Experimental report on CH-2705 Nov / Dec 2008 9 shifts.
Development of dispersive differenc es phasing using multi-heavy atom c ompounds including use of heavy wat er in protein powder diffraction.

Introduction

Protein powder diffraction continues to excite strong international interest. There are a variety of applications, including industrial protein characterisation, such as polymorphs of insulin [1]. In addition we offer our view [2] that:- "an especially exciting application would be extending structure determination to yet smaller crystal samples, which would otherwise be outside the range of X-ray data collection from a protein microcrystal (typically 20 microns [3]). In effect, in the powder case, the sample volume is not restricted, unlike the microcrystal case, offering a strategy for getting around X-radiation damage." In ref [2], we outlined a $\Delta f'$ Powder Dispersive Difference (PDD) approach to phasing. We initially pursued this at Daresbury SRS beamlines 2.3 [2] and then 9.1. We have now extended these experiments at ID31 (beamtime award CH-2705), which allowed extremely rapid data acquisition compared with Daresbury, as well as the capability for `freeze trapping' of samples, and where we obtained exciting and encouraging results with K_2PtBr_6 bound to specific sites in lysozyme as test.

During these experiments at ID31:-

We were able to show:-

(i) that the freezing of the K_2PtBr_6 soaked HEWL lysozyme samples was essential, since the bleaching of samples over a period of a few hours, observed at Daresbury SRS 9.1, led to the disappearance of the K2PtBr6 from the binding site. Thus, all samples at ESRF ID31were prepared as rapidly as possible, and then frozen, ready for data collection at 80 K. Moreover, the freezing of samples extended their life time in the ID31 X-ray beam, in terms of radiation damage, up to about eight hours.

(ii) We developed an optimised data collection strategy, and where we collected data on six K_2PtBr_6 soaked HEWL lysozyme samples including at the Pt LIII and the Br K absorption edges, and where clear differences in the intensities of peaks on the diffraction patterns compared with that of the native lysozyme were immediately visible.

(iii) We also collected powder diffraction data on the K_2PtBr_6 alone ie as a model compound at wavelengths around the Pt LIII edge.

(iv) We have been able to show the presence of $PtBr6^{2-}$ at the two expected binding sites in lysozyme using Fo-Fc omit maps; see fig 1 Samples 1 and 5, similar results obtained for the other samples.

(v) Moreover, in both isomorphous (for most samples) and $\Delta f'$ difference Patterson maps (for sample 5 only), the highest non-origin peak was found to be at site 1; for the latter see fig 2.

(vi) In parallel we have extended our preparatory single crystal (Cu Kalpha rotating anode RAXIS IV) experiment to freeze quench the K_2PtBr_6 bound to HEWL after 10 minutes soaking to a study at 90 minutes K_2PtBr_6 soaking and to K_2PtBr_6 170 minutes soaking, each followed by freeze

quench. These time-resolved analytical experiments show that a further optimization of the soak time to favour a larger percentage occupancy at each of the two binding sites is possible. Combining these with what we have learnt with the ID31 experiments that the sample 5 dispersive difference Patterson showed site 1 Harker peaks suggests that by soaking longer we can hope to get a more reproducible sample to sample behaviour.

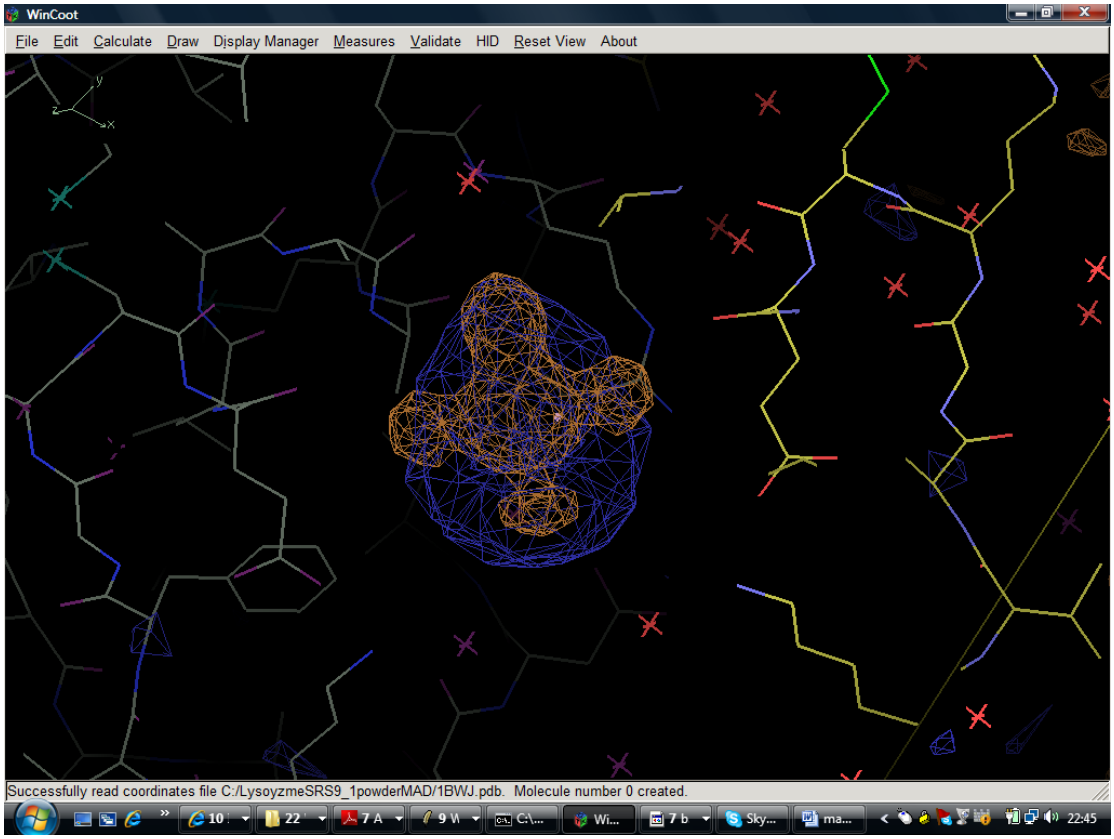
References

[1] I. Margiolaki and J. P. Wright Acta Cryst. (2008). A64, 169-180.

[2] J.R. Helliwell, M. Helliwell and R.H. Jones (2005) Acta Cryst A61, 568-574.

[3] B. Hedman, K.O. Hodgson, J.R. Helliwell, R. Liddington and M.Z. PNAS.USA (1985) 82, 7604-7607.

Fig 1 (a) ID31 K_2PtBr_6 soaked HEWL lysozyme sample 1 refined in REFMAC against 1LZ8 cryo model HEWL [CCP4 'ESRF_ID31_21_DELFWT.MAP' file] (blue) and 10 minutes freeze quench K_2PtBr_6 soaked HEWL lysozyme 1.7Å single crystal study (brown), both maps contoured at 3 sigma.



(b) ID31 K_2PtBr_6 soaked HEWL lysozyme sample 5 higher resolution calculation (3.25 Å resolution); REFMAC DELF map (in green) contoured from 2.1 sigma to show square plane and axial bromines at site 1 details. In brown 10 minutes freeze quench K2PtBr6 HEWL single crystal study contoured at 3 sigma:-

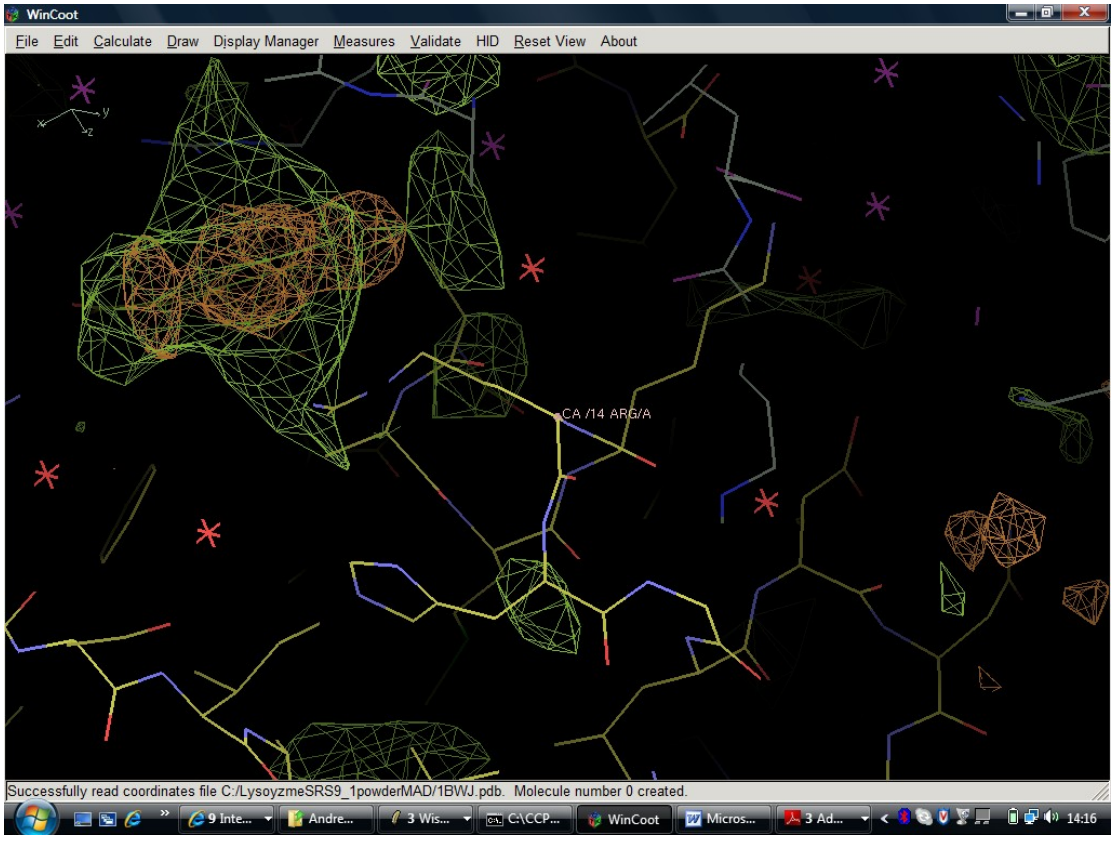


Figure 2 ID31 K_2PtBr_6 soaked HEWL lysozyme Sample 5 delta f ' ie dispersive difference Patterson calculated at 5 Å resolution gives biggest non-origin peak being at site 1.

(a) Peak heights

C:/id31_8keV/ESRF_ID31_206_peaks

Click on line to add/remove initial #

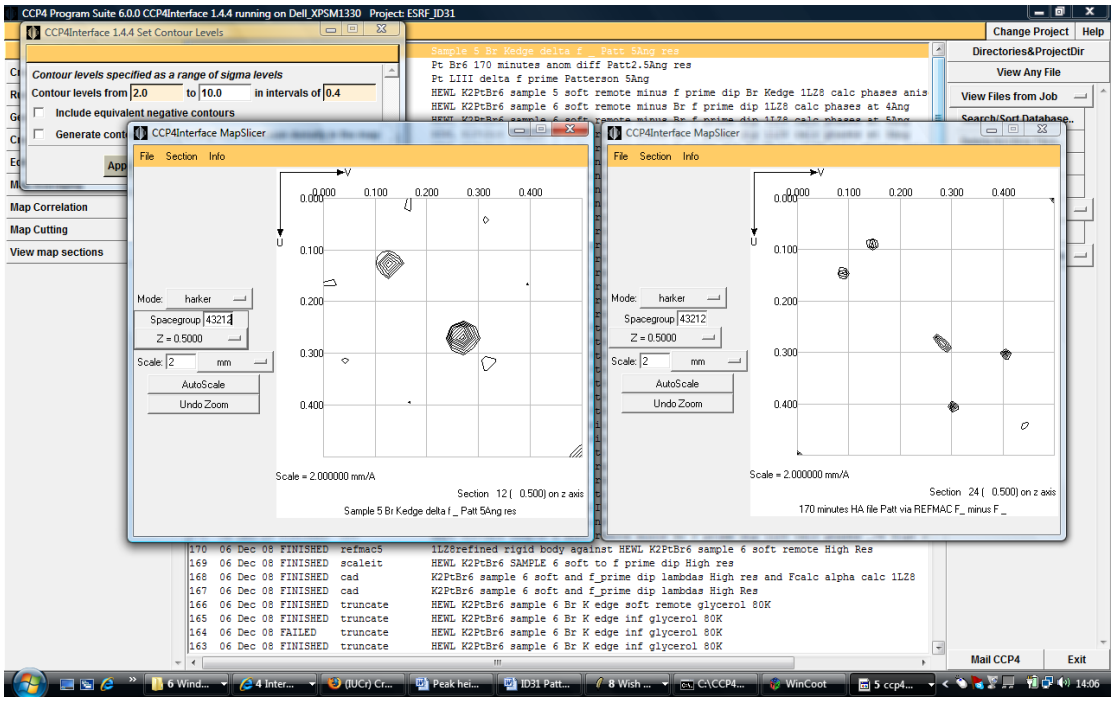
```
GRID   48   48   24
CELL    79.0700    79.0700    37.0600    90.0000    90.0000    90.0000
ATOM1    Ano    0.0000   0.0000   0.0000        23.42   0.0 BFAC   20.0
ATOM2    Ano    0.2679   0.2679   0.5000         5.26   0.0 BFAC   20.0
ATOM3    Ano    0.1270   0.1270   0.5000         4.07   0.0 BFAC   20.0
ATOM4    Ano    0.5000   0.5000   0.5000         3.52   0.0 BFAC   20.0
ATOM5    Ano    0.4257   0.1454   0.0000         3.28   0.0 BFAC   20.0
ATOM6    Ano    0.0000   0.4568   0.1653         3.21   0.0 BFAC   20.0
ATOM7    Ano    0.2709   0.2709   0.3208         3.08   0.0 BFAC   20.0
ATOM8    Ano    0.0000   0.0000   0.3889         3.07   0.0 BFAC   20.0
```

(b) Known coordinates:-
Pt 1 x=-0.14 y= 0.24 z=0.25
Harker peaks include u=0.28 v=0.28 w=0.5

Pt2 x =0.08 y= 0.05 z = 0.81
Harker peaks include u=0.16 v=0.1 w=0.5

(c) left ID31 Dispersive difference Patterson Harker sections, calculated at 5 Åresolution, plotted and compared with, right, single crystal '170 minutes soak', calculated at 2.5Å, anomalous ie F^+ - F^- anom difference Patterson, individual Harker sections as labelled.

w=0.5:-



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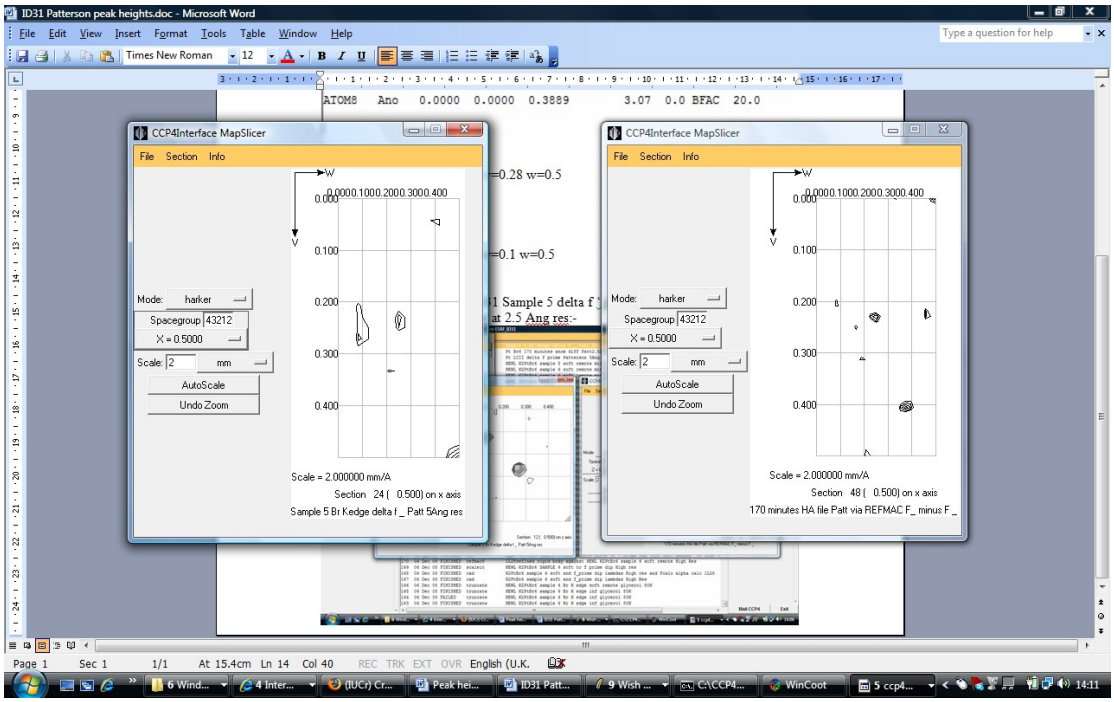
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CCP4Interface MapSlicer
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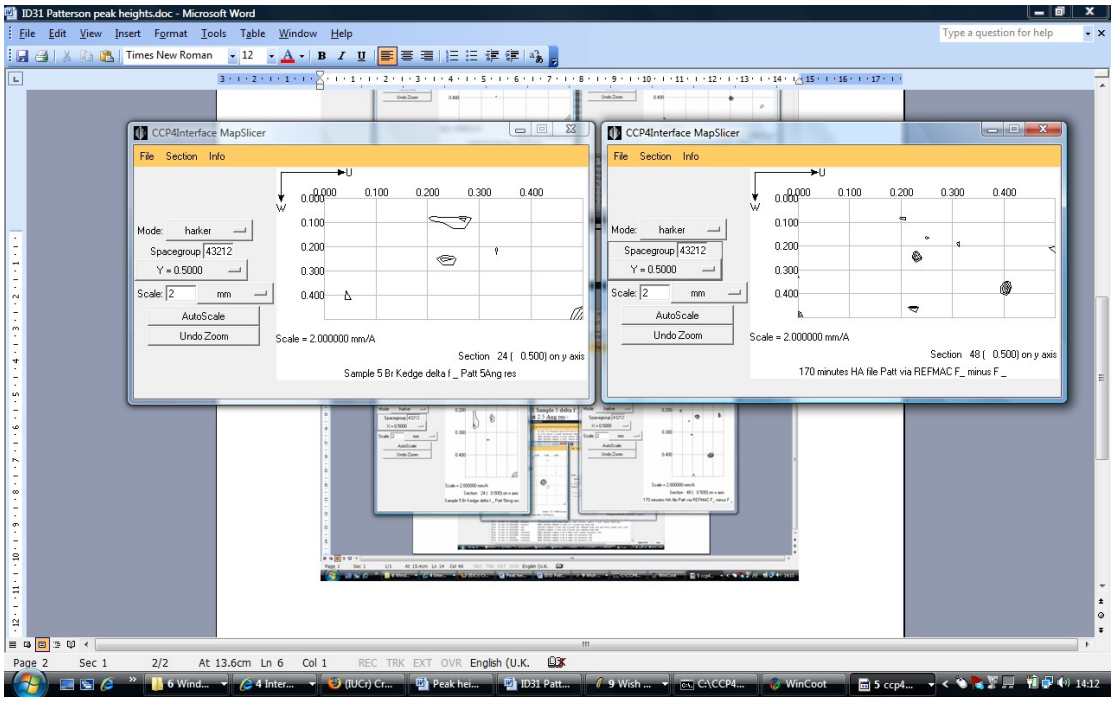
AutoScale
Undo Zoom
Scale = 2.000000 mm/A
Section 24 (0.500) on x axis
Sample 5 Br Kedge delta f _ Patt 5Ang res
Section 48 (0.500) on x axis
170 minutes HA file Patt via REFMAC F_ minus F _

V=0.5:-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Scale = 2.000000 mm/A
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Section 48 (0.500) on y axis
170 minutes HA file Patt via REFMAC F_ minus F _

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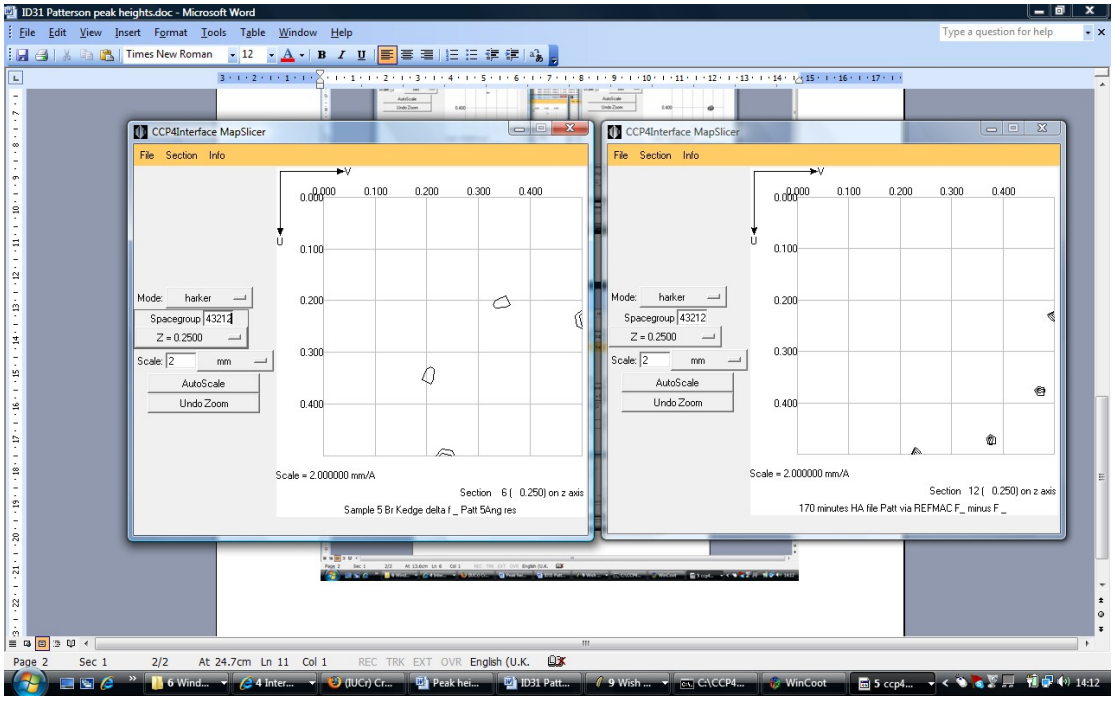
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Scale = 2.000000 mm/A
Section 6 (0.250) on z axis
Sample 5 Br Kedge delta f _ Patt 5Ang res
CCP4Interface MapSlicer
File Section Info
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AutoScale
Undo Zoom
Scale = 2.000000 mm/A
Section 12 (0.250) on z axis
170 minutes HA file Patt via REFMAC F_ minus F _
Page 2 Sec 1 2/2 At 24.7cm Ln 11 Col 1
English (U.K.)
14:12