**Protein Crystallisation at York**

**Ongoing developments & personal stories**

Shirley M Roberts (on behalf of the group)

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### Scrambling around for crystals

- **Human IODA2 (a member of the IODA family)** of calcium-modulated binding proteins has a role in inflammation and host parasite response and is linked to major diseases such as diabetes, cataract, rheumatoid arthritis and rheovirus.
- Similar to other 100 protein, IODA2 binds two in addition to calcium, the zinc binding enhances the calcium affinity by a factor of 100.

- **Crystalisation did not occur after 0.5-3mM zinc was added** directly to 10mg/ml protein and a range of methods indicate the protein was aggregating at this zinc conc. However, when IODA2 was diluted to 0.1 molar (5-6M) it buffer to 0.5 molar aqueous and concentrated to 10 mg/ml, crystals were obtained. The procedure was repeated three times to achieve complete saturation with zinc whilst avoiding aggregation problems.

- **The protein/complex crystal grew in two crystal form** (P22212 and P222) which diffraction to 1.2 and 1.7 A resolution respectively. The zinc complex structure represented an explanation for the two-shown 1.5mM zinc increase in calcium affinity and provided insight into the role of a calcium-salt complex in the transition of IODA2 from a dimer through a trimer to a hexameter.

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### Structural Biology at York: various robotics facilitate the workflow

- **Preparation of crystallisation screens:**
  - Stop-kiss (Taran Lazarus).
  - Protein Molecule bot (Taran Lazarus Ltd).

- **Scrambling around for crystals:**
  - **Crystallisation Screens used in YSB:**
    - Index from Hampton Research (HR).
    - Silver Keto and Helix-HT (HR).
  - **Crystallisation Screens and 1.2, 2, and 3 mM zinc (HR).**
  - **Crystal Screens 1 and 2, Hired in York (HR).**
  - **Pick from Molecular Dicordance (MD) Clear Screen Scan 1 and 2 (MD).**
  - **PAPA (MD).**
  - **Morphos (HR).**
  - **Variation of the YSB Index from Hampton Research (HR).**
  - **ICSD and ICSD (ICSD).**
  - **Molecule and Protein crystal Screens used in YSB (HR).**
  - **Ammonium Sulfate index (HR).**

- **Picking up the crystals:**
  - **Setting up the cryotransfation:**
    - **Cryotransfer Imager:**
      - **ThermoFluo: an aid in protein ligand studies:**
        - A fluorescence-based thermal assay (ThermoFluo) is being used to assign the affinity and identification of ligands which bind to protein.
        - An environmentally sensitive fluorescent dye, such as SYPRO orange, is used to monitor the unfolding of the protein as the temperature is increased. Using a sXRC machine e.g. Synergy H4 Multiwell, the temperature increase and the protein unfolding is then an increase in excised hydrophobic residue proteins seen in an increase in fluorescence. The fluorescence spectra can be used to calculate the melting temperature, Tm, of the protein.

- **The finding of ligand crystals:**
  - Final step of the process is to identify crystals that can stabilise the protein and therefore increase the melting temperature. **In the presence of the protein in a propionic acid (PA), the melting temperature of the protein, TolB, was found to increase 2°C.**

- **The technique can also test the crystallisation of proteins if problems occur.** The melting temperature is monitored for protein as a sign of different buffers, ligand and additives to identify conditions in which the protein is most stable

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### Protein heterogeneity uncovered by ESI-MS

- **Enzyme was selected for crystallisation and bisected:**
  - It proved easy to express and purify and was amenable to X-ray crystallography using various synthetic scaffolds and fragments.

- **Subsequent optimisation and subsequent optimisation yielded small metal-like crystals:**
  - The low space group results in a non-merohedric crystal.

- **Unification was achieved after cloning the gene and expressing it in both active sites:**
  - Further optimisation for crystallisation led to a non-merohedric crystal used in combination with high affinity ligands resulting in crystals suitable for data collection and X-ray crystallography.

- **Identification of the enzyme:**
  - **ThermoFluo:**
    - A fluorescence-based thermal assay is being used to assign the affinity and identification of ligands which bind to protein.
    - An environmentally sensitive fluorescent dye, such as SYPRO orange, is used to monitor the unfolding of the protein as the temperature is increased. Using a sXRC machine e.g. Synergy H4 Multiwell, the temperature increase and the protein unfolding is then an increase in excised hydrophobic residue proteins seen in an increase in fluorescence. The fluorescence spectra can be used to calculate the melting temperature, Tm, of the protein.

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### Ongoing developments & personal stories

- **A cross-counterintuitive solution to a Mn problem:**
  - **The growth conditions for a large protein produced ([69S]Mn ATP) was examined:**
    - It was found that manganese concentration and addition is required.
    - The manganese concentration and addition was required to stabilise the protein.

- **Preparation of crystallisation screens:**
  - **Stop-kiss (Taran Lazarus).**
  - **Protein Molecule Bot (Taran Lazarus Ltd).**

- **Scrambling around for crystals:**
  - **Crystallisation Screens used in YSB:**
    - **Index from Hampton Research (HR).**
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  - **Crystallisation Screens and 1.2, 2, and 3 mM zinc (HR).**
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  - **Setting up the cryotransfer:**
    - **Cryotransfer Imager:**
      - **ThermoFluo: an aid in protein ligand studies:**
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- **The finding of ligand crystals:**
  - **Enzyme was selected for crystallisation and bisected:**
    - It proved easy to express and purify and was amenable to X-ray crystallography using various synthetic scaffolds and fragments.

- **Correlation was found between the crystal space group and the protein crystallography:**
  - The low space group results in a non-merohedric crystal.

- **Unification was achieved after cloning the gene and expressing it in both active sites:**
  - Further optimisation for crystallisation led to a non-merohedric crystal used in combination with high affinity ligands resulting in crystals suitable for data collection and X-ray crystallography.

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