determination of GPCRs

Crystallisation of GPCRs using vapor diffusion or lipidic cubic phases.

First Mosquito-LCP developed by Schertler

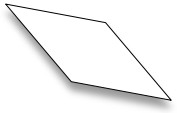


β1-adrenergic receptor crystals grown in lipidic cubic phase (Rock-Imager, Formulatrix) observed under bright light (a) or using cross polarization (b). Inset: Differential Interference Microscopy (DIC).

β1-adrenergic receptor purification and crystallisation: Brückner & Schertler *Methods in Enzymol* 2013.

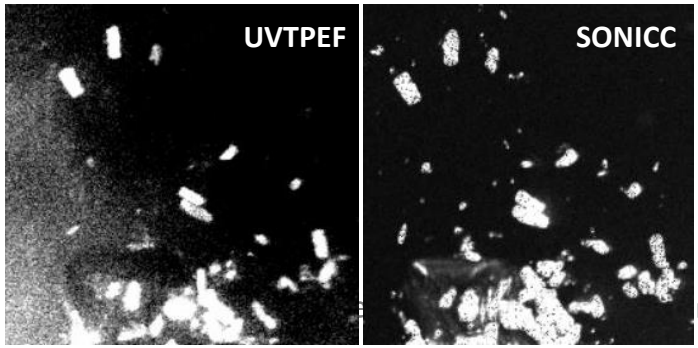
GPCR crystals characterisation

NANOCRYSTALS (submicron)



protein crystal having at least one dimension smaller than a micron.

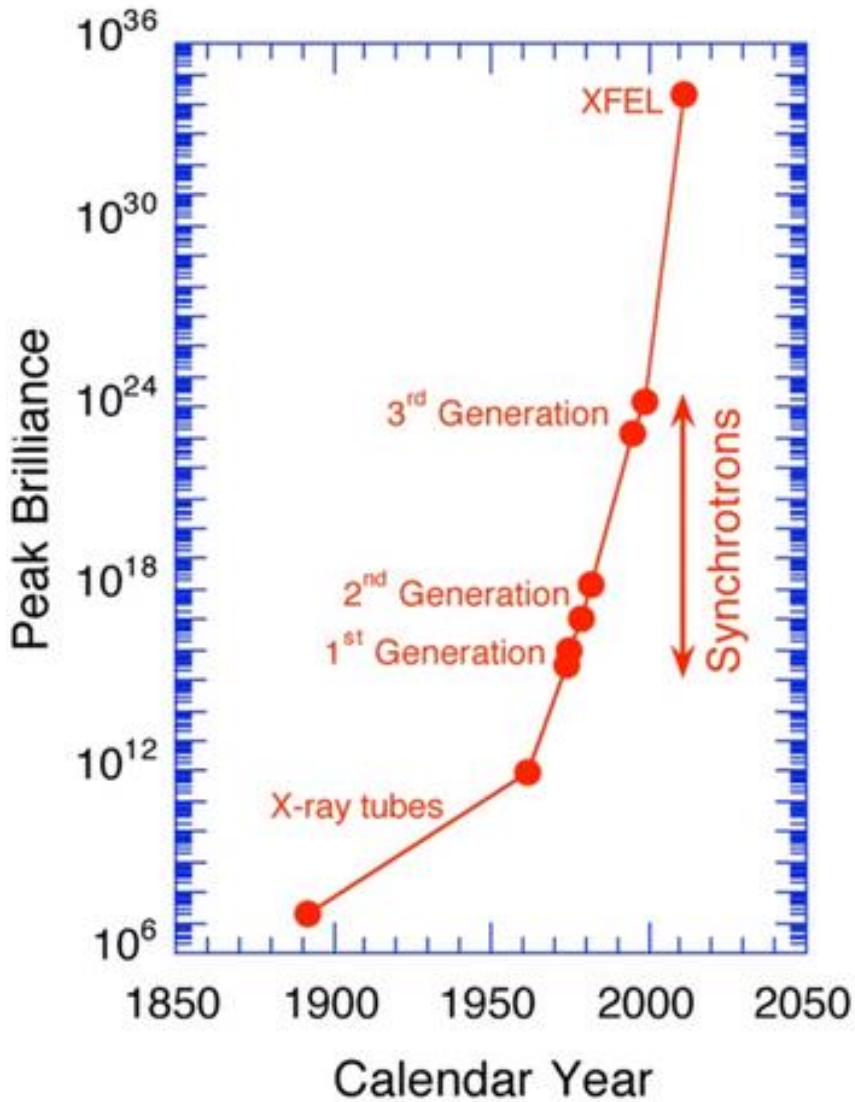
PRODUCTION	DETECTION	ANALYSIS
Adapted Screenings	Rock Imager	Powder diffraction
Nucleation Control	DIC-Microscopy	Electron diffraction
Dynamic Light Scattering	UV/Fluorescence	Microfocus
	SONICC	Nanodiffraction
	In plate diffraction	XFEL
	Streek seeding	



measure by Cornelius Gati, CFEL/DESY

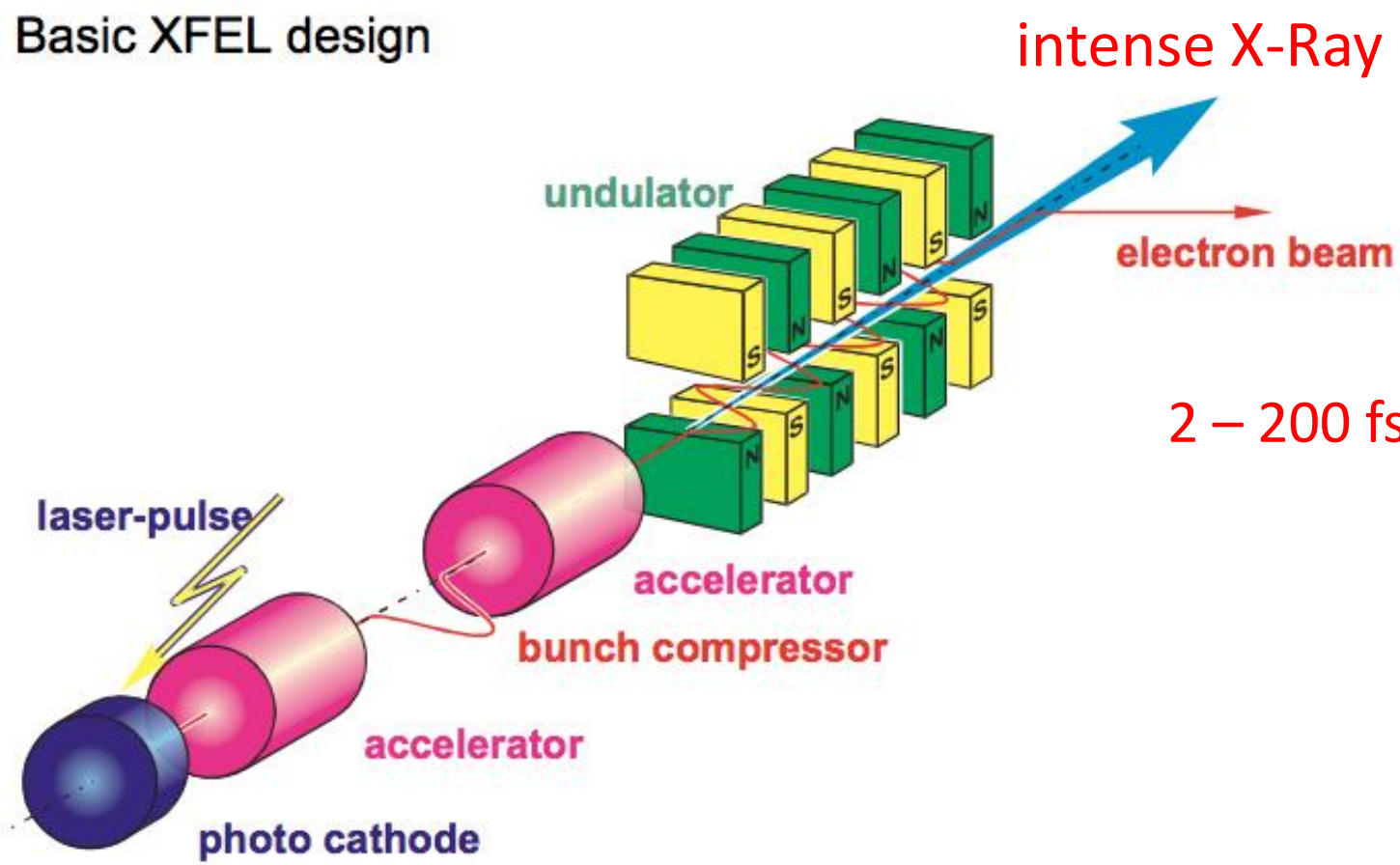
Optimisation of Crystal Size? No. Nanocrystallography!

the X-Ray Free Electron Laser Brilliance



the XFEL Design

Incredibly short and intense X-Ray Pulses

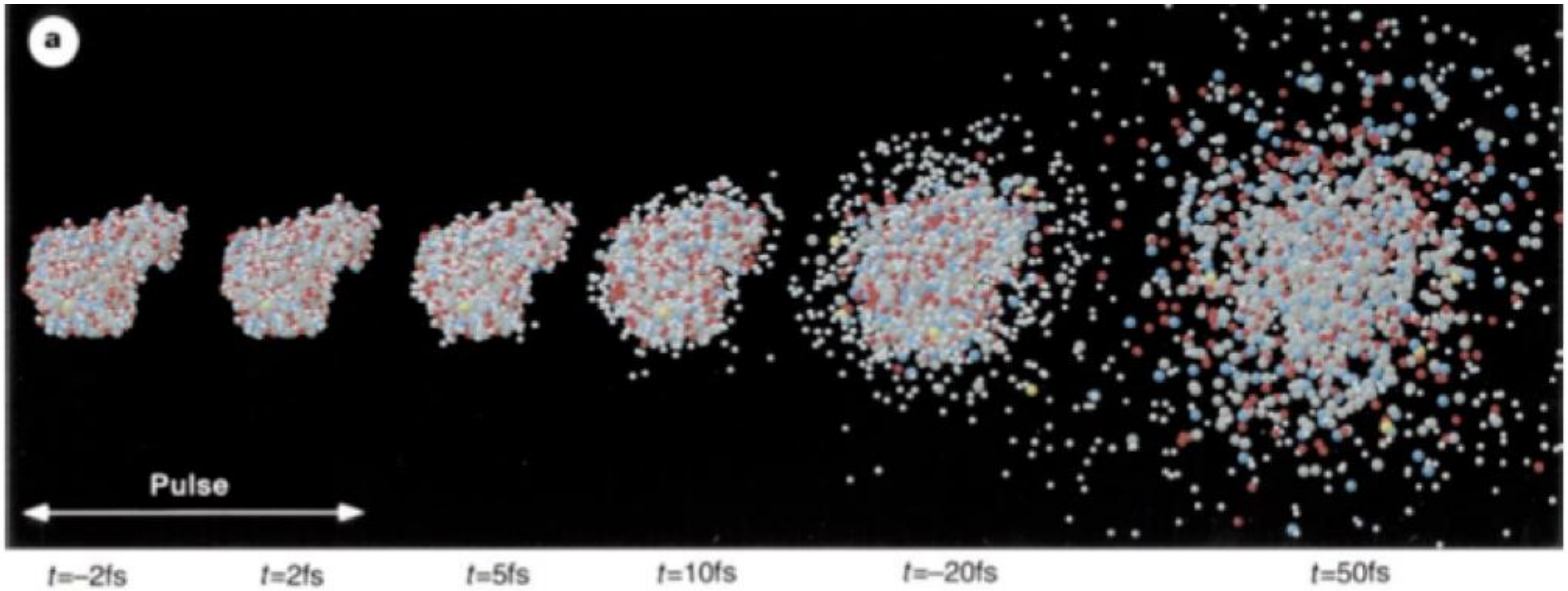


2 – 200 fsec

the short Pulses of the XFEL

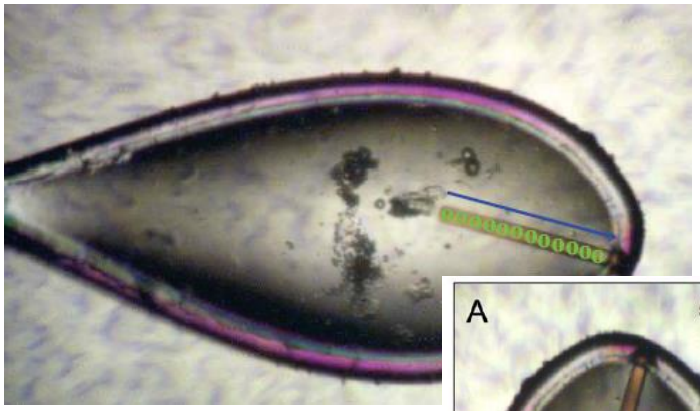
help to circumvent radiation damage

Simulation of the Explosion Dynamics of Biomolecules (C, N, O)

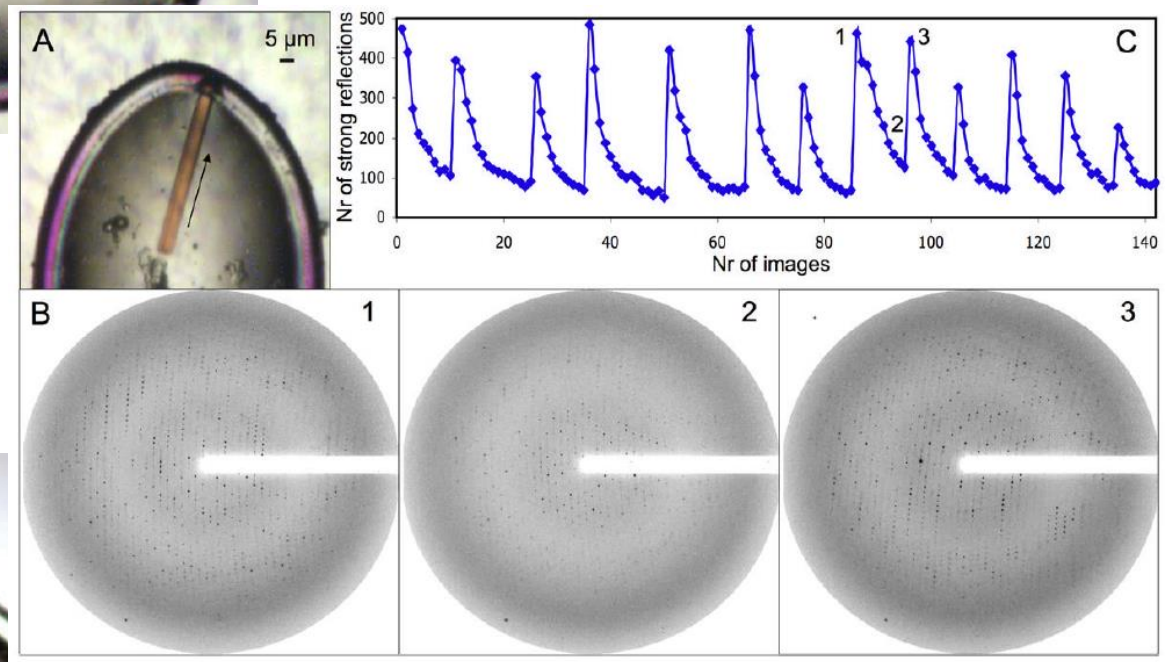


Neutze et al., Nature 2000

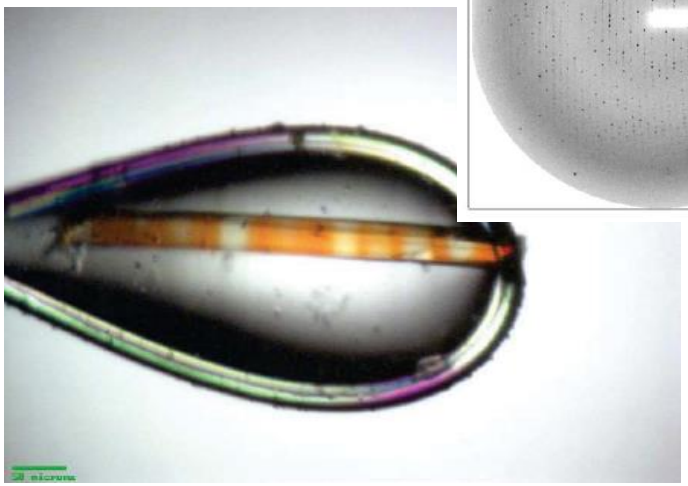
Radiation Damage at the Synchrotron & Microdiffraction



Dark-state Rhodopsin crystals



Standfuss & Schertler, 2007
Riekel & Schertler, 2005



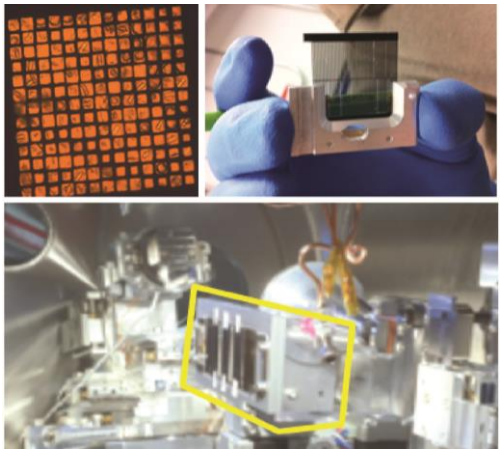
Edwards & Schertler, unpublished

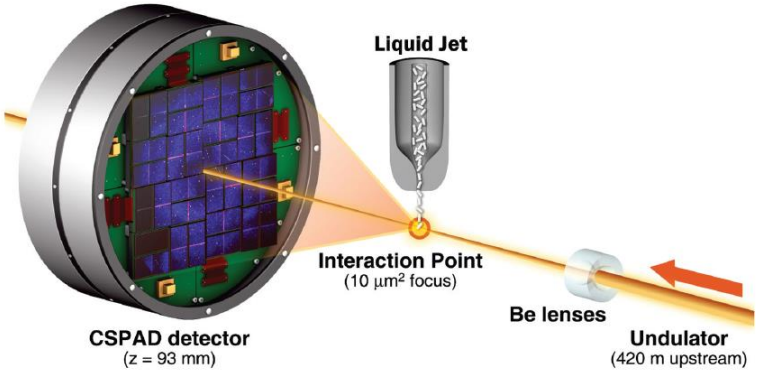
Serial Femtosecond Crystallography (SFX)

Parameter	40-fs pulses	5-fs pulses	SLS RT data 3
Wavelength	1.32 Å	1.32 Å	0.9997 Å
X-ray focus (μm^2)	~10	~10	~100 × 100
Pulse energy/fluence at sample	600 μJ/4 × 10^{11} photons per pulse	53 μJ/3.5 ×10^{10} photons per pulse	n.a./2.5 × 10^{10} photons/s
Dose (MGy)	33.0 per crystal	2.9 per crystal	0.024 total
Dose rate (Gy/s)	8.3 × 10^{20}	5.8 × 10^{20}	9.6 × 10^{2}
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$
Unit cell length (Å), $\alpha = \beta = \gamma = 90°$	$a = b$ =79, c = 38	$a = b$ = 79, c = 38	$a = b$ = 79.2, c = 38.1
Oscillation range/exposure time	Still exp./40 fs*	Still exp./5 fs*	1.0°/0.25 s
No. collected diffraction images	1,471,615	1,997,712	100
No. of hits/indexed images	66,442/12,247	40,115/10,575	n.a./100
Number of reflections	n.a.	n.a.	70,960
Number of unique reflections	9921	9743	9297
Resolution limits (Å)	35.3–1.9	35.3–1.9	35.4–1.9
Completeness	98.3% (96.6%)	98.2% (91.2%)	92.6% (95.1%)
$I/\sigma(I)$	7.4 (2.8)	7.3 (3.1)	18.24 (5.3)
R_{split}	0.158	0.159	n.a.
R_{merge}	n.a.	n.a.	0.075 (0.332)
Wilson B factor	28.3 $Å^2$	28.5 $Å^2$	19.4 $Å^2$
R-factor/R-free	0.196/0.229	0.189/0.227	0.166/0.200
Rmsd bonds, Rmsd angles	0.006 Å, 1.00°	0.006 Å, 1.03°	0.007 Å, 1.05°
PDB code	4ET8	4ET9	4ETC

*Electron bunch length

Boutet et al, 2012

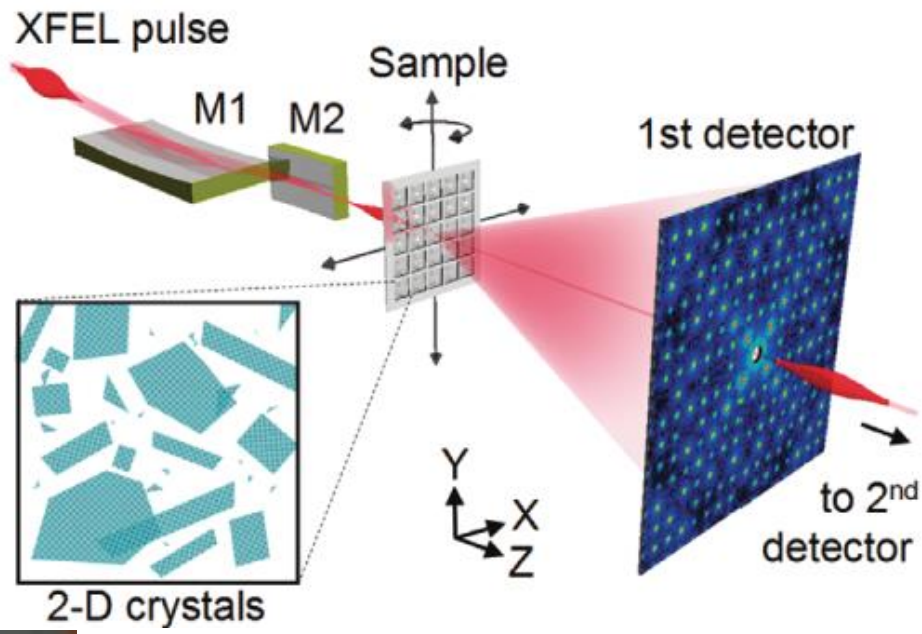


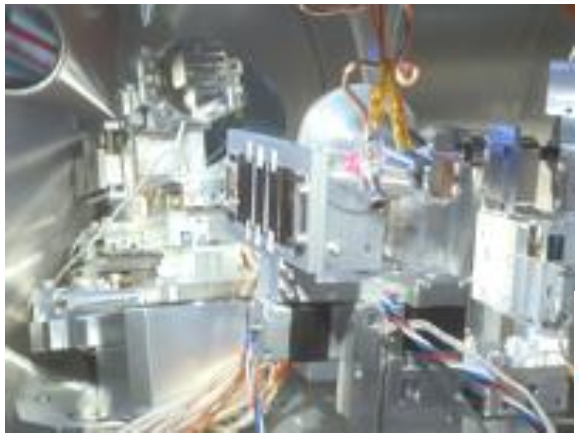


Serial Femtosecond Crystallography (SFX)

2D-crystals
of
bacteriorhodopsin

(display only one or
a few lattices)



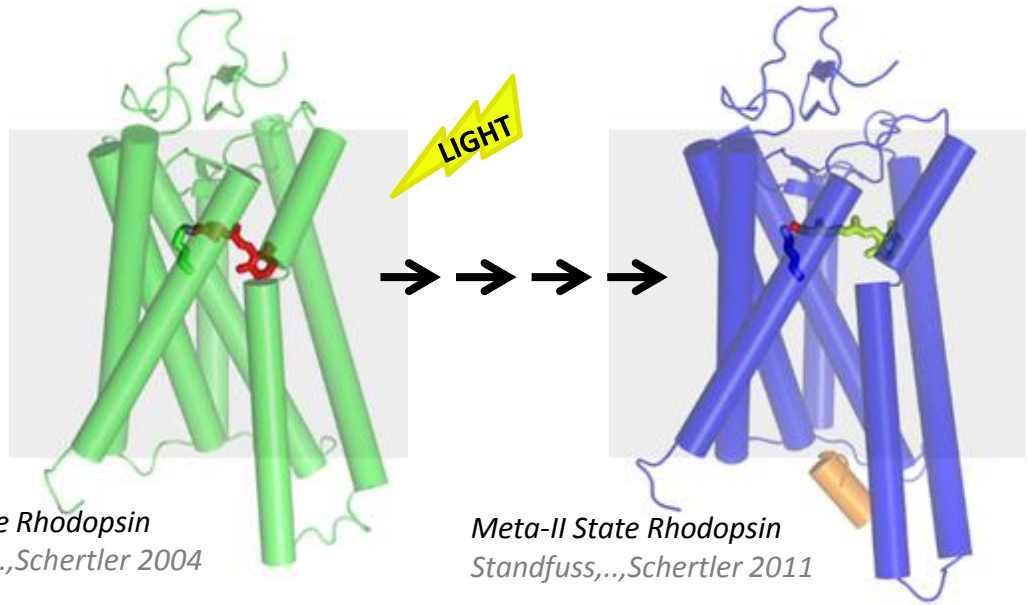


diffraction patterns with a
resolution less than 9 Å !

Tsai, Schertler and Li unpublished & JMB 2013

Time-Resolved Pump Probe SFX

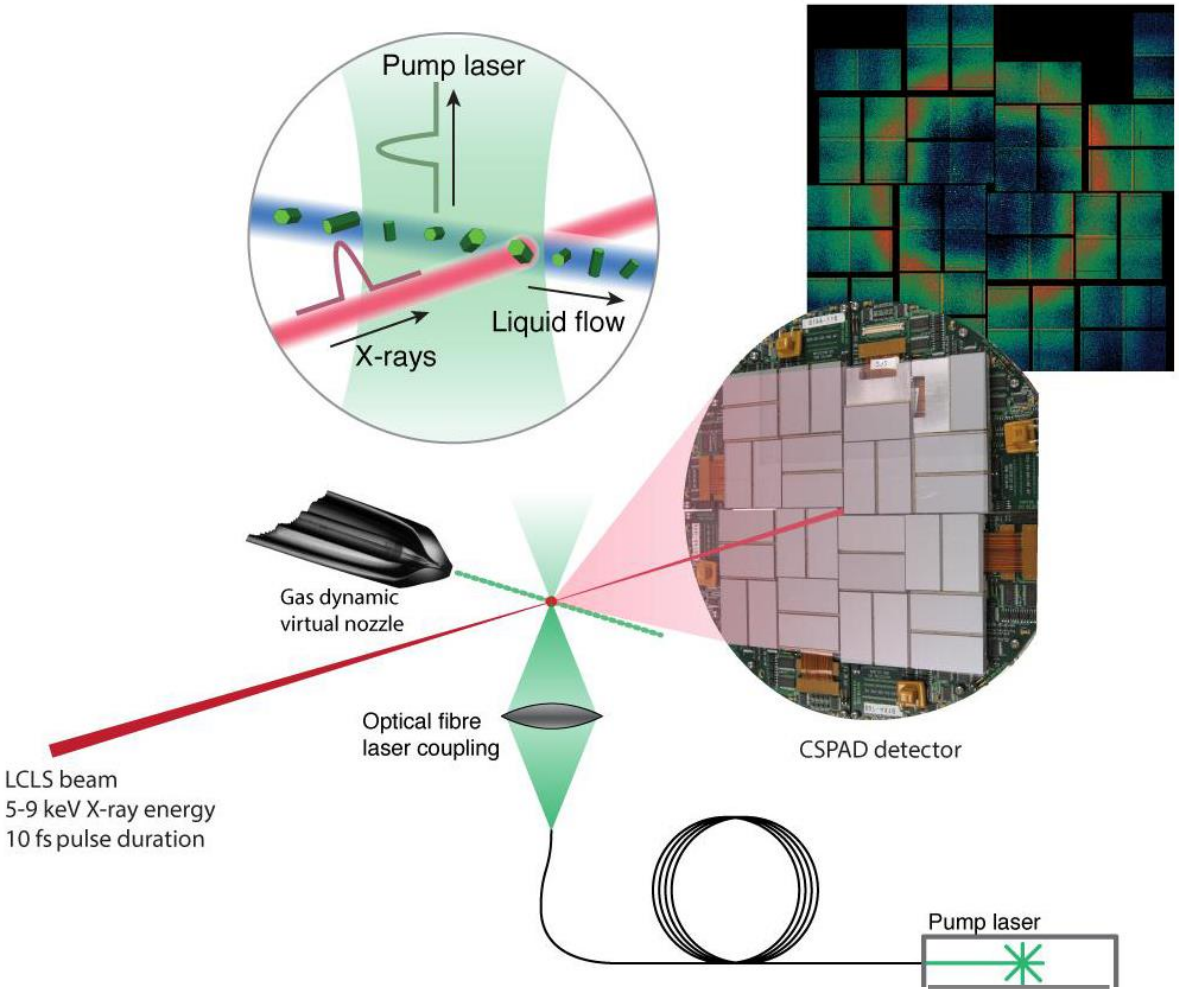
- Rhodopsin Photoactivation -



Dark State Rhodopsin
Edwards,..,Schertler 2004

Meta-II State Rhodopsin
Standfuss,..,Schertler 2011

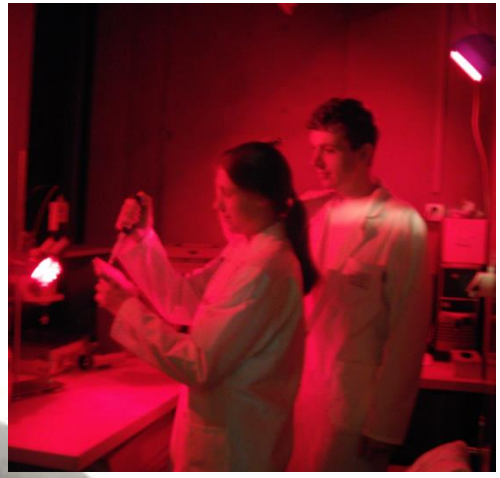
Time-Resolved Pump Probe SFX

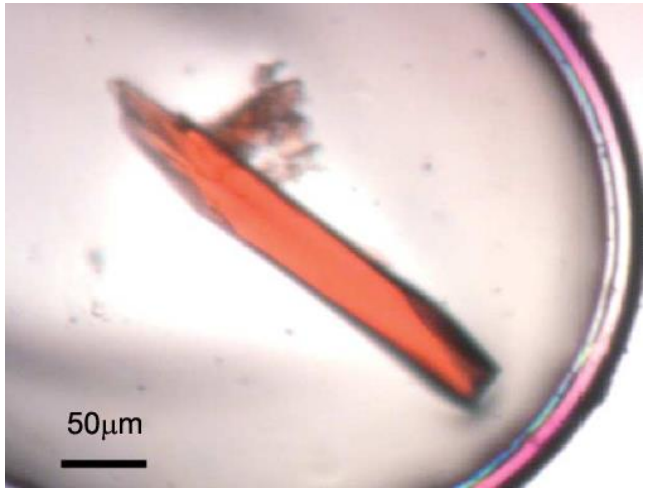


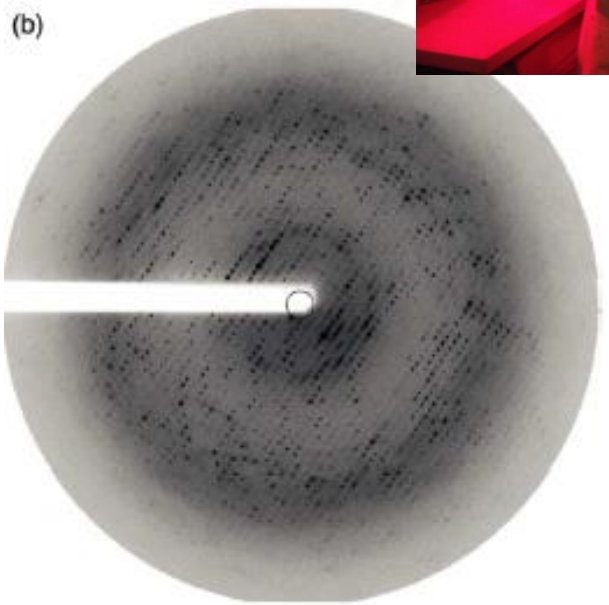
Aquila, … & Chapman, Nature 2012; modified at CFEL (Barty)

Routine Wild-Type Rhodopsin Crystallisation

Crystallisation of wild-type rhodopsin in the dark state.

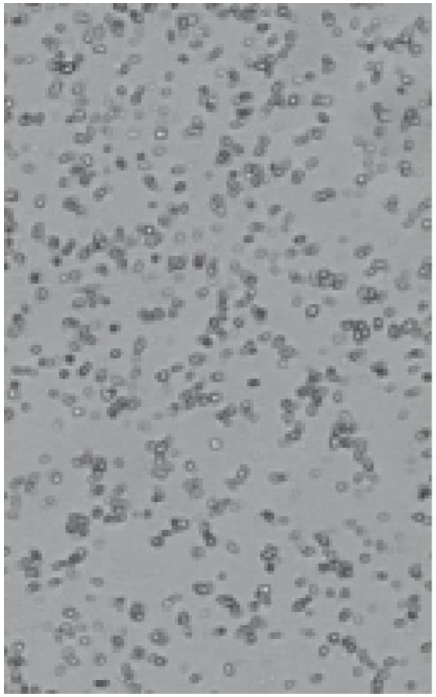






Edwards & Schertler, J.Mol.Biol. 2004

Lysozyme Nanocrystallisation

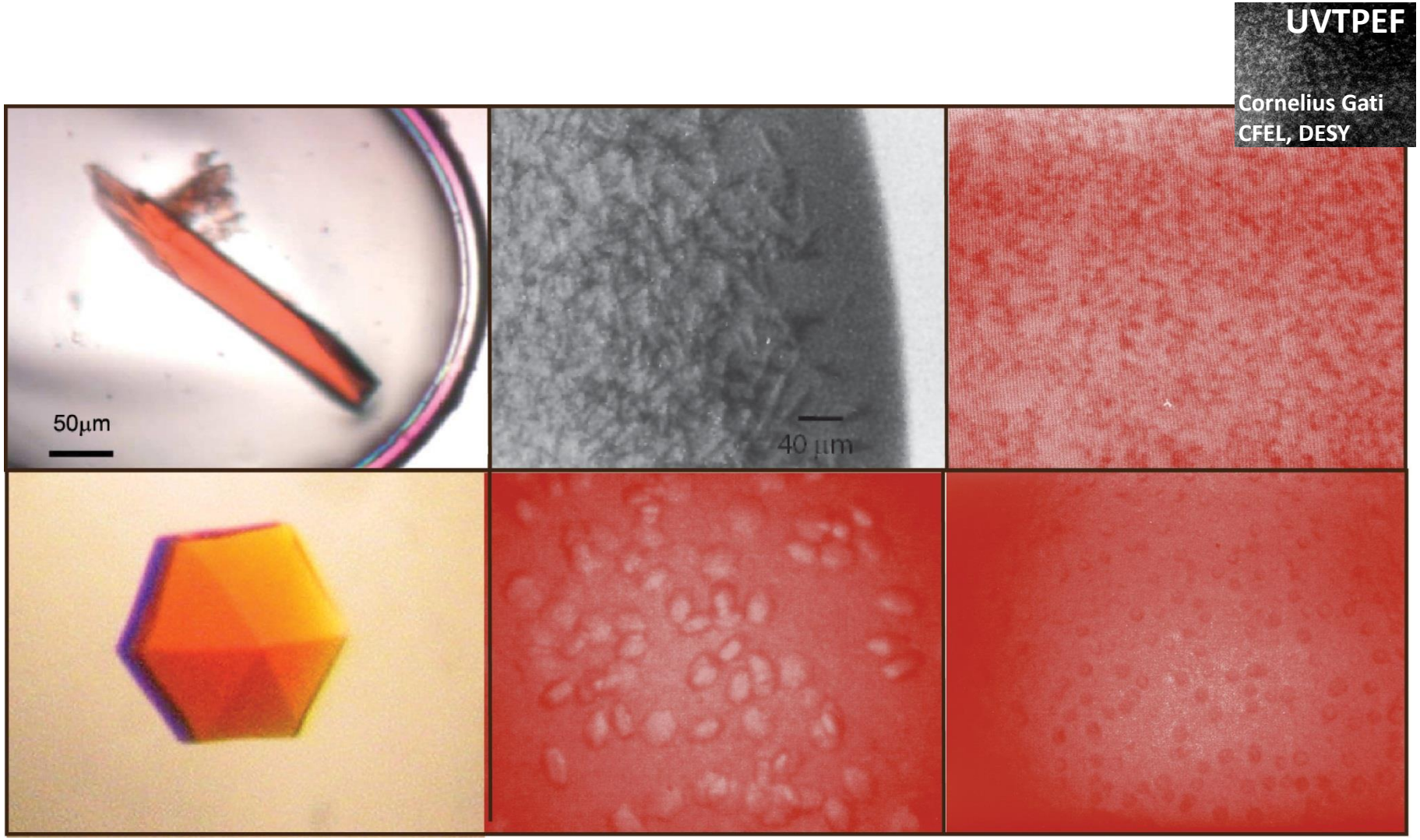


Schlichting et al, Curr.Op.Strcut.Biol. 2012

“about 1.5 million individual “snapshot” diffraction patterns.
About 4.5% of the patterns classified as crystal hits, 18.4% of which were indexed.”

Boutet et al, 2012

Producing a Nanocrystal suspension for XFEL experiment



conclusion

Too Small Crystals?

→ Serial Femtosecond Crystallography at the Free Electron Laser.

! First structure of a GPCR solved at the FEL using SFX: *Wacker et al, Thesis, unpublished results.*

Dynamics in Structural Biology?

→ Time-resolved Pump Probe SFX Experiment at the XFEL.

Acknowledgments:

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PhD at CFEL

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Rafael Abela and team

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Crystallisation Facility
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FP7-PEOPLE-2012-ITN
SEVENTH FRAMEWORK PROGRAMME

Thanks for your Attention!