CUI THE HAMBURG CENTRE FOR ULTRAFAST IMAGING

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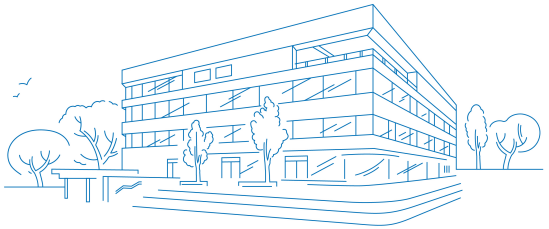
Protein Crystallography at XFELs
Or
What are XFELs good for? And when would I (not) want to use one?

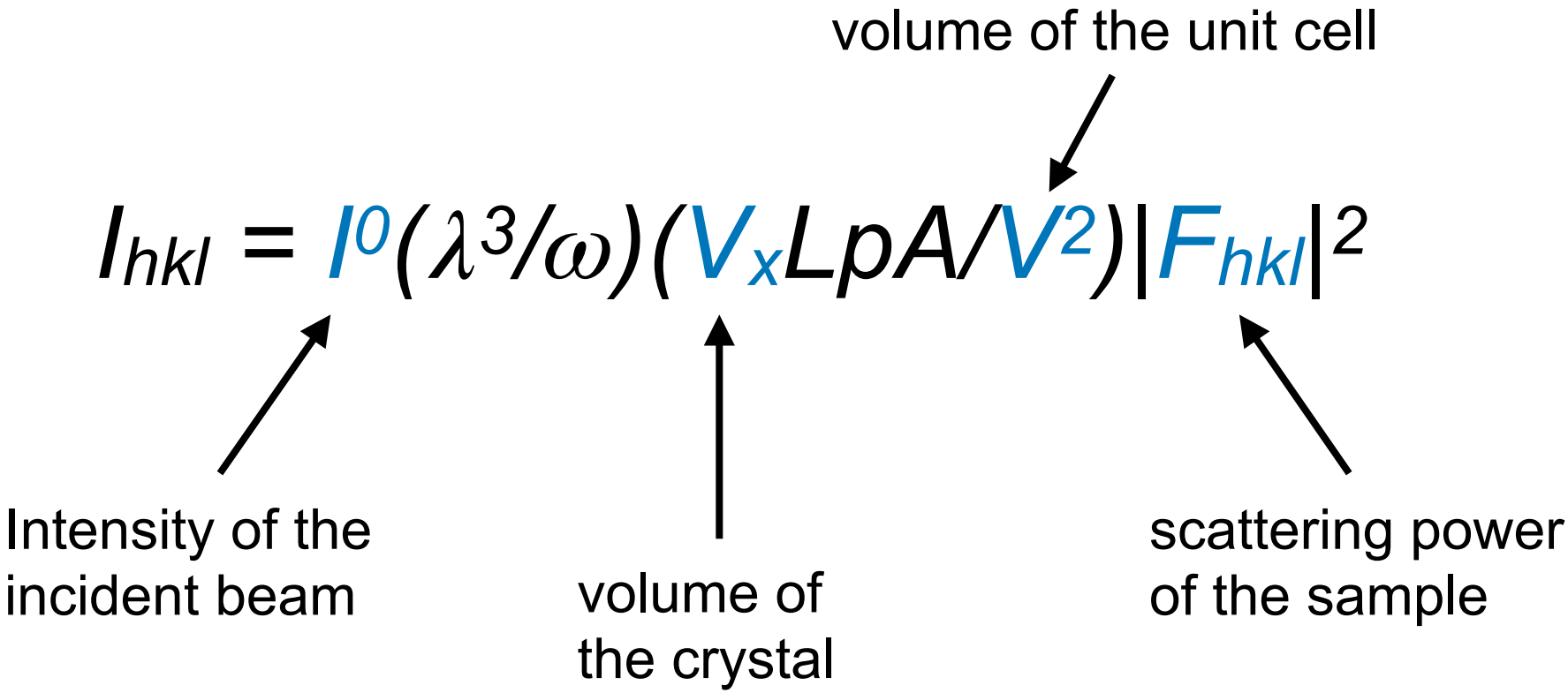
Arwen Pearson

T-REX



- We determine macromolecular structures to understand how biology works
- Macromolecules are nanoscopic objects
  - Sensitive to radiation damage
- Use ensemble methods to determine high resolution structures
  - Able to spread dose (absorbed energy) over the sample
  - Increased signal to noise

- How to increase signal?
- Signal is dependent on both the source properties & the sample!



- How to increase signal?
- Signal is dependent on both the source properties & the sample!

volume of the unit cell

$$I_{hkl} = I^0(\lambda^3/\omega)(V_x L p A/V^2)|F_{hkl}|^2$$

Intensity of the incident beam

volume of the crystal

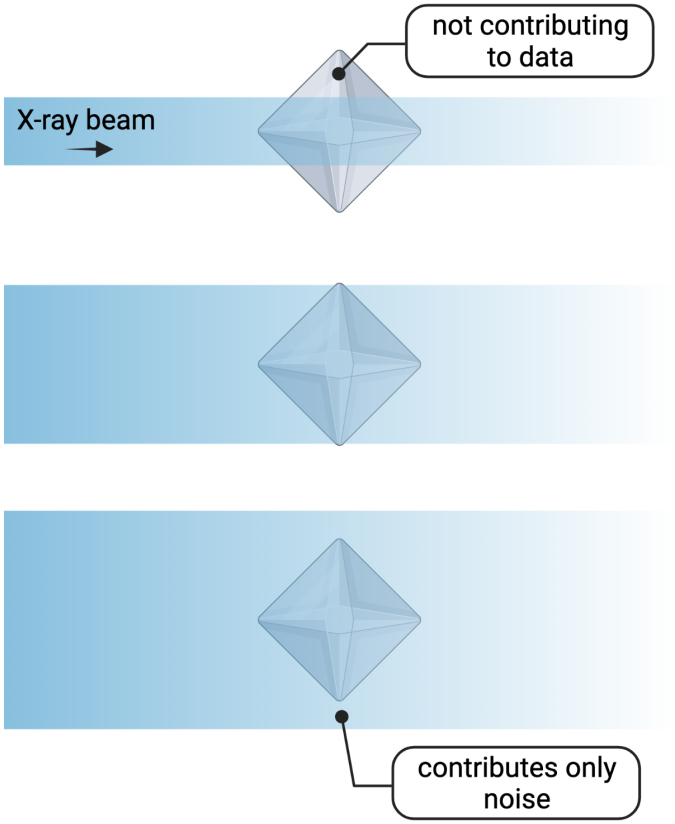
scattering power of the sample

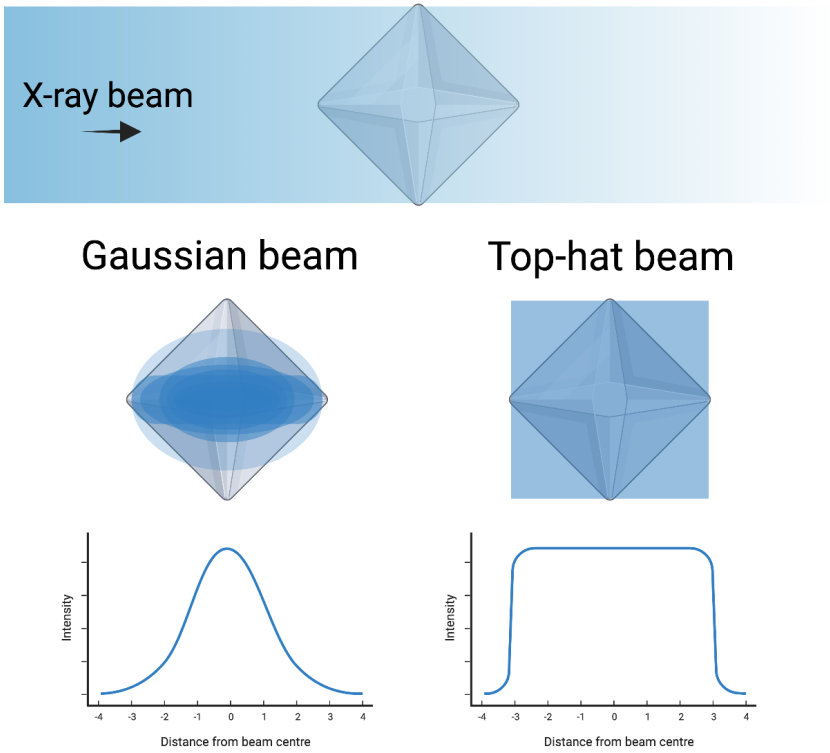
Properties of the sample that can't be easily changed

- The evolution of macromolecular crystallography has been focused on
    - Bigger and better crystals
    - Brighter and brighter X-ray beams

There is no substitute for optimised sample prep and good experimental design!

- It is worth putting in the effort to get beautiful large crystals
- And then designing the experiment to get the best data from them.





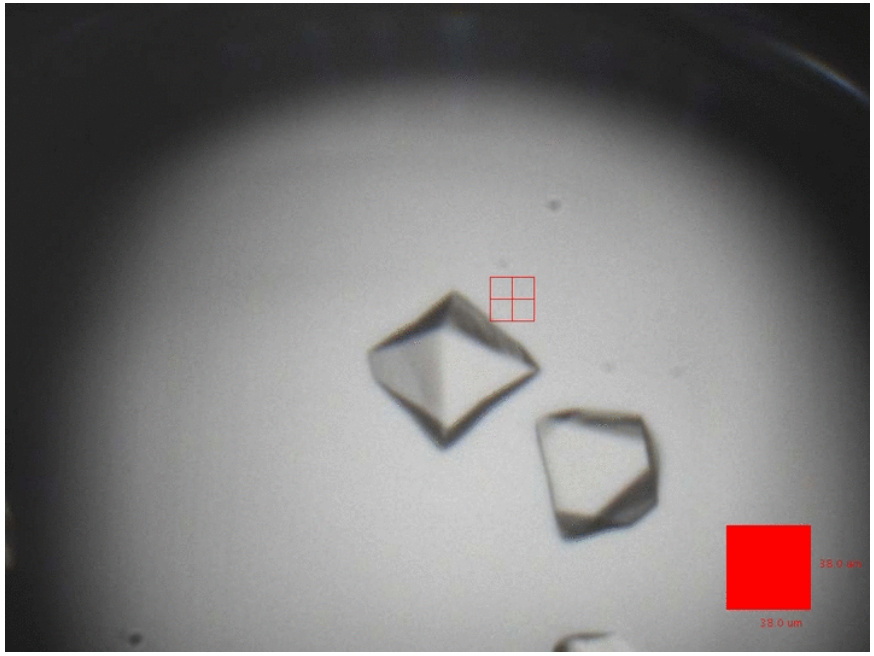
Nick Pearce

There is no substitute for optimised sample prep and good experimental design!

- It is worth putting in the effort to get beautiful large crystals
- And then designing the experiment to get the best data from them
- If you can't get beautiful big crystals
    - More X-ray intensity will help
        - But only up to a point

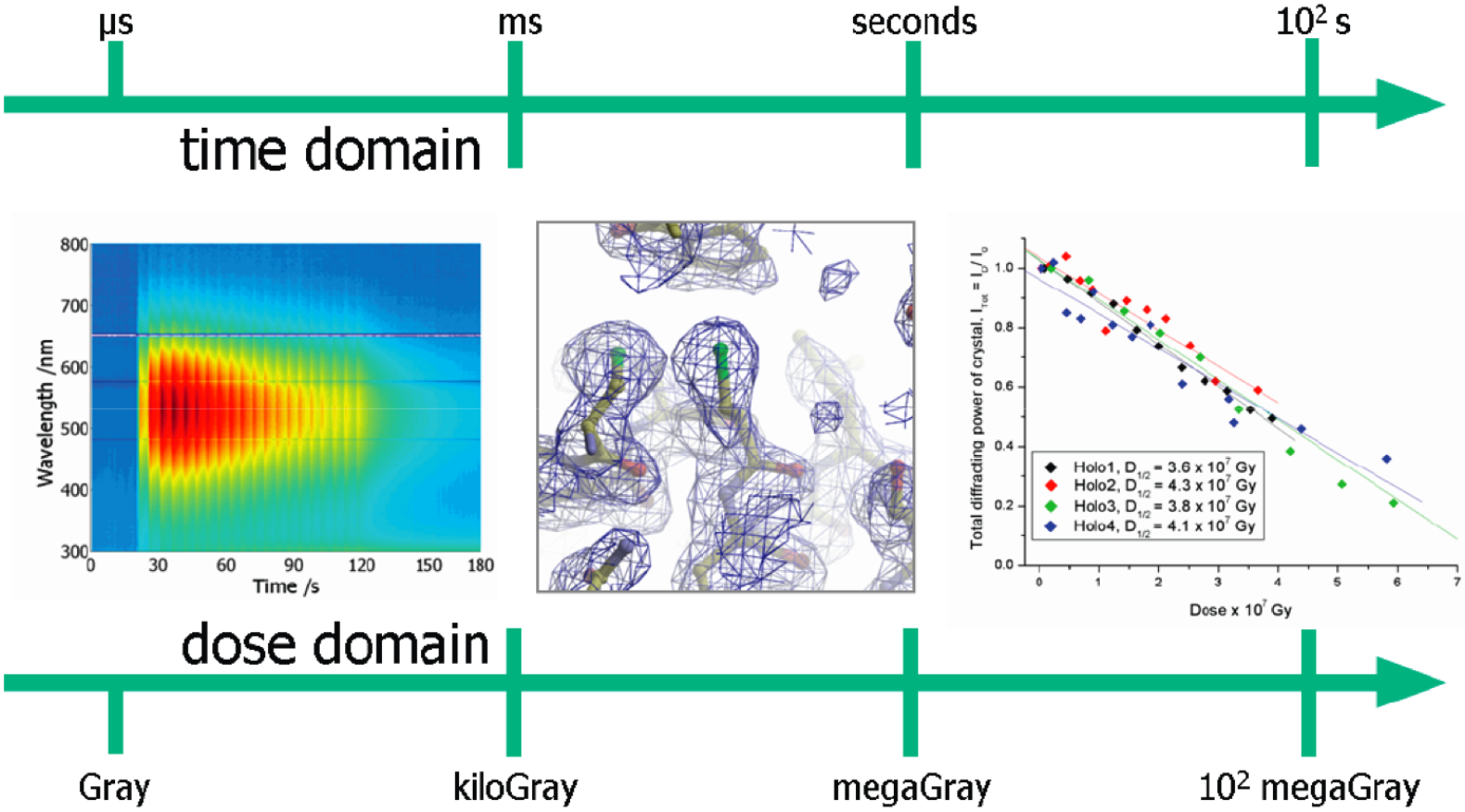
We need to talk about radiation damage

- How you deliver your photons matters
  - Radiation damage causes rapid changes in your crystal



We need to talk about radiation damage

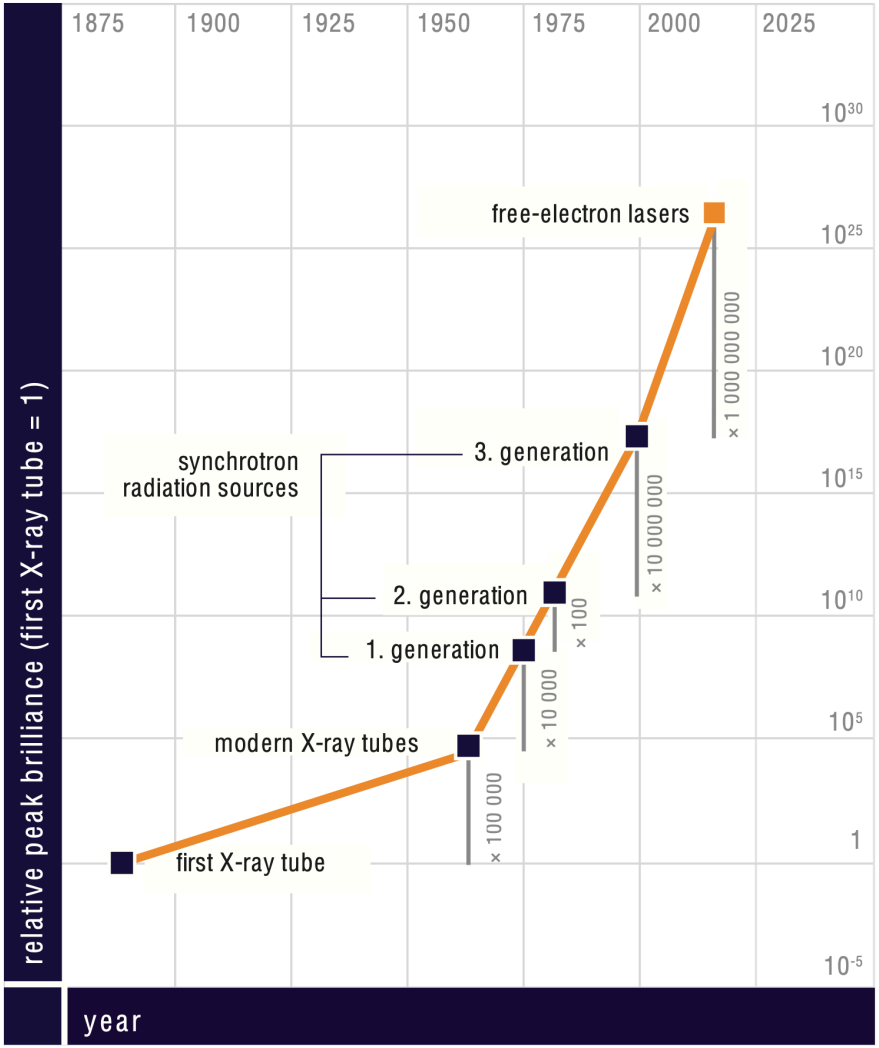
- How you deliver your photons matters
  - Radiation damage causes rapid changes in your crystal
  - These begin as soon as X-rays hit the sample



Robin Owen

First key advantage of XFELs and synchrotrons

- Both synchrotrons and XFELs are pulsed sources



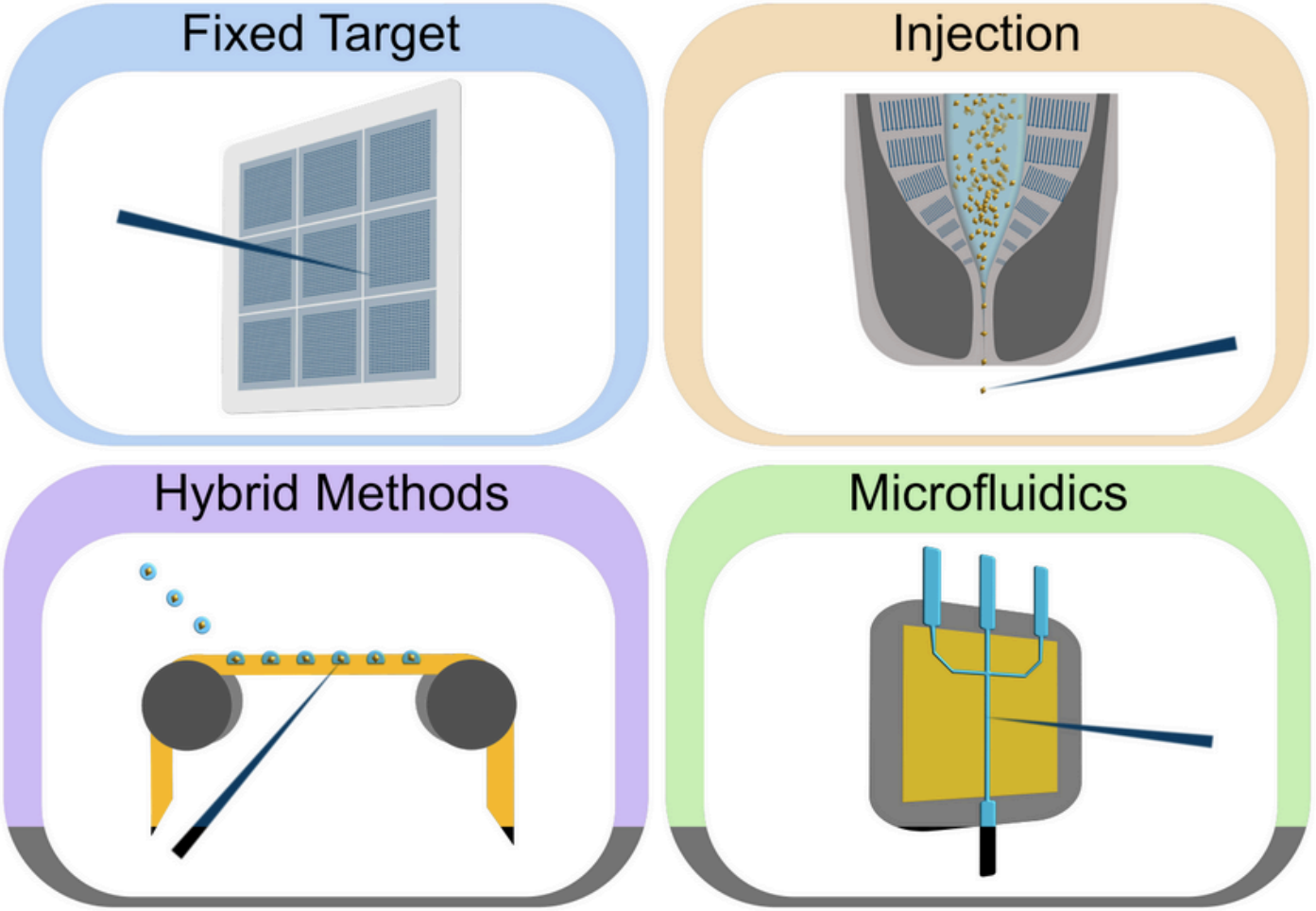
- XFELs can deliver in a single pulse the same intensity as a synchrotron in ~ second
- This makes a big difference to radiation damage
- Diffract and destroy - SFX

SFX experiments

- SFX experiments are becoming increasingly accessible
  - MFX endstation at LCLS
    - Standardised sample environment
    - Closer to the "synchrotron experience"
- Challenges
  - Sample
    - Crystal size
    - Crystal quantity
  - Sample delivery
    - New sample constantly needed
    - Sample delivery should match photon delivery

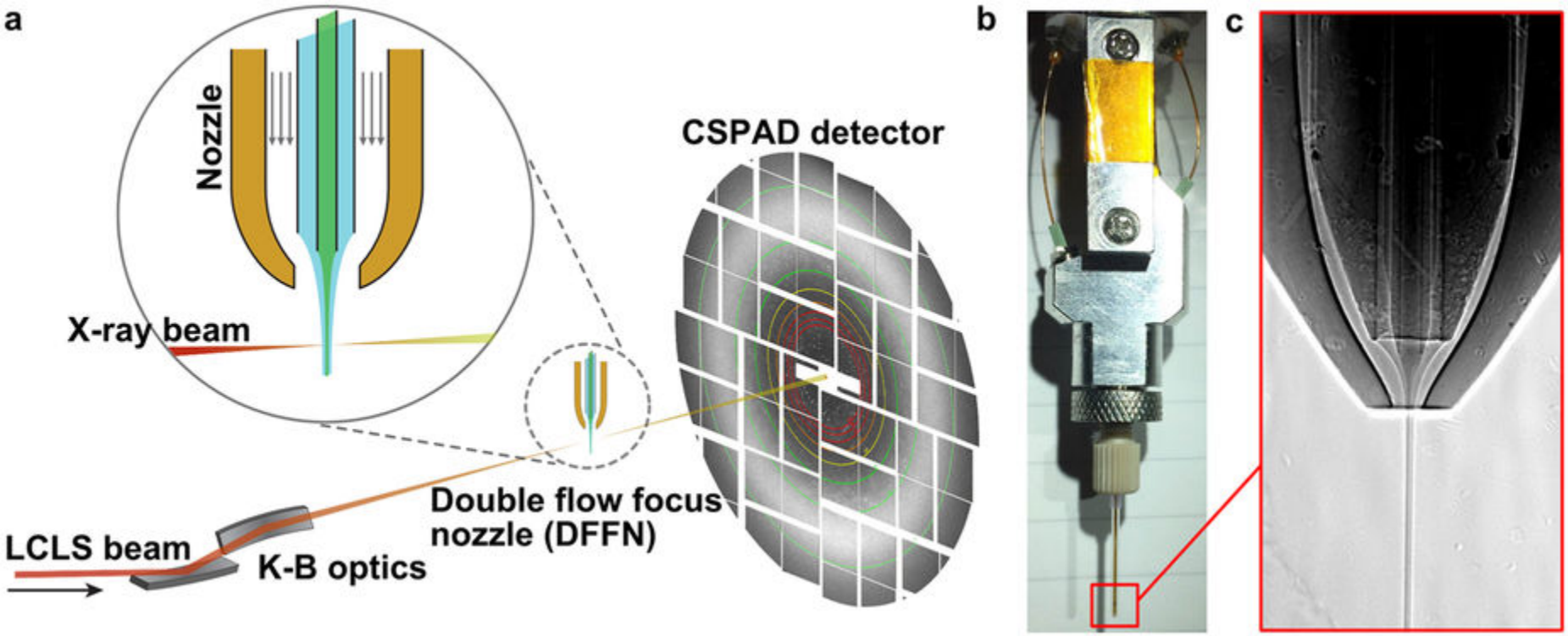
SFX experiments

- SFX experiments are becoming increasingly accessible
  - Sample delivery should be matched to your experiment



Pedram Mehrabi, Curr. Opin. Struct. Biol. 2020

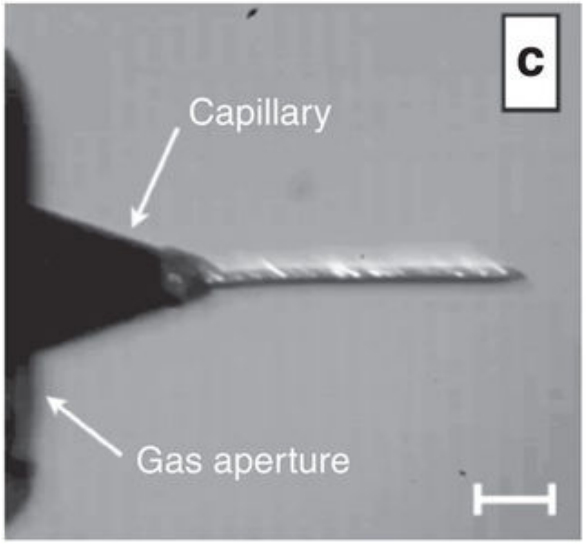
- Jets

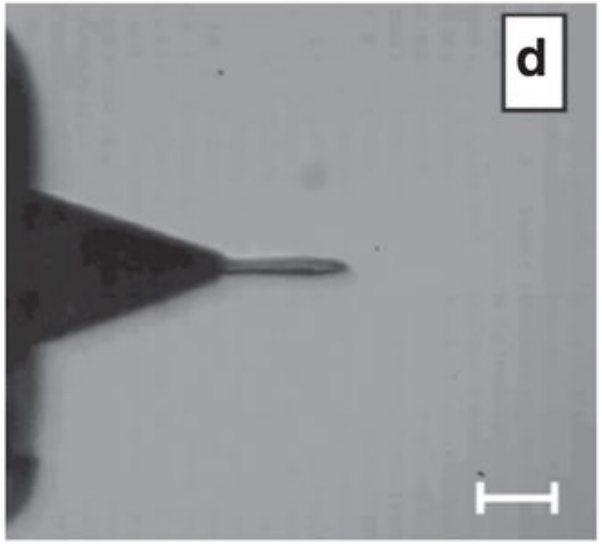


- Sample delivery can be very fast, but is stochastic
- can use a LOT of sample
- need a way to stop the crystals settling

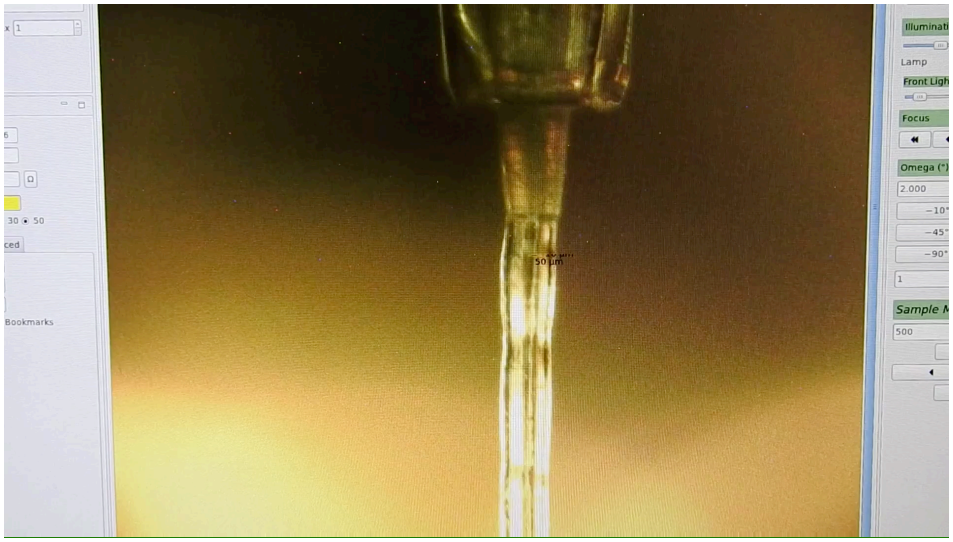
Oberthuer, Dominik http://dx.doi.org/10.1038/srep44628

Viscous Jets





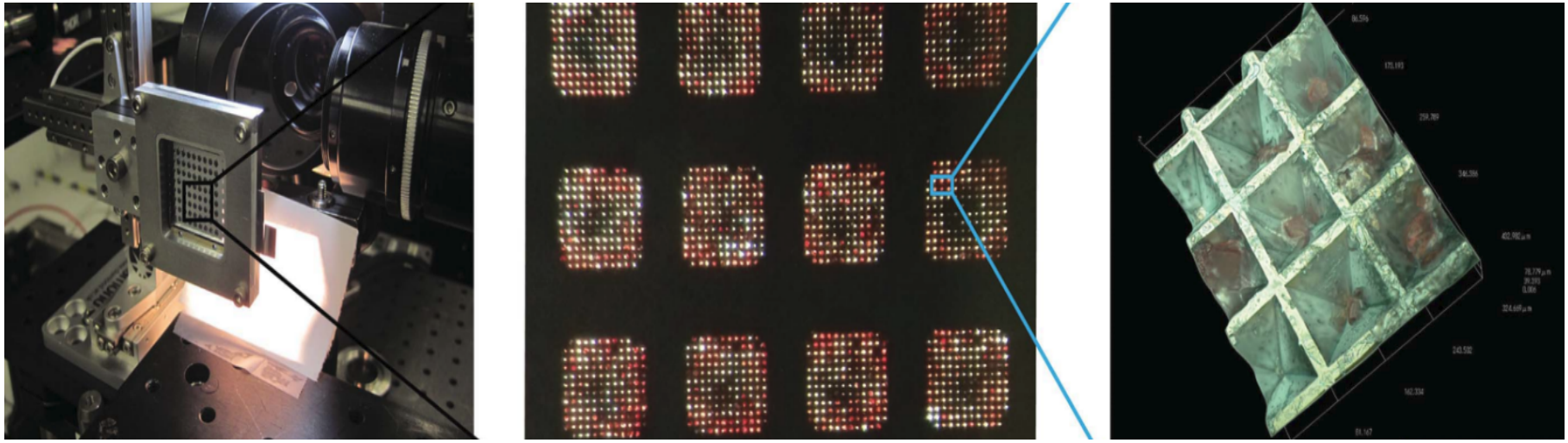
Uwe Weierstall Nature Comms (2014) doi:10.1038/ncomms4309



- First demonstrated with LCP
- Can also use “grease” and other polymers
- Sample delivery is slow - matches well to the rep rate of the LCLS and SACLA
- Also works well at synchrotrons
- Vital to test compatibility of media with YOUR sample

Kovascova et al., IUCrJ, 2017

Fixed/Solid Targets

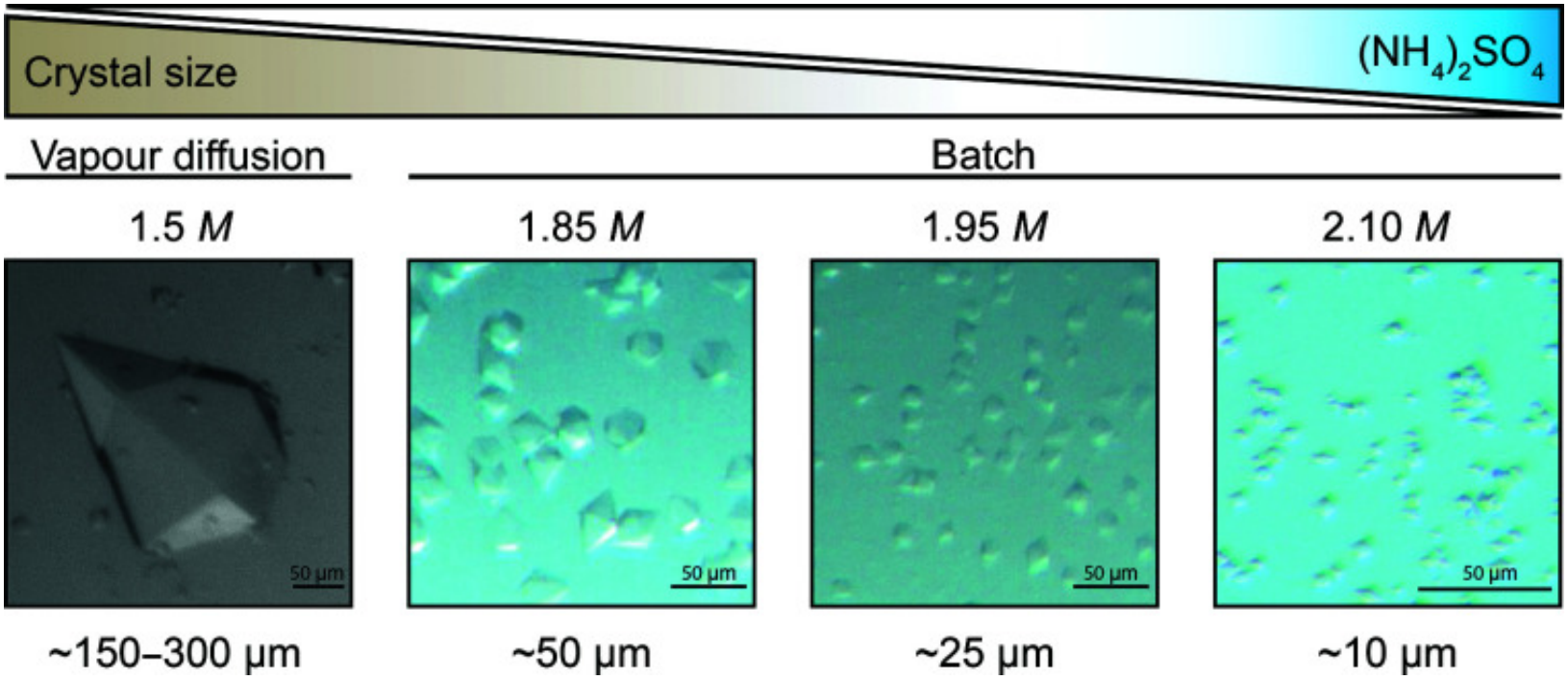


Oghbaey et al 2016, Acta Cryst. D

- Samples can be presented randomly or in a defined array
  - if defined can achieve near 100 % hit rates
  - useful for cases where sample is limited
  - Background can be minimised

SFX experiments

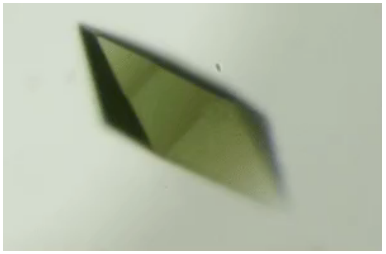
- SFX experiments are becoming increasingly accessible
  - Need sufficient amounts of micro crystals
  - Batch crystallisation is the way to go
    - Seeding
    - Careful control of lab conditions

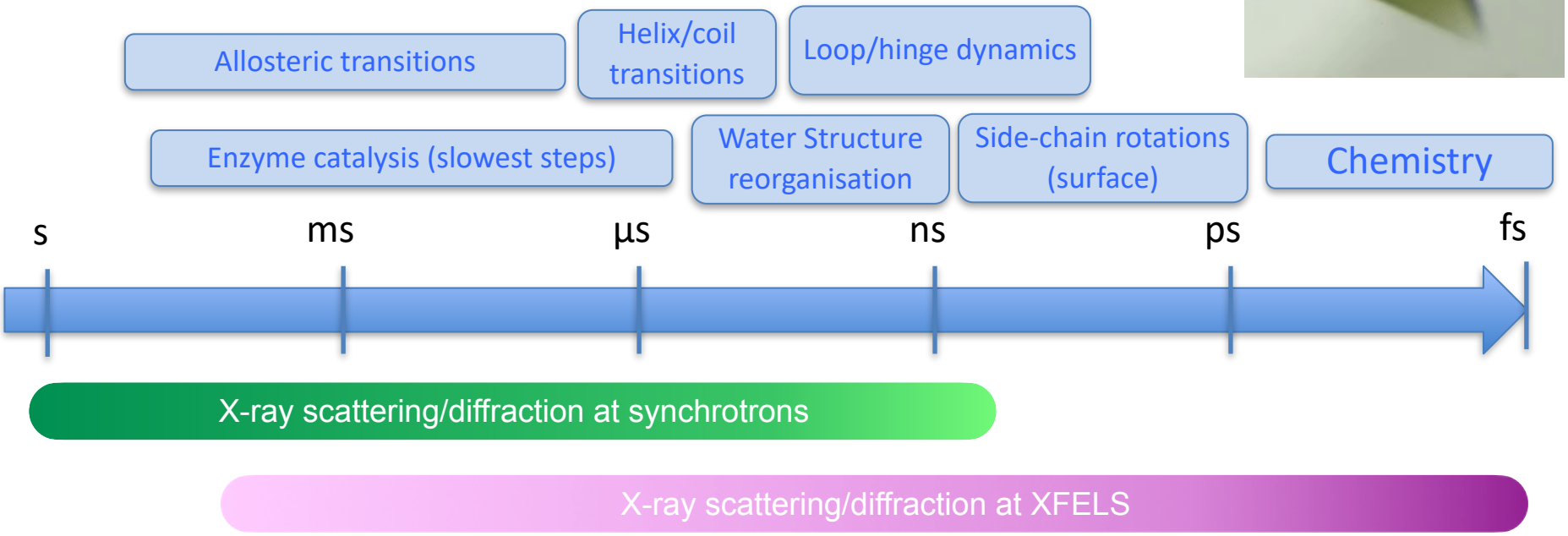


Claudia Stohrer, Acta Cryst D. 2021

What about time-resolved experiments?

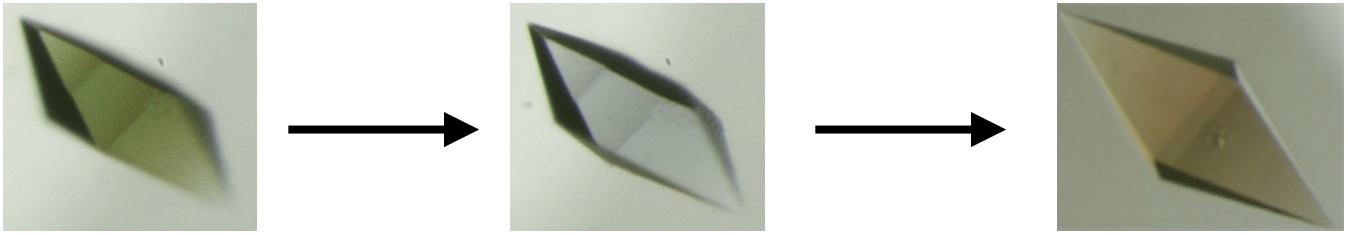
- [protein] in crystals ≈ [protein] in the cell
- many proteins retain catalytic activity in the crystal
- if there are no large conformational changes during catalysis, many proteins **remain** crystalline during turnover





Don’t make your “experimental” life harder than it needs to be!

- Trapping experiments



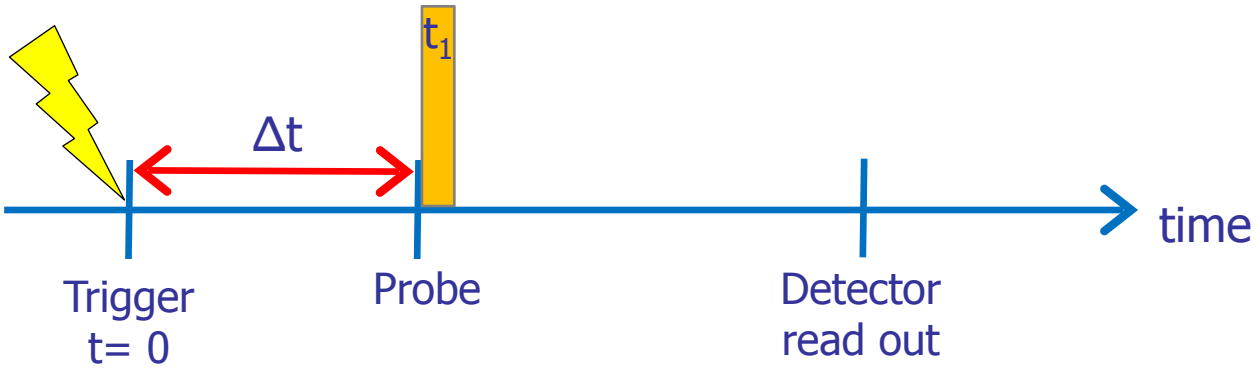
- Set up your experiment to arrest the reaction at a certain point
- Can work for many intermediates regardless of the time-scale on which they form and decay
- If this kind of experiment is possible and answers your question - don’t make things any more complicated!

Trapping experiments don't answer my question - what now?

- I can't trap the state I'm interested in
  - no way to mechanistically trap it
  - it forms too fast/is too shortlived for cryo-trapping
- I want to know about transitions between reaction intermediates
- In this case you need to do a true time-resolved experiment

Muybridge, Stanford

All time-resolved experiments essentially follow the same scheme



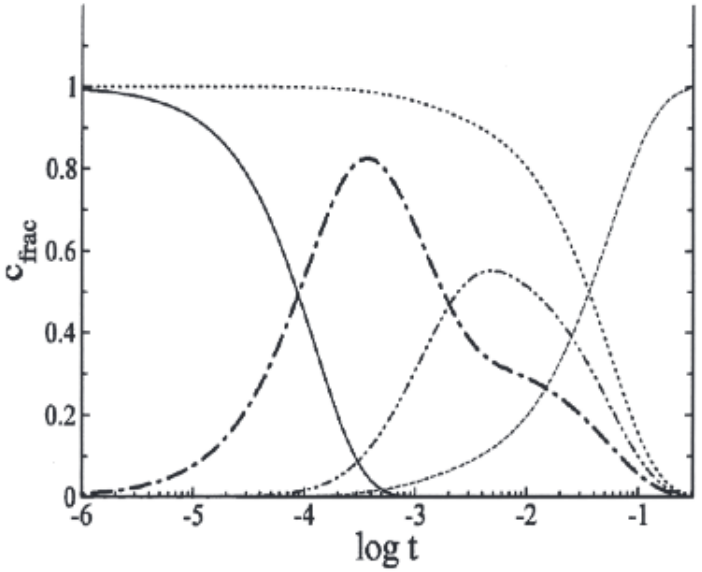
Time resolution is determined by the slowest of:

- Pump pulse width (or speed of reaction imitation event)
- Timing between the pump and probe
- Probe pulse width

But really, it's all about signal to noise, so anything you can do to increase signal and reduce background will help.

Why do I still need to worry about signal-to-noise?

- A time-resolved experiment results in a series of datasets recorded at different time-points
- There is always inherent heterogeneity in these data
  - Fractional excitation
  - Mixtures of different intermediate states



- Need enough data to be sure that you can really see any differences
- The lower the fraction of your sample reacting the more data you need

Schmidt et al., Methods in Molecular Biology 305 115-154 (2005)

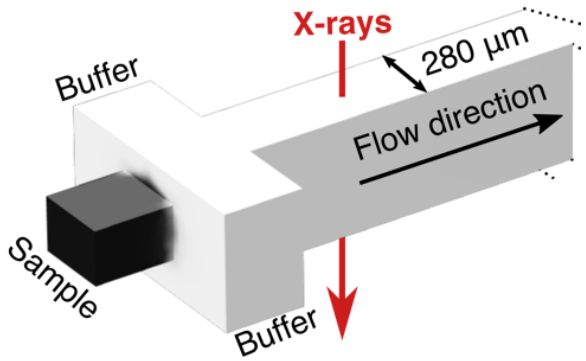
How do I get my samples all doing the same thing

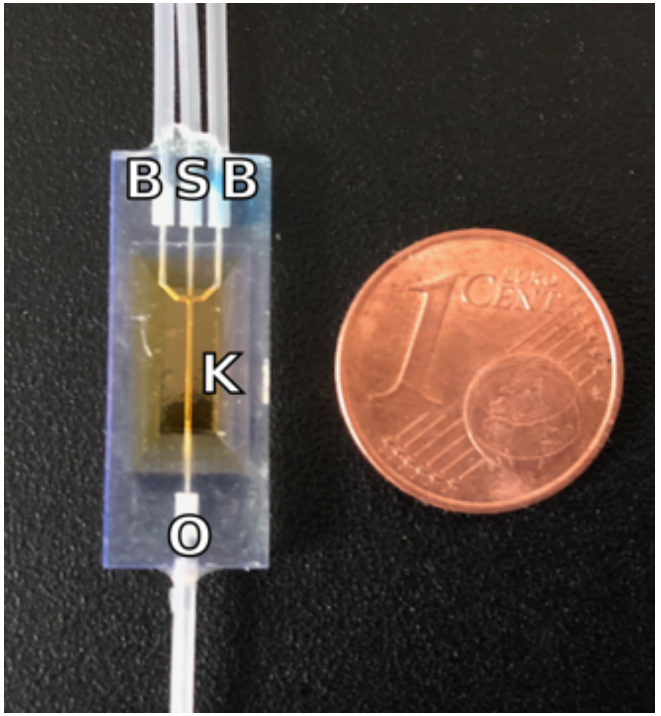
Thelwell

- There are two basic ways to initiate a reaction
  - Mixing
  - Photoactivation

Rapid mixing

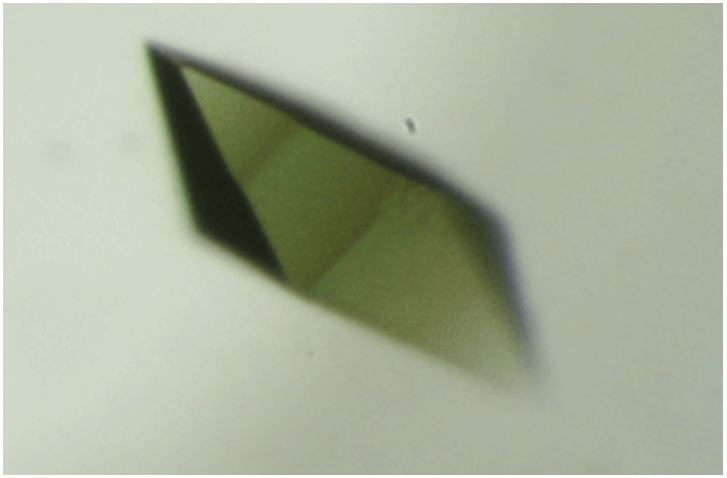
- 3D printed
- channel size: 300x300 µm
- sample consumption: 5 µl/h
- delay times: 100 ms – 3 s





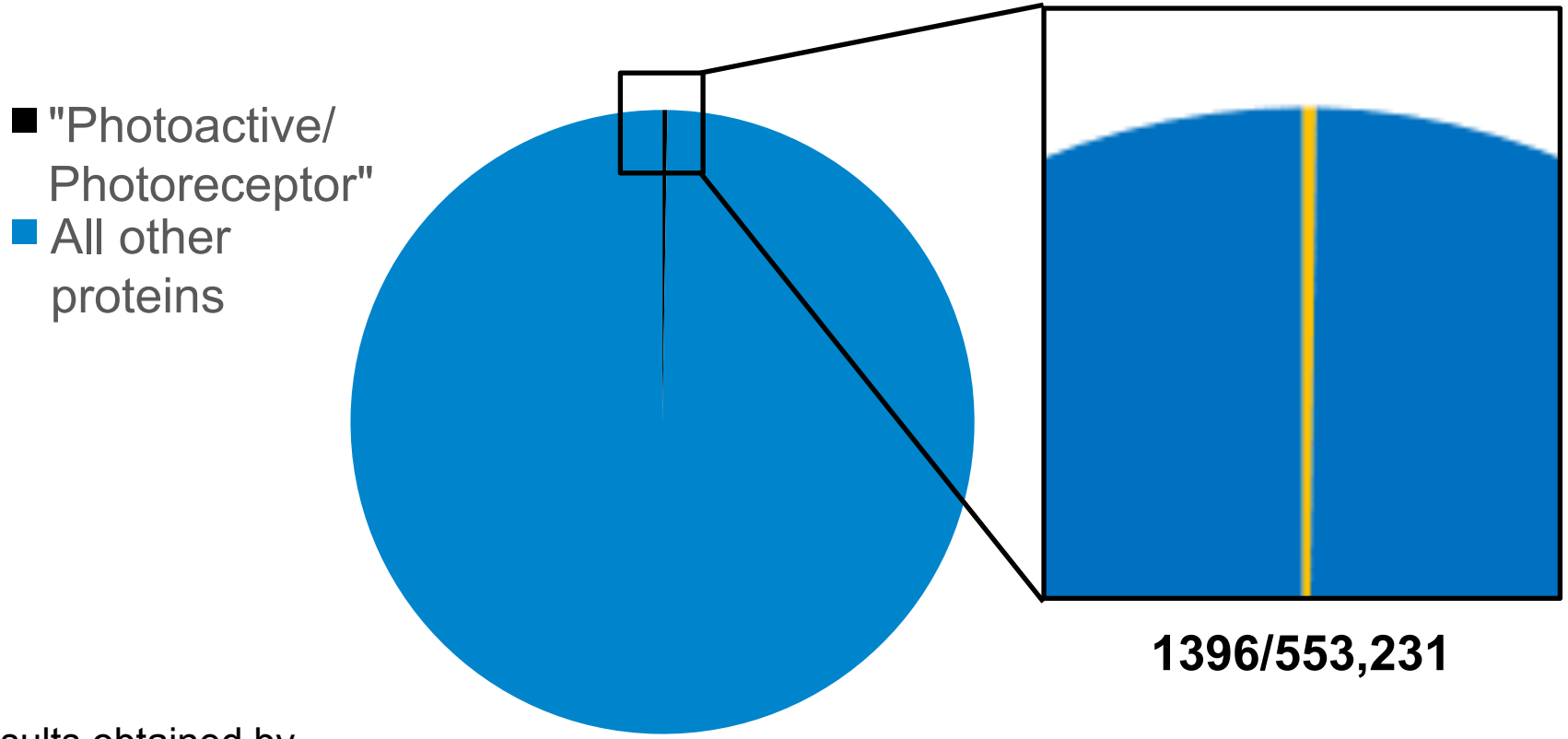
Monteiro et al. (2020) IUCrJ **7** 207-219

- **Achievable time-resolution in mixing depends on the critical depth**
  - This defines the maximum distance a ligand can diffuse for the diffusion rate to still be faster than the process you're interested in
- Depending on the reaction rate of the species you are looking at AND the buffer conditions this can be extremely variable



- Using small samples and rapid mixing methods time-resolutions ~ 1 ms can be achieved.

- Fast reactions (< ms) we need to trigger by light
  - T-jump via IR pulse (ns)
  - Photoisomerisation/direct photocleavage (few fs)

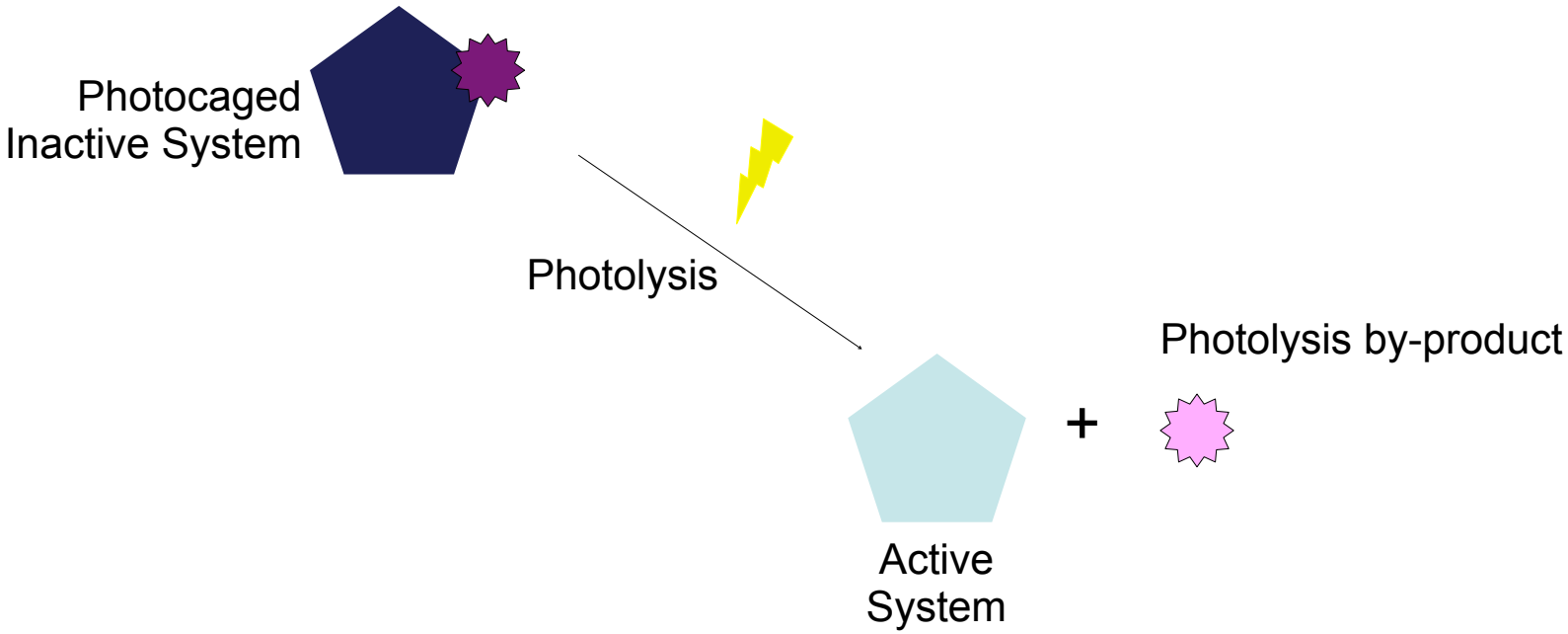


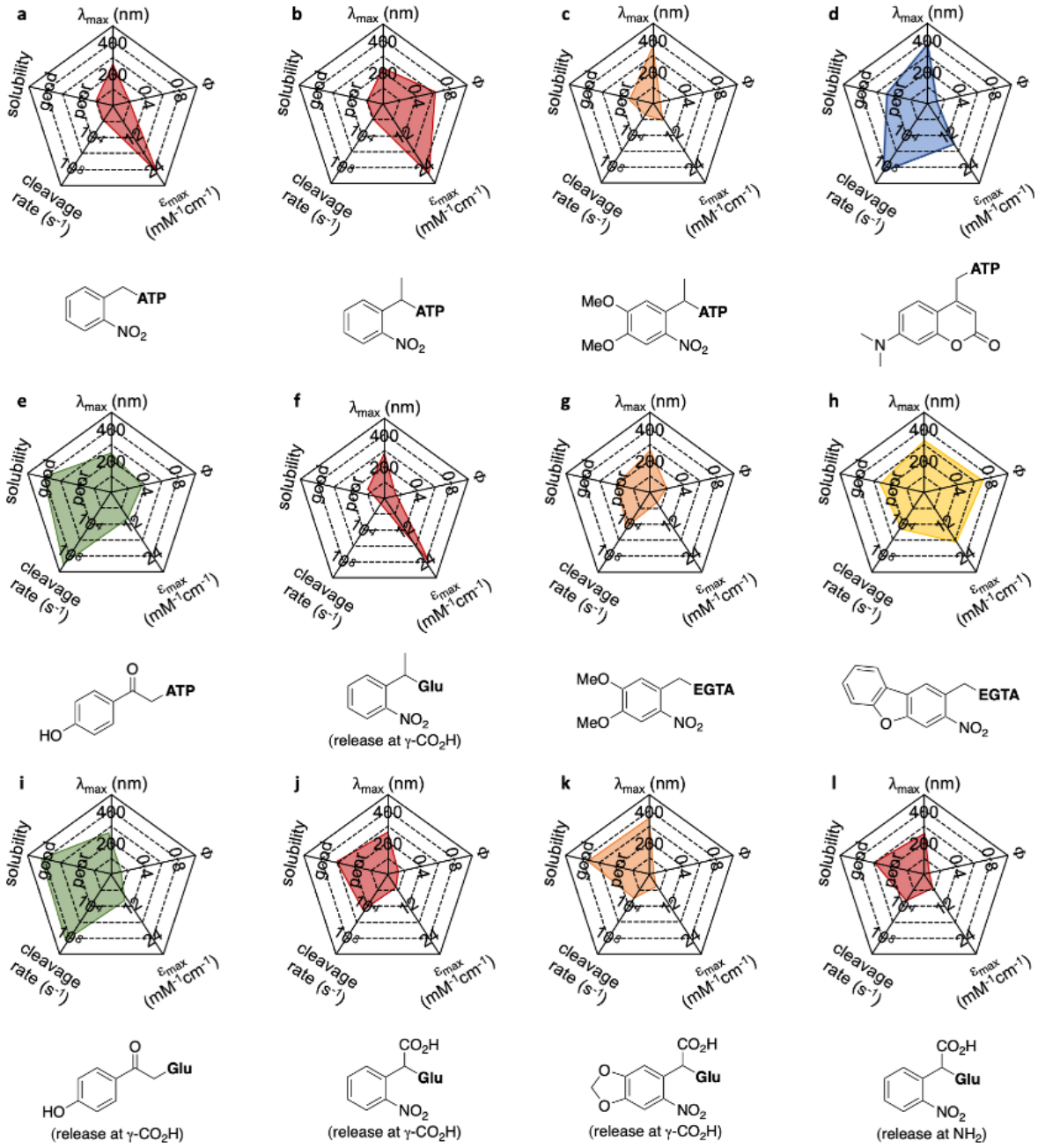
Results obtained by searching manually curated SwissProt entries for keywords “photoactive” or “photoreceptor.”

By approximation, only 0.25% of proteins are photoactive!!

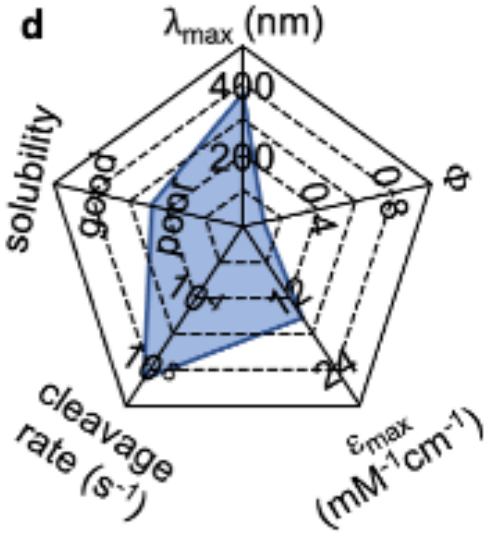
Mike Thompson, UCSF

- Fast reactions (< ms) we need to trigger by light
  - T-jump via IR pulse (ns)
  - Photoisomerisation/direct photocleavage (few fs)
  - Photocaging (ns-ms)





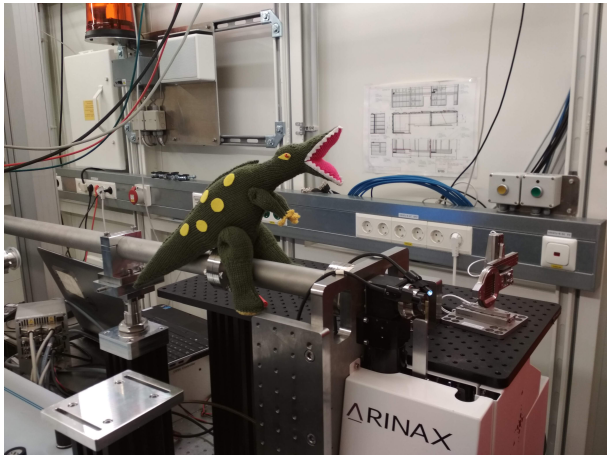
- Choosing a photo cage means balancing a set of properties

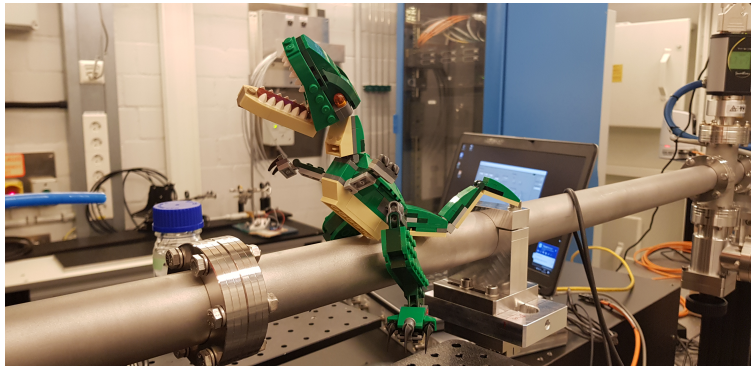


Diana Monteiro, CCP4 SW 2020

Final practicalities

- How much data do I need?
  - Recommend 5-10k diffraction patterns for a good quality dataset
    - Better to have fewer time points and better data than *vice versa*!
- Test, test, test
  - Take advantage of synchrotrons to test crystal quality
    - Cryo and RT
    - Use static soaks to check turnover
- Choose your weapon carefully
  - Synchrotrons and XFELs are complementary
  - Crystallography *might* not be the best approach!





T-REX