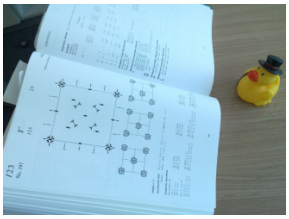
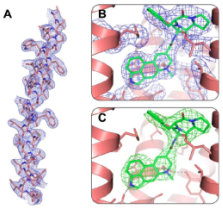
Crystallography and molecular imaging using X-ray lasers.

Fundamentals and applications

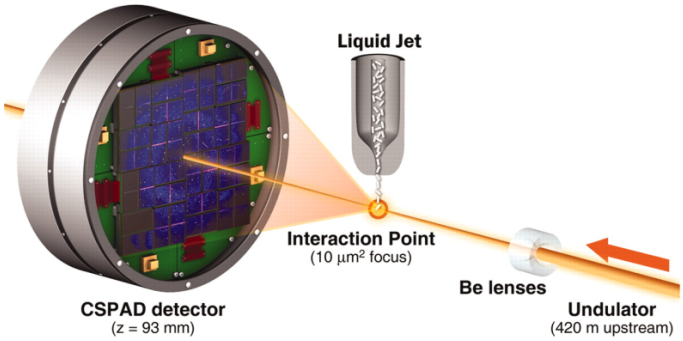




Thomas A. White
CERN Accelerator School
Hotel Scandic Emporio, Hamburg
9th June 2016

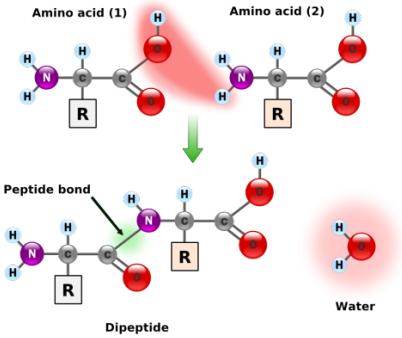
HELMHOLTZ ASSOCIATION | CFEL SCIENCE | DESY

What is this all about?

- Study the **structure and function of proteins** ...
- ... using a **hard X-ray free-electron laser** ...
- ... by using **many small crystals** of the protein.



What is a protein?



(plus ligands, saccharides, the odd metal ion and other bits and pieces)

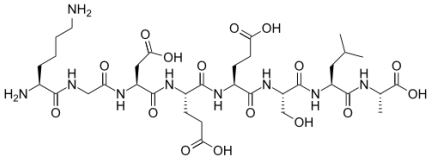
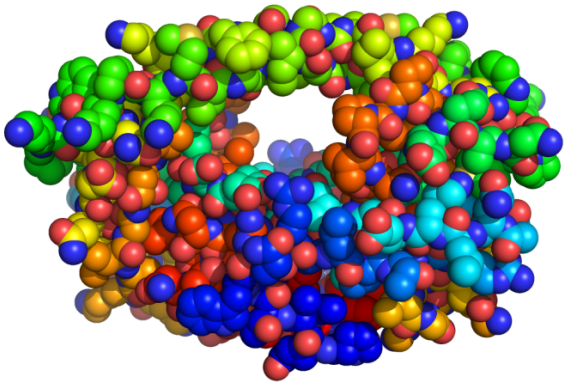


Image: https://en.wikipedia.org/wiki/File:Peptidformationball.svg

Why study the structure of proteins?

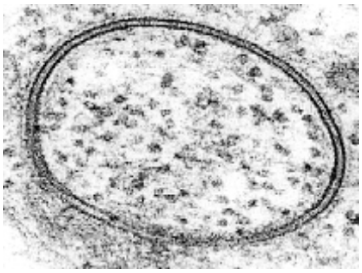
> The function of proteins is determined by their structure, not elemental composition.
> All proteins have the same empirical formula (ratio of elements).

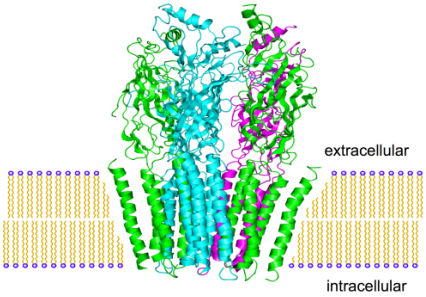


More? http://pdb.rcsb.org/motm/6

Why study the structure of proteins?

> Membrane proteins are particularly difficult to study.





Nicotinic acetylcholine receptor (nAChrR)

Images: https://en.wikipedia.org/wiki/File:Annular_Gap_Junction_Vesicle.jpg
https://en.wikipedia.org/wiki/File:NAchR_2BG9.png

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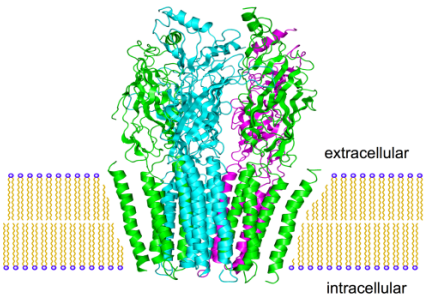

Nicotinic acetylcholine receptor (nAChrR)

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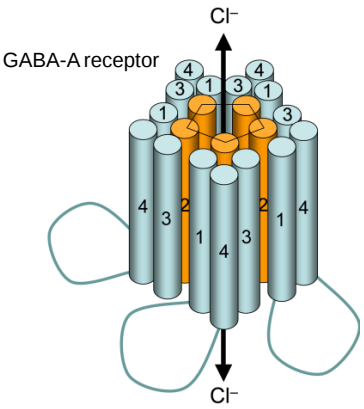
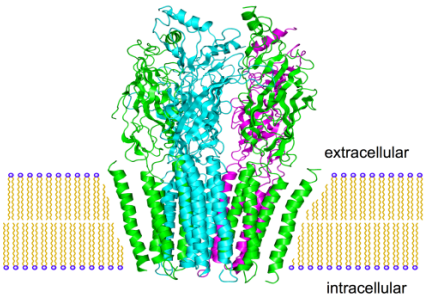




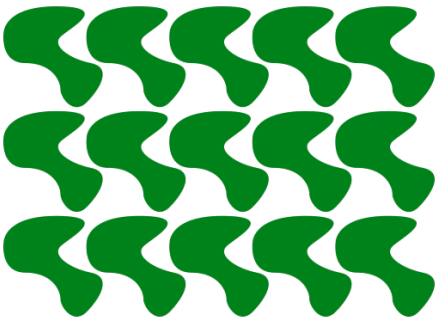
Nicotinic acetylcholine receptor (nAChrR)

Images: https://en.wikipedia.org/wiki/File:NAchR_2BG9.png
https://en.wikipedia.org/wiki/File:NAchR_2BG9.png

Studying proteins using X-rays

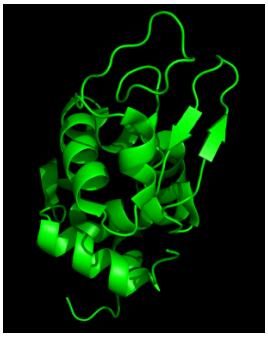
> What is a crystal?

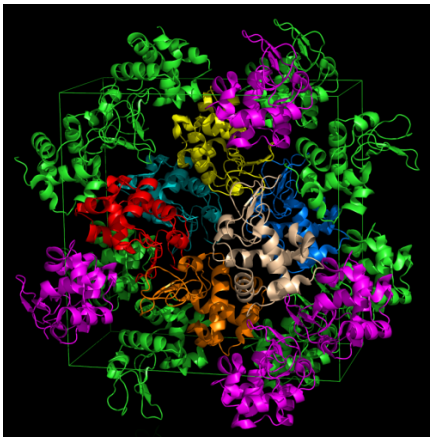
A molecule of "blob"



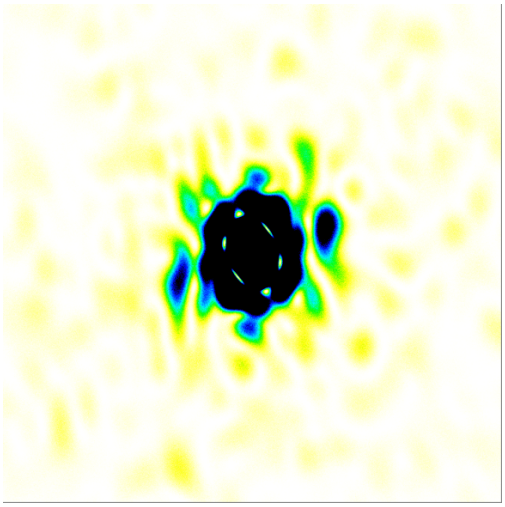
A crystal of "blob"

Studying proteins using X-rays



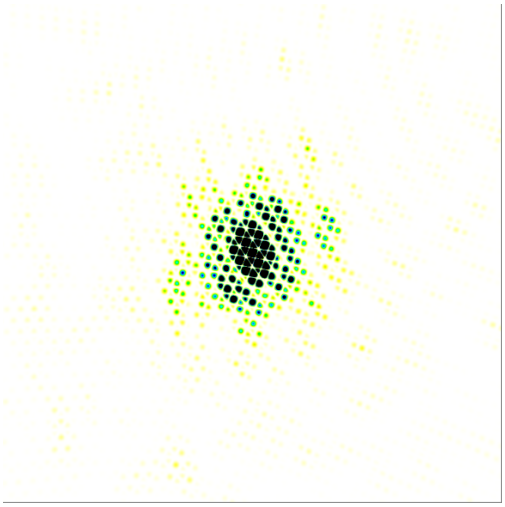


Studying proteins using X-rays



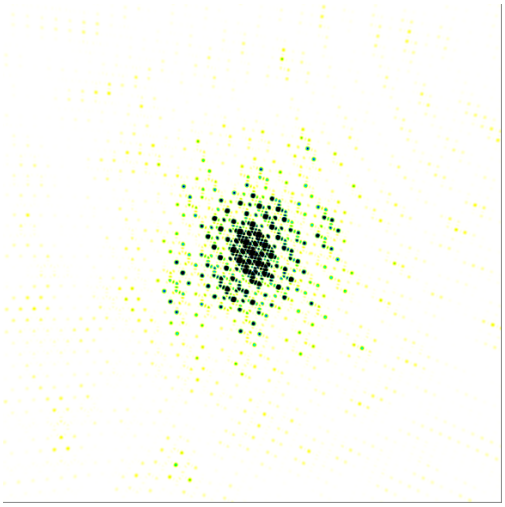
1 molecule

Studying proteins using X-rays



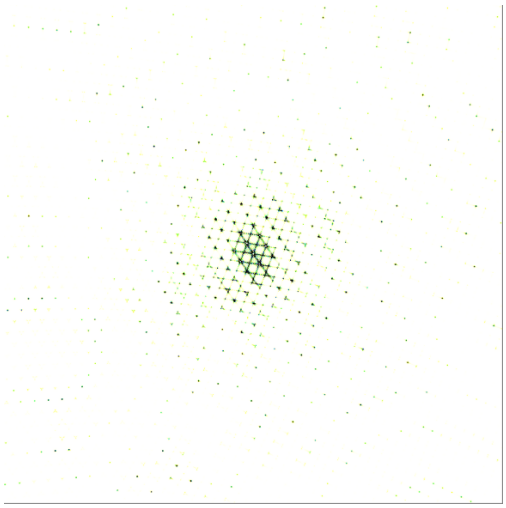
2x2x2 lattice

Studying proteins using X-rays



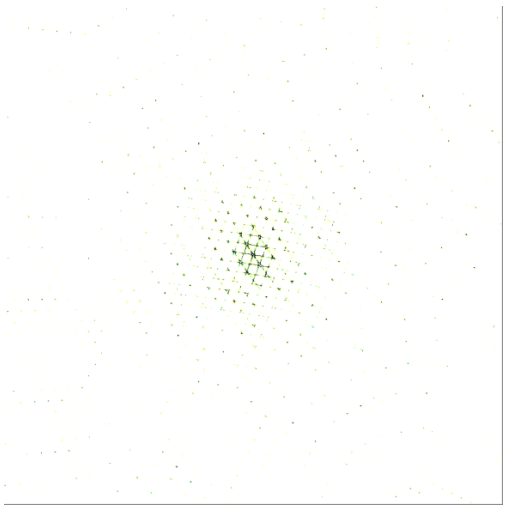
3x3x3 lattice

Studying proteins using X-rays



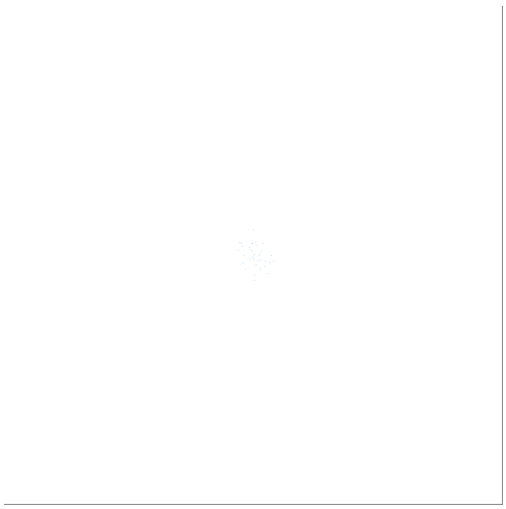
10x10x10
lattice

Studying proteins using X-rays



20x20x20
lattice

Studying proteins using X-rays



1 molecule

Studying proteins using X-rays

> We would prefer to use single molecules rather than crystals, but the signal level is very low:

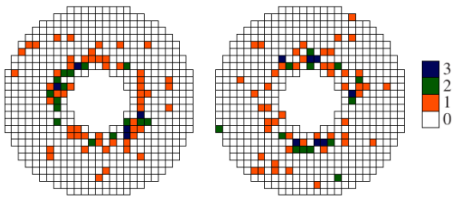


FIG. 1. (Color online) The same or different? Two simulated measurements (noisy diffraction patterns) in a single-particle imaging experiment, where color (white, orange, green, and blue) represents recorded photon counts (0, 1, 2, and 3). Are the differences in the measurements purely statistical, or do they reflect a different view (orientation) of the particle?

⟶ Loh and Elser, Phys. Rev. E 80 (2009) 026705

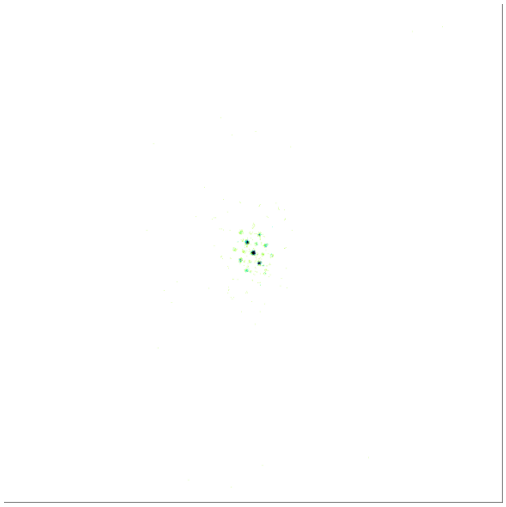
Studying proteins using X-rays

1 molecule

Studying proteins using X-rays

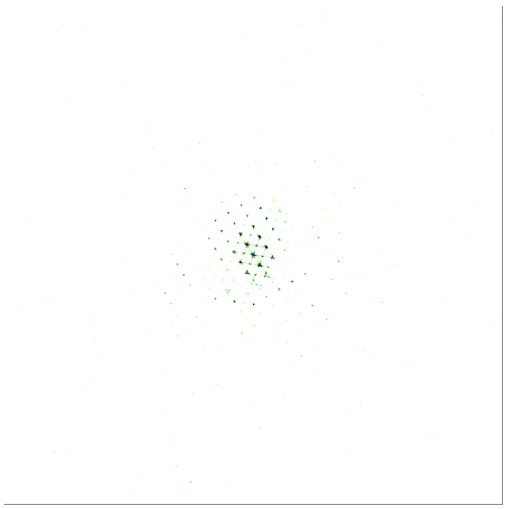
2x2x2 lattice

Studying proteins using X-rays



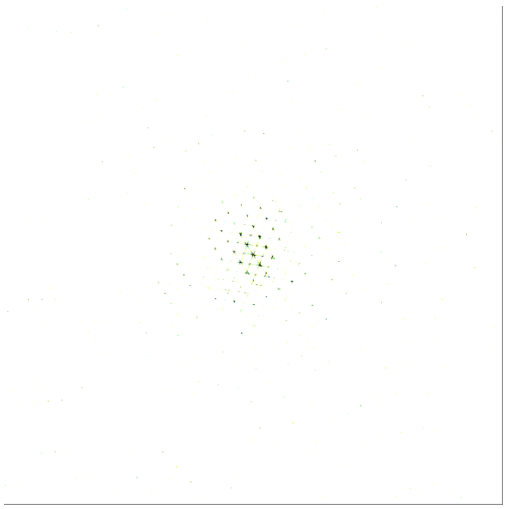
3x3x3 lattice

Studying proteins using X-rays



10x10x10 lattice

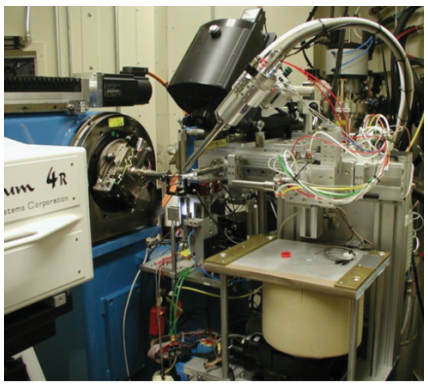
Studying proteins using X-rays



20x20x20 lattice

Crystallographic data collection

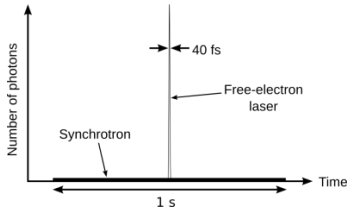
> Goniometer, one crystal (or a few crystals) for entire dataset

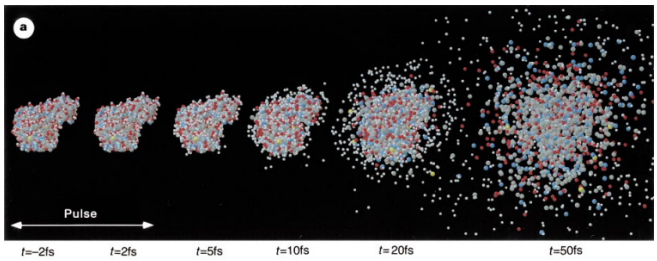


Why use a free-electron laser?

Very short, very intense pulses of X-rays mean we can "side-step" radiation damage.

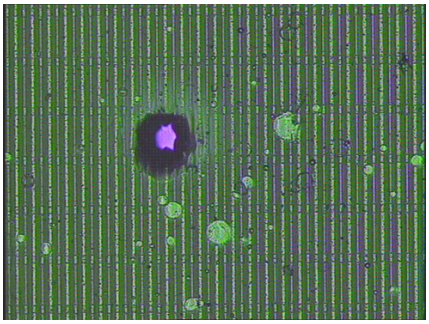
R. Neutze et al., "Potential for biomolecular imaging with femtosecond X-ray pulses", Nature 406 (2000) p752.

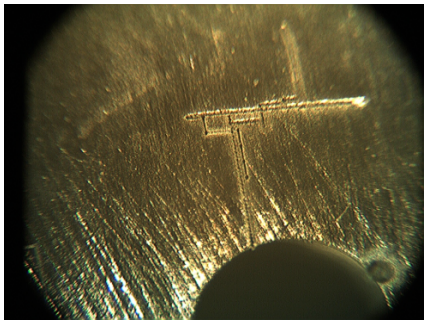




Radiation damage

> X-rays damage crystals
(... and the detector, and the shroud, and ...)

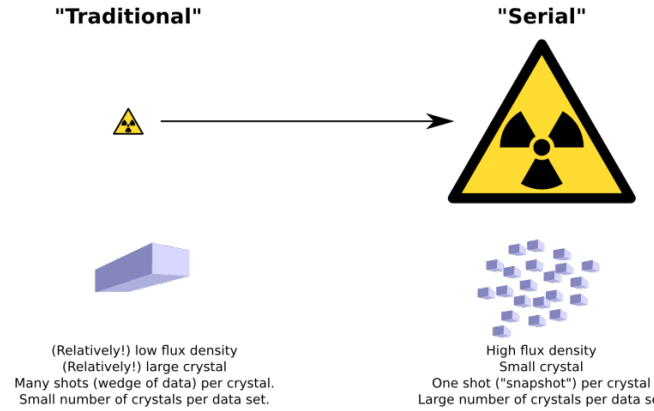




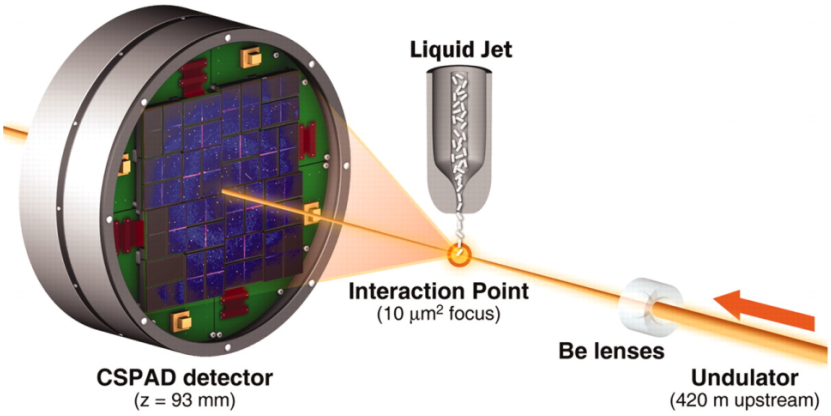
G. Weidenspointner et al., Proc. SPIE (2011) 80780U

Multi-crystal data collection

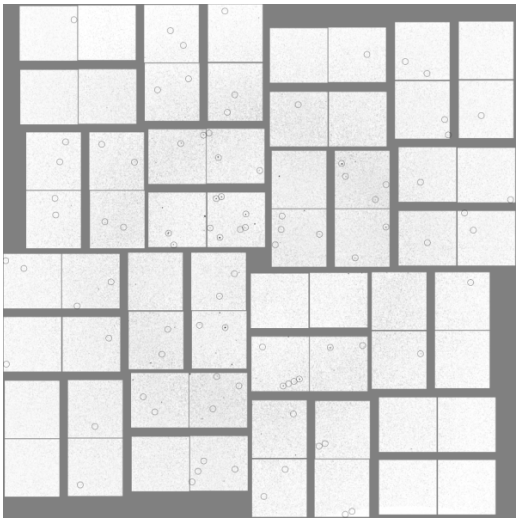
- We want to study more and more difficult proteins.
- Size of crystals down, intensity up, damage up, number of crystals up.
- Limit: one diffraction pattern per crystal: "serial crystallography".



Serial crystallography in practice

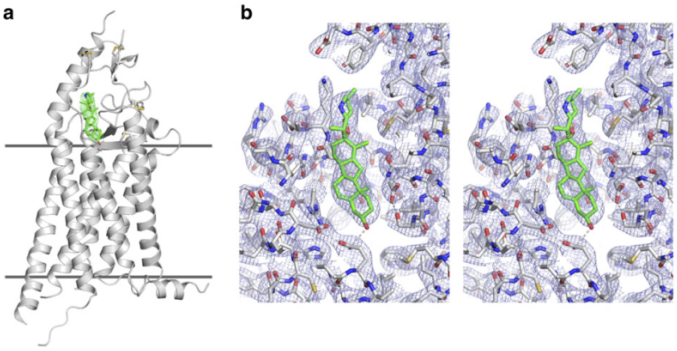


Serial crystallography in practice



Example: Human receptors

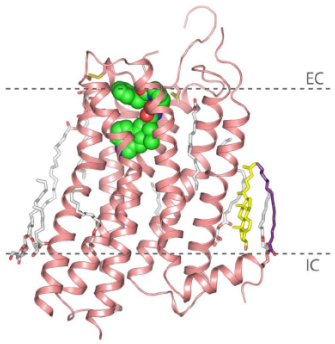
> Weierstall et al., Nature Communications 5:3309 (2014)



"Smoothened" receptor

Example: Human receptors

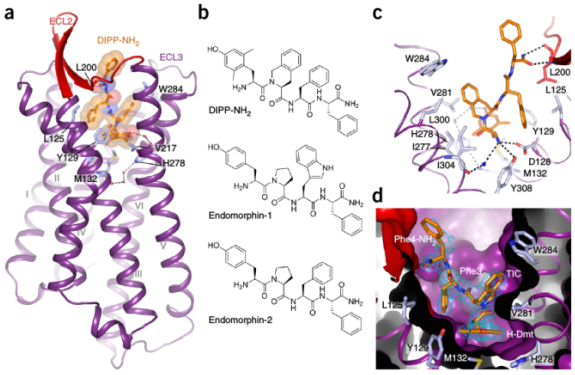
> Liu et al., Science 342 (2013) p1521



5HT2B receptor

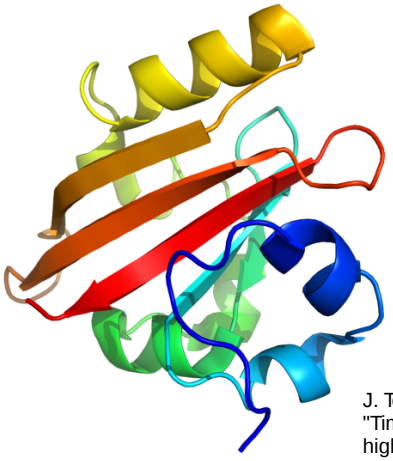
Example: Human receptors

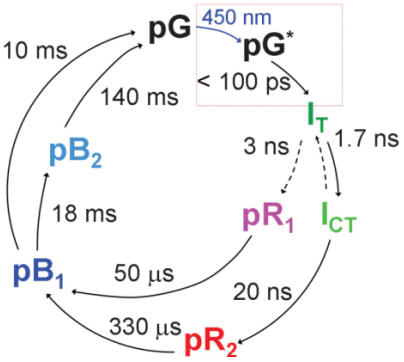
> Fenalti et al., Nature Structural and Molecular Biology 22 (2015) p265



Delta-opioid receptor

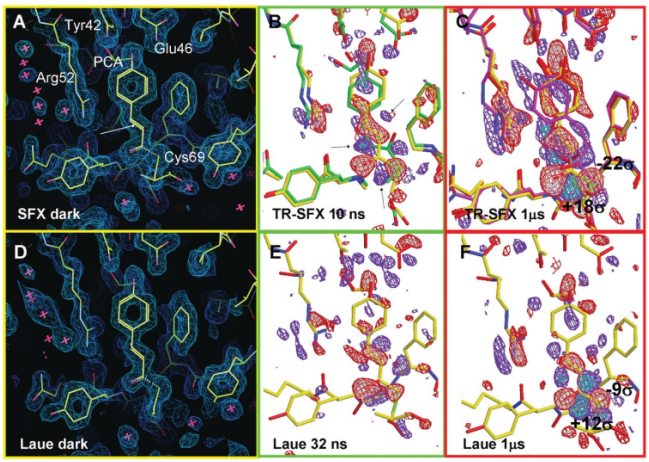
Photoactive Yellow Protein



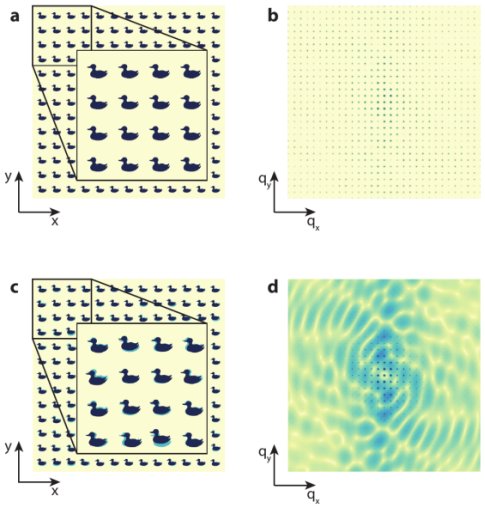


J. Tenboer, S. Basu, N. Zatsepin, K. Pande et al. "Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein". Science 346 (2014) p1242

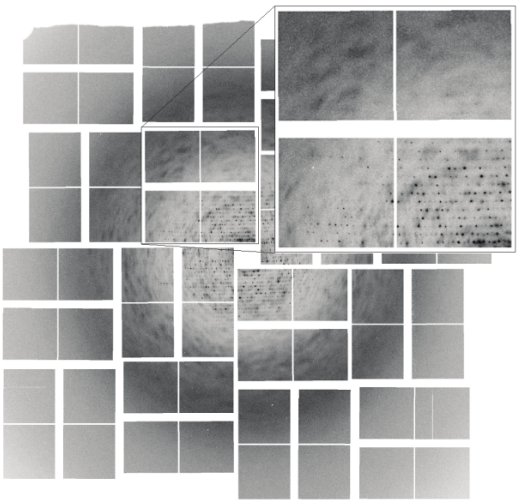
Photoactive Yellow Protein



Molecular Imaging



Molecular Imaging



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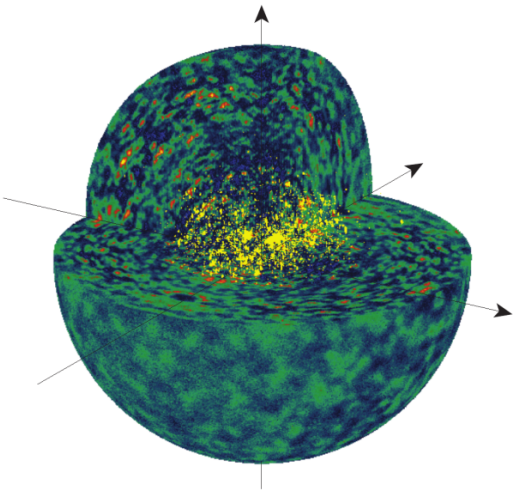

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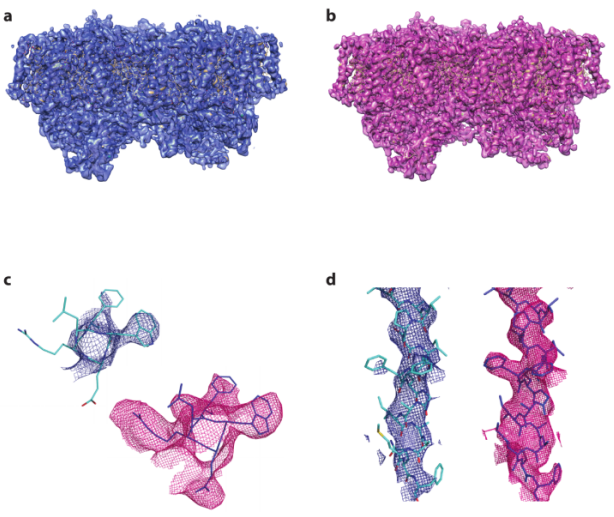

Useful references

First FEL crystallography experiment:
H. N. Chapman, P. Fromme et al., Nature 470 (2011) p73

Liquid jet sample injector ("Gas dynamic virtual nozzle"):
D. P. DePonte, U. Weierstall et al., J. Phys. D: Appl. Phys. 41 (2008) 195505

Lipidic cubic phase extrusion injector:
U. Weierstall, D. James, C. Wang et al., Nature Communications 5:3309 (2014)

Molecular imaging using imperfect crystals:
K. Ayyer, O. M. Yefanov, D. Oberthür et al., Nature 550 (2016) p202

Loads more references on CrystFEL website: **https://www.desy.de/~twhite/crystfel**

Email me: **taw@physics.org**