Collecting data on Microfocus beam lines

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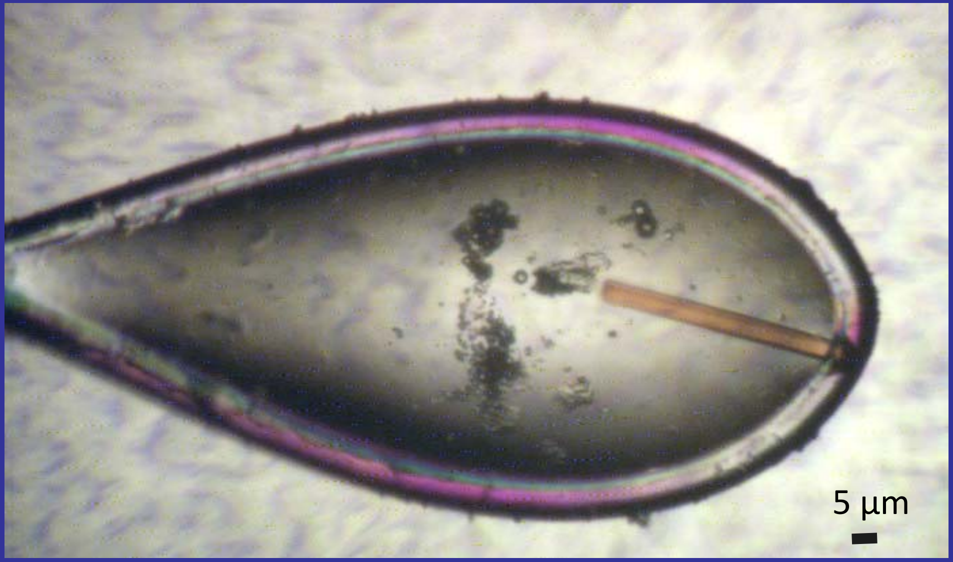
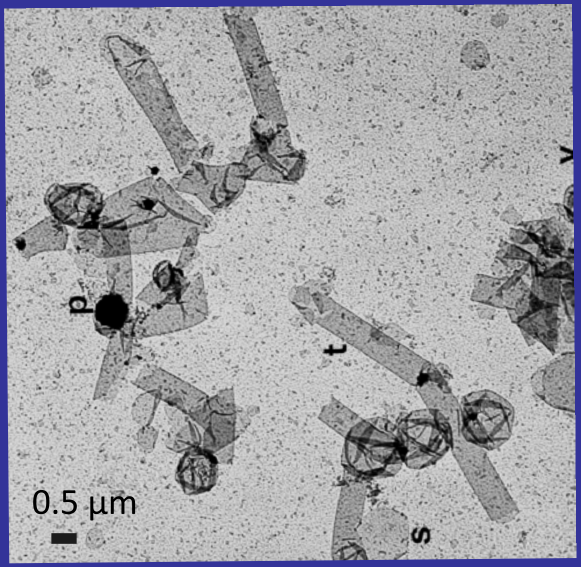
Why microfocus?

Rhodopsin; 3D and 2D crystal comparison





3D

2D

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Microfocus is useful for difficult targets

Membrane proteins; our targets are eukaryotic

- difficult to express
- low yield
- difficult to purify, need detergents and lipids
- difficult to crystallise
- lipid cubic phase

But crystallisation robotics now give more chances with less protein

Microfocus is useful for difficult targets

Rhodopsin;

- smaller crystals diffracted better than large ones
- first good diffraction found on ID13 in 1997
- general tendency for thin needles, anisotropic in needle direction

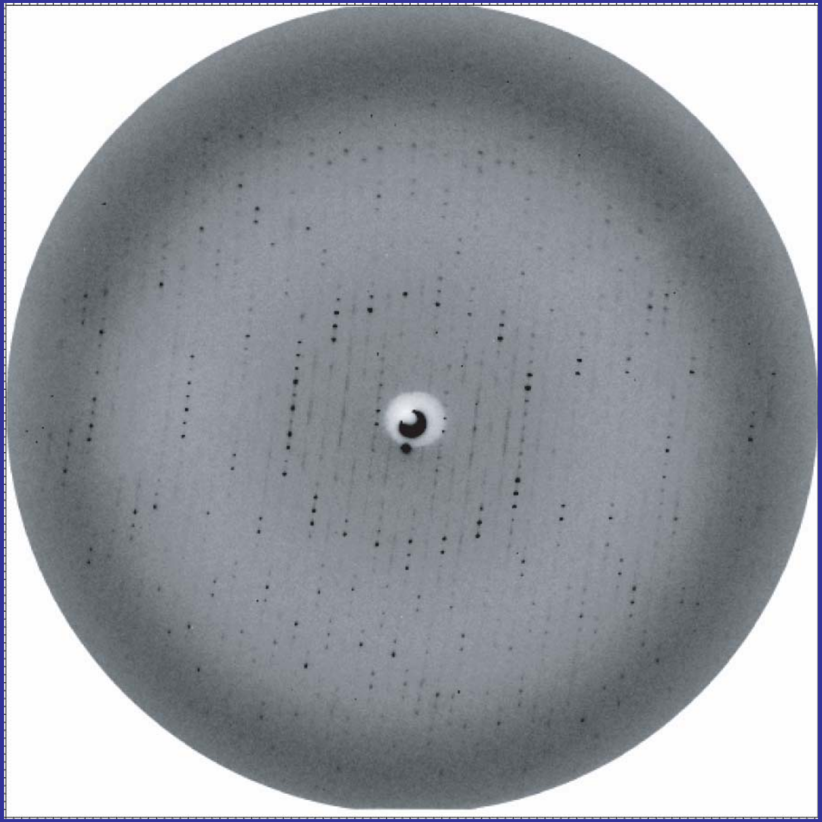
Collecting data on Microfocus beam lines

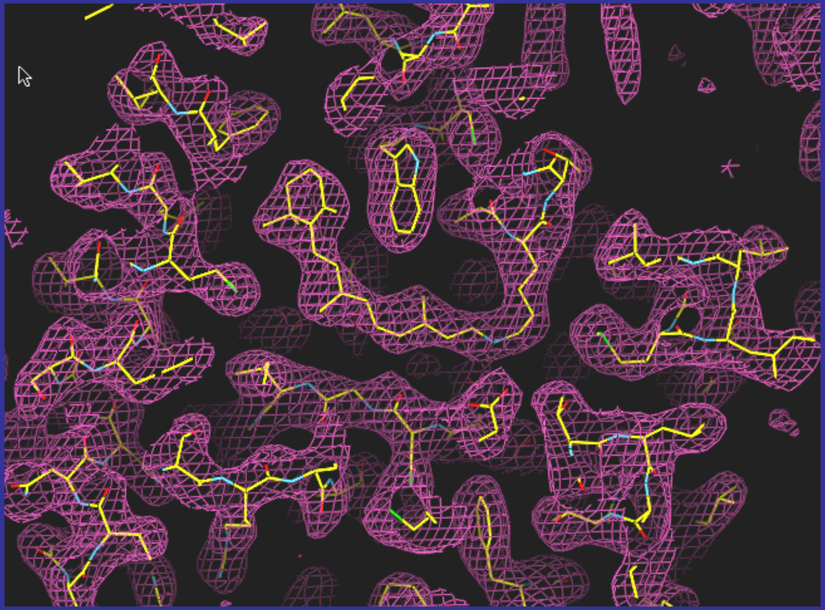
ID13; Manfred Burghammer, Christian Riekel

ID23-2; David Flot, Sean McSweeney

The GPCR bovine rhodopsin - 1999

first good diffraction with 10μm beam

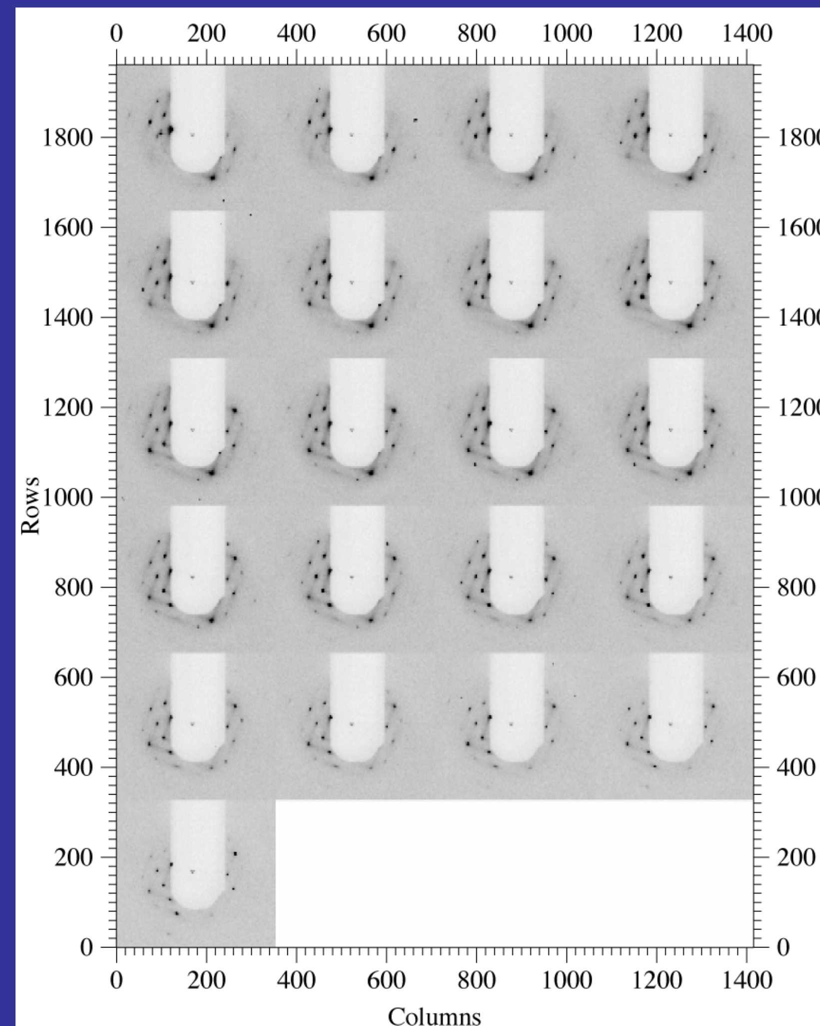




Li *et al*, JMB 2004

Human beta2 adrenoceptor micro crystal
ID13 5μm beam

Initial hits



Brian Kobilka, Stanford

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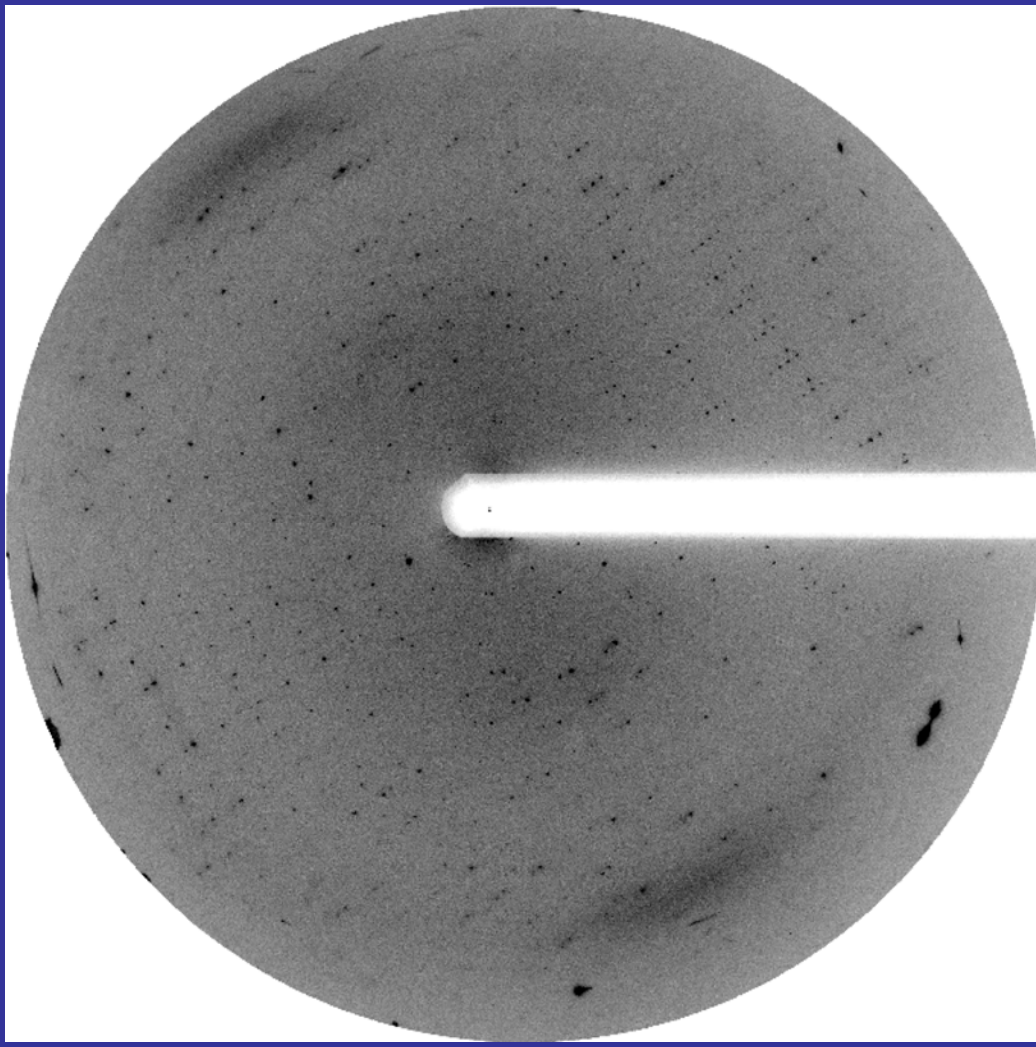
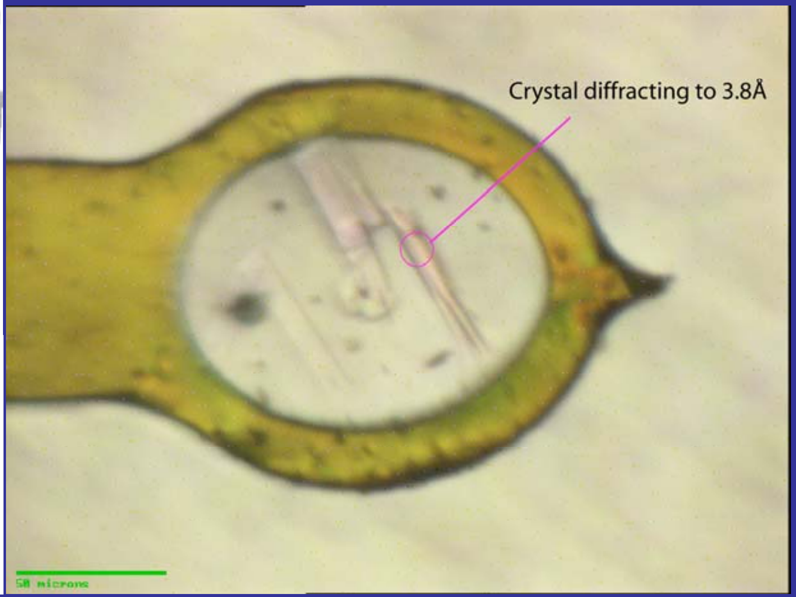
FAB-beta2 complex

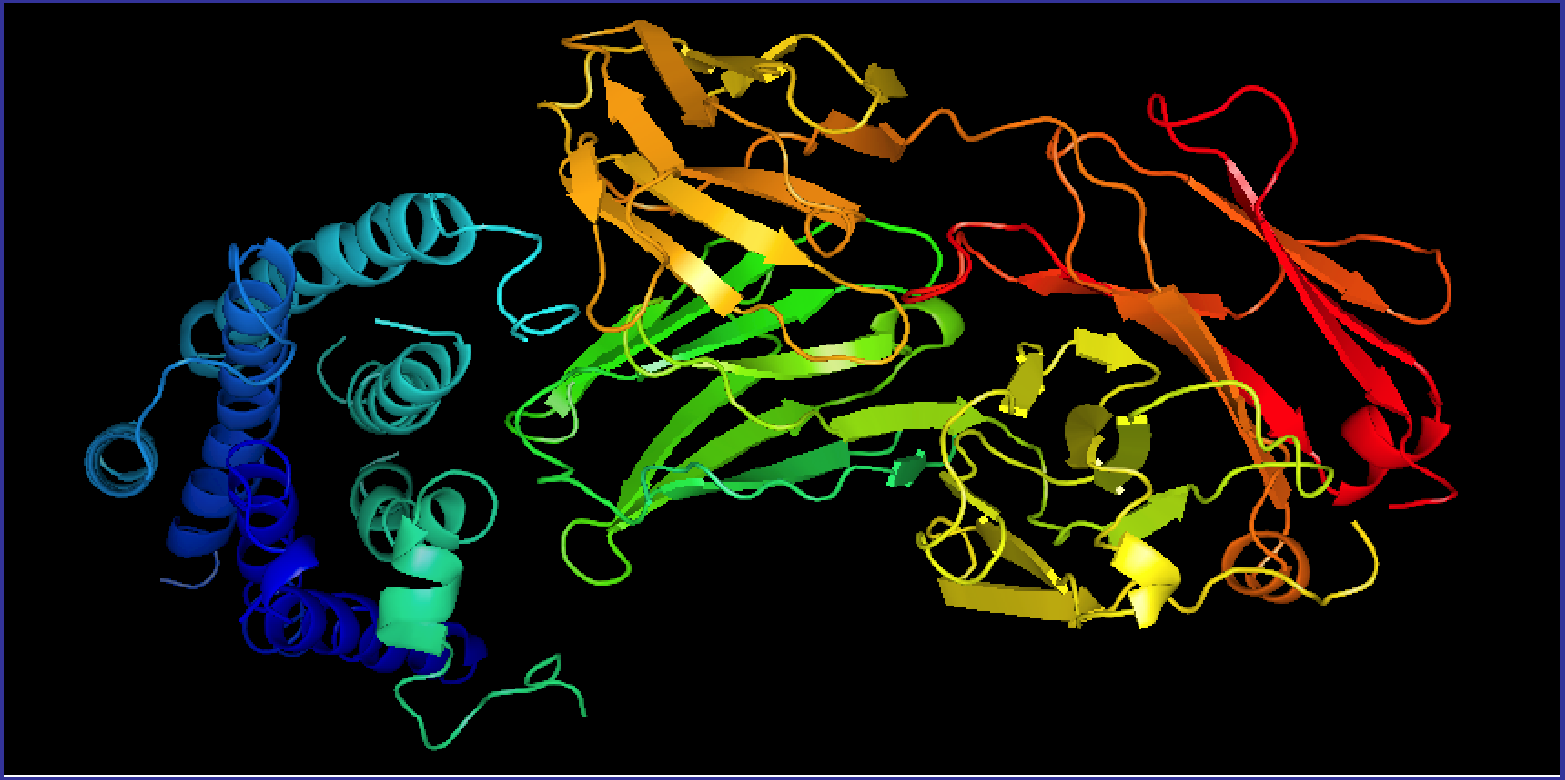


Diffraction is slightly better than 3.8 Å



Fab-beta 2 crystal in loop

Structure of human beta2 in complex with the antibody fragment



Rasmussen *et al*, Nature 2007

Microbeams improve signal to noise by excluding solvent diffraction

Beam size; 200 µm vs 10 µm

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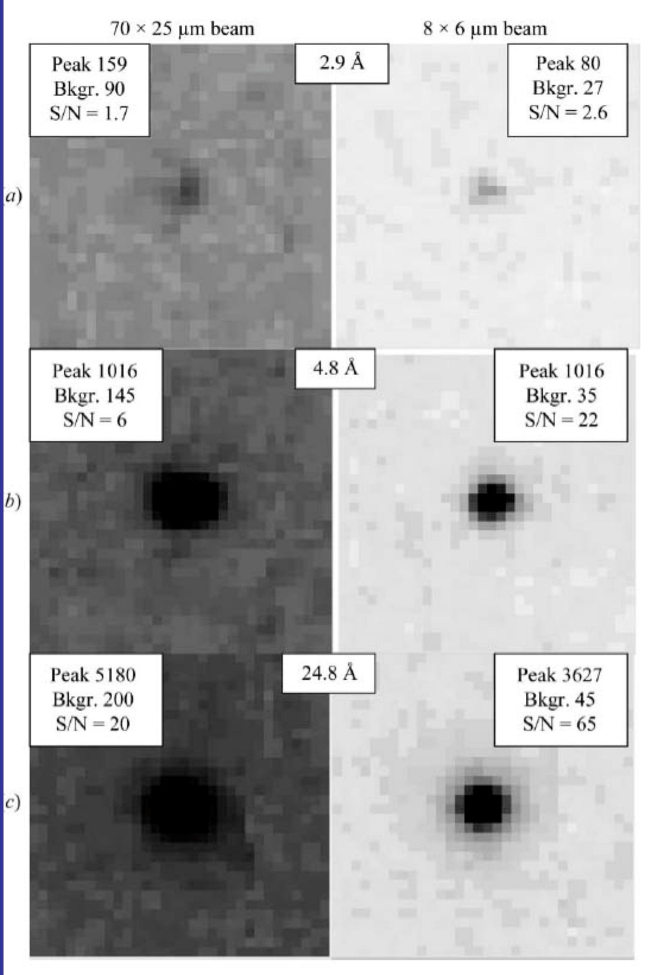
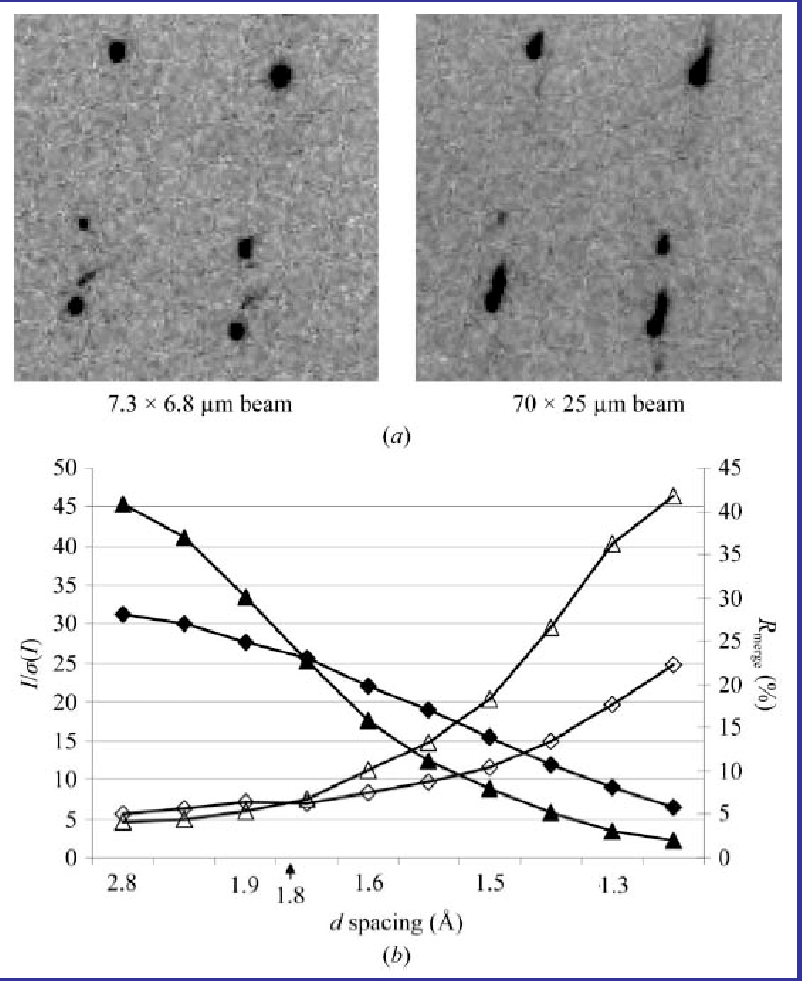
Small vs large beam

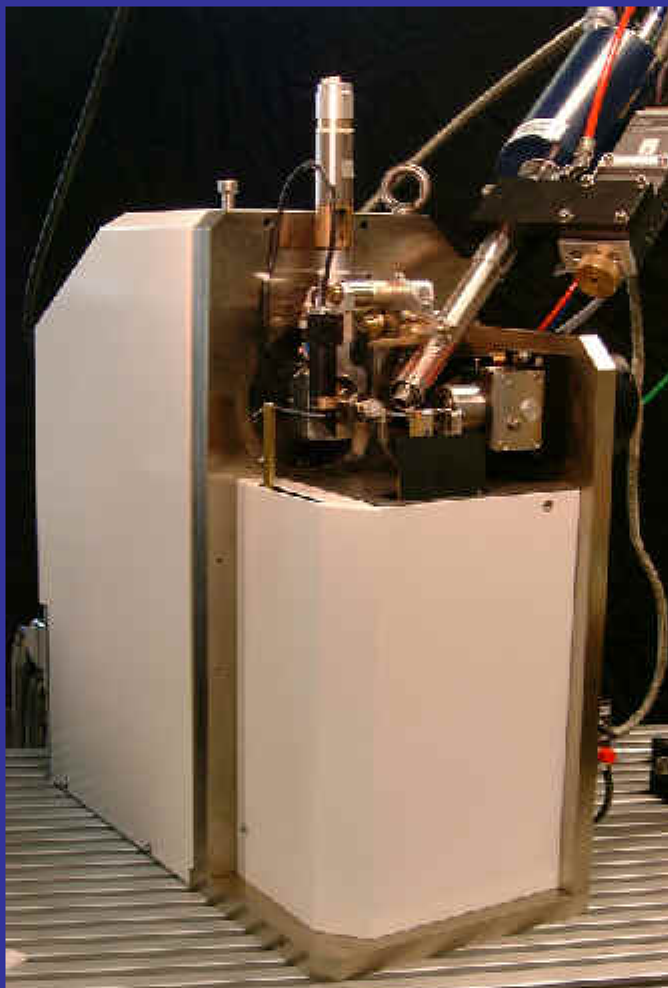




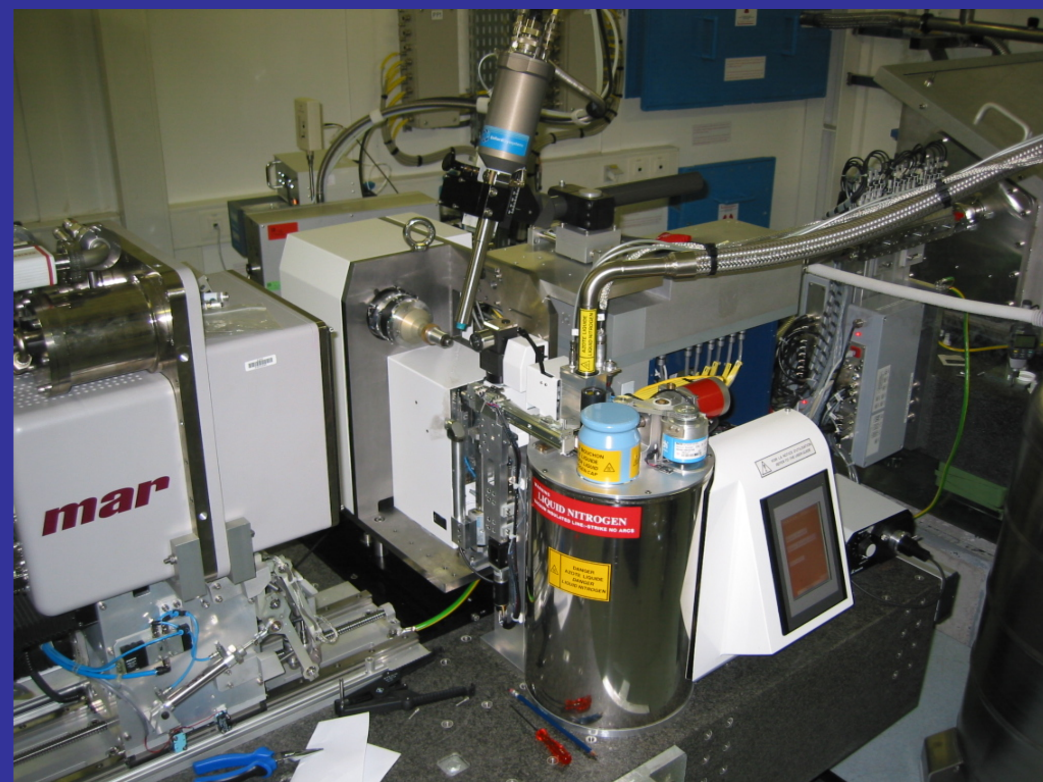
Sanishvili et al, Acta Crys. D 2008

Microfocus beam line set ups

ID13/EMBL

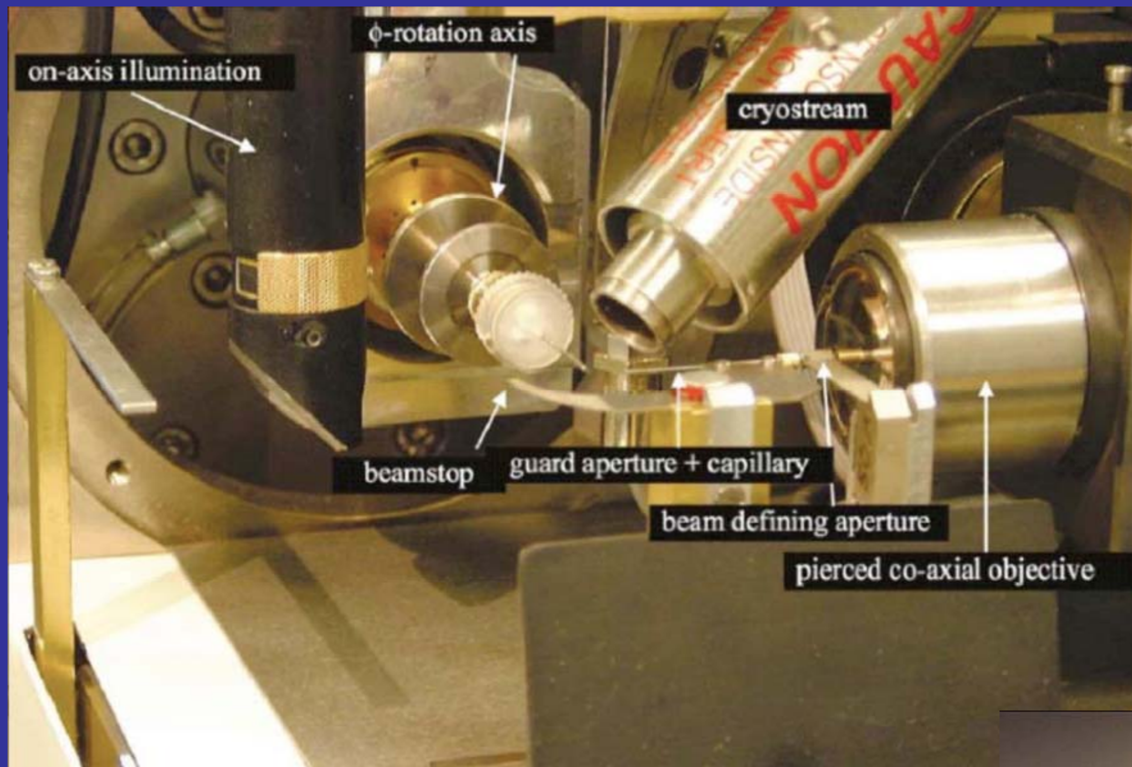


ID23-2



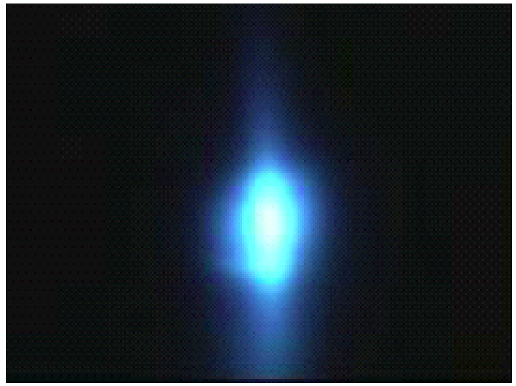
Cusack et al, 1998 / Perrakis et al, 1999

Sample environment

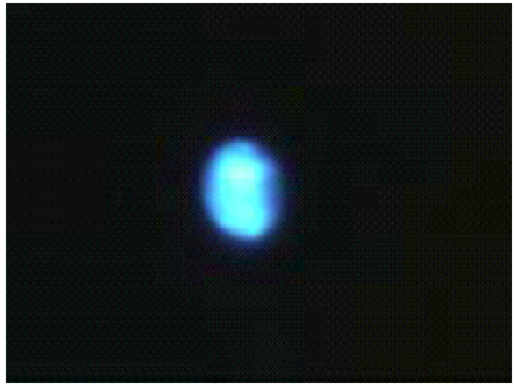


ID13; showing first micro-diffractometer

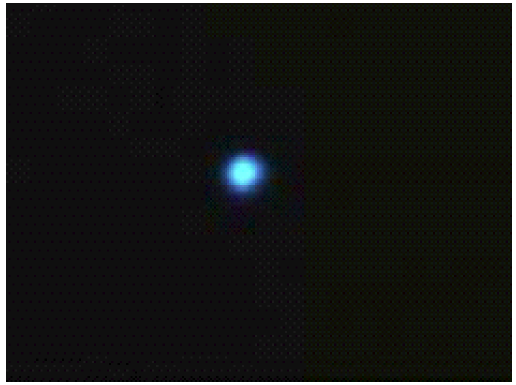
Beam shaping with guard tube and aperture



Focussed beam
No shaping



100 µm cleaning aperture



30 µm definition aperture +
100 µm cleaning aperture

Detectors

- Mar CCD

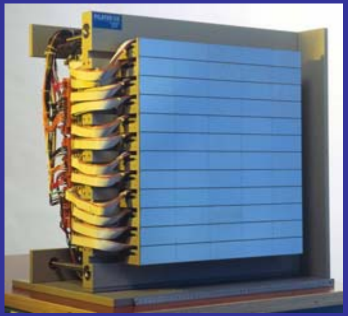
  ID13, ID23, SLS

- FreLon

  ID13

- Pilatus - Pixel detector

  SLS



Important to have fast readout, especially for scanning procedures

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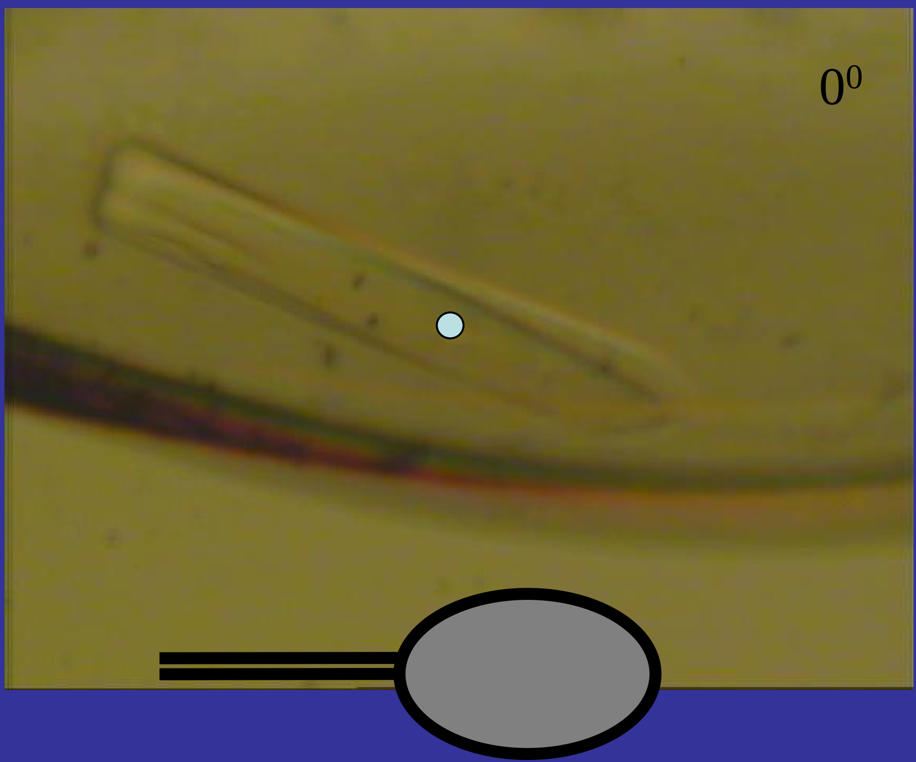
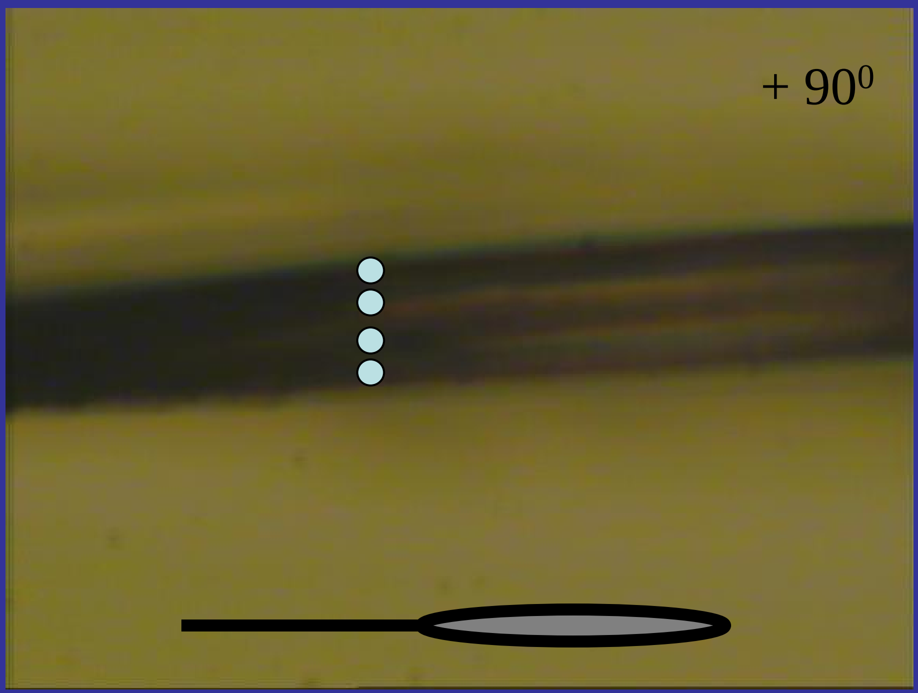
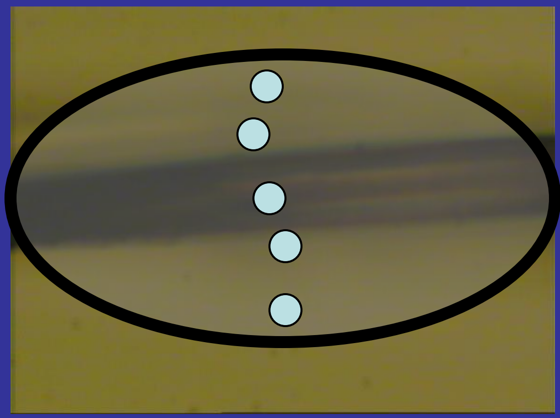
Practicalities- centring crystals is essential with microbeams





Lens effect creates a problem for accurate centring

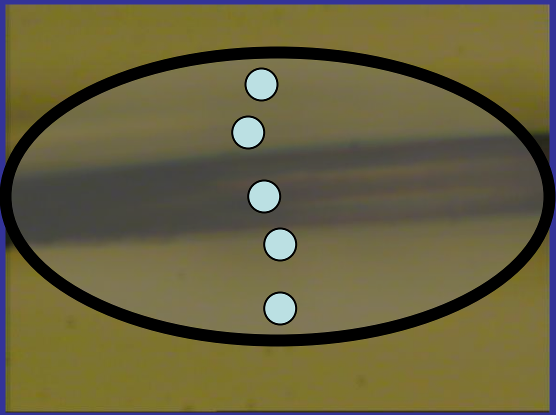


Sample optimisation

- Cryo buffers tend to create viscous solutions
- Must avoid big fat loops of solution

  especially in very small < 0.2mm loops
- Convex solution can causes stress on the needle
- Better to have larger loops

  with thin film very flat parallel to loop
- Use flatter loops such as LithoLoops or MiTeGen loops



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Benefits

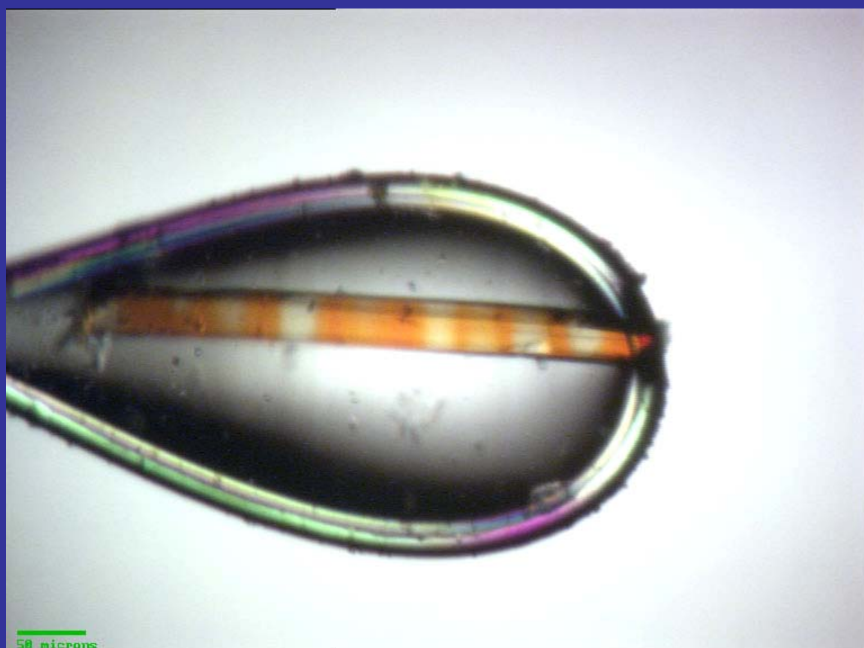
- Heterogeneous crystals - choose only best parts
- Many wedge opportunities
- Variable order in different positions leads to variable mosaicity
- Radiation damage; after a limited number of frames can change position
- Multiple crystals can be checked in same loop

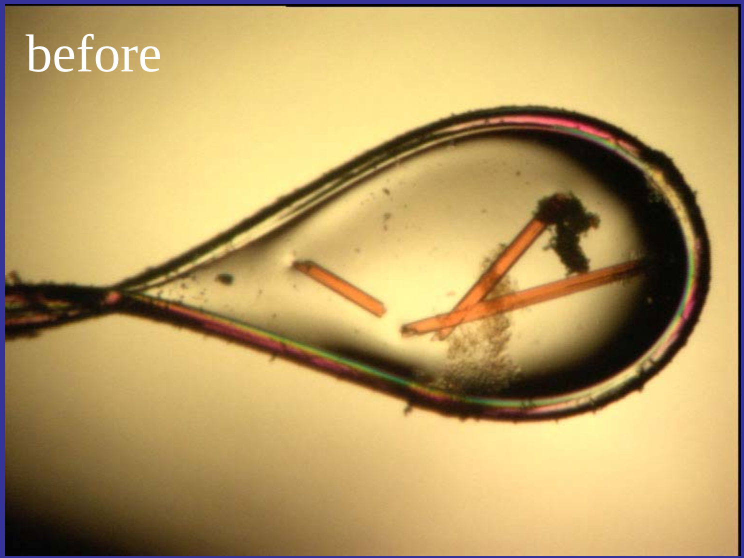
Problems

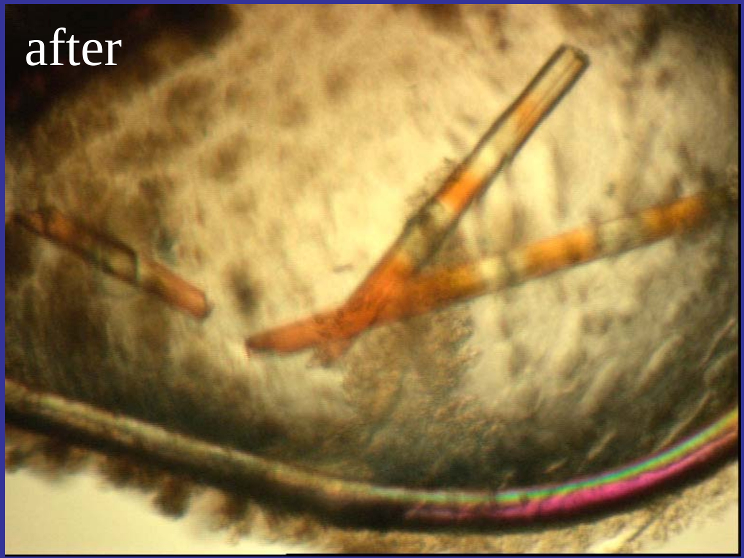
- Splitting
- Many independent wedges to merge
- Radiation damage
- Data anisotropy - weak diffraction due to misalignment
- Non -isomorphous
- Scaling problems

Localised beam damage

Bovine rhodopsin crystals



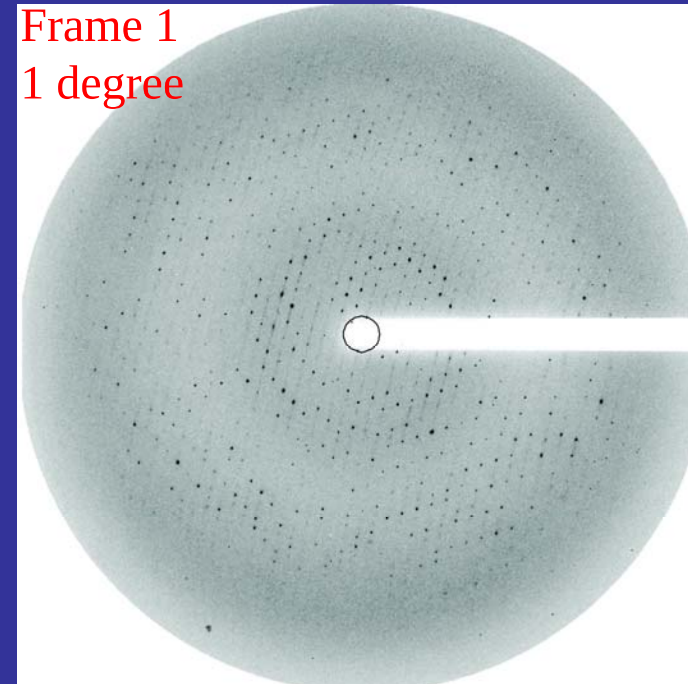


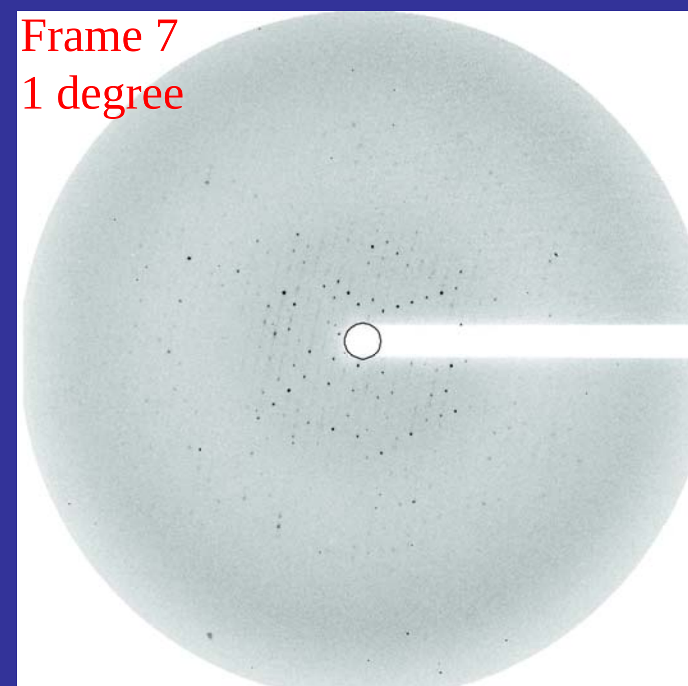


Data collection using ID13 Microfocus beamline

5 micrometer beam on a
5 micrometer recombinant rhodopsin crystal

12 data wedges were collected from
Different positions on a single needle





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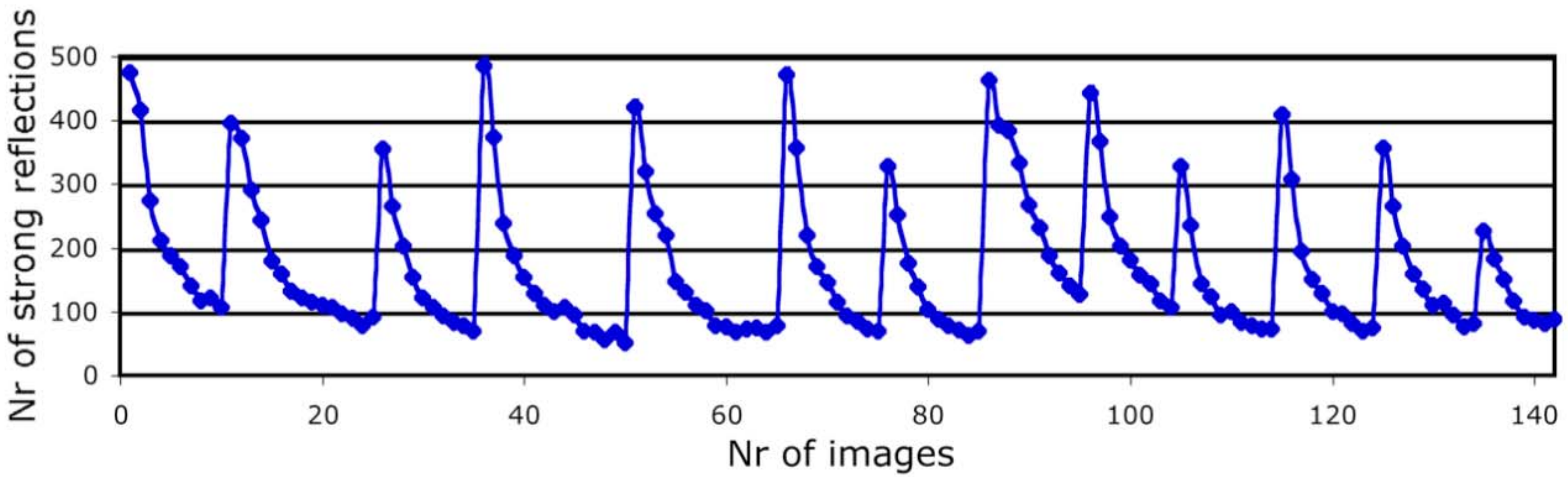
Merging of data wedges from different positions



- Merge as much data as possible without including too much radiation damage
- Find an acceptable compromise

Standfuss et al, JMB 2007

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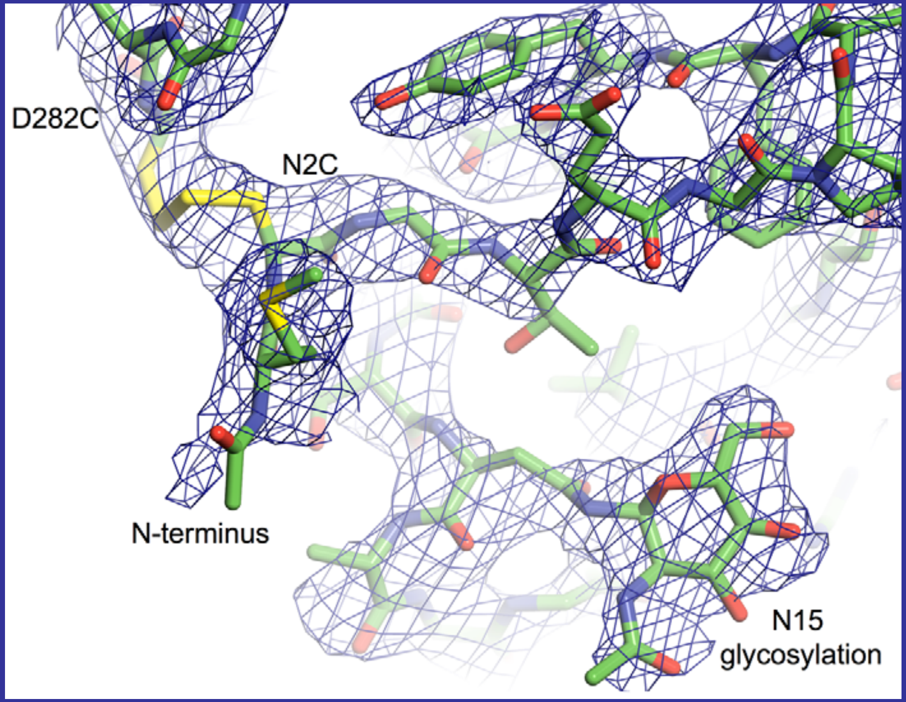
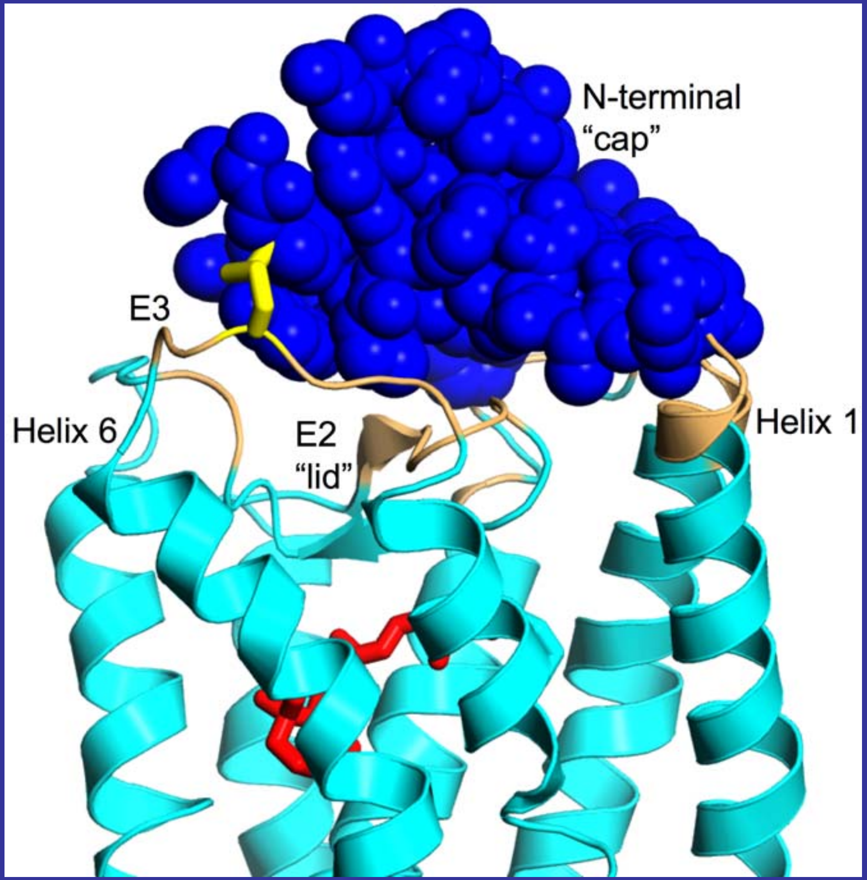
Opsin stabilising disulphide bond fixes the N-terminal cap over the lid



Radiation sensitive disulphide bridge is visible



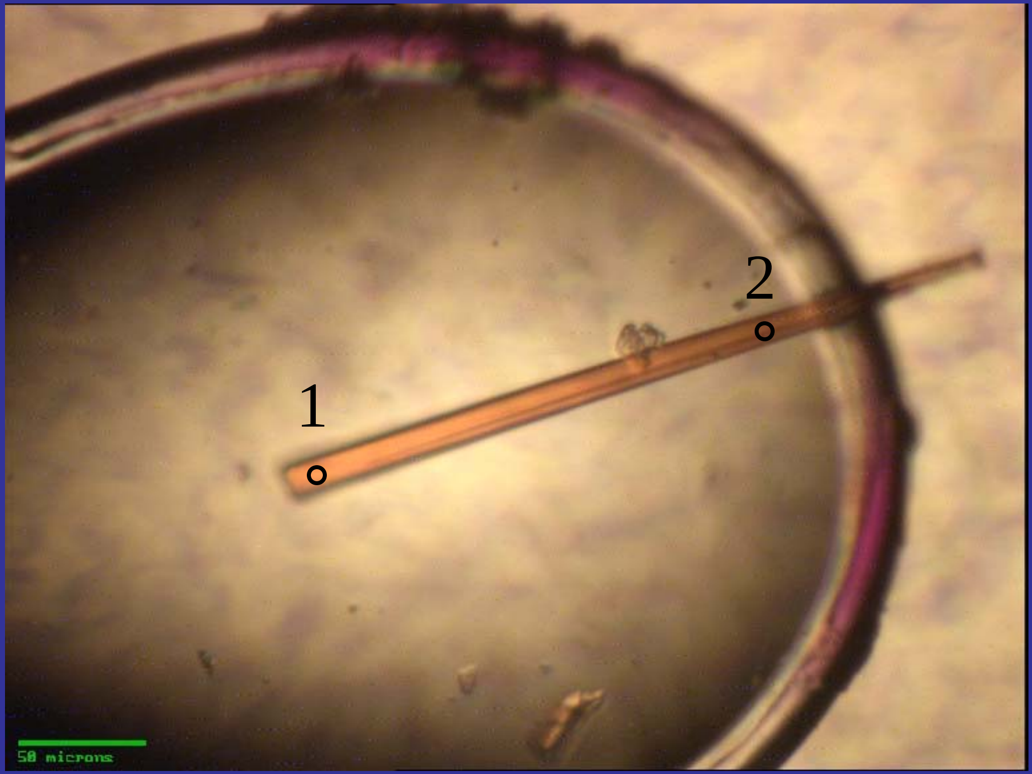
First structure of a recombinant GPCR

Standfuss et al, JMB 2007

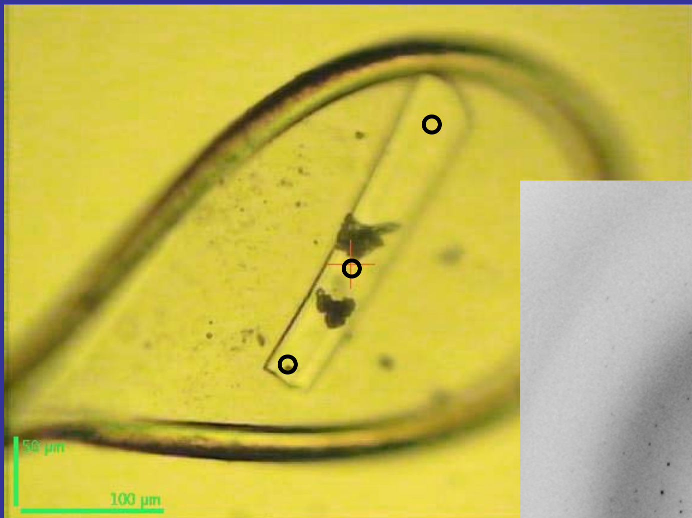
Macros to automate collection

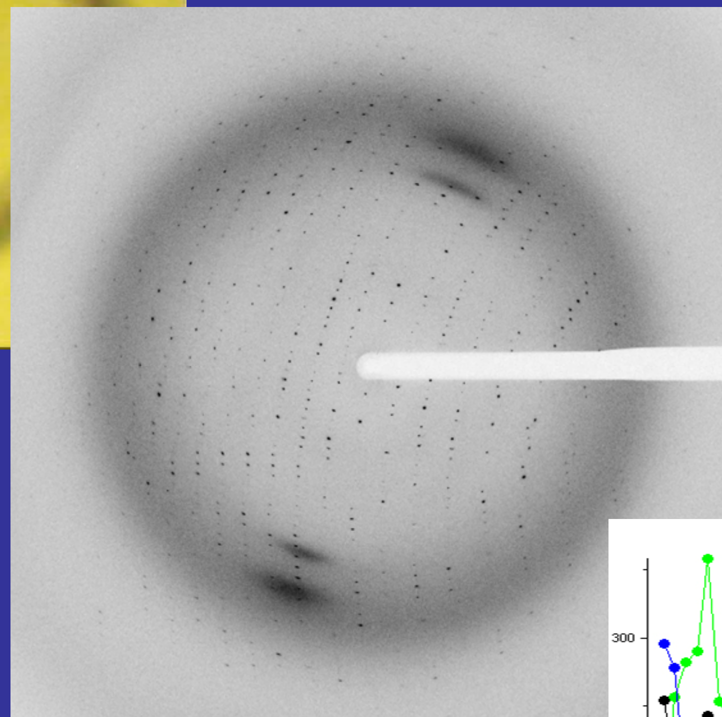
ID23-2- David Flot

Macro for collecting data along a needle -'helical data collection'.

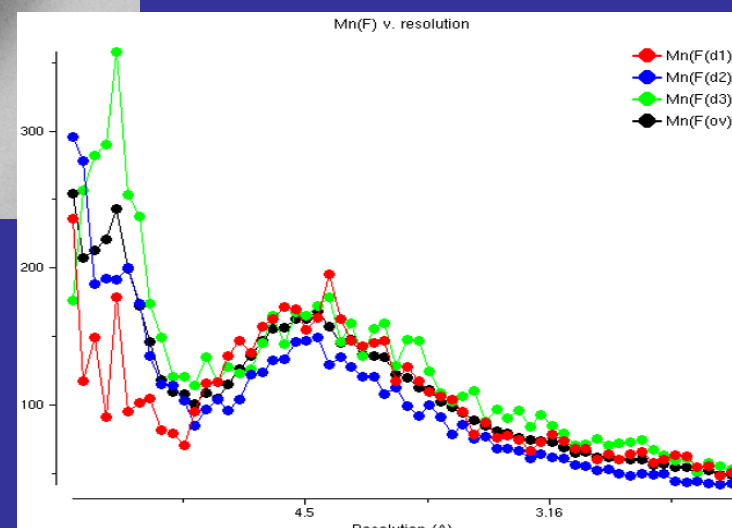


Beta1 adrenergic receptor; data collection at ID23-2





Data isotropic !



Space group P1
Cell parameters: a=55.5Å, b=86.8Å, c=95.50Å
α=67.60, β=73.30, γ=85.80

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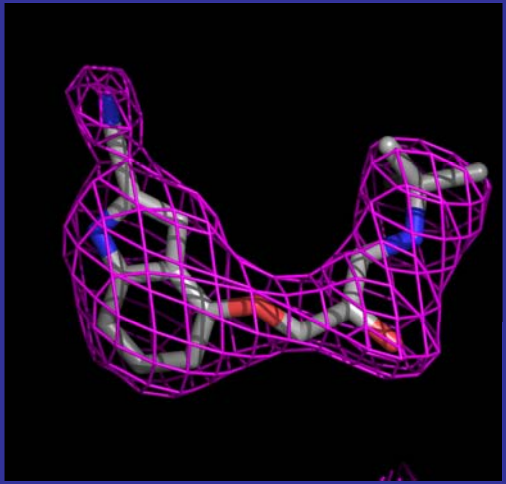
Structure of Beta1 adrenergic receptor

Ligand - beta blocker
Cyanopindolol



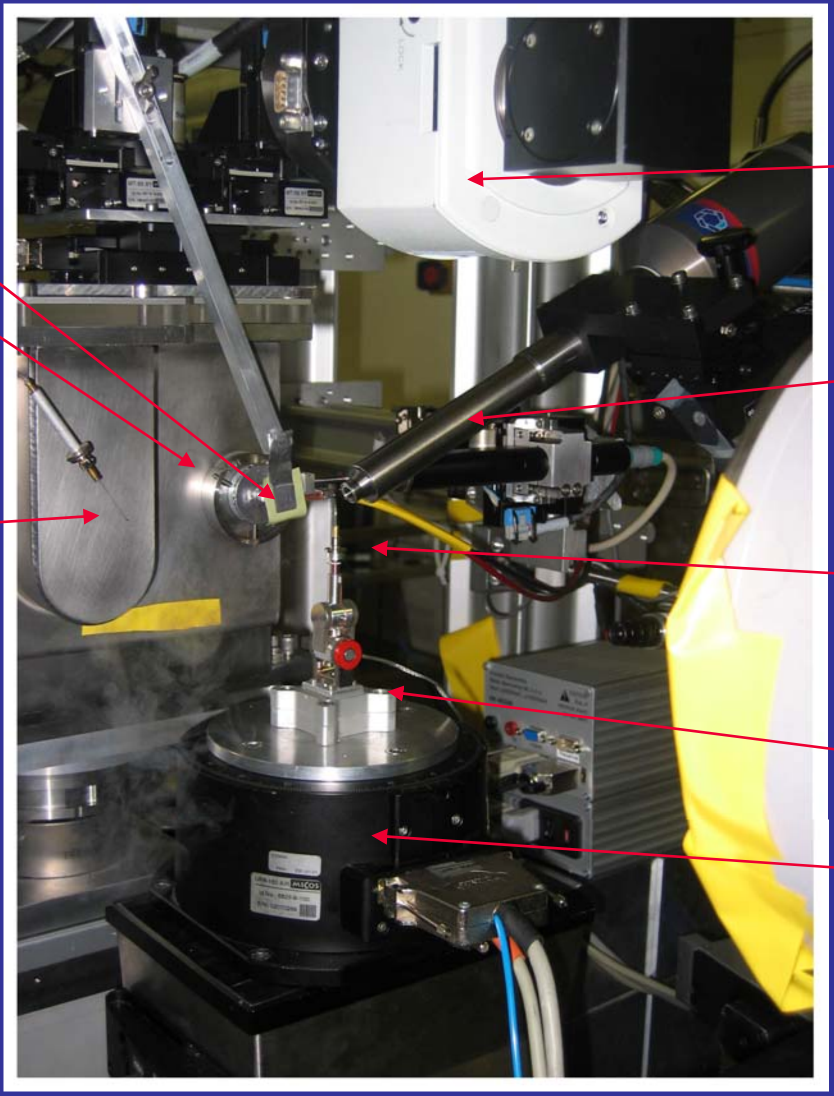
Warne et al,
Nature 2008

The future…

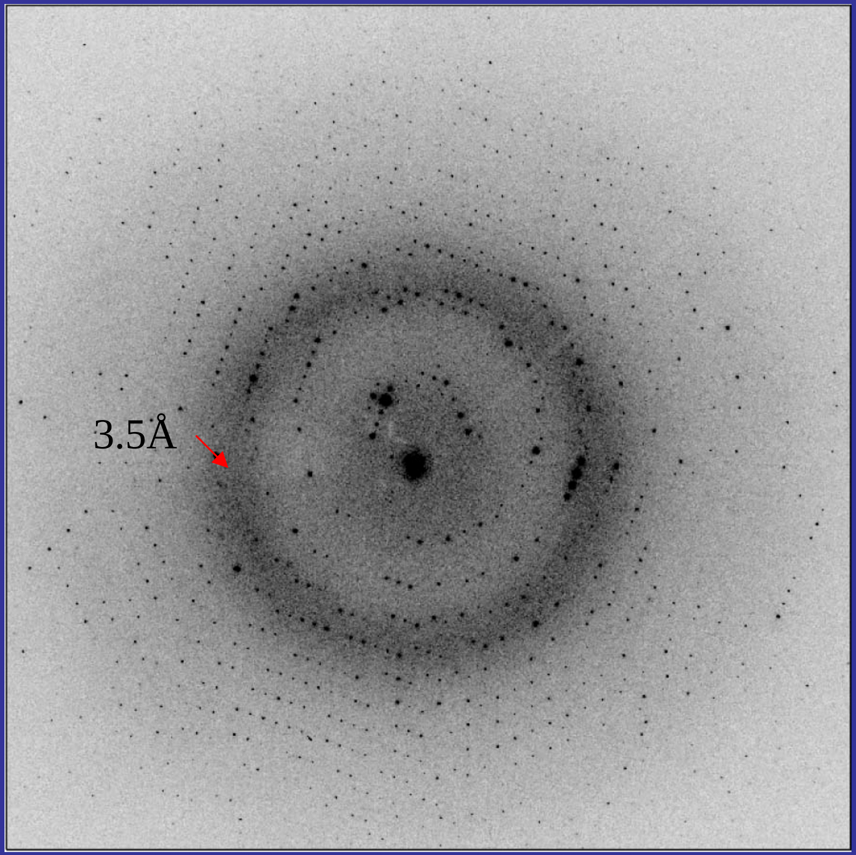
- Nano beams
- Scanning procedures
- Random data collection

Scanning micro-diffractometer

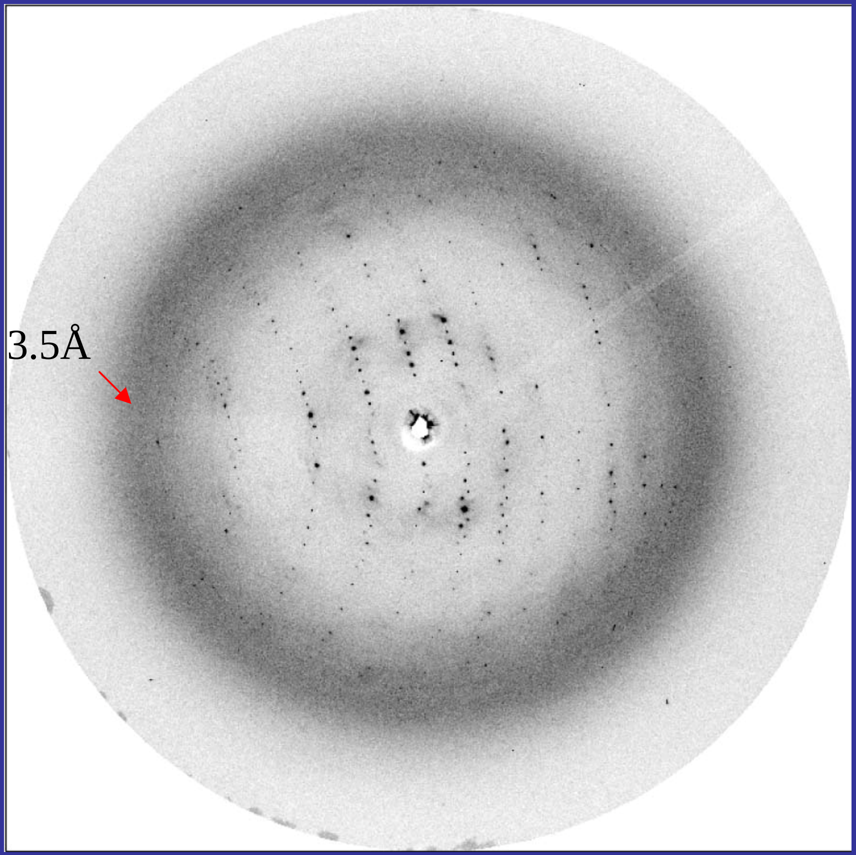
1 µm beam set up on ID13



1µm patterns at ID13

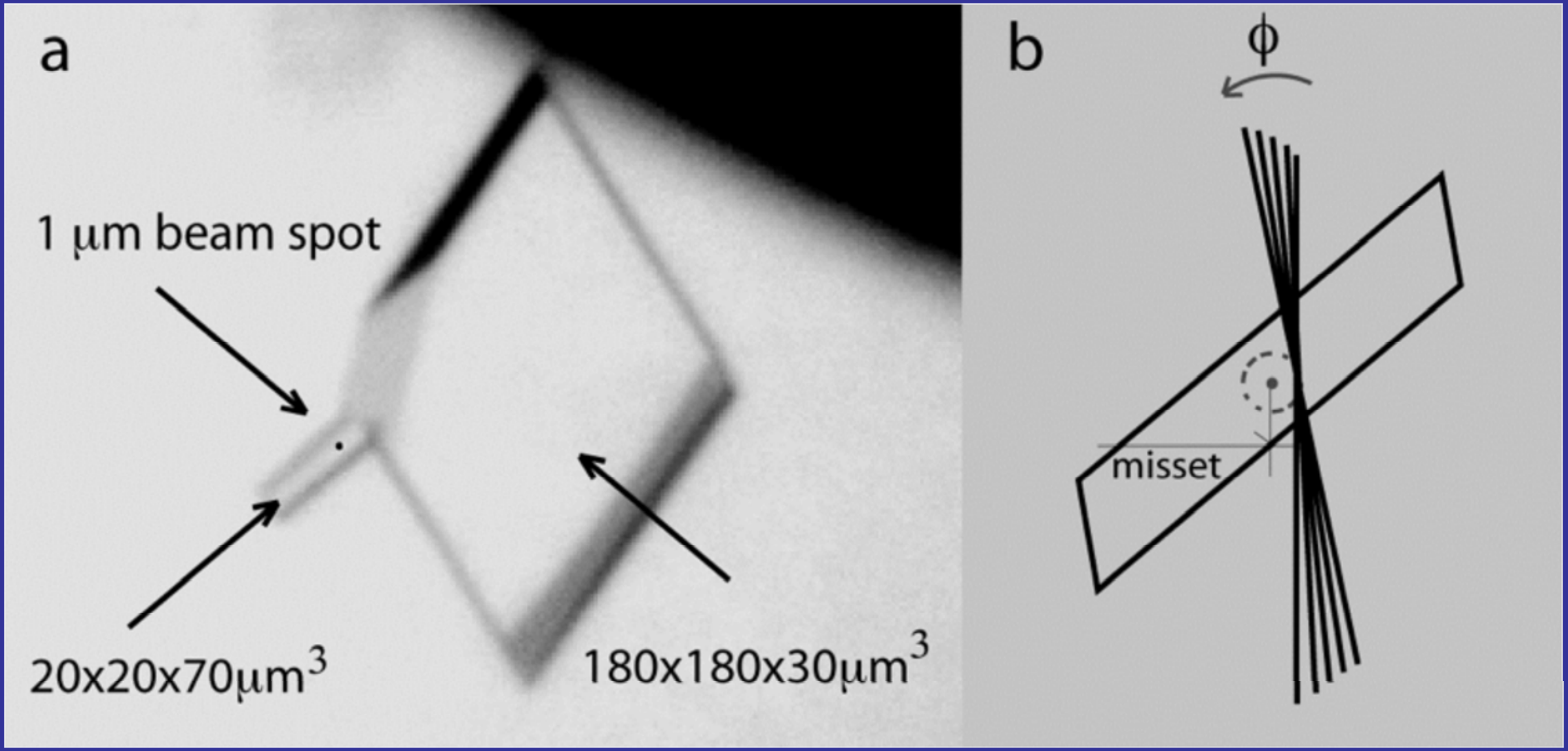


Lysozyme



Beta1 adrenergic receptor

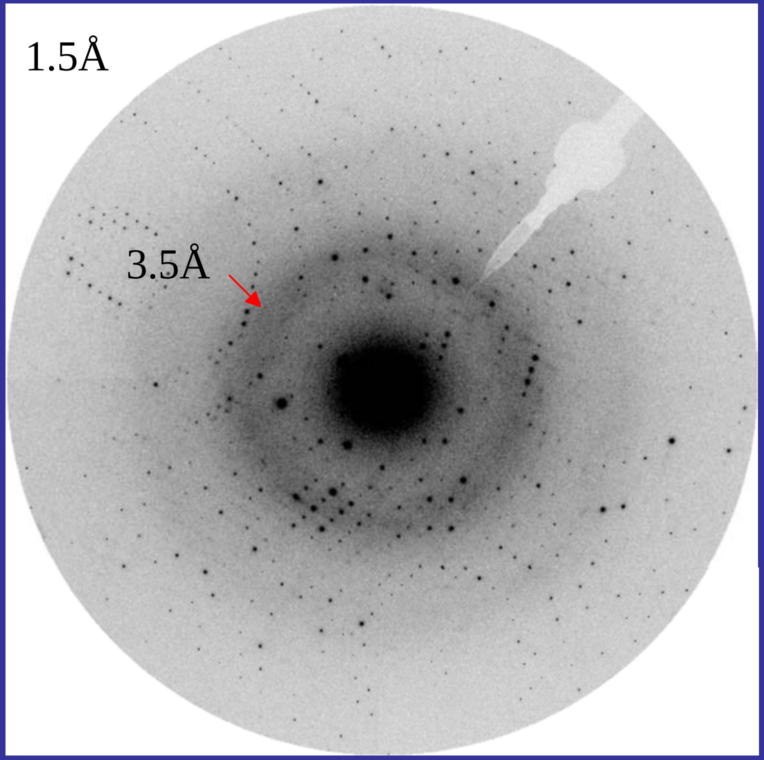
First structure with a 1µm X-ray beam at ID13

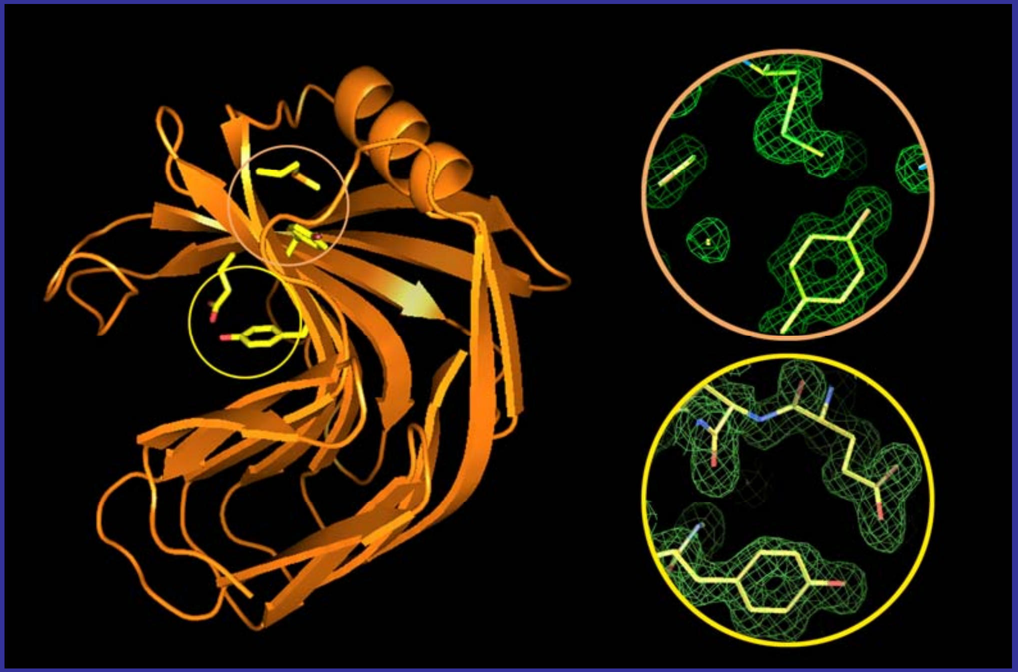


Xylanase

Moukhametzianov *et al*, Acta. D 2008

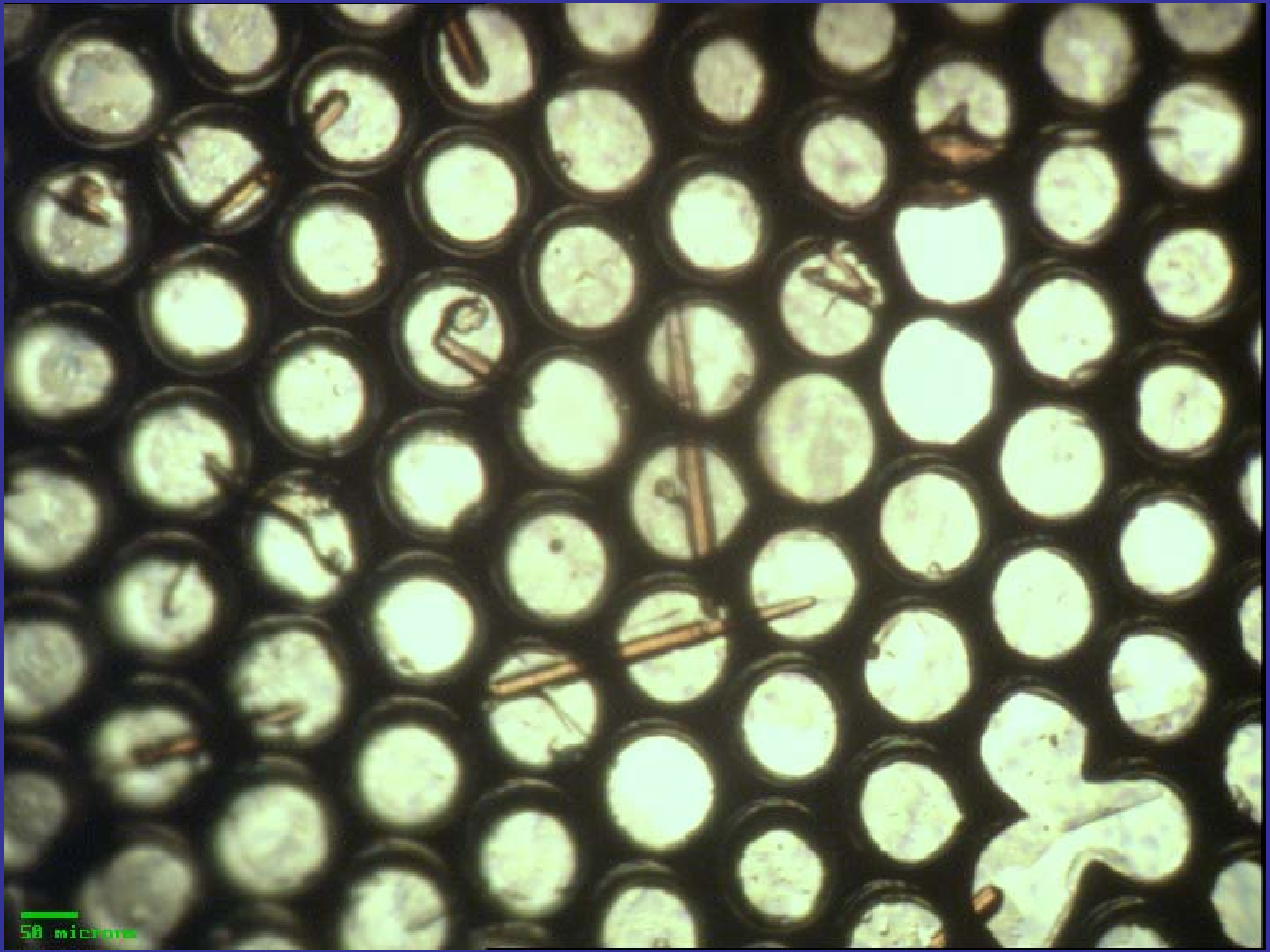
Structure of Xylanase with a 1µm beam





Moukhametzianov *et al*, Acta. D 2008

Carbon grid mount with rhodopsin crystals



Carbon film support for x-ray diffraction of protein crystals

Acknowledgements

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Gebhard Schertler

Jade Li - Bovine rhodopsin

Joerg Standfuss, Dan Oprian - Rhodopsin mutant

Brian Kobilka - Human Beta 2 adrenergic receptor

Tony Warne, Maria Serrano, Chris Tate, Andrew Leslie - Turkey Beta 1 adrenergic receptor

Rouslan Moukhametzianov, Maikel Fransen - Xylanase

ESRF:

ID13 Manfred Burghammer

ID23-2 David Flot

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