

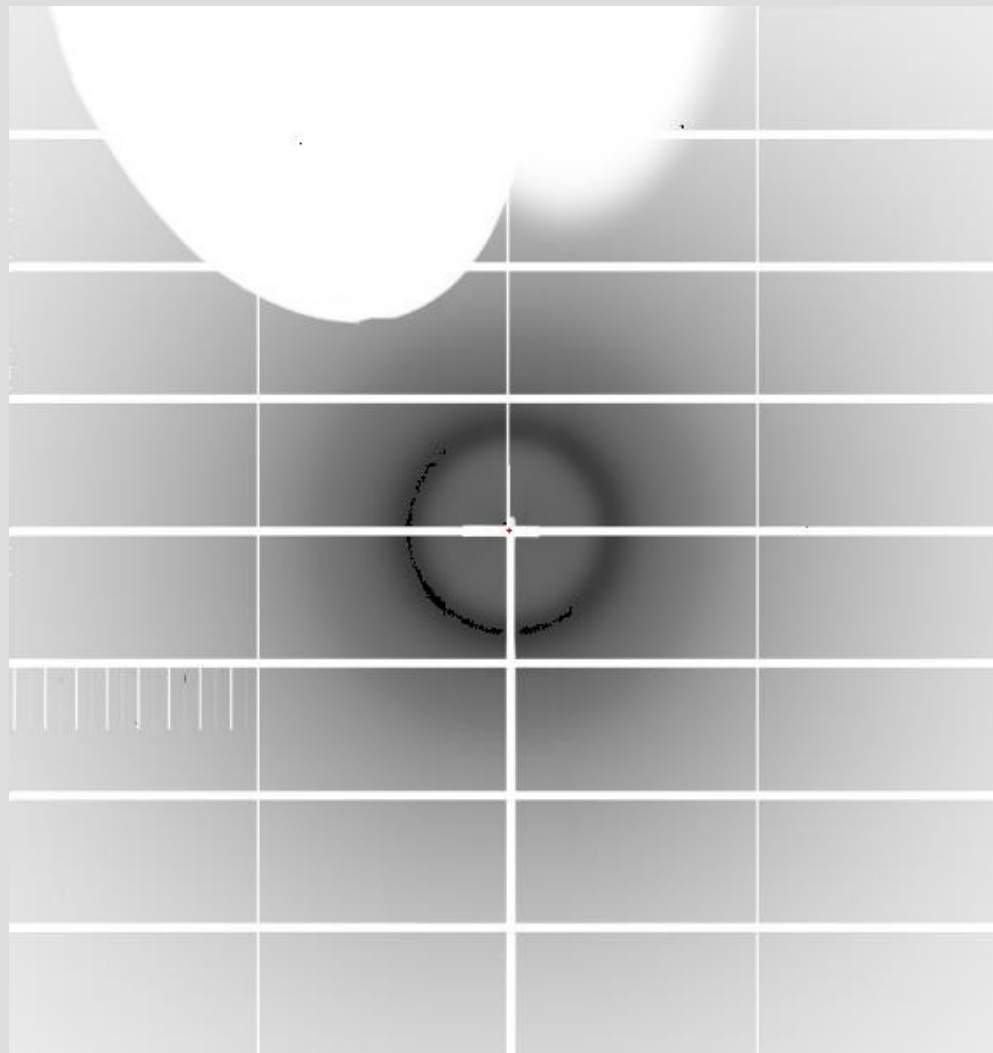
GΦL status

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DESY: P14 Visit 6-7 May 2019 (1)

- **Lessons:**
 - We must always have access to the MxCUBE code running on the beamline before the visit
 - Detector positioning is handled in an unusual way at P14
 - Quick and dirty fix during the visit
 - The Abstract Beamline Interface contract was broken
 - Workflow-generated collection sequences were new to the beamline: images were lost
 - Initial configuration is still hard
 - the camera transforms the coordinate system
 - a software solution is possible
 - Pin shadowing is a problem

Unmodelled shadow



DESY: P14 Visit 6-7 May 2019 (2)

- Achievements:
 - Translation calibration
 - Diffractometer calibration (using cubic insulin and thaumatin)
 - Shadowing images
- This is the furthest that we have got on the initial beamline visit so far.

ALBA: XALOC visit (28-29 May 2019)

- **Diffractionmeter calibration**
 - Our first use of germanate crystals within the MxCUBE collaboration (rather than DLS)
- **First scientific use of the GΦL Workflows**
 - Native strategies on ligand-soaked crystals

Diffractionmeter calibration (1)

- Wrong wavelength used
 - Too much absorption, led to crystal decay
 - Inexperience with handling germanate - next time it will be done better
- Crystal quality variable?
- Centring at many (κ, φ) values
 - We still have not cracked the retention of centring on a mini-Kappa at multiple orientations
 - Unsure of the relative contributions of pin shrinkage and mini-Kappa mechanics

Diffraction calibration (2)

- Shadowing still a problem
 - Pin shadowing more unpredictable than goniostat shadowing
 - Careful sample preparation needed
 - Beamstop shadowing also caused problems
 - Edge effects gave spurious spots
 - Had to be corrected by hand in processing
- Nevertheless, the processing succeeded
 - Some parameters needed to be adjusted to their most permissive values
- Improvements possible for next time

Native data collection strategies

- Strategies specifically designed for known symmetry and orientation of each sample
 - Dose calculation added to MxCUBE by Rasmus, with input from Leigh Carter (GΦL) and Gleb (EMBL-HH)
- Samples provided by José Márquez (EMBL-Grenoble)
 - 5 datasets collected: 2 apo, 3 on ligand-soaked crystals
 - One ligand-soaked crystal had approximately-doubled unit cell dimensions:
 - $a_2 = 2.0 a_1$; $c_2 = 2.1 c_1$

Dose budget in GΦL Workflow UI

The screenshot displays the GΦL Workflow parameters window, titled "GΦL Workflow parameters <@mx3_deb9_qt4_gphl>". The window is divided into several sections:

- Data collection plan:** A text area showing acquisition details:

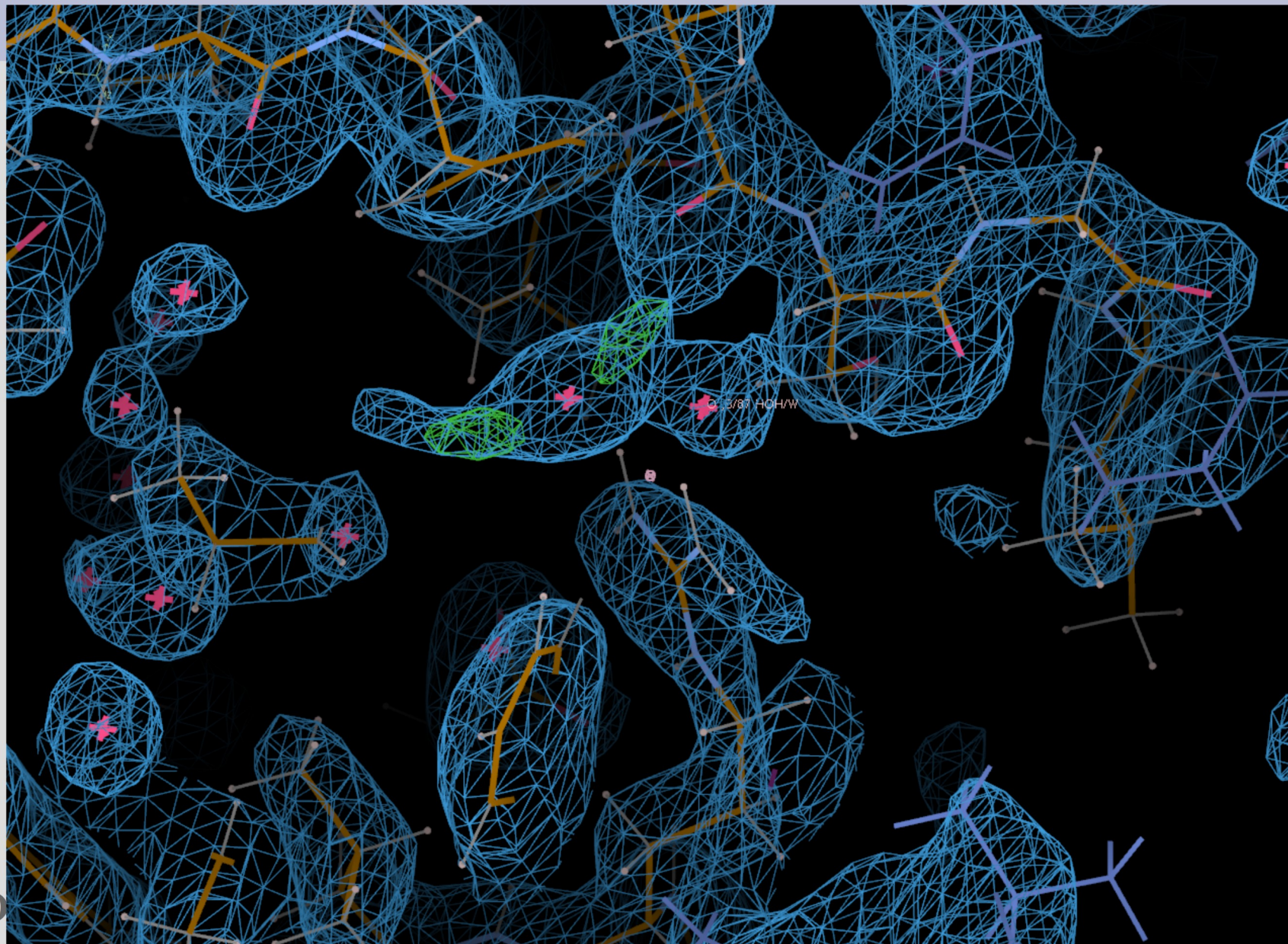
```
Geometric strategy :
- Acquisition      : 720.0 degrees
Total rotation    : 720.0 degrees

Orientation: kappa= 78.3, kappa_phi= -22.0
- sweep phi= 8.9, width= 360.0 degrees

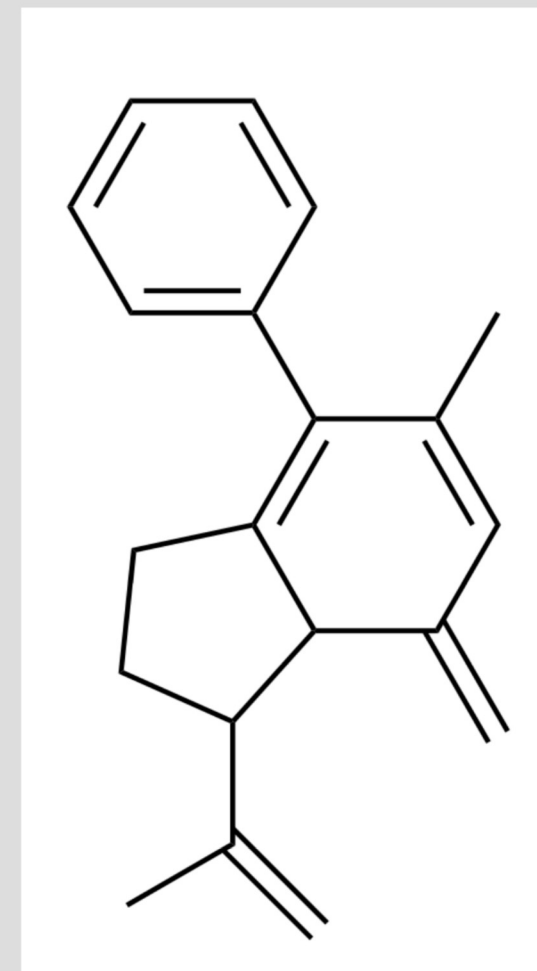
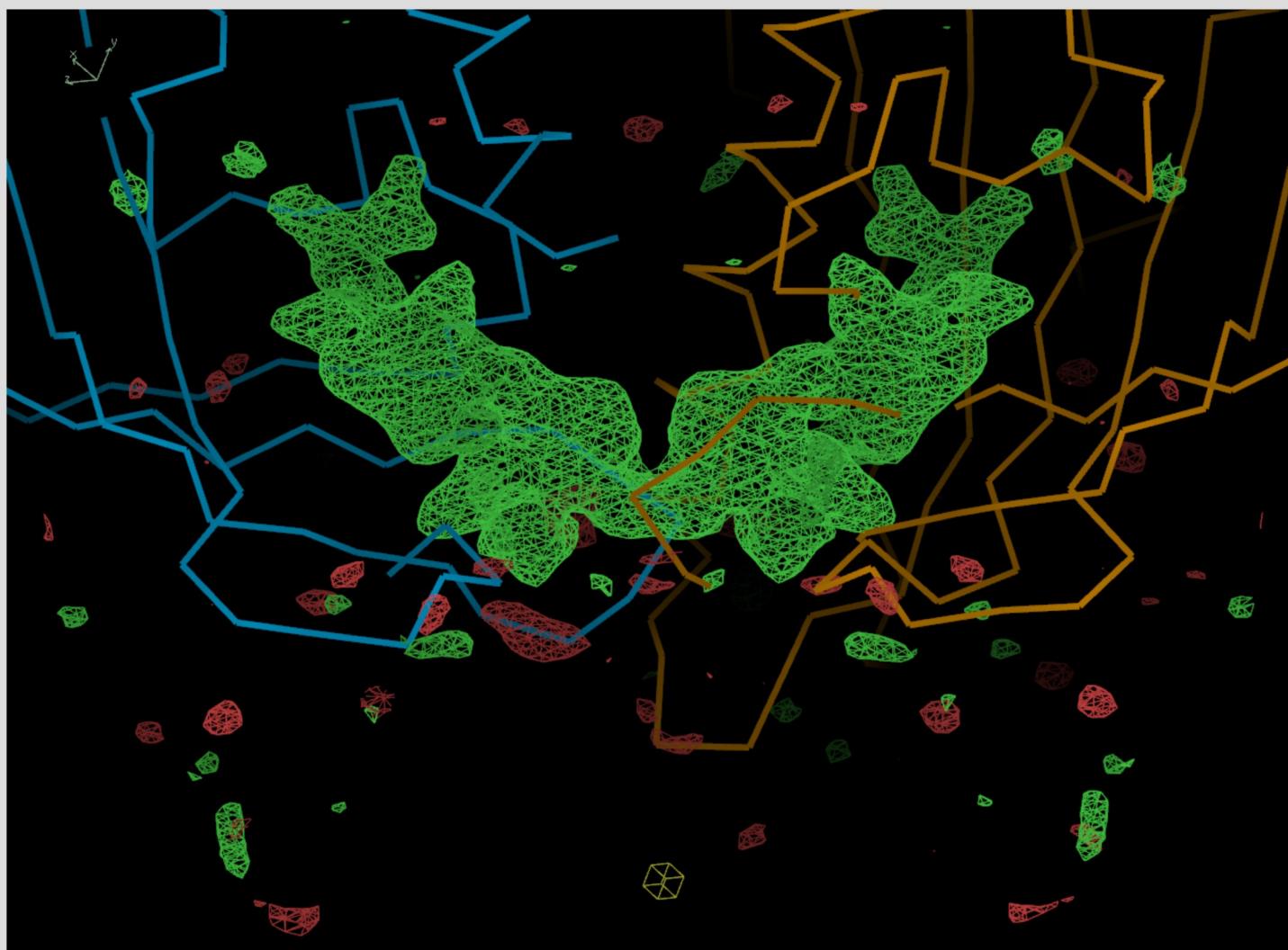
Orientation: kappa= 81.2, kappa_phi= 156.4
- sweep phi= -172.3, width= 180.0 degrees
- sweep phi= 7.7, width= 180.0 degrees
```
- Parameters:** A grid of input fields for various experimental settings:

Oscillation range	0.1	Detector resolution (Å)	1.700
Exposure Time (s)	0.0400	Acquisition beam energy (keV)	12.7000
Transmission (%)	8.99	Wedge width (deg)	15
Experiment duration (s)	288.0	<input checked="" type="checkbox"/> (Re)centre crystal before acquisition start?	
Rotation speed (deg/s)	2.5	<input type="checkbox"/> (Re)centre crystal at the start of each scan?	
Dose budget (MGy)	9.5		
% of dose budget required	100.01		
- Video Feed:** A live video window showing a sample. A red line indicates a 180° rotation. A timestamp "11:57:34" is visible in the bottom right corner of the video area.
- Control Panel:** A vertical toolbar on the right side of the video feed with buttons: Centre, Save, Line, Grid, Focus, Snapshot, Refresh, Align, Select all, Clear all, and Auto.
- Bottom Panel:** Additional controls including "Continue" and "Abort" buttons, and dropdown menus for "Aperture" (set to 5μ) and "Phase" (set to Transfer).

Good map quality



Ligand identification



Diffraction Anisotropy

- The GΦL workflows have an initial characterisation data collection to derive the orientation matrix
 - Normally 6° , but increased to 12° for this ALBA visit
- With Ian Tickle, re-processed characterisation datasets with STARANISO
 - Can diffraction anisotropy be predicted before the crystal's dose budget has been used?

Diffraction Anisotropy

- An example:

Collection	Diffraction limits	B11, B33
Char	1.7, 2.2	22, 51
Main	1.5, 2.0	25, 52

- The symmetry in this case is high, so the direction of anisotropy is constrained.
- The strongest and weakest diffracting directions have been correctly identified from the characterisation dataset alone; for this symmetry that is all that is needed.

- More investigation with low-symmetry systems is needed.