Femtosecond Protein Nanocrystallography

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Collaborators

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The CAMP Team

The CAMP instrument was designed and commissioned by the Max Planck CFEL Advanced Study Group

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Key Advantages

- Needs only **small crystals** (sub-micron sized).
- Can use **radiation-sensitive samples**.
  - Illumination is **fast** and crystals are not reused (*serial crystallography*). Specimen damage as it is conventionally understood is **irrelevant**.
- **Extra information** arises from the coherence of the beam across the entire crystal.
- Offers **high time resolution** when doing pump-probe experiments, and can study **irreversible reactions**.

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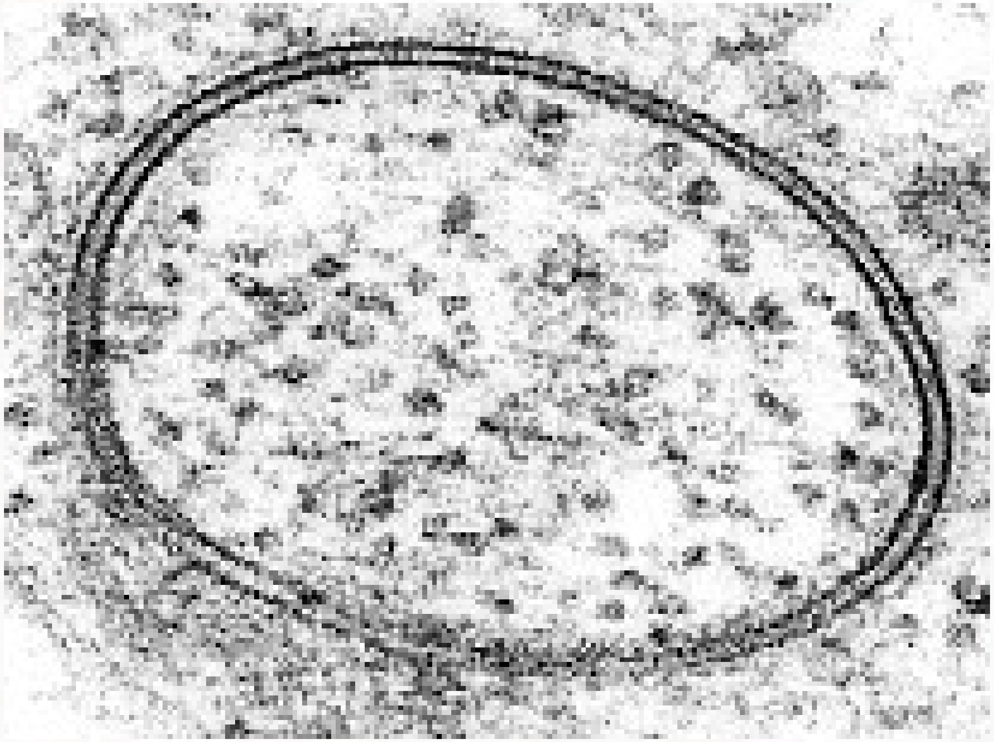
Big proteins...

- Life relies on separating “inside” from “outside”.



Membrane thickness: about 5-6 nm.

Image: http://en.wikipedia.org/wiki/File:Annular_Gap_Junction_Vesicle.jpg (public domain)

Big proteins...

- Transmembrane proteins control (amongst many other things) what may cross the membrane.

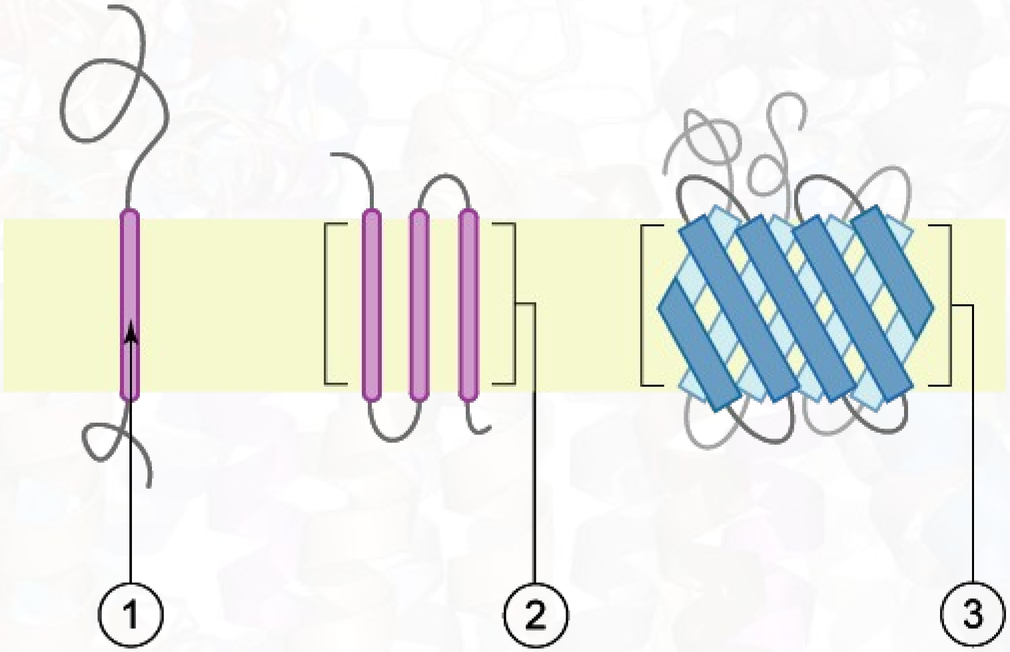
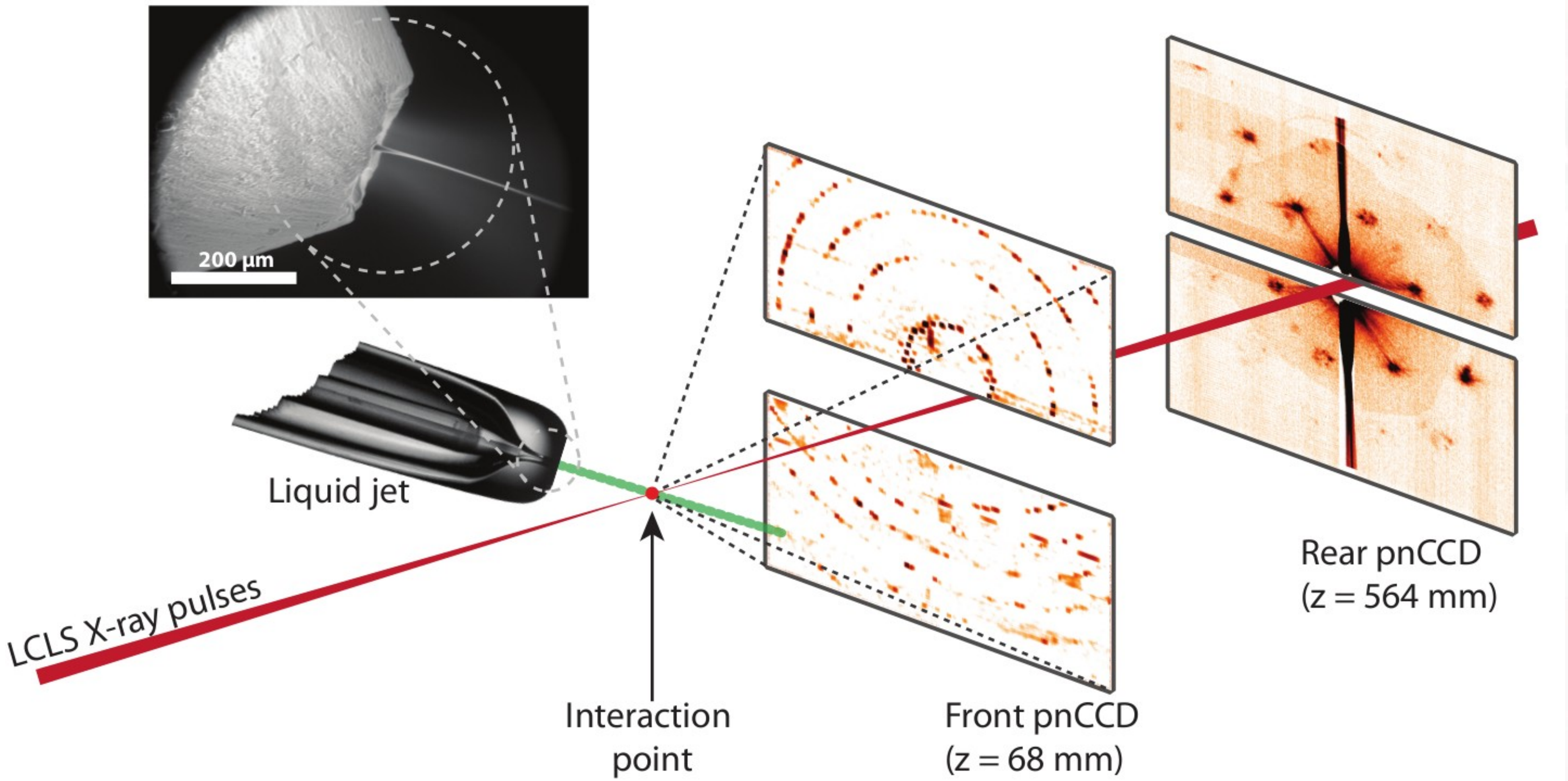


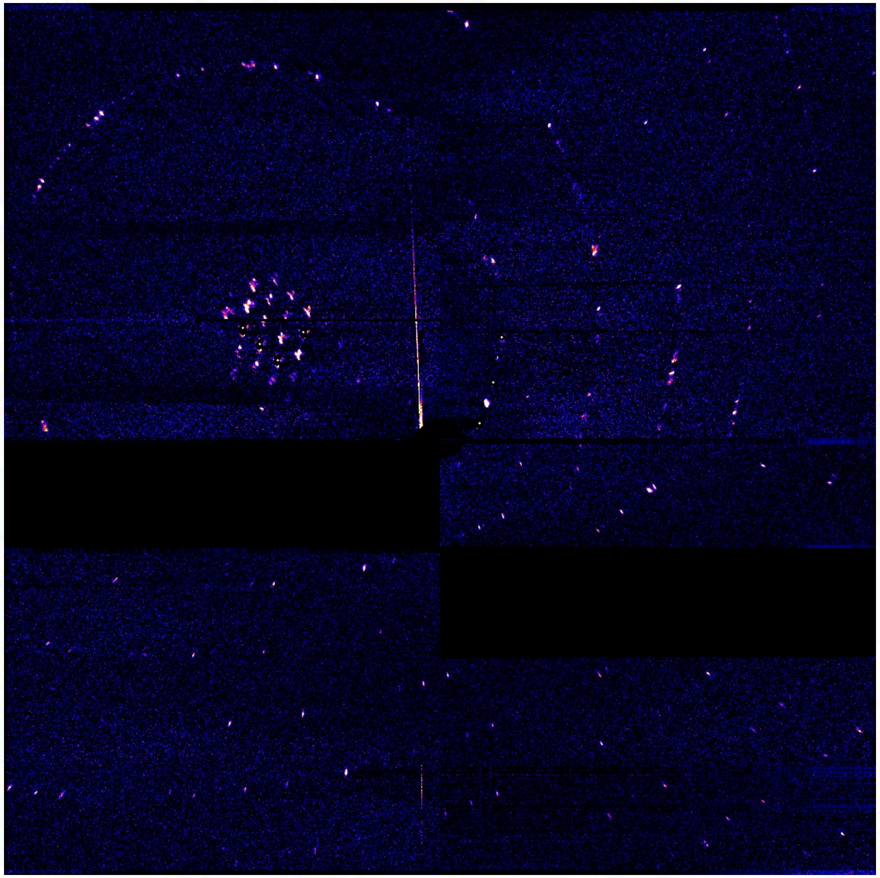
Image: http://en.wikipedia.org/wiki/File:Polytopic_membrane_protein.png CC-BY-SA

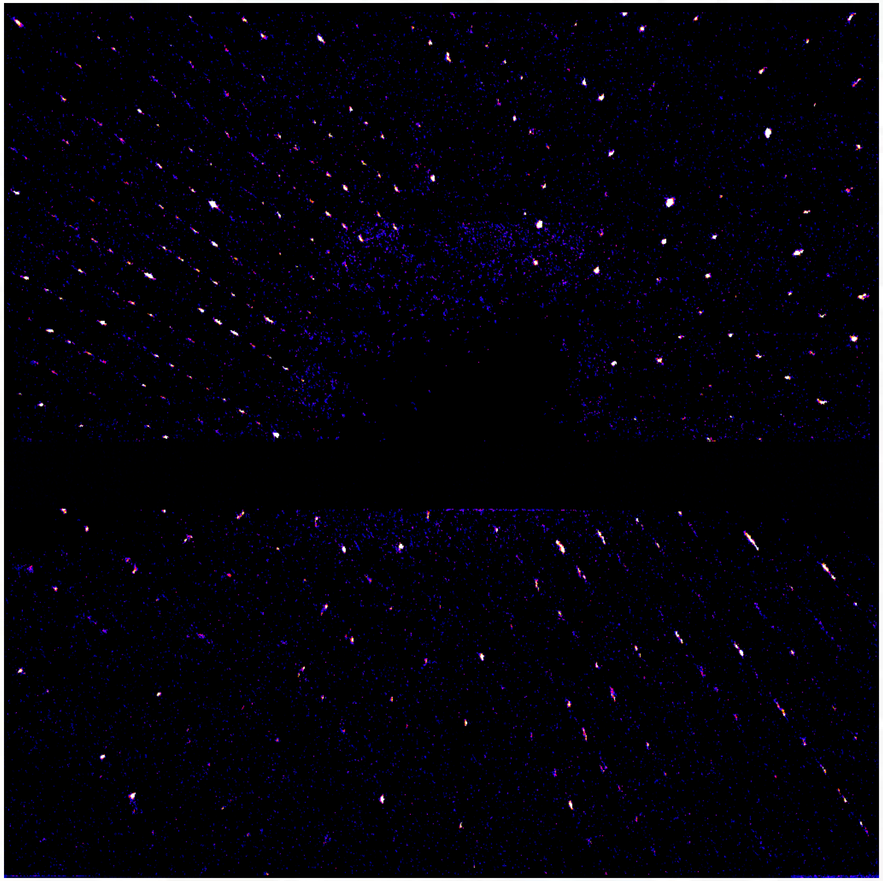
Experimental Setup



LCLS, AMO beamline, 2 keV, 70 fs pulse duration.

Diffraction Patterns





The “Monte Carlo” Method

- By measuring a dataset with very high redundancy (>1000 measurements per independent reflection), we get accurate intensities despite the lack of rotation:

Kirian et al., Optics Express **18** (2010) p5713-5723

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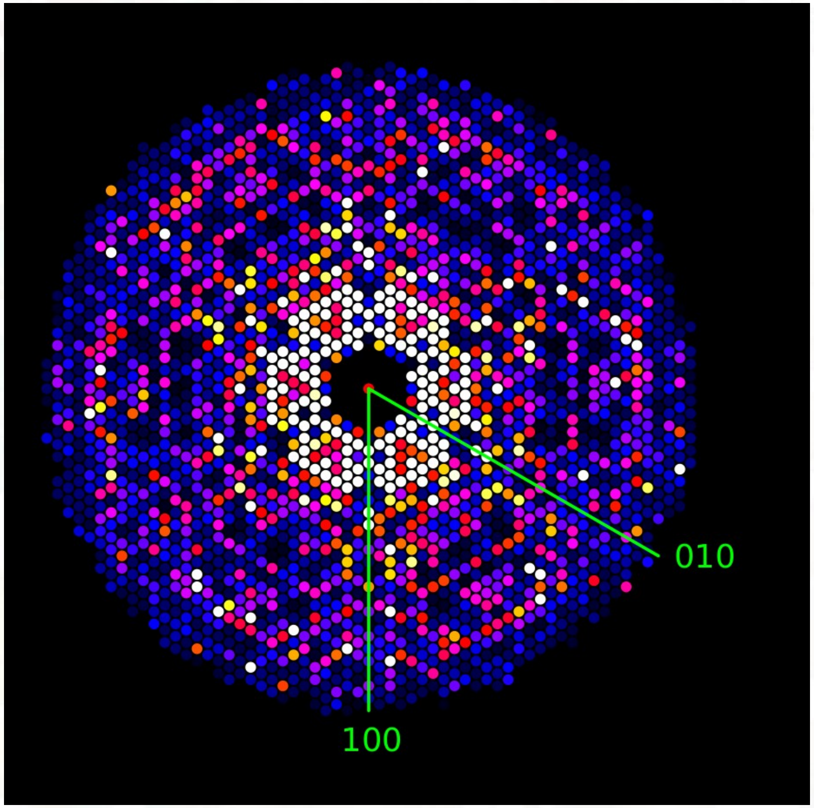
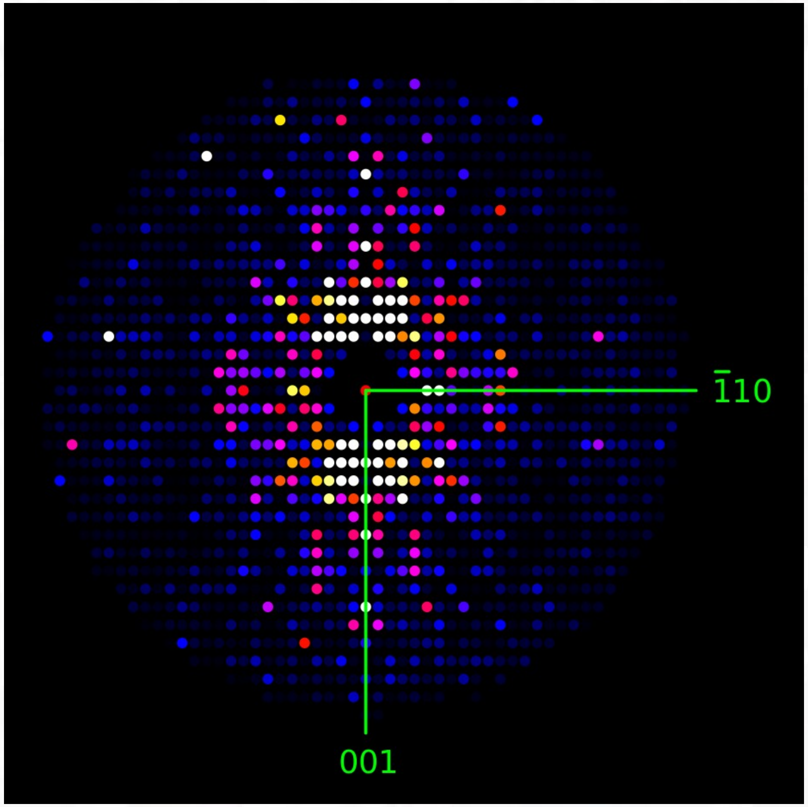
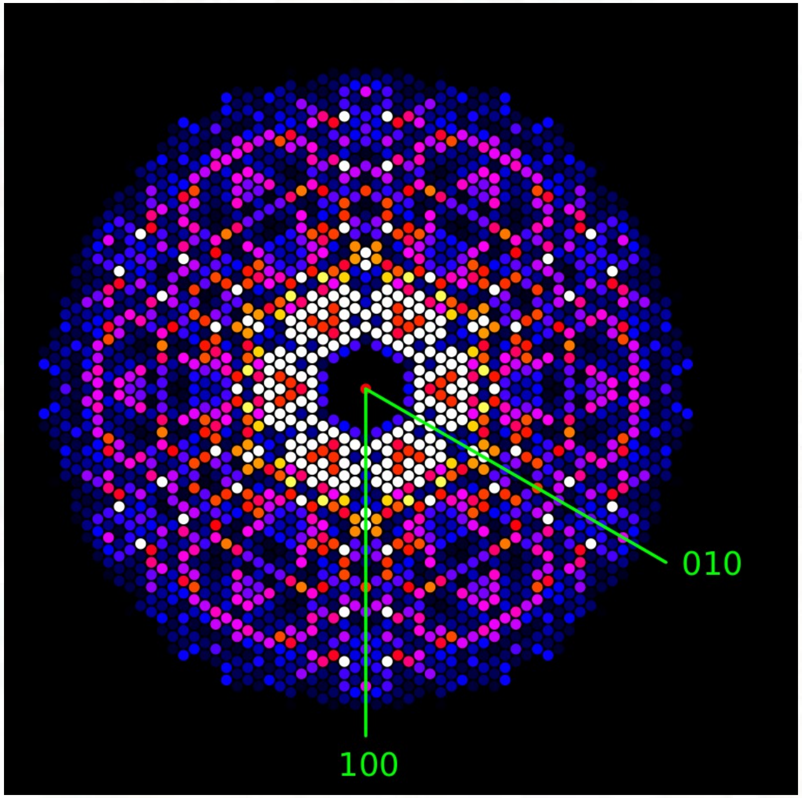
Merging of Intensities

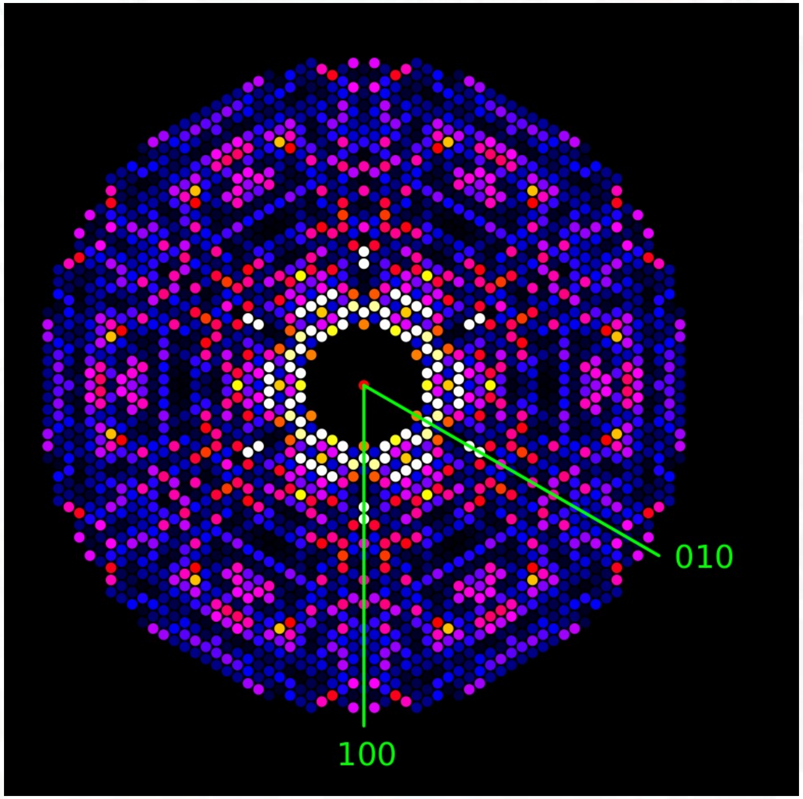
- If the symmetry of the crystal comes through in the final results, things are probably not going too badly.





Merging of Intensities

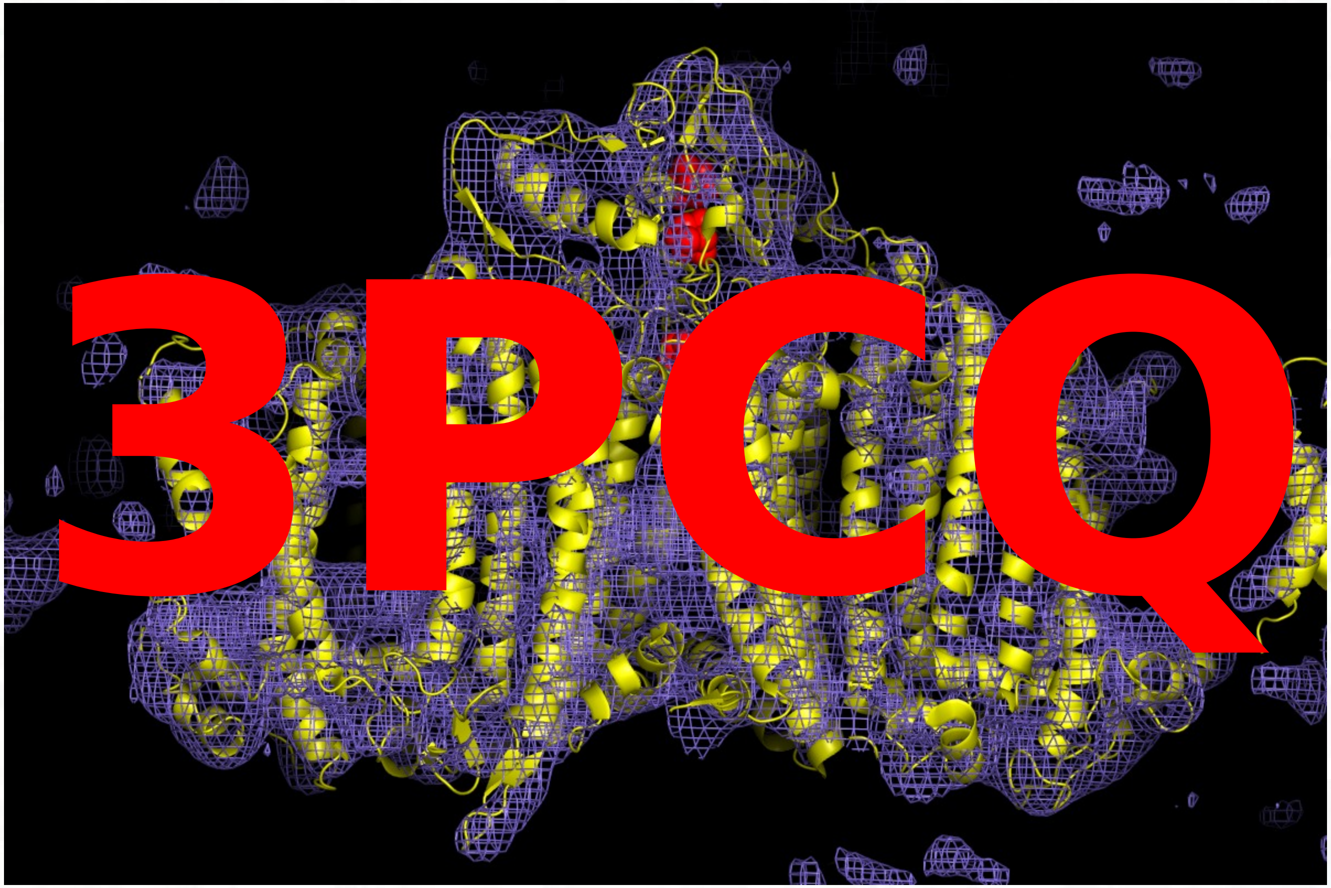




- Another way to evaluate the quality of the data is to compare against synchrotron data, but beware...

Phasing the Data

- At this point, our data goes into the conventional MX analysis pipeline.



Some numbers from “3PCQ”...

- **Number of crystals:** 15,445
- **Crystal size**: 0.2 – 2 µm
- **Data frames per crystal:** 1
- **Temperature:** room temperature
- **Hydration:** Fully hydrated, in mother liquor

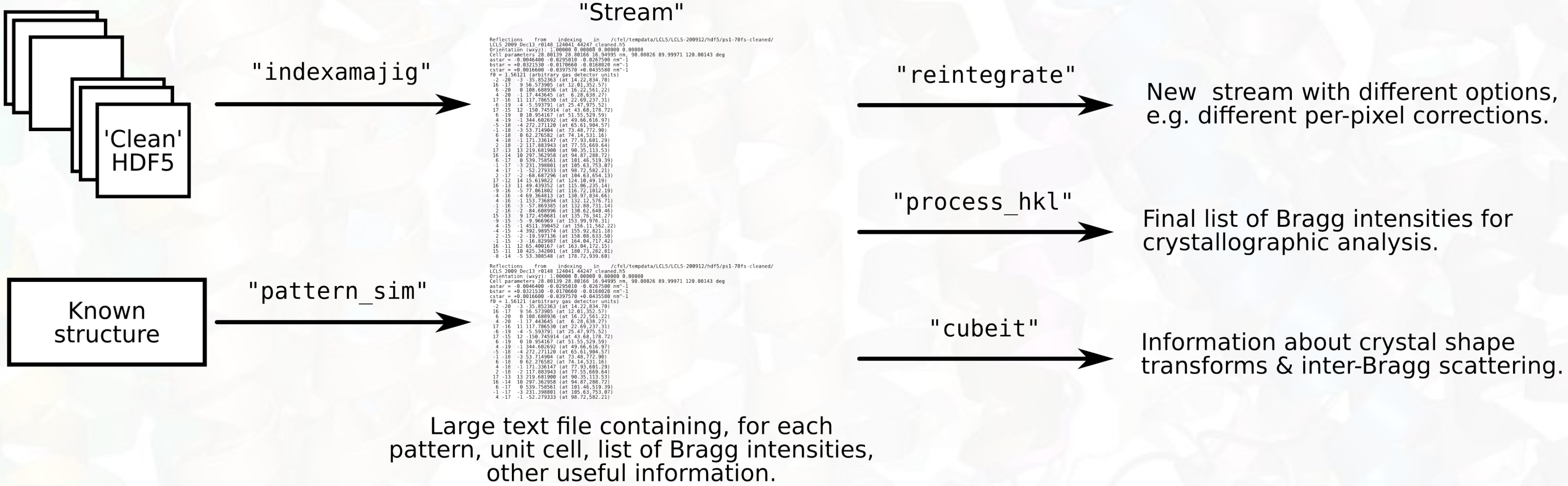
- **Exposure time:** 70 fs
- **Number of images collected:** ~ 1,800,000
- **Oscillation angle:** zero
- **Data reduction software:** CrystFEL

- **X-ray energy (per exposure):** random
- **X-ray energy (mean):** 2 keV
- **X-ray bandwidth:** ~ 0.1%

Anton Barty, CFEL

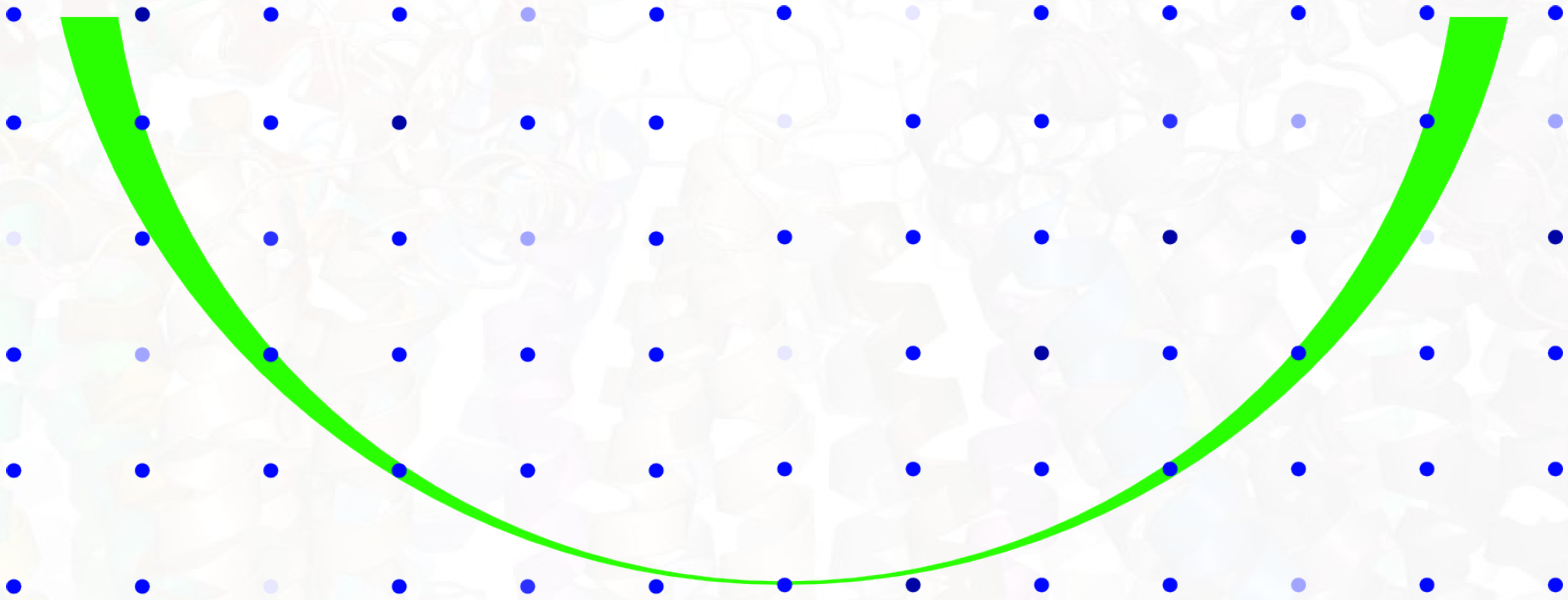
Software: CrystFEL

- Suite of programs which share code and file formats, “CCP4 style”.
- It's easy to interchange real and simulated input.
- Time consuming steps are multi-threaded.



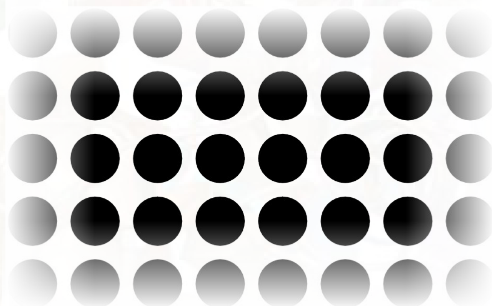
Reciprocal Space

- Section through reciprocal lattice with Ewald sphere overlaid.

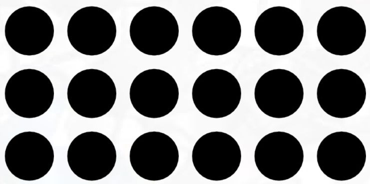


Reciprocal Space

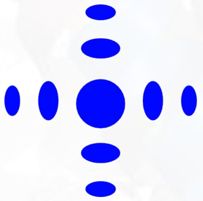
- Truncation of crystal lattice leads to “truncation rods” at each point of the reciprocal lattice.



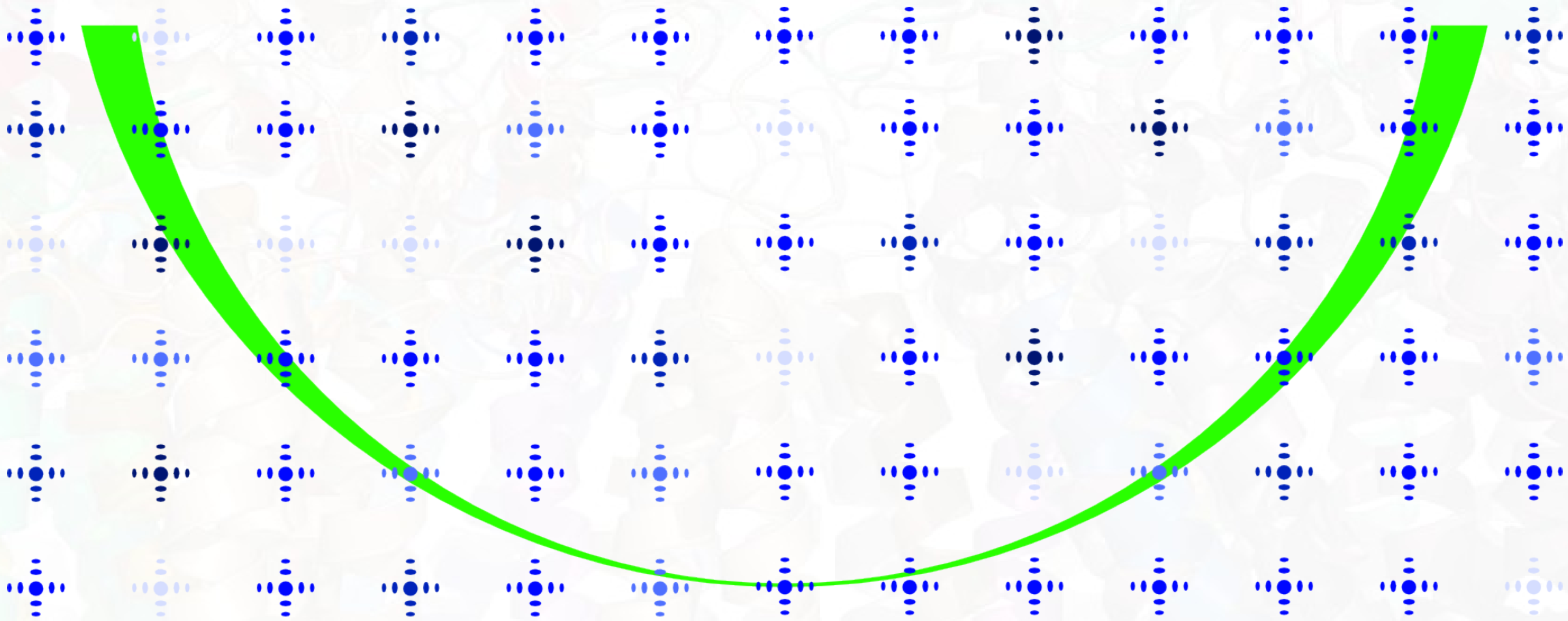
Infinite lattice



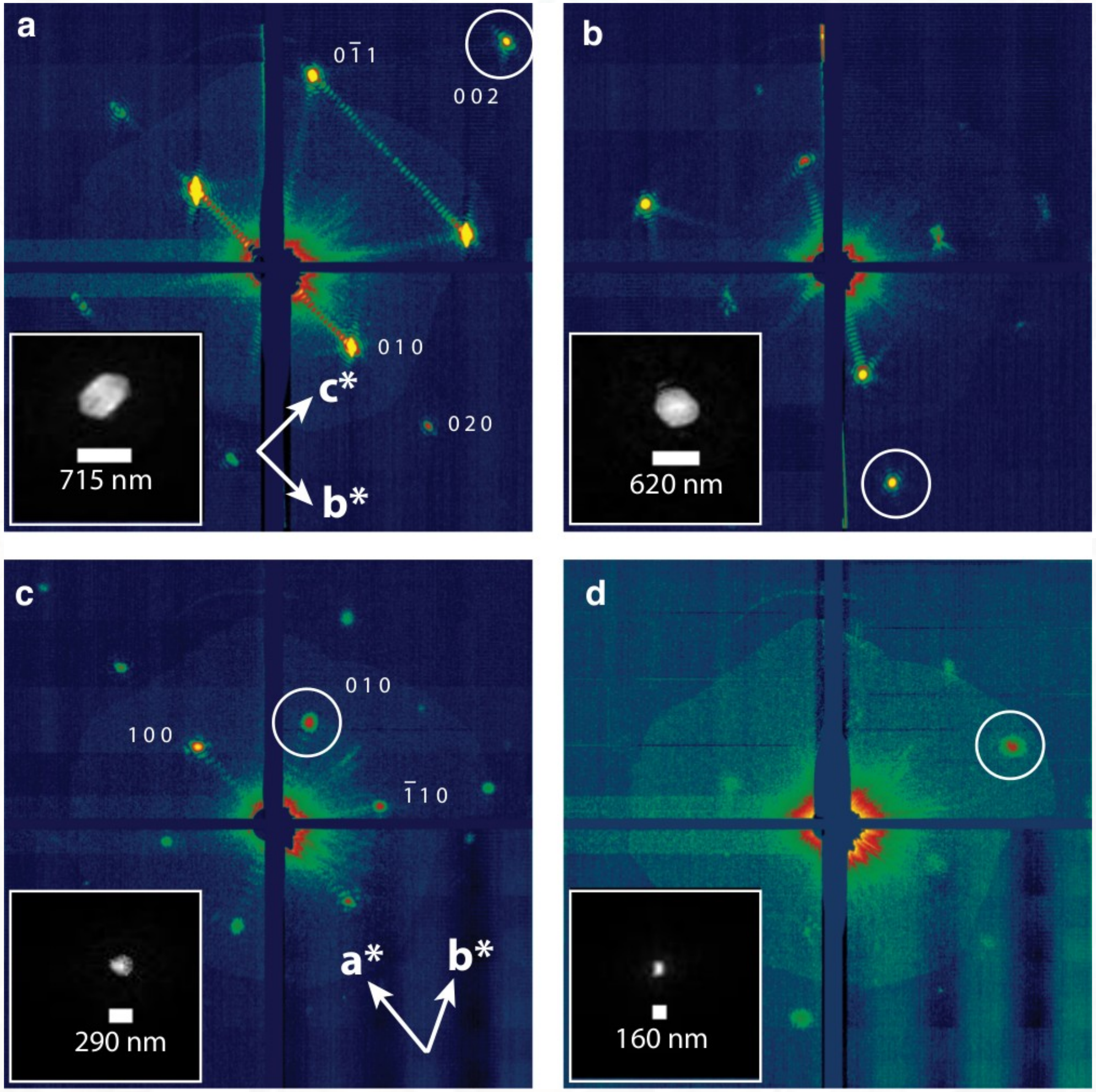
Truncated lattice



Reciprocal Space



Peak Shapes – Rear Detector



Andrew Martin, CFEL

Specimens

- **Photosystem 1**
- **Lysozyme**
- **R. Viridis reaction centre** (light/dark)
- **Elastase**
- **Proteinase K**
- **Cathepsin B** (glycosylated form)
- **Photosystem 1–ferredoxin cocrystals** (light/dark)
- **Photosystem 2** (light/dark)
- **Haemoglobin**

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The next year or two

- Develop analysis algorithms – get even better results.
- New injector technology – waste less sample.
- Use unique information (between Bragg peaks) to solve structures in new ways.
- Demonstrate conventional phasing methods: SAD/MAD etc.
- ... solve lots of otherwise inaccessible structures!

Looking ahead to 2015...

High repetition rate FELs, combined with detectors which can keep up, can make this go a **lot** faster...

Conclusions

- The **feasibility** of doing crystallography in the "diffract and destroy" regime using a femtosecond laser has been demonstrated.
- Even at this early stage, **new structural information** is being obtained.
- There are many more exciting things to try...

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Main publication

Chapman et al., Nature (2011) – out on the 3rd Feb

Injector

DePonte et al., J. Phys. D **41**, 195505 (2008)

CAMP instrument

Strüder et al., Nuclear Instruments and Methods in Physics Research A **614** (2010) 483-496

Monte Carlo integration

Kirian et al., Optics Express **18** (2010) 5713–5723

PDB entry 3PCQ

Poster #130 (page 25), Koopmann et al.

... and many more in preparation ...!

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