# Micro and nano-focused X-ray beams, 4th generation synchrotron sources, X-ray Free Electron Lasers :

## current and future opportunities in structural biology.

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## Outline :

**1: X-ray macromolecular crystallography in an equation-free nutshell**

2: Conventional micro-crystallography at synchrotron-radiation (SR) sources

3: Serial micro-crystallography (XFELs and SR sources)

4: Serial nano-crystallography (XFEL sources)

5: Coherent X-ray diffraction and direct phasing of structural information

## Why resorting to the use of X-rays ?

Because their wavelength commensurates with atomic dimensions

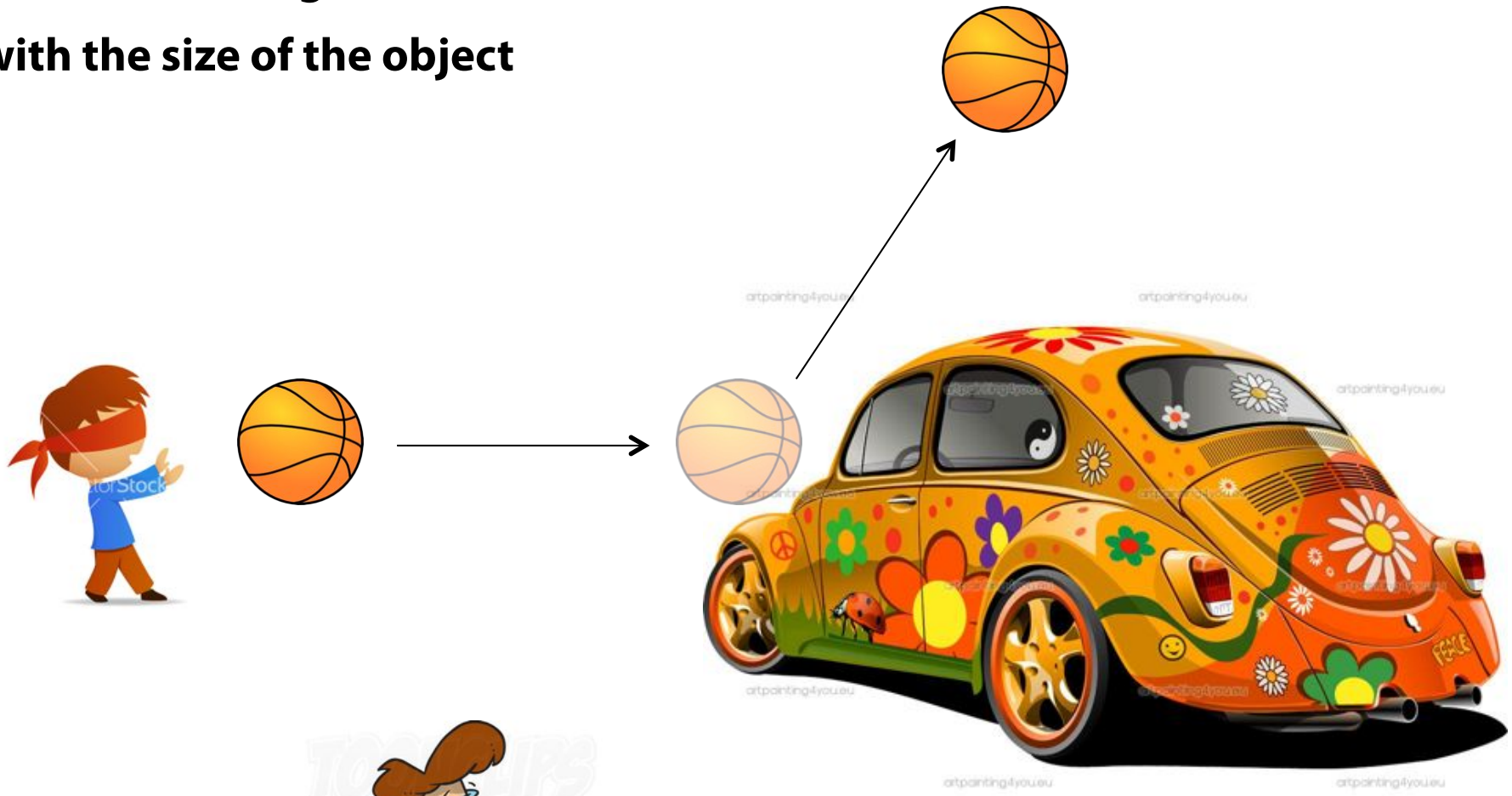
# Size of biological objects studied in structural biology

| Objects | Size | Imaging method | | wavelength |
|---|---|---|---|---|
| Bacteria | few µms | optical microscopies |  | UV-visible (200-800 nm) |
| Virus, Protein complexes | 10 - 1000 Å (1 - 100 nm) | electron microscopies |  | < 1 nm |
| Proteins, Small molecules, Atoms, | 1 - 100 Å | X-ray crystallography |  | 0.5 - 2 Å |

**To “see” an object, we need a light**

**whose wavelength commensurates**

**with the size of the object**



Perfect !!

**To “see” an object, we need a light whose wavelength commensurates with the size of the object**

Too large, completely useless !!!!

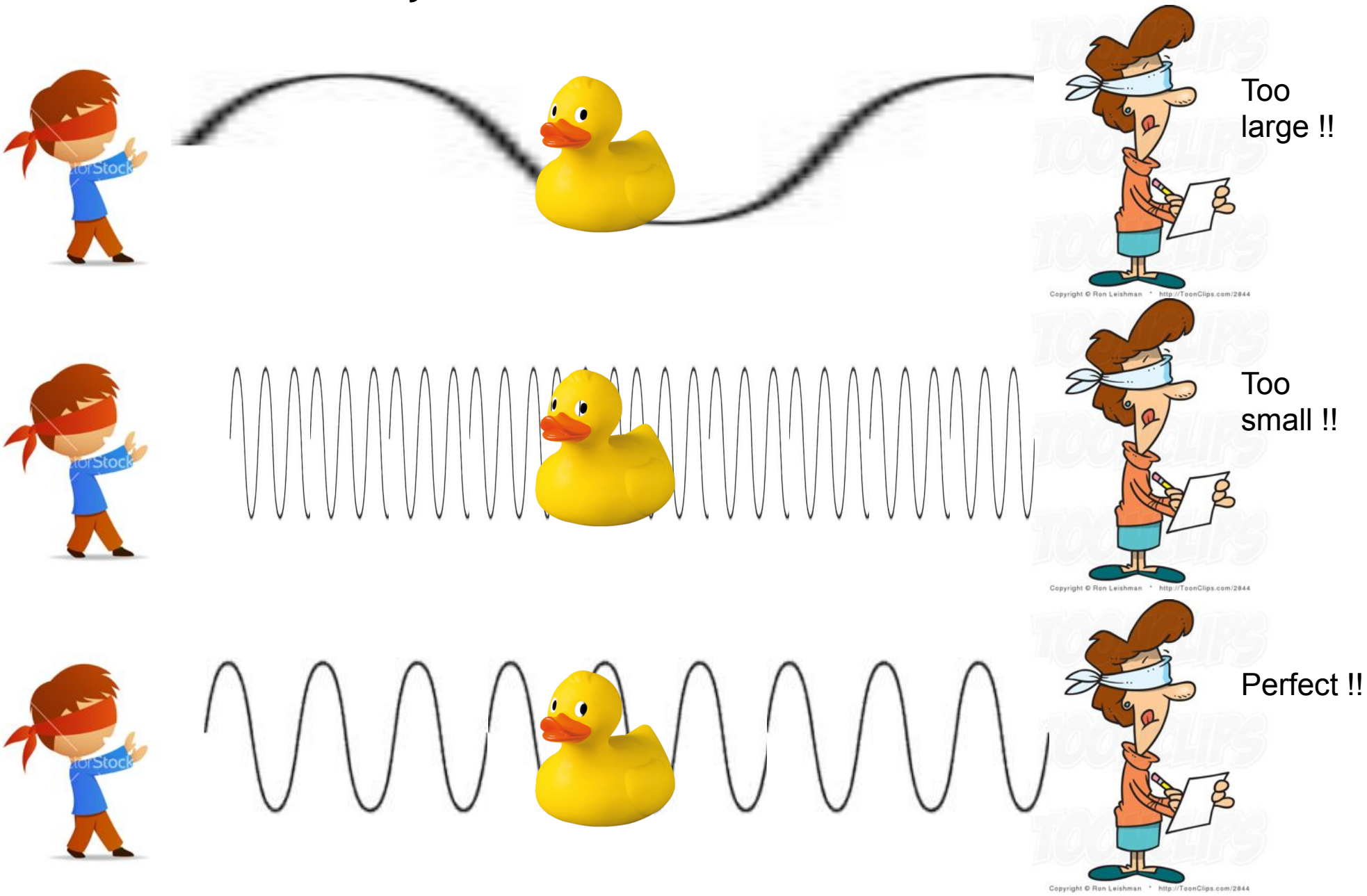
**To “see” an object, we need a light**

**whose wavelength commensurates**

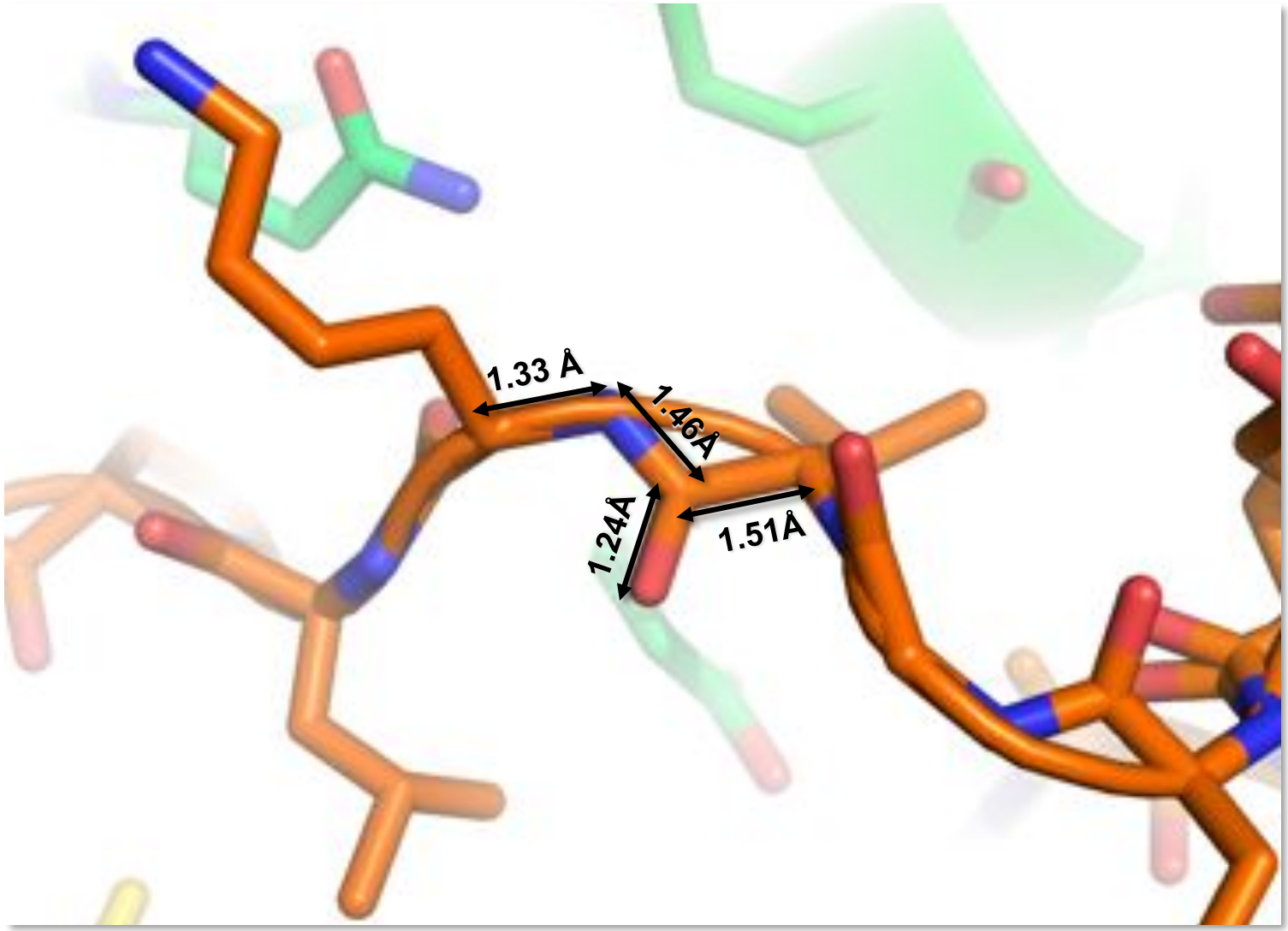
**with the size of the object**

Too small, counter-productive !!!

**To "see" an object, we need a light whose wavelength commensurates with the size of the object**

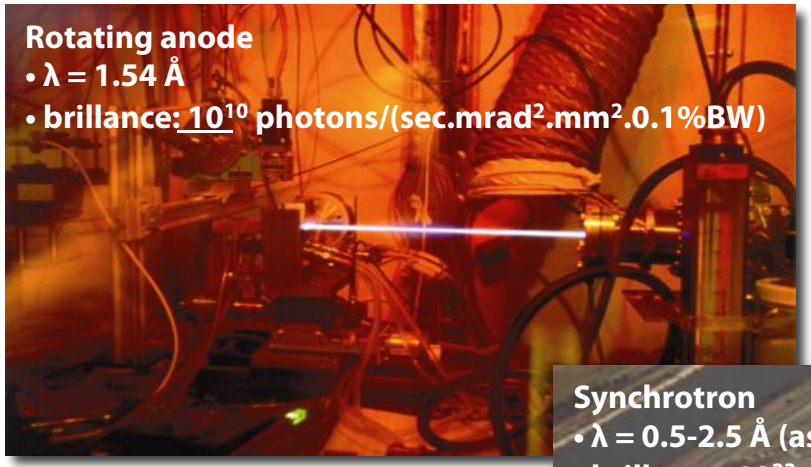


**To "see" an object, we need a light whose wavelength commensurates with the size of the object**

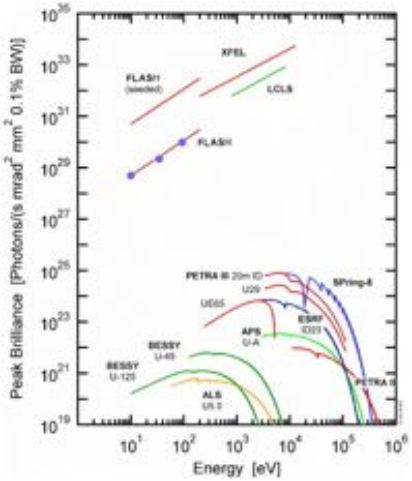



**X-rays are very well suited to probe biological materials at atomic resolution**

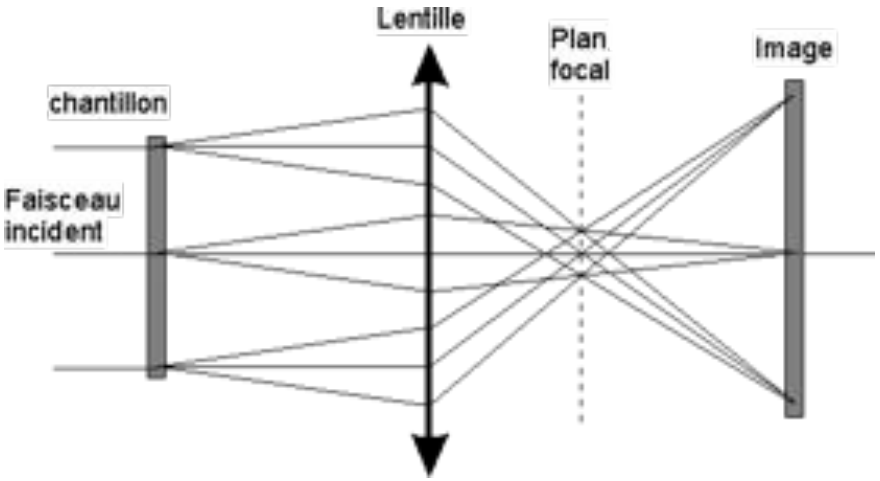
# X-ray wavelength and sources generally used in crystallography





# General principle of lens-based imaging




• A point from the object scatters incident light.

• Emitted light is intercepted by a lens, which re-focuses it as a point on the detector, hence providing an optical image.

• Light is an electromagnetic wave: it has an intensity and a phase.

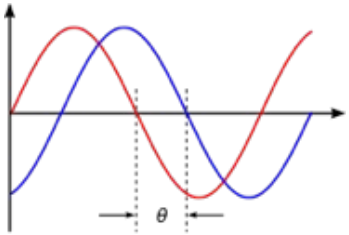
• Refocusing by a lens allows preserving both the intensity and the phase.

• Waves from the incident light can display different phases: **phase difference is angular** :

θ = 0 : waves are **in phase**

θ = π : waves have **opposed phases** (180°)

θ = π/2: waves are in phase **quadrature** (90°)



# There are no lens that can refocus X-rays better than 5-10 nm

- Emitted waves cannot be refocused :

We therefore need to work in the reciprocal space.

- In diffraction experiment, we only measure intensities and the phase information is lost:

→Computation will be required to get it back

→The mathematical operation that allows going from the real space to the reciprocal space is the **Fourier transform**

## First things first : what is the reciprocal space ?

Intuitive answer :

an inversion of the real space

Educated answer :

the Fourier transform of the real space

Answer in a crystallography exam :

the scattering space, as opposed to the sample space

## Why even bother about … the reciprocal space ?

Intuitive answer :

because you have personal issues

Educated answer :

because when it comes to small objects, it makes things easier

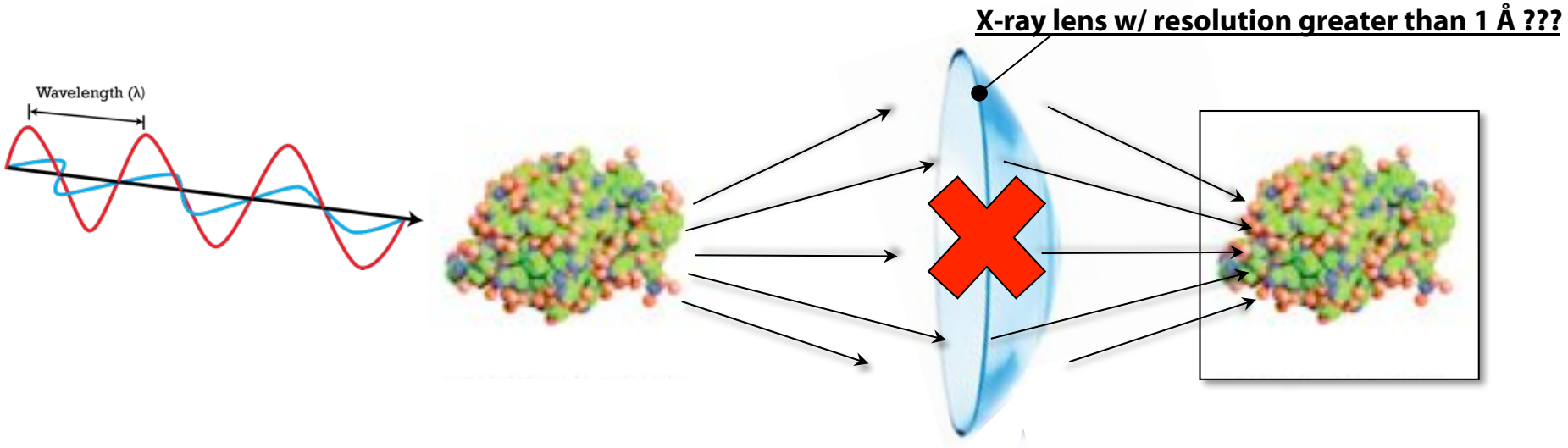
(**e.g., when you try measuring the thickness of a hair**)

Answer in a crystallography exam :

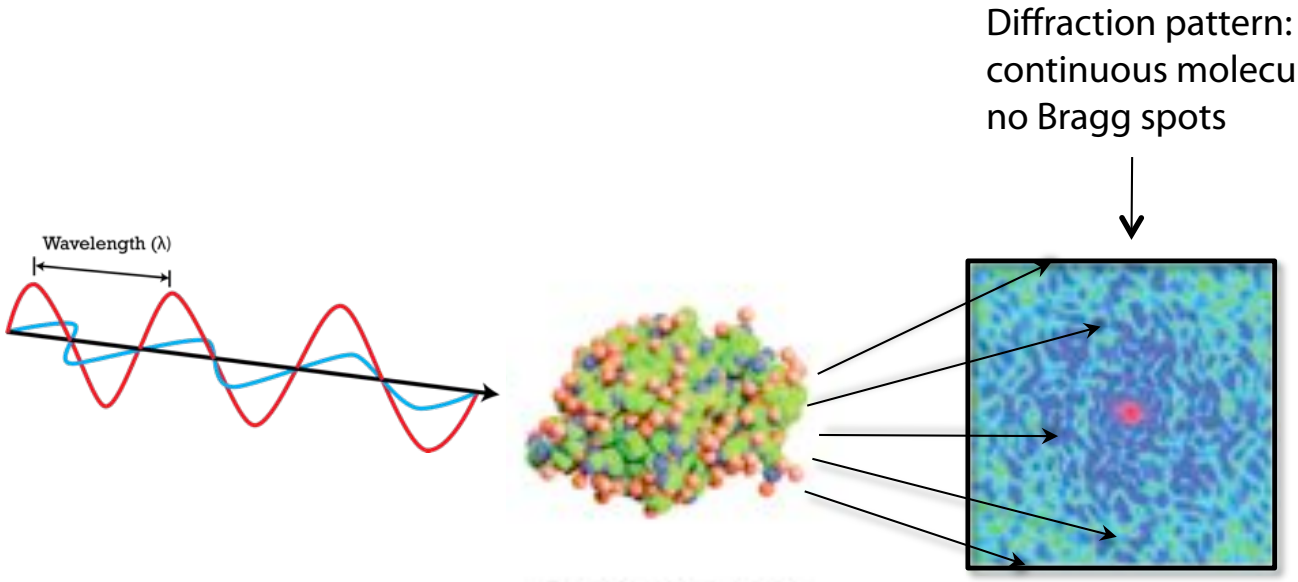
because we don't have X-rays lenses focusing below 10 nm,

and therefore cannot refocus X-ray scattering from biological samples

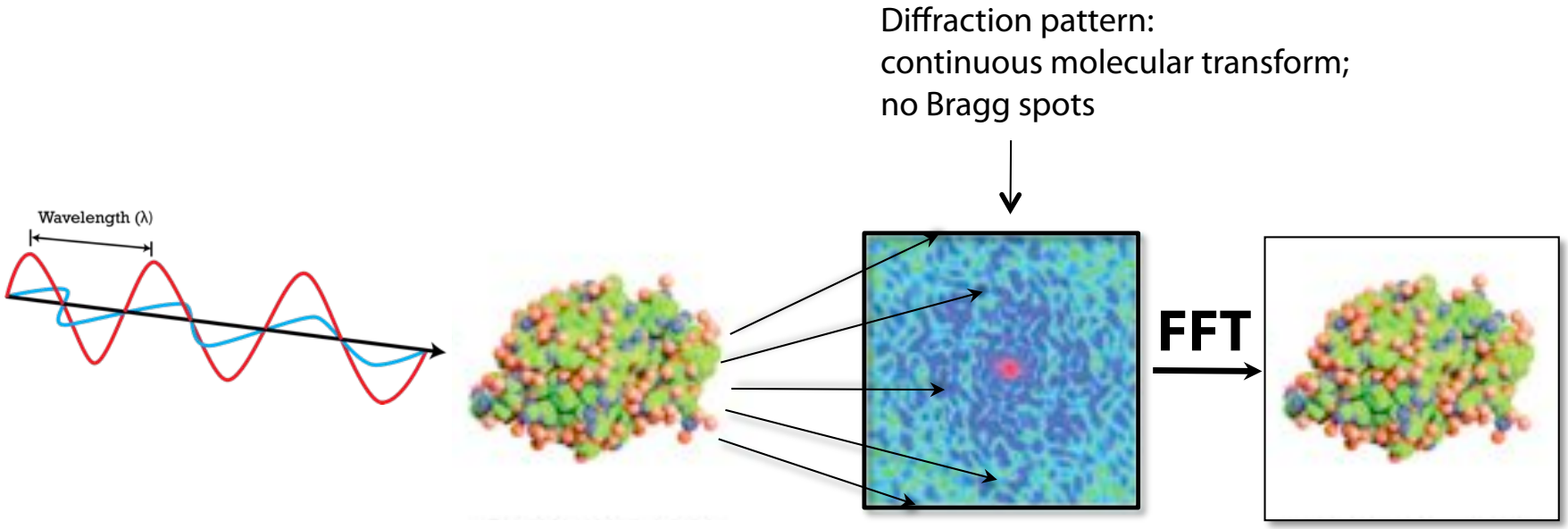
# X-ray scattering from a molecule cannot be refocused



# X-ray scattering needs to be recorded in the reciprocal space



# X-ray scattering is about probing the structure without a lens

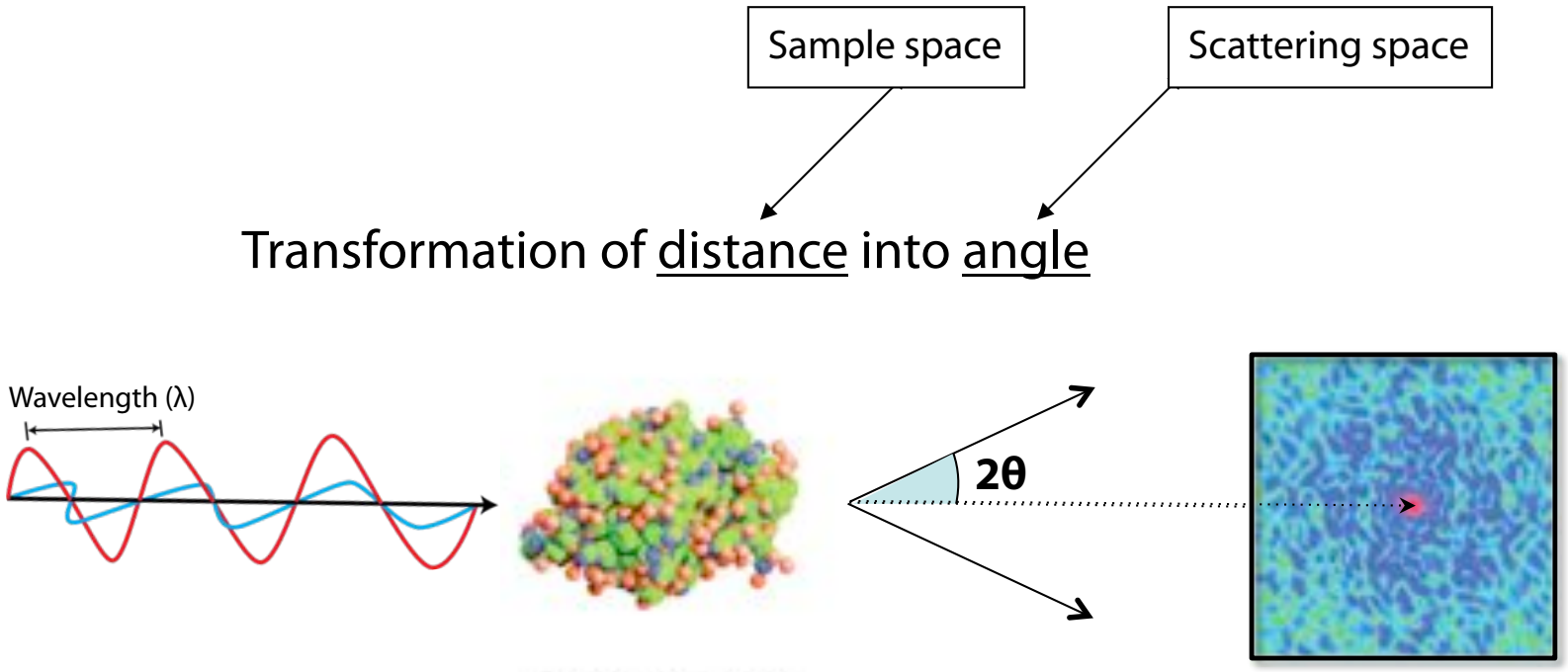



**Sample space**

**Scattering space**

**Image space**

# X-ray scattering from a molecule




## Sample space

## Scattering space

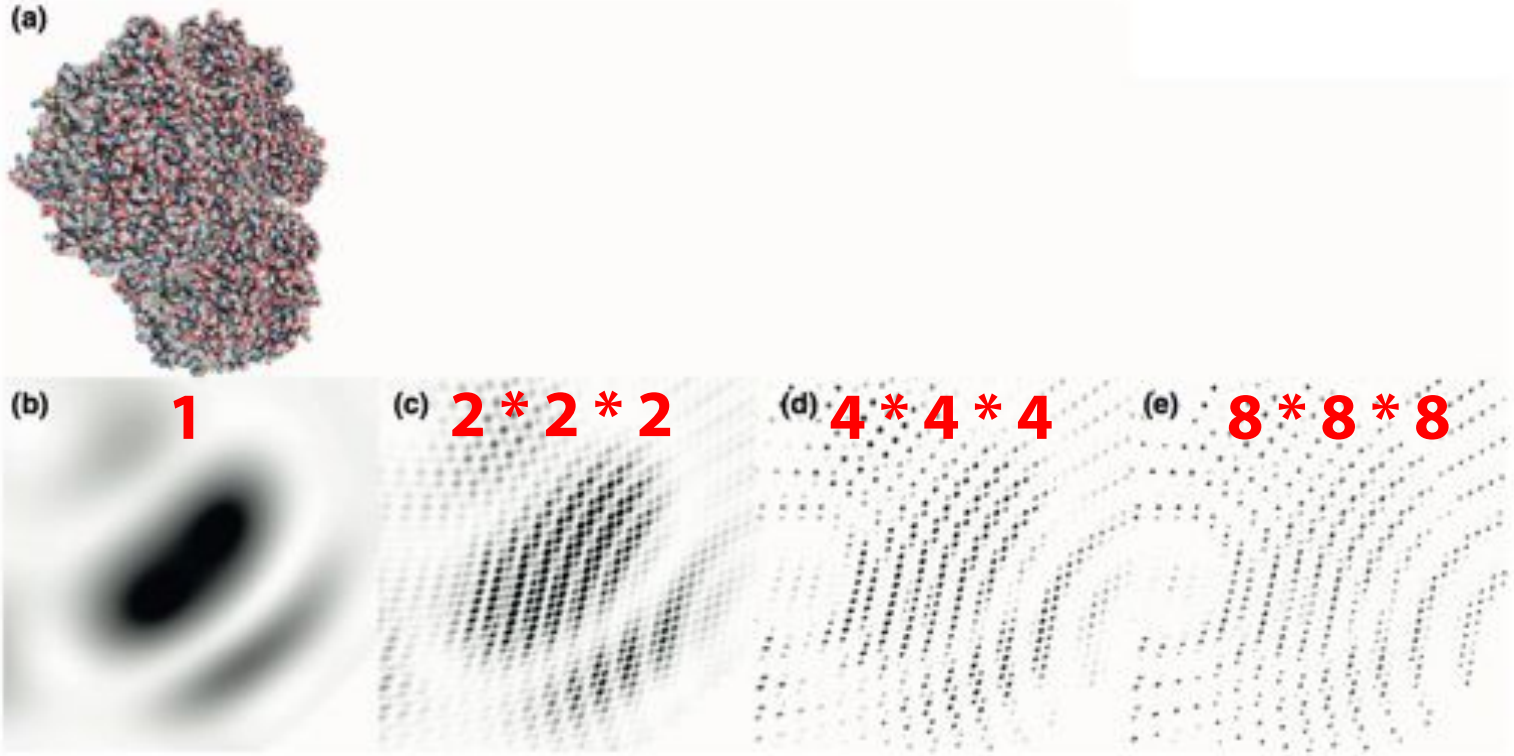
Diffraction Pattern : contains all the contrast relevant information at the resolution of λ/2sin(θ)

# Restrictions to the use of X-rays

X-ray/matter interaction is weak (1/10000 photons)

We use crystals as a means to amplify the signal ($2.10^{14}$ symmetry-related molecules)

The Bragg law applies : constructive interferences only if $2*d*\sin(\theta) = \lambda$

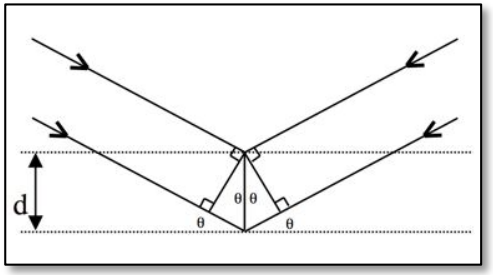


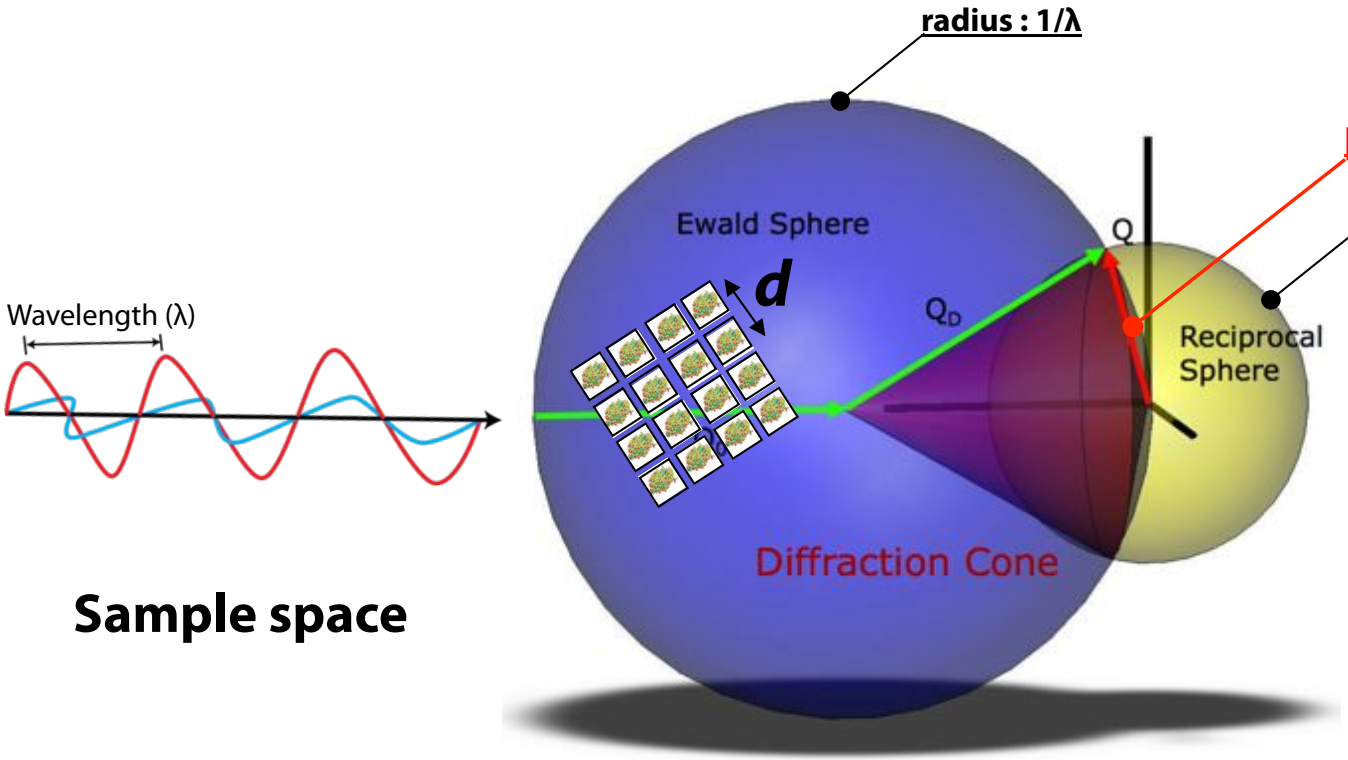

continuous molecular transform; no Bragg spots

Bragg spots and fringes between spots

Schlichting & Miao, 2012, *Curr Opin Struct Biol*

# X-ray scattering from a crystal




## Sample space

## Bragg's law

**Constructive interferences only if :**

$2*d*\sin(\theta) = \lambda$

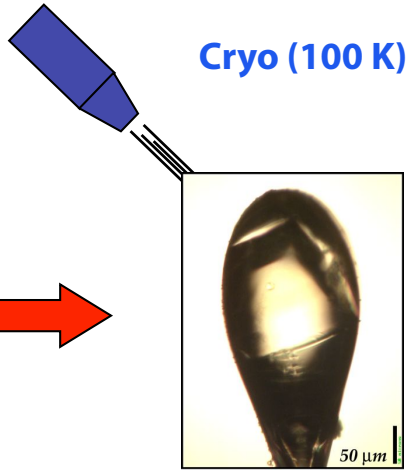
$d/2 = \sin(\theta)/\lambda$

- **distance** vs. **angle** "reciprocal" **relation**
- **fundamental unit** is not θ, but **sin(θ)/λ**

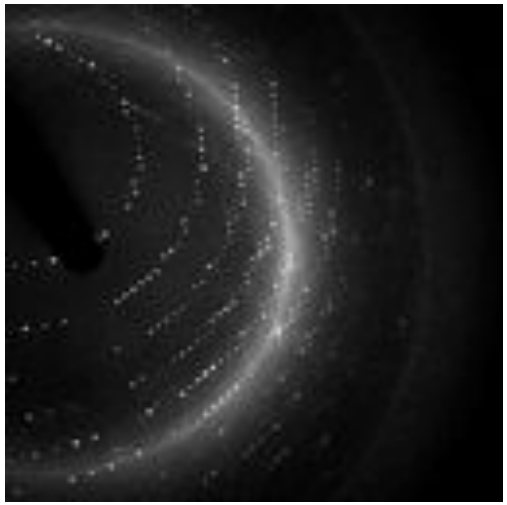
# X-ray protein crystallography

**X-ray source**
**(e.g. ESRF, Soleil, Petra-3,…)**

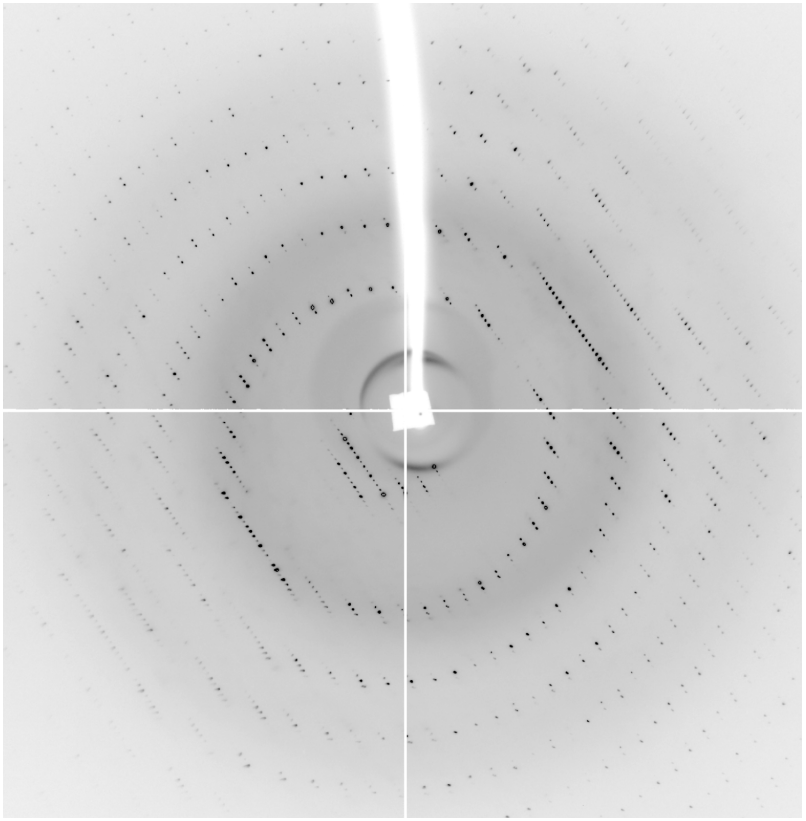
**Cryo (100 K)**




**Crystal of your favorite protein**



# A typical diffraction pattern



- The crystal is characterized by symmetry-operators :

The protein molecules are "**symmetry-related**" within the crystal (real-space)

- This symmetry also applies in the reciprocal space :

**Equivalent diffraction spots must be merged**

- We can then attribute indices to the spots :

**H, K, L**

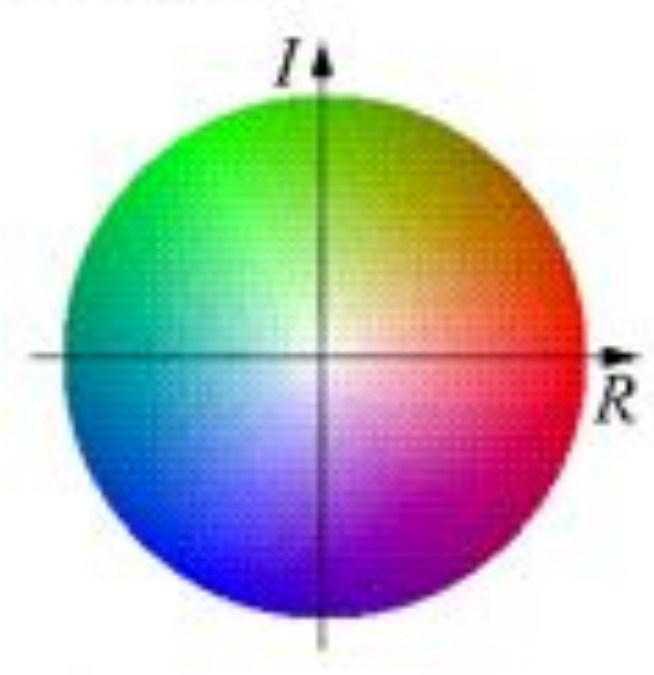
• The intensity of a diffraction spot is proportional to the square of its amplitude : $I \alpha F^2$

• F is a structure factor - it has *h, k, l* indices in the reciprocal space : $F_{hkl}$

• $F_{hkl}$ is a complex number of the reciprocal space

Its "Fourier transform" is the density at point *x, y, z* in the real space : $\rho_{xyz}$

$$\rho_{x,y,z} = 1/V \, . \, \Sigma\Sigma\Sigma |F_{hkl}| \, . \, \exp \, [i\varphi_{hkl} - 2\pi i.(hx + ky + lz)]$$

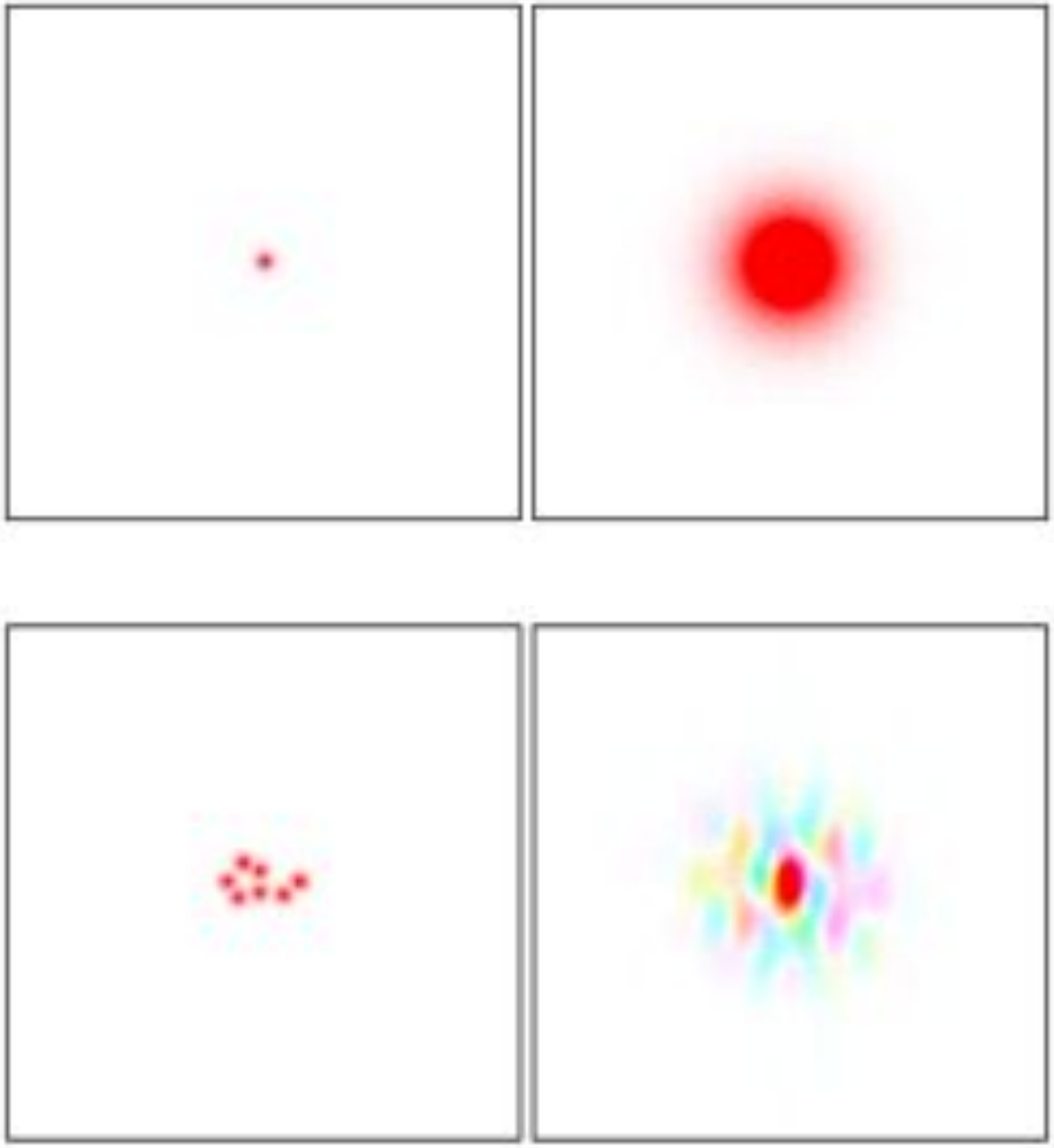



Amplitude = Brightness/Saturation

Phase = Hue/Color

http://www.ysbl.york.ac.uk/~cowtan/fourier/

## What is the Fourier transform of an atom or a protein ?



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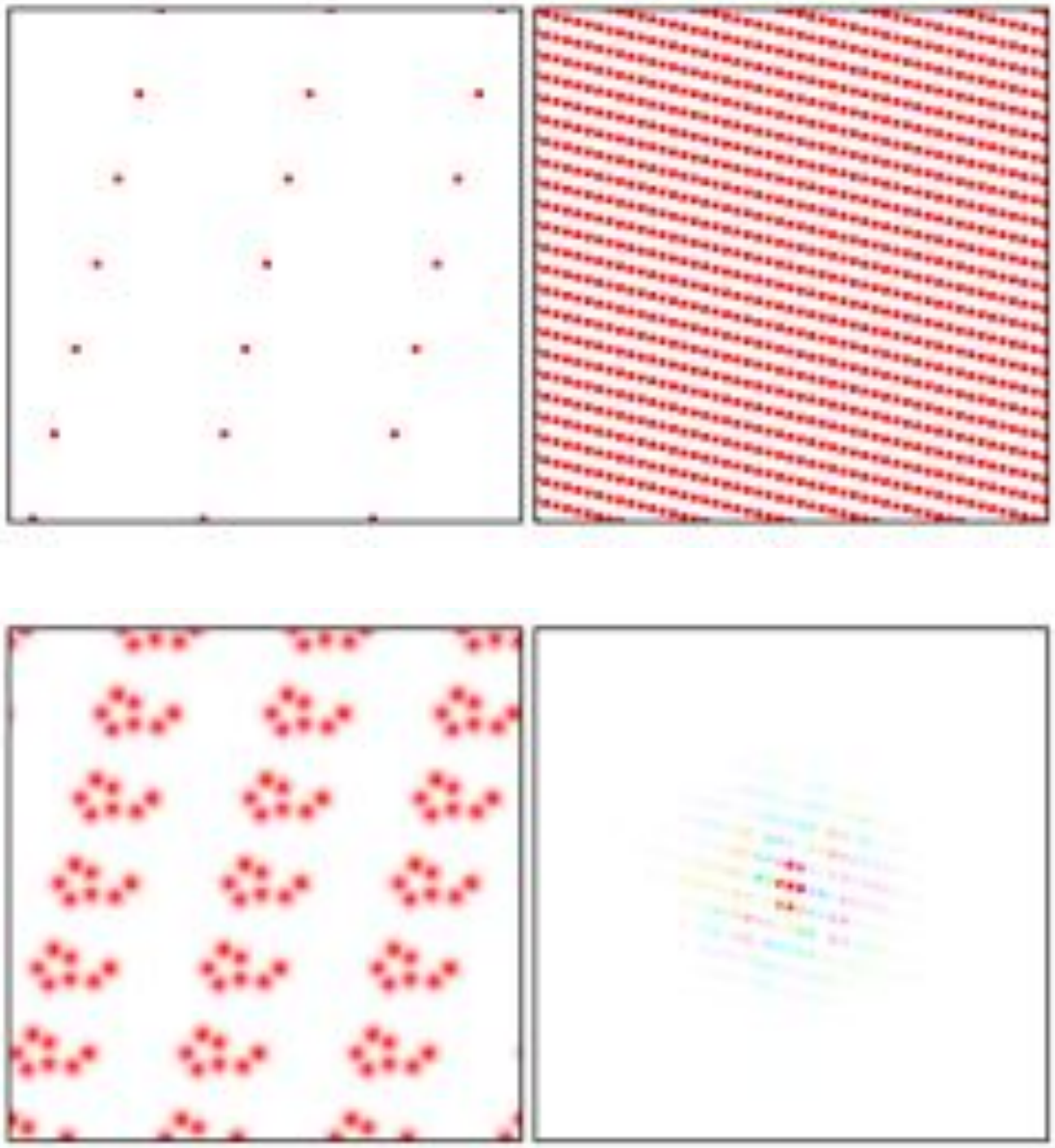
## What is the Fourier transform of an atom or protein crystal ?



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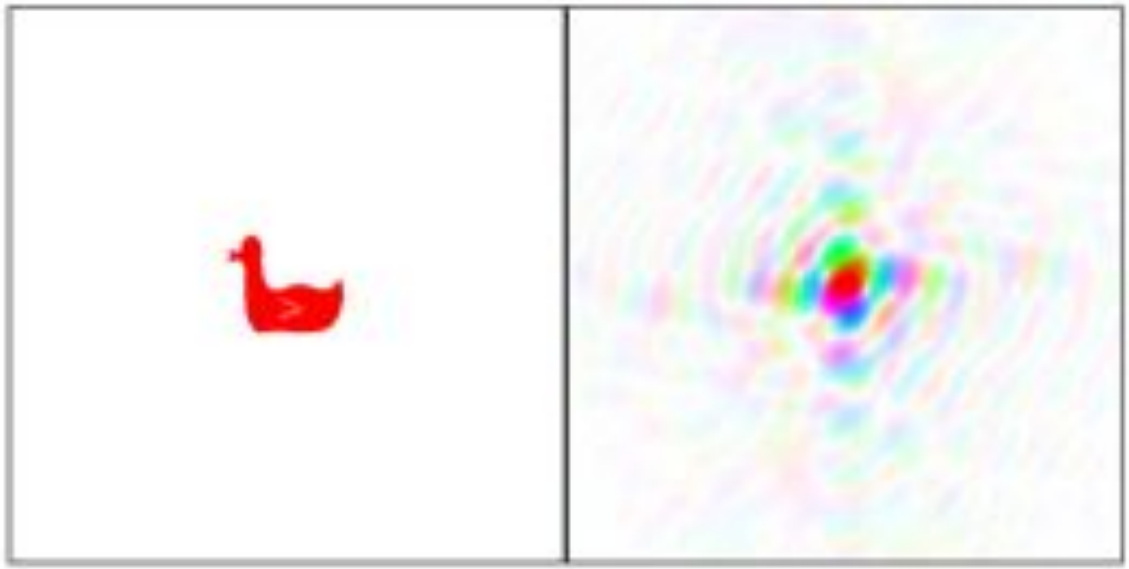
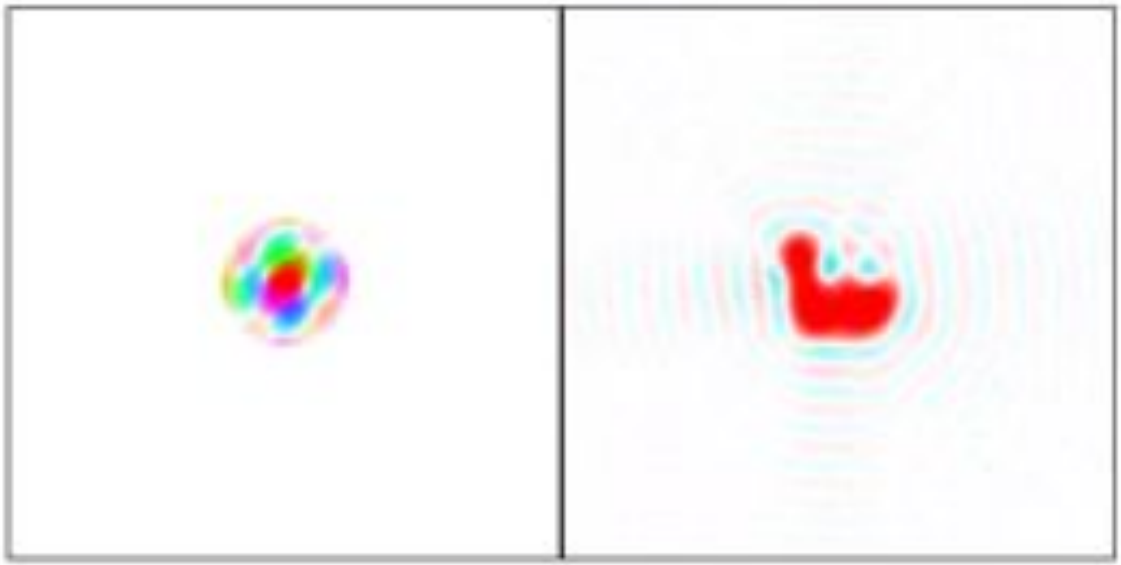
# Why do we need high resolution data ?



Fourier Transform



Reverse
Fourier Transform

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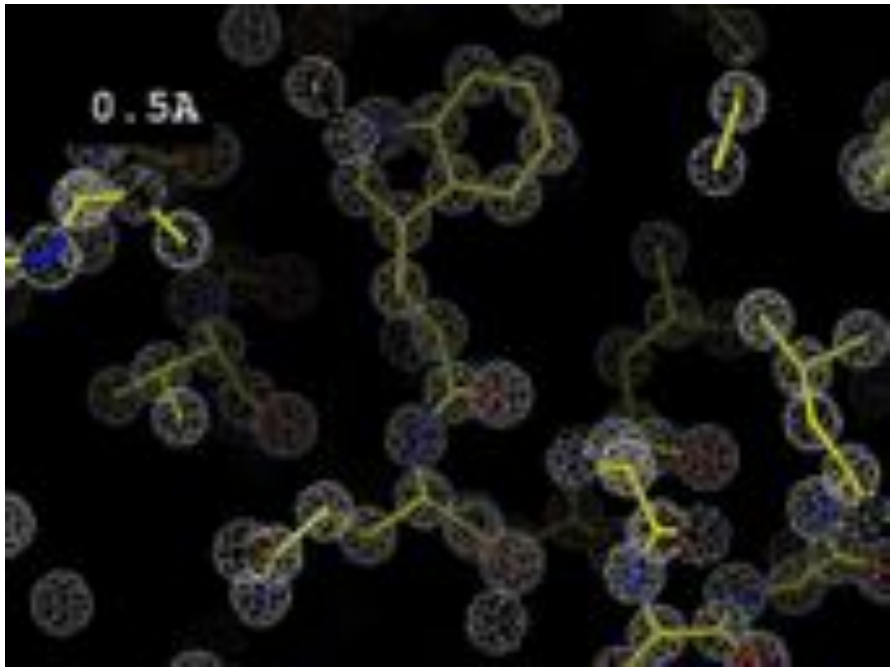
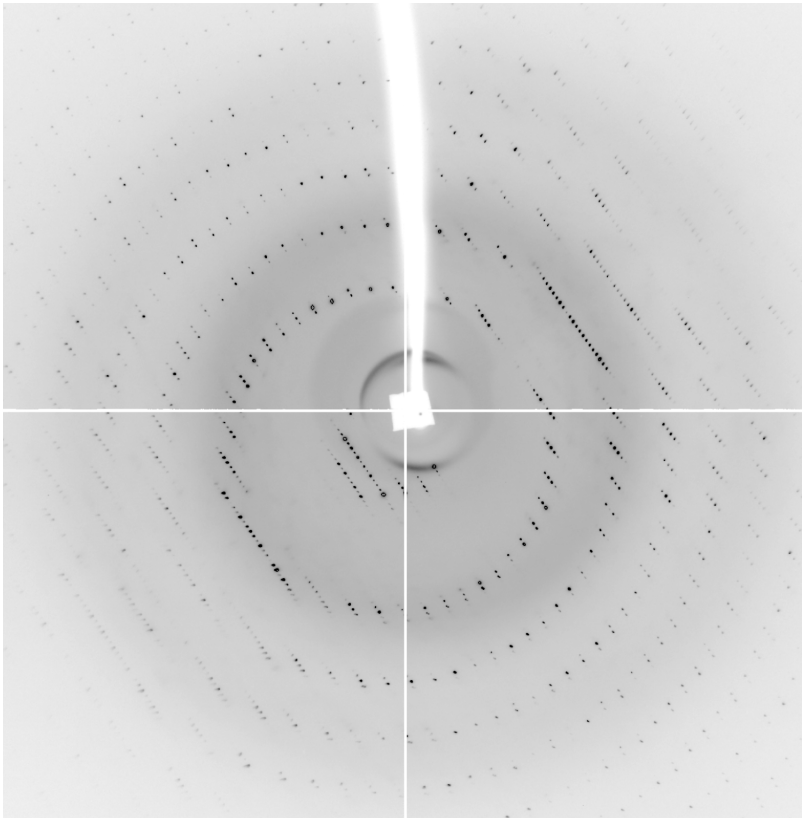
## Why do we need high resolution data ?



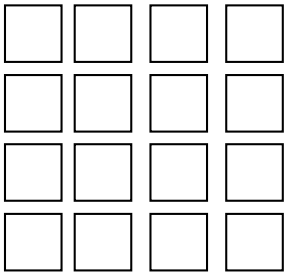

http://ucxray.berkeley.edu/~jamesh/movies/

# Why don’t we always get high resolution data ?

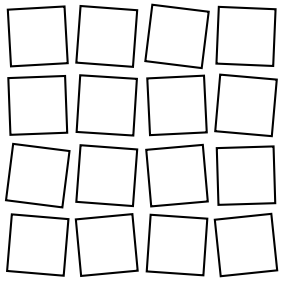


• The crystal isn’t perfect but displays intrinsic mosaicity :

The **quality of the diffraction data** depends on the crystal

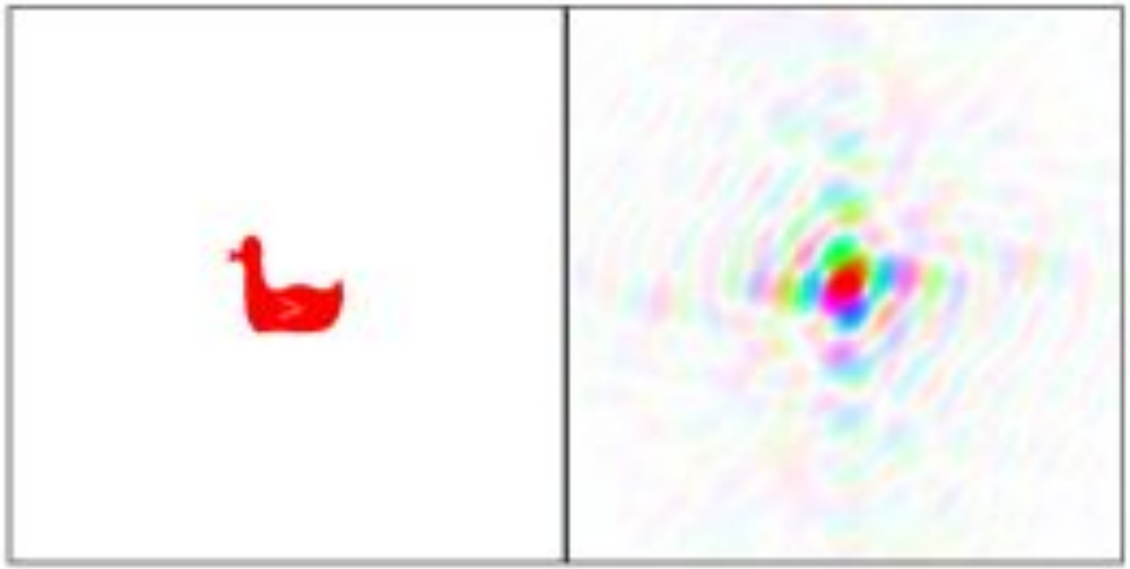


Crystal of good quality

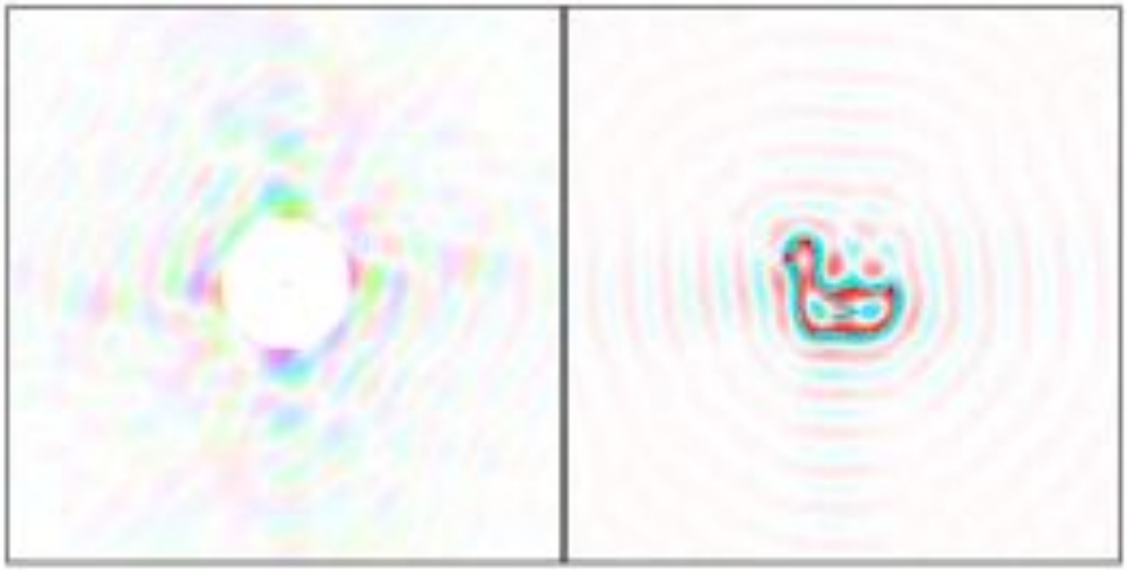


Crystal of bad quality

## Why do we need low resolution data ?



Fourier Transform



Reverse
Fourier Transform

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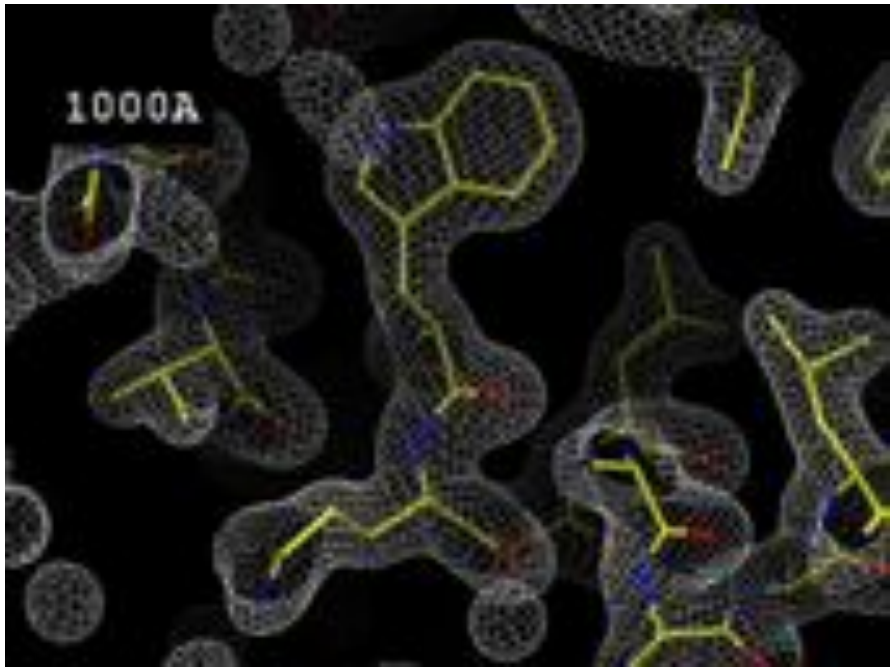
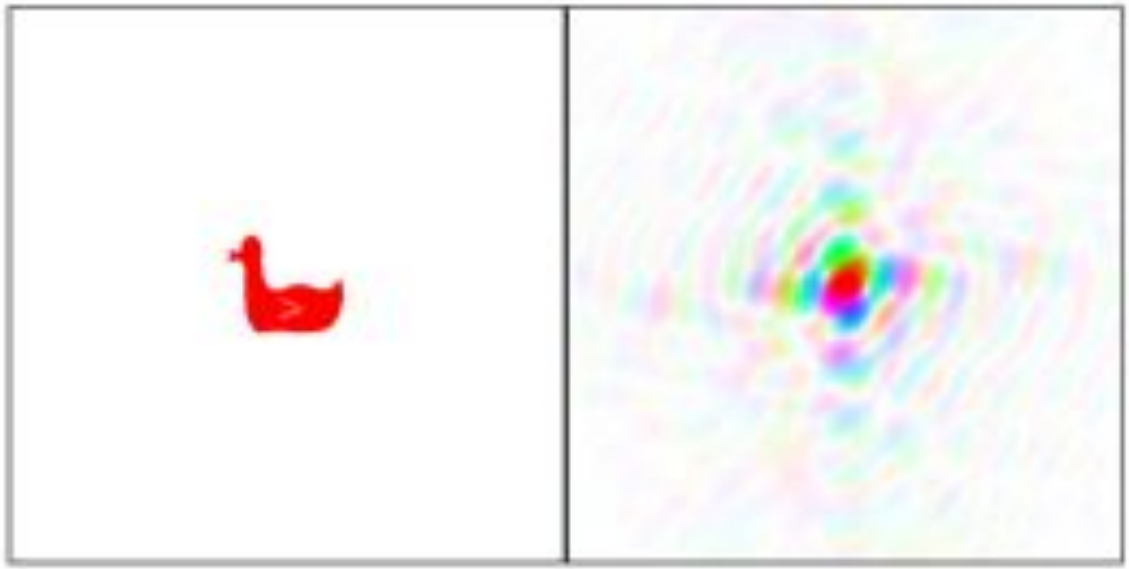
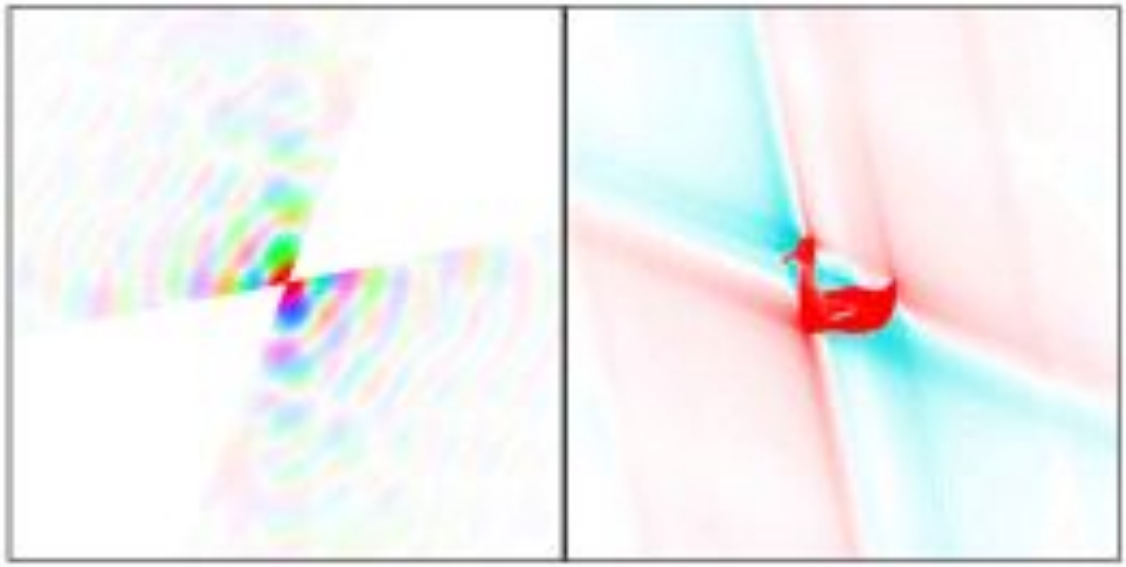



http://ucxray.berkeley.edu/~jamesh/movies/

## Why do we need 100 % completeness ?



Fourier Transform



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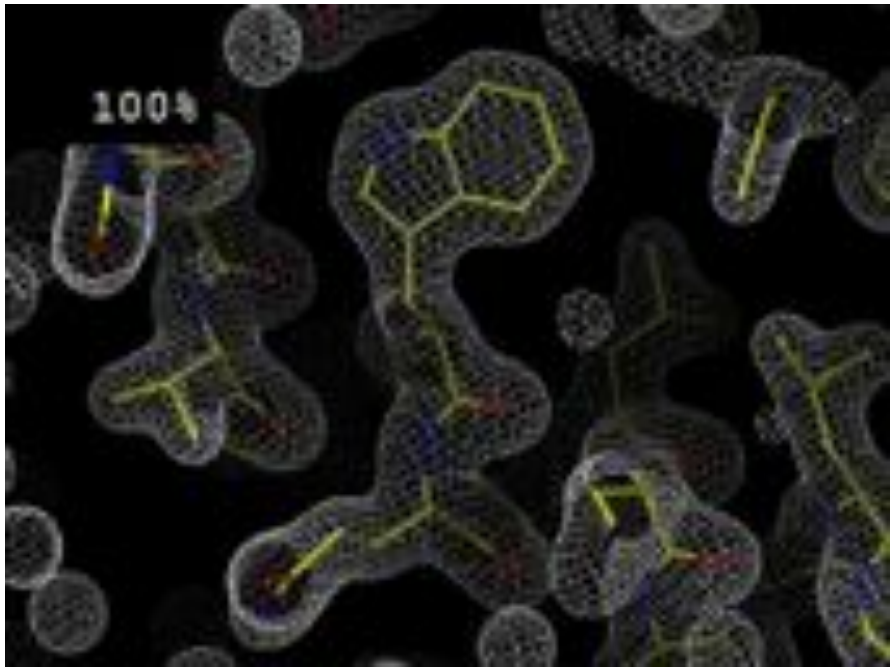


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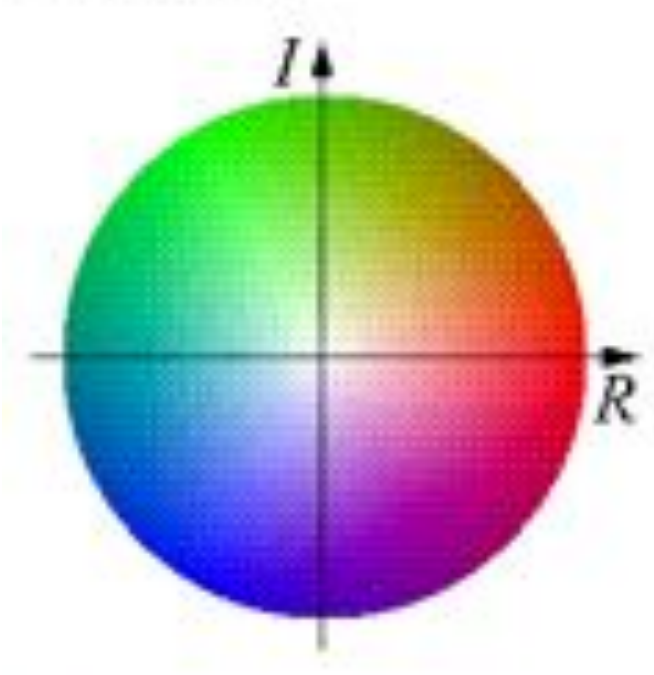


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Phase = Hue/Color

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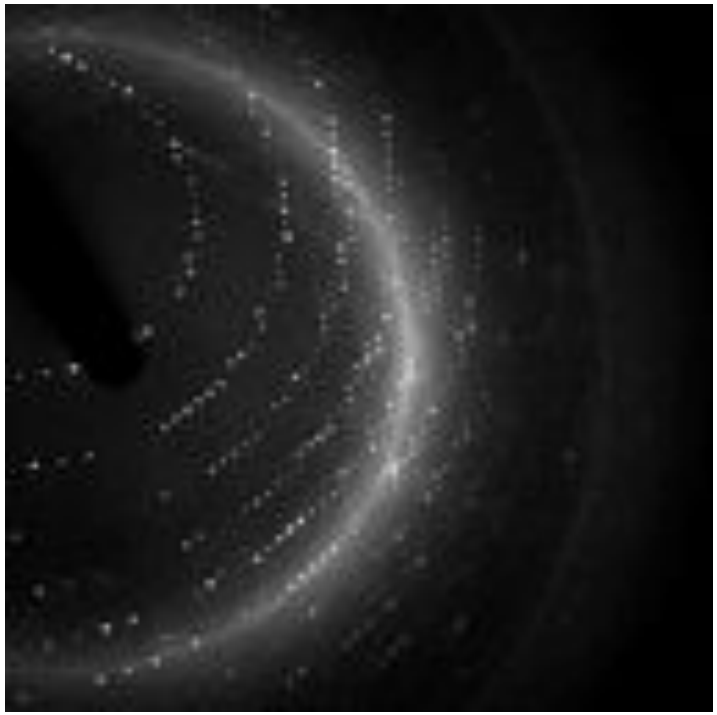


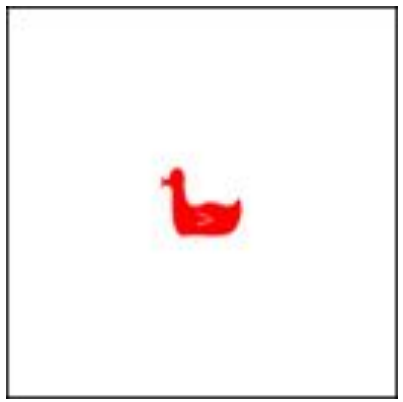
Amplitude = Brightness/Saturation

Phase = Hue/Color ?

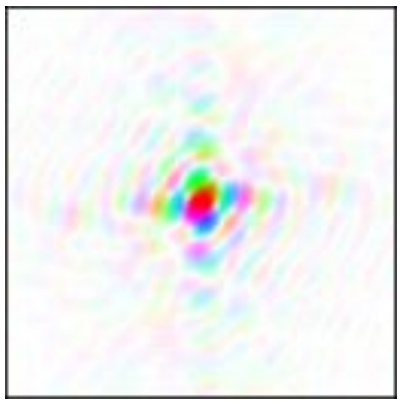
We only measure amplitudes ; the phase information is lost !!!

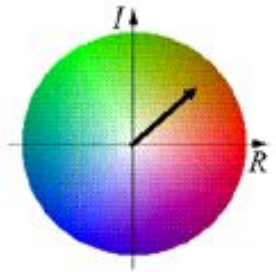
http://www.ysbl.york.ac.uk/~cowtan/fourier/

## Why are phases so important ?



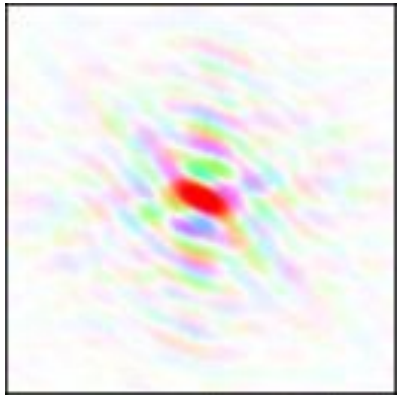
Fourier Transform



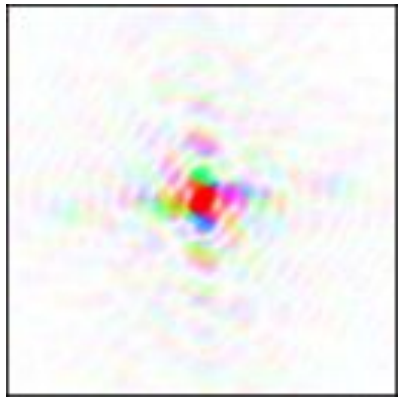



$(A_D, \Phi_D)$

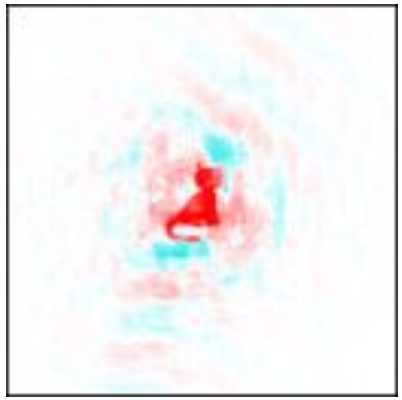
Fourier Transform



$(A_C, \Phi_C)$

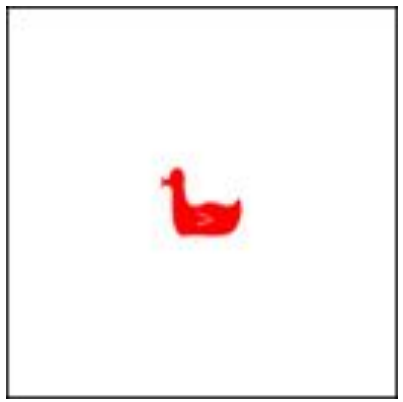


Reverse Fourier Transform

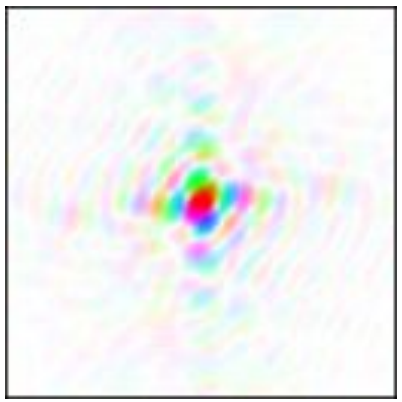


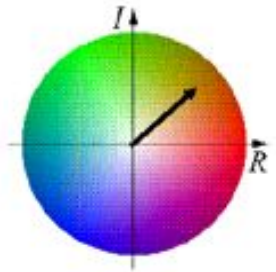
$(A_D, \Phi_C)$

## Why are phases so important ?



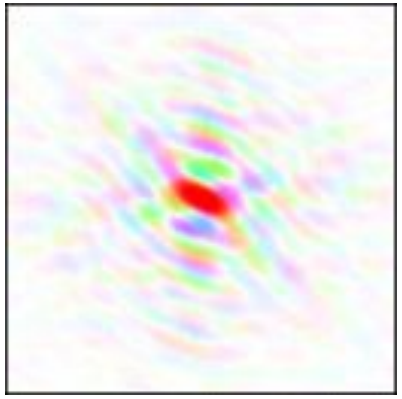
Fourier Transform



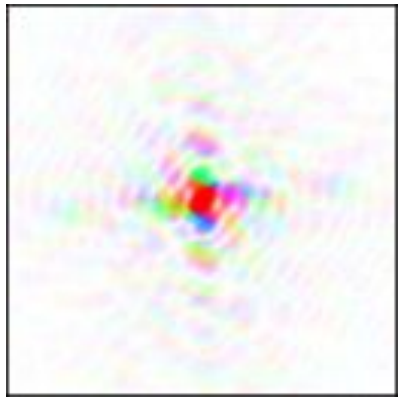



$(A_D, \Phi_D)$

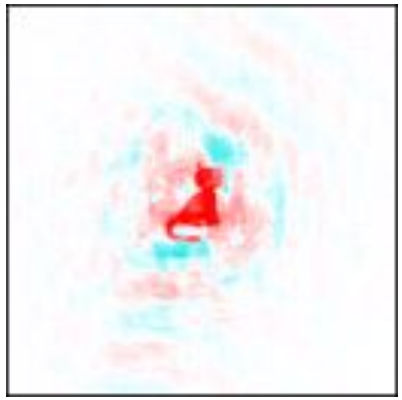
Fourier Transform



$(A_C, \Phi_C)$



Reverse Fourier Transform



$(A_D, \Phi_C)$

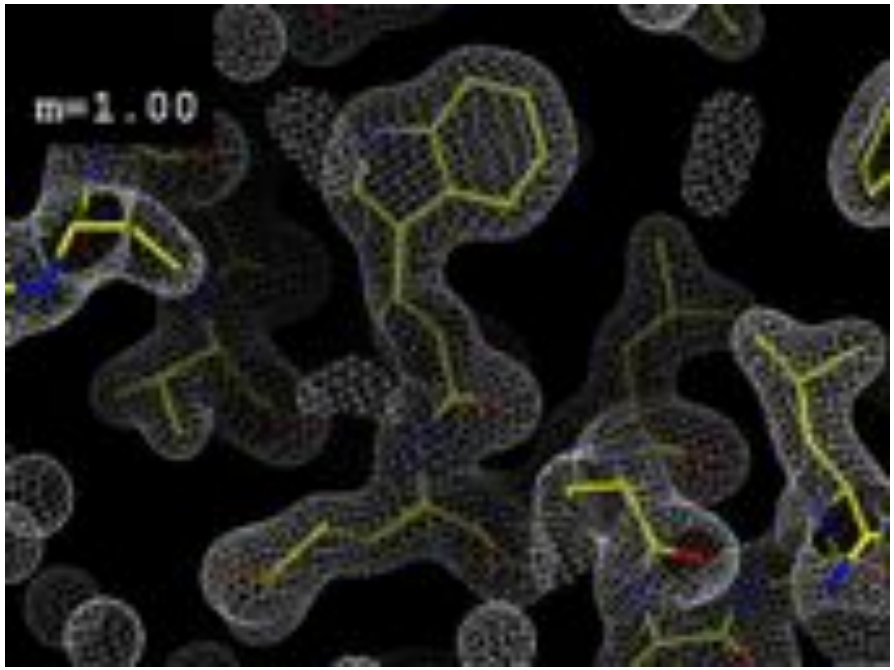
|F| Jerome Karl

Φ Jerome Karl

|F| Herb Hauptman

Φ Herb Hauptman

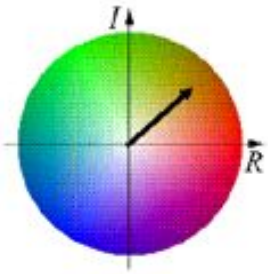
**Why are phases so important ?**




$$\mathbf{m = \cos(\Delta\varphi)}$$

$\Delta\varphi$ = phase error (°)

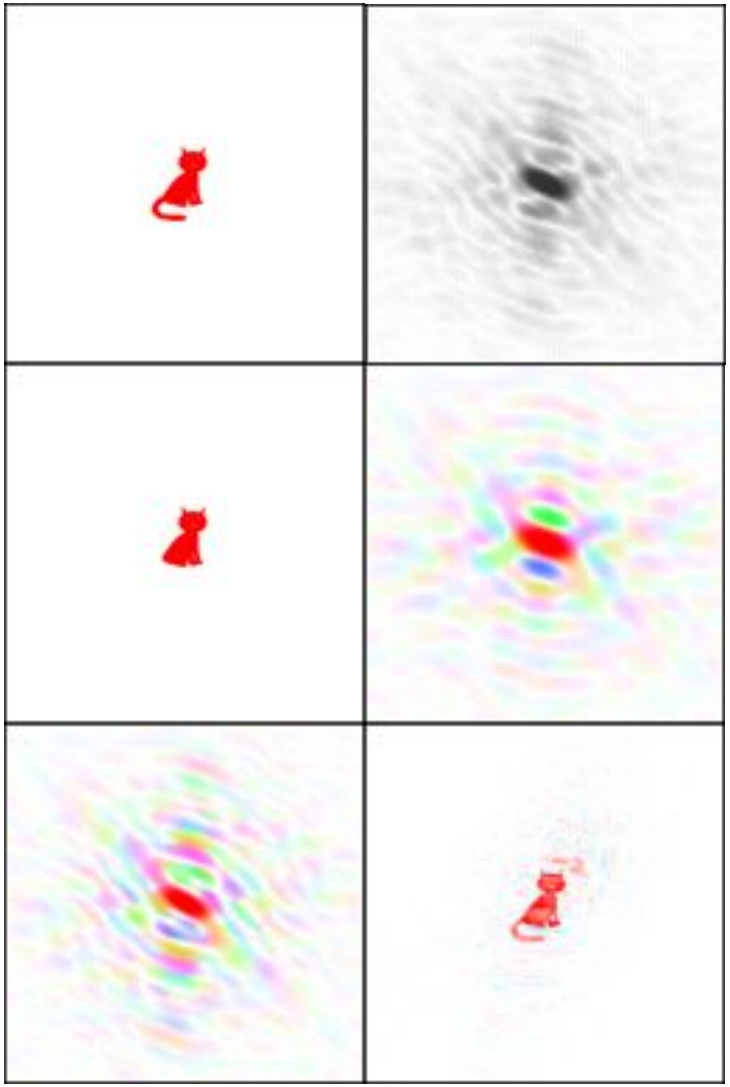
## How can we solve the phase problem ?




Perturb the structure and diffraction pattern : use of heavy atoms or anomalous scatterers

**Guess the phases : Molecular Replacement**

## How is molecular replacement possible ?



Measured data : only intensities (amplitude), no phase information.

$(A_{obs})$

Something known to be similar.

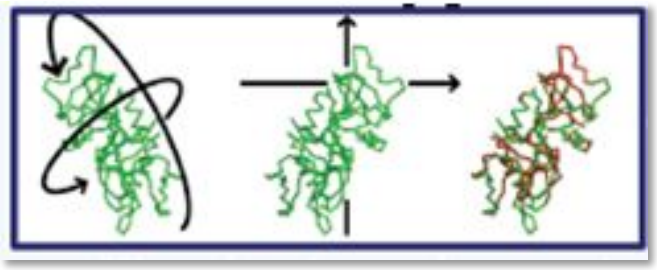
We can calculate phases and amplitudes via Fourier transform

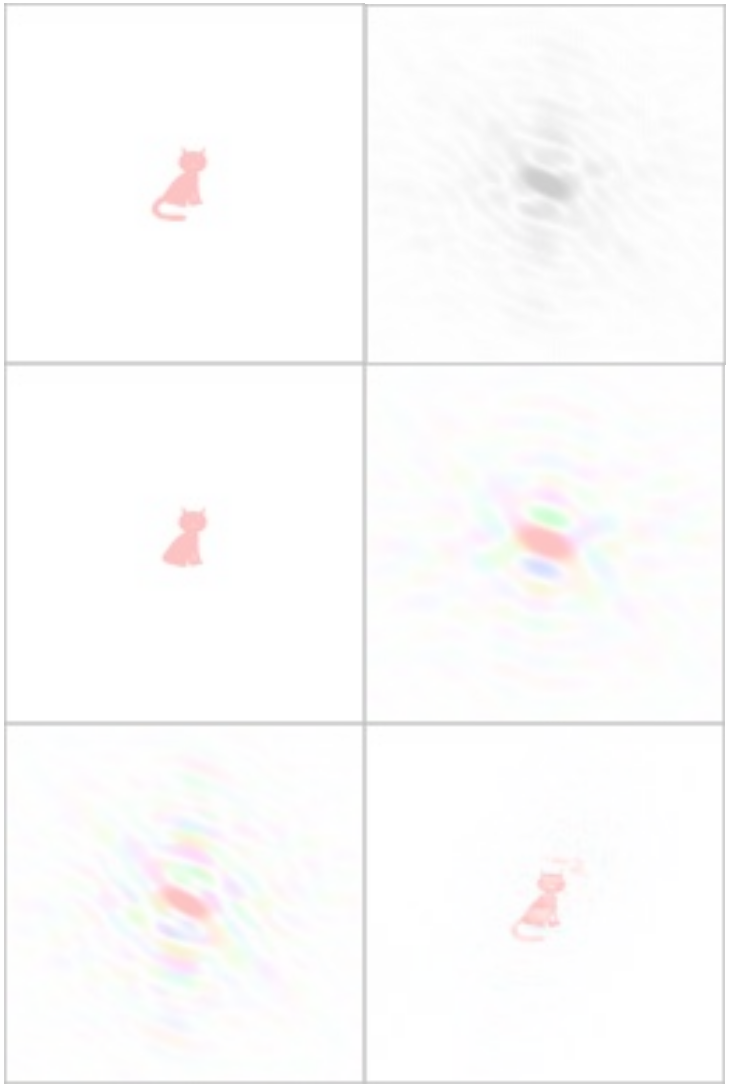
$(A_{calc}, \Phi_{calc})$

Reverse Fourier transform using measured intensities and phases from similar structure

$(A_{obs}, \Phi_{calc})$

**In practice, …**





Measured data : only intensities (amplitude), no phase information.

$(A_{obs})$

Something known to be similar.

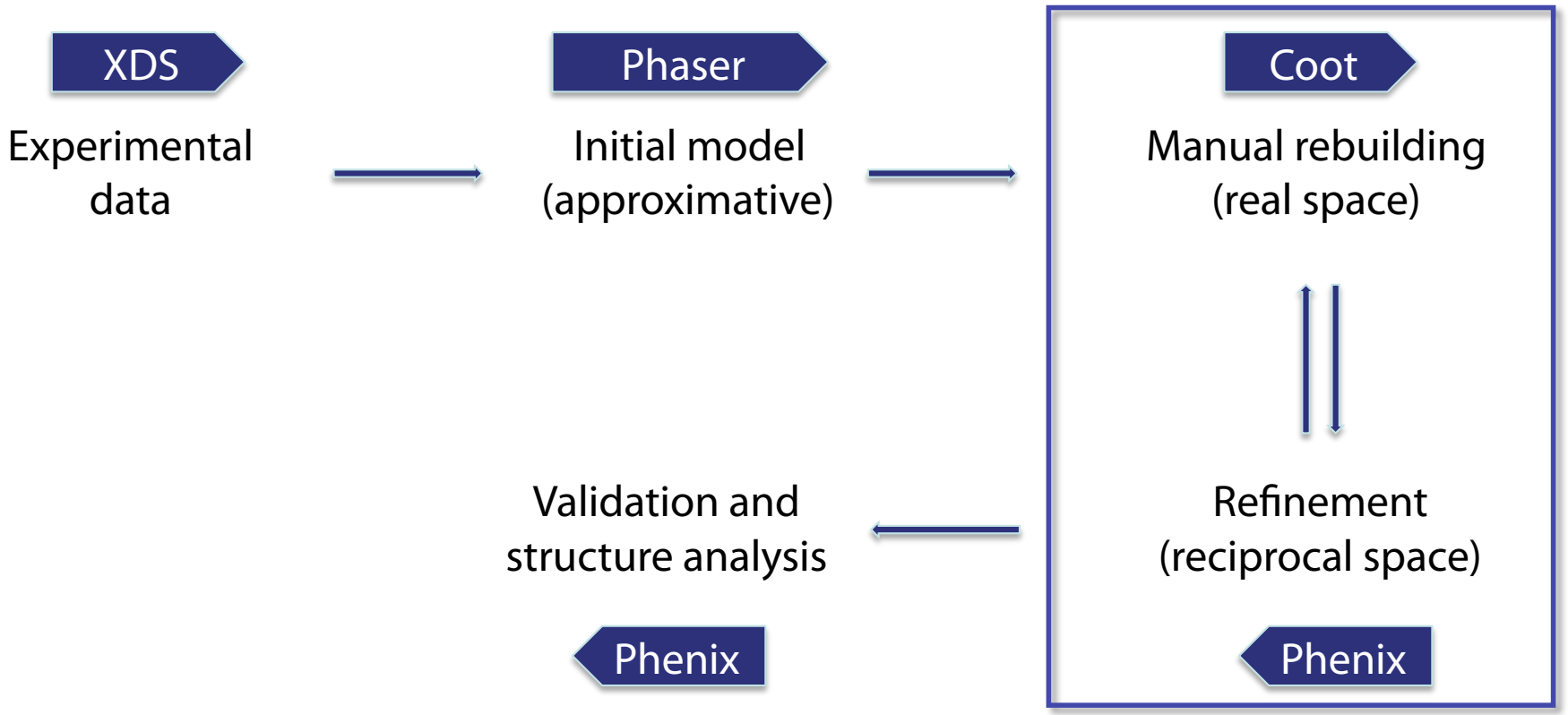
We can calculate phases and amplitudes via Fourier transform

$(A_{calc}, \Phi_{calc})$

Reverse Fourier transform using measured intensities and phases from similar structure

$(A_{obs}, \Phi_{calc})$

**Structure determination flowchart**



## Structure refinement

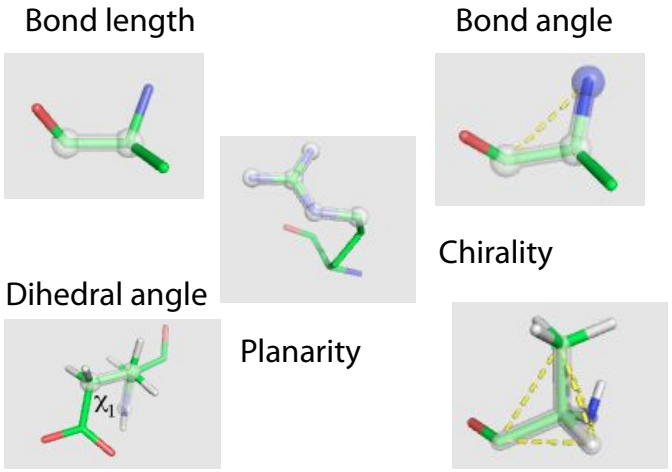
To refine your structure in reciprocal space, you need a weighted function taking into account :

- the geometry of your model (1,2)
(covalent bonds and angles, van der Waals and electrostatic interactions, etc …)

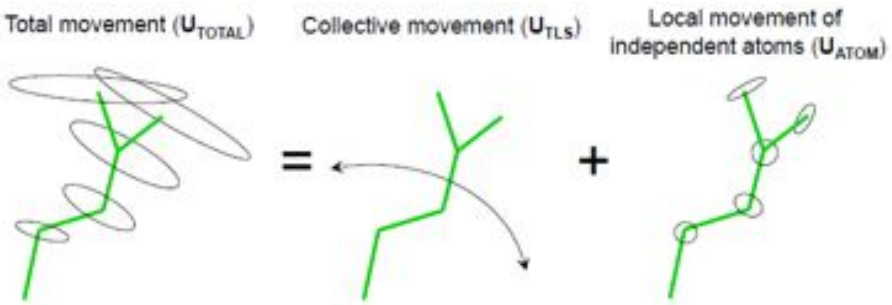
- the agreement with the X-ray experimental data
***(the model which fits the data best)***

- the likelihood of your model
***(the accuracy of such a model)***

(1) Atomic coordinates (x, y, z)



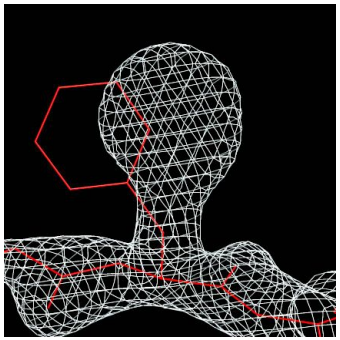

(2) Atomic displacement parameters

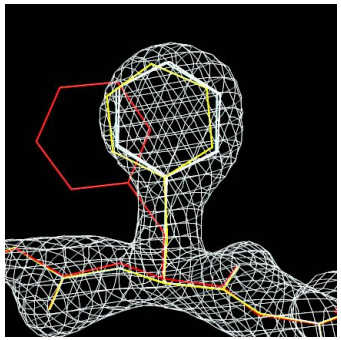



TLS restraints B factor

## Structure refinement

From the refined model, better phases, therefore a more reliable electron density map, allowing to build a better model :





**The best model is the one which has the highest probability given a set of observations and a certain prior knowledge.**

**Outline :**

1: X-ray macromolecular crystallography in an equation-free nutshell

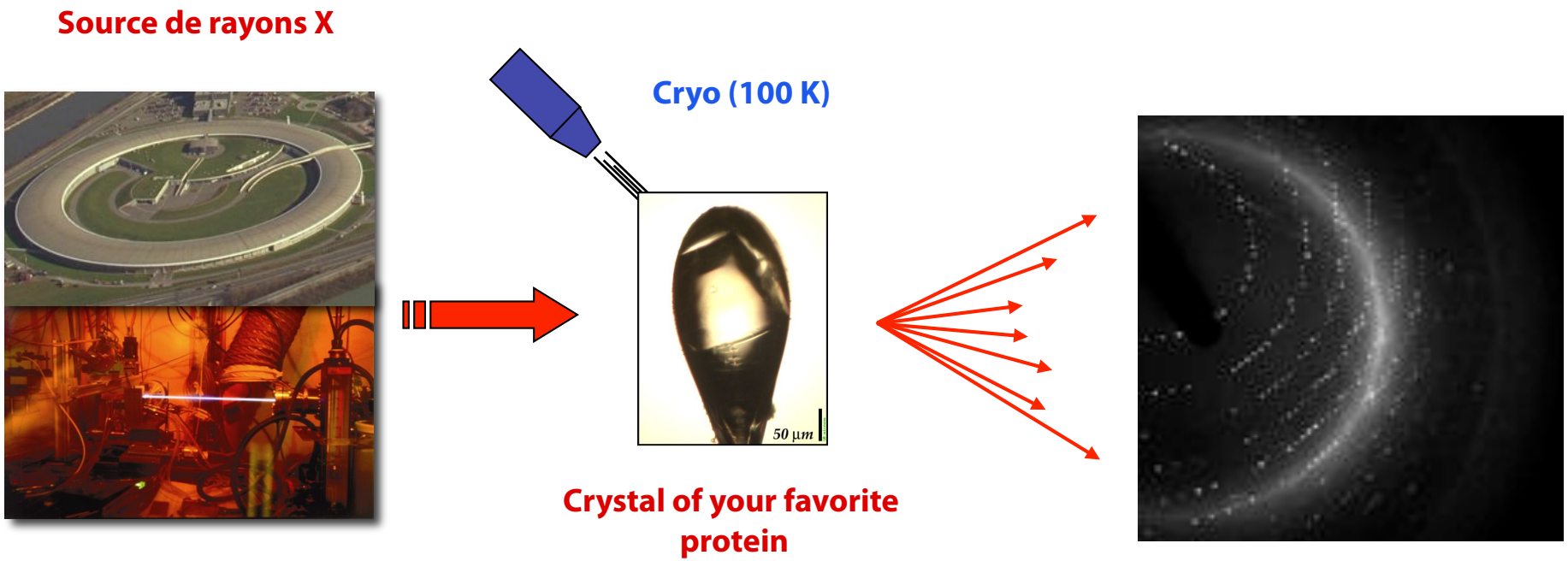
**2: Conventional micro-crystallography at synchrotron-radiation (SR) sources**

3: Serial micro-crystallography (XFELs and SR sources)

4: Serial nano-crystallography (XFEL sources)

5: Coherent X-ray diffraction and direct phasing of structural information

# Conventional crystallography : use of oscillation for data collection

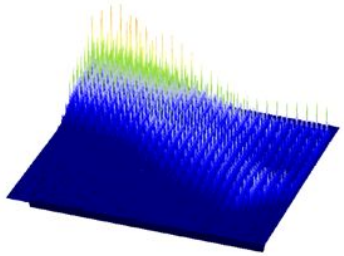


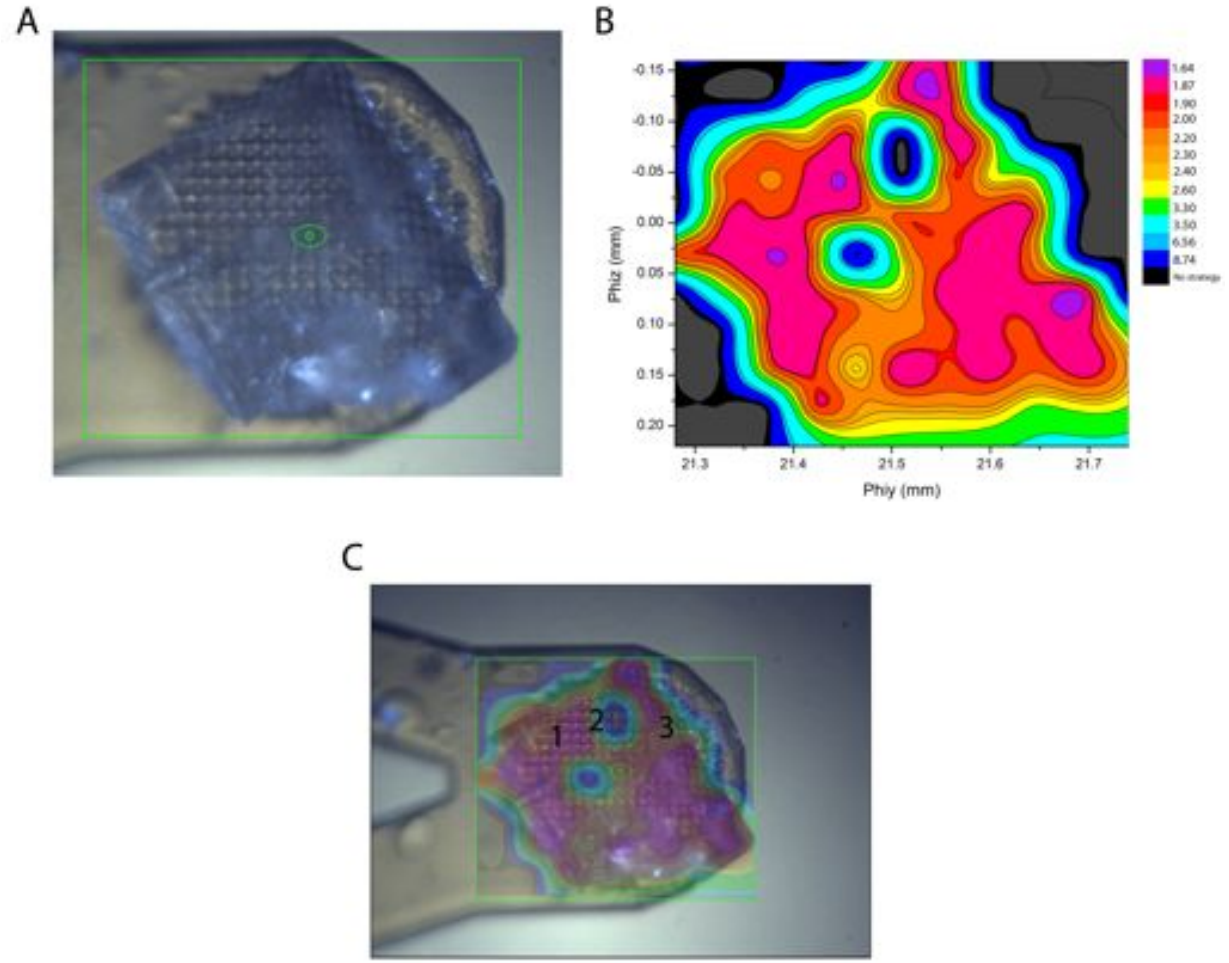
# When is it necessary to resort to micro/nano crystallography ?

## Heterogeneity in crystal quality

*Beam radius: 10 - 2.5 µm*

e.g. crystals of membrane proteins, ribosome, etc.



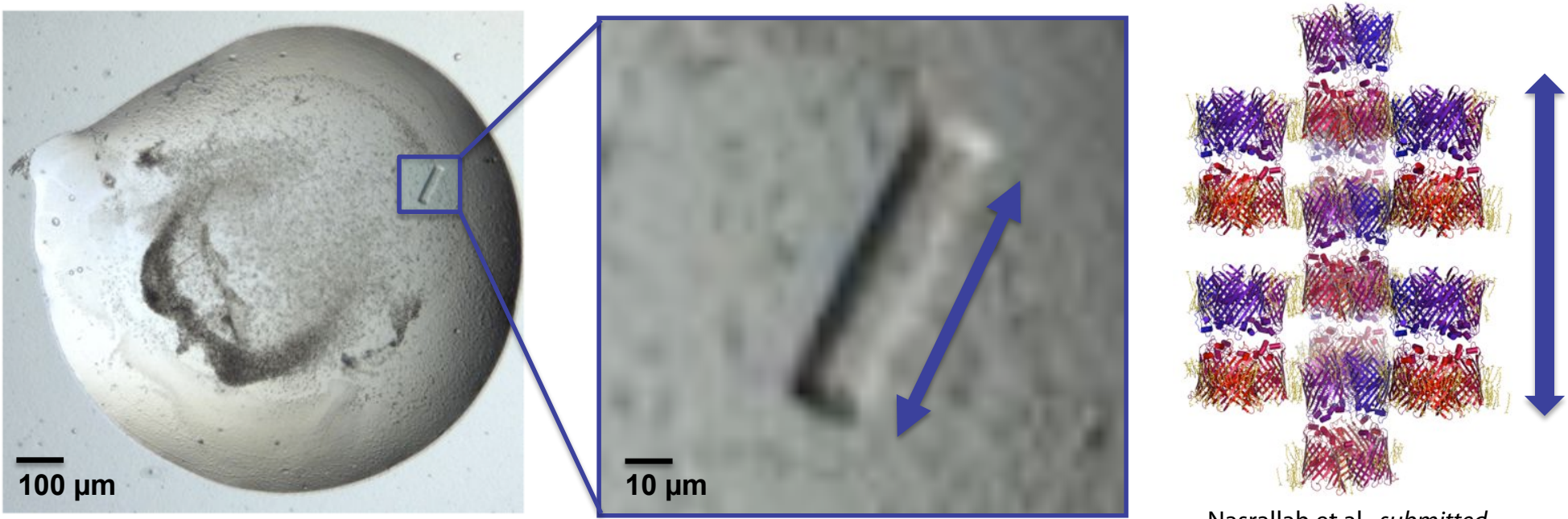



http://www.esrf.eu/UsersAndScience/Experiments/MX/About_our_beamlines/mesh_helical/Cartography2.png

**Small crystals**

*Beam radius: 1 - 2.5 µm*

e.g. crystals of membrane proteins, ribosome, etc.

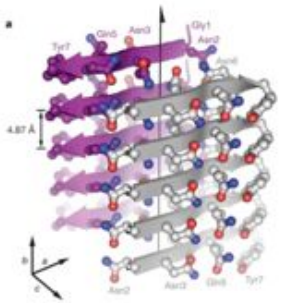



Nasrallah et al., *submitted*

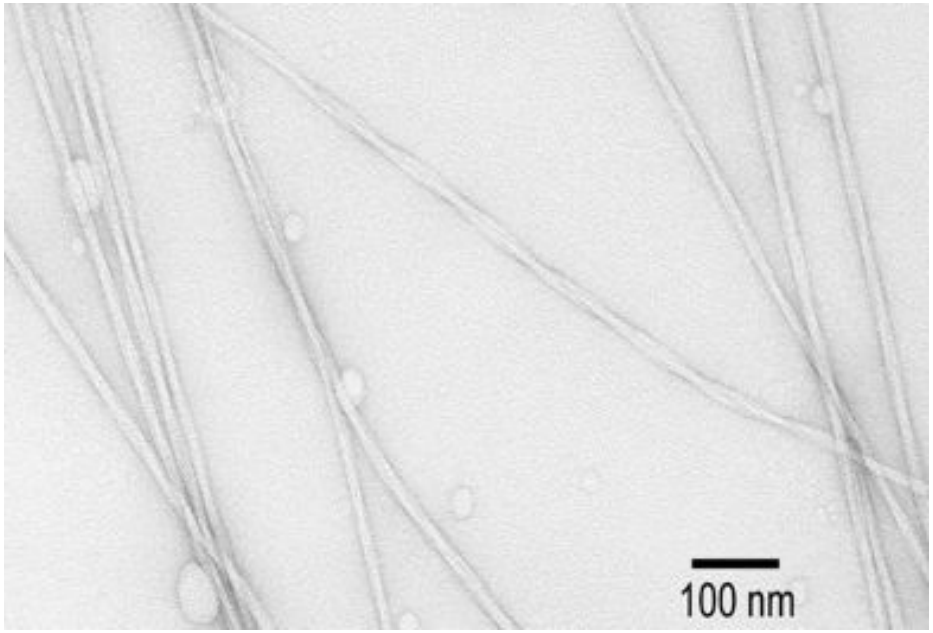
## Micro- and nano-crystals

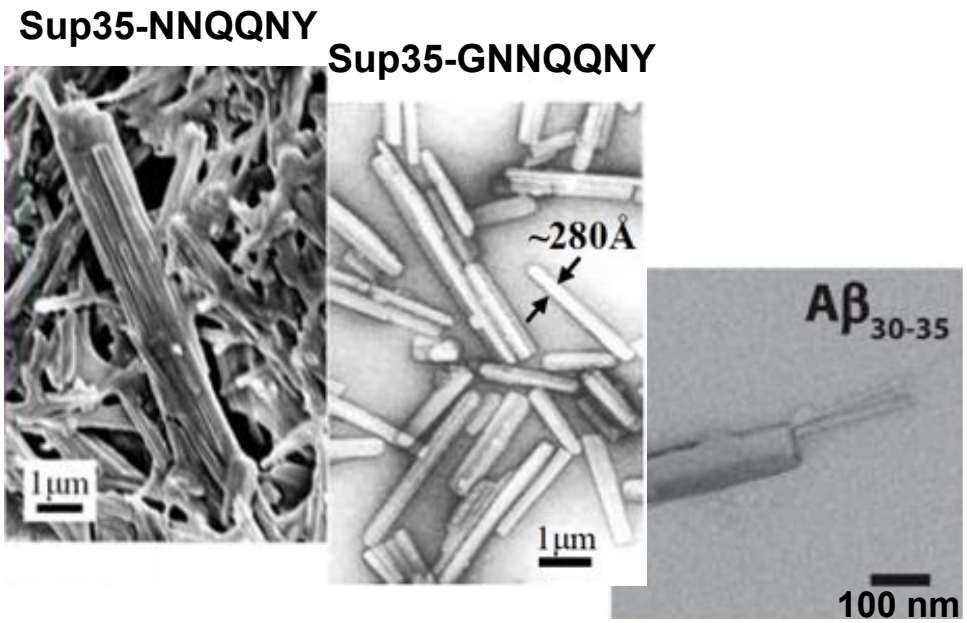
*Beam radius: 0.1- 2.5 µm_*

e.g. crystals of membrane proteins, proteins complexes, amyloid proteins



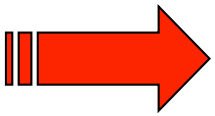
Nelson *et al.*, 2005, *Nature*



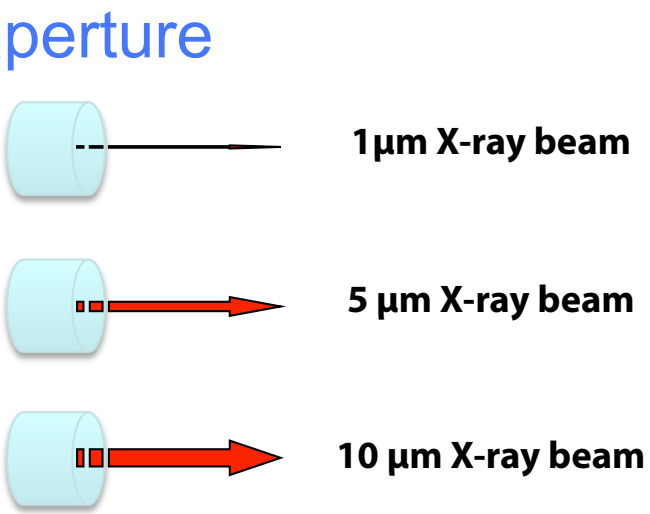



# Micro/nano-beam & micro/nano-focused beams are not the same thing

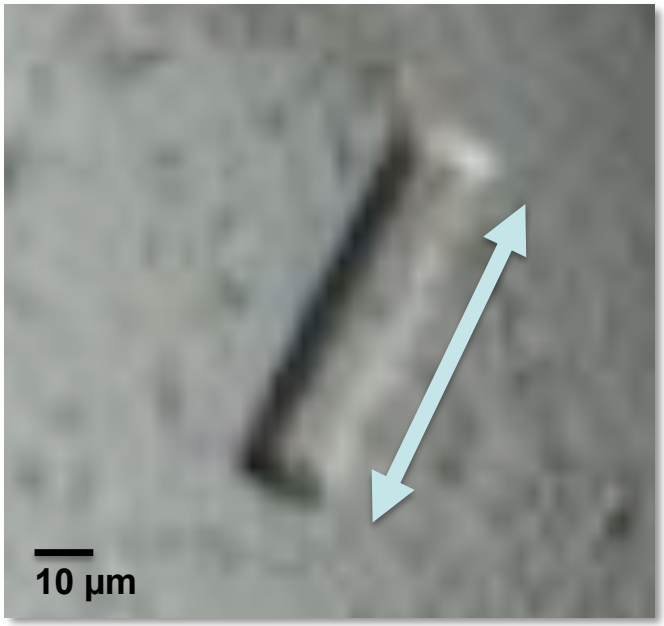
## **Micro-beam** vs. micro-focused beam



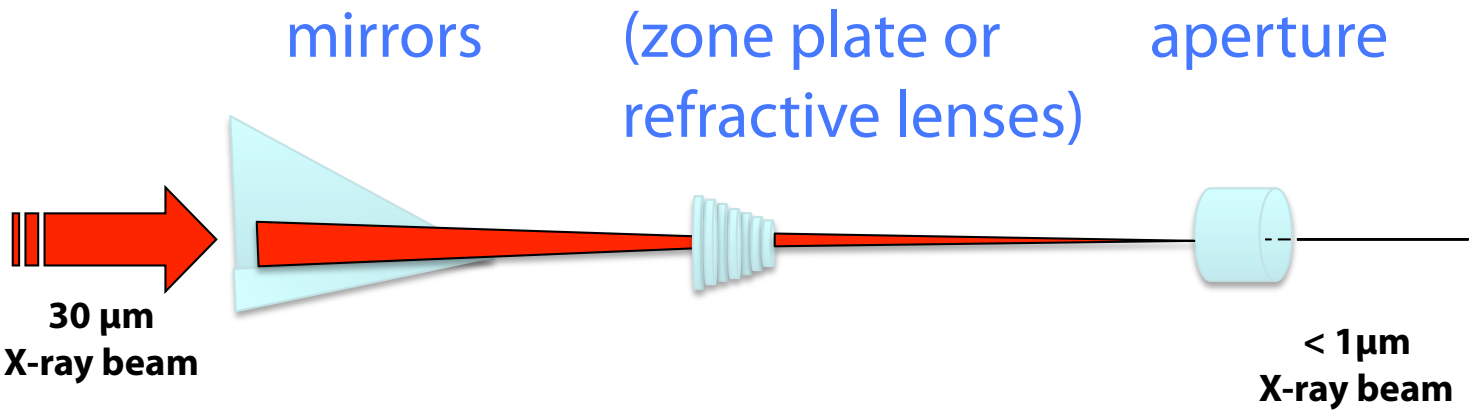
30 µm
X-ray beam



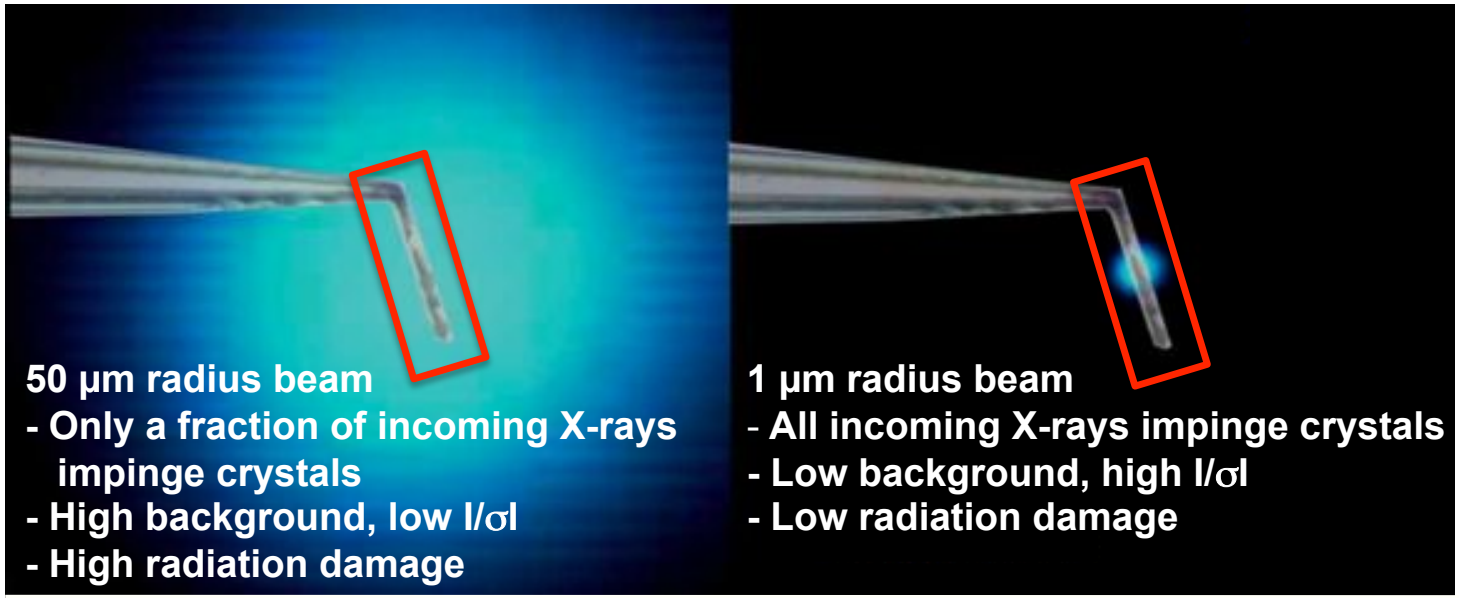

Suited for **small crystals** or for crystals displaying **heterogeneity in diffraction quality**



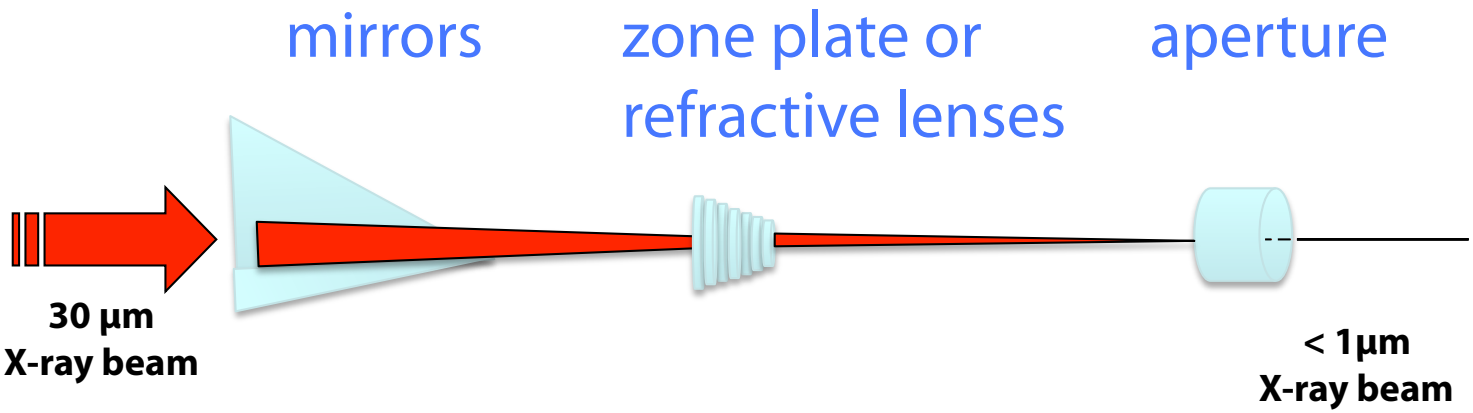
# Micro-beam vs. micro-focused beam



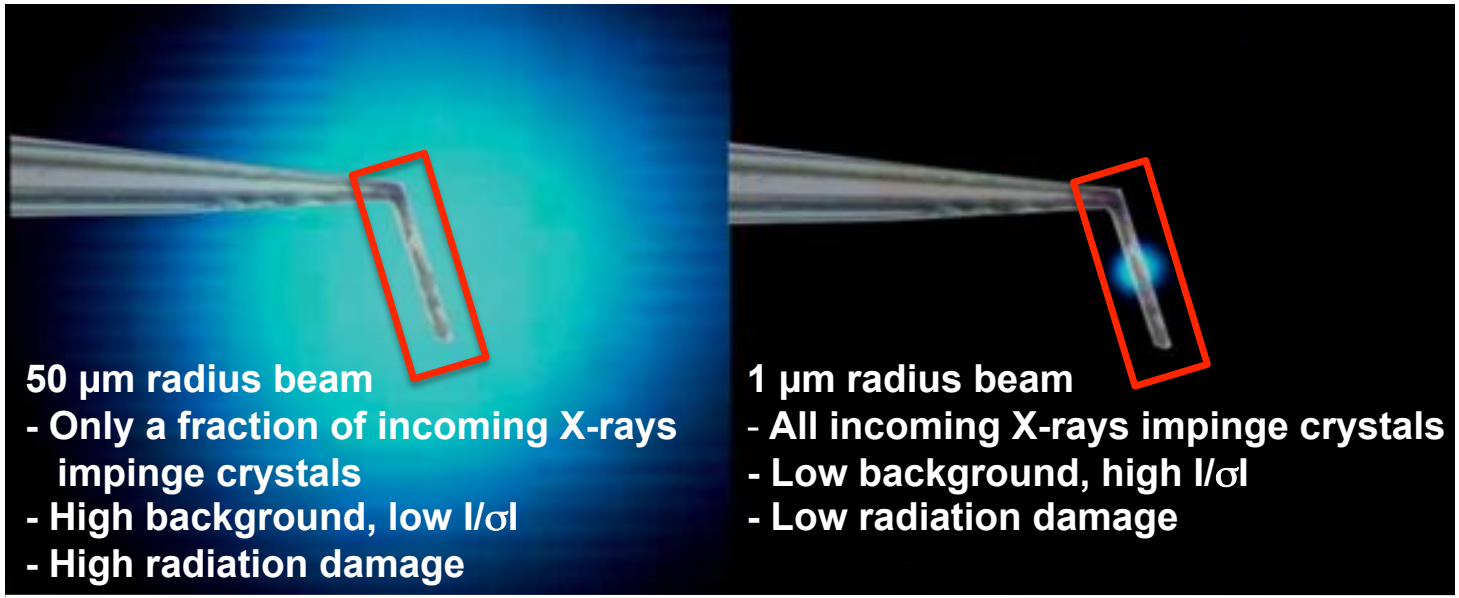

Suited for
**micro-crystals.**



# Micro-beam vs. micro/**nano**-focused beam

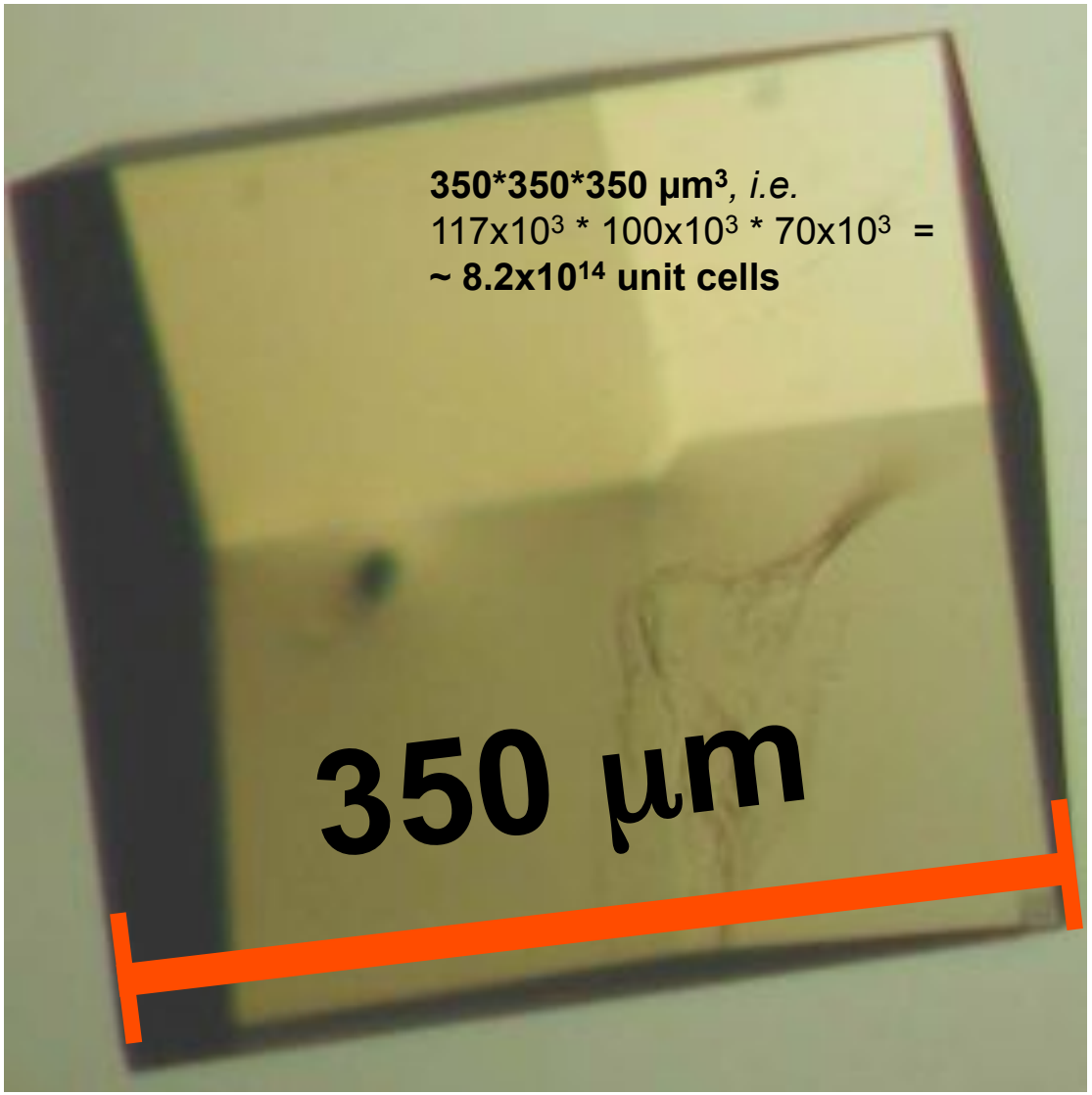


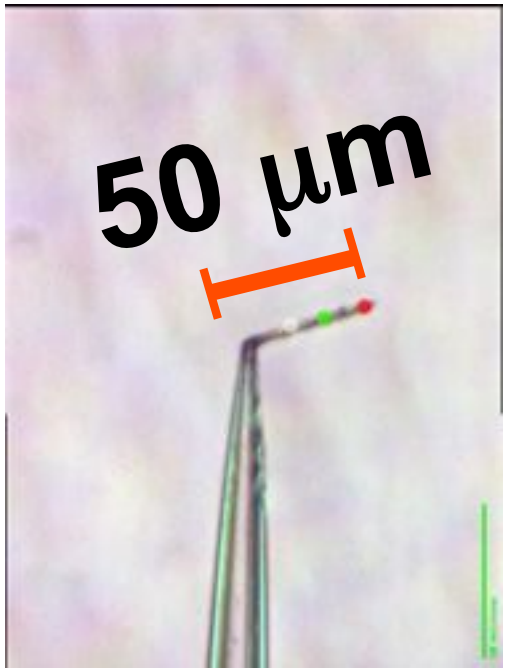

Suited for
**micro/nano**
**-crystals.**



**To collect oscillation data, crystals must be mounted & centered beforehand**

## Conventional size *vs.* microcrystals



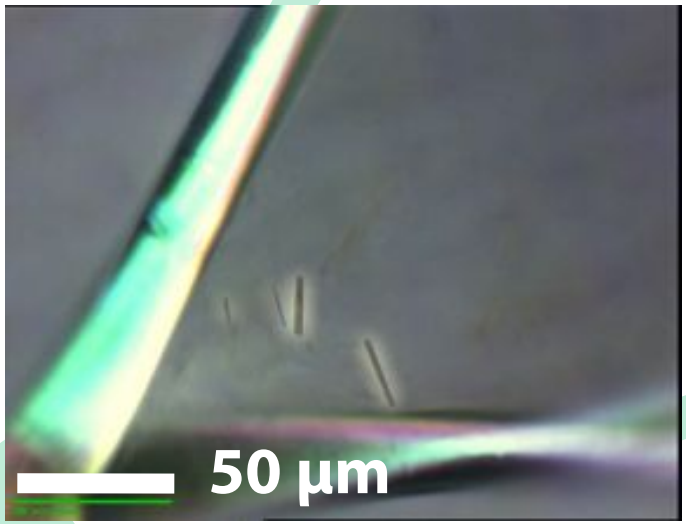

**2*2*50 µm³**, *i.e.*
0.9x10³ * 0.9x10³ * 100x10³ =
**8.1x10¹⁰ unit cells total**

***10,000 LGNY microcrystals could fit in this lysozyme crystal***

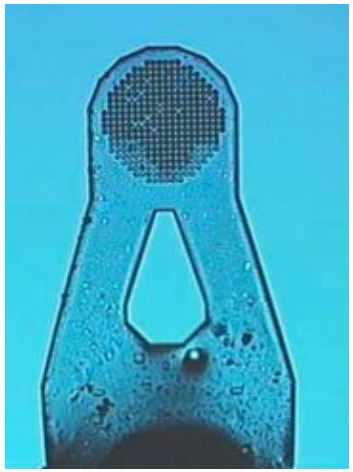
# How can we mount and centre a microcrystal ?

## Loop vs. mesh

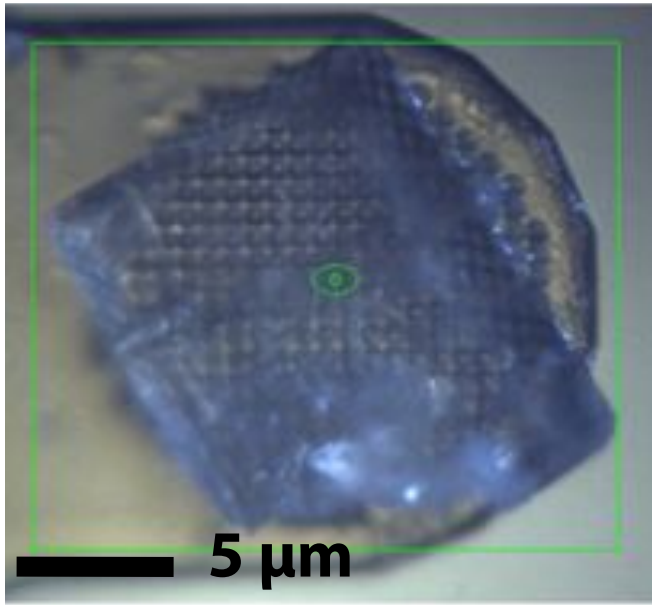
loop




- Background scattering from loop
- Crystal is hidden in the loop fiber and is difficult to centre

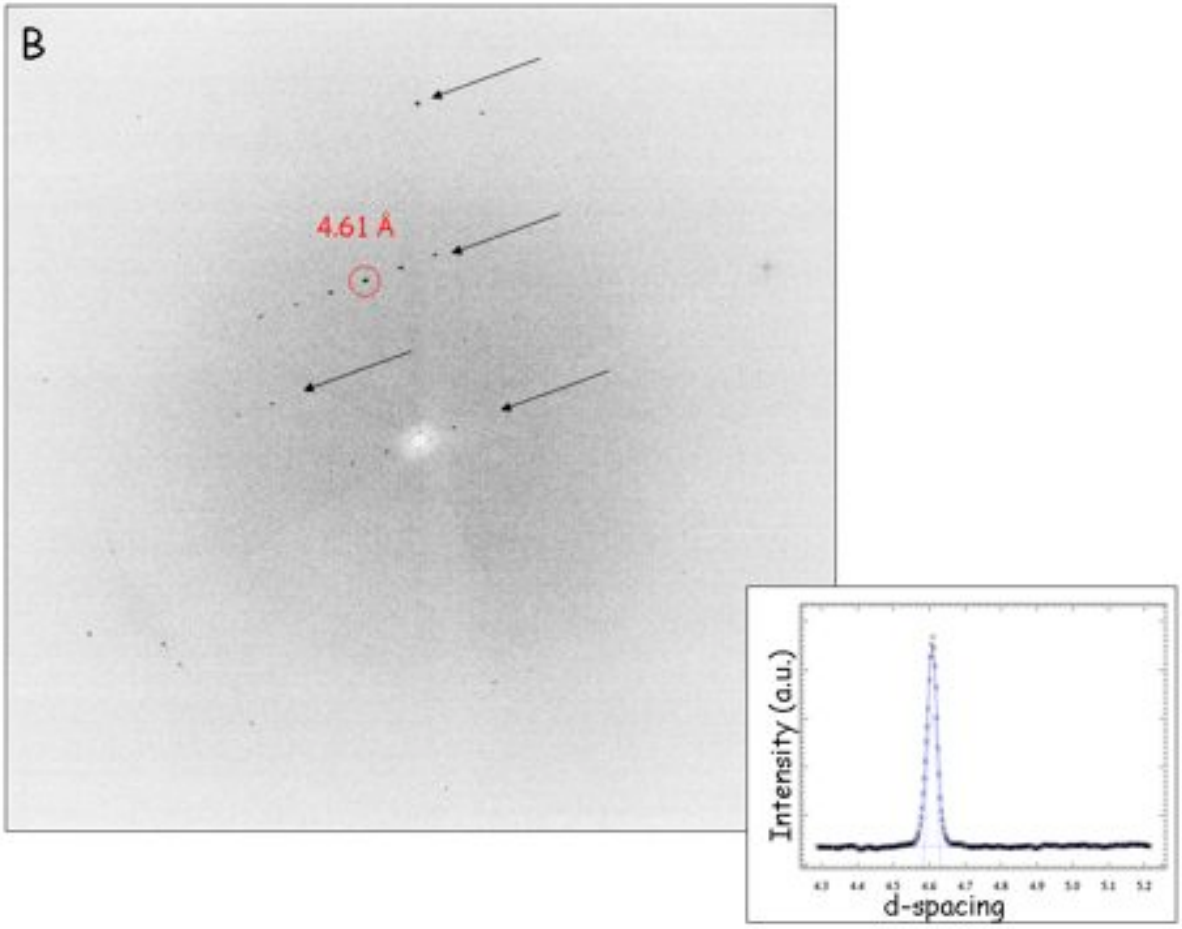


µ-mesh




- Reduced background scattering
- Easier to see & center crystal
- Improved diffracted intensities

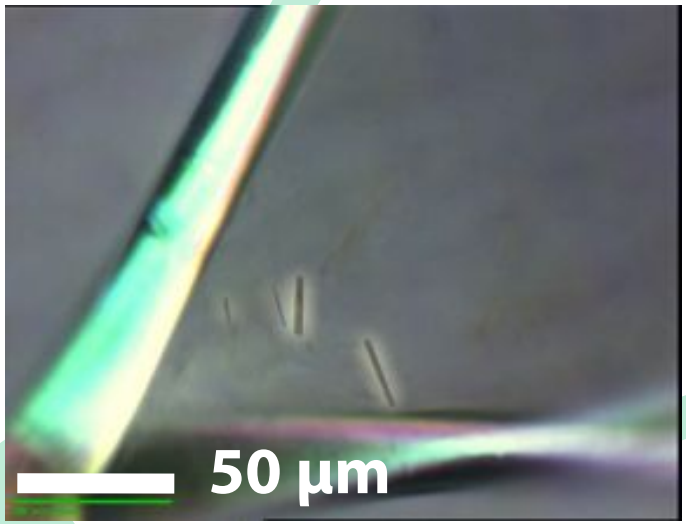
# Loop vs. mesh


KLIMY peptide needles

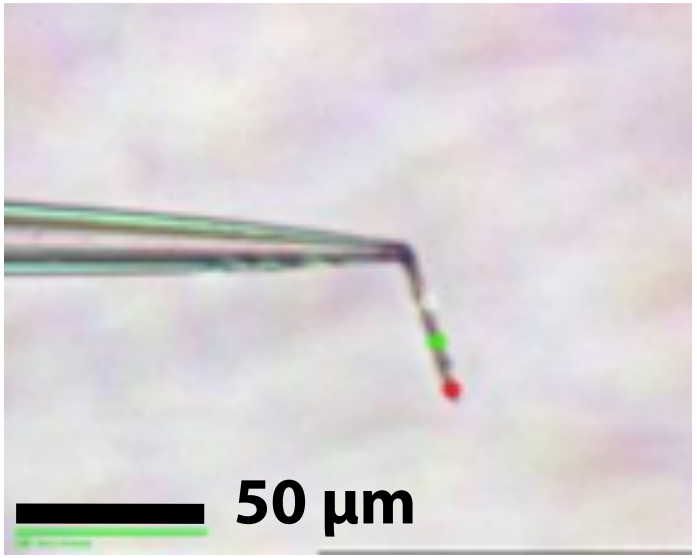
# Loop vs. capillaries

## loop




- Background scattering from loop
- Crystal is hidden in the loop fiber and is difficult to centre

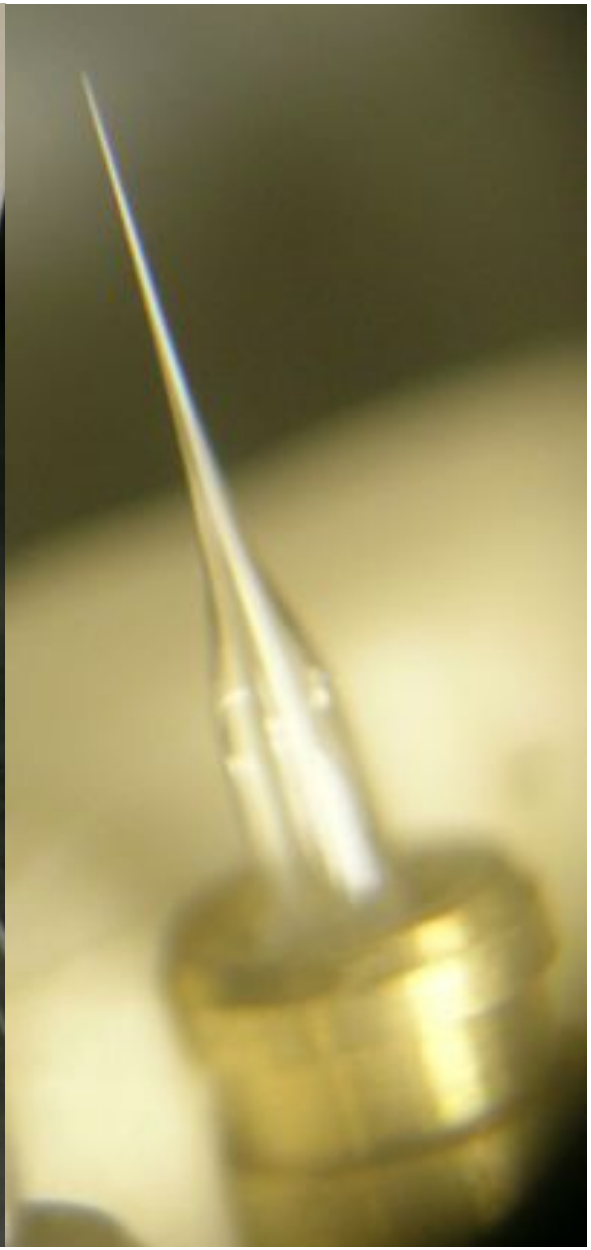
## glass µ-capillary




- No background scattering
- Easy to see & center crystal
- Improved diffracted intensities

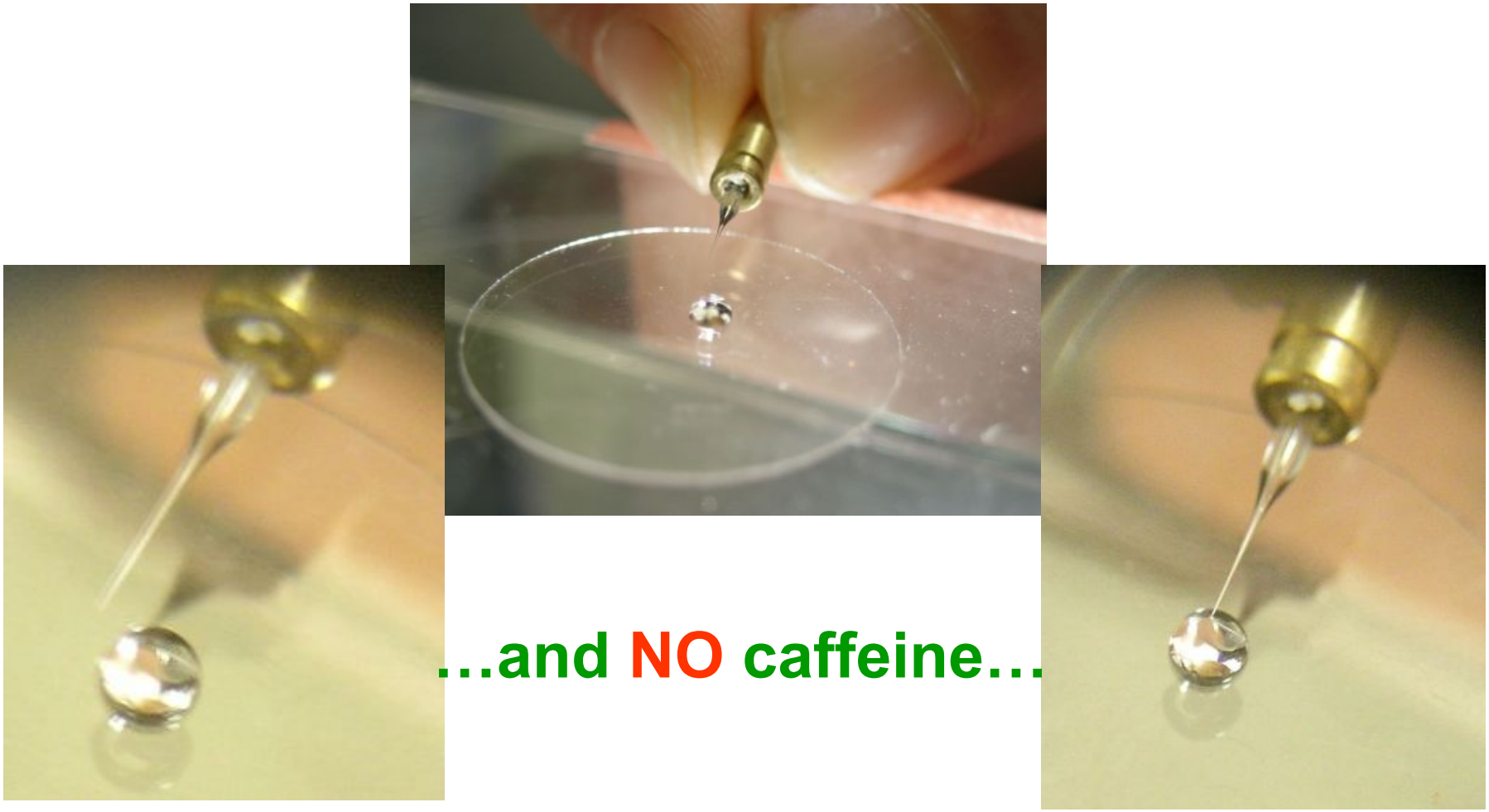
# How to mount a crystal on a glass µ-capillary ?

# Crystal Mounting

## Crystal Mounting

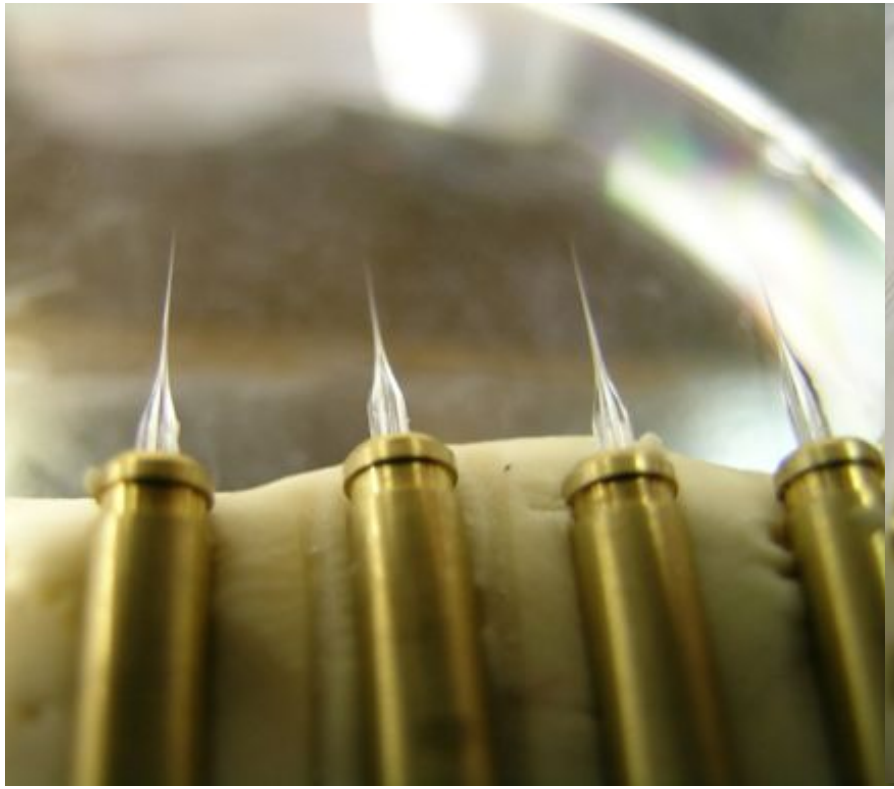
**Fish the microcrystals out of the drop using > 10x**



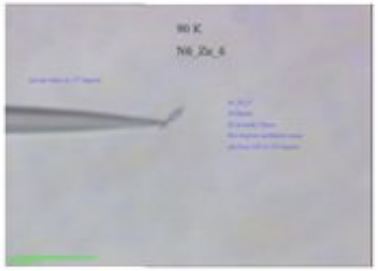
**…and NO caffeine…**

**30x(….and repeat, and patience…...)**

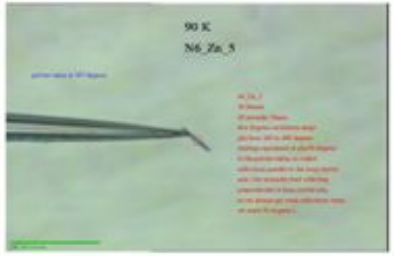
# Crystal Evaluation under microscope

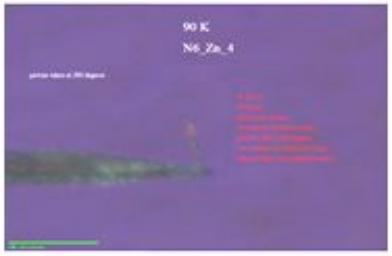


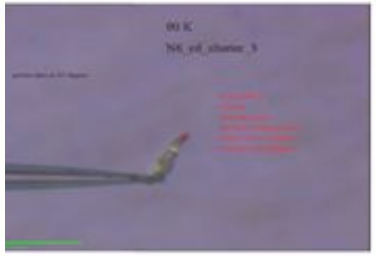
# Screen through dozens of crystals for diffraction quality

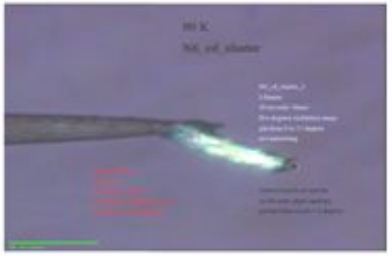


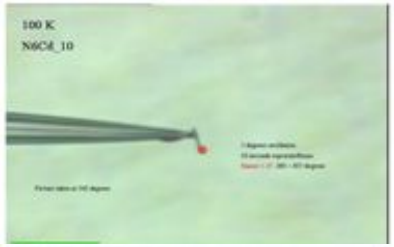


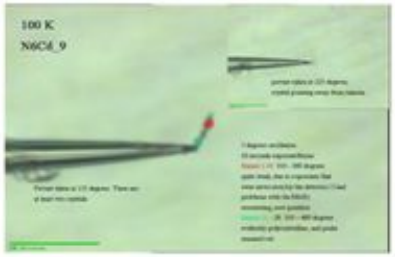


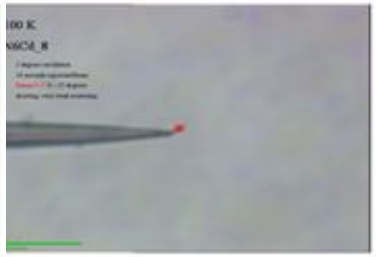



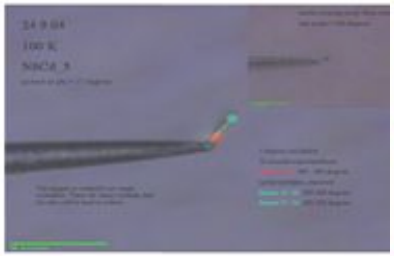



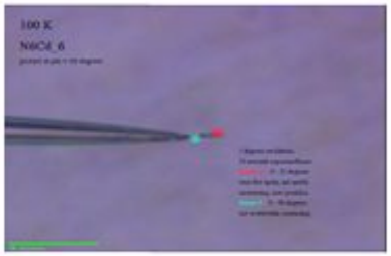



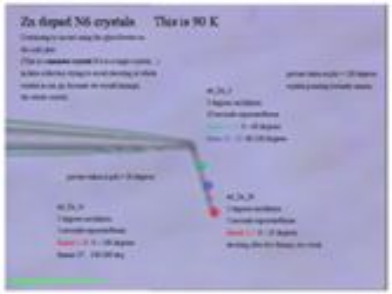



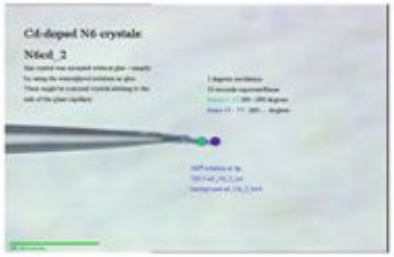



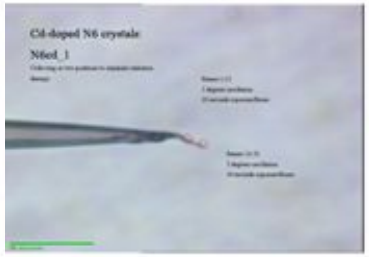



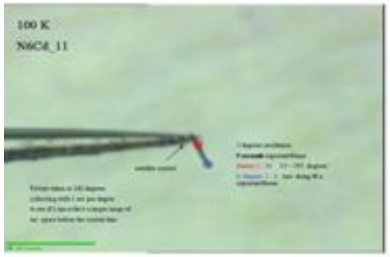



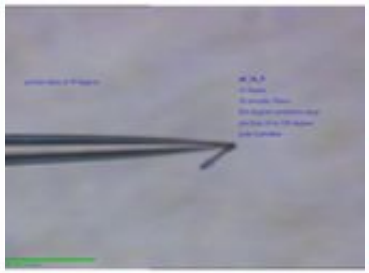



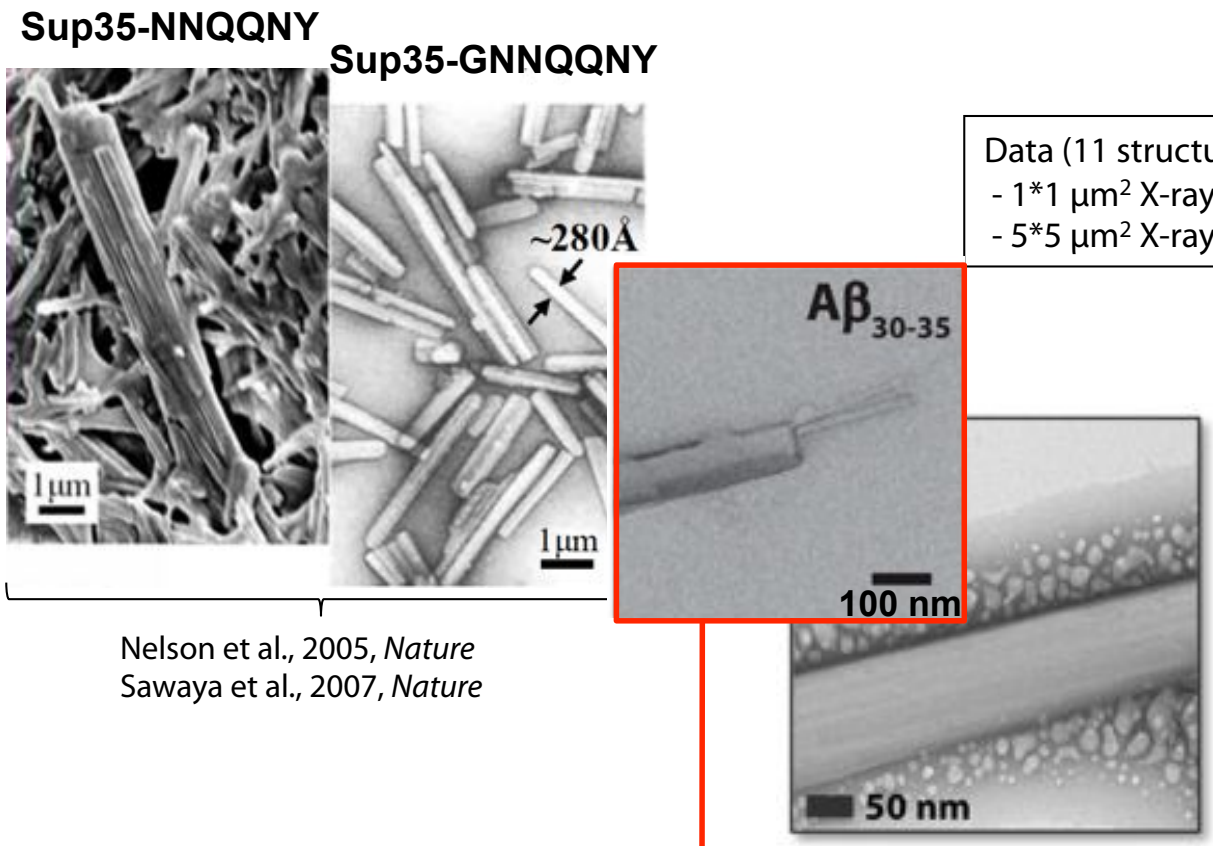




*etc …*

# Can structures be solved from such small crystals ?



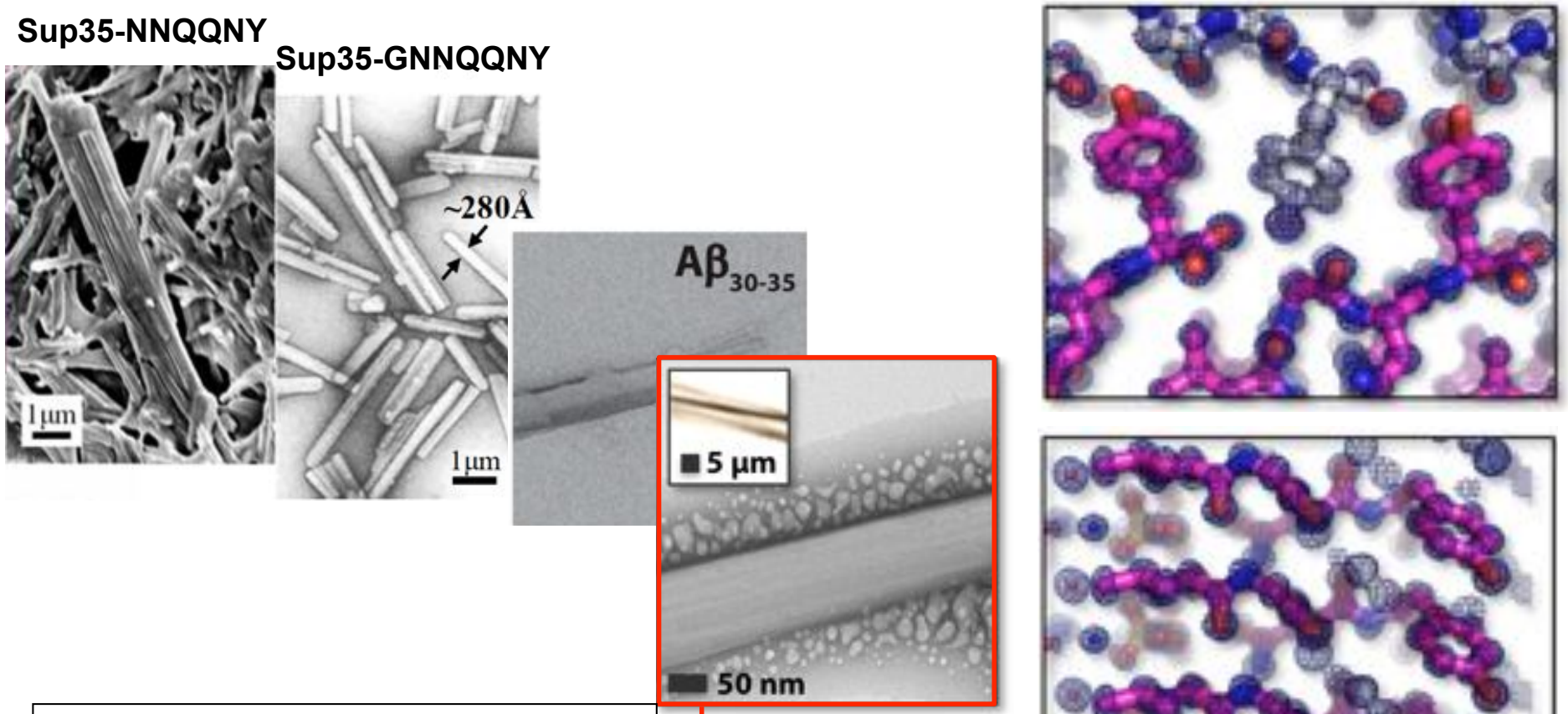

Nelson et al., 2005, *Nature*
Sawaya et al., 2007, *Nature*

Data (11 structures) collected at ESRF using :
- 1*1 µm² X-ray beam of ID13EH2
- 5*5 µm² X-ray beam of ID23EH3

Molecular basis for Aβ polymorphism

Colletier, Laganowsky, Zhao, Soriaga, Landau, Goldschmidt, Cascio, Sawaya & Eisenberg (2011). *PNAS*, 108, 16938-16943

# Can such small crystals diffract to high resolution ?



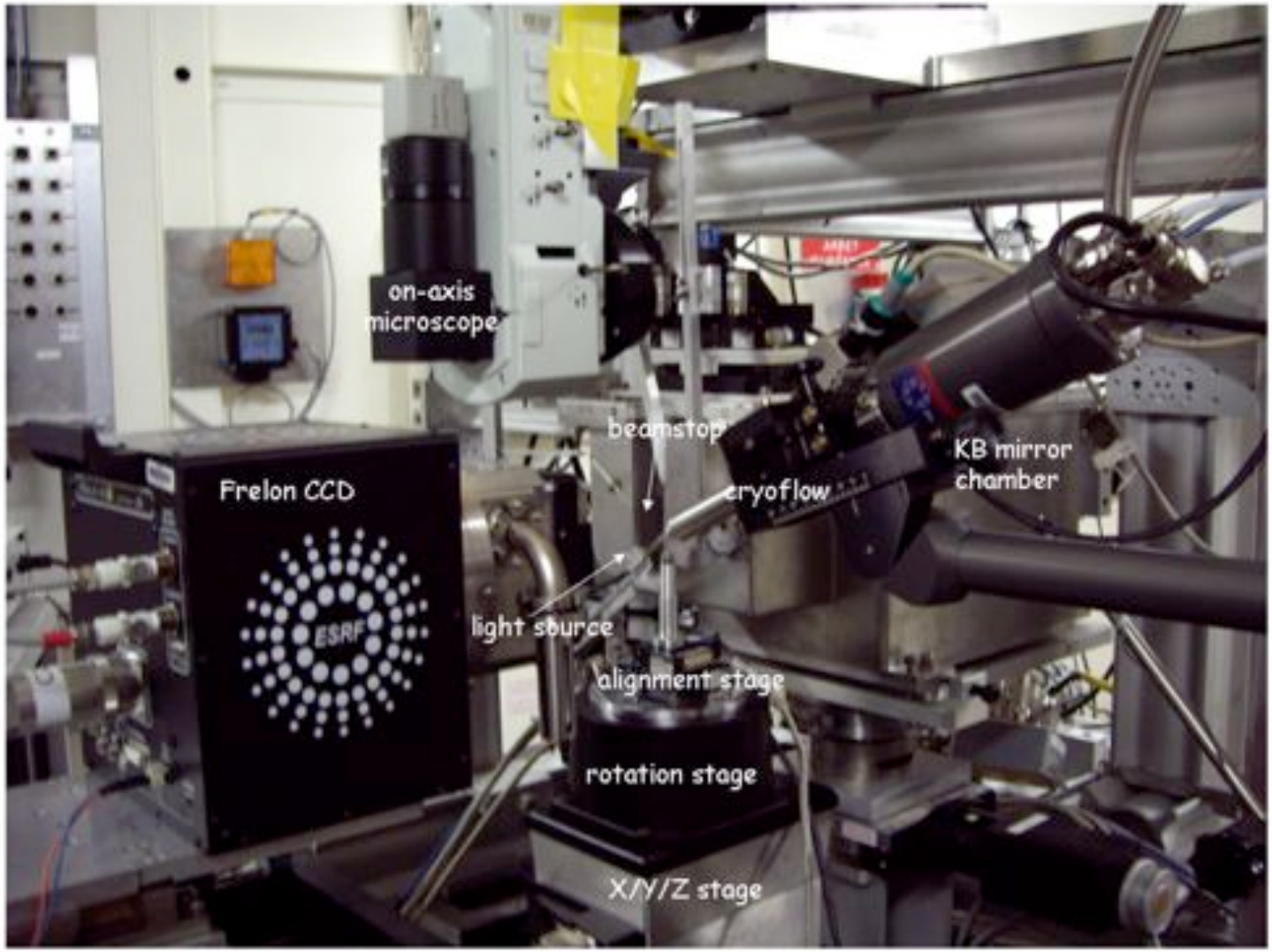

Data (2 structures) collected at ESRF using :
- 5*5 µm$^2$ X-ray beam of ID23EH3

Molecular basis for the self-association of Omp-Pst1 & Omp-Pst2

Nasrallah, Lethier, Tran, Fenel, Song, Schoehn, Bolla, Vivaudou, Pagès, Weik, Winterhalter, Colletier (*submitted*)

# Macromolecular micro-crystallography at ESRF ?

**- 1*1 µm² X-ray beam of ID13EH2**
- 5*5 µm² X-ray beam of ID23EH3



**What if crystals are either too small or too fragile,**

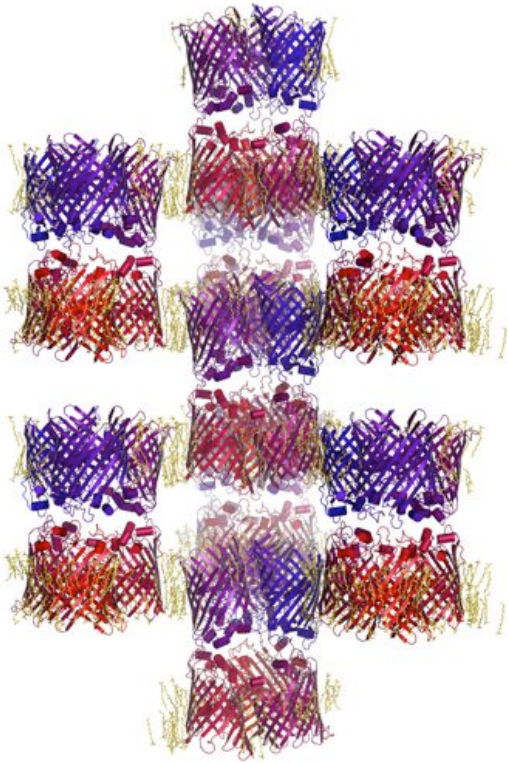
**and thence unamenable to mounting ?**

*e.g. membrane proteins, amyloid proteins, protein complexes,*

The essential limitation to obtaining well-ordered, large crystals of such objects resides in the limited number of strong and specific contacts that can be established between them inside the crystal.

Thus, they are fragile by nature.

In addition, cryo-protection may tamper with crystal contacts, requiring that data be collected at room-temperature.



**What if crystals are either too small or too fragile,**

**and thence unamenable to mounting ?**

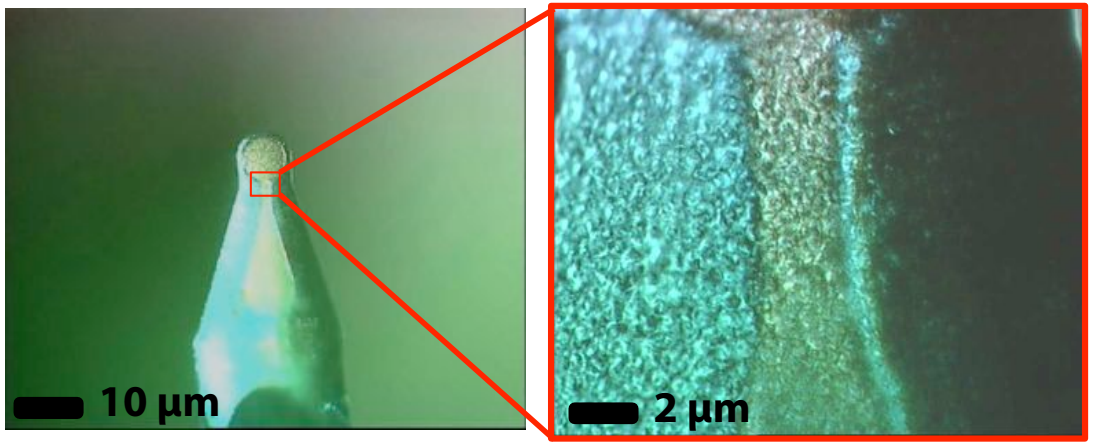
*e.g. naturally occurring nano-crystals (< 1µm)*

**1 – Cryo-protection is not an option**

* issues w/ ice nanocrystallites
* issues w/ background scattering from cryo stream

**2 – Oscillation data collection is not an option**

* goniometer's sphere of confusion (1-5 µm) >> larger than the crystal
* camera resolution ≤ the size of the crystal
* optical effects due to solvent & cryo stream
* loops & meshes >> than the samples; µ-capillary not adapted



## Outline :

1: X-ray macromolecular crystallography in an equation-free nutshell

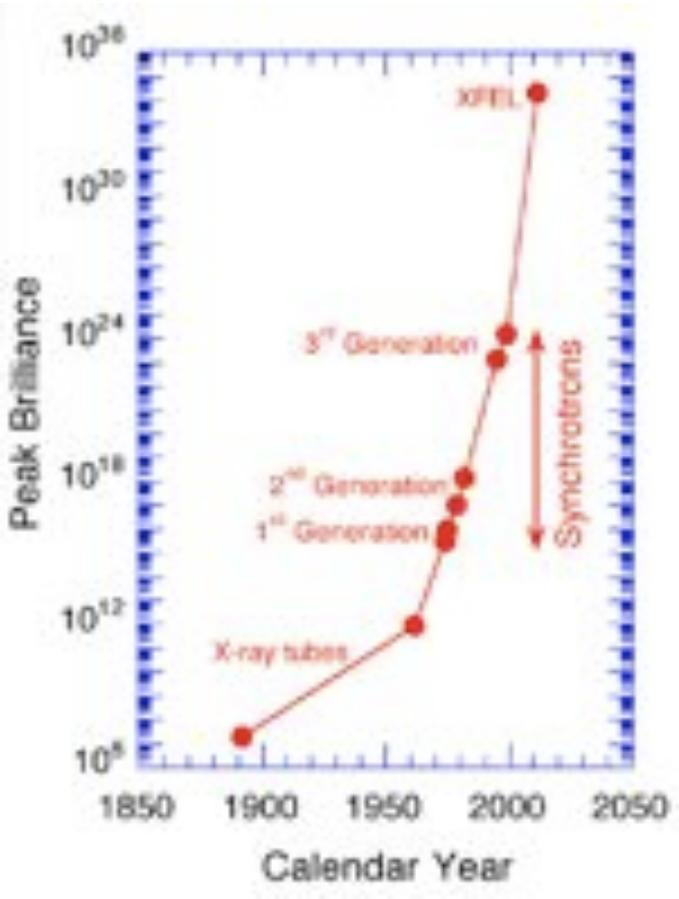
2: Conventional micro-crystallography at synchrotron-radiation (SR) sources

**3: Serial micro-crystallography (XFELs and SR sources)**

4: Serial nano-crystallography (XFEL sources)

5: Coherent X-ray diffraction and direct phasing of structural information

# Seminal serial crystallography experiments performed at XFELs

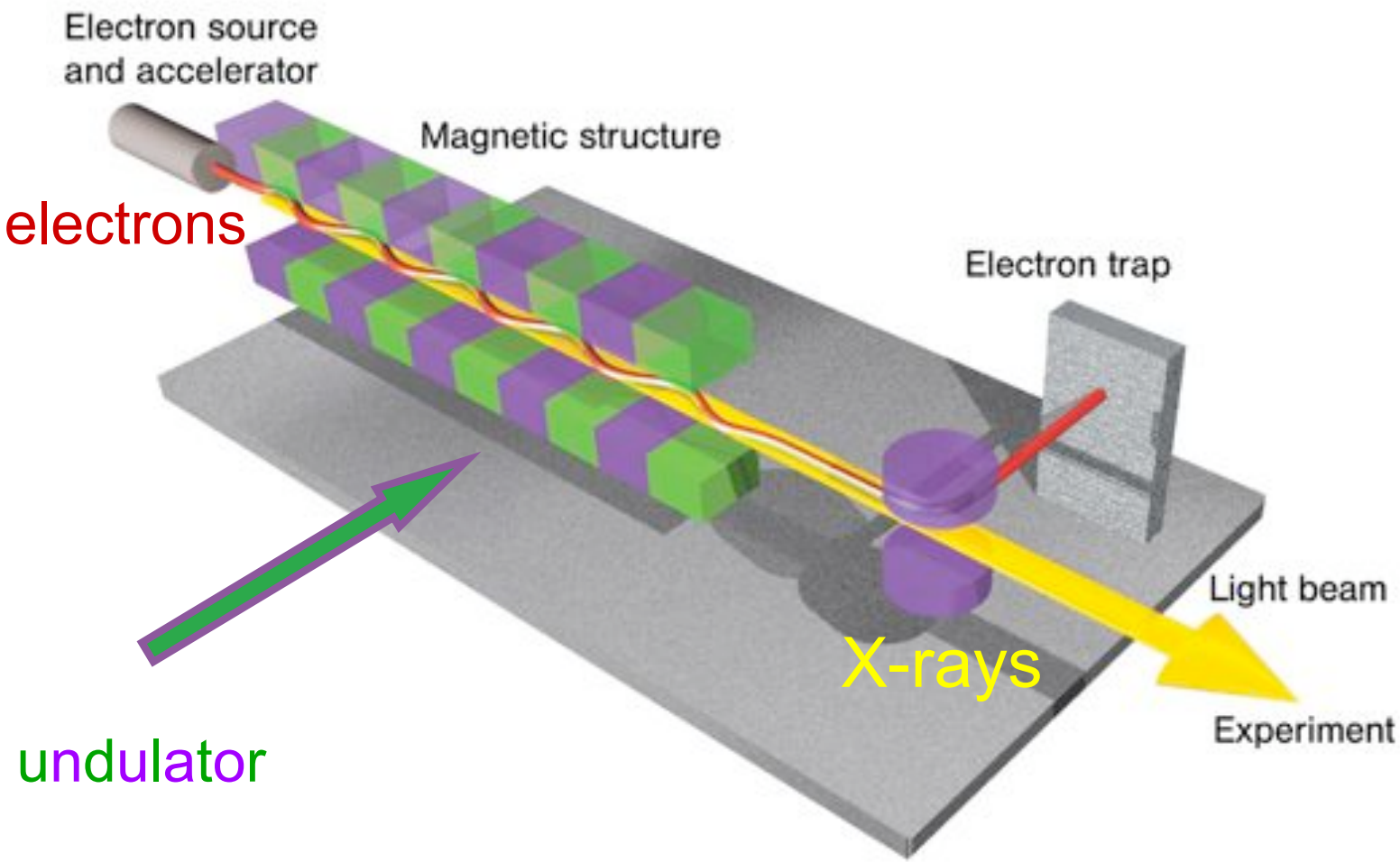




$10^{10}$ times more brilliance
than 3rd generation synchrotron

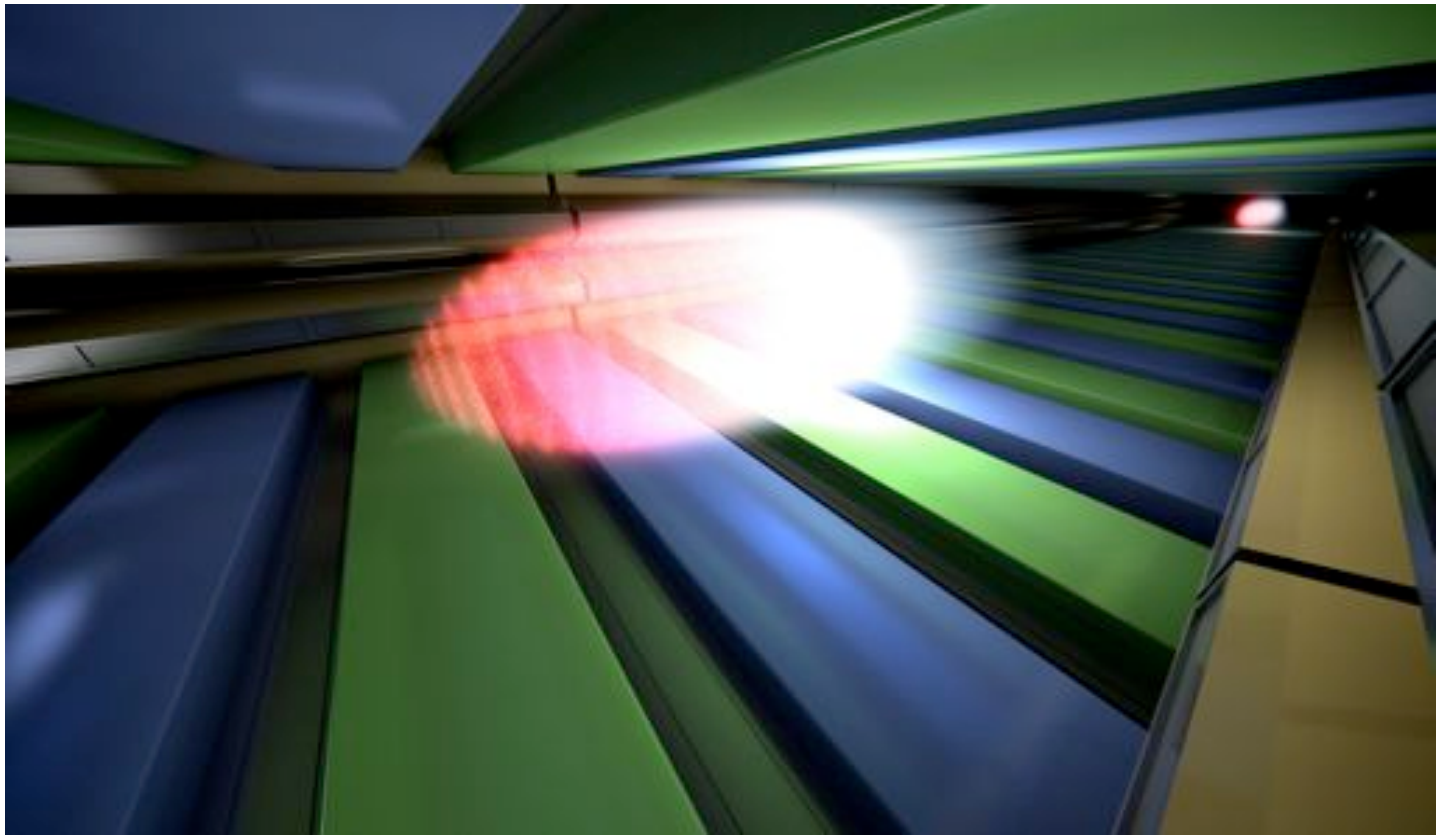
Boutet et al., 2012, Science
Redecke et al., 2012, Science
Chapman et al., 2011, Nature

## Principle behind XFELs

Idea of FEL: J. M. J. Madey (1971) J.Appl. Phys.42, 19061



**Self-Amplified Spontaneous Emission (SASE)** due to continuous interaction of emitted X-rays with the electron bunch over the full undulator length



SASE in long undulator (100 m) leads to micro-bunching of electron macro-bunches

Micro-bunches are separated by a distance equal to one radiation wavelength:

**Coherent wave is emitted with very high brilliance**

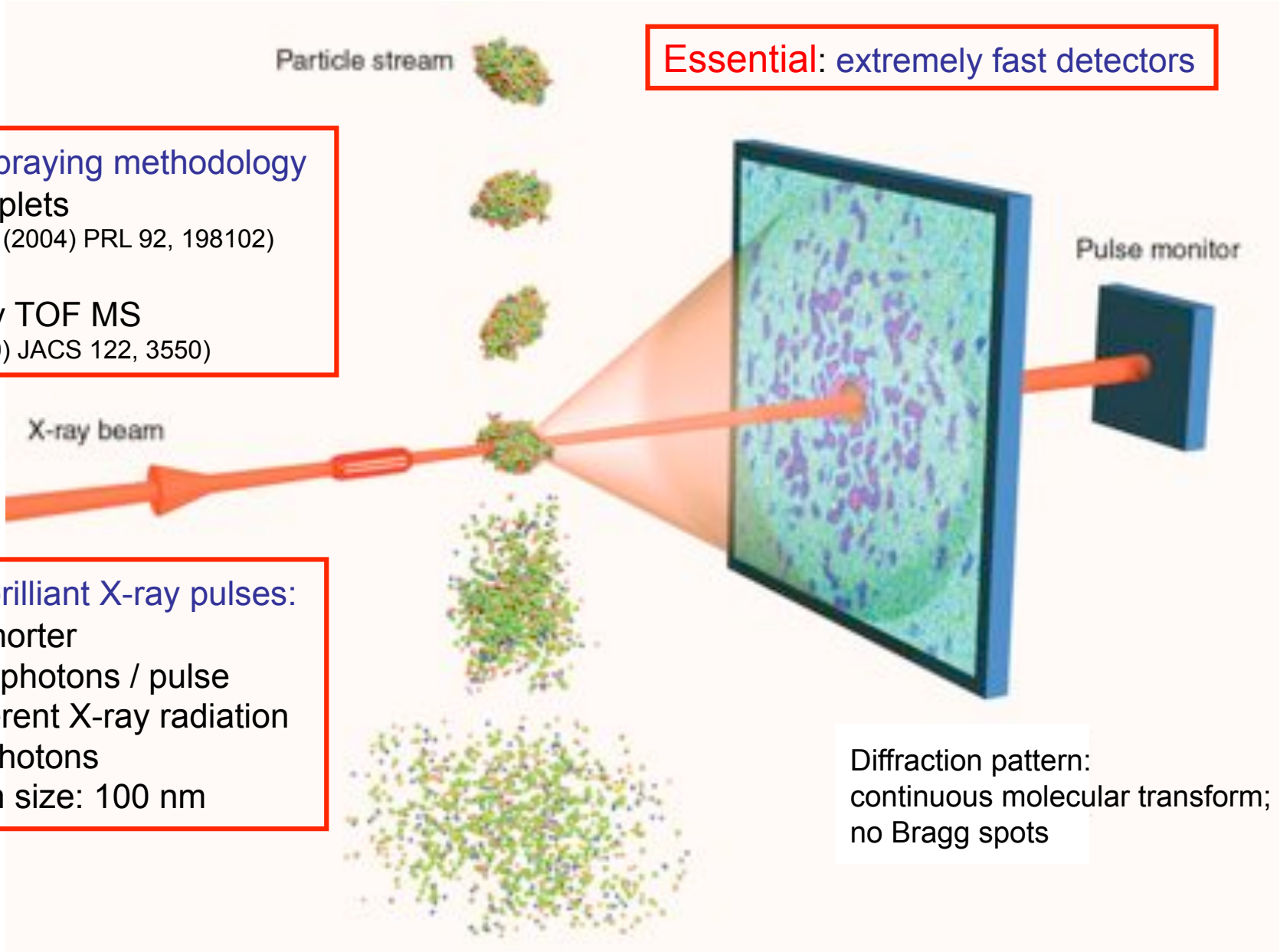
# Diffraction before desctruction imaging

Essential: Spraying methodology
-liquid He droplets
(Spence & Doak (2004) PRL 92, 198102)

- Electrospray TOF MS
(Tito et al. (2000) JACS 122, 3550)

Essential: brilliant X-ray pulses:
- 100 fs or shorter
- giving $10^{12}$ photons / pulse
- giving coherent X-ray radiation
- giving 1Å photons
- X-ray beam size: 100 nm

Essential: extremely fast detectors




Diffraction pattern:
continuous molecular transform;
no Bragg spots

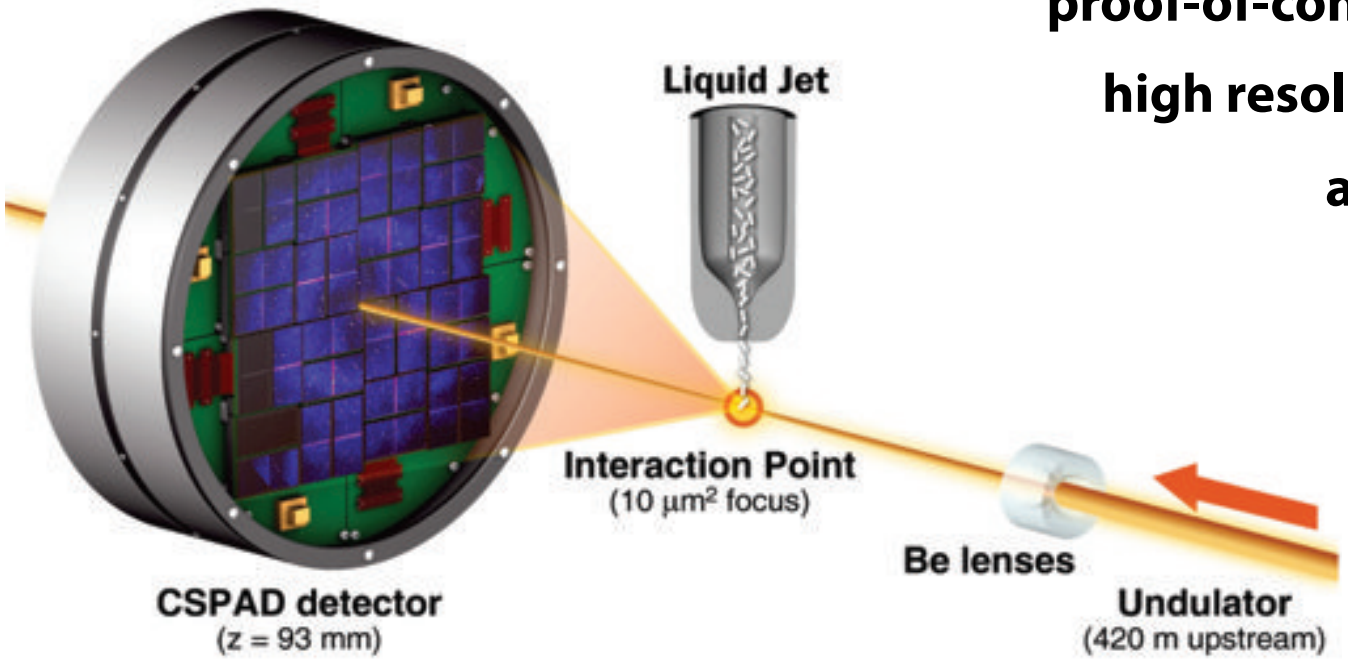
Image: Gaffney & Chapman (2007) Science 316, 1444

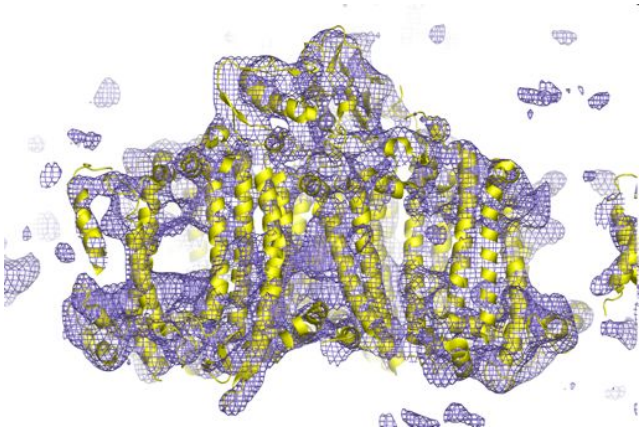
## Serial femtosecond crystallography (SFX):

**proof-of-concept on photosystem I,**

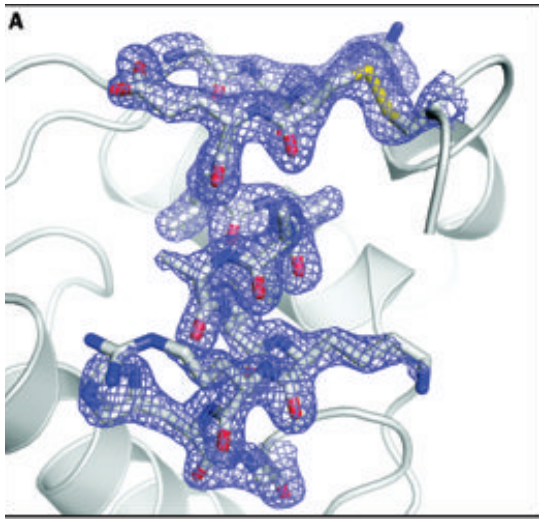
**high resolution data on lysozyme**

**and *T. brucei* cathepsin B**

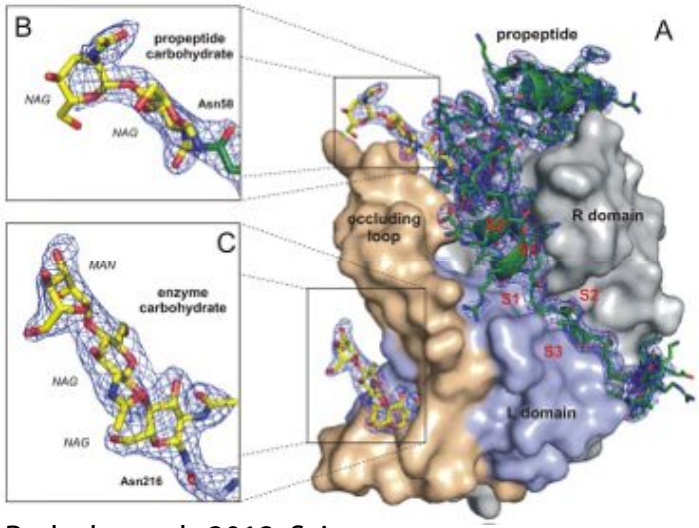


Chapman et al., 2011, Nature




Boutet et al., 2012, Science;

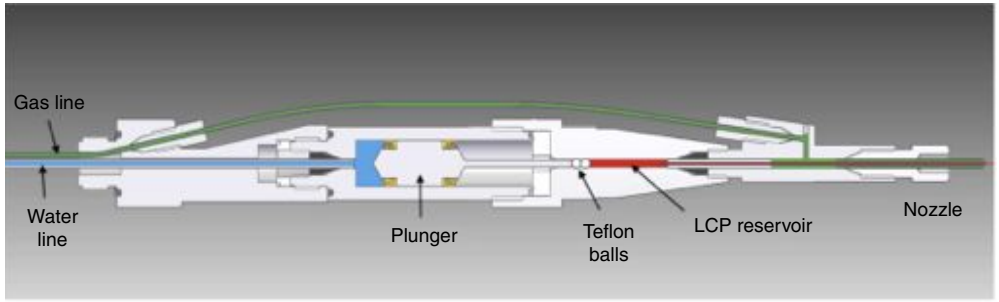


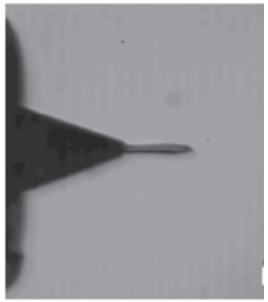

Redecke et al., 2012, Science

# Main limitations of SFX: beamtime availibility and sample consumption

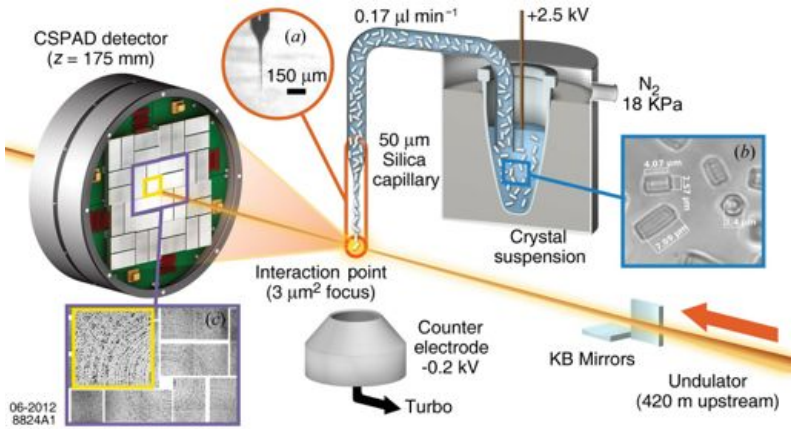
- 2 new XFELs under construction : European XFEL (2016) and SwissFEL (2017)

- Alternative approaches to the gas-focused liquid jet (14 mL per 12 hours):
  - Lipid cubic phase injector: 1-200 µL/12 hours, but LCP may affect diffraction
  - Nanoflow electrospinning injector: 149 µL/ 12 hours, but no salt…



Weierstall et al., (2014), Nat. Comm.




Sierra et al., (2012), Acta Cryst D

# Is serial crystallography amenable to synchrotron sources ?

- *T. brucei* cathepsin B crystals (CatB) mounted in a nylon loop at 110 K
  (Gati et al, 2014, IUCR J)

- Lysozyme crystals mounted in a poly-vinyle chip
  (Zarrine-Afsar et al., 2012, Acta D)

→Statistics of 'acceptable' quality
→Oscillation data collection

# Is REAL serial crystallography amenable to synchrotron sources ?

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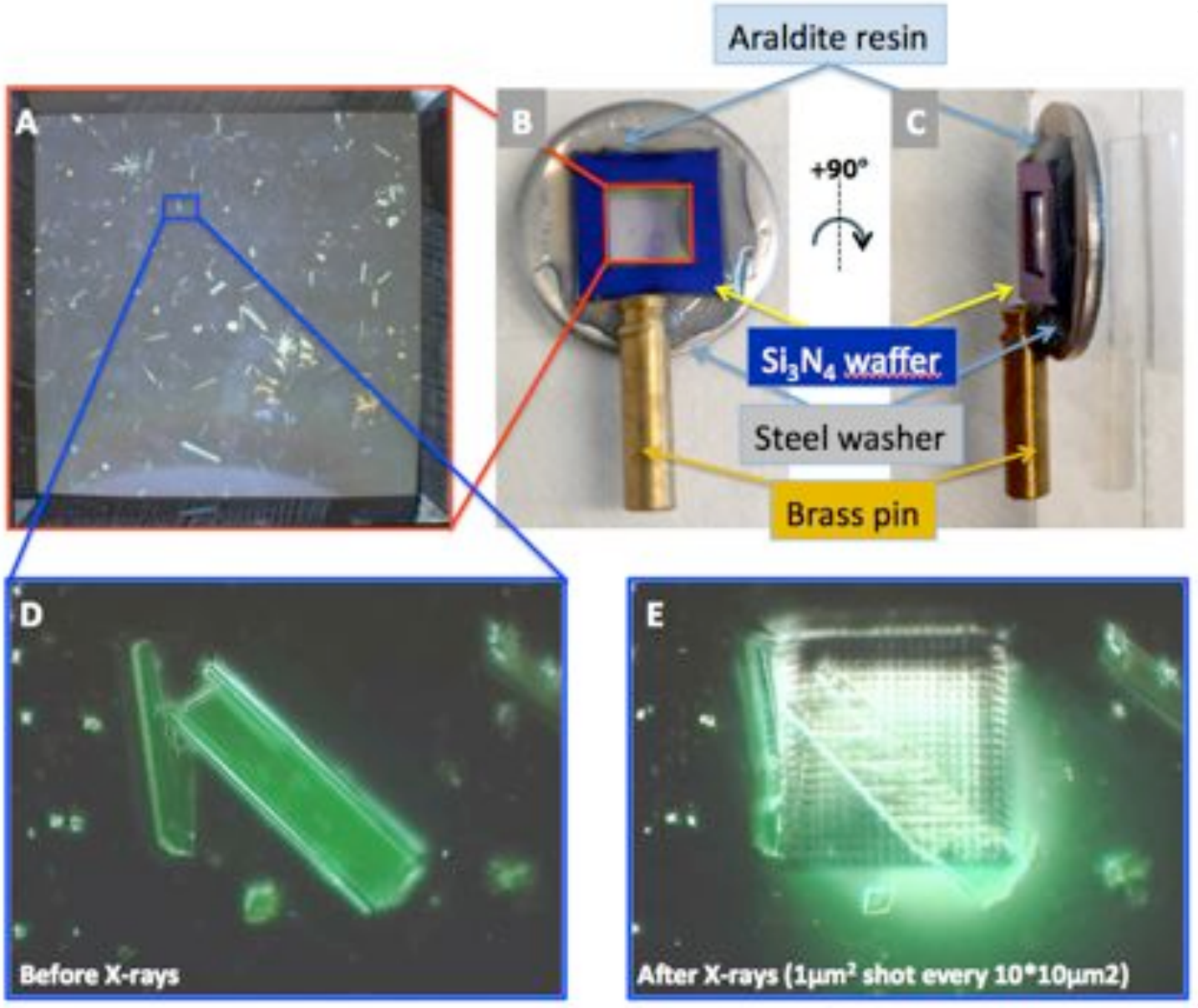
- Lysozyme crystals mounted in a poly-vinyle chip
(Zarrine-Afsar et al., 2012, Acta D)

→Statistics of 'acceptable' quality
→Oscillation data collection

→ We need high-quality data
→ We need an handling-free & centering-free methodology

**Crystals can be presented to the X-ray beam on solid support and data collected in raster-scanning mode (no oscillation)**




We collect tens of thousands of diffraction patterns…

Sometimes, we hit a crystal…

Sometimes, we hit solvent…

We need to split the difference !!!

Coquelle, Brewster, Kapp, Shilova, Weinhausen, Sauter, Burghammer, Colletier (*in preparation*)

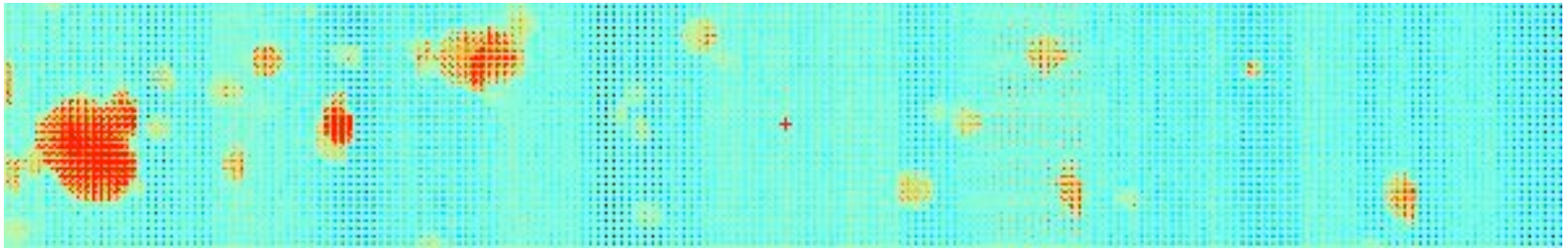
## Sorting the good from the bad ?

"Hit-finding" is a major challenge in serial crystallography

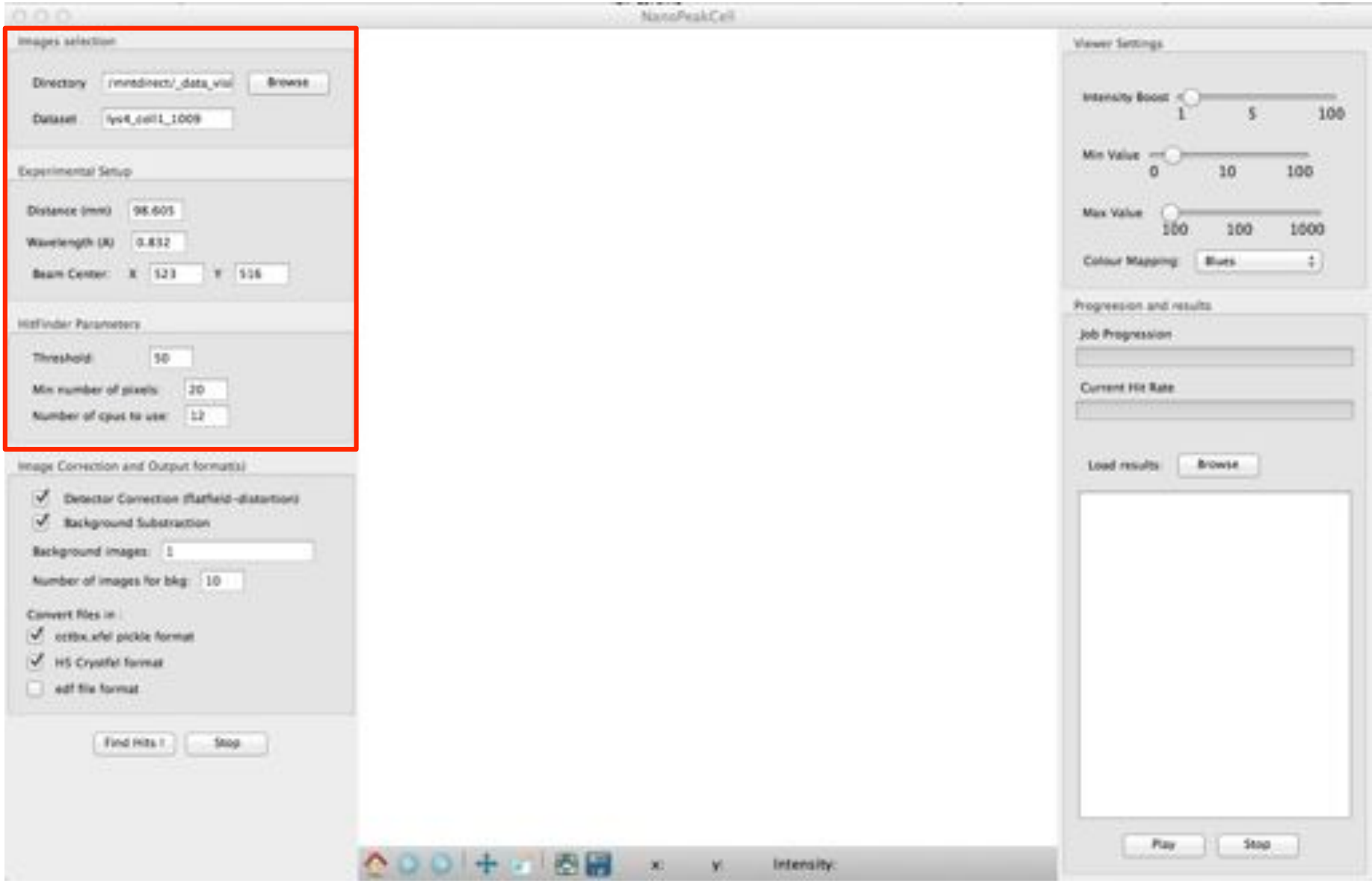
At XFELs, approaches have been developed (Cheetah, Apple.Py),
yet they are not suited for conventional X-ray crystallography data formats
(edf, cbf, smv, mccd, …)

Also, these approaches do not include background subtraction nor
background classification and scaling.

→ **If it isn't available, do it yourself !!!**

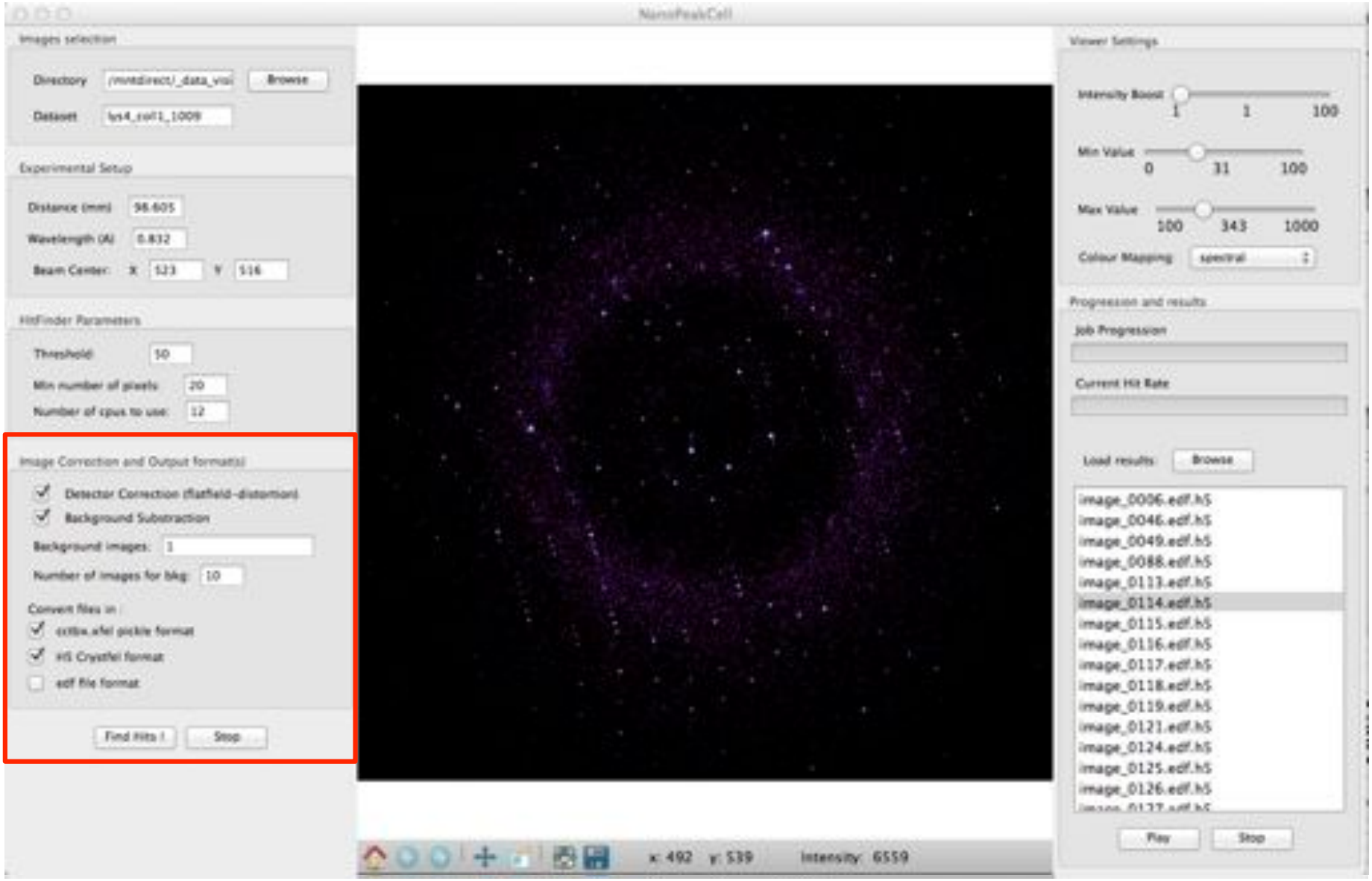


# NanoPeakCell : a hit finder that understands data need to be corrected…




Coquelle, Brewster, Kapp, Shilova, Weinhausen, Sauter, Burghammer, Colletier (*in preparation*)

# NanoPeakCell : a hit finder that prepare data for further processing…




Coquelle, Brewster, Kapp, Shilova, Weinhausen, Sauter, Burghammer, Colletier (*in preparation*)

# Proof of concept on lysozyme :

| Parameter | "micro" | | "nano" | |
|---|---|---|---|---|
| Resolution | 50 – 1.7 Å | | 50 – 1.5 Å | |
| Beam size [µm (v) * µm (h)] | 1.5 * 2.5 | | 0.150 * 0.175 | |
| Beam divergence [mrad] | 1.0 | | 0.326 | |
| Wavelength [Å] | 0.954 | | 0.832 | |
| Beam fluence [ph/s] | $1.00 \times 10^{11}$ | | $1.70 \times 10^{10}$ | |
| Flux density [ph/s/mm$^2$] | $2.67 \times 10^{19}$ | | $6.48 \times 10^{17}$ | |
| Brilliance [ph/s/mm$^2$/mrad$^2$] | $2.67 \times 10^{22}$ | | $6.09 \times 10^{20}$ | |
| Bandwidth [Å] | $< 1.00 \times 10^{-4}$ | | $< 1.00 \times 10^{-4}$ | |
| Exposure time per frame [s] | 0.2 | | 0.1 | |
| Dose [MGy] per crystal | 2.70 | | 24.72 | |
| Dose rate [MGy/s] | 27.0 | | 240.7 | |
| Space Group | $P4_32_12$ | | | |
| Indexing software | CrystFEL | cctbx.xfel | CrystFEL | cctbx.xfel |
| Unit cell length [Å], α=β=γ=90° | a=b=78.0 Å c= 38.3 | a=b=78.0 Å c= 38.4 | a=b=78.0 Å c= 38.5 | a=b=78.0 Å c= 38.4 |
| # collected diffraction images | 69,319 | | 139,985 | |
| # hits/indexed images | 6999/3576 | | 45172/35446 | |
| # reflections | 5997182 | | 3564462 | |
| # unique reflections | 13546 | | 19613 | |
| | | | | |
| Completeness [%] | 100.0 (100) | | 100.0 (100) | |
| Redundancy | 442.7 (60.6) | | 133.3 (47.8) | |
| I/σI | 5.58 (3.71) | | 11.23 (8.52) | |
| Rsplit | 18.34 (32.30) | | 6.18 (21.28) | |
| Rsym (I) | 25.28 (44.49) | | 8.70 (21.72) | |
| Rsym (F) | 14.00 (22.82) | | 5.42 (10.81) | |
| CC$^{1/2}$ [%] | 0.99 (0.81) | | 0.99 (0.54) | |
| Wilson B [Å$^2$] | 21.71 | | 11.07 | |
| Rfree [%] | 29.97 | | 25.11 | |
| Rfact [%] | 23.82 | | 21.52 | |
| Rmsd bonds [Å] | 0.007 | | 0.008 | |
| Rmsd angles [°] | 1.040 | | 1.040 | |

**Table 1.** SFX and synchrotron data and refinement statistics

| 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/ $4 \times 10^{11}$ ph/pulse | 53 µJ/$3.5 \times 10^{10}$ ph/pulse | N.A./ $2.5 \times 10^{10}$ ph/s |
| Dose [MGy] | 33.0 per crystal | 2.9 per crystal | 0.024 total |
| Dose rate [Gy/s] | $8.3 \times 10^{20}$ | $5.8 \times 10^{20}$ | $9.6 \times 10^{2}$ |
| Space group | $P4_32_12$ | $P4_32_12$ | $P4_32_12$ |
| Unit cell length [Å], α=β=γ=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 |
| # collected diffraction images | 1471615 | 1997712 | 100 |
| # of hits/indexed images | 66442 /12247 | 40115/10575 | n.a./100 |
| Number of reflections | n.a. | n.a. | 70960 |
| 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/σ(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 ** Highest resolution shell: 2.0-1.9 Å

*** $R_{split}$ as defined in (*15*) $R_{split} = \left(1/\sqrt{2}\right) \cdot \left( \sum_{hkl} \left| I_{hkl}^{even} - I_{hkl}^{odd} \right| \Big/ \frac{1}{2} \sum_{hkl} \left| I_{hkl}^{even} + I_{hkl}^{odd} \right| \right)$

**** Statistics from XDS (*19*) ***** Calculated with TRUNCATE (*20*)
****** Calculated with PHENIX (*21*)

From Boutet et al., 2012, Science

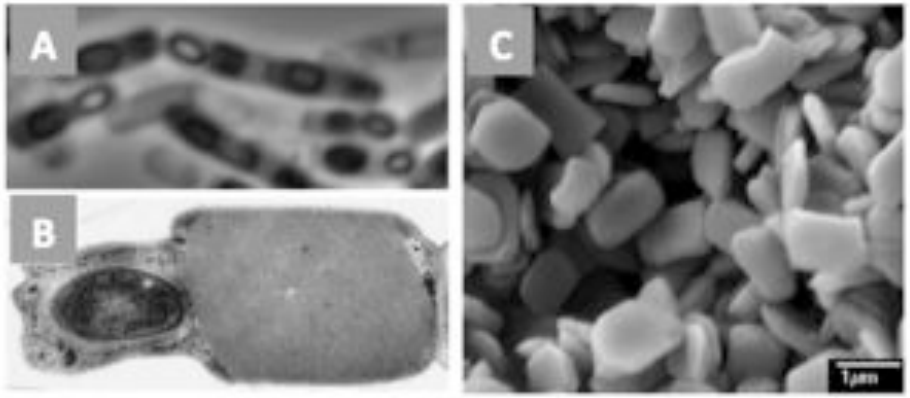
- Proof of feasibility for serial synchrotron crystallography (SSX) using micro (1.5 µm) or nano (150 nm) focused beams

- Data of comparable or better quality than that produced by XFELs (…), yet using much less frames.

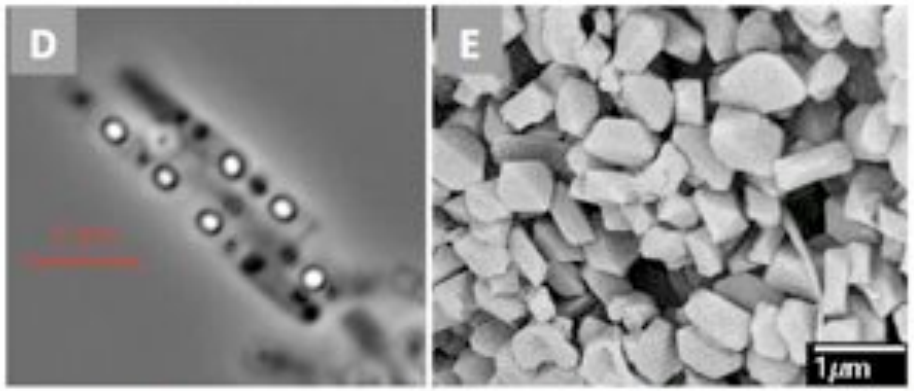
- An comparatively economic approach: ~ 5µL of concentrated crystals instead of tens of mL at XFELs.

Coquelle, Brewster, Kapp, Shilova, Weinhausen, Sauter, Burghammer, Colletier (*in preparation*)

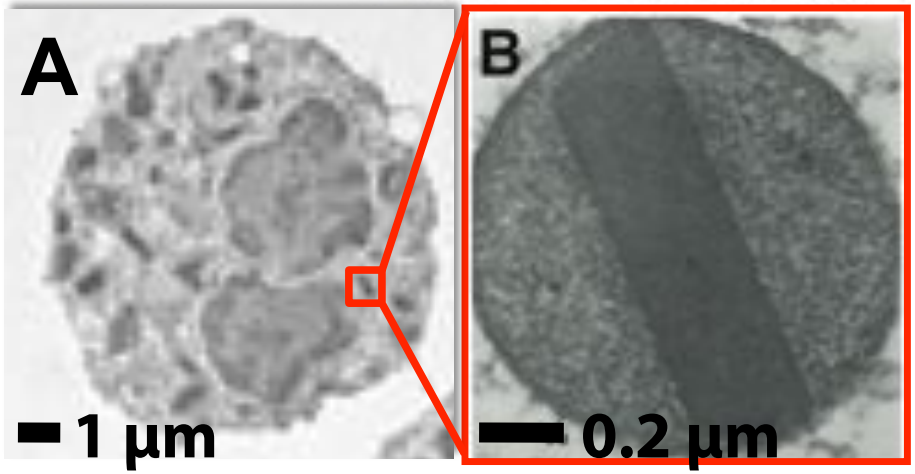
**Can this approach be used to characterize naturally-occuring nano-crystals ?**




Nano-crystals of Cry3A in
*Bacillus thuringiensis* cells




Nano-crystals of B1nAB in
*Bacillus thuringiensis* cells

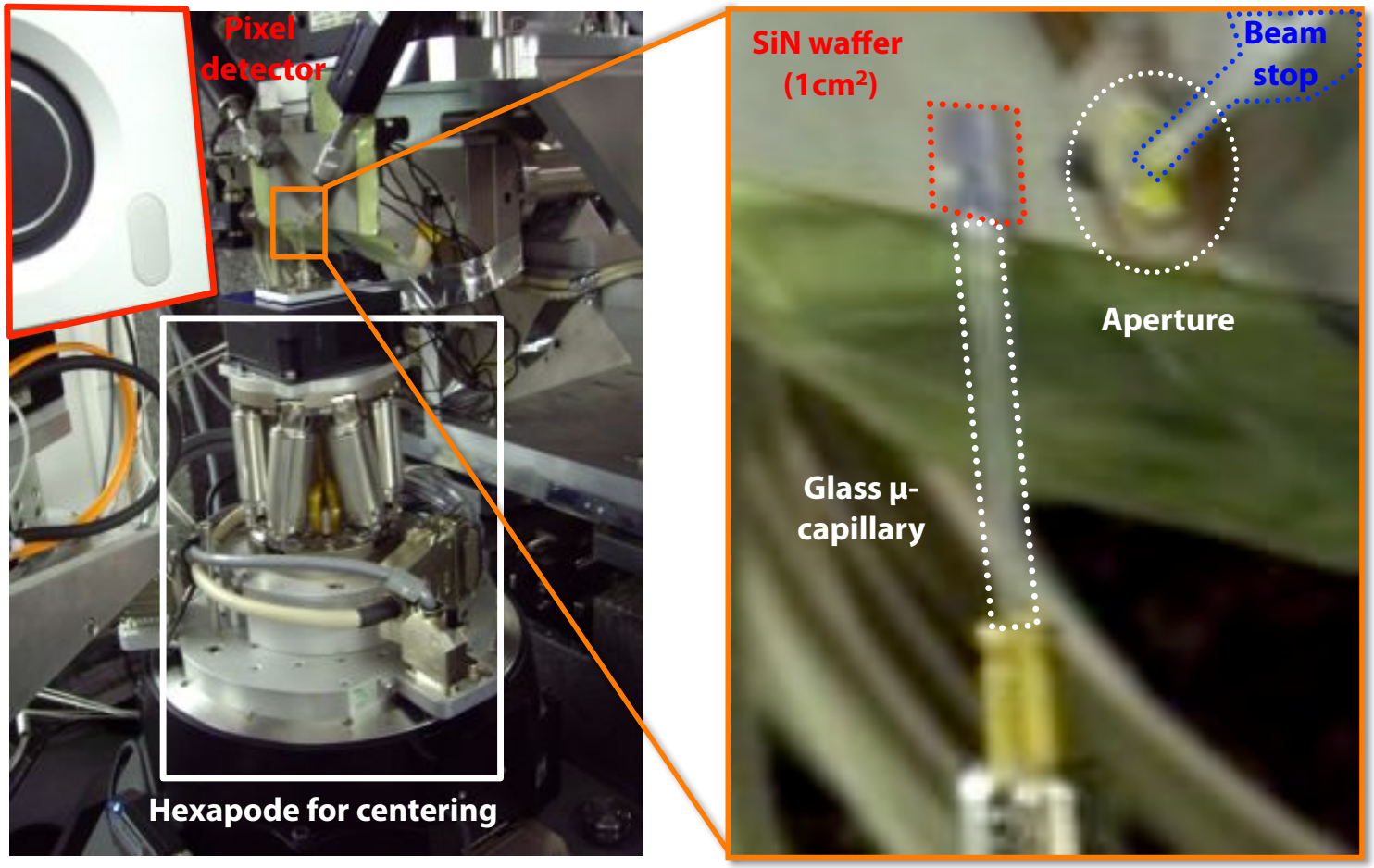



Nano-crystals of major basic protein
in human eosinophilic granulocytes

# Macromolecular nano-crystallography at ESRF

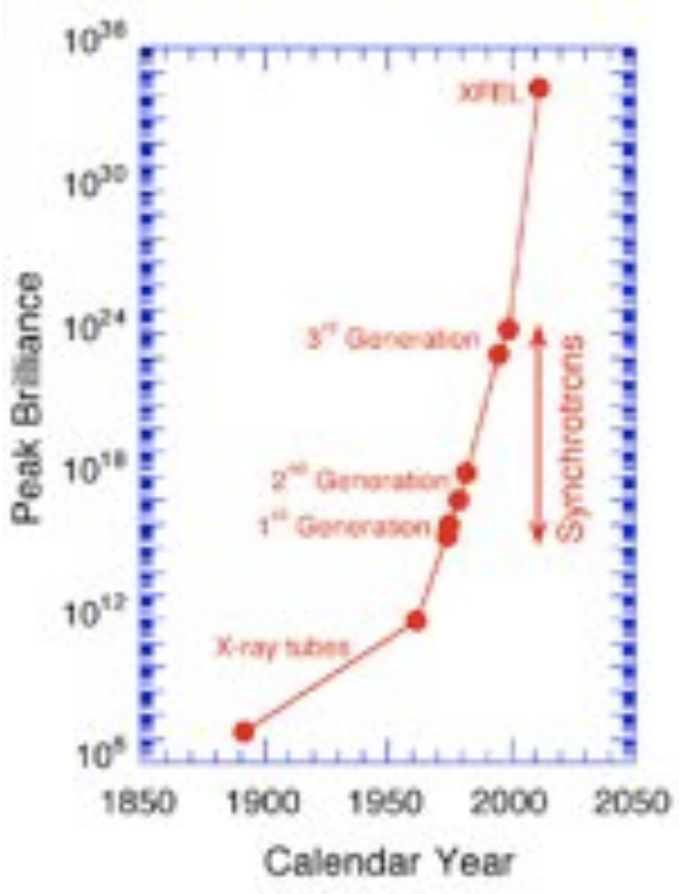
Manfred Burghammer & Christian Riekel

**50*50 to 200*200 nm2**
**X-ray beam on ID13EH3**



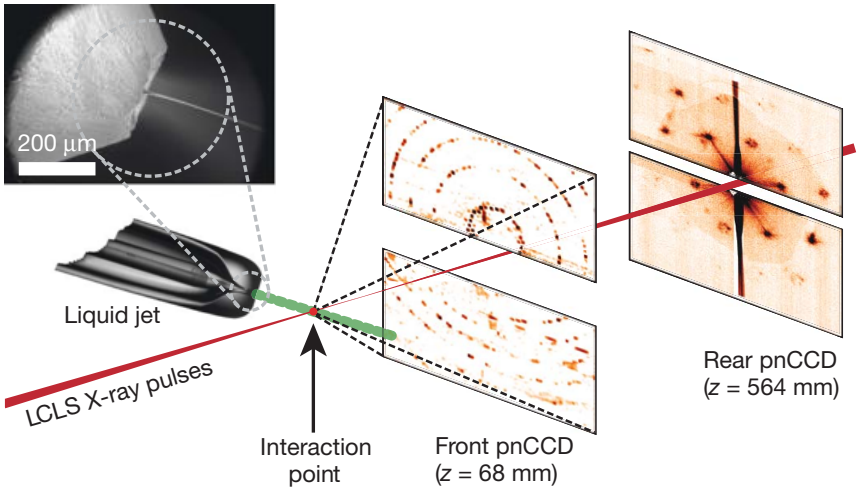
**Long story short: impossibly small nanocrystals are not amenable to SSX.**

***We need to resort to SFX at XFELs : the ultra-short nature of the pulses will allow observing diffraction before destruction occurs***




**XFELs deliver 10-10000 times more X-rays in 50 fs than at ESRF in 1s…**

# Liquid jet consumes 14 ml of sample ($10^{10}$-$10^{11}$ crystals/ml) per 12h …

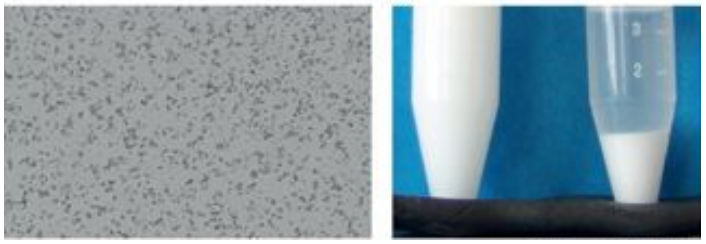



**Sample is sprayed (in general…)**

- Crystallography:
  Liquid droplets
  (Spence & Doak (2004) PRL 92, 198102)

- Coherent X-ray imaging
  Electrospray TOF MS
  (Tito et al. (2000) JACS 122, 3550)

## Feasible for lysozyme …



Schlichting & Miao (2012) *Curr Opin Struct Biol*

## But not (humanely) feasible for naturally occuring human nanocrystals

NB: cells are purified from the blood of donors
(patients with hyper-eosinophilic auto-immune diseases)

# Phasing of SFX data is notoriously difficult….

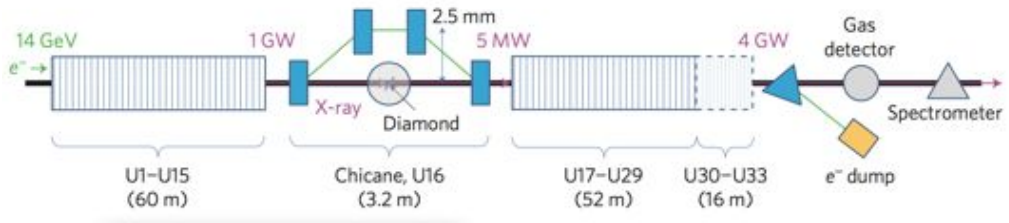
•To date, only Schlichting and coll. have succeeded in phasing SFX data

Barends et al, 2014, *Nature*

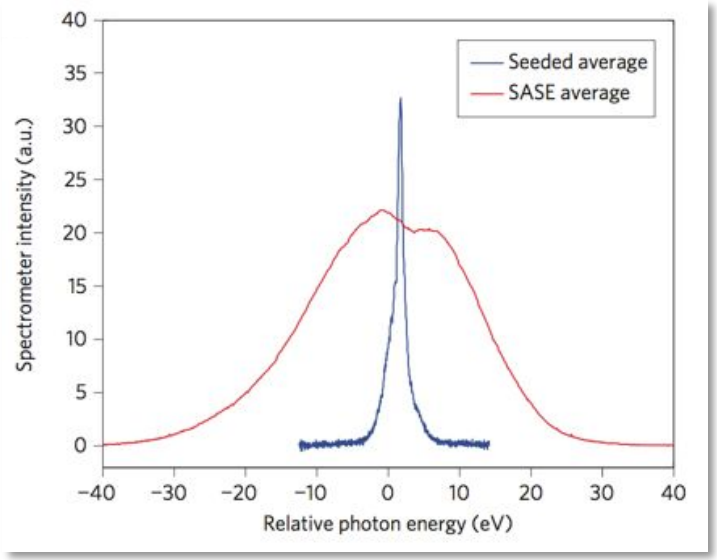
•Amidst reasons :

- Lack of isomorphism between crystals (> 10,000 ≠ crystals)
- Energy jittering between XFEL pulses
- Large bandwidth

**Could be solved by self-seeding**




Amann et al., 2012, *Nat. Photonics*
Ding *et al.*, 2010, *Phys. Rev. ST Accel. Beams*



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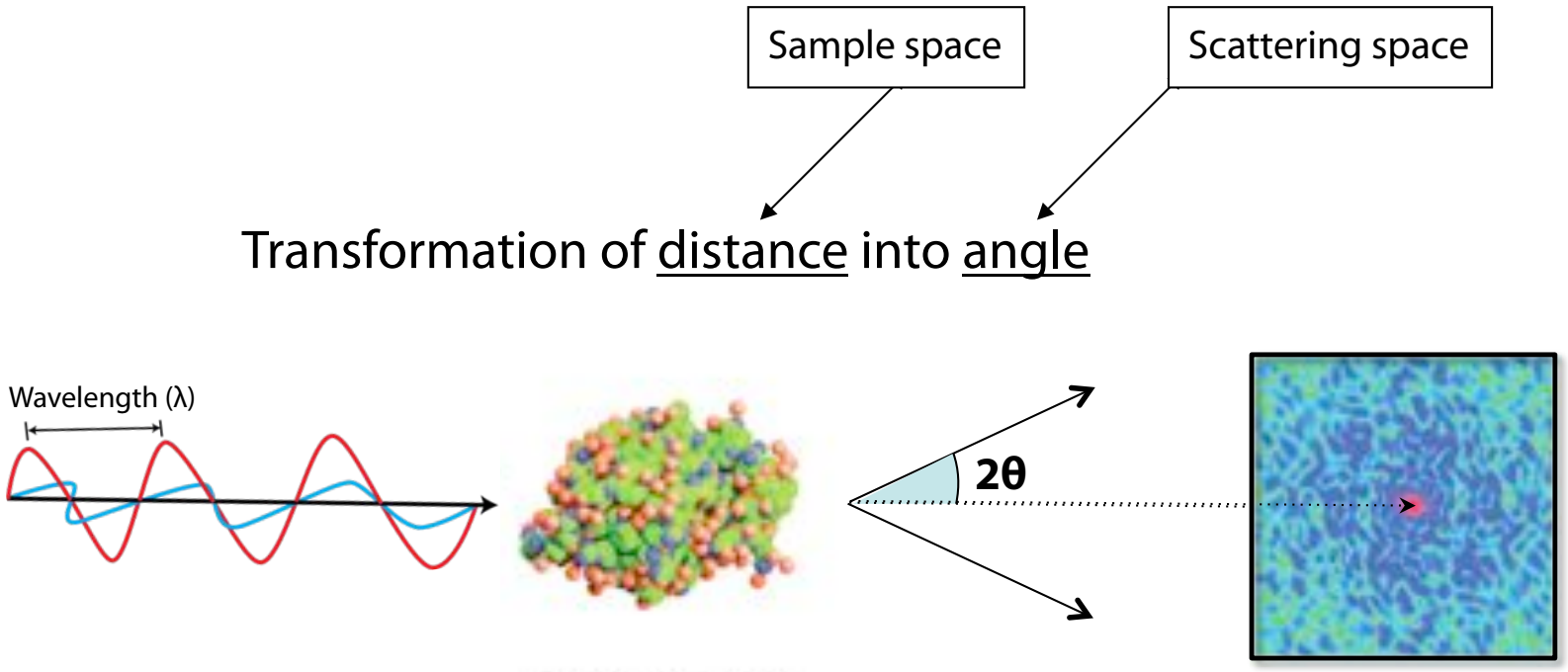
3: Serial micro-crystallography (XFELs and SR sources)

4: Serial nano-crystallography (XFEL sources)

**5: Coherent X-ray diffraction and direct phasing of structural information**

# X-ray scattering from a molecule

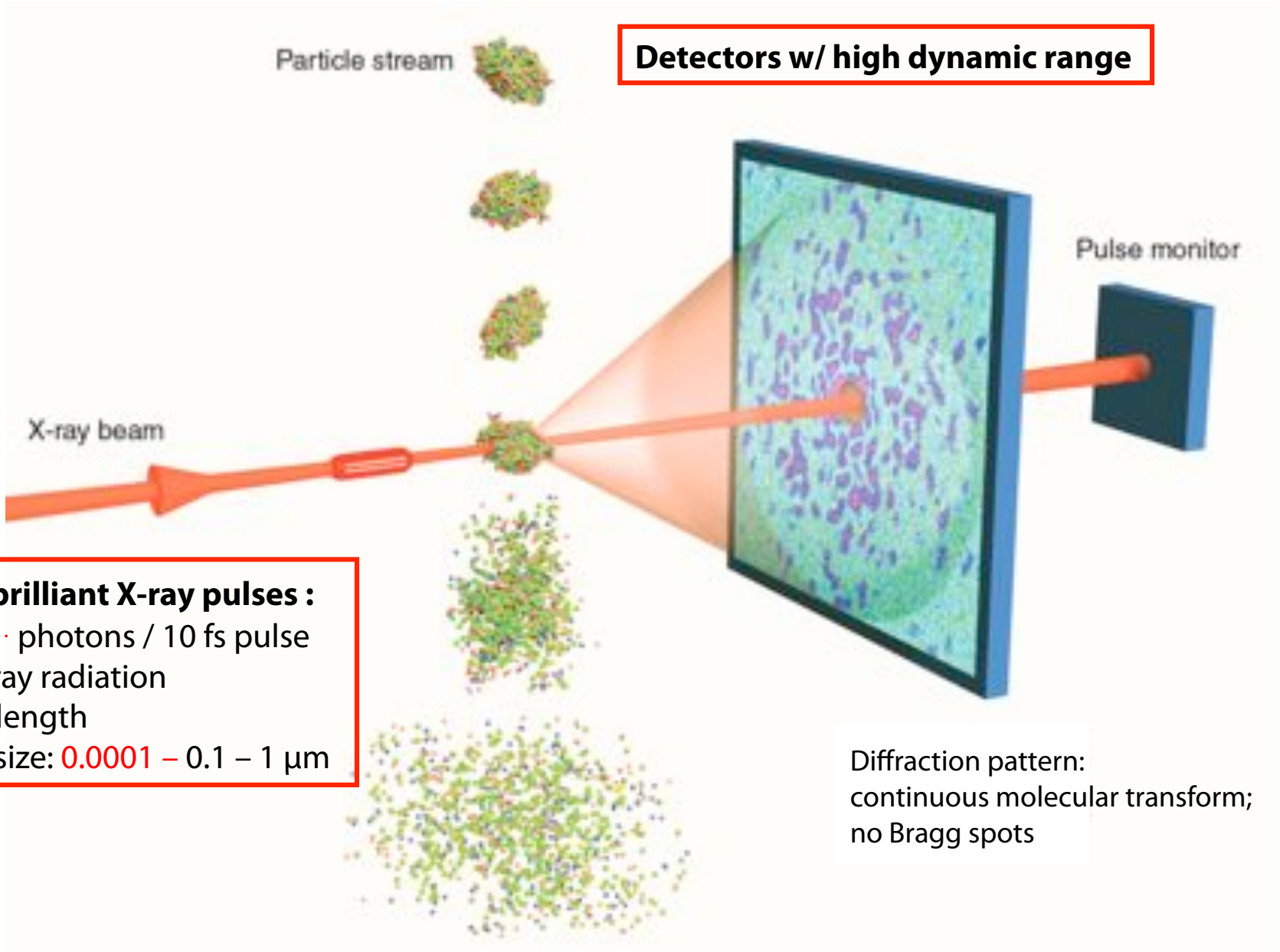



## Sample space

## Scattering space

Diffraction Pattern : contains all the contrast relevant information at the resolution of λ/2sin(θ)

## **Ideal** case :




**Extremely brilliant X-ray pulses :**
- giving $10^{12\text{-}\cdots}$ photons / 10 fs pulse
- coherent X-ray radiation
- 1 - 8 Å wavelength
- X-ray beam size: 0.0001 – 0.1 – 1 µm

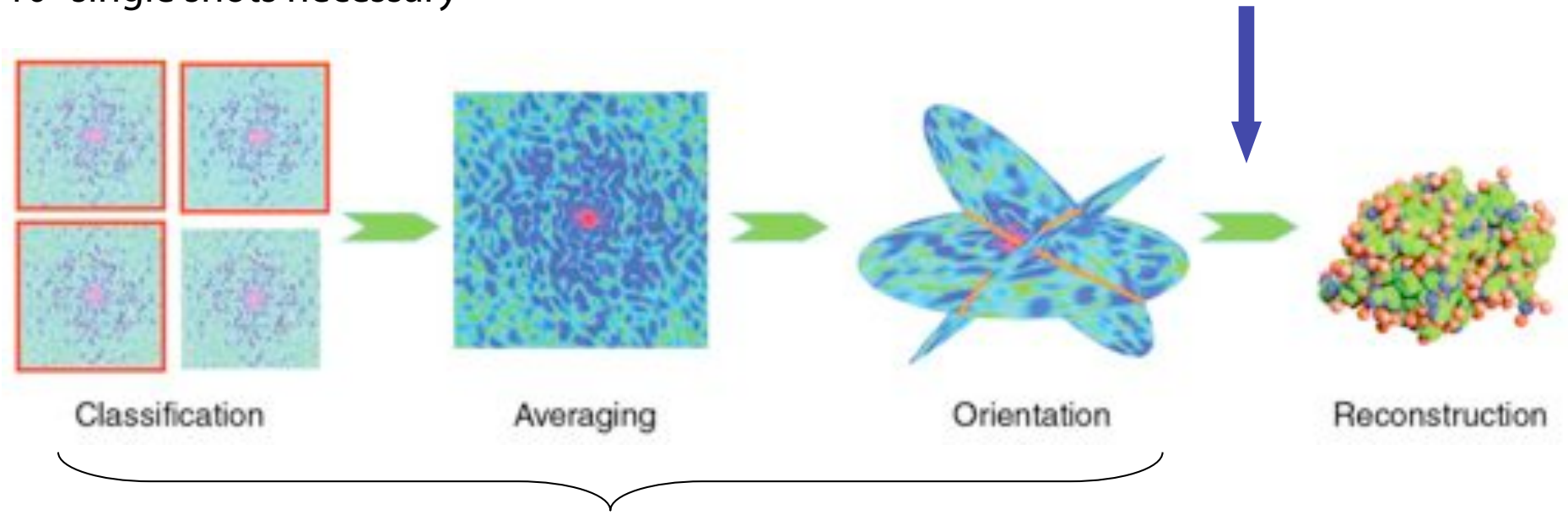
Diffraction pattern:
continuous molecular transform;
no Bragg spots

Gaffney & Chapman (2007) Science 316, 1444

# Single particle imaging by coherent X-ray imaging : direct phasing

Essential: Direct phase retrieval by the oversampling technique
(Miao et al. (2001) PNAS 98, 6641)

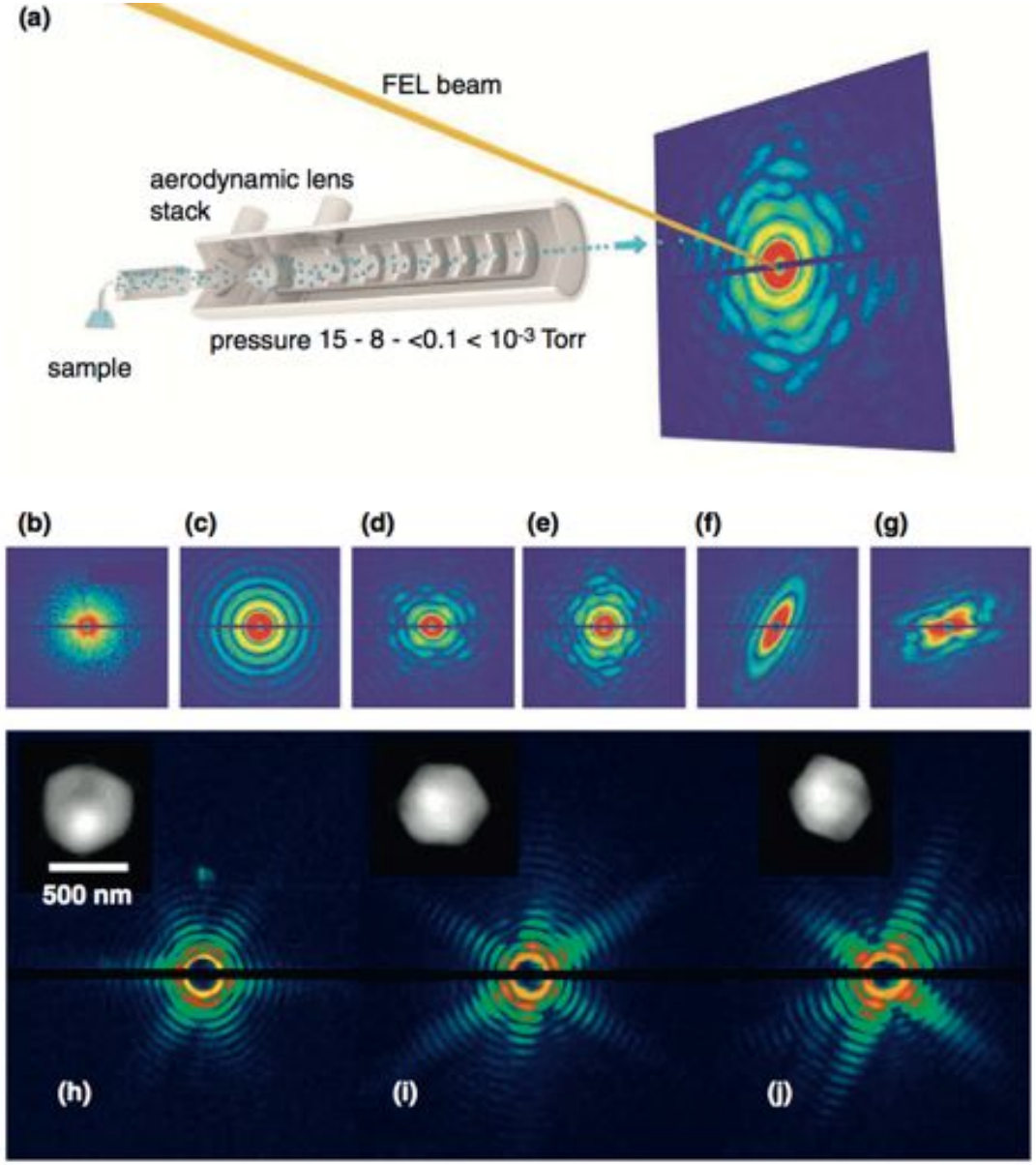
$10^7$ single shots necessary




Essential: Methods from single particle EM

Gaffney & Chapman (2007) Science 316, 1444

# Single particle imaging of a 500 nm particle : done !!!



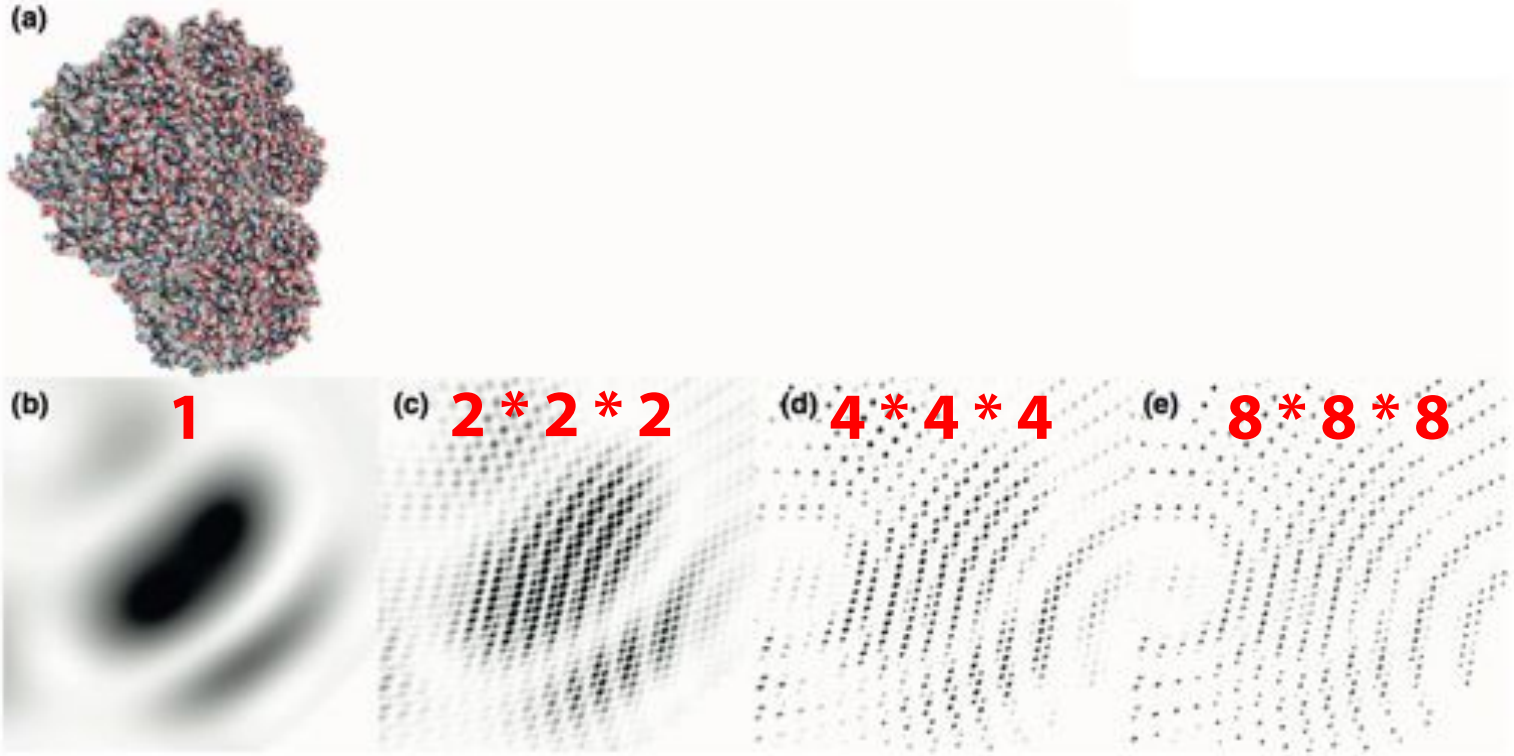

Seibert et al., 2011, *Nature*
Schlichting & Miao, 2012, *Curr Opin Struct Biol*

## But protein are too small --- not enough scattering for direct phasing !!!

X-ray/matter interaction is weak (1/10000 photons)

We use crystals as a means to amplify the signal ($2.10^{14}$ symmetry-related molecules)

The Bragg law applies : constructive interferences only if $2*d*\sin(\theta) = \lambda$




continuous molecular transform; no Bragg spots

Bragg spots and fringes between spots

Schlichting & Miao, 2012, *Curr Opin Struct Biol*

**When using nanocrystals, convolution of the continuous fourier transform of the object with that of the lattice**

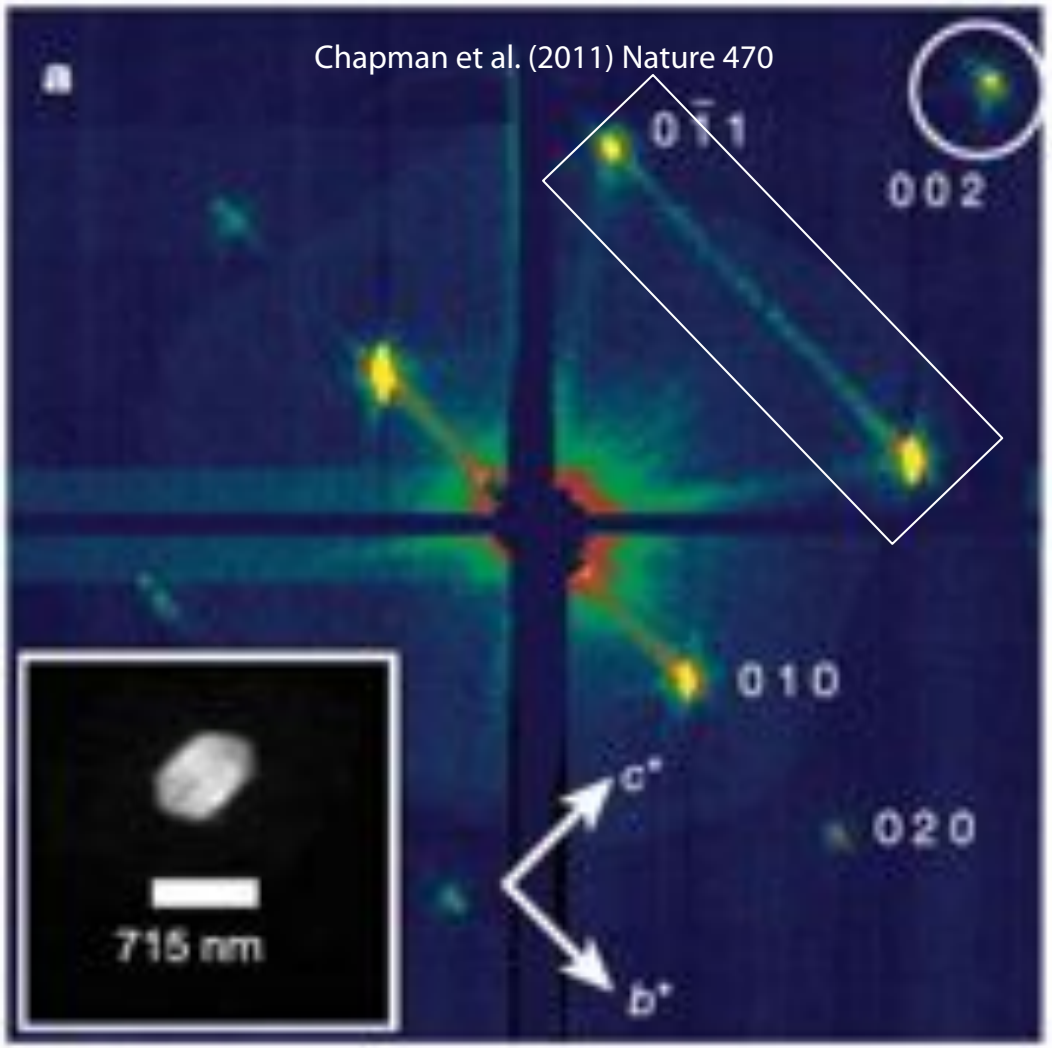
e.g. Photosystem I ($P6_3$)

- unit cell : 281*281*165 $Å^3$ α=β= 90°; γ=120°
- 8 Å wavelength, 1 $\mu m^2$ beamsize, 15,000 frames

**N unit cells gives rise to N-2 fringes between neighbouring Bragg peaks.**
(fringe-spacing is finer by a factor of 1/N than the Bragg spacing)

⟶ **34 unit cells**

**Information between bragg peaks could be used to phase the structural information**

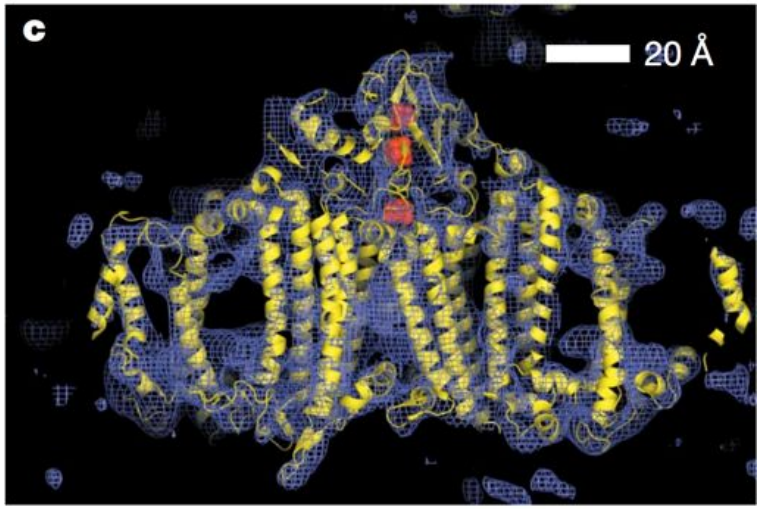


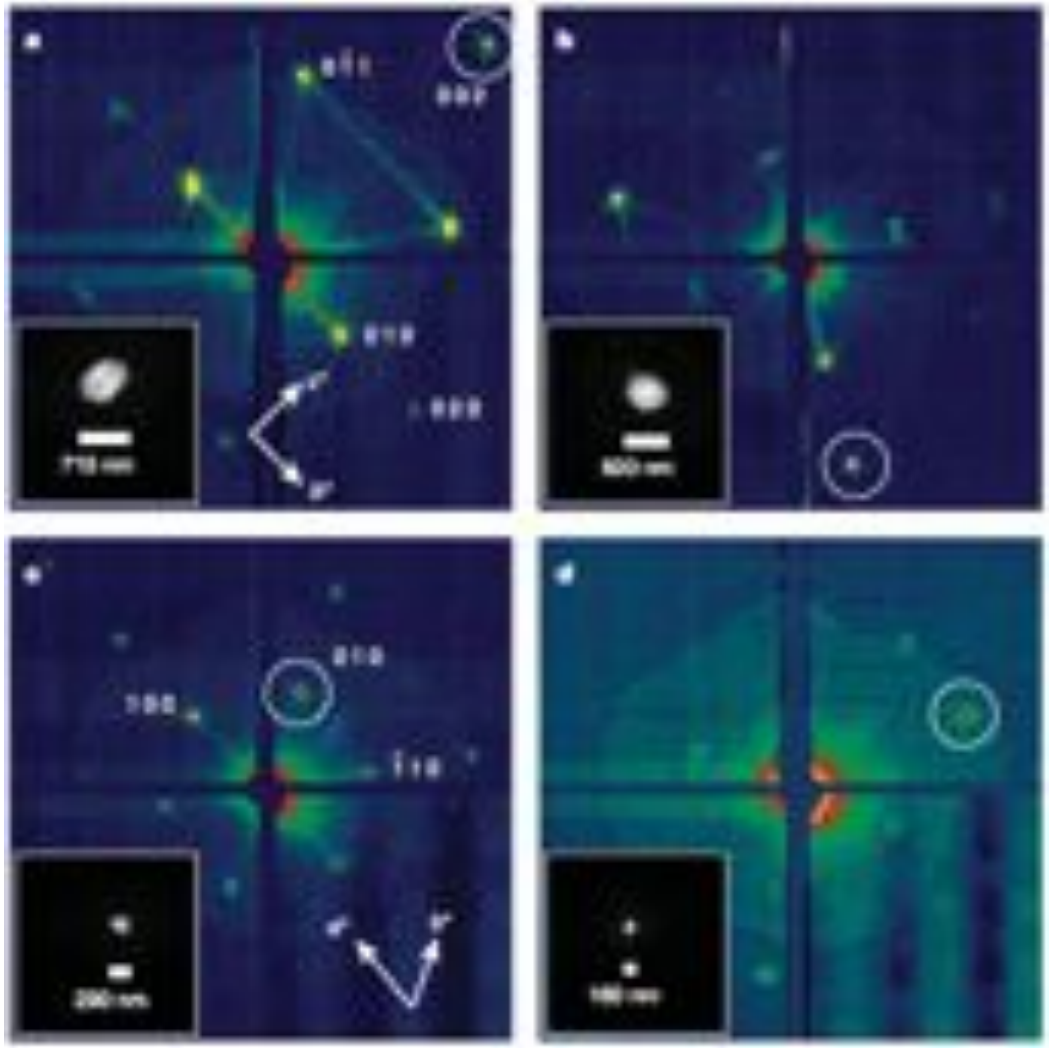
**Best use of XFEL for now : (time-resolved) micro-crystallography :**

e.g. Photosystem I ($P6_3$)

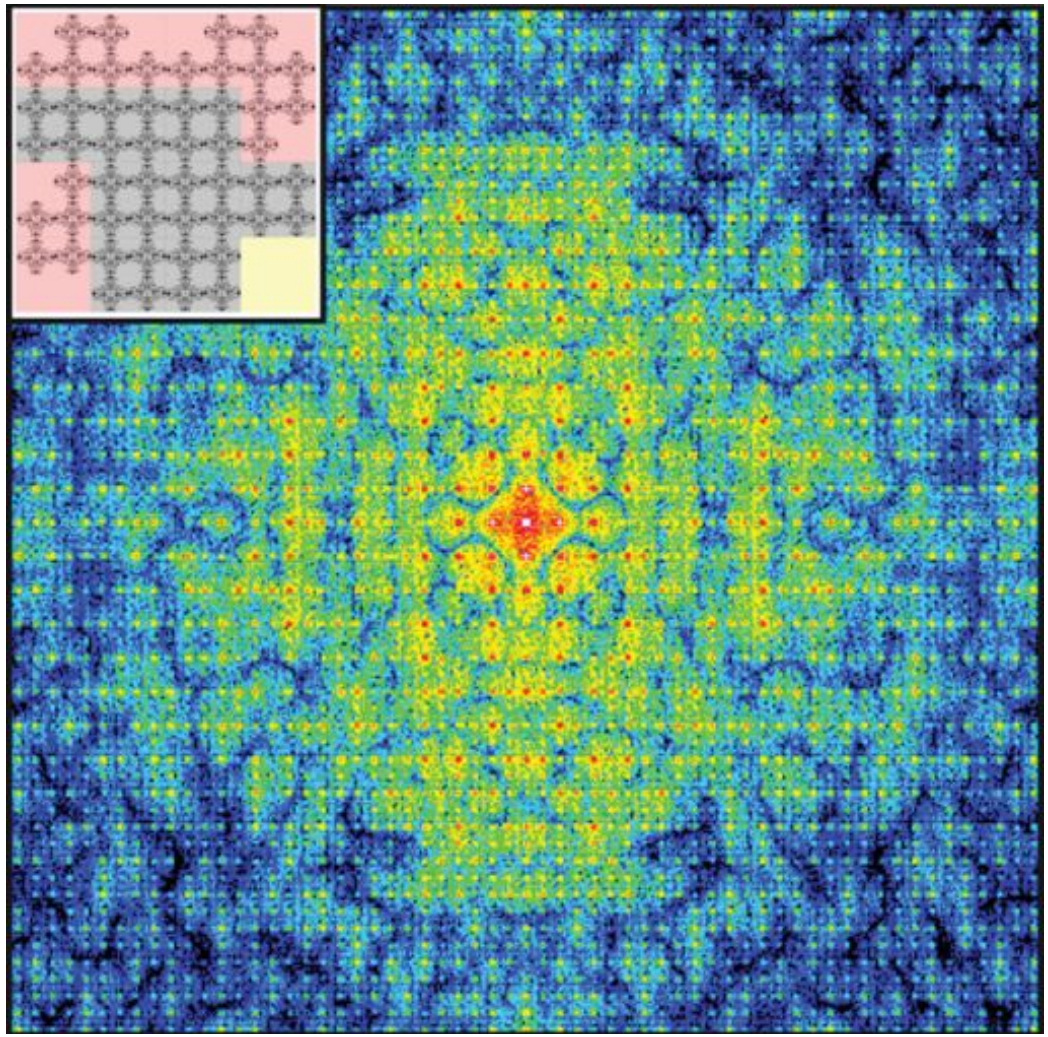
- unit cell : 281*281*165 $Å^3$
  $\alpha=\beta= 90°; \gamma=120°$
- 8 Å wavelength, 1 $\mu m^2$ beamsize, 15,000 frames

**N unit cells gives rise to N-2 fringes between neighbouring Bragg peaks.**
(fringe-spacing is finer by a factor of 1/N than the Bragg spacing)



# Information between bragg peaks could be used to phase structural information



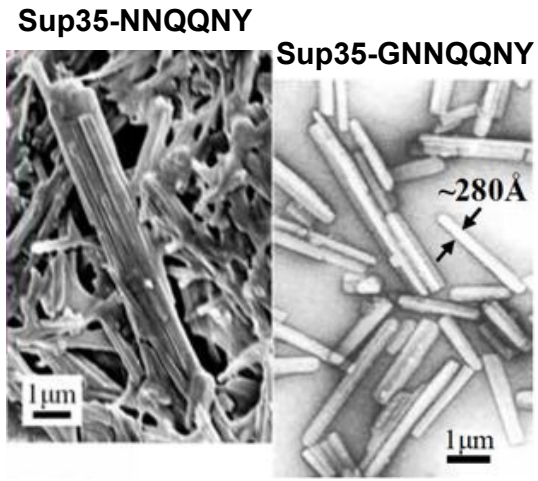
Rodriguez & Miao, 2014, IUCR J.

## Some proteins form nano-crystals *in vitro :*

*e.g. membrane proteins, amyloid proteins, protein complexes…*

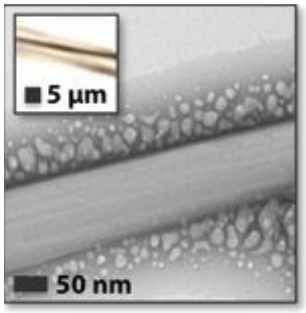
- Check out whitish precipitates in crystallization drops

**Do TEM on your drops !!!** ⟶




Nelson et al., 2005, *Nature*
Sawaya et al., 2007, *Nature*

- Keep faith… (0.8 Å data from a nano-crystal after 8h of "fish-mount-shoot" routine)




Nasrallah *et al., submitted*

- Refrain from avoidance of the feasible by invocation of the impossible

**Shoot first, think later !!!**

**Some proteins form nano-crystals *in vivo:***

- Opens a new era in structural biology, in which the **structures** of macromolecules are probed directly in their **cellular environments**

- **XFEL** >>> synchrotron radiation because crystals are small

- In the near future: $4^{th}$ generation synchroton sources : $10^4$ increase in flux !!!

→ may become feasible at synchrotron too ?

**Serial crystallography *:***

- Already feasible at synchroton sources (use NanoPeakCell !!!)

- Serial crystallography **is important** for macromolecular objects that are hard to crystallize :
  - *** membrane proteins**
  - *** protein complexes**

- **Time-resolved crystallography** : pump-and-probe experiments on the fs-ms timescale

## In conclusion *:*

- **It's a great time to be doing structural biology !!!!**
  (mass spec., spectroscopists, crystallographers, programmers, biologists, engineers…)

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