Biological nanocrystallography using FELs – an overview

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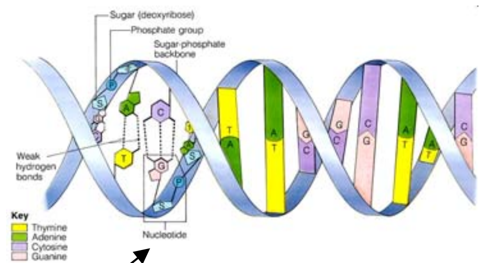
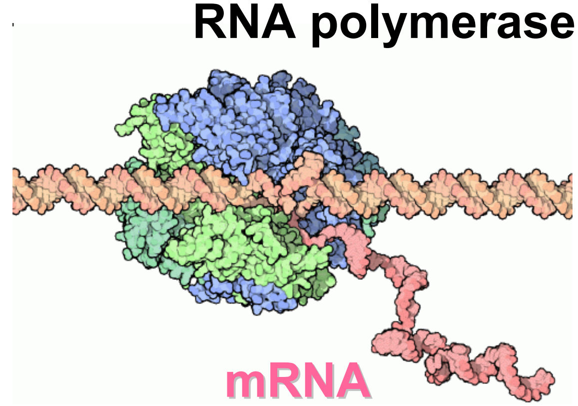
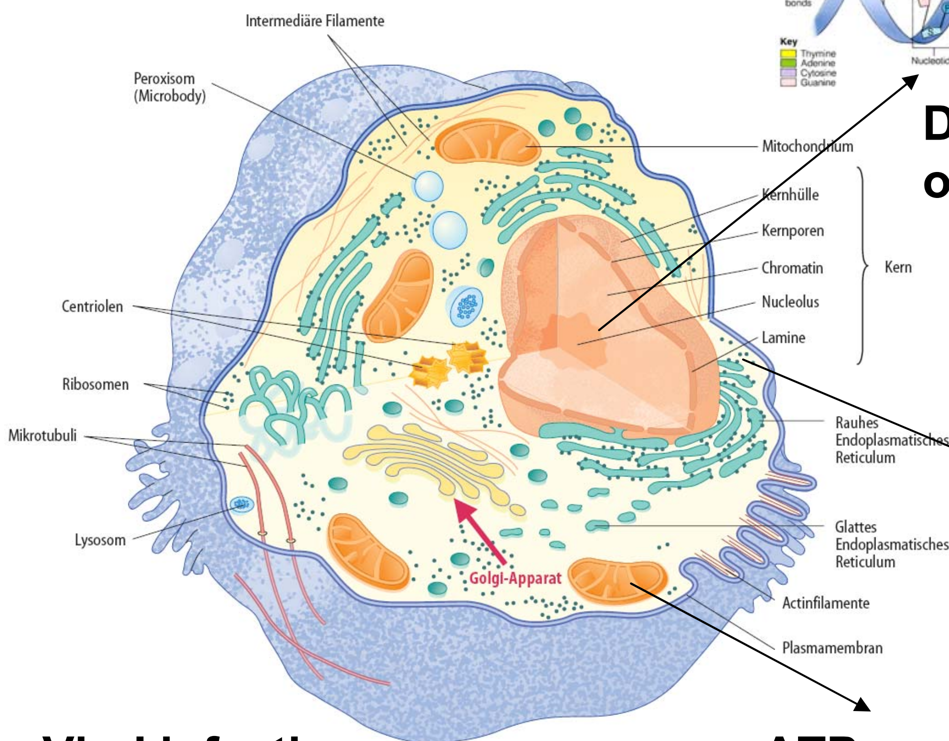
Cells are the basis of life



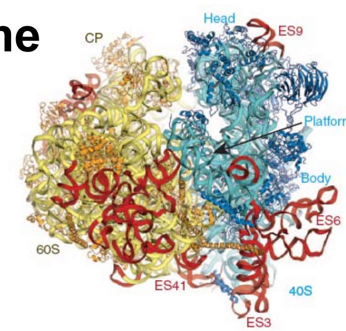
Transcribed by RNA polymerase



DNA: storage of genetic info

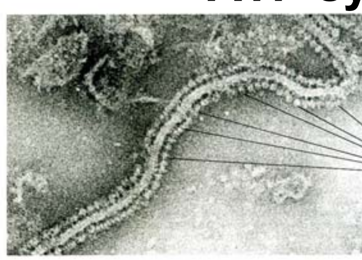


Translated and synthesized in protein: ribosome



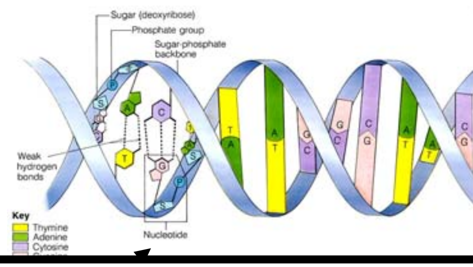
Viral infection

ATP synthesis



F_1/F_0 ATP synthase

Cells are the basis of life



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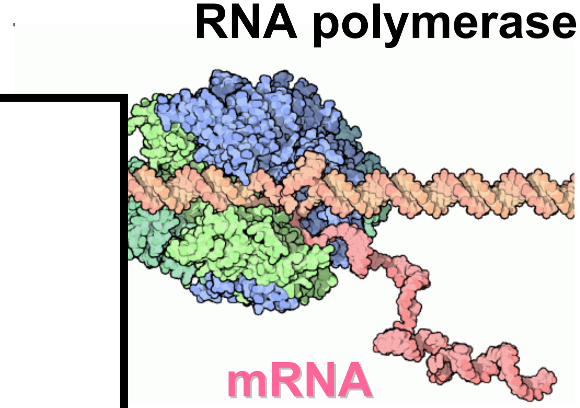
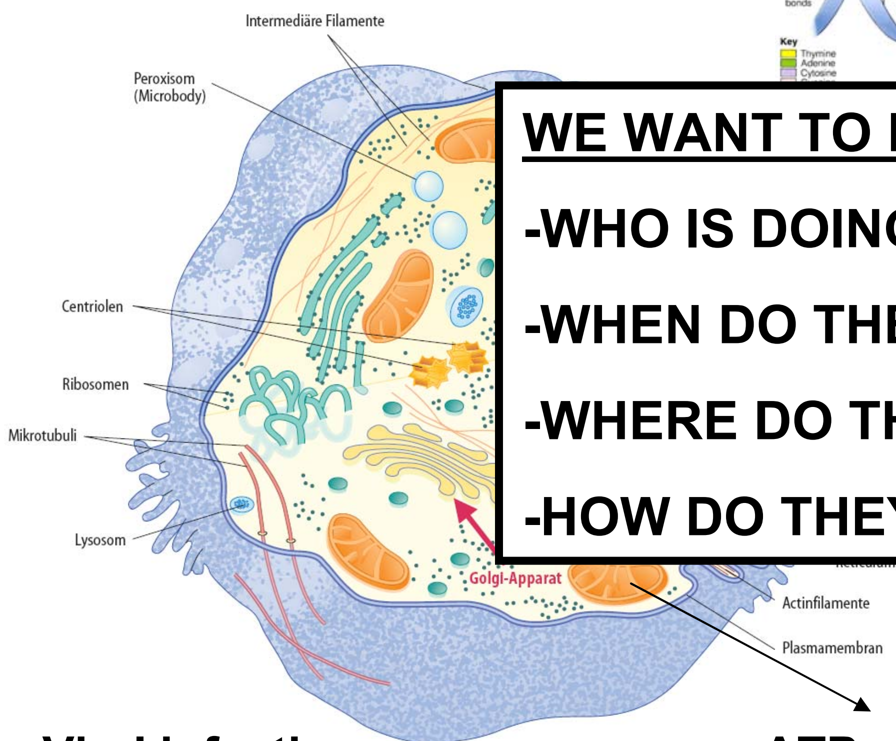




<u>WE WANT TO KNOW:</u>

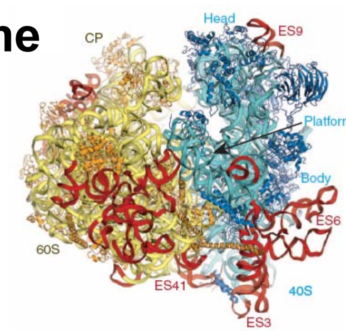
-WHO IS DOING WHAT ?

-WHEN DO THEY DO IT ?

-WHERE DO THEY DO IT ?

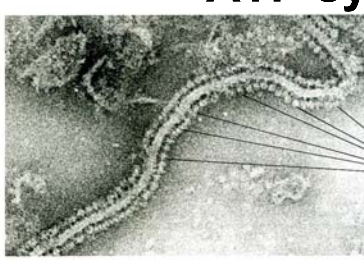
-HOW DO THEY DO IT ?

l synthesized
osome



Viral infection

ATP synthesis



F_1/F_0 ATP synthase

X-ray Crystallography

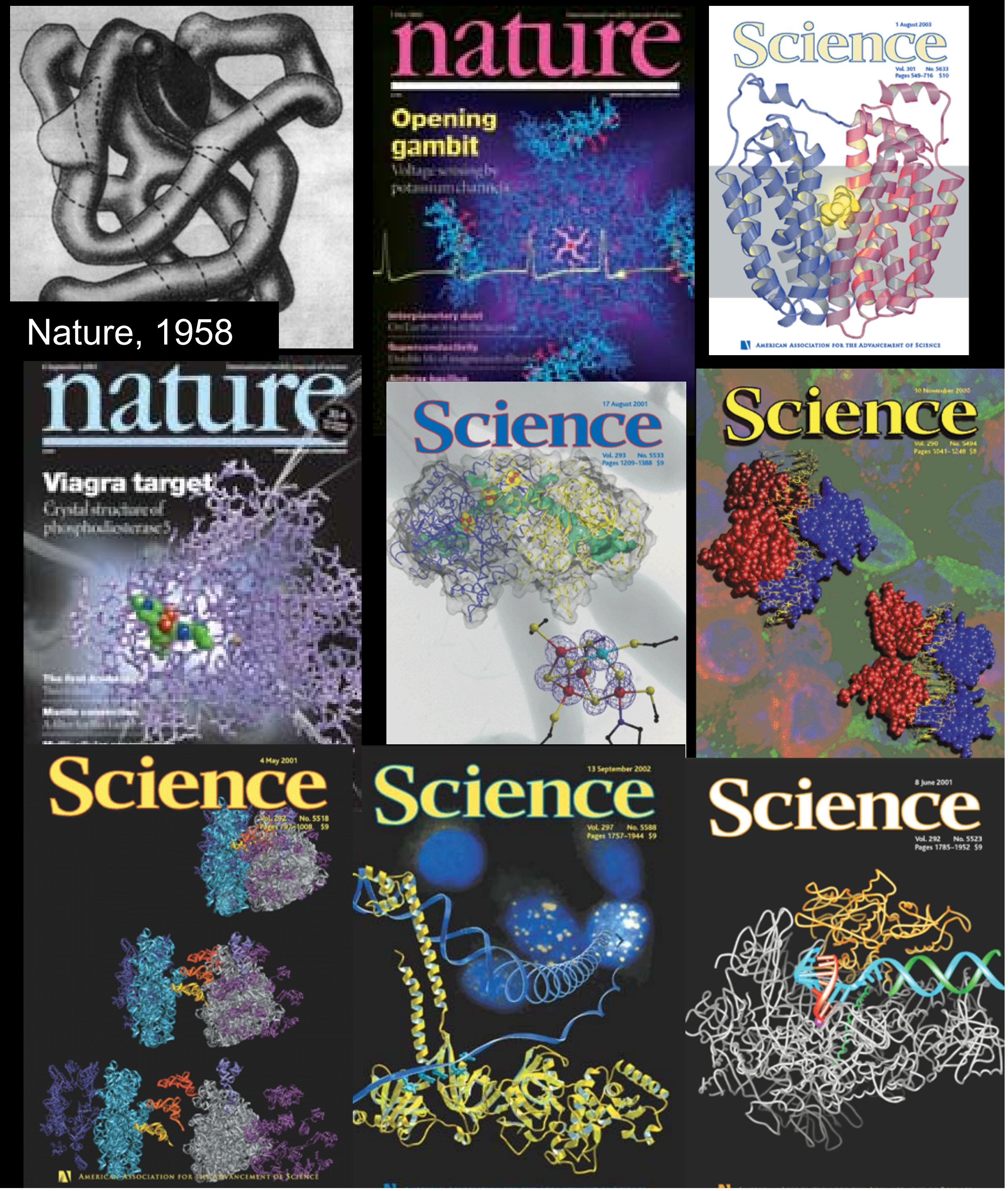
Elucidation of structures of macromolecules with the aim of understanding the chemical mechanisms underlying biological function.

Applications

1. Cell & Molecular Biology
2. Chemistry & Chemical Physics
3. Drug Discovery

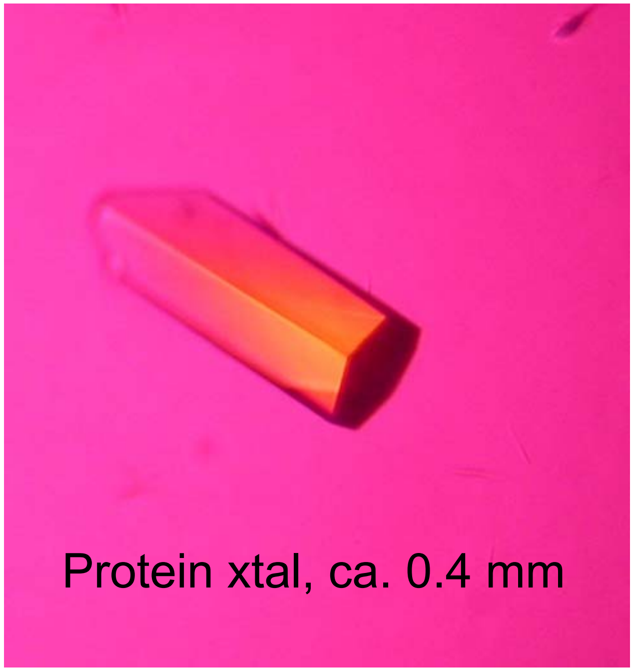
Advantages

1. Mature discipline that continues at a high level of achievement.
   X-ray structures: ~43,066
   (NMR structures: ~ 7,285)
2. Imaging method with a mol. size limit > 10^6 Daltons
3. Facilitated by synchrotron sources



Nature, 1958

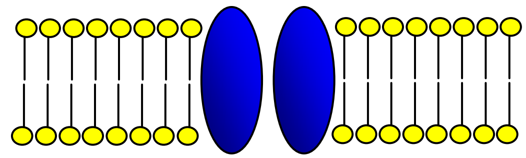
Protein Crystallography requires well-ordered, macroscopic crystals



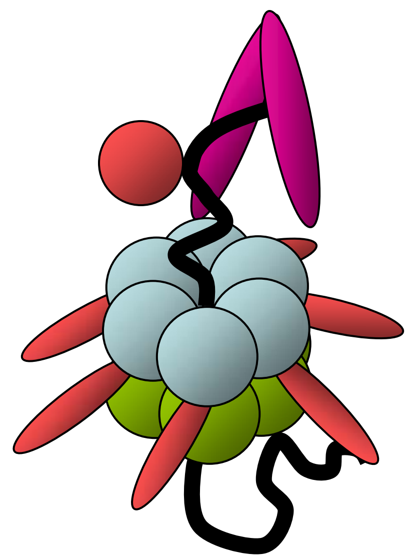
Protein xtal, ca. 0.4 mm

<u>**Particularly difficult to crystallize:**</u>

-membrane proteins, glycosylated proteins
(extremely interesting, many drug targets are membrane proteins and/or are glycosylated)



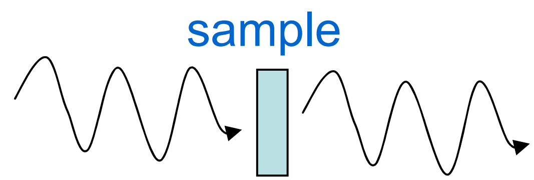
-large complexes
(important to understand the whole cell)



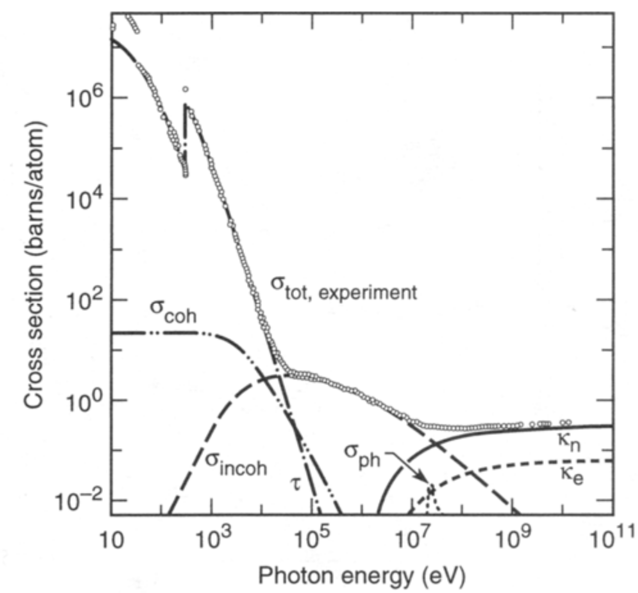
Can we get away without (macroscopic) crystals?
-single particles?
-nanocrystals?
-other ordered scaffolds?

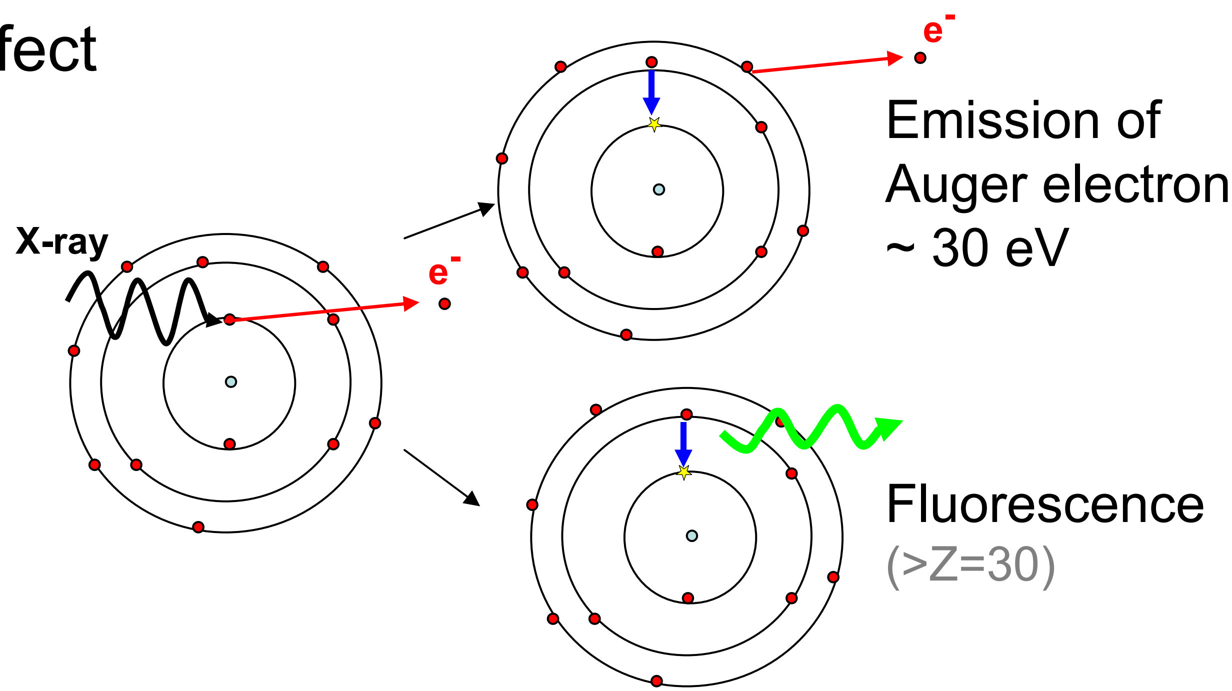
Nine out of ten X-ray photons cause radiation damage – can we reduce radiation damage?

At 12 keV (λ=1.03 Å)



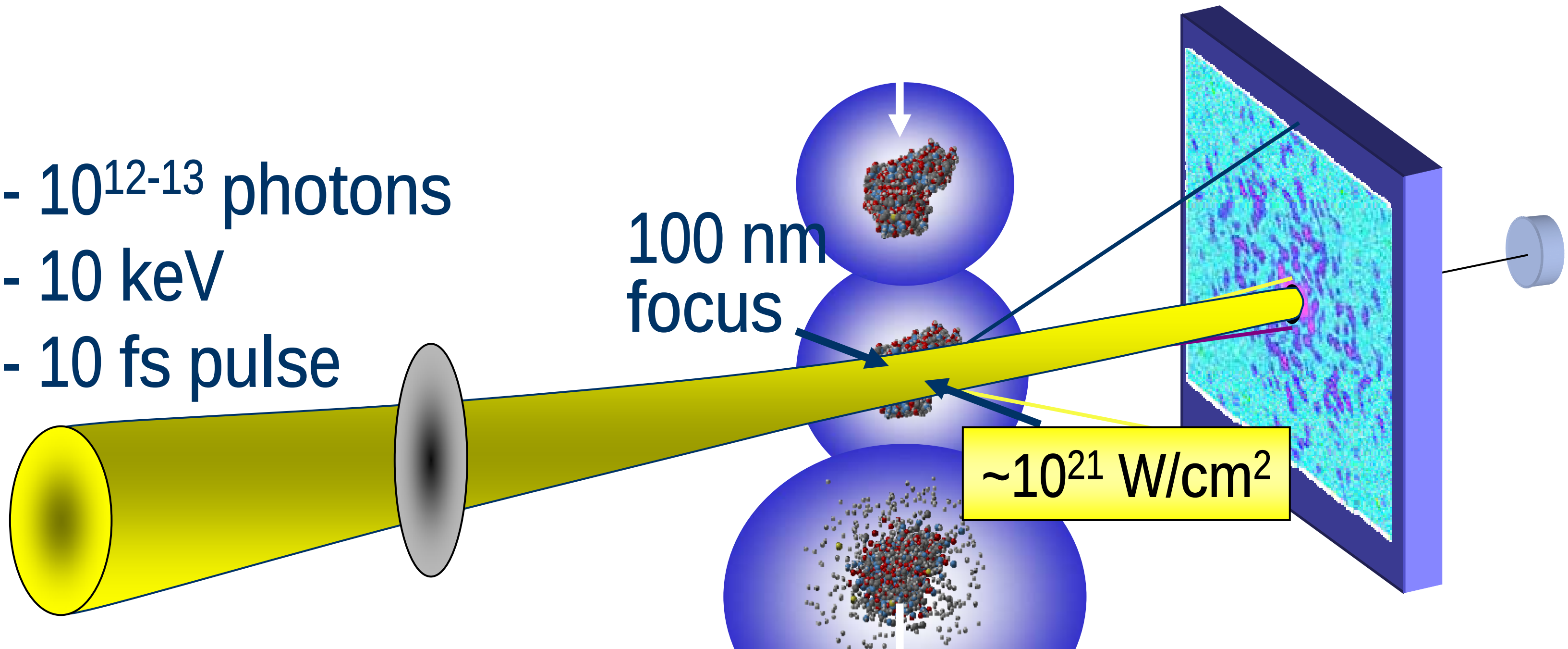
- 10% Rayleigh scattering
- 10% Compton effect
- 80% Photoelectric effect

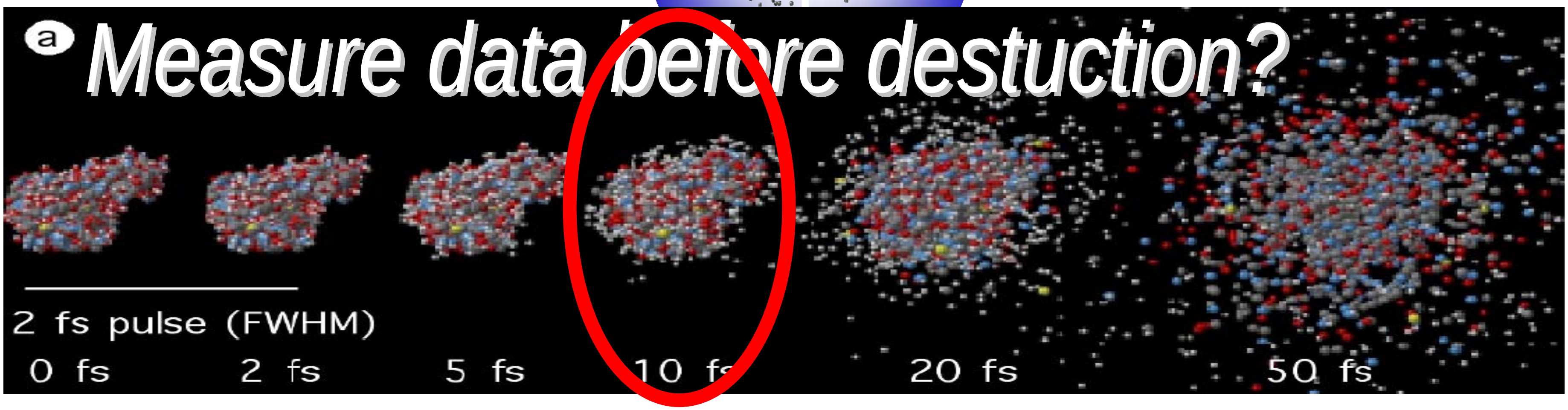




Coherent Diffractive Imaging

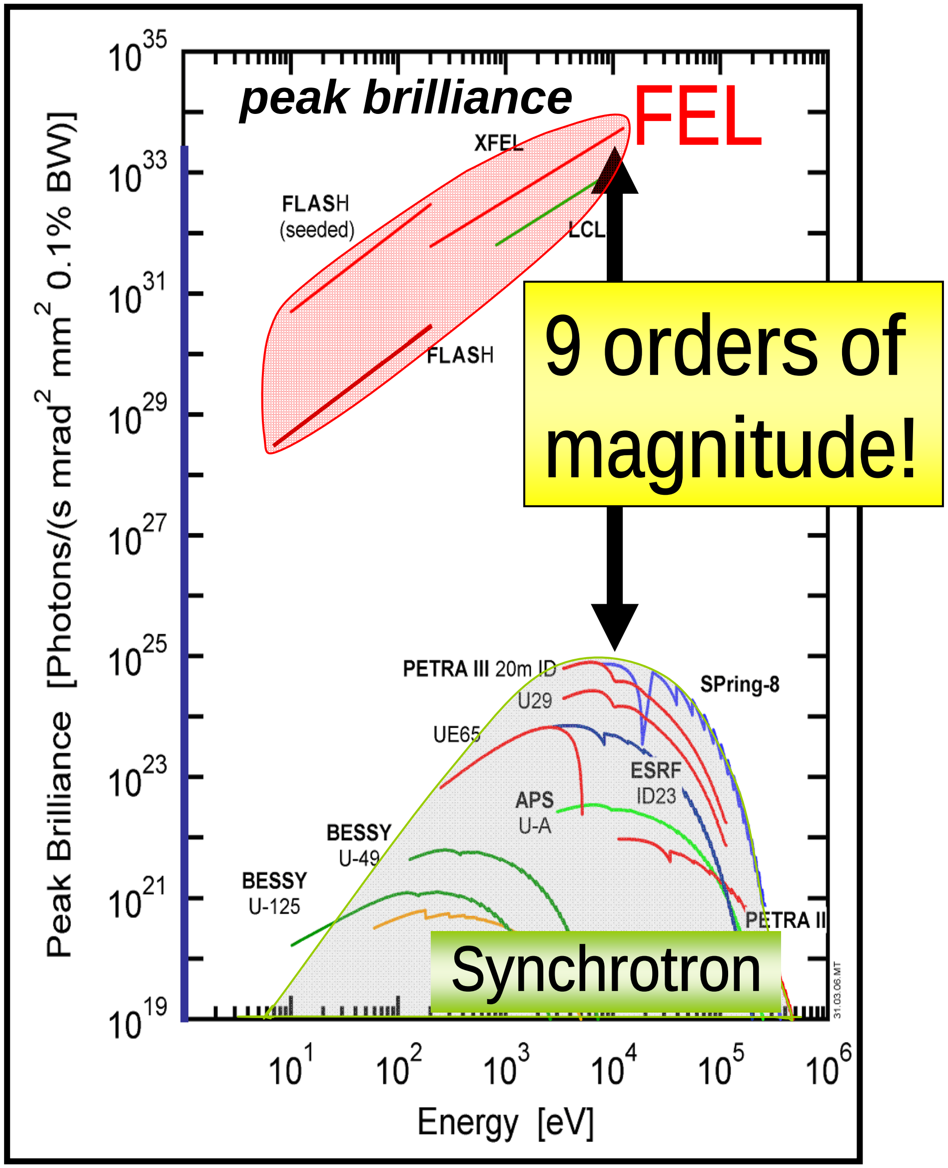
- $10^{12\text{-}13}$ photons
- 10 keV
- 10 fs pulse





Calculations in vacuum, Neutze et al., Nature 2000

Free Electron Lasers



- FLASH: 2005
- Fermi: 2009
- LCLS: 2009
- SCSS: 2011…
- XFEL: 2016
- KVI, Shanghai, …

- **$10^{12\text{-}13}$ photons: ~ 10 fs pulses**
- **repetition rate: now 120 Hz**
- **photon energies: 10 keV**
- **transversally: fully coherent**

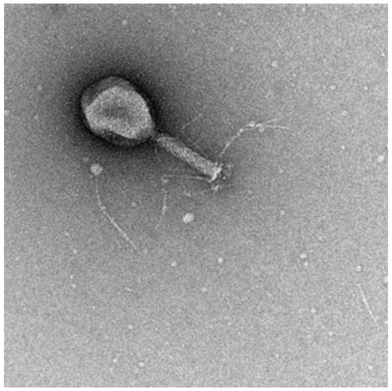
FLASH – Free Electron Laser Hamburg

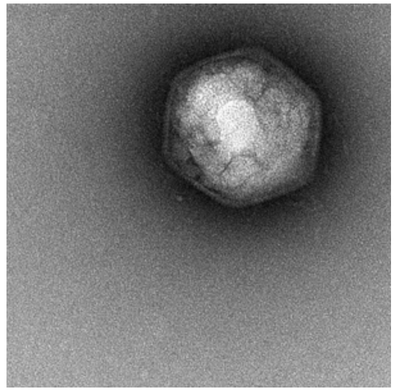
0.2 keV, 6 nm wavelength

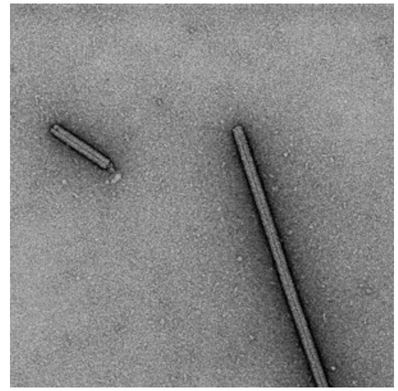
LCLS – Linear Coherent Light Source Stanford

Lasing at 1.5 keV observed in May 2009

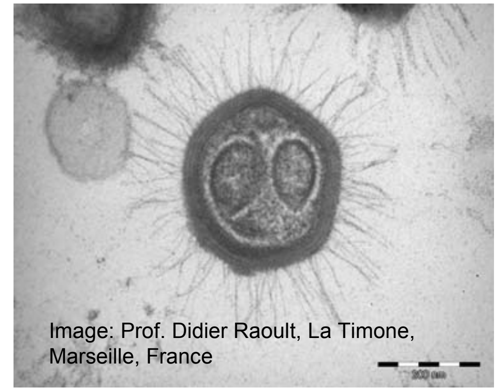
Some biological model systems for nanodiffraction/diffractive imaging:



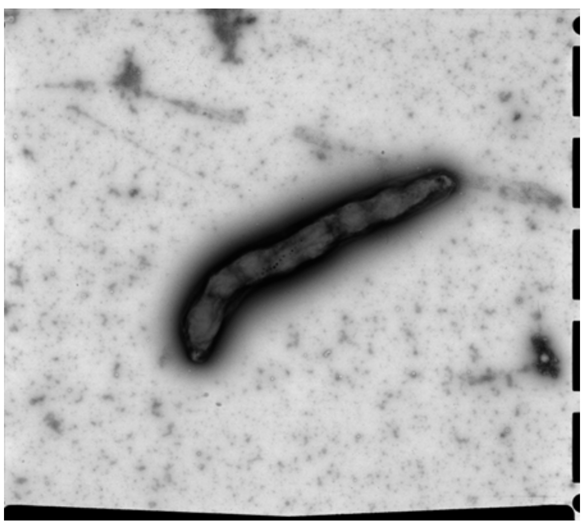




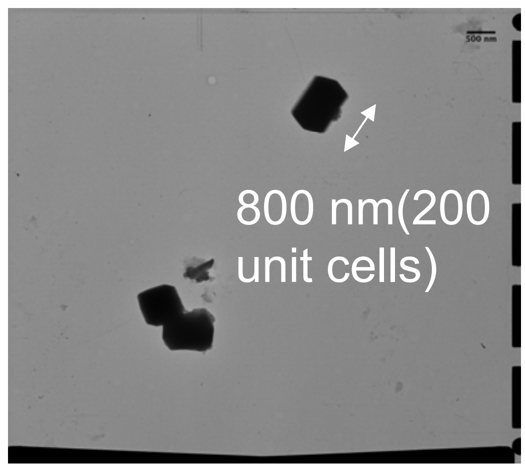
Various virus particles (Heidelberg)



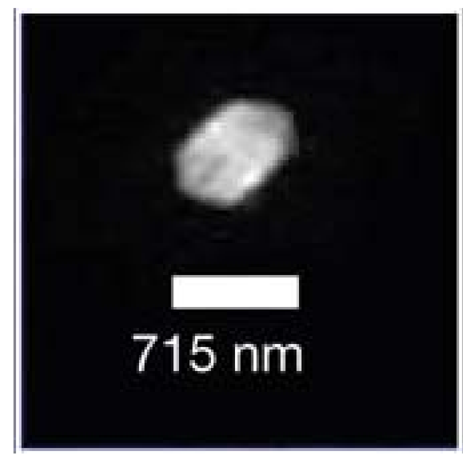
Mimivirus
(Janos Hajdu group)



Magnetotactic bacteria
(Heidelberg)

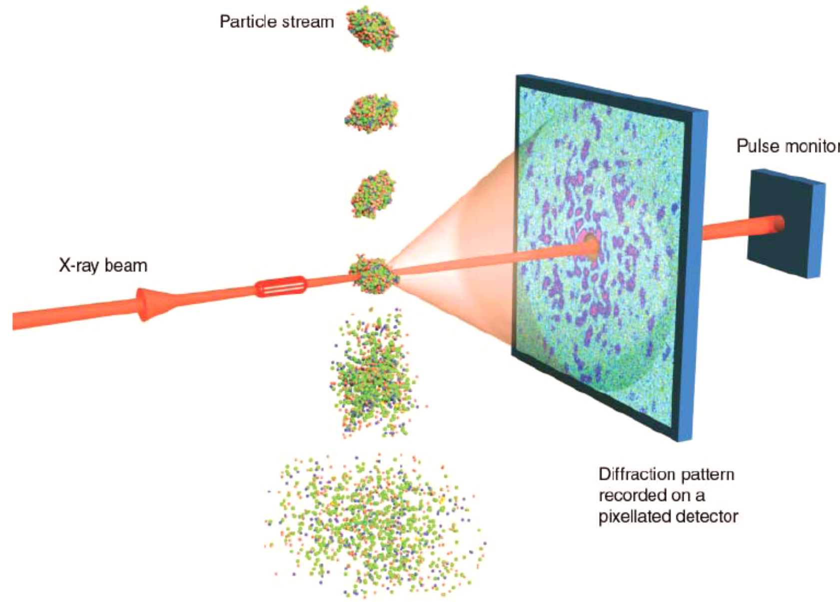


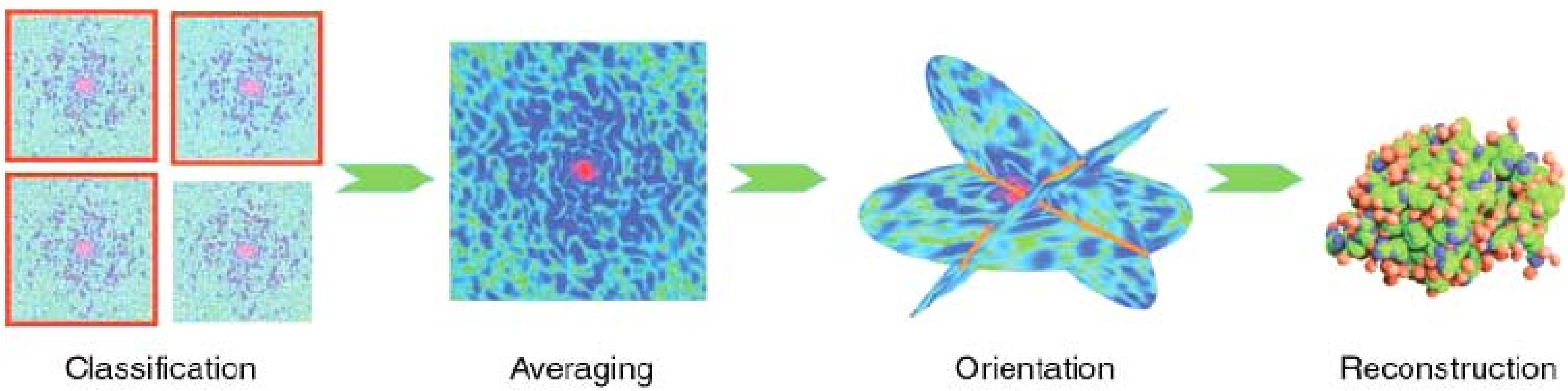
lysozyme nanoxtals
(Heidelberg)



Photosystem I nanocrystals
(Petra Fromme group,
U. of Arizona)

Diffractive imaging of particles



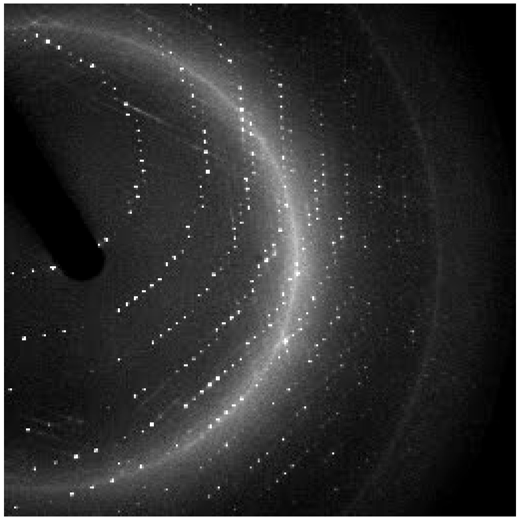


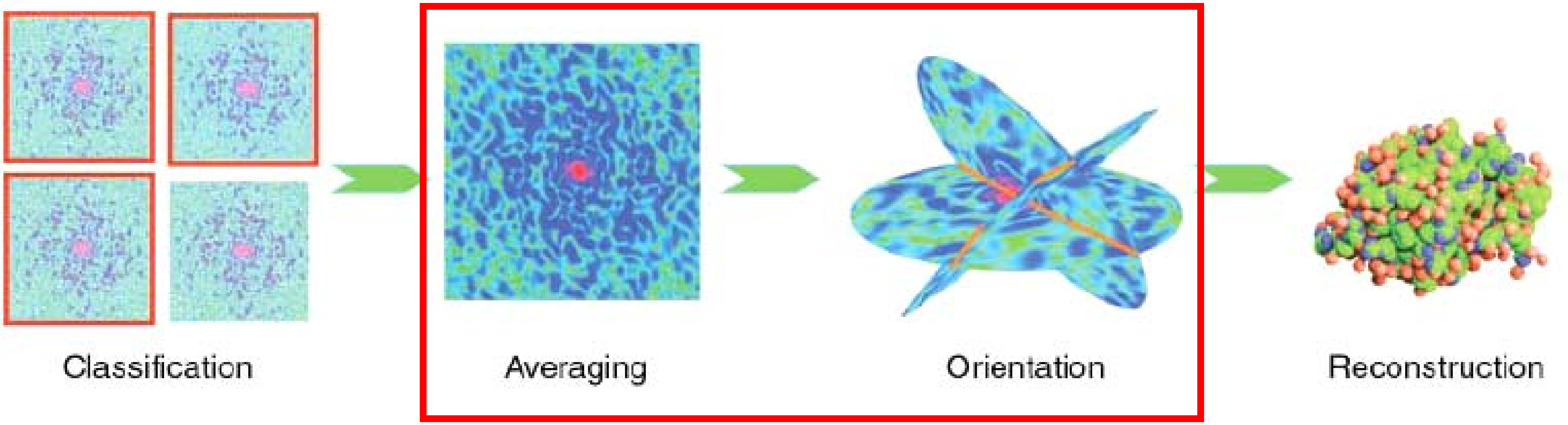
Data evaluation
3D reconstruction

Gaffney & Chapman, Science 2007

THE HUGE ADVANTAGE OF CRYSTALS:
BRAGG PEAKS!

1. BRAGG PEAKS MAKE HIT FINDING EASY
2. NOTHING BEATS A CRYSTAL IN TERMS OF SIGNAL/NOISE
3. DISCRETE, LATTICE CHARACTER OF REC. SPACE SOLVES ORIENTATION PROBLEM

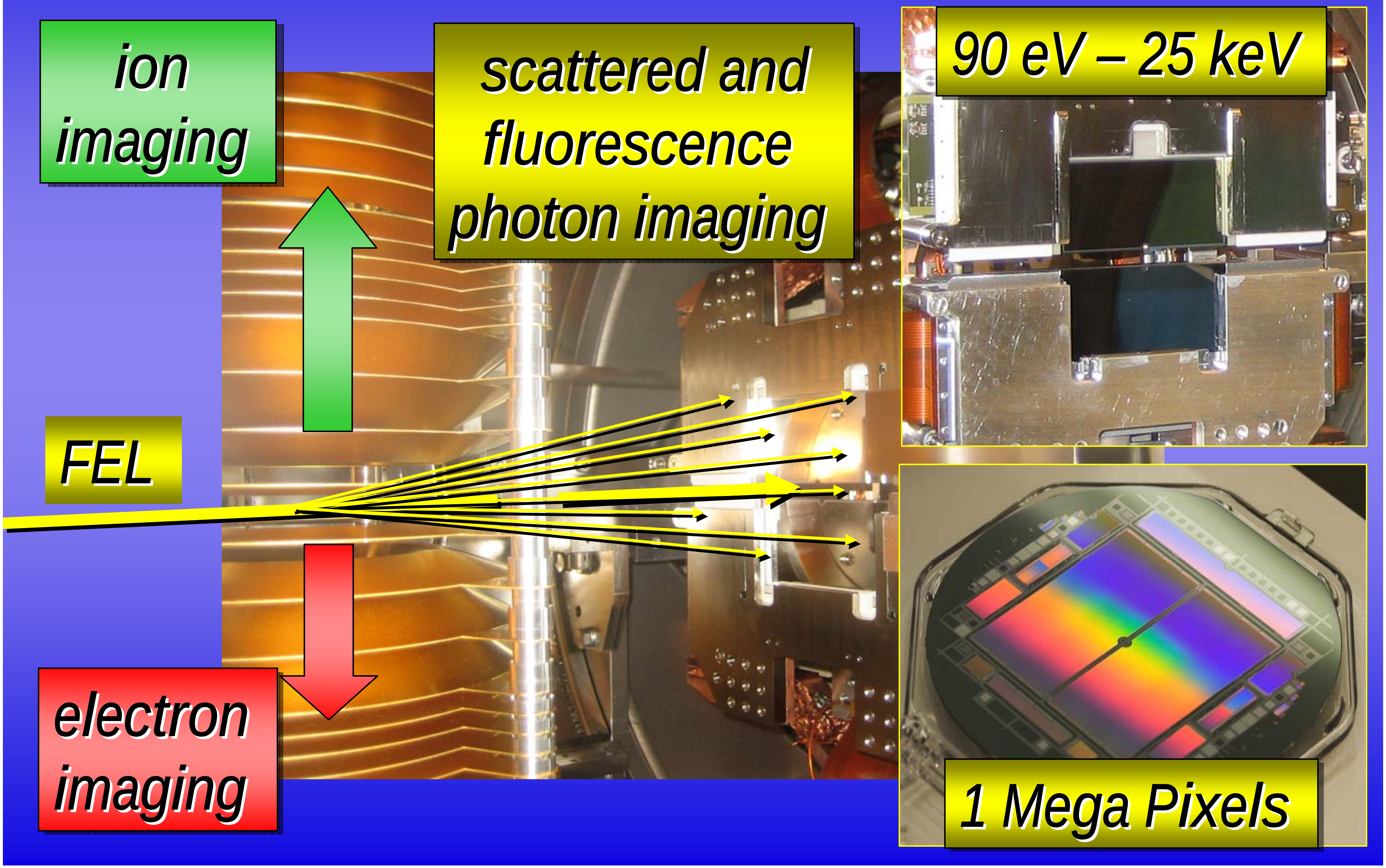




Data evaluation
3D reconstruction

Gaffney & Chapman, Science 2007

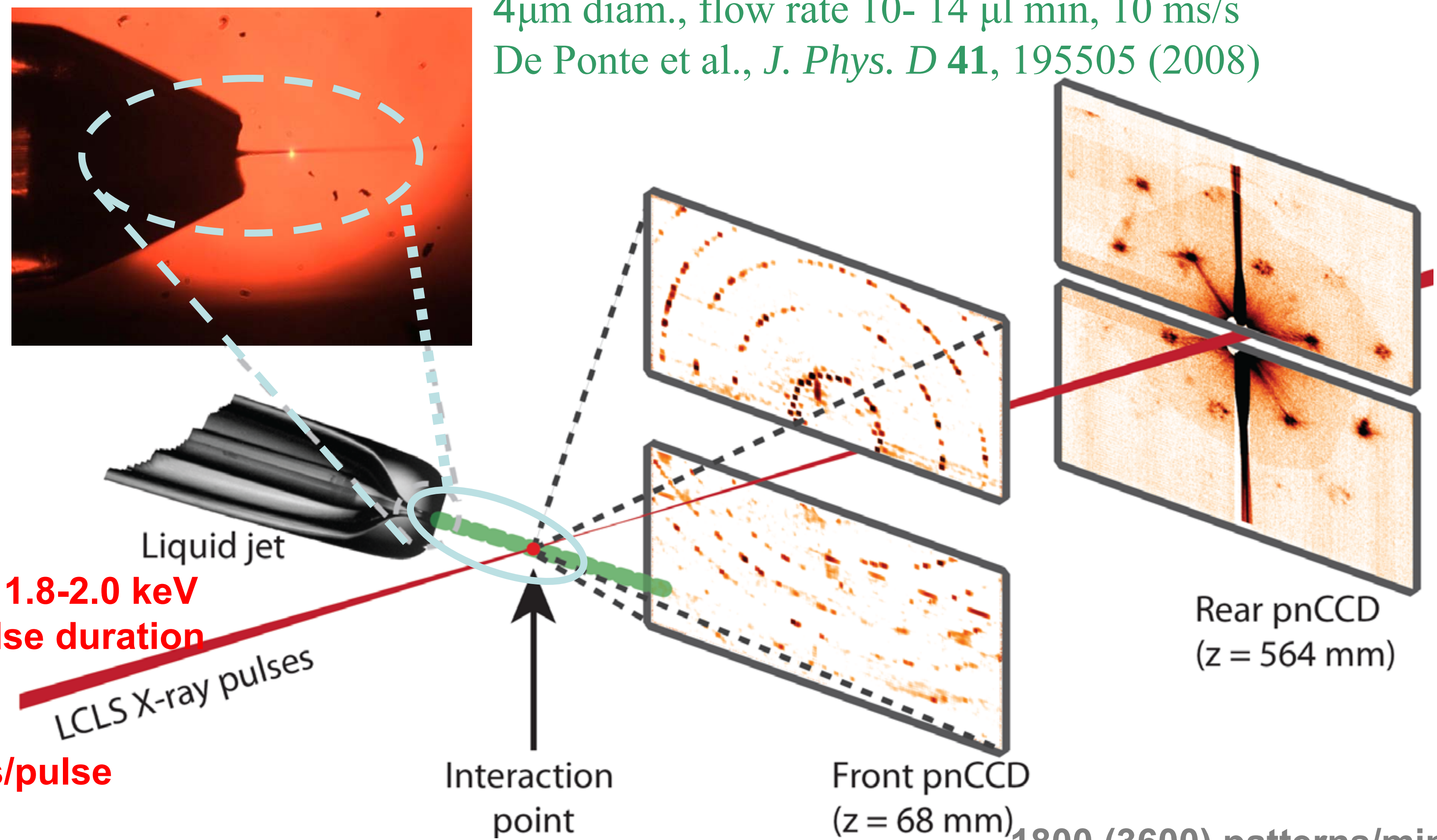
CFEL-ASG Multi Purpose



First serial femtosecond crystallography experiments at LCLS/AMO/CAMP

Chapman et al
Nature 470: 73 (2011)

Gas focussed liquid jet:
4µm diam., flow rate 10- 14 µl min, 10 ms/s
De Ponte et al., *J. Phys. D* **41**, 195505 (2008)



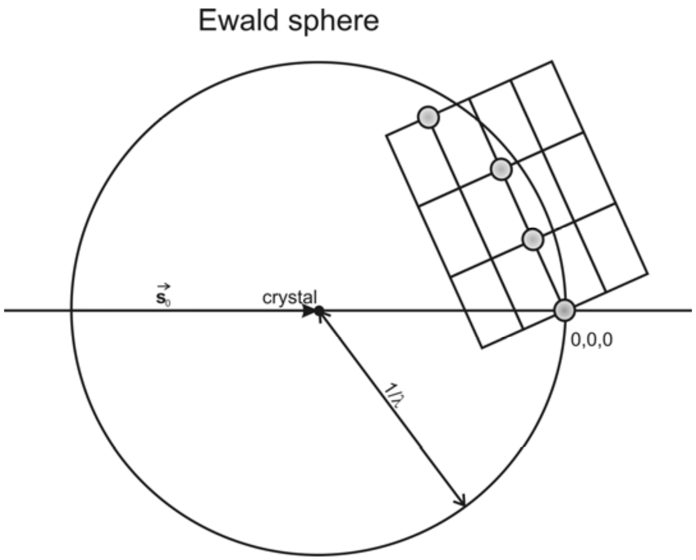
λ =6.2-6.9 Å, 1.8-2.0 keV
10-300 fs pulse duration
30 (60) Hz

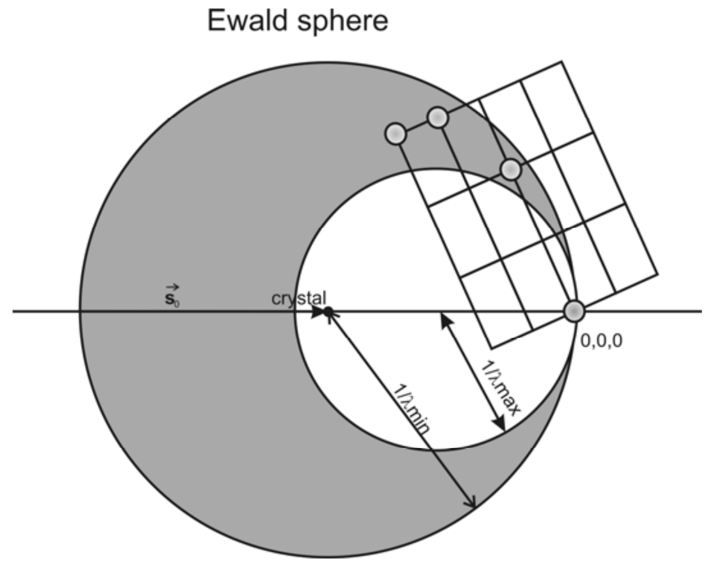
10^{12} photons/pulse
900 J/cm^2
Dose/pulse: 300-700 MGy

~ 7 µm diam.
~ 4 µm diam.

1800 (3600) patterns/min
5 TB in one night

Indexing and integrating reflections: conventional methods

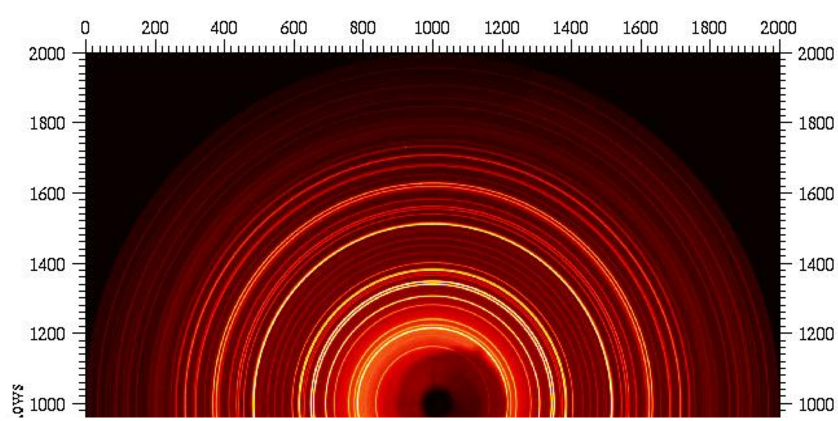


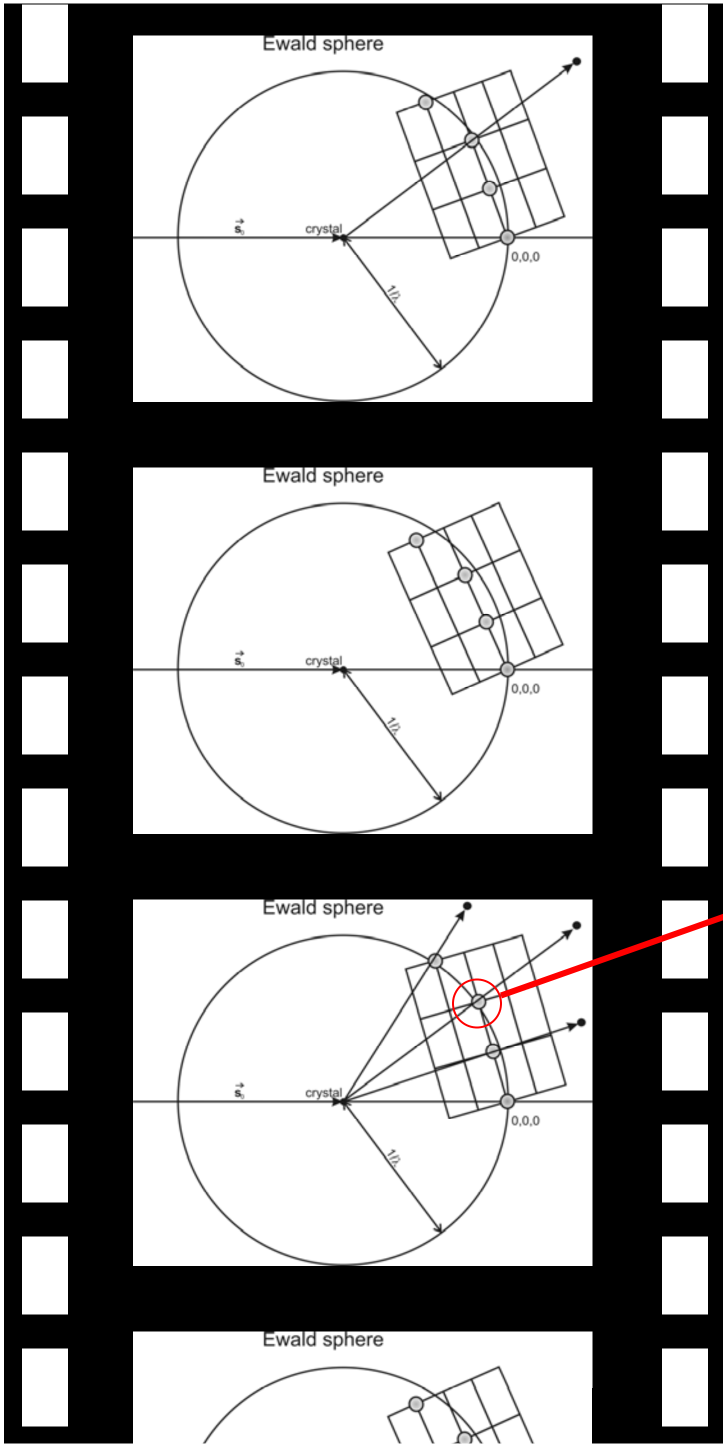


Rotation method
-rotate xtal over finite range
-calculate orientation matrix from observed spot positions
Can fully integrate whole reflections!

Laue method
-use polychromatic radiation
-calculate orientation matrix from observed spot positions
Can fully integrate whole reflections!

Powder method:
-Rotate powder of many xtals
-assign hkl from scattering angle of reflections *(if unique!)*
Fully integrates whole reflections!

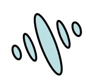


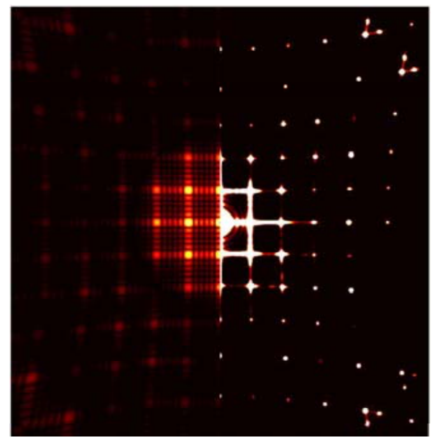


Serial femtosecond crystallography

- Numerous shots of different crystals with possibly different sizes
- No *a priori* control over orientation
- Crystals effectively stand still during a 300 fs pulse
-Only part of reflection intersects Ewald sphere ("partials", no "fullies")
- Fringes rather than neat spots

6x6x6 unit cells





200x200x200 unit cells

(Simulation software by Wolfgang Kabsch)

It is possible to do a *Monte Carlo* integration over multiple *indexed* femtosecond images and obtain a dataset of fully integrated reflections

Kirian *et al* (2010), *Optics Express*,**18**, 5713-5723:

“Extra” features allow sizing and phasing of nanocrystals

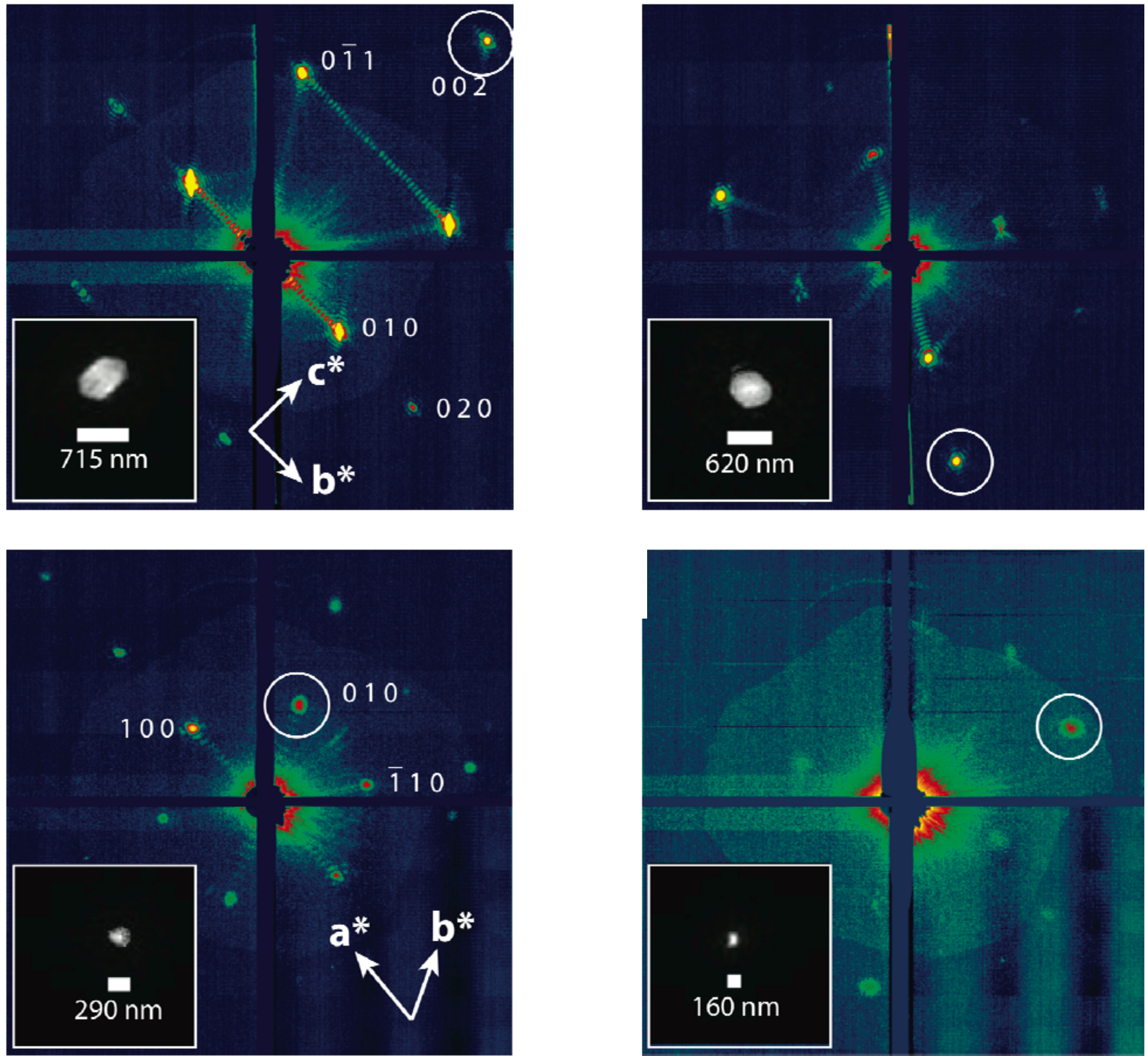
N **unit cells**

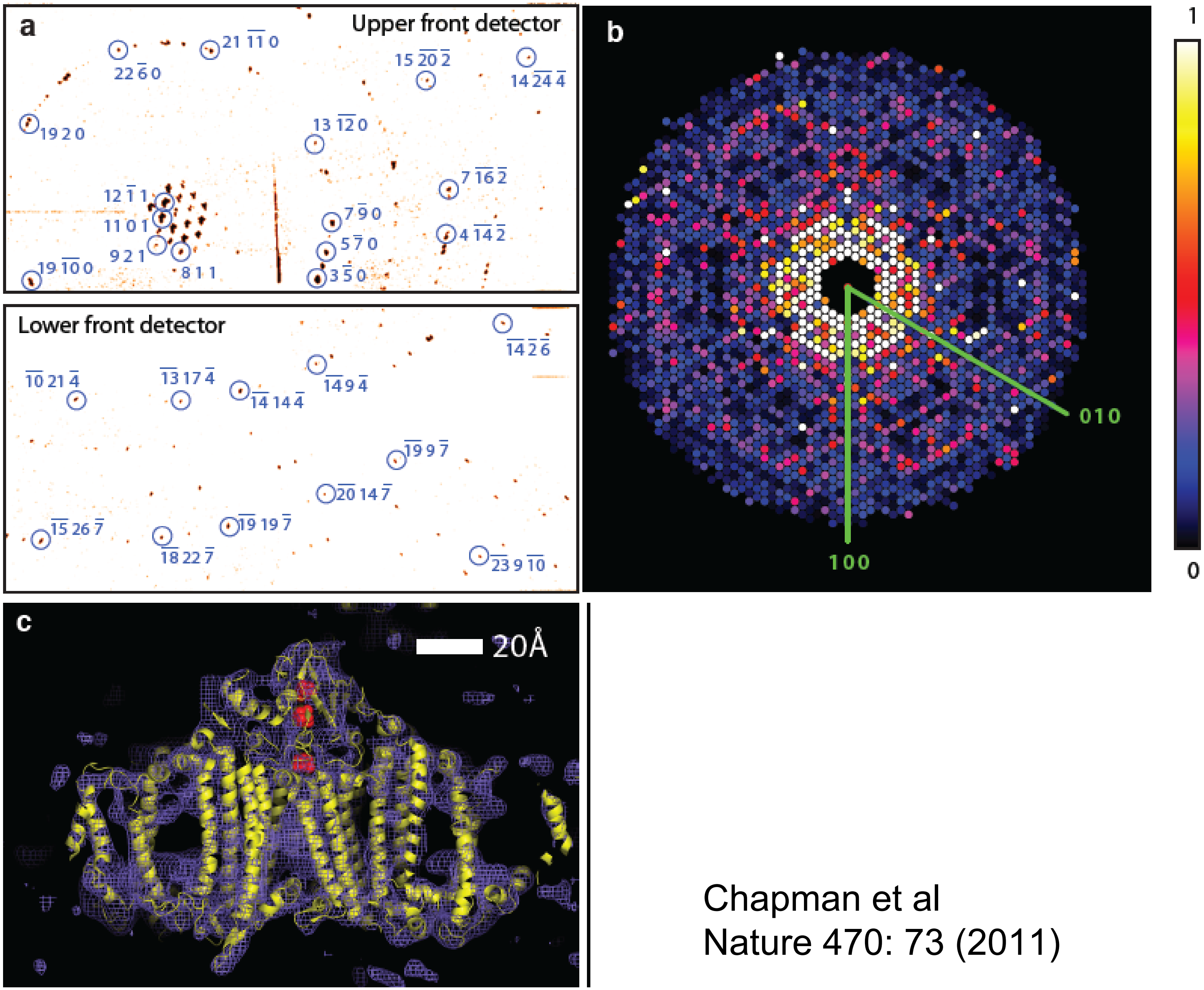
→ *N* -2 **fringes,**

Intensity:

$$I \propto \frac{\sin^2 N\left(\pi\vartheta\lambda/b\right)}{\sin^2\left(\pi\vartheta\lambda/b\right)}$$

Chapman et al
Nature 470: 73 (2011)





Chapman et al
Nature 470: 73 (2011)

So what's the *bad* news?

- Hit rates are low, + only a fraction of hits indexable
- → the method needs:
  - 1-to-several ml of highly concentrated (yoghurt-like!) suspension of microcrystals (hit rates are low, for high resolution many 10,000s images needed)

How do you make that much protein?

(usual yields are in the 0.1-1mg range for membrane proteins..., very difficult to produce, not stable!)

Can you make nanocrystals of it?

(*how do you know you have them?*

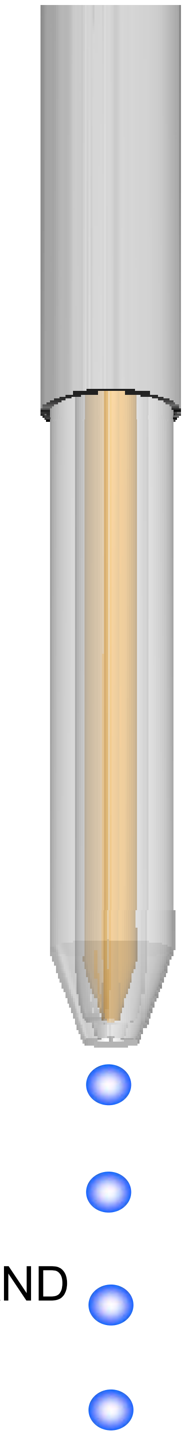
how do you know they are any good?

testing them can only be done at the FEL...)

(*can you inject them?*

PEG/salts may clog the nozzle.....)

.......?



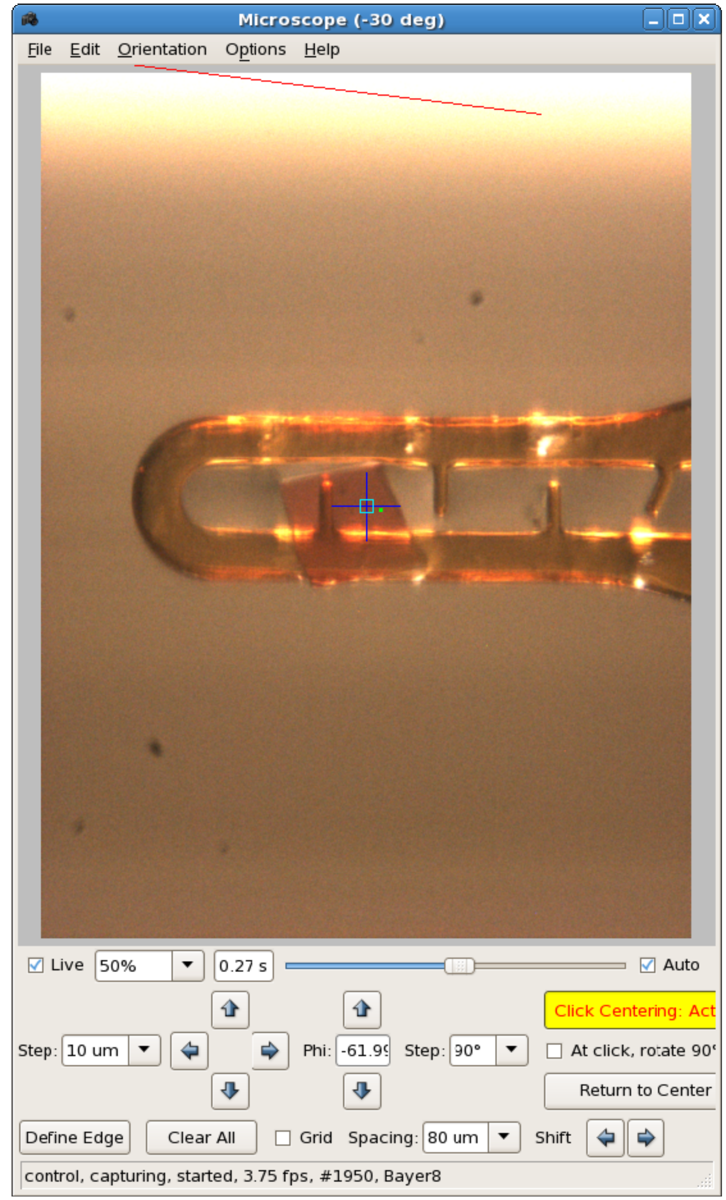
DROPLET-ON-DEMAND
TO SAVE SAMPLE ?
HIGH PULSE RATE?

What if you want to do "normal" xtallography, *e.g.* for time-resolved studies?

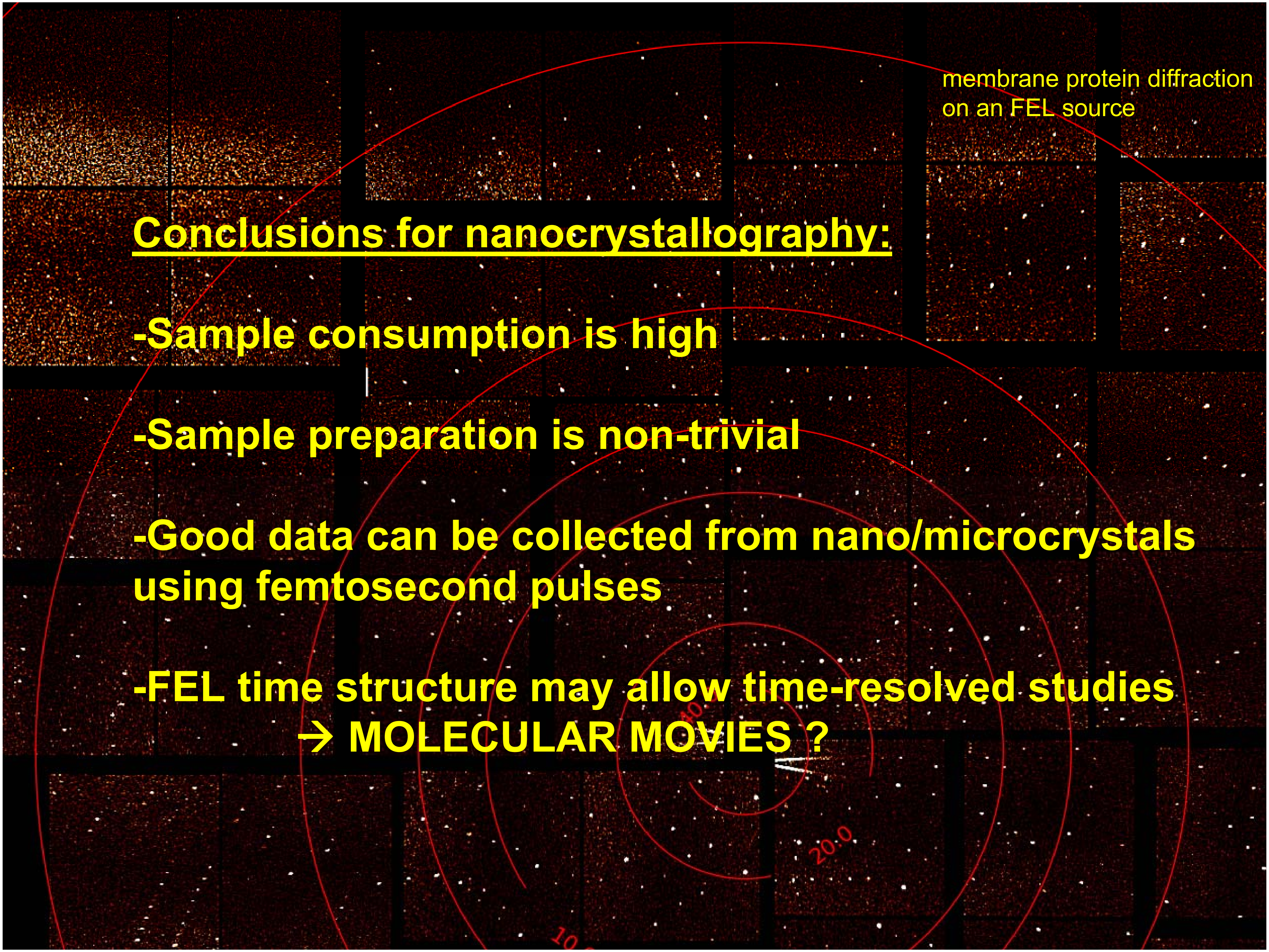
-serial nanocrystal data processing is based on averaging large numbers of exposures

-Preliminary experience with larger xtals (fewer exposures, "regular" xtallography) shows:

PULSE-TO-PULSE REPRODUCIBILITY IS EVERYTHING!!!
(Intensity, coherence, spectrum – seeding?)



CO-myoglobin xtal
LCLS X-ray pump-probe
Dec. 2010



membrane protein diffraction on an FEL source

Conclusions for nanocrystallography:

-Sample consumption is high

-Sample preparation is non-trivial

-Good data can be collected from nano/microcrystals using femtosecond pulses

-FEL time structure may allow time-resolved studies
→ MOLECULAR MOVIES ?

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MPI Semiconductor Lab	Nils Kimmel, Georg Weidenspointner, Daniel Pietschner, Günter Hauser, Sven Herrmann, Gerhard Schaller, Florian Schopper, Robert Andritschke, **Lothar Strüder**
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Advanced Light Source	James Holton, Stefano Marchesini
NIH	Friedrich Schotte, Hyun Sun Cho, **Philip Anfinrud**