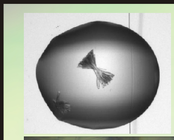
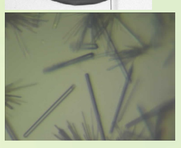
Protein Crystallisation at York
ongoing developments & personal stories

Shirley Roberts (on behalf of the group)

York Structural Biology Laboratory

X14 Carbohydrate binding domain

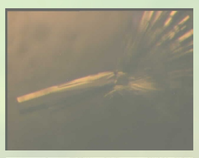




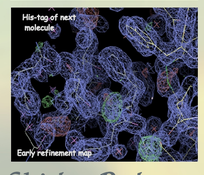
First crystals obtained during evaluation of the JSCG screen (SPINE). No crystals grew in the Index screen's identical solution. The JSCG condition was pH 6.5, Index was pH 7 as written. This "error" in pH was crucial to repeating the crystallisation!

Se-met protein, produced with DTT in buffers and TCEP to protect protein from selenium oxidation failed to crystallise, with or without seeding. Hg and Pt soaks did not produce derivatives.

Se-met. protein prepared without reducing agents did crystallise, the structure was solved by SAD. An internal disulphide bond affecting crystallisation contacts was found which the DTT probably disrupted.



However the his-tag binds in the carbohydrate binding region of the adjacent molecule so ligand binding using this crystal form is unlikely to be successful.



Shirley Roberts

Dehydration of crystals to improve diffraction

Mil (*M. Thermoautotrophicum* MP4-like protein)

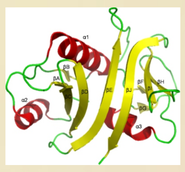
Solubility of this protein is sensitive to temperature and salt concentration.

- Increase and solubility improves.
- Decrease and protein precipitates.

Crystals grew in Peg 3350 or 2kMME, protein was 11mg/ml in buffer plus 0.5M NaCl. Crystal diffraction was only to 7Å.

Some crystals diffracted to 3Å when 2.1M NaCl was used with mother liquor and protein buffer in the cryoprotectant, but crystals split and cracked in this cryo.

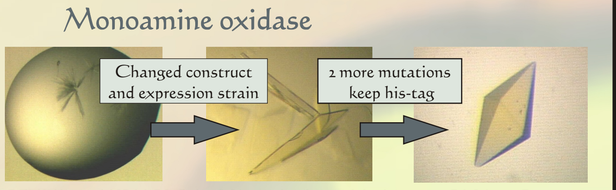
The quality of the crystal diffraction improved dramatically after 2 hours partial dehydration over mother liquor including 2.1M NaCl. Crystals could then be cryo-cooled straight from the drop (Ng et al, reference 1).



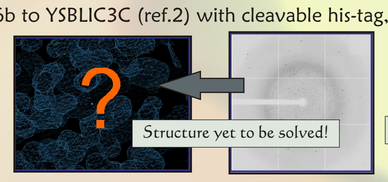
Leong C.Ng

When at first you don't suceed... keep trying!

Monoamine oxidase



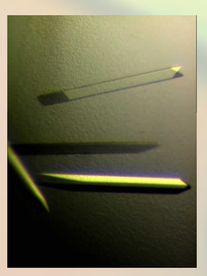
PET16b to YSBLIC3C (ref.2) with cleavable his-tag, BL21 to Rosetta.



Kate Atkin

A glycosyl transferase

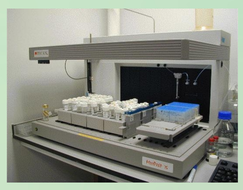
GTM had to be expressed as a GST fusion protein but precipitated when the GST tag was removed. The intact fusion protein was plated out in crystallisation trials together with low levels of protease (enterokinase) to cleave off the tag. Crystals grew which were optimised and data collected.

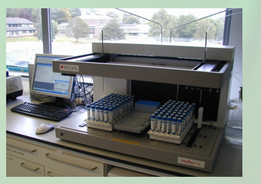


Carlos Martinez Fleites

Facilitating Protein Crystallisation: The York Setup

Mixing and dispensing of crystallisation screens



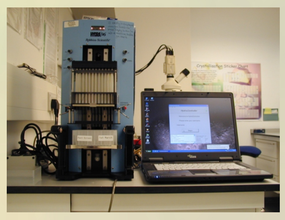


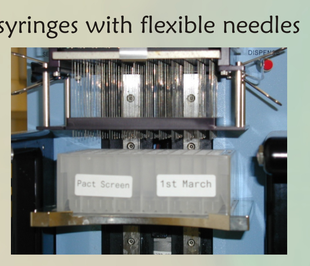
Single and Dual arm miniprep robot (Tecan UK Ltd)

- Stocks for mixing home-made screens are made from our own laboratory shelf chemicals and colour-coded according to the screen to be mixed.
- Customised lids with rubber inserts allow needle entry with minimal evaporation.
- Hampton 1 (ref. 3) and Hampton 2, CSS 1 and 2 (ref. 4), Peg-Ion, 25% Peg-Ion, Stura Footprint (ref. 5), A screen in development (based on Page et al, ref. 6) and a buffer screen, (ref. 7) are all prepared.
- The Index, Pact and JCSG commercial screens are bought in 10ml batches from Hampton Research and Molecular Dimensions Ltd and dispensed by the dual-arm Tecan.

Transfer of screen solutions to crystallisation plates

96 hamilton syringes with flexible needles

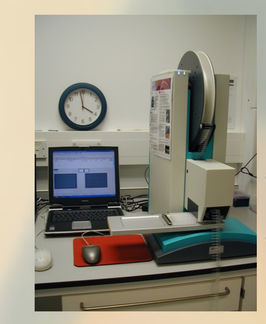


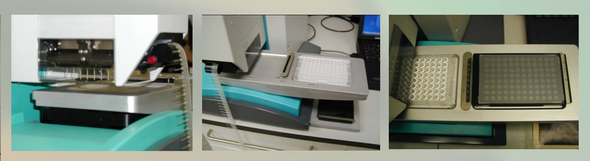


Favourite plate: MRC Wilden

Reconditioned Hydra (Alpha Helix, London)

Protein Crystallisation Trials





- Disposable needles lead to fast set-ups. Hanging and sitting drops are possible.
- Nanolitre drops 150nl+150nl and/or 150nl+100nl routinely used for screening. Larger drops can be used, up to 1.2µl per dispense, for optimisation.

Mosquito (TTP Labtech UK)

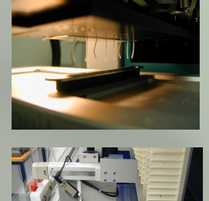
Crystal Growth monitoring and imaging

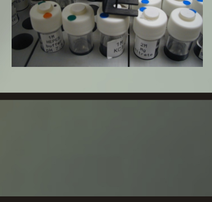
BioStore (BioTom, Evry France - taken over by RECIF Tech., France)

- Initial problems getting this robot working have only been solved by dedicated efforts of a senior technician at YSBL, Simon Grist.
- Users can chose to view the results of crystallisation trials from their computers, rather than hours at the microscope.
- YSBL is involved in implementing PiMS (data management from target to crystallisation, ref. 8) & ALICE (automated crystal recognition software, ref. 9).

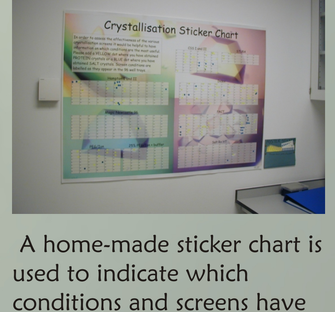
Robot Problems

Setting up the crystallisation robotics at York has not been without its problems and has required a significant technical commitment especially when robots arrive in a "develop on site" collaboration. However, thankfully, crashing needles and suicidal robotic arms are now a thing of the past at York - unless someone puts their tray in the wrong way round!





Screen Evaluation



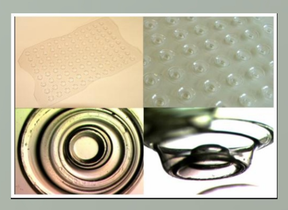
A home-made sticker chart is used to indicate which conditions and screens have grown protein (and salt!) crystals.

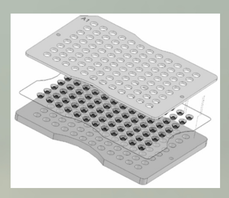
Shirley Roberts

A New Platform for Structural Biology

A microplate (ref. 10) has been developed designed to be used with new, expensive precipitants being synthesized at the University of York.

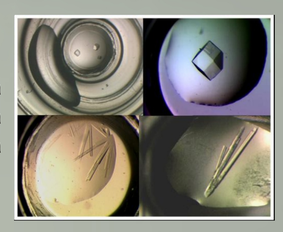
Only 1.2µl mother liquor is needed in the circular well and 150nl protein + 150nl mother liquor in the central ledge.





Magnetic upper and lower holders clamp the microplate during set-up, (mosquito robot).

Crystal nucleation and growth rates are greatly increased with crystals often forming within hours!

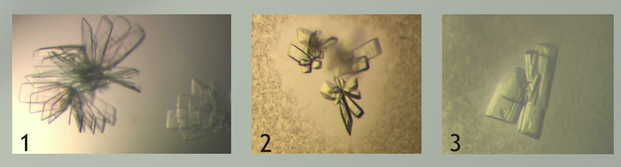


Marek Brzozowski and Justyna Korczyńska (with TTPLabtech).

Buffers, an important variable that can be a problem!

A glycoside hydrolase

*Bt*Man2A was crystallised in Peg 3350, 0.2M NaBr with 0.1M bis-tris propane as buffer (Victoria Money, ref.11). Structure solution revealed a molecule of bis-tris propane in the active site, attempts to obtain ligand complexes with these crystals failed. Eventually crystals were obtained in the same conditions without buffer (1) but the crystals did not diffract. Crystals grown in the conditions plus Hepes pH7 (and various other buffers) did not diffract either. (2)



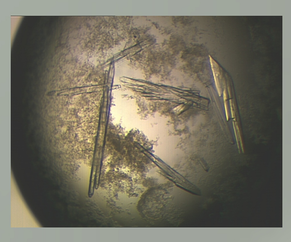
However crystals grown in the same conditions with 0.1M Mes pH6.4 did diffract to 2Å (3). Ligand complexes have been successfully obtained by soaking these crystals. So, in conclusion, even if you have crystals that don't diffract, it is always worth varying pH and the type of buffer used and trying again!

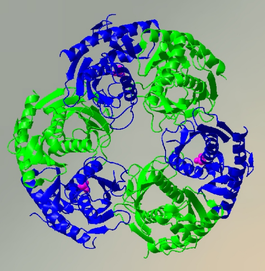
Wendy Offen

Using co-expression and additives in crystallisation

Exosome core complex of *M. Thermoautotrophicum*

- *Mth*Rrp41 and *Mth*Rrp42 form a heterodimer which trimerises to form a hexamer
- *Mth*Rrp41 was insoluble when expressed individually.
- Co-expression produced a soluble protein complex.
- Crystals grew in clusters that could not be tested for diffraction.
- Crystal growth was improved by adding 5% isopropanol and data were collected to 2.65Å.





Drop of crystals used in data collection. Ugly but quality!

Leong C.Ng

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References:
1) Ng, C.L., et al (2005) EMBO Rep **6**, 140-146.
2) Alzari, P.M. et al (2006) Acta Cryst. D**62** 1103-1113
3) Jancarik, J. and Kim, S.H. (1991) J. Appl. Cryst. **24**, 409-411
4) Andrzej Marek Brzozowski and Julia Walton (2000) J. Appl. Cryst **34**, 97-101
5) Stura, E.A. et al (1992) J. Cryst. Growth **122**, 273-285
6) Page, R. et al (2003) Acta Cryst. D**59**, 1028-10
7) Jancarik, J. et al (2004) Acta Cryst D**60**, 1670-1673
8) http://www.pims-lims.org
9) http://www.ysbl.york.ac.uk/people/19.htm (Julie's work web pages).
10) Korczyńska, J. et al (2007) Acta Cryst. **D63**, 1009-1015.
11) Tailford, L.E. et al (2007) JBC **282**(15), 11291-11299

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