ESRF	**Experiment title:** The crystal structure analysis of *Aleura aurantia* Lectin (AAL) cocrystallized with $HgC1_2$	**Experiment number:** LS-804
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Report: Background: Multiwavelength anomalous dispersion (MAD) data was taken on *Aleuria aurantia* lectin (AAL) cocrystallized with $HgCl_2$. The protein consistently forms large prisms which diffract to high resolution with little radiation damage seen on a rotating anode or at the Photon Factory (PF), Tsukuba Japan, but no isomorphous derivative had been found. MAD data was therefore necessary. Positions of the mercury sites had never been confirmed, as AAL:Hg cocrystals and native AAL form in different crystal systems, orthorhombic C2221 or C222 and hexagonal $P6_{1(5)}22$, respectively, rendering difference Patterson maps meaningless. X-ray absorption fluorescence spectroscopy (XAFS) on the cocrystals had, however, given the anticipated Hg absorbtion near 1.0086Å.

Experimental procedures: XAFS data was taken on ESRF BM14, beamline 19 with the kind assistance of A Thompson, and 3 wavelengths were chosen for crystallographic intensity data collection at 12.2878, 12.3499 and 13.5012keV (1.00901Å, 1.00394Å and 0.918326Å, referred to as Ll , L3 and L4, respectively). Crystallographic data was recorded on a 34.5mm diameter MAResearch area detector at 350mm. Significant radiation damage was observed, therefore, data was taken in 15" blocks with inverse mates at all 3 wavelengths before translating or changing the crvstal. One block of data at one wavelength took about 40

minutes, thus Bijvoet pairs were recorded within 20 minutes of each other. A total of 105" of data plus 105" of inverse mate data was obtained on 6 crystals with significant overlap.

Data analysis: Data analyzed with DENZO and SCALEPACK' gave R_{merge} values for individual crystals at each wavelength ranging from 5.2 to 6.9% at 2.5Å resolution. Merging 2 of the 6 crystals, crystals 1 and 3, gave the best combined statistics (table I), and further analysis was limited to those data.

Table 1: Statistics for diffraction data merging 2 crystals with 2σ cutoff

Data set	R_{merge}	completeness	redundancy
L1	5.9%	96.1%	7.1
L3	5.8%	96.6%	7.2
L4	6.2%	96.3%	6.3

Patterson analysis showed possible preliminary positions only from low density vectors which appeared far down the peak list found by CCP4's RSPS program. High density Patterson peaks were inconsistent and observed only on a single Harker section with no corresponding self vector peaks appearing on other sections, ie: strong peaks which appeared on the v=O Harker section did not have matching vectors on the u=O and w=O (C222) or $w=1/_2$ (C2221) sections, and similarly for strong peaks on u or v sections. Furthermore, positions of consistent weak peaks from any one data set differed between Bijvoet and MAD analyses and between different wavelengths, with many sitting on special positions. From all possible analyses, 3 heavy metal positions from C2221 MAD data using Ll and L3 refined best, however, very poor figures of merit and phasing power were observed. As AAL is active as a dimer with 6 monomers expected in the asymmetric unit, 3 peaks are reasonable for metal binding at a dimer interface. However, difference Fourier analysis using any one of the 3 positions found from the Ll/L3 MAD Patterson maps failed to confirm the 2 other peaks.

Conclusion: The positions of the mercury atoms in the AAL:Hg cocrystals which give rise to XAFS signal could not be reasonably assigned from the present Patterson analysis. Remeasurement of the diffraction data may be necessary.

References:

[1]Z Otwinowski & W Minor (1997) Methods *in Enzymology 276:307-326.*

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