VMXm: A new micro/nanofocus protein crystallography beamline at Diamond

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Introduction

- Target proteins are getting more complex, leading to smaller and more disordered crystals
- Can design ideal experiment to give optimal data quality

Current Limits

- 2.2 Å data can be collected from 1 µm³ crystals (~700 well diffracting crystals)
- 3 Å data can be collected from 5 µm³ membrane protein crystals grown in LCP (~35 crystals, grid scanned first for centring)
- From theoretical calculations a complete 2 Å dataset can be collected from a single 1 µm³ lysozyme crystal (Holton and Frankel, 2010)
- Discrepancies between theory and experiment
Current Limits

• Dose tolerance of samples cannot be changed – Henderson/Garman limit fixed

• Reduce dose on sample to measure given data quality:
  • Reduce experimental background
  • Cleaner sample mounting
  • Improve analysis for weak and multicrystal data
  • Record rotation data to improve data quality
  • Visualization of micron and sub-micron crystals
VMXm Aims

• Improve signal to noise by reducing background:
  • Sample environment under vacuum
  • Crystals mounted with minimal liquid
  • Reduce beamsize to match that of the crystal
• Standard rotation data collection on samples down to 500 nm
  • Alignment without the need for X-ray raster scanning
• Optimise sample alignment, sample cooling and data analysis for micron and sub-micron crystals
• Data collections using minimal amounts of sample
VMXm Specifications

- 6 – 28 keV energy range
- 0.3 – 10 μm (v) & 0.5 – 5 μm (h)
- Flux
  - $\sim 10^{13} \text{ ph/s in } 0.3 \times 5 \text{ μm (v x h)}$
  - $>10^{11} \text{ ph/s } 0.3 \times 0.5 \text{ μm (v x h)}$
- initially monochromatic beam
- polychromatic beam as an upgrade path
Sample Environment

- **In vacuo** sample environment - reduce X-ray background to a minimum
- Cryo-stage - preserve sample *in vacuo*
- Standard on-axis optical microscope + SEM for sample visualization and alignment
- High stability goniometry - permit rotation data to be measured from micro and nanocrystals
- Serial data collection also possible
SEM
Sample XZ stages
Isoalted cold-stage and gripper
Fluorescence detector
Sample holder
OAV port
Beamstop XY
Goniometer
SED
Sample Preparation

Ideal sample mounting:

- Reduce background to increase signal-noise:
  - Air path around sample
  - Sample mount
  - Solvent surrounding crystal
- Multiple crystals per mount
- Cryo-EM style sample preparation
  - Grids
  - Blotting
  - Plunge freezing
- SEM for sample characterisation
Cryo-EM Grids

Support film

Grid

3 mm
Sample Characterisation
Sample Mounting
RT Measurements in Vacuum

No sample
300 mA, 12.4 keV, 100 % transmission, 0.5s exposure
RT Measurements in Vacuum

Shot through SiN window containing 1M NaAc pH 3.0, 6% PEG 6000, 20% NaCl
500 nm windows x 2, ~10-20 um solvent thickness
300 mA, 12.4 keV, 100% transmission, 0.5s exposure
First User Experiments

- Dr. Ivo Tews and Rachel Bolton
  - Institute of Life Sciences, School of Biological Sciences, University of Southampton

- FutA
  - Space group P21
  - Unit cell 39.2, 77.7, 47.7, 90.0, 97.9, 90.0
  - Approx. crystal size 5 - 10 µm
  - Measured ~5 – 10 degs per crystal

- The first grid measured at cryo temperatures, in vacuum, using a 0.8 x 2.8µm X-ray beam produced beautiful diffraction
FutA: 12.423 keV; 0.1 s, 0.1 deg, 10 % transmission (~5x10^{10} ph/s)

11.45 Å
FutA: 12.423 keV; 0.1 s, 0.1 deg, 10% transmission (~5x10^{10} ph/s)
## Merging statistics by resolution bin

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Refined FW, PHWT map from REFMAC viewed in COOT

Data from 13 FutA crystals integrated and scaled with DIALS and analysed further using CCP4

~80% complete data to 2.4 Å
CPV Ld14

- Spacegroup I23
- Unit cell $a=103$ Å
- Approx crystal size 3 – 4 µm
**CPV Ld14:** 12.423 keV; 0.1 s, 0.1 deg, 100% transmission (~$5 \times 10^{11}$ ph/s)
Acknowledgements

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Dave Butler
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Leon Adams
Simon Lay
Hugo Shiers
Guenther Rehm
Chris Bloomer

Gwyndaf Evans
Jose Trincao
Emma Beale
Adam Crawshaw

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