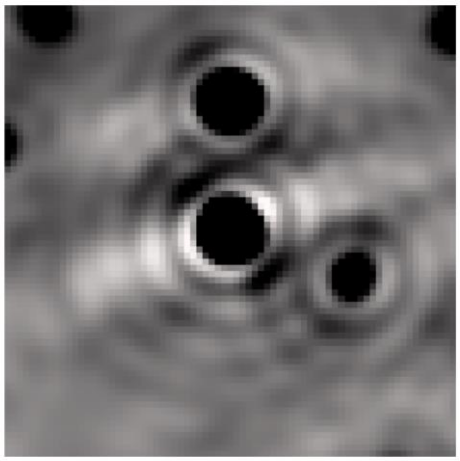
Leucippus Examples

Fourier Truncation Effects

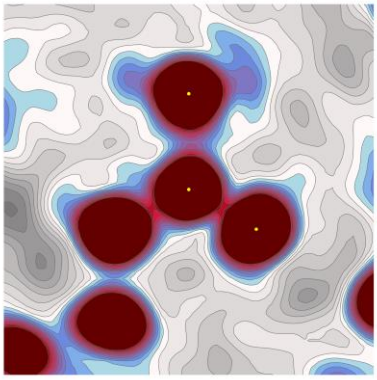
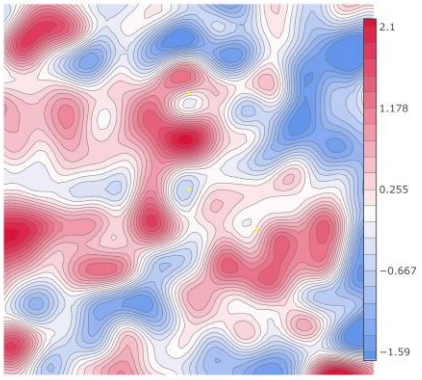


Link to 1yk4 rings

In ultra-high resolution electron density, there can be a visible effect of Fourier truncation in the form of rings around the heavier atoms which leads some to suggest that only balanced density is reliable for making inferences (Afonine et al, 2004), by which they mean Fo-Fc, suggesting 2Fo-Fc is not reliable. In the structure 1yk4, the deposition paper mentions the observation of these ring artefacts (Bönisch et al, 2005). An example of this is shown below with the iron in the centre. It demonstrates the possibility of the observed "unbalanced" density around truncation rings being calculation artefacts. Of course, the balanced density will only be free of the calculation artefact if they exactly balance out in the observed and modelled density.

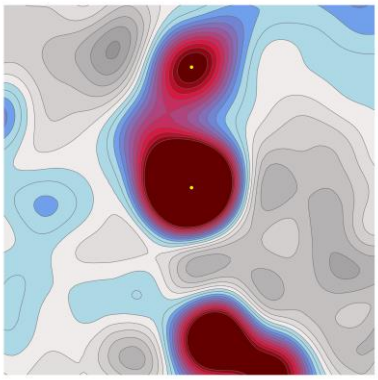
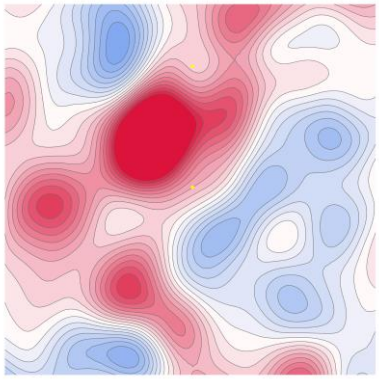
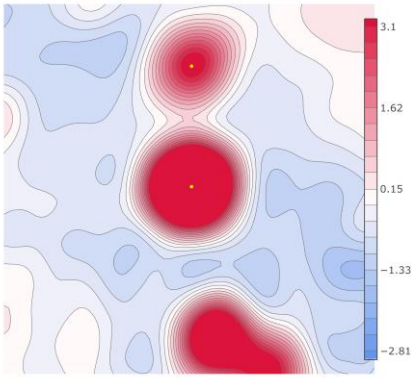
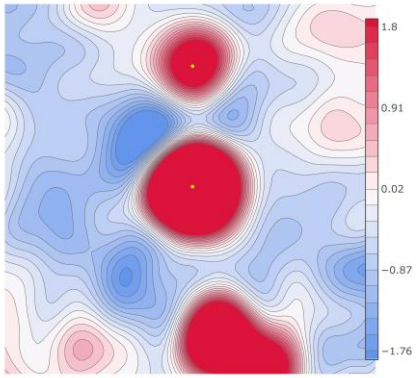
Bond Electrons

The 1yk4 deposition paper suggests that bond electrons are visible between most of the main chain CA-C bonds (Bönisch et al, 2005). One example is show below for residue 6, a cysteine close to the iron core. The plots shown are the 2Fo-Fc density and the Fo-Fc difference density.

Density for CYS 6	Difference for CYS 6
	
Density with bond electrons link	Difference density link

Negative Density

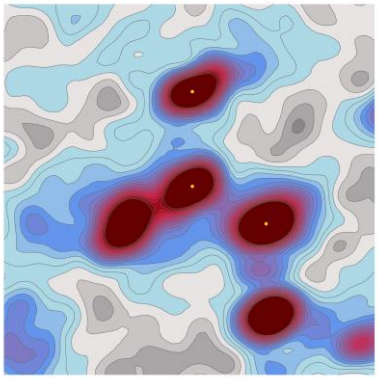
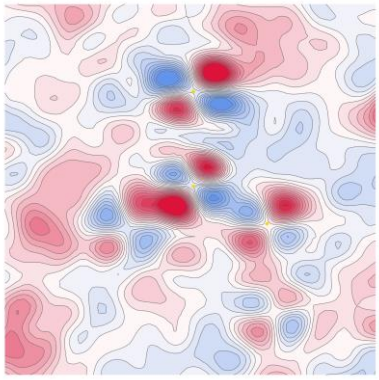
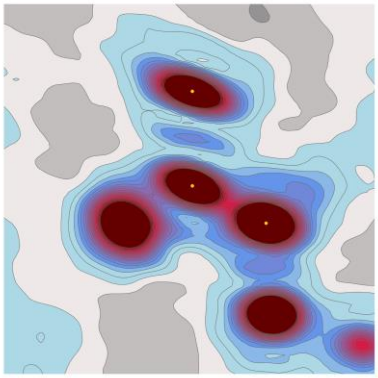
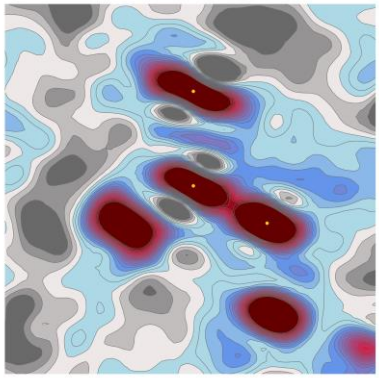
The four views below all show the same frame of density with different multiples of Fo and Fc. The paper about this structure, 3u7z, suggests that the large volume of difference density between CYS100SG and LYS128NZ suggests a missing atom – they suggest oxygen, and that it is a NOS switch (Wensien et al, 2021).

2Fo-Fc shows CYS100SG in the centre and LYS128NZ above. Link 	Fo-Fc shows a large volume of difference density suggestive of a missing atom in the model. Link 
Fo, the observed density. Link 	Fc, the calculated density, notice that there is a large volume of negative density where the difference density is positive. Link 

The difference density is the observed density MINUS the calculated density, so when subtracting a negative number there will be positive density. The apparent missing oxygen could be a calculation artefact or perhaps there is a scaling problem.

Distortion of density

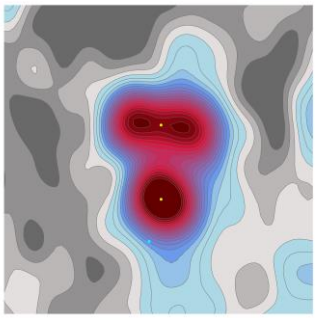
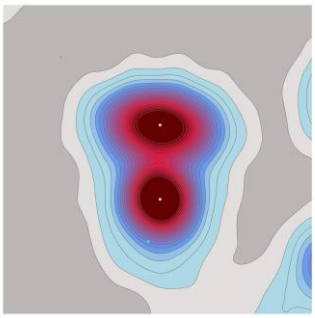
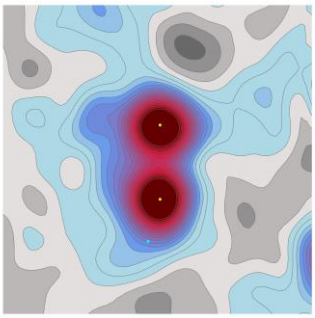
The combination of observed and calculated density introduces the possibility of mutual distortion between the 2 patterns that change the nature of the density quite fundamentally. In the ultrahigh resolution structure of crambin for 1ejg (Jelsch et al, 2000), this example shows how the Fo and Fc combinations change the apparent anisotropic nature of the density. Each frame is the same with different Fo Fc multiples. This shows the peptide bond between A39 THR and A40 CYS.

2Fo-Fc Link 	Fo-Fc Link 
Fo – Link 	Fc – Link 

There is clearly a significant distortion from the effect of the model's shape. In addition to the apparent change in anisotropy, the difference density has lobe areas that could be interpreted as meaningful but might be better interpreted as model artefacts.

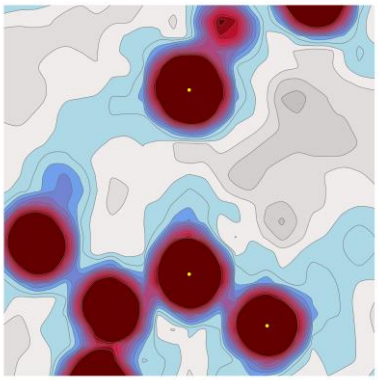
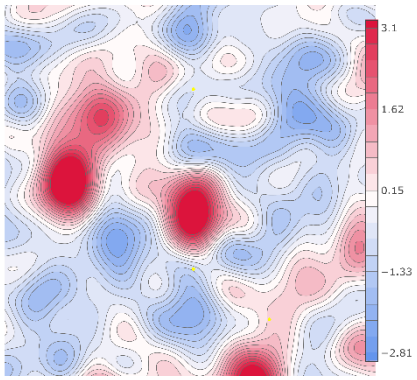
Occupancy

The ability to see the maxima in the density aids interpretation. In this example, where the 2Fo-Fc suggests what appears to be a multiple occupancy, the Fo and Fc densities themselves do not suggest this. All the views are the same with different Fo-Fc multiples. This is structure 6e6o (Finke & Marsh, released 7/8/2019, no deposition paper) and shows the carbonyl oxygen in SER A17.

2Fo-Fc -Link	Fo – Link	Fc – Link
		

Hydrogen Bonds

Ultrahigh resolution x-ray structures can facilitate identification of hydrogen bonds. This can be through apparent density or through difference density, when the positive difference density implies something in reality that was not modelled – aka hydrogen. For this example, I chose a structure for which I knew there were reported hydrogens in the difference density, 1r6j (Kang et al, 2004) and I used Leucippus to try to find an example of hydrogen in the difference density. I arbitrarily navigated to residue 201. I used the **Neighbours** tool to find that 265 N was within hydrogen bonding distance of 201 O, so I navigated the atoms to include 210O and 265N and looked at the difference density between. But, negative density in the Fc is a possible problem.

2Fo-Fc – Link	Fo-Fc – Link
	

References

Afonine, P. V., Lunin, V. Y., Muzet, N., & Urzhumtsev, A. (2004). On the possibility of the observation of valence electron density for individual bonds in proteins in conventional difference maps. Acta Crystallographica Section D Biological Crystallography, 60(2), 260– 274. https://doi.org/10.1107/S0907444903026209

Bönisch, H., Schmidt, C. L., Bianco, P., & Ladenstein, R. (2005). Ultrahigh-resolution study on Pyrococcus abyssi rubredoxin. I. 0.69 Å X-ray structure of mutant W4L/R5S. Acta Crystallographica Section D Biological Crystallography, 61(7), 990–1004. https://doi.org/10.1107/S090744490501293X

Jelsch, C., Teeter, M. M., Lamzin, V., Pichon-Pesme, V., Blessing, R. H., & Lecomte, C. (2000). Accurate protein crystallography at ultra-high resolution: Valence electron distribution in crambin. Proceedings of the National Academy of Sciences, 97(7), 3171–3176. https://doi.org/10.1073/pnas.97.7.3171

Kang, B. S., Devedjiev, Y., Derewenda, U., & Derewenda, Z. S. (2004). The PDZ2 Domain of Syntenin at Ultra-high Resolution: Bridging the Gap Between Macromolecular and Small Molecule Crystallography. Journal of Molecular Biology, 338(3), 483–493. https://doi.org/10.1016/j.jmb.2004.02.057

Wensien, M., von Pappenheim, F.R., Funk, LM. et al. A lysine–cysteine redox switch with an NOS bridge regulates enzyme function. Nature 593, 460–464 (2021). https://doi.org/10.1038/s41586-021-03513-3