The application of Free Electron Lasers to Biology: Playing with retinal proteins and GPCRs

Wir schaffen Wissen – heute für morgen

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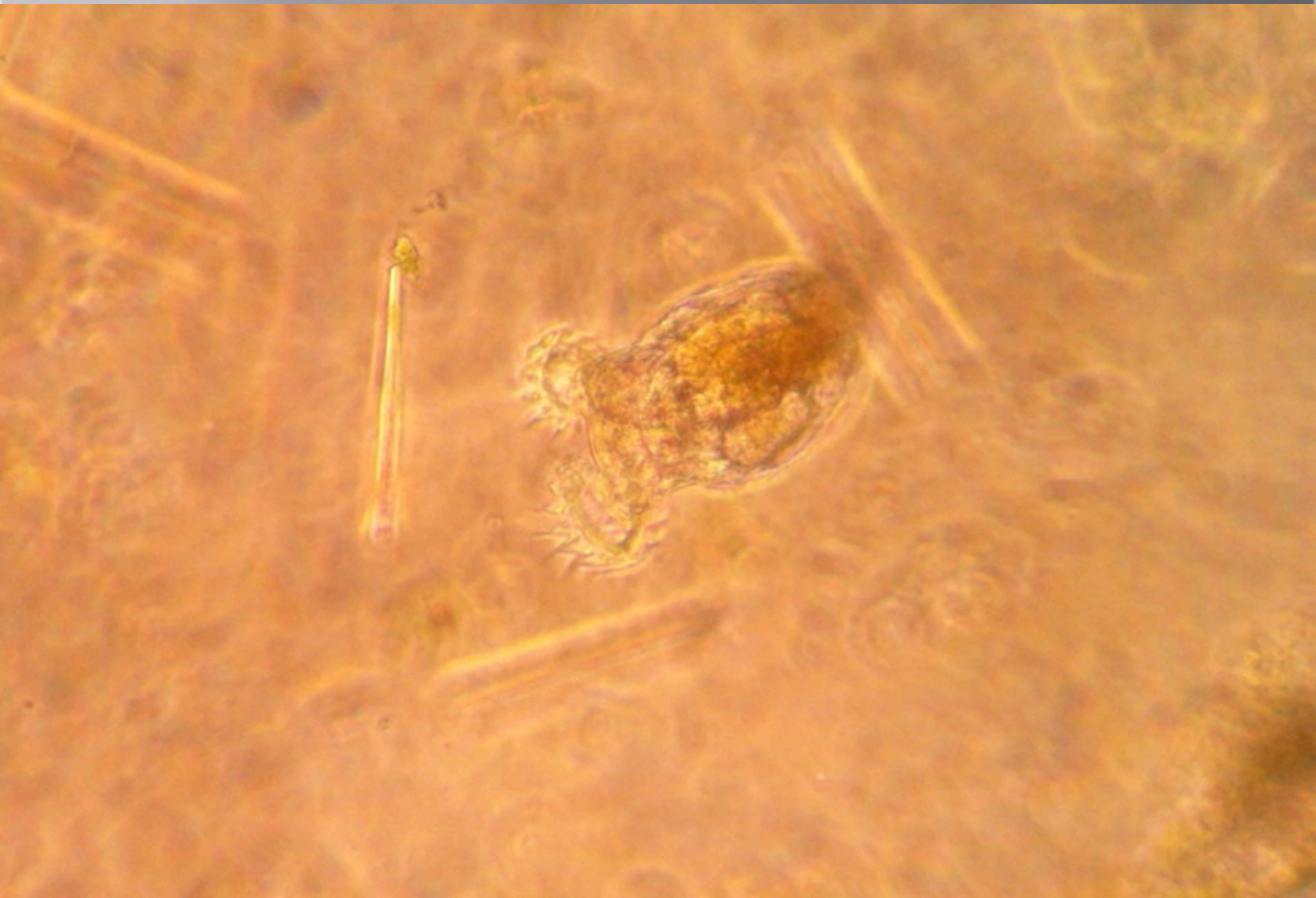
Professor for Structural Biology ETH Zürich
Laboratory of Biomolecular Research
Switzerland

Paul Scherrer Institute PSI

Biology is Dynamic and has many Time Domains



Time domains in Biology

Evolution million of years

Human life cycle 80 years

Circadian day night rhythm one day

Cell division hours

Enzyme activation milliseconds

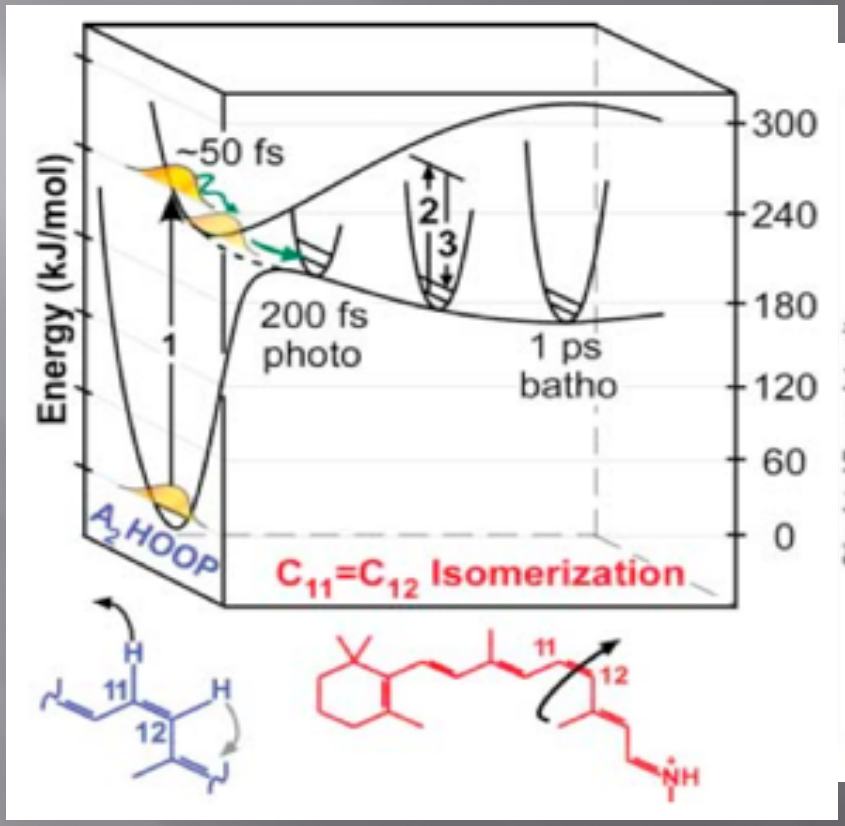
Protein motion conformational change microseconds

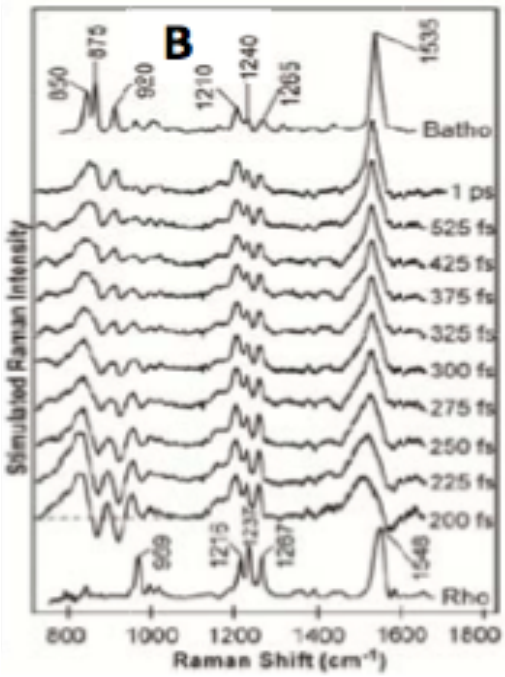
Formation of early photoproduct in vision picoseconds

Retinal isomerization femtoseconds

Photon capture, orbital rearrangements attoseconds

The catalytic step in vision





- The quantum yield and stereo selectivity
- is decided in 200 femtoseconds!!!

Membrane proteins are key drug targets

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Genome Reviews

Curated versions of EMBL entries for complete genome sequences

IPI

A top-level guide to the main databases that describing higher eukaryotic proteomes

EBI › Databases › Integr8

Integr8 : Proteome analysis:

Search for species [ ] Go! Search for gene/protein [ ] in H.sapiens Go!

Selected species *H.sapiens* Change scope

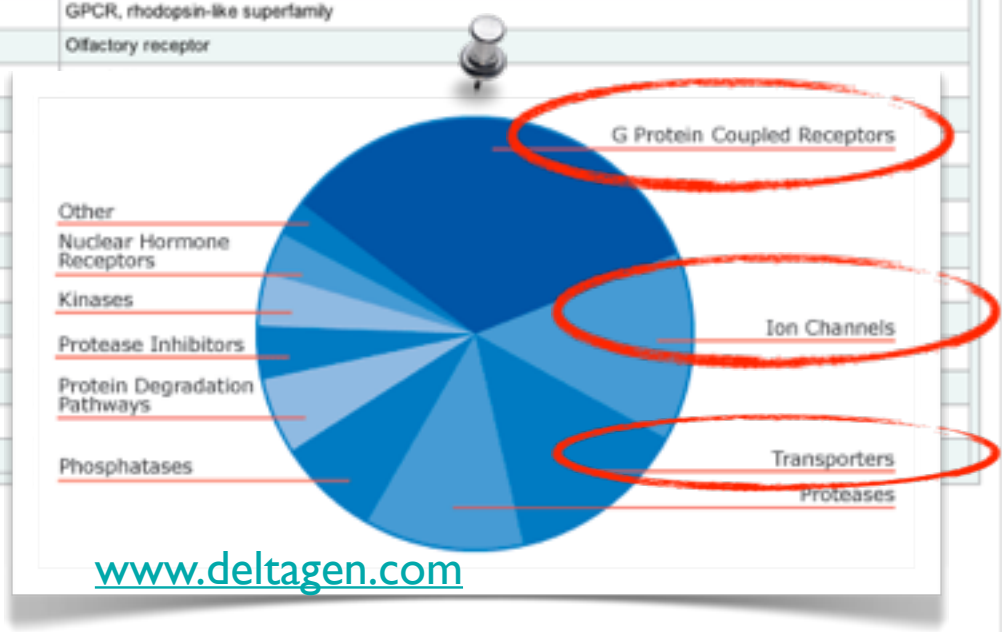
InterPro | Comparative | CluSTr | GO | Structure

Select analysis: 15 most common families

InterPro

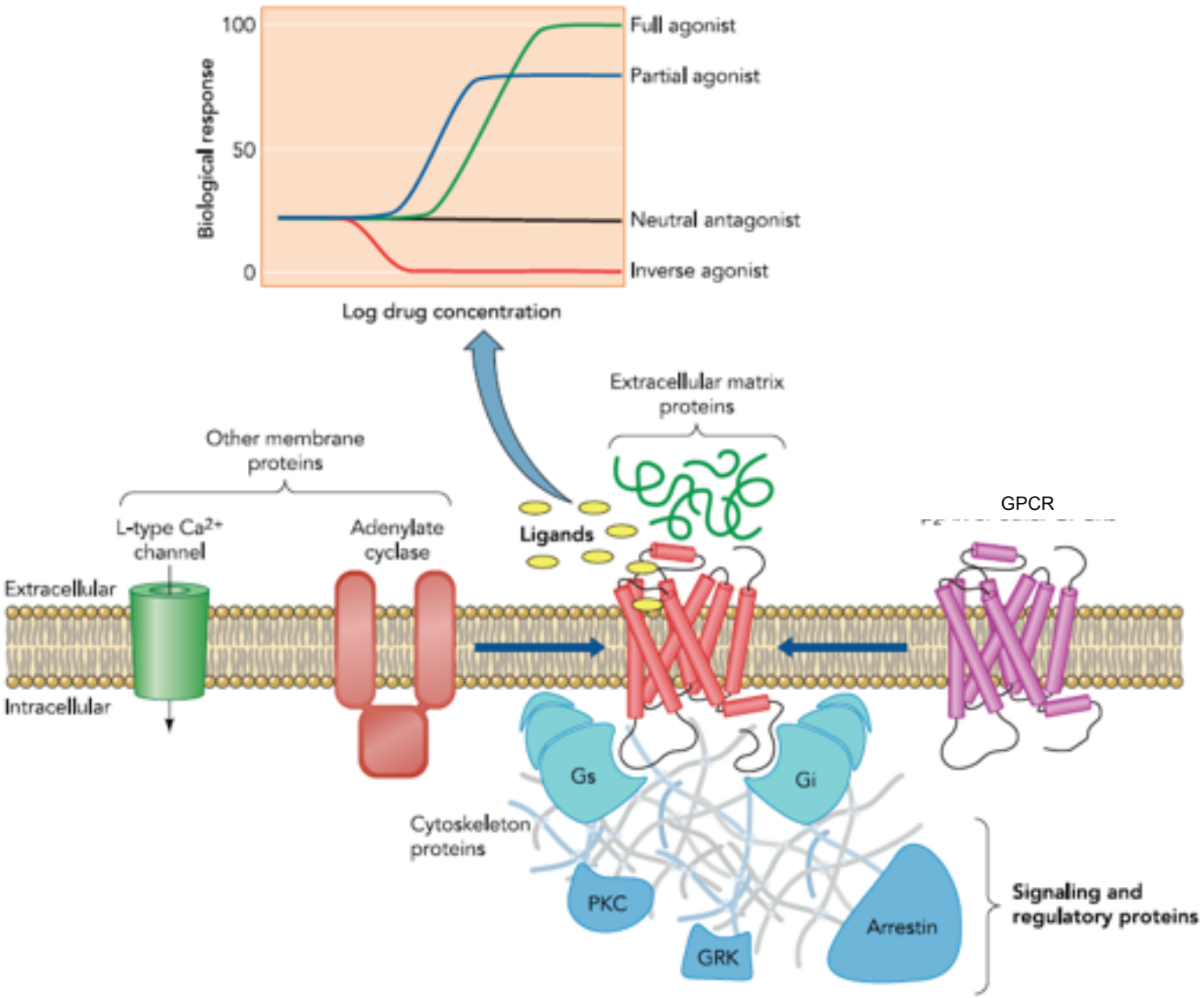
15 most common families for H.sapiens

InterPro	Proteins matched	Name
IPR000276	848	GPCR, rhodopsin-like
IPR017452	842	GPCR, rhodopsin-like superfamily
IPR000725	532	Olfactory receptor
IPR001806	169	
IPR013753	127	
IPR007114	105	
IPR001664	88	
IPR011701	86	
IPR001128	76	
IPR000832	69	
IPR002494	64	
IPR002198	64	
IPR003579	63	
IPR004000	59	
IPR001993	59	



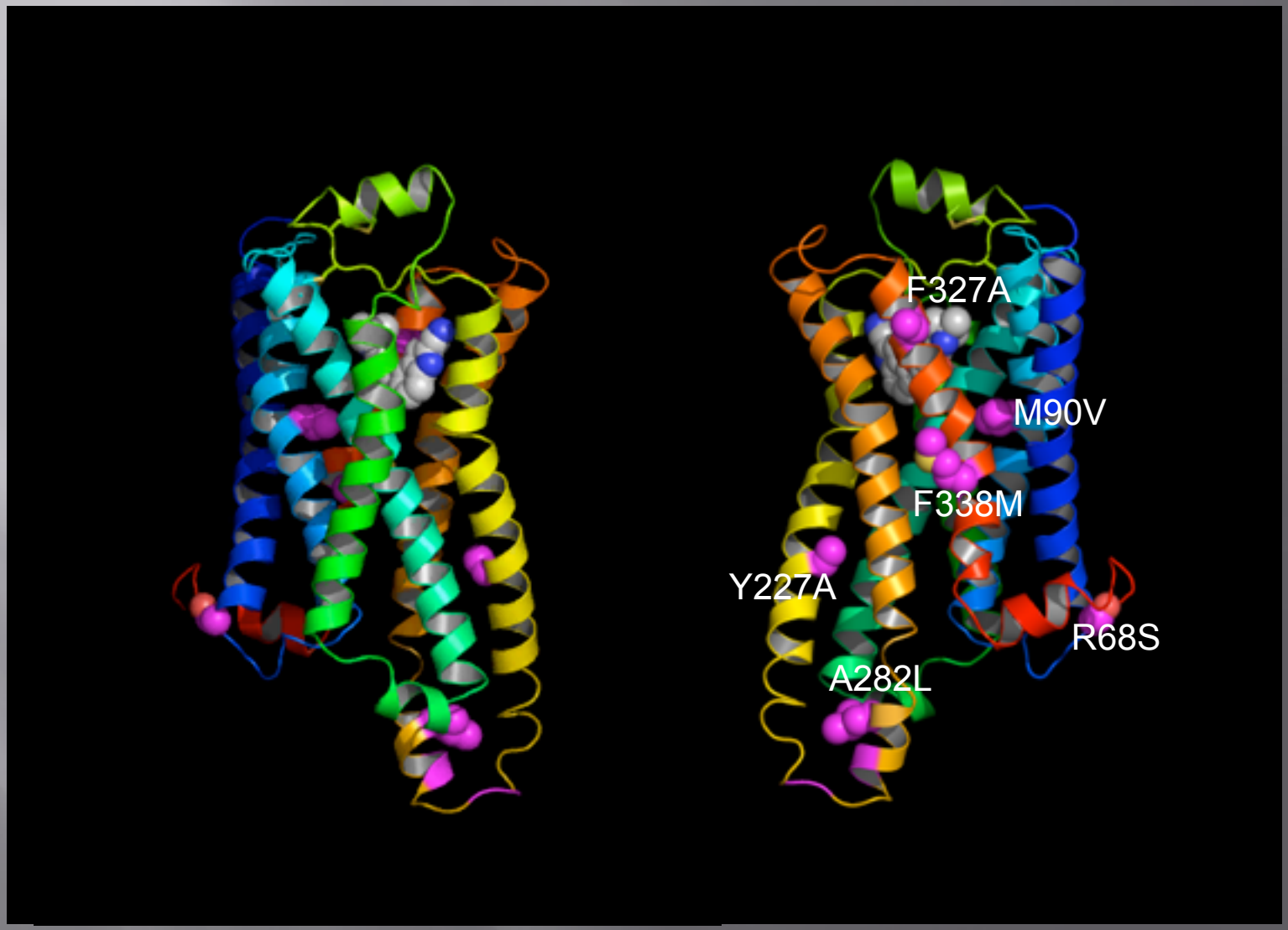
www.deltagen.com

G-ProteinCoupled Receptors GPCRs



Deupi & Kobilka, 2010

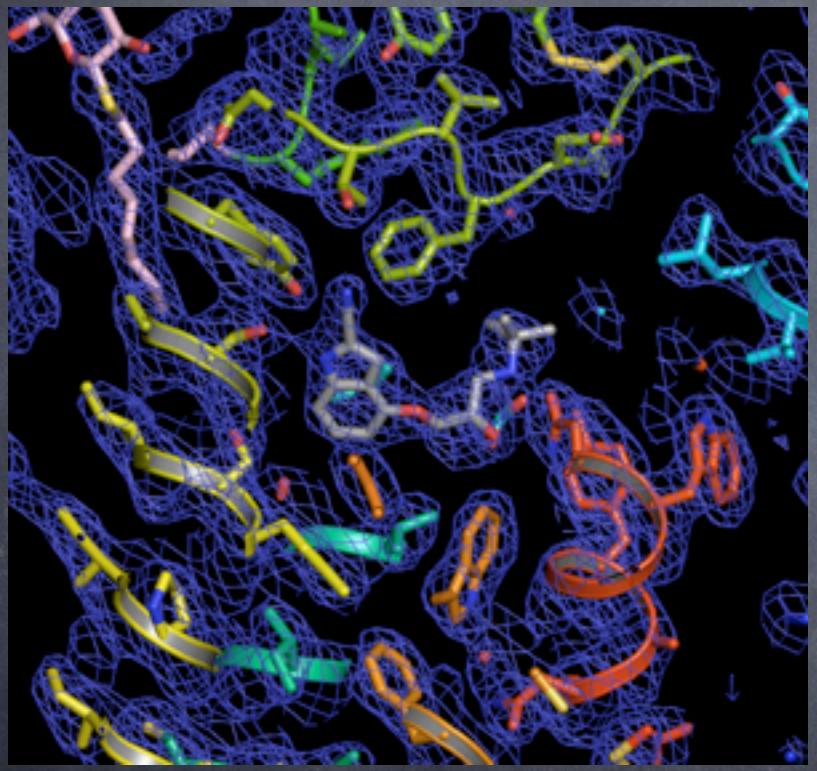
Beta1 Adrenergic Receptor
C3 deletions and stabilizing mutations

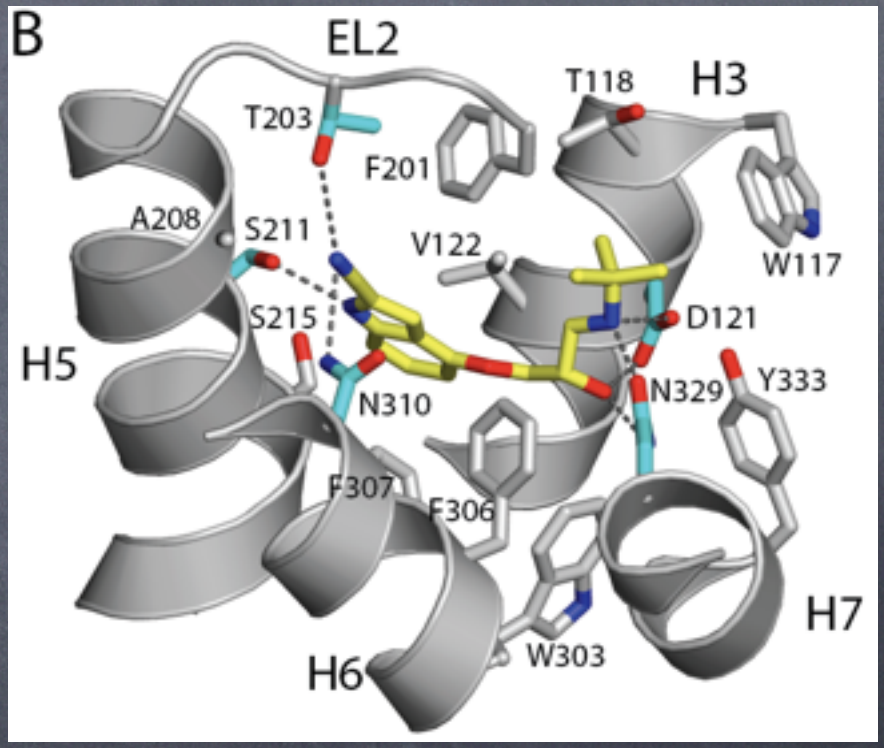


Moukhametzianov and Schertler PNAS 2011

How does a ligand bind to a GPCR?

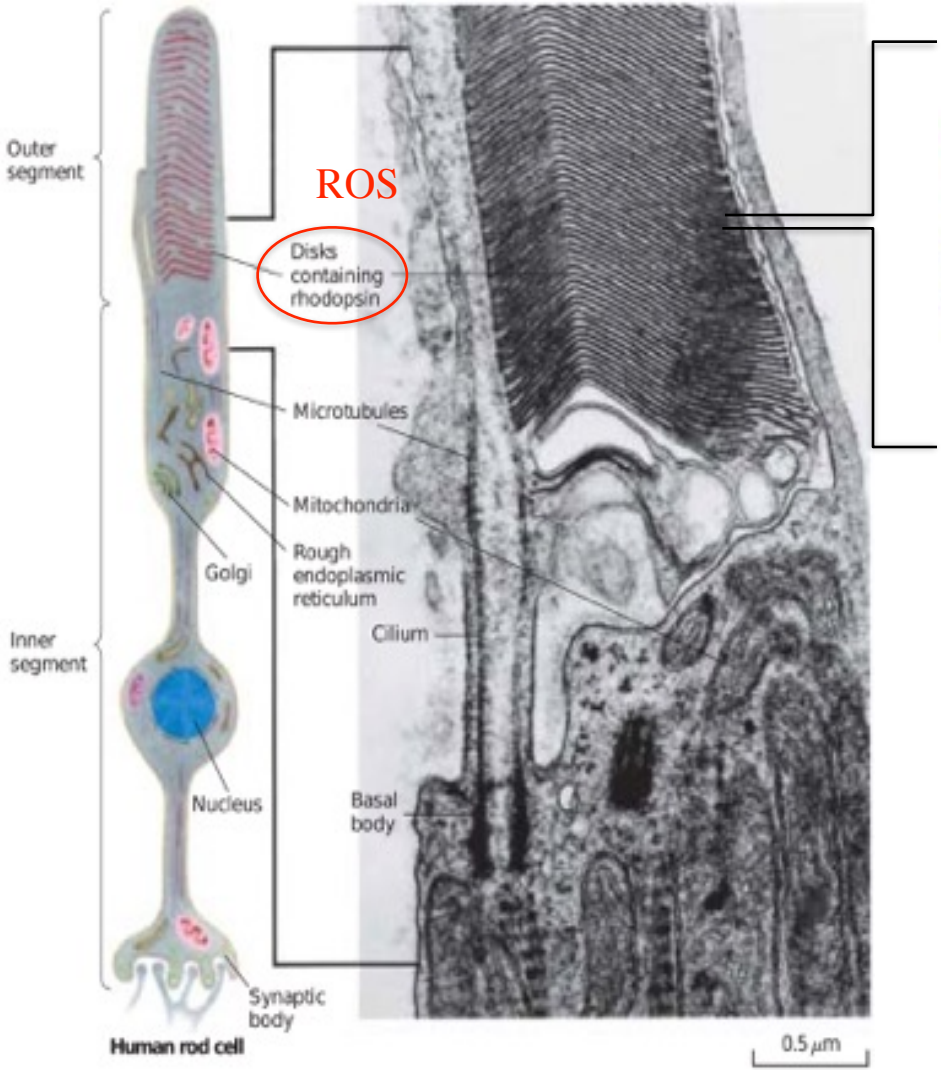
Structure of stabilized beta 1 adrenergic receptor



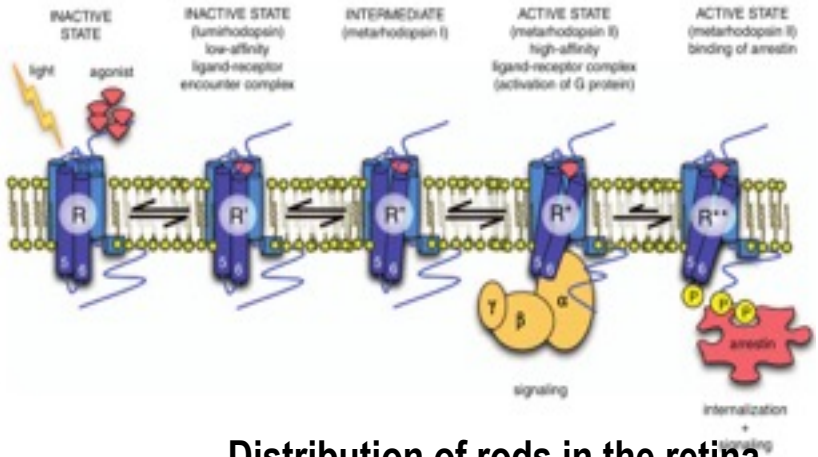


Warene, Tate and Schertler Nature 2008

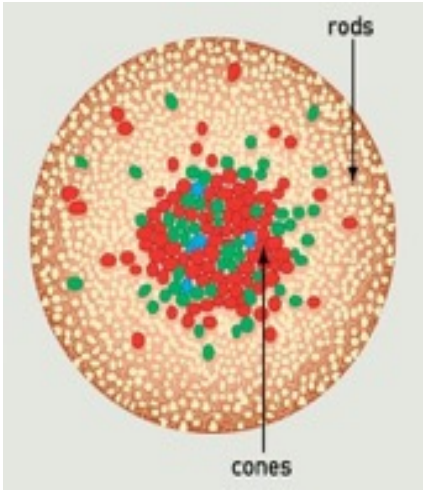
Rhodopsin in the Visual System



Rhodopsin activation states



Distribution of rods in the retina



Lodish *et al.*, 2003, Deupi & Standfuss, 2011

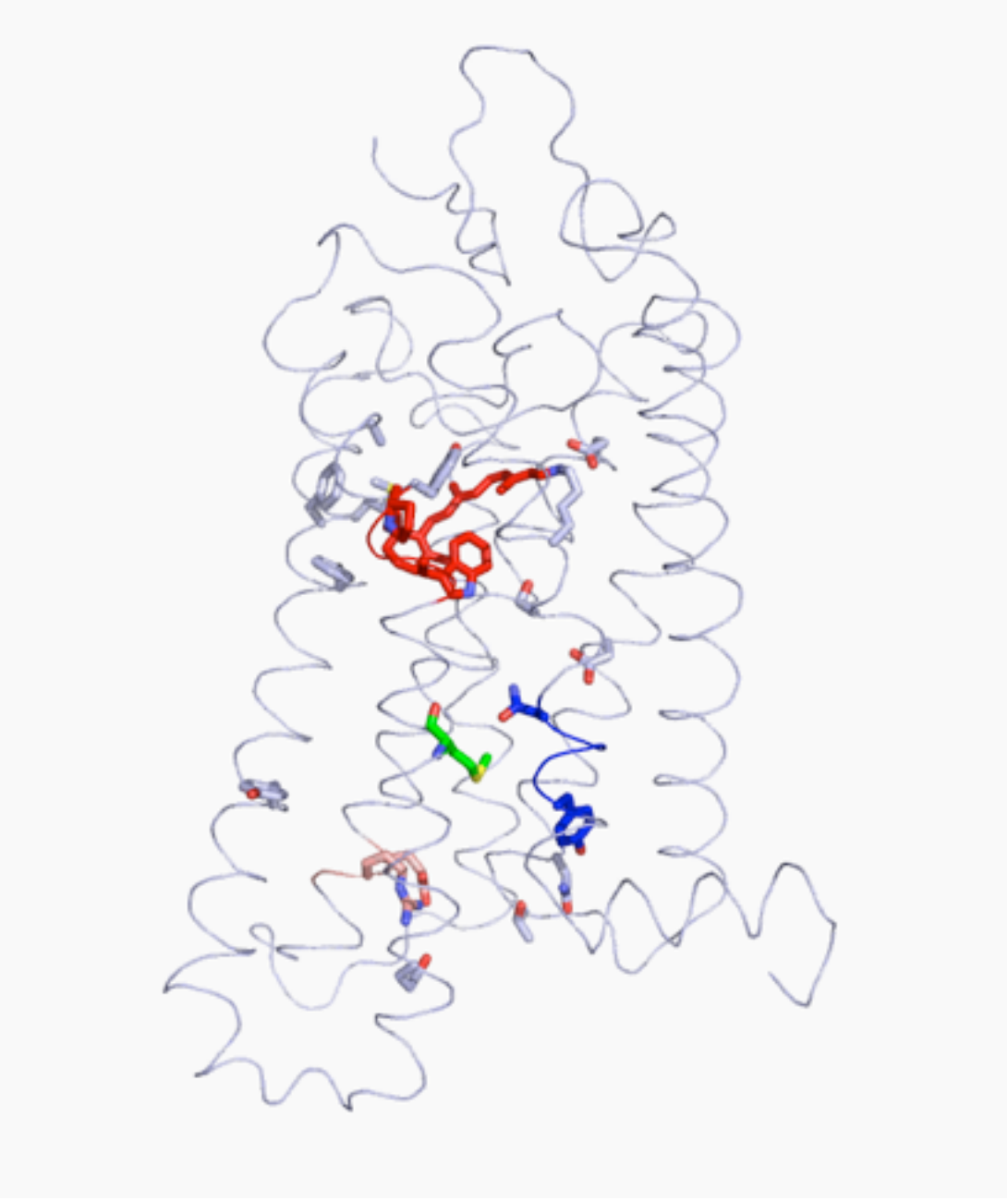
GPCR Activation: Rhodopsin

Retinal and CWxP

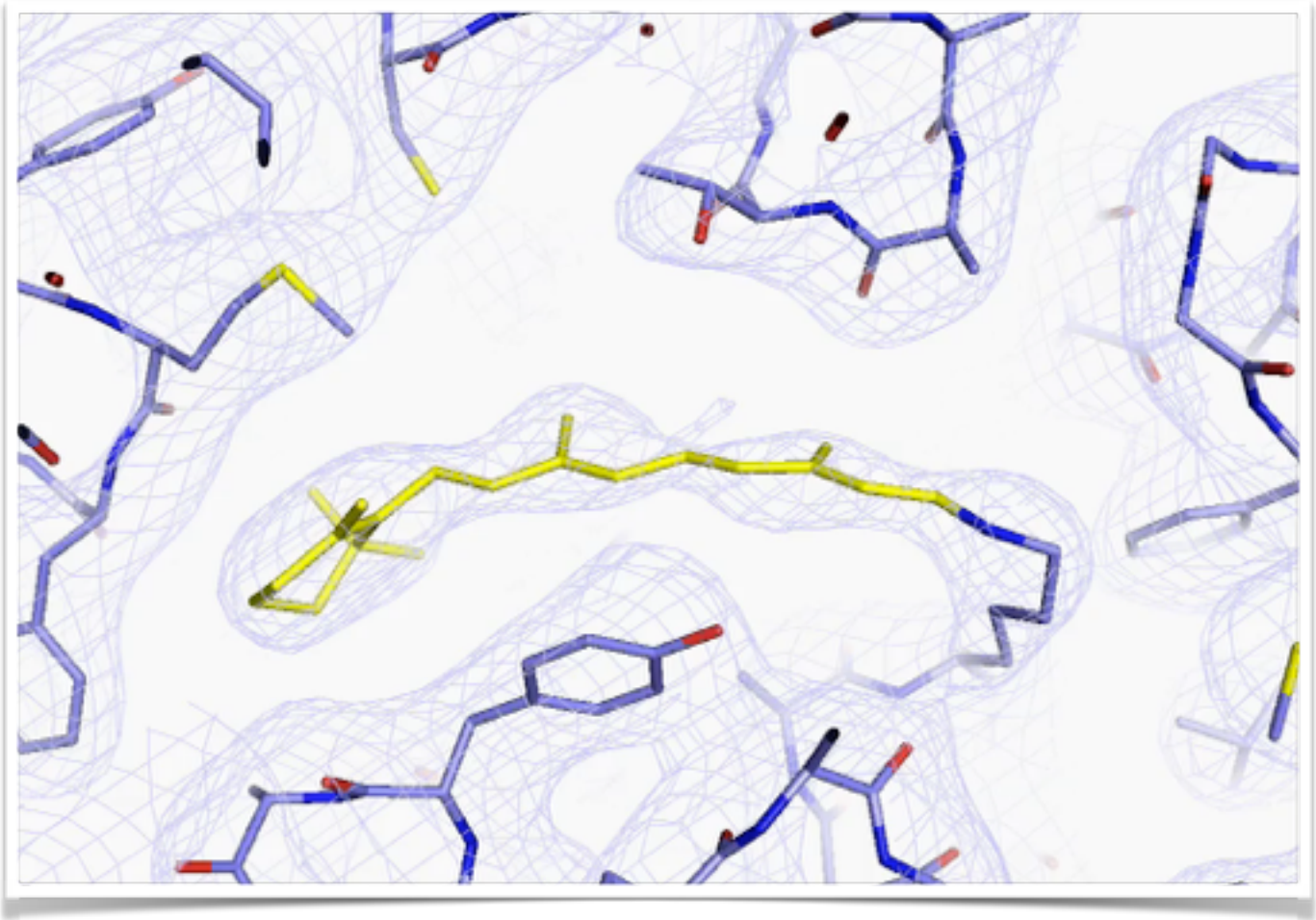
NPxxY

E(D)RY

GαCT



M257Y rhodopsin contains all-*trans* retinal covalently bound as in active Rhodopsin: Meta-rhodopsin II

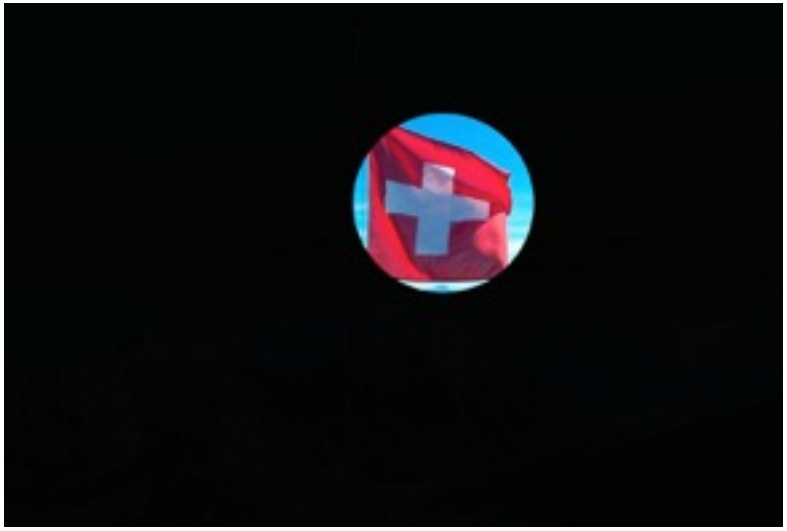


Deupi *et al., Proc. Natl. Acad. Sci.*, 2012

A structural basis for hereditary blindness

Normal Vision

Retinitis Pigmentosa (RP)



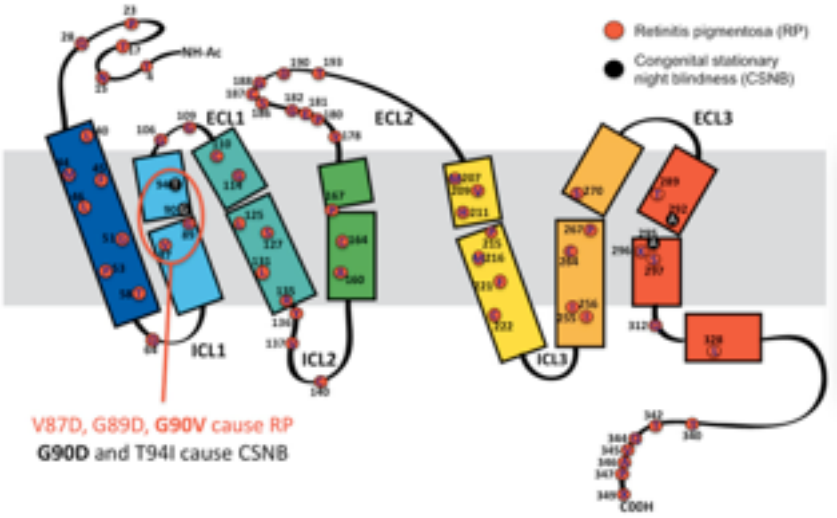
Retinitis pigmentosa (RP) is a hereditary disease leading to initial night blindness with slow progression towards complete loss of sight

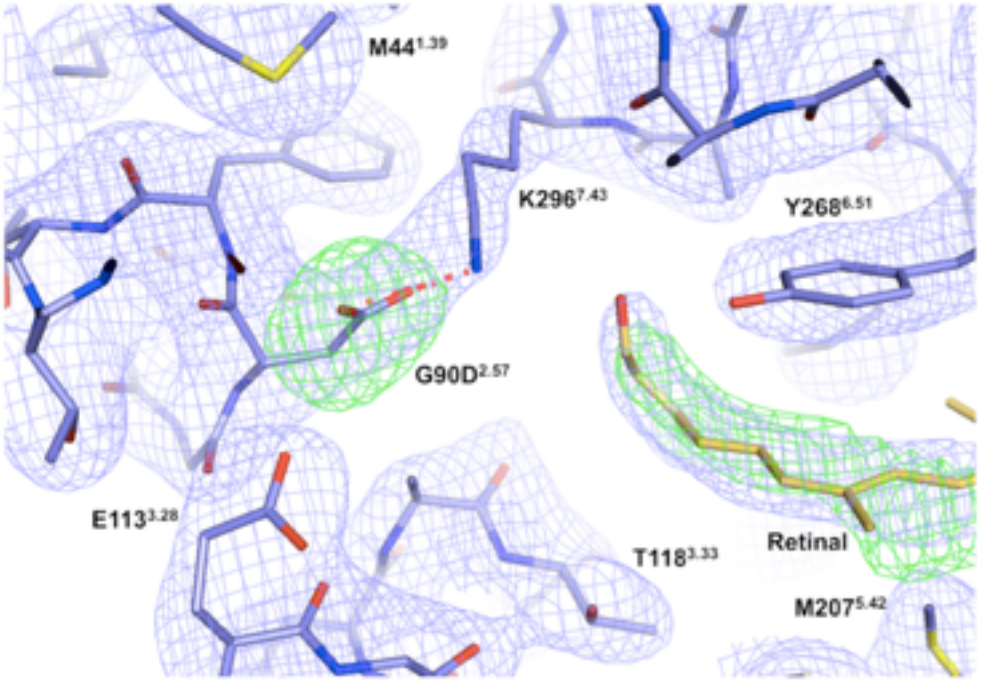
- Occurrence 1:4000 at birth
- Rho gene disease origin for 25% of RP patients
- 90% of mutations are misfolding mutations
- Four mutations result in a non-progressive night blindness phenotype (CSNB)
- Oral application of small molecules (Vitamin A plamitate, Safranal) reduces retina degeneration in mice and human RP patients

Ankita Singhal

Insights into congenital stationary night blindness based on the structure of active G90D rhodopsin

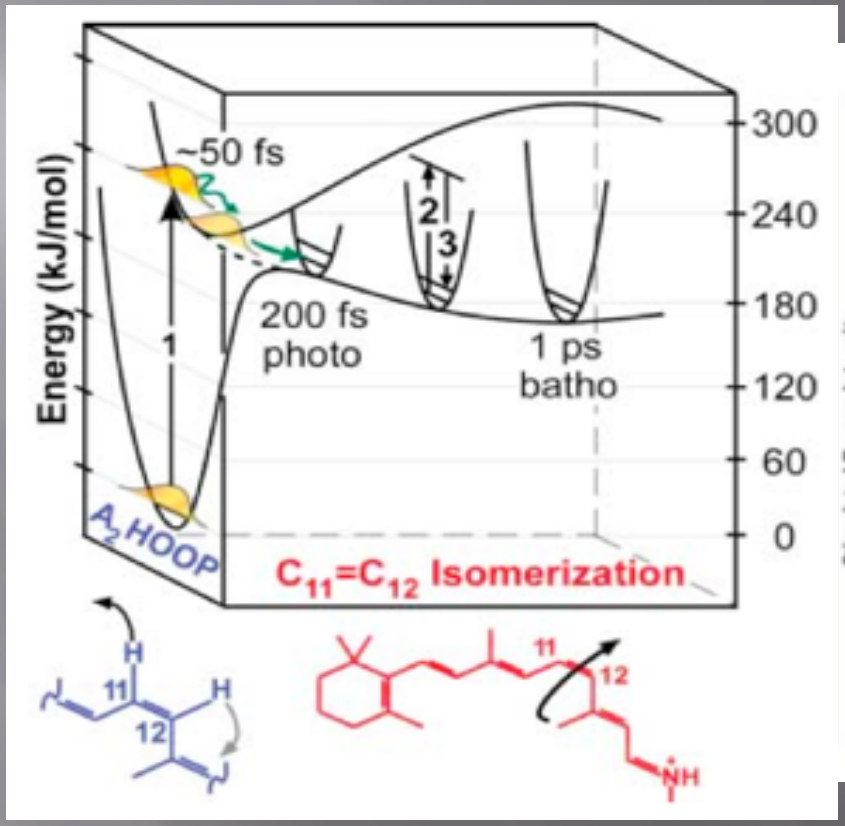
Ankita Singhal[1], Martin K. Ostermaier[1], Sergey A. Vishnivetskiy[2], Valérie Panneels[1], Kristoff T. Homan[3], John J. G. Tesmer[3], Dmitry Veprintsev[1], Xavier Deupi[1,4], Vsevolod V. Gurevich[2], Gebhard F.X. Schertler[1,5] and Joerg Standfuss[1,*]

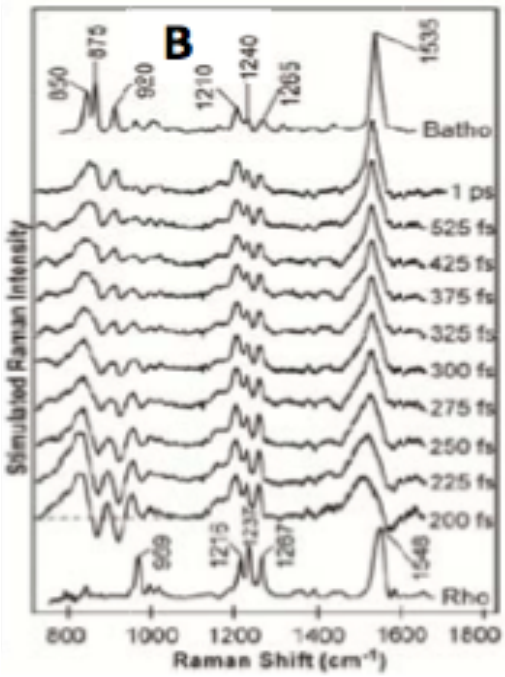




Singhal *et al., EMBO Rep. 2013*

The catalytic step in vision





- The quantum yield and stereo selectivity
- is decided in 200 femtoseconds!!!

Why Pharmacology needs SwissFEL

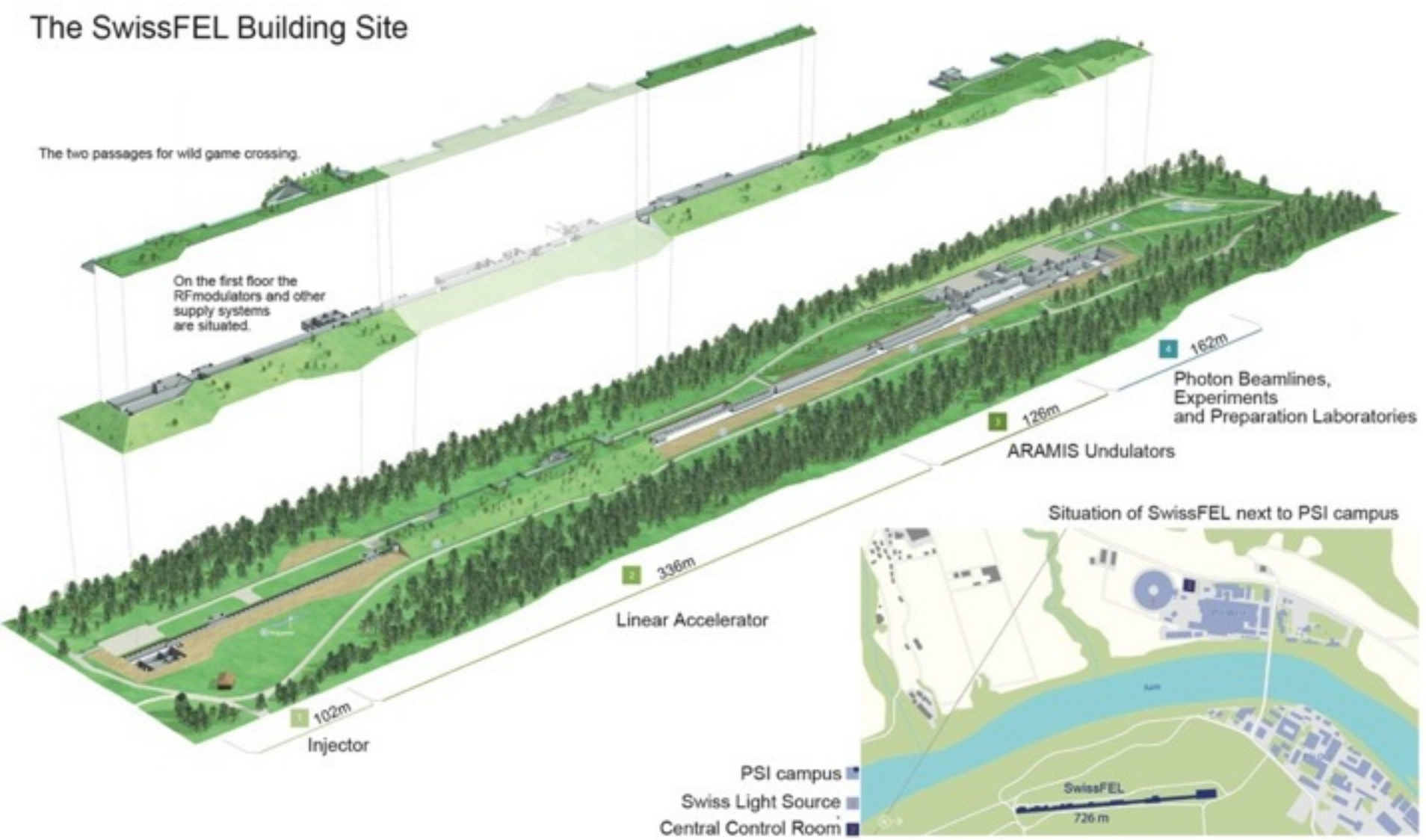
→Methods to look at unique structures on a wide range of length scales and time scales are necessary to obtain a building plan of biological signaling machines!

→Dynamic atomic information not static structure is necessary for understanding the pharmacology of drug targets!

→Understanding catalytic effect of proteins means to observe the changes to the dynamic energetic landscapes introduced by the the bound substrate or ligand.

→Quantification of the dynamic mixture of different conformations of Receptors, Channels and Transporters in solution can explain drug action in more precise detail.

→Using the right kind of experiments all these questions can be addressed with the Free Electron Laser SwissFEL in the future.

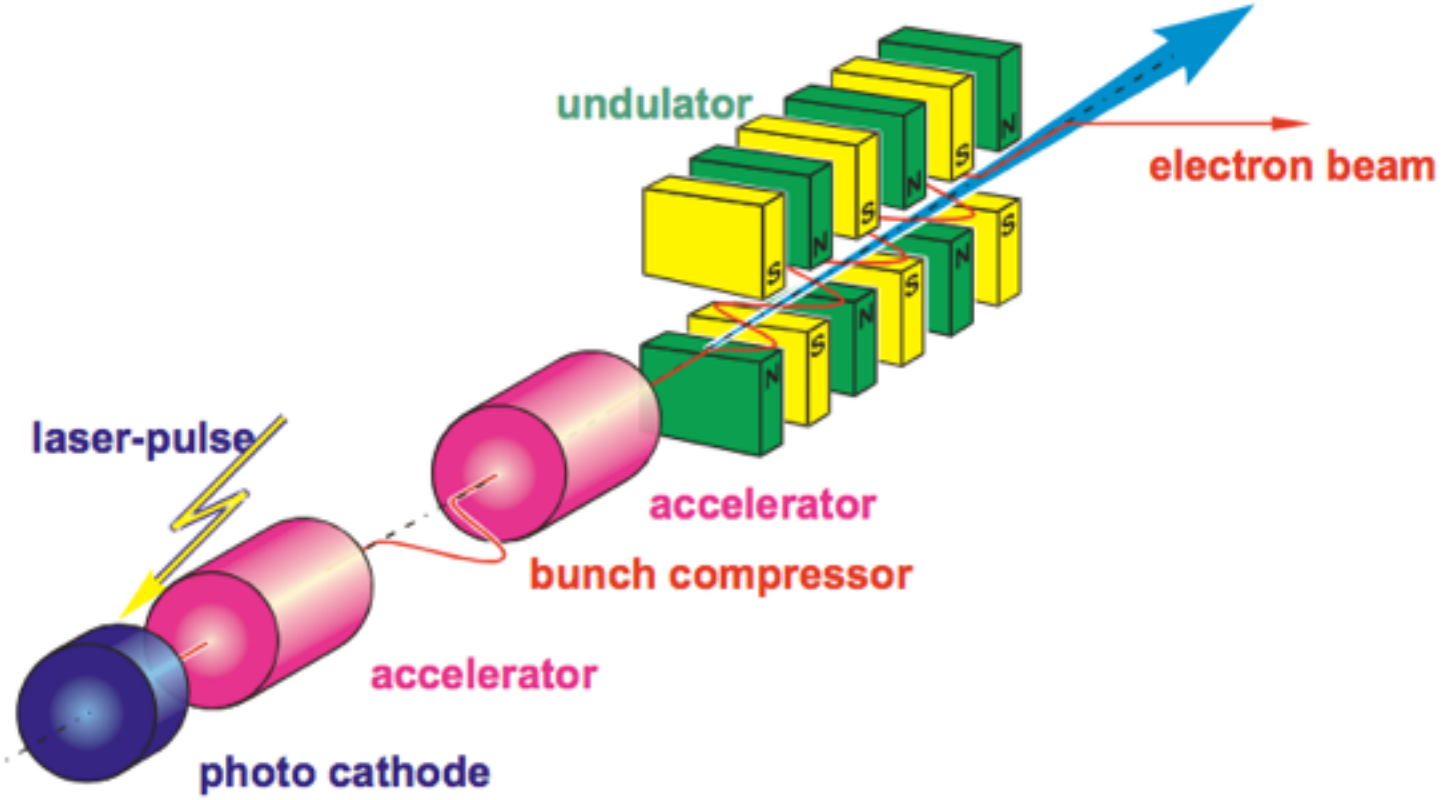

The SwissFEL Building Site
The two passages for wild game crossing.
On the first floor the RFmodulators and other supply systems are situated.
1 102m
Injector
2 336m
Linear Accelerator
3 126m
ARAMIS Undulators
4 162m
Photon Beamlines, Experiments and Preparation Laboratories
Situation of SwissFEL next to PSI campus
SwissFEL
726 m
PSI campus
Swiss Light Source
Central Control Room

SwissFEL: making a incredibly intense Femtosecond Puls

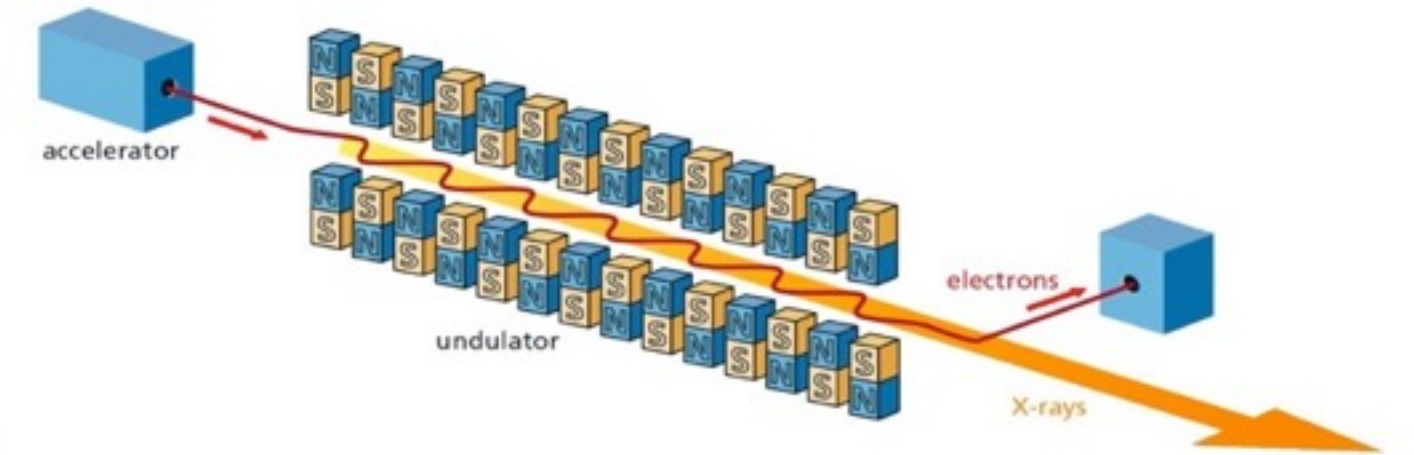
PAUL SCHERRER INSTITUTE

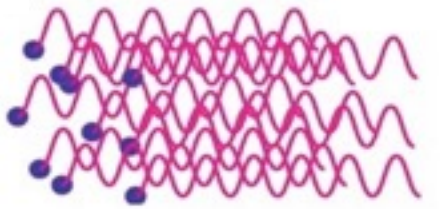
Incredibly short and
intens and coherent X-ray puls

Basic XFEL design

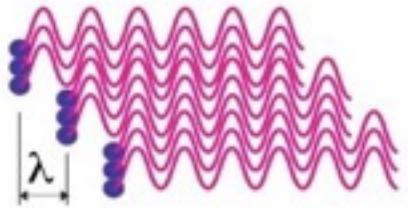


The XFEL How it Works



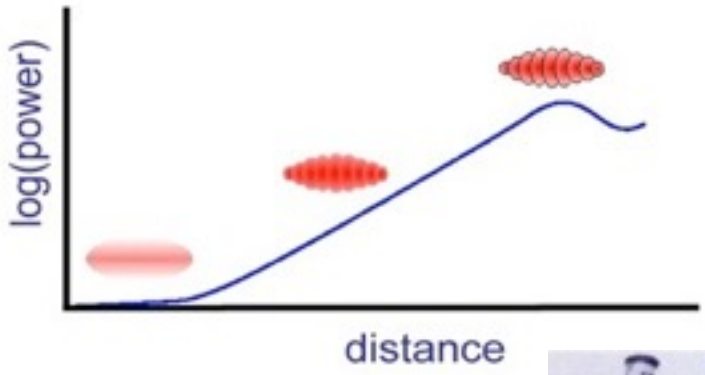


$P = N\,P_1$



$E = N\,E_1$

$P = N^2\,P_1$



SASE: "self amplified spontaneous emission"

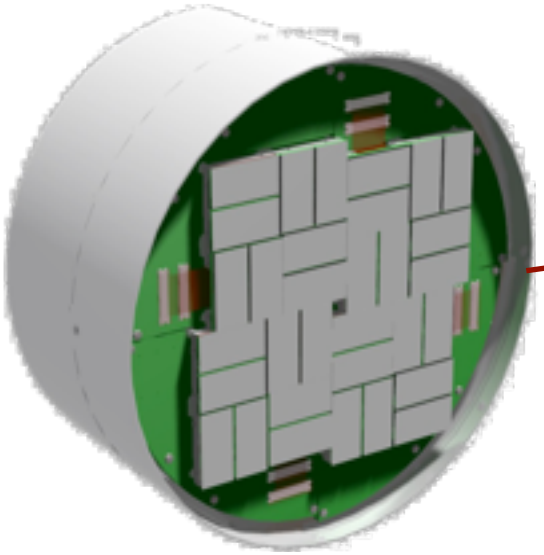
The LCLS is the world's first hard X-ray laser

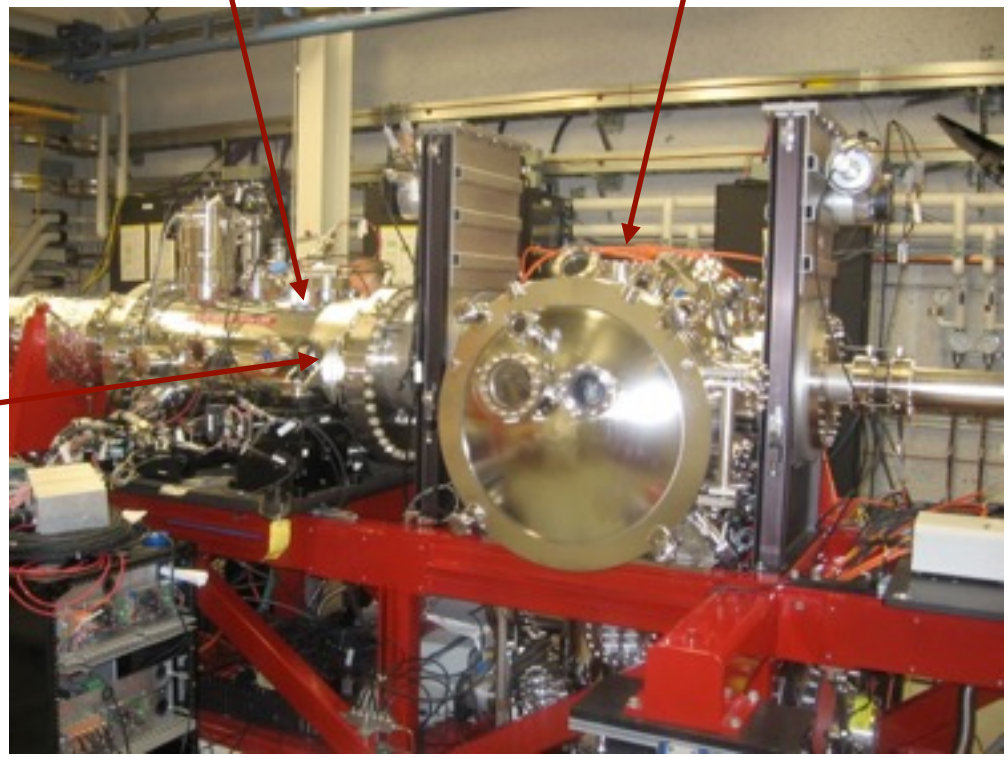
First lasing in April 2009. The LCLS at Stanford is the world's first hard X-ray laser. It produces 9 kV X-rays (1.4 Ang) in 5 - 200 fs pulses, about 1E12 photons per pulse.

Newly Commissioned CXI Hutch Optimized

Cornell-SLAC Pixel Area Detector

- 10 micron pixels
- 1.5 megapixels
- tiled design

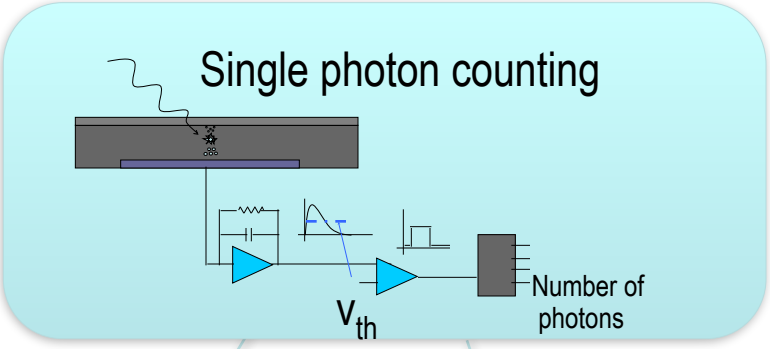


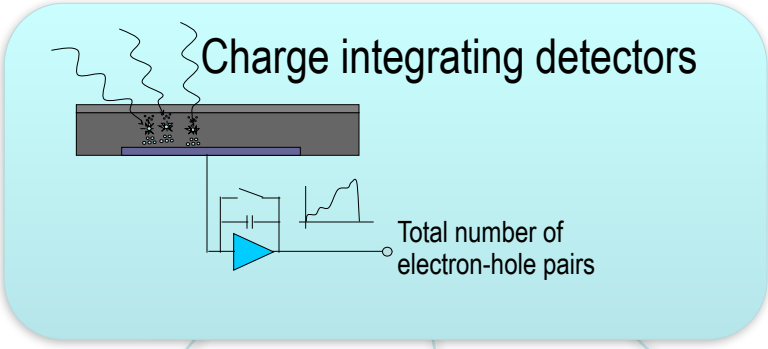


SLAC NATIONAL ACCELERATOR LABORATORY

X-ray Detector Development

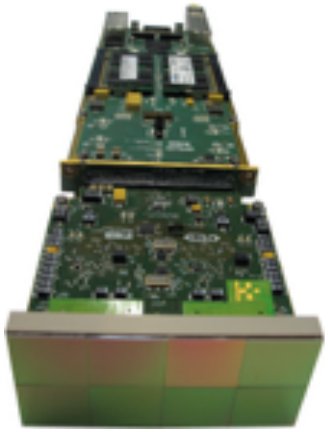
Synchrotron detectors





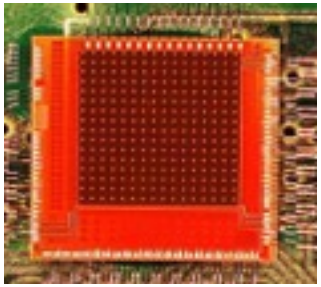
Mythen II

Eiger

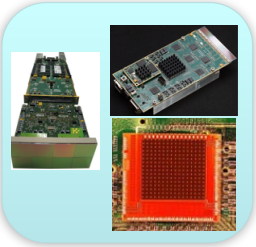


EU-XFEL, SwissFEL: Gotthard

EU-XFEL: AGIPD



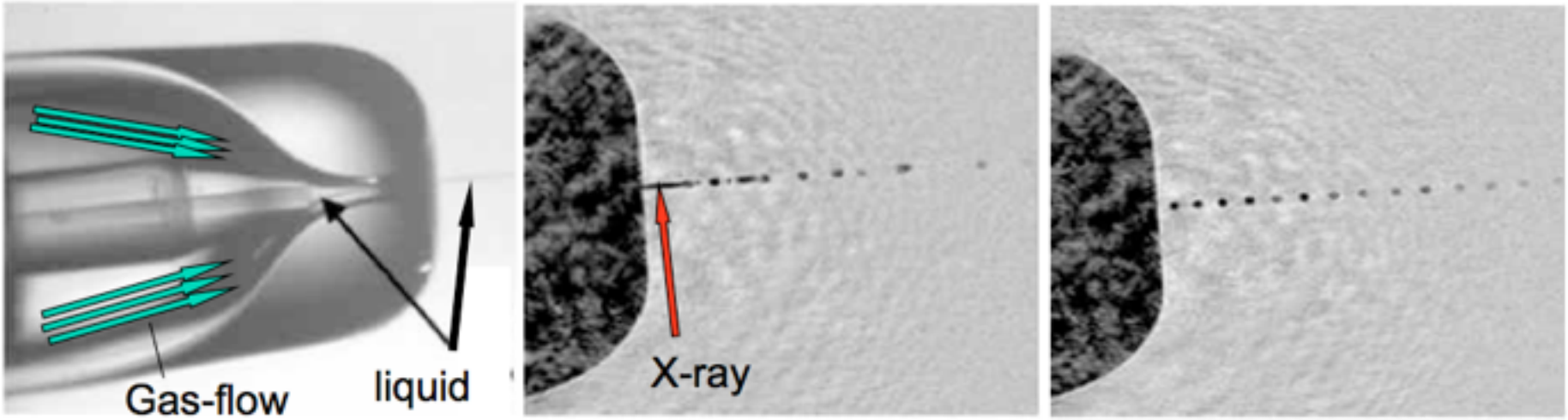
SwissFEL: Jungfrau



DESY PAUL SCHERRER INSTITUT universitätbonn UHH PAUL SCHERRER INSTITUT DESY

Droplet Injector for Biological Suspensions

The new Aerojet technique provides very fine liquid stream of micrometer size



Aerojet source. Left: Liquid cone visible generated by converging gas flow.

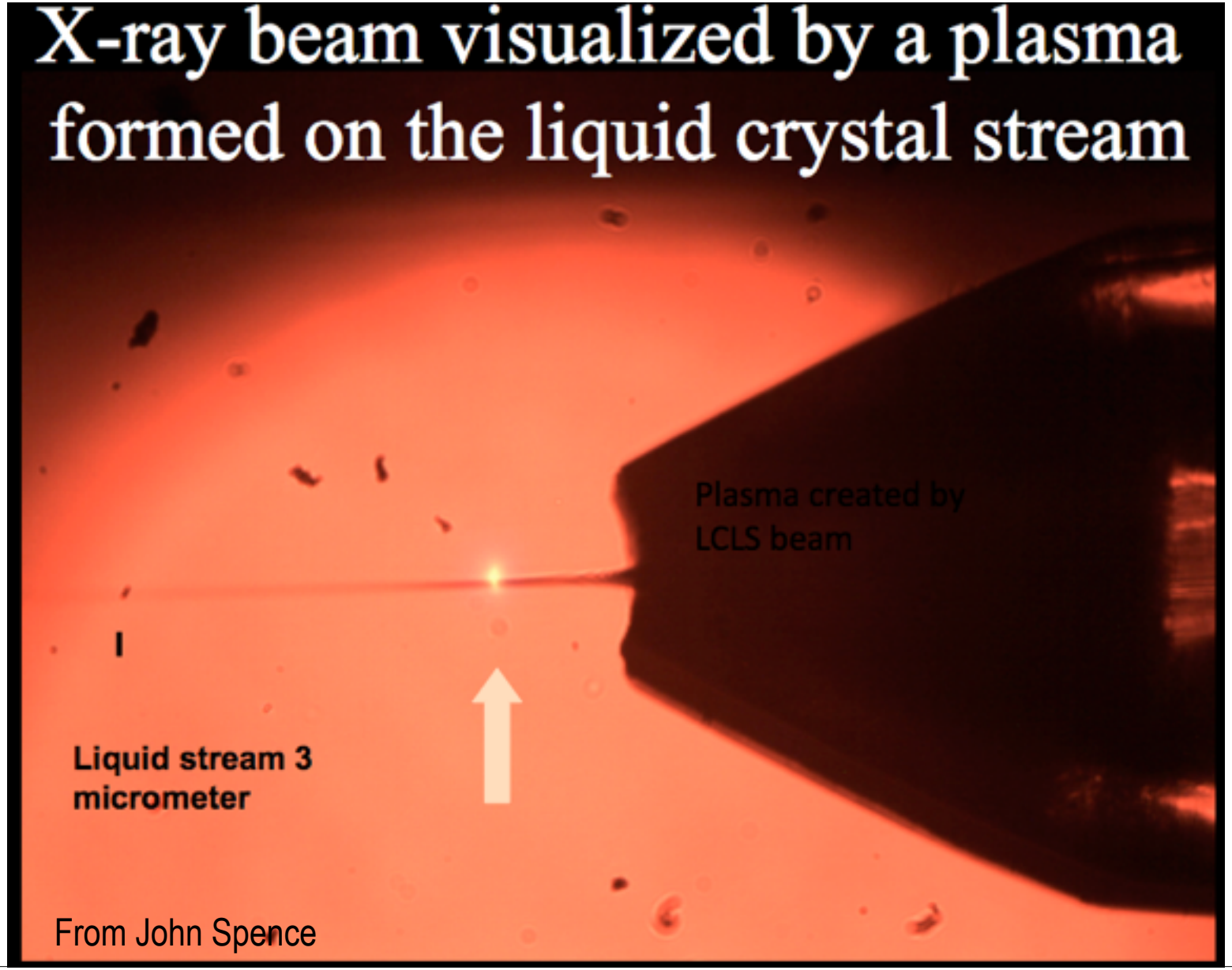
Middle: Untriggered breakup into droplets.

Right: Triggered breakup into droplets.

Droplet speed about 10m/sec. Trigger frequency: 170kHz.

From John Spence

Femtosecond nano crystallography



A Strategy for FEL Biology

Samples generated from live systems from biologists:

Suspensions:

3D nano and microcrystals
Large protein assemblies:
Virus particles, Virus shells
Protein/RNA assemblies:
Spliceosome, Signalosome, Ribosome
Protein solutions and Membrane proteins in detergent:

WAXS experiments
Ultrafast photochemistry and photobiology
Serial femtosecond crystallography (SFX)

Jet sample injectors:

Fast Jet system:
Fast pump-probe, WAXS and SFX
Slow Jet injector:
For viscous and jelly samples
LCP-Jet membrane protein SFX

Supported 2D sections:

Tissue sections
2D Membrane protein crystals
Tubular protein assemblies
Tubulin and helically arranged membrane protein tubes
3D Nanocrystals on a support

Nano-beam with cryo-2D scanning stage:

2D crystallography
Element selective topography
Helical 3D reconstruction
Characterization of nanocrystals
Crystallography with very small amounts

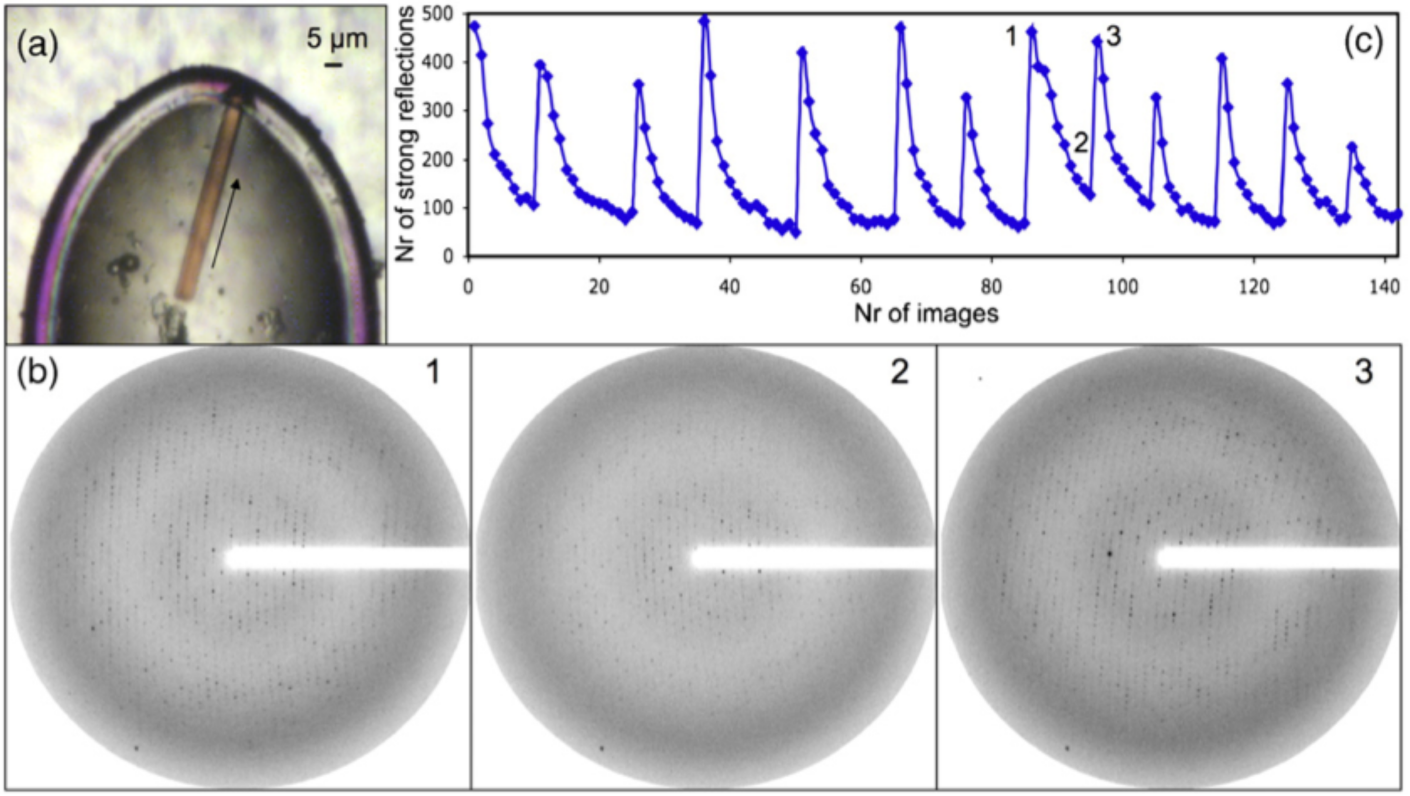
3D Blocks of tissues:

Retina
Bone
Muscle
Brain tissue
Block of frozen yeast
3D-printed cell assemblies

Nano-beam with cryo-3D scanning stage:

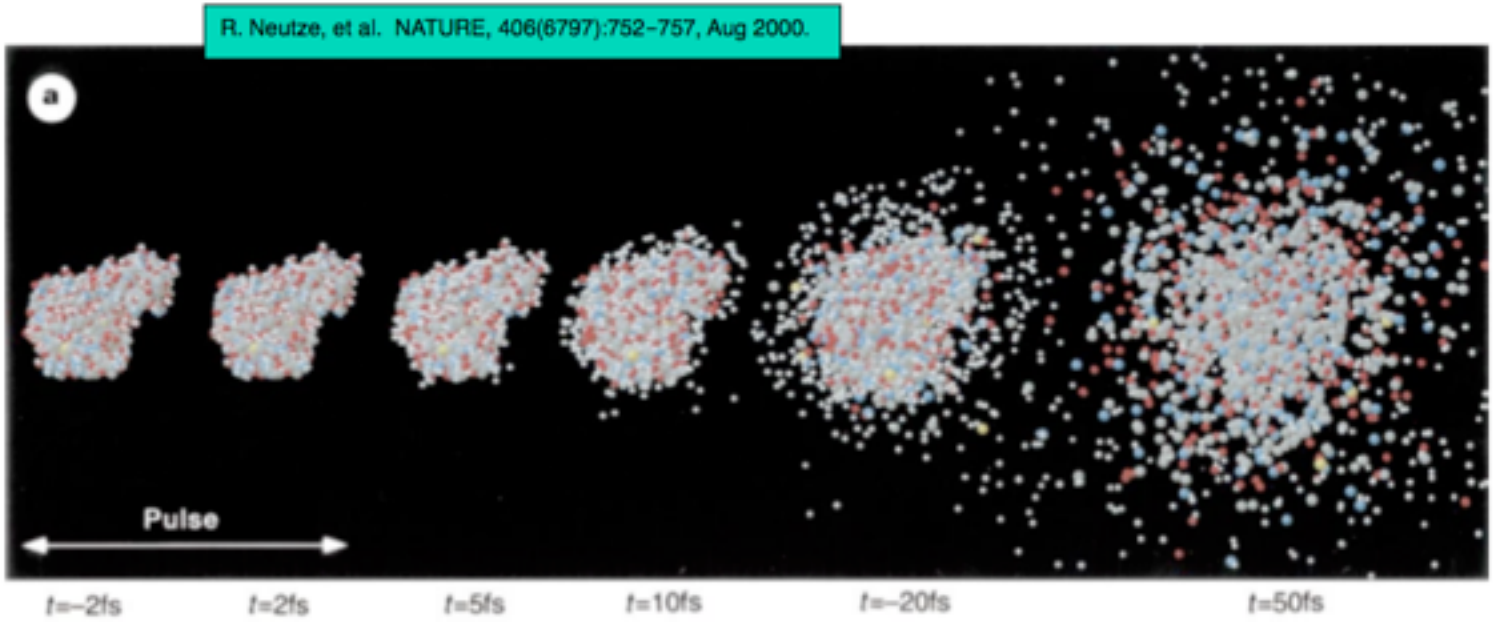
3D element selective topography
Element selective imaging
Direct or holographic imaging
Tomography

Radation Damage in X-Ray Diffraction and EM is limiting Structural Biology



Outrunning Radiation Damage in Crystallography

Simulation of the Explosion dynamics of biomolecules (C,N,O):

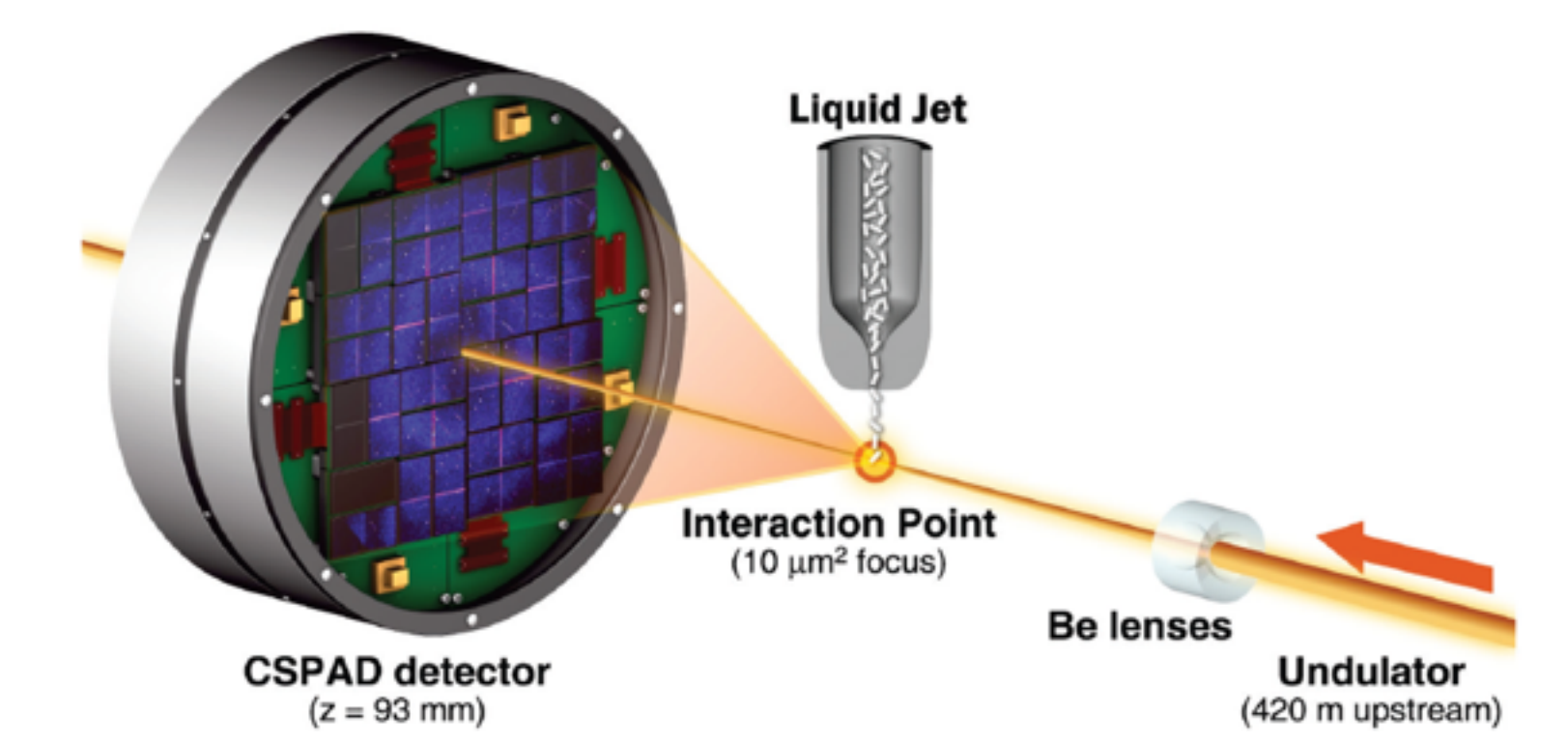


Tricks:

1) When the light is not on then we can not see what is happening

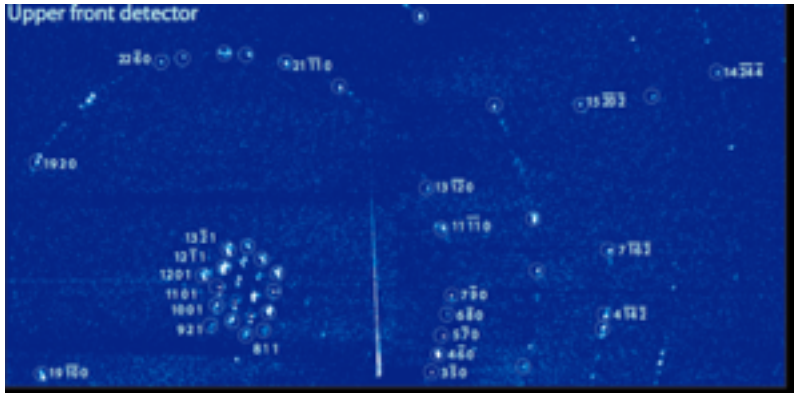
2) Only crystalline ordered material has enough average signal to be detected

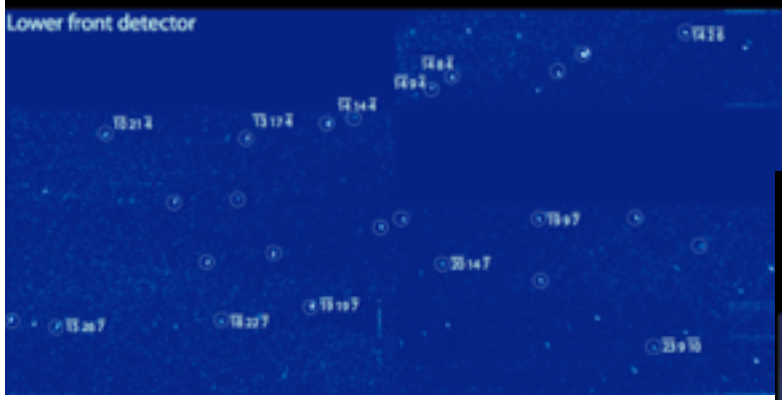
3D CRYSTALLOGRAPHY WITH FELS



Boutet et al, 2012

Femtosecond nano crystallography

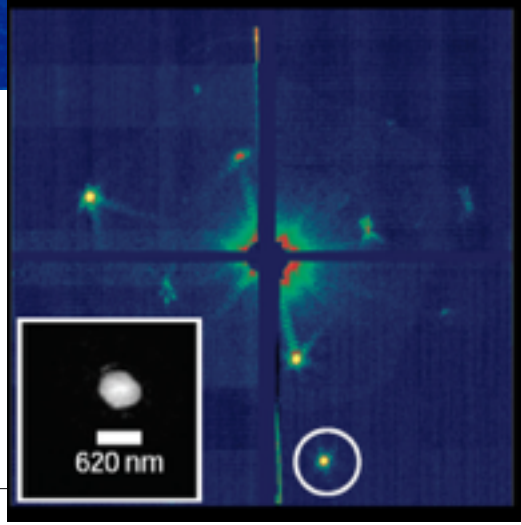


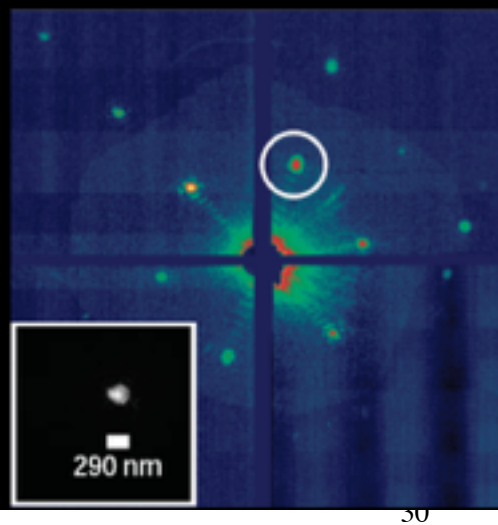


Example of one diffraction pattern indexed with MOSFLM

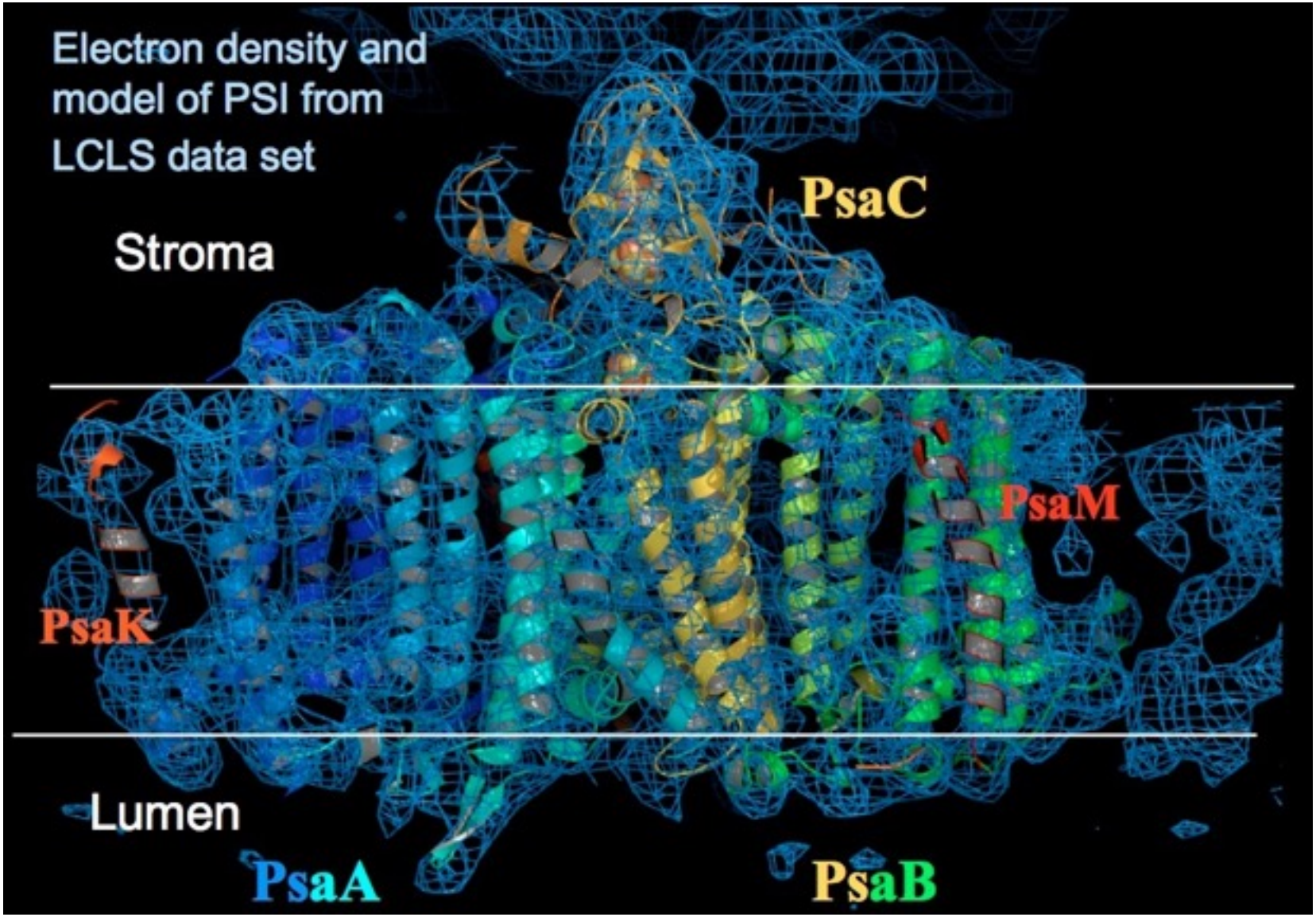
From
Petra Fromme

Examples of the determination of crystal size by evaluation of the shape transforms





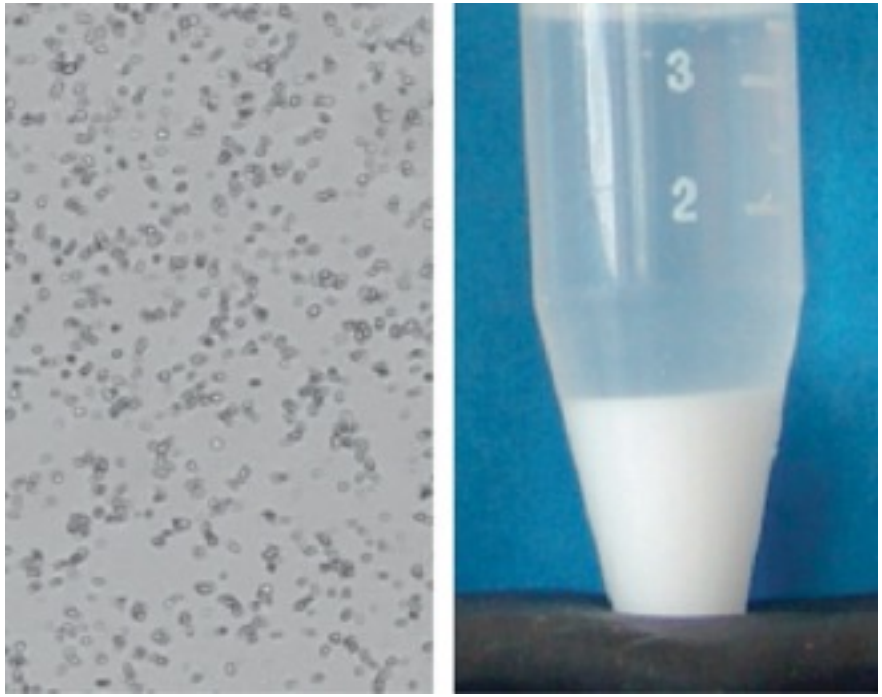
Structure of Photosystem I from XFEL Data



From Petra Fromme and John Spence

Serial Femtosecond Crystallography (SFX)

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.”

Ilme Schlichting MPI Heidelberg

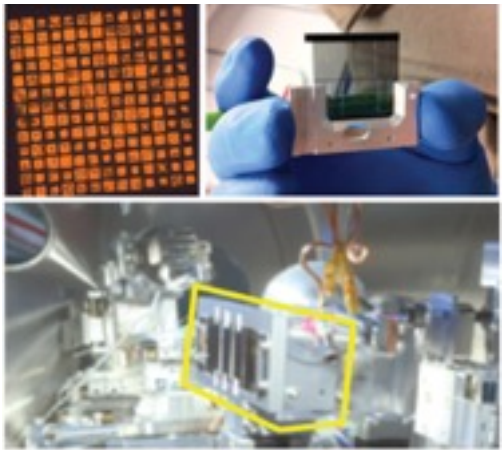
Boutet et al, 2012

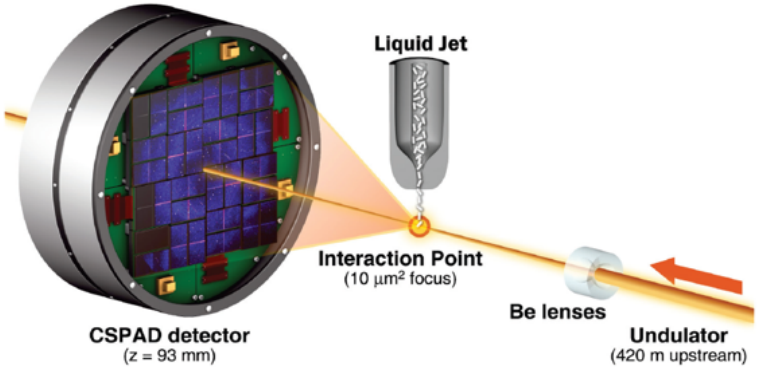
Serial Femtosecond Crystallography (SFX)

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





Femtosecond Nano Crystallography

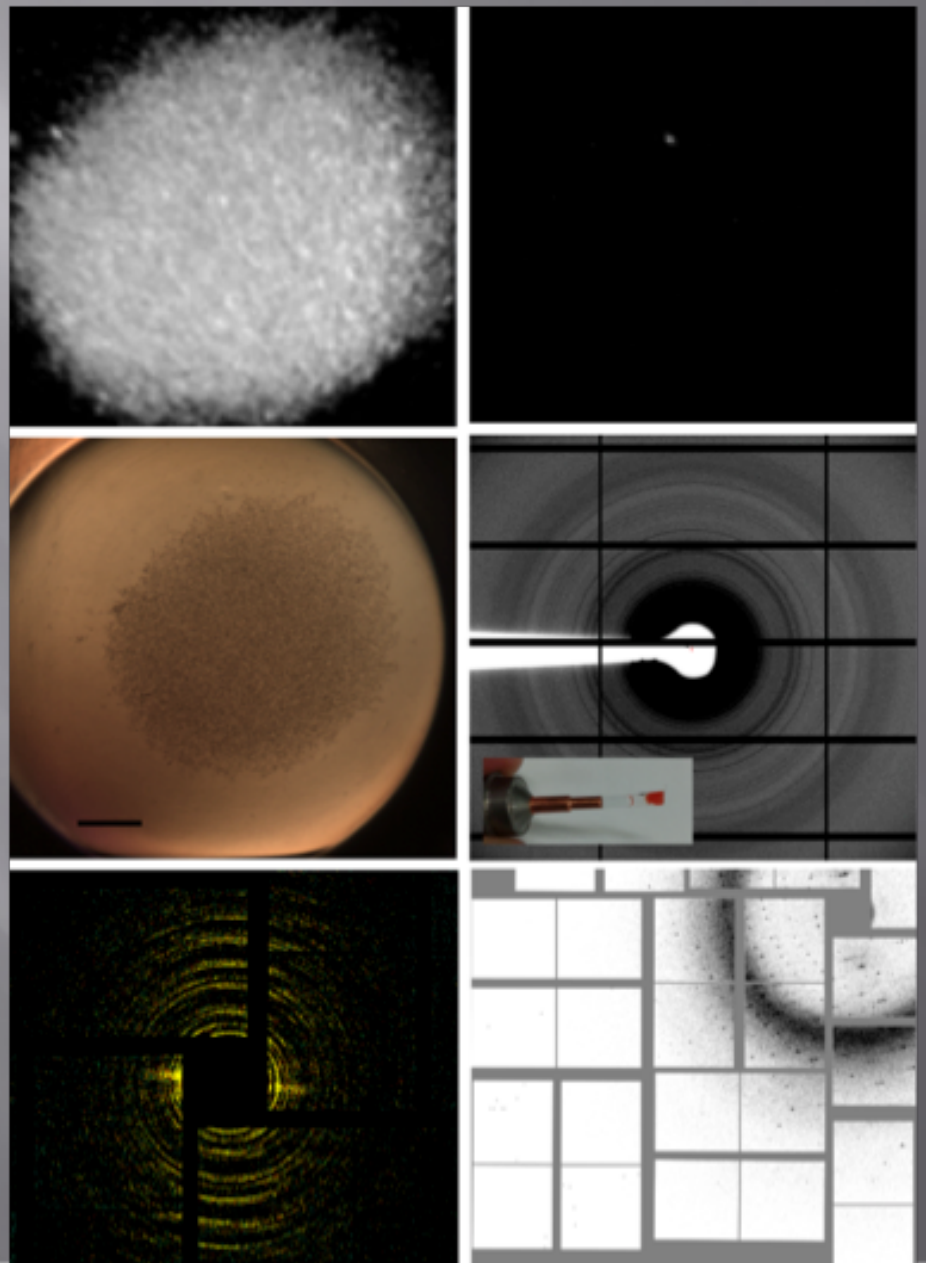
→Fully hydrated nanocrystals gave excellent diffraction patterns at room temperature

→Pulses from 2 to 60 Femtoseconds were useful to outrun radiation damage and no degradation of patterns was detectable when nanocrystals were used

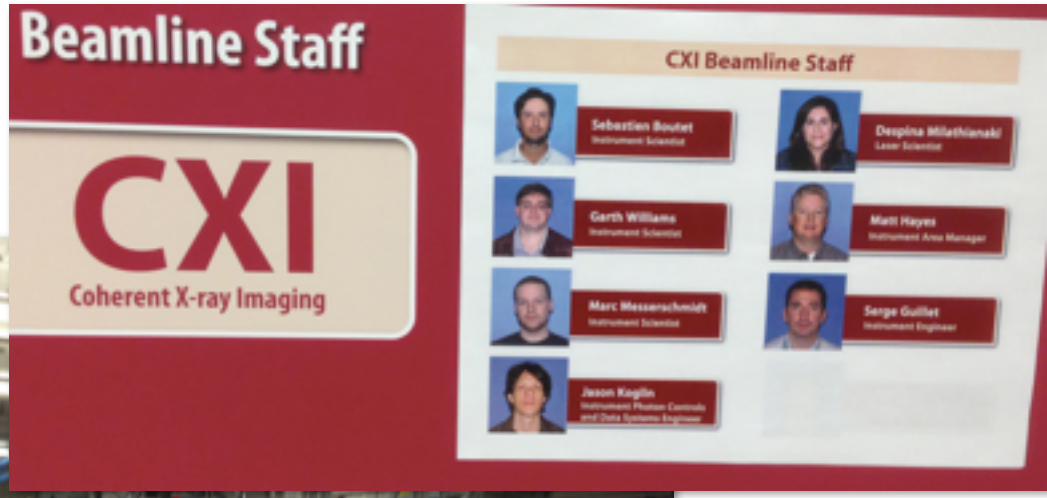
→The method of Femtosecond X-ray crystallography will revolutionize dynamic structural biology

→Difficult but medically very interesting targets like membrane proteins are very well suited for femtosecond nano crystallography

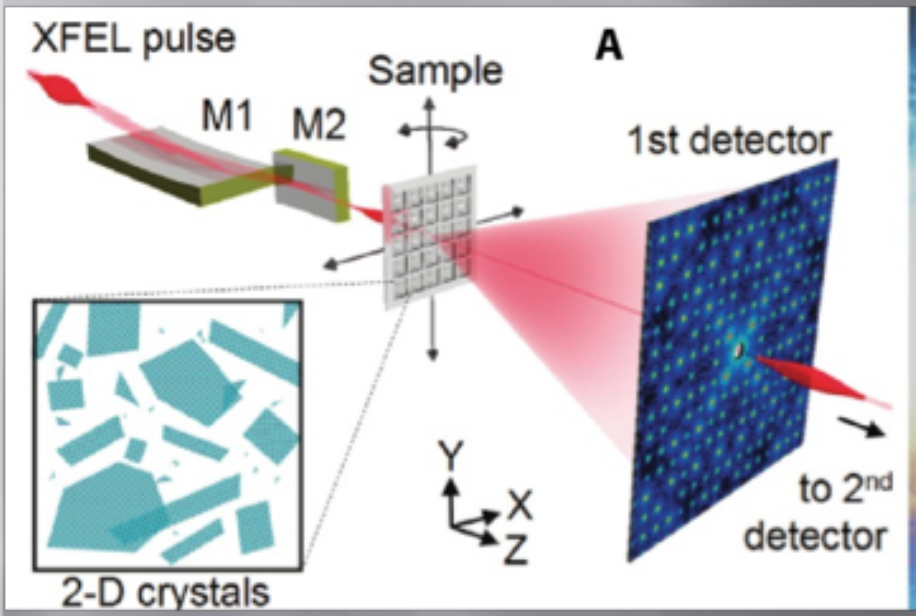
Working with micro and nano crystals

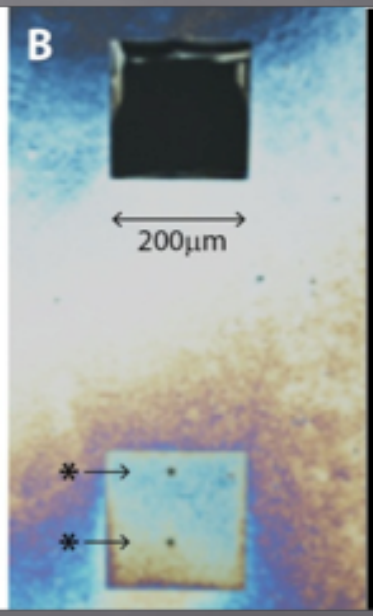


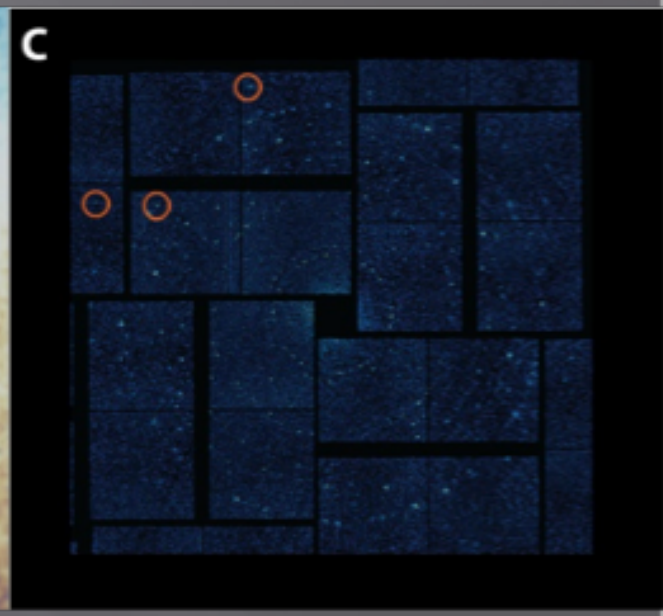
Coherent X-ray Diffraction Imaging: CXI



Diffraction from 2D crystals on a



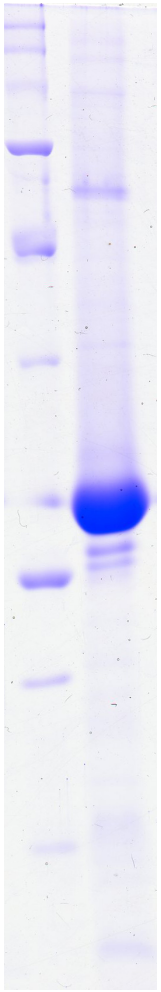




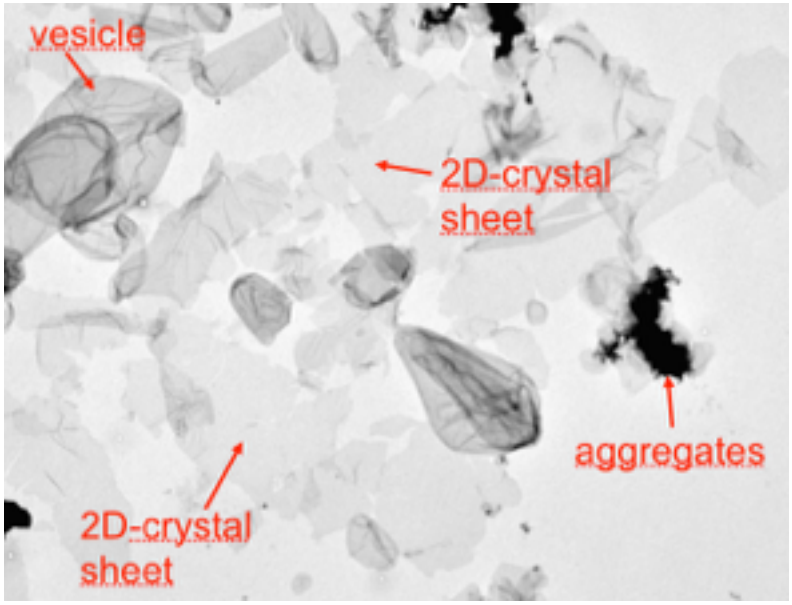
2D crystals of Voltage Gaited Channel

- **3D structure of Channel in 2 Dimensional crystals obtained by cryo-EM**
- Prepared in the Biomolecular Research Laboratory at PSI
- by Ching Ju Tsai with Xiao Dan Li project start 2010

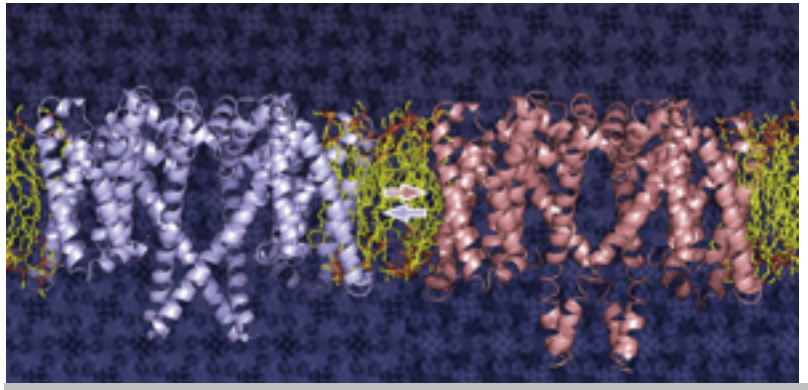
Cryo-EM projection structure of 2D crystal

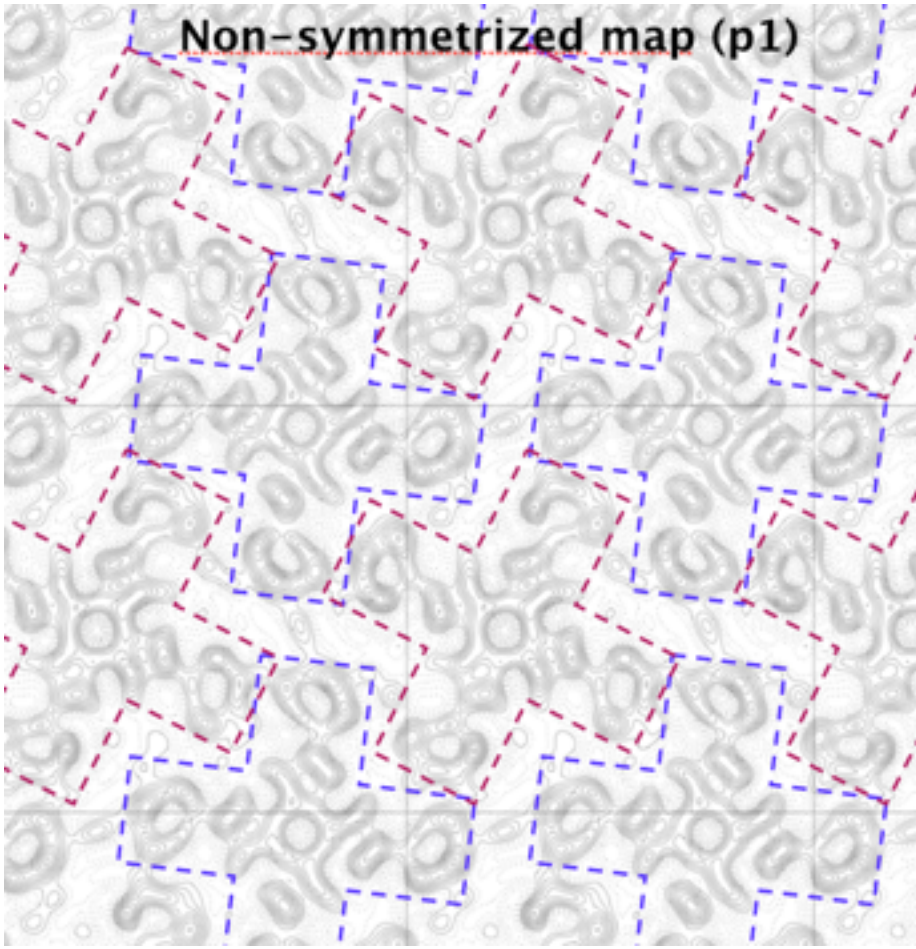


Purified channel



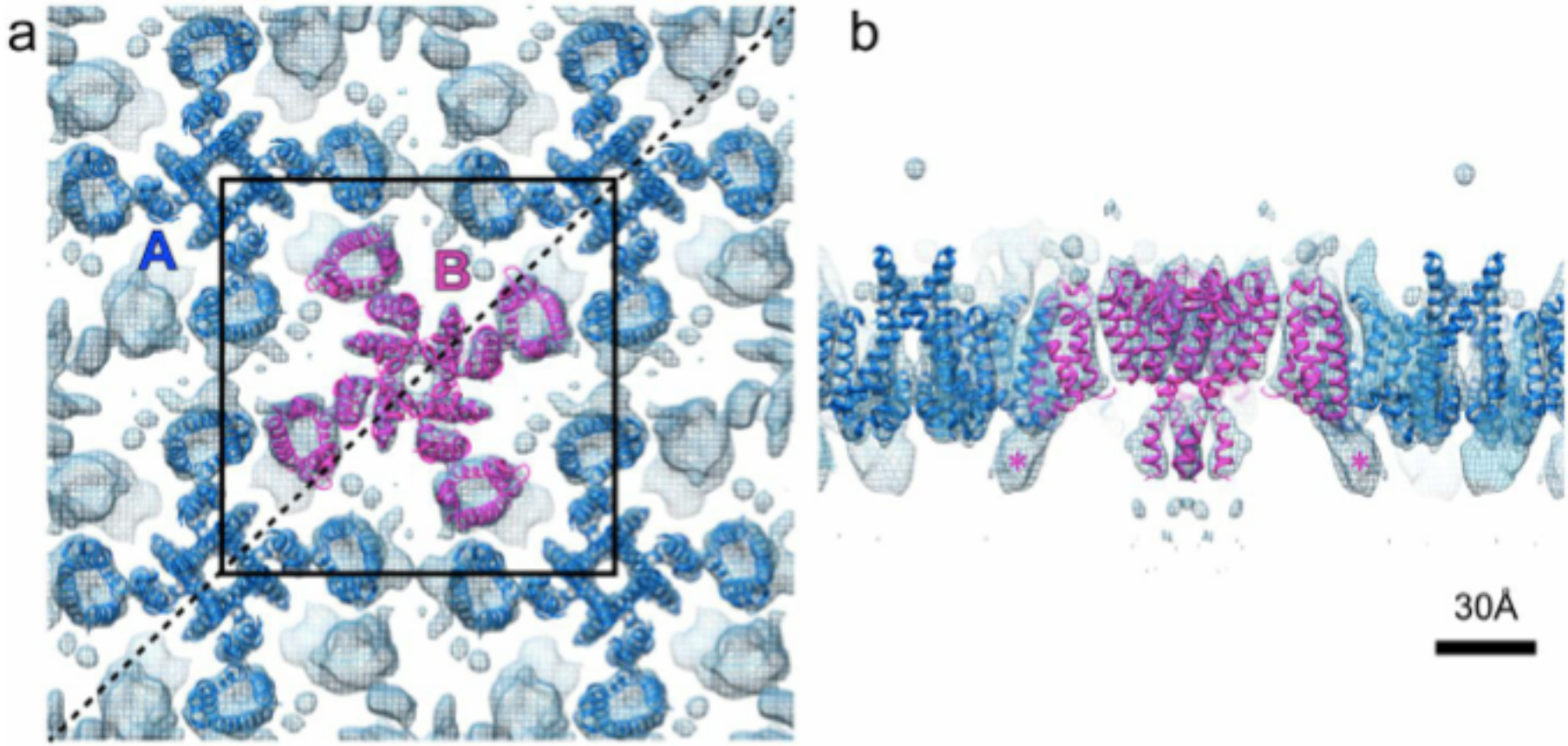
Suspension of 2D crystal on carbon film





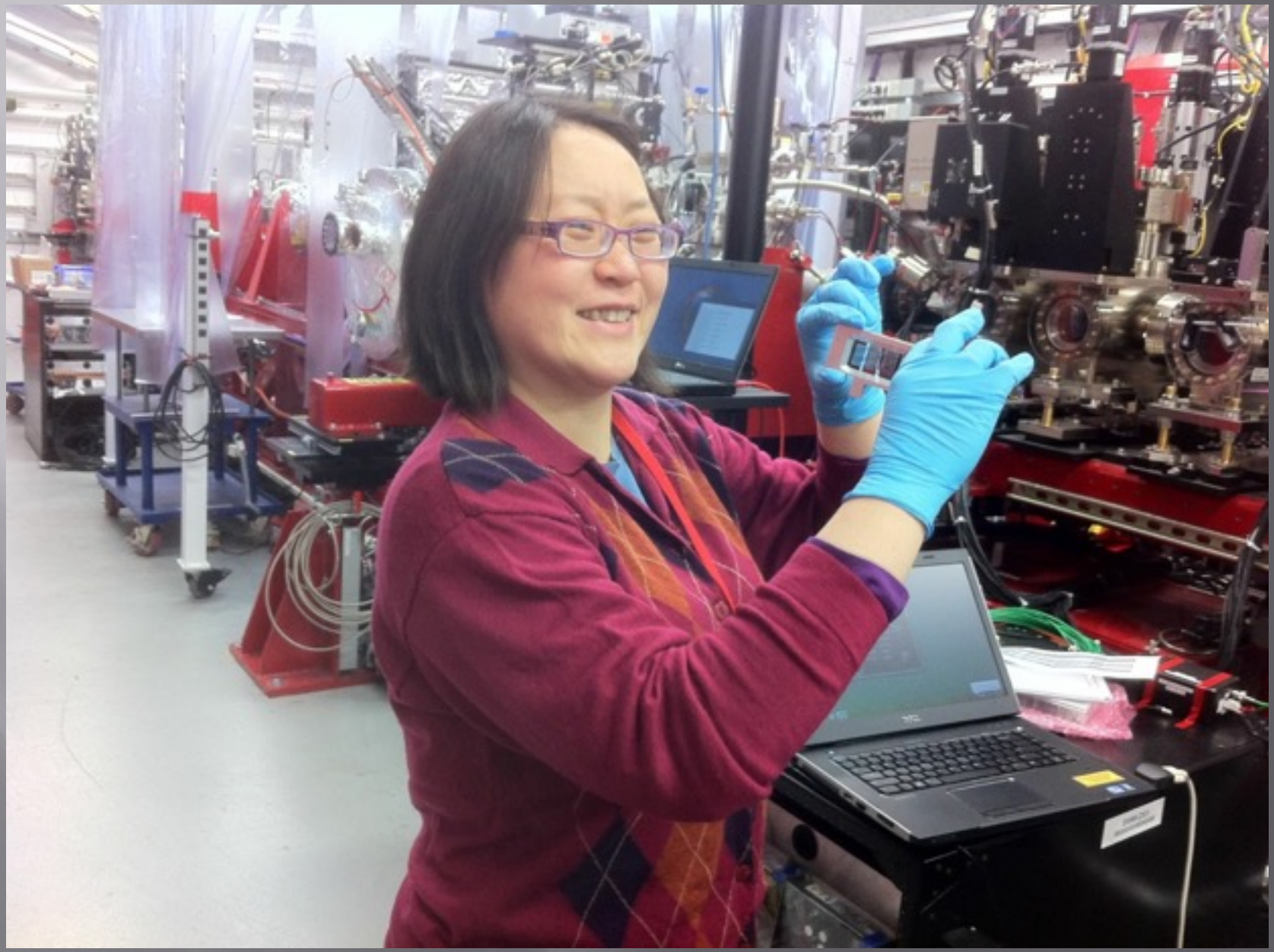
Tsai, C-J, et al, Two Alternative Conformations of a Voltage-Gated Sodium Channel, J Mol Biol (2013),

3D structure of Voltage Gaited Channel

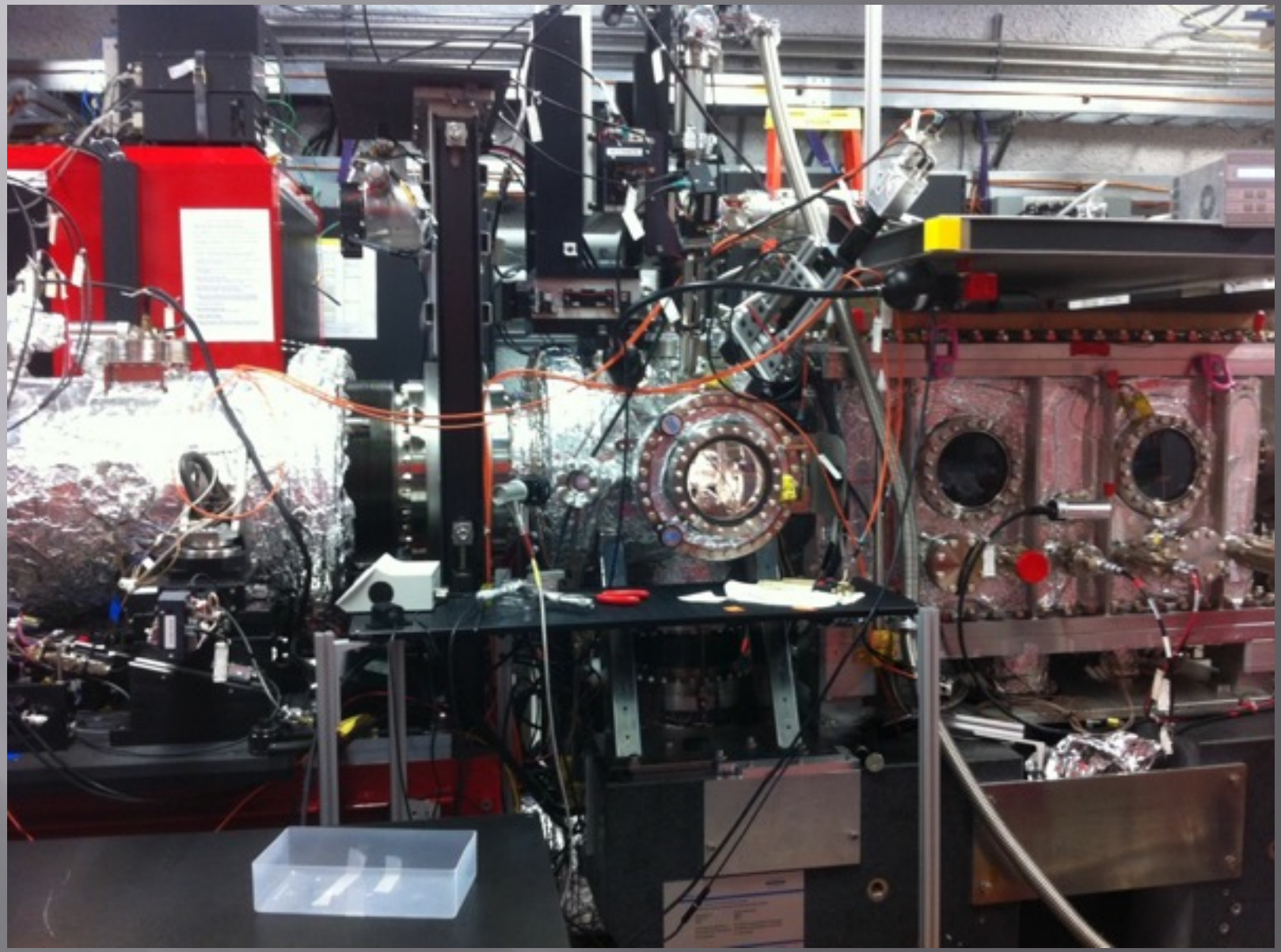


3D Cryo-EM structure from 2D crystal of a channel

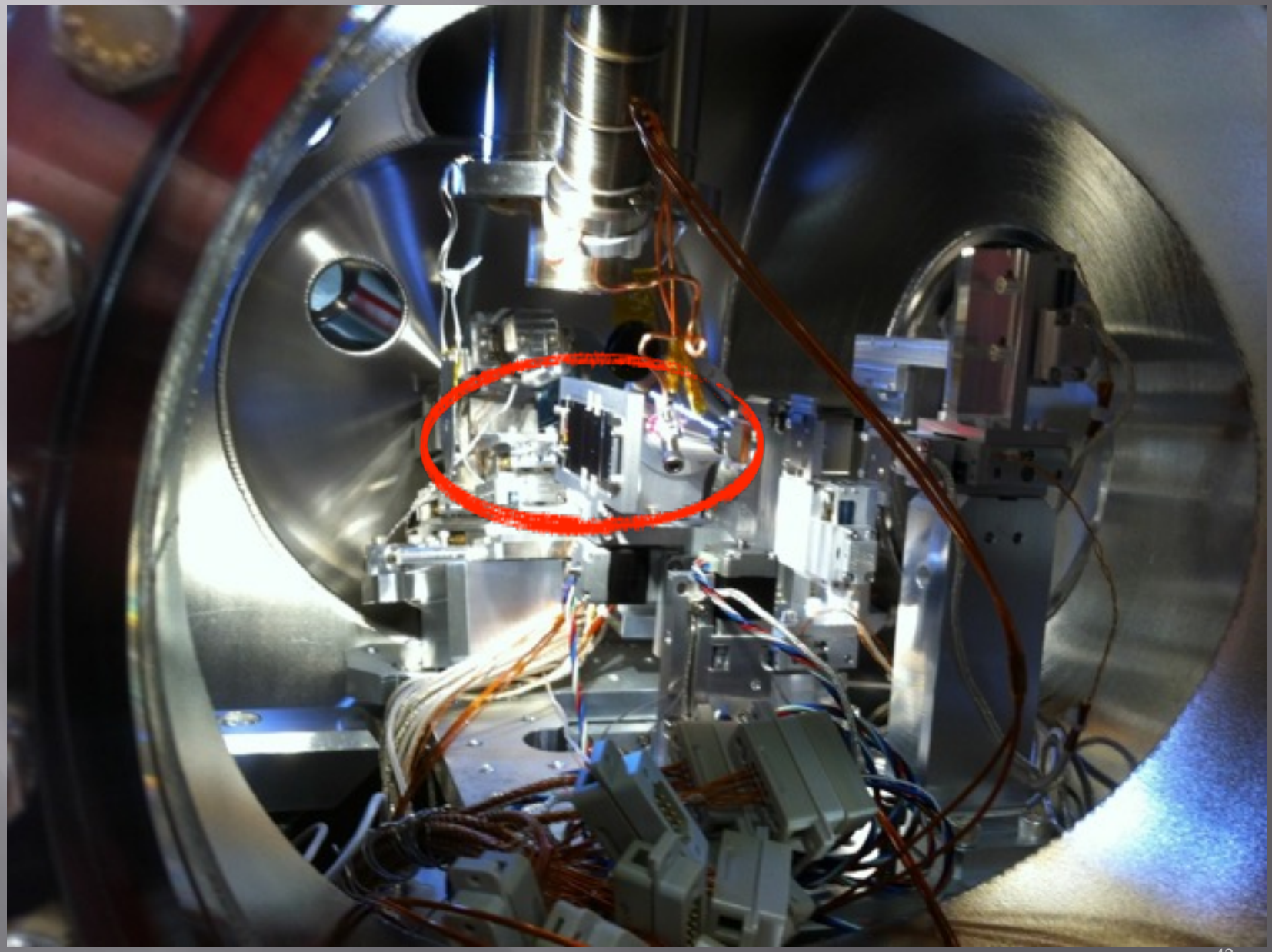
Ching Ju Tsai with solid suppor



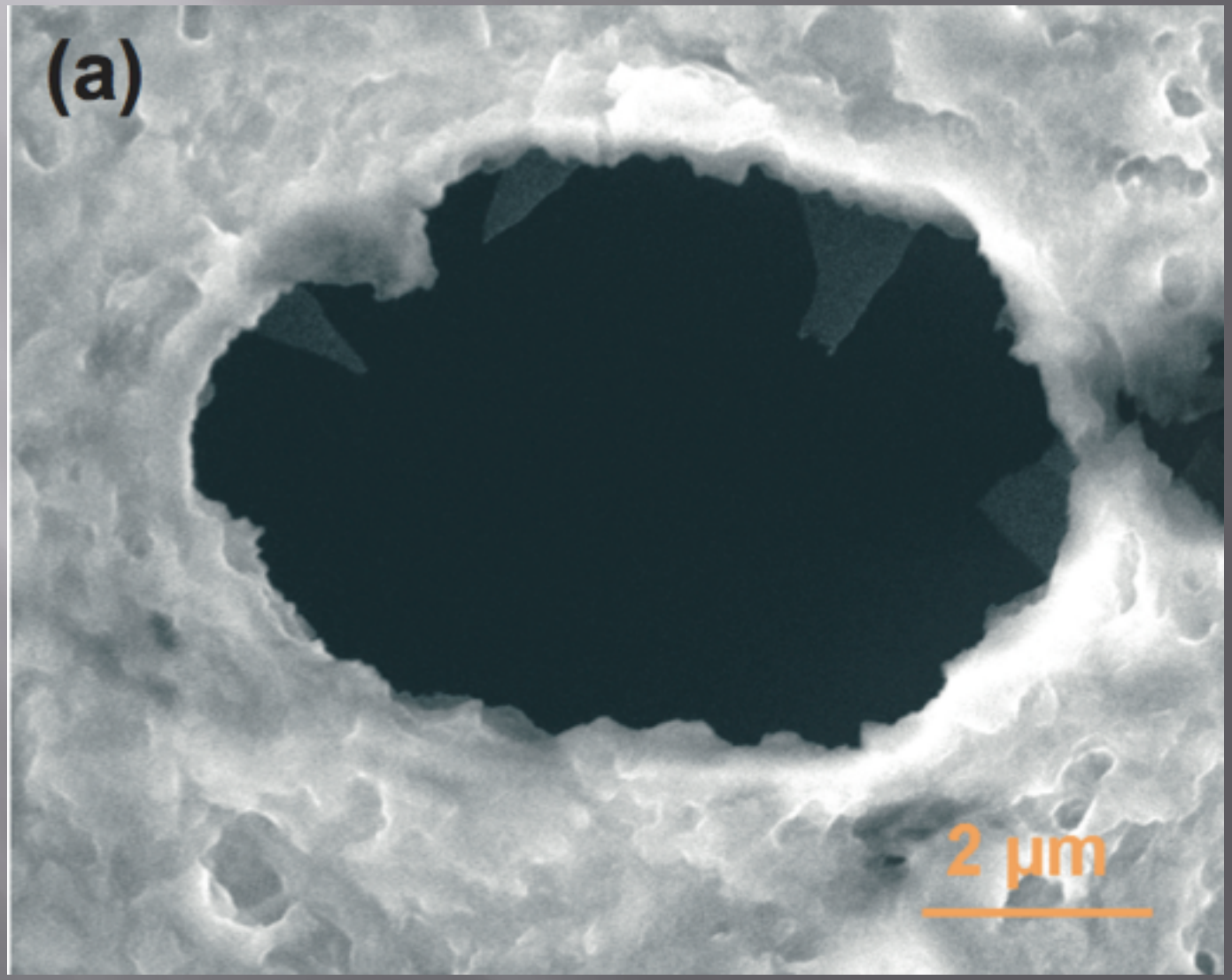
X-ray Free Electron Laser end station



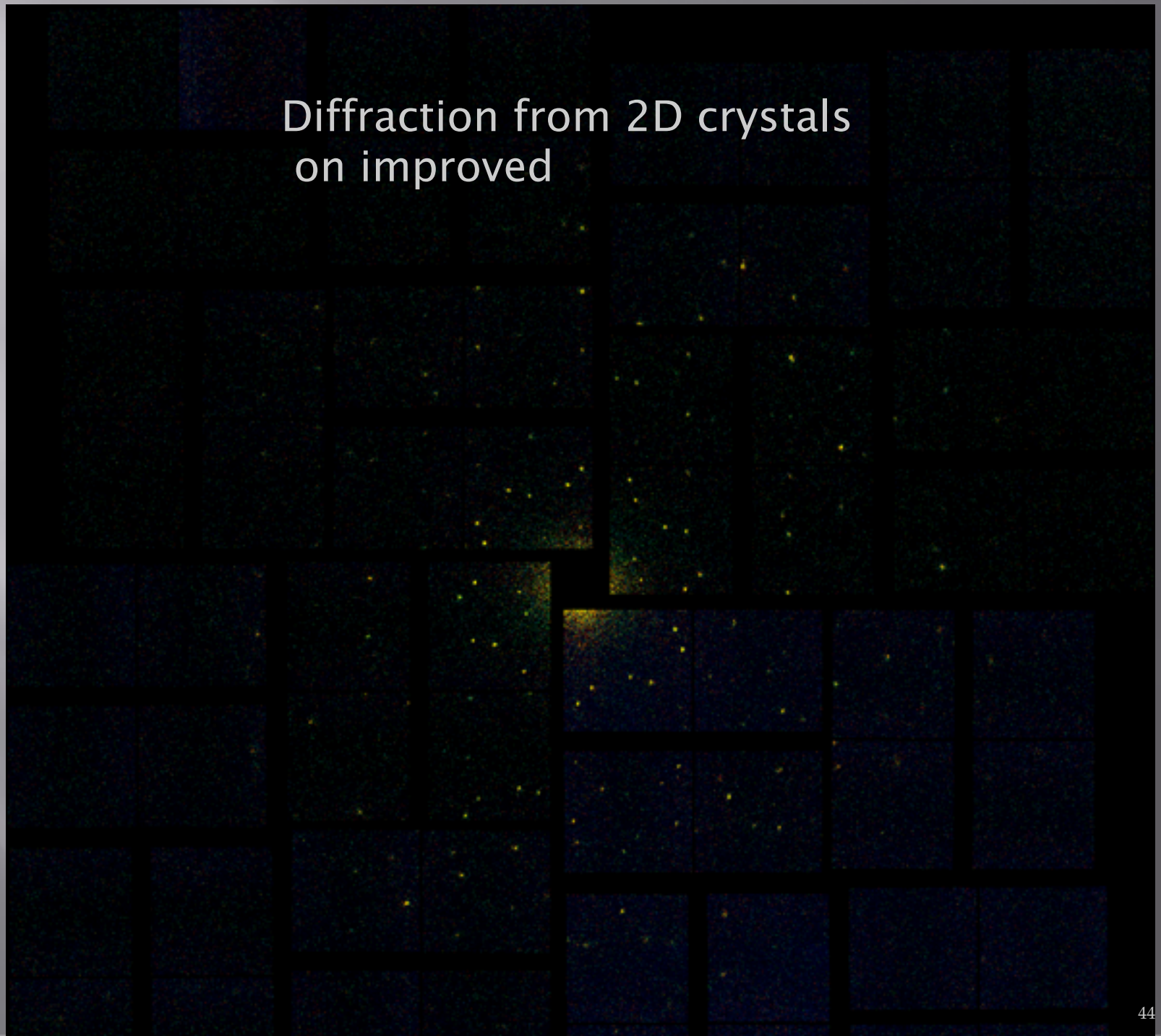
Solid support in vacuum chamber

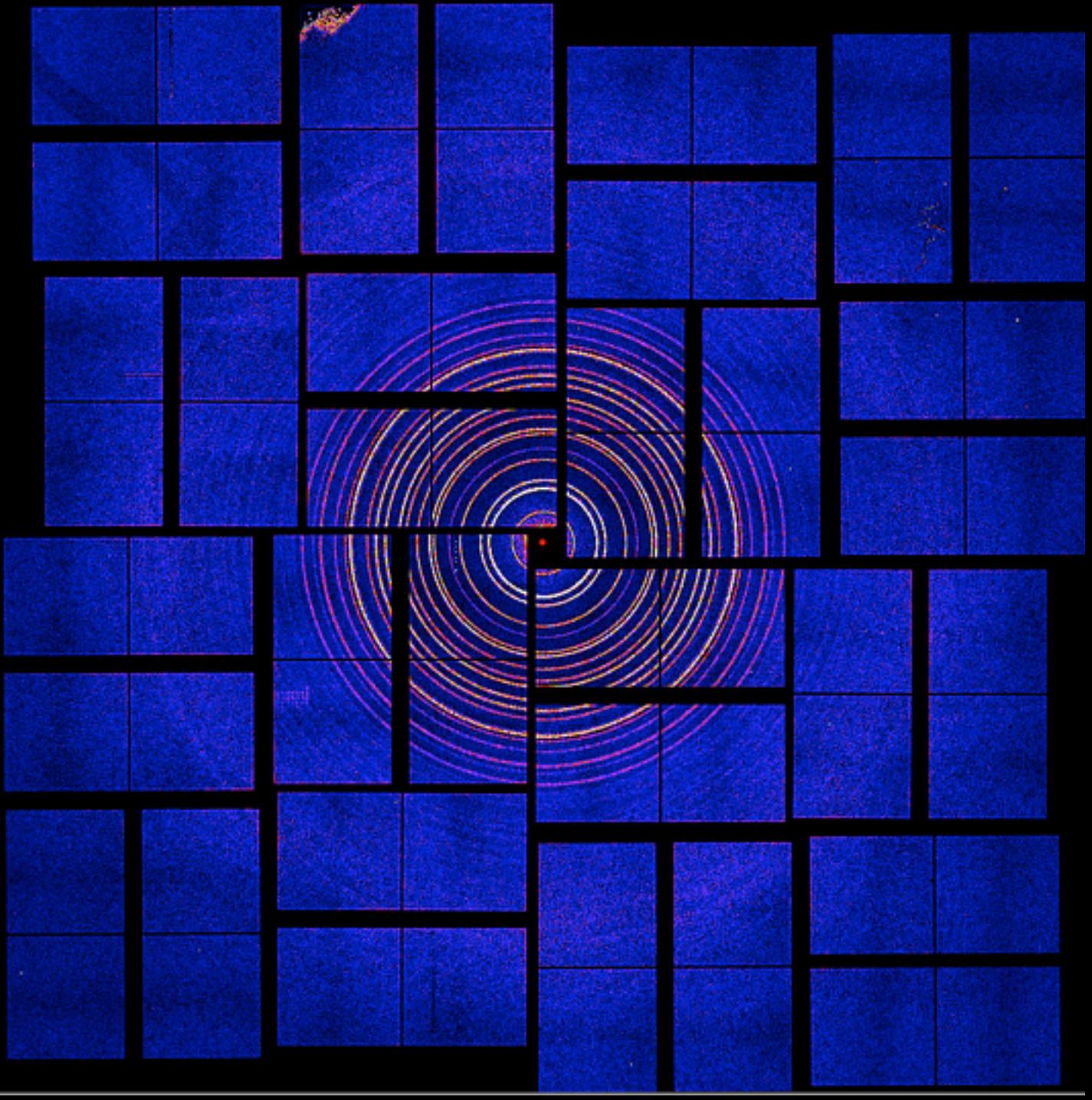


Devastating impact of a X-FEL

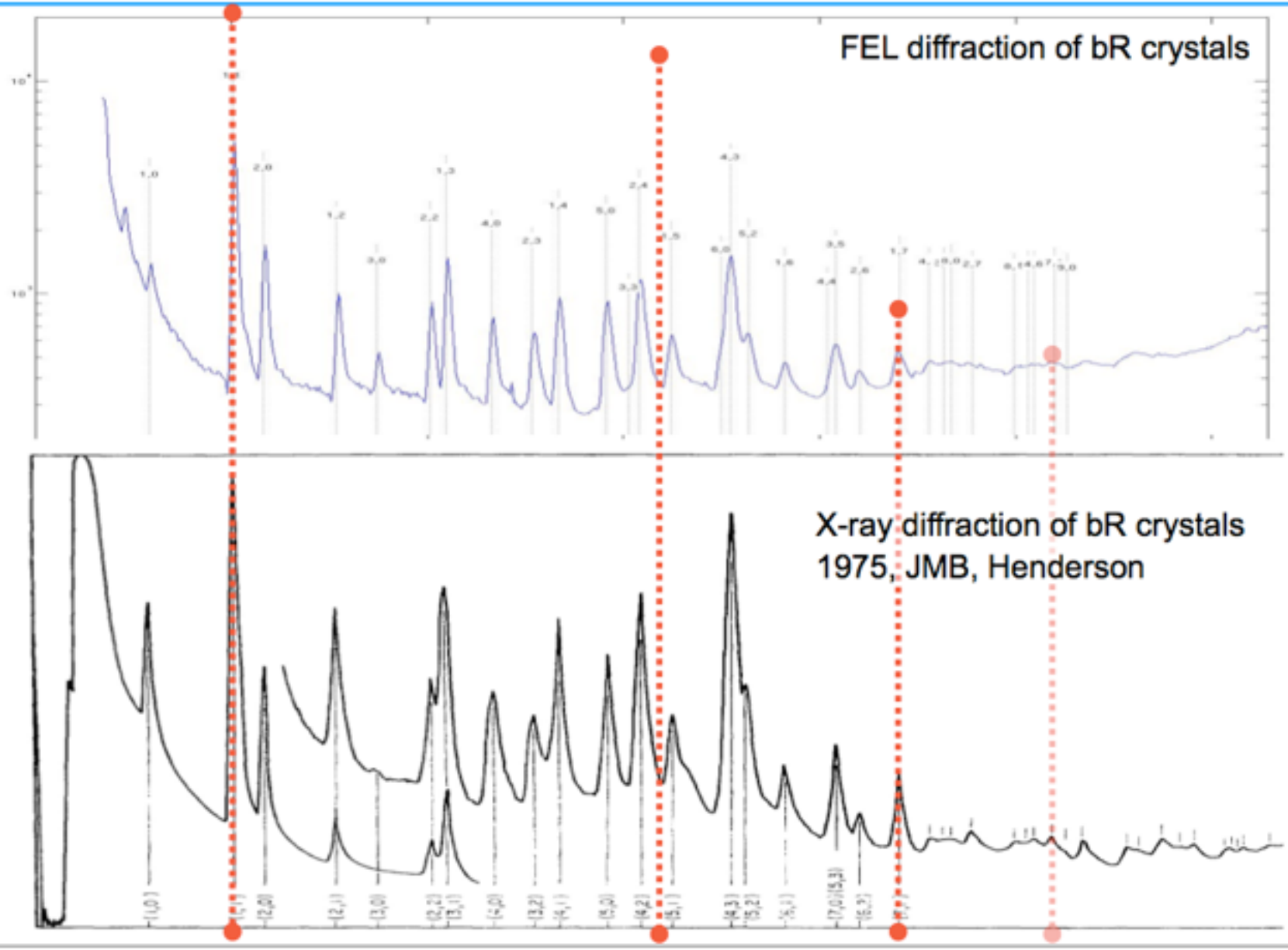


Diffraction from 2D crystals
on improved





Run 0165
Averaged pattern from 104 images


FEL diffraction of bR crystals
X-ray diffraction of bR crystals
1975, JMB, Henderson

First Publikation on D2 Membrane Protein Crystal X-ray diffraction

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Femtosecond X-ray diffraction from two-dimensional protein crystals

Matthias Frank,[a]* David B. Carlson,[b] Mark S. Hunter,[a] Garth J. Williams,[c] Marc Messerschmidt,[c] Nadia A. Zatsepin,[d] Anton Barty,[e] W. Henry Benner,[a] Kaiqin Chu,[f] Alexander T. Graf,[a] Stefan P. Hau-Riege,[a] Richard A. Kirian,[e] Celestino Padeste,[g] Tommaso Pardini,[a] Bill Pedrini,[g] Brent Segelke,[a] M. Marvin Seibert,[c] John C. H. Spence,[d] Ching-Ju Tsai,[g] Stephen M. Lane,[f] Xiao-Dan Li,[g] Gebhard Schertler,[g] Sebastien Boutet,[c] Matthew Coleman[a] and James E. Evans[h,b]*

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X-ray diffraction patterns from two-dimensional (2-D) protein crystals obtained using femtosecond X-ray pulses from an X-ray free-electron laser (XFEL) are presented. To date, it has not been possible to acquire transmission X-ray diffraction patterns from individual 2-D protein crystals due to radiation damage. However, the intense and ultrafast pulses generated by an XFEL permit a new method of collecting diffraction data before the sample is destroyed. Utilizing a diffract-before-destroy approach at the Linac Coherent Light Source, Bragg diffraction was acquired to better than 8.5 Å resolution for two different 2-D protein crystal samples each less than 10 nm thick and maintained at room temperature. These proof-of-principle results show promise for structural analysis of both soluble and membrane proteins arranged as 2-D crystals without requiring cryogenic conditions or the formation of three-dimensional crystals.

First Publikation on D2 Membrane Protein Crystal X-ray diffraction

Submitted to Phil. Trans. R. Soc. B - Issue

7 Å resolution in protein 2D-crystal X-ray diffraction at LCLS

Bill Pedrini*[1], Ching-Ju Tsai[1], Guido Capitani[1], Celestino Padeste[1], Mark S. Hunter[2], Nadia A. Zatsepin[4], Anton Barty[5], W. Henry Benner[2], Sébastien Boutet[6], Geoffrey K. Feld[2], Stefan P. Hau-Riege[2], Richard A. Kirian[5], Christopher Kupitz[4], Marc Messerschmitt[6], John I. Ogren[7], Tommaso Pardini[2], Brent Segelke[2], Garth J. Williams[6], John C. H. Spence[4], Rafael Abela[1], Matthew Coleman[2], James E. Evans[3], Gebhard Schertler[1], Matthias Frank†[2], and Xiao-Dan Li‡[1]

[1]Paul Scherrer Institute, 5232 Villigen PSI, Switzerland
[2]Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA, 94550, USA
[3]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, 3335 Innovation Blvd., Richland, WA, 99354, USA
[4]Arizona State University, 300 E. University Dr., Tempe, AZ, 85287, USA
[5]Center for Free-Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany
[6]Linac Coherent Light Source, 2575 Sand Hill Road, Menlo Park, CA, 94025, USA
[7]Physiscs Departement, Boston University, 590 Commonwealth Ave, Boston, MA, 02215, USA

January 22, 2014

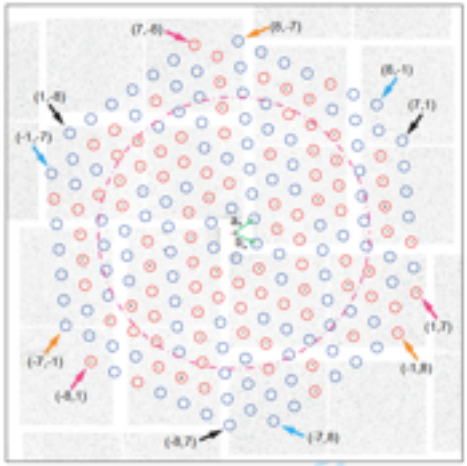


Figure 1: Example of a diffraction image from a single bR 2D crystal. The dashed ring corresponds to 10 Å resolution. The circles mark the positions of expected diffraction peaks at lower than 7 Å resolution. The precise orientation of the peak lattice was derived from the positions of the prominent, easily identifiable peaks encircled in red. The basis vectors (a_r, b_r) of the 2D reciprocal space lattice are shown as green arrows. The small arrows mark the position of peaks in the classes ((7, 1)) (black), ((1, 7)) (magenta), ((−7, −1)) (orange) and ((−1, −7)) (cyan), each class consisting of three equivalent peaks.

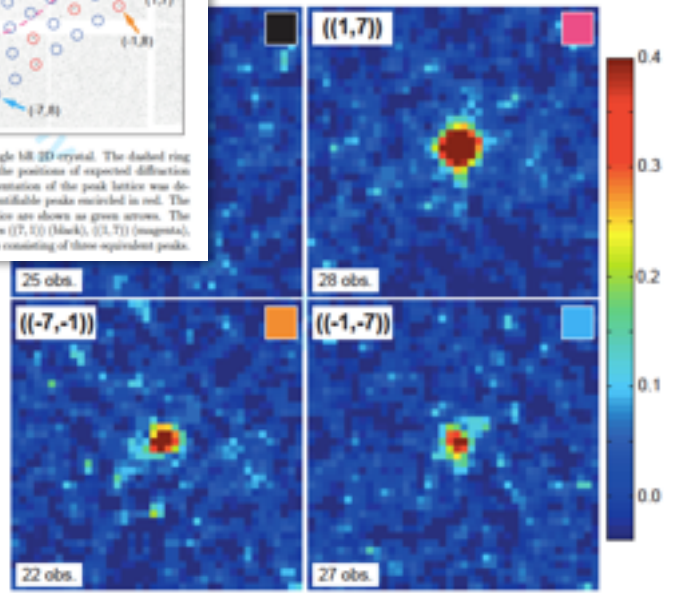
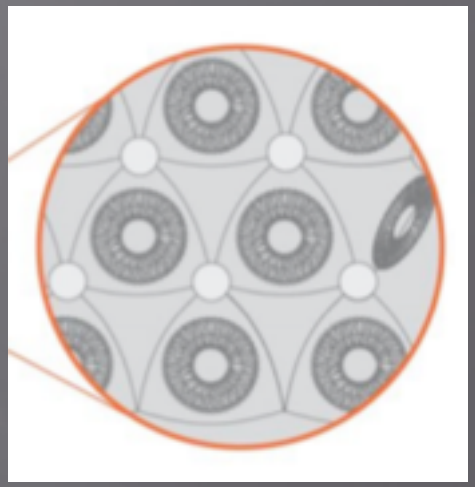


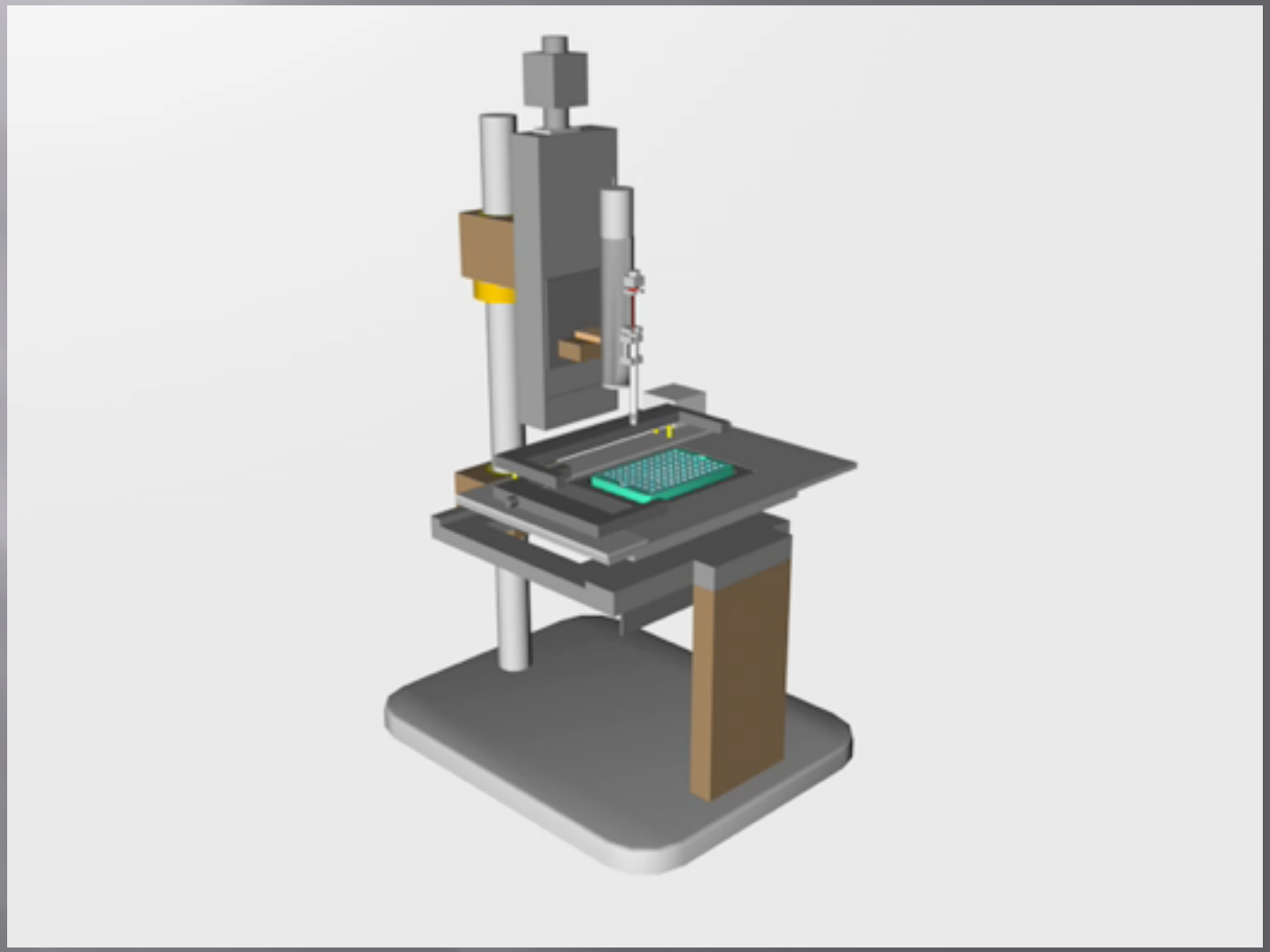
Figure 3: Examples of "image sector sums" (see text) for the four peak classes ((7, 1)), ((−7, −1)), ((1, 7)) and ((−1, −7)), all at 7.2 Å resolution. For each peak, the number of observations is indicated, and the color in the small box at the top right of each panel corresponds to that of the arrows in Figure 1. The intensity color scale is the same for all four panels. Maximum intensity is about 40 times the background noise, calculated as the average on all image sector sums of the local noise level measured away from the central peak region.

Lipidic Cubic Phase Crystallization

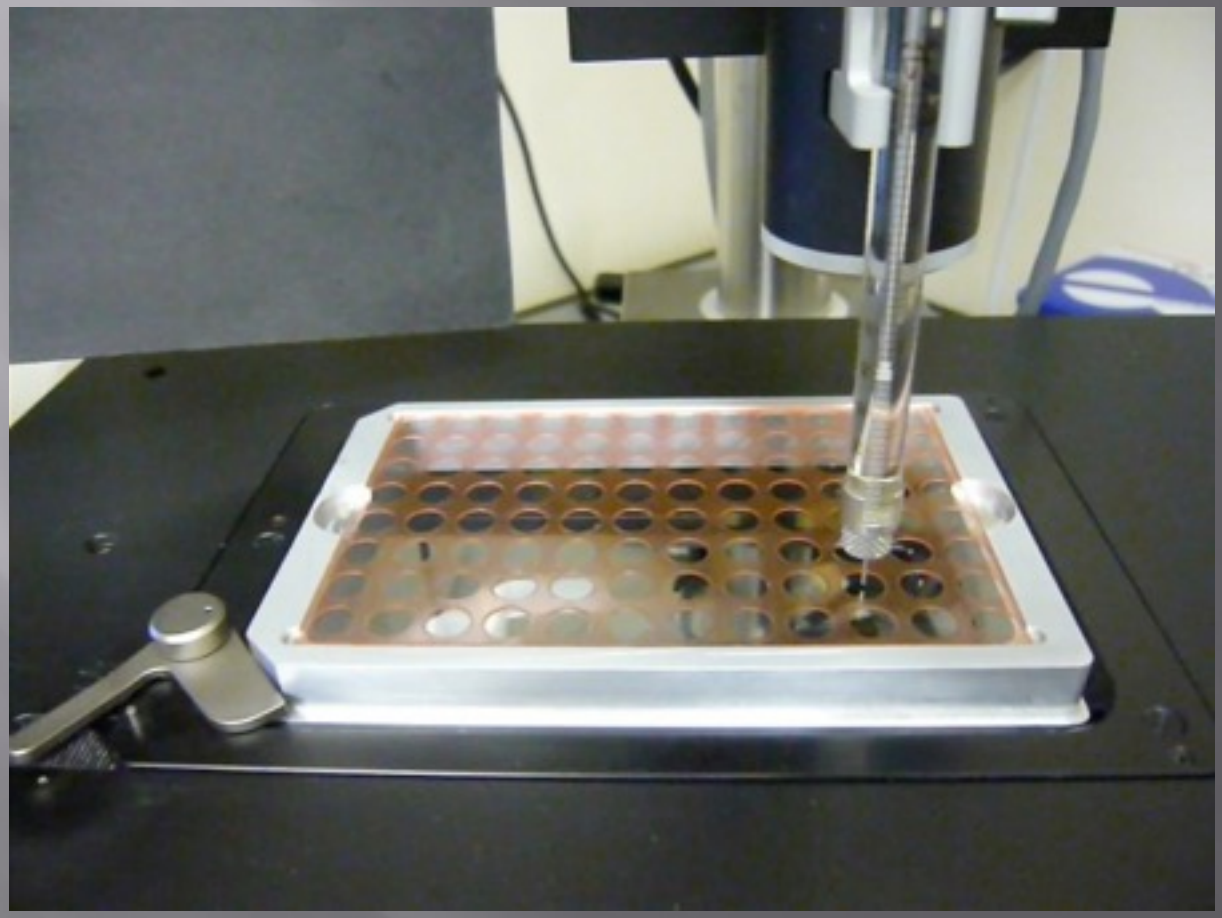


- Our membrane protein structures have often moderate resolution
- Lipidic Cubic Phase (LCP) crystallization produces often membrane protein crystals with Type I packing of molecules
- The new crystal forms can show increased order
- This way can get better resolution membrane protein structures
- Still a number of technical issues in practice

MRC Robotic Cubic Lipidic Phase Dispendser



100nL dispense on UV transparent 96 well plate



TTP LabTech launches dedicated instrument for lipidic cubic phase screening

TTP LabTech are pleased to announce the launch of mosquito® LCP. Developed in collaboration with senior researchers from the MRC, UK (Gebhard Schertler and Pat Edwards), this instrument facilitates the automation and increased throughput of lipidic cubic phase (LCP) crystallisation set-ups. This novel automation system enables LCP screening to be performed accurately and with ease.

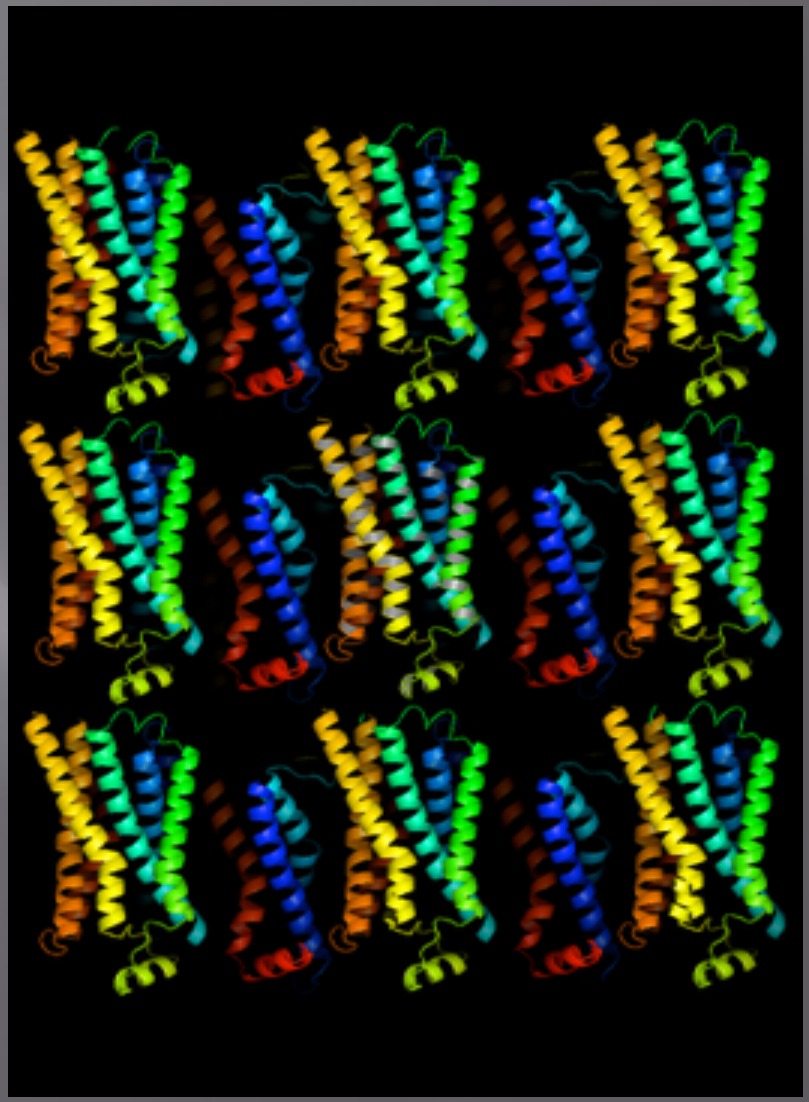
The LCP technique for crystallising membrane proteins can be difficult and time-consuming to set up by hand as it utilises highly viscous lipid mesophases to reconstitute proteins. TTP LabTech has overcome these problems with mosquito® LCP, a dedicated pipetting instrument for automated LCP screening set-up. This new product boasts the full functionality of the renowned mosquito product, whilst incorporating technical innovations specific for LCP techniques.

Using a positive displacement syringe with automated tip positioning, mosquito LCP provides accurate and repeatable dispensing of the LCP drops. The precise positioning of the LCP material also facilitates automated imaging of membrane protein crystals in a range of high density plate types. Mosquito® LCP provides significant benefits over manual processes due to the use of its unique disposable tip technology. For the precipitant additions step, this not only guarantees zero cross-contamination, but negates the need for time-consuming tip washing. Subsequently, high throughput rates of more than eight 96- well plates per hour are easily achieved and evaporation of the dispensed LCP is minimised.

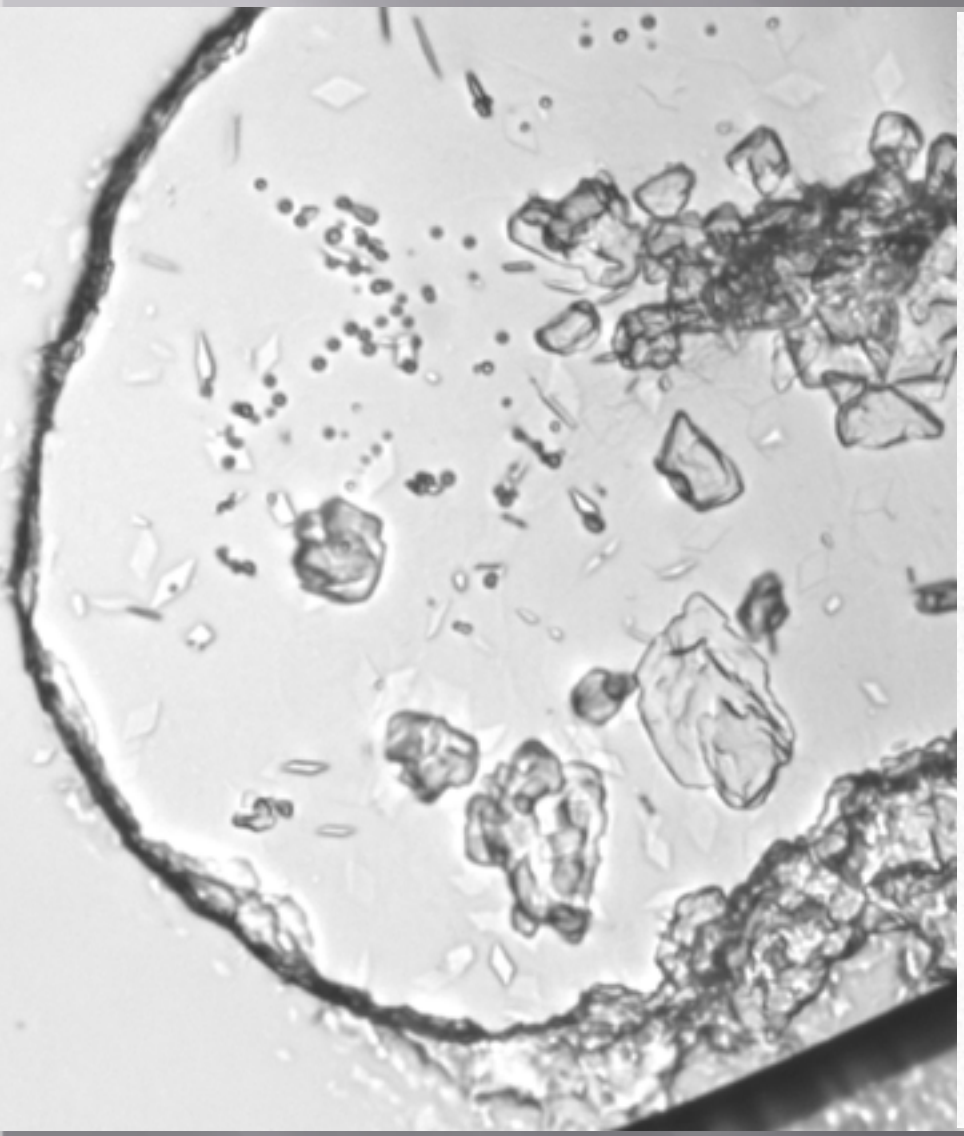
'The mosquito® is already being used extensively for automated, high throughput protein crystallisation in sitting drop, hanging drop and micro batch applications,' commented Gebhard Schertler from the MRC 'This new instrument now extends the applications of the mosquito® to the LCP technique. The mosquito® LCP will be an invaluable addition to any membrane protein crystallisation laboratory.'

www.ttplabtech.com

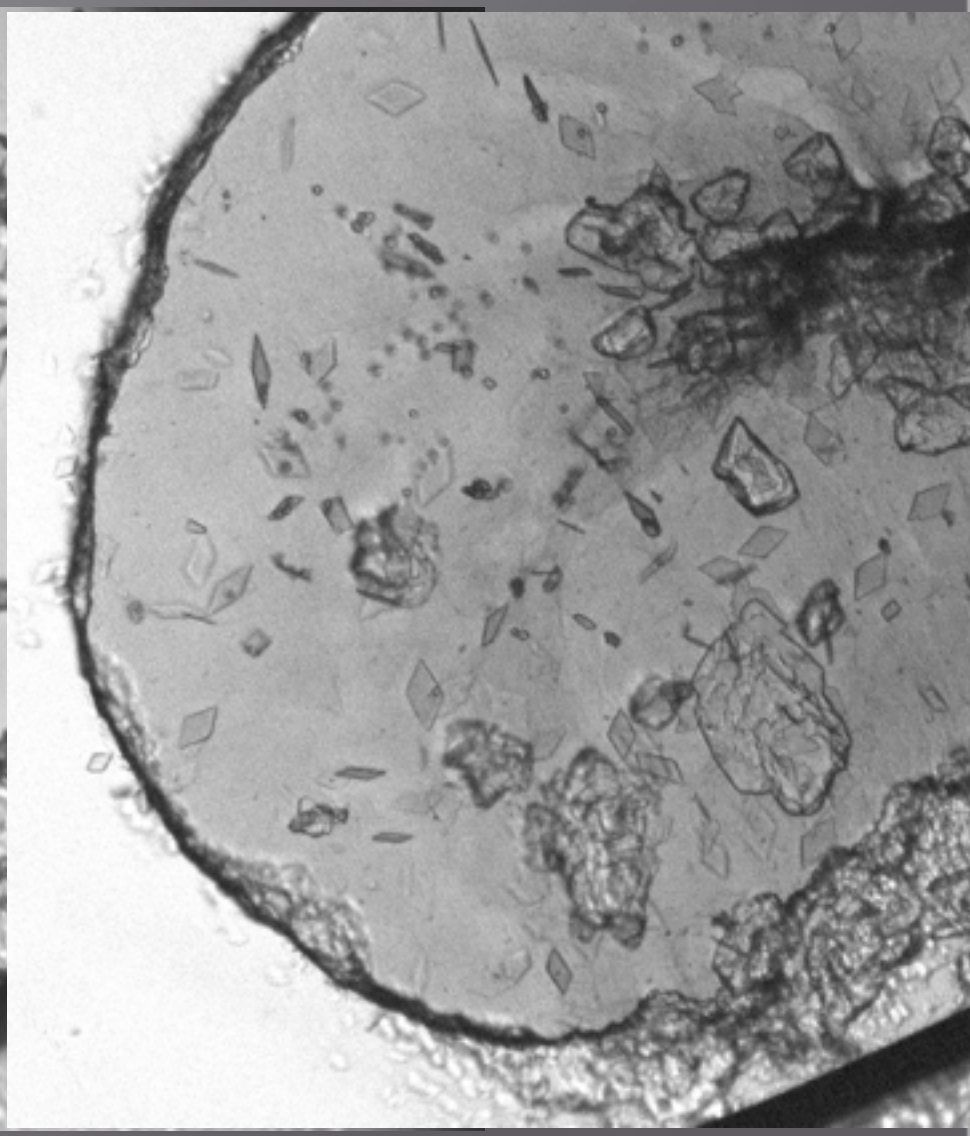
european pharmaceutical review digital



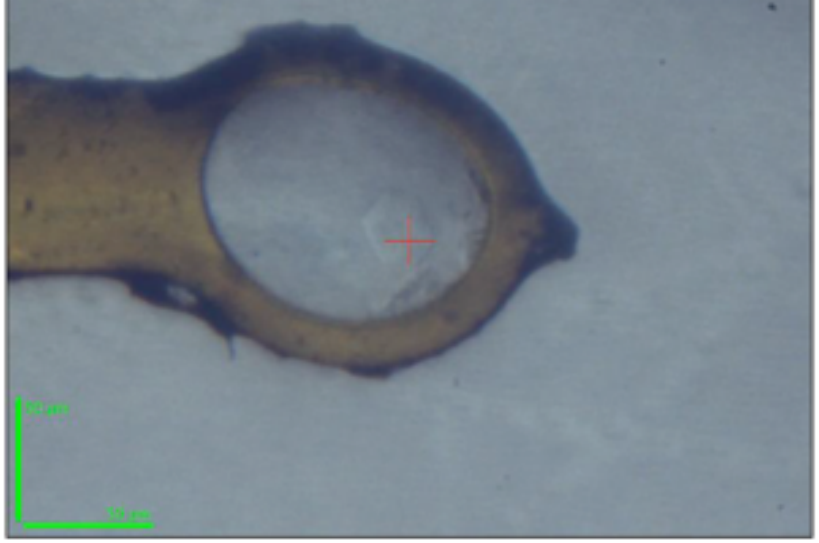
LCP crystals of stabilised beta 1 adrenergic receptor

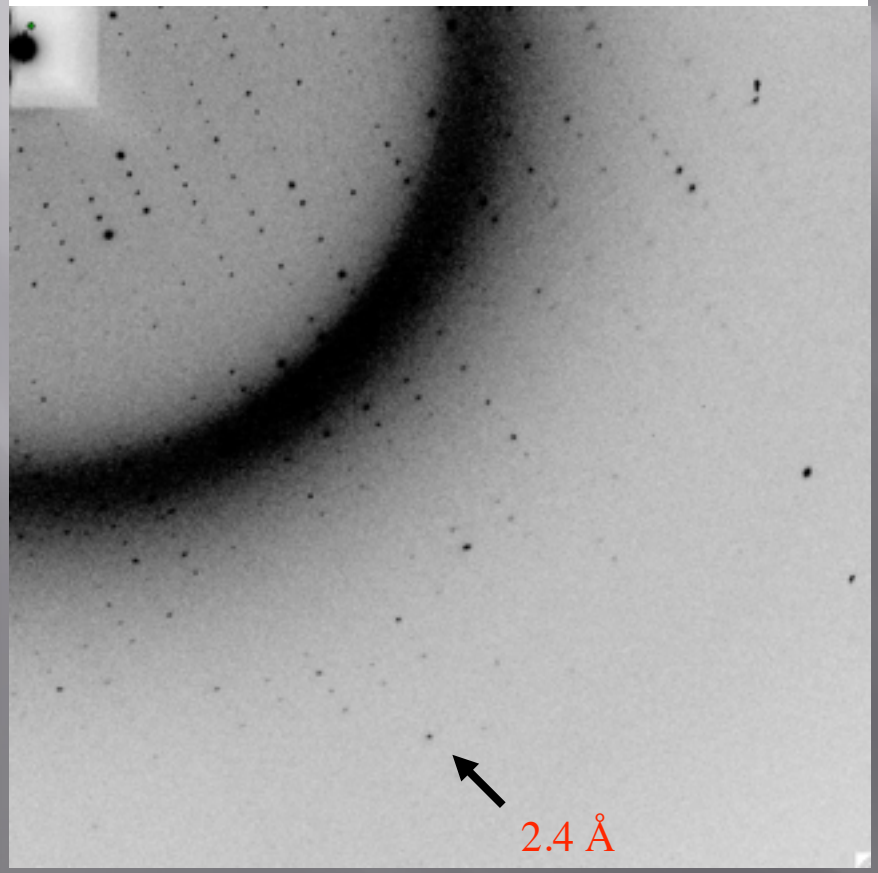


Visible light

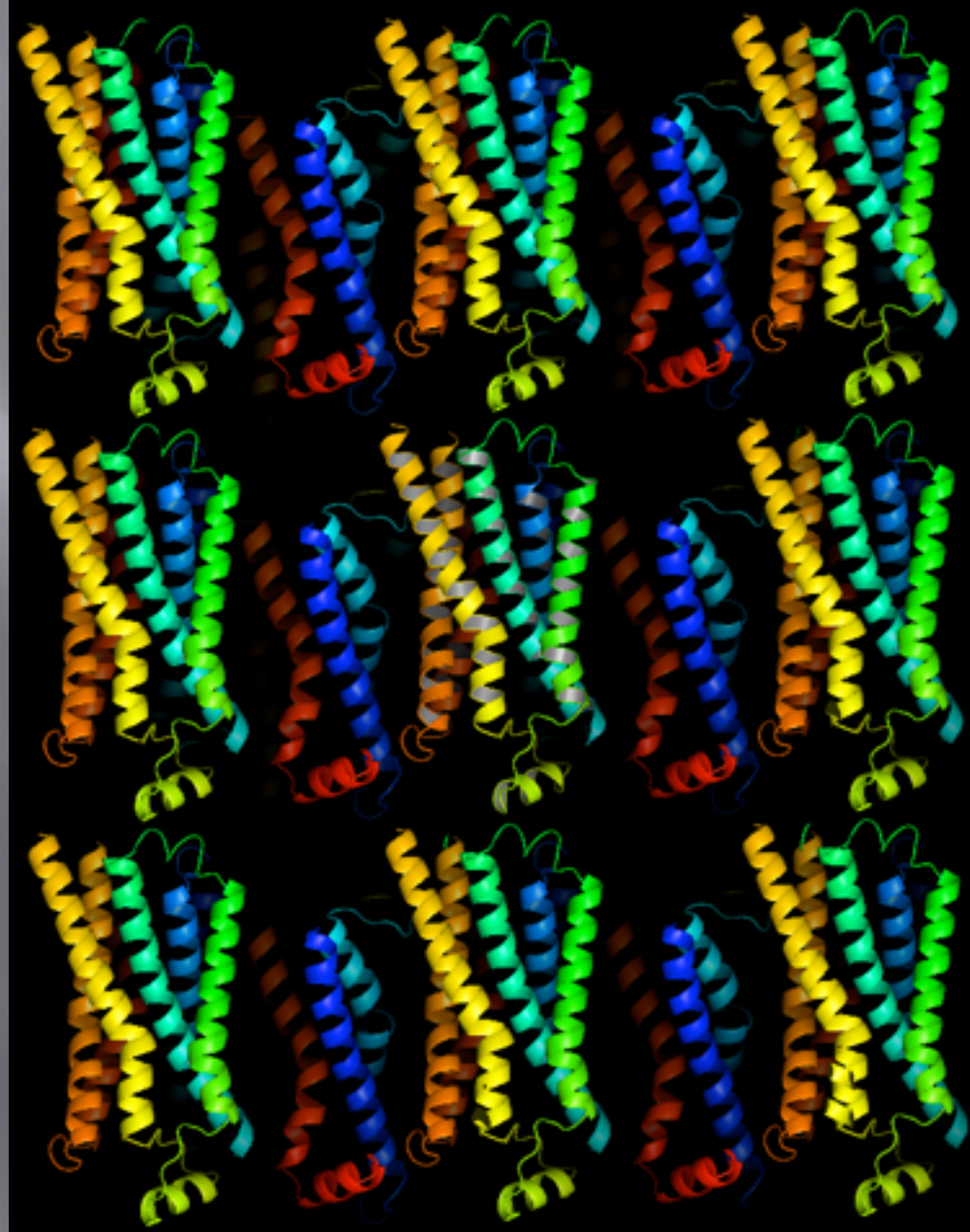


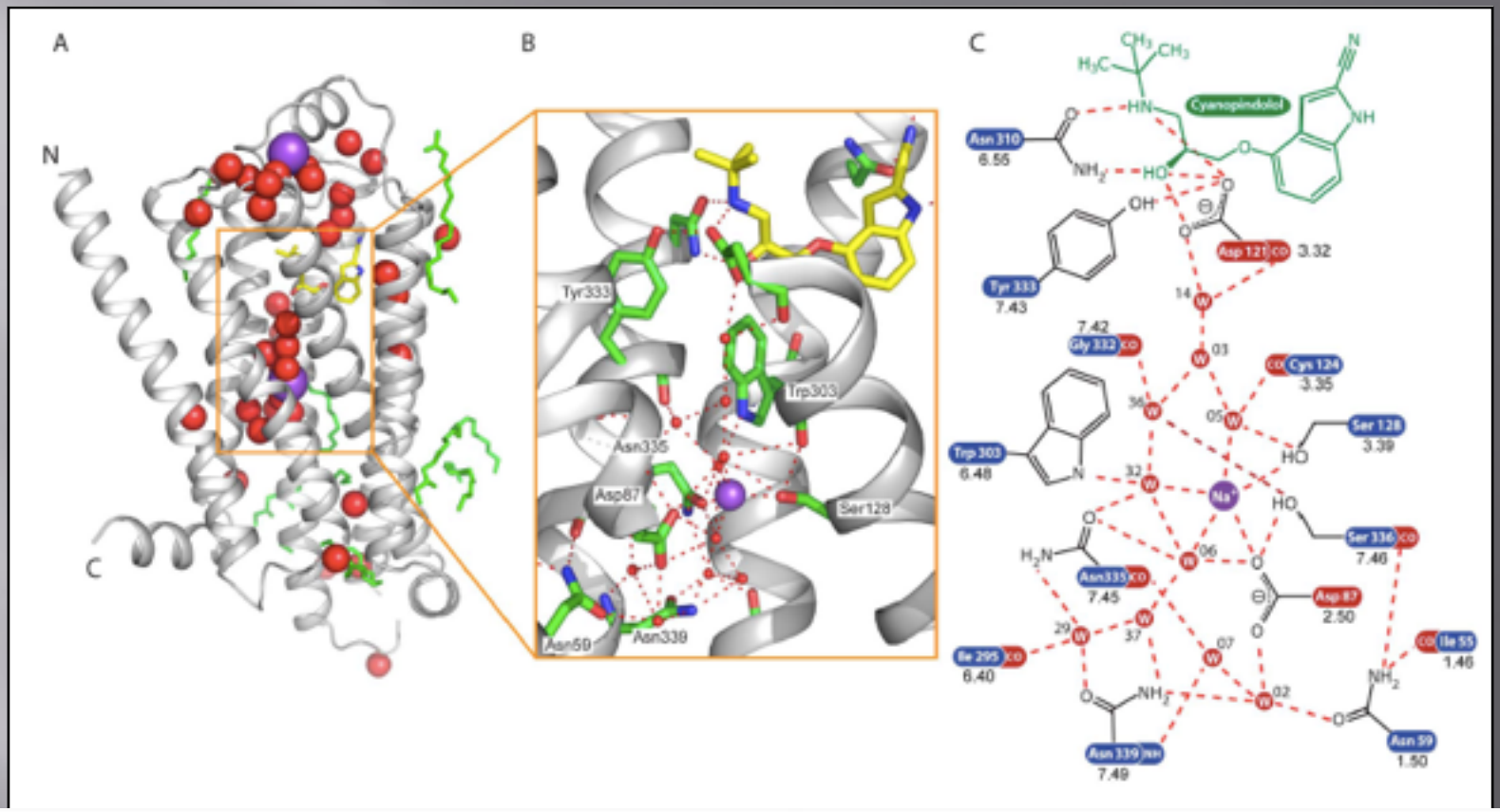
UV image 280 nm





Stabilisd beta 1 adrenergic receptor
Type I membrane protein crystal packing





The 2.1 Å Resolution Structure of Cyanopindolol-Bound β_1-Adrenoceptor Identifies an Intramembrane Na^+ Ion that Stabilises the Ligand-Free Receptor

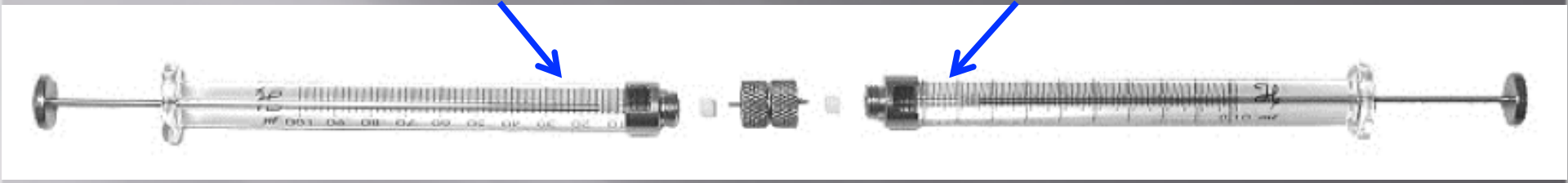
Jennifer L. Miller-Gallacher[¤a], Rony Nehmé[¤], Tony Warne, Patricia C. Edwards, Gebhard F. X. Schertler[¤b¤c], Andrew G. W. Leslie, Christopher G. Tate*

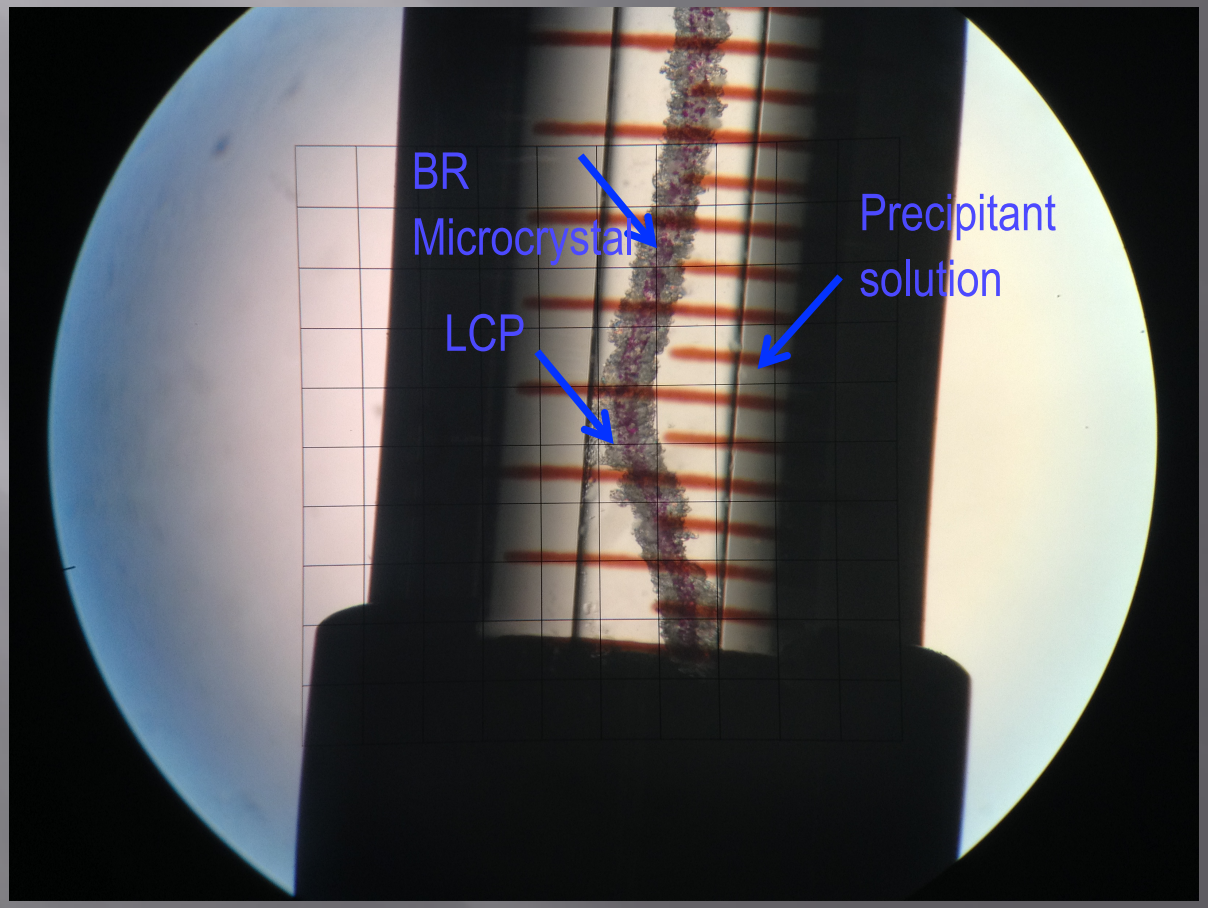
Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, Cambridgeshire, United Kingdom

Lipidic Cubic Phase crystallization for SFX

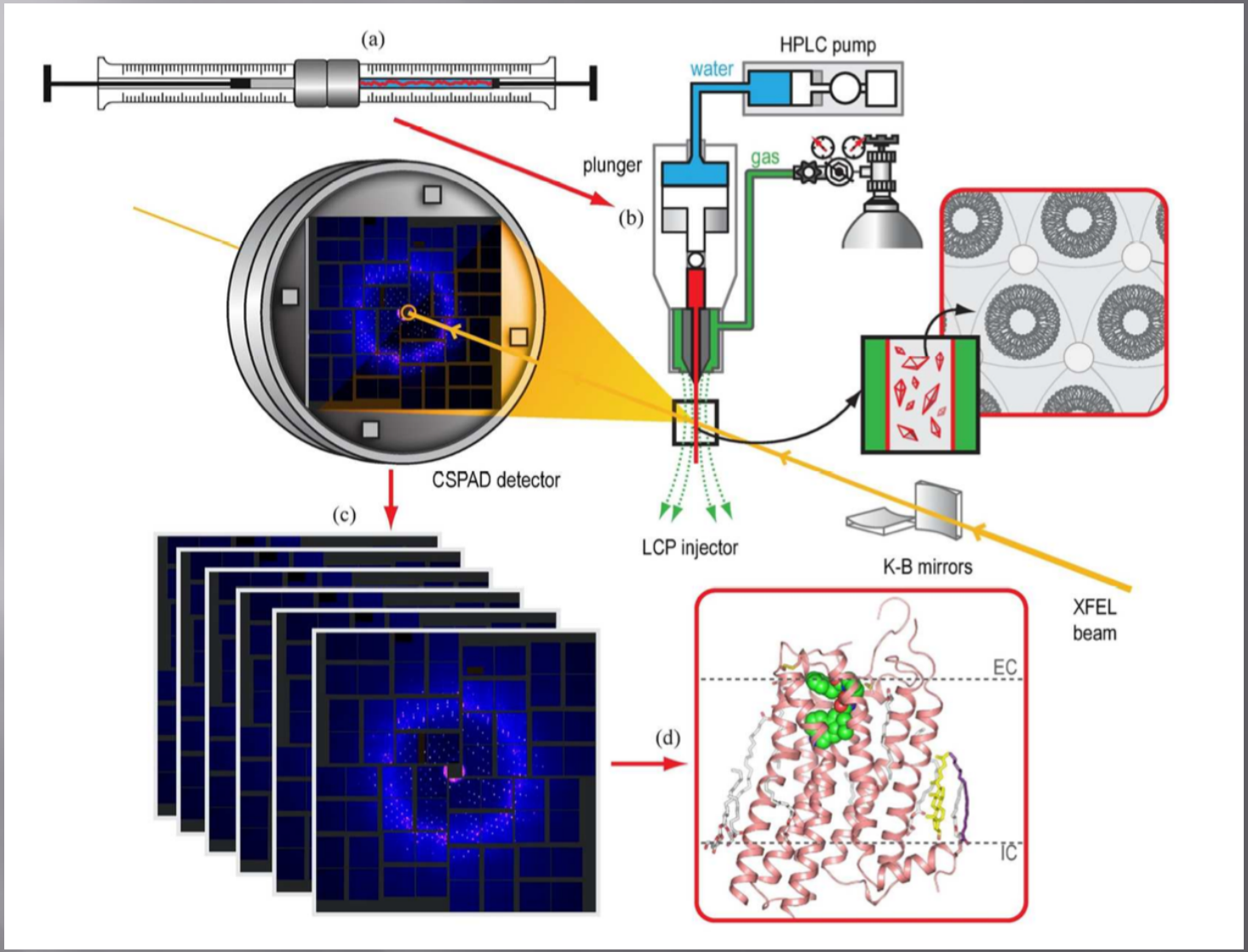
Protein-LCP

Precipitant solution





LCP Jet

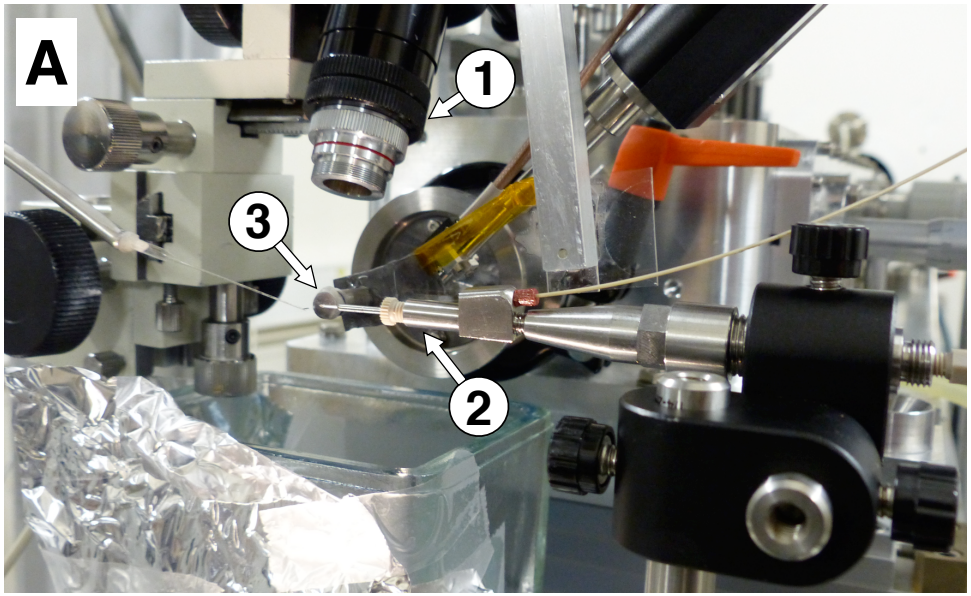


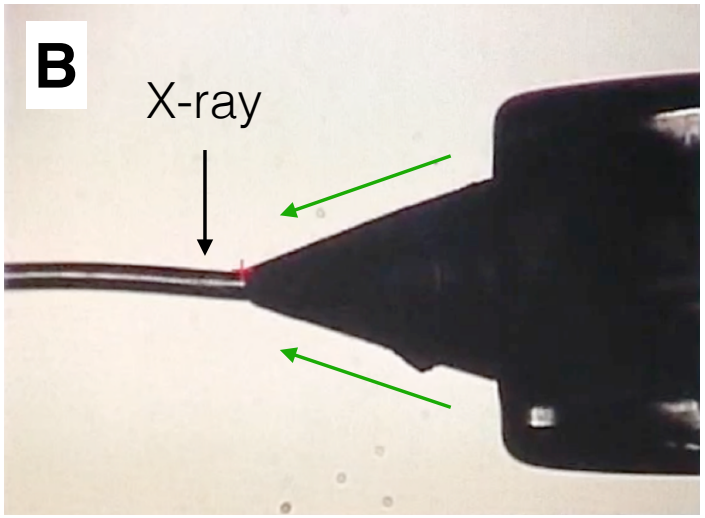
AIP Review of Scientific Instruments

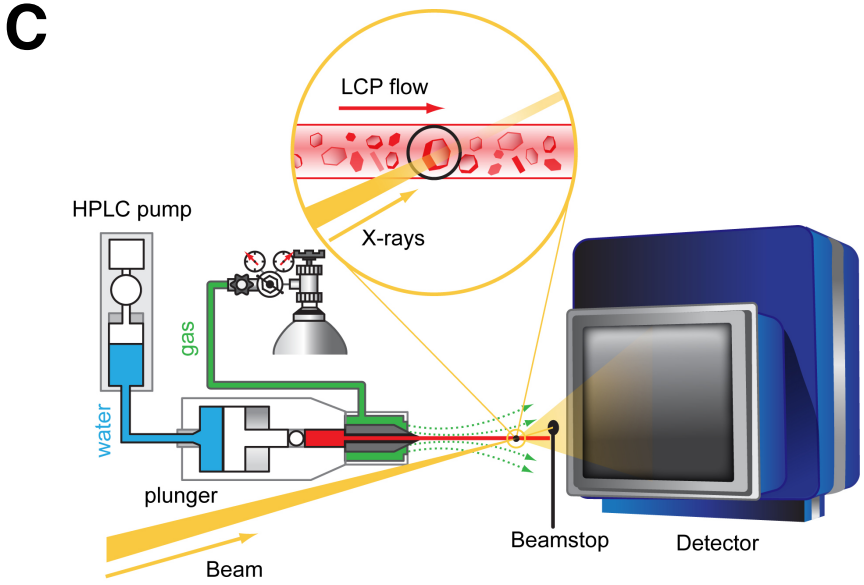
Injector for scattering measurements on fully solvated biospecies

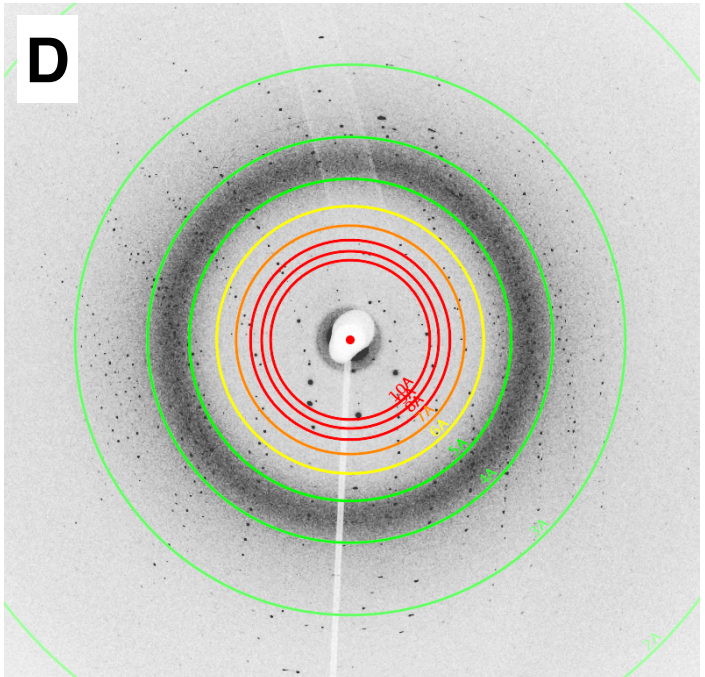
U. Weierstall, J. C. H. Spence, and R. B. Doak

Lipidic cubic phase (LCP) Jet injector ESRF

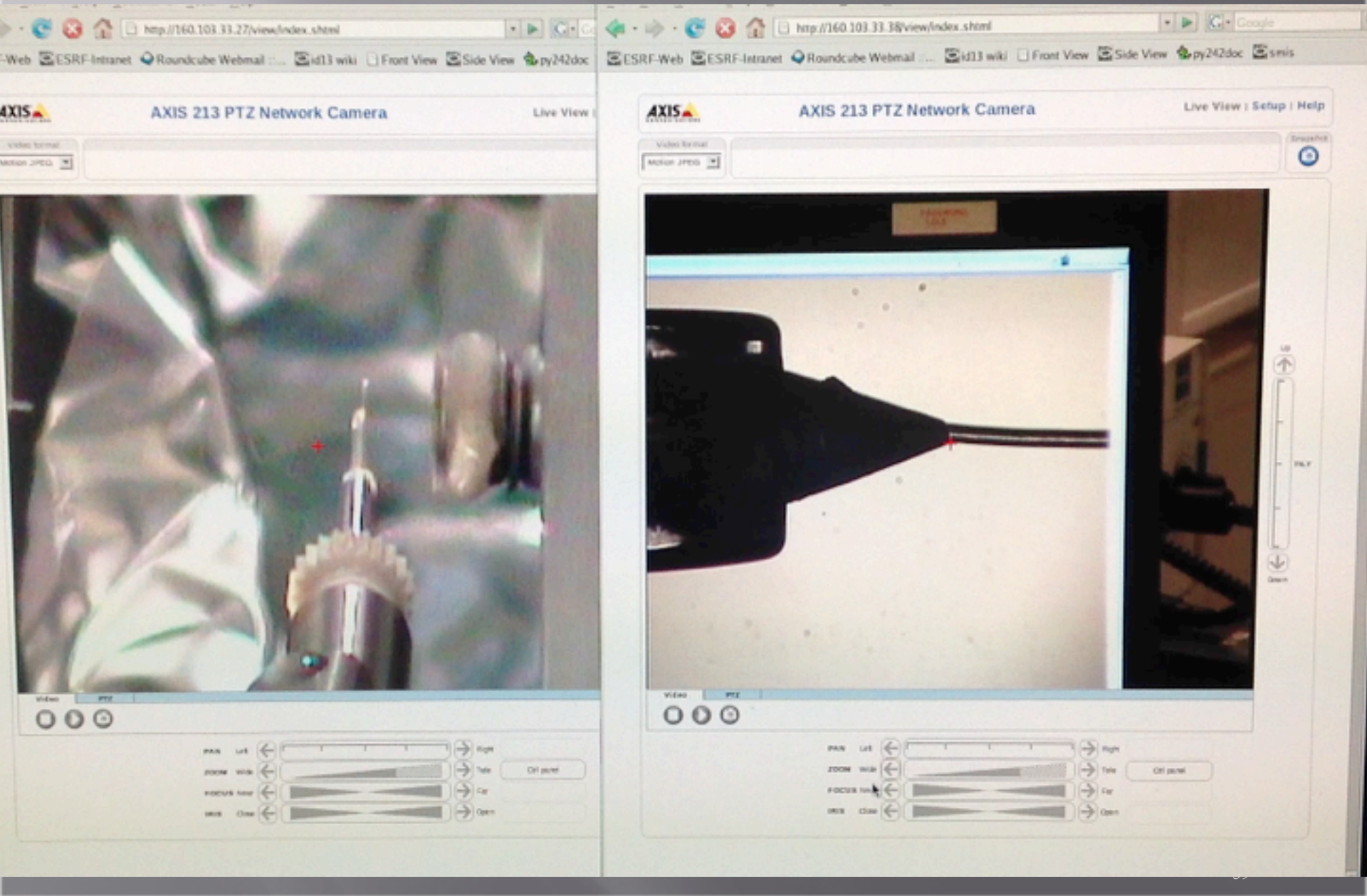




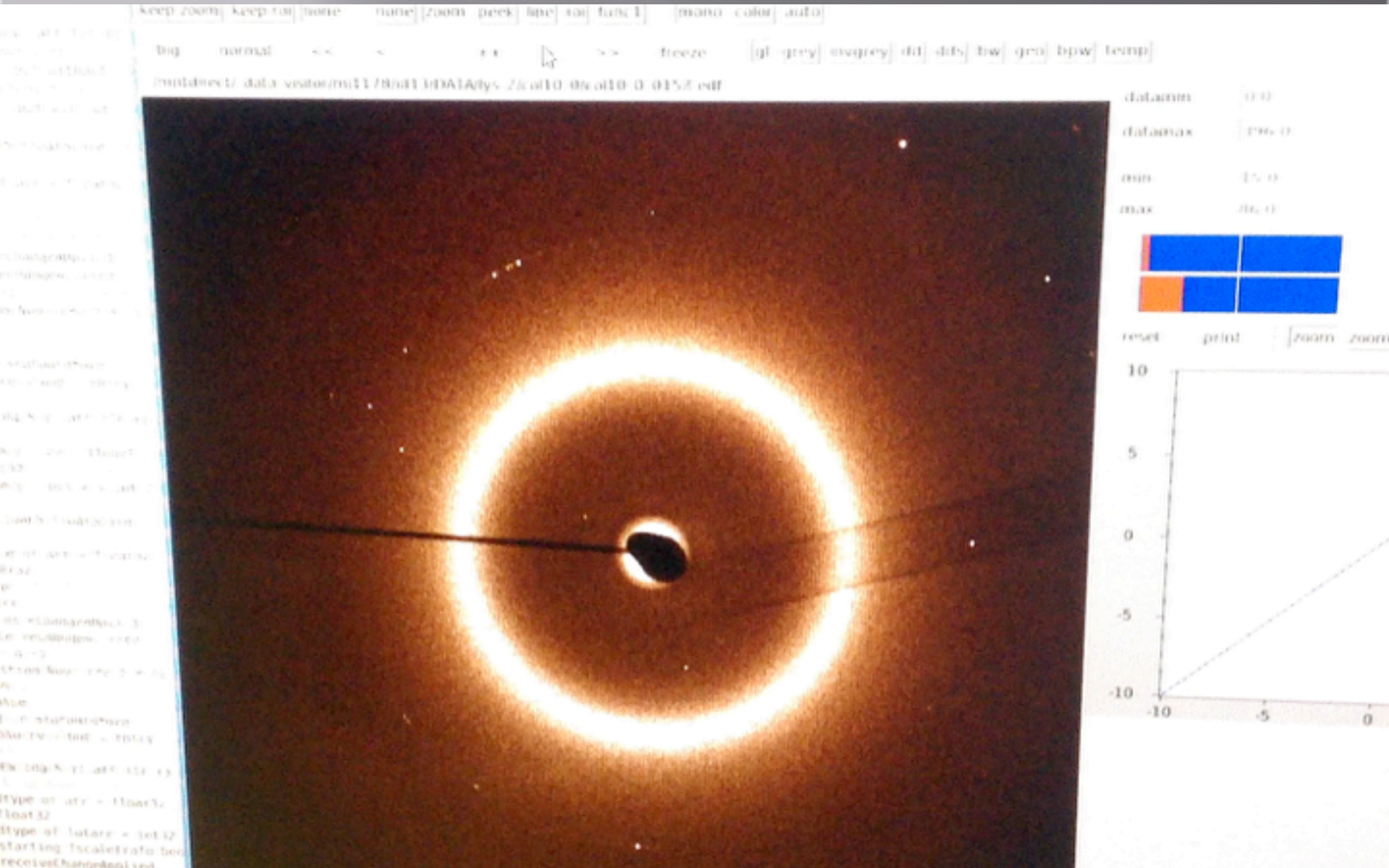




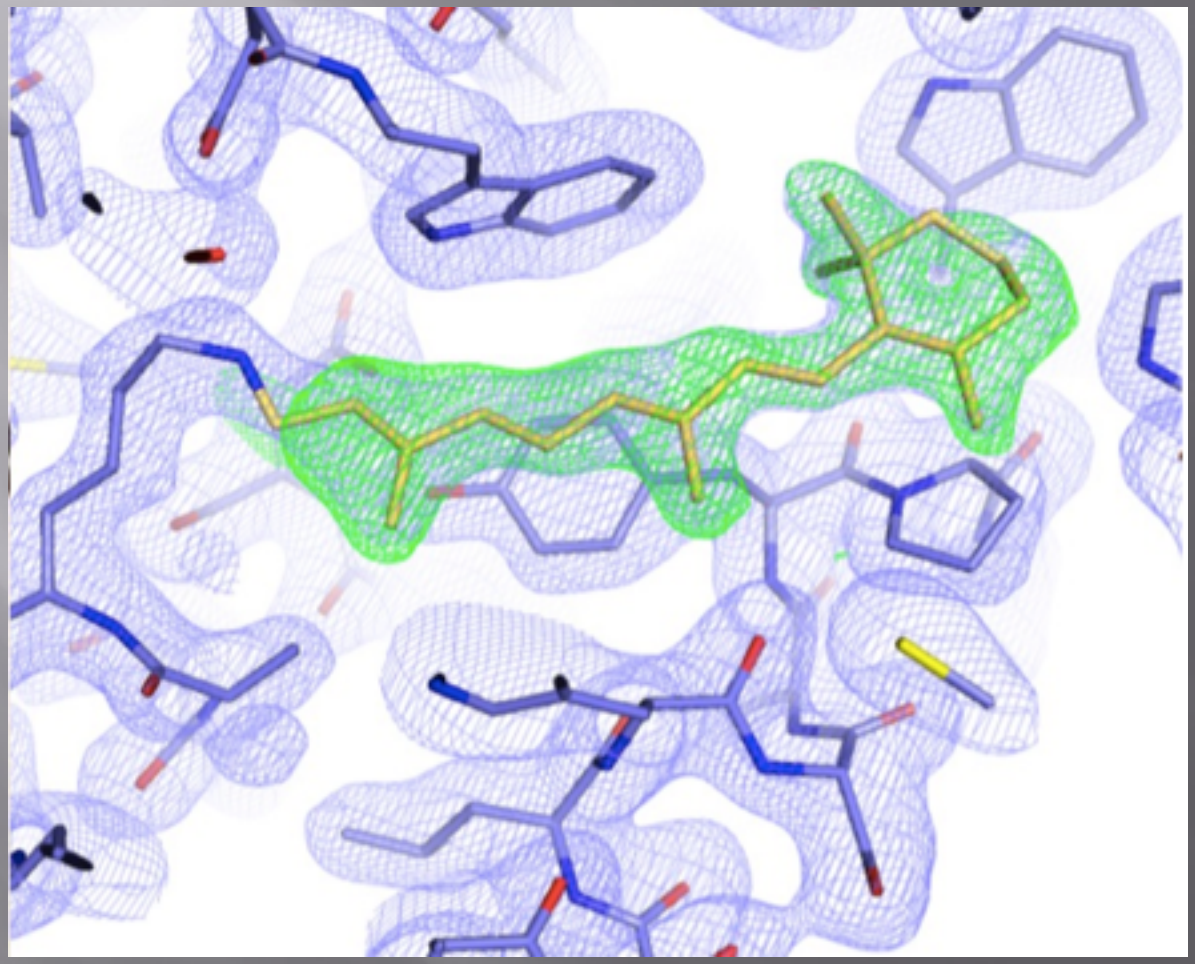
LCP Jet


AXIS 213 PTZ Network Camera
Live View
AXIS 213 PTZ Network Camera
Live View | Setup | Help

Data collection with LCP Jet
NANOMEM consortium

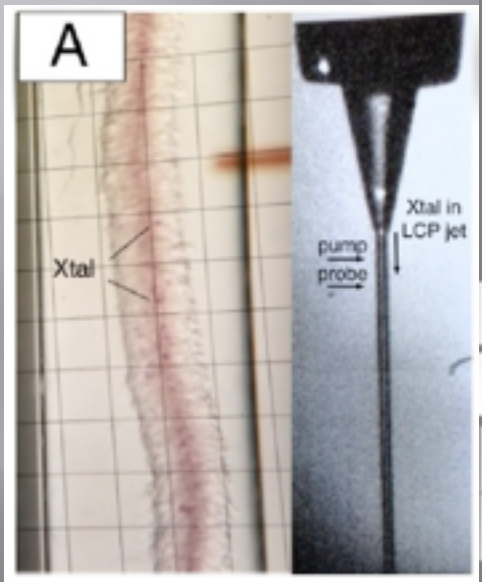


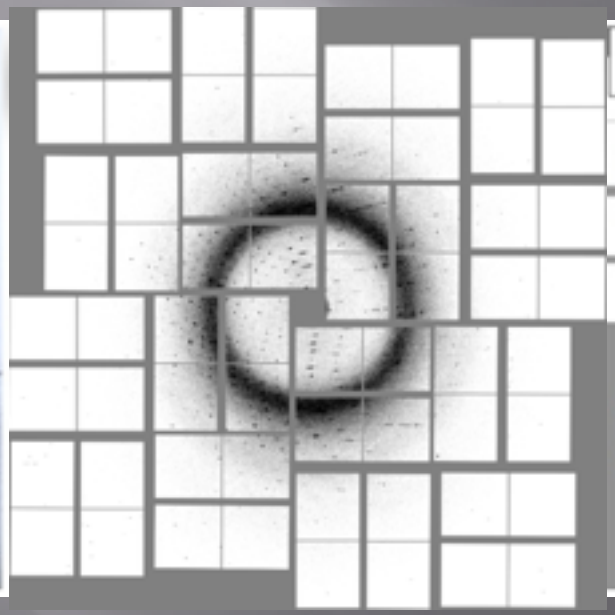
Bacteriorhodopsin structure assembled from single shot diffraction

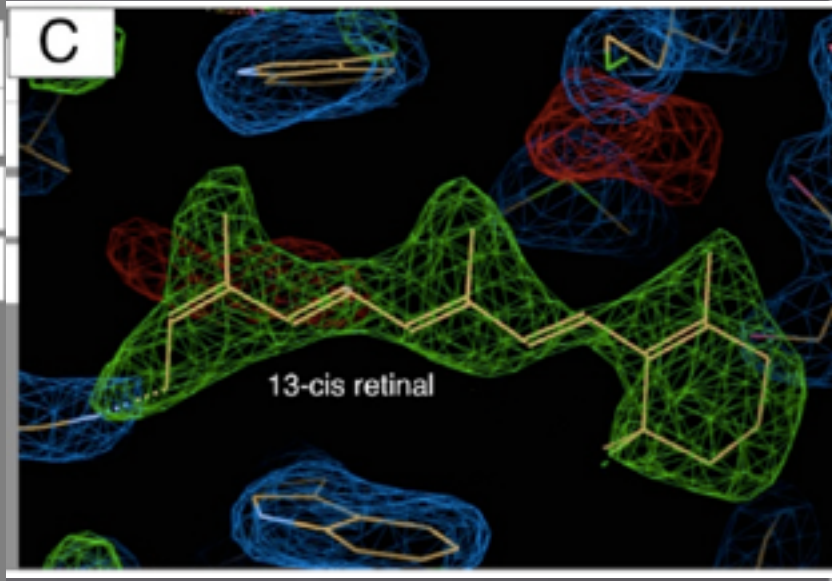


NANOMEM Consortium

Bacteriorhodopsin diffraction in LCP at LCLS Stanford







LCP Jet

FEL BR diffraction

Retinal omit map BR FEL

Femtosecond photo isomerization in Rhodopsin crystals

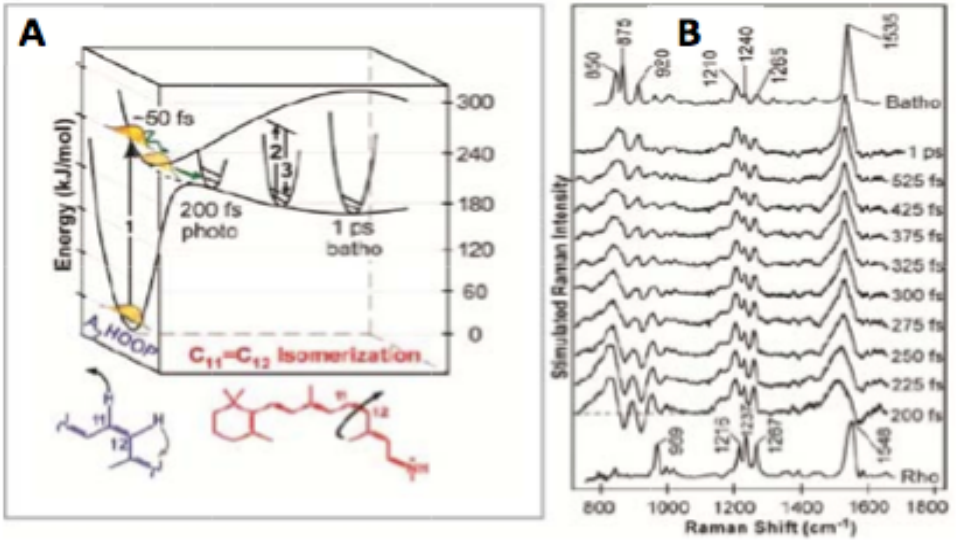
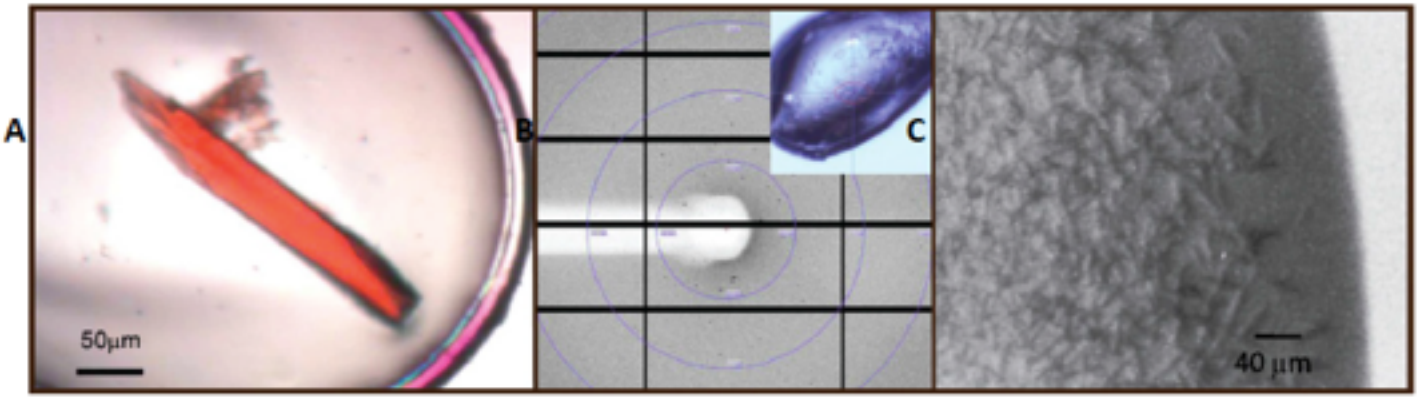
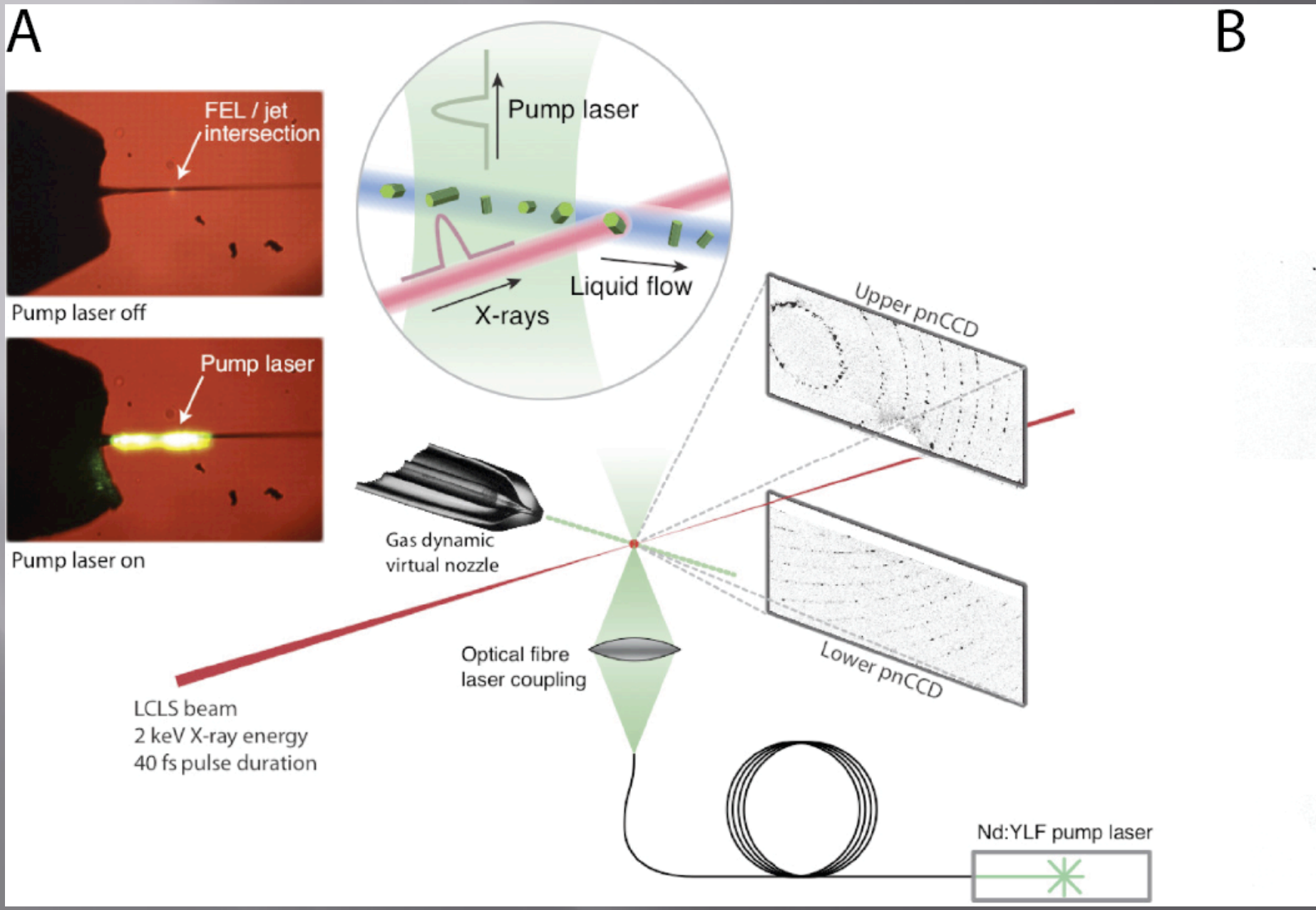


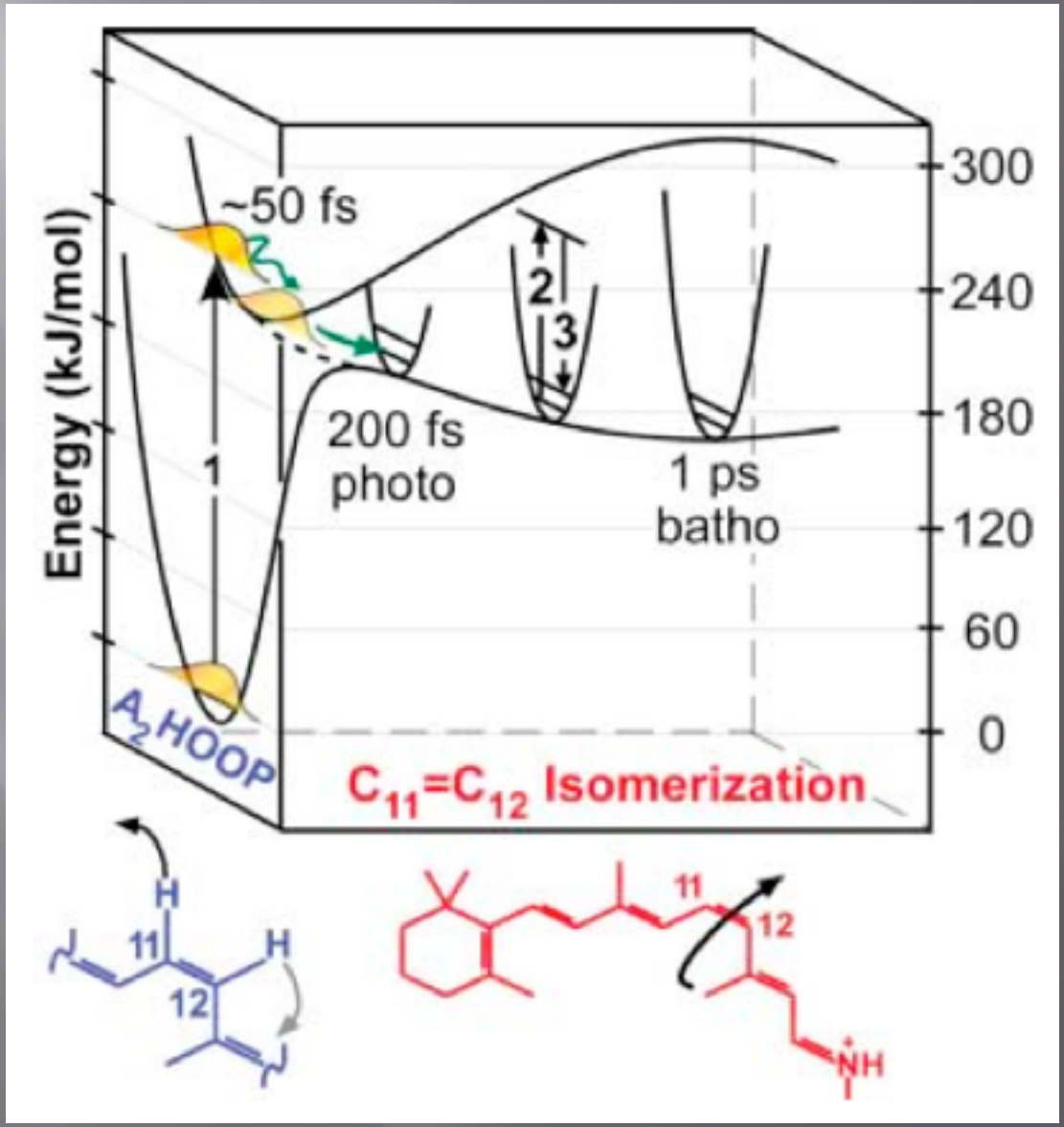
Fig.1. Femtosecond-photoisomerisation of retinal during the first event of vision. **A.** Multidimensional representation of the isomerization coordinate of retinal analyzed by coherent Raman vibrational spectroscopy[17]. The cis-retinal photo-rhodopsin and the all-trans retinal bathorhodopsin states are reached after 200fs and 1ps, respectively. **B.** Time-resolved femtosecond stimulated Raman spectra of rhodopsin[17] in the ground-state (Rho) and in the trapped bathorhodopsin (Batho) state.



X-ray Free Electron Laser Pump probe experiment

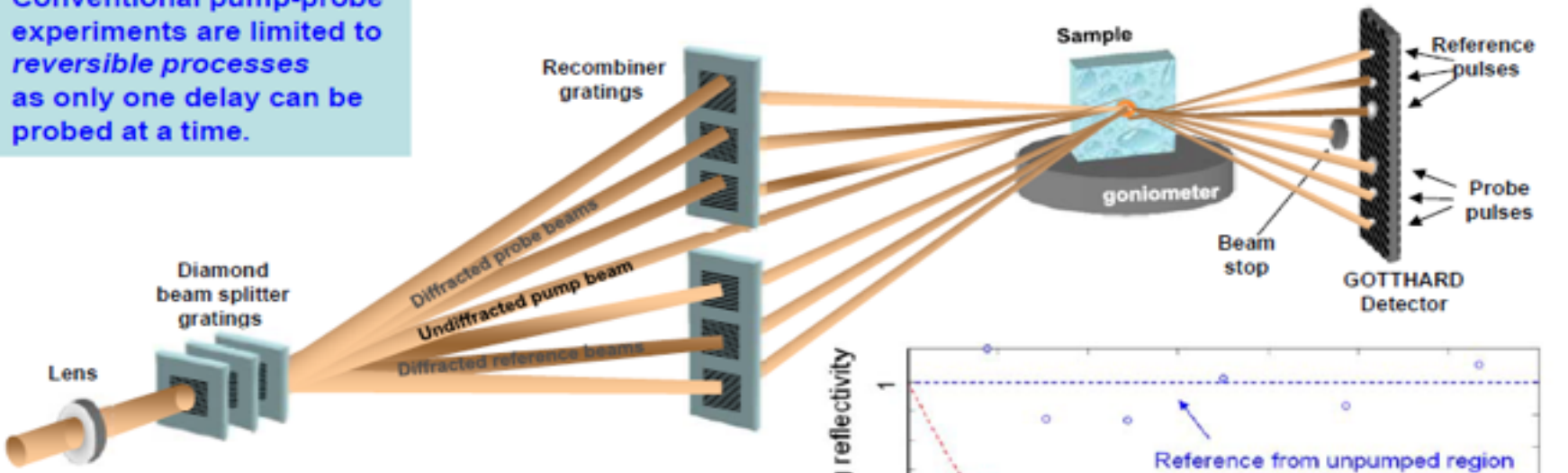


The catalytic step in vision



Delay line for x-ray pump-probe experiments

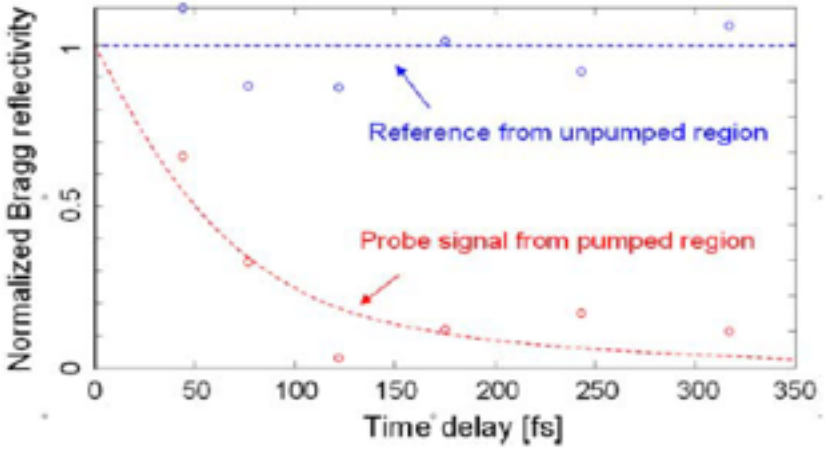
Conventional pump-probe experiments are limited to *reversible processes* as only one delay can be probed at a time.



A novel x-ray delay line based on diffraction gratings creates *several probe beams* with different delays.

Allows for observation of *irreversible processes* with femtosecond resolution.

First *„single-pump multi-probe"* measurements demonstrated at LCLS.



Bi <111> Bragg reflectivity measured with XFEL radiation at 4.5 keV photon energy .

Ch. David et. al.

The Future of Structural Biology

Micro and nano- diffraction with micro focus beam lines will stay essential.

Dynamic of biological structures is essential.

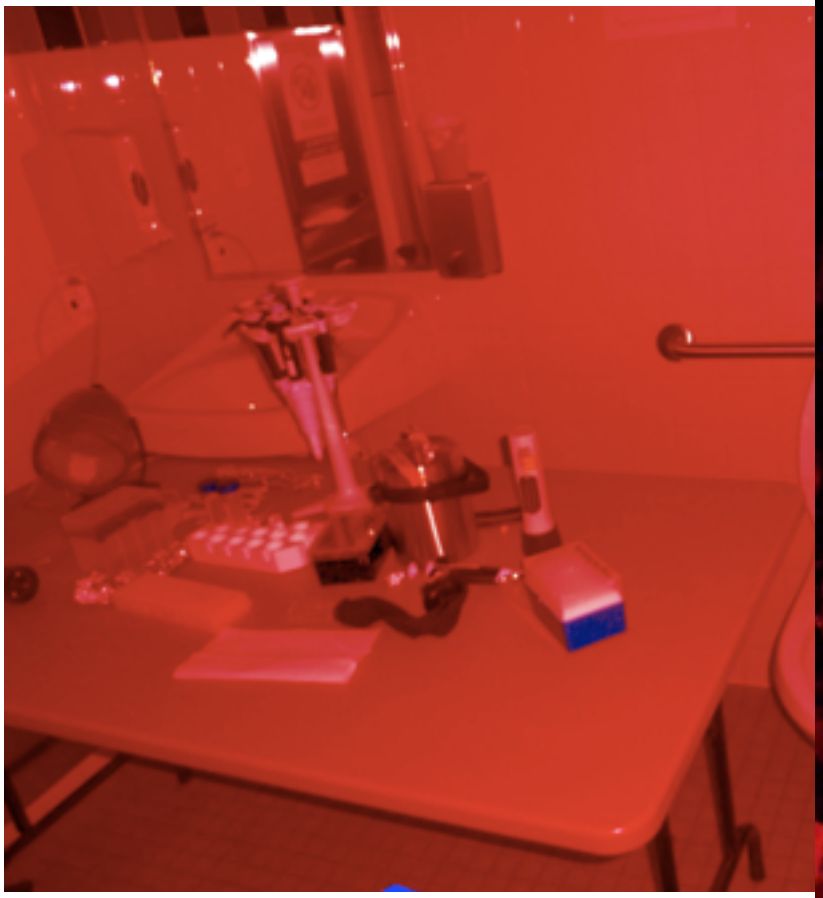
X-ray Free Electron Lasers will change the way we work also on synchrotrons.

Kinetic Crystallography will become more aces sable with FELs

Part of the LB 32 Team at LCLS CXI

Richar Neutze and the part of the measuring team
from PSI, Arrizona, and Hamburg and Goeteborg

Dark Complications



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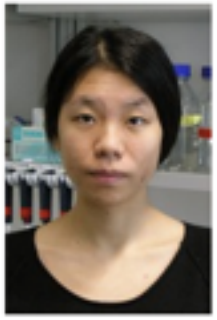

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