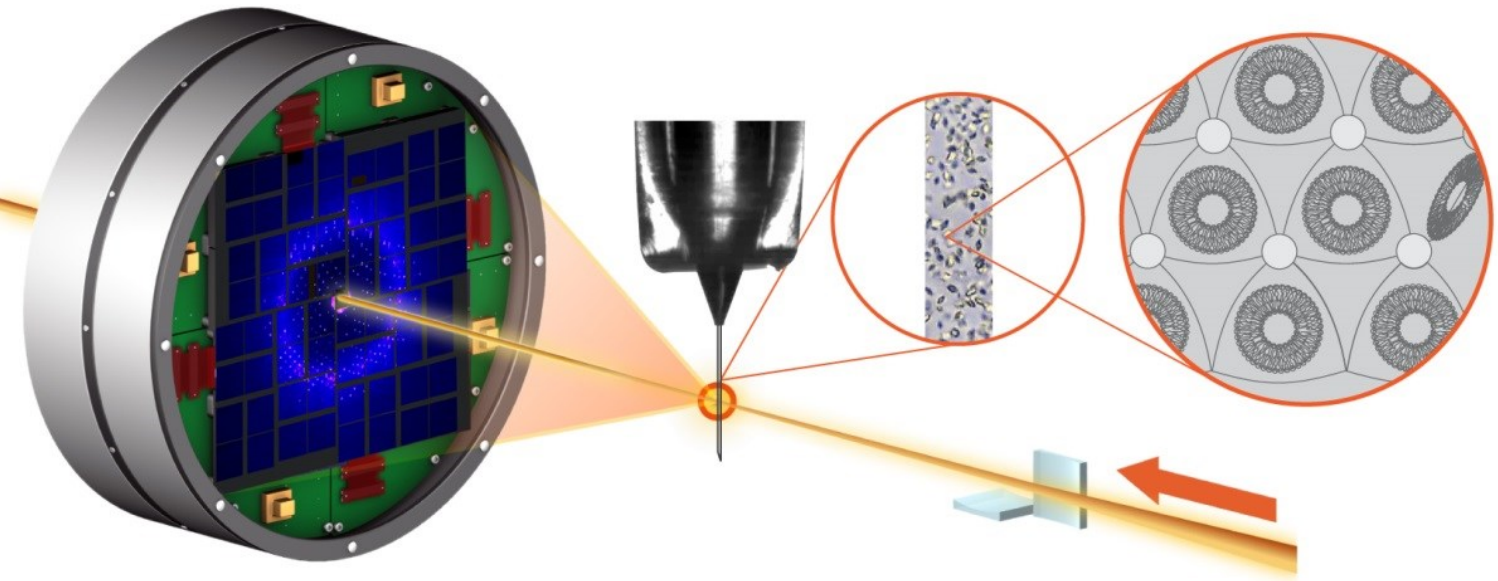
Serial femtosecond crystallography of GPCRs



Cornelius Gati
Center for Free-Electron Laser Science / DESY

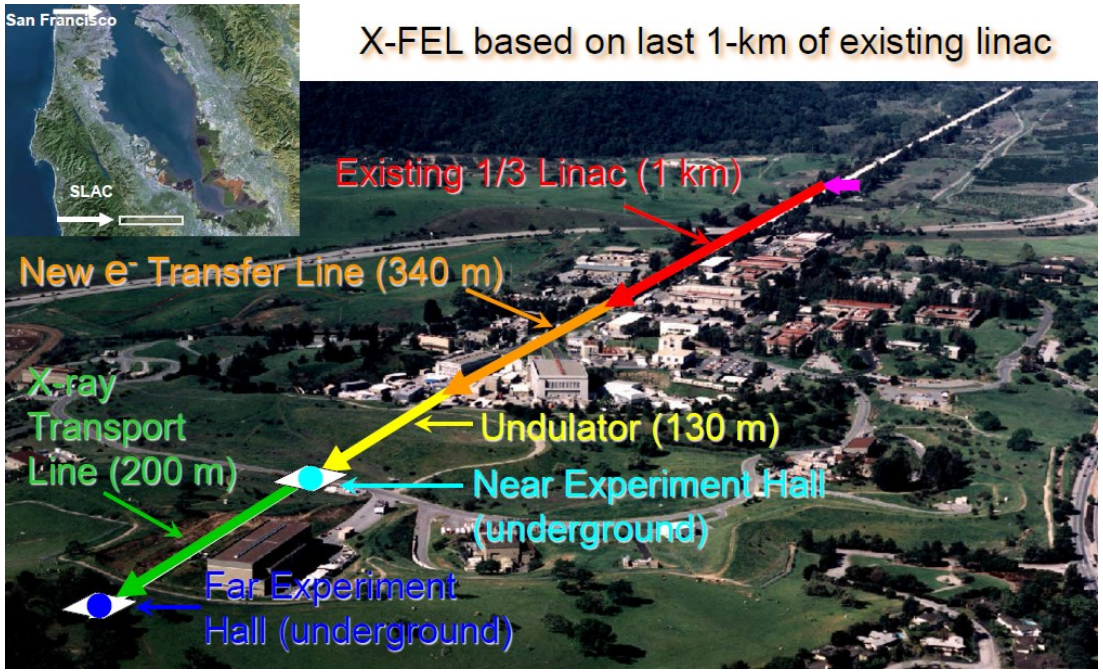
02.06.2015, SyNeW Workshop, Utrecht

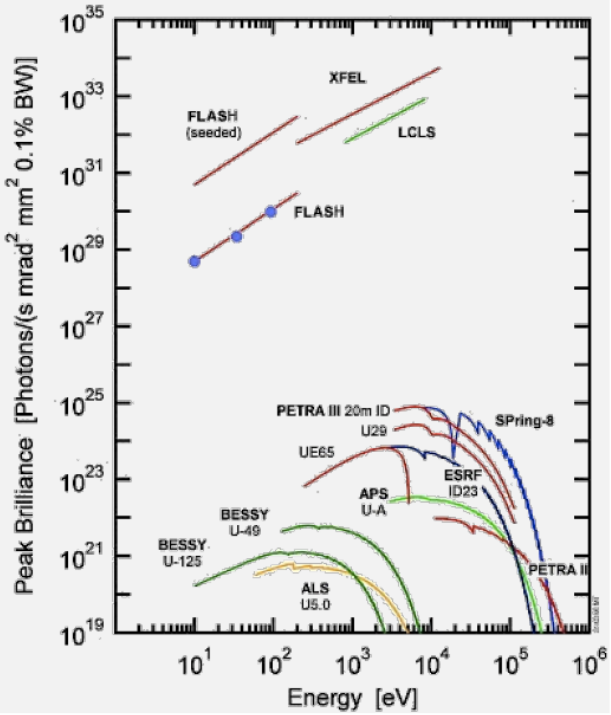
HELMHOLTZ ASSOCIATION

CFEL SCIENCE

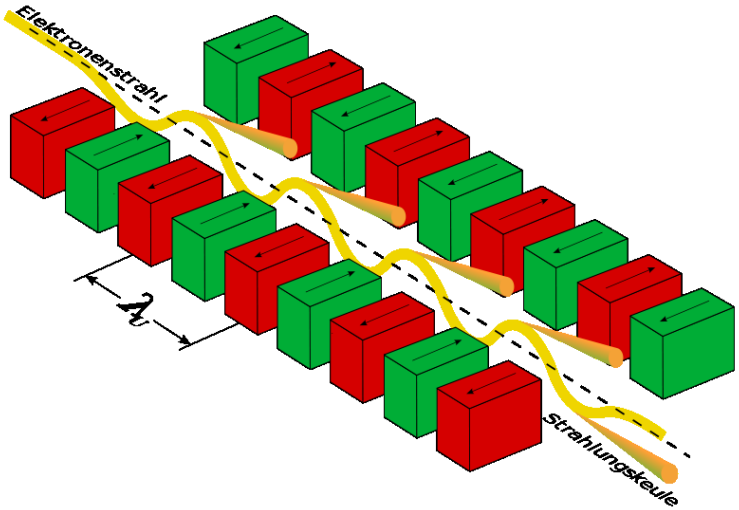
DESY

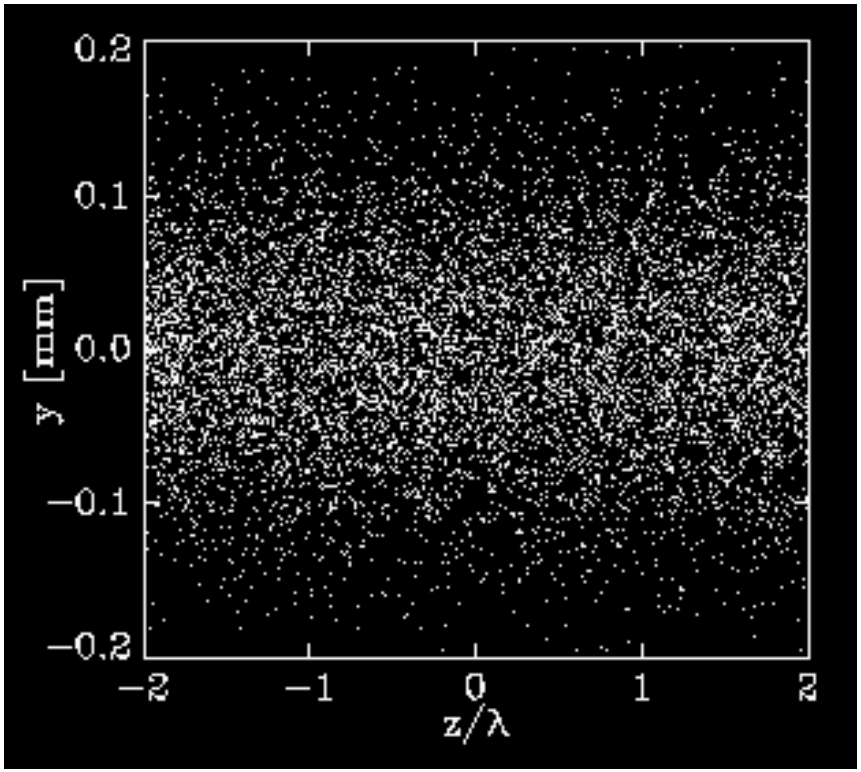
Linac Coherent Light Source

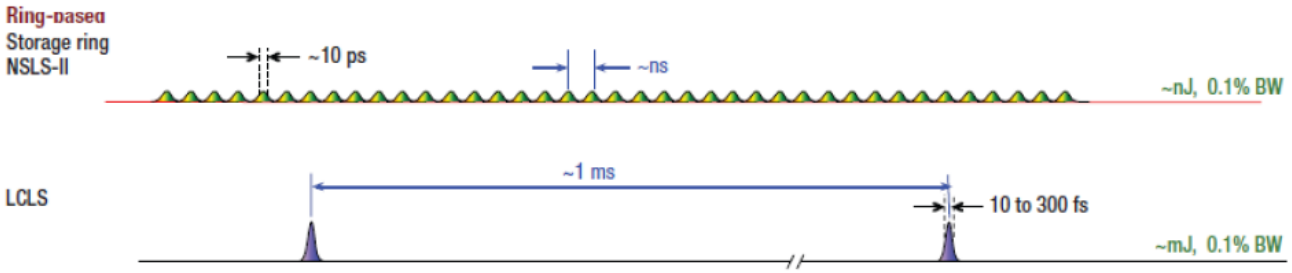




The SASE effect

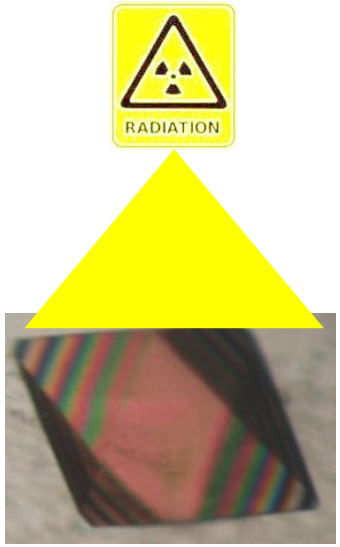






What is serial crystallography?

‘Conventional’ crystallography



20-1000 µm

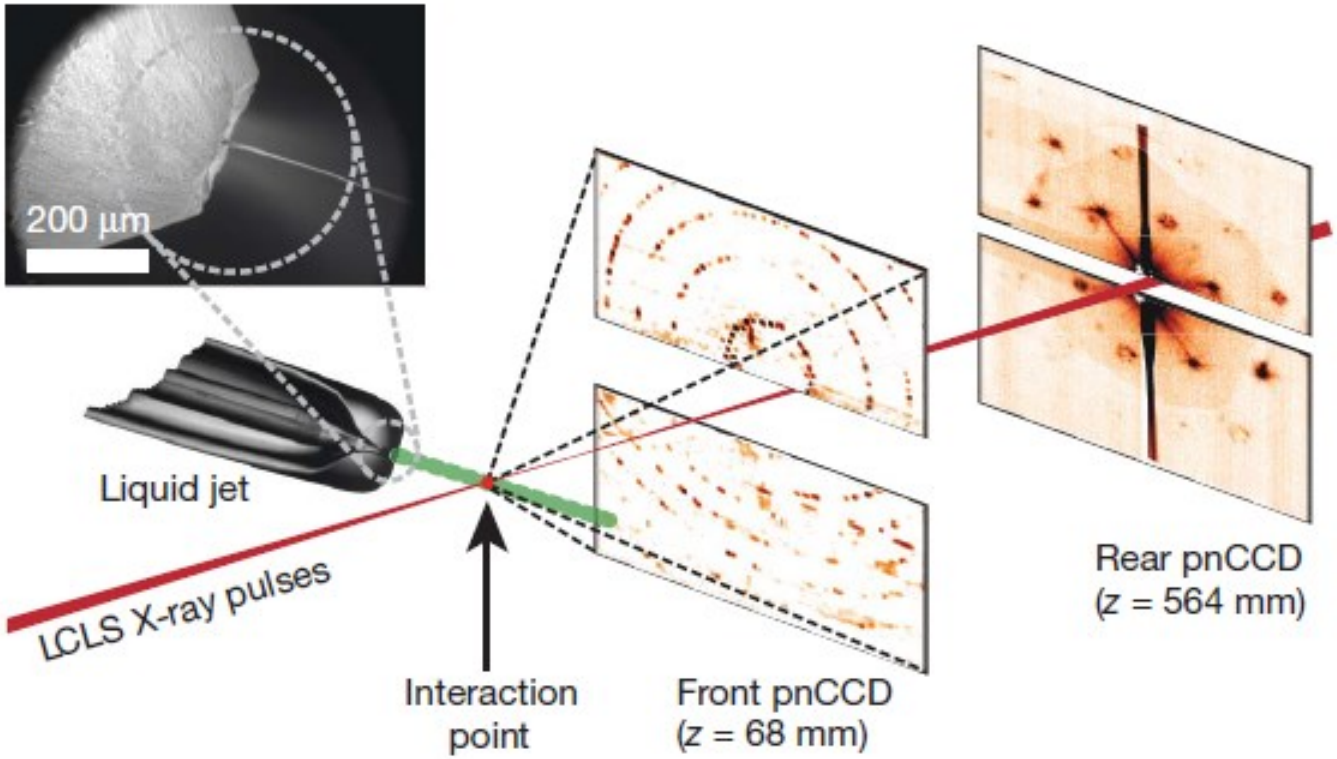
one or few crystals
(relatively!) large crystals
(relatively!) low flux density
oscillation series
centering

Serial crystallography

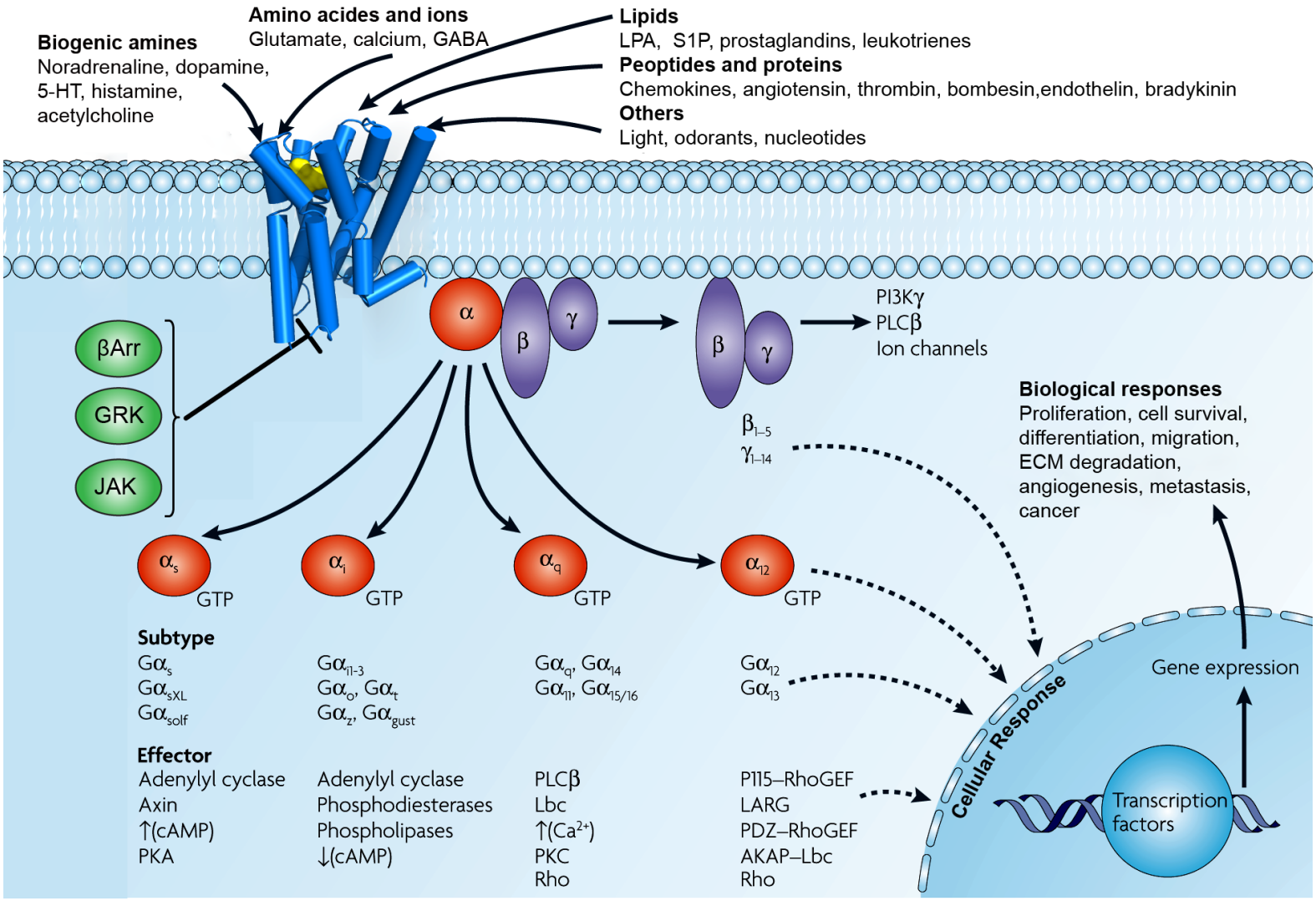
0.2-5 µm

dozens to thousands of crystals
small crystals
high flux density
single or few pattern per crystal
random orientation

Serial Femtosecond Crystallography

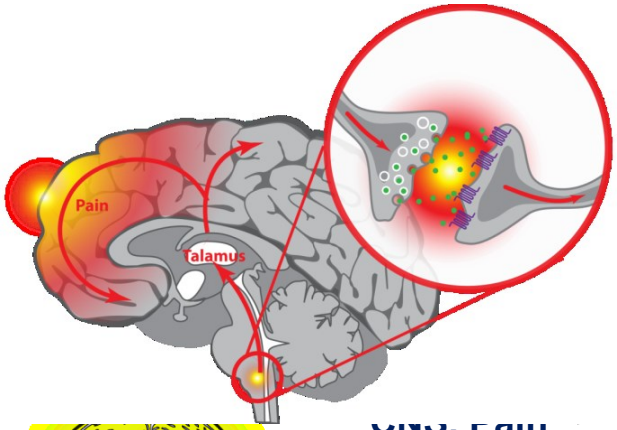


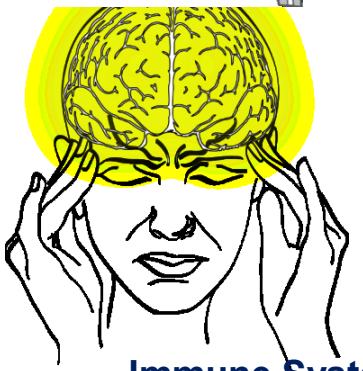
Chapman et al. 2011 (Nature)



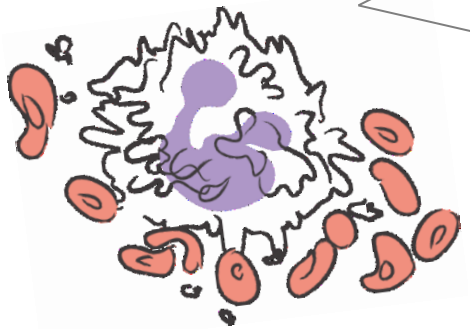
Nature Reviews Cancer **7**, 79-94 (February 2007)

GPCR Biology

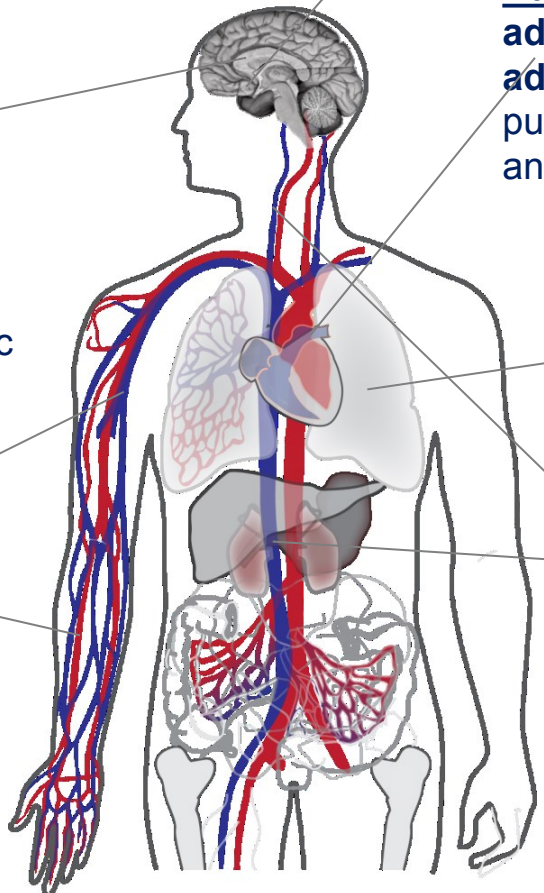


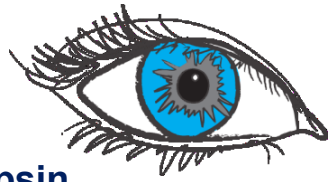


CNS, Pain
acetylcholine, **dopamine**, **serotonin**, **opioid**, cannabinoid, etc

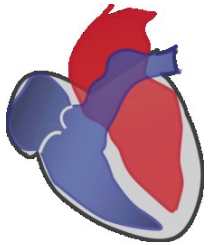


Immune System, HIV, Cancer
cytokine, sphingosine





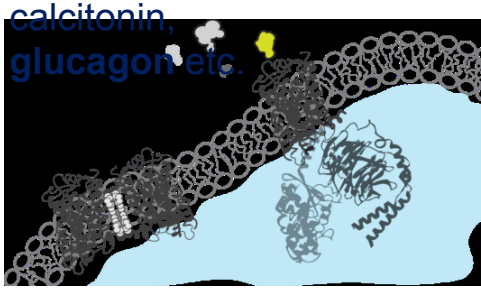
Vision
rhodopsin



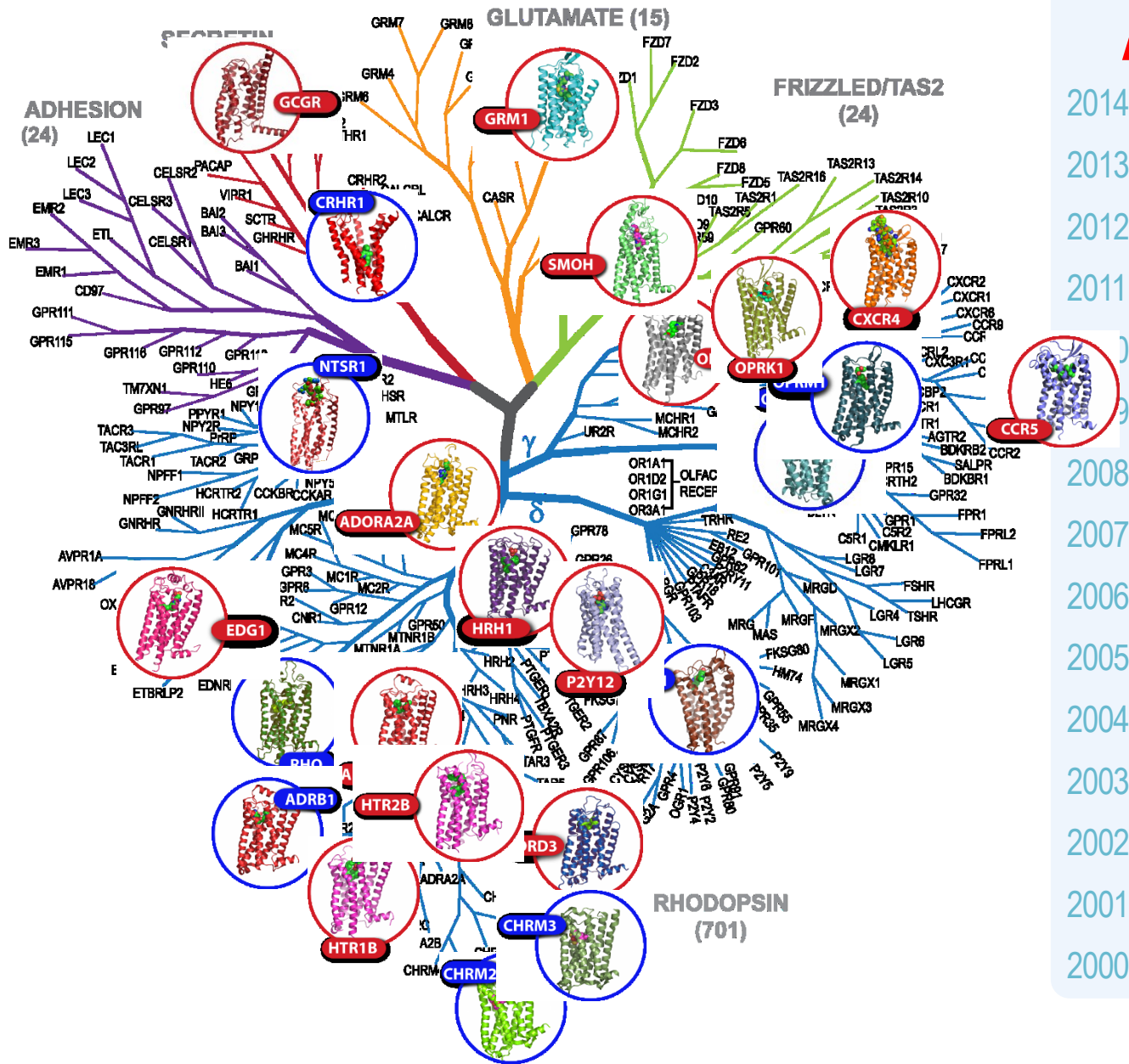
Cardiovascular
adrenergic, **adenosine**, purinergic, angiotensin, etc

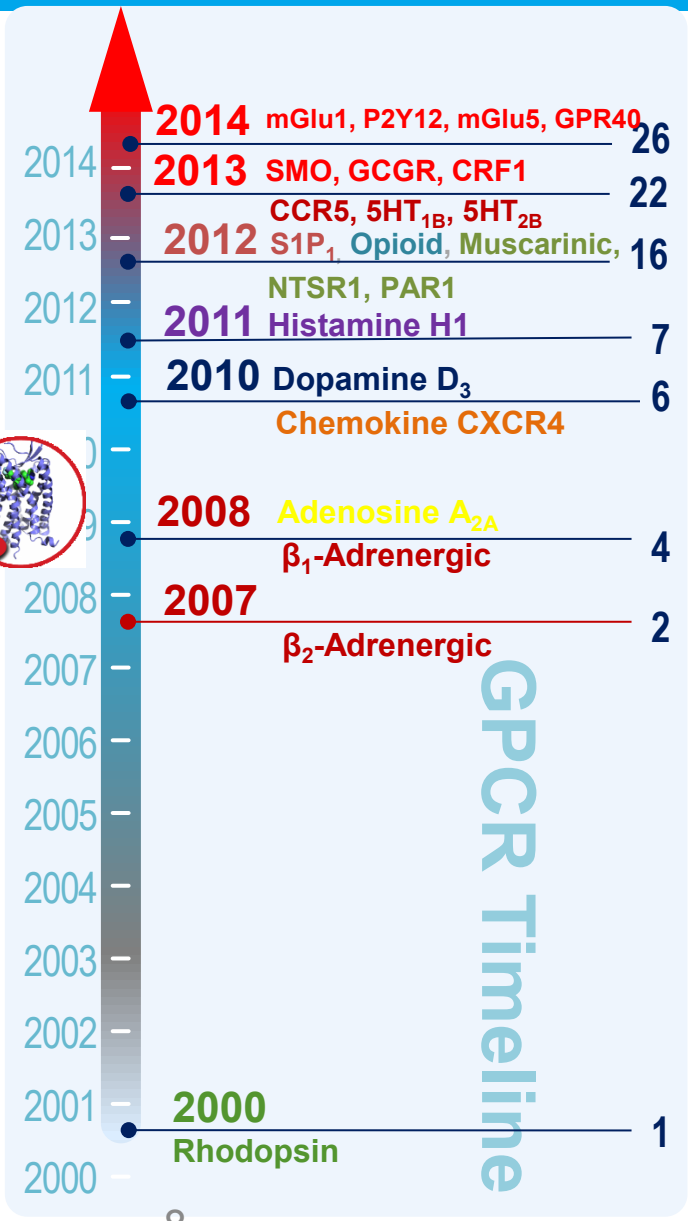
Allergy
histamine

Metabolic
parathyroid, THS, calcitonin, **glucagon** etc.

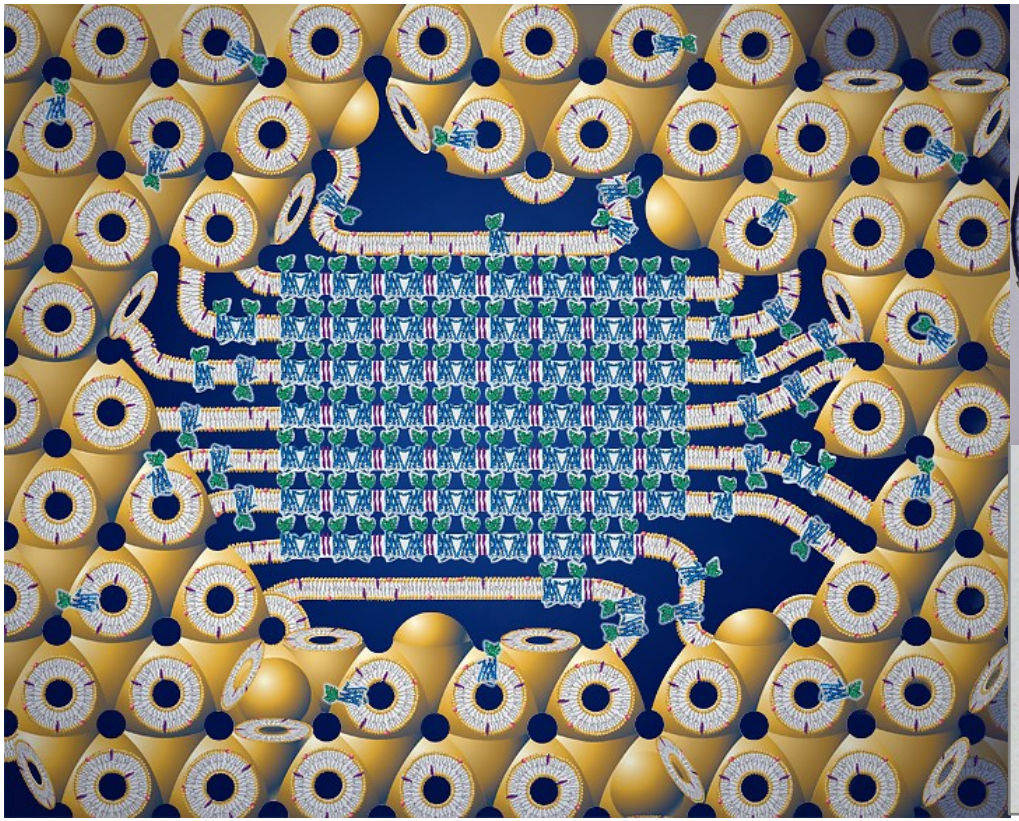


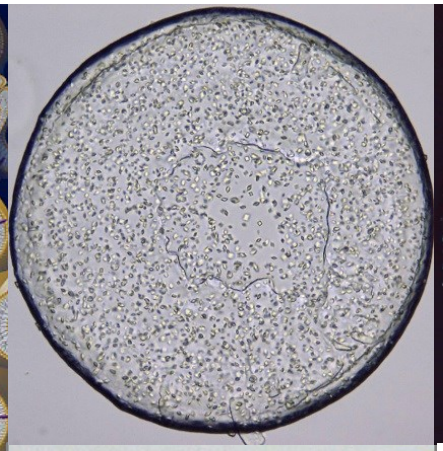
GPCR Structure Timeline

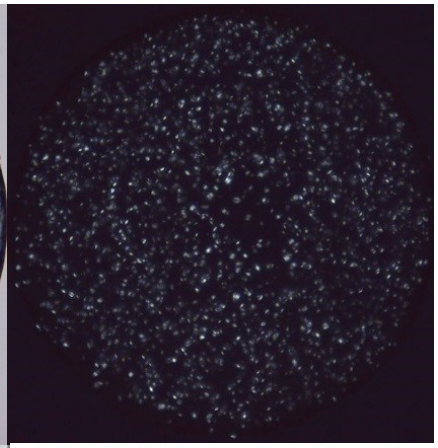


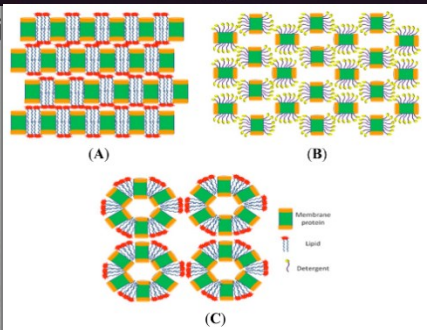


Lipidic Cubic Phase crystallization



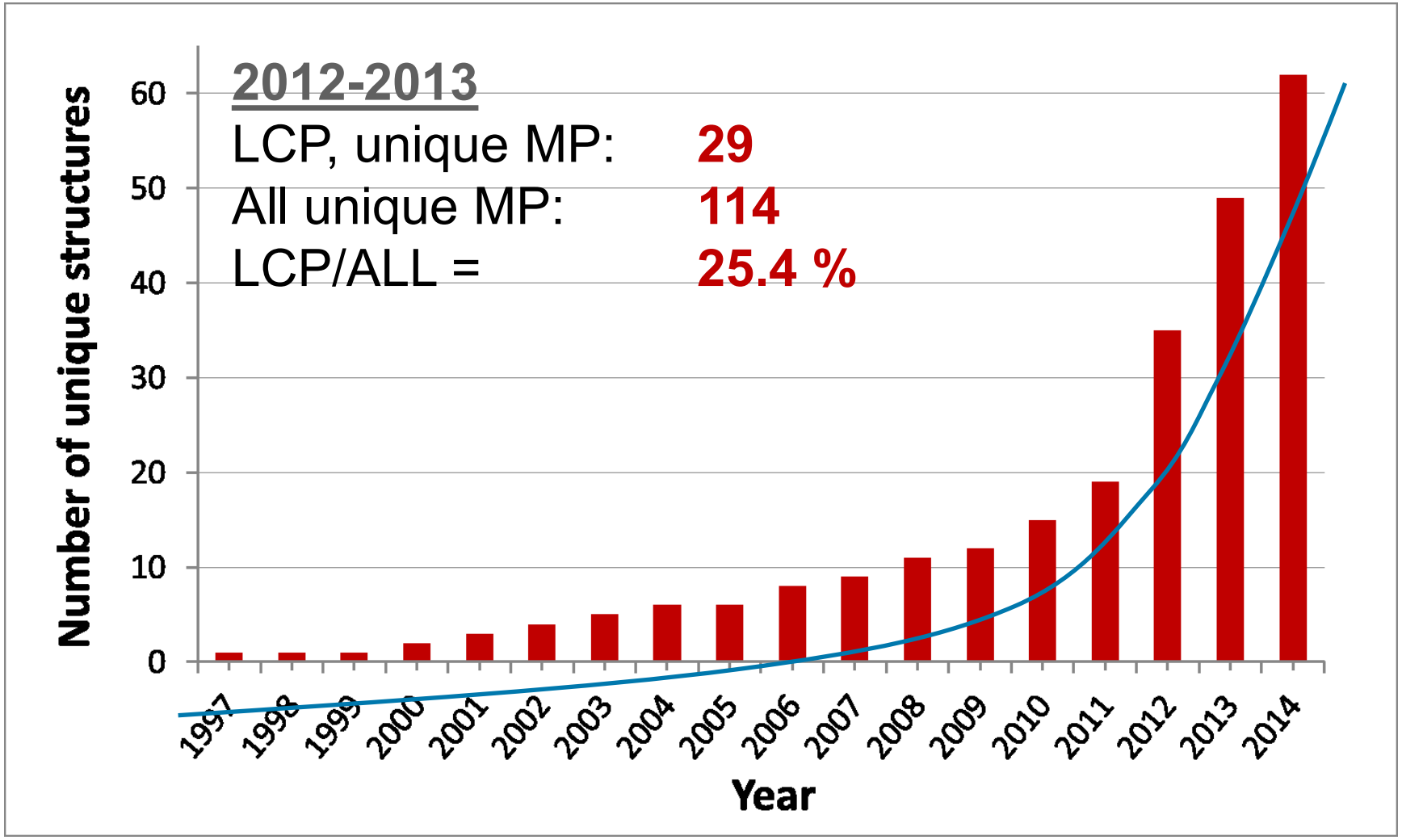






- Native-like membrane environment
- Transparent, Viscous, Gel-like
- High crystal nucleation rate (**high density of small crystals**

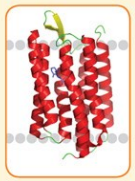
LCP - on the rise



LCP unique MP: **62** ALL unique MP: **504** LCP/ALL = **12.3 %**

Total structures: 185 Unique structures: 62

LCP STRUCTURES

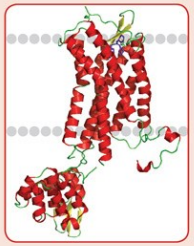


Microbial Rhodopsins:

bR	1.43 Å (1M0K)
hR	1.70 Å (2JAF)
sR II	2.10 Å (1H68)
sR II/ tr	1.93 Å (1H2S)
sR	2.00 Å (1XIO)
ARII	3.20 Å (3AM6)
ChR	2.30 Å (3UG9)
ESR	2.30 Å (4HYJ)

G Protein-Coupled Receptors:

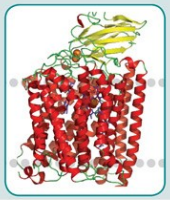
β_2AR	2.40 Å (2RH1)
A_{2A}AR	1.80 Å (4EIY)
CXCR4	2.50 Å (3ODU)
D3R	3.15 Å (3PBL)
H1R	3.10 Å (3RZE)
β_2AR/Gs	3.20 Å (3SN6)
$S1P_1$	2.80 Å (3V2Y)
M2R	3.00 Å (34ON)
M3R	3.40 Å (4DAJ)
NTSR1	2.80 Å (4GRV)



κOR	2.90 Å (4DJH)
μOR	2.80 Å (4DKL)
NOP	3.00 Å (4EA3)
δOR	3.40 Å (4EJ4)
PAR1	2.20 Å (3VW7)
$5HT_{2B}$	2.70 Å (4IB4)
$5HT_{1B}$	2.70 Å (4IAR)
SMO	2.45 Å (4JKV)
GCGR	3.30 Å (4L6R)
CRF1	2.98 Å (4K5Y)
CCR5	2.71 Å (4MBS)

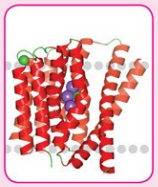
Heme-Copper Oxidases:

ba3	1.80 Å (3S8F)
caa3	2.36 Å (2YEV)

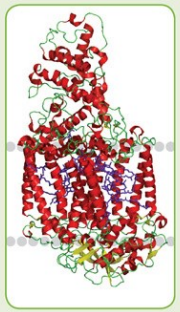


Transporters:

NCX	1.90 Å (3V5U)
CAX	2.30 Å (4KPP)
VCX1	2.30 Å (4K1C)
POT	1.90 Å (4IKV)
MATE	2.40 Å (3VVN)



Photosynthetic Proteins:



RC, *R Sph*
2.2 Å (2GNU)

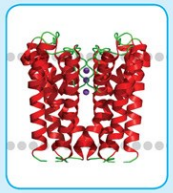
LH2
2.45 Å (2FKW)

RC, *R viridis*
1.86 Å (2WJN)

Enzymes:

DgkA
2.05 Å (3ZE3)

Ion Channels:

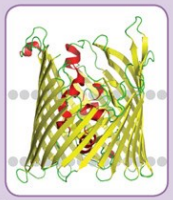


KvLm
3.10 Å (4H33)

Peptides:

gD
1.08 Å (2Y5M)

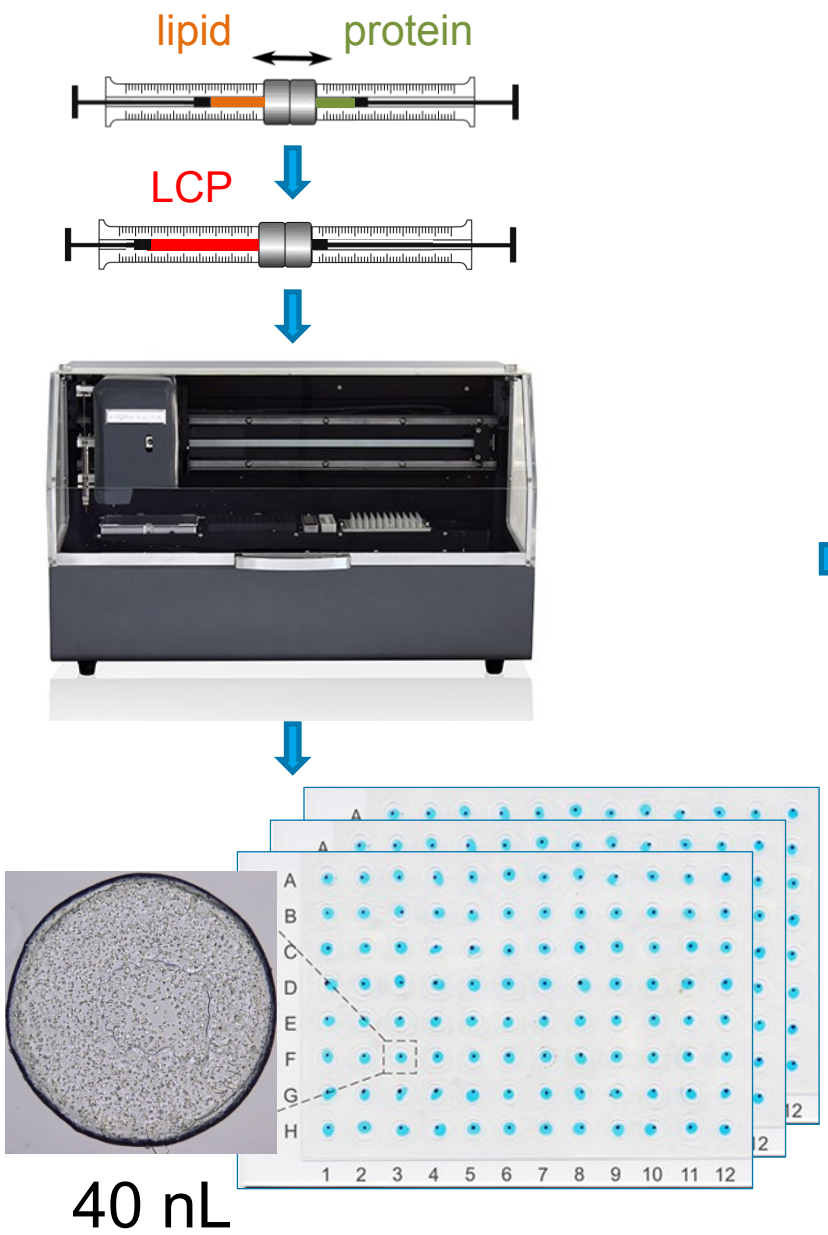
Outer Membrane Proteins:

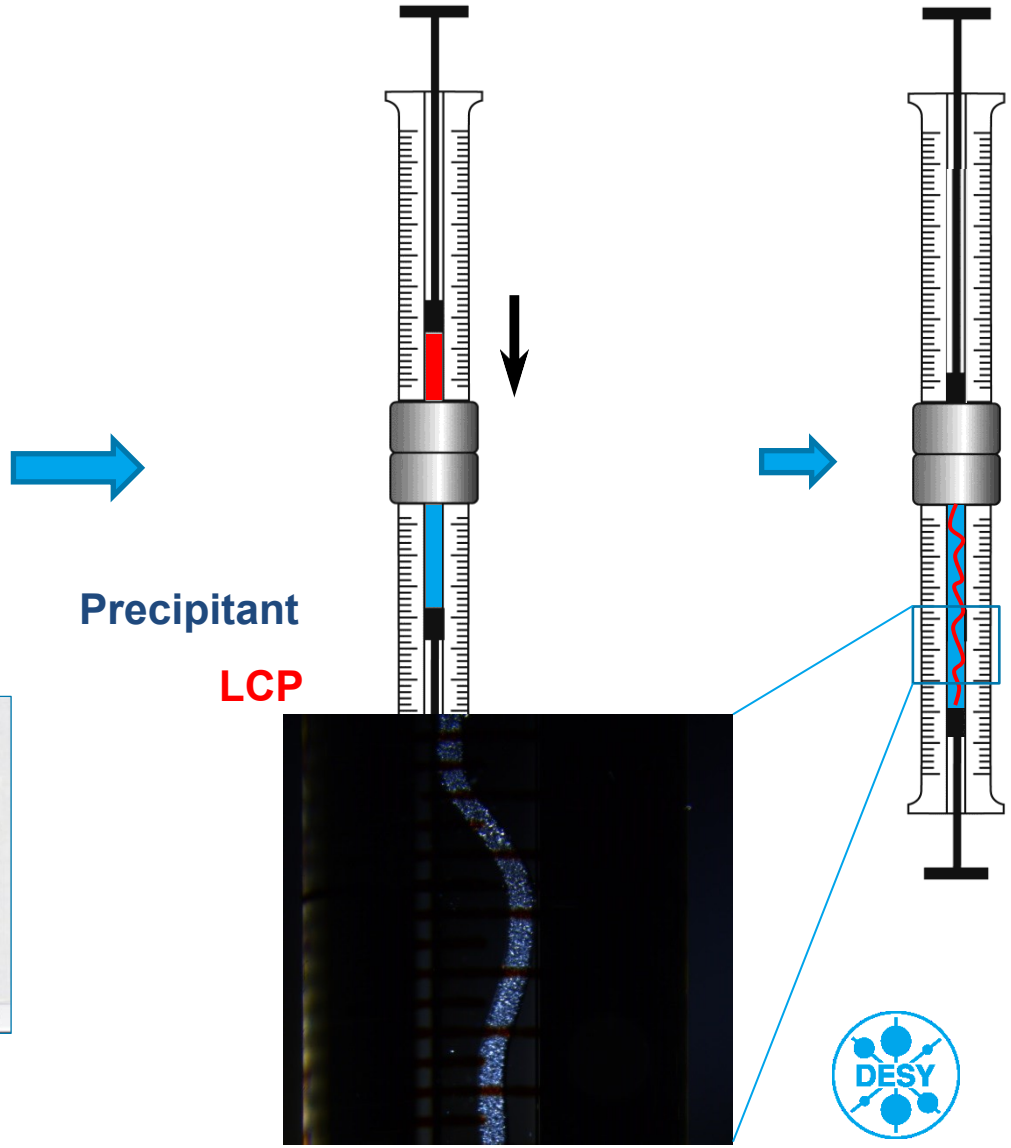


BtuB	1.95 Å (2GUF)
OpcA	1.95 Å (2VDF)
OmpF	1.90 Å (3POQ)
Intimin	1.80 Å (4E1S)
Invasin	1.80 Å (4E1T)
AlgE	1.90 Å (4AFK)

Sample preparation for LCP-SFX

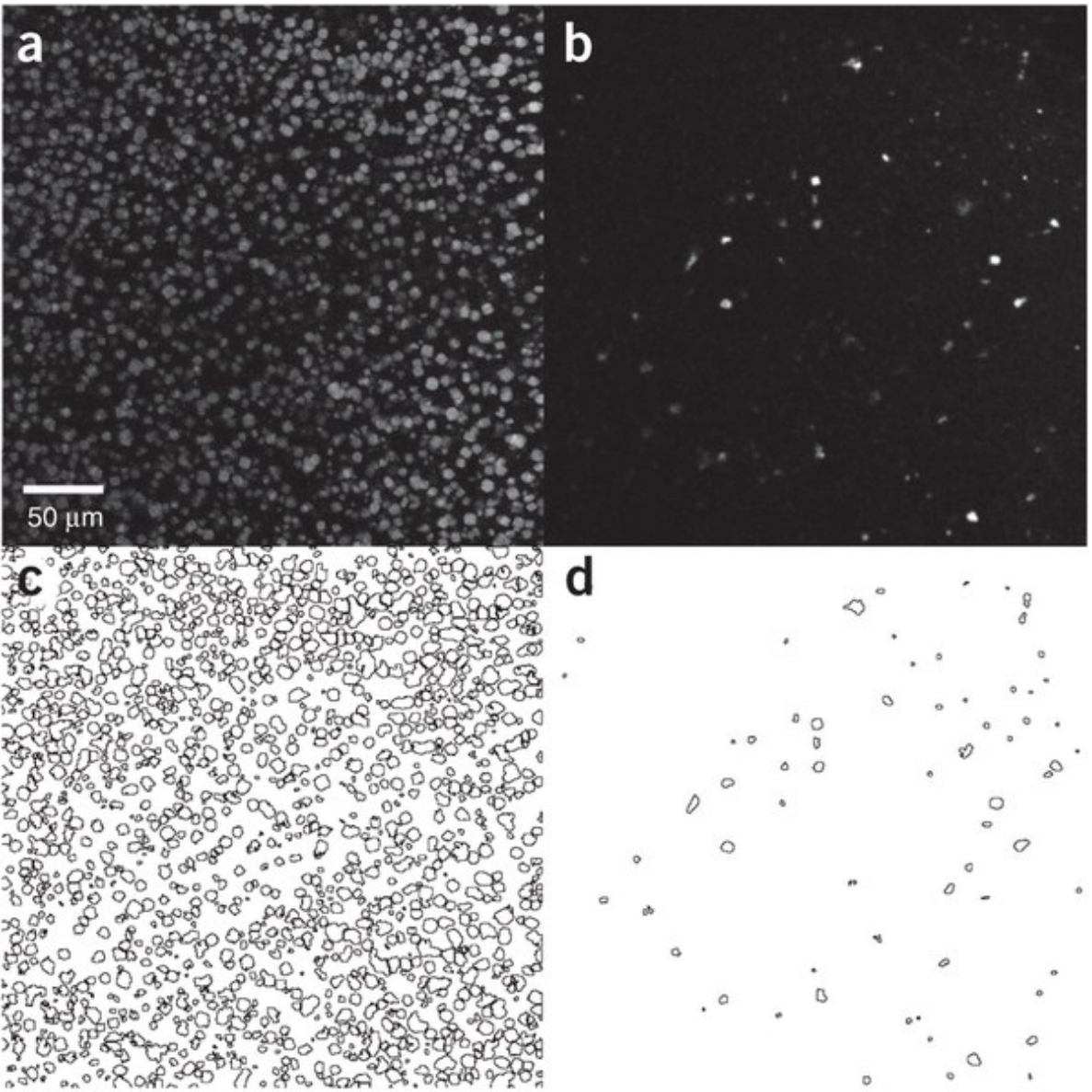
Liu et al. **2014**, Nature Protocols 9:2123





Crystal density assessment

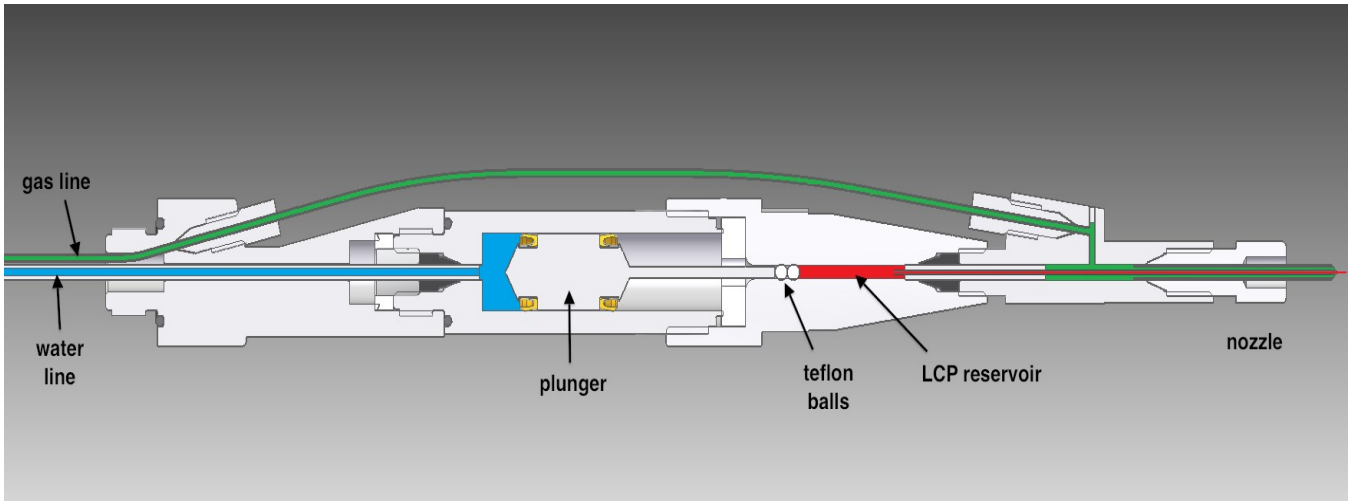
Liu et al. **2014**, Nature Protocols 9:2123



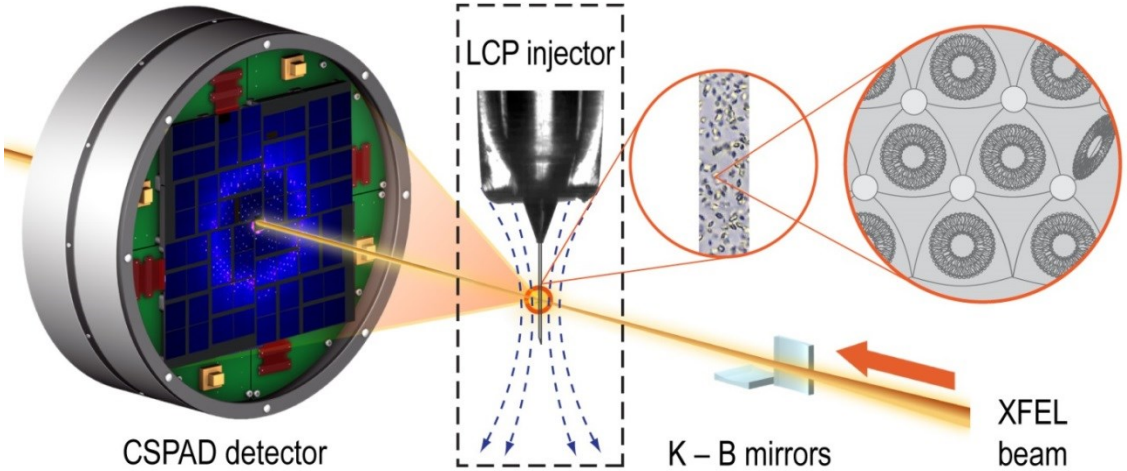
(a,b) Single-slice UV-TPEF images (depth of field ~20 µm) of two preparations of lysozyme crystals embedded in LCP captured by SONICC imager (Formulatrix).

(c,d) Outlines of microcrystals from images in a,b, obtained by an automatic crystal detection and counting algorithm, implemented using the ImageJ program (http://imagej.nih.gov/ij/).

LCP injector

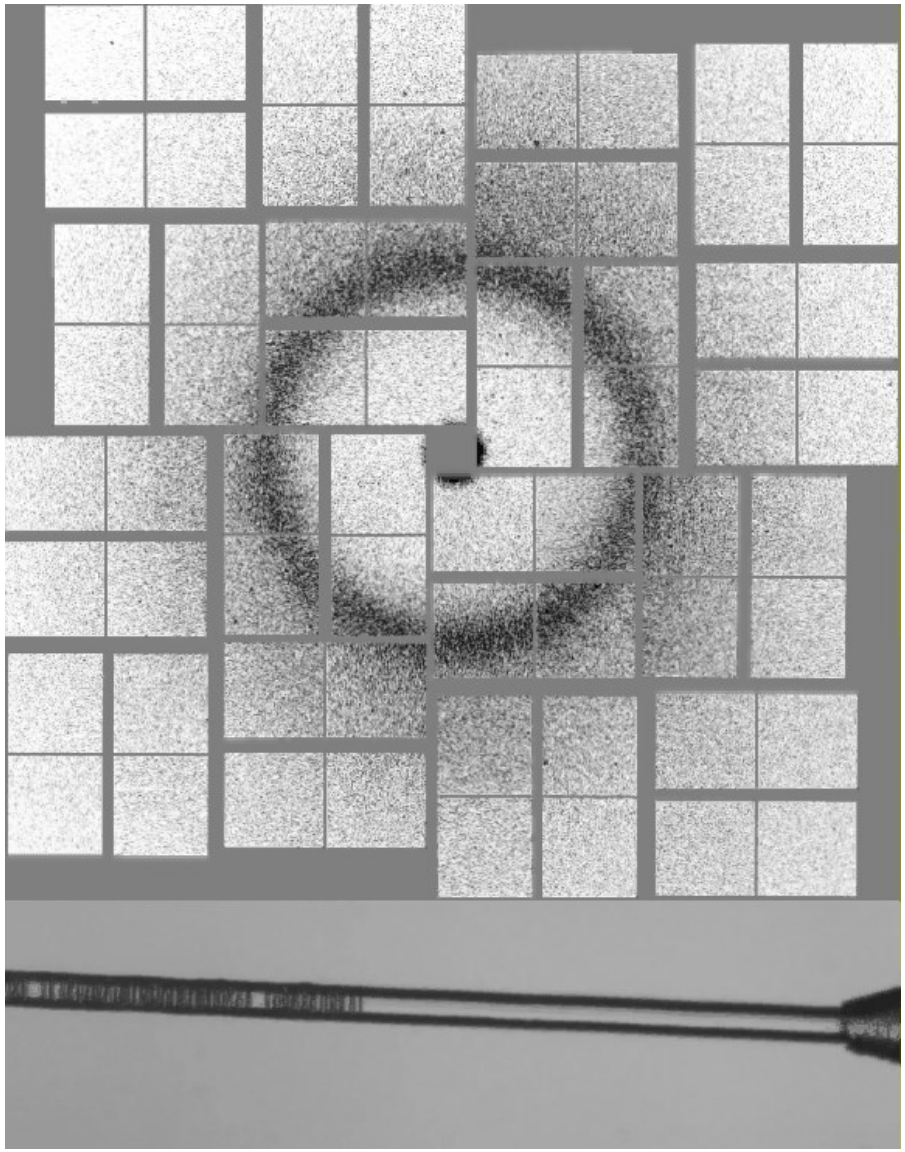


Weierstall et al., **2014** Nat Commun 5:3309



Diameter: 10-50 μm
Flow velocity: ~2.5 mm/s
Flow rate: ~10 – 300 nL/min
Distance between shots: ~20 μm

LCP-SFX data collection

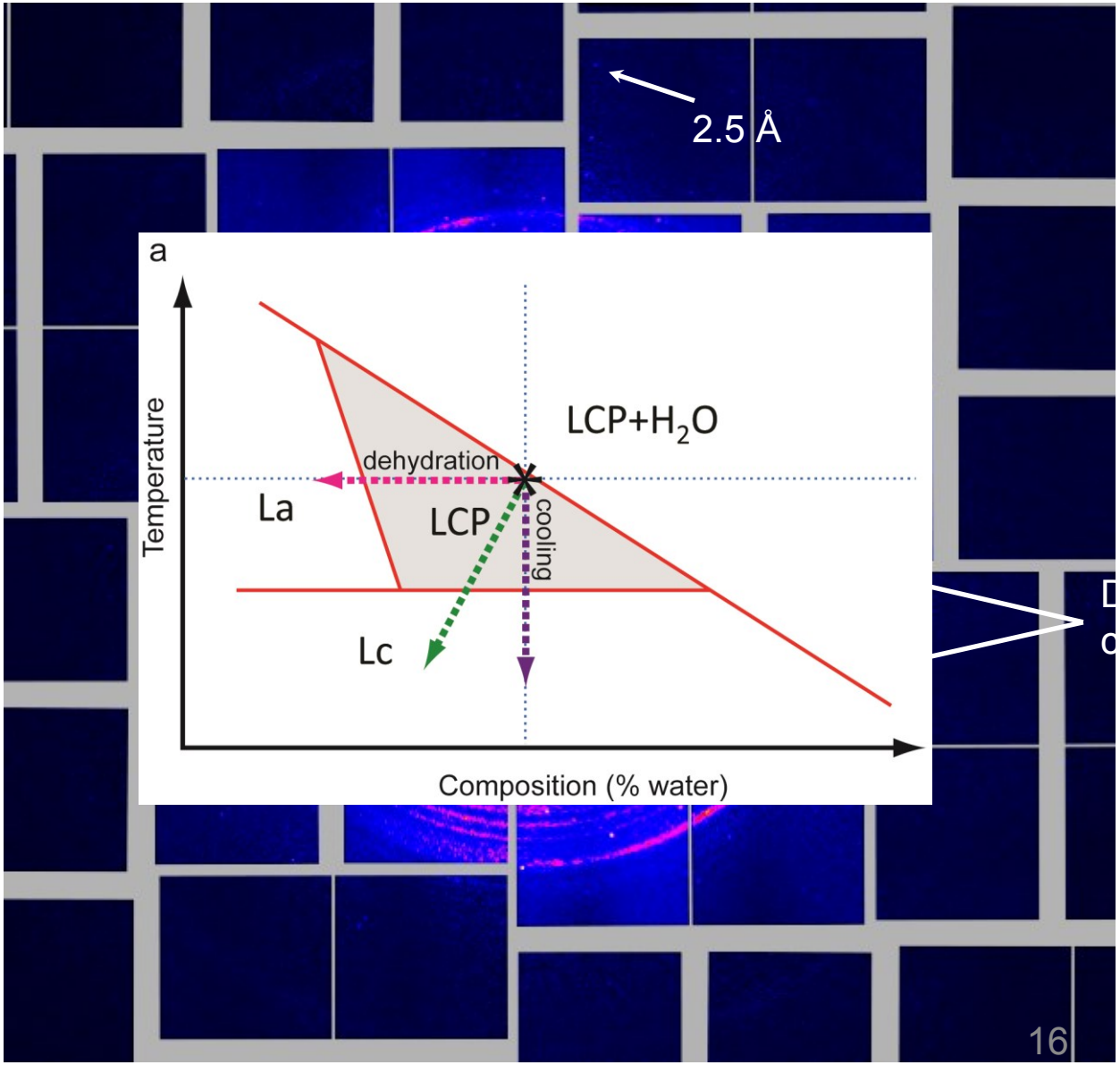


Slow motion video (slowed down 30x)
XFEL pulses attenuated to 1% of intensity

Diameter:	50 μm
Flow velocity:	2.5 mm/s
Flow rate:	300 nL/min
Distance between shots:	20 μm

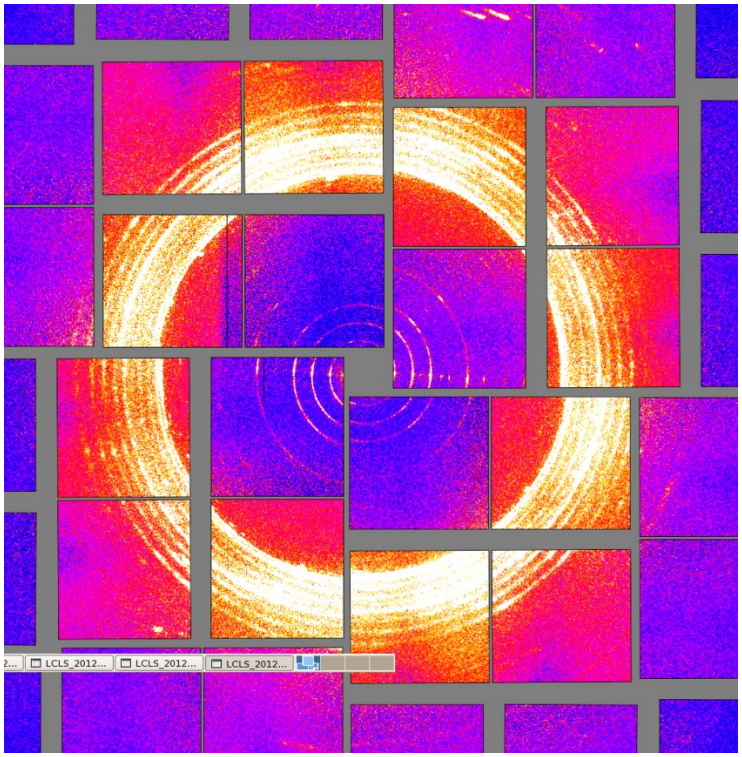
C. Stan *et al*

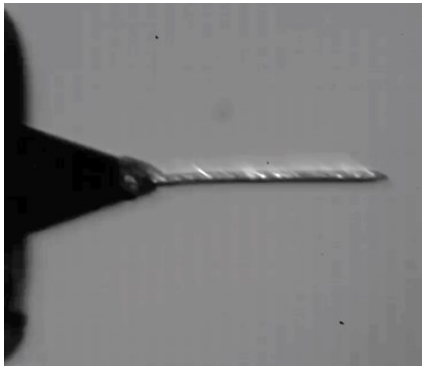
Adenosine A_{2A} receptor (LCLS, Feb, 2012)



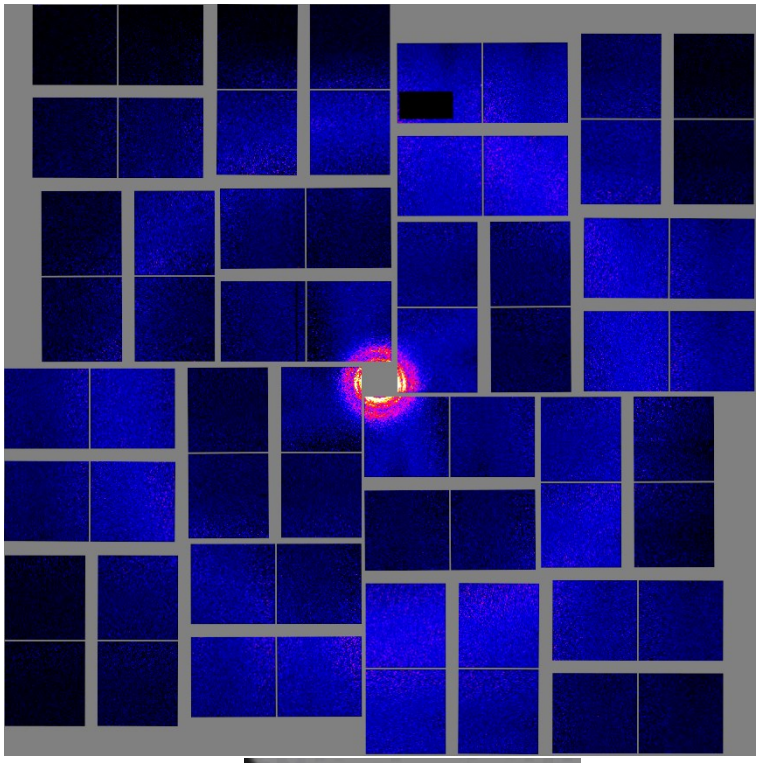
New LCP host lipid eliminates strong wide-angle powder diffraction background (LCLS, June, 2012)

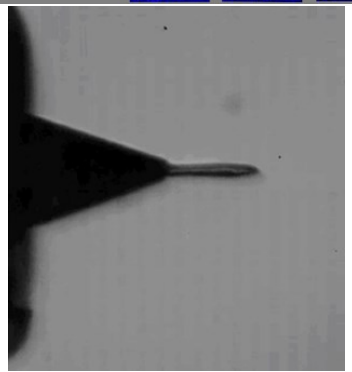
LCP host lipid: **9.9 MAG**





LCP host lipid: **7.9 MAG**

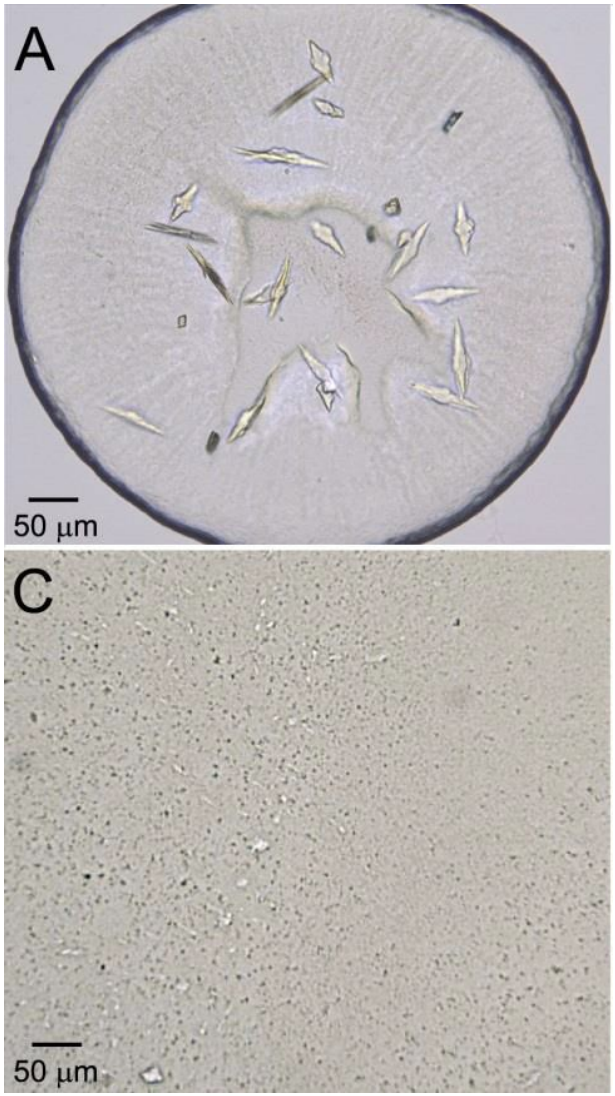




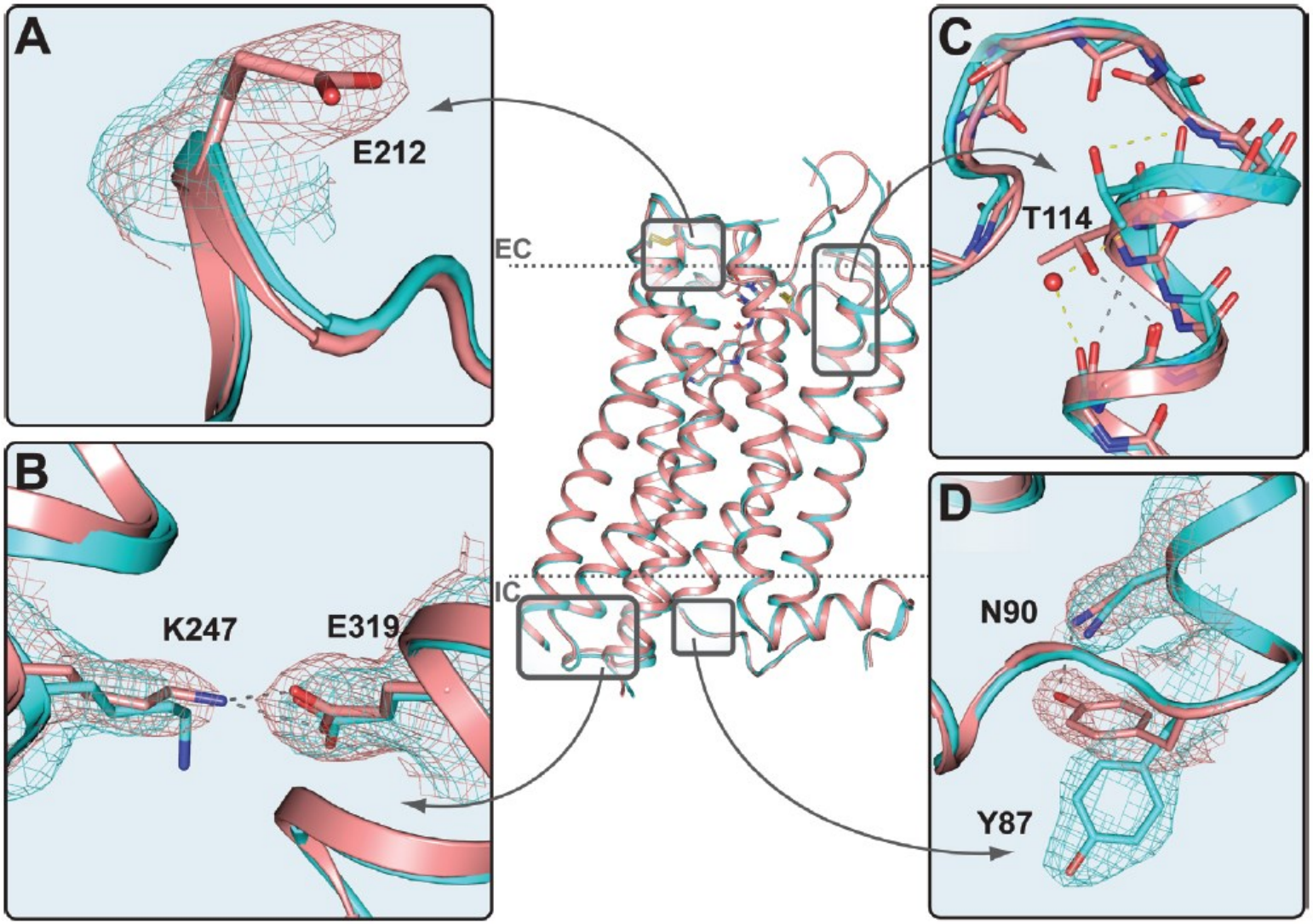
5-Hydroxytryptamine-2B (5-HT_{2B})

- Serotonin receptor
- CNS / behavioral
- cardiovascular
- immune system regulation
- recreational drugs

- 2.7 Å synchrotron structure available (Wacker et al. 2013)



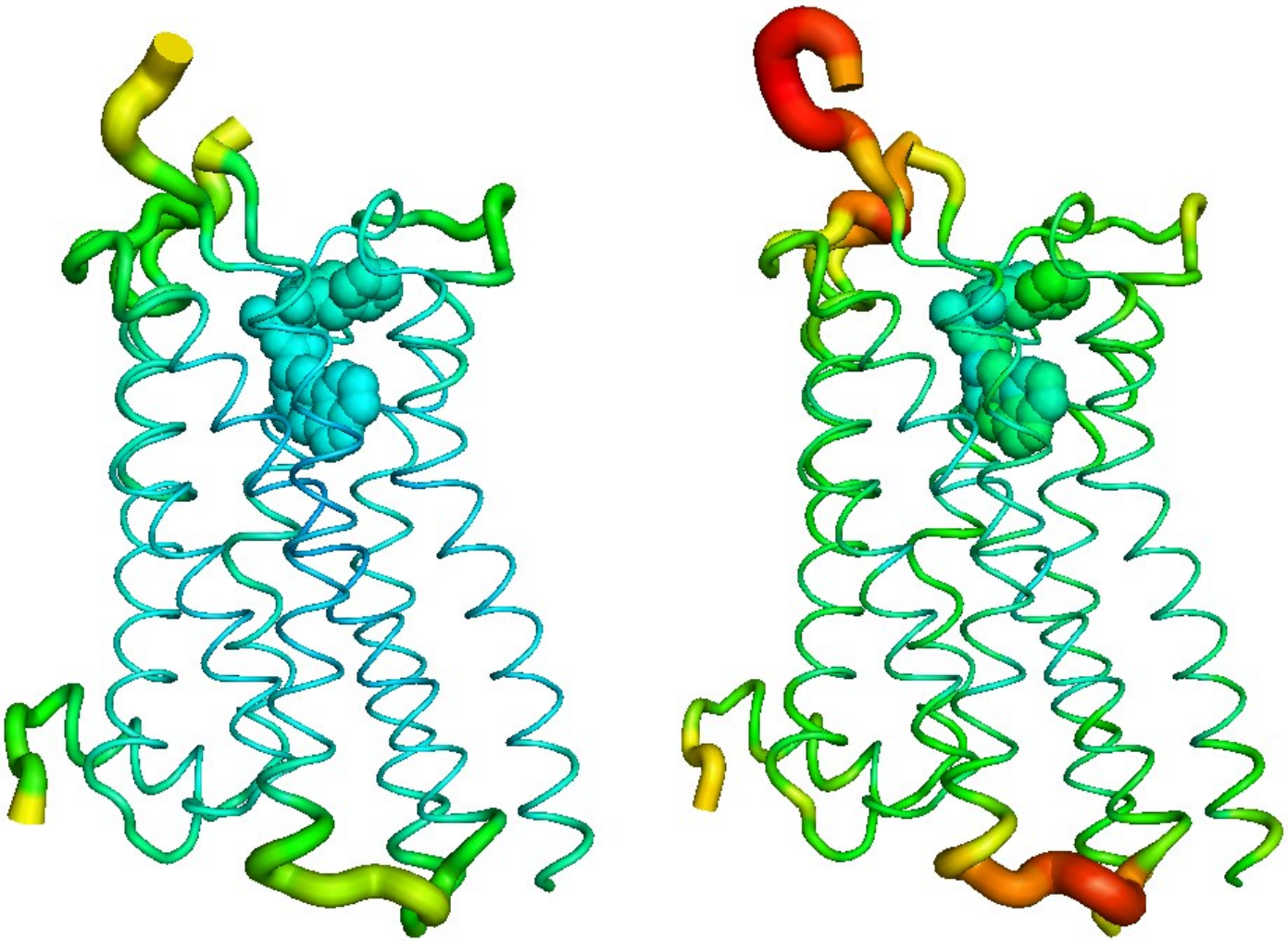
Differences 5-HT_{2B}-SYN and -XFEL model



Cyan: Synchrotron Magenta: XFEL

Liu *et al*, Science (2013)

Differences 5-HT_{2B}-SYN and -XFEL B-factors



Synchrotron

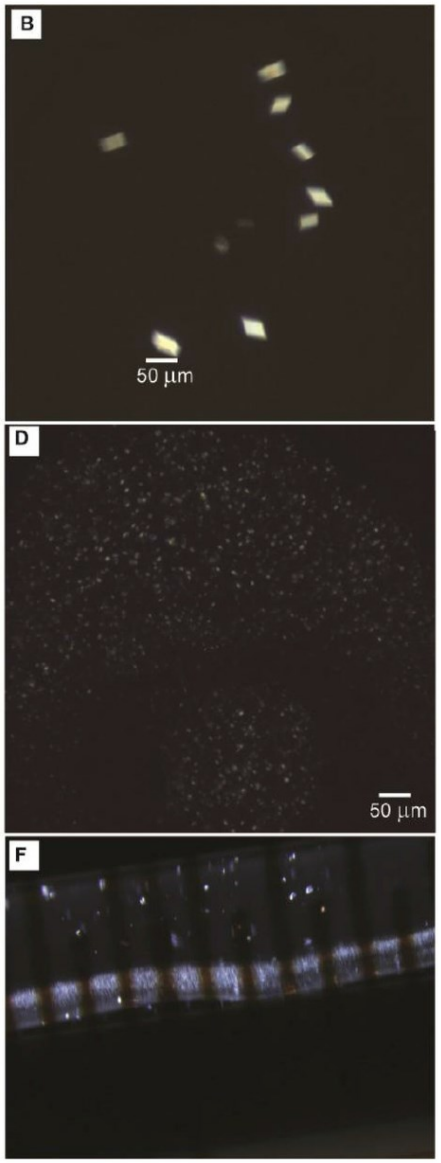
XFEL

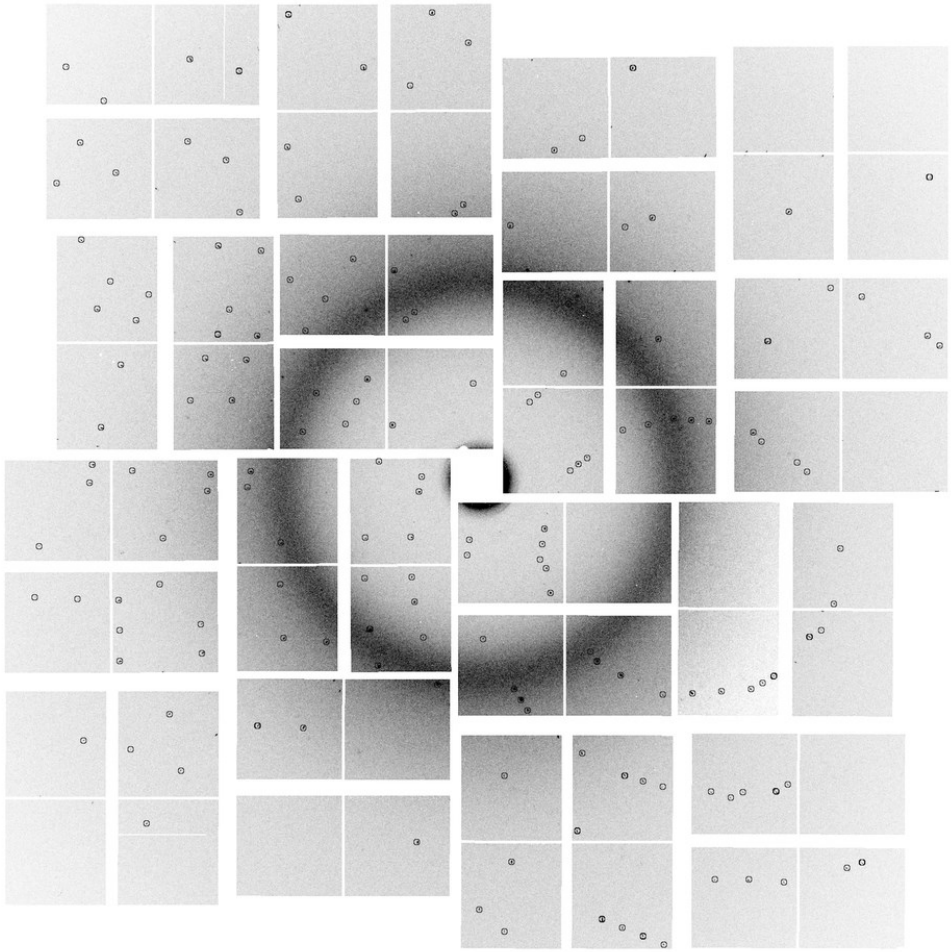
Liu *et al,* Science (2013)

Bi-functional peptide DIPP-NH_2 for delta-opioid (δ-OR) receptor

- ORs (**μ-, κ-, δ-,** NOP) involved in management of pain, mood states / neurophysiological processes
- Regulated by opioid peptides, (e.g. endomorphins, etc.)
- Alkaloid opiates are the most widely used analgesics for treatment of moderate to severe pain
- Single compounds with a mixed δ-OR antagonist/μ-OR agonist function have beneficial properties
- 1.8 Å DOR/naltrindole synchrotron structure was available

Crystals of δOR in complex with bi-functional peptide DIPP-NH_2





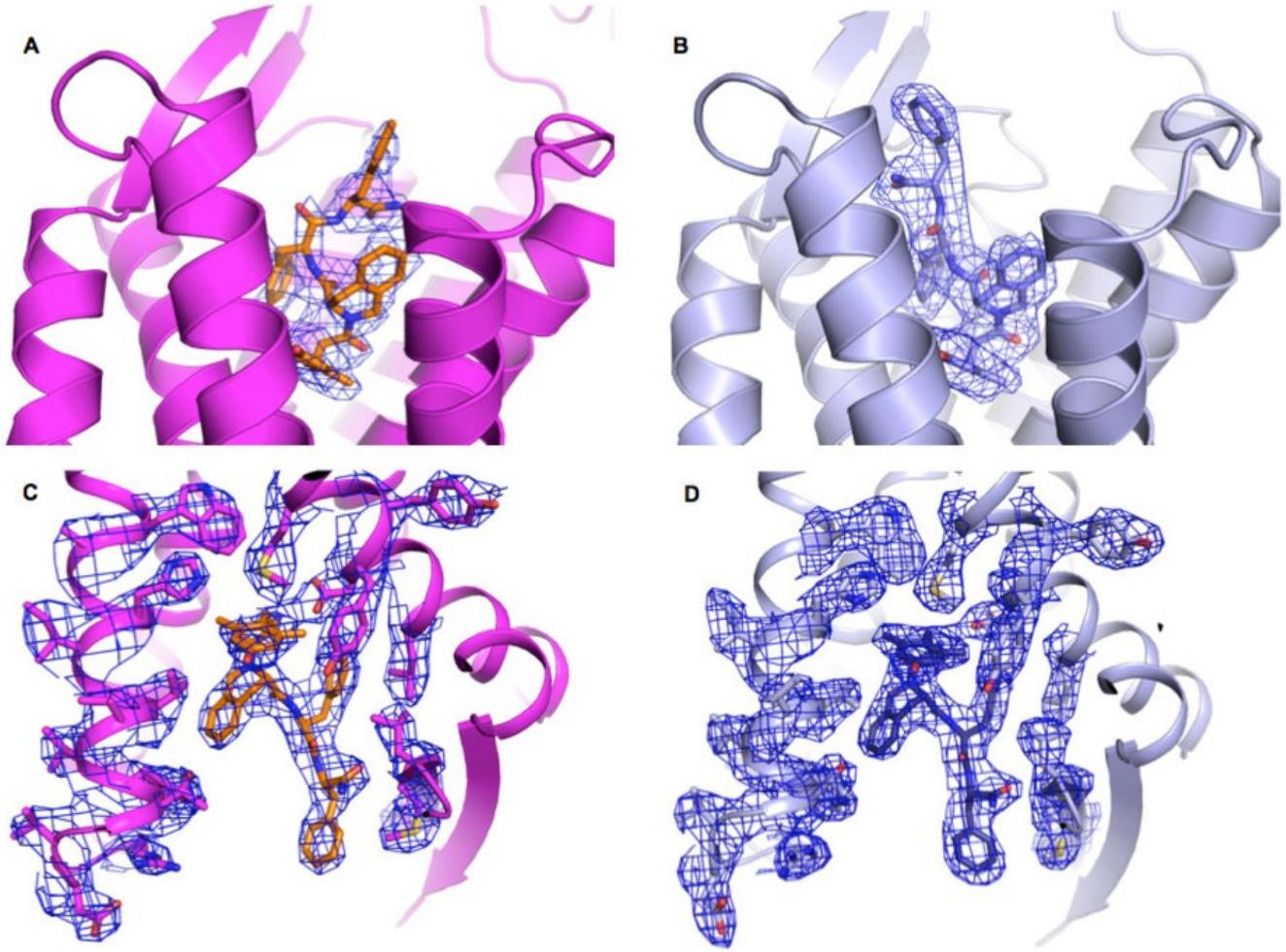
Fenalti *et al*, NSMB (2015)

Data collection statistics

	Synchrotron		XFEL	
Data collection				
Space group	C 1 2 1		C 1 2 1	
Cell dimensions				
a, *b*, *c* (Å)	160.55, 86.12, 94.68		156.2, 89.3, 96.4	
α, β, γ (°)	90.0, 92.2, 90.0		90.0, 92.3, 90.0	
Resolution (Å)	40.0-3.30 (3.51-3.30)*		33.5-2.70 (2.80-2.70) *	
R_{merge} or R_{split}	0.173 (0.875) R_{merge}		0.118 (0.879) R_{split}	
$I / \sigma I$	11.7 (1.9)		6.0 (1.3)	
Completeness (%)	96.3 (97.3)		100 (100)	
Redundancy	4.3 (4.4)		560 (209)	
Refinement				
Resolution (Å)	37.97-3.28		33.45-2.70	
No. reflections	17,904		34,653	
R_{work} / R_{free}	0.239 / 0.273		0.212 / 0.230	
No. atoms	A	B	A	B
Protein	2,921	2,901	3,039	3,044
DIPP-NH_2	49	49	49	49
Na^+	0	0	1	1
Lipids and other	14	0	80	79
B-factors				
Protein	93.1	96.1	66.3	66.5
DIPP-NH_2	93.8	101.5	62.7	56.8
Na^+	N/A	N/A	66.2	66.1
Lipids and other	92.5	N/A	74.3	77.8
r.m.s. deviations				
Bond lengths (Å)	0.007		0.005	
Bond angles (°)	1.1		0.9	

δOR in complex with bi-functional peptide DIPP-NH_2

Synchrotron



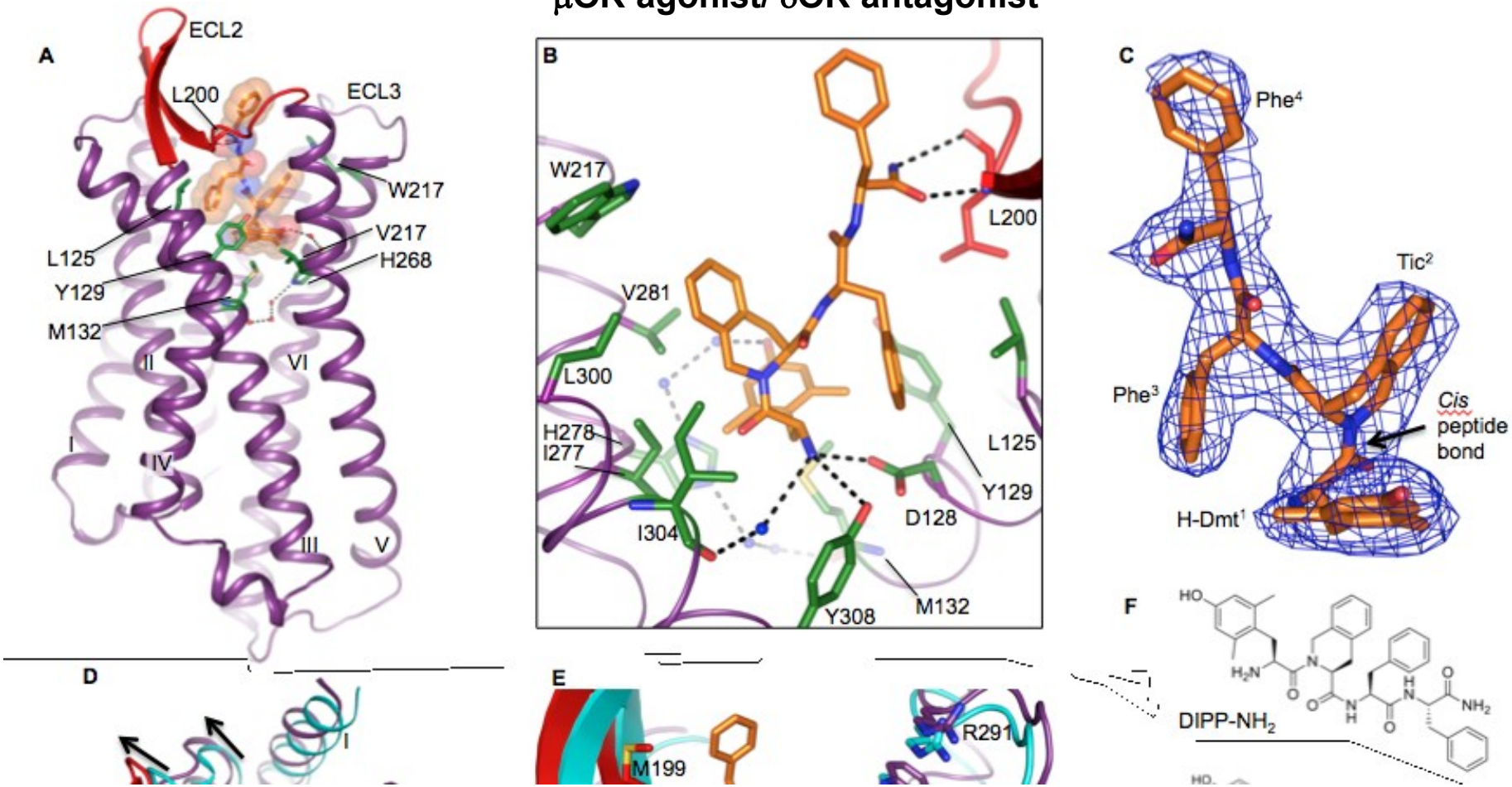
XFEL

Fenalti *et al*, NSMB (2015)

Structure of δOR in complex with bi-functional peptide DIPP-NH_2

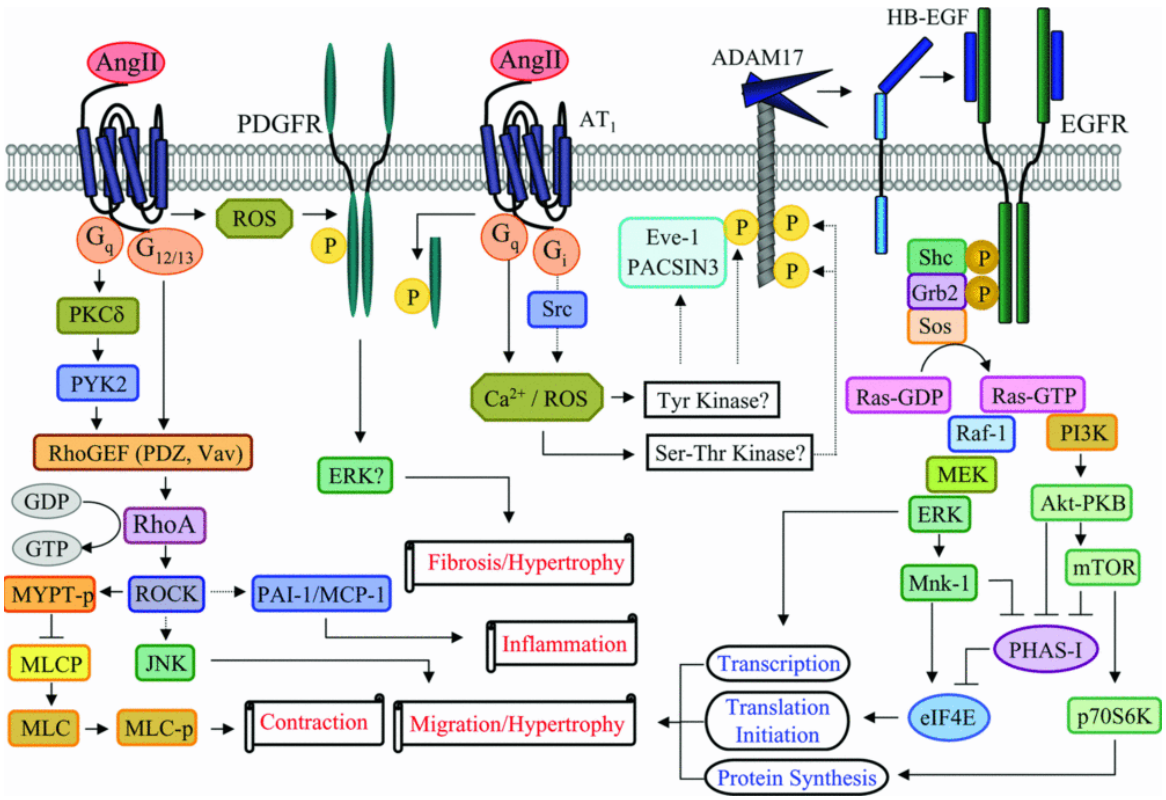
Synchrotron: 3.3 Å
XFEL: 2.6 Å

μOR agonist/ δOR antagonist



Fenalti et al, NSMB (2015)

AT1 mediated signaling cascades



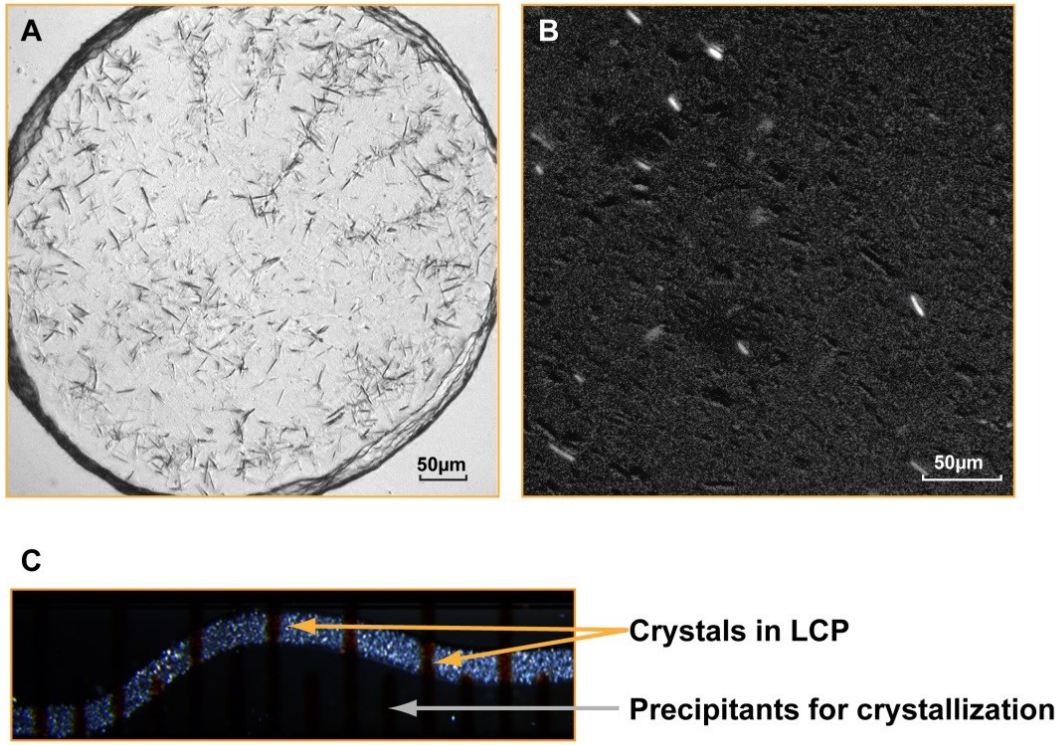
Hypertension:

- **Renin inh.**
- **ACE inh.**
- **ARBs**

- **Angiotensin II type 1 receptor (AT_1R) is a primary regulator for blood pressure maintenance**
- **G protein independent β-arrestin mediated signaling by AT_1R confers cardio-protective benefits**
- **ZD7155, a high affinity antagonist and precursor to the anti-hypertensive drug candesartan**

Higuchi *et al*, Clinical Science (2007)

AT1R data collection

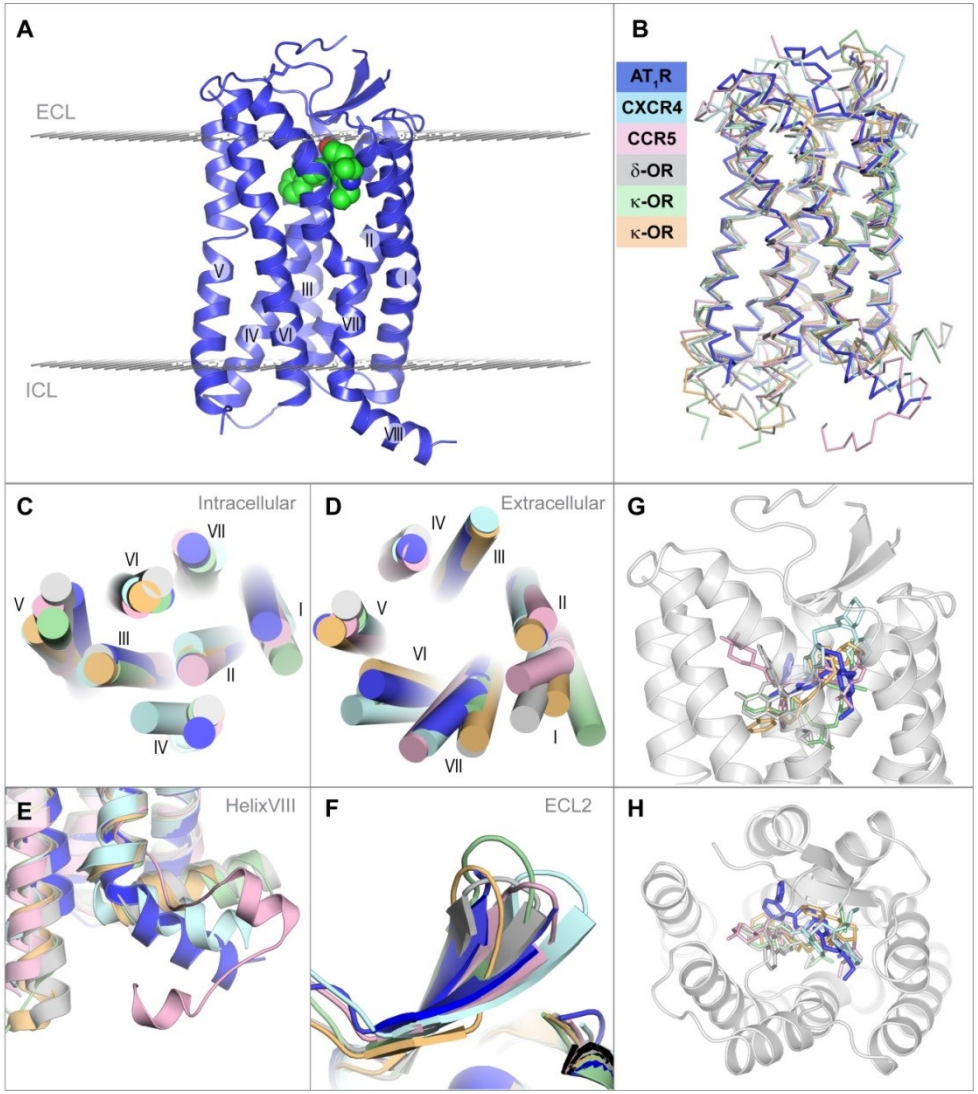


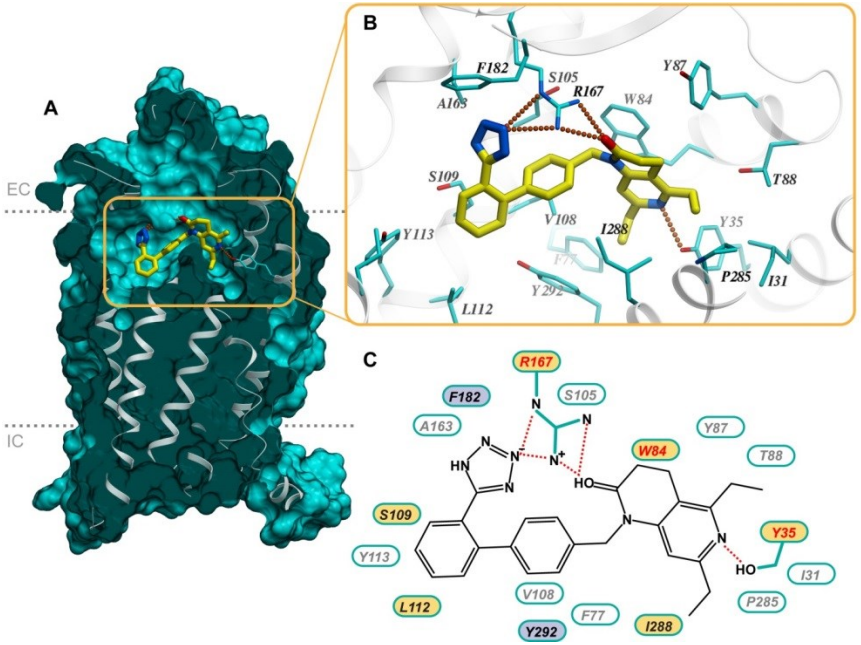
A. Bright field image
B. UV fluorescence image
C. Crystal sample in LCP syringe

AT_1R-ZD7155-XFEL	
Data collection	
Temperature (K)	294
Wavelength (Å)	1.56
Beam size (µm)	1.5×1.5
Average crystal size (µm)	10×2×2
Flux (ph/pulse) / Pulse duration (fs)	$1 \cdot 10^{11}$ / 36
Max dose per crystal (MGy)	75
Space group	*C*2
Unit cell parameters *a,b,c* (Å); β (°)	72.8, 41.0, 167.7; 99.4
Number of collected frames	2,764,739
Number of hits / indexed images	457,275 / 73,130
Number of total / unique reflections	14,415,424 / 11,190
Resolution (Å) [a]	32.64 – 2.90 (3.00 - 2.90)
Completeness (%)	100.0 (100.0)
Multiplicity	1,288 (215)
I/σ(I)	8.2 (0.84)
CC* [b]	0.999 (0.872)
R_{split} (%) [c]	9.8 (140)
Refinement	
Resolution (Å)	32.64 – 2.90
Number of reflections / test set	11,167 / 576
R_{work} / R_{free} (%)	22.8 / 27.4
Number of atoms	
Receptor / BRIL	3,077
Ligand	33

Zhang *et al,* Cell (2015)

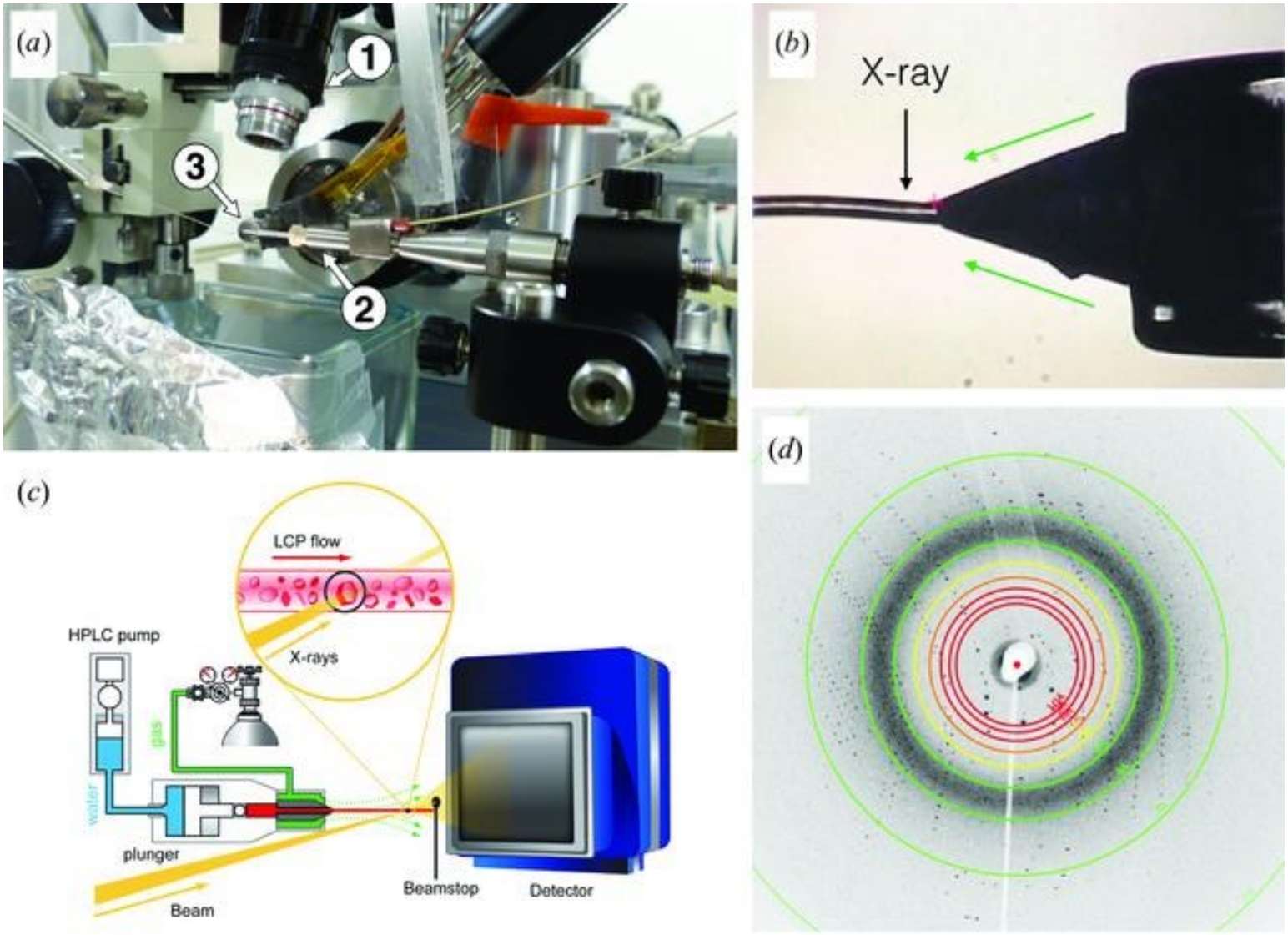
AT1R structure and ZD7155 binding mode





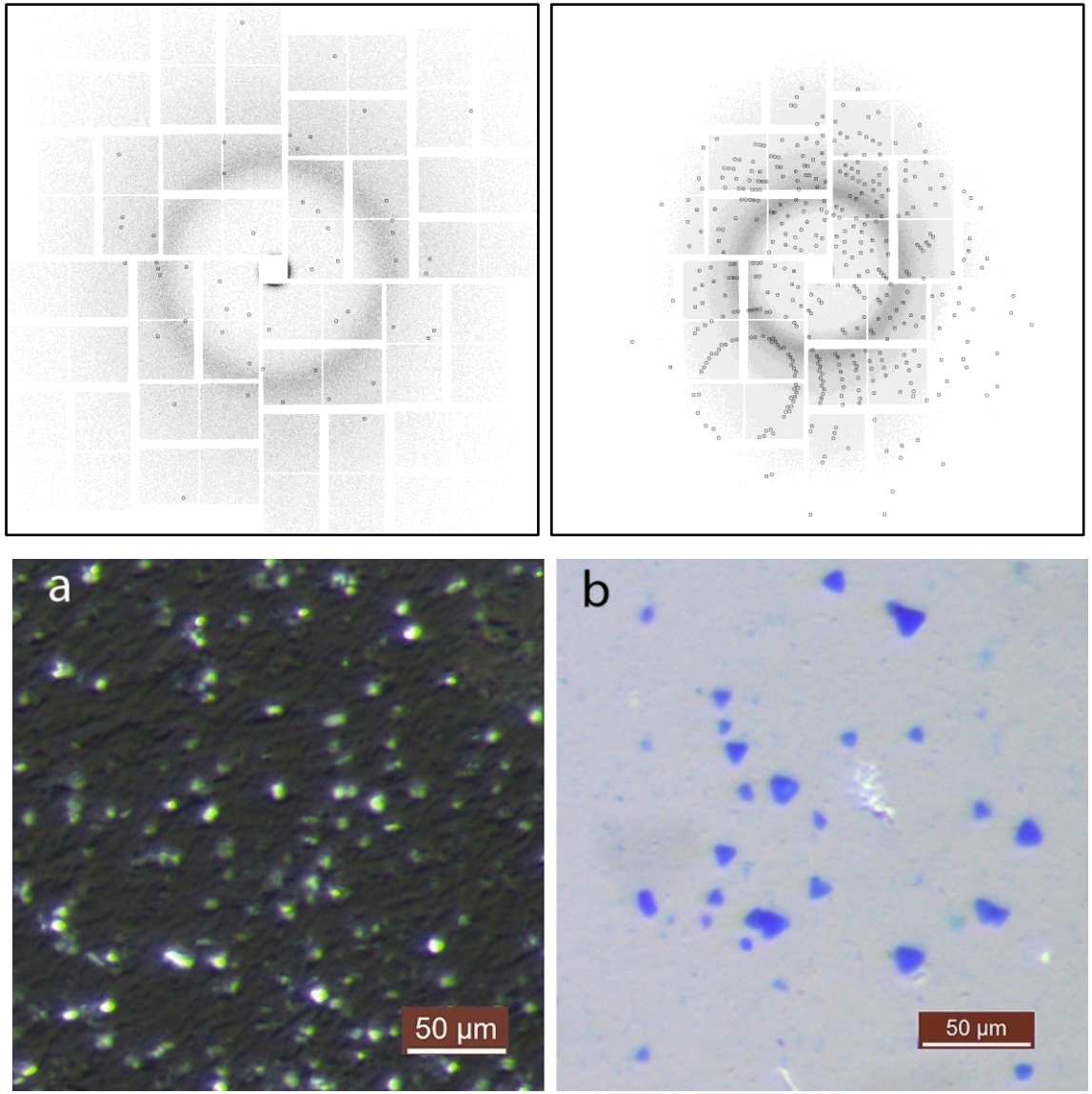
Zhang *et al*, Cell (2015)

Serial crystallography at synchrotron



Nogly et al. *IUCrJ*, 2015

LCP-SFX with Soluble Protein Crystals



Lysozyme (14 kDa) **Phycocyanin (~200 kDa)**

Fromme *et al.*
To be submitted

Summary

- Established LCP-SFX
  - Small crystals (<10 μm)
  - Ambient temperatures
  - No harvesting
  - Low sample consumption (<0.3 mg protein)
  - Increased resolution with respect to synchrotron data
  - Works with soluble protein crystals

- First human membrane protein at RT (serotonin receptor)
- Recognition of bi-functional peptide (opioid receptors)
- Novel GPCR structure (angiotensin receptor AT1R)

Future Directions

- Further improvements (smaller crystals, lower protein consumption, faster data collection)
- High resolution (< 2 Å) RT GPCR structure
- High dynamic range detectors needed to collect data from samples with high salt content
- Experimental phasing
- Structures of complexes
- Molecular movies

Acknowledgements

Wei Liu, Andrii Ishchenko, Daniel Wacker, Chong Wang, Gye Won Han, Gus Fenalti, Seva Katritch, Haitao Zhang, Linda Johansson, Ben Stauch, Alex Batyuk, Ray Stevens, **Vadim Cherezov**

THE SCRIPPS RESEARCH INSTITUTE

Trinity College, Dublin

Martin Caffrey, Dianfan Li

USC

Bryan Roth
Patrick Giguere
X.-P. Huang

UNC SCHOOL OF MEDICINE

Henry Chapman
Tom White, Anton Barty
Markus Metz

CFEL SCIENCE

ARIZONA STATE UNIVERSITY

Uwe Weierstall, Petra Fromme
John Spence, Raimund Fromme
Shibom Basu, Daniel James, Dingjie Wang. Nadia Zatsepin, Chris Kupitz, Ingo Grotjohann, Garrett Nelson, Marc Hunter

SLAC NATIONAL ACCELERATOR LABORATORY

Sebastien Boutet
Garth Williams, Claudiu Stan, Andy Aquila, Marc Messerschmidt

DESY

Thank you!